Expanded View Figures

Figure EV1. SFI1 localization at centrioles revealed by expansion microscopy.

A Representative confocal image of expanded U2OS centrioles stained with α/β-tubulin (αβTub, magenta) and SFI1 (green, home-made antibody). White arrowheads point to the SFI1 signal at mature centrioles and white arrows highlight the SFI1 signal at procentrioles. M stands for mature centrioles and P for Procentrioles. Scale bar: 200 nm.

B Representative confocal image of expanded RPE-1 centrioles stained for α/β-tubulin (αβTub, magenta) and SFI1 (green, home-made antibody). Arrowheads point to the SFI1 signal at mature centrioles while the thin arrows highlight the SFI1 signal at procentrioles. M stands for mature centrioles and P for procentrioles. Scale bar: 200 nm.

C, D Representative confocal image of expanded RPE-1 centrioles treated with siCT (C) or siSFI1 (D), stained with α/β-tubulin (αβTub, magenta) and SFI1 (green, home-made antibody). White arrowheads point to the SFI1 signal at the distal end of centrioles while red arrowhead points to a faint proximal signal. Scale bar: 200 nm.

E Relative SFI1 intensity in the indicated conditions showing a significant decrease in siSFI1-treated cells.

F Representative confocal image of expanded U2OS centrioles stained with α/β-tubulin (αβTub, magenta) and SFI1 (green, commercial antibody). White arrowheads point to the SFI1 signal at mature centrioles and white arrows highlight the SFI1 signal at procentrioles. M stands for mature centrioles and P for procentrioles. Scale bar: 200 nm.

G Representative confocal image of expanded RPE-1 centrioles stained for α/β-tubulin (αβTub, magenta) and SFI1 (green, commercial antibody). White arrowheads indicate the SFI1 signal at mature centrioles and white arrows highlight the SFI1 signal at procentrioles. M stands for mature centriole and P for procentriole. Scale bar: 200 nm.

H–K Representative confocal image of expanded RPE-1 centrioles treated with siCT (H, I) or siSFI1 (J, K), stained with α/β-tubulin (αβTub, magenta) and SFI1 (green, commercial antibody). White arrowheads point to SFI1 signal at the distal end of centrioles while red arrowhead points to a faint proximal signal. Scale bars: 200 nm (H, J) and 100 nm (I, K).

L, M Representative widefield images of expanded centrioles from U2OS cells treated with siSFI1. Cells are stained for α/β-tubulin (αβTub, magenta) and SFI1 (green) allowing the quantification of the siSFI1 efficiency which can lead to either total SFI1 depletion (No SFI1, L) or incomplete SFI1 depletion (Partial SFI1, M).

N–P Representative widefield images of expanded U2OS centrioles treated with siCT or siPOC5, stained with α/β-tubulin (αβTub, magenta) and POC5 (green, N), Cetn2/3 (green, O) or SFI1 + POC5 (green, P). White arrowheads indicate the remaining proximal belt of POC5 sometimes observable in siPOC5 treated cells when depletion is incomplete (N, middle panel). Note that Centrin behavior seems to follow POC5 upon POC5 depletion (O, middle panel). Asterisks indicate the presence of the distal dot of Centrin and SFI1 in POC5-depleted cells. Scale bar: 200 nm.

Q Quantification of the siPOC5 efficiency at centrosomes.

R Percentage of depleted centrioles (without POC5 staining) containing SFI1 as a distal dot in siCT and siPOC5 treated cells.

Data information: Average ± SD, *N*, statistical analysis: (E) siCT (area under the curve): 0.79 ± 0.2, siSFI1 (area under the curve): 0.55 ± 0.3. $N = 24$ for siCT and 50 for siSFI1 from 4 independent experiments. Mann–Whitney test (***$P < 0.0001$). (Q) siCT = 96.4% ± 5.4; siPOC5 = 43.8% ± 11.6, $N = 5$ independent experiments (100 cells/experiment). Mann–Whitney test (**$P = 0.002$). (R) siCT = 94.5% ± 6.4; siPOC5 = 92.5% ± 6.4. $N = 2$ independent experiments (100 cells/experiment). Mann–Whitney test ($P = 0.667$).

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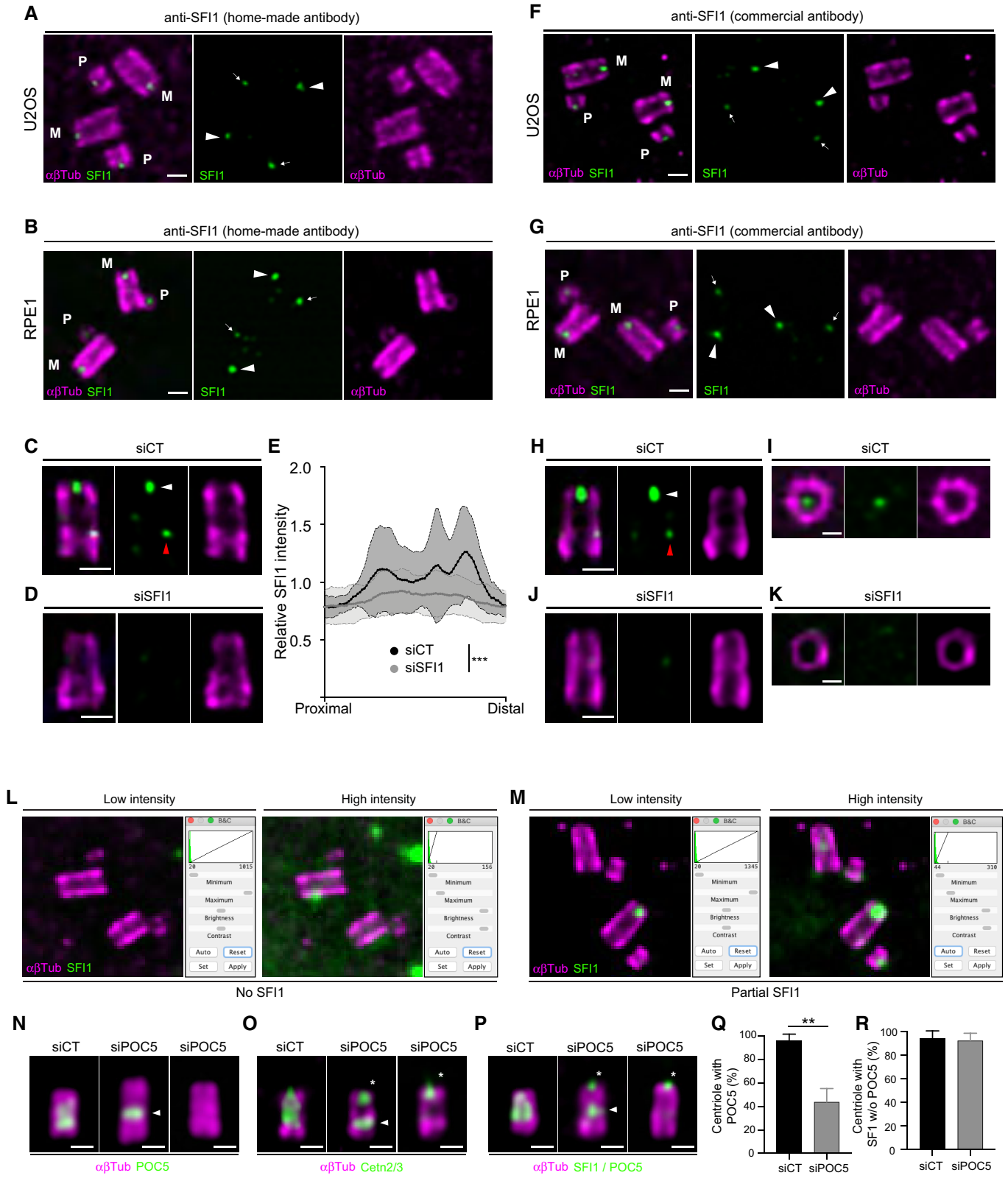

Figure EV1.

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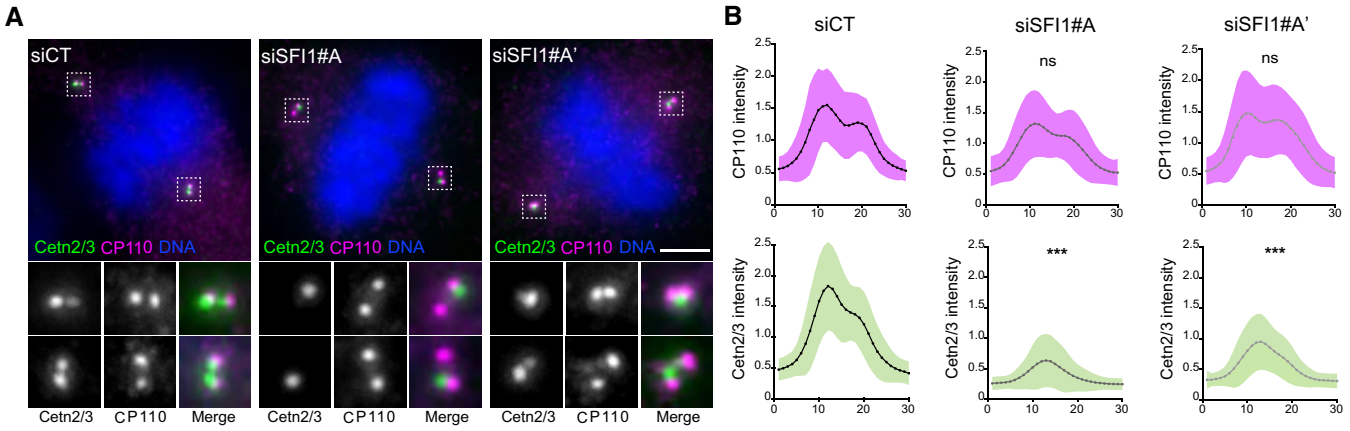

Figure EV2. SFI1 depletion alters centriolar Centrin position but not CP110.

A Representative confocal images of mitotic control and SFI1-depleted RPE1-1 cells stained for Centrin (Cetn2/3, green) and CP110 (magenta). Scale bar: 5 μm.

B CP110 (magenta) and Centrin (green) relative integrated intensities from a plot profile across the 2 centrioles in control and SFI1-depleted cells (siSFI1#A and siSFI1#A′ correspond to two different siRNAs, see material and methods). Average ± SD, *N*, statistical analysis: siCT (area under the curve) = CP110: 1 ± 0.3, Cetn2/3: = 0.99 ± 0.5. siSFI1#A (area under the curve) = CP110: 0.9 ± 0.3, Cetn2/3: 0.4 ± 0.1. siSFI#A′ (area under the curve) = CP110: 1 ± 0.3, Cetn2/3: 0.5 ± 0.2. *N* = 60 cells from three independent experiments. Unpaired *t*-test (***$P < 0.0001$).

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Figure EV3. Characterization of SFI1 depletion in HeLa and U2OS.

A, B Representative widefield images of expanded centrioles from HeLa cells treated with siCT or siSFI1#A. Cells are stained with Tubulin (magenta) and SFI1 (green, A) or Cetn2/3 (gray, B). Yellow asterisks show the absence of SFI1 (A) and Cetn2/3 (B) at the distal tip of the centriole in siSFI1-treated cells. Scale bar: 250 nm. Quantifications show the similar loss of SFI1 and Cetn2/3 in SFI1-depleted cells.

C Representative widefield images of expanded centrioles from HeLa cells treated siSFI1#A and stained for tubulin (magenta) and SFI1 (green). Note the abnormal shape and structural alteration of the centriole in SFI1-depleted cells. Yellow asterisks show the absence of SFI1. Red arrowheads indicate abnormal centrioles. Scale bar: 250 nm.

D Quantification of the percentage of duplicating centrioles in the indicated conditions.

E Percentage of abnormal centrioles in the indicated conditions.

F, G Representative widefield images of expanded centrioles from HeLa cells treated with siCT or siSFI1#B. Cells are stained with Tubulin (magenta) and SFI1 (green, F) or Cetn2/3 (gray, G). Yellow asterisks show the decreased intensity of SFI1 (F) and Cetn2/3 (G) at the distal tip of the centriole in siSFI1-treated cells. Scale bar: 250 nm.

H Quantification of the efficiency of the siSFI1 shows a mild loss of SFI1 in these conditions.

I Quantification of the percentage of duplicating centrioles in the indicated conditions.

J Percentage of abnormal centrioles in the indicated conditions.

K, L Quantification of the signal intensities of SFI1 (K) and Cetn2/3 (L) in HeLa cells treated with siCT or siSFI1#B showing a marked decrease of both SFI1 and Cetn2/3 at the level of mature and procentriole. However, the complete disappearance of the signal was rarely observed (see panel H).

M, N Representative widefield images of expanded centrioles from U2OS cells treated with siCT or siSFI1#B. Cells are stained for tubulin (magenta) and SFI1 (green, M) or Cetn2/3 (gray, N). Yellow asterisks show the decreased intensity of SFI1 (M) and Cetn2/3 (N) at the distal tip of the centriole in siSFI1-treated cells. Scale bar: 250 nm.

O Quantification of the efficiency of the siSFI1 shows a mild loss of SFI1 in these conditions.

P Quantification of the percentage of duplicating centrioles in the indicated conditions.

Q Percentage of abnormal centrioles in the indicated conditions.

R, S Quantification of the signal intensities of SFI1 (R) and Cetn2/3 (S) in U2OS cells treated with siCT or siSFI1#B showing a notable decrease of both SFI1 and Cetn2/3 at the level of mature and procentriole. However, the complete disappearance of the signal was rarely observed (see panel O).

Data information: Average ± SD, *N*, statistical analysis: (A) siCT = Intact SFI1: 97.2% ± 3.9, Partial SFI1: 2.8% ± 3.9, No SFI1: 0% ± 0, siSFI1 = Intact SFI1: 11.3% ± 7.6, Partial SFI1: 32.2% ± 12.6, No SFI1: 56.5% ± 5. *N* = 2 independent experiments (> 50 centrioles per experiment). Two-way ANOVA (***$P < 0.0001$). (B) siCT = Intact Cetn2/3: 95% ± 7.1, Partial Cetn2/3: 5% ± 7.1, No Cetn2/3: 0% ± 0, siSFI1 = Intact Cetn2/3: 14.4% ± 14.9, Partial Cetn2/3: 36.2% ± 8.7, No Cetn2/3: 49.4% ± 6.3. *N* = 2 independent experiments (> 50 centrioles per experiment). Two-way ANOVA (***$P < 0.0001$). (D) siCT: 49.9% ± 7, siSFI1#A: 60.7 ± 6.8. *N* = 3 independent experiments (> 50 cells/experiment). Unpaired *t*-test ($P = 0.13$). (E) siCT: 2.1% ± 1.9, siSFI1#A: 21.1% ± 2.6. *N* = 3 independent experiments (> 50 cells/experiment). Unpaired *t*-test (***$P = 0.0005$). (H) siCT = Intact SFI1: 97.4% ± 4.4, Partial SFI1: 2.6% ± 4.4, No SFI1: 0% ± 0, siSFI1 = Intact SFI1: 84.9% ± 4.3, Partial SFI1: 15.1% ± 4.3, No SFI1: 0% ± 0. *N* = 3 independent experiments (> 50 centrioles per experiment). Two-way ANOVA (***$P = 0.0002$). (I) siCT: 47.6% ± 2.2, siSFI1#B: 57.1 ± 6.8. *N* = 3 independent experiments (> 50 centrioles per experiment). Unpaired *t*-test ($P = 0.08$). (J) siCT: 1.2% ± 2.3, siSFI1#B: 2.3 ± 2.3. *N* = 3 independent experiments (> 50 centrioles per experiment). Unpaired *t*-test ($P = 0.63$). (K) siCT mature: 1.0 ± 0.13, siSFI1#B mature: 0.69 ± 0.06, siCT procentriole: 1.0 ± 0.10, siSFI1#B procentriole: 0.70 ± 0.10. *N* = 58, 47, 45, 57 for siCT mature, siSFI1#B mature, siCT procentriole and siSFI1#B procentriole respectively, from three independent experiments. One-way ANOVA (***$P < 0.0001$ in all conditions). (L) siCT mature: 1.0 ± 0.12, siSFI1#B mature: 0.69 ± 0.13, siCT procentriole: 1.0 ± 0.12, siSFI1#B procentriole: 0.70 ± 0.07. *N* = 37, 34, 37, 40 for siCT mature, siSFI1#B mature, siCT procentriole and siSFI1#B procentriole respectively, from three independent experiments. One-way ANOVA (***$P < 0.0001$ in all conditions). (O) siCT = Intact SFI1: 100% ± 0, Partial SFI1: 0% ± 0, No SFI1: 0% ± 0, siSFI1#B=Intact SFI1: 86.2% ± 1.5, Partial SFI1: 12.5% ± 1.6, No SFI1: 2% ± 2.8. *N* = 3 independent experiments (> 50 centrioles per experiment). Two-way ANOVA (***$P = 0.0001$). (P) siCT: 44.1% ± 4.7, siSFI1#B: 51.8 ± 3.3. *N* = 3 independent experiments (> 50 centrioles per experiment). Unpaired *t*-test ($P = 0.08$). (Q) siCT: 1.1% ± 1.2, siSFI1#B: 1.3 ± 1.5. *N* = 3 independent experiments (> 50 centrioles per experiment). Unpaired *t*-test ($P = 0.86$). (R) siCT mature: 1.0 ± 0.10, siSFI1#B mature: 0.70 ± 0.09, siCT procentriole: 1.0 ± 0.11, siSFI1#B procentriole: 0.70 ± 0.08. *N* = 100, 83, 74, and 57 for siCT mature, siSFI1#B mature, siCT procentriole, and siSFI1#B procentriole respectively, from three independent experiments. One-way ANOVA (***$P < 0.0001$ in all conditions). (S) siCT mature: 1.0 ± 0.12, siSFI1#B mature: 0.58 ± 0.11, siCT procentriole: 1.0 ± 0.13, siSFI1#B procentriole: 0.60 ± 0.12. *N* = 58, 48, 51, 41 for siCT mature, siSFI1#B mature, siCT procentriole, and siSFI1#B procentriole respectively, from three independent experiments. One-way ANOVA (***$P < 0.0001$ in all conditions).

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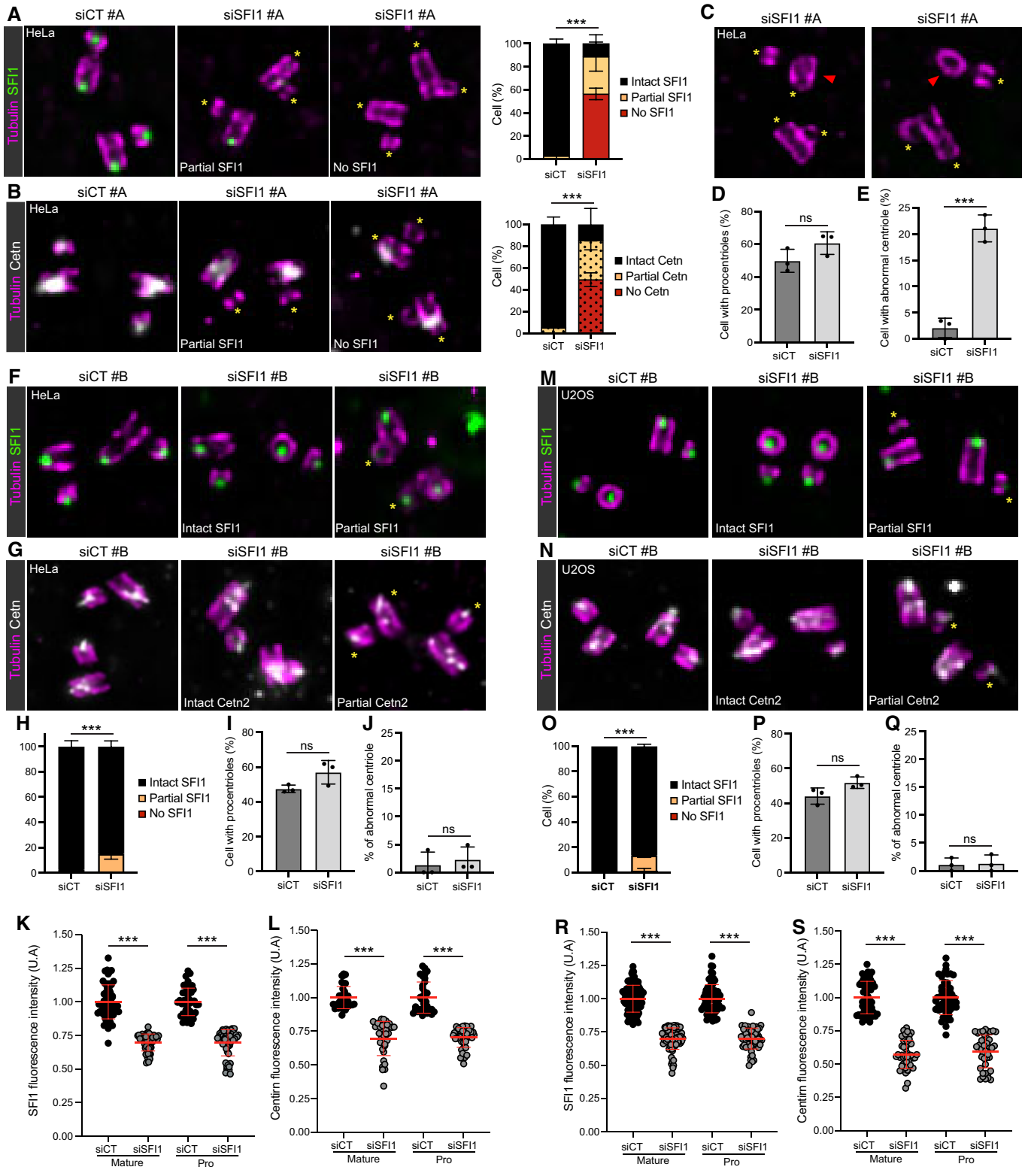

Figure EV3.

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Figure EV4. Gallery of defective centrioles in SFI1-depleted RPE-1 cells.

A, B Confocal images of expanded centrioles from SFI1-depleted RPE-1 stained for α/β-tubulin (magenta). Top view (top panel) and side view (bottom panels) of broken centriole (A) and abnormal but not broken (B) stained for (α/β-tubulin, magenta) and SFI1 (green). Scale bar: 200 nm.

C, D Confocal images of expanded centrioles from SFI1-depleted RPE-1 stained for α/β-tubulin (magenta) and CP110 (yellow). Top view (top panel) and side view (bottom panels) of broken centriole (C) and abnormal but not broken (D) stained for (α/β-tubulin, magenta) and CP110 (yellow). Scale bar: 200 nm.

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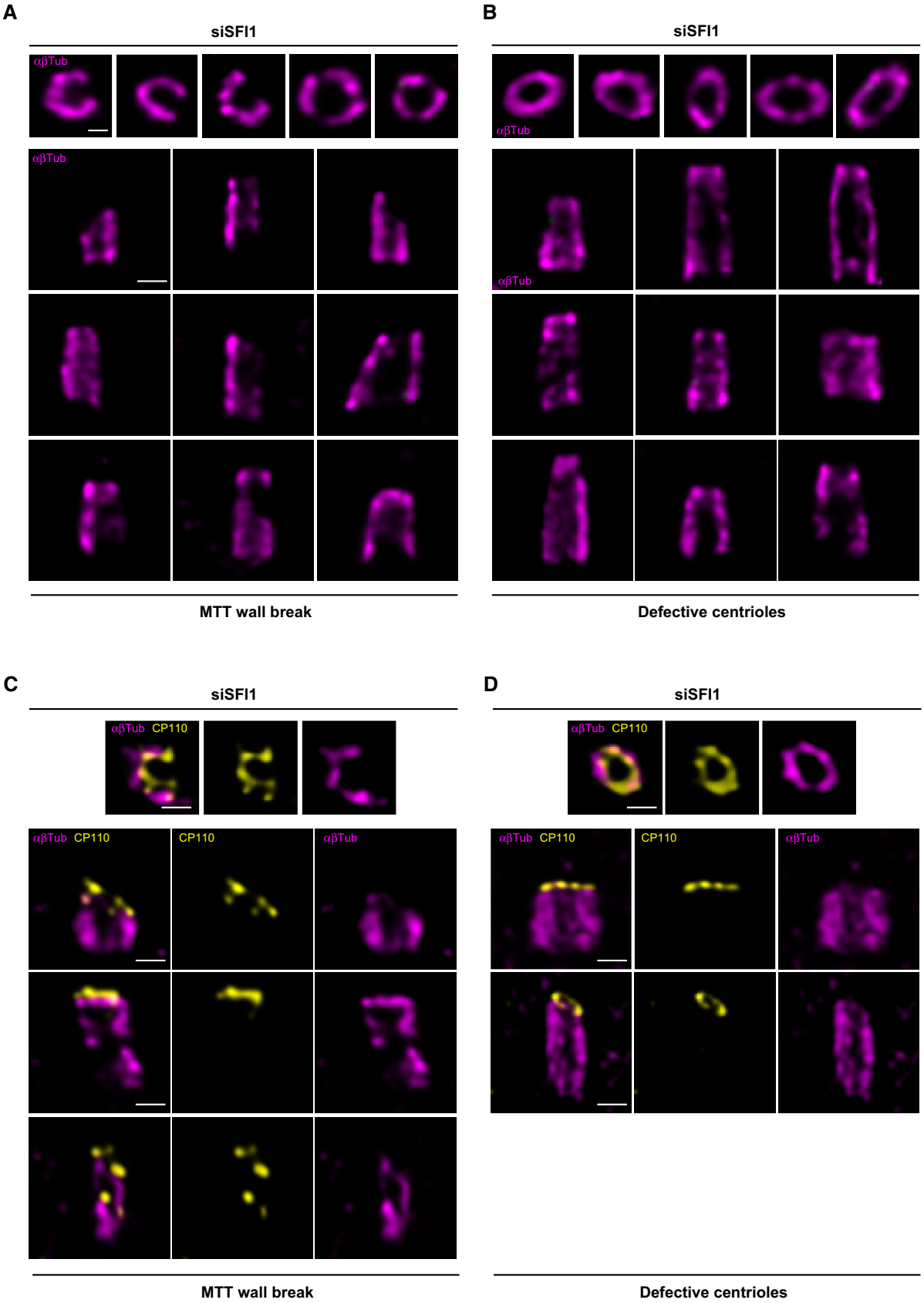

Figure EV4.

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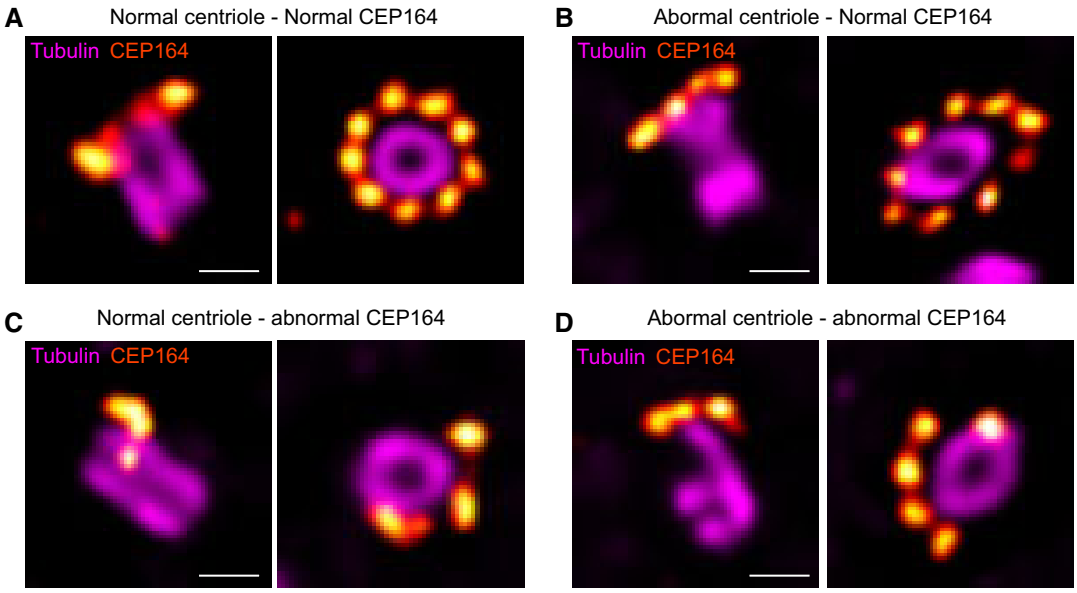

Figure EV5. CEP164 disorganization in SFI1-depleted RPE-1.

A–D Representative widefield images of expanded centrioles in siSFI1-depleted cells stained for tubulin (magenta) and CEP164 (red hot). Each panel shows a different combination of alterations such as normal centriole with normal CEP164 (A), abnormal centriole with normal CEP164 (B), normal centriole with abnormal CEP164 (C), and abnormal centriole with abnormal CEP164 (D). Scale bars: 250 nm.

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