Supplemental figure 7: The zinc-ribbon domain of Csl4p is required for cytoplasmic exosome-mediated mRNA decay. A. To investigate what domains of Csl4p are required for exosome-mediated mRNA decay we used the four viable Csl4p truncations in a *his3-nonstop* growth assay. This assay exploits a mutant *HIS3* gene that is missing a stop codon. The resulting aberrant mRNA is rapidly degraded by the cytoplasmic exosome. This results in low levels of His3p, which results in a failure to grow in the absence of histidine[1]. Note that the interpretation of this assay is counter-intuitive: A defect in nonstop mRNA decay results in growth due to stabilization of the *his3-nonstop* mRNA, while normal nonstop mRNA decay result in a failure to grow. The two truncations that are missing the zinc-ribbon domain of Csl4p have a defect in nonstop mRNA decay (*i.e.* allow growth), while the two truncations that have the zinc-ribbon domain of Csl4p do not have a defect in nonstop mRNA decay. Thus, these results confirm that the zinc-ribbon domain is specifically required for cytoplasmic exosome-mediated nonstop mRNA decay. **B.** Yeast contains two general pathways for mRNA decay. One pathway requires Dcp1p (and other proteins), while the alternative pathway requires the cytoplasmic exosome[2,3]. We employed a strain that has a single amino acid change in Csl4p that inactivates cytoplasmic functions of the exosome, but does not affect nuclear functions of the exosome (*csl4-G253E*)[4] combined with a temperature sensitive mutation in *DCP1* (*dcp1-2*). The *dcp1-2 csl4-G253E* double mutant is viable at room temperature because the decapping enzyme is active, but this strain fails to grow at 30^{o}C and above, because both the decapping enzymes and the cytoplasmic exosome are inactive[4]. Growth at 37^{o}C is restored to the *dcp1-2 csl4-G253E* strain upon introduction of either full length Csl4p, or the two viable truncations that contain the zinc-ribbon domain. In contrast, the

two viable truncations that are missing the zinc-ribbon domain do not restore growth at 37^{o}C. These results confirm that the zinc-ribbon domain is required for cytoplasmic exosome-mediated mRNA decay. Importantly, this assay monitors general mRNA decay, and unlike the other two assays is not specific to nonstop mRNA decay[1]. Thus, these results indicate that the zinc-ribbon domain is required for cytoplasmic exosome-mediated decay of both normal and nonstop mRNAs.

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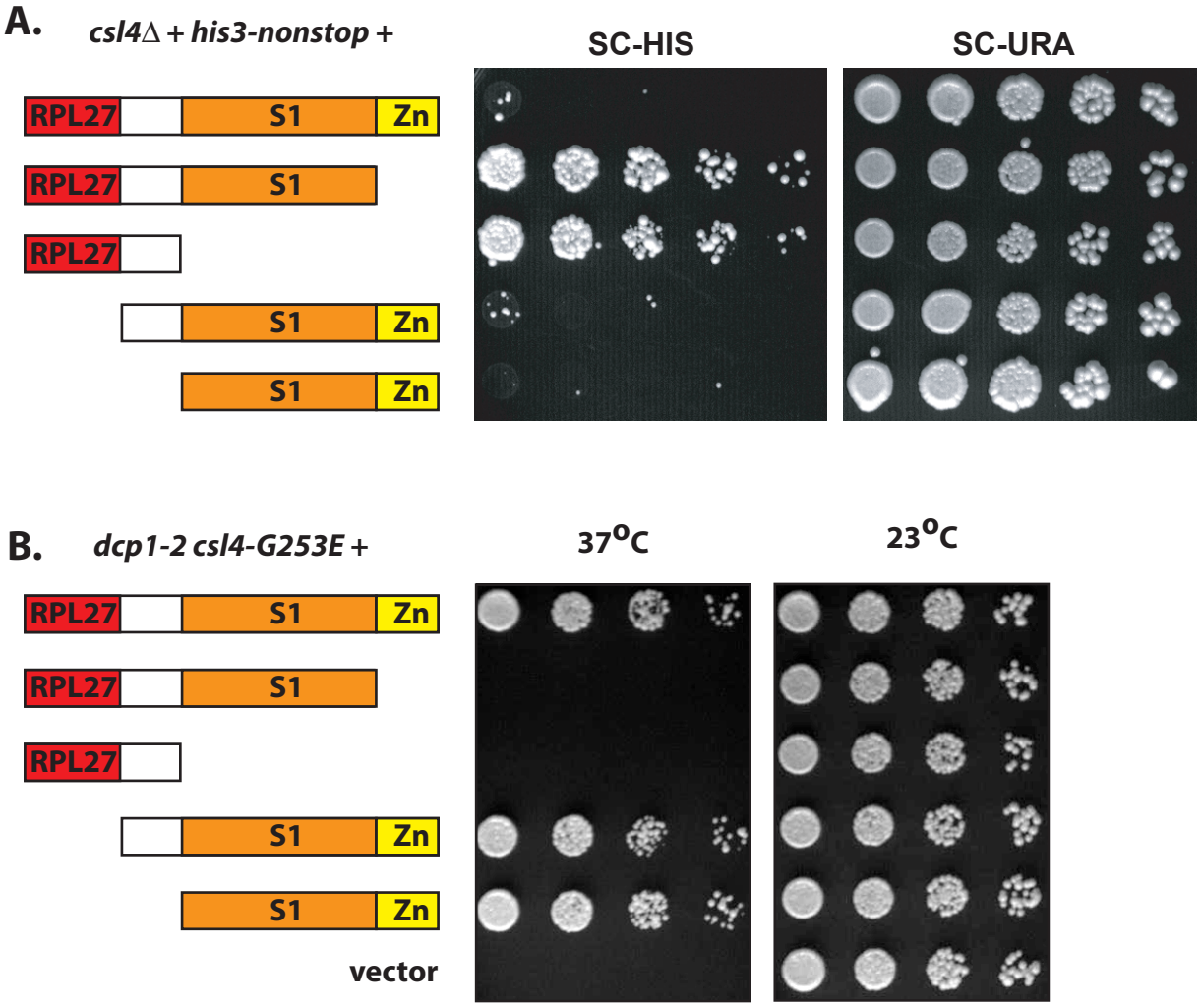
A.
csl4Δ + his3-nonstop +
SC-HIS
SC-URA
RPL27
S1
Zn
RPL27
S1
RPL27
S1
Zn
S1
Zn
B.
dcp1-2 csl4-G253E +
37°C
23°C
RPL27
S1
Zn
RPL27
S1
RPL27
S1
Zn
S1
Zn
vector