Supplementary Information

Pro/Ala/Ser (PAS) peptides as an alternative to polyethylene glycol (PEG) for the surface shielding of lipid nanoparticles (LNPs) carrying therapeutic mRNA

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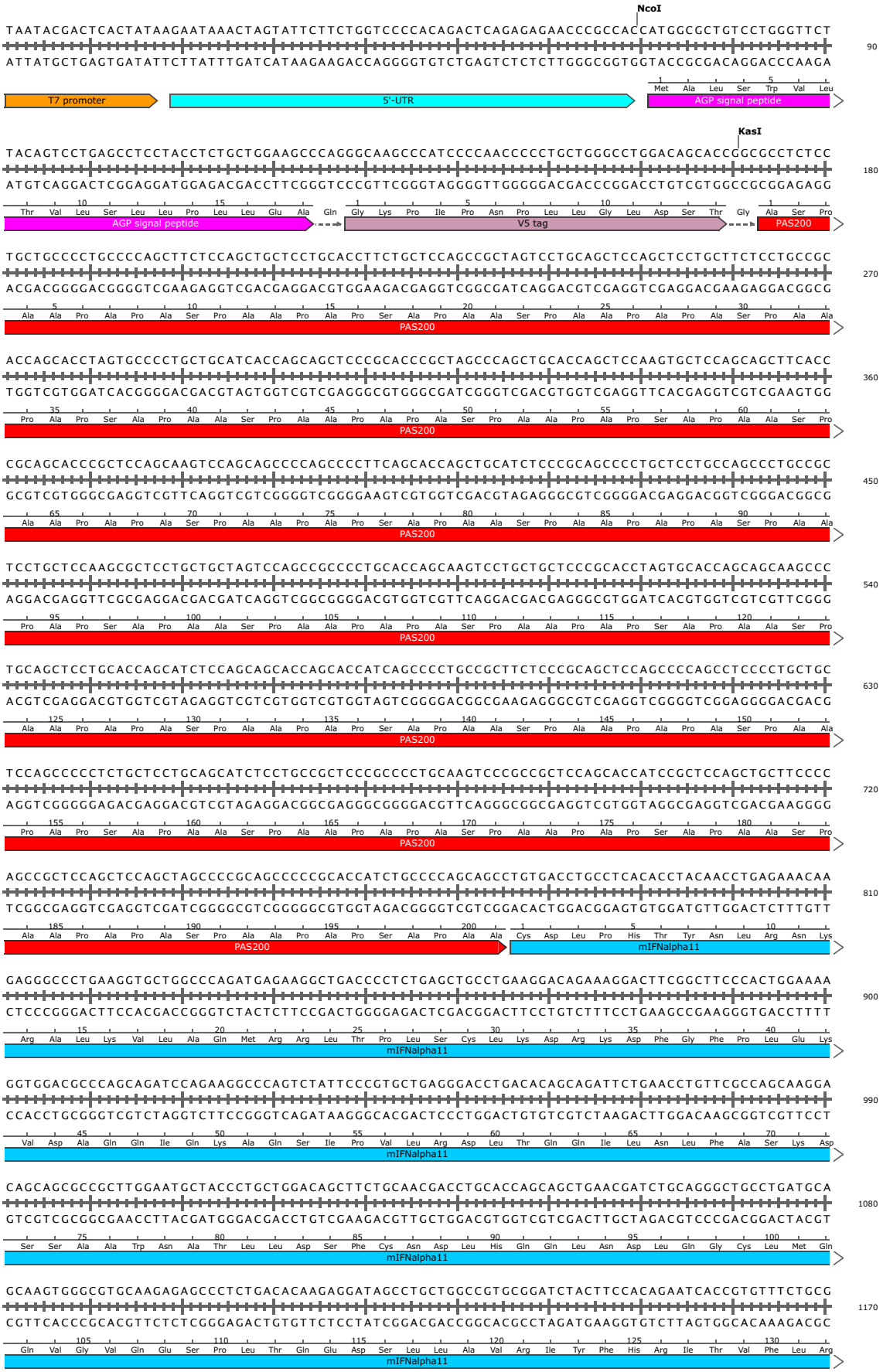
NcoI
KasI
T7 promoter
5'-UTR
AGP signal peptide
V5 tag
PAS200
mIFNalpha11

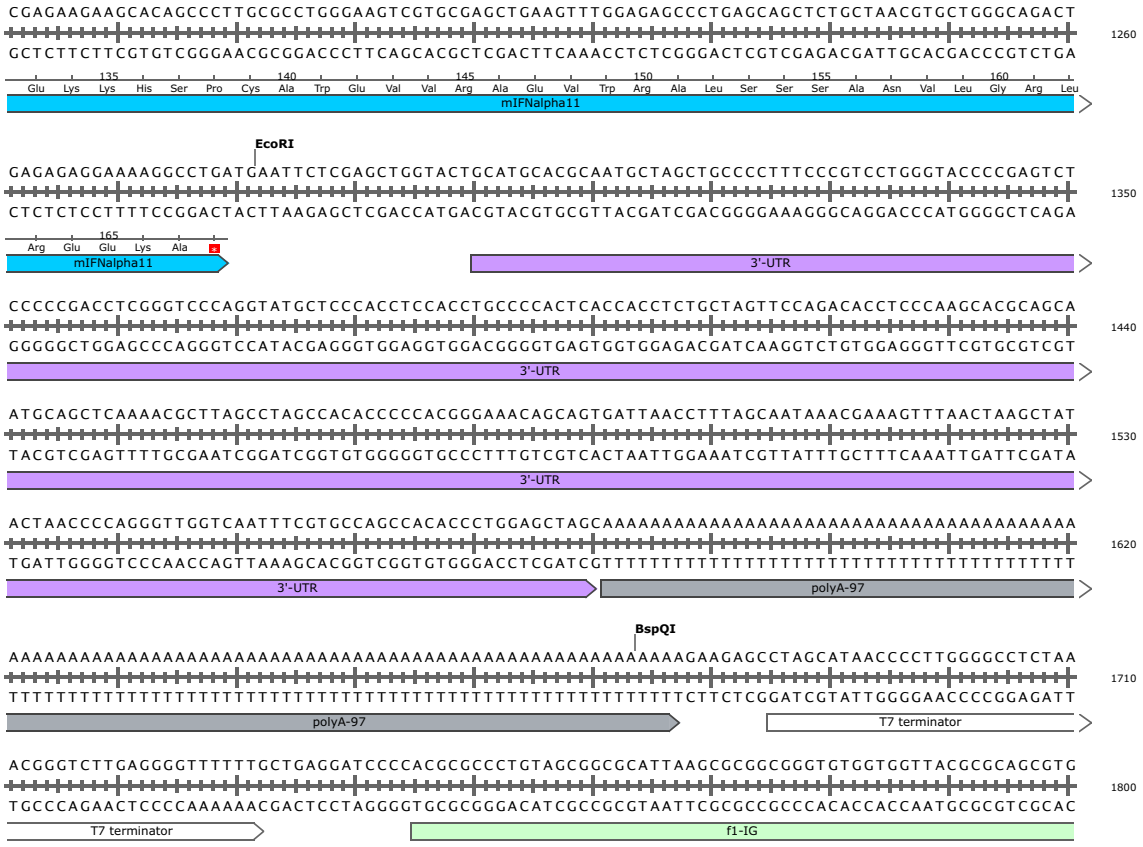

Figure S1: Sequence of the coding region for the AGP-V5-PAS200-mIFNα11(97A) transcript as well as flanking regulatory regions. The encoded fusion protein comprises the N-terminal AGP signal peptide, followed by the V5-tag, a non-repetitive gene cassette encoding 201 PAS residues, and the mature part of the mIFNα11 cytokine. The regulatory elements include the bacterial T7 promoter, eukaryotic 5'- and 3'-untranslated regions (UTRs), a DNA-encoded poly-A tail comprising 97 adenine nucleotides (ending with a *Bsp*QI/*Sap*I restriction site), and the T7 transcriptional terminator.

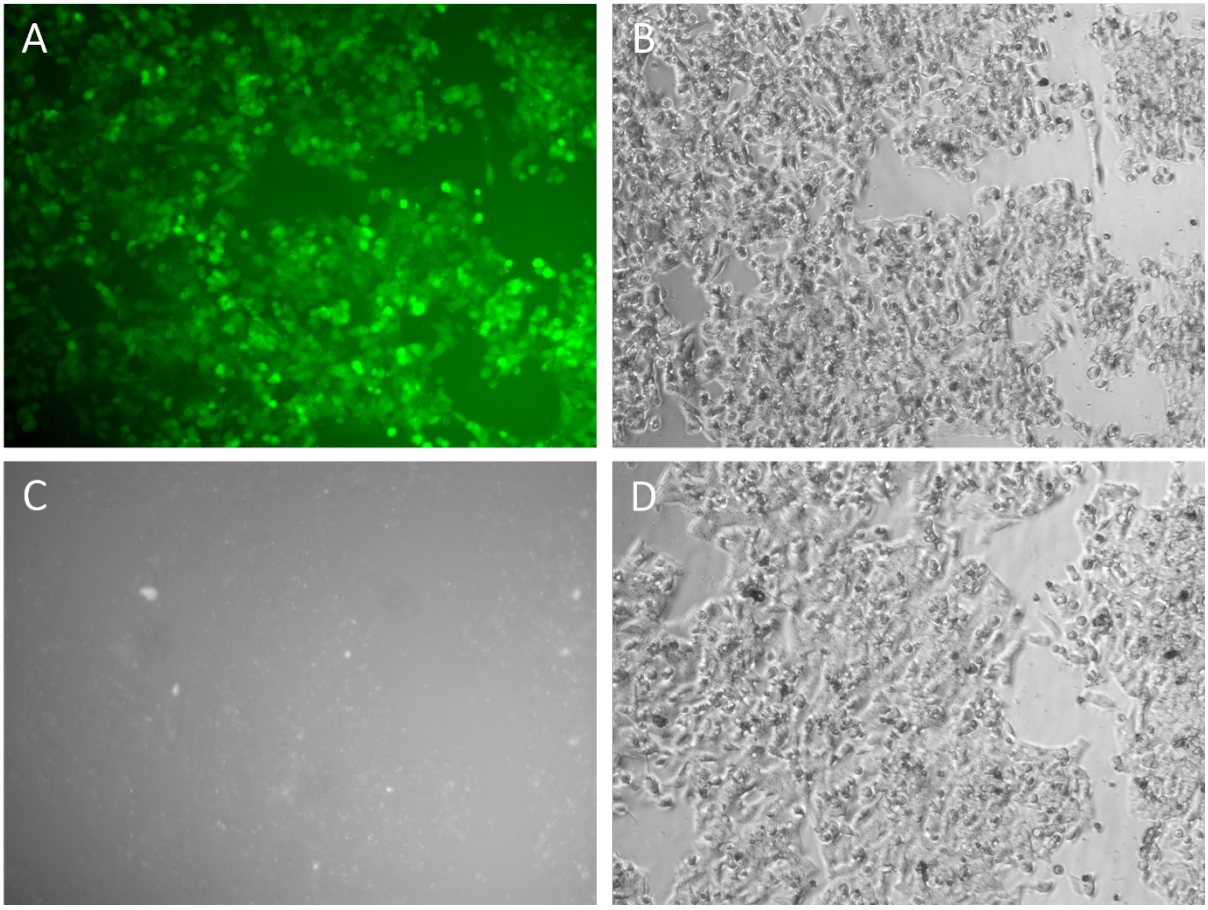

Figure S2: Treatment of HepG2 cells with LNPs delivering the eGFP mRNA as cargo. HepG2 cells (2×10^6 cells in 3 mL culture volume) were grown in 6-well culture plates and treated with mRNA-LNPs encoding eGFP. (A) After 24 h at 37 °C, images were recorded of the cell monolayer by an Axiovert 40 CFL Inverted Fluorescence Microscope (Carl Zeiss, Oberkochen, Germany) using the bandpass filter 470/40 for excitation of eGFP and the 515 emission filter to detect its fluorescence emission. (B) Bright field image from the same image section as in (A). (C and D) Untreated HepG2 cells were imaged as in (A) and (B).

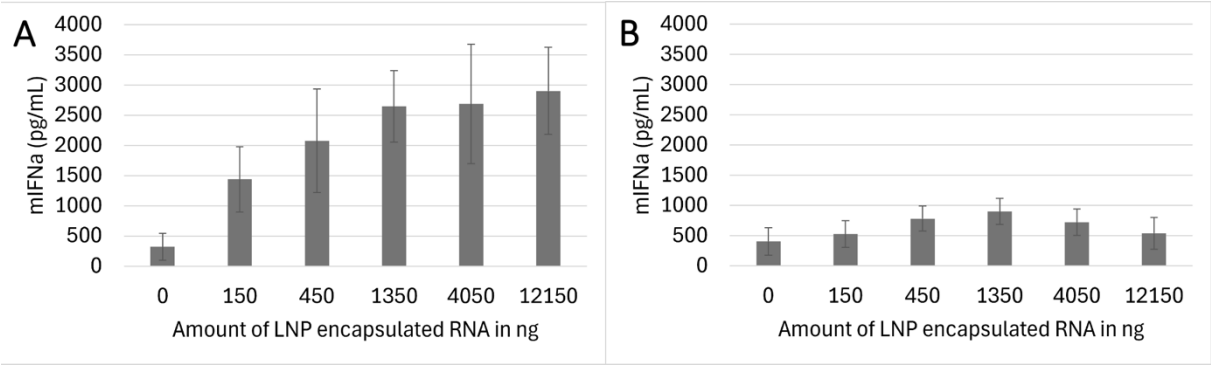

Figure S3. Comparison of V5-PAS200-mIFNα11 secretion from HepG2 cells transfected with LNPs charged with AGP-V5-PAS200-mIFNa11(40A) mRNA and prepared either with the PAS-DTAs or the PEG2000-C-DMG lipid. HepG2 cells (3 mL culture volume with 2×10^6) were treated with different amounts of the mRNA-LNPs and incubated at 37 °C for 24 h. (A and B) The concentration of the V5-PAS200-mIFNα11 protein in the cell-free supernatants was quantified by sandwich ELISA. The dilution series indicate that PAS-LNPs induce the secretion of higher amounts of AGP-V5-PAS200-mIFNa11(40A) in comparison to PEG-LNPs. Of note, for both preparations the signals are much lower than those obtained for LNPs charged with AGP-V5-PAS200-mIFNa11(97A) mRNA (see Figure 8).