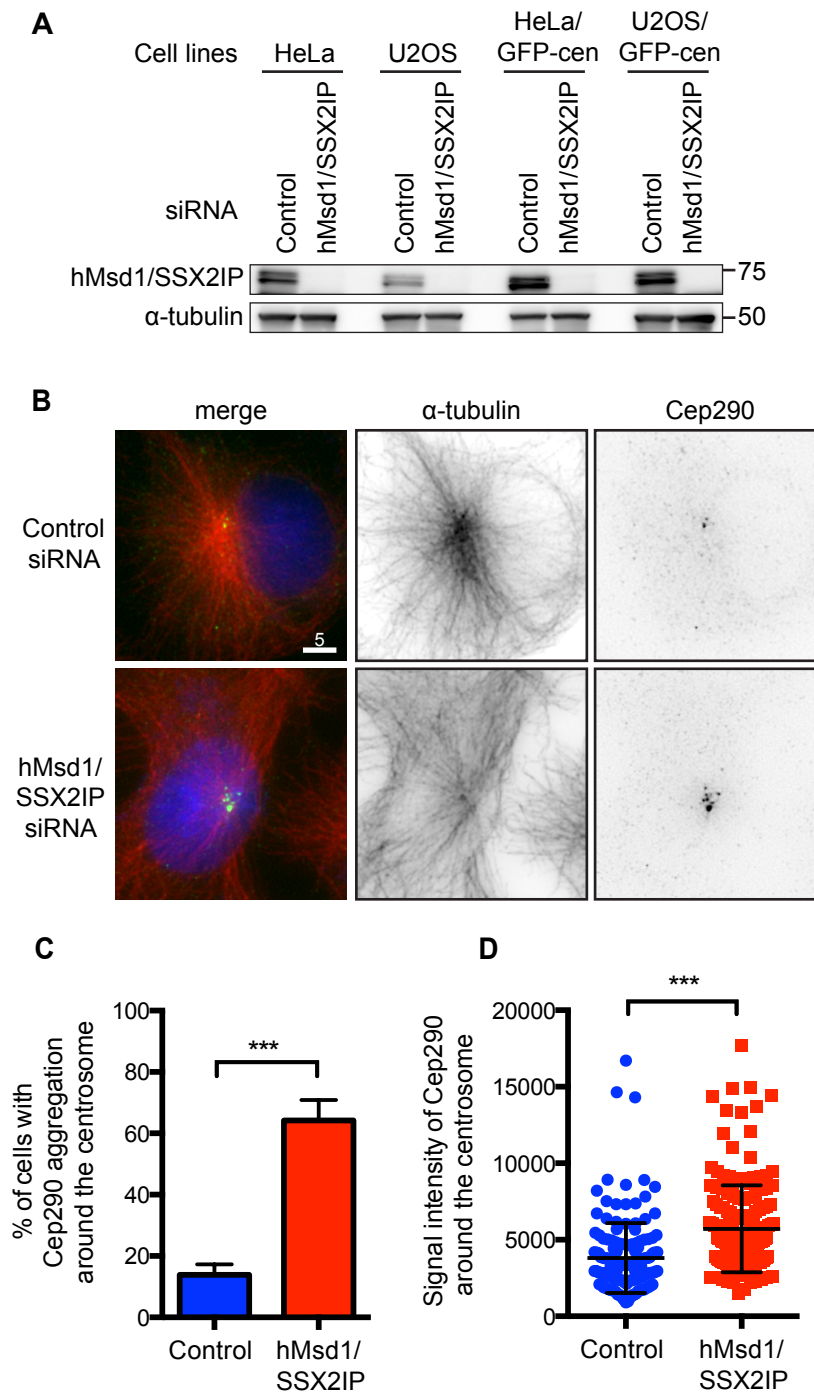


## **Supplemental Material**

### **Centriolar satellites- and hMsd1/SSX2IP-dependent microtubule anchoring is critical for centriole assembly**

Akiko Hori<sup>1</sup>, Christopher J. Peddie<sup>2</sup>, Lucy M. Collinson<sup>2</sup>, and Takashi Toda<sup>1</sup>

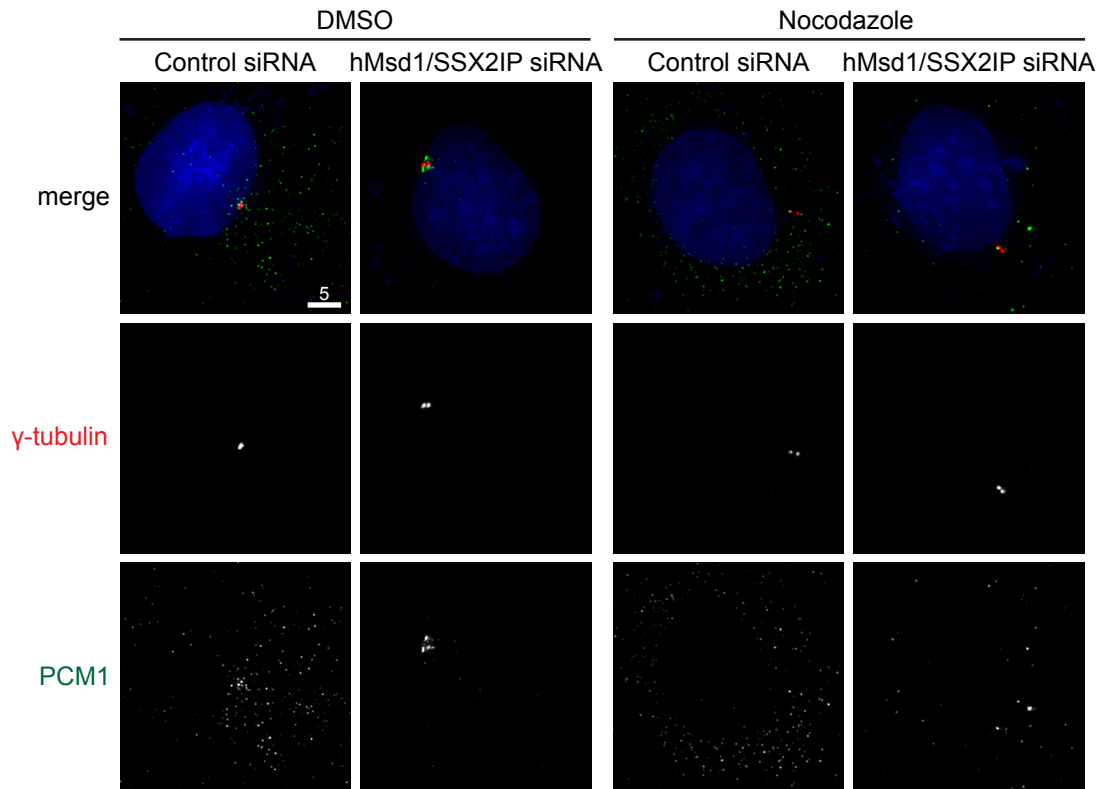
## Supplemental Figure S1 Hori A et al.



### Supplemental Figure S1. Evaluation of siRNA-mediated hMsd1/SSX2IP depletion and confirmation that hMsd1/SSX2IP depletion leads to Cep290 aggregation around the centrosome concomitant with microtubule disorganization.

(A) Immunoblotting of protein extracts prepared from HeLa, HeLa stably expressing centrin-GFP, U2OS, or U2OS stably expressing centrin-GFP cells. These cells were treated with control or hMsd1/SSX2IP siRNA and immunoblotting was performed with antibodies specific to hMsd1/SSX2IP and  $\alpha$ -tubulin. The positions of molecular weight markers (kDa) are shown on the right. (B) U2OS cells were transfected with control or hMsd1/SSX2IP siRNA and 48 h later immunostained with anti- $\alpha$ -tubulin (red) and anti-Cep290 antibodies (green). DAPI staining (blue) is also included in merged images (left). Scale bar, 5  $\mu$ m. (C) Quantification of cells displaying Cep290 aggregation around the centrosome. The data represent the mean+SD (>300 cells derived from three independent experiments, n=3). \*\*\*P<0.0001. (D) Quantification of Cep290 signal intensities around the centrosome. 25 pixel squares around centrosome were measured. Data represent the mean+SD (>100 cells, n=3). \*\*\*P<0.0001.

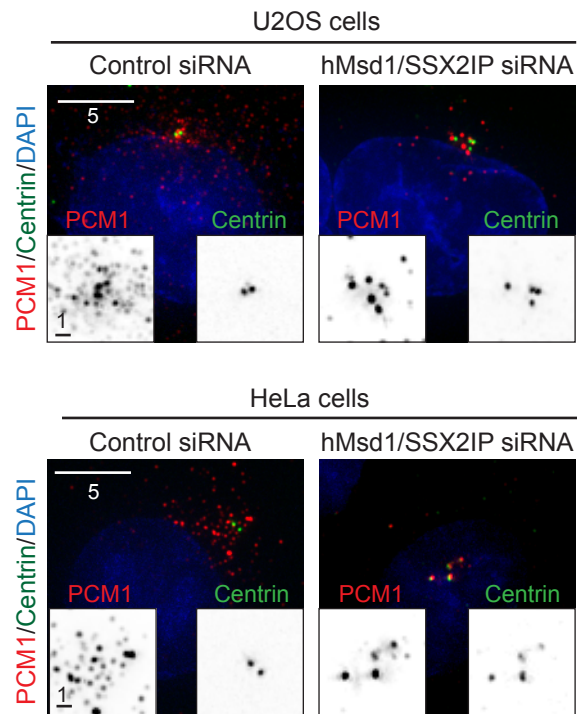
## Supplemental Figure S2 Hori A et al.



### Supplemental Figure S2. PCM1 aggregation in hMsd1/SSX2IP-depleted cells requires microtubules.

U2OS cells were transfected with control or hMsd1/SSX2IP siRNA and 48 h later treated with DMSO (left) or 20  $\mu$ M Nocodazole (right) for 2 h, fixed and immunostained with antibodies against PCM1 (green) and  $\gamma$ -tubulin (red). DNA was stained with DAPI (blue). Scale bars, 5  $\mu$ m.

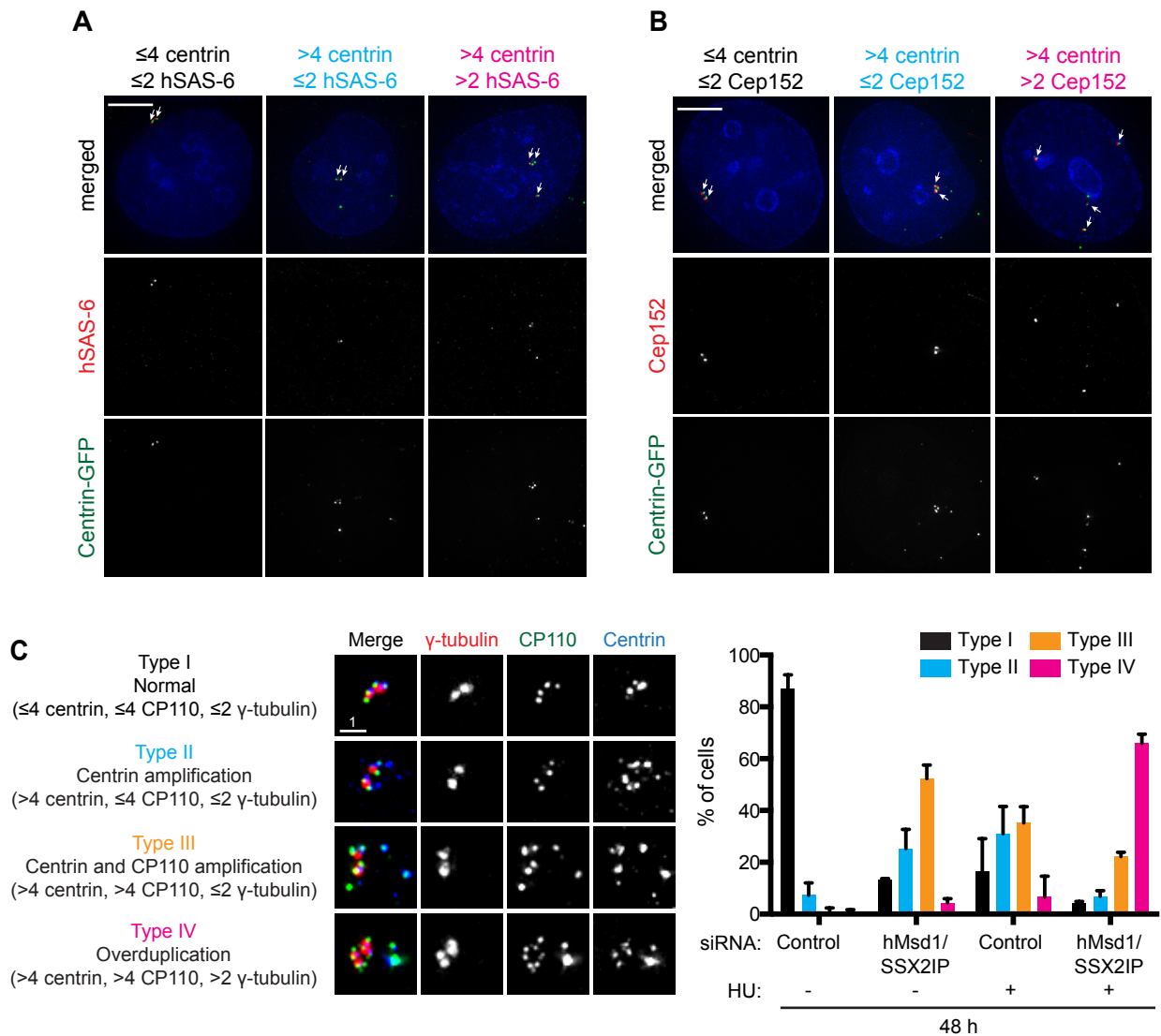
## Supplemental Figure S3 Hori A et al.



**Supplemental Figure S3. The emergence of supernumerary centrin foci upon hMsd1/SSX2IP depletion is independent of ectopically expressed GFP-centrin.**

U2OS (top) or HeLa cells (bottom) were transfected with control (left) or hMsd1/SSX2IP siRNAs (right). 48 h after transfection, cells were fixed and immunostained with anti-centrin (green) and anti-PCM1 antibodies (red). DNA was stained with DAPI (blue). Enlarged images around the centrosomal region are shown in the bottom two insets. Scale bars, 5  $\mu\text{m}$  and 1  $\mu\text{m}$  (bottom insets).

## Supplemental Figure S4 Hori A et al.



### Supplemental Figure S4: hMsd1/SSX2IP depletion promotes centrosome overduplication in HU arrested U2OS cells

(A) and (B). HU was added to U2OS cells stably expressing centrin-GFP and simultaneously transfected with control or hMsd1/SSX2IP siRNA. 48 h and 72 h after transfection, cells were fixed and stained with antibodies against hSAS-6 (A) or Cep152 (B). Three representative types of cells are shown;  $\leq 4$ centrin/ $\leq 2$  hSAS-6 (A), or Cep152 (B),  $> 4$  centrin/ $\leq 2$  hSAS-6 (A), or Cep152 (B) and  $> 4$  centrin/ $> 2$  hSAS-6 (A) or Cep152 (B). Scale bars, 5  $\mu$ m. (C) HU was added to U2OS cells stably expressing centrin-GFP and simultaneously transfected with control or hMsd1/SSX2IP siRNA. 48 h after transfection, cells were fixed and stained with antibodies against CP110 (green) and  $\gamma$ -tubulin (red). Left, the representative types of cells are shown; Type I:  $\leq 4$  centrin,  $\leq 4$  CP110,  $\leq 2$   $\gamma$ -tubulin (normal); Type II:  $> 4$  centrin,  $\leq 4$  CP110,  $\leq 2$   $\gamma$ -tubulin (centrin amplification), Type III:  $> 4$  centrin,  $> 4$  CP110,  $\leq 2$   $\gamma$ -tubulin (centrin and CP110 amplification) and Type IV:  $> 4$  centrin,  $> 4$  CP110,  $> 2$   $\gamma$ -tubulin (overduplication). Right, quantification of the percentage of cells representing the four types of centrin/CP110/ $\gamma$ -tubulin patterns is shown. Data represent the mean+SD ( $> 200$  cells,  $n=2$ ).