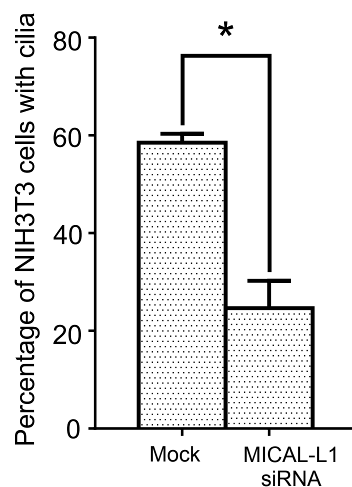
Supplemental Fig. 1

A



B

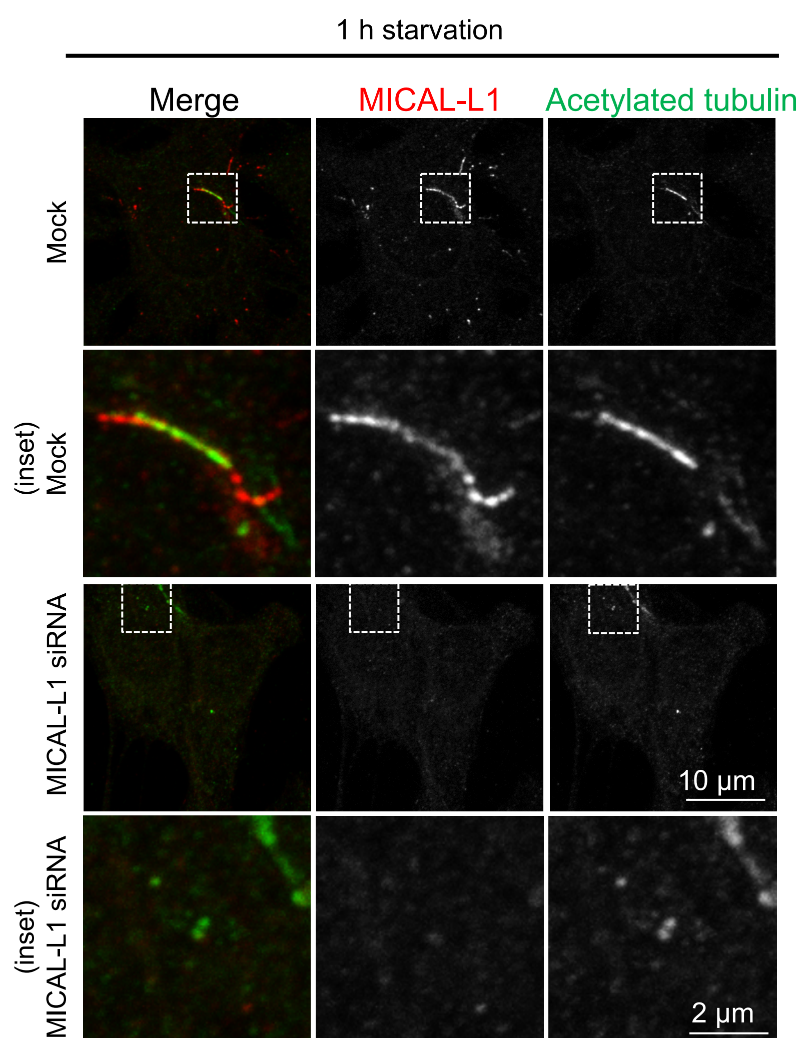


Fig S1. MICAL-L1 knock-down impairs ciliogenesis in NIH3T3 cells and its immunostaining at the basal body and cilia is specific.

(A) Mock-treated or MICAL-L1 siRNA-treated NIH3T3 cells were serum starved for 24 h. Once knock-down efficacy was ascertained by immunoblotting, the cells were then fixed and immunostained with an antibody that recognizes acetylated tubulin. The percentages of Mock-treated and MICAL-siRNA-treated cells that generated cilia upon serum-starvation were quantified and presented as a bar graph. Error bars denote standard deviation. * $p < 0.05$.

(B) Mock-treated or MICAL-L1 siRNA-treated cells were serum starved for 1 h to induce ciliogenesis, and then fixed and immunostained with antibodies to acetylated tubulin (green) and MICAL-L1 (red). Marker bars, 10 μm and 2 μm (insets). The absence of MICAL-L1 staining on the acetylated tubulin-marked basal bodies observed upon MICAL-L1-depletion highlights the specificity of the anti-MICAL-L1 antibody.

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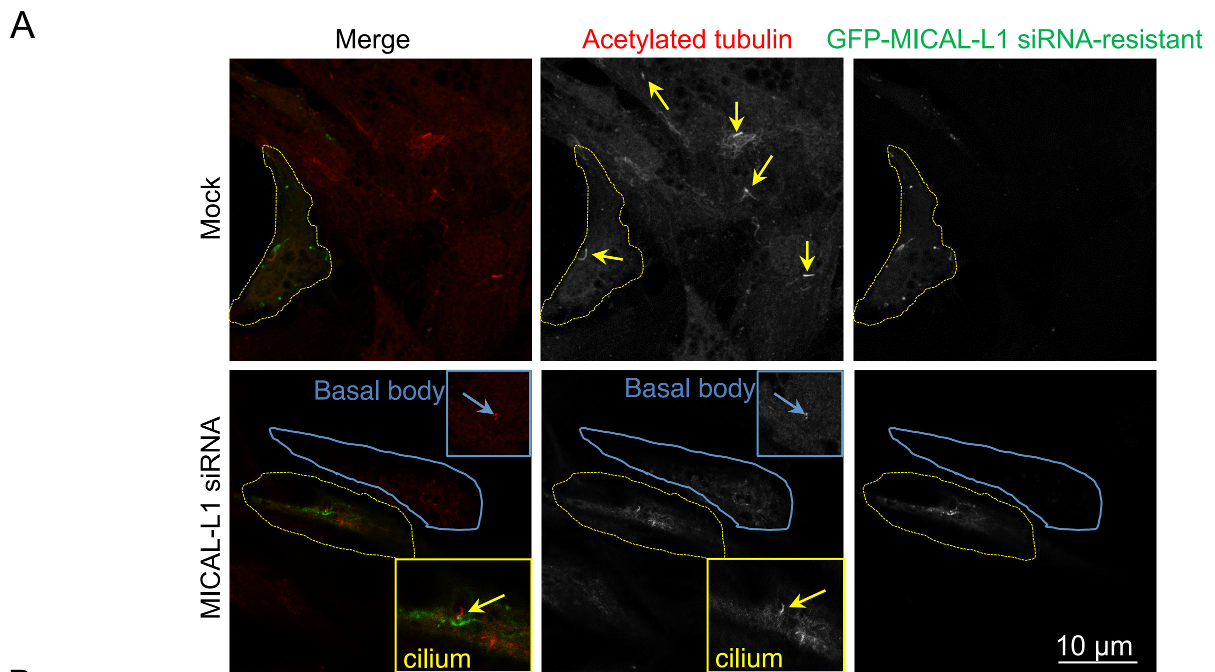
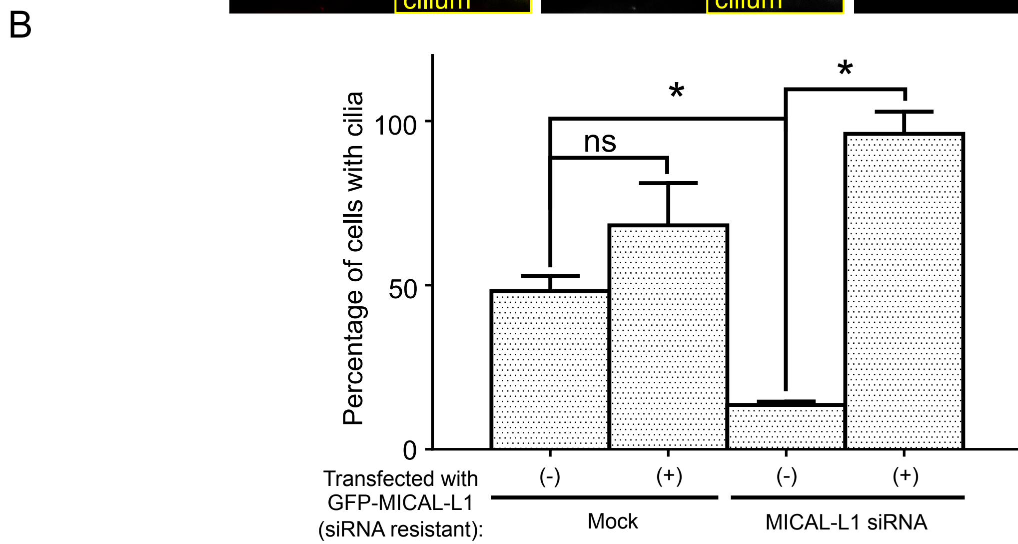
Supplemental Fig. 2





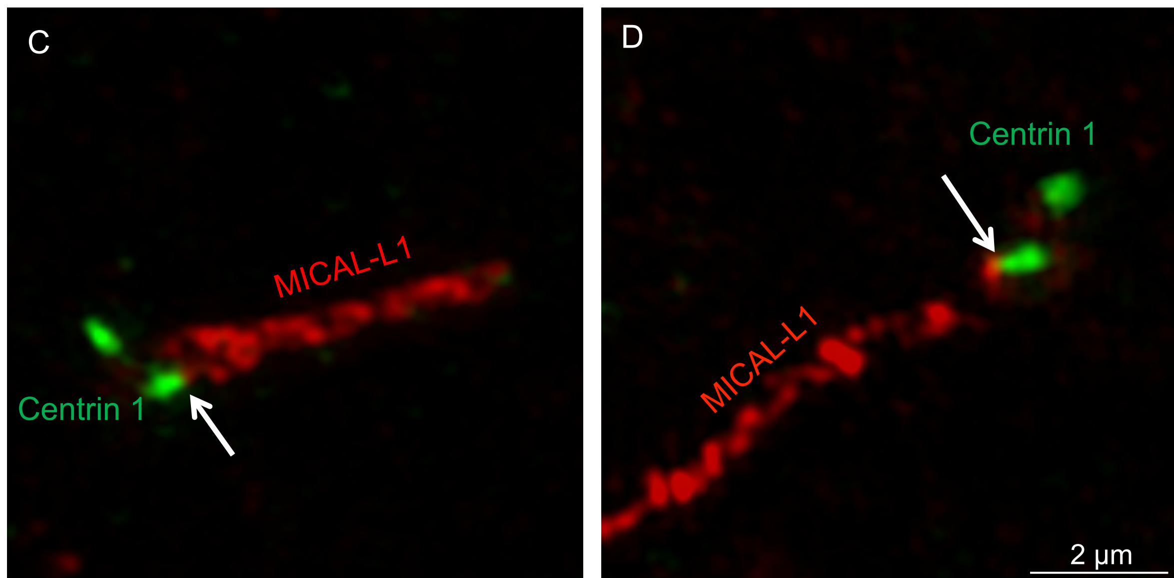


Fig. S2. Expression of a siRNA-resistant GFP-MICAL-L1 rescues the cilia defect observed upon MICAL-L1 knock-down, and MICAL-L1 localizes primarily to the distal side of the m-centriole.

(A) Mock-treated or MICAL-L1 siRNA-treated NIH3T3 cells were serum starved for 24 h. Both sets of cells were transfected with a wild-type GFP-MICAL-L1 plasmid engineered with subtle changes that do not affecting coding, but render the construct resistant to the siRNA. 24 h after transfection, the cells were fixed and immunostained with antibodies against acetylated tubulin, and analyzed by confocal microscopy. Dashed yellow outlines depict cells transfected with the GFP-MICAL-L1 siRNA-resistant plasmid. Multiple yellow arrows mark cilia in the Mock-treated cells, and the single yellow arrow in the yellow inset for the MICAL-L1 siRNA rescue cell denotes the generation of a cilium in a transfected cell. The blue dashed outlines mark a MICAL-L1 siRNA-treated cell lacking a primary cilium. The blue insets depict the basal body from that cell (blue arrow), but a different z-section is portrayed than the main image where the cilium is in focus. After initially demonstrating efficacy of MICAL-L1 KD by immuoblot analysis and showing the dramatic decrease in ciliation in the absence of MICAL-L1 but not with control siRNA (from 50% ciliation to <13%), subsequent experiments relied on decreased ciliation to verify KD.
Bar, 10 μm.

(B) Bar graph representing quantification of the data depicted in A. Data are derived from three independent experiments. Error bars denote standard deviation. * $p < 0.05$, ns, not significant.**(C-D)** RPE-1 cells were serum starved for 24 h, and then fixed and subjected to immunostaining with antibodies against MICAL-L1 (red) and Centrin 1 (green). Sr-SIM imaging was used to capture serial z-sections, and an orthogonal maximal projection is shown. Arrows mark areas of “contact” between MICAL-L1 and the Centrin 1 marker of the distal region of the m-centriole. Marker bar, 2 μm.

Supplemental Fig. 3

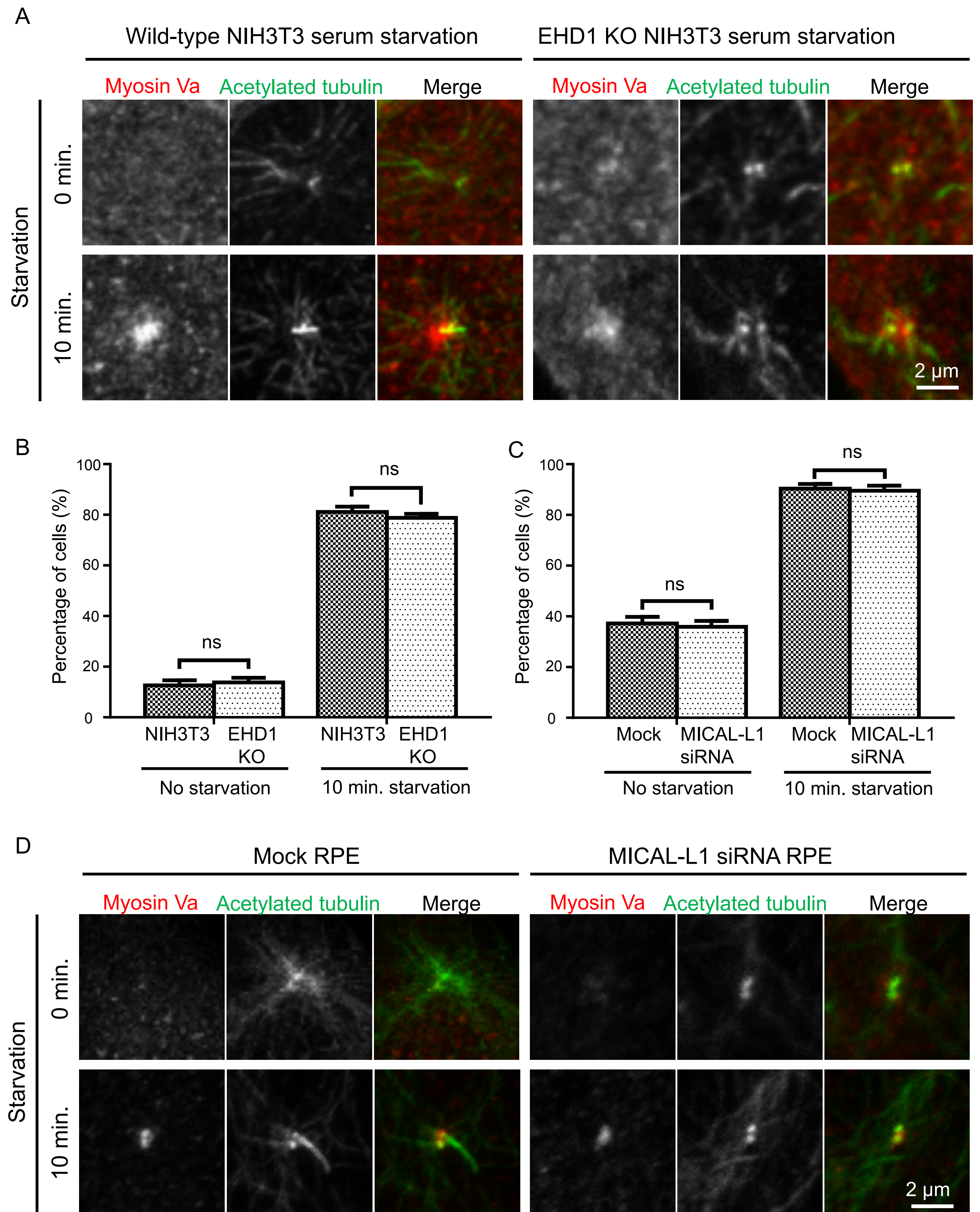


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Fig. S3. EHD1- and MICAL-L1-depletion do not impact Myosin Va-positive vesicles.
(A-B) Wild-type or CRISPR/Cas9 EHD1 knockout NIH3T3 cells were either fixed directly from culture or following 10 min. of serum starvation. Cells were then immunostained for Myosin Va (red) and acetylated tubulin (green). Myosin Va-positive vesicles were recruited to the basal body in the presence of absence of EHD1 (A). Bar graph shows the percentage of cells displaying Myosin Va recruitment to the basal body (B). $n = 3$ independent experiments, >100 cells per experiment were quantified. Error bars denote standard deviation. ns, not significant ($p > 0.05$). Bar, 2 µm.

(C-D) Mock- or MICAL-L1-depleted cells were either fixed directly from culture or following 10 min. of serum-starvation. Cells were then immunostained for Myosin Va (red) and acetylated tubulin (green). Bar graph shows the percentage of cells displaying Myosin Va recruitment to the basal body (C). Myosin Va-positive vesicles were recruited to the basal body in the presence of absence of MICAL-L1 (D). $n = 3$ independent experiments, >100 cells per experiment were quantified. Error bars denote standard deviation. ns, not significant ($p > 0.05$). Bar, 2 µm.

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Supplemental Fig. 4

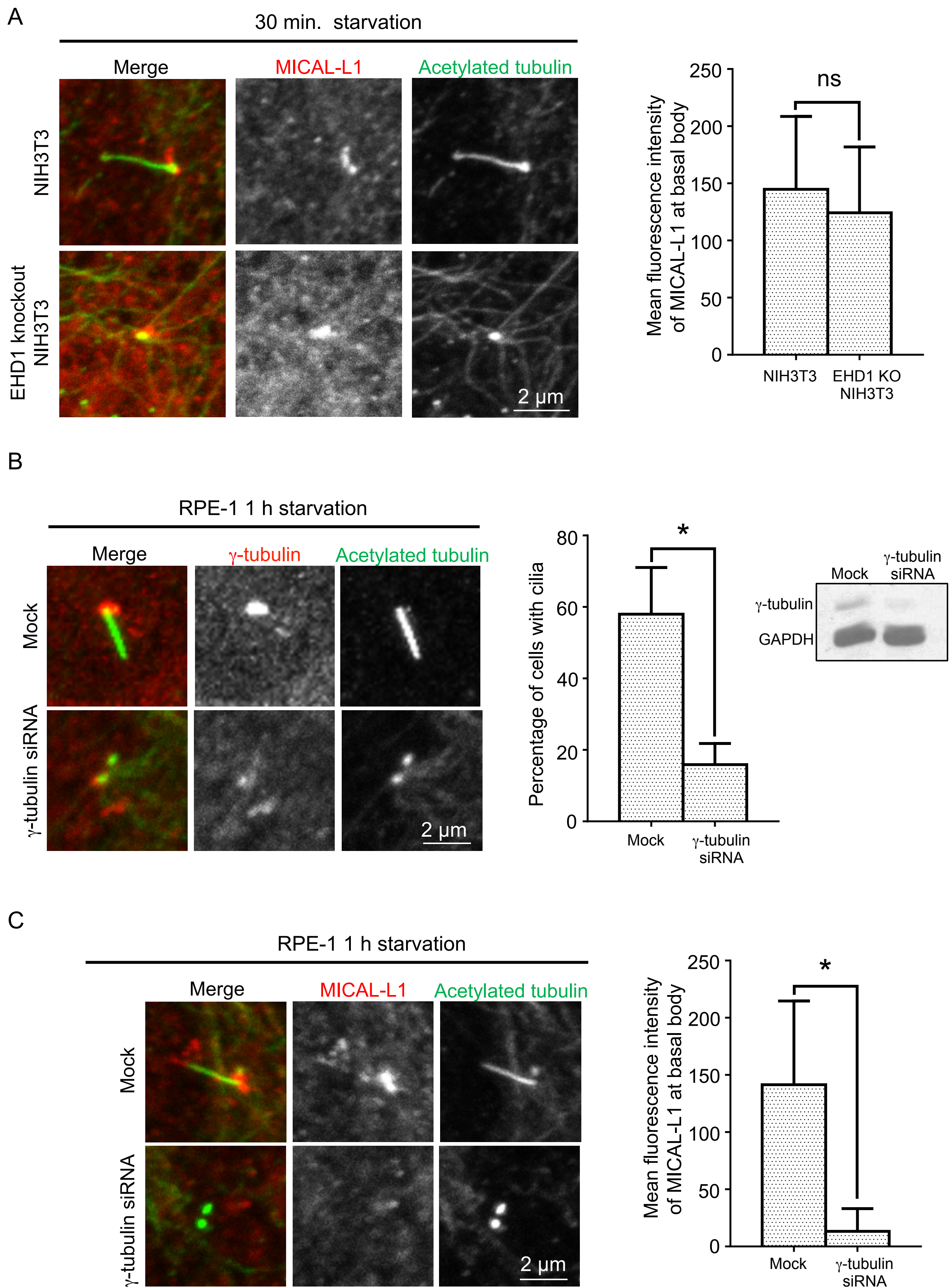

Fig. S4. γ-tubulin-depletion prevents recruitment of MICAL-L1 to the basal body.
(A) Wild-type or CRISPR/Cas9 EHD1 knockout NIH3T3 cells were serum starved for 30 min., and then subject to fixation and immuostaining with antibodies directed against MICAL-L1 (red) or acetylated tubulin (green), and imaged by confocal microscopy. The mean fluorescence intensity of MICAL-L1 at the basal body was measured by NIH Image J software, and plotted for each cell type. Error bars denote standard deviation from 3 independent experiments, and statistical significance was defined as $p<0.05$. Ns, not significant. Bar, 2 μm.

(B) RPE-1 cells were Mock-treated or treated with γ-tubulin siRNA for 48 h, and then serum starved for 1 h. Efficacy of γ-tubulin knock-down was validated by immunoblotting, and the cells were fixed and immunostained with antibodies directed at γ-tubulin (red) and acetylated tubulin (green). The percentage of Mock- and γ-tubulin knock-down cells displaying cilia was counted from 3 independent experiments. Error bars denote standard deviation. * $p < 0.05$.

(C) RPE-1 cells were Mock-treated or treated with γ-tubulin siRNA for 48 h, and then serum starved for 1 h. The cells were fixed and immunostained with antibodies directed at MICAL-L1 (red) and acetylated tubulin (green), and imaged by confocal microscopy. The mean fluorescence intensity of MICAL-L1 at the basal body was measured by NIH Image J software, and plotted for each cell type. Error bars denote standard deviation from 3 independent experiments, and statistical significance was defined as $p < 0.05$. Bar, 2 μm.

Table S1. Supplemental Antibody Information

Antibodies	Host	Manufacturer	Catalogue #	Application	Dilution
Acetylated tubulin	Mouse	Sigma	T7451	IF	1:100
Acetylated tubulin	Rabbit	Cell signaling	5335	IF	1:100
CP110	Rabbit	ProteinTech	12794-1-AP	IF	1:200
γ-tubulin	Mouse	Sigma	T5326	IF	1:200
γ-tubulin	Rabbit	Abcam	Ab11317	IF, IB, IP	1:100 (IF) 1:2000 (IB)
GST-HRP	Goat	Genscript	A01380	IB	1:1000
Myosin Va	Rabbit	Novus	NBP1-92156	IF	1:500
Pericentrin	Rabbit	Abcam	Ab4448	IF	1:1000
GAPDH-HRP	Mouse	ProteinTech	HRP60004	IB	1:5000
Actin	Mouse	Novus	NB600-535	IB	1:5000
GFP	Rabbit	Pierce	PA1-980-A	IP	
Mouse HRP light chain only	Goat	Jackson	115-035-174	IB	1:7000
Rabbit HRP	Donkey	GE Healthcare	NA934V	IB	1:5000
Mouse Alexa 488	Goat	Molecular Probe	A11029	IF	1:500
Rabbit Alexa 568	Goat	Molecular Probe	A11036	IF	1:500
Mouse Alexa 568	Rabbit	Molecular Probe	A11061	IF	1:500
Rabbit Alexa 488	Goat	Molecular Probe	A11034	IF	1:500
Rabbit Alexa 405	Goat	Thermo	A31556	IF	1:500
Syndapin 2	Rabbit	Sigma	SAB1101494	IB	1:1000