

Expanded View Figures

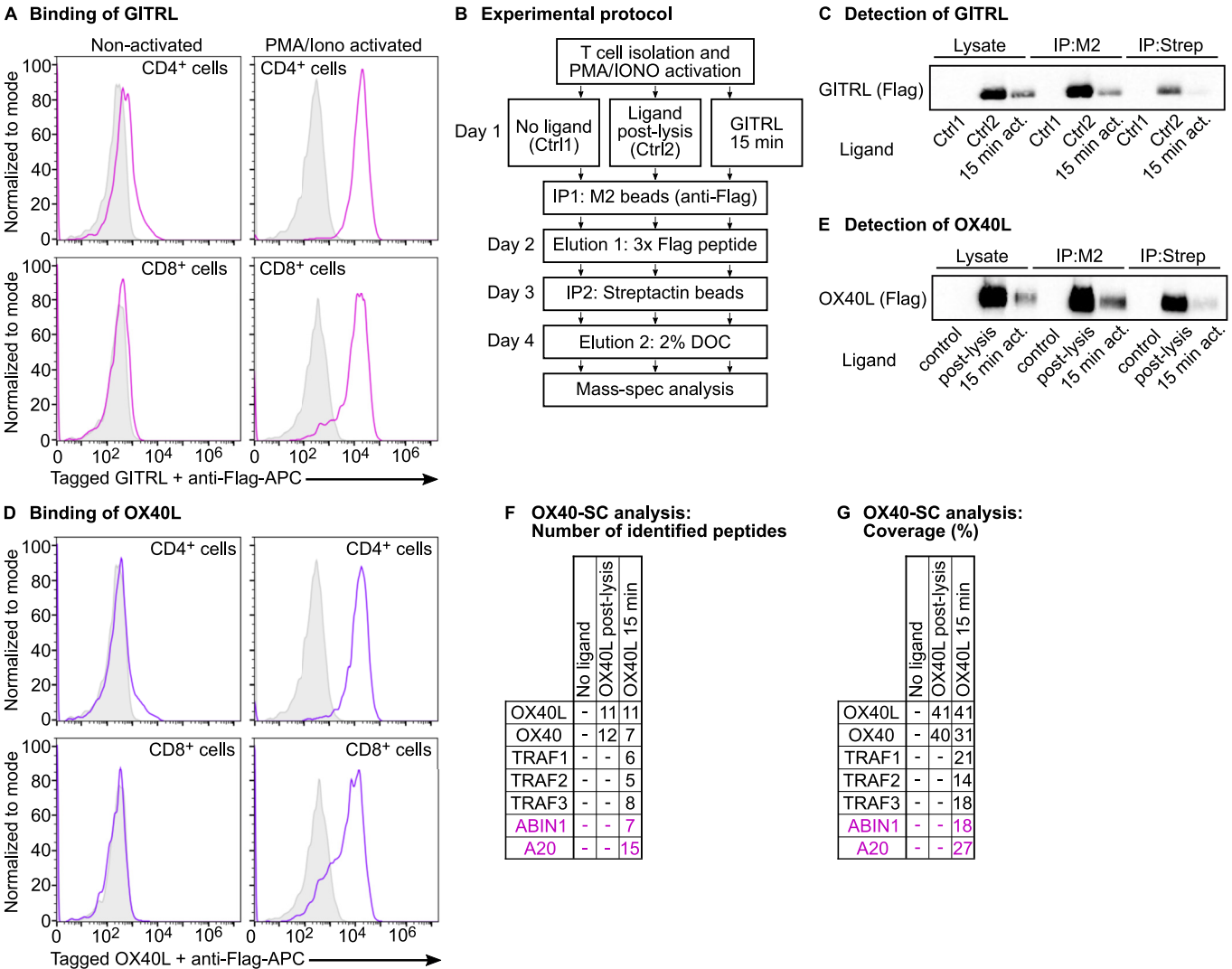


Figure EV1. Analysis of the proximal GTR and OX40 signaling complex (SC).

(A) Primary murine T cells were pre-activated with PMA/ionomycin for 72 h or not and stained with the anti-CD4 and anti-CD8 antibodies, recombinant GITRL followed by anti-FLAG antibody. A representative experiment out of 3 biological replicates in total. (B) Illustration of the protocol for the identification of the composition of GTR and OX40 signaling complexes. (C) Detection of the recombinant ligand in the lysate and after the first and second affinity purification step by immunoblotting. A representative experiment out of 3 biological replicates in total. (D) Primary murine T cells were pre-activated with PMA/ionomycin for 72 h or not and stained with the anti-CD4 and anti-CD8 antibodies, recombinant OX40 followed by anti-FLAG antibody. A representative experiment out of 3 biological replicates in total. (E-G) Primary murine T cells were pre-activated with PMA/ionomycin for 72 h and stimulated with the recombinant OX40L for 15 min. The cells were lysed and the OX40-SC was isolated via tandem affinity purification and samples were analyzed by mass spectrometry. (E) Detection of the recombinant ligand in the lysate and after the first and second affinity purification step by immunoblotting. Results of the protein identification are shown as the number of peptides (F) and the coverage (G). A single experiment was performed.

A *Abin1* alleles used in this study

Knock-out allele first - Genetrap (GT)



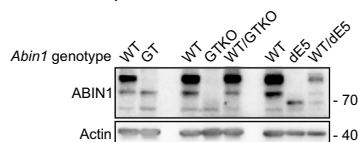
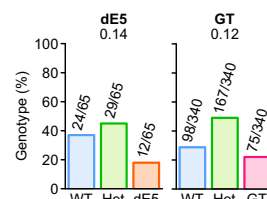
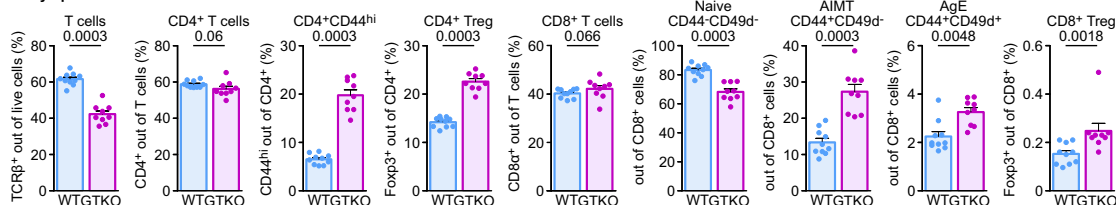
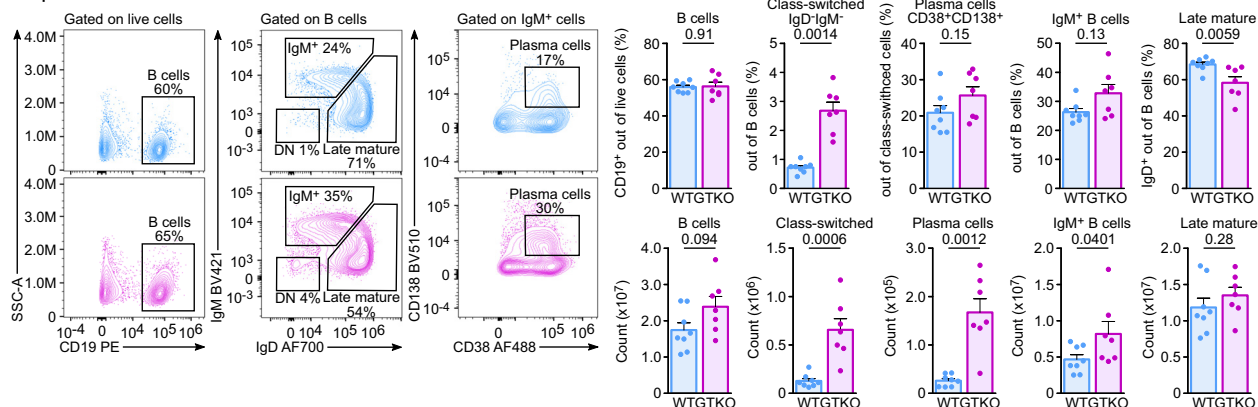
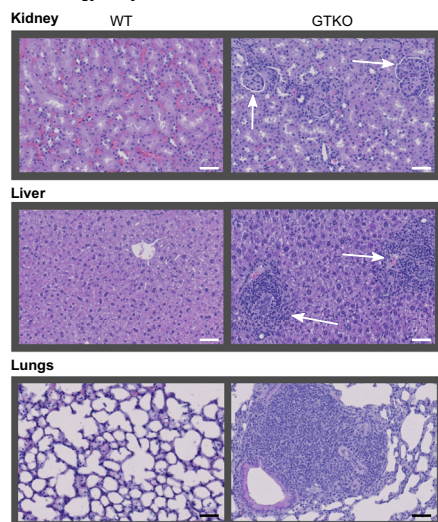
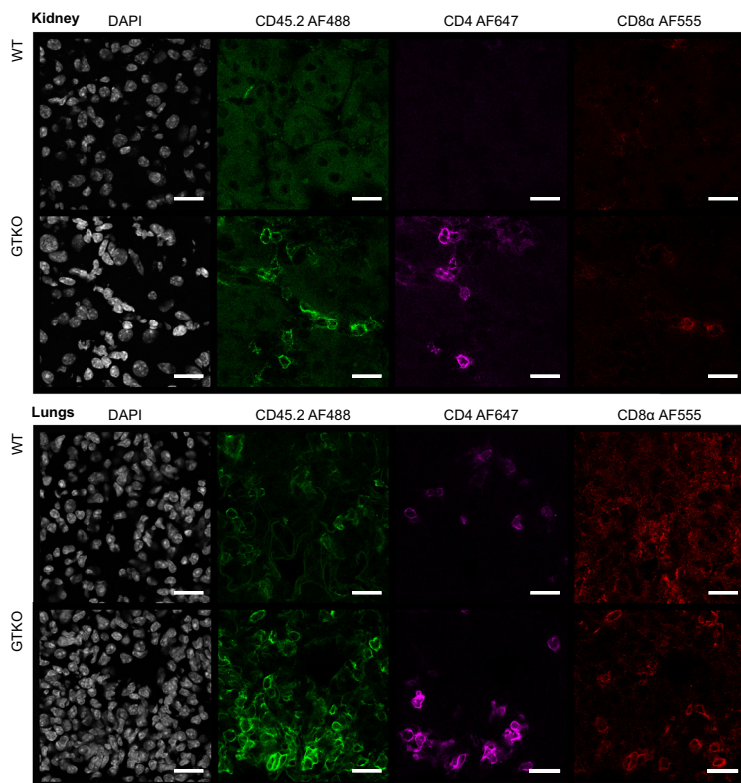
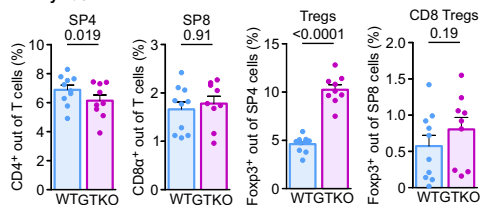
Floxed allele



dE5 (presumed knockout)



Genetrap/knock-out (GT/KO)

**B** Expression of ABIN1 in T cells**C** Born ratio**D** Lymph nodes**E** Spleen**F** Histology analysis**G** Immunofluorescence**H** Thymus

◀ **Figure EV2. Characterization of *Abin1*^{GT/GT} (GT), *Abin1*^{dE5/dE5} (dE5), and *Abin1*^{GTKO/GTKO} (GTKO) mice.**

(A) An overview of *Abin1* alleles used in this study. (B) Immunoblot analysis of ABIN1 in GT, GTKO, dE5 mice and corresponding littermate controls. A representative experiment is shown. The experiment was performed in 5 (GT), 3 (GTKO), or 2 (dE5) biological replicates. (C) Heterozygous *Abin1*^{WT/dE5} or *Abin1*^{WT/GT}, respectively, were bred and the genotype of the offspring was determined upon weaning. The frequencies and numbers of pups with particular genotypes are indicated. $n = 340$ (GT) or 65 (dE5) offspring mice per group in total from 16 (GT) or 2 (dE5) breedings. (D) Lymph node cells were stained with indicated antibodies and analyzed by flow cytometry. Aggregate results of the abundance of indicated subsets are shown, $n = 10$ (WT) or 9 (GTKO) mice per group. (E) Splenocytes were stained with indicated antibodies and analyzed by flow cytometry. Representative dot plots and aggregate results of the frequency of indicated subsets are shown. $n = 10$ (WT) or 9 (GTKO) mice per group. (F) Histological analysis using H&E staining of indicated organs of 20–26 week-old mice. Arrows pointing to inflammatory foci. Representative staining out of 4 mice per group in total. (G) Cryosections of lungs and kidneys of WT and GTKO mice were stained with indicated antibodies and DAPI (nuclei) and analyzed by confocal fluorescence microscopy. Representative sections out of 4 mice per group in total. (H) Fixed and permeabilized thymocytes from WT and GTKO mice were stained with indicated antibodies and analyzed by flow cytometry. $n = 10$ (WT) or 9 (GTKO) mice per group. Data information: In (D, E, H), data are represented as mean + SEM and p values are indicated. In (F, G), the scale bars indicate 20 μm and 50 μm , respectively. Statistical significance was determined by a binomial test (A) or two-tailed Mann-Whitney test (D, E, H).

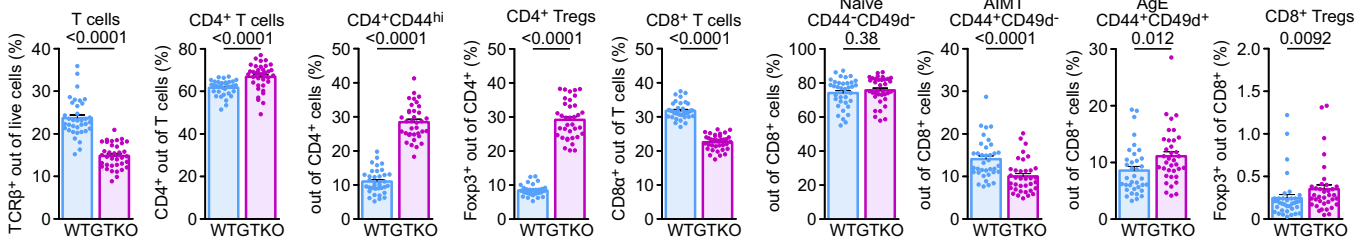
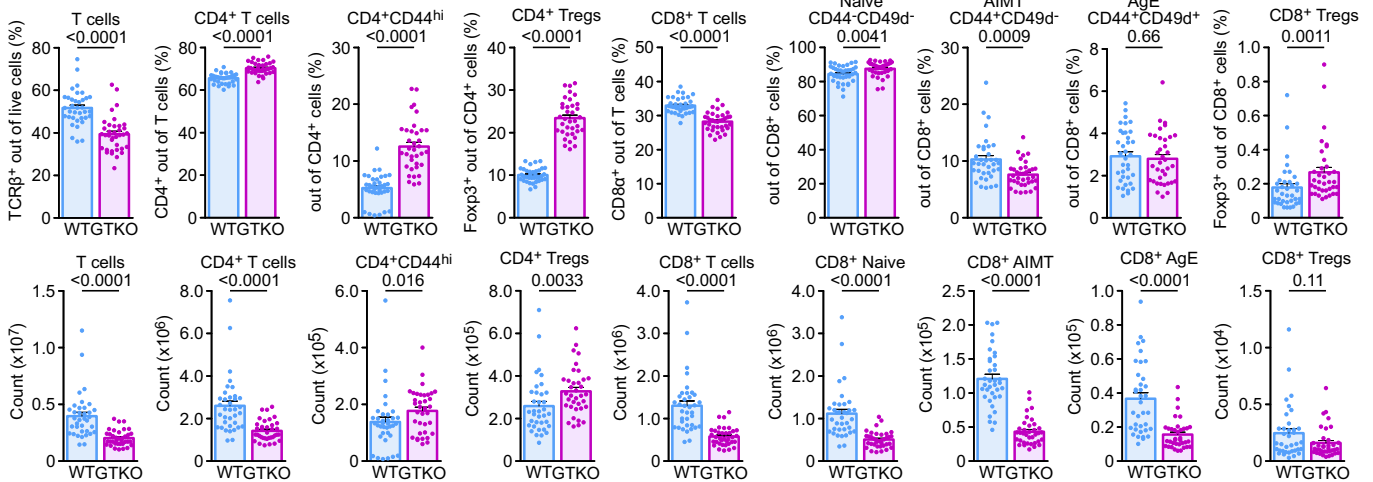
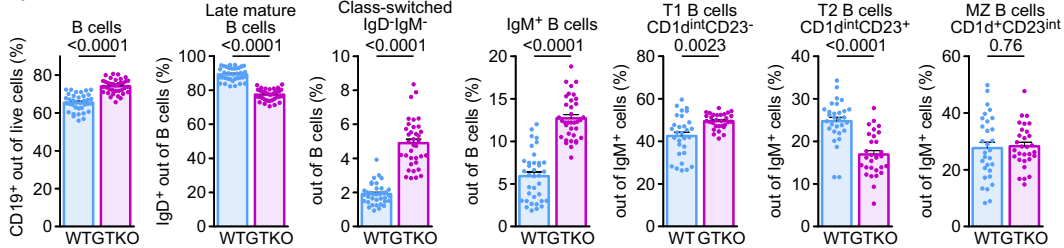
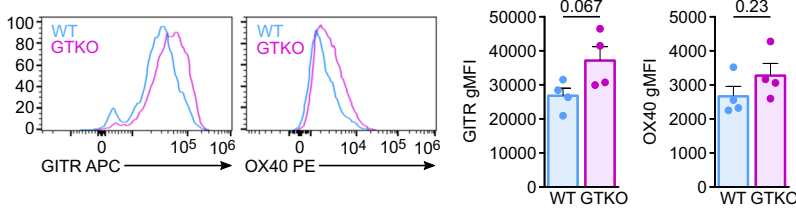
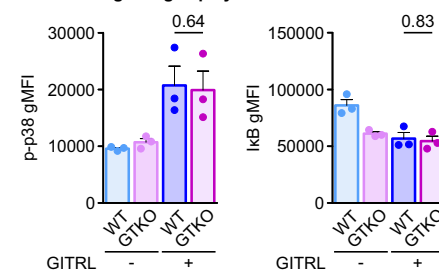
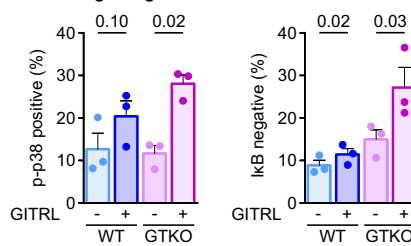
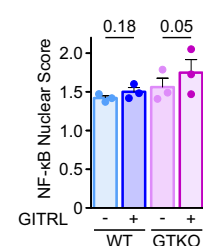
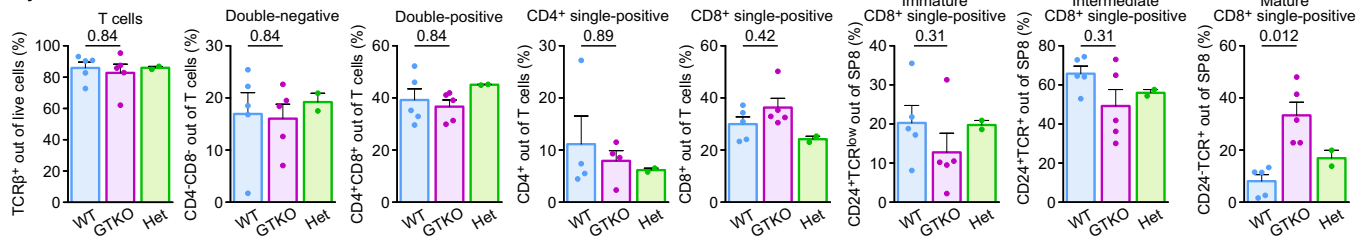
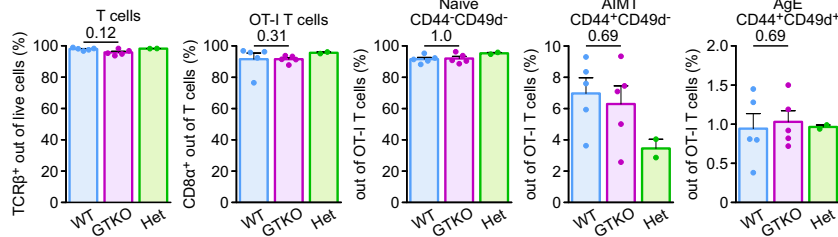
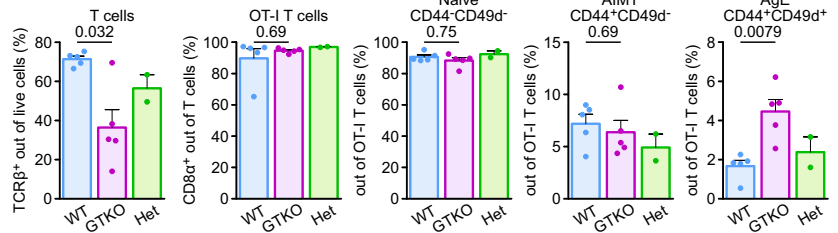
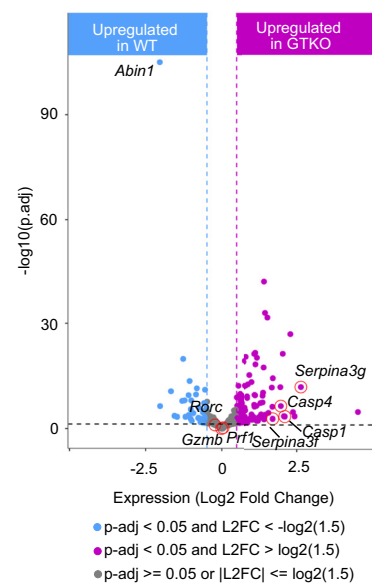
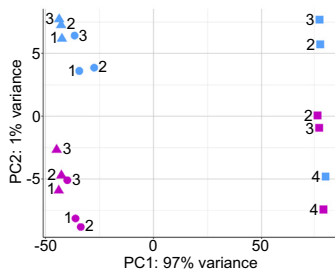
A Mixed bone marrow chimeras**Spleen****Lymph nodes****B Mixed bone marrow chimeras****Spleen****C CD4⁺ Foxp3⁺ T regulatory cells****D GITR signaling in polyclonal CD8⁺ T cells****E GITR signaling in OT-I T cells****F NF-κB translocation**

Figure EV3. Intrinsic roles of ABIN1 in T cells.

(A, B) The experiment shown in Fig. 3A,B. Aggregate results of the frequency and absolute counts of indicated subsets of T cells (A) or B cells (B). $n = 36$ mice per group. (C) Lymph node cells from WT or *Abin1*^{GTKO/GTKO} mice were analyzed by flow cytometry. The expression of GITR and OX40 on CD4⁺ FOXP3⁺ T cells is shown. Representative histograms and aggregate data are shown. $n = 4$ mice per group. (D) The experiment shown in Fig. 3D. Lymph node cells from WT or *Abin1*^{GTKO/GTKO} mice were pre-activated with PMA/ionomycin and stimulated with GITRL or left untreated (controls). Indicated activation pathways were analyzed by flow cytometry. Aggregate results of phospho-p38 and I κ B levels in CD8⁺ T cells. $n = 4$ mice per group. (E, F) The experiment shown in Fig. 3E. Lymph node cells from WT or *Abin1*^{GTKO/GTKO} OT-I *Rag2*^{KO/KO} mice were pre-activated with PMA/ionomycin and stimulated with GITRL or left untreated and analyzed by flow cytometry (E) or flow imaging (F). $n = 3$ mice per group. (E) The Percentage of phospho-p38 and I κ B positive cells is shown. (F) The nuclear translocation score for NF- κ B is shown. Data information: In (A–E), the data are presented as mean $\pm$ SEM and P values are indicated. Statistical significance was determined by two-tailed Mann-Whitney test (A, B) or two-tailed Student's t test (C, D) or one-tailed paired t test (E, F).

A *Abin1*^{GTKO/GTKO} *OT-I* *Rag2*^{KO/KO}**Thymus****Lymph nodes****Spleen****C** Transcriptomic analysis of non-activated T cells**B** Principle component analysis (all samples)

Principle component analysis (only activated samples)

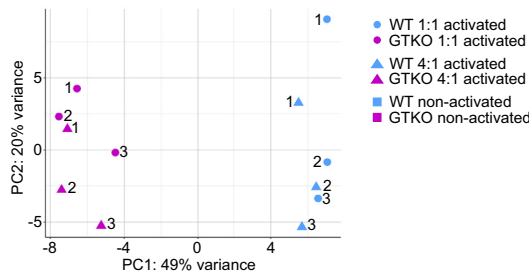
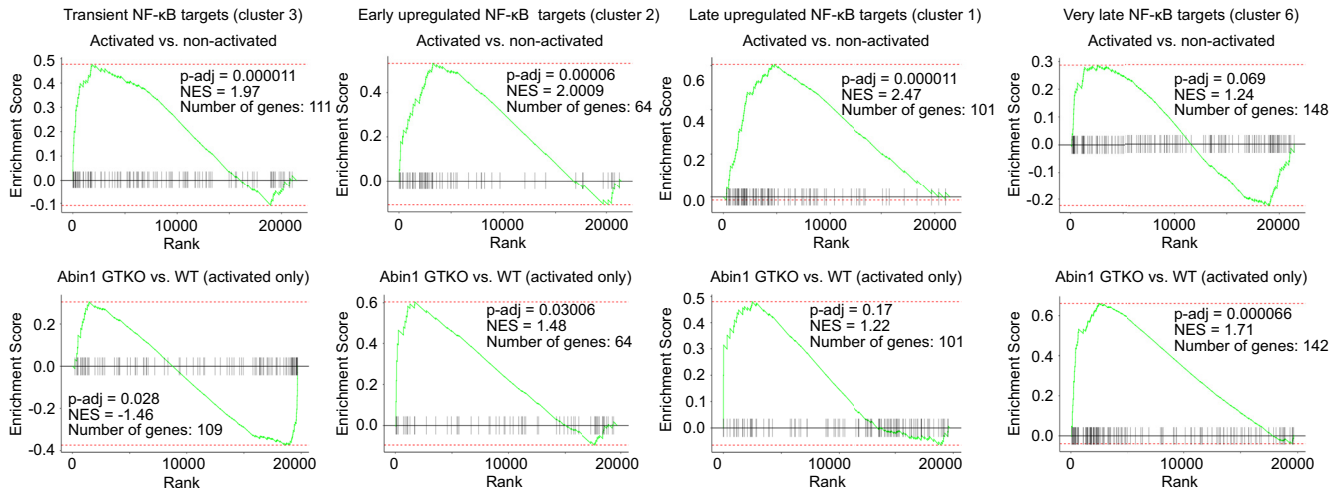
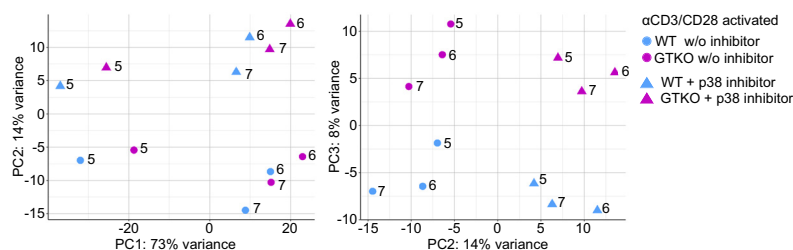
**D** Gene set enrichment analysis (gene sets from Zhao et al. 2023)

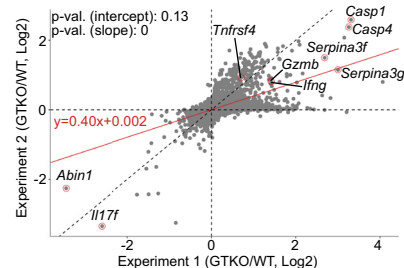

Figure EV4. Characterization of *Abin1*^{GTKO/GTKO} OT-I mice.

(A) Cells from lymph nodes, spleen, and thymus from *Abin1*^{WT/WT} OT-I *Rag2*^{KO/KO} (WT), *Abin1*^{GTKO/GTKO} OT-I *Rag2*^{KO/KO} (GTKO), and *Abin1*^{WT/GTKO} OT-I *Rag2*^{KO/KO} (HET) were stained with indicated antibodies and analyzed by flow cytometry. Aggregate results of the frequency of indicated subsets are shown. $n = 5$ (WT and GTKO) or 2 (HET) mice per group. (B) Principal component analysis of the RNAseq experiment shown in Fig. 4C,D. (C) A volcano plot showing the differences in gene expression between non-activated WT and GTKO T cells from the experiment shown in Fig. 4C,D. (D) Four clusters of NF- κ B responsive genes with distinct expression kinetics after the antigenic signaling were taken from a study by Zhao et al (Zhao et al, 2023). Using these gene lists, we performed a gene set enrichment analysis for the contrast between activated vs. non-activated OT-I T cells (upper lane) and activated GTKO vs. activated WT OT-I T cells (bottom lane) using the RNAseq data shown in Fig. 4C,D. Data information: In (A), the data are presented as mean $\pm$ SEM. The statistical significance was calculated using two-tailed Mann-Whitney test (A), Wald test with Benjamini-Hochberg multiple testing correction (C), or Weighted Kolmogorov-Smirnov test (D).

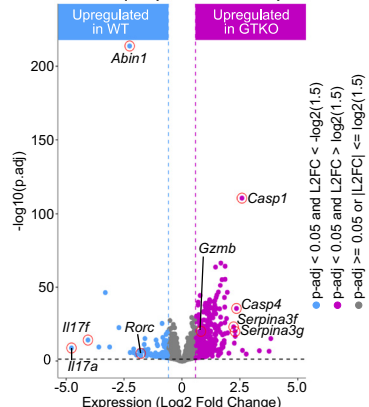
A Principle component analysis



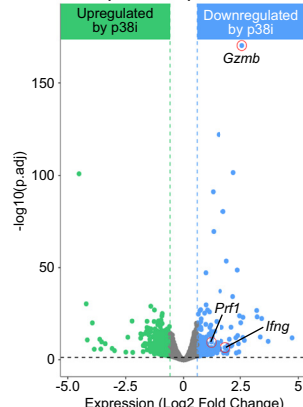
B Comparison of activated WT vs GTKO from two experiments



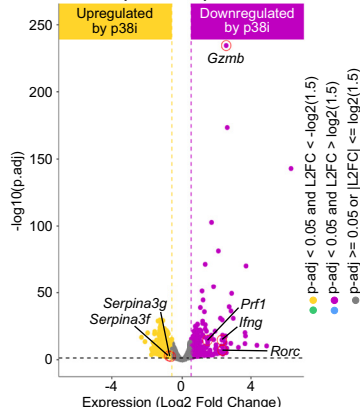
C GTKO vs WT (samples with and w/o p38i combined)



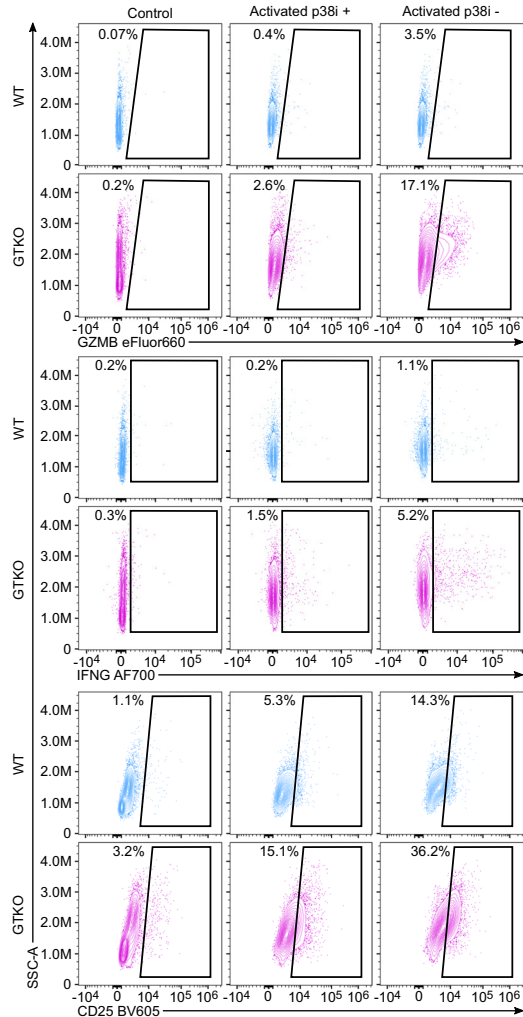
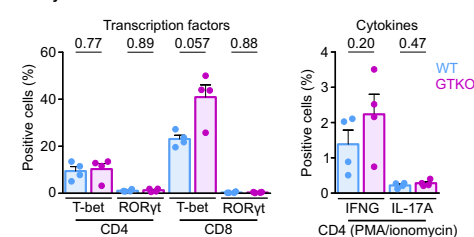
D WT cells: +p38i vs w/o p38i



GTKO cells: +p38i vs w/o p38i



E OT-I T cells

F Polyclonal CD4⁺ and CD8⁺ T cells

◀ **Figure EV5. Analysis of signaling pathways in ABIN1-deficient T cells.**

(A–D) The same RNAseq experiment as presented in Fig. 5E,F. $n = 3$ biological replicates. (A) Principal component analysis of the samples. (B) Comparison of these experiments and the previous set of experiments (presented in Fig. 4C,D) by plotting the fold changes of activated *Abin1*^{GTKO/GTKO} OT-I *Rag2*^{KO/KO} (GTKO) vs. *Abin1*^{WT/WT} OT-I *Rag2*^{KO/KO} (WT) T cells in both sets of experiments. (C) A volcano plot showing expression changes of activated T cells treated p38 MAPK inhibitor (12.5 μ M) for combined WT and GTKO samples. (D) A volcano plot showing expression changes of activated T cells treated or non-treated with p38 MAPK inhibitor (12.5 μ M) for WT and GTKO samples separately. (E) Lymph node cells from WT and GTKO OT-I mice from the experiment shown in Fig. 5G. Representative dot plots showing the expression of indicated markers are shown. $n = 7$ (WT), or 8 (GTKO) mice per group. (F) Lymph node cells from WT or GTKO polyclonal mice were analyzed by flow cytometry for the expression of indicated transcription factors (left) or activated with PMA/ionomycin for 4 h and analyzed by flow cytometry for the production of indicated cytokines (right). $n = 4$ mice per group. Data information: In (F), data are presented as mean $\pm$ SEM. The statistical analysis was calculated using two-tailed Wald test with Benjamini-Hochberg multiple testing correction (C, D) or two-tailed Student's *t* test (F).

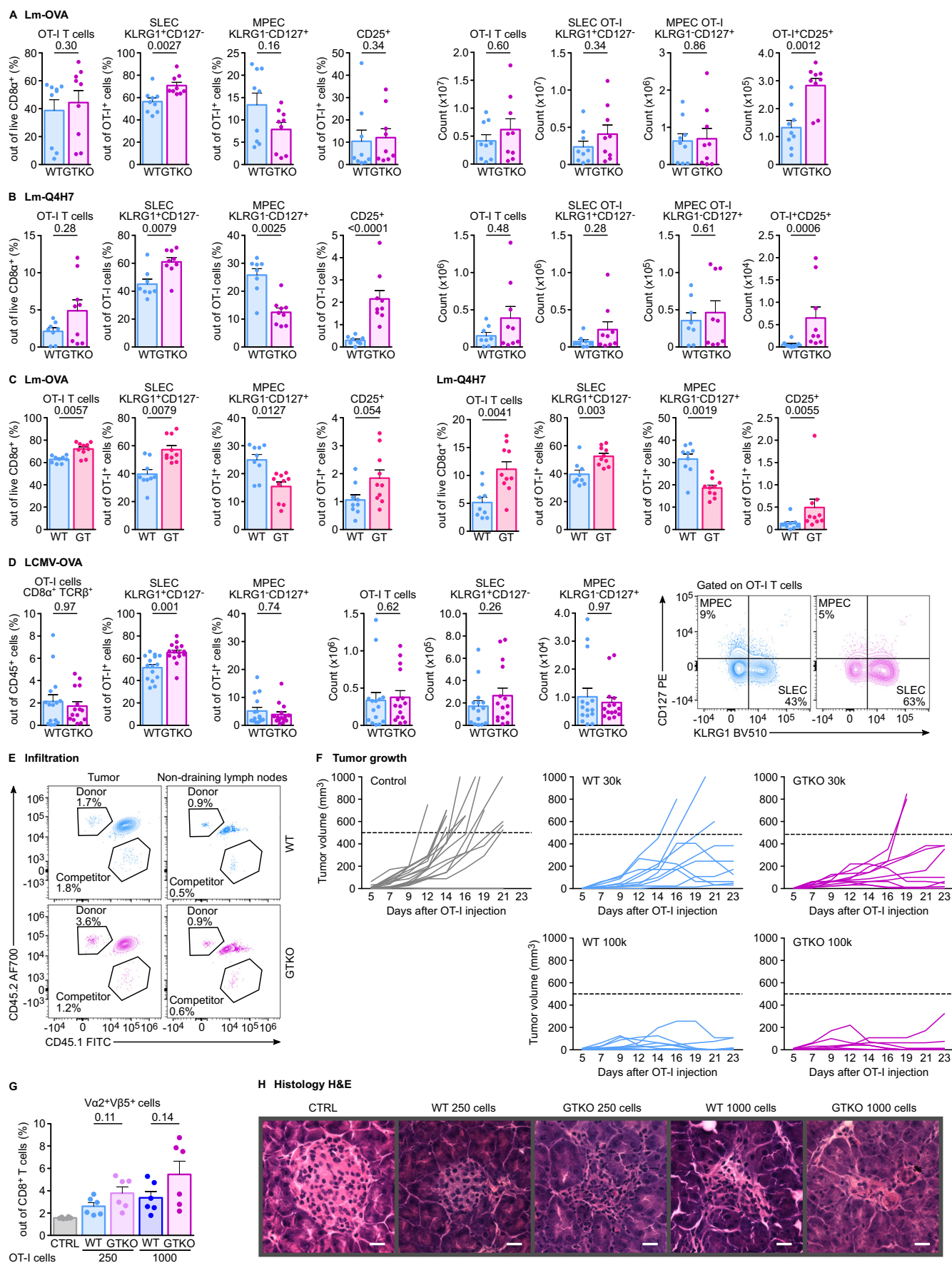


Figure EV6. ABIN1 regulates T-cell responses in vivo.

(A, B) The experiments as shown in Fig. 6A,B with identical protocol, but using a transfer of 5×10^4 T cells. (A) $n = 9$ mice per group. (B) $n = 8$ (WT), or 9 (GTKO) mice per group. Quantified frequencies and counts of indicated subsets of donor cells are shown. (C) 1×10^5 OT-I cells from WT or *Abin1*^{GTKO/GTKO} OT-I *Rag2*^{KO/KO} (GT) mice were adoptively transferred to Ly5.2 hosts that were infected with Lm-OVA. Splenocytes were analyzed by flow cytometry on day 6 post infection. The frequency of indicated subsets is shown. $n = 9$ (WT) or 10 (GT) mice per group. (D) 1×10^4 OT-I cells from WT or *Abin1*^{GTKO/GTKO} OT-I *Rag2*^{KO/KO} mice were adoptively transferred to the *CD3e*^{KO/KO} hosts that were infected with LCMV expressing OVA peptide. Splenocytes were analyzed by flow cytometry on day 5 post infection. Representative dot plots and quantified frequencies and absolute counts of indicated subsets of donor cells are shown. $n = 15$ mice per group. (E) The experiment shown in Fig. 6C. A representative experiment for tumor and non-draining lymph nodes. (F) The experiment shown in Fig. 6D. The tumor growth in individual mice is shown. The dashed line represents the endpoint of the experiment (tumor volume 500 mm³). The genotype and number of transferred OT-I *Rag2*^{KO/KO} T cells are indicated. (G) In the experimental autoimmune assay (Fig. 6F), splenocytes of the host mice were analyzed by flow cytometry on day 6. The frequency of Va2⁺ Vβ5⁺ cells among all CD8⁺ T cells was quantified as a proxy for donor OT-I T cells. $n = 6$ mice per group. (H) Pancreatic cryosections (same experiments as shown in Fig. 6I) were stained with hematoxylin and eosin and imaged by microscopy. Data information: In (A–D, G), data are presented as mean ± SEM. In (H), the scale bars represent 20 μm. The statistical significance was calculated using two-tailed Mann–Whitney test (A–D) or two-tailed Student's *t* test (G).