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SUPPLEMENTARY INFORMATION

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Tet2 and Tet3 in B cells are required to repress CD86 and prevent autoimmunity

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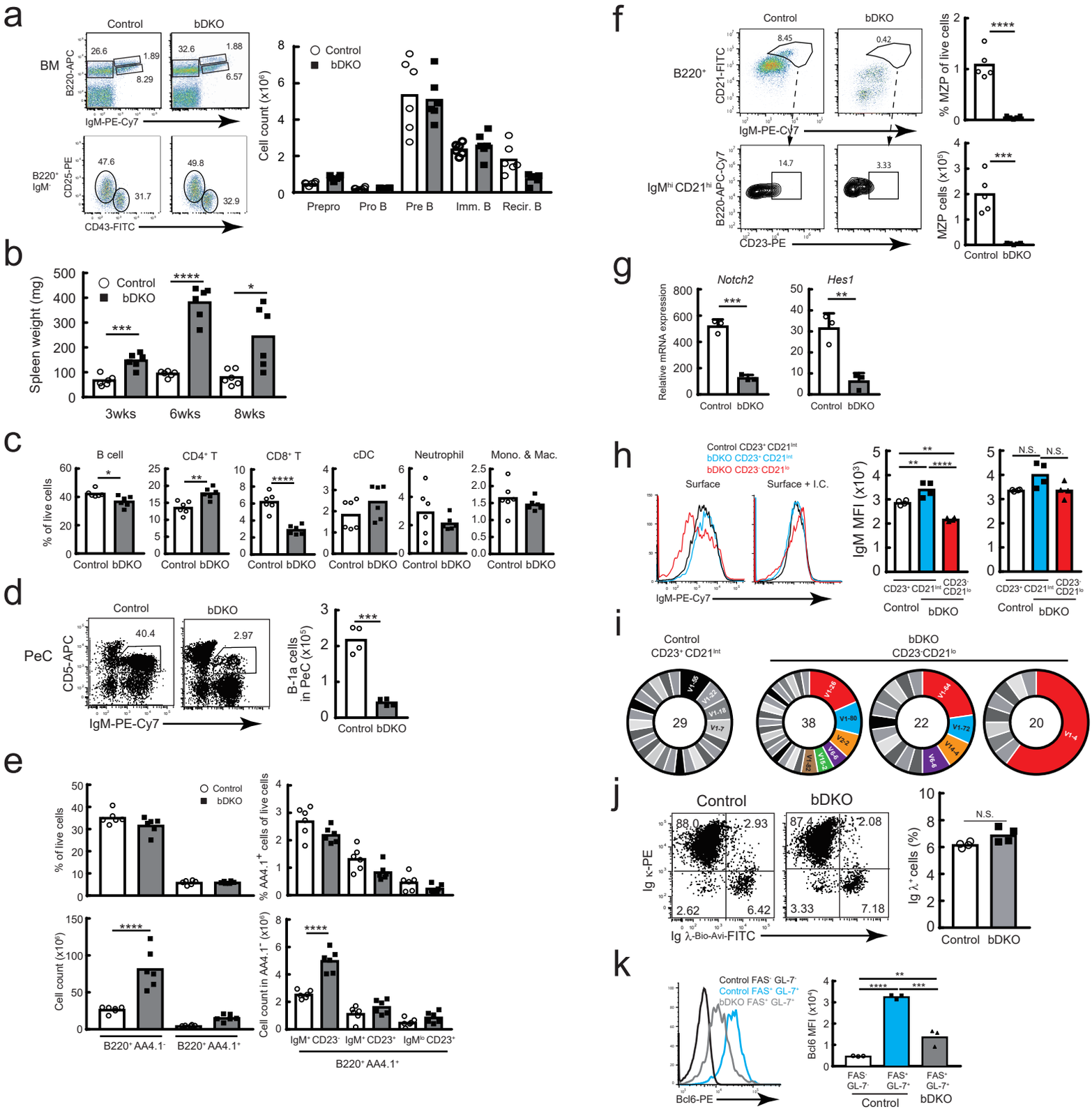
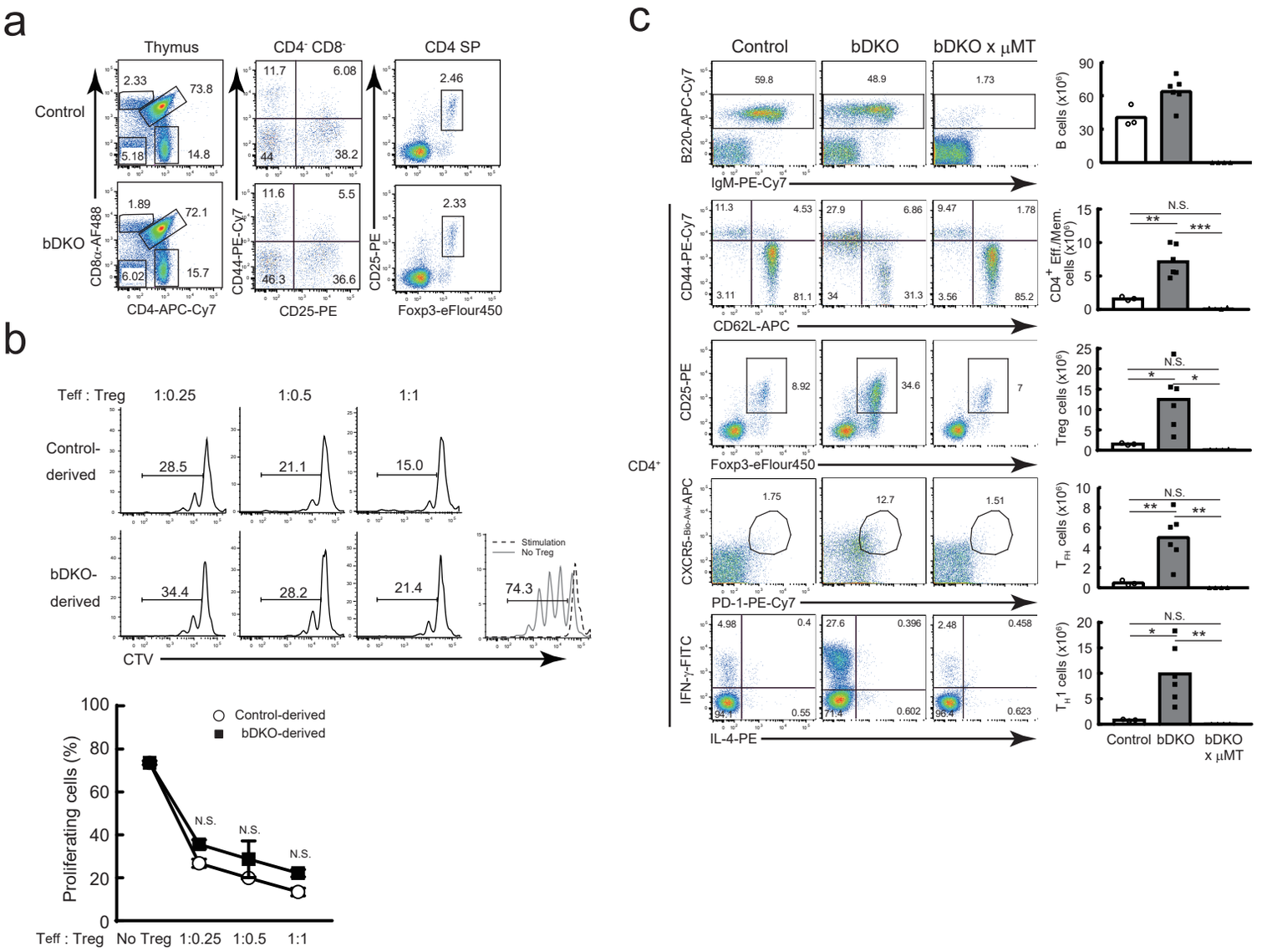


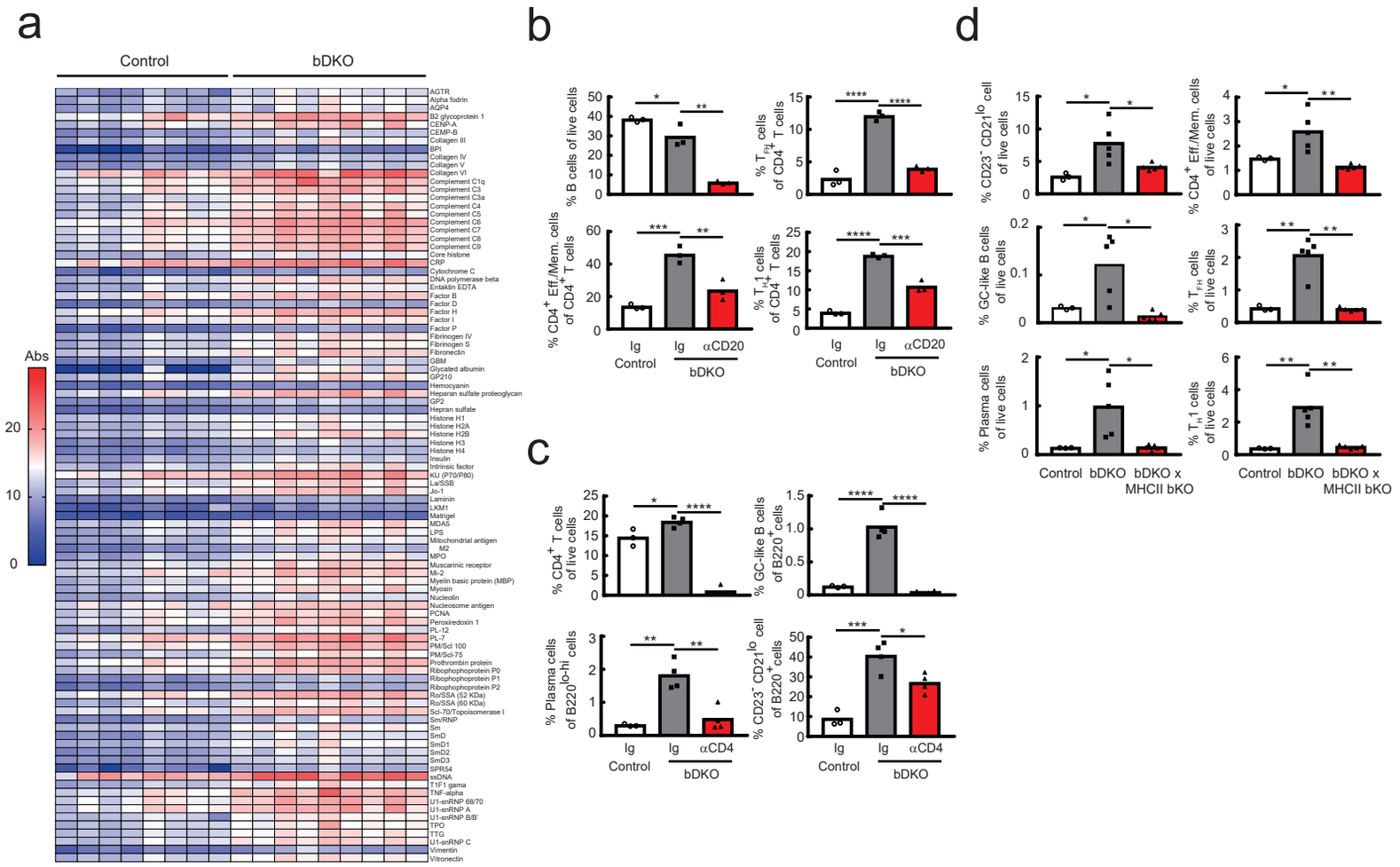
Supplementary figure 1 Deletion efficiency of Tet2 and Tet3 and cellular analysis in bDKO mice

a, Percentages (Left panels) and absolute number (Right panel) of developing B cells in BM isolated from 6-week-old control and bDKO mice (n=6 each): prepro B (B220$^+$ IgM$^-$ CD25$^-$ CD43$^+$ CD19$^-$), pro B (B220$^+$ IgM$^-$ CD25$^-$ CD43$^+$ CD19$^+$), pre B (B220$^+$ IgM$^-$ CD25$^+$ CD43$^-$), immature B (B220^{Int} IgM$^+$) and recirculating B (B220^{hi} IgM$^+$) cells. **b**, Spleen weight of 3, 6 and 8-week-old control (n=6) and bDKO (n=6) mice. **c**, Frequency of splenic B cells, CD4$^+$ T cells, CD8$^+$ T cells, cDCs, Neutrophils, Mono.& Mac. from 6-week-old control (n=6) and bDKO (n=6) mice. **d**, Representative flow plot for IgM and CD5 expression by peritoneal cavity (PeC) lymphocytes from control and bDKO mice (Left panels). Absolute numbers of IgM$^+$ CD5$^+$ B-1a cells from individual mice (n=4, Right panel). **e**, Frequency (Upper panels) and number (Lower panels) of splenic mature (B220$^+$ AA4.1$^-$), transitional (B220$^+$ AA4.1$^+$) B and transitional B cell subsets of control and bDKO mice (n=6 each). **f**, Frequency and number of MZ precursors (MZPs) (B220$^+$ IgM^{hi} CD21^{hi} CD23$^+$) of 6-week-old control (n=5) and bDKO (n=5) mice. Data are representative of two (f) or three (a, b, c, d, e) experiments. **g**, Notch2 and Hes1 mRNA expressions in splenic B220$^+$ AA4.1$^+$ cells of control and bDKO mice (n=3 each). Data are mean ± S.D. **h**, Representative histogram showing surface (Left histogram) and surface plus intracellular (I.C.) (Right histogram) IgM expression in Fo and B220$^+$ CD23$^-$ CD21^{lo} cells of 6-week-old control and bDKO mice (n=4 each). **i**, Immunoglobulin heavy chain usage of AA4.1$^-$ CD23$^+$ CD21^{Int} and AA4.1$^-$ CD23$^-$ CD21^{lo} splenocytes of control (n=1) and bDKO (n=3) mice, respectively. The individual V segments was indicated, in case the same V segments was found in more than two clones. **j**, Representative plot (Left panels) for Ig κ and Ig λ in immature BM B cells (B220^{Int} IgM$^+$) and the frequency (Right panel) of Ig λ$^+$ immature B cells of 6-week-old control (n=4) and bDKO (n=4) mice. Data are representative of three experiments (h, j). **k**, Comparison of expression level of intracellular Bcl6 expression detected in Peyer's patch control B220$^+$ FAS$^+$ GL-7$^+$, B220$^+$ FAS$^-$ GL-7$^-$ and splenic DKO B220$^+$ FAS$^+$ GL-7$^+$ cells (n=3 each). Data are representative of two experiments. Significance was determined by one-way ANOVA followed by Sidak's multiple comparison test (a), unpaired two-tailed Student's t-test (b, c, d, f, g, j) or one-way ANOVA followed by Tukey's multiple comparison test (e, h, k). (*<0.05, **<0.01, ***<0.001, ****<0.0001, N.S.: not significant)



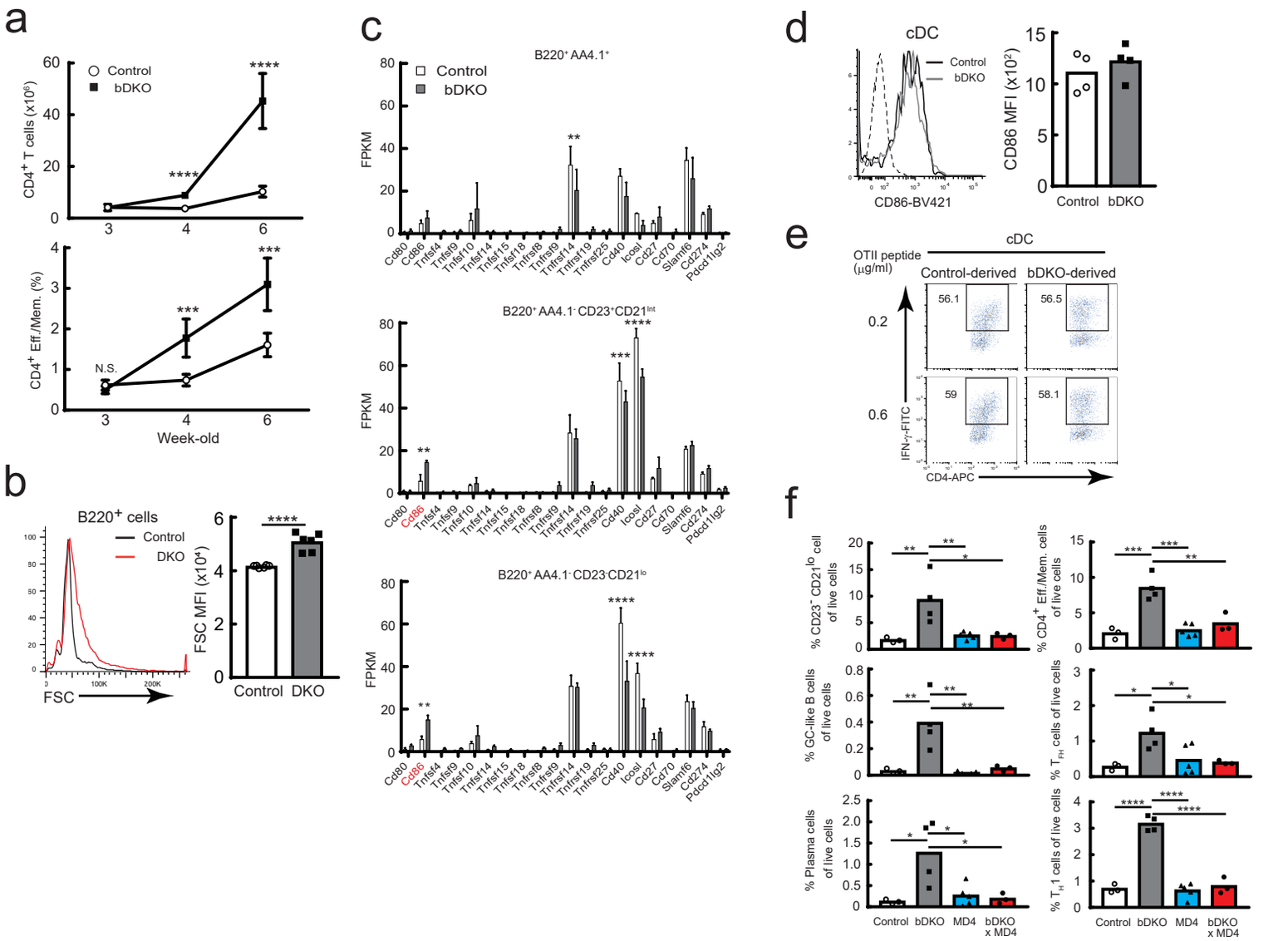
Supplementary figure 2 Normal T cell development and Treg function *in vitro*

a, Frequencies of developing T cells in the thymus of 6-week-old control and bDKO mice: shown are CD4 single positive (CD4 SP), CD8 single positive (CD8 SP), double positive (DP), double negative [DN; DN1 ($CD44^+$ $CD25^-$), DN2 ($CD44^+$ $CD25^+$), DN3 ($CD44^-$ $CD25^+$), DN4 ($CD44^-$ $CD25^-$)] and Treg ($CD25^+$ $Foxp3^+$ in CD4 SP) cells. Data are representative of three experiments. **b**, Suppression assay of $CD4^+$ T cells co-cultured with Treg cells derived from 6-week-old control and bDKO mice (n=3 each). Data are mean ± S.D. and representative of two experiments. **c**, Representative flow plots of splenic B, $CD4^+$ Eff./Mem., Treg, Tfh and Th1 cells derived from control (n=3), bDKO (n=6) and bDKO x μMT mice (n=4) (Left panels). Absolute numbers of these lymphocyte populations are shown (Right panels). Data are representative of three experiments. Significance was determined by two-way ANOVA followed by Sidak' s (b) and one-way ANOVA followed by Tukey' s (c) multiple comparison test. (*<0.05, **<0.01, ***<0.001, N.S.: not significant)



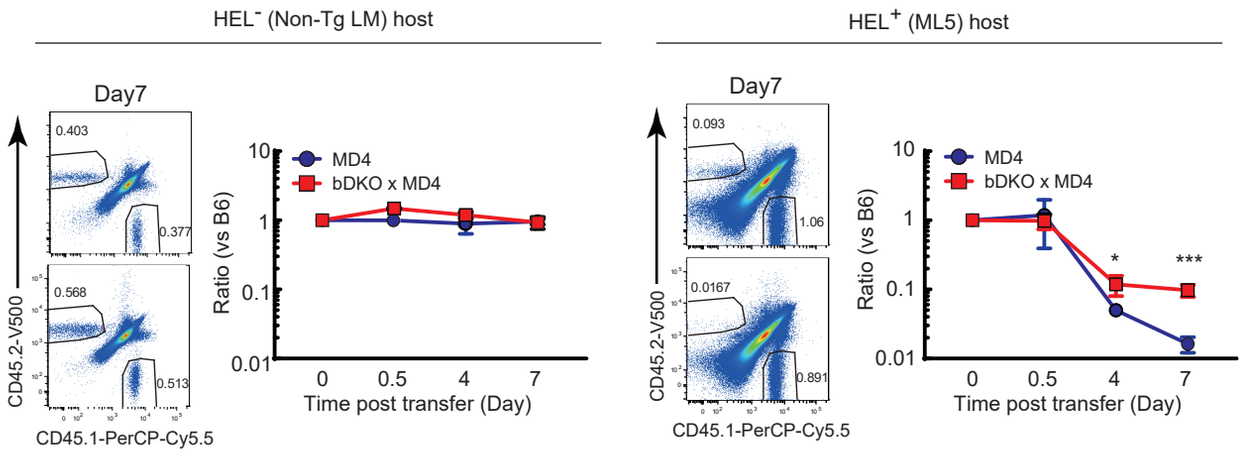
Supplementary figure 3 Polyreactive B cells against self-antigens in bDKO mice

a, Polyreactivity of serum isolated from control (n=8) and bDKO mice (n=9) tested by autoantibody array assay. The assay between groups were conducted using the R package 'limma' and multiple comparisons correction was performed (BH-adjusted p value < 0.05 between tested groups). The statistically significant autoantibody data was used for heatmap generation. **b**, Frequency of B, $CD4^+$ Eff./Mem., Tfh and Th1 cells derived from isotype control Ig-treated control (n=3), -bDKO (n=3) and anti-CD20 mAb-treated bDKO mice (n=3). **c**, Frequency of $CD4^+$ T, plasma, GC-like B and $CD23^-$ $CD21^{lo}$ cells derived from Ig-treated control (n=3), -bDKO (n=4) and anti-CD4 mAb-treated bDKO mice (n=4). Data are representative of two experiments (b, c). **d**, Frequency of $CD23^-$ $CD21^{lo}$, GC-like B, plasma, $CD4^+$ Eff./Mem., Tfh and Th1 cells derived from control (n=3), bDKO (n=5) and bDKO x MHCII bKO mice (n=5). Data are representative of three experiments. Significance was determined by one-way ANOVA followed by Tukey' s multiple comparison test (b, c, d). (*<0.05, **<0.01, ***<0.001, ****<0.0001)



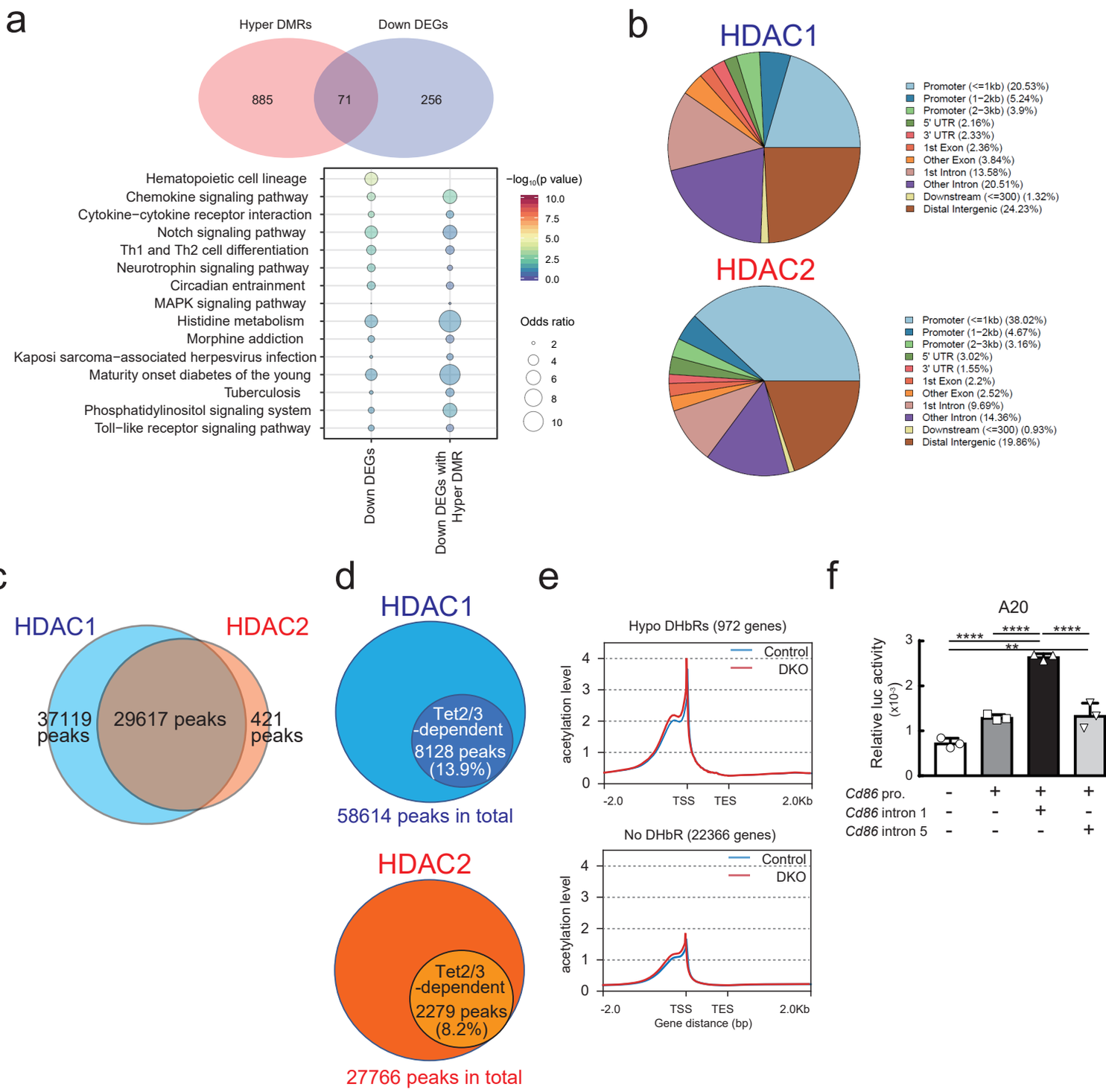
Supplementary figure 4 Time course of CD4⁺ T cell activation and DC activity in bDKO mice

a, Absolute number of total CD4⁺ T cells (Left panel) and the frequency of CD4⁺ Eff./Mem. cells (Right panel) in 3, 4, and 6-week-old control and bDKO mice (n=5-6). Data are mean ± S.D. and representative of three experiments. **b**, Cell size of splenic B220⁺ cells of control and bDKO mice, detected by FSC [control (n=6) and bDKO (n=6)] **c**, RNA-seq expression of selected genes encoding co-stimulatory molecules in B220⁺ AA4.1⁺, B220⁺ AA4.1⁻ CD23⁺ CD21^{int} and B220⁺ AA4.1⁻ CD23⁻ CD21^{lo} B cells isolated, by FACS, from spleen of 4-week-old age matched control and bDKO mice (n=3 each) determined. Data are mean ± S.D. **d**, representative histogram showing CD86 expression on cDC in 6-week-old control and bDKO mice (Left panel). CD86 MFI of individual mice (n=4 each) is shown (Right panel). **e**, Percentage of IFN-γ⁺ CD4⁺ cells derived from co-culture of OTII T cells with cDCs purified from control and bDKO mice in the presence of OTII peptide. **f**, Frequency of CD23⁻ CD21^{lo}, GC-like B, plasma, CD4⁺ Eff./Mem., Tfh and Th1 cells derived from 6-week-old control (n=3), bDKO (n=4), MD4 Tg (n=5) and bDKO x MD4 (n=3) mice. Data are representative of three independent experiments (b, d, e, f). Significance was determined by two-way ANOVA followed by Sidak' s multiple comparison test (a, c), unpaired two-tailed Student' s t-test (b, d) and one-way ANOVA followed by Tukey' s multiple comparison test (f). (*<0.05, **<0.01, ***<0.001, ****<0.0001, N.S.: not significant)



Supplementary figure 5 Persistence of Tet2/Tet3-deficient B cells in a peripheral tolerance model

Representative flow plot for CD45.1/1 B6 B cells (internal control) and CD45.2/2 MD4 or MD4-DKO B cells in non-Tg LM (Left panels, n=3) or ML5 mice (Right panels, n=3-4). The ratio of MD4 or MD4-DKO B cell vs control B cells is shown before (0 day) and 0.5, 4 and 7 days after transfer. Data are mean ± S.D. and representative of three independent experiments. Significance was determined by two-way ANOVA followed by Sidak' s multiple comparison test. (*<0.05, ***<0.001)



Supplementary figure 6 Genome-wide analyses for HDAC binding and modifications of DNA methylation and H3 acetylation in DKO B cells

a, Integrative analysis of downDEGs [analyzed byDESeq2 (FDR<0.01)] with hyper DMRs (Upper panel). Enrichment GO terms analyzed by KEGG enrichment analysis for gene sets indicated (Lower panel). For statistics, one-sided Fisher' s exact test of EnrichR R package was used. Data are representative of one experiment with at least two biological replicates for bisulfite-seq and RNA-seq (n=3 each for control and DKO). **b**, Genome-wide distribution of HDAC1/HDAC2 peaks of control B cells. **c**, Overlapping peaks of HDAC1 and HDAC2 of control B cells. **d**, Frequency of Tet2/Tet3-dependent and -independent binding of HDAC1 and HDAC2. **e**, Line plots of acetylation level of H3K9/14 at gene loci associated with hypo DHbRs and no DHbR. Data are representative of one experiment with at least two biological replicates for bisulfite- and ChIP-seq. **f**, Luciferase assay with the A20 B lymphoma (n=3) transduced with luciferase reporter plasmids [promoter less pGL-3, pGL-3 containing the Cd86 promoter (-1287/+303), pGL-3-promoter/intron 1 (+29822/+31053) and pGL-3-promoter/intron 5 (+57634/+588001)]. Data are mean ± S.D. and representative of three experiments. Significance was determined by one-way ANOVA followed by Tukey' s multiple comparison test (f). (**<0.01, ****<0.0001)