



Supplementary Figure 1

(a) Complete immunoblot of lactococcal plasma membrane vesicles containing MsbA-MD, MsbA-WT, MsbA- Δ K382, or without MsbA protein (control), utilized in Fig. 1a. (b) Complete immunoblot of right side-out membrane vesicles (RSOVs) and inside-out membrane vesicles (ISOVs) containing MsbA-WT or MsbA-MD before and after exposure to protein kinase K (\pm PK) in the external buffer, utilized in Fig. 1b. (c) Complete immunoblot of proteoliposomes containing purified MsbA-DED, MsbA-MD, MsbA- Δ K382 or MsbA-WT, or without MsbA proteins (control), utilized in Fig. 2a. (d) Complete immunoblot of proteoliposomes containing purified MsbA-WT after exposure to PK in the external buffer at 37 °C or 0 °C, and before exposure to PK at 0 °C, utilized in Fig. 2b. The migration of molecular mass markers (kDa) is indicated.