LEGENDS FOR SUPPLEMENTAL FIGURES

Supplemental Figure 1.

Representative images of MDA-MB-231 (A) and 4T1 (B) tumor sections stained for F4/80 (green) and Gr-1 (red). Most of the F4/80$^+$ cells were macrophages as they were Gr-1$^-$(green cells). White arrowheads point at few F4/80$^+$Gr-1$^+$ monocytes (yellow). Staining with the individual antibodies as well as merged images are shown. These are magnified images of the staining patterns shown in Figure 3F and 3I, respectively, plus staining for individual antibodies as well as the merge images are also shown for further details. Size bar, 146 µm.

Supplemental Figure 2.

Representative images of MDA-MB-231 (A) and 4T1 (B) tumor sections stained for F4/80 (green) and CD206 (red). Staining for individual antibodies as well as the merge images are also shown for further details. Most of the F4/80$^+$ cells were polarized to the M2 phenotype since they were CD206$^+$ (yellow+orange cells). Size-bar, 146 µm.

Supplemental Figure 3.

Loss of Kindlin-2 inhibits macrophage recruitment to MDA-MB-231 tumors. (A) Representative images of MDA-MB-231-Scram (Top) and K2-CRISPR (Bottom) tumor sections stained for F4/80 (green) and CD206 (red). Most of the F4/80$^+$ cells were polarized to the M2 phenotype since they were CD206$^+$ (yellow + orange cells). The staining was performed in Scram and K2-CRISPR tumors of roughly similar size (volume-normalized pairs), where the smallest Scram tumor (268 mm^3) and the largest K2-CRISPR tumor (180 mm^3) were analyzed. The Scram Size bar, 146 µm. (B) Quantification of macrophage-specific areas. Data are expressed as means ± SEM. *p=0.02, n=3 fields.

Supplemental Figure 4.

Loss of Kindlin-2 inhibits macrophage recruitment to tumors. The 4T1 model. (A) Tumors generated from inoculation of Scram or K2-CRISPR 4T1 cells into the mammary fat pads of BALB/C mice. Tumor volume after removal (means± SD) is also shown (*, p =0.45, n=5 mice). To obtain tumors of similar size, two variables were introduced in this experiment: (i) the number of K2-CRISPR 4T1 cells inoculated was 3 times that of the Scram 4T1 cells; and (ii) the K2-CRISPR tumors were removed ~3-weeks after removal of the Scam tumors. (B) Representative images of 4T1-Scram (Top) and K2-CRISPR (Bottom) tumor sections stained for macrophages with anti-F4/80 (green) and anti-CD206 (red). Most of the $F4/80^{+}$ cells were polarized to the M2 phenotype since they were $CD206^{+}$ (yellow + orange cells). Size-bar, 146 µm. (C) Quantification macrophage-specific $F4/80^{+}$ areas in tumors. Data shown means ± SEM (*$p<0.001$, n=5 mice). (D) Quantitative-real time RT-PCR of CSF-1, CSF1R, EGF and EGFR transcripts in tumors derived Scram or K2-CRISPR 4T1 cells. GAPDH was used for normalization. Data are the means ± SD (n=5 mice,**,$p<0.05$; Student's t-test).

Supplemental Figure 5.

Kaplan-Meier (KM, http://kmplot.com/analysis/) plot correlating survival of 3951 breast cancer patients with CSF-1 expression levels.