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Supplemental Material to:

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A non-canonical mode of microtubule organization operates throughout pre-implantation development in mouse

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Figure S1

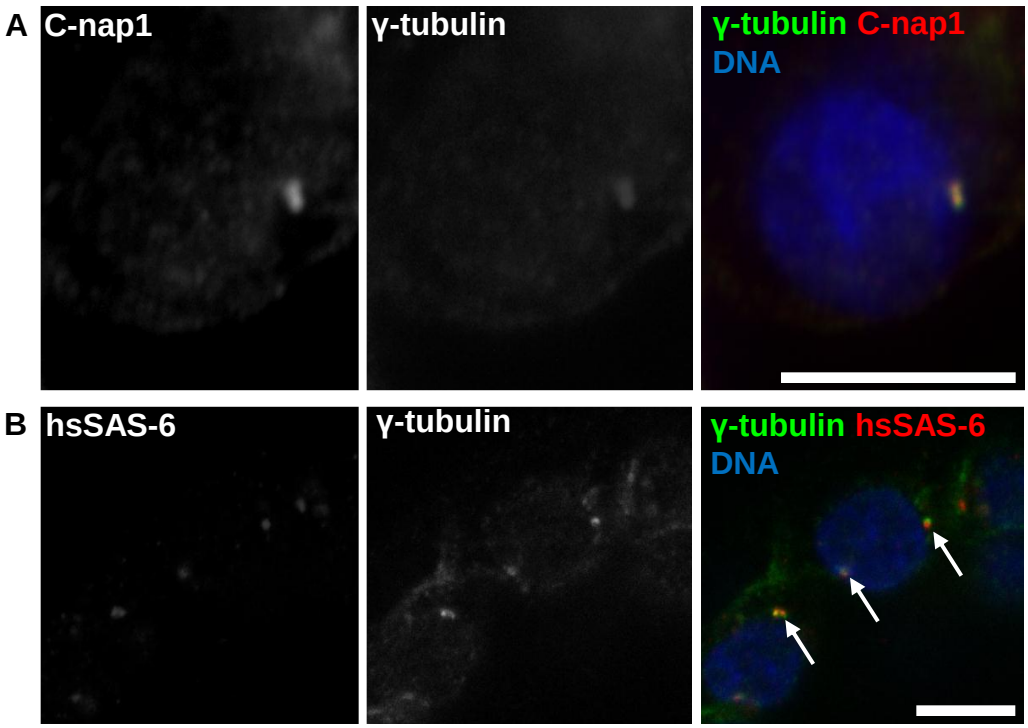

A
C-nap1
γ-tubulin
γ-tubulin C-nap1
DNA
B
hsSAS-6
γ-tubulin
γ-tubulin hsSAS-6
DNA

Figure S2

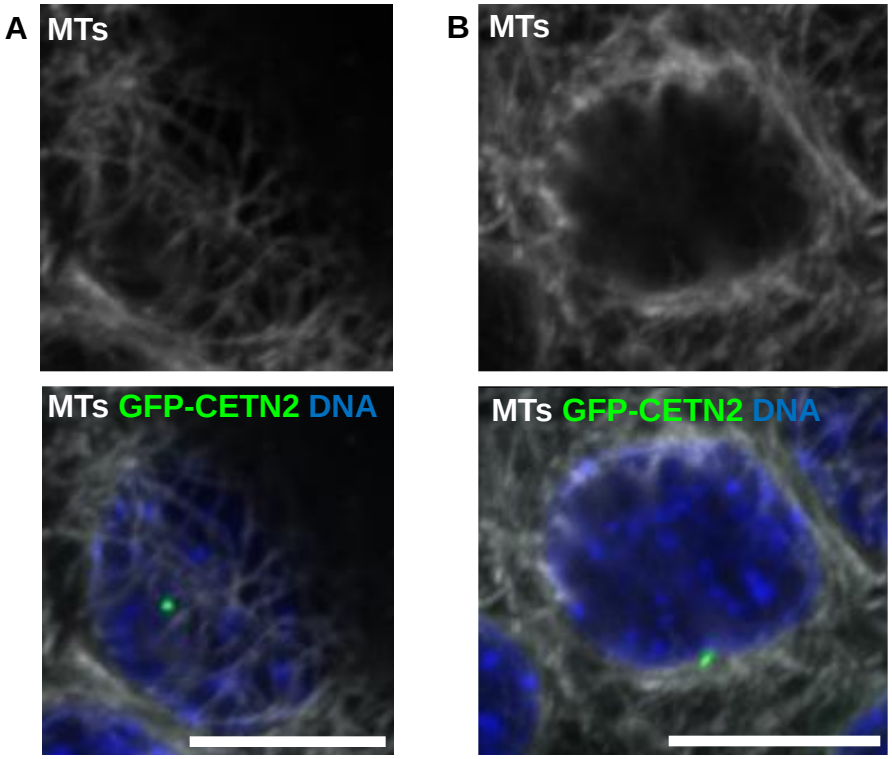


Figure S3

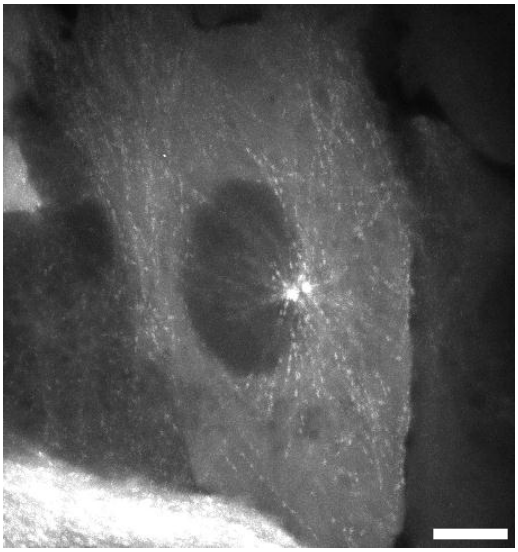


Figure S4

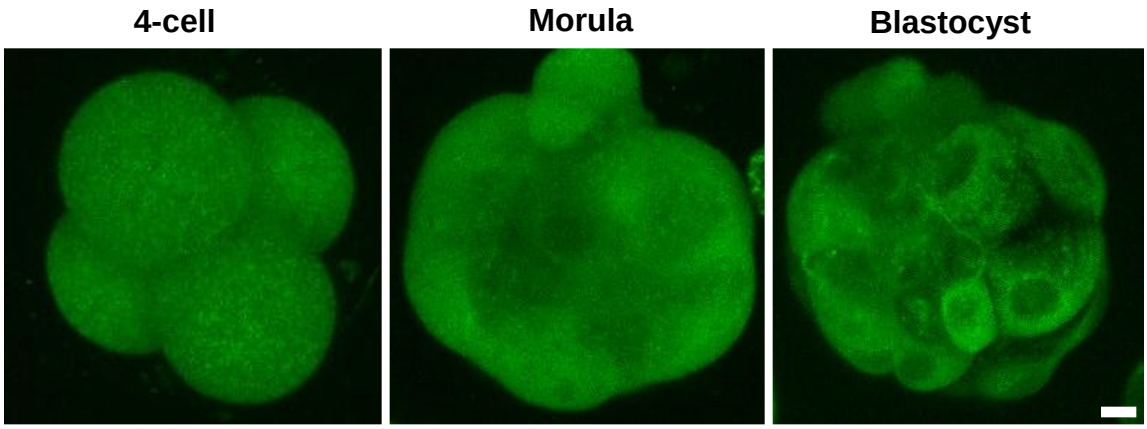


Figure S5

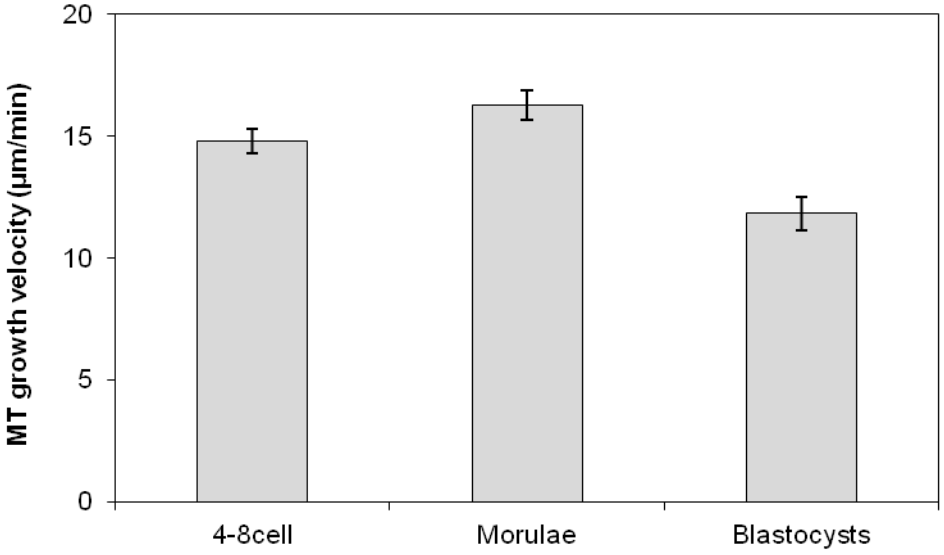
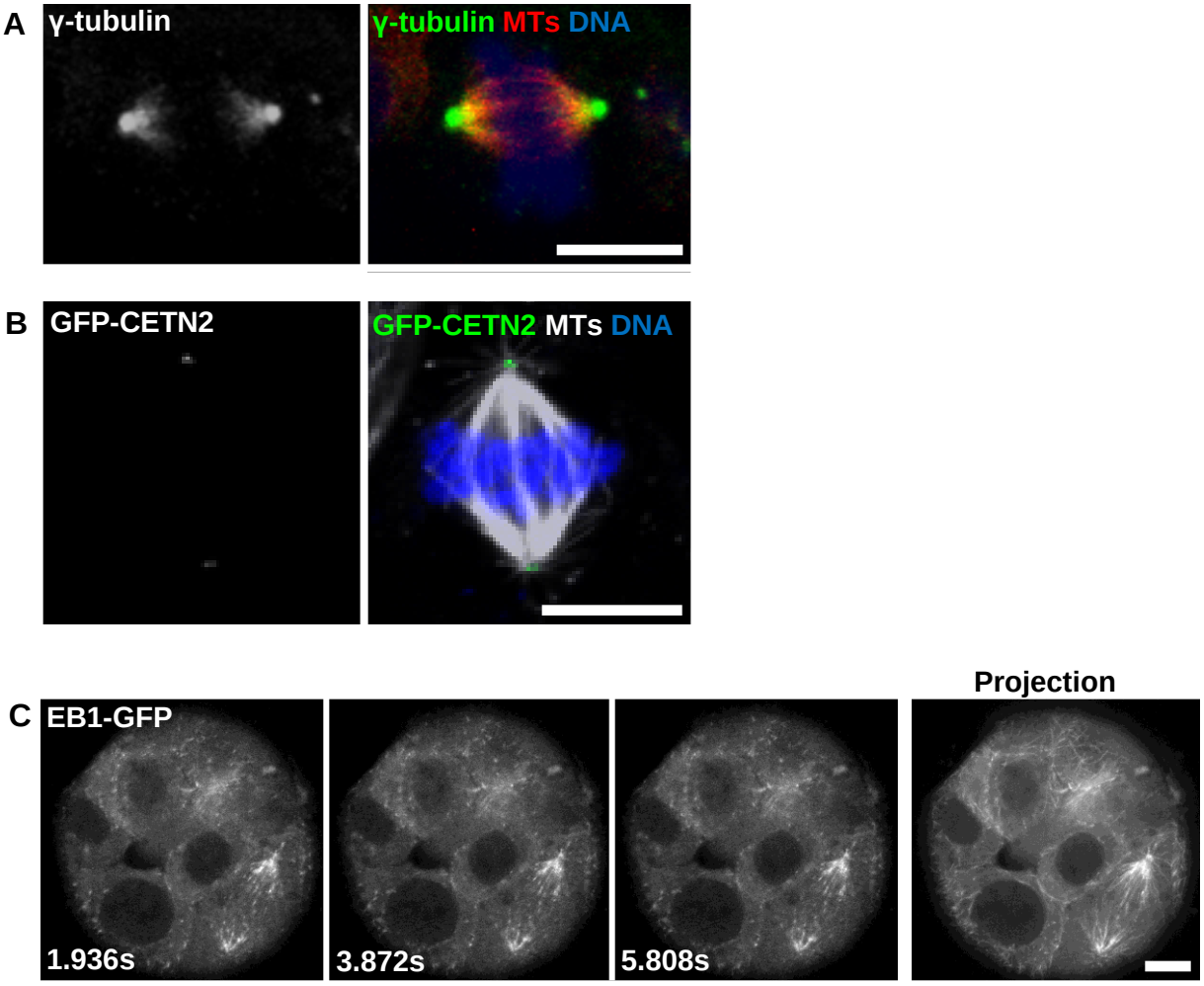

MT growth velocity (µm/min)
20
15
10
5
0
4-8cell
Morulae
Blastocysts

Figure S6


A
γ-tubulin
γ-tubulin MTs DNA
B
GFP-CETN2
GFP-CETN2 MTs DNA
C
EB1-GFP
1.936s
3.872s
5.808s
Projection

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Immunolocalisation of known centrosomal proteins to centrosomes in blastocysts. Antibodies used are (A) mouse anti-C-nap1 (BD biosciences) and (B) rabbit anti-hsSAS-6 (gift from Pierre Gönczy [46]). Scale 10μm.

Figure S2. Additional examples of centriole positioning relative to the MT network in embryos. Note in S2A the centrioles are located at a MT-sparse site, as was the case for the vast majority of centrioles examined. S2B shows an example in which the centrosome lies in close apposition to a thickening of MTs. Centrioles were judged to be in apposition to a MT thickening in 62/433 of cases (~14%). Scale 10μm.

Figure S3. Example of an EB1::GFP-expressing somatic cultured cell. Images from a movie generated by Lynn Cassimeris (Bethlehem, PA) of an LLCPK (porcine kidney epithelial) cell stably expressing EB1::GFP. Note that the centrosome is easily identifiable as an enrichment of EB1::GFP fluorescence adjacent to the nucleus, and that EB1::GFP comets emanate radially from that major MTOC. Scale 10μm.

Figure S4. (A) Z-projection images of EB1::GFP in early embryos. Note the absence of a major accumulation of EB1::GFP such as is seen in EB1::GFP-expressing somatic cells. Scale 10μm.

Figure S5. Analysis of MT growth velocity in 4-8-cell, morula, and blastocyst stage embryos. Tracks were identified and velocities measured using *plusTipTracker* software. 14589-19707 tracks were examined from 20-21 movies per developmental stage. Data shown is an average of the mean velocity calculated from each movie examined. Blastocysts were significantly different from both morulae and 4-8 cell embryos (ANOVA + Tukey-Kramers, $P<0.01$).

Figure S6. Centrosomes localise to spindle poles in embryonic mitosis. (A) Typical images of mitotic spindles in blastocysts. Centrosomes labelled with γ-tubulin antibodies (top panel), or GFP::CETN (bottom). Note that centrosomes are located at spindle poles. (B) EB1::GFP analysis of a blastocyst spindle. Scale 10μm.