Supplementary Information

Differential Transcriptional Programming of Class-Specific B Cell Memory by T-bet and RORα

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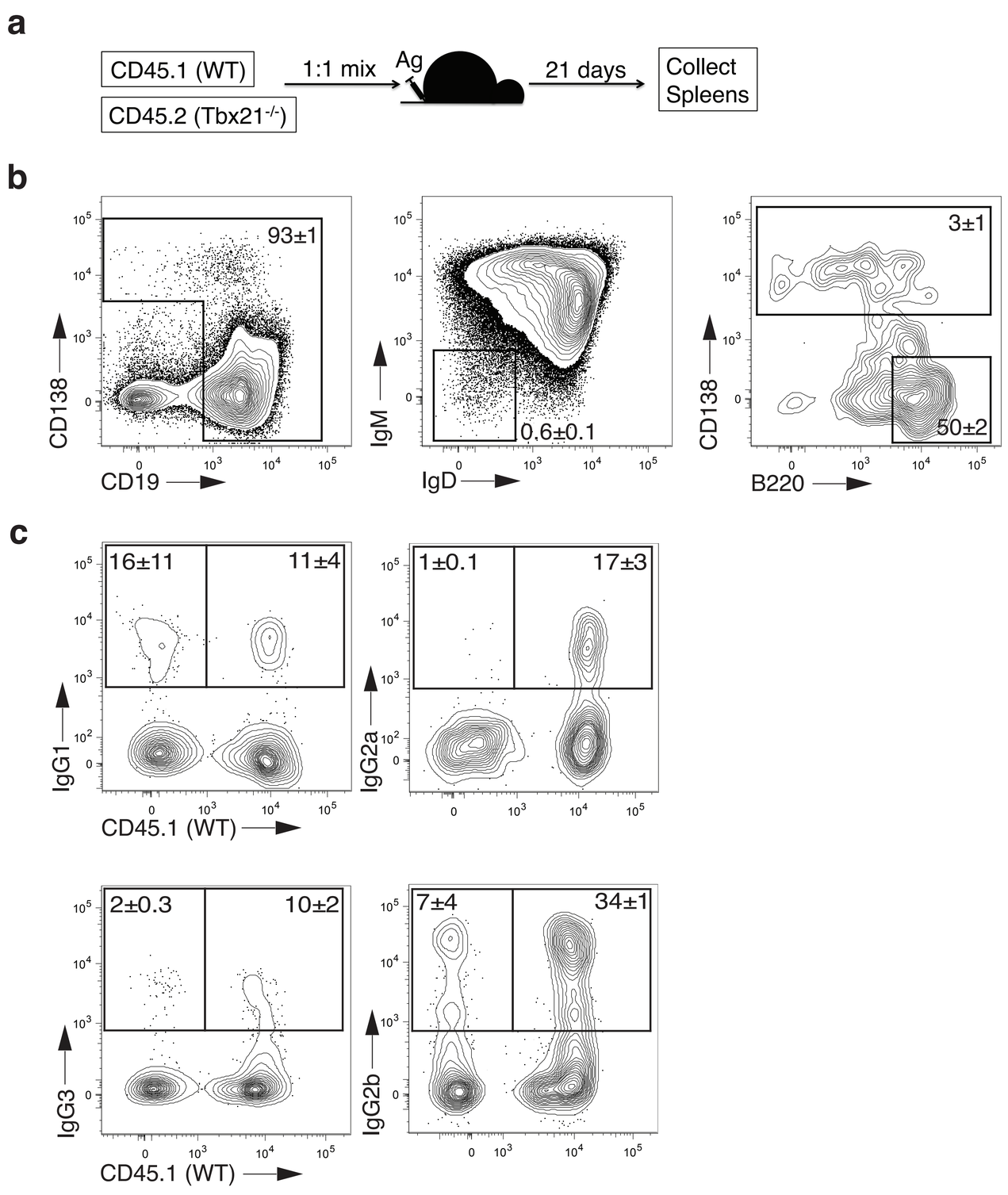
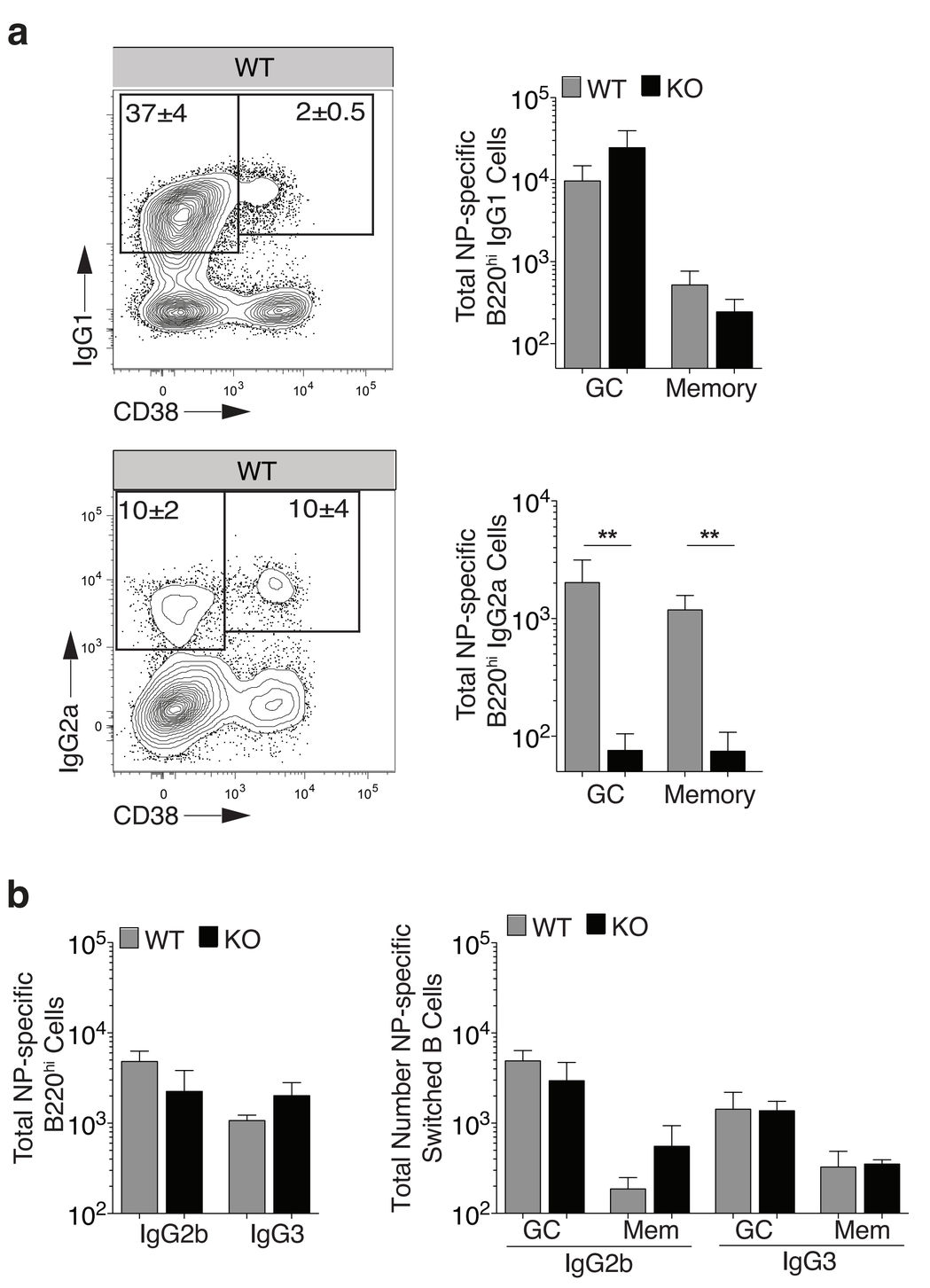


Supplementary Figure 1. Intact germline knockout pre-transfer

(a) Schematic for generation of peripheral chimeras using Tbx21$^{-/-}$ (CD45.2) and wt (CD45.1) donors.

(b) Gating strategy showing B cell gate (CD19^{+} or CD138^{+}) already gated on Gr1^{-}CD4^{-}CD8^{-}, Switch gate (IgM^{-}IgD^{-}) and B220^{hi} or Plasma cell (CD138^{+}) gate.

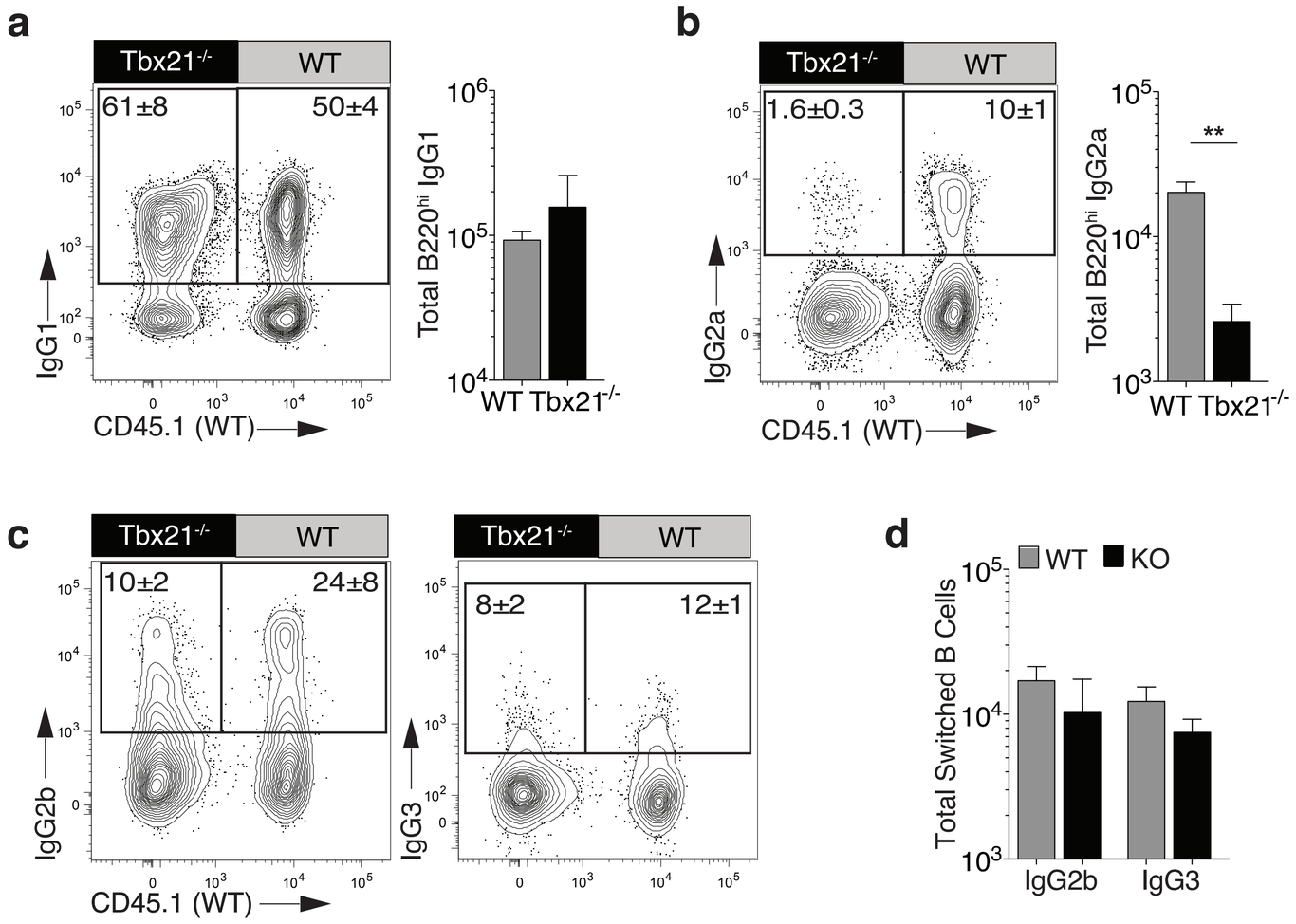
(c) Rag1$^{-/-}$ mice received 1:1 mixture of splenocytes from Tbx21$^{-/-}$ (CD45.2) and wt (CD45.1) donors. Representative expression of isotype staining pre-transfer. Frequency of isotypes is mean±sem of same congenic compartment, n=5, and on B cells (Gr1-CD4-CD8-CD138-CD19+B220hi) that are switched (IgM-IgD-).



Supplementary Figure 2. NP-specific response in peripheral chimeras

(a) Splenic NP-specific response in Rag1-/- mice 21 days after receiving 1:1 mixture of splenocytes from CD45.1 (WT) and Tbx21-/- donors following by an immunization of NP-KLH in adjuvant. Frequency and total cell number of NP-specific switched B cells ($Gr1^{-}CD4^{-}CD8^{-}$ CD19 or $CD138^{+}$ $IgM^{-}IgD^{-}$ NP^{+}) . n=5 $Rag1^{-/-}$ mice. ** P value<0.01 (Mann-Whitney Test).

(b) Total switched NP-specific $B220^{hi}$ B cells that are $IgG2b^{+}$ or $IgG3^{+}$ from same experiment as in part (a). Frequency represent percentage of same congenic compartment.



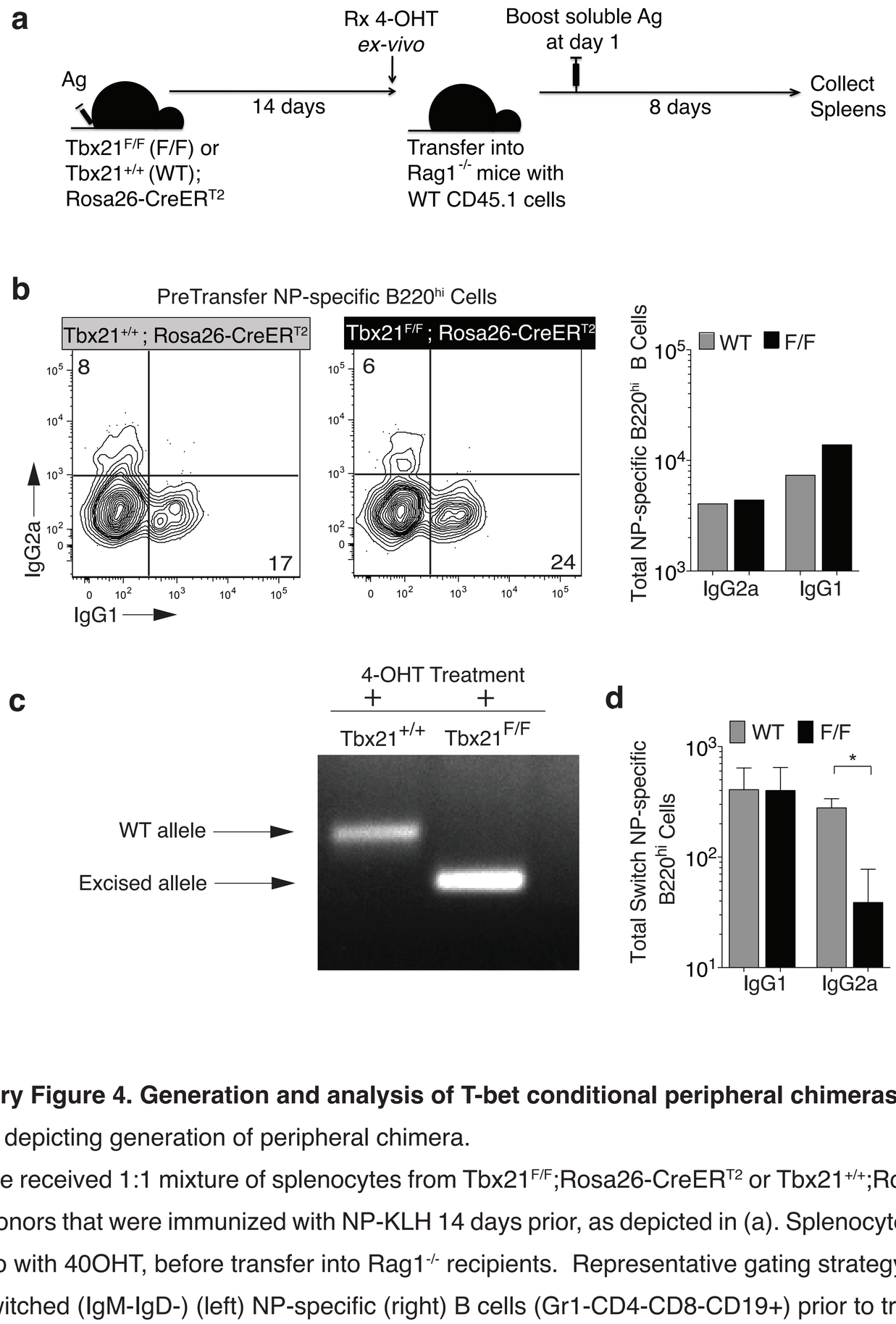
Supplementary Figure 3. Polyclonal B cell response in peripheral chimera

(a) Rag1$^{-/-}$ mice received 1:1 mixture of splenocytes from Tbx21$^{-/-}$ (CD45.2) and wt (CD45.1) donors. Representative IgG1 expression 21 days after immunization in polyclonal B cell (Gr1-CD4-CD8-CD138-CD19+B220^{hi}) switched (IgM-IgD-) compartment (left). Mean±sem; n=5 Rag1$^{-/-}$ mice.

(b) Representative frequency IgG2a expression 21 days after immunization in polyclonal switched B cell compartment (left) and total numbers (right). Isotype frequencies are displayed as percentage of same congenic compartment. Mean±sem; n=5 Rag1$^{-/-}$ mice. ** P value<0.01 (Mann Whitney Test).

(c) Representative frequency of isotype expression 21 days after immunization in polyclonal switched B cell compartments. Mean±sem; n=5 Rag1$^{-/-}$ mice.

(d) Total numbers from (c). Mean±sem; n=5 Rag1$^{-/-}$ mice.



Supplementary Figure 4. Generation and analysis of T-bet conditional peripheral chimeras

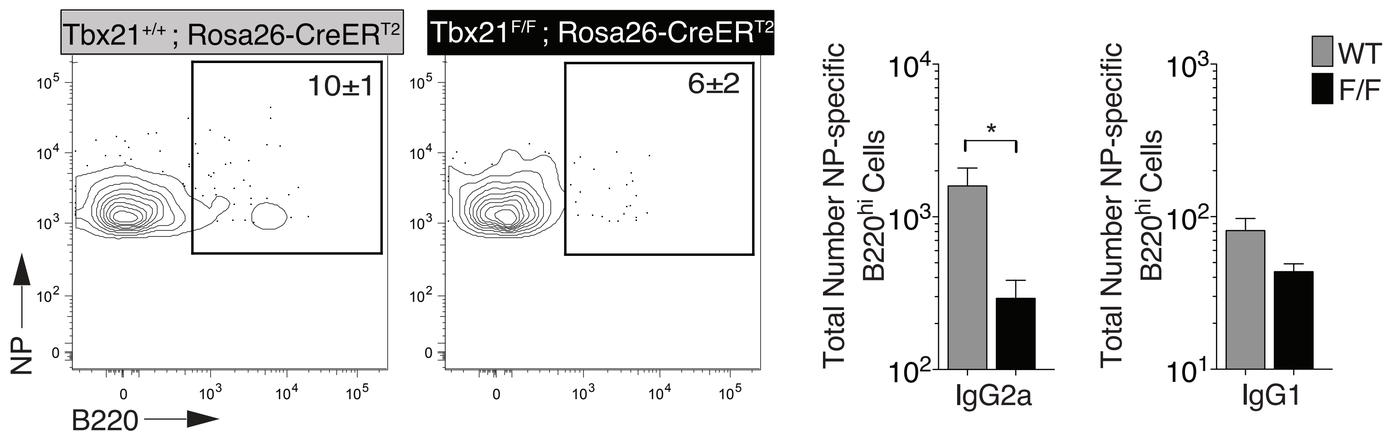
(a) Schematic depicting generation of peripheral chimera.

(b) Rag1$^{-/-}$ mice received 1:1 mixture of splenocytes from Tbx21$^{F/F}$;Rosa26-CreER^{T2} or Tbx21$^{+/+}$;Rosa26-CreER^{T2} (CD45.2) or wt (CD45.1) donors that were immunized with NP-KLH 14 days prior, as depicted in (a). Splenocytes from these animals were treated ex-vivo with 40OHT, before transfer into Rag1$^{-/-}$ recipients. Representative gating strategy to identify and enumerate the input of switched (IgM-IgD-) (left) NP-specific (right) B cells (Gr1-CD4-CD8-CD19+) prior to transfer.

(c) 1x10^{6} splenocytes that were treated with 4-OHT from (b) were set aside in ex vivo culture for 24 hours. PCR on genomic DNA from cells to assess Tbx21 excision following drug treatment.

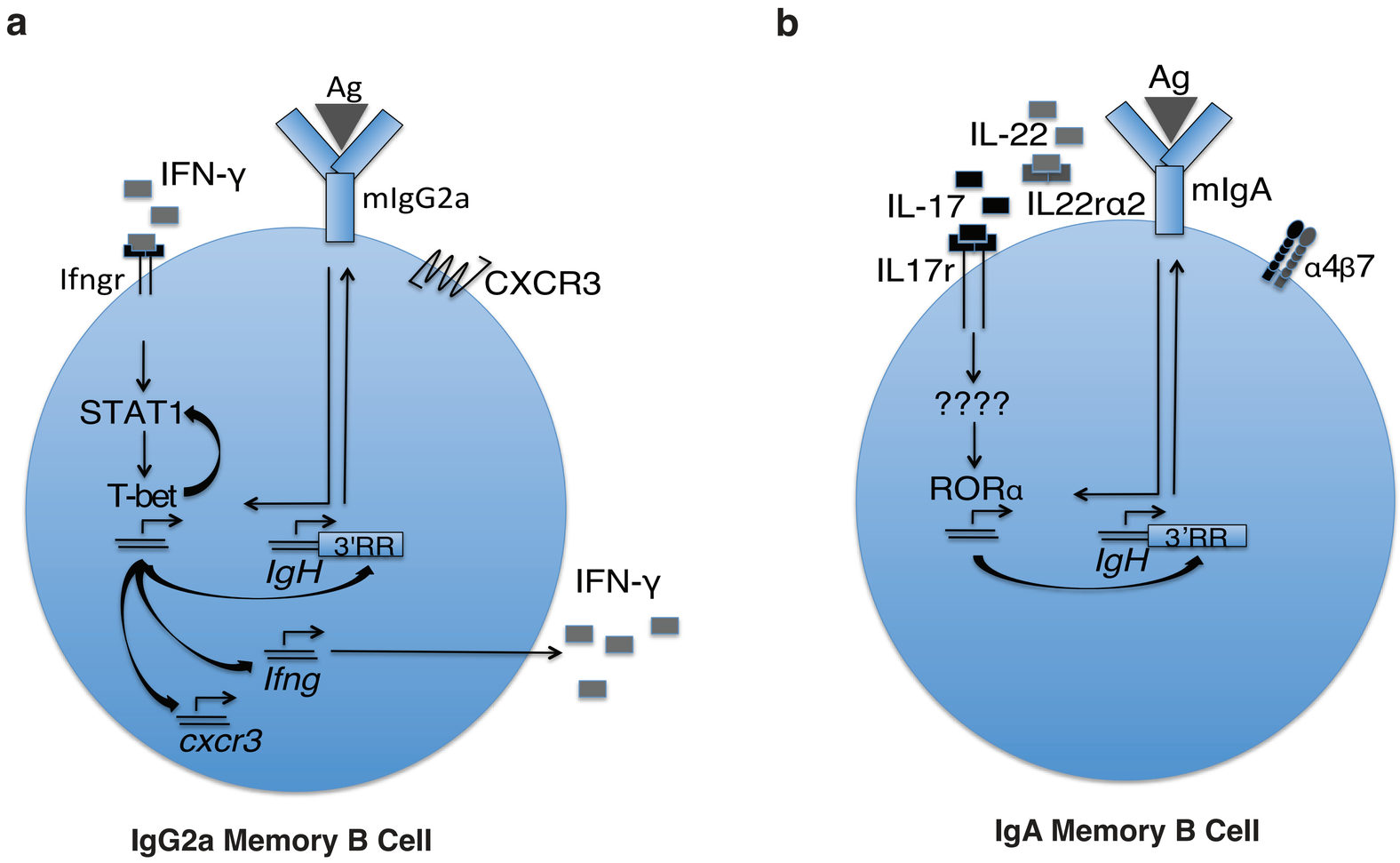
(d) Setup similar to that depicted in (a), except immunized donors were left for 6 months prior to splenocyte transfer into Rag1$^{-/-}$ recipients. Rag1$^{-/-}$ mice received 1:1 mixture of splenocytes from Tbx21F/F;Rosa26-CreERT2 (CD45.2) or wt (CD45.1) donors that were immunized with NP-KLH 6 months prior. Total isotype-specific B220^{hi} switched B cells obtained by gating as in (b) and derived from Tbx21F/F;Rosa26-CreERT2 (F/F) or CD45.1 (wt) donors. Mean±sem; n=3 Rag1$^{-/-}$ mice,

* P value<0.05 (Mann Whitney Test).



Supplementary Figure 5. Analysis of T-bet conditional peripheral chimeras following plasma cell and naive B cell depletion

Tbx21F/F;Rosa26-CreERT2 or Tbx21$^{+/+}$;Rosa26-CreER^{T2} (CD45.2) donors were immunized with NP-KLH and rested for >8 weeks. Splenocytes were treated ex-vivo with 4-OHT or vehicle, and then FACS-purified to deplete splenocytes of plasma cells (CD138-) and naive B cells (IgM-IgD-). Splenocytes were then transferred into Rag1-/- recipients, and boosted with soluble NP-KLH 1 day after transfer. Frequency (left panels) and total number (right panels) of NP-specific isotype-specific cells (Gr1-CD4-CD8- IgM-IgD- CD19+CD138- NP+IgG2a+) in the spleen 4 days after IP boost. Mean±sem, n=3 Rag1$^{-/-}$ mice, *P<0.05.



Supplementary Figure 6. Model of class-specific transcriptional programming of memory B cells.

(a) Schematic depicting model of unique IgG2a memory B cell transcriptional programming.

(b) Schematic depicting model of unique IgA memory B cell transcriptional programming.

Supplementary Table 1. Primers Sets

Name	Sense	5' to 3'
T-bet	Sense	TTTCCAAGAGACCCAGTTCATTG
T-bet	Anti-sense	ATGCGTACATGGACTCAAAGTT
GAPDH	Sense	AGGTCGGTGTGAACGGATTTG
GAPDH	Anti-sense	TGTAGACCATGTAGTTGAGGTCA
Post-switch Forward	Sense	GACCTCTCCGAAACCAGGC
Post-switch IgG2a	Anti-sense	GGGCCAGTGGATAGACCGA
Post-switch IgG1	Anti-sense	GGATCCAGAGTTCCAGGTCACT
Post-switch IgA	Anti-sense	GAGCTGGTGGGAGTGTCAGTG
Stat1	Sense	TCACAGTGGTTCGAGCTTCAG
Stat1	Anti-sense	GCAAACGAGACATCATAGGCA
Rad51	Sense	AAGTTTTGGTCCACAGCCTAT
Rad51	Anti-sense	CGGTGCATAAGCAACAGCC
cxcr3	Sense	TACCTTGAGGTTAGTGAACGT
cxcr3	Anti-sense	CGCTCTCGTTTTCCCCATAAT
Ifng	Sense	ATGAACGCTACACACTGCATC
Ifng	Anti-sense	CCATCCTTTTGCCAGTTCCTC
Il2rb	Sense	TGGAGCCTGTCCCTCTACG
Il2rb	Anti-sense	TCCACATGCAAGAGACATTGG
KSR	Sense	GCACCAAGTGCTCAGTGTCTA
KSR	Anti-sense	CTGAAGCGTGGGTAGCTGTT
MAPK1	Sense	CAGGTGTTCGACGTAGGGC
MAPK1	Anti-sense	TCTGGTGCTCAAAAGGACTGA
CDK6	Sense	GGCGTACCCACAGAAACCATA
CDK6	Anti-sense	AGGTAAGGGCCATCTGAAAAC
Runx1	Sense	GCAGGCAACGATGAAAACTAC
Runx1	Anti-sense	GCAACTTGTGGCGGATTTGTA
CALM2	Sense	AGCTTCGCCATGTGATGACAA
CALM2	Anti-sense	TCTTCGTAGTTTACCTGACCG
JMJD1a	Sense	CAGCAACTCCATCTAGCAAGG
JMJD1a	Anti-sense	TGTTCTCGGTACTTCAGGTTTT
CCL3	Sense	TTCTCTGTACCATGACACTCT
CCL3	Anti-sense	CGTGGAATCTTCCGGCTGTAG
Psgl1	Sense	CCCGGTGAAGCAATGTCTG
Psgl1	Anti-sense	AGTAGTTCCGCACTGGGTACA
Tbet Outer	Sense	CAATGTGACCCAGATGATCG
Tbet Outer	Anti-sense	GTTGACAGTTGGGTCCAGGT
GAPDH Outer	Sense	CCGCATCTTCTTGTGCAGT
GAPDH Outer	Anti-sense	CACCCCATTTGATGTTAGTGG
IFNg Outer	Sense	CTGATCCTTTGGACCCTCTG
IFNg Outer	Anti-sense	GCTGATGGCCTGATTGTCTT
FoxP3	Sense	CCCATCCCCAGGAGTCTTG
FoxP3	Anti-sense	ACCATGACTAGGGGCACTGTA
Rorgt	Sense	TTCACCCCACCTCCACT
Rorgt	Anti-sense	TTGTCCCCACAGATCTTGC
Rora	Sense	CGCATTGATGGATTTATGGAG
Rora	Anti-sense	TCGCATACTTCCCGTCAAAG
Gata3	Sense	CTCGGCCATTCGTACATGGAA

Name	Sense	5' to 3'
Gata3	Anti-sense	GGATACCTCTGCACCGTAGC
Tgfbrl	Sense	TCTGCATTGCACTTATGCTGA
Tgfbrl	Anti-sense	AAAGGGCGATCTAGTGATGGA
TgfbrII	Sense	AATAGGACCATCCATCCACTG
TgfbrII	Anti-sense	TCACATCGCAAAACTTGCACA
Ifngrl	Sense	TGACTATGCACGGTCAAAAGA
Ifngrl	Anti-sense	CGACCTTTCCCTTTAGGCACA
IfngrII	Sense	CTTCCAGCAATGACCCAAGAC
IfngrII	Anti-sense	TCCAGCAACCTATGCCAAGAG
IL17rc	Sense	GCTGCCTGATGGTGACAATGT
IL17rc	Anti-sense	TGGACGCAGGTACAGTAAGAAG
ILL22ra2	Sense	AAGCATTGCCTTCTAGGTCTC
ILL22ra2	Anti-sense	ACGAGCTGGTTGTATTTCTGT
STAT4	Sense	GCAGCCAACATGCCTATCCA
STAT4	Anti-sense	GCAGACACTTTGTGTTCCACA
SMAD2	Sense	AAGCCATCACCACTCAGAATT
SMAD2	Anti-sense	CACTGATCTACCGTATTTGCT
SMAD3	Sense	CACGCAGAACGTGAACACC
SMAD3	Anti-sense	GGCAGTAGATAACGTGAGGGA
SMAD4	Sense	AGCCGTCCTTACCCACTGAA
SMAD4	Anti-sense	GGTGGTAGTGCTGTTATGATG
DAXX	Sense	TGCACTGTTCTTAAAGCTCAC
DAXX	Anti-sense	GGCTCTGTGGGAGGGTTAG
BCL6	Sense	CCGGCACGCTAGTGATGTT
BCL6	Anti-sense	TGTCTTATGGGCTCTAAACTGCT
PRDM1	Sense	TTCTCTTGGAAAAACGTGTGGG
PRDM1	Anti-sense	GGAGCCGGAGCTAGACTTG
Itpr1	Sense	ATTTGTTCTCTGTATGCGGAGG
Itpr1	Anti-sense	AGCTTAAAGAGGCAGTCTCTGA
Hif1a	Sense	GGGGAGGACGATGAACATCAA
Hif1a	Anti-sense	GGGTGGTTTCTTGTACCCACA

Supplementary Methods.

Flow Cytometry

Draining lymph nodes and spleen were removed from unimmunized or immunized animals and single cell suspensions in PBS with 5% (vol/vol) FBS prepared. 4 x 10^8 cells per mL were incubated for 15 min with anti-CD16/32 (Fc block, 2.4G2) followed for 45 min at 4^{o}C with fluorophore-labeled or biotin-labeled monoclonal antibodies: allophycocyanin-conjugated anti-CD138 (281-2), allophycocyanin-Cy7-conjugated anti-CD19 (1D3), phycoerythrin-conjugated anti-CD138 (281-2), phycoerythrin texas red-conjugated anti-B220 (RA3-6B2), fluorescein isothiocyanate-conjugated anti-IgG1 (A85-1), anti-IgG3 (R40-8L), Horizon V500-conjugated anti-CD8 (53-6.7) and anti-CD4 (Rm4-5), biotin-conjugated anti IgG2a^b (5.7) [all from BD Biosciences]. Phycoerythrin-conjugated anti-T-bet (eBio4B10), Phycoerythrin-cy7-conjugated anti-CD45.1 (A20), Phycoerythrin-cy7-conjugated anti-CXCR3 (CXCR3-173), fluorescein isothiocyanate-conjugated anti-CD45.2 (104), Biotin-conjugated anti-IgG2b (RMG2b-1), Biotin-conjugated anti-CD45.2 (104), and Biotin-conjugated anti-IgA (RMA-1) [all from Biolegend, Inc]. Alexa Fluor 700-conjugated anti-CD38 (90), phycoerythrin-cy5-conjugated anti-Gr-1 (Ly6G/C, RB68C5) [all from eBiosciences, Inc]. Allophycocyanin-conjugated anti-NP, phycoerythrin-conjugated NP, peridinin-chlorophyll protein-cy5.5-conjugated anti-IgM (331.12), Pacific Blue-conjugated IgD (11.26) [all from MMW Lab]. Immunoglobulin-specific antibodies were added in a separate step for 45 min at 4^{o}C with normal mouse serum (1:50) before other reagents were added. Streptavidin-conjugated Qdot 655 (Invitrogen) were used as a second step visualization reagent. For intracellular staining, cells were fixed, permeabilized, and stained using the protocol in the Foxp3 Staining Buffer Set (eBioscience). Cells were washed and re-suspended in PBS/FBS and analyzed with a FACSAria III with FACSDiva software (BD Biosciences). Data were analyzed with FlowJo software (Tree Star, Inc).

Quantitative PCR

cDNA was prepared as described previously (51). Briefly, 5000 cells were sorted directly into lysis buffer (Qiagen) and mRNA was purified using RNeasy Kit (Qiagen). cDNA was generated using the First-Strand Synthesis System for RT-PCR (Invitrogen) using random hexamers. SYBR Green qPCR was conducted using the Platinum SYBR Green Supermix UDG reaction mix (Invitrogen) on the StepOnePlus Real-time

PCR system and analyzed using the Step One Software (Applied Biosystems). Primers were used at a final concentration of 0.25μM and can be found in Supplementary Table 1. GAPDH was used as an endogenous control. For measurement of T-bet expression levels, naïve B cells were assigned a value of 1, and the relative expression assigned accordingly. For measurement of STAT1 and IgG2a post-switch mRNA levels, cells receiving 4-OHT treatment were assigned a value of 1, and the relative expression assigned accordingly. For cells treated with RORa siRNA or SR1001, cells receiving the drug or siRNA were assigned a value of 1, and the relative expression assigned accordingly. For single cell qPCR on mRNA, single cells were directly sorted into 2x Reaction buffer containing SSIII RT Platinum Taq found in the SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase Kit (Invitrogen) and containing outside primers for IFNg, T-bet, and GAPDH at a final concentration of 0.25μM. 96-well plates were placed in PCR cycler for 15 minutes at 50C, followed by 2 minutes at 95C. 22 cycles of 15 seconds at 95C followed by 4 minutes at 60C were then performed. 1μL of product was used in a standard qPCR reaction described above.

References

51. Fazilleau N *et al., Nat Immunol*. **10**, 375-84, (2009).