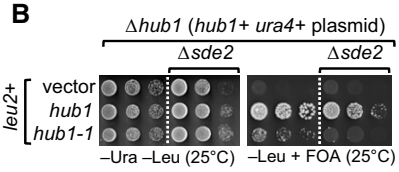
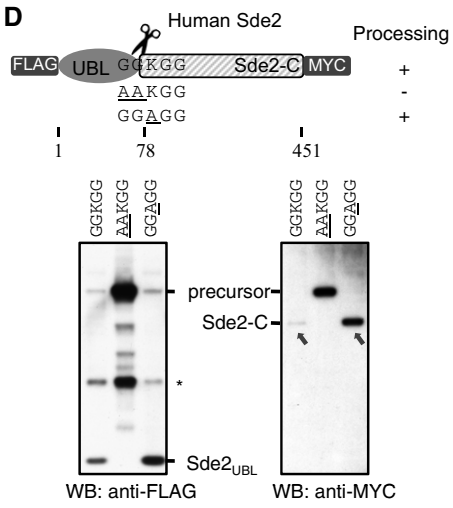
Expanded View Figures

A

Negative genetic interactors of *hub1-1*

Systematic ID	Gene
SPBC18H10.10C	*saf4*
SPCP1E11.07C	*cwf18*
SPCC364.02C	*bis1*
SPAC1952.06C	-
SPBC32F12.05C	*cwf12*
SPAC31G5.18C	***sde2***
SPBC19C2.14	*smd3*
SPAC30D11.09	*cwf19*
SPAC9.13C	*cwf16*
SPBC1289.11	*spf38*





C

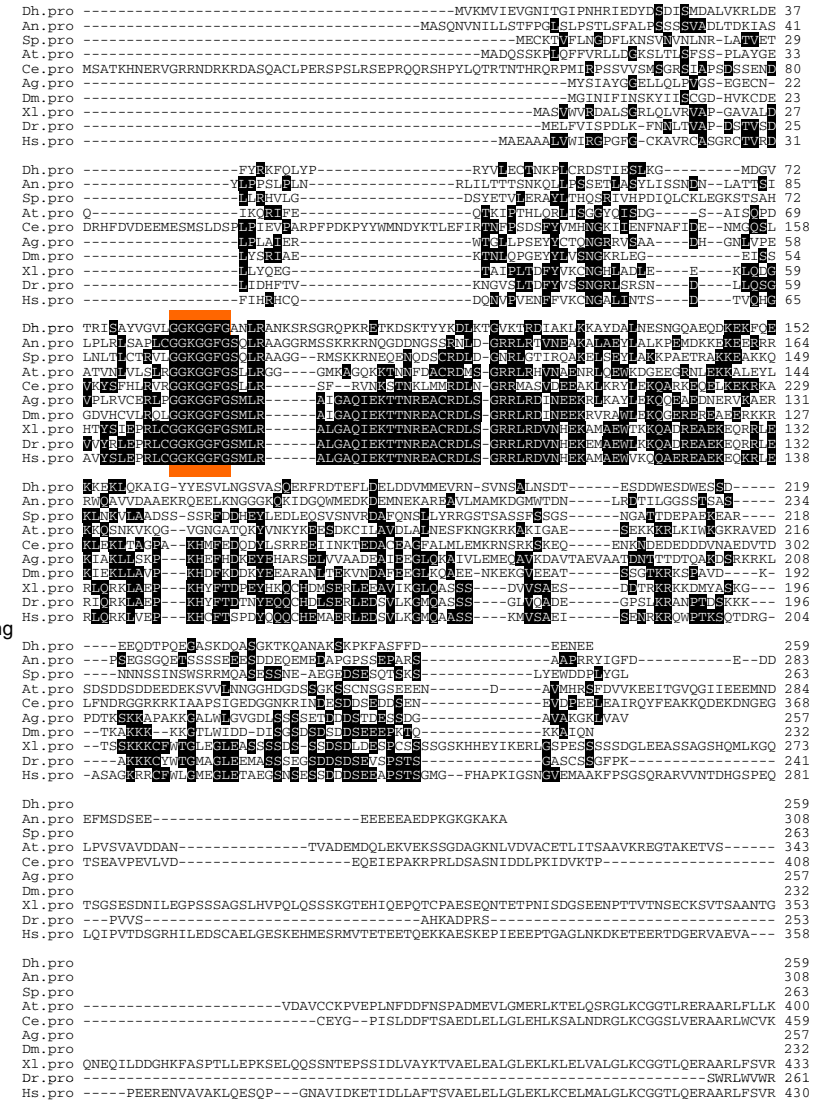


Figure EV1. Sde2 is processed like ubiquitin and genetically interacts with ubiquitin-like protein Hub1.

A Genetic interactors of *hub1*. *hub1-1* is the temperature-sensitive *hub1(I42S)* mutant reported by Yashiroda and Tanaka (2004). A genetic screen was performed with *hub1-1* and the haploid deletion library of non-essential genes in *Schizosaccharomyces pombe*. *hub1-1* was synthetically sick with the deletion mutants of given genes including *sde2*. Among the top hits, genes with relevance to pre-mRNA splicing are shown.

B Confirmation of the negative genetic interaction between *hub1-1* and Δ*sde2* mutants. *sde2* gene, from ATG to the stop codon, was deleted in a *S. pombe Δhub1* strain, which was kept viable with a *ura4*$^+$ marked *hub1* expression plasmid with its own promoter and terminator. The resultant strain was transformed with *leu2*$^+$ marked expression plasmids expressing wild-type (WT) *hub1* or the *hub1-1* mutant. Fivefold serial dilutions of cells from these transformations were spotted on indicated agar plates. Plates were incubated at 25°C. 5-fluoroorotic acid (FOA) (1 g/l of media) was used to shuffle-out *ura4*$^+$ plasmid. Thus, cells growing on −Leu + FOA plates will have *hub1* mutants in Δ*sde2* background.

C Alignment of Sde2 protein orthologs from different eukaryotes. Abbreviations used are (respective NCBI protein accession numbers are given in parentheses) as follows: Dh, *Debaryomyces hansenii* (XP_458854); An, *Aspergillus niger* (XP_001391007); Sp, *Schizosaccharomyces pombe* (NP_594019); At, *Arabidopsis thaliana* (NP_192009); Ce, *Caenorhabditis elegans* (NP_506378); Ag, *Anopheles gambiae* (XP_321833); Dm, *Drosophila melanogaster* (NP_651207); Xl, *Xenopus laevis* (NP_001084858); Dr, *Danio rario* (AAH93198); Hs, *Homo sapiens* (NP_689821). The sequence marked in orange indicates most conserved region in Sde2 orthologs.

D Expression of human Sde2 (C1orf55) protein in U2OS cells. Constructs with sequences encoding 3FLAG epitope tag at the N-terminus of *HsSDE2* gene and single *MYC* epitope tag at its C-terminus under *CMV* (cytomegalovirus) promoter were used. With WT Sde2, Sde2-C would start with lysine (KGG. . .), whereas with Sde2 GGAGG mutant, Sde2-C would start with alanine (AGG. . .). Asterisk indicates antibody cross reactivity signal. Arrows indicate the *Hs*Sde2-C protein formed after processing of the GGAGG mutant precursor accumulated to higher levels than the protein formed from the wild-type precursor.

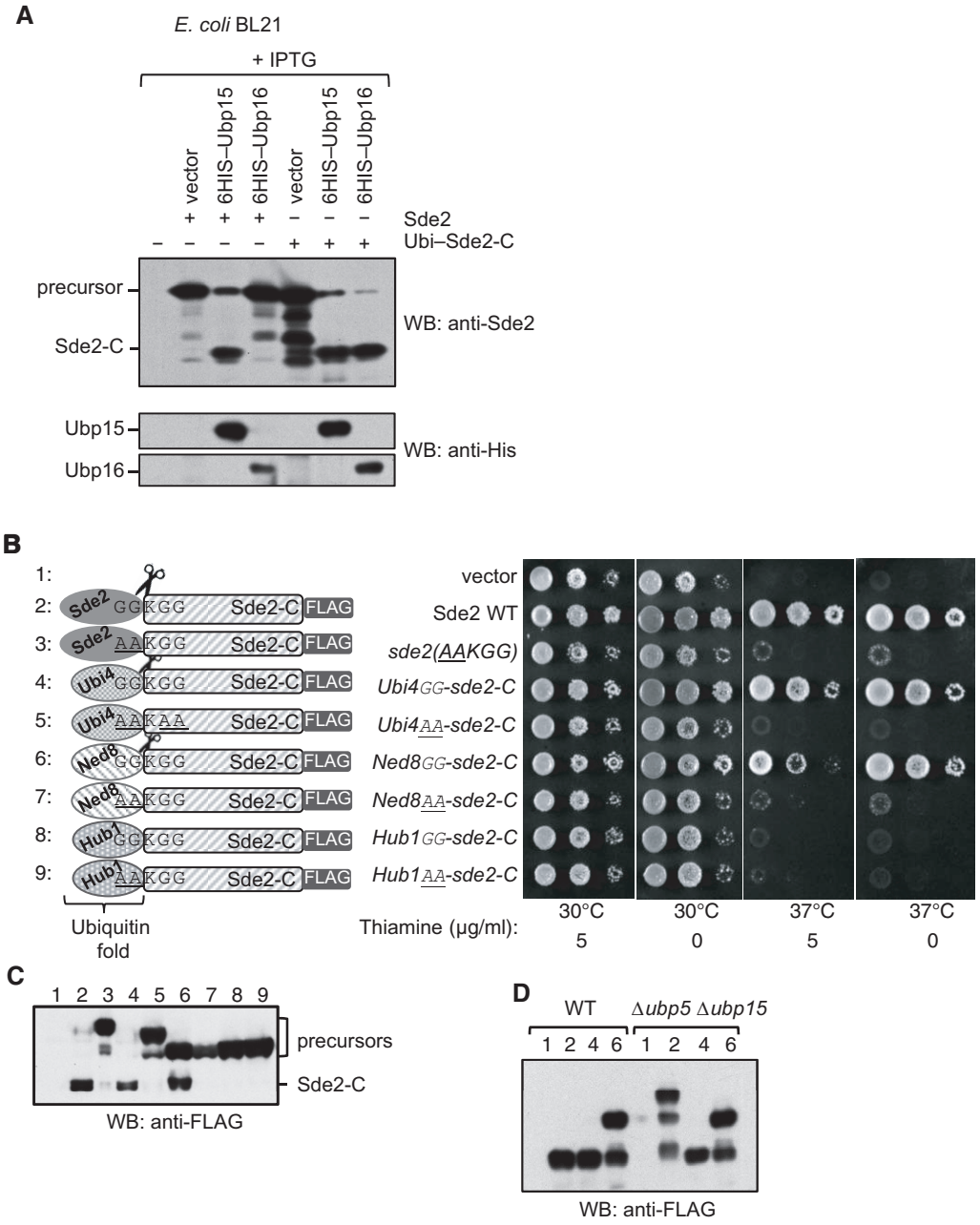


Figure EV2. Replacement of Sde2 UBL fold with other ubiquitin-like folds.

A A USP domain-containing DUB Ubp16 does not process Sde2. Experiment similar to Fig 2D. Processing of ubiquitin–Sde2-C fusion by the proteases was used as control. Anti-HIS immunoblotting was used to monitor expression of the proteases.

B $Sde2_{UBL}$ can be replaced with the UBLs ubiquitin and Ned8. Complementation of growth defects of *Schizosaccharomyces pombe Δsde2* strain with indicated fusion constructs. All constructs with a C-terminal 3FLAG tag were expressed under the thiamine-repressible *nmt81* promoter. Expressed proteins are N-terminal fusions of the ubiquitin-folds of various UBLs (Ubiquitin/Ubi4, Ned8 or Hub1) with Sde2-C. The GGKGG motif was kept at the junctions in the fusion proteins. After processing of the UBLs, Sde2-C would start with a lysine.

C Anti-FLAG immunoblot assay of fusion proteins shown in (B).

D Processing of ubiquitin- and Ned8–Sde-C chimeras was not affected in *Δubp5 Δubp15* double mutant. Expression of the clones used in (B) and indicated with numerical was monitored by immunoblotting using anti-FLAG antibody.

Source data are available online for this figure.

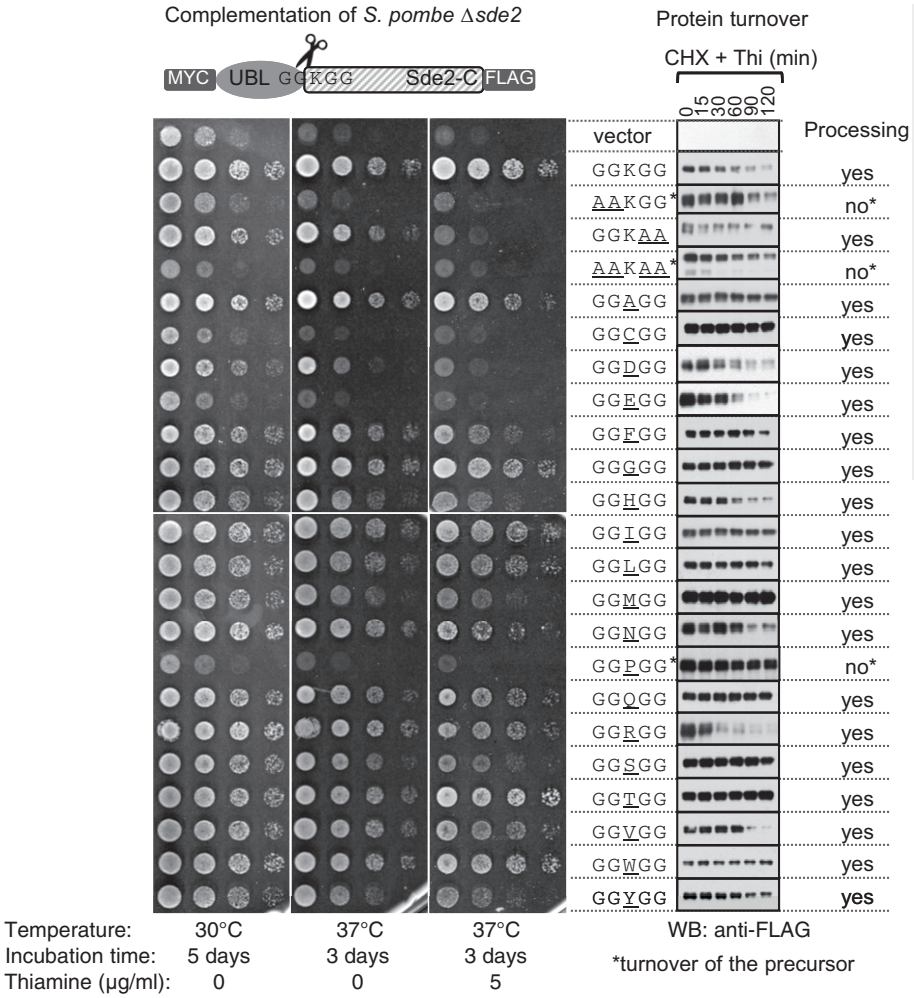


Figure EV3. Sde2-C is a substrate of the N-end rule pathway.

Complementation of growth defects of *Schizosaccharomyces pombe Δsde2* strain (left) with indicated constructs (middle), and protein turnover assays (right). The constructs are as in Fig 1B. Spot assay was performed as in Fig 1C. For protein turnover assays, transcription from the *nmt81* promoter was repressed by adding 1.7 μg/ml thiamine, and translation was halted by 100 μg/ml of cycloheximide. Cells harvested from equal volume of cultures at indicated time intervals were processed for immunoblot analysis with anti-FLAG antibody. Asterisks mark Sde2 variants defective in processing; therefore, in these cases, turnover of the precursor has been analysed.

Source data are available online for this figure.

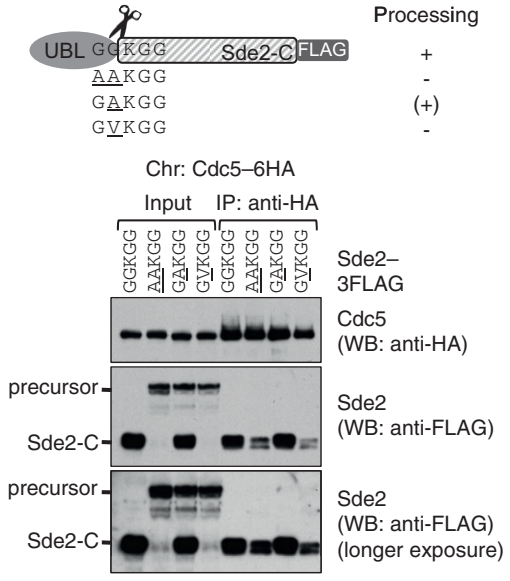


Figure EV4. Sde2 UBL is inhibitory for the incorporation of Sde2-C into the spliceosome.

Sde2 variants in the schematics were transformed into *Schizosaccharomyces pombe Δsde2* with Cdc5-6HA chromosomal tag. Anti-HA IP was carried out to monitor Co-IP of Sde2-C and precursor. Only processed Sde2-C, but not the precursor, associates with Cdc5.

Source data are available online for this figure.

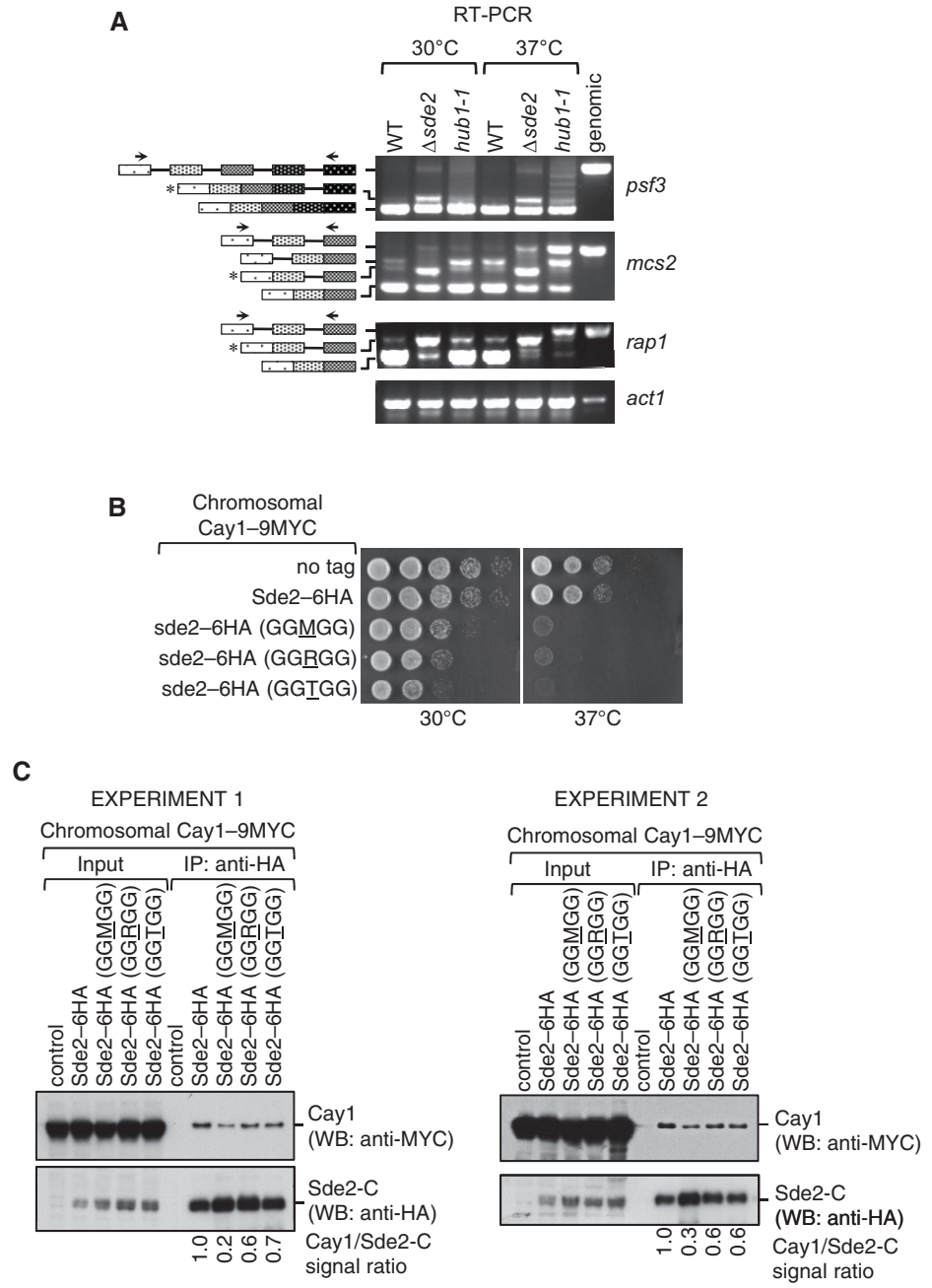


Figure EV5. Sde2 and Hub1 have distinct splicing targets and N-terminal lysine of Sde2-C is crucial for interaction with Cactin.

A RT–PCR assays to monitor accumulation of intron-containing transcripts in *Δsde2* and *hub1-1* mutants. Assay is similar to Fig 5B.

B Growth phenotypes of chromosomal Sde2 lysine mutants on rich media at 30°C and 37°C.

C Association of Cactin/Cay1 with lysine mutants of Sde2-C. Experiments are performed with proteins expressed from chromosomally tagged strains. IP was performed using anti-HA beads followed by immunoblot assays with anti-MYC antibody to monitor Co-IP of Cay1. Anti-HA antibody was used to monitor IP efficiency of Sde2 mutants. Numbers indicate ratio of Cay1 to Sde2 (MYC/HA) signals obtained from ImageJ quantification of immunoblots. Results from two independent biological replicate experiments are shown.

Source data are available online for this figure.