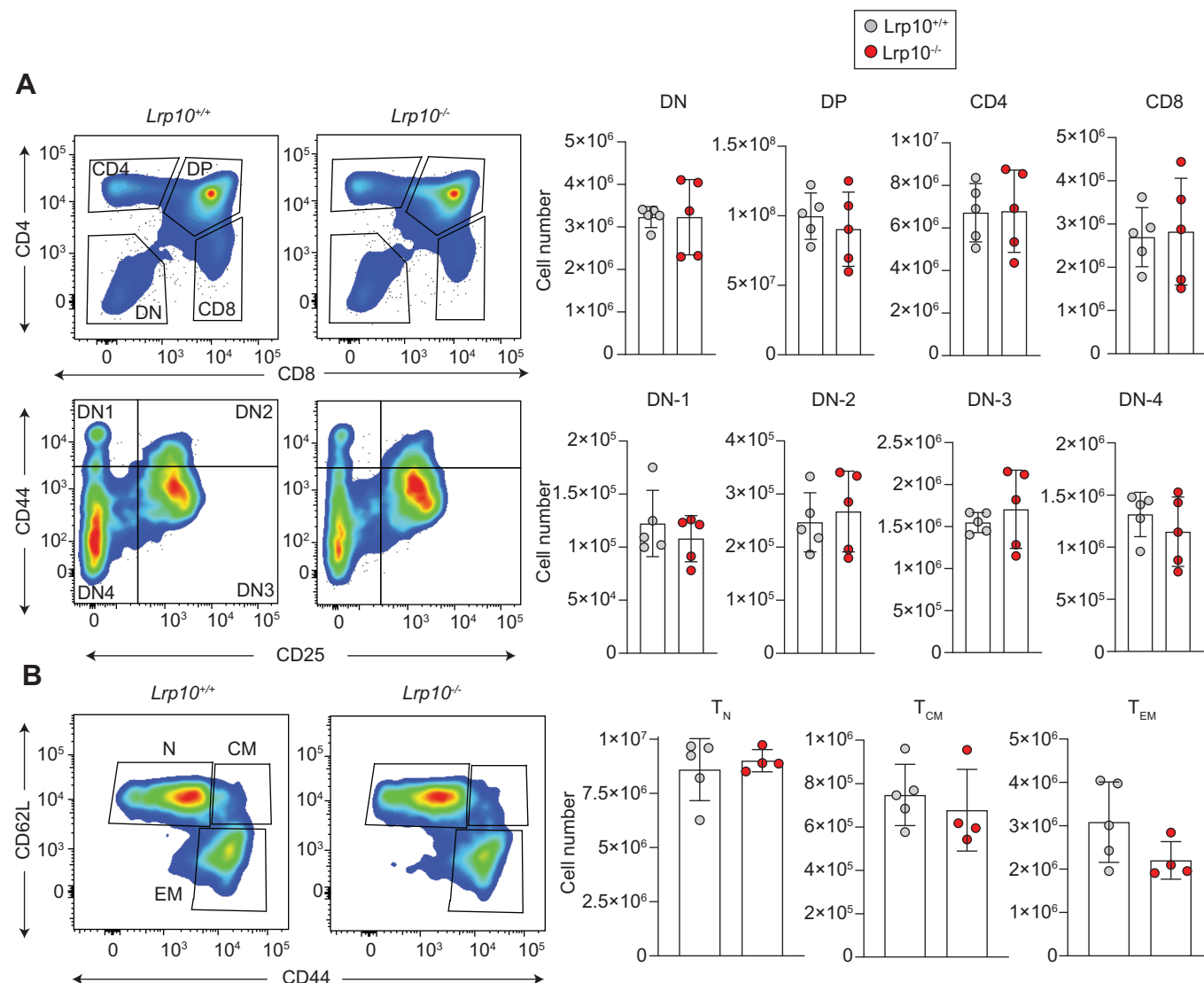


Expanded View Figures



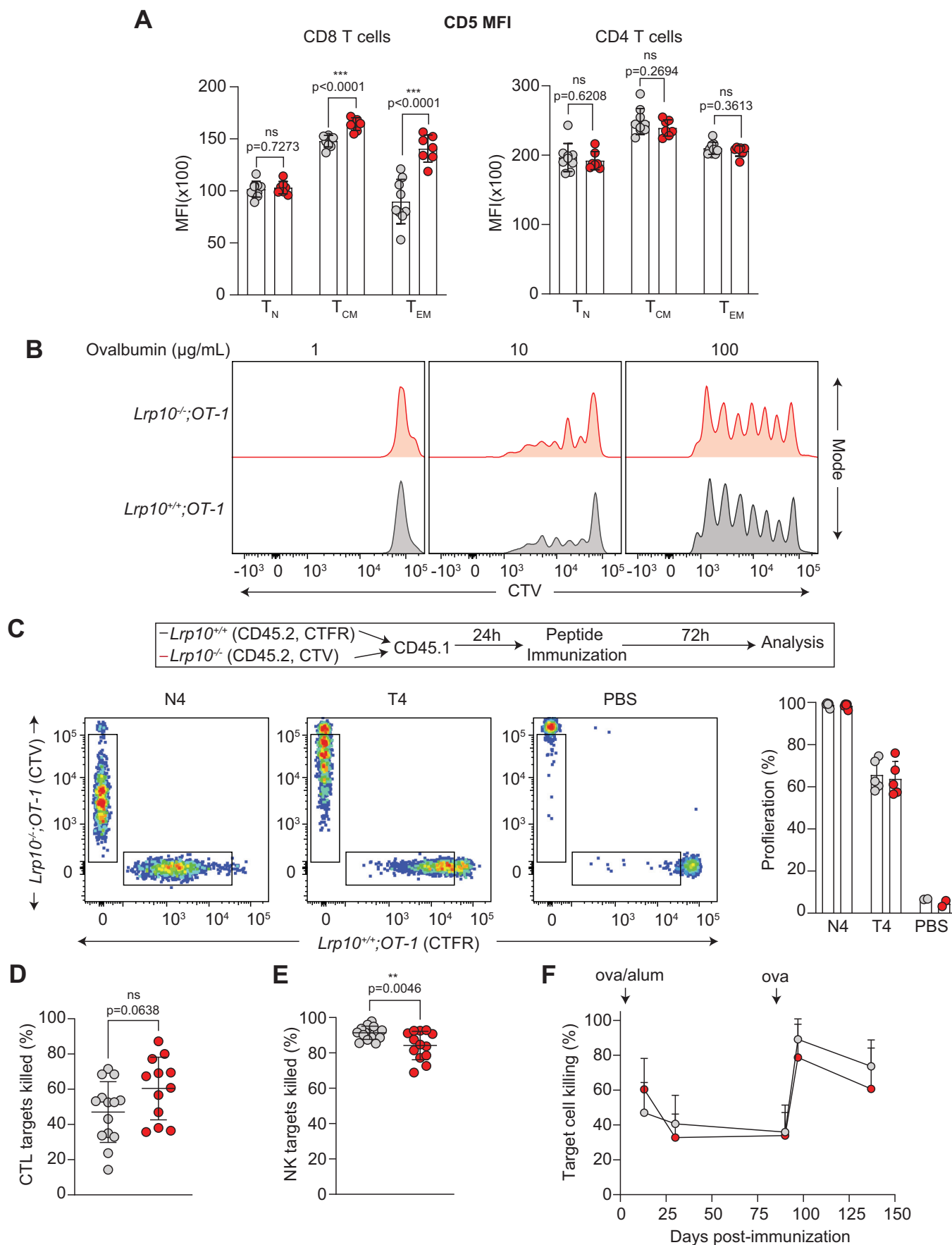


Figure EV2. TCR sensitivity and cytotoxic activity in *Lrp10*^{-/-} mice.

(A) Cell surface CD5 expression in splenic CD8 and CD4 T-cell subsets. (B) Proliferation of *Lrp10*^{+/+};OT-1 or *Lrp10*^{-/-};OT-1 cells 72 h after co-culture with *Lrp10*^{+/-} dendritic cells pulsed with the indicated concentrations of ovalbumin. (C) 10⁶ *Lrp10*^{+/-};OT-1 and *Lrp10*^{-/-};OT-1 cells were labeled with CTFR and CTV, respectively, and injected into unirradiated recipients. 24 h later mice were immunized with SIINFEKL (N4), SIITFEKL (T4), or PBS. Cell proliferation was measured 72 h after immunization based on dye dilution. (D) In vivo CTL cytotoxicity in *Lrp10*^{+/-} and *Lrp10*^{-/-} mice 12 days after immunization with ovalbumin and aluminum adjuvant (ova/alum). *N* = 14 *Lrp10*^{+/-} and *n* = 12 *Lrp10*^{-/-} mice. (E) In vivo NK cytotoxicity assay in *Lrp10*^{+/-} and *Lrp10*^{-/-} mice injected with labeled MHC-I-deficient target cells. *N* = 15 *Lrp10*^{+/-} and *n* = 13 *Lrp10*^{-/-} mice. (F) Serial in vivo cytotoxicity assays in the mice from (E) after immunization with ova/alum. Mice were given a boost of ova protein alone on day 90. *N* = 14 *Lrp10*^{+/-} and *n* = 12 *Lrp10*^{-/-} mice at each timepoint. Data information: In bar graphs, symbols represent individual mice (biological replicates), horizontal bars indicate mean values, and error bars show SD. In (F), symbols indicate the mean values and error bars show SD. Data from (B) is representative of two separate in vitro stimulation experiments. Data from (D, E) were replicated twice on separate cohorts of at least 10 mice. Data from (C, F) are from one immunization experiment each with the indicated *n*. *P* values were calculated by two-tailed unpaired *t* tests (A, D, E). No statistical testing was performed in (C, F). Significant *P* values were flagged as follows: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *P* values > 0.05 were considered to be not significant (ns).

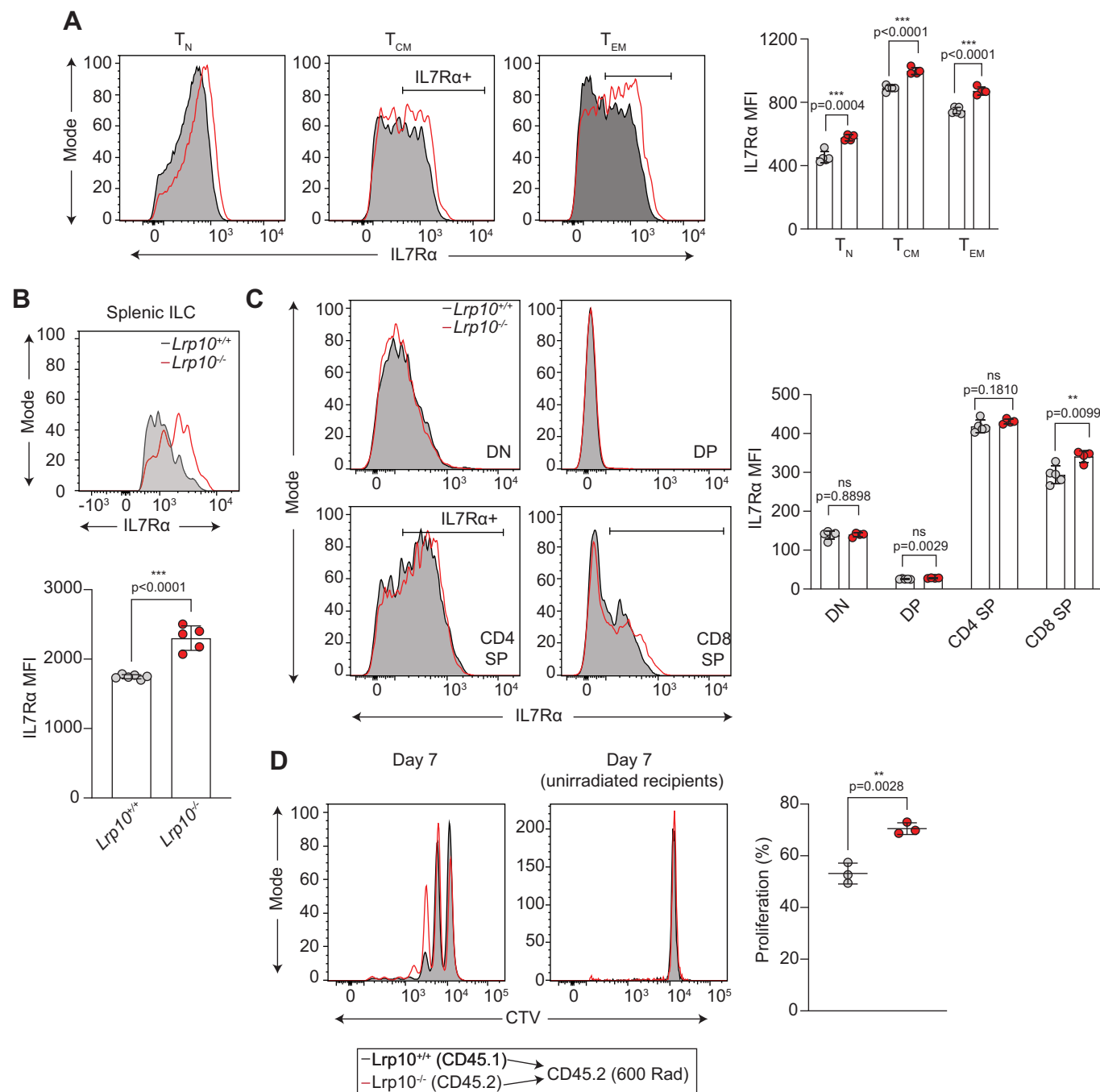


Figure EV3. IL7R expression on CD4 T cell, ILC, and thymocyte subpopulations.

(A) Cell surface IL7R expression in CD4 subpopulations. (B) Cell surface IL7R expression on Lin-CD11b-IL7R + NK1.1 + NKp46+ splenic ILCs. Lin: CD3, B220, CD11c. (C) Cell surface IL7R expression on thymic T-cell subpopulations. (D) Representative FACS plots of homeostatic expansion of $Lrp10^{+/+}$ and $Lrp10^{-/-}$ CD4 T cells labeled with CTV and injected in sub-lethally irradiated and unirradiated recipients. The bar graph shows proliferation of CD4 T cells transplanted into irradiated recipients. Proliferation was determined based on the fraction of cells that underwent at least one cell division. Data information: In bar graphs, symbols represent individual mice (biological replicates), horizontal bars indicate the mean, and error bars show SD. Data from (D) are from one CD4 adoptive transfer experiment with three irradiated recipients and one unirradiated recipient. P values were calculated by two-tailed unpaired t tests. Significant p values were flagged as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. P values > 0.05 were considered to be not significant (ns).

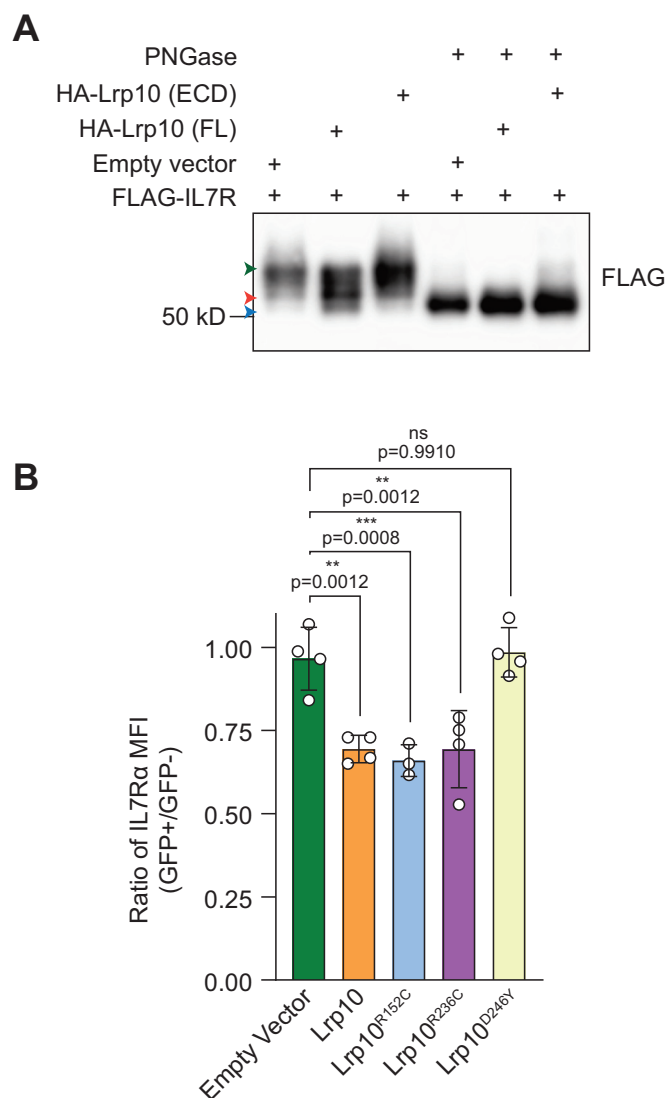


Figure EV4. Effect of Lrp10 variants on IL7R glycosylation and cell surface expression.

(A) IP of IL7Rα-FLAG from transfected HEK 293 T cells under denaturing conditions in the context of empty vector, Lrp10-HA (FL), or Lrp10-HA (ECD) followed by de-glycosylation with PNGase-F. (B) Normalized cell surface IL7R expression on activated *Lrp10*^{-/-} CD8 T cells infected with MSCV retroviruses encoding GFP-only, Lrp10^{WT}-IRES-GFP, Lrp10^{R132C}-IRES-GFP, Lrp10^{R235C}-IRES-GFP, or Lrp10^{D246Y}-IRES-GFP (*chowmein* allele). IL7R levels on GFP⁺ cells in each sample were normalized to levels on the GFP⁻ population. Data information: In (B), the symbols show the results of three or four separate retroviral transductions (biological replicates), the horizontal bars indicate mean values, and error bars show SD. *P* values were calculated by one-way ANOVA with Dunnett's multiple comparisons test. Significant *P* values were flagged as follows: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *P* values > 0.05 were considered to be not significant (ns).

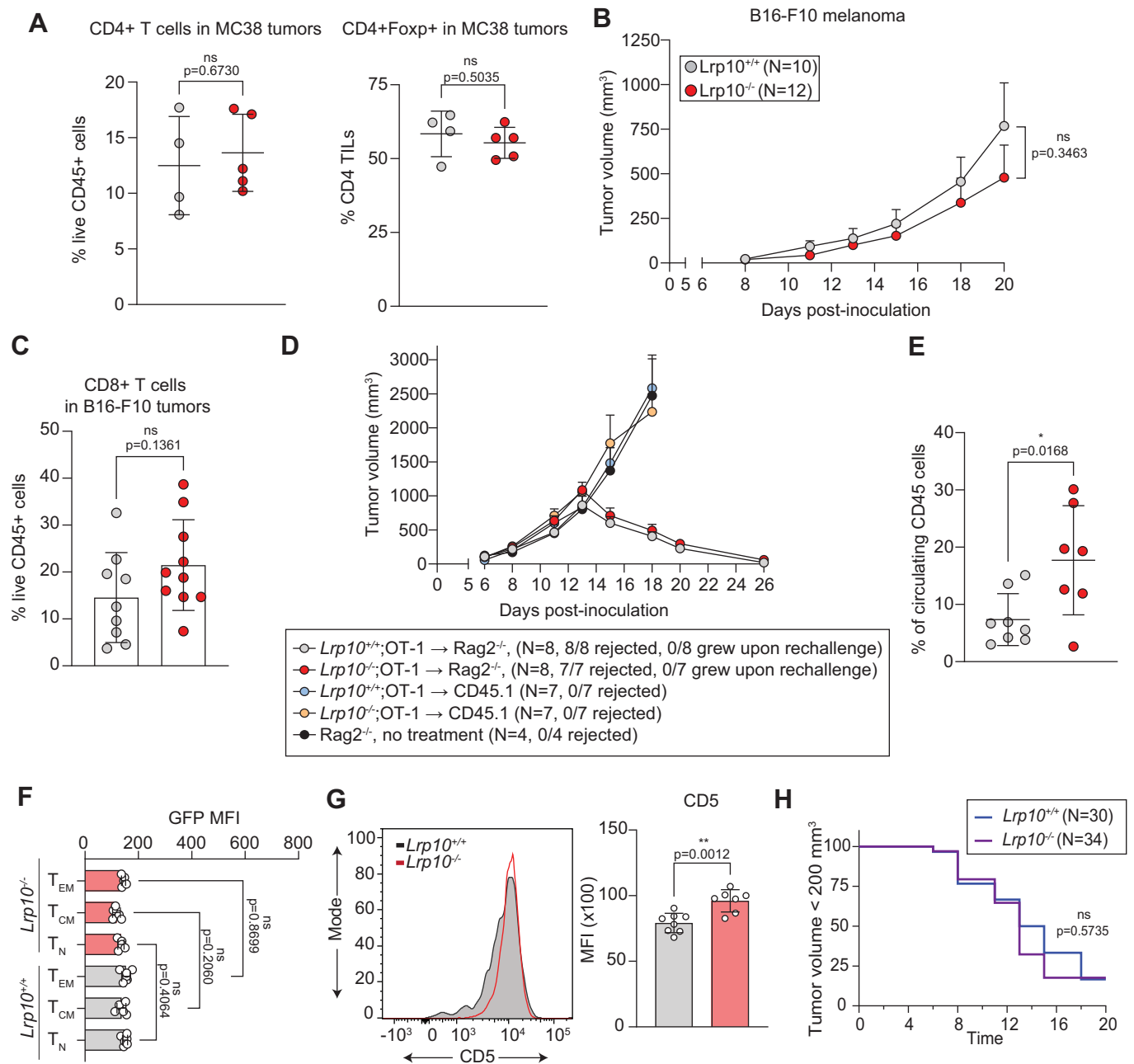


Figure EV5. Effect of *Lrp10* deletion on tumor growth in poorly immunogenic tumors and during adoptive transfer.

(A) Frequency of total CD4 T cells and CD4⁺Foxp3⁺ Tregs in total CD4 that were infiltrating D18 MC38 tumors on *Lrp10*^{+/+} and *Lrp10*^{-/-} mice. (B) Tumor growth of B16F10 melanoma cells injected subcutaneously into *Lrp10*^{+/+} and *Lrp10*^{-/-} mice. (C) Frequency of CD8 T cells infiltrating B16F10 tumors on D20. (D) Adoptive transfer of *Lrp10*^{+/+};OT-1 or *Lrp10*^{-/-};OT-1 cells into the indicated recipients on D6 after inoculation with B16-ova cells. The number of Rag2^{-/-} recipients that rejected the primary tumor and were resistant to tumor rechallenge 30 days later is indicated. (E) The frequency of *Lrp10*^{+/+};OT-1 or *Lrp10*^{-/-};OT-1 cells in the peripheral blood of Rag2^{-/-} recipients 30 days after primary tumor rejection. (F) GFP MFI in splenic CD8 T-cell subsets from *n* = 6 naive *Lrp10*^{+/+};Nur77^{GFP} and *n* = 5 *Lrp10*^{-/-};Nur77^{GFP} mice. (G) Representative FACS plot and CD5 MFI in T_{CM} phenotype cells from MC38 tumors on D18. (H) Frequency of B16F10 melanoma tumor progression in *Lrp10*^{+/+} and *Lrp10*^{-/-} mice treated with 10 mg/kg anti-PD1 across three separate cohorts with the indicated number *n* per genotype. Mice were said to have progressed if tumor volume exceeded 200 mm³. Data information: In bar graphs, symbols represent individual mice (biological replicates), horizontal bars indicate the mean, and error bars show SD. In (B, D), symbols represent the mean value and error bars show SEM. Results shown in (A, B, D, E, G) were replicated twice (A, D, E, G) or three times (B) in separate cohorts of at least three (A) or five mice (B, D, E, G) per genotype. Results shown in (C) are combined from two separate experiments. *P* values calculated with two-tailed unpaired *t* tests in (A, B, C, E, G), one-way ANOVA with Tukey's test (F), and log-rank (Mantel-Cox) test (H). Significant *P* values were flagged as follows: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. *P* values > 0.05 were considered to be not significant (ns).