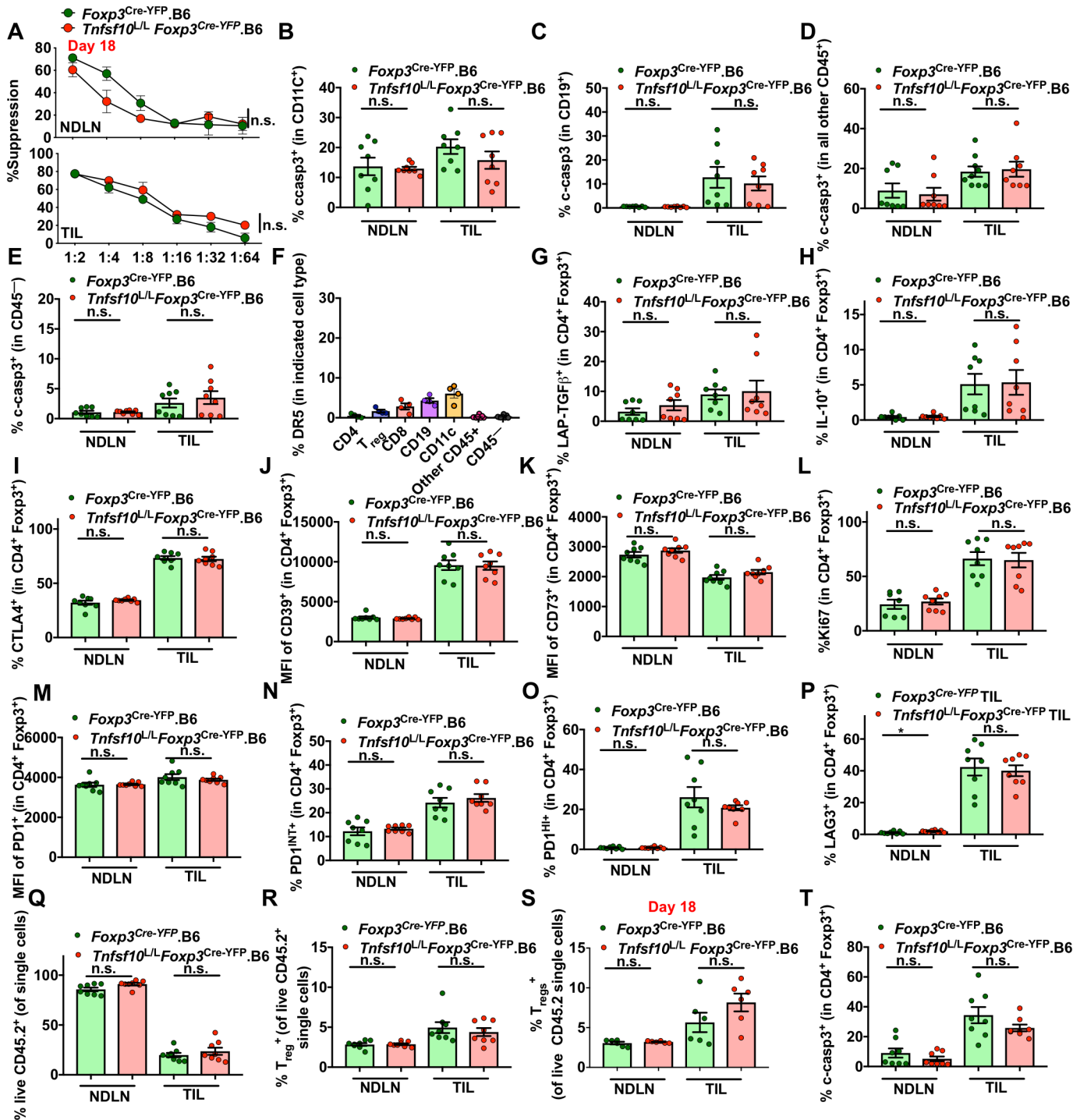
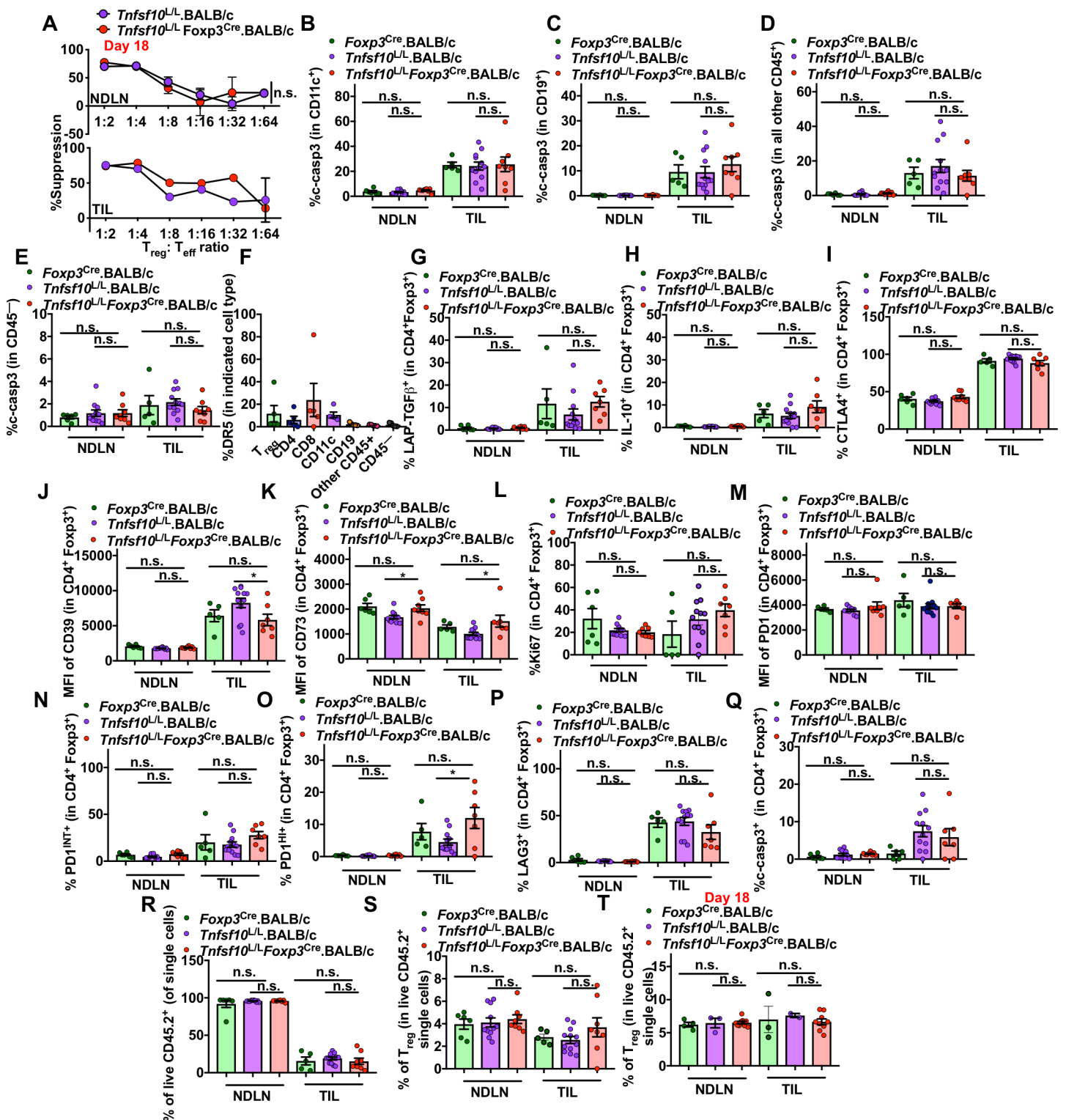


# Supp Figure 1 Dadey et al



**Supplemental Figure 1. No change in cell death in other populations and maintenance of other T<sub>reg</sub> suppressive molecules in C57BL/6 mice** (A) *Foxp3*<sup>Cre-YFP</sup>.B6 and *Tnfsf10*<sup>L/L</sup> *Foxp3*<sup>Cre-YFP</sup>.B6 mice were injected with 125,000 B16 i.d. and T<sub>regs</sub> were isolated on Day 18 from NDNL and TIL to perform a classical microsuppression assay. (B) *Foxp3*<sup>Cre-YFP</sup>.B6 and *Tnfsf10*<sup>L/L</sup> *Foxp3*<sup>Cre-YFP</sup>.B6 mice were injected with 125,000 B16 i.d. and TCRβ<sup>-</sup>CD11c<sup>+</sup> cells were stained for percent expression of cleaved-caspase3 (c-casp3). (C) TCRβ<sup>-</sup>CD19<sup>+</sup>, (D) CD45<sup>+</sup>TCRβ<sup>-</sup>CD19<sup>-</sup>CD11c<sup>-</sup> cells and (E) CD45<sup>-</sup> cells were stained for percent expression of c-casp3. (F) Cell populations were stained for percent positive surface DR5 expression. (G) Tabulated LAP-TGFβ and (H) IL-10 percent expression on T<sub>regs</sub>. (I) %CTLA4 expression on gated T<sub>regs</sub>. (J) Gated MFI of CD39 on T<sub>regs</sub> (K) Gated MFI of CD73 on T<sub>regs</sub> (L) % Ki67 on T<sub>regs</sub> (M) MFI of PD-1 in PD-1<sup>+</sup> T<sub>regs</sub>. (N) %PD-1 intermediate in T<sub>regs</sub> (O) %PD-1 high in T<sub>regs</sub> (P) %LAG3 on T<sub>regs</sub> (Q) % live CD45.2<sup>+</sup> on gated single cells (R) %CD4<sup>+</sup> Foxp3<sup>+</sup> T<sub>regs</sub> in live CD45.2<sup>+</sup> single cells at Day 12 (S) %CD4<sup>+</sup> Foxp3<sup>+</sup> T<sub>regs</sub> in live CD45.2<sup>+</sup> single cells at Day 18 (T) %CD4<sup>+</sup> Foxp3<sup>+</sup> T<sub>regs</sub> were gated for percent positive expression of c-casp3. Data in (A-E) (G-T) is representative of 2 experiments with 6-8 mice/group. (F) is representative of 1 experiment with 4 mice/group. 2-way ANOVA (A) was used. Student unpaired t test (B-E, G-T) was used. (ns, not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001).

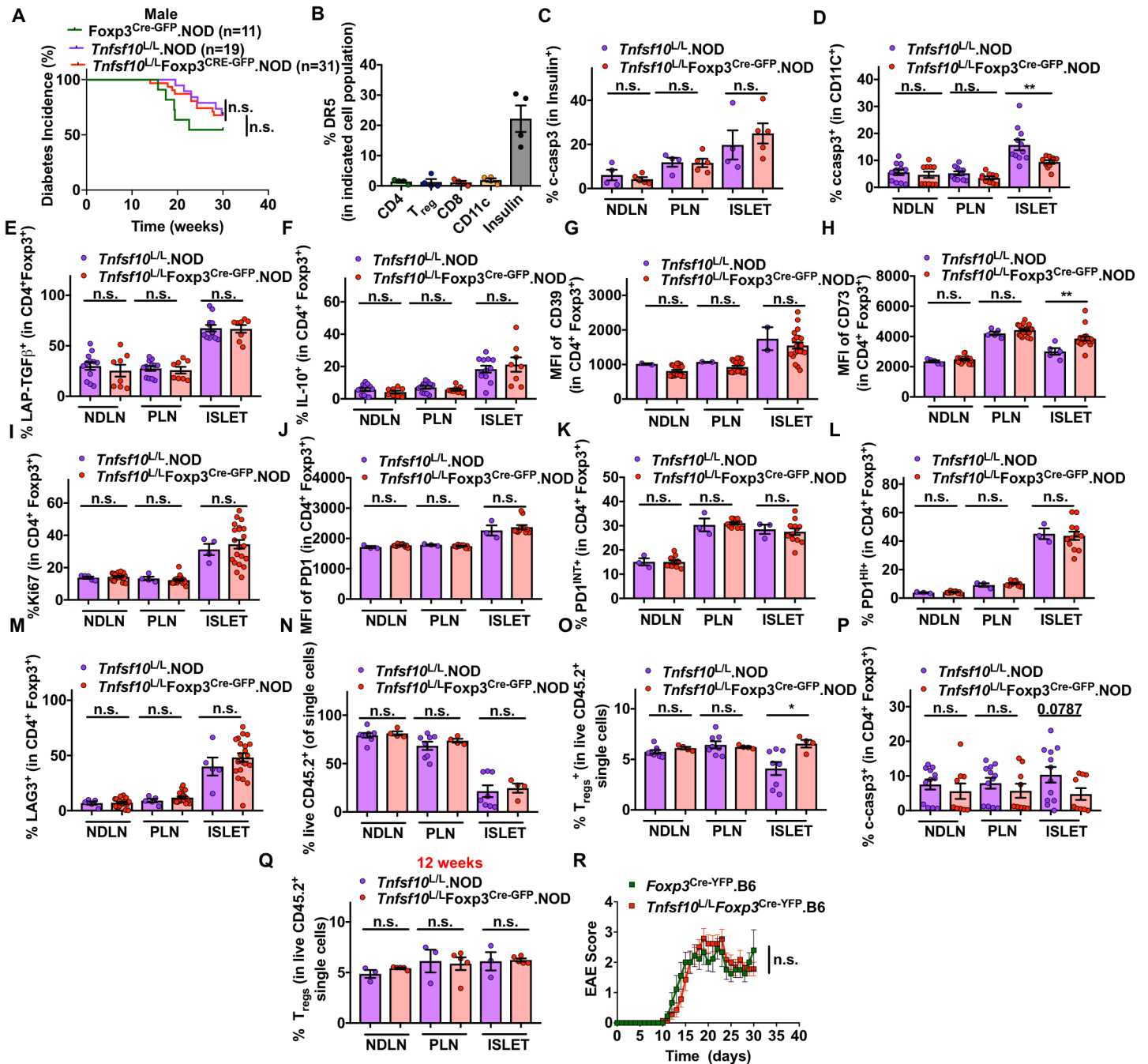
## Supp Figure 2 Dadey et al



### Supplemental Figure 2. No change in cell death in other populations and maintenance of other $T_{reg}$ suppressive molecules in BALB/c mice

(A)  $Tnfsf10^{L/L}.BALB/c$  and  $Tnfsf10^{L/L}.Foxp3^{Cre}.BALB/c$  mice were injected with 125,000 CT26 s.c. and  $T_{reg}$ s were isolated on Day 18 from NDNL and TIL to perform a classical microsuppression assay. (B)  $Foxp3^{Cre}.BALB/c$ ,  $Tnfsf10^{L/L}.BALB/c$ ,  $Tnfsf10^{L/L}.Foxp3^{Cre}.BALB/c$  mice were injected with 125,000 CT26 s.c. and TCRβ-CD11c<sup>+</sup> cells were stained for percent expression of cleaved-caspase3 (c-casp3). (C) TCRβ-CD19<sup>+</sup>, (D) CD45<sup>+</sup>TCRβ-CD19-CD11c<sup>-</sup> cells and (E) CD45<sup>-</sup> cells were stained for percent expression of c-casp3. (F) Cell populations were stained for percent positive surface DR5 expression. (G) Tabulated LAP-TGFβ and (H) IL-10 percent expression on  $T_{reg}$ s (I) %CTLA4 expression on gated  $T_{reg}$ s. (J) Gated MFI of CD39 on  $T_{reg}$ s (K) Gated MFI of CD73 on  $T_{reg}$ s. (L) %Ki67 on  $T_{reg}$ s (M) MFI of PD-1 in PD-1<sup>+</sup>  $T_{reg}$ s (N) %PD-1 intermediate in  $T_{reg}$ s (O) %PD-1 high in  $T_{reg}$ s (P) %LAG3<sup>+</sup> on  $T_{reg}$ s (Q) %CD4<sup>+</sup>Foxp3<sup>+</sup>  $T_{reg}$ s were gated for percent positive expression of c-casp3. (R) % live CD45.2<sup>+</sup> on gated single cells (S) %CD4<sup>+</sup>Foxp3<sup>+</sup>  $T_{reg}$ s in live CD45.2<sup>+</sup> single cells at Day 12 (T) %CD4<sup>+</sup>Foxp3<sup>+</sup>  $T_{reg}$ s in live CD45.2<sup>+</sup> single cells at Day 18. Data in (A) is representative of 1 experiment with 2-3 mice/group pooled. (B-E, G-S) is representative of 2 experiments with 6-12 mice/group. Data in (F, T) is representative of 1 experiment with 2-9 mice/group. 2-way ANOVA (A) was used. Student unpaired t test (B-E, G-T) was used. (ns, not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001).

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## Supplemental Figure 3. $T_{reg}$ -restricted deletion of *Tnfsf10* effects cell death of CD11c<sup>+</sup> and $T_{regs}$ in the diabetic islet

(A) Diabetes onset monitored in  $Tnfsf10^{L/L}.Foxp3^{Cre-GFP}.NOD$  males and co-caged controls. (B) Islets from 10 week old female  $Foxp3^{Cre-GFP}.NOD$  mice were stained for surface DR5 on indicated cell populations. (C) Insulin<sup>+</sup> cells isolated from  $Tnfsf10^{L/L}.NOD$  and  $Tnfsf10^{L/L}.Foxp3^{Cre-GFP}.NOD$  female mice were stained for percent expression of cleaved-caspase3 (c-casp3). (D) TCRβ-CD11c<sup>+</sup> cells were stained for percent expression of c-casp3. (E) Tabulated LAP-TGFβ and (F) IL-10 percent expression on  $T_{regs}$  (G) Gated MFI of CD39 on  $T_{regs}$  (H) Gated MFI of CD73 on  $T_{regs}$ . (I) %Ki67 on  $T_{regs}$  (J) MFI of PD-1 in PD-1<sup>+</sup>  $T_{regs}$  (K) %PD-1 intermediate in  $T_{regs}$  (L) %PD-1 high in  $T_{regs}$  (M) %LAG3 on  $T_{regs}$  (N) % live CD45.2<sup>+</sup> on gated single cells (O) %CD4<sup>+</sup> Foxp3<sup>+</sup>  $T_{regs}$  in live CD45.2<sup>+</sup> single cells at 10 weeks (P) %CD4<sup>+</sup> Foxp3<sup>+</sup>  $T_{regs}$  were gated for percent positive expression of c-casp3. (Q) %CD4<sup>+</sup> Foxp3<sup>+</sup>  $T_{regs}$  in live CD45.2<sup>+</sup> single cells at 12 weeks. (R) EAE scoring in  $Tnfsf10^{L/L}.Foxp3^{Cre-YFP}.B6$  mice and co-caged controls.

Data in (A) is representative of >3 experiments with 11-31 mice/group. (B) is representative of 1 experiment with 4 mice/group. (C-P) is representative of 2 experiments with 4-19 mice/group. Data in (Q) is representative of 1 experiment with 3-5 mice/group. Data in (R) is representative of 3 experiments with 9-14 mice/group. Statistics were determined using Log-rank (Mantel Cox) test (A) and Student unpaired t test (C-Q) and 2-way ANOVA (R). (ns, not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001).