PNAS

Supporting Information for

In situ production and secretion of proteins endow therapeutic benefit against psoriasiform dermatitis and melanoma

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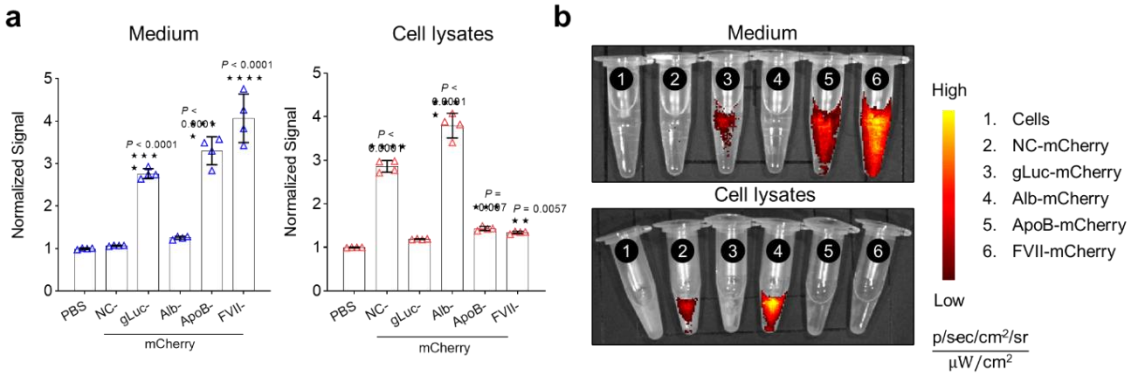


Fig. S1. Optimal signal peptide (SP) drives mCherry secretion effectively in Huh7 cells. (A), Quantification of mCherry fluorescence in cell lysates and medium at 72 h after transfection. Data presented as mean±s.e.m. (n=4 biologically independent samples). (B), Cell lysates and medium were imaged by IVIS. Huh7 cells in 96-well plate were treated by Lipofectamine 2000/pDNA (50ng per well). At 72 hours, mCherry signal was quantified by plate reader or captured by IVIS. A two-tailed unpaired t-test was used to determine the significance (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001). WT, Wild Type; NC, Negative Control; Alb, human Albumin; ApoB, human Apolipoprotein B; gLuc, Gaussia luciferase; FVII, human Factor VII.

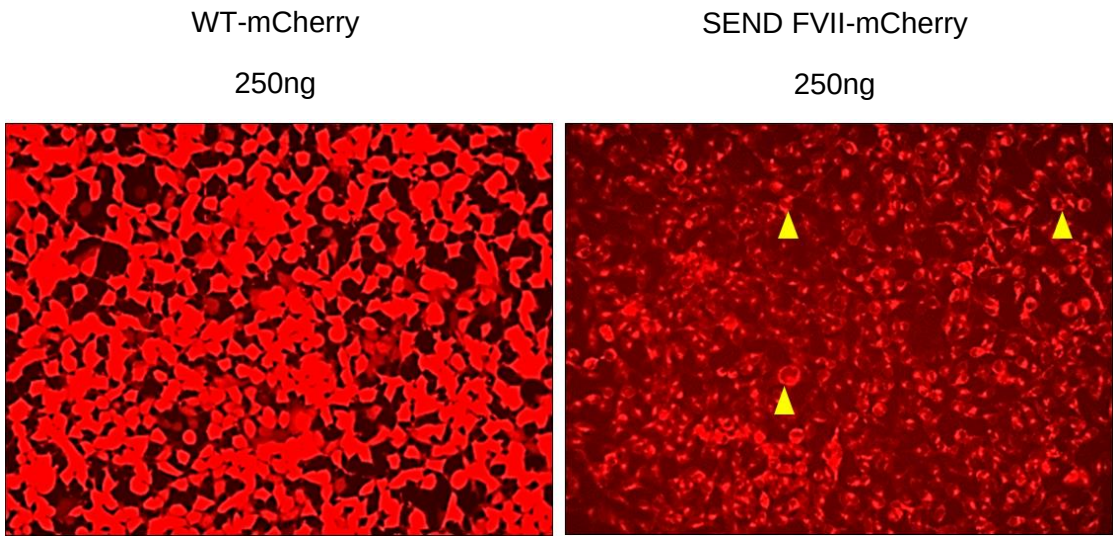


Fig. S2. The FVII SP drives effective mCherry secretion validated by mRNA delivery. Images of HEK293T cells treated with mRNA formulations for 24 h (exposure time, 1/6s). These images from Figure 2d have been reproduced here to show the “circle-like” mCherry distribution (indicated by yellow arrows) in FVII-mCherry mRNA formulation group, which was similar with pDNA delivery shown in Figure 1h.

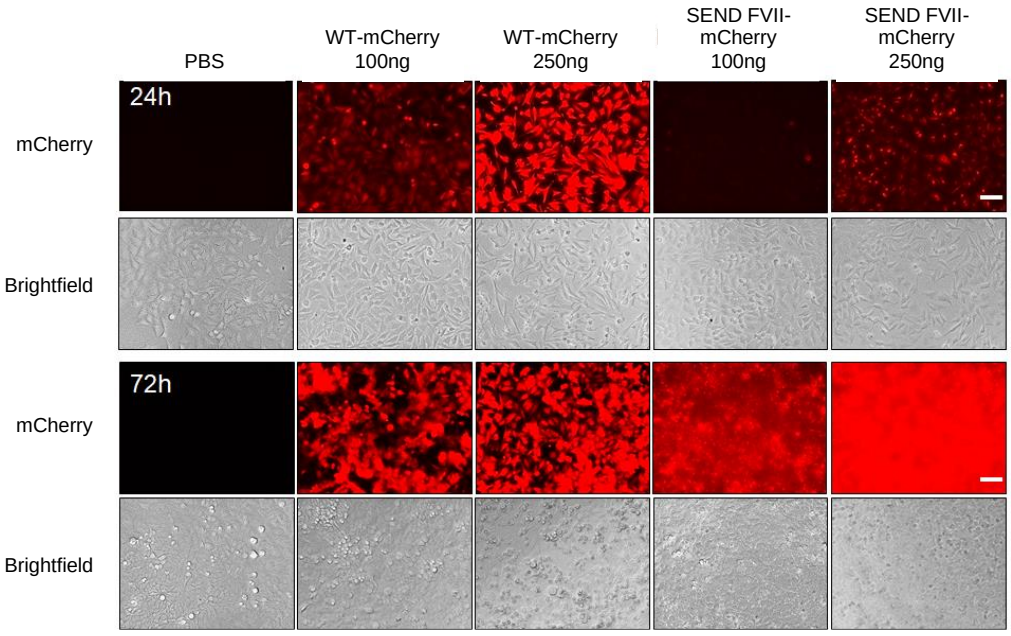


Fig. S3. The FVII SP drives effective mCherry secretion confirmed in HeLa cells following mRNA delivery. Obvious mCherry signal was observed in the medium at day 3 treated with FVII-mCherry mRNA formulation. HeLa cells in 96-well plate were treated by mRNA LNPs with different doses and imaged with a Keyence microscope (exposure time, 1/6s for 24h and 1/15s for 72h).

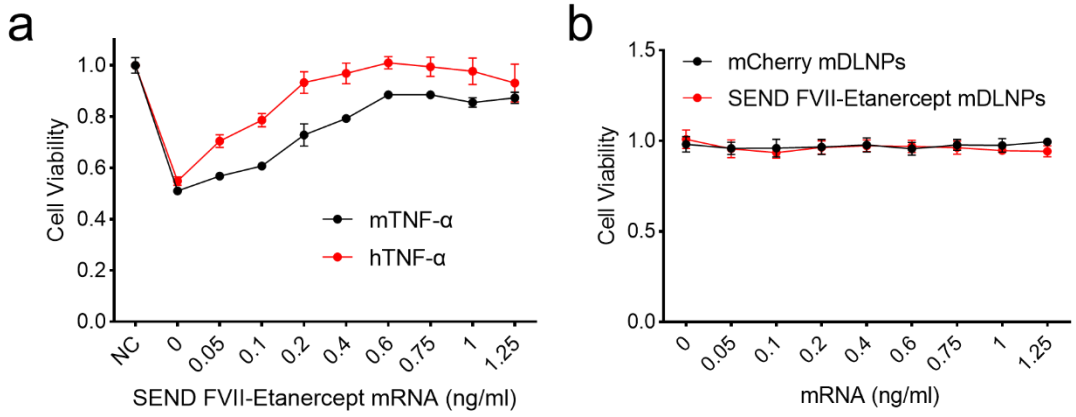
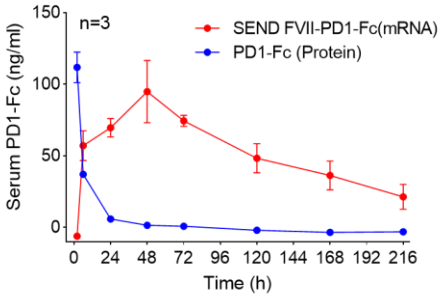


Fig. S4. The FVII-etanercept mRNA formulation rescued the cytotoxicity induced by TNF-α. (A), Dose-dependent rescue with LNP delivery. L929 cells were pretreated by FVII-etanercept mRNA LNPs with mRNA doses of 0 – 1.25 ng/mL. After two days, cells were challenged by TNF-α with dose of 0.02 ng/mL and fixed Actinomycin of 1ug/mL. After another 24h, cell viability was detected. (B), No significant cytotoxicity was observed by mRNA formulation only.



	PD1 Protein		SEND FVII-PD1 mRNA	
	Mean	SD	Mean	SD
AUC(0-t) (ng/mL*h)	1051.48	125.408	11845.509	3026.195
MRT(0-t) (h)	10.032	6.296	85.625	10.972
t1/2z (h)	13.326	0	99.196	64.358
Tmax (h)	2	0	56	13.856
Cmax (ng/mL)	111.761	18.49	100.598	27.761

Fig. S5. IV administration of FVII-PD1 mRNA LNPs enabled >11-fold increase in total area under the curve (AUC), >7-fold enhancement in half-life (t1/2), and a 28-fold extension of mean residence time (MRT) as compared to IV administration of PD1-Fc protein. For pharmacokinetic comparison between PD1 protein and mRNA LNPs after single dosing, mice were I.V. injected with dose of 0.5 mg/kg mRNA LNP formulation or proteins. Serum was collected at different time points and PD1 was quantified by ELISA kit.

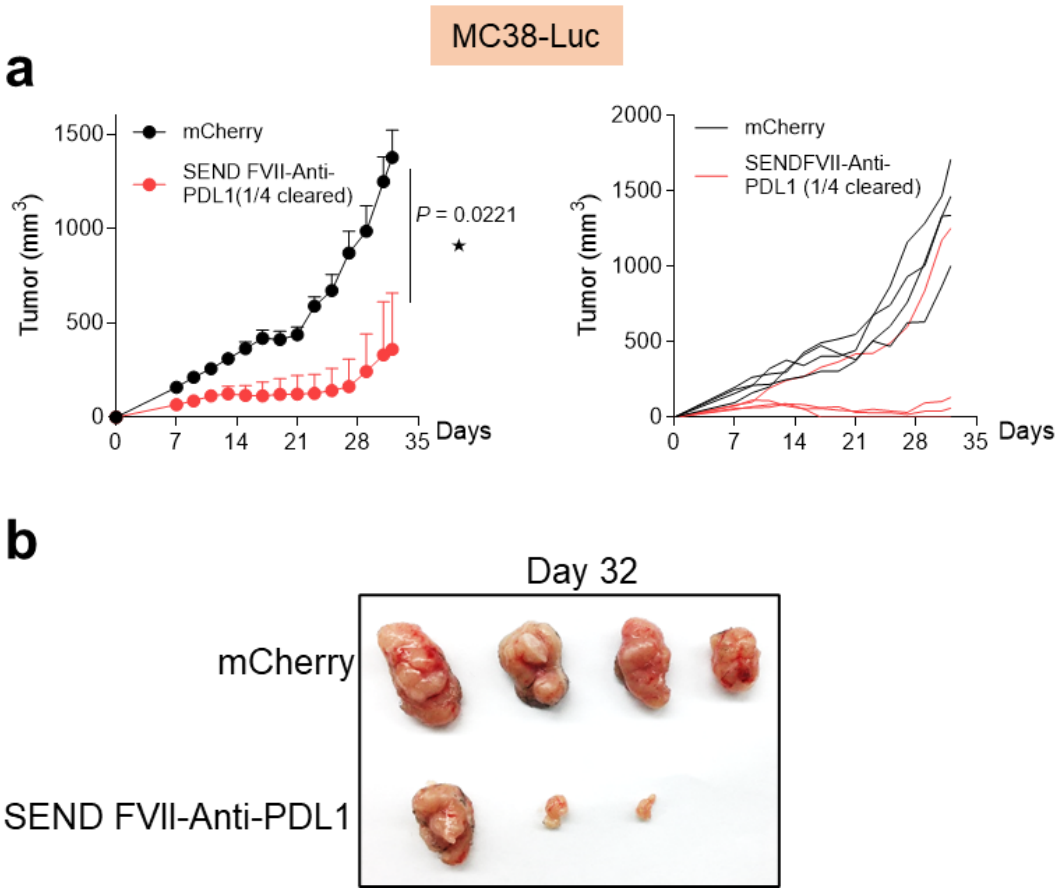


Fig S6. Tumor immunotherapy by FVII-Anti-PDL1 mRNA LNPs. (A), Tumor growth of MC38-Luc model during treatments by mRNA formulations. (B), Tumor images at the day 32 (1/4 cleared). (*P < 0.05)

Table S1. Design of signal peptide-modified mCherry sequences.

Sample ID	AA Sequence (SP + mCherry)	SP Sources	Predicted SP (?)
WT-mCherry	MVSKG…	N/A	No
NC-mCherry	MEDAKNIKKGPAPFYPLEDGMVSKG…	GenBank: CAB91857.1	No
Alb-mCherry	MKWVTFISLLFLFSSAYSMVSKG…	GenBank: AAA98797.1	Yes
ApoB-mCherry	MDPPRPALLALLALPALLLLLLAGARAMVSKG…	NCBI Ref.: NP_000375.3	Yes
gLuc-mCherry	MGVKVLFALICIAVAEAMVSKG…	GenBank: BAR71165.1	Yes
FVII-mCherry	MVSQALRLLCLLLGLQGCLAMVSKG…	GenBank: AAP33841.1	Yes

Sample ID	AA Sequence (SP + hEPO)
WT-hEPO	MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPD TKVNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLR ALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR*
NSP-hEPO	MAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYAWKRMEVGQQAVEVWQGL ALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKEAISPPDAASAAPLRTITADT FRKLFRVYSNFLRGKLKLYTGEACRTGDR*
SEND FVII-hEPO	MVSQALRLLCLLLGLQGCLAAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNFYA WKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLLRALGAQKE AISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLKLYTGEACRTGDR*

Table S2. Design of signal peptide-modified hEPO sequences.

Sample ID	AA Sequence (SP + Therapeutic)
SEND FVII-etanercept	MGVHECPAWLWLLLSLLSLPLGLPVLGLPAQVAFTPYAPEPGSTCRLREYYDQTAQMCCSKCSPGQH AKVFCTKTSDTVCDSCEDSTYTQLWNWVPECLSCGSRCSSDQVETQACTREQNRICTCRPGWYCALS KQEGCRLCAPLRKCRPGFGVARPGTETSDVVCKPCAPGTFSNTTSSTDICRPHQICNVVAIPGNASMD AVCTSTSPTRSMAPGAVHLPQPVSTRSQHTQPTPEPSTAPSTSFLLPMGPSPPAEGSTGDEPKSCDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
SEND FVII-anti-PDL1	MGVHECPAWLWLLLSLLSLPLGLPVLGMVSQALRLLCLLLGLQGCLAEVQLVESGGGLVQPGGSLRLS CAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCARRHWPGGFDYWGQGTLVTVSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSLSASV GDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISSLQPEDFA TYYCQQYLYHPATFGQGTKVEIKEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGSHHHHHH

Table S3. Design of signal peptide-modified therapeutics (etanercept and anti-PDL1)