Cell Reports, Volume *22*

Supplemental Information

Age-Associated Decline in Thymic B Cell Expression of Aire and Aire-Dependent Self-Antigens

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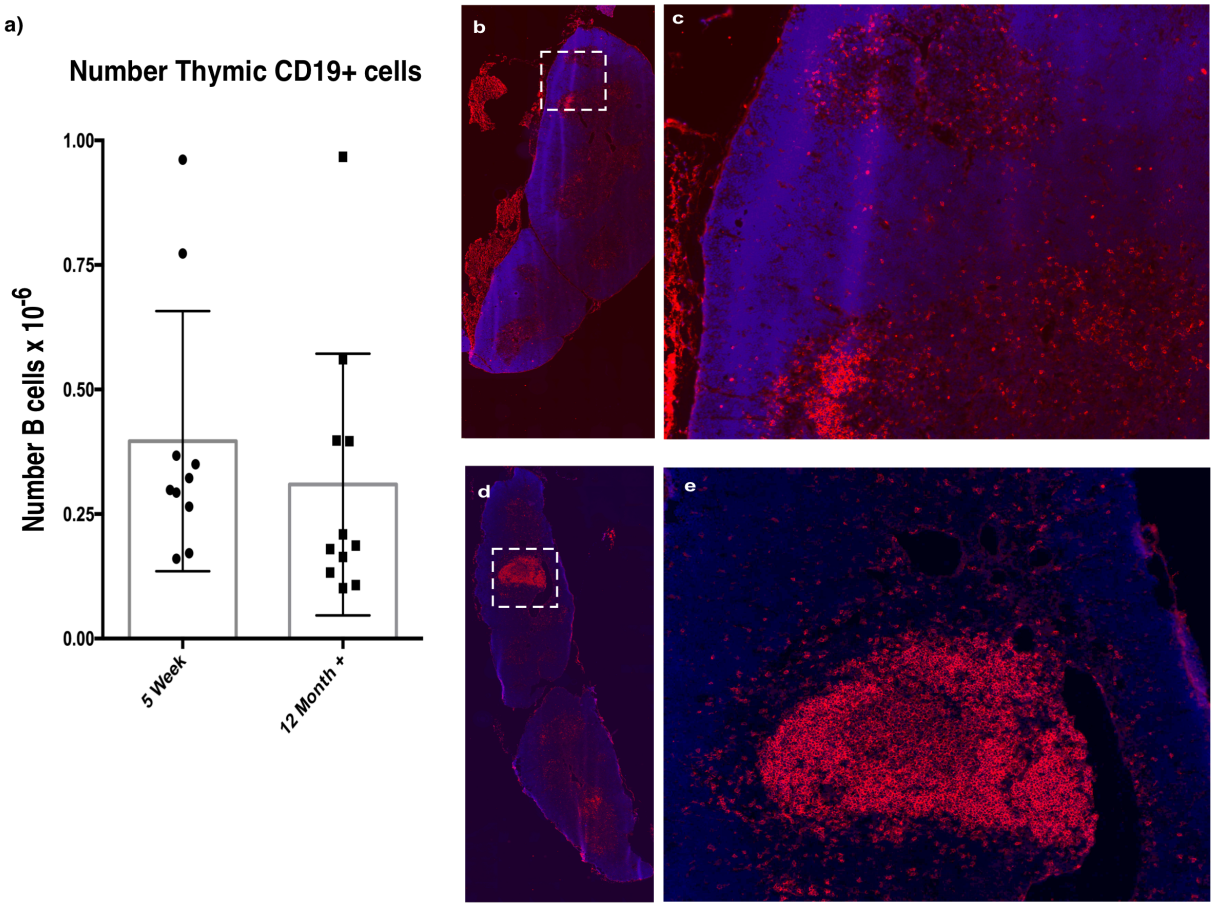
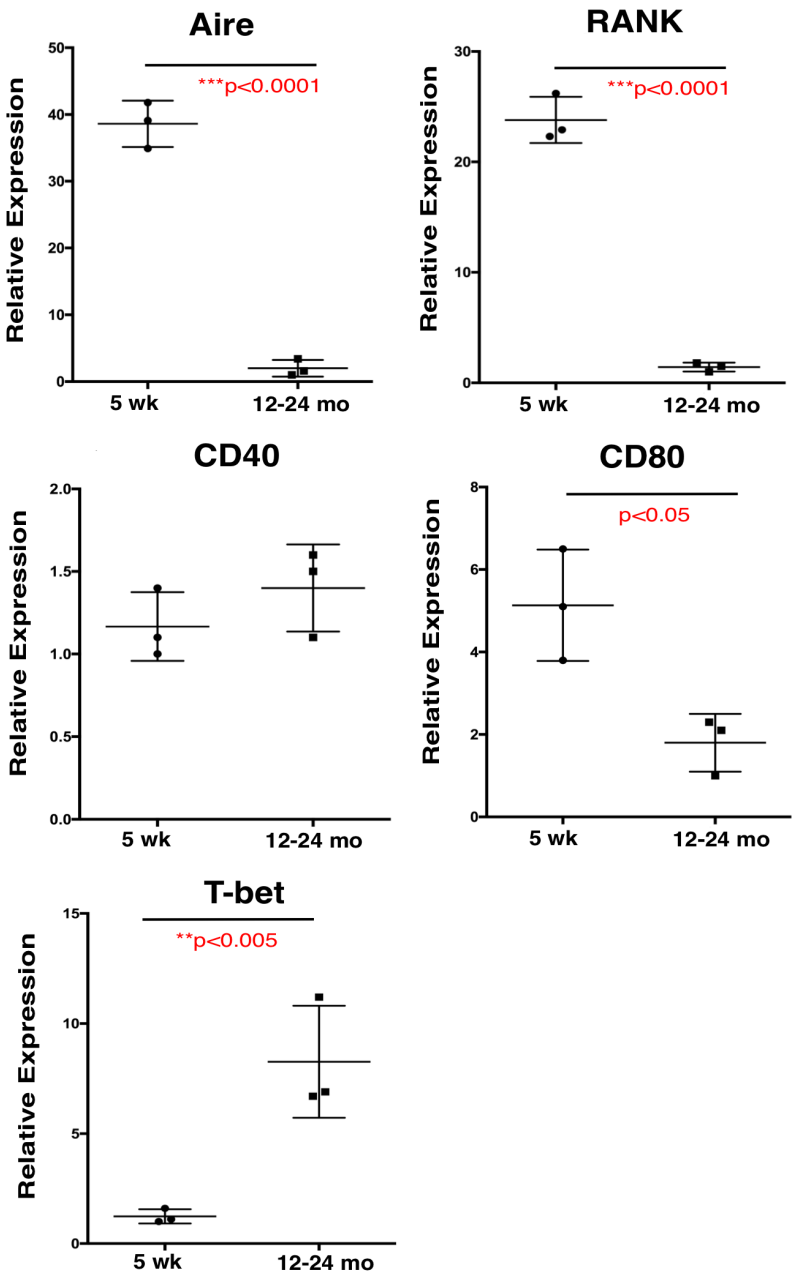
Figure S1. B cell number and localization in the aged mouse thymus. Related to Figure 1.



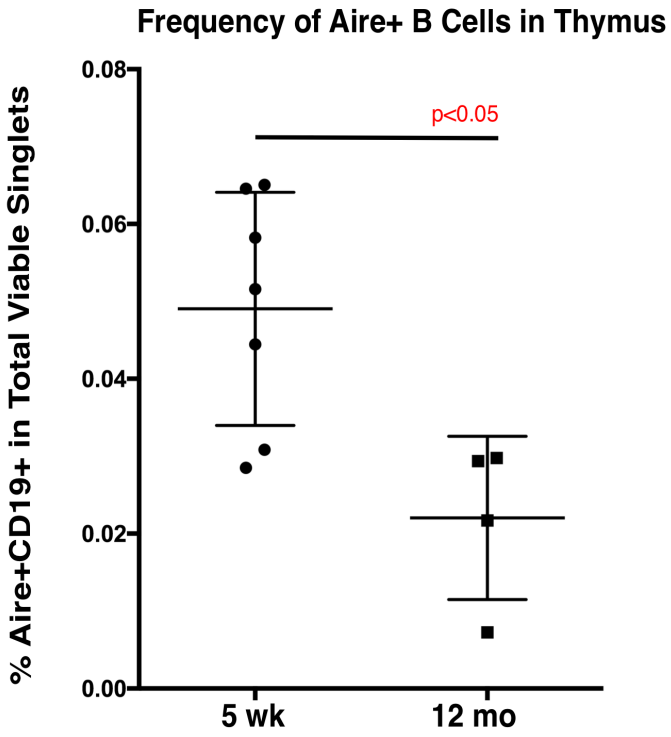
Supplemental Figure 1. B cell number and localization in the aged mouse thymus. a) The number of CD19+ B cells in thymus in young (5 week, n= 10) and aged (12-24 month, n=11) mouse thymus. Bars indicate mean ± SEM. b) Anti-B220 (red), and DAPI (blue) stains identify B cells and nuclei, respectively. B cells increase in frequency in 12 month-old mice (b-e). Stitched composite 40X images were assembled (b,d) to identify the spatial/anatomical organization of thymic B cells in 12 month-old mice. Thymic B cells are most common in medulla, but are also present in cortex less frequently. Panels c and e are higher magnification images from the inset boxes in b and d, respectively. Panels b-e represent 2 individual 12 month-old mice.

Figure S2. Validation of RNAseq by qPCR. Related to Figure 2.



Supplemental Figure 2. Validation of RNA-seq by qPCR. Relative expression levels of Aire, RANK, CD40, CD80, and T-bet were measured by qPCR to confirm RNA-seq results in intrathymic B cells described in Figure 2, normalized to Hprt. Bars indicate mean ± SEM. P-values calculated using student's t-test.

Figure S3. Diminished frequency of Aire expressing thymic B cells in aged mice. Related to Figure 4.



Supplemental Figure 3. Diminished frequency of Aire expressing thymic B cells in aged mice. a) Frequency of Aire-GFP+ B cells in total viable, singlet thymus cells in 5 week old (n=7) and 12 month old (n=4) Adig reporter mice. Bars indicate means and standard deviation. P-values calculated using unpaired two-tailed Student's t-test.

Table S1. Expression of Aire-dependent B cell-specific genes in human and murine thymic B cells. Related to Figure 3.

Human Aire-Dependent B cell-specific Gene List				Mouse Aire-Dependent B cell-specific Gene List			
Gene Symbol	Young Mean	Old Mean	p value (limma)	Gene Symbol	Young Mean	Old Mean	p value (limma)
HLA-B	1547.958	1705.964	6.657E-01	A630023P12Rik	8411.812	2525.442	2.085E-04
NLRC5	92.291	133.245	3.094E-02	Stard9	4608.144	6209.816	1.233E-01
OAS2	84.705	39.954	4.082E-03	G630030J09Rik	1037.906	1096.159	6.671E-01
PHF11	44.179	45.412	9.731E-01	Vwa3b	931.758	1590.937	1.509E-01
PARP12	41.641	36.048	2.881E-01	Gm10382	865.037	1303.538	1.864E-01
DDX60	39.690	18.345	2.046E-02	1600012P17Rik	502.689	12.151	4.276E-08
GPR114	39.546	47.613	3.157E-01	Flt3	286.122	96.890	7.524E-05
ZBP1	34.151	24.359	2.013E-01	H2-Q7	274.756	221.847	2.907E-02
HSPH1	25.613	17.108	2.658E-01	Gm16315	259.682	80.185	5.330E-03
RSAD2	24.805	13.674	3.712E-02	Zbtb32	253.086	442.974	2.541E-01
CSF2RB	23.833	21.660	6.924E-01	Tet2	236.196	185.398	3.249E-03
FNIP1	12.691	12.230	7.847E-01	Gm17034	167.378	76.755	5.532E-01
TET2	12.607	9.146	1.400E-01	Gatsl3	94.560	31.791	2.483E-05
SLC5A3	10.755	4.457	1.938E-02	Nlrc5	92.896	73.244	5.542E-02
ZBTB32	9.410	46.717	1.306E-04	Ctsw	79.631	17.457	3.639E-04
PIK3R6	9.156	10.183	7.641E-01	Aire	73.472	8.124	9.067E-08
AHR	8.456	9.344	4.584E-01	St8sia1	73.425	8.652	1.504E-06
OBSCN	7.506	5.992	3.553E-01	Atp10a	62.356	17.126	3.169E-04
SSPN	7.412	20.217	7.154E-02	Zbp1	57.560	44.828	1.079E-01
ATP2B4	6.954	16.945	2.840E-03	Hsph1	55.356	30.757	7.306E-02
STK38L	6.656	7.312	5.894E-01	Oas2	41.858	12.046	3.048E-04
ARHGAP19	5.970	5.673	6.940E-01	Cdh17	36.628	12.501	2.977E-06
HK2	4.805	1.327	3.670E-02	Mx1	35.501	11.388	8.179E-04
GATSL3	4.430	6.092	2.075E-01	Stxbp1	33.622	20.905	8.832E-04
SOCS3	3.922	1.378	5.109E-02	Rprm	27.757	4.905	3.037E-05

IL2RB	3.820	2.592	2.973E-01	Stk38l	25.856	21.481	9.798E-02
PKD2	3.506	1.287	2.748E-02	Slfn1	24.499	4.909	1.526E-04
RAG1	2.972	2.267	2.691E-01	Rag1	23.969	12.384	3.724E-02
CISH	2.758	1.294	2.469E-01	Ahr	22.857	8.561	7.985E-07
CTSW	2.733	2.018	4.006E-01	Oasl2	21.813	8.976	2.753E-04
CD3E	2.716	2.404	3.219E-01	Sspn	20.386	10.493	7.209E-04
FLT3	2.400	2.324	5.743E-01	Ddx60	17.870	5.147	5.849E-05
RUNX2	2.362	0.400	5.110E-02	Gbp7	17.849	14.800	1.077E-01
SOCS2	2.160	1.124	2.853E-01	Atp2b4	16.824	10.951	5.661E-02
SLC24A1	1.692	2.055	6.247E-01	Socs3	15.860	17.888	3.789E-01
LAMP3	1.601	0.156	4.937E-02	Fnip1	15.310	13.320	4.548E-02
SYN3	1.187	2.094	5.674E-01	2810429I04Rik	14.990	5.834	4.396E-06
CD8A	1.042	0.983	6.701E-01	Nek2	14.877	6.455	1.369E-04
GBP7	0.707	2.467	5.331E-04	Il2rb	13.549	6.172	1.071E-03
EML5	0.672	0.011	1.362E-01	1700071M16Rik	13.160	12.151	7.058E-01
ATP10A	0.625	0.942	4.017E-01	Gm16184	13.160	12.151	7.058E-01
TP73	0.548	0.015	4.297E-01	4930594C11Rik	12.733	13.579	4.476E-01
AIRE	0.531	0.461	1.273E-02	Pik3r6	10.925	3.145	1.792E-05
NEK2	0.405	3.822	1.173E-02	Cish	9.207	2.713	4.064E-05
DBNDD1	0.394	0.025	7.246E-01	Cd3e	8.786	6.913	3.070E-01
RAPGEF4	0.332	0.026	1.484E-02	Hk2	8.729	4.803	1.237E-04
SEMA6A	0.251	0.104	1.559E-01	Pvr	8.320	5.574	5.110E-03
GAS2L3	0.111	0.003	6.891E-01	Rsad2	6.509	1.870	1.650E-04
CDH17	0.105	0.327	4.883E-01	Lamp3	6.253	2.010	1.199E-06
ST8SIA1	0.103	0.383	8.306E-01	Cd8a	5.995	5.790	5.649E-01
GGN	0.086	0.824	9.307E-01	Grik2	5.320	0.093	4.577E-04
VASN	0.061	0.572	3.088E-01	Parp12	5.287	1.711	7.778E-04
ACSBG1	0.060	0.085	4.090E-05	Arhgap19	4.316	3.266	2.825E-01

VWA3B	0.047	0.002	1.180E-04	Sema6d	4.071	1.677	2.163E-04
FAM196B	0.041	0.009	6.439E-05	Eml5	3.876	4.525	1.098E-01
MAP3K15	0.029	0.002	1.445E-01	Acsbg1	3.011	0.385	1.782E-06
STXBP1	0.028	0.014	1.597E-01	Slc5a3	2.943	2.427	1.374E-01
SEMA6D	0.026	0.002	3.919E-01	Rapgef4	2.786	7.120	3.371E-05
TFCP2L1	0.020	0.006	1.540E-01	Socs2	2.347	0.507	3.443E-04
GRIK2	0.012	0.002	7.619E-01	Siglech	2.227	0.427	2.845E-06
RANBP17	0.006	0.001	4.176E-01	Iigp1	2.134	5.590	1.252E-02
RPRM	0.005	0.002	5.126E-01	Map3k15	2.107	3.751	3.400E-02
SCEL	0.002	0.009	5.108E-01	Gas2l3	1.958	1.002	8.010E-02
LIF	0.002	0.002	7.272E-01	Runx2	1.362	1.625	3.018E-01
				Scel	1.213	0.176	1.012E-04
				Csf2rb2	1.111	1.063	5.990E-01
				Ggn	1.080	0.458	3.262E-04
				Trp73	0.902	0.642	8.222E-02
				Pkd2	0.598	0.110	3.586E-06
				Syn3	0.396	0.841	7.067E-02
				Vasn	0.335	0.172	1.020E-02
				Lif	0.180	0.025	1.014E-03
				Obscn	0.173	0.188	6.482E-01
				Gm9855	0.165	0.160	9.945E-01
				Ranbp17	0.156	0.421	2.855E-02
				Fam196b	0.058	0.050	8.996E-01
				Dbndd1	0.035	0.011	2.268E-01
				Slc24a1	0.018	0.010	5.133E-01
				Sema6a	0.011	0.013	9.230E-01

Supplemental Table 1. List of Aire-dependent B cell-specific genes identified by Yamano et al. with average RNAseq expression values (RPKM) in young (3 month, 5months, 4 years, n=3) and aged (42-61 years, n=3) human thymic B cells and in young (5 week, n=7) and aged (12-24 month, n=5) murine thymic B cells. P-value calculated with *limma*.

Supplemental Experimental Procedures

Immunofluorescent microscopy

Thymi removed after euthanasia, embedded in ice-cold OCT (Fisher Scientific), and immediately frozen. 5 micron transverse sections were fixed in ice-cold acetone, stained with Alexa 594-conjugated anti-B220 antibody and then mounted in Prolong Gold (Molecular Probes) containing 0.25μg/ml DAPI. Images were captured on a fluorescent microscope using mercury illumination.