Curing autoimmune diabetes in mice with allogeneic islet and hematopoietic cell transplantation after CD117 antibody-based conditioning

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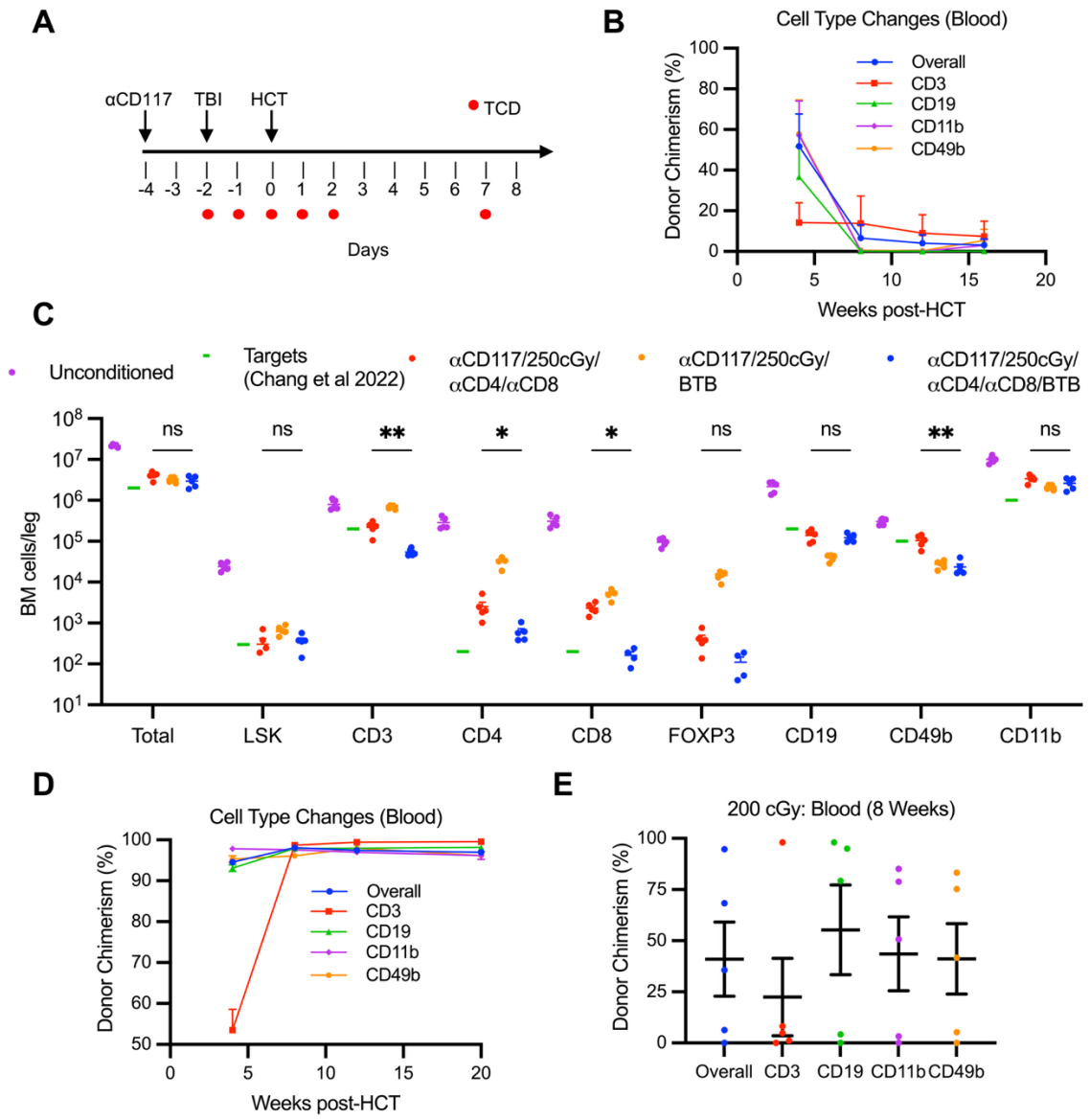
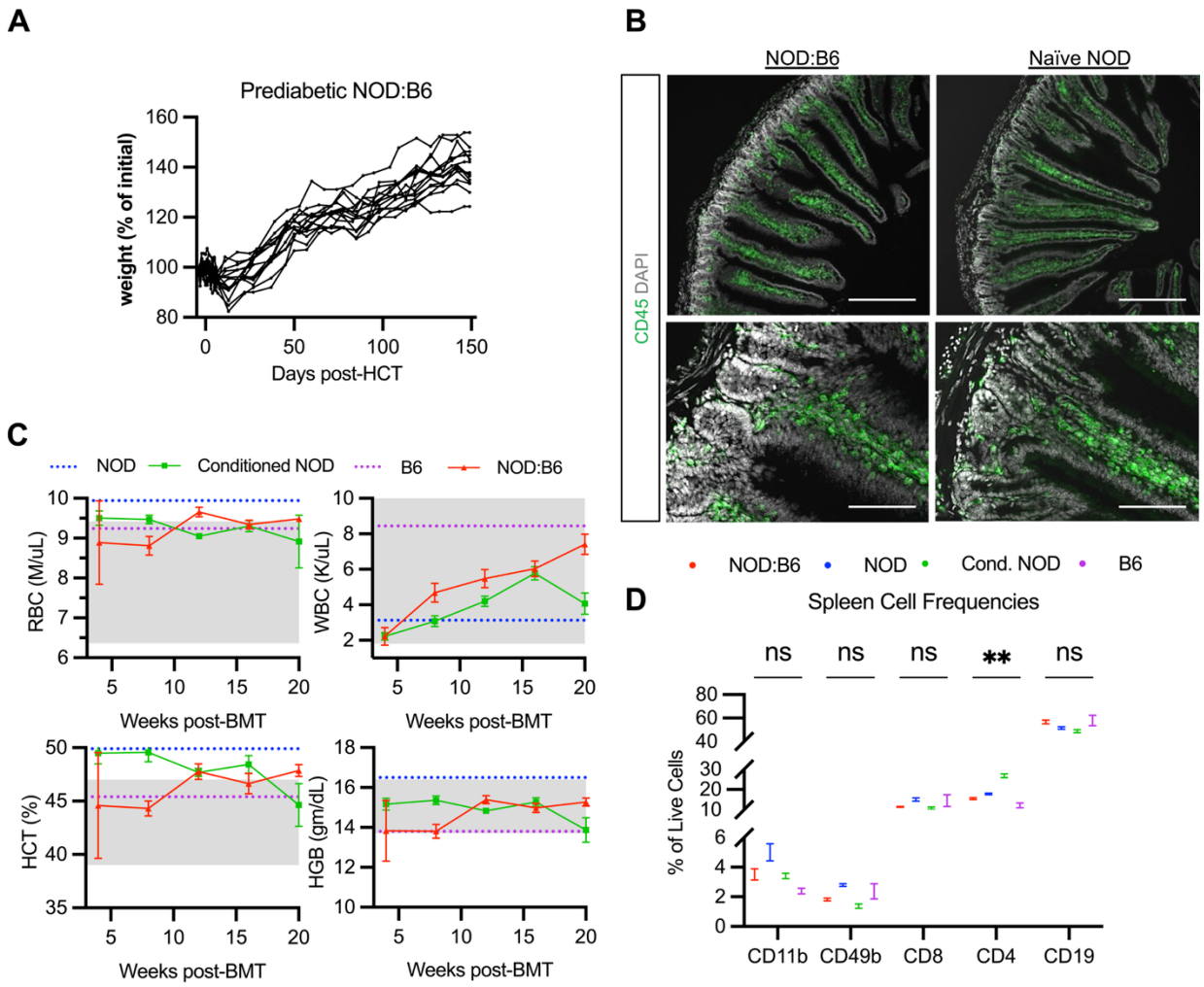
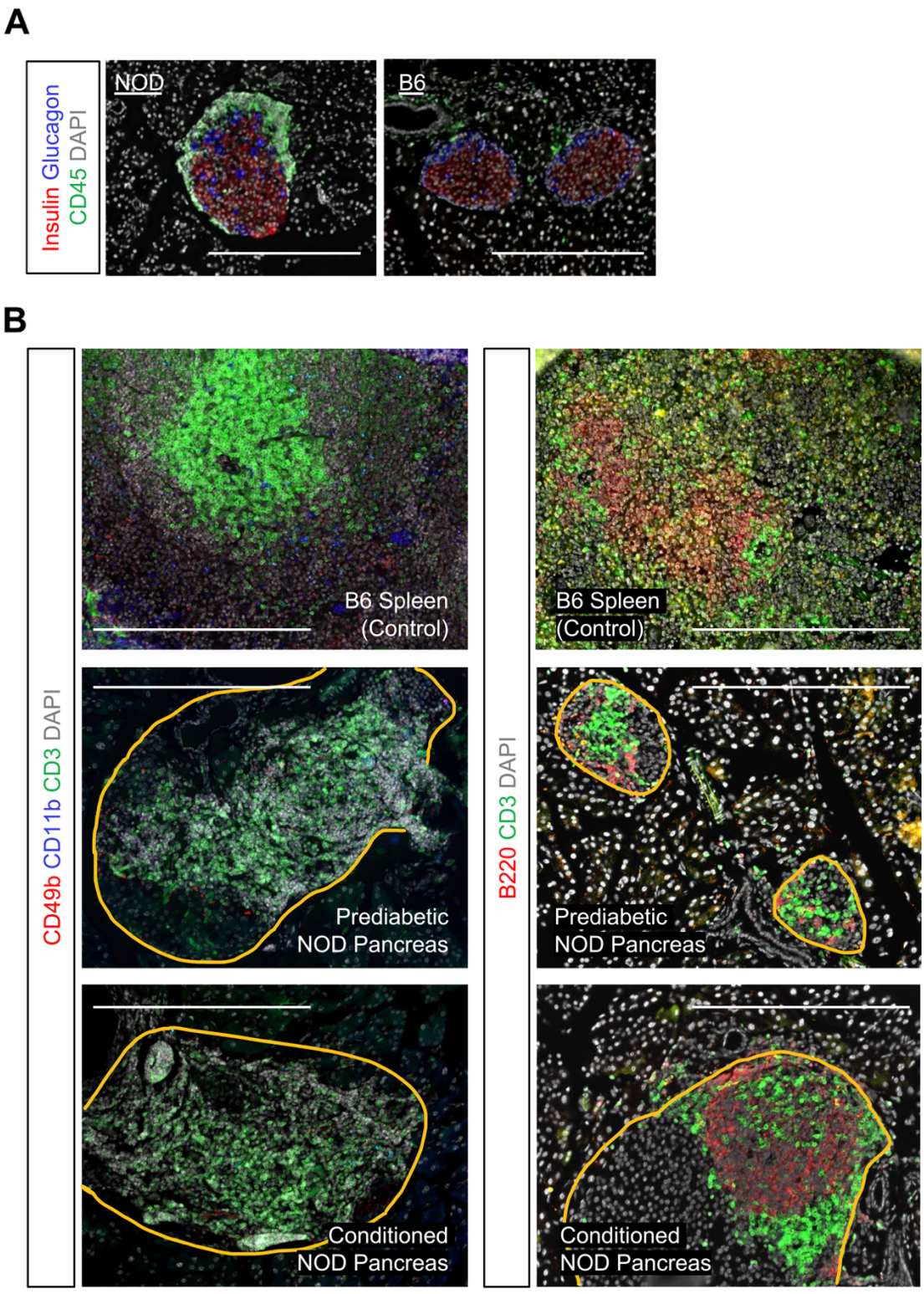
Supplemental Materials



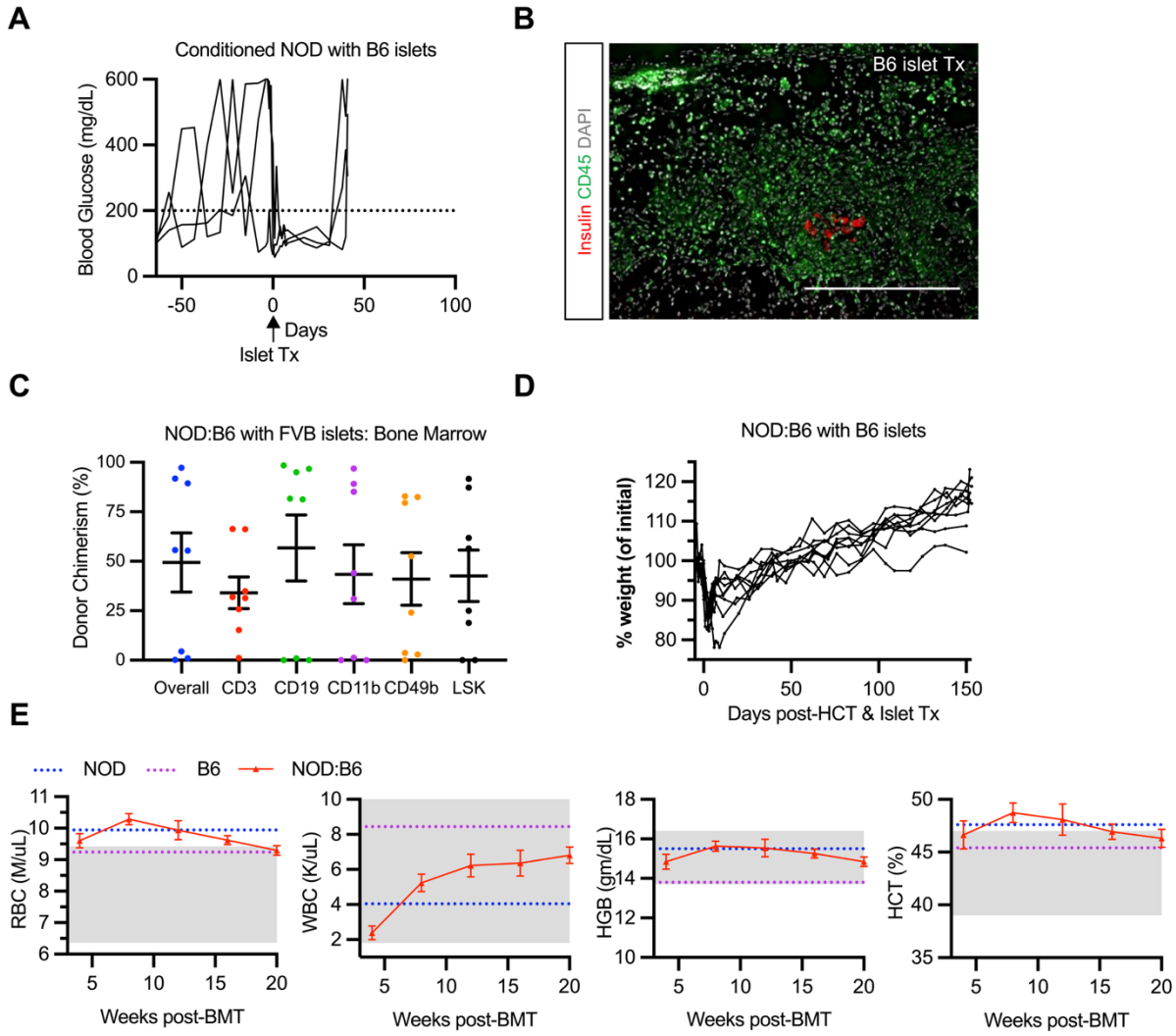
Supplemental Figure 1. Non-myeloablative conditioning requires JAK inhibition to permit durable allogeneic donor chimerism in a radioresistant NOD model. (A) Reduced intensity conditioning regimen without JAK inhibition. 500µg αCD117 is administered on day -4, 300cGy TBI on day -2, 600µg/300µg of αCD4/CD8 on days -2 through +2 and day +7. On day 0, mice are transplanted with 30E6 WBM cells or 1.5E6 HSPCs after TCD treatment. Mixed chimerism is analyzed every 4 weeks afterwards, in peripheral blood. (**B**) Longitudinal chimerism analysis of peripheral blood after conditioning (without JAK inhibition) and HCT depicting overall, $CD3^+$ T cell, $CD19^+$ B cell, $CD11b^+$ myeloid cell, and $CD49b^+$ NK cell donor ($CD45.2^+$) chimerism ($n = 5$). (**C**) Bone marrow (BM) absolute cell count analysis at Day 0 after conditioning regimen containing αCD117, 250 cGy TBI, and αCD4/CD8 with (blue, Figure 1A) or without (red, Supplemental Fig. 1A) JAK inhibitor baricitinib (BTB), as well as αCD117, 250 cGy TBI, and BTB without TCD (orange). Approximate target values for depletion are shown in green from studies in C57BL/6 mice in Chang et al 2022, and unconditioned NOD in purple. (**D**) Chimerism analysis of peripheral blood over 20 weeks post-HCT with 2.5E6 B6 HSPCs and conditioning with JAK inhibition ($n = 5$). (**E**) Chimerism analysis of peripheral blood at 8 weeks post-HCT with 30E6 B6 WBM cells and conditioning with JAK inhibition and XRT reduced to 200 cGy ($n = 5$). (B-E) Data are represented with mean ± SEM. $*P < 0.05$; $**P < 0.01$; ns = not significant. *P* values were calculated using Mann-Whitney U tests. TBI = total body irradiation; HCT = hematopoietic cell transplant; TCD = T cell depletion; HSC = hematopoietic stem cell; LSK = Lin^- $cKit^+$ $Sca\text{-}1^+$



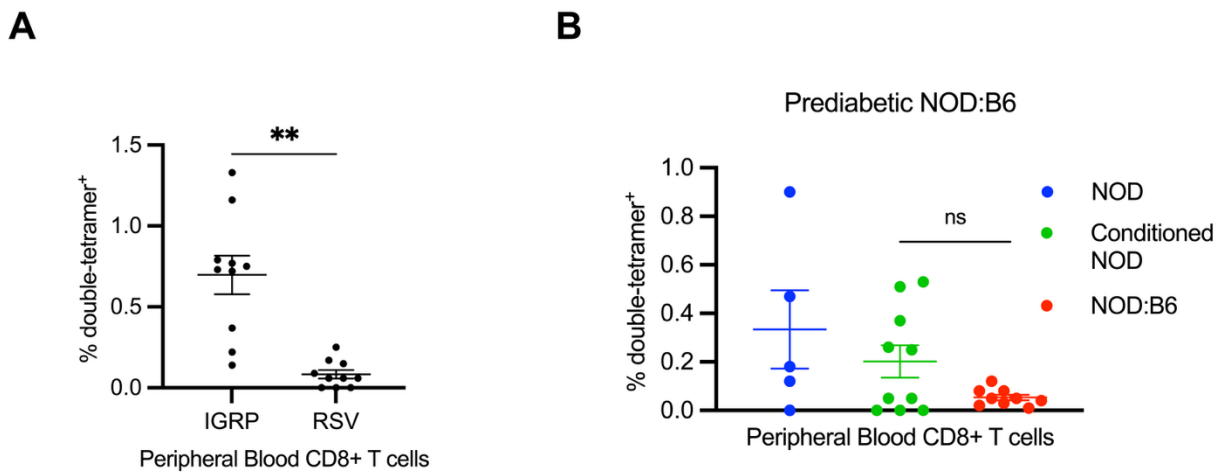
Supplemental Figure 2. Functional status of prediabetic mixed chimeras after non-myeloablative conditioning and HCT. (**A**) Weight after HCT of prediabetic NOD:B6 as a percentage of initial weight prior to conditioning start (n = 15, sum of 3 independent experiments). (**B**) Representative host duodenal histology of prediabetic NOD:B6 chimeras (n = 14) at 20 weeks post-HCT compared to naïve NOD, stained for CD45. Scale bars = 200µm (top) or 50µm (bottom). (**C**) Complete blood count values for prediabetic NOD:B6 mice after conditioning and HCT (red; n = 10) vs. prediabetic NOD mice after conditioning only (green; n = 10). Blue dotted lines show averages from naïve prediabetic NOD mice (n = 5) and purple dotted lines show averages from naïve B6 mice (n = 9). Gray boxes show reference ranges as provided with hematology analyzer equipment (see Methods). WBC = white blood cell; RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit. (**D**) Myeloid, NK, $CD8^+$ T, $CD4^+$ T, and B cell frequencies in spleens of prediabetic NOD:B6, naïve NOD, conditioned NOD, and naïve B6 mice (n = 9-14). Data are represented with mean ± SEM. P values were calculated using Mann-Whitney U tests. $^{**}P < 0.01$; ns = not significant



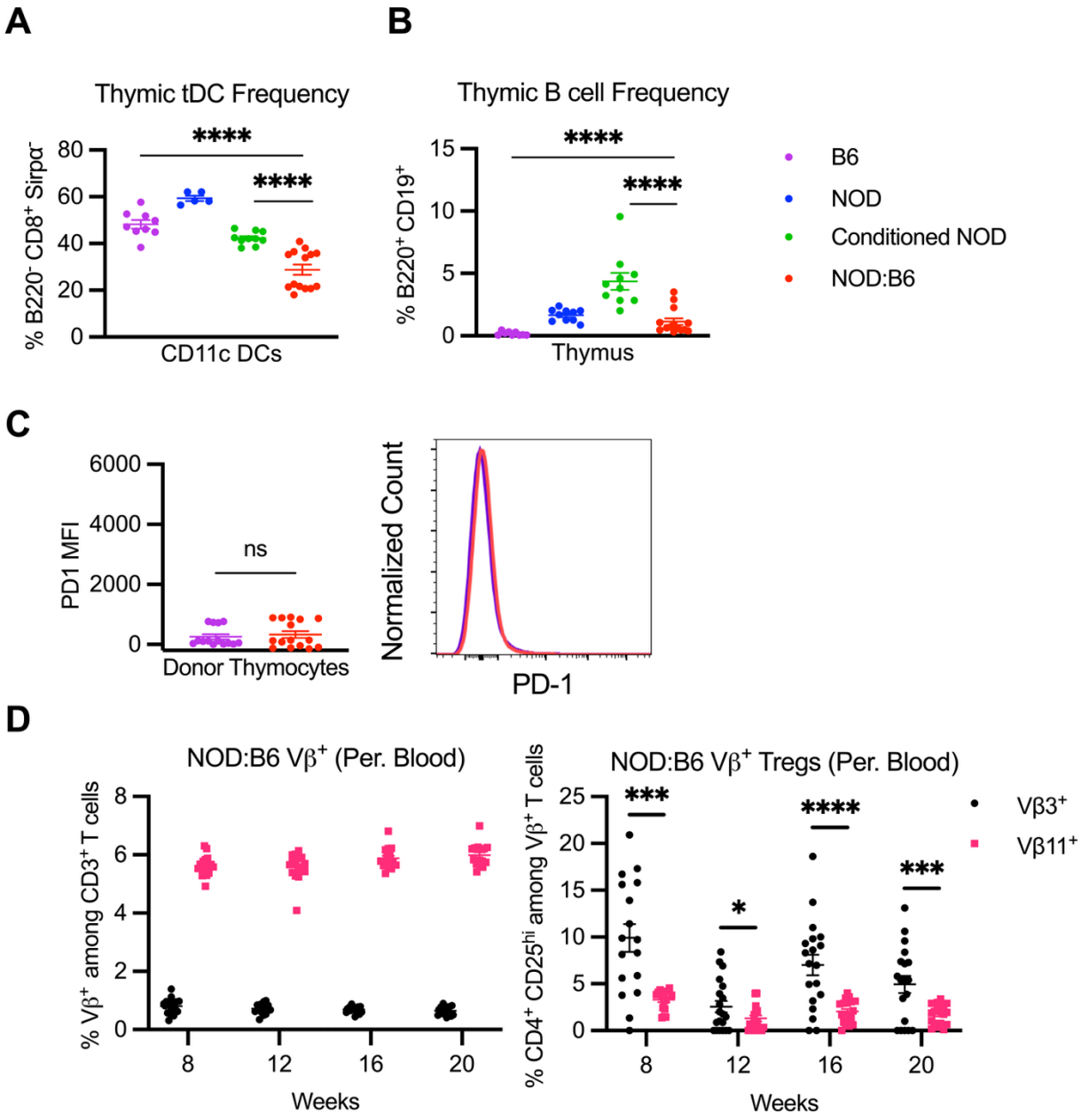
Supplemental Figure 3. Insulitis is present in conditioned mice but not in mixed chimeras. (**A**) Representative pancreatic histology of prediabetic NOD at 8 weeks compared to naïve B6 after immunostaining for insulin, glucagon, and CD45. (n = 4-6; scale bars = 200μm). (**B**) Representative pancreas histology of naïve prediabetic NOD at 12 weeks, or conditioned prediabetic NOD (20 weeks after conditioning), immunostained as indicated for CD3, CD49b and CD11b, or CD3 and B220 (n = 3 each, scale bars = 200μm, islets circled in yellow). B6 spleen included as a staining control.



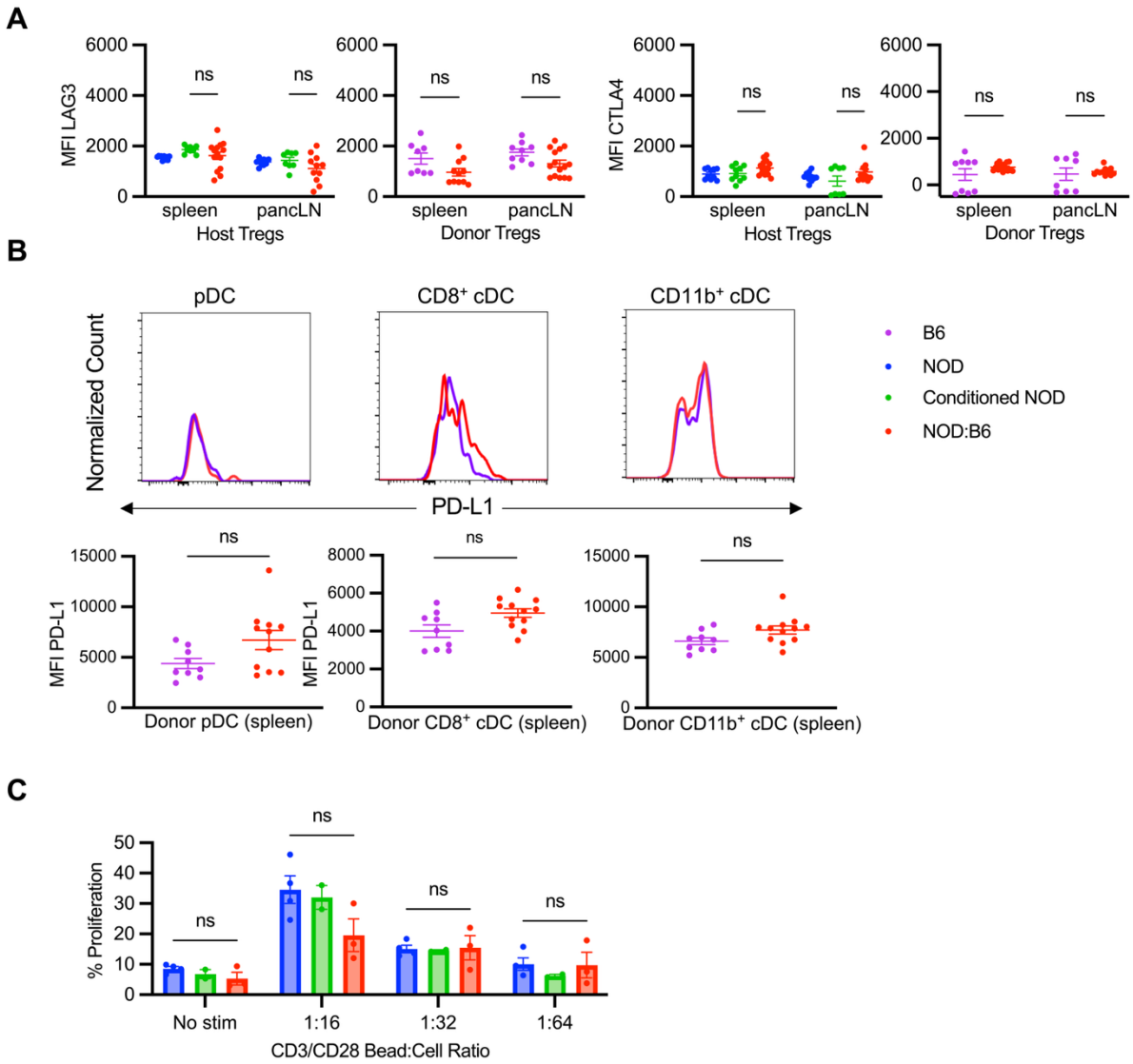
Supplemental Figure 4. Functional status of diabetic mixed chimeras after non-myeloablative conditioning, HCT, and islet transplantation. (A) Non-fasting blood glucose of conditioned diabetic NOD mice without HCT that received B6 islets (n = 3). Dotted line (200 mg/dL) indicates normoglycemia threshold. **(B)** B6 islet graft 5 weeks after transplantation in conditioned diabetic NOD, stained for insulin and CD45 (n = 3). Scale bar = 200µm (**C**) Bone marrow chimerism at endpoint (islet rejection) in diabetic NOD:B6 mice that received FVB islets, including Lin$^-$ cKit$^+$ Sca-1$^+$ (LSK) HSC chimerism (n = 8, sum of 3 independent experiments). (**D**) Weight after HCT and B6 islet transplantation of diabetic NOD:B6 as a percentage of initial weight prior to conditioning start (n = 11, sum of 3 independent experiments). (**E**) Complete blood count values for diabetic NOD:B6 mice after conditioning, HCT, and B6 islet transplantation (red, n = 9). Blue dotted lines show averages from naïve diabetic NOD mice (n = 5) and purple dotted lines show averages from naïve B6 mice (n = 9). Gray boxes show reference ranges as provided with hematology analyzer equipment (see Methods). WBC = white blood cell; RBC = red blood cell; HGB = hemoglobin; HCT = hematocrit. Data are represented with mean ± SEM.



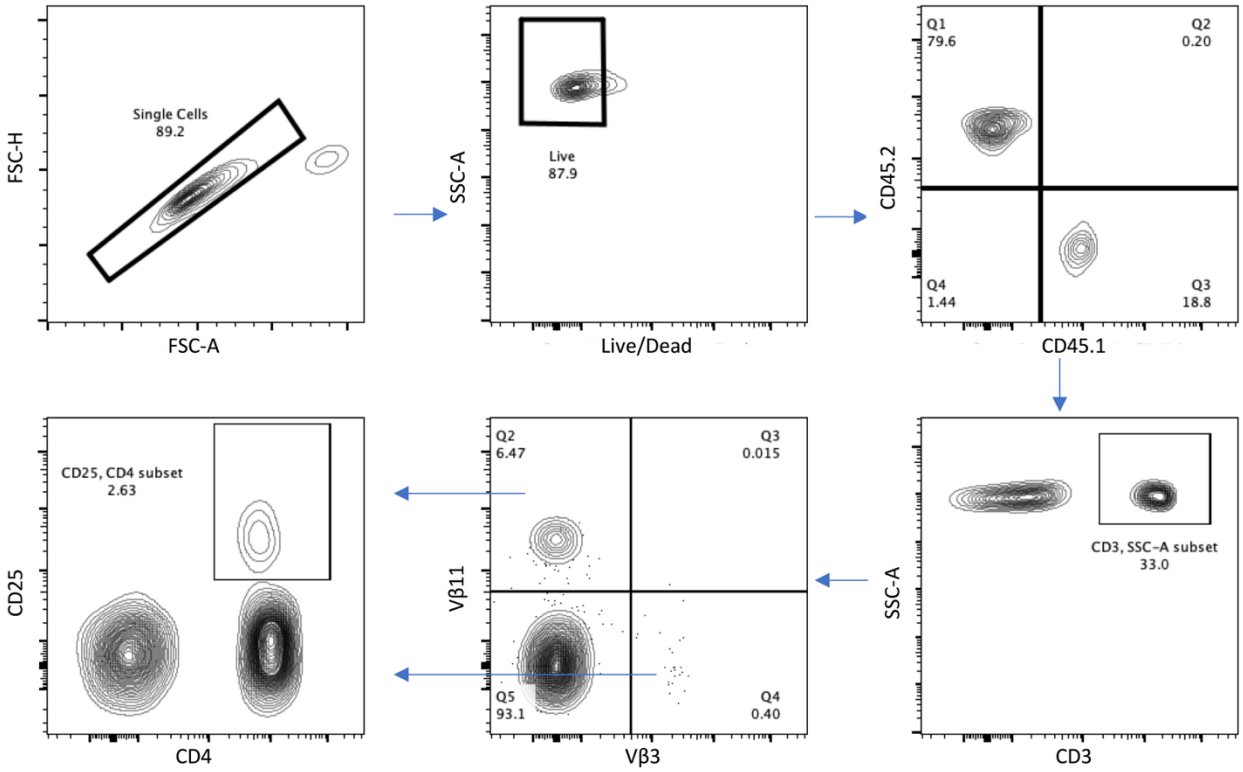
Supplemental Figure 5. Autoreactive T cells can be tracked using islet autoantigen specific tetramer analysis. (**A**) Islet autoantigen IGRP-double-tetramer$^+$ frequency compared to irrelevant RSV-double-tetramer$^+$ frequency among peripheral blood CD8$^+$ T cells in diabetic NOD (n = 10). (**B**) Frequency of IGRP-double-tetramer$^+$ autoreactive cells among CD3$^+$ CD8$^+$ T cells in peripheral blood of prediabetic NOD and prediabetic NOD:B6 at 20 weeks post-conditioning and HCT (n = 5-10). Data are represented with mean ± SEM. P values were calculated using Wilcoxon matched-pairs signed rank test (A) or Mann-Whitney U tests with Holm-Šídák correction for multiple comparisons (B). **$P < 0.01$; ns = not significant



Supplemental Figure 6. Donor antigen presenting cell presence is associated with thymic deletion of host and donor T cells. (**A**) Frequency of thymus-resident DCs (tDC; B220$^-$ CD8$^+$ Sirpα$^-$) among CD11c$^+$ DCs in naïve B6 controls, naïve prediabetic NOD, conditioned prediabetic NOD controls at 20 weeks post-conditioning, and prediabetic NOD:B6 at 20 weeks post-conditioning and HCT (n = 9-14). (**B**) Frequency of B cells among thymocytes in naïve B6 controls, naïve prediabetic NOD, conditioned prediabetic NOD controls at 20 weeks post-conditioning, and prediabetic NOD:B6 at 20 weeks post-conditioning and HCT (n = 9-14). (**C**) Representative histogram and mean ± SEM of median fluorescence intensity (MFI) of PD-1 expressed by CD45.2$^+$ donor thymocytes in prediabetic NOD:B6 compared to B6 controls (n = 9-14). (**D**) Frequency of Vβ3$^+$ and Vβ11$^+$ T cells among CD3$^+$ T cells in peripheral blood of prediabetic NOD:B6 from 8 to 20 weeks post-HCT (left). Frequency of CD4$^+$ CD25^{hi} Tregs among Vβ3$^+$ or Vβ11$^+$ T cells in peripheral blood of prediabetic NOD:B6 from 8 to 20 weeks post-HCT (right) (n = 14). Data are represented with mean ± SEM. P values were calculated using Mann-Whitney U tests with Holm-Šídák correction for multiple comparisons (A-C) or Wilcoxon matched-pairs signed rank test (D). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. DC = dendritic cell



Supplemental Figure 7. Peripheral tolerance mechanisms contribute to anergy of peripheral host effector cells. (**A**) Median fluorescence intensity (MFI) of LAG3 and CTLA4 expressed by CD45.1$^+$ host Tregs in prediabetic NOD:B6 spleen and pancLN compared to NOD and conditioned NOD controls (n = 9-14). MFI of LAG3 and CTLA4 expressed by CD45.2$^+$ donor Tregs in NOD:B6 spleen and pancLN compared to B6 controls (n = 9-14). **(B)** Representative histogram and mean ± SEM of median fluorescence intensity (MFI) of PD-L1 expressed by CD45.2$^+$ donor plasmacytoid DCs (pDCs), CD8$^+$ conventional DCs (cDCs), and CD11b$^+$ cDCs in prediabetic NOD:B6 spleen compared to B6 controls (n = 9-10). (**C**) Proliferation of host CD4$^+$ splenic T cells from prediabetic NOD:B6 compared to NOD and conditioned NOD controls after isolation and incubation in vitro with CD3/CD28 stimulation beads at decreasing dilutions (1:16 to 1:64) compared to unstimulated. Data are represented with mean ± SEM. P values were calculated using Mann-Whitney U tests with Holm-Šídák correction for multiple comparisons. ns = not significant. DC = dendritic cell



Supplemental Figure 8. Gating strategy for Vβ subsets. Lymphocytes were gated on FSC-A vs. SSC-A (not shown), then subset to single cell live donor ($CD45.2^+$) or host ($CD45.1^+$) $CD3^+$ T cells. These were further subset into $V\beta3^+$ and $V\beta11^+$ T cells. $CD4^+$ $CD25^+$ Tregs were gated on both $V\beta3^+$ and $V\beta11^+$ T cell subsets.

Supplemental Table 1

Primary Antibody	**Dilution**	**Company**	**Catalog**
Rat α-Mouse CD3	1:200	Biolegend	100202
Rat α-Mouse CD45	1:200	Biolegend	103102
Guinea pig α-insulin	1:500	Dako	A0564
Rabbit α -glucagon	1:200	ThermoFisher Scientific	PA5-88091
Secondary Antibody	**Dilution**	**Company**	**Catalog**
AF647 Goat α-Guinea Pig	1:100	ThermoFisher Scientific	A-21450
AF555 Donkey α-Rabbit	1:100	ThermoFisher Scientific	A-31572
AF594 mouse α-Rat	1:200	Biolegend	408207
AF488 Mouse α-Rat	1:200	Biolegend	408211
AF594 Donkey α-Guinea Pig	1:1000	Millipore Sigma	SAB4600096
AF488 Donkey α-Guinea Pig	1:1000	Millipore Sigma	SAB4600033
Conjugated Antibody	**Dilution**	**Company**	**Catalog**
AF594 Rat α-Mouse CD45.2	1:100	Biolegend	109850
AF488 Rat α-Mouse CD45.1	1:100	Biolegend	110717

Supplemental Table 2

Antibody	Clone	Color	Catalog	Company
CD45.1	A20	PerCP-Cy-5.5	110728	Biolegend
CD45.1	A20	BV685	110743	Biolegend
CD45.2	104	Pacific Blue	109820	Biolegend
CD3	17A2	AF488	100210	Biolegend
CD3	17A2	AF700	100216	Biolegend
CD3	17A2	PE-Cy7	100220	Biolegend
CD3	17A2	FITC	100204	Biolegend
CD4	RM4-4	PE	116006	Biolegend
CD4	RM4-4	BV421	100563	Biolegend
CD8	53-6.7	BV510	100752	Biolegend
CD8	53-6.7	FITC	100705	Biolegend
CD11b	M1/70	BV605	101257	Biolegend
CD11b	M1/70	FITC	101206	Biolegend
CD25	PC61.5	PE-Cy5	102010	Biolegend
CD19	6D5	PE-Cy7	115520	Biolegend
CD49b	DX5	APC	108910	Biolegend
Gr-1	R B6-8C5	FITC	108406	Biolegend
TER-119	TER-119	FITC	116206	Biolegend
CD11c	N418	BV421	117329	Biolegend

CD172a (SIRPa)	P84	APC	144013	Biolegend
CD317 (PDCA-1)	927	PE	127009	Biolegend
MHCII (I-A/I-E)	M5/114.15.2	PE-Cy7	107629	Biolegend
CD274 (PDL1)	10F.9G2	PE/Dazzle 594	124323	Biolegend
B220	RA3-6B2	FITC	103206	Biolegend
CD62L	MEL-14	PE-Cy5	104410	Biolegend
CD44	IM7	BV605	103047	Biolegend
CD304 (Nrp1)	3E12	PE	145203	Biolegend
CD73	TY/11.8	PE/Dazzle 594	127234	Biolegend
FR4	12A5	PE-Cy7	125012	Biolegend
Helios	22F6	AF488	137213	Biolegend
FOXP3	150D	AF647	320013	Biolegend
CD39	Duha59	PE/Fire 640	143818	Biolegend
CD19	6D5	AF700	115528	Biolegend
TCR-beta	H57-597	AF700	109224	Biolegend
ICOS (CD278)	C398.4A	BV650	313549	Biolegend
LAG3 (CD223)	C9B7W	BV711	125243	Biolegend
CTLA4 (CD152)	UC10-4B9	BV421	106311	Biolegend
PD1	29F.1A12	BV750	135263	Biolegend
CD25	3C7	PE-Cy7	101915	Biolegend
CD4	GK1.5	AF700	100429	Biolegend
CD4	GK1.5	Pacific Blue	116008	Biolegend
CD117	2B8	APC	17-1171-82	ThermoFisher Scientific

Sca-1	D7	PE-Cy7	25-5981-81	ThermoFisher Scientific
TCR Vβ11	RR3-15	PE	139004	Biolegend
TCR Vβ3	REA646	APC	130-109-895	Miltenyi Biotec