Supplementary Figure 1 | Alternative gating strategy for the identification of T_{FH} cells. (a) Representative lymphocyte gates for the identification of T_{FH} cells. T_{FH} cells were analyzed by FACS in the spleens of wild type (upper panel) and DKO (Def6^{tm/tr}Swap-70^{--}) (lower panel) mice. After gating on CD4^+ T helper cells (left panel), CD4^+PD1^{hi}CXCR5^{hi} cells (middle panel) were defined as T_{FH} cells (CD4^+CXCR5^{hi}PD1^{hi}CD44^+). All the T_{FH} cells in this study were evaluated by using this gating strategy. (b-e) Quantification of the frequencies and numbers of T_{FH} cells (CD4^+CXCR5^{hi}PD1^{hi}CD44^+Foxp3^+) after excluding follicular regulatory T cells (CD4^+CXCR5^{hi}PD1^{hi}CD44^+Foxp3^+) based on Foxp3 staining (right upper panel). (e,f)(CD4^+CXCR5^{hi}PD1^{hi}CD44^+Foxp3^+) in young (b,e,f) and aging (c,d) mice of the indicated genotypes.
Supplementary Figure 2 | Spontaneous accumulation of GC B cells and plasma cells in DKO mice. GC B cells were analyzed by FACS in the spleens from young (6-11-week-old) (a-d) and aging (>24 week-old) wt and DKO (Def6frfr, Swap-70−/−) mice (e-h). WT (open circle), DKO (closed circle). (a) Representative FACS plots for GL7 and FAS gated on B220+ cells from young mice. (b) Quantification of GC B cells in young mouse (n=16-17). (c) Representative FACS plots for B220 and CD138 gated on live cells from young mice. (d) Quantification of plasma cells in young mouse (n=16-17). (e) Representative FACS plots for GL7 and FAS gated on B220+ cells in aging mice. (f) Quantification of GC B cells in aging mice (n=11-12). (g) Representative FACS plots for B220 and CD138 gated on live cells from aging mice. (h) Quantifications of plasma cells in aging mice (n=8-9). Each dot represents an individual mouse. Error bars indicate mean ± s.d., p value by unpaired t-test.
Supplementary Figure 3 | T<sub>FH</sub> cells are localized in the germinal center and produce IFN-γ and IL-21 in aging DKO mice. (a) Quantification of T<sub>FH</sub> cells in germinal centers from wt mice 8 days post-immunization with NP-CGG and young DKO unimmunized mice. (b) Immunofluorescence images of splenic sections from aging (>24 weeks old) wt and DKO (Def6<sup>Δ/Δ</sup> Swap-70<sup>−/−</sup>) mice. Representative images from two independent experiments with 2 to 3 mice each. PNA, green; PD1, red; CD3, magenta. Scale bar, 100μm. (c-e) Representative FACS plots showing intracellular stains of IL-21 and IFN-γ (d) or IL-21 and IL-17 (e) gated on either CD4<sup>+</sup> T cells or CXCR5<sup>hi</sup>PD1<sup>hi</sup>CD4<sup>+</sup> T cells (c) in the spleens from aging (>24 week-old) mice.
Supplementary Figure 4 | Bcl6 mRNA and protein levels in wt and DKO T cells. (a) Gating strategy for the sorting of naïve T cells for in vitro culture (top panel) and representative FACS plots for CD44 and PD1 (bottom left panel) or FACS histograms of Bcl6 expression (bottom right panel) gated on naïve T cells (CD4+CD44+PD1−) from spleens of young mice (6-11-week old) of the indicated genotypes. Data are representative of two independent experiments. (b) The abundance of Bcl6 protein in Fig. 3a was quantified and normalized to Lamin B. The levels of Bcl6 in the wt samples were set as 100%. (c) Naïve T cells from wt, Def6 heterozygous (Def6+/−) and Swap−70−/− mice were stimulated with anti-CD3 and anti-CD28 for three days. Nuclear extracts were evaluated by Western blotting with antibodies to Bcl6 and IRF4. HDAC1 was used as a loading control (top panel). Quantification of Bcl6 signal intensity is shown (bottom panel). (d) Western blots showing the phosphorylation of Stat1, Stat3, and Stat4 in wt and DKO T cells stimulated with anti-CD3 and anti-CD28 for three days. (e) Naïve T cells from wt and DKO mice were stimulated with anti-CD3 and anti-CD28 in the presence of IL-12 and anti-IL-4 (Til1) for three days (top panel). Nuclear extracts were evaluated by Western blotting with antibodies against Bcl6 and T-bet. HDAC1 was used as a loading control (bottom panel). Quantitative RT-PCR analysis of the expression of Bcl6 and T-bet mRNA. The data were normalized relative to β-Actin mRNA expression. Data are representative of two independent experiments. (f) Schematic representation showing the organization of Bcl6 primary transcript. Exons are shown as boxes numbered 1-10, whereas introns are shown as flexed lines. The shaded rectangular boxes indicate the protein-coding regions. Bcl6 mRNA was quantified using primer pair 1 in Fig. 3b and primer pair 2 in Supplementary Fig. 4g. (g) Quantitative RT-PCR analysis of the expression of Bcl6 mRNA in naïve T cells stimulated with anti-CD3 and anti-CD28 for three days. The data were normalized relative to Hprt mRNA expression. (h) Autoradiography of SDS-PAGE gel to detect [35S]-labeled proteins from nuclear or cytoplasmic extracts of T cells cultured for 3 days followed by metabolic labeling with [35S]methionine/cysteine for 1 hr. Uncropped, full-size scans of autoradiogram shown in Fig. 3e. Data are representative of two independent experiments. NE; nuclear extract, CE; cytoplasmic extract.
Supplementary Figure 5 | Analysis of Bcl6 mRNA expression and mTORC1 targets. (a) The abundance of Bcl6 protein in the Western blots of Fig. 4a was quantified and normalized to Lamin B. The levels of Bcl6 in the wt control were set as 100%. (b) Quantitative RT-PCR analysis of the expression of Bcl6 mRNA in sorted naïve wt or DKO T cells cultured as described in Figure 3. The data were normalized relative to β-Actin mRNA expression and are representative of two independent experiments. (c) The phosphorylation levels of 4E-BP proteins in Fig. 4b were quantified and normalized to Lamin B. The ratios of the levels of p-4E-BP to Lamin B are shown. (d) Western blot analysis of the levels of p-S6K1 in extracts from wt or DKO T cells. The phosphorylation levels of S6K1 protein were quantified and normalized to S6K1. The levels of p-S6K1 in the vehicle-treated wt control were set as 100%. (e) Quantification of the frequencies and numbers of T$_{FH}$ cells (CD4$^+$CXCR5$^{hi}$PD1$^{hi}$) in wt, DKO, and BXSX-Yaa mice (n=3-4). Each dot represents an individual mouse. Error bars indicate mean ± s.d., p value by unpaired t-test. (f) Representative FACS plots for CD44 and PD1 (top panel) or FACS histograms showing p-4E-BP expression (bottom panel) gated on naïve T cells (CD4$^+$CD44$^-$PD1$^-$) in spleen from young (4-14-week-old) mice of the indicated genotype. Data are representative of at least two independent experiments. (g) Representative FACS plots for CXCR5 and PD1 (top panel), BTLA and PD1 (bottom panel), or FACS histograms showing p-4E-BP expression (right panel) gated on indicated T cells in spleens from mice that were immunized 5 days earlier. Data are representative of two independent experiments. (h) The abundance of Bcl6 protein in the Western blots of Fig. 4f were quantified and normalized to HDAC1. Data are representative of two independent experiments. (i) Quantitative RT-PCR analysis of the expression of Bcl6 mRNA in sorted naïve wt or DKO T cells cultured as indicated in Figure 3 and treated with vehicle control or the indicated dose of 4EGI-1 for the final 24 hours of the three-day culture. The data were normalized relative to hprt mRNA expression and are representative of two independent experiments.
Supplementary Figure 6 | Mapping of the interaction of DEF6 with p62.
(a) Schematic representation showing protein interaction domains of SQSTM1/p62 and the deletion mutants utilized in the mapping experiments. PB1; a Phox and Bem1p domain, ZZ; an atypical zinc finger, TBS; TRAF6 binding sequence, UBA; a carboxy-terminal ubiquitin-associated domain. (b) 293T cells were cotransfected with HA-DEF6 (FL) and either Flag-p62 (FL 1-440), Flag-p62 (128-440), Flag-p62 (225-440), Flag-p62 (267-440), Flag-p62 (1-386), Flag-p62 (1-266), or Flag-p62 (1-224) expression constructs. HA-DEF6 immunoprecipitates were analyzed by Western blotting with the indicated antibodies to determine the interaction between DEF6 and various p62 deletion mutants. aa; amino acid, FL; full length.
Supplementary Figure 7 | Regulation of DKO T_FH cells by mTORC1 in vivo. (a-d) Analysis of the spleens from aging (>35 week-old) DKO mice treated with vehicle control or rapamycin (3 mg kg\(^{-1}\)) daily for 10 days. Combined data from two independent experiments. (n=9 for the control mice and n=8 for rapamycin treated mice). (a) Quantification of CD4\(^+\) T cells. (b) Representative FACS plots for GL7 and FAS gated on B220\(^+\) cells. (c) Quantification of GC B cells (GL7\(^+\)FAS\(^+\)B220\(^+\)). (d) Quantifications of B220\(^+\) B cells. (e) Representative FACS plots for CXCR5 and PD1 on CD4\(^+\) T cells from CD4\(^{cre}\) or CD4\(^{cre}\)raptor\(^{f/f}\) mice that were either unimmunized (top panel) or immunized with NP-KLH in alum 7 days previously (bottom panel). The data are representative of two independent experiments (n=5-6). (f) Quantification of T_FH cells (CD4\(^+\)CXCR5\(^{hi}\)PD1\(^{hi}\)) (n=5-6) in CD4\(^{cre}\) and CD4\(^{cre}\)raptor\(^{f/f}\) mice. Mice were either unimmunized or immunized with NP-KLH in alum 7 days previously. Combined data from two independent experiments. Each dot represents an individual mouse. Error bars indicate mean ± s.d., p value by two-tailed t-test. (g,h) Quantification of CD4\(^+\) T cells in the spleens of wt, CD4\(^{cre}\)raptor\(^{f/f}\), DKO, and CD4\(^{cre}\)raptor\(^{f/f}\)/DKO mice (n=10-14). Combined data from four independent experiments. Each dot represents an individual mouse. Error bars indicate mean ± s.d., p value by two-tailed t-test. (i) Quantification of CD4\(^+\) T cells producing IFN-γ and IL-21 in the spleens of wt, CD4\(^{cre}\)raptor\(^{f/f}\), DKO, and CD4\(^{cre}\)raptor\(^{f/f}\)/DKO mice analyzed by intracellular flow cytometry. Data were combined from two independent experiments. (j,k) Quantification of B220\(^+\) B cells in the spleens of wt, CD4\(^{cre}\)raptor\(^{f/f}\), DKO, and CD4\(^{cre}\)raptor\(^{f/f}\)/DKO mice (n=10-14). Combined data from four independent experiments.
Supplementary Figure 8 | Localization of Def6 and P62. (a) Immunofluorescence images of Def6 and p62 in T\textsubscript{FH} cell and naive T cells sorted from wt mice that were immunized with NP-CGG 7 days earlier. Representative images from two independent experiments. Def6, green; p62, red; DAPI, Blue. Scale bar, 50µm
Supplementary Figure 9 | Uncropped images of the original scans of Western blots. Uncropped, full-size scans of immunoblots shown in Fig. 3a, Fig. 3c, Fig. 4a, Fig. 4b, and Fig. 4f.
Supplementary Figure 10 | Uncropped images of the original scans of Western blots.
Uncropped, full-size scans of immunoblots shown in Fig. 5a-i.
Supplementary Figure 11 | Uncropped images of the original scans of western blots. Uncropped, full-size scans of immunoblots shown in Fig. 6b, Fig. 6c, Supplementary Fig. 4c, Supplementary Fig. 4d, Supplementary Fig. 4e, and Supplementary Fig. 5d.