Supplemental Materials

Molecular Biology of the Cell

Copeland et al.

Figure S1. FHDC1 associates with the Golgi ribbon, but not cytoplasmic microtubules, in spreading cells. NIH 3T3 fibroblasts were fixed 1 hour, 2 hours and 4 hours after plating and stained for endogenous FHDC1 and either GM130 or α-tubulin. In A, C, and E the plane of focus is centered on the Golgi ribbon while in B, D and F the plane of focus is centered on microtubules at the cell periphery. (A,B) One hour after plating, the Golgi is likely disconnected from the centrosome (Kisurina-Evgen'eva and Onishchenko, 2004); FHDC1 is primarily associated with the Golgi and nascent Golgi-derived MTs, but is not associated with microtubules at the cell periphery. (C,D) Two hours after plating, the cells are starting to spread, FHDC1 is associated with the Golgi and with MTs at the same plane of focus, but is still not found on the peripheral microtubules. (E,F) Four hours after plating, the cells are fully spread and FHDC1 is concentrated at the Golgi and can now be seen on MTs at the same plane of focus as the bulk of the cytoplasmic microtubules at the cell's periphery. Scale bar=10 μm.

Figure S2. Brefeldin A inhibits FHDC1 and CLASP2 recruitment to microtubules during recovery from nocodazole treatment. (A) In untreated cells FHDC1 (red) and CLASP2 (green) are concentrated on perinuclear microtubules (α-tubulin, white) likely associated with the Golgi. (B) Cells were treated for 2 hours with 2.5 μg/ml nocodazole and 5.0 μg/ml brefeldin A. The Golgi is dispersed and both FHDC1 and CLASP2 are distributed throughout the cytoplasm. (C) Cells were allowed to recover after nocodazole washout for 120 minutes in the presence of brefeldin A. FHDC1 does not recover its filamentous staining pattern and maintains its punctate cytoplasmic distribution. CLASP2 also fails to recover and does not accumulate on perinuclear microtubules. (D) Cells were allowed to recover for 120 minute s in the absence of BFA where both FHDC1 and CLASP2 regain their association with the microtubule network and accumulate in the presumed vicinity of the Golgi. Scale bar=10 μm. Note that for optimal CLASP2 staining cells were fixed in 4% paraformaldehyde in PHEM buffer instead of cold methanol. (E) In untreated cells FHDC1 (red) and CLASP2 (green) are concentrated on microtubules associated with the Golgi (GM130, white). (F) In cells treated with nocodazole and brefeldin A the Golgi is dispersed throughout the cytoplasm and FHDC1 and CLASP2 have a punctate cytoplasmic distribution. (G) Cells were allowed to recover after nocodazole washout for 120 minutes in the presence of brefeldin A. FHDC1 does not recover its filamentous staining pattern and CLASP2 fails to accumulate on perinuclear microtubules at the Golgi. (H) Cells were allowed to recover for 120 minute s in the absence of BFA. Both FHDC1 and CLASP2 regain their association with the microtubule network and accumulate in the vicinity of the Golgi. Scale bar=10 μm. Note that for optimal CLASP2 staining cells were fixed in 4% paraformaldehyde in PHEM buffer.

Figure S3. Characterization of FHDC1.I180A. (A) Transient expression of mCherry-tagged FHDC1 protein (red) induces formation of thin actin stress fibers (green). (B) Transient expression of FHDC1.I180A does not induce stress fiber formation. (C) Expression of full-length FHDC1 induces activation of the actin-responsive MRTF/SRF reporter gene 3D.ALuc, an indirect measure of G-actin depletion and F-actin accumulation. Expression of FHDC1.I180A does not induce activation of the reporter gene above background levels. Reporter activation is expressed relative to an SRF-VP16 control fusion protein. N=3, error bars=SEM. (D) Alignment of amino acid residues 168-192 of human FHDC1 with FHDC1 homologues from other species and the corresponding region of the FH2 domain of mDia1. The red star

indicates the position of the conserved isoleucine residue mutated to alanine in FHDC1.I180A. (E, F) Transient expression of FHDC1 or FHDC1.I180A (red) is sufficient to induce microtubule acetylation (white). F-actin is shown in green in merged image (right panel). Scale bar=10 μm. (G) Quantification of data presented in (E,F), N=3, >100 cells counted per experiment. Error bars = SEM.

Figure S4. FHDC1 is a potent inducer of Golgi dispersion. (A) Expression of mCherry has no effect on Golgi morphology as revealed by GM130 staining. (B) Expression of full-length FHDC1 induced Golgi dispersion in nearly all transfected cells. (C) Expression of a constitutively active FH1+FH2 derivative (codons 1-485) of FHDC1 fails to induce Golgi dispersion in the majority of transfected cells. (D) Expression of a constitutively active FH1+FH2 derivative (codons 449-1091) of FMNL1 fails to induce dispersion in the majority of cells. (E) Expression of a constitutively active FH1+FH2 derivative (codons 567-1255) of mDia1 fails to induce dispersion in the majority of cells. (F) Expression of a constitutively active FH1+FH2 derivative (codons 419-1272) of INF2 fails to induce dispersion in the majority of cells. Scale bar=10 μm. (G) Quantification of the data shown in (A-F). PNR= Perinuclear Golgi Ribbon. N=3, >100 cells counted per experiment. Error bars=SEM.

Figure S5. FHDC1 expression does not affect LAMP1 or Calreticulin distribution. The ER marker pEYFP-ER (Clontech) and full-length FHDC1 were transiently expressed in NIH 3T3 cells. (A,B) Co-expression of FHDC1 (red) does not affect distribution of the ER marker (green) in cells where the Golgi (white) is clearly dispersed. (C,D) Co-expression of FHDC1 (red) does not affect distribution of EYFP-ER (green) in cells where FHDC1 has induced re-organization of the microtubule network (white). The lysosome marker LAMP1-RFP (Sherer et al., 2003) and full-length FHDC1 were transiently expressed in NIH 3T3 cells. (E,F) Co-expression of FHDC1 (green) does not affect distribution of the LAMP1 marker (red) in cells where the Golgi (white) is clearly dispersed. (G,H) Co-expression of FHDC1 (green) does not affect distribution of the LAMP1 marker (red) in cells where FHDC1 has induced cell elongation and re-organization of the microtubule network (white). Scale bar=10 μm.

Figure S6. GM130 depletion affects a subset of FHDC1 activities. (A,B) NIH 3T3 cells were transfected with control siRNA duplexes or duplexes targeting GM130 to knockdown GM130 expression. Distribution of endogenous FHDC1 protein (red) is not affected in control cells, but is less filamentous and more punctate in GM130 knockdown cells (green). (C,D) NIH 3T3 cells were transfected with control siRNA duplexes or duplexes targeting GM130 to knockdown GM130 expression. Full-length FHDC1 was transiently expressed in NIH 3T3 cells that were previously transfected with (C) control siRNA duplexes or (D) duplexes targeting GM130 to knockdown GM130 expression. (C) In control cells FHDC1 expression (red) induces Golgi dispersion (white) and microtubule acetylation (green). (D) In GM130 knockdown cells, FHDC1 expression is sufficient to induce microtubule acetylation. Scale bar=10 μm. (E) Quantification of the data shown in (C&D). (F) Whole cell lysates from GM130 knockdown (k/d), control transfected cells (scr) and untransfected cells (-) were immunoblotted separately for either GM130 or FHDC1 and then each blot stripped and re-probed for α-tubulin. Scr=scrambled control; k/d=GM130 knockdown cells. N=3, >100 cells counted per experiment. Error bars=SEM.

Figure S7. FHDC1 binds F-actin and microtubules directly. (A) Full-length FHDC1 protein (0.75 μM) was incubated with pre-assembled MTs (0.8 μM) for 30 minutes at room temperature and the reaction products were visualized by immunofluorescence as previously described (Young et al., 2008). Incubation with FHDC1 (red) resulted in assembly of a bundled MT array (white). (B) Incubation with bovine serum albumin (BSA) did not induce MT bundling. (C) FHDC1 (0.75 μM) and BSA were incubated with pre-assembled MTs (0.8 μM) as indicated and then centrifuged at 100,000 x g. Equivalent samples of the supernatant (s) and pellet (p) were subjected to SDS-PAGE and visualized by Coomassie blue staining. Full-length FHDC1, but not BSA, co-sediments with pre-assembled MTs. In the absence of MTs, both FHDC1 and BSA remain in the supernatant. (D) FHDC1 (0.75 μM) was incubated with pre-assembled F-actin (2.0 μM) and the reaction products were visualized by immunofluorescence using anti-FHDC1 and anti-actin antibodies. Incubation with FHDC1 (red) resulted in F-actin bundling (green). (E) Increasing concentrations of FHDC1 (0.375, 0.75, 1.5 μM) were incubated with pre-assembled F-actin (2.0 μM) for 10 minutes at room temperature and then centrifuged for 10 minutes at 16,000 x g. Equivalent samples of the supernatant and pellet fractions were subjected to SDS-PAGE and visualized by Coomassie blue staining. Incubation with FHDC1 bundles F-actin and recruits it to the pelleted fraction. (F) MTs (0.8 μM) and F-actin (2.0 μM) were incubated with FHDC1 (0.75 μM) and the reaction products were visualized by immunofluorescence as above. FHDC1 (red) recruits F-actin (green) and MTs (white) into a bundled complex. (G) MTs (0.8 μM) and F-actin (2.0 μM) were incubated together and the reaction products visualized by immunofluorescence. In the absence of FHDC1 MTs and F-actin do not bundle or co-align. Scale bar=20 μm for all images.

Figure S1

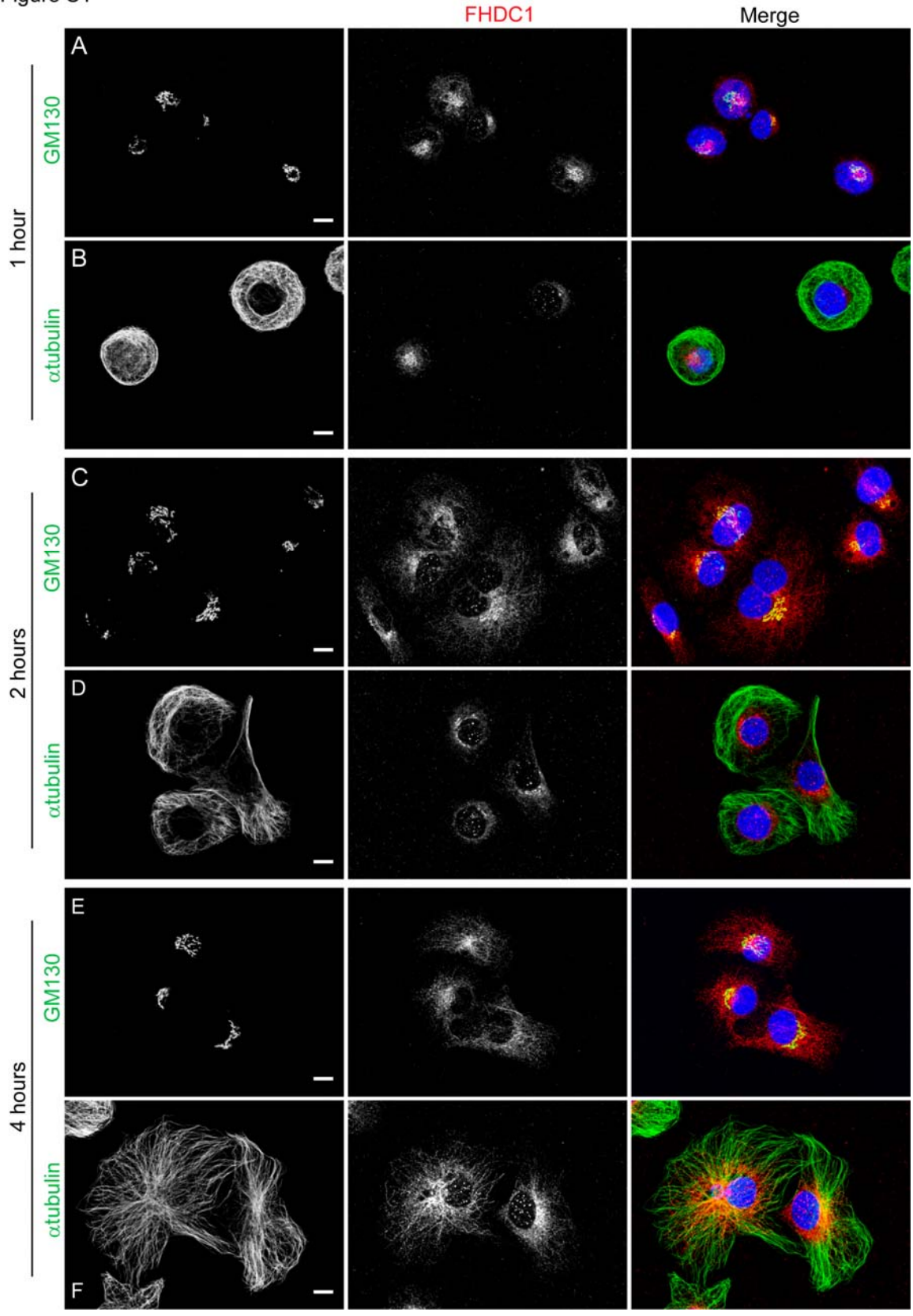

FHDC1
Merge
A
B
C
D
E
F
1 hour
2 hours
4 hours
GM130
αtubulin
GM130
αtubulin
GM130
αtubulin

Figure S2

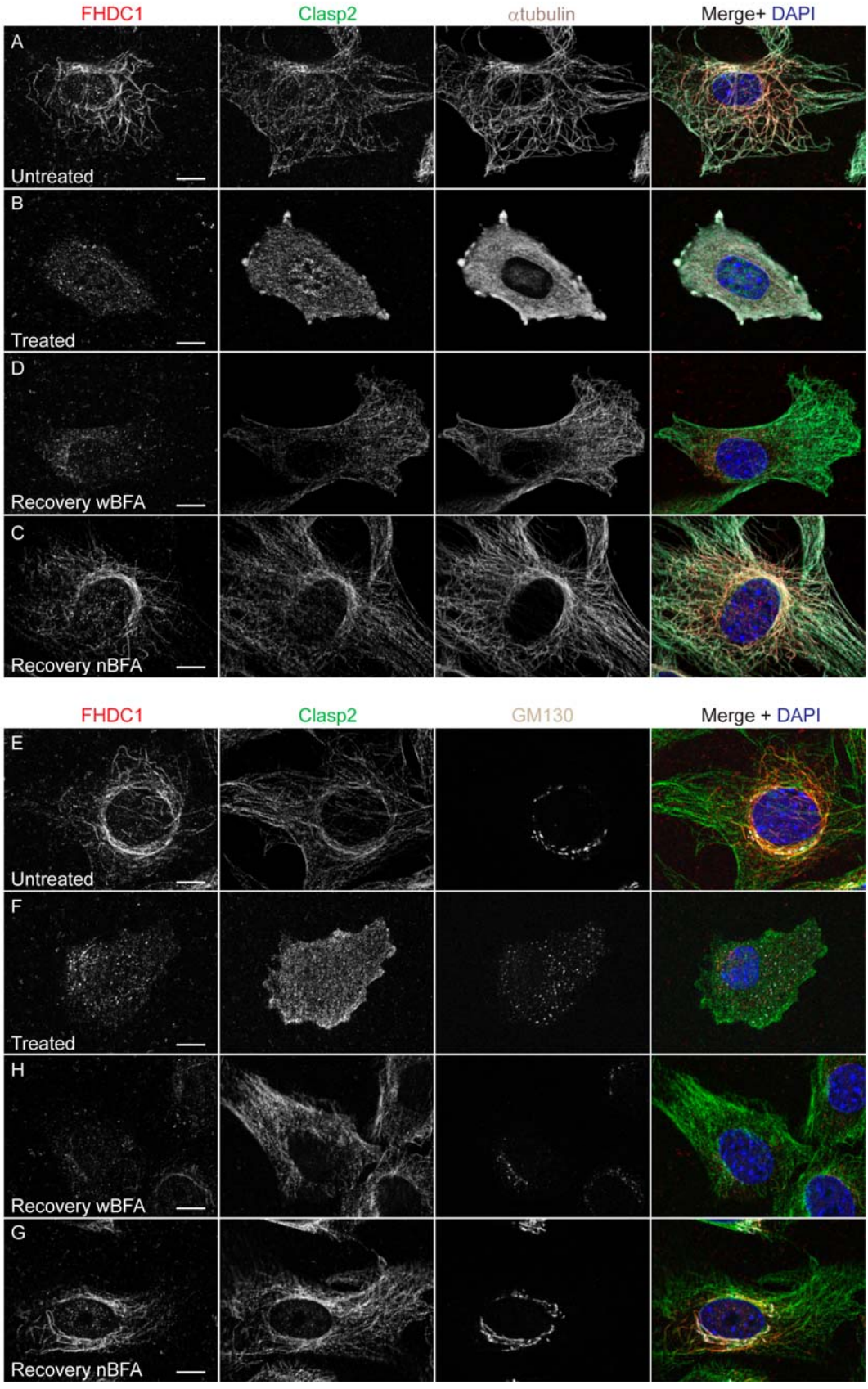

FHDC1
Clasp2
αtubulin
Merge+ DAPI
A
Untreated
B
Treated
D
Recovery wBFA
C
Recovery nBFA
FHDC1
Clasp2
GM130
Merge + DAPI
E
Untreated
F
Treated
H
Recovery wBFA
G
Recovery nBFA

Figure S3

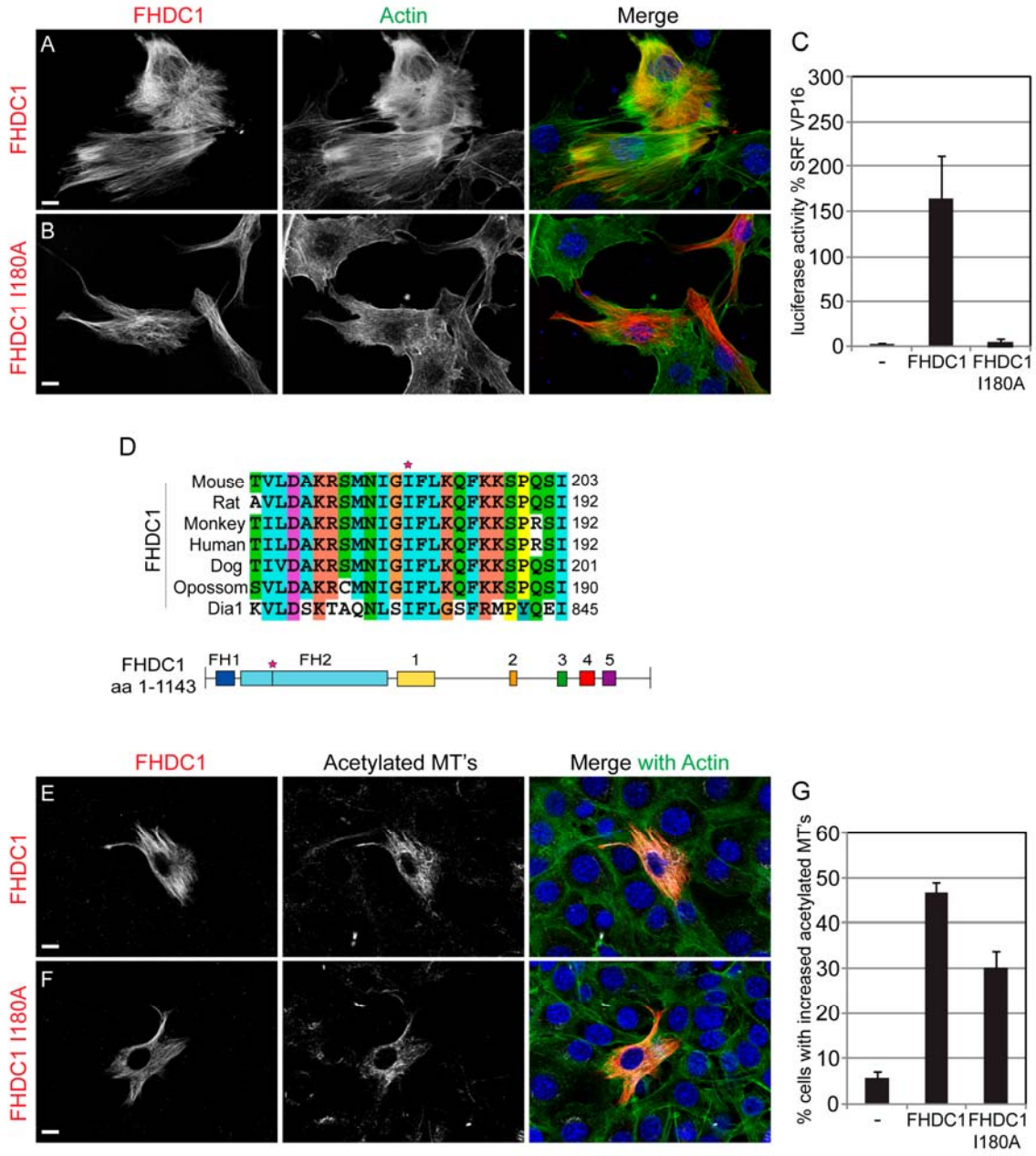
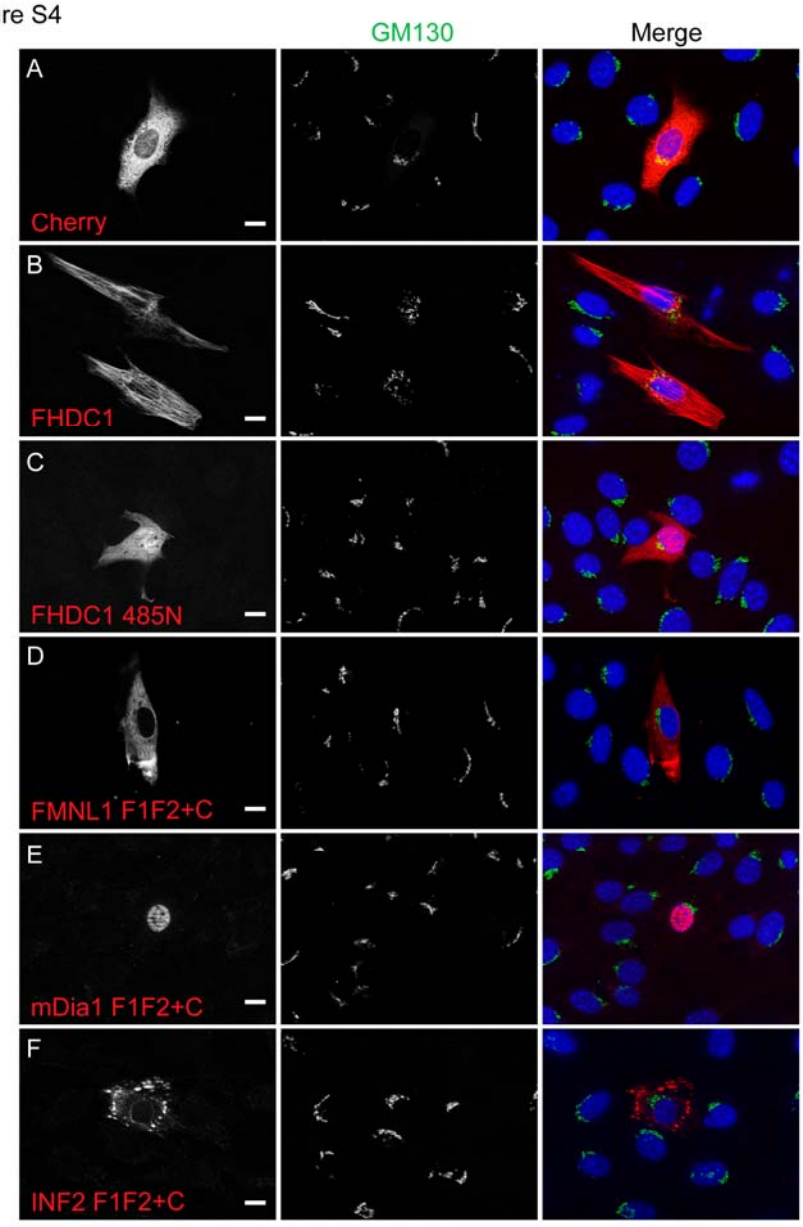

FHDC1
Actin
Merge
A
B
FHDC1
FHDC1 I180A
C
luciferase activity % SRF VP16
300
250
200
150
100
50
0
-
FHDC1
FHDC1 I180A
D
FHDC1
Mouse TVLDAKRSMNIGIFLKQFKKSPQSI 203
Rat AVLDAKRSMNIGIFLKQFKKSPQSI 192
Monkey TILDAKRSMNIGIFLKQFKKSPRSI 192
Human TILDAKRSMNIGIFLKQFKKSPRSI 192
Dog TIVDAKRSMNIGIFLKQFKKSPQSI 201
Opossom SVLDAKRCMNIGIFLKQFKKSPQSI 190
Dia1 KVLDSKTAQNLSIFLGSFRMPYQEI 845
FHDC1
aa 1-1143
FH1
FH2
1
2
3
4
5
FHDC1
Acetylated MT's
Merge with Actin
E
F
FHDC1
FHDC1 I180A
G
% cells with increased acetylated MT's
60
50
40
30
20
10
0
-
FHDC1
FHDC1 I180A

Figure S4


GM130
Merge
A
Cherry
B
FHDC1
C
FHDC1 485N
D
FMNL1 F1F2+C
E
mDia1 F1F2+C
F
INF2 F1F2+C

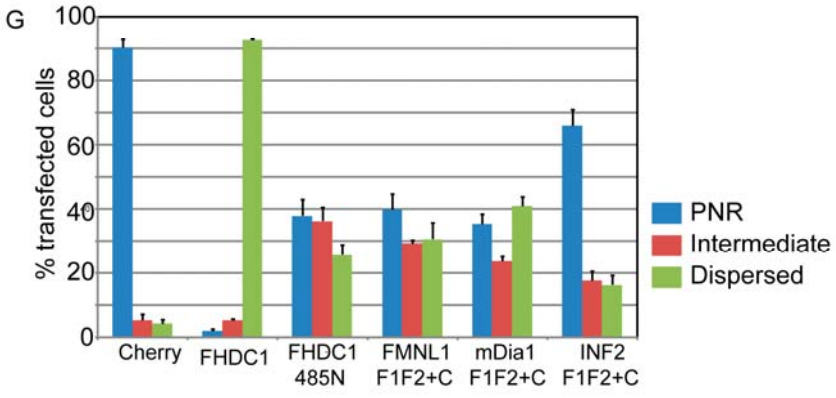

G
100
80
60
40
20
0
% transfected cells
Cherry
FHDC1
FHDC1 485N
FMNL1 F1F2+C
mDia1 F1F2+C
INF2 F1F2+C
PNR
Intermediate
Dispersed

Figure S5

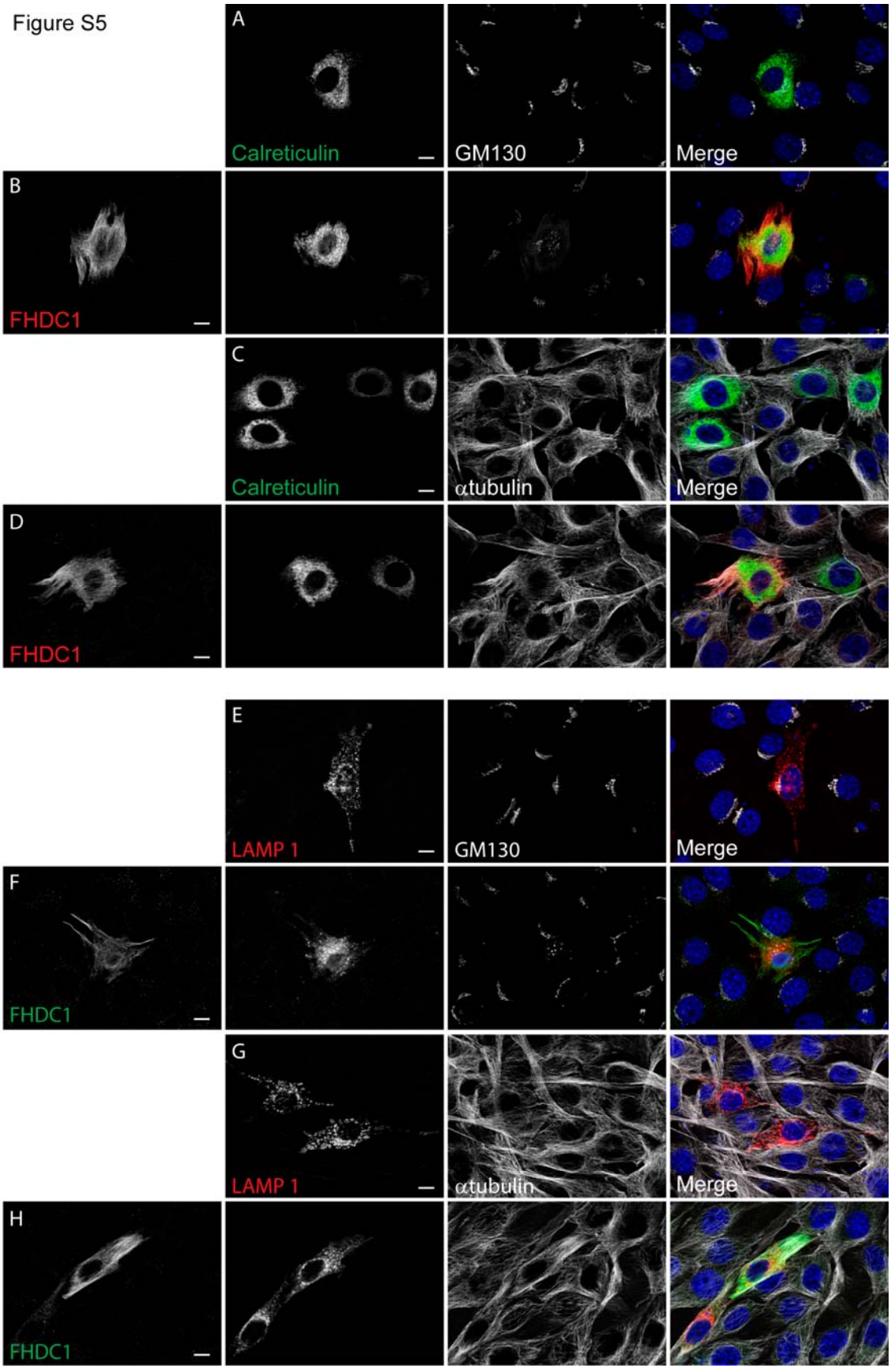
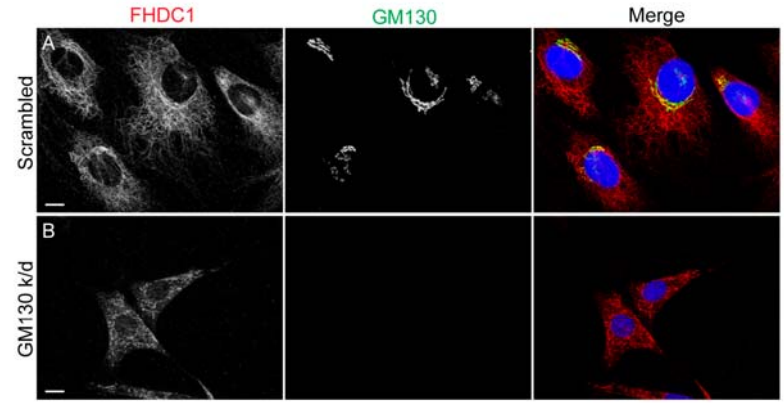
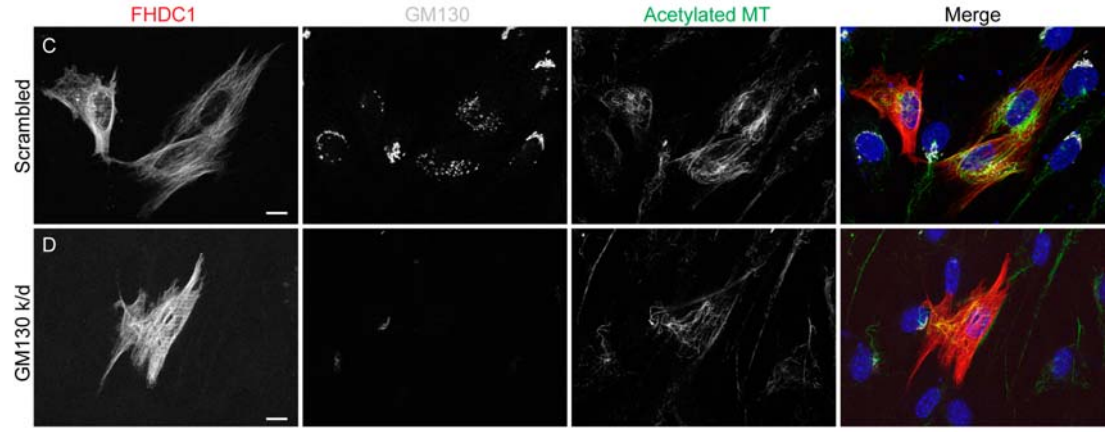
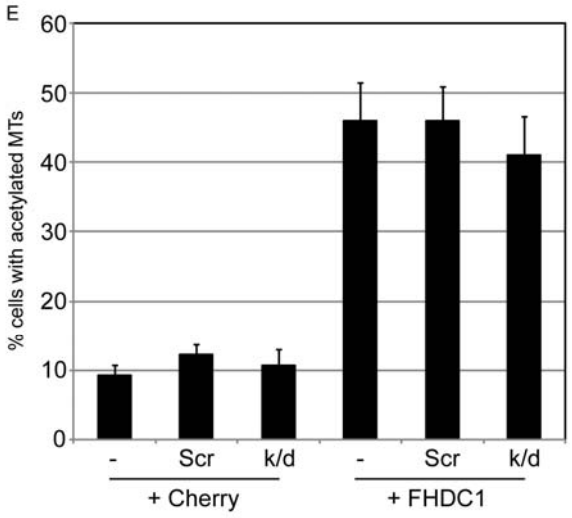

A
Calreticulin
GM130
Merge
B
FHDC1
C
Calreticulin
αtubulin
Merge
D
FHDC1
E
LAMP 1
GM130
Merge
F
FHDC1
G
LAMP 1
αtubulin
Merge
H
FHDC1

Figure S6


FHDC1
GM130
Merge
A
Scrambled
B
GM130 k/d


FHDC1
GM130
Acetylated MT
Merge
C
Scrambled
D
GM130 k/d


E
% cells with acetylated MTs
60
50
40
30
20
10
0
-
Scr
k/d
-
Scr
k/d
+ Cherry
+ FHDC1

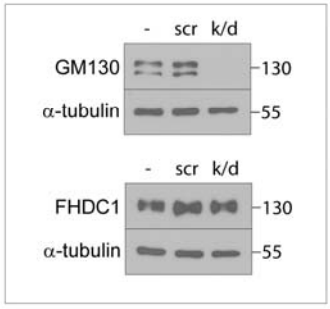
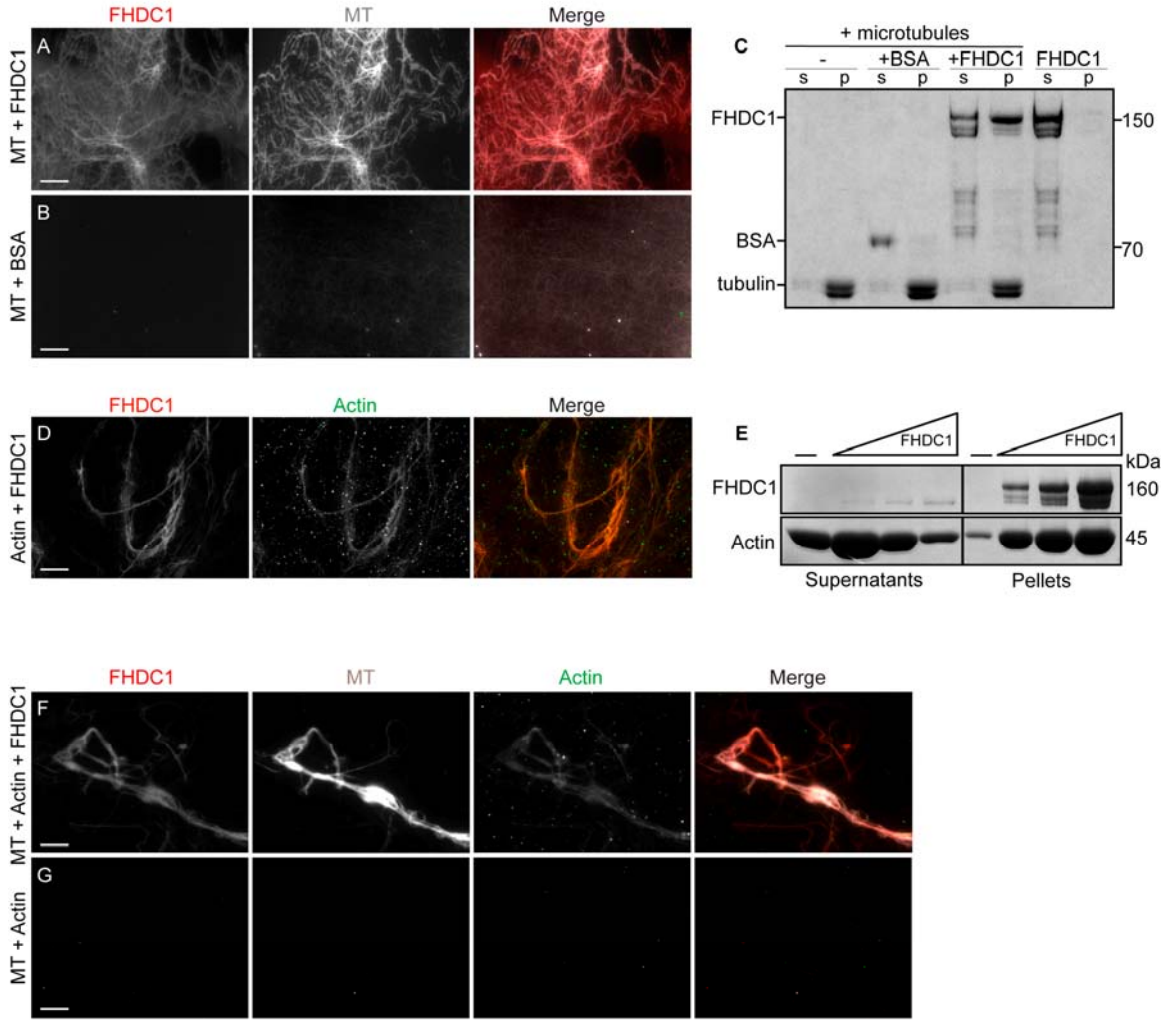

F
-
scr
k/d
GM130
130
α-tubulin
55
-
scr
k/d
FHDC1
130
α-tubulin
55

Figure S7


FHDC1
MT
Merge
A
MT + FHDC1
B
MT + BSA
C
+ microtubules
-
+BSA
+FHDC1
FHDC1
s p s p s p s p
FHDC1
BSA
tubulin
150
70
FHDC1
Actin
Merge
D
Actin + FHDC1
E
FHDC1
FHDC1
kDa
FHDC1
160
Actin
45
Supernatants
Pellets
FHDC1
MT
Actin
Merge
F
MT + Actin + FHDC1
G
MT + Actin