



Figure S1: Biophysical characterization of OMVs prepared from pure cultures of LTEE clones (A) OMVs size distribution profiles as measured using Dynamic Light Scattering (DLS). (B) Principle and standard curve of the ANS assay used to quantify total lipid amount in OMVs preparations. This fluorescence assay is based on 1-Anilinonaphthalene-8-Sulfonic Acid (ANS) that behaves as a fluorochrome exclusively when bound to lipidic membranes. Ex.: excitation; Em.: emission. The standard curve used for the assay was obtained using various amounts of artificial liposomes built with a mix of Phosphatidyl Ethanolamine (PE)/Phosphatidyl Glycerol (PG)/Cardiolipin (CL) at a 40/40/20 molar ratio. (C) Summary of the data obtained from DLS and ANS assays that were used for calculation as explained in the material and methods section. The presented data are representative of 6 experiments for the ancestral strain (Anc, REL606) but only one for the 50K-evolved clones (*).