

SUPPLEMENTARY TABLES

Table S1. Yeast strains used in this study.

| Strain | genotype | reference |
|------------------------|---|----------------------------------|
| BY4741 | <i>MATa his3 leu2 met15 ura3</i> | (SHOEMAKER <i>et al.</i> 1996) |
| IH1793; ATCC#204279 | <i>MATa lys1</i> | (MICHAELIS AND HERSKOWITZ 1988) |
| SM2331 | <i>MATa trp1 leu2 ura3 his4 can1 mfa1-Δ1 mfa2-Δ1</i> | (CHEN <i>et al.</i> 1997) |
| yWS304 | <i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ydj1Δ::KAN^R</i> | (GIAEVER <i>et al.</i> 2002) |
| yWS1632 | <i>MATa his3 leu2 met15 ura3 ram1Δ::KAN^R</i> | (GIAEVER <i>et al.</i> 2002) |
| yWS1635 | <i>MATa his3 leu2 met15 ura3 ydj1Δ::KAN^R ste14Δ::KAN^R</i> | (HILDEBRANDT <i>et al.</i> 2016) |
| yWS2542 | <i>MATa his3 leu2 met15 ura3 ydj1Δ::NAT^R ram1Δ::KAN^R</i> | This study |
| yWS2544 | <i>MATa his3 leu2 met15 ura3 ydj1Δ::NAT^R</i> | This study |

Table S2. Plasmids used in this study.

| gene | identifier | genotype | reference |
|-------------|-------------------|--|------------------------------------|
| vector | pRS315 | <i>CEN LEU2</i> | (SIKORSKI AND HIETER 1989) |
| | pRS316 | <i>CEN URA3</i> | (SIKORSKI AND HIETER 1989) |
| YDJ1 | pWS942 | <i>CEN URA3 YDJ1 (CASQ)</i> | (HILDEBRANDT <i>et al.</i> 2016) |
| | pWS1132 | <i>CEN URA3 YDJ1 (SASQ)</i> | (HILDEBRANDT <i>et al.</i> 2016) |
| | pWS1246 | <i>CEN URA3 YDJ1 (CTLM)</i> | (HILDEBRANDT <i>et al.</i> 2016) |
| | pWS1286 | <i>CEN URA3 YDJ1 (CVIA)</i> | (HILDEBRANDT <i>et al.</i> 2016) |
| | pWS1339 | <i>CEN URA3 YDJ1 (AQCASQ)</i> | This study |
| | pWS1341 | <i>CEN URA3 YDJ1 (VACASQ)</i> | This study |
| | pWS1373 | <i>CEN URA3 YDJ1 (CQSQ)</i> | This study |
| | pWS1372 | <i>CEN URA3 YDJ1 (CAAQ)</i> | This study |
| | pWS1343 | <i>CEN URA3 YDJ1 (CASA)</i> | This study |
| | pWS1402 | <i>CEN URA3 YDJ1 (CAIQ)</i> | This study |
| | pWS1403 | <i>CEN URA3 YDJ1 (CALQ)</i> | This study |
| | pWS1404 | <i>CEN URA3 YDJ1 (CAMQ)</i> | This study |
| | pWS1409 | <i>CEN URA3 YDJ1 (CAVQ)</i> | This study |
| | pWS1623 | <i>CEN URA3 ydj1::NAT^R</i> | This study |
| | various | <i>CEN URA3 YDJ1-Cxxx variants (n=153)</i> | This study |
| MFA1 | pWS610 | <i>CEN LEU2 MFA1 (CVIA)</i> | (KRISHNANKUTTY <i>et al.</i> 2009) |
| | pWS1587 | <i>CEN LEU2 MFA1 (CRVG)</i> | This study |
| | pWS1588 | <i>CEN LEU2 MFA1 (CVLS)</i> | This study |
| | pWS1589 | <i>CEN LEU2 MFA1 (CVLG)</i> | This study |
| | pWS1590 | <i>CEN LEU2 MFA1 (CTLC)</i> | This study |
| | pWS1591 | <i>CEN LEU2 MFA1 (CSVM)</i> | This study |
| | pWS1616 | <i>CEN LEU2 MFA1 (CRIW)</i> | This study |
| | pWS1617 | <i>CEN LEU2 MFA1 (CALT)</i> | This study |
| | pWS1618 | <i>CEN LEU2 MFA1 (CAVA)</i> | This study |
| | pWS1619 | <i>CEN LEU2 MFA1 (CWGG)</i> | This study |
| | pWS1620 | <i>CEN LEU2 MFA1 (CSIS)</i> | This study |
| | pWS1621 | <i>CEN LEU2 MFA1 (CSFN)</i> | This study |
| | pWS1622 | <i>CEN LEU2 MFA1 (CQIS)</i> | This study |
| | pWS1627 | <i>CEN LEU2 MFA1 (CALL)</i> | This study |
| | pWS1628 | <i>CEN LEU2 MFA1 (CVCL)</i> | This study |
| | pWS1629 | <i>CEN LEU2 MFA1 (CAGF)</i> | This study |

Table S3. PCR oligonucleotides used to mutate *YDJ1* and *MFA1* genes.

| Gene | Oligo | Mutation | sequence (5' → 3') ^c |
|-------------|---------------------|-----------------|---|
| Plasmid UTR | oWS219 ^a | NA ^b | TGACCATGATTACGCCAAGC |
| <i>YDJ1</i> | oWS983 | AQCASQ | AACTATGATTCCGATGAAGAAGAACAAGGTGGCGAAGGTGcTCAA TGT GCATCTCAATGATTTTCT |
| | oWS984 | VQCASA | GAAGAAGAACAAGGTGGCGAAGGTGTTCAA TGT GCATCTgcATGATTTTCTTGATAAAAAAAGATCA |
| | oWS985 | VACASQ | TATGATTCCGATGAAGAAGAACAAGGTGGCGAAGGTGTTgcA TGT GCATCTCAATGATTTTCTTGA |
| | oWS986 | Cxxx | GATTCCGATGAAGAAGAACAAGGTGGCGAAGGTGTTCAA TGc nnnnnnnnnTGATTTTCTTGATAAAAAAAGA |
| | oWS990 | VQCQSQ | TCCGATGAAGAAGAACAAGGTGGCGAAGGTGTTCAA TGT caAagcCAATGATTTTCTTGATAAAAAAAGA |
| | oWS991 | VQCAAQ | GATGAAGAAGAACAAGGTGGCGAAGGTGTTCAA TGT GCAgCTCAATGATTTTCTTGATAAAAAAAGA |
| | oWS993 | VQCAIQ | TCCGATGAAGAAGAACAAGGTGGCGAAGGTGTTCAA TGT GcTatcCAATGATTTTCTTGATAAAAAAAGATCA |
| | oWS994 | VQCALQ | TCCGATGAAGAAGAACAAGGTGGCGAAGGTGTTCAA TGT GcTctcCAATGATTTTCTTGATAAAAAAAGATCA |
| | oWS995 | VQCAMQ | TCCGATGAAGAAGAACAAGGTGGCGAAGGTGTTCAA TGT GcTatgCAATGATTTTCTTGATAAAAAAAGATCA |
| | oWS996 | VQCAVQ | TCCGATGAAGAAGAACAAGGTGGCGAAGGTGTTCAA TGT GcTgtcCAATGATTTTCTTGATAAAAAAAGATCA |
| <i>MFA1</i> | oWS1145 | CRIW | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc agaATTtgTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1153 | CRVG | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc agagTTGgTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1155 | CALT | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc GcTtTgaCTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1156 | CAVA | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc GcTgTTGCTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1157 | CAGF | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc GcTggTtTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1166 | CVLS | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc GTTtTgtCTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1167 | CVLG | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc GTTtTgGgTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1168 | CALL | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc GcTtTgtgTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1169 | CTLC | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc acTtTgtgTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1170 | CSVM | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc tcTgTTatgTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1211 | CSIS | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc tcTATTtCTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1210 | CWGG | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc tggggTGgTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1212 | CVCL | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc GTTtgTtgTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1213 | CSFN | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc tcTtTaaTTAGTTTCTGCGTACAAAAACGCGT |
| | oWS1214 | CQIS | AACTATATTATCAAAGGTGTCTTCTGGGACCCAGCA TGc caaATTtCTTAGTTTCTGCGTACAAAAACGCGT |

^a Reverse PCR oligonucleotide paired with *YDJ1* and *MFA1* mutagenic oligonucleotides.

^b NA – not applicable

^c n reflects random incorporation of A, C, G or T during synthesis; lowercase letters indicate positions different than the wildtype *YDJ1* or *MFA1* sequences in pWS942 and pWS610, respectively; bold TGT and TGC represent the position of the cysteine codon within the Cxxx encoding sequence.

Table S4. Categorization of 153 sequences recovered by Ydj1p-based thermoselection.

| CASQ-like^a | | | CASQ-like (weak) | | CVIA-like |
|------------------------------|-----------------------|-----------------------|-------------------------|-----------------------|-------------------------|
| CAAG | CISN ^b | CSHV | CAHI | CLSI ^b | CAGF |
| CAGH | CISS ^b | CSQS | CAPQ | CLYT | CALL |
| CAGQ ^b | CKSQ | CSQT | CAVG | CMYS ^b | CALT |
| CAGT | CKYS ^b | CSQV | CDFI | CNQH | CAVA |
| CAGV (2) | CLAS | CSQY | CDLG | CNYQ | CQIS |
| CANI | CLNQ ^b | CSST (3) ^c | CDLT | CPVD ^b | CRIW |
| CAPL | CLST | CSSV (4) ^c | CDMS | CRSQ | CRVG |
| CASQ | CNAQ | CSTI | CDMV | CRSV | CSFN |
| CASS | CNSS ^b | CSTS ^b | CDQM | CRTA | CSIS (2) |
| CASV | CNTV | CSTT (2) | CDVG | CSAV (2) ^c | CTLC |
| CATG | CPNV | CTES ^c | CDVV | CSFE ^b | CVCL |
| CAVH | CPQI | CTGV | CEVG ^b | CSGV | CVLG |
| CAVQ ^b | CPSA | CTHA | CEYG | CSPI | CVLS (2) |
| CDID | CPST | CTPG ^b | CFAL | CSSM | CWGG ^b |
| CDLN ^b | CPSV ^b | CTPI | CFNG | CTAY | CVIA-like (weak) |
| CDTH | CRGS (2) ^c | CTPS ^b | CFNS | CTIR ^b | CSVM |
| CEHL | CRGV (2) | CTSH | CGGW | CTPV | |
| CESV (3) | CRHA ^b | CTSN | CGLH | CTSI (2) | |
| CEVQ | CRNV ^b | CTSQ ^b | CGNS | CTSV | |
| CGAV | CRPL | CTYS (2) | CGQN ^b | CVAT | |
| CGHA ^b | CRST ^b | CVAN (2) ^c | CGSH ^b | CVGI | |
| CGLD | CSAG | CVDH ^b | CHTQ | CVGL (2) | |
| CGLL | CSAI | CVES | CIGF ^b | CVGV | |
| CGQV (2) | CSAN | CVGS | CIQN ^b | CVGY ^b | |
| CGVE (2) ^c | CSAS | CVNT | CIYG | CVQT ^b | |
| CGVQ ^b | CSEQ | CVSA | CKIE | CVSI | |
| CHTG ^b | CSHA | CYSV ^c | CKYA | CVTS | |
| CHVD | | | CLNV | CVVR | |

^a Groups are based on thermotolerance score; see Figure S2 for average score of individual sequences; see Table 1 for distribution of scores. The CASQ-like group includes the CASQ and CAVQ sequences independently identified during screening. Values after certain sequences represent the number of instances the indicated sequence was recovered as a hit (if greater than one). Unless otherwise noted, the sequence was recovered using the *ydj1Δ ste14Δ* background.

^b This sequence was identified using the *ydj1*Δ background.

^c This sequence was identified independently using the *ydj1*Δ and *ydj1*Δ *ste14*Δ backgrounds.

Table S5. Categorization of yeast Cxxx proteins by predicted prenylation and cleavage status.

| Locus ID | Gene | Motif | PS ^b | CS |
|---|--------------------------|-------|-----------------|----|
| Strongly Prenylated / Strongly Cleaved | | | | |
| YML116W | <i>ATR1</i> | CTVA | 0 | 3 |
| YLR229C | <i>CDC42^a</i> | CAIL | 0 | 2 |
| YIR032C | <i>DAL3</i> | CIII | 0 | 2 |
| YIR007W | <i>EGH1</i> | CVIS | 0 | 3 |
| YML006C | <i>GIS4</i> | CAIM | 0 | 3 |
| YJL062W | <i>LAS21</i> | CALD | 0 | 1 |
| YDR461W | <i>MFA1^a</i> | CVIA | 0 | 3 |
| YNL145W | <i>MFA2^a</i> | CVIA | 0 | 3 |
| YOR101W | <i>RAS1^a</i> | CIIC | 0 | 3 |
| YNL098C | <i>RAS2^a</i> | CIIS | 0 | 3 |
| YJL204C | <i>RCY1</i> | CCIM | 0 | 2 |
| YCR027C | <i>RHB1</i> | CSIM | 0 | 2 |
| YPR165W | <i>RHO1^a</i> | CVLL | 0 | 2 |
| YNL090W | <i>RHO2^a</i> | CIIL | 0 | 2 |
| YIL118W | <i>RHO3^a</i> | CTIM | 0 | 3 |
| YKR055W | <i>RHO4^a</i> | CIIM | 0 | 3 |
| YNL180C | <i>RHO5^a</i> | CVIL | 0 | 2 |
| YGR152C | <i>RSR1^a</i> | CTIL | 0 | 2 |
| YBL061C | <i>SKT5</i> | CVIM | 0 | 3 |
| YJR086W | <i>STE18^a</i> | CTLM | 0 | 3 |
| YLR090W | <i>XDJ1</i> | CCIQ | 0 | 2 |
| YCR004C | <i>YCP4</i> | CTVM | 0 | 3 |
| YKL196C | <i>YKT6^a</i> | CIIM | 0 | 3 |
| YDL009C | | CAVS | 0 | 3 |
| YDL022C-A | | CSII | 0 | 1 |
| YFL066C | | CCVC | 0 | 2 |
| YGL082W | | CVIM | 0 | 3 |
| YJL118W | | CCCS | 0 | 1 |
| YJR128W | | CMMI | 0 | 1 |
| YNL234W | | CSIM | 0 | 2 |
| YOL014W | | CIIL | 0 | 2 |
| YPL191C | | CVVM | 0 | 3 |
| Strongly Prenylated / Weakly Cleaved | | | | |
| YGR282C | <i>BGL2</i> | CDFS | 0 | 0 |
| YDR261C | <i>EXG2</i> | CASL | 0 | -1 |
| YNL106C | <i>INP52</i> | CDPN | 0 | -3 |
| YJR107W | <i>LIH1</i> | CSGL | 0 | -2 |
| YKL176C | <i>LST4</i> | CNAG | 0 | -2 |
| YBL049W | <i>MOH1</i> | CKCT | 0 | -1 |
| YKR048C | <i>NAP1^a</i> | CKQS | 0 | -1 |
| YDL065C | <i>PEX19^a</i> | CKQQ | 0 | -1 |
| YBL018C | <i>POP8</i> | CKCI | 0 | -1 |
| YBR087W | <i>RFC5</i> | CCLD | 0 | 0 |
| YMR060C | <i>SAM37</i> | CKYI | 0 | -2 |
| YGL169W | <i>SUA5</i> | CIQF | 0 | 0 |
| YJR066W | <i>TOR1</i> | CPFW | 0 | -2 |
| YKL203C | <i>TOR2</i> | CPFW | 0 | -2 |
| YNL064C | <i>YDJ1^a</i> | CASQ | 0 | 0 |
| YDR034W-B | | CDVF | 0 | 0 |
| YFL065C | | CCPS | 0 | -1 |
| YHL049C | | CCPS | 0 | -1 |
| YMR265C | | CSNA | 0 | -1 |
| YMR272W-B | | CMYV | 0 | -1 |
| YOL164W-A | | CIHH | 0 | -2 |
| YPR203W | | CCPS | 0 | -1 |
| Locus ID | Gene | Motif | PS | CS |
| Weakly Prenylated / Strongly Cleaved | | | | |
| YOR257W | <i>CDC31</i> | CTDS | -1 | 1 |
| Weakly Prenylated / Weakly Cleaved | | | | |
| YGR068C | <i>ART5</i> | CDDD | -1 | -3 |
| YGL263W | <i>COS12</i> | CNDV | -1 | -1 |
| YBR042C | <i>CST26</i> | CFIF | -1 | 0 |
| YBR033W | <i>EDS1</i> | CFFN | -1 | -1 |
| YCR020C | <i>PET18</i> | CYNA | -1 | -2 |
| YOR242C | <i>SSP2</i> | CIDL | -1 | 0 |
| YML041C | <i>VPS71</i> | CRNR | -1 | -4 |
| YJL059W | <i>YHC3</i> | CRME | -1 | 0 |
| YBR096W | | CSEI | -1 | -1 |
| YIL134C-A | | CAPY | -1 | -2 |
| YPL109C | | CPNY | -1 | -3 |
| YDL186W | | CHHD | -1 | -4 |
| YMR187C | | CKGE | -1 | -4 |
| Not Prenylated | | | | |
| YMR300C | <i>ADE4</i> | CADY | -2 | -1 |
| YPR093C | <i>ASR1</i> | CHDE | -3 | -3 |
| YOR299W | <i>BUD7</i> | CYDA | -2 | -1 |
| YDR301W | <i>CFT1</i> | CQ GK | -3 | -5 |
| YOR031W | <i>CRS5</i> | CEKC | -2 | -2 |
| YHR053C | <i>CUP1-1</i> | CSGK | -2 | -4 |
| YHR055C | <i>CUP1-2</i> | CSGK | -2 | -4 |
| YNL255C | <i>GIS2</i> | CPKA | -2 | -1 |
| YDR528W | <i>HLR1</i> | CTRK | -4 | -3 |
| YML075C | <i>HMG1</i> | CIKS | -2 | 0 |
| YOR231W | <i>MKK1</i> | CWKD | -3 | -4 |
| YMR158W | <i>MRPS8</i> | CRVK | -2 | -1 |
| YMR023C | <i>MSS1</i> | CIGK | -2 | -3 |
| YDR307W | <i>PMT7</i> | CLAK | -2 | -2 |
| YGL045W | <i>RIM8</i> | CDDY | -2 | -3 |
| YDR257C | <i>RKM4</i> | CVKK | -4 | -3 |
| YBR150C | <i>TBS1</i> | CVKM | -2 | 0 |
| YBR209W | | CSKP | -4 | -4 |
| YKL069W | | CVFK | -2 | -1 |
| YLR154C-G | | CDGP | -2 | -5 |
| YOR034C-A | | CQRK | -5 | -5 |

^a Known to be farnesylated, geranylgeranylated or highly likely to be prenylated based on similarity to a homolog.

^b PS – in-house algorithm prenylation score; CS – in-house algorithm cleavage score; see Materials and Methods for description of scoring systems.

| Species | % Identity | COOH-terminus |
|------------------------------|-------------------|----------------------|
| <i>S. cerevisiae</i> (Ydj1p) | 100.0 | ...VQCASQ |
| <i>K. lactis</i> | 73.2 | ...VQCASQ |
| <i>A. gossypii</i> | 71.4 | ...VQCASQ |
| <i>S. pombe</i> | 55.6 | ...VQCAQQ |
| <i>N. crassa</i> | 53.1 | ...VQCASQ |
| <i>M. oryzae</i> | 52.0 | ...VQCASQ |
| <i>G. gallus</i> | 51.5 | ...VQCAHQ |
| <i>M. musculus</i> | 50.9 | ...VQCAHQ |
| <i>H. sapiens</i> | 50.5 | ...VQCAHQ |
| <i>D. rerio</i> | 50.1 | ...VQCAHQ |
| <i>X. tropicalis</i> | 50.1 | ...VQCAHQ |
| <i>O. sativa</i> | 48.1 | ...VQCAQQ |
| <i>A. thaliana</i> | 47.3 | ...VQCAQQ |
| <i>C. elegans</i> | 45.7 | ...VRCQHQ |

Figure S1. COOH-terminal sequences of Ydj1p and related homologs.

“Ydj1” was used as a query to retrieve related sequences and overall percent identity scores from the Homologene database (<http://www.ncbi.nlm.nih.gov/homologene>). Only the last 6 amino acids of each homolog are shown. The retrieved list of homologs was culled to reduce over-representation of mammalian homologs and to limit each species to one representative when multiple entries were found. When multiple entries were observed, the homolog with the highest identity score relative to Ydj1p was retained.

FIGURE S2a. Berger et al.

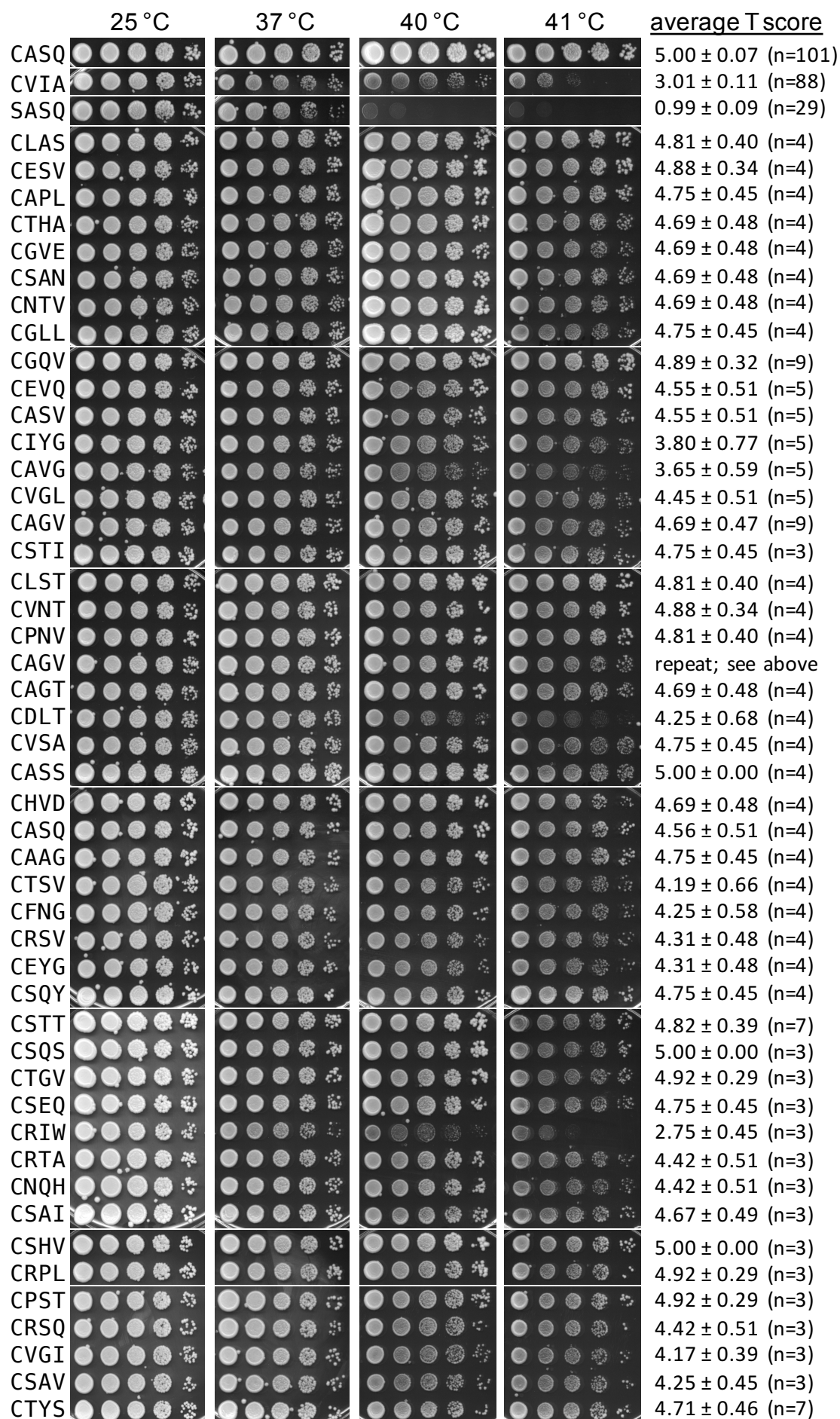


FIGURE S2b. Berger et al. (continued)

| | 25 °C | 37 °C | 40 °C | 41 °C | average T score |
|------|-------|-------|-------|-------|-----------------------|
| CASQ | | | | | 5.00 ± 0.07 (n=101) |
| CVIA | | | | | 3.01 ± 0.11 (n=88) |
| SASQ | | | | | 0.99 ± 0.09 (n=29) |
| CTYS | | | | | 4.71 ± 0.46 (n=7) |
| CDVV | | | | | 4.31 ± 0.48 (n=4) |
| CSQV | | | | | 4.69 ± 0.48 (n=4) |
| CVGS | | | | | 4.50 ± 0.52 (n=4) |
| CDLG | | | | | 4.25 ± 0.68 (n=4) |
| CSSM | | | | | 4.13 ± 0.62 (n=4) |
| CNAQ | | | | | 4.50 ± 0.52 (n=4) |
| CTPI | | | | | 4.75 ± 0.45 (n=4) |
| CAVH | | | | | 4.50 ± 0.52 (n=4) |
| CVAT | | | | | 3.94 ± 0.57 (n=4) |
| CGGW | | | | | 4.00 ± 0.63 (n=4) |
| CDVG | | | | | 4.44 ± 0.51 (n=4) |
| CDQM | | | | | 4.31 ± 0.48 (n=4) |
| CTSI | | | | | 3.94 ± 0.44 (n=4) |
| CGNS | | | | | 3.88 ± 0.50 (n=4) |
| CDID | | | | | 4.63 ± 0.50 (n=4) |
| CDTH | | | | | 4.94 ± 0.25 (n=4) |
| CTAY | | | | | 3.75 ± 0.58 (n=4) |
| CVGV | | | | | 4.31 ± 0.48 (n=4) |
| CRGV | | | | | 4.50 ± 0.52 (n=4) |
| CAHI | | | | | 4.25 ± 0.45 (n=4) |
| CVTS | | | | | 3.88 ± 0.34 (n=4) |
| CNYQ | | | | | 4.13 ± 0.34 (n=4) |
| CGAV | | | | | 5.00 ± 0.00 (n=4) |
| CSQT | | | | | 4.83 ± 0.39 (n=3) |
| CTSH | | | | | 4.75 ± 0.45 (n=3) |
| CFAL | | | | | 3.92 ± 0.67 (n=3) |
| CTPV | | | | | 4.25 ± 0.45 (n=3) |
| CAPQ | | | | | 4.33 ± 0.49 (n=3) |
| CSPI | | | | | 4.00 ± 0.00 (n=3) |
| CKSQ | | | | | 4.67 ± 0.49 (n=3) |
| CSAS | | | | | 4.83 ± 0.39 (n=3) |
| CVAN | | | | | 4.89 ± 0.31 (n=7) |
| CSAG | | | | | 4.88 ± 0.34 (n=4) |
| CSHA | | | | | 4.81 ± 0.40 (n=4) |
| CSSV | | | | | 4.75 ± 0.44 (n=7) |
| CDMS | | | | | 4.06 ± 0.25 (n=4) |
| CLYT | | | | | 4.06 ± 0.25 (n=4) |
| CSGV | | | | | 4.31 ± 0.48 (n=4) |
| CATG | | | | | 5.00 ± 0.00 (n=4) |
| CEHL | | | | | 4.75 ± 0.45 (n=4) |
| CSTT | | | | | repeat; see panel S2c |
| CPSA | | | | | 4.75 ± 0.45 (n=4) |
| CDMV | | | | | 3.94 ± 0.44 (n=4) |
| CPQI | | | | | 4.50 ± 0.52 (n=4) |
| CHTQ | | | | | 4.25 ± 0.45 (n=4) |
| CLNV | | | | | 4.44 ± 0.51 (n=4) |
| CGLD | | | | | 4.94 ± 0.25 (n=4) |

FIGURE S2c. Berger et al. (continued)

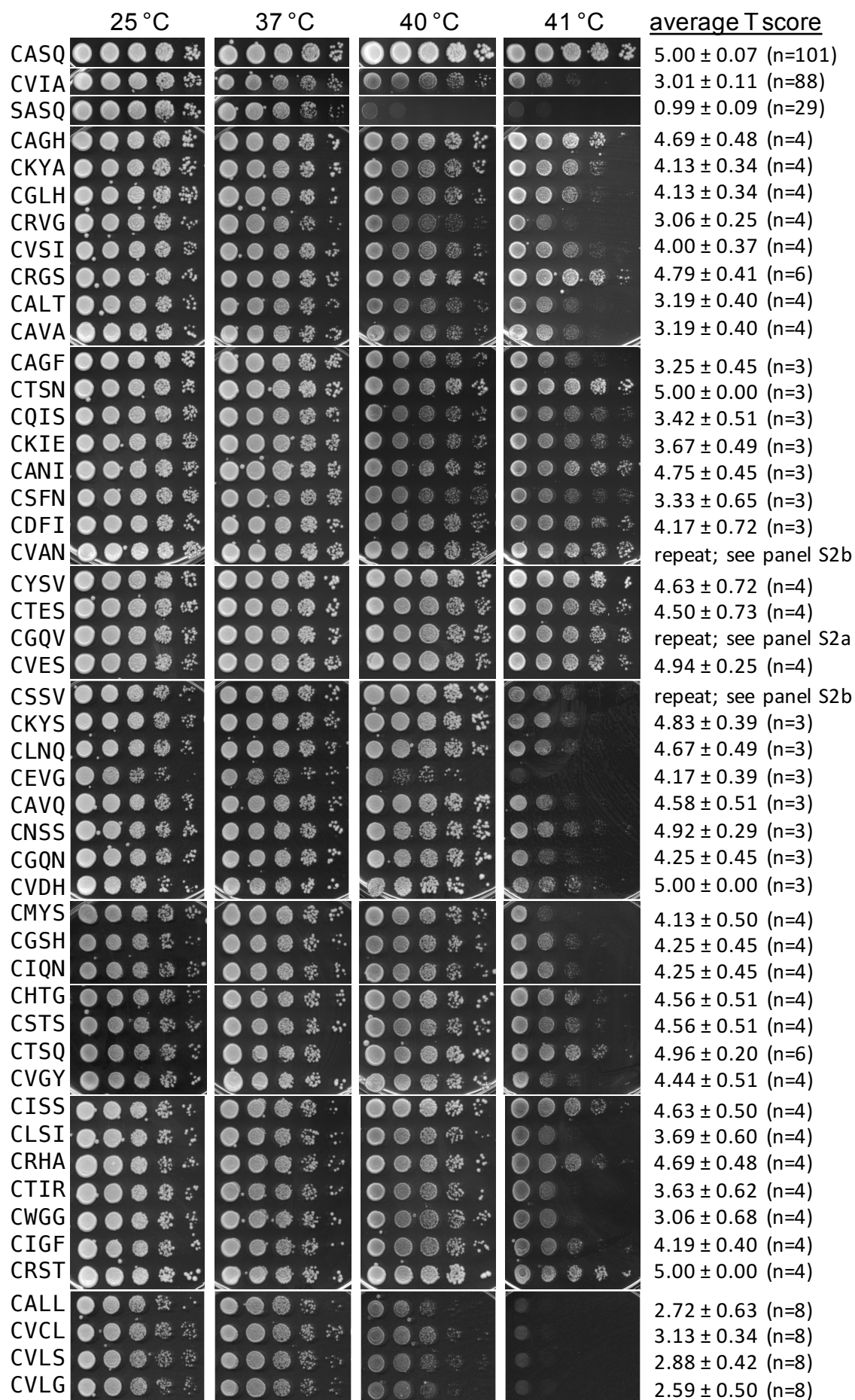


FIGURE S2d. Berger et al. (continued)

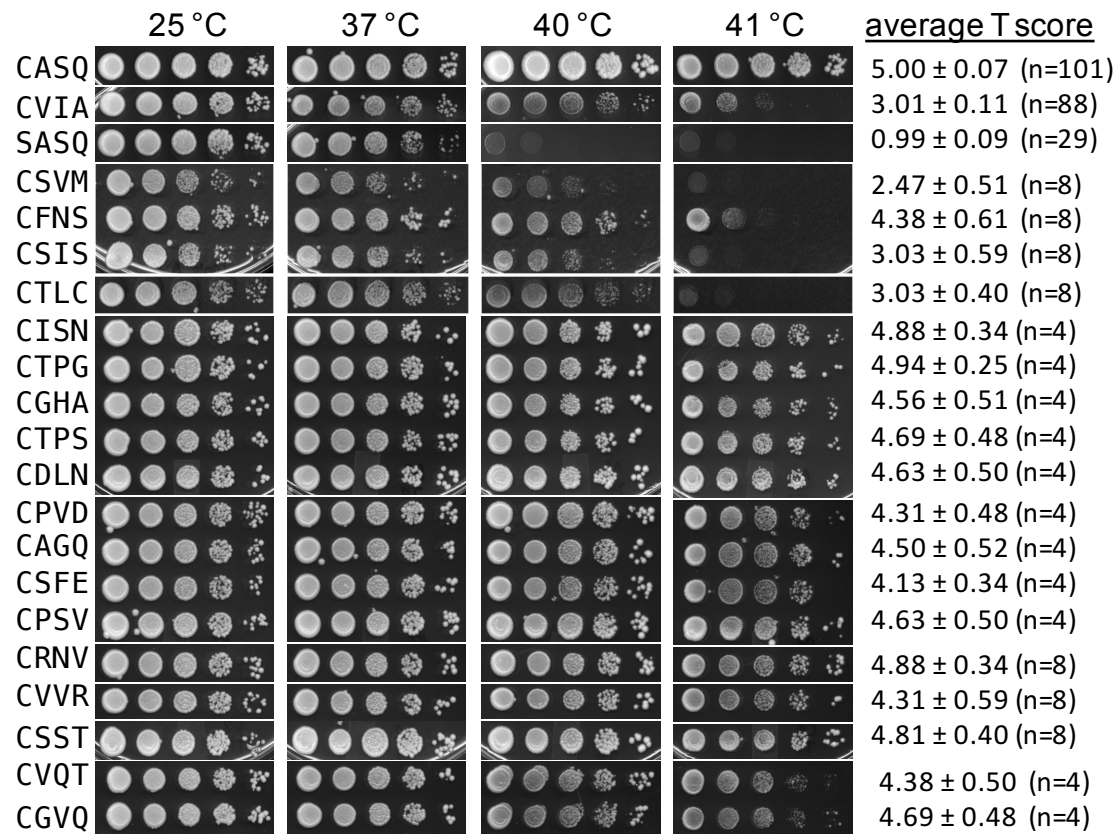


Figure S2. Phenotypes and isoprenylation status of Ydj1p Cxxx mutants identified by thermotolerance selection.

Ydj1p Cxxx mutants were evaluated for their ability to support growth of a *ydj1Δ* strain at indicated temperatures as described for Figure 1. For clarity, only one representative dilution series is shown for each Cxxx mutant, including the reference controls that are replicated on different pages associated with this data set. The dilution series associated with the first 8 Ydj1p Cxxx mutants were used for Figure 2D. The thermotolerance profile of each dilution series was scored by 4 independent observers (range 1-5), where SASQ, CVIA and CASQ controls were set to values of 1, 3 and 5, respectively. Multiple replicates (not shown) were scored for each mutant, and scores were averaged to generate a thermotolerance (T) score and standard deviation. The total number of replicates evaluated follows the T score. Two independent yeast transformant colonies were scored for most analyses (n=136); only one transformant was scored in the remaining cases (n=17).

FIGURE S3. Berger et al.

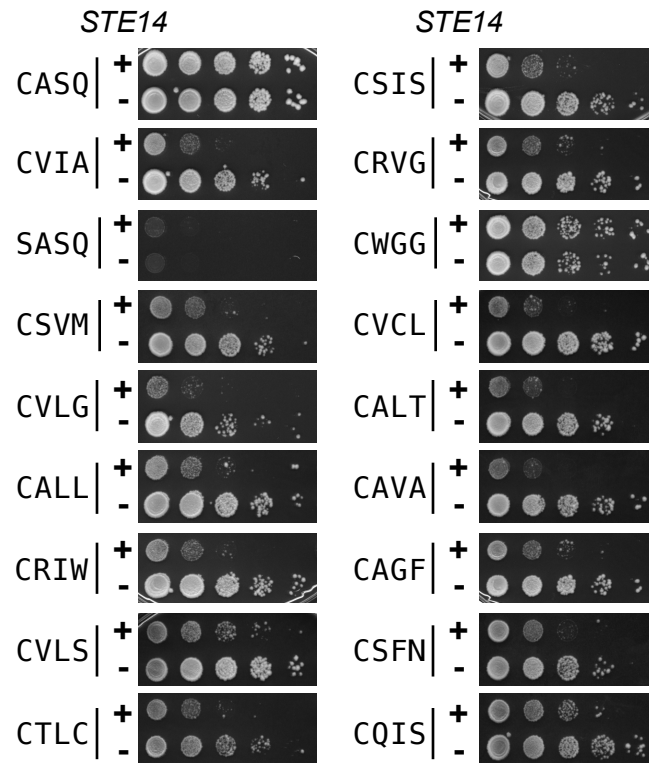


Figure S3. *Impact of carboxylmethylation on thermotolerance properties of CVIA-like Ydj1p Cxxx mutants.*

The indicated Ydj1p Cxxx variants were expressed in *ydj1Δ* yeast with or without the Ste14p isoprenylcysteine methyltransferase (+ and -, respectively) and assessed for thermotolerance as described for Figure 1C, except that recovery at room temperature was for two days instead of one. Only the 41 °C condition is shown.

FIGURE S4. Berger et al.

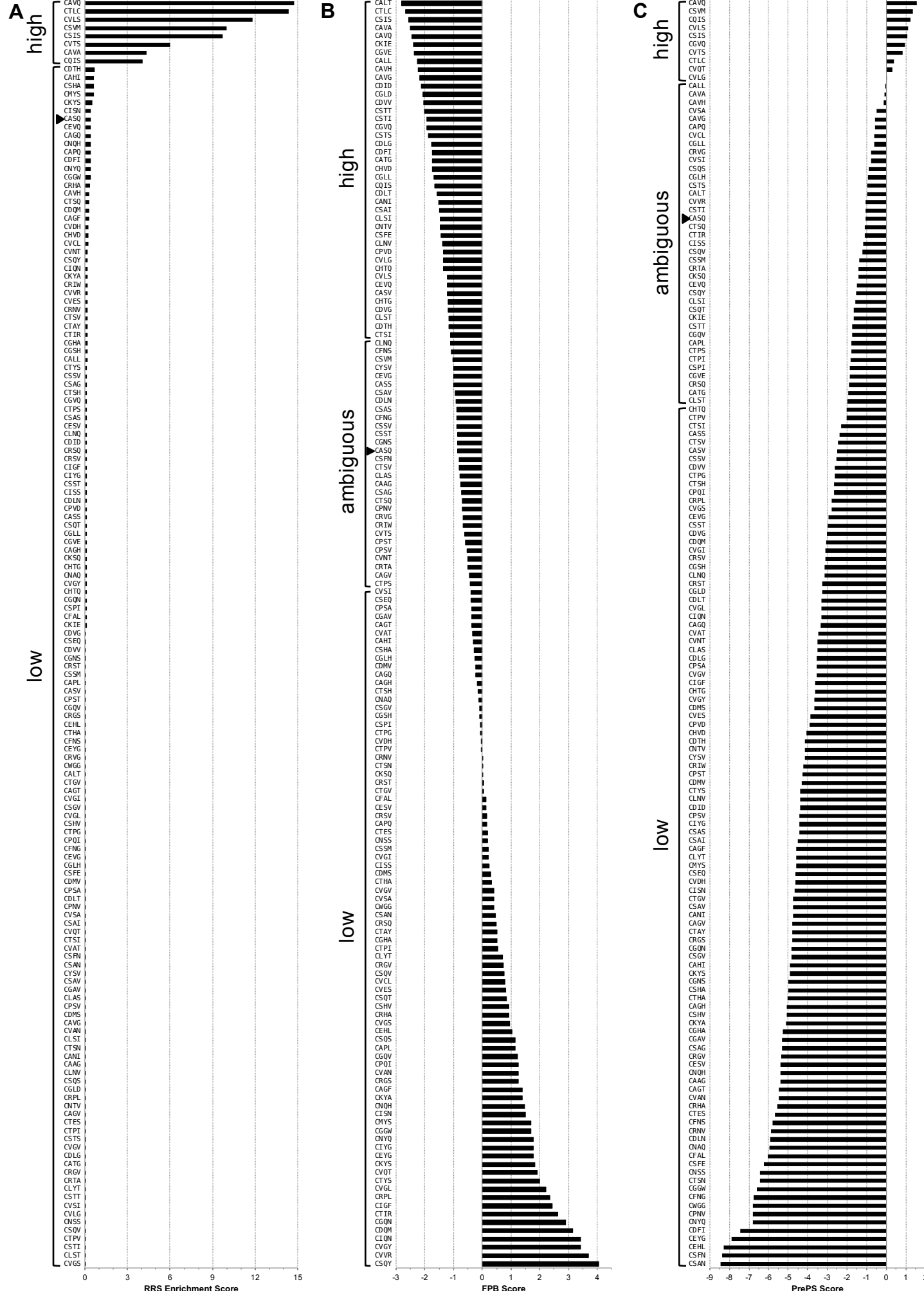


Figure S4. *The predicted prenylation potential of individual Ydj1p-based hits.*

A) Hits were matched to a score derived from a Ras-based enrichment strategy for prenylatable sequences and graphed based on score (Stein *et al.* 2015). Hits are binned into 2 categories: high enrichment in screen (i.e. score greater than 3), and low enrichment (i.e. score less than 1); intermediate scores were not observed (i.e. score 1-3). **B)** Hits were matched to a score derived using a prenylation prediction algorithm (London *et al.* 2011). Hits are binned into 3 probability categories: high (i.e. scores less than -1.1); ambiguous (i.e. scores -1.1 to -0.4); low (i.e. scores greater than -0.4). **C)** Hits were matched to a score derived using the PrePS server (London *et al.* 2011). Hits are binned into 3 probability categories: high (i.e. scores more than 0); ambiguous (i.e. scores between 0 and -2); low (i.e. scores less than -2). For all graphs, scores were plotted such that sequences with the highest prenylation probability are at the top of each graph; the relative position of CASQ is noted (triangle).