Mucosal CD8 memory T Cells are selected in the periphery by an MHC Class I molecule

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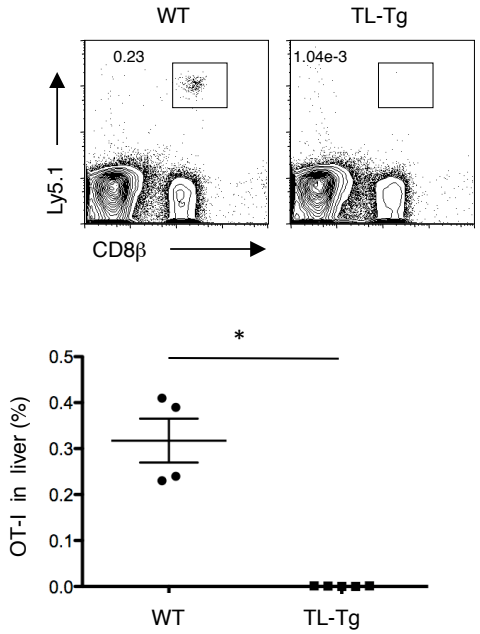
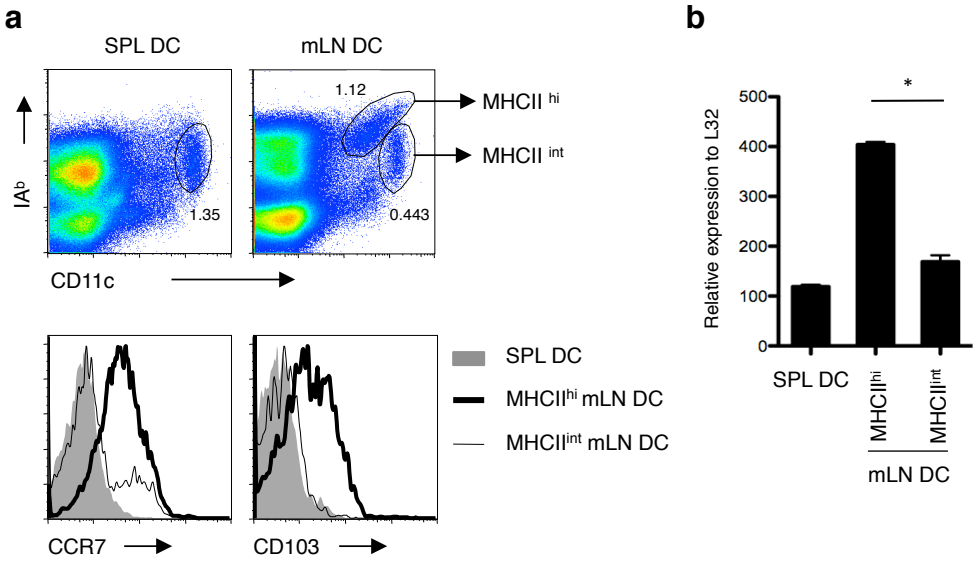
Huang et al, Supplementary Figure 1



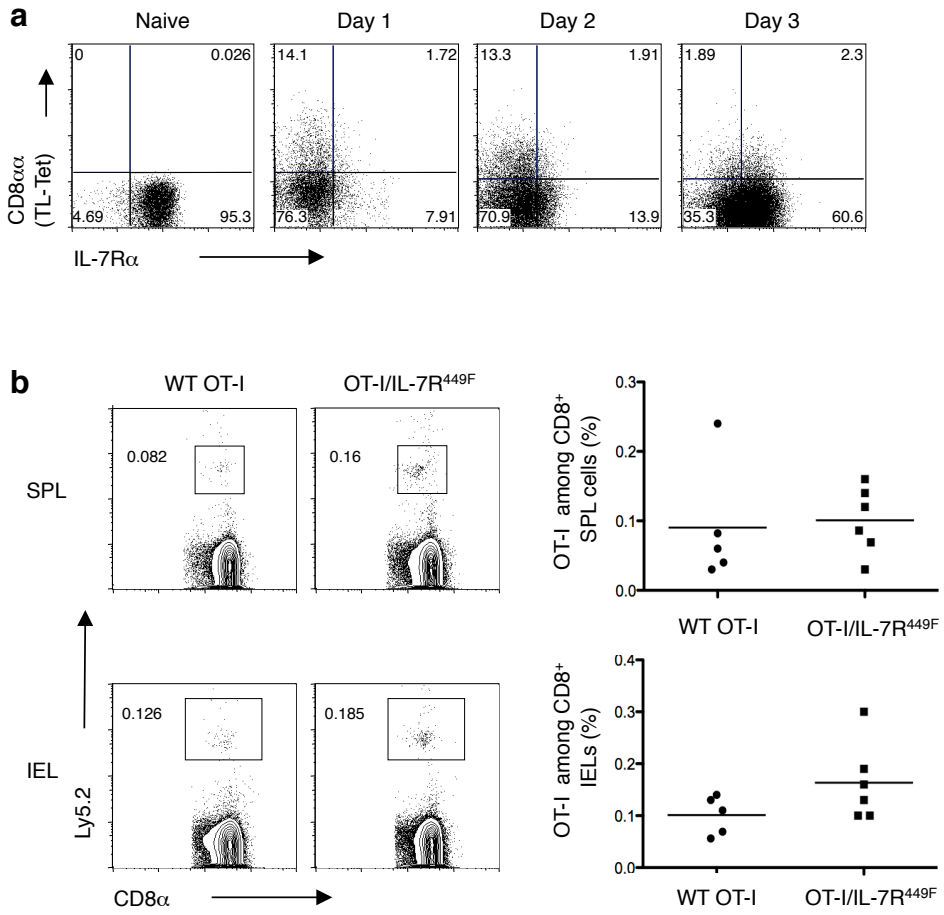
Supplementary Figure 1. TL deletes activated CD8αβ$^+$ T cells. 1 × 10^6 naïve Ly5.1$^+$ OT-I cells were transferred into WT or TL-Tg recipients. One day after transfer, mice were orally infected with 1 × 10^9 ActA$^-$ Lm-OVA. Memory CD8$^+$ OT-I cells in the livers were analyzed 2 month p.i.. Graph depicts pooled data ± s.e.m.. * $P < 0.001$ (unpaired *t*-test). Data are representative of two independent experiments

Huang et al, Supplementary Figure 2



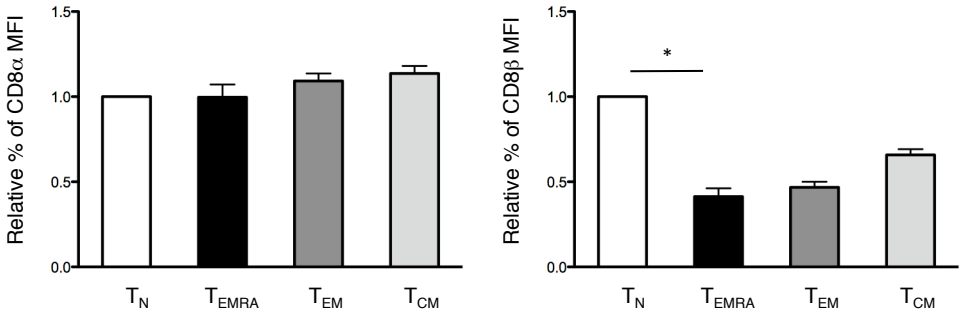
Supplementary Figure 2. MHC class II^{hi}, CD11c^{+} MLN DC have the phenotype of CCR7^{+} CD103^{+} migratory DC. (**a**) mLN contain a distinct MHC class II^{hi} DC subset that is absent in the spleen (dot plots). Surface expression of CCR7 and CD103 were analyzed on CD11c $^{+}$MHC II^{+} DCs from SPL and CD11c^{+}MHC II^{hi} or CD11c^{+}MHC II^{int} DCs from mLN (histograms). Data are representative from at least three independent experiments. (**b**) Spleen or mLN DCs were sorted based on the CD11c and MHC II expression and used for mRNA quantification for TL expression by qRT-PCR. * $P < 0.05$ (unpaired *t*-test). Data are representative from three independent experiments.

Huang et al, Supplementary Figure 3



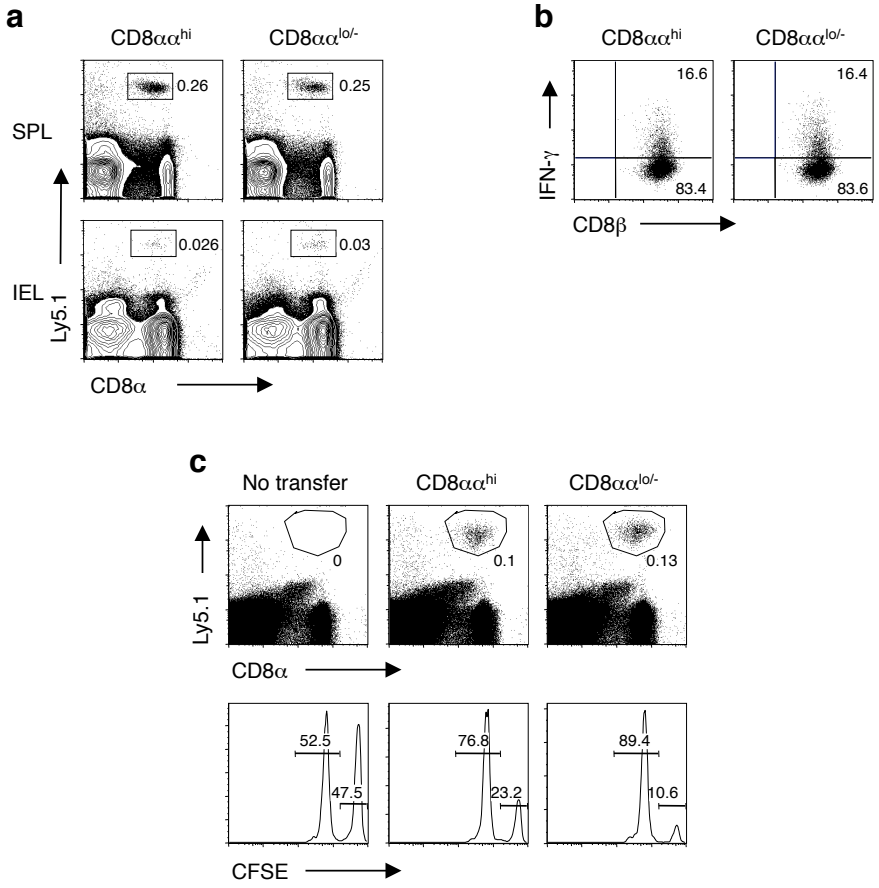
Supplementary Figure 3. Affinity-based selective programming of memory precursor cells does not require IL-7R signals. (**a**) Naïve $CD8^+$ OT-I cells were cultured in the presence of APC (MEC.B7.SigOVA). CD8αα and IL-7Rα expression on OT-I cells was determined by flow cytometry at different time points. (**b**) 1×10^4 naïve $CD8^+$ WT OT-I cells or $IL\text{-}7Ra^{449F}$ OT-I cells were adoptively transferred into $Ly5.1^+$ WT recipient mice. 1 d after transfer, mice were orally infected with 1×10^9 $ActA^-$ Lm-OVA. Donor OT-I cells were tracked in the spleens and IEL 2 months p.i.. Data are representative of two independent experiments.

Huang et al, Supplementary Figure 4



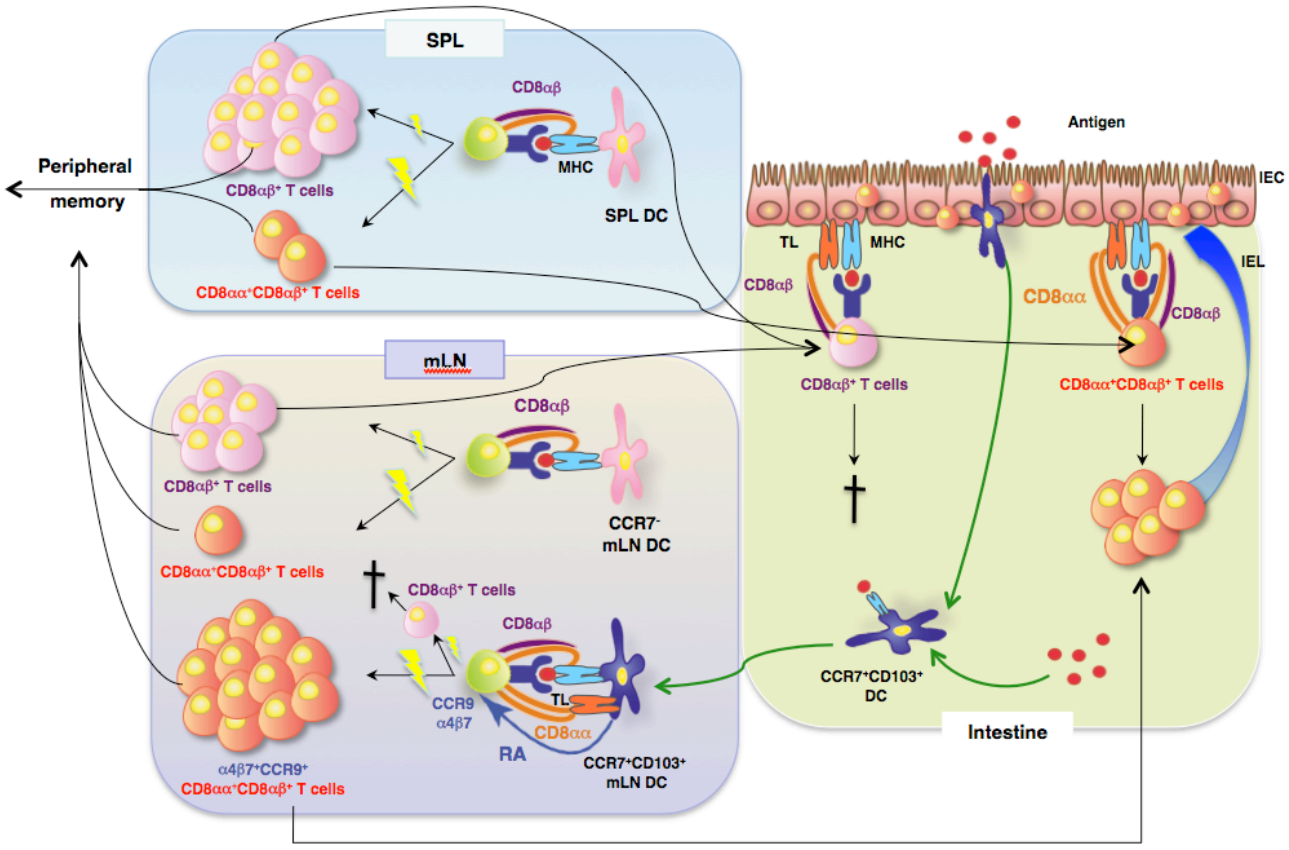
Supplementary Figure 4. Expression of CD8α and CD8β on human $CD8^+$ T cell subsets. The bar graphs show the relative percentage of CD8α and CD8β mean fluorescence intensity (MFI) in human $CD8^+$ T cell subsets ($n = 9$). * $P < 0.01$ (unpaired *t*-test).

Huang et al, Supplementary Figure 5



Supplementary Figure 5. Characterization of *in vitro* activated CD8αα^{hi} and CD8αα$^{lo/-}$ CD8αβ^{+} T cells. (**a**) Naïve Ly5.1^{+} CD8^{+} OT-I cells were cultured in the presence of APC (MEC.B7.SigOVA). After two days' culture, CD8αα^{hi} and CD8αα$^{lo/-}$ OT-I cells were sorted and cultured for 3 more days *in vitro*. Then 0.5 × 10^{6} CD8αα^{hi} or CD8αα$^{lo/-}$ were adoptively transferred into B6 recipients. 3 d after transfer, effector OT-I cell were tracked in the spleen and IEL. (**b**) Sorted CD8αα^{hi} and CD8αα$^{lo/-}$ OT-I cells were directly stimulated with $OVA_{257-264}$ peptide for 5 h and intracellular staining for IFN-γ was performed. (**c**) 3 d after transfer of CD8αα^{hi} or CD8αα$^{lo/-}$, *in vivo* cytotoxicity assay was performed in recipient mice. B6 splenocytes labeled as CFSE^{hi} and pulsed with $OVA_{257-264}$ peptide were used as target cells and splenocytes labeled as CFSE^{low} and pulsed with the control peptide were used as control target cells. Data are representative from three independent experiments.

Huang et al, Supplementary Figure 6



Supplementary Figure 6. Proposed model for the roles of TL and CD8αα on selection of memory CD8αβ⁺ T cells. CD8αα expression is selectively induced on high affinity/avidity primary effector CD8αβ⁺ T cells and further enhanced by RA released by mucosal migratory (CCR7⁺) DC, which also promote gut-tropism (α4β7⁺CCR9⁺) of the effector T cells. Activated migratory DC express the CD8αα high affinity ligand, TL, which when interacting with CD8αβ on activated T cells leads to TICD. High affinity primary effector cells that induce CD8αα, escape TICD by sequestering TL away from CD8αβ via CD8αα leading to affinity-based selective survival. TL constitutively expressed on the intestinal epithelial cells (IEC) mediates affinity maturation of the mucosal T_{EM} and eliminates low affinity/avidity primary and secondary effector cells that home to the gut and fail to induce CD8αα.