Breuer et al., http://www.jcb.org/cgi/content/full/jcb.201005065/DC1

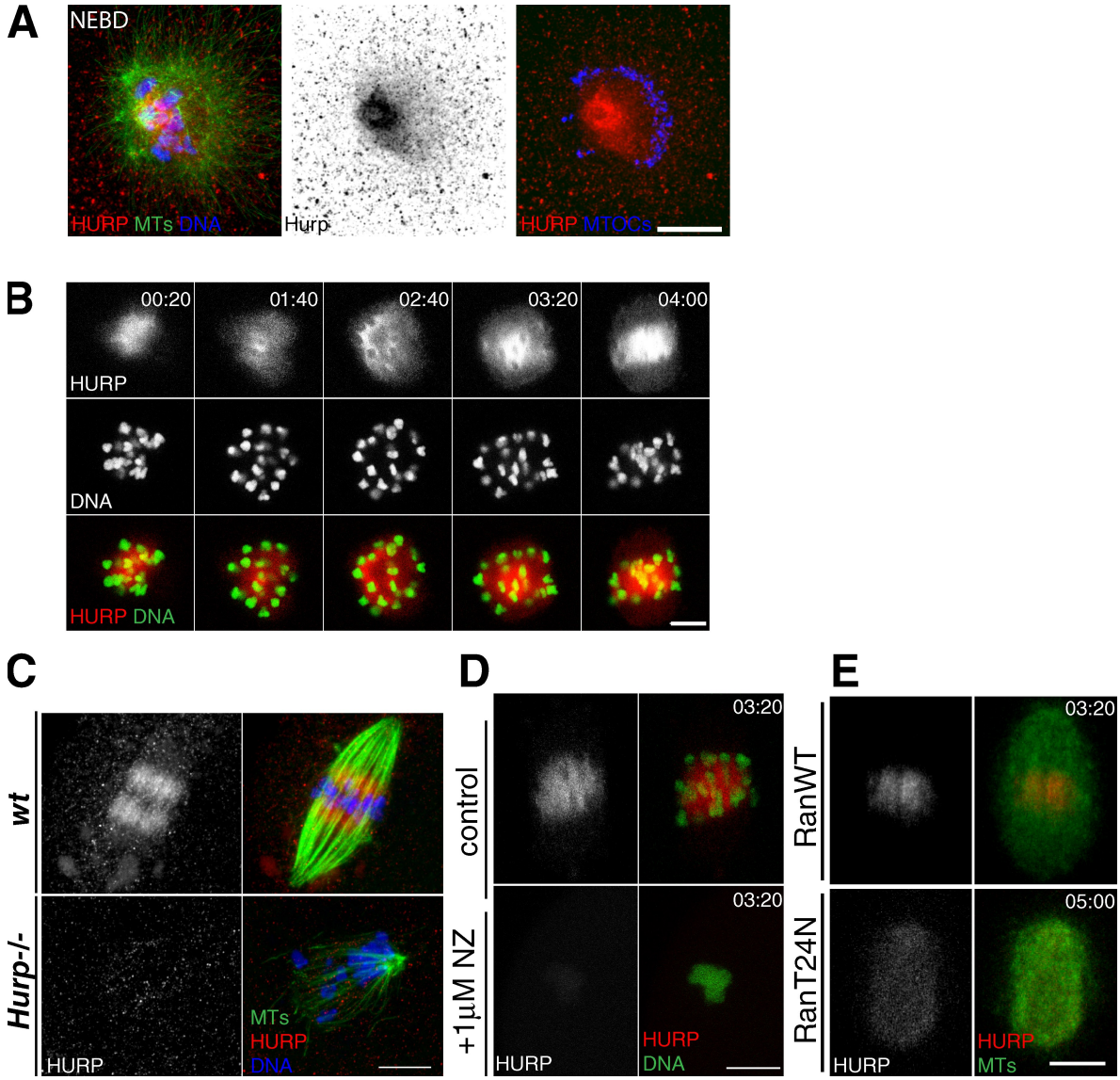


Figure S1. **HURP labels MTs in the vicinity of chromosomes and is undetectable in *Hurp*-deficient oocytes, and its localization depends on the presence of MTs and RanGTP.** (A) HURP localizes between the chromosomes and MTOCs. An oocyte was observed at NEBD and labeled for HURP, MTs (left), and pericentrin (blue, right). (B) HURP is enriched around chromosomes in early prometaphase. Images of live oocytes expressing H2B-RFP and GFP-HURP. (C) HURP cannot be detected in *Hurp*$^{-/-}$ oocytes. Images of *wt* and *Hurp*$^{-/-}$ oocytes stained with Hoechst (blue), tubulin for MTs, and HURP. (D) HURP localization depends on MTs. Images of live oocytes expressing GFP-HURP and H2B-RFP. Nocodazole (NZ) was added after NEBD. (E) RanGTP is required for HURP accumulation at the spindle center. Live oocytes expressing GFP-HURP, EB3-mCherry together with RanWT (top), or with inactive RanT24N (bottom). All times are given in hours and minutes after NEBD. Bars, 10 µm.

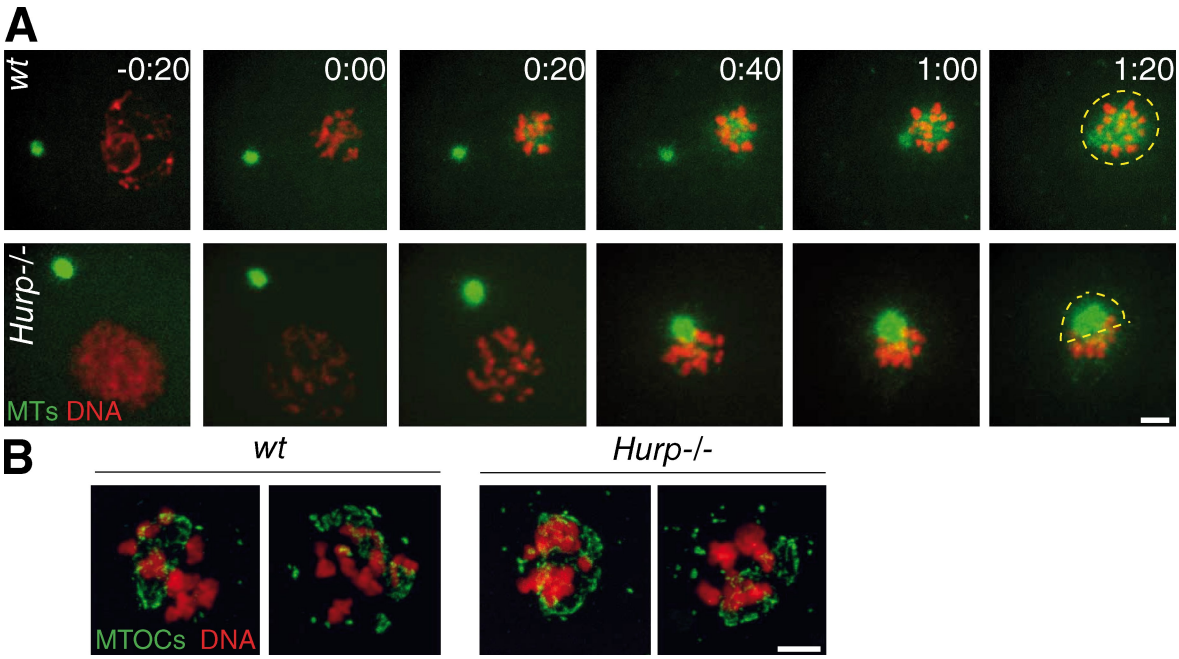


Figure S2. **MTOC distribution in *Hurp*-deficient oocytes is comparable with *wt* at very early steps of meiotic maturation.** (A) MTOC distribution in prophase I is comparable in *wt* versus $Hurp^{-/-}$. Oocytes express EB3-GFP (green) and H2B-RFP (red). MTs appear as cytoplasmic aster, which is progressively captured by the chromatin and incorporated symmetrically in *wt* or asymmetrically in $Hurp^{-/-}$ oocytes later on (area highlighted with the yellow dashed lines). (B) MTOC distribution in *wt* versus $Hurp^{-/-}$ oocytes is similar at NEBD. MTOCs were labeled using pericentrin (green) and DNA. All times are given in hours and minutes after NEBD. Bars, 10 µm.

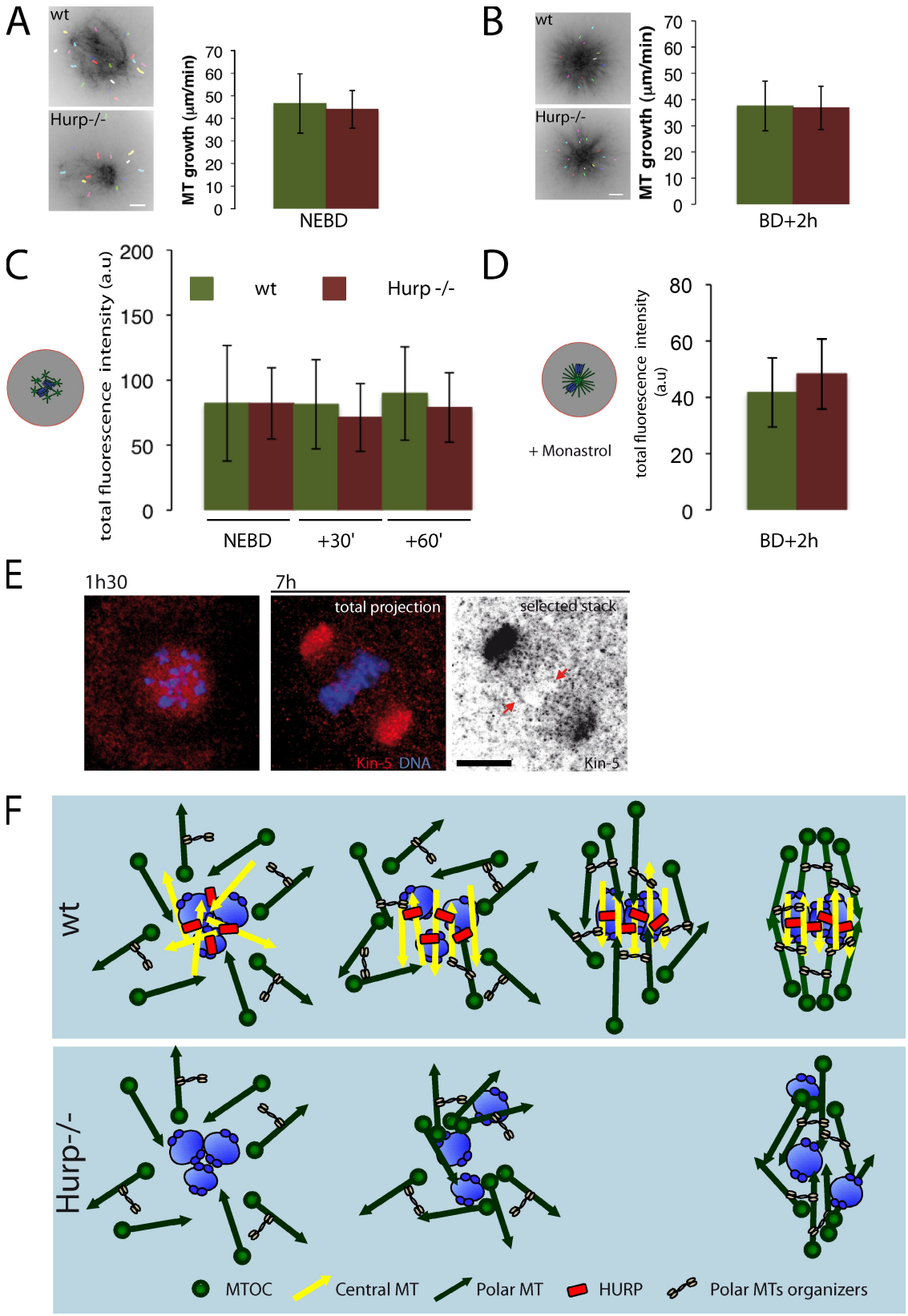
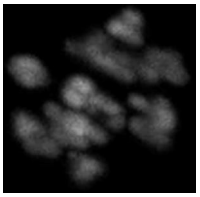
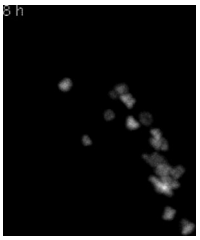


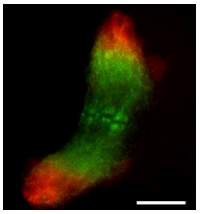
Figure S3. **MT growth at NEBD is not affected in *Hurp*-deficient oocytes.** (A) MT growth at NEBD is not affected in *Hurp*$^{-/-}$. Growing MT plus ends were tracked manually (colored tracks) in live oocytes expressing EB3-GFP and H2B-RFP (wt: *n* = 112; and *Hurp*$^{-/-}$: *n* = 67). The velocity was assessed in the chromosome vicinity at NEBD. No significant difference was observed between groups for growth at MTOCs (P = 0.1505). Each picture is the maximum projection of 20 images taken over 5 s. Bar, 5 µm. (B) MT growth in monasters is not affected in *Hurp*$^{-/-}$. The velocity was assessed in the chromosome vicinity after addition of 100 µM monastrol (right; NEBD + 2 h). No significant difference of MT growth was observed between the two groups (P = 0.5226). Bar, 5 µm. (C) Total oocyte fluorescence intensity is similar in *wt* and *Hurp*$^{-/-}$ oocytes. The fluorescence signal intensity of MTs from live oocytes (*wt* and



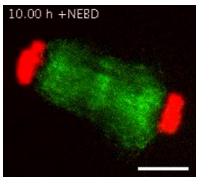
Video 1. **Chromosome segregation in a *wt* oocyte.** Time-lapse video showing a live *wt* oocyte expressing H2B-RFP starting from NEBD + 7 h. Maximal Z projections of 10 confocal sections (Z step: 4 µm). Time is given in hours after NEBD. Acquisition is every 20 min.



Video 2. **Lagging chromosomes at anaphase I in a *Hurp*$^{-/-}$ oocyte.** Time-lapse video showing a live *Hurp*$^{-/-}$ oocyte expressing H2B-RFP starting from NEBD + 7 h. Maximal Z projections of 10 confocal sections (Z step: 4 µm). Time is given in hours after NEBD. Acquisition is every 20 min.

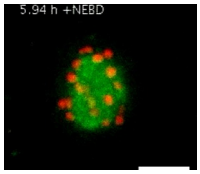


Video 3. **Dynamic localization of HURP in vivo.** Time-lapse video showing a live oocyte expressing EB3-mCherry (green) and GFP-HURP (red) throughout meiotic maturation. Maximal Z projections of nine confocal sections taken every 3 µm. HURP alone is on the left, and the merge with EB3 is on the right. Time is given in hours after NEBD. Acquisition is every 15 min. Bar, 10 µm.

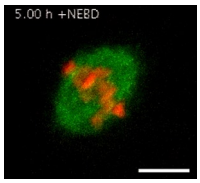


Video 4. **Meiotic maturation in a *Hurp*$^{+/-}$ oocyte.** Time-lapse video showing a live *Hurp*$^{+/-}$ oocyte expressing EB3-GFP (green) and H2B-RFP (red) throughout meiotic maturation. Maximal Z projections of 10 confocal sections (Z step: 4 µm). Time is given in hours after NEBD. Acquisition is every 20 min. Bar, 10 µm.

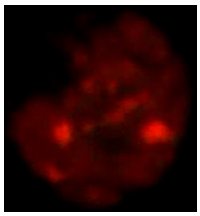
Hurp$^{-/-}$*)* expressing EB3-GFP and H2B-RFP was assessed in the whole cytoplasm in time intervals after NEBD. The MT signal was not different in *Hurp*$^{-/-}$ oocytes (n = 9) versus *wt* (n = 10) oocytes with nonsignificant p-values (P = 0.9959, 0.485, and 0.4717). (D) MT fluorescence intensity is similar in *wt* and *Hurp*$^{-/-}$ oocytes after addition of 100 µM monastrol (NEBD + 2 h). The MT signal was not different in *Hurp*$^{-/-}$ oocytes (*wt*: n = 9; and *Hurp*$^{-/-}$: n = 7). P-value for monasters is nonsignificant (P = 0.3057). Error bars represent standard deviation. (E) Kinesin-5 labels MTs in the vicinity of chromosomes in prometaphase I and in spindle poles and interpolar MTs in MI (red arrows, right). Prometaphase and MI oocytes labeled for Kinesin-5 and chromosomes. Bar, 10 µm. (F) Model of HURP function in meiosis I spindle assembly. At NEBD, MTOCs and MTs are randomly organized around the chromosomes (blue). HURP, possibly acting in concert with Kinesin-5, promotes the assembly of interpolar MTs (light green) in the central region of the spindle close to chromosomes. This array promotes MTOC sorting, pole formation, and bipolarity maintenance. HURP-independent factors (gray) participate in spindle pole organization. In *Hurp*$^{-/-}$ oocytes (bottom), the latter solely contribute to form an unstable bipolar structure. a.u., arbitrary units.



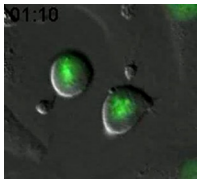
Video 5. **Meiotic maturation in a *Hurp*$^{-/-}$ oocyte with polar body extrusion.** Time-lapse video showing a live *Hurp*$^{-/-}$ oocyte expressing EB3-GFP (green) and H2B-RFP (red) throughout meiotic maturation. Maximal Z projections of 10 confocal sections (Z step: 4 µm). Time is given in hours after NEBD. Acquisition is every 20 min. Bar, 10 µm.



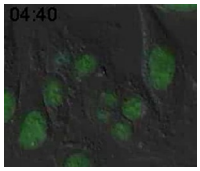
Video 6. **Meiotic maturation in a *Hurp*$^{-/-}$ oocyte with prolonged metaphase I.** Time-lapse video showing a live *Hurp*$^{-/-}$ oocyte expressing EB3-GFP (green) and H2B-RFP (red) throughout meiotic maturation. Maximal Z projections of 10 confocal sections (Z step: 4 µm). Time is given in hours after NEBD. Acquisition is every 20 min. Bar, 10 µm.



Video 7. **GFP-HURP overexpression.** Time-lapse video showing a live *wt* oocyte overexpressing GFP-HURP and expressing H2B-RFP. Maximal Z projections of 10 confocal sections (Z step: 4 µm). Acquisition is every 30 min.



Video 8. **Mitosis in control U2OS cells overexpressing PLK4.** Time-lapse video showing live control (luciferase RNAi) U2OS cells expressing GFP-H2B and with inducible overexpression of PLK4, which undergo bipolar division. Time is given in hours and minutes. Acquisition is every 10 min.



Video 9. **Mitosis in HURP-depleted U2OS cells overexpressing PLK4.** Time-lapse video showing live HURP-depleted U2OS cells expressing GFP-H2B and with inducible overexpression of PLK4, which undergo multipolar divisions. Time is given in hours and minutes. Acquisition is every 10 min.