# *Dual Toll-like Receptor Targeting Liposomal Spherical Nucleic Acids Supplemental Information*

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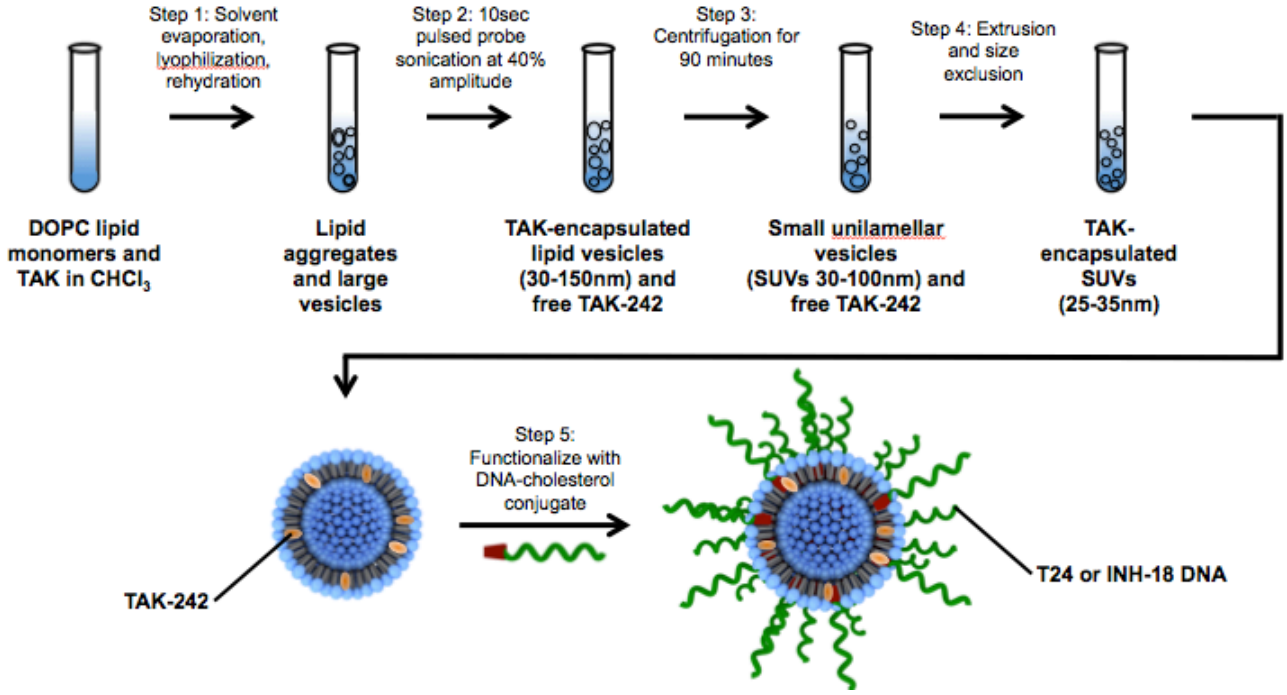
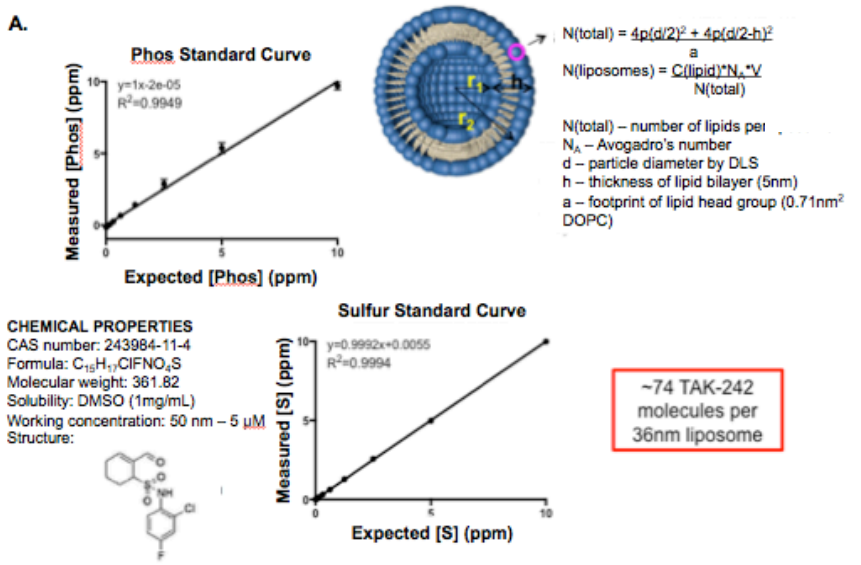


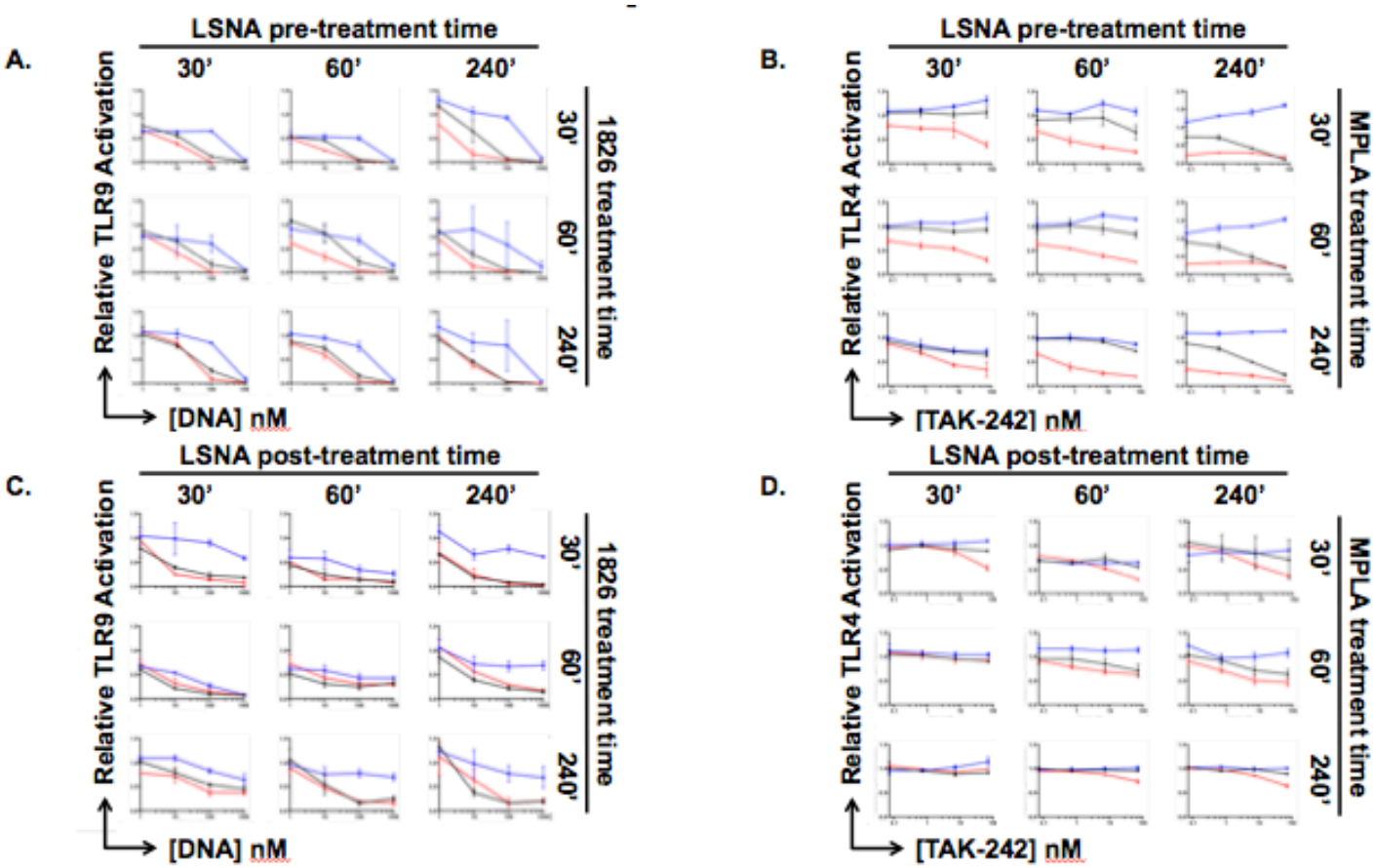

**SI Figure 1.** Synthesis steps for TAK-encapsulated LSNAs. Lipid films with or without TAK-242 were rehydrated in buffer and extruded through a series of increasingly smaller pores to created uniform, small unilamellar vesicles (SUVs). Once the desired SUV size was achieved, cholesterol-terminated DNA was mixed with SUVs, allowing functionalization of the liposome surface with oligonucleotides through hydrophobic interactions.



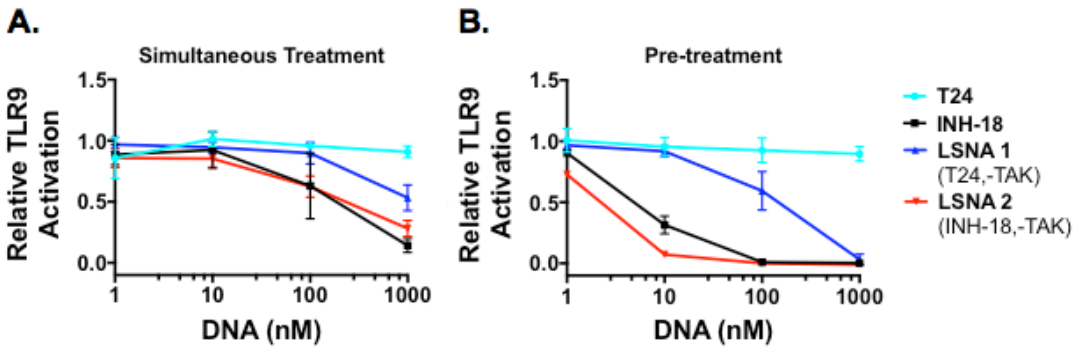

B.

| Sample | 1 hour | 8 hour |
|---|---|---|
| Pre-SEC SUV | 9.8% | 3.7% |
| Post-SEC SUV | 17.2% | 8.3% |
| Free TAK-242 | 31.2% | 56% |
| LSNA 4 | 6.4% | 8.8% |

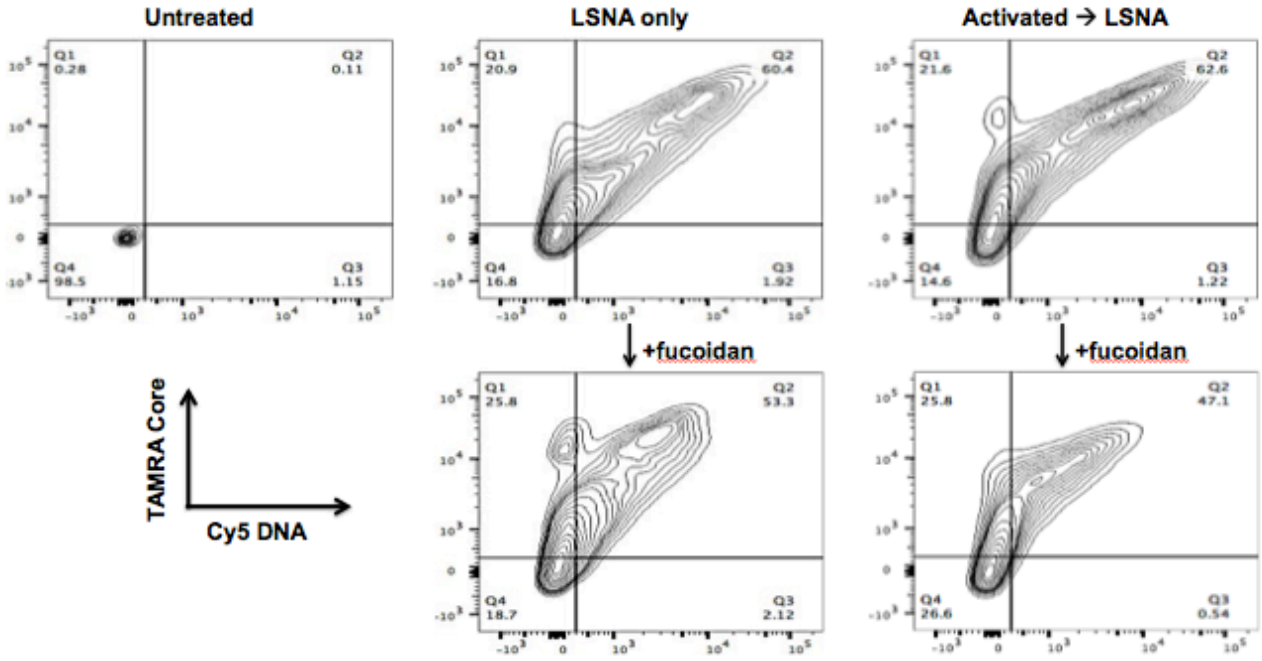
**SI Figure 2.** Example standard curves for calculating phosphorous (lipid, A) and sulfur (TAK-242, B) concentrations (A). Table shows percent loss of TAK-242 from particles during dialysis (B).



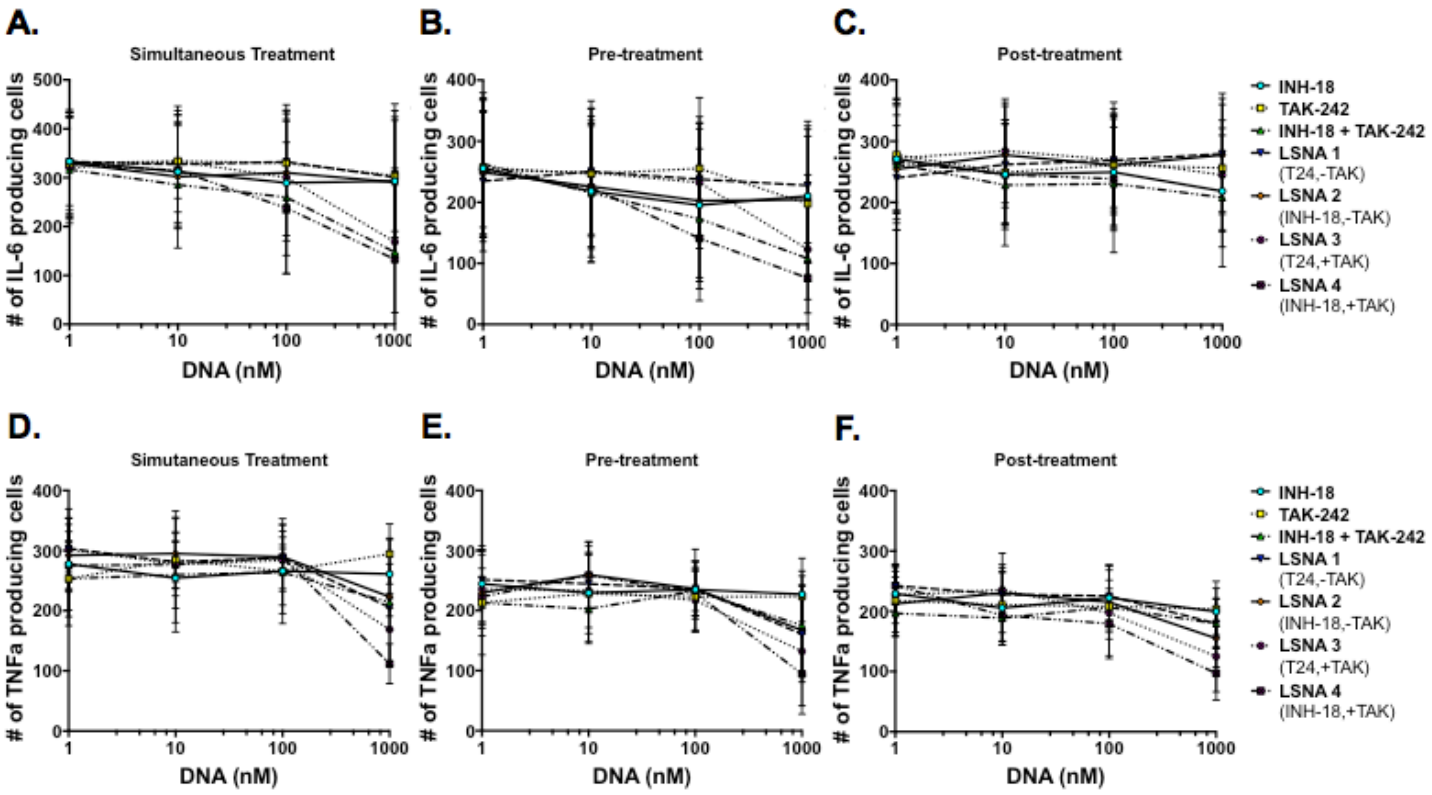

**SI Figure 3.** All LSNA pre- and post-treatment conditions. Longer exposure to either agonist or antagonists enhanced the effect of each treatment. TLR9 graphs (A,C) show the comparison between free INH-18 (black), LSNA1 (T24 LSNA, blue), and LSNA2 (INH-18 LSNA, red). TLR4 graphs (B,D) show the comparison between free TAK-242 (black), LSNA1 (T24, no TAK-242, blue), and LSNA3 (T24 with TAK-242, red).



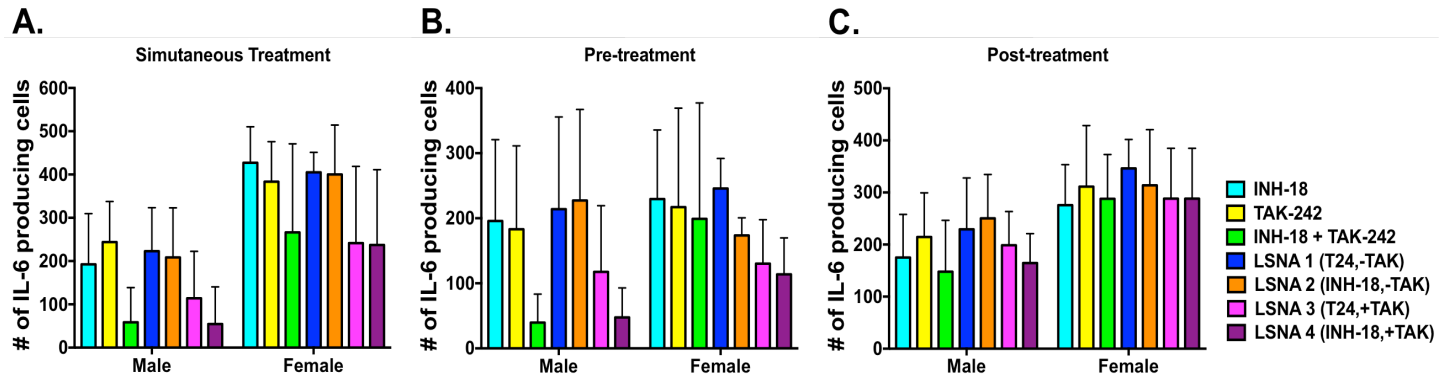

**SI Figure 4.** LSNA structure may be inherently TLR9 inhibitory. When HEK-Blue mTLR9 cells are treated with agonists and antagonists simultaneously (A), or when antagonists are used as a pre-treatment (B), the non-targeting LSNA (LSNA1) demonstrates TLR9 inhibitory activity whereas the linear T24 sequence does not.




**SI Figure 5**. Activation of thioglycollate-elicited mouse peritoneal macrophages does not affect overall LSNA uptake. Fucoidan, a scavenger receptor A inhibitor, reduces uptake of dual fluorophore-labeled LSNAs.




**SI Figure 6.** Peritoneal macrophage cytokine production over a range of inhibitor concentrations. IL-6 production during simultaneous treatment (A), pre-treatment (B), and post-treatment (C); TNFα production during simultaneous treatment (D), pre-treatment (E), and post-treatment (F).




**SI Figure 7**. IL-6 production by male and female peritoneal macrophages. Although TLR4/9-stimulated male and female peritoneal macrophages both produced similar levels of IL-6, male peritoneal macrophages seemed more sensitive to antagonist and LSNA treatment than female macrophages. This trend was observed most noticeably during simultaneous treatment and pre-treatment conditions.