

## Supporting Information to

### ***In vivo* delivery of plasmid DNA by lipid nanoparticles: the influence of ionizable cationic lipids on organ-selective gene expression**

Azizah Algarni<sup>1</sup>, Emily H. Pilkington<sup>1</sup>, Estelle J. A. Suys<sup>1</sup>, Hareth Al-Wassiti<sup>1</sup>, Colin W. Pouton<sup>1,\*</sup> and Nghia P. Truong<sup>1,\*</sup>

<sup>1</sup> *Department of Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC 3052, Australia*

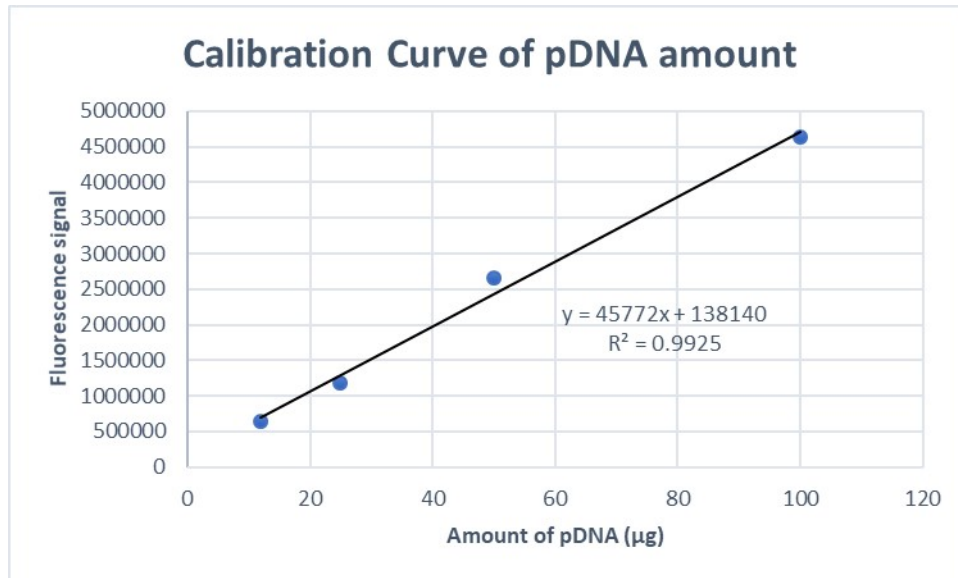
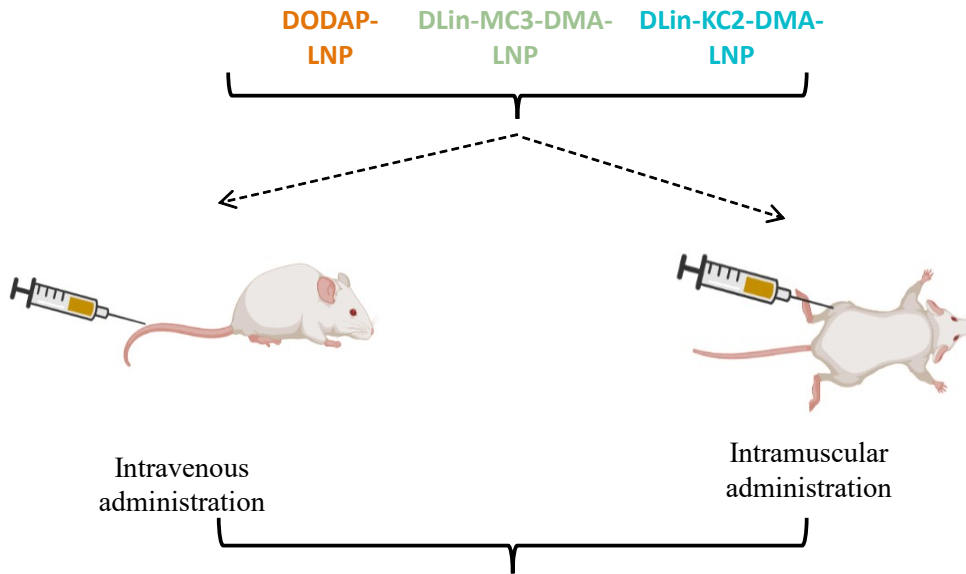


Figure S1: pDNA calibration curve for quantifying the amount of pDNA release used in a model endosome escape assay.

Table S1. DNA release data used for characterizing endosome escape.

LNPs	pH 5.5				pH 7.4				Normalised pDNA ( $\mu\text{g}$ )
	Well 1	Well 2	Well 3	Average pDNA ( $\mu\text{g}$ )	Well 1	Well 2	Well 3	Average pDNA ( $\mu\text{g}$ )	
DODAP	818235	924716	831596	15.7	911757	765569	779916	14.9	0.9
DLin-MC3-DMA	1002148	1142357	1105145	20.6	369077	335467	310735	4.4	16.3
DLin-KC2-DMA	2022236	1801152	2072839	38.7	291778	306796	293450	3.5	35.3

## A) Administration of LNPs



## B) Luminescence quantification and imaging

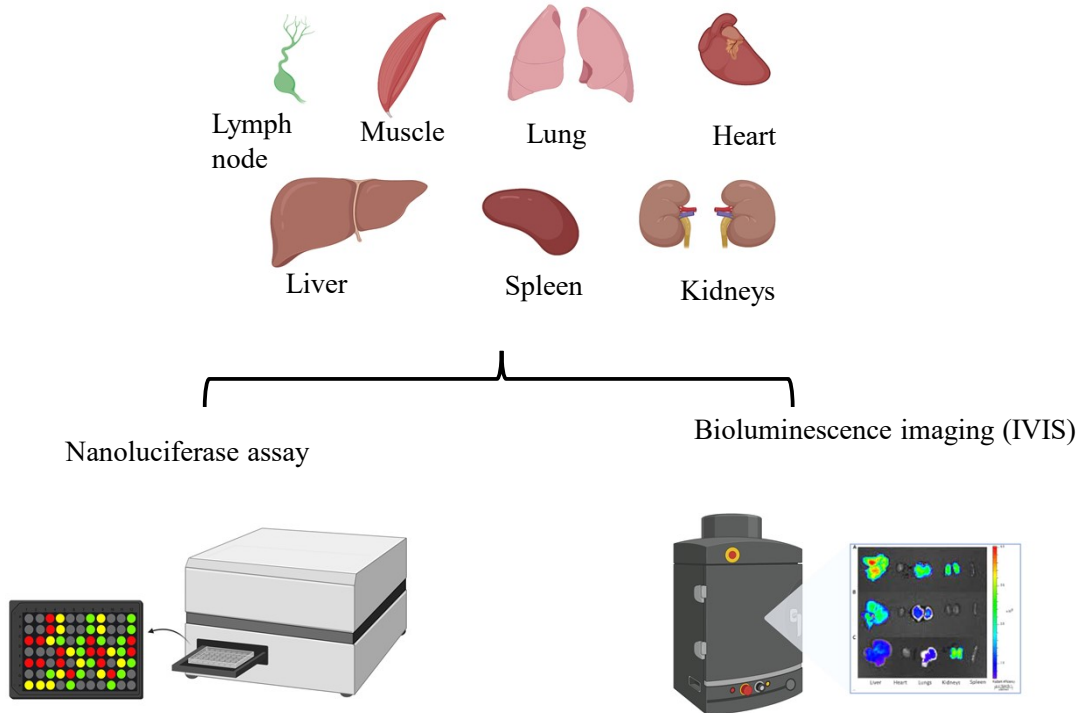


Figure S2. Schematic of lipid nanoparticles (LNPs) encapsulating Nanoluciferase encoding plasmid (pNL 1.1 CMV) for *in vivo* delivery screening. (A) Different LNP formulations were administered intravenously and intramuscularly to Balb/c mice. (B) Organs were harvested 24 h post-injection, and nLuc plasmid delivery and expression were quantified using both Nanoluciferase assay and whole-organ luminescence imaging.

Table S2. Physicochemical properties and encapsulation efficiency of pDNA LNPs.

N/P ratio	<b>DODAP</b>			
	<b>Mean size (nm)</b>	<b>PDI</b>	<b>ZP (mV)</b>	<b>EE%</b>
<b>3</b>	176 ± 17	0.2 ± 0.1	-36.9 ± 4	54 ± 10
<b>6</b>	122.4 ± 2.4	0.1 ± 0.02	-13.5 ± 3	97 ± 2
<b>9</b>	113.6 ± 3	0.1 ± 0.03	-10 ± 4	95 ± 4
	<b>DLin-MC3-DMA</b>			
	<b>Mean size (nm)</b>	<b>PDI</b>	<b>ZP (mV)</b>	<b>EE%</b>
<b>3</b>	156 ± 12	0.3 ± 0.1	-34.6 ± 5	55 ± 3.2
<b>6</b>	135 ± 1.5	0.1 ± 0.03	-12 ± 5	97 ± 2
<b>9</b>	123.9 ± 3	0.1 ± 0.05	-9 ± 3.7	97 ± 3
	<b>DLin-KC2-DMA</b>			
	<b>Mean size (nm)</b>	<b>PDI</b>	<b>ZP (mV)</b>	<b>EE%</b>
<b>3</b>	166.8 ± 19	0.5 ± 0.1	-27 ± 4	77 ± 7.3
<b>6</b>	120.4 ± 5	0.1 ± 0.02	-13.9 ± 3	95 ± 5
<b>9</b>	104.9 ± 3	0.1 ± 0.02	-7 ± 3	94 ± 4

Table S3. Nano luciferase protein expression/mg of tissues following IM administration of nLuc pDNA LNPs.

	<b>DODAP</b>		<b>DLin-MC3-DMA</b>		<b>DLin-KC2-DMA</b>	
	<b>RLU/mg</b>	<b>SD</b>	<b>RLU/mg</b>	<b>SD</b>	<b>RLU/mg</b>	<b>SD</b>
<b>DLN</b>	6809	1278	94761	17345	132350	74969
<b>non-DLN</b>	1705	1838	14297	3903	79637	13277
<b>Muscle</b>	240776	44265	307453	56326	691888	64440
<b>Heart</b>	704	330	1070	133	3550	3338
<b>Lung</b>	372	47	361	39	3745	2974
<b>Liver</b>	3296	1037	3296	3514	13782	11933
<b>Spleen</b>	437	47	5315	2000	22050	13047
<b>Kidneys</b>	805	283	1761	407	12525	6658

Table S4. Nano luciferase protein expression/mg of tissues following IV administration of nLuc pDNA LNPs

	<b>DODAP</b>		<b>DLin-MC3-DMA</b>		<b>DLin-KC2-DMA</b>	
	<b>RLU/mg</b>	<b>SD</b>	<b>RLU/mg</b>	<b>SD</b>	<b>RLU/mg</b>	<b>SD</b>
<b>Inguinal Lymph Node</b>	629	234	1035	458	12623	2280
<b>Heart</b>	472	180	2304	740	3353	1632
<b>Lung</b>	546	284	785	239	245	243
<b>Liver</b>	11935	8246	5936	4226	37260	16332
<b>Spleen</b>	5023	3887	24357	9804	69827	10536
<b>kidney</b>	607	240	4091	1553	8627	1886