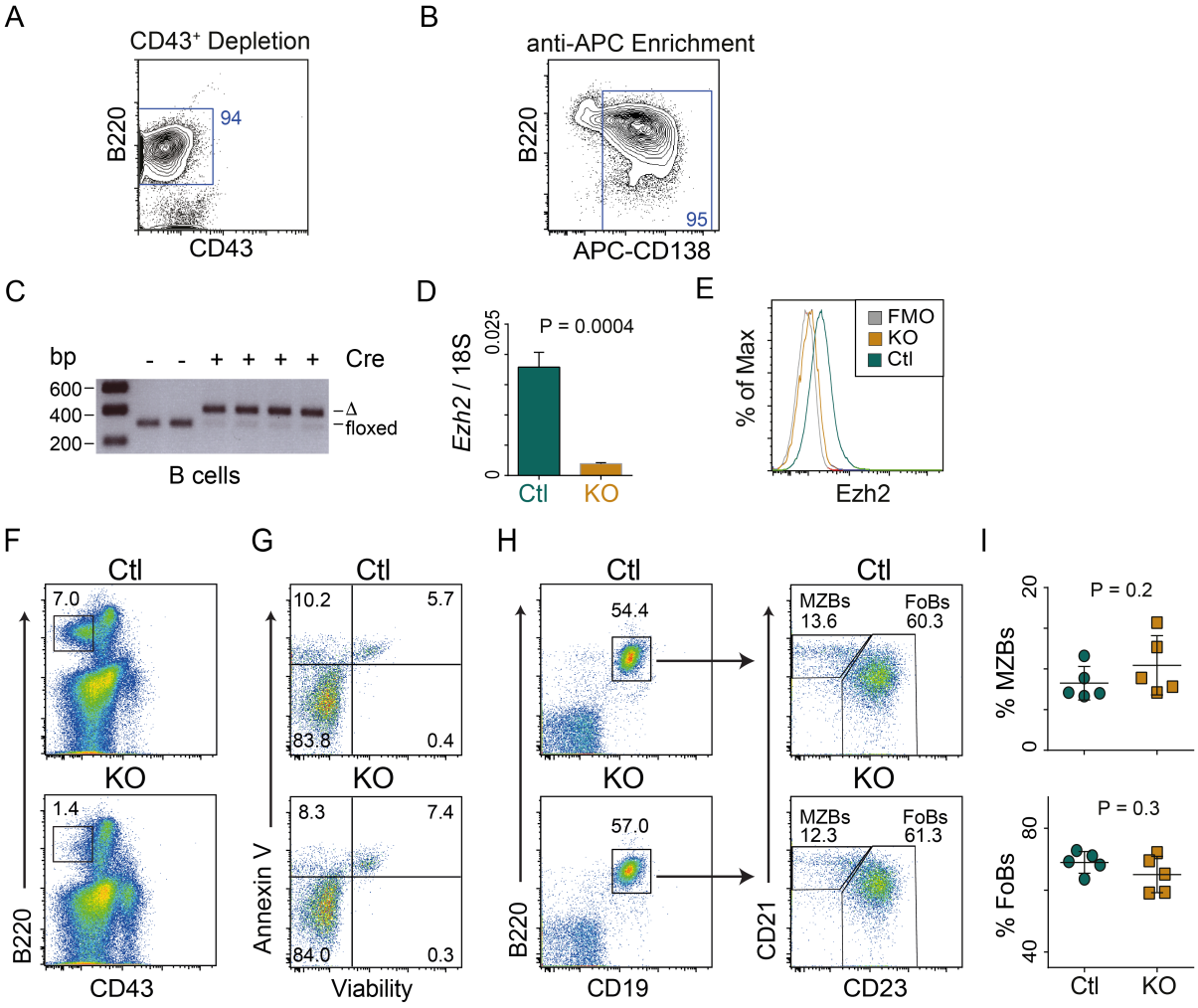
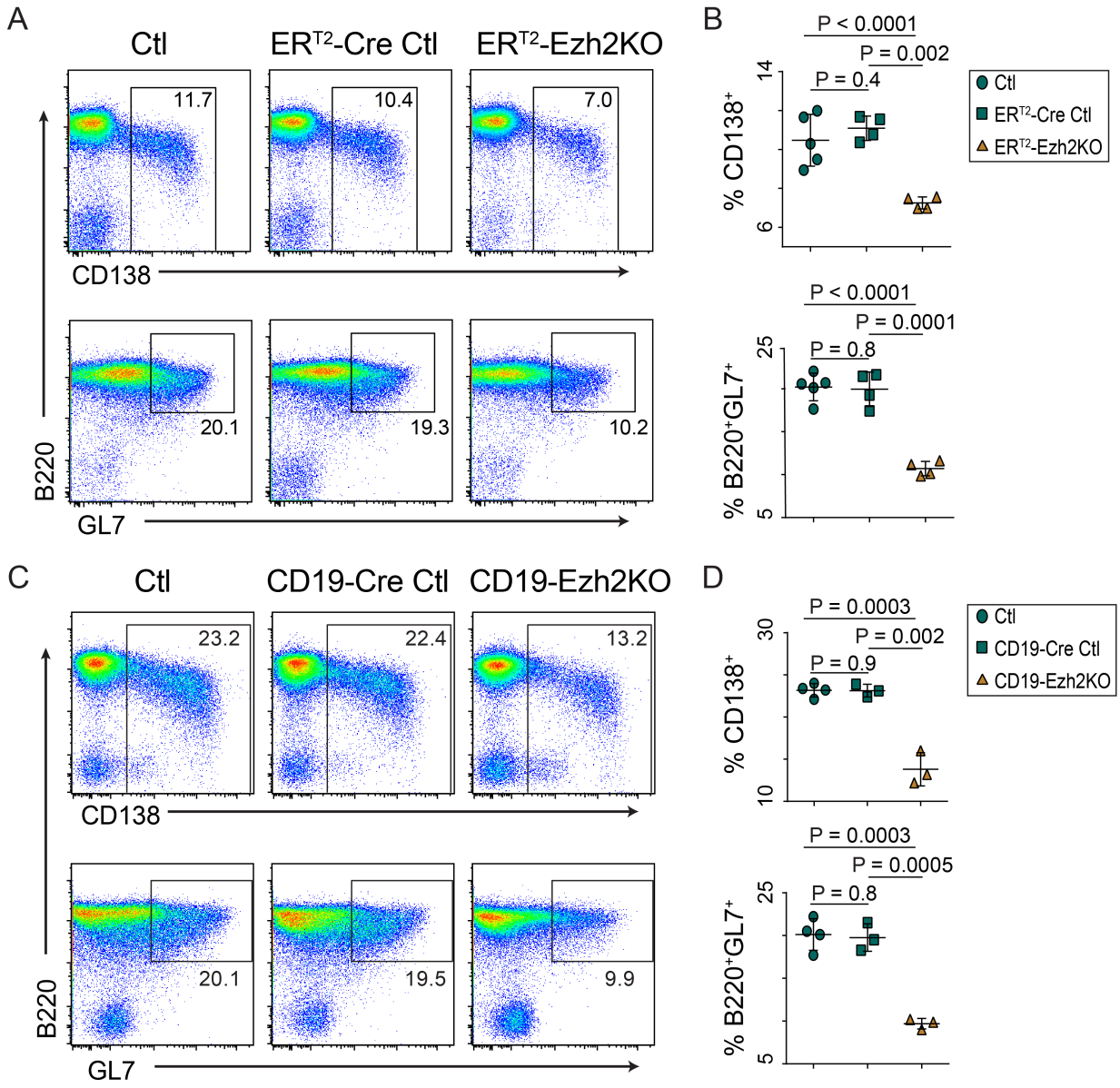
Supplemental Figure 1

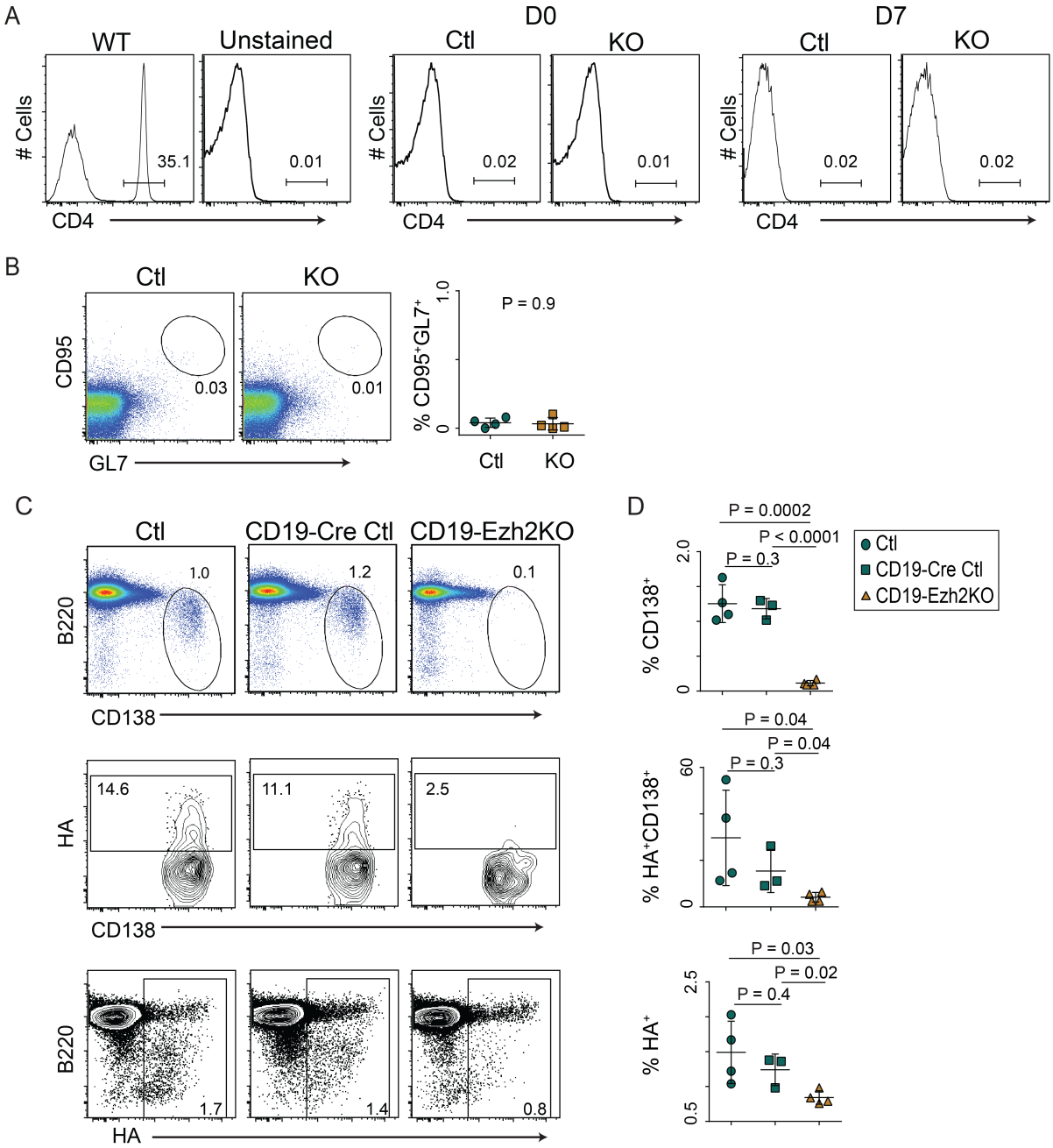


Supplemental Figure 1. EZH2 is dispensable for mature B cell survival.
(A) Purity of B cells following magnetic depletion of CD43^{+} splenic cells. **(B)** Purity of CD138^{+} ASC following anti-APC magnetic enrichment of APC-CD138 stained splenocytes. **(C)** *Ezh2*$^{fl/fl}$ (Ctl) and *Ezh2*$^{fl/fl}$*Rosa26*$^{CreERT2/+}$ (KO) mice were injected with tamoxifen daily from day 0 to day 4 and B cells purified at day 10 and genotyped by PCR using primers specific for the floxed (lower) or deleted (upper) alleles. **(D)** mRNA levels of *Ezh2* in splenic B cells after tamoxifen treatment as in **C** measured by RT-qPCR and expressed as percentage of 18S ribosomal RNA. Data is the mean ±SD of eight biological replicates. **(E)** Protein levels of EZH2 in splenic B cells after tamoxifen treatment measured by flow cytometry. Data is representative of two individual experiments. **(F)** The frequency of pre-B cells (Thy1.1^{-}F4/80^{-}CD11c^{-}B220^{+}CD43^{-}) in the bone marrow of mice treated as in **C**. **(G)** Viability of splenic B cells populations treated as in **C**. **(H)** Marginal zone (Thy1.1^{-}F4/80^{-}CD11c^{-}B220^{+}CD19^{+}CD21^{+}CD23^{-}) and follicular B cell (Thy1.1^{-}F4/80^{-}CD11c^{-}B220^{+}CD19^{+}CD21$^{mid/-}$CD23^{+}) populations from mice treated as in **C** are identified. Data is representative of four experiments. **(I)** Mean ±SD is shown for populations identified in **H**. Significance was determined by Student's two-tailed T-test.



Supplemental Figure 2. The CRE recombinase allele and hypomorphic CD19 does not alter ASC differentiation in response to LPS.

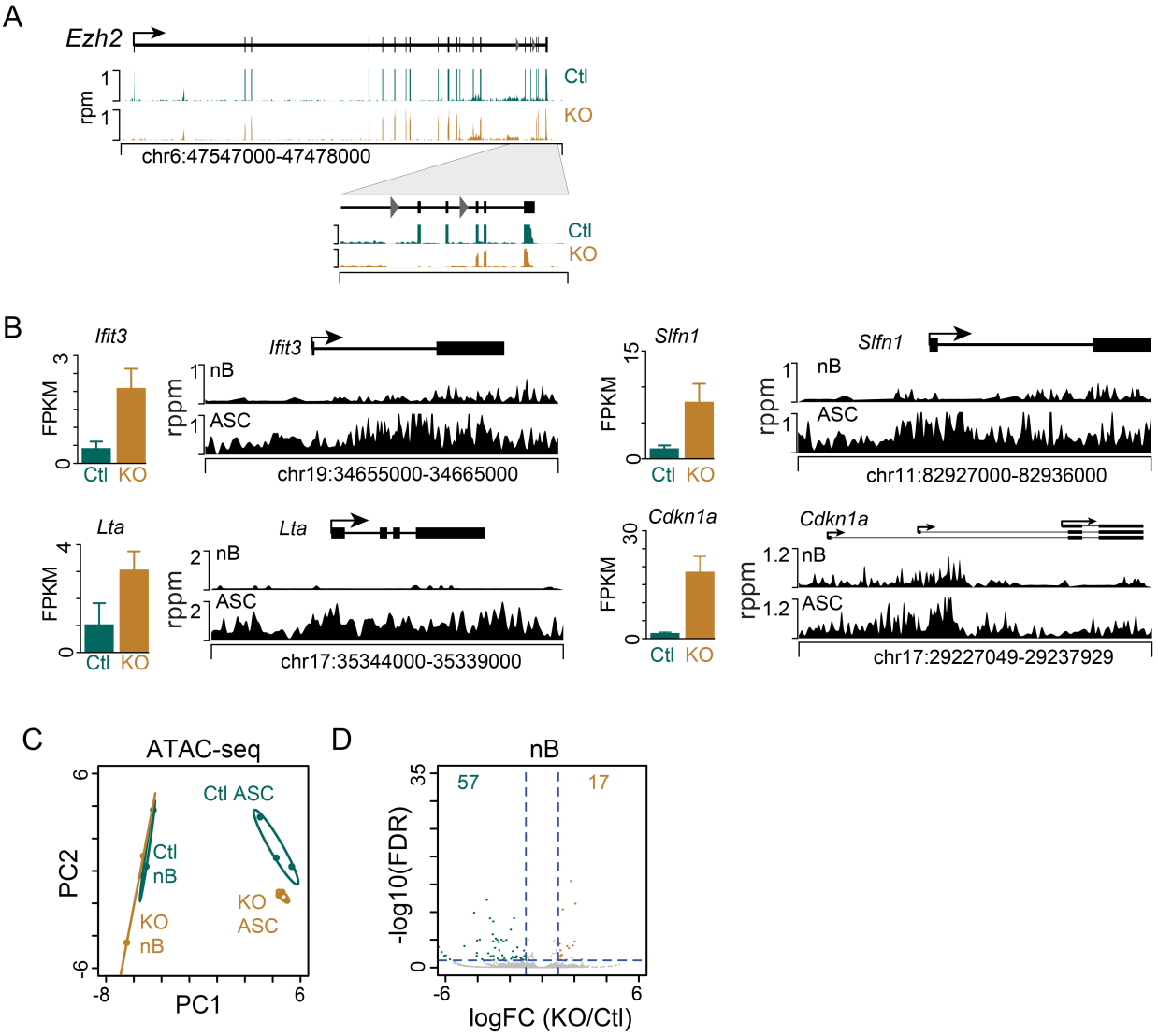
(A) *Ezh2*$^{fl/fl}$ (Ctl), *Ezh2*$^{fl/+}$*Rosa26*$^{CreERT2/+}$ (ER^{T2}-Cre Ctl), and *Ezh2*$^{fl/fl}$*Rosa26*$^{CreERT2/+}$ (ER^{T2}-Ezh2KO) mice were treated with tamoxifen, inoculated with 50 μg LPS, and splenic ASC and actB cells identified by flow cytometry three days later. **(B)** Quantification of ASC and actB cell frequencies from **A**. Each point represents an individual animal with the mean ±SD shown. **(C)** *Ezh2*$^{fl/fl}$ (Ctl), *Ezh2*$^{+/+}$*Cd19*$^{Cre/+}$ (CD19-Cre Ctl), and *Ezh2*$^{fl/fl}$*Cd19*$^{Cre/+}$ (CD19-Ezh2KO) mice were inoculated with 50 μg LPS and splenic ASC and actB cells identified by flow cytometry three days later. **(D)**. Quantification of ASC and actB frequencies from **C**. Each point represents an individual animal with the mean ±SD shown. All data is representative of two independent experiments containing cohorts of 3-5 mice per genotype. Significance was determined by Student's two-tailed T-test.



Supplemental Figure 3. Reduced HA-specific B cell differentiation at day 7 in response to influenza in CD4 T cell deficient mice.

Ezh2$^{fl/fl}$ (Ctl) and *Ezh2*$^{fl/fl}$*Cd19*$^{Cre/+}$ (KO) mice were treated with anti-CD4 antibodies at day -3 and -1 prior to infection with the A/PR8/34 (PR8) strain of influenza. **(A)** Frequency of CD4^{+} T cells in Ctl and KO mice at day 0 and day 7 following infection. **(B)** Frequency of CD95^{+}GL7^{+} germinal center B cells in the brachial LN. Representative flow plot is shown on the left and summarized as mean ±SD on the right. **(C)** Ctl, *Ezh2*$^{fl/+}$*Cd19*$^{Cre/+}$ (CD19-Cre Ctl), and *Ezh2*$^{fl/fl}$*Cd19*$^{Cre/+}$ (CD19-Ezh2KO) were mice treated as in **A**. Representative populations of CD138+ ASC (top), HA tetramer^{+} ASC (middle), and HA tetramer^{+} lineage negative (Thy1.1^{-}F4/80^{-}CD11c^{-}) cells (bottom). **(D)** Quantitation of populations in E displayed as mean ±SD. Data is representative of two individual experiments containing cohorts of 3-5 mice per genotype. Significance was determined by Student's two-tailed T-test.

Supplemental Figure 4



Supplemental Figure 4. Unique chromatin accessibility landscape in *Ezh2*-deficient ASC. **(A)** Genome plot of the *Ezh2* locus showing the RNA-seq read depth for *Ezh2*$^{fl/fl}$ (Ctl) and *Ezh2*$^{fl/fl}$*Rosa26*$^{CreERT2/+}$ (KO) ASC following tamoxifen treatment and LPS inoculation. RNA-seq data represents the mean of three biological replicates of Ctl and KO ASC. The location of LoxP sits are indicated. rpm, reads per million. **(B)** RNA-seq expression and genome plot depicting the H2K37me3 profile for the gene locus in nB and ASC. FPKM, fragments per kilobase per million; rppm, reads per peak per million. **(C)** Principal component (PC) analysis of ATAC-seq data from *Ezh2*$^{fl/fl}$ (Ctl) and *Ezh2*$^{fl/fl}$*Rosa26*$^{CreERT2/+}$ (KO) nB and ASC from tamoxifen treated bone marrow chimeras three days following LPS inoculation. **(D)** Differentially accessible regions (DAR) between Ctl and KO nB described in **C**.