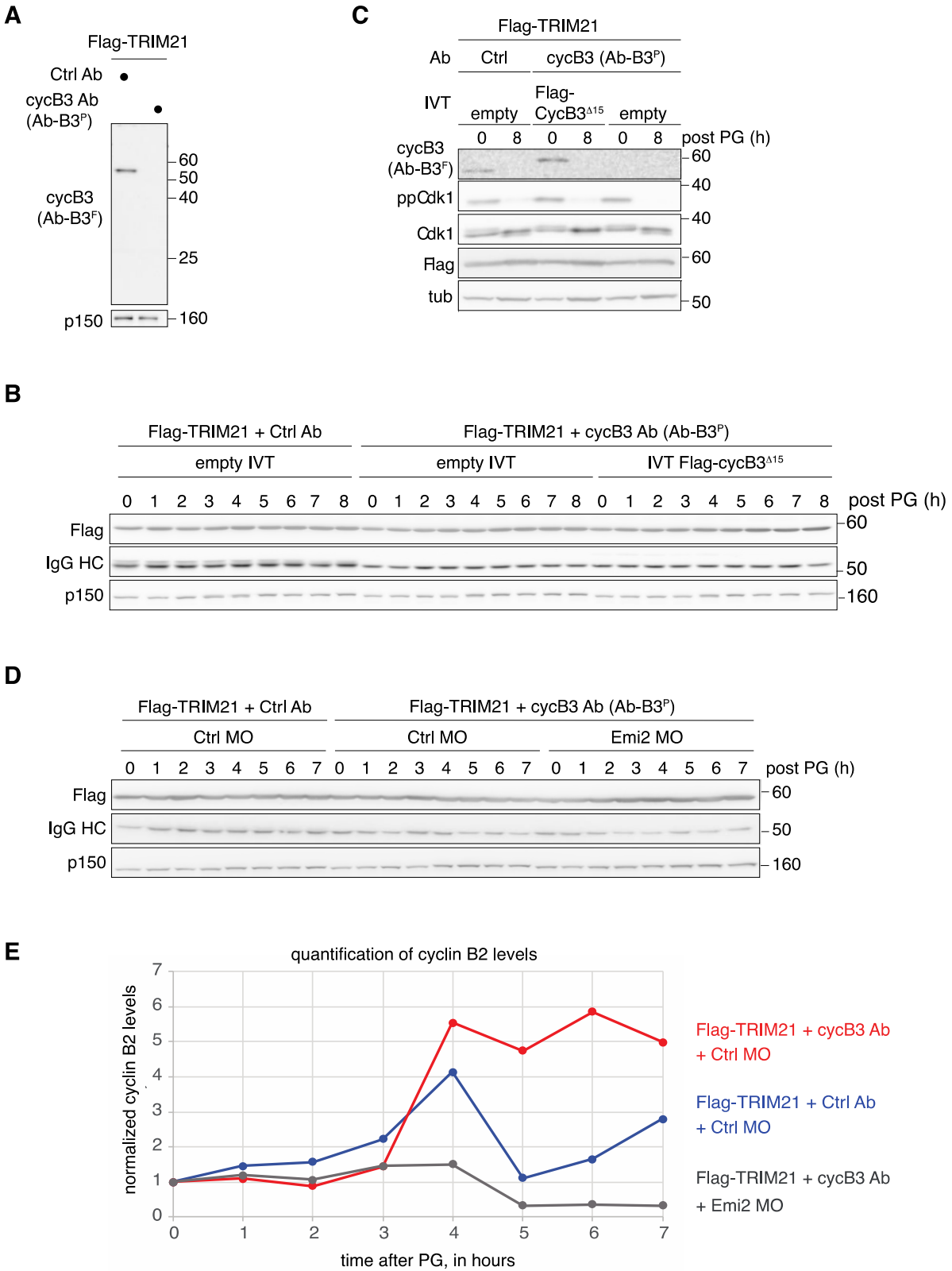


Supplemental Figure 1 (related to Figures 1 and 3). Schematic comparison of meiotic maturation in mouse and frog oocytes.

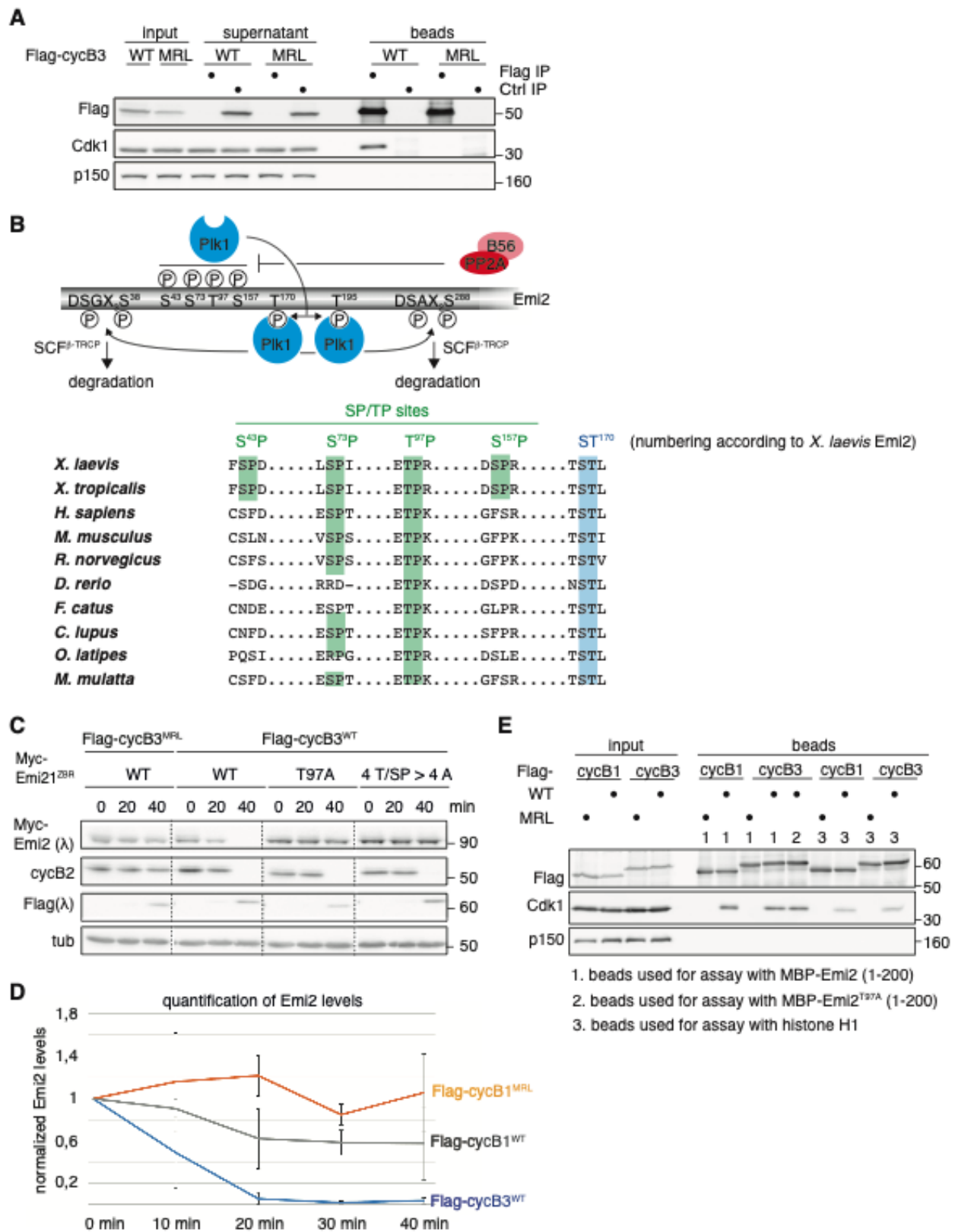
Immature mouse and frog oocytes are arrested at prophase of meiosis I. Meiotic resumption is induced by washing oocytes in medium without dbcAmp (mouse) or by treating oocytes with progesterone (PG, frog). Germinal vesicle breakdown (GVBD) can directly be observed by light-microscopy in mouse oocytes, whereas the appearance of a white spot in the animal hemisphere of the oocytes serves as indirect readout for frog oocytes. Hours (h) and minutes (min) relative to GVBD are indicated. For mouse oocytes, subsequent meiotic phases can be monitored in real-time by live microscopy to follow chromosome movements and extrusion of the PB. Chromosome spreads allow further assessment of cell

cycle stage (chromosome pairs (bivalents) in metaphase I, that segregate into paired sister chromatids (dyads) in anaphase I; in metaphase II, dyads that segregate into single sister chromatids in anaphase II.) Since opacity prevents live cell imaging of frog oocytes, Western blot analysis of indicated cell cycle regulators serves as readout for meiotic maturation in this organism. Loss of inhibitory phosphorylation of Cdk1 (ppCk1) and appearance of activating phosphorylation of MAPK (pMAPK) are used as markers for meiotic resumption. Retarded mobility of the APC/C subunit Cdc27 due to phosphorylation serves as readout for meiosis I. PB extrusion (PBE), cyclin B2 degradation, and downshift of APC/C mark exit from MI. Entry into meiosis II is accompanied by re-phosphorylation of APC/C. Mature oocytes await fertilization arrested at metaphase of meiosis II, also termed cytostatic factor- (CSF-) arrest. Spindles are in magenta, chromosomes in blue. Metaphase I arrest in *Ccnb3*^{-/-} mouse oocytes is indicated by an arrowhead.



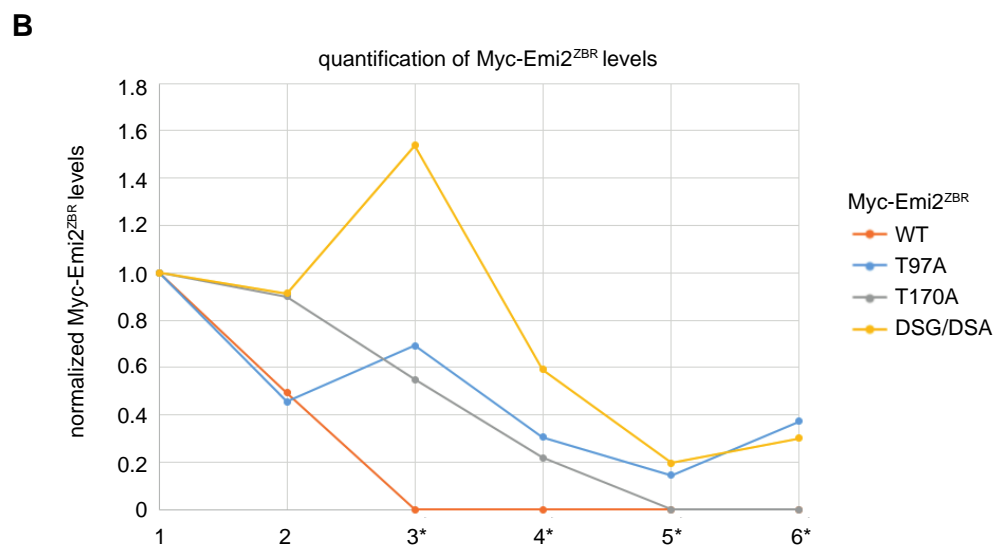
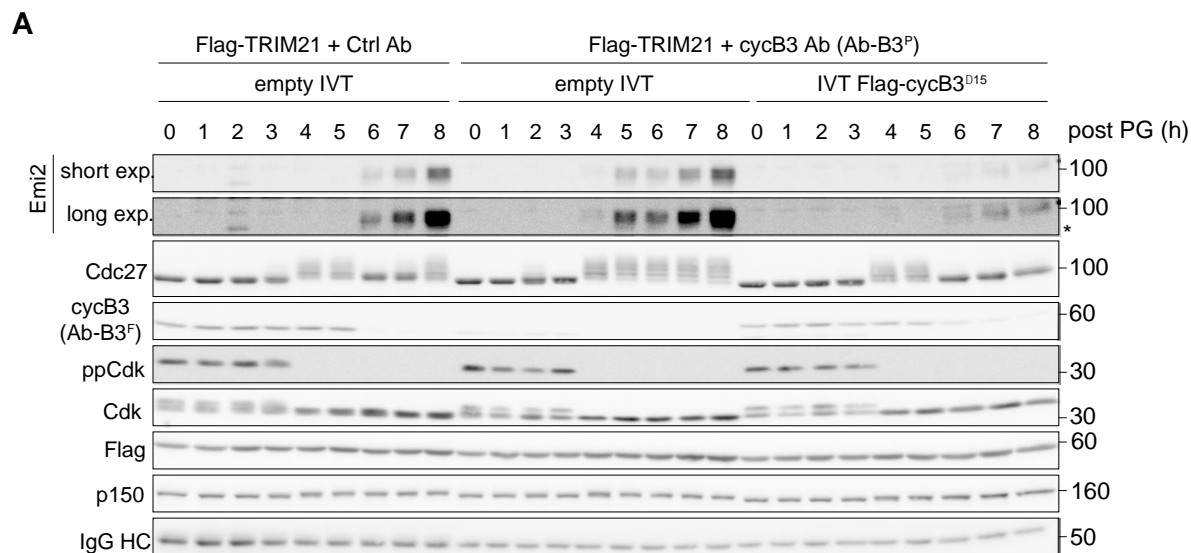
Supplemental Figure 2 (related to Figure 3). Validation of *Xenopus* cyclin B3 tools.

(A) Validation of cyclin B3 antibodies by TRIM-Away. Prophase I arrested oocytes were injected with Flag-TRIM21 mRNA and control (Ctrl) or cyclin B3 (cycB3) peptide Ab (aa 6-26, Ab-B3^P). 23 hours post injection, oocytes were lysed and immunoblotted for cyclin B3 using the antibody raised against an N-terminal fragment of cyclin B3 (aa 1-150, Ab-B3^F). p150 serves as loading control. **(B)** Western blot (WB) showing expression of Flag-TRIM21, injected Ctrl or Ab-B3^P Ab (IgG HC: IgG heavy chain), and p150 as loading control for Figure 2B. WB is representative of experiments performed in triplicate. **(C)** Prophase I arrested oocytes were injected with Flag-TRIM21 mRNA, control (Ctrl) or cyclin B3 peptide Ab (Ab-B3^P), and empty *in vitro* translation (IVT) reaction or with IVT Flag-tagged *X.l.* cyclin B3^{Δ15} and analysed by WB. Of note, cyclin B3^{Δ15} lacks the first 15 aa and is not recognized by the peptide antibody Ab-B3^P, which was used to deplete endogenous cyclin B3 by TRIM-Away. Tubulin serves as loading control. **(D)** WB showing expression of Flag-TRIM21, injected Ctrl or Ab-B3^P Ab (IgG HC: IgG heavy chain), and p150 as loading control for Figure 2D. **(E)** Quantification of the cyclin B2 signal shown in Figure 2D. **(A-D)** Data are representative of independent biological triplicate experiments.



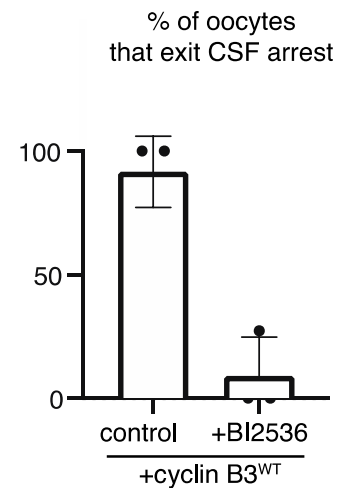
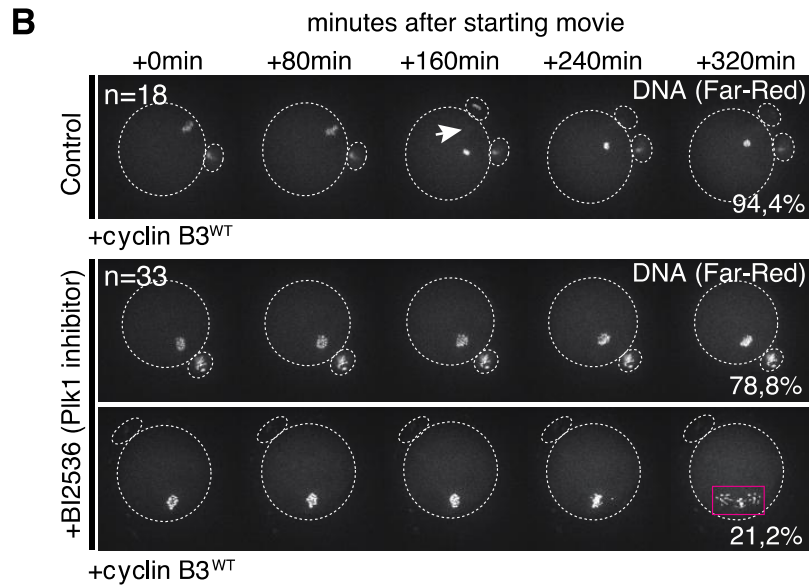
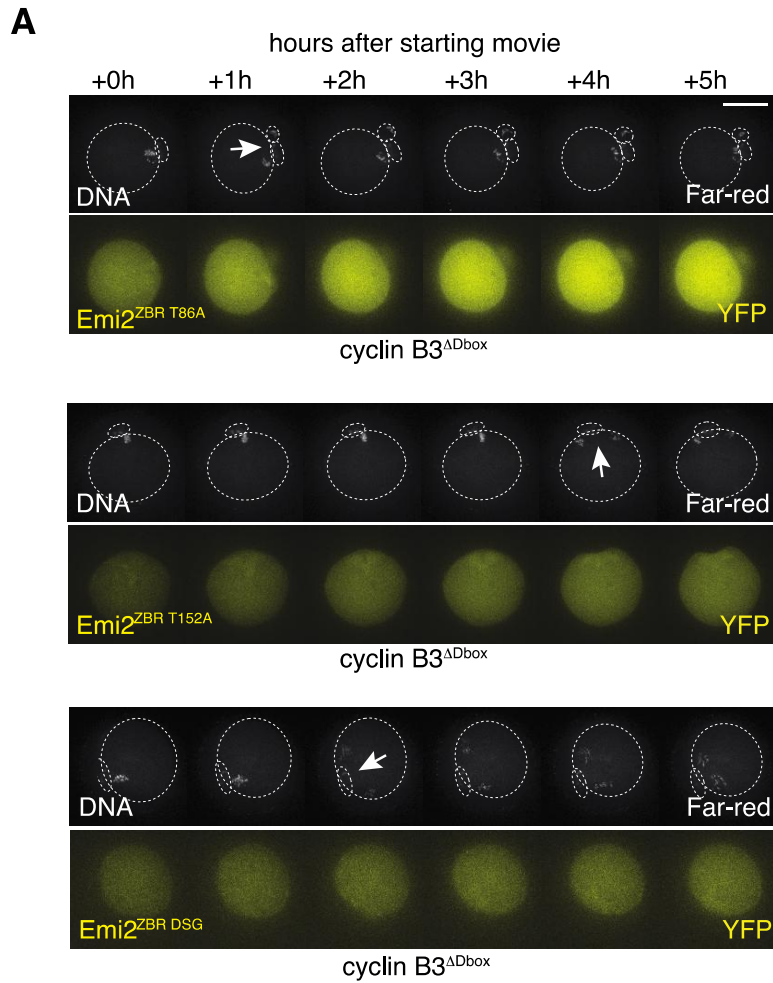
Supplementary Figure 3 (related to Figure 4). T97 is critical for cyclin B3-mediated Emi2 destabilization.

(A) Flag-tagged WT or MRL mutant *X.l.* cyclin B3 (cycB3) was expressed from mRNA in *Xenopus* CSF extract followed by immunoprecipitation using α -Flag or control (Ctrl) antibodies. Input, supernatant, and bead samples were analysed as indicated. p150 serves as loading control. **(B)** Upper panel: scheme of Emi2 regulation during CSF-arrest to maintain constant MPF activity despite ongoing cyclin-B1 synthesis. See text and PMID: 21871841 for details. Lower panel: sequence alignment of Emi2 orthologues from different species. Numbering refers to *X.l.* Emi2. Green and blue sites refer to Cdk1 and Plk1 sites, respectively. **(C)** *Xenopus* CSF egg extract containing IVT Myc-tagged Emi2^{ZBR} variants (WT: wildtype; T97A mutant; 4T/SP>4A: S43A, S73A, T97A and S157A) was supplemented with mRNA encoding Flag-tagged WT or MRL mutant *X.l.* cyclin B3 and analysed by immunoblotting. Where indicated, extract samples were treated with lambda phosphatase (λ) prior to immunoblotting. Tubulin serves as loading control. **(D)** Quantification of the Emi2 signal shown in Figure 4D. All error bars indicate means \pm SD of three independent experiments. **(E)** CSF extract was supplemented with mRNA encoding Flag-tagged WT or MRL mutant *X.l.* cyclin B1 (cycB1) or cyclin B3 followed by anti-Flag immunoprecipitation. WB analyses of input samples and immunoprecipitates (beads), which were used for the *in vitro* kinase assay (Figure 3E) using MBP/His-tagged Emi2 (aa 1-200) WT (S43A, S73A and S157A, labeled 1), T97A (S43A, S73A, S157A and T97A, labeled 2) or histone H1 (labeled 3) as substrate. p150 serves as loading control. (A, C, E) Data are representative of triplicate experiments.



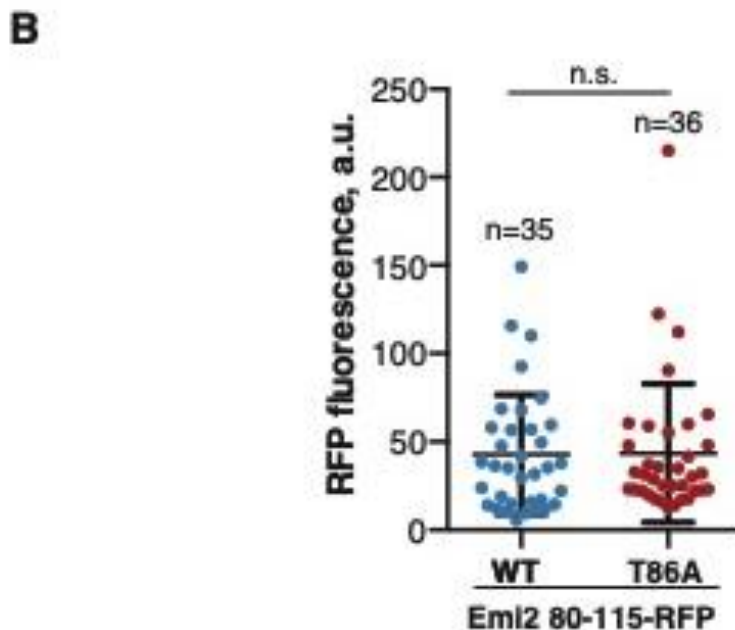
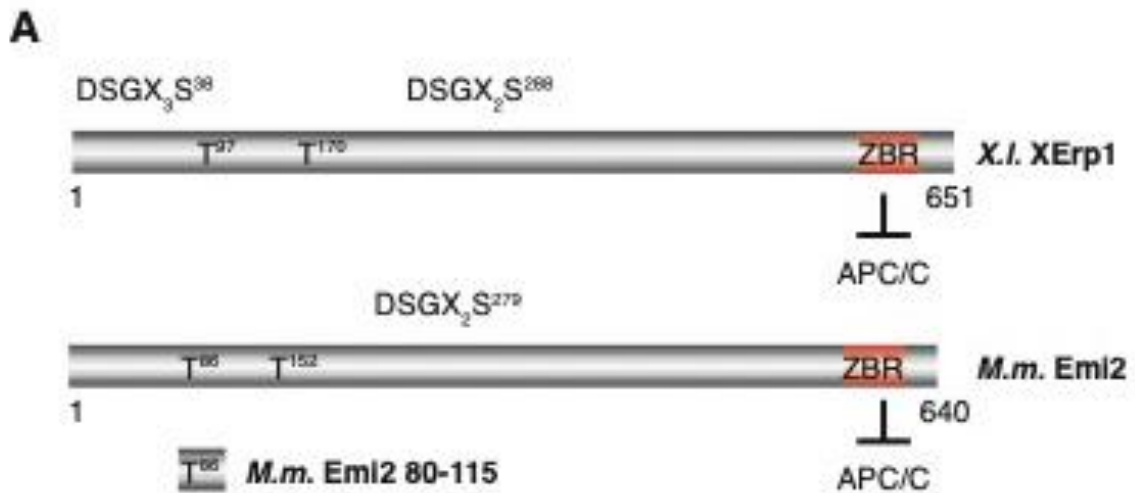
Supplementary Figure 4 (related to Figure 5). Emi2 accumulates prematurely in *Xenopus* oocytes lacking cyclin B3.

(A) *Xenopus* prophase I oocytes were injected with Flag-TRIM21 mRNA and Ctrl Ab or Ab-B3^P and empty or *X.l.* Flag-cyclin B3^{Δ15} IVT. 18 hours later, PG was added, and oocytes were immunoblotted at indicated time points. To correlate Emi2 accumulation with the appearance of phosphorylation-dependent cell cycle markers, samples were – in contrast to figure 5A – not treated with phosphatase. p150 serves as loading control. Asterisk marks unspecific band. IgG HC: injected Ab heavy chain. The experiment was performed in triplicate and a representative WB is depicted. **(B)** Quantification of the IVT Myc-Emi2^{ZBR} signal shown in Figure 5C. Asterisks mark time points where inhibitory Cdk1 phosphorylation was absent, i.e., time points where oocytes have/had? resumed meiosis. See figure 5C.



Supplementary Figure 5 (related to Figure 6). Live imaging of Cyclin B3-induced Emi2 degradation and CSF-release.

(A) mRNAs coding for the indicated Emi2^{ZBR}-YFP mutants were co-injected with cyclin-B3^{ΔDbox} or cyclin-B3^{ΔDbox MRL} mRNAs in CSF-arrested mouse oocytes. Time-lapse acquisitions were done every 20 minutes. Selected images in the YFP channel (1z section) and overlays of the Far-red channel (15 z-sections, chromosomes) are shown, anaphase II is indicated with an arrow. Number of oocytes analysed is indicated in Figure 5C. Note: The 2nd PB was not always clearly visible, hence it is not necessarily indicated. **(B)** CSF-arrested mouse oocytes expressing WT cyclin B3 were followed by time-lapse imaging in absence or presence of 50 nM of the Plk1 inhibitor BI2536. SiR-DNA was used to visualize chromosomes. Anaphase II in control oocytes is indicated with arrowhead, anaphase-like movements with a rectangle. Scale bar: 50μm. Oocytes that exited CSF-arrest or with perturbed segregation were quantified (Error bars indicate mean ± SD). n= number of oocytes from 3 independent experiments, means of each experiment indicated as dots.



Supplementary Figure 6 (related to Figure 7). Emi2 fragment comprising T86 causes metaphase I arrest in mouse oocytes

(A) Schematic representation of Mouse (*M.m.*) and *Xenopus* (*X.l.*) Emi2. Of note, the Emi2 fragment 80-115 lacks the APC/C inhibitory ZBR domain, but contains T86 (corresponding to T97 in frog), the Cdk1/cyclin B3 site critical for cyclin B3-mediated degradation of Emi2. **(B)** Quantification of RFP-signal in oocytes that had resumed meiosis expressing WT or T86A Emi2⁸⁰⁻¹¹⁵-RFP. a.u.: arbitrary units, n indicates number of oocytes analysed, bar and whiskers are means and \pm SD, n.s.: not significant, according to Mann-Whitney test.

Cloned into pCS2 plasmid	<i>M. mutans</i> 186A	Cloning nucleotides	PCR with Phusion Polymerase					
Cloned into pRN3 plasmid	<i>M.m.</i> Emi2 ZBR (C573A) YFP	CCCGAGGTGCCAATCCCCTGCTAAGTACC	Pfu PCR mutagenesis					
		GCAGGCTTTAAAGCTTCATCAGTAAACAGTG						
	<i>M.m.</i> Emi2 ZBR T86A YFP	ACTTTGTGAGGCTCCTAAACTCAG		CTTGAAACACTCCGAGGATTTGAGTC				
		CATTAGTTACCAGCGGATAAAAAACAG			GTAATCAATGGTCGGCTATTTTTGTC			
	<i>M.m.</i> Emi2 ZBR T152A YFP	TTGCAACCTTAGCTGGGACAAATCAG		AAGCCATTGTCTTCAGGTGAAAAAGAC		Q5 site-directed mutagenesis kit (NEB, E05545)		
		AACCTTAGCTGGGACAAATCAGAAGATTTCTC			GCAAAAGCCATTGTCTTCAGGTGAAAAAGACTG			
	<i>M.m.</i> Emi2 DSG first mutation, S275N	TCATGACCTCCTCCGAGGAC		CATGGAATTCAGATCTGCCAAAGT			In-Fusion HD cloning kit (Ozyme, 639648)	
		<i>M.m.</i> Emi2 DSG second mutation, S279N			GATCTGAATTCATGAGCCAGAATTTGTGAGACT			
	<i>M.m.</i> Emi2 80-115 aa-RFP				CGGAGGAGGTCATGATGCTTTGTGAATCAGAATCCCC			GATATTAGCAAGGATgCgCgCGCTATCgCgTGTGGATTGGATGGTG
		<i>X.l.</i> Cyclin B3 MRL			CACCATCCAATCCACAgcGATAGCCgCgCgATCCTTGCTAATATC			
	<i>X.l.</i> Emi2 S33N (DSG)			CTTCAGGTGCGCAGGACAaTGGCTACAGTGACTCTCTAAAG	CTTTAGAGAGTCACTGTAGCCATGTCTGCGACACTGAAG			
		<i>X.l.</i> Emi2 S284N S288N (DSA)		GTAAGTGAAGGACaCGCATTTCACaaTCTTAGCCTGGAT		ATCCAGGCTAAGAttGTGAAATGCGtGTCTCAGTTAC		
<i>X.l.</i> Emi2 T170A	ATAGCCACCAGCgCTTTAAAGACAGAG		CTCTGTCTTTAAAGcGCTGGTGGCTAT					
	<i>X.l.</i> Emi2 T97A	CTCCACTTTATGAAGCCCTCGAGTTGGG		CCCAACTCGAGGGGcTTCATAAAGTGGAG				
<i>M.m.</i> Emi2		attagccggccaATGGACTCCTCTGCTGTAC	aatggcgccTCAGAGCGTTTTAAGTTCCGC					
	<i>M.m.</i> Emi2 C573A	GATGAAGCTTTAAAGCCTgCCCCGAGGTGCCAATCC		GGATTGGCACCTCGGGgCAGGCTTTAAAGCTTCATC				
<i>M.m.</i> Emi2 T152A		TTAGTTACCAGCgCGATAAAAAACAGA	TCTGTTTTTATCGcGCTGTAATAA					

		TCTGAGTTTAGGAGcCTCACAAGTTCTG	
	<i>M.m.</i> Emi2 S275N S279N	TTACACCTGAAGACAaTGGCTTTTGCaacCTTAGCTGGGACAAA	
		TTGTCCCAGCTAAGgttGCAAAAGCCAtGTCTTCAGGTGTAA	

Supplementary Table 1. List of oligonucleotides used for cloning. Related to STAR Methods.