Supplemental Materials

Molecular Biology of the Cell

Copeland et al.

Figure S1. FHDC1 over-expression affects cilia assembly. (A,B) Constitutively active derivatives of the indicated formins were expressed in NIH 3T3 fibroblasts by transient transfection. The effects on ciliogenesis were assessed as in figure 1. FHDC1 was the only formin tested that affected either cilia length or the number of cells with cilia when over-expressed. N=3, >100 cells counted per experiment. Error bars = SEM.**FHDC1 is recruited to primary cilia. (C)**GFP was expressed in control cells by transient transfection. The transfected cells were fixed forty eight hours later and the effects on ciliogenesis were assessed by immunofluorescence using an anti-acetylated tubulin antibody. GFP was not enriched in the primary cilia. **(D)** Flag-tagged FHDC1 (red) was co-expressed with GFP (green) by transient transfection and effects on ciliogenesis were assessed as before. In all ciliated cells the exogenous FHDC1 has clearly accumulated along the length of the primary cilia, while GFP was not obviously present.

Figure S2. The FH1 and FH2 domains of FHDC1 induceF-actin assembly but are not sufficient to affect ciliogenesis. The indicated FHDC1 derivatives (red) were expressed by transient transfection. The effects on cilia assembly were assessed as in Figure 3. The effects on F-actin assembly were assessed by phalloidin staining (green). **(A)** mCherry expressing control cells assemble cilia. **(B)** FHDC1 over-expression induces F-actin assembly and inhibits cilia formation in the majority of transfected cells. **(C)** FHDC1.I180A over-expression has more moderate effects on cilia assembly and does not induce F-actin accumulation. **(D, E)** Over-expression of FHDC1.958N and 485N is sufficient to induce F-actin accumulation, but does not inhibit cilia assembly. **(F)** FHDC1.485C does not induce F-actin assembly and does not inhibit cilia assembly.
FHDC1-induced Golgi dispersion is not associated with inhibition of ciliogenesis. The indicated FHDC1 derivatives (red) were expressed by transient transfection. The effects on cilia assembly were assessed as in Figure 3. The effects on Golgi dispersion were assessed using the Golgi marker GalT-GFP (green). **(G)** mCherry expressing control cells assemble cilia. **(H)** FHDC1 over-expression induces Golgi dispersion in the majority of cells. Cells with dispersed Golgi still exhibited cilia elongation. **(I)** FHDC1.I180A over-expression does not induce Gogi dispersion and has more moderate effects on cilia assembly. **(J,K)** Over-expression of FHDC1.958N and 485N does not affect Golgi dispersion or cilia assembly.

Figure S3. F-actin is present in the cilia of FHDC1 over-expressing cells. FHDC1 and FHDC1.I180A were expressed in NIH 3T3 cells by transient transfection and the effects on F-actin accumulation and cilia assembly were assessed by immunofluorescence. Untransfected cells were used as a control. To minimise concerns regarding bleed-through between channels, F-actin was detected with Alexa488 phalloidin (green), anti-acetylated tubulin staining was detected with AMCA donkey anti-mouse (blue) and the flag tagged FHDC1 was detected with rabbit anti-flag and Cy5 donkey anti-rabbit secondary antibody (white).**(A)** F-actin is not detected in cilia of untransfected cells. **(B)**FHDC1.I180A (white) is recruited to the cilia.F-actin is not detected in cilia of FHDC1.I180A over-expressing cells. **(C,D)**F-actin (green) is readily detected by phalloidin staining in the elongated cilia of FHDC1 (white) over-expressing cells.

Figure S4. FHDC1 depletion affects centrosome morphology. (A,B) As in figure 8, siRNA-mediated knockdown was used to deplete FHDC1 expression in both parental NIH 3T3 cells and the partial FHDC1 knockout cell-line. The effects on centrosome morphology were assessed by γ-tubulin staining (green). FHDC1-depleted cells do not have obvious perinuclear γ-tubulin puncta. N=1, >100 cells counted per experiment.

Figure S5. Identification of FHDC1-interacting proteins using BioID. (A-C) Characterization of BirA*-FHDC1. (A) BirA*-FHDC1 over-expression induces Golgi dispersion similar to the myc-tagged protein, as previously reported. **(B,C)**BirA*-FHDC1 over-expression reduces the number of ciliated cells while inducing cilia elongation in cells where cilia are present.**(D) Initial validation of putative FHDC1 interacting proteins.** BirA*-FHDC1 was expressed alone or with either GFP or GFP-Cep170. Following transfection the cells were treated with exogenous biotin for 24 hours prior to lysis. Cleared cell lysates were prepared and the relevant proteins were immunoprecipitated. BirA*-FHDC1 expression was confirmed by immunoblotting (IB) for the encoded myc tag (FHDC1 input blot). Recovery of the indicated immunoprecipitated proteins was confirmed by IB;the blot was stripped and probed with Streptavidin HRP to detect biotinylation (Eluate blots). The immunoprecipitatedCep170 wasefficiently biotinylated by co-expression with BirA*-FHDC1 whileGFP alone was not.

Figure S6. FHDC1 over-expression disrupts the centriole maturation cycle. FHDC1, FHDC1.I180A or mCherrywere expressed by transient transfection and the effects on centrosome morphology (Centrin2-GFP, green) and cilia assembly were assessed by immunofluorescence (anti-acetylated tubulin, white). **(A)**mCherry (red) expression does not affect centriole number nor the distance between centrioles as detected using co-expression of centrin2-GFP (green). **(B-D)**FHDC1 (red) over-expression induces centriole separation and supernumerary centrioles in both ciliated and non-ciliated cells. **(E)** FHDC1.I180A (red) over-expression does not affect centriole number nor their separation. **(F)** Quantification of data shown in (F-I). N=3, >100 cells counted per sample. Error bars=SEM.

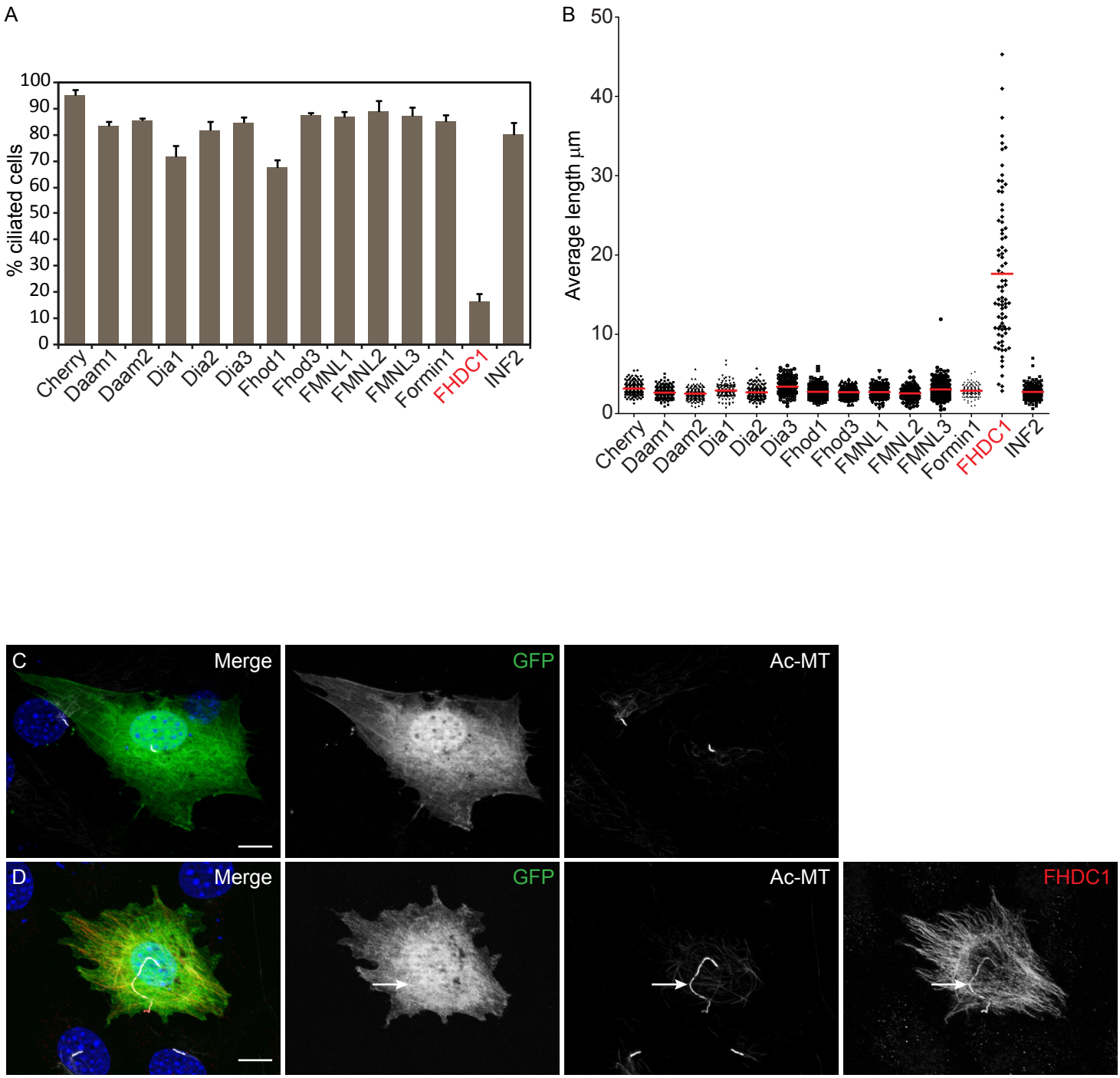

A
% ciliated cells
100
90
80
70
60
50
40
30
20
10
0
Cherry
Daam1
Daam2
Dia1
Dia2
Dia3
Fhod1
Fhod3
FMNL1
FMNL2
FMNL3
Formin1
FHDC1
INF2
B
Average length µm
50
40
30
20
10
0
Cherry
Daam1
Daam2
Dia1
Dia2
Dia3
Fhod1
Fhod3
FMNL1
FMNL2
FMNL3
Formin1
FHDC1
INF2
C
Merge
GFP
Ac-MT
D
Merge
GFP
Ac-MT
FHDC1

Figure S1.

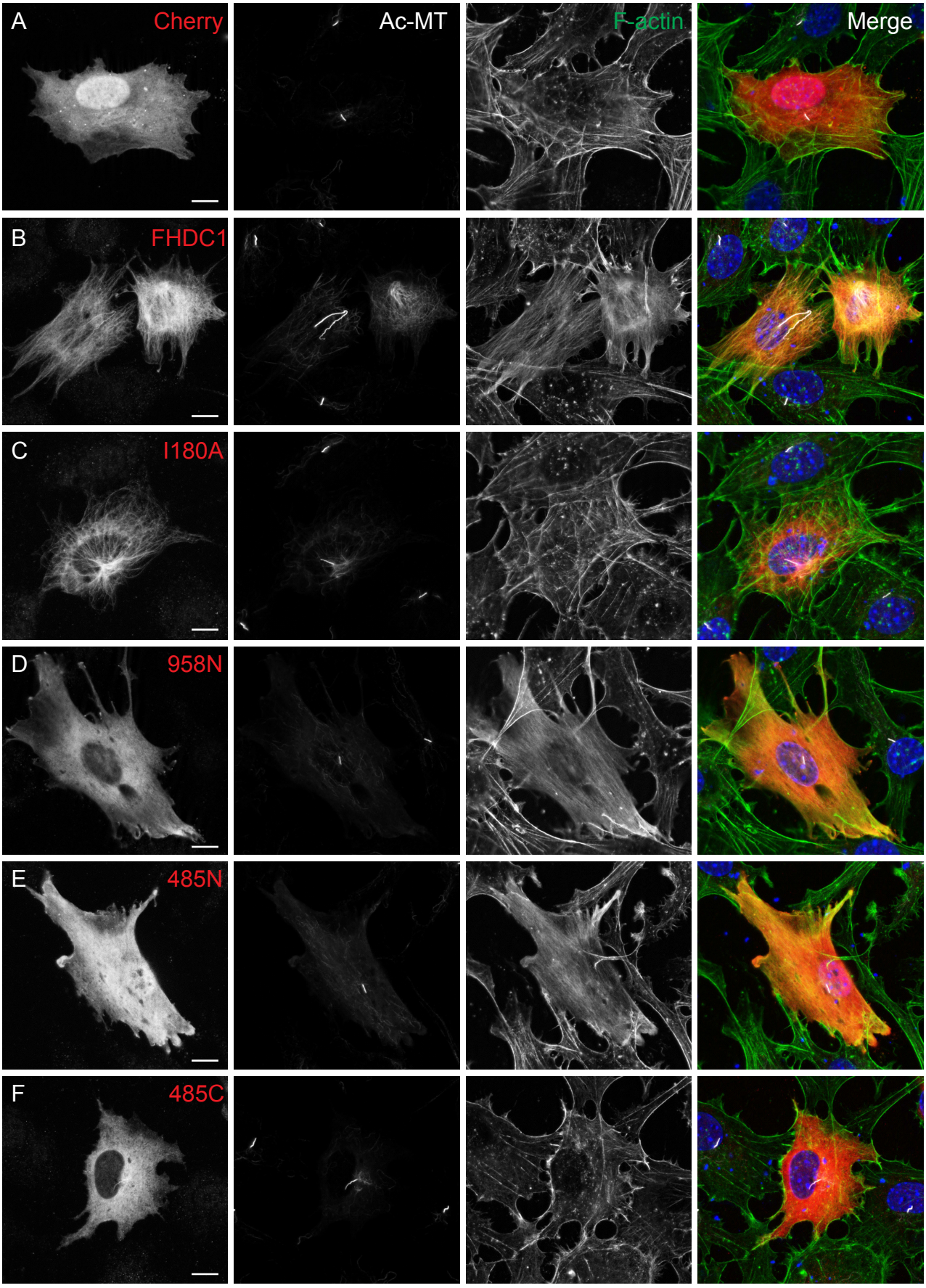


Figure S2

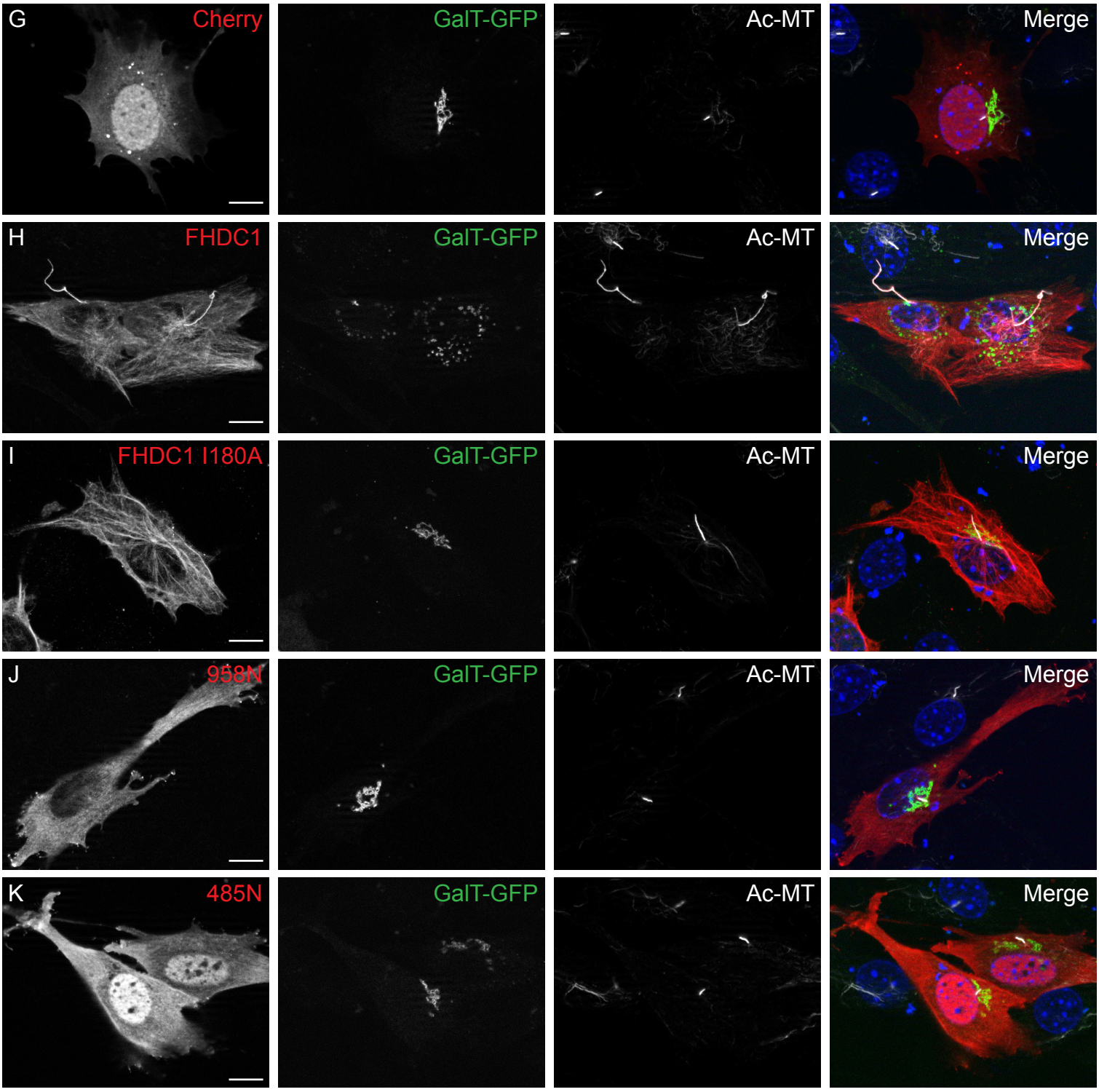

G
Cherry
GalT-GFP
Ac-MT
Merge
H
FHDC1
GalT-GFP
Ac-MT
Merge
I
FHDC1 I180A
GalT-GFP
Ac-MT
Merge
J
958N
GalT-GFP
Ac-MT
Merge
K
485N
GalT-GFP
Ac-MT
Merge

Figure S2

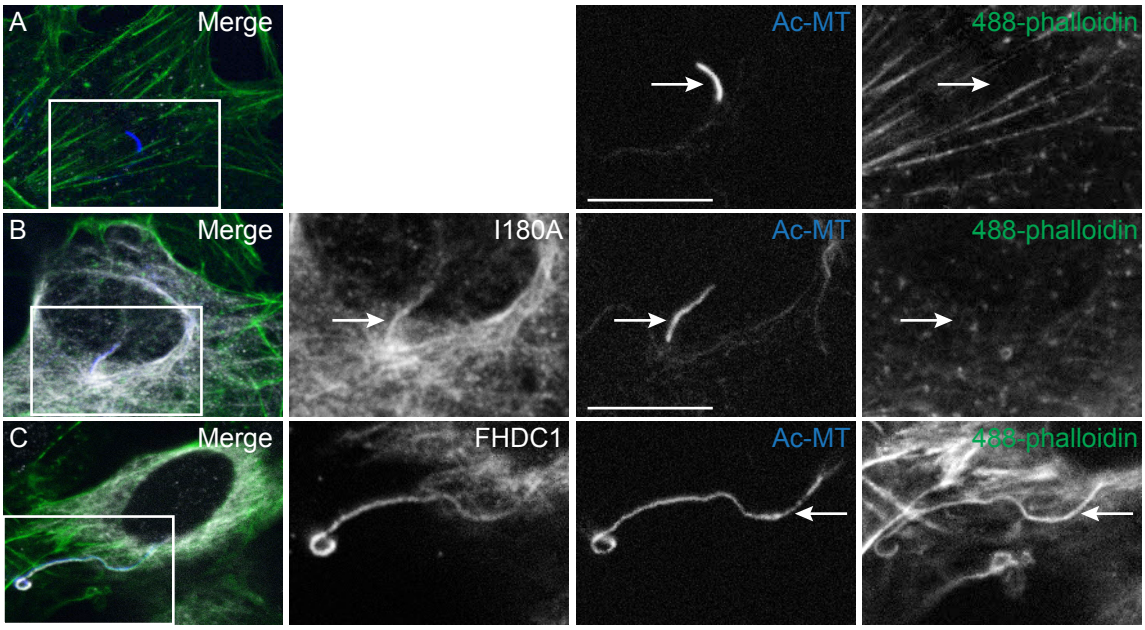

A
Merge
Ac-MT
488-phalloidin
B
Merge
I180A
Ac-MT
488-phalloidin
C
Merge
FHDC1
Ac-MT
488-phalloidin

Figure S3

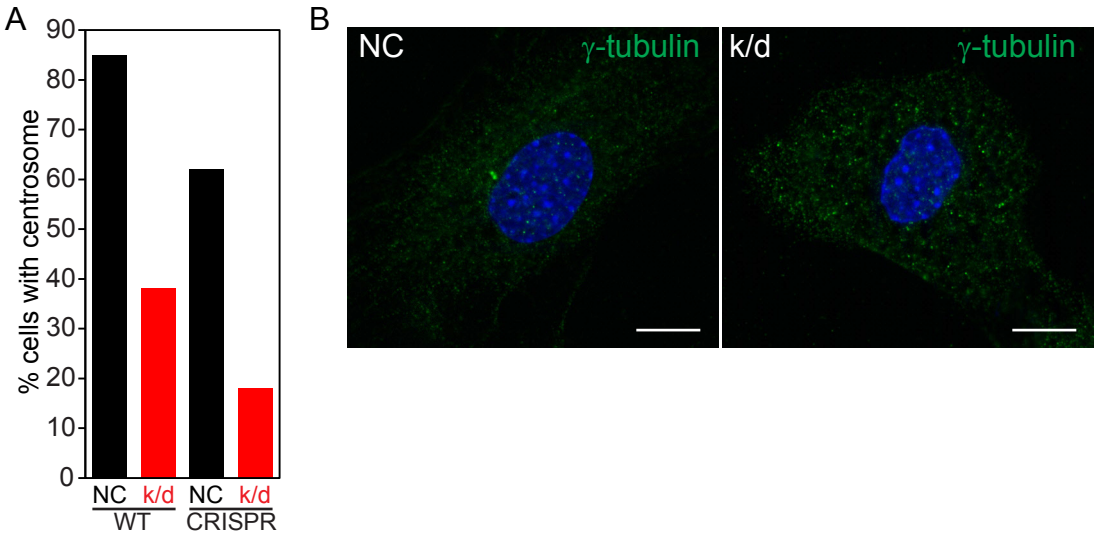

A
B
% cells with centrosome
90
80
70
60
50
40
30
20
10
0
NC
k/d
NC
k/d
WT
CRISPR
NC
γ-tubulin
k/d
γ-tubulin

Figure S4

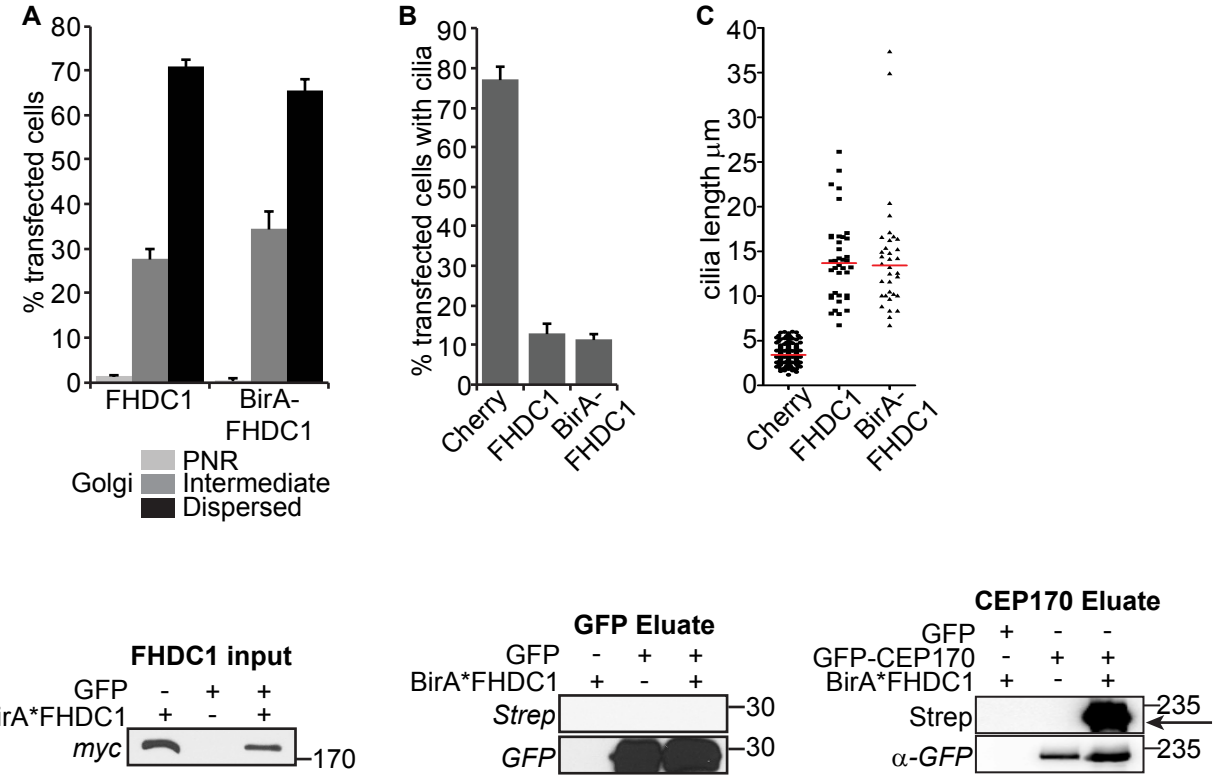
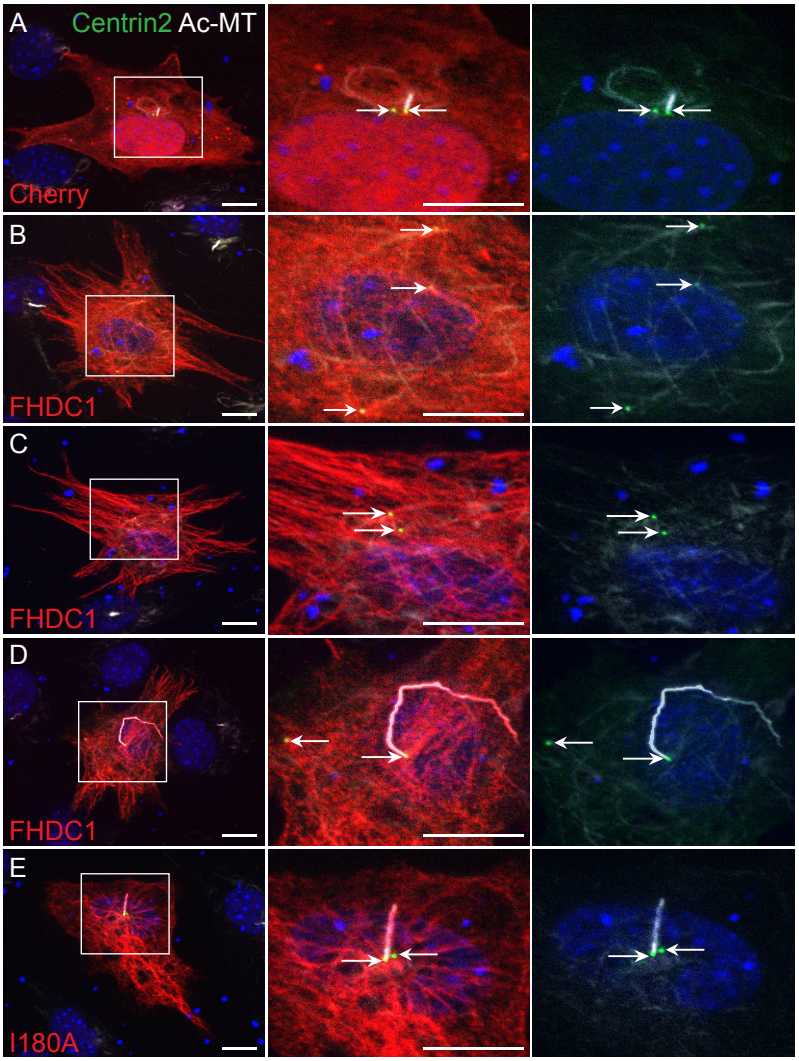
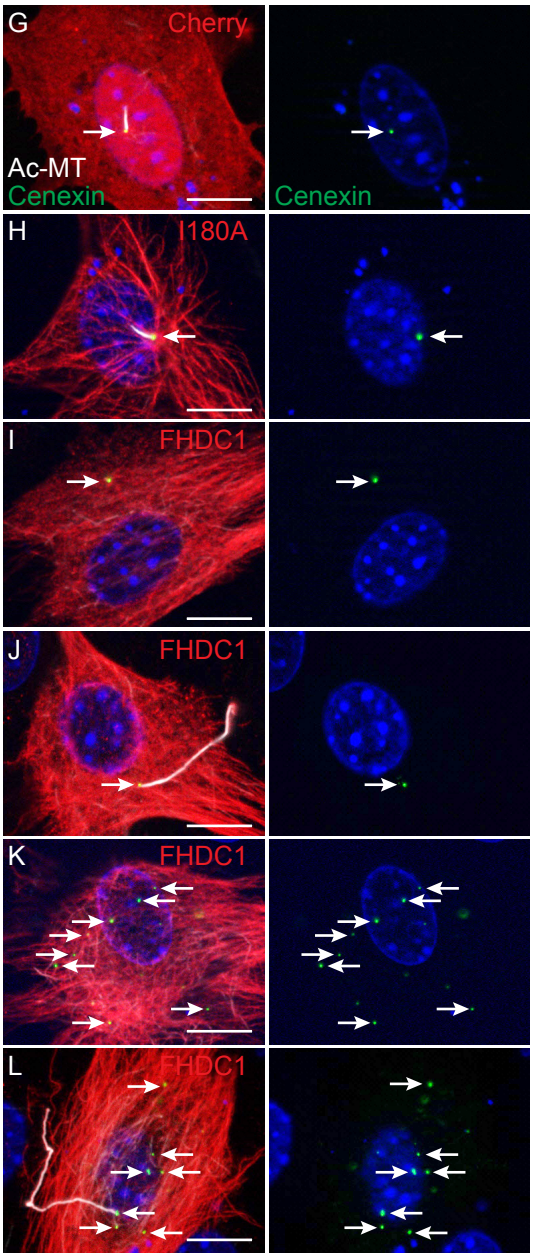
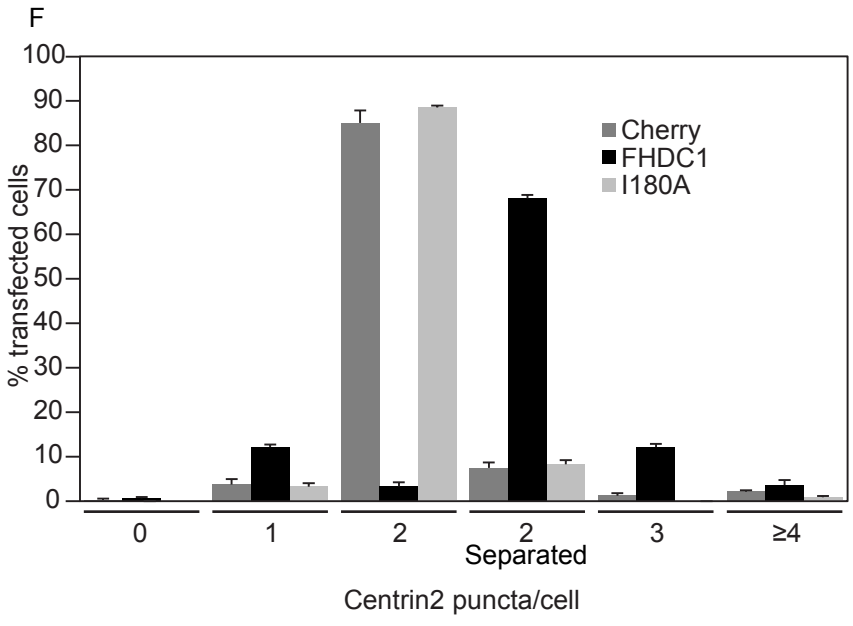

A
% transfected cells
80
70
60
50
40
30
20
10
0
FHDC1
BirA-FHDC1
Golgi
PNR
Intermediate
Dispersed
B
% transfected cells with cilia
90
80
70
60
50
40
30
20
10
0
Cherry
FHDC1
BirA-FHDC1
C
cilia length µm
40
35
30
25
20
15
10
5
0
Cherry
FHDC1
BirA-FHDC1
D
FHDC1 input
GFP - + +
BirA*FHDC1 + - +
myc
170
GFP Eluate
GFP - + +
BirA*FHDC1 + - +
Strep
30
GFP
30
CEP170 Eluate
GFP + - -
GFP-CEP170 - + +
BirA*FHDC1 + - +
Strep
235
α-GFP
235

Figure S5.


A
Centrin2 Ac-MT
Cherry
B
FHDC1
C
FHDC1
D
FHDC1
E
I180A


G
Cherry
Ac-MT
Cenexin
Cenexin
H
I180A
I
FHDC1
J
FHDC1
K
FHDC1
L
FHDC1


F
% transfected cells
100
90
80
70
60
50
40
30
20
10
0
Cherry
FHDC1
I180A
0
1
2
2
3
≥4
Separated
Centrin2 puncta/cell

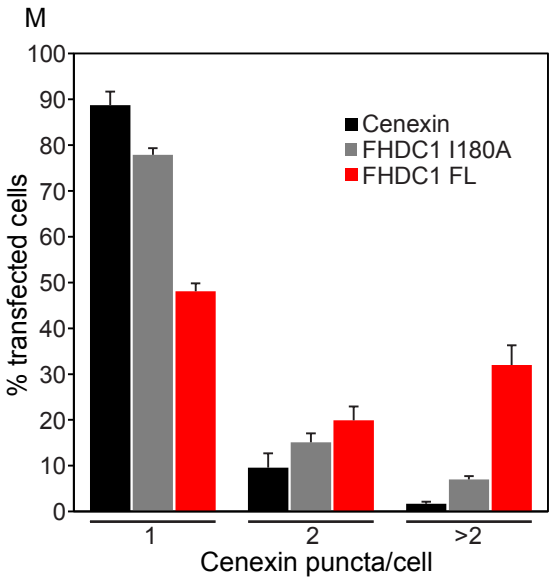

M
% transfected cells
100
90
80
70
60
50
40
30
20
10
0
Cenexin
FHDC1 I180A
FHDC1 FL
1
2
>2
Cenexin puncta/cell

Figure S6