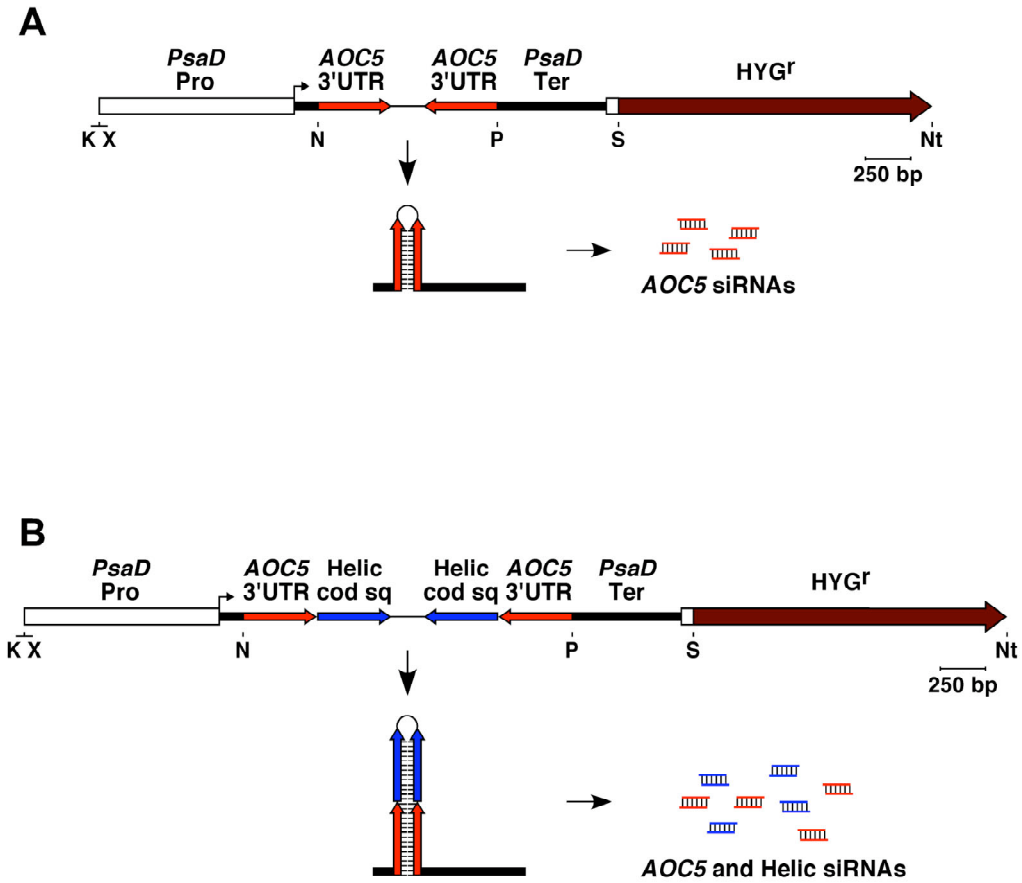
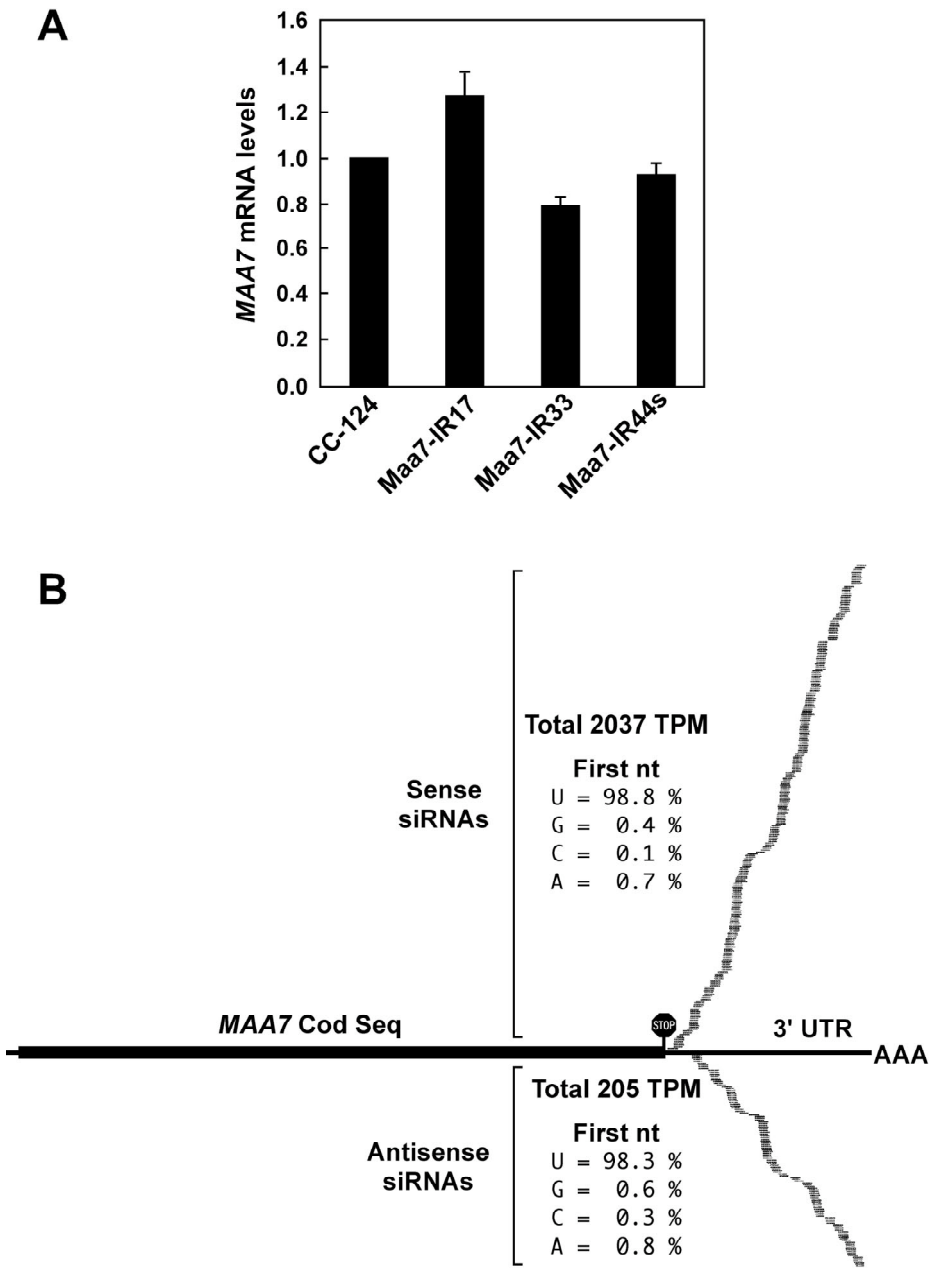


Supplemental Figure 1. Schematic diagrams of inverted repeat (IR) transgenes used to induce RNA interference (RNAi) in *Chlamydomonas*.

(A) Diagram of the construct triggering *AOC5* silencing. A 380-bp fragment, corresponding to the *AOC5* 3' UTR, was cloned in forward and reverse orientations flanking a DNA spacer and placed under the control of *PsaD* (encoding a Photosystem I subunit) regulatory sequences. This IR transgene was designed to generate, upon transcription, an RNA containing a double-stranded stem loop structure that can be processed into small interfering RNAs (siRNAs). The previously engineered *Aminoglycoside Phosphotransferase* gene (*aph7*), conferring resistance to hygromycin B (HYG^r) (Berthold et al., 2002), was placed immediately downstream from the *AOC5* IR transgene. Restriction sites: K, *Kpn*I; N, *Nco*I; Nt, *Not*I; P, *Pst*I; S, *Spe*I; X, *Xba*I.

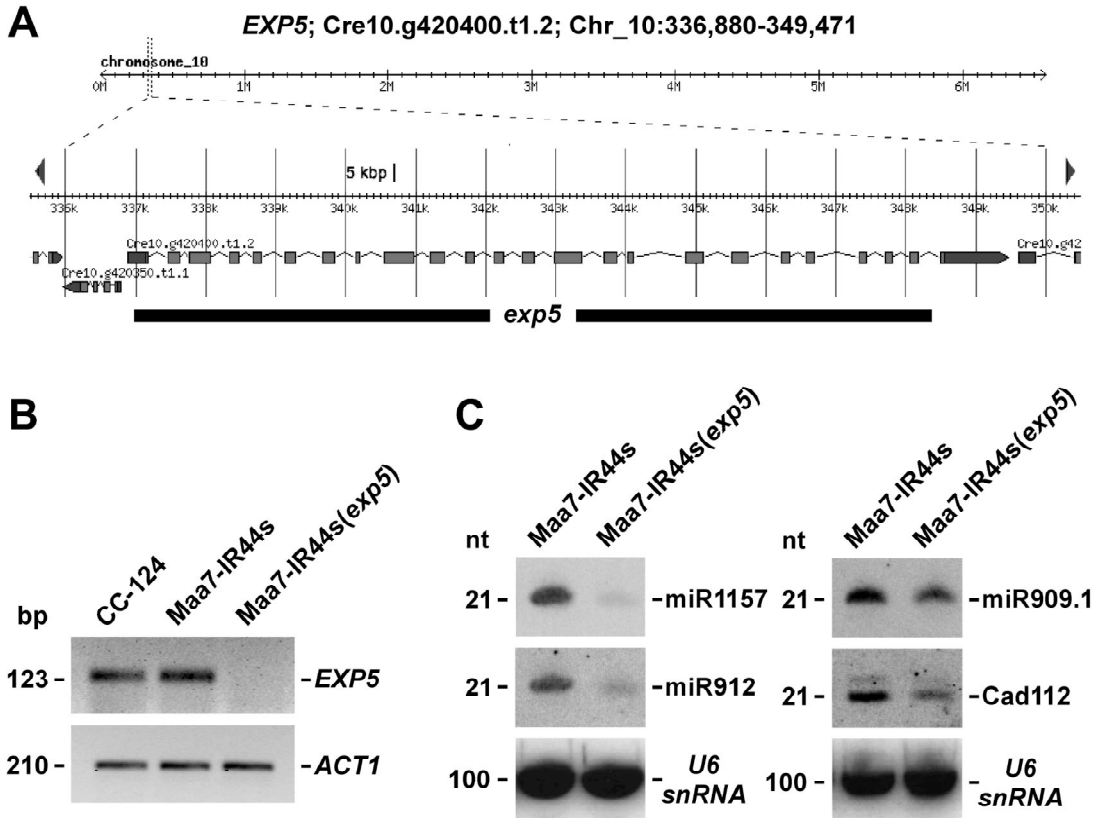
(B) Diagram of the construct triggering *AOC5* and *Cre16.g662000* (encoding a putative RNA helicase) silencing. A 400-bp fragment, corresponding to the *Cre16.g662000* coding sequence (Helic cod sq), was cloned in sense and antisense orientations, flanking a DNA spacer, in between the arms of the *AOC5* 3' UTR inverted repeat. This tandem IR transgene was designed to generate a double-stranded RNA transcript that can be processed into both *AOC5* and *Cre16.g662000* (Helic) siRNAs.



Supplemental Figure 2. Abundance of the *MAA7* (encoding tryptophan synthase β subunit) mRNA and siRNAs in transgenic strains undergoing RNA-mediated silencing.

(A) *MAA7* transcript levels in Maa7-IR transgenic strains and the CC-124 untransformed wild type strain. Total cell RNA was separated in agarose gels under denaturing conditions and hybridized to the *MAA7* coding sequence. The same blots were re-probed with the *ACT1* (encoding actin) coding sequence. Radioactive signal intensities from phosphorimager images were quantified with Imagequant TL software and the *MAA7* transcript levels normalized to those of the *ACT1* mRNA for each sample. For illustration purposes, the normalized *MAA7* amount in CC-124 was set to 1.0 and the remaining samples adjusted accordingly in the bar graph. The values represent the average of four independent experiments +/- SEM.

(B) Small interfering RNAs (siRNAs) matching perfectly to the *MAA7* mRNA 3' UTR in strain Maa7-IR44s. The location and abundance (as transcripts per million, TPM) of sense and antisense siRNAs are shown on the diagram. The nucleotide frequency at the 5' end of the siRNAs is also indicated.

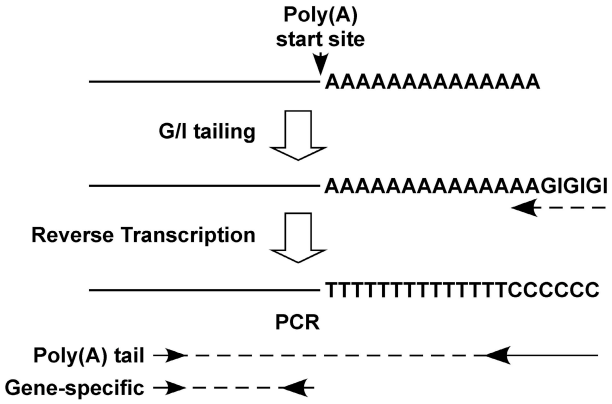


Supplemental Figure 3. A *Chlamydomonas Exportin 5* deletion mutant shows reduced steady-state levels of several endogenous microRNAs.

(A) Diagram of the *Cre10.g420400* gene, encoding the *C. reinhardtii* exportin 5 ortholog. The dark horizontal bar indicates the extent of the deletion in the Maa7-IR44s(*exp5*) mutant.

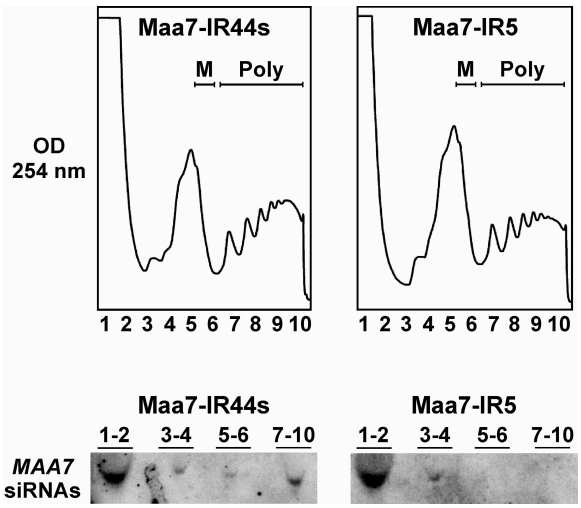
(B) Reverse Transcriptase (RT)-PCR analysis of *Exportin 5* steady-state mRNA levels in the indicated strains. Amplification of *ACT1* transcripts is shown as an input control. The panels show reverse images of agarose gel fractionated RT-PCR products corresponding to representative results out of three independent experiments. Reactions using RNA not treated with reverse transcriptase as the template were employed as a negative control (data not shown). CC-124, wild type strain; Maa7-IR44s, CC-124 transformed with an IR transgene targeting the 3' UTR of *MAA7* for silencing; Maa7-IR44s(*exp5*), Maa7-IR44s strain containing the *Cre10.g420400* deletion.

(C) RNA gel blot analyses of small RNAs isolated from the indicated strains and detected with probes specific for several *Chlamydomonas* miRNAs. Cad112, candidate miRNA 112. The same filters were re-probed with the U6 small nuclear RNA sequence as a control for equivalent loading of the lanes.



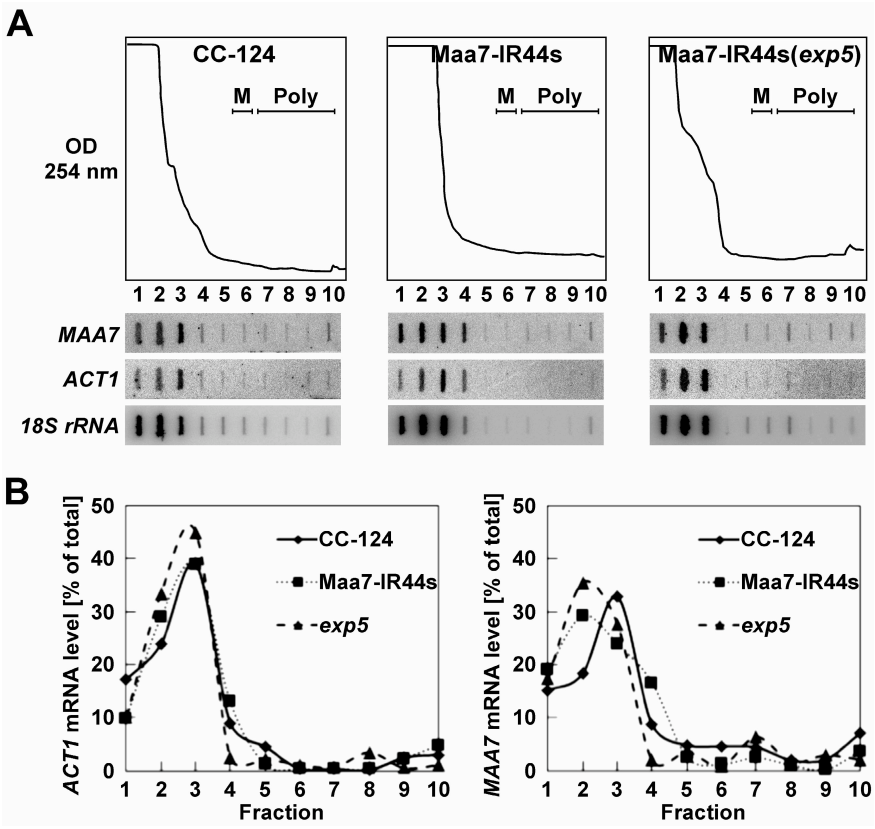
Supplemental Figure 4. Schematic diagram of the G/I tailing protocol used to examine mRNA poly(A) tail length.

A limited number of guanosine and inosine residues were first added to the 3' ends of transcripts by poly(A) polymerase. Tailed RNAs were then converted to DNA by reverse transcription using the newly added G/I tails and 2 nucleotides of the endogenous poly(A) tail as the priming sites. Finally, PCR amplification products were generated using two primer sets. A gene-specific forward and reverse primer set, designed to anneal upstream of the polyadenylation site, was used to produce a specific fragment that serves as a control for the gene of interest. The gene-specific forward primer and a universal reverse primer were used to generate another PCR product that includes the poly(A) tail of the gene of interest. After separating the PCR products on an agarose gel, the poly(A) tail length of the gene of interest can be determined by subtracting from the length of the poly(A) PCR product the length of the universal reverse primer and the distance of the gene-specific forward primer to the known polyadenylation start site.



Supplemental Figure 5. Distribution of *MAA7* siRNAs in sucrose density gradients of the Maa7-IR44s and Maa7-IR5 strains, assessed by RNA gel blot hybridization.

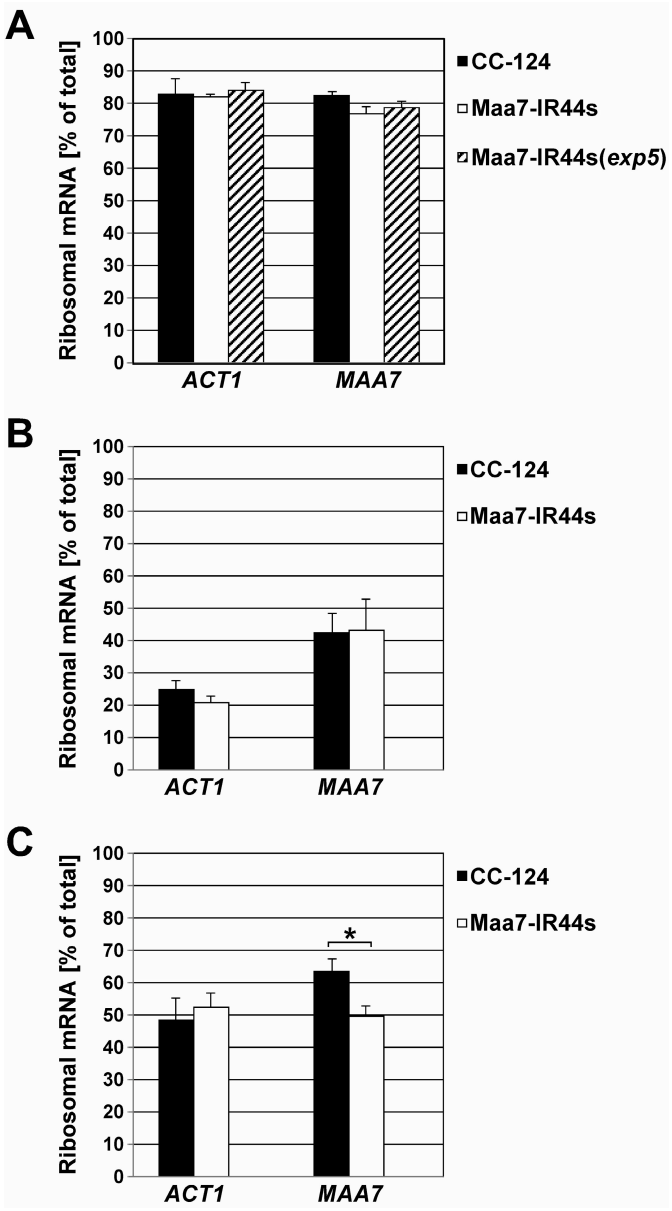
Numbers above the blots indicate pooled gradient fractions. The upper panels show typical polyribosome profiles of the indicated strains treated with 150 μg/ml cycloheximide throughout lysis and ultracentrifugation procedures. M, monoribosomes; Poly, polyribosomes. Maa7-IR5, strain expressing a *MAA7* IR transgene that induces target mRNA degradation (Rohr et al., 2004). Please note than in order to improve isolation of small RNAs cell extracts were separated through low salt sucrose gradients, as previously described (Djikeng et al., 2003).



Supplemental Figure 6. Migration of *MAA7* and *ACT1* transcripts in sucrose density gradients when examining cell extracts in the presence of 50 mM EDTA.

(A) Typical polyribosome profiles of the indicated strains. EDTA disrupts ribosome subunit association and the expected location of monosomes (M) and polyribosomes (Poly) is indicated on the graphs (upper panels). The distribution of the *MAA7*, *ACT1*, and *18S rRNA* transcripts in the gradient fractions was examined by slot blot hybridization (lower panels).

(B) Distribution of *ACT1* and *MAA7* mRNAs across the EDTA sucrose density gradients for the CC-124, Maa7-IR44s, and Maa7-IR44s(*exp5*) strains. The values represent the average of two independent experiments.

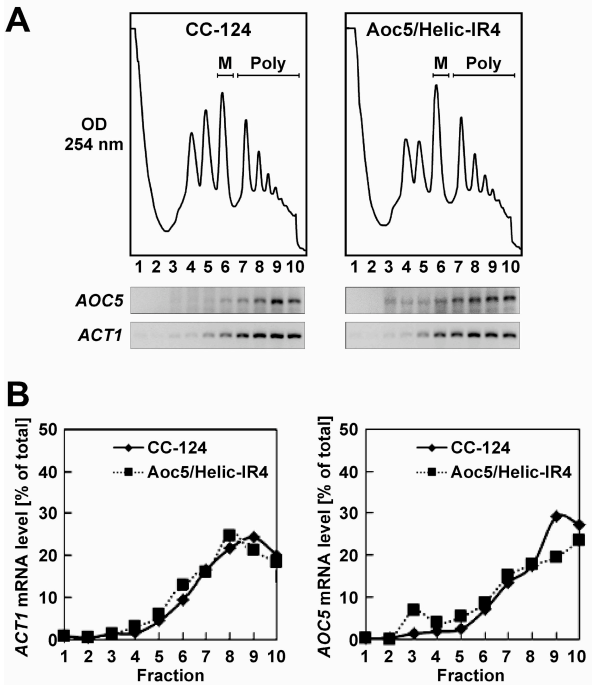


Supplemental Figure 7. Ribosome occupancy, the fraction of a specific mRNA associated with ribosomes, for the *MAA7* and *ACT1* transcripts after separation on sucrose density gradients.

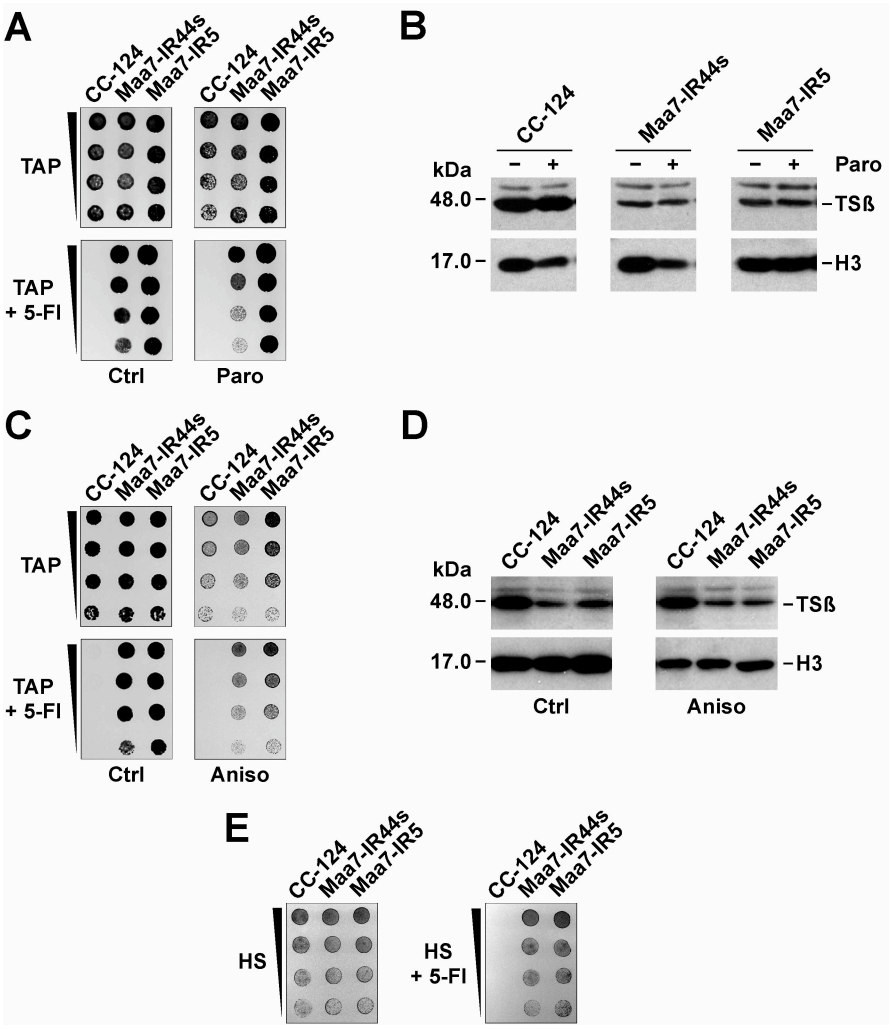
(A) Ribosome occupancy in the indicated strains treated with 150 μg/ml cycloheximide throughout lysis and ultracentrifugation procedures. The values represent the average of three independent experiments +/- SEM.

(B) Ribosome occupancy in the indicated strains after sucrose density gradient ultracentrifugation of cell extracts subjected to ribosome run-off in the absence of cycloheximide. The values represent the average of three independent experiments +/- SEM.

(C) Ribosome occupancy in the indicated strains after sucrose density gradient ultracentrifugation of cells extracts subjected to ribosome run-off in the presence of 30 μg/ml cycloheximide. The values represent the average of three independent experiments +/- SEM. Samples marked with an asterisk are significantly different ($P<0.05$) in a two tailed Student's *t*-test.



Supplemental Figure 8. IR-repressed *AOC5* transcripts are moderately depleted from polyribosomal fractions after ribosome run-off assays in the presence of low concentrations of cycloheximide.
(A) Typical polyribosome profiles of the indicated strains treated with 30 μg/ml cycloheximide throughout lysis and ultracentrifugation procedures (upper panels). M, monoribosomes; Poly, polyribosomes. The distribution of the *AOC5* and *ACT1* transcripts in the gradient fractions was examined by RT-PCR (lower panels).
(B) Distribution of *ACT1* and *AOC5* mRNAs across polyribosome profiles of the CC-124 and Aoc5/Helic-IR4 strains.



Supplemental Figure 9. TSβ protein synthesis from the *MAA7* transcript, subjected to siRNA-mediated translation repression, is not affected by treatment with paromomycin, anisomycin, or by slow growth on minimal medium.

(A) Growth and survival of the indicated strains on Tris-Acetate-Phosphate medium without (upper panels) or with (lower panels) 7 μM 5-fluoroindole supplemented with solvent (Ctrl) or with a sub-lethal concentration of paromomycin (Paro, 1.5 μg/ml). Cells grown to logarithmic phase in TAP medium were serially diluted, 5 μl-aliquots spotted on plates, and incubated for 7 to 15 days under dim lights. Maa7-IR5, strain expressing a *MAA7* IR transgene that induces target mRNA degradation (Rohr et al., 2004). Please note that this strain also carries an aminoglycoside 3'-phosphotransferase transgene and is therefore insensitive to paromomycin.

(B) Immunoblot analyses of TSβ and histone H3 protein levels. Cells from the indicated strains were cultured for 18 h in liquid TAP medium alone or containing 0.8 μg/ml paromomycin. Proteins corresponding to equal numbers of cells were loaded per lane. Since aminoglycoside 3'-phosphotransferase inactivates paromomycin in Maa7-IR5, the antibiotic has no effect on histone H3 accumulation (and on overall protein synthesis) in this strain.

(C) Growth and survival of the indicated strains on Tris-Acetate-Phosphate medium without (upper panels) or with (lower panels) 7 μM 5-fluoroindole supplemented with solvent (Ctrl) or with a sub-lethal concentration of anisomycin (Aniso, 1.0 μg/ml).

(D) Immunoblot analyses of TSβ and histone H3 protein levels. Cells from the indicated strains were cultured for 18 h in liquid TAP medium supplemented with solvent (Ctrl) or containing 0.6 μg/ml anisomycin. Proteins corresponding to equal numbers of cells were loaded per lane.

(E) Growth and survival of the indicated strains in High Salt (HS) minimal medium without (left panel) or with (right panel) 7 μM 5-fluoroindole. Cells grown to logarithmic phase in HS medium were serially diluted, 5 μl-aliquots spotted on plates, and incubated for 7 to 15 days under dim lights.

Supplemental References:

Berthold, P., Schmitt, R., and Mages, W. (2002). An engineered *Streptomyces hygroscopicus* aph 7" gene mediates dominant resistance against hygromycin B in *Chlamydomonas reinhardtii*. Protist **153:** 401-412.

Djikeng, A., Shi, H., Tschudi, C., Shen, S., and Ullu, E. (2003). An siRNA ribonucleoprotein is found associated with polyribosomes in *Trypanosoma brucei*. RNA **9:** 802-808.

Rohr, J., Sarkar, N., Balenger, S., Jeong, B.-r., and Cerutti, H. (2004). Tandem inverted repeat system for selection of effective transgenic RNAi strains in *Chlamydomonas*. Plant J. **40:** 611-621.