Supplementary Information

Supplementary Figure 1

(A) Immunoprecipitated C7orf43-LAP expressed in HEK293 cells was incubated with GST-alone and GST-tagged Rabin8 full-length. Representative results from three independent experiments are shown.

(B) Immunoprecipitated GFP-alone, GFP-tagged C7orf43-LAP, GFP-Rab11aQ70L, and GFP-Rab11aS25N expressed as described in (A) were incubated with recombinant GST-Rabin8. Representative results from two independent experiments are shown.

Supplementary Figure 2

The phylogenetic tree for C7orf43/TRAPPC14 for the following BLAST identified proteins: *N.nippon*, XP_009472339.1; *M.musculus*, NP_694801.2; *D.rerio*, XP_001339329.2; *H.sapiens*, NP_060745.3; *X.tropicalis*, NP_001121523.1, *T.rubripes*, XP_003970822.1; *N.vectensis*, XP_001632706.1. A tree was constructed using these sequences on MacVector software (Version 15.5.0). Values over branches, or branch lengths, represent evolutionary distances. Method: Neighbor Joining; Best Tree; tie breaking = Systematic Distance: Uncorrected ("p") Gaps distributed proportionally.

Supplementary Figure 3

Multiple sequence alignment of the C7orf43 orthologs from Fig. S2 was performed using ClustalW in MacVector software (Version 15.5.0). MSF file was generated and imported into BoxShade. A glycine-rich region from residues 53 to 134 is also well conserved in mammals.

Supplementary Figure 4

(A) Serum fed RPE-1 cells transiently expressing C7orf43-LAP for 24h, fixed and stained with antibodies for the Golgi marker GM130. Nuclei were stained with DAPI. Images are representative from two independent experiments. Scale bar =10 µm. (B) Panels show representative images of RPE-1 cells transiently expressing C7orf43-LAP and tRFP-Rabin8 for ~24h and serum-starved for the last 1h followed by fixation with -20^{o}C methanol for 10min. Antibody staining for Myosin-Va and gamma-tubulin (centriole marker) and imaging was as in (A) without triton in the incubation buffers. Representative images are shown from two independent experiments. Scale bar top panel = 10 µm, bottom panel = 1 µm. (C) Representative images of RPE-1 cells treated as described in Fig. 3B showing C7orf43-LAP and tRFP-Rabin8 colocalization in the distal region of cilia (marked by ^{Ac}tub). The ciliary localization of exogenously expressed proteins was observed in two cells out of a total of n>100 ciliated cells from three independent experiments. Scale bar = 2 µm. (D) Plot of GFP-Rabin8 centrosomal accumulation from RPE-1 GFP-Rabin8 cells treated with siControl, siC7orf43#1, siC7orf43#2, or siC7orf43#1+#2 and starved for 1-2h as described in Fig. 3C and 3D. Means ± s.e.m are shown from three independent experiments with n>120 cells counted in total. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Supplementary Figure 5

Multiple sequence alignment of the *S.cerevisiae* Trs65 (KZV11397.1) with human, mouse and frog sequences of C7orf43/TRAPPC14 from Fig. S2 was generated using Muscle matrix in MacVector software (Version 15.5.0) and alignment was rendered as in Fig S3. Stretches of higher homology are shown with a pink bar. In a parallel analysis, Clustal Omega (ClustalW matrix with

default settings) was used to calculate % identity. *S.cerevisiae* Trs65 displayed 20.4%, 20.4%, and 20.3% identity to human, mouse and frog C7orf43/TRAPPC14, respectively.

Supplementary Figure 6

(A)Western blot from HEK293 cell lysate treated with RNAi for a Control, C7orf43/TRAPPC14, TRAPPC3, -C9, or -C10 and blotted with TRAPPC and ß-actin antibodies. Representative results from three independent experiments are shown. (B) Immunoprecipitation analysis of transiently-expressed GFP after siControl and siTRAPPC10 treatments as described in Fig. 6A. GFP was transfected into cells 24h after RNAi treatment. Immunoblots were probed with antibodies as indicated. Protein levels compared to siControl treatments were determined by densitometry analysis and are shown below blots normalized for actin levels. Representative results from three independent experiments are shown.

Supplementary Figure 7

(A)Immunoblot of RPE-1 cells depleted of FBF-1, CEP83, and CEP164 following 72h RNAi treatments and probed with specified antibodies. Representative blot from two independent experiments are shown. (B) Immunoblot of immunoprecipitated HA-luciferase or HA-C7orf43/TRAPPC14 with LAP-CEP83. Representative results from two independent experiments performed in HEK293 are shown.

Supplementary Table S1: siRNAs used in this study

Gene	SiRNA	Sense sequence	Company (Cat no.)
hC7orf43	siC7orf43 #1	ACAAGATTGCCAAGCGCGA	Dharmacon (J-016464-19/ OTP)
hC7orf43	siC7orf43#2	AGAGGGTGGTGATGGCTAA (targeting 3'UTR)	Dharmacon (J-Custom/ OTP)
hCEP83	siCEP83 #1	GAATCTAGATGAAGAGGTA	Dharmacon (J-021034-18 OTP)
hCEP83	siCEP83 #2	AGGTGAAGTTGGTGACTCA (Reference 1) (targeting 3'UTR)	Dharmacon (Custom/ OTP)
hCEP164	siCEP164 #1	CAGGTGACATTTACTATTTCA (Reference 2)	Dharmacon (Custom/ OTP)
hCEP164	siCEP164 #2	AAGAAGATACAGGAAGCTCAA (Reference 2)	Dharmacon (Custom/ OTP)
hFBF1	siFBF1 #1	GGAACAACTGCACGAGAAA	Dharmacon (J-030807-18/ OTP)
hFBF1	siFBF1 #2	GGTTGGGCCTCAAGGACGA	Dharmacon (J-030807-19/ OTP)
hTRAPPC10	siTRAPPC10	GGTTAATAGTGATAGTTGA (Reference 3)	Dharmacon (J-008621-09/ OTP)

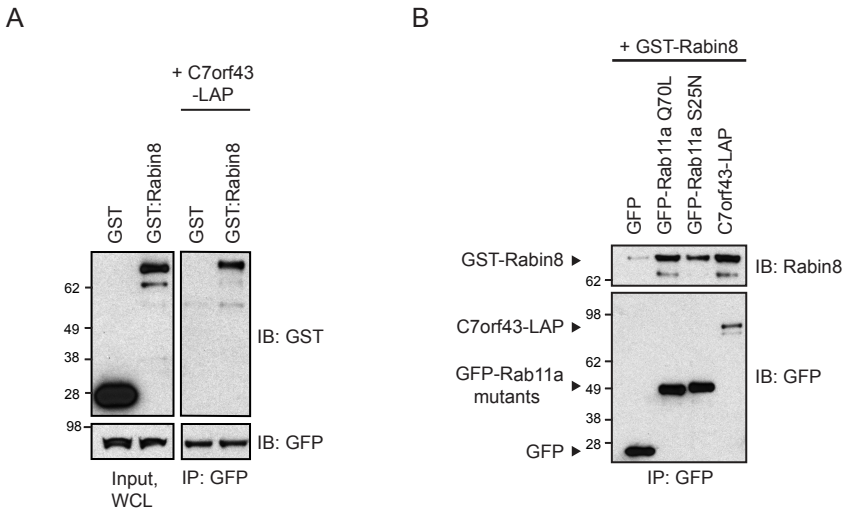
hTRAPPC9	siTRAPPC9	GAAAGTCAGCAACTAATCA (Reference 3)	Dharmacon (J- Custom/ OTP)
hTRAPPC3	siTRAPPC3	TCAGGCGGATTGAGGACAA (Reference 3)	Dharmacon (J-017649-12/ OTP)

Reference 1: Joo K*, et al.* (2013) CCDC41 is required for ciliary vesicle docking to the mother centriole. *Proc Natl Acad Sci U S A* 110(15):5987-5992.

Reference 2: Schmidt KN*, et al.* (2012) Cep164 mediates vesicular docking to the mother centriole during early steps of ciliogenesis. *J Cell Biol* 199(7):1083-1101.

Reference 3: Westlake CJ*, et al.* (2011) Primary cilia membrane assembly is initiated by Rab11 and transport protein particle II (TRAPPII) complex-dependent trafficking of Rabin8 to the centrosome. *Proc Natl Acad Sci U S A* 108(7):2759-2764.

Figure S1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GFP
GFP-Rab11a Q70L
GFP-Rab11a S25N
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GST-Rabin8
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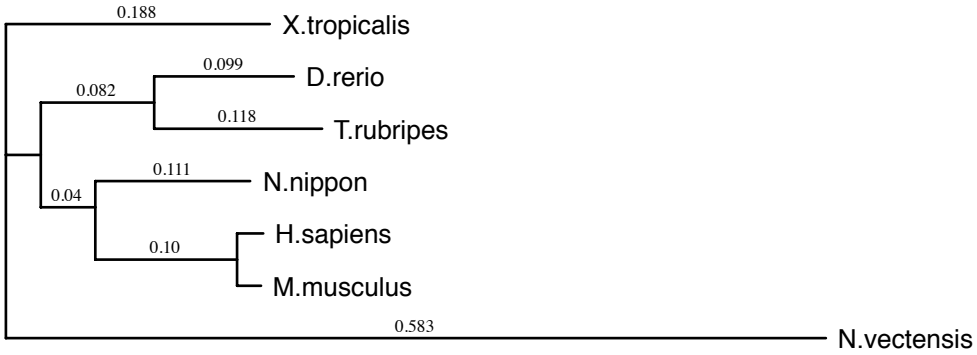
Figure S2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D.rerio
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T.rubripes
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N.nippon
0.04
H.sapiens
0.10
M.musculus
0.583
N.vectensis

Figure S3


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X.tropicalis      1 ----MESQCDYSMIFPACPLLP----SDLPVRYRALPRRNHLYLGETLRFLLVLRLRSAG
D.rerio           1 MVLMMESQCEYEMYFPAVPISD----LSDPAKYRTLPRRSHLYLGETVRFLLVLRSQSAS
T.rubripes        1 ----MESQCEYYMYFPAVPITD----LSDPARYRTLPRRSHLYLGETVRFLLVLRCRDGA
N.nippon          1 ------------------------------------------------------------
N.vectensis       1 ----MAGVKLDSNFFIFLPHIS----TREPVSDIEKGPRYSCFVGELLHFFLIAQFRGKG

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M.musculus       56 VGAGVGG---------GAGLASRGAWTELATSLAALASVSAGGA---------------L
X.tropicalis     53 ERPQERA---------GTEASAVGEASQESPGSEAFSWAPLAAS---------------L
D.rerio          57 GSSDGSC---------GSEQHSSRSWRELAGSLSAVASVSPGDS---RQRTQPLYHDYHS
T.rubripes       53 GTPADHCPARNCTPSFGTELASSQAWRELAGSLCAVASVSPGESSRHRSSHYPHHHDYQS
N.nippon          1 ---------------------------------MAPPRVSPGGT---------------E
N.vectensis      53 DSTDGVR--------------EWQNRLLRLNTCVSVSCVDLASR----------------

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M.musculus       92 PGCGSAGDQD--ADPPGGGDPGGGG--------LFRGCSPLLTHGQG----PATSGGATT
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T.rubripes      113 SGDEANDDGEEDYIAAAEAAIAALGSRVDSRCRSFRDCKPLLIHNSSGTATREFRRAPVQ
N.nippon         13 AGGDRDGDLDGTGDDTGSCASLEPT--------VFRDCRALLTHSQG----PPGRP-AAG
N.vectensis      83 ---------------SDECQQIKSG---------FVKCYAVSSFRNE----KIIDSPHDS

H.sapiens       138 LPVEEPIVST-DEVIFPLTVSLDRLPPGTPKAKIVVTVWKREIEAPEVRDQG-----YLR
M.musculus      138 LPVEEPIVST-DEVIFPLTVSLDRLPPGTPKAKIVVTVWKREVEAPEVRDQG-----YLR
X.tropicalis    136 TPVTDPVVSS-DEVIFPLSVSLDRLPPGTVKAKIVVTVWKRDTEQSRVRTFG-----YRS
D.rerio         161 SPVDEPVVLS-DEVIFPLTVSLDKLPVNTLKVKIIVTVWKQEEEKAEIQEHG-----YLS
T.rubripes      173 SPLDEPVVLT-DEVIFPLTVSLDKLPVNTLKVKVMVTVWKREAEKAEVQELG-----YLS
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N.vectensis     115 TKVHCPIITSRGDIIYPLTASLDVLPALSKRIKLSVNVWTQELSAPVNVTNGLQWSNYVD

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T.rubripes      227 VLQQCEPTHTFRHDLNTFKAQVSTTLTVLPPPTVHCKQMTVSGKHLAVLKVLSGSSQEEI
N.nippon        115 LLQTRAPAHVFRQEQGAFKAQVSTLLTVLPPPVVRCRQLTVSGKYLTVLKVLNGWSQEEI
N.vectensis     175 YFINNDPDDALGDLQTPFFCHVNATLPVVSPPTVRGKFTTATGKHYVAIEVINSLG-EPV

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M.musculus      252 SIWDIRILPNFNASYLPVMPDGSVLLVDNVCHQSGEVSMGSFCRLPGTSGYFPCPLSALE
X.tropicalis    250 SICDVRILPNFNANYLPVMPDGSVLLVDNVCHQSGDITMASFLRLHSASSQLPSRLGSLE
D.rerio         275 CVRDVKILPNFNASYLPMMPDGSVLLVDNVCHQSGEVAMASFYRMDSESSHLPSMLSALE
T.rubripes      287 SIRDIRILPNLNASYLPMMPDGSVLLVDNVCHQSGEVGMASYCRVDSLASHLPTMLSTLE
N.nippon        175 SLWDVQILPNFNASYLPVMPDGSVLLVDDVCHHSGEVPVCAFCRVASAGSACPCALSALE
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Figure S3 cont.

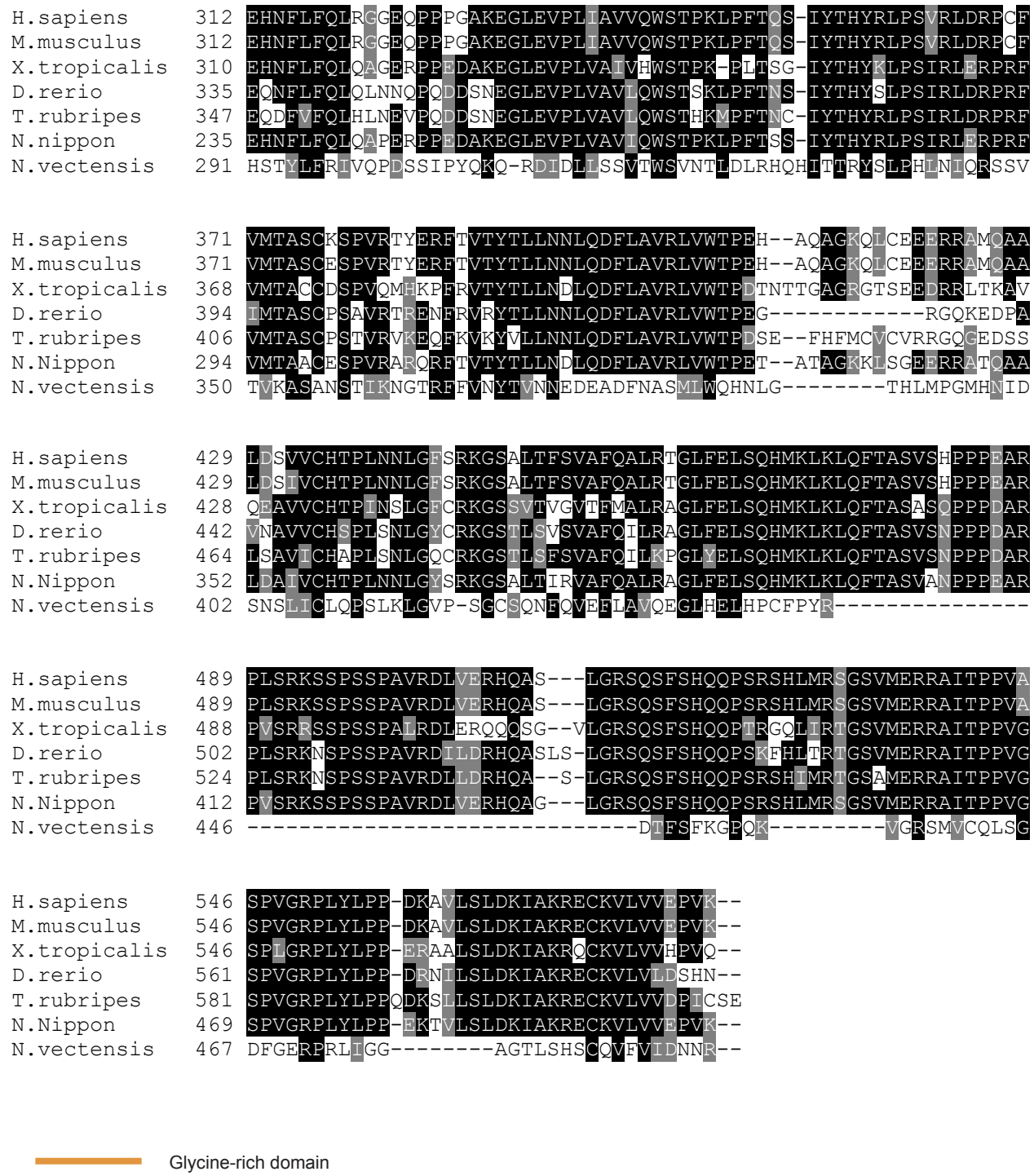
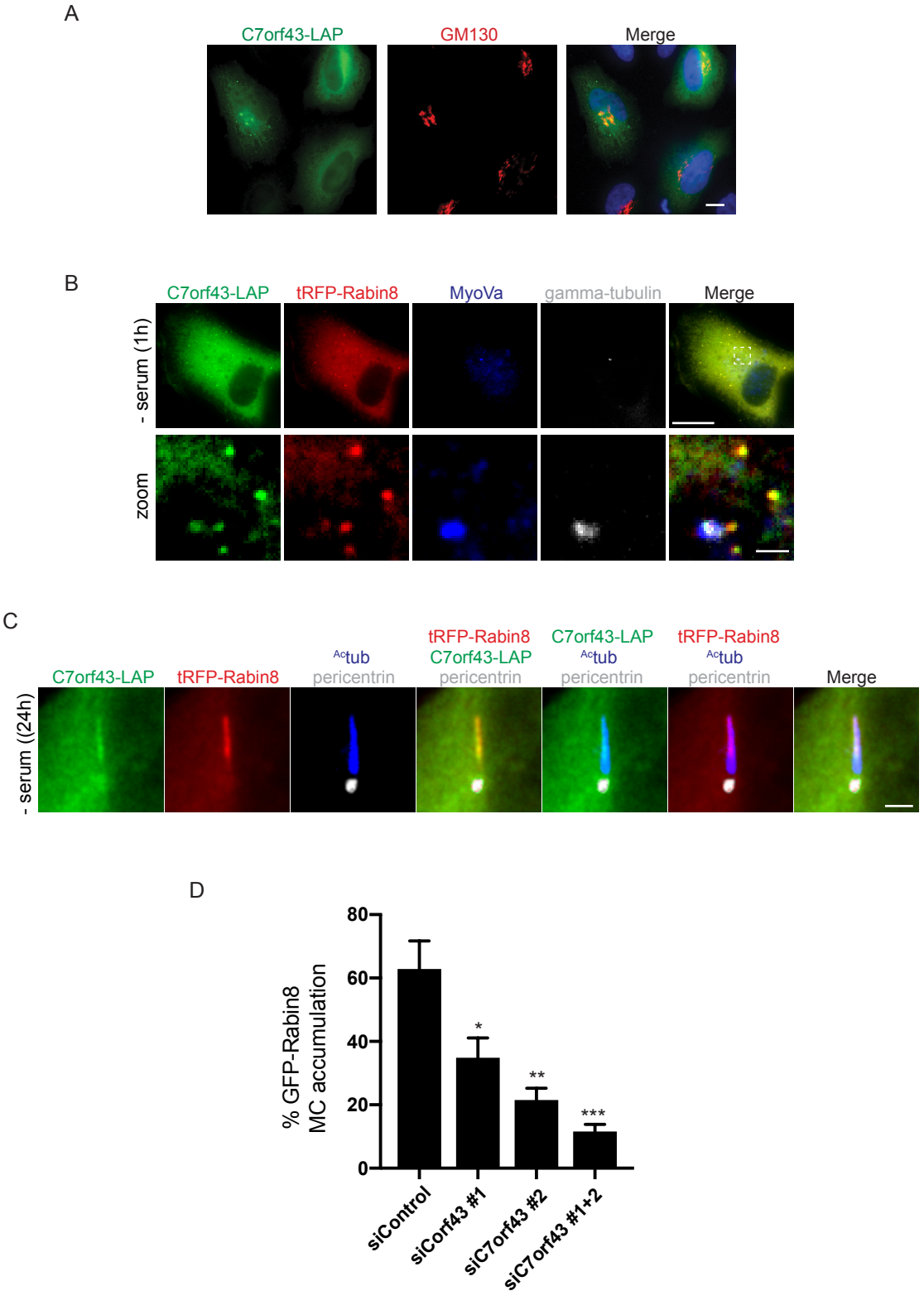


Figure S4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C
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tRFP-Rabin8
Actub
pericentrin
tRFP-Rabin8
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pericentrin
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tRFP-Rabin8
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pericentrin
Merge
- serum ((24h)
D
% GFP-Rabin8
MC accumulation
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0
*
**
***
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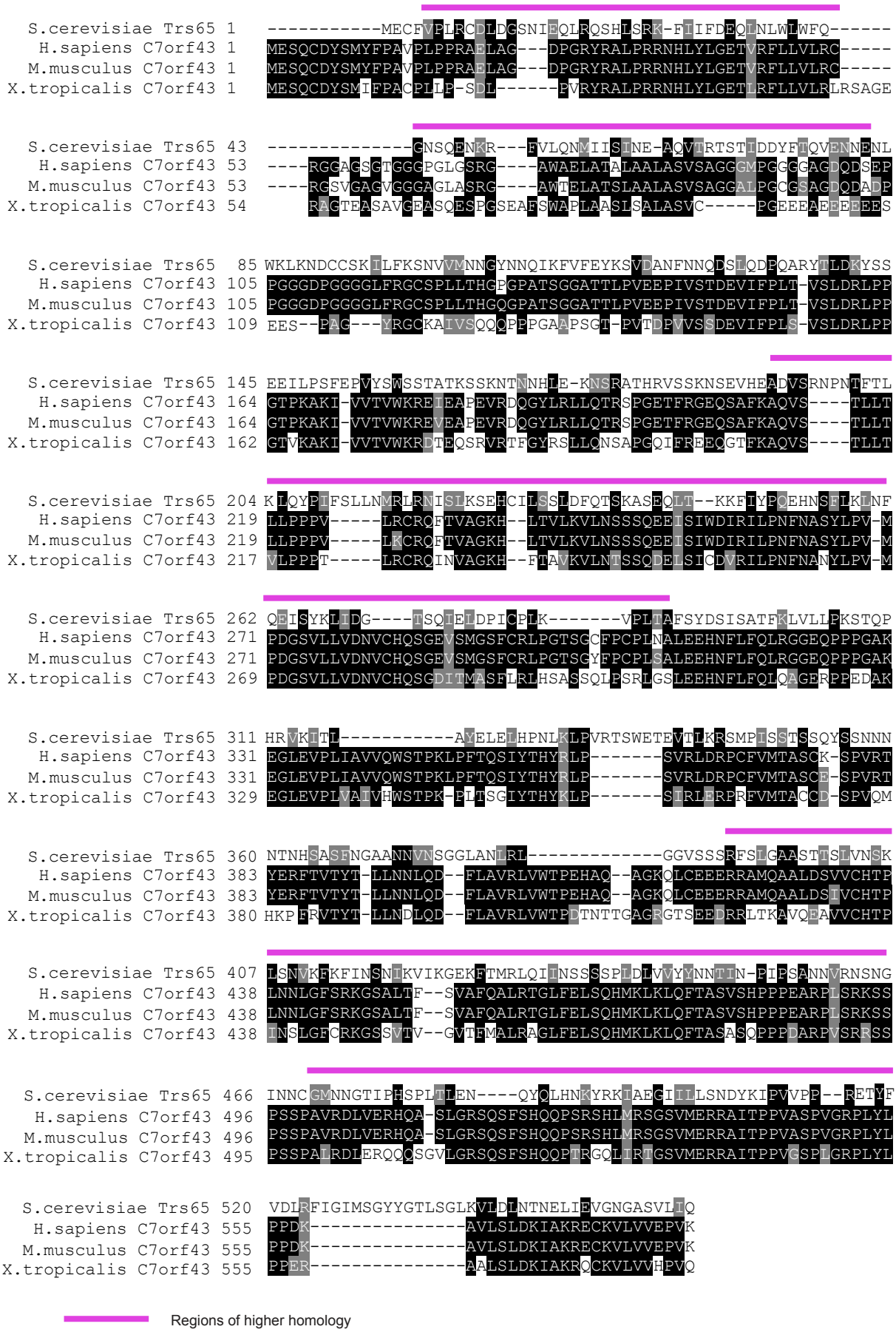
Figure S5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Regions of higher homology

Figure S6

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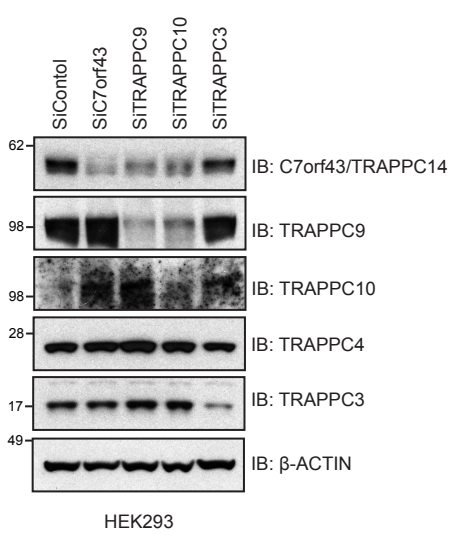
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17
IB: TRAPPC3
49
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HEK293

B

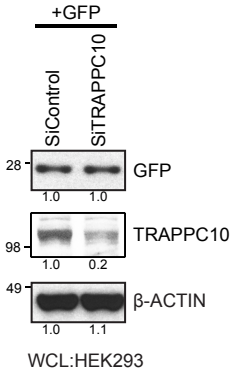
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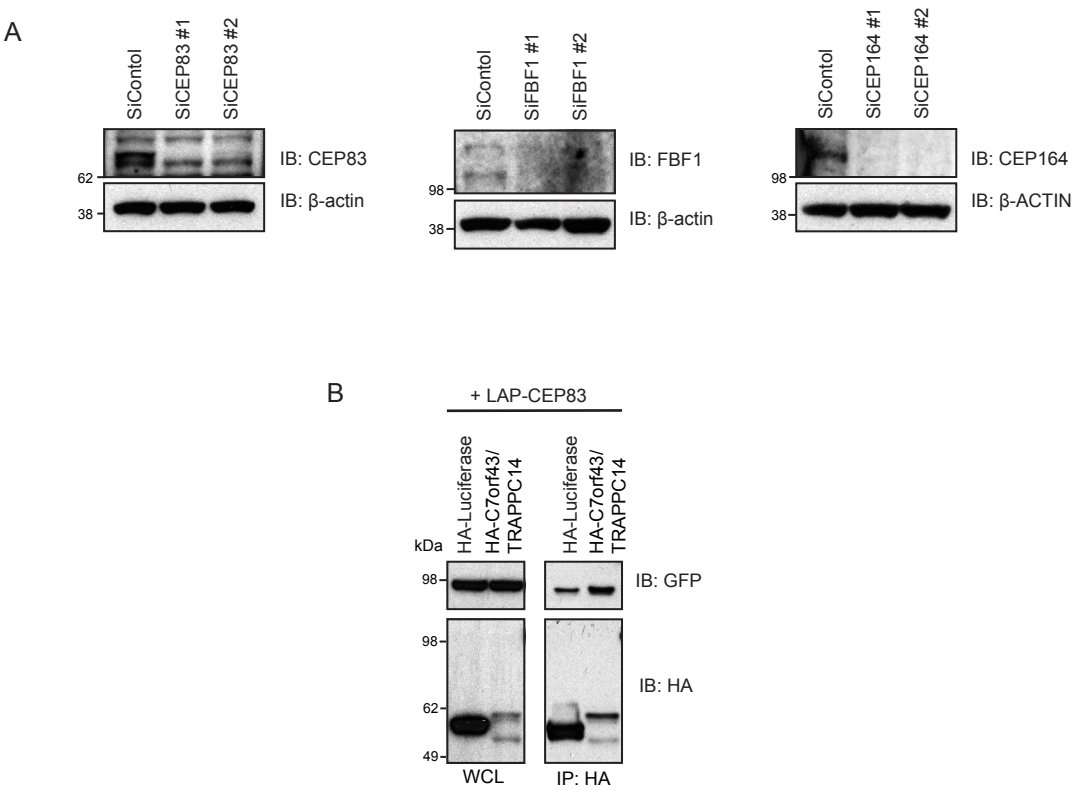
Figure S7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38
IB: FBF1
IB: β-actin
SiContol
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SiCEP164 #2
98
38
IB: CEP164
IB: β-ACTIN
B
+ LAP-CEP83
kDa
HA-Luciferase
HA-C7orf43/ TRAPPC14
HA-Luciferase
HA-C7orf43/ TRAPPC14
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98
62
49
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WCL
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