Supplementary Figure legends

Supplementary Figure S1 (related to Fig 1): hMsd1/SSX2IP is a component of the centrosome and centriolar satellites

(A) hMsd1 protein levels are constant during the cell cycle. HeLa cell extracts were prepared from asynchronous proliferating cells (AS) or cells treated with the DNA replication initiator mimosine (G1) or hydroxyurea (HU, G1/S), 6 h after release from HU block (G2/M) or Nocodazole (M) for 24 h. Immunoblotting was performed with antibodies against hMsd1, Plk1 (marker for cell cycle progression), and α-tubulin (loading control).

(B) hMsd1 localises to the centrosome and centriolar satellites during interphase in U2OS cells. U2OS cells were immunostained with antibodies against hMsd1 (green) and γ-tubulin (red, top), or hMsd1 (green) and PCM1 (red, bottom). DNA was stained with DAPI (blue).

(C) Ectopically produced hMsd1 localises to the centrosome and centriolar satellites. HeLa cells were transfected with hMsd1-EGFP, fixed and immunostained with antibodies against GFP (green) and γ-tubulin (red, top) or GFP (green) and PCM1 (red, bottom). DNA was stained with DAPI (blue). The three right-hand panels show magnifications of the boxed areas on the left. Yellow arrows mark the centrosome, whilst white arrowheads show centriolar satellites in which hMsd1-EGFP and PCM1 colocalise.

(D) Centriolar satellite localisation of hMsd1 requires microtubules. U2OS cells were treated with DMSO (top) or 20 μM Nocodazole (bottom) for 2 h, fixed and immunostained with antibodies against hMsd1 (green) and α-tubulin (red). DNA was stained with DAPI (blue). Arrows show the centrosome.

(E) Dynein function is essential for hMsd1 localisation to the pericentriolar region. HeLa cells were transfected with plasmids containing Myc-tagged p50-dynamitin (obtained from Andrew Fry) and 24 h after immunostained with antibodies against anti-Myc (green), hMsd1 (orange) and γ-tubulin (magenta, left) or anti-Myc (green), PCM1 (magenta) and γ-tubulin (orange, right). DNA was stained with DAPI (blue). Enlarged images corresponding to the centrosomal areas (squares on the far left panels) are shown on

the right. Squares labelled 1 show untransfected cells (Myc signal-negative), whilst squares labelled 2 show transfected cells (Myc signal-positive).

(F) Pericentriolar localisation of hMsd1 is dependent on PCM1. HeLa cells stably expressing centrin-GFP (centriole marker, obtained from Michel Bornens, Institut Curie) were transfected with control (top) or PCM1-targeting siRNAs (bottom) for 48 h, fixed and immunostained with antibodies against hMsd1 (red). Centrosomal areas (centrin-GFP, green) are marked with arrows.

(G) Interaction between hMsd1 and γ-tubulin or PCM1 upon microtubule depolymerisation. HeLa cells were transfected with plasmids expressing GFP-hMsd1 or GFP-PCM1 (obtained from Andreas Merdes, CNRS/Université de Toulouse) for 24 h, and treated with Nocodazole for an additional 2 h. Immunoprecipitation was performed with a GFP-antibody, followed by immunoblotting with antibodies against GFP, γ-tubulin and hMsd1 (left) or PCM1 (right).

(H) Interaction between hMsd1 and γ-tubulin or PCM1 during the cell cycle. Cell-cycle specific extracts were prepared from HeLa cells (transfected with hMsd1-GFP) as in Fig S1A. Immunoprecipitation was performed with an anti-GFP antibody, followed by immunoblotting with antibodies against PCM1, γ-tubulin, Plk1 (marker for cell cycle progression) and GFP. Note that hMsd1 binds to γ-tubulin during the whole cell cycle, whilst binding to PCM1 is not detected during mitosis. hMsd1 appears to interact with Plk1 in a mitosis specific manner, the mechanism of which remains to be addressed. The positions of molecular weight markers (kDa) are shown on the right (A,G.H). Asterisk denotes non-specific bands (A). Scale bars, 5 μm (B,C, left, D-F), 1 μm (C, the three panels from the right, E, the two panels from the right).

Supplementary Figure S2 (related to Figure 2): hMsd1/SSX2IP is essential for the anchoring of interphase microtubules independent of microtubule nucleation and Ninein recruitment

(A) γ-tubulin and Pericentrin are retained in hMsd1-depleted cells. Control (top) or hMsd1 siRNA treated U2OS cells (bottom) were immunostained 48 h after transfection with antibodies against hMsd1 (red) and γ-tubulin (green, left) or hMsd1 (red) and Pericentrin (green, right). DNA was stained with DAPI (blue).

(B) Quantification of signal intensities of γ-tubulin, Pericentrin, NEDD1 and CDK5RAP2 at the centrosome. Ratio represents signal intensities of indicated proteins located to the centrosome relative to those at the cell periphery. softWoRx software was used for quantification. Error bars represent SD (n=3, >200 cells).

(C) Microtubule anchoring, but not nucleation, is defective in PCM1- or Ninein-depleted cells. U2OS cells were transfected with indicated siRNA for 48 h, and incubated with 20 μM Nocodazole for an additional 2 h. After drug washout, individual cells were fixed at 0, 5 and 30 min time points, and immunostained with an antibody against α-tubulin. Enlarged images corresponding to cells marked with arrows in the left-hand panels are shown on the right.

(D) The differential centrosomal localisation of hMsd1 and Ninein. U2OS cells were stained with antibodies against hMsd1 (red) and Ninein (green). DNA was stained with DAPI (blue). The centrosomal area (enlarged) is shown on the bottom.

(E) Ninein localises normally to the centrosome upon hMsd1 depletion. U2OS cells were transfected with control (top), hMsd1 (middle) or PCM1 targeted siRNAs (bottom) and immunostained with antibodies against Ninein (green) and α-tubulin (red). DNA was stained with DAPI (blue). Individual images were created by projections with image stacks using the DeltaVision microscope system (softWoRx 3.3.0; Applied Precision). PCM1 depletion led to a reduced level of Ninein at the centrosome, whilst hMsd1-depleted cells displayed Ninein signals even stronger than control cells, the reason for which is currently not explored. Scale bars, 5 μm (A,C,D, top,E), 1 μm (D, bottom).

Supplementary Figure S3 (related to Figure 3): Different domains within hMsd1/SSX2IP are responsible for the cellular localisation and interaction with γ-tubulin and PCM1

(A) Schematic of hMsd1 deletion constructs and summary of their localisation and Interaction with γ-tubulin and PCM1. Colocalisation was determined by analysing HeLa and U2OS cells transfected with each GFP-tagged hMsd1 deletion construct. Cen, centrosome; C.S., centriolar satellites.

(B) Interaction between GFP-tagged hMsd1 deletion constructs and γ-tubulin or PCM1. Coimmunoprecipitation was performed using cell extracts prepared from HeLa cells expressing individual GFP-tagged hMsd1 deletion constructs with an anti-GFP antibody. Immunoprecipitates were immunoblotted with antibodies against GFP, γ-tubulin and PCM1. The positions of molecular weight markers (kDa) are shown on the right.

Supplementary Figure S4 (related to Figure 4): hMsd1/SSX2IP depletion results in various spindle defects and misaligned chromosomes without affecting microtubule nucleation activities.

(A) A reconstruction of centrosomal hMsd1 and γ-tubulin localisation. Representative images obtained from mitotic HeLa cells transfected with hMsd1-EGFP are shown (rotated 45 degrees). Cells were immunostained with antibodies against γ-tubulin (blue) and α-tubulin (red). GFP signals are shown in green. Images of 80 sections (each 0.2 μm thick) were reconstructed using the Metamorph software (Molecular Devices, LLC). hMsd1 localises to the broad pericentriolar material in a non-regular pattern that encompasses γ-tubulin signals.

(B) Spindle microtubules of hMsd1-depleted cells are shorter. Left: HeLa cells were transfected with control and hMsd1 siRNA for 48 h, treated with MG132 (5 μM) for an additional 3 h, fixed and stained with antibodies against γ-tubulin (green) and α-tubulin (red). DNA was stained with DAPI (blue). Right: Quantification of spindle length.

(C) Quantification of various spindle defects upon hMsd1 depletion. HeLa cells were transfected with control or hMsd1 siRNA for 48 h, treated with MG132 (5 μM) for an additional 3 h, fixed and immunostained with antibodies against γ-tubulin and α-tubulin. DNA was stained with DAPI. Mitotic spindles and chromosomes were observed as in Fig 4A-C.

(D) Tilted spindle phenotypes are observed in hMsd1-depleted cells plated on fibronectin-coated coverslips. Cells were treated with control or hMsd1 siRNA and plated on coverslips that were coated (right) or not coated (left) with fibronectin. Spindle angles were measured as in Fig 4A-C.

(E) Reduced astral microtubules in hMsd1 depleted cells. Left: Control (top) and hMsd1 siRNA-treated mitotic HeLa cells (bottom) were stained with α-tubulin (red) and EB1 (green). DNA was stained with DAPI (blue). Areas where astral microtubules emanate towards the cell cortex (upper part) are enlarged in the two right-hand side panels. Right: Quantification of signal intensities of EB1.

(F) γ-tubulin and Pericentrin are retained in hMsd1-depleted mitotic cells. HeLa cells were transfected with control or hMsd1 siRNAs, fixed and immunostained with antibodies against hMsd1 (red) and γ-tubulin (green, top) or hMsd1 (red) and Pericentrin (green, bottom). DNA was stained with DAPI (blue).

(G, H) Microtubule depolymerisation and regrowth assay in mitotic cells. HeLa cells were transfected with control or hMsd1 siRNA for 48 h, followed by treatment with 20 μM Nocodazole for an additional 2 h. After drug washout, cells were fixed at 0, 5 and 30 min time points and immunostained with α-tubulin (red) and γ-tubulin (green). DNA was stained with DAPI (blue). Note that hMsd1-depleted cells displayed a characteristic morphology of tilted spindles with chromosome misalignment (30 min). An arrow marks astral microtubules, whilst circles show the centrosomal areas (G). Quantification of microtubule intensities around the centrosome (circles) 5 min after microtubule regrowth (H, left) and the percentage of cells containing spindle tilt 30 min later (right, spindles displaying angles larger than 20° were assigned as tilted spindles) are shown. Error bars

represent SD (C, n=3, >200 cells; E,H, n=2, >100 cells). The box-and-whisker plot indicates the minimum and maximum values, 25th and 75th percentiles, and the median (B, n=3, >200 cells, D, n=2, >200 cells). **p<0.001, ***p<0.0001, n.s. not significant (two-tailed unpaired student's t-test) in (C-E,H). Scale bars, 5 μm (A, top,B,E, left,F,G), 1 μm (A, bottom,E, the two panels from the right),

Supplementary Figure S5 (related to Figure 5): hMsd1/SSX2IP is essential for ciliogenesis and zebrafish Msd1 localises to the centrosome and the basal body.

(A) Centrioles are not overduplicated upon hMsd1 knockdown. hTERT-RPE-1 cells stably expressing centrin-GFP (obtained from Alexey Khodjakov, Wadsworth Center) were transfected with control (top) or hMsd1 siRNA (bottom), and 48 h later were fixed and immunostained with an antibody against hMsd1 (red). DNA was stained with DAPI (blue). Enlarged centrosomal areas (centrin-GFP, green) are shown in insets.

(B) Quantification of the number of centrin-GFP dots.

(C-F) Centrosome targeting C-terminal half of hMsd1 (hMsd1-C-PACT) rescues defects in microtubule disorganisation and faulty ciliogenesis in hMsd1-depleted hTERT-RPE-1 cells. hTERT-RPE-1 cells treated with hMsd1 siRNA were transfected with plasmids containing EGFP-tagged PACT or hMsd1-C-PACT. After 24 h, cells were immunostained with antibodies against α-tubulin (red) and detyrosinated tubulin (Glu-tubulin, magenta). DNA was stained with DAPI (blue). Enlarged images corresponding to the centrosomal/basal body region (squares, left) are shown in the far right panels (C). Quantification of the percentage of cells containing focussed microtubule asters (D) or cilia (E) or measurement of cilia length in each ciliated cells (F) is also shown.

(G) Localisation of zebrafish Msd1 in the early embryos (40% epibody). RNA encoding Msd1-GFP (green) and membrane-RFP (red) was coinjected into the wild-type embryo at the one-cell stage and subjected for staining with γ-tubulin (red) for the centrosome. The centrosomal area in a single cell (arrows) is shown enlarged in the bottom insets.

Due to low sensitivity, we could not judge whether Msd1-GFP localised to centriole satellites under this condition.

(H) Zebrafish Msd1 localises to the basal body in the Kupffer's vesicle. RNA encoding Msd1-GFP (green, middle) was injected into the wild-type embryos, and the embryo was fixed at the 8-somite stage for visualising the cilia (stained with anti-acetylated α-tubulin, red, right) in the Kupffer's vesicle. Bottom insets show enlarged images of a single cilium (arrows). Error bars represent SD (B, n=3, >200 cells, D-F, n=2, >100 cells). **p<0.001, ***p<0.0001 (two-tailed unpaired student's *t*-test). Scale bars, 10 μm (G,H), 5 μm (A,C), 1 μm (A, insets, C, right).

Supplementary Figure S6: Prolonged treatment of hMsd1/SSX2IP siRNA leads to other centrosomal defects including centrosome fragmentation

(A) Evaluation of siRNA-mediated hMsd1 depletion. Immunoblotting of protein extracts prepared from HeLa (left), U2OS (middle) or hTERT-PRE-1 cells (right) that were treated with individual siRNAs (control, hMsd1, No.1, or No.2) was performed with antibodies specific to hMsd1, γ-tubulin and α-tubulin. Note that No.1 and No.2 oligonucleotides were used to knock down hMsd1/SSX2IP by Barenz et al. (2013)[S][1]. The positions of molecular weight markers (kDa) are shown on the right.

(B) Quantification of the percentage of cells containing focussed microtubule asters. U2OS cells were treated with individual siRNAs and immunostained with an antibody against α-tubulin after 48 h or 96 h.

(C) Quantification of spindle angles. HeLa cells were treated with individual siRNAs and immunostained with antibodies against γ-tubulin and α-tubulin. Spindle angles were measured as in Fig 4C after 48 h or 96 h. Spindles displaying angles larger than 20° were assigned as spindle tilt.

(D) Quantification of the number of γ-tubulin foci. The number of γ-tubulin foci were counted using samples prepared in (C). Cells exhibiting more than two γ-tubulin foci

were assigned as those showing ectopic γ-tubulin foci. Error bars represent SD (B-D, n=2, >100 cells).