SUPPLEMENTAL MATERIAL

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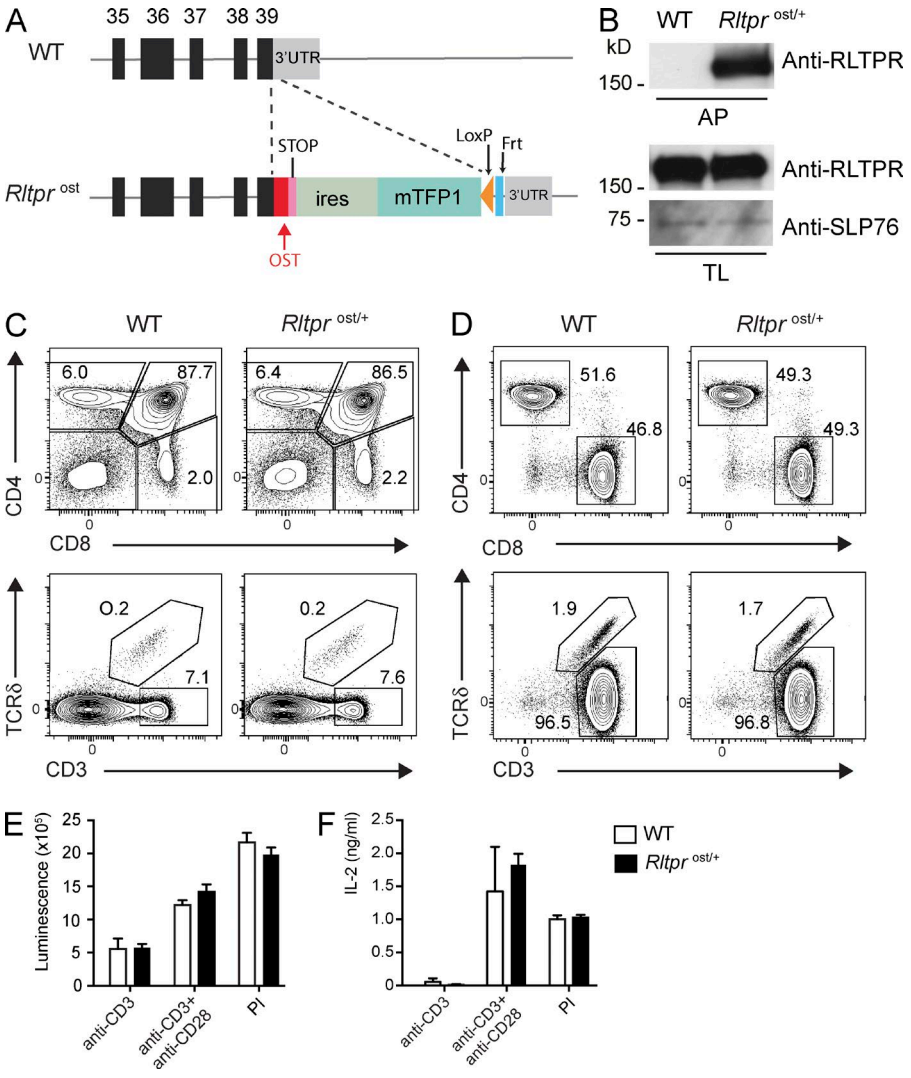

Figure S1. **Generation and validation of knock-in mice expressing endogenous RLTPR proteins tagged with a One-STrEP-tag (OST) sequence.** (A) Schematic representation of the 3′ end of the *Rltpr* WT (top) and *Rltpr*^{ost} allele (bottom). Exons 35–39 are shown as filled black boxes and numbered, and the 3′UTR is shown as a gray box. The structure of the *Rltpr*^{ost} allele is shown after FLP-mediated excision of the Frt-*neo*^{r}-Frt and Frt3-puro^{r}-Frt3 cassettes. An OST sequence was added in frame at the end of exon 39 and followed by a STOP codon. The 3′ UTR also contained an IRES-mTFP1 cassette, and a LoxP and an Frt sequences. A LoxP sequence is also present in the intron located between exons 23 and 24 (not depicted). (B) Immunoblot analysis of equal amounts of lysates of thymocytes from WT and *Rltpr*$^{ost/+}$ mice subjected to affinity purification (AP) on Strep-Tactin-Sepharose beads, followed by elution of proteins with D-biotin, and probed with the EM-53 anti-RLTPR antibody. Also shown is an immunoblot analysis of equal amounts of total lysates (TL) of thymocytes from WT and *Rltpr*$^{ost/+}$ mice probed with anti-RLTPR and anti-SLP76 (loading control). (C) Flow cytometry analysis of thymocytes from WT and *Rltpr*$^{ost/+}$ mice. Thymocytes were analyzed for expression of CD4 and CD8 (top) and of TCRδ and CD3 (bottom) and numbers in quadrants indicate percent CD4^{+}CD8^{+} double-positive, CD4^{+} single-positive, and CD8^{+} single-positive thymocytes (top), and percent TCRαβ^{+} and TCRγδ^{+} thymocytes (bottom). (D) Flow cytometry analysis of splenocytes from WT and *Rltpr*$^{ost/+}$ mice. Splenocytes were analyzed for expression of the same markers as thymocytes. Numbers in quadrants indicate percent CD4^{+} T and CD8^{+} T cells (top), and percent TCRαβ^{+} and TCRγδ^{+} T cells (bottom). (E) Negatively purified CD4^{+} T cells from spleen of WT and *Rltpr*$^{ost/+}$ mice were activated for 48 h in vitro with plate-bound anti-CD3 (9 µg/ml) in the presence or absence of soluble anti-CD28 (1 µg/ml) or with PMA and ionomycin (PI), and the ATP present in the culture medium assessed by luminescence as a measure of the extent of cell proliferation. (F) IL-2 in supernatants of purified WT and *Rltpr*$^{ost/+}$ CD4^{+} T cells activated for 48 h, as in E. Data are representative of at least two experiments with three mice per group (E and F; mean and SEM).

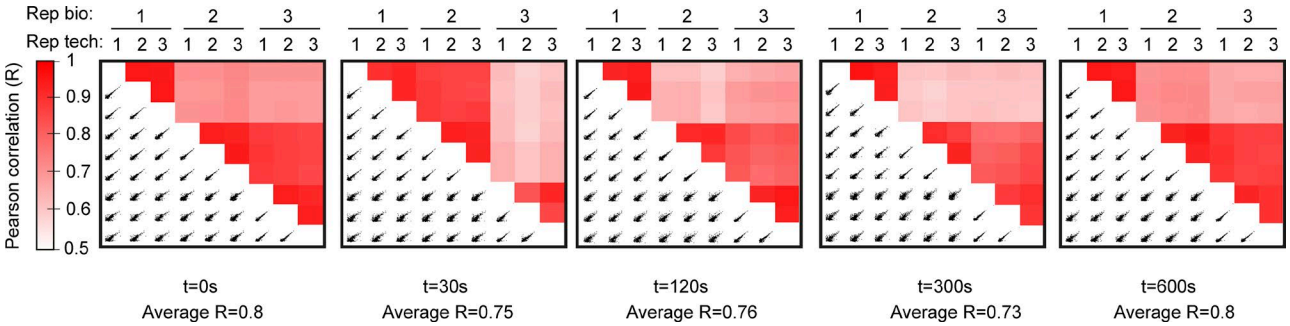

Figure S2. **Assessment of biological and technical variability across AP-MS samples.** The variability between AP-MS samples corresponding to a given condition of activation was estimated by computing the Pearson correlation coefficient from log transformed raw intensities of all detected proteins for all pairs of technical and biological replicates (denoted as Rep. Tech. and Rep. Bio., respectively). For each condition of activation (i.e., before (t = 0 s) or after T cell activation for t = 30 s, t = 120 s, t = 300 s, and t = 600 s), scatter plots of log-transformed raw intensities for all pairs of technical and biological replicates are represented along with the corresponding Pearson correlation coefficients (R). The average Pearson correlation coefficient across all pairs of technical and biological replicates (denoted as Average R) is also indicated for each time point.

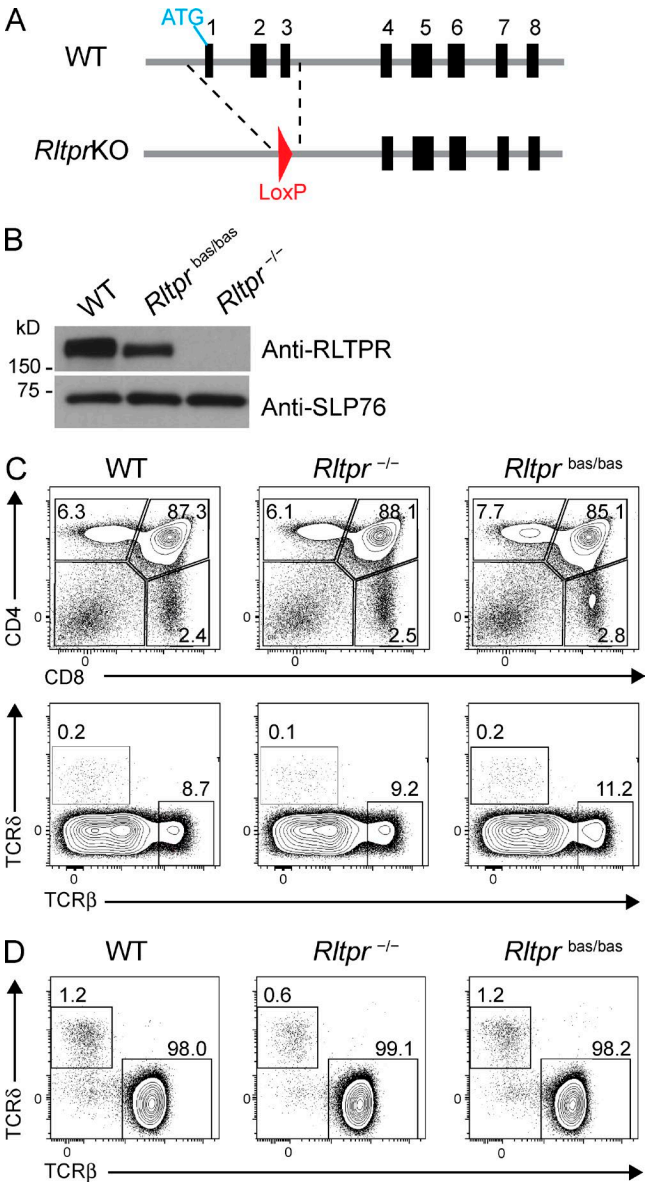

Figure S3. **Generation and validation of RLTPR-deficient mice.** (A) Schematic representation the 5′ region of *Rltpr* WT (top) and *Rltpr*$^{-}$ (bottom) alleles. Exons 1–8 are shown as filled black boxes and the position of the start codon (ATG) is specified. The structure of the *Rltpr*$^{-}$ allele is shown after CRE-mediated excision of the ACN cassette (Bunting et al., 1999). (B) Analysis of RLTPR expression in T cells from WT, *Rltpr*$^{bas/bas}$, and *Rltpr*$^{-/-}$ mice. Equal amounts of proteins from total lysates were analyzed by immunoblots probed with the EM-53 anti-RLTPR antibody and with anti-SLP76 (loading control). (C) Thymocytes from WT, *Rltpr*$^{bas/bas}$, and *Rltpr*$^{-/-}$ mice were analyzed for expression of CD4 and CD8 and of TCRβ and TCRδ and numbers in quadrants indicate percent CD4^{+}CD8^{+} double-positive thymocytes, CD4^{+} single-positive thymocytes and CD8^{+} single-positive thymocytes (top), and percent TCRαβ^{+} or TCRγδ^{+} thymocytes (bottom). (D) T cells from the spleen of WT, *Rltpr*$^{bas/bas}$, and *Rltpr*$^{-/-}$ mice were analyzed for expression of TCRβ and TCRδ. Numbers in quadrants indicate percent TCRαβ^{+} and TCRγδ^{+} T cells. Data are representative of at least two experiments with three mice per group.

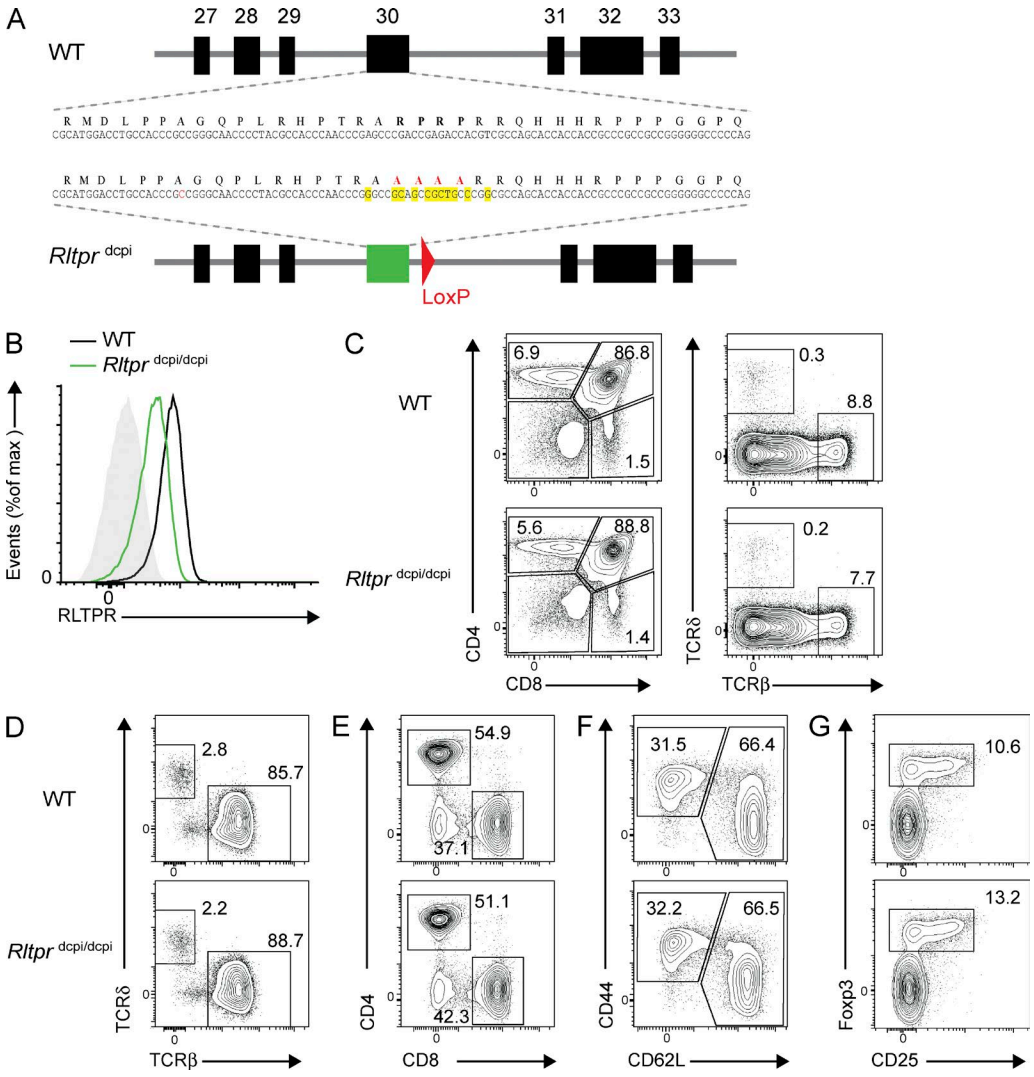

Figure S4. **Generation and validation of *Rltpr*^{dcpi} mice.** (A) Schematic representation of the WT *Rltpr* (top) and *Rltpr*^{dcpi} (bottom) alleles encompassing exons 27–33 (shown as black boxes). The structure of the *Rltpr*^{dcpi} allele is shown after CRE-mediated excision of the ACN cassette. The residual LoxP site is shown in red and exon 30, containing the mutated CPI motif, is highlighted in green. Also shown are the nucleotide sequences present in WT and mutated exon 30 and their corresponding amino acid sequences. Mutated bases are highlighted in yellow and the resulting mutated amino acids in red. (B) Expression of RLTPR in splenic T cells from WT and *Rltpr*^{dcpi} mice. Gray shaded curves, negative control based on staining of T cells from RLTPR-deficient mice. (C) Thymocytes from WT, *Rltpr*$^{dcpi/dcpi}$ mice were analyzed for expression of CD4 and CD8 and of TCRβ and TCRδ and numbers in quadrants indicate percent CD4$^+$CD8$^+$ double-positive thymocytes, CD4$^+$ single-positive thymocytes, and CD8$^+$ single-positive thymocytes (left), and percent TCRαβ$^+$ or TCRγδ$^+$ thymocytes (right). (D) T cells from the spleen of WT, and *Rltpr*$^{dcpi/dcpi}$ mice were analyzed for expression of TCRβ and TCRδ. Numbers in quadrants indicate percent TCRαβ$^+$ and TCRγδ$^+$ T cells. (E) T cells from the spleen of WT, and *Rltpr*$^{dcpi/dcpi}$ mice were analyzed for expression of CD4 and CD8. Numbers in quadrants indicate percent CD4$^+$ T and CD8$^+$ T cells. (F) CD4$^+$ T cells from WT, and *Rltpr*$^{dcpi/dcpi}$ spleens were analyzed for expression of CD44 and CD62L. Numbers in quadrants indicate percent naive (CD44^{lo}CD62L^{hi}) and effector memory (CD44^{hi}CD62L^{lo}) CD4$^+$ T cells. (G) CD4$^+$ T cells from WT, and *Rltpr*$^{dcpi/dcpi}$ spleens were analyzed for expression of Foxp3 and CD25. Numbers in quadrants indicate percent T reg cells. Data are representative of at least two experiments with three mice per group.

Table S1 (available as an Excel file) shows the list of proteins associated with RLTPR in resting thymocytes and in thymocytes stimulated with pervanadate for 30, 120, 300, and 600 s.

REFERENCE

Bunting, M., K.E. Bernstein, J.M. Greer, M.R. Capecchi, and K.R. Thomas. 1999. Targeting genes for self-excision in the germ line. *Genes Dev.* 13:1524–1528. http://dx.doi.org/10.1101/gad.13.12.1524