SUPPORTING INFORMATION

Selective Transfection of a Transferrin Receptor-Expressing Cell Line with DNA-Lipid Nanoparticles

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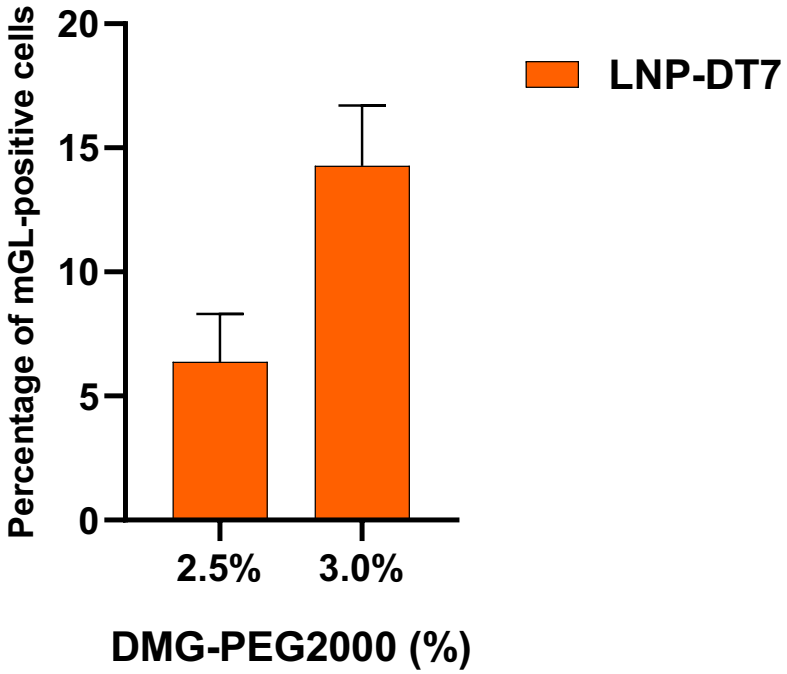

Figure S1. Percentage of mGL-positive CHO-TRVb-hTfR1 cells using two different LNP-DT7 formulations, one with 2.5% DMG-PEG2000 and the other with 3% DMG-PEG2000. CHO-TRVb-hTfR1 cells were transfected with 600 ng of mGL-pDNA encapsulated in LNP-DT7. The percentage of mGL transfected cells was evaluated by flow cytometry. Data were represented as mean ± SD of biological replicates (n = 3).

Table S1. Characterization of LNP-Mal and LNP-DT7 at different DMG-PEG2000 percentages (top section) and different N/P ratios (bottom section) showing size (intensity-weighted), PDI, ζ-potential, EE%, and yield.

Different DMG-PEG2000 percentages

		Size (nm)			PDI			ζ-Potential (mV)			EE (%)			Yield (%)		
DMG-PEG2000 %	LNP	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1	LNP-Mal	123.2	112.6	117.2	0.091	0.071	0.072	7.6	5.0	6.4	90	92	88	81	83	65
2	LNP-Mal	125.5	98.6	100.4	0.097	0.097	0.078	5.9	5.9	5.6	79	91	87	97	97	78
2.5	LNP-Mal	121.9	96.5	94.2	0.118	0.141	0.120	8.9	8.4	10.3	73	84	91	88	99	99
3	LNP-Mal	98.9	92.6	91.3	0.105	0.119	0.096	5.7	7.6	4.2	85	87	87	93	91	99
5	LNP-Mal	88.4	82.1	83.5	0.084	0.085	0.100	0.0	2.3	1.5	28	57	66	77	90	97
1	LNP-DT7	agg	agg	agg	agg	agg	agg	agg	agg	agg	agg	agg	agg	agg	agg	agg
2	LNP-DT7	176.7	158.8	154.7	0.097	0.162	0.180	-6.8	-6.7	-6.8	61	86	93	82	74	75
2.5	LNP-DT7	177.0	161.3	172.5	0.115	0.150	0.187	-9.5	-8.3	-8.1	66	72	78	83	80	84
3	LNP-DT7	158.2	149.0	148.2	0.099	0.176	0.161	-6.0	-3.1	-6.6	80	79	81	83	88	96
5	LNP-DT7	136.6	145.8	149.6	0.070	0.051	0.039	-8.5	-7.4	-8.8	28	46	50	79	81	91

Different N/P ratios keeping DMG-PEG2000 at 3%

		Size (nm)			PDI			ζ-Potential (mV)			EE (%)			Yield (%)		
N/P ratio	LNP	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
4	LNP-Mal	106.2	103.3	98.3	0.105	0.096	0.076	4.1	2.2	5.6	45	73	71	93	96	90
6	LNP-Mal	98.9	92.6	91.3	0.105	0.119	0.096	5.7	7.6	4.2	85	87	87	93	91	99
8	LNP-Mal	105.1	88.1	84.8	0.138	0.125	0.121	3.1	3.1	3.1	69	88	86	74	97	98
10	LNP-Mal	98.8	82.1	85.6	0.140	0.112	0.142	7.1	5.5	4.6	73	87	88	69	92	97
4	LNP-DT7	210.2	218.3	199.0	0.302	0.182	0.174	-7.2	-9.1	-10.3	41	61	63	88	79	80
6	LNP-DT7	158.2	149.0	148.2	0.099	0.176	0.161	-6.0	-3.1	-6.6	80	79	81	83	88	96
8	LNP-DT7	172.6	133.5	144.7	0.091	0.215	0.200	-5.2	-8.3	-9.1	64	79	79	67	76	80
10	LNP-DT7	150.8	110.9	113.9	0.152	0.288	0.235	-5.8	-4.3	-4.0	68	86	82	59	83	99

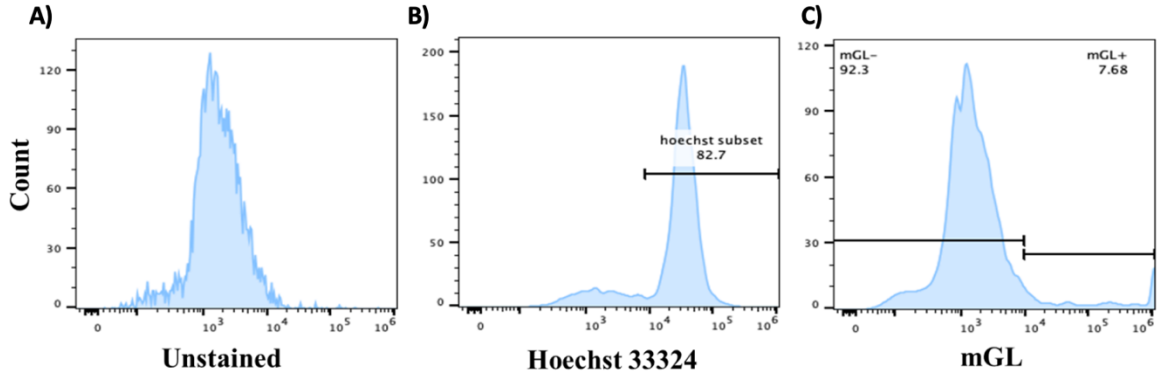

Figure S2. Flow cytometry gating strategy to determine the percentage of mGL-positive cells in the *in vitro* transfection experiments. A) Histogram of unstained CHO-TRVb (-neo or -hTfR1) cells. B) The Hoechst-positive population was identified (FL9: filter 450/40), and the gating strategy was established by counting 2000 events. C) mGL-positive cells (FL1: 525/40) were pre-gated with Hoechst 33324.

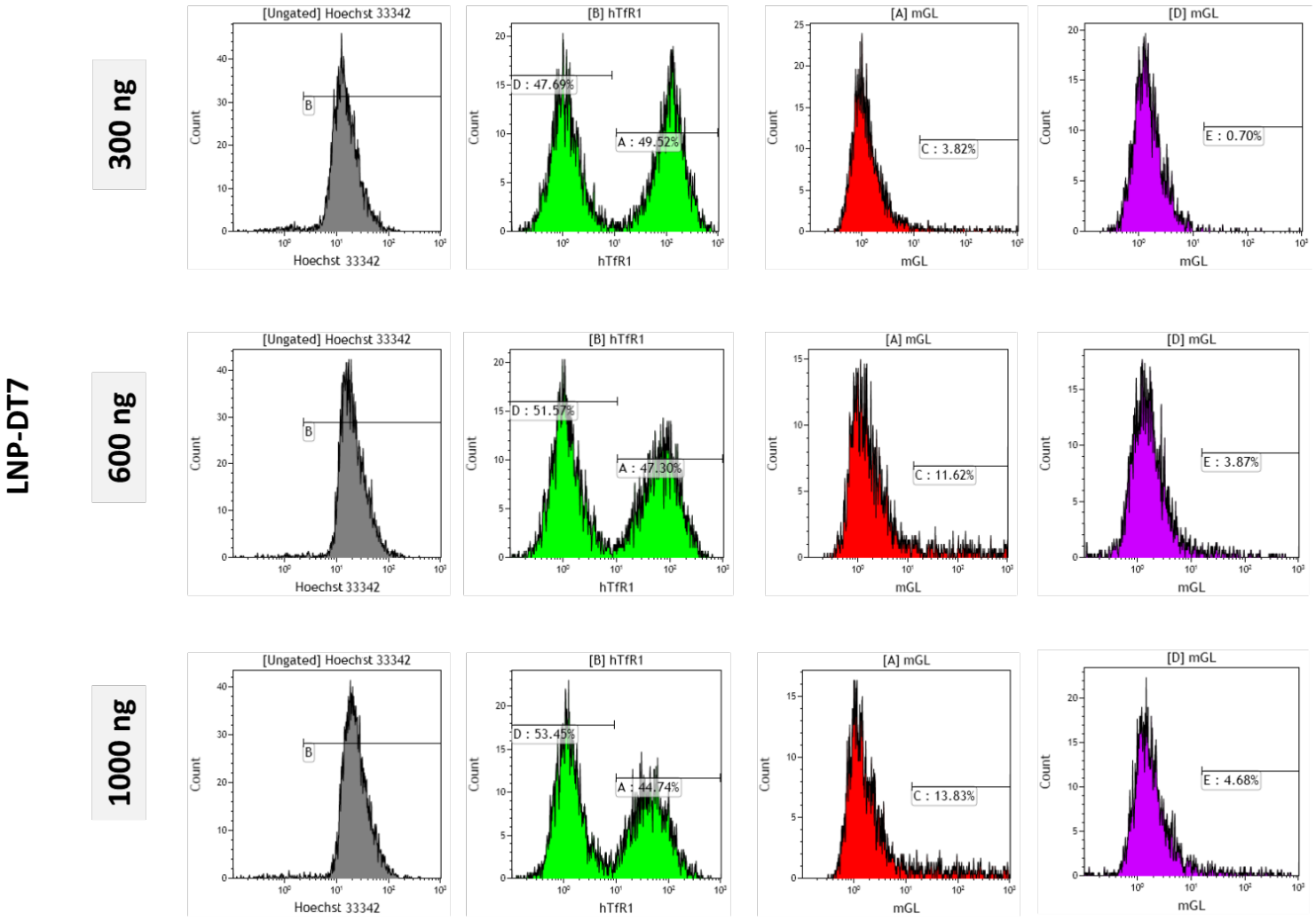

Figure S3. Representative flow cytometry data for the transfection experiments using a 1:1 mixture of CHO-TRVb-neo and CHO-TRVb-hTfR1 cells at a pDNA concentration of 300, 600, and 1000 ng/well encapsulated in LNP-DT7. A total of 5000 events were acquired in Gallios Beckman Coulter. The cells were first gated in Hoechst 33342 positive cells, a second gate on the expression of the hTfR1 receptor detected by the fluorescent mouse anti-hTfR1 (CD71) monoclonal antibody (OKT9 (OKT-9)), PerCP-eFluorTM 710, eBiosciencesTM, and followed by a third gate on the expression of the mGL.

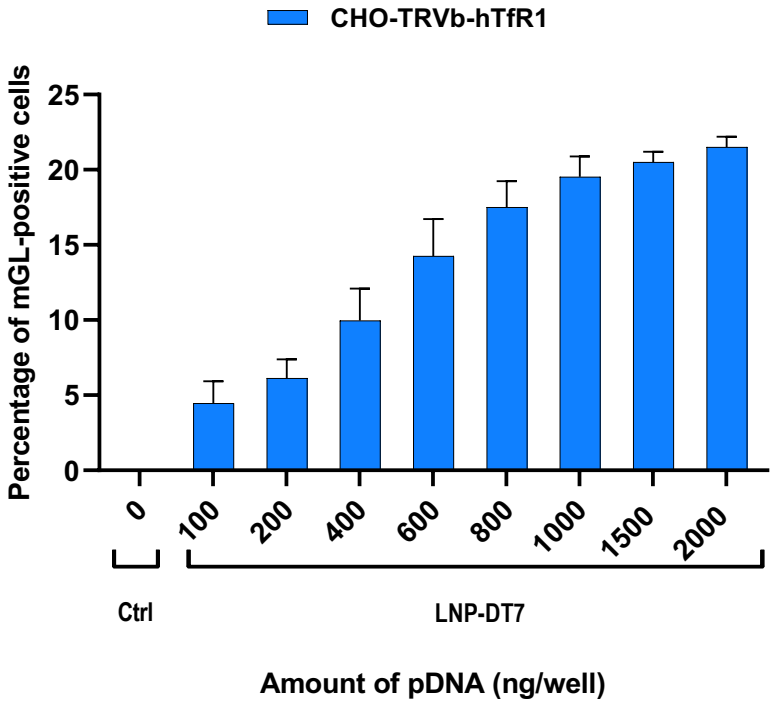

Figure S4. Percentage of mGL-positive CHO-TRVb-hTfR1 cells at different concentrations of pDNA encapsulated in LNP-DT7. CHO-TRVb-hTfR1 cells transfected with 0, 100, 200, 400, 600, 800, 1000, 1500, and 2000 ng of mGL-pDNA encapsulated in LNP-DT7. The percentage of mGL transfected cells was evaluated by flow cytometry. Data were represented as mean ± SD of biological replicates (n = 3 for 0, 100, 200, 400, 600, 800, 1000; and n = 2 for 1500 and 2000).

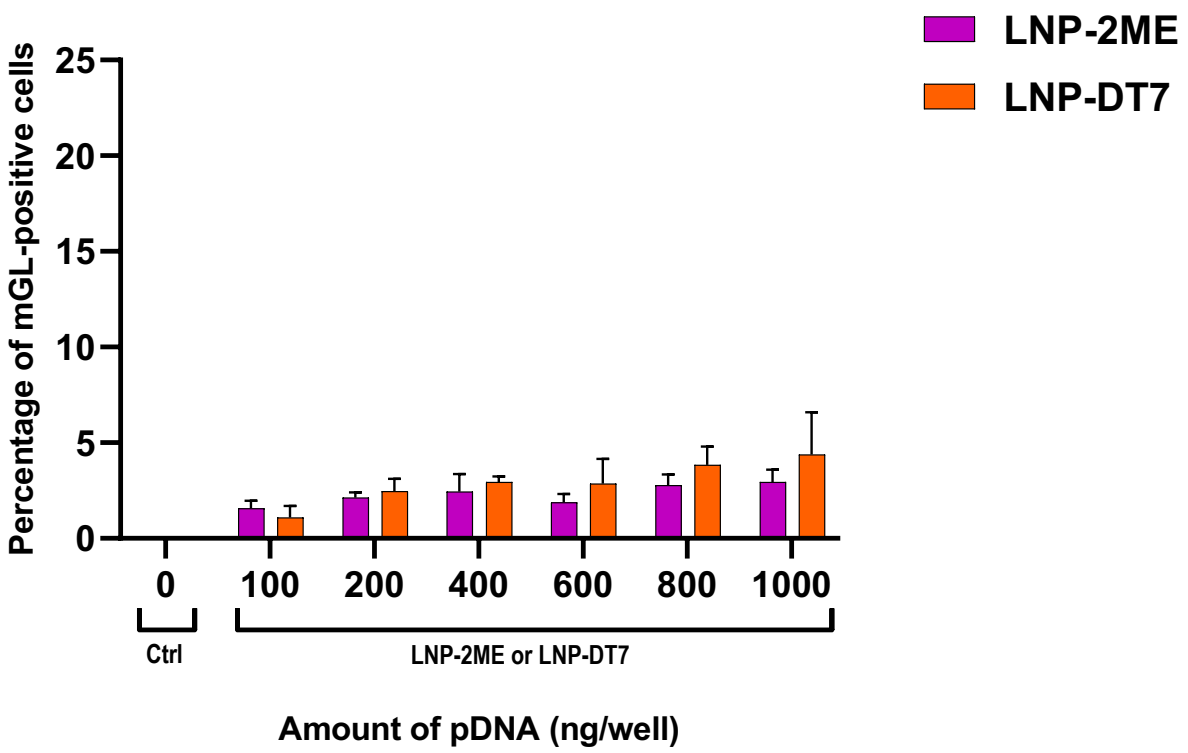

Figure S5. Percentage of mGL-positive CHO-TRVb-neo cells at different concentrations of pDNA encapsulated in LNP-2ME and LNP-DT7. CHO-TRVb-neo cells were transfected with 0, 100, 200, 400, 600, 800, and 1000 ng of mGL-pDNA encapsulated in LNP-2ME (non-targeting LNPs) and LNP-DT7 (active targeting LNPs). The percentage of mGL transfected cells was evaluated by flow cytometry by counting 10,000 events.

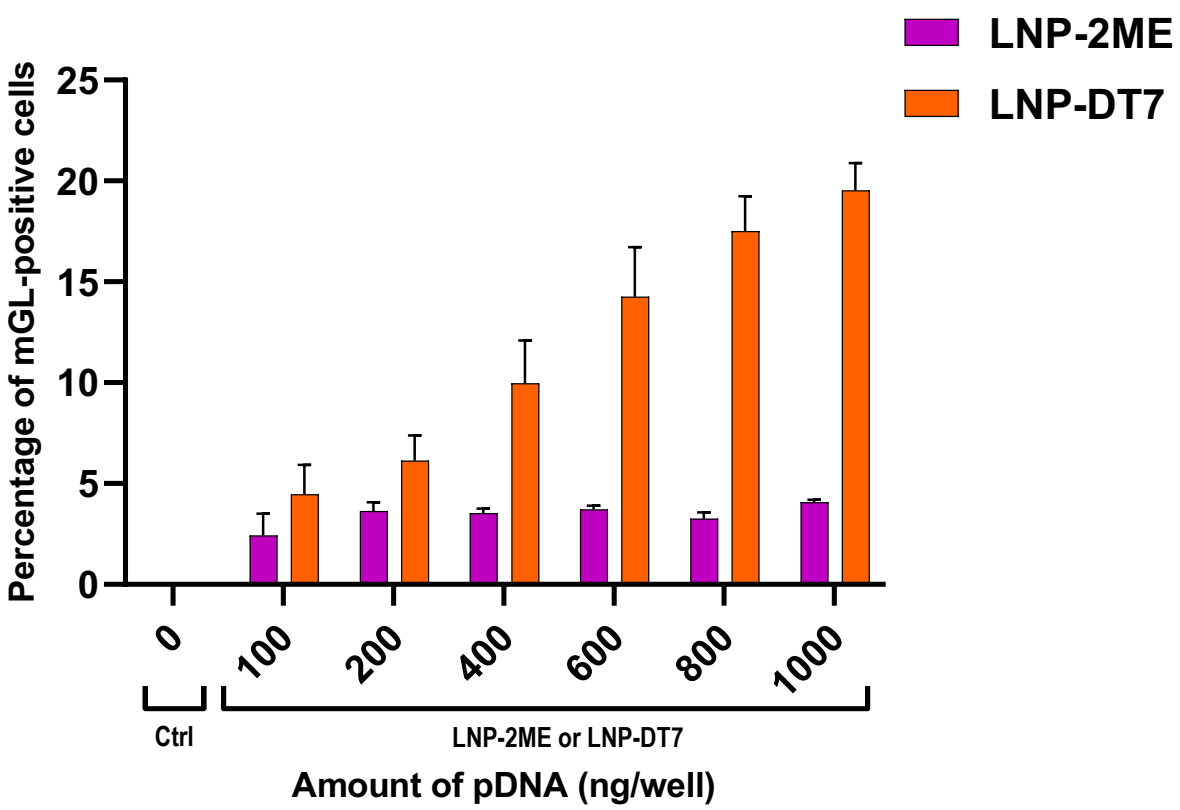

Figure S6. Percentage of mGL-positive CHO-TRVb-hTfR1 cells at different concentrations of pDNA encapsulated in LNP-2ME and LNP-DT7. CHO-TRVb-hTfR1 cells were transfected with 0, 100, 200, 400, 600, 800, and 1000 ng of mGL-pDNA encapsulated in LNP-2ME (non-targeting LNPs) and LNP-DT7 (active targeting LNPs). The percentage of mGL transfected cells was evaluated by flow cytometry by counting 10,000 events.