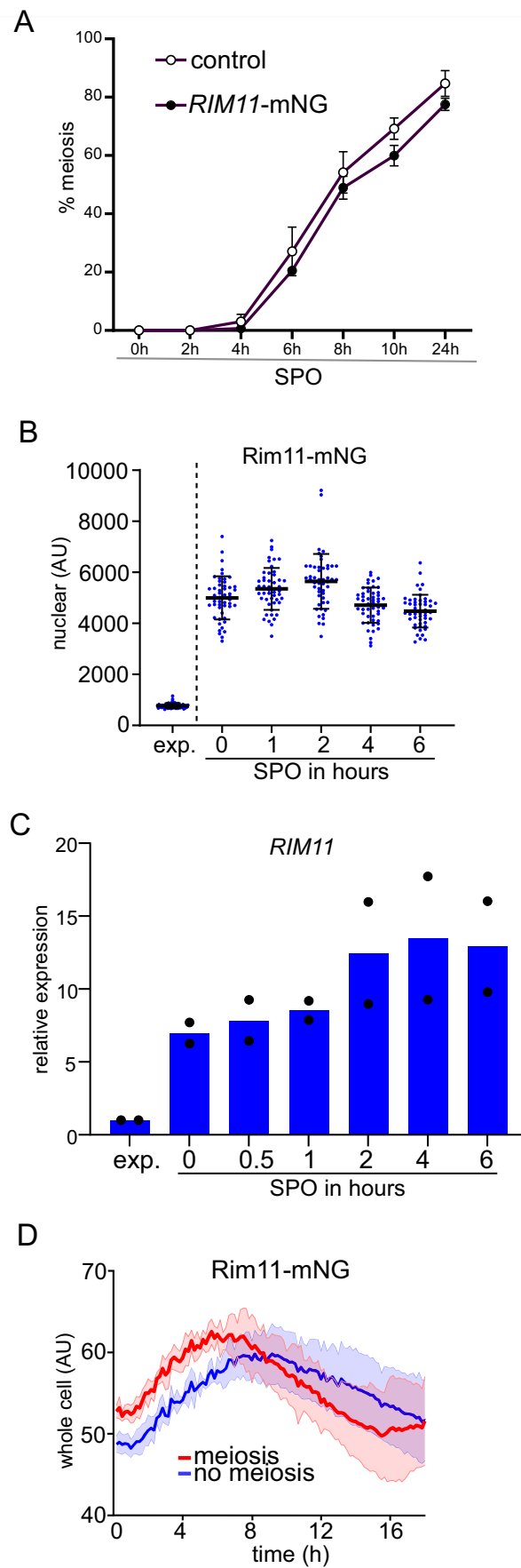


Expanded View Figures

Figure EV1. Rim11 expression and localization dynamics prior and during meiosis.

(A) Onset of meiosis in WT (7794) and cells harboring *RIM11* tagged with mNeonGreen (*RIM11*-mNG) (FW10297). Cells were induced to sporulate, and samples were taken at the indicated time points, fixed, and stained with DAPI. Cells that contained two more DAPI masses were considered to have entered meiosis. The error bars represent the mean + SEM of $n = 3$, and least $n = 100$ cells per biological repeat were quantified. (B) Nuclear concentrations of Rim11 in exponential growth (exp.) and in cells induced to enter meiosis in SPO and expressing *RIM11*-mNG + *HTB1*-mCh (FW10297). At least $n = 50$ cells per condition were quantified. The error bars represent the mean + SD. (C) *RIM11* expression determined by RT-qPCR in WT cells (FW1511) grown until the exponential phase (exp.) and cells induced to enter meiosis. Samples were normalized to the time point 0 h. $n = 2$ biological repeats were performed. The *RIM11* signals were normalized over *ACT1*. (D) Quantification of whole-cell concentrations of Rim11-mNG using live-cell imaging setup using the strain described in (A). Indicated are the mean traces of cells that entered meiosis (meiosis) and cells that did not enter meiosis (no meiosis). Shown are the Rim11-mNG cytoplasmic concentrations.



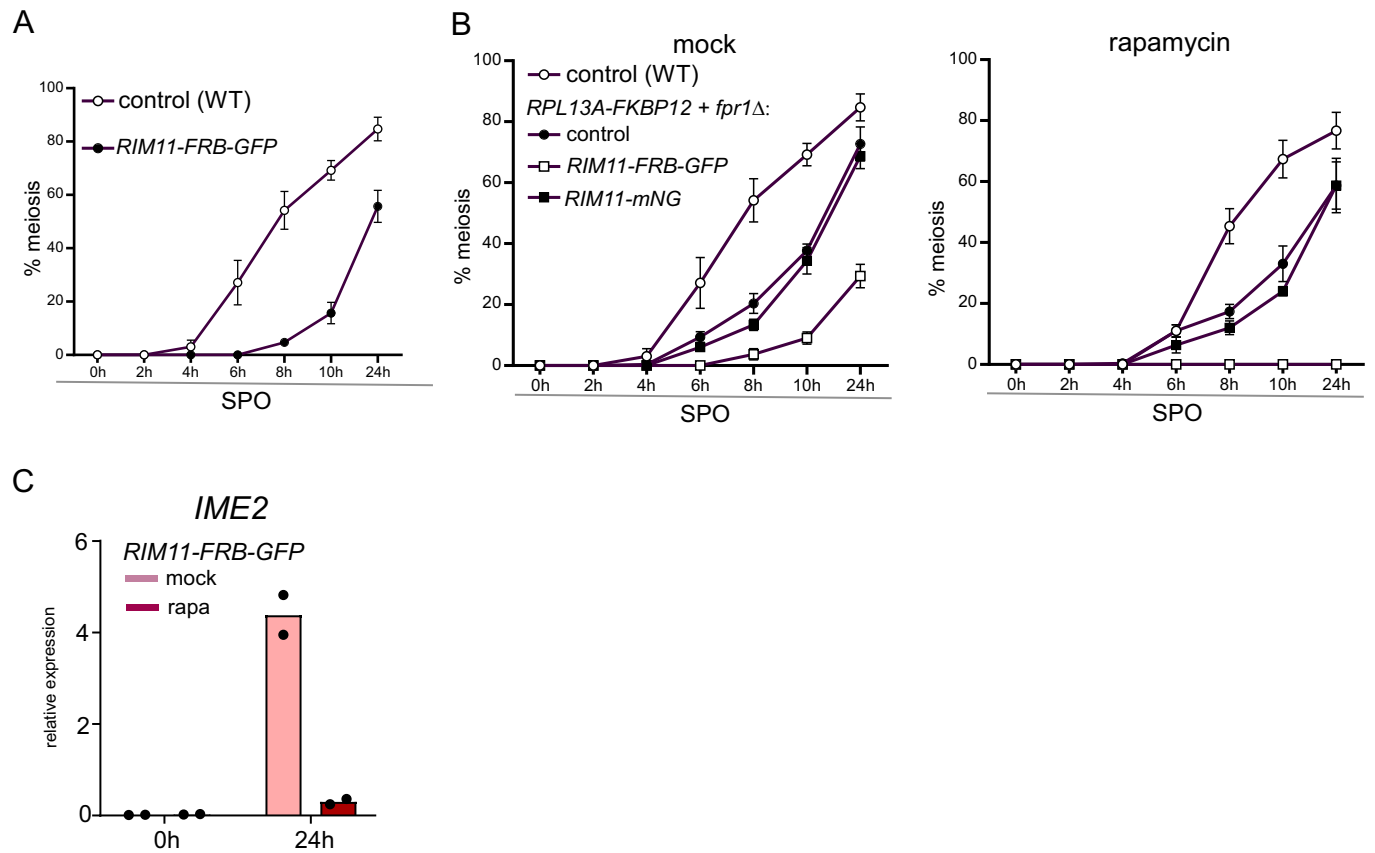


Figure EV2. Rim11 nuclear localization is required for meiosis.

(A) Onset of meiosis in WT (7794) and *RIM11-FRB-GFP* (FW11208). Cells were induced to sporulate, and samples were taken at the indicated time points, fixed, and stained with DAPI. Cells that contained two more DAPI masses were considered to have entered meiosis. The error bars represent the mean + SEM of $n = 3$, and at least $n = 100$ cells per biological repeat were quantified. (B) Onset of meiosis in WT cells (FW1511), and in cells harboring *RPL13A-FKBP12* and *fpr1Δ* (FW11257) by itself or together with *RIM11-FRB-GFP* (FW11124) or *RIM11-mNG* (FW11126). Cells were mock-treated or untreated with rapamycin at 0 h in SPO. The mean and SEM of 3 independent repeats is shown. (C) *IME2* expression was determined in *RIM11-FRB-GFP* (FW11124) after 24 h in SPO in mock and rapamycin-treated cells.

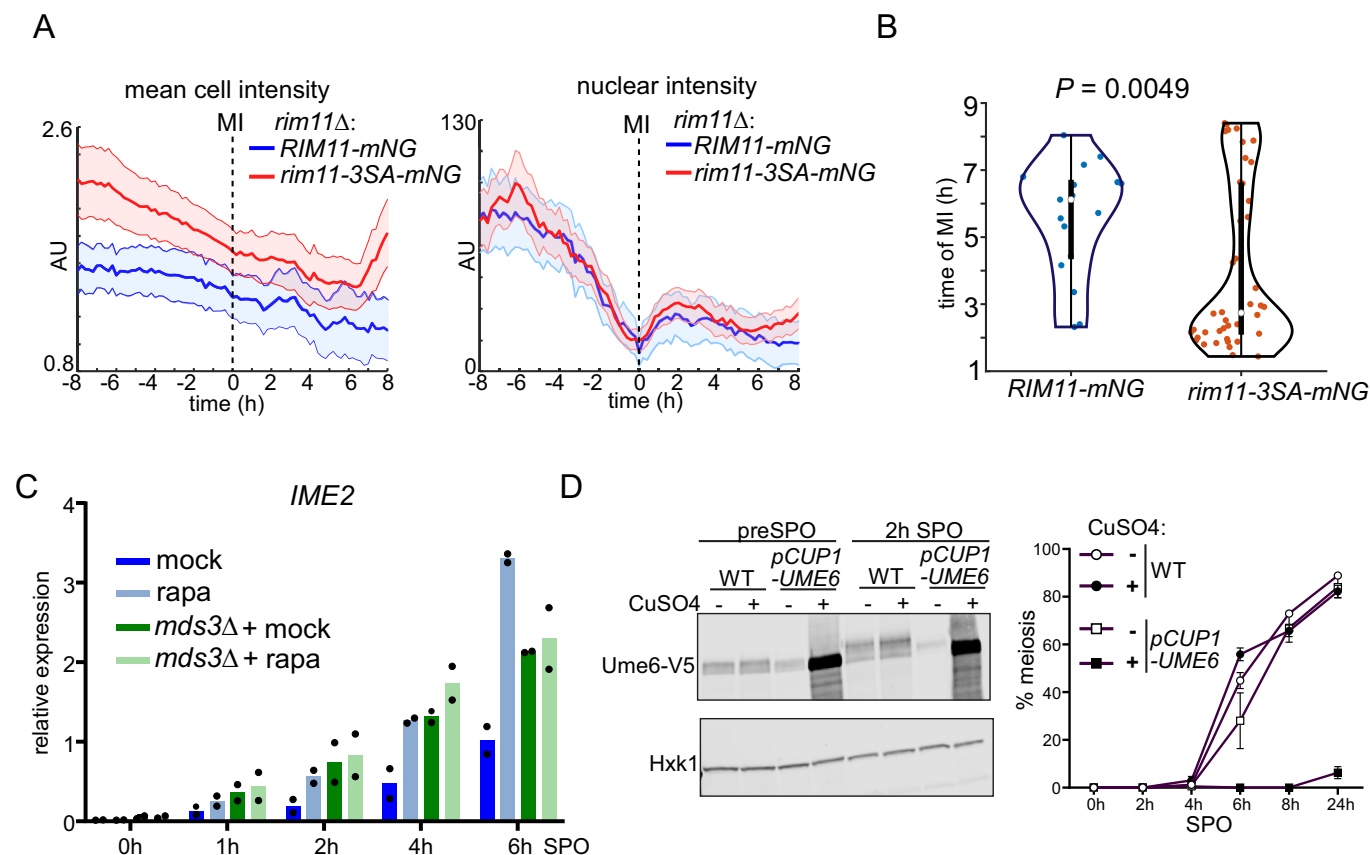
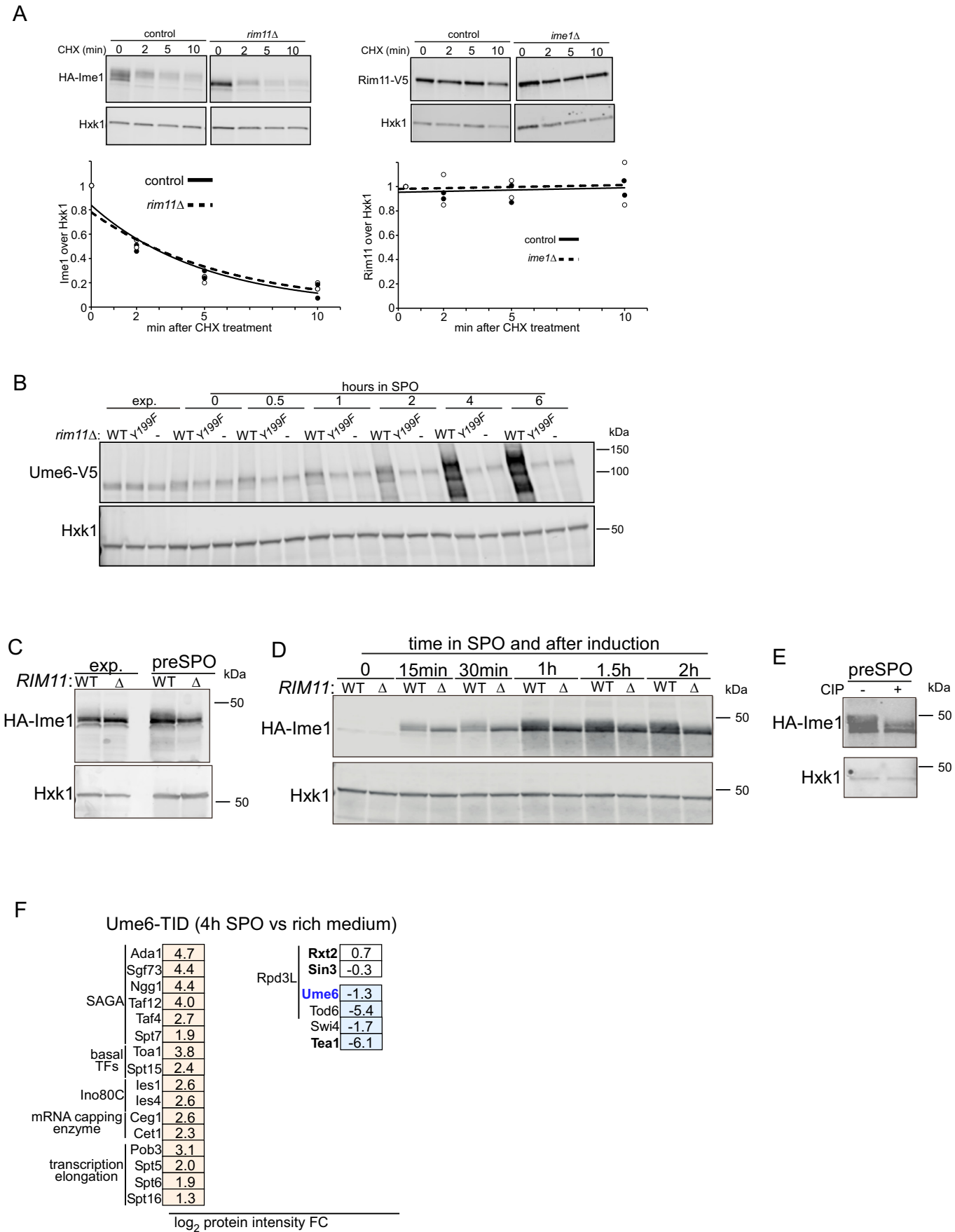


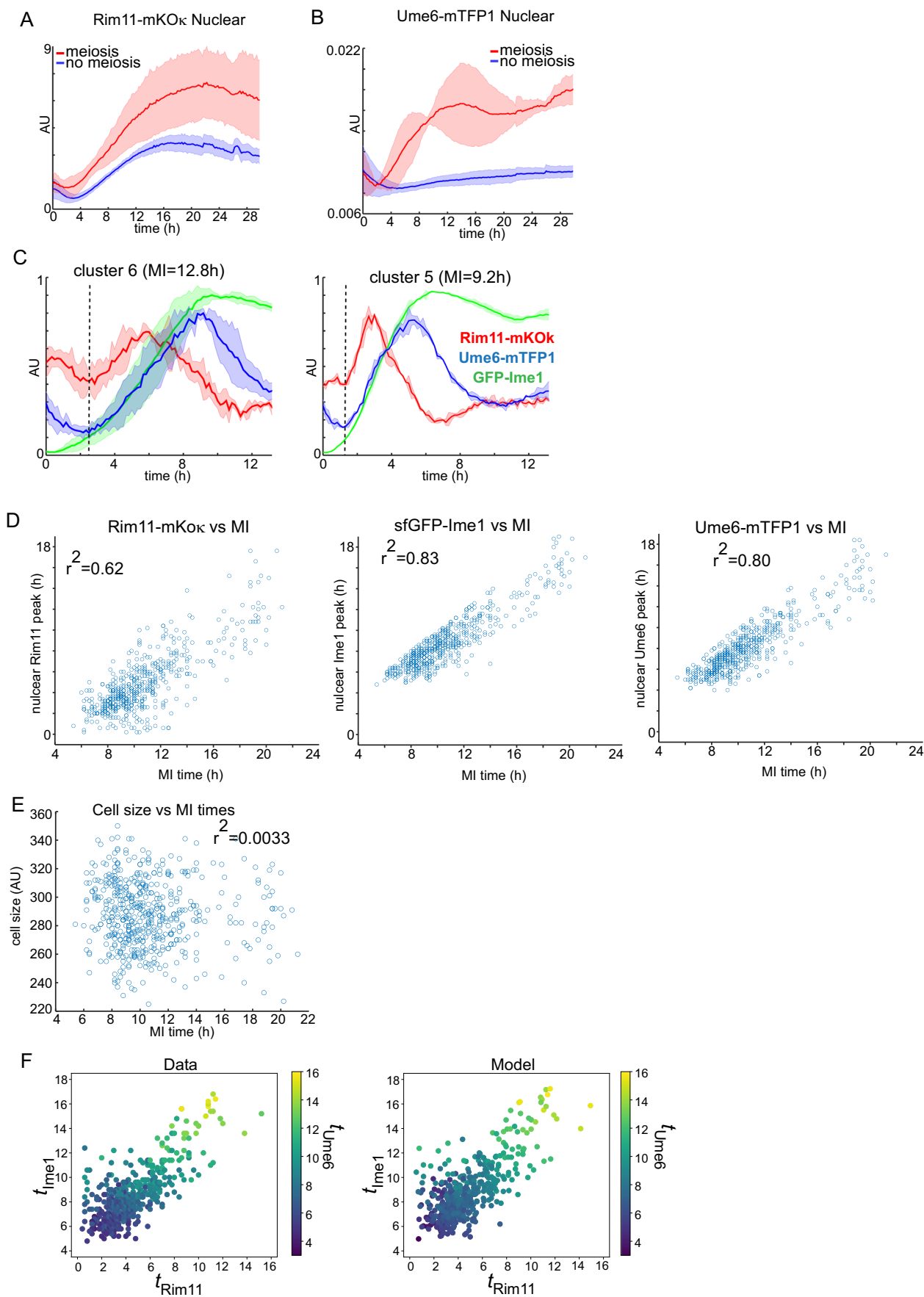
Figure EV3. PKA and TORC1 control Rim11 via distinct mechanisms.

(A) Quantification of live-cell imaging of Rim11-mNG and rim11-3SA-mNG. Shown are whole-cell concentration (left) and nuclear intensity (right). Cells harboring *rim11Δ* expressing a single-copy integration plasmid harboring *RIM11-mNG* and *rim11-3SA-mNG* (FW10776 and FW10778). Time points were aligned according to the MI division. At least $n = 500$ cells were quantified. The bold colored line represents the mean, and the 95% intervals are highlighted by the thin colored lines. (B) The same experiment as in A but showing the timing of meiosis in Rim11-mNG and rim11-3SA-mNG. $N = 17$ (Rim11-mNG) and $n = 52$ (rim11-3SA-mNG) cells were quantified. The minima and maxima represent the whole distribution range, the center dot the median of the distribution, the bounds of the box represents limits of the 1.5 interquartile range, whiskers represent the whole distribution leaving out the outliers using a 1.5 interquartile outlier criterion. The area around the whisker plot shows the shape of the distribution represented as the kernel density. (C) *IME2* expression in cells induced to enter meiosis in WT and *mds3Δ* that were either untreated or treated with rapamycin (FW10297 and FW10718). *IME2* expression signals were normalized over *ACT1*. The mean signals of $n = 2$ biological repeats are shown. (D) Expression and onset of meiosis in *UME6-V5* (FW1208) and *pCUP1-UME6-V5* (FW10562). Cells were grown till the presporulation medium, treated with CuSO₄ or untreated, shifted to SPO. Samples were taken at the indicated time points for western blot and DAPI staining. Membranes were probed with anti-V5 antibodies and Hxk1 antibodies as a loading control (left). Cells that contained two more DAPI masses were considered to have entered meiosis. The error bars represent the mean + SEM of $n = 3$, and at least $n = 100$ cells per biological repeat were quantified (right).



◀ **Figure EV4. Ime1 is required for Rim11-directed Ume6 phosphorylation.**

(A) Relative protein stability of Ime1 and Rim11 in control and in *rim11Δ* or *ime1Δ* cells, respectively. *pCUP-HA-IME1* (control and *rim11Δ*, FW2444 and FW10373) or *RIM11-V5* (control and *ime1Δ*, FW10446 and FW11682) cells were induced to enter meiosis. At 2 h in SPO Ime1 expression was induced with CuSO₄. At 4 h cells were treated with cycloheximide (CHX). Samples were taken at the indicated time points for western blotting. Membranes were probed with anti-HA or anti-V5 antibodies. As a loading control Hxk1 was used. Relative quantification with respect Hxk1 and the 0-hour time point is shown for *n* = 2 biological repeats. (B) Ume6-V5 expression and migration as determined by western blotting in *rim11Δ* expressing an integration plasmid harboring *RIM11-mNG* or *rim11-Y199F-mNG* cells induced to enter meiosis (FW11186, FW11184, FW10033). Membranes were probed with anti-V5 antibodies and Hxk1 antibodies. (C-E) Ime1 expression and migration in exponential growth, and in cells induced to enter meiosis. Ime1 tagged with HA was expressed from the *CUP1* promoter and induced in WT and *rim11Δ* cells (FW2444, FW10373). Samples were taken at the indicated time points, and probed with anti-HA antibodies, and Hxk1 antibodies as a control. In E protein extracts from samples grown in presporulation medium were treated with alkaline phosphatase (CIP). (F) Proximity labeling using Ume6-TID comparing 4 h SPO to rich medium conditions. Log₂ protein intensity fold change (FC) comparing 4 h SPO to rich medium. Proteins involved in transcription or that are known to interact with Ume6 are shown.



◀ **Figure EV5. Single-cell analysis and modeling reveals the timing dynamics Rim11, Ime1, Ume6 and meiosis.**

Live-cell imaging of the dynamics of Rim11-mKO_κ, sfGFP-Ime1, Ume6-mTFP in meiotic and non-meiotic cells (FW11243). (A) Nuclear concentrations of Rim1-mKO_κ in meiotic and non-meiotic cells. (B) Nuclear concentrations of Rim11-mTFP in meiotic and non-meiotic cells. (C) Mean traces of Rim11-mKO_κ, sfGFP-Ime1, and Ume6-mTFP nuclear intensity for clusters 6 (left), and 5 (right) described in Fig. 7D. (D) Scatter plot showing the single-cell data comparing the timing of Rim11-mKO_κ peak versus MI (left), sfGFP-Ime1 peak versus MI (middle), Ume6-mTFP peak versus MI (right). (E) Scatter plot showing the single-cell data comparing the timing of cell size versus MI. (F) Time of Ume6-mTFP peak as a function of sfGFP-Ime1 and Rim11-mKO_κ peak timings for the cells that enter meiosis. The left panel shows the results from the time series data, while the right panel illustrates our model predictions. Besides the peak time in Ime1 and Rim11, the model reproduces the amplitudes of the peaks in Ime1 and Rim11 as well as the final value of Rim11 in the single-cell data. The plot contains 461 out of 524 cells—of the remaining 63 cells, most were excluded because the maximal values in Ime1 and Rim11 of the model lie outside the first 18 h after $t = 0$.