Expanded View Figures

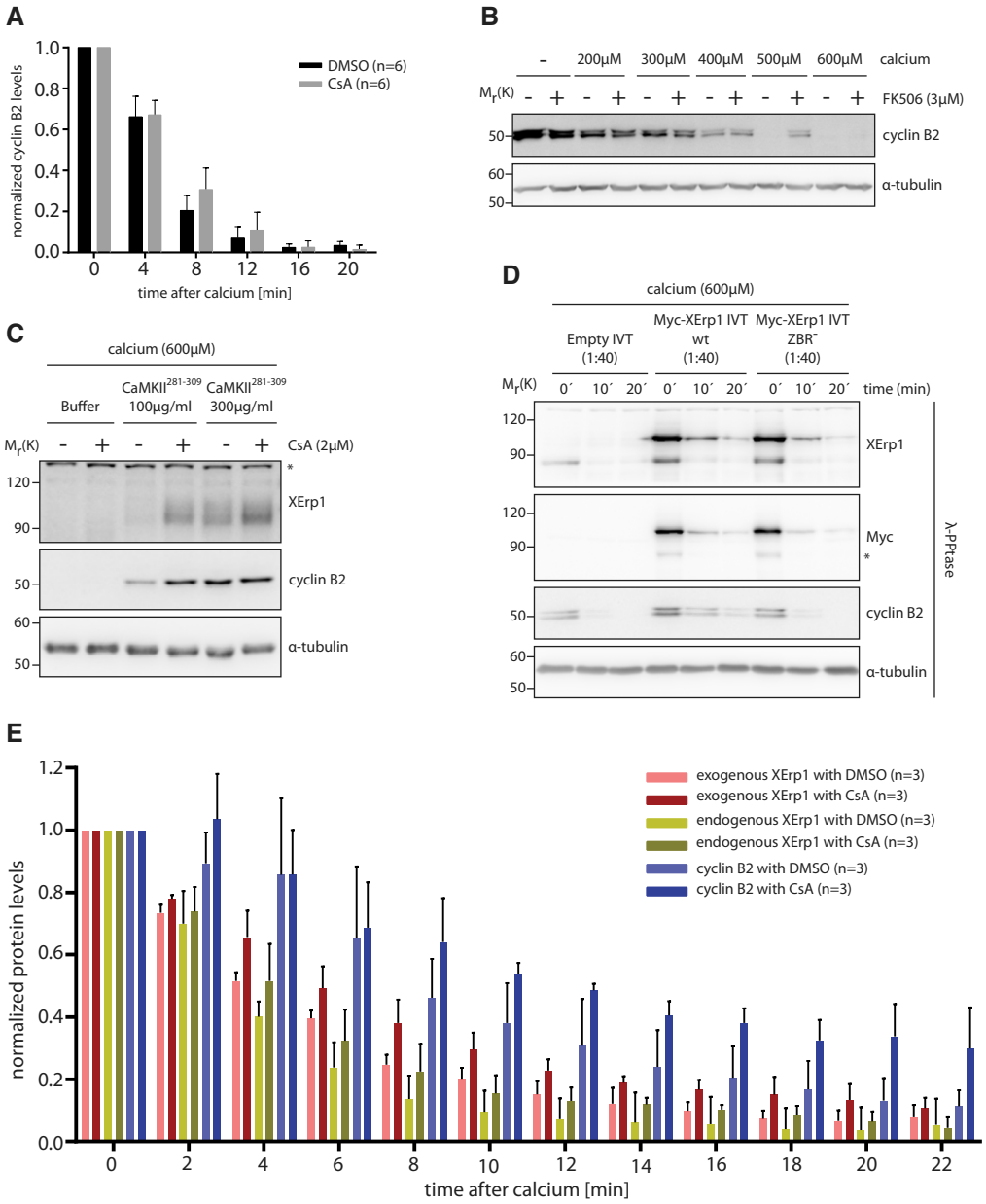

Figure EV1.

◀ **Figure EV1. Calcineurin affects cyclin B2 and XErp1 degradation at meiotic exit.**

A Quantification of cyclin B2 degradation in DMSO- or CsA-treated CSF extracts after calcium addition as shown in Fig 1B. Values were normalized to $t = 0$ min and are given as mean ± standard deviation of six independent biological replicates. The *P*-value for the 8 min timepoint (DMSO/CsA) is 0.08 (unpaired two-sided *t*-test with unequal variance).

B CSF extract was treated with DMSO or FK506. Both reactions were divided and supplemented with the indicated amounts of calcium or H_2O. After 20 min, samples were taken and immunoblotted for cyclin B2. The cyclin B2 membrane was stripped and reprobed for α-tubulin.

C CSF extract was treated with control buffer or the indicated amounts of CaM Kinase II Inhibitor 281–309 ($CaMKII^{281-309}$). Extracts were divided and treated with DMSO or CsA before addition of calcium. After 15 min, samples were taken and immunoblotted for XErp1 and cyclin B2. The cyclin B2 membrane was stripped and reprobed for α-tubulin. Asterisk indicates unspecific bands.

D CSF extract was treated with Myc-XErp1 IVT that was either wild-type or mutated to alanine at C583 (ZBR^-). An empty IVT not expressing XErp1 was added as control. Meiotic exit was induced by addition of calcium, and samples were taken at the indicated time points, treated with λ-phosphatase and immunoblotted for XErp1, the Myc-tag, cyclin B2, and α-tubulin. Asterisk indicates unspecific bands.

E Quantification of exogenous XErp1, endogenous XErp1, and cyclin B2 in DMSO- or CsA-treated CSF extracts after calcium addition as shown in Fig 1E. Values were normalized to $t = 0$ min and are given as mean ± standard deviation of three independent biological replicates. The *P*-values for the 8 min timepoint are 0.07 (exo. XErp1, DMSO/CsA), 0.26 (endo. XErp1, DMSO/CsA), and 0.17 (cyclin B2, DMSO/CsA) using an unpaired two-sided *t*-test with unequal variance.

Source data are available online for this figure.

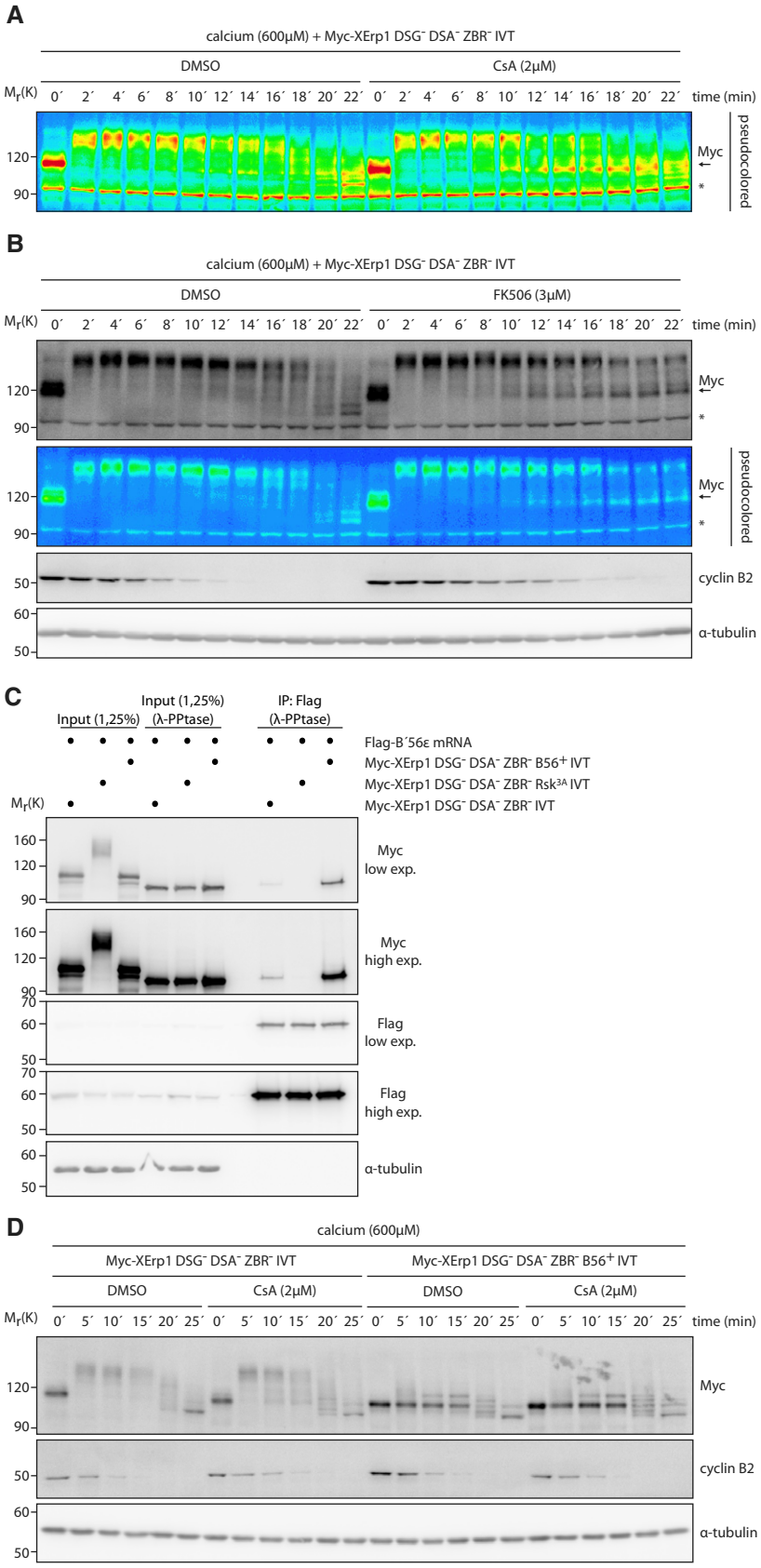

Figure EV2. Calcineurin affects the phospho-regulation of XErp1.

A Pseudocolor representation of the Myc immunoblot shown in Fig 2B. Asterisk indicates unspecific bands.

B CSF extract was supplemented with Myc-XErp1 DSG⁻ DSA⁻ ZBR⁻ (S33N S38N S284N S288N C583A) IVT. The reaction was divided and treated with DMSO or FK506. Both reactions were treated with calcium, and samples were taken at the indicated time points. Samples were immunoblotted for the Myc-tag, cyclin B2, and α-tubulin. A pseudocolored representation of the Myc immunoblot is shown. Arrow marks the meiotic phosphorylation state of Myc-XErp1. Asterisk indicates unspecific bands.

C CSF extract was treated with mRNA encoding Flag -B′56ε. The extract was divided and treated with Myc-XErp1 IVT carrying the indicated combinations of the mutations DSG⁻ DSA⁻ ZBR⁻ (S33N S38N S284N S288N C583A), Rsk^{3A} (S335A T336A S342A), and $B56^{+}$ (S335P L337I R340E G341E S342E). Flag-B′56ε was immunoprecipitated, and the input and pellet (IP: Flag) samples were treated with λ-phosphatase as indicated. The samples were immunoblotted for the Myc-tag, the Flag-tag, and α-tubulin. Low and high exposures are shown for the Myc-tag and the Flag-tag.

D CSF extract was supplemented with Myc-XErp1 IVT carrying the indicated combinations of the mutations DSG⁻ DSA⁻ ZBR⁻ (S33N S38N S284N S288N C583A) and $B56^{+}$ (S335P L337I R340E G341E S342E). Both reactions were divided and treated with DMSO or CsA. Calcium was added, and samples were taken at the indicated time points. Samples were immunoblotted for the Myc-tag, cyclin B2, and α-tubulin.

Source data are available online for this figure.

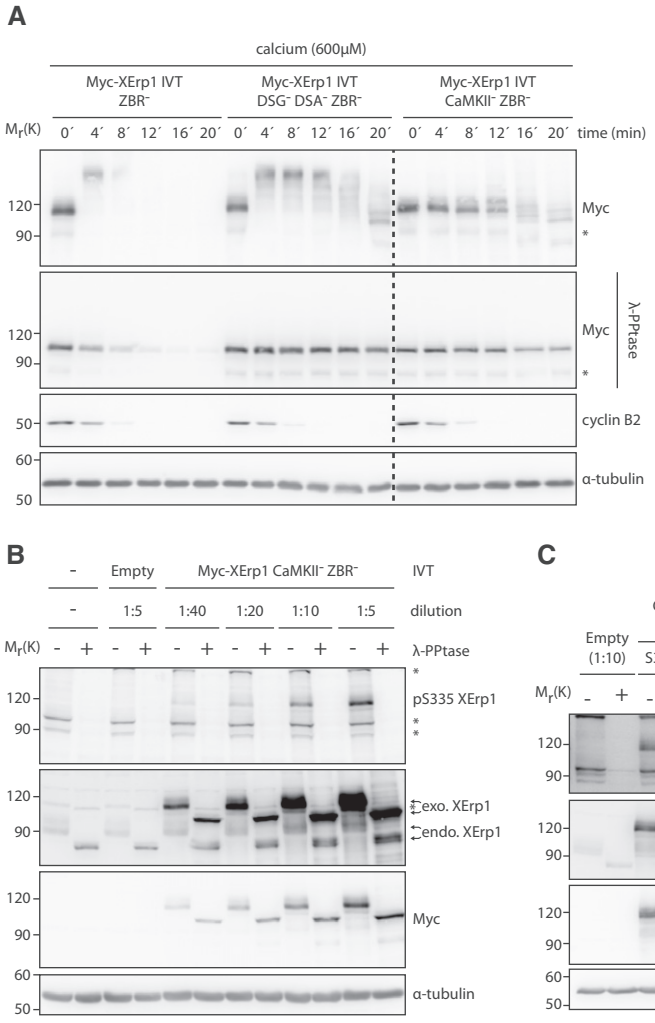

Figure EV3. Characterization of pSer335 XErp1 antibody.

A CSF extract was treated with Myc-XErp1 IVT carrying the indicated combinations of the mutations DSG⁻ (S33N S38N), DSA⁻ (S284N S288N), ZBR⁻ (C583A) and CaMKII⁻ (T195A). Calcium was added, samples were taken at the indicated time points and as indicated treated with λ-phosphatase. Samples were immunoblotted for the Myc-tag and cyclin B2. The cyclin B2 membrane was stripped and reprobed for α-tubulin. Asterisk indicates unspecific bands. Several lanes were removed at the dashed line.

B CSF extract was treated with Myc-XErp1 CaMKII⁻ ZBR⁻ (T195A C583A) IVT at the indicated dilutions. An empty IVT not expressing XErp1 and an untreated condition were used as controls. Samples were taken, treated as indicated with λ-phosphatase and immunoblotted for XErp1, pSer335 XErp1, and α-tubulin. The XErp1 membrane was stripped and reprobed for the Myc-tag. Asterisks indicate unspecific bands.

C CSF extract was treated with Myc-XErp1 CaMKII⁻ ZBR⁻ (T195A C583A) IVT that was either wild-type or mutated to alanine at Ser335. An empty IVT reaction not expressing Myc-XErp1 served as control. Samples were taken, treated as indicated with λ-phosphatase and immunoblotted for XErp1, the Myc-tag, pSer335 XErp1, and α-tubulin. Asterisks indicate unspecific bands.

Source data are available online for this figure.

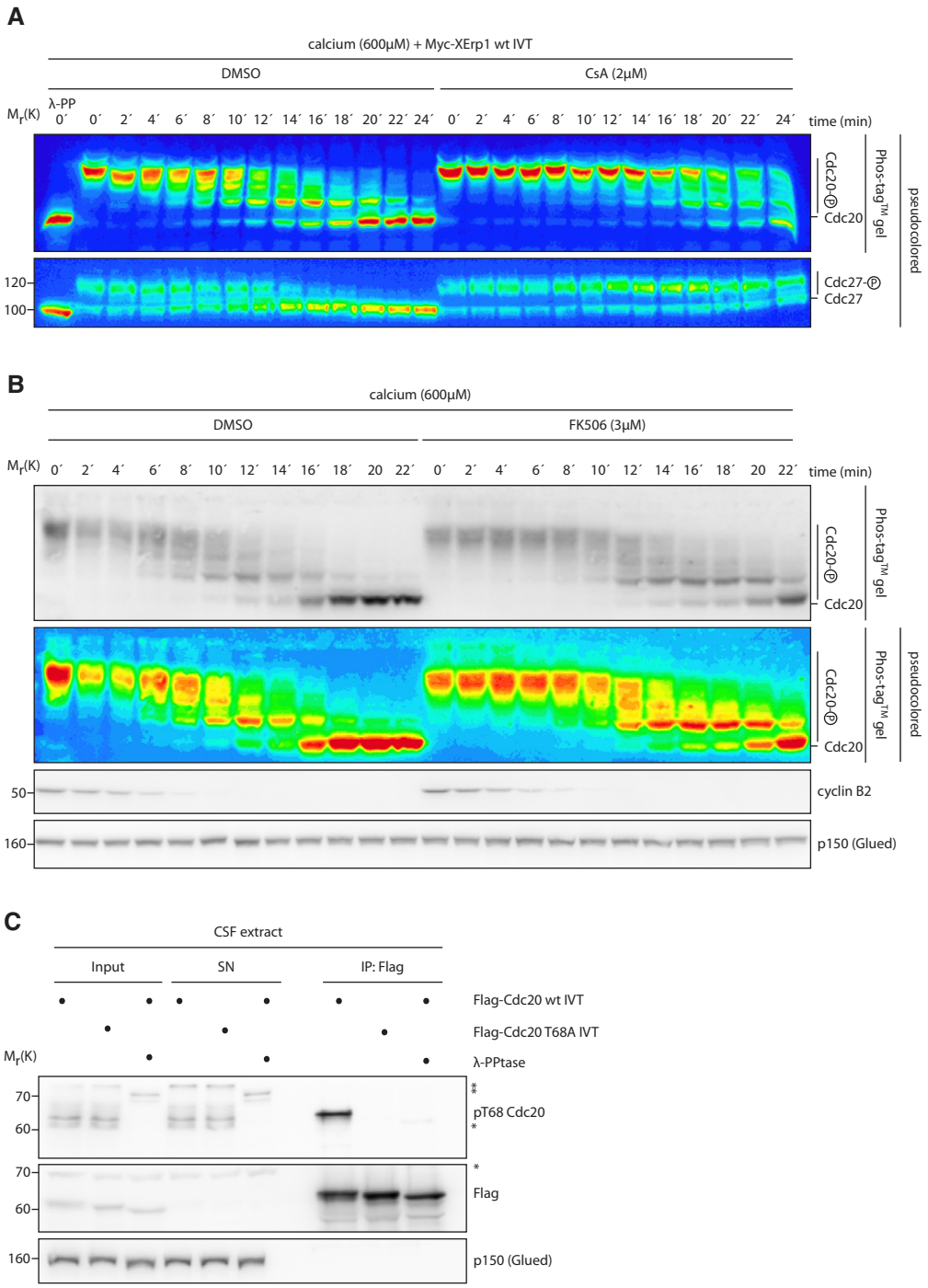

Figure EV4. Calcineurin affects the phospho-regulation of Cdc20.

A Pseudocolor representation of the Cdc20 Phos-tag™ and the Cdc27 immunoblots shown in Fig 3B.

B CSF extract was treated with DMSO or FK506. Calcium was added, and samples were taken at the indicated time points. Samples were resolved by either conventional or Phos-tag™ SDS–PAGE and immunoblotted for Cdc20, cyclin B2, and p150(Glued). A pseudocolored representation of the Cdc20 Phos-tag™ immunoblot is shown.

C CSF extract was supplemented with Flag-Cdc20 IVT that was either wild-type or mutated to alanine at Thr68. Flag-Cdc20 was re-isolated by α-Flag immunoprecipitation. Samples and immunoprecipitates were treated with λ-phosphatase as indicated. Input, supernatant (SN), and pellet (IP: Flag) samples were immunoblotted for the Flag-tag, pThr68 Cdc20, and p150 (Glued). Asterisks indicate unspecific bands.

Source data are available online for this figure.