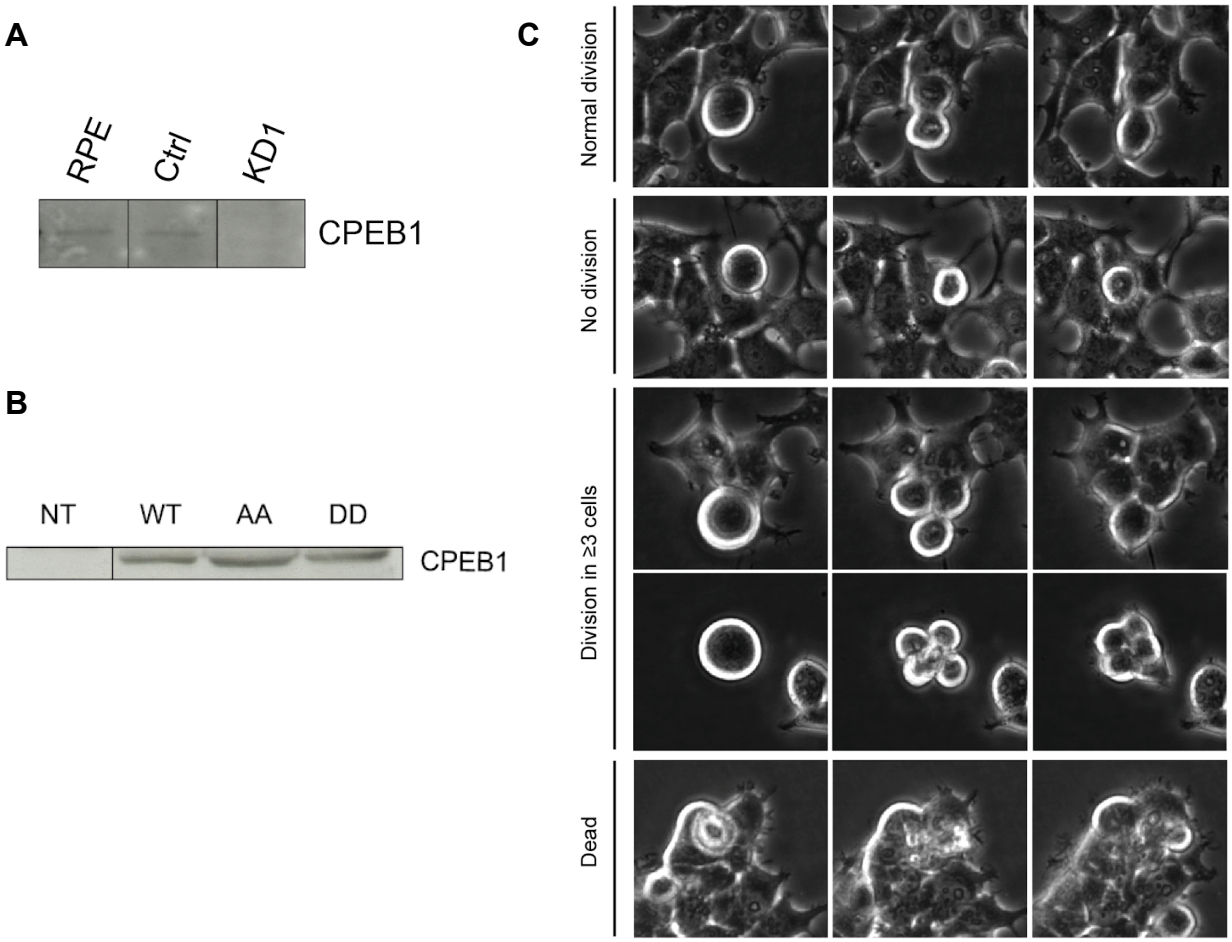


FIGURE S1. **(A)** Western Blot showing the efficiency of CPEB1 depletion in Fig. 1B and E. **(B)** Overexpression of CPEB1 WT and the AA and DD mutants in Fig. 1F, shown by Western Blot. Conditions correspond to non-transfected cells (NT), cells transfected with wild type CPEB1 (WT), cells transfected with the non-phosphorylated CPEB1 (AA), or cells transfected with the phosphor-mimetic CPEB1 mutant (DD). **(C)** Representative still images from the movies illustrating each one of the phenotypes scored in Fig. 1F.

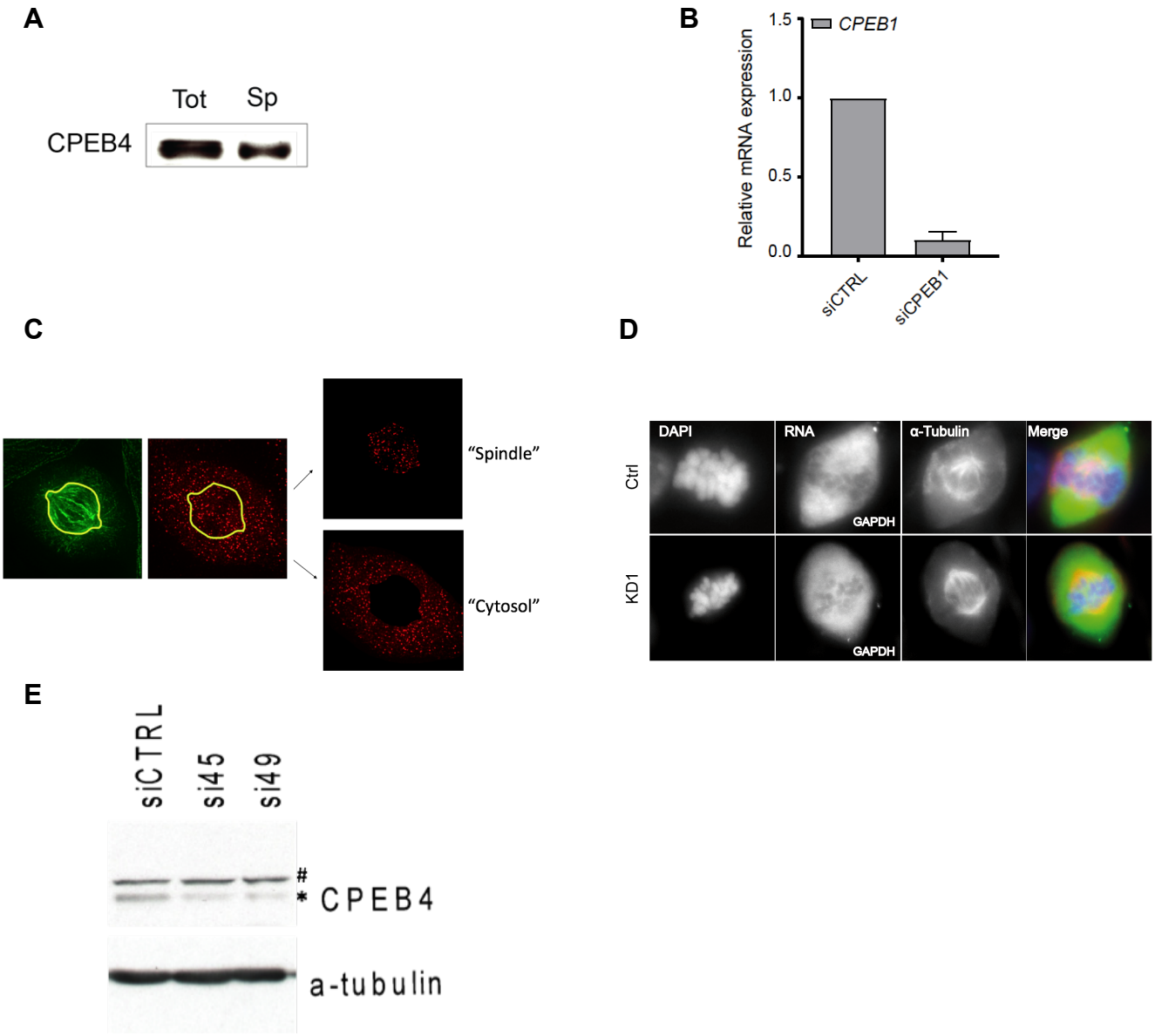
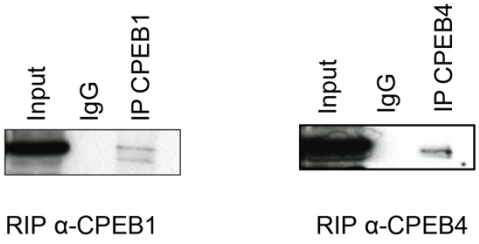
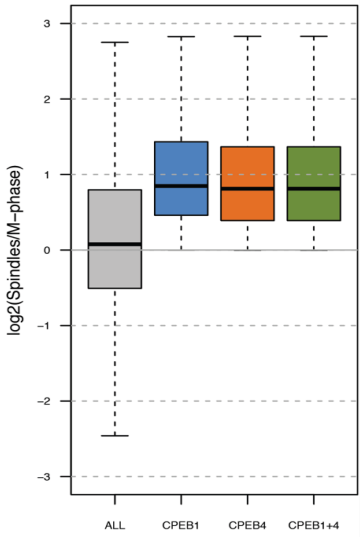


FIGURE S2. (A) Western Blot analysis of total cell extracts (Tot) or in purified mitotic spindles (Sp) for CPEB4 in Hela S3 cells. **(B)** RT-qPCR analysis of U2OS cells treated with siRNA Control (siCTRL) or siRNA CPEB1 (siCPEB1). Average fold change (calculated as a value of 2^-ΔΔCt) of three independent experiments is shown here. Data were normalized to siCTRL, which was set to 1. **(C)** Representative image for showing the delineation of the spindle area to be quantified in Figure 2F. **(D)** FISH images of *GAPDH* mRNA of cells infected with shRNAs control (Ctrl) or against CPEB1 (KD1). Chromosomes and spindles were visualized by DAPI staining and α-tubulin immunofluorescence, respectively. **(E)** Efficiency of two different siRNAs against CPEB4 checked by Western Blot of CPEB4 in hTERT-RPE1 cells. α-tubulin is used as a loading control. *specific CPEB4 band; # nonspecific band.

A



B



C

Category	GO-Term	Examples
CPEB1 + Spindle	cell cycle	PDS5B, BUB3, CDC25A, CCNA2, CCNB1, NCAPD2, PCN T, PLK1, RCC2
CPEB4 + Spindle	cell cycle	LATS2, MAD1L1, NSL1, NIPBL, CLASP1, CLASP2, FZR1, KIF2C, LZTS2, RCC1, STAG1, PSMD11, SKP2, ZW10, CENPE, UBE2I, ZC3HC1, NUP37

D

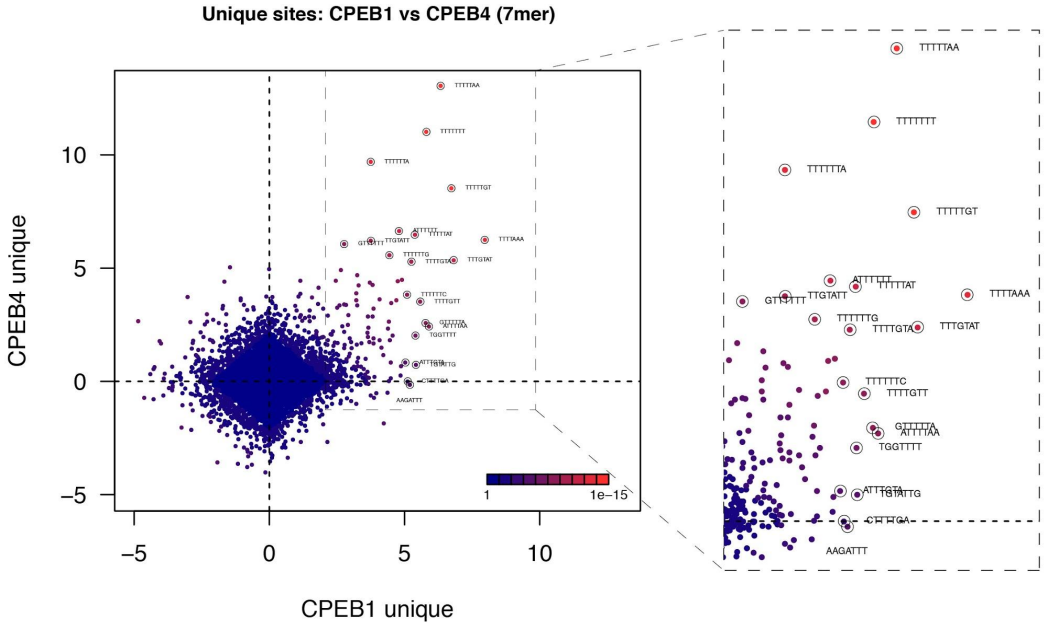


FIGURE S3. (A) Western Blots for RNA-IPs with α-CPEB1 (left) or α-CPEB4 (right) antibodies in Hela S3 cells. IgG antibodies were used as negative controls. **(B)** Plot showing log2 fold enrichment in spindles compared M-phase in all mRNAs or mRNAs bound by CPEB1, CPEB4 or both CPEBs. **(C)** Examples of cell cycle–related spindle-localized mRNA targets of CPEB1 or CPEB4.**(D)** Naïve discovery of enriched seven-nucleotide motives (7mers) in CPEB4 and CPEB1 bound 3' UTRs.

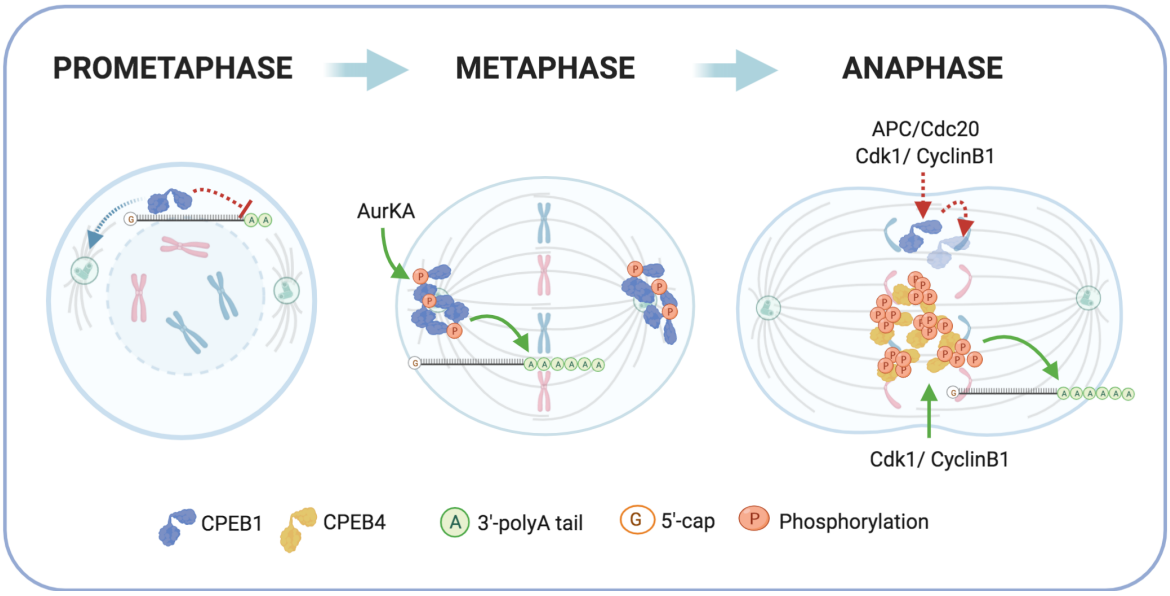


FIGURE S4. Proposed model for CPEB1 and CPEB4 sequential actions on the mitotic spindle. Created with BioRender.com.

TABLE S1. List for spindle-associated, CPEB1-bound and CPEB4-bound mRNAs.

TABLE S2. Gene Ontology terms Biological Processes for spindle-associated, CPEB1-bound and CPEB4-bound mRNAs.