A

|                | Tof2 360-440 | Net1 486-552 |
|----------------|--------------|--------------|
| Csm1:          | -            | +            |
| Input (10%)    | +            | +            |
| Bound          | +            | +            |
| Bound hi-exp   | +            | +            |

B

**Tof2^{368-404} peptide binding**

| Protein                  | Peptide                        | $K_f$ (μM) |
|--------------------------|--------------------------------|------------|
| Csm1^{150-190}; Ulp2^{205-344}, Tof2^{368-404} | Tof2 WT                       | >100       |
| Csm1^{150-190}; Ulp2^{205-344}, Tof2^{368-404} | Tof2 F396A                     | >100       |
A  

Complete  

-URA  

**URA3 reporter**

**Genotype**

- WT
- leu² NTS1
- leu² NTS1 ulp²-F839D
- leu² NTS1 tof²-F396A
- leu² NTS1 tof²-F396A ulp²-F839D

B  

Complete  

-URA  

**URA3 reporter**

**Genotype**

- WT
- leu² NTS1
- leu² NTS1 smt3-allR
- leu² NTS1 ulp²-F839D
- leu² NTS1 smt3-allR ulp²-F839D
**Supplementary Table 1** – Proteins associated with Ulp2 identified by mass spectrometry

Ulp2 vs *ulp2-781Δ*

| Gene | Median ratio (HZY4162/HZY4160) | # of peptides found |
|------|-------------------------------|---------------------|
| ADH1 | 1.422                         | 3                   |
| ALD6 | 2.234                         | 3                   |
| RPL12B|RPL12A | 0.852                     | 3                   |
| RPL21A|RPL21B  | 0.853                     | 3                   |
| RPL26B|RPL26A  | 0.694                     | 3                   |
| RPL29 | 0.649                         | 3                   |
| RPL32 | 0.89                          | 3                   |
| RPL34A|RPL34B | 0.879                     | 3                   |
| RPL36A|RPL36B | 0.622                     | 3                   |
| RPL6B|RPL6A | 0.778                     | 3                   |
| RPS17B|RPS17A | 0.857                     | 3                   |
| RPS18A|RPS18B | 0.833                     | 3                   |
| RPS19B|RPS19A | 1.009                     | 3                   |
| RPS20 | 1.406                         | 3                   |
| RPS25A|RPS25B | 0.847                     | 3                   |
| RPS4B|RPS4A  | 0.758                     | 3                   |
| RPS7B | 1.251                         | 3                   |
| RPS8A|RPS8B | 0.593                     | 3                   |
| SIK1  | 0.941                         | 3                   |
| SRO9 | 0.928                         | 3                   |
| SSA2  | 0.614                         | 3                   |
| STM1  | 0.894                         | 3                   |
| THI20 | 0.743                         | 3                   |
| UBA4 | 1.74                          | 3                   |
| VMA2  | 0.736                         | 3                   |
| CSM1  | 19.7975                      | 4                   |
| ENO1|ENO2 | 1.124                     | 4                   |
| LRS4 | 225.7                         | 4                   |
| RPL14B|RPL14A | 0.6675                    | 4                   |
| RPL20A|RPL20B | 0.783                     | 4                   |
| RPL25 | 0.787                         | 4                   |
| RPL9A|RPL9B  | 0.8895                    | 4                   |
| RPS0A|RPS0B  | 1.3935                    | 4                   |
| RPS11B|RPS11A  | 1.0045                    | 4                   |
| RPS14A|RPS14B | 1.0165                    | 4                   |
| RPS1A|RPS1B  | 0.97                      | 4                   |
| RPS6B|RPS6A  | 0.802                     | 4                   |
| PDB1 | 1.136                         | 5                   |
| RPA34 | 0.844                         | 5                   |
| RPL18B|RPL18A | 0.656                     | 5                   |
| RPL28 | 0.683                         | 5                   |
| RPP0 | 0.936                         | 5                   |
| RPS5 | 1.037                         | 5                   |
| Gene                      | Median ratio (HZY4162/HZY4160) | # of peptides found |
|---------------------------|---------------------------------|---------------------|
| RPS9B|RPS9A                  | 0.825                  | 5                   |
| SSA1|SSA2                     | 0.828                  | 5                   |
| RPL10                     | 0.798                  | 6                   |
| RPL19B|RPL19A                  | 0.8325                 | 6                   |
| RPL8A|RPL8B                   | 0.73                   | 6                   |
| RPS13                     | 0.776                  | 6                   |
| RPS15                     | 0.8145                 | 6                   |
| RTF1                      | 0.8425                 | 6                   |
| SAM2|SAM1                     | 0.999                  | 6                   |
| TDH3                      | 1.509                  | 6                   |
| TDH3|TDH1|TDH2                  | 1.1495                 | 6                   |
| TDH3|TDH2                     | 1.0305                 | 6                   |
| TEF2|TEF1                     | 1.71                   | 6                   |
| RPL13A|RPL13B                  | 0.67                   | 7                   |
| RPL2A|RPL2B                   | 0.699                  | 7                   |
| RPS24A|RPS24B                  | 0.647                  | 7                   |
| RPS7B|RPS7A                   | 1.05                   | 7                   |
| RPL3                      | 0.857                  | 8                   |
| RPS16B|RPS16A                  | 0.784                  | 8                   |
| RPS3                      | 0.9595                 | 10                  |
| RPS7A                     | 1.0595                 | 10                  |
| SSB1|SSB2                     | 0.9065                 | 10                  |
| RPL4A|RPL4B                   | 0.773                  | 11                  |
| ATG20                     | 0.415                  | 13                  |
| CDC19                     | 1.841                  | 16                  |
| KSP1                      | 0.237                  | 17                  |
| LEU1                      | 0.717                  | 20                  |
| ULP2                      | 2.031                  | 23                  |
**Supplementary Table 2** - Sumoylated proteins identified by mass spectrometry

*ulp2-781Δ vs wild-type*

| Gene   | ORF       | Median ratio (HZY4068/HZY2101) | # of peptides found |
|--------|-----------|---------------------------------|---------------------|
| ABF1   | YKL112W   | 1.075                           | 43                  |
| ABP1   | YCR088W   | 0.863                           | 5                   |
| AOS1   | YPR180W   | 0.711                           | 11                  |
| ASF2   | YDL197C   | 0.797                           | 8                   |
| AZF1   | YOR113W   | 0.777                           | 7                   |
| BDF1   | YLR399C   | 0.966                           | 9                   |
| BDP1   | YNL039W   | 0.451                           | 34                  |
| BIR1   | YJR089W   | 0.6635                          | 52                  |
| BOP3   | YNL042W   | 0.7345                          | 26                  |
| BRF1   | YGR246C   | 0.524                           | 22                  |
| BRN1   | YBL097W   | 0.363                           | 33                  |
| CBF1   | YJR060W   | 1.226                           | 9                   |
| CBF2   | YGR140W   | 1.3735                          | 12                  |
| CDC11  | YJR076C   | 0.894                           | 13                  |
| CDC14  | YFR028C   | 4.235                           | 13                  |
| CDC3   | YLR314C   | 0.829                           | 40                  |
| CDC48  | YDL126C   | 0.959                           | 31                  |
| CET1   | YPL228W   | 0.432                           | 27                  |
| CIN5   | YOR028C   | 1.1325                          | 6                   |
| CRZ1   | YNL027W   | 0.795                           | 6                   |
| CTI6   | YPL181W   | 0.737                           | 11                  |
| CYC8   | YBR112C   | 0.839                           | 6                   |
| DIG1   | YPL049C   | 0.488                           | 5                   |
| EBP2   | YKL172W   | 0.44                            | 7                   |
| ENO2   | YHR174W   | 1.822                           | 5                   |
| FOB1   | YDR110W   | 0.53                            | 24                  |
| GCN5   | YGR252W   | 0.728                           | 13                  |
| GCR2   | YNL199C   | 0.508                           | 11                  |
| HIR2   | YOR038C   | 0.4335                          | 4                   |
| HMO1   | YDR174W   | 0.4175                          | 12                  |
| HMS1   | YOR032C   | 0.5915                          | 4                   |
| HPC2   | YBR215W   | 0.571                           | 30                  |
| HSP104 | YLL026W   | 1.654                           | 3                   |
| HTA2|HTA1  | YBL003C|YDR225W | 0.6495 | 6     |
| HTB2|HTB1  | YBL002W|YDR224C | 0.675  | 13    |
| Gene      | ORF                  | Median ratio (HZY4068/HZY2101) | # of peptides found |
|-----------|----------------------|---------------------------------|---------------------|
| IPP1      | YBR011C              | 1.72                            | 9                   |
| ISW1      | YBR245C              | 0.731                           | 9                   |
| ISW1|ISW2 | YBR245C|YOR304W | 0.6475 | 4 |
| ISW1|ISW2 | YBR245C|YOR304W | 0.6475 | 4 |
| MAD1      | YGL086W              | 0.844                           | 14                  |
| MCD1      | YDL003W              | 0.438                           | 12                  |
| MCM2      | YBL023C              | 0.7665                          | 12                  |
| MCM21     | YDR318W              | 3.661                           | 4                   |
| MCM22     | YJR135C              | 2.505                           | 3                   |
| MCM3      | YEL032W              | 0.5435                          | 8                   |
| MCM6      | YGL201C              | 0.785                           | 7                   |
| MET4      | YNL103W              | 0.5495                          | 10                  |
| MLP1      | YKR095W              | 0.7665                          | 82                  |
| MLP2      | YIL149C              | 0.685                           | 50                  |
| MRP8      | YKL142W              | 1.059                           | 14                  |
| NET1      | YJL076W              | 2.695                           | 127                 |
| NFI1      | YOR156C              | 1.02                            | 6                   |
| NGG1      | YDR176W              | 0.808                           | 11                  |
| NPL6      | YMR091C              | 0.699                           | 4                   |
| NUP2      | YLR335W              | 1.019                           | 33                  |
| NUP60     | YAR002W              | 1.299                           | 5                   |
| OKP1      | YGR179C              | 1.6395                          | 4                   |
| PAF1      | YBR279W              | 0.719                           | 12                  |
| PDC1      | YLR044C              | 3.774                           | 15                  |
| PGK1      | YCR012W              | 1.1195                          | 40                  |
| POB3      | YML069W              | 0.752                           | 19                  |
| POL30     | YBR088C              | 0.456                           | 18                  |
| PRP45     | YAL032C              | 1.259                           | 27                  |
| RAD16     | YBR114W              | 0.661                           | 8                   |
| RAP1      | YNL216W              | 0.946                           | 43                  |
| REB1      | YBR049C              | 0.751                           | 37                  |
| RPA190    | YOR341W              | 0.308                           | 4                   |
| RPA43     | YOR340C              | 0.519                           | 13                  |
| RPB4      | YJL140W              | 0.649                           | 17                  |
| RPC37     | YKR025W              | 0.427                           | 17                  |
| RPC53     | YDL150W              | 0.6045                          | 36                  |
| RPC82     | YPR190C              | 0.2095                          | 6                   |
| RPO21     | YDL140C              | 0.534                           | 25                  |
| RPO26     | YPR187W              | 0.587                           | 11                  |
| RRP5      | YMR229C              | 0.877                           | 3                   |
| Gene   | ORF         | Median ratio (HZY4068/HZY2101) | # of peptides found |
|--------|-------------|---------------------------------|---------------------|
| RSC1   | YGR056W     | 0.6145                          | 4                   |
| RSC2   | YLR357W     | 0.669                           | 28                  |
| RSC58  | YLR033W     | 0.676                           | 16                  |
| RSC8   | YFR037C     | 0.779                           | 32                  |
| RVB1   | YDR190C     | 0.809                           | 13                  |
| SGF73  | YGL066W     | 0.723                           | 7                   |
| SHS1   | YDL225W     | 0.768                           | 21                  |
| SIR3   | YLR442C     | 0.539                           | 4                   |
| SIR4   | YDR227W     | 0.6965                          | 74                  |
| SIZ1   | YDR409W     | 0.6485                          | 22                  |
| SKO1   | YNL167C     | 1.017                           | 20                  |
| SLO15  | YBR156C     | 0.6265                          | 14                  |
| SMC2   | YFR031C     | 0.512                           | 7                   |
| SMC3   | YJL074C     | 0.337                           | 7                   |
| SMC4   | YLR086W     | 0.456                           | 23                  |
| SMC5   | YOL034W     | 0.89                            | 15                  |
| SMC6   | YLR383W     | 1.085                           | 6                   |
| SNF5   | YBR289W     | 0.56                            | 3                   |
| SPC24  | YMR117C     | 0.876                           | 9                   |
| SPN1   | YPR133C     | 0.532                           | 11                  |
| SPP41  | YDR464W     | 1.0545                          | 70                  |
| SPT15  | YER148W     | 0.718                           | 8                   |
| SPT5   | YML010W     | 0.556                           | 39                  |
| SPT7   | YBR081C     | 0.718                           | 25                  |
| STB3   | YDR169C     | 0.9255                          | 22                  |
| STE12  | YHR084W     | 0.5835                          | 6                   |
| STH1   | YIL126W     | 0.635                           | 7                   |
| SUB2   | YDL084W     | 1.7535                          | 4                   |
| SUM1   | YDR310C     | 0.7265                          | 38                  |
| SWC3   | YAL011W     | 0.734                           | 9                   |
| SWI3   | YJL176C     | 0.601                           | 15                  |
| SWI4   | YER111C     | 0.66                            | 5                   |
| TAF12  | YDR145W     | 0.737                           | 3                   |
| TAL1   | YLR354C     | 4.7845                          | 14                  |
| TEC1   | YBR083W     | 0.524                           | 15                  |
| TFC3   | YAL001C     | 0.7035                          | 6                   |
| TFC6   | YDR362C     | 0.63                            | 8                   |
| TFC7   | YOR110W     | 0.584                           | 13                  |
| TFG1   | YGR186W     | 0.518                           | 35                  |
| Gene  | ORF     | Median ratio (HZY4068/HZY2101) | # of peptides found |
|-------|---------|-------------------------------|--------------------|
| TKL1  | YPR074C | 4.758                         | 3                  |
| TOA1  | YOR194C | 0.5715                        | 6                  |
| TOF2  | YKR010C | 1.9595                        | 42                 |
| TOP1  | YOL006C | 0.441                         | 10                 |
| TOP2  | YNL088W | 0.387                         | 12                 |
| TUP1  | YCR084C | 0.601                         | 53                 |
| TYE7  | YOR344C | 0.381                         | 11                 |
| UAF30 | YOR295W | 0.746                         | 9                  |
| UBA2  | YDR390C | 1.0605                        | 24                 |
| UBC9  | YDL064W | 0.968                         | 15                 |
| UPC2  | YDR213W | 0.668                         | 4                  |
| VID21 | YDR359C | 0.734                         | 10                 |
| VPS72 | YDR485C | 0.726                         | 23                 |
| WTM1  | YOR230W | 0.545                         | 18                 |
| YMR111C | YMR111C | 1.073                         | 44                 |
Supplementary Table 3 - Sumoylated proteins identified by mass spectrometry
csm1Δ vs wild-type

| Gene   | ORF       | Median ratio (JLY1061/HZY2101) | # of peptides found |
|--------|-----------|---------------------------------|---------------------|
| ABF1   | YKL112W   | 0.682                           | 33                  |
| AOS1   | YPR180W   | 1.238                           | 3                   |
| BDP1   | YNL039W   | 0.511                           | 22                  |
| BIR1   | YJR089W   | 0.326                           | 14                  |
| BOP3   | YNL042W   | 0.419                           | 26                  |
| BRF1   | YGR246C   | 0.509                           | 3                   |
| BRN1   | YBL097W   | 0.124                           | 4                   |
| BUD4   | YJR092W   | 0.703                           | 11                  |
| CDC11  | YJR076C   | 0.827                           | 9                   |
| CDC14  | YFR028C   | 10.741                          | 9                   |
| CDC3   | YLR314C   | 0.916                           | 33                  |
| CDC48  | YDL126C   | 0.576                           | 40                  |
| CET1   | YPL228W   | 0.501                           | 20                  |
| CRZ1   | YNL027W   | 0.321                           | 17                  |
| CST6   | YIL036W   | 0.402                           | 18                  |
| CTI6   | YPL181W   | 0.219                           | 7                   |
| CYC8   | YBR112C   | 0.462                           | 19                  |
| DIG1   | YPL049C   | 0.3425                          | 6                   |
| EDE1   | YBL047C   | 0.491                           | 21                  |
| GCN5   | YGR252W   | 0.1985                          | 4                   |
| GCR1   | YPL075W   | 0.382                           | 5                   |
| GCR2   | YNL199C   | 0.143                           | 6                   |
| GIN4   | YDR507C   | 2.464                           | 7                   |
| HAP1   | YLR256W   | 0.153                           | 13                  |
| HIR2   | YOR038C   | 0.48                            | 13                  |
| HMS1   | YOR032C   | 0.116                           | 13                  |
| HPC2   | YBR215W   | 0.455                           | 33                  |
| HTB2| HTB1     | YBR002W|YDR224C | 0.829 | 6 |
| ISW1   | YBR245C   | 0.583                           | 5                   |
| ITC1   | YGL133W   | 0.575                           | 3                   |
| MCD1   | YDL003W   | 2.0535                          | 6                   |
| MET4   | YNL103W   | 0.1965                          | 18                  |
| MLP1   | YKR095W   | 2.0695                          | 104                 |
| MLP2   | YIL149C   | 2                               | 21                  |
| MOT1   | YPL082C   | 0.3705                          | 22                  |
| MRC1   | YCL061C   | 0.566                           | 8                   |
| Gene | ORF     | Median ratio (JLY1061/HZY2101) | # of peptides found |
|------|---------|-------------------------------|-------------------|
| MRP8 | YKL142W | 1.302                         | 8                 |
| NET1 | YJL076W | 7.647                         | 131               |
| NFI1 | YOR156C | 0.2745                        | 4                 |
| NGG1 | YDR176W | 0.147                         | 13                |
| NUP2 | YLR335W | 1.443                         | 3                 |
| NUT1 | YGL151W | 0.3465                        | 4                 |
| PAF1 | YBR279W | 0.458                         | 3                 |
| PGK1 | YCR012W | 0.81                          | 24                |
| POB3 | YML069W | 0.658                         | 21                |
| POL30| YBR088C | 0.161                         | 15                |
| PRP45| YAL032C | 2.041                         | 17                |
| RAP1 | YNL216W | 0.591                         | 5                 |
| RIF1 | YBR049C | 1.02                          | 13                |
| RIF1 | YBR275C | 0.497                         | 3                 |
| RIS1 | YOR191W | 0.228                         | 3                 |
| RPB4 | YJL140W | 0.48                          | 11                |
| RPC37| YKR025W | 0.242                         | 12                |
| RPC53| YDL150W | 0.48                          | 21                |
| RPC82| YPR190C | 0.4315                        | 8                 |
| RPO21| YDL140C | 0.549                         | 33                |
| RPO26| YPR187W | 0.45                          | 3                 |
| RRP5 | YMR229C | 1.331                         | 3                 |
| RSC2 | YLR357W | 0.3145                        | 8                 |
| RSC8 | YFR037C | 0.359                         | 15                |
| SCS2 | YER120W | 0.6225                        | 12                |
| SHS1 | YDL225W | 0.937                         | 31                |
| SIN3 | YOL004W | 0.3095                        | 8                 |
| SIR3 | YLR442C | 0.421                         | 35                |
| SIR4 | YDR227W | 0.345                         | 34                |
| SIZ1 | YDR409W | 0.594                         | 12                |
| SKO1 | YNL167C | 0.475                         | 19                |
| SMC3 | YJL074C | 2.08                          | 8                 |
| SMC4 | YLR086W | 0.107                         | 10                |
| SNF2 | YOR290C | 0.27                          | 8                 |
| SPA2 | YLL021W | 0.793                         | 35                |
| SPP41| YDR464W | 0.321                         | 21                |
| SPT5 | YML010W | 0.356                         | 29                |
| SPT7 | YBR081C | 0.171                         | 44                |
| STB3 | YDR169C | 0.634                         | 23                |
| STH1 | YIL126W | 0.1975                        | 10                |
| Gene  | ORF     | Median ratio (JLY1061/HZY2101) | # of peptides found |
|-------|---------|--------------------------------|---------------------|
| STU1  | YBL034C | 0.878                          | 3                   |
| SUM1  | YDR310C | 0.331                          | 57                  |
| SWI3  | YJL176C | 0.377                          | 13                  |
| SWR1  | YDR334W | 0.217                          | 3                   |
| TEC1  | YBR083W | 0.261                          | 23                  |
| TFG1  | YGR186W | 0.503                          | 34                  |
| TOA1  | YOR194C | 0.404                          | 7                   |
| TOF2  | YKR010C | 3.892                          | 21                  |
| TOP2  | YNL088W | 0.6925                         | 52                  |
| TUP1  | YCR084C | 0.39                           | 51                  |
| TYE7  | YOR344C | 0.086                          | 15                  |
| UBA2  | YDR390C | 0.709                          | 27                  |
| UBC9  | YDL064W | 0.9795                         | 16                  |
| UPC2  | YDR213W | 0.344                          | 10                  |
| VHR1  | YIL056W | 0.2805                         | 12                  |
| VPS72 | YDR485C | 0.244                          | 9                   |
| YCS4  | YLR272C | 0.1355                         | 12                  |
| YMR111C | YMR111C | 0.3475                         | 18                  |
| ZEO1  | YOL109W | 0.86                           | 3                   |
## Supplementary Table 4 - Crystallographic data collection and refinement statistics

| Data collection       | Sc Ulp2821-847:Csm169-181 | Sc Ulp2825-844-Tof2384-400 :Csm169-181 |
|-----------------------|---------------------------|---------------------------------------|
| **Resolution (Å)**    | 44.0 – 2.14               | 42.0 – 1.30                           |
| **Wavelength (Å)**    | 1.18 Å                    | 0.98 Å                                |
| **Space Group**       | P4_2_2                    | C2                                    |
| **Unit Cell Dimensions (a, b, c) Å** | 46.74, 46.74, 124.64        | 71.68, 56.93, 40.99                  |
| **Unit cell Angles (a,b,g)°** | 90, 90, 90                | 90, 121.06, 90                        |
| **I/s (last shell)**  | 53.9 (2.0)                | 10.1 (1.1)                            |
| **1R_{sym} (last shell)** | 0.053 (1.318)              | 0.068 (2.281)                         |
| **2R_{meas} (last shell)** | 0.055 (1.369)              | 0.08 (2.698)                          |
| **3CC_{1/2} (last shell)** | 0.699                     | 0.418                                 |
| **Completeness (last shell) %** | 98.7 (98.2)                | 96.4 (92.7)                           |
| **Number of reflections** | 111900                    | 122421                                |
| **unique**            | 8129                      | 33373                                 |
| **Multiplicity (last shell)** | 13.8 (13.4)               | 3.7 (3.5)                             |
| **Refinement**        |                           |                                       |
| **Resolution (Å)**    | 44.0 – 2.14               | 35.0 – 1.30                           |
| **No. of reflections** | 8102                      | 33342                                 |
| **working**           | 7292                      | 31797                                 |
| **free**              | 810                       | 1545                                  |
| **4R_{work} (last shell) (%)** | 23.45 (31.46)             | 19.20 (44.39)                         |
| **4R_{free} (last shell) (%)** | 27.82 (34.49)             | 21.71 (51.18)                         |
| **Structure/Stereochemistry** |                        |                                       |
| **No. of atoms**      | 1019                      | 2355                                  |
| **solvent**           | 20                        | 85                                    |
| **hydrogen**          | 0                         | 1114                                  |
| **r.m.s.d. bond lengths (Å)** | 0.003                     | 0.023                                 |
| **r.m.s.d. bond angles (°)** | 0.565                     | 1.735                                 |
| **5SBGrid Data Bank ID** | 327                       | 398                                   |
| **6Protein Data Bank ID** | 5V1A                      | 5V3N                                  |

\(^{1}R_{sym} = \frac{\sum_{j} |I_{j} - \langle I \rangle|}{\sum_{j} I_{j}}, \) where \(I_{j}\) is the intensity measurement for reflection \(j\) and \(\langle I \rangle\) is the mean intensity for multiply recorded reflections.
where $h_j$ is a single intensity measurement for reflection $h$, $\langle h \rangle$ is the average intensity measurement for multiply recorded reflections, and $n$ is the number of observations of reflection $h$.

$^{3}CC_{1/2}$ is the Pearson correlation coefficient between the average measured intensities of two randomly-assigned half-sets of the measurements of each unique reflection (Karplus & Diederichs (2012) Science 336:1030-1033). $CC_{1/2}$ is considered significant above a value of ~0.15.

$^{4}R_{\text{work, free}} = \sum \frac{|F_{\text{obs}}| - |F_{\text{calc}}|}{|F_{\text{obs}}|}$, where the working and free $R$-factors are calculated using the working and free reflection sets, respectively.

$^{5}$Diffraction data for each structure have been deposited with the SBGrid Data Bank (https://data.sbgrid.org) with the noted accession codes.

$^{6}$Coordinates and structure factors for each structure have been deposited with the Protein Data Bank (http://www.pdb.org) with the noted accession codes.
**Supplementary Table 5** - Net1 and Tof2 peptides identified from *HF-smt3-I96R ulp2A*

| Peptide identified                        | Protein | K-e-GG position |
|-------------------------------------------|---------|-----------------|
| K.AKNES AQIDR.Q                          | NET1    |                 |
| K.ASN TSITK.S                            | NET1    |                 |
| K.DIN SKP DPR.N                          | NET1    |                 |
| K.DISL HSLK.G                            | NET1    |                 |
| K.DISL HSL KGS V PKD SK.I                | NET1    |                 |
| K.DVF N VNNIVR.V                         | NET1    |                 |
| K.EDGT IIN GTIE DDGNDND NDVE DTTVR.I     | NET1    |                 |
| K.EGNVQL PKPS AND K.LK.D                 | NET1    |                 |
| K.EGNVQL PKPS AND KL KD LK.A            | NET1    |                 |
| K.EGP SSP AS IL PAK.A                    | NET1    |                 |
| K.EL KEGP SSP AS IL PAK.A                | NET1    |                 |
| K.FLL FT KPTN TLL NL SDE I IDK.C         | NET1    |                 |
| K.FLL FT KPTN TLL NL SDE I ID K CEK.M    | NET1    |                 |
| K.GSV VP VKD SK.I                       | NET1    |                 |
| K.GTTS FNEE GN R.K                      | NET1    |                 |
| K.GTTS FNEE GN RK.N                     | NET1    |                 |
| K.IEAP SP SVN KK.I                       | NET1    |                 |
| K.IIN KEV DEER.N                        | NET1    |                 |
| K.IK [242.14] SSIVEEDI VSR.S            | NET1    | K268            |
| K.IK SSIVEEDI VSR.S                     | NET1    |                 |
| K.INAT PD KIP VT QL MDM SPP SVK.S       | NET1    |                 |
| K.ISEQ MAK.S                            | NET1    |                 |
| K.IVSN NSDD DE EDIGER.S                 | NET1    |                 |
| K.KK PK SGG F AS LIK.D                  | NET1    |                 |
| K.KK PK SGG F AS LIK.D                  | NET1    |                 |
| K.KK PK SGG F AS LIK DF K.K             | NET1    |                 |
| K.KP SGG F AS LIK DF K.K                | NET1    |                 |
| K.KR PP GT TTT TT TIR.S                 | NET1    |                 |
| K.KSA V SESS VT N SK.I                  | NET1    |                 |
| K.KSQA EPS GIVE PK.R                    | NET1    |                 |
| K.LNN GSP QSV PQ QQIQ PSSG VL.R         | NET1    |                 |
| K.LQV VL VPP SL QAT MP IQ FG Y GPTIA ESS Q LL PNR.T | NET1 |                |
| Peptide identified | Protein | K-e-GG position |
|--------------------|---------|-----------------|
| K.MIEGDDTDLPQWFK.G | NET1    |                 |
| K.MTDHLKEGNVQLPKPSANDK.L | NET1 |                 |
| K.MTDHLKEGNVQLPKPSANDKLK.D | NET1 |                 |
| K.MTDHLKEGNVQLPKPSANDKLKDLK.A | NET1 |                 |
| K.NEIDLDDSAPVSLYK.S | NET1 |                 |
| K.NESAQIDR.Q | NET1 |                 |
| K.NMSVPNNGPDKDISLHSLK.G | NET1 |                 |
| K.NMSVPNNGPDKDISLHSLKGSVVPVKDSK.I | NET1 |                 |
| K.NSKPYTTVLNK.D | NET1 |                 |
| K.NSPLGDAMPHNVHLAELPK.A | NET1 |                 |
| K.RAAQLLAGAK.K | NET1 |                 |
| K.RMTNFLDDNQVR.E | NET1 |                 |
| K.SANIGGEDLNK.K | NET1 |                 |
| K.SANIGGEDLNKK.A | NET1 |                 |
| K.SASAALGKK.K | NET1 |                 |
| K.SAVSESSVTNSK.I | NET1 |                 |
| K.SFYPNSNK.K | NET1 |                 |
| K.SKTTSNPSSILHDLPR.K | NET1 |                 |
| K.SQAEPSGIVEPK.R | NET1 |                 |
| K.SSIVEEDIVSR.S | NET1 |                 |
| K.SSLETIVEK.K | NET1 |                 |
| K.SSLETIVEKK.S | NET1 |                 |
| K.TKAKNESAQIDR.Q | NET1 |                 |
| K.TLVPPGIISNEK.N | NET1 |                 |
| K.TTSNPSSILHDLPR.K | NET1 |                 |
| K.TTSNPSSILHDLPRK.V | NET1 |                 |
| K.VETKPAQASSFPVVGGSPSVATK.G | NET1 |                 |
| K.VRPSLSSSLSDLVSR.G | NET1 |                 |
| L.FTKPTNTLLNLSDEIIDKCEK.M | NET1 |                 |
| L.QDNSGCDLDPDFLVDVFNVNNIVR.V | NET1 |                 |
| L.SLDNSGCDLDPDFLVDVFNVNNIVR.V | NET1 |                 |
| L.SLDNSGCDLDPDFLVDVFNVNNIVR.V | NET1 |                 |
| Peptide identified | Protein | K-ε-GG position |
|--------------------|---------|-----------------|
| M.AQSAGDASLQYANLR.S | NET1 | |
| R.AAQLLAGAK.K | NET1 | |
| R.EKEDTNKLEKEILPTIPHDQPIALLSSDK.S | NET1 | |
| R.EKEDTNKLEKEILPTIPHDQPIALLSSDKSNGTLK.S | NET1 | |
| R.ISEIEKELK.K | NET1 | |
| R.ISEIEKELK[242.14]EGPSSPASILPAK.A | NET1 | K380 |
| R.ISEIEKELKEGPSSPASILPAK.A | NET1 | |
| R.ISSGIDAGKK.I | NET1 | |
| R.IVPQDSDSSFPK.S | NET1 | |
| R.IVPQDSDSSFPKSDLFK.M | NET1 | |
| R.KFLLFTKPTNTLLNLSDEIIIDKCEK.M | NET1 | |
| R.KPPVTTPR.I | NET1 | |
| R.KSSELETIVEK.K | NET1 | |
| R.KSSELETIVEKK.S | NET1 | |
| R.KVRLPSLSSLSDLVSR.G | NET1 | |
| R.LLSGTPIMSTMTPNR.V | NET1 | |
| R.LLSGTPIMSTMTPNRVTLTGQR.V | NET1 | |
| R.MTNFLDDNQVR.E | NET1 | |
| R.QIYPQSSK.I | NET1 | |
| R.RVVVTTPR.E | NET1 | |
| R.RVVVNTPREVR.S | NET1 | |
| R.SANVSFTPSYFNQSR.F | NET1 | |
| R.SATNGSMR.V | NET1 | |
| R.SATVDPDKTK.Q | NET1 | |
| R.SFLPPPTQPQSPPR.I | NET1 | |
| R.SKLLNGSPQSVPQPPQIQIPSSGVLR.I | NET1 | |
| R.SQSSIADNNGSPVK.N | NET1 | |
| R.SQSSIADNNGSPVKNSPLGDAMPHNVLAELPK.A | NET1 | |
| R.SSKEAPPSVNNK.I | NET1 | |
| R.TNMAQSAGDASLQYANLR.S | NET1 | |
| R.VILKNEIDLDDAPVSLYK.S | NET1 | |
| R.VTLTGQR.V | NET1 | |
| Peptide identified                             | Protein | K-ε-GG position |
|------------------------------------------------|---------|-----------------|
| R.VVSEHAHK.N                                   | NET1    |                 |
| R.VVSEHAHKNELVFSAS.A                           | NET1    |                 |
| R.VVSEHAHKNELVFSASASSSSFANGGTAAVTAQDINR.K      | NET1    |                 |
| R.VVVNTPREPVR.S                                 | NET1    |                 |
| S.ASSSSFANGGTAAVTAQDINRKPPVTTPR.I              | NET1    |                 |
| S.FTPSYFNQSR.F                                  | NET1    |                 |
| F.TDGDNTLLLQLSNEILTKFDR.L                      | TOF2    |                 |
| K.ASMGFRDINSDDLDSVSFSNDIENAVQSTQSTK.N          | TOF2    |                 |
| K.CPMPLTSTVVASNVHKDVK.D                        | TOF2    |                 |
| K.CPMPLTSTVVASNVHKDVKDHAR.A                    | TOF2    |                 |
| K.DIFANAGKPPNAASTIK.V                          | TOF2    |                 |
| K.EDLSSISNKDTMHLIAK.S                          | TOF2    |                 |
| K.EFPDKSLGAASSTSHAK.D                          | TOF2    |                 |
| K.ESIEIVSLQDR.H                                | TOF2    |                 |
| K.FDRLYPNFK.E                                  | TOF2    |                 |
| K.FDRLYPNFKESIEIVSLQDR.H                       | TOF2    |                 |
| K.FLHFTDGDNTLLLQLSNEILTK.F                     | TOF2    |                 |
| K.FLHFTDGDNTLLLQLSNEILTKFDR.L                  | TOF2    |                 |
| K.GDDLPLNDKDIGENC.R                            | TOF2    |                 |
| K.GVSLETKHFDDPNTIISGGEK.F                      | TOF2    |                 |
| K.GVSLETKHFDDPNTIISGGEKFAK.F                   | TOF2    |                 |
| K.HFDDPNTIISGGEK.F                             | TOF2    |                 |
| K.HFDDPNTIISGGEK[242.14]FAK.F                  | TOF2    | K570            |
| K.HFDDPNTIISGGEKFAK.F                          | TOF2    |                 |
| K.HFDDPNTIISGGEKF[242.14]FGK.I                 | TOF2    | K573            |
| K.ISKEDLSSISNKDTMHLIAK.S                       | TOF2    |                 |
| K.KASMGFR.D                                    | TOF2    |                 |
| K.KASMGFRDINSDDLDSVSFSNDIENAVQSTQSTK.N         | TOF2    |                 |
| K.KFLHFTDGDNTLLLQLSNEILTK.F                    | TOF2    |                 |
| K.KFLHFTDGDNTLLLQLSNEILTKFDR.L                 | TOF2    |                 |
| K.KGDDLPLNDKDIGENC.R                            | TOF2    |                 |
| Peptide identified                                      | Protein | K-ε-GG position |
|---------------------------------------------------------|---------|-----------------|
| K.KGDDLPLNDKDIGENCRR.I                                 | TOF2    |                 |
| K.KISKEDLSSSNKDMHLIA.K.S                               | TOF2    |                 |
| K.LMRDPVDN.SK.D                                        | TOF2    |                 |
| K.NGQAIPSSLER.T                                        | TOF2    |                 |
| K.NVVSPPFFPEK.E                                        | TOF2    |                 |
| K.NVVSPPFFPEK[242.14]ELNNR.L                           | TOF2    | K462            |
| K.NVVSPPFFPEKELNNR.L                                   | TOF2    |                 |
| K.NVVSPPFFPEKELNNRLHQS.QGK.E                           | TOF2    |                 |
| K.RAESKDLDLL.R.N                                       | TOF2    |                 |
| K.RKNGQAIPSSLER.T                                      | TOF2    |                 |
| K.RKTDDVGSK.V                                          | TOF2    |                 |
| K.RNSITEPYYQ.G.K.F                                     | TOF2    |                 |
| K.SITSK[242.14]GVSLET.K.H                              | TOF2    | K550            |
| K.SLGAASSTSHAK.D                                       | TOF2    |                 |
| K.SLGAASSTSHAKDV.K.I                                   | TOF2    |                 |
| K.SRVSTPLMNEILPLASKYDALNKEK.C                          | TOF2    |                 |
| K.SSLKNNFINK.S                                         | TOF2    |                 |
| K.TSHGPAGNSNGKPMLDVDDNEINT.K                            | TOF2    |                 |
| K.VQNKGDDLPLNDKDIGENC.R                                 | TOF2    |                 |
| K.YDALNKEK.C                                           | TOF2    |                 |
| R.AESKDLDLL.R.N                                        | TOF2    |                 |
| R.DINSDLSDSFSN.SDIENAVQSTQST.K.N                       | TOF2    |                 |
| R.FKPTGETK.V                                           | TOF2    |                 |
| R.FKPTGETK[242.14]VQKR.N                              | TOF2    | K521            |
| R.IEAFSDEEDFNEDNDRA.DSFNNS.K                           | TOF2    |                 |
| R.IEAFSDEEDFNEDNDRA.DSFNNS.K.A                         | TOF2    |                 |
| R.KKISKEDLSSN.KDMHLIA.K.S                              | TOF2    |                 |
| R.KSSLEIK.V                                            | TOF2    |                 |
| R.KTDDVGSK.V                                           | TOF2    |                 |
| R.KTDDVGSK[242.14]VIEF.K                               | TOF2    | K589            |
| R.KTDDVGSKU.VIEF.K                                     | TOF2    |                 |
| R.LHQS.QGK[242.14]EALFR.L                             | TOF2    | K474            |
| R.LHQS.QGKEALFR.L                                      | TOF2    |                 |
| R.LQIVL.VPPSAQDIITFLER.L                               | TOF2    |                 |
| Peptide identified                                      | Protein | K-e-GG position |
|----------------------------------------------------------|---------|-----------------|
| R.LVEKEFPDK.S                                           | TOF2    |                 |
| R.LVEKEFPDK[242.14]SLGAASSTSHAK.D                       | TOF2    | K488            |
| R.LVEKEFPDK[242.14]SLGAASSTSHAKDVK.I                   | TOF2    | K488            |
| R.LVEKEFPDKSLGAASSTSHAK.D                               | TOF2    |                 |
| R.LVEKEFPDKSLGAASSTSHAKDVK.I                           | TOF2    |                 |
| R.LVEKEFPDKSLGAASSTSHAKDVK[242.14]IQETIR.K             | TOF2    | K503            |
| R.LYPNFKESIEIVSLQDR.H                                    | TOF2    |                 |
| R.NQHISLLQLAR.Q                                         | TOF2    |                 |
| R.NSITEPYYGK.F                                          | TOF2    |                 |
| R.RIEAFSDEEDFNETDNDRADSFINNSK.K                         | TOF2    |                 |
| R.RIEAFSDEEDFNETDNDRADSFINNSKK.A                        | TOF2    |                 |
| R.SLKDIFANAGKPPNAASTIK.V                                | TOF2    |                 |
| R.SLKDIFANAGKPPNAASTIK[242.14]VVK.L                    | TOF2    | K622            |
| R.SLKDIFANAGKPPNAASTIKVVK.L                            | TOF2    |                 |
| R.SSDFINYLPNCK.K                                        | TOF2    |                 |
| R.SSDFINYLPNCKK.F                                       | TOF2    |                 |
| R.VSTPLMNEILPLASK.Y                                     | TOF2    |                 |
| R.VSTPLMNEILPLASKYDALNK.E                               | TOF2    |                 |
| R.VSTPLMNEILPLASKYDALNKEK.C                             | TOF2    |                 |
| V.SFNSDIENAVQSTQSTK.N                                   | TOF2    |                 |
Supplementary Table 6 - Modified lysine residues identified for Tof2 and Net1

| Protein | In protein | Identified by MS | Containing GG remnant (K-ε-GG) | Protein sequence coverage |
|---------|------------|-----------------|-------------------------------|--------------------------|
| Net1    | 122        | 77              | 2                             | 69.5%                    |
| Tof2    | 80         | 52              | 10                            | 62.4%                    |
Supplementary Table 7 – Yeast strains list

All strains are derivatives of W303 and share the same markers as A2587 unless otherwise noted. All strains with HF-SMT3 are isogenic to HZY2101 and derived from S288c. Strains with 2-micron plasmid removed, cir0, are as indicated (See methods).

| Strain number | Relevant genotype                                                                 | Reference        |
|---------------|-----------------------------------------------------------------------------------|------------------|
| HZY2101       | MATa HF-SMT3 sml1Δ::TRP1 arg4Δ ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgl hom3-10 ade2Δ ade8, cir0 (2-micron removed) | 3                |
| HZY4068       | MATa ulp2-781Δ::HIS3 HF-SMT3                                                      | This work        |
| JLY1061       | MATa csm1Δ::natMX4 HF-SMT3                                                        | This work        |
| HZY4171       | MATa TOF2-His6-3xHA::His3MX6 HF-SMT3                                             | This work        |
| JLY881        | MATa TOF2-His6-3xHA::His3MX6 ulp2-781Δ::natMX4 HF-SMT3                           | This work        |
| JLY892        | MATa TOF2-His6-3xHA::His3MX6 ulp2Δ::natMX4 HF-SMT3                               | This work        |
| HZY3721       | MATa NET1-His6-3xHA::kanMX6 HF-SMT3                                              | This work        |
| HZY3725       | MATa NET1-His6-3xHA::kanMX6 ulp2Δ::HIS3 HF-SMT3                                  | This work        |
| HZY1905       | MATa NET1-His6-3xHA::kanMX6 ulp2-781Δ::HIS3 HF-SMT3                              | This work        |
| JLY1013       | MATa TOF2-His6-3xHA::His3MX6 csm1Δ::natMX4 HF-SMT3                               | This work        |
| JLY1321       | MATa TOF2-His6-3xHA::His3MX6 ulp2-2A(F827,839A)::kanMX6 HF-SMT3                   | This work        |
| JLY1323       | MATa TOF2-His6-3xHA::His3MX6 ulp2-F827D::kanMX6 HF-SMT3                          | This work        |
| JLY1326       | MATa TOF2-His6-3xHA::His3MX6 ulp2-F839D::kanMX6 HF-SMT3                          | This work        |
| JLY963        | MATa TOF2-His6-3xHA::His3MX6 ulp2-781Δ::HIS3 HF-SMT3                             | This work        |
| JLY966        | MATa TOF2-His6-3xHA::His3MX6 ulp2-781Δ::HIS3 HF-SMT3                             | This work        |
| JLY969        | MATa TOF2-His6-3xHA::His3MX6 ulp2-781Δ::HIS3 HF-SMT3                             | This work        |
| JLY1277       | MATa TOF2-His6-3xHA::His3MX6 ulp2-781Δ::HIS3 HF-SMT3                             | This work        |
| JLY1294       | MATa TOF2-His6-3xHA::His3MX6 ulp2-781Δ::HIS3 HF-SMT3                             | This work        |
| JLY1374       | MATa TOF2-His6-3xHA::His3MX6 ulp2-781Δ::HIS3 HF-SMT3                             | This work        |
| JLY1379       | MATa TOF2- His6-3xHA::His3MX6 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work        |
| JLY1380       | MATa TOF2- His6-3xHA::His3MX6 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work        |
| JLY1382       | MATa TOF2- His6-3xHA::His3MX6 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work        |
| JLY1383       | MATa TOF2- His6-3xHA::His3MX6 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work        |
| JLY1390       | MATa TOF2- His6-3xHA::His3MX6 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work        |
| JLY1401       | MATa TOF2- His6-3xHA::His3MX6 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work        |
| JLY1396       | MATa TOF2- His6-3xHA::His3MX6 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work        |
| DMY2798 (JLY786) | MATa leu2::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | 18               |
| Strain number | Relevant genotype                                                                 | Reference |
|--------------|----------------------------------------------------------------------------------|-----------|
| DMY2804 (JLY787) | MATα RDN1-NTS2::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1       | 18        |
| DMY2800 (JLY788) | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1       | 18        |
| JLY810        | MATα leu2::mURA3 ulp2Δ::natMX4                                                 | This work |
| JLY812        | MATα RDN1-NTS2::mURA3 ulp2Δ::natMX4                                             | This work |
| JLY814        | MATα RDN1-NTS1::mURA3 ulp2Δ::natMX4                                             | This work |
| JLY816        | MATα leu2::mURA3 ulp2-707Δ::natMX4                                             | This work |
| JLY819        | MATα RDN1-NTS2::mURA3 ulp2-707Δ::natMX4                                         | This work |
| JLY822        | MATα RDN1-NTS1::mURA3 ulp2-707Δ::natMX4                                         | This work |
| JLY826        | MATα leu2::mURA3 ulp2-781Δ::natMX4                                             | This work |
| JLY828        | MATα RDN1-NTS2::mURA3 ulp2-781Δ::natMX4                                         | This work |
| JLY830        | MATα RDN1-NTS1::mURA3 ulp2-781Δ::natMX4                                         | This work |
| JLY832        | MATα leu2::mURA3 ulp2-873Δ::natMX4                                             | This work |
| JLY834        | MATα RDN1-NTS2::mURA3 ulp2-873Δ::natMX4                                         | This work |
| JLY836        | MATα RDN1-NTS1::mURA3 ulp2-873Δ::natMX4                                         | This work |
| JLY838        | MATα leu2::mURA3 sir2Δ::natMX4                                                 | This work |
| JLY840        | MATα RDN1-NTS2::mURA3 sir2Δ::natMX4                                             | This work |
| JLY842        | MATα RDN1-NTS1::mURA3 sir2Δ::natMX4                                             | This work |
| JLY1333       | MATα leu2::mURA3 ulp2-F827Δ::kanMX6                                            | This work |
| JLY1335       | MATα leu2::mURA3 ulp2-F839Δ::kanMX6                                            | This work |
| JLY1337       | MATα leu2::mURA3 ulp2-2A(F827,839A)::kanMX6                                    | This work |
| JLY1339       | MATα RDN1-NTS1::mURA3 ulp2-F827Δ::kanMX6                                        | This work |
| JLY1341       | MATα RDN1-NTS1::mURA3 ulp2-F839Δ::kanMX6                                        | This work |
| JLY1344       | MATα RDN1-NTS1::mURA3 ulp2-2A(F827,839A)::kanMX6                                | This work |
| JLY1074       | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work |
| JLY1045       | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work |
| JLY1391       | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work |
| JLY1393       | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work |
| JLY912        | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work |
| JLY932        | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work |
| JLY934        | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work |
| JLY937        | MATα RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work |
| Strain number | Relevant genotype                                                                 | Reference  |
|---------------|-----------------------------------------------------------------------------------|------------|
| JLY1164       | MATα ulp2Δ::natMX4 VRTEL::URA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work  |
| JLY1166       | MATα sir2Δ::natMX4 VRTEL::URA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work  |
| DMY3149       | leu2::mURA3 csm1Δ::KAN^R                                                        | 18         |
| DMY3157       | RDN1-NTS1::mURA3 csm1Δ::KAN^R                                                   | 18         |
| DMY3153       | RDN1-NTS2::mURA3 csm1Δ::KAN^R                                                   | 18         |
| KDC171        | leu2::mURA3 csm1-L161D::kanMX6                                                  | 23         |
| KDC186        | RDN1-NTS1::mURA3 csm1-L161D::kanMX6                                              | 23         |
| KDC179        | RDN1-NTS2::mURA3 csm1-L161D::kanMX6                                              | 23         |
| DMY2845       | leu2::mURA3 tof2Δ::KAN^R                                                        | 18         |
| DMY2847       | RDN1-NTS1::mURA3 tof2Δ::KAN^R                                                   | 18         |
| DMY2849       | RDN1-NTS2::mURA3 tof2Δ::KAN^R                                                   | 18         |
| KDC290        | leu2::mURA3 tof2-F396A::kanMX6                                                  | This work  |
| KDC294        | RDN1-NTS1::mURA3 tof2-F396A::kanMX6                                              | This work  |
| KDC291        | RDN1-NTS2::mURA3 tof2-F396A::kanMX6                                              | This work  |
| JLY1377       | MATα tof2-F396A-His₅₀-3xHA::kanMX6 leu2::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work  |
| JLY1384       | MATα tof2-F396A-His₅₀-3xHA RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work  |
| JLY1411       | MATα slx5Δ::natMX4 tof2-F396A-His₅₀-3xHA::kanMX6 leu2::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work  |
| JLY1397       | MATα slx5Δ::natMX4 tof2-F396A-His₅₀-3xHA RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0 | This work  |
| JLY1507       | MATα smt3-allR::kanMX6 leu2::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0(2μm removed) | This work  |
| JLY1509       | MATα smt3-allR::kanMX6 RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0(2μm removed) | This work  |
| JLY1513       | MATα smt3-allR::kanMX6 ulp2-F839D::natMX4 mURA3::leu2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0(2μm removed) | This work  |
| JLY1514       | MATα smt3-allR::kanMX6 ulp2-F839D::natMX4 RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0(2μm removed) | This work  |
| JLY1519       | MATα tof-F396A::kanMX6 ulp2-F839D::natMX4 mURA3::leu2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0(2μm removed) | This work  |
| JLY1520       | MATα tof-F396A::kanMX6 ulp2-F839D::natMX4 RDN1-NTS1::mURA3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+ cir0(2μm removed) | This work  |
| HZY4162       | MATα ULP2-TAF::kanMX6 sml1Δ::TRP1 arg4Δ ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgI hom3-10 ade2Δ ade8 | This work  |
| HZY4160       | MATα ulp2-781Δ-TAF::kanMX6 sml1Δ::TRP1 arg4Δ ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgI hom3-10 ade2Δ ade8 | This work  |
| HZY4280       | MATα CSM1-PreScission-Tev-3HA ULP2-TAF::kanMX6 sml1Δ::TRP1 arg4Δ ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgI hom3-10 ade2Δ ade8 | This work  |
| HZY4286       | MATα CSM1-PreScission-Tev-3HA ulp2-F893D-TAF::kanMX6 sml1Δ::TRP1 arg4Δ ura3-52 leu2Δ1 trp1Δ63 his3Δ200 lys2ΔBgI hom3-10 ade2Δ ade8 | This work  |