# *Bacteroides fragilis* Polysaccharide A Induces IL-10 Secreting B and T cells that Prevent Viral Encephalitis

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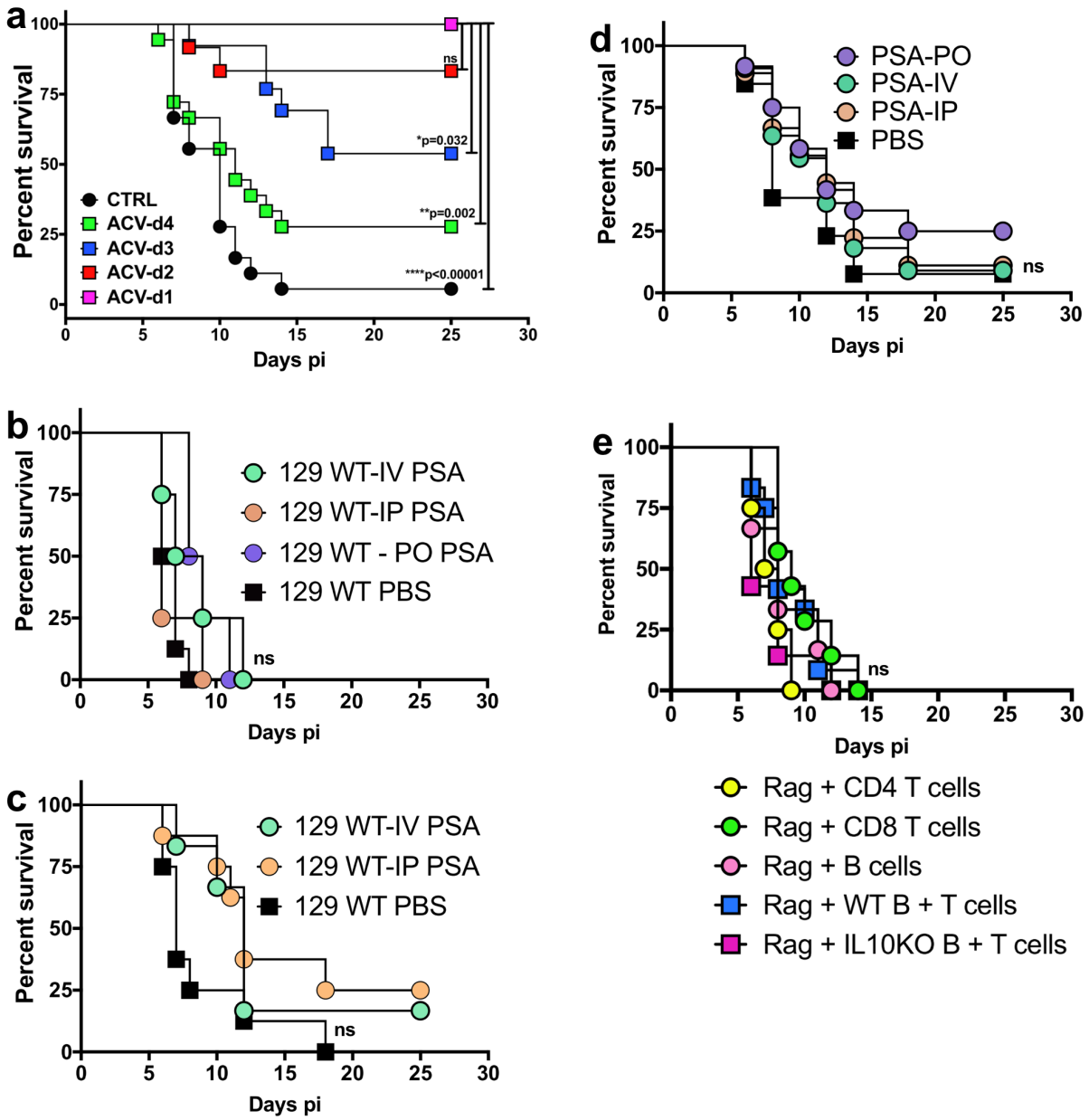
**Supplementary Figures.**

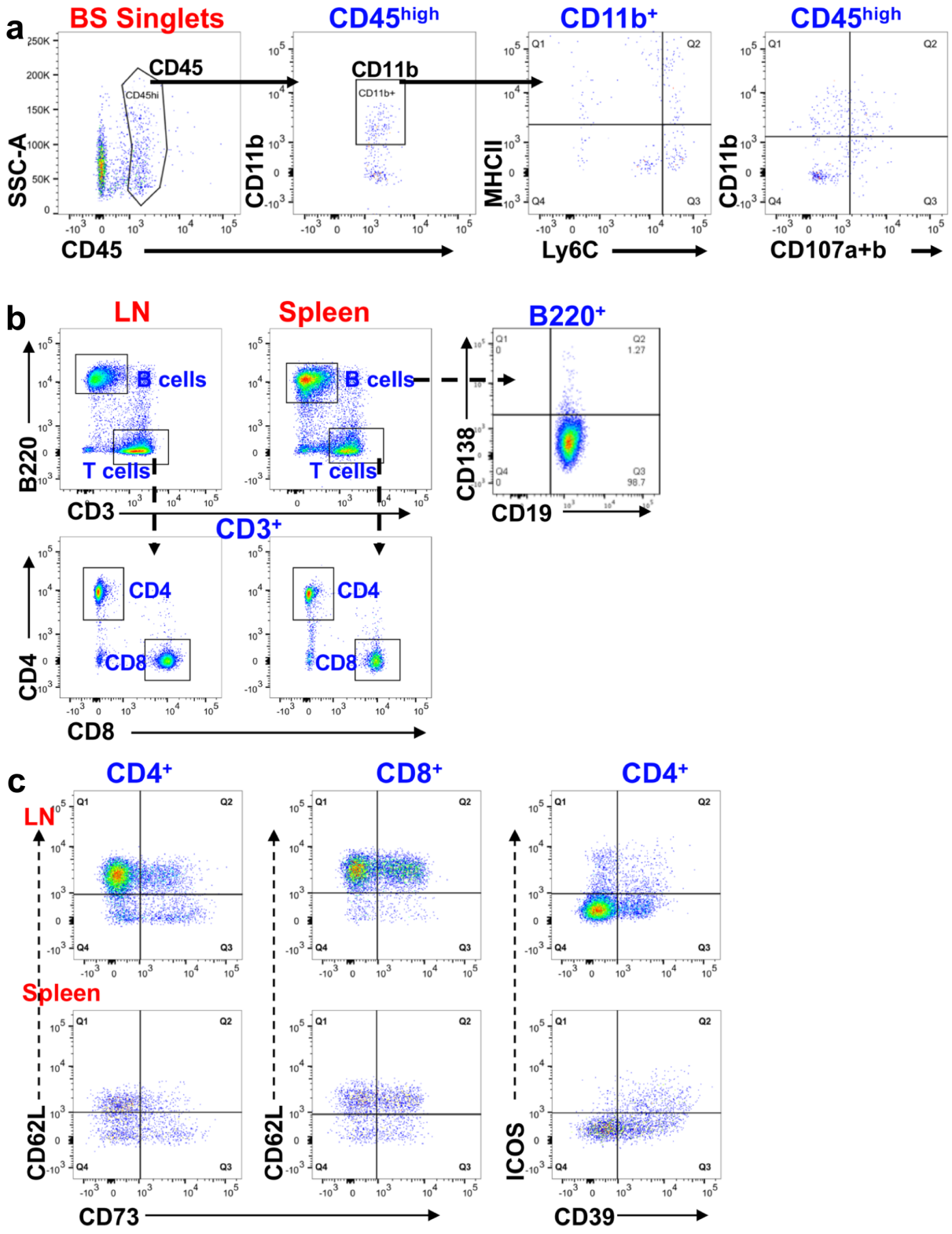
**Supplementary Figure 1.**



**Supplementary Figure 1. Only prophylactic oral administration of PSA protects against HSE in a murine model with delayed ACV treatment.**

**(a)** HSV infected 129 WT mice were given ip injections of ACV (1.25 mg/mouse) or PBS (CTRL) from the indicated time points for a week and monitored for HSE symptoms and survival (n=8-18 mice per group). HSV infected 129 WT mice given ACV from day 4 pi for a week by ip injections were also treated with PSA (50μg) or PBS by (**b)** ip, iv or oral (po) routes on days 1, 2 and 4 pi or (**c)** were pre-treated with 6 doses of PSA or PBS by ip or iv injections and monitored for survival, (n=6-8 mice per group, ns-not significant). (**d)** Another group of WT mice were pre-treated with PSA by the ip, iv or oral (po) routes but not given ACV after infection (n= 9-13 / group), ns=not significant. (**e)** PBS treated naïve Rag mice that were adoptively transferred with WT CD4 or CD8 T cells or WT B cells or WT B + T cells or IL-10KO B + T cells were infected with HSV and given daily ACV injections for a week starting on day 4 pi, (n= 6-12), ns= not significant. All statistical analysis was performed using log rank Mantel Cox survival test. See **Figures 1** and **5**.

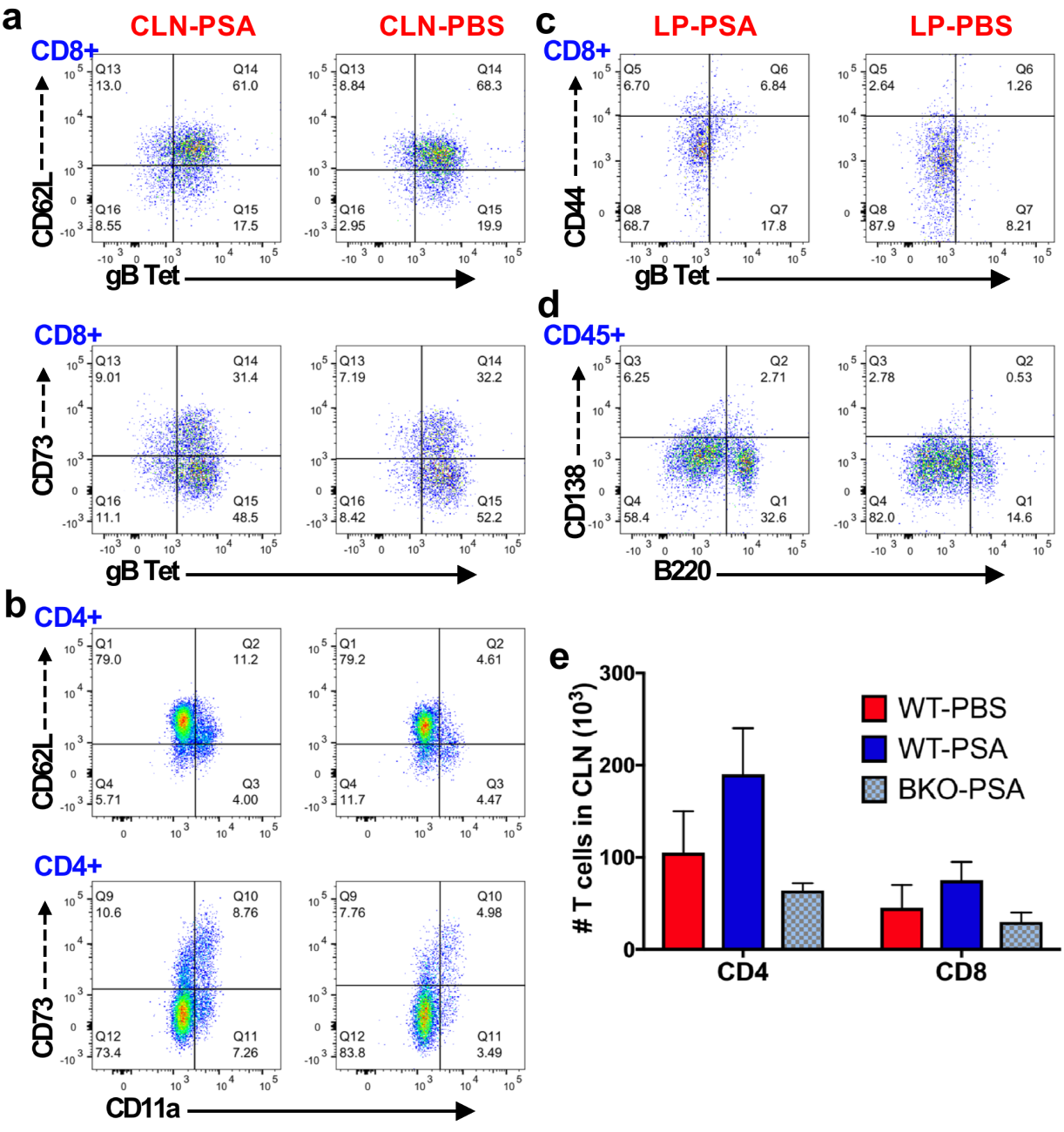
**Supplementary Figure 2.**



**Supplementary Figure 2. Gating Strategy for analysis of cells in the brainstem (BS), lymph nodes and spleen.**

**(a)** Mononuclear cells (live, singlets) isolated from the BS of infected mice at day 6 pi were analyzed for CD45 expression (left); $CD45^{high}$ gated cells were analyzed for $CD11b^{+}$ myeloid cells (left middle); $CD45^{high}CD11b^{+}$ gated cells were analyzed for MHC II and Ly6C expression to determine IM (right middle), PMN are MHC $II^{-}$ $Ly6C^{int}$ $Ly6G^{+}$; $CD45^{high}$ gated cells were analyzed for degranulation of $CD11b^{+}$ cells by surface CD107a+b expression (right plot). This gating strategy was used to identify cells in the BS of WT and Rag mice and shown in **Figure 2, 4e-f, 5d-e** and **Supplementary Figures 5c**. (**b)** Mononuclear live singlet cells isolated from lymph nodes (LN: left plot) and spleen (middle plot) gated using lymphocyte gate were analyzed for B220 and CD3 expression, $B220^{+}$ gated cells were confirmed for CD19 expression (top right); $CD3^{+}$ gated T cells were analyzed for CD4 and CD8 expression (bottom panel). This gating strategy was used to identify and enumerate cells in LN and spleens of WT mice in **Figures 3, 4a-b, 6c-e** and **Supplementary Figures 2c, 3, 4c-d, 5a-b, 5d, 7, 8b-c and 1**0. **(c)** T cells from LN (top) and spleen (bottom) were gated on CD4 (left plot) or CD8 (middle plot) shown above in **(b)** and analyzed for CD73 and CD62L or ICOS and CD39 (right plot: CD4); See **Figures 2 and 3**.

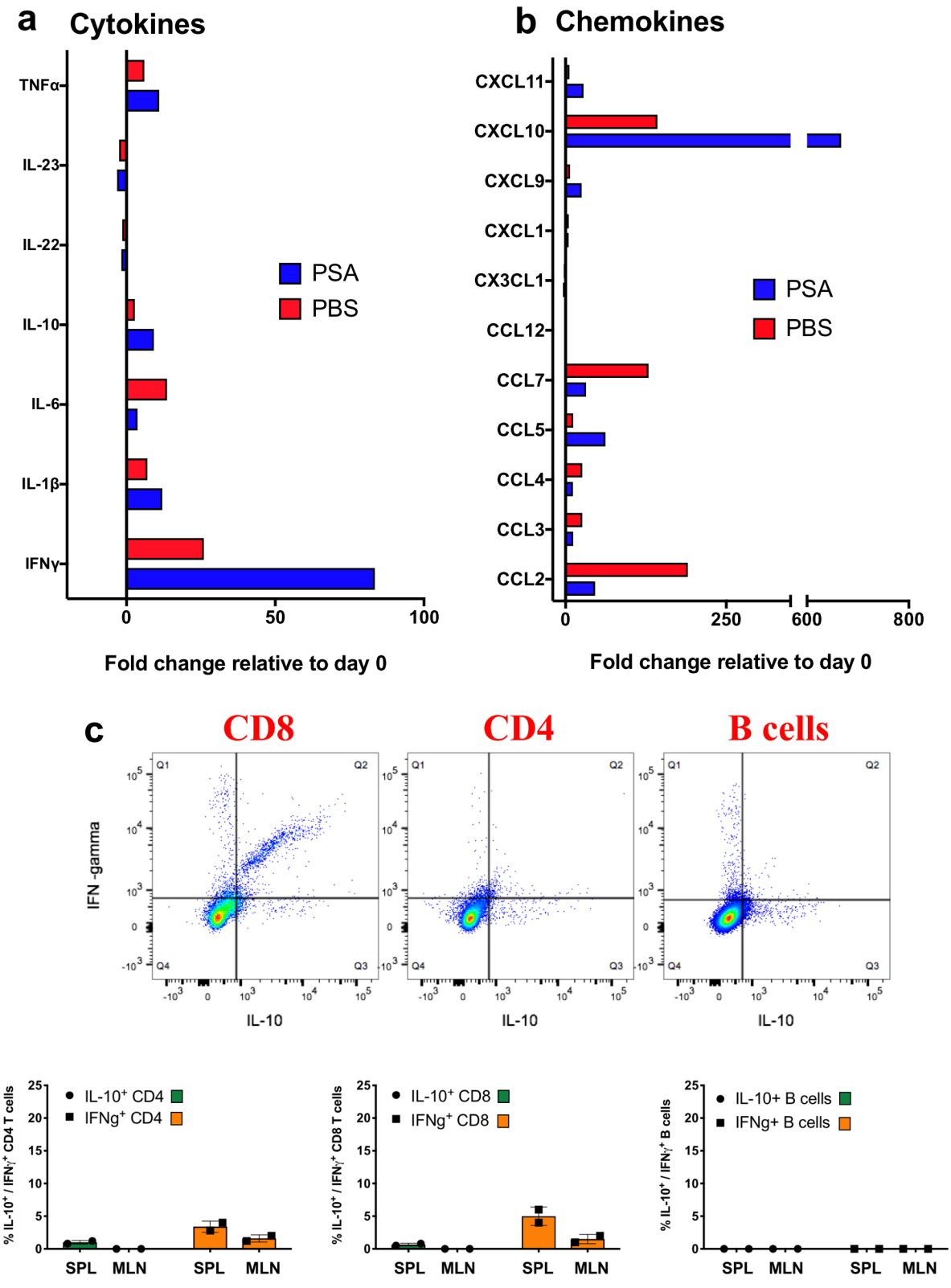
**Supplementary Figure 3.**



**Supplementary Figure 3. PSA increases expansion of antigen specific T cells in lymphoid organs.**

The draining cervical LN (CLN) isolated at day 6 pi from PSA or PBS pre-treated HSV infected, ACV treated WT mice were analyzed for accumulation of **(a)** CD8 T cells reactive to HSV $gB_{498\text{-}505}$ tetramer (gB Tet) and CD62L expression (top panel) or CD73 (bottom panel), **(b)** CD4 T cells expressing CD11a (as a surrogate marker for antigen experience) and CD62L (top panel) or CD73 (bottom panel). Naïve CD4 T cells do not express CD11a. **(c)** Mononuclear cells isolated from Lamina Propria (LP) at day 6 pi gated on $CD45^+$ $CD8^+$ T cells were analyzed for HSV $gB_{498\text{-}505}$ tetramer reactivity (gB Tet) and CD44 expression, and **(d)** $CD45^+$ cells were analyzed for plasma cells as determined by CD138 and $B220^{low}$ phenotype. **(e)** Bar plots shows total numbers of CD4 and CD8 T cells in the CLN of HSV infected 129 WT mice treated with PBS or PSA and 129 BKO mice treated with PSA (n=6 mice). All data show mean +/- SEM. (See **Figure 3 and 4**).

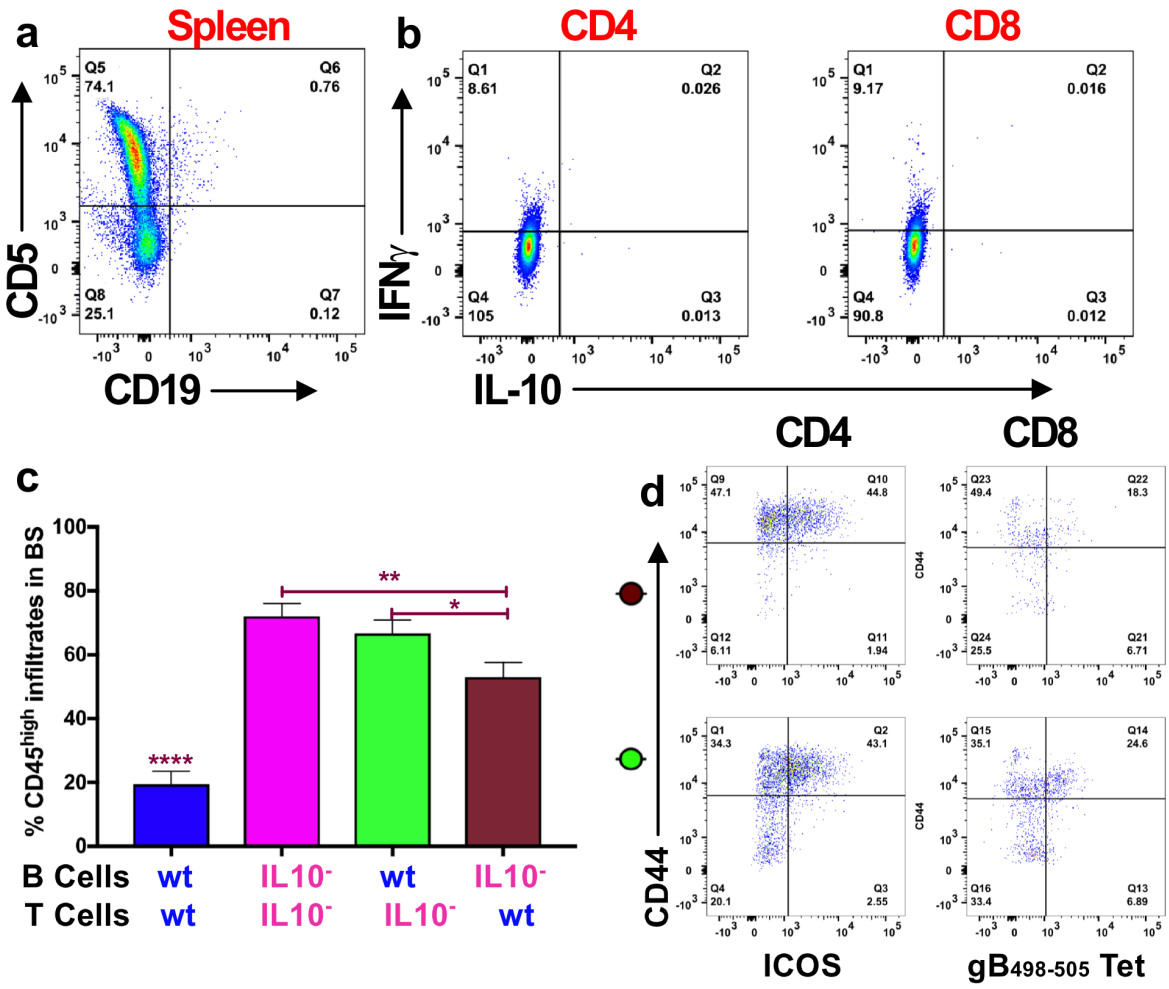
**Supplementary Figure 4.**



**Supplementary Figure 4. Cytokine and Chemokine expression in the CNS and lymphoid organs.**

**(a)** Cytokine and **(b)** chemokine expression in the BS of PSA (blue) or PBS (red) treated mice (n=3) at day 6 post HSV infection relative to uninfected mice (day 0); all mice were given ACV from day 4 pi. **(c)** Splenocytes were isolated at day 6 pi from PSA or PBS pre-treated HSV infected ACV treated WT mice and analyzed for intracellular IFNγ and IL-10 secretion following PMA and Ionomycin stimulation for 5 hours. Flow cytometry plots show CD8 (left) and CD4 (middle) T cell and B cell (right) gated cells. (**d)** Bar plots show CD4 (left) and CD8 T cells (middle) and B cells (right) isolated from spleens and MLN of WT mice treated with 6 doses of PSA orally that were analyzed prior to HSV infection for IL-10 and IFNγ secretion, n=2 experiments. All data show mean +/- SEM. See **Figure 4**.

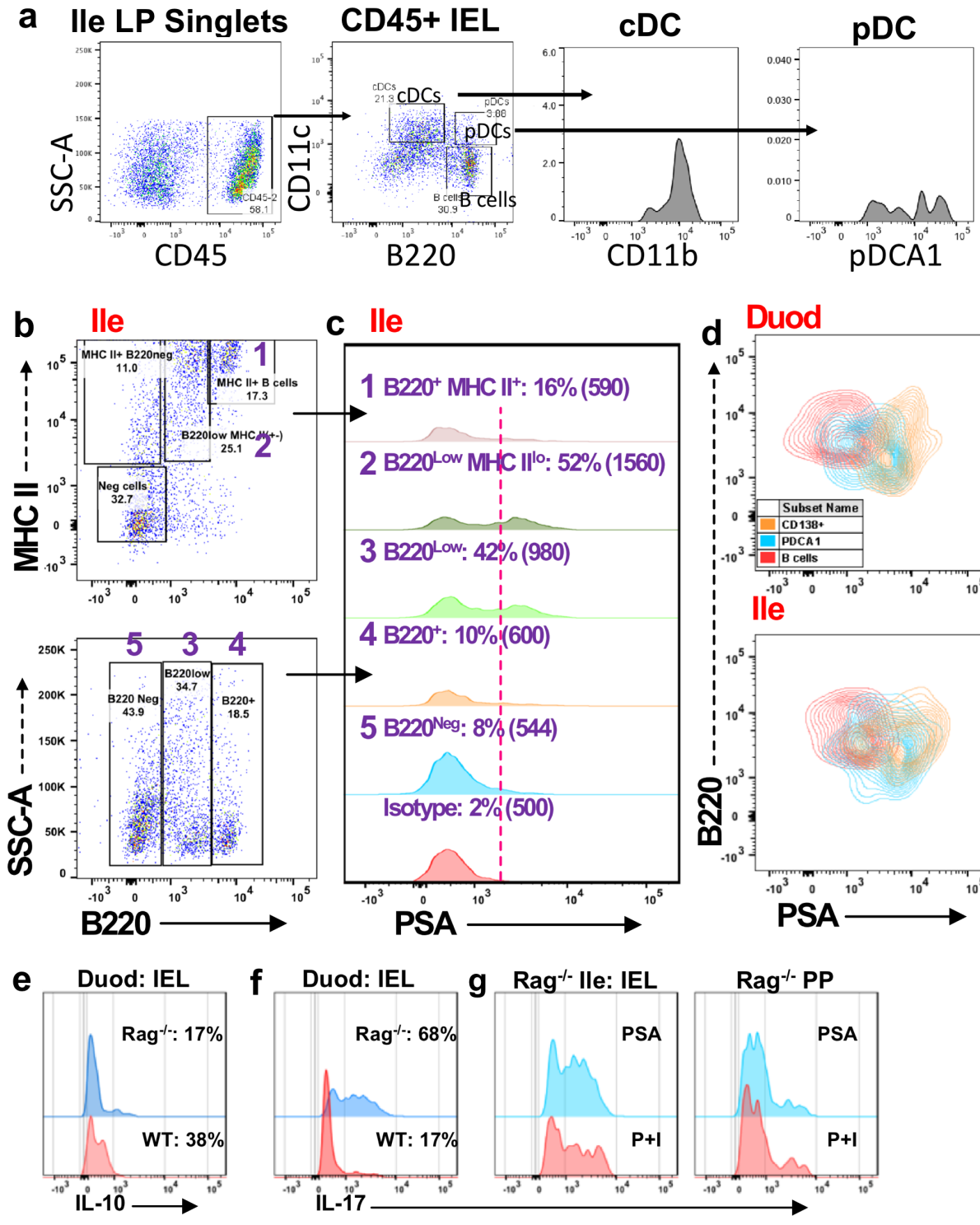
**Supplementary Figure 5.**



**Supplementary Figure 5. PSA requires B cells to induce IL-10 secreting CD4 T cells.**

**(a)** Flow cytometry plots show (left plot) complete depletion of $CD19^+$ B cells but not T cells ($CD5^+$) in the spleens of BKO mice at day 6 pi. To deplete B cells, WT mice were treated with αCD20 mAb given by ip injection every 10 days, beginning prior to PSA treatment and continued throughout infection (see also **Fig. 5b**). (**b)** Intracellular IFNγ and IL-10 expression by CD4 (middle plot) and CD8 (right plot) T cells isolated from spleens of BKO mice at day 6 pi following 5 hr stimulation with PMA + Ionomycin. (**c)** Bar plots show % $CD45^{high}$ cells infiltrating the BS of Rag recipients of WT B + T cells (blue), IL10KO B + T cells (magenta), WT B + IL10KO T cells (green) and IL10KO B + WT T cells (brown) at day 6 pi (see **Fig. 5d-e**), *p<0.05, **p<0.01, ****p<0.0001, as determined by ordinary one way ANOVA with Turkeys multiple comparisons tests. All data show mean +/- SEM. **(d)** Flow cytometry plots of spleens taken at day 6 pi from 129 Rag recipient mice of mixed transfers of IL-10KO B + WT T cells (top row: brown circle) and WT B + IL10KO T cells (bottom row: green circle) that were pre-treated with PSA prior to HSV infection show CD44 expression by (left plot) CD4 T cells and ICOS or (right plot) virus specific CD8 T cells reactive to HSV $gB_{498\text{-}505}$ tetramer (See **Figure 5d-e**).

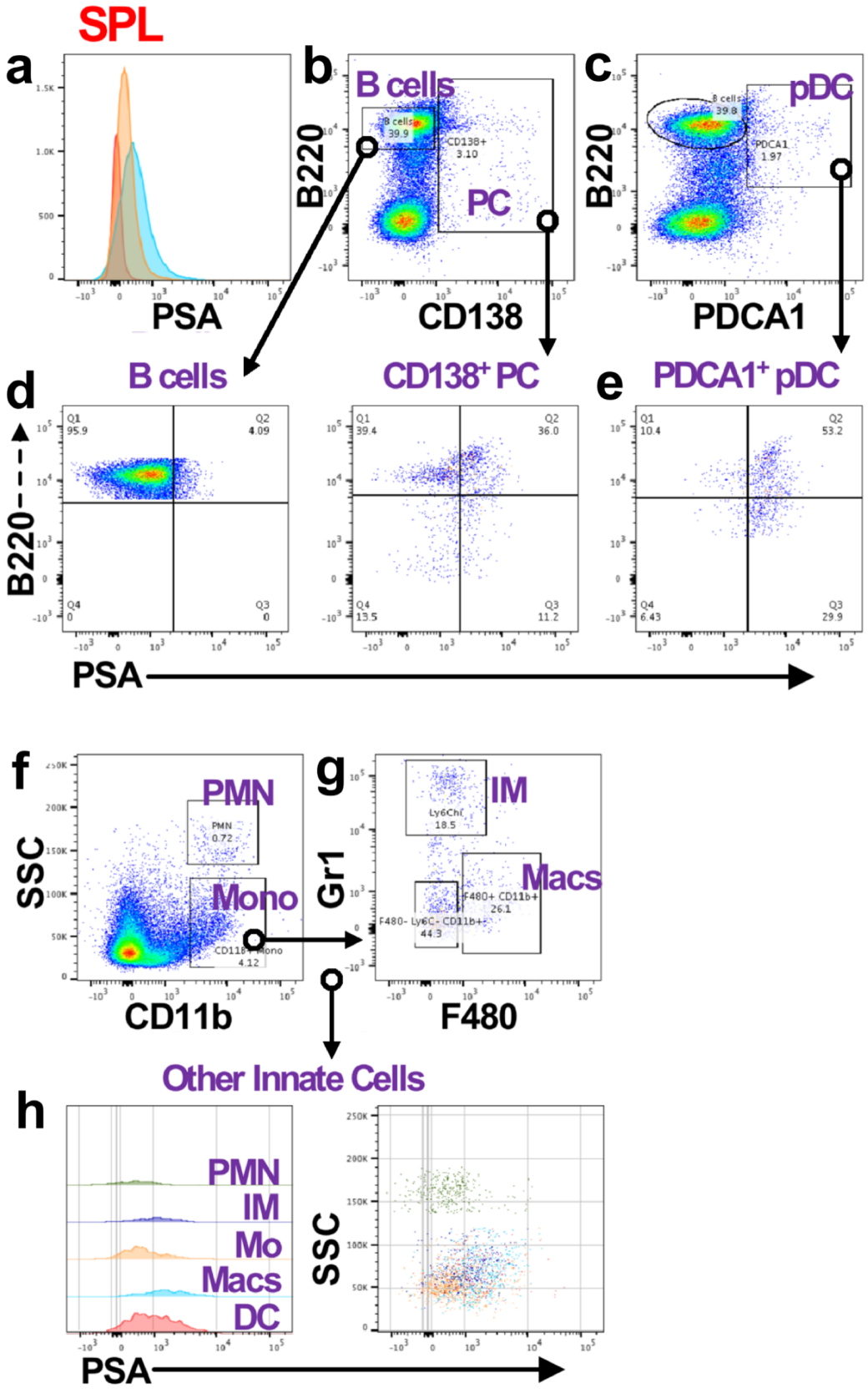
**Supplementary Figure 6.**



**Supplementary Figure 6. PSA binding studies for gut resident B cells and IL-10 secretion by WT but not Rag gut cells.**

**(a)** Gating strategy used to analyze Ileal $CD45^+$ mononuclear singlet IEL cells (left plot) to show CD45 $B220^+$ B cells, $B220^+$ $CD11c^+$ pDCs and $B220^-$ $CD11c^+$ cDCs (left middle plot). cDCs were confirmed by expression of CD11b (right middle histogram) while pDCs were confirmed by pDCA1 expression (right histogram). The gating strategy shown in **a** was used to identify immune cell types in the duodenum and ileum of WT and Rag mice as shown in **Figure 6** and **Supplementary Figures 6, 8a and 9b-c**. **(b)** Gating strategy used to analyze Ileal $CD45^+$ mononuclear singlet cells to show $B220^+$ cells with MHC II expression (top) and $B220^+$, $B220^{low}$ and $B220^-$ cells (bottom). **(c)** Gated populations represented by numbers 1-5 from the top and bottom plots in **(b)** were analyzed for binding to PSA, numbers show % PSA reactive while numbers in parenthesis show intensity of binding to PSA as denoted by MFI. **(d)** $CD45^+$ singlet mononuclear cells isolated from duodenum (Duod: top) and ileum (Ile: bottom) were gated on B cells, $CD138^+$ PCs and $PDCA1^+B220^+$ pDCs as shown in **(a)** and analyzed for binding to PSA. **(e)** $CD45^+$ IEL isolated from Duod of Rag (blue line) and WT (red line) mice (left 2 plots) showing IL-10 or **(f)** IL-17 secretion; **(g)** IL-17 secretion by Ile $CD45^+$ IEL (left) and PP cells (right) isolated from $Rag^{-/-}$ mice following stimulation with PSA (blue line) or PMA + Ionomycin (P+I: red line). See **Figure 6**.

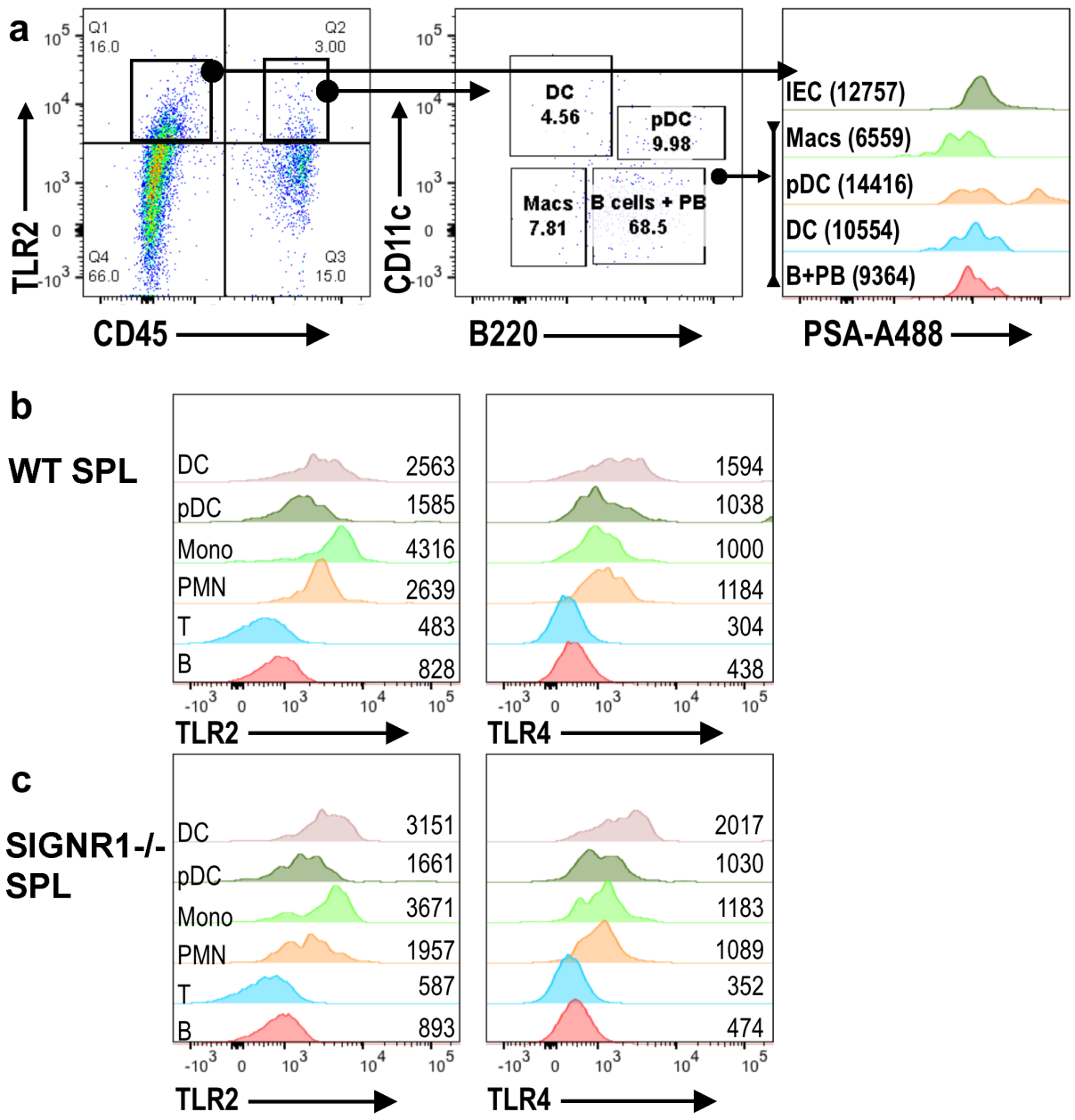
**Supplementary Figure 7.**



**Supplementary Figure 7. PSA binding studies by spleen subsets.**

**(a), Top row**, left histogram: Splenocytes from naïve WT mice were analyzed for binding to fluorescent conjugated PSA after pre-blocking with unlabeled PSA (yellow line) or PBS (blue line). Unlabeled cells: red line. Gating strategy to distinguish **(b)** $B220^{+}$ B cells and $CD138^{+}B220^{low}$ plasma cells (PC), **(c)** $B220^{+}PDCA1^{+}CD11c^{+}$ pDCs subsets (see arrows for primary gate) were analyzed for binding to PSA in **(d)** B cells (left) and PC (right), and **(e)** pDC. Gating strategy to analyze Innate cells including **(f)** $CD11b^{+}SSC^{high}Ly6G^{+}$ PMN and $SSC^{low}CD11b^{+}$ monocytes and **(g)** $SSC^{low}CD11b^{+}$ gated $F480^{+}CD11b^{+}$ macrophages (Macs) and $SSC^{low}Gr1^{high}F480^{-}$ IM (right plot). **(h)** Gated populations from plots **f and g** (see arrows for primary gate) were analyzed for PSA binding in **h**. This gating strategy was also used to identify spleen cell populations in **Supplementary Figure 8b-c**.

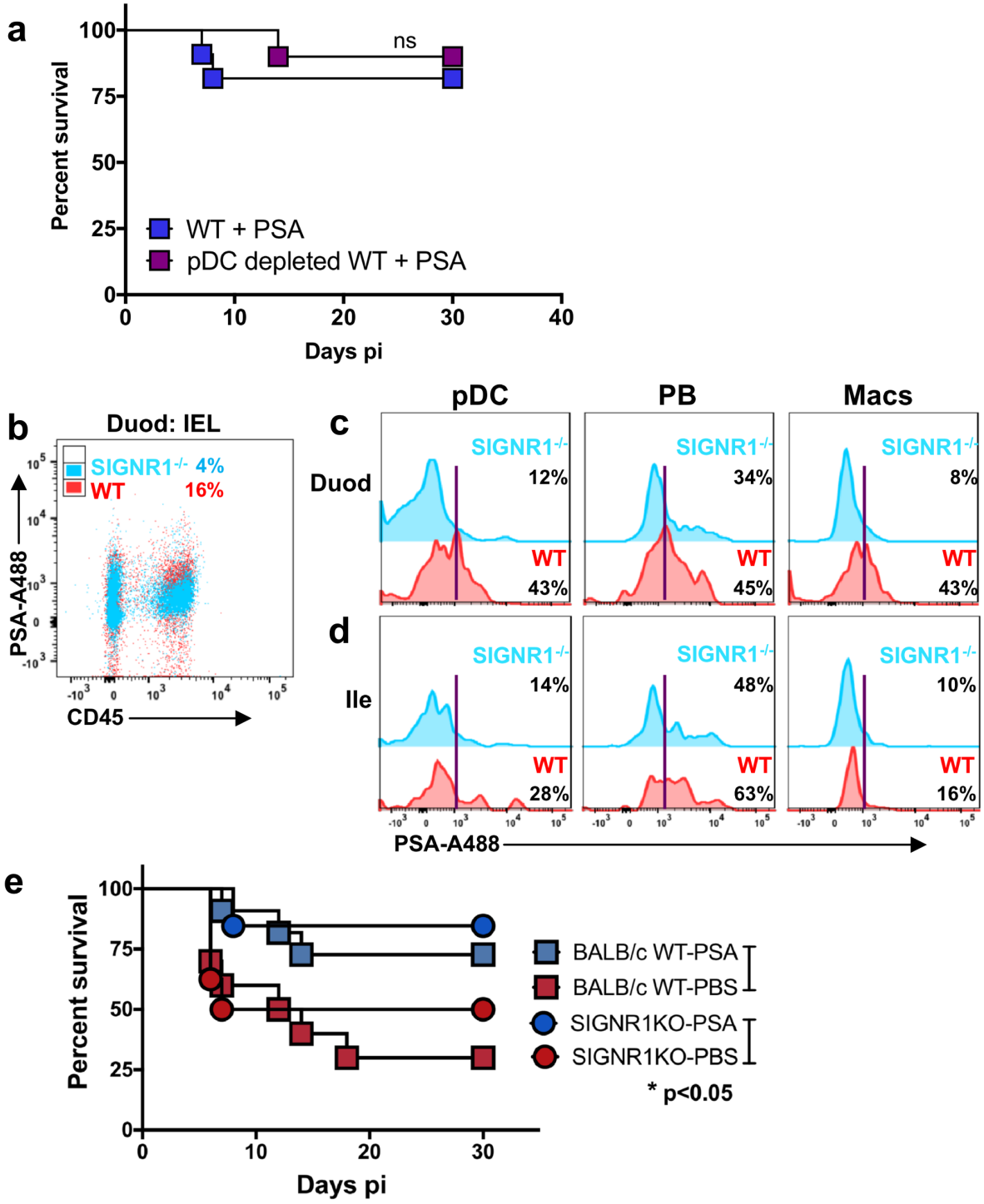
**Supplementary Figure 8.**



**Supplementary Figure 8. TLR2 expressing cells bind PSA.**

**(a)** Mononuclear live singlet cells isolated from duodenum of WT mice were analyzed for TLR2 and CD45 expression (left plot). $CD45^+$ $TLR2^+$ gated IEL cells were analyzed for CD11c, B220 and CD11b expression (middle plot); Gates show $CD11c^+B220^-$ cDCs, $B220^+CD11c^+$ pDCs, $B220^+CD11c^-CD19^+$ B cells + PB and $B220^-CD11c^-CD11b^+$ macrophages which were probed for binding to PSA (right plot), see **Supplementary Figure 6a** for gating strategy. $TLR2^+$ $CD45^-$ IEC (left plot) were also probed for binding to PSA in the right plot. **(b-c)** Various spleen cell subsets including B220- CD11c+ cDCs, PDCA1+CD11c+ pDCs, $SSC^{low}$ CD11b+ monocytes/macs, $SSC^{high}$ CD11b+ PMN, CD3+ T cells and B220+CD19+ B cells from **(b)** BALB/c WT and **(c)** SIGNR1KO mice were probed for TLR2 (left) and TLR4 (right) expression, see **Supplementary Figure 7** for gating strategy.

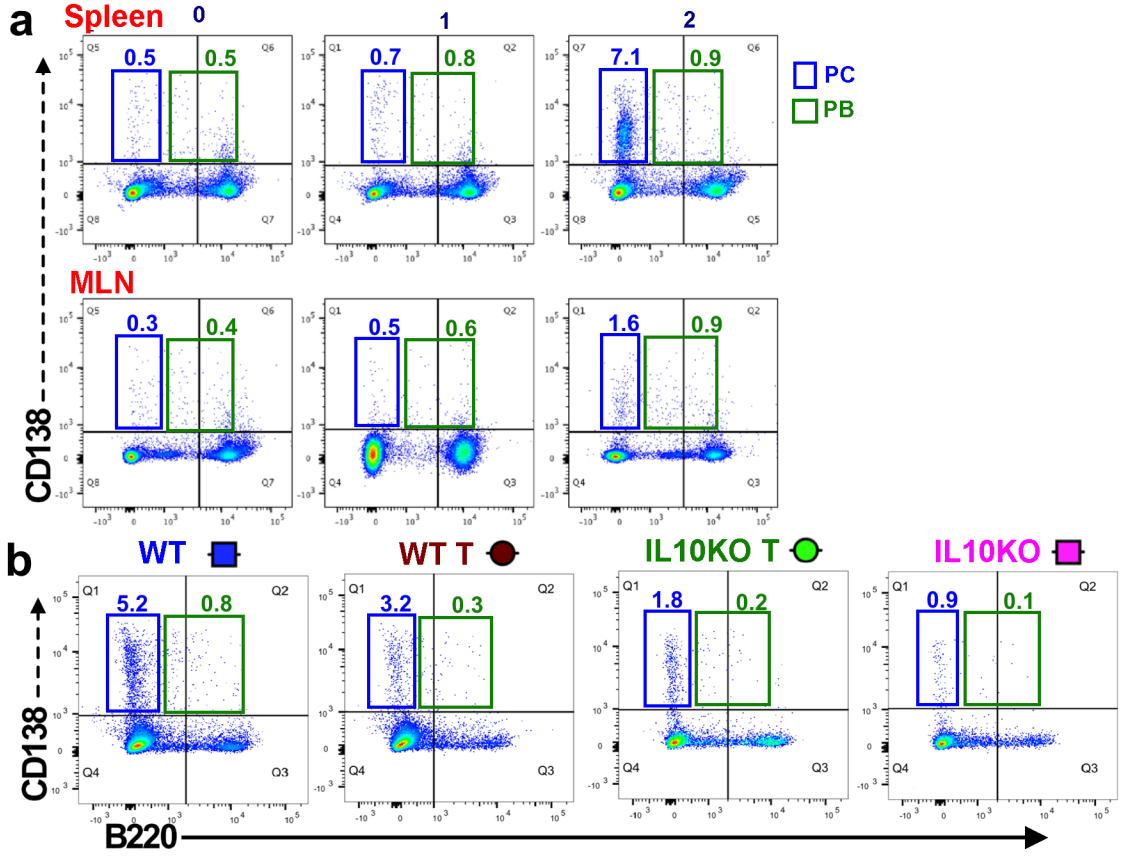
**Supplementary Figure 9.**



**Supplementary Figure 9. Depletion of pDCs or SIGNR1 does not alter PSA mediated protection.**

**(a)** WT mice were depleted of pDCs using anti-PDCA1 mAb prior to PSA pretreatment, and continued through HSV infection, mAb given every 3-4 days throughout experiment, n=10-12, ns=not significant. **(b)** IEL isolated from duodenum (Duod) of WT or SIGNR1$^{-/-}$ mice were analyzed for binding to PSA-A488. **(c-d)** pDCs (left), PB (middle) and F480$^{+}$CD11c$^{-}$ macrophages (right) in the IELs isolated from **(c)** Duod and **(d)** Ile of WT (red line) and SIGNR1$^{-/-}$ (blue line) mice were analyzed for reactivity to PSA-A488. **(e)** SIGNR1KO or WT littermates were pre-treated with PSA or PBS as in Fig. 1a, infected with HSV 17+ ($8x10^4$ PFU) by corneal scarification and treated with ACV from day 4 pi for one week; mice were monitored for symptoms of HSE and mortality (n=8-12), *$p<0.05$ as determined by log rank Mantel Cox test.

**Supplementary Figure 10.**



**Supplementary Figure 10. T cell derived IL-10 essential for accumulation of plasma cells in the LN.**

**(a)** Plasma cell ($CD138^{+}B220^{-}$ PC) and plasmablast ($CD138^{+}B220^{+}$ PB) accumulation in the spleens (top) and MLN (bottom) of WT mice prior to PSA treatment (left), after one week with 3 doses (1: middle) and after 2 weeks with 6 doses (2: right plot). Blue box labeled PC shows plasma cells and green box shows plasmablasts (PB). **(b)** Accumulation of PC and PB at day 6 pi in spleens of Rag recipients of WT B + T cells (WT: blue), IL10KO B + WT T cells (WT T: brown), WT B + IL10KO T cells (IL10KO T: green), and IL10KO B + T cells (IL10KO: magenta) that had been given 6 doses of PSA prior to HSV infection and ACV treatment. See **Figure 5** for key to symbols.