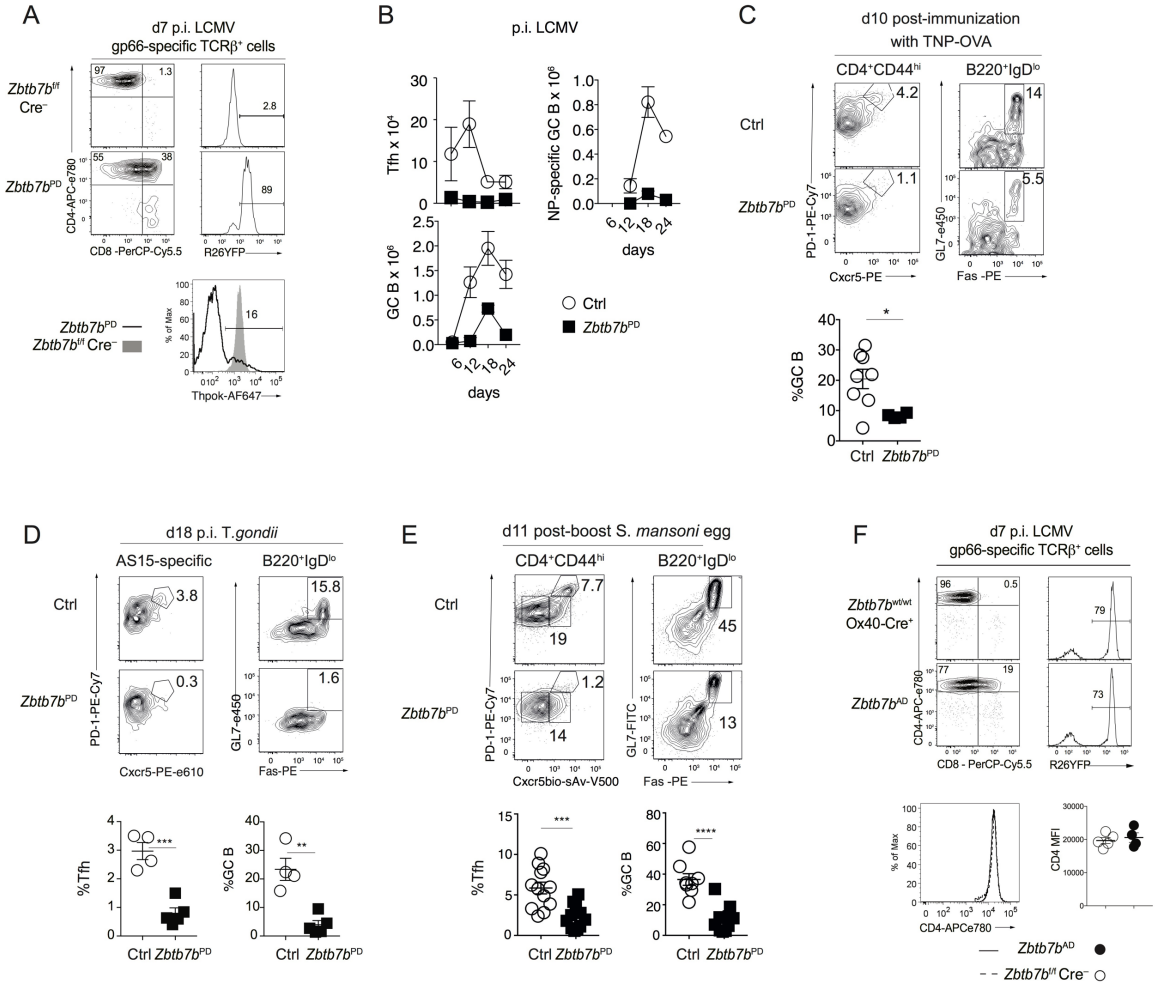
Supplemental Figure 1, Related to Figure 1

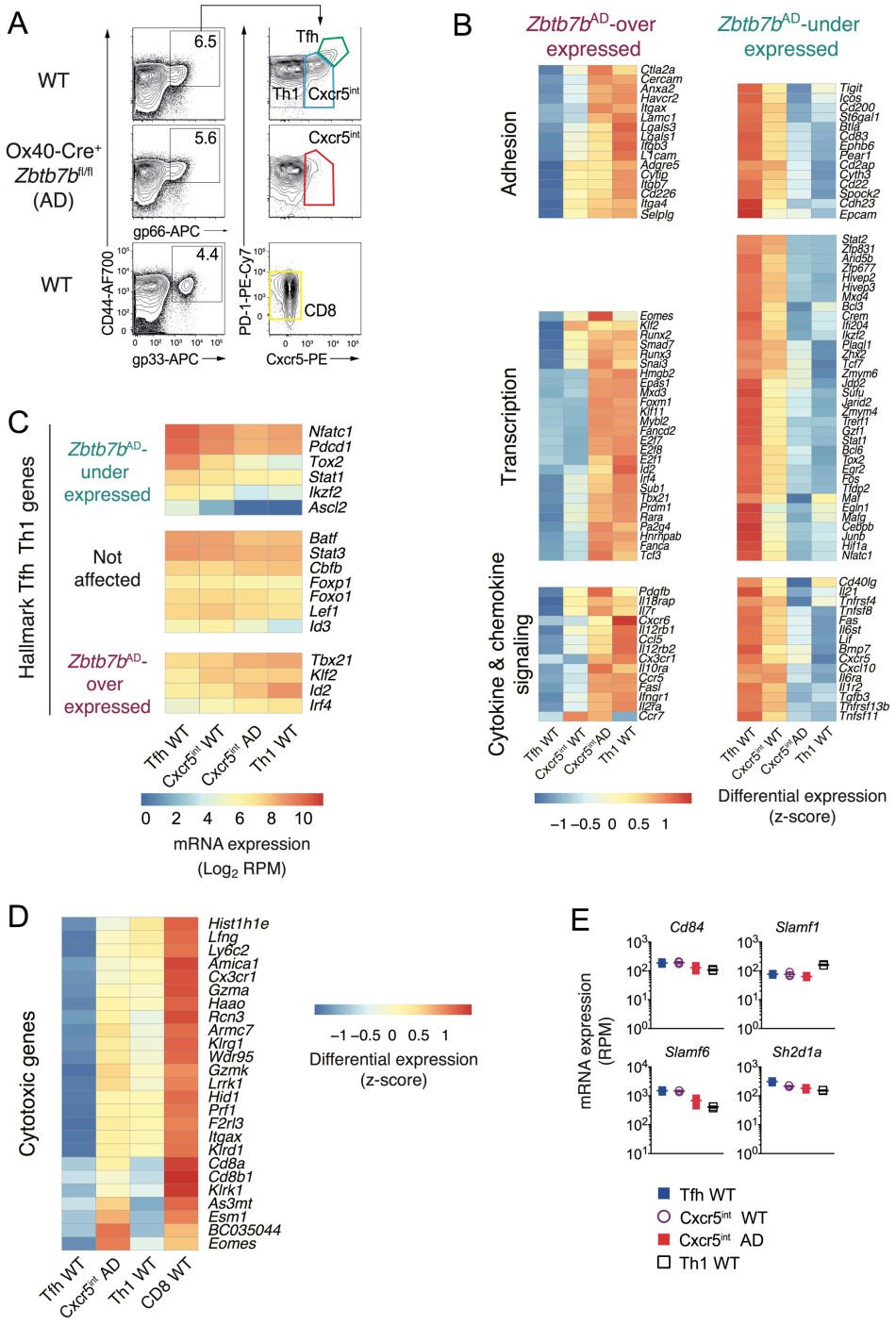


Supplemental Figure 1 (related to Figure 1). Thpok deletion impairs Tfh and GC B differentiation in response to multiple immunogens.

(**A**) (top) Representative plots show expression of CD4 and CD8 (left) or of *Rosa26*^{YFP} (R26YFP, right) as a reporter for Cre recombinase activity, on TCRβ^{+} I-A^{b}-gp66 tetramer binding (gp66) spleen cells from *Zbtb7b*^{PD} and *Zbtb7b*$^{fl/fl}$ Cre^{-} littermate control mice at d7 p.i. LCMV. (bottom) Representative histograms of Thpok expression in *Zbtb7b*^{PD} or control (Cre^{-}) gp66-specific T cells (number represents percentage of *Zbtb7b*^{PD} cells expressing intra-cellular Thpok protein. (**B**) Enumeration of Tfh, B220^{+}IgD^{lo} Fas^{+}GL7^{+} total GC B and NP-specific GC B cells in the spleen of *Zbtb7b*^{PD} or control mice infected with LCMV and analyzed at the indicated times p.i. Data is from one experiment with 4 (control) and 3 (*Zbtb7b*^{PD}) mice for each time point. (**C**) *Zbtb7b*^{PD} or control mice were immunized intra-peritoneally with 100μg TNP-OVA and analyzed 10 days post immunization. Contour plots (top) gated on indicated populations identify Tfh (left, not YFP-gated) and GC B (right) subsets as defined in Fig. 1AB; data is representative of 4 experiments. Graph (bottom) indicates percent of GC B cells (Ctrl, n=8, *Zbtb7b*^{PD}, n=4

mice) * P = 0.03. (**D**) *Zbtb7b*^{PD} or control mice were orally infected with 10 *T. gondii* cysts. Contour plots (top) indicate spleen Tfh (left, gated on *T. gondii* AS15 peptide-specific CD4^{+} T cells) and GC B cells, both defined as in Fig. 1AB, at d18 p.i. Data is representative of two experiments summarized in graphs (bottom, each symbol represents an individual mouse); ** P = 0.001 *** P = 0.0004 (Student t-test). (**E**) *Zbtb7b*^{PD} and control mice were injected at day 1 and 14 with inactivated S. *mansoni* eggs and analyzed 11-13 days after the second injection. Contour plots (top) show Cxcr5 vs. PD1 expression gated on CD44^{hi} CD4^{+} spleen T cells and Fas vs. GL7 expression gated on B220^{+} IgD^{lo} spleen B cells. Data is representative of three experiments, summarized in graphs (bottom; control, n=8; *Zbtb7b*^{PD} , n=10 mice); ***P < 0.005 **** P<10^{-4} (Student t-test). (**F**) (top) contour plots of CD4 and CD8 expression on gp66-specific T cells from *Zbtb7b*^{AD} and Ox40-Cre^{+} *Zbtb7b*$^{wt/wt}$ control mice; (bottom) Histogram overlays (left) and graph summary (MFI, right) of CD4 expression in d7 p.i. gp66-specific cells.

Supplemental Figure 2, Related to Figure 3

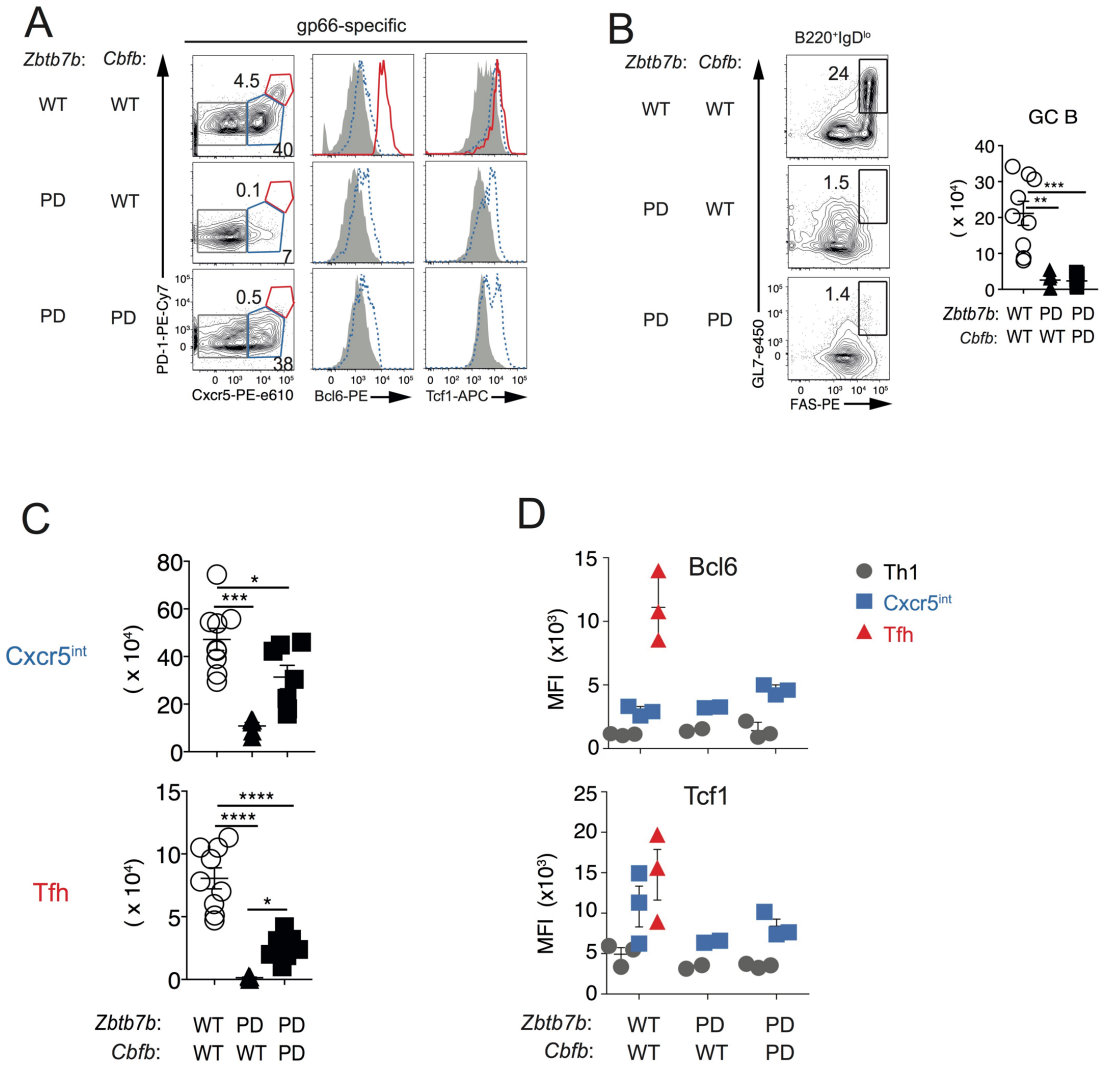


Supplemental Figure 2 (related to Figure 3). Impact of Thpok on the Tfh cell transcriptome.

(**A**) Cell purification strategy for RNAseq experiments shown in Fig. 3. CD44^{hi} gp66-specific CD4^{+} Rosa26YFP^{+} T cells from *Zbtb7b*^{AD} or Ox40-Cre^{+} *Zbtb7b*$^{wt/wt}$ controls, and CD44^{hi} gp33-specific CD8^{+} T cells from Ox40-Cre^{+} *Zbtb7b*$^{wt/wt}$ control mice, were sorted

from spleen cells at d8 p.i. with-LCMV. (**B**) Heatmaps show row-standardized (z-scores of average RPM values, scale at bottom) mRNA expression of genes part of adhesion (top) transcription (middle) or cytokine signaling (bottom) Gene Ontology signatures and showing a 2-fold or greater differential expression between wild-type Tfh and *Zbtb7b*^{AD} Cxcr5^{int} CD4^{+} T cells. For each heatmap, data is shown for wild-type Tfh and Cxcr5^{int} (first two columns) and Th1 cells (fourth column) and *Zbtb7b*^{AD} Cxcr5^{int} cells (AD, third columns). (**C**) Impact of *Zbtb7b* disruption on genes involved in Tfh differentiation. Heatmaps show the normalized mRNA expression (Log_2 of average RPM values, scale at bottom) in cell subsets as in (B). (**D**) Heatmap shows row-standardized expression of cytotoxic signature genes, defined by 2-fold or greater expression in wild-type LCMV-specific CD8^{+} T cells than in wild-type Th1 CD4^{+} T cells. Data is shown as in (B) for wild-type Tfh and Th1 CD4^{+} T cells (first and third columns), *Zbtb7b*^{AD} Cxcr5^{int} CD4^{+} T cells (AD, second column) and wild-type LCMV gp33-specific CD8^{+} T cells (fourth column). (**E**) Plots show expression of genes involved in SLAM adhesion-signaling displayed as in Fig. 3C.

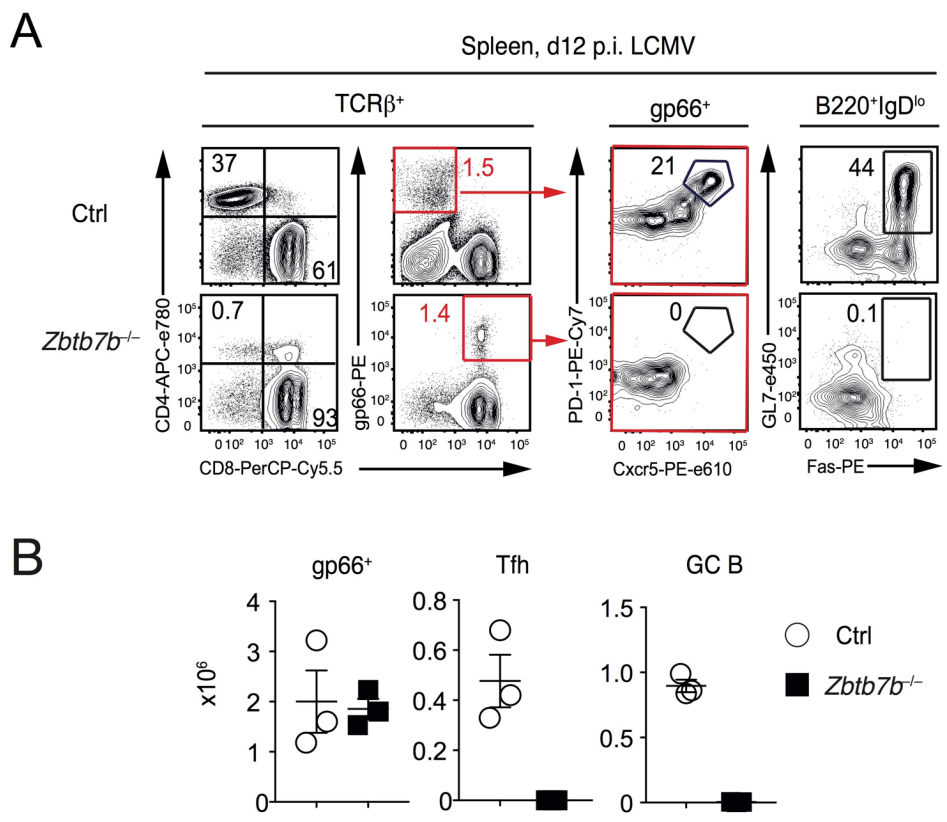
Supplemental Figure 3, Related to Figure 4



Supplemental Figure 3 (related to Figure 4). Runx disruption fails to rescue the Tfh differentiation of Thpok-deficient CD4⁺ T cells.

(**A**) Contour plots (left) of Cxcr5 vs. PD1 expression on gp66-specific spleen CD4⁺ T cells define Tfh (red), Cxcr5^{int} cells (dotted blue) and Th1 (grey) subsets that are analyzed for intra-cellular expression of Bcl6 and Tcf1 in overlaid histograms (middle and right). Data is representative of two independent experiments, with a total of 9 (Ctrl), 5 (*Zbtb7b*^{PD}) and 7 (*Zbtb7b*^{PD} *Cbfb*^{PD}) mice. (**B**) Contour plots (left) identify GC B cells; data is from the same experiment as in (A) and is summarized in graph (right). (**C**) Graphs summarize cell numbers from (A). (**D**) Quantification (MFI) of intra-cellular protein expression in gates defined in (A). Each symbol represents a separate mouse; **** P<0.0001, ***P<0.0003, **P<0.005, *P<0.05 (Student t-test).

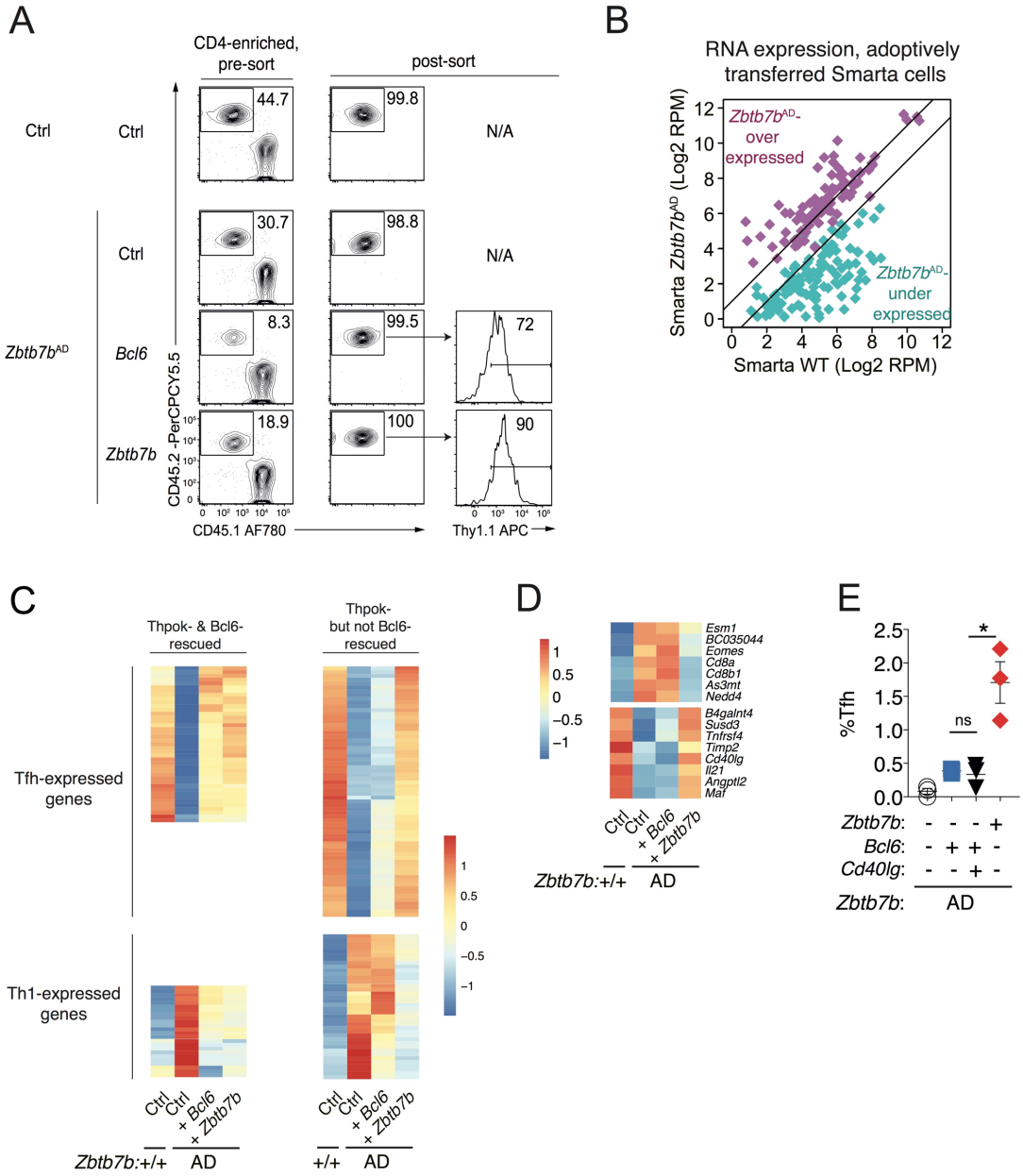
Supplemental Figure 4, Related to Figure 6



Supplemental Figure 4 (related to Figure 6). Germline Thpok inactivation prevents Tfh and GC B cell differentiation.

(**A**) *Zbtb7b*$^{-/-}$ and control littermates were analyzed d12 p.i with LCMV. Left two columns show CD4 and CD8 expression and CD8 expression vs. I-A^{b}-gp66-tetramer binding gated on TCRβ^{+} cells. gp66-specific cells (red box) are analyzed for Cxcr5 vs. PD1 expression to define Tfh cells (third column). The right column shows Fas vs. GL7 expression on B220^{+} IgD^{lo} spleen B cells. Note that germline deletion of *Zbtb7b* redirects MHC II-restricted T cells into the CD8^{+}-lineage (Vacchio and Bosselut, 2016). (**B**) Graphs summarize one experiment with 3 mice of each genotype and show numbers of indicated cell subsets in each genotype. Data is representative of three independent experiments.

Supplemental Figure 5, Related to Figure 7



Supplemental Figure 5 (related to Figure 7). Bcl6-independent impact of Thpok on the Tfh transcriptome.

(**A**) Sorting strategy of Smarta CD4$^+$ T cells generated as in Fig. 6A and prepared for RNAseq. Panels show pre-sort and post-sort populations for CD45 allele expression (left and center columns, respectively). Right histograms show Thy1.1 expression of CD45.2$^+$ sorted populations. Data is shown for Smarta cells that were either wild-type (Ctrl) or *Zbtb7b*^{AD} and had been transduced with retroviral vectors expressing Thy1.1 (as a reporter for transduction) and either no additional protein (Ctrl) or Bcl6 or Thpok (Bcl6 and Thpok "add-back"). Ctrl vector-transduced cells (top two rows) were not sorted for

Thy1.1 expression. (**B**) Scatter plot displays mRNA expression (Log_2 of average RPM values) in adoptively transferred, control vector-transduced Smarta cells, either wild-type (x-axis) or *Zbtb7b*^{AD} (y-axis) and processed as in (A). Data is shown on the gene set defined in Fig. 3B by a 4-fold or greater differential expression in wild-type Tfh compared to *Zbtb7b*^{AD} Cxcr5^{int} cells. Each symbol represents one gene and is color-coded as in Fig. 3B. Lines show 2-fold differential gene expression. (**C**) Heatmap shows row-standardized mRNA expression (z-scores of average RPM values, scale on the right) of 169 Tfh- or Th1-signature genes that were Thpok-dependent in "add-back" experiments (see Methods). (**D**) Heatmap shows row-standardized mRNA expression (z-scores of average RPM values, scale on the right) on relevant Thpok signature genes (see Methods). (**E**) Wild-type or *Zbtb7b*^{AD} Smarta CD4$^+$ T cells were retrovirally transduced with the indicated vectors and processed for analyses of response to LCMV as shown in Fig. 6A. Graph summarizes percent of Tfh cells among transduced Smarta *Zbtb7b*^{AD} cells; data is from one experiment with 3 mice from each transduction. Significance determined by Student t-test; * $P<0.02$.