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Supplementary information

Structurally derived universal mechanism for the catalytic cycle of the tail-anchored targeting factor Get3

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Structurally derived universal mechanism for the catalytic cycle of the tail-anchored targeting factor Get3

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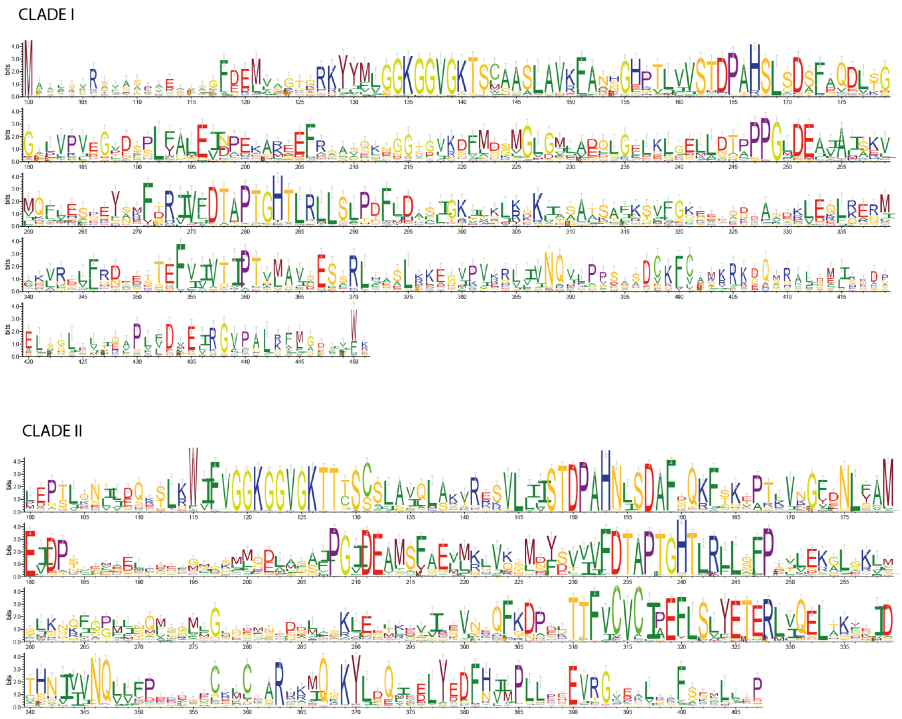

Fig. S1: **Conservation across clades of of eukaryotic Get3s**
Sequence alignments for each clade are visualized using HMM logo plots where residues size correlate to prevalence in the alignment (Clade I, *top* and Clade II, *bottom*) [1]. There are no distinct motif differences between the clades.

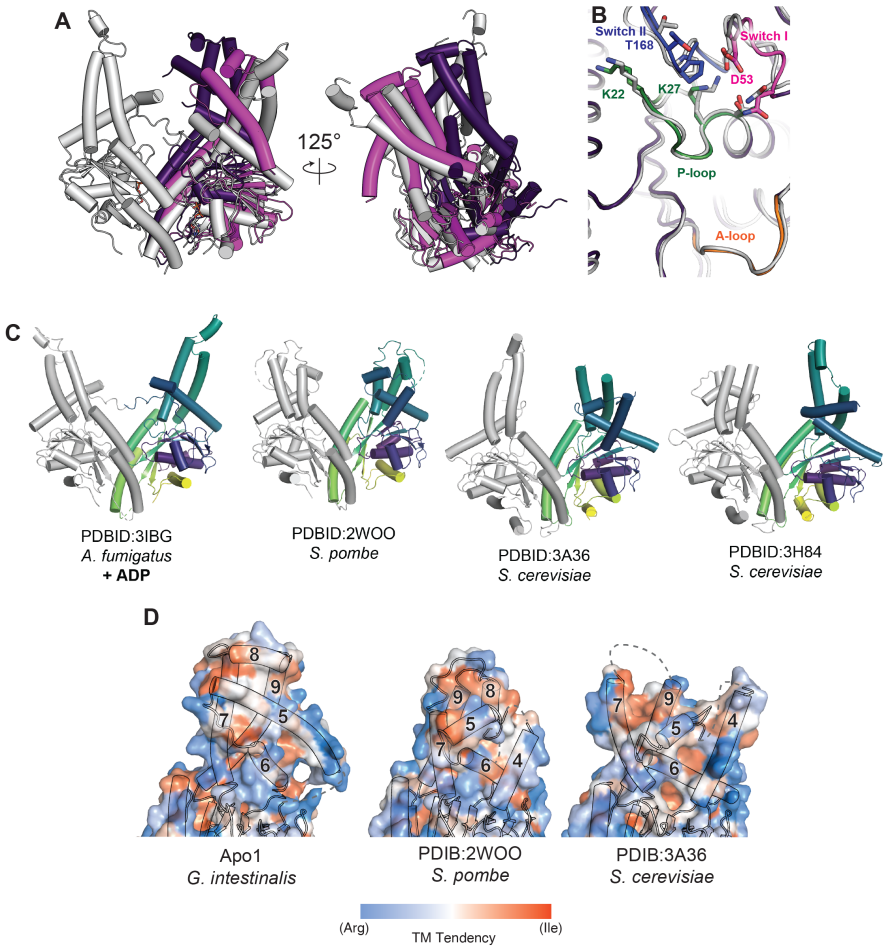

Fig. S2: **Comparison of apo *Gi*Get3 with various structures of opisthokont Get3 homologs in the 'open' state.**

A) Two views of *Gi*Get3 apo1 (*magenta*) and apo2 (*purple*) aligned with chain A of *Af*Get3 (*grey*, PDBID:3IBG) [2]. *B)* A comparison of the active sites of *Gi*Get3 apo2 (*purple*) and *Af*Get3 (*grey*). The P-loop, A-loop, and Switch I & II are colored as in Fig. 2. *C*) Cartoon representations of fungal Get3 homologs in the open state (PDBIDs:3IBG, 2WOO, 3A36, & 3H84). Chain A is colored in grey and chain B is colored from N- to C-terminus using the viridis color map (*purple to yellow*). Species and ligands are specified below the PDBID numbers. *D*) Surface and cartoon representations of the CBD of 2WOO, 3A36, and Apo1 where residues are colored by hydrophobicity using the TM Tendency scale [3] and helices are numbered as in Fig. 2

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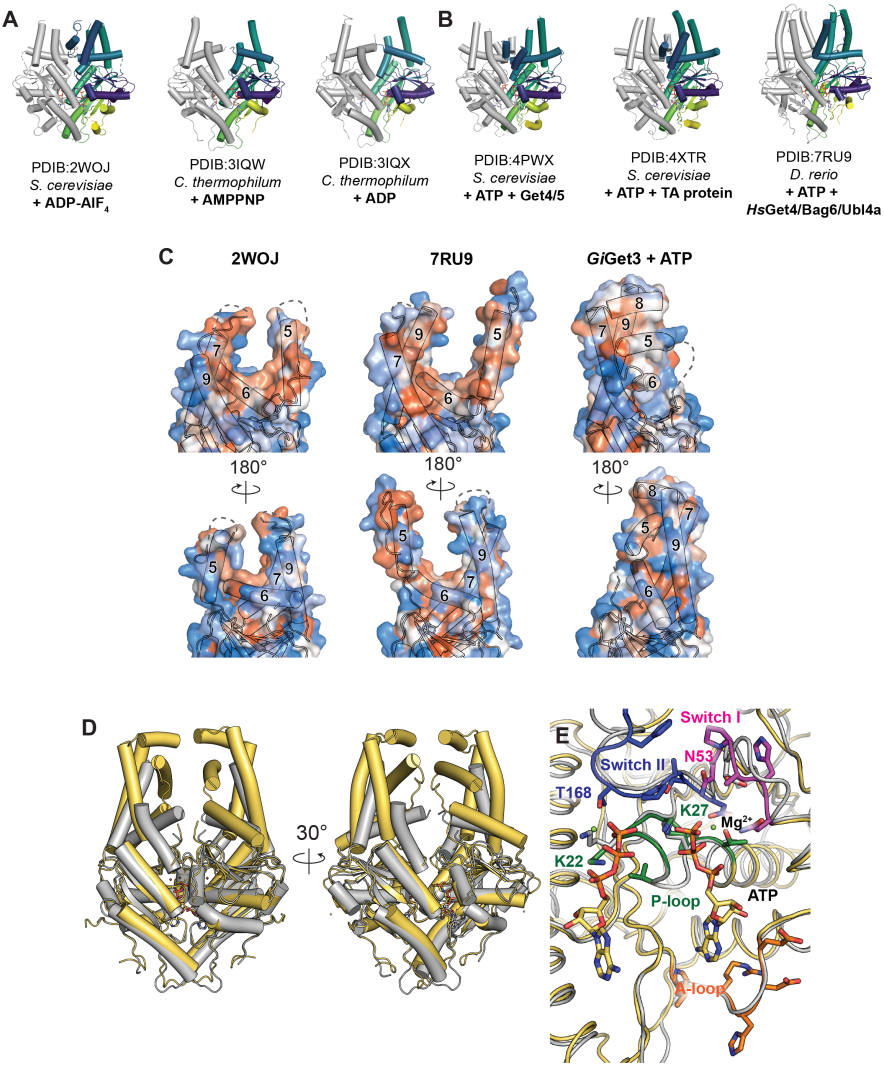

Fig. S3: **Comparison of ATP-bound *Gi*Get3 to structures of opisthokont Get3s in the 'closed' state.**

Cartoon representation of *A*) fungal Get3s bound to nucleotide in the 'closed' state (PDBIDs:2WOJ, 3IQW, and 3IQX) [4, 5] and *B*) opisthokont Get3s in complex with other GET components (PDBIDS:4PWX, 4XTR, and 7RU9) [6–8]. For *A* & *B*, chain A is colored in grey and chain B is colored from N- to C-terminus using the viridis color map (*purple to yellow*). Species and ligands are specified below the PDBID numbers. *C*) Two views of surface and cartoon representations of the CBD of 2WOJ, 7RU9, and *Gi*Get3$_{D53N}$·ATP where residues are colored by hydrophobicity using the TM Tendency scale [3] and helices are numbered as in Extended Data Fig. 3. *D)* Two views of *Gi*Get3$_{D53N}$· ATP (*yellow*) and *Ct*Get3·AMPPNP (*grey*) aligned by chain A. *E*) A comparison of the active sites of *Gi*Get3 (*yellow*) and *Ct*Get3 (*grey*). Nucleotide and important catalytic loops are shown as sticks and colored as in Extended Data Fig. 3.

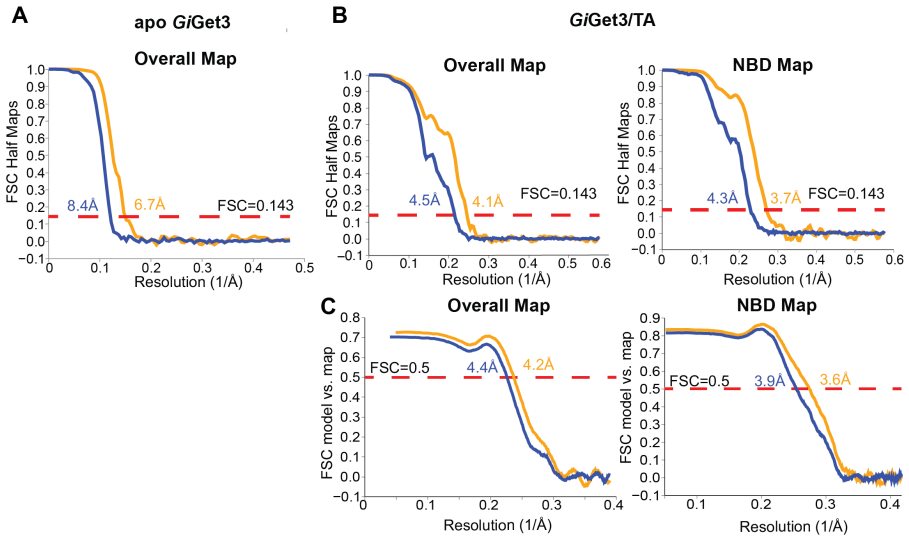

Fig. S4: **SPA map and model quality**
The Gold-standard Fourier Shell Correlation curves for 'masked' (*orange*) and 'unmasked' (*blue*) half maps used to build the (*A*) *Gi*Get3 and (*B*) *Gi*Get3/TA complex maps from RELION 3.1.2. While a complete model could be built into the overall map, a focused refined map of the NBD improved the local resolution and provided more detail. Resolution was determined using an FSC=0.143 criterion. *C*) The FSC curves of the model vs. map for the 'unmasked' (*blue*) and 'masked' (*orange*) *Gi*Get3/TA complex model. Overall refers to the overall map and NBD refers to the focused refined map. Resolution was determined using an FSC=0.5 criterion.

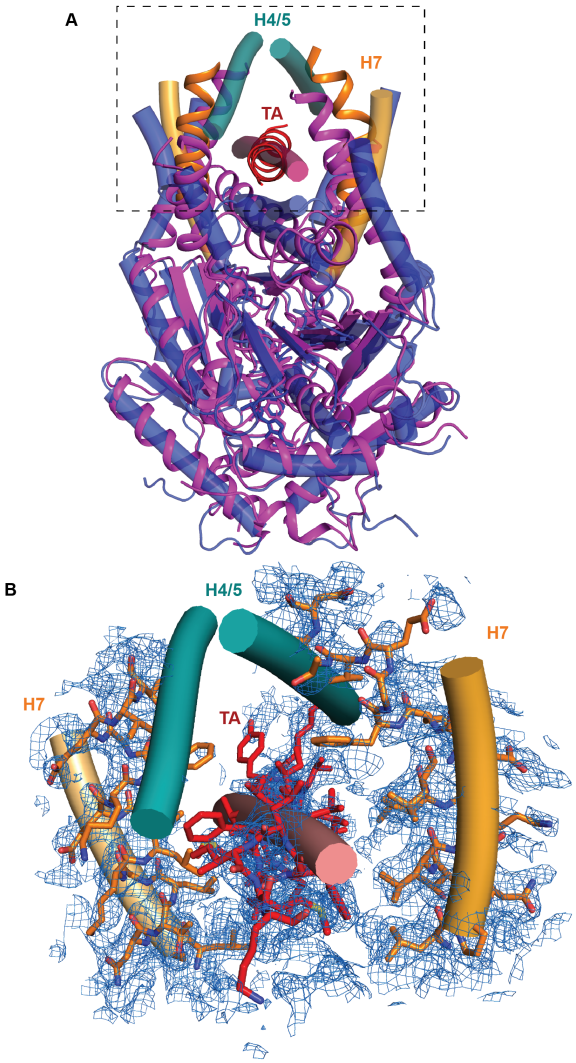

Fig. S5: **Comparison of Get3 bound to the TA protein before and after hydrolysis**
A comparison of the CBD of the Get3/TA complex (PDBID:4XTR) before (ribbons and sticks) and after hydrolysis (cylinders). *A*) Overlay of the crystal structure of *Sc*Get3·D57N bound to the TMD of the TA protein, Pep12, (*magenta*, ribbon) and our EM structure of *Gi*Get3 bound to the TMD of the TA protein, Bos1 (*blue*, cylinders). Helices of interest are colored as they are in (*B*), H7 in orange, H4/5 in teal, and the TA protein in red. *B*) Density from the crystal structure of the transition state yeast complex contoured to 0.8 sigma [7] with the corresponding model displayed as sticks (PDBID:4XTR). The aligned SPA-EM model of the post-hydrolysis *Gi*Get3/TA complex is shown as cylinders. The overlay demonstrates how the TA protein is fully protected in the post-hydrolysis form with H4/5 and this conformation is not compatible with the transition state density. There lacks room for H4/5 in the hydrophobic groove in the transition state which requires the opening of *Gi*Get3 CBD when bound to TA protein [9].

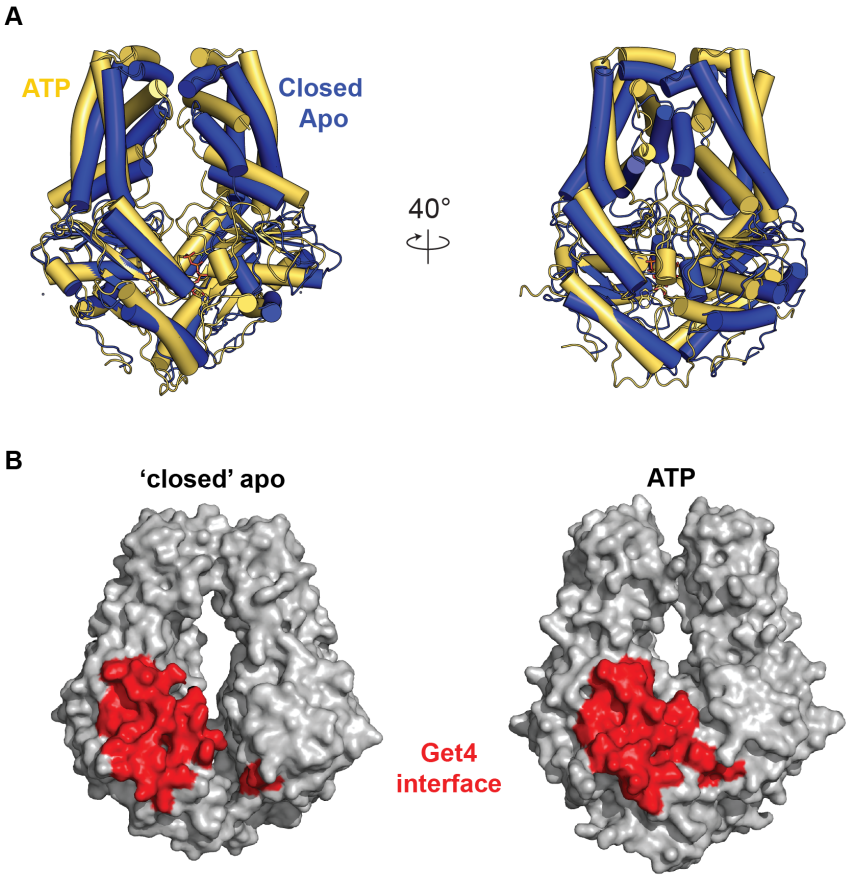

Fig. S6: **Comparison of ATP-bound and the closed apo conformations of *Gi*Get3**
A) Two views of an alignment of the *Gi*Get3$_{D53N}$·ATP (*yellow*) and 'closed' apo *Gi*Get3 (*blue*). *B*) A surface representation of the 'closed' apo and *Gi*Get3$_{D53N}$·ATP with residues that bind to Get4 colored in red. Note that the full interface requires ATP.

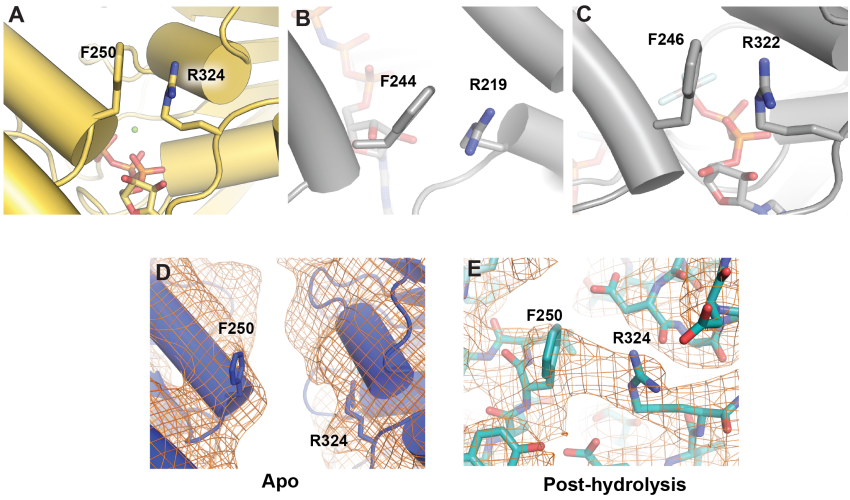

Fig. S7: **A cation-pi interaction helps to stabilize the 'closed' conformation**
A close up view of Phe_{250} and Arg_{324} (shown as sticks) for the *A) Gi*Get3$_{D53N}$ with ATP, *B) Ct*Get3·AMPPNP (PDBID:3IQW), and *C) Sc*Get3·ADP-AlF_4 (PDBID:2WOJ), *D*) closed *Gi*Get3 apo form where the two residues are too far apart to form this stabilizing interaction, and *E*) post-hydrolysis *Gi*Get3/TA bound forms where the two residues are close enough to form a cation-pi interaction. Density for the EM structures (*D,E*) is shown filtered to a 7.5 sigma.

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