С А В d10 post-immunization d7 p.i. LCMV p.i. LCMV with TNP-OVA ap66-specific TCR6+ cells B220⁺lgD^₀ CD4+CD44^h NP-specific GC B x 106 30 1.01 Zbtb7b^t 0.8 Cre 20 Tfh x 10⁴ Ctrl 0.6 0.4 10 0.2 Zbtb7b 89 0.0 0 Zbtb7b^P 2.5 6,2,82× 2.0 è CD8 -PerCP-Cv5 5 0 10° 10° D26VED davs GC B x 10⁶ 1.5 Fas -PE Cxcr5-PE 1.0 O Ctrl 0.5 40 ■ Zbtb7b^{PD} Zbtb7b^{PD} Zbtb7b^{t/f}Cre-0.0 30-8 %GC B 6,2,82 độ Độ 20-Thpok-AF647days 10 0 Ctrl Zbtb7bPD d11 post-boost S. mansoni egg d7 p.i. LCMV D d18 p.i. T.gondii Е F gp66-specific TCRβ⁺ cells B220⁺lgD^₀ CD4+CD44h B220⁺lgD¹⁰ AS15-specific 15.8 3.8 Ctrl Ctrl Zbtb7b^{wt/w} Ox40-Cre 19 1.6 -PE-Cy7 0.3 1.2 Zbtb7b^{PD} Zbtb7b^₄D Zhth7h^{PD} 5-1-1 ğ 14 P0-1-H CD8 - PerCP-Cy5.5 104 105 R26YEP Fas-PE -Cxcr5-PE-e610 0 10³ 10⁴ 10⁶ Cxcr5bio-sAv-V500 15 80 40 60 %GC B 30 C 10 6GC B ğ %Tfh %Tfh 40 20 2 $0 \ 10^2 \ 10^3$ CD4-APCe780 o Zhth7h^{AD} Ctrl Zbtb7bPC Ctrl Zbtb7bPD Ctrl Zbtb7bPD Ctrl Zbtb7bPC - - Zbtb7b^{t/t}Cre- O

1 Supplemental Figure 1, Related to Figure 1

2

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

5 (A) (top) Representative plots show expression of CD4 and CD8 (left) or of Rosa26^{YFP} 6 (R26YFP, right) as a reporter for Cre recombinase activity, on TCRβ⁺ I-A^b-gp66 tetramer 7 binding (gp66) spleen cells from Zbtb7b^{PD} and Zbtb7b^{fl/fl} Cre⁻ littermate control mice at 8 d7 p.i. LCMV. (bottom) Representative histograms of Thpok expression in $Zbtb7b^{PD}$ or 9 control (Cre⁻) gp66-specific T cells (number represents percentage of Zbtb7b^{PD} cells 10 expressing intra-cellular Thpok protein. (B) Enumeration of Tfh, B220⁺IgD¹⁰ Fas⁺GL7⁺ total GC B and NP-specific GC B cells in the spleen of Zbtb7b^{PD} or control mice infected 11 12 with LCMV and analyzed at the indicated times p.i. Data is from one experiment with 4 13 (control) and 3 ($Zbtb7b^{PD}$) mice for each time point. (C) $Zbtb7b^{PD}$ or control mice were 14 immunized intra-peritoneally with 100µg TNP-OVA and analyzed 10 days post 15 immunization. Contour plots (top) gated on indicated populations identify Tfh (left, not 16 YFP-gated) and GC B (right) subsets as defined in Fig. 1AB; data is representative of 4

17 experiments. Graph (bottom) indicates percent of GC B cells (Ctrl, n=8, Zbtb7b^{PD}, n=4

- 18 mice) * P = 0.03. (**D**) Zbtb7b^{PD} or control mice were orally infected with 10 T. gondii
- 19 cysts. Contour plots (top) indicate spleen Tfh (left, gated on T. gondii AS15 peptide-
- 20 specific CD4⁺ T cells) and GC B cells, both defined as in Fig. 1AB, at d18 p.i. Data is
- 21 representative of two experiments summarized in graphs (bottom, each symbol represents
- 22 an individual mouse); ** P = 0.001 *** P = 0.0004 (Student t-test). (E) Zbtb7b^{PD} and
- 23 control mice were injected at day 1 and 14 with inactivated S. mansoni eggs and analyzed
- 24 11-13 days after the second injection. Contour plots (top) show Cxcr5 vs. PD1 expression
- 25 gated on CD44^{hi} CD4⁺ spleen T cells and Fas vs. GL7 expression gated on B220⁺ IgD^{lo}
- 26 spleen B cells. Data is representative of three experiments, summarized in graphs
- 27 (bottom; control, n=8; $Zbtb7b^{PD}$, n=10 mice); ***P < 0.005 **** P<10⁻⁴ (Student t-test).
- 28 (F) (top) contour plots of CD4 and CD8 expression on gp66-specific T cells from
- 29 Zbtb7b^{AD} and Ox40-Cre⁺ Zbtb7b^{wt/wt} control mice; (bottom) Histogram overlays (left) and
- 30 graph summary (MFI, right) of CD4 expression in d7 p.i. gp66-specific cells.

Zbtb7b^{AD}-over Zbtb7b^{AD}-under A В Tfh 6.5 expressed expressed WT Th1 Cxcr5" Adhesion 5.6 Cxcr5^{int} Ox40-Cre⁺ Zbtb7b^{fl/fl} (AD) gp66-APC 4.4 PE-CV7 CD44-AF700 WΤ CD8 Grem Ifi204 Ikzf2 Plagi Zhx2 Tcf7 Zmyr Jdp2 Sufu 6 102 103 104 105 0 10³ 104 10 gp33-APC -. Cxcr5-PE Transcription С Nfatc1 Pdcd1 Tox2 Stat1 Ikzf2 Ascl2 Zbtb7b^{AD}-Hallmark Tfh Th1 genes under expressed Batf Stat3 Cbfb Foxp1 Foxo1 Lef1 Id3 Not Cytokine & chemokine affected frsf4 signaling Tbx21 Klf2 Id2 Irf4 Zbtb7b^{AD}over Tgfb3 Tnfrsf13b Tnfsf11 expressed LILL VACUUM CACION AD ThINT CXCIST WT THINK to CACIONAD THINK AN WY N. CA 4 2 Differential expression 2 4 6 8 10 (z-score) 0 -1-0.5 0 0.5 1 mRNA expression (Log₂ RPM) D Е Hist1h1e Lfng Ly6c2 Amica1 Cx3cr1 Gzma Haao Cd84 Slamf1 10 103 10² 10² mRNA expression (RPM) 10¹ 10 aao cn3 Cytotoxic genes 10 10 -1-0.5 0 0.5 1 Slamf6 Sh2d1a Differential expression 10' Gzml 103 (z-score) 10³ 10 F2rl3 Itgax Klrd1 Cd8a 10 10 10¹ 100 Cd8b1 Klrk1 As3mt Esm1 BC035044 Eomes Tfh WT O Cxcr5^{int} WT Cxcr5^{int} AD THONT CALL AD AN AN □ Th1 WT

31 Supplemental Figure 2, Related to Figure 3

32

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

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

- 38 from spleen cells at d8 p.i. with-LCMV. (B) Heatmaps show row-standardized (z-scores
- 39 of average RPM values, scale at bottom) mRNA expression of genes part of adhesion
- 40 (top) transcription (middle) or cytokine signaling (bottom) Gene Ontology signatures and
- 41 showing a 2-fold or greater differential expression between wild-type Tfh and $Zbtb7b^{AD}$
- 42 Cxcr5^{int} CD4⁺ T cells. For each heatmap, data is shown for wild-type Tfh and Cxcr5^{int}
- 43 (first two columns) and Th1 cells (fourth column) and Zbtb7b^{AD} Cxcr5^{int} cells (AD, third
- 44 columns). (C) Impact of *Zbtb7b* disruption on genes involved in Tfh differentiation.
- 45 Heatmaps show the normalized mRNA expression (Log₂ of average RPM values, scale at
- 46 bottom) in cell subsets as in (B). (D) Heatmap shows row-standardized expression of
- 47 cytotoxic signature genes, defined by 2-fold or greater expression in wild-type LCMV-
- 48 specific CD8⁺ T cells than in wild-type Th1 CD4⁺ T cells. Data is shown as in (B) for
- 49 wild-type Tfh and Th1 CD4⁺ T cells (first and third columns), *Zbtb7b*^{AD} Cxcr5^{int}CD4⁺ T
- 50 cells (AD, second column) and wild-type LCMV gp33-specific CD8⁺ T cells (fourth
- 51 column). (E) Plots show expression of genes involved in SLAM adhesion-signaling
- 52 displayed as in Fig. 3C.



53 Supplemental Figure 3, Related to Figure 4

54

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

57 (A) Contour plots (left) of Cxcr5 vs. PD1 expression on gp66-specific spleen CD4⁺ T

58 cells define Tfh (red), Cxcr5^{int} cells (dotted blue) and Th1 (grey) subsets that are analyzed

59 for intra-cellular expression of Bcl6 and Tcf1 in overlaid histograms (middle and right).

- 60 Data is representative of two independent experiments, with a total of 9 (Ctrl), 5
- 61 $(Zbtb7b^{PD})$ and 7 $(Zbtb7b^{PD} Cbfb^{PD})$ mice. (**B**) Contour plots (left) identify GC B cells;
- 62 data is from the same experiment as in (A) and is summarized in graph (right). (C)
- 63 Graphs summarize cell numbers from (A). (D) Quantification (MFI) of intra-cellular
- 64 protein expression in gates defined in (A). Each symbol represents a separate mouse;

65 **** P<0.0001, ***P<0.0003, **P<0.005, *P<0.05 (Student t-test).

Α Spleen, d12 p.i. LCMV TCRβ⁺ B220⁺lgD^{Io} gp66+ 37 1.5 21 Ctrl 61 10 0.7 10 **PC-e780** 1.4 0 0.1 ۲ Ω 104 Û 10 10 Zbtb7b^{_/_} 10 GL7-e450 Щ 비 103 gp66-PD-1. CD4-/ 10 0 10³ 10⁴ 10² 10³ 10⁴ 0 102 10 10² 10³ 10⁴ 10² 10³ 10⁴ 10 0 10⁵ 0 CD8-PerCP-Cv5.5 Fas-PE Cxcr5-PE-e610 В Tfh GC B gp66+ 4 0.8 1.5 Ctrl \bigcirc 0.6 3 x10⁶ 1.0 Zbtb7b^{-/-} 2 0.4 0.5 1 0.2 0 0 0

66 Supplemental Figure 4, Related to Figure 6

67

68 Supplemental Figure 4 (related to Figure 6). Germline Thpok inactivation prevents 69 Tfh and GC B cell differentiation.

- 70 (A) $Zbtb7b^{--}$ and control littermates were analyzed d12 p.i with LCMV. Left two
- 71 columns show CD4 and CD8 expression and CD8 expression vs. I-A^b-gp66-tetramer
- 72 binding gated on TCR β^+ cells. gp66-specific cells (red box) are analyzed for Cxcr5 vs.
- 73 PD1 expression to define Tfh cells (third column). The right column shows Fas vs. GL7
- 74 expression on B220⁺ IgD¹⁰ spleen B cells. Note that germline deletion of *Zbtb7b* redirects
- 75 MHC II-restricted T cells into the CD8+-lineage (Vacchio and Bosselut, 2016). (B)
- 76 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 independentexperiments.



79 Supplemental Figure 5, Related to Figure 7

80

81 Supplemental Figure 5 (related to Figure 7). Bcl6-independent impact of Thpok on 82 the Tfh transcriptome.

83 (A) Sorting strategy of Smarta CD4⁺ T cells generated as in Fig. 6A and prepared for

84 RNAseq. Panels show pre-sort and post-sort populations for CD45 allele expression (left

85 and center columns, respectively). Right histograms show Thy1.1 expression of CD45.2⁺

86 sorted populations. Data is shown for Smarta cells that were either wild-type (Ctrl) or

87 *Zbtb7b*^{AD} and had been transduced with retroviral vectors expressing Thy1.1 (as a

88 reporter for transduction) and either no additional protein (Ctrl) or Bcl6 or Thpok (Bcl6

89 and Thpok "add-back"). Ctrl vector-transduced cells (top two rows) were not sorted for

- 90 Thy1.1 expression. (**B**) Scatter plot displays mRNA expression (Log₂ of average RPM
- 91 values) in adoptively transferred, control vector-transduced Smarta cells, either wild-type
- 92 (x-axis) or $Zbtb7b^{AD}$ (y-axis) and processed as in (A). Data is shown on the gene set
- 93 defined in Fig. 3B by a 4-fold or greater differential expression in wild-type Tfh
- 94 compared to Zbtb7b^{AD} Cxcr5^{int} cells. Each symbol represents one gene and is color-coded
- 95 as in Fig. 3B. Lines show 2-fold differential gene expression. (C) Heatmap shows row-
- 96 standardized mRNA expression (z-scores of average RPM values, scale on the right) of
- 97 169 Tfh- or Th1-signature genes that were Thpok-dependent in "add-back" experiments
- 98 (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
- 100 Methods). (E) Wild-type or Zbtb7b^{AD} Smarta CD4⁺ T cells were retrovirally transduced
- 101 with the indicated vectors and processed for analyses of response to LCMV as shown in
- 102 Fig. 6A. Graph summarizes percent of Tfh cells among transduced Smarta $Zbtb7b^{AD}$
- 103 cells; data is from one experiment with 3 mice from each transduction. Significance
- 104 determined by Student t-test; * P<0.02.