

## SUPPLEMENTAL INFORMATION

### **Melanophilin Stimulates Myosin-5a Motor Function by Allosterically Inhibiting the Interaction between the Head and Tail of Myosin-5a**

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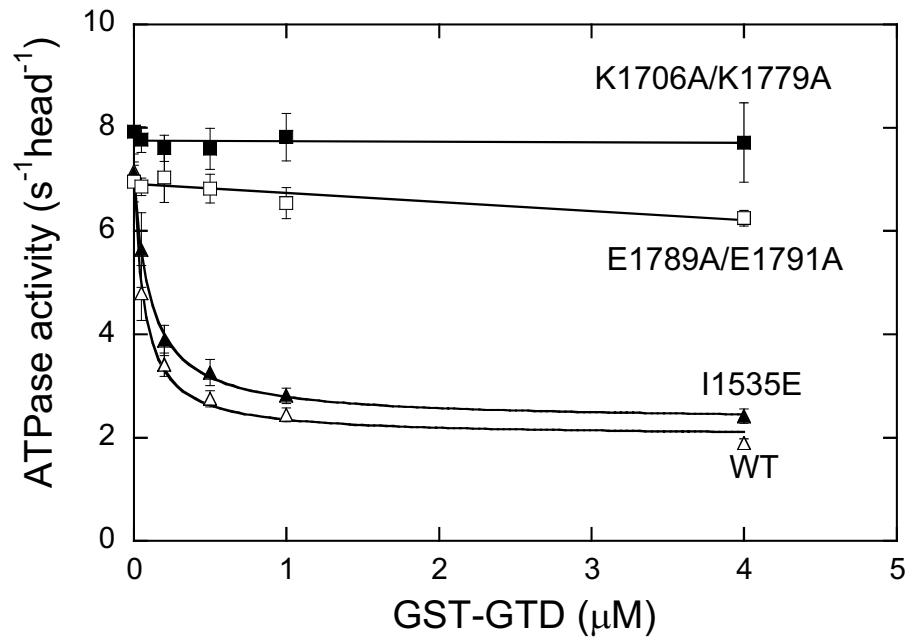
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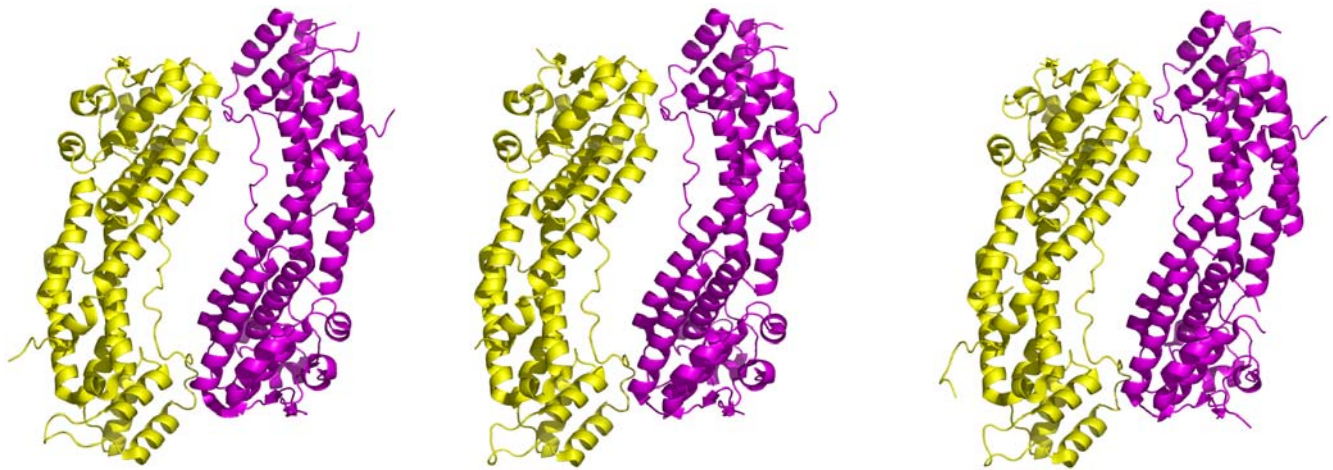
## Supplemental Figure 1



**Figure S1. The effects of GTD mutants on the Myo5a-HMM ATPase activity.** The Myo5a-HMM ATPase activity was strongly inhibited by GST-GTD-WT and -I1535E mutant. In contrast, the Myo5a-HMM ATPase activity was not inhibited by GST-GTD-K1706A/K1779A mutant and only weakly inhibited by GST-GTD-E1789A/E1791A mutant. The ATPase assays were conducted in 20 mM MOPS-KOH, pH 7.0, 0.1 M NaCl, 1 mM MgCl<sub>2</sub>, 1 mM DTT, 0.25 mg/ml BSA, 12  $\mu\text{M}$  CaM, 0.5 mM ATP, 2.5 mM phosphoenolpyruvate, 20 U/ml pyruvate kinase, 1 mM EGTA, 40  $\mu\text{M}$  actin, ~30 nM Myo5a-HMM, and 0 - 4  $\mu\text{M}$  GST-GTD. Data are means  $\pm$  std from three independent assays.

## Supplemental Figure 2

A

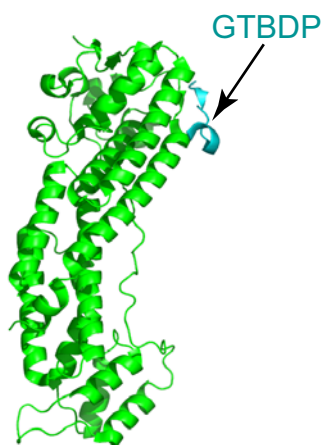


Mouse Myo5a-GTD  
(PDB ID: 3WB8)

Human Myo5a-GTD  
(PDB ID: 4LX1)

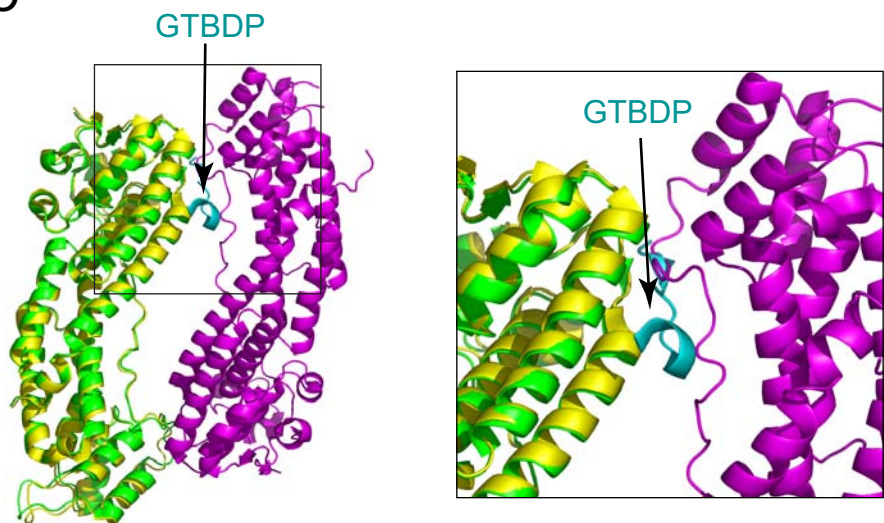
Human Myo5a-GTD  
(PDB ID: 4J5L)

B



Human M5a-GTD/GTBDP  
(PDB ID: 4LX2)

C



Superimposed human M5a-GTD and M5a-GTD/GTBDP  
(PDB IDs: 4LX1, 4LX2)

**Figure S2. GTBDP binds to the interface of Myo5a-GTD dimer.** (A) The crystal structures of human Myo5a-GTD and mouse Myo5a-GTD. There are 4 dimers in the crystal structure of mouse Myo5a-GTD and only one dimer (chain a and c) is shown here. (B) The crystal structure of human Myo5a-GTD/GTBDP complex. (C) Superimposed structures of human Myo5a-GTD (shown in yellow and magenta) and Myo5a-GTD/GTBDP complex (shown in green and cyan).