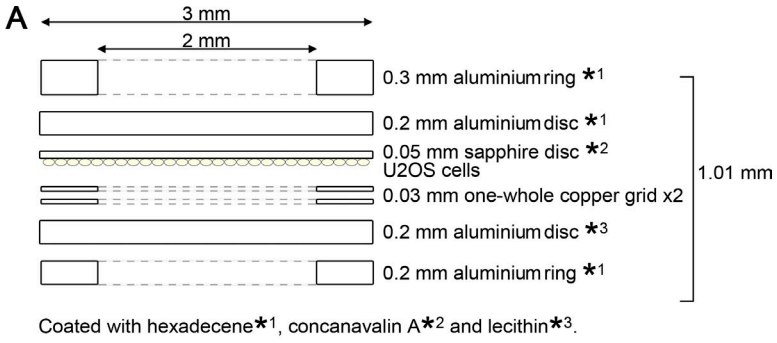
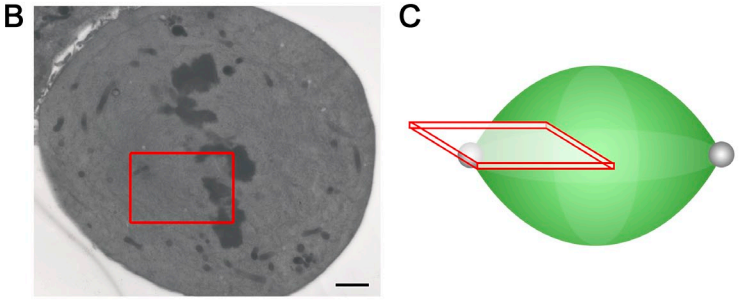
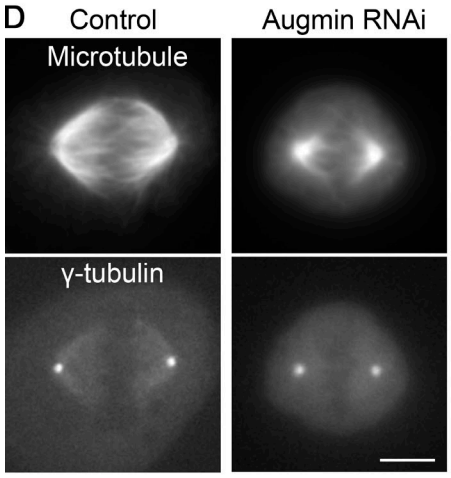
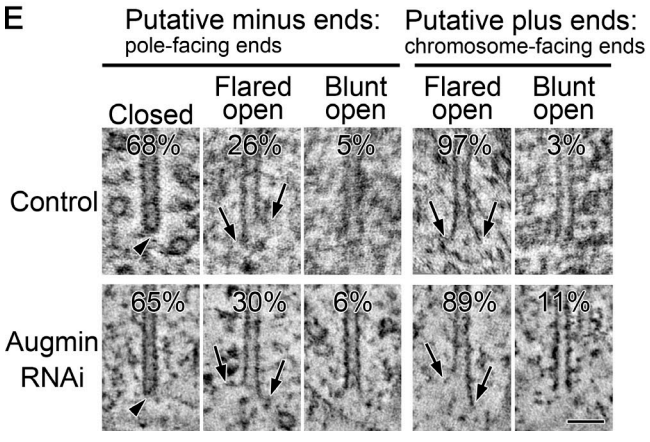
Kamasaki et al., http://www.jcb.org/cgi/content/full/jcb.201304031/DC1

Figure S1. **HPF followed by freeze substitution of human cells cultivated on a sapphire disc for electron tomography.** (A) A diagram of a specimen sandwich in the sample holder designed for this study. (B) Electron micrograph of a semithick section of a metaphase cell after HPF/freeze substitution. A red box indicates the region imaged by electron tomography. Bar, 2 µm. (C) Illustration of the final volume reconstructed from a metaphase spindle. (D) Confirmation of the augmin phenotype in U2OS cells. Note that the γ-tubulin signal inside the spindle is specifically reduced after RNAi. Bar, 10 µm. (E) MT end structures. Arrowheads point to closed ends, whereas arrows indicate flared protofilaments. Note that we observed almost no closed minus ends that faced the chromosomes (only 1 out of 1,788). The frequency of the blunt open end appeared to increase after augmin knockdown; however, the difference was not statistically significant with the current sample numbers. Bar, 50 nm.









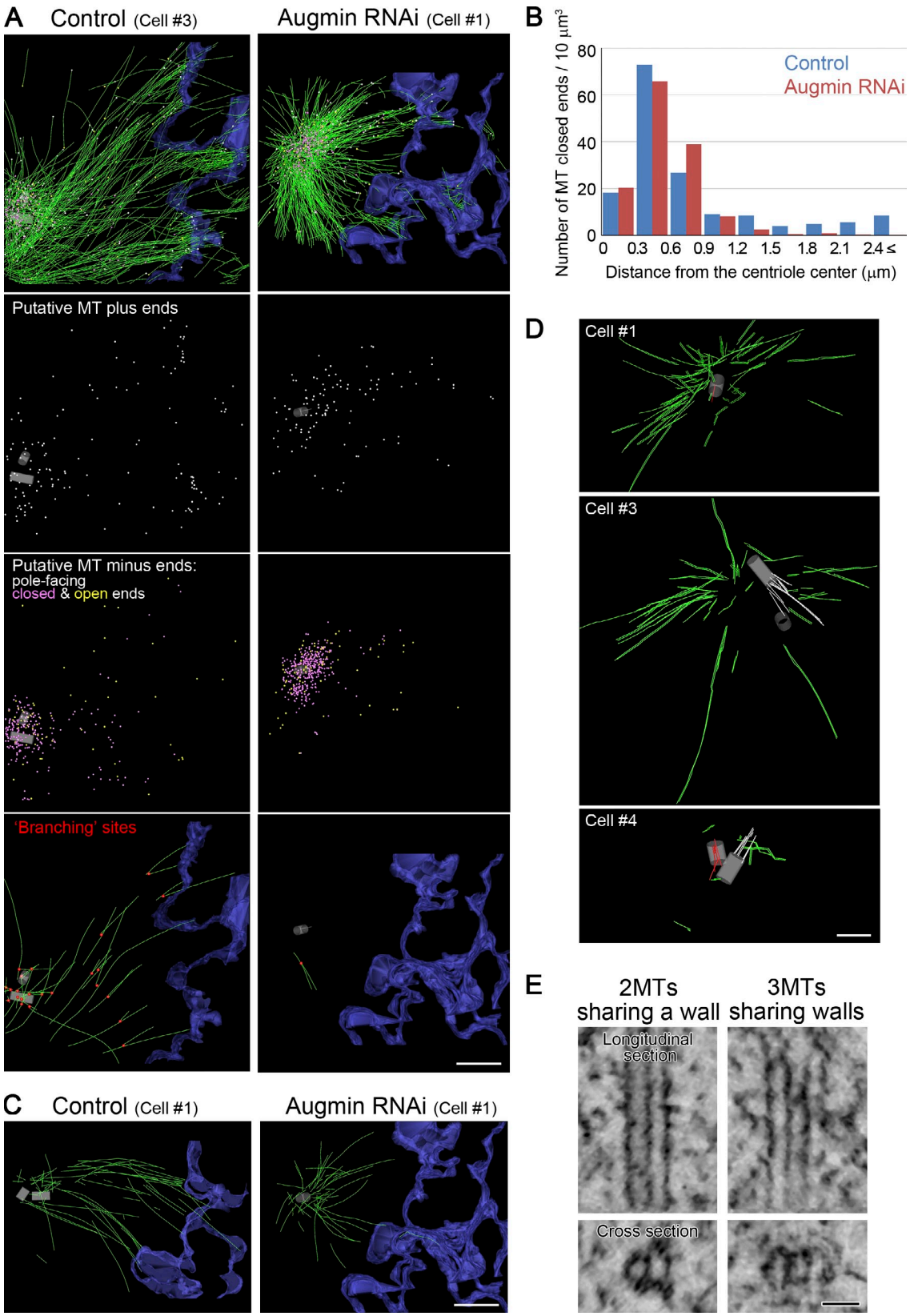


Figure S2. **MT organization and end distribution.** (A) Other control and RNAi-treated cells displayed in a manner identical to Fig. 1 (A–D). (B) Distribution of closed MT ends in the spindle. Reduction of the closed MT ends was evident in the body of the spindle after augmin knockdown. Combined data from three control or four augmin knockdown cells are displayed. Information on each cell is provided in Tables S1 and S2. (C) Models displaying only the MTs with both ends in the reconstructed volume. MTs and chromosomes are colored green and blue, respectively. The centrioles are displayed as gray cylinders. (D and E) In three out of four augmin knockdown cells, abnormal centriolar MTs were observed. For example, we observed (a) overly long centriolar MTs extended from the distal centriole end in two cells (white), (b) MTs within the lumen of two centrioles (red), and (c) two or three centrosomal MTs that appear to share a wall (D, green, and E). Bars: (A and C) 1 μm; (D) 0.5 μm; (E) 30 nm.

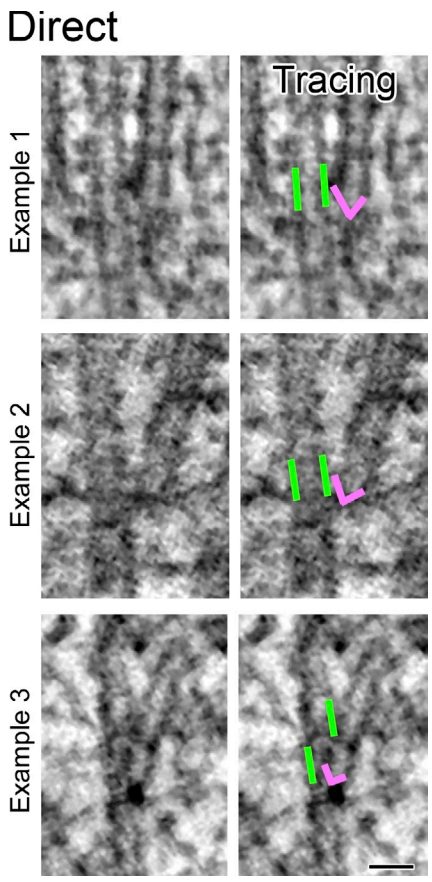
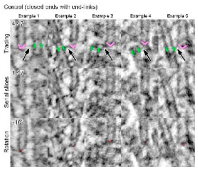
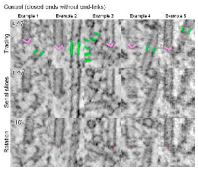


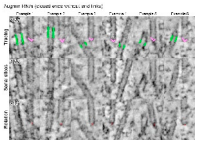
Figure S3. **Three other examples of the direct end-links.** Manual tracings of mother MT wall (green; a part of the wall was marked) and the capped daughter MT ends (magenta) are displayed on the right. Bar, 30 nm.



Video 1. **Rod-shaped end-links found in control cells.** Five examples of the rod-shaped end-link between an MT closed end and the lattice of an adjacent MT. Displayed are seven serial slices (1.4-nm-thick slice/frame) and ±10° Y-rotation images (1°/frame). Arrows indicate the end-links on the view where they are most visible. Red crosses in the bottom row indicate tips of closed ends and rotation axes. Images at the top display manual traces of a part of the mother MT wall (green) and the capped end of a daughter MT (magenta). The 3DMOD program in the IMOD software package was used for producing tomographic slices and rotating tomograms. ImageJ software (National Institutes of Health) was used for making the video from image sequences.



Video 2. **End-link–free closed ends in control cells.** Five examples of the closed MT end that has no end-links with an adjacent MT in control cells. Displayed are seven serial slices (1.4-nm-thick slice/frame) and ±10° Y-rotation images (1°/frame). Red crosses in the bottom row indicate tips of closed ends and rotation axes. Images at the top display manual traces of a part of the mother MT wall (green) and the capped end of a daughter MT (magenta) at a sampling plane where MT ends are most clearly seen. The 3DMOD program in the IMOD software package was used for producing tomographic slices and rotating tomograms. ImageJ software was used for making the video from image sequences.



Video 3. **End-link–free closed ends in augmin knockdown cells.** Six examples of the closed MT end that has no end-links with an adjacent MT in augmin knockdown cells. Displayed are seven serial slices (1.4-nm-thick slice/frame) and ±10° Y-rotation images (1°/frame). Red crosses in the bottom row indicate tips of closed ends and rotation axes. Images at the top display manual traces of a part of the mother MT wall (green) and the capped end of a daughter MT (magenta) at a sampling plane where MT ends are most clearly seen. The 3DMOD program in the IMOD software package was used for producing tomographic slices and rotating tomograms. ImageJ software was used for making the video from image sequences.

Table S1. **Analysis summary of control cells**

Parameters	Control cells				Total	Mean ± SD
	Cell #1	Cell #2[a]	Cell #3	Cell #4		
Volume size (X × Y × Z µm)	6 × 4 × 0.7	4 × 4 × 0.6	5 × 6 × 0.6	6 × 6 × 0.5	57 μm^3	–
Number of MTs	703	487	926	1,015	3,131	–
Total MT length (µm)	619	381	810	838	2,648	–
Number of MTs (/μm^2) passing through the plane 2.5 µm from the centriole center	97	–	61	55	–	71 ± 23
Number of closed ends	324	51	208	228	811	–
Number of closed ends/10 μm^3	254	56	118	127	–	139 ± 83
Number of closed ends near the centriole[b]/10 μm^3	203	–	99	121	–	141 ± 55
Number of closed ends away from the centriole[c]/10 μm^3	51	–	19	6	–	26 ± 23
Number of pole-facing open ends (flared, blunt)	111 (71, 40)	30 (30, 0)	63 (52, 11)	170 (161, 9)	374 (314, 60)	–
Number of chromosome-facing open ends (flared, blunt)	136 (130, 6)	43 (40, 3)	137 (135, 2)	158 (156, 2)	474 (461, 13)	–
Number of end-links (rod shaped, direct)	15 (11, 4)	10 (6, 4)	23 (12, 11)	23 (8, 15)	71 (37, 34)	–
Number of end-links/10 μm^3	12	11	13	13	–	12 ± 1
Number of end-links near the centriole[c]/10 μm^3	8	–	8	11	–	9 ± 2
Number of end-links away from the centriole[c] /10 μm^3	4	–	5	2	–	4 ± 1
Percentage of end-links/closed ends	5	20	11	10	–	11 ± 6
Percentage of end-links/closed ends, near the centriole[b]	4	–	8	9	–	7 ± 3
Percentage of end-links/closed ends, away from the centriole[c]	8	–	26	36	–	24 ± 15
Number of end-links/1-mm MT	24	26	28	27	–	27 ± 2

Minus signs indicate data were not analyzable or not significant.
[a]The tomographic volumes of this cell did not contain centrioles.
[b]<1.5 µm from the centriole center.
[c]≥1.5 µm from the centriole center.

Table S2. **Analysis summary of augmin knockdown cells**

Parameters	Augmin knockdown cells				Total	Mean ± SD	P-value[c]
	Cell #1	Cell #2	Cell #3	Cell #4			
Volume size (X × Y × Z µm)	6 × 4 × 0.7	6 × 4 × 0.7	5 × 6 × 0.7	4 × 7 × 0.7	71 µm^3	–	–
Number of MTs	828	463	874	1,028	3,193	–	–
Total MT length (µm)	578	424	696	951	2,649	–	–
Number of MTs (/µm^2) passing through the plane 2.5 µm from the centriole center	31	47	14	42	–	34 ± 15	0.05
Number of closed ends	314	112	237	314	977	–	–
Number of closed ends/10 µm^3	180	86	123	146	–	134 ± 39	0.9
Number of closed ends near the centriole[a]/10 µm^3	175	84	121	146	–	131 ± 38	0.8
Number of closed ends away from the centriole[b]/10 µm^3	5	2	3	0	–	2 ± 2	0.08
Number of pole-facing open ends (flared, blunt)	81 (61, 20)	41 (40, 1)	213 (175, 38)	201 (176, 25)	536 (452, 84)	–	–
Number of chromosome-facing open ends (flared, blunt)	106 (94, 12)	60 (57, 3)	109 (87, 22)	140 (132, 8)	415 (370, 45)	–	–
Number of end-links (rod shaped, direct)	1 (0, 1)	1 (0, 1)	5 (3, 2)	4 (3, 1)	11 (6, 5)	–	–
Number of end-links/10 µm^3	0.6	0.8	2.6	1.9	–	1.4 ± 1.0	0.0001
Number of end-links near the centriole[a]/10 µm^3	0.6	0.8	2.6	1.9	–	1.4 ± 1.0	0.001
Number of end-links away from the centriole[b]/10 µm^3	0	0	0	0	–	0 ± 0	0.0004
Percentage of end-links/closed ends	0.3	0.9	2.1	1.3	–	1.1 ± 0.8	0.02
Percentage of end-links/closed ends near the centriole[a]	0.3	0.9	2.2	1.3	–	1.2 ± 0.8	0.01
Percentage of end-links/closed ends, away from the centriole[b]	0	0	0	0	–	0 ± 0	0.02
Number of end-links/1-mm MT	2	2	7	4	–	4 ± 2	0.0001

Minus signs indicate data were not analyzable or not significant.
[a]<1.5 µm from the centriole center.
[b]≥1.5 µm from the centriole center.
[c]Based on *t* test against the control.