Supplementary Informations

IDO-orchestrated crosstalk between pDCs and Tregs inhibits Autoimmunity

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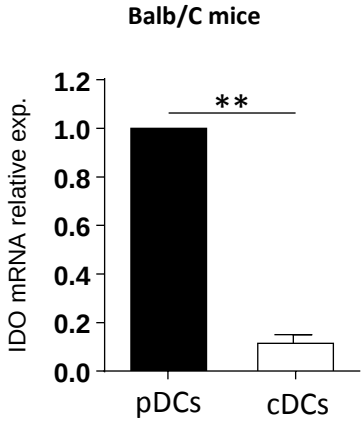
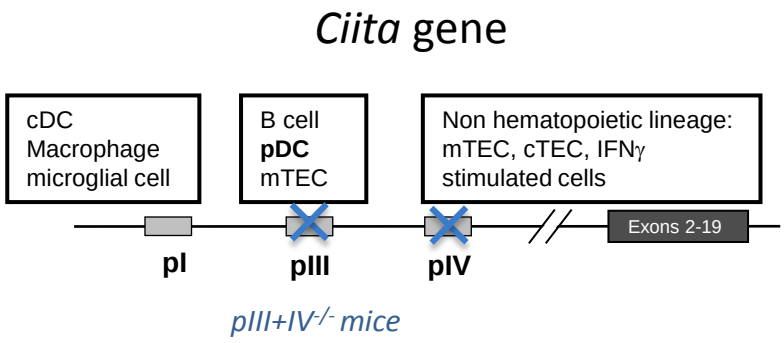
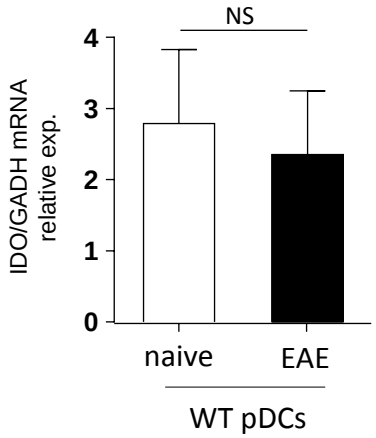


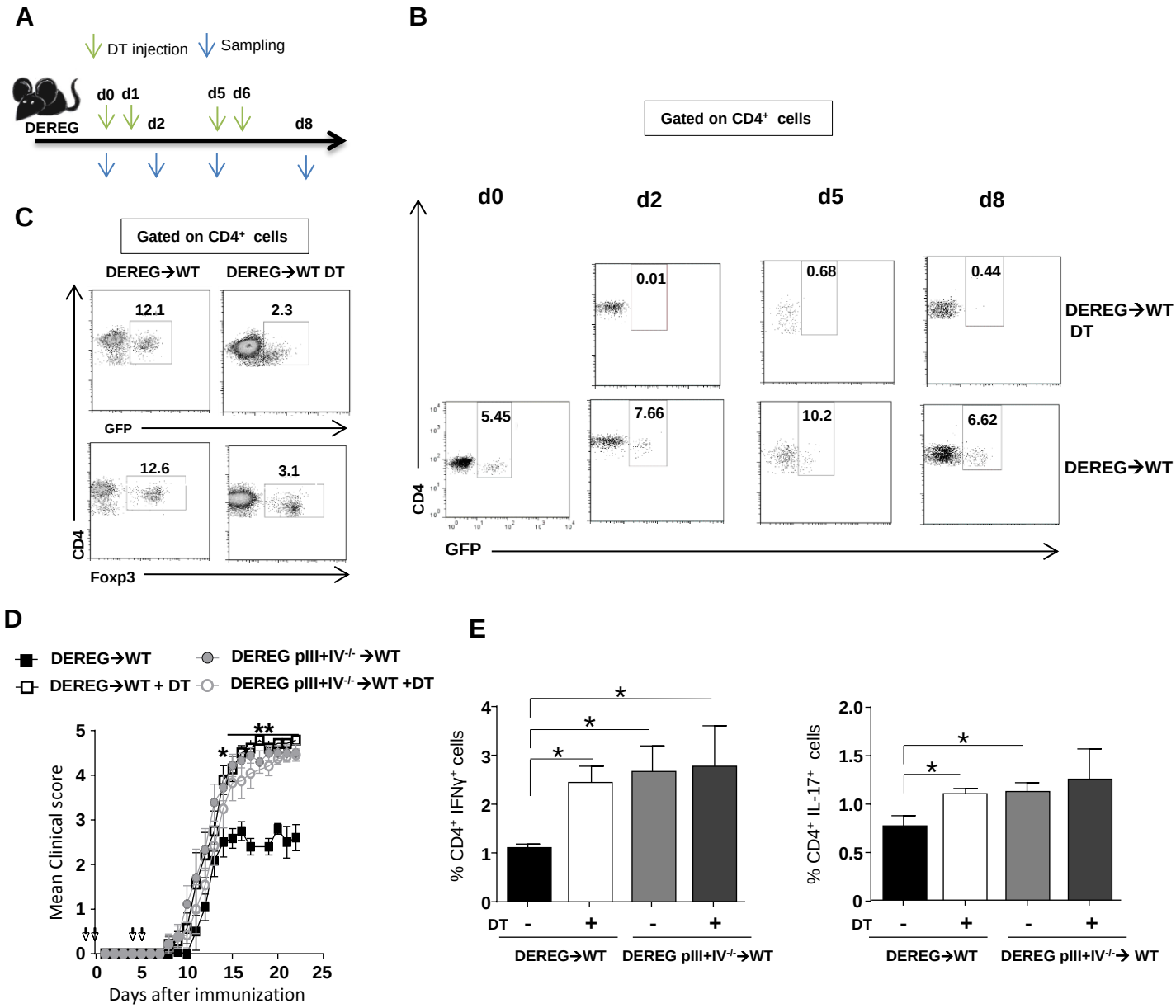
Supplementary Figure 1. IDO expression by pDCs sorted from LNs of Balb/c mice. (A) pDCs and cDCs were selectively sorted from total skin LNs of Balb/C mice by flow cytometry and mRNA extraction was performed. IDO mRNA expression in pDCs and cDCs was determined by qPCR. Error bars depict mean ± SEM. Two-tailed Mann-Whitney test was used. ** $P < 0.01$.



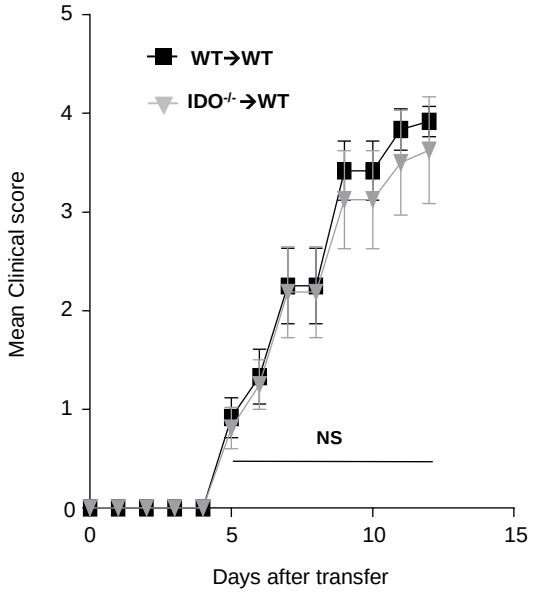
Supplementary Figure 2. Mice selectively lacking MHCII expression on pDCs. In mice, CIITA gene expression is driven by three different cell specific promoters pI, pIII and pIV. pIII+IV$^{-/-}$ mice carry the deletion of promoters pIII and pIV. mTECs: medullary thymic epithelial cell, cTECs: cortical TEC. (B-E) WT→WT and pIII+IV$^{-/-}$→WT chimeric mice were generated by transfer of either WT or pIII+IV$^{-/-}$ bone marrow precursor cells in lethally irradiated WT recipient mice.



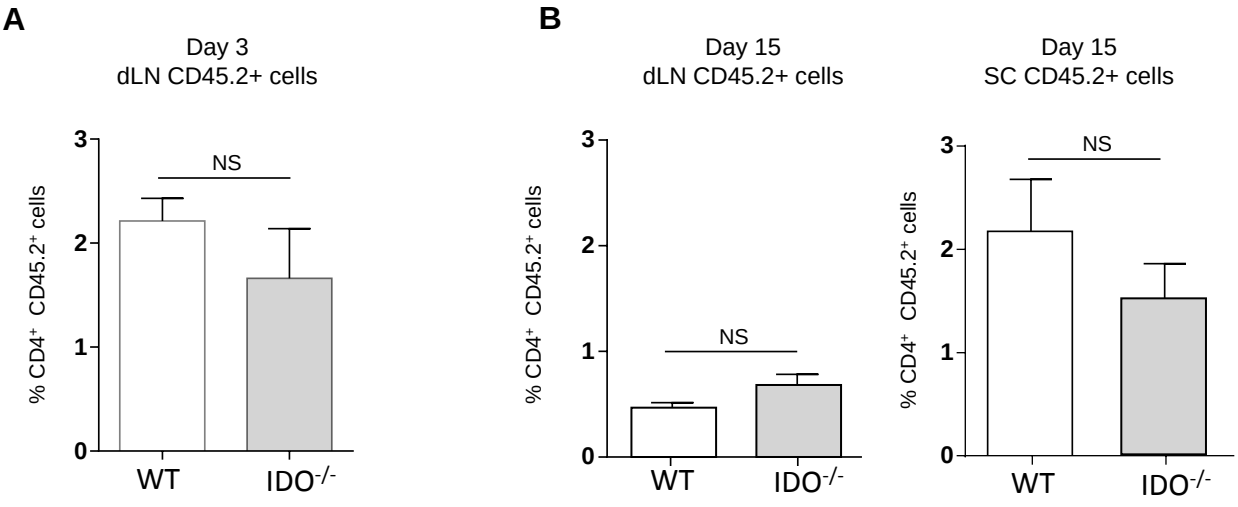
Supplementary Figure 3. IDO expression by pDCs sorted from LN of naïve and EAE mice. pDCs were selectively sorted by flow cytometry from total skin LNs of naive C57Bl/6 WT mice or from draining LNs of EAE C57Bl/6 WT mice at day 10 after immunization. IDO mRNA expression in pDCs was determined by qPCR and expressed relatively compare to GAPDH expression in the same cells. Error bars depict mean ± SEM. Two-tailed Mann-Whitney test was used. NS = Non significant.



Supplementary Figure 4. pDC-induced Tregs suppress encephalitogenic T cell priming in lymph nodes. DT was injected or not in DEREG→WT BM chimeric mice at d0, d1, d5 and d6. (A) Experimental design is shown. Treg depletion was assessed (B) in dLN at d8 based on $CD4^+$ $Foxp3^+$ staining and (C) in blood at d0, d2, d5 and d8 by following $CD4^+$ GFP^+ cells. Flow cytometry data are shown. (D and E) EAE was induced in DEREG→WT chimeric mice treated (□) or not (■) with DT (arrows) and DEREG x pIII+$IV^{-/-}$ →WT chimeric mice treated (○) or not (●) with DT as before (D) Clinical scores were followed daily (two-way ANOVA with Bonferroni post Hoc test). (E) Frequency of $IFN\gamma^+$ and $IL\text{-}17^+$ $CD4^+$ T cells in dLN at d9 (one-way ANOVA with Bonferroni post Hoc test). (A-E) Data are representative of at least 2 independent experiments with 6-8 mice per group. Error bars depict mean ± SEM. * $P < 0.05$, ** $P< 0.01$, *** $P< 0.001$.



Supplementary Figure 5. IDO deficiency does not affect EAE effector phase. Passive EAE was induced by transferring MOG_{35-55} primed 2D2 effector T cells in WT→WT (■) and $IDO^{-/-}$→WT (▼) BM chimeras. Clinical scores were followed daily. Error bars represent mean ± SEM (2-way ANOVA with Bonferroni's post-Hoc test). NS = Non significant.



Supplementary Figure 6. Tregs primed in WT and IDO deficient mice migrate in LNs and SC with similar efficiency upon adoptive transfer in EAE mice. $CD4^+CD25^{hi}$ ($CD45.2^+$) cells were purified from dLNs of WT:WT and $IDO^{-/-}$:WT BM chimeras 10 days after EAE induction and transferred into WT $CD45.1^+$ recipients immunized for EAE the day after. Donor T cell frequencies among $CD4^+$ T cells were analysed after gating on $CD45.2^+$ cells in (**A**) dLNs (day 3) and (**B**) in dLNs and SC (day 15) of recipient mice. Results are representative of 2 independent experiments with 3 mice per group. Error bars depict mean ± SEM. Two-tailed Mann-Whitney test was used. NS = Non significant.