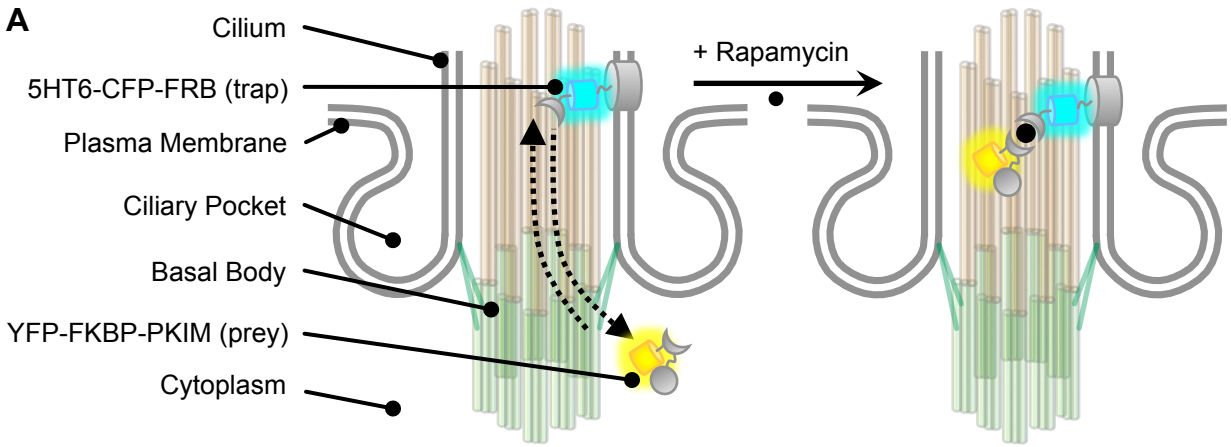
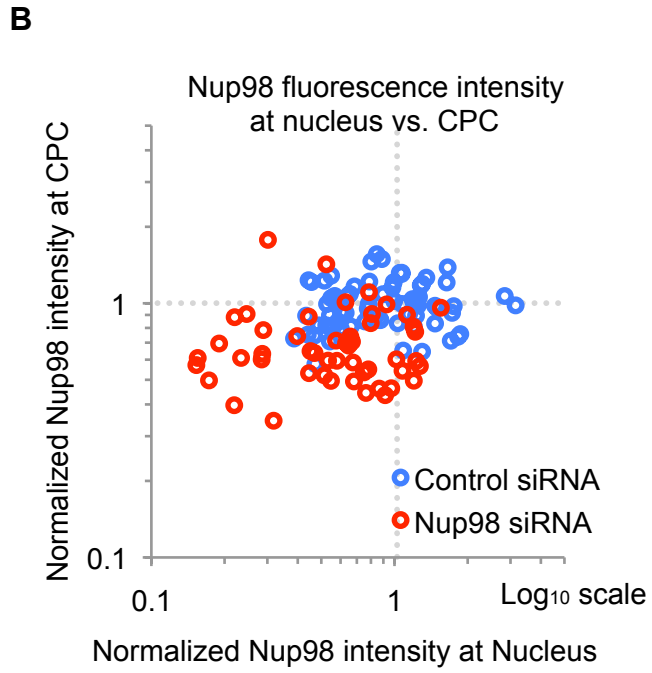
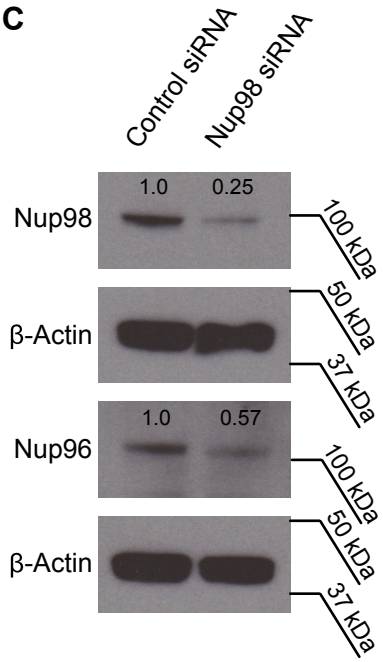
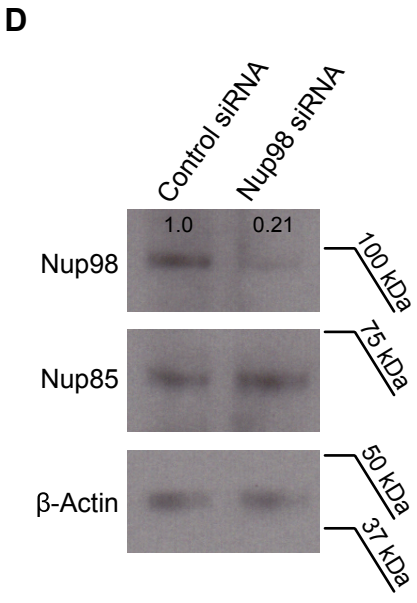

A
Cilium
5HT6-CFP-FRB (trap)
Plasma Membrane
Ciliary Pocket
Basal Body
YFP-FKBP-PKIM (prey)
Cytoplasm
+ Rapamycin


B
Nup98 fluorescence intensity
at nucleus vs. CPC
Normalized Nup98 intensity at CPC
1
0.1
0.1
1
Log10 scale
Control siRNA
Nup98 siRNA
Normalized Nup98 intensity at Nucleus


C
Control siRNA
Nup98 siRNA
Nup98
1.0
0.25
100 kDa
β-Actin
50 kDa
37 kDa
Nup96
1.0
0.57
100 kDa
β-Actin
50 kDa
37 kDa


D
Control siRNA
Nup98 siRNA
Nup98
1.0
0.21
100 kDa
Nup85
75 kDa
β-Actin
50 kDa
37 kDa

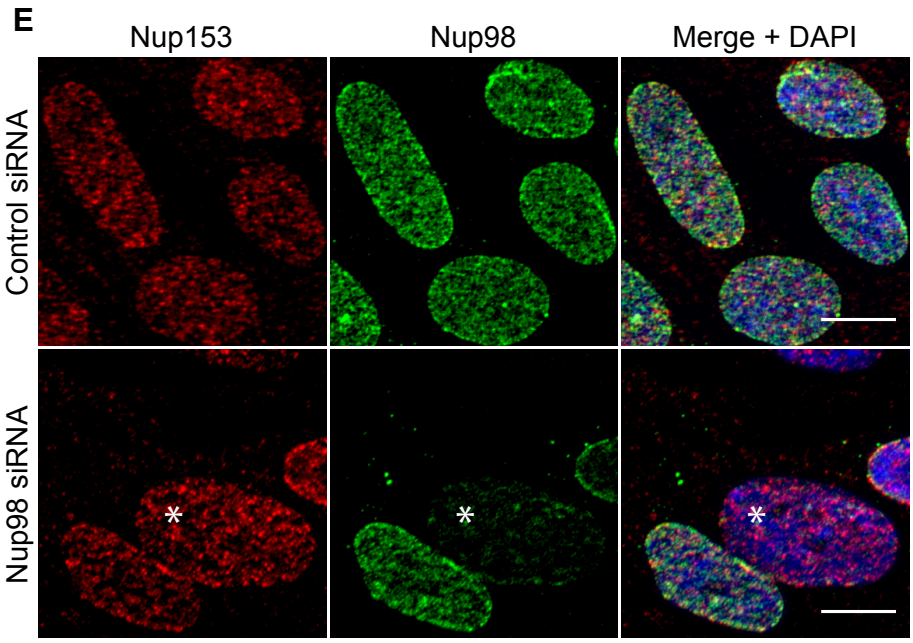

E
Nup153
Nup98
Merge + DAPI
Control siRNA
Nup98 siRNA

Figure S1: The knockdown of Nup98 does not cause major architectural changes to the nucleus – see also Figures 1 & 2 (A) Schematic of how rapamycin induces the dimerization of the 5HT6-CFP-FRB diffusion trap and the YFP-FKBP prey construct. (B) The fluorescence intensities of Nup98 at the nucleus vs. the CPC are plotted against each other for cells treated with control or Nup98 siRNAs to show that Nup98 siRNA treatment decreases Nup98 intensity at both the nucleus and the base of the cilium. (C) The knockdown of Nup96 is much less than the knockdown of Nup98, most likely due to the much longer half-life of Nup96. (D) The protein levels of Nup85 do not decrease after Nup98 knockdown. (E) Qualitative assessment of cells stained with both Nup153 and Nup98 antibodies shows that Nup98 knockdown (cell indicated by *) does not cause a reduction in the pore density at the nucleus.
Scale bars = 10 μm.

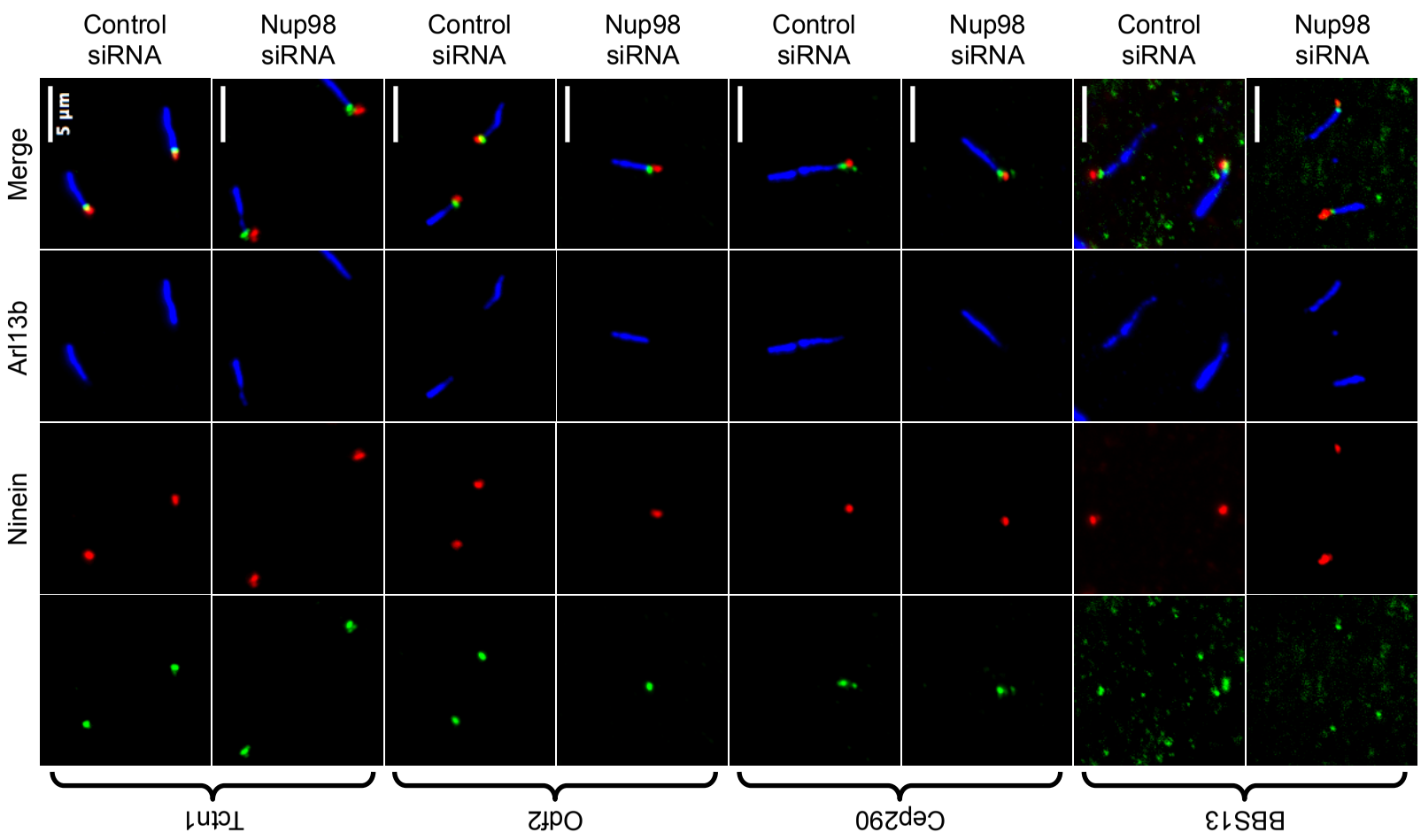

Control siRNA
Nup98 siRNA
Control siRNA
Nup98 siRNA
Control siRNA
Nup98 siRNA
Control siRNA
Nup98 siRNA
5 µm
Merge
Arl13b
Ninein
Tctn1
Odf2
Cep290
BBS13

Figure S2: Nup98 knockdown does not cause a loss of transition zone markers – see also Figure 2. Transition zone markers Tctn1 or BBS13 and basal body components Odf2 and Cep290 localize normally in cells depleted of Nup98. Scale bars = 5 μm.

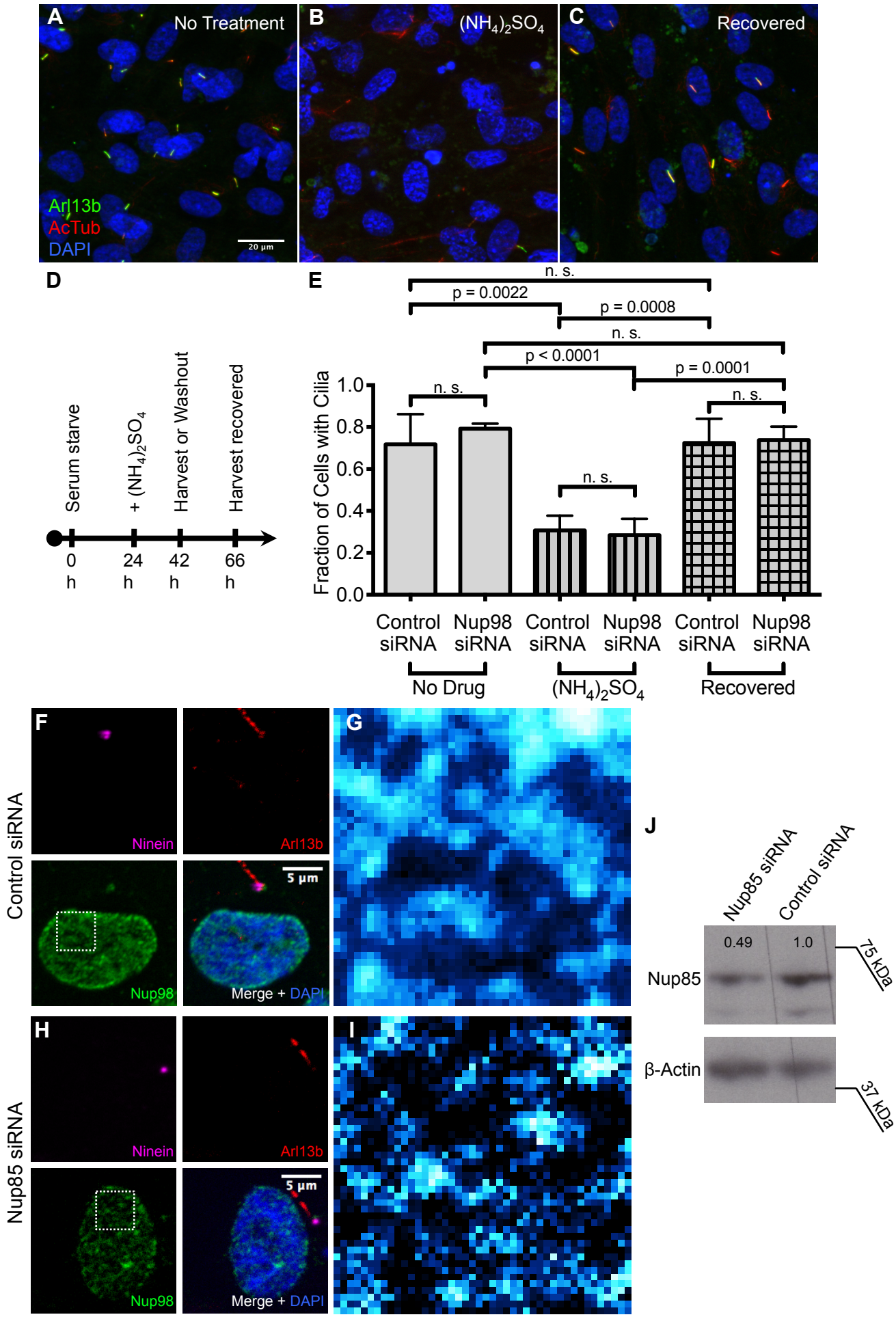

A
No Treatment
B
$(NH_4)_2SO_4$
C
Recovered
Arl13b
AcTub
DAPI
20 μm
D
Serum starve
+ $(NH_4)_2SO_4$
Harvest or Washout
Harvest recovered
0 h
24 h
42 h
66 h
E
n. s.
p = 0.0022
p = 0.0008
n. s.
p < 0.0001
p = 0.0001
n. s.
n. s.
n. s.
Fraction of Cells with Cilia
1.0
0.8
0.6
0.4
0.2
0.0
Control siRNA
Nup98 siRNA
Control siRNA
Nup98 siRNA
Control siRNA
Nup98 siRNA
No Drug
$(NH_4)_2SO_4$
Recovered
F
Control siRNA
Ninein
Arl13b
Nup98
Merge + DAPI
5 μm
G
H
Nup85 siRNA
Ninein
Arl13b
Nup98
Merge + DAPI
5 μm
I
J
Nup85 siRNA
Control siRNA
0.49
1.0
Nup85
75 kDa
β-Actin
37 kDa

Figure S3: Nup98 is not required for cilia biogenesis after deciliation – see also Figure 4. Deciliation by $(NH_4)_2SO_4$ shock effectively removes cilia as counted by Arl13b and Acetylated Tubulin staining. (A) before treatment (B after deciliation (C) after 24 hour recovery. (D) timeline laying out the experimental procedure. (E) Quantification of cilia numbers before deciliation, after deciliation, and after recovery in cells treated with a control or Nup98 siRNA. Knockdown of Nup98 causes no change in cilia numbers at any step. (F-I) Nup85 siRNA treatment decreases Nup98 localization to the nuclear envelope. (J) Western blot and quantification of Nup85 knockdown after 7 days of Nup85 siRNA treatment.
Error bars are S.E.M. p values were calculated by unpaired t test. Western blots were quantified in ImageJ.

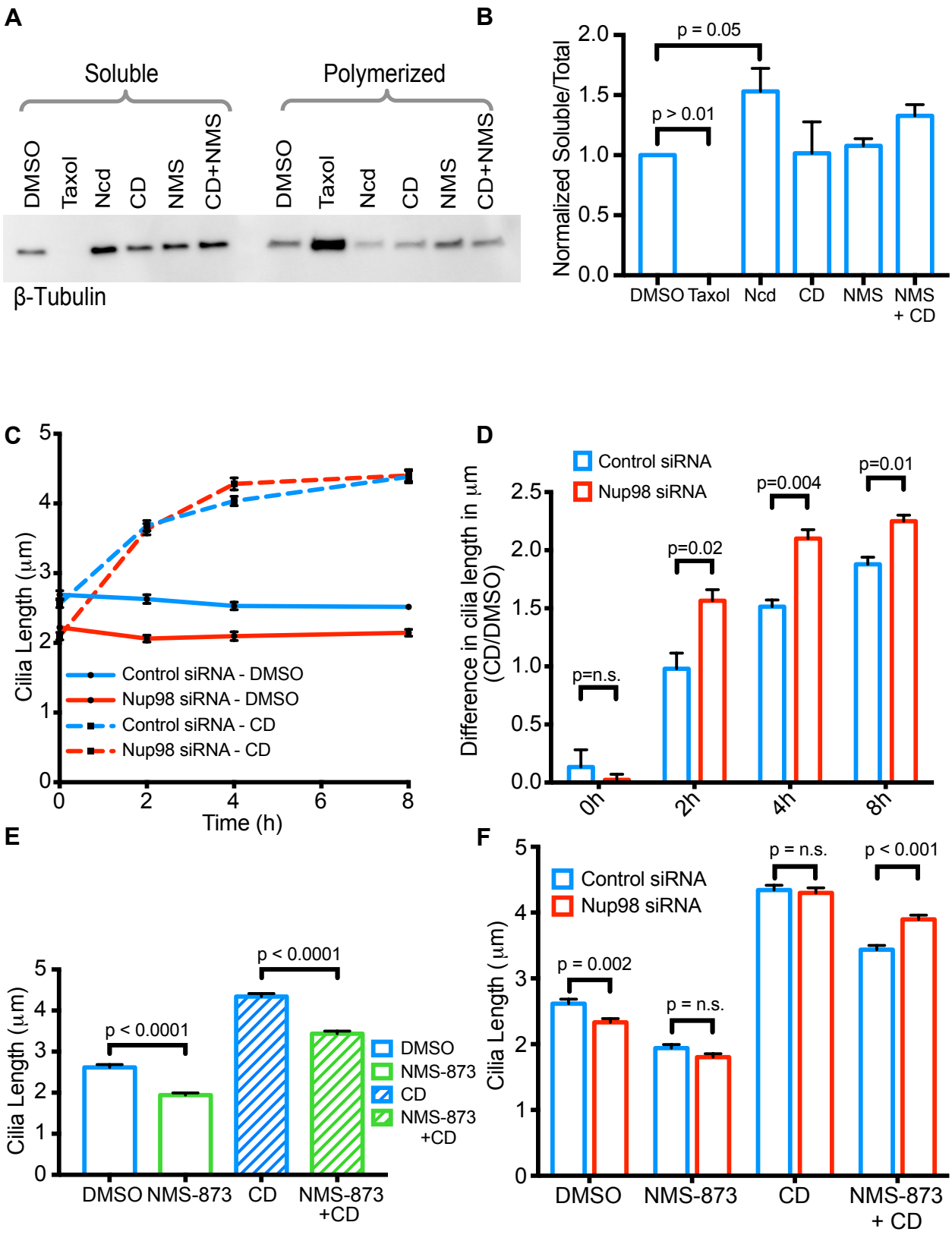

A
Soluble
Polymerized
DMSO
Taxol
Ncd
CD
NMS
CD+NMS
β-Tubulin
B
Normalized Soluble/Total
p = 0.05
p > 0.01
DMSO Taxol Ncd CD NMS NMS + CD
C
Cilia Length (μm)
Time (h)
Control siRNA - DMSO
Nup98 siRNA - DMSO
Control siRNA - CD
Nup98 siRNA - CD
D
Difference in cilia length in μm (CD/DMSO)
Control siRNA
Nup98 siRNA
p=n.s.
p=0.02
p=0.004
p=0.01
0h
2h
4h
8h
E
Cilia Length (μm)
p < 0.0001
p < 0.0001
DMSO
NMS-873
CD
NMS-873 +CD
F
Cilia Length (μm)
Control siRNA
Nup98 siRNA
p = 0.002
p = n.s.
p = n.s.
p < 0.001
DMSO NMS-873 CD NMS-873 + CD

Figure S4: cytochalasin D treatment extends cilia length – see also Figure 3. (A) Representative western blot of soluble and polymerized fractions of tubulin in cells treated for 4h with the drugs used in this study. (B) quantification of soluble/polymerized tubulin after drug treatments. (C, D) The cilia-lengthening effect of CD treatment is increased upon knockdown of Nup98, consistent with increased diffusion through the base of the cilium, when Nup98 levels are decreased. (E) Treatment of cells with NMS-873, which has been shown to destabilize anterograde IFT particles, reduces cilia length in control RPE cells, and lessens the cilia lengthening effects of CD. (F) When Nup98 is knocked down by siRNA and cells are treated with CD, partial inhibition of IFT with NMS-873 is significantly less effective at reducing cilia length when compared to control cells, also consistent with diffusion, rather than IFT, being the primary mode of macromolecular entry into the ciliary matrix, when Nup98 is reduced.
Error bars are S.E.M. p values were calculated by unpaired t-test.