SUPPLEMENTAL LEGENDS

Supplemental Figure S1. Identification of Sumo-enriched genes. (A) Proteins marked by FLAG-tagged Sumo are specifically pulled down by anti-FLAG beads. Cells expressing untagged Sumo ("*SMT3*") or FLAG-tagged Sumo ("*FLAG-SMT3*") were grown to log phase, after which cell lysates were incubated with anti-FLAG beads. Sumoylated proteins were visualized by Western blotting with anti-FLAG antibodies. **(B)** Snapshots of the Sumo ChIP-seq dataset showing Sumo enrichment of *CDC19*, *PMA1*, *ADH1*, and the *GAL1/10* promoter. **(C)** Examples of Sumo peaks that were improperly assigned to the wrong ORF. **(D)** Reduced protein sumoylation in the *ubc9-1* mutant even at permissive temperature. *UBC9* and *ubc9-1* strains were grown to log phase at the permissive temperature of 30°C and cellular proteins were purified by TCA protein extraction. Sumo, Ubc9 and the septin Cdc11 (loading control) were visualized by Western blotting with specific antibodies. The level of Ubc9p is substantially reduced in the *ubc9-1* mutant, resulting in a strong reduction in overall protein sumoylation. **(E)** The level of Sumo at *tRNA* genes and RPGs is dependent upon *UBC9*. WT cells or *ubc9-1* mutants were grown to log phase and the level of FLAG-tagged Sumo at $tRNA^{W}$, *RPL35A*, *ACT1* and an untranscribed region on chromosome V (*ChV*) was analyzed by ChIP using FLAG antibodies. Error bars indicate SEM of three independent experiments. **(F)** The Sumo defect at RPGs and tRNA genes in *ubc9-1* mutants is rescued by a plasmid containing WT *UBC9*. ChIPs were performed as in *(E)*.

Supplemental Figure S2. Ubc9 and TORC1 cooperate in expression of RPGs. (A) The expression of nearly all RPGs is reduced in *ubc9-1* mutants compared to WT cells. y-axis shows the relative expression levels of each RPG (ratio of WT cells over

ubc9-1 mutants). Data were derived from RNA-seq experiments. **(B**) The global transcriptome of RNAPII-transcribed genes is not affected by either Sumo or rapamycin. Data were derived from RNA-seq experiments. Differences between groups were tested with a two-sided Mann-Whitney U test. **(C)** Inhibition of TORC1 specifically increases sumoylation at RPGs both in WT cells and in *ubc9-1* mutants. WT cells and *ubc9-1* mutants expressing FLAG-tagged Sumo were treated with 100 nM rapamycin for 30 min, after which the level of Sumo at the indicated genes was determined by ChIP. Error bars indicate SEM of three independent experiments. **(D)** Inhibition of TORC1 results in a reduction in Sumo levels at *tDNA*^{w} and in reduced expression levels of immature *tRNA*^{w}. WT cells expressing FLAG-tagged Sumo were treated with 100 nM rapamycin for 30 min, after which RNA levels were analyzed by qPCR, while Sumo levels were determined by ChIP. Error bars indicate SEM of three independent experiments. **(E)** Inhibition of TORC1 results in increased Sumo levels at *RPL35A,* while reducing *RPL35A* expression levels. The same samples as in (*D*) were analyzed with primers specific for *RPL35A*. Error bars indicate SEM of three independent experiments.

Supplemental Figure S3. Rap1 is the molecular target of Sumo. (A) Heat maps showing co-localization of Rap1 and Sumo at RPGs. The density of Sumo and Rap1 ChIP-seq reads were plotted 2 kb upstream and 2 kb downstream of the TSS of RPGs. Green indicates high signal, red no signal. *Asterisk*, RPGs that do not bind Rap1 also do not contain Sumo at the promoter region. **(B)** Metagene analysis showing that the Sumo and Rap1 peaks do not perfectly overlap at Rap1-enriched sites of non-RPGs. **(C)** ChIP-seq snapshots of the promoter region of *SNR47* shows non-overlapping Sumo and Rap1 peaks. **(D)** Depletion of Rap1 results in specific reduction of *RPL35A*

expression levels. Cells were grown to log-phase in galactose, washed and incubated in glucose for the indicated times. Expression levels of *ACT1* and *RPL35A* were determined by RT-qPCR. Error bars indicate SEM. **(E)** Overview of the semi-native formaldehyde-fixation MS method. **(F)** Increased presence of Rap1 in the fraction of sumoylated proteins after rapamycin treatment. Cells expressing HIS_6-FLAG-tagged Sumo were grown to log-phase, treated for 30 min with either DMSO or 100 nM rapamycin and the levels of sumoylated proteins were determined by quantitative MS. **(G)** Tagging Sumo with a HIS_6-FLAG tag does not affect Rap1 expression levels. Cells expressing untagged Sumo ("*SMT3*"), and *HIS_6-FLAG-SMT3* cells (treated with either DMSO or 100 nM rapamycin for 30 min) were lysed and Rap1 levels were analyzed by Western blotting using Rap1 antibodies. **(H)** Western blot analysis of the samples used in Figure 4E and 4F, showing depletion of endogenous Rap1 (HA-tagged) and expression of the plasmid-borne *RAP1* and *rap1* alleles (GFP-tagged) after transferring cells from galactose to glucose-containing medium. **(I)** ChIP analysis of the levels of endogenous HA-Rap1 at RPG promoters, both before and after transferring cells from galactose to glucose-containing medium (the same samples were used as in Figure 4E). Error bars, SEM. **(J)** ChIP analysis of the level of HA-tagged wild-type Rap1 and GFP-tagged mutant Rap1 at the promoter of RPGs, both before and after transferring cells from galactose to glucose-containing medium (the same samples were used as in Figure 4E). Error bars, SEM.

Supplemental Figure S4. The interaction between Rap1 and the basal transcription machinery is dependent on Sumo but not TORC1. (A) Metagene analysis of RNAPII ChIP-seq data, showing that RNAPII levels at RPGs are specifically reduced in the *ubc9-1* mutant, particularly after a 30 min treatment with

100 nM rapamycin (*left*). In contrast, RNAPII levels at the top 2860 most highly transcribed genes remained unchanged under these conditions (*right*). **(B)** Inhibiting protein translation with either rapamycin or cycloheximide induces increased sumoylation of several high-molecular weight proteins. Cells were treated with 100 nM rapamycin or 200 μg/ml cycloheximide for the indicated times and whole cell lysates were subjected to Western blotting with Sumo antibodies (*upper panels*). Equal protein loading was confirmed by BioRad TGX stain-free gel electrophoresis, which was visualized with UV light (TGX UV, *lower panels*). **(C)** Sumo levels increase at the Rap1 binding site following inhibition of protein synthesis by rapamycin or cycloheximide treatment. Cells expressing Flag-tagged Sumo were treated with 100 nM rapamycin or 200 μg/ml cycloheximide for the indicated times and the level of Sumo at the Rap1 binding sites of *RPL35A* and *RPL43A* was determined by ChIP with Flag antibodies. *ACT1*, which does not recruit Rap1, was used as a control. **(D)** Ubc9 is part of a homeostatic feedback loop. Under optimal growth conditions, TORC1 and Ubc9 promote transcription of RPGs, which is important for the cellular translational capacity (*left panel*). However, when the translational capacity is compromised, a homeostatic feedback loop is induced that activates TORC1 (Urban et al. 2007) and which increase the activity of Ubc9 towards Rap1 (*right panel*).