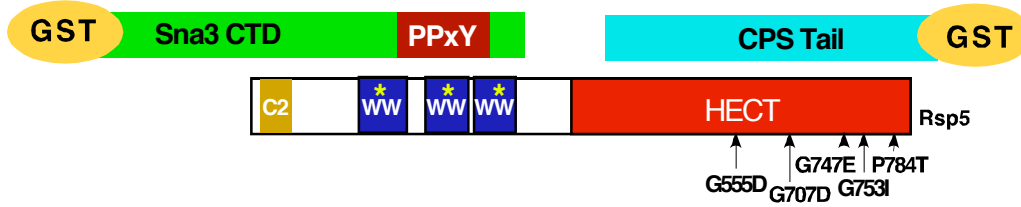
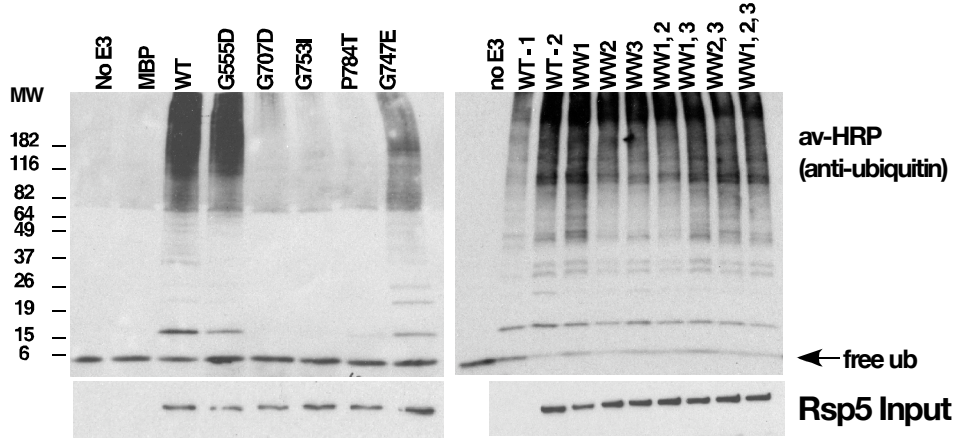


**Supplemental Figure 1: Characterization of mutant Rsp5 binding and ubiquitination defects *in vitro***

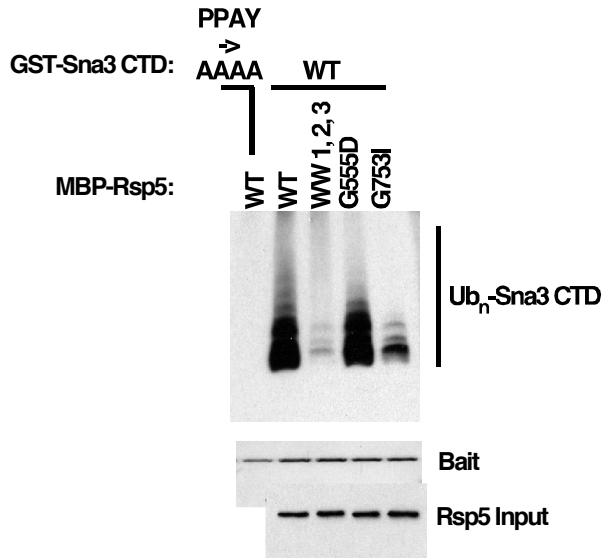
**A.**



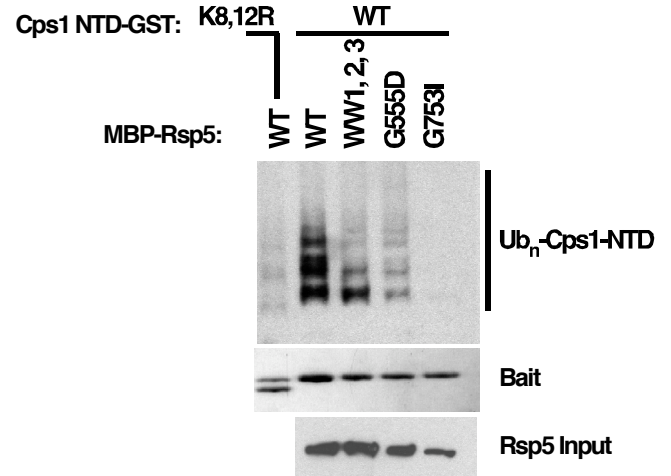
**B.**



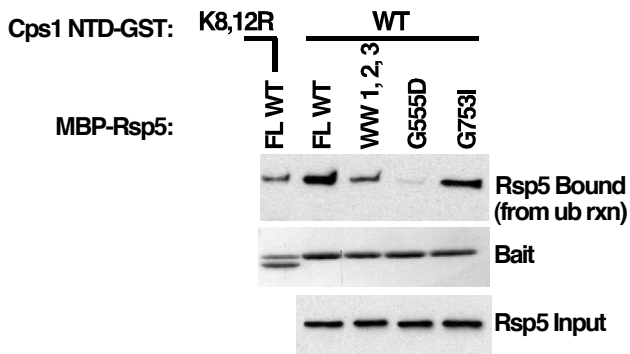
**C.**



**D.**

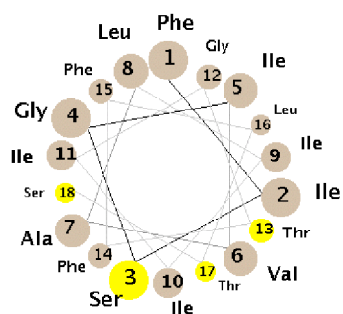


**E.**



Supplemental Figure 2: Analysis of Cps1 TMD mutants

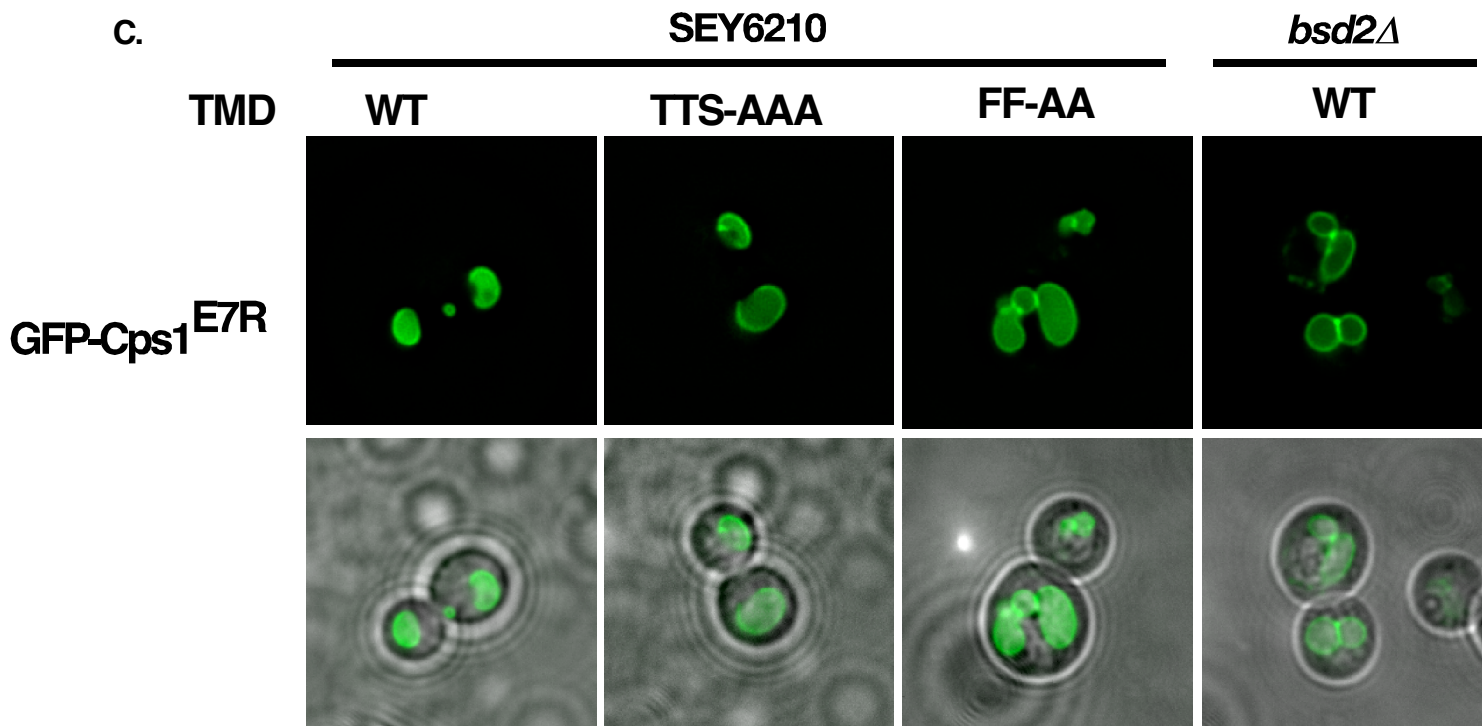
A



B

Sso1 RCWLIVFAIIVVVVVVVVPAVVK  
 Cps1 RAFISGIVALIIIGTFFLTSGLH  
 Phm5 KSLIAILSTCVLFFLVFIIGAK  
 Sec12 KFFTNFILIVLLSYILQFSYK

C



Supplemental Table 1: HECT domain proteins

HERC2\_DROME  
HERC2\_MOUSE  
HERC2\_HUMAN  
HECD1\_HUMAN  
TRIPC\_HUMAN  
HUL4\_ASHGO  
HUL4\_YEAST  
UBE3A\_HUMAN  
UBE3A\_MOUSE  
HECD2\_MOUSE  
HECD2\_PONPY  
HECD2\_HUMAN  
HERC5\_HUMAN  
HERC3\_HUMAN  
HUL5\_YEAST  
UBE3C\_HUMAN  
UBE3C\_MOUSE  
K0317\_HUMAN  
K0317\_MOUSE  
UPL1\_ARATH  
UPL2\_ARATH  
HUWE1\_HUMAN  
HUWE1\_RAT  
TOM1\_ASHGO  
TOM1\_YEAST  
PTR1\_SCHPO  
TOM1\_NEUCR  
SUDX\_DROME  
ITCH\_MOUSE  
ITCH\_HUMAN  
1nd7\_A  
WWP1\_HUMAN  
WWP1\_MOUSE  
WWP2\_HUMAN  
WWP2\_MOUSE  
SMUF1\_DROME  
SMUF2\_HUMAN  
SMUF1\_XENLA  
SMUF1\_HUMAN  
SMUF1\_MOUSE  
NEDD4\_DROME  
NED4L\_HUMAN  
NED4L\_PONPY  
NEDD4\_HUMAN  
NEDD4\_MOUSE

NEDD4\_RAT  
PUB2\_SCHPO  
RSP5\_YEAST  
PUB1\_SCHPO  
PUB3\_SCHPO