Supplemental Material for

IFNGR/STAT1 Signaling in Recipient Hematopoietic Antigen Presenting Cells Suppresses Graft versus Host Disease

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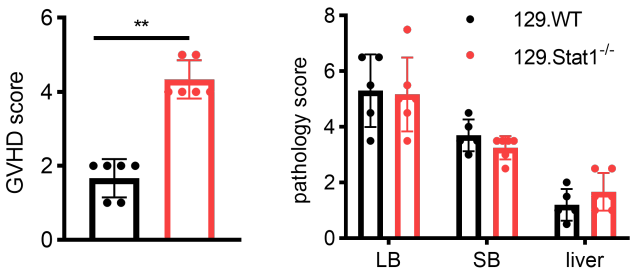
1. Supplemental Methods

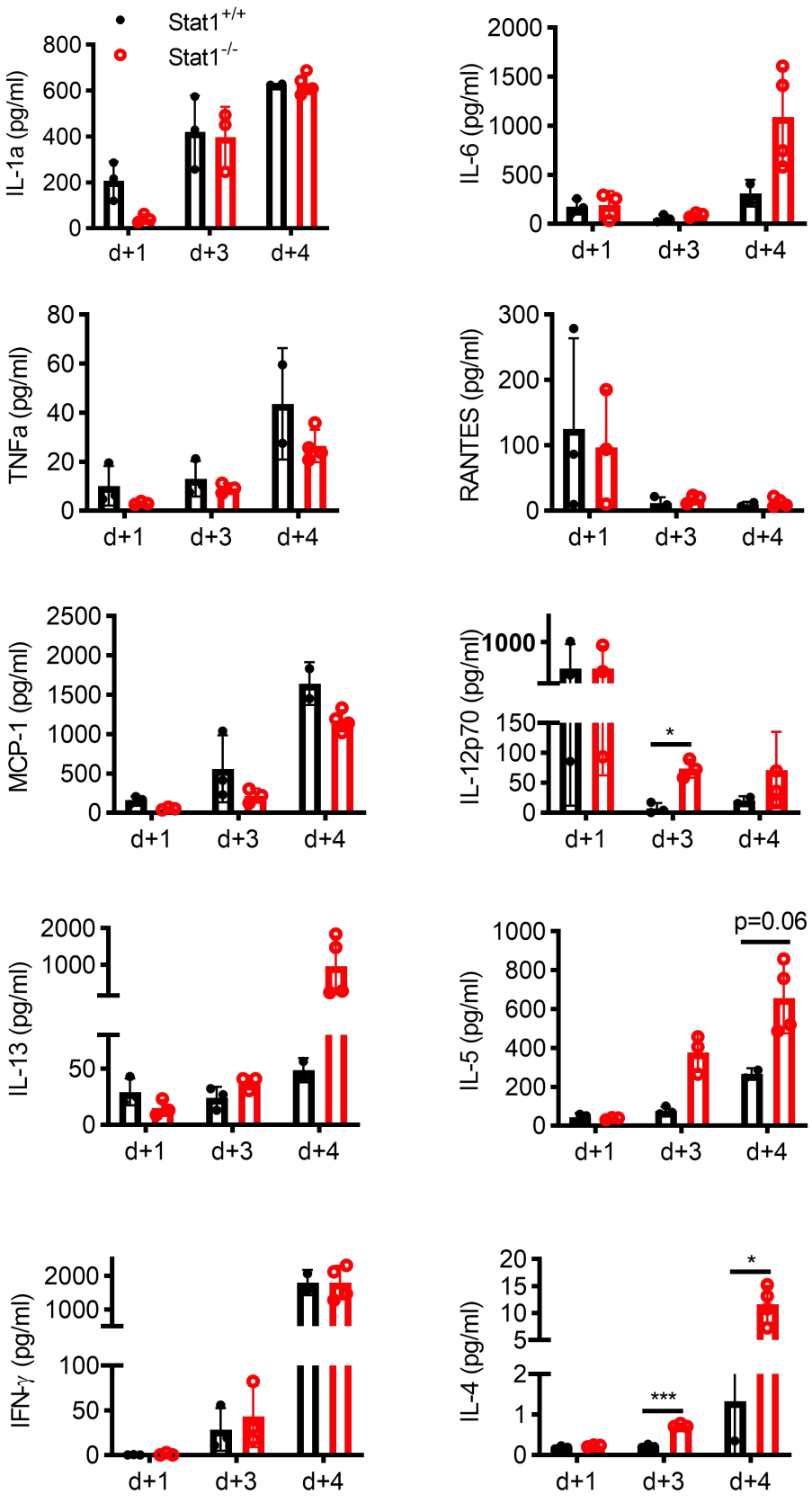
Supplemental Table I Antibodies

Marker	Flurochrome	Clone	Manufacturer	Catalog #	Dilution
CD4	PE-CY5	H129.19	BD Biosciences	553654	1:500
CD8a	Alexa Fluor700	53-6.7	BD Biosciences	557959	1:500
CD62L	PE	MEL-14	Biolegend	104408	1:500
CD44	APC-CY7	IM7	Biolegend	103028	1:500
CD11b	PE-CY7	M1/70	BD Biosciences	552850	1:500
CD11c	Percpcy5.5	HL3	BD Biosciences	560584	1:100
B220	APC-CY7	RA3-6B2	BD Biosciences	552094	1:500
H-2K^{b}	BV421	AF6-88.5	BD Biosciences	562942	1:100
H-2D^{d}	BV786	34-2-12	BD Biosciences	742465	1:100
I-Ab	FITC/PE	AF6-120.1	BD Biosciences	553551/553552	1:500
CD86	PE	GL1	BD Biosciences	553692	1:100
PD-L1	APC	MIH5	BD Biosciences	564715	1:500
CD74	FITC	In-1	BD Biosciences	561941	1:500
IFN-g	PE	XMG1.2	BD Biosciences	554412	1:100
CD25	PE-CY7	PC61	BD Biosciences	552880	1:200
Y-Ae	FITC	ebio-YAe	eBioscience	11-5741-82	1:100
IL-17A	Alexa Fluor488	TC11-18H10	BD Biosciences	560220	1:100
FOXP3	Alex Fluor488	FJK-16s	eBioscience	53-5773-82	1:100

A B

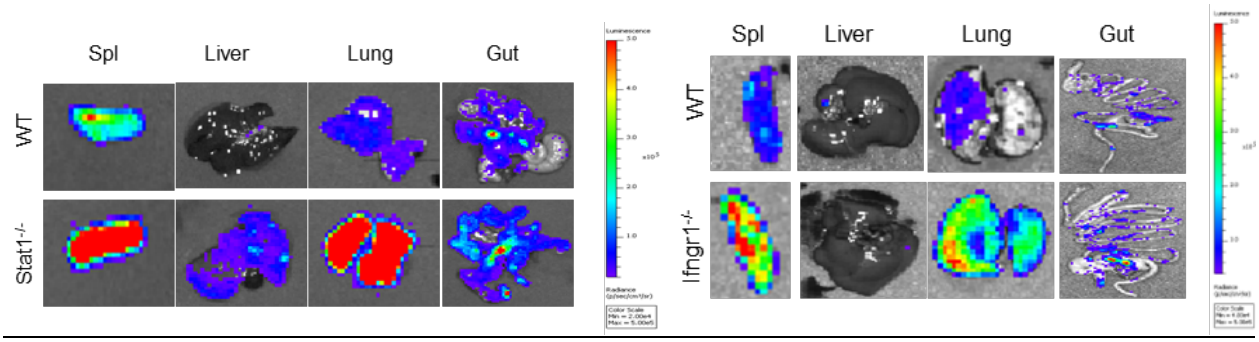


Suppl. Fig 1. Exacerbated morbidity measured by GVHD score in STAT1 deficient host. A) Increased GVHD scores day+4 post-BMT are shown in 129.Stat1$^{-/-}$ host receiving 5x10^6 BMC and 10x10^6 splenocytes from BALB/c mice. Mann-Whitney U test ** $p<0.01$. **B)** Histopathological scores in target organs, including large bowel (LB), small bowel (SB), and liver, was shown. Two independent experiments were combined.



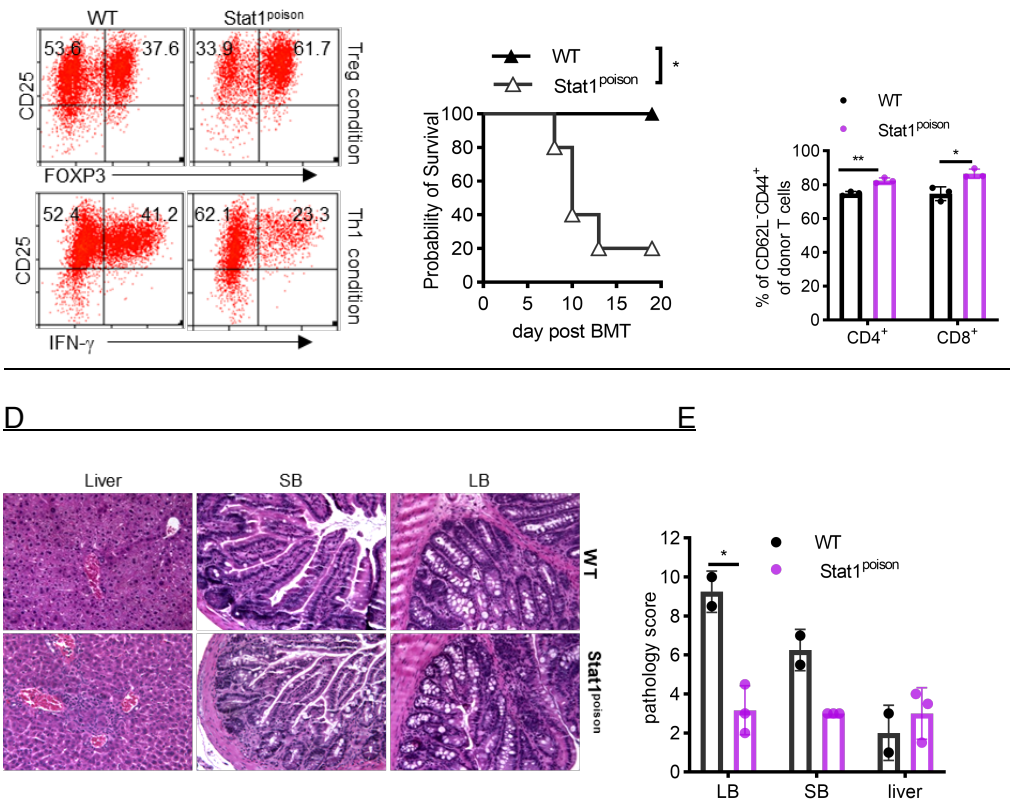
Suppl. Fig 2. Inflammatory cytokine profiles in Stat1$^{-/-}$ recipient mice in comparison with wild-type recipient mice.

GVHD was induced in the fully MHC-mismatched (BALB/c [H2^{d}] to 129Sv [H2^{b}]) strain combination. Lethally irradiated (1,044 cGy) 129.Stat1$^{-/-}$ or 129.Stat1$^{+/+}$ mice received 5x10^{6} BMC and 1x10^{7} splenocytes from BALB/c mice. Serum cytokine profiles were studied on day+1, +3, and d+4 post-BMT with 2–4 mice/group at each time point. Serum levels of individual animals are shown. Horizontal bars denote the mean cytokine serum concentration of the group. Bar graphs represent the mean ± SEM, p values were calculated 2-way ANOVA and Sidak correction for multiple comparisons * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



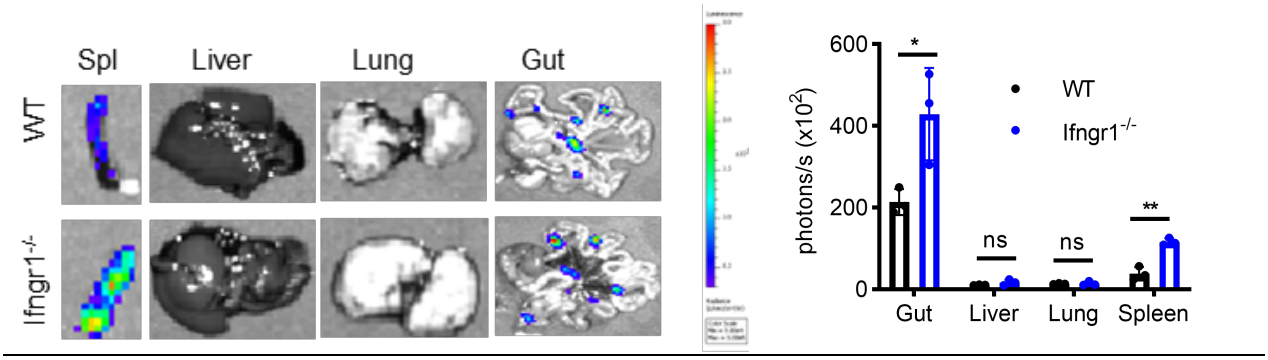
Suppl. Fig 3. Increased alloreactive donor lymphocyte expansion and infiltration in recipient mice with IFNGR or STAT1 deficiency.

A) BLI analysis of the *in vivo* expansion of alloreactive donor BALB/c-luc T cells and target organ infiltration (spleen, liver, lung, and gut) in B6 (WT) or B6.Stat1$^{-/-}$ recipients on day+6 post-BMT. Representative results of one of 3 independent experiments with 3-4 animals per group are shown. **B)** Fully MHC-mismatched GVHD induction following lethal irradiation in B6 (WT) vs. B6.Ifngr$^{-/-}$ recipients using 1x10^{7} splenocytes and 5x10^{6} BMCs from BALB/c-luc mice. Infiltration and expansion of BALB/c-luc lymphocytes in the organs of recipient animals were monitored on day +7 post-BMT using BLI. Representative results of bar graphs in Fig.1D and Fig.1H.



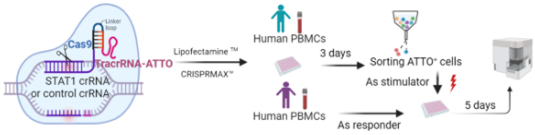
Suppl. Fig 4. Characterization of B6.Stat1^{Poison} mice

A) Enhanced T_{reg} development and reduced Th1 differentiation in B6.Stat1^{poison} mice. CD4 T cells from wild-type B6 (B6.SJL) or B6.Stat1^{poison} mice were assessed for CD25 expression and Foxp3 or IFN-γ intracellular staining following three days of culture under Th1 or T_{reg} conditions. **B**) GVHD was induced in the fully MHC-mismatched (BALB/c [H2^{d}] to B6 [H2^{b}]) strain combination. Wild-type B6 or B6.Stat1^{poison} mice were lethally irradiated with 1,075rad and received 3x10^{6} BALB/c BMC and 3.0x10^{6} splenocytes. Survival analysis following induction of GVHD (Median survival time (MST) not reached vs. 11 days, log-rank test p=0.014) in wildtype compared to B6.STAT1^{poison} mice. Representative data are from 3 similar experiments with 5 animals per group. **C**) On day +8 post-BMT, animals were euthanized, and splenocytes were analyzed by flow cytometry for the activation status of donor-derived CD4^{+} and CD8^{+} cells based on CD62L^{-}CD44^{+}. Representative results of 3 independent experiments are shown with 5 mice per group. **D, E)** GVHD target organs were isolated on day+8 post-BMT. Histology with HE staining (100x) and pathology scores of the liver, small bowel (SB), and large bowel (LB) are shown. Bar graphs represent the mean ± SEM, p values were calculated by 2-way ANOVA with Sidak correction for multiple comparisons * p<0.05, ** p<0.01.



Suppl. Fig 5. Donor lymphocyte expansion in target organs in recipient mice with IFNGR deficiency in comparison to wild-type recipients on day+4 post -BMT. Fully MHC-mismatched GVHD induction following lethal irradiation in WT vs. B6.Ifngr$^{-/-}$ recipients using 1x10^{7} splenocytes from BALB/c-luc mice. A, B) Infiltration and expansion of BALB/c-luc T lymphocytes in recipient animals' spleen, liver, lung, and gut were monitored on day+4 post-BMT using BLI (n=3 mice/group). A summary bar graph shows representative results of one of 2 independent experiments with 3 animals/group. Bar graphs represent the mean ± SEM, p values were calculated by 2-way ANOVA with Sidak correction for multiple comparisons * $p<0.05$, ** $p<0.01$.

A



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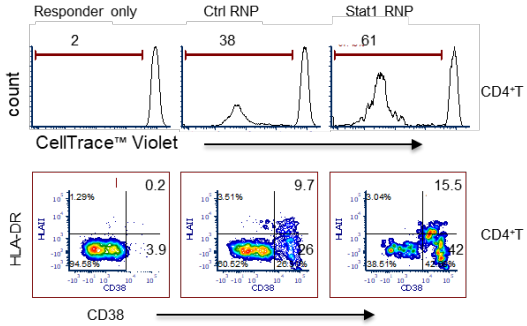
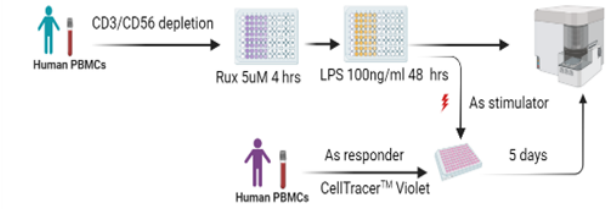


Suppl. Fig 6. Knockdown of Stat1 in human PBMCs by CRISPR/Cas9 enhances their allo-stimulatory capacities.

A) Schematic of experimental procedure. **B)** Allo-CD4⁺ T cell proliferation and activation were detected by flow cytometry after 4 days of coculturing of stimulator and responder cells with a ratio of 1:1. Representative result of 2 independent experiments with two different donors.

A



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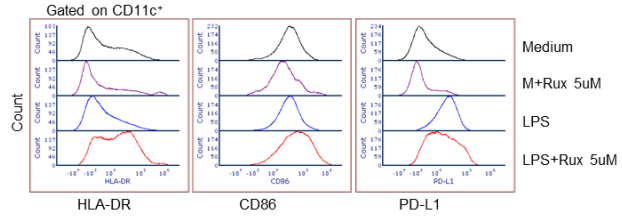

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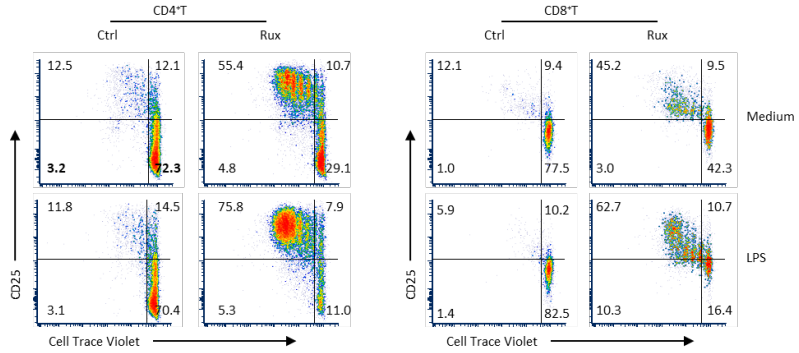
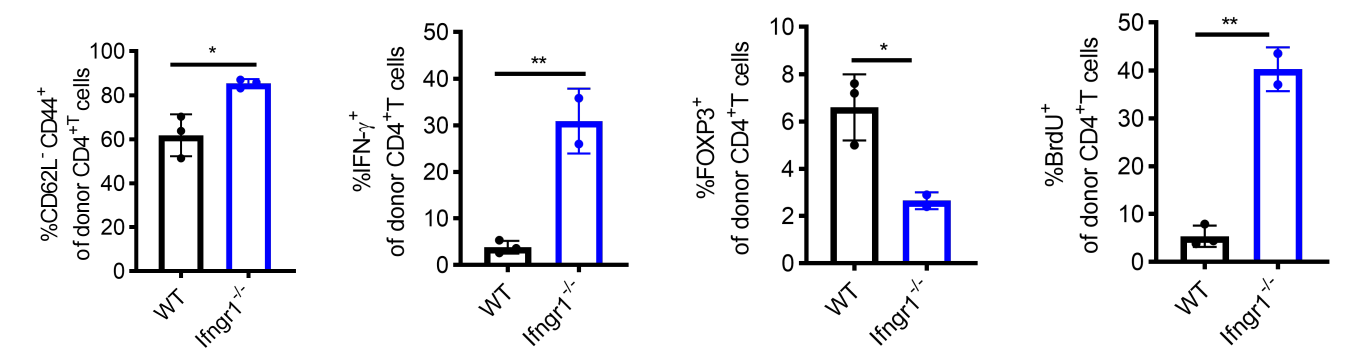
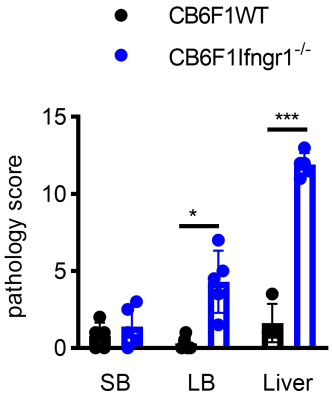


Suppl. Fig 7. JAK inhibitor Ruxolitinib (Rux) treated hPBMCs have increased HLA II, CD86 and reduced PD-L1 expression on CD11C⁺ cells, and enhanced allo-stimulatory capacity. A) Experimental design. B) Phenotype of Rux (5uM) treated human PBMCs 48hrs after LPS stimulation. **C)** Allo-human PBMCs labeled with CellTrace-Violet were cocultured with Rux and LPS (100ng/ml) treated cells for 4 days, and CD25 expression and Violet dilution on CD4⁺ and CD8⁺ T cells were studied by FACS. Representative of 2 independent experiments.



Suppl. Fig 8. Enhanced activation of TEa-donor T cells in CB6F1 recipient mice with IFNGR-deficiency. GVHD was induced in the B6 to CB6F1 mouse model. TEa splenocytes ($1x10^7$) recognizing Ea52-68 peptide presented by I-A^b were i.v. injected into WT or Ifngr$^{-/-}$ CB6F1 mice. Thereafter, mice were injected i.p. with BrdU. On day +7, the mice were euthanized. Splenocytes were harvested and assessed for T cell activation ($CD62L^-$ and CD44 expression) in donor CD4 and CD8 T cells (**A),** Th1(**B**), T_{reg} (**C**) differentiation by staining for IFN-γ and FOXP3 in CD4 T cells and $CD4^+$donor T cell proliferation (**D**) by BrdU incorporation. Bar graphs represent the mean ± SEM, p values were calculated by 2-tailed t test * $p<0.05$, ** $p<0.01$.

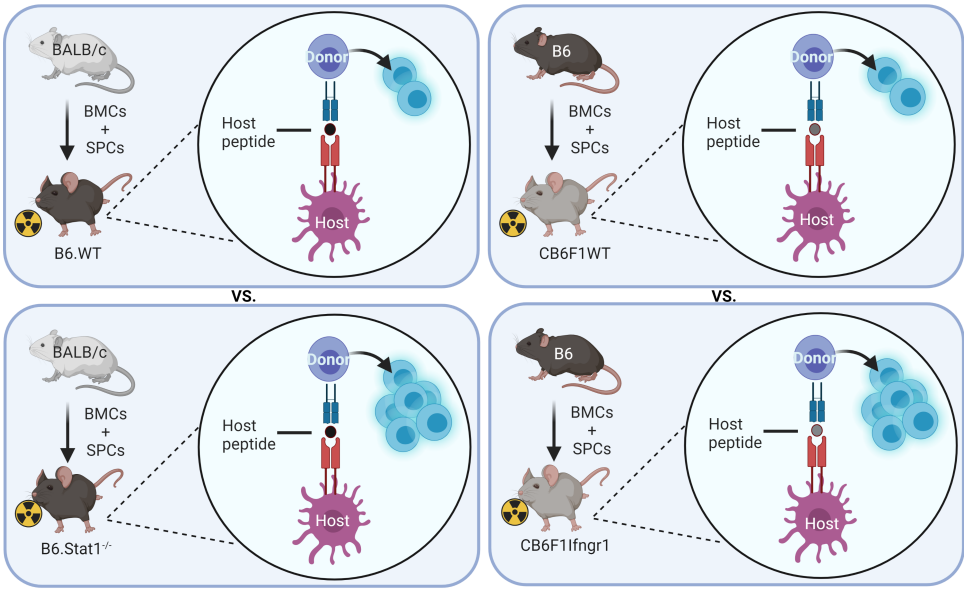


Suppl. Fig 9. Enhanced inflammation in GVHD target tissues in CB6F1 recipient mice with IFNGR deficiency.

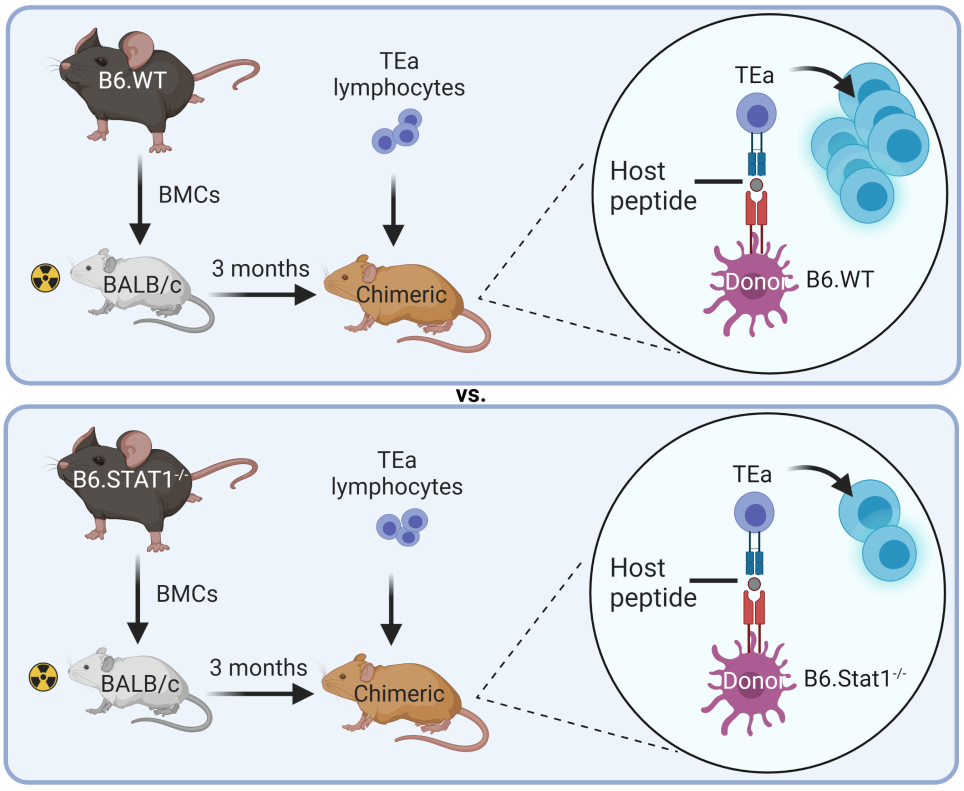
GVHD was induced in the P →F1 (B6.SJL[$H2^b$] →CB6F1 [$H2^{bxd}$]) mouse model using wild-type or Ifgnr$^{-/-}$ recipients receiving $5x10^6$ BMCs and $2.5x10^7$ splenocytes from B6 mice without irradiation. GVHD target organs were isolated on day +30 post-BMT. Pathology scores of the small intestine (SB), colon (LB), and liver are shown. Representative of 2 independent experiments. Bar graphs represent the mean ± SEM, p values were calculated by 2-way ANOVA with Sidak correction for multiple comparisons * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Suppl. Fig. 10. Defective IFNGR/STAT1 signaling leads to enhanced endogenous but compromised exogenous Ag presentation (Created with BioRender.com)

A. Enhanced direct endogenous Ag presentation by the recipient APCs with defective IFNGR/STAT1 signaling in the BALB/→B6 MHC-mismatched BMT setting (left) and in the haplotype MHC-mismatched B6 to CB6F1 BMT model (right).



B. Compromised indirect host Ag presentation by the donor-derived APCs with defective STAT1 signaling (B6 →BALB/c) chimeric mice as recipients. Proliferation and activation of the adoptively transferred TEa lymphocytes were used as readouts of the indirect Ag presentation.



C. Presentation of exogenous OVA protein was compromised in Stat1$^{-/-}$ B6 mice (left); in contrast, constitutively expressed endogenous ovalbumin Ag presentation was enhanced in the Stat1$^{-/-}$ host (right).