

Supplementary Figure 1. PreproCPY translocates slowly in sec61-3 mutant cells. MWY63 (sec61-3) cells containing pMW346 (preproCPY) were grown at 30°C to an  $OD_{600nm}$  of 0.2 then shifted to 17°C for 2 hours prior to labelling with  $10\mu\text{Ci}$  of  $^{35}\text{S}$  Methionine Cysteine mix  $/OD_{600nm}$  for 20 minutes. These pulse-labelled cells were then chased following the addition of 2mM each of Methionine and Cysteine plus  $10\mu\text{g/ml}$  cycloheximide. Cell extracts were prepared after either 0, 20, 40, 60, 90 or 120 minutes of chase (as indicated) and immunoprecipitated CPY analysed by SDS-PAGE as described in materials and methods. As a control, wildtype cells expressing native CPY were labelled following 30 minutes pretreatment with Tunicamycin in order to accumulate unglycosylated proCPY as a migration control .