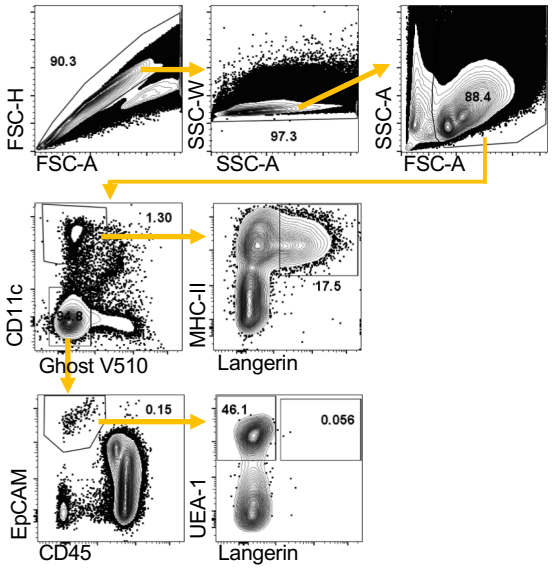

Figure S1. Thymic epithelial cells in neonatal NOD mouse do not express Langerin. 2-day old NOD wild-type neonatal thymus was harvested and digested with the enzyme Liberase TH. Thymic cells were assessed for Langerin expression in DCs (CD11c+MHCII+) and medullary thymic epithelial cells (CD11c-CD45-EpCAM+UEA1+). Ghost V510 = Live/Dead stain.

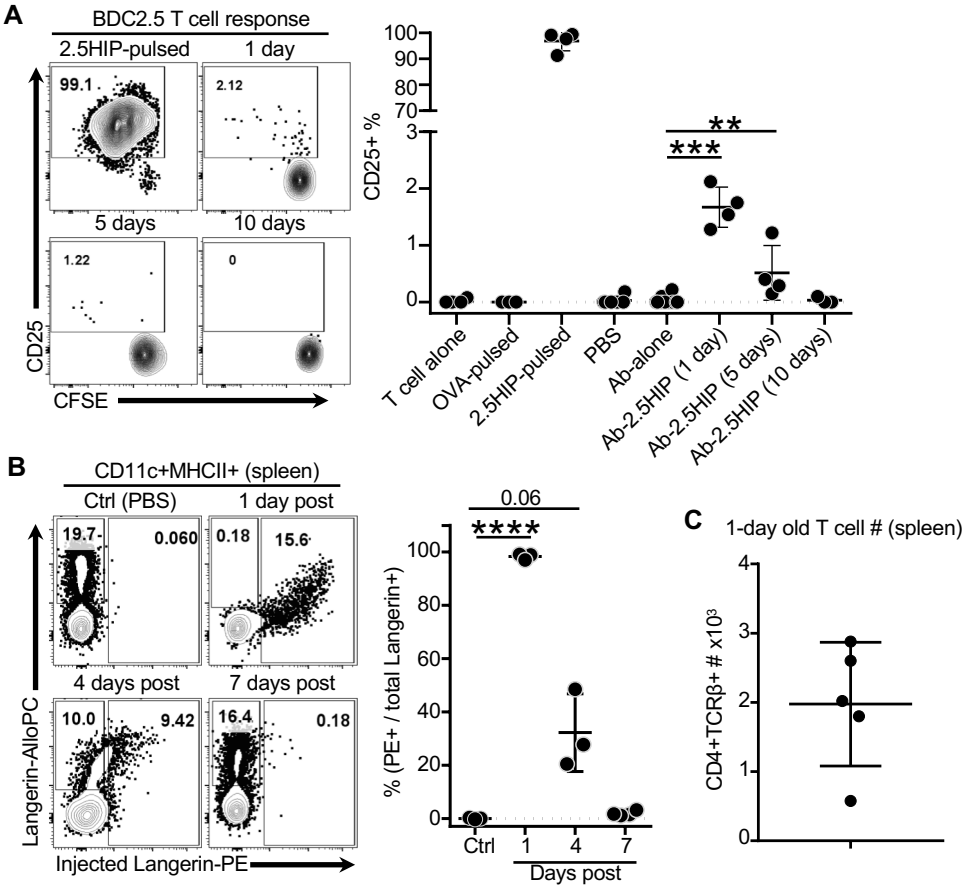

Figure S2. Duration of peptide presentation by neonatal thymic Langerin+ DCs after Ab-2.5HIP treatment. (A) 1-2 day old NOD wild-type neonates were treated with 2.5µg of Ab-2.5HIP, Ab-alone, or PBS. At 1-, 5-, or 10-days after treatment, thymic CD11c+ cells were isolated and co-cultured with BDC2.5 T cells for 48 hours. T cell activation (CD25 expression) was measured as an indicator of peptide presentation. (data representative of 2 experiments; n=3-7 per group, each dot represents an animal; in the OVA-pulsed and 2.5HIP-pulsed group, BDC2.5 T cells were co-cultured with thymic CD11c+ cells from untreated mice but pulsed with OVA 323-339 or 2.5HIP in culture). (B) Assessing in vivo targeting of peripheral Langerin+ DCs by anti-Langerin antibody. 2-day old NOD neonates were given 2.5µg of anti-Langerin antibody conjugated to the fluorophore PE. Spleens were harvested at 1-, 4-, and 7-days post-treatment and co-stained with Langerin antibody conjugated to allophycocyanin (Langerin-AlloPC). Frequency of Langerin+ DCs targeted by the injected antibody is calculated as PE+/total Langerin+ (data pooled from 2 independent experiments; gated on MHCII+CD11c+; Ctrl = untreated; n=3-4 per group). (C) total CD4 T cell number in the spleen of 1-day old NOD mice. Mean ± SD. Mann-Whitney U Test, **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$

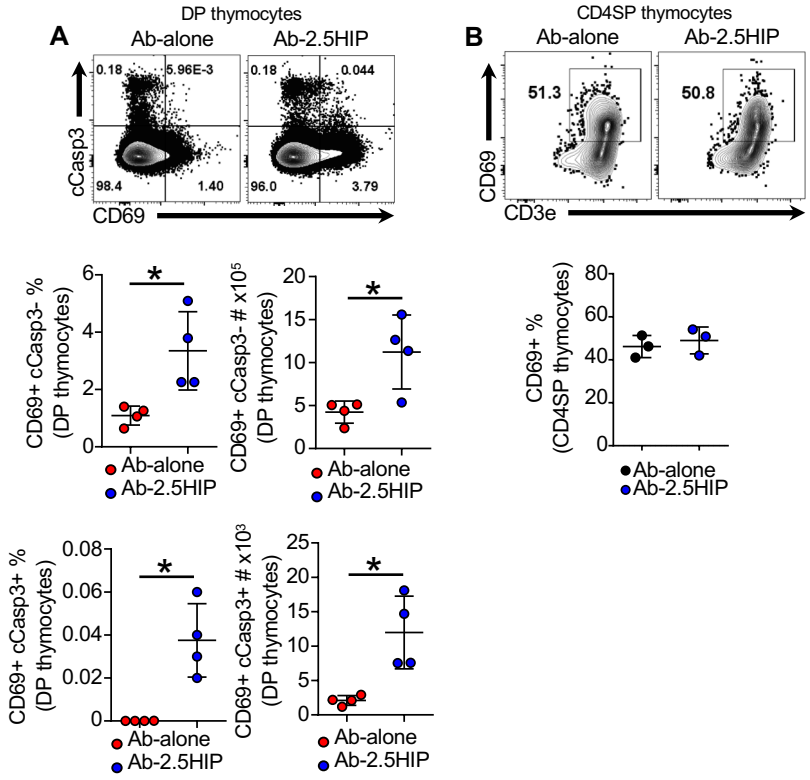

Figure S3. Assessing CD69 expression in BDC2.5 DP and CD4SP thymocytes post Ab-2.5HIP treatment. 2-day old BDC2.5 mice were treated with 2.5µg of Ab-2.5HIP, or Ab-alone. 72 hours post-treatment, thymocytes were assessed for expression of CD69. (A) CD69 and cleaved Caspase3 (cCasp3) expression in CD4+CD8+ (DP) thymocytes (n=4 per group). (B) CD69 expression in CD4+CD8- (CD4SP) thymocytes (n=3 per group). Mean ± SD, Mann-Whitney U Test, * $p<0.05$

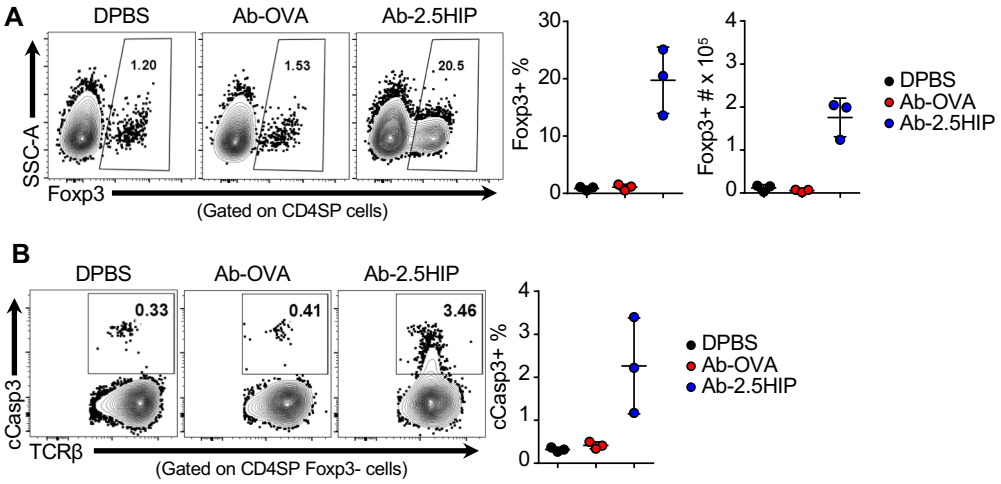

Figure S4. Anti-Langerin-OVA does not affect T cell central tolerance in BDC2.5 thymocytes. 2-day old BDC2.5 mice were treated with 2.5µg of Ab-2.5HIP, Ab-OVA, or DPBS. 72 hours post-treatment, thymocytes were assessed for expression tTreg development and thymocyte deletion. (A) Representative flow plot and quantification of Foxp3+ cell percentage and number within CD4SP TCRβ+. (B) Representative flow plot of apoptotic (cleaved Caspase3+ = cCasp3+) cell percentage within CD4SP TCRβ+ Foxp3- thymocytes. n=3 per group. Mean ± SD, Mann-Whitney U Test.

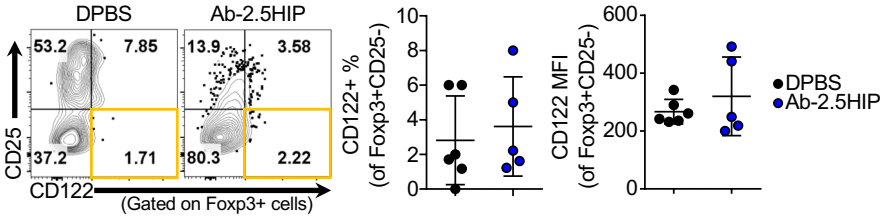

Figure S5. No upregulation in the expression of IL-2 Receptor β (CD122) in Ab-2.5HIP-induced Foxp3+CD25- tTregs. 1-2-day old BDC2.5 mice were treated with 2.5μg of Ab-2.5HIP, or DPBS. 72 hours post-treatment, Foxp3+CD25- CD4SP thymocytes were assessed for expression of CD122 (IL-2 Receptor β chain). n=5-6 mice per group. Mean ± SD, Mann-Whitney U Test.

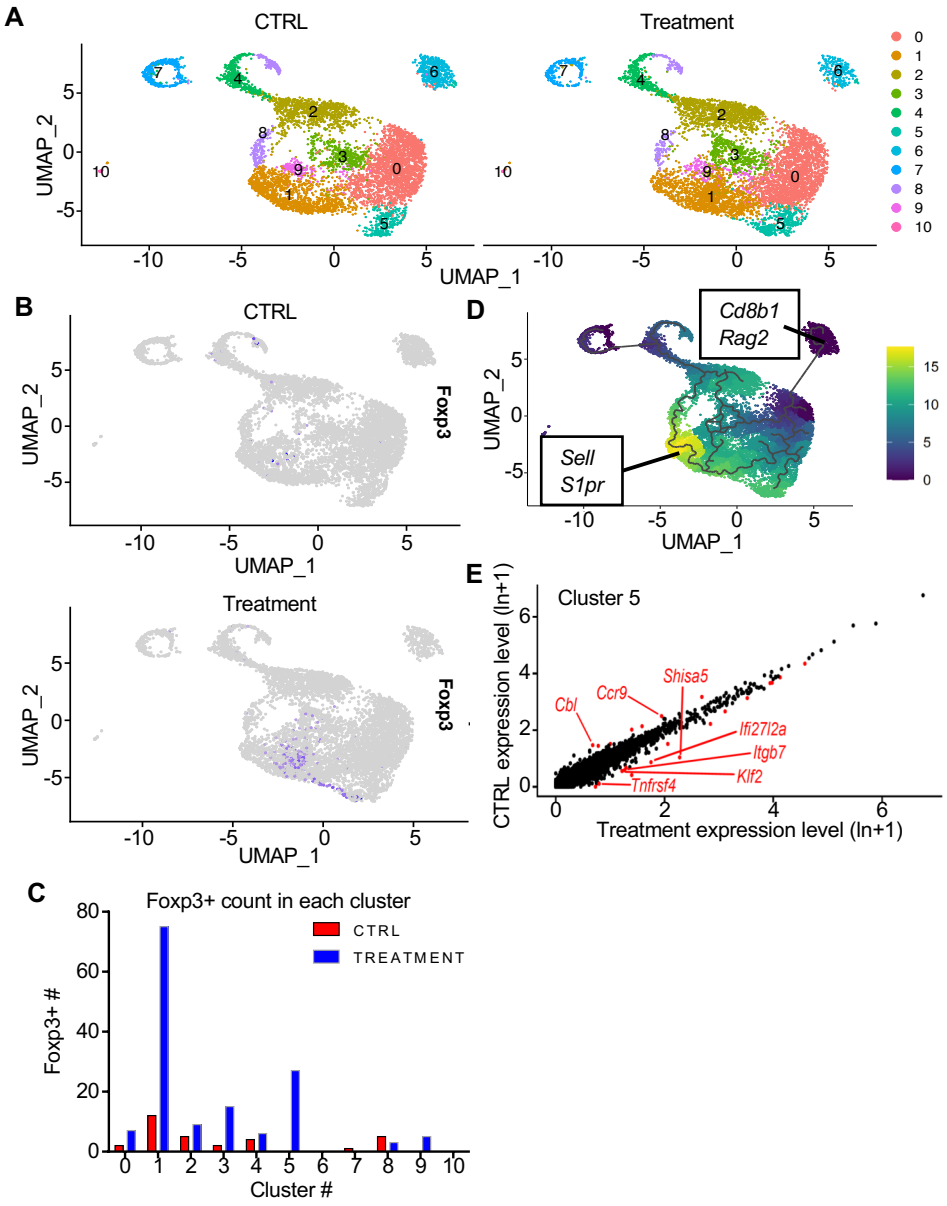

Figure S6. scRNAseq identifies Ab-2.5HIP-induced tTregs and possible survival mechanism. (A) UMAP plot of sorted thymocytes (CD4SP and CD4+CD8-intermediate population) from BDC2.5 thymus treated with either Ab-alone (CTRL) or Ab-2.5HIP (Treatment) identifies 11 clusters. (B) Distribution of Foxp3+ cells across all clusters. (C) Foxp3+ cell count in each cluster. (D) Pseudo-time analysis indicating developmental relationship amongst the clusters. Blue = most immature cells. Yellow = most mature cells. (E) Differential gene expression analysis between CTRL and Treatment groups within cell cluster 5.

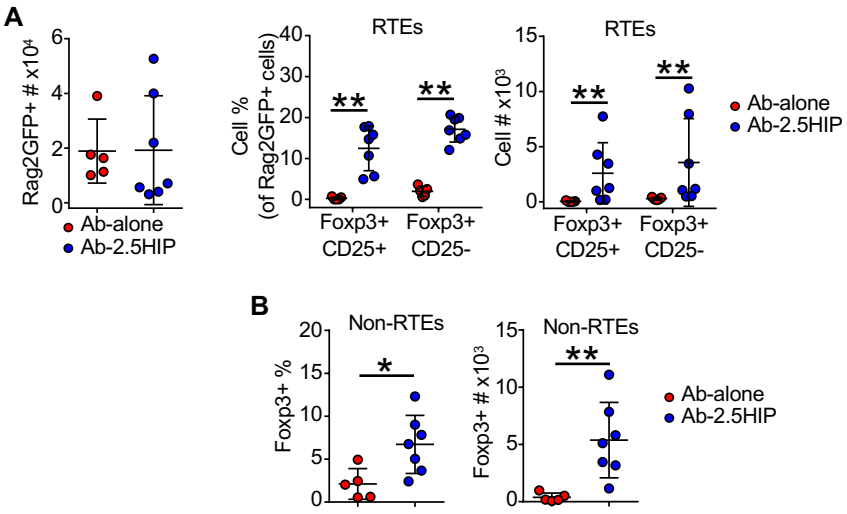

Figure S7. Analysis of Recent Thymic Immigrants (RTEs) of neonatal BDC2.5 spleen 6-days after Ab-2.5HIP treatment. (A) Quantification of total RTE numbers in the spleen 6-days after Ab-2.5HIP treatment (left). Analysis of Treg population within the RTE population revealed an increase in both the CD25+ and CD25- subsets (right). (B) Analysis of Treg frequency and number in the Non-RTE population. n=5-7 per group. Mean ± SD, Mann-Whitney U Test, ** $p<0.01$, * $p<0.05$

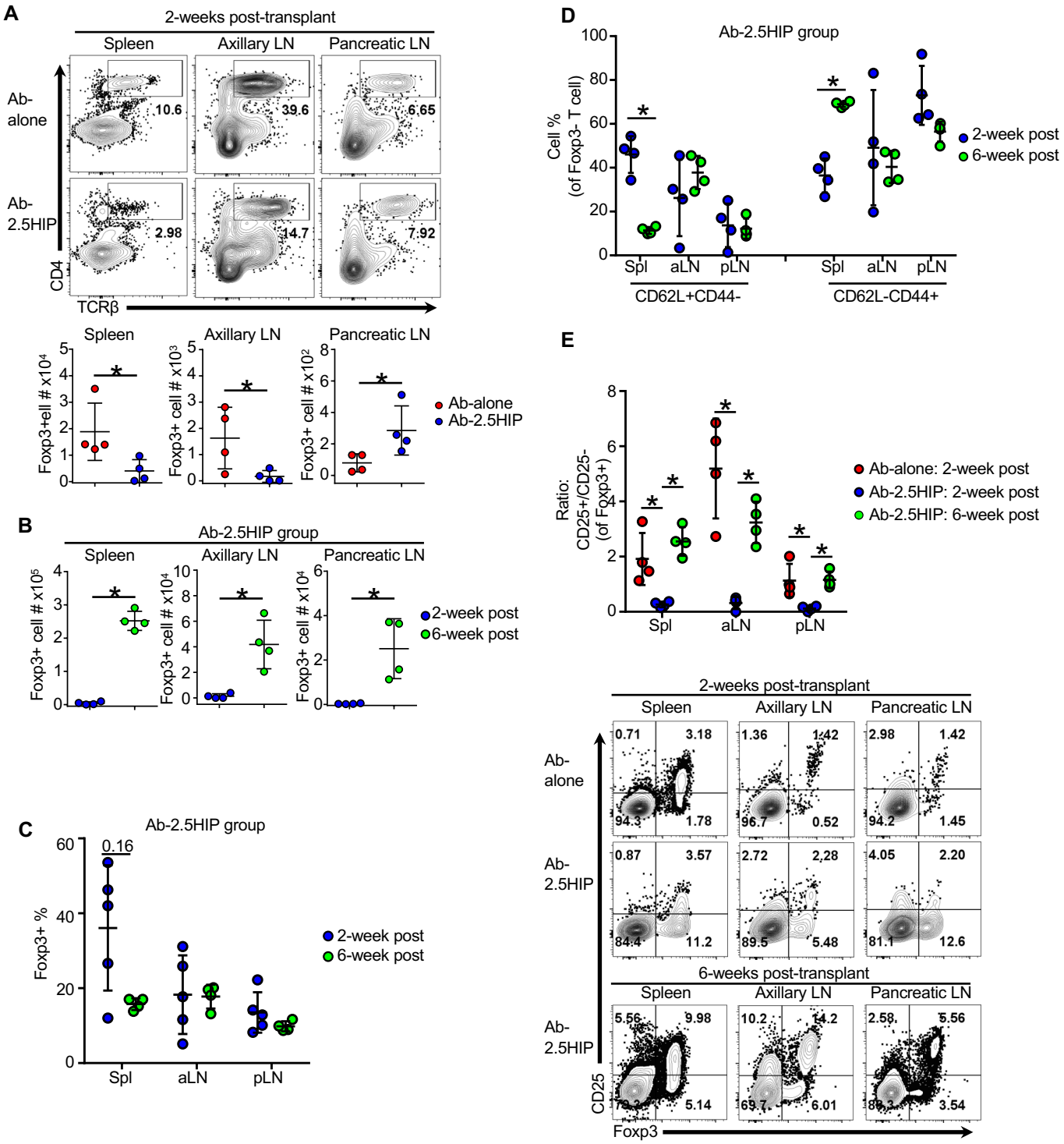

Figure S8 Characterization of peripheral donor BDC2.5 T cell phenotype at 2- and 6-weeks after thymus transplant. (A) top: flow plot of donor T cell frequency in lymphoid organs. bottom: quantification of Foxp3+ cell number in the spleen, axillary LN, and pancreatic LN (n= 4 per group) 2-weeks post-transplant. (B) Comparison between Foxp3+ cell number at 2- and 6-weeks post-transplant in the spleen, axillary LN, and pancreatic LN of recipients of the Ab-2.5HIP-treated thymi. (C) Comparison between Foxp3+ cell frequency at 2- and 6-weeks post-transplant in the spleen (Spl), axillary LN (aLN), and pancreatic LN (pLN) of recipients of the Ab-2.5HIP-treated thymi. (D) Comparison of naïve T cell (CD62L+CD44-) and effector T cell (CD62L-CD44+) frequencies between 2- and 6-weeks post-transplant in the Spl, aLN, and pLN of recipients of the Ab-2.5HIP-treated thymi. (E) Quantification of the ratio of CD25+/CD25- within the Foxp3+ population at 2 and 6-weeks. n=4-5 per group. Mann-Whitney U Test, * $p<0.05$.