Supplementary information

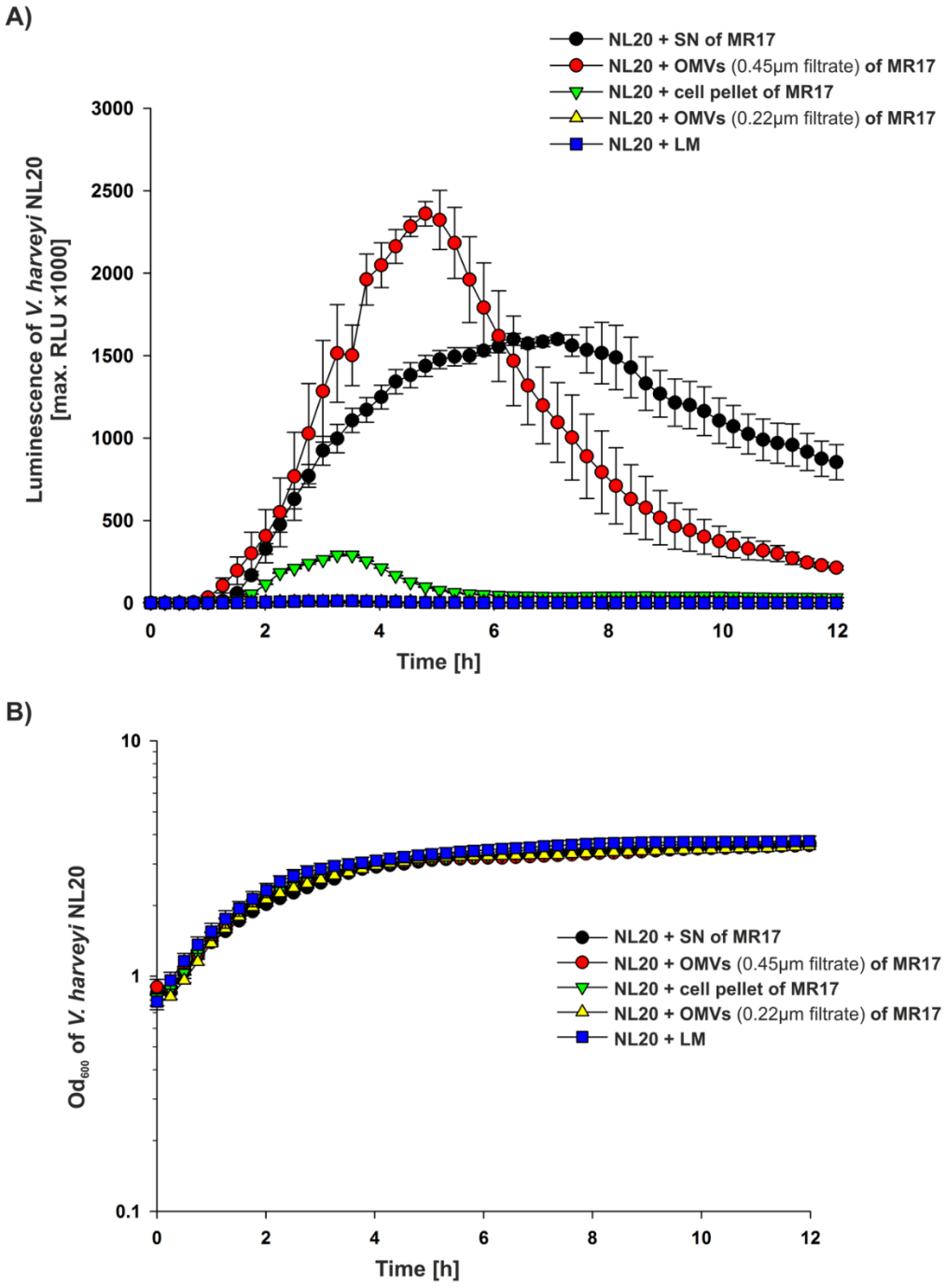

Fig. S1: Time courses of growth and luminescence of the *V. harveyi* reporter strain NL20. The indicated fractions of the strain MR17 were added to the reporter strain NL20 and luminescence production (**A**) and growth (**B**) were monitored over time. MR17 synthesizes only CAI-1 and NL20 recognizes only CAI-1. Error bars represent the standard deviations of data from three different experiments. RLU, relative light units expressed in counts per second per milliliter per OD_{600}. SN, culture supernatant; OMVs (0.45 µm filtrate), filtrate of SN through 0.45 µm filter; OMVs (0.22 µm filtrate), filtrate of SN additionally through 0.22 µm filter; LM, cell free culture medium as control.

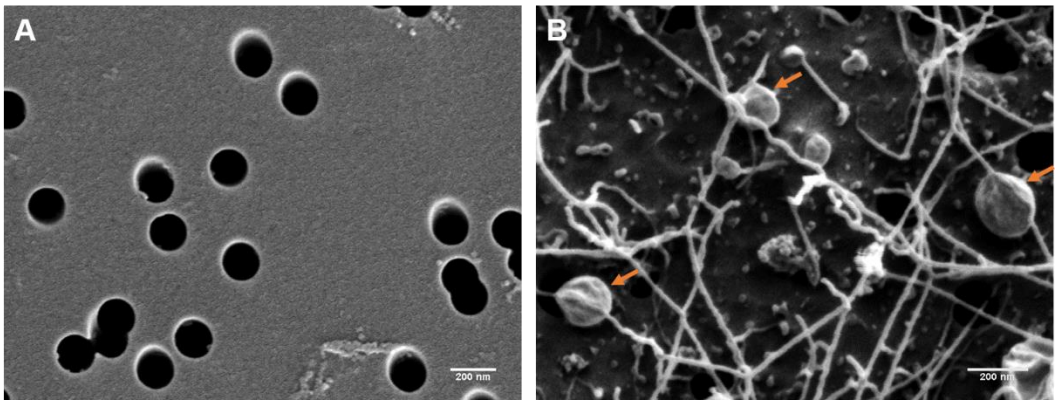

Fig. S2: Large OMVs are retained on the surface of a 0.22-µm PVDF filter after filtration. (**A**) Surface of an unused 0.22 µm filter with uniform pores. (**B**) Scanning electron micrograph of a 0.22 µm PVDF filter membrane after filtration of the OMV-containing 0.45 µm filtrate. Different sizes of OMVs and flagella are trapped on the membrane. Scale bar = 200 nm.

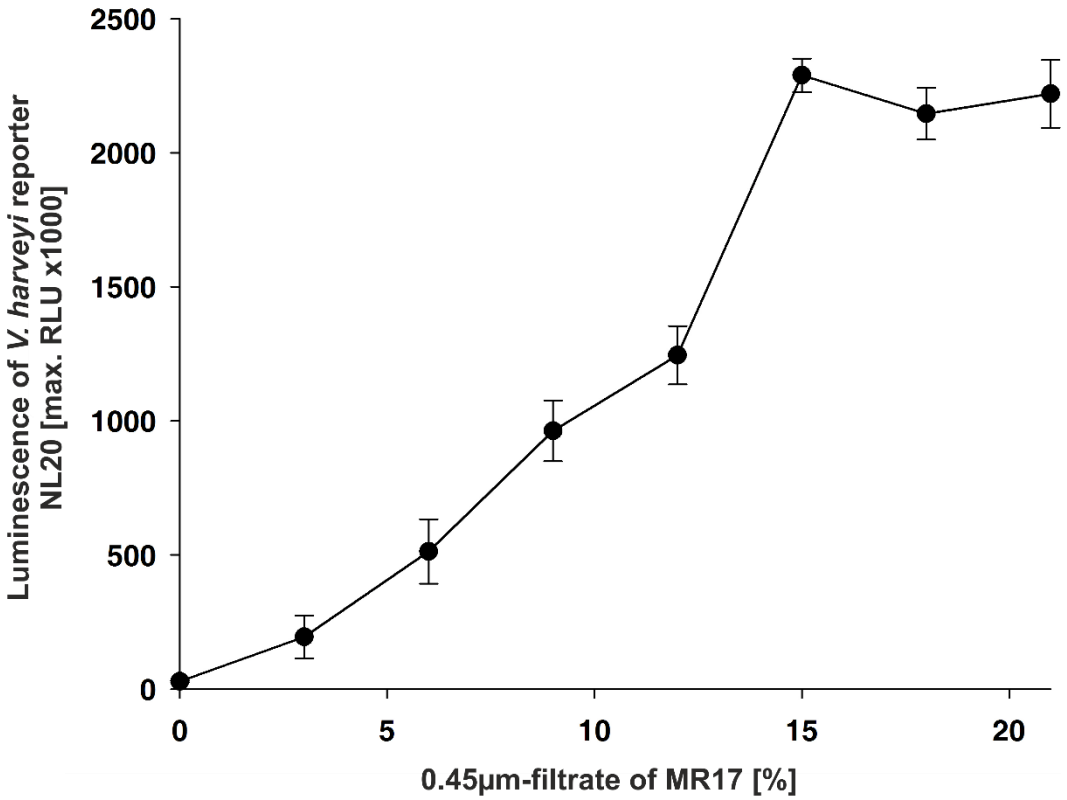

Fig. S3: Relative quantification of CAI-1 in OMVs using *V. harveyi* reporter strain NL20. The indicated volume fractions of the OMV-containing 0.45 µm filtrate of strain MR17 were added to the reporter strain NL20 and luminescence production was monitored over time as readout for QS activation. MR17 synthesizes only CAI-1 and reporter strain NL20 recognizes only CAI-1. Error bars represent the standard deviations of data from three different experiments. RLU, relative light units expressed in counts per second per milliliter per OD_{600}. OMVs (0.45 µm filtrate), filtrate of MR17 SN through 0.45 µm filter. %, percentage of 0.45 µm filtrate of strain MR17 added to reporter strain.

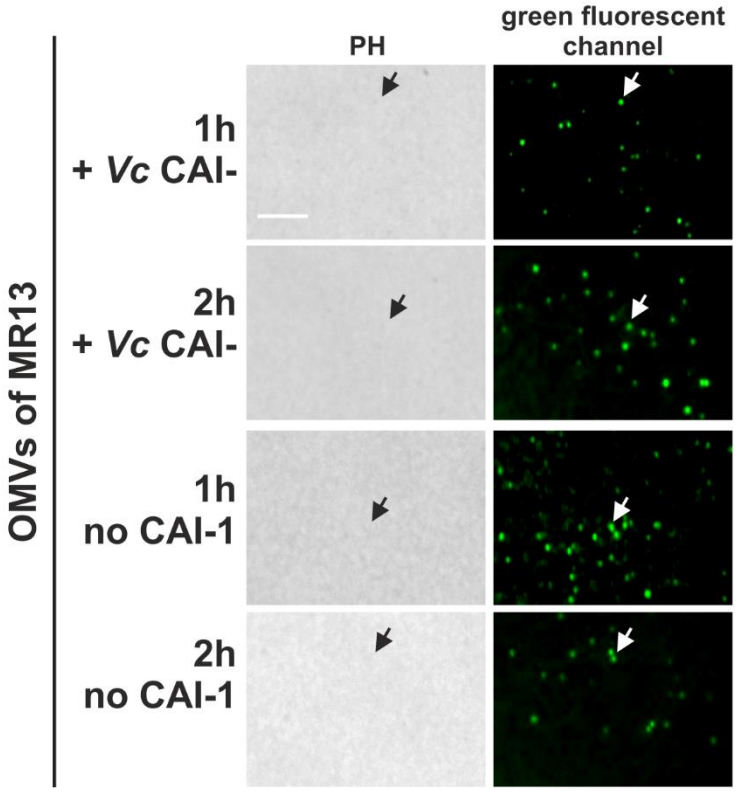

Fig. S4: OMV production is not stimulated by the presence of exogenous CAI-1. Images of fluorescently labelled OMVs. *V. harveyi* strain MR13 does not produce CAI-1, but is able to release OMVs after 24 h of cultivation. Addition of 10 µM *Cholerae* CAI-1 [(*S*)-3-hydroxy-tridecan-4-one] (= *Vc* CAI-1) for up to 2 h did not stimulate OMV formation. Isolated OMVs were stained with the green fluorescent MitoTrackerFM dye, which stains lipids, and imaged using a green fluorescent channel. Each white arrow indicates a fluorescent OMV and the black arrow shows the respective position in the phase-contrast image. PH, phase-contrast images; green fluorescence images of stained OMVs. Scale bar = 5 µm.