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## Supplemental information

# Thymus antibody-secreting cells possess an interferon gene signature and are preferentially expanded in young female mice

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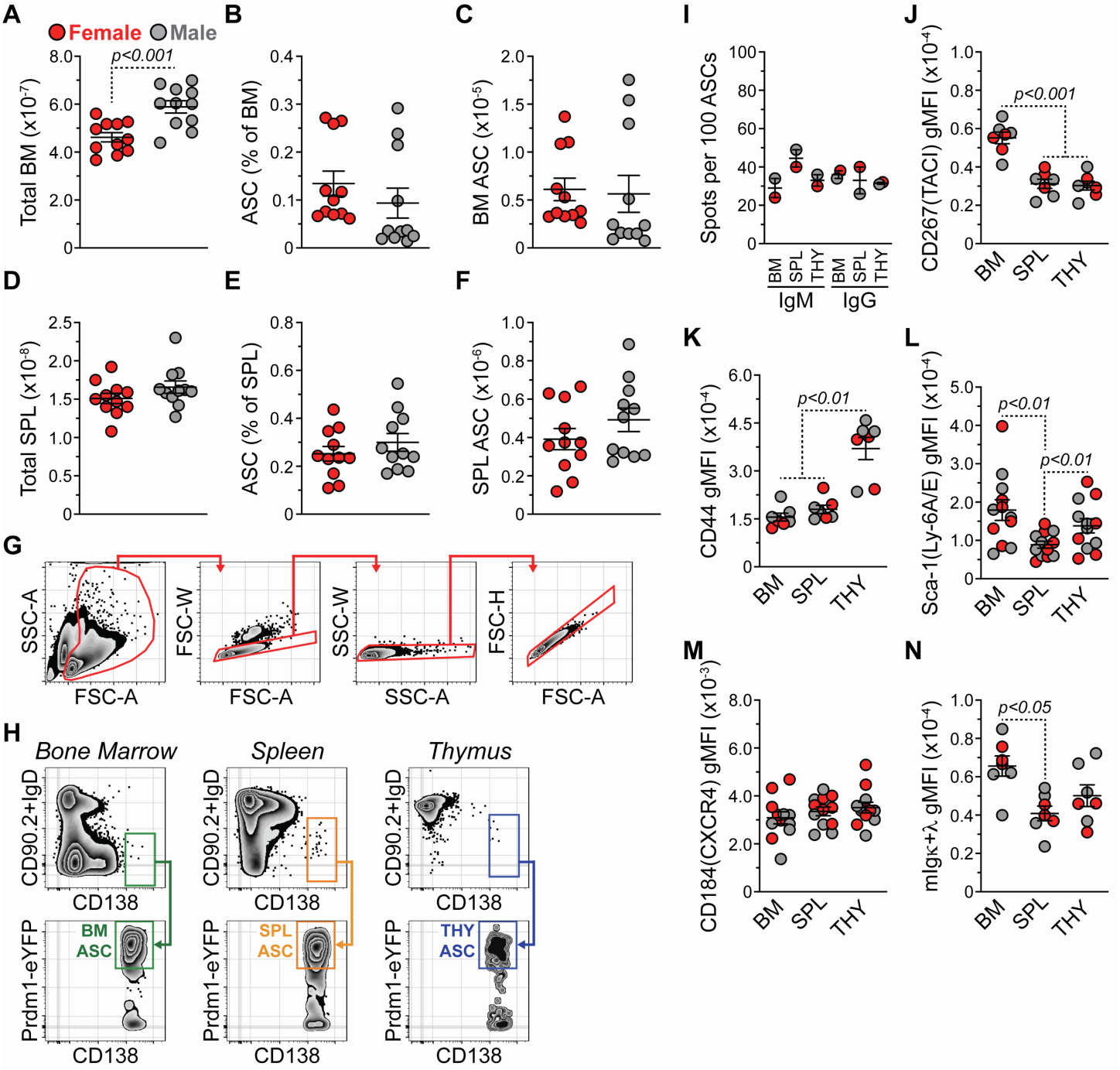


**Figure S1. Thymus antibody-secreting cells express expected surface markers. Related to Figure 1.**
(A) Total BM cell numbers. (B) Percentages of BM ASCs. (C) Numbers of BM ASCs. (D) Total SPL cell numbers. (E) Percentages of SPL ASCs. (F) Numbers of SPL ASCs. (G) Representative flow cytometry plots depicting live cell and singlet gating based upon various SSC and FSC parameters. (H) Representative flow cytometry plots illustrating gating of ASCs from BM, SPL and THY. ASCs were defined as $CD138^{HI}$ $IgD^{-/LO}$ $CD90.2^{-/LO}$ $Prdm1\text{-}eYFP^{+}$. (I) Numbers of IgM and total IgG spots per 100 BM, SPL and THY ASCs plated per ELISpot assay. (J-N) Flow cytometry geometric mean fluorescence intensities (gMFIs) of (J) CD267(TACI), (K) CD44, (L) Sca-1(Ly-6A/E), (M) CD184(CXCR4) and (N) mIgκ+λ on the surface of ASCs from BM, SPL and THY. (A-F, I-N) Symbols represent individual mice. Horizontal lines represent mean ± SEM. (A-F) Unpaired Student's t-Test. Female: n = 11; Male: n = 11. (I) Female: n = 1 pool of 3 mice; Male: n = 1 pool of 3 mice. (J-N) One-way ANOVA with Tukey's correction. (J-K, N) Female: n = 3; Male: n = 4. (L-M) Female: n = 6; Male: n = 6.

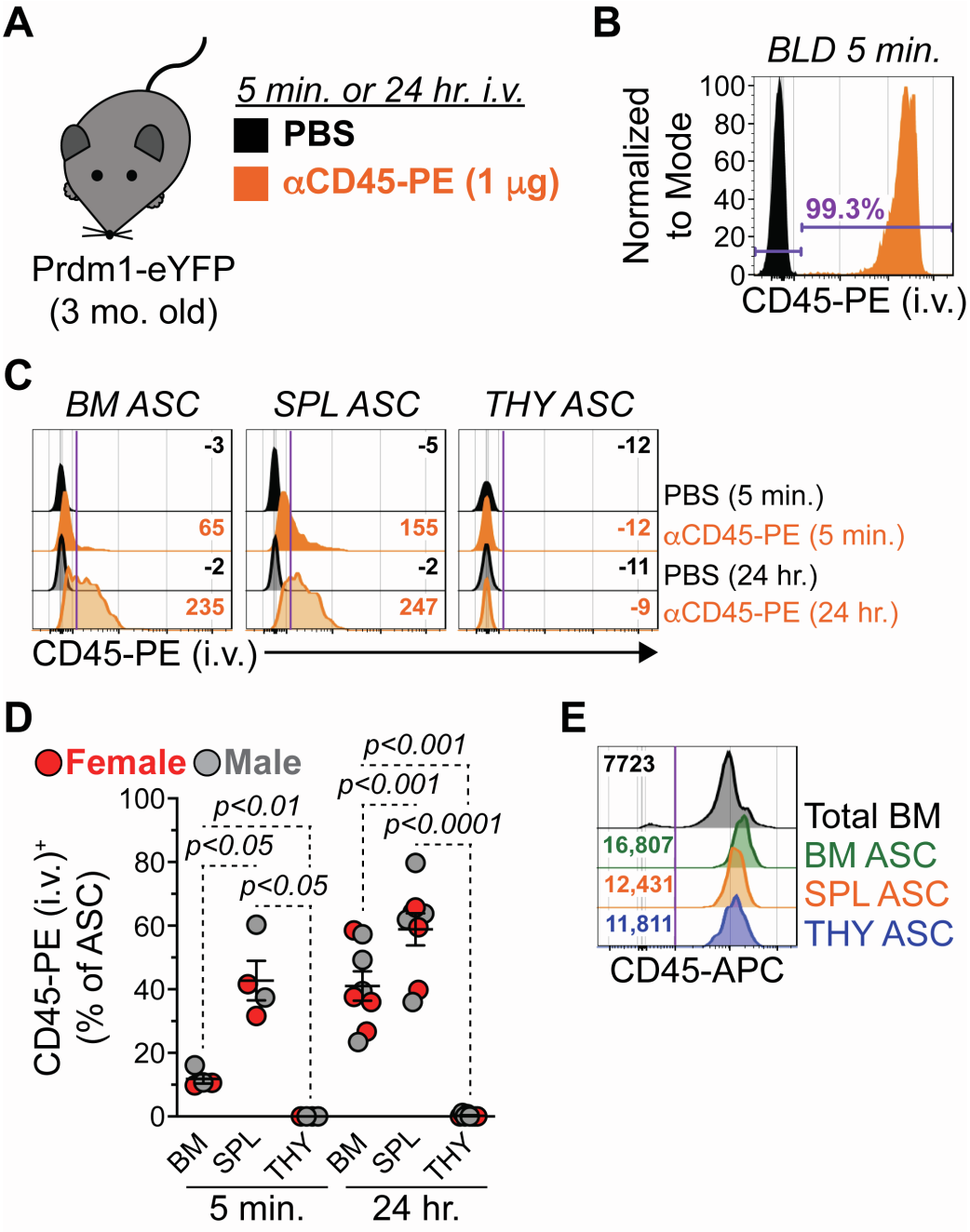


**Figure S2. Thymic antibody-secreting cells are not recent thymic immigrants. Related to Figure 2.**
(A) Young (3 mo. old) female and male Prdm1-eYFP mice received i.v. injections through the retro-orbital (r.o.) sinus containing either PBS or αCD45-PE Abs (1 μg). Animals were euthanized and assessed for αCD45-PE labeling 5 min. or 24 hr. post-injection. (B) Flow cytometry histograms overlaying CD45-PE fluorescence of total peripheral BLD cells from animals that received either PBS or αCD45-PE for 5 min. Number in plot indicates percentage of total peripheral BLD cells positive for CD45-PE (i.v.) staining 5 min. post-injection of αCD45-PE. (C) Flow cytometry plots depicting αCD45-PE (i.v.) labeling of ASCs from BM, SPL and THY. Staining from PBS-treated mice and mice that received αCD45-PE Abs for 5 min. or 24 hr. is shown for comparison. Purple vertical lines added to CD45-PE (i.v.) histograms show cut-offs for positive staining. Numbers in plots indicate gMFI. (D) Percentages of BM, SPL and THY ASCs labeled with αCD45-PE 5 min. or 24 hr. post-injection. Symbols represent individual mice. Horizontal lines represent mean ± SEM. One-way ANOVA with Tukey's correction. 5 min.: n = 4; 24 hr.: n = 8. (E) Flow cytometry histogram showing CD45-APC *ex vivo* staining of total BM cells and ASCs from BM, SPL and THY. Purple vertical line added to CD45-APC histogram shows cut-off for positive staining. Numbers in plots indicate gMFI.

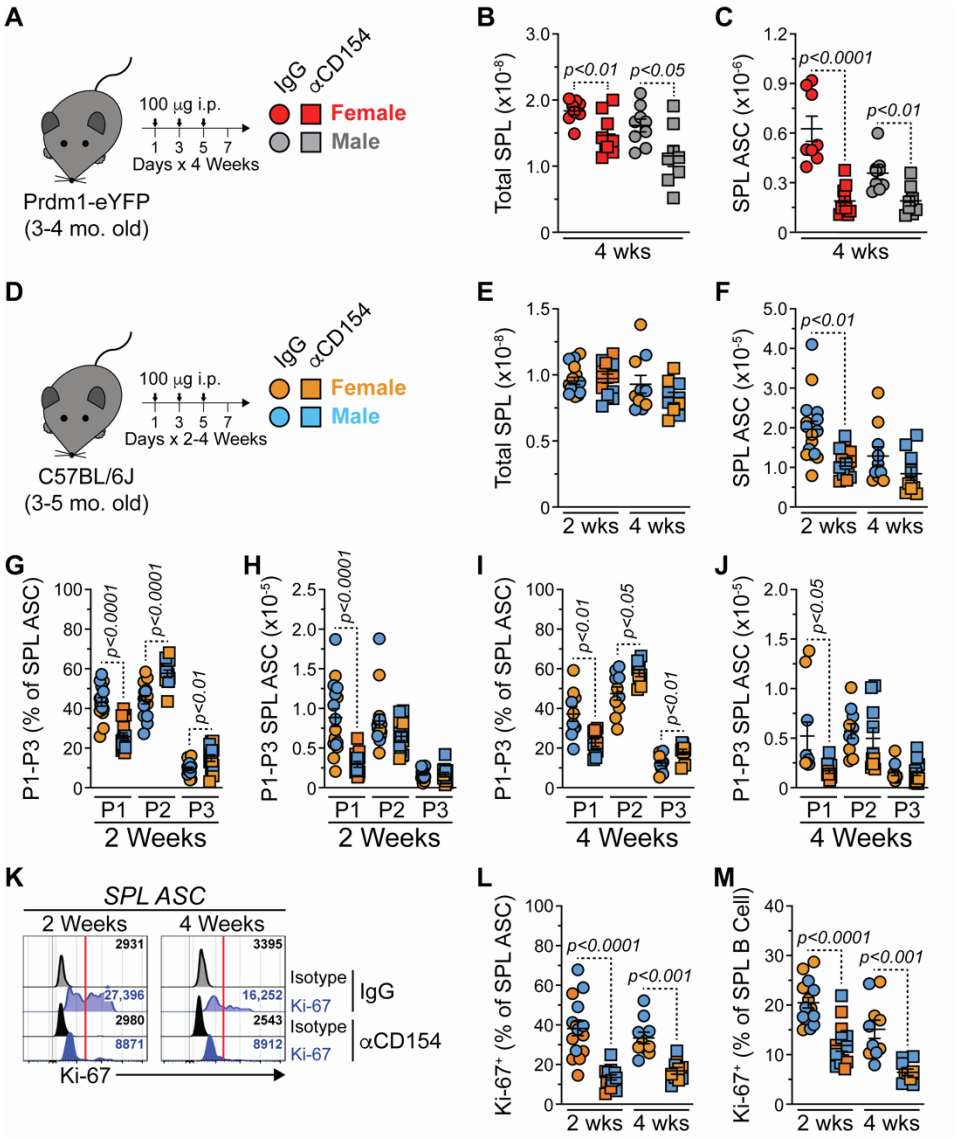


**Figure S3. Blockade of CD154(CD40L)-derived signals reduces proliferative antibody-secreting cells in the spleen. Related to Figure 3.**

(A) Young (3-4 mo. old) Prdm1-eYFP female and male mice received 1200 μg total of hamster IgG isotype control or anti-mouse CD154(CD40L) (MR-1) Abs. Administration was split evenly amongst 12-total i.p. injections (100 μg per injection, 100 μl volume) over a 4-week span. (B-C) Total SPL and SPL ASC numbers in Prdm1-eYFP mice treated with hamster IgG isotype control or anti-mouse CD154 Abs for 4 weeks. (D) Young (3-5 mo. old) C57BL/6J female and male mice were treated as in (A) over a 2- or 4-week span. (E-F) Total SPL and SPL ASC numbers in C57BL/6J mice treated with hamster IgG isotype control or anti-mouse CD154 Abs for 2 or 4 weeks. (G-H) C57BL/6J P1-P3 (G) percentages within SPL ASCs and (H) cell numbers following 2 weeks of IgG isotype control or anti-mouse CD154 treatment. (I-J) C57BL/6J P1-P3 (I) percentages within SPL ASCs and (J) cell numbers following 4 weeks of IgG isotype control or anti-mouse CD154 treatment. (K) Flow cytometry overlays showing isotype control and Ki-67 intracellular staining of SPL ASCs from mice following 2 or 4 weeks of IgG isotype control or anti-mouse CD154 treatment. Red vertical lines added to histograms show cut-offs for positive staining. Numbers in plots indicate gMFI. (L-M) Percentages of Ki-67$^{+}$ cells within SPL (L) ASCs and (M) B cells following 2 or 4 weeks of IgG isotype control or anti-mouse CD154 treatment. (B-C, E-F, G-J, L-M) Symbols represent individual mice. Horizontal lines represent mean ± SEM. (B-C) Unpaired Student's t-Test. Female IgG: n = 8; Female αCD154: n = 10; Male IgG: n = 9; Male αCD154: n = 8. (E-F, G-J, L-M) Unpaired Student's t-Test. 2 weeks Female IgG: n = 8; 2 weeks Female αCD154: n = 7; 2 weeks Male IgG: n = 8; 2 weeks Male αCD154: n = 7; 4 weeks Female IgG: n = 5; 4 weeks Female αCD154: n = 5; 4 weeks Male IgG: n = 5; 4 weeks Male αCD154: n = 5.

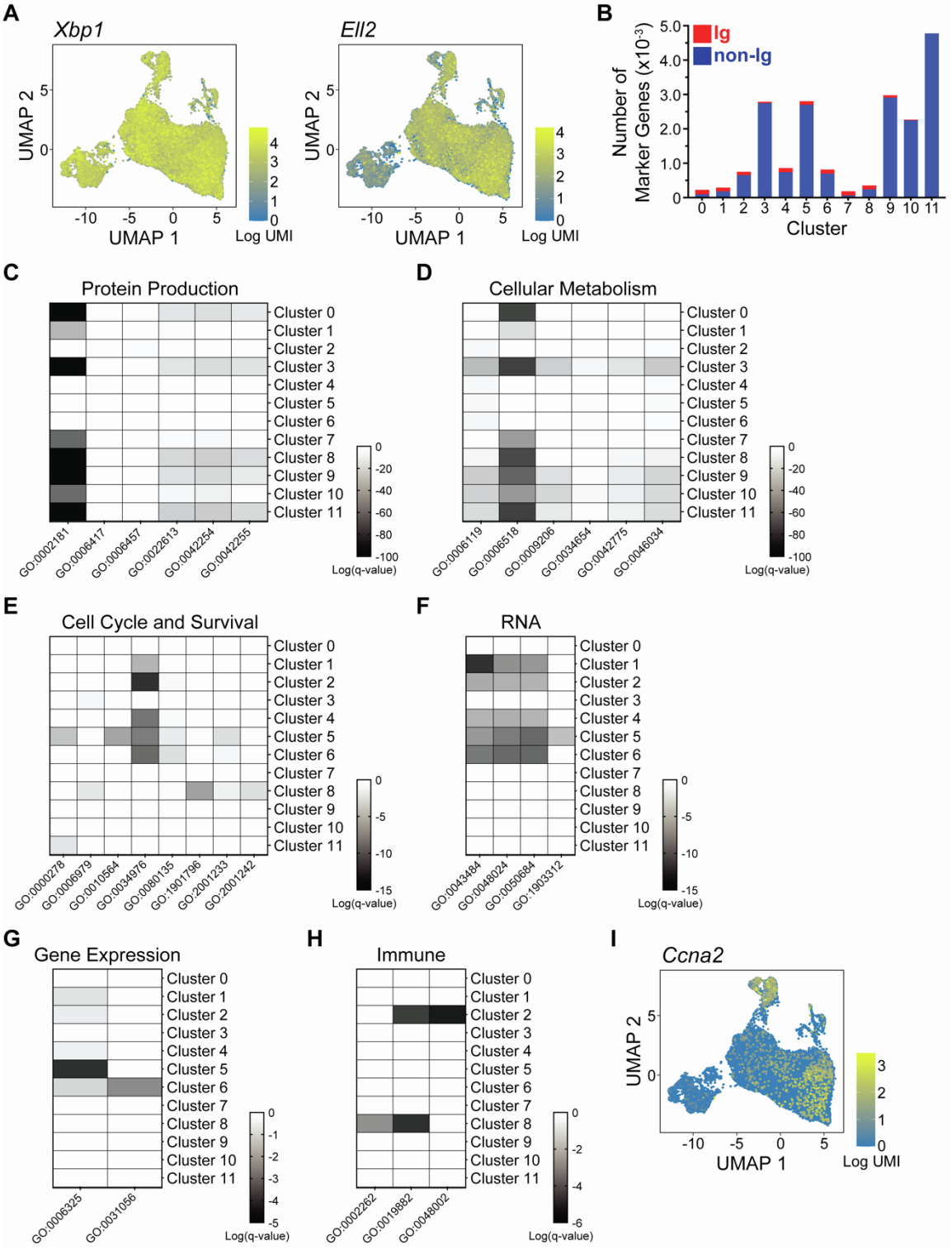


**Figure S4. Gene ontology analysis of antibody-secreting cell clusters. Related to Figure 5.**
(A) UMAP plots showing log-normalized unique molecular identifier (UMI) counts for *Xbp1* and *Ell2*. (B) Numbers of cluster marker genes that were Ig-related or non-Ig-related. Ig-related genes consisted of those encoding either V, D and J segments or constant regions for heavy and light chains. (C-H) Heatmaps depicting Log(q-value) statistical significance for cluster marker gene association with selected (C) Protein Production, (D) Cellular Metabolism, (E) Cell Cycle & Survival, (F) RNA, (G) Gene Expression and (H) Immune GO categories. Values derived from Metascape analyses presented in Table S2. (I) UMAP plot showing log-normalized UMI counts for *Ccna2*.

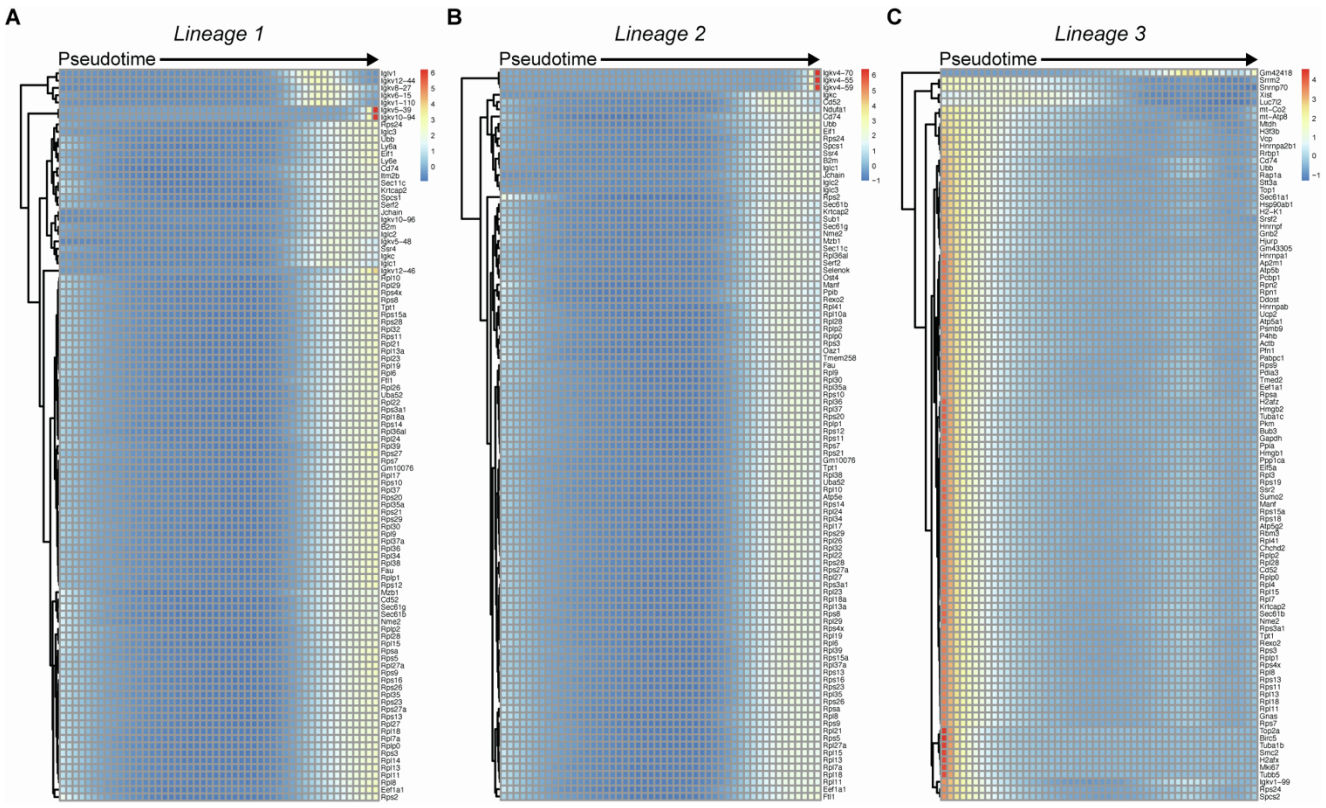


**Figure S5. Antibody-secreting cell pseudotime lineages demonstrate progressive changes in gene expression. Related to Figure 5.**

(A-C) Heatmaps showing row-normalized, smoothed gene expression of the top 100 lineage-associated genes for (A) *Lineage 1*, (B) *Lineage 2* and (C) *Lineage 3*. Pseudotime progresses from left-to-right.

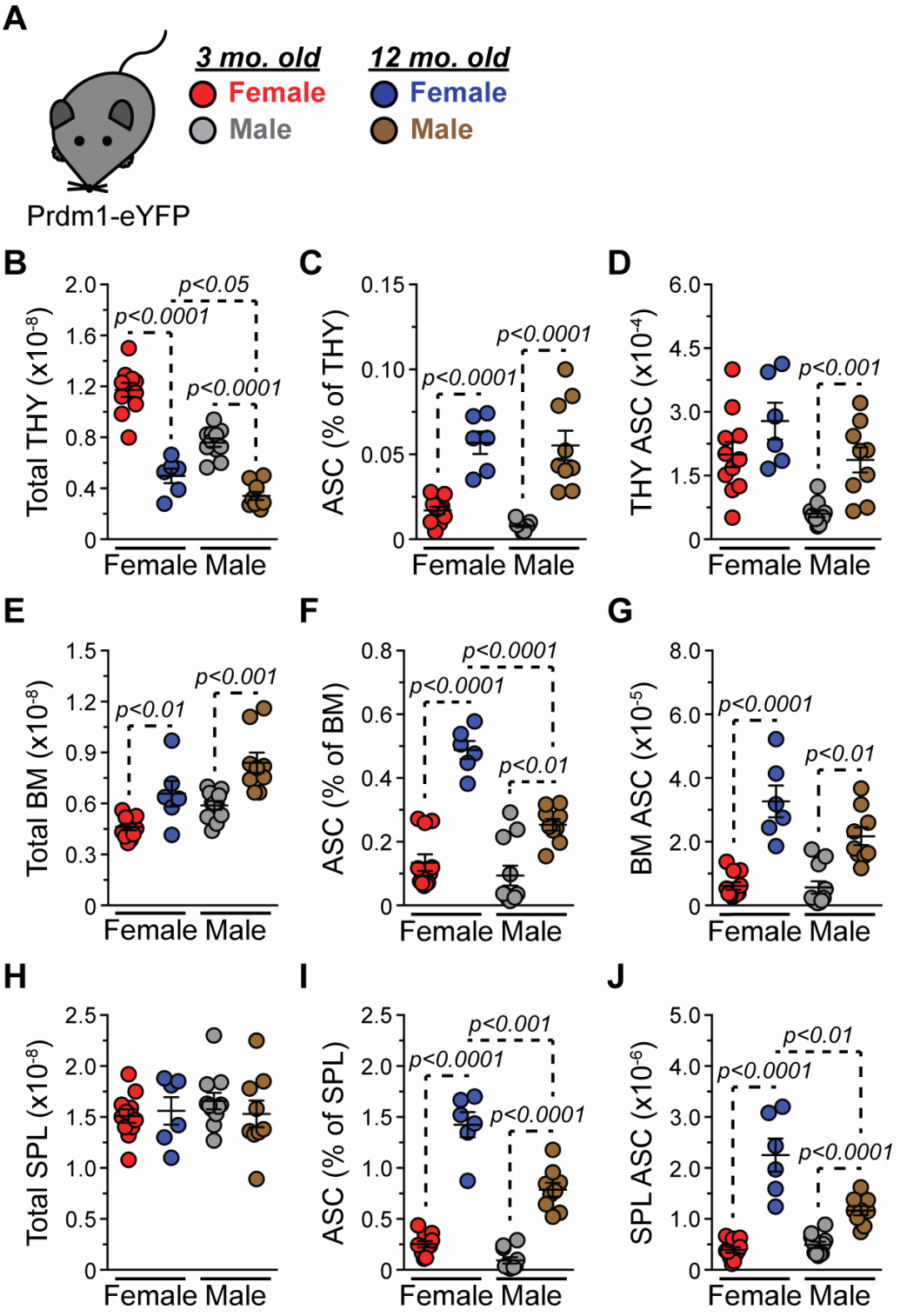


**Figure S6. Quantification of antibody-secreting cells in middle-aged bone marrow, spleen and thymus. Related to Figure 7.**

(A) Middle-aged (12 mo. old) female and male Prdm1-eYFP mice were assayed for ASCs in BM, SPL and THY. Middle-aged data compared to that of young (3 mo.) mice generated in Figure 1. (B) Total THY cell numbers. (C-D) THY ASC (C) percentages and (D) numbers. (E) Total BM cell numbers. (F-G) BM ASC (F) percentages and (G) numbers. (H) Total SPL cell numbers. (I-J) SPL ASC (I) percentages and (J) numbers. (B-J) Symbols represent individual mice. Horizontal lines represent mean ± SEM. Unpaired Student's t-Test. Female 3 mo.: n = 11; Male 3 mo.: n = 11; Female 12 mo.: n = 6; Male 12 mo.: n = 9.

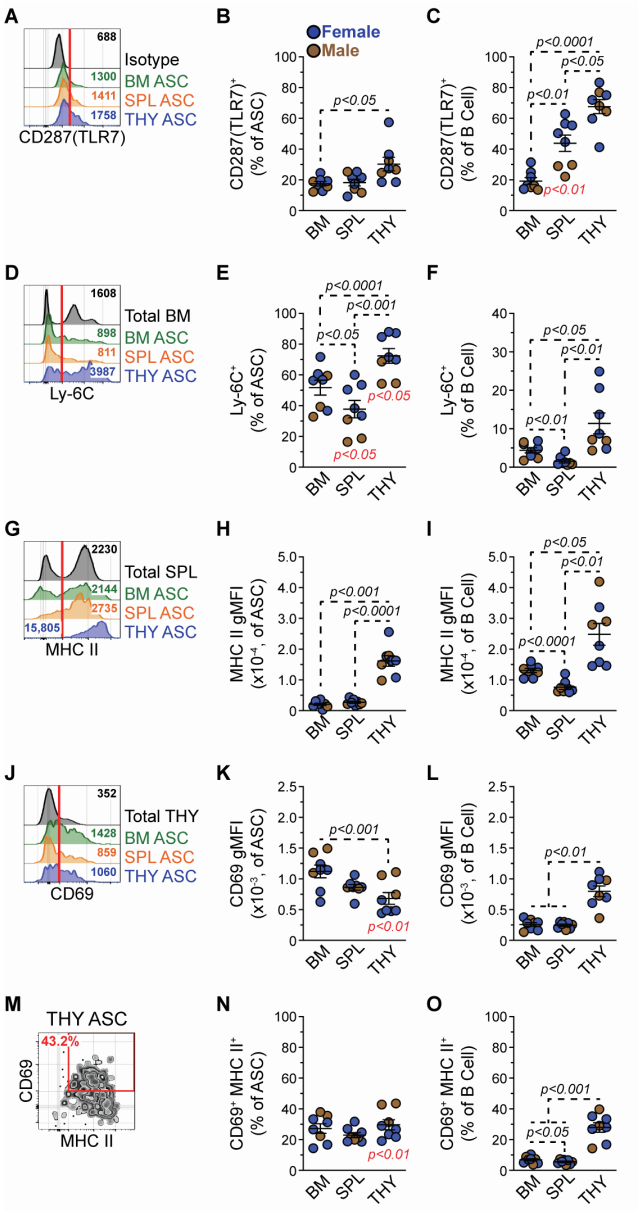


**Figure S7. Middle-aged thymus antibody-secreting cells demonstrate upregulation of selected interferon-related factors. Related to Figure 7.**

(A) Representative flow cytometry histograms depicting intracellular CD287(TLR7) staining of BM, SPL and THY ASCs. Isotype control staining of BM ASCs is shown for comparison. Red vertical line added shows cut-off for positive staining. Numbers in plots indicate gMFI. (B-C) Percentages of CD287(TLR7)$^{+}$ cells within (C) ASCs and (C) CD45R(B220)$^{+}$ CD19$^{+}$ CD138$^{-/LO}$ B cells from BM, SPL and THY. (D) Representative flow cytometry histograms depicting Ly-6C surface staining of BM, SPL and THY ASCs. Total BM is shown for comparison. Red vertical line added shows cut-off for positive staining. Numbers in plots indicate gMFI. (E-F) Percentages of Ly-6C$^{+}$ cells within (E) ASCs and (F) CD45R(B220)$^{+}$ CD19$^{+}$ CD138$^{-/LO}$ B cells from BM, SPL and THY. (G) Representative flow cytometry histograms depicting MHC II surface staining of BM, SPL and THY ASCs. Total SPL is shown for comparison. Red vertical line added shows cut-off for positive staining. Numbers in plots indicate gMFI. (H-I) MHC II gMFIs of (H) ASCs and (I) CD45R(B220)$^{+}$ CD19$^{+}$ CD138$^{-/LO}$ B cells from BM, SPL and THY. (J) Representative flow cytometry histograms depicting CD69 surface staining of BM, SPL and THY ASCs. Total THY is shown for comparison. Red vertical line added to show cut-off for positive staining. Numbers in plots indicate gMFI. (K-L) CD69 gMFIs of (K) ASCs and (L) CD45R(B220)$^{+}$ CD19$^{+}$ CD138$^{-/LO}$ B cells from BM, SPL and THY. (M) Representative flow cytometry plot depicting CD69 and MHC II dual surface staining on THY ASCs. (N-O) Percentages of CD69$^{+}$ MHC II$^{+}$ cells within (N) ASCs and (O) CD45R(B220)$^{+}$ CD19$^{+}$ CD138$^{-/LO}$ B cells from BM, SPL and THY. (B-C, E-F, H-I, K-L, N-O) Symbols represent individual mice. Horizontal lines represent mean ± SEM. Unpaired Student's t-Test for comparisons within sex indicated by red p-values. One-way ANOVA with Tukey's correction for comparisons between organs. Female: n = 5; Male: n = 3.