Supplemental Information

$CD4^+$ T Cells and CD40 Participate in Selection and Homeostasis of Peripheral B Cells

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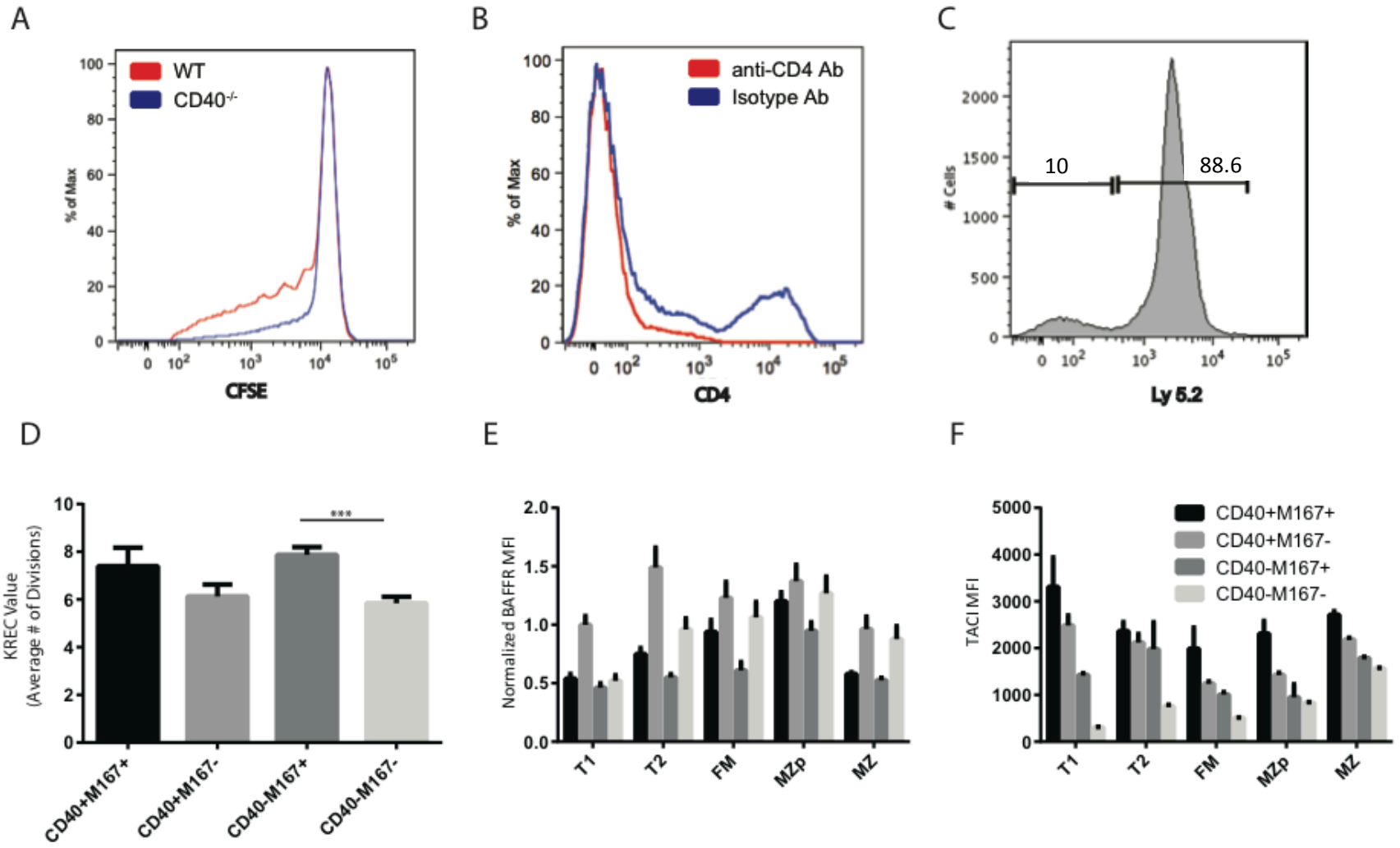
Supplemental Inventory

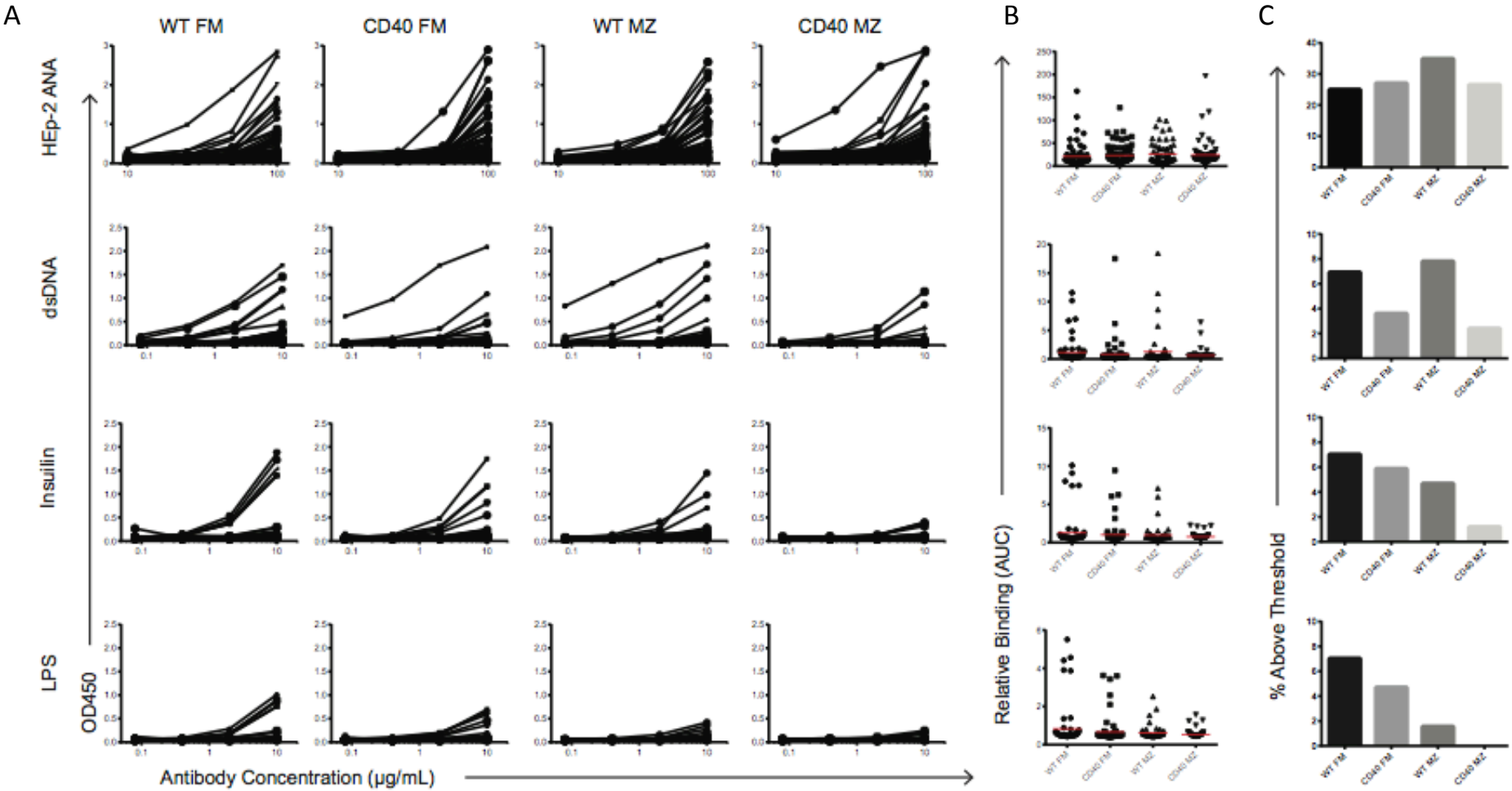
Supplemental Figures

Supplemental Figure 1



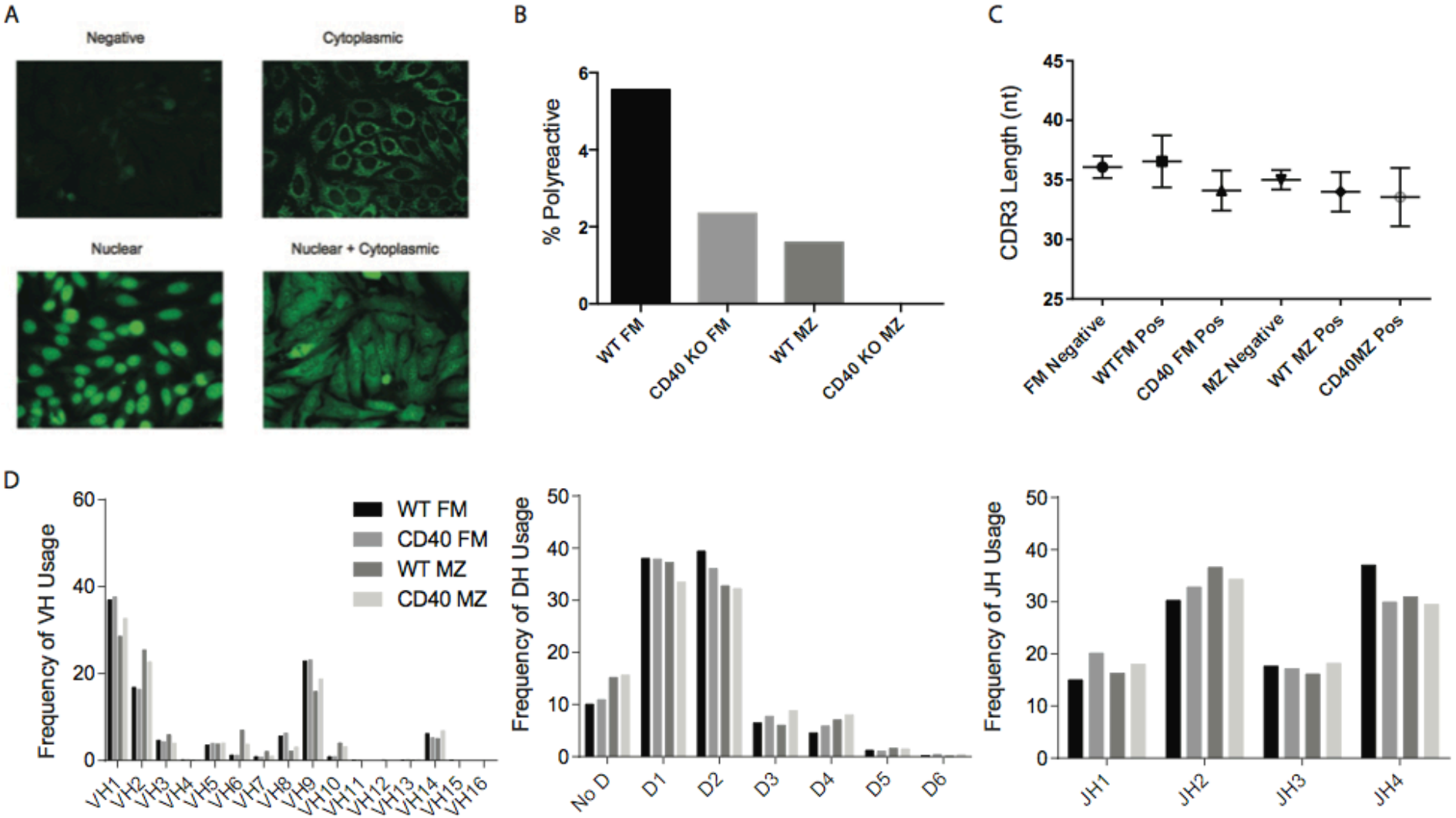
Supplemental Figure 1: CD40 modulates development of self-reactive transgenic B cells. (A) Representative CFSE histograms of WT and CD40$^{-/-}$ splenic B cells 5-7 days post-transplant measuring homeostatic proliferation as described in Figure 1 (B) Representative FACs plot measuring efficiency of CD4 depletion after 4 weeks of weekly treatment with anti-CD4 or isotype antibody (C) Representative FACS plots measuring purity of mixed bone marrow (90:10 ratio of CD40 KO (Ly5.2): WT (Ly5.1)) prior to transplantation (D) KRECs assay in cells sorted from M167-Tg WT/CD40$^{-/-}$ mixed BM chimeras based on both CD40 expression and Id-specific antibody binding, surface expression of BAFFR (E) and TACI (F) in mixed BM chimeras, gated based on CD40 and Id-antibody. Error bars show SEM, ***p<0.0005, data representative of at least two experiments.

Supplemental Figure 2



Supplemental Figure 2: Repertoire Analysis of WT and CD40 KO Mice. (A-C): ELISA specificity results of BCRs cloned from WT vs. CD40KO mice. Antibody specificity assessed with an ELISA panel including HEp-2 ANAs, dsDNA, insulin, and LPS. (A)*:* OD450 results are shown with each line representing an individual cloned antibody. Total number of BCR clones are: 72 WT FM, 85 CD40 KO FM, 63 WT MZ, and 83 CD40 KO MZ. (B) Area under the curve (AUC) was calculated for each individual clone and plotted with mean AUC depicted by red lines. (C) Bar graphs show percentage of clones within each subset considered positive based on threshold cutoff OD450 values. Data pooled from three independent single-cell sorts.

Supplemental Figure 3



Supplemental Figure 3: BCR Cloning and Sequence Analysis of WT and CD40 KO Naïve B cell repertoire (A) HEp-2 IFAs, showing negative, cytoplasmic, nuclear, and nuclear plus cytoplasmic patterns. (B) Total percentage of polyreactive BCR clones. Clones are defined as polyreactive if found Hep-2 ANA positive as well as positive for 2 other self-antigens by ELISA (C) CDR3 length of ANA+ or Negative WT and CD40KO FM and MZ cloned BCRs (D) High throughput BCR heavy chain sequencing of FM and MZ subsets sorted from WT and CD40 KO mice. VDJ gene usage in FM And MZ B cell subsets.

Supplemental Table 1

	Total Sequences	Functional Sequences	Unique Clonotypes
WT FM	42481	20167	8102
CD40 FM	49220	22864	9060
WT MZ	43338	22216	4305
CD40 MZ	46553	24319	9368
Ly5.1 FM	36878	25612	1897
Ly5.2 FM	36803	25748	3303
Ly5.1 MZ	32267	24191	1180
Ly5.2 MZ	31182	21881	2310
Vk8	19474	16298	10083
Vk8xCD40-/-	25382	20943	8753

Table S1. Tabulation of BCR Clones Obtained for High-throughput Sequencing, Related to Figure 7. FM and MZ subsets were sorted by FACS from WT and CD40 KO mice, from WT/CD40 KO mixed BM chimeras (Ly5.1/Ly5.2 gates), and from Vk8 light chain transgenic and Vk8 x CD40 KO mice. Sorted FM and MZ subsets are each composed of 4 independent samples from 3 pooled mice. Vk8 and Vk8CD40$^{-/-}$ samples are CD43-depleted splenocytes from two samples taken from 3 pooled mice.