

PNAS

www.pnas.org

Supplementary Information for

Tumor Cell Lysate-Loaded Immunostimulatory Spherical Nucleic Acids as Therapeutics for Triple Negative Breast Cancer

Cassandra E. Callmann,^{1,2} Lisa E. Cole,^{1,2} Caroline D. Kusmierz,^{1,2} Ziyin Huang,^{2,3} Dai Horiuchi,^{4,5} and Chad A. Mirkin^{1,2,3,5*}

*Prof. Chad A. Mirkin

Email: chadnano@northwestern.edu

This PDF file includes:

Figures S1 to S6
Table S1

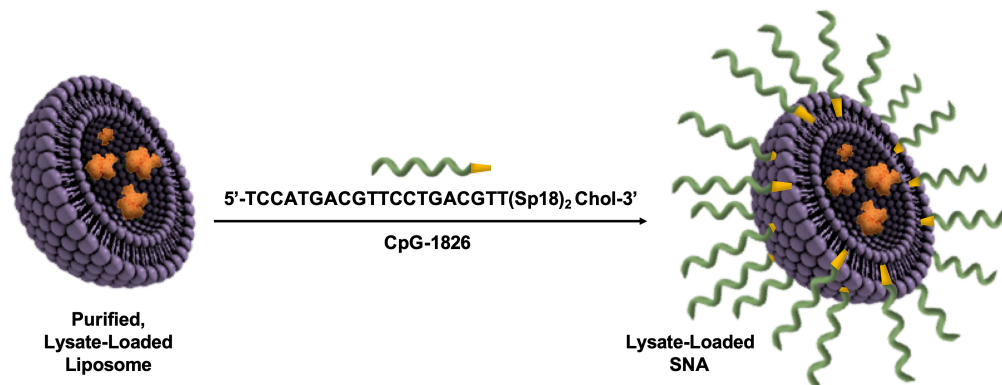


Fig. S1. Lysate-loaded SNA formation from lysate-loaded liposomes. CpG-1826 is 3'-modified with cholesterol to induce bilayer intercalation.

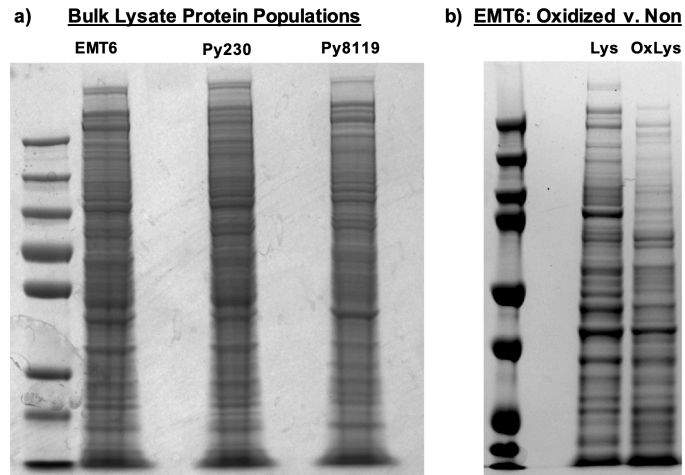


Fig. S2. Western blot of (a) lysates isolated from EMT6, Py230, and Py8119, and (b) lysates isolated from non-oxidized (left lane) and oxidized EMT6 cells (right lane).

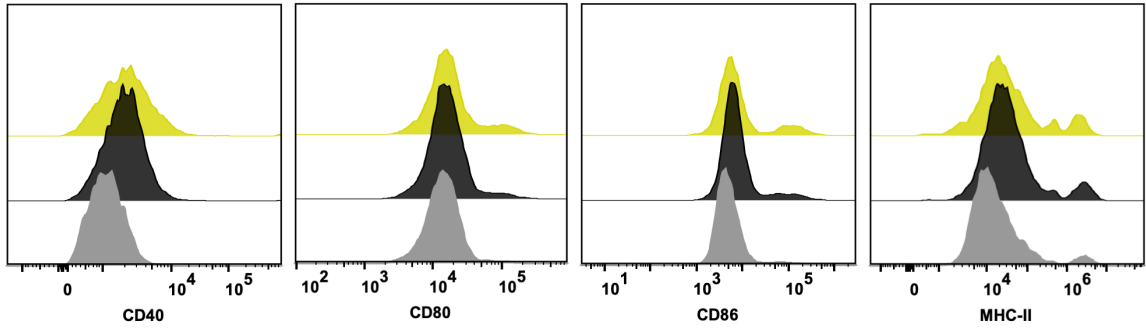


Fig. S3. Representative histograms for data presented in **Figure 4**.

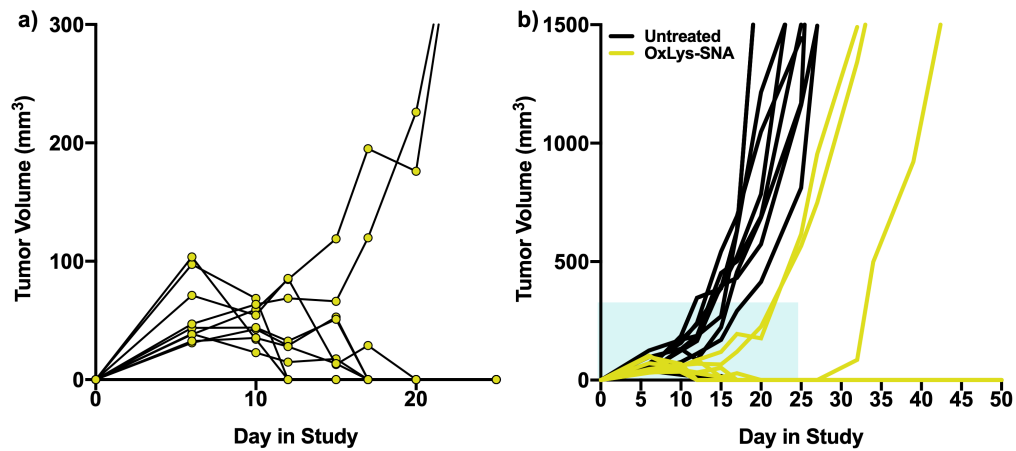


Fig. S4. (a) Individual tumor growth (spider) plots of animals administered OxLys-SNA from day 0 to day 25 post-inoculation. At day 20, all but 2 animals receiving OxLys-SNA treatment experienced complete tumor remission. (b) Spider plots of animals administered OxLys-SNA (yellow lines) or saline (black lines) out to day 50 post-inoculation. All saline-treated animals succumbed to tumor burden before the first OxLys-SNA treated animal. Blue box denotes the region in panel a. NOTE: the three animals administered OxLys-SNA that ultimately succumb to tumor burden (as depicted in the spider plots presented here) cause the average tumor growth curve presented in Figure 5c to appear as though tumors grow and then be rejected. This is a consequence of large variations in tumor volume between the non-responders and responders. This variation is eliminated when the animals are removed from the study once they pass the tumor volume survival threshold, thus causing the average tumor growth to decrease later in the study.

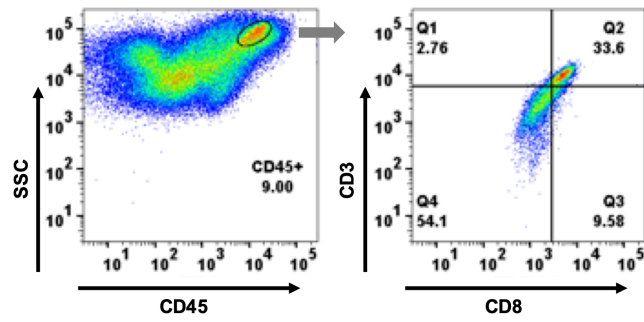


Fig. S5. Representative example of the CD8+ (cytotoxic) T cell population at the tumor site at day 11 post-inoculation.

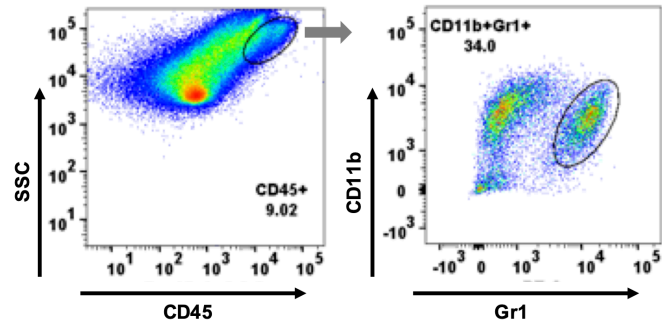


Fig. S6. Representative example of the MDSC population at the tumor site at day 11 post-inoculation.

Table S1. Zeta potential analyses of Lys-SNAs and OxLys-SNAs.

| <u>TNBC Line</u> | <u>as liposomes</u> | | <u>as SNAs</u> | |
|------------------|----------------------------|-----------------|----------------------------|-----------------|
| | <u>zeta potential (mV)</u> | <u>St. Dev.</u> | <u>zeta potential (mV)</u> | <u>St. Dev.</u> |
| Py230 | -7.05 | 1.42 | -23.45 | 2.75 |
| Py8119 | -4.28 | 3.80 | -27.83 | 1.68 |
| EMT6 | -5.37 | 1.14 | -28.01 | 1.52 |
| Ox-EMT6 | -5.66 | 1.84 | -35.25 | 0.08 |