YMTHE, Volume *25*

Supplemental Information

Safe Recombinant Outer Membrane Vesicles that Display M2e Elicit Heterologous Influenza Protection

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SUPPLEMENTAL INFORMATION

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Table S1: Primers used for PCR

Primer	Sequence (5'- 3')	Source
gutQ-F	GTCGATAAGCTGATTACCGACGC	Mamat 2015[1]
gutQ-R	GTGAAACTATTCGTCAGGCACTGG	Mamat 2015[1]
nlpI-F	ATTTACGCCGCGCATGTGTTTAG	This paper
nlpI-R	AGGGCCGTATCCGTCTGAGC	This paper

1. Mamat, U, Wilke, K, Bramhill, D, Schromm, AB, Lindner, B, Kohl, TA, *et al.* (2015). Detoxifying Escherichia coli for endotoxin-free production of recombinant proteins. *Microb. Cell Fact.* **14**: 1–15.

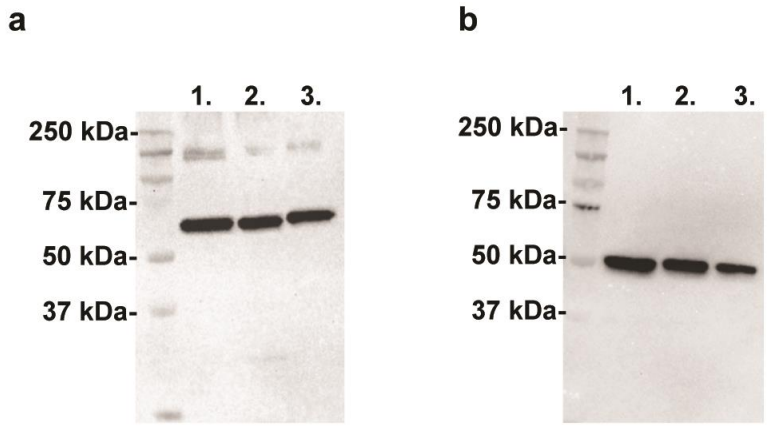


Figure S1: (**a**) Western blot of rOMVs expressing ClyA-GFP developed using anti-GFP antibody. Lane 1: ClyA-GFP CC rOMVs (2 µg), lane 2: ClyA-GFP BL21 rOMVs (2 µg), lane 3: ClyA-GFP purified protein (0.125 µg). (**b**) Western blot of rOMVs expressing influenza-based antigen ClyA-M2e4xHet developed using anti-His tag antibody. Lane 1: ClyA-M2e4xHet CC rOMVs (1 µg), lane 2: ClyA-M2e4xHet Nsl rOMVs (1 µg), lane 3: ClyA-M2e4xHet purified protein (0.2 µg). Both blots developed using chemiluminescence and imaged with Bio-Rad ChemiDoc Touch Imaging System (Bio-Rad, Hercules, CA).

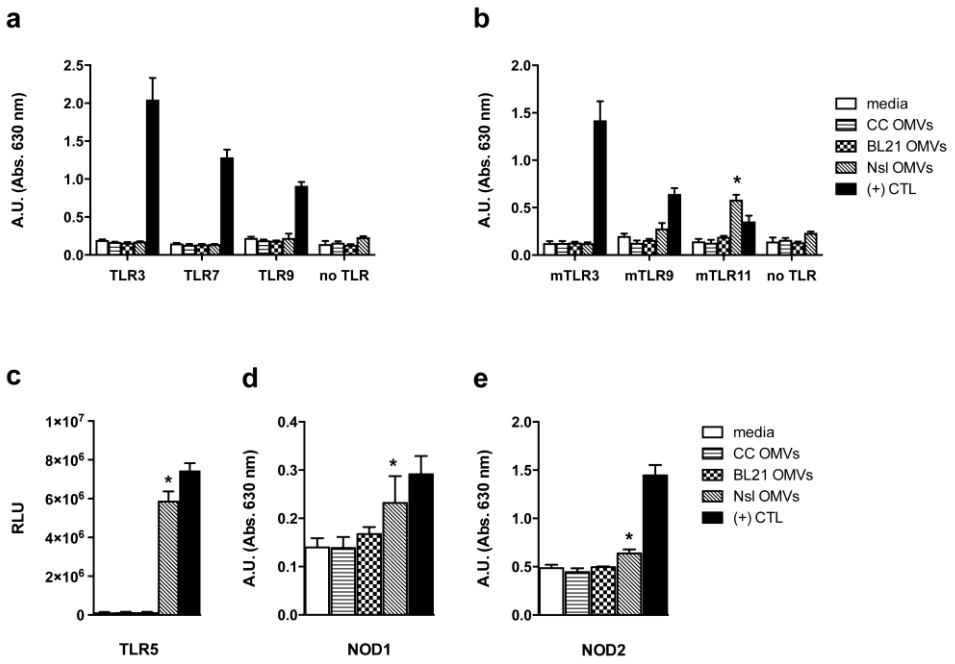
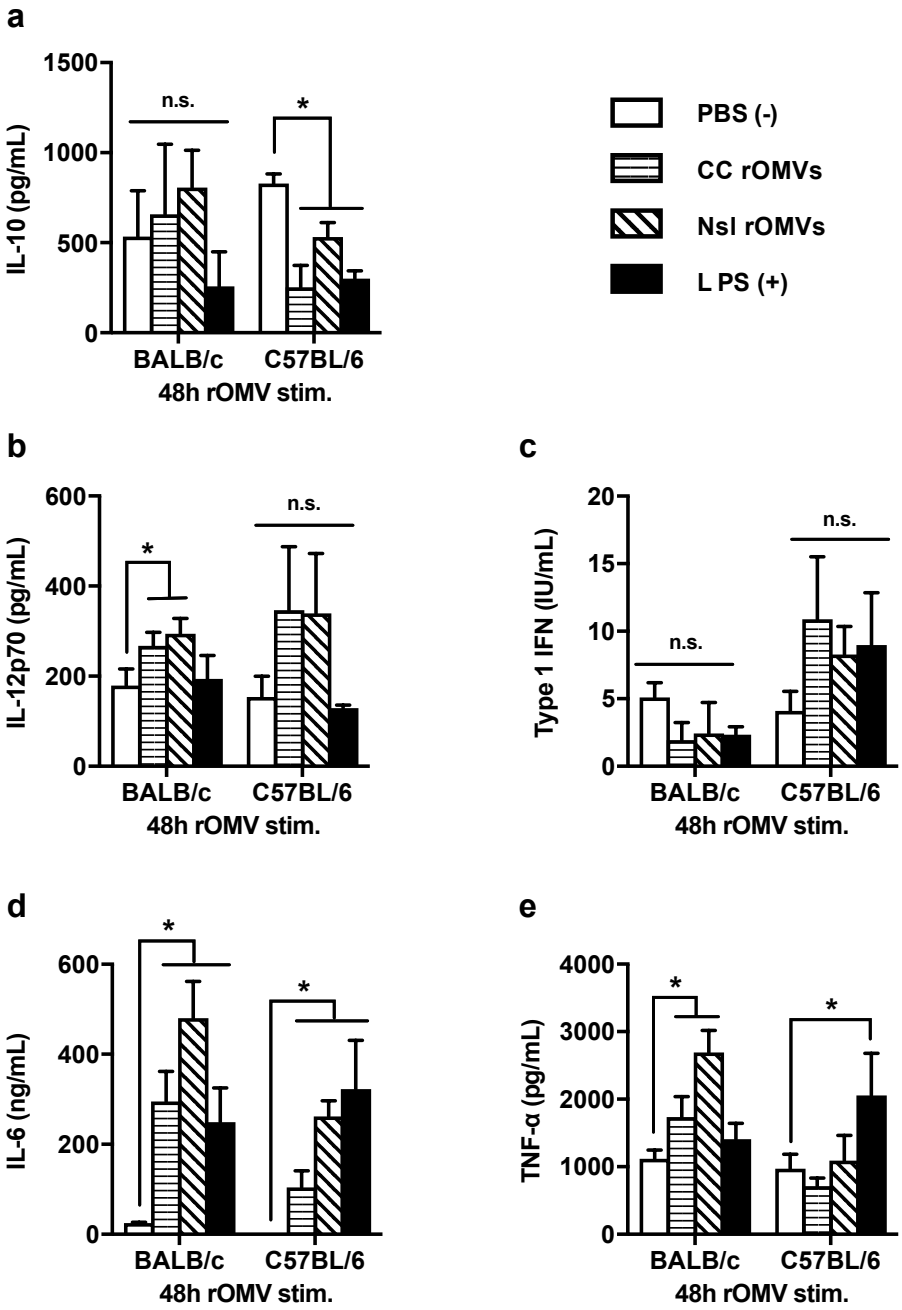


Figure S2: PRR signaling in response to rOMVs. (**a**) HEK-Blue™ KD-TLR5 cells were transfected with human TLR2, 3, 7, and 9, then stimulated with CC, Nsl, and BL21 rOMVs (100 ng/mL). (**b**) HEK-Blue™ KD-TLR5 cells were transfected with murine TLR2, 3, 9, and 11, then stimulated with CC, Nsl, and BL21 rOMVs (100 ng/mL). (**c**) HEK-293 cells were transfected with a 5xNF-kβ-luciferase reporter plasmid and TLR5, then stimulated with CC, Nsl, and BL21 rOMVs (100 ng/mL). (**d, e**) HEK-293 Blue™ murine NOD1 (**d**) and NOD2 (**e**) reporter cells were stimulated with CC, Nsl, and BL21 rOMVs (100 ng/mL). Graphs analyzed using ANOVA followed by multiple comparisons to stimulation caused by media using Dunnett method of correction, * $p<0.01$. Error bars represent mean + SD (n=4).



S3: BMDCs were isolated from BALB/c and C57BL/6 mice, then stimulated with CC rOMVs (100 ng/mL), NsI rOMVs (100 ng/mL), LPS (100 ng/mL) or PBS. Media was collected 48h post stimulation and analyzed for IL-10 (**a**), IL-12p70 (**b**), type I IFN (**c**), IL-6 (**d**), and TNF-α (**e**). Error bars represent standard deviation. Samples analyzed via ANOVA followed by Holm multiple comparison test * $p<0.05$ (n=3).

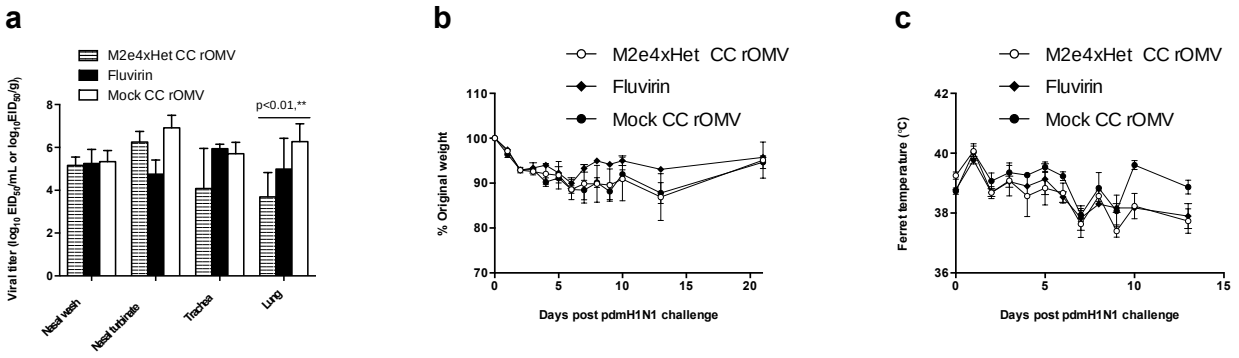


Figure S4: (**a**) Day 3 post influenza exposure, ferrets were assessed for viral titers. Groups analyzed with 2-way ANOVA, followed by multiple comparisons to mock CC rOMV using Bonferroni method of correction (n=3) ** p<0.01. (**b,c**) Weight loss (**b**) and temperature (**c**) were monitored in ferrets post influenza (pdmH1N1) exposure.