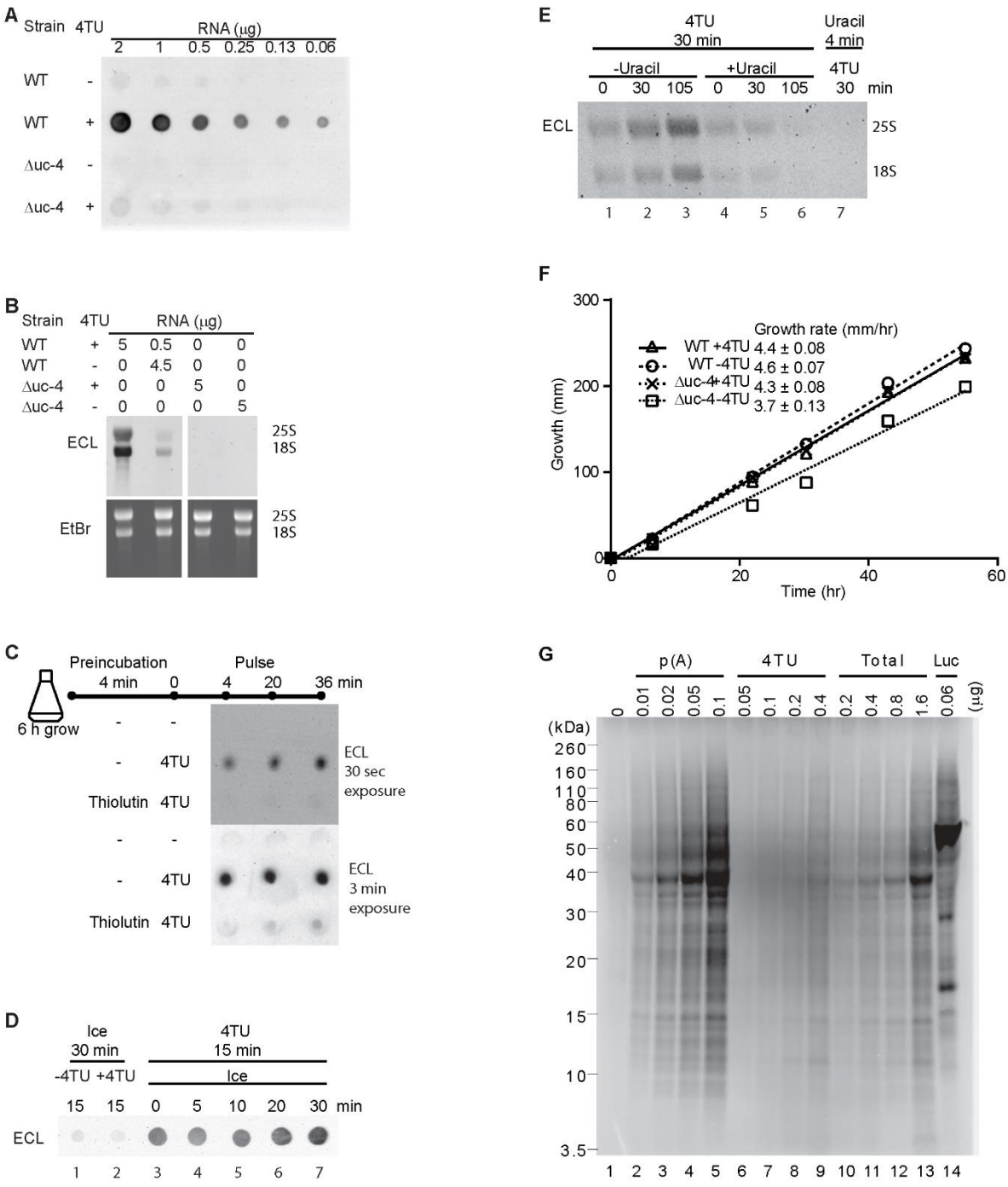

Figure S3 Validation of the 4TU pulse-chase protocol.

(A) ECL detection of biotinylated RNA prepared from wt or *Δuc-4 N. crassa* cultures germinated for 6 hr and incubated for 15 min with 0.2 mM 4TU (+) or without 4TU (-). Total RNA was prepared and biotinylated. The biotinylation probe is designed to react with the thio-group in 4TU-labeled

RNA. Serial dilutions of biotinylated RNA were spotted directly on nylon membrane at the indicated amounts. (B) Biotinylated RNA (5 μg/lane) was subjected to denaturing agarose gel electrophoresis and then transferred to membrane. The presence of biotinylated-4TU RNA was assessed using streptavidin coupled to horseradish peroxidase and incubation with ECL reagent. *25S* and *18S* rRNA bands stained with ethidium bromide are shown below. (C) Thiolutin blocks incorporation of 4TU into RNA. *N. crassa* wt cultures were germinated for 6 hr and treated with thiolutin (0.5 μg/ml) or not treated. Then 0.2 mM 4TU was added to cultures and samples were taken 4, 20 and 36 min following addition of 4TU. Total RNA was prepared and biotinylated; 1 μg of biotinylated RNA was spotted on nylon membrane and biotinylated 4TU RNA detected by ECL. Top panel, 30 sec exposure; bottom panel, 3 min exposure. (D) Chilling of cultures on ice reduces incorporation of 4TU. Lanes 1 and 2: 6 hr germinated wt cultures were placed on ice for 30 min; then 0.2 mM 4TU was added (lane 2) or not added (lane 1) and incubation on ice continued for 15 min; incorporation of 4TU into RNA was examined by dot-blotting and ECL. Lanes 3-7: a 6 hr germinated wt culture was incubated with shaking for 15 min at 30°C with 0.2 mM 4TU; then uracil was added to 10 mM and the culture quick-chilled by mixing with 7 ml VM/2% sucrose/10 mM uracil that had been pre-frozen in a 50 ml conical centrifuge tubes. The chilled culture was placed on ice and cells were harvested by filtration at 0, 5, 10, 20 and 30 min time points. For ECL analyses in lanes 1-7, total RNA was prepared and biotinylated; 1 μg of biotinylated RNA was spotted on nylon membrane and biotinylated 4TU RNA detected by ECL. (E) Lanes 1-6: Cultures of conidia from wt were germinated for 5.5 hr and then were treated with 0.2 mM 4TU for 30 min. Then 10 mM uracil was added to cultures using a 0.25M stock prepared in DMSO (+uracil, lanes 4-6) or the equivalent volume of DMSO was added (- uracil, lanes 1-3). Cells were harvested 0, 30 and 105 min later by filtration. In a separate analysis (lane 7), a 7 hr germinated culture were pretreated with 10 mM uracil for 4 min, then incubated with 0.2 mM 4TU for an additional 30 min, and then harvested by filtration. For lanes 1-7, total RNA was prepared and biotinylated; 2.5 μg of biotinylated RNA was subjected to denaturing agarose gel electrophoresis and then transferred to membrane. The presence of biotinylated-4TU *25S* and *18S* RNA was detected with ECL reagent. (F) Effects of 4TU on growth in race tubes. wt and *Δuc-4* strains were grown in race tubes containing VM/1.5% sucrose/2% agar with or without 0.2mM 4TU at 30°C with 12:12 hr L:D for 60 hr. The growth front was marked twice daily and the growth rate was calculated from triplicate experiments. (G) *In vitro* translation of 4TU RNA. Indicated amounts of *N. crassa* poly(A) RNA (Lanes 2-5), 4TU RNA (Lanes 6-9), total RNA (Lanes 10-13) and *in vitro* transcribed synthetic *luc* mRNA that was capped and polyadenylated (Lane 14) were used as templates for *in vitro* translation in micrococcal nuclease-treated wt *N.crassa* cell extracts (Wu *et al.* 2012). A translation reaction with no added template is shown in Lane 1. [^{35}S]Met-labeled translation products were separated on a 12% NuPAGE Bis-Tris Gel (Invitrogen NP0343) and detected by phosphorimaging.