Supplemental materials

Targeted immunotherapy of triple-negative breast cancer by aptamer-engineered NK cells

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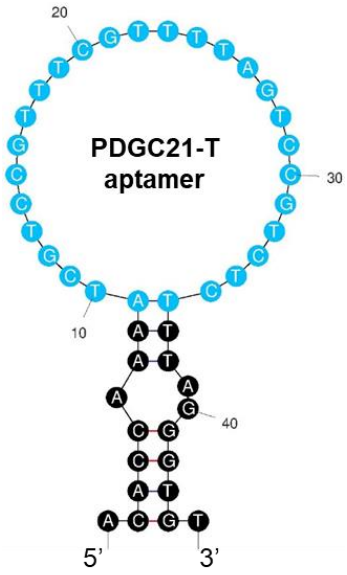

Figure S1. Sequence of PDGC21-T aptamer and its secondary structure predicted by NUPACK software (temp = 37°C; Na+ = 137 mM; Mg++ = 5.5 mM).

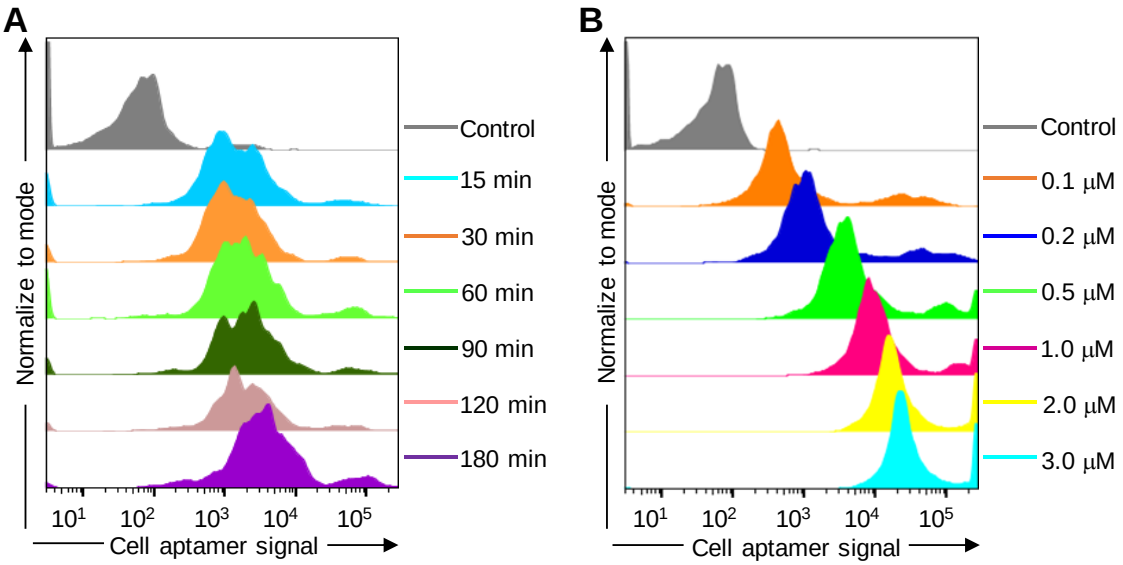

Figure S2. Optimizing ApEn-NK production conditions. **(A)** Time course study of aptamer anchoring reaction. To produce ApEn-NK, NK-92 cells were incubated with aptamer-anchor structure (1 μM final concentration) and resultant ApEn-NK cells were harvested at different time points (15 to 180 min). Cellular signals derived from surface-anchored aptamers were monitored by flow cytometry analysis. **(B)** Dose course study of aptamer-anchor structures. NK-92 cells were incubated with aptamer-anchor structures at different final concentrations ranging from 0.1 μM to 3 μM for 30 min as indicated. Resultant ApEn-NK were harvested, and cellular signals derived from surface-anchored aptamers were quantified by flow cytometry. Notably, to keep the balance of high efficacy of anchoring reaction and minimal impact on NK cell functions, ApEn-NK cells were produced with 1 μM aptamer-anchor structure (final concentration) and 30 min reaction time in this study.

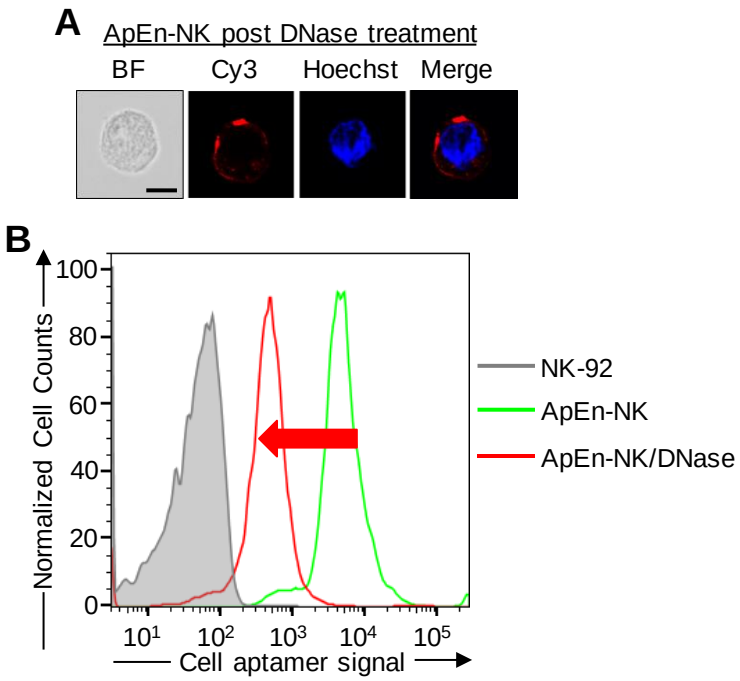

Figure S3. To validate aptamer anchoring on surface of cells, the produced ApEn-NK were treated with 250 U/mL DNase in DPBS at 37 °C for 30 min to digest aptamer sequences on the exterior of the cell membranes. Cells were then harvested and stained with Hoechst dye (blue) to highlight nuclei for cell tracking purpose. **(A)** Confocal microscopy revealed significant decrease in cellular aptamer signals (red) on the treated ApEn-NK. Scale bar: 10 μm. **(B)** Quantitative flow cytometry confirmed decrease in cellular aptamer signals post DNase treatment.

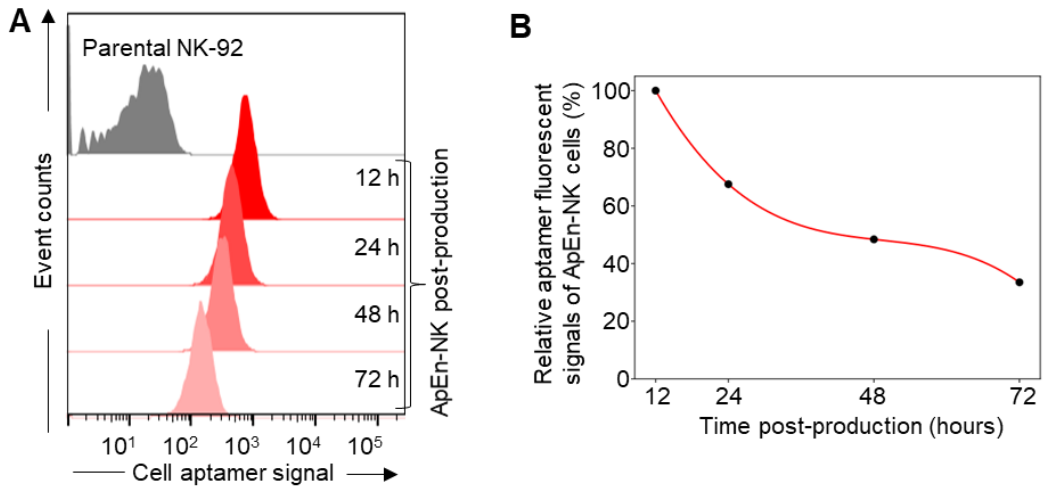

Figure S4. Additional stability assay of ApEn-NK post-production. **(A)** The surface-anchored aptamer signals of ApEn-NK cells were kinetically measured by flow cytometry up to 72 hr post-production. The parental NK-92 cells were used as the baseline control. **(B)** Previous study revealed minimal changes in cellular aptamer signals of ApEn-NK from 0 to 10 h post-production (Figure 2D). Additional time course study demonstrated that ApEn-NK products were relatively stable in culture medium containing 10% FBS at 37°C, with remaining of cellular aptamer signal 67.5% at 24 h, 48.4% at 48 h, and 33.5% at 72 h post-production. The experiments were repeated in triplicates with similar results.

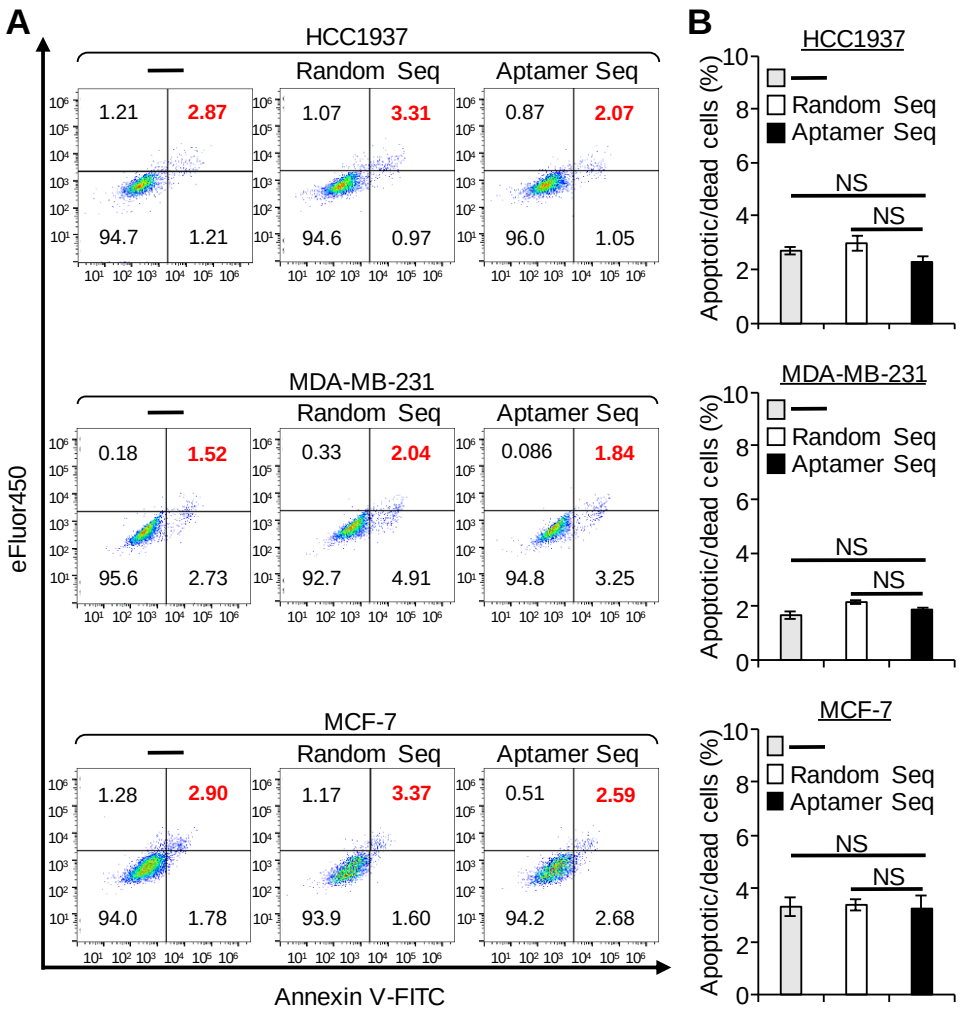

Figure S5. PDGC21-T aptamer treatment had no cellular effects on breast cancer cells. Cultured TNBC cell lines (HCC1937 and MDA-MB-231) and non-TNBC cell line (MCF-7) were treated with PDGC21-T aptamer sequence alone at 100 nM final concentration for 24 h. Random sequences were used in control group under the same condition. The treated cells were harvested and then stained with Annexin V/eFluor450 as described in Figure 5. Flow cytometry analysis demonstrated that PDGC21-T aptamer treatment had no effect on apoptosis and/or death rates (%) of TNBC or non-TNBC cells **(A)**. All experiments were repeated in triplicates and the data were shown as mean ± S.D. **(B)**. NS: not significant.