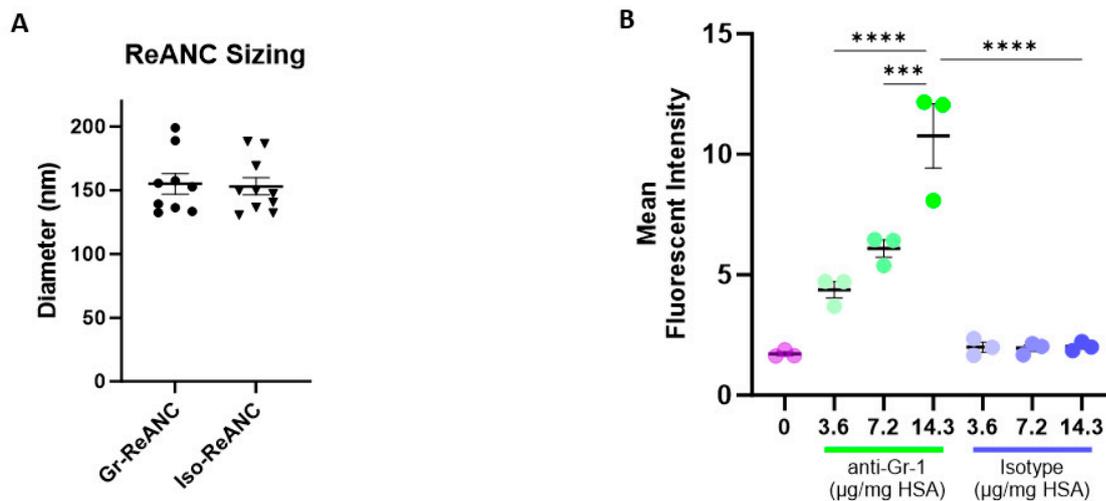
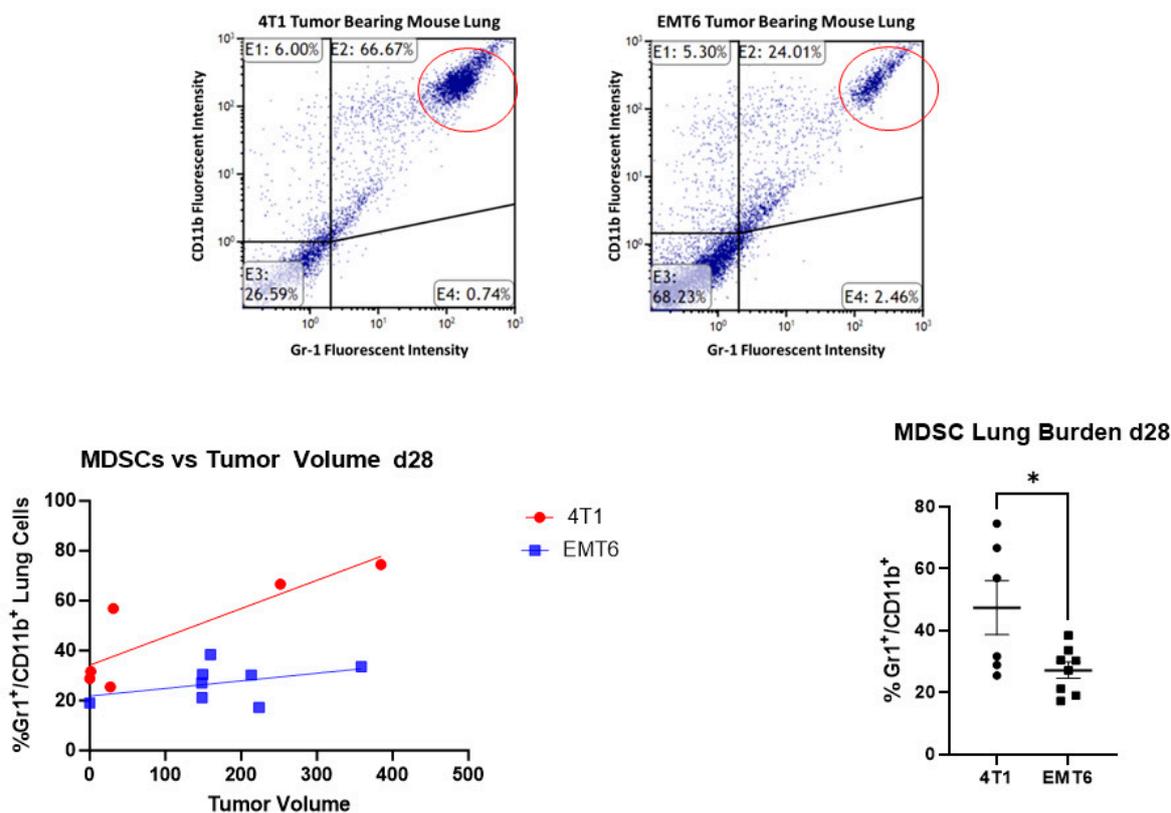


# Supplementary Materials: Early Detection of Myeloid-Derived Suppressor Cells in the Lung Pre-Metastatic Niche by Shortwave Infrared Nanoprobes

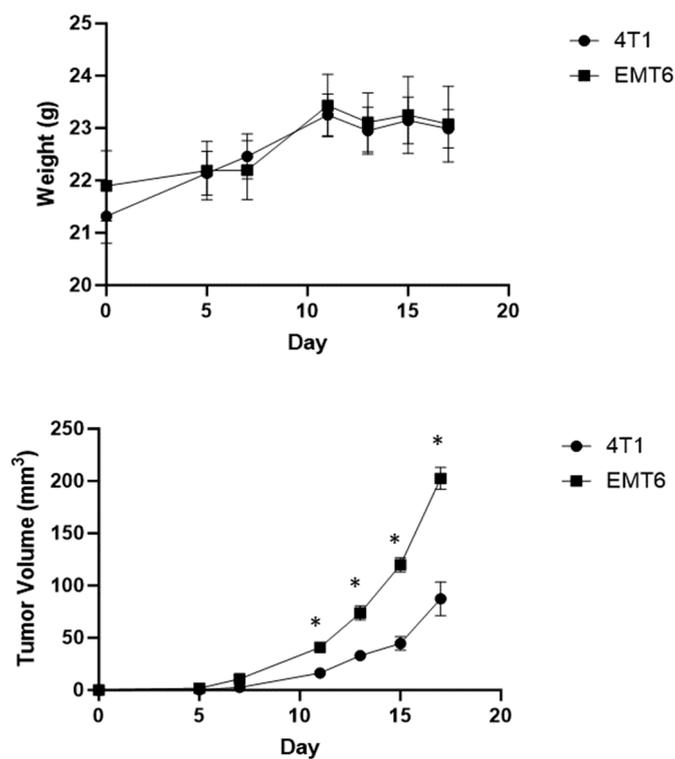
Jake N. Siebert, Jay V. Shah, Mei Chee Tan, Richard E. Riman, Mark C. Pierce, Edmund C. Lattime, Vidya Ganapathy and Prabhas V. Moghe



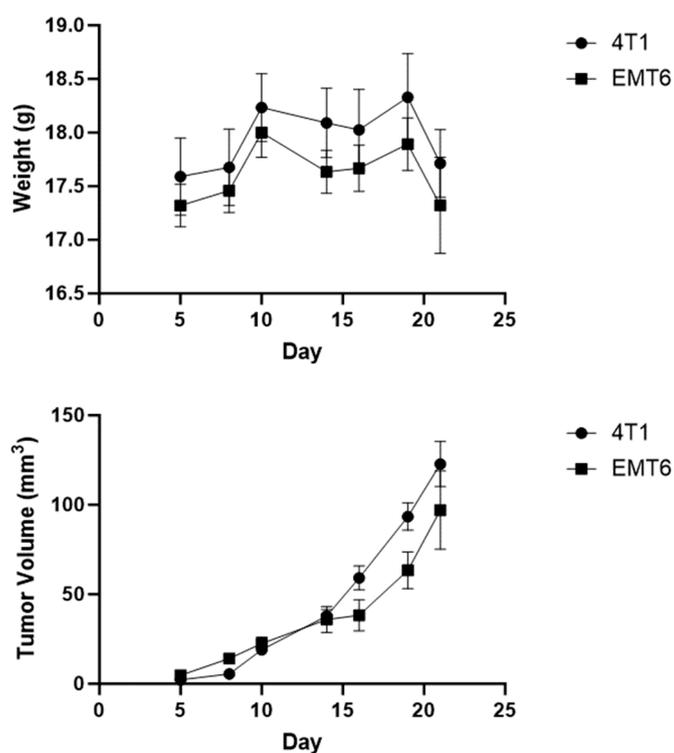
**Figure S1.** IgG-ReANC Sizing and IgG-ANC MDSC Binding Assay. **A)** The hydrodynamic diameters of Gr-ReANCs and Iso-ReANCs were determined by dynamic light scattering (DLS). The average hydrodynamic diameter was  $155.0 \pm 8.0$  nm and  $153.0 \pm 6.7$  nm for Gr-ReANCs (PDI: 0.17) and Iso-ReANCs (PDI: 0.15). **B)** MDSC binding of Gr-ANCs and Iso-ANCs assessed by flow cytometry with IgG loading concentrations of 3.6 to  $14.3 \mu\text{g mg}^{-1}$  HSA.



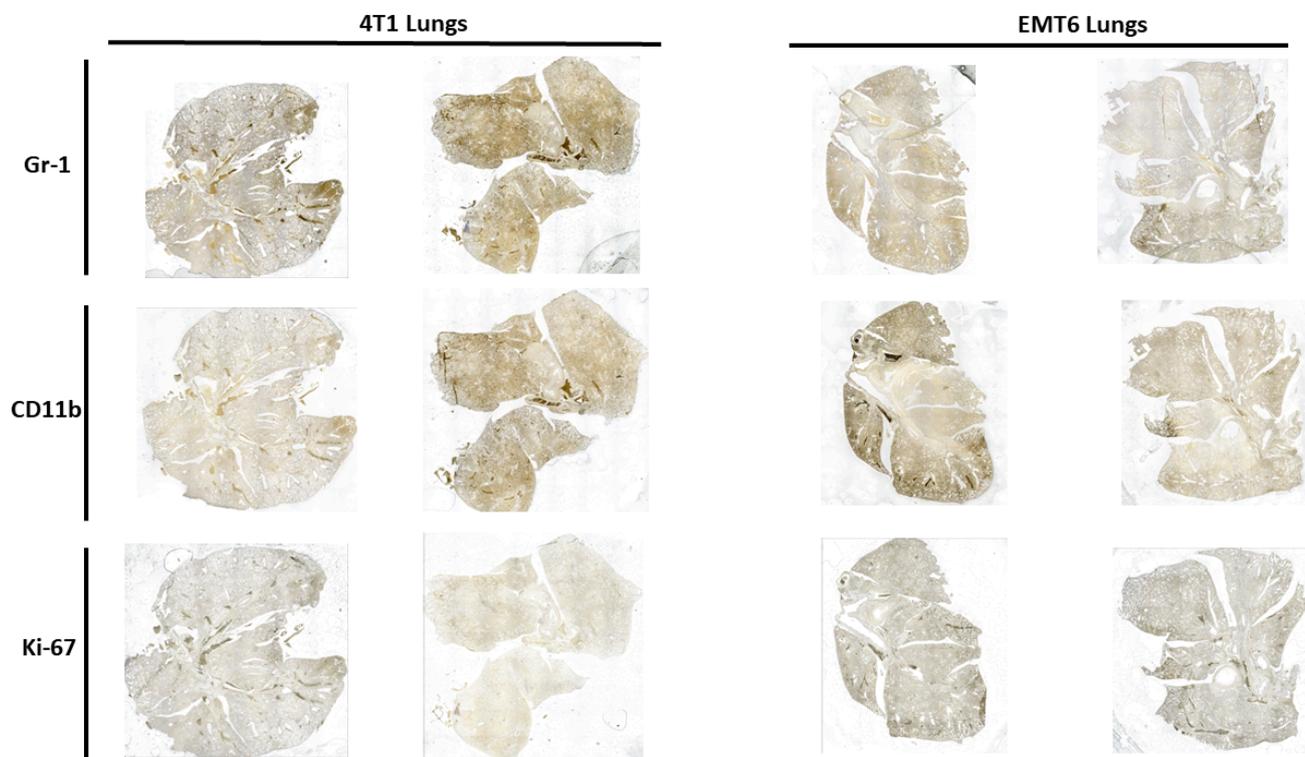
**Figure S2.** Characterization of MDSC lung infiltration in two murine syngeneic mammary fat pad models of breast cancer, 4T1 and EMT6, with spontaneous lung metastasis. **Top:** Flow cytometry gating of single cell lung suspensions stained for Gr-1<sup>+</sup> and CD11b<sup>+</sup> cells in both 4T1 and EMT6 mice. 66.7% of cells stained positive for both markers in 4T1 mice compared to EMT6 mice. **Bottom:** Flow cytometry results on day 28 post-inoculation demonstrates significantly increased MDSC lung infiltration in 4T1 mice.



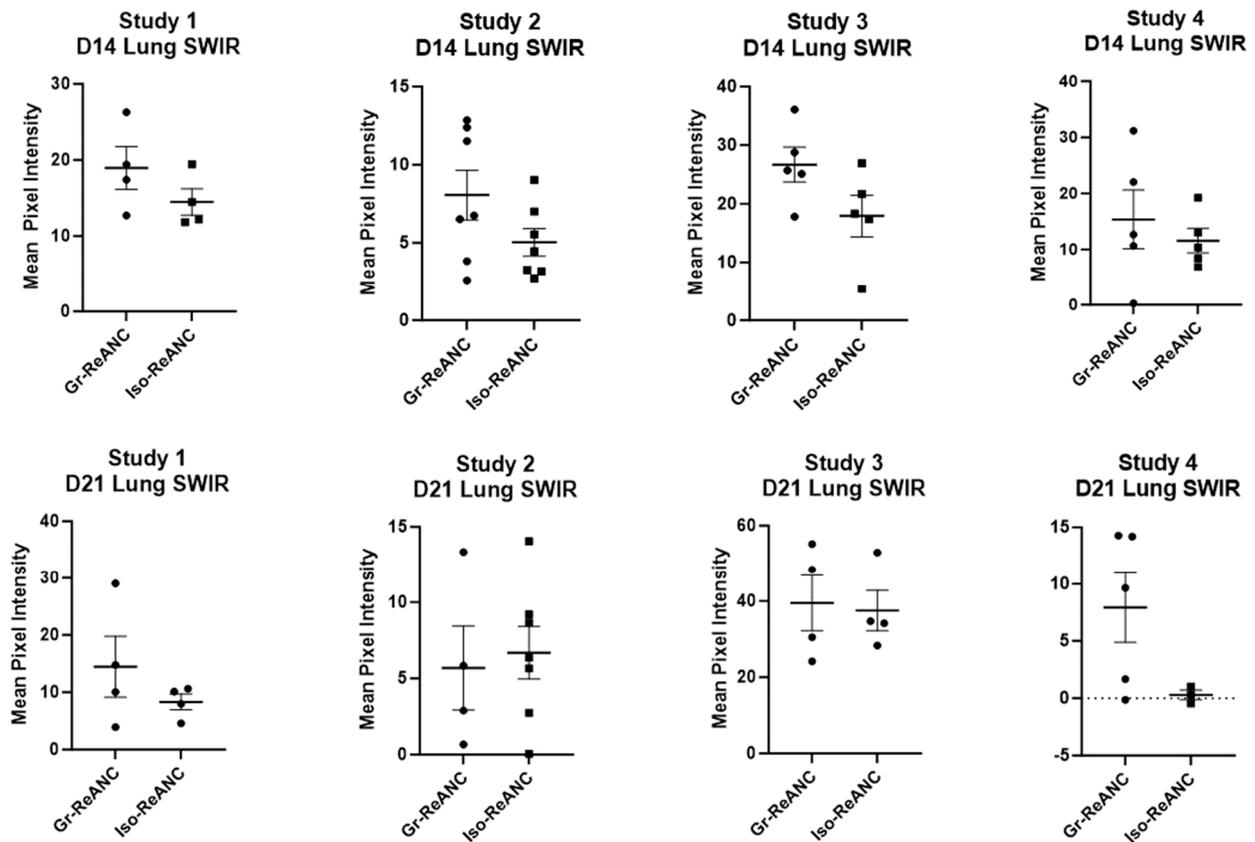
**Figure S3.** Athymic mouse weights and tumor growth. **Top:** Mouse weights for EMT6 and 4T1 tumor bearing athymic nude mice from day 0 to day 17. **Bottom:** 4T1 and EMT6 tumor volumes, EMT6 tumors are significantly larger than 4T1 tumors from day 11 on ( $p < 0.0001$ ).



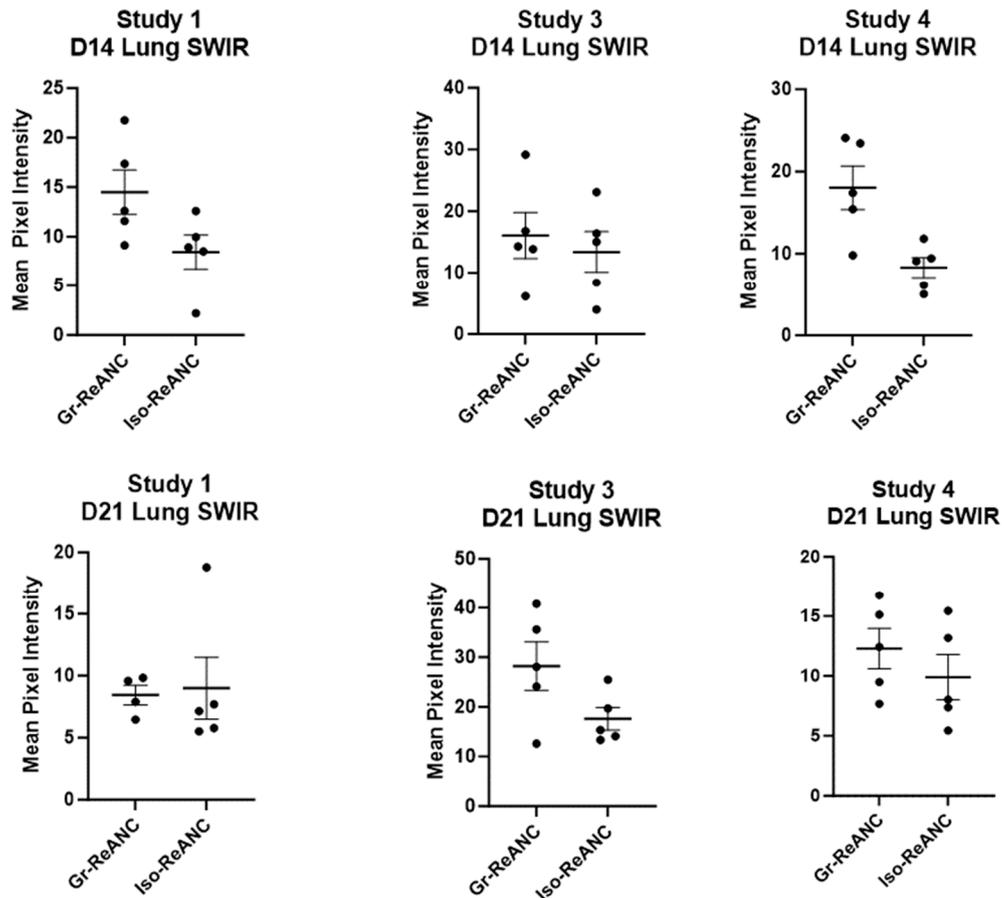
**Figure S4.** Balb/c mouse weights and tumor growth. **Top:** Average mouse weights during the course of study 3 in Balb/c mice. **Bottom:** Average volume of 4T1 and EMT6 tumors during study 3, no difference in tumor volume on day 14 ( $p=0.799$ ) or day 21 ( $p=0.327$ ).



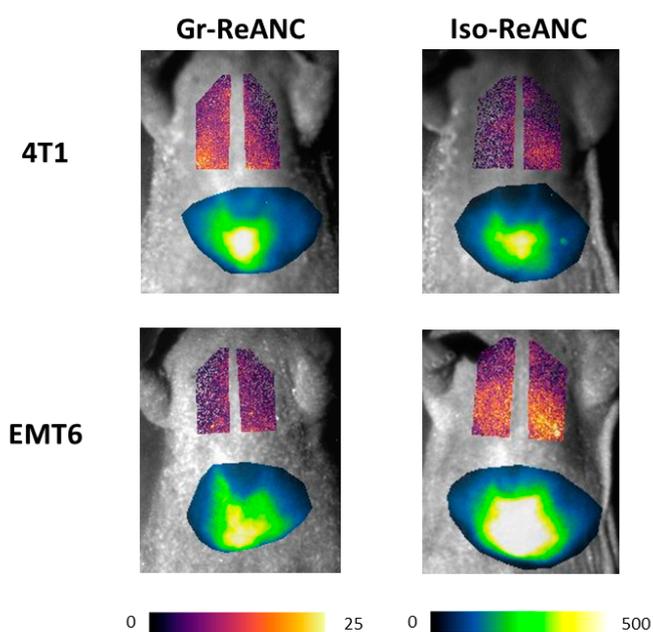
**Figure S5.** Representative lung samples from 4T1 and EMT6 tumor bearing mice stained for MDSCs, Gr-1 and CD11b, and proliferation marker Ki-67. Positive staining appears brown.



**Figure S6.** Individual study results from 4T1 mice on day 14 (top) and day 21 (bottom). Individual study results demonstrate a consistent increase in SWIR signal with Gr-ReANCs compared to Iso-ReANCs on day 14 across all studies. Day 21 results demonstrate variability in differences between Gr-ReANC and Iso-ReANC.



**Figure S7.** Individual study results from EMT6 tumor bearing mice on day 14 (top) and day 21 (bottom). Results show variability in relative differences between Gr-ReANC SWIR signal and Iso-ReANCs at both day 14 and day 21.



**Figure S8.** False color SWIR images of athymic mice with 4T1 or EMT6 tumors treated with either Gr-ReANCs or Iso-ReANCs, corresponding to the quantified pixel intensity data from Figure 3A. The results show increased SWIR intensity in 4T1 tumor bearing mice treated with Gr-ReANCs compared to Iso-ReANCs and EMT6 tumor bearing mice treated with Gr-ReANCs. There is no difference between EMT6 mice treated with Gr-ReANCs and Iso-ReANCs.