Supplementary Information

A Novel DNA Aptamer for Dual Targeting of Polymorphonuclear Myeloid-derived Suppressor Cells and Tumor Cells

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AUTHOR INFORMATION

The authors declare no competing financial interest.

All authors have given approval to the final version of this manuscript.

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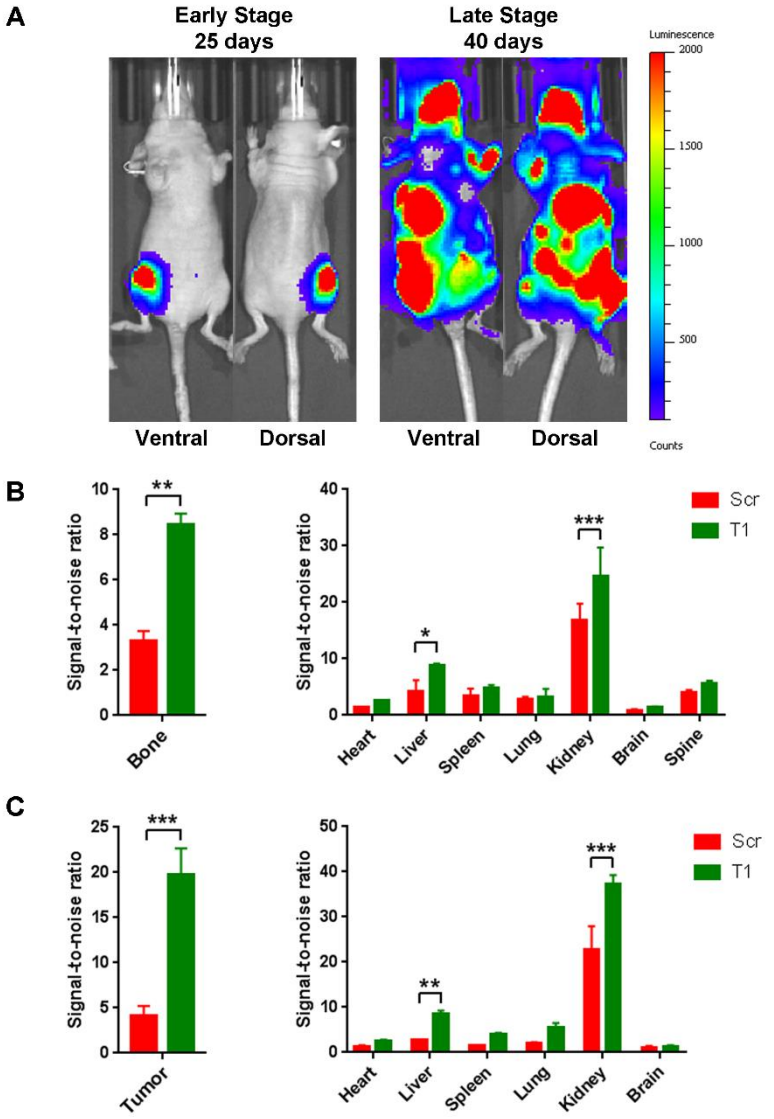
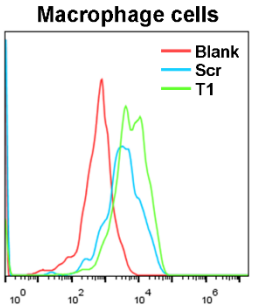
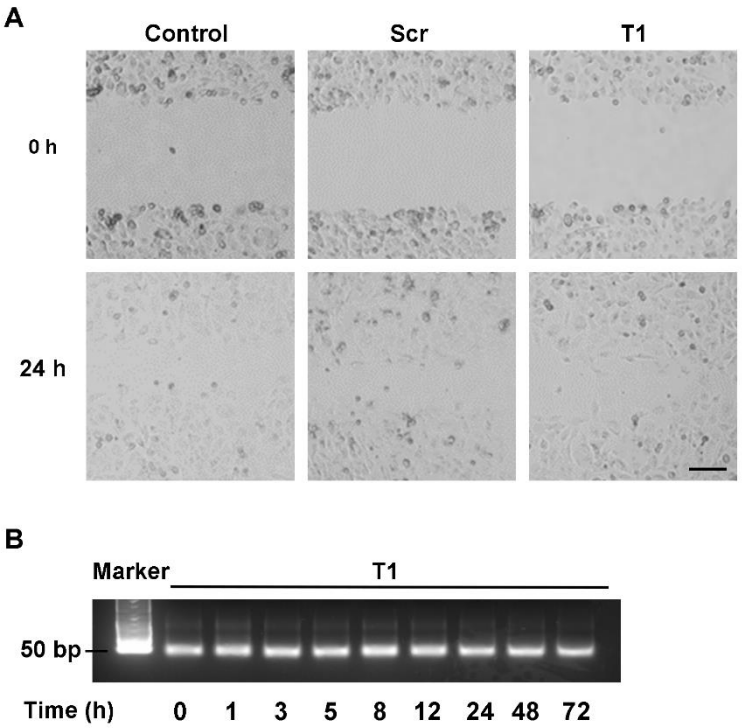


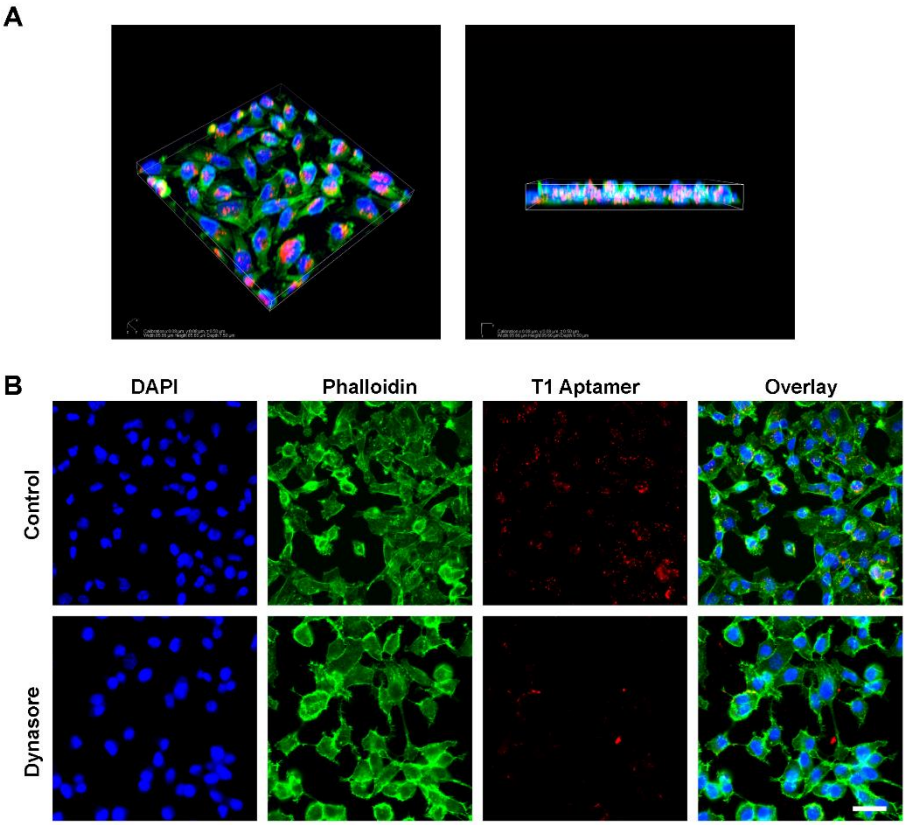
Supplementary Figure 1. Validation of T1 aptamer tumor accumulation in an orthotopic and metastatic MDA-MB-231 breast cancer mouse model. (**A**) Representative bioluminescent images of breast cancer (luciferase expressing) bone metastases. (**B, C**) Aptamer biodistribution in a metastatic (**B**) and orthotopic (**C**) model. Data is presented as mean ± s.d. (n = 3). *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.



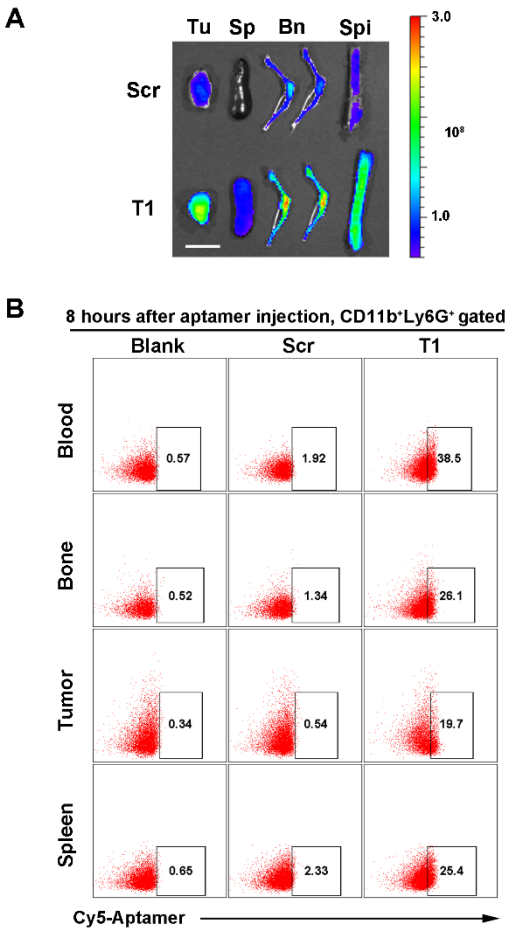
Supplementary Figure 2. Representative flow cytometry graph of Cy5-labeled Scr and T1 aptamer binding (ice incubation) to macrophage cells ($CD11b^{+}F4/80^{+}$) isolated from MDA-MB-231 breast cancer tumors.



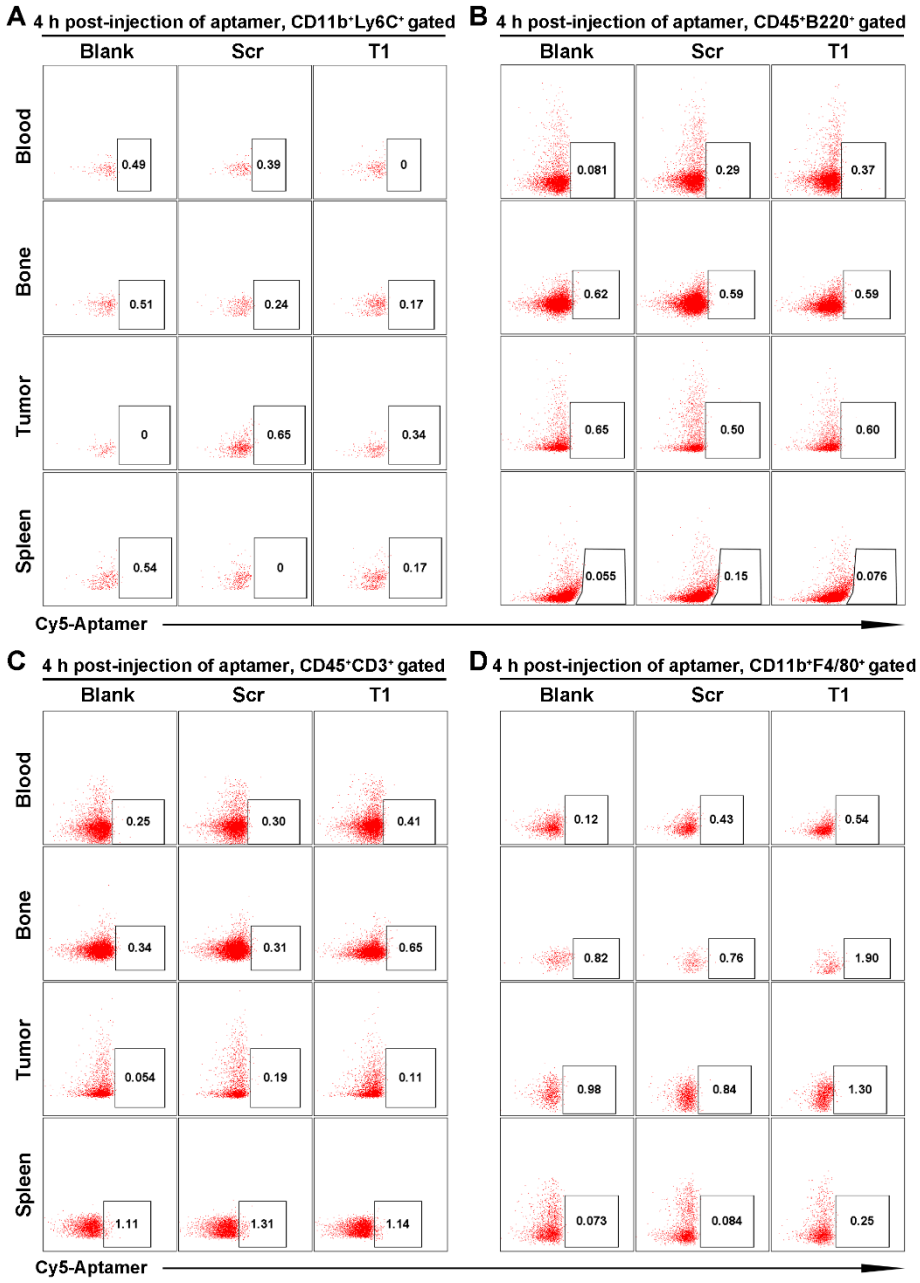
Supplementary Figure 3. T1-induced effects on cell migration and T1 stability. (**A**) Scratch assay in MDA-MB-231 cells. Scale bar, 50 μm. (**B**) T1 stability evaluated by agarose gel electrophoresis. T1 was incubated in 2% fetal bovine serum (FBS) at 37 ℃ for various time points.



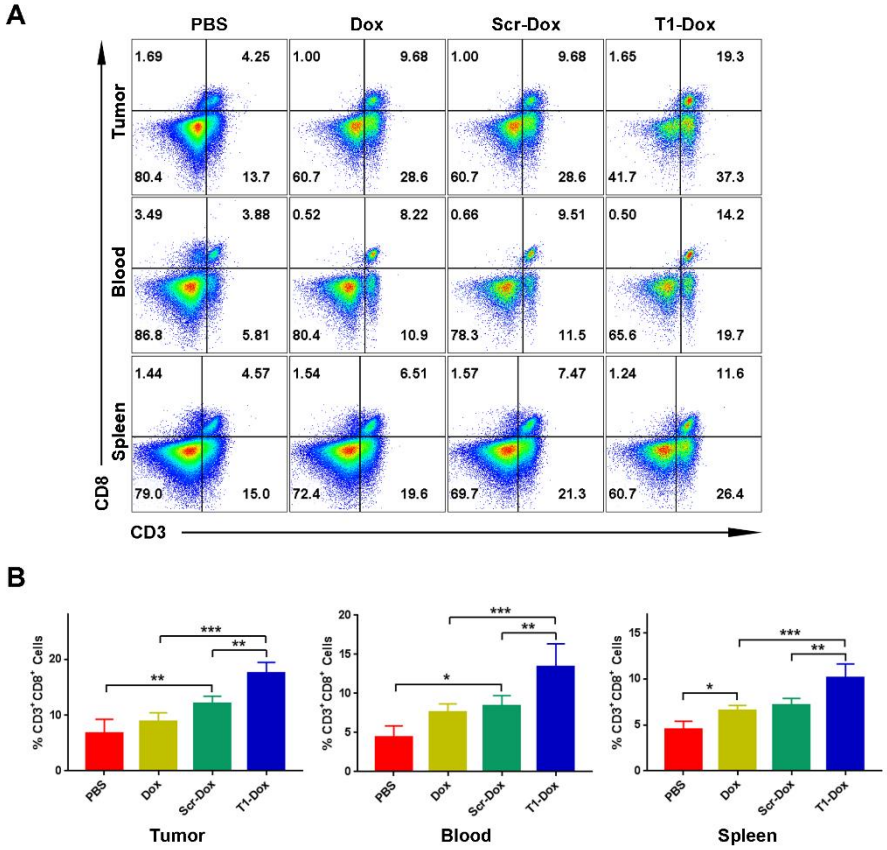
Supplementary Figure 4. Confocal fluorescence microscopy images of T1 uptake in MDA-MB-231 cells. T1 aptamer (red), phalloidin (green), and DAPI (blue). (**A)** Representative 3D image. (**B**) Representative images of control cells and dynasore-treated cells. Scale bar, 20 μm.



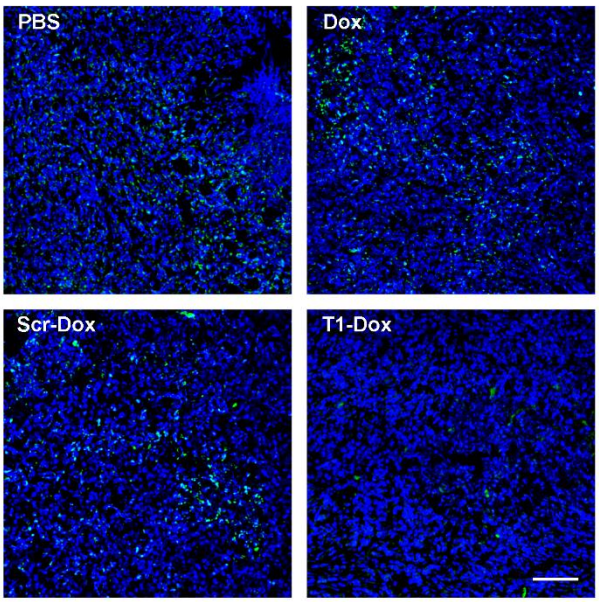
Supplementary Figure 5. Evaluation of T1 binding targets in a 4T1 orthotopic breast cancer model. Cy5-labeled aptamers were intravenously administered and analysis was performed 8 h post-injection. (**A**) Fluorescent images of organs captured with the IVIS 200 spectrum imaging system. Organs: Tu, tumor; Sp, spleen; Bn, bones; Sp, spine. Scale bar, 1 cm. (**B**) Representative flow cytometry graphs of PMN-MDSCs in blood, bone, tumor, and spleen samples.



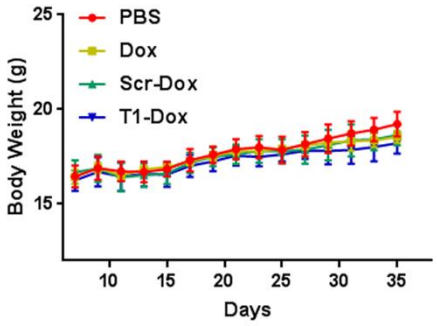
Supplementary Figure 6. Binding of T1 to immune cells in mice bearing 4T1 orthotopic breast cancer tumors. Cy5-labeled aptamers were intravenously administered and analysis was performed 4 h post-injection (**A-D**) Representative flow cytometry graphs of M-MDSCs (A), B cells (B), T cells (C), and macrophages (D) from blood, bone, tumor, and spleen samples.



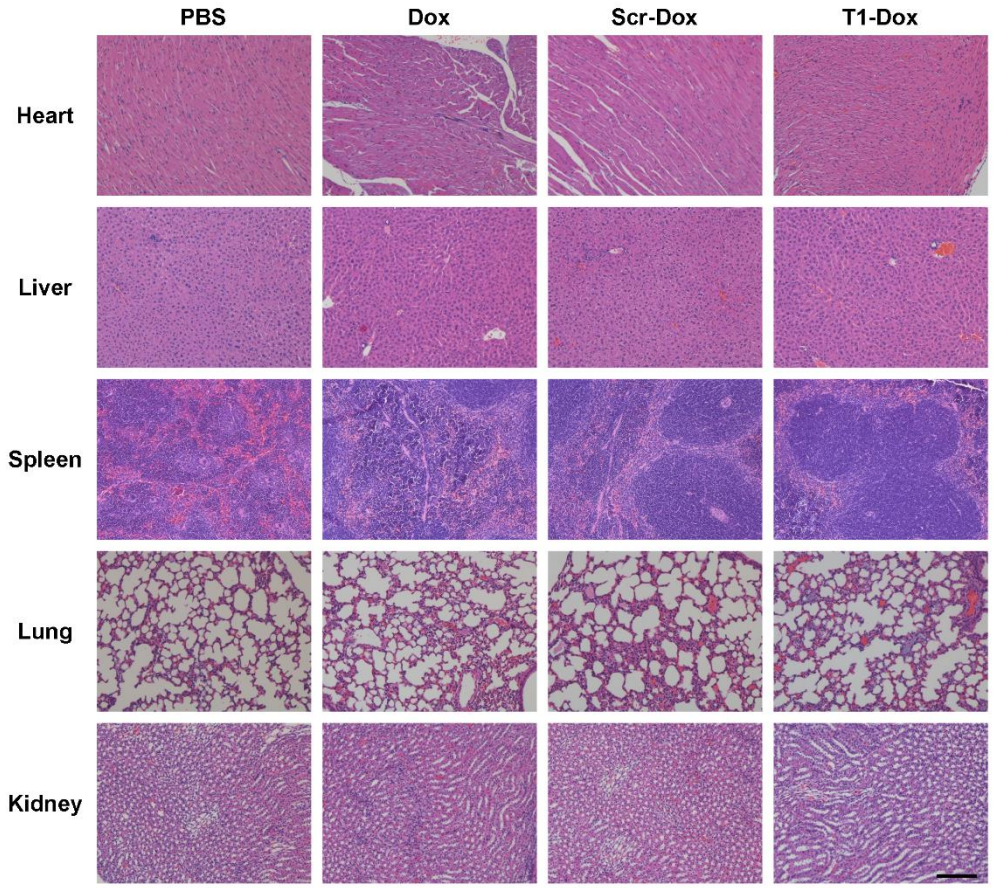
Supplementary Figure 7. Cytotoxic T cells ($CD3^+CD8^+$) in mice bearing 4T1 orthotopic breast cancer tumors. PBS, Dox, Scr-Dox, or T1-Dox (3 mg/kg) was administered intravenously on day 7, 14, 21, and 28 post-injection of cancer cells. (**A**) Representative flow cytometry graphs of cytotoxic T cells in tumor, blood, and spleen samples. (**B**) Statistical representation of flow cytometry results. Data is presented as mean ± s.d. (n = 5). *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.



Supplementary Figure 8. Immunofluorescence staining of Ki-67 in orthotopic 4T1 breast cancer tumors treated with T1-Dox. PBS, Dox, Scr-Dox, or T1-Dox (3 mg/kg) was administered intravenously on day 7, 14, 21, and 28 post-injection of cancer cells. Ki-67 (green) and DAPI (blue). Scale bar, 100 μm.



Supplementary Figure 9. Mouse body weight in response to T1-Dox treatment in a 4T1 orthotopic breast cancer model. PBS, Dox, Scr-Dox, or T1-Dox (3 mg/kg) was administered intravenously on day 7, 14, 21, and 28 post-injection of cancer cells. Data is presented as mean ± s.d. (n = 8).



Supplementary Figure 10. Hematoxylin and eosin (H&E) staining of major organs following T1-Dox treatment in a 4T1 orthotopic breast cancer model. PBS, Dox, Scr-Dox, or T1-Dox (3 mg/kg) was administered intravenously on day 7, 14, 21, and 28 post-injection of cancer cells. Scale bar, 200 μm.