# Supplemental Materials

Molecular Biology of the Cell

Turn *et al*.

**Figure S1: Generation of *Elmod1* KO, *Elmod3* KO, and DKO MEFs.** (**A, B**) Diagrams show the open reading frames encoding ELMOD1 and ELMOD3 proteins under the exons and where the guides target in the exons and corresponding protein sequences. Note that all the guides generated were made to target upstream of the ELMOD domain, increasing the likelihood that if any protein product is made, it is non-functional. The locations of the ELMOD domains are shown under the open reading frames. (**C**) The names and alleles of each of the cloned KO lines used are shown. The two *Elmod1* KO, two *Elmod3* KO, and two DKO lines used in all studies are indicated in bold, though all cloned KO lines are shown here as all were tested in at least some of the reported assays and yielded the same results as the two studied most thoroughly.

**Figure S2: Cellular markers unchanged in *Elmod1*, *Elmod3*, or DKO cells.** KO MEFs display no evidence of changes in staining of nuclear speckles (**A**: SC35), nuclei (**B**: Hoechst), mitochondria (**C**: HSP60), microtubules (**D**: $\alpha$-tubulin), f-actin (**E**: phalloidin), or Golgi (**F**: GM130). (**G**) Because of later evidence of changes in traffic of specific proteins from the Golgi, we examined other Golgi markers and again observed no differences between WT and KO cells. Here, cells were co-stained for the ARF GEF BIG2 (aka ARFGEF2) and Golgin-97, or separately for $\beta$-COP (bottom panels). Cells were fixed with 4% PFA, permeabilized with 0.1% Triton X-100, and stained for the indicated markers in two lines each of WT, *Elmod1* KO, *Elmod3* KO, and DKO. Screening was repeated at least in triplicate. Representative images of WT and KO cells were collected at 100X magnification using widefield microscopy, and images were processed via FIJI imaging software. Scale bar = 10 µm.

**Figure S3: *Elmod1 KO*, *Elmod3 KO*, or DKO MEFs exhibit no evident defects in cell cycle, rootlet morphology, or centrosome separation. (A)** Two lines each of WT, *Elmod1* KO, *Elmod3* KO, and DKO MEFs growing in log phase were collected, stained for propidium iodide, and examined for DNA content by flow cytometry, as described under Methods. At least 10,000 cells were analyzed per sample, and data were processed via FloJo software. This experiment was repeated in triplicate with each of the eight cell lines, and no consistent differences were evident. Representative graphs are shown. **(B)** The same eight cell lines described in **(A)** were serum starved for 24 hrs prior to fixation and were co-stained for rootletin and ARL13B. The experiment was repeated at least three times for each cell line. Images of WT and DKO cells are shown were collected at 100X magnification using widefield microscopy. Note that one of the DKO cells shown displays ARL13B staining in its cilium, to highlight that while ciliation and ARL13B staining of cilia are each strongly reduced, each can be found. Scale bar = 10 µm. **(C)** These same cell lines were grown to ~75% confluence, fixed, and stained for $\gamma$-tubulin to mark centrosomes. Images were collected, and FIJI measuring tool was used to quantify the percentage of cells with centrosomes farther than 2µm apart. Results were plotted in GraphPad software as box-and-whisker plots. ns = $p>0.05$, calculated via One-Way ANOVA with multiple comparisons.

**Figure S4: ELMOD1-myc is expressed to much higher levels than is ELMOD1-myc in MEFs.** WT cells were transfected with plasmids directing the expression of either empty vector, ELMOD1-myc, or ELMOD3-myc, as indicated above each lane. The next day, cells were collected, and total cell lysates were generated, as described under Methods. Equal protein (40 $\mu$g/lane) was loaded on each lane of a 13% polyacrylamide gel and later transferred onto nitrocellulose membranes for 90 min at 60V. The membrane was then stained with Ponceau S (left panel) or with anti-myc antibody and developed using enhanced chemiluminescence (right panel). Molecular weight standards were run in a separate lane, and sizes are indicated between panels.

**Figure S5: Neither *Elmod1* nor *Elmod3* KO alters the extent of CEP164 recruitment and CP110 release from the basal body, despite the decrease in ciliation.** Two lines each of WT, *Elmod1* KO, *Elmod3* KO, and DKO cells were serum starved for 24 hours

before fixation and staining for either (**A**) CEP164 or (**B**) CP110 and γ-tubulin, as described under Methods. The presence of CEP164 and CP110 were scored in 100 cells per cell line in duplicate, binning cells as having either 0, 1, or 2 centrosomes positive for CEP164 or CP110. Data were graphed using GraphPad Prism. Error bars = SEM. (**C**) Representative widefield images of cilia from cells stained for CEP290 and Ac Tub are shown, with the genotypes of the cells shown at the top. Scale bar = 10 μm.

**Figure S6: Gli3 recruitment to cilia is unaffected by deletion of *Elmod1* or *Elmod3*.** The standard eight cell lines were serum starved for 24 hr before fixation and co-staining for Gli3 and Ac Tub. This experiment was repeated at least in triplicate with consistent lack of changes. Representative images from each genotype were collected at 100x magnification via widefield microscopy. Scale bar = 10 μm.

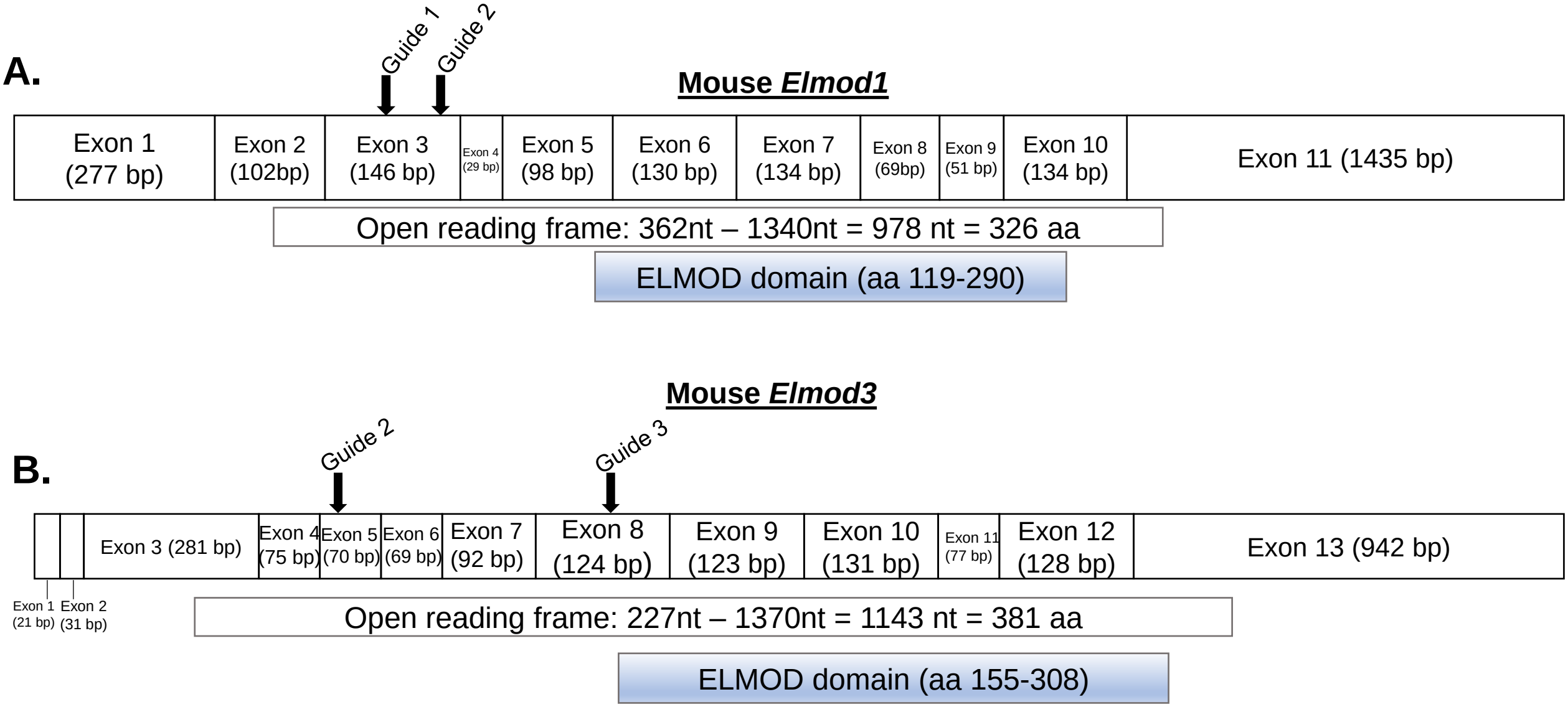
**Figure S7: IFT140 colocalizes with Rootletin in both WT and KO cells**. The standard eight cell lines were serum starved for 24 hours before being fixed with ice-cold methanol and stained for IFT140 and rootletin (to mark the ciliary rootlet). Representative images from each genotype were collected at 100x magnification via widefield microscopy. Scale bar = 10 μm.

**Figure S8: IFT88 and IFT140 localization to cilia in ELMOD1/3 KO lines is unchanged.** Eight working cell lines that were serum-starved for 24 hours were fixed with 4% PFA and stained for Ac Tub (to mark cilia) and either IFT88 (**A**) or IFT140 (**B**). Representative images from each genotype were collected at 100x magnification via widefield microscopy. Scale bar = 10 μm.

**Figure S9: Number and sizes of focal adhesions are unchanged in ELMOD1 and ELMOD3 KO lines.** The eight working cell lines were tested for changes in focal adhesion architecture via immunofluorescence, staining for total β-integrin and active (ligand-bound) β-integrin using the activation-specific antibody 9EG7. Image quantification was performed using FIJI software, applying the same conditions to subtract background and to set thresholds for each sample. Data were processed via GraphPad prism software, and statistical significance was assessed via One-Way ANOVA. N = 3. Error bars = SEM. * = $p<0.05$ as calculated by One-Way ANOVA with multiple comparisons.

**Figure S10: Expression of myc-tagged ELMOD1 and ELMOD3 in WT MEFs.** Cells were transfected with either ELMOD1-myc or ELMOD3-myc. Twenty-four hours after transfection, cells were fixed with 4% PFA and stained for myc to mark transfected cells and phalloidin to stain the actin cytoskeleton. Images were collected via widefield microscopy. Representative images are shown though there is variation in apparent localization of each protein in each population so specific localization was not pursued. Scale bar = 10 μm.

**Figure S11: ARL13B expression is reduced in *Elmod1* KO, *Elmod3* KO, and DKO lines**. Two lines each of WT, *Elmod1* KO, *Elmod3* KO, and DKO cells were serum starved for 24 hours before fixation and staining for ARL13B and acetylated tubulin. Images were collected via widefield microscopy at 100x magnification, imaging at least 28 cilia per genotype. Images were processed via CiliaQ plugin in FIJI imaging software, measuring the average intensity of ARL13B along the center line of each cilium. Data were graphed as box-and-whisker plots using GraphPad. Statistical significance was assessed via One-Way ANOVA, performing multiple comparisons. ns = not significant, * = $p<0.05$.


A.
Guide 1
Guide 2
Mouse Elmod1
Exon 1 (277 bp)
Exon 2 (102bp)
Exon 3 (146 bp)
Exon 4 (29 bp)
Exon 5 (98 bp)
Exon 6 (130 bp)
Exon 7 (134 bp)
Exon 8 (69bp)
Exon 9 (51 bp)
Exon 10 (134 bp)
Exon 11 (1435 bp)
Open reading frame: 362nt – 1340nt = 978 nt = 326 aa
ELMOD domain (aa 119-290)
B.
Mouse Elmod3
Guide 2
Guide 3
Exon 1 (21 bp)
Exon 2 (31 bp)
Exon 3 (281 bp)
Exon 4 (75 bp)
Exon 5 (70 bp)
Exon 6 (69 bp)
Exon 7 (92 bp)
Exon 8 (124 bp)
Exon 9 (123 bp)
Exon 10 (131 bp)
Exon 11 (77 bp)
Exon 12 (128 bp)
Exon 13 (942 bp)
Open reading frame: 227nt – 1370nt = 1143 nt = 381 aa
ELMOD domain (aa 155-308)

# Figure S1 A, B

**C.**

| Clone ID | Lab Name | Guide | Alleles |
|---|---|---|---|
| ***Elmod1*$^{-/-}$ #1** | **A12,1_1,1,1** | 1 | Both alleles: 1bp deletion (C); …VMRKLTEGVNCKGFATAPNLELPEP* |
| ***Elmod1*$^{-/-}$ #2** | D4,2_1,2,5 | 2 | 1: 1 bp deletion (A); … CELQRICYGTNLELPEP*<br>2: 2 bp deletion (AA); …CELQRICYGTTWSFQNHEN* |
| *Elmod1*$^{-/-}$ #3 | E2,2_1,1,1 | 1 | Both alleles: 4 bp deletion (CGGA); …VMRKLTGVNCKGFATAPNLELPEP* |
| *Elmod1*$^{-/-}$ #4 | G1,2_1,1,1 | 1 | Both alleles: 1bp deletion (C); …VMRKLTEGVNCKGFATAPNLELPEP* |
| *Elmod1*$^{-/-}$ #5 | H4,2_1,2,5 | 2 | Both alleles: 2bp deletion (AA); …CELQRICYGTTWSFQNHEN* |
| ***Elmod3*$^{-/-}$ #1** | **H8,1_3,2,7** | 2 | 1: 128 bp deletion; 2: 218 bp deletion; each results in loss of 21/23 amino acids of exon 5,<br>and loss of nucleotides in the following intron that may also alter splicing |
| ***Elmod3*$^{-/-}$ #2** | **C6,1_3,3,11** | 3 | 1: 1 bp deletion (G); … TNHGILQALTQKPMGGSQVVRTPRALGVWGRPRSEPPRSPLAVVCISHALALSFR IL*<br>2: 4 bp deletion (GGCA); … TNHGILQALTKPMGGSQVVRTPRALGVWGRPRSEPPRSPLAVVCISHALALSFRIL* |
| *Elmod3*$^{-/-}$ #3 | F1,1_3,2,7 | 2 | Both alleles: 1 bp insertion (T); **…**ISELDEPWHSPGPDGRNQWVAARW* |
| *Elmod3*$^{-/-}$ #4 | C2,2_3,2,7 | 2 | Both alleles: 1 bp insertion (T); …ISELDEPWHSPGPDGRNQWVAARW* |
| *Elmod3*$^{-/-}$ #5 | A10,1_3,3,11 | 3 | Both alleles: 1 bp deletion (G); …ALTQKPMGGSQVVRTPRALGVWGRPRSEPPRSPLAVVCISHALALSFRIL* |
| *Elmod3*$^{-/-}$ #6 | B6,1_3,3,11 | 3 | Both alleles: 1 bp deletion (G); …ALTQKPMGGSQVVRTPRALGVWGRPRSEPPRSPLAVVCISHALALSFRIL* |
| *Elmod3*$^{-/-}$ #7 | D7,1_3,3,11 | 3 | Both alleles: 1 bp insertion (G); …ALTGRNQWVAARW* |
| *Elmod3*$^{-/-}$ #8 | E5,2_3,3,11 | 3 | Both alleles: 1 bp deletion (G); …ALTQKPMGGSQVVRTPRALGVWGRPRSEPPRSPLAVVCISHALALSFRIL* |
| *Elmod3*$^{-/-}$ #9 | A10,3_3,3,11 | 3 | 1: 1 bp insertion (G); **…**ALTGRNQWVAARW*<br>2: 1 bp insertion (G), 2 bp deletion (AC); ALGQKPMGGSQVVRTPRALGVWGRPRSEPPRSPLAVVCISHALALSFRIL* |
| **DKO #1** | **C12,G7_3,2,7** | 2 | Both alleles: 101 bp deletion; results in deletion of the first 19 amino acids of exon 5,<br>and 43 nucleotides of the preceding intron, likely disrupting proper splicing |
| **DKO #2** | **G7,G1_3,2,7** | 2 | Both alleles: 1 bp insertion (T); …ISELDEPWHSPGPDGRNQWVAARW* |

# Figure S1 C

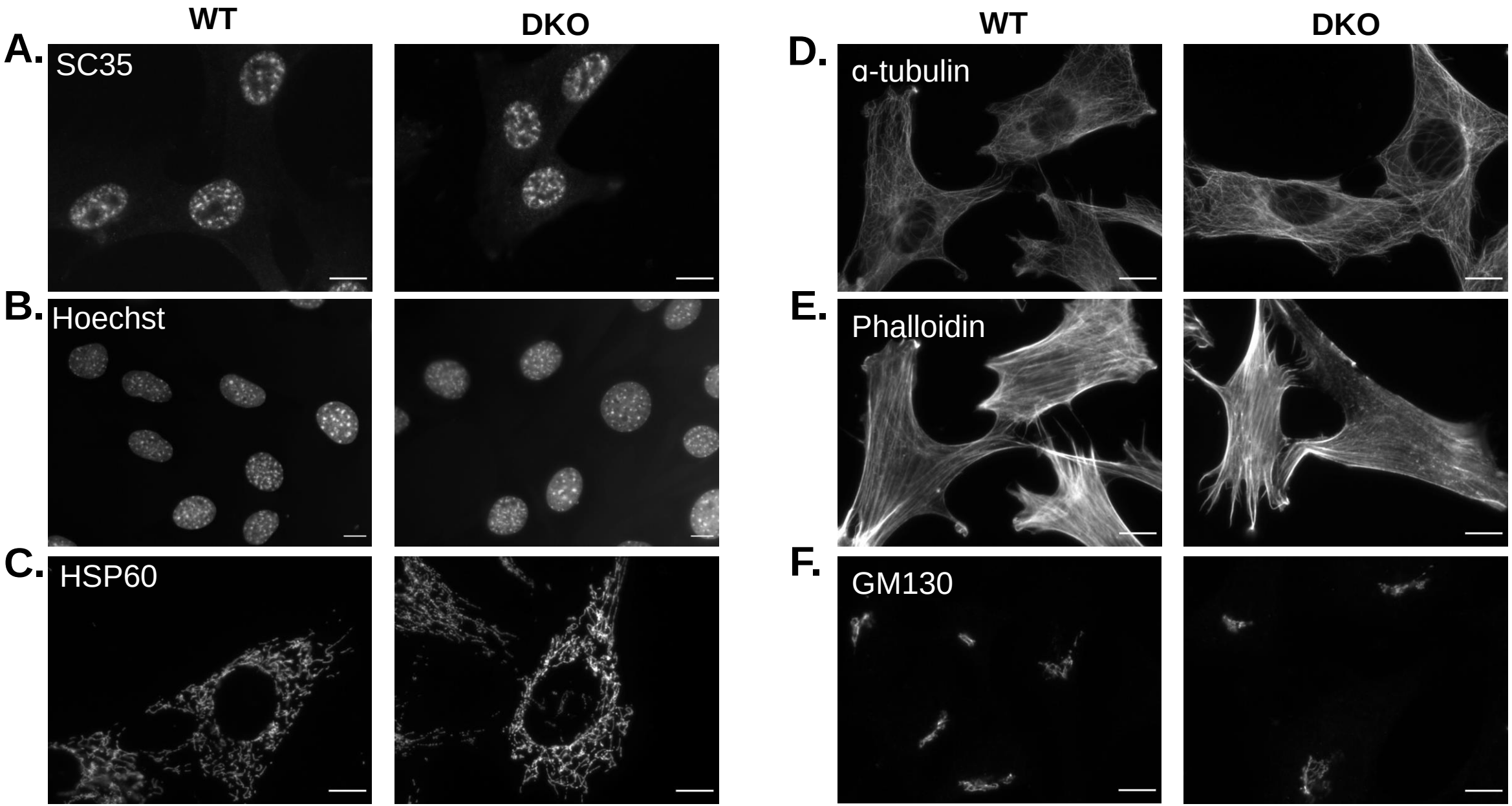

WT
DKO
A.
SC35
B.
Hoechst
C.
HSP60
WT
DKO
D.
α-tubulin
E.
Phalloidin
F.
GM130

**Figure S2 A-F**

G.

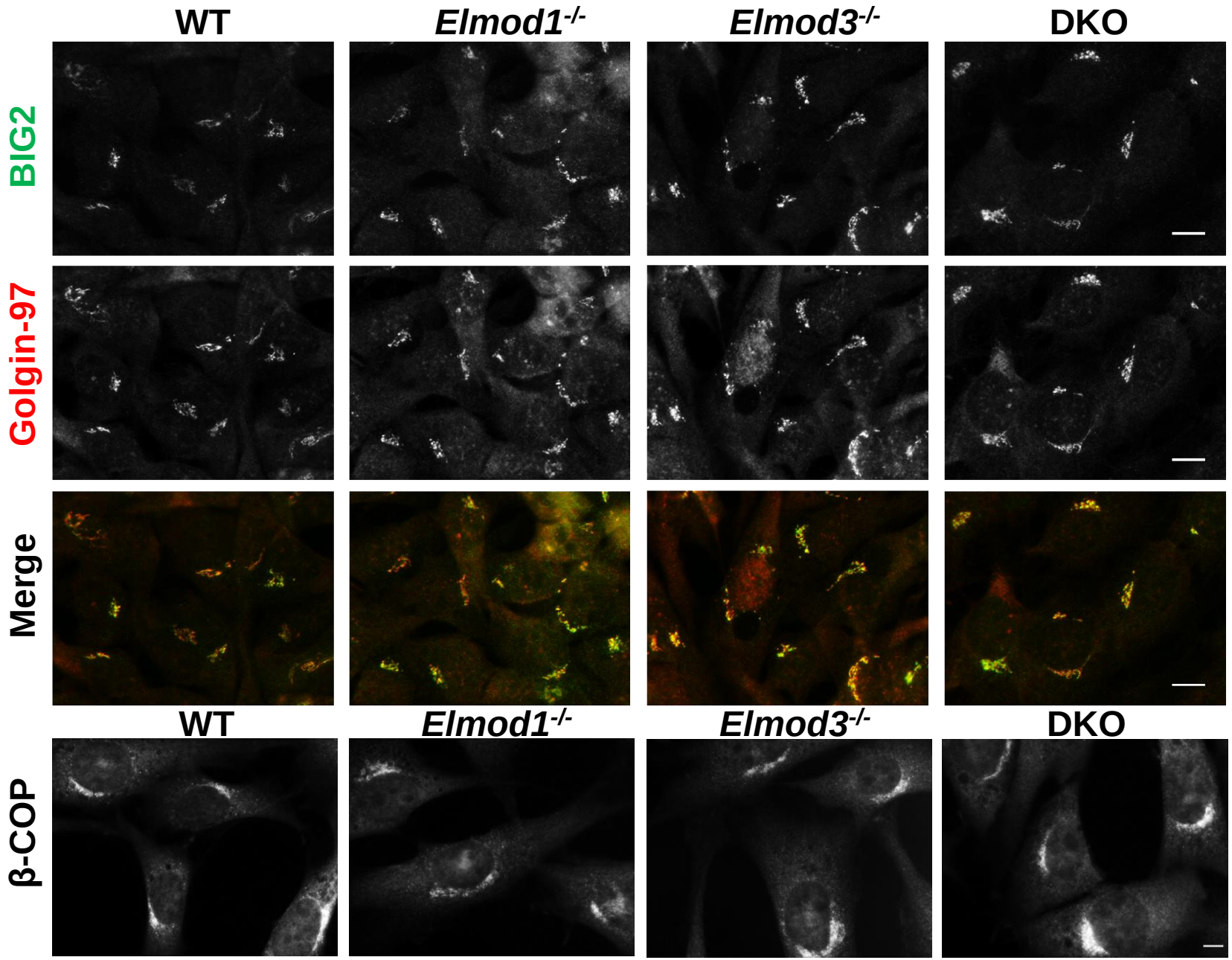
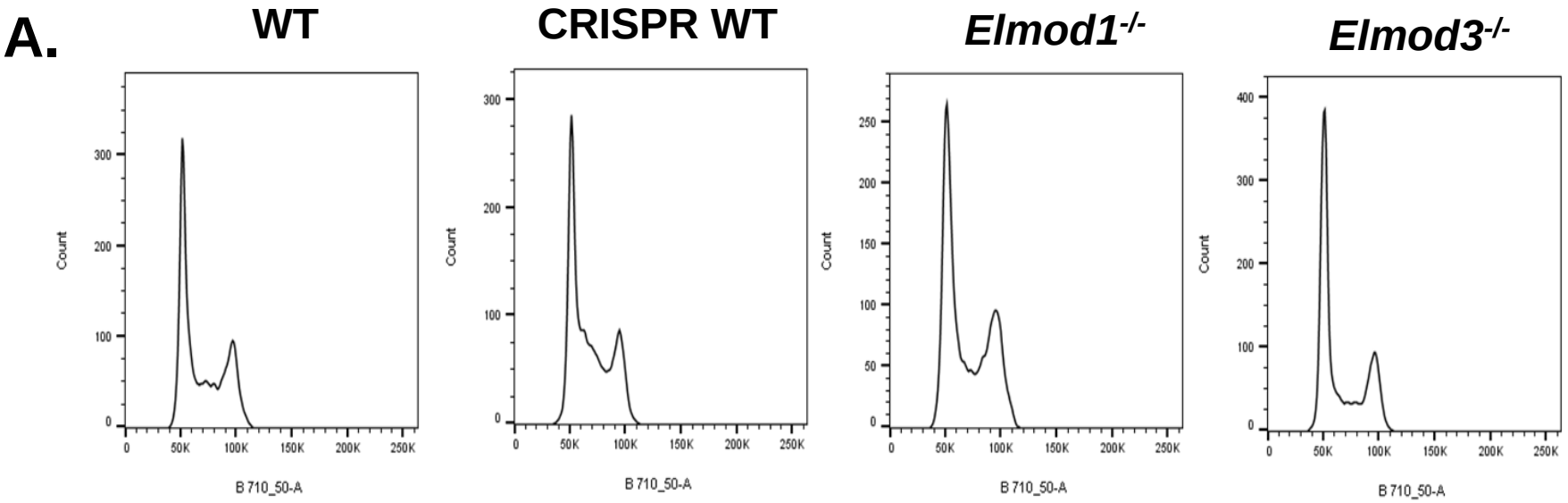
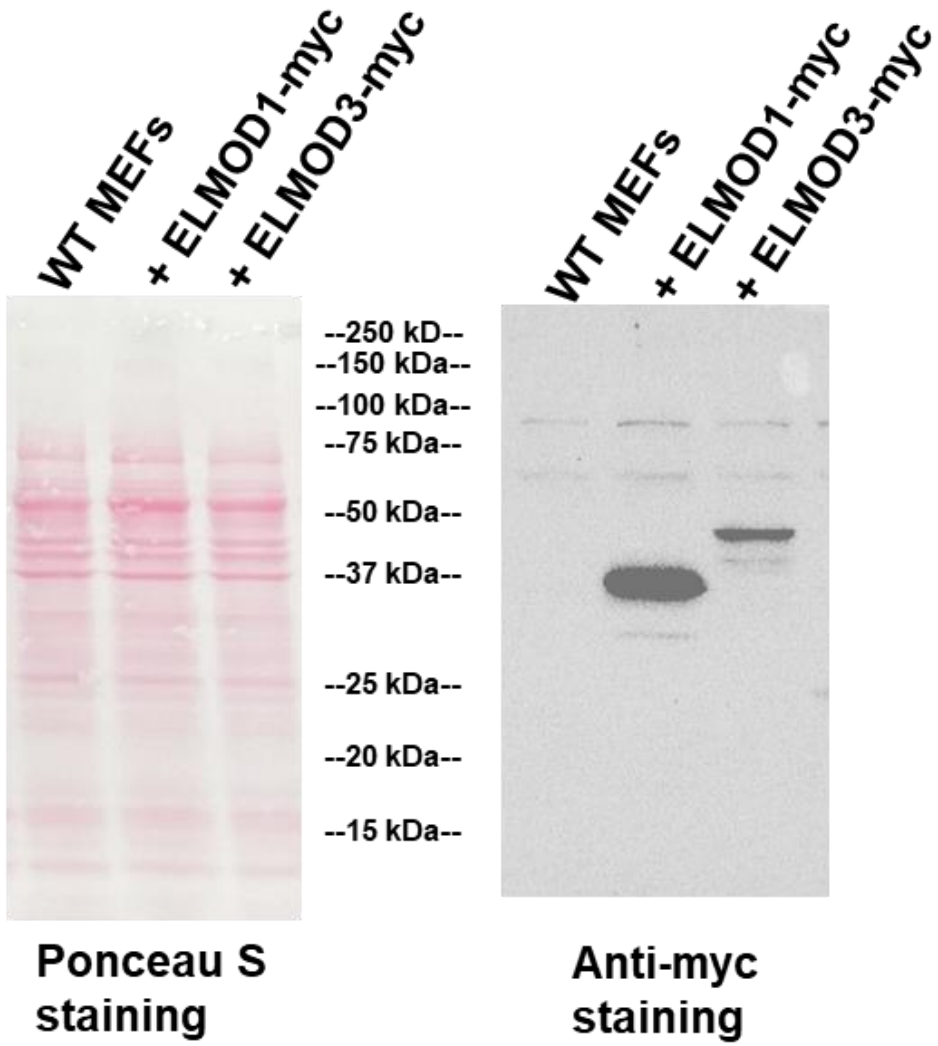

WT
Elmod1-/-
Elmod3-/-
DKO
BIG2
Golgin-97
Merge
WT
Elmod1-/-
Elmod3-/-
DKO
β-COP

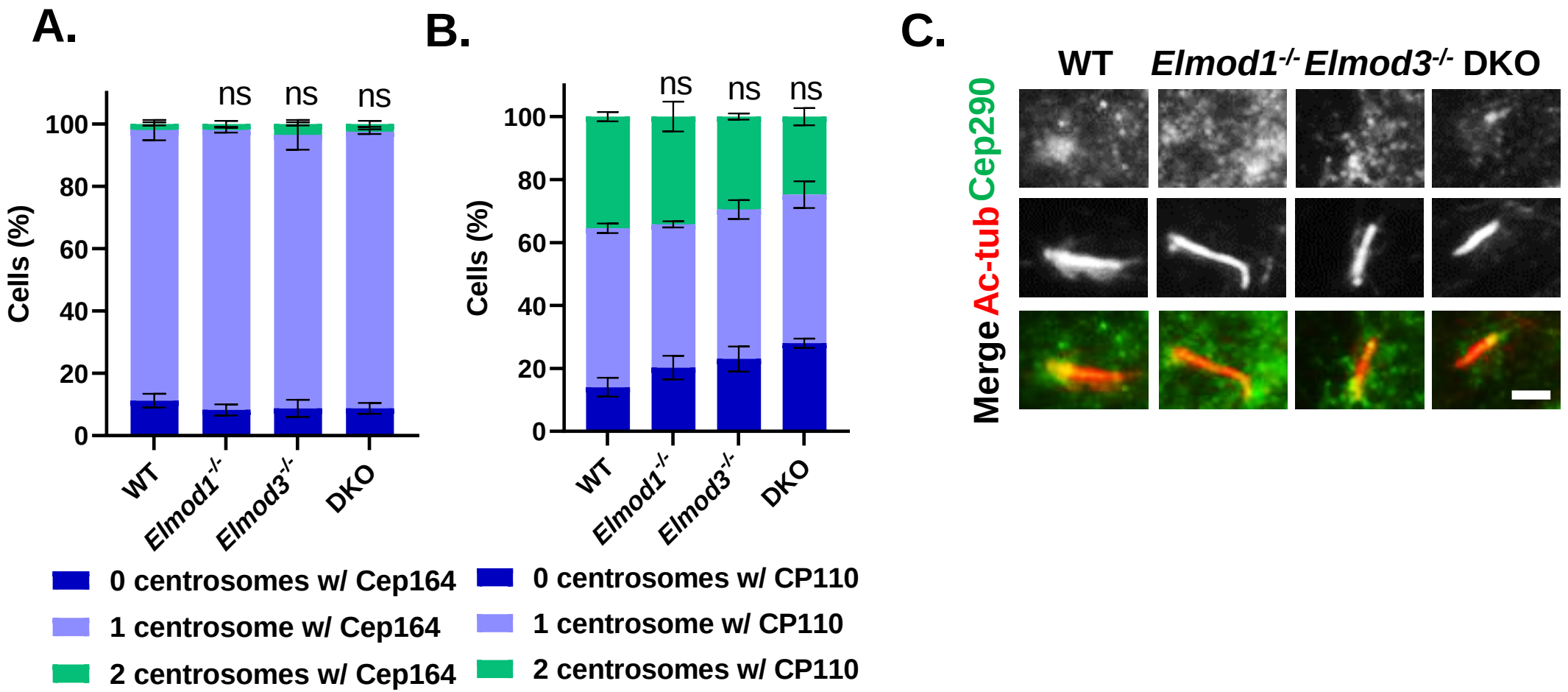
Figure S2 G


A.
WT
CRISPR WT
Elmod1-/-
Elmod3-/-
Count
B 710_50-A
B.
WT
DKO
Rootletin
ARL13B
C.
Cells w/ centrosomes >2µm apart (%)
100
80
60
40
20
0
ns
ns
ns
WT
Elmod1-/-
Elmod3-/-
DKO

# Figure S3 A,B


WT MEFs
+ ELMOD1-myc
+ ELMOD3-myc
WT MEFs
+ ELMOD1-myc
+ ELMOD3-myc
--250 kD--
--150 kDa--
--100 kDa--
--75 kDa--
--50 kDa--
--37 kDa--
--25 kDa--
--20 kDa--
--15 kDa--
Ponceau S staining
Anti-myc staining

# Figure S4


A.
B.
C.
ns
Cells (%)
100
80
60
40
20
0
WT
Elmod1-/-
Elmod3-/-
DKO
0 centrosomes w/ Cep164
1 centrosome w/ Cep164
2 centrosomes w/ Cep164
0 centrosomes w/ CP110
1 centrosome w/ CP110
2 centrosomes w/ CP110
Merge Ac-tub Cep290

# Figure S5 A-C

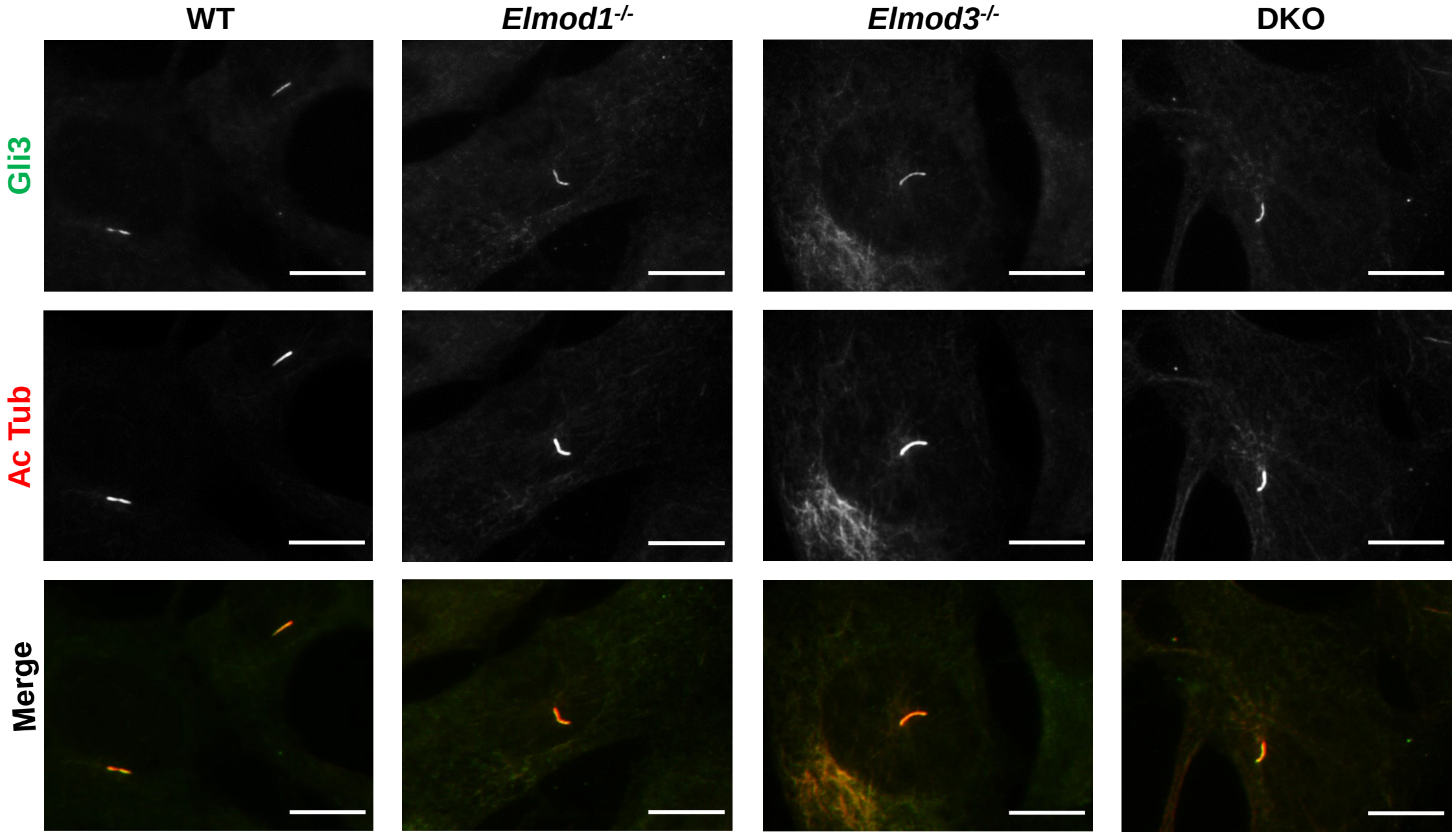
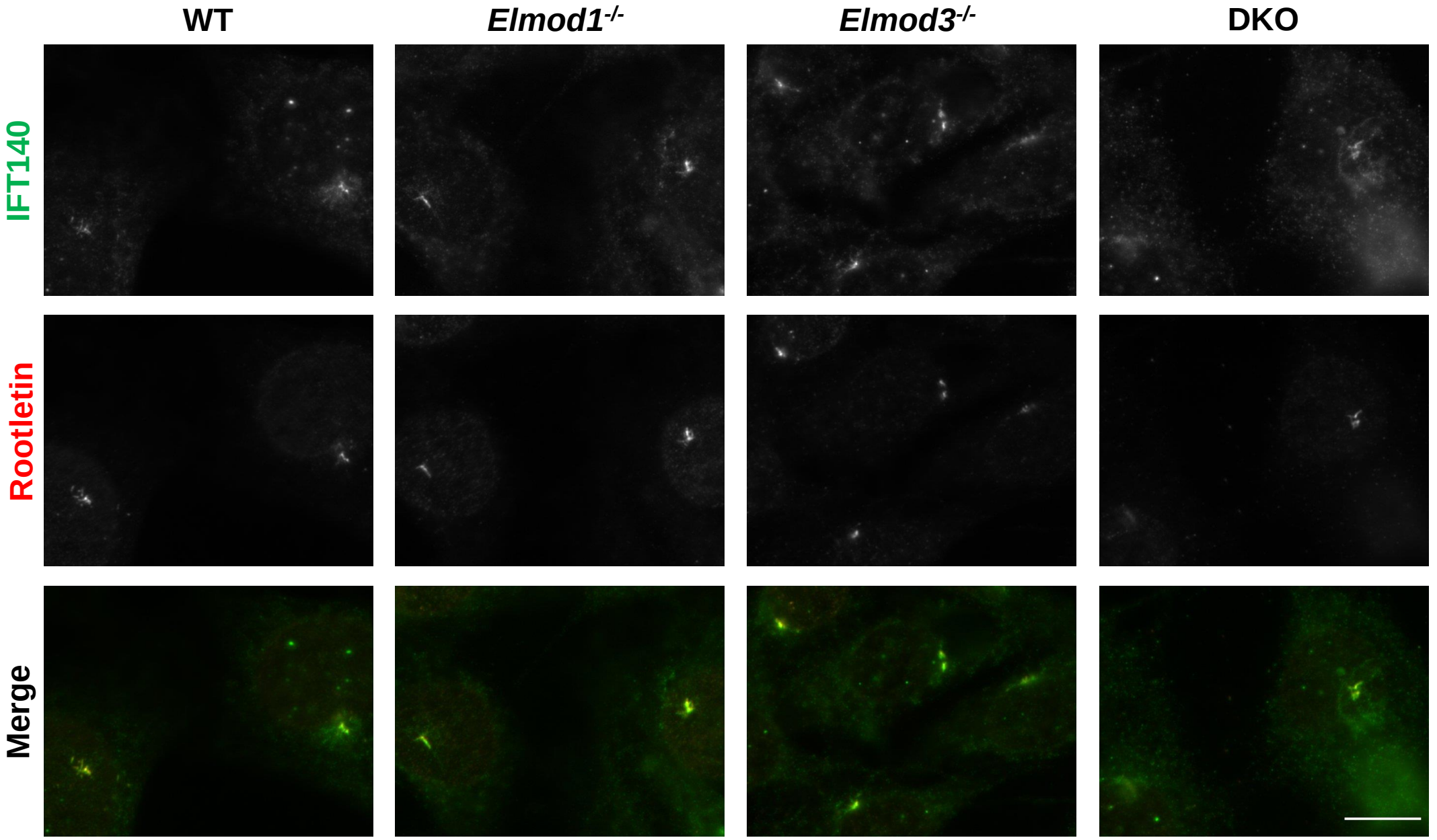
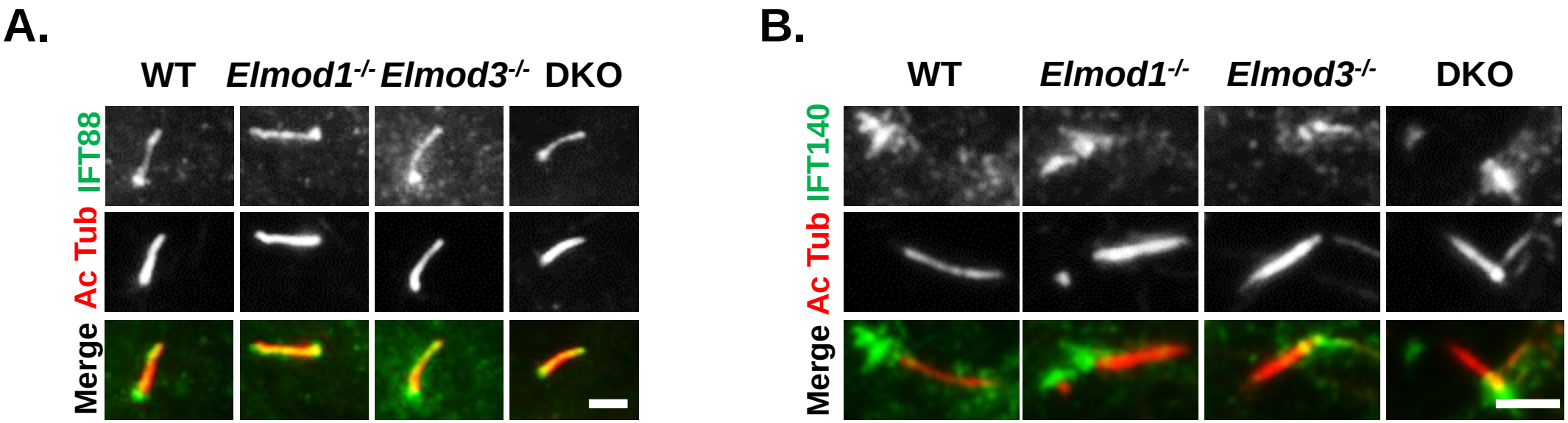


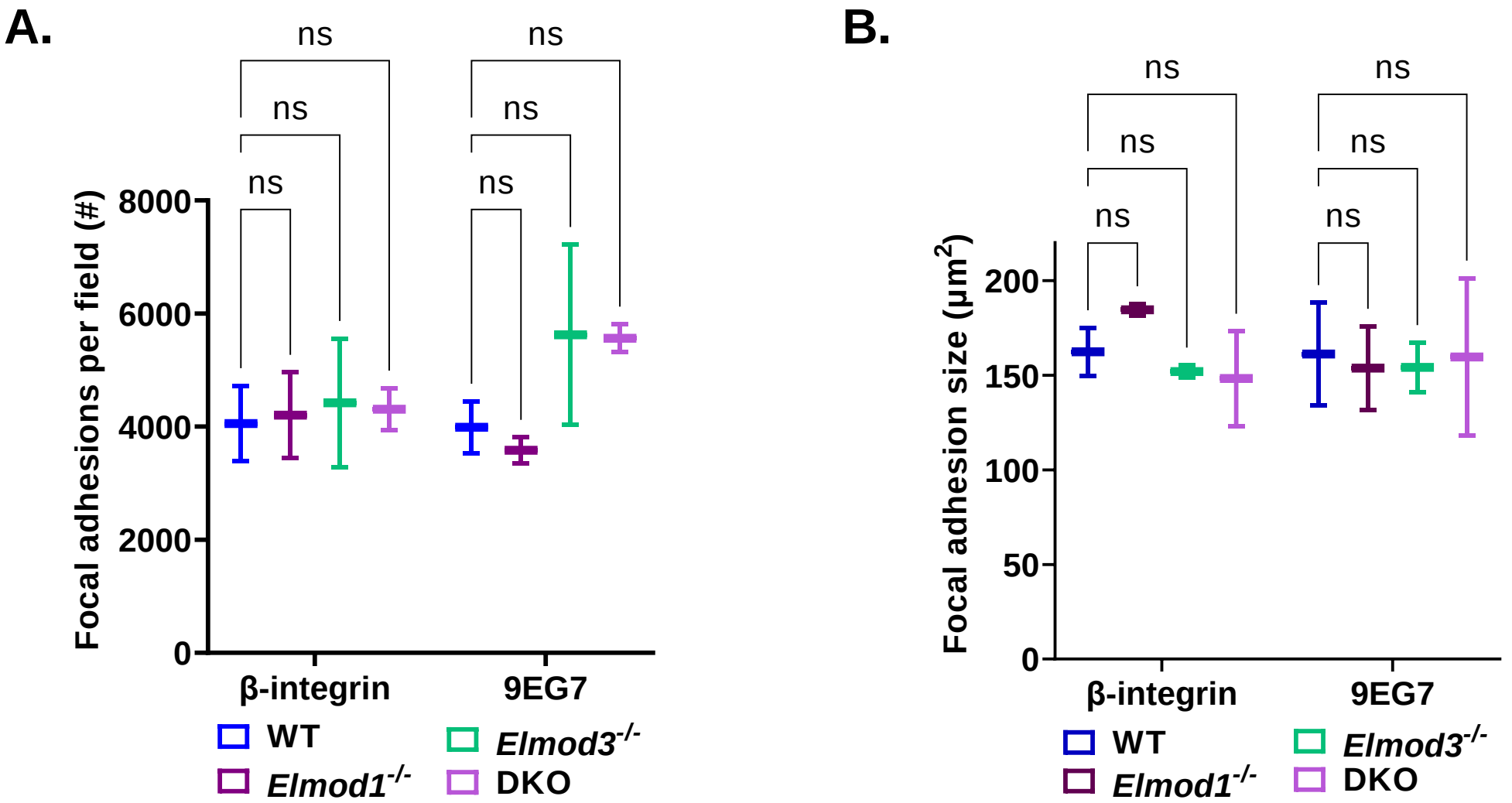

Figure S6




# Figure S7


A.
WT
Elmod1-/-
Elmod3-/-
DKO
IFT88
Ac Tub
Merge
B.
WT
Elmod1-/-
Elmod3-/-
DKO
IFT140
Ac Tub
Merge

# Figure S8


A.
ns
ns
ns
ns
ns
ns
Focal adhesions per field (#)
8000
6000
4000
2000
0
β-integrin
9EG7
WT
Elmod1-/-
Elmod3-/-
DKO
B.
ns
ns
ns
ns
ns
ns
Focal adhesion size (µm2)
200
150
100
50
0
β-integrin
9EG7
WT
Elmod1-/-
Elmod3-/-
DKO

# Figure S9 A, B

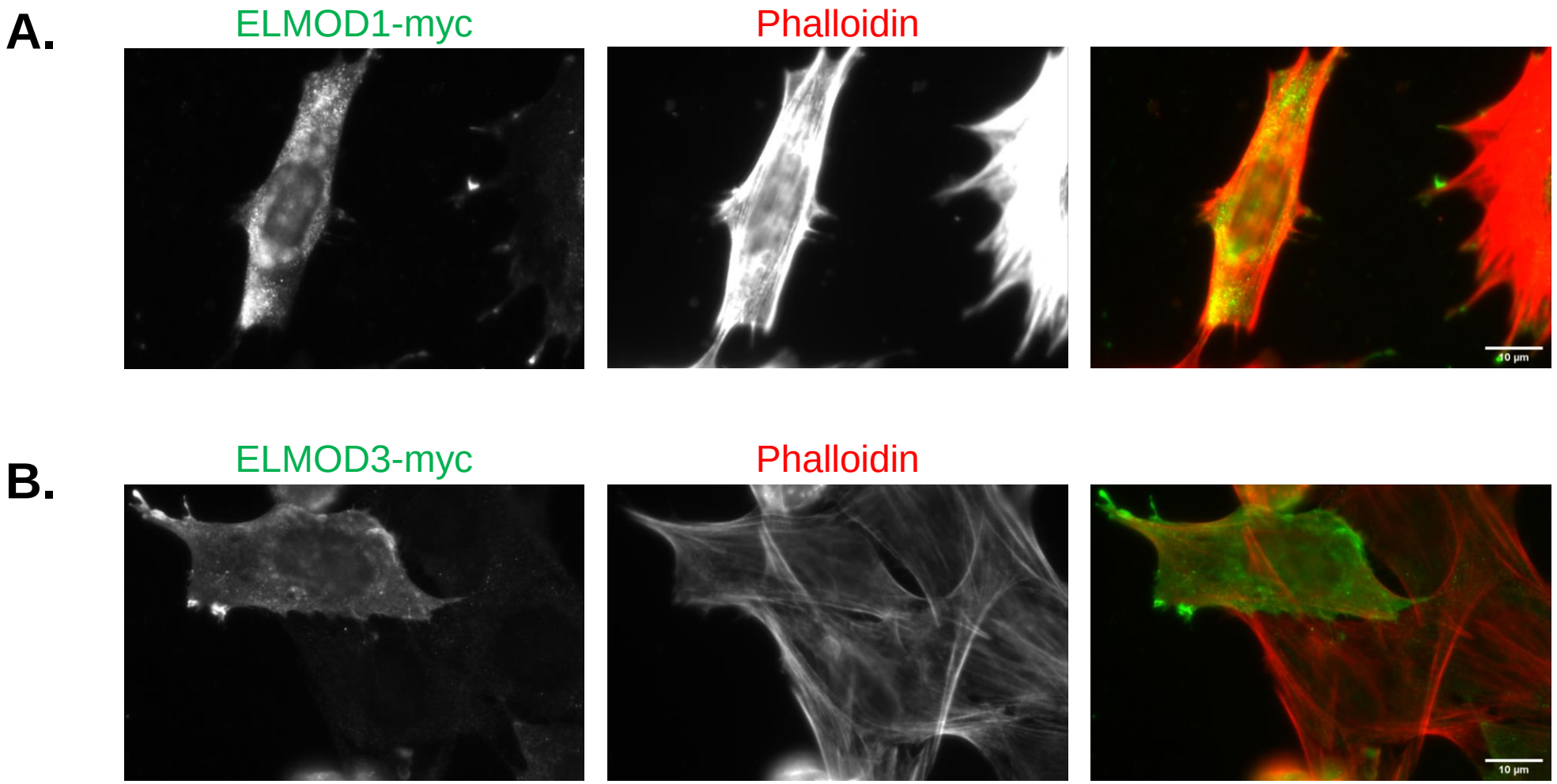
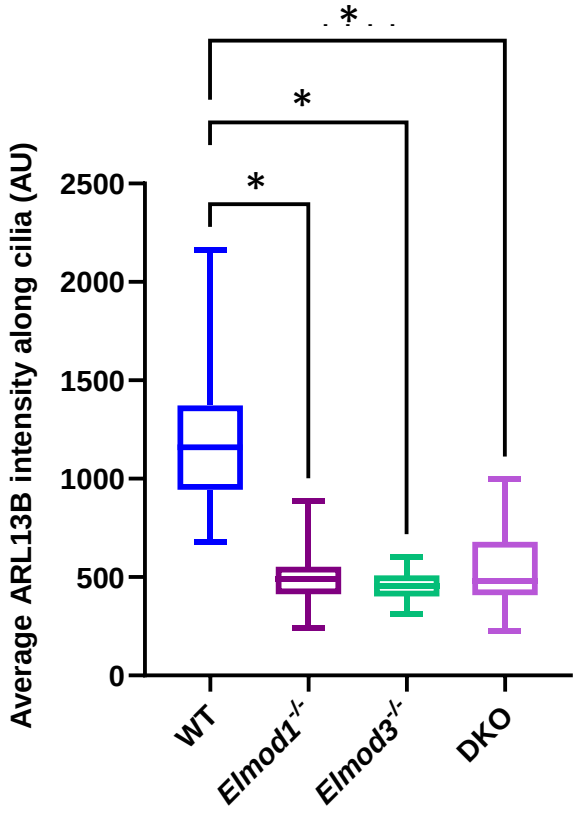



**Figure S10**


*
*
*
Average ARL13B intensity along cilia (AU)
2500
2000
1500
1000
500
0
WT
Elmod1-/-
Elmod3-/-
DKO

# Figure S11