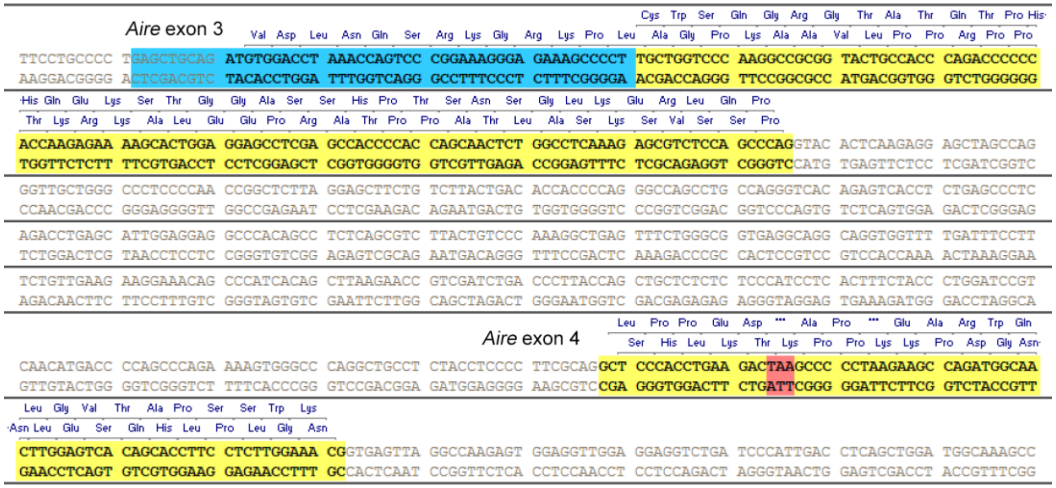
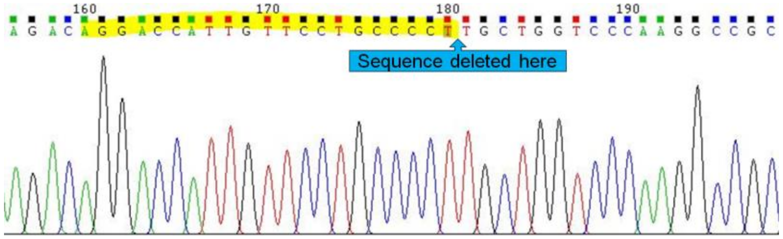
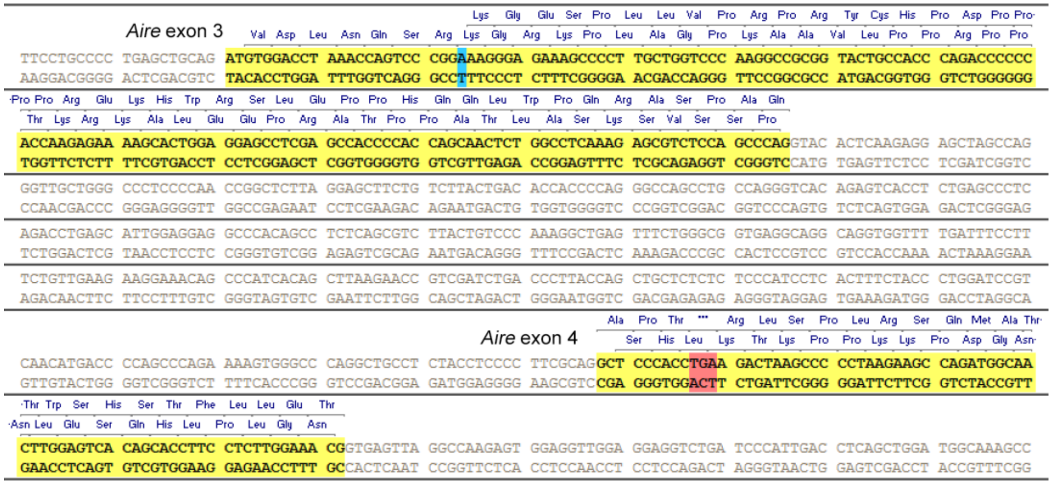
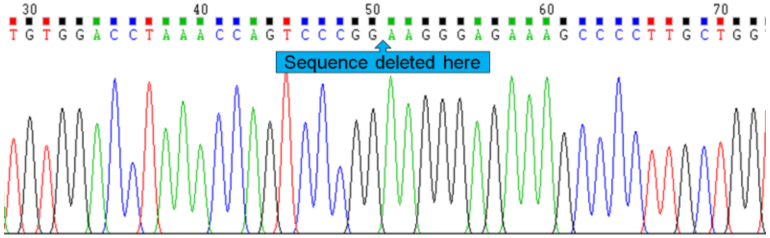
SUPPLEMENTAL INFORMATION

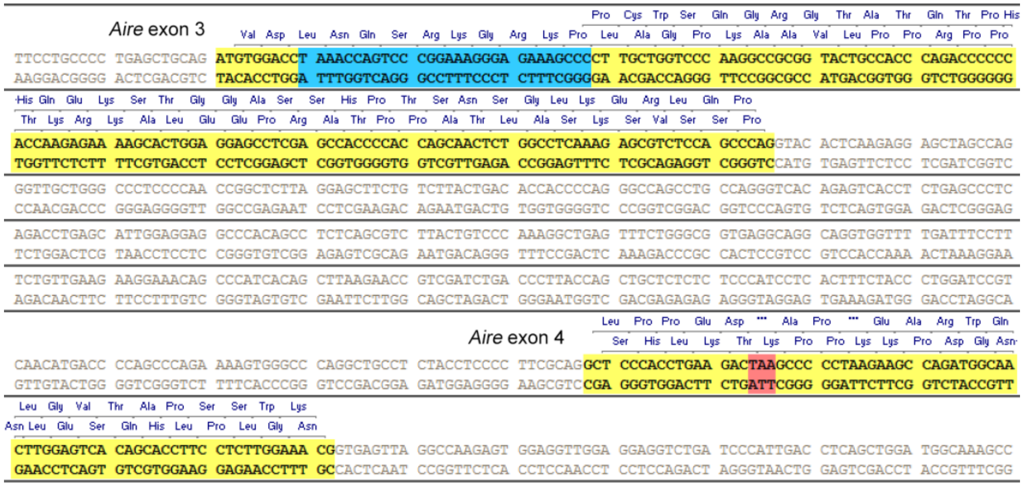
SUPPLEMENTAL TABLES

Table S1. *Aire*$^{-/-}$ CH12 Cell Clones, Related to Figure 4 and Figure 5.

Clone	Mutation(s) and alleles	Sequencing histogram
43	Allele 1  Cys Trp Ser Gln Gly Arg Gly Thr Ala Thr Gln Thr Pro His· *Aire* exon 3 Val Asp Leu Asn Gln Ser Arg Lys Gly Arg Lys Pro Leu Ala Gly Pro Lys Ala Ala Val Leu Pro Pro Arg Pro Pro TTCCTGCCCC TGAGCTGCAG ATGTGGACCT AAACCAGTCC CGGAAAGGGA GAAAGCCCCT TGCTGGTCCC AAGGCCGCGG TACTGCCACC CAGACCCCCC AAGGACGGGG ACTCGACGTC TACACCTGGA TTTGGTCAGG GCCTTTCCCT CTTTCGGGGA ACGACCAGGG TTCCGGCGCC ATGACGGTGG GTCTGGGGGG ·His Gln Glu Lys Ser Thr Gly Gly Ala Ser Ser His Pro Thr Ser Asn Ser Gly Leu Lys Glu Arg Leu Gln Pro Thr Lys Arg Lys Ala Leu Glu Glu Pro Arg Ala Thr Pro Pro Ala Thr Leu Ala Ser Lys Ser Val Ser Ser Pro ACCAAGAGAA AAGCACTGGA GGAGCCTCGA GCCACCCCAC CAGCAACTCT GGCCTCAAAG AGCGTCTCCA GCCCAGGTAC ACTCAAGAGG AGCTAGCCAG TGGTTCTCTT TTCGTGACCT CCTCGGAGCT CGGTGGGGTG GTCGTTGAGA CCGGAGTTTC TCGCAGAGGT CGGGTCCATG TGAGTTCTCC TCGATCGGTC GGTTGCTGGG CCCTCCCCAA CCGGCTCTTA GGAGCTTCTG TCTTACTGAC ACCACCCCAG GGCCAGCCTG CCAGGGTCAC AGAGTCACCT CTGAGCCCTC CCAACGACCC GGGAGGGGTT GGCCGAGAAT CCTCGAAGAC AGAATGACTG TGGTGGGGTC CCGGTCGGAC GGTCCCAGTG TCTCAGTGGA GACTCGGGAG AGACCTGAGC ATTGGAGGAG GCCCACAGCC TCTCAGCGTC TTACTGTCCC AAAGGCTGAG TTTCTGGGCG GTGAGGCAGG CAGGTGGTTT TGATTTCCTT TCTGGACTCG TAACCTCCTC CGGGTGTCGG AGAGTCGCAG AATGACAGGG TTTCCGACTC AAAGACCCGC CACTCCGTCC GTCCACCAAA ACTAAAGGAA TCTGTTGAAG AAGGAAACAG CCCATCACAG CTTAAGAACC GTCGATCTGA CCCTTACCAG CTGCTCTCTC TCCCATCCTC ACTTTCTACC CTGGATCCGT AGACAACTTC TTCCTTTGTC GGGTAGTGTC GAATTCTTGG CAGCTAGACT GGGAATGGTC GACGAGAGAG AGGGTAGGAG TGAAAGATGG GACCTAGGCA Leu Pro Pro Glu Asp *** Ala Pro *** Glu Ala Arg Trp Gln *Aire* exon 4 Ser His Leu Lys Thr Lys Pro Pro Lys Lys Pro Asp Gly Asn· CAACATGACC CCAGCCCAGA AAAGTGGGCC CAGGCTGCCT CTACCTCCCC TTCGCAGGCT CCCACCTGAA GACTAAGCCC CCTAAGAAGC CAGATGGCAA GTTGTACTGG GGTCGGGTCT TTTCACCCGG GTCCGACGGA GATGGAGGGG AAGCGTCCGA GGGTGGACTT CTGATTCGGG GGATTCTTCG GTCTACCGTT Leu Gly Val Thr Ala Pro Ser Ser Trp Lys ·Asn Leu Glu Ser Gln His Leu Pro Leu Gly Asn CTTGGAGTCA CAGCACCTTC CTCTTGGAAA CGGTGAGTTA GGCCAAGAGT GGAGGTTGGA GGAGGTCTGA TCCCATTGAC CTCAGCTGGA TGGCAAAGCC GAACCTCAGT GTCGTGGAAG GAGAACCTTT GCCACTCAAT CCGGTTCTCA CCTCCAACCT CCTCCAGACT AGGGTAACTG GAGTCGACCT ACCGTTTCGG	
	Allele 2  Lys Gly Glu Ser Pro Leu Leu Val Pro Arg Pro Arg Tyr Cys His Pro Asp Pro Pro· *Aire* exon 3 Val Asp Leu Asn Gln Ser Arg Lys Gly Arg Lys Pro Leu Ala Gly Pro Lys Ala Ala Val Leu Pro Pro Arg Pro Pro TTCCTGCCCC TGAGCTGCAG ATGTGGACCT AAACCAGTCC CGGAAAGGGA GAAAGCCCCT TGCTGGTCCC AAGGCCGCGG TACTGCCACC CAGACCCCCC AAGGACGGGG ACTCGACGTC TACACCTGGA TTTGGTCAGG GCCTTTCCCT CTTTCGGGGA ACGACCAGGG TTCCGGCGCC ATGACGGTGG GTCTGGGGGG ·Pro Pro Arg Glu Lys His Trp Arg Ser Leu Glu Pro Pro His Gln Gln Leu Trp Pro Gln Arg Ala Ser Pro Ala Gln Thr Lys Arg Lys Ala Leu Glu Glu Pro Arg Ala Thr Pro Pro Ala Thr Leu Ala Ser Lys Ser Val Ser Ser Pro ACCAAGAGAA AAGCACTGGA GGAGCCTCGA GCCACCCCAC CAGCAACTCT GGCCTCAAAG AGCGTCTCCA GCCCAGGTAC ACTCAAGAGG AGCTAGCCAG TGGTTCTCTT TTCGTGACCT CCTCGGAGCT CGGTGGGGTG GTCGTTGAGA CCGGAGTTTC TCGCAGAGGT CGGGTCCATG TGAGTTCTCC TCGATCGGTC GGTTGCTGGG CCCTCCCCAA CCGGCTCTTA GGAGCTTCTG TCTTACTGAC ACCACCCCAG GGCCAGCCTG CCAGGGTCAC AGAGTCACCT CTGAGCCCTC CCAACGACCC GGGAGGGGTT GGCCGAGAAT CCTCGAAGAC AGAATGACTG TGGTGGGGTC CCGGTCGGAC GGTCCCAGTG TCTCAGTGGA GACTCGGGAG AGACCTGAGC ATTGGAGGAG GCCCACAGCC TCTCAGCGTC TTACTGTCCC AAAGGCTGAG TTTCTGGGCG GTGAGGCAGG CAGGTGGTTT TGATTTCCTT TCTGGACTCG TAACCTCCTC CGGGTGTCGG AGAGTCGCAG AATGACAGGG TTTCCGACTC AAAGACCCGC CACTCCGTCC GTCCACCAAA ACTAAAGGAA TCTGTTGAAG AAGGAAACAG CCCATCACAG CTTAAGAACC GTCGATCTGA CCCTTACCAG CTGCTCTCTC TCCCATCCTC ACTTTCTACC CTGGATCCGT AGACAACTTC TTCCTTTGTC GGGTAGTGTC GAATTCTTGG CAGCTAGACT GGGAATGGTC GACGAGAGAG AGGGTAGGAG TGAAAGATGG GACCTAGGCA Ala Pro Thr *** Arg Leu Ser Pro Leu Arg Ser Gln Met Ala Thr· *Aire* exon 4 Ser His Leu Lys Thr Lys Pro Pro Lys Lys Pro Asp Gly Asn· CAACATGACC CCAGCCCAGA AAAGTGGGCC CAGGCTGCCT CTACCTCCCC TTCGCAGGCT CCCACCTGAA GACTAAGCCC CCTAAGAAGC CAGATGGCAA GTTGTACTGG GGTCGGGTCT TTTCACCCGG GTCCGACGGA GATGGAGGGG AAGCGTCCGA GGGTGGACTT CTGATTCGGG GGATTCTTCG GTCTACCGTT ·Thr Trp Ser His Ser Thr Phe Leu Leu Glu Thr ·Asn Leu Glu Ser Gln His Leu Pro Leu Gly Asn CTTGGAGTCA CAGCACCTTC CTCTTGGAAA CGGTGAGTTA GGCCAAGAGT GGAGGTTGGA GGAGGTCTGA TCCCATTGAC CTCAGCTGGA TGGCAAAGCC GAACCTCAGT GTCGTGGAAG GAGAACCTTT GCCACTCAAT CCGGTTCTCA CCTCCAACCT CCTCCAGACT AGGGTAACTG GAGTCGACCT ACCGTTTCGG	

53

Allele 1



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His Gln  Glu  Lys  Ser  Thr  Gly  Gly  Ala  Ser  Ser  His  Pro  Thr  Ser  Asn  Ser  Gly  Leu  Lys  Glu  Arg  Leu  Gln  Pro
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CCAACGACCC GGGAGGGGTT GGCCGAGAAT CCTCGAAGAC AGAATGACTG TGGTGGGGTC CCGGTCGGAC GGTCCCAGTG TCTCAGTGGA GACTCGGGAG

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TCTGTTGAAG AAGGAAACAG CCCATCACAG CTTAAGAACC GTCGATCTGA CCCTTACCAG CTGCTCTCTC TCCCATCCTC ACTTTCTACC CTGGATCCGT
AGACAACTTC TTCCTTTGTC GGGTAGTGTC GAATTCTTGG CAGCTAGACT GGGAATGGTC GACGAGAGAG AGGGTAGGAG TGAAAGATGG GACCTAGGCA

                                                                Leu  Pro  Pro  Glu  Asp  ***  Ala  Pro  ***  Glu  Ala  Arg  Trp  Gln
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 Leu  Gly  Val  Thr  Ala  Pro  Ser  Ser  Trp  Lys
Asn Leu  Glu  Ser  Gln  His  Leu  Pro  Leu  Gly  Asn
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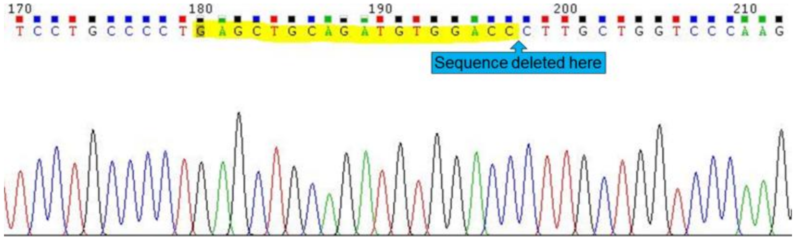
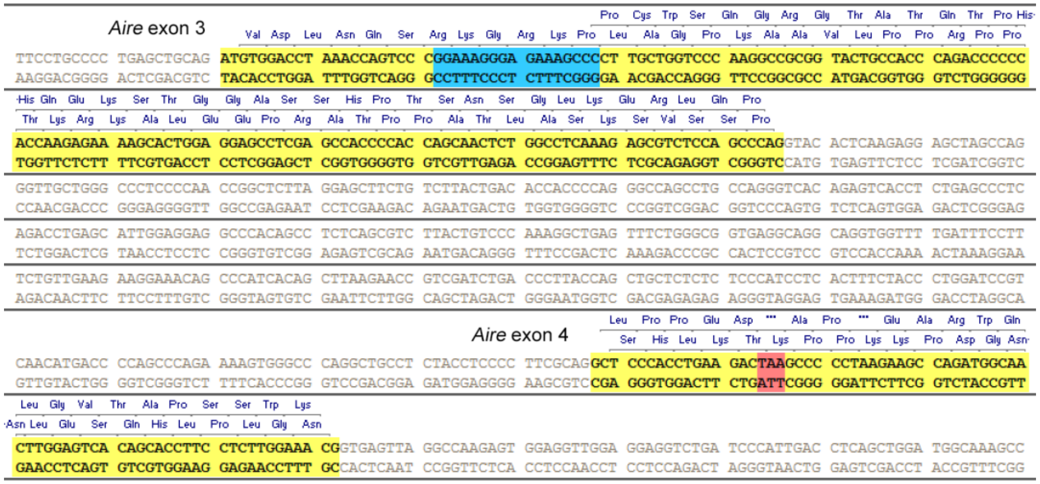


Allele 2



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 Leu  Gly  Val  Thr  Ala  Pro  Ser  Ser  Trp  Lys
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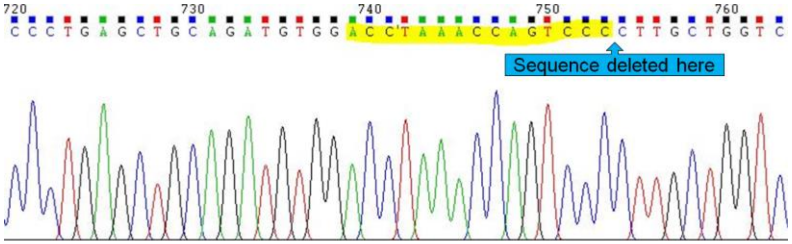


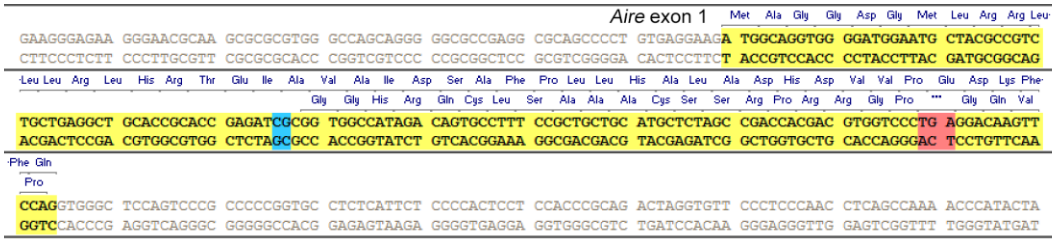
69	Both alleles  *Aire* exon 1 Met Ala Gly Gly Asp Gly Met Leu Arg Arg Leu GAAGGGAGAA GGGAACGCAA GCGCGCGTGG GCCAGCAGGG GGCGCCGAGG CGCAGCCCCT GTGAGGAAGA TGGCAGGTGG GGATGGAATG CTACGCCGTC CTTCCCTCTT CCCTTGCGTT CGCGCGCACC CGGTCGTCCC CCGCGGCTCC GCGTCGGGGA CACTCCTTCT ACCGTCCACC CCTACCTTAC GATGCGGCAG Leu Leu Arg Leu His Arg Thr Glu Ile Ala Val Ala Ile Asp Ser Ala Phe Pro Leu Leu His Ala Leu Ala Asp His Asp Val Val Pro Glu Asp Lys Phe Gly Gly His Arg Gln Cys Leu Ser Ala Ala Ala Cys Ser Ser Arg Pro Arg Arg Gly Pro *** Gly Gln Val TGCTGAGGCT GCACCGCACC GAGATCGCGG TGGCCATAGA CAGTGCCTTT CCGCTGCTGC ATGCTCTAGC CGACCACGAC GTGGTCCCTG AGGACAAGTT ACGACTCCGA CGTGGCGTGG CTCTAGCGCC ACCGGTATCT GTCACGGAAA GGCGACGACG TACGAGATCG GCTGGTGCTG CACCAGGGAC TCCTGTTCAA Phe Gln Pro CCAGGTGGGC TCCAGTCCCG CCCCCGGTGC CTCTCATTCT CCCCACTCCT CCACCCGCAG ACTAGGTGTT CCCTCCCAAC CTCAGCCAAA ACCCATACTA GGTCCACCCG AGGTCAGGGC GGGGGCCACG GAGAGTAAGA GGGGTGAGGA GGTGGGCGTC TGATCCACAA GGGAGGGTTG GAGTCGGTTT TGGGTATGAT	

Table S2. Human AIRE and AID Constructs, Related to Figure 4 and Figure 5.

(**A**) Human AIRE constructs in pcDNA3.1(–)/Myc-His

Name	Description	Remaining region	MW with tag (kDa)
WT	Full length	1-545	60.7
M1	ΔPHD2	1-430	48.8
M2	ΔPHD1, PHD2	1-298	35
M3	ΔSAND, PHD1, PHD2	1-181	23
M4	ΔCARD	101-545	49
M5	ΔCARD, ΔNLS	181-545	41
M6	ΔNLS	1-100, 181-545	52.5
M7	NLS only	101-181	12

(**B**) Human AID constructs in pFLAG-CMV2

Name	Description	Remaining region	MW with tag (kDa)
WT	Full length	1-198	26.3
M1	E58A	1-198	26.3
M2	NLS of AID replaced with NLS of nucleoplasmin	1-198	26.3
M3	ΔCatalytic domain	1-54, 95-198	21.4
M4	ΔAPOBEC-like and ΔNES domains	1-94	13.8
M5	ΔNLS	1-8, 27-198	24
M6	G23S	1-198	26.3

Table S3. Cloning Primers Used to Generate *Aire*$^{–/–}$ CH12 Clones and AIRE and AID Mutant Molecules, Related to Figure 4 and Figure 5.

(A) Primers for cloning human AIRE constructs into pcDNA3.1(–)/Myc-His

Name	Description	Primer name and sequence (5'-3')
WT	Full length	–
M1	ΔPHD2	`D430_R AGGAGCCAGGTTCTGCTGACC` `Hind-Myc_F GAAAGCTTTCTAGAACAAAAACTCATCTCA`
M2	ΔPHD1, PHD2	`D298_R CTCGTCCTCATTCTTCTGGTGGAG` `Hind-Myc_F GAAAGCTTTCTAGAACAAAAACTCATCTCA`
M3	ΔSAND, PHD1, PHD2	`D181_R AATCCCGTTCCCGAGTGGAAG` `Hind-Myc_F GAAAGCTTTCTAGAACAAAAACTCATCTCA`
M4	ΔCARD	`EcoRV-ATG_R CATGGTGAATTCTGCAGATATCCAGC` `D101_F CCCAAAGATGTGGACCTCAGCC`
M5	ΔCARD, ΔNLS	`EcoRV-ATG_R CATGGTGAATTCTGCAGATATCCAGC` `D181_F ATTCAGACCATGTCAGCTTCAGTCCA`
M6	ΔNLS	`D100_R GAAGCTGTCCAGGATGGGCTG` `D181_F ATTCAGACCATGTCAGCTTCAGTCCA`
M7	NLS only	`D181_R AATCCCGTTCCCGAGTGGAAG` `Hind-Myc_F GAAAGCTTTCTAGAACAAAAACTCATCTCA`

(B) Primers for cloning human AID constructs into pFLAG-CMV2

Name	Description	Primer name and sequence (5'-3')
M1	E58A	`AID_F ATGGACAGCCTCTTGATGAACCG` `AID_R AAGTCCCAAAGTACGAAATGCGTC`

M2	NLS of AID replaced with NLS of nucleoplasmin	`npNLS_top`
		`AAAAGGCCGGCGGCCACGAAAAAGGCCGGCCAGGCAAAAAAGAAAAAG`
		`npNLS_bottom`
		`CTTTTTCTTTTTTGCCTGGCCGGCCTTTTTCGTGGCCGCCGGCCTTTT`
		(`npNLS_top` and `npNLS_bottom` were annealed together before ligation with the PCR product of the primer pair AID8_R and AID27_F.)

M3	ΔCatalytic domain	`AID54_R GCCGTTCTTATTGCGAAGATAACCA` `AID95_F GCCGACTTTCTGCGAGGGA`
M4	ΔAPOBEC-like and ΔNES domains	`AID94_R CACATGTCGGGCACAGTCGTAG` `TAG_F TAGACTGAAACTTTTTTGGGGGAGGG`

M5	ΔNLS	`AID8_R CCGGTTCATCAAGAGGCTGTCC` `AID27_F ACCTACCTGTGCTACGTAGTGAAGAGGC`
M6	G23S	`AID22_R CTTAGCCCAGCGGACATTTTTGA` `AIDS23_F AGTCGGCGTGAGACCTACCTGTG`

(C) Cloning primers for generating pCMV-Tag1-mAire-GFP plasmids

Description	**Primer name and sequence (5’-3’)**
Full length Aire	–
AireΔNLS	`D106_R GTCCACATCTTTTGGGAAGCCG` `D182_F ATTCAGACCATGGCAGCTTCTGTC`
Full length eGFP	`GFP_F ATGGTGAGCAAGGGCGAGGAG` `GFP_R CTTGTACAGCTCGTCCATGCCG`
Aire of AireΔNLS in pCMV-Tag1 vector	`TGA-SalI_F TGATGACAGGTCGACCTCGAGC` `AIRE_R GGAAGAGAAGGGTGGTGTCTCGG`

(D) Oligos used to clone sgRNA into targeting vectors

Description	**Oligo name and sequence (5’-3’)**
Aire$^{-/-}$ CH12 clone 69	`_Top CACCGGCACCGCACCGAGATCGCGG`
	`_Bottom AAACCCGCGATCTCGGTGCGGTGCC`
Aire$^{-/-}$ CH12 clones 43 and 53	`_Top CACCGACCTAAACCAGTCCCGGAAA`
	`_Bottom AAACTTTCCGGGACTGGTTTAGGTC`

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. AIRE Is Expressed Specifically in GC B Cells of Human and Mouse Secondary Lymphoid Organs, Related to Figure 1.

(A) Immunofluorescence analysis of the thymus of a healthy donor for EpCAM, AIRE and DNA. Bars: 20 μm.

(B and C) Immunofluorescence analysis of tonsillar tissues of healthy donors for IgD, AIRE, CD19, Pax5, Bcl-6 and DNA. The dotted lines mark the boundary between tonsil follicular mantle zone and the follicle. Arrow heads point to follicular IgD$^+$ plasmablasts (B) and Pax5$^+$Bcl-6$^+$AIRE$^+$ GC B cells (C). Bars: 15 μm (B) and 30 μm (C).

(D) Flow cytometric analysis of AIRE expression in human peripheral blood naive (IgD$^+$CD27$^–$), MZ (IgD$^+$CD27$^+$), switched memory (IgD$^–$CD27+), double-negative (IgD$^–$CD27$^–$) B cells, and transitional (CD24^{hi}CD38^{hi}), mature (CD24^{int}CD38^{int}), memory (CD24^{hi}CD38$^–$) B cells and plasma cells (CD24$^–$CD38^{hi}).

(E) Immunofluorescence analysis of the thymic tissue of a B6 mouse for UEA-1, AIRE and DNA, and the splenic tissue of a B6 mouse immunized with 3 doses of sheep red blood cells (SRBCs) for IgD, Aire, CD19 and DNA, and Bar: 20 μm.

(G) Flow cytometric gating strategy for identifying mouse splenic non-GC (CD19$^+$B220$^+$GL7$^–$FAS$^–$), GC (CD19$^+$B220$^+$GL7$^+$FAS$^+$) B cells and plasma cells (CD19^{lo}B220^{lo}CD138$^+$).

(H) Genotypes and Aire expression in ILN, splenic, peripheral blood and peritoneal B cells of a litter of *Aire*^{Adig} mice after 1 dose of i.p. SRBC immunization with or without CFA.

(I) Percentage of GFP$^+$ B cells (mean ± SEM) in splenic GC B cells of *Aire*^{Adig} transgene-positive mice (n = 5) after 1 dose of i.p. SRBC immunization with CFA. The dotted line indicates of mean value of GFP$^+$ B cells in splenic non-GC B cells of these mice.

(J) AIRE expression in mouse peripheral blood, splenic, MLN, PP, peritoneum and thymic B cells of B6.*Aire*^{Adig} mice after 1 dose of i.p. SRBC immunization without CFA. The data are representative of 6 B6.*Aire*^{Adig} and 6 B6 mice that were age- and sex-matched and housed in the same SPF room.

Figure S2. *Aire*$^{+/+}$ and *Aire*$^{–/–}$ Donor BM and B Cells Had a Similar Phenotype Before Transfer, Related to Figure 3.

(A) Flow cytometric analysis of CD45.1^{+} *Aire*$^{+/+}$ and CD45.2^{+} *Aire*$^{–/–}$ donor BM before and after B220 cell depletion.

(B) Flow cytometry analysis of splenic naive resting B cells that were purified from the spleens of primary μMT chimeras of CD45.1^{+} *Aire*$^{+/+}$ and CD45.2^{+} *Aire*$^{–/–}$ BM and used as donor B cells for the secondary μMT chimeric hosts. The ratio of CD45.2^{+} *Aire*$^{+/+}$ and CD45.2^{+} *Aire*$^{–/–}$ splenic B cells were adjusted to be 1:1 prior to the secondary transfer.

(C) Representative purity of CD45.2^{+} *Aire*$^{+/+}$ and CD45.2^{+} *Aire*$^{–/–}$ littermate donor B cells before adoptive transfer into μMT hosts.

(D) Cell surface expression of CD21, CD23, CD38, CD40, CD62L, CD80, CD86, CD93, I-A^{b}, BAFF-R and IgM and IgD on purified CD45.2^{+} *Aire*$^{+/+}$ and CD45.2^{+} *Aire*$^{–/–}$ littermate donor B cells before adoptive transfer, as determined by flow cytometry.

(E) NP$_{8}$-to-NP$_{36}$ binding ratios (mean ± SEM) of pre-immune splenic naive resting donor B cells of CD45.2^{+} *Aire*$^{–/–}$, CD45.2^{+} *Aire*$^{+/+}$ and CD45.1^{+} *Aire*$^{+/+}$ mice, by 1-way ANOVA with Tukey's post hoc test.

(F) Percentage of GL7$^+$FAS$^+$ GC B cells in the spleens of μMT recipients of either *Aire*$^{+/+}$ or *Aire*$^{-/-}$ B cells that were immunized i.p. with NP$_{32}$-KLH. Flow cytometry was performed 4 d after the last immunization.

(G) Cell surface expression of the co-stimulatory or co-inhibitory molecules CD80, CD86, PD-L1, PD-L2 and ICOSL on GL7$^+$FAS$^+$ GC B cells in the spleens of μMT recipients after immunizations. Shaded histograms indicate the staining using isotype-matched control antibodies.

(H and I) Percentage of splenic PD-1$^+$CXCR5$^+$ T$_{FH}$ cells and PD-1$^+$CXCR5$^+$Foxp3$^+$CD25$^+$ T$_{FR}$ cells in the spleens of immunized μMT recipients. The results shown represent 4 experiments, each consisting of B cells from 3–5 age- and sex-matched littermate donor mice and 6–8 age- and sex-matched littermate μMT recipient mice.

(J and K) Flow cytometric and statistical analyses of the percentages of total and intravascular B cells in thymic cells of μMT mice that received donor B cells after all the immunizations with NP$_{32}$-KLH. Age and sex-matched unimmunized μMT mice were included as controls. The data are represented as mean ± SEM.

(L) The sorting and sequencing strategies for *Aire*$^{+/+}$ and *Aire*$^{-/-}$ donor B cells in μMT recipients after immunizations with NP$_{32}$-KLH. NP-specific B cells were sorted based on NP$_{36}$ binding.

Figure S3. *Aire*$^{+/+}$ and *Aire*$^{-/-}$B Cells Showed Similar Proliferation and Apoptosis *in vitro*, Related to Figure 3.

(A and B) CFSE dilution in purified splenic B cells from age- and sex-matched littermate donor *Aire*$^{+/+}$ and *Aire*$^{-/-}$ mice treated with medium (Control) or 5 μg/ml anti-CD40 and 100 ng/ml IL-4 for 4 or 6 d. Non-viable cells were excluded from the analysis.

(C) Statistical comparison of the percentage (mean ± SEM) of CFSE^{lo} *Aire*$^{+/+}$ vs. *Aire*$^{–/–}$ splenic B cells ($n = 3$) after 4 or 6 days of stimulation with 5 µg/ml anti-CD40 and 100 ng/ml IL-4, by 2-tailed unpaired t-test. The results represent 3 independent experiments.

(D) Apoptosis of *Aire*$^{+/+}$ or *Aire*$^{–/–}$ B cells treated with medium (Control) or 500 ng/ml CD40L and 100 ng/ml IL-4 for 3 or 7 d, as determined by Annexin V and 7-AAD staining by flow cytometry. All results shown are representative of 3 experiments, each consisting of cells from 2–3 age- and sex-matched littermate *Aire*$^{+/+}$ and *Aire*$^{–/–}$ mice.

Fig. S4. Validation of *Aire*$^{–/–}$ CH12 Cell Clones, Related to Figure 4.

(A) Verification of *Aire* mutations in CH12 clones by PCR using primers that only anneal to the WT sequence, giving no amplification in clones 43, 47 and 53. Clone 47 has a 3-bp deletion in both *Aire* alleles causing a single amino acid deletion, and hence was not used in experiments.

(B) Verification of *Aire* mutations in both alleles of CH12 clone 69 by PCR showing no amplification using primer pair #2 which anneals to the WT but not the mutated sequence. Primer pair #1 amplifies a sequence immediately downstream of the mutation site, and primer pair #3 is specific for the single-stranded repair template used in CRISPR.

(C) Western Blot analysis of AIRE protein expression in WT and *Aire*$^{–/–}$ CH12 cells.

(D) Flow cytometric analysis of apoptosis by Annexin V and 7-AAD staining of WT and *Aire*$^{–/–}$ CH12 cells treated with medium (Control) or anti-CD40, TGF-β1 and IL-4 for 3 d.

(E) Percentages of late (Annexin V^{+}7-AAD^{+}) and early (Annexin V^{+}7-AAD$^{–}$) apoptotic cells (mean ± SEM) in WT and *Aire*$^{–/–}$ CH12 cells treated with medium (Control) or anti-CD40, TGF-β1 and IL-4 for 3 d. $^{*}P < 0.05$, by 2-tailed t-test. The data in D and E represent 4 experiments.

Fig. S5. AIRE and AID Co-localize in the Nuclei of GC B Cells, Related to Figure 5.

(A–E) Imaging flow cytometry of AIRE and AID in tonsillar IgD^{-}CD38^{+} GC, IgD^{+}CD38^{-} naive, IgD^{-}CD38^{-} switched memory B cells, IgD^{-}CD38^{hi} switched PCs and IgD^{+}CD38^{+} founder GC (FGC) B cells of a healthy donor. DNA was counter stained with DAPI. Samples stained with isotype-matched control antibodies were used to define the fluorescence baseline for AIRE and AID. Four representative cells in each population stained with AIRE and AID or with isotype control antibodies were shown. Bars: 7 μm.

Figure S6. AID Interacts with AIRE in B Cells, Related to Figure 5 and Figure 6.

(A) Co-IP of AIRE and AID in tonsillar CD19^{+} total, IgD^{+} naive, and FGC and CD19^{+}IgD^{-} GC and memory B cells of a healthy donor after treatment of the cell lysates with DNAse I. PCR amplification of β-Actin gDNA in DNAse I-treated or untreated cells was also performed.

(B) Co-IP of AIRE and AID in splenic B cells of immunized WT or *Aicda*$^{-/-}$ mice. The data represent 2 experiments.

(C) Co-IP of AID with pSer5-Pol II, total Pol II, Spt5 and Aire in WT and *Aire*$^{-/-}$ CH12 cells after 72 h of treatment without or with anti-CD40, TGF-β and IL-4. The results represent 3 experiments.

Figure S7. AIRE Deficiency in B Dells Impairs Skin T$_{H}$17 Immunity against *C. albicans*, Related to Figure 7.

(A) Levels of autoantibodies (mean ± SEM) binding to IL-17A, IL-17F and IL-22 in the sera of μMT recipient mice of *Aire*$^{+/+}$ or *Aire*$^{-/-}$ donor B cells 4 d after infection. $^{*}P < 0.05$, $^{**}P < 0.01$, by 1-tailed unpaired *t*-test.

(B) Flow cytometric gating strategy for identifying mouse skin viable T cells after *ex vivo* re-stimulation. T cells downregulation CD3 or TCR after *ex vivo* stimulation with PMA and ionomycin; thus CD3$^+$ or TCRβ^+ events were gated for analysis. This gate also included TCR$\gamma\delta^+$ T cells, which were CD3$^+$.

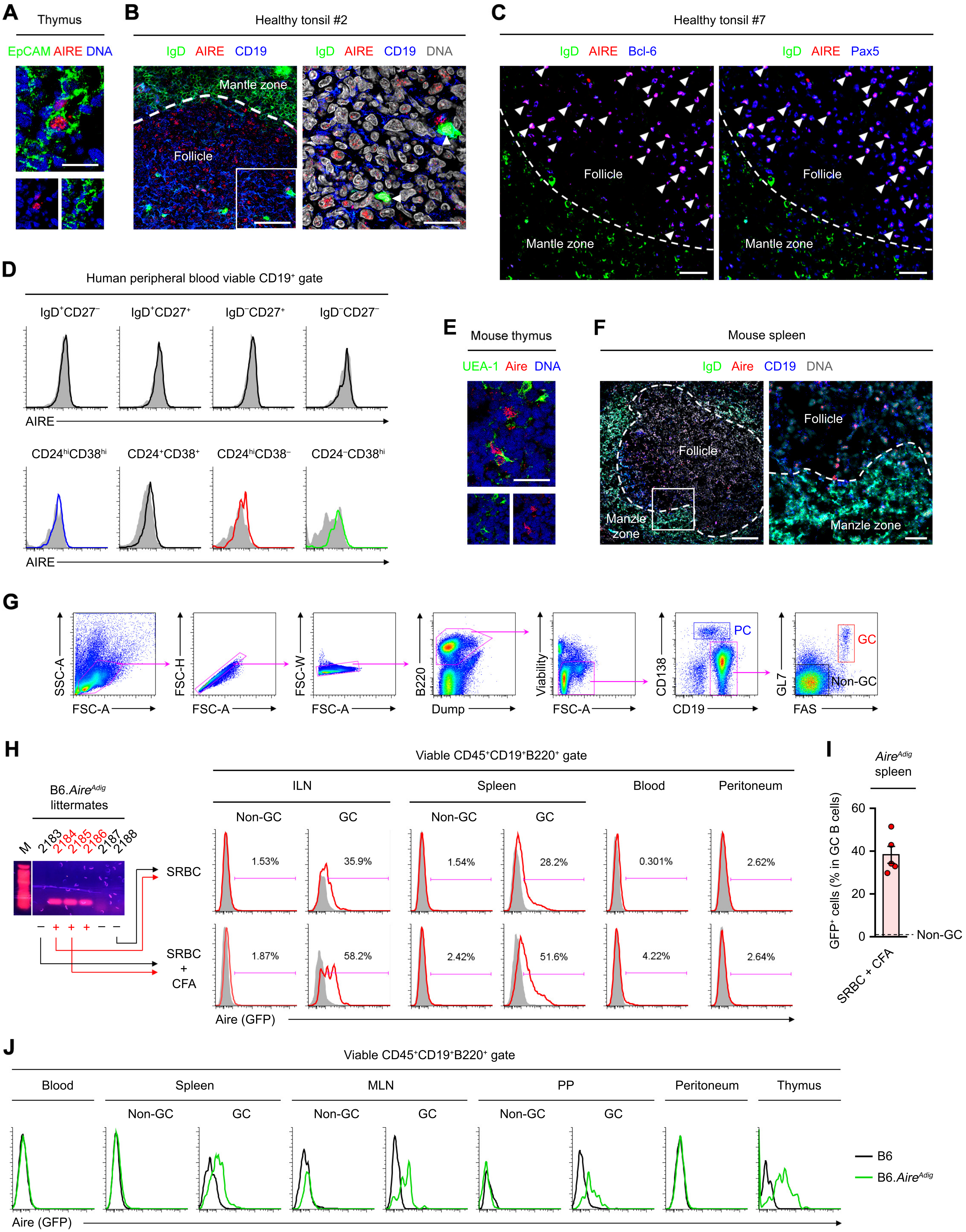

A
Thymus
EpCAM AIRE DNA
B
Healthy tonsil #2
IgD AIRE CD19
IgD AIRE CD19 DNA
Mantle zone
Follicle
C
Healthy tonsil #7
IgD AIRE Bcl-6
IgD AIRE Pax5
Follicle
Mantle zone
D
Human peripheral blood viable CD19⁺ gate
IgD⁺CD27⁻
IgD⁺CD27⁺
IgD⁻CD27⁺
IgD⁻CD27⁻
AIRE
CD24hiCD38hi
CD24⁺CD38⁺
CD24hiCD38⁻
CD24⁻CD38hi
AIRE
E
Mouse thymus
UEA-1 Aire DNA
F
Mouse spleen
IgD Aire CD19 DNA
Follicle
Manzle zone
G
SSC-A
FSC-A
FSC-H
FSC-A
FSC-W
FSC-A
B220
Dump
Viability
FSC-A
CD138
CD19
PC
GL7
FAS
GC
Non-GC
H
B6.AireAdig littermates
M
2183
2184
2185
2186
2187
2188
– + + + – –
SRBC
SRBC + CFA
Viable CD45⁺CD19⁺B220⁺ gate
ILN
Spleen
Blood
Peritoneum
Non-GC
GC
Non-GC
GC
1.53%
35.9%
1.54%
28.2%
0.301%
2.62%
1.87%
58.2%
2.42%
51.6%
4.22%
2.64%
Aire (GFP)
I
AireAdig spleen
GFP⁺ cells (% in GC B cells)
60
40
20
0
Non-GC
SRBC + CFA
J
Viable CD45⁺CD19⁺B220⁺ gate
Blood
Spleen
MLN
PP
Peritoneum
Thymus
Non-GC
GC
Non-GC
GC
Non-GC
GC
Aire (GFP)
B6
B6.AireAdig

Figure S1

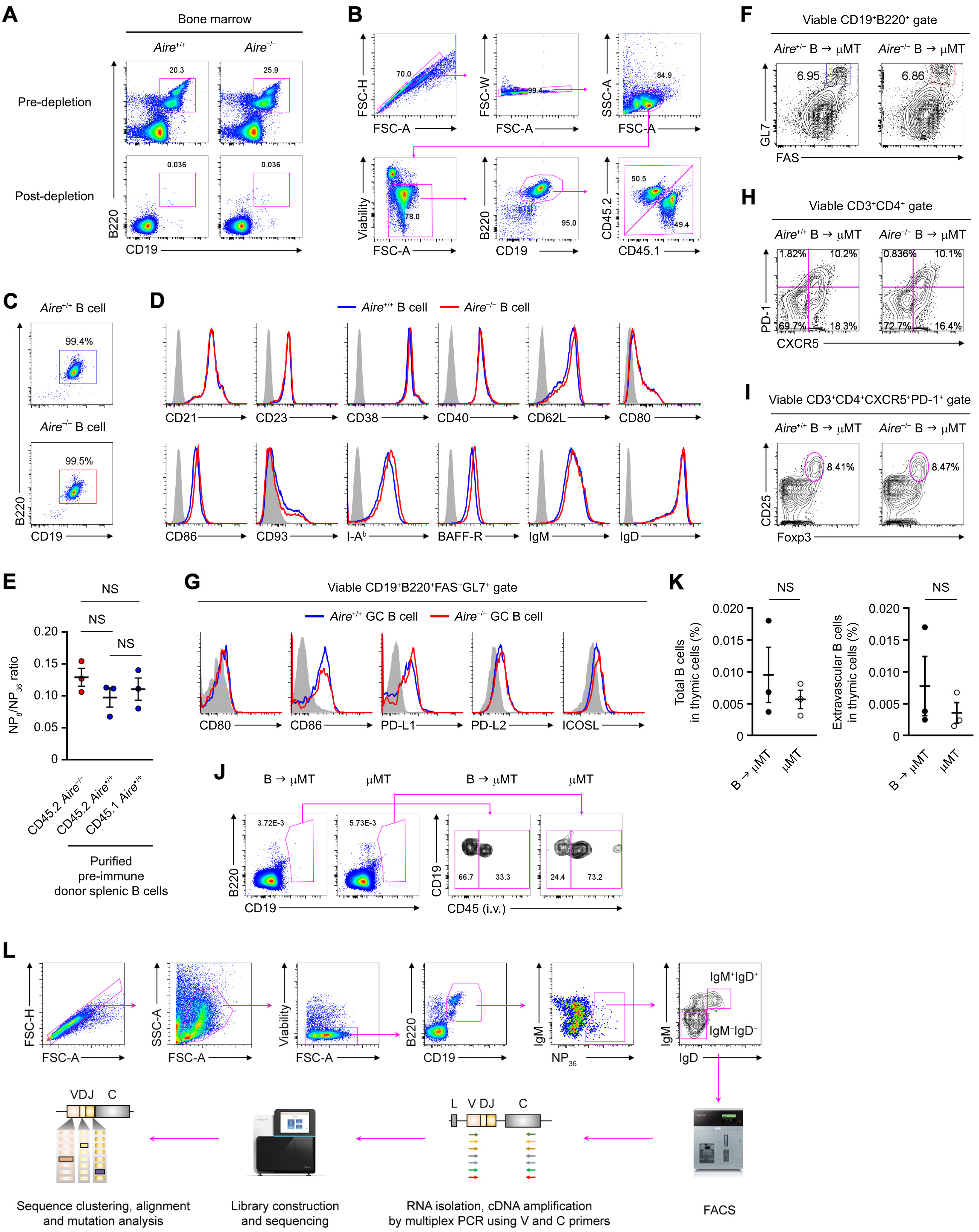

A
Bone marrow
Aire+/+
Aire−/−
Pre-depletion
Post-depletion
20.3
25.9
0.036
0.036
B220
CD19
B
FSC-H
FSC-A
70.0
FSC-W
99.4
SSC-A
84.9
Viability
78.0
B220
CD19
95.0
CD45.2
CD45.1
50.5
49.4
C
Aire+/+ B cell
99.4%
Aire−/− B cell
99.5%
B220
CD19
D
Aire+/+ B cell
Aire−/− B cell
CD21
CD23
CD38
CD40
CD62L
CD80
CD86
CD93
I-Ab
BAFF-R
IgM
IgD
E
NS
NS
NS
NP8/NP36 ratio
0.20
0.15
0.10
0.05
0
CD45.2 Aire−/−
CD45.2 Aire+/+
CD45.1 Aire+/+
Purified pre-immune donor splenic B cells
F
Viable CD19+B220+ gate
Aire+/+ B → μMT
Aire−/− B → μMT
6.95
6.86
GL7
FAS
G
Viable CD19+B220+FAS+GL7+ gate
Aire+/+ GC B cell
Aire−/− GC B cell
CD80
CD86
PD-L1
PD-L2
ICOSL
H
Viable CD3+CD4+ gate
Aire+/+ B → μMT
Aire−/− B → μMT
1.82%
10.2%
69.7%
18.3%
0.836%
10.1%
72.7%
16.4%
PD-1
CXCR5
I
Viable CD3+CD4+CXCR5+PD-1+ gate
Aire+/+ B → μMT
Aire−/− B → μMT
8.41%
8.47%
CD25
Foxp3
J
B → μMT
μMT
B → μMT
μMT
3.72E-3
5.73E-3
B220
CD19
66.7
33.3
24.4
73.2
CD19
CD45 (i.v.)
K
NS
NS
Total B cells in thymic cells (%)
Extravascular B cells in thymic cells (%)
0.020
0.015
0.010
0.005
0
B → μMT
μMT
L
FSC-H
FSC-A
SSC-A
FSC-A
Viability
FSC-A
B220
CD19
IgM
NP36
IgM+IgD+
IgM−IgD−
IgM
IgD
VDJ
C
L
V DJ
C
Sequence clustering, alignment and mutation analysis
Library construction and sequencing
RNA isolation, cDNA amplification by multiplex PCR using V and C primers
FACS

Figure S2

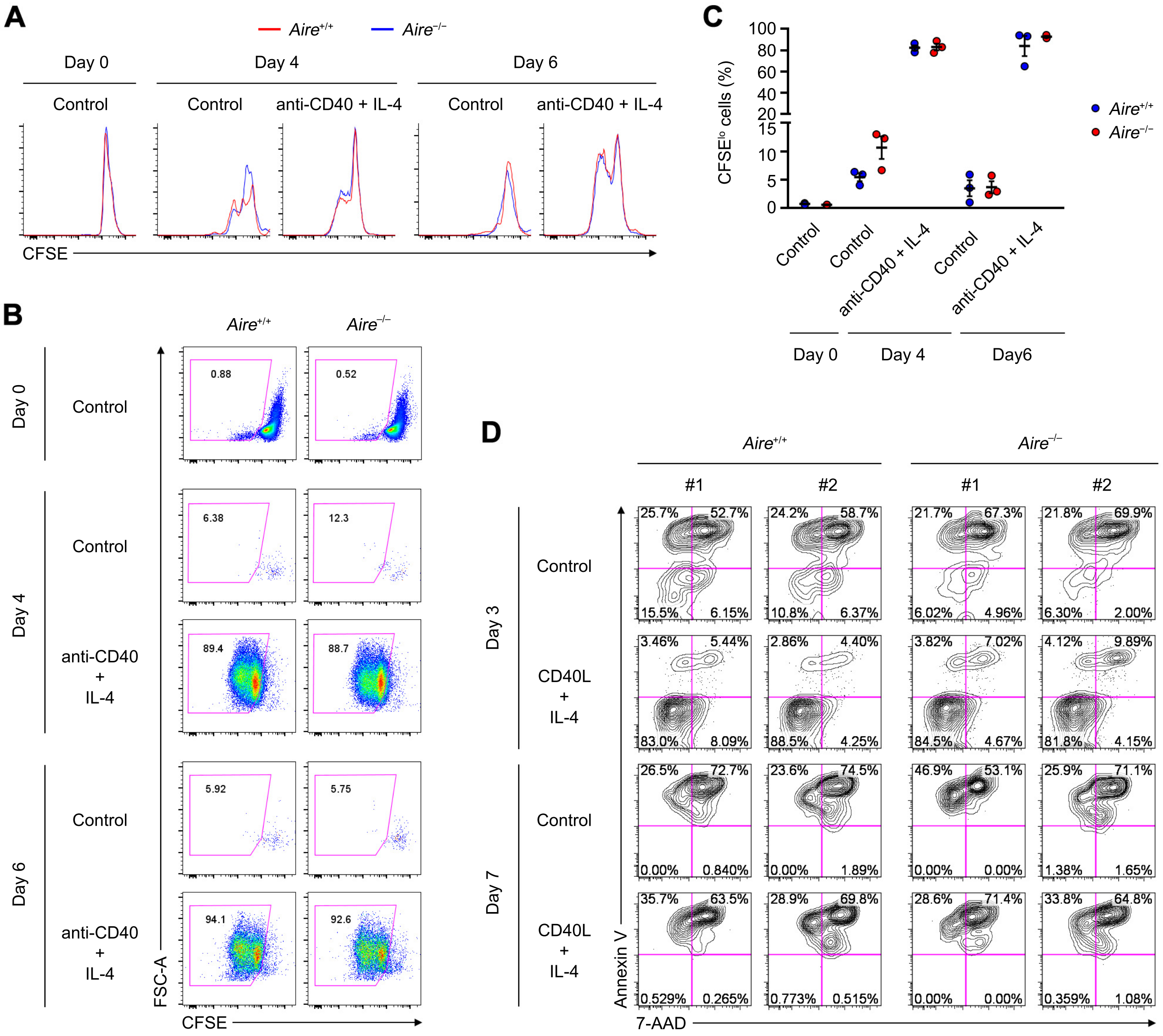

A
Aire+/+
Aire−/−
Day 0
Day 4
Day 6
Control
Control
anti-CD40 + IL-4
Control
anti-CD40 + IL-4
CFSE
B
Aire+/+
Aire−/−
Day 0
Control
0.88
0.52
Day 4
Control
6.38
12.3
anti-CD40 + IL-4
89.4
88.7
Day 6
Control
5.92
5.75
anti-CD40 + IL-4
94.1
92.6
FSC-A
CFSE
C
CFSElo cells (%)
100
80
60
40
20
15
10
5
0
Aire+/+
Aire−/−
Control
Control
anti-CD40 + IL-4
Control
anti-CD40 + IL-4
Day 0
Day 4
Day6
D
Aire+/+
Aire−/−
#1
#2
#1
#2
Day 3
Control
25.7%
52.7%
15.5%
6.15%
24.2%
58.7%
10.8%
6.37%
21.7%
67.3%
6.02%
4.96%
21.8%
69.9%
6.30%
2.00%
CD40L + IL-4
3.46%
5.44%
83.0%
8.09%
2.86%
4.40%
88.5%
4.25%
3.82%
7.02%
84.5%
4.67%
4.12%
9.89%
81.8%
4.15%
Day 7
Control
26.5%
72.7%
0.00%
0.840%
23.6%
74.5%
0.00%
1.89%
46.9%
53.1%
0.00%
0.00%
25.9%
71.1%
1.38%
1.65%
CD40L + IL-4
35.7%
63.5%
0.529%
0.265%
28.9%
69.8%
0.773%
0.515%
28.6%
71.4%
0.00%
0.00%
33.8%
64.8%
0.359%
1.08%
Annexin V
7-AAD

Figure S3

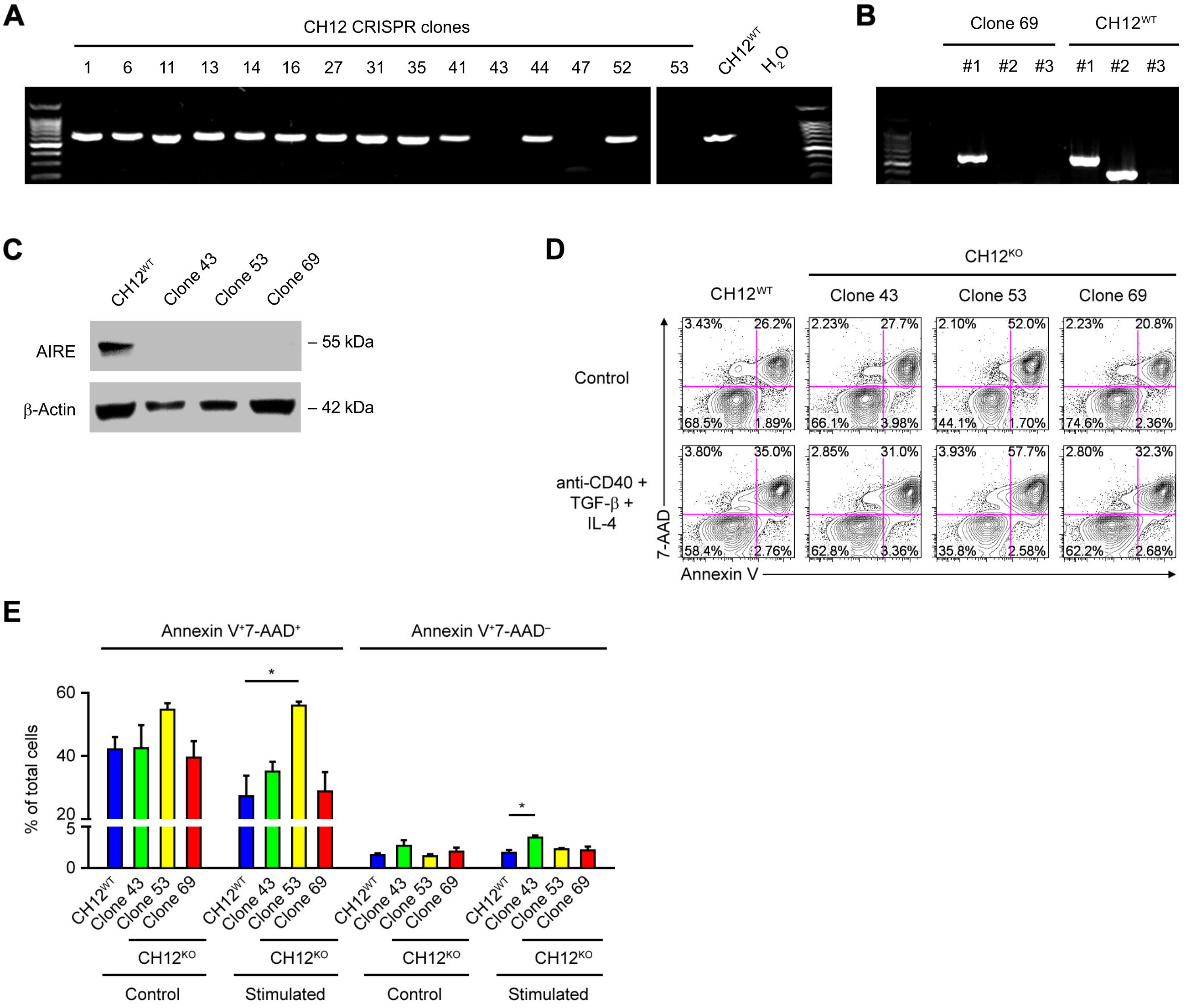

A
CH12 CRISPR clones
1
6
11
13
14
16
27
31
35
41
43
44
47
52
53
CH12WT
H2O
B
Clone 69
CH12WT
#1
#2
#3
#1
#2
#3
C
CH12WT
Clone 43
Clone 53
Clone 69
AIRE
β-Actin
– 55 kDa
– 42 kDa
D
CH12KO
CH12WT
Clone 43
Clone 53
Clone 69
Control
anti-CD40 + TGF-β + IL-4
7-AAD
Annexin V
3.43%
26.2%
68.5%
1.89%
2.23%
27.7%
66.1%
3.98%
2.10%
52.0%
44.1%
1.70%
2.23%
20.8%
74.6%
2.36%
3.80%
35.0%
58.4%
2.76%
2.85%
31.0%
62.8%
3.36%
3.93%
57.7%
35.8%
2.58%
2.80%
32.3%
62.2%
2.68%
E
Annexin V+7-AAD+
Annexin V+7-AAD−
% of total cells
60
40
20
5
0
*
*
CH12WT
Clone 43
Clone 53
Clone 69
CH12KO
Control
Stimulated
Control
Stimulated

Figure S4

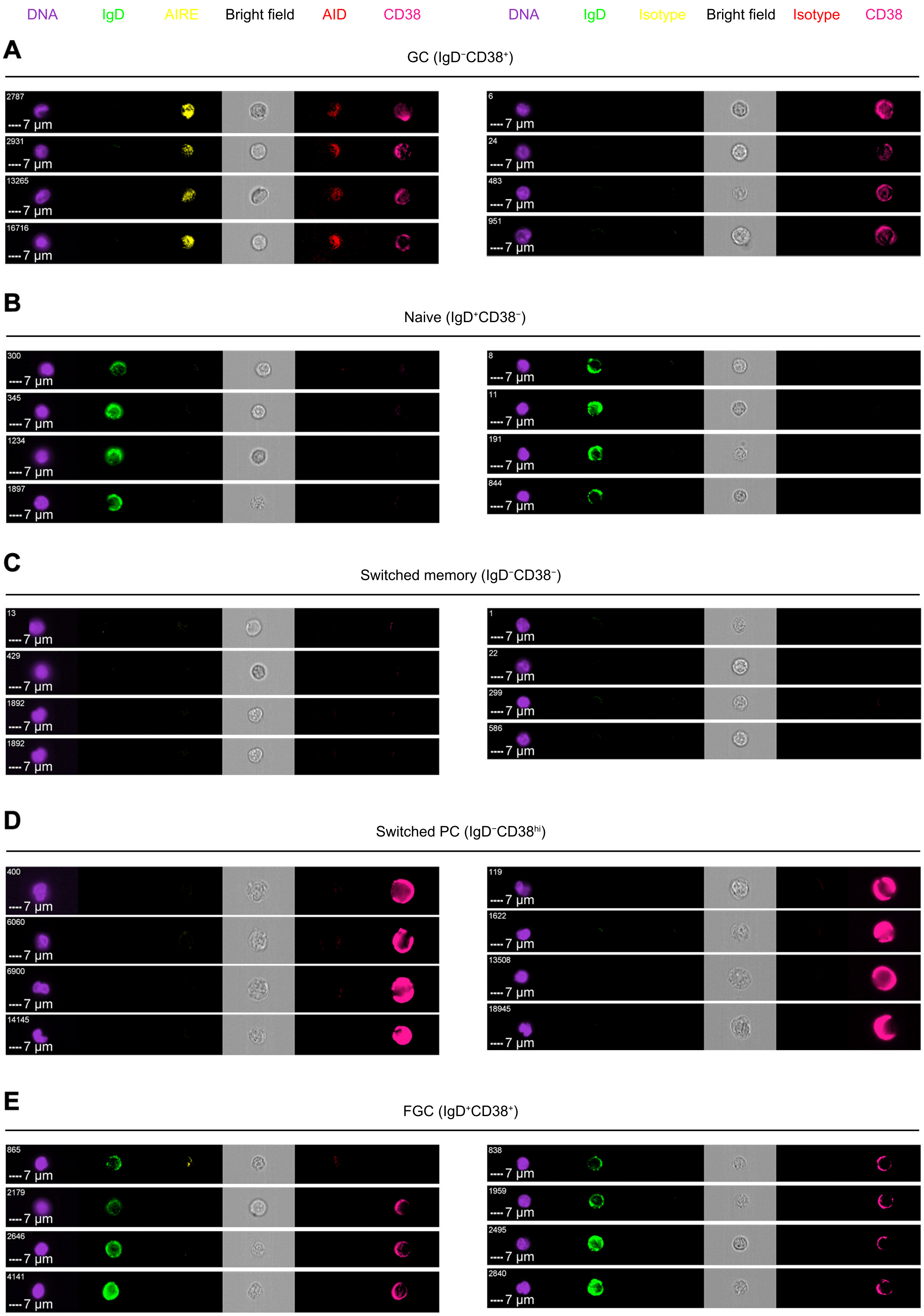

DNA
IgD
AIRE
Bright field
AID
CD38
DNA
IgD
Isotype
Bright field
Isotype
CD38
A
GC (IgD$^-$CD38$^+$)
2787
2931
13265
16716
6
24
483
951
B
Naive (IgD$^+$CD38$^-$)
300
345
1234
1897
8
11
191
844
C
Switched memory (IgD$^-$CD38$^-$)
13
429
1892
1892
1
22
299
586
D
Switched PC (IgD$^-$CD38^{hi})
400
6060
6900
14145
119
1622
13508
18945
E
FGC (IgD$^+$CD38$^+$)
865
2179
2646
4141
838
1959
2495
2840
7 µm

Figure S5

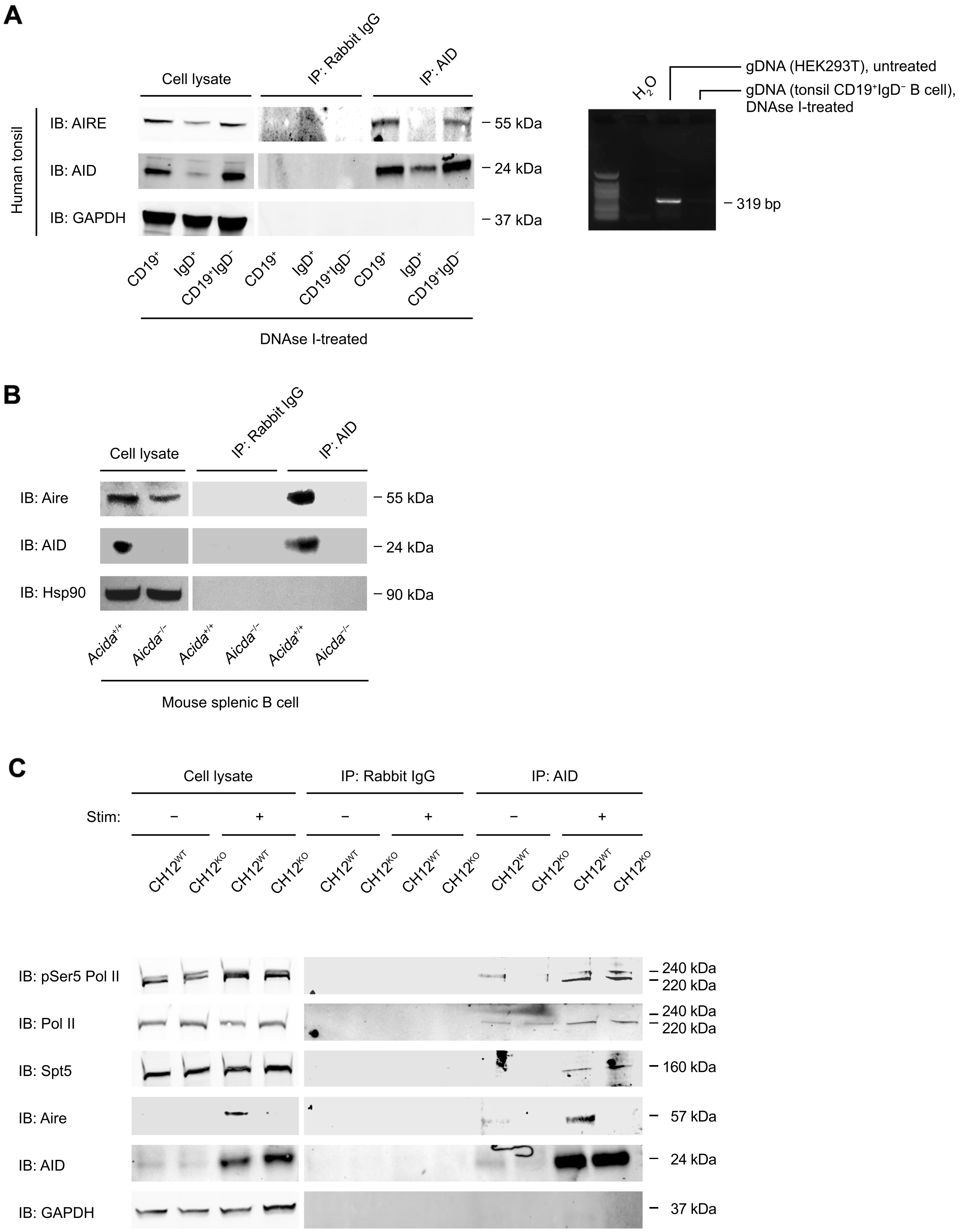

A
Cell lysate
IP: Rabbit IgG
IP: AID
Human tonsil
IB: AIRE
IB: AID
IB: GAPDH
– 55 kDa
– 24 kDa
– 37 kDa
CD19⁺
IgD⁺
CD19⁺IgD⁻
CD19⁺
IgD⁺
CD19⁺IgD⁻
CD19⁺
IgD⁺
CD19⁺IgD⁻
DNAse I-treated
H₂O
gDNA (HEK293T), untreated
gDNA (tonsil CD19⁺IgD⁻ B cell), DNAse I-treated
– 319 bp
B
Cell lysate
IP: Rabbit IgG
IP: AID
IB: Aire
IB: AID
IB: Hsp90
– 55 kDa
– 24 kDa
– 90 kDa
Acida⁺/⁺
Aicda⁻/⁻
Acida⁺/⁺
Aicda⁻/⁻
Acida⁺/⁺
Aicda⁻/⁻
Mouse splenic B cell
C
Cell lysate
IP: Rabbit IgG
IP: AID
Stim:
–
+
–
+
–
+
CH12ᵂᵀ
CH12ᴷᴼ
CH12ᵂᵀ
CH12ᴷᴼ
CH12ᵂᵀ
CH12ᴷᴼ
CH12ᵂᵀ
CH12ᴷᴼ
CH12ᵂᵀ
CH12ᴷᴼ
CH12ᵂᵀ
CH12ᴷᴼ
IB: pSer5 Pol II
IB: Pol II
IB: Spt5
IB: Aire
IB: AID
IB: GAPDH
– 240 kDa
– 220 kDa
– 240 kDa
– 220 kDa
– 160 kDa
– 57 kDa
– 24 kDa
– 37 kDa

Figure S6

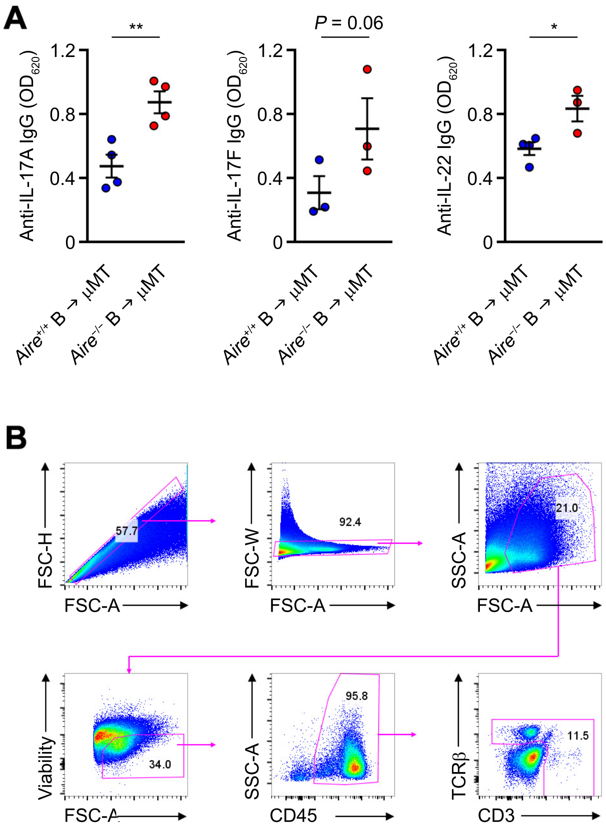

A
**
Anti-IL-17A IgG (OD620)
1.2
0.8
0.4
0
Aire+/+ B → μMT
Aire−/− B → μMT
P = 0.06
Anti-IL-17F IgG (OD620)
1.2
0.8
0.4
0
Aire+/+ B → μMT
Aire−/− B → μMT
*
Anti-IL-22 IgG (OD620)
1.2
0.8
0.4
0
Aire+/+ B → μMT
Aire−/− B → μMT
B
FSC-H
FSC-A
57.7
FSC-W
FSC-A
92.4
SSC-A
FSC-A
21.0
Viability
FSC-A
34.0
SSC-A
CD45
95.8
TCRβ
CD3
11.5

Figure S7