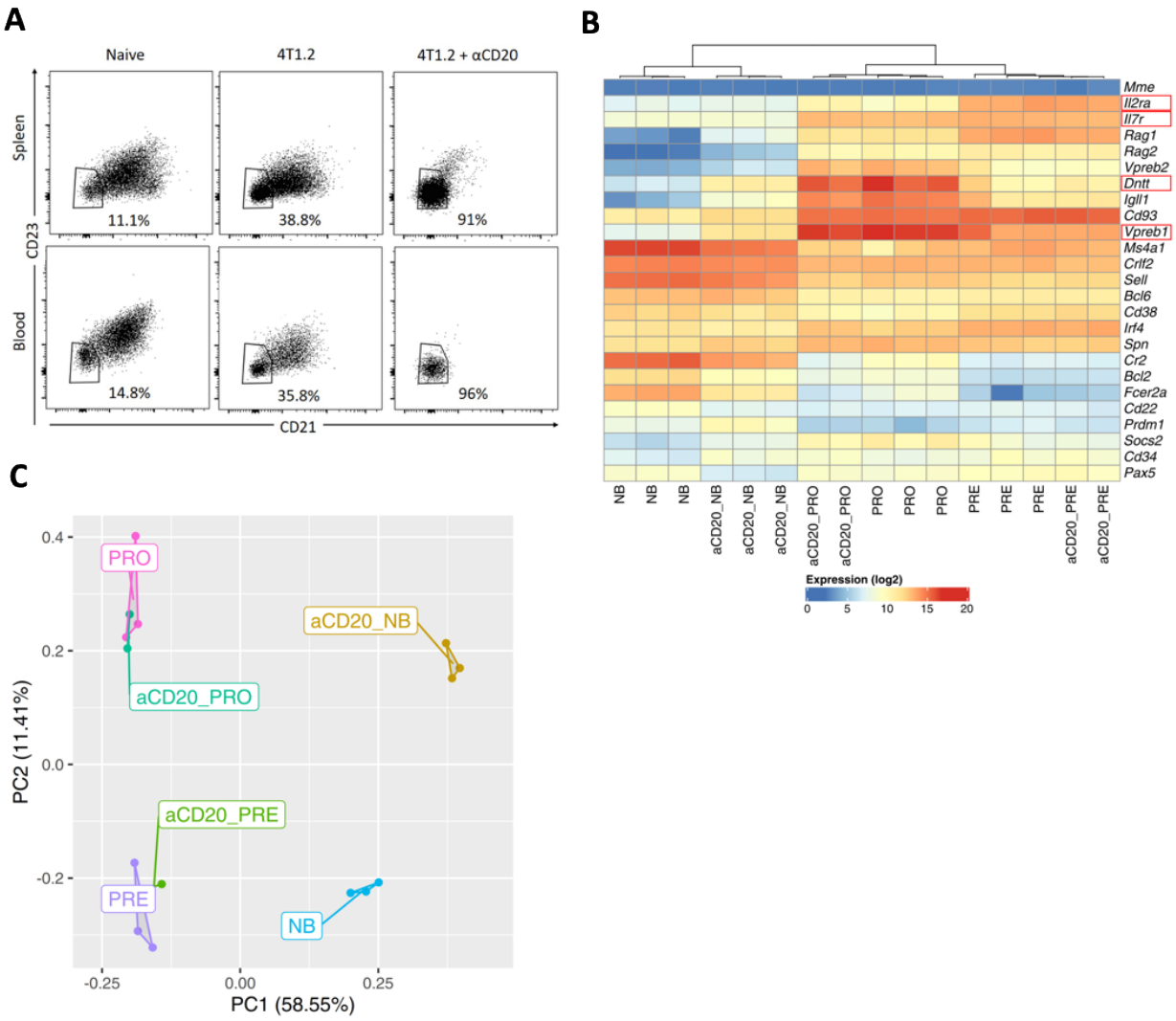
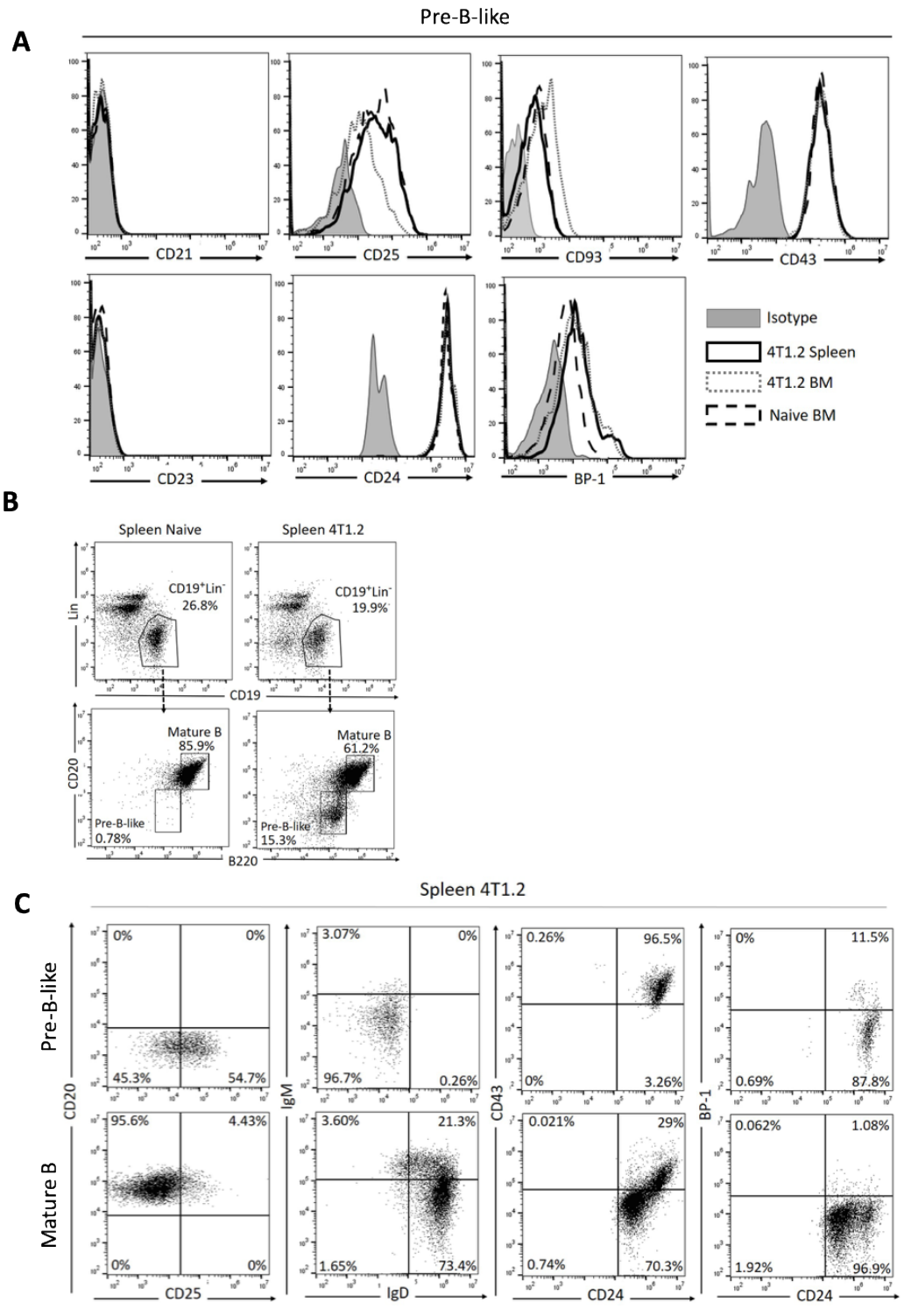
SUPPLEMENTAL DATA: Ragonnaud et al.

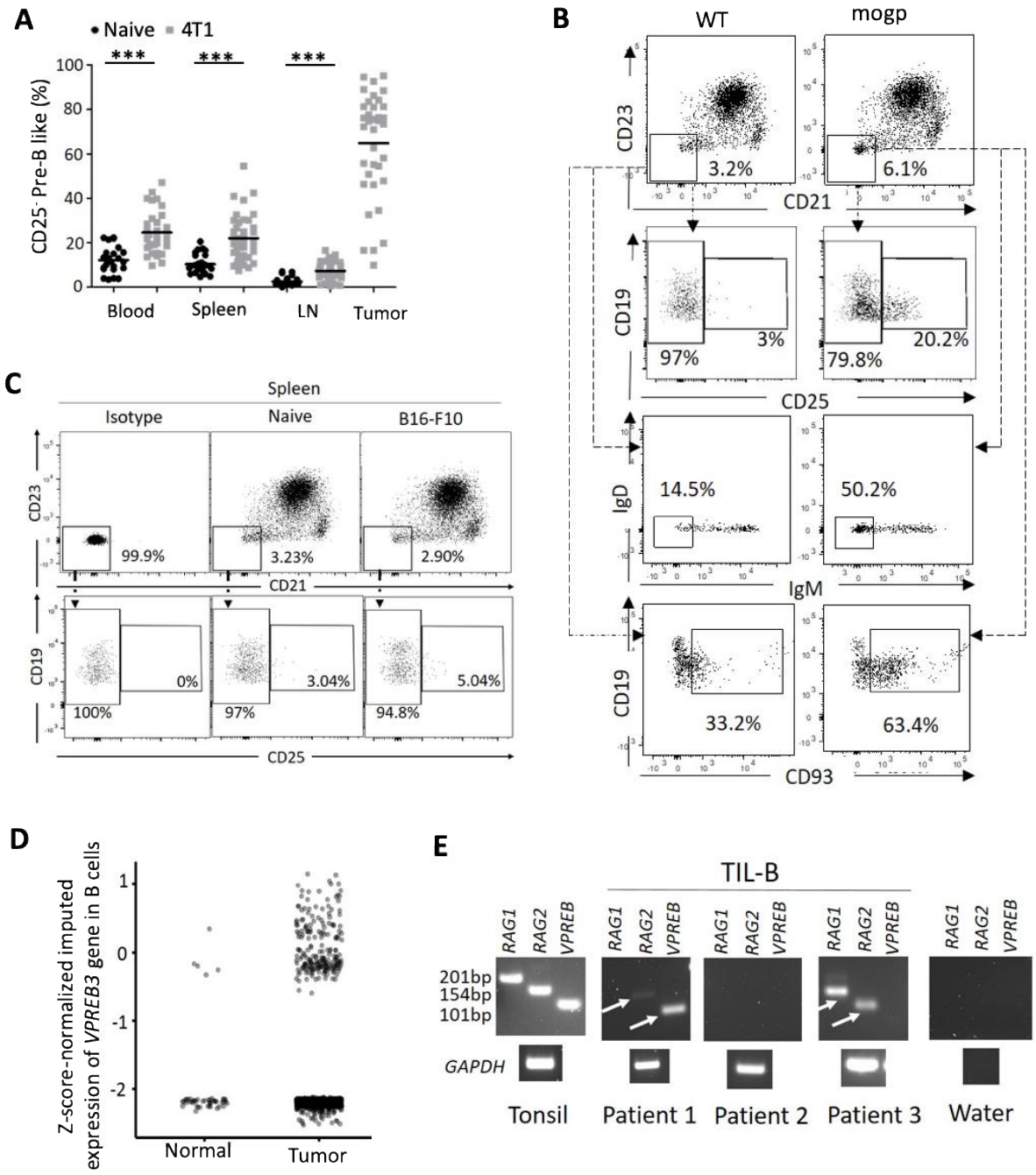


Supplementary Figure 1. Anti-CD20 Ab enriches for BM B-cell precursor-like cells in blood and spleen of mice with 4T1 cancer. Shown are representative FACS dot plots and frequency of pre-B-like cells ($CD25^+$ and $CD25^-CD23^-CD21^-CD19^+$) in the blood and spleen of mice without (naïve) and with 4T1 cancer after mock or anti-CD20 Ab treatment (n=4, **A**). Expression profile of splenic B cells from cancer-free mice treated with anti-CD20 Ab (aCD20_NB) differs from that of BM B-cell precursors (PRO, $CD25^-IgM^-CD19^+$ and PRE, $CD25^+IgM^-CD19^+$) as shown in the heat map (**B**) and PCA analysis (**C**, n=2–3). NB, total splenic B cells from naïve mouse treated with control IgG antibody.



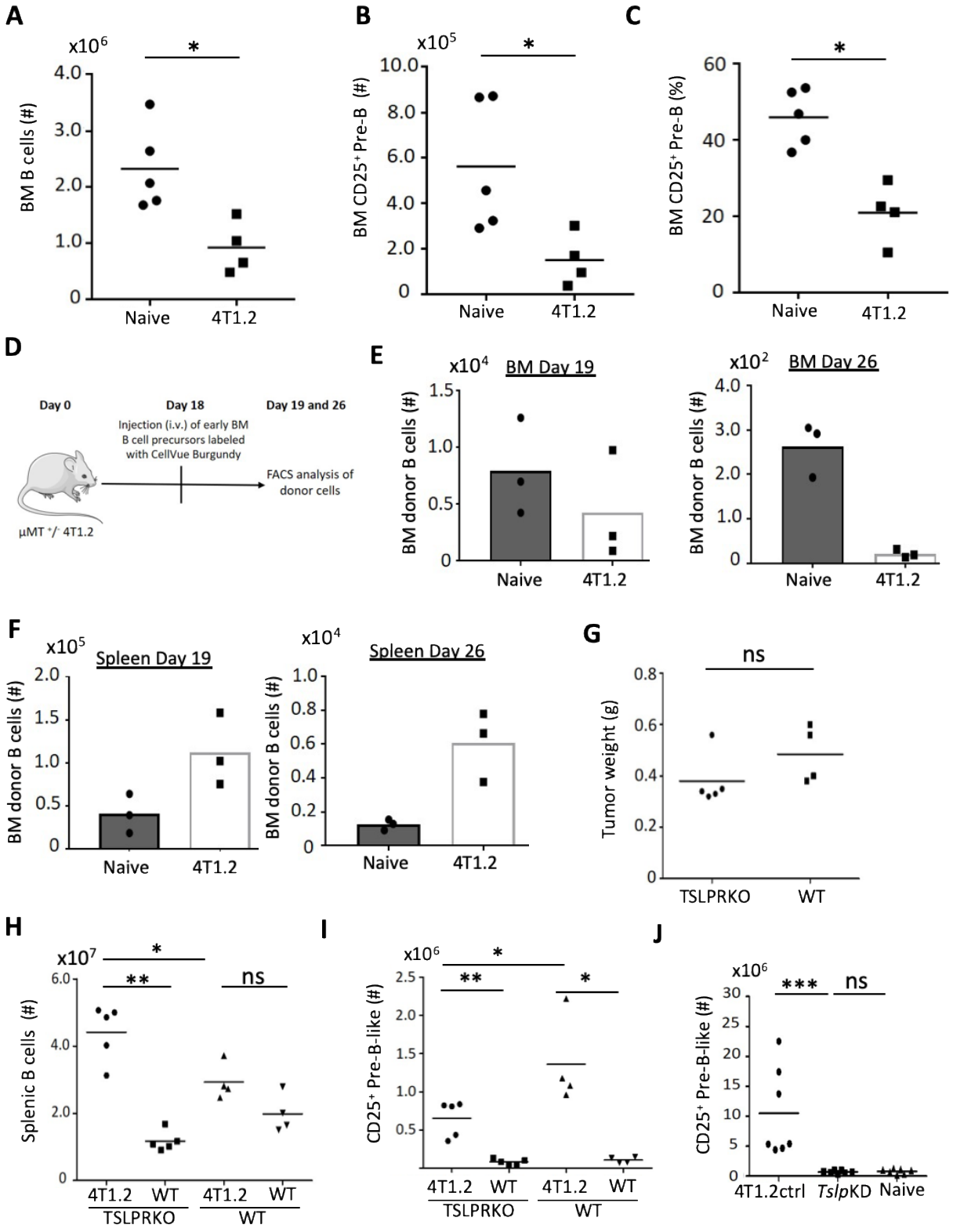
Supplementary Figure 2. Characterization of circulating pre-B-like cells. **A,** Histograms show expression of surface markers in splenic pre-B-like cells ($CD25^{+}$ and $CD25^{-}$ $CD20^{-/Lo}B220^{Int}$ $CD23^{-}CD21^{-}CD19^{+}$) from mice with 4T1.2 cancer (solid lines) as compared to BM of mice with 4T1.2 cancer (grey dashed lines) or without cancer (dark dashed lines). Isotype control staining (filled in grey). **B** and **C,** Representative FACS dot plots showing the frequency of pre-B-like cells in the spleen of naïve and 4T1 cancer-bearing mice (**B**) and the expression levels of IgM, IgD, CD43, CD24, and BP-1 in circulating pre-B-like cells and mature B cells ($CD20^{+}B220^{Hi}$) from a spleen of mice with 4T1.2

cancer. **A–C,** Cells were gated on single and viable cells and then in $CD19^{+}$ Lin^{-} (Lin, Lineage). Data are representative of at least 2 independent experiments (n>4).



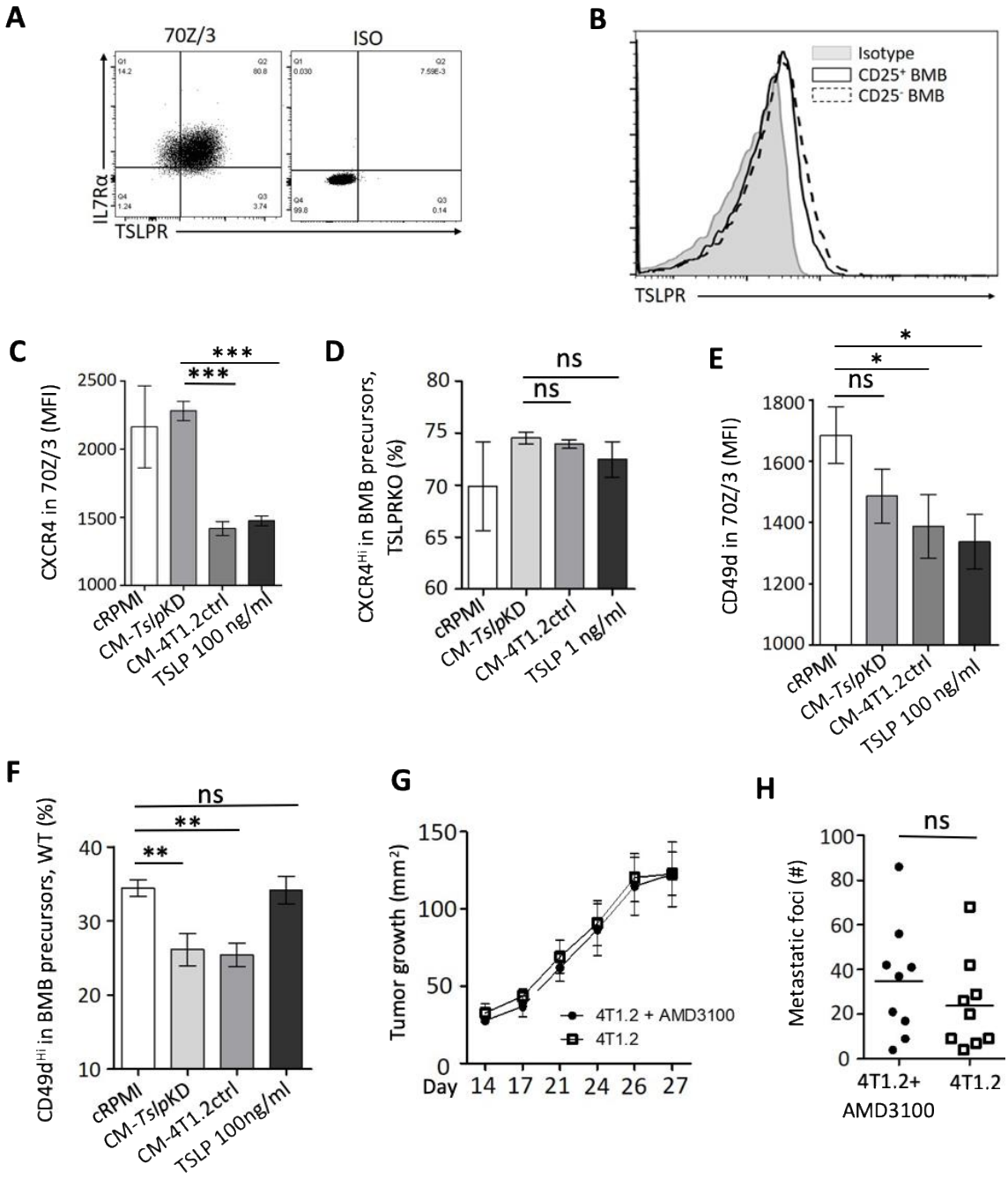
Supplementary Figure 3. Circulating pre-B-like cells accumulate in mice and humans with cancer. A, Frequency of $CD25^{-}$ pre-B-like cells in the blood, spleen, LN, and primary tumor

(Tumor) of naive (Naive, n=19) and 4T1 cancer-bearing BALB/c mice (4T1, n=32). **B** and **C,** Mice with spontaneous ovarian cancer (mogp, **B**), but not with B16-F10 melanoma (**C**), increase pre-B-like cells in circulation. Shown are representative FACS dot plots of $CD25^{+}$ and $CD25^{-}$ pre-B-like cells expressing indicated surface markers in spleen of C57BL/6 (Naive or WT) and mogp mice. **D,** In single cell RNAseq data by Azizi et al. (39), *VPREB3* is upregulated in B cells infiltrated breast cancer (BC) as compared to adjacent healthy breast tissue (p=0.08, Wilcox rank test, n=1176). **E,** RT/PCR analysis confirmed that *RAG1*, *RAG2*, and *VPREB* genes are expressed in human BC-infiltrating B cells (TIL-B, n=3) as in children tonsil (n=1). PCR positive bands are indicated with white arrows. In **A** and **D**, each symbol is for a single mouse and human. P-values are calculated with Mann-Whitney Wilcoxon t-test (**A**).

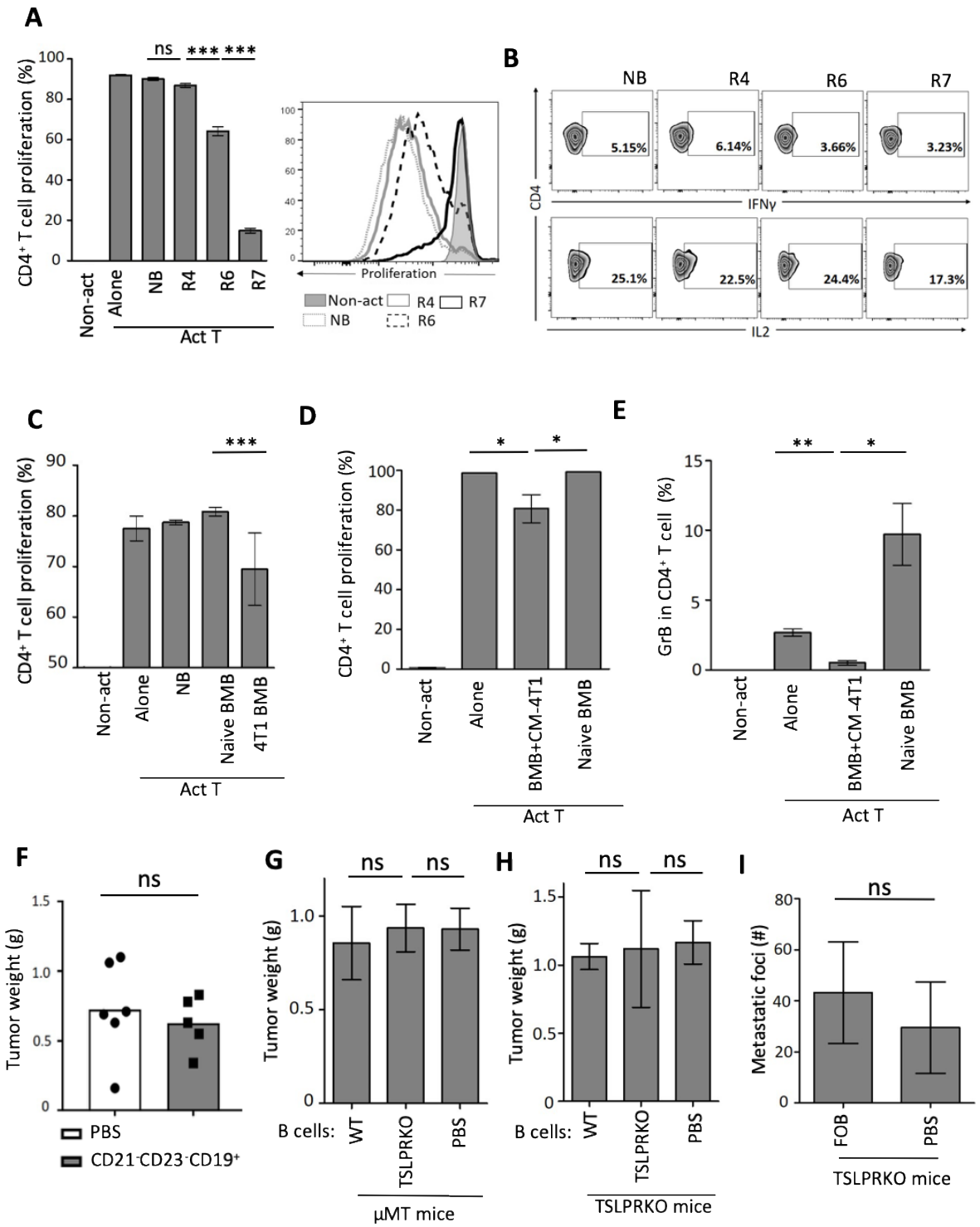


Supplementary Figure 4. Cancer affects BM B cell homing. 4T1.2 cancer-bearing mice decrease CD19$^+$ B cells and CD25$^+$ pre-B cells in BM (numbers, **A** and **B**, and frequency, **C**) as compared to cancer-free mice (Naive, n=4–5). Results are reproduced at least twice. **D–F** show results of in vivo tracking of FACS-sorted BM B-cell precursors (CD25$^+$ and CD25$^-$ from BM

naïve BALB/c, labeled with burgundy) after i.v. transfer into congenic μMT mice with and without 4T1.2 cancer (Naive and 4T1.2, respectively, n=3). Donor cells are quantified at days 19 and 26 after transfer in BM (**E**) and spleen (**F**). **G-I,** Despite comparable tumor progression (**G**), but significant increase of splenic $CD19^+$ B cells in mice with 4T1.2 cancer (**H**), the TSLPR KO impairs accumulation of $CD25^+$ pre-B-like cells as compared with WT mice (**I**, n=5-4). The accumulation of circulating $CD25^+$ pre-B-like cells in BALB/c mice is induced by challenge with 4T1.2ctrl, but not *Tslp*KD, 4t1.2 cells (n=7, **J**). In **A**–**C** and **E**–**J**, each symbol is for a single mouse and p-values were calculated with Mann-Whitney Wilcoxon t-test.

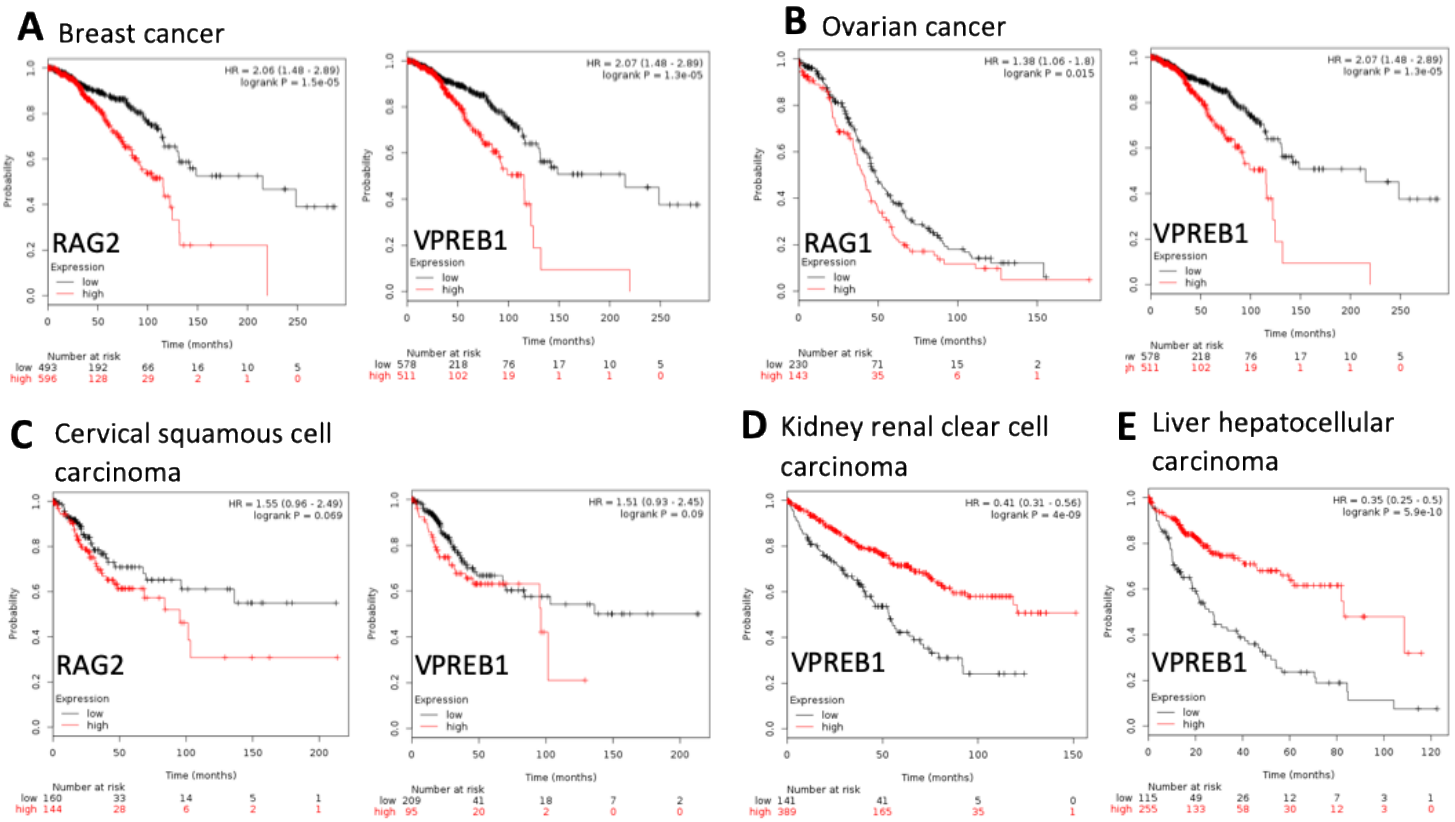


Supplemenrary Figure 5. Cancer-produced TSLP impairs BM retention of B-cell precursors. Representative FACS dot plots (**A**) and histogram (**B**) show expression of TLSPR on 70Z/3 cells (**A**) and primary BM $CD25^+$ and $CD25^-$ B-cell precursors from naïve mice (n=2–3, **B**) as compared to isotype control Ab (ISO, **A**). 70Z/3 cells (**C** and **E**) or primary BM B-cell precursors (**D** and **F**) were cultured overnight with TSLP 9 indicated concentration) or CM-*Tslp*KD and CM-4T1.2Ctrl (16% in cRPMI) to evaluate surface expression of CXCR4 (**C** and **D**) or CD49d (**E** and **F**). Tumor growth (mm^2, **G**) and lung metastasis (**H**) are not affected by inhibition of CXCR4 signaling in mice with 4T1.2 cancer (4T1.2 + AMD3100). P-values are from Welch's t-test (**C-F)** and with Mann-Whitney Wilcoxon (**H**).



Supplementary Figure 6. 4T1 cancer uses pre-B-like cells to generate tBregs (A–E), which promote metastasis (F–I). Shown are representative data from in vitro $CD4^+$ T cell suppression assay with pre-B-like cells from spleen (R6 and R7, **A**) or with BM B cells (both $CD25^+$ and $CD25^-$ IgM^- B-precursors, BMB, **C**) from mice with 4T1 cancer (4T1.2 BMB) or without cancer (NB). Control cells were FOB (R4) and BMB from naïve mice. Act T/alone and non-act are for stimulated with anti-CD3/28 beads or mock-stimulated T cells, respectively. The R7 and, at a

lesser extent, R6 inhibit expression of IFNγ and IL-2 in $CD4^+$ T cells (**B**). To demonstrate that 4T1 cancer confers B-cell precursors with a regulatory activity, naïve mouse BM B-cell precursors (BMB) are cultured in CM-4T1 or in cRPMI for 7 days (**D** and **E**). Only CM-4T1-treated BMB suppressed proliferation (**D**) and granzyme B expression (**E**) in $CD4^+$ T cells in in vitro suppression assay. Primary 4T1.2 tumor progression is not affected by i.v. injection of pre-B-like cells (from spleen of syngeneic mice with 4T1.2 cancer) in BALB/c mice pretreated with 20 μg MK886 to eliminate endogenous tBregs (n=5). Similarly, tumor weight is not changed in μMT (**G**) or TSLPR KO (**H**) mice after transfer of B cells from WT or TSLPR KO mice with 4T1.2 cancer (n=4). Adoptive transfer of FOB from naïve mice does not increase lung metastasis in TSLPR KO mice bearing 4T1.2 cancer (n=4–5, **I**). In **F**, each symbol is for a single mouse and in **G**–**I**, mean ± SD. P-values were calculated with one-way ANOVA followed by Tukey test (**A**), Welch's t-test (**D**–**E**), and Mann-Whitney Wilcoxon t-test (**C**, **F**–**I**).



Supplementary Figure 7. The presence of pre-B-like cells can be associated with both poor and good disease outcomes in patients with cancer. Shown is Kaplan-Meier survival curves of patients with different cancers containing high levels of indicated BM B-cell precursor-associated genes in RNA-seq of patients' primary tumor database. Expression of VPREB1 (**A**–**E**), RAG1 (**B**), and RAG2 (**A**–**C**) was associated ($p<0.05$) with lower survival in human breast (**A**) and ovarian cancer (**B**) and cervical squamous cell carcinoma (**C**) but higher survival in kidney renal clear cell carcinoma (**D**) and liver hepatocellular carcinoma (**E**).