

Supplementary Figure 3. The effect on Apm4 binding and Mid2 localization of a Y278 mutation in the Mid2 cytoplasmic tail. (A) GST alone; GST tagged Apm4 ; His-tagged Mid2 or Mid2 Y278 were prepared as described and their binding interaction tested using pull down assays on beads with 20 μ M Mid2. Apm4 is clearly seen on a Generon NUVView gel, along with a faint band for Mid2 (arrow). Mid2 was detected using anti-His tag antibodies after western blotting. This band was verified as Mid2 using mass spectrometry. (B) Mid2-GFP and Mid2-Y278A-GFP were transformed into cells otherwise lacking *mid2* (KAY53) or both *mid2* and *apm4* (KAY1700). Localization after 1 hour incubation with alpha factor was observed microscopically.

