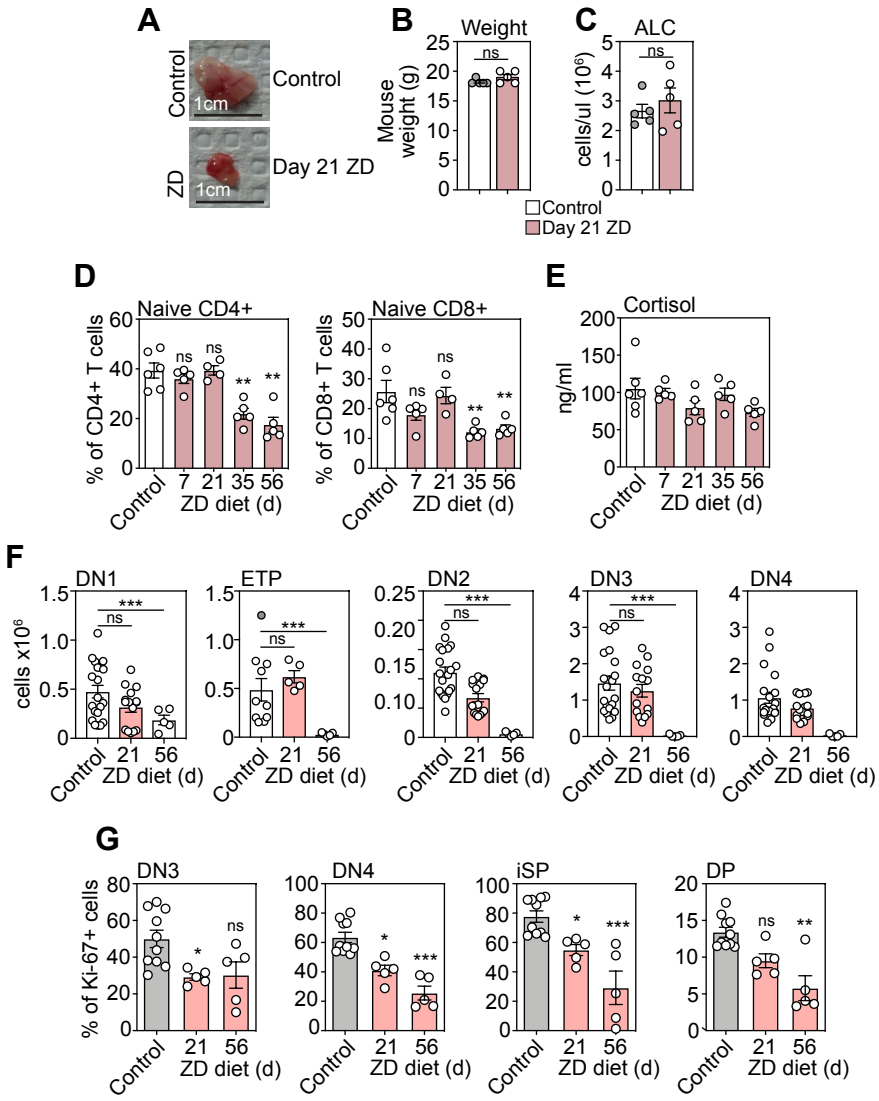
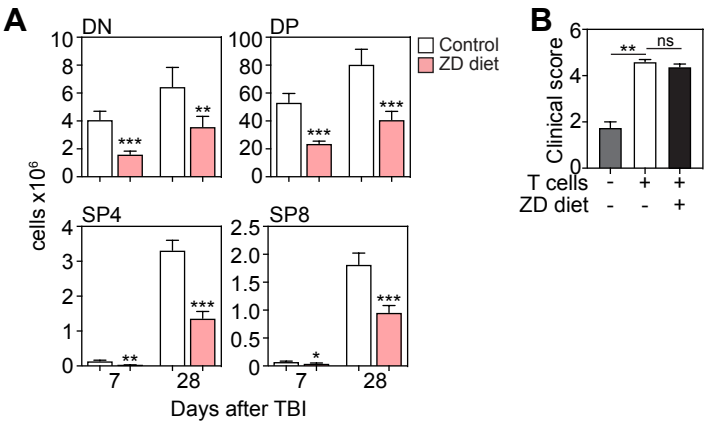
Figure S1



Supplementary Figure 1

6-8-week-old female C57BL/6 mice were fed a normal or Zn-deficient (ZD) diet for up to 56 days. **A,** Photos of the thymus from mice fed either control diet or ZD diet for 21 days. **B,** Weight of thymuses isolated after 21 days of ZD diet. **C,** Absolute lymphocyte counts (ALC) on the peripheral blood after 21 days of ZD diet. **D,** Proportion of naïve CD4+ or CD8+ T cells (as a proportion of total CD4+ or CD8+ T cells) after 1, 3, 5, or 8 weeks of ZD diet. **E,** Concentration of cortisol in serum of mice that had received control diet (ctrl), and after 1, 3, 5, and 8 weeks of ZD diet. **F,** Number of $CD44^{+}CD25^{-}$ DN1, $CD44^{+}CD25^{-}c\text{-}kit^{+}$ ETP, $CD44^{+}CD25^{+}$ DN3, or $CD44^{-}CD25^{-}CD90^{+}$ DN4 (untreated, n=24, combined from animals harvested alongside either day 21 or day 56 mice; day 21, n=15 over three independent experiments; day 56, n=10 over two independent experiments). **G,** Proportion of Ki-67+ events gated on CD4-CD8-Thy1+ CD25+CD44- DN (DN3), CD4-CD8-Thy1+ CD25-CD44- DN (DN4), CD3-CD4-CD8+ (iSP), CD4+CD8+ (DP) from untreated mice or after 21 or 56 days of ZD.Graphs represent mean ± SEM, each dot represents a biologically independent observation. *, $p<0.05$; **, $p<0.01$; *** $p<0.001$

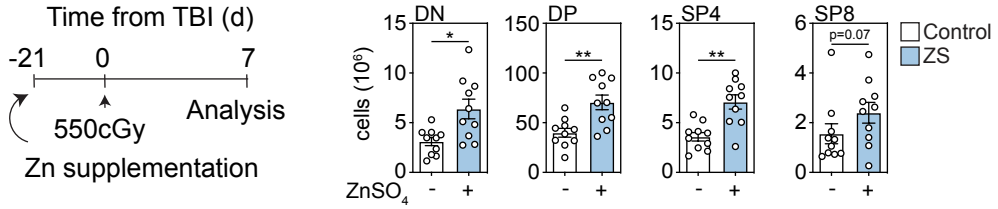
Figure S2



Supplementary Figure 2

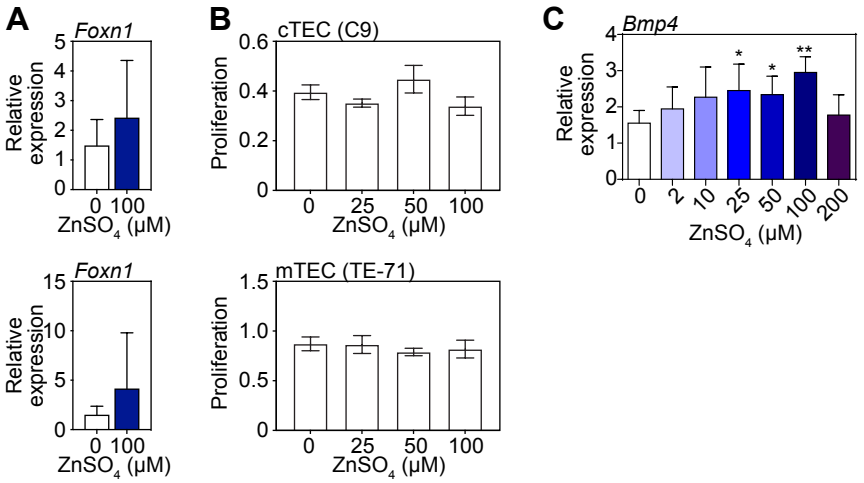
A, 6-8 week-old female C57BL/6 mice were fed a normal or ZD diet for 21 days at which point mice were given a sublethal dose of total body irradiation (TBI, 550cGy). Absolute number of DN, DP, SP4, and SP8 thymocyte subsets was calculated on day 7 and day 28 after TBI. **B**, 6 wo BALB.B mice were fed a normal or ZD diet for 21 days after which they were transplanted with $5x10^6$ TCD BM from B6 mice. GVHD groups were given a dose of 2×10^6 purified T cells. GVHD clinical score on day 7. Graphs represent mean ± SEM, each dot represents a biologically independent observation. *, $p<0.05$; **, $p<0.01$; *** $p<0.001$.

Figure S3



Supplementary Figure 3
6-8-week-old C57BL/6 female mice were given supplemental Zn in drinking water (300mg/kg/day $ZnSO_4$) for 21 days at which point mice were given 550 cGy TBI. Mice were maintained on Zn-supplemented drinking water for the duration of the study. Absolute number of DN, DP, SP4, and SP8 thymocyte subsets was calculated on day 7 after TBI. Graphs represent mean ± SEM, each dot represents a biologically independent observation. *, $p<0.05$; **, $p<0.01$; *** $p<0.001$.

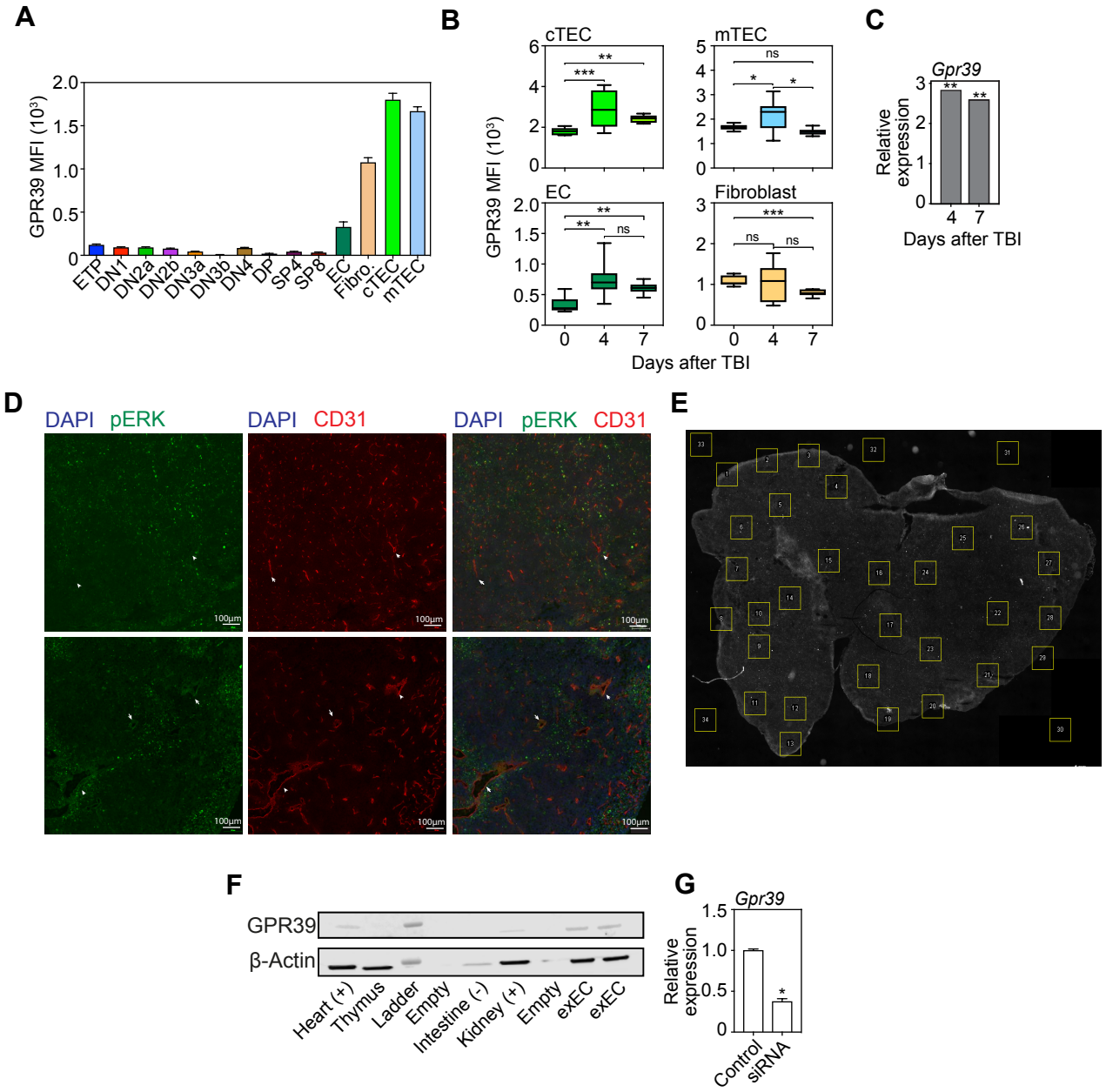
Figure S4



Supplementary Figure 4

A, Thymic epithelial cell lines (C9, cTEC; TE-71, mTEC) were cultured with 100μM ZnSO4 for 24h when Foxn1 expression was quantified by qPCR (n=3 independent experiments). **B,** C9 or TE-71 cells were incubatyed with graded doses of ZnSO4 for 24h after which proliferation was assessed. **C,** exEC cells were incubated with graded doses of ZnSO4 for 24h after which Bmp4 expression was quantified by qPCR (n=3-6 independent experiments). Graphs represent mean ± SEM, each dot represents a biologically independent observation (n=3 independent experiments). *, $p<0.05$; **, $p<0.01$; *** $p<0.001$.

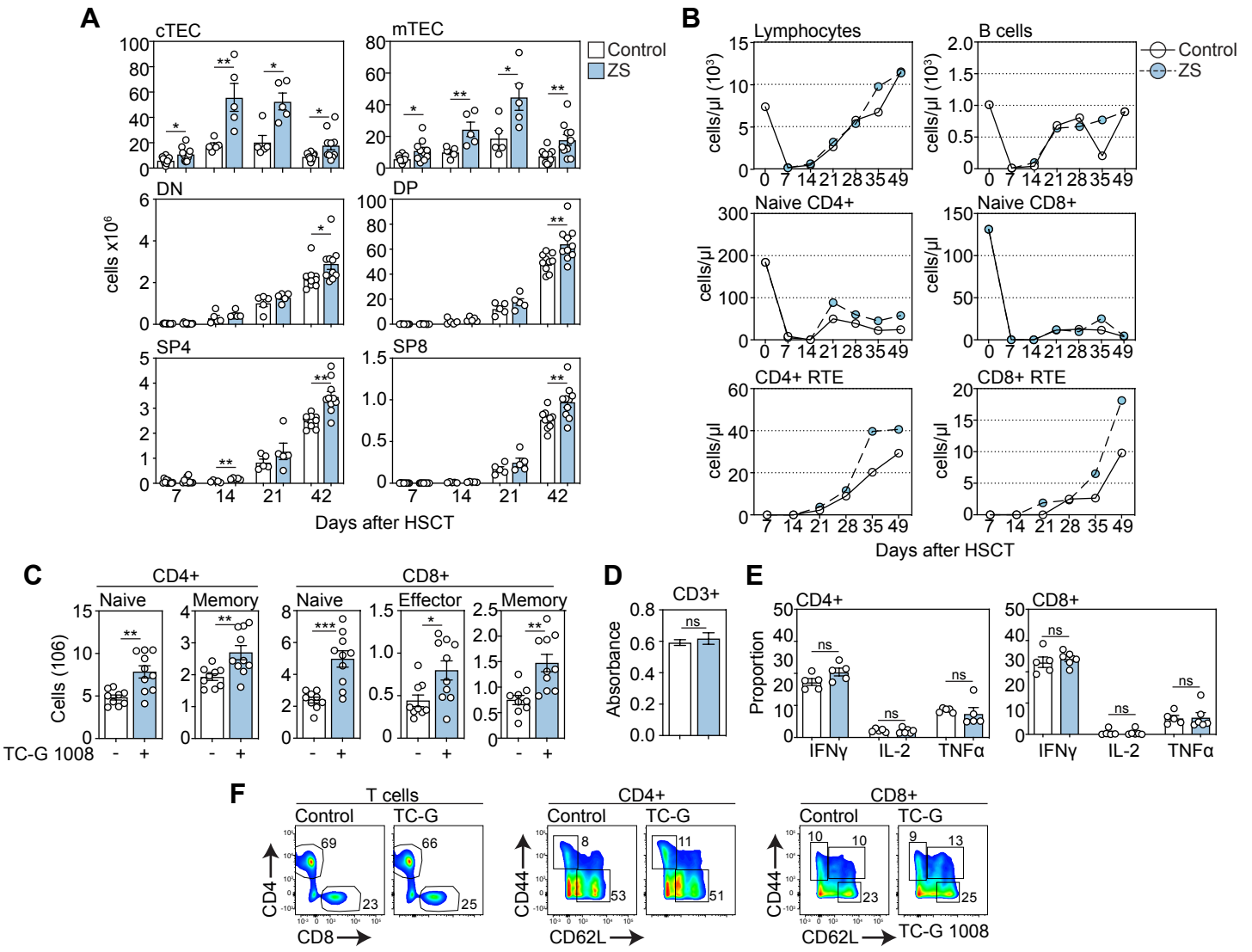
Figure S5



Supplementary Figure 5

A, GPR39 expression (MFI) across subsets in the thymus by flow cytometry at baseline. **B,** Expression of GPR39 on cTECs, mTECs, ECs and Fibroblasts at days 0, 4, and 7 after TBI. **C,** Thymic non-hematopoietic stromal cells were isolated from 6-week-old female C57BL/6 mice using CD45 MACS cell depletion at days 0, 4, and 7 after a single dose sub-lethal total body radiation (SL-TBI) and microarray analysis performed as previously described (Wertheimer et al., 2018) (GSE106982). Displayed is the differential gene expression fold-change of Gpr39 comparing day 4 to day 0 or day 7 to day (n=3; CD45- cells were pooled from 3-4 mice/n). **D,** ERK phosphorylation (pERK) *in situ* assessed at steady state. Co-expression of pERK (green) and VECAM (CD31, red) in thymic endothelial cells (yellow). The white arrowheads higlight the endothelial cells **E,** Regions of interest (ROI) of the same size were created to measure the intensity of pERK signal/ROI across different timepoints. The background was used as blank to be subtracted. The same number of ROI/-sample was acquired from different slices of thymic tissue. **F,** Western blot showing expression of GPR39 on whole thymus tissue and in thymic exECs. Skeletal muscle was used as negative control and intestine as positive control. **G,** Gpr39 expression measured by qPCR in exECs after silencing with siRNA. Graphs represent mean ± SEM.

Figure S6



Supplementary Figure 6

6-8-week-old male C57BL/6 mice were given supplemental Zn in drinking water (300mg/kg/day ZnSO4) for 21 days at which point mice were given a lethal dose of TBI (2 x 550cGy) along with T cell depleted BM from female C57BL/6 mice. Mice were maintained on ZnSO4 in drinking water for the duration of the study (n=5-10/group/timepoint combined from two independent experiments). **A,** Total number of cTECs, mTECs, DN, DP, SP4, and SP8 thymocytes at days 7, 21, 28, and 42 after allo-HCT. **B,** Numbers of lymphocytes, B cells, CD4+ and CD8+ naive and RTEs in peripheral blood. **C,** Numbers of CD4+ and CD8+ subpopulations on spleen (n=6/group, from two separate experiments). **D-E,** CD3+ T cells were isolated from spleen at day 40 after HSCT and stimulated for 72h with anti-CD3 and anti-CD28. **D,** Proliferation of all T cells (n=6/group, from two separate experiments). **E,** Intracellular cytokine staining (ICS) for IFNγ, IL2, and TNFα on CD4+ and CD8+ (n=5/group, from two separate experiments). **F,** Phenotype of T cells on lymph nodes harvested at day 40 after HSCT from mice treated with vehicle or TC-G1008 (see the main text). Concatenated flow plots from n=5 biological replicates are shown. Graphs represent mean ± SEM, each dot represents a biologically independent observation. *, p<0.05; **, p<0.01; *** p<0.001.