Supplementary Data – Figures

Supplementary Data – Tables

Table S1. DASE analysis at the *CCDC170/C6orf97-ESR1* locus *(P<0.05)*

Table S2. Stratification of BioID hits based on filtering potential contaminants

Table S3. Final BioID hits

Figure S1. CCDC170 does not colocalize with markers for mitochondria, endosomes, lysosomes and endoplasmic reticulum. GFP-CCDC170 localization was examined in HeLa cells for each of four organelle marker (in red): anti-AIF(mitochondria), anti-EEA1(endosomes), LysoTracker (lysosomes), and anti-calreticulin (endoplasmic reticulum). The nuclei are shown in blue (DAPI) as a reference. For the mitochondrial and endosome markers, antibodies from the Organelle Localization IF Antibody Sampler Kit (Cell Signaling Technology, #8653) were used at the recommended dilutions. For lysosome detection, LysoTracker Red (Invitrogen/Molecular Probes) was used. For the endoplasmic reticulum marker, anti-calreticulin (abcam ab92516) was used at the recommended dilution.

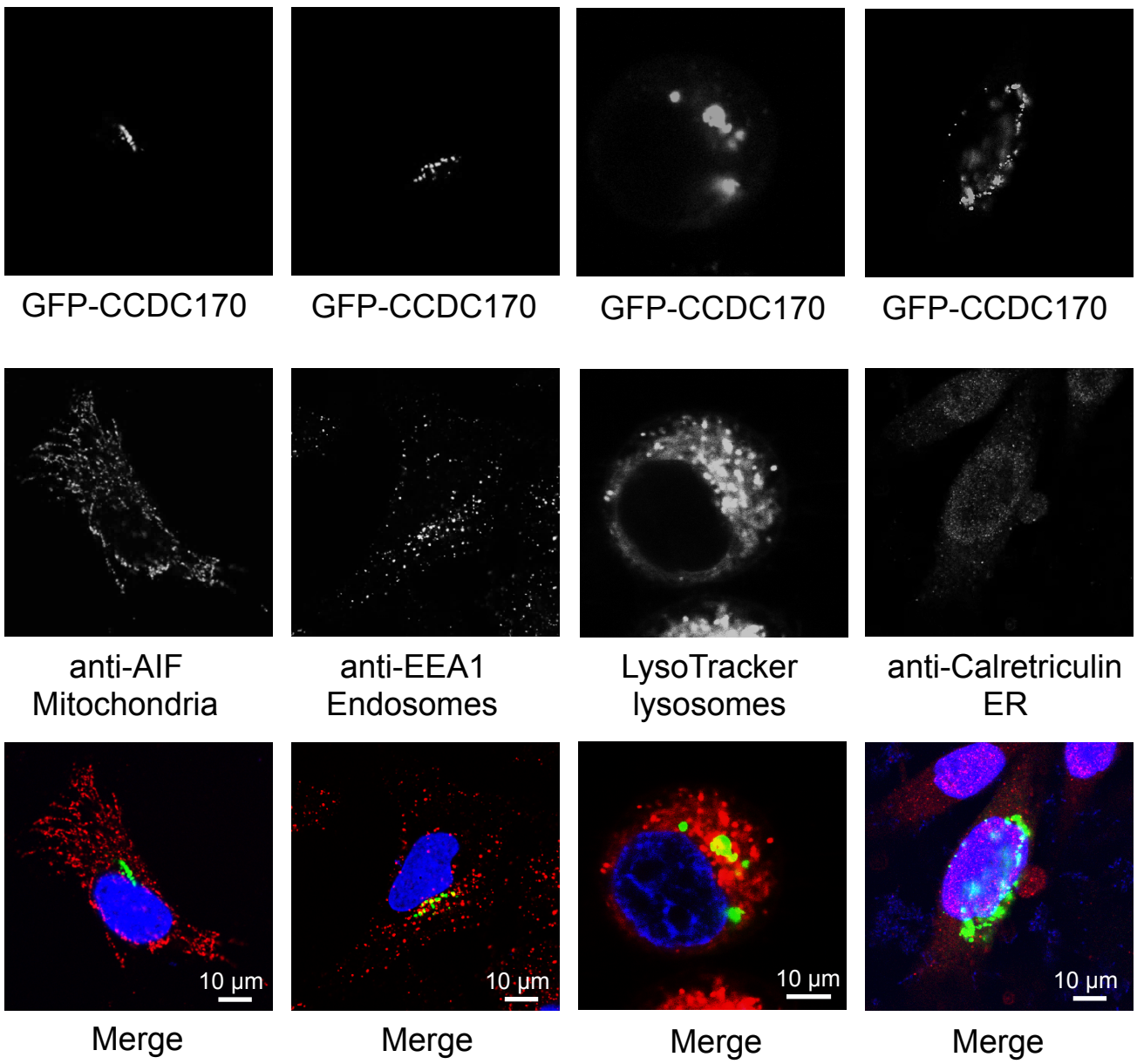


Figure S2. Untagged CCDC170 shows similar localization to the N-terminal GFP fusion. HeLa cells were transfected with either N-GFP-CCDC170 or untagged CCDC170. The untagged protein was detected using anti-CCDC170 (abcam, # ab97814) and goat anti-rabbit secondary (red) (Alexa 594 conjugated, Cell Signaling #8889).

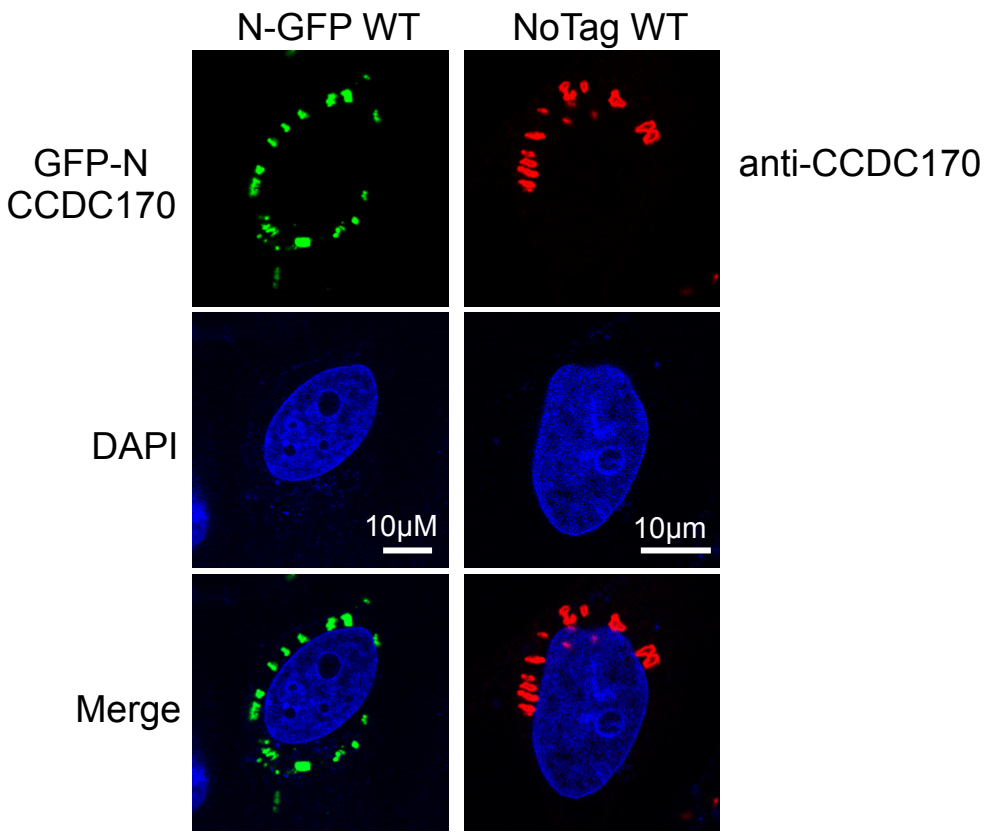


Figure S3. GFP-tagged CCDC170 localizes with Golgi cisternae-like structures. Localization of CCDC170 was examined by EM in HeLa cells transiently expressing GFP-CCDC170. GFP-tagged CCDC170 localized with Golgi cisternae-like structures. Two examples are shown.

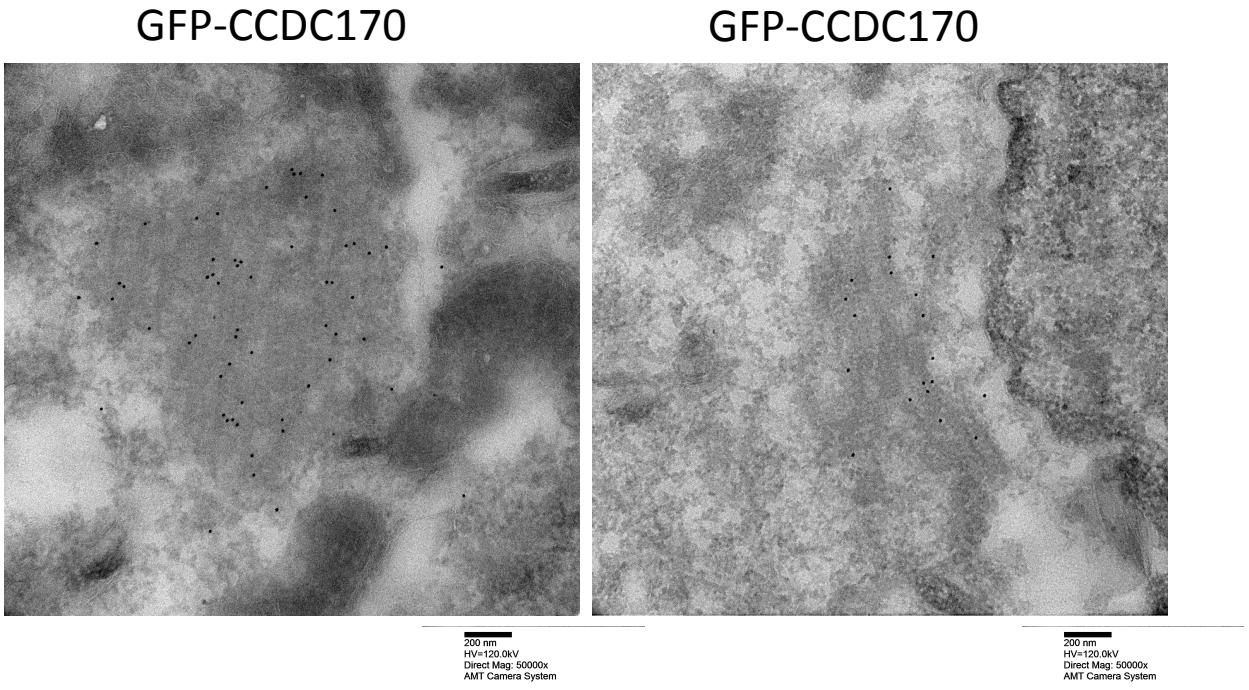
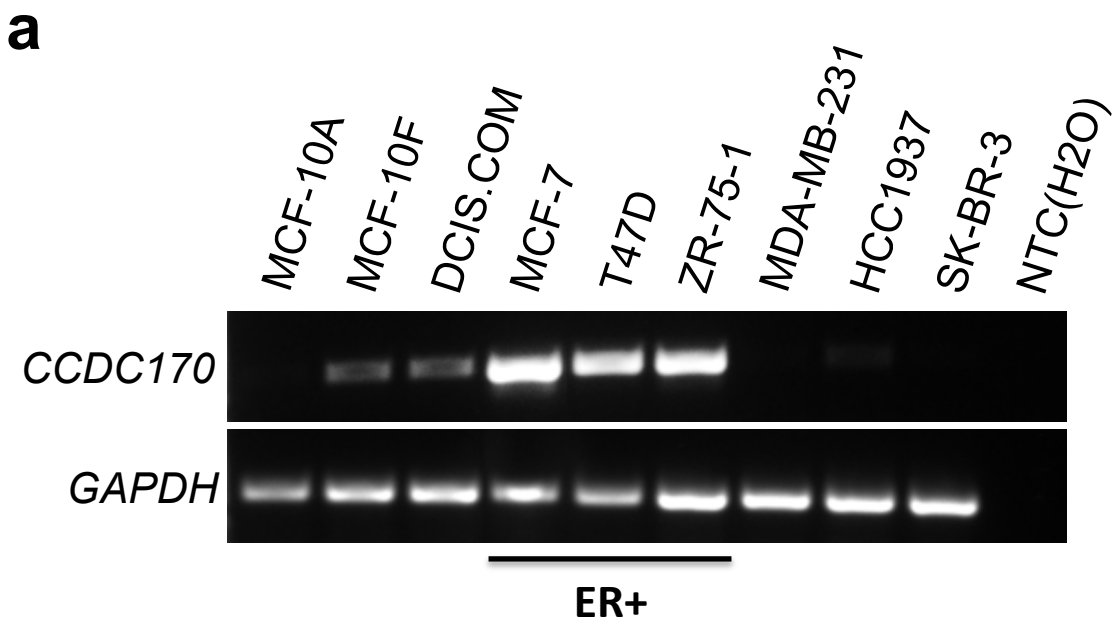
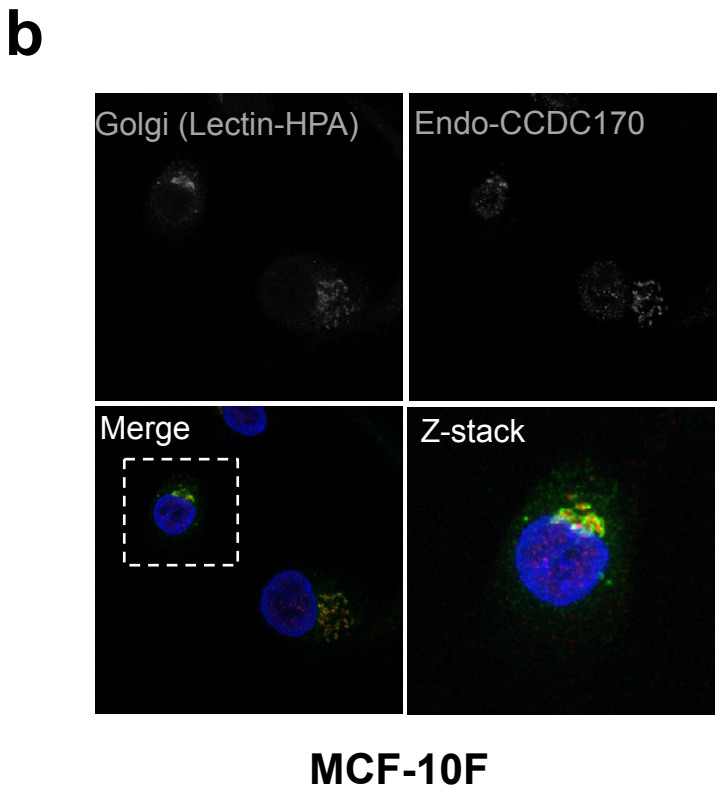


Figure S4. Localization and expression of endogenous CCDC170 in breast cells. a. Evaluation of CCDC170 mRNA expression in two benign breast cell lines (MCF-10A and MCF-10F) and ten breast cancer cell lines by RT-PCR. **b.** Subcellular localization of endogenous CCDC170 in normal MCF-10A cells. Lectin-HPA was used to detect the Golgi. CCDC170 protein was detected using rabbit anti-CCDC170 (1:100, HPA027114,Sigma-Aldrich). **c.** Representative IF images of CCDC170 in ER+ breast cancer cells using rabbit anti-CCDC170, as in Panel B.





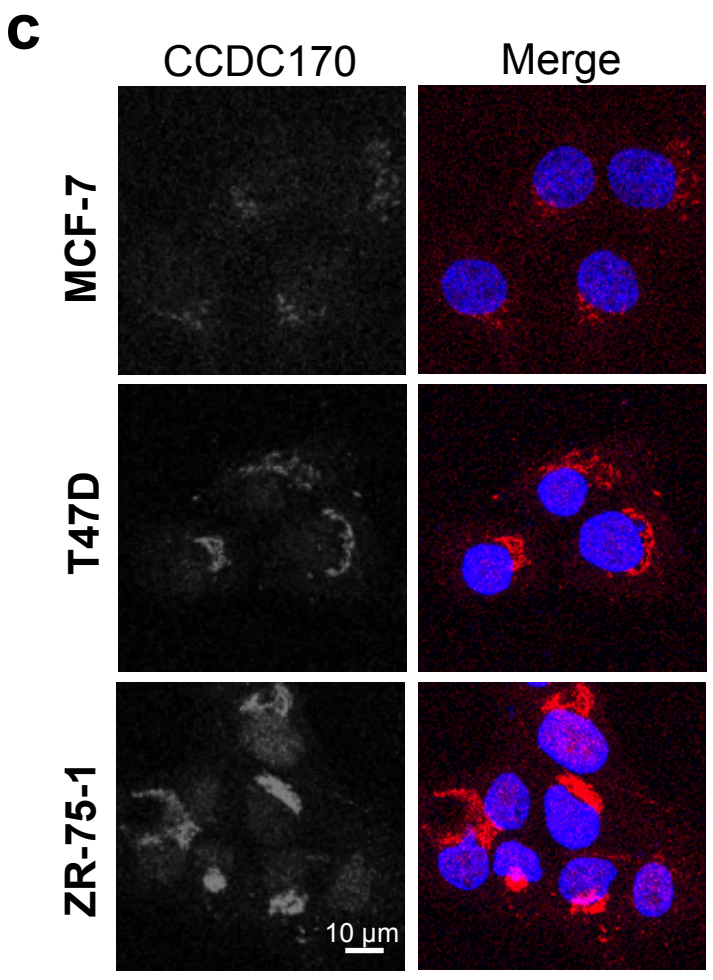


Figure S5. Endogenous CCDC170 is associated with MT in MCF-10F. MCF10F cells were fixed and stained with α-tubulin (red) and CCDC170 (green) antibodies (#T5168, Sigma-Aldrich and HPA027114, Sigma-Aldrich, respectively). In addition to Golgi localization (**Figure S4b**), endogenous CCDC170 associates with MTs. As is the case with CCDC165, we also noted some apparent accumulation at the cell periphery (Sato et al, 2014).

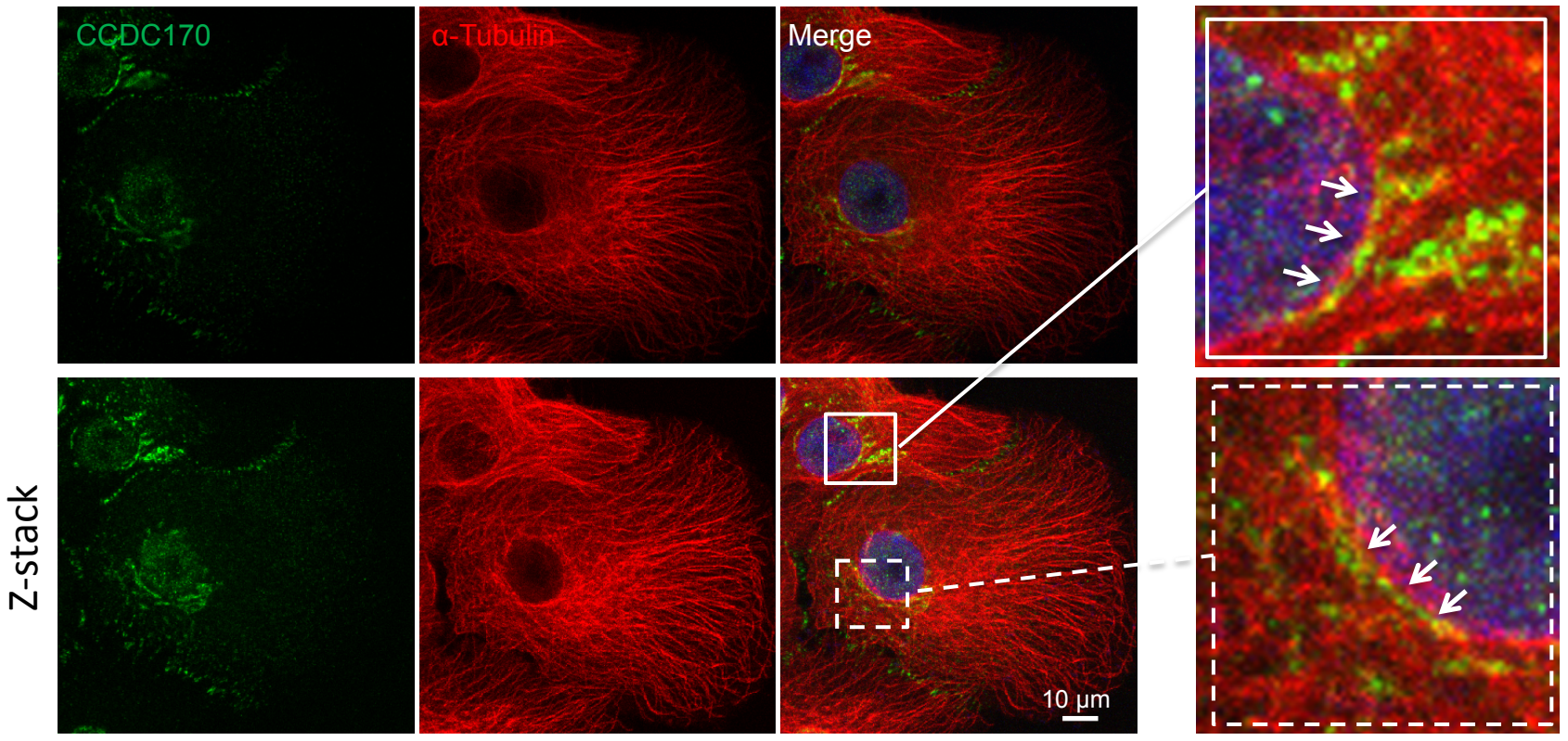


Figure S6. HeLa or U2OS cells were transfected with the indicated GFP-CCDC170 constructs. Cells were fixed and stained with anti-tubulin. Confocal imaging was carried out as described in the Methods.

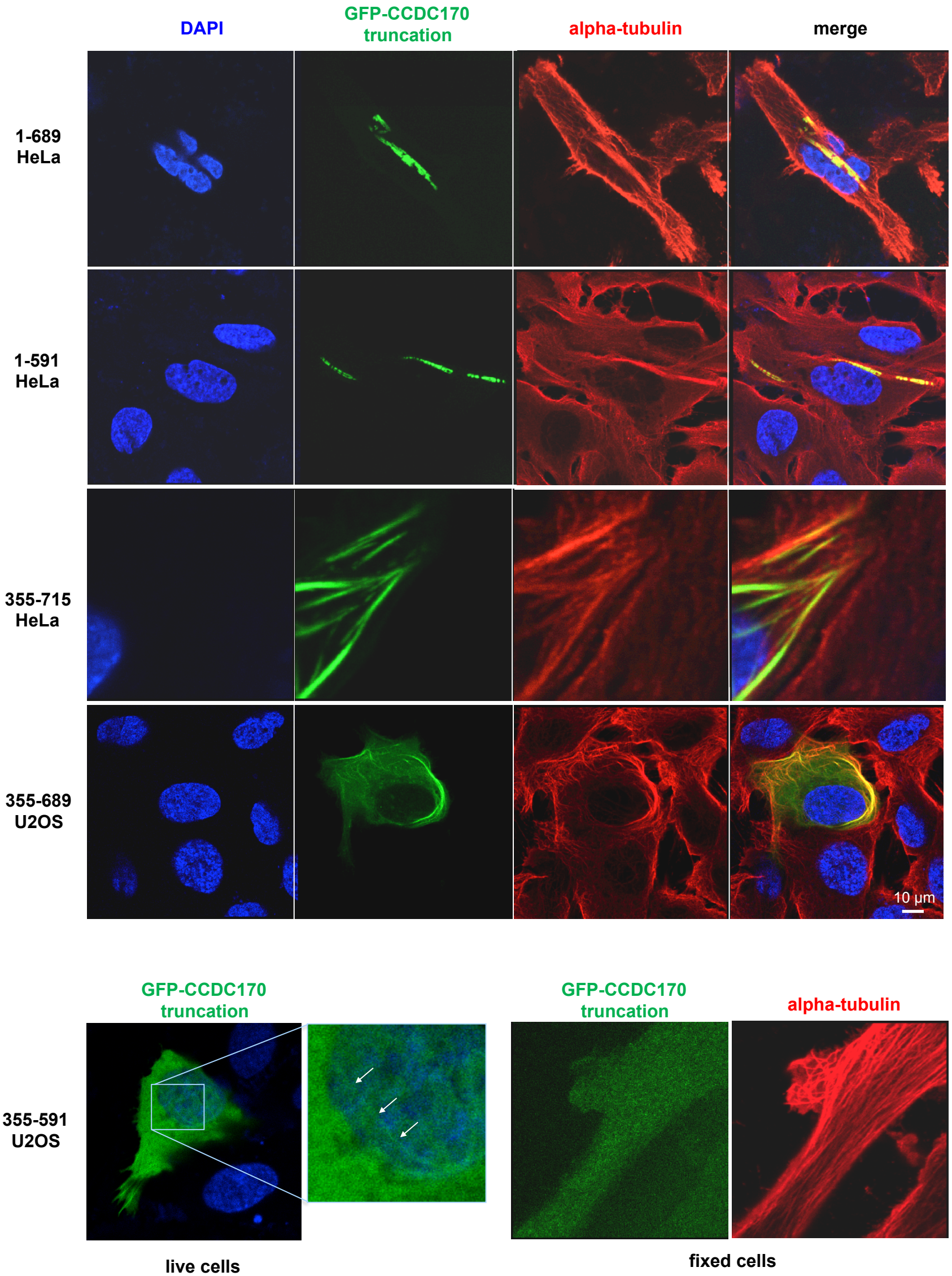
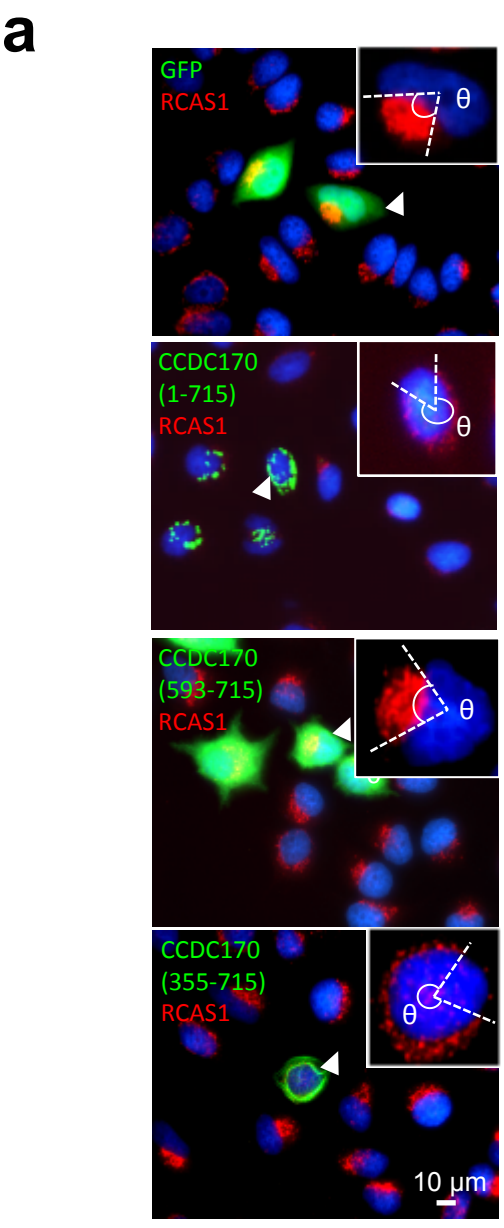


Figure S7. CCDC170 overexpression promotes elongation or fragmentation of the Golgi ribbon structure in CCDC170-inducible MCF-7 Tet-On cells. a. To examine whether overexpression of GFP-CCDC170 promoted reorganization of the Golgi, inducible MCF-7 Tet-On cells expressing GFP-CCDC170 or truncated forms (355-715, and 593-715) were treated with doxycycline at 500ng/ml. Non-fused GFP was used as a negative control. The angle of Golgi spreading was observed by staining with anti-RCAS1. **b**. Graph shows the distribution of cells with different ranges of Golgi ribbon angle (Θ: 0° - 360°) with respect to expression of non-fused GFP, GFP-CCDC170, GFP-593-715 and GFP-355-715 [n=100 for each construct; vs. non-fused GFP (180°-360°); *: P<0.01, **: P<0.001].



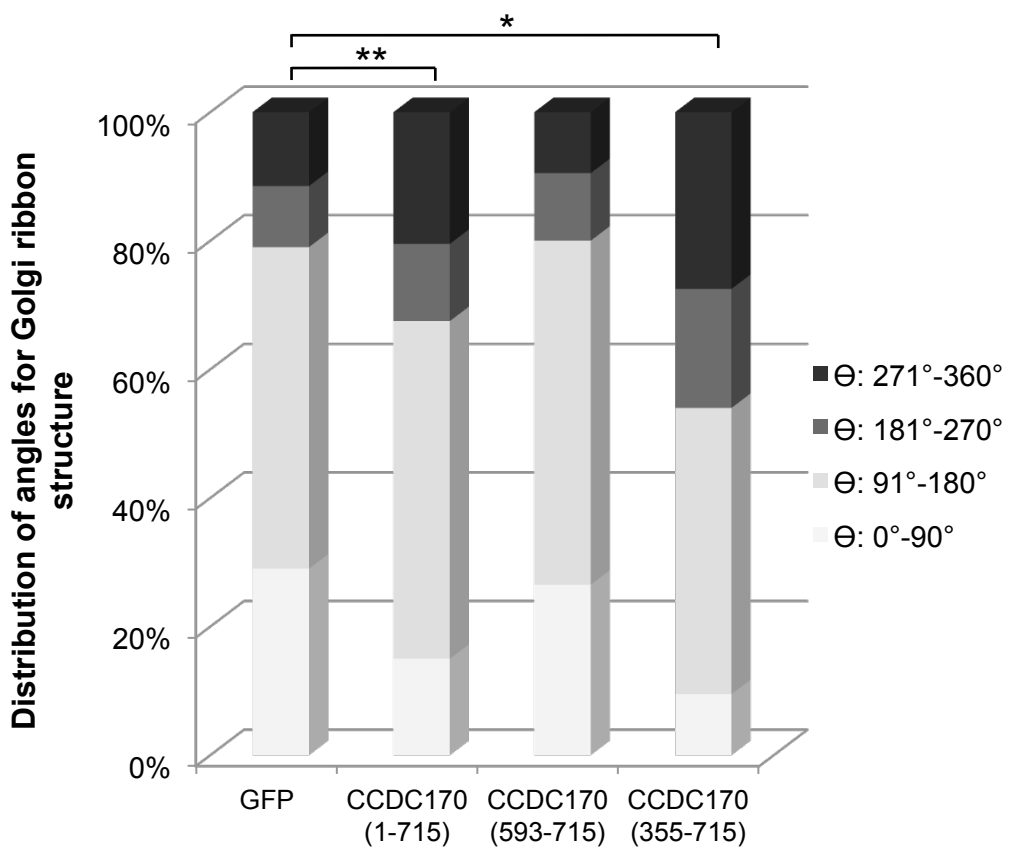
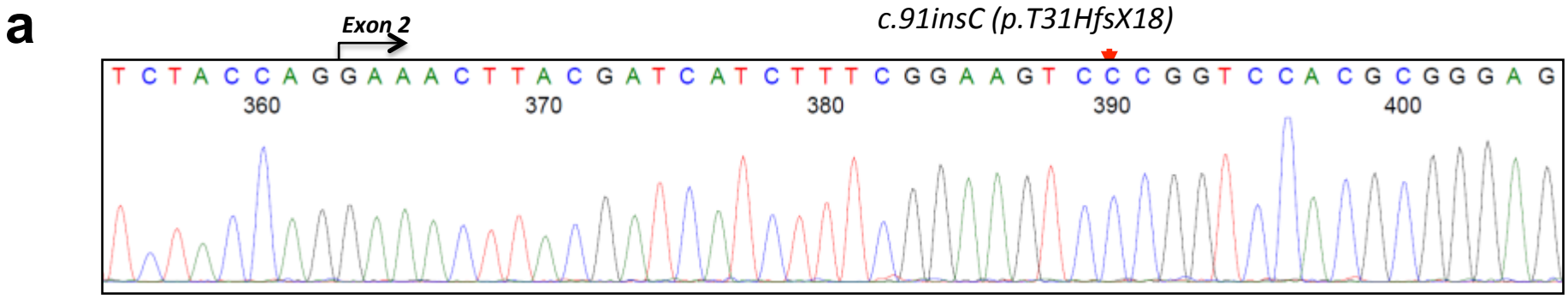
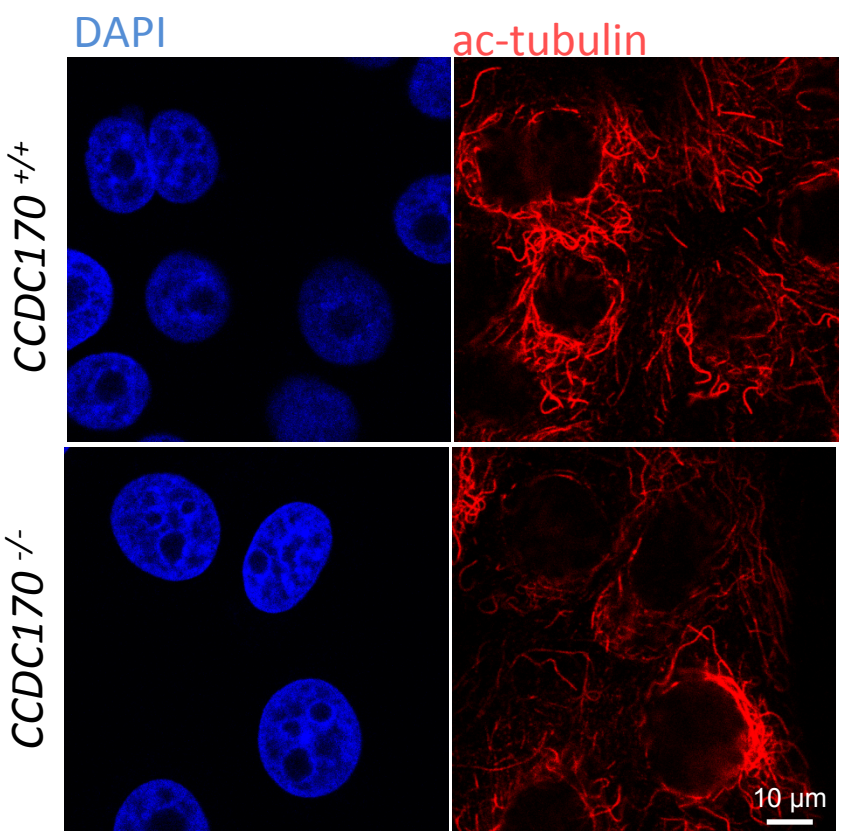


Figure S8. Ac-α-tubulin levels are decreased in MCF-7 *CCDC170*$^{-/-}$ cells. a. *CCDC170* knock-out MCF-7 cells were generated using CRISPR/Cas9 technology. The presence of out-of-frame insertions/deletions (indels) in CCDC170 alleles, was assessed and verified by Sanger DNA sequencing. The cell clone shown has a single C insertion at both alleles. **b**. Representive images show ac-a-tubulin staining of MCF-7-*CCDC170*$^{+/+}$ or *CCDC170*$^{-/-}$ cells. **c.** CCDC170, ac-tubulin, and alpha-tubulin levels were examined by SDS-PAGE/immunoblot analysis.





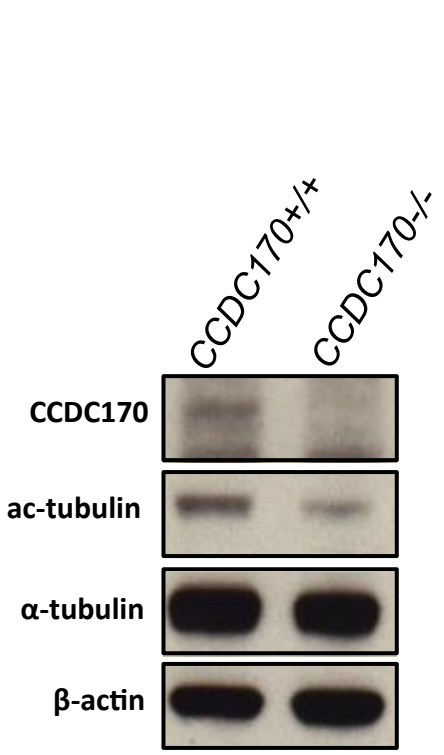


Figure S9. Co-staining of ac-α-tubulin and α-tubulin in the CCDC170-expressing cells. HeLa cells were transfected with GFP-CCDC170 and after 20 hours were treated with nocodazole for four hours to depolymerize MTs and observe potential CCDC170-induced resistance to nocodazole-induced MT depolymerization. Cells were then fixed and stained with anti-GFP (green), anti-α-tubulin (red) or anti-ac- α-tubulin (purple). The GFP-CCDC170, a-tubulin, and ac-a-alpha tubulin signals largely overlapped. The a-tubulin and ac-a-alpha tubulin formed punctated segments corresponding to regions of punctated CCDC170 accumulation.

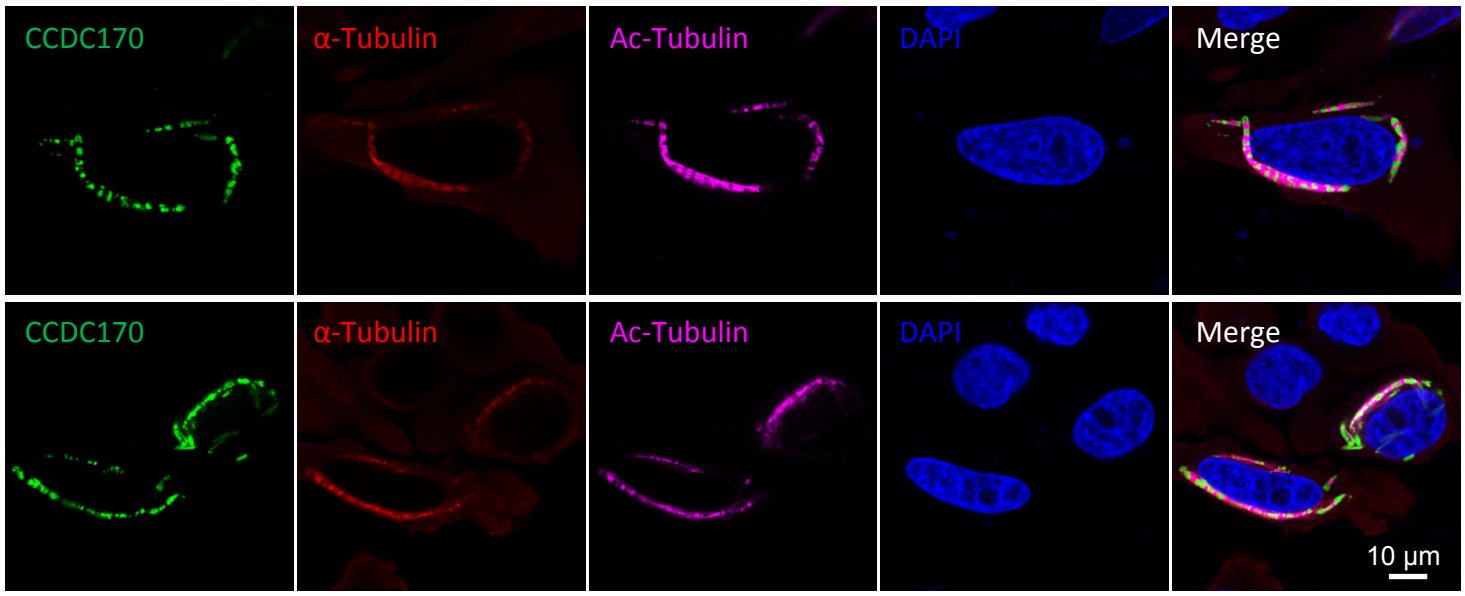
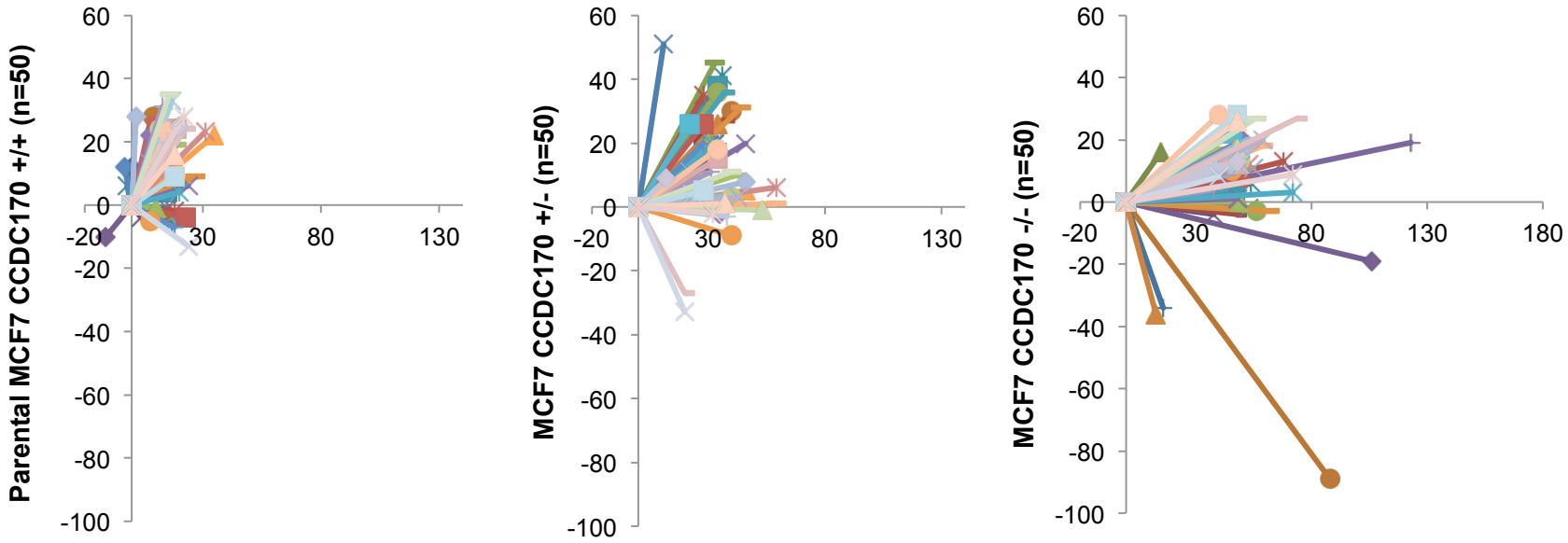


Figure S10. CCDC170 protein levels affect the rate of 2D cell migration. Cell migration was compared (see **Figure 6**) in MCF-7-*CCDC170*$^{+/+}$, MCF-7-*CCDC170*$^{+/-}$, and MCF-7-*CCDC1*$^{+-/-}$ cells. Directional migration of each cell was quantified as the direct distance from start to end point and the average distances were measured by ImageJ (**a**) and compared by *t*-test (**b**) (n =50; *: P<0.01, **: P<0.001).

a



b

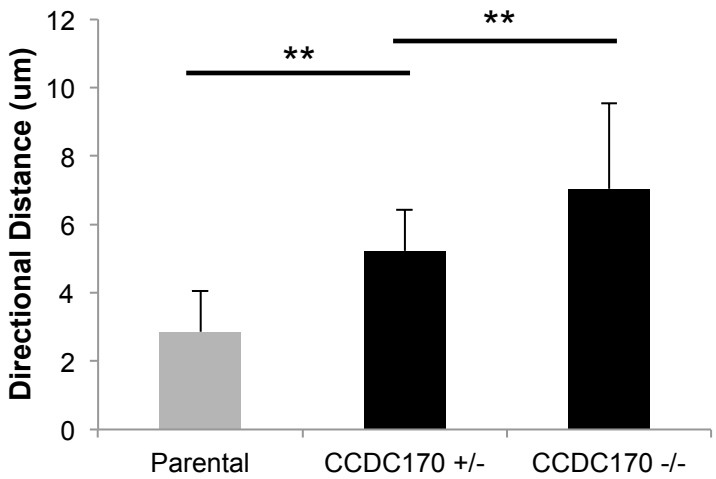
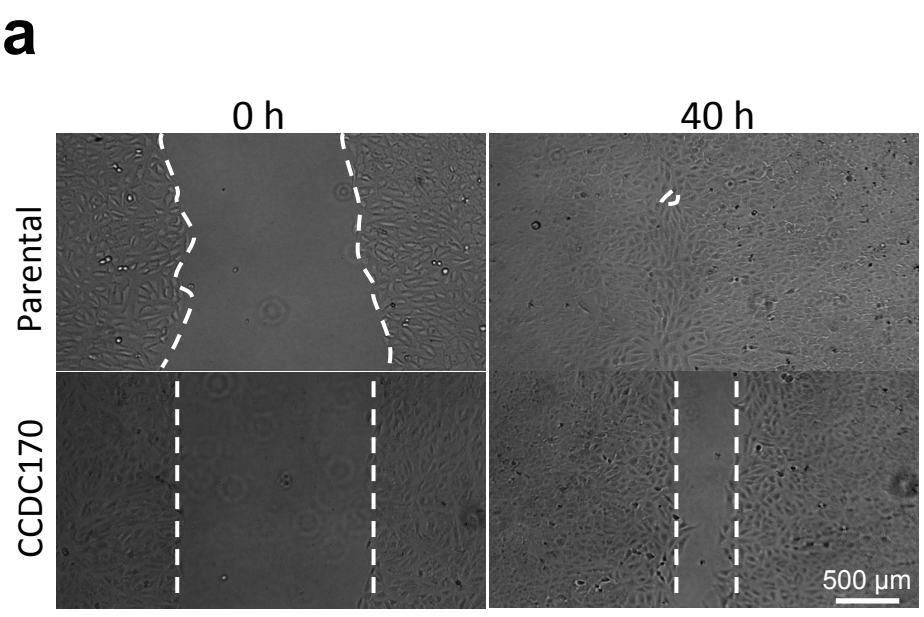


Figure S11. CCDC170 mediates Golgi-associated cell migration in U2OS cells. a. The relative wound closure of parental and GFP-CCDC170-expressing cells was quantified from 6 different fields for 72 hours. The images are representative of wound healing for each group. White dotted lines labeled the wound edges. **b.** The wound healing process was evaluated for 40 hours and the relative wound areas (vs. 0 h) are shown as mean ±SD (n =3).



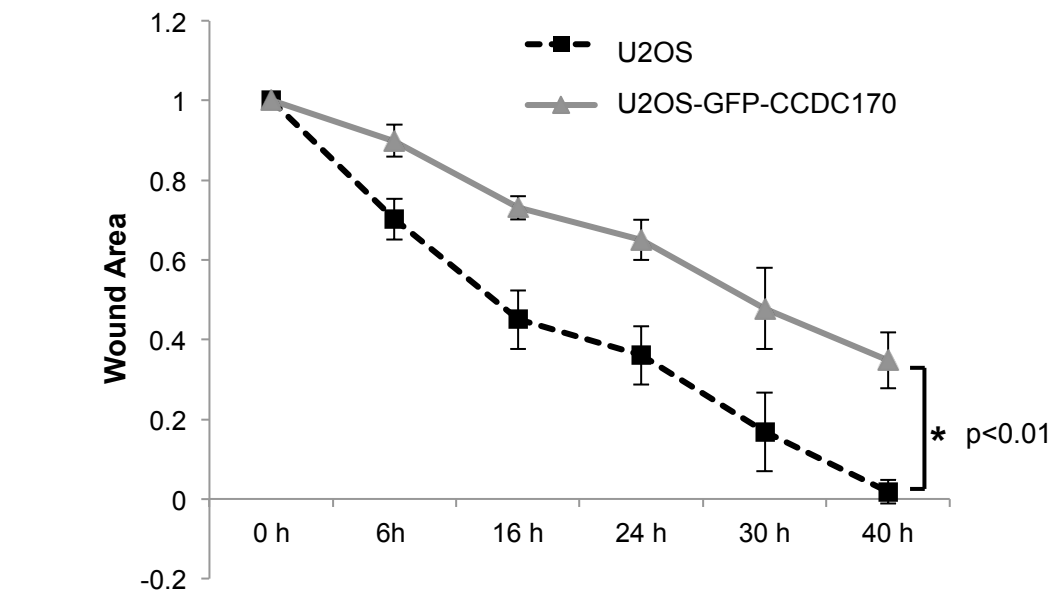
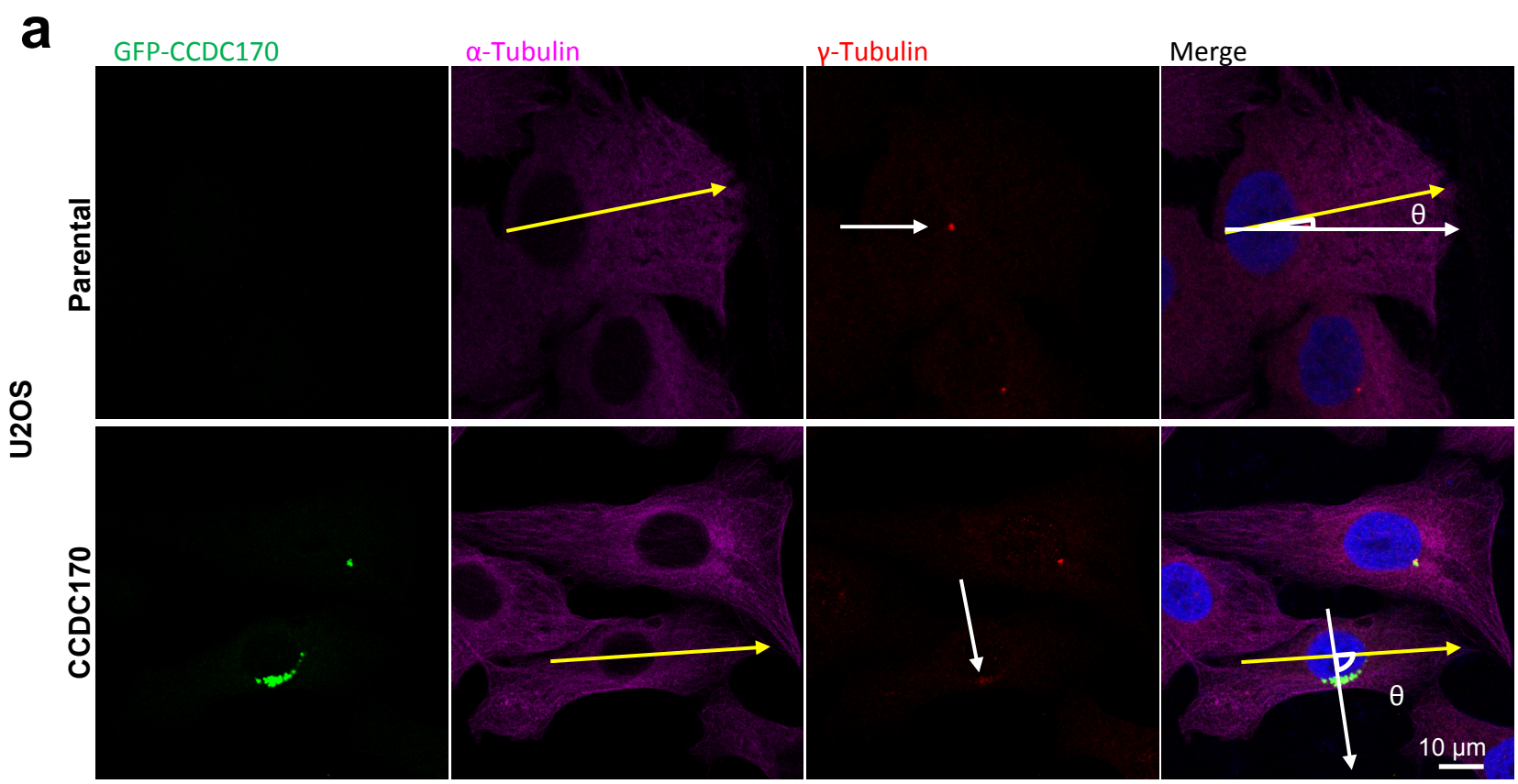
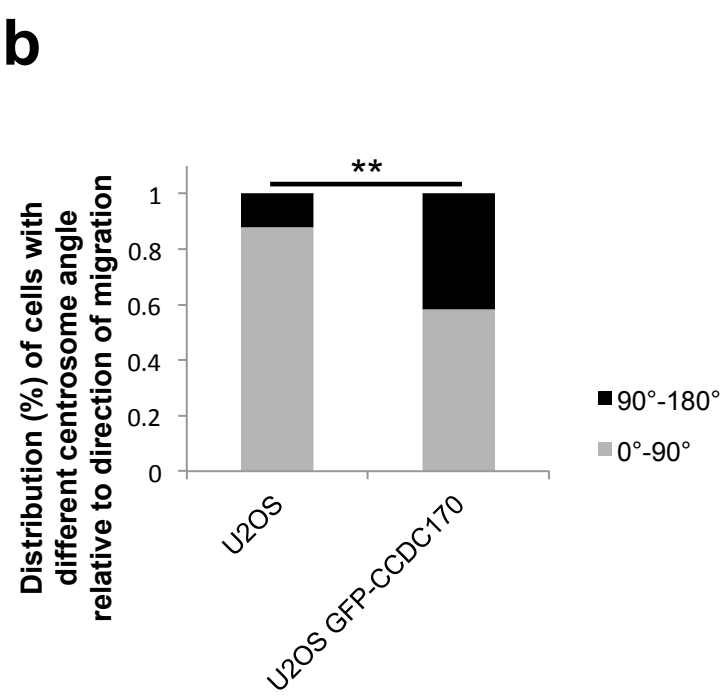


Figure S12. Evidence that centrosome polarization might be defective in CCDC170-overexpressing cells. a. The effects of CCDC170 on the positioning of the centrosome with respect to the migratory leading edge of the nucleus were examined using U2OS cells stably overexpressing GFP-CCDC170. Directional migration of U2OS cells was initiated by wounding the monolayer and directional migration is defined as facing the wound. After cells began migrating into the wound, the cells were fixed and stained with anti-tubulin (cell outline), anti-gamma-tubulin (centrosome), and DAPI (nucleus). The yellow arrows point toward the wound (directional cell migration), while the white arrow describes the center of the nuclear-centrosome axis. In parental migrating U2OS cells, the directional cell migration and nuclear-centrosome axis largely overlapped, and an angle of 0 to 90° between the two was defined as the normal range. An angle of greater than 90° was defined as an uncoupling of centrosome positioning at the leading edge of nucleus. The angle between was measured using Image J software. **b.** Quantitative results the distribution (%) of cells with different angles relative to direction of migration (*: P<0.01). Parental cells were compared to GFP-overexpressing cells (left). High and low GFP-CCDC170-expressing cells were also compared (right).





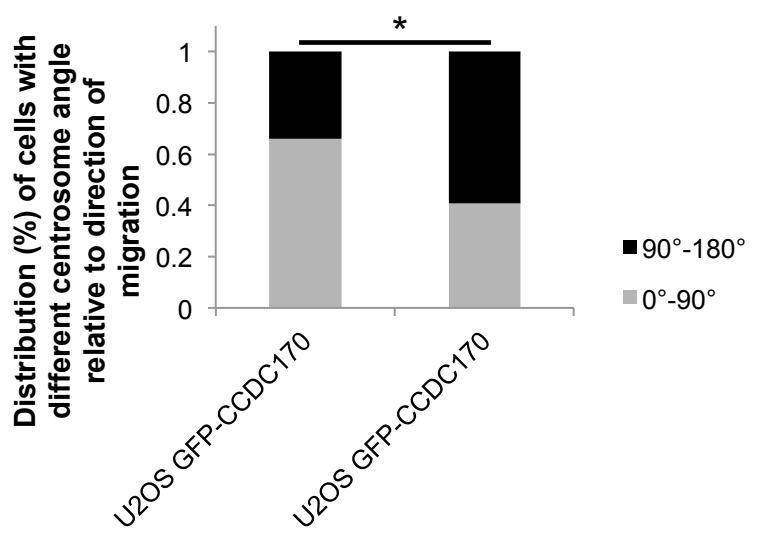
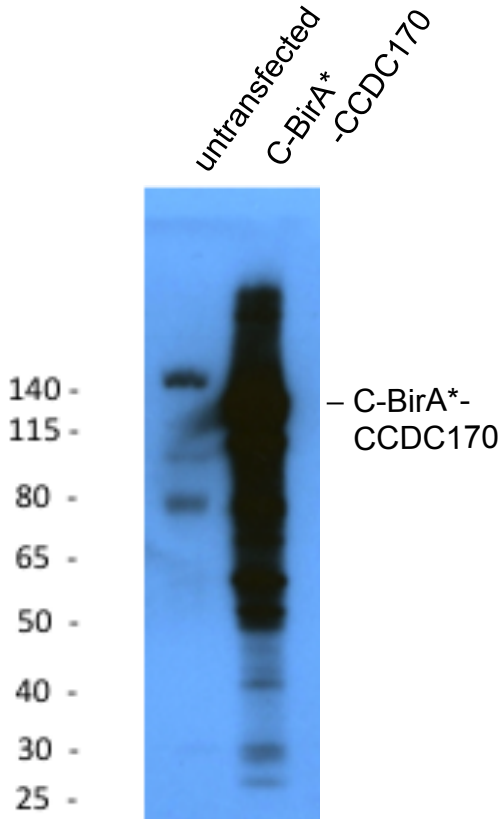


Figure S13. Detection of candidate CCDC170-interacting protein partners by BioID.

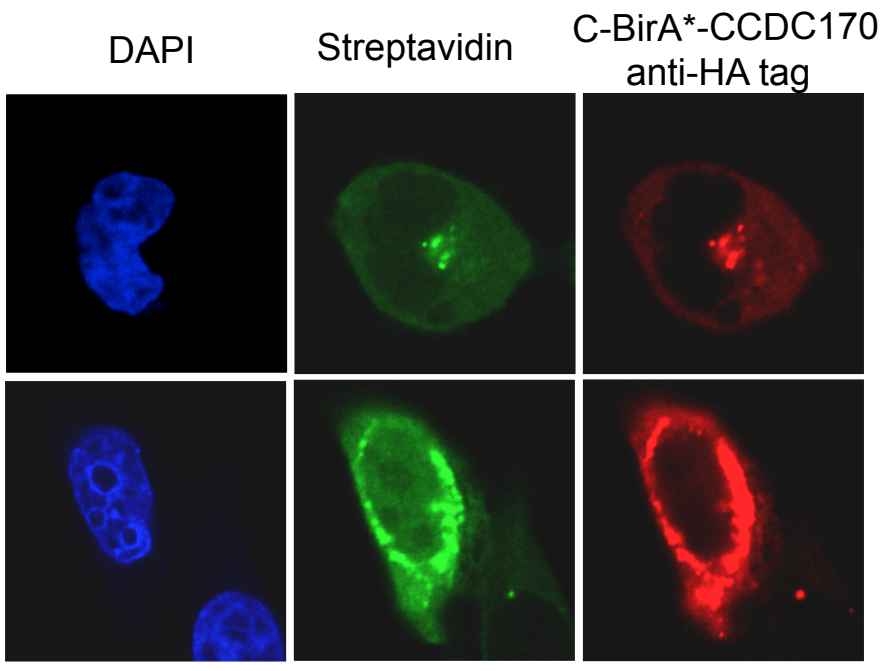
a

Detection of biotinylated proteins after C-BirA*-CCDC170 transfection by streptavidin western blot



b

Localization and biotinylation activity of C-BirA*-CCDC170



c

BioID identification of candidate binding partners of CCDC170 in Hela cells

CCDC170 bait proteins transfected

1. C-terminal BirA* fusion
2. C-terminal BirA* fusion

duplicate transfection

3. N-terminal BirA* fusion
4. Untransfected control

Isolate biotinylated proteins on beads

Duplicate mass spec analysis

Absent in control and spectral counts ≥ 2:

Present in at least one out of two duplicate experiments using the C-terminal BirA* fusion

217 proteins detected

Eliminated proteins detected with a parallel nuclear bait protein and parallel control. Eliminated proteins belonging to protein families detected in control experiments:
Ribosomal proteins, arg-ser rich, arg-glu-rich,RNA helicase, chromodomain helicase,heat shock proteins, histones, E3 ubiquitin ligases,elongation factors, hnRNP proteins, DNA topoisomerase, translational initiation factors, keratin, nucleolar proteins, splicing factors, prolyl isomerases, snRNP proteins, RNA binding proteins. *MT and actin-associated proteins were filtered in the subsequent steps.*

62 proteins detected

Eliminated proteins detected with unrelated BirA* bait proteins:

Fredriksson et al. PLoS One. **10,** 2015 (tight junctions)
Roux et al. J. Cell Biol. **196**:801, 2012 (nuclear lamina)
Kim et al. Proc. Natl. Acad. Sci. USA. **111**: E2453, 2014 (nuclear pore)

Eliminated proteins detected in greater than 25% of mass spec control experiments:

Mellacheruvu et al. Nat. Methods **10**:730, 2013 (contaminant repository)

32 proteins detected

32 proteins detected

Microtubule 6
Golgi 6
Centrosome 6
Other 14

Figure S14. CCDC170 co-localizes with AKAP9. HeLa cells were transfected with GFP-CCDC170 and after 24 hours cells were fixed and AKAP9 was detected (red). Representative image shows that GFP-CCDC170 and AKAP9 partially co-localize in perinuclear regions.

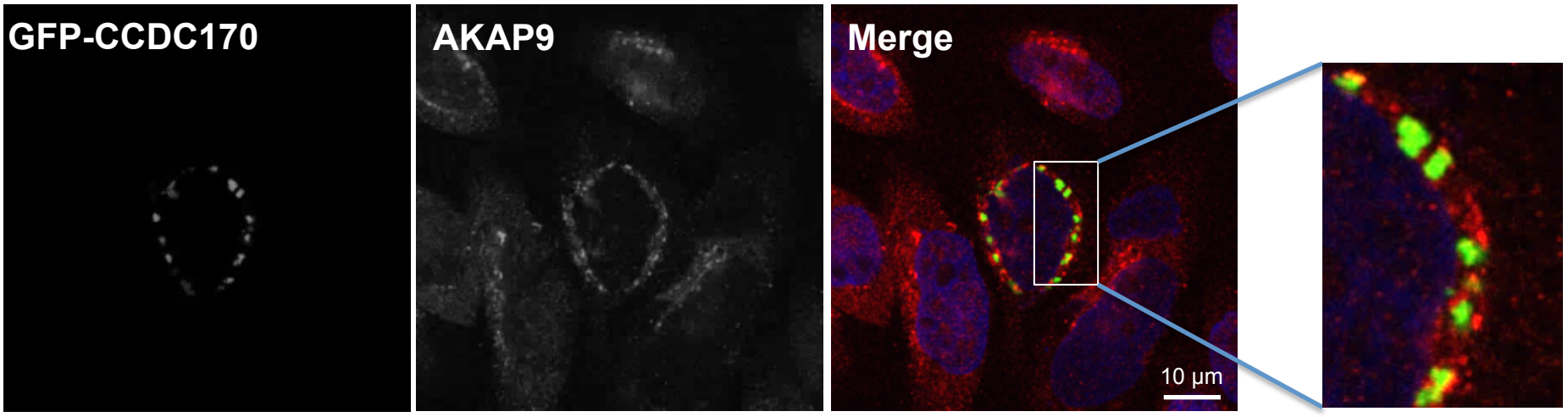


Table S1: DASE analysis at the *CCDC170/C6orf97-ESR1* locus *(P<0.05)*

SNP	Chr	GeneSymbol	GeneLocation	DASE (Log2)	P-value
rs4870034	6	*CCDC170/C6orf97*	CODING	1.136642433	8.39E-05
rs953767	6	*CCDC170/C6orf97*	CODING	0.590031636	0.023981986
rs12205837	6	*CCDC170/C6orf97*	CODING	2.342557529	0.017967093
rs6929137	6	*CCDC170/C6orf97*	CODING	1.301953718	0.020275714
rs3734804	6	*CCDC170/C6orf97*	CODING	1.179238392	0.002558726
rs3734805	6	*CCDC170/C6orf97*	UTR	1.046868051	0.037197929
rs6932260	6	*CCDC170/C6orf97*	UTR	1.150589227	0.001553989
rs6932603	6	*CCDC170/C6orf97*	UTR	1.14750246	0.002603497
rs6906130	6	*CCDC170/C6orf97*	UTR	1.161502999	0.003848164
kgp11470953	6	*ESR1*	UTR	0.230758202	0.018936968
rs2077647	6	*ESR1*	CODING	0.691217936	0.000178424
rs2228480	6	*ESR1*	CODING	0.976295931	0.016562028
kgp6150604	6	*ESR1*	UTR	0.582381734	4.42E-05

Table S2: Stratification of BioID hits based on filtering potential contaminants

rank/216	gene symbol	Golgi, Microtubules, Centrosome	Survived strict filter	accession	spectral counts control	spectral counts 1	spectral counts 2 (primary ranking)	detection with BirA* fused to the N-terminus of CCDC170 bait (spectral counts)	description	localization (relevant function/localiztion)	contaminant repository	nuclear pore	tight junction
1	CCDC170			Q8IYT3	0	385	874.5	986	Coiled-coil domain-containing protein 170 OS=Homo sapiens GN=CCDC170 PE=2 SV=3				
4	**PCM1**	✔	**y**	**Q15154**	**0**	**26.5**	**59**	**y (16)**	**Pericentriolar material 1 protein OS=Homo sapiens GN=PCM1 PE=1 SV=4**	**Centrosome**	**31/411**	**n**	**n**
9	**SEC16A**	✔	**y**	**O15027**	**0**	**8**	**24.5**	**n**	**Protein transport protein Sec16A OS=Homo sapiens GN=SEC16A PE=1 SV=3**	**Golgi and cytoplasm**	**41/411**	**n**	**n**
12	TUBA4A		n	P68366	0	0	18	y (39)	Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1	Microtubules	377/411	n	n
13	**ERC1**	✔	**y**	**Q8IUD2**	**0**	**6.5**	**17.5**	**n**	**ELKS/Rab6-interacting/CAST family member 1 OS=Homo sapiens GN=ERC1 PE=1 SV=1**	**Golgi (mouse) and cytoplasm**	**44/411**	**n**	**n**
14	**APC**	✔	**y**	**P25054**	**0**	**8**	**17.5**	**n**	**Adenomatous polyposis coli protein OS=Homo sapiens GN=APC PE=1 SV=2**	**Microtubules**	**18/411**	**n**	**n**
18	HSPA1L		n	P34931	0	0	14	n	Heat shock 70 kDa protein 1-like OS=Homo sapiens GN=HSPA1L PE=1 SV=2	Cytoplasm (Heat shock protein)	389/411	n	n
19	**MAP4**	✔	**y**	**P27816**	**0**	**2.5**	**13**	**y (16)**	**Microtubule-associated protein 4 OS=Homo sapiens GN=MAP4 PE=1 SV=3**	**Microtubules**	**86/411**	**n**	**n**
27	**CALD1**		**y**	**Q05682**	**0**	**7.5**	**8.5**	**y (22)**	**Caldesmon OS=Homo sapiens GN=CALD1 PE=1 SV=3**	**Actin- and myosin-binding**	**55/411**	**n**	**n**
25	MYH9		n	P35579	0	8	8.5	y (73)	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	Cytoplasm, Actin filaments (Motor protein)	203/411	n	n
30	**ALMS1**	✔	**y**	**Q8TCU4**	**0**	**3**	**8**	**y (6)**	**Alstrom syndrome protein 1 OS=Homo sapiens GN=ALMS1 PE=1 SV=3**	**Centrosome**	**17/411**	**n**	**n**
32	CRK		n	P46108	0	6	7.5	n	Adapter molecule crk OS=Homo sapiens GN=CRK PE=1 SV=2	Plasma membrane (Oncogene adapter protein)	24/411	y	y
37	**CEP170**	✔	**y**	**Q5SW79**	**0**	**5.5**	**7**	**y (4)**	**Centrosomal protein of 170 kDa OS=Homo sapiens GN=CEP170 PE=1 SV=1**	**Centrosome**	**46/411**	**n**	**n**
39	**LIMCH1**		**y**	**Q9UPQ0**	**0**	**2.5**	**7**	**n**	**LIM and calponin homology domains-containing protein 1 OS=Homo sapiens GN=LIMCH1 PE=1 SV=4**	**Cytoplasm**	**22/411**	**n**	**n**
38	VIM		n	P08670	0	4.5	7	y (12)	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4	Cytoskeleton (intermediate filament)	257/411	n	y
46	ACTA1		n	P68133	0	0	6.5	y (67)	Actin, alpha skeletal muscle OS=Homo sapiens GN=ACTA1 PE=1 SV=1	Cytoskeleton	354/411	n	n
47	ACTG2		n	P63267	0	0	6.5	n	Actin, gamma-enteric smooth muscle OS=Homo sapiens GN=ACTG2 PE=1 SV=1	Cytoskeleton	351/411	n	n
48	ACTC1		n	P68032	0	0	6.5	y (67)	Actin, alpha cardiac muscle 1 OS=Homo sapiens GN=ACTC1 PE=1 SV=1	Cytoskeleton	354/411	n	n
49	ACTA2		n	P62736	0	0	6.5	y (67)	Actin, aortic smooth muscle OS=Homo sapiens GN=ACTA2 PE=1 SV=1	Cytoskeleton	351/411	n	n
54	**GAPVD1**		**y**	**Q14C86**	**0**	**1**	**6**	**n**	**GTPase-activating protein and VPS9 domain-containing protein 1 OS=Homo sapiens GN=GAPVD1 PE=1 SV=2**	**Cytoplasm**	**33/411**	**n**	**n**
55	**PHLDB2**	✔	**y**	**Q86SQ0**	**0**	**3.5**	**6**	**n**	**Pleckstrin homology-like domain family B member 2 OS=Homo sapiens GN=PHLDB2 PE=1 SV=2**	**Cytoplasm**	**5/411**	**n**	**n**
63	**HAUS6**	✔	**y**	**Q7Z4H7**	**0**	**3.5**	**4.5**	**n**	**HAUS augmin-like complex subunit 6 OS=Homo sapiens GN=HAUS6 PE=1 SV=2**	**Centrosome**	**22/411**	**n**	**n**
65	**TNS3**		**y**	**Q68CZ2**	**0**	**7**	**4.5**	**y (4)**	**Tensin-3 OS=Homo sapiens GN=TNS3 PE=1 SV=2**	**Focal adhesions**	**11/411**	**n**	**n**
66	**FAM21B**		**y**	**Q5SNT6**	**0**	**2.5**	**4.5**	**n**	**WASH complex subunit FAM21B OS=Homo sapiens GN=FAM21B PE=2 SV=2**	**Vessicles**	**24/411**	**n**	**n**
68	**ARFGAP1**	✔	**y**	**Q8N6T3**	**0**	**1.5**	**4.5**	**y (2)**	**ADP-ribosylation factor GTPase-activating protein 1 OS=Homo sapiens GN=ARFGAP1 PE=1 SV=2**	**Golgi**	**24/411**	**n**	**n**
69	**EXOC4**		**y**	**Q96A65**	**0**	**0.5**	**4.5**	**y (3)**	**Exocyst complex component 4 OS=Homo sapiens GN=EXOC4 PE=1 SV=1**	**Vesicles**	**43 / 411**	**n**	**n**
64	FAM21A		n	Q641Q2	0	2.5	4.5	n	WASH complex subunit FAM21A OS=Homo sapiens GN=FAM21A PE=2 SV=3	Vessicles	24/411	n	n
74	**MISP**	✔	**y**	**Q8IVT2**	**0**	**3**	**4**	**n**	**Mitotic interactor and substrate of PLK1 OS=Homo sapiens GN=MISP PE=1 SV=1**	**Plasma membrane**	**20/411**	**n**	**n**
75	**CKAP5**	✔	**y**	**Q14008**	**0**	**1**	**4**	**n**	**Cytoskeleton-associated protein 5 OS=Homo sapiens GN=CKAP5 PE=1 SV=3**	**Cytoplasm (Microtubule assembly)**	**84/411**	**n**	**n**
77	TAGLN2		n	P37802	0	6.5	4	y (3)	Transgelin-2 OS=Homo sapiens GN=TAGLN2 PE=1 SV=3	Cytoskeleton (Actin filament-binding)	156/411	n	n
80	SRP72		n	O76094	0	0.5	4	y	Signal recognition particle subunit SRP72 OS=Homo sapiens GN=SRP72 PE=1 SV=3	Cytoplasm (Signal-recognition particle)	65/411	n	n
83	**DAB2**		**y**	**P98082**	**0**	**2**	**3.5**	**n**	**Disabled homolog 2 OS=Homo sapiens GN=DAB2 PE=1 SV=3**	**Vessicles**	**13/411**	**n**	**n**
96	**CLINT1**	✔	**y**	**Q14677**	**0**	**0.5**	**3.5**	**n**	**Clathrin interactor 1 OS=Homo sapiens GN=CLINT1 PE=1 SV=1**	**Vesicles (Golgi)**	**40/411**	**n**	**n**
97	**SKA3**	✔	**y**	**Q8IX90**	**0**	**0.5**	**3.5**	**n**	**Spindle and kinetochore-associated protein 3 OS=Homo sapiens GN=SKA3 PE=1 SV=2**	**Cytoplasm**	**15/411**	**n**	**n**
101	**ABLIM1**		**y**	**O14639**	**0**	**0**	**3.5**	**n**	**Actin-binding LIM protein 1 OS=Homo sapiens GN=ABLIM1 PE=1 SV=3**	**Cytoplasm**	**29/411**	**n**	**n**
95	ARHGAP21	✔	n	Q5T5U3	0	0.5	3.5	y (6)	Rho GTPase-activating protein 21 OS=Homo sapiens GN=ARHGAP21 PE=1 SV=1	Cytoskeleton (Actin filaments, recruited to Golgi membranes)	21/411	n	n
112	**AKAP9**	✔	**y**	**Q99996**	**0**	**1**	**3**	**y (5)**	**A-kinase anchor protein 9 OS=Homo sapiens GN=AKAP9 PE=1 SV=3**	**Golgi**	**5/411**	**n**	**n**
122	**CEP152**	✔	**y**	**O94986**	**0**	**0**	**3**	**n**	**Centrosomal protein of 152 kDa OS=Homo sapiens GN=CEP152 PE=1 SV=4**	**Centrosome**	**6/411**	**n**	**n**
126	**COPA**	✔	**y**	**P53621**	**0**	**1.5**	**2.5**	**y (23)**	**Coatomer subunit alpha OS=Homo sapiens GN=COPA PE=1 SV=2**	**Golgi**	**86/411**	**n**	**n**
143	**CCDC14**		**y**	**Q4VAA8**	**0**	**0**	**2.5**	**n**	**Coiled-coil domain-containing protein 14 OS=Homo sapiens GN=CCDC14 PE=1 SV=3**	**Centrosome**	**2/411**	**n**	**n**
136	DYNC1H1	✔	n	Q14204	0	1	2.5	y (20)	Cytoplasmic dynein 1 heavy chain 1 OS=Homo sapiens GN=DYNC1H1 PE=1 SV=5	Cytoplasm, Centrosome (Microtuble motor protein)	131/411	n	n
139	EPPK1		n	P58107	0	1.5	2.5	y (21)	Epiplakin OS=Homo sapiens GN=EPPK1 PE=1 SV=2	Cytoskeleton	126/411	n	n
142	KIF2A	✔	n	O00139	0	0.5	2.5	y (2)	Kinesin-like protein KIF2A OS=Homo sapiens GN=KIF2A PE=1 SV=3	Cytoskeleton (Microtubule motor protein, Centrosome)	33/411	n	n
149	KIF5B	✔	n	P33176	0	0	2.5	y (13)	Kinesin-1 heavy chain OS=Homo sapiens GN=KIF5B PE=1 SV=1	Cytoskeleton (Microtubule motor protein, Centrosome)	97/411	n	n
153	BOD1L1		n	Q8NFC6	0	0	2.5	y (4)	Biorientation of chromosomes in cell division protein 1-like 1 OS=Homo sapiens GN=BOD1L1 PE=1 SV=2	Nucleus	37/411	n	n
164	**LIMA1**		**y**	**Q9UHB6**	**0**	**1**	**2**	**y (2)**	**LIM domain and actin-binding protein 1 OS=Homo sapiens GN=LIMA1 PE=1 SV=1**	**Cytoskeleton, Actin filaments**	**76/411**	**n**	**n**
170	**CAMSAP1**	✔	**y**	**Q5T5Y3**	**0**	**0.5**	**2**	**y (5)**	**Calmodulin-regulated spectrin-associated protein 1 OS=Homo sapiens GN=CAMSAP1 PE=1 SV=2**	**Microtubules**	**16/411**	**n**	**n**
174	**CAMSAP2**	✔	**y**	**Q8DAD1**	**0**	**1**	**2**	**n**	**Calmodulin-regulated spectrin-associated protein 2 OS=Homo sapiens GN=CAMSAP2 PE=1 SV=3**	**Microtubule ends**	**12/411**	**n**	**n**
179	**CASC5**	✔	**y**	**Q8NG31**	**0**	**0**	**2**	**n**	**Protein CASC5 OS=Homo sapiens GN=CASC5 PE=1 SV=3**	**Nucleus**	**24/411**	**n**	**n**
181	**RTN4**		**y**	**Q9NQC3**	**0**	**0**	**2**	**n**	**Reticulon-4 OS=Homo sapiens GN=RTN4 PE=1 SV=2**	**Endoplasmic reticulum**	**64/411**	**n**	**n**
191	**DIAPH3**	✔	**y**	**Q9NSV4**	**0**	**0**	**2**	**y (4)**	**Protein diaphanous homolog 3 OS=Homo sapiens GN=DIAPH3 PE=1 SV=4**	**Microtubules**	**14/411**	**n**	**n**
158	CCT7	✔	n	Q99832	0	2	2	y (18)	T-complex protein 1 subunit eta OS=Homo sapiens GN=CCT7 PE=1 SV=2	Cytoplasm (Chaperone for actin and tubulin)	172/411	n	n
159	CLTC	✔	n	Q00610	0	1.5	2	y (31)	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5	Vesicles (trans-Golgi network membranes)	180/411	n	n
160	AP3D1	✔	n	O14617	0	3.5	2	n	AP-3 complex subunit delta-1 OS=Homo sapiens GN=AP3D1 PE=1 SV=1	Vesicles (Golgi)	73/411	n	n
166	FAM21C		n	Q9Y4E1	0	2.5	2	n	WASH complex subunit FAM21C OS=Homo sapiens GN=FAM21C PE=1 SV=3	Vesicles	24/411	n	n
187	CCT4	✔	n	P50991	0	0	2	y (14)	T-complex protein 1 subunit delta OS=Homo sapiens GN=CCT4 PE=1 SV=4	Cytoplasm (Chaperone for actin and tubulin)	186/411	n	n
192	NONO		n	Q15233	0	0	2	y (13)	Non-POU domain-containing octamer-binding protein OS=Homo sapiens GN=NONO PE=1 SV=4	Nucleus	220/411	y	n
203	PFN1		n	P07737	0	1	1.5	y (20)	Profilin-1 OS=Homo sapiens GN=PFN1 PE=1 SV=2	Cytoplasm (Actin-binding)	148/411	n	n
205	NAT10	✔	n	Q9H0A0	0	4.5	1.5	y (23)	N-acetyltransferase 10 OS=Homo sapiens GN=NAT10 PE=1 SV=2	Nucleus	70/411	n	n
206	COPG1	✔	n	Q9Y678	0	2.5	0.5	y (7)	Coatomer subunit gamma-1 OS=Homo sapiens GN=COPG1 PE=1 SV=1	Vesicles (Golgi)	78/411	n	n
207	COPG2	✔	n	Q9UBF2	0	2.5	0.5	y (6)	Coatomer subunit gamma-2 OS=Homo sapiens GN=COPG2 PE=1 SV=1	Vesicles (Golgi)	67/411	y	n
208	CCT5	✔	n	P48643	0	2.5	0	y (11)	T-complex protein 1 subunit epsilon OS=Homo sapiens GN=CCT5 PE=1 SV=1	Cytoplasm (Chaperone for actin and tubulin)	182/411	n	n

Red - Suspected major contaminant

Black Bold - survived strict filter

Black - did not survive strict filtering (detected in unrelated nuclear data sets, not shown)

Table S3: Final BioID hits

protein	relevant localization or function	source of localization/functional information: Human Protein Atlas (HPA), GeneCards (GC), or primary literature
PCM1	Centrosome	HPA
SEC16A	Golgi and cytoplasm	HPA
ERC1	Golgi (mouse) and cytoplasm	HPA
APC	Microtubules	Mimori-Kiyosue Y, Shiina N, Tsukita S. J Cell Biol. 2000 148:505-18.
MAP4	Microtubules	HPA
CALD1	Actin- and myosin-binding	HPA
ALMS1	Centrosome	HPA
CEP170	Centrosome	HPA
LIMCH1	Cytoplasm	HPA(Cytoplasm), GC (Actin binding)
GAPVD1	Cytoplasm	HPA
PHLDB2	Cytoplasm	HPA (Cytoplasm), GC (Microtubule regulation)
HAUS6	Centrosome	HPA
TNS3	Focal adhesions	HPA
FAM21B	Vessicles	HPA
ARFGAP1	Golgi	HPA
EXOC4	Vesicles	HPA (Nucleus), GC (Vessicles)
MISP	Plasma membrane	HPA
CKAP5	Cytoplasm (Microtubule assembly)	HPA, Al-Bassam J, Chang F. Trends Cell Biol. 2011 21:604-14.
DAB2	Vessicles	HPA
CLINT1	Vesicles (Golgi)	HPA (Vessicles), GC (Golgi)
SKA3	Cytoplasm	HPA (Cytoplasm), GC (Kinetochore-microtubule interface)
ABLIM1	Cytoplasm	HPA (Cytoplasm), GC (Actin filament binding)
AKAP9	Golgi	HPA (Golgi), GC (Centrosome)
CEP152	Centrosome	HPA
COPA	Golgi	HPA
CCDC14	Centrosome	HPA
LIMA1	Actin filaments	HPA
CAMSAP1	Microtubules	GC
CAMSAP2	Microtubule ends	HPA
CASC5	Nucleus	HPA (Nucleus), GC (Kinetochore-microtubule attachment)
RTN4	Endoplasmic reticulum	HPA
DIAPH3	Microtubules	HPA

RED- Golgi 6
GREEN - Microtubules 6
BLUE - Centrosome 6
BLACK - Other 14