Kurischko et al., http://www.jcb.org/cgi/content/full/jcb.201011061/DC1



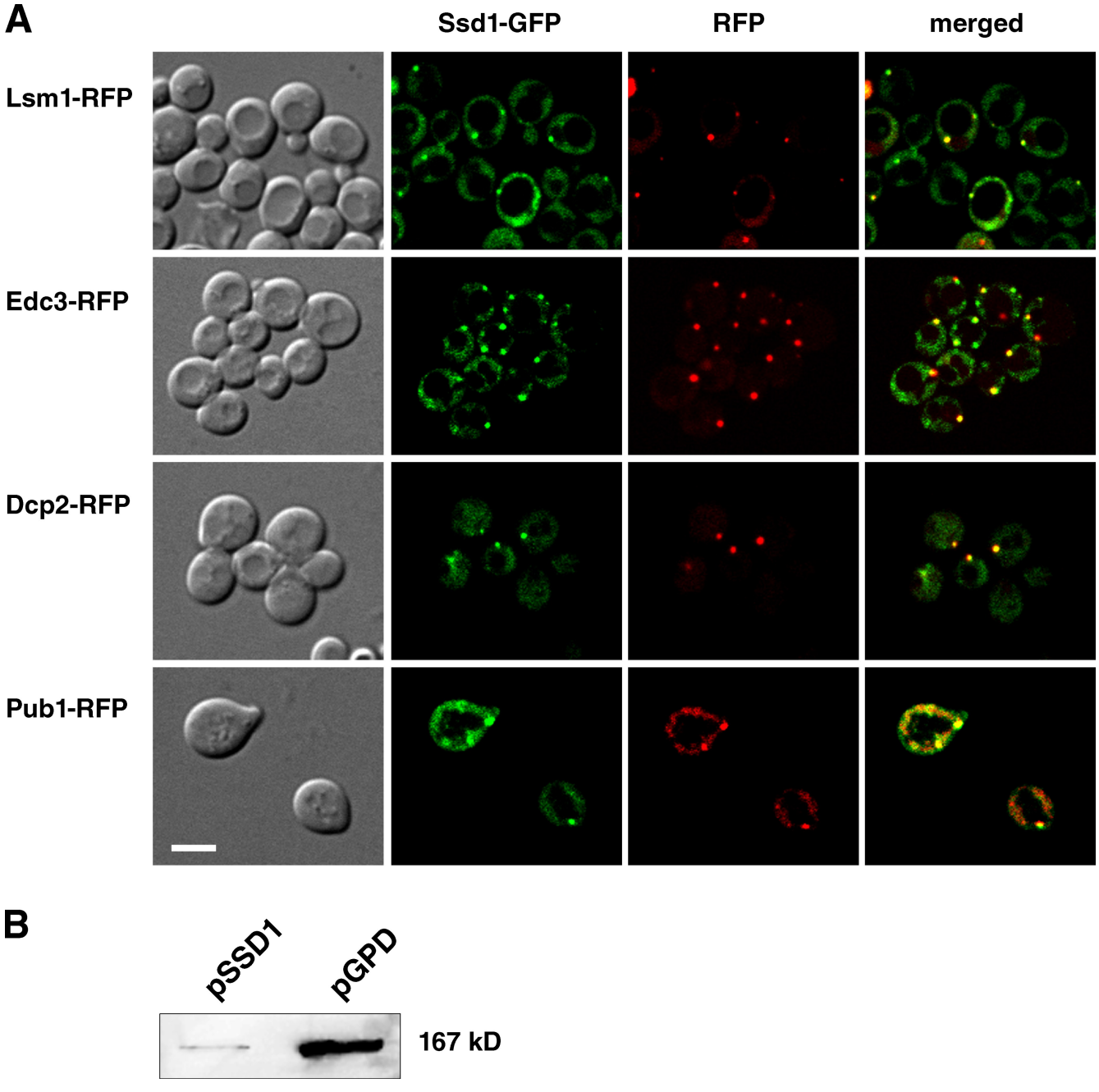


Figure S1. **Physiologically expressed Ssd1 colocalizes with P-bodies in glucose-depleted cells.** (A) Cells expressing Ssd1-GFP under control of its physiological promoter and RFP-tagged P-body and stress granule proteins were transferred to glucose-depleted medium for 10 min and analyzed by spinning disk confocal microscopy. The RFP-tagged proteins (Edc3, Lsm1, Dcp2, and Pub1) were encoded by plasmids (see Table II). Most (~90%), but not all Ssd1 puncta colocalize with P-body and stress granule proteins. All images represent single optical sections. Bars, 2 µm. (B) Ssd1 immunoblot showing comparative analysis of Ssd1 expression from GPD promoter vs. endogenous promoter. Cells expressing integrated Ssd1-GFP and plasmid-borne GPD promoter-driven Ssd1-GFP (FLY1593 and FLY2184 + FLE1019) were TCA precipitated and processed for immunoblots as described in Materials and methods. Based on immunoblot analysis, GPD-driven Ssd1 (on low copy plasmid) is expressed approximately sevenfold higher than physiologically expressed Ssd1.

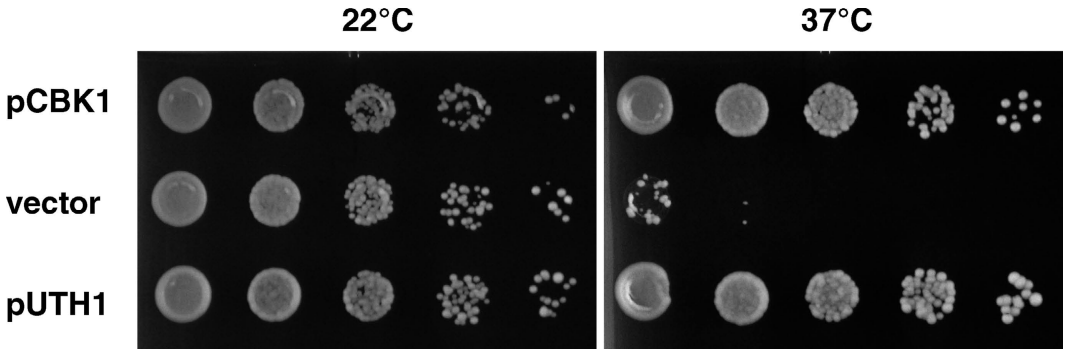


Figure S2. ***UTH1* is a dosage suppressor of *cbk1-8* mutants.** Serial dilution of conditional *cbk1-8* mutant cells (FLY2884) containing empty vector, *CBK1*, and high copy *UTH1* plasmid (pGP564, FLE1171, pGP564-UTH1).

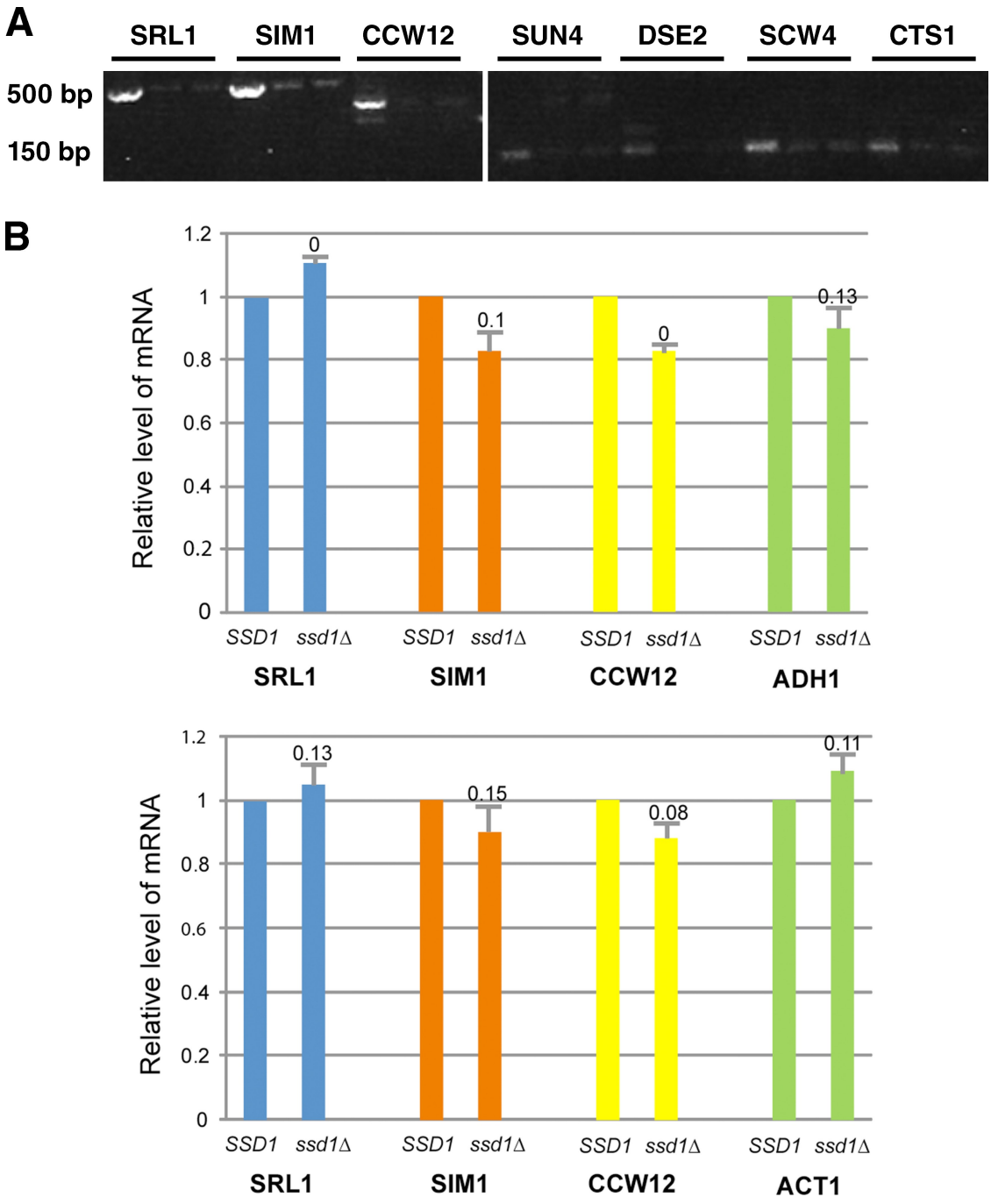


Figure S3. **Ssd1 precipitates *SRL1*, *SIM1*, *CCW12*, *SUN4*, *DSE2*, *SCW4*, and *CTS1* mRNAs and does not affect steady-state mRNA levels.** (A) Strain FLY2184 was transformed with either pGPD-SSD1-GFP (FLE1019; lane 1 for each gene) or pGPD-SSD1$^{1\text{-}520}$-GFP, which lacks the RNA-binding domain (FLE1020; lane 2). Lane 3 represents the untransformed strain. Ssd1-GFP was immunoprecipitated with an anti-GFP monoclonal antibody. The presence of the precipitated mRNAs was shown by RT-PCR. (B) Steady-state levels of *SRL1*, *CCW12*, and *SIM1* mRNA in *SSD1* and *ssd1Δ* cells are indistinguishable. Quantitative RT-PCR was performed as described in Materials and methods. The relative levels of query mRNA transcripts were compared with that of *ACT1* mRNA (top) and *ADH1* mRNA (bottom).

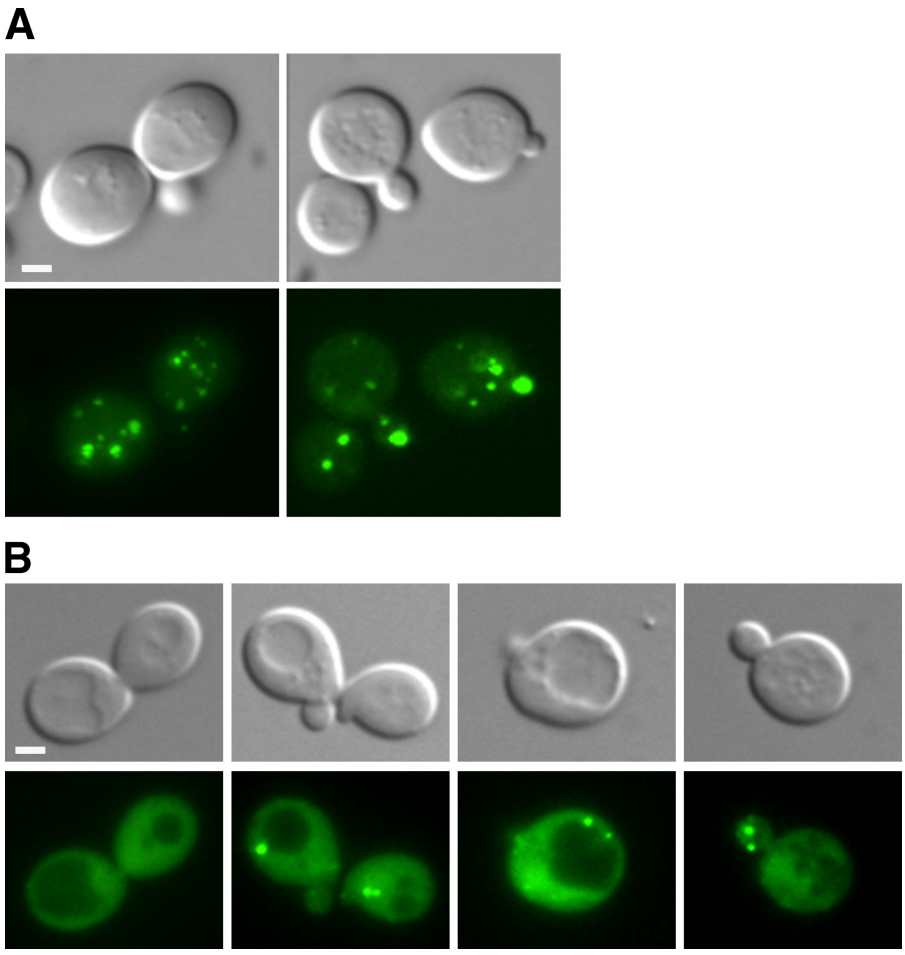


Figure S4. **Ssd1-9A, but not Ssd1-9A-RBDΔ expression causes SRL1 mRNA to localize to P-bodies.** (A) Cells expressing GAL-inducible Ssd1-9A-HA and *SRL1* mRNA-GFP (FLY3196 with plasmid FLE1208) were transferred to galactose medium for 1–2 h and *SRL1* mRNA localization was observed by wide-field fluorescence microscopy. (B) Representative images of SRL1 mRNA distribution in *ssd1Δ* cells expressing GPD promoter-driven Ssd1-9A-RBDΔ (FLE1278). The distribution of SRL1 mRNA localization in Ssd1-9A-RBDΔ cells is: no mRNA spots 15.6%, M 15.9%, D 25.3%, M+D 29.6%, multiple faint spots 13.6% (*n* = 257 cells). All images represent single optical sections. Bars, 2 μm.

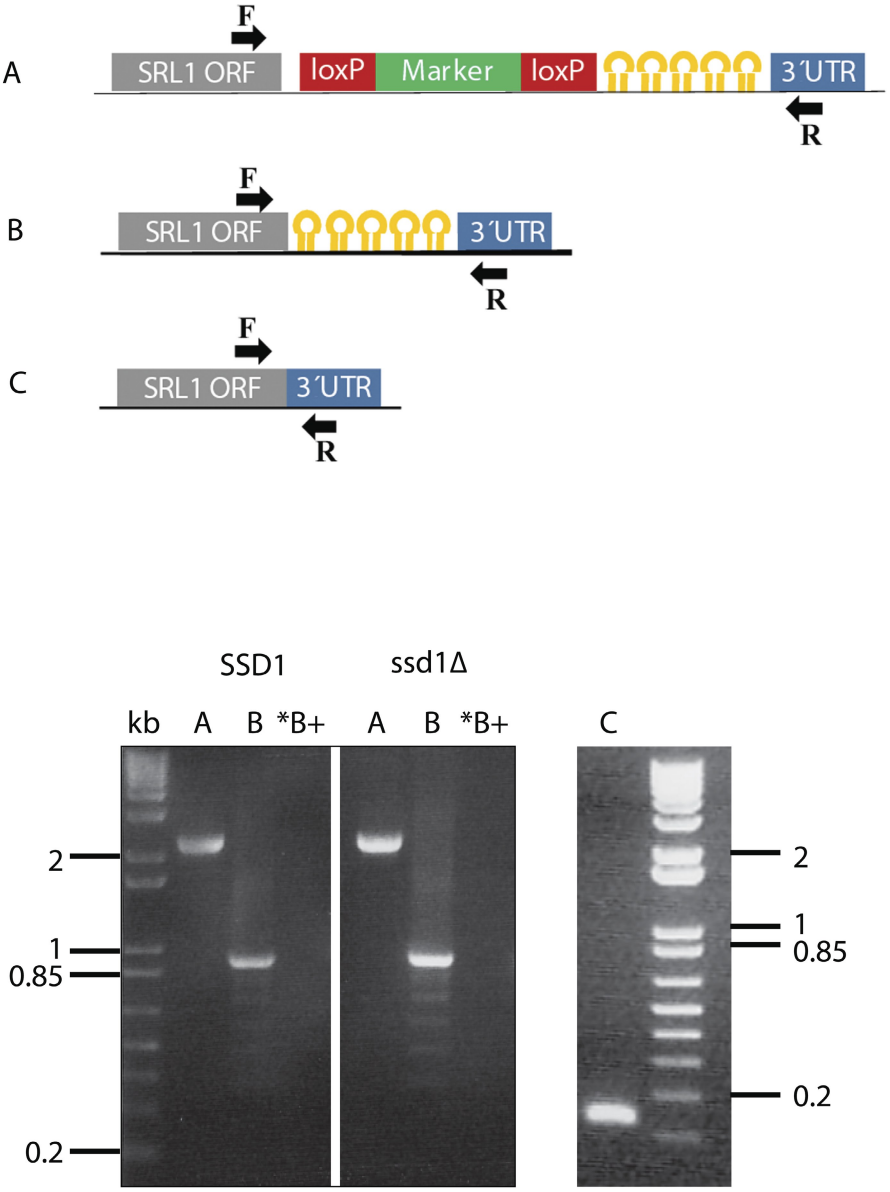


Figure S5. **Confirmation of *SRL1* mRNA tag expression in wild-type and *ssd1Δ* cells.** *SRL1* mRNA was tagged as described in Haim et al. (2007). The sequential steps are shown: (A) Integration of the MS2-CP-GFP cassette; (B) Loop-out of the selective marker; (C) *SRL1* locus without integration of the cassette. Each step was monitored by RT-PCR and reveals different sizes of the PCR products. There is no detectable difference in the expression of *SRL1* mRNA in *SSD1* and *ssd1Δ* strains.

## Reference

Haim, L., G. Zipor, S. Aronov, and J.E. Gerst. 2007. A genomic integration method to visualize localization of endogenous mRNAs in living yeast. *Nat. Methods*. 4:409–412.