## **Supplementary Materials**

## In vitro characterization of the phage lysis protein MS2-L

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MKTRFPQQSQ QTPASTNRR PFKHEDYPCR RQQRSSTLY LIFLAIFLSK FTNQLLLSLL EAVIRTVTT QQLLT MS2-Lp<sub>23</sub> MKHEDYPCR RQQRSSTLY LIFLAIFLSK FTNQLLLSLL EAVIRTVTT QQLLT MS2-Lp<sub>26</sub> MDYPCR RQQRSSTLY LIFLAIFLSK FTNQLLLSLL EAVIRTVTT QQLLT MS2-Lp<sub>26</sub> MCR RQQRSSTLY LIFLAIFLSK FTNQLLLSLL EAVIRTVTT QQLLT MS2-Lp<sub>26</sub> MQQRSSTLY LIFLAIFLSK FTNQLLLSLL EAVIRTVTT QQLLT MS2-Lp<sub>26</sub> MS2-Lp<sub>29</sub> MQQRSSTLY LIFLAIFLSK FTNQLLLSLL EAVIRTVTT QQLLT MS2-Lp<sub>35</sub>

**Supplementary Figure 1.** Amino acid sequences of MS2-L derivatives. Full-length MS2-L was gradually truncated at the N-terminally located soluble domain. The transmembrane region, predicted by the interactive protein feature visualization tool PROTTER (ETH Zürich), is highlighted in yellow. All constructs were synthesized with a C-terminal StrepII-tag separated by a short linker (MS2-L-GTGG-WSHPQFEK).



**Supplementary Figure 2.** CF expression and purification of detergent solubilized MS2-L derivatives. All proteins were CF synthesized at a previously determined Mg<sup>2+</sup> optimum of 20 mM. (A) Exemplary P-CF expression. After harvesting, the pellets were suspended in S30 buffer C in the respective RM volume and 1.5  $\mu$ L of the sample was analyzed on a discontinuous 4%-11 % Tris-Tricine SDS-PAGE. Overexpression bands are framed by red boxes. Other bands appearing on the SDS-PAGE correspond to co-precipitated proteins from the S30 lysate; (B) Exemplary StrepII-purified MS2-L derivatives after D-CF expression with detergent Brij78.



**Supplementary Figure 3.** Co-translational insertion of MS2-L and MS2-Lp<sub>23</sub> into NDs. CF reactions were supplemented with increasing concentrations of preformed NDs. After expression, the samples were centrifuged and pellets were suspended in S30 buffer C in the respective RM volume. 3  $\mu$ L of pellet and supernatant fractions were separated by SDS-PAGE and the amount of peptide in both fractions was determined by densitometry of immunoblots. The combined signal was normalized to 1 and the relative amounts of solubilized peptide/ND complexes were calculated. Error bars represent the SEM. (A) MS2-L; DMPC (n = 6 for 5, 10, 20, 40, 60  $\mu$ M; n = 4 for 0  $\mu$ M; n = 3 for 80  $\mu$ M), DMPG (n = 4 for 0, 20, 40, 60, 80  $\mu$ M; n = 3 for 5, 10  $\mu$ M); (B) MS2-Lp<sub>23</sub>; DMPC (n = 3 for all concentrations), DMPG (n = 3 for 0  $\mu$ M, n = 4 for 10  $\mu$ M, n = 5 for 20, 40, 60  $\mu$ M and n = 6 for 5  $\mu$ M).



**Supplementary Figure 4.** Representative growth curves of Lemo21 (DE3) cells expressing MS2-L derivatives. Liquid cultures were inoculated with freshly plated transformed cells and induced with 1 mM IPTG at early log phase (t = 0). Cell growth was monitored over time at the indicated time points after induction.