Supplementary information

Cellular and Molecular Life Sciences

Requirement of NPHP5 in the hierarchical assembly of basal feet associated with basal bodies of primary cilia

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A.

No serum

Cell line	% of Ki67 -ve cells	% of Ki67 +ve cells	% of cells with 1 CP110 dot	% of cells with 2/4 CP110 dots	% of non-ciliated cells	% of ciliated cells
RPE-1	78 ± 4	22 ± 4	77 ± 3	23 ± 3	27 ± 2	73 ± 2
ARPE-19	80 ± 4	20 ± 4	78 ± 6	22 ± 6	27 ± 2	73 ± 2
HK-2	43 ± 2	57 ± 2	41 ± 1	59 ± 1	63 ± 4	37 ± 4
HeLa	36 ± 5	64 ± 5	31 ± 2	69 ± 2	69 ± 3	31 ± 3
U2OS	0 ± 0	100 ± 0	4 ± 1	96 ± 1	100 ± 0	0 ± 0
PC-3	6 ± 2	94 ± 2	6 ± 1	94 ± 1	99 ± 2	1 ± 2
MCF-7	7 ± 3	93 ± 3	10 ± 1	90 ± 1	95 ± 1	5 ± 1
DU-145	20 ± 1	80 ± 1	4 ± 1	96 ± 1	100 ± 0	0 ± 0
SAOS-2	18 ± 2	82 ± 2	4 ± 1	96 ± 1	100 ± 0	0 ± 0

B.

Serum

Cell line	% of Ki67 -ve cells	% of Ki67 +ve cells	% of cells with 1 CP110 dot	% of cells with 2/4 CP110 dots	% of non-ciliated cells	% of ciliated cells
RPE-1	27 ± 3	73 ± 3	22 ± 3	78 ± 3	81 ± 1	19 ± 1
ARPE-19	27 ± 2	73 ± 2	23 ± 1	77 ± 1	81 ± 1	19 ± 1
HK-2	18 ± 2	82 ± 2	13 ± 1	87 ± 1	89 ± 1	11 ± 1
HeLa	9 ± 1	91 ± 1	8 ± 1	92 ± 1	94 ± 2	6 ± 2
U2OS	0 ± 0	100 ± 0	4 ± 2	96 ± 2	100 ± 0	0 ± 0
PC-3	0 ± 0	100 ± 0	2 ± 2	98 ± 2	100 ± 0	0 ± 0
MCF-7	3 ± 1	97 ± 1	4 ± 1	96 ± 1	99 ± 1	1 ± 1
DU-145	2 ± 1	98 ± 1	3 ± 1	97 ± 1	100 ± 0	0 ± 0
SAOS-2	2 ± 1	98 ± 1	4 ± 1	96 ± 1	100 ± 0	0 ± 0

Figure S1: Cell lines vary in their ability to enter quiescence, form basal bodies, and ciliate

RPE-1, ARPE-19, HK-2, HeLa, U2OS, PC-3, MCF-7, DU-145, and SAOS-2 cells grown in the absence or presence of serum were stained with antibodies against CP110, Ki67 or glutamylated tubulin (GT335). The percentage of Ki67 negative versus positive cells, the percentage of cells with 1 versus 2/4 CP110 dots, and the percentage of non-ciliated versus ciliated cells are presented. At least 100 cells for each condition were scored, and the mean and standard error of three independent experiments are presented.

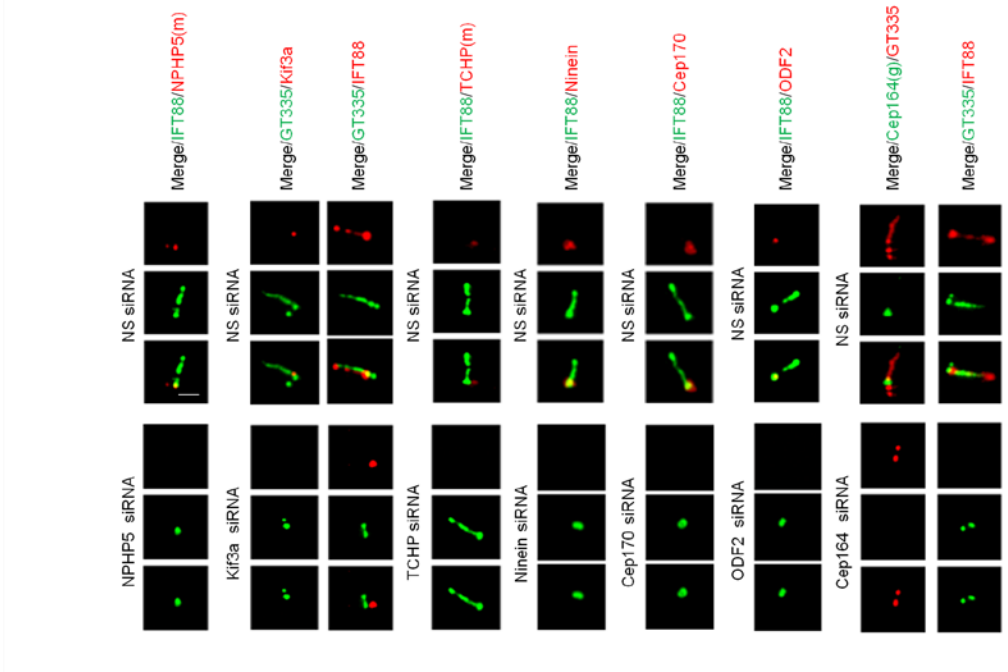

Figure S2: Ablation of BF components inhibits ciliogenesis without affecting recruitment of IFT88

Quiescent RPE-1 cells transfected with NS (non-specific) or the indicated siRNAs targeting SDA/BF components (NPHP5, Kif3a, TCHP, ninein, Cep170, ODF2) or a DA/TF component (Cep164) were stained with the indicated antibodies. Scale bar, 1 µm.

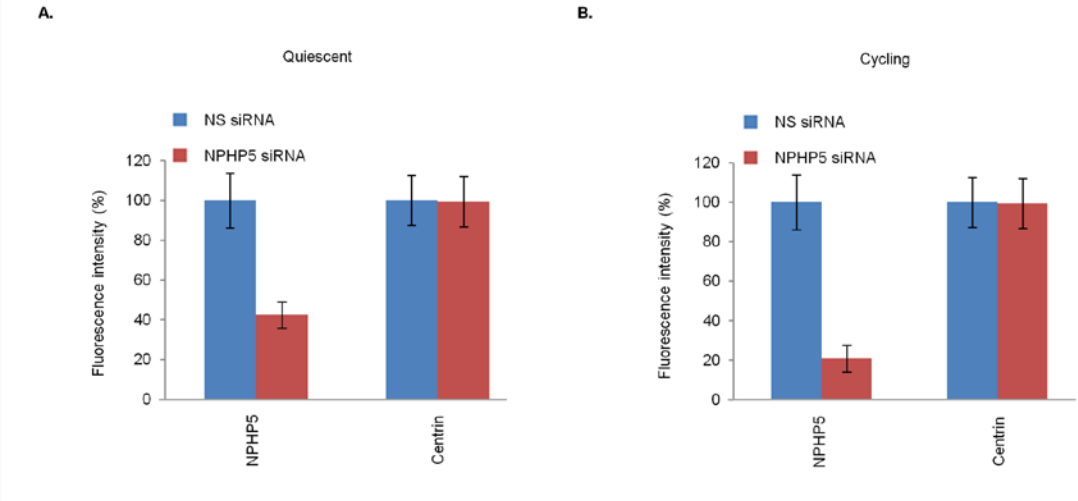

Figure S3: Knockdown efficiency of NPHP5 in cycling versus quiescent RPE-1 cells

A) Quiescent or **B)** cycling RPE-1 cells transfected with NS (non-specific) or NPHP5 siRNAs were stained with antibodies against NPHP5 and centrin. Fluorescence intensities of NPHP5 and centrin at the centrosome were quantitated and set to 100% in NS siRNA-transfected cells. For quantitation, at least 20 cells for each condition were analyzed, and the mean and standard error of three independent experiments are presented.

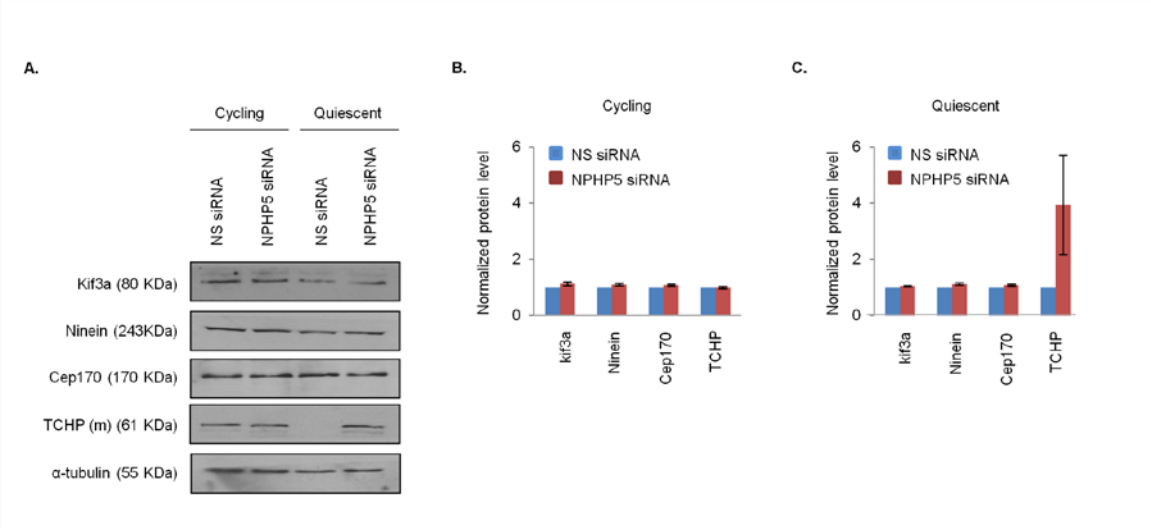

Figure S4: NPHP5 destabilizes TCHP in quiescent RPE-1 cells

A) Lysates from cycling or quiescent RPE-1 cells transfected with NS (non-specific) or NPHP5 siRNAs were analyzed by immunoblotting with the indicated antibodies. α-tubulin was used as a loading control. **B)** Normalized protein levels of Kif3a, ninein, Cep170 and TCHP in cycling RPE-1 cells based on three independent experiments. **C)** Normalized protein levels of Kif3a, ninein, Cep170 and TCHP in quiescent RPE-1 cells based on three independent experiments.

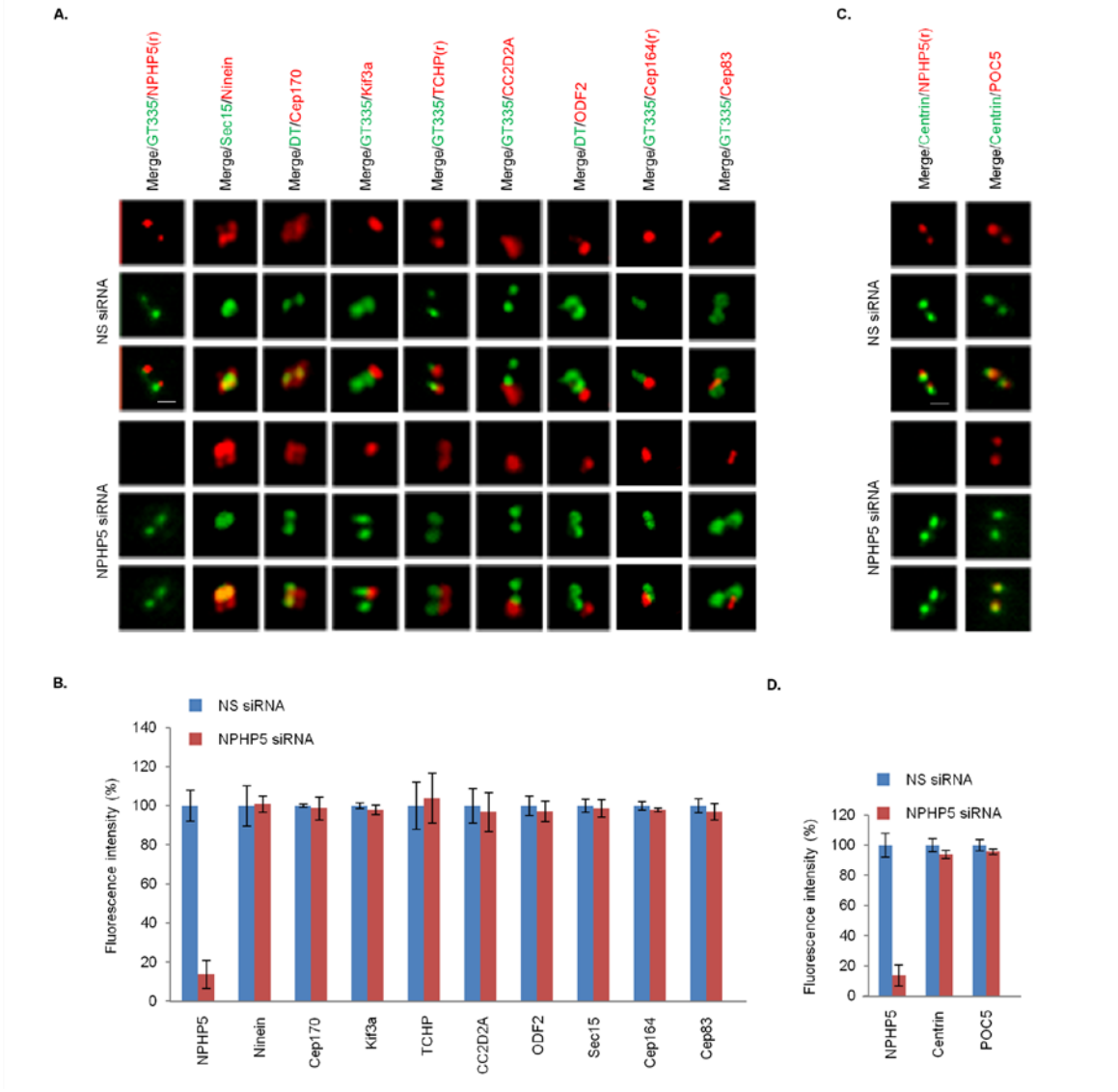

Figure S5: NPHP5 does not affect SDA assembly in cycling RPE-1 cells

A, C) Cycling RPE-1 cells transfected with NS (non-specific) or NPHP5 siRNAs were stained with the indicated antibodies. Scale bar, 1 µm. **B, D)** Fluorescence intensities of various proteins at the centrosome were quantitated and set to 100% in NS siRNA-transfected cells. For quantitation, at least 20 cells for each condition were analyzed, and the mean and standard error of three independent experiments are presented.

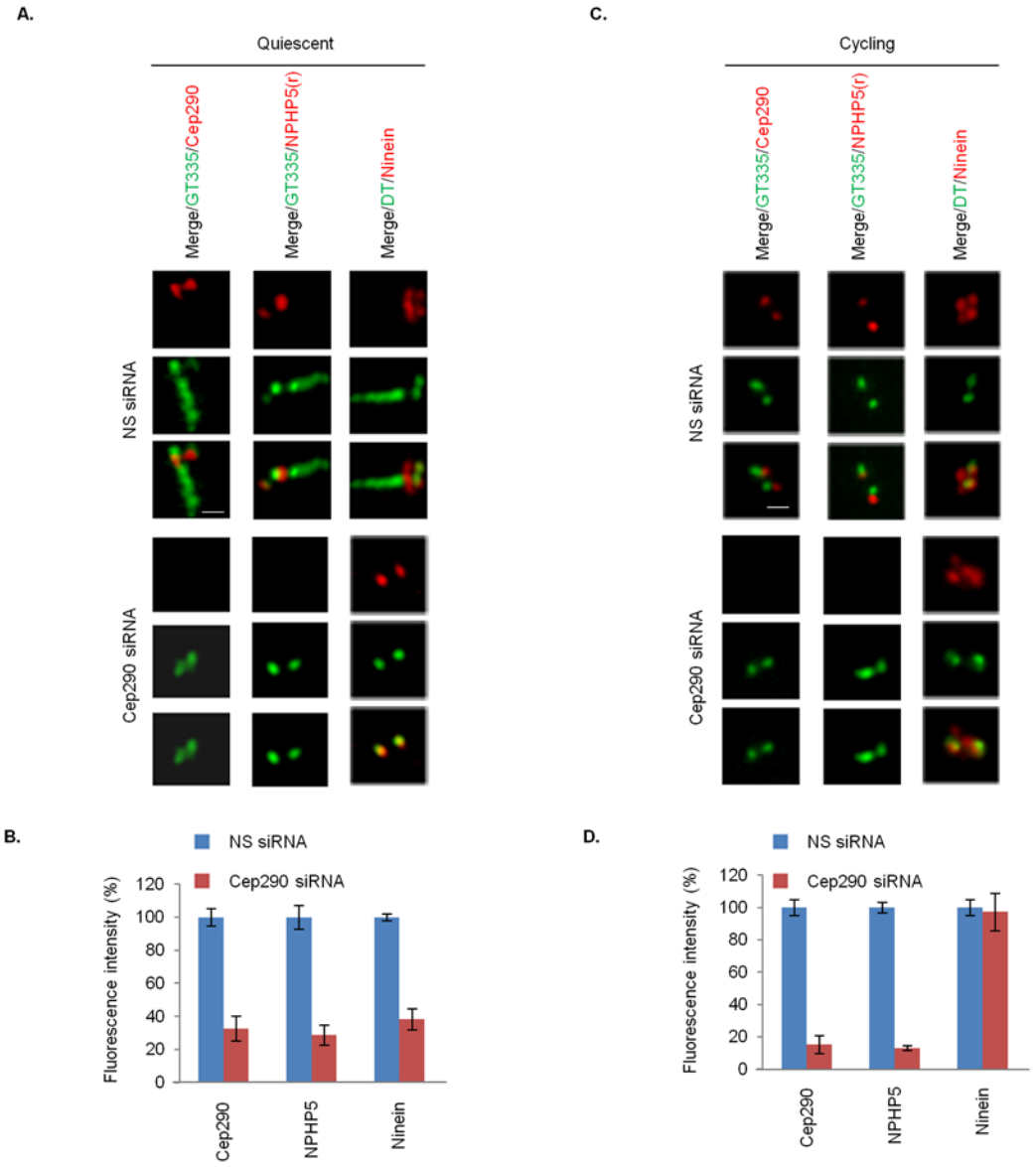

Figure S6: Cep290 specifically recruits ninein to BF but not SDAs

A) Quiescent or **C)** cycling RPE-1 cells transfected with NS (non-specific) or Cep290 siRNAs were stained with the indicated antibodies. Scale bar, 1 µm. **B, D)** Fluorescence intensities of various proteins at the centrosome were quantitated and set to 100% in NS siRNA-transfected cells. For quantitation, at least 20 cells for each condition were analyzed, and the mean and standard error of three independent experiments are presented.

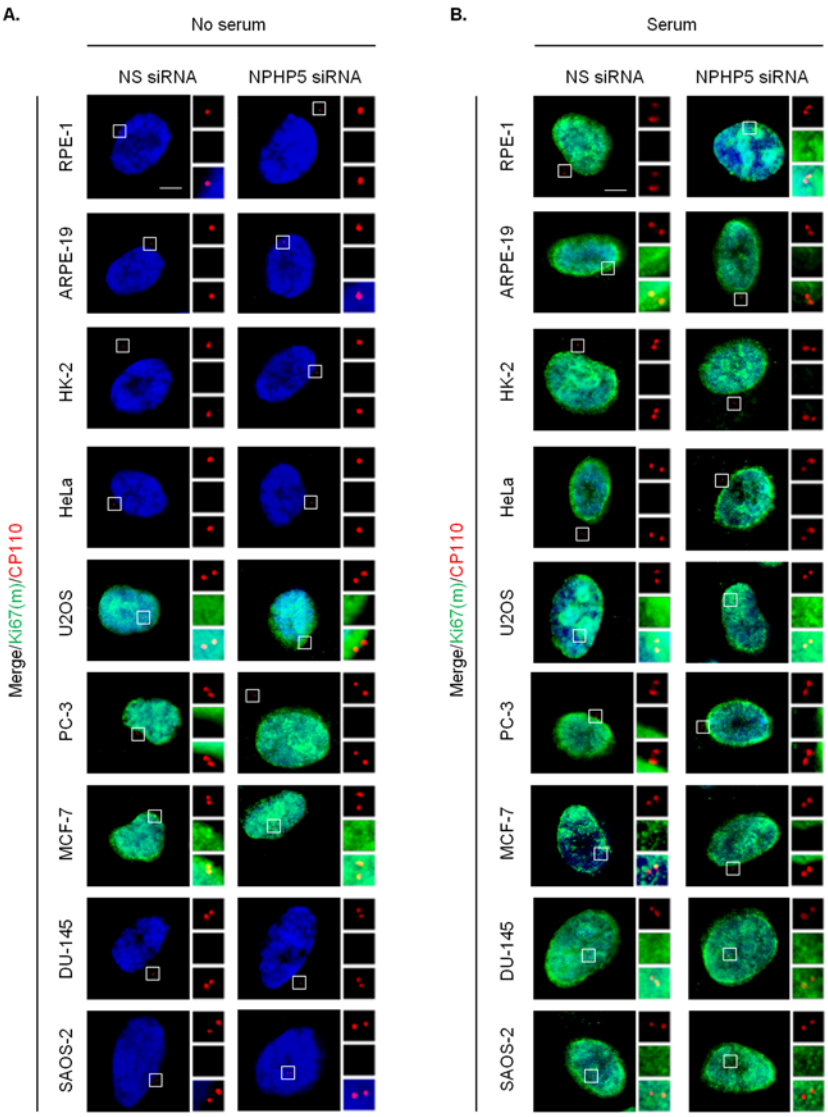

Figure S7: Ablation of NPHP5 does not affect entry into quiescence or basal body formation in RPE-1, ARPE-19, HK-2, and HeLa cells

RPE-1, ARPE-19, HK-2, HeLa, U2OS, PC-3, MCF-7, DU-145, and SAOS-2 cells transfected with NS (non-specific) or NPHP5 siRNAs and grown in the absence or presence of serum were stained with antibodies against CP110 and Ki67, and with DAPI (blue). Scale bar, 2 µm.

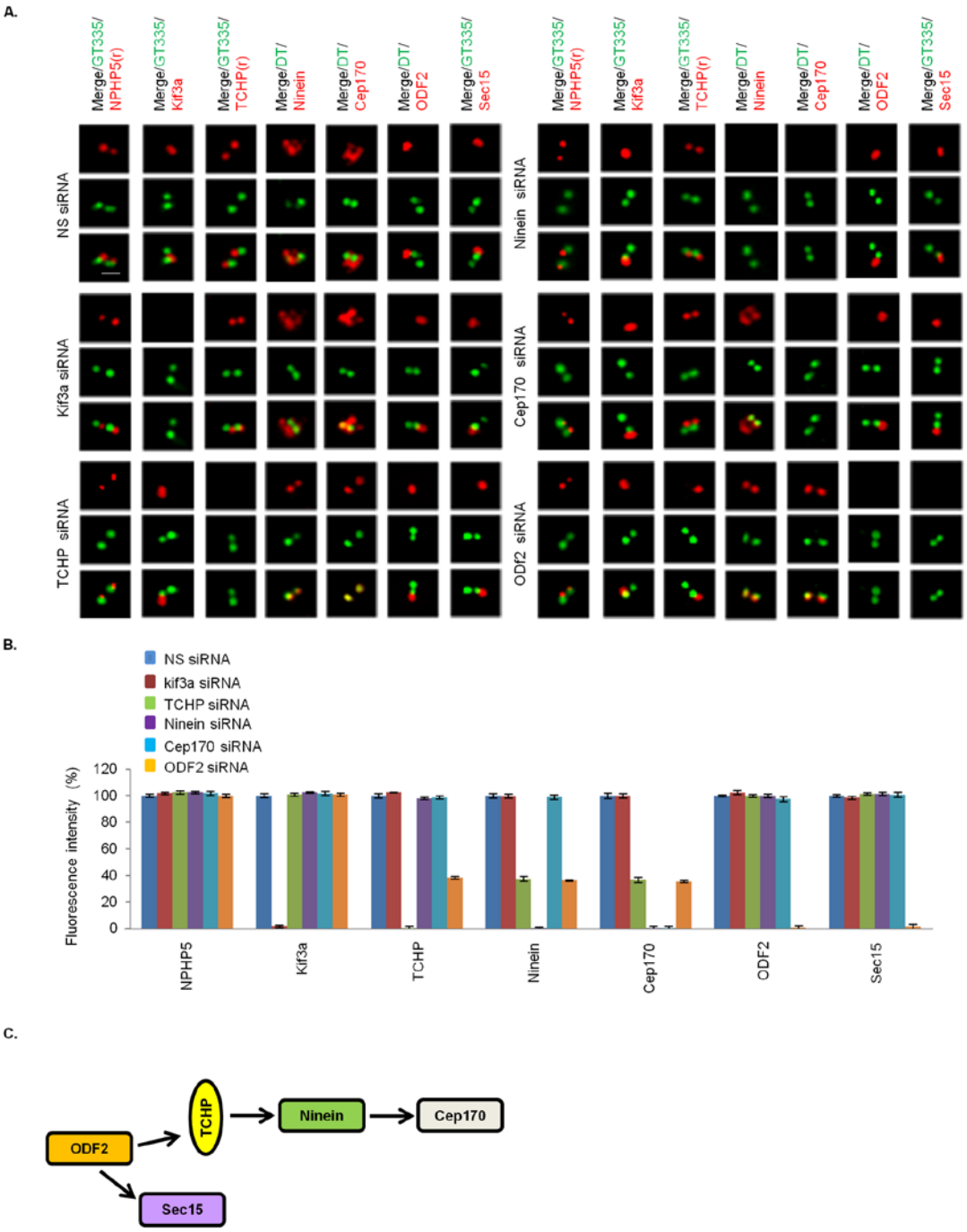
A.
Merge/GT335/ NPHP5(r)
Merge/GT335/ Kif3a
Merge/GT335/ TCHP(r)
Merge/DT/ Ninein
Merge/DT/ Cep170
Merge/DT/ ODF2
Merge/GT335/ Sec15
NS siRNA
Kif3a siRNA
TCHP siRNA
Ninein siRNA
Cep170 siRNA
ODf2 siRNA
B.
NS siRNA
kif3a siRNA
TCHP siRNA
Ninein siRNA
Cep170 siRNA
ODF2 siRNA
Fluorescence intensity (%)
120
100
80
60
40
20
0
NPHP5
Kif3a
TCHP
Ninein
Cep170
ODF2
Sec15
C.
ODF2
TCHP
Ninein
Cep170
Sec15

Figure S8: Hierarchical assembly of SDAs

A) Cycling RPE-1 cells transfected with NS (non-specific) or the indicated siRNAs targeting SDA components (Kif3a, TCHP, ninein, Cep170, ODF2) were stained with the indicated antibodies. Scale bar, 1 μm. **B)** Fluorescence intensities of various proteins at the centrosome were quantitated and set to 100% in NS siRNA-transfected cells. For quantitation, at least 20 cells for each condition were analyzed, and the mean and standard error of three independent experiments are presented. **C)** Schematic model of SDAs assembly.