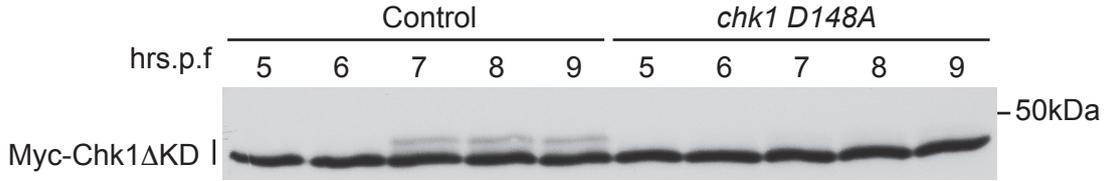
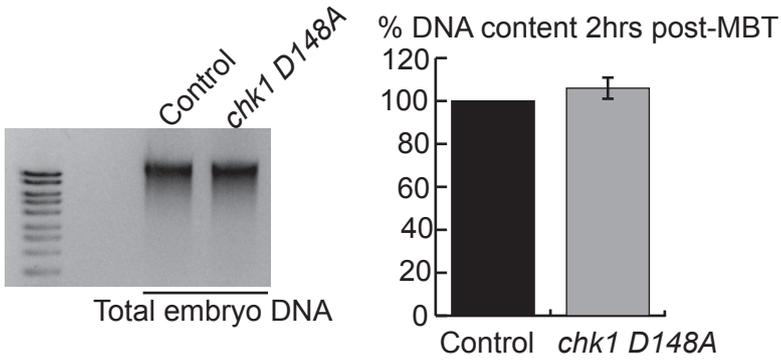


Figure S1

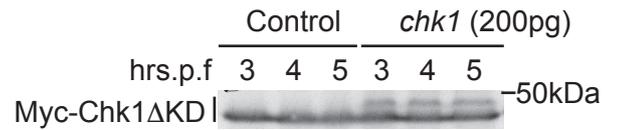
A. Chk1 D148A overexpression inhibits phosphorylation of a Chk1 target



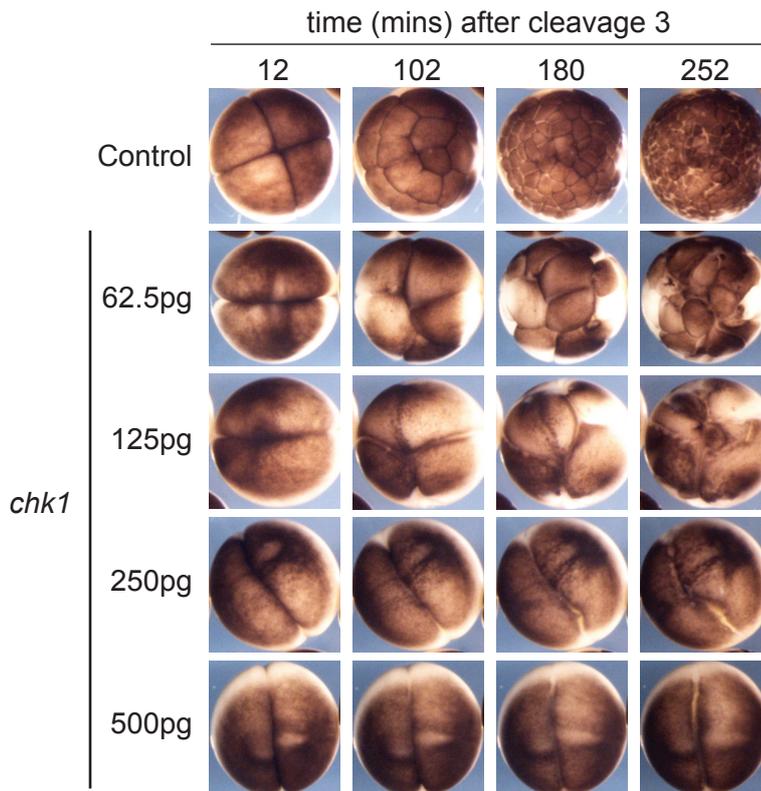
B. Chk1 D148A over-expression alone does not cause an increase in DNA content after the MBT



C. Wild type Chk1 over-expression causes premature Chk1 activation



D. Chk1 is a potent inhibitor of cell cycle progression in the early embryo



E. Drf1 or Dbf4 over-expression does not affect Chk1 activation

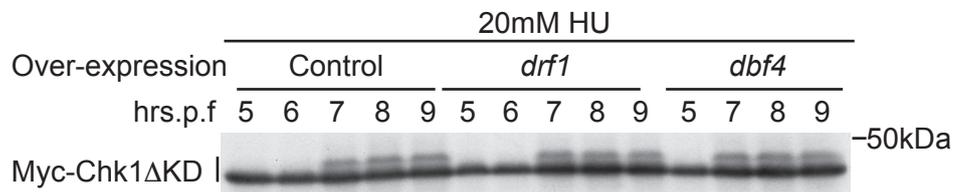


Figure S2.

Only over-expression of *treslin*, *recq4* and *cut5* together with the *chk1 D148A* mutant permits fast MBT cycles

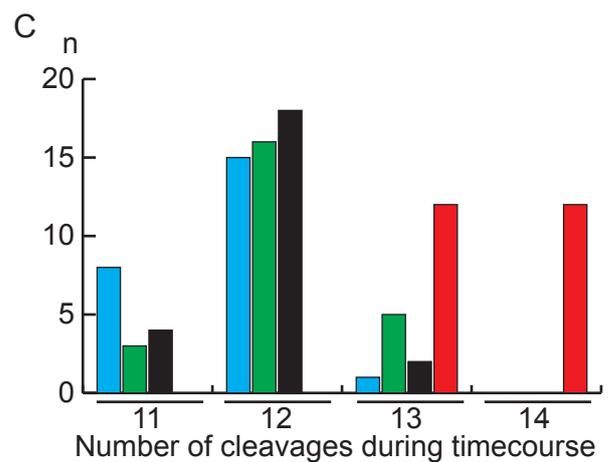
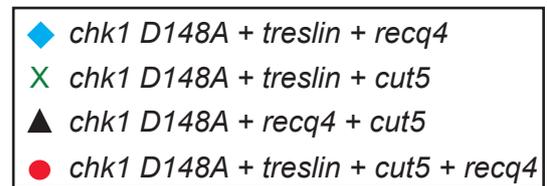
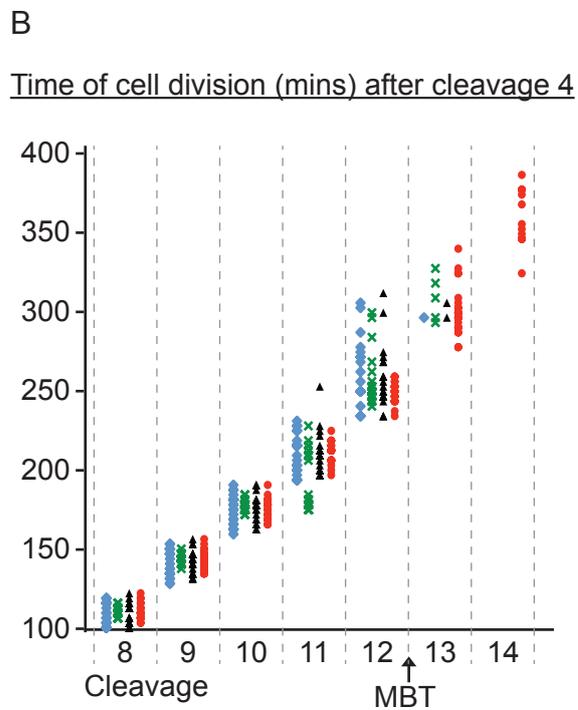
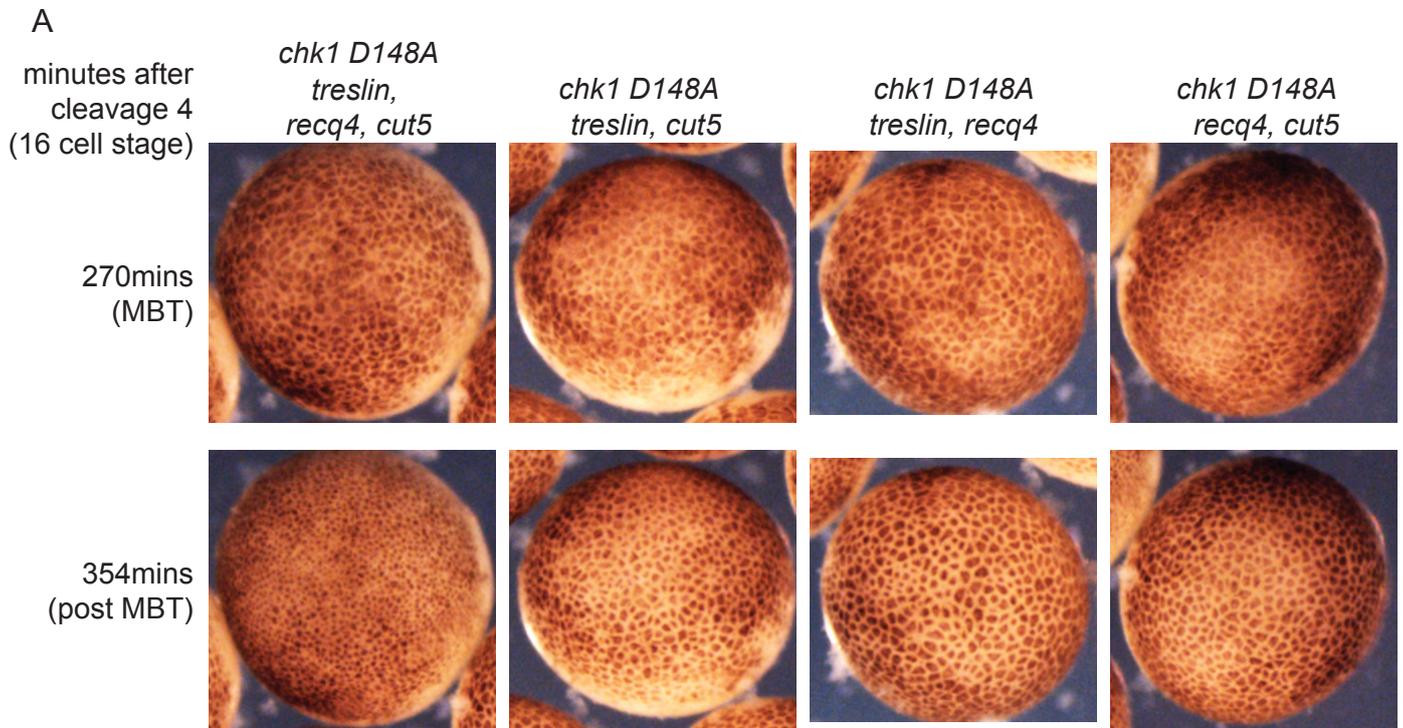


Figure S3. Inhibition of DNA replication by injection of aphidicolin is sufficient to block the cell division, pre-MBT.

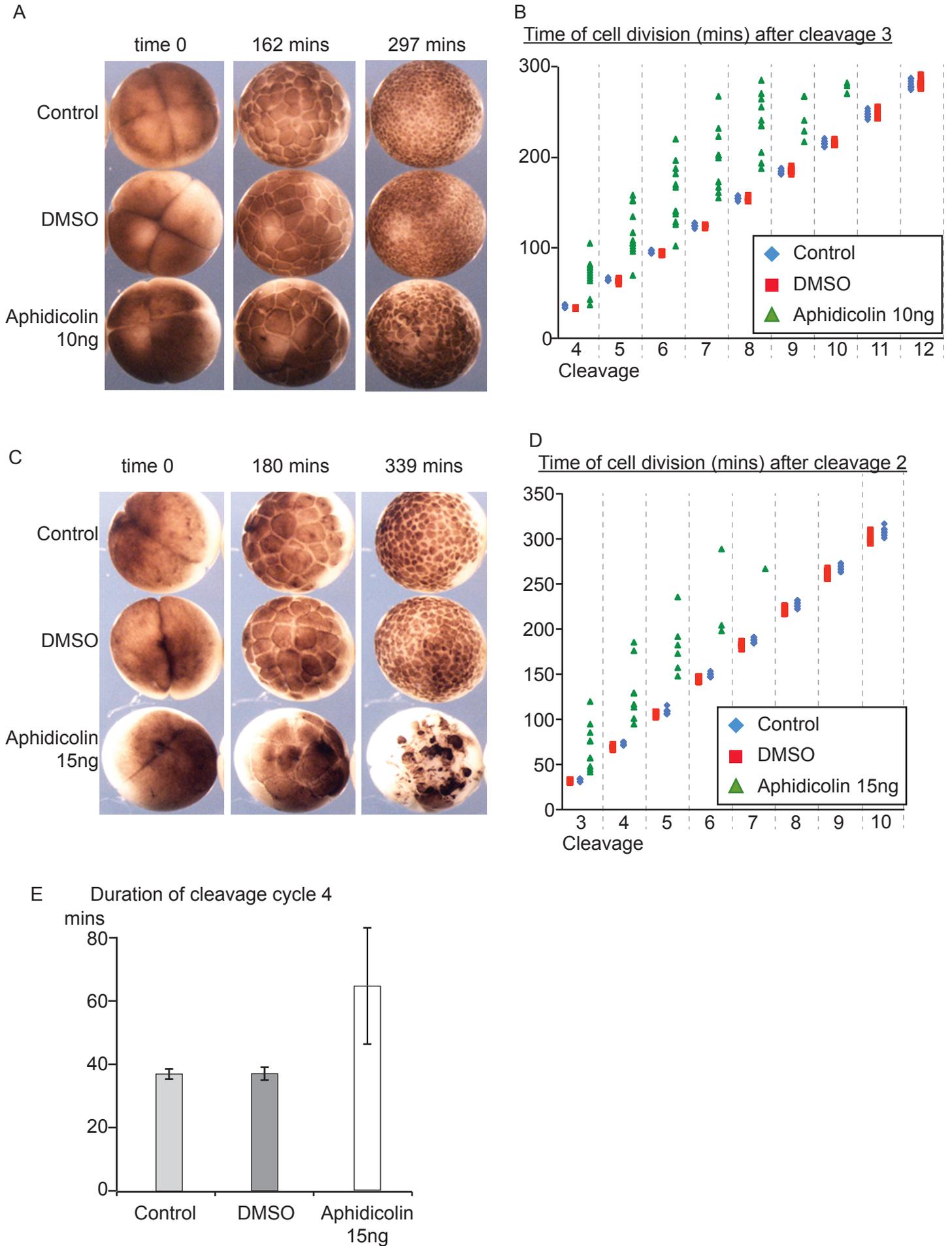


Figure S4.

Over-expression of *cdk1-AF* does not prevent Chk1-mediated elongation of pre-MBT cycles

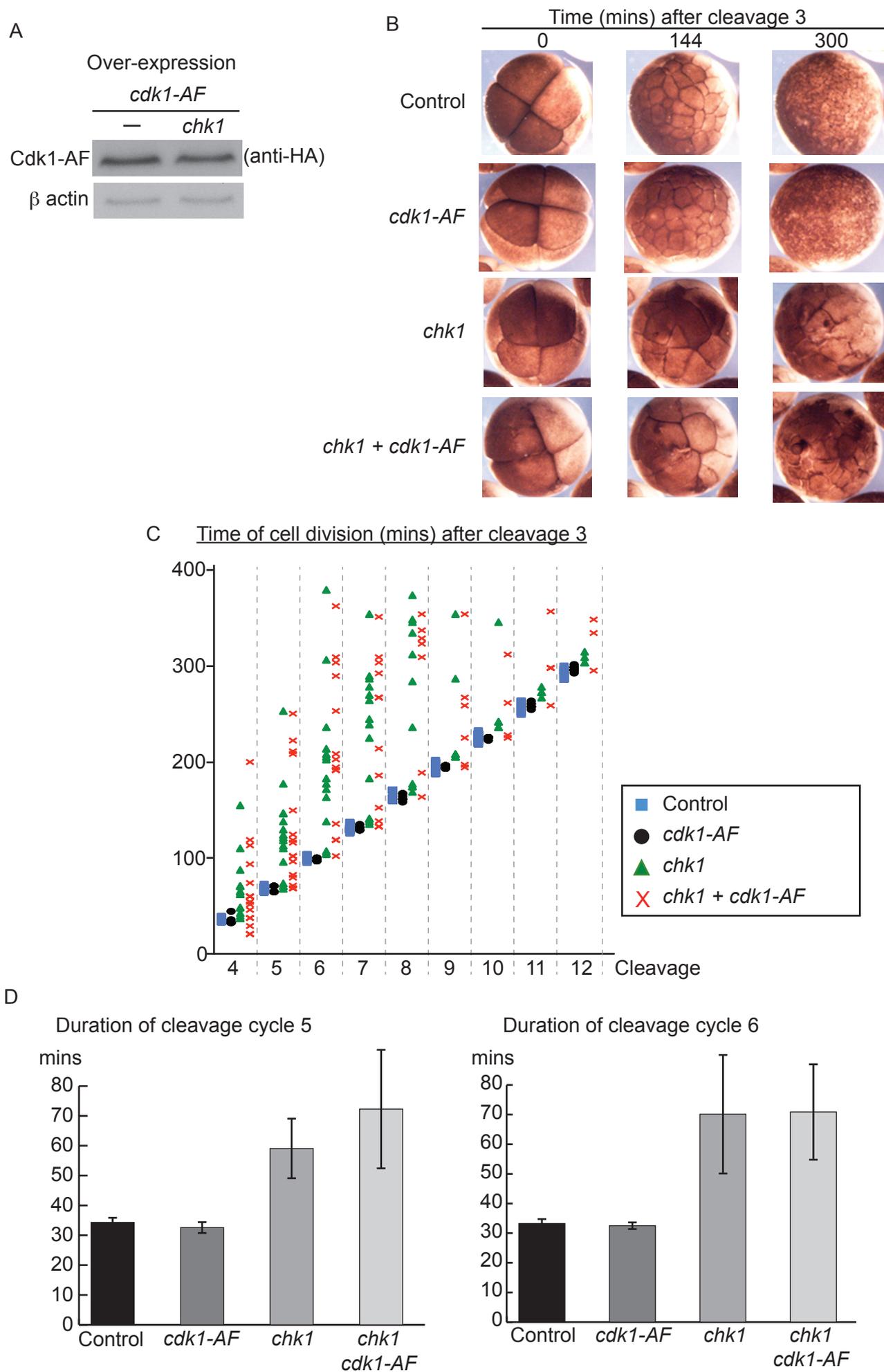


Figure S5

Over-expression of *cdk1-AF* does not affect the elongation of the cell cycle at the MBT

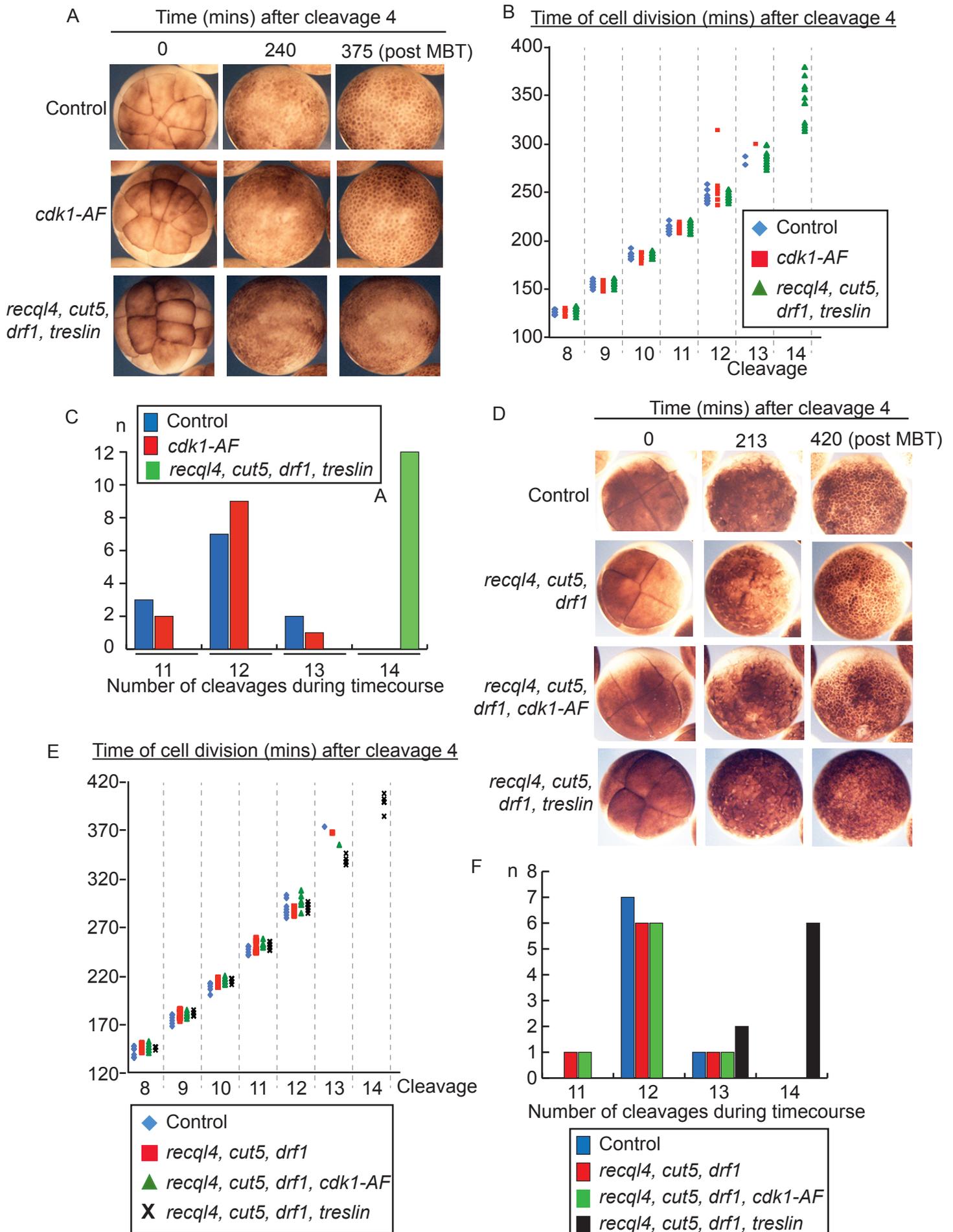


Figure S6

Over-expression of  $\beta$  *trcp* causes earlier Drf1 degradation and premature cell cycle elongation

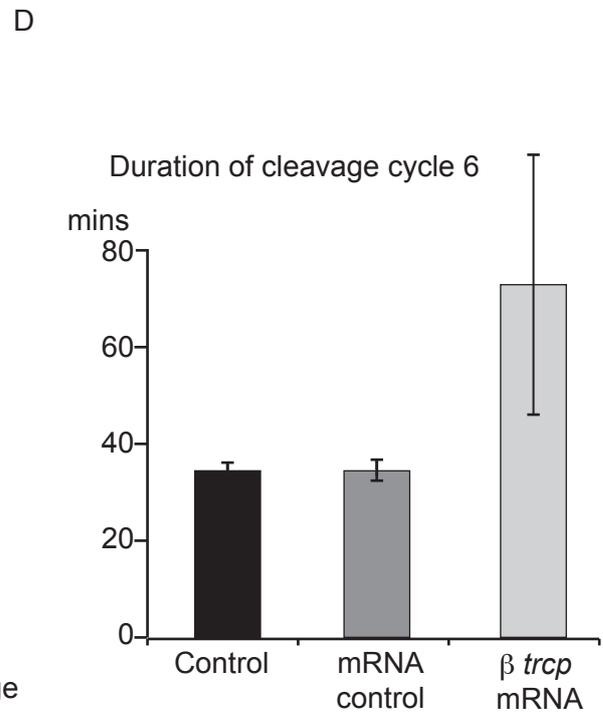
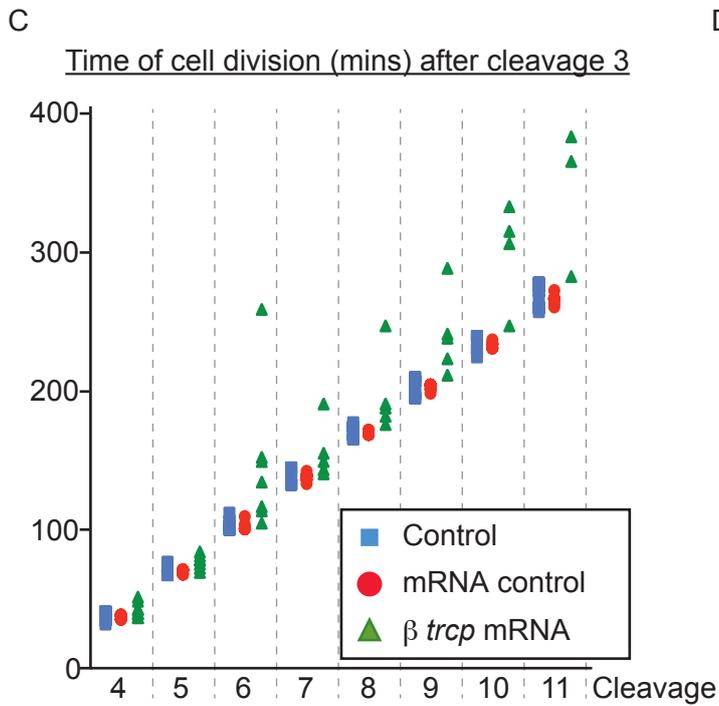
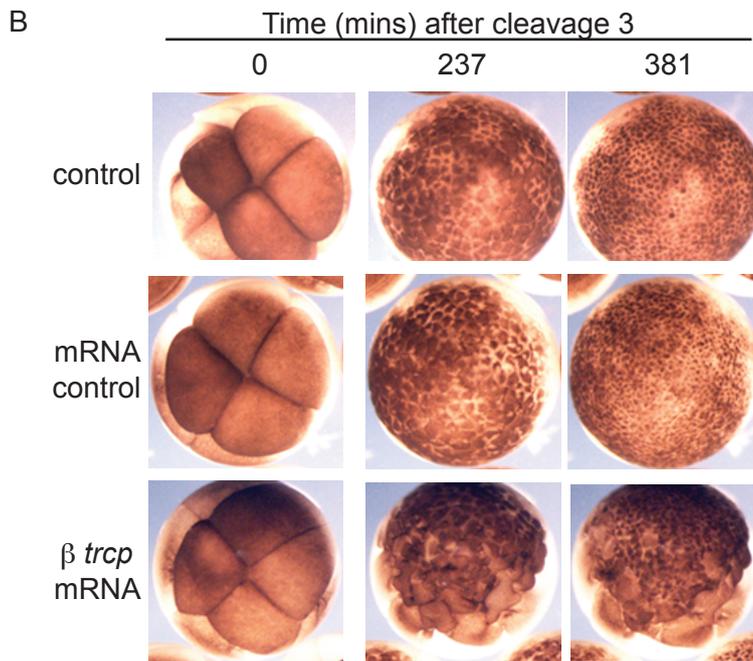
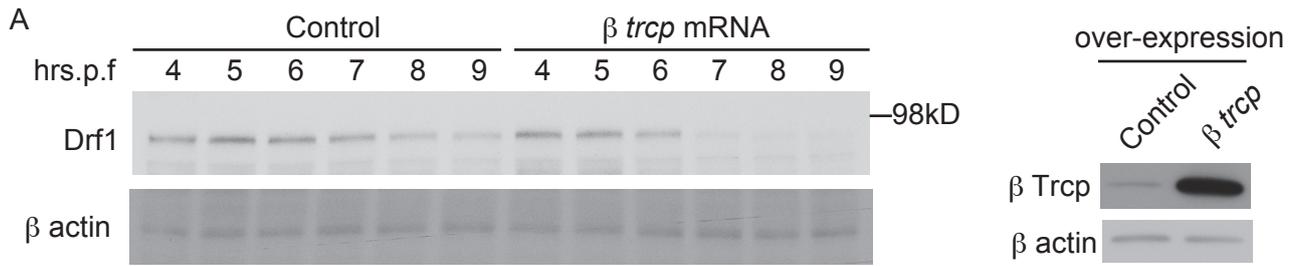
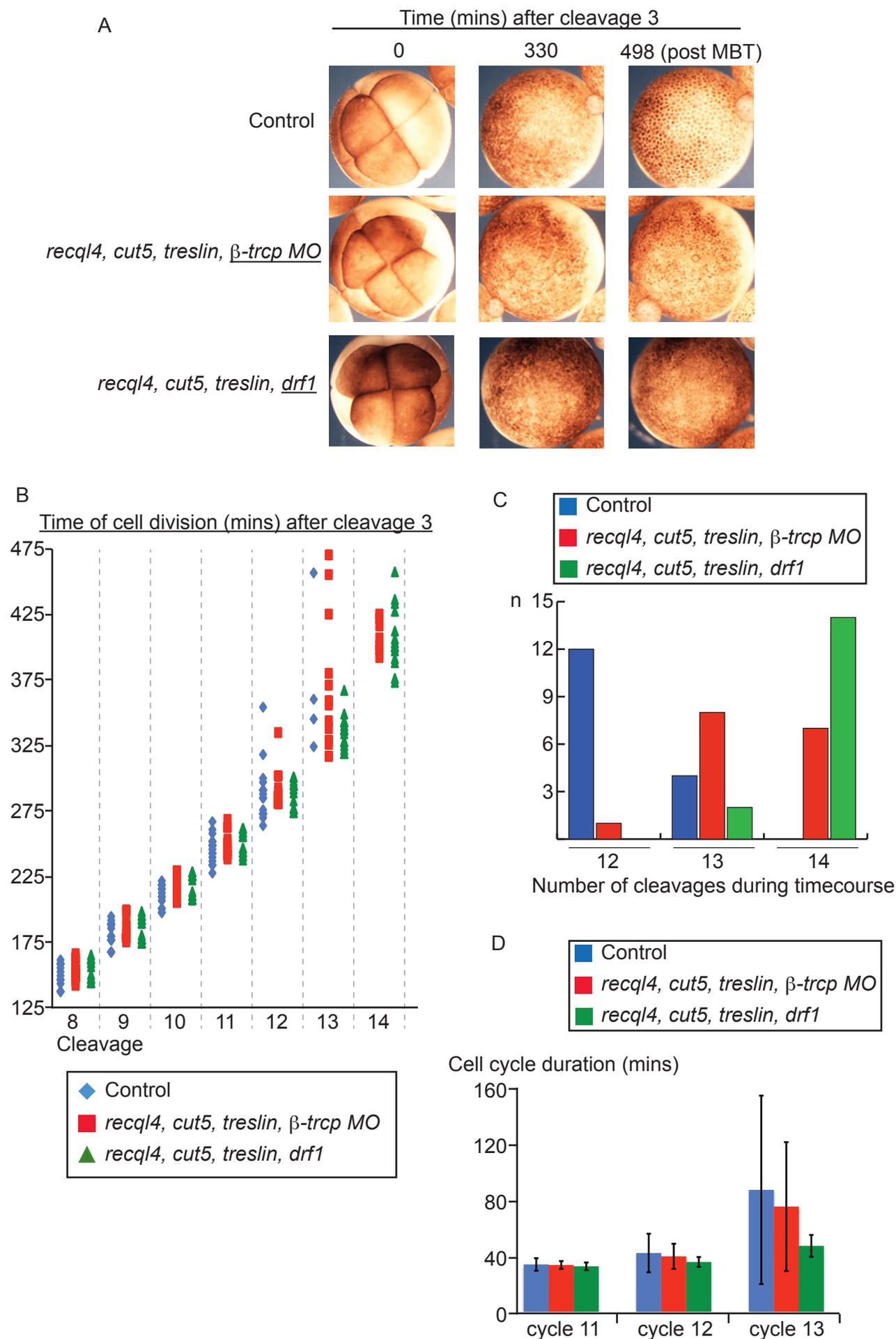


Figure S7

Unlike over-expression, stabilisation of Drf1 by down-regulation of  $\beta$ -trcp is not sufficient to allow rapid, synchronous divisions at the MBT.



## Supplementary figure legends

**Figure S1.** Chk1 inactivation blocks phosphorylation of downstream targets and Chk1 over-expression inhibits cell cycle progression in the early embryo. Related to Figure 1 and 3.

**A.** A Myc tagged fragment of Chk1 lacking the kinase domain (Chk1 $\Delta$ KD) is phosphorylated by endogenous Chk1, resulting in a mobility shift in SDS-PAGE. This is an anti-myc Western blot of Chk1 $\Delta$ KD from staged embryos at the indicated number of hours post fertilisation (hrs.p.f). Embryos were injected at the one cell stage either with water (control) or with mRNA corresponding to the *chk1* dominant negative mutant (D148A).

**B.** The DNA content of embryos 2hrs post MBT, injected at the one-cell stage with either water or *chk1 D148A* mRNA, was quantified on agarose gels (left) using ImageJ. The DNA content of 3 embryos was averaged and the control embryo DNA content set to 1 (right). Data are represented as mean  $\pm$  SD

**C.** As in A except wild type *chk1* mRNA (200pg) was injected.

**D.** Images of embryos injected at the 1-cell stage either with water (control) or with increasing amounts of wild type *chk1* mRNA. Images are from time-lapse movies of these embryos at the indicated times after cleavage 3 (8 cell embryo).

**E.** As in A except embryos were incubated with 20mM HU with or without injection of mRNA for *drf1* or *dbf4*.

**Figure S2.** Over-expression of Treslin, Recq4 and Cut5 is required for rapid cell divisions at the MBT in embryos expressing Chk1 D148A. Related to Figure 2.

**A.** Embryos were injected in both blastomeres at the 2-cell stage with the indicated mRNA or water (control) and followed by time-lapse imaging. The 4<sup>th</sup> division, generating the 16-cell embryo was set to time zero. See also Movie S3.

**B.** The division of individual cells from A were followed throughout the movie. Each time-point represents the division of a single cell. Cleavages 4-7 are excluded for simplicity. n=24 cells from 6 embryos for each condition.

**C.** Total number of divisions each cell in B undergoes until the end of the time-lapse movie. The colour code is the same for both B and C.

**Figure S3.** Injection of aphidicolin is sufficient to block cell division before the MBT. Related to Figure 3.

**A.** Water (control), DMSO or aphidicolin was injected in both blastomeres at the 2-cell stage and followed by time-lapse imaging. The 3<sup>rd</sup> division, generating the 8-cell embryo was set to time zero.

**B.** The division of individual cells from A were followed throughout the movie. Each time-point represents the division of a single cell. n=12 cells from 3 embryos for each condition.

**C,D** as A,B.

**E.** Quantitation of the average duration of cell cycle 4 from C,D. n=12 cells from 3 embryos for each condition. Data are represented as mean  $\pm$  SD, which indicates the synchrony of cell divisions.

**Figure S4.** Over-expression of Cdk1-AF does not prevent premature cell cycle lengthening after over-expression of Chk1. Related to Figure 3.

**A.** Anti HA western blot of N-terminally HA tagged Cdk1-AF from embryos 3 hrs.p.f.

**B.** Water (control), *chk1* mRNA, *cdk1-AF* mRNA or both mRNAs were injected in both blastomeres at the 2-cell stage and followed by time-lapse imaging. Total mRNA injected for *chk1* was 50pg, while for *cdk1-AF* it was 500pg. The 3rd division, generating the 8-cell embryo was set to time zero.

**C.** The division of individual cells from B were followed throughout the movies. Each time-point represents the division of a single cell. n=16 cells from 4 embryos for each condition.

**D.** Quantitation of the average duration of cell cycle 5 (left) and cycle 6 (right) from C. Data are represented as mean  $\pm$  SD, which indicates the synchrony of cell divisions.

**Figure S5.** Over-expression of Cdk1-AF does not affect the duration of the cell cycle at the MBT. Related to Figure 3.

**A.** Embryos were injected in both blastomeres at the 2-cell stage with *cdk1-AF* mRNA (1ng total), the four limiting replication factors (300pg each total) or water (control) and followed by time-lapse imaging. The 4<sup>th</sup> division, generating the 16-cell embryo was set to time zero.

**B.** The division of individual cells from A were followed throughout the movies. Each time-point represents the division of a single cell. n=12 cells from 3 embryos for each condition.

**C.** Total number of divisions each cell in B undergoes until the end of the time-lapse movie. The colour code is the same for both B and C.

**D.** Embryos were injected in both blastomeres at the 2-cell stage with mRNAs or water (control) as indicated and followed by time-lapse imaging. The amounts of injected mRNAs were 500ng total for *cdk1-AF* and 300pg each total for *drf1*, *recq4*, *cut5* and *treslin*. The 4<sup>th</sup> division, generating the 16-cell embryo was set to time zero.

**E.** The division of individual cells from D were followed throughout the movies. Each time-point represents the division of a single cell. n=8 cells from 2 embryos for each condition.

**F.** Total number of divisions each cell in E undergoes until the end of the time-lapse movie. The colour code is the same for both E and F.

**Figure S6.** Over-expression of  $\beta$ -Trcp causes premature cell cycle elongation.

Related to Figure 4.

**A.** (right) Anti  $\beta$ -Trcp western blot from stage 7 embryos (just before MBT) after injection at the 1 cell stage with water (control) or 1ng of  *$\beta$ -trcp* mRNA. (left) Anti-Drf1 western blot from staged embryos.

**B.** Water (control), antisense  *$\beta$ -trcp* mRNA (mRNA control) or  *$\beta$ -trcp* mRNA were injected in both blastomeres at the 2-cell stage and followed by time-lapse imaging. The total amount of injected mRNA was 5ng. The 3rd division, generating the 8-cell embryo was set to time zero.

**C.** The division of individual cells from B were followed throughout the movies. Each time-point represents the division of a single cell. n=8 cells from 2 embryos for each condition.

**D.** Quantitation of the average duration of cell cycle 6 from C. Data are represented as mean  $\pm$  SD, which indicates the synchrony of cell divisions.

**Figure S7.** Over-expression of Drf1 is required to allow rapid, synchronous divisions at the MBT. Related to Figure 7D

**A.** Embryos were injected in both blastomeres at the 2-cell stage with mRNAs, anti- *$\beta$ -trcp* morpholinos (MO) or water (control) as indicated and followed by time-lapse imaging. The total amounts of injected mRNAs/MO were *drf1*, *recq4*, *cut5* and *treslin* (300pg each),  *$\beta$ -trcp* morpholinos (80ng). The 3rd division, generating the 8-cell embryo was set to time zero.

**B.** The division of individual cells from A were followed throughout the movies. Each time-point represents the division of a single cell. n=16 cells from 4 embryos for each condition.

**C.** Total number of divisions each cell in B undergoes until the end of the time-lapse movie. The colour code is the same for B-D.

**D.** Quantitation of the average duration of cell cycles 11-13 from B. Data are represented as mean  $\pm$  SD, which indicates the synchrony of cell divisions. Notably only over-expression of Drf1 (together with the other 3 limiting factors) allows for rapid and synchronous cleavages in cycles 12/13.