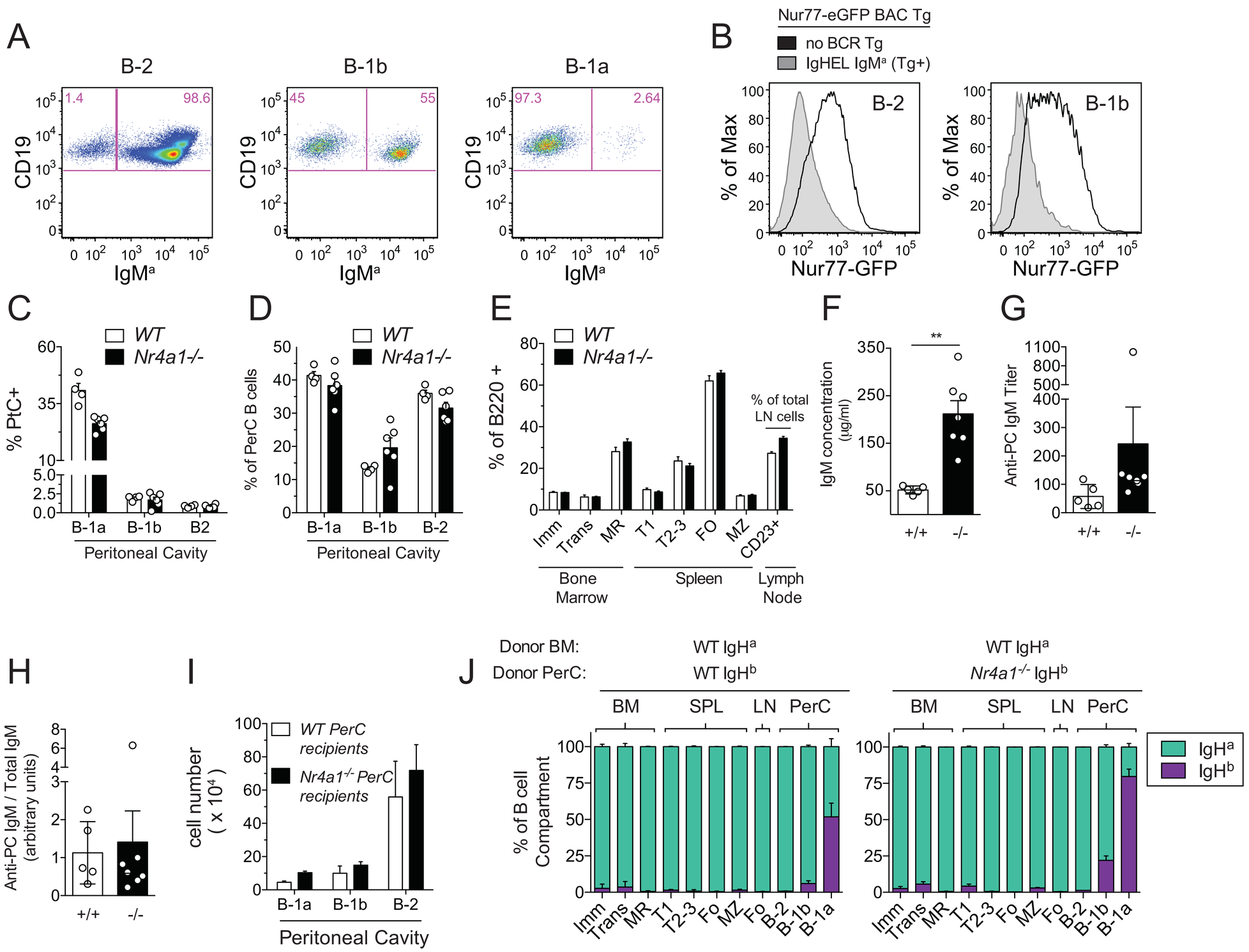
Supplemental Figure 1



Supplemental Figure 1.

(A) PerC cells from IgHEL BCR Tg Nur77-eGFP reporter mice were stained to identify BCR Tg expression (IgM^a) in B cell subsets (as gated in 1D). Plots show representative gating to detect Tg+ cells in each B cell compartment. Data are quantified in 1E.

(B) Nur77-eGFP mice with or without IgHEL BCR Tg were stained and gated as in 1D, and (A) above. Histograms depict GFP expression in Tg+ and Tg- reporter B cells. Data are quantified in 1F.

(C) Graph depicts mean % PtC+ cells (as gated in 2A) among B-1a, B-1b, and B-2 compartments (as gated in 1A) in WT and Nr4a1-/- mice +/- SEM.

(D) Graph depicts % B-1a, B-1b, and B-2 cells (as gated in 1A) in PerC from WT and Nr4a1-/- mice +/- SEM.

(E) Graph depicts % B cell subsets in BM, Spleen, and LN of WT and Nr4a1-/- mice +/- SEM. CD23+ LN cells are plotted as % of total LN. Imm = IgM+IgD-; Trans = $IgM^{hi}IgD^{int}$; MR = $IgD^{hi}IgM^{var}$; T1 = AA4.1+CD23-$CD21^{lo}$; T2-3 = AA4.1+CD23+$CD21^{int}$; Fo = AA4.1-CD23+$CD21^{int}$; MZ = $CD21^{hi}CD23^{int}$.

(F) Sera from WT and *Nr4a1-/-* mice were collected at 16 weeks of age and correspond to those analyzed in 3B. Graph shows IgM concentration quantified by ELISA +/- SEM.

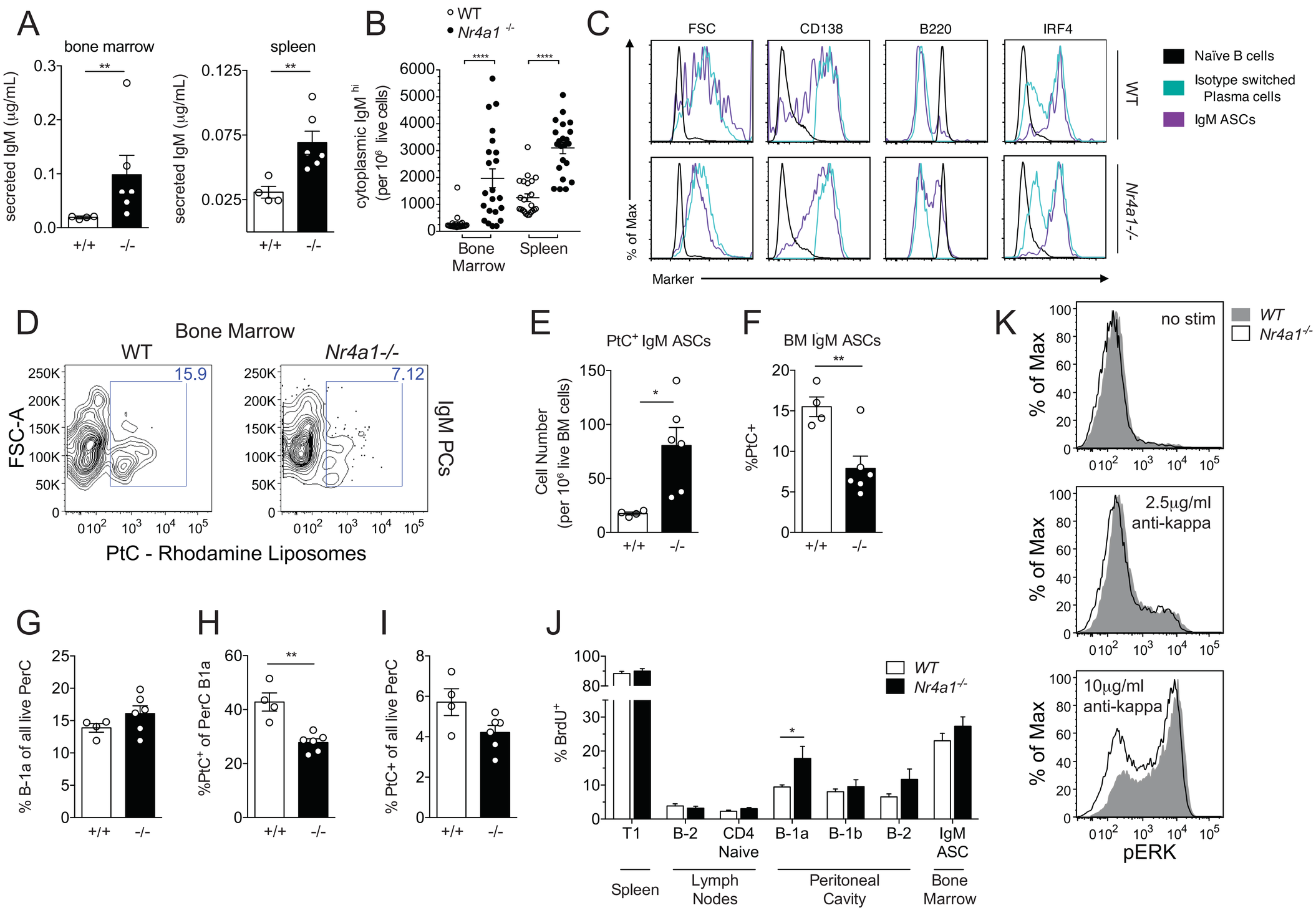
(G) Sera described in above were quantified for PC-specificity by ELISA. Graph shows mean IgM PC relative concentration +/- SEM.

(H) PC-specific IgM titers (as in D) were normalized for individual mice to total IgM (as in C). Graph depicts mean ratio +/- SEM.

(I, J) Reconstitution of radiation chimeras generated as in 3C was assessed after 10 weeks. (I) Graph depicts absolute cell counts of PerC B cell subsets in recipients of either WT or Nr4a1-/- PerC cells +/- SEM for N=4 biological replicates. (J) Graph depicts mean % IgM^a and IgM^b within each B cell compartment +/- SEM for N=4 biological replicates.

P-values in this figure were calculated using unpaired t-test, with Welch's correction applied to F.

Supplemental Figure 2



Supplemental Figure 2.
(A) Bone marrow and spleen cells from WT and KO mice were cultured for 24 hours ($2.5x10^5$ cells/ 200μl) and supernatants were collected. Graphs represent mean IgM concentration assessed by ELISA +/- SEM.
(B) Graphs depict mean number of IgM ASCs per 10^6 cells +/- SEM from BM and spleen of N=22 WT and KO mice analyzed as gated in 4A.
(C) Representative histograms of naïve B cells (B220hi, IC-IgM-low), isotype switched PCs (CD138hi, IgM-, B220-low), and IgM ASCs (as gated in 4A) from WT and KO mice were overlayed to display FSC, surface CD138 and B220, and intra-cellular Irf4 expression.
(D) Bone marrow from WT and KO mice was stained with PtC-liposomes and anti-IgM prior to fixation, followed by intra-cellular IgM. Representative plots gated on IgM ASCs as in 4A gated to identify PtC+ ASCs. Negative gates set relative to isotype-switched PCs which do not bind PtC.
(E-I) Graphs depict mean +/- SEM: (E) number of PtC+ IgM ASCs (as gated in S2D); (F) % PtC+ IgM ASCs (as gated in S2D); (G) % B-1a cells in PerC; (H) % PtC+ cells among B-1a compartment; (I) % PtC+ cells in PerC.
(J) Samples described in 5F were analyzed for BrdU incorporation in lymphocyte subsets. Graph depicts mean % BrdU+ cells in each subset +/- SEM in N= 5 biological replicates.
(K) PerC cells from WT and KO mice were stimulated with varying doses of anti-kappa for 5 minutes, fixed, permeabilized and stained to detect B-1a cells and intra-cellular pErk. Histograms show pErk expression in kappa+ B-1a cells and are representative of 2 independent experiments.
Data in A, E-I from same N=4 WT and N=6 KO mice and correspond to those analyzed by ELISPOT in 4D, and by FACS in 5A-E. P-values in this figure were calculated using unpaired t-test except Welsh's correction applied for B, E. Mann-Whitney test was used for A, B (for non-normal sample distributions).