Supporting Information

High Density Lipoprotein-Mimicking Nanodiscs for Chemo-Immunotherapy against Glioblastoma Multiforme.

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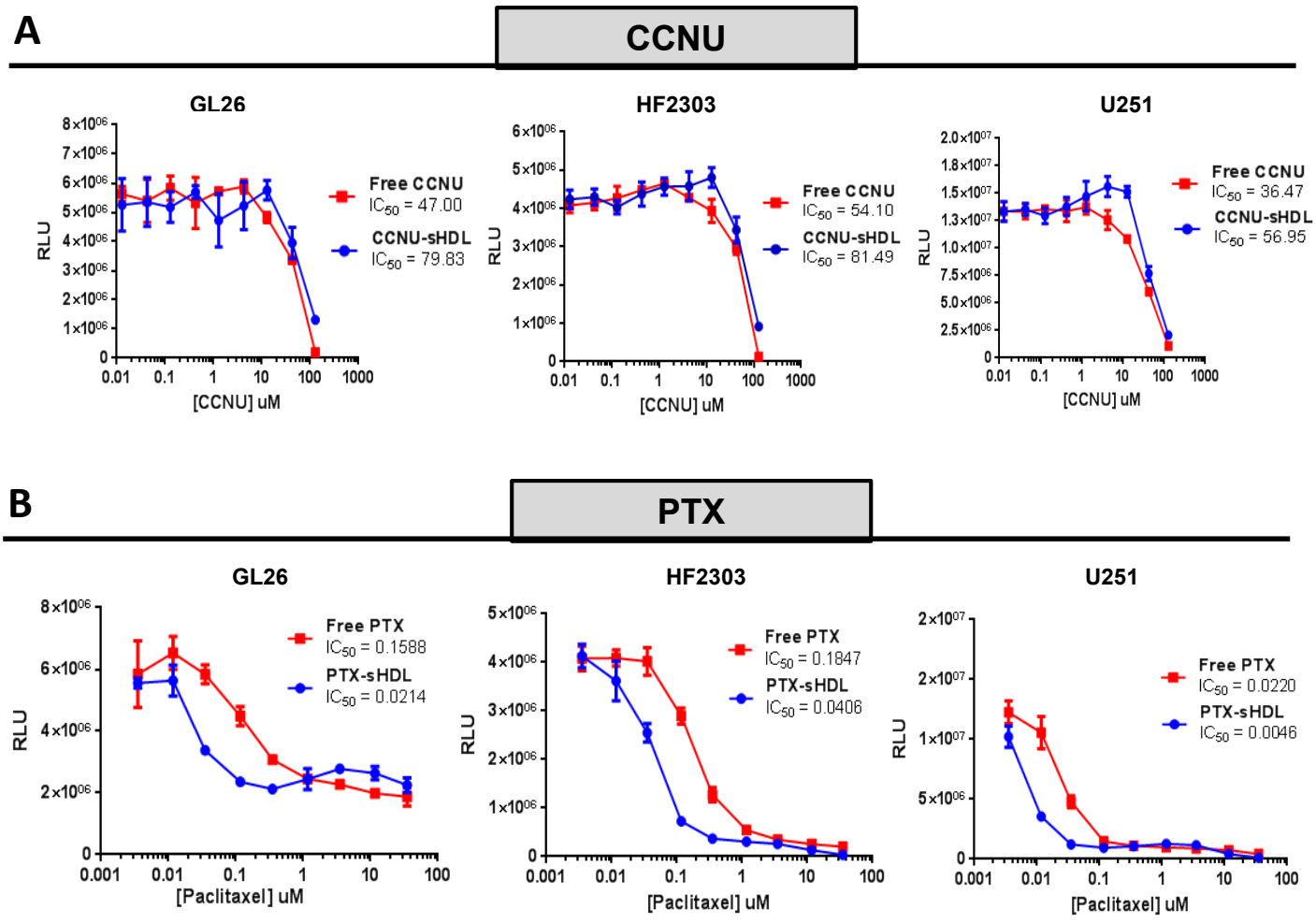
1 Supplementary Table

4 Supplementary Figures

Supplemental Table 1: Characterization Summary of different 22A sHDL-mimicking Nanodiscs.

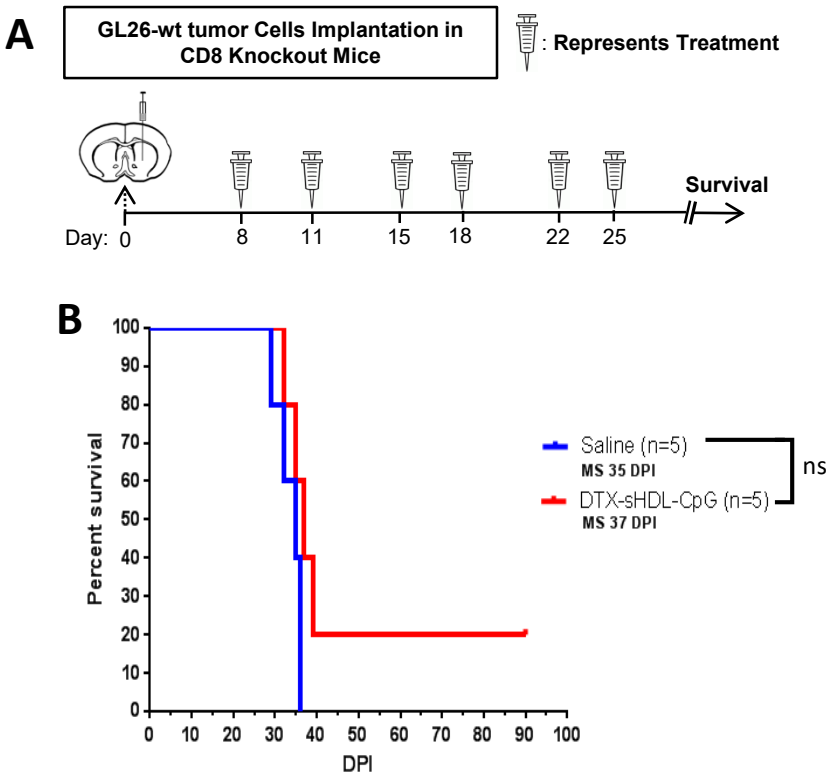
Group	Formulations (weight ratio)	RT[a] (min)	Particle Size (nm)	PDI[b]	Purity
A	22A: DMPC: POPC: PTX (1:1:1:0.06)	7.6	12.1 ± 0.3	0.07 ± 0.01	99.2%
B	22A: DMPC: POPC:DTX (1:1:1:0.06)	7.5	11.2 ± 0.4	0.15 ± 0.01	99.2%
C	22A: DMPC: POPC: CCNU (1:1:1:0.06)	7.4	10.3 ± 0.1	0.05 ± 0.02	98.5%
D	22A: SM (1:2)	7.9	9.5 ± 0.4	0.11 ± 0.01	99.0%
E	22A SM: DTX (1:2:0.05)	7.9	9.9 ± 0.1	0.07± 0.01	97.6%
F	22A: SM: DTX (1:2:0.1)	7.9	9.4 ± 0.2	0.13 ± 0.06	97.2%
G	22A: DPPC: DTX (1:1:0.1)	7.8	8.9 ± 0.1	0.15 ± 0.01	97.3%
H	22A: DPPC: SM: DTX (1:1:1:0.1)	7.8	9.4 ± 0.1	0.14 ± 0.01	98.0%
I	22A: DMPC: POPC: DTX (1:1:1:0.1)	7.4	11.1 ± 0.2	0.14 ± 0.02	99.7%
J	22A: SM: DTX: CpG (1:2:0.05:0.0075)	8.1	8.6 ± 0.1	0.13 ± 0.02	97.9%

Supplemental Figure 1



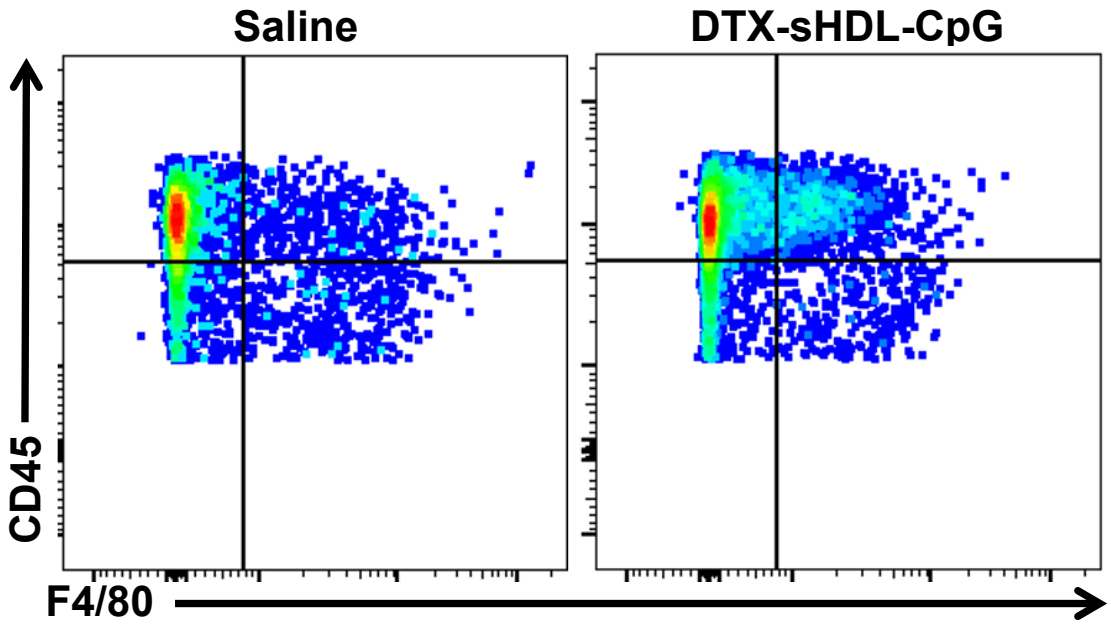
Supplemental Figure 1: Cytotoxicity of chemotherapeutic loaded HDL-mimicking nanodiscs. **(A-B)** Dose response curves for mouse (GL26) and human (HF2303, U251) glioma cells treated with free (Panel A) CCNU, (Panel B) PTX; HDLs loaded with CCNU, PTX; or empty HDLs of equivalent HDL concentration to the chemotherapeutic loaded-HDLs. Cells were incubated for 48 hours at indicated doses, then cell viability was evaluated. Bars represent $\pm$ SEM corresponding to three technical replicates.

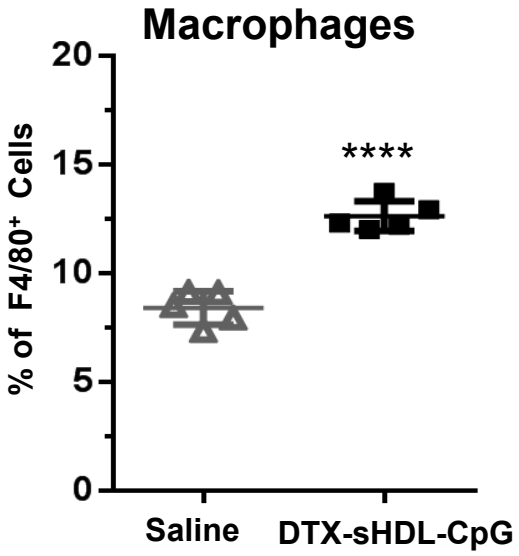
Supplemental Figure 2



Supplemental Figure 2: Intratumoral DTX-sHDL-CpG treatment for CD8 knockout mice. **(A)** CD8 knockout mice were implanted with GL26-wt tumors into the right striatum and they were treated intratumorally with saline or 0.5mg/Kg-DTX-sHDL-CpG nanodiscs on 8, 11, 15, 18, 22 and 25 days post tumor implantation. (**F**) Kaplan-Meier survival analysis of saline (n=5) or DTX-sHDL-CpG (n=5) treated CD8 knockout mice. Data were analyzed using the log-rank (Mantel-Cox) test (MS= median survival; ns= non-significant).

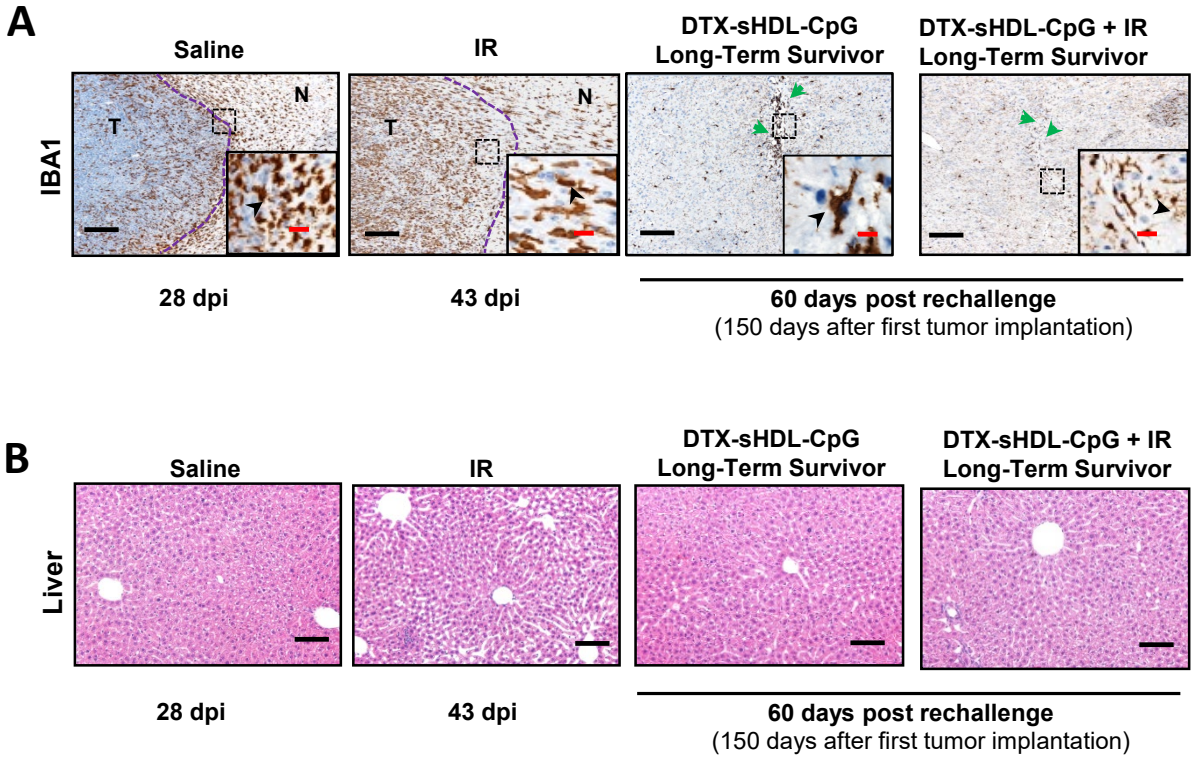
Supplemental Figure 3





Supplemental figure 3: Chemo-immunotherapy enhances macrophage responses within GBM TME. The percent of macrophages ($CD45^+/F4/80^+$) within the TME of GL26-OVA tumor bearing mice were compared between saline and sHDL-CpG-DTX treatment groups at 26 days post tumor implantation. Representative flow plots for each group are displayed. ****$p < 0.0001$; unpaired t-test. Bars represent mean ± SEM (n=5 biological replicates).

Supplemental Figure 4



Supplemental figure 4: Histopathological assessment of brains and livers from mice treated with chemo-immunotherapy. (A) Paraffin embedded 5μm brain sections from saline (28 dpi), IR (43 dpi), and long term survivors from DTX-sHDL-CpG and DTX-sHDL-CpG + IR treatment groups (60dpi after rechallenge with GL26 cells). Paraffin embedded 5μm brain sections from each treatment group were stained for IBA1. Low magnification panels show normal brain (N) and tumor (T) tissue (black scale bar = 100 μm). Green arrows in the low magnifications panels indicate scar tissue. Black arrows in the high magnification panels (red scale bar = 20 μm) indicate positive staining for the areas delineated in the low magnification panels. **(B)** H&E staining of 5μm paraffin embedded liver sections from saline (28 dpi), IR (43 dpi), and long term survivors from DTX-sHDL-CpG and DTX-sHDL-CpG + IR treatment groups (60dpi after tumor rechallenged with GL26 cells) (black scale bar =200μm).