

Receptor-tuned Interleukin-2 Mutein Decouples Treg Expansion from ILC2-driven Cutaneous Inflammation

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Abstract

Low-dose interleukin-2 (IL-2) and IL-2 muteins are being developed to expand regulatory T cells (Tregs) for autoimmune disease therapy, but injection site reactions (ISRs) remain frequent and poorly understood. Here we show IL2Mut24, a murine surrogate of IL-2 receptor α (IL-2R α , CD25)-biased IL-2 mutein, enhances Treg expansion yet paradoxically exacerbates cutaneous inflammation compared to wild-type IL-2. Using immunodeficient mice, antibody blockade and adoptive transfer, we identify group 2 innate lymphoid cells (ILC2s) as key drivers of IL-2-induced skin inflammation, defining a CD25-dependent innate activation axis that constrains immune tolerance. In cynomolgus monkeys, the CD25-biased IL-2 mutein efavaleukin alfa promotes greater peripheral Treg expansion than aldesleukin (recombinant human IL-2) but is accompanied by transient increases in IL-5 and dose-dependent ISRs, indicating conservation of this innate inflammatory program across species. To improve the therapeutic window, we engineer receptor clamps by linking IL2Mut24 to antibodies against CD25 to restrict IL-2 access to CD25. This receptor-tuned IL-2 preserves Treg selectivity, suppresses ISRs, and outperforms IL2Mut24 in experimental autoimmune encephalomyelitis by restraining Th17 responses. These findings reveal a conserved innate mechanism underlying IL-2–associated toxicity and establish receptor-tuning as a strategy to improve the safety and efficacy of IL-2–based immunotherapy.

Introduction

Interleukin-2 (IL-2) is a pleiotropic cytokine that orchestrates immune activation and immune tolerance (1). Its biological outcome is dictated by differential expression of the IL-2 receptor (IL-2R) across immune subsets. IL-2 signals through either an intermediate-affinity receptor composed of IL-2R β (CD122) and the common γ chain (γ c), or a high-affinity receptor that additionally includes IL-2R α (CD25) (2). Regulatory T cells (Tregs) constitutively express CD25, allowing them to efficiently scavenge limited IL-2 through the high-affinity IL-2R and maintain immune tolerance (3). This receptor hierarchy underlies low-dose IL-2 strategies designed to preferentially expand Tregs in autoimmune and inflammatory diseases (4, 5).

Despite consistent Treg expansion in clinical studies, injection-site reactions (ISRs) remain among the most common adverse events reported across IL-2-based therapy programs (6-11). These reactions present as erythema, induration or painful nodules at the site of administration and occasionally necessitate dose reductions or treatment discontinuation (12, 13). However, the cellular mechanisms driving IL-2-associated cutaneous inflammation remain poorly defined despite their prevalence.

To enhance Treg selectivity and improve pharmacokinetics (PK), engineered IL-2 molecules incorporate attenuating mutations and long-acting formats such as PEGylation (14) or fragment crystallizable (Fc)-fusion constructs including efavaleukin alfa (AMG592) (15). The V91K substitution in AMG592 reduces signaling potency and increases dependence on CD25 engagement, thereby favoring Treg expansion over activation of effector T cells and NK cells (15, 16). IL2Mut24, a murine IL-2 mutein bearing mutations

at the IL-2R β interface, similarly confers CD25 bias and enhances Treg selectivity *in vivo* (17). Whether such CD25-biased designs modify inflammatory signaling within tissues and thereby influence ISR liability remains unresolved.

Although IL-2 was initially believed to primarily act on T cells and natural killer (NK) cells, recent studies have identified group 2 innate lymphoid cells (ILC2s) as an additional IL-2-responsive population (6, 18). Upon activation, ILC2s can express high levels of CD25 and produce type 2 cytokines such as IL-4, IL-5 and IL-13, contributing to allergic inflammation and immune homeostasis (19). Low-dose IL-2-anti-IL-2 antibody complexes activate ILC2s and IL-5-dependent eosinophilia (6, 7), and wild-type (WT) IL-2 can induce CD25-dependent activation of innate immune cells including ILC2s, promoting cutaneous inflammation (7). These observations implicate CD25-dependent innate pathways in IL-2-driven inflammatory responses.

In this study, by directly comparing wild-type and CD25-biased IL-2 variants in mice and cynomolgus monkeys, we uncover a CD25-dependent innate inflammatory axis driven by ILC2 activation that constrains IL-2 tolerability. We further show that receptor-guided engineering can uncouple immune regulation from inflammatory toxicity, establishing a design principle for next-generation cytokine therapies.

Results

CD25-biased IL2Mut24 amplifies cutaneous inflammation

To model and investigate IL-2-associated ISRs observed clinically, C57BL/6 mice were injected subcutaneously (s.c.) twice weekly for four weeks with PBS or mouse surrogate Fc-fused IL-2 proteins (Fc-IL2Mut24 or Fc-IL2WT) (Figure 1A). Repeated dosing with Fc-IL2Mut24 induced severe cutaneous inflammation characterized by

erythema, swelling, hair loss, and occasional ulceration, resulting in significantly elevated ISR scores (Figure 1B, C). In contrast, Fc-IL2WT induced only mild to moderate reactions, and PBS-treated controls displayed no visible inflammation (Figure 1B, C). A similar differential response to Fc-IL2Mut24 versus Fc-IL2WT was also observed in BALB/c mice (Supplemental Figure 1A-C).

PK analysis revealed prolonged plasma persistence of Fc-IL2Mut24 relative to Fc-IL2WT (Figure 1D). Tissue biodistribution analysis over 72 hours after a single injection further demonstrated sustained accumulation of Fc-IL2Mut24 in both injection site and non-injection site skin (Figure 1E, F). These findings suggest that enhanced and prolonged local exposure contributes to the severity and persistence of Fc-IL2Mut24-induced cutaneous inflammation.

To assess the impact of host immune context in the induction of ISRs, we compared responses across Th1-biased C57BL/6 (20), Th2-biased BALB/c (20) and autoimmune-prone C3H/HeJ strains (21). Fc-IL2Mut24 induced ISRs in BALB/c at a low dose (28.9 pmol/dose), while C57BL/6 mice were largely resistant (Supplemental Figure 2A-C). At a higher dose (289 pmol/dose), both strains developed substantial inflammation without significant changes in body weight, with C57BL/6 mice exhibiting greater ISR severity (Supplemental Figure 2C, D). C3H/HeJ mice developed particularly severe ISRs following Fc-IL2Mut24 treatment, including progressive hair loss and ulceration, accompanied by >10% body weight loss (Supplemental Figure 2E-G).

Collectively, these results demonstrate that CD25-biased IL-2 mutein amplifies cutaneous inflammatory responses across genetically distinct backgrounds, with severity

modulated by dose and host immune context. Therefore, Fc-IL2Mut24-induced ISR provides a reproducible model of IL-2-associated skin toxicity.

CD25-biased IL2Mut24 drives parallel expansion of Tregs and type 2 innate immune responses

To define the immune dynamics underlying the distinct ISR profiles of Fc-IL2Mut24 and Fc-IL2WT, we performed a time-course analysis following a single subcutaneous administration of 116 pmol selected from a previously established Treg-expanding range in C57BL/6J mice (Figure 2A) (17). At day 2 post-injection, Fc-IL2WT increased serum IL-6, TNF and IL-4 relative to PBS and Fc-IL2Mut24, consistent with its broader pro-inflammatory activity (Figure 2B–D). In contrast, Fc-IL2Mut24 treatment induced minimal IL-6 or TNF but selectively elevated serum IL-5, suggesting a distinct cytokine bias toward type 2 immunity (Figure 2E).

Analysis of splenic compartments revealed parallel Treg and ILC2 kinetics. Both populations expanded following Fc-IL2Mut24 treatment, peaked at day 4, and contracted toward baseline by day 7, with greater day 4 responses than those induced by Fc-IL2WT (Figure 2F, G). Although less abundant overall, ILC2s showed a particularly pronounced Fc-IL2Mut24 response, whereas Fc-IL2WT induced a smaller response that persisted through day 7. Eosinophils followed similar kinetics, peaking at day 4 and declining by day 7 (Figure 2H). The earlier rise in serum IL-5 is consistent with activation of the ILC2–eosinophil axis preceding maximal cellular expansion.

Collectively, these data indicate that sustained CD25-biased IL-2 signaling drives a temporally distinct immunological trajectory, characterized by transient Treg expansion followed by preferential amplification of ILC2 and eosinophilic responses. This temporal

shift toward type 2-skewed innate activation may underlie the development of ISRs during sustained IL-2 exposure.

Fc-IL2Mut24 induces a CD25-dependent type 2 inflammatory program in skin

To define the local immune response associated with IL-2-induced ISR, we characterized ILC subsets and cytokines in injection-site skin from C57BL/6 mice treated with PBS, Fc-IL2WT, or Fc-IL2Mut24. At 69 pmol, a pharmacodynamically active Treg-expanding dose, Fc-IL2Mut24 also induced more severe ISR than Fc-IL2WT (Figure 3A) (22). Total ILC numbers increased numerically following IL-2 treatment, most prominently with Fc-IL2Mut24 (Figure 3B). CD25⁺ frequencies increased selectively among ILC2s, with the strongest response to Fc-IL2Mut24, but were unchanged among ILC1s and ILC3s (Supplemental Figure 3A–C). Both IL-2 proteins increased cutaneous CD25⁺ Tregs and ILC2s: Treg accumulation was comparable while Fc-IL2Mut24 elicited greater ILC2 accumulation, leading to a lower Treg:ILC2 ratio (Figure 3C–E). A similar ILC2-biased response was observed in the lung, where ILC2s were the predominant expanding ILC population and showed the most pronounced increase in CD25 expression following IL-2 treatment (Supplemental Figure 3D–G).

Skin cytokine profiling showed treatment-associated elevation in TNF, IFN γ , IL-6 and IL-5 (Supplemental Figure 3H–K). Only Fc-IL2WT significantly increased IL-4 relative to PBS, whereas IL-13 induction did not differ between IL-2 molecules (Supplemental Figure 3L–M). Among type 17-associated cytokines, IL-17A and IL-23 showed upward trends, whereas IL-17A/F and IL-22 were unchanged (Supplemental Figure 3N–Q). Together, these findings identify ILC2s as the most CD25-responsive ILC subset in injection-site skin and support a CD25-dependent type 2 inflammatory program.

To distinguish the requirement for CD25-dependent signaling from that of the IL-5-eosinophil effector axis, C57BL/6 mice were treated with Fc-IL2Mut24 in combination with anti-IL-5, anti-CD25, or isotype control antibodies (Figure 3F). IL-5 neutralization modestly reduced ISRs, whereas anti-CD25 completely prevented ISRs (Figure 3G, Supplemental Figure 4A). Histopathology confirmed that only anti-CD25 significantly reduced cutaneous inflammation (Figure 3H; Supplemental Figure 4B–C). Consistently, cytokine profiling of skin tissue showed minimal reduction in IL-1 β , TNF and IL-5 following anti-IL-5 treatment, but near-complete suppression with anti-CD25 (Supplemental Figure 4D–F). Splenic immune profiling further revealed that anti-IL-5 treatment selectively eliminated eosinophils whereas anti-CD25 reduced both ILC2s and eosinophils (Figure 3I, J). Thus, CD25-dependent signaling is a central requirement for Fc-IL2Mut24-induced ISR, whereas the IL-5-eosinophil axis contributes to, but does not fully account for, the inflammatory response, consistent with previous reports (7).

ILC2 drive IL-2-induced cutaneous inflammation independent of adaptive immunity

To determine whether adaptive immunity was required for ISR development, *Rag2*^{-/-} mice, which lack T and B cells, were treated with IgG-IL2Mut24, generated by linking IL2Mut24 to a non-targeting mouse IgG (Supplemental Figure 5A). IgG-IL2Mut24 induced ISRs in C57BL/6 mice similarly to Fc-IL2Mut24 (Supplemental Figure 5B, C). Notably, *Rag2*^{-/-} mice developed more severe ISRs than immunocompetent C57BL/6, indicating that innate immune cells are sufficient to mediate ISR and that Tregs may partially restrain pathology in C57BL/6 mice (Supplemental Figure 5B, C). In contrast, *Rag2*^{-/-} γ c^{-/-} mice, which lack all CD132 (γ c)-dependent lineages including ILC2s, failed

to develop ISR upon IgG-IL2Mut24 treatment, indicating that γ c-dependent innate lymphocytes are required for ISR development (Supplemental Figure 5B–C) (23).

To directly assess the contribution of Tregs in restraining ISR severity, T cells from Foxp3-DTR donor mice were transferred into *Rag2*^{-/-} recipients before repeated IgG-IL2Mut24 administration (Supplemental Figure 6A), allowing selective depletion of Foxp3⁺ Tregs within a reconstituted T cell compartment after ISR onset. Diphtheria toxin (DT)-mediated Treg depletion resulted in progressive worsening of ISR and substantial body weight loss compared with non-depleted recipients (Supplemental Figure 6B–D). These findings indicate that Tregs within the transferred T cell population limit ISR progression and systemic morbidity, although their presence was insufficient to prevent ISR development.

To define the cytokine pathways contributing to Fc-IL2Mut24-induced ISR independently of adaptive immunity, we next performed antibody-blockade studies in *Rag2*^{-/-} mice, with anti-CD25 included as a control (Figure 4A, Supplemental Figure 7A). Neutralization of IFN γ , IL-17A, or IL-4 did not attenuate ISR, whereas IL-5 blockade provided partial protection and IL-13 blockade produced the greatest attenuation among the cytokines tested (Figure 4B, Supplemental Figure 7B–D). Consistent with its effect on ISR severity, IL-13 blockade reduced IFN γ , TNF, IL-6, and IL-1 β in injection-site skin and decreased the frequency of CD25⁺ ILC2s without significantly altering total ILC2 numbers (Figure 4C–D, Supplemental Figure 7E–H). Anti-CD25 also completely prevented ISR development in *Rag2*^{-/-} mice (Supplemental Figure 7B–C). Together, these findings identify CD25-dependent innate activation as a key driver of ISR, with IL-13 acting as a prominent effector and the IL-5-eosinophil axis serving as a contributory amplifier.

To determine which γ c-dependent innate lymphocyte population could restore susceptibility to ISRs, IL-33-expanded ILC2s harvested from the lungs and IL-15 plus IL-12-expanded ILC1s and NK cells isolated from the livers of *Rag2*^{-/-} mice were adoptively transferred into *Rag2*^{-/-} γ c^{-/-} mice, followed by Fc-IL2Mut24 administration (Figure 4E, Supplemental Figures 8A–B, 9A–C) (24, 25). In addition, *Rag2*^{-/-} mice receiving Fc-IL2Mut24 served as a control (Figure 4E). Among the populations we tested, only engrafted ILC2s restored ISR development in *Rag2*^{-/-} γ c^{-/-} recipient mice, whereas transfer of neither ILC1s nor NK cells did (Figure 4F, Supplemental Figures 8C and 9D–E). Inflammation in ILC2-reconstituted recipients localized to injection sites, unlike the more widespread reactions observed in *Rag2*^{-/-} mice receiving Fc-IL2Mut24 (Supplemental Figure 8C). The transferred ILC2s were functionally active, as evidenced by downstream eosinophil expansion (Figure 4G–I). Notably, Fc-IL2Mut24 not only expanded eosinophils but also recruited neutrophils in ILC2-transferred *Rag2*^{-/-} γ c^{-/-} mice (Figure 4J). Consistent with these cellular changes, elevated levels of IL-5, IL-6, TNF and Keratinocyte Chemoattractant/Growth-Regulated Oncogene (KC/GRO) were detected in the skin lysates of *Rag2*^{-/-} mice, as well as ILC2-recipient *Rag2*^{-/-} γ c^{-/-} mice upon Fc-IL2Mut24 injection (Supplemental Figure 8D–G).

Collectively, these findings establish CD25-dependent innate signaling as a critical driver of IL-2-induced cutaneous inflammation. CD25⁺ ILC2s function as upstream initiators of a cytokine network that recruits eosinophils and neutrophils, defining an innate inflammatory circuit that constrains IL-2 tolerability.

CD25-biased IL-2 mediates innate activation and cutaneous inflammation in cynomolgus monkeys

To assess whether the CD25-dependent innate activation axis identified in mice is conserved in a translational setting, we evaluated AMG592, a human IL-2 mutein engineered to preferentially engage CD25, in cynomolgus monkeys (16). Aldesleukin (recombinant human IL-2) was included as a clinical comparator to distinguish the pharmacodynamic (PD) consequences of CD25-biased versus conventional IL-2 receptor engagement (Figure 5A).

In study 118075, male cynomolgus monkeys received two treatment cycles of AMG592 or aldesleukin. Expansion of circulating Tregs was observed following the second dosing cycle, which used higher doses, reaching maximal levels 5 to 8 days later (days 18, 19 and 21) (Figure 5B). AMG592 also induced a marked increase in serum IL-5 levels following second dosing, peaking around days 17-18 during the second cycle and resolving thereafter (Figure 5C). Across the tested dose range, AMG592 produced a higher Treg response and IL-5 peak than aldesleukin (Figure 5B, C). However, aldesleukin induced broader cytokine responses, including IFN γ , GM-CSF, IL-10, and IL-6, whereas AMG592 triggered minimal increases in these cytokines (Figure 5D–G). Together, these data indicate that CD25-biased IL-2 signaling is associated with selective IL-5 induction followed by robust Treg expansion, consistent with observations in the murine model (Figure 2).

In a separate repeated-dose toxicology study (study 118065), histopathologic evaluation of subcutaneous injection sites after three months of weekly AMG592 dosing in cynomolgus monkeys demonstrated dose-dependent inflammatory changes localized to the administration site (Figure 5H, I). Epidermal remodeling, characterized by minimal-to-mild hyperkeratosis and acanthosis, increased in both incidence and severity with dose

and mirrored findings observed in murine models. In the dermis, mid- and high-dose animals exhibited increased mononuclear cell infiltrates, with occasional mixed-cell infiltrates at higher exposures. These findings were generally minimal to mild in severity and are consistent with localized immune activation associated with pharmacologic activity.

Together, these data indicate that CD25-biased IL-2 engagement in non-human primates recapitulates the transient type II innate activation and localized inflammatory responses observed in murine models, supporting cross-species conservation of this CD25-dependent inflammatory axis.

Epitope-specific CD25 engagement tunes IL-2 signaling strength and tissue immune responses

To modulate CD25-dependent IL-2 signaling, we generated anti-CD25-IL2Mut24 fusion proteins by fusing IL2Mut24 to three monoclonal antibodies (mAbs) recognizing distinct CD25 epitopes with different antagonistic properties and affinities: 7D4 (non-antagonist, $K_D=0.3$ nM), PC61 (partial antagonist, $K_D=0.3$ nM), and 3C7 (full antagonist, $K_D=7.6$ nM) (Figure 6A) (26). IgG-IL2Mut24 served as a control. Structural modeling revealed that the PC61 epitope partially overlaps with the IL-2-CD25 interface, consistent with partial antagonism, while the 7D4 epitope does not (Supplemental Figures 10A–C). We hypothesized that intramolecular fusion of IL2Mut24 to anti-CD25 antibodies would enforce local co-engagement of CD25 while competitively limiting high-affinity receptor assembly, thereby quantitatively modulating downstream signaling in a clone-dependent manner. This ‘receptor clamp’ strategy was designed to favor the engagement of CD25⁺ Tregs while limiting maximal IL-2 signaling capacity.

IL-2 bioactivity was first assessed in proliferation assays using CD25⁺ CTLL-2 cells, an IL-2-dependent mouse T cell line (Figure 6B). All IL-2 constructs induced dose-dependent proliferation, with magnitude following the rank: Fc-IL2WT > Fc-IL2Mut24 = IgG-IL2Mut24 > 7D4-IL2Mut24 = 3C7-IL2Mut24 > PC61-IL2Mut24. To determine whether this effect required intramolecular colocalization, anti-CD25 antibodies were co-delivered with IgG-IL2Mut24 (Figure 6C). For 7D4 and 3C7, free antibody co-delivery recapitulated their respective fusion activities. In contrast, free PC61 completely blocked IL-2 signaling likely due to its high affinity and antagonism. Thus, IL-2 signaling amplitude is tunable according to antibody epitope and affinity.

Because CD25 expression on ILC2s is dynamically regulated and can be rapidly upregulated following activation (27), we first assessed its kinetics in primary mouse ILC2s. Fc-IL2WT, Fc-IL2Mut24, and IgG-IL2Mut24 progressively increased CD25 surface intensity over 8 hours, whereas PC61-IL2Mut24 and 3C7-IL2Mut24 resulted in more limited induction (Supplemental Figure 11A), consistent with an IL-2-driven amplification of ILC2 responsiveness. In proliferation assays, IgG-IL2Mut24 induced robust, dose-dependent ILC2 proliferation, whereas 7D4-, PC61-, and 3C7-IL2Mut24 elicited minimal responses (Figure 6D). Free PC61 or 7D4 co-delivered with IgG-IL2Mut24 similarly suppressed proliferation, whereas 3C7 retained partial activity (Figure 6E). Together, these findings indicate that receptor tuning constrains activation-associated CD25 induction and ILC2 proliferation.

The IL2Mut24 fusions displayed broadly similar serum profiles, with peak concentrations at 24 hours followed by a decline through 72 hours, although Fc-IL2WT showed substantially lower concentrations (Supplemental Figure 11B). IL-2 fusion

proteins were detectable in both injection site and non-injection site skin at 72 hours, but varied among constructs: 7D4- and 3C7-IL2Mut24 showed relatively high levels, while PC61-IL2Mut24 was comparable to Fc-IL2Mut24 and IgG-IL2Mut24 at both injection and non-injection sites (Supplemental Figure 11C). In contrast, Fc-IL2WT remained low in both compartments. Together, these findings indicate broadly comparable systemic exposure and varying tissue distribution among the IL2Mut24 fusion proteins.

To evaluate *in vivo* PD activity, C57BL/6J mice received 69 pmol of each IL-2 fusion or co-delivered anti-CD25 antibody with IgG-IL2Mut24 (Figure 6F). IgG-IL2Mut24, PC61-IL2Mut24, and 3C7-IL2Mut24 increased Treg frequencies and absolute numbers across spleen, mesenteric lymph nodes (mLNs), lung, and visceral adipose tissue (VAT) on Day 4 (Figure 6G, H; Supplemental Figure 11D–I). In contrast, 7D4-IL2Mut24 produced weaker Treg expansion. Co-delivered PC61 and IgG-IL2Mut24 failed to expand Tregs, whereas 3C7 co-delivery retained activity in terms of Treg frequency in secondary lymphoid tissues but was less effective in peripheral compartments than the corresponding fusion (Supplemental Figure 11D–I). This suggests the importance of molecular fusion for coordinated delivery and CD25 engagement.

In the repeated-dose ISR model, both 7D4-IL2Mut24 and PC61-IL2Mut24 significantly reduced ISR severity, whereas 3C7-IL2Mut24 induced inflammation comparable to IgG-IL2Mut24 (Figure 6I–J, Supplemental Figure 12A). IgG-IL2Mut24 and PC61-IL2Mut24 were further selected for detailed cutaneous profiling because they represent benchmark versus attenuated IL-2 signaling. A murine IgG-LALAPG control did not induce ISR (Supplemental Figure 12B), arguing against a non-specific inflammatory effect of the Fc-containing protein scaffold. Consistent with markedly reducing ISR

severity, PC61-IL2Mut24 maintained the increase in cutaneous CD25⁺ FoxP3⁺ Tregs comparable to IgG-IL2Mut24, while markedly reducing the frequency of CD25⁺ ILC2s and lowering TNF and IFN γ in injection site skin (Supplemental Figure 12C–H). PC61-IL2Mut24 reduced CD25 surface intensity on both Tregs and ILC2s compared with IgG-IL2Mut24, although CD25 induction remained more pronounced in Tregs (Supplemental Figure 12E–F). Together, these findings indicate that PC61-IL2Mut24 preferentially constrains the cutaneous ILC2 response while maintaining Treg expansion.

Collectively, these results validate the receptor clamp concept and show that epitope-specific fusion of IL2Mut24 to anti-CD25 enables quantitative and tissue-relevant modulation of IL-2 signaling, demonstrating that tuning CD25 engagement can preserve Treg expansion while limiting inflammatory toxicity.

Receptor-tuned IL-2 reshapes the cutaneous immune transcriptional landscape

To define how receptor tuning alters local immune responses, we performed single-cell RNA sequencing of injection-site tissue. BALB/c-Foxp3-GFP mice received a single s.c. dose of Fc-IL2Mut24 or PC61-IL2Mut24, and injection-site skin was harvested on day 4. Cell suspensions were sorted into three populations for scRNAseq: CD45⁺TCR β ⁺GFP⁺(Treg), CD45⁺TCR β ⁺GFP⁻ and CD45⁺ TCR β ⁻ (Supplemental Figure 13A).

scRNAseq of these compartments revealed treatment-specific remodeling across cell populations. Within the CD45⁺TCR β ⁺GFP⁻ cell population, Fc-IL2Mut24 increased the Th2 fraction with a modest rise in Type 1 Cytotoxic T cells (Tc1) versus isotype control (Supplemental Figure 13B, C). In contrast, PC61-IL2Mut24 decreased Th2/Tc1 and preferentially enriched CCR7⁺CD4⁺ and CCR7⁺CD8⁺ populations. Within the Treg

compartment (CD45⁺TCRβ⁺GFP⁺), Fc-IL2Mut24 increased the relative representation of activated and cycling states, whereas PC61-IL2Mut24 retained a substantial activated Treg population but shifted the composition toward a more resting and less proliferative phenotype (Figure 7A, B, Supplemental Figure 13D). In the CD45⁺TCRβ⁻ fraction, Fc-IL2Mut24 shifted composition away from B cell lineages toward ILC2 with a concomitant rise in ILC1s, whereas PC61-IL2Mut24 restrained ILC2 enrichment and preserved higher B cell representation (Figure 7C, D, Supplemental Figure 13E).

Integrated Principal Component Analysis (PCA) and heatmaps of selected differentially expressed genes revealed treatment-specific transcriptional remodeling in skin Tregs and ILC2s. In Tregs, both Fc-IL2Mut24 and PC61-IL2Mut24 separated from isotype, with PC61-IL2Mut24 exhibiting a tempered profile relative to Fc-IL2Mut24 (Figure 7E). Fc-IL2Mut24 strongly induced interferon-responsive and cell-cycle programs, including *Ifitm1/2/3*, *Aspm*, *Kif15* and *Clsn*, consistent with enrichment of activated and cycling Treg states (Figure 7F) (22, 28-31). PC61-IL2Mut24 retained a substantial activated Treg population but showed reduced expression of these interferon-responsive and proliferative genes, together with a distinct module marked by *Epas1* and *Penk*, suggesting a less proliferative, tissue-adapted state (Figure 7F) (32-34). In ILC2s, all groups diverged primarily along PC1 (22% variance), and PC61-IL2Mut24 occupied a position between isotype and Fc-IL2Mut24, indicating distinct treatment-associated transcriptional states (Figure 7G). Consistently, Fc-IL2Mut24 upregulated inflammatory, stress-response and chemotaxis-associated signatures in ILC2s (e.g., *Hspa1a*, *Ifitm1/2/3*, *Nlrp3*, *Ccl5*) (31, 35-38). PC61-IL2Mut24 attenuated many of these changes while inducing a distinct module marked by *Smpd3a*, *Fchs2*, *Rab18*, *Vamp3*, together

with cytoskeletal genes such as *Tuba1c*, *Tubb4b* and *Palld*, indicating altered immunometabolic, membrane-trafficking and cytoskeletal programs (Figure 7H) (39-42). Thus, PC61-IL2Mut24 qualitatively reprogrammed, rather than simply normalized, the ILC2 response. In contrast, CD4⁺ and CD8⁺ conventional T cells showed no clear separation among conditions (Supplemental Figure 13F–G).

Collectively, these data demonstrate that Fc-IL2Mut24 markedly remodeled the skin immune landscape, expanding activated/proliferative Tregs, Th2/Tc1 subsets, and inflammatory ILC2 programs. In contrast, PC61-IL2Mut24 preserved activated Treg enrichment while limiting Treg cycling and constraining ILC2 enrichment and inflammatory programming. These findings indicate that quantitative modulation of CD25 engagement differentially reshapes adaptive and innate immune compartments *in vivo*.

Receptor-tuned IL-2 enhances suppression of EAE onset

To evaluate whether receptor tuning improves IL-2-mediated immune tolerance *in vivo*, we evaluated PC61-IL2Mut24 in experimental autoimmune encephalomyelitis (EAE), a Treg-sensitive, CD4⁺ T cell-mediated model of central nervous system autoimmunity (43). Mice received i.p. injections of 69 pmol of isotype control, IgG-IL2Mut24, or PC61-IL2Mut24 on an “all” regimen (days –2, 0, 2, 6, 9, 13, 16), PC61-IL2Mut24 on an “early” regimen (days 2 and 6), or PC61-IL2Mut24 on a “late” regimen (days 16 and 19) relative to EAE induction, and anti-CD4 served as a control (Figure 8A). Both IgG-IL2Mut24 and PC61-IL2Mut24 administered on the “all” regimen significantly reduced clinical EAE scores and weight loss compared with isotype control, with PC61-IL2Mut24 demonstrating superior efficacy (Figure 8B-C). In addition, a clear regimen response was observed: PC61-IL2Mut24 “all” treatment significantly reduced cumulative

EAE scores compared with “early” treatment, which in turn outperformed “late” treatment, the latter showing no therapeutic benefit (Figure 8D). PC61-IL2Mut24 also reduced maximal clinical scores more effectively than IgG-IL2Mut24, indicating enhanced therapeutic efficacy (Supplemental Figure 14A). Analysis of CNS-infiltrating T cells at study termination revealed that both IgG-IL2Mut24 and PC61-IL2Mut24 markedly reduced T cell infiltration (Figure 8E, Supplemental Figure 14B) and decreased the frequency of IFN γ ⁺ CD4⁺ T cells, with PC61-IL2Mut24 producing the more pronounced effect (Figure 8F). Only PC61-IL2Mut24 significantly reduced IL-17A⁺ CD4⁺ T cells (Figure 8G), whereas IgG-IL2Mut24 preferentially increased CD25 and 4-1BB expression on conventional CD4⁺ T cells (Figure 8H, Supplemental Figure 14C). In addition, PC61-IL2Mut24 significantly reduced the frequency of MOG-Tetramer⁺ CD4⁺ T cells (Figure 8I) while increasing the proportion of Tregs within the MOG-Tetramer⁺ CD4 T cells compared with isotype-treated mice and increasing PD-1 expression on CD4⁺ Tregs (Figure 8J, Supplemental Figure 14D).

Together, these results show that receptor-tuned PC61-IL2Mut24 confers superior disease control compared to IgG-IL2Mut24 and that early initiation with sustained dosing is required for durable immunomodulation in EAE. This enhanced efficacy was associated with limited activation of pathogenic conventional CD4⁺ T cells while preserving Treg responses. These findings support quantitative tuning of CD25-dependent IL-2 signaling as a strategy to shift antigen-specific immunity toward tolerance.

DISCUSSION

Our study reveals an unanticipated divergence between Treg-selective IL-2 engineering and local inflammatory outcomes, highlighting a key challenge in developing

next-generation IL-2 therapies. IL2Mut24 is a CD25-biased IL-2 mutein designed to preferentially expand Tregs and Fc-IL2Mut24 confers prolonged systemic and tissue exposure. Despite this regulatory design, Fc-IL2Mut24 paradoxically exacerbates cutaneous inflammation compared with Fc-IL2WT. Because these molecules were compared at the same molar dose but were not matched for systemic or cutaneous exposure, the difference in ISR severity cannot be attributed solely to CD25 bias; altered receptor pharmacology and prolonged exposure likely both contribute. These findings highlight a key therapeutic dilemma: although Treg expansion promotes immune tolerance, concurrent activation of CD25-expressing ILC2s can drive type 2 inflammation, particularly in barrier tissues such as the skin (6, 15, 17).

Notably, cynomolgus monkey studies indicate that this safety-efficacy trade-off may extend across species. As the toxicology study of AMG592 included vehicle but not a matched protein control, nonspecific injection-related effects cannot be fully excluded; however, these studies were not designed to resolve tissue ILC2 activation directly. Thus, the concordance between Treg expansion, IL-5 induction, and localized pathology supports but does not definitively establish a pharmacology-driven, ILC2-dependent mechanism in non-human primates. These observations remain relevant to the clinical development of CD25-biased IL-2 therapies such as AMG592 and rezpegaldesleukin (14, 15). Our data raise the possibility that sustained CD25 engagement may couple regulatory expansion with local innate immune activation, potentially limiting the therapeutic window of CD25-biased IL-2 therapies in humans.

Tregs are reported to suppress inflammation partially by acting as an IL-2 “sink”: constitutive CD25 expression enables them to outcompete effector T cells and ILCs for

limited IL-2 (44). However, sustained ligand availability may erode this advantage as effector T cells and ILC2s upregulate CD25 and become increasingly responsive to IL-2 (27, 35, 36, 45). Consistent with this model, Fc-IL2Mut24 engaged both cutaneous Tregs and ILC2s, with CD25 expression increasing on ILC2s following activation. The greater ISR severity in *Rag2*^{-/-} mice and the subsequent selective Treg-depletion study indicated that Tregs restrain but do not fully prevent inflammation when IL-2 remains available to activated innate populations. PC61-IL2Mut24 instead preserved cutaneous Treg enrichment while reducing ILC2 responses and ISR. The EAE findings extend this principle to a CD4⁺ T cell-driven autoimmune setting, in which early and sustained PC61-IL2Mut24 treatment limited inflammatory CD4⁺ Tcon cell especially Th17 responses while maintaining Treg activation and increasing Treg representation within the MOG-specific cells. Together, these observations suggest that effective tolerance requires both preservation of regulatory responses and quantitative restriction of IL-2 signaling in the dominant pathogenic CD25⁺ population. The precise basis of this differential control remains to be established, as we did not directly assess the susceptibility of activated ILC2s and Tcons to Treg-mediated suppression.

Mechanistically, our data identify an ILC2-centered, but not ILC2-exclusive, model of IL-2-induced cutaneous inflammation. Sustained CD25-dependent signaling appears to preferentially amplify ILC2 responses and engage a broader inflammatory network, whereas adaptive immunity is dispensable for ISR development. The limited effects of IFN γ or IL-17A blockade, together with the failure of ILC1 or NK-cell reconstitution to restore ISR, argue against dominant roles for these pathways under the conditions tested. Nevertheless, because ILC2-specific depletion was not performed and other cell-specific

functions were not comprehensively examined, contributions from additional immune populations cannot be excluded.

The divergent blockade phenotypes are consistent with the distinct although overlapping biology of ILC2 cytokines: IL-5 primarily supports eosinophil development and survival; IL-13 preferentially engages structural-cell programs associated with inflammatory tissue remodeling; and IL-4 has broader activity on hematopoietic cells and can support macrophage-mediated repair, although IL-4 and IL-13 share signaling through the type II IL-4 receptor (46). The incomplete protection afforded by IL-5 blockade indicates that ISR extends beyond the IL-5-eosinophil axis (47, 48). IL-13 emerged as a prominent mediator in this model and may participate in a tissue feedback circuit that sustains the CD25⁺ ILC2 state. The accompanying neutrophil recruitment and increases in KC/GRO, IL-6, and TNF suggest secondary engagement of myeloid and tissue-resident compartments. Because our single-cell analysis was restricted to CD45⁺ cells and whole-skin lysates do not resolve mediator sources, the contributions of keratinocytes, fibroblasts and other stromal populations remain undefined. Thus, ILC2s are best viewed as central mediators within a multicellular inflammatory circuit linking sustained IL-2 signaling to barrier-tissue pathology.

To overcome the safety-efficacy trade-off, we developed anti-CD25-IL2Mut24 “receptor-clamp” fusions that modulate IL-2 receptor engagement at the cellular interface. Our data support a model in which epitope-dependent CD25 engagement acts as a signal modulator controlling IL-2 signaling strength. Structural modeling showed that the PC61 epitope partially overlaps the IL-2–CD25 interface. Consistent with a configuration-dependent mechanism, unconjugated PC61 completely blocked IgG-IL2Mut24 activity,

whereas covalently linked PC61-IL2Mut24 retained attenuated agonist activity. The distinct behaviors of the 7D4, PC61, and 3C7 constructs indicate that epitope, affinity, molecular configuration, and exposure collectively determine signaling output, rather than antagonistic classification alone. PC61-IL2Mut24 markedly reduced ISR despite broadly comparable measured exposure to IgG-IL2Mut24 fusions, suggesting that exposure alone does not fully account for its improved tolerability. Its preservation of Treg responses with minimal primary ILC2 proliferation supports a signaling-threshold model in which constitutive CD25 expression enables Tregs to respond to attenuated signaling, whereas ILC2s may require stronger or more sustained signals for activation. Single-cell analysis further showed preservation of activated Tregs alongside a constrained and qualitatively remodeled ILC2 response.

Several limitations and translational questions warrant consideration. The murine ISR model intentionally amplifies local inflammation through repeated dosing at a single site and should therefore be viewed as a mechanistic stress-test model rather than a direct reproduction of the typically moderate and transient reactions observed clinically. Neither the Fc-IL2WT versus Fc-IL2Mut24 comparison nor the receptor-clamp studies were formally exposure matched; therefore, equivalence in area under the curve (AUC), peak tissue concentration, receptor occupancy, and signaling duration was not established. We also did not perform ILC-specific depletion, and lineage-reconstitution experiments used cytokine-expanded, preconditioned cells rather than naïve populations. The scRNAseq studies were performed in BALB/c-Foxp3-GFP mice, whereas most mechanistic ISR experiments used C57BL/6-background mice; therefore, strain-specific transcriptional responses and differences in cellular composition cannot be excluded. In

addition, the cynomolgus studies had small group sizes, lacked a matched protein control, and did not directly assess tissue ILC2s. Although PC61-IL2Mut24 attenuated exaggerated IL-2–driven ILC2 responses without reducing ILC2s below baseline, dedicated challenge studies will be needed to determine whether receptor tuning affects host defense. Validation in primary human Tregs, ILC2s, and skin systems using human receptor-clamp molecules will also be required to establish the translational relevance of the proposed signaling-threshold mechanism.

Collectively, our findings identify a CD25-dependent, ILC2-centered inflammatory circuit in mice and suggest that related pharmacology may contribute to IL-2-associated local inflammation in non-human primates. By quantitatively tuning cytokine-receptor engagement, receptor-clamp IL-2 fusions provide a framework for preserving regulatory benefit while limiting activation of inflammatory CD25⁺ populations. More broadly, these results illustrate how control of signaling amplitude, receptor geometry, and cellular context can reshape immune outcomes and inform the design of next-generation cytokine therapies.

METHODS

Sex as a biological variable

Cynomolgus monkey studies included both male and female samples. All mouse experiments were performed with female mice at an age of 6-21 weeks.

Mice

C57BL/6, BALB/c, *Rag2*^{-/-}, *Rag2*^{-/-} γ *c*^{-/-}, Foxp3-reporter/DTR, and other indicated mouse strains were obtained from commercial vendors and maintained under specific pathogen-free conditions. Detailed strain information and husbandry conditions are provided in Supplemental Methods.

IL-2 treatment and injection-site reaction studies

Mice received the indicated doses of Fc-IL2WT or IL2Mut24 fusion proteins by subcutaneous injection, with dose and schedule specified in the figure legends. For pharmacodynamic studies, mice received a single dose. For injection-site reaction (ISR) studies, mice were dosed twice weekly for up to 4 weeks and monitored longitudinally for body weight and local reactions. ISR severity was assessed using a composite scoring system incorporating lesion size, bleeding, skin thickening, and inflammation. Detailed dosing and scoring criteria are provided in Supplemental Methods.

Pharmacokinetics and tissue biodistribution

C57BL/6J mice received a single subcutaneous dose of Fc-IL2WT or IL2Mut24 fusion proteins (289 pmol/dose). Plasma and/or skin from injection and non-injection sites were collected at the indicated time points. IL-2 fusion protein concentrations were quantified

using a customized electrochemiluminescence immunoassay. Additional sample-processing and assay details are provided in Supplemental Methods.

Cytokine blockade

To evaluate pathways contributing to Fc-IL2Mut24–induced ISR, C57BL/6J or *Rag2*^{-/-} mice were treated with Fc-IL2Mut24 together with blocking antibodies against IL-4, IL-5, IL-13, IFN γ , IL-17A, or CD25, or with an isotype control. Antibodies were administered weekly beginning with the first Fc-IL2Mut24 dose. ISR development was monitored longitudinally.

ILC expansion and adoptive transfer

ILC2s were expanded from IL-33–treated *Rag2*^{-/-} donor mice, whereas ILC1s and NK cells were isolated from *Rag2*^{-/-} mice treated with IL-12 plus IL-15. Purified cells were transferred subcutaneously and/or intradermally into *Rag2*^{-/-} γ C^{-/-} recipients, which were subsequently treated with Fc-IL2Mut24. ISR incidence and severity were assessed longitudinally. Detailed expansion, purification, and transfer procedures are provided in Supplemental Methods.

Treg depletion

Rag2^{-/-} mice received T cells isolated from Foxp3-DTR-mCherry mice and were subsequently treated with IgG-IL2Mut24. Following ISR development, Tregs were depleted by administration of diphtheria toxin, and ISR severity and body weight were monitored longitudinally. Generation and validation of the Foxp3-DTR-mCherry mouse strain are described in Supplemental Methods.

Tissue processing and flow cytometry

Single-cell suspensions were prepared from spleen, lymph nodes, lung, visceral adipose tissue, central nervous system, and skin using tissue-specific mechanical and/or enzymatic dissociation. Cells were stained with viability, surface, and intracellular markers and analyzed by flow cytometry. Absolute cell numbers were determined using counting beads. Data were acquired on BD flow cytometers and analyzed using FlowJo. Representative gating strategies are shown in Supplemental Figure 15, and antibodies are listed in Supplemental Table 1. Detailed tissue processing and staining procedures are provided in Supplemental Methods.

Single-cell RNA sequencing

Skin leukocytes were enriched and multiplexed using oligonucleotide-conjugated antibodies before flow-cytometric sorting. Sorted populations were processed using the 10x Genomics Chromium Single Cell 5' platform and sequenced on an Illumina NovaSeq X. Reads were processed using Cell Ranger and analyzed in R using Seurat. Low-quality cells were removed before normalization, dimensionality reduction, clustering, and cell-type annotation based on canonical markers.

Pseudo-bulk analyses were performed using DESeq2, and differential-state analyses were performed using muscat. Differentially regulated genes were identified using the criteria described in Supplemental Methods. Cell-type signatures and differential-expression results are provided in Supplemental Tables 2 and 3.

Cytokine measurements

Serum and skin lysates were collected from treated mice, and cytokine concentrations were measured using Meso Scale Discovery (MSD) electrochemiluminescence assays . IL-13 was additionally quantified by ELISA. Detailed sample preparation and assay conditions are provided in Supplemental Methods.

Histopathology

Full-thickness injection-site skin was formalin-fixed, paraffin-embedded, sectioned, and stained with H&E. Sections were evaluated by a board-certified veterinary pathologist using predefined criteria for epidermal, dermal, pannicular, and subcutaneous changes. Composite pathology scores were generated from individual tissue-layer severity scores. Detailed grading criteria are provided in Supplemental Methods.

Generation and characterization of IL-2 molecules

Mouse IL2WT and IL2Mut24 fusion proteins were generated as previously described (17). IL2Mut24 contained N103R and V106D substitutions in addition to manufacturability substitutions shared with IL2WT. Recombinant proteins were expressed in CHO cells, purified by Protein A chromatography followed by size-exclusion chromatography, and characterized for purity, molecular mass, and endotoxin content. Additional construct and analytical details are provided in Supplemental Methods.

IL-2 bioactivity assays

IL-2 bioactivity was assessed using IL-2–dependent CTLL-2 cells and purified mouse ILC2s. Cells were stimulated with serial dilutions of IL-2 constructs, and proliferation was quantified by [³H]-thymidine incorporation. IL-2–induced CD25 upregulation on ILC2s was

measured by flow cytometry. Additional purification and assay details are provided in Supplemental Methods.

Experimental autoimmune encephalomyelitis

EAE was induced in female C57BL/6 mice by immunization with MOG_{35–55} in complete Freund's adjuvant followed by pertussis toxin. Mice were monitored daily for body weight and neurological disease using a 0–5 clinical scoring system. Detailed immunization and scoring procedures are provided in Supplemental Methods.

Cynomolgus monkey studies

In Study 118075, cynomolgus monkeys received subcutaneous aldesleukin or AMG592 according to the schedules indicated in Figure 5A. Peripheral blood was collected longitudinally for immunophenotyping and serum cytokine analysis.

In Study 118065, male and female cynomolgus monkeys received AMG592 at 10, 30, or 100 µg/kg subcutaneously once weekly for 3 months. Injection sites were collected at scheduled necropsy for histopathologic evaluation. Additional study design, sampling, and assay details are provided in Supplemental Methods.

Statistics

All data were analyzed using GraphPad Prism. For multiple group comparisons (e.g. flow cytometry data), one-way ANOVA followed by Sidak's multiple comparisons post hoc test was applied. For multiple-group comparisons over time (e.g. weight over time, ISR score over time), two-way ANOVA (or mixed-effects analysis in case of missing values) followed by Sidak's multiple comparisons post hoc test was applied. For non-parametric

longitudinal EAE clinical scores, a mixed-effects model with Tukey's multiple comparisons post hoc test was applied. Significance thresholds were as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). All error bars represent the standard error of the mean (SEM) unless otherwise noted.

Study approval

All mouse studies were approved by the Institutional Animal Care and Use Committee (IACUC) of Amgen Inc. or the Charles River Accelerator and Development Lab (CRADL; Charles River Laboratories). All studies involving cynomolgus monkeys were approved by the IACUC of Charles River Laboratories, Inc. (Study 118065) or MPI Research (Study 118075). All studies were conducted in accordance with the U.S. Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*.

Data availability

Values for all data points in graphs are reported in the Supporting Data Values file. All data and code needed to evaluate and reproduce the results in the paper are present in the paper and/or the Supplementary Materials. Materials will be made available to the scientific community by completion of a material transfer agreement. Requests can be made at www.amgen.com/partners/academic-collaborations/new-requests. The scRNAseq data generated in this study are available through GEO under accession number: GSE316048.

Manuscript information

Author contributions

W.D., A.S., C.M., and M.T. conceptualized the study and designed the experiments, A.S., C.M., M.T., A.V., S.L., R.P., S.M. performed the experiments, Y.J. and X.L. performed RNA-seq analysis, S.W. and H.S.T. designed the tool molecules and provided structural modeling, R.R.H. provided pathology reviews. M.F. was the study director of NHP studies. W.D., A.S., C.M. and M.T. wrote the manuscript with input from all authors.

AI disclosures

ChatGPT (OpenAI, GPT5.6) was used for language editing and organization during manuscript preparation (March-September, 2026). All AI-assisted content was reviewed and verified by the authors, who take responsibility for the final manuscript.

Conflict of interest

All authors are current or former employees of Amgen Inc. and own stock in Amgen Inc.

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Figure 1

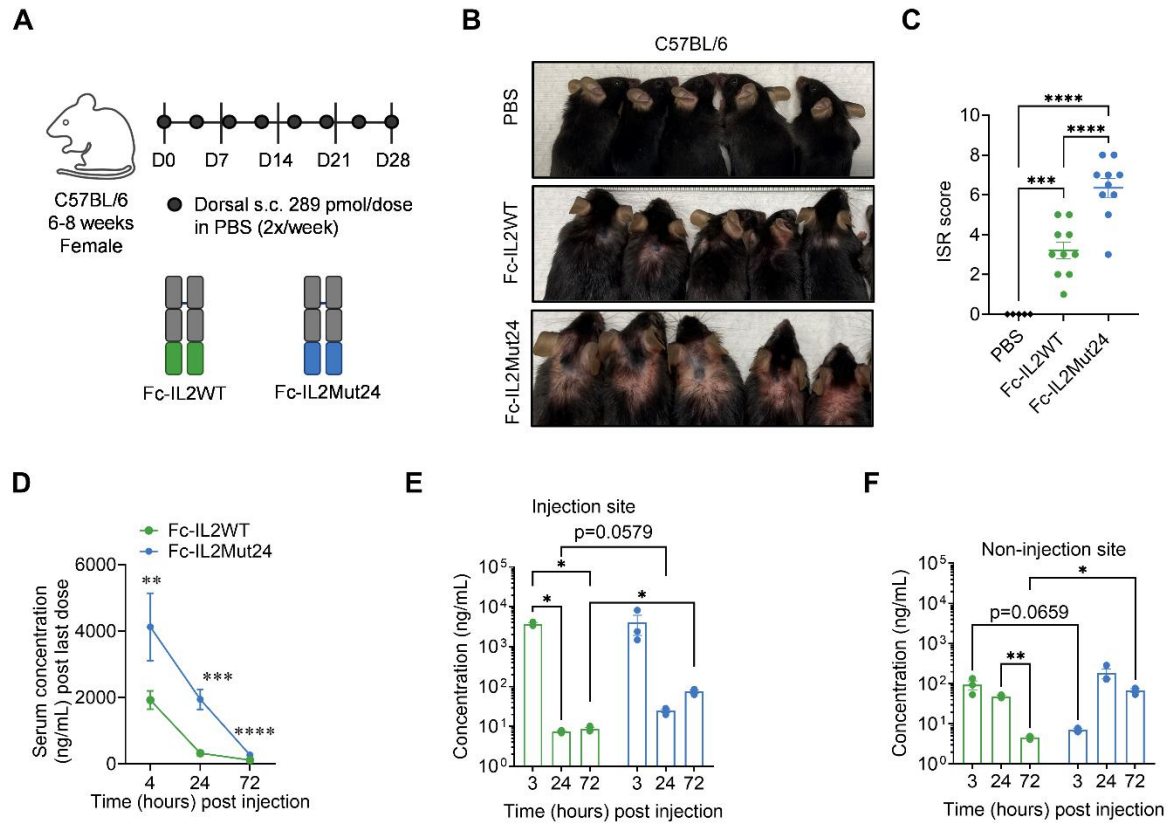


Figure 1. Fc-IL2Mut24 induces severe ISRs associated with prolonged systemic and cutaneous exposure. 6-8-week-old female C57BL/6 mice received dorsal s.c. dosing of either Fc-IL2WT or Fc-IL2Mut24 289 pmol (~25 μ g/dose/mouse in PBS) or PBS alone twice per week for four weeks. **(A)** Schematic representation of experimental design. **(B)** Representative photograph of dorsal ISR (n=5 mice per group). **(C)** ISR score (n=5 mice in PBS, n=10 mice each in Fc-IL2WT and Fc-IL2Mut24). **(D)** Fc-IL2WT and Fc-IL2Mut24 plasma concentrations (ng/mL) at 3, 24 and 72 hours post a single dose as detected by MSD (25 μ g/dose, n=5 mice per condition at 3 and 24 hours and n=10 mice per condition at 72 hours). **(E-F)** Concentrations (ng/mL) of Fc-IL2WT and Fc-IL2Mut24 detected from skin samples in injection-site **(E)** and non-injection site **(F)** at 3, 24 and 72 hours post single dose as detected by MSD (n=3 mice per timepoint and per condition). **(C-F)** Data shown as mean $\pm$ SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's post hoc test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 2

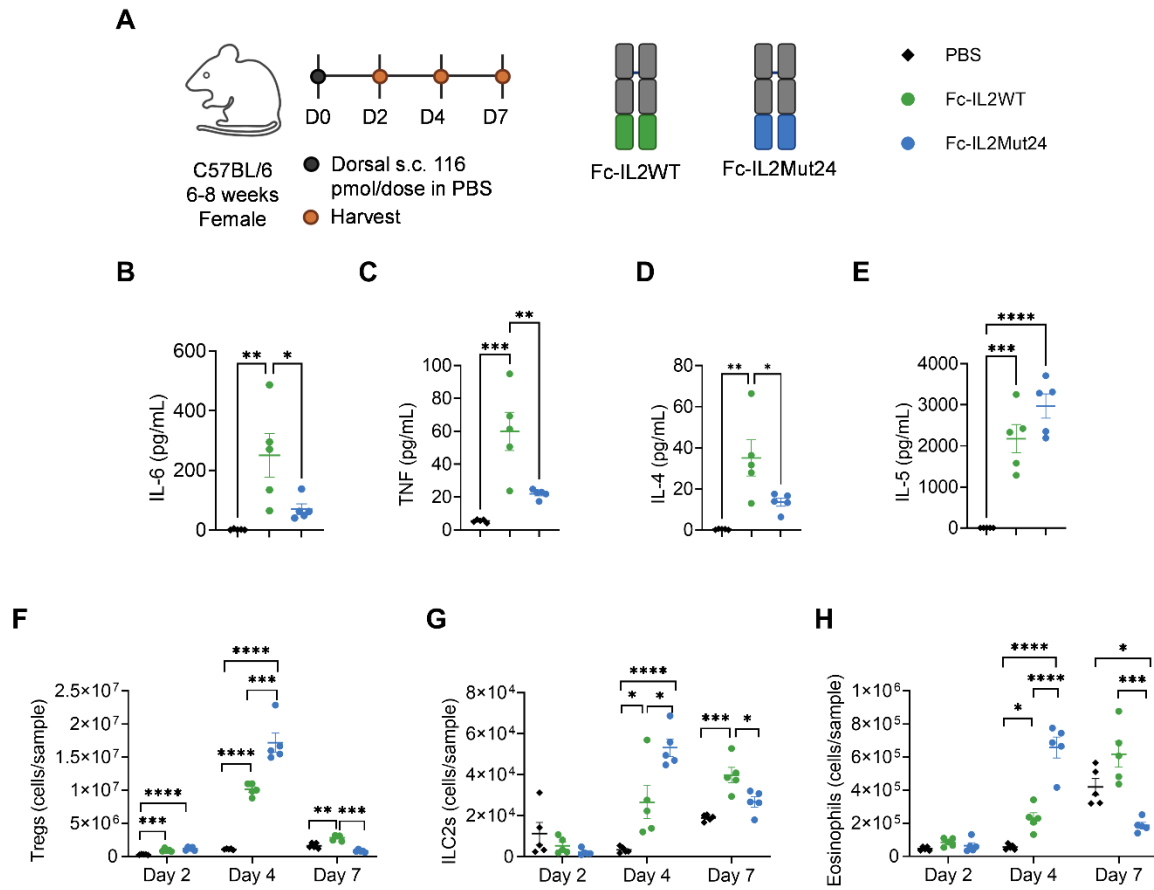


Figure 2. Fc-IL2Mut24 induces Treg expansion but concomitantly amplifies ILC2 and eosinophil responses. 6-8-week-old female C57BL/6 mice received dorsal s.c. dosing of Fc-IL2WT, or Fc-IL2Mut24 at 116 pmol (~10 µg/dose/ mouse), or PBS alone on day 0. Mice were euthanized on days 2, 4, and 7 for serum cytokine analysis by MSD and splenic cell analysis by flow cytometry. **(A)** Schematic representation of experimental design. **(B-E)** Concentrations (pg/ml) of IL-6 **(B)**, TNF **(C)**, IL-4 **(D)** and IL-5 **(E)** in serum on day 2. **(F-H)** Treg **(F)**, ILC2s **(G)** and eosinophil **(H)** counts (cells per sample) in spleen on days 2, 4 and 7. Data are representative of three independent experiments. Data shown as mean±SEM from n=5 mice per condition and per timepoint. Two-tailed ordinary one-way ANOVA followed by Sidak's post hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 3

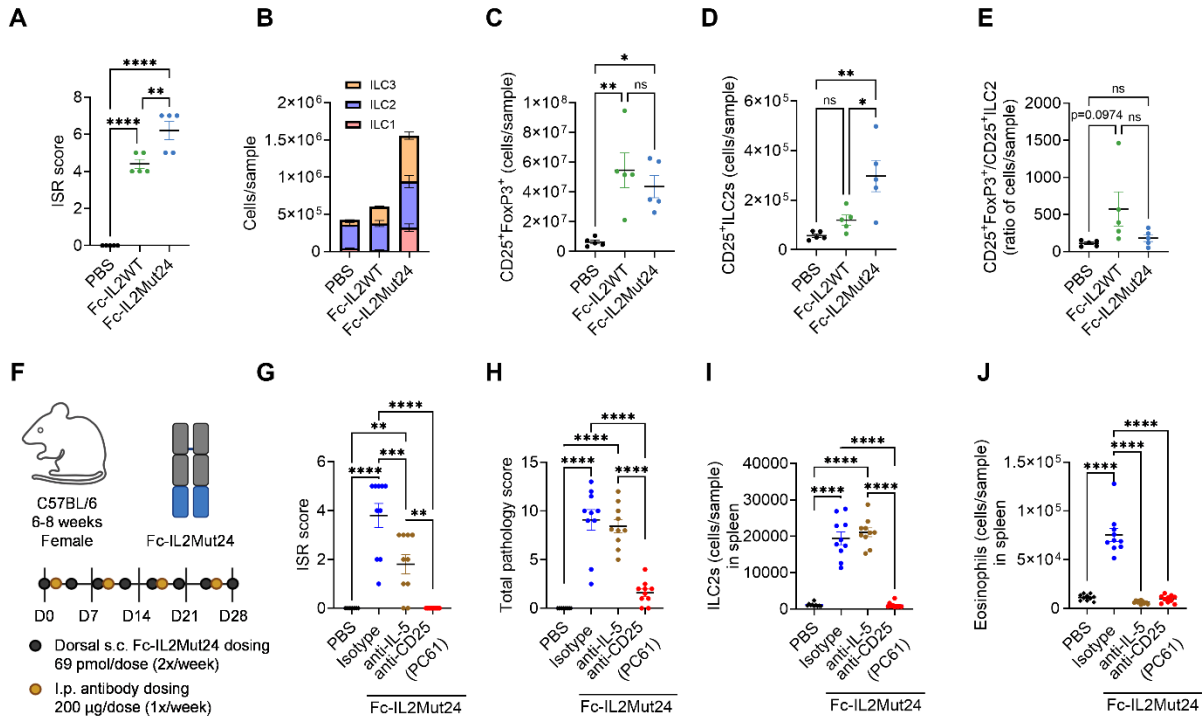


Figure 3. Fc-IL2Mut24 induces a CD25-dependent type 2 inflammatory program in skin. (A-E) C57BL/6 mice (n=5 mice per group) received dorsal s.c. dosing twice weekly for four weeks with PBS, 69 pmol (~6.5 µg/dose/mouse) Fc-IL2WT or Fc-IL2Mut24. **(A)** ISR scores at endpoint. **(B)** ILC1, ILC2 and ILC3 cell counts per sample in skin at endpoint represented as stacked bar graph, as analyzed by flow cytometry. **(C-D)** CD25⁺ FoxP3⁺ cells **(C)**, CD25⁺ ILC2s **(D)** in skin at endpoint, shown as cell counts per sample, as analyzed by flow cytometry, and ratio thereof **(E)**. **(F-J)** C57BL/6 mice (n=10 mice per group) received dorsal s.c. dosing twice weekly for four weeks with 69 pmol (~6.5 µg/dose/mouse) Fc-IL2Mut24 and co-treated once weekly with 200 µg anti-IL-5, anti-CD25 or isotype control. **(F)** Schematic representation of experimental design. **(G)** ISR scores at endpoint. **(H)** Skin pathology scores at endpoint. **(I-J)** ILC2 counts (cells/sample, **I**) and eosinophil counts (cells/sample, **J**) in spleen as analyzed by flow cytometry. Data shown as mean ± SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc; only selected comparisons are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Figure 4

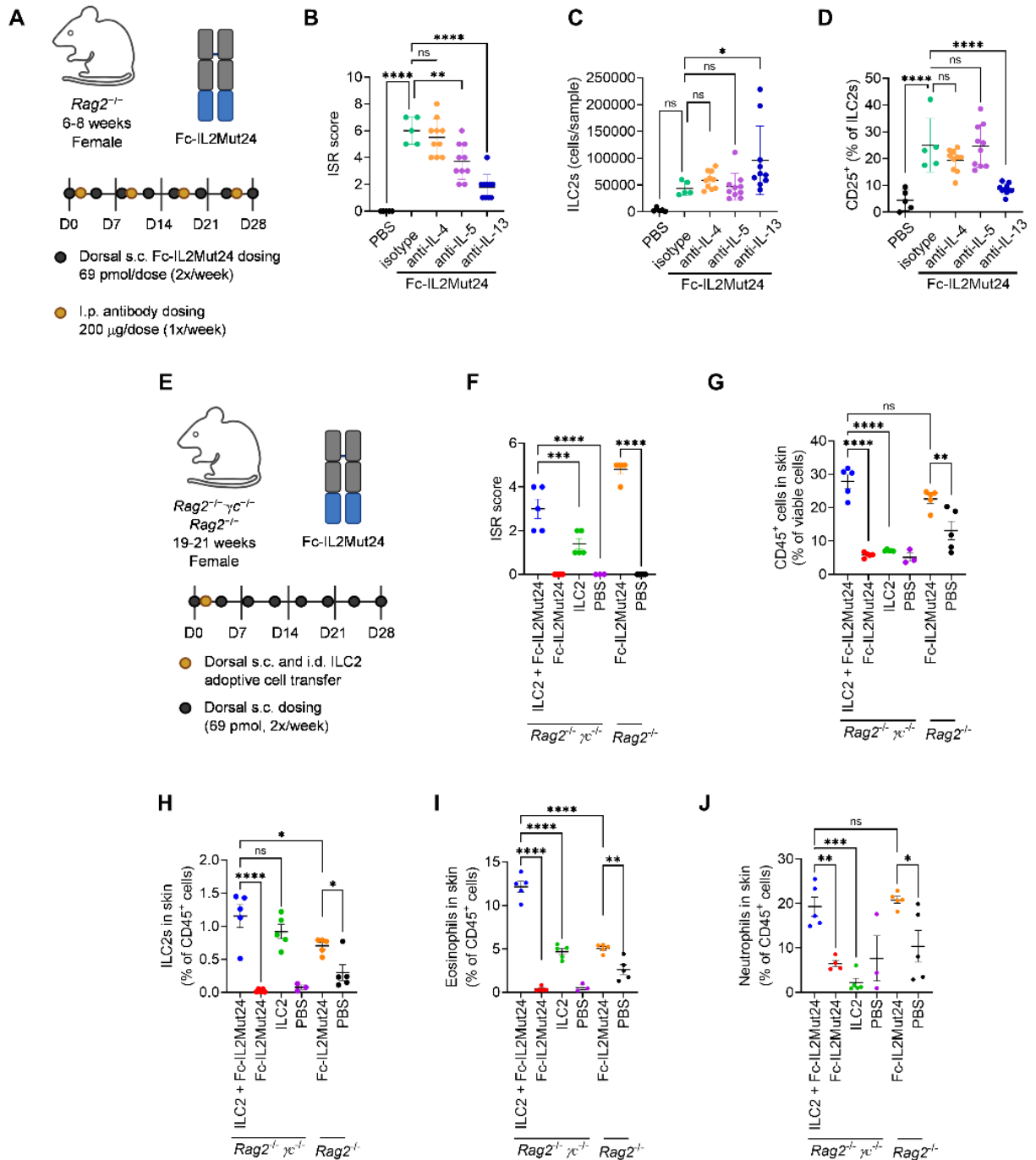


Figure 4. ILC2s are sufficient for IL-2-induced cutaneous inflammation. (A-D) *Rag2*^{-/-} mice (n=5 mice in PBS and isotype and n=10 mice in all other groups) received dorsal s.c. dosing twice weekly for four weeks with 69 pmol (~6.5 μg/dose/mouse) Fc-IL2Mut24 and co-treated once weekly with 200 μg anti-IL-4, anti-IL-5, anti-IL-13 or isotype control. (A) Schematic representation of experimental design. (B) ISR scores at endpoint. (C)

ILC2 counts (absolute number of cells per sample) and **(D)** frequency of CD25⁺ cells among ILC2s in skin as analyzed by flow cytometry. **(E-J)** *RAG2*^{-/-}*γC*^{-/-} mice (n=3 mice in PBS groups, n=5 mice per group in all other groups) received both s.c. and i.d. adoptive transfer of 2x10⁶ ILC2 cells and were then treated subcutaneously twice weekly for four weeks with 69 pmol Fc-IL2Mut24. *RAG2*^{-/-} mice (n=5) were treated in parallel as a control. **(E)** Schematic representation of experimental design. **(F)** ISR scores at endpoint. **(G)** Frequency of CD45⁺ among live cells, **(H-J)** Frequency of ILC2s **(H)**, eosinophils **(I)** and neutrophils **(J)** in CD45⁺ cells in skin at endpoint analyzed by flow cytometry. Data are representative of two independent experiments. Data shown as mean ± SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc; only selected comparisons are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Figure 5

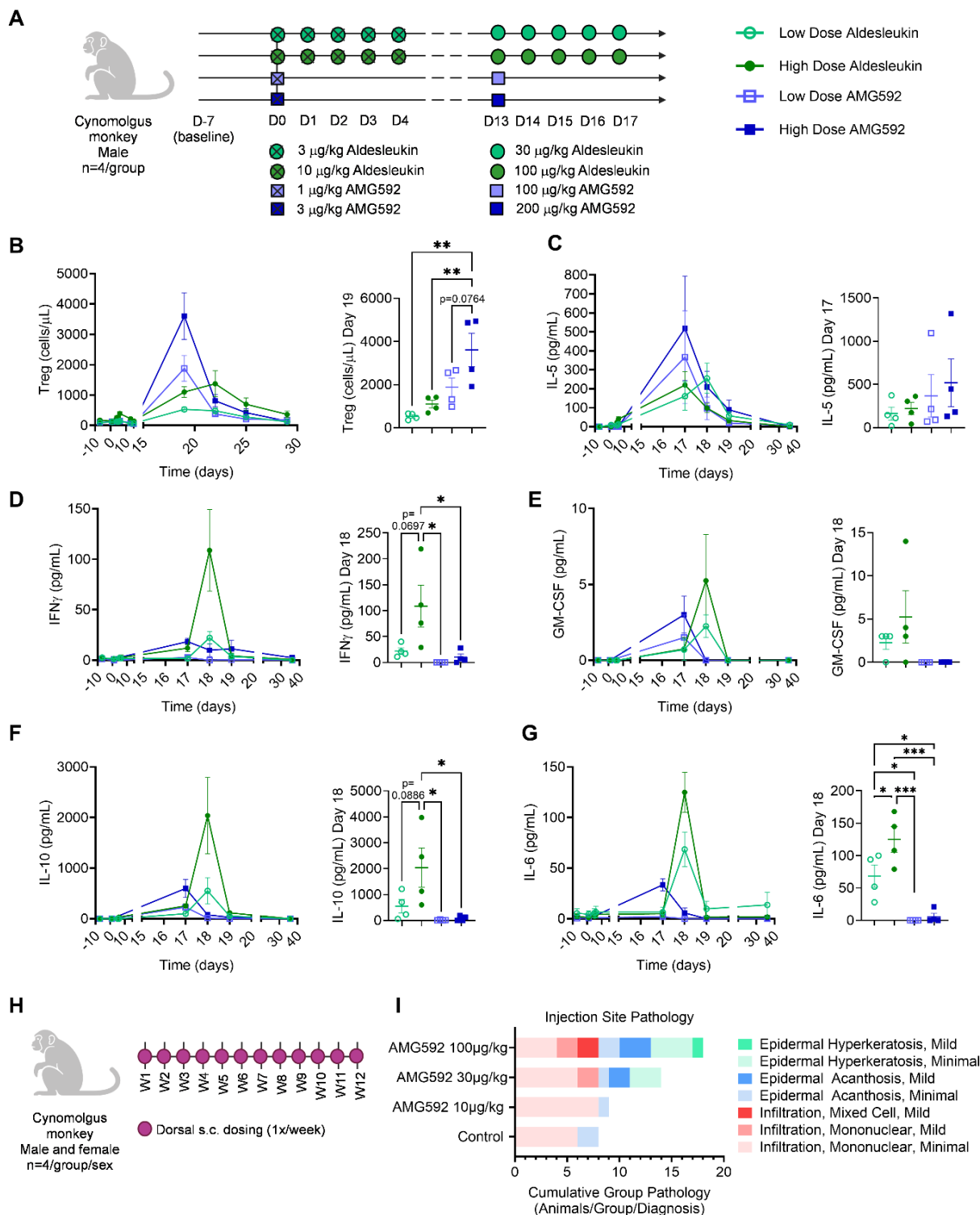


Figure 5. AMG592 expands Tregs but induces IL-5 production and injection-site pathology in Cynomolgus monkeys. (A-G) Male cynomolgus monkeys (n = 4 per

group) received subcutaneous dosing of low or high doses of aldesleukin (3 or 10 µg/kg on days 0-4 followed by 30 or 100 µg/kg on days 13-17) or AMG592 (1 or 3 µg/kg on day 0 followed by 100 or 200 µg/kg on day 13). Blood samples were collected at the indicated time points for PD analysis. **(A)** Schematic representation of study 118075. **(B)** Circulating Tregs (gated as CD3⁺CD4⁺FoxP3⁺CD25⁺ cells prior to dosing and CD3⁺CD4⁺FoxP3⁺ cells after dosing) as assessed by flow cytometry. **(C-G)** Serum cytokine concentrations following treatment: IL-5 **(C)**, IFN γ **(D)**, GM-CSF **(E)**, IL-10 **(F)**, and IL-6 **(G)**. Peak values (day 19 in **B**, day 17 in **C**, day 18 in **D-G**) shown in right panels. Data shown as mean $\pm$ SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc; only selected comparisons are shown. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. **(H-I)** Cynomolgus monkeys (n=4 male and 4 female per group) received s.c. AMG592 (100 µg/kg, 30 µg/kg or 10 µg/kg) or vehicle control once per week for twelve weeks. Injection site skin was collected on day 92 after the first dose. **(H)** Schematic representation of study 118065. **(I)** Cumulative histopathology at the dorsal subcutaneous injection site. Bars represent the total number of animals per group exhibiting each lesion category and severity. Observed changes included epidermal hyperkeratosis and acanthosis (minimal or mild), as well as dermal inflammatory infiltrates (mononuclear or mixed-cell, minimal or mild).

Figure 6

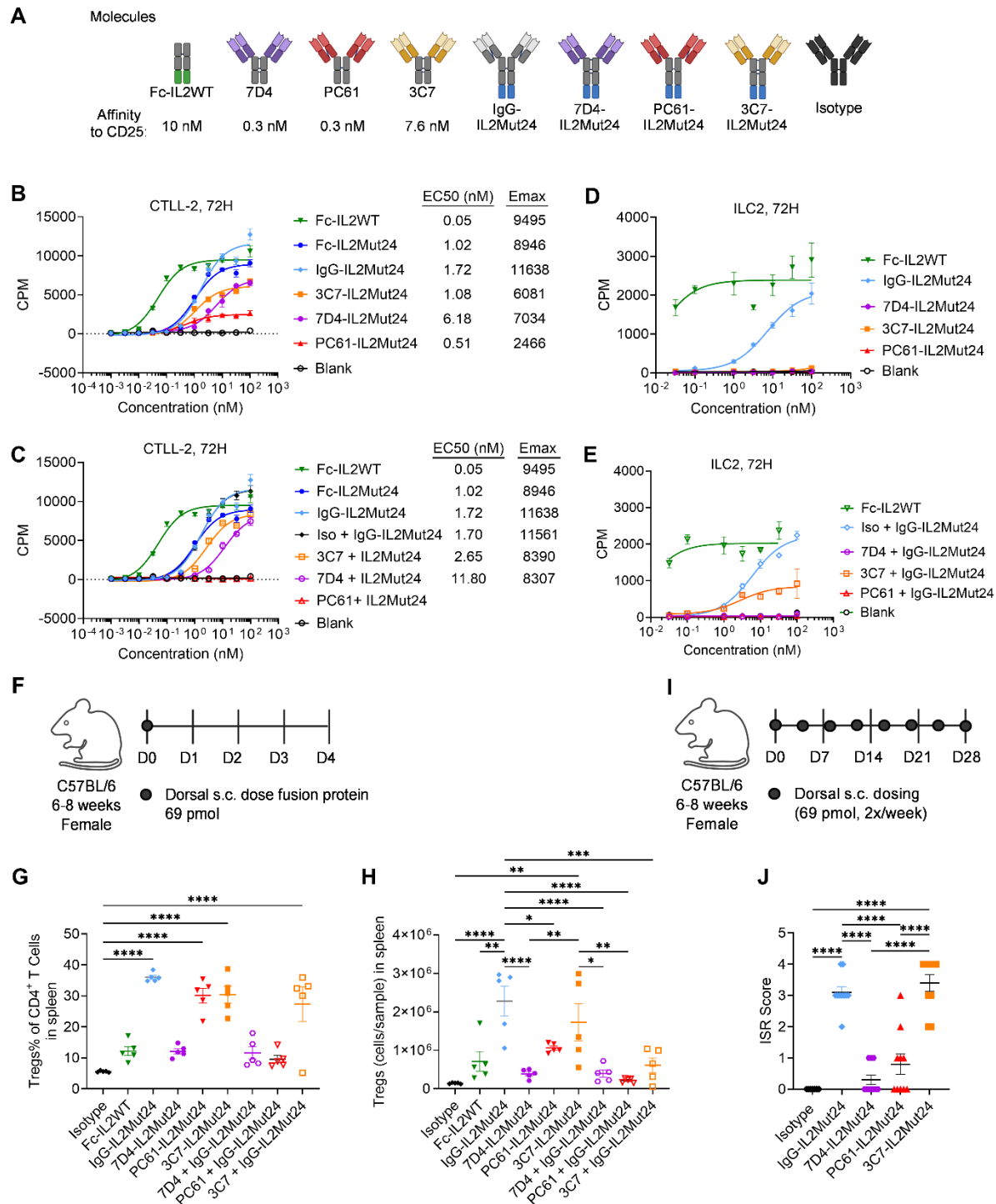


Figure 6. Epitope-specific CD25 engagement tunes IL-2 signaling strength and tissue immune responses. (A–E) Proliferation assays using CTLL-2 cells (B, C) or purified ILC2s (D, E) treated with serial dilutions of IL-2 fusion proteins or controls. (A) Schematic of Fc-IL2WT and anti-CD25–IL2Mut24 fusion proteins; indicated values

denote binding affinities for mouse CD25. **(B, D)** Proliferative responses to 7D4-IL2Mut24, PC61-IL2Mut24, 3C7-IL2Mut24, Fc-IL2WT, Fc-IL2Mut24, and IgG-IL2Mut24. **(C, E)** Responses to equimolar mixtures of unconjugated anti-CD25 antibodies with IgG-IL2Mut24. **(B,C)** Data are shown as mean $\pm$ SEM of three technical replicates from one representative experiment of three. **(D, E)** Data are from one experiment. **(F-H)** C57BL/6J mice (n=5/group) received 69 pmol of the indicated fusion proteins or matched combinations of unconjugated anti-CD25 antibodies with IgG-IL2Mut24 and were analyzed on day 4. **(F)** Schematic representation of experimental design. **(G-H)** Frequency **(G)** and cell counts per sample **(H)** of CD25⁺FOXP3⁺ Treg cells within the CD4⁺ T cell compartment in spleen as analyzed by flow cytometry. **(I-J)** C57BL/6J mice were treated subcutaneously twice per week for four weeks with 69 pmol of indicated protein (n=10 mice per group). **(I)** Schematic representation of experimental design. **(J)** ISR scores at endpoint. Data shown as mean $\pm$ SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc test, only selected comparisons are shown. *p < 0.05, **p < 0.01, ***p < 0.001, **** p < 0.0001.

Figure 7

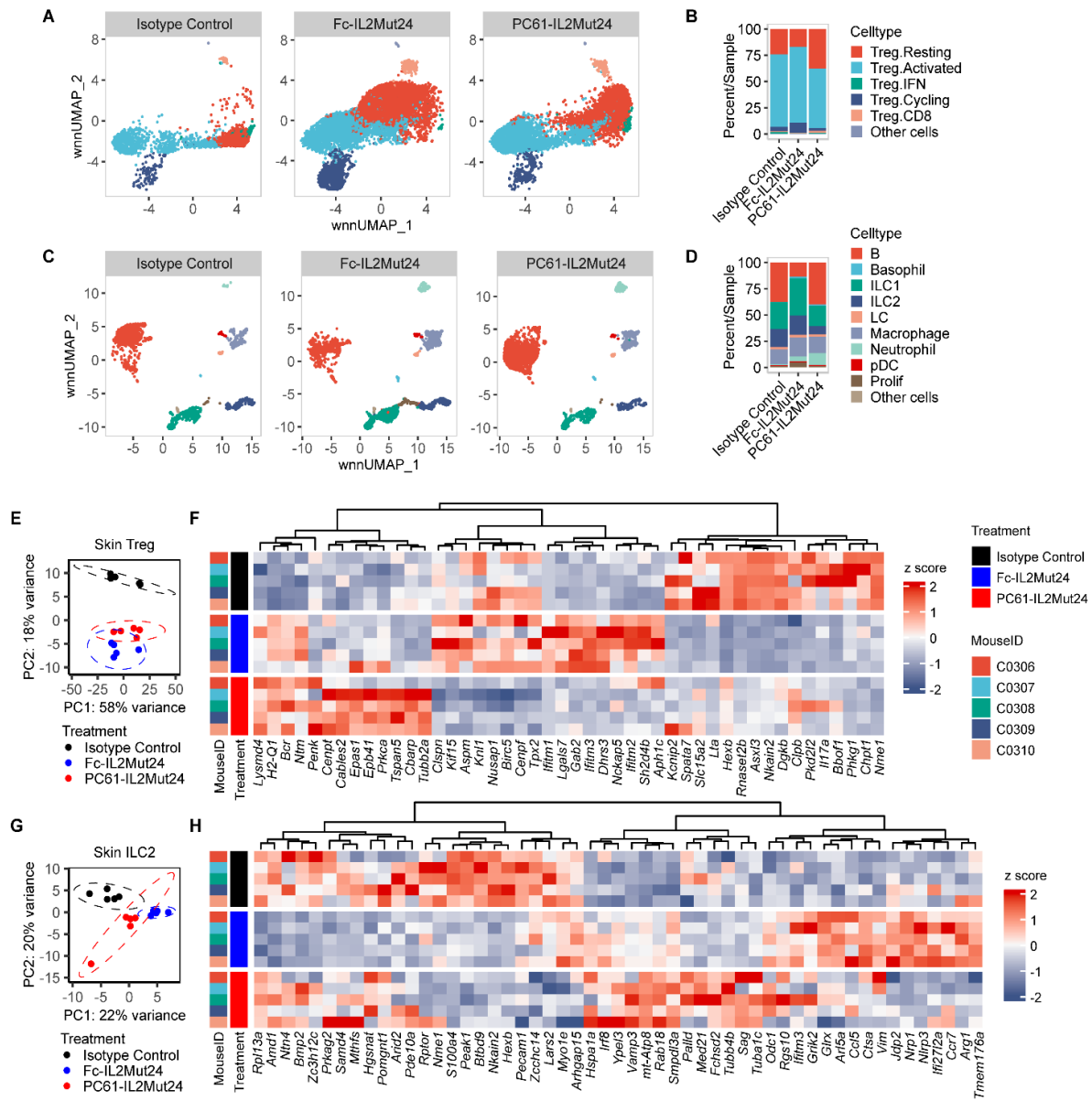


Figure 7. Fc-IL2Mut24 and PC61-IL2Mut24 differentially remodel the cutaneous immune transcriptional landscape. BALB/c-FoxP3-GFP reporter mice received a single dorsal subcutaneous dose of isotype control, Fc-IL2Mut24 or PC61-IL2Mut24 (69 pmol). Skin samples were collected and processed on day 4 after dosing. Uniform Manifold Approximation and Projection (UMAP) of Treg cell subsets from the CD45⁺TCRβ⁺GFP⁺ sorted samples (**A**) and CD45⁺TCRβ⁻GFP⁻ immune cell populations (**C**) in skin. Average proportion of different Treg cell subsets from the CD45⁺TCRβ⁺GFP⁺ sorted samples (**B**) and different immune cell subsets from the CD45⁺TCRβ⁻GFP⁻ sorted samples in each condition (**D**) in skin. Principal component analysis (PCA) of genes expressed by Tregs (**E**) and ILC2s (**G**) with 95% confidence intervals marked by dashed circles. Heatmap of top 50 genes differentially expressed by Tregs (**F**) and ILC2s (**H**) across isotype, Fc-IL2Mut24, and PC61-IL2Mut24 treatment groups in skin.

Figure 8

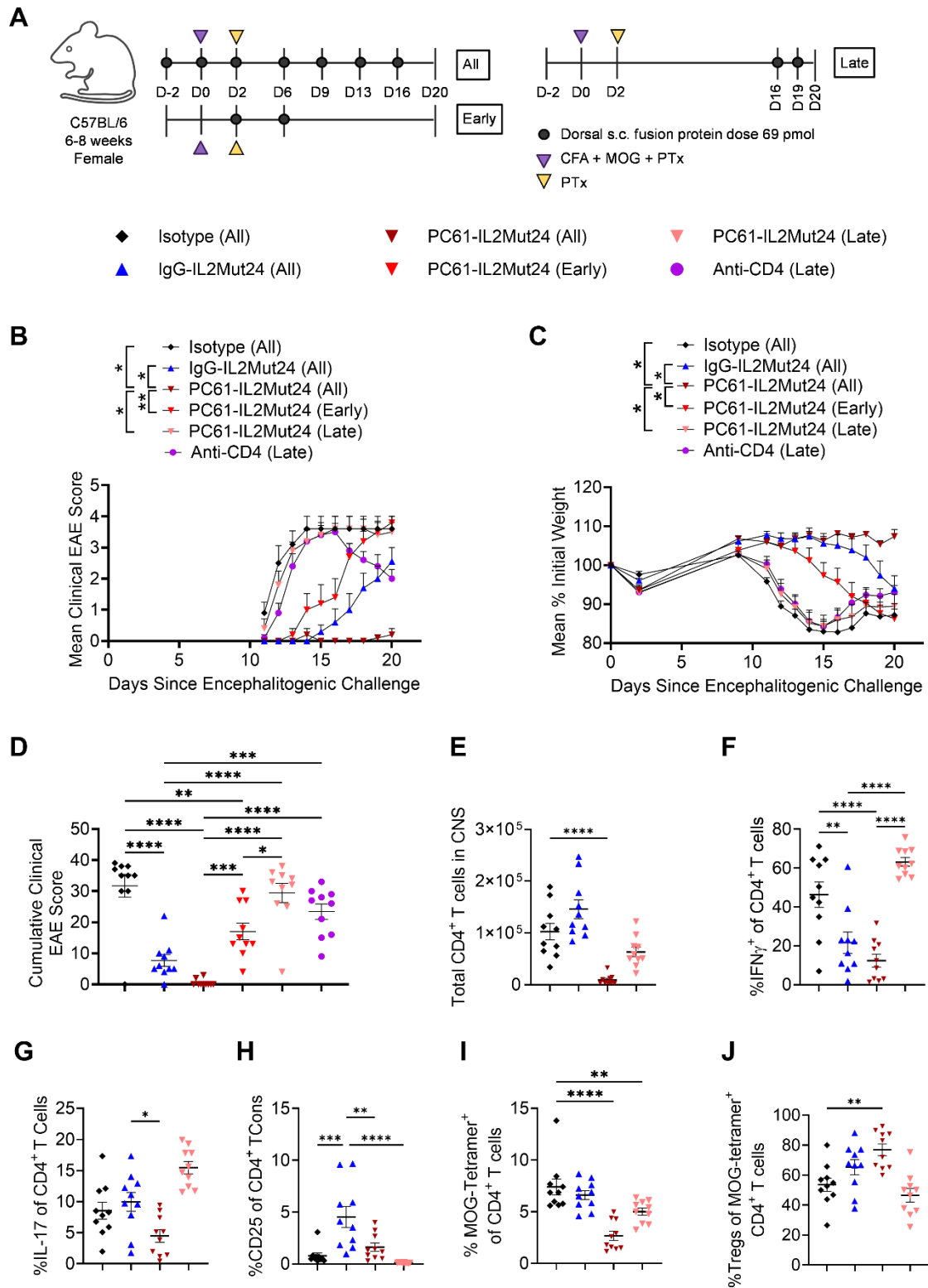


Figure 8. PC61-IL2Mut24 outperforms IgG-IL2Mut24 in suppression of EAE onset. C57BL/6J mice (n = 10) were treated i.p. with 100 μ L PBS containing isotype, PC61-IL2Mut24, IgG-IL2Mut24, or anti-CD4 on schedules: “All” (Days -2, 0, 2, 6, 9, 13, 16), “Early” (Days 2, 6), or “Late” (Days 16, 19). EAE was induced on Day 0 using 200 μ g MOG₃₅₋₅₅ emulsified in CFA, and 120 ng pertussis toxin was administered i.p. on Days 0 and 2. **(A)** Schematic representation of experimental design. **(B)** Clinical EAE scores over time (days). **(C)** Body weight over time (days) as % change from initial weight. **(D)** Cumulative EAE scores over time (days). **(E-J)** At study endpoint, CNS-infiltrating cells were isolated and stained with MOG₃₅₋₅₅-IA^b Tetramer. Total CD4⁺ T cell counts **(E)**, frequency of IFN γ ⁺ **(F)**, IL-17A⁺ **(G)**, CD25⁺ **(H)**, MOG-Tetramer⁺ **(I)** cells among CD4⁺ cells, frequency of FoxP3⁺ cells among MOG-Tetramer⁺ CD4⁺ T cells **(J)** in the CNS as analyzed by flow cytometry. Data are shown as mean $\pm$ SEM from n=10 mice per group. Data are representative of two independent experiments. **(B-C)** EAE scores and body weights were analyzed using mixed-effects models with Tukey’s multiple-comparisons test. PC61-IL2Mut24 (All) reduced EAE scores versus isotype on days 5-13 (*), IgG-IL2Mut24 on days 11-13 (*), PC61-IL2Mut24 (Early) on days 10-13 (**), and PC61-IL2Mut24 (Late) on days 5-13 (*). Body weight was greater versus isotype on days 3-13 (*), IgG-IL2Mut24 on day 13 (*), PC61-IL2Mut24 (Early) on days 10-13 (*), and PC61-IL2Mut24 (Late) on day 3 and days 5-13 (*). *P < 0.05; **P < 0.01; **(D-J)** Two-tailed ordinary one-way ANOVA followed by Sidak’s post hoc test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

SUPPLEMENTAL METHODS

Mice

Female (6-12 weeks old) C57BL/6NCrI and BALB/cAnNCrI mice were purchased from Charles River Laboratories; B6.129S6-Rag2^{tm1Fwa} N12, C57BL/6NTac.Cg-Rag2^{tm1Fwa} Il2rg^{tm1Wjl} mice and C57BL/6NTac were obtained from Taconic Biosciences; and B6.Cg-Foxp3tm2Tch/J, C.Cg-Foxp3tm2Tch/J, C3H/HeJ, and C57BL/6J mice were purchased from The Jackson Laboratory. All mice were housed under specific pathogen-free (SPF) conditions at CRADL. Animals were maintained in individually ventilated cages under controlled environmental conditions (temperature: 22 ± 2°C, relative humidity: 50-60%) on a 12-hour light/dark cycle. Mice had ad libitum access to autoclaved food and water and were acclimated for at least five days prior to experimentation.

In Vivo Treatments and Interventions

Mice received either high (25 µg/289 pmol), optimal (10 µg/116 pmol), low (6.5 µg/69 pmol) or ultra-low dose (2.5 µg/28.9 pmol) of IL-2 molecules as specified in figure legends. For single dose pharmacodynamic (PD) studies, animals received (10 µg/116 pmol) per mouse of either Fc-IL2WT or Fc-IL2Mut24 via s.c. injection. Control mice were given an equal volume of vehicle (PBS). For the preclinical model of injection-site reaction (ISR), mice were dosed subcutaneously twice weekly for a total of eight doses with either high (25 µg/289 pmol) or low (6.5 µg/69 pmol) dose of either Fc-IL2WT or Fc-IL2Mut24, unless specified otherwise in figure legends. Development of lesions at the injection site was observed around the 4th-5th dose. Mice were monitored twice per week for body weight changes and ISRs symptoms.

Pharmacokinetics (PK) and Tissue Biodistribution

C57BL/6J mice were administered a single high dose (289 pmol) of Fc-IL2WT or IL2Mut24 fusion proteins by subcutaneous injection over the shoulder region. Blood samples were collected at 4, 24, and 72 hours post-injection into SARSTEDT Microvette® K3 EDTA plasma separation tubes. Plasma was isolated by centrifugation and stored at -70 ± 10 °C until analysis.

Skin samples from injection site and non-injection site from lower back were excised, weighed and transferred into bead-containing Precellys® soft tissue homogenizing CK14 tubes (Bertin Instruments). Lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 0.1% Triton X-100, pH 7.4) supplemented with cOmplete™ Mini EDTA-free protease inhibitor tablets (Roche; cat# 11836170001) was added at a ratio of 200 mg tissue per mL buffer. Tissues were homogenized using a Precellys® homogenizer with one cycle consisting of three 30-second homogenization steps separated by 15-second rest intervals. Samples were maintained on ice immediately before and after homogenization to preserve protein integrity.

IL-2 fusion protein concentrations in plasma and skin homogenates were quantified using a customized electroluminescence immunoassay on a MESO SECTOR S600 instrument (Meso Scale Discovery). In short, biotinylated anti-mouse IL-2 antibody (R&D Systems; BAF402) was used as the capture reagent, and ruthenylated goat anti-mouse IgG antibodies (Jackson ImmunoResearch; Cat#115-005-205 and 115-005-206) were used for detection. Analyte concentrations were interpolated from standard curves using Watson LIMS™ Non-GLP Prod. Data acquisition and analysis were performed using Discovery Workbench 4.0 software (MSD).

Injection Site Reaction Scoring

All mice were given a physical examination of the injection site once per week. The scoring system was modified from ulcerative dermatitis (49), atopic dermatitis (50) and psoriasis (51) scoring. Briefly, the size of the affected area at the injection site, including visible hair-coat loss, was evaluated by measuring the maximal continuous length of the largest lesion in one dimension only. The lesion size was scored as follows: 1, <1 cm; 2, 1-2 cm; 3, 2-3 cm; and 4, greater than 3 cm. The bleeding characteristics were identified as 1, hemorrhage only; 2, slight active bleeding; 3, hemorrhage and moderate to severe active bleeding (>2 mm). Skin thickness was evaluated by skin desquamation and induration: 1 mild, minor dry spot with minimal skin change; 2, moderate, flaking dry spots with visible skin thickness; 3 severe, flaking across a large surface area with notable thickness and tight ridges. Inflammation was assessed based on tissue edema and erythema and scored as follows: 1, inflamed eyelid or skin; 2, inflamed eyelid and skin; and 3, involvement of additional tissues, such as the head region. When inflammation involved the head region, swollen eyelids, excessive ocular discharge, and possible crusting were also recorded. The overall injection-site severity index was calculated as the sum of the individual component scores.

In vivo antibody blockade during IL2Mut24-induced injection site reactions

For cytokine-blockade studies, *Rag2*^{-/-} mice received 69 pmol Fc-IL2Mut24 by dorsal subcutaneous injection twice weekly for four weeks. Mice were co-treated by intraperitoneal injection with 200 µg/mouse of anti-IL-4 (clone 11B11; BioXCell, BE0045), anti-IL-5 (clone TRFK5; BioXCell, BE0198), anti-IL-13 (clone 8H8; InvivoGen, mil13-mab9-1), anti-IFN γ (clone XMG1.2; BioXCell, BE0055), anti-IL-17A (clone 17F3;

BioXCell, BE0173), anti-CD25 (clone PC-61.5.3; InvivoGen, mcd25c1-mab10-1), or rat IgG1 κ anti-horseradish peroxidase isotype control (clone HRPN; BioXCell, BE0088). Antibodies were administered once weekly beginning on day 0, concurrently with the first Fc-IL2Mut24 dose. Injection site reactions were monitored throughout the study.

ILC2, ILC1, and NK cell expansion and adoptive transfer to *Rag2*^{-/-} γ *c*^{-/-} Mice

For ILC2 expansion, female *Rag2*^{-/-} donor mice were injected intraperitoneally with 4 μ g recombinant human IL-33 (BioLegend, Cat. 581808) in 100 μ L PBS on days 0, 2, 4, and 6. On day 8, lungs were harvested and digested for 1 hour at 37°C with Liberase TL (Roche, Cat. 05401020001; 250 μ g/mL) and DNase I (Sigma-Aldrich, Cat. D4263-1VL; 1 μ g/mL). Digests were filtered through 70- μ m strainers, treated with ACK lysis buffer (Gibco, Cat. A10492-01) and enriched using the Mouse ILC2 Isolation Kit (STEMCELL Technologies, Cat. 19842). Purified ILC2s were expanded for 3 days at 0.5×10^6 cells/mL in complete RPMI containing recombinant human IL-2 (20 ng/mL; PeproTech, Cat. 200-02).

For ILC1 and NK-cell expansion, female *Rag2*^{-/-} donor mice received 6 μ g recombinant mouse IL-15 (PeproTech, Cat. 210-15) and 1 μ g recombinant mouse IL-12 (PeproTech, Cat. 210-12) intraperitoneally every other day for five doses. One day after the final dose, livers were harvested and digested for 30 minutes at 37°C with Liberase TL (250 μ g/mL) and DNase I (1 μ g/mL). Leukocytes were enriched using a 30%/70% Percoll gradient, and ILC1s and NK cells were purified by flow-cytometric sorting.

Rag2^{-/-} γ *c*^{-/-} recipients received either 2×10^6 ILC2s (Figure 4) in the initial ILC2 reconstitution experiment or 3×10^5 ILC2s, ILC1s, or NK cells (Supplemental Figure 9) in

the matched lineage-comparison experiment. The lower cell number was used in the latter experiment because sufficient numbers of ILC1s and NK cells could not be obtained with the isolation and expansion protocol to support transfer of 2×10^6 cells per recipient.

Treg depletion and assessment of IL2Mut24-induced toxicity

Female *Rag2*^{-/-} mice (6-8 weeks old) received 1×10^7 T cells isolated from the spleens of Foxp3-DTR-mCherry knock-in mice (Mouse T Cell Isolation Kit, STEMCELL, cat#19851) by retro-orbital injection in 50 μ L PBS. Mice were subsequently administered 69 pmol IgG-IL2Mut24 subcutaneously in 100 μ L PBS twice weekly for three weeks. Following the final IgG-IL2Mut24 dose and after ISR onset, Tregs were depleted by intraperitoneal administration of unnicked diphtheria toxin from *Corynebacterium diphtheriae* (lyophilized powder; Sigma-Aldrich/SAFC) at 500 ng/mouse in 100 μ L PBS on days 21, 24, 28, 36, and 51. ISR severity and body weight were monitored longitudinally.

Foxp3-DTR-mCherry mice

Foxp3-DTR-mCherry knock-in mice were generated on a C57BL/6J background by Biocytogen using CRISPR/Cas9-mediated insertion of an IRES-DTR-1 $\times$ G4S-mCherry cassette between Foxp3 exon 14 and the 3' untranslated region. The targeting design was based on Foxp3 transcript 204 (NM_001199347.1). Founder mice were crossed with C57BL/6J mice, and germline transmission was confirmed by PCR, sequencing, and Southern blotting. Functional validation confirmed specific co-expression of FOXP3 and mCherry. Intraperitoneal administration of diphtheria toxin at 100, 200, or 500 ng per mouse resulted in complete Treg depletion in the spleen and inguinal lymph nodes within

12 hours. Depletion was maintained for up to 72 hours, after which the Treg population began to reconstitute.

Tissue Processing and Flow Cytometry

The spleen, lung, visceral adipose tissue (VAT), lymph nodes (LN), central nervous system (CNS), and skin tissues were dissected from mice and placed into PBS (Gibco) containing 2% FBS. The lung, VAT and CNS tissues were cut into small pieces and incubated in media containing 2 mg/mL collagenase (Millipore Sigma, Cat#11088858001) and RNase-free DNase (Sigma-Aldrich, Cat# D4263-1VL) and incubated at 37°C for 30-60 min under agitation, filtered over a 70 µm cell strainer (Corning), and washed with wash buffer (PBS+ 1 mM EDTA + 2% FBS) to obtain a single-cell suspension. CNS was further enriched using Percoll gradient. CNS suspension was resuspended in 6 mL of 37% Percoll, overlaid on top of 6 mL of 70% Percoll, and centrifuged at 2,000 RPM for 20 min with no brake at 4°C. The interphase containing the enriched leukocytes was collected and used. For spleen and LN processing, the tissues were placed on 70 µm filter and gently smashed using the back of a syringe plunger. The splenocytes were then lysed with 2 mL of ACK lysis buffer (Gibco, Cat#A10492-01) for 5 min and neutralized with wash buffer, followed by another round of washing. For skin tissue processing, subcutaneous fat was scraped off using a scalpel, minced to a paste and digested in a buffer containing RPMI, 250 µg/ml Liberase TL Research Grade (Roche, Cat# 5401020001) and 1 µg/ml Deoxyribonuclease I from bovine pancreas (Sigma-Aldrich, Cat# D4263-1VL) per 2x2 cm of skin for 90 mins-2 hours at 37°C. The tissues were then strained on a 70 µm filter, pelleted at 4°C and washed once. The obtained single cell suspensions of tissues were then used for further downstream analysis. Cell Stimulation

Cocktail plus Protein Transport Inhibitors (eBioscience™, cat#00-4975-93) reagent was used for cell stimulation (for 4 hours, at 37°C). Cells were washed prior to staining. For flow cytometry staining, cells were preincubated for 10 min with anti-mouse CD16/32 (Biolegend, Cat# 101301) to block mouse Fc receptor and then surface stained with cocktails (50 µl) of fluorochrome-conjugated antibodies in Brilliant Stain Buffer (BD Biosciences, Cat# 563794) with 1:1,000 LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Invitrogen, Cat# L34957) for 20 min. For intracellular staining, samples were fixed and permeabilized and stained with Foxp3/Transcription Factor Staining Buffer Set (eBioscience, Cat#00-5523-00) per manufacturer's directions. To obtain cell counts, CountBright™ Plus Absolute Counting Beads (Invitrogen, Cat# C36950) were added to samples immediately before flow cytometric analysis. Samples were acquired on Symphony Cytometer (BD) using BD FACSDiva software and then analyzed using the FlowJo software. Representative gating strategies for flow cytometric analysis are summarized in Supplemental Figure 15. The list of flow cytometry antibodies used in this study can be found in Supplementary Table 1.

scRNAseq Analysis

For scRNA seq, skin cell suspensions were enriched with Percoll density gradient centrifugation (Cytiva, Cat# 17144003) gradient. Briefly, cells were resuspended in 6 mL of 37% Percoll, overlaid on top of 6 mL of 70% Percoll, and centrifuged at 800 x g for 20 min with no break at 4°C. The interphase containing the enriched leukocytes was collected for downstream processing.

Before staining, cells were preincubated for 20 min with anti-mouse CD16/32 (Biolegend, Cat# 101301). For sample multiplexing, each individual sample from mice

within the same treatment group was incubated with a cocktail of TotalSeq™-C Mouse Universal Cocktail, V1.0 (Biolegend, Cat#199903) plus one TotalSeq™-C Hashtag antibody (Biolegend, 2 µL/sample, Hashtags C0306 (Cat#155871), C0307 (Cat#155873), C0308 (Cat#155875), C0309 (Cat#155877), and C0310 (Cat#155879)) at 4°C for 15 min. Samples were washed with BD Pharmingen™ Stain Buffer (BD Biosciences, Cat#554657) and were stained for CD45-BV786 (Biolegend, Cat#103149), TCRβ-PE (BD Biosciences, Cat#561081) and LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit (Invitrogen, Cat#L34976) at 4°C for 15 min before sorting. Within each treatment group, samples were pooled and sorted on a FACS Aria (BD Biosciences) into three fractions: CD45⁺TCRβ⁺ GFP⁺, CD45⁺TCRβ⁺GFP⁻, CD45⁺TCRβ⁻GFP⁻. Sorted cells were captured in droplet emulsions using Chromium X (10x Genomics) and libraries were constructed using the 10x Genomics Chromium Next GEM Single Cell 5' Kit v2 (10x Genomics, Cat#1000263) and Chromium Next GEM Single Cell 5' Gel Bead Kit v2 (10x Genomics, Cat# 1000264) according to manufacturer's instructions. Amplified 5' Gene Expression, V(D)J and Cell Surface Protein libraries were profiled using the 4200 TapeStation System (Agilent Technologies) and pooled at 10:1:1 ratio. Pooled samples were sequenced on Illumina NovaSeq X (UCSF, Center for Advanced Technology (CAT)) using a 28-10-10-90 asymmetric read configuration. A minimal sequencing depth of 50,000 reads/cell was targeted for each Gene Expression Library.

Raw data were aligned to the mouse reference genome mm10 and GRCm38 V(D)J using a custom workflow of Cell Ranger (v7.1.0) that supports sample demultiplexing and 5' immune profiling. The filtered output matrices were imported into R (4.1.0) for downstream analysis.

The Seurat R package (version 4.3.0) was used for further analysis of the single-cell data. Low-quality cells were removed by excluding those with $\geq 10\%$ mitochondrial UMIs, ≤ 200 detected genes, or UMI counts outside the 2.5-97.5 percentile of the library-size distribution. RNA and ADT count matrices were normalized using the “LogNormalize” and “CLR” methods, respectively. The top 2000 variable genes were identified with “FindVariableFeatures.” Data was scaled (“ScaleData”) before PCA reduction (“RunPCA”), and graph-based clustering was performed via “FindMultiModalNeighbors” and “FindClusters” function at a resolution of 0.5, 0.8, 1.0 and 2.0. Dimensionality reduction was performed with “RunUMAP” function and visualized by Uniform Manifold Approximation and Projection (UMAP). Cell subclusters were annotated to known biological cell types using canonical marker genes (Supplemental Figure 13C-E). Gene signatures used to define cell populations are provided in Supplementary Table 2 (22).

For principal component analysis of ILC2, Tregs, CD4⁺ and CD8⁺ conventional T cells, the “AggregateExpression” function was used to sum together gene counts of all the cells from each treatment group for each cell type. Summed gene counts were extracted from the pseudo-bulk single-cell samples to perform “DESeq” analysis using the DESeq2 package (v1.34.0). PCA matrix was obtained by “plotPCA” after VST transformation, and the results were visualized by ggplot2 (v3.5.2).

Differential state analysis was performed using the muscat package (v1.0.1) to identify differentially regulated genes in each cell type between (1) Fc-IL2Mut24 and isotype control, (2) PC61-IL2Mut24 and isotype control and (3) PC61-IL2Mut24 and Fc-IL2Mut24 groups. Mitochondrial, ribosomal, histone, TCR and predicted genes were excluded from the analysis. The 120 most significantly up- or down-regulated genes

(adjusted local p -value < 0.05) were selected, duplicate genes were removed, and heatmaps were generated using the ComplexHeatmap (v2.6.2). A complete list of the up to top 120 significantly differentially regulated genes can be found in Supplementary Table 3.

Cytokine Measurements

For serum collection, whole blood was collected via cardiac puncture using a sterile 1 mL syringe and 25G needle. Blood was transferred into serum separator tubes (SST) without anticoagulants (BD Microtainer, Cat#365967). Samples were allowed to clot at room temperature for 30 minutes, followed by centrifugation at 2,000 g for 10 minutes at 4°C. The serum supernatant was carefully collected without disturbing the clot. Sera were aliquoted into sterile microcentrifuge tubes and stored at -80°C until cytokine analysis.

For skin lysate preparation, skin samples were harvested from euthanized mice, rinsed briefly in ice-cold PBS to remove blood and debris, and snap-frozen in liquid nitrogen or stored at -80°C until processing. Frozen skin tissues were weighed and placed in pre-chilled lysing matrix tubes (tubes containing ceramic beads (Precellys, Cat#10011151). Lysis buffer (Gibco, Cat#A10492-01) supplemented with protease and phosphatase inhibitor (Thermo Scientific, Cat# 78842) was added at 10 μ L per mg of tissue. Samples were homogenized using a bead homogenizer (Precellys Evolution Homogenizer, P000062-PEVO0-A) for 2 $\times$ 30 seconds at 6.5 m/s with a 5-minute interval on ice between cycles to prevent overheating. Homogenates were centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was collected, and total protein concentration

was determined using a Pierce BCA protein assay (Thermo Fisher Scientific, Cat#23227). Aliquots of clarified lysate were stored at -80°C until analysis.

The concentration of cytokines in mouse serum samples and mouse skin lysate samples was analyzed with V-PLEX Proinflammatory Panel 1 Mouse Kit (MSD, Cat#K15048D) per manufacturer's instructions. Electroluminescence was measured with a MESO SECTOR S600 instrument and analyzed using Discovery Workbench 4.0 (MSD). IL-13 was measured by ELISA using the Mouse IL-13 ELISA Kit (Invitrogen, cat#BMS6015) according to the manufacturer's instructions.

Histopathological Analysis

Freshly collected full-thickness intrascapular skin samples were fixed in 10% neutral buffered formalin for a minimum of 24 hours and post-fixed in ethanol. Samples were paraffin-embedded, sectioned at a standard thickness of 4 µm, and stained with hematoxylin and eosin (H&E) at Cureline, Inc. (South San Francisco, CA). Digital scanning of stained slides was performed to facilitate virtual assessment by a board-certified veterinary pathologist. Two non-serial skin sections per animal were reviewed for each animal and scored using a 5-point scale for grading disease severity according to predefined criteria for inflammation, cellular infiltrate, hyperplasia, fibroplasia, and structural integrity. Each anatomical skin layer-epidermis, dermis, panniculus, and subcutis-was assessed and assigned a severity grade. The sum of individual layer scores (potential maximum score of 20) constituted the Total (composite) Pathology Score, representing the overall grade of histopathological changes for each animal. Scores were defined as follows: Normal intrascapular skin; Grade 1 pathology (minimal epidermal

hyperplasia (acanthosis of $<100\ \mu\text{m}$) and hyperkeratosis, minimal mixed dermal inflammation, minimal mixed inflammatory cell infiltrates within the panniculus, and a normal subcutis); Grade 2 pathology (mild epidermal hyperplasia (acanthosis of $<150\ \mu\text{m}$) and hyperkeratosis (with crusts, intraepithelial pustules and spongiosis), mild diffuse dermal inflammation with foci of subepidermal fibrosis, mild pannicular mixed inflammatory cell infiltrates with perivascular cuffing, and minimal to mild diffuse mixed inflammatory infiltrates in the subcutis); Grade 3 pathology (similar, but more frequent and severe features (including ulcerations) within the epidermis, moderate mixed dermal inflammation with prominent subepidermal fibrosis, moderate pannicular mixed inflammation and bridging fibroplasia, and mild to moderate diffuse mixed inflammatory infiltrates in the subcutis); Grade 4 pathology (similar, but more severe features of inflammation including regional epithelial separation and full thickness ulceration of the epidermis, marked mixed inflammation with focal to diffuse fibroplasia (loss of adnexa), marked pannicular inflammation and fibroplasia with reduced adipocytes, and moderate diffuse mixed inflammatory infiltrates with foci of neutrophil aggregation in the subcutis); Grade 5 pathology (severe) was not observed in this study.

Design and generation of tool molecules

All recombinant proteins used in this study included monoclonal antibodies (mAbs) and IL-2 fusion proteins. The IL-2 proteins included mouse IL2WT (1-149, T3A, P51T, C140A) and IL2Mut24 (1-149, T3A, P51T, N103R, V106D, C140A), each fused to the C-terminus of mouse Fc domains or mAbs (17). Residue numbering was based on position 1 as the first residue after sequence peptide cleavage. The substitutions T3A, P51T, and

C140A were introduced to improve construct manufacturability and did not alter the biological activity of IL2WT or IL2Mut24 (17).

DNA fragments were ordered from either IDT (Coralville, IA) or Twist Bioscience (South San Francisco, CA). The gene fragments were assembled using Golden-Gate cloning technology into proprietary mammalian expression vectors compatible with the piggyBac (PB) transposase system (52). All constructs were verified by Sanger DNA sequencing.

All recombinant molecules were stably expressed in CHOK1 (Amgen Inc.) suspension cells using transposase-based technology described previously (52). Protein titers of conditioned media (CM) were estimated by Octet quantitation assays (Protein A Biosensors, ForteBio cat#18-0004) or SDS-PAGE analysis (Invitrogen, NuPAGE™ Bis-Tris Midi Protein Gels).

All recombinant proteins were captured via pre-packed protein A columns (Cytiva, HiTrap™ MabSelect™ SuRe) on AKTA FPLC systems. The CMs obtained using the expression procedure described above were loaded and washed on columns equilibrated in TBS (20 mM Tris, 150 mM NaCl, pH 7.5). Captured proteins were eluted with 0.5% acetic acid, 150 mM NaCl, pH 3.5. The eluents were further polished by size exclusion chromatography (SEC) (HiLoad Superdex 200 pg, Cytiva, Cat#28989336) in HBS (HEPES: 30 mM HEPES; 150 mM NaCl; pH 7.6). The final purified proteins were formulated in HBS or A52Su (Acetate: 10 mM NaOAc; 9% Sucrose; pH 5.2) for storage and filtered with 0.2 µm filters with polysulfone membrane (Cytiva, Cat#50-206-3123). Protein concentrations were determined by Nanodrop spectrophotometry for

concentration (NanoDrop-8000, Thermo Fisher Scientific), and all samples were stored at -70°C.

Protein purity was assessed by analytical SEC on an Agilent 1200 series HPLC, using either: (1) a Zenix-C SEC-300 column (3 μ m, 300 $\times$ 4.6 mm; Sepax, Cat#233300-4630), or (2) a TSK Gel 3000SWXL column (7.8 mm I.D. $\times$ 30 cm; TOSOH Bioscience, Cat#0008541). The Zenix-C column was operated at 0.45 mL/min and 12-minute runtime, using a mobile phase consisting of 100 mM sodium phosphate and 250 mM NaCl at pH 6.8. The TSK Gel column was run at 1 mL/min, with a mobile phase consisting of 50 mM phosphate buffer and 0.4 M sodium perchlorate at pH 6.8. For comparison, protein purity was also assessed by SDS-PAGE.

Intact mass analysis was performed using an Agilent 1200 HPLC system coupled with Agilent 6230 TOF mass spectrometer. Non-reduced samples were prepared to contain 5 μ g of protein in 0.05% formic acid in water. Reduced samples were prepared by heating the 5 μ g of sample in 4M guanidine hydrochloride and 16.7 mM TCEP at 60°C for 30 min, then chilling briefly before adding formic acid to a final concentration of 0.05% in water. Glycosylated samples were deglycosylated by reduced or non-reduced Rapid PNGase F as described previously (New England Biolabs, reduced: Cat#P0704S, non-reduced: Cat#P0711S)(52). 2-5 μ g of proteins were injected onto a 2.1 mm $\times$ 75 mm C4 reverse phase column (Mac-mod) equilibrated and washed by 0.1% formic acid in water. Proteins within each sample were separated using a gradient of 5-95% buffer B (0.1% formic acid in acetonitrile) at a flow rate of 0.75 mL/min for 12 minutes prior to MS analysis. The full MS scan was obtained over the m/z range of 500-3500.

The endotoxin content of purified proteins was measured using Endosafe LAL cartridges and the Nexgen-MCS system (Charles River). All samples used in this study contained endotoxin less than 0.2 EU/mg.

CTLL-2 and ILC2 Proliferation and ILC2 CD25 Upregulation Assays

To assess IL-2 bioactivity, [³H]-thymidine incorporation assays were performed using IL-2-dependent CTLL-2 cells (ATCC, Cat# TIB-214™) or purified mouse ILC2s. CTLL-2 cells were maintained in complete RPMI-1640 supplemented with 10% FBS, 1 mM sodium pyruvate, 1× non-essential amino acids, 10 mM HEPES, 1× penicillin-streptomycin, and recombinant human IL-2 (PeproTech, Cat# 200-02) at a density of 0.2-1 × 10⁶ cells/mL. Cells were washed and deprived of IL-2 overnight before use.

For ILC2 isolation, *Rag2*^{-/-} mice were administered 4 µg IL-33 five times over one week. Lungs were harvested, and ILC2s were purified using the Mouse ILC2 Enrichment Kit (STEMCELL Technologies, Cat# 19842). Purified ILC2s were expanded at 0.5 × 10⁶ cells/mL in complete RPMI containing 20 ng/mL IL-2 for 4 days, washed twice, and cultured overnight in IL-2-free T-cell medium before use.

CTLL-2 cells (10,000 cells/well) or ILC2s (80,000 cells/well) were plated in flat-bottom 96-well plates and stimulated with half-log serial dilutions of each IL-2 construct from 100 nM to 32 pM. After 48 hours, cells were pulsed with 1 µCi/well [³H]-thymidine for an additional 18 hours. Cells were harvested onto filter membranes, and incorporated radioactivity was quantified using a MicroBeta² scintillation counter. Results were reported as counts per minute (CPM).

To assess CD25 upregulation, IL-33-expanded ILC2s were cultured directly with IL-2 constructs at 1 nM. CD25 surface expression was measured by flow cytometry at 0, 2, 4, and 8 hours with anti-CD25 (clone: 7D4).

Active Induction of EAE

Experimental autoimmune encephalomyelitis (EAE) was induced in female C57BL/6 mice (8-14 weeks old) by subcutaneous immunization with MOG₃₅₋₅₅ peptide emulsified in complete Freund's adjuvant (CFA). Mice received a total of 200 μ L of CFA (1 mg/mL MOG₃₅₋₅₅; 1 to 5 mg killed mycobacterium tuberculosis H37Ra/mL; Hooke Laboratories, Cat#EK-2110), administered at two sites (100 μ L per flank; right and left hind flanks). On the day of immunization (day 0) and again on day 2, mice were intraperitoneally (i.p.) injected with 120 ng of pertussis toxin (PTx, Hooke Laboratories, Cat#BT-0105) diluted in sterile saline. Anti-CD4 (Clone GK1.5, BioXCell, cat#BE0003-1) was used as a control in the study. Following immunization, mice were monitored daily for body weight and clinical signs of EAE using the following scoring system: 0 indicated no clinical signs; 0.5, partial limp tail or body tremors; 1, limp tail with normal gait; 2, limp tail and hind limb weakness (waddling gait); 3, partial hind limb paralysis; 4, complete hind limb paralysis; and 5, severe forelimb paralysis, moribund condition, or humane endpoint. Body weights were normalized to the day 0 value (set as 100%) and tracked daily as a percentage change. The mean clinical EAE score was calculated as the average of daily scores across the disease course for each mouse. Mice that reached a score of 5 were euthanized and excluded from further clinical score and weight analysis from that point forward.

Cynomolgus Monkey Studies

Animals and Ethical Compliance

Naïve cynomolgus monkeys (*Macaca fascicularis*) were housed under controlled environmental conditions with a 12-hour light/dark cycle and provided environmental enrichment according to institutional standard operating procedures. Monkeys were individually housed for intensive observation following dosing and pair- or group-housed thereafter when compatible. Animals were monitored daily for clinical signs and overall health throughout the study.

Study Design and Dosing

In study 118075, male cynomolgus monkeys (n = 4 per group) received either five daily s.c. injections of 3 or 10 µg/kg aldesleukin on days 0 to 4 followed by 30 or 100 µg/kg on days 13-17) or a single s.c. injection of 1 or 3 µg/kg AMG592 on day 0 and 100 or 200 µg/kg on day 13 (Figure 5A).

In study 118065, monkeys were assigned to treatment groups (n = 4 per sex per group) and administered s.c. injections of AMG592 (10, 30 or 100 µg/kg) once weekly for three months (total of 12 doses) (Figure 5H).

Blood collection, immunophenotyping, serum cytokine analysis

In study 118075, for immunophenotyping, peripheral blood was collected via the femoral vein or artery in K₂EDTA tubes pre-dose (days -15, -8, and day 0 prior to 1st dose) and on days 3, 4, 5, 12, 14, 18, 21, 24, and 28. For cytokine analysis, whole blood was collected at the following time points and processed to serum: pre-dose (days -8 and day

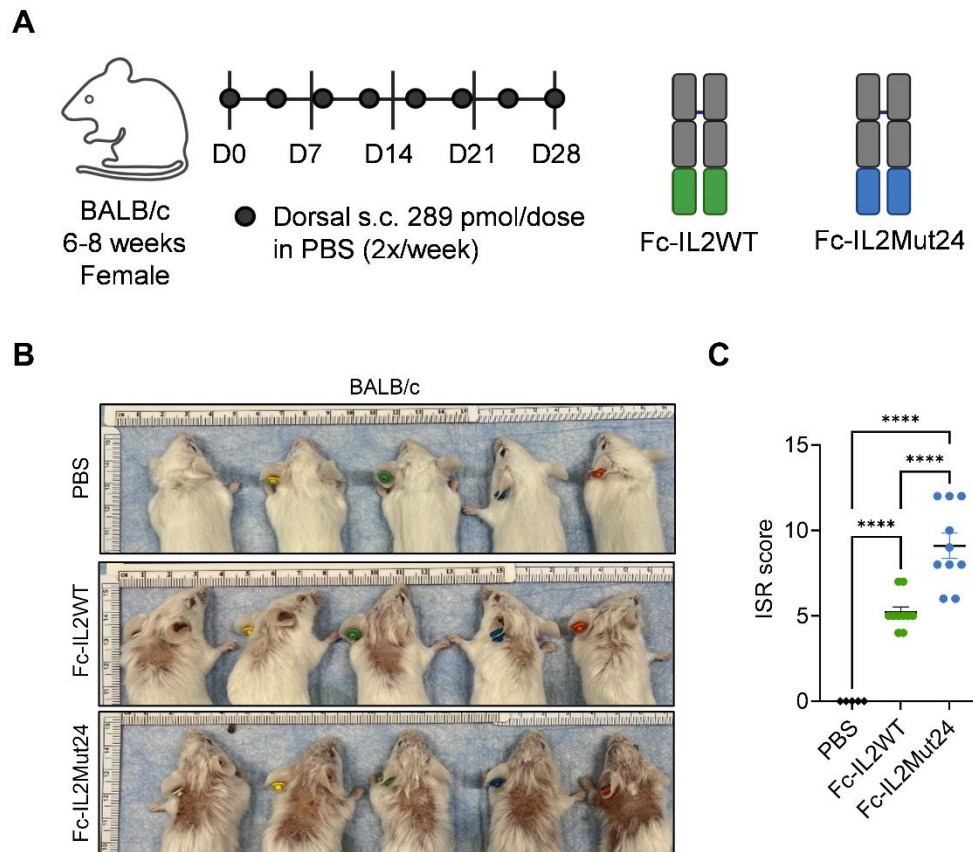
0 prior to dosing), days 4, 5, 16, 17, 18, and 35. Serum cytokines were quantified using Milliplex®MAP Non-HumanPrimate Cytokine Magnetic Bead Panel (Millipore, Cat# PRCYTOMAG-40K-10) according to manufacturer instructions. Cytokine concentrations were determined relative to standard curves generated using a 5 parameter logistic non-linear regression fitting curve. The following cytokines were measured: IFN γ , GM-CSF, TNF, IL1- β , IL-2, IL-5, IL-6, IL-10, IL-12p40, IL-13, and IL-17. Cytokines detected above the assay limit were shown in Figure 5C-G.

Clinical Monitoring and Injection-Site Pathology

In study 118065, at scheduled necropsy (day 92), the designated subcutaneous injection site was collected for histopathologic evaluation. Tissues were fixed, processed, and stained with hematoxylin and eosin according to standard toxicologic pathology procedures, and lesions were evaluated by a board-certified veterinary pathologist and graded by severity (e.g., minimal or mild).

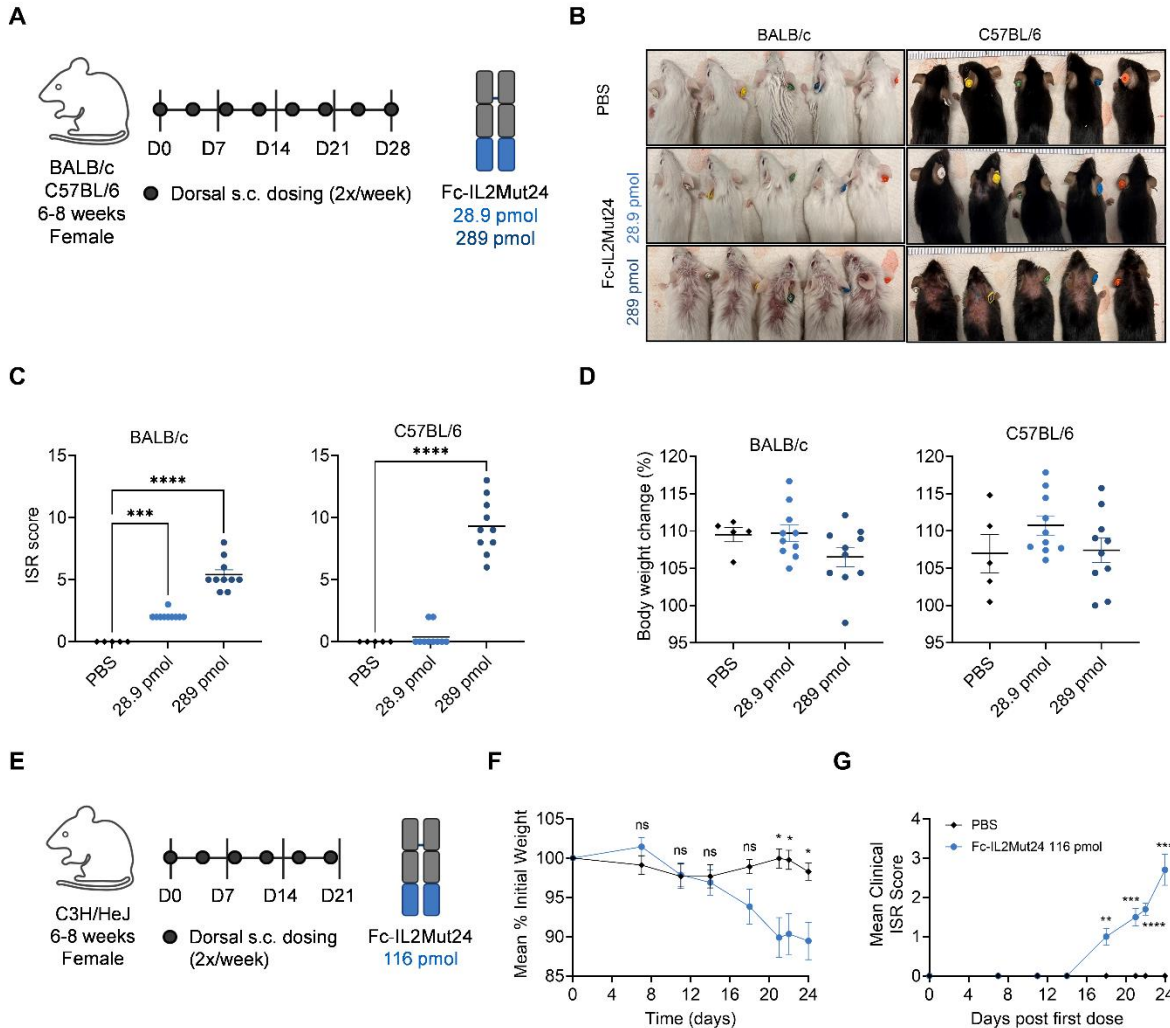
SUPPLEMENTAL FIGURES

Supplemental Figure 1



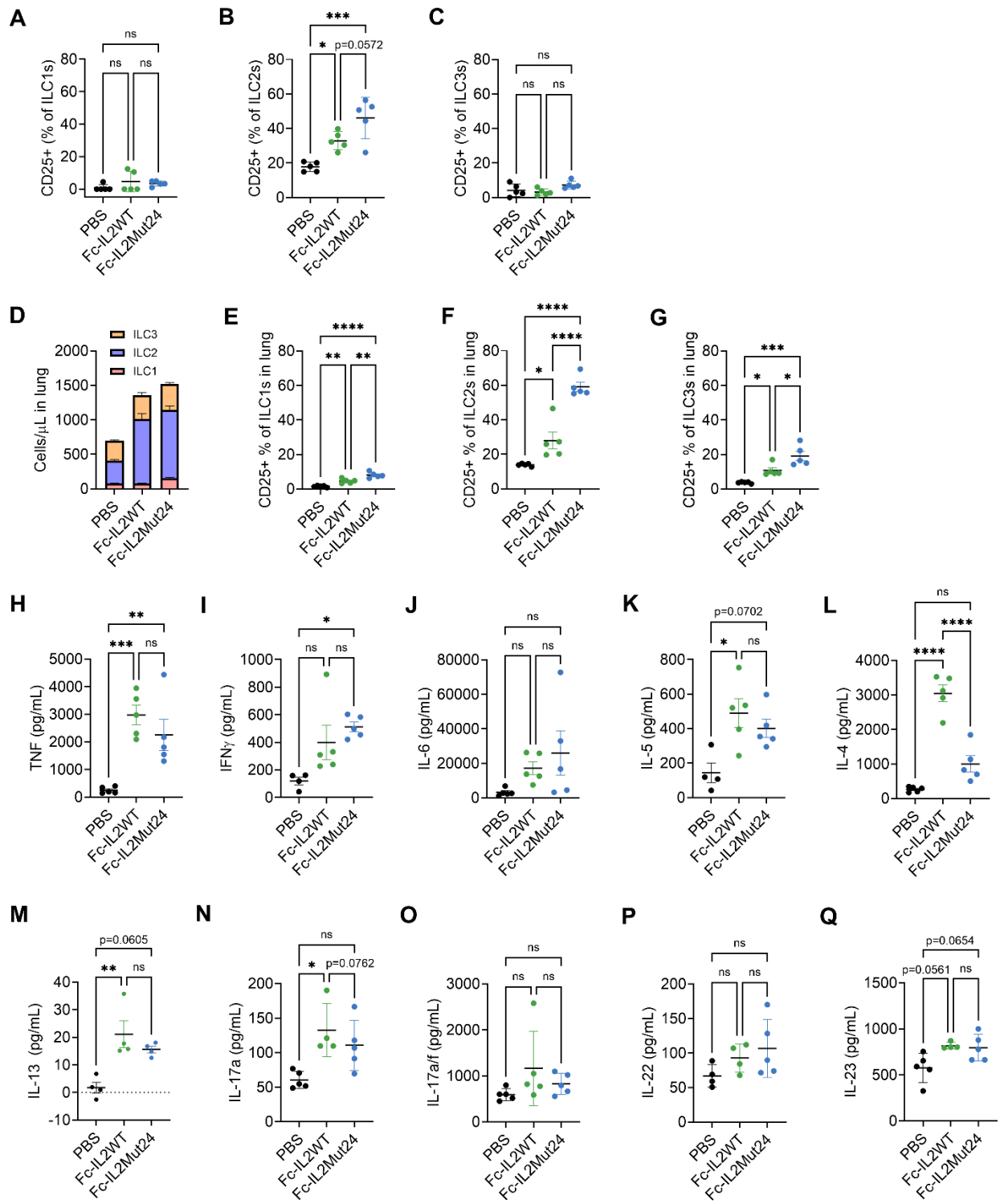
Supplemental Figure 1. Fc-IL2Mut24 induces severe ISRs associated with prolonged systemic and cutaneous exposure. 6-8-week-old female BALB/c mice received dorsal s.c. dosing of either Fc-IL2WT or Fc-IL2Mut24 289 pmol (~25 μ g/dose/mouse in PBS) or PBS alone twice per week for four weeks. **(A)** Schematic representation of experimental design. **(B)** Representative photograph of dorsal ISR (n=5 mice per group). **(C)** ISR score (n=5 mice in PBS, n=10 mice each in Fc-IL2WT and Fc-IL2Mut24). Two-tailed ordinary one-way ANOVA followed by Sidak's post hoc test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplemental Figure 2



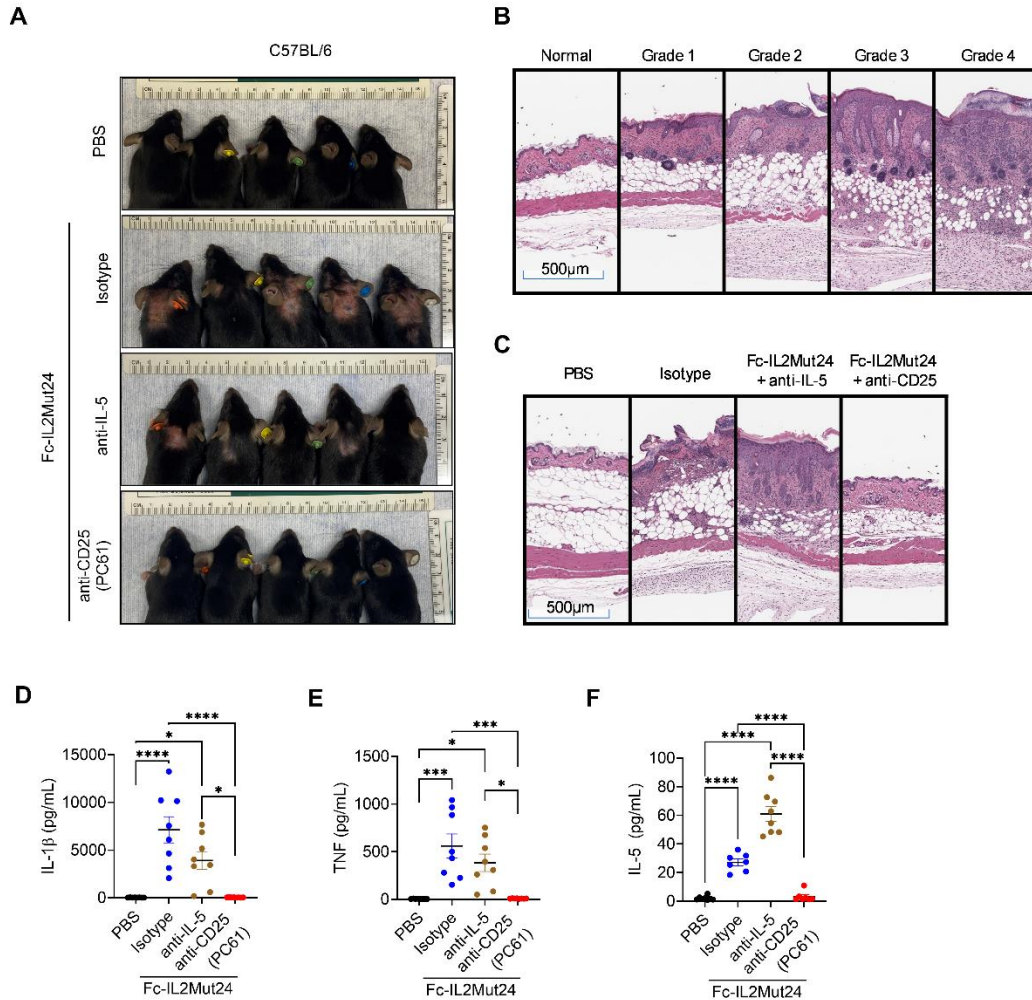
Supplemental Figure 2. Fc-IL2Mut24 induced ISRs across BALB/c, C57BL/6J, and C3H/HeJ. (A-D) BALB/c (left) and C57BL/6J (right) mice were treated s.c. twice weekly for four weeks with PBS, or 28.9 pmol or 289 pmol Fc-IL2Mut24. (A) Schematic representation of experimental design. (B) Representative images from n=5 mice per strain after eight doses of Fc-IL2Mut24. (C) ISR score, (D) Body weight change from day 0 (%) at endpoint. (E-G) In a separate cohort, C3H/HeJ mice were immunized with 116 pmol Fc-IL2Mut24 twice weekly for three weeks, while a control group remained naïve and untreated. (E) Schematic representation of experimental design. (F) Body weight over time (days) as % change from initial weight. (G) ISR score over time (days). (C-D) n=5 mice per strain in the PBS condition, n=10 mice per strain in the 28.9 pmol and 289 pmol Fc-IL2mut24 conditions. (F-G) n=10 mice per condition. Data shown as mean $\pm$ SEM. (C) Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc test; (F) Two-tailed mixed effects analysis followed by Sidak's multiple comparisons post hoc test. (G) Two-way ANOVA followed by Sidak's multiple comparisons post hoc test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplemental Figure 3



Supplemental Figure 3. Fc-IL2Mut24 induces a CD25-dependent type 2 inflammatory program. (A-G) C57BL/6 mice (n=5 mice per group) received dorsal s.c. dosing twice weekly for four weeks with PBS, 69 pmol (~6.5 µg/dose/mouse) Fc-IL2WT, or Fc-IL2Mut24. **(A-C)** Frequency of CD25⁺ cells among ILC1s **(A)**, ILC2s **(B)** and ILC3s **(C)** in skin at endpoint, as analyzed by flow cytometry. **(D)** ILC1, ILC2 and ILC3 cell counts per sample in lung at endpoint represented as stacked bar graph, as analyzed by flow cytometry. **(E-G)** Frequency of CD25⁺ cells among ILC1s **(E)**, ILC2s **(F)** and ILC3s **(G)** in lung at endpoint, as analyzed by flow cytometry. **(H-Q)** Cytokine levels of TNF **(H)**, IFN γ **(I)**, IL-6 **(J)**, IL-5 **(K)**, IL-4 **(L)**, IL-13 **(M)**, IL-17A **(N)**, IL-17A/F **(O)**, IL-22 **(P)**, and IL-23 **(Q)**, shown as concentration (pg/mL) from endpoint skin homogenates as detected by MSD. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplemental Figure 4

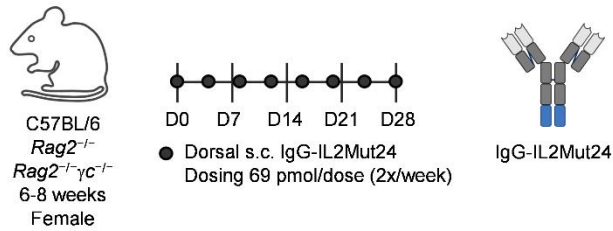


Supplemental Figure 4. Anti-CD25 blockade reduces IL-2-induced skin pathology.

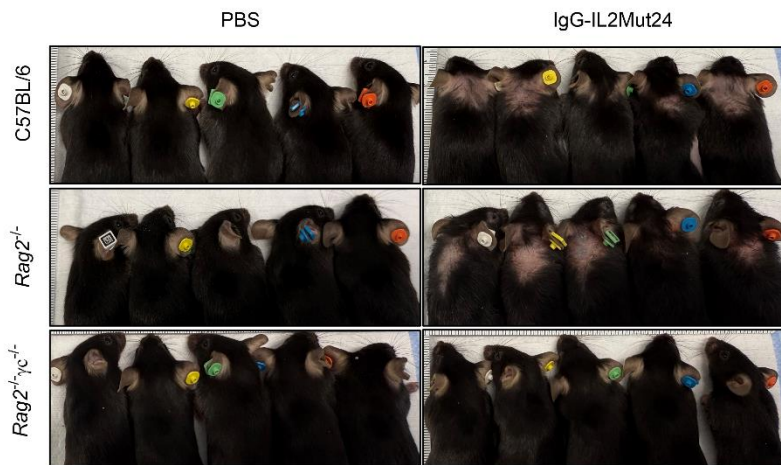
(A-G) C57BL/6 mice (n=10 mice per group) received dorsal s.c. dosing twice weekly for four weeks with PBS or 69 pmol (~6.5 μg/dose/mouse) Fc-IL2Mut24 and co-treated once weekly with 200 μg anti-IL-5, anti-CD25 or isotype control. **(A)** Representative dorsal images (n=5 mice per group). **(B)** Representative H&E staining images showing range of ISR severity in C57BL/6J mice following eight doses of Fc-IL2Mut24 (scale bar = 500 μm, 4x magnification). **(C)** Representative H&E staining images from one mouse per treatment condition (scale bar = 500 μm, 4x magnification) from the experiment shown in Figure 3H. Mice were selected to represent the mean pathology score for their respective treatment conditions (PBS = 0, isotype = 9, anti-IL-5 = 8, anti-CD25 = 1.5). **(D-F)** Inflammatory cytokine levels of IL-1β **(D)**, TNF **(E)**, IL-5 **(F)**, shown as concentration (pg/mL) from endpoint skin homogenates as detected by MSD. Data shown as mean ± SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc test; only selected comparisons are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplemental Figure 5

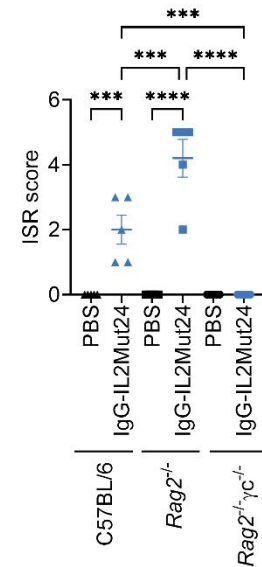
A



B



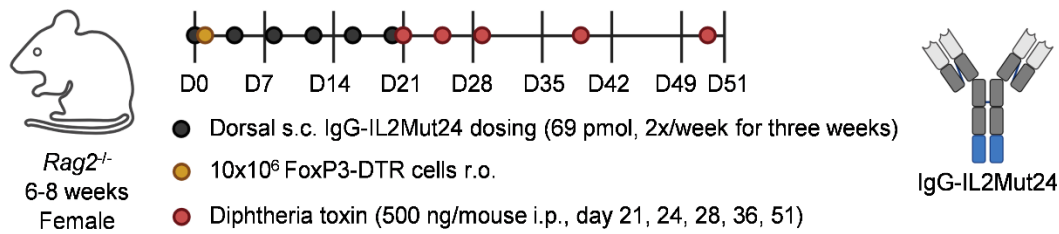
C



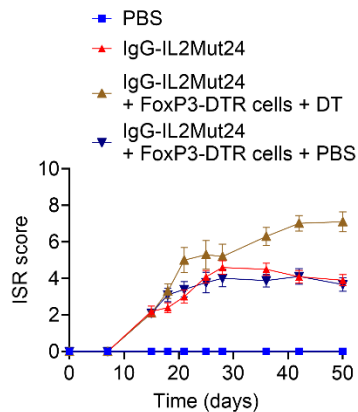
Supplemental Figure 5. γ c deficiency protects from IL-2-induced injection site reaction. (A-C) C57BL/6NTac, *RAG2*^{-/-} or *RAG2*^{-/-} γ *c*^{-/-} (n=5 mice per group) were treated subcutaneously twice weekly for four weeks with 69 pmol IgG-IL2Mut24. **(A)** Schematic representation of experimental design. **(B)** Dorsal images. **(C)** ISR scores at endpoint. Data shown as mean $\pm$ SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc; only selected comparisons are shown. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

Supplemental Figure 6

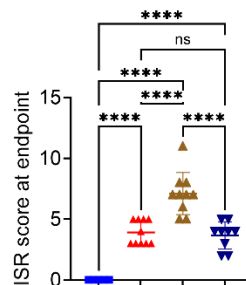
A



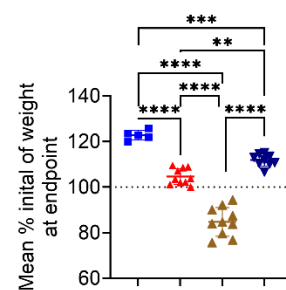
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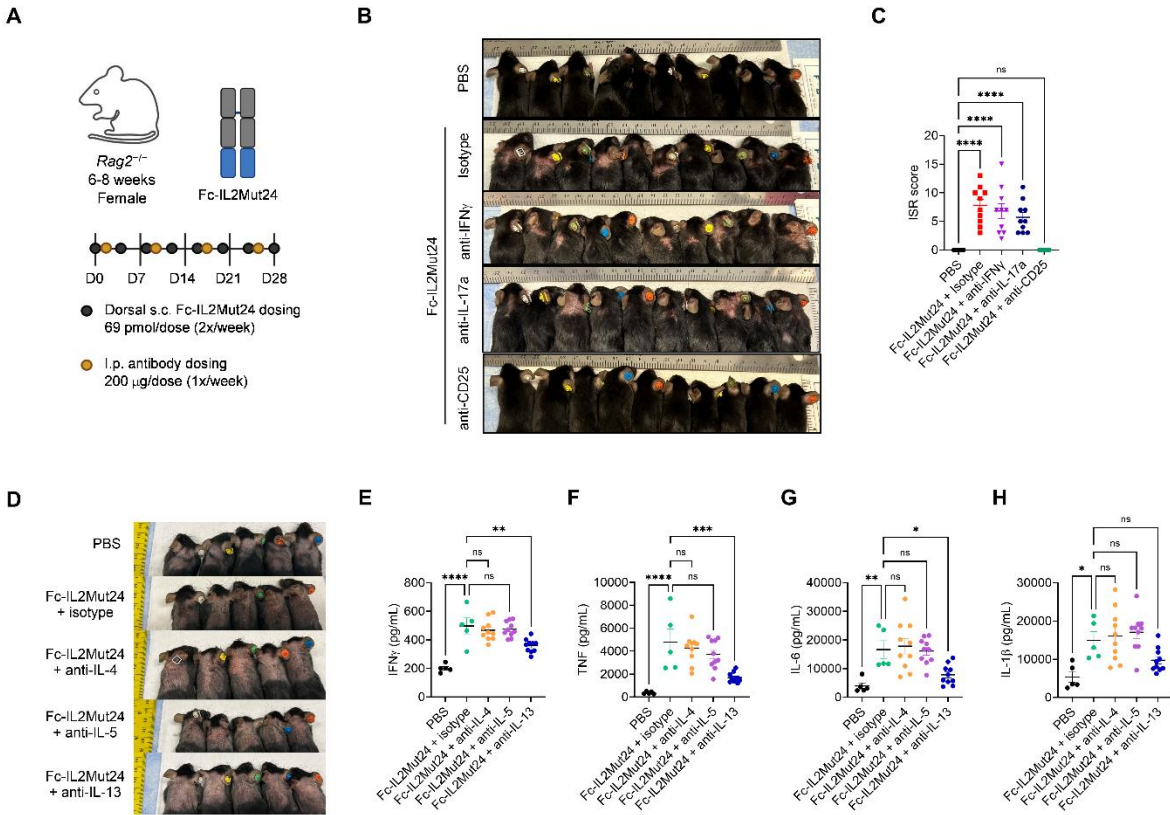
D



Supplemental Figure 6. Treg depletion exacerbates IL2Mut24-induced ISR and weight loss in immune-reconstituted mice.

(A-D) 6-8-week-old female *Rag2^{-/-}* mice were transferred retro-orbitally with 10 million FoxP3-DTR cells (enriched T cells) in 50 μ L of PBS and received 69 pmol of IgG-IL2Mut24 in 100 μ L of PBS s.c. twice per week for three weeks. Diphtheria toxin (500ng/mouse in 100 μ L PBS, i.p. on days 21, 24, 28, 36, and 51) was administered following the last dose of IgG-IL2Mut24 (after ISR onset). **(A)** Schematic representation of experimental design. **(B)** ISR scores over time (days) at the injection site. **(C)** ISR scores at the injection site at endpoint. **(D)** Body weight at endpoint as % change from initial weight. $n=5$ mice in the PBS condition and $n=10$ mice in all other conditions. Data shown as mean $\pm$ SEM. Two-tailed ordinary one-way ANOVA analysis followed by Sidak's multiple comparisons post hoc test in **C** and **D**; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

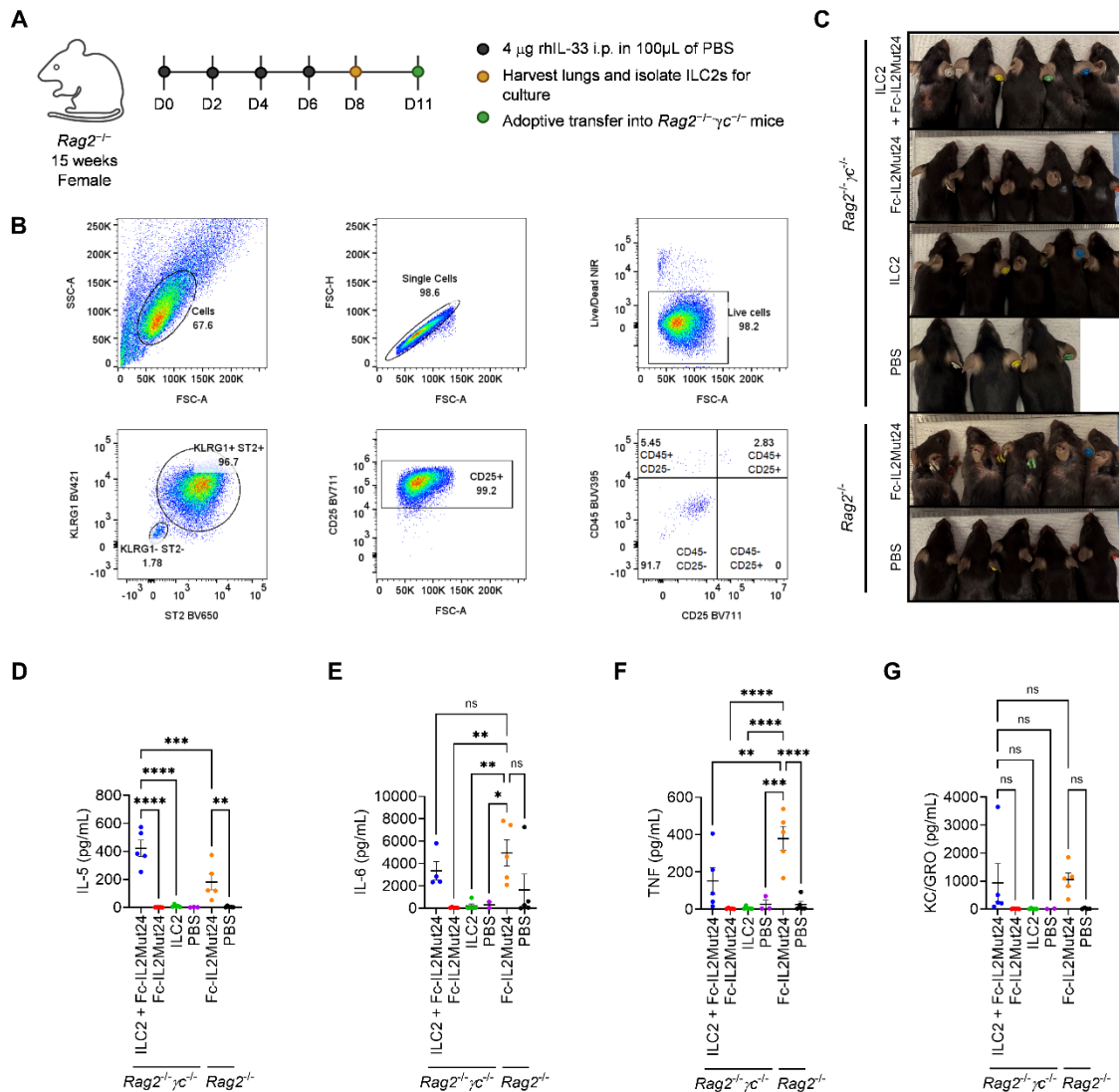
Supplemental Figure 7



Supplemental Figure 7. Effects of CD25 and cytokine blockade on Fc-IL2Mut24-induced ISR in *Rag2*^{-/-} mice.

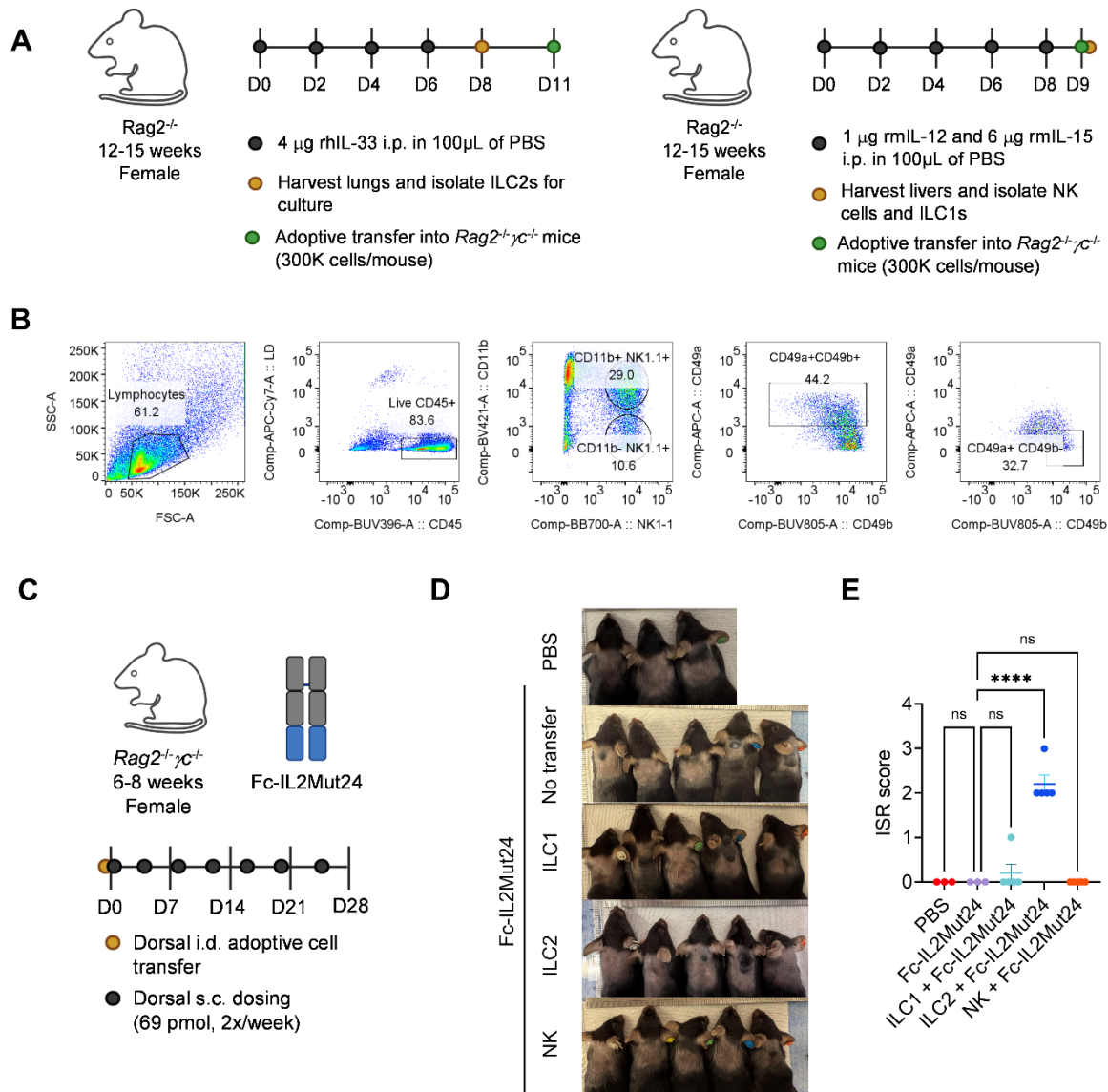
(A-C) *Rag2*^{-/-} mice (n=10 mice per group) received dorsal s.c. dosing twice weekly for four weeks with PBS or 69 pmol (~6.5 µg/dose/mouse) Fc-IL2Mut24 and co-treated once weekly with 200 µg anti-IFN γ , anti-IL-17a, anti-CD25 or isotype control. (A) Schematic representation of experimental design. (B) Representative dorsal images (n=5 mice per group). (C) ISR scores at endpoint. (D-H) *Rag2*^{-/-} mice (n=5 mice in PBS and isotype groups and n=10 mice in all other groups) received dorsal s.c. dosing twice weekly for four weeks with 69 pmol (~6.5 µg/dose/mouse) Fc-IL2Mut24 and co-treated once weekly with 200 µg anti-IL-4, anti-IL-5, anti-IL-13 or isotype control. (D) Representative dorsal images (n=5 mice per group). Mice were shaved for better visualization of the injection site. (E-H) Cytokine levels of IFN γ (E), TNF (F), IL-6 (G) and IL-1 β (H), shown as concentration (pg/mL) from endpoint skin homogenates as detected by MSD. Data shown as mean±SEM. Two-tailed ordinary one-way ANOVA analysis followed by Sidak's multiple comparisons post hoc test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplemental Figure 8



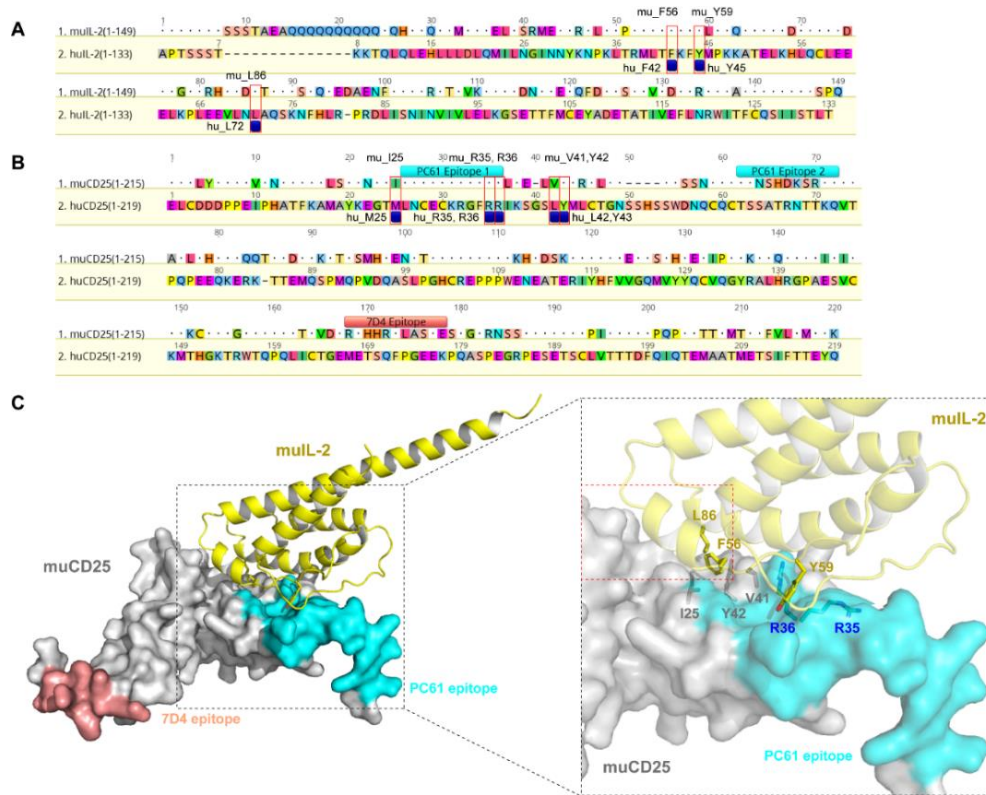
Supplemental Figure 8. Characterization of IL-33-expanded ILC2s and skin cytokine responses after transfer. (A) Schematic representation of experimental design for IL-33-mediated ILC2 expansion in *RAG2*^{-/-} mice. (B) Flow cytometry analysis of cultured ILC2 cells prior to adoptive transfer to recipient mice. (C) Representative dorsal images (n=5 mice per group) from the experiment shown in Figure 4F. (D-G) Concentration (pg per 50 µL of skin lysate) of IL-5 (D), IL-6 (E), TNF (F) and KC/GRO (G) at endpoint. n=3 mice in PBS group and 5 mice in all other groups. Data shown as mean ± SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc test; only selected comparisons are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplemental Figure 9



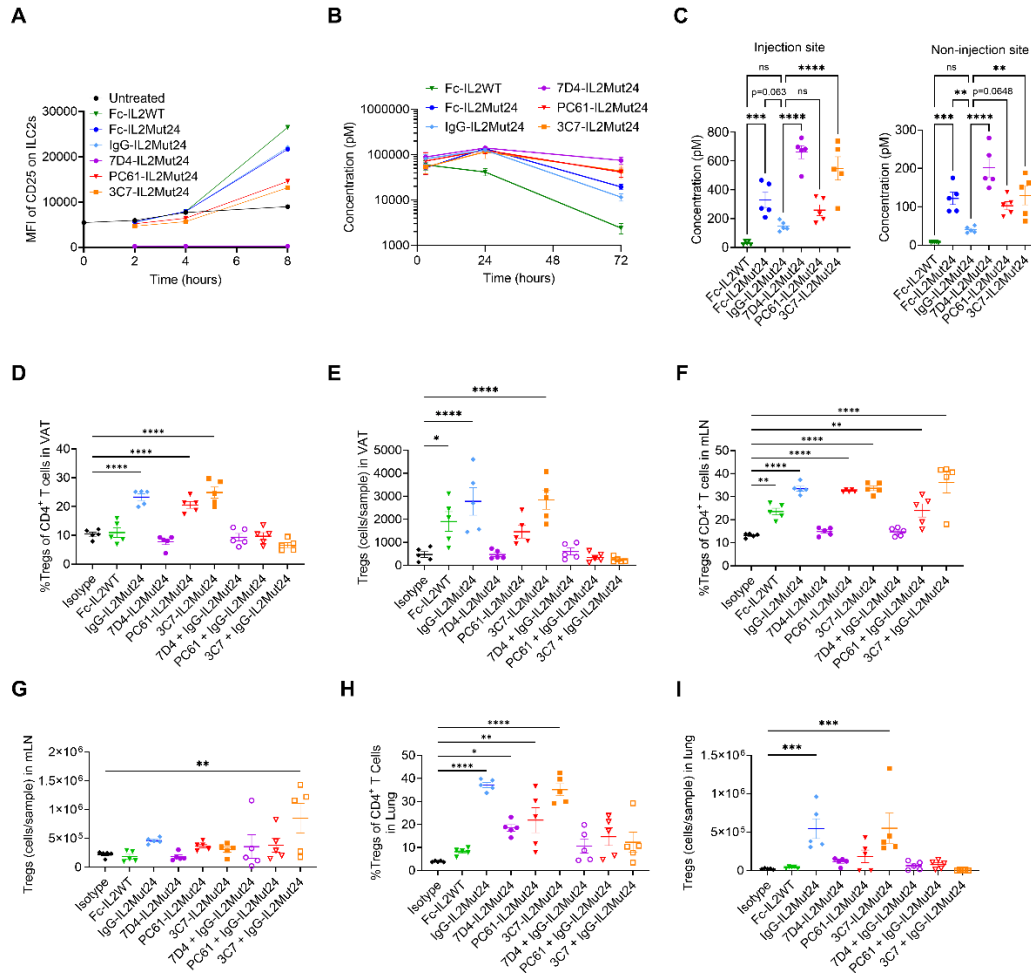
Supplemental Figure 9. Characterization of IL-12+IL-15-expanded ILC1s and NK cells and ISRs after adoptive transfer. (A) Schematic representation of experimental design for IL-33-mediated ILC2 expansion and IL-12 + IL15-mediated ILC1 expansion in *RAG2*^{-/-} mice. **(B)** Gating strategy for cell sorting of ILC1s and NK cells for adoptive transfer to recipient mice. ILC1s were sorted as live CD45⁺CD11b⁺NK1.1⁺CD49a⁺CD49b⁻. NK cells were sorted as live CD45⁺CD11b⁺NK1.1⁺CD49a⁺CD49b⁺ cells. **(C)** Schematic representation of experimental design for adoptive transfer of 3x10⁵ ILC1, ILC2 and NK cell. **(D)** Representative dorsal images (n=5 mice per group). Mice were shaved for better visualization of the injection site. **(E)** ISR scores at endpoint. n=3 mice in PBS group and 5 mice in all other groups. Data shown as mean ± SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc test; only selected comparisons are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplemental Figure 10



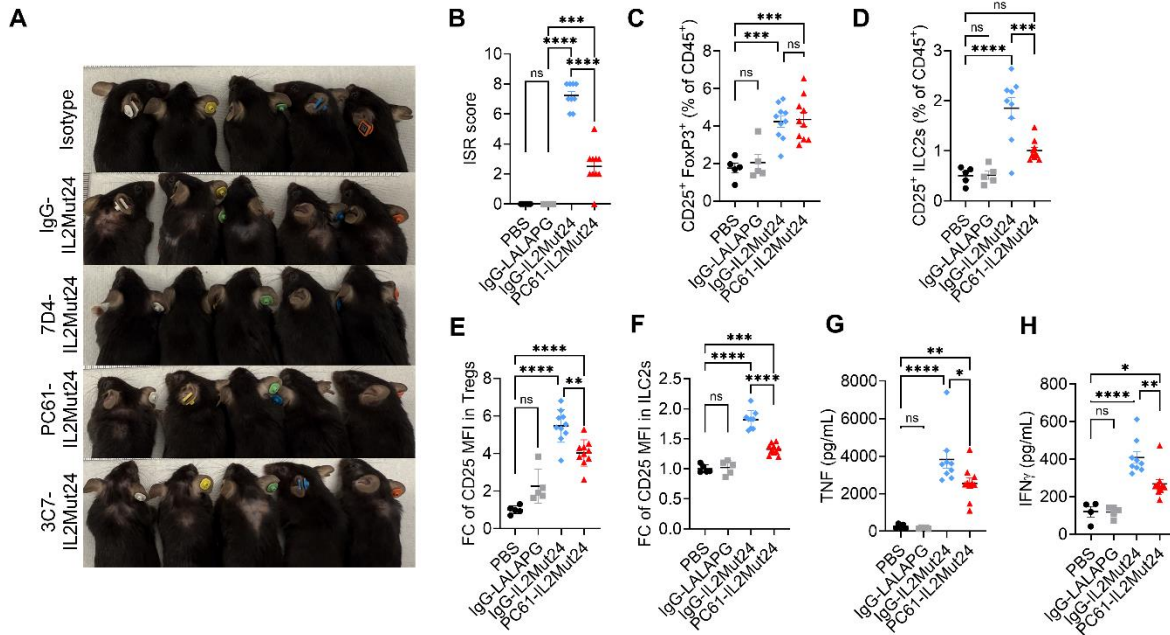
Supplemental Figure 10. The partial blocker PC61 epitope partially overlaps with the IL-2 and CD25 interface. (A-B) Sequence alignment of human (hu) and mouse (mu) IL-2 **(A)** and CD25 **(B)**. (Geneious version 10.2 created by Biomatters). Numbering begins at the first amino acid following signal sequence cleavage. Key interface residues, identified from the crystal structure of huIL-2 and huCD25 (PDB: 1z92), are boxed and labeled (53). Corresponding residues on muIL-2 and muCD25 were inferred using an internally modified version of AlphaFold2 Multimer-modeled structure and sequence alignment. PC61 and 7D4 epitopes, as described in a patent (54), are indicated with cyan and salmon bars, respectively. **(C, left)** The complex structure of muIL-2 and muCD25 modeled by an internally modified AlphaFold2 Multimer. MuIL-2 is shown in cartoon representation (yellow), and muCD25 is shown in surface representation (gray). Loop regions of muCD25 (70-95 and 176-215) with low predicted Local Distance Difference Test (pLDDT) scores are omitted for clarity. PC61 and 7D4 epitopes on muCD25 are shown in cyan and salmon, respectively. **(C, right)** Close-up view of the boxed region on the left. Key interface residues are shown in stick representation. Residues on muIL2 and muCD25 are colored yellow and cyan, respectively. The PC61 epitope partially overlaps with the muIL-2 and muCD25 interface. One hydrophobic patch-comprising R35 and R36 of muCD25 (corresponding to R35 and R36 in huCD25) interacts with Y59 on muIL2 (Y45 in huIL2) and lies within the PC61 epitope (53). A second hydrophobic patch-forming a recessed pocket of I25, V41, and Y42 on muCD25 (M25, L42, and Y43 in huCD25) interacts with F56 and L86 on muIL2 (F42 and L72 in huIL2) and lies adjacent to the PC61 epitope (53). The N-terminal region of muCD25, which also interacts with muIL2, lies outside the PC61 epitope (red dotted box).

Supplemental Figure 11



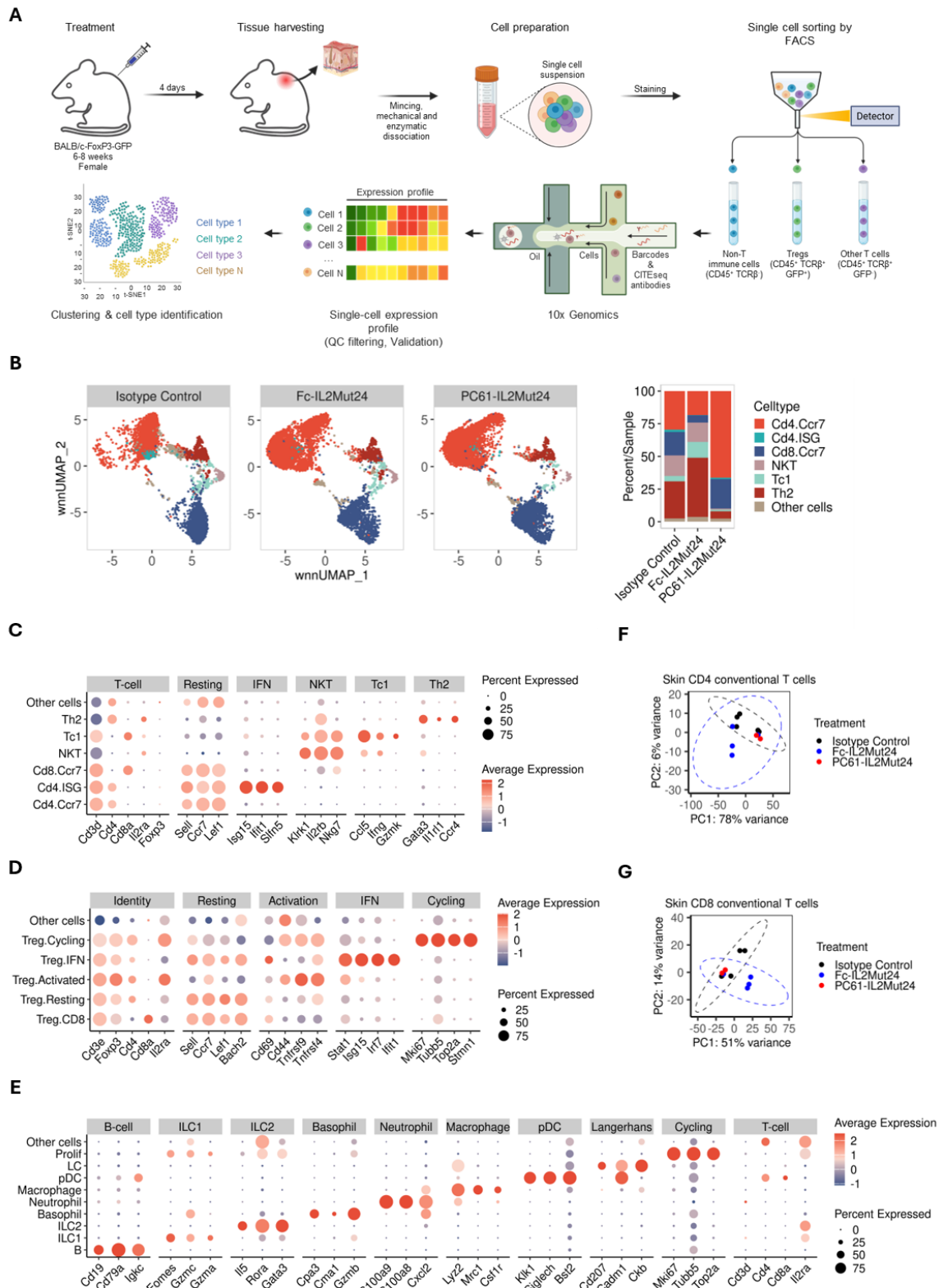
Supplemental Figure 11. Skin biodistribution and tissue response of IL2Mut24 fusion proteins. (A) ILC2s were isolated from the lungs of IL-33-treated *Rag2*^{-/-} mice. Mean fluorescence intensity (MFI) of CD25 on IL-33-expanded ILC2s treated *in vitro* with 1 nM each of Fc-IL2WT and the IL2Mut24 fusion proteins over time (hours) as analyzed by flow cytometry using anti-CD25 (clone:7D4). (B) Fc-IL2WT and IL2Mut24 fusion proteins plasma concentrations (pM) at 3, 24 and 72 hours post injection as detected by MSD (n=5 mice/condition). (C) Concentrations (pM) of Fc-IL2WT and the IL2Mut24 fusion proteins detected in skin samples from injection-site and non-injection site at 72 hours post injection by MSD (n=5 mice per condition, one representative experiment of two). (D-I). 6-8-week-old female C57BL/6J mice were injected subcutaneously with 69 pmol of either isotype, 7D4-IL2Mut24, PC61-IL2Mut24, 3C7-IL2Mut24, IgG-IL2Mut24, Fc-IL2WT, or matched combinations of unconjugated anti-CD25 clones (7D4, PC61, or 3C7) co-injected with IgG-IL2Mut24. Mice were euthanized on day 4 post-injection. Frequency (D, F, H) and absolute counts per sample (E, G, I) of CD25⁺ FOXP3⁺ cells within the CD4⁺ T cell compartment (referred to as Tregs) in visceral adipose tissues (VAT) (D, E), mesenteric lymph nodes (mLN) (F, G), and lungs (H, I) as analyzed by flow cytometry. n=5 mice per group from one representative experiment of two.

Supplemental Figure 12



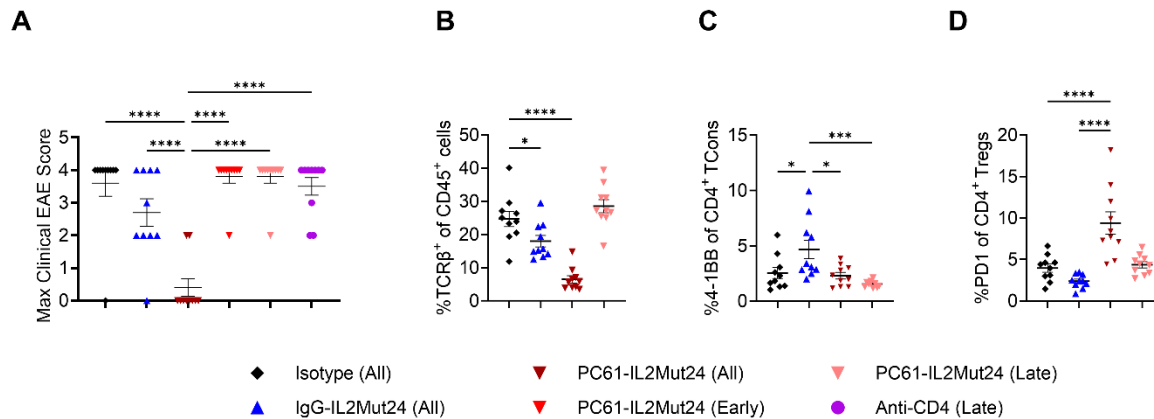
Supplemental Figure 12. PC61-IL2Mut24 reduces ISR by constrains the cutaneous ILC2 response. (A) Representative dorsal images (n=5 mice per group) from experiment shown in Figure 6I-J. (B-H) Independent experimental repetition of setup shown in Figure 6I, with n=5 mice in PBS and IgG-LALAPG groups and n=10 mice in IgG-IL2Mut24 and PC61-IL2Mut24 groups. (B) ISR scores at endpoint. (C-D) Frequency of CD25⁺ FoxP3⁺ cells (C) and CD25⁺ ILC2s (D) among CD45⁺ cells in skin, as analyzed by flow cytometry. (E-F) CD25 MFI fold changes in Tregs (E) and ILC2s (F) compared to PBS. (G-H) TNF and IFN γ shown as concentration (pg/mL) from endpoint skin homogenates as detected by MSD. Data shown as mean $\pm$ SEM. Two-tailed ordinary one-way ANOVA followed by Sidak's multiple comparisons post hoc test, only selected comparisons are shown. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplemental Figure 13



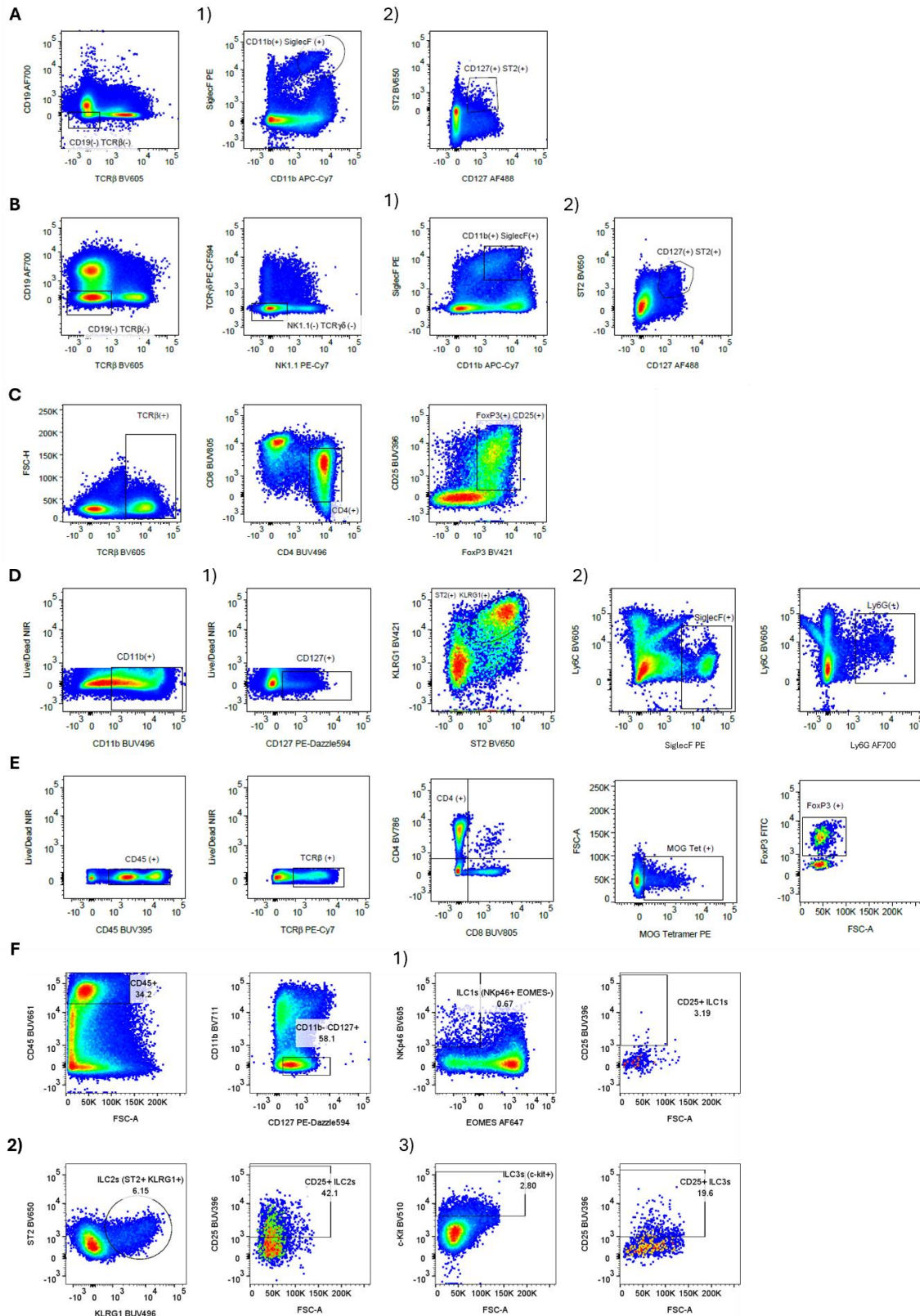
Supplemental Figure 13. Fc-IL2Mut24 and PC61-IL2Mut24 alter the skin immune transcriptional landscape. BALB/c-FoxP3-GFP mice (n=5 mice/group) received a single dorsal subcutaneous dose of isotype control, Fc-IL2Mut24 or PC61-IL2Mut24 (69 pmol). Skin samples were collected and processed on day 4 after dosing. **(A)** Schematic representation of experimental design and downstream skin sample processing methods. **(B)** Uniform Manifold Approximation and Projection (UMAP) of CD45⁺TCR β ⁺GFP⁻ sorted samples (left). Average proportion of different populations from the CD45⁺TCR β ⁺GFP⁻ sorted skin samples in each condition (right). **(C-E)** Cell type markers in the CD45⁺TCR β ⁺GFP⁻ **(C)**, CD45⁺TCR β ⁺GFP⁺ **(D)** and CD45⁺TCR β ⁻GFP⁻ **(E)** sorted populations. Bubble size shows the frequency (%) of cells expressing each gene, while color gradient shows gene expression levels. **(F-G)** Principal component analysis (PCA, % confidence interval = 95) of genes expressed by conventional CD4⁺ T cells **(F)** and CD8⁺ T cells **(G)** in skin.

Supplemental Figure 14



Supplemental Figure 14. PC61-IL2Mut24 outperforms IgG-IL2Mut24 in suppression of EAE onset. C57BL/6J mice ($n = 10$) were treated i.p. with 100 μ L PBS containing isotype, PC61-IL2Mut24, IgG-IL2Mut24, or anti-CD4 on schedules: “All” (Days -2, 2, 6, 9, 13, 16), “Early” (Days 2, 6), or “Late” (Days 15, 19). EAE was induced on Day 0 using 200 μ g MOG₃₅₋₅₅ emulsified in CFA, and 120 ng pertussis toxin was administered i.p. on Days 0 and 2. **(A)** Maximum EAE scores. **(B-D)** At study endpoint, CNS-infiltrating cells were isolated and stained with MOG₃₅₋₅₅-IAb Tetramer. Frequency of 4-1BB $^+$ cells among CD4 $^+$ conventional T cells **(C)** and PD-1 $^+$ cells among CD4 $^+$ Tregs **(D)** in the CNS as analyzed by flow cytometry. Data are shown as mean $\pm$ SEM from $n=10$ mice per group. Data are representative of two independent experiments. Two-tailed ordinary one-way ANOVA followed by Sidak’s post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Supplemental Figure 15:



Supplemental Figure 15. Mouse flow cytometry gating strategies. Representative gating strategies used for mouse flow-cytometric analyses in this study. **(A)** For Figure 2H, eosinophils were identified as CD11b⁺SiglecF⁺ cells among live CD45⁺CD19⁻TCRβ⁻ cells (**A1**). For Figure 2G, ILC2s were identified as CD127⁺ST2⁺ cells among live CD45⁺CD19⁻TCRβ⁻ cells (**A2**). **(B)** For Figure 3J, eosinophils were identified as CD11b⁺SiglecF⁺ cells among live CD45⁺CD19⁻TCRβ⁻NK1.1⁻TCRγδ⁻ cells (**B1**); for Figure 3I, ILC2s were identified as CD127⁺ST2⁺ cells from the same parent population (**B2**). **(C)** Tregs were analyzed within live CD45⁺TCRβ⁺CD4⁺ cells using FoxP3 and CD25. Figure 2F used FoxP3⁺ Tregs; Figure 3C, Figure 6G–H, Supplemental Figure 11D–I, and Supplemental Figure 12C used CD25⁺FoxP3⁺ Tregs. CD25 MFI in Tregs was quantified in Supplemental Figure 12E. **(D)** Downstream gating used for Figure 4H–J is shown: ILC2s were ST2⁺KLRG1⁺ among live CD45⁺CD11b⁻CD127⁺ cells (**D1**); eosinophils were SiglecF⁺ among live CD45⁺CD11b⁺Ly6C⁻ cells and neutrophils were Ly6G⁺ among live CD45⁺CD11b⁺Ly6C⁻SiglecF⁻ cells (**D2**). **(E)** The upstream CNS T-cell gating used for Figure 8E–J and Supplemental Figure 14B–D is shown. CD4⁺ T cells were identified among live CD45⁺TCRβ⁺ cells, MOG-tetramer⁺ cells within CD4⁺ T cells, and FoxP3⁺ Tregs within MOG-tetramer⁺ CD4⁺ T cells. **(F)** ILC1s, ILC2s, and ILC3s were identified among live CD45⁺CD11b⁻CD127⁺ cells as NKp46⁺EOMES⁻ (**F1**), ST2⁺KLRG1⁺ (**F2**), and c-Kit⁺ (**F3**), respectively. These gates were used for Figure 3B, Figure 4C–D, Supplemental Figure 3A–G, and Supplemental Figure 12D; CD25 MFI in ILC2s was quantified in Supplemental Figure 12F.

Supplementary Table 1

Antibody	Isotype	Reactivity	Conjugate	Clone	Supplier	Catalog #
CD45	Rat IgG2b,κ	Mouse	BV786	30-F11	BioLegend	103149
TCRβ	Armenian hamster IgG2,λ1	Mouse	BV605	H57-597	BD Biosciences	562840
CD19	Lewis IgG2a, κ	Mouse	Alexa Fluor 700	1D3	BD Biosciences	557958
CD11b	DA/HA IgG2b, κ	Mouse/human	APC-Cy7	M1/70	BD Biosciences	557657
CD127	Rat IgG2b,κ	Mouse	Alexa Fluor 488	SB/199	BD Biosciences	561533
NK1.1	C3H x BALB/c IgG2a, κ	Mouse	PE-Cy7	PK136	BD Biosciences	552878
CD4	Lewis IgG2b, κ	Mouse	BUV496	GK1.5	BD Biosciences	612952
CD8	LOU/M IgG2a, κ	Mouse	BUV805	53-6.7	BD Biosciences	612898
KLRG1	Syrian Hamster IgG2, κ	Mouse	BV421	2F1	BD Biosciences	562897
ST2	Rat IgG2a, κ	Mouse	BV650	U29-93	BD Biosciences	745403
SiglecF	LOU/M IgG2a, κ	Mouse	PE	E50-2440	BD Biosciences	562068
γδ TCR	Armenian Hamster IgG2, κ	Mouse	PE-CF594	GL3	BD Biosciences	563532
FoxP3	Rat IgG2b, κ	Mouse	FITC	MF-14	BD Biosciences	126406
CD25	Rat IgG1, λ	Mouse	BV711	PC61	BD Biosciences	740714
CD45	LOU/M IgG2b, κ	Mouse	BUV395	30-F11	BD Biosciences	564279
TCRβ	Armenian Hamster IgG2, λ1	Mouse	BV421	H57-597	BD Biosciences	562839
CD4	Rat IgG2a, κ	Mouse	BV786	RM4-5	BioLegend	100551
CD8α	LOU/M IgG2a, κ	Mouse	PE-Cy7	53-6.7	BD Biosciences	561097
FoxP3	Rat IgG2b, κ	Mouse	BV421	MF-14	BD Biosciences	126419
CD25	OFA IgG1, λ	Mouse	BUV395	PC61	BD Biosciences	564022
CD11b	DA/HA IgG2b, κ	Mouse	BUV495	M1/70	BD Biosciences	749864
CD45.2	IgG2a, κ	Mouse	BUV805	104	BD Biosciences	569200
Ly6C	Rat IgM, κ	Mouse	BV605	AL-21	BD Biosciences	563011
TCR	Armenian Hamster IgG	Mouse	AF488	H57-597	BioLegend	109215
Ly6G	Lewis IgG2a, κ	Mouse	AF700	1A8	BD Biosciences	561236
CD127	Rat IgG2a, κ	Mouse	PE-Dazzle594	A7R34	BioLegend	135032
CD45	Rat IgG2b, κ	Mouse	BUV661	30-F11	BD Biosciences	612975
CD4	Rat IgG2a, κ	Mouse	BUV496	RM4-5	BD Biosciences	741050
FoxP3	Rat IgG2a, κ	Mouse/rat	Alexa Fluor 488	FJK-16s	Invitrogen	535773
NKp46	Rat IgG2a, κ	Mouse	BV605	29A1.4	BD Biosciences	564069

EOMES	Rat IgG2b, λ	Mouse	AF647	W17001A	BioLegend	157703
CD11b	Rat IgG2b, κ	Mouse/human	BV711	M1/70	BioLegend	101242
c-Kit	Rat IgG2b, κ	Mouse	BV510	2B8	BioLegend	105839
KLRG1	Syrian hamster IgG	Mouse	BUV496	2F1	Invitrogen	364-5893-82
CD25	Rat IgM, κ	Mouse	FITC	7D4	BD Biosciences	553071
4-1BB	Syrian Hamster IgG	Mouse	BV421	17B5	BD Biosciences	570317
PD-1	Rat IgG2a, κ	Mouse	BV605	29F.1A12	Biolegend	135220
IFNγ	Rat IgG1, κ	Mouse	APC	XMG1.2	Biolegend	505810
CD49a	Armenian Hamster IgG	Mouse	APC	HMa1	BioLegend	142606
CD49b	Armenian Hamster IgG1, κ	Mouse	BV805	HMa2	BD Biosciences	741962
T-cell I-A ^b MOG ₃₅₋₅₅ tetramer	NA	Mouse	PE	NA	MBL International	TS-M704-1
CD3	Mouse BALB/c IgG1, λ	NHP	PerCP	SP34-2	BD Biosciences	552851
CD4	Mouse BALB/c IgG1, κ	NHP	BV605	L200	BD Biosciences	562843
FoxP3	Mouse IgG1, κ	NHP	PE	206D	BioLegend	320108
CD25	Mouse IgG2b, κ	NHP	APC	4E3	Miltenyi	130-092-858

Supplementary Table 2

Cell type	Markers
B cell	<i>Cd19, Cd79a, Igkc, Mzb1, Ms4a1</i>
Basophil	<i>Mcpt8, Fcer1A, Cd200r3, Cpa3, Cma1</i>
ILC	<i>Gzmb, Prf1, Klrb1, Fgfbp2, Klrf1, Gzmm, Gzmb, Il2Rb, Klrd1, Il7r, Ifng, Tnf</i>
ILC1	<i>Eomes, Tnfrsf9, Klrb1C, Ncr1, Itga1, Il1r1, Gzmc</i>
ILC2	<i>Kit, Thy1, St2, Il5, Rora, Icos, Gata3</i>
ILC3	<i>Kit, Thy1, Il1r1, Il23r</i>
LC	<i>Ckb, Timd4, Cd207</i>
Macrophage	<i>Fcgr2a, Csf1r, Sat1, Lyz2, Mrc1</i>
Neutrophil	<i>S100a8, S100a9, Fpr2, Cxcr2, Prok2, Ffar2, Fcgr3b, Cxcl2</i>
pDC	<i>Clec4c, Jchain, Gzmb, Siglech, Klk1, Cadm1</i>
Prolif	<i>Mki67</i>
CD4 T cell	<i>Cd4, Cd3d</i>
Cd4.Ccr7	<i>Ccr7, Lef1, Sell</i>
Cd4.ISG	<i>Isg15, Ifit1</i>
Th2	<i>Gata3, Il4, Il13, Il1r1, Ccr4</i>
CD8 T cell	<i>Cd8a, Cd3d</i>
Cd8.Ccr7	<i>Ccr7, Lef1, Sell</i>
Tc1	<i>Ccl5, Ifng, Gzmk, Gzmb</i>
NKT	<i>Cd3d, Nkg7, Il2rb, Klrk1</i>
Treg	<i>Cd3d, Foxp3, Il2ra</i>
Treg.Ctla4	<i>Ctla4, Tnfrsf9, Stat3, Fth1</i>
Treg.II31ra	<i>Il31ra</i>
Treg.Itgb7	<i>Itgb7</i>
Treg.Lef1	<i>Lef1, Tcf7</i>
Treg.Ms4a4	<i>Ms4a4b, Evl</i>
Treg.Prolif	<i>Mki67</i>
Treg.Tnfrsf4	<i>Tnfrsf4, Eomes, Egr3</i>
Treg.Tox	<i>Tox</i>
Treg.Cd8	<i>Cd8a, Cd8b</i>

Supplementary Table 3

	gene	cluster_id	logFC	p_adj.loc	logCP M	p_val	p_adj.glb	comparison
1	<i>Amd1</i>	ILC2	-1.70	3.23E-05	6.71	7.49E-08	1.04E-05	Fc-IL2Mut24 vs Isotype Control
2	<i>Zc3h12c</i>	ILC2	-1.54	2.59E-04	7.06	1.32E-06	1.08E-04	Fc-IL2Mut24 vs Isotype Control
3	<i>Nlrp3</i>	ILC2	2.64	6.04E-04	5.92	5.49E-06	3.35E-04	Fc-IL2Mut24 vs Isotype Control
4	<i>Nme1</i>	ILC2	-1.77	6.78E-04	5.82	6.35E-06	3.71E-04	Fc-IL2Mut24 vs Isotype Control
5	<i>Nrp1</i>	ILC2	2.49	1.90E-03	5.71	2.80E-05	1.20E-03	Fc-IL2Mut24 vs Isotype Control
6	<i>Ctsa</i>	ILC2	1.65	2.06E-03	5.95	3.31E-05	1.33E-03	Fc-IL2Mut24 vs Isotype Control
7	<i>Arl5a</i>	ILC2	1.61	6.16E-03	5.97	1.53E-04	4.32E-03	Fc-IL2Mut24 vs Isotype Control
8	<i>Samd4</i>	ILC2	-2.05	8.91E-03	5.06	2.74E-04	6.59E-03	Fc-IL2Mut24 vs Isotype Control
9	<i>Hspa1a</i>	ILC2	2.83	1.08E-02	7.53	3.81E-04	8.38E-03	Fc-IL2Mut24 vs Isotype Control
10	<i>Pde10a</i>	ILC2	-2.49	1.25E-02	5.89	4.73E-04	9.90E-03	Fc-IL2Mut24 vs Isotype Control
11	<i>Prkag2</i>	ILC2	-1.59	1.39E-02	5.65	5.89E-04	1.16E-02	Fc-IL2Mut24 vs Isotype Control
12	<i>Mthfs</i>	ILC2	-1.64	1.50E-02	5.16	6.86E-04	1.30E-02	Fc-IL2Mut24 vs Isotype Control
13	<i>Grik2</i>	ILC2	2.44	1.77E-02	6.16	8.70E-04	1.52E-02	Fc-IL2Mut24 vs Isotype Control
14	<i>Ccl5</i>	ILC2	3.34	2.06E-02	6.16	1.14E-03	1.82E-02	Fc-IL2Mut24 vs Isotype Control
15	<i>Pomgnt1</i>	ILC2	-2.08	3.12E-02	6.78	2.08E-03	2.77E-02	Fc-IL2Mut24 vs Isotype Control
16	<i>Ifitm3</i>	ILC2	1.73	3.42E-02	6.17	2.38E-03	3.03E-02	Fc-IL2Mut24 vs Isotype Control
17	<i>Ntn4</i>	ILC2	-2.38	3.51E-02	4.78	2.47E-03	3.11E-02	Fc-IL2Mut24 vs Isotype Control
18	<i>Irf8</i>	ILC2	1.84	3.55E-02	5.74	2.51E-03	3.14E-02	Fc-IL2Mut24 vs Isotype Control
19	<i>Arid2</i>	ILC2	-1.72	3.76E-02	5.70	2.71E-03	3.33E-02	Fc-IL2Mut24 vs Isotype Control
20	<i>Ccr7</i>	ILC2	2.02	4.53E-02	6.94	3.55E-03	4.00E-02	Fc-IL2Mut24 vs Isotype Control
21	<i>mt-Atp8</i>	ILC2	2.60	2.15E-11	9.58	5.74E-15	2.37E-12	PC61-IL2Mut24 vs Isotype Control
22	<i>Hexb</i>	ILC2	-1.51	2.37E-06	12.80	1.27E-09	1.11E-07	PC61-IL2Mut24 vs Isotype Control
23	<i>S100a4</i>	ILC2	-1.35	3.89E-06	9.79	3.12E-09	2.33E-07	PC61-IL2Mut24 vs Isotype Control
24	<i>Btbd9</i>	ILC2	-1.43	9.99E-05	8.73	1.34E-07	5.95E-06	PC61-IL2Mut24 vs Isotype Control

25	<i>Nkain2</i>	ILC2	-1.33	2.16E-04	8.40	3.47E-07	1.35E-05	PC61-IL2Mut24 vs Isotype Control
26	<i>Peak1</i>	ILC2	-1.43	5.10E-04	7.68	1.36E-06	4.46E-05	PC61-IL2Mut24 vs Isotype Control
27	<i>Smpd13a</i>	ILC2	1.49	1.75E-03	6.99	6.54E-06	1.66E-04	PC61-IL2Mut24 vs Isotype Control
28	<i>Lars2</i>	ILC2	-1.30	2.42E-03	12.70	1.36E-05	3.05E-04	PC61-IL2Mut24 vs Isotype Control
29	<i>Rptor</i>	ILC2	-1.39	2.83E-03	7.56	1.97E-05	4.16E-04	PC61-IL2Mut24 vs Isotype Control
30	<i>Hspa1a</i>	ILC2	3.55	5.12E-03	7.53	4.25E-05	7.93E-04	PC61-IL2Mut24 vs Isotype Control
31	<i>Vim</i>	ILC2	1.09	5.42E-03	11.10	4.68E-05	8.58E-04	PC61-IL2Mut24 vs Isotype Control
32	<i>Nme1</i>	ILC2	-1.93	1.05E-02	5.82	9.93E-05	1.59E-03	PC61-IL2Mut24 vs Isotype Control
33	<i>Vamp3</i>	ILC2	1.72	1.35E-02	5.93	1.52E-04	2.27E-03	PC61-IL2Mut24 vs Isotype Control
34	<i>Ypel3</i>	ILC2	1.41	1.35E-02	6.41	1.40E-04	2.12E-03	PC61-IL2Mut24 vs Isotype Control
35	<i>Zcchc14</i>	ILC2	-2.65	1.35E-02	5.59	1.58E-04	2.33E-03	PC61-IL2Mut24 vs Isotype Control
36	<i>Rab18</i>	ILC2	1.62	1.40E-02	5.96	1.83E-04	2.61E-03	PC61-IL2Mut24 vs Isotype Control
37	<i>Tuba1c</i>	ILC2	1.53	1.46E-02	6.63	2.02E-04	2.82E-03	PC61-IL2Mut24 vs Isotype Control
38	<i>Pecam1</i>	ILC2	-1.79	1.46E-02	6.80	1.95E-04	2.74E-03	PC61-IL2Mut24 vs Isotype Control
39	<i>Odc1</i>	ILC2	1.42	3.54E-02	8.61	7.66E-04	8.46E-03	PC61-IL2Mut24 vs Isotype Control
40	<i>Rgs10</i>	ILC2	2.11	3.59E-02	5.75	8.21E-04	8.90E-03	PC61-IL2Mut24 vs Isotype Control
41	<i>mt-Atp8</i>	ILC2	1.06	6.79E-05	9.58	1.82E-08	1.32E-05	PC61-IL2Mut24 vs Fc-IL2Mut24
42	<i>Nlrp3</i>	ILC2	-3.96	1.58E-03	5.92	8.44E-07	2.80E-04	PC61-IL2Mut24 vs Fc-IL2Mut24
43	<i>Nrp1</i>	ILC2	-3.69	3.73E-03	5.71	3.99E-06	9.18E-04	PC61-IL2Mut24 vs Fc-IL2Mut24
44	<i>Rpl13a</i>	ILC2	0.95	6.84E-03	8.29	1.19E-05	2.21E-03	PC61-IL2Mut24 vs Fc-IL2Mut24
45	<i>Arg1</i>	ILC2	-2.28	3.92E-02	6.82	1.05E-04	1.16E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
46	<i>Amd1</i>	ILC2	1.17	4.02E-02	6.71	1.18E-04	1.26E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
47	<i>Sag</i>	ILC2	1.60	4.25E-02	6.08	2.83E-04	2.35E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
48	<i>Palld</i>	ILC2	1.52	4.25E-02	9.92	1.86E-04	1.74E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
49	<i>Hgsnat</i>	ILC2	1.44	4.25E-02	5.92	3.58E-04	2.74E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
50	<i>Fchsd2</i>	ILC2	1.36	4.25E-02	7.11	2.06E-04	1.87E-02	PC61-IL2Mut24 vs Fc-IL2Mut24

51	<i>Bmp2</i>	ILC2	1.12	4.25E-02	7.63	2.65E-04	2.24E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
52	<i>Med21</i>	ILC2	1.03	4.25E-02	7.21	3.77E-04	2.81E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
53	<i>Tubb4b</i>	ILC2	0.91	4.25E-02	7.60	2.15E-04	1.93E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
54	<i>Glr3</i>	ILC2	-0.91	4.25E-02	8.02	3.42E-04	2.65E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
55	<i>Arhgap15</i>	ILC2	-0.92	4.25E-02	8.48	3.69E-04	2.77E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
56	<i>Tmem176a</i>	ILC2	-1.02	4.25E-02	7.08	3.37E-04	2.64E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
57	<i>Myo1e</i>	ILC2	-1.08	4.25E-02	8.35	2.23E-04	1.99E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
58	<i>Jdp2</i>	ILC2	-1.44	4.25E-02	7.37	3.90E-04	2.89E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
59	<i>Ifi272a</i>	ILC2	-3.09	4.25E-02	7.20	3.62E-04	2.74E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
60	<i>Zcchc14</i>	ILC2	-2.28	4.68E-02	5.59	4.50E-04	3.22E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
61	<i>Nkain2</i>	Treg	-2.67	1.29E-11	7.07	1.64E-15	1.29E-11	Fc-IL2Mut24 vs Isotype Control
62	<i>Asx13</i>	Treg	-2.48	4.64E-09	5.48	1.18E-12	4.64E-09	Fc-IL2Mut24 vs Isotype Control
63	<i>Rnaset2b</i>	Treg	-2.52	9.79E-08	6.69	6.22E-11	9.79E-08	Fc-IL2Mut24 vs Isotype Control
64	<i>Ifitm3</i>	Treg	3.22	1.26E-06	6.57	1.93E-09	1.26E-06	Fc-IL2Mut24 vs Isotype Control
65	<i>Dgkb</i>	Treg	-2.33	3.09E-06	4.08	5.50E-09	3.09E-06	Fc-IL2Mut24 vs Isotype Control
66	<i>Bbof1</i>	Treg	-3.20	1.25E-04	4.02	7.32E-07	1.25E-04	Fc-IL2Mut24 vs Isotype Control
67	<i>Gab2</i>	Treg	2.41	3.31E-04	2.70	2.65E-06	3.31E-04	Fc-IL2Mut24 vs Isotype Control
68	<i>Ifitm1</i>	Treg	11.10	7.09E-04	3.69	7.02E-06	7.09E-04	Fc-IL2Mut24 vs Isotype Control
69	<i>Pkd2l2</i>	Treg	-2.24	7.93E-04	5.59	8.27E-06	7.93E-04	Fc-IL2Mut24 vs Isotype Control
70	<i>Lgals7</i>	Treg	1.68	9.92E-04	4.95	1.10E-05	9.92E-04	Fc-IL2Mut24 vs Isotype Control
71	<i>Ifitm2</i>	Treg	2.46	9.94E-04	6.94	1.11E-05	9.94E-04	Fc-IL2Mut24 vs Isotype Control
72	<i>Spata7</i>	Treg	-2.92	1.60E-03	5.02	2.10E-05	1.60E-03	Fc-IL2Mut24 vs Isotype Control
73	<i>Slc15a2</i>	Treg	-2.81	2.54E-03	5.57	4.57E-05	2.54E-03	Fc-IL2Mut24 vs Isotype Control
74	<i>Ntm</i>	Treg	2.37	2.88E-03	2.98	5.37E-05	2.88E-03	Fc-IL2Mut24 vs Isotype Control
75	<i>Dhrs3</i>	Treg	1.48	9.13E-03	3.67	3.04E-04	9.13E-03	Fc-IL2Mut24 vs Isotype Control
76	<i>Kcnp2</i>	Treg	-2.93	1.43E-02	3.47	6.16E-04	1.43E-02	Fc-IL2Mut24 vs Isotype Control

77	<i>Nckap5</i>	Treg	2.20	3.26E-02	4.11	2.39E-03	3.26E-02	Fc-IL2Mut24 vs Isotype Control
78	<i>Aph1c</i>	Treg	2.03	3.29E-02	3.55	2.45E-03	3.29E-02	Fc-IL2Mut24 vs Isotype Control
79	<i>Il17a</i>	Treg	-4.17	3.33E-02	3.28	2.53E-03	3.33E-02	Fc-IL2Mut24 vs Isotype Control
80	<i>Sh2d4b</i>	Treg	1.66	4.77E-02	2.95	4.54E-03	4.77E-02	Fc-IL2Mut24 vs Isotype Control
81	<i>Nme1</i>	Treg	-2.45	3.30E-06	6.07	8.39E-10	3.30E-06	PC61-IL2Mut24 vs Isotype Control
82	<i>H2-Q1</i>	Treg	1.35	1.04E-05	7.85	2.24E-08	1.04E-05	PC61-IL2Mut24 vs Isotype Control
83	<i>Tpx2</i>	Treg	-2.45	2.37E-04	3.40	1.14E-06	2.37E-04	PC61-IL2Mut24 vs Isotype Control
84	<i>Nusap1</i>	Treg	-2.62	2.57E-04	3.94	1.30E-06	2.57E-04	PC61-IL2Mut24 vs Isotype Control
85	<i>Birc5</i>	Treg	-2.62	7.30E-04	4.94	6.67E-06	7.30E-04	PC61-IL2Mut24 vs Isotype Control
86	<i>Cenpf</i>	Treg	-2.51	1.15E-03	3.65	1.27E-05	1.15E-03	PC61-IL2Mut24 vs Isotype Control
87	<i>Bcr</i>	Treg	1.20	1.79E-03	4.16	2.39E-05	1.79E-03	PC61-IL2Mut24 vs Isotype Control
88	<i>Clspn</i>	Treg	-2.33	1.94E-03	3.47	2.78E-05	1.94E-03	PC61-IL2Mut24 vs Isotype Control
89	<i>Phkg1</i>	Treg	-2.83	2.31E-03	5.33	3.72E-05	2.31E-03	PC61-IL2Mut24 vs Isotype Control
90	<i>Chpt1</i>	Treg	-2.48	2.49E-03	3.58	4.47E-05	2.49E-03	PC61-IL2Mut24 vs Isotype Control
91	<i>Cables2</i>	Treg	1.16	2.54E-03	5.14	4.61E-05	2.54E-03	PC61-IL2Mut24 vs Isotype Control
92	<i>Kn1l</i>	Treg	-2.39	2.79E-03	3.39	5.21E-05	2.79E-03	PC61-IL2Mut24 vs Isotype Control
93	<i>Lta</i>	Treg	-2.38	3.28E-03	4.22	6.75E-05	3.28E-03	PC61-IL2Mut24 vs Isotype Control
94	<i>Prkca</i>	Treg	1.22	3.88E-03	9.11	9.13E-05	3.88E-03	PC61-IL2Mut24 vs Isotype Control
95	<i>Lysmd4</i>	Treg	1.32	4.35E-03	3.15	1.07E-04	4.35E-03	PC61-IL2Mut24 vs Isotype Control
96	<i>Ntm</i>	Treg	2.09	6.78E-03	2.98	2.15E-04	6.78E-03	PC61-IL2Mut24 vs Isotype Control
97	<i>Cenpt</i>	Treg	1.18	9.15E-03	3.73	3.72E-04	9.15E-03	PC61-IL2Mut24 vs Isotype Control
98	<i>Epas1</i>	Treg	1.60	2.84E-02	4.30	2.22E-03	2.84E-02	PC61-IL2Mut24 vs Isotype Control
99	<i>Tubb2a</i>	Treg	2.06	2.85E-02	7.65	2.24E-03	2.85E-02	PC61-IL2Mut24 vs Isotype Control
100	<i>Epb41</i>	Treg	1.15	3.46E-02	6.95	3.12E-03	3.46E-02	PC61-IL2Mut24 vs Isotype Control
101	<i>Nkain2</i>	Treg	1.35	4.33E-07	7.07	5.51E-11	4.33E-07	PC61-IL2Mut24 vs Fc-IL2Mut24
102	<i>Hexb</i>	Treg	1.52	1.85E-06	10.80	4.70E-10	1.85E-06	PC61-IL2Mut24 vs Fc-IL2Mut24

103	<i>Ifitm3</i>	Treg	-3.04	6.07E-06	6.57	2.31E-09	6.07E-06	PC61-IL2Mut24 vs Fc-IL2Mut24
104	<i>Asx13</i>	Treg	1.25	6.80E-05	5.48	3.46E-08	6.80E-05	PC61-IL2Mut24 vs Fc-IL2Mut24
105	<i>Kif15</i>	Treg	-2.02	6.98E-04	3.24	9.76E-07	6.98E-04	PC61-IL2Mut24 vs Fc-IL2Mut24
106	<i>Gab2</i>	Treg	-2.04	2.59E-03	2.70	4.56E-06	2.59E-03	PC61-IL2Mut24 vs Fc-IL2Mut24
107	<i>Dgkb</i>	Treg	1.38	3.11E-03	4.08	6.21E-06	3.11E-03	PC61-IL2Mut24 vs Fc-IL2Mut24
108	<i>Ifitm1</i>	Treg	-5.98	3.11E-03	3.69	6.57E-06	3.11E-03	PC61-IL2Mut24 vs Fc-IL2Mut24
109	<i>Clspn</i>	Treg	-2.42	5.22E-03	3.47	1.39E-05	5.22E-03	PC61-IL2Mut24 vs Fc-IL2Mut24
110	<i>Tpx2</i>	Treg	-1.93	6.36E-03	3.40	2.45E-05	6.36E-03	PC61-IL2Mut24 vs Fc-IL2Mut24
111	<i>Nusap1</i>	Treg	-1.91	1.22E-02	3.94	6.49E-05	1.22E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
112	<i>Birc5</i>	Treg	-2.13	1.26E-02	4.94	7.09E-05	1.26E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
113	<i>Tspan5</i>	Treg	1.22	1.44E-02	5.62	9.37E-05	1.44E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
114	<i>Cenpf</i>	Treg	-1.96	2.07E-02	3.65	1.97E-04	2.07E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
115	<i>Bbof1</i>	Treg	1.93	2.46E-02	4.02	2.62E-04	2.46E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
116	<i>Tubb2a</i>	Treg	2.58	2.81E-02	7.65	3.42E-04	2.81E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
117	<i>Clpb</i>	Treg	1.24	3.33E-02	3.46	4.87E-04	3.33E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
118	<i>Aspm</i>	Treg	-2.39	3.45E-02	3.06	5.22E-04	3.45E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
119	<i>Cbarp</i>	Treg	1.20	3.86E-02	5.48	6.67E-04	3.86E-02	PC61-IL2Mut24 vs Fc-IL2Mut24
120	<i>Penk</i>	Treg	1.92	4.25E-02	7.41	7.94E-04	4.25E-02	PC61-IL2Mut24 vs Fc-IL2Mut24