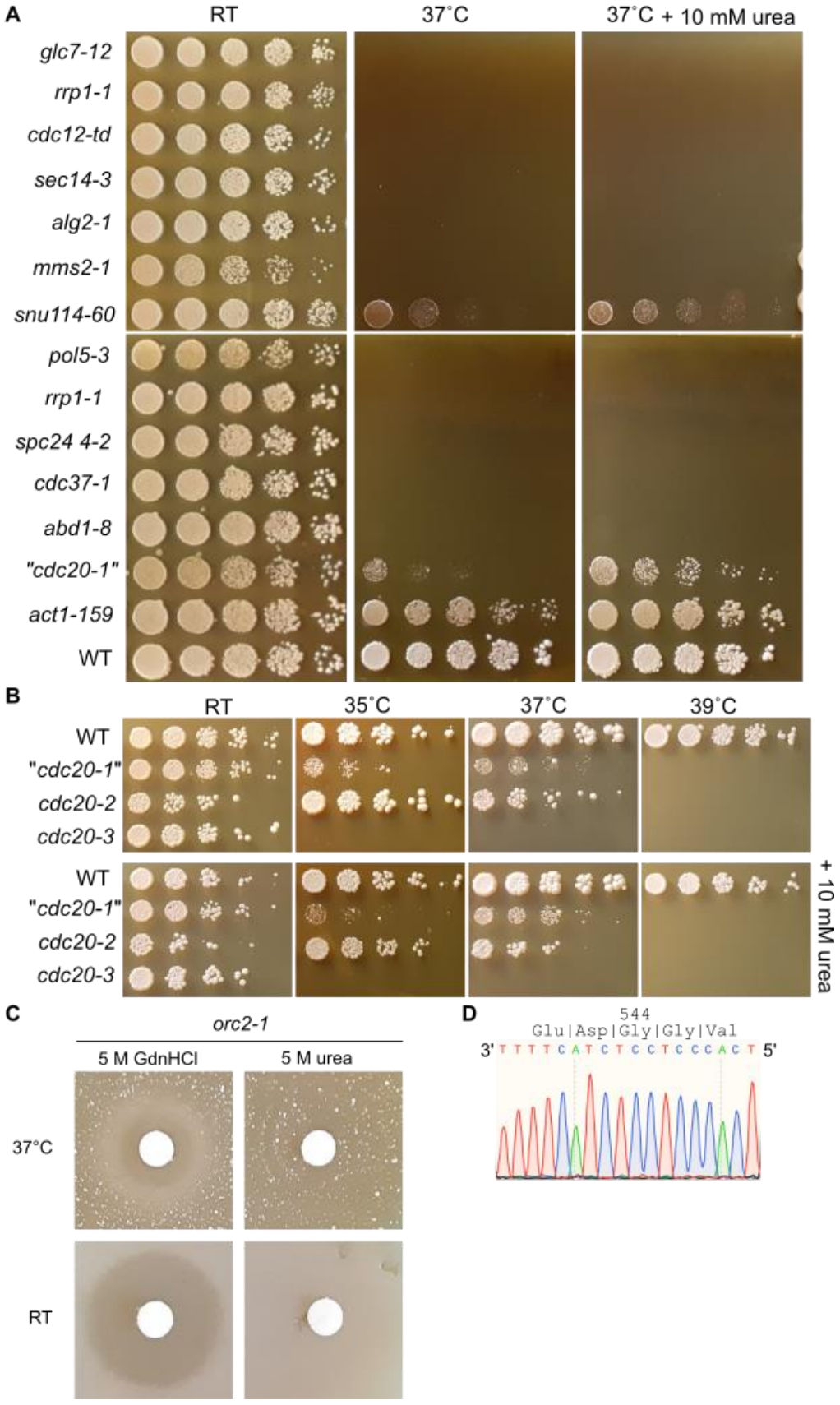

Figure S5. Failure of urea to rescue TS mutants (part I). (A) Mutants from the Boone TS collection scored as possible examples of urea rescue based on robotic screening were serially diluted and spotted on rich (YPD) medium with or without 10 mM urea and incubated at the indicated temperatures. “WT”, BY4741. Note that the

bottom row of images is of the same plates as the top, but was cropped and moved to preserve figure space. "*cdc20-1*" is the strain isolated from the well in the TS collection that should have contained the *cdc20-1* mutant, but subsequent PCR and sequencing of the *CDC20* coding region revealed WT sequence at the location of the Gly 544 codon that is mutated in *cdc20-1* (see panel D). (B) As in (A) but with the "*cdc20-1*", *cdc20-2*, and *cdc20-3* mutants from the Boone TS collection and incubated at additional temperatures. (C) A lawn of *orc2-1* cells (strain CBY08224) was spread on rich (YPD) plates and a paper disc soaked in 5 M GdnHCl or 5 M urea was placed in the center of the plate, after which the plates were incubated at the indicated temperatures. (D) Chromatogram showing dideoxy sequencing results of a PCR product made from genomic DNA of the strain found in the well of the Boone TS collection where the *cdc20-1* mutant (substitution G544R) should have been. Sequencing of PCR products from elsewhere in the *CDC20* coding region of this strain revealed no mutation, whereas sequencing of the same products from the *cdc20-2* and *cdc20-3* strains revealed mutations causing substitutions G360S and E494K, respectively (data not shown).