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Supplemental Information

The Ciliopathy-Associated Cep104 Protein

Interacts with Tubulin and Nek1 Kinase

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Table S1, related to Table 2

Human TOG Cep104³⁹²⁻⁶⁷⁶ 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 α=90.0 β=91.8 a=52.1 b=53.3 c=155.4 α=90				
Chine Cent dimensions (11)	γ=90.0	γ=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 $I/\sigma I$ (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 Cep104⁷⁴⁶⁻⁸⁷⁵ 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	a=73.9 b=80.5 c=118.3	a=73.9 b=80.5 c=118.3
	α=90.0 β=90.0 γ=90.0	α=90.0 β=90.0 γ=90.0	α=90.0 β=90.0 γ=90.0
Resolution (Å)	80.5 - 2.2	80.5 - 2.2	80.5 - 2.2
Completeness	99.2 / 99.7 / 98.1	99.3 / 99.8 / 98.2	99.4 / 99.5 / 98.5
(overall / inner / outer shell)			
Rmerge (overall / inner /	0.139 / 0.069 / 1.512	0.114 / 0.067 / 1.017	0.198 / 0.067 / 1.976
outer shell)			
Rpim (overall / inner / outer	0.046 / 0.024 / 0.510	0.038 / 0.025 / 0.342	0.065 / 0.024 / 0.657
shell)			
Mean I/oI (overall / inner /	11.3 / 30.5 / 1.9	12.0 / 28.3 / 2.5	9.6 / 28.4 / 1.8
outer shell)			
Multiplicity (overall / inner /	9.9 / 8.5 / 9.4	10.1 / 8.7 / 9.6	10.1 / 8.7 / 9.8
outer shell)			
Zn found / expected	6 / 16		

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 α -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 pulldown (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 Δ ZNF (Cep104 $^{\Delta746-880}$), 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 µm.

Figure S5, related to Figure 3A Binding of CP110⁹⁰⁶⁻⁹³⁶ 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 CP110⁹⁰⁶⁻⁹³⁶ or *Danio rerio* STIL⁴⁰⁴⁻⁴⁴⁸. Please note that *Danio rerio* STIL⁴⁰⁴⁻⁴⁴⁸ 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.

Homology model of Cep104 N-term. domain



Α











A

Residues mutated in this study



В

• Residues mutated in this study

		440	460	480	500	520	540	560
Homo Sapiens	EPEPLTEKALREASSAIDVLC	TLVAEAYCKTW	SYREDALLALSKKLMEMPVGTPK	EDIKNTLRASVELVRR	AIKDIVTSVFOASLKILKMIITO	TPKHKLSKLETAHCVERT	IPVLLTRTCDS SARLRVTA	ANFIOEMALFKEVKSLOIIP
Pan paniscus	EPEPLTEKALREASSAIDVLG	TLVAGAYCKTW	SYREDALLALSKKLMEMPVGTPK	EDLKNTLRASVELVRR	AIKDIVTSVFOASLKLLKMIITO	YIPKHKLSKLETAHCVERT	I P V L L T R T G D S SAR L R V T A	ANFIDEMALFKEVTSLOIIP
Pan troglodytes	EPEPLTEKALREASSAIDVLC	TLVAGAYCKTW	SYREDALLALSKKLMEMPVGTPK	EDLKNTLRASVFLVRR	AIKDIVTSV FQASLKLLKMIITO	YIPKHKLSKLETAHCVERT	I P V L L T R T G D S S A R L R V T A	ANFIQEMALFKEVTSLQIIP
Gorilla gorilla	EPEPLTEKALREASSAVDVLG	TLVAGAYSKTW	SYREDALLALYKKLMEMPVGTPK	EDLKNTLRASVFLIRR	A I K D I V T S V FQ A S L K L L K M I I T I	YIPKHKLSKLETAHCVERT	I P V L L T R T G D S S A R L R V A A	ANFIQEMALFKEVKSLQIIP
Macaca fascicularis	EPEPLTEKALREASSAIDVLG	TLVAGAYSKTW	SYREDALLALYKKLMEMPVGTPK	EDLKNTLRASIFLIRR	A I K D I V T S V FQ A S L K L L K M I I T C	YIPKHKLSKLETAHCVERT	I P V L L T R T G D S S A R L R V T A	ANFIQEMALFKEVKSLQIIP
Macaca mulatta	EPEPLTEKALREASSAIDVLG	TLVAGAYSKTW	SYREDALLALYKKLMEMPVGTPK	EDLKNTLRASIFLIRR	A I K D I V T S V FQ A S L K L L K M I I T C	YIPKHKLSKLETAHCVERT	I P V L L T R T G D S S A R L R V T A	ANFIQEMALFKEVKSLQIIP
Papio anubis	EPEPLTEKALREASSAIDVLGI	TLVAGAYSKTW	SYREDALLALYKKLMEMPVGTPK	EDLKNTLRASIFLIRR	AIKDIVTSVFQASLKLLKMIITO	YIPKHKLSKLETAHCVERT	IPVLLTRTGDS SARLRVTA	ANFIQEMALFKEVKSLQIIP
Callithrix Jacchus	EPEPLIENAL REASSAIDVLG	TLUACAYSKIW	STREDALLALTKKLMEIPVGIPK	EDIKNILKASIFLVKK	AIKDIVISVEQASLKLLKMIIIC	TIPKHKLIKLEIAHLVEKI	VPVLLIKIGDSSAKIRVIA	ANFIQEMALFKEVKSLQIIP
	DPEPLTERAL PEASSALDVLC	TLVACAVSETW	SYDEDALLALYKYI MEMPUCTOK	EDIVNTIDASICIOD	ATK DIVISVEQASIVILIEMITTE	VIPVHVISVIETTHEVERT	VPVLLTPTCDSSAPLPTIA	SNCIDEMAL EVEVESTOILE
Trichechus manatus latirostris	EPEPLTEKAL REASSALDVLC	TIVACAYSKTW	SYREDALLALYKKIMEMPTGTPK	FDIKNMIRASVELIRR	AVKDIVTSVEDASIKIIKMIITO	VIPKHKISKIFTSHCVFRT	IPTIIT RTCDS SVRIBILS	SNEIDEMALEKEVKSLOLIP
Loxodonta africana	EPEPLTEKALREASSAIDVLC	TLVAGAYSKTW	SYREDALLALHKKLMEMPVGTPK	EDLKNMLRASVELIRR	AMKDIVTSVFOASLKLLKMVITC	YIPKHKLSKLETSHCVERI	VPPLLARTCDSSTRLRIIA	SNEIDEMALEKEVKSLOIIP
Otolemur garnetti	EPEPLTEKALREASSAIDVLC	TLVAGAYSKTW	SYREDALLALYKQLMEMPIGTPK	EDLKNILRASIFLIRR	AIKDIVSPVFQASLKLLKMIITO	YIPKHRLGKLEATHCVERT	I.P.I.L.T.R.T.G.D.S.S.A.R.L.R.VMA	VNFIQEMALFKEVKSFQIIP
Ceratotherium simum	EPEPLTEKALREASSAVDVLC	SLVAGAYSKTW	SYREDALLALYKQLMEMPVGTPK	EDLKSMLRASVFLIRR	A I R D I V T P V F Q A S L K L L K M I I T C	YIAKHKLSKLETTHCVERT	I P I L L T R T G D S S A R L R V I A	SNFVQEMALFKEVKSLQIIP
Chrysochloris asiatica	EPEPLTEKVLREASSAIDVFG	TLVAGAYSKTW	SYREDALLALYKQLMEMPIGTPK	EDLKNMLRASVFLIRR	A V K D I V T S V FQ A S L K L L K M I I T C	YIPKHKLSKLETTHCVERT	I P V L L T R T G D S S T R L R I I A	SNFIQEMAL FKEVKSLQIIP
Dasypus novemcinctus	EPEPLTEKAAREASSAVDVLG	ALVAGAY SKTW	SYREDALLALYKKLMEMPVETPK	EDLKNMLRASVFLIRR	ALRDIVTSVFQASLKLLKMIITO	YIPKHKLSKLEATHEVERT	IPFLLTRTGDSSARLRIIA	SNFIQEMALFKEIKPLQIIP
Canis lupus familiaris	EPEPLTEKALREASPAVDVLG	SLVAGAYSKTW	SYREDALLALYKQLMETPAGTPK	EELKSMLRASIFLVRR	AIRDIVTPVFQASLKLLKMIITC	YIPKHKLSKLETAHCVERT	I P V L L T R T G D S SAR L R V I A	SNFIQEMALFKEVRSLQIIP
Equus caballus Elembantulus educardii	EPEPLTERAL REASSAVUVLG	ALVAGATSKIW	SHREDALLALYKULMEMPVGTPK	DDIVNMIDACICLIPP	AJKDIVIPVPQASLKLLKMIIIC	TIPKHKLSKLEIIHLVEKI	IPTLLTRTCDSSARLKVIA	SNFVQEMALFKEVKSLQIIP
Saimiri boliviensis boliviensis	EPEPLTERALPEASSALDVLC	TLVACAVSKTW	SYREDALLALYERIMEIDVCTTK	EDIKNTIPASIELVPP	ALK DIVISVEOASLULIVALITO	VIPENELIKIETAHEVERT	IPVILITETCOS SAPUPUTA	ANCINCIMALEKEVESLOLIP
Odobenus rosmarus divergens	EPEPLTEKALREAGPAVDVLG	SLVAGAYSKTW	SYREDALLALYKREMETPAGTPK	EELK SMLRASIFLVRR	ALROIVTPVFOASLKLLKMIITO	YIPKHKLSKLETAHCVERT	IPILLTRTCDS SARLRVIA	SNEIDEMALEKEVRSLOIIP
Mustela putorius furo	EPEPLTEKALREAGPAVDVLG	SLVAGAYSRTW	SYREDALLALYKRLMETPTGTPK	EELKSTLRASVFLVRR	ALRDIVTPVFQASLKLLKMMITC	YIPKHKLSKLETAHCVERT	I P V L L T R T G D S S A R L R V V A	SNFIDEMALFKEVKALOIIP
Jaculus jaculus	EPEPLTEKALREASSAIEVLGI	TLVAGAYSKTW	SYREDALLALYKKLMEMPVETPK	EDLKNILRAAVFLIRR	A I K D I V T S V F Q A S L K L L K M I I T C	YIPKHKLSKLETTHCVERA	V P A L L A R T G D S S A R L R V M A	LNFIQEMALFKEVRSLQIIP
Mesocricetus auratus	EPEPLTEKALREASSAIDTLG	ALVAGAYSKTW	SCREDALLALYKKLMEMPVGTQK	EDLKNILRASVFLVRR	ATK DIVTAV FQASLKLLKMIITO	YIPKHKLSKLETTHCVERT	VPLLLTRTGDSSARLRVIA	LNFIQEMALFKEVKSLQLIP
Orcinus orca	EPEPLTEKALREASSAVDVLC	ALVAGAYSKTW	SHREDALLALYKQLMDAPAATPK	EDLKSTLRAAVFIIRR	AVRDIVTPVFQASLKLLKMLITC	YIPKHKLGKLETTHCVERT	I P V L L T R T G D S S A R L R V I A	SNFIQEMALFKEVKSLQLIP
Ictidomys tridcemlineatus	EP EP LSEKALREASSAVDVLG	TLVAGAYSKTW	SCREDALLGLCRRLMEVPVGTPK	EDVKSMLRASVFLVRR	AMKDIVTSVFQASLKLLKMMITC	Y I P K HQLSKLETTHEVERT	I PALLARTGDSSTRLRIMA	SNFIQEMAL FREVEPLOTIP
Capra bircus	EPEPLTEVALPEASSAVDVLC	ALVAGATSKTW	SHREDALLTLHKOLADTPVGTPK	EDIN SMIDAAVELVDD	AIRDIVIEVOASLALLEMALITA	VIPENELGELETAHEVERT	IPVILT PTCDS SAPL PVMA	SNEIDEIALEVEVKSLOVID
Ochotona princeps	EPEPLTEKAL REASSALDVICE	SIVAGAYSKTW	SYREDALLALYKRMMEMPVGTPK	EDIKNTIRASIELIRA	AIKDIVTSVEDASIKILKMVITO	Y IPRHRIGRI ETTHOVERT	VPVLLART GDS SCRIRVIA	CNEIDELALEKEVSSLOMIP
Pantholops hodgsonii	EPEPLTEKALREASSAVDVLG	ALVAGAYSKTW	SHREDALLTLHKQLADTPVGTPK	EDLKSMLRAAVFLVRR	AIRDIVTPVFQASLKLLKMIITO	YIPKHKLGRLETAHCVERT	I P V L L T R T G D S SAR L R VMA	SNFIGEIALFKEVKSLOVIP
Microtus ochrogaster	EPEPLTEKALREASSAIDILGI	PLVAGAYSKTW	SCREDALLALYQKLMEMPVGTQK	EDLKNMLRASVFLIRR	A I K D I V T S V FQA S L K L V K M I I T C	YIPKHKLSKLETTHCVERT	VPLLLARTGDSSARLRVMA	LNFIQEMALFKEVKSLQLIP
Pteropus alecto	EPEPLTEKALREASAAVDVLG	TLVAGAYSRTW	SHREAALLALHKRLMEAPVGTPK	EDLRGLLRAAVFLIRR	A I R D I V T P V F Q A S L K L L K M V I T C	YIPKHKLGKLETMHCVERT	I P V L L T R T G D S S A R L R V V A	SNFIQEMALFKEVKSLQMIP
Bos taurus	EPEPLTEKALREASSAVDVLG	ALVAGAYSKTW	SHREDALLTLHKQLTDTPVGTPK	EDLKSTLRAAVFLVRR	AIRDIVTPVFQASLKLLKMIITC	YIPKHKLGRLETAHCVERT	I P V L L T R T G D S SAR L R VMA	SNFIQEIALFKEVKSLQVIP
Alluropoda melanoleuca	EP EP LAEKALREAGPAVDVLGI	SLVAGAYSKIW	SYREDALLALHKQLMETPAGSPK	EELKSTLRASVFLVRR	ATROVVTPVFQASLKLLKMTTTC	Y IPKHKLSKLEIAHCVERI	IPVLLIRIGDS SARLRVIA	SNFTQEMALFKEVRSLQTTP
Felis catus	EPEPLTEKAL PEASAAVDVICE	ALVACAYSKTW	SYREDALLALCKOLMOMPACAPK	FELKNVLPASIFLVPP	AVPDIVTOVEOASIKIIKMIITO	VIPKHKI SKI ETAHOVERT	VPVILTPTCDSSAPLPVIA	SNEIDEMALEKEVRSIOLIP
Heterocephalus glaber	EPEPLTEKALREAGATIDALG	TLVAGAYSKTW	S FREDALLALYKKLTEMPAGTPK	EDAKNTLRAAVELIHR	AIKDVVTSVFOVSLKLLKMLVTO	YIPKHKLGKLETAHCVERT	VPGLLARTGDSCTRLRLAA	SNFIDELALCEEVRALOMVP
Mus musculus	EPEPLTEKALREASAAIDTLC	ALVAGAY 5KMW	SCREDALLALYKKLMEMPVGTQK	EDLKNMLRASVFLIRR	AIKDIVASVFQASLKLLKMIITO	YIPKHKLGKLDTTHCVERA	FP L L L AR AGD S SAR LR VMA	LNFIGEMALFKEVKSLQLIP
Cavia porcellus	QPEALTEKALREASAAIDALGE	TLVAGAYSKTW	SCREDALLALYRKLMEMPAGTPK	EDAKNMLRASVFLIRR	A I K D I V T S V F HACLK L L K M L L T C	YIPKHKLGKLETAHCVERT	LP S L L A R T G D S S S R L R V A A	CNFIQEMALCK EVRALQLVP
Cricetulus griseus	EPEPLTEKALREAGSTIDTLG	ALVAGAYSKTW	SCREDALLALYKKLMEMPVGTQK	EDLKNILRASVFLIRR	ATKDIVTSVFQASL <mark>K</mark> LL <mark>K</mark> MIITO	YIPKHKLSKLETTHCVERT	VPLLLTRTGDSSARLRVIA	LNFIQEMALFNEVKSLQLIP
Sarcophilus harisii	EPEPLTEKAMREASSTIEVLGE	TLVAGAYSKTW	SYREDALLSLYNKLMTMPIGTSK	EDLKNLLRASIFLIRR	AIKDLVPSVFQASLKLLKMIITC	IY I P K H K L G K P E T T L S V E K T	LPNLLSRTGDSSARLRIIA	SNFIQELALCKEIKPLQIIP
Kattus norvegicus	EP EP LTEKALREASSAIDTLG	ALVAGAYSKMW	SCREDALLALYKRLMEMPVGTQK	EDLKNMLRASVFLIRR	A IKDIVISVEQASLKLLKMI ITC	Y IPKHKLGKLDITYCVERA	I P L L LART GDS SAR LRVMA	LNFIDEMALFKEVRSLOLIP
Camelus ferus	EPEPLTERVIPEASSAVDVIC	ALVACAVSPIW	SHPECALLALY FOLSET DUCT DE	EDIKNTIPASVELIPP	A I P D I V T P V E O A SI VII V MII T C	VIPVHVISVIETAHOVERT	VPLILAPTOTSAPIPSTA	SNEIDEMALCKEVRSLOUIP
Monodelphis domestica	EPEPLTEKALREASSTIDVICE	TIVACAYSKTW	SYREDALLSLYNRLMATPVGTPK	FOLKNULRAAIFLIRE	AIKDLVPSVFOTSLKLLKMIITC	YIPKHKLCKPETTVTVEKT	LPNLLTRTGDS SARLRIIA	SNFIDELALCKEIKPLOIIP
Octodon degus	EPEPLTEKALREAGTAIDVLC	SLVAGAYSKTW	SCRENALLALYEKLMEVPAGAPK	EDAKNMLRASVFLTRR	AIKDIVTSVFQASLKLLKMLLTO	YVPKHKLSKLETSHCVERT	LPALLARTGDS SAR LRGMA	SNFIGEMALCKEVRALOVVP
Alligator sinensis	EPEPLTEKALREASPAIEVFG	ALVAGAYSKTW	SYREDALLAIYKKLMEISVSTPK	EDLKNMLRAAVFLVRR	ATK DIVSSVFQASLK LLKMIITO	YIPKHKLGKLETAHCVDRI	LPNLLSRTGDSSTRLRIVA	SNFIQEMALCNEVKPLHIIP
Alligator mississippiensis	EPEPLTEKALREASPAIEVFG	ALVAGAYSKTW	SYREDALLAIYKKLMEISVSTPK	EDLKNMLRAAIFLIRR	A T K D I V S S V FQ A S L K L L K M I I T C	YIPKHKLGKLETAHCVDRI	LPNLLSRTGDSSTRLRIVA	SNFIQEMALCNEVKPLHIVP
Sorex araneus	EPEPLTEKAWREAGSVIEVLGI	TLVAGAYSRTW	SYREDALLALSKQLAGMPVGAPR	EELRNALRAAIFLVRR	AIRDVVTPVFQASLKLLKMLVTC	FVPKHRLGRPEAAHCVERT	I P A L L A R T G D S S A R L R L M A	SNFIQELALSKEVRSLQIVP
Myotis davidii Analis satalinansis	EPEPLTEKALREASSATEVLGE	ALVAQAYSKTW	SFREDALLTLYQQLVNTPVGTPK	EDLKGLLRAAVFLIRR	STRDIVTPVFQASLKVLKMLLTC	YVPKHKLSKLEITHCVERT	VPVLLARTGDS SARLRAMA	SNFIQEMALCKEVKSLQMVP
Anons caronnensis Meleagris gallopavo	EPEPLIENALKEASAAIEAFG	GLVAGAT SKIW	STREUSLEATTKKMMEVSPSTPK	COLKSLERGAIFLIVE	ATK DIVSSVENASLKLLKMITT	VUP VHVI CVI ET SHCVEVT	IPCLISPTCDSSSPLPIMA	ANFIQEMALCNEVEP LQIIP
Gallus gallus	EPEPLSEKALPEASPALEVEC	ALVSCAVSKSW	SYREDALLAVYKKIMEMSVSTPK	EDLENMLRAAIELVER	AIKDIVSSVEDASIKIIKMIITO	YVPKHKLGKLETSHCVFKT	LPGLLSBTGDSSSRIPIVA	AKFIDEMALWSEVKPLOIVP
Myotis lucifuqus	EPEPLTEKALREASSALEVLC	ALVAQAYSKTW	SFREDALLTLHQQLVDTPVGTPK	EDLKGLLRAAVELIRR	SIRDIVTPVFQASLKVLKMLLTC	Y I PRHKLSKLETTHCVERT	VPVLLARTGDS SARLRVMA	SNEIGEMALCKEVKSLOMVP
Anas platyrhynchos	EPEPLTEKALREASPAIEVFC	ALVSGAYSKTW	SYREDALLAVYQK LMEMSVSTPK	EDLKNMMRAAIFLVRR	AIKDIVSSVFQASLKLLKMIVTO	YIPKHKLCKQETSHCVEKT	LP SLLSRTGDSSSRLRLVA	SNFIQEMALCSEVKPLQIVP
Myotis brandtii	EPEPLTEKALREASSAIEVLC	ALVAQAYSKTW	SFREDALLTLHQQLVDTPVGTPK	EDLKGLLRAAVFLIRK	SIRDIVTPVFQASLKVLKMLLTC	YIPKHKLSKLETTHCVERT	VPVLLARTGDSSARLRVMA	SNFIQEMALCKEVKSLQMVP
Melopsittacus undulatus	EPEPLSEKALREASPALEVFC	ALVSGAFSKTW	SYREDALLAVYKKLMEVSVTTPK	EDLKNMLRAAIFLVRR	A SKDIVP SVFQASLKLLKMIITO	YIPKHKLGKLETSHCVEKT	LPNLLSRTGDSSSRLRLVA	SNFIQEMALCSEVKPLQIVP
Falco peregrinus	EPETLTEKALREASPAIEVFGI	ALVSCAYSKTW	SYREDALLAVYKKLMEMSVNTPK	DDLKNMLRAAIFLVRR.	ATEDIVSSVFQAALKLLKMIITO	PKHKLGKLETSHCVEKT	LPNLLSRTGDSSLRLRLMA	SNFIDEMALCSEVKPLQIVP
Faico cherrug Columba livia	LALD EASPALEVEG	ALVSCATSKTW	SYDEDALLAVYKKLMEMSVNTPK	DDLKNMLRAATELVRR	A LEDIVS SVEQAALNEL MITTO	VIPKHKI CPLETSYCUEVT	LPNLISPTCDS SPLEIL	STEIDEMALSSEVEPLOIVE
Xenopus tropicalis	EPEPLTEKALREAGFALEVEG	ALVAGAY SKTW	SYREDALLAVSKKLADIASCMSK	DDLKNLLBAAMFLVBB	ALKOKYTSYFOASLELLKMILT	YIPKHKLGKGETTHCVEKC	LPDLLVRTGDSTARHRLIS	SNEIDEMALEKEVKPLOILP

B (continued)

• Residues mutated in this study

	570	590	610	630	650	670
Homo Sapiens	SYLVQPL	ANSSVHLAMSQMGLLARLLKDLG	TGSSGFTIDNVMKFSVSAL	HRVYEVRETAVRI LDMYRQ	HQASILEYLPPDDSNTRRNILY	KTIFEGFAKID
Pan paniscus	SYLVQPL	KANSSVHLAMSQMGLLARLLKDLG	TGSSGFTIDNVMKFSVSAL	E HR VY EV R ET AVR I I L DMY RQ	HQASILEYLPPDDSNTRRNILY	KTIFEGFAKID
Pan troglodytes	SYLVQPL	KANSSVHLAMSQMGLLARLLKDLG	TGSSGFT I DNVMK FSVSAL	EHRVYEVRETAVRIILDMYRQ	HQASILEYLPPDDSNTRRNILY	KTIFEGFAKID
Macaca fascicularis	SYLVOPL	TNSSVHLAMSOMGLIARLIKDIG	TCSSCETIONVMEESVSSL	EHRVY EVRETAVRI I DMYRO	HOASILETLEPPDDSNIKKNILT	KTIFEGEAKID
Macaca mulatta	SYLVOPL	KTNSSVHLAMSOMGLLARLLKDLG	TGSSGFTIDNVMKFSVSSL	HRVYEVRETAVRILLDMYRO	HOASILEYLPPDDSNTRKNILY	KTIFEGEAKID
Papio anubis	SYLVQPL	KANSSVHLAMSQMGLLARLLKDLG	TGSSGFTIDNVMKFSVS5L	EHRVYEVRETAVRIILDMY RQ	HQASILEYLPPDDSNTRKNILY	KTIFECFAKID
Callithrix jacchus	SYLVQPL	KANSSVHLAMSQMDLLARLLKDLG	TGNSGFTTDNVMKFSVSAL	E HR VY EV R ET AVR I I L DMY RQ	HQASILEYLPPDDNNTRKNILY	KTIFDGFAKID
Nomascus leucogenys	SYLVQPL	KANSSVHLAMSQMGLLARLLKDLG	TGSSGFTIDNVMKFSVSAL	EHRVYEVRETAVRIILDMYRQ	HQASILEYLPPDDSNTRKNILY	KTIFEGFAKID
Iupaia chinensis	SYLVQPL	KANSSVHLAMSQMDLLARLLKDLG	T G N S G F T I D N V M R F S V S A L	EHRVYEVRETAVRIILDMYKH	HQAYILEYLPPDDNNTRKNVLY	KTIFEGFAKID
Loxodonta africana	TYLVOPI	KANSSTHLAMSOMDI LARLIKDIG	TEGSCETTONYMRESVSAL	EHR VY EVR ETAVRILLDMYKO	HRAFILEVIPPDDNNTRKNILY	KTIEFCEAKID
Otolemur garnetti	SYLVOPL	KANSSAHLAMSOMELLARLLKDLG	TESSCETVONVMKESVSAL	EHRVYEVRETAVRI LDMYKO	HOAFVLEYLPPDDSNTRKNVLY	KTIFEGFAKID
Ceratotherium simum	SYLVQPL	KANSSTHLAMSQMALLAR LLKDLG	TENTGFTVDNVMKFSVSAL	EHRVYEVREIAVRI I LDMY KQ	HGASVLEYLPPDDSTTRKNILY	KTIFEGFAKID
Chrysochloris asiatica	TYLVQPL	KANSSTHLAMSQMDLLARLLKDLC	TEGSGFTTDSVMRFSVSAL	EHRVYEVRETAVRIILDLYRR	YRTLILEYLPPDDSNTRKNVLY	KTIFEGFAKID
Dasypus novemcinctus	SYLVQPL	KANSSAHLAMSQTALLARLLRDLG	TGASGETLDSVMKESVSAL	EHRVYEVRETAVRIILDMYRQ	HQTFILEYLPPDDSSTRRNVLY	KTIFEGFAKID
Canis lupus familiaris	SYLVOPE	KANCSTHLAMSOMSLLARLLKDLG	GTTGFTTDNVMKFSVSAL	EHRVYEVRETAVRVILDMYKQ	HRAFILEYLPPDDSTTRKNVLY	KTIFEGFAKID
Elephantulus edwardii	TYLVOPL	ANSSTHLAMSOLDILAR LENDLG	TECSCET I DNVMKESVSAL	EHRYTEVRETAVE I I DMY PO	HRAFVI FVI PPDDSSTPKNILY	KTIEECEAKID
Saimiri boliviensis boliviensis	SYLVOPL	KANSSVHLAMSHMDLLARLLKDLG	TGSSGFTVDNVMKFSVSAL	HRVYEVRETAVRIILDMYRO	HOASILEYLPPDDSNTRRNILY	KTIEDGEAKID
Odobenus rosmarus divergens	SYLVQPL	KANSSNHLAMSQIGLLAR LLKDLG	TRGTGFTIDNVMKFSVSTL	EHRVY EVRETAVRVILDMY KO	HRAFILEYLPPDDNTIRKNVLY	KTIFEGFAKID
Mustela putorius furo	SHLVQPL	KANTSNHLAM SQVGLLAR LLKDLG	TGSSGFSVDSVMKFSVSAL	E HR VY EV R E A A V R V I L DMY KQ	HRALILDYLPPDDTTTRKNVLY	KTIFEGFAKID
Jaculus jaculus	SYLVQPL	K TNA STHLAM SQVDLLAR LLRDLG	TGSSGFTVDSVMKFAVGAL	EHR VY EVR EMAVR VILDMY KQ	HPALTLEHLPPDDS5TRRNLLY	KTIFEGFAKID
Mesocricetus auratus	SYLVQPL	KANASVHLAMSQVDLLSRLLRDLG	TENSGETVDNVMKFALSAL	EHKVYEVRETAVRVILDMYRQ	HPALTLEHLPPEDSSTRRNLLY	KAIFEGFAKID
Urcinus orca	SYLVOPL		TC STC ETVDSVMR FAVRAL	EHREHKVRDSAVGMILDLYAQ	HRALVLECLPPEESAIRKSVLY	TIEFCEARID
Ovis aries	SYLVOPI	KANSSTHLAMSOMALLARLED) G	TEGAGETYDSVMRESVOAL	EHRYREV REALVENILDI YAO	HRAIVIEVIPPDDSTTRRNVIV	KTIFFGFAKID
Capra hircus	SYLVOPL	KANSSTHLAMSOMALLAR LLRDLG	TEGAGETVDSVMRESVOAL	HRVREVREAAVGMILDLYAO	HRALVLEYLPPDDSATRRNVLY	KMIFEGFAKID
Ochotona princeps	SYLVQPL	KASSSTHLAMSQMDLLAR LLRDLG	TEGSGFTVDSVMKFSVSAL	E HR VY E V R E A A V R V V L E L Y Q R	HPASVLEYLPAEDSSARRSVLY	KTLFEGFARVD
Pantholops hodgsonii	SYLVQPL	KANSSTHLAMSQMALLAR LLRDLC	TEGAGETVDSVMRFSVQAL	EHRVREVREAAVGMILDLYTQ	HQALVLEYLPPDDSATRRNVLY	KTIFEGFAKID
Microtus ochrogaster	SYLVQPL	KP NA STHLAM SQVDLLAR LLRDLG	TGNSGFTVDSVMKFALNAL	EHKVYEVRETAVRIJLDLYRQ	HPALTLEHLPPEDSSTRRNLLY	KAIFEGIAKID
Pteropus alecto	SYLVOPL	KANAPTHLATSRMELLARLLKDLG	TEGSCETVDDVMK FAVGAL	EMRVYEVRETAVRVILDMYEQ	HRAPVLDYLPPDDCAARRNVLY	KILFEGFAKIE
Ailuropoda melanoleuca	SYLVOPI		TCCTCETIONVMEESVSAL	EHRVKEVREAAVGMILDLTAQ	HRAEILEVIPPDDSTIRKNVLT	KTIEFCEAKID
Chinchilla lanigera	SYLVOPL	KPNCSAHLAMSOVDLLARLLRDLG	TAGSGETVDSVMKFAVSAL	EHRVY EVRETAVRY I LDMY A E	HOALVLEYLPPDNSSLRRNLLY	KSIFEGEARID
Felis catus	PYLVQPL	KANSSAHLAMSQMDLLARLLRDLG	TGNTGFTVDNVMKFSVSAL	EHRVYEVRETAVRI I LDMY KQ	HRAFILEYLPPDDNTTRKNVLY	KTIFDGFAKID
Heterocephalus glaber	TYLVQPL	K P S C S A R L A M S Q M D L L A R L L R D L G	TEGTGFSVDSVMRFAVSAL	E HR V Y E V R E T A V R V I L D M Y AQ	HQALVLEFLP PDD S ST RRNLLY	KTIFEGFARID
Mus musculus	SYLVQPL	KANASVHLAMSQVDLLARLLRDLG	TESSGFTVDNVMKFAVSAL	EHRVYEVRETAVRIILDMYRQ	HPALT LEHLPPDDSAT RRNLLY	KAIFEGFAKID
Cavia porcellus	AYLVQPL	KANCSAHLAMSQVDLLARLLRDLC	TENSCETVONVAK FAVRAL	EHRVYEVRETAVRVILDMYTR	HQALVLEYLPPDDSGMRRNLLY	KTIFEGFARID
Sarcophilus harisii	IVIVOPI	KONSSTHLAMSOTDLLARLIKDIC	TENSCETVONVMRESVSAL	EHRLY FIREIAIKILLOMYKT	HRTIILEVIPMODONTPKNILY	KTIEFCEAKID
Rattus norvegicus	SYLVOPL	KTNASVHLAMSOVDLLARLLRDLG	TEGSGETVDNVMKEAVSAL	HRVYEVRETAVRIJLDMYRO	HPALTLEHLPPDDSTTRRNLLY	KALFEGEAKID
Chrysemys picta bellii	VHLVQPL	KPNSPTHLAMSQVDLVER LLKDLC	TENSGETIONVMREATGAL	EHRVYEV RDTALRIIFDMY RQ	HQAIILDYLPPDDANTRKNVLY	KTLFDGFTKID
Camelus ferus	AYLVQPL	KAGSSAHLAI SQMALLAR LLRDLG	TEGAGETVDSVMKESARAL	EHRVPEVREAAVRLILGLYAQ	HRAAVLEHLPPADSATRKNVLY	KTIFECFAKID
Monodelphis domestica	VHLIQPL	KQNSSTHLAMSQTDLLARLLKDLG	TENSGETVDNVMRESVSAL	EHRIFEIREIAIKIILDMYKK	HRTLILEYLPADDGNARKNILY	KTLFEGFAKID
Octodon degus	SYLVQPL	KPNCSAHLAMSQVDLLARLLRDLG	MAGSGFTVDSVMKFAVSAL	EHR VHEVREMAVRLILDMY AE	HRALVLEHLPPDDSSTRKNLLY	TIFEGLARID
Alligator mississippiensis	HLVHPL	KPNSPTHLAMSOVELVERLLKNLG	TENSCETVONIMERATCAL	EHRVY EVEDTAL RIJEDMY RO	HRTVILNYLPPDDANTRKNVLY	KTLEDGETKID
Sorex araneus	PYLLOPL	KANASPHLAL SQMSLLAR LLRDLG	TESEGFPPDAVMKFTVSAL	EHRVY EVR ETAVOVILDLYRO	HRPVVLEHMPPDDAATRKNVLY	RTIFEGFARID
Myotis davidii	SYLVQPL	KANAPAHLAL SQVALL SRLLRDLG	TOSTGETVENVMTETVRAL	HRVLQVREMAVCVILDLYRQ	RGSVVLEYLPPDDSISRKNILF	KTIFEGFAEID
Anolis carolinensis	AHLVQPL	KPNSPTHLSMSQVELVERLLKDLG	TONT GET VONVMR FATGAL	EHRVYEVRETALRIILDLYRQ	HRSAVLDYLPPDDANTRKNVLY	KTLFDGFAKID
Meleagris gallopavo	VHLVQPL	SNSPTHLAMSRVELVECLLKEMG	ENSGET I SNAMK FATGAL	ENRVY EVROVALRIIFDMYRK	HKAAILEYLPPDDASIRKTVLY	TLEDGETKID
Myotis lucifucus	SYLVOPI	ANASAHLAI SOVALI SPLIPDIC	TOST CETVENVMK FATGAL	CHRYLOVE EMAYCYLLDLYRO	HR SVVIEVIPPDDSISPUNIE	TIFECEAFID
Anas platvrhvnchos	VHLYOPI	KPNSP I HLAMSOVELVECT LKDMC	TENSGETISNYMKEATCAL	EHRVYEVRDVALRILEDMYRK	HKAAILEYLPPDDASIRKTVLY	KTLEDGEAKID
Myotis brandtii	SYLVQPL	ANASAHLALSQVALLSRLLRDLC	TDST GFTVENVMK FAVRAL	HRVLQVREMAVCVILDLYRQ	HRSVVLEYLPPDDSISRKNILF	KTIFEGFAEID
Melopsittacus undulatus	VHLVQPL	KPNSAPHLAMSQVELVQCLLRDMG	TENSGETISNVMKEATGAL	EHRVYEVRDVALQIIFDMYSK	HRAAVLEYLPPDDATIRKTVLY	KTLFDGFTKID
Falco peregrinus	VHLVQPL	KPNSPRR LAMSQAELVEY LLKEMG	TENSGETITNVMKFATGAL	EHRVYEVRDAALRIIFDMYRK	HKAAVLEYLPPDDASIRKTVLY	KTLFDGFTKID
Falco cherrug	VHLVQPL	KPNSPRRLAMSQAELVEYLLKEMG	TENSGETITNVMKFATGAL	EHRVYEVRDAALRIIFDMYRK	HKAAVLEYLPPDDASIRKTVLY	KTLFDCFTKID
Xenopus tropicalis	THIVOP	PTMPSRIAO SOVDIJENU VEC	VESSOET VDNVMK FAVCAL	ENTISEVEDIAALKITIFAMYKM	HP SOVI FYMPT FOSSAPYNILY	KTLEFCFAKID



Figure S3, related to Figure 2A



PPP6R3

SRP68

PSMC2

NOTCH2

DBN1

SCIN

SEC16A

PSMD12

CEP290

TCP1

KTN1

NEDD1

PA2G4

EIF4G2

SPAG9

CSDE1

RPAP3

PIBF1

TACC1

TMF1

DST

RANBP2

HNRPK

EIF2A

PLS3

ENO1

STIP1

PXN

VCL

SPAG5

KIF5B

SNX1

ERBB2IP

PPP1R12A

GOLGB1

CCDC85C

ZYX

SLK

TJP2

SMG7

TRIM9

RASSF8

CCDC66

XIAP

IFT74

EEF2

FKBP4

SYAP1

CCT2

RAI14

EPB41L2

KIAA1524

ZC3H15

WDR44

FAM21A

EIF4ENIF1 EPS15

DNAJC7

REPS1

CLMN

SLAIN2

B0FTY2_HUMAN

B4DTX5_HUMAN

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





Figure S5, related to Figure 3A



