

**Supplemental Information**

**The Ciliopathy-Associated Cep104 Protein**

**Interacts with Tubulin and Nek1 Kinase**

**Caezar Al-Jassar, Antonina Andreeva, Deepak D. Barnabas, Stephen H. McLaughlin, Christopher M. Johnson, Minmin Yu, and Mark van Breugel**

## **Table S1, related to Table 2**

**Human TOG Cep104<sup>392-676</sup> SeMet MAD dataset analysis.**

Beamline	ID29 (ESRF)	
Space Group	P21	
Wavelength (Å)	0.97916 (peak)	0.97923 (inflection)
Unit Cell dimensions (Å)	a=51.8 b=53.3 c=154.4 $\alpha$ =90.0 $\beta$ =91.8 $\gamma$ =90.0	a=52.1 b=53.3 c=155.4 $\alpha$ =90.0 $\beta$ =92.0 $\gamma$ =90.0
Resolution (Å)	49.5 – 2.3	49.54 – 2.3
Completeness (overall / inner / outer shell)	99.6 / 99.1 / 99.5	99.5 / 98.7 / 99.5
Rmerge (overall / inner / outer shell)	0.145 / 0.045 / 1.050	0.127 / 0.034 / 0.987
Rpim (overall / inner / outer shell)	0.069 / 0.031 / 0.456	0.060 / 0.025 / 0.426
Mean I/oI (overall / inner / outer shell)	9.2 / 30.4 / 1.8	10.2 / 32.2 / 2.1
Multiplicity (overall / inner / outer shell)	6.9 / 6.5 / 7.1	6.9 / 6.5 / 7.1
Se sites found / expected	15 / 22 (16 SeMet residues are visible in the electron density map)	

## **Table S2, related to Table 2**

**Human ZNF Cep104<sup>746-875</sup> S763E Zn MAD dataset analysis.**

Beamline	I04 (Diamond Light Source)		
Space Group	P212121		
Wavelength (Å)	1.28295 (peak)	1.28321 (inflection)	1.21984 (remote)
Unit Cell dimensions (Å)	a=73.9 b=80.5 c=118.3 $\alpha$ =90.0 $\beta$ =90.0 $\gamma$ =90.0	a=73.9 b=80.5 c=118.3 $\alpha$ =90.0 $\beta$ =90.0 $\gamma$ =90.0	a=73.9 b=80.5 c=118.3 $\alpha$ =90.0 $\beta$ =90.0 $\gamma$ =90.0
Resolution (Å)	80.5 – 2.2	80.5 – 2.2	80.5 – 2.2
Completeness (overall / inner / outer shell)	99.2 / 99.7 / 98.1	99.3 / 99.8 / 98.2	99.4 / 99.5 / 98.5
Rmerge (overall / inner / outer shell)	0.139 / 0.069 / 1.512	0.114 / 0.067 / 1.017	0.198 / 0.067 / 1.976
Rpim (overall / inner / outer shell)	0.046 / 0.024 / 0.510	0.038 / 0.025 / 0.342	0.065 / 0.024 / 0.657
Mean I/oI (overall / inner / outer shell)	11.3 / 30.5 / 1.9	12.0 / 28.3 / 2.5	9.6 / 28.4 / 1.8
Multiplicity (overall / inner / outer shell)	9.9 / 8.5 / 9.4	10.1 / 8.7 / 9.6	10.1 / 8.7 / 9.8
Zn found / expected	6 / 16		

**Figure S1, related to Figure 1A****Homology model of the N-terminal APC10-like domain of Cep104.**

A) Ribbon presentation of the N-terminal domain of Cep104 (rainbow-coloured from N- to C-terminus) together with an equivalent representation as molecular surface, coloured according to Consurf evolutionary conservation score from unconserved (cyan) to highly conserved (burgundy). **B)** Equivalent view onto the IFT25-IFT27 (pdb 2yc2) and the APC10-HSL1 (pdb 5g04) complexes. Note that IFT27 and HSL1 engage their corresponding APC10(-like) domain at a region that is conserved in the N-terminal, APC10-like domain in Cep104.

**Figure S2, related to Figure 1B-D****Multiple sequence alignments and structural analyses of Cep104's TOG domain.**

**A)** A structure-based, multiple sequence alignment of Cep104 TOG and other TOG domains with experimentally determined high-resolution structures (pdb 4ffb Stu2 TOG1, pdb 4g3a Mast/Orbit TOG1, pdb 2qk1 Stu2 TOG2, pdb 2qk2 Msps TOG2, pdb 4qmj ch-TOG TOG4, pdb 2of3 Zyg9 TOG3, pdb 3woy CLASP2 TOG2). Residues that were mutated in this study are marked with green circles. Structurally equivalent residues are shown in upper case. **B)** Multiple sequence alignment of Cep104 TOG of closely related homologs. Residues that were mutated in this study are marked with green circles. **C)** The tubulin-binding interface of Cep104 TOG is conserved and conformationally similar to other tubulin binding TOG domains. Globally fitted overlay of the ribbon representation of the Cep104 TOG structure with other TOG domains with experimentally determined high-resolution structures. Helices are displayed as cylinders. The top view onto the tubulin-binding interface reveals similar positions of the  $\alpha$ -helices and their connecting loops, while that is not observed when the domain is viewed from the bottom. Used PDB codes were: 4ffb (Stu2 TOG1), 2of3 (Zyg9 TOG3), 2qk1 (Stu2 TOG2), 2qk2 (Msps TOG2), 3woy (CLASP2 TOG2), 4g3a (Mast/Orbit TOG1), 4qmj (ch-TOG TOG4). **D)** Cep104 TOG structure displayed as molecular surface coloured according to Consurf evolutionary conservation score from unconserved (cyan) to highly conserved (burgundy). View onto the tubulin-binding interface (left) or onto the bottom of the TOG domain (right).

**Figure S3, related to Figure 2A****Candidates identified as putative Cep104 (proximity) interactors in BioID and cross-linking experiments.**

**A)** Top, Coomassie stained SDS-PAGE gel showing the elutions from a Cep104 pull-down (employing site-specific biotinylation of Avitag-WT BirA tagged Cep104 *in vivo*) in the presence or absence of cross-linking. The gel corresponds to ~10% of the material that was subjected to mass-spectrometric analysis. Bottom, Proteins identified by mass-spectroscopy that showed a ten-fold or higher enrichment of exclusive unique peptide counts compared to the control. **B)** Top, Coomassie stained SDS-PAGE gel showing ~10% of the elution from a Cep104 BioID experiment (biotinylation of proteins in the vicinity of BirA\* tagged Cep104 *in vivo*). The remainder of the elution was subjected to mass-spectrometric analysis. Bottom, proteins with a ten-fold or higher enrichment of exclusive unique peptide counts compared to the control.

**Figure S4, related to Figure 2B****The Cep104 ZNF domain is necessary for the Cep104-Nek1 interaction *in vivo*.**

Representative fluorescence micrographs of a re-routing and capture assay (Wong and

Munro, 2014) in U2OS cells with Nek1 and Cep104 constructs. N-terminally mCherry-tagged Cep104 or Cep104 $\Delta$ ZNF (Cep104 $^{\Delta 746-880}$ ), fused at their C-terminus to the HA tag and the outer-mitochondrial membrane targeting transmembrane domain of monoamine oxidase (MAO) were co-transfected with GFP-Nek1 into U2OS cells, fixed after 24 hours and visualized using a confocal microscope. A mitochondrial stain (MitoTracker Deep-red, ThermoFisher Scientific) and DAPI were used to mark mitochondria and DNA respectively. Please note that ectopically expressed GFP-Nek1 reroutes only to the mitochondrially anchored Cep104 when Cep104's ZNF domain is present. Scale bars, 5  $\mu$ m.

**Figure S5, related to Figure 3A**

**Binding of CP110 $^{906-936}$  and Nek1 to the Cep104 ZNF array is mutually exclusive.**

Right: Western blot showing a pull-down experiment with GST-Cep104 ZNF beads and lysates from Hek293 cells transiently overexpressing 3xFlag-tagged human Nek1 in the presence of an excess of recombinant CP110 $^{906-936}$  or *Danio rerio* STIL $^{404-448}$ . Please note that *Danio rerio* STIL $^{404-448}$  did not transfer/Ponceau-stain well as revealed by the Coomassie-stained SDS-PAGE gel of the input material shown on the left.

**Figure S6, related to Figure 4**

**Multiple sequence alignment and structural comparison of Cep104's ZNF domain.**

**A)** Marked with coloured lines and circles are the zinc-coordinating residues and the residues that constitute the three hydrophobic clusters that mediate the packing of the individual ZNFs into a globular domain. Indicated in red is residue S763 that was mutated (S763E) to increase solubility of the ZNF domain. **B)** The globular arrangement of the Cep104 ZNF array is novel. Comparison of the Cep104 ZNF domain with a similar ZNF array from TRAF6 (ribbon presentation, pdb 3hcs).

### **Supplementary References**

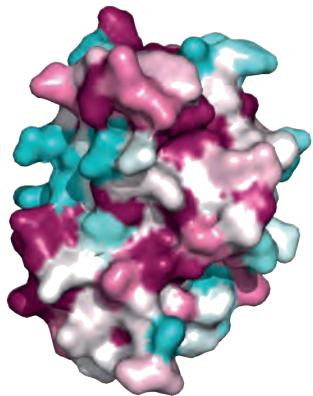
Wong, M., and Munro, S. (2014). Membrane trafficking. The specificity of vesicle traffic to the Golgi is encoded in the golgin coiled-coil proteins. *Science* (New York, NY) **346**, 1256898.

## Figure S1, related to Figure 1A

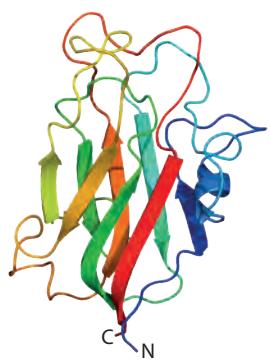
**A**

Homology model of  
Cep104 N-term. domain

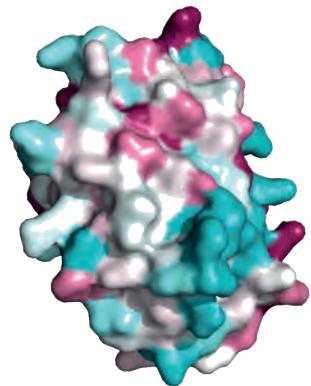
Conservation Scale  
1 2 3 4 5 6 7 8 9  
Variable Average Conserved



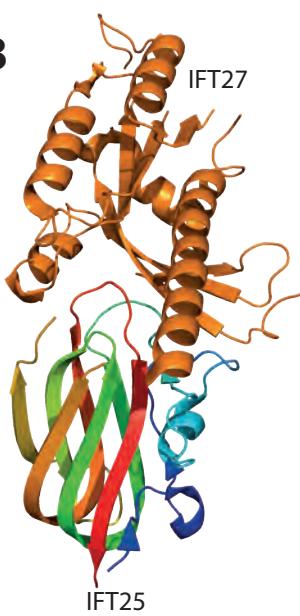
180°  
↻



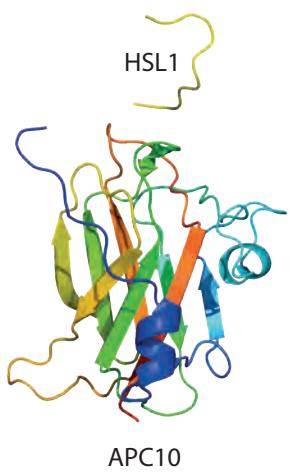
180°  
↻



**B**



IFT27



APC10

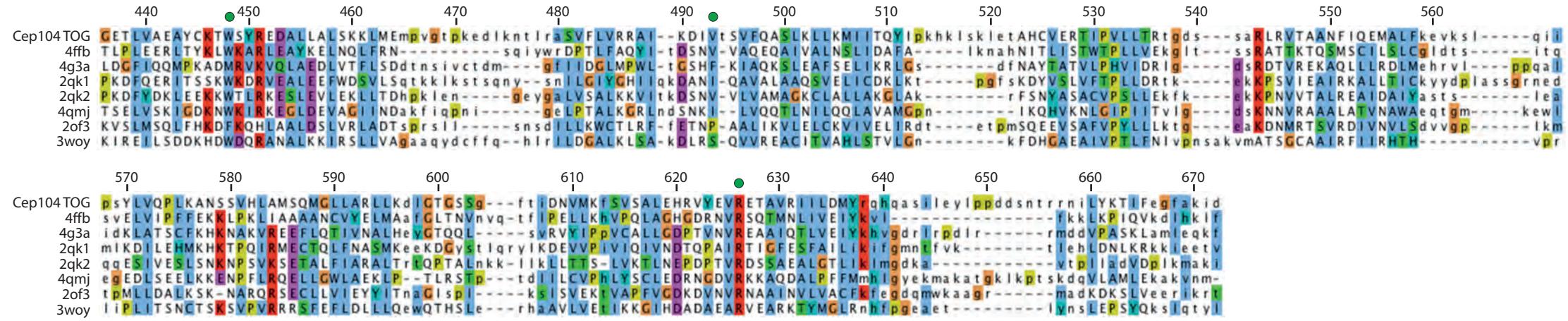


N C

## Figure S2, related to Figure 1B-D

**A**

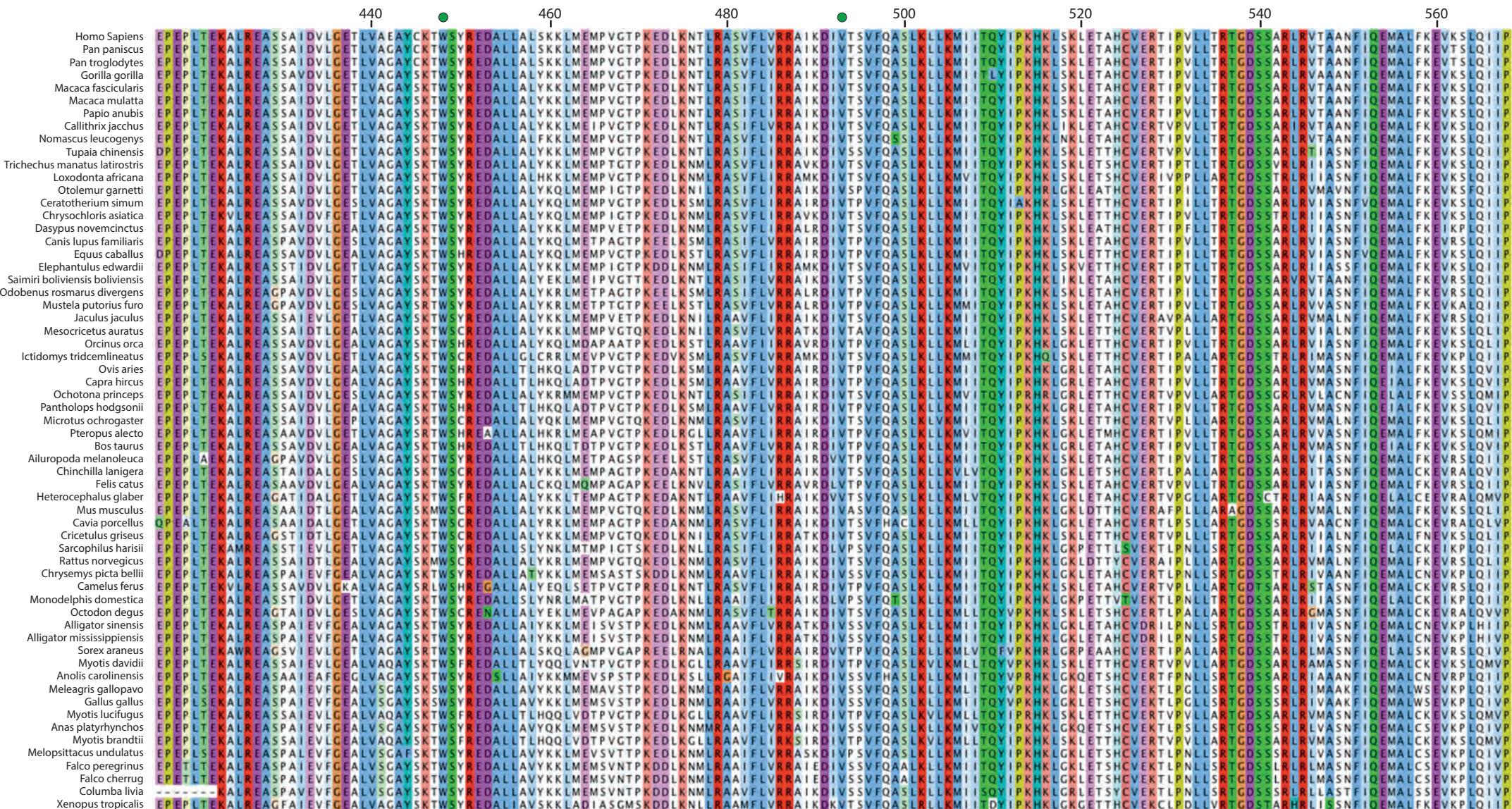
● Residues mutated in this study



**Figure S2, related to Figure 1B-D**

**B**

● Residues mutated in this study



# Figure S2, related to Figure 1B-D

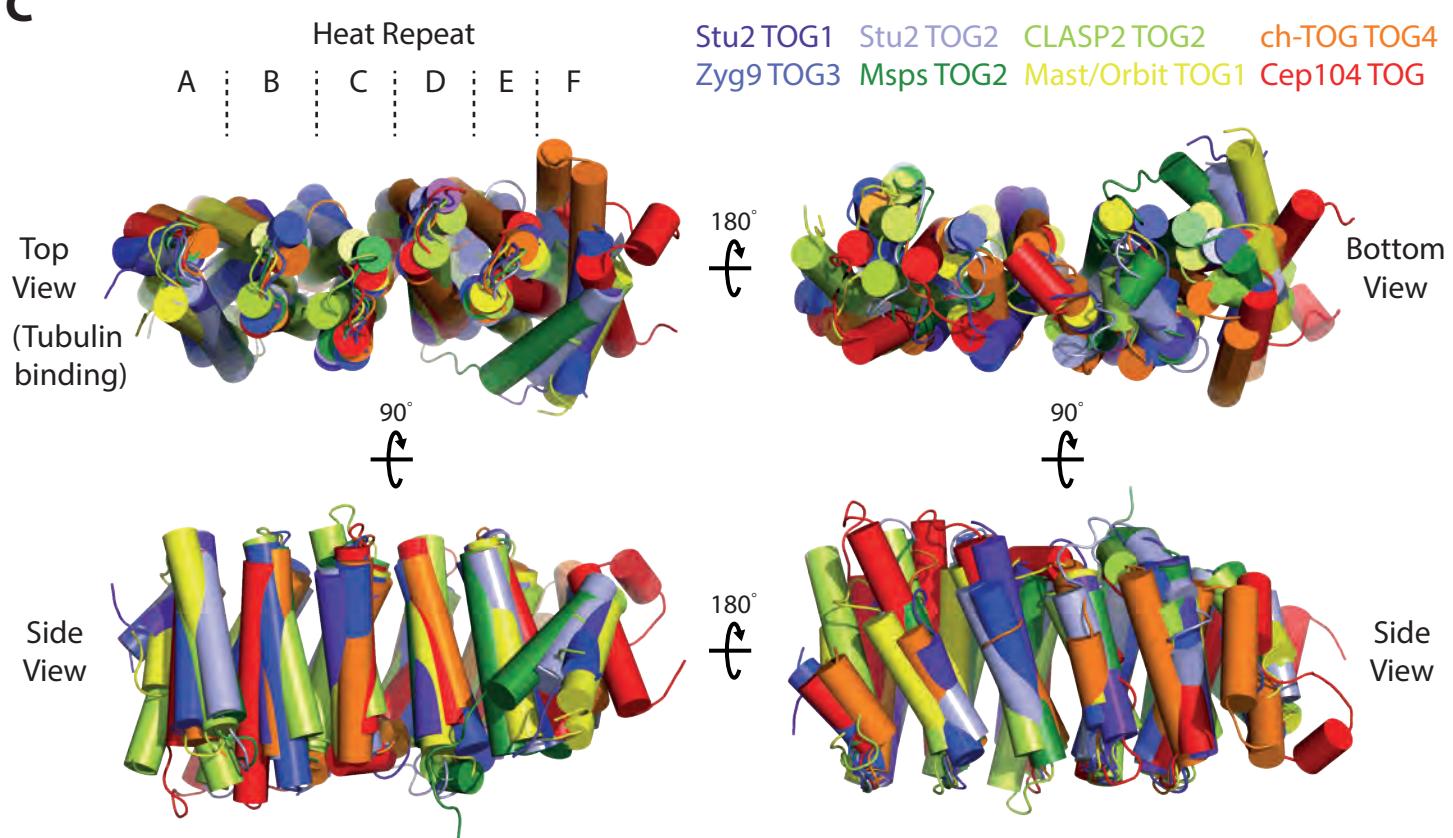
## B (continued)

● Residues mutated in this study

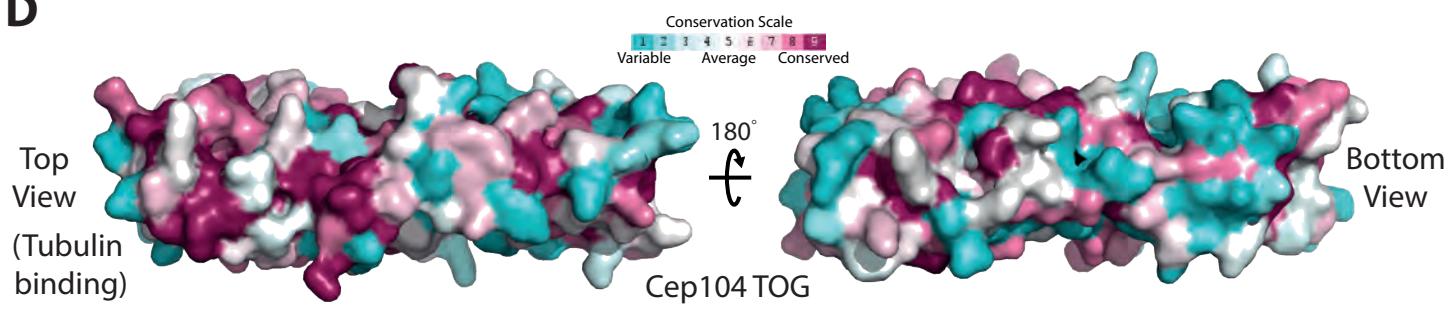
	570	590	610	630	650	670
Homo Sapiens	S Y L V Q P L K A N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Pan paniscus	S Y L V Q P L K A N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Pan troglodytes	S Y L V Q P L K A N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Gorilla gorilla	S Y L V Q P L K A N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Macaca fascicularis	S Y L V Q P L K T N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Macaca mulatta	S Y L V Q P L K T N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Papio anubis	S Y L V Q P L K A N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Callithrix jacchus	S Y L V Q P L K A N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Nomascus leucogenys	S Y L V Q P L K A N S S V H L A M S Q M G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A S I   L E Y L P P D S N T R R N I   L Y K T I   F E G F A K   D		
Tupaia chinensis	S Y L V Q P L K A N S S V H L A M S Q M D	L L A R L L K D L G T G N S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y K H H Q A Y I   L E Y L P P D D N N T R K N V   L Y K T I   F E G F A K   D		
Trichechus manatus latirostris	S Y L V Q P L K A N S S T H L A M S Q M D	L L A R L L K D L G T E G S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y K Q H R A F I   L E Y L P P D D N N T R K N I   L Y K T I   F E G F A K   D		
Loxodonta africana	S Y L V Q P L K A N S S T H L A M S Q M D	L L A R L L K D L G T E G S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y K Q H R A F I   L E Y L P P D D N N T R K N I   L Y K T I   F E G F A K   D		
Otolemur garnetti	S Y L V Q P L K A N S S A H L A M S Q M E	L L A R L L K D L G T E S S G F T	I D F T V D N V M K F S V S A L E H R V Y E V R E	I A V R I   L D M Y K Q H Q A F V   L E Y L P P D D S N T R K N V   L Y K T I   F E G F A K   D		
Ceratotherium simum	S Y L V Q P L K A N S S T H L A M S Q M D	L L A R L L K D L G T E N T G F T	I D F T V D N V M K F S V S A L E H R V Y E V R E	I A V R I   L D M Y K Q H G A S V   L E Y L P P D D S T T R K N I   L Y K T I   F E G F A K   D		
Chrysochloris asiatica	S Y L V Q P L K A N S S T H L A M S Q M D	L L A R L L K D L G T E G S G F T	I D F T D S V M R F S V S A L E H R V Y E V R E	T A V R I   L D M Y R R Y R T L I   L E Y L P P D S N T R K N V   L Y K T I   F E G F A K   D		
Dasypus novemcinctus	S Y L V Q P L K A N S S A H L A M S Q T A	L L A R L L K D L G T G A S G F T	I D F T D S V M R F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H Q T F I   L E Y L P P D D S T R R N V   L Y K T I   F E G F A K   D		
Canis lupus familiaris	S Y L V Q P L K A N C S T H L A M S Q M S	L L A R L L K D L G T G T C F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R V   L D M Y K Q H R A F I   L E Y L P P D D S T T R K N V   L Y K T I   F E G F A K   D		
Equus caballus	S Y L V Q P P F K A K S P H V A L S Q V A	L L A R L L K D L G L E C S G L T A D S V M K	F S V S A L E H R V Y E V R E	T A V T I   L D M Y K Q H G A S V   L E Y L P P D D S T T R K N V   L Y K T I   F E G F A K   D		
Elephantulus edwardii	S Y L V Q P L K A N S S T H L A M S Q M D	L L A R L L K D L G T E G M G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H R A F V   L E Y L P P D D S S T R K N V   L Y K T I   F E G F A K   D		
Saimiri boliviensis boliviensis	S Y L V Q P L K A N S S T H L A M S Q M D	L L A R L L K D L G T E G S G F T	I D N V M K F S V S A L E H R V Y E V R E	T A V R I   L D M Y R Q H A S I   L E Y L P P D D S N T R R N I   L Y K T I   F E G F A K   D		
Odobenus rosmarus divergens	S Y L V Q P L K A N S S N H L A M S Q I G L	L L A R L L K D L G T R G T G F T	I D N V M K F S V S T L E H R V Y E V R E	T A V R V   L D M Y K Q H R A F I   L E Y L P P D D O N T I R K N V   L Y K T I   F E G F A K   D		
Mustela putorius furo	S Y L V Q P L K A N T S H N L A M S Q V G	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	A A V R V   L D M Y K Q H R A F I   L E Y L P P D D T T R K N V   L Y K T I   F E G F A K   D		
Jacalus jacalus	S Y L V Q P L K T N A S T H L A M S Q V D	L L A R L L K D L G T G S S G F T	I D N V M K F S V S A L E H R V Y E V R E	A A V R V   L D M Y K Q H R A F I   L E Y L P P D D T T R K N V   L Y K T I   F E G F A K   D		
Mesocricetus auratus	S Y L V Q P L K A N A S V H L A M S Q V D L	L S R L L R D L G T E N S G F T	I D N V M K F A L S A L E H R V Y E V R E	T A V R V   L D M Y R Q H P A L T   L E Y L P P D D S T T R R N L L Y K A   F E G F A K   D		
Orcinus orca	S Y L V Q P L K A N S S A H L A M S Q M A L L	L S R L L R D L G T E O G M F T	I D N V M K F A L S A L E H R V Y E V R E	T A V R V   L D M Y R Q H P A L T   L E Y L P P D D S T T R R N L L Y K A   F E G F A K   D		
Ictidomys tridecemlineatus	S Y L V Q P L K A N V C S A H L A M S Q M D	L L A R L L K D L G T G S T G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R I   L D M Y R Q H Q A L T   L E Y L P P D D S S T R R N I   L Y K T I   F E G F A K   D		
Ovis aries	S Y L V Q P L K A N S S T H L A M S Q M A L	L L A R L L K D L G T E G A G F T	I D N V M K F A V A S L E H R V Y E V R E	A A V G M   L D M Y A Q H R A L V   L E Y L P P D D S T T R R N V   L Y K T I   F E G F A K   D		
Capra hircus	S Y L V Q P L K A N S S T H L A M S Q M A L L	L L A R L L K D L G T E G A G F T	I D N V M K F A V A S L E H R V Y E V R E	A A V G M   L D M Y A Q H R A L V   L E Y L P P D D S T T R R N V   L Y K T I   F E G F A K   D		
Pantherops hodgsonii	S Y L V Q P L K A N S S T H L A M S Q M A L L	L L A R L L K D L G T E G A G F T	I D N V M K F A V A S L E H R V Y E V R E	A A V G M   L D M Y T Q H Q A L V   L E Y L P P D D S T T R R N V   L Y K T I   F E G F A K   D		
Microtus ochrogaster	S Y L V Q P L K P N A S T H L A M S Q V D L	L L A R L L K D L G T G N S G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R V   L D M Y R Q H P A L T   L E H L P P D S T T R R N L L Y K A   F E G F A K   D		
Pteropus alecto	S Y L V Q P L K L A N A P T H L A T S R M E	L L A R L L K D L G T E G S G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R V   L D M Y E Q H R A P V   L D Y L P P D D C A A R V   L E Y L P P D D S T T R R N V   L Y K T I   F E G F A K   D		
Bos taurus	S Y L V Q P L K A N S S T H L A M S Q M A L L	L L A R L L K D L G T E G A G F T	I D N V M K F A V A S L E H R V Y E V R E	A A V G M   L D M Y A Q H R A L V   L E Y L P P D D S T T R R N V   L Y K T I   F E G F A K   D		
Ailuropoda melanoleuca	S Y L V Q P L K T N S S T H L A M S Q M G L	L L A R L L K D L G T G G T F T	I D N V M K F A V A S L E H R V Y E V R E	A A V G M   L D M Y A Q H R A F I   L E Y L P P D D S T T R R N V   L Y K T I   F E G F A K   D		
Chinchilla lanigera	S Y L V Q P L K P N C S A H L A M S Q V D	L L A R L L K D L G T G A S G F T	I D N V M K F A V A S L E H R V Y E V R E	A A V G M   L D M Y A Q H R A F I   L E Y L P P D D S T T R R N V   L Y K T I   F E G F A K   D		
Felis catus	S Y L V Q P L K A N S S A H L A M S Q M D L	L L A R L L K D L G T G N T G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R V   L D M Y K Q H R A F I   L E Y L P P D D N T T R K N V   L Y K T I   F D G F A K   D		
Heterocephalus glaber	S Y L V Q P L K P S C S A R L A M S Q M D L	L L A R L L K D L G T G T G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R V   L D M Y A Q H Q A L V   L E F L P P D D S T R R N L L Y K A   F E G F A K   D		
Mus musculus	S Y L V Q P L K A N A S V H L A M S Q M D L	L L A R L L K D L G T G T G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R V   L D M Y A Q H Q A L V   L E F L P P D D S T R R N L L Y K A   F E G F A K   D		
Cavia porcellus	S Y L V Q P L K A N C S A H L A M S Q V D L	L L A R L L K D L G T G T G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R V   L D M Y T R H Q A L V   L E Y L P P D D S G M R R N   L L Y K A   F E G F A K   D		
Cricetulus griseus	S Y L V Q P L K S N V S H L A M S Q V D L	L L A R L L K D L G T G T G F T	I D N V M K F A L S A L E H R V Y E V R E	T A V R V   L D M Y R Q H P A L T   L E H L P P D D S T R R N L L Y K A   F E G F A K   D		
Sarcophilus harisii	S Y L V Q P L K Q N S S T H L A M S Q T D L	L L A R L L K D L G T G T G F T	I D N V M K F A L S A L E H R V Y E V R E	T A V R V   L D M Y K T H R T L I   L E Y L P M D D G N T R K N I   L Y K T I   F E G F A K   D		
Rattus norvegicus	S Y L V Q P L K T N A S V H L A M S Q V D L	L L A R L L K D L G T G T G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R V   L D M Y R Q H P A L T   L E H L P P D D S T T R R N L L Y K A   F E G F A K   D		
Chrysemys picta bellii	S Y L V Q P L K P N S P T H L A M S Q V D L	L L A R L L K D L G T G T G F T	I D N V M K F A V A S L E H R V Y E V R E	T A V R V   L D M Y R Q H P A L T   L E H L P P D D S T T R R N L L Y K A   F E G F A K   D		
Camelus ferus	A Y L V Q P L K A C G S S A H L A M S Q M A L L	L L A R L L K D L G T G T G F T	I D N V M K F A S A R A L E H R V Y E V R E	A A V R L I   L G L Y A Q H R A A V   L E H L P P A D S A T R K N V   L Y K T I   F E G F A K   D		
Monodelphis domestica	V H L I Q P L K Q N S S T H L A M S Q T D L	L L A R L L K D L G T G T G F T	I D N V M K F A S A R A L E H R V Y E V R E	A A V R L I   L G L Y A Q H R A A V   L E H L P P A D S A T R K N V   L Y K T I   F E G F A K   D		
Octodon degus	S Y L V Q P L K P N C S A H L A M S Q V D L	L L A R L L K D L G T G T G F T	I D N V M K F A S A R A L E H R V Y E V R E	A A V R L I   L G L Y A Q H R A A V   L E H L P P A D S A T R K N V   L Y K T I   F E G F A K   D		
Alligator mississippiensis	I H L V H P L K P N S P T H L A M S Q V E L	L L A R L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I D L Y R Q H R T I I   L N Y L P P D D A N T R K N V   L Y K T I   F E G F A K   D			
Sorex araneus	P Y L L Q P L K A N A S A H L A M S Q M S L	L L A R L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I D L Y R Q H R T V I   L N Y L P P D D A N T R K N V   L Y K T I   F E G F A K   D			
Myotis davidii	S Y L V Q P L K A N A P A H L A M S Q V A L L	L S R L L R D L G T D S T G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I D L Y R Q H R V L Q V R E M A V C V   I D L Y R Q H R S V V   L E Y L P P D D S I S R K N I   L F K T I   F E G F A E   D			
Anolis carolinensis	A H L V Q P L K P N S P T H L S M S Q V E L	L V E R L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I D L Y R Q H R V L Q V R E M A V C V   I D L Y R Q H R S V V   L E Y L P P D D S I S R K N I   L F K T I   F E G F A E   D			
Meleagris gallopavo	V H L V Q P L K S N S P T H L A M S R V E L	L V E C L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I D M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A K   D			
Gallus gallus	V H L V Q L L K P N S P T H L A M S R V E L	L V E C L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I D M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A K   D			
Myotis lucifugus	S Y L V Q P L K A N A S A H L A M S Q V A L L	L V E Y L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I F G M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A K   D			
Anas platyrhynchos	V H L V Q P L K P N S P R R L A M S Q A E L	L V E Y L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I F G M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A E   D			
Melopsittacus undulatus	V H L V Q P L K P N S P R R L A M S Q A E L	L V E Y L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I F G M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A E   D			
Falco peregrinus	V H L V Q P L K P N S P R R L A M S Q A E L	L V E Y L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I F G M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A E   D			
Falco cherrug	V H L V Q P L K P N S P R R L A M S Q A E L	L V E Y L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I F G M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A E   D			
Columba livia	V H L V Q P L K P N S P R R L A M S R V E L	L V E Y L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I F G M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A E   D			
Xenopus tropicalis	T H L V Q P L K P T M P S R L A Q S Q V D L L E	L V E Y L L K N L G T E N S G F T	I D N V M K F A T G A L E H R V Y E V R D A L R I   I F G M Y R K H K A A I   L E Y L P P D D A S I R K T V   L Y K T I   F E G F A E   D			

## Figure S2, related to Figure 1B-D

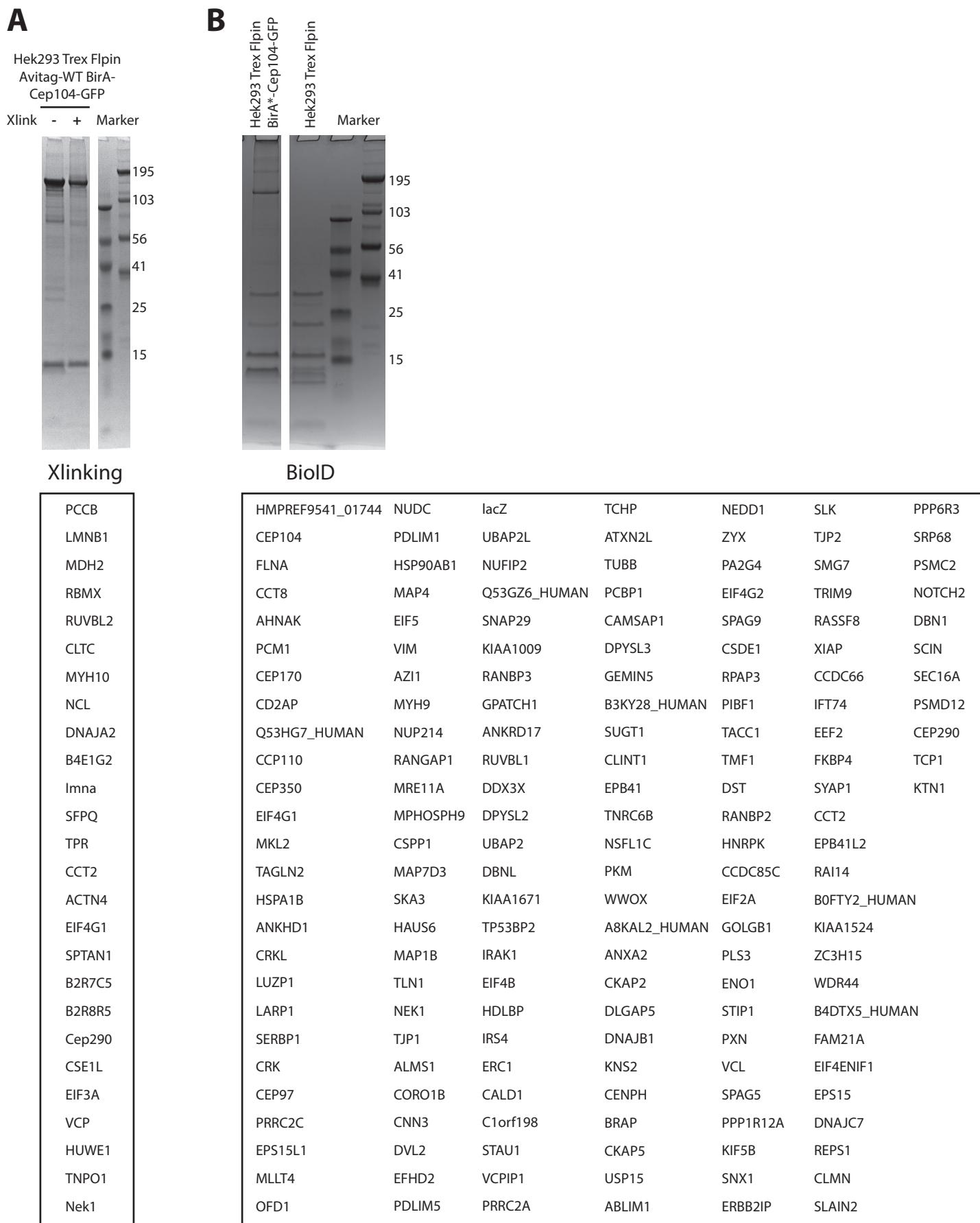
C



D

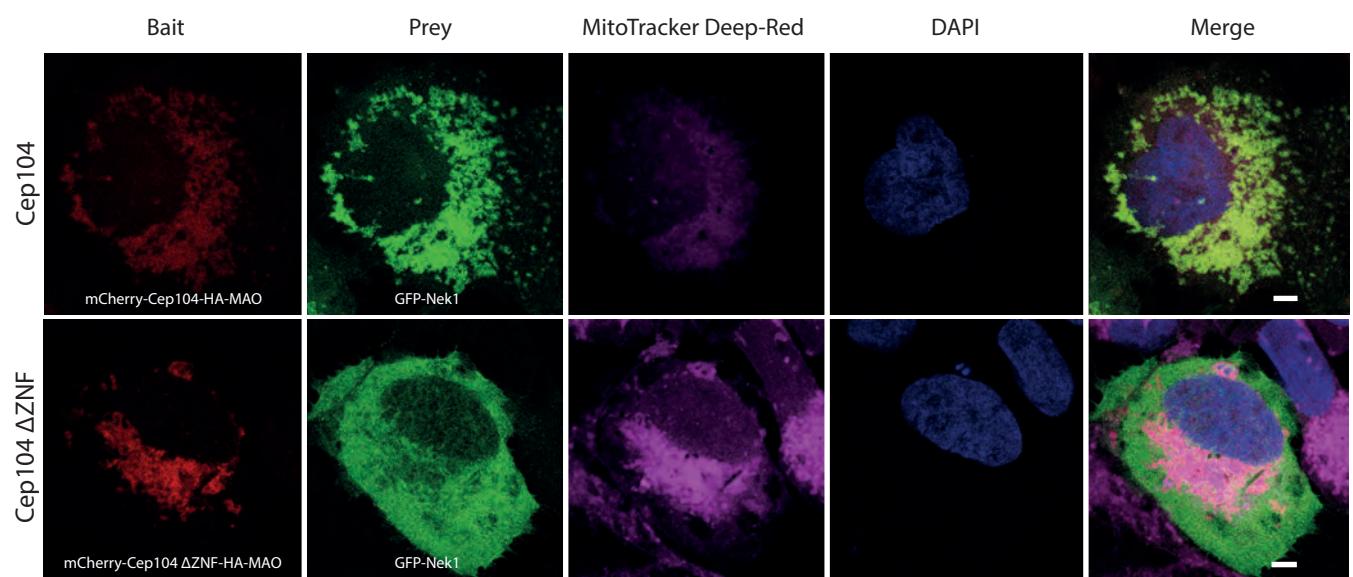


## Figure S3, related to Figure 2A

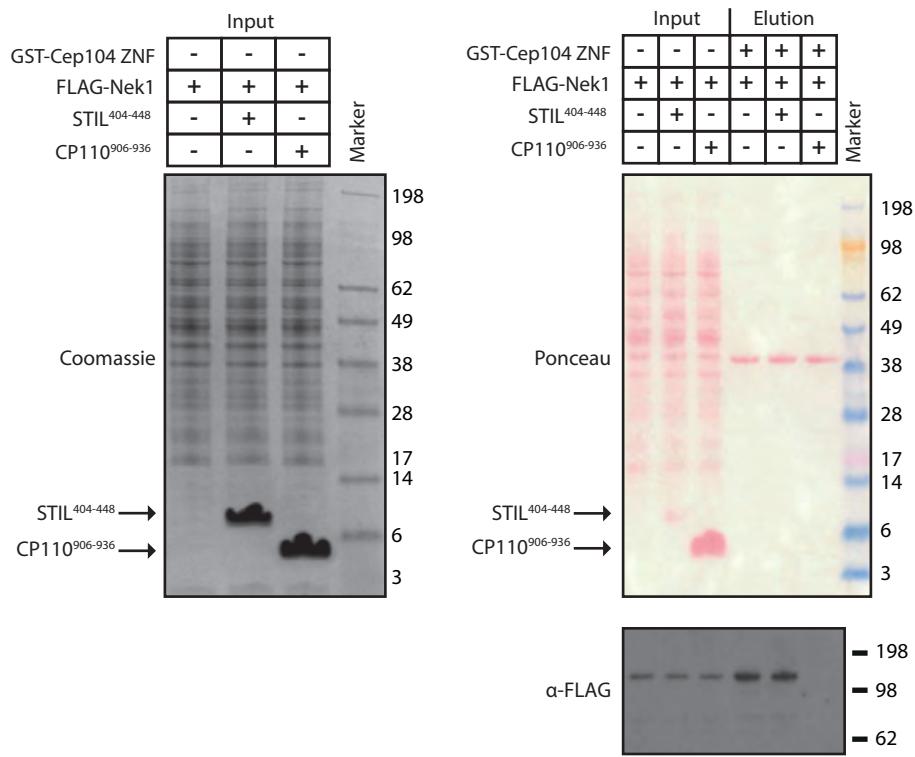


Hits with  $\geq 10$ -fold enrichment of exclusive, unique peptide counts compared to control

**Figure S4, related to Figure 2B**

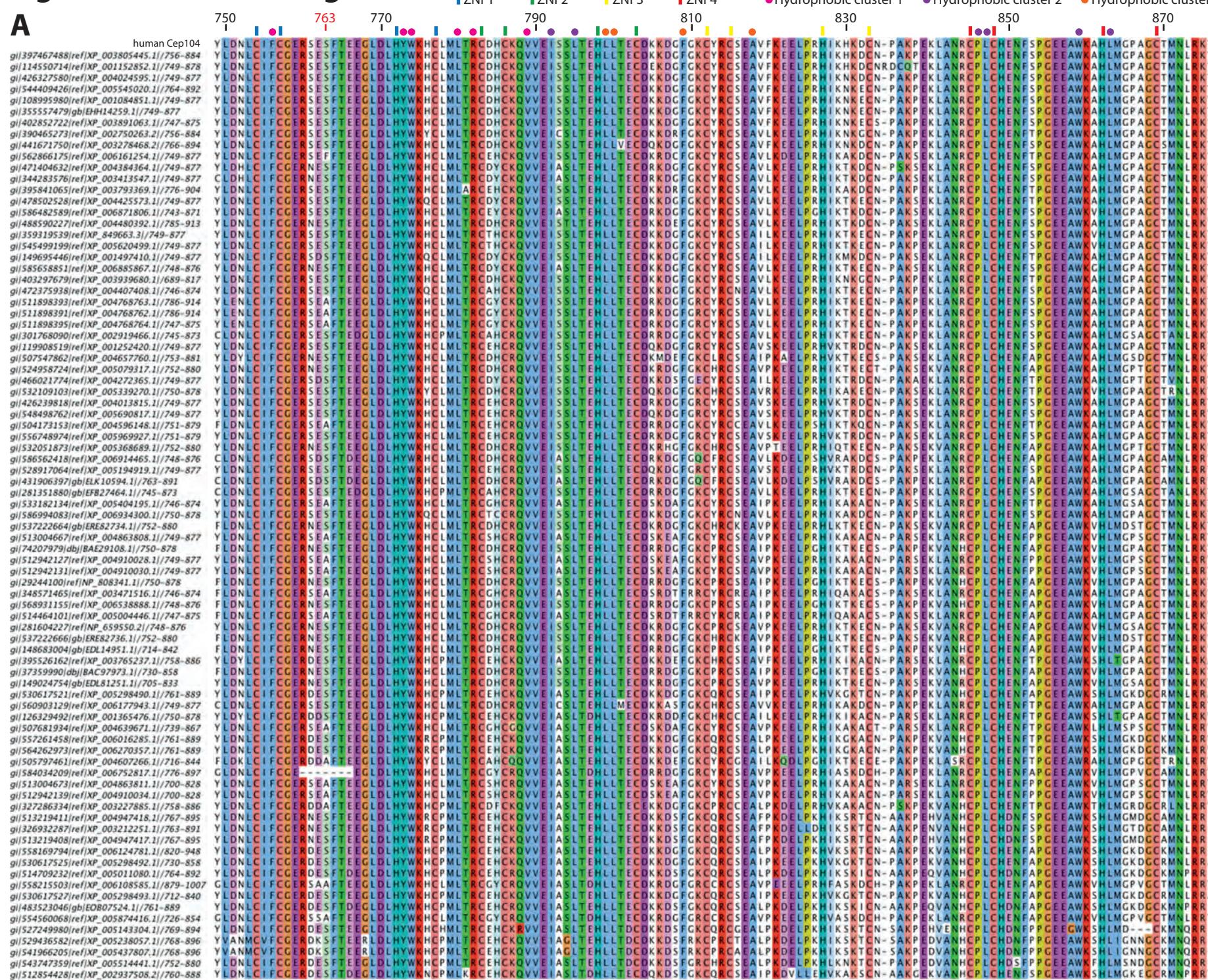


## Figure S5, related to Figure 3A



# Figure S6, related to Figure 4

**A**



**Figure S6, related to Figure 4**

**B**

