SUPPLEMENTARY INFORMATION

Simultaneous targeting of CD44 and EpCAM with a bispecific aptamer effectively inhibits intraperitoneal ovarian cancer growth

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Running Title: Suppressing ovarian cancer growth by bispecific CD44-EpCAM aptamer

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Figure S1. Effect of unpaired base linkers between aptamer and double stranded adaptor on activity of CD44-EpCAM aptamer. (A-C) schematic of CD44-EpCAM and no linker controls.(A) Linker containing CD44-EpCAM; (B) CD44-EpCAM-No linker-1, two “U”s (underlined) were added to the 3-termini of EpCAM aptamer-adaptor to pair with two “A”; (C) CD44-EpCAM-No linker-2, two “U”s were added to 3-termini of EpCAM aptamer-adaptor to pair with two “A”, and three single base AAU (blue) by which the unpaired base linker between EpCAM aptamer and double stranded adaptor were removed. (D) Comparison of the activities of single base linker-containing CD44-EpCAM aptamer and no linker controls. OVCAR8 cells were treated with varying concentrations of CD44-EpCAM aptamer and no linker controls for 72h. The removal of all single base linkers between aptamer and double stranded adaptor (CD44-EpCAM no linker-2) has resulted about 95% activity loss, slightly activity (5% cell killing activity) was detected at 4µM, and the other concentrations did not show any efficacy of cytotoxicity. The control with only removal of the linker between CD44 aptamer and double stranded adaptor (CD44-EpCAM No linker-1) showed 25% of cytotoxicity at 2µM and 53% at 4µM. The cytotoxicity of CD44-EpCAM No linker-1 is significantly lower than linker containing CD44-EpCAM which has 77% of cytotoxicity at 2µM and 95% at 4µM.
Figure S2. Evaluation of nonspecific binding of CD44-EpCAM at the concentration of 2µM. HEK293T were blocked with 500µg/ml sperm DNA, 500µg/ml yeast tRNA and 5% bovine serum albumin in TBS/0.05% tween buffer for 20min, then stained with Cy5 labeled- MG aptamer (1µM) and CD44-EpCAM (1µM or 2µM). There is no detectable difference of Cy5 intensity between 1µM and 2µM of CD44-EpCAM.