Supplementary Material

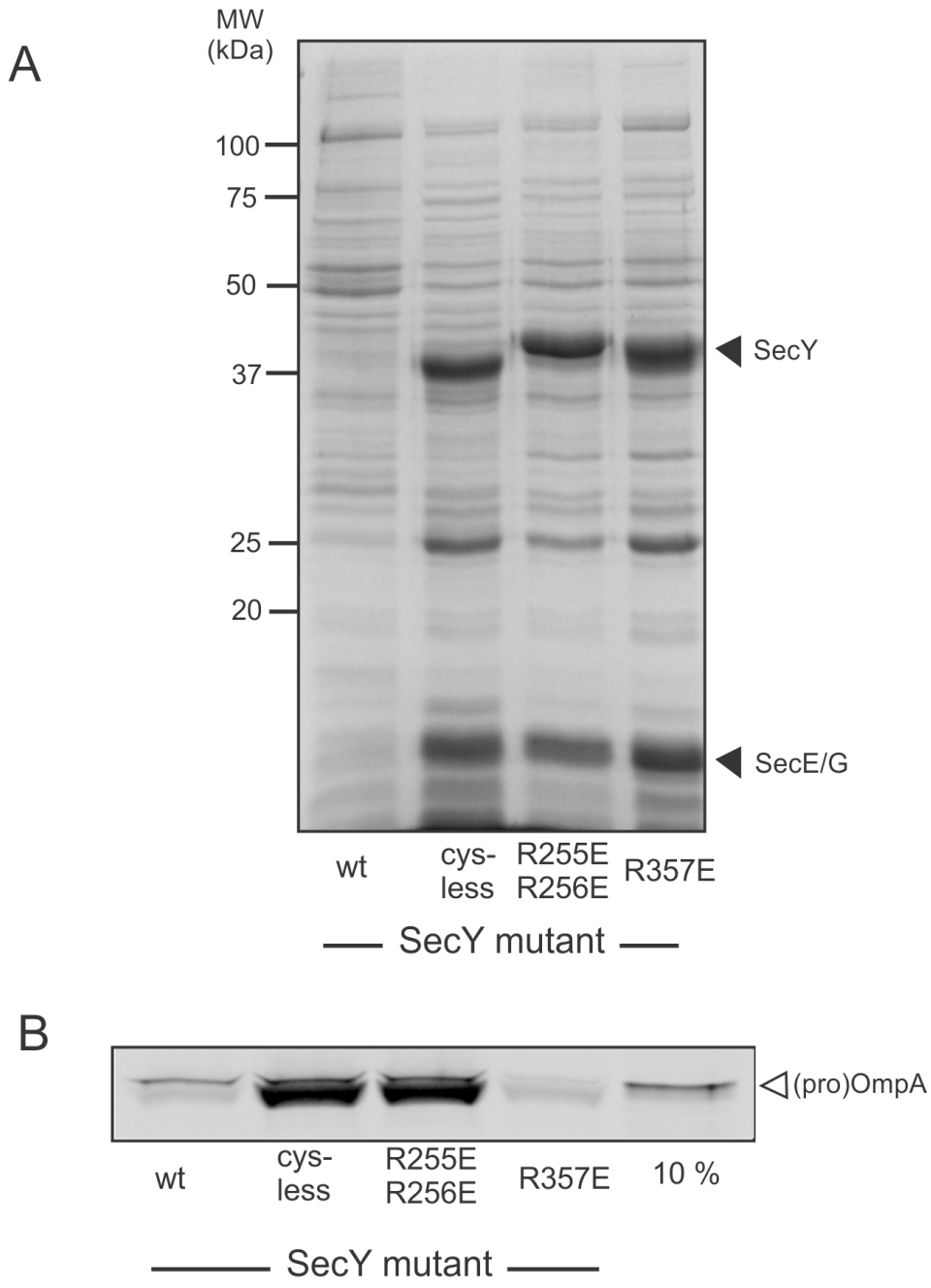

Fig S1. Overexpression of SecYEG mutants. (A) IMVs containing endogenous SecYEG levels or overexpressed Cys-less SecYEG, SecY(R255E,R256E)EG or SecY(R357E)EG was analyzed by SDS-PAGE and Coomassie brilliant blue staining. (B) Translocation of fluorescein labeled proOmpA(C290S) into IMVs containing the indicated overexpressed SecYEG mutants.

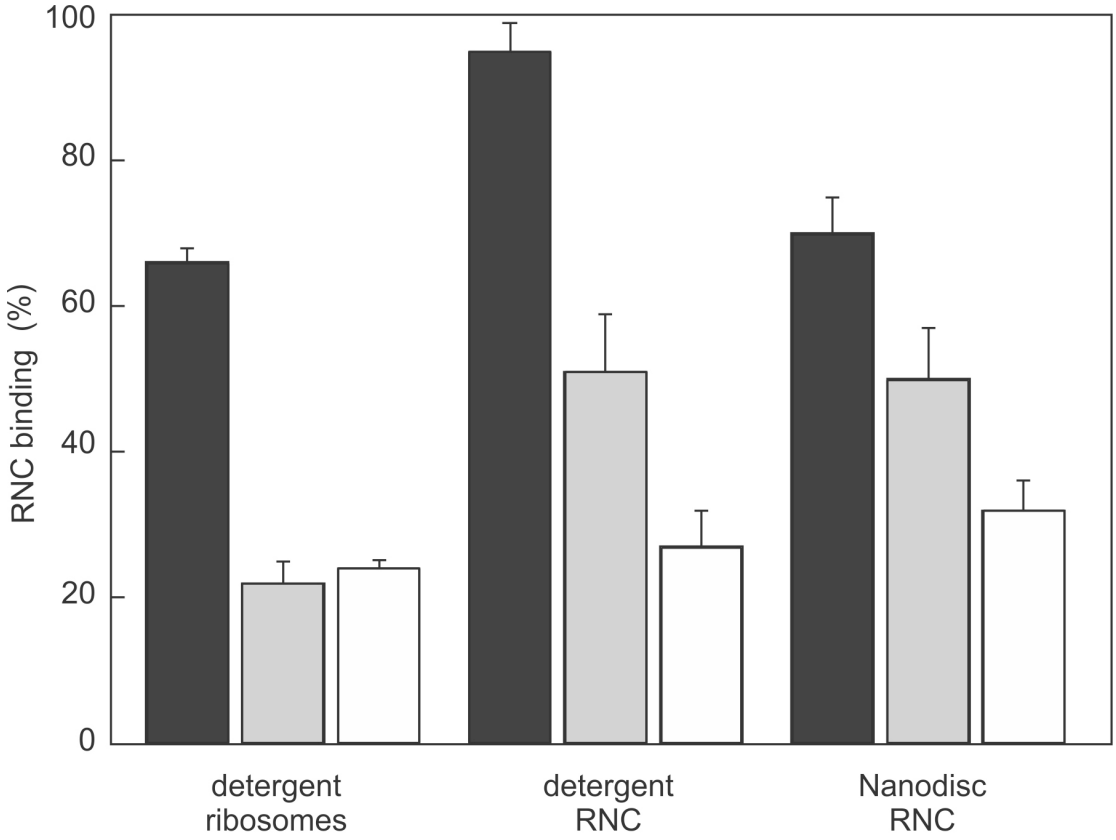

Fig. S2. Interaction between ribosomes and RNCs and purified SecYEG mutants. Fluorescence correlation spectroscopy (FCS) was used to quantify binding of ribosomes and RNCs to SecY(L148C)EG-AlexaFluor 488 (black bars), and its derivatives bearing the SecY mutations R357E (grey bars) or R255E,R256E (white bars). Binding was analyzed both for detergent-solubilized and nanodisc-reconstituted SecYEG. SecYEG was reconstituted into nanodiscs in parallel preparations using SecYEG:MSP:lipid ratio of 1:8:200, and fraction 15 of size-exclusion chromatography was used for the binding assay. Analysis of FCS data is described in the main text.

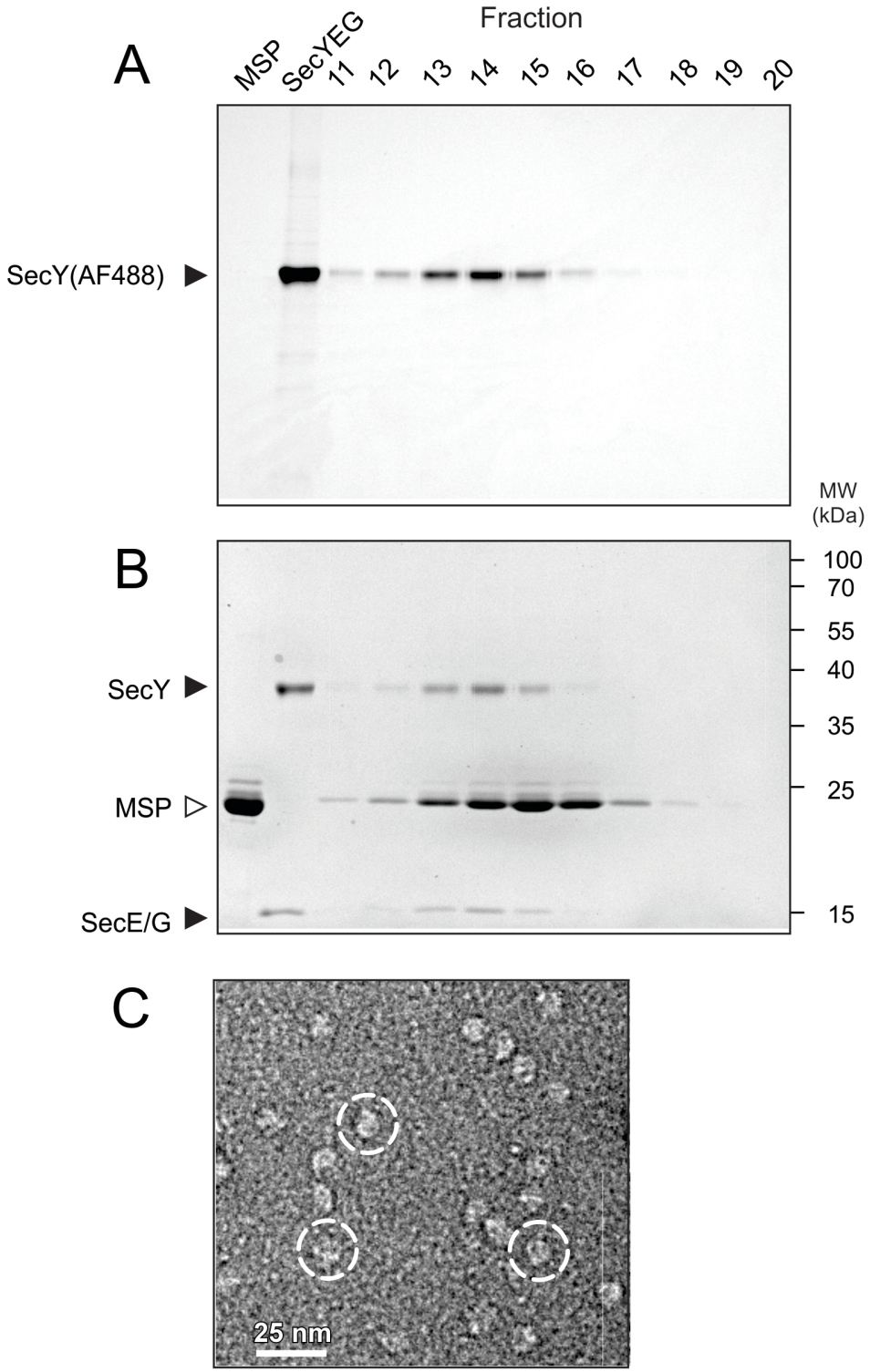

Fig. S3. Reconstitution of SecYEG in nanodiscs. SecY(L148C)EG conjugated with AlexaFluor 488 fluorophore (AF488) was reconstituted with phospholipids in presence of MSP1D1 scaffold protein (MSP). MSP-encapsulated nanodiscs were isolated using size-exclusion chromatography, and collected in 1 mL fractions. SDS-PAGE confirmed that SecYEG and MSP co-eluted in fractions 12 to 16, as visualized by (A) fluorescence and (B) Coomassie staining. (C) Individual nanodiscs (Fraction 15) were imaged using cryo-electron microscopy, and showed as circular entities of ~10 nm diameter (encircled in white).

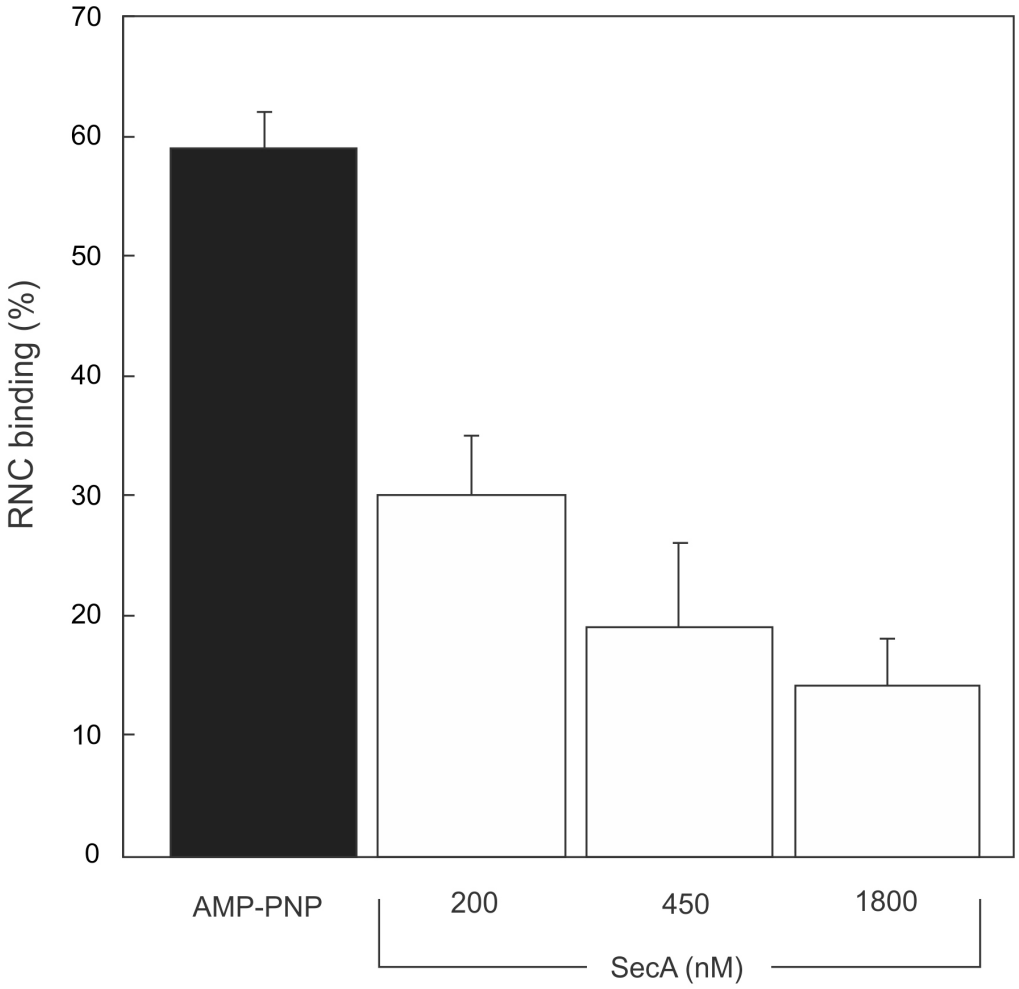

Fig. S4. AMP-PNP-stimulated SecA binding abolishes SecYEG:RNC interactions. FCS was used to quantify RNC binding to nanodisc-reconstituted SecY(L148C)EG-AlexaFluor 488 in the absence and presence of increasing amounts of SecA. AMP-PNP at 5 mM was used to promote SecA binding to the translocon. In presence of SecA an up to 3-fold reduction in SecYEG:RNC binding was observed.