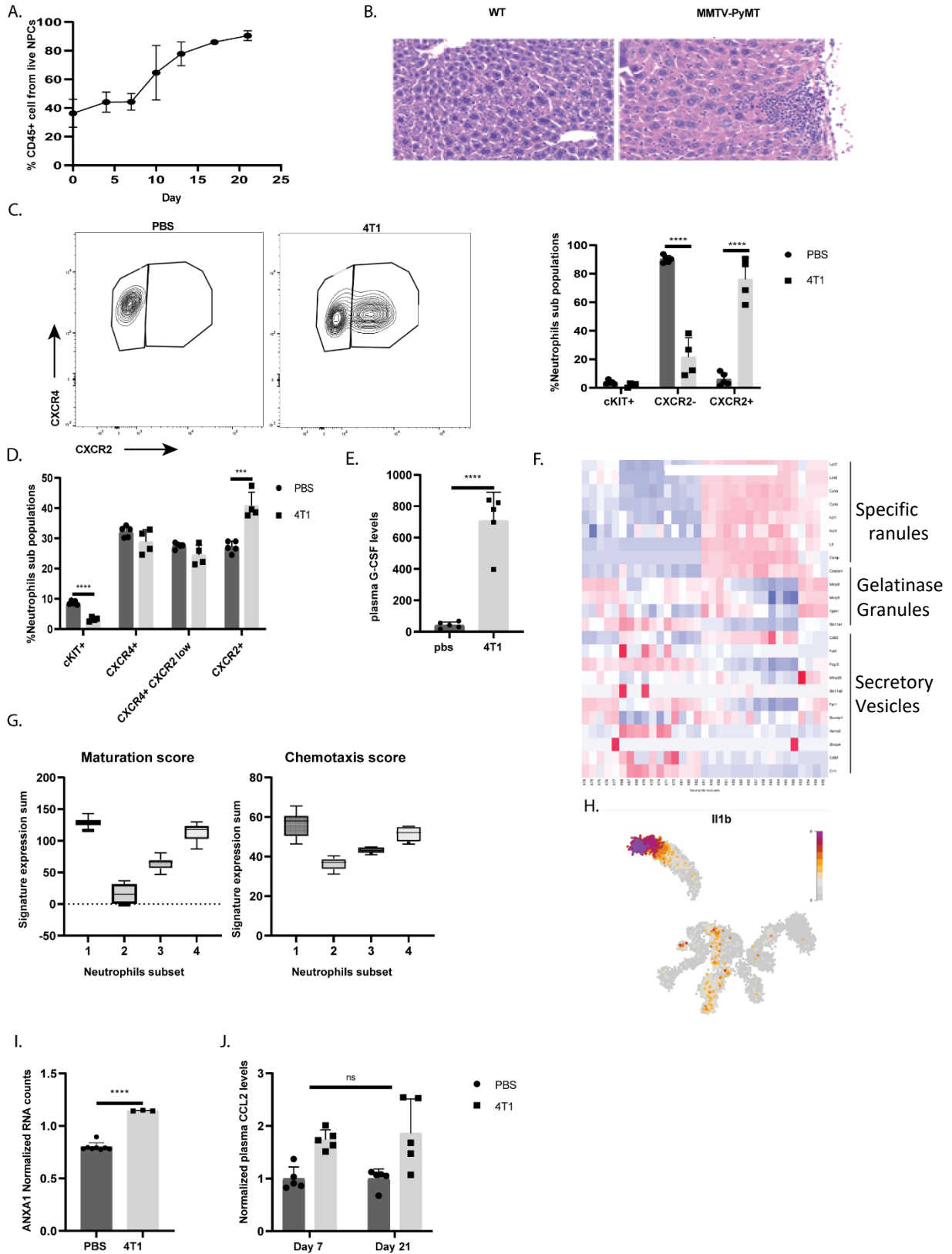
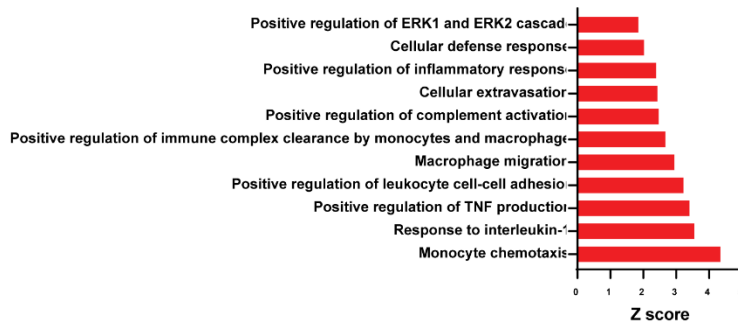


Supplementary Figure S2: Mature innate immune cells infiltrate the liver during early BC carcinogenics



K.



Supplementary Figure 2: Mature innate immune cells infiltrate the liver during early BC carcinogenesis. A.

FACS analysis of liver non-parenchymal cells (NPC) shows a gradual elevation in CD45⁺ cells in BC-bearing mice (days 0,4,7,10,14,17 and 21. n = 2 for each time point). **B.** MMTV –PyMT BC genetic model shows immune cells infiltration to the liver. **C.** Left panel-FACS analysis of liver neutrophils subpopulations shows a significant elevation in CXCR2⁺ neutrophils in BC-bearing mice compared to WT-PBS injected mice, as quantified in the Right panel (WT n = 5, 4T1 n = 4, student T-Test). P<0.0001. **D.** FACS analysis of bone marrow neutrophils subpopulations shows a significant elevation in CXCR2⁺ and a reduction in cKIT⁺ neutrophils in BC-bearing mice compared to WT-PBS injected mice, (WT n = 5, 4T1 n = 4, student T-Test). P values: cKIT<0.0001, CXCR2 =0.0004. **E.** Multiplex ELISA immunoassay demonstrates increased levels of G-CSF in BC-bearing mice (n = 5, student T-Test). P<0.0001 **F.** Heatmap of the expression levels of granules-related genes in each meta-cell for meta-cells annotated as neutrophils. **G.** A maturation score (Left panel) and chemotaxis score (Right panel) for each neutrophils subset (based on annotations) were calculated and are presented as the sum of the expression level of each gene in each meta-cell. Box plots show mean and std. **H.** Single-cell RNAseq reveals expression of IL1b in neutrophils-1 subset. **I.** Normalized levels of scRNA of ANXA1 from macrophages at day 21. P<0.0001 **J.** ELISA assay demonstrates a non-significant interaction effect between tumor progression and elevation of CCL2 in the plasma of BC mice relative to WT-PBS injected mice (n=5, 2-way ANOVA) P = 0.0034. **K.** Pathway analysis of bulk RNA from livers of BC-bearing mice demonstrates elevation in migration-related pathways.