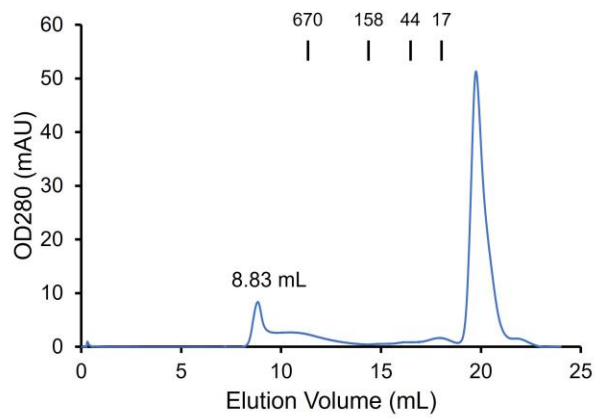
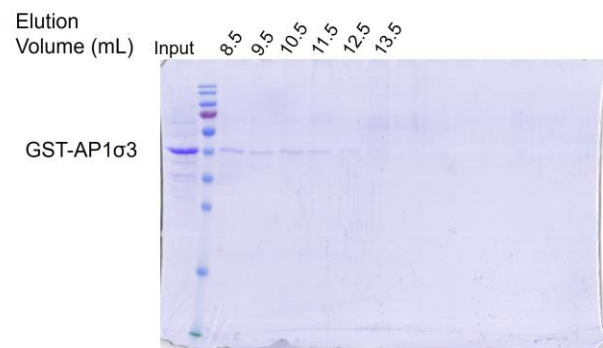


Supplementary Figure 1 AAGAB N-terminal region is a pseudoGTPase domain

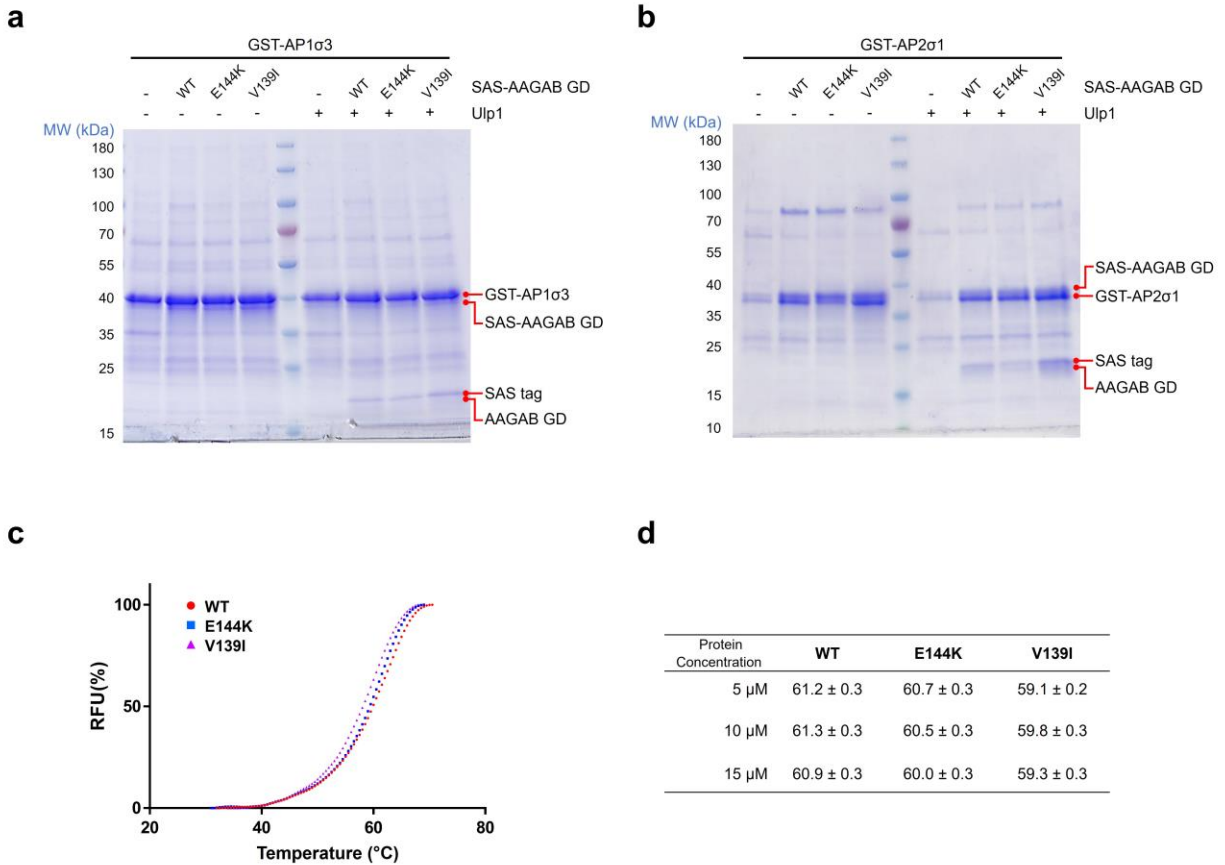
(a) Sequence alignment of human AAGAB N-terminal region with human HRas. Identical and similar residues are marked by *, : and .. The five G motifs in HRas are underlined, and consensus residues are in bold. α helices and β strands are highlighted in cyan and pink, respectively. (b) Size-exclusion chromatography (SEC) coupled multi-angle 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).

a**b**

Supplementary Figure 2 AAGAB psGD assists in σ subunit folding

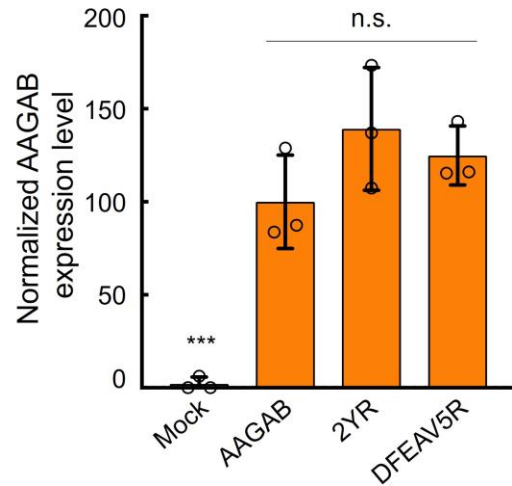
(a) Size exclusion chromatography profile of GST-AP1 σ 3 on a Superdex 200 10/300 column. Protein standards with known molecular weight are marked at the top. (b) SDS-PAGE of elution fractions in (a).

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; AP2 σ 1: P53680; AP1 σ 1: P61966; AP1 σ 2: P56377; AP4 σ 1: 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-AP1 σ 3 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-AP2 σ 1 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-AP2 σ 1 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 \pm 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.