Suppl. Fig. 2

Α 120 - Rpt1 wt + Mock - Rpt1 K222S + Mock 100 -Rpt1 wt +S5b FLAG-Rpt1 wt FLAG-Rpt1 K222S FLAG-Rpt1 wt FLAG-Rpt1 K222S + Mock + HA-S5b + HA-S5b + Mock - Rpt1 K222S + S5b 80 CHX 3 6 9 9 (hr) 0 3 6 9 0 3 6 9 0 0 36 60 $IB : \alpha$ -FLAG 40 $IB: \alpha - HA$ IB : α - β actin 20 0 3 6 9 0 В 120 -- + - - Rpt6 wt + Mock - Rpt6 K 196S + Mock 100 | --- Rpt6 wt + PAAF1 FLAG-Rpt6 K196S FLAG-Rpt6 K196S FLAG-Rpt6 wt FLAG-Rpt6 wt - Rpt6 K222S + PAAF1 + Mock + Mock + HA-PAAF1 + HA-PAAF1 80 (hr) CHX 0 3 6 9 0 3 6 9 3 69 0 3 6 9 0 60 $IB : \alpha$ -FLAG 40 $IB: \alpha - HA$ 20 IB : α - β actin

Fig. 2. The stability of the wild-type or Walker A mutant version of Rpt1 and Rpt6 is not affected by co-expression of the cognate chaperones. HeLa cells were transfected with the expression constructs of wild-type and Walker A mutant Rpt1 (A) or Rpt6 (B) with or without the expression vector for HA-S5b and HA-PAAF1 as indicated. Twenty-four hours after transfection, cycloheximide was added to all samples. Cells were harvested at the time points shown and the expression levels of Rpts were determined by Western blotting with anti-FLAG antibody. In all cases, actin levels were measured as a loading control. The graphs in the right panels illustrate densitometric analysis of FLAG signals.

0

0

3

6

9