Supplemental figure 5: Unlike Csl4p, each of the Rrp4p and Rrp40p domains is required for the essential exosome function. A. Rrp4p has an N-terminal RPL27-like domain (red), a central S1 domain (orange) and a C-terminal KH domain (green). **B.** an *rrp4Δ* strain complemented by full length *RRP4* on a plasmid with a *URA3* marker was transformed with *LEU2* 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. **C.** Like Rrp4p, Rrp40p has an N-terminal RPL27-like domain (red), a central S1 domain (orange) and a C-terminal KH domain (green). **D.** an *rrp40Δ* strain complemented by full length *RRP40* on a plasmid with a *URA3* marker was transformed with *LEU2* plasmids encoding the depicted Rrp40p truncations. Failure to grow on 5FOA indicates that the truncated Rrp40p can not carry out the essential function of the exosome. These results were confirmed in growth assays using *GAL::rrp4* and *GAL::rrp40* strains[1,2]: None of the truncations restored growth in the presence of glucose (data not shown).

One reason why the Rrp4p and Rrp40p truncations might be inviable is that they might not be expressed as stable proteins. To test this, we tagged all eight truncations and the full length proteins with a TAP tag. Western blotting analysis showed that each of the N-terminal truncations of Rrp4p is expressed at levels similar to that of wild type, while the C-terminal truncations were expressed at somewhat lower levels (data not shown). We conclude that the failure to complement the *rrp4Δ* or *GAL::rrp4* does not correlate with a failure to express these truncations. Most of the Rrp40p truncations were expressed at levels significantly lower than the full length RRP40p (data not shown). There are at least two possible explanations for the reduced expression of some of the truncations. One

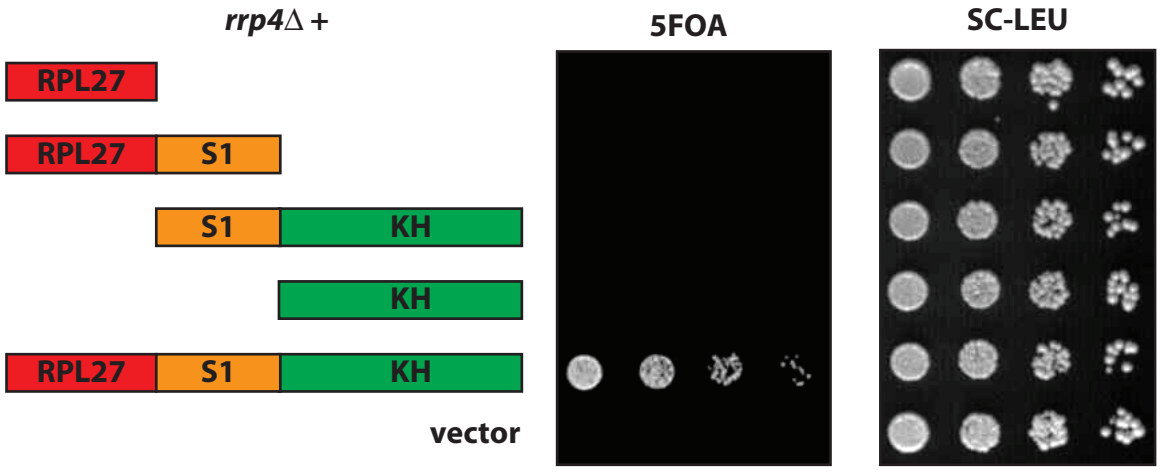
hypothesis is that the truncations are inherently unstable, and that the low expression is the cause of the failure to complement. The alternative hypothesis is that the truncated protein is not assembled into the exosome complex, and that the free subunit is rapidly degraded (thus the low expression is a consequence of the failure to complement). Regardless of the detailed explanation, the reduced expression level of some Rrp4p and Rrp40p truncations does not affect any of our major conclusions.

1. Allmang, C. et al. The yeast exosome and human PM-Scl are related complexes of 3' --> 5' exonucleases. *Genes Dev* **13**, 2148-58 (1999).
2. Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M. & Tollervey, D. The exosome: a conserved eukaryotic RNA processing complex containing multiple 3'-->5' exoribonucleases. *Cell* **91**, 457-66. (1997).

A.


Rrp4p:
RPL27
S1
KH

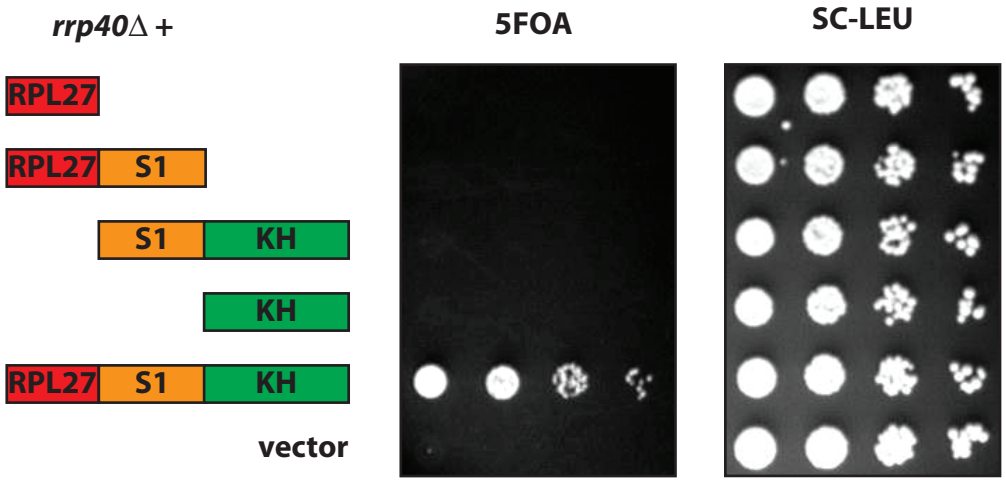
B.


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

C.


Rrp40p:
RPL27
S1
KH

D.


rrp40Δ +
5FOA
SC-LEU
RPL27
RPL27
S1
S1
KH
KH
RPL27
S1
KH
vector