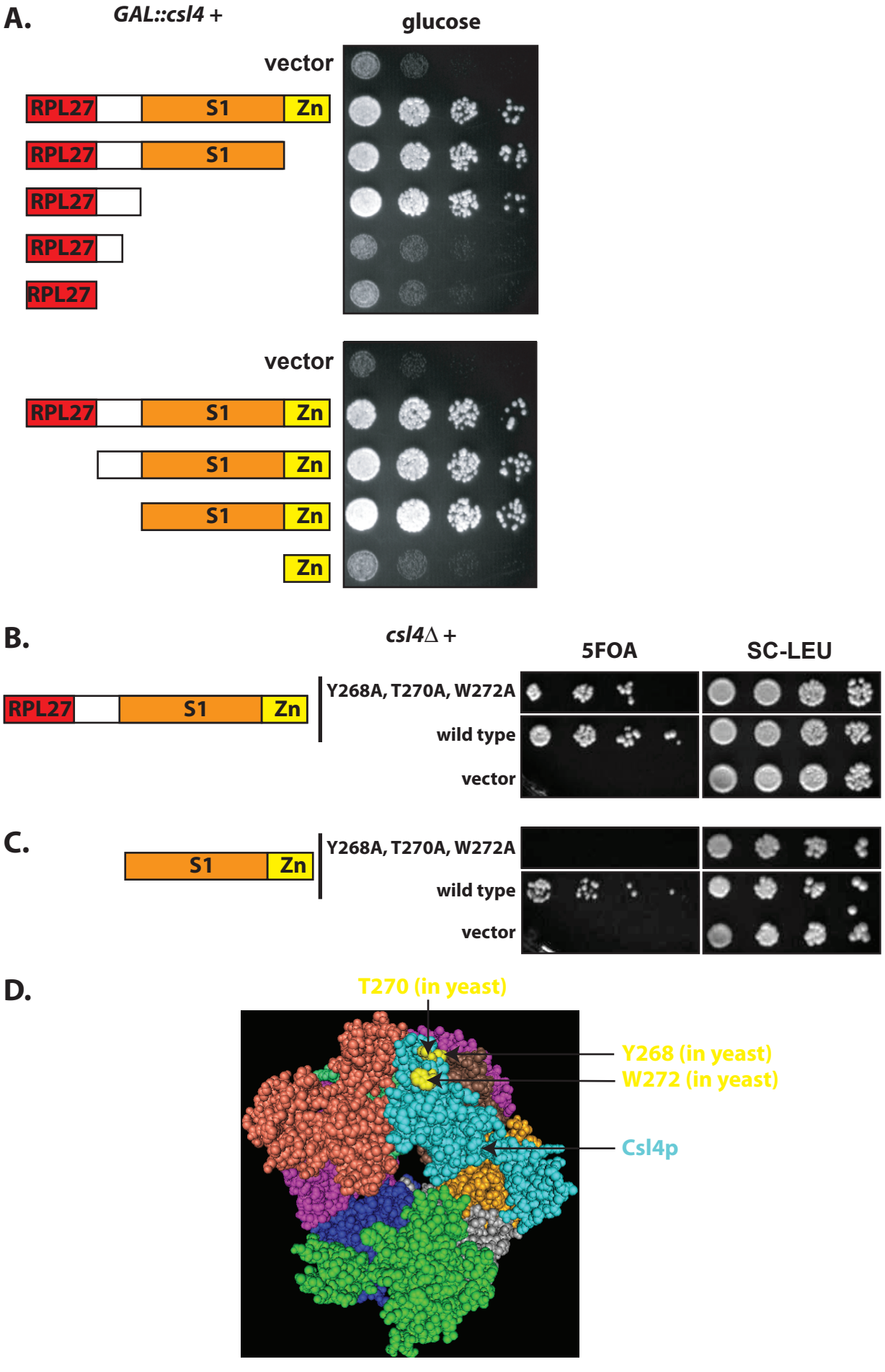
Supplemental figure 4: The essential Csl4p does not contain any essential domains. **A.** The finding that an essential protein does not contain any essential domains is highly unusual. We therefore sought to confirm this conclusion using a strain with Csl4p expressed from a galactose-regulated promoter (*GAL::csl4* strain[1]). This strain expresses Csl4p when grown on galactose, but Csl4p expression is repressed in the presence of glucose, resulting in slow growth. This strain was transformed with plasmids encoding each of the depicted Csl4p truncations. Growth in the presence of glucose indicates that truncated Csl4p can carry out the essential function of the exosome. Importantly, the results exactly matched the results of the *csl4Δ* plasmid shuffle: a plasmid containing just the RPL27-like domain and linker or a plasmid containing the S1 domain and zinc-ribbon domain restored full growth in the presence of glucose, whereas either an empty vector or further truncations did not. Therefore, these results confirm that the essential Csl4p does not contain any essential domains. Instead the RPL27-like and S1 or Zn-ribbon domains appear to be redundant for the essential function of the exosome. **B.** Csl4p homologues from 16 diverse eukaryotes and 11 archaea were aligned. This revealed several residues that are surface exposed in the human exosome crystal structure and are conserved in the eukaryotic exosome, but not in the archaeal exosome. Since the eukaryotic exosome interacts with many cofactors, but the archaeal exosome is not known to require cofactors, such residues may be binding sites for cofactors, and were targeted for mutagenesis. Changing Y268, T270 and W272 to A in full length Csl4p has no effect on growth. **C.** These same mutations are lethal in combination with deletion of the RPL27-like domain. **D.** The corresponding residues in the human exosome are highlighted in yellow. Individual subunits of the human exosome are indicated with distinct colors (light

blue for Csl4p). Although the triple mutant is presented as a separate panel in B and C, all three strains were grown on the same plate under identical conditions, but several irrelevant strains on this same plate are not shown.

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).


A.
GAL::csl4 +
glucose
vector
RPL27
S1
Zn
RPL27
S1
RPL27
RPL27
RPL27
vector
RPL27
S1
Zn
S1
Zn
S1
Zn
Zn
B.
csl4Δ +
5FOA
SC-LEU
RPL27
S1
Zn
Y268A, T270A, W272A
wild type
vector
C.
S1
Zn
Y268A, T270A, W272A
wild type
vector
D.
T270 (in yeast)
Y268 (in yeast)
W272 (in yeast)
Csl4p