

```

protein 69  S M E G T V K P L K A L S A K Y N F L L F E D R K F A D I G
WT 1  TCCATGGAGGGCACAGTTAAGCCGCTAAAGGCATTATCCGCCAAGTACAATTTTTACTCTTCGAAGACAGAAAATTTGCTGACATTGGT
rec.WT 1  .....A..T....C..G....T....T.....C.....T.....
rec.R235A 1  .....A..T....C..G....T....T.....C.....T.....

protein 99  N T V K L Q Y S A G V Y R I A E W A D I T N A H G V V G P G
WT 91  AATACAGTCAAATTCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGGCAGACATTACGAATGCACACGGTGTGGTGGGCCAGGT
rec. WT 91  ..C..T....G.....T....T.....T.....T.....T.....T..T..A.....T.....T.....T.....T...
rec.R235A 91  ..C..T....G.....T....T.....T.....T.....T.....T..T..A.....T.....T.....T.....T...

protein 129 I V S G L K Q A A E E V T K E P R G L L M L A E L S C K G S
WT 181 ATTGTTAGCGGTTTGAAGCAGGCGGGCGGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAATTGTCATGCAAGGGCTCC
rec. WT 181 .....G....A....A....A....G..G....T.....A....T....A....G..T....A.....A....T
rec.R235A 181 .....G....A....A....A....G..G....T.....A....T....A....G..T....A.....A....T

protein 159 L A T G E Y T K G T V D I A K S D K D F V I G F I A Q R D M
WT 271 CTAGCTACTGGAGAATATACTAAGGGTACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTATCGGCTTTATTGCTCAAAGAGACATG
rec. WT 271 .....A....T....C....A....A....T.....A.....G....C.....C....A.....T...
rec.R235A 271 .....A....T....C....A....A....T.....A.....G....C.....C....A.....T...

protein 189 G G R D E G Y D W L I M T P G V G L D D K G D A L G Q Q Y R
WT 361 GGTGGAAGAGATGAAGGTTACGATTGGTTGATTATGACACCCGGTGTGGGTTTAGATGACAAGGGAGACGCATTGGGTCAACAGTATAGA
rec. WT 361 .....T....C.....T.....A.....T.....T....G..C....A.....T.....G....C...
rec.R235A 361 .....T....C.....T.....A.....T.....T....G..C....A.....T.....G....C...

protein 219 T V D D V V S T G S D I I I V G R G L F A K G R D A K V E G
WT 451 ACCGTGGATGATGTGGTCTCTACAGGATCTGACATTATTATTGTTGGAAGAGGACTATTTGCAAAGGGGAGGGATGCTAAGGTAGAGGGT
rec. WT 451 ..T.....T.....T.....T....A..A.....G.....
rec.R235A 451 ..T.....T.....T.....T....A..A.....GCT.....

```

Figure S1. Sequence of “recoded” *URA3* genes used for CRISPR-Cas9-based integration into the yeast genome. “protein”, the amino acid sequence of WT Ura3 starting with residue 69. “WT”, a portion of the DNA sequence of WT *URA3* corresponding to the synthetic sequences below that were used as donor templates for recombination. The sequence corresponding to the guide RNA targeting Cas9 cleavage is italicized. Periods indicate no change in the donor sequences relative to WT.