Methods for the rescuing experiment

To test whether overexpression of Sro7p rescues the deletion of EXO84 and EXO70, two strains were generated: GY1264 and GY2180 (Table I). In GY1264, the endogenous EXO84 gene was disrupted by HIS3 and was supplemented with a CEN URA3 EXO84 plasmid as a balancer. Likewise, in GY2180, the endogenous EXO70 was replaced by HIS3 and was supplemented with a CEN URA3 EXO70. For SRO7 overexpression, a 2μ LEU2 SRO7 plasmid was transformed into GY1264 and GY2180. As positive controls, CEN plasmids containing LEU2 EXO84 or LEU2 EXO70 were also transformed to GY1264 and GY2180, respectively. The 2μ LEU2 vector was transformed into the two host strains as a negative control. SC Leu plates containing uracil and 5-FOA (1 mg/ml at final concentration) were used to test whether the cells, with high-copy SRO7, were able to survive after losing the CEN URA3 balancer plasmid carrying EXO84 or EXO70.