Fig. S1

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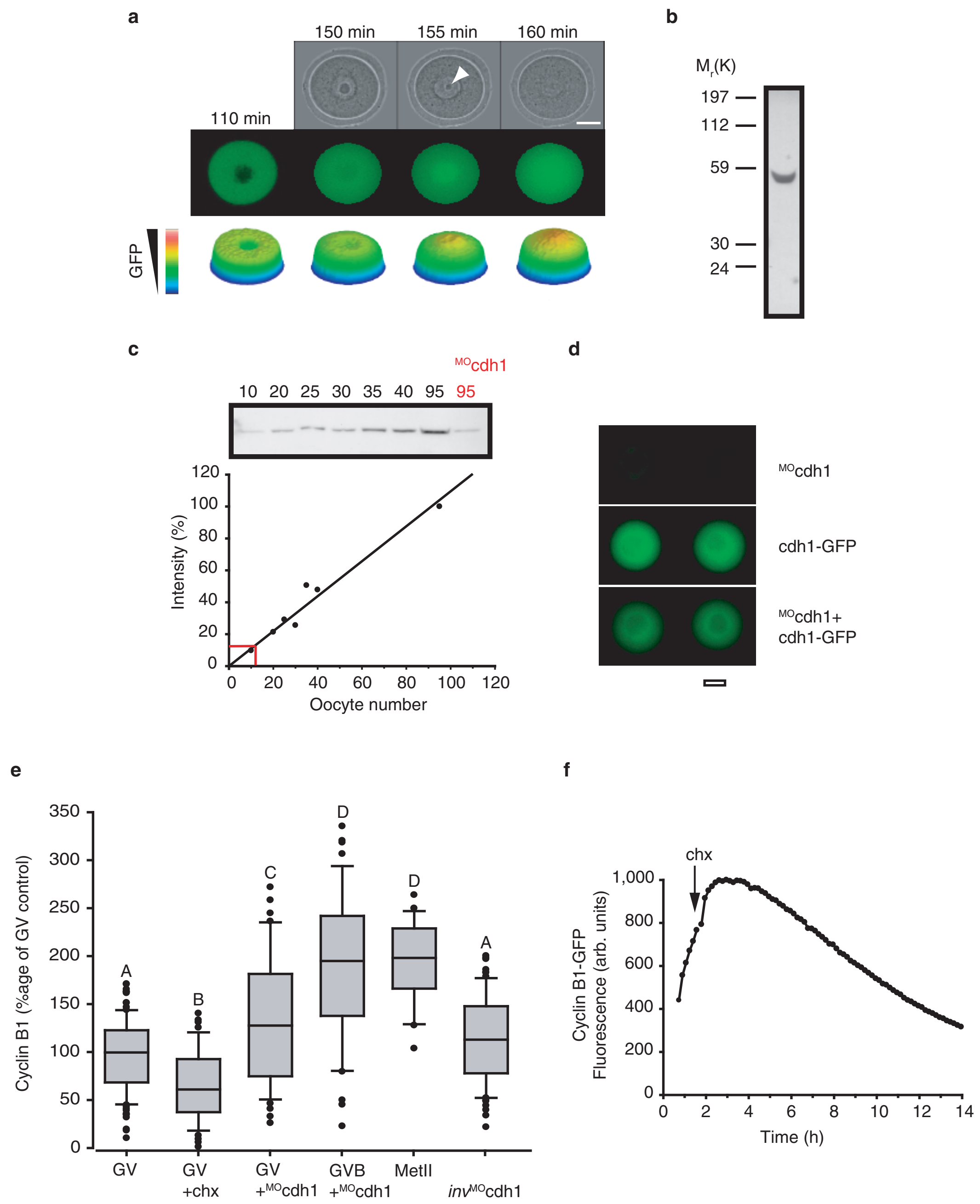

Figure S1. (a) GVB induced by injection of cyclin B1-GFP (at t= 0 min) observed by brightfield (top row); and corresponding cyclin B1-GFP fluorescence by confocal (110 min; middle row) or epifluorescence (150-160 min; middle row) and their corresponding topographical representations (bottom row) at the times indicated. GVB ends with the disappearance of the nucleolus (arrowhead). Media contained 1μM milrinone. Bar is 20μm. **(b)** Western blot of cdh1 in 75 GV arrested oocytes using a monoclonal antibody to cdh1 (ab3242, Abcam). Molecular weight markers are indicated. The same molecular weight band was obtained using a second cdh1 monoclonal AR38 (not shown). **(c)** Western blot for cdh1 in increasing numbers of GV arrested oocytes (between 10 and 95) and oocytes microinjected with cdh1 morpholino (n=95) and then cultured for 24 h. Densitometric analysis of bands (100% intensity corresponding to 95 control oocytes) versus oocyte number, with the red lines indicating the densitometric value for morpholino-treated oocytes. The morpholino reduced cdh1 levels by about 90%. A further blot of just 75 control and ^{MO}cdh1-injected oocytes confirmed the effectiveness of the knockdown. **(d)** Fluorescence images of oocytes injected with ^{MO}cdh1, cdh1-GFP, or ^{MO}cdh1 + cdh1-GFP. The morpholino to cdh1 was not able to block expression of exogenously added cdh1 cRNA, demonstrating that cdh1 cRNA rescue following endogenous gene knockdown is possible. Scale bar is 50μm. **(e)** Endogenous cyclin B1 measued by immunofluorescence in fixed and permeablised oocytes. GV oocytes were cultured for 14 h in milrinone-containing medium alone (GV; n=143); with cycloheximide (GV+chx; n=49); with injection of ^{MO}cdh1 (n=91); with injection of *inv*^{MO}cdh1 (n=79); or without milrinone (MetII; n=23). Oocytes injected with ^{MO}cdh1 were grouped into those still GV arrested (GV + ^{MO}cdh1; n=48) or those having undergone GVB (GVB + ^{MO}cdh1; n=43). Oocytes cultured without milrinone had matured, extruded a first polar body and so were classified as at metaphase II. Immunofluorescence levels were normalised with respect to GV controls. Data is presented in Box and Whisker format: the 25th and 75th percentile form the box with the mean marked as a line, the 10th and 80th percentile form the whiskers and the outlying points are plotted individually. Different letters denotes significantly different (p=0.05; 1-way ANOVA with Fisher's post-hoc test). **(f)** Representative degradation profile of cyclin B1-GFP in a GV oocyte following the translation of cyclin B1-GFP cRNA and the addition of cycloheximide (chx) to block further synthesis. The oocyte had been microinjected 24 h previously with *inv*^{MO}cdh1, a control morpholino for cdh1, to act as a sham-knockdown for cdh1.

Supplementary Information

Table S1. Percent loss in GFP signal for various cRNA constructs

cRNA injection	cdh1 rescue?	Percent loss in fluorescence[a] mean±sd (n)
GFP	NA	0± 4 (16)
Cyclin B1-GFP	NA	72±13 (13)
Cyclin B1-GFP +^{MO}cdh1	-	6± 3 (14)
	+	79± 5 (12)[c]
Cyclin B1-GFP +*inv*^{MO}cdh1	NA	69± 9 (16)
Cdc20-GFP	NA	54±15 (18)
Cdc20-GFP +MG132	NA	22±12 (15)[b]
Cdc20-GFP +MeUb	NA	23±11 (17)[b]
Cdc20-GFP +^{MO}cdh1	-	9± 3 (15)
	+	70± 9 (18)[c]
Securin^{dm}-GFP	NA	70±18 (11)
Securin^{dm}-GFP +^{MO}cdh1	-	7± 4 (16)
	+	76±10 (15)[c]

[a] Following cycloheximide addition or cdh1 cRNA microinjection. Cdh1 cRNA was microinjected in rescue experiments only (+); for all others degradation rates following addition of cyclohexmide are given. The relative fall in GFP fluorescence was calculated 14 h later.

[b] Statistically different from Cdc20-GFP alone (Chi-Squared, $p<0.05$).

[c] Statistically different from non-rescue (Chi-Squared, $p<0.01$).

NA, not applicable. Rescue experiments were performed following ^{MO}cdh1 addition only.

Supplementary Information.

METHODS

All chemicals were from Sigma-Aldrich and the UK unless stated otherwise

Oocyte collection and culture

GV oocytes were collected from four to six week old MFI mice (Harlan) mice 44-52 h after intraperitoneal injection of 7.5 IU pregnant mares' serum gonadotrophin (Calbiochem). For bench handling, microinjections, and imaging experiments oocytes, were cultured in medium M2. For long-term incubation, GV stage oocytes were cultured in MEM (Gibco) with 20% foetal calf serum in a 5% CO_2 humidified incubator at 37°C. Milrinone (1μM) was used to arrest GV oocytes when necessary.

cRNA and morpholinos

Cyclin B1,Δ90 cyclin B1, securin^{dm} and cdc20 were made as described previously [6,7], using a modified pRN3 vector designed to produce protein C-terminally coupled to MmGFP [11]. cRNA was synthesised using T3 mMESSAGE mMACHINE (Ambion), and dissolved in nuclease-free water to a concentration of approximately 1 μg/μl before microinjection.

The following morpholinos (Gene Tools LLC, OR, USA) were used at a micropipette concentration of 1.5mM: ^{MO}cdh1, 5'-CCTTCGCTCATAGTCCTGGTCCATG-3'; and *inv*^{MO}cdh1, 5'-GTACCTGGTCCTGATACTCGCTTCC-3'.

Microinjections and imaging

All microinjections into oocytes were made on the heated stage of a Nikon TE300 inverted microscope as described previously [7]. Briefly, micropipettes were inserted into cells using the negative capacitance overcompensation facility on an electrophysiological amplifier. This procedure ensures a very high rate of cell survival. A single 0.1-0.3% volume injection was achieved using a timed injection on

a Pneumatic PicoPump (World Precision Instruments). Brightfield and GFP images were recorded by epi-fluorescence and a Sony Interline MicroMax CCD camera. Confocal sections were performed on a LeicaSP2. Metamorph and Metafluor software (Universal Imaging Corp., PA, USA) were used for image capture and data analysis.

Westerns

Oocytes were washed in phosphate-buffered saline with 1% polyvinylpyrrolidone (PBS+PVP), and heated at 95°C for 5 mins with 5x sample buffer (0.06M TRIS-HCl, pH 6.8, 2%SDS, 10% glycerol 0.025% bromophenol blue and 5% mercaptoethanol). Proteins were separated on a XCell Blot Module (Invitrogen) using a 10% NuPage Bis-Tris precast gel (Invitrogen) and a MOPS running buffer. Proteins were blotted onto polyvinylidenedifluoride membrances. Anti-cdh1 ab3242 (Abcam, UK) was used for Western Blotting at 1:200. We confirmed the presence of cdh1 in GV oocytes using a second cdh1 antibody (AR38; gift of Drs J Gannon and T Hunt, CRUK). Standard ECL techniques (Amersham) were used for detection according to manufacturer's instructions.

Immunofluorescence

Oocytes were fixed with 3.7% paraformaldehyde in PBS+PVP and then transferred to the same containing 2% Triton-X100. Fixing and permeabilising were for 30 mins at room temperature. Oocytes were then washed extensively before incubation with 1:400 anti-cyclin B1 (ab72; Abcam). Following further washes oocytes were incubated with 1:20 TRITC labelled rabbit ant-mouse IgG (Dako). These incubations were at 37°C in PBS+PVP. Whole cell epi-fluorescence was used to measure cyclin B1 levels using Metamorph software.

Statistical analysis

All 1-way ANOVA statistical analysis was performed using Minitab release 14, with a 95% confidence level and Fisher's post-hoc analysis. Yates' correction was used for Chi-squared analysis.