











AAGAB Irc6p	INLCVVPNKFLVTAEIAES FDLYIDSCK <mark>EIPVWVEEFITP</mark> ::* : **:	COAPYYYYFDSTOKSGL <mark>DSYSSWLPLAKAW</mark> LPE CCEPLRNYMAGIILLYDIROTKPOELLHOFMIAAHRNT * * * * * * * * * * * * * * * * * * *	108 113
AAGAB Irc6p	VMILVODRV-SEDGIN <mark>ROKAO</mark> VVLANV <mark>N</mark> EEV <mark>EQDEID-ELNE</mark> *:: ::* *: : :	SNCI KHGE USELSPEELPEEDDDFPESTGVKKIVOA IMSNAFTNVIIIII VIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	165 172
AAGAB Irc6p	INANVWSNVVMK- 1 IDTHDWLNCEVQP 1 :::: * * ::	77 85	

DLIVEVTSNDA



57 53

а

е

g

Supplementary Figure 1 AAGAB N-terminal region is a pseudoGTPase domain

(a) Sequence alignment of human AAGAB N-terminal region with human HRas. Identical and similar residues are marked by *, : and .. The five G motifs in HRas are underlined, and consensus residues are in bold. α helices and β strands are highlighted in cyan and pink, respectively. (b) Size-exclusion chromatography (SEC) coupled multiangle light scattering (SEC-MALS) profile of AAGAB G domain (GD). Blue trace: refractive index (RI) of AAGAB normalized to 1; orange trace: calculated MW across the AAGAB GD peak. (c) AAGAB GD (blue), GBP2 GD (red), and SUMO (gray) are mixed with mant-GDP and changes in mant-GDP fluorescence are monitored. (d) SEC profile of AAGAB GD E144K mutant from a HiLoad 16/600 column. Protein standards with known molecular weight are marked at the top. (e) Superposition of the two GD^{E144K} molecules mol A (gold) and mol B (yellow-green) in the asymmetric subunit. Arrows point to the two loops with different conformations. (f) Structure superposition of AAGAB psGD (gold) with HRas GDP Mg (gray, PDB ID 4Q21). (g) Sequence alignment of human AAGAB N-terminal region with yeast Irc6p. Identical and similar residues are marked by *, : and ... α helices and β strands are highlighted in cyan and pink, respectively. (h) Structure superposition of AAGAB psGD (gold) with yeast Irc6p (pale cyan, PDB ID 3UC9).



Supplementary Figure 2 AAGAB psGD assists in σ subunit folding

(a) Size exclusion chromatography profile of GST-AP1 σ 3 on a Superdex 200 10/300 column. Protein standards with known molecular weight are marked at the top. (b) SDS-PAGE of elution fractions in (a).



Supplementary Figure 3 AAGAB psGD utilizes a unique interface to interact with sigma adaptins

(a) Surface presentation of AAGAB psGD:AP1 σ 3 complex oriented the same as in Figure 3a left panel. psGD and AP1 σ 3 are colored gold and violet, respectively. (b) Superposition of AAGAB psGD in psGD:AP1 σ 3 complex (gold), AAGAB GD^{E144K} molecule A (light blue), and AAGAB GD^{E144K} molecule B (lemon). (c) AAGAB psGD:AP1 σ 3 interaction between psGD α 4 and AP1 σ 3 H1 (left panel). The presentation in (c) is rotated ~ 160 ° along y axis from in (a). The key residues psGD I132 and AP1 σ 3 L40 and S41 are shown as sticks in the zoom-in view (right panel). (d) Sequence alignment of human AP1 σ 3, AP2 σ 1, AP1 σ 1, AP1 σ 2, and AP4 σ 1. UniProt ID: AP1 σ 3: Q96PC3; AP2σ1: P53680; AP1σ1: P61966; AP1σ2: P56377; AP4σ1: Q9Y587. Residues interacting with psGD are in bold and color blue (hydrophilic residues) or orange (hydrophobic residues). * identical residues; : similar residues. (e) Structures of HRas:Sos-1 (PDB ID: 1BKD), HRas: RalGDS (PDB ID: 1LFD), and HRas:RafRBD (PDB ID: 4G0N). The orientations of HRas molecules are very similar to that in Figure 3g. HRas molecules are colored gray, while Sos-1, RalGDS, and RafRBD are colored in greencyan. Switch I and II regions of HRas are highlighted in red. (f) Nickel pull-down gel of GST-AP1σ3 co-expressed with His₆-SUMO tagged WT or SAS tagged mutant AAGAB psGD. AR: A168R; 2YR: Y53R/Y54R; DEF3R: D151R/F153R/E155R. Ulp1 protease treatment is used to confirm the identity of His₆-SUMO-AAGAB psGD^{WT} and SAS-AAGAB psGD mutants. (g) Nickel pull-down gel of GST-AP2o1 co-expressed with His₆-SUMO tagged WT or SAS tagged mutant AAGAB psGD. AR: A168R; 2YR: Y53R/Y54R; DEF3R: D151R/F153R/E155R. Ulp1 protease treatment is used to confirm the identity of His₆-SUMO-AAGAB psGD^{WT} and SAS-AAGAB psGD mutants. Note that the molecular weights of GST-AP2o1 and His₆-SUMO-AAGAB psGD are too close to separate on SDS-PAGE.



Supplementary Figure 4 Characterization of PPKP1-related psGD mutants E144K and V139I

(a) GST pull-down gel of GST-AP1 σ 3 co-expressed with SAS tagged AAGAB psGD WT, E144K, or V139I mutant. Ulp1 protease treatment is used to confirm the identity of SAS-AAGAB psGD. (b) GST pull-down gel of GST-AP2 σ 1 co-expressed with SAS tagged AAGAB psGD WT, E144K, or V139I mutant. Ulp1 protease treatment is used to confirm the identity of SAS-AAGAB GD. (c) A representative thermostability assay of AAGAB psGD WT, E144K, and V139I at the protein concentration of 5 μ M. The fluorescence values are normalized to the maximum value at the highest temperature point for each psGD protein. (d) Summary of the melting temperatures (*T*m) for AAGAB psGD WT, E144K, and V139I at three different protein concentrations.



Supplementary Figure 5 Expression levels of AAGAB WT and psGD mutants in cells

The expression levels of indicated protein were normalized to WT AAGAB samples. Data are presented as mean±s.d., n=3. n.s., P>0.05. P values were calculated using one-way ANOVA and Dunnett's multiple comparison test. The representative immunoblots are shown in Figure 3e.