

Supplementary Materials for
**Peptide-guided lipid nanoparticles deliver mRNA to the neural retina of
rodents and nonhuman primates**

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Sci. Adv. **9**, eadd4623 (2023)
DOI: 10.1126/sciadv.add4623

The PDF file includes:

Figs. S1 to S6
Legend for table S1

Other Supplementary Material for this manuscript includes the following:

Table S1

A

Peptide sequence of top candidates against 661w cells

661w Top five peptide candidates

Peptide Number slide	One letter code	Linker seq	Three-letter (targeting sequence)	Color	pI (Isoelectric point)
42	SPALHFL	GGGS[C]	SerProAlaLeuHisPheLeu	Orange	6.63
43	SNLAAFP	GGGS[C]	SerAsnLeuAlaAlaPhePro	Cyan	5.02
50	MPVAVYR	GGGS[C]	MetProValAlaValTyrArg	Blue	7.25
52	LAFHRMP	GGGS[C]	LeuAlaPheHisArgMetPro	Red	7.4
54 (54)	HLSSLTP	GGGS[C]	HisLeuSerSerLeuThrPro	Yellow	6.63

B

Peptide Name	Acidic	Basic	Neutral	Hydrophobic
MH42	0%	9%	45%	46%
MH43	0%	0%	55%	45%
MH50	0%	9%	36%	55%

Figure S1. Structural and Bioinformatic properties of top- and *in vivo*-tested candidate sequences with affinity to 661w cone cells. **(A)** 661w top binders with sequence and isoelectric focusing points. **(B)** Relevant characteristics of peptides that were synthesized for testing.

A Peptide sequence of top candidates against ARPE19 cells

APRE-19 Top five peptide candidates

Peptide Number slide	One letter code	Linker seq	Three-letter (Targeting sequence)	Color	pI (Isoelectric point)
3	DGPPRKP	GGGS[C]	AspGlyProProArgLysPro	Magenta	7.25
50	MPVAVYR	GGGS[C]	MetProValAlaValTyrArg	Blue	7.25
52	LAFHRMP	GGGS[C]	LeuAlaPheHisArgMetPro	Red	7.40
54 (54)	HLSSLTP	GGGS[C]	HisLeuSerSerLeuThrPro	Yellow	6.63
57	ATGPRSV	GGGS[C]	AlaThrGlyProArgSerVal	Lime	7.25

B

Peptide Name	Acidic	Basic	Neutral	Hydrophobic
MH3	8%	17%	67%	8%
MH50	0%	9%	36%	55%

Figure S2. Structural and Bioinformatic properties of top- and *in vivo*-tested candidate sequences with affinity to ARPE-19 cells. **(A)** ARPE-19 top binders with sequence characteristics. **(B)** Relevant characteristics of peptides that were synthesized for testing.

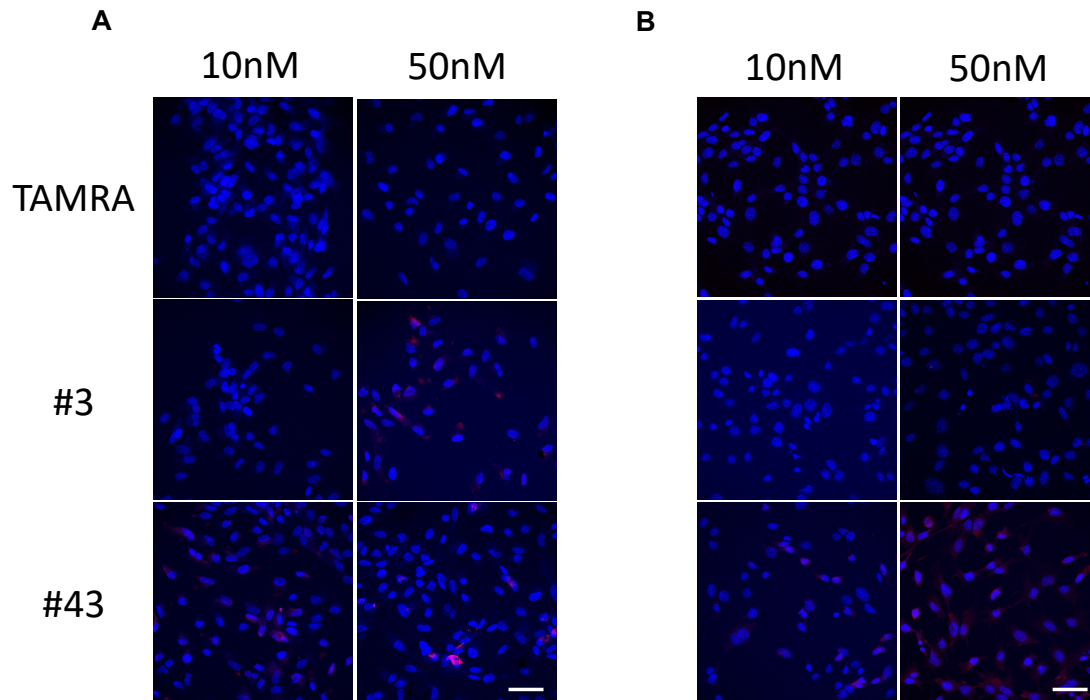


Figure S3. Cell uptake of TAMRA-labelled peptide candidates. Confocal microscopy images of **(A)** hARPE19 and **(B)** 661w cone cells incubated with 10nM and 50nM peptides MH3 and MH43. TAMRA dye used as negative control. Scale bars represent 50 μ m.

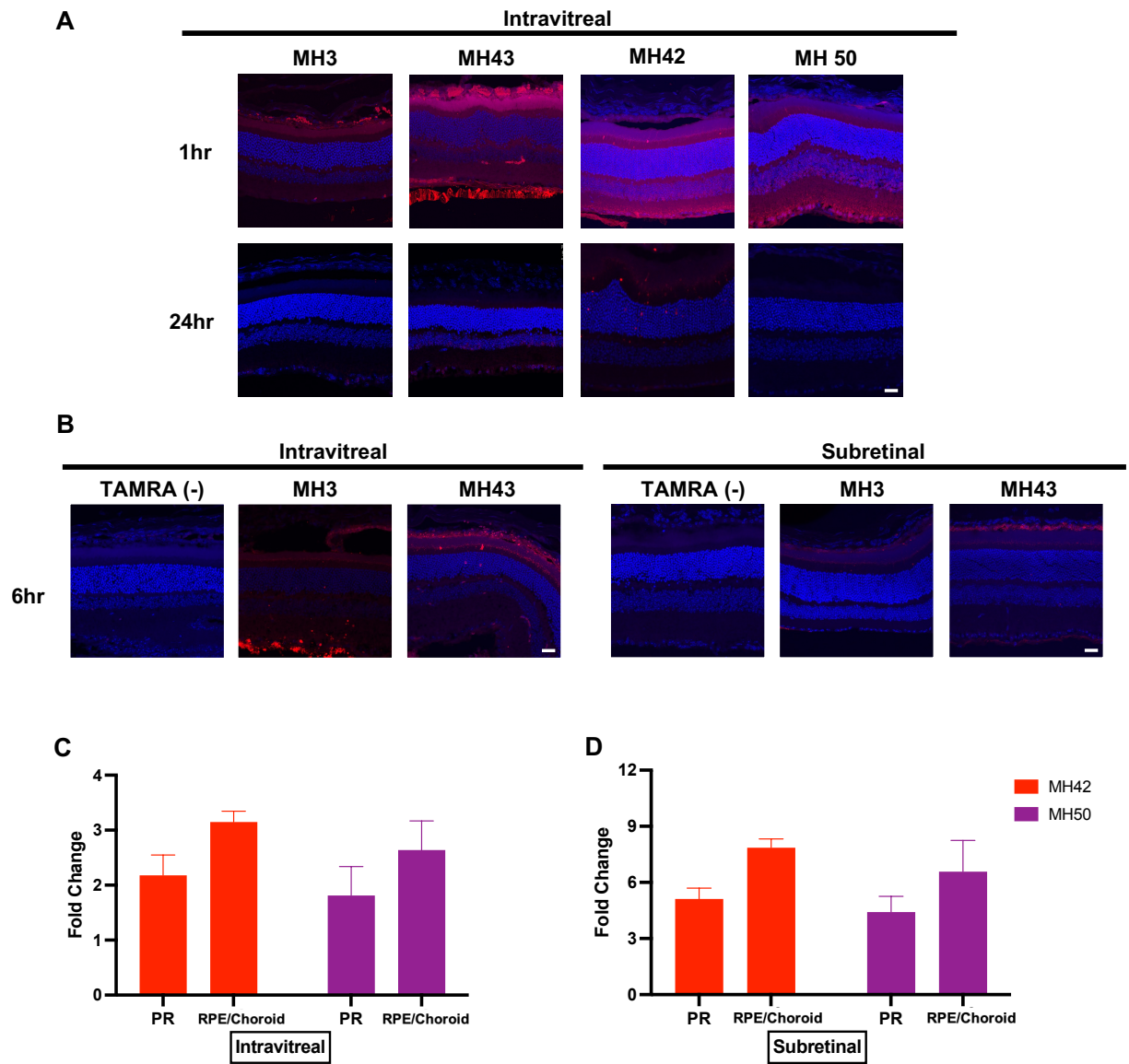


Figure S4. TAMRA-tagged peptide uptake in BALB/c mice. **(A)** Representative confocal microscopy images of BALB/c mice eyes intravitreally injected with peptides MH3, MH43, MH42 and MH50 collected at 1hr and 24hrs. **(B)** Representative confocal images of TAMRA- MH3 and -MH43 delivered intravitreally and subretinally. Scale bar represents 25 μ m. **(C-D)** Mean fluorescence intensity fold-change of peptide uptake in vivo of MH42 and MH50 over TAMRA control; n = 4-8 eyes per group.

A

← PEG amount in LNPs (Mol%) →

PEG	LNPm-0%	LNPm-0.15%	LNPm-0.3%	LNPm-0.6%	LNPm-0.9%	LNPm-1.2%
DMG-PEG 2000	1.5	1.35	1.2	0.9	0.6	0.3
DSPE-PEG (2000) Maleimide	-	0.15	0.3	0.6	0.9	1.2
Total	1.5	1.5	1.5	1.5	1.5	1.5

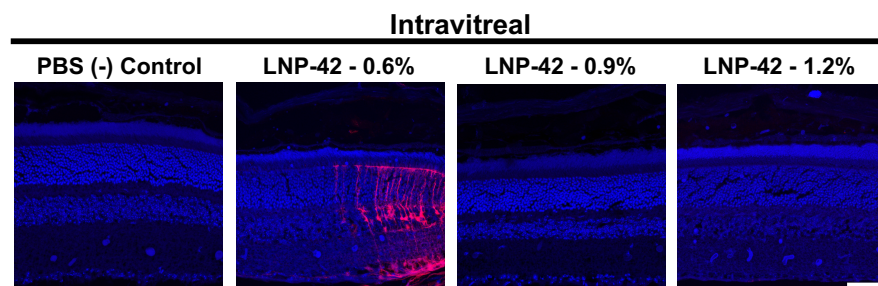
B

Figure S5. Ratios of peptide surface density and Ai9 *in vivo* injections. **(A)** Functionalized PEG component in LNP formulations with different peptide densities and **(B)** Confocal microscopy images of PBS injected control as well as 0.6-1.2% formulations tested. Scale bar represents 50 μm .

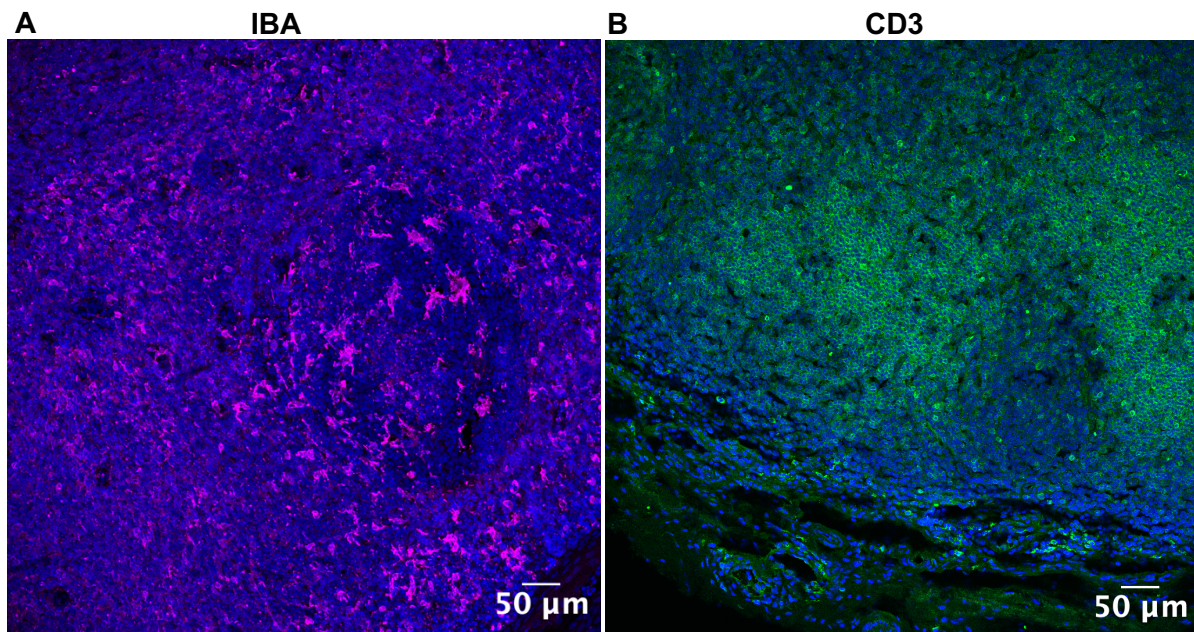


Figure S6. (A) Confocal image of rhesus tonsil stained with IBA-1 (microglia, magenta) and DAPI (blue). **(B)** Confocal image of rhesus tonsil stained with CD3 (T cells, green) and DAPI (blue). Slides were stained alongside retinal cross-sections to serve as positive controls.

Supplemental Excel File 1 (Table S1). Schrödinger™ QikProp output values for pharmaceutically relevant properties for peptide sequences isolated. Peptide sequences ordered with added linker (blue). The supplementary excel file is available as a supplementary document.