

**The conserved  $\beta$ -hairpin of the SUI1 domain is a dual-function structural module governing translation initiation and ribosome recycling in yeast**

Kseniya A. Zamyatnina, Valery N. Urakov, Inna A. Volynkina, Elena A. Stolboushkina, Evgeny S. Gerasimov, Leonid M. Kats, Vitaly V. Kushnirov, Piotr A. Kamenski, Sergey E. Dmitriev

**Supplementary Table S1. DNA oligonucleotides used for cloning and strain construction**

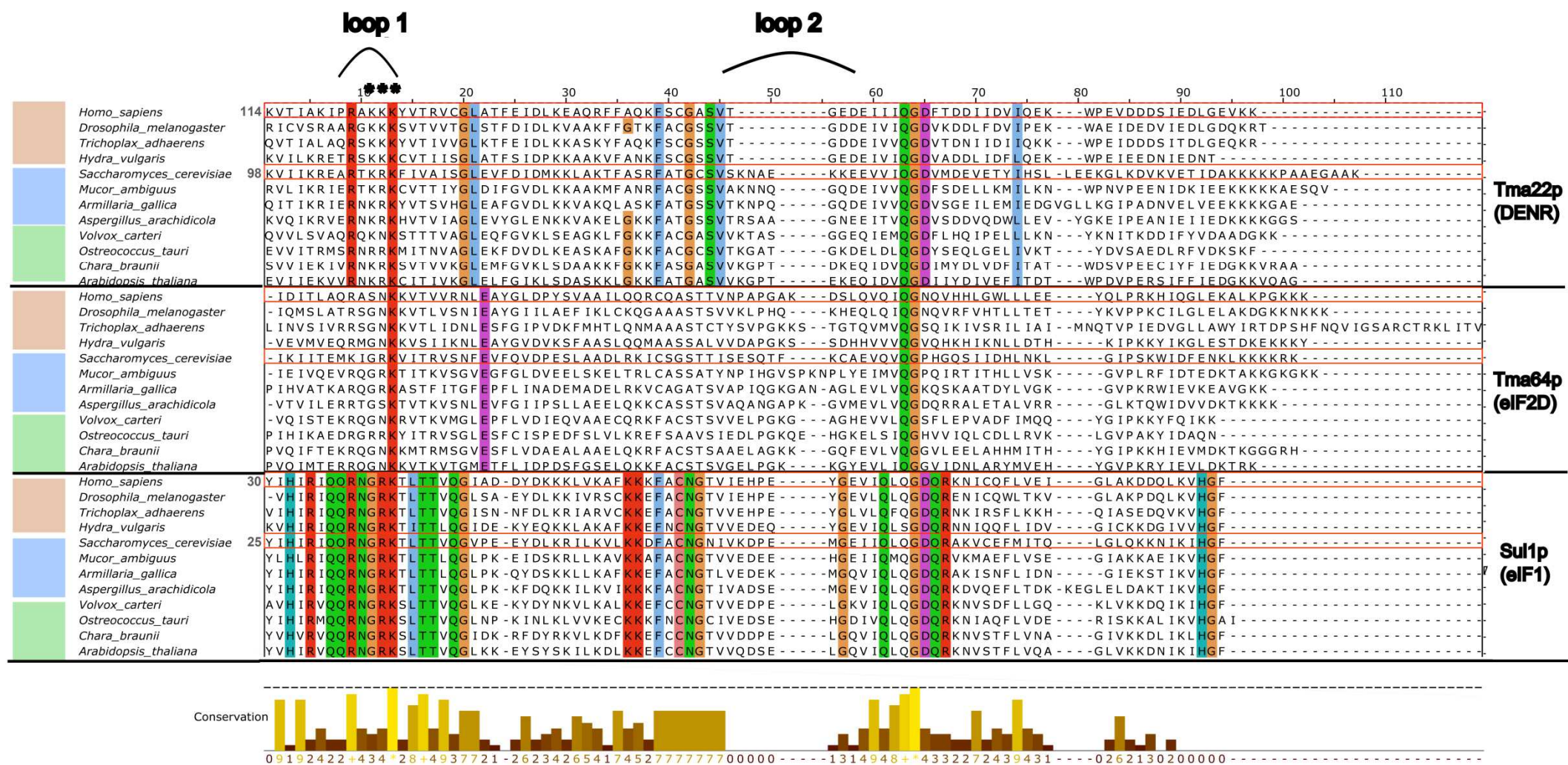
| Oligonucleotide        | Nucleotide sequence (5' to 3')                                                                                                 |
|------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| Tma22_for              | GGCCCATATGTTAAGAGAAGTCATCTATTG                                                                                                 |
| Tma22_rev              | GCCGGGATCCTTACTTGGCAGCTCCTTCTG                                                                                                 |
| F_K110E_TMA22          | GCTAGGACCAAGAGAGAATTTATCGTCGCTATC                                                                                              |
| R_K110E_TMA22          | GATAGCGACGATAAATTCTCTCTTGGTCCTAGC                                                                                              |
| F_eIF1                 | GGCCCATATGTCCATTGAGAATCTGAAATC                                                                                                 |
| R_eIF1_HindIII         | GCCGAAGCTTTAAAACCCATGAATTTTAATG                                                                                                |
| TMA22rev (1-75)        | GCCGGGATCCTTAGTCCTTTTCCAACCTTTTC                                                                                               |
| F_chimera              | GGAAAAGGACTTGCTGCATATTCGTATCCAAC                                                                                               |
| R_chimera              | GTTGGATACGAATATGCAGCAAGTCCTTTTCC                                                                                               |
| TMA20-HIS3-F           | ATATAAAAGACAAAGACGGTAAACTAAAACAGCAGAGAGGAACGTTTTAAGAGCTTGGTGAGC                                                                |
| TMA20-HIS3-R           | TGTAGCAGATGGATAGTAATATAGTGTTGACGGCTCCGTTTGTATCGAGTTCAAGAGAAAAAAA<br>AAGAA                                                      |
| TMA22cassDir           | CCCAAGGAAACAGTTCAAGAG                                                                                                          |
| TMA22-108AEA_Rev       | AAGCTTCAGCGGTCCTAGCTTCTCTTTTAATGA                                                                                              |
| TMA22cassRev           | TAAAAAGTCCTTTTCTCCCAGAAC                                                                                                       |
| TMA22-108AEA_Dir       | GCTAGGACCGCTGAAGCTTTTATCGTCGCTATCTCTGGG                                                                                        |
| TMA22-95X_Dir          | GCTAAGAAGTAAGCAATCCATAAATATGTAATAGCA                                                                                           |
| TMA22-95X_Rev          | TATTTATGGATTGCTTACTTCTTAGCTAATTCTCTTTGTTCC                                                                                     |
| TMA22-d2-90_Rev        | GCTTCTTAGCTAACATTTTATGCAATATGCTTTTCT                                                                                           |
| TMA22-d2-90_Dir        | GCATAAAATGTTAGCTAAGAAGCTGTCATCGAA                                                                                              |
| ChimYlplac_F2.0        | ACCAAAAGCTTAACAAAATGTTAAGAGAAGTCATCTATTGTGG                                                                                    |
| ChimYlplac_R2.0        | CCACACATATGTTAAAACCCATGAATTTTAATGTTT                                                                                           |
| Prom22Hind_F           | CCACAAAGCTTACTGACGTGAACCTGCACCATG                                                                                              |
| Prom22Hind_R           | CCACAAAGCTTTTTATGCAATATGCTTTTCTTTAGTTT                                                                                         |
| TMA22_A                | CTGACGTGAACCTGCACCATG                                                                                                          |
| TMA22D2                | GAAGGTTTCAGAGAAGGAACG                                                                                                          |
| Kan_B                  | GTATATGAAAGAAGAACCTCAGTG                                                                                                       |
| TMA22_B                | TTTTCAGCAAGTCCTTTTCCAAC                                                                                                        |
| BamHI_Fluc_fw          | GCAACGGATCCAACAACAACAACAACAACAATGGAAGACGCCAAAAACATAAAG                                                                         |
| PstI_AgeI_Fluc_rev     | GCAACCTGCAGGCATACACCGGTACGGCGATCTTTCCGC                                                                                        |
| AgeI_Nluc_rein_fw      | GCAACACCGGTGAATTATGACTGTCTTCACACTCGAAGATTTCGTT                                                                                 |
| PstI_Nluc_rev          | GCAACCTGCAGTTACGCCAGAATGCGTTCCG                                                                                                |
| PstI_AgeI_P2A_Fluc_rev | GCAACCTGCAGGCATACACCGGT <b>TGGACCTGGATTCAATTCAACATCACCAGCCAATTTCAACAA<br/>AGAAAAATTAGTAGCACCACCAGAACCAGT</b> CACGGCGATCTTTCCGC |
| AgeI_Nluc_fw           | GCAACACCGGTATGACTGTCTTCACACTCGAAGATTTCGTT                                                                                      |
| PstI_Fluc_rev          | GCCCTGCAGTTACACGGCGATCTTTCCG                                                                                                   |
| BamHI_uORF_Nluc_fw     | GCCGGATCCAACAACAACAACAACAACAATGACACAAACACAATGACTGTCTTCACACTCGAAGAT<br>TTCGTT                                                   |
| BamHI_noORF_Nluc_fw    | GCCGGATCCAACAACAACAACAACAACAAGTACACAAACACAATGACTGTCTTCACACTCGAAGAT<br>TTCGTT                                                   |
| HindIII_ADH1term_fw    | GCAACAAGCTTATAAGCGAATTTCTTATGATTTATGATTTTATTATT                                                                                |

|                      |                                                                                   |
|----------------------|-----------------------------------------------------------------------------------|
| HindIII_CYC1term_rev | GCAACAAGCTTGGCCGCAAATTAAA                                                         |
| TMA22_fw             | CCCAAGGAAACAGTTCAAGAGCTAAACTAAAGAAAAGCATATTGCATAAAATGTTAAGAGAAGTC<br>ATCTATTGTGGA |
| TMA22_rv             | GAAATTCGCTATGGATTGCTTACTTGGCAGCTCCTTCTG                                           |
| ADH1term_fw          | AGCAATCCATAGCGAATTTCTTATGATTTATGATTTTATTATTAAAT                                   |
| TEFterm_rv           | TAAAAAGTCCTTTTCTCCCAGAACGGTGCTATTACATATTTATGGATTGCCAGTATAGCGACCAG<br>CATTCA       |

Restriction sites are underlined. The sequence corresponding to the 2A peptide from Equine rhinitis B virus is marked in bold.

**Supplementary Table S2. Yeast strains used in this study**

| Name    | Genotype                                                                                                       | Source                            |
|---------|----------------------------------------------------------------------------------------------------------------|-----------------------------------|
| BY4741  | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>                                                                       | Dharmacon                         |
| 328     | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma20Δ::KanMX4</i>                                                        | Dharmacon                         |
| 6812    | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma22Δ::KanMX4</i>                                                        | Dharmacon                         |
| FZY793  | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma64Δ::HygMX4</i>                                                        | Young et al., 2018                |
| YDY10   | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma64Δ::HygMX4 tma20Δ::KanMX4</i>                                         | Young et al., 2018                |
| YDY12   | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma64Δ::HygMX4 tma22Δ::KanMX4</i>                                         | Young et al., 2018                |
| LSBS001 | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma22Δ::KanMX4 tma20::His3</i>                                            | This study                        |
| LSBS002 | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma64Δ::HygMX4 tma22Δ::KanMX4<br/>tma20::His3</i>                         | This study                        |
| LSBS003 | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma64Δ::HygMX4 tma22Δ::KanMX4 tma22-<br/>K108A_R109E_K110A</i>            | This study                        |
| LSBS004 | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma64Δ::HygMX4 tma22Δ::KanMX4 tma22-<br/>K110E</i>                        | This study                        |
| LSBS005 | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma64Δ::HygMX4 tma22Δ::KanMX4 tma22-<br/>95*</i>                          | This study                        |
| LSBS006 | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 tma64Δ::HygMX4 tma22Δ::KanMX4 tma22-<br/>L2-E90del</i>                    | This study                        |
| W303    | <i>MATa/MATα {leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15} [phi+]</i>                                  | A gift from<br>Svyatoslav Sokolov |
| KAZ001  | <i>MATa/MATα {leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15} [phi+], tma22-<br/>sui1</i>                 | This study                        |
| KAZ002  | <i>MATa/MATα {leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15} [phi+] tma22Δ ::<br/>KanMX4,</i>            | This study                        |
| KAZ003  | <i>MATa/MATα {leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15} [phi+] tma22Δ ::<br/>KanMX4, tma22-sui1</i> | This study                        |



**Supplementary Figure S1.** Alignment of amino acid sequences of SUI1 domains from Tma22p/DENR, Tma64p/eIF2D, and Sui1p/eIF1 proteins from a representative set of eukaryotic organisms. Animal, fungi, and plant species are color-coded. For Tma22p/DENR and Sui1p/eIF1 from human and budding yeast, starting residue numbers are shown. Conserved residues are highlighted according to amino acid properties, and the positions of  $\beta$ -hairpin loops 1 and 2 are marked above the alignment. Alignment was generated using ESPrnt 3.