JEM

Supplemental material

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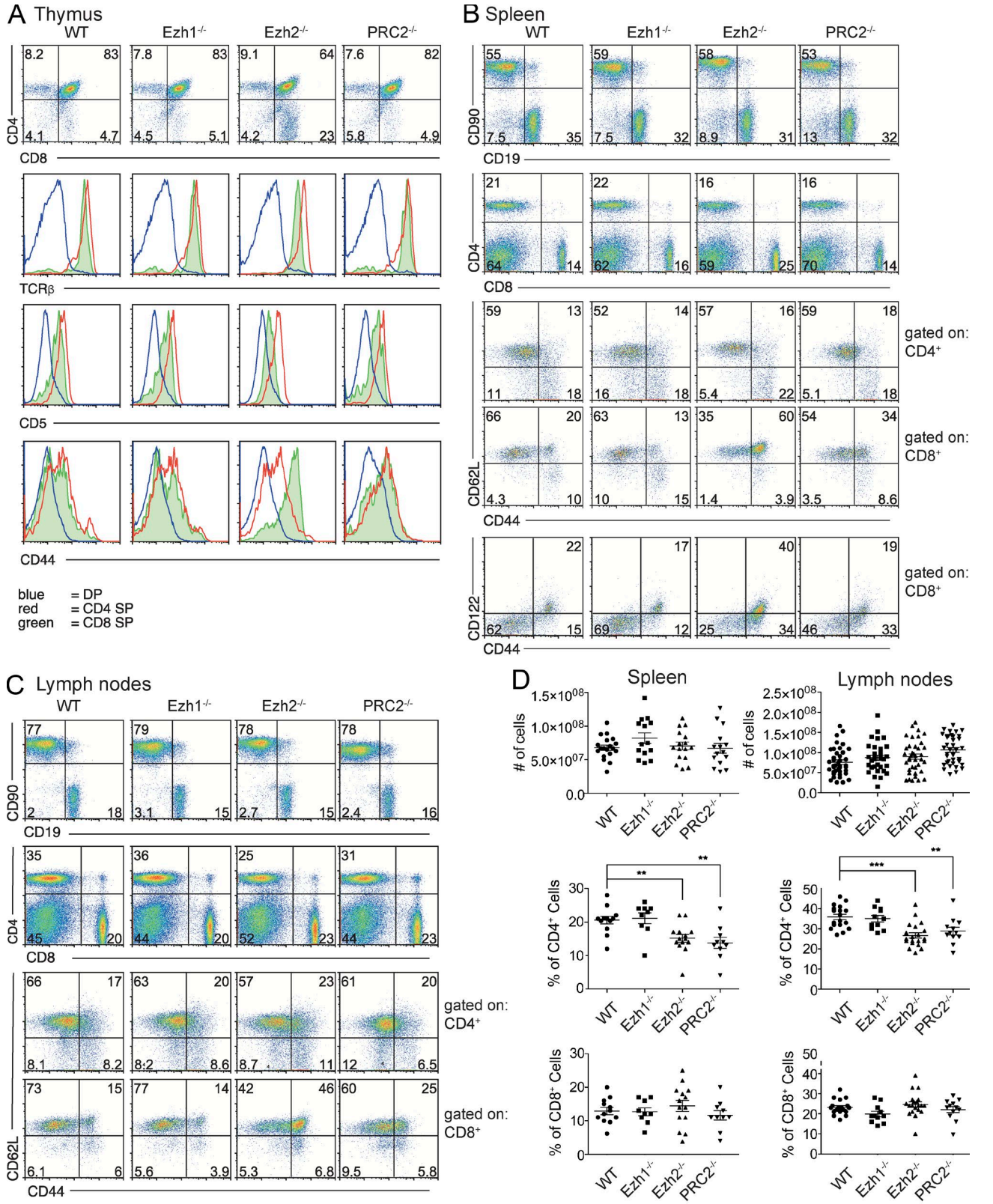

Figure S1. **Development and maintenance of T cells is not impaired by deficiency in Ezh1, Ezh2, or PRC2. (A–C)** Cells isolated from thymus (A), spleen (B), or lymph nodes (C) of WT, conventional Ezh1 knockout (Ezh1$^{-/-}$), Ezh2$^{fl/fl}$; CD4-cre (Ezh2$^{-/-}$), and Ezh1$^{-/-}$; Ezh2$^{fl/fl}$; CD4-cre (PRC2$^{-/-}$) mice were analyzed by FACS for the expression of indicated surface markers. Numbers in quadrants indicate percent of cells in each population. **(D)** Absolute cell numbers in spleen and lymph nodes (top) and percent of CD4$^+$ T cells (middle) and CD8$^+$ T cells (bottom) are shown. Each symbol represents and individual mouse. All data are representative of more than three independent experiments (n = 3–6), and significance was determined by the unpaired Student's t test: **, P ≤ 0.01; and ***, P ≤ 0.001. Error bars show mean ± SEM.

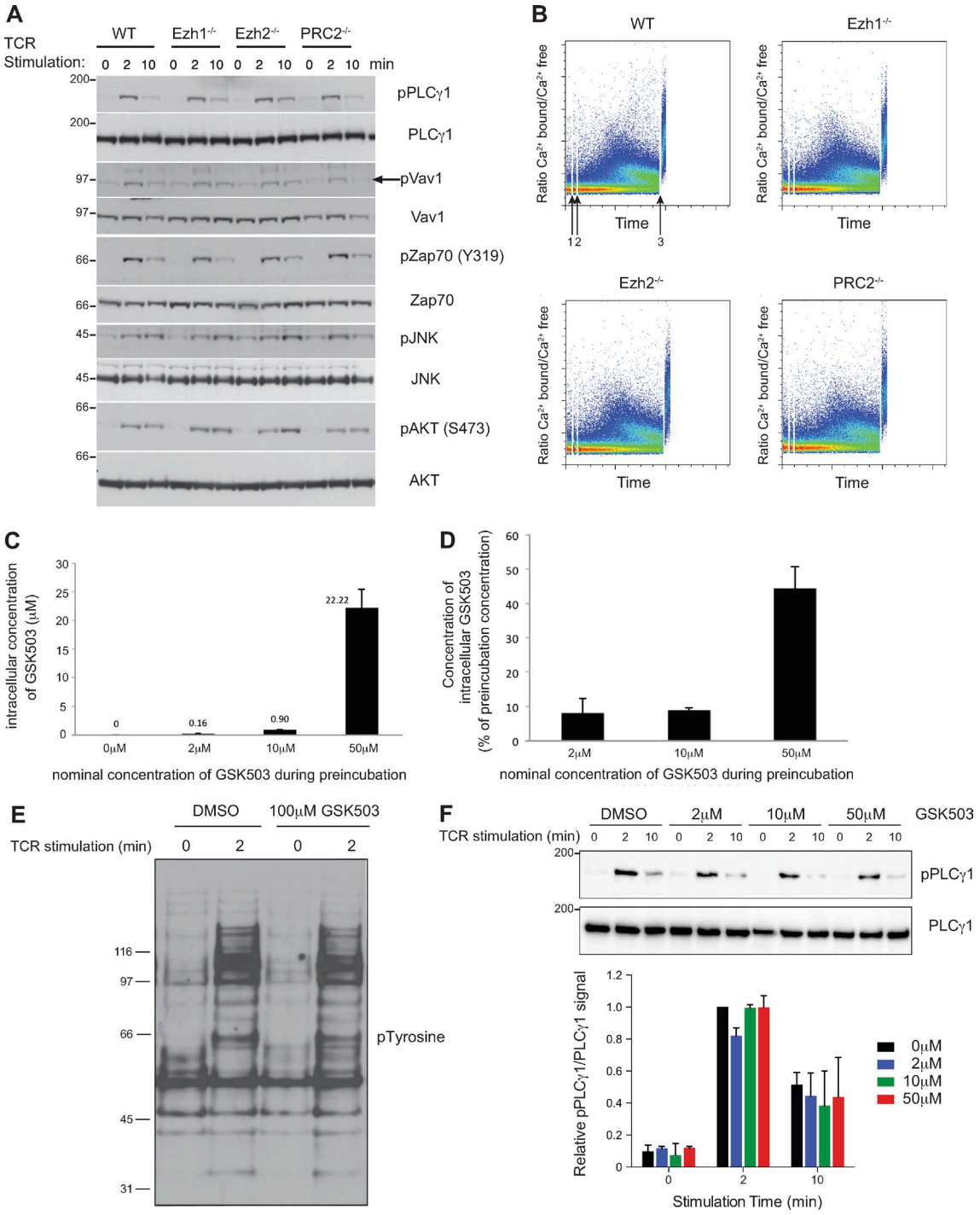

Figure S2. **Proximal signaling events downstream of the TCR are not affected by deficiency in Ezh1, Ezh2, or PRC2. (A)** Phosphorylation and expression levels of the indicated proteins in naive and TCR triggered CD4+ T cells was measured by Western blotting using phospho-specific or protein-specific antibodies. Cells were stimulated by a combination of anti-CD3 and anti-CD28 antibodies for the indicated periods of time. **(B)** Calcium mobilization was measure by FACS analysis of Indo-1 loaded splenic CD4+ T cells. The addition of anti-CD3 antibody (1), the addition of cross-linking antibody (2), and the addition of ionomycin (3). Preincubation of CD4+ T cells with GSK503 at 0°C is sufficient to achieve active intracellular concentrations of GSK503. **(C)** Mass spectrometry analysis of intracellular concentrations of GSK503 after preincubation for 1 h on ice with the indicated amounts of inhibitor. **(D)** The bar graph indicates the intracellular concentration of GSK503 as percent of the preincubation concentration of GSK503. **(E)** Total tyrosine phosphorylation in naive and TCR-triggered WT CD4+ T cells pretreated in the absence (DMSO) or presence of the indicated amounts of the Ezh2 inhibitor GSK503 was measured by Western blotting using a pan-phospho-tyrosine–specific antibody. Cells were stimulated by a combination of anti-CD3 and anti-CD28 antibodies for the indicated time periods. **(F)** Phosphorylation of PLCγ1 (pPLCγ1) in naive and TCR-triggered WT CD4+ T cells pretreated in the absence (DMSO) or presence of the indicated amounts of the Ezh2 inhibitor GSK503 was measured and normalized to the total amount of PLCγ1. Cells were stimulated by a combination of anti-CD3 and anti-CD28 antibodies for the indicated time periods. The bar graph shows the quantification of these measurements. All data are representative of three independent experiments. Error bars show mean ± SEM. Molecular mass is indicated in kilodaltons.

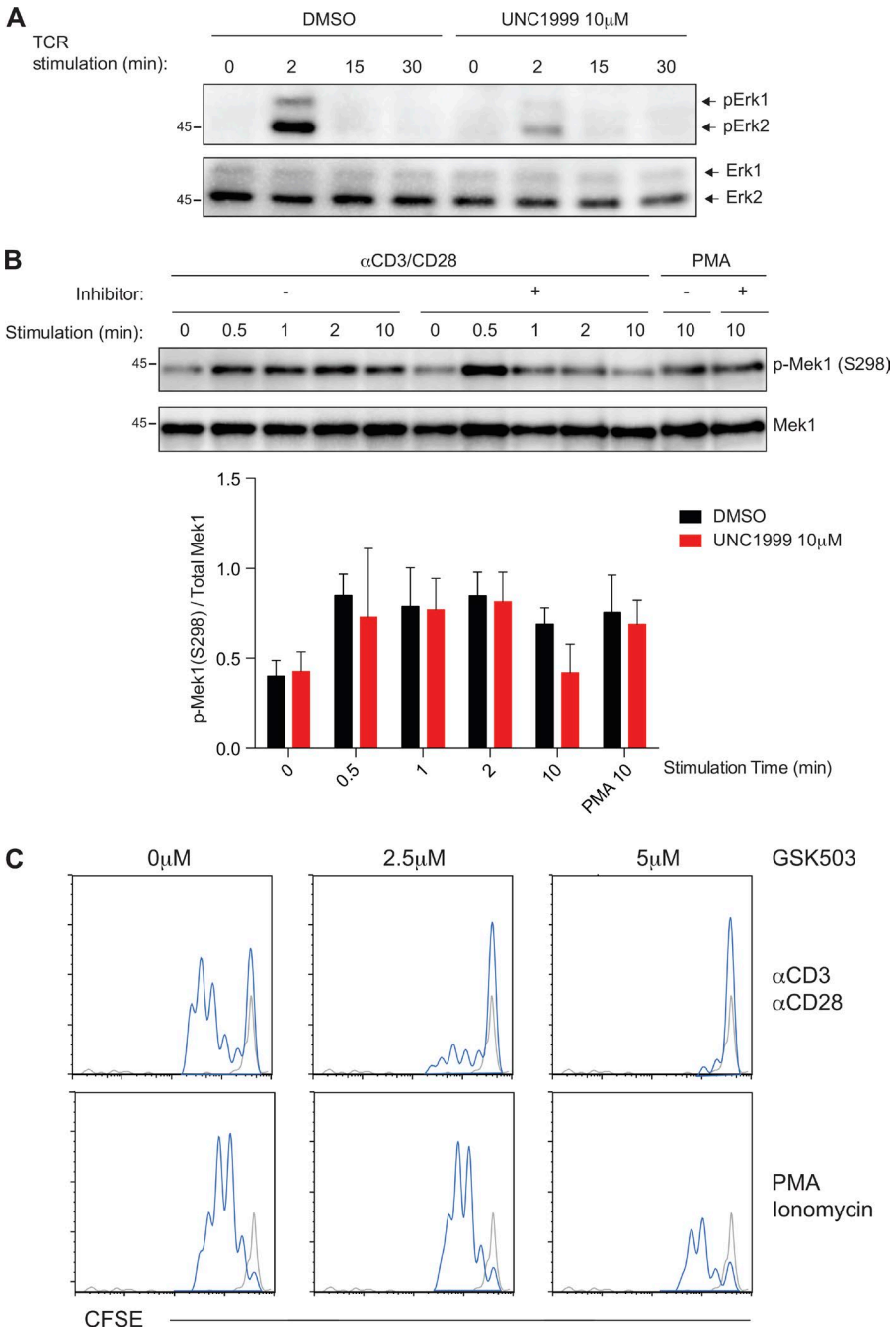

Figure S3. **PRC2 inhibition does not affect the kinetics of Erk1/2 phosphorylation or regulate Mek1 S298 phosphorylation upon TCR stimulation.** **(A)** TCR-mediated Erk1/2 phosphorylation in the presence of 10 μM UNC1999 or DMSO. WT CD4⁺ T cells were stimulated by a combination of anti-CD3 and anti-CD28 antibodies for the indicated periods of time. **(B)** WT CD4⁺ T cells were stimulated by a combination of anti-CD3 and anti-CD28 antibodies for the indicated periods of time in the presence of 10 μM UNC1999 or DMSO. The level of pMek1 S298 were measured and normalized to the total amount of Mek1. The bar graph shows the quantification of these measurements. Error bars show mean ± SEM. **(C)** Pharmacological PRC2 inhibition blocks TCR-induced T cell proliferation in vitro. Histograms show the pattern of CFSE dilution of CD4⁺ T cells stimulated with anti-CD3 and anti-CD28 (top) or PMA and ionomycin (bottom) for 72 h in the presence of indicated amounts of the Ezh2 inhibitor GSK503. The histograms marked in gray and blue show the amount of CFSE in nonstimulated cells or stimulated cells, respectively. All data are representative of three independent experiments. Molecular mass is indicated in kilodaltons.