Expanded View Figures

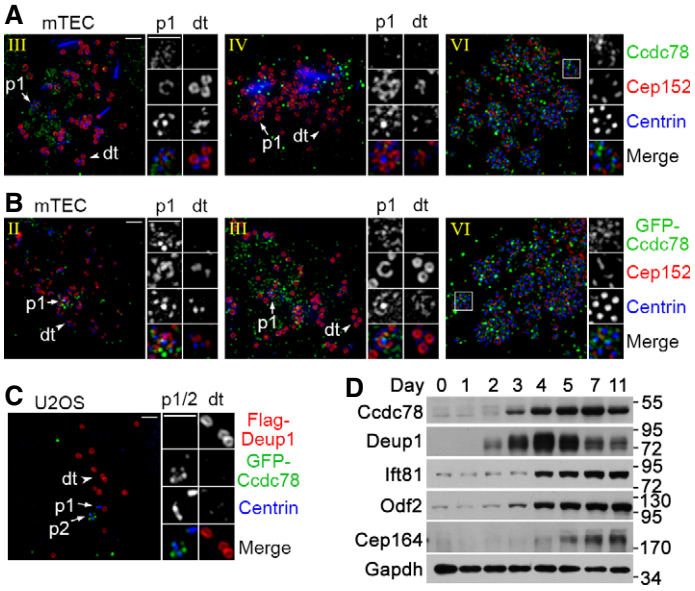
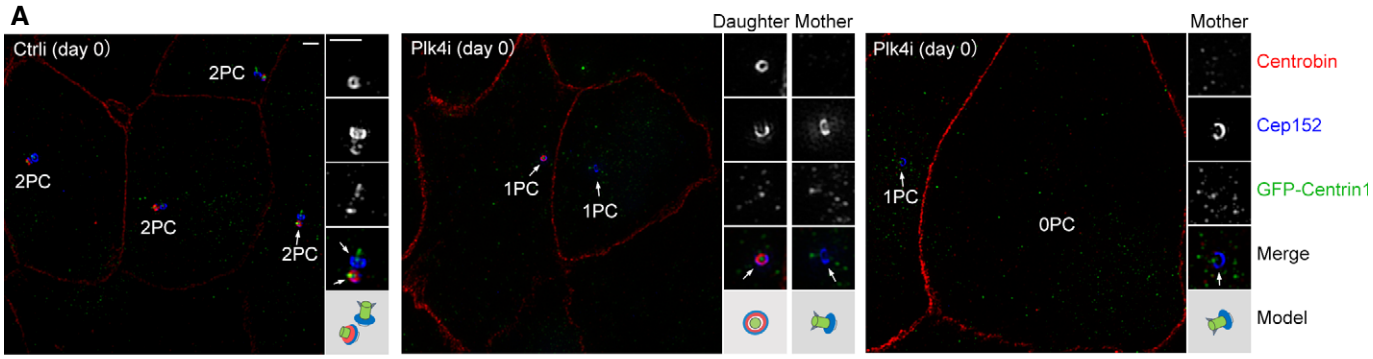


Figure EV1. Mammalian Ccdc78 is not a deuterosome protein.

A, B Neither endogenous nor exogenous murine Ccdc78 localized to deuterosomes in mTECs. Cultured mTECs were fixed at day 3 post-ALI (A) or transfected with lentivirus at day −1 to express GFP-Ccdc78, followed by fixation at day 3 (B). After immunostaining to visualize the indicated proteins, the samples were imaged with SIM. Parental centrioles (p1; arrows), typical deuterosomes (dt; arrowheads), and typical regions with basal bodies (framed) are magnified 2× to show details. Scale bar, 1 μm.

C GFP-Ccdc78 did not localize to Flag-Deup1-induced deuterosome-like structures in U2OS cells. U2OS cells were co-transfected to express Flag-Deup1 and GFP-Ccdc78 for 48 h and fixed for immunostaining. Parental centrioles (p1/p2; arrows) and typical deuterosome-like structures (dt; arrowheads) are magnified 2× to show details. Scale bar, 1 μm.

D Ccdc78 and Deup1 had different expression patterns in differentiating mTECs. Cultured mTECs were induced to undergo multiciliation from day 0 and collected at the indicated time. Gapdh served as loading control.



B

Plk4-depleted ependymal progenitors with 1 PC

	Exp.1	Exp.2	Exp.3	Total
Parental centrioles analyzed	43	44	33	120
Centrobin-positive centrioles	5	5	0	10

Figure EV2. The daughter centriole accounts for a small portion of the single parental centriole in Plk4-depleted ependymal progenitors.

A Identification of the daughter centriole through Centrobin. Ependymal progenitors treated as in Fig 4B were fixed at day 0 and immunostained for Centrobin and Cep152. GFP-Centrin1 served as both infection and centriole markers. Zo-1 was visualized in the same channel with Centrobin to label the cell boundaries because their antibodies were from mouse and their staining patterns did not overlap. Parental centrioles are denoted by arrows. An illustration is provided for each set of the magnified (2×) images. Scale bar, 1 μm.

B Quantification for Centrobin-positive parental centrioles in three independent experiments.

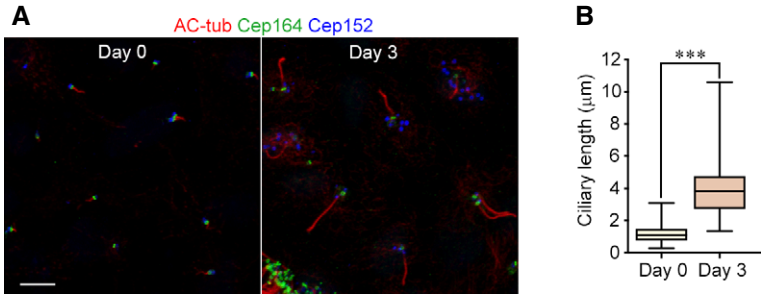


Figure EV3. Ciliary length increases dramatically during the differentiation of ependymal progenitors.

A Cilia in the progenitors (day 0) and mEPCs (day 3). Ependymal progenitors treated as in Fig 4B were fixed and immunostained to visualize acetylated tubulin (AC-tub; a cilia marker), Cep164 (a marker for mother centriole or basal body), and Cep152 (a marker for parental centriole and deuterosome). Scale bar, 5 μm.

B Quantification for the ciliary length. The results were from three independent experiments. At least 105 cilia were measured in each experiment and condition. The bottom and top of the box represent the 25th and 75th percentiles, respectively. The band is the median. The ends of the whiskers indicate the maximum and minimum of the data. Two-tailed unpaired Student's *t*-test: ***$P < 0.001$.

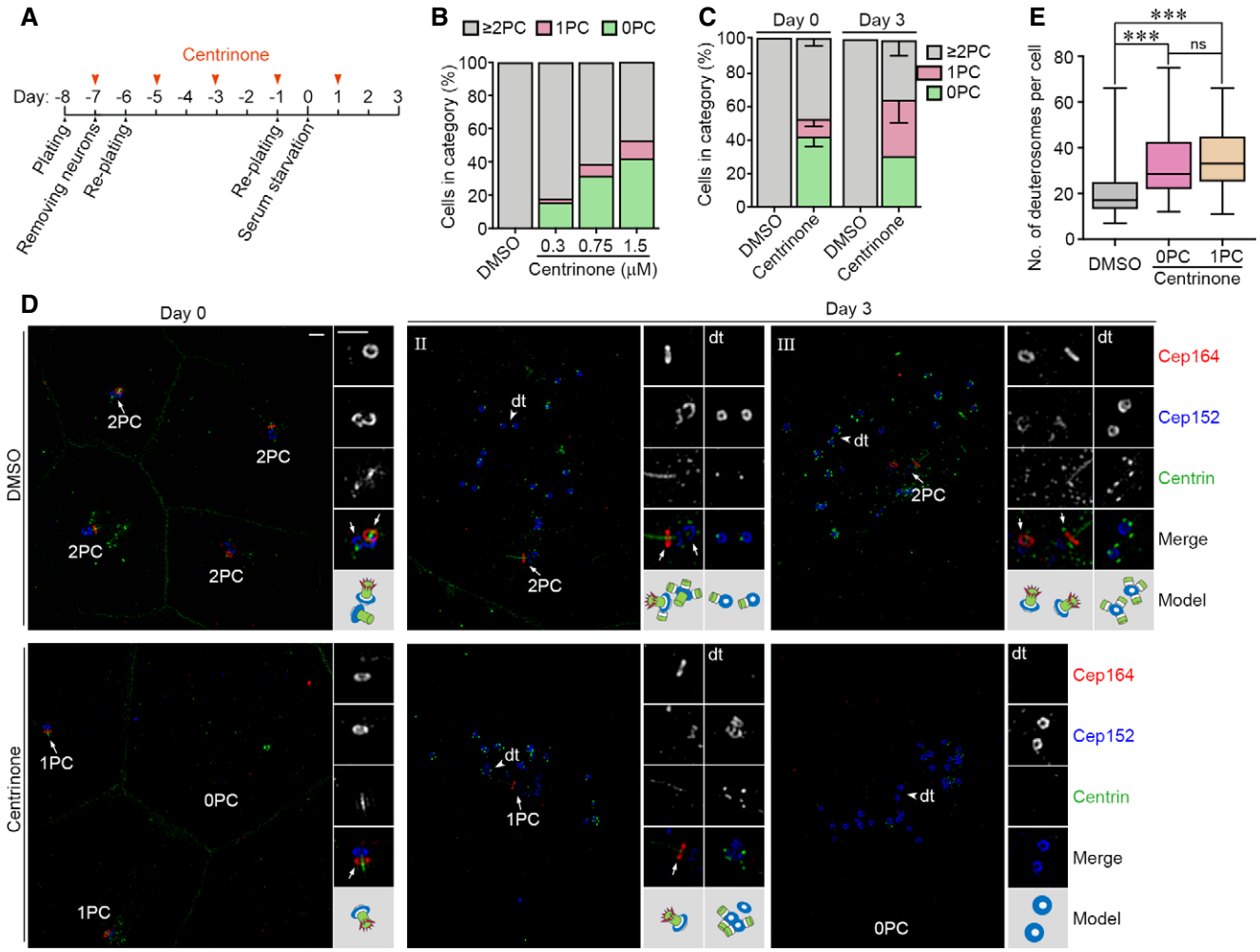


Figure EV4. Depletion of parental centrioles with centrinone does not affect deuterosome formation.

A Experimental design.

B Effects of different centrinone concentrations on parental centrioles. Mouse ependymal progenitors treated as in (A) with 0.3–3.0 μM centrinone were fixed at day 0 and immunostained as in (D). Parental centrioles were identified based on the co-staining patterns of Cep152, Centrin, and Cep164. The histograms represent mean values from one experiment, and at least 89 cells were scored in each condition. The cells treated with 3.0 μM of centrinone died massively and were not analyzed.

C Parental centriole contents of the cells treated with 1.5 μM centrinone, based on the co-staining patterns of Cep152, Centrin, and Cep164 (D). The histograms represent mean values from three independent experiments, and at least 482 cells at days 0 and 100 deuterosome-containing cells at day 3 were scored in each experiment and condition. Error bars represent SD.

D Typical cells treated with DMSO or centrinone, immunostained for Cep152, Cep164, Centrin, and Zo-1. Zo-1 and Centrin were immunostained in the same channel because their antibodies were both from mouse and their staining patterns did not overlap. Magnified images (2×) show details of representative parental centrioles (arrows) and deuterosomes (dt; arrowheads). An illustration is provided for each set of the magnified images. Scale bar, 1 μm.

E Box plots for deuterosome numbers per cell at day 3. Deuterosomes were scored as ring-shaped structures decorated by Cep152 but excluding parental centrioles (D). At least 100 deuterosome-containing cells from three independent experiments were scored. The bottom and top of the box represent the 25th and 75th percentiles, respectively. The band is the median. The ends of the whiskers indicate the maximum and minimum of the data. Two-tailed unpaired Student's *t*-test: ns, no significance; ***$P < 0.001$.