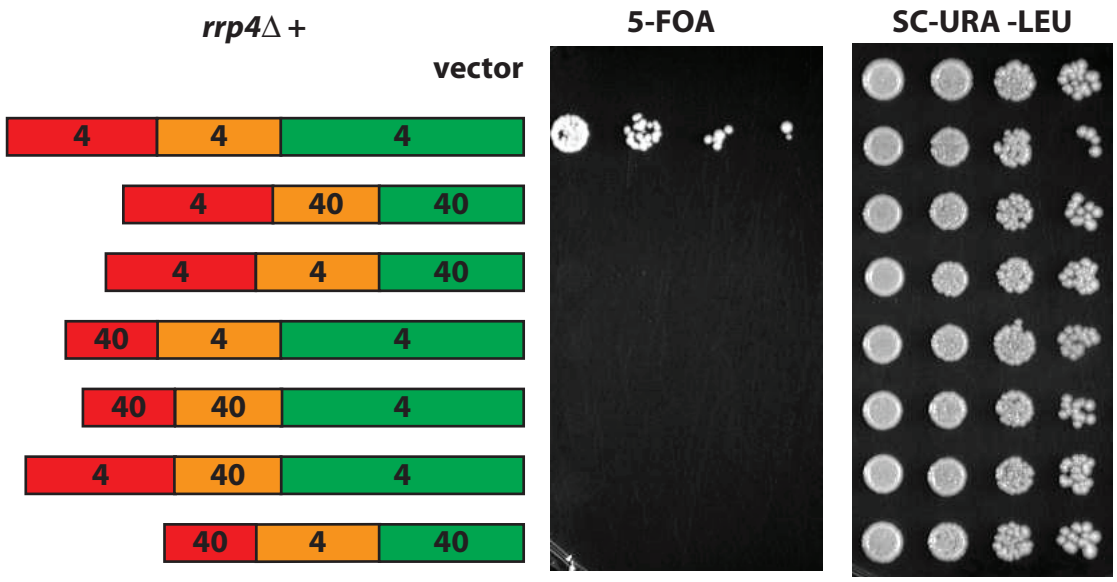
Supplemental figure 6: The domains of Rrp4p and Rrp40p can not be replaced by their paralogous domain, or expressed as two separate proteins. To test whether each of the domains of Rrp4p and Rrp40p had distinct functions, we generated chimaeras where one or two of the domains were replaced by the paralogous domains. **A.** An *rrp4Δ* strain complemented by full length *RRP4* on a plasmid with a *URA3* marker was transformed with *LEU2* plasmids encoding the six possible chimaeras. Failure to grow on 5FOA indicates that the chimeric proteins can not carry out the essential function of the exosome. **B.** The same was done using an *rrp40Δ* strain. **C.** Co-expressing all of the domains of Rrp4p as two separate proteins does not complement an *rrp4Δ*. The cap proteins are thought to be important for exosome structure because they provide bridging contacts between neighboring PH-ring subunits. For Rrp4p this bridging function is mediated through contacts of the RPL27-like domain and S1 domain with Rrp41p and the S1 and KH domains with Rrp42p (Figure 4A). The hypothesis that these bridging contacts are important predicts that co-expressing the Rrp4p domains as separate proteins will not complement an *rrp4Δ*. We tested this prediction with two experiments. In one experiment we expressed the RPL27-like domain of Rrp4p as one protein and the S1 and KH domains as a second protein (top row). In the alternative experiment, we expressed the RPL27-like and S1 domains as one protein, and the KH domain as a second protein (second row). Neither one of these combinations resulted in significant growth. These results indicate that the mere expression of all three domains is not sufficient for growth, but that they need to be expressed as one protein, as predicted for a protein that provides bridging contacts. An *rrp4Δ* strain complemented by full length Rrp4p on a plasmid with a *URA3* marker, was transformed with *LEU2* and *HIS3* plasmids encoding each of the

depicted Rrp4p truncations. Failure to grow on 5FOA indicates that the truncated Rrp4p can not carry out the essential function of the exosome.

A.


rrp4Δ +
vector
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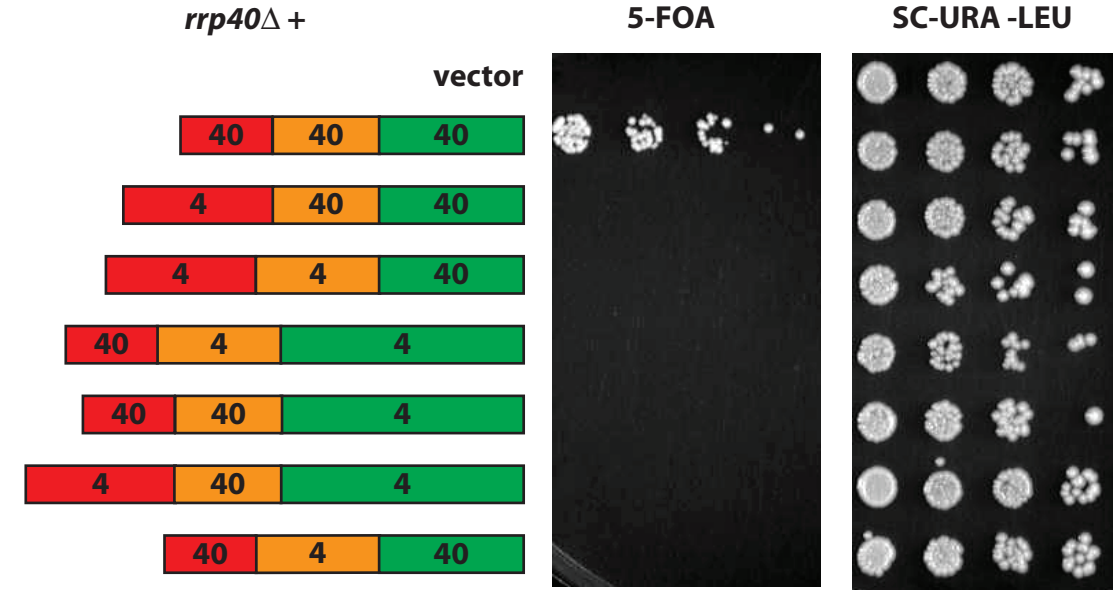
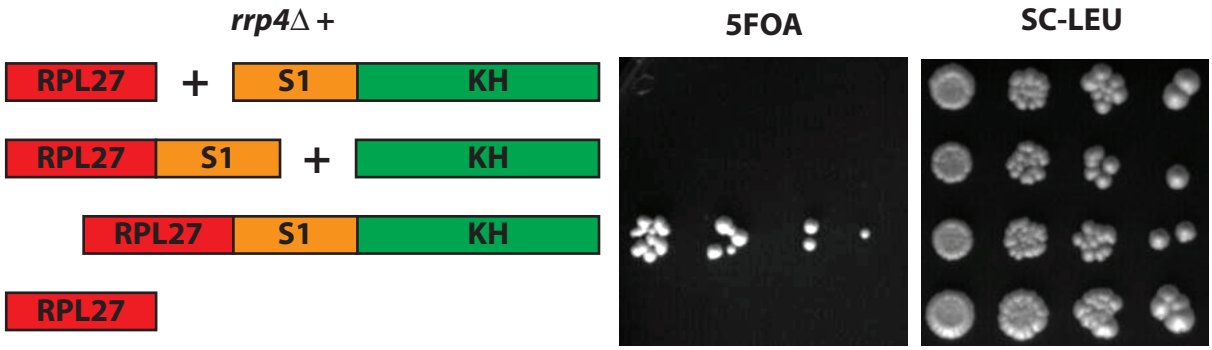
4 40 40
4 4 40
40 4 4
40 40 4
4 40 4
40 4 40
5-FOA
SC-URA -LEU

B.


rrp40Δ +
vector
40 40 40
4 40 40
4 4 40
40 4 4
40 40 4
4 40 4
40 4 40
5-FOA
SC-URA -LEU

C.


rrp4Δ +
RPL27 + S1 KH
RPL27 S1 + KH
RPL27 S1 KH
RPL27
5FOA
SC-LEU