Supplementary Methods

Hela cells were transiently transfected with p-eYFPc1 (Clontech), which expresses the GFP 10c variant (with mutation S65G, V68L, S72A, and T203Y), or with vectors expressing Venus or Citrine. Two days post-transfection, cells were fixed (4% paraformaldehyde in PBS for 20 minutes), washed and mounted in PBS, and imaged with a MetaLsm confocal microscope (Zeiss).

To purify YFP proteins, 293T cells were transiently transfected with appropriate vectors, and 2 days post-transfection, cells were lysed in HNTG for 20 minutes at 4°C. Lysates were centrifugated 10 minutes at 20,000g, and soluble proteins were added on sepharose beads coated with anti-GFP antibodies (Roche). Following 2h incubation at 4°C, beads were washed four times and mounted in PBS. HNTG is Hapes 20mM pH7,9; NaCl 150 mM; MgCl2 1mM; EGTA 1mM; Triton 1%; Glycerol 10%; protease inhibitors (Roche).