Supporting Information

Bankston et al. 10.1073/pnas.1201997109

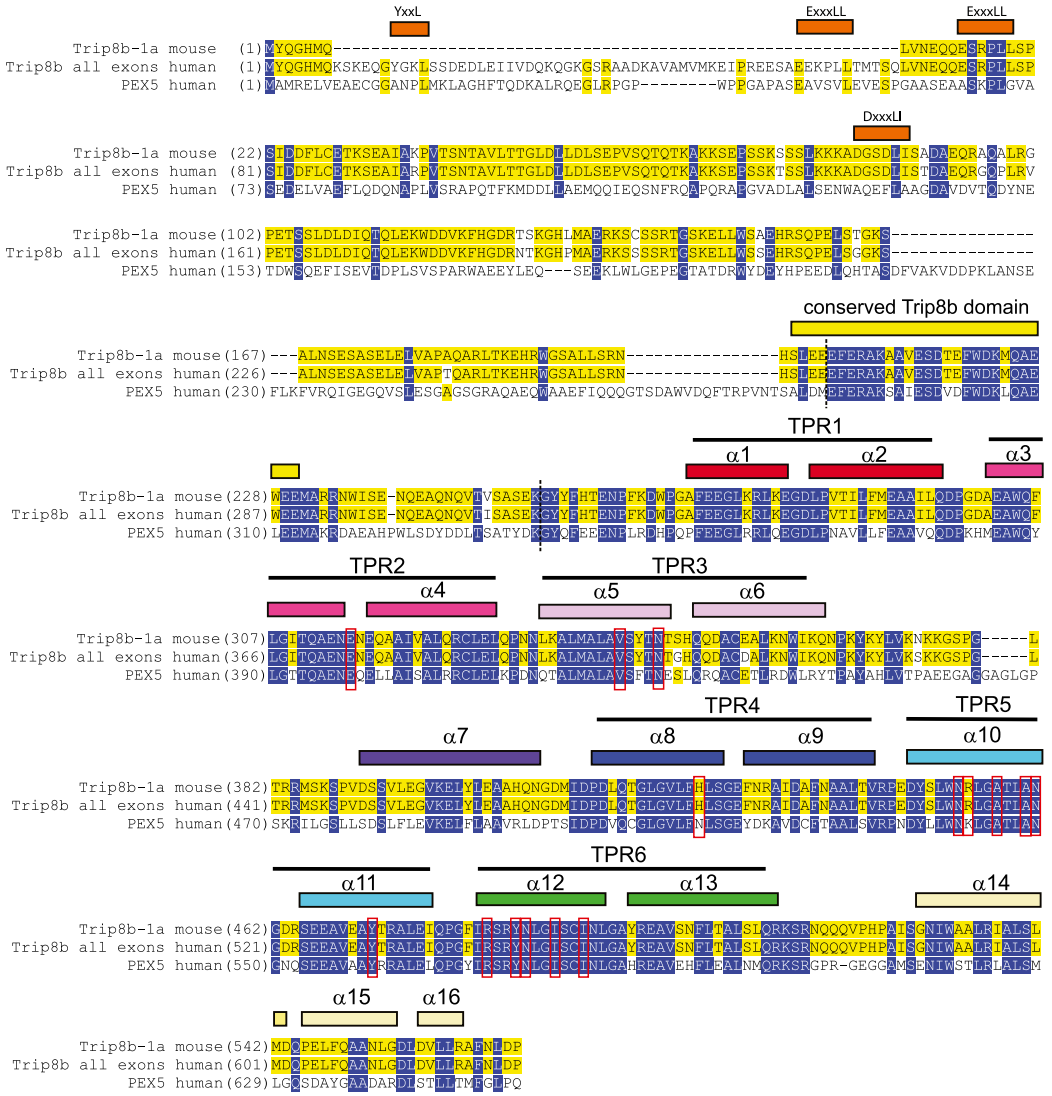

Fig. S1. Sequence alignment of tetratricopetide repeat-containing Rab8b-interacting protein TRIP8b and the related protein peroxin-5 (PEX5). The numbering system in this study is based on isoform TRIP8b-1a (top row). The second row shows the sequence of all potential exons of TRIP8b and does not represent a real protein. The bottom row shows the sequence of the related PEX5 protein. Orange bars denote four trafficking motifs that are known to recruit distinct sets of proteins involved in membrane protein trafficking. The yellow bar denotes the TRIP8b conserved domain, a region that is 100% conserved in all orthologs of TRIP8b, which is thought to be involved in binding to the cyclic nucleotide-binding domain of hyperpolarization-activated cyclic nucleotide-gated channels. The remaining colored bars denote the helices found in the X-ray crystal structure, and the colors match the color scheme in Fig. 4*A*. Dashed lines denote the start of the TRIP8b-1aΔ1–205 and TRIP8b-1aΔ1–254 proteins.

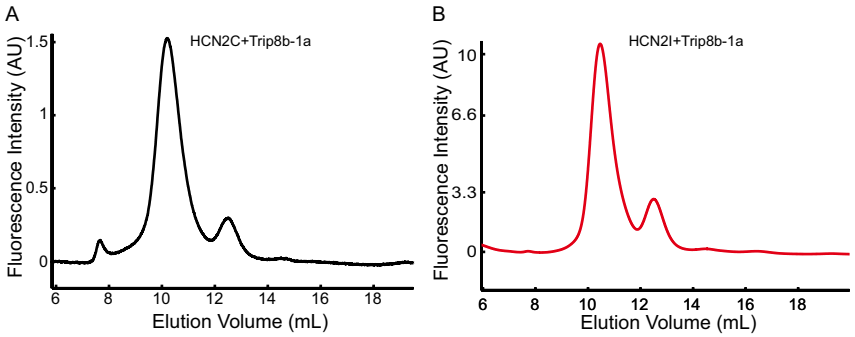

Fig. S2. TRIP8b-1a/HCN2 remains intact after affinity purification. (*A*) Fluorescence-detection size-exclusion chromatography (FSEC) results for the TRIP8b-1a/HCN2C complex after purification on a Ni^{2+} column and removal of the EGFP and histidine tags. A fluorescence signal, excited at 280 nm and measured at 350 nm, arises from the tryptophans present in TRIP8b-1a. The peak at 10.2 mL corresponds to the peak that represents the complex, and the smaller peak at 12.5 mL corresponds to a small fraction of unbound TRIP8b-1a. No peak for HCN2C is seen because there are no tryptophans in the protein. (*B*) FSEC results for the TRIP8b-1a/HCN2I complex after purification on a Ni^{2+} column and removal of the EGFP and histidine tags. The peak at 10.4 mL corresponds to the peak that represents the complex, and the smaller peak at 12.7 mL corresponds to a small fraction of unbound TRIP8b-1a.

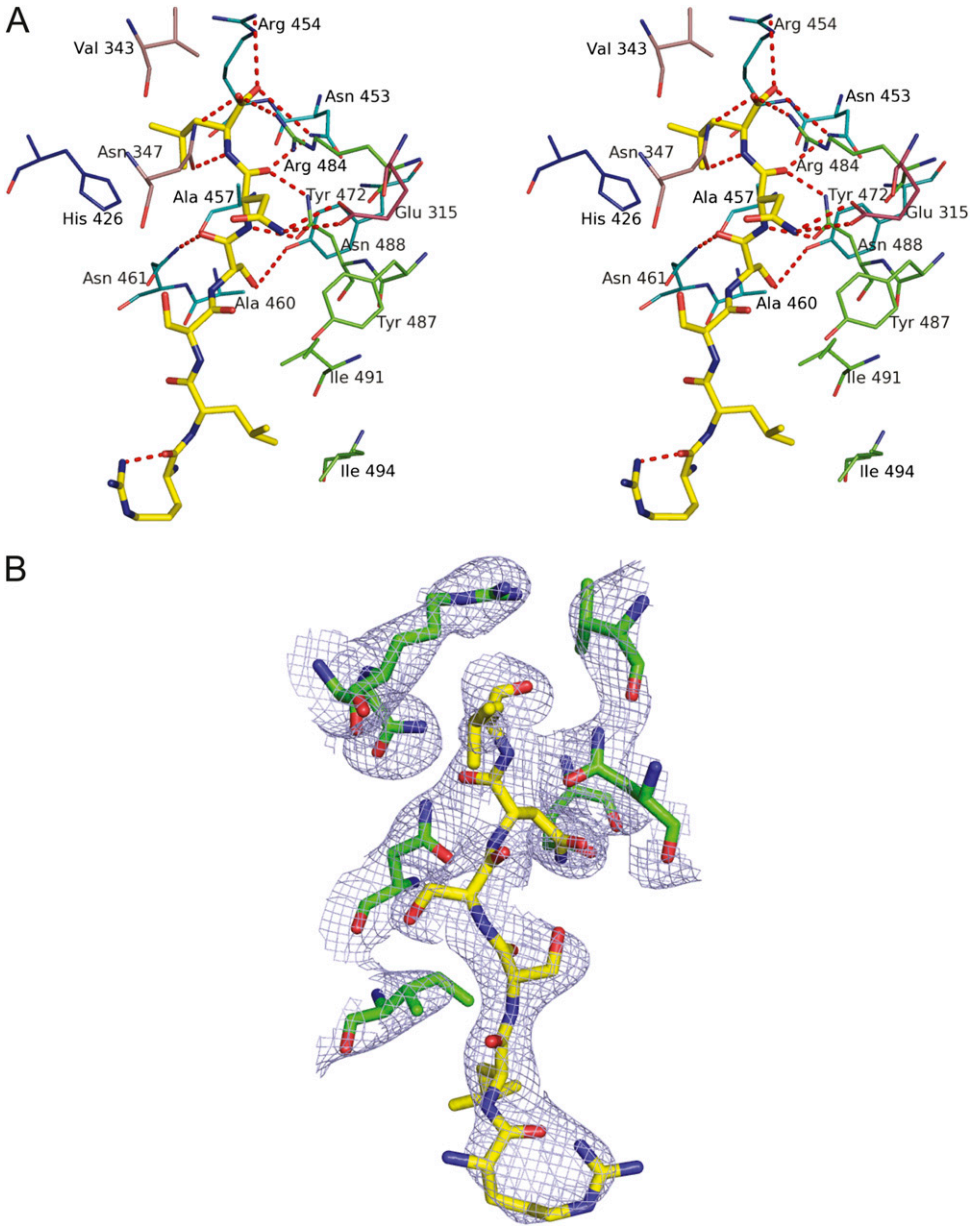

Fig. S3. (*A*) Stereoview of the major residues involved in the binding site for TRIP8b and HCN2. The HCN2 peptide is rendered as sticks and colored by element, and the residues on TRIP8b are rendered as lines and colored by elements. The colors of the carbons on TRIP8b match the color scheme of the ribbon representation in Fig. 4*A*. Polar contacts are shown as dashed red lines. (*B*) 2Fo-Fc "omit" map of the HCN2 peptide and some of the interacting residues on Trip8b.

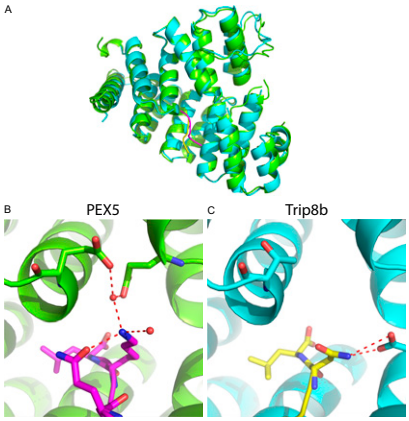

Fig. S4. Overlay of the PEX5 and TRIP8b structures. (*A*) The structures of TRIP8b (cyan) and PEX5 (accession code 1FCH, green) overlayed. (*B*) Binding site for the side chain of the lysine at position −2 of PTS1. Red spheres represent two of the water molecules seen in the PEX5 structure. The green residues are from PEX5, and the magenta residues are from its cargo PTS1. (*C*) Binding site for the side chain of the asparagine at position −2 in HCN2 channels. TRIP8b is shown in blue and HCN2 is shown in yellow.

Table S1. Data collection and refinement statistics

	Trip8b-1a
Data collection	
Space group	$P4_12_12$
Molecules/asymmetric unit	1
Cell dimensions	
a,*b*,*c* (Å)	72.32, 72.32, 146.74
α,β,γ (°)	90, 90, 90
Wavelength (Å)	1.0
Resolution (Å)*	48.91–3.00 (3.16–3.00)
R_{merge} (%)†	9.20 (99.4)
$I/\sigma I$	15.1 (2.6)
Completeness (%)	100.0 (100.0)
Redundancy	13.0 (13.7)
Refinement	
Resolution (Å)	48.29–3.00
No. reflections	8364
R_{work}/R_{free}	0.231/0.284
No. atoms	2,260
β-Factor	109.70
rms deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.136
Ramachandran plot statistics	
% favored	95
% allowed regions	5
% disallowed	0

*Numbers in parentheses in this row define the highest resolution shell of data.
†Numbers in parentheses in this row are the statistics for the highest resolution shell of data.