Supporting Information

Wu et al. 10.1073/pnas.1110415108

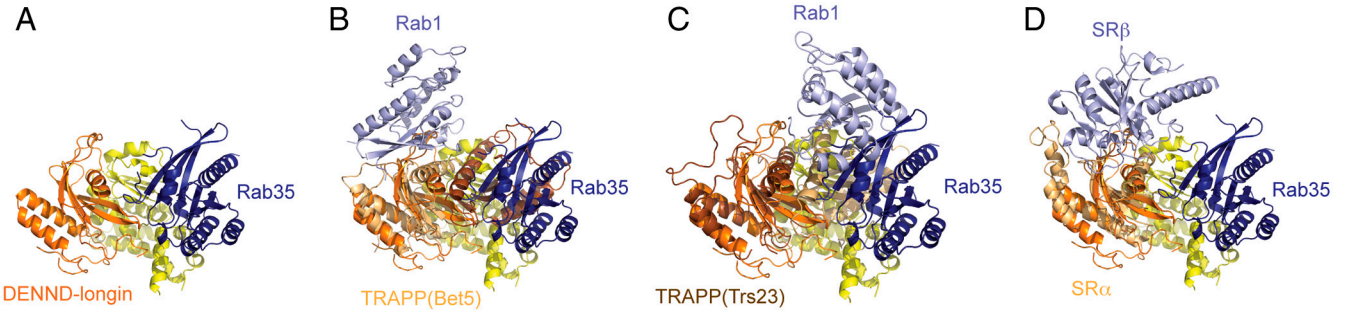

Fig. S1. Longin domains use different surfaces to interact with small GTPases. (*A*) The Rab35/DENND1B complex. The Rab is blue, the DENND1BS N-terminal longin domain orange, and the DENND1BS C terminus yellow. (*B*) DENND1BS/Rab35 as in *A*, with the Rab1/Bet5/Trs23 assembly from the Rab1/TRAPP structure (PDB ID code 3CUE). The Bet5 longin domain is light orange, that of Trs23 is brown, and Rab1 is light blue. The Rab1/TRAPP subcomplex is oriented so that the Bet5 longin domain is superimposed onto the longin module of DENND1B. Rab1 and Rab35 do not superimpose because the Rabs interact with different surfaces in the longin domain. (*C*) As in *B*, except that the longin domain of Trs23 in the Rab1/TRAPP subcomplex is superimposed on the DENND1B N terminus. (*D*) A subcomplex of the signal recognition particle receptor comprising a longin domain is subunit α (light orange) and a small Ras-like GTPase in subunit β (PDB ID code 1NRJ, light blue), oriented so that the α subunit is superimposed on the DENND1B longin module.

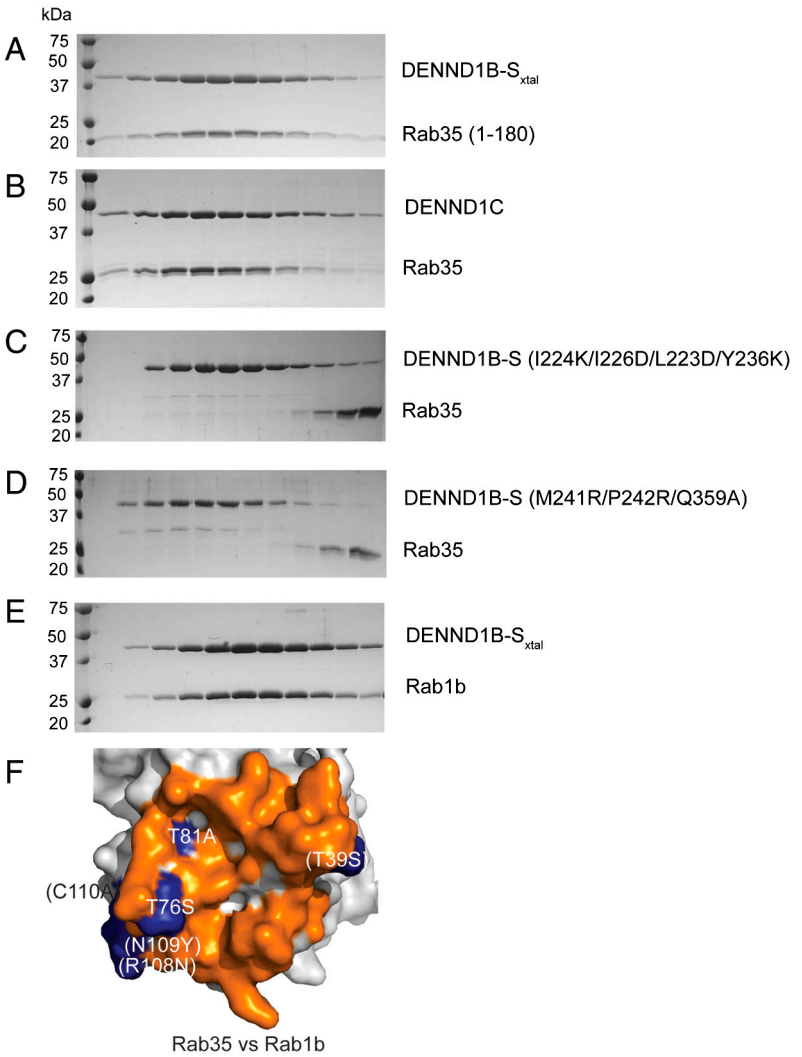

Fig. S2. Complex formation between DENN-domain constructs and Rabs. The DENN-domain construct and Rab were mixed in the presence of EDTA, then run on a Superdex75 sizing column (GE Healthcare). Fractions from the column were analyzed by SDS/PAGE gel. The DENN domain and Rab comigrate on the column when a stable complex forms. (*A*) The DENND1B-S construct used for crystallization forms complex with Rab35 (residues 1–180). (*B*) DENND1C (1–424) forms a stable complex with Rab35. (*C* and *D*) The DENND1B-S mutants (I224K/I226D/L233D/Y236K and M241R/P242R/Q359A) do not comigrate with Rab35. (*E*) DENND1B-S$_{xtal}$ comigrates with Rab1b. (*F*) The Rab35 surface that interacts with DENND1B is highly conserved in Rab1b. The DENND1B footprint—Rab35 surface residues within 5 Å of DENND1B—is colored orange, and residues in Rab35 within 10 Å of DENND1B that are different in Rab1b are indicated (blue). Residues further than 5 Å from DENND1B are in parentheses.

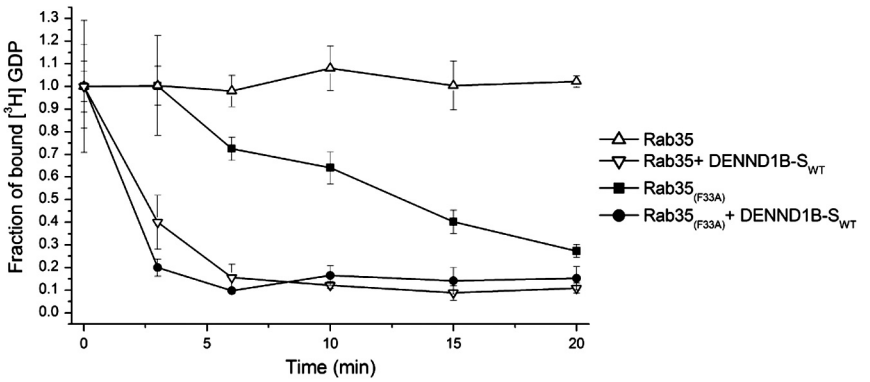

Fig. S3. DENND1B-S facilitates nucleotide exchange for the Rab35 F33A mutant as well as wild-type Rab. Nucleotide release was measured by quantitating the release of tritiated GDP (45 Ci/mmol, PerkinElmer) using a filter-based binding assay as in ref. 1. The reaction was performed at 10 °C in the presence of 0.2 μM of the indicated Rab and 0.4 μM DENND1B-S.

1 Cai Y, et al. (2008) The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. *Cell* 133:1202–1213.

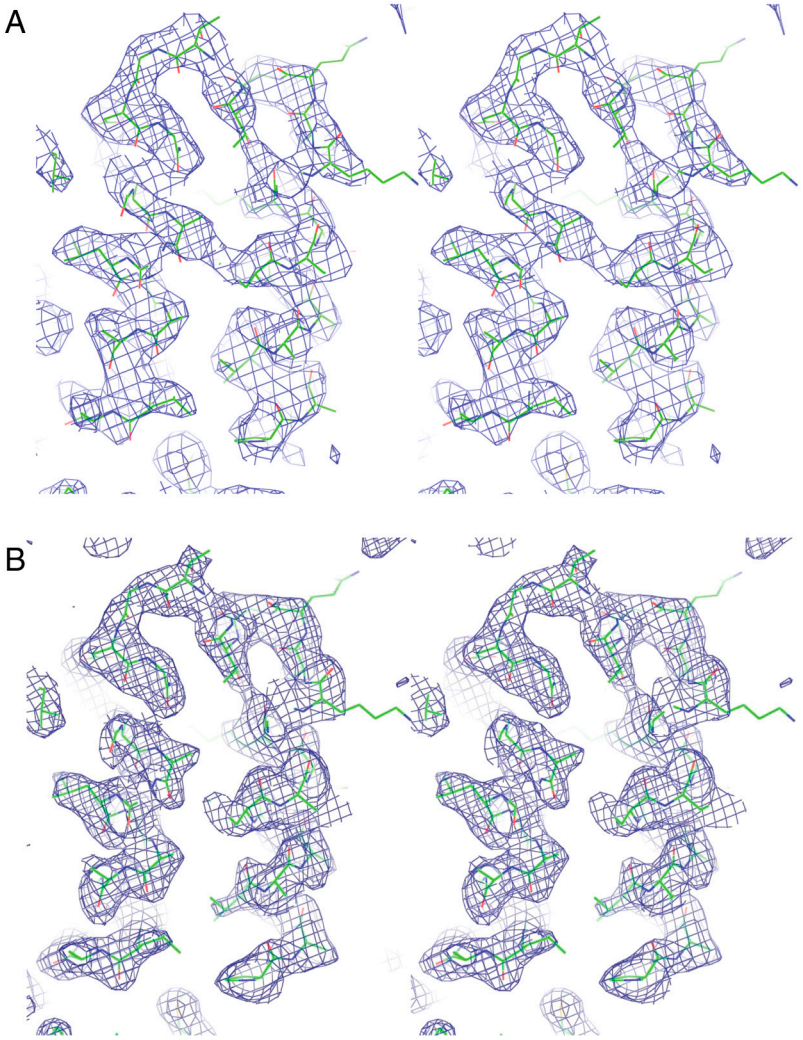

Fig. S4. Stereo images for an equivalent region of the SAD map and the final 2fo-fc map. (*A*) The experimental map is contoured at 1.0σ. (*B*) The 2fo-fc map is also contoured at 1.0σ.

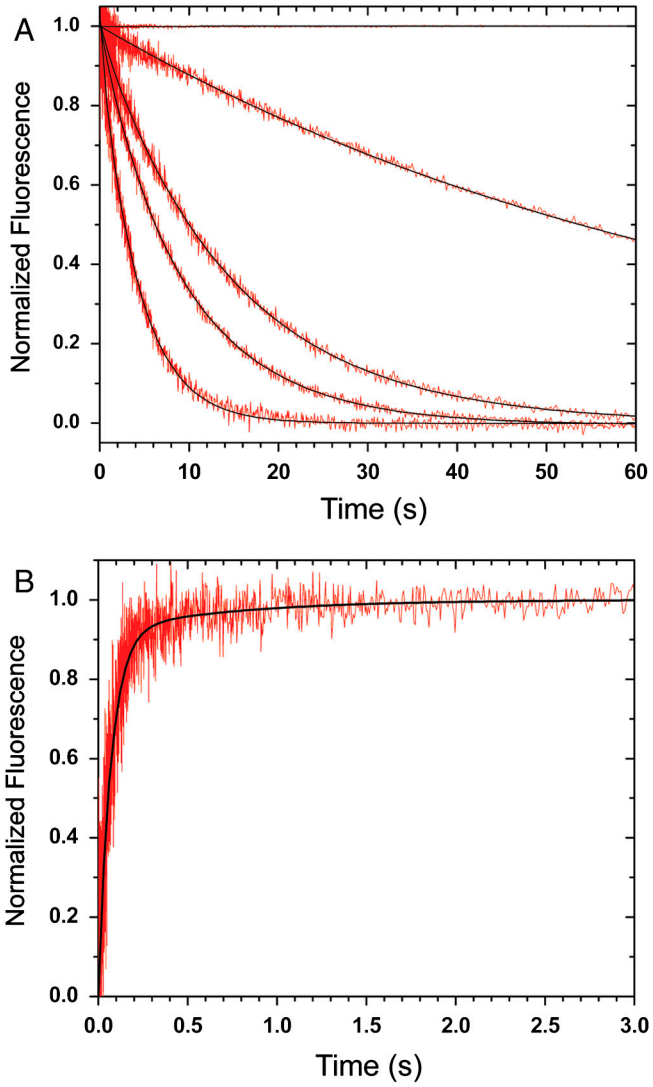

Fig. S5. Representative time courses of dissociation or association of mantGDP from Rab35. (*A*) Time course of mantGDP release from Rab35 stimulated by DENN domains. For these experiments, 0.25 μM mantGDP loaded Rab35 was quickly mixed (upper to lower curve) with 0 μM DENN domain, 4 μM DENND1C (1–424), 2.5, 4, or 8 μM DENND1B-S_{WT}. The solid line is the best fit to a single exponential function with a slow linear component, which generates the observed rate constant (k_{obs}). These data were used for preparation of Fig. 1*B*. (*B*) Time course of mantGDP association with nucleotide-free Rab35. For this experiment, 0.5 μM nucleotide-free Rab35 was quickly mixed (within 10 min, following addition of 5 mM EDTA at 25 °C) with 5 μM mantGDP. The solid line is the best fit to a double exponential function. These data were used to prepare Fig 3*H*.