Supporting Information

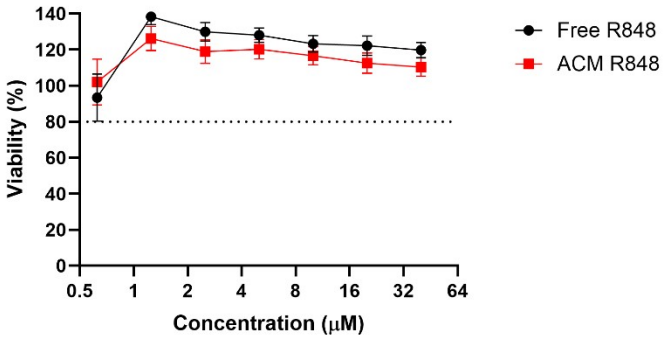
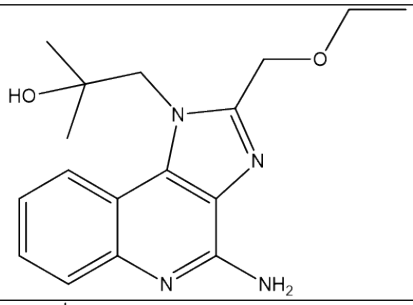
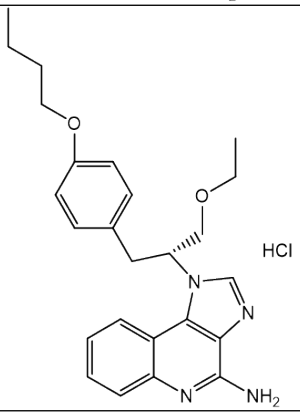
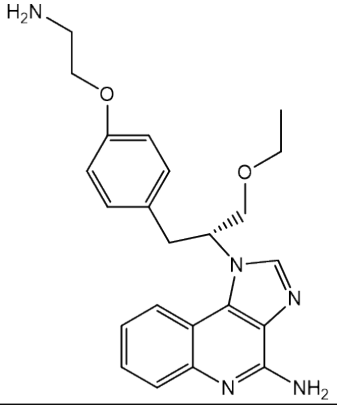


Figure S1. *In vitro* cytotoxicity of R848 and ACM-R848 in PBMC after 24 h incubation at 37°C.

Table S1. Physicochemical properties of the TLR7/8 agonists

TLR7/8 agonists	M_w	Structure	Log P
R848	314.38		1.72[1]
3M-060	455.00		6.43*
3M-060A	405.5		NA

- The Log P value of 3M-060 is a calculated value (cLogP) determined using ACD/ChemSketch software
- NA indicates not available

Table S2. *In vitro* immunogenicity properties of the 3M-060 in HEK293 cells expressing either TLR7 or TLR8[2]

Cytokine	HEK293 cells minimum effective concentration (MEC) (μM)	
	Expressing TLR7	Expressing TLR8
IL-8	≤ 0.01	3.3

Table S3. *In vitro* immunogenicity properties of the TLR7/8 agonists in PBMCs

TLR7/8 agonists	Human PBMCs minimum effective concentration (MEC) (μM)	
	TNF-α	IFN-γ
R848	0.37	1.1
3M-060	≤ 0.01	≤ 0.01

Table S4. Physicochemical properties of the empty polymersomes

	PBD-PEO/DOTAP conc. (mM)	Z average (nm)	PDI	Zeta potential (mV)	Endotoxin level (EU/mL) *
Empty polymersomes	10	134.4± 4.5	0.16	29.6 ± 0.6	0.2**

*: Endotoxin level corresponds to empty polymersomes formulation stock solution.
** empty polymersomes was prepared under strict endotoxin-controlled conditions.

Table S5. Primers for qPCR

Entries	Gene	Forward primer	Reverse primer
1	CD8a	CCGTTGACCCGCTTTCTGT	TTCGGCGTCCATTTTCTTTGG
2	CD4	AGGTGATGGGACCTACCTCTC	GGGGCCACCACTTGAACTAC
3	FoxP3	CACCTATGCCACCCTTATCCG	CATGCGAGTAAACCAATGGTAGA
4	GZMB	CCACTCTCGACCCTACATGG	GGCCCCCAAAGTGACATTTATT
5	IFNG	GCCACGGCACAGTCATTGA	TGCTGATGGCCTGATTGTCTT
6	IL10	AGCCTTATCGGAAATGATCCAGT	GGCCTTGTAGACACCTTGGT
7	TNFa	CCTGTAGCCCACGTCGTAG	GGGAGTAGACAAGGTACAACCC
8	TGFB1	CTTCAATACGTCAGACATTCGGG	GTAACGCCAGGAATTGTTGCTA
9	PD-1	ACCCTGGTCATTCACTTGGG	CATTTGCTCCCTCTGACACTG
10	LAG3	CTGGGACTGCTTTGGGAAG	GGTTGATGTTGCCAGATAACCC
11	FasL	TGGGTAGACAGCAGTGCCAC	GCCCACAAGATGGACAGGG
12	IL12A	CAATCACGCTACCTCCTCTTTT	CAGCAGTGCAGGAATAATGTTTC
13	IL12B	GTCCTCAGAAGCTAACCATCTCC	CCAGAGCCTATGACTCCATGTC
14	IL6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
15	IRF7	GAGACTGGCTATTGGGGGAG	GACCGAAATGCTTCCAGGG
16	IFNA1	TAATTCCTACGTCTTTTCTTT	TATGCCTGATCCCTGAACAGT
17	GAPDH	GGTCATCCATGACAACTTTGG	GGATGCAGGGATGATGTTCT

References

1. R. Fu, D. Meng, X. Han, L. Liu, C. Chen, R. Zheng, Y. Zhao, X. Zhang, X. Lin, M. Hasani, Y. Song, T. Liang, D. Wen, H. Li, I. Yavuz, H. Feng, Z. Gu, Z. Li, S. Li, F. Liu and Y. Yang, *Sci Adv*, 2025, **11**, eadp2757.
2. D. Smirnov, J. J. Schmidt, J. T. Capecchi and P. D. Wightman, *Vaccine*, 2011, **29**, 5434-5442.