

**Supplemental Information for:**

**Cytosolic Ca<sup>2+</sup> transients during pulsed focused ultrasound generate reactive oxygen species and cause DNA damage in tumor cells**

Robert B. Rosenblatt<sup>1</sup>, Joseph A. Frank<sup>1, 2</sup>, and Scott R. Burks<sup>1,\*</sup>

<sup>1</sup>Frank Laboratory, Department of Radiology and Imaging Sciences, NIH Clinical Center, Bethesda, MD, 20892

<sup>2</sup>National Institute of Biomedical Imaging and Bioengineering, Bethesda, MD 20892

\*Address Correspondence to:

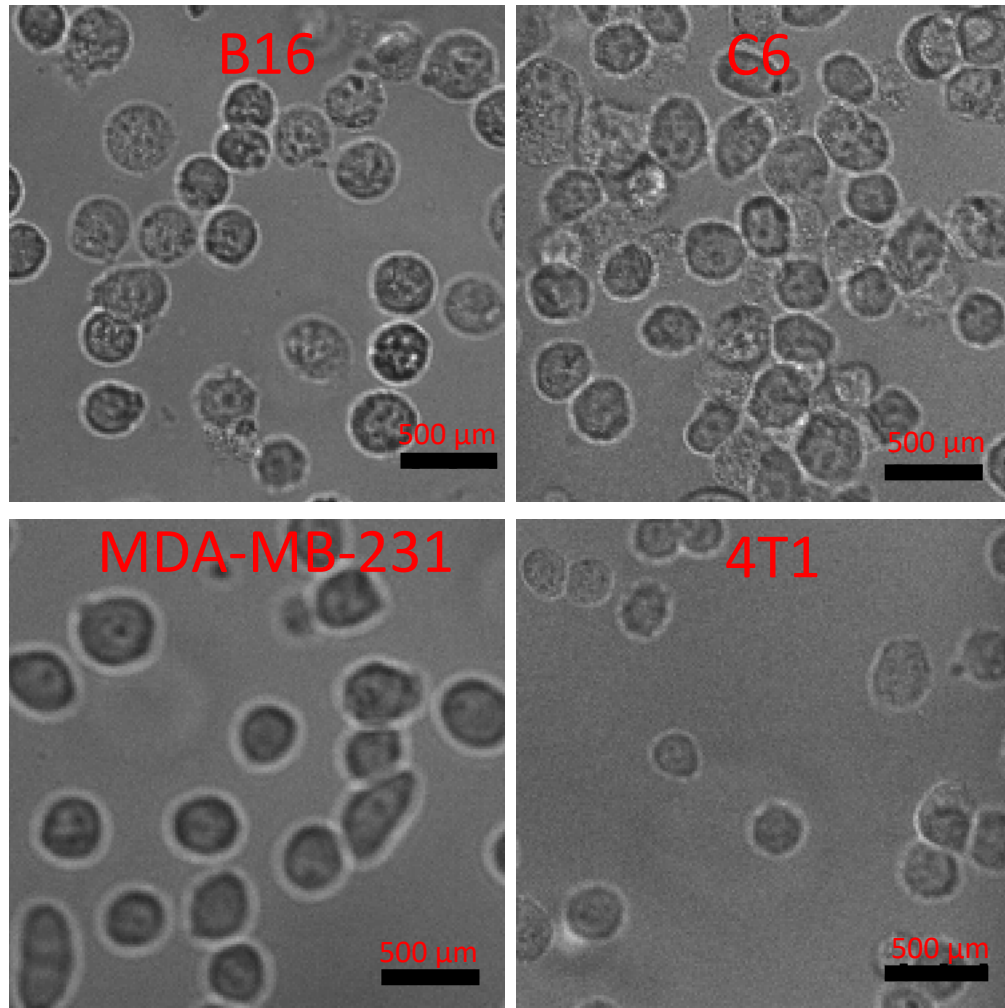
Scott R. Burks, Ph.D.

10 Center Dr., RmB1N256

Bethesda, MD 20892

Ph: (301) 594-2368

[scott.burks@nih.gov](mailto:scott.burks@nih.gov)



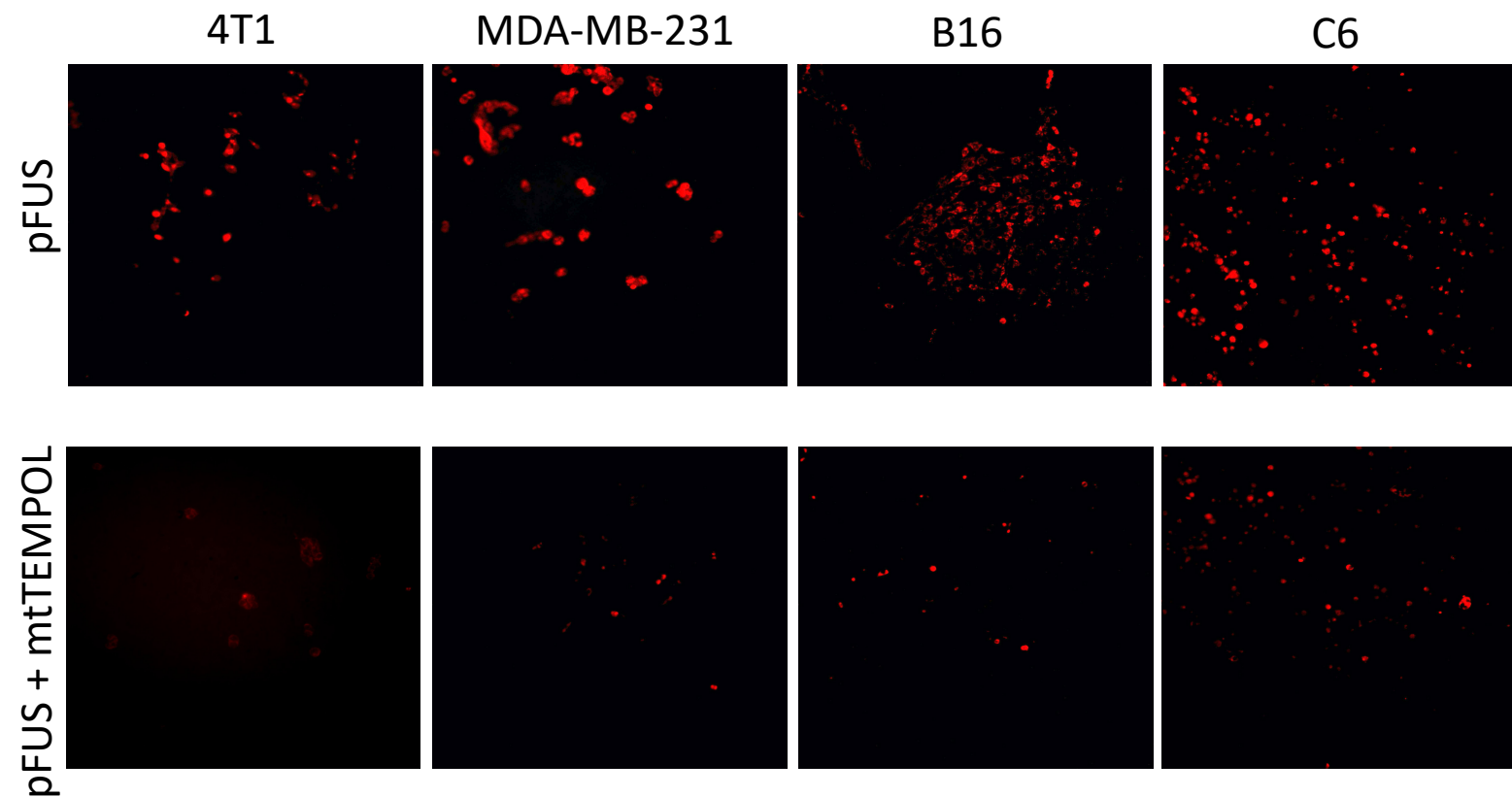
| Cell Line  | Average Cell Diameter (μM) | SD   |
|------------|----------------------------|------|
| B16        | 20.97                      | 1.18 |
| C6         | 16.87                      | 2.35 |
| MDA-MB-231 | 27.17                      | 3.98 |
| 4T1        | 26.07                      | 1.86 |

**Supplemental Figure 1. Means and variance of cell diameters to estimate intracellular**

**volumes.** Diameters (n = 100 per cell type) were measured across 3 plates of cells for each type

and then used to calculate spherical volumes of cells using the equation:  $V = \frac{4}{3}\pi r^2$  where V

is volume in  $\mu\text{m}^3$  and r is radius in  $\mu\text{m}$ .



**Supplemental Figure 2. mtTEMPOL neutralizes superoxide formation following pFUS to tumor cells.** Cells were given pFUS in the presence or absence of mtTEMPOL (20  $\mu$ M) and allowed to incubate for 2 hr before live cell imaging on an epifluorescent microscope. Measurements were repeated in triplicate with similar results.