

Base-modified Factor VIII mRNA delivery with Galactosylated lipid nanoparticles as a protein replacement therapy for Haemophilia A

Porkizhi Arjunan^{a,b#}, Gokulnath Mahalingam^{a#}, Priyanka Sankar^{a#}, Durga Kathirvelu^a,
Sevanthy Suresh^a, Sandya Rani^a, Kumarasamypet M Mohankumar^a, Saravanabhavan
Thangavel^a, Srujan Marepally^{a*}

a Centre for Stem Cell Research (CSCR) (a unit of inStem, Bengaluru), CMC Campus, Vellore,
632002, TN, India

b Manipal Academy of Higher Education, Manipal, Karnataka 576104, India.

PA, GM, and PS contributed equally to this work

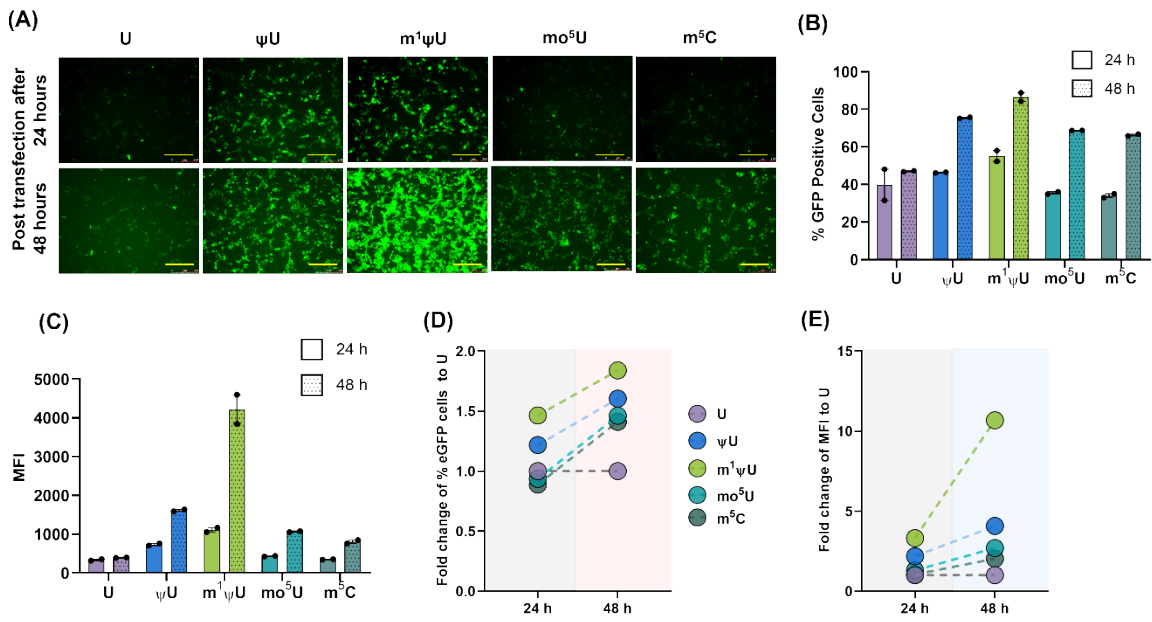


Figure S1: Functional analysis of unmodified and base modified eGFP mRNAs in HEK-293T cell lines. After transfection in HEK-293T cells, depth of translation efficiencies between unmodified and base modified eGFP mRNAs were determined by fluorescent microscopy and the scale bar indicates 250 μm (A) and % of eGFP positive cells (B) and MFI (C) were quantified by flow cytometry at 24 and 48 hours. Fold change of % eGFP positive cells (D) and MFI (E) between unmodified and base modified of eGFP mRNAs. (n = 2); (statistical significance compared to U-eGFP mRNA given in Table S1).

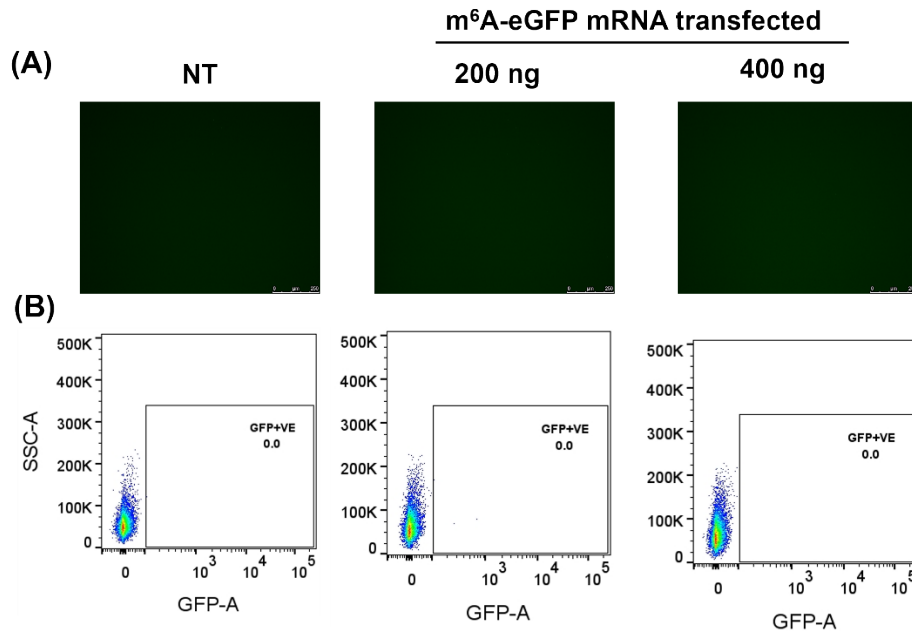


Figure S2: Transfection of m⁶A eGFP mRNA in HEK-293T cell line (A) Fluorescent images and (B) GFP positive population (analysed by flow cytometry) of non-transfected (NT) HEK-293T cells in comparison with HEK-293T cells transfected with m⁶A eGFP mRNA 2 days post-transfection.

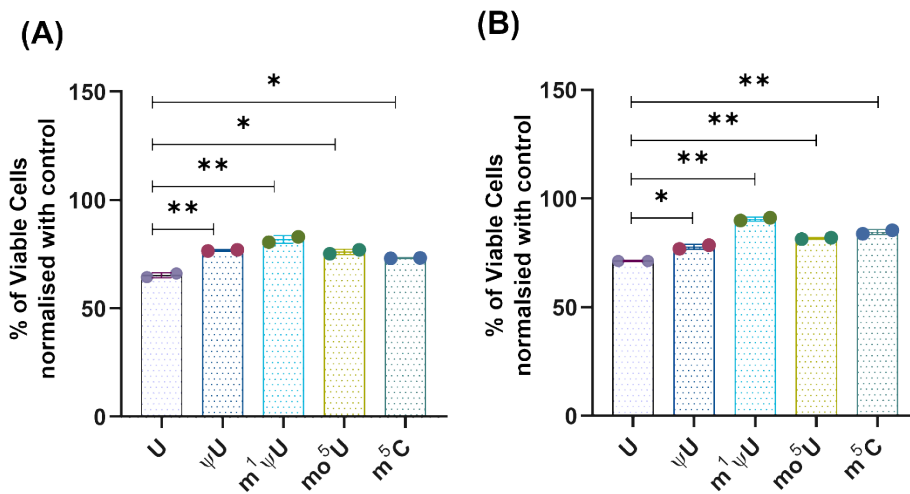


Figure S3: Toxicity levels of each base-modified eGFP mRNAs were analysed in SK-HEP-1 (A) and Hepa 1-6 (B) cell lines by MTT assay after 48 hours post-transfection. * $p \leq 0.05$ ** $p \leq 0.01$ compared with U-eGFP mRNA.

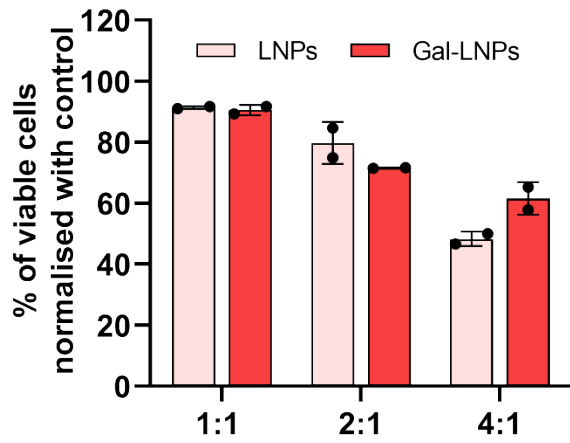


Figure S4: Toxicity levels of LNP and Gal-LNP in SK-HEP-1 cell lines by MTT assay after 48 hours post-transfection (n=2).

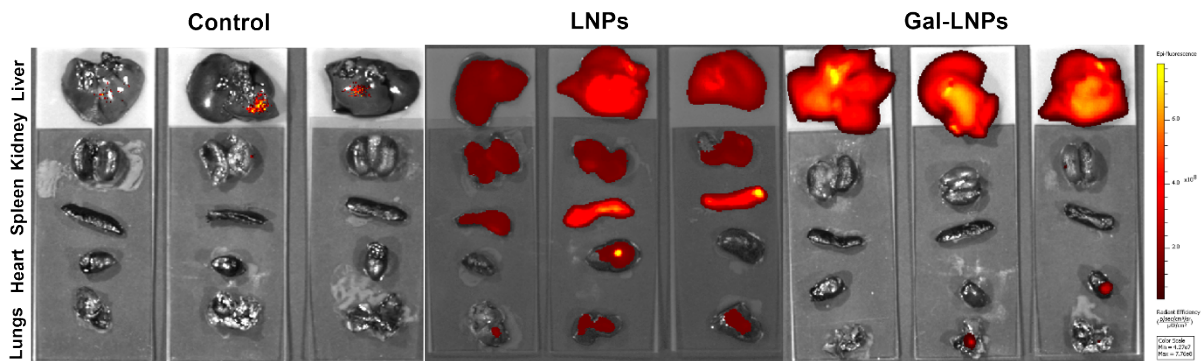


Figure S5: Localisation of LNP and Gal-LNP in mice evaluated by *In-Vivo* Imaging System (IVIS) (n=3).

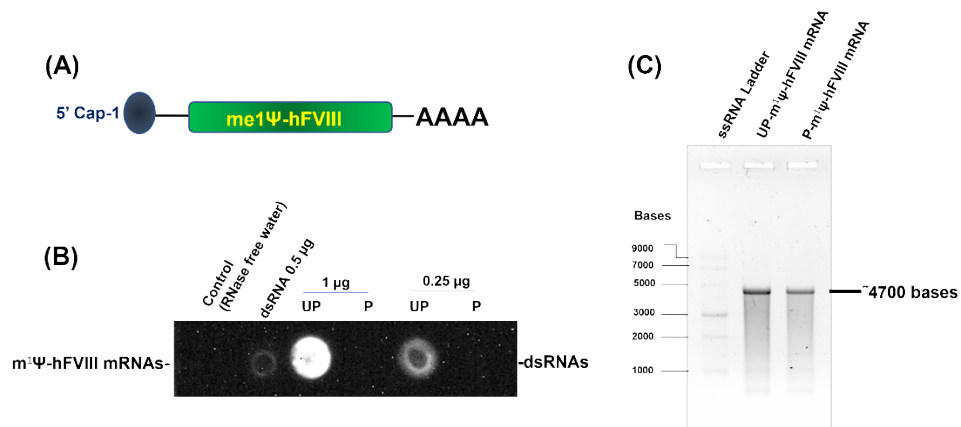


Figure S6: Schematic diagram of m¹ΨU- modified FVIII mRNA (A). The RNA Dot-Blot analysis of purified m¹ΨU- modified FVIII mRNAs (B). The integrity of synthesized (unpurified, UP) and dsRNA removed (purified, P) m¹ΨU- modified FVIII mRNAs were analysed in RNA agarose electrophoresis (C).

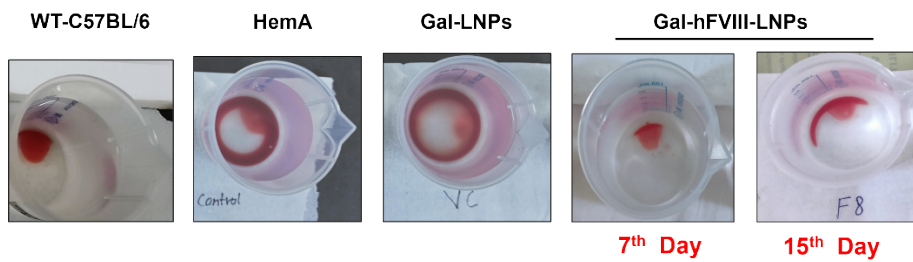


Figure S7: Photographic images of tail vein transverse assay.

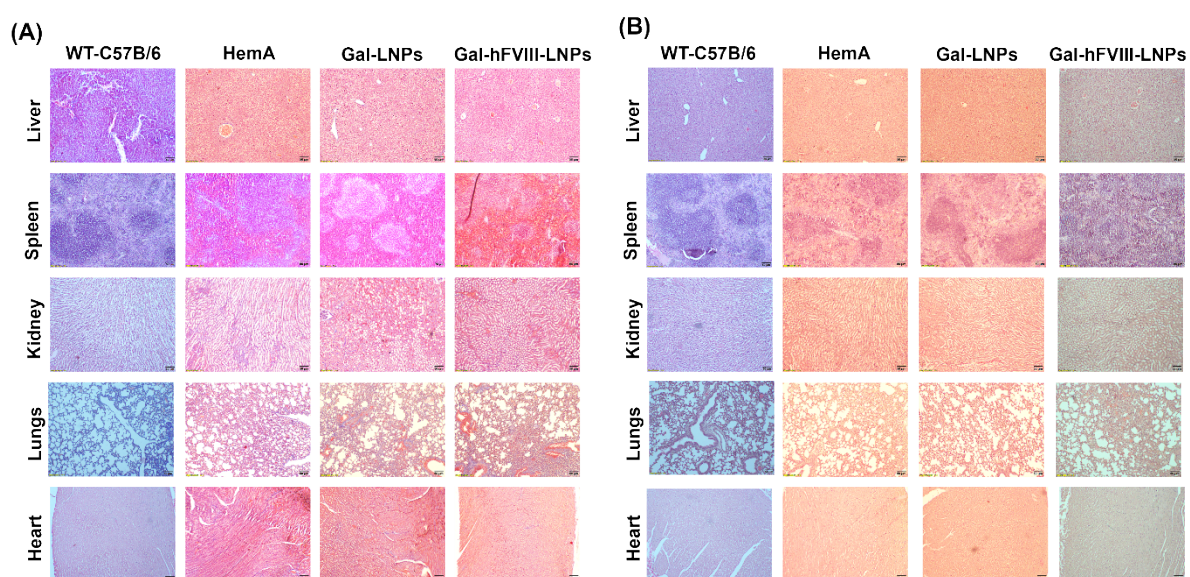


Figure S8: Mason trichrome staining of WT-C57B/6, HemaA, Gal-LNPs -Vehicle control (VC) and Gal-hFVIII-LNPs (A) Haemoxyl and Eosin staining (B).

Post-Transfection After	24 hrs				48 hrs			
	Ψ U	$m^1\Psi$ U	mo^5 U	m^5 C	Ψ U	$m^1\Psi$ U	mo^5 U	m^5 C
% GFP Positive Cells (Fig. S1B)	NS	NS	NS	NS	***	**	***	***
Fold Change of % GFP Positive Cells (Fig. S1D)	NS	NS	NS	NS	****	**	***	**
MFI (Fig. S1C)	**	**	*	NS	***	**	***	*
Fold Change of MFI (Fig. S1E)	*	*	NS	NS	****	*	****	**

Table S1: Statistical significance computed using Multiple t-tests for HEK-293T cells transfected with base modified eGFP mRNAs. Each data point was compared with corresponding data for Unmodified (U) eGFP mRNAs. (n = 2); NS – Non-Significant $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$). Related to Figure S1.

Post-Transfection After	24 hrs				48 hrs			
	Ψ U	m ¹ Ψ U	mo ⁵ U	m ⁵ C	Ψ U	m ¹ Ψ U	mo ⁵ U	m ⁵ C
% GFP Positive Cells (Fig. 2B)	*	**	**	*	**	***	***	*
Fold Change of % GFP Positive Cells (Fig. 2D)	*	***	**	*	*	*	*	*
MFI (Fig. 2C)	*	***	NS	*	**	****	NS	*
Fold Change of MFI (Fig. 2E)	NS	**	***	*	***	****	*	*

Table S2: Statistical significance computed using Multiple t-tests for SK-HEP-1 cell lines transfected with modified eGFP mRNAs. Each data point was compared with corresponding data for U-eGFP mRNA. (n = 2); NS - Non-Significant p > 0.05, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001). Related to Figure 2.

Post-Transfection After	24 hrs				48 hrs			
	Ψ U	m ¹ Ψ U	mo ⁵ U	m ⁵ C	Ψ U	m ¹ Ψ U	mo ⁵ U	m ⁵ C
% GFP Positive Cells (Fig. 3B)	NS	*	NS	NS	**	***	*	**
Fold Change of % GFP Positive Cells (Fig. 3D)	*	NS	NS	NS	**	**	**	****
MFI (Fig. 3C)	**	**	NS	*	**	***	*	**
Fold Change of MFI (Fig. 3E)	*	***	NS	****	**	***	**	***

Table S3: Statistical significance computed using Multiple t-tests for Hepa 1-6 cell lines transfected with modified eGFP mRNA. Each data point was compared with corresponding data for U-eGFP mRNA. (n = 2); NS - Non-Significant p > 0.05, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001). Related to Figure 3.