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Supplemental Data

Abrogation of TGFβ Signaling in Mammary Carcinomas Recruits Gr-1+CD11b+ Myeloid Cells that Promote Metastasis

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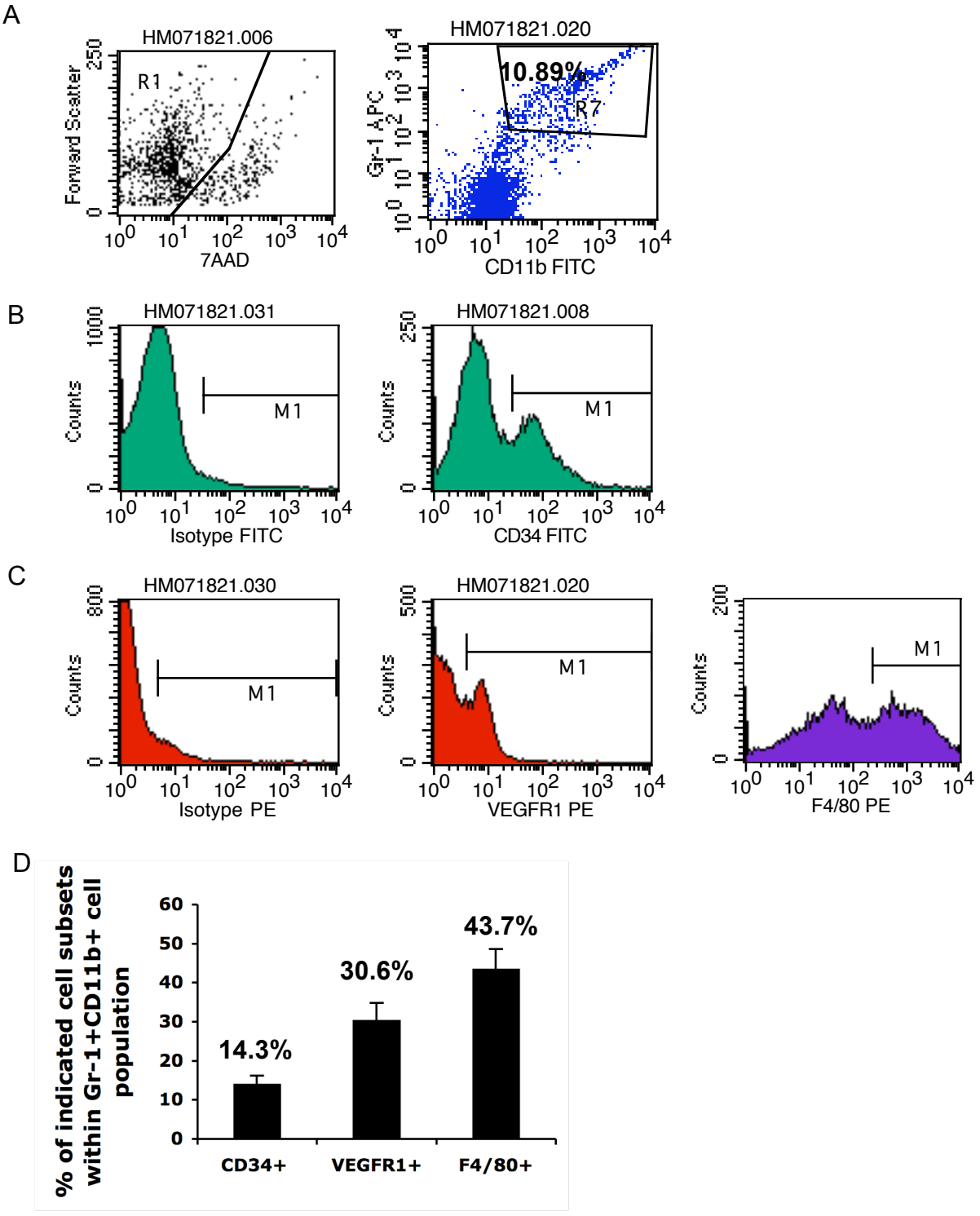
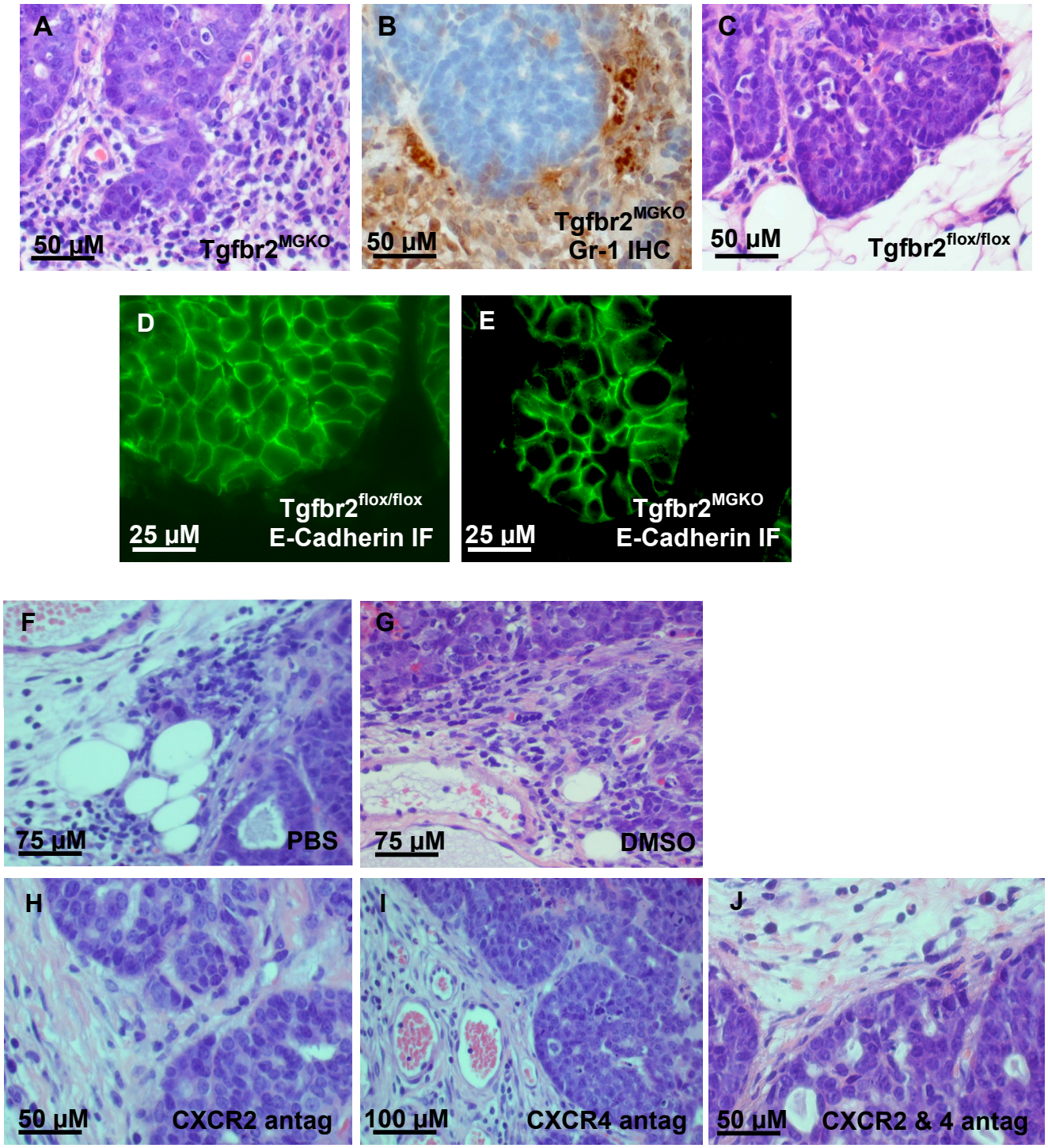


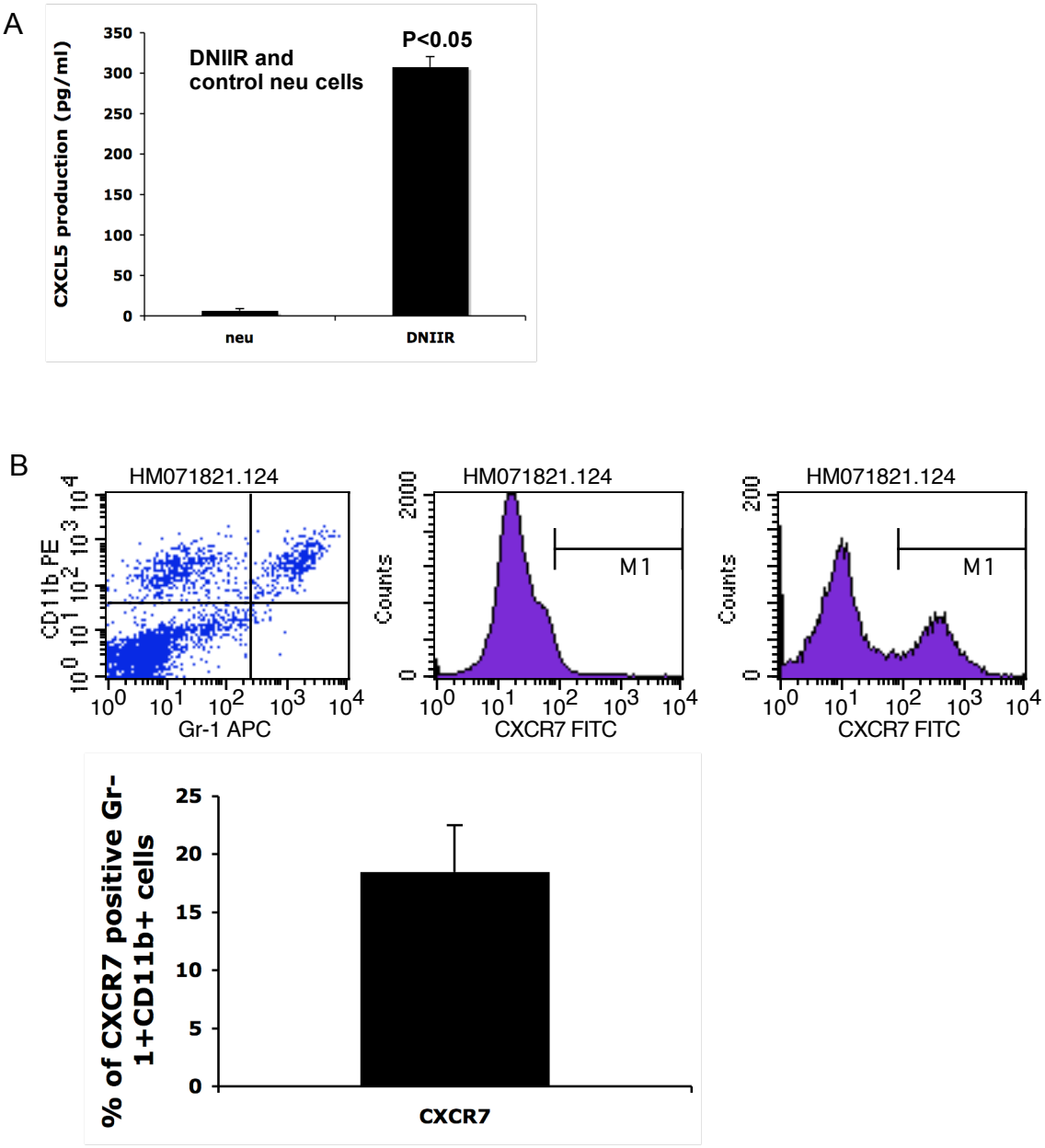
Supplementary figure 1: Characterization of tumor infiltrating Gr-1+CD11b+ myeloid cells by flow cytometry analysis. Single cell suspensions were prepared from 4T1 tumor tissues and stained with fluorescence labeled antibodies as indicated. **A.** Gate was set on live cells (7AAD negative, R1) and Gr-1 and CD11b double positive Gr-1+CD11b+ cells (R7). **B.** Histogram of CD34 positive cells within Gr-1+CD11b+ cell population (right panel), with FITC-isotype as a control (left panel). **C.** Histogram of

VEGFR1 cells (middle panel) and F4/80 positive cells (right panel) within Gr-1+CD11b+ cell population, with PE-isotype as a control (left panel). Shown is one of the 4 mice analyzed. **D.** Percentage of positive cells for indicated cell surface marker within Gr-1+CD11b+ cell population is quantitated and plotted as Y axis. Results are presented as the mean ± SE.



Supplementary figure 2: **A.** A representative invasive front of PyVmT/Tgfbr2^{MGKO} tumors at early stage, compared with PyVmT/Tgfbr2$^{flox/flox}$ control tumors (C) at similar tumor stage or similar tumor size by H&E staining. **B.** Infiltration of Gr-1+CD11b+ cells in PyVmT/Tgfbr2^{MGKO} tumors by IHC of Gr-1. **F-J** PyVmT/Tgfbr2^{MGKO} primary tumor invasion with blockade of CXCR2 or CXCR4 alone or both. Tumors were removed, fixed in 10% formalin and stained for H&E. Shown is representative microscopy of

control tumors (F: PBS; G: DMSO), CXCR2 antagonist treated tumors (H), CXCR4 antagonist treated tumors (I), and tumors treated with both drugs (J). Scale bars are indicated in the figures.



Supplementary figure 3: **A.** Elevated production of CXCL5 in a mammary carcinoma cell culture supernatant of dominant-negative type II receptor expression in combination with c-Neu expression by ELISA, in comparison with the control carcinoma cells expressing only c-Neu. **B.** Flow cytometry analysis of CXCR7 expression in infiltrating Gr-1+CD11b+ myeloid cells from 4T1 tumors. Left panel: Quadrant of Gr-1 staining and CD-11b staining in single cell suspension from whole 4T1 tumors. Gr-1+CD11b+ cells are located in right top quadrant is. Right panel: histogram of CXCR7 expression gated on Gr-1+CD11b+ double positive cells. Middle panel: histogram of CXCR7 expression gated on Gr-1CD11b double negative cells, used as a control. Shown is one of the 4 mice analyzed. Quantitative data from total of 4 mice is shown in the lower bar graph. Results are presented as the mean ± SE.