Supplementary Tables for

**Conserved Sequence Preferences Contribute to Substrate Recognition by the Proteasome**

Houqing Yu1,2, Amit K. Singh Gautam1, Shameika R. Wilmington1,2, Dennis Wylie3, Kirby Martinez-Fonts1,2, Grace Kago1, Marie Warburton1, Sreenivas Chavali4, Tomonao Inobe5, Ilya J. Finkelstein1, M. Madan Babu4, and Andreas Matouschek1,2

1Department of Molecular Biosciences, The University of Texas at Austin, Austin, TX 78712

2Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208

3Center for Computational Biology and Bioinformatics, The University of Texas at Austin, Austin, TX 78712

4Medical Research Council Laboratory of Molecular Biology, Cambridge, UK.

5Frontier Research Core for Life Sciences, University of Toyama, 3190 Gofuku, Toyama-shi, Toyama 930-8555, Japan

To whom correspondence should be addressed: Andreas Matouschek, Department of Molecular Biosciences, The University of Texas at Austin, 2506 Speedway Stop A5000, Austin, TX 78712, Tel.: (512) 232-4045; Fax: (512) 471-1218; Email: matouschek@austin.utexas.edu

**Table S1.** Initiation region sequences.

|  |  |  |
| --- | --- | --- |
| name | tail | amino acid sequences |
| A | 35 | LRYQPLLRISQNCEAAILRASQTRLNTIHHHHHH |
| B | ODC | FPPEVEEQDDGTLPMSCAQESGMDRHPAACASARINV |
| C | polyG | GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG |
| D | GRR | EIKDKEEVQRKRQKLMPNFSDSFGGGSGAGAGGGGMFGSGGGGGGTGSTGPGYSFPH |
| E | SRR | RSSSTSSDSGSSSSSSASSSSGSSSTSSDSGSSSSSSASSSSGSSSTSSDSGSSSSSSASSSSGSSSTSSDSGSSSSSSASSSSGSGTMKHGT |
| F | NB | RSKRIKCPDCEPFCNKRGSKRIKCPDCEPFCNKRGSKRIKCPDCEPFCNKRGS |
| G | NS | RSLIEEVRHRLKTTENSGSLIEEVRHRLKTTENSGSLIEEVRHRLKTTENSGS |
| H | SP1 | RSSLLTEVETPGSSLLTEVETPGSSLLTEVETPGSSLLTEVETPGSSLLTEVETP |
| I | SP2 | RSPESMREEYRKEGSPESMREEYRKEGSPESMREEYRKEGSPESMREEYRKEGSPESMREEYRKE |
| J | SPmix | RSPESMREEYRKEGSSLLTEVETPGSPESMREEYRKEGSSLLTEVETPGSPESMREEYRKE |
| K | SNS | RSPESMREEYRKEGSKRIKCPDCEPFCNKRGSPESMREEYRKE |
| L | NBS | RSKRIKCPDCEPFCNKRGSKRIKCPDCEPFCNKRGSPESMREEYRKE |
| M | DRR | RSDEDDDEDDDDDDDDEDDDDEGSDEDDDEDDDDDDDDEDDDDEGSGTMKHEYSIVSPK |
| N | eRR | GGGGAWLLPVSLVRRRTTLAPNTQTASPRALADS |
| O | PEST | GGSGGTLQMLPESEDEESYDTESEFTEFTEDELPYDDCVFGGQRLTL |
| P | Su9 | MASTRVLASRLASQMAASAKVARPAVRVAQVSKRTIQTGSPLQRAYSS |
| Q | no tail | N/A |

**Table S2.** Additional initiation region sequences analyzed in Figure 8.

|  |  |  |
| --- | --- | --- |
| **No** | **SEQUENCE** | **YFP/RFP** |
| 1 | RIPYRRYPRRRGPPRNYQQNYQNSESGEKNEGSESAPEGQAQQRRPYRRRRFPARVN | 0.27±0.09 |
| 2 | RIPKTVHGAEFYANKKHKGLPLNDANSRLQQNNSRHNLQEHNIDSSPCSEDSHARVN | 1.2±0.3 |
| 3 | RIPEKPYICKAPGCTKRYTDPSSLRKHVKTVHGAEFYANKKHKGLPLNDANSRARVN | 0.3±0.1 |
| 4 | RIPKNEGSESAPEGQAQQRRPYRRRRFPPYYMRRPYGRRPQYSNPPVQGEVMEARVN | 0.17±0.01 |
| 5 | RIPKSNAGMKHGTMDTESKKDPEGCDKSDDKNTVNLFGKVIETTEQDQEPSEAARVN | 5.5±0.8 |
| 6 | RIPTVGNGEVTLTYATGTKEESAGVQDNLFLEKAMQLAKRHANALFDYAVTGDARVN | 4.5±0.5 |
| 7 | RIPLGKMLGTSSPSIKSESDISSSNHHLVNGVRASDSLLTYSPDDLAENLNLDARVN | 0.6±0.1 |
| 8 | RIPDSTISTATTALALQARRNPAGTKWMEHVKLERLKQVNGMFPRLNPILPPKARVN | 0.14±0.01 |
| 9 | RIPAKRIVGSDSSPSNNNAGLHSQNSSLNSYTNMMRNINDEQLITEVIKSFKRARVN | 0.16±0.04 |
| 10 | RIPFRRGPPRQRQPREDGNEEDKENQGDETQGQQPPQRRYRRNFNYRRRRPENARVN | 0.6±0.2 |
| 11 | RIPDDKNTVNLFGKVIETTEQDQEPSEATVGNGEVTLTYATGTKEESAGVQDNARVN | 0.19±0.05 |
| 12 | RIPTGPGYSFPHYGFPTYGGITFHPGTTKSNAGMKHGTMDTESKKDPEGCDKSARVN | 2.6±0.9 |
| 13 | RIPPYYMRRPYGRRPQYSNPPVQGEVMEGADNQGAGEQGRPVRQNMYRGYRPRARVN | 0.21±0.02 |
| 14 | RIPQQDLSNTTSKREECLQVKTVKAEKPMTSQPSPGGQSSCSSQQSPISNYSNARVN | 1.0±0.3 |
| 15 | RIPPPPRDSGSHSQSRSPGRPTQGALGEQQDLSNTTSKREECLQVKTVKAEKPARVN | 0.7±0.2 |
| 16 | RIPKSTGPSPNQHSMNLNTSVLYSDEVLIQKVIKSLNINSNISICDSLGRTLLARVN | 0.16±0.02 |
| 17 | RIPSFPYGQPQYGVAGQYPYQLPKNNYNYYQTQNGQEQQSPNQGVAQHSEDSQARVN | 1.2±0.5 |
| 18 | RIPIQKVIKSLNINSNISICDSLGRTLLHLACLKNYSSLVYTLIKKGARVNDIARVN | 0.11±0.03 |
| 19 | RIPDSFGLTPLHFACISGDPKIIKMLLNCKVNYSLRSHNGLTAREVFIANHIHARVN | 0.13±0.01 |
| 20 | RIPHLACLKNYSSLVYTLIKKGARVNDIDSFGLTPLHFACISGDPKIIKMLLNARVN | 0.15±0.01 |
| 21 | RIPAEAANVTGPGGVPVQGSKYAADRNHYRRYPRRRGPPRNYQQNYQNSESGEARVN | 0.9±0.2 |
| 22 | RIPNGNASASTIGGSVLARQRFRGRLQTKGINSSTIMLCNIPESNRTFGISELARVN | 0.16±0.03 |
| 23 | RIPDGWNCDDDVDVADLPIVLRAMVNIGNGNASASTIGGSVLARQRFRGRLQTARVN | 0.19±0.02 |
| 24 | RIPNSYTNMMRNINDEQLITEVIKSFKRNNNLSTVNLSMCDVRGRTLLHLAAFARVN | 0.11±0.03 |
| 25 | RIPDARNIAKRIVGNDSPDSGTNGNSCSKSTGPSPNQHSMNLNTSVLYSDEVLARVN | 0.33±0.03 |
| 26 | RIPGADNQGAGEQGRPVRQNMYRGYRPRFRRGPPRQRQPREDGNEEDKENQGDARVN | 4.57±0.01 |
| 27 | RIPMTSQPSPGGQSSCSSQQSPISNYSNSGLELPLTDGGSIGDLSAIDETPIMARVN | 0.4±0.1 |
| 28 | RIPQSQQPQSQQSPQSQKQGNNVAAQQYYMYQNQFPGYSYPGMFDSQGYAYGQARVN | 0.21±0.07 |
| 29 | RIPNNENNNDDLPQEIEINDNKKAIFTYVDDTDRQLIELALQIVGLKMNGKLEARVN | 0.26±0.09 |
| 30 | RIPLQQNNSRHNLQEHNIDSSPCSEDSHLGKMLGTSSPSIKSESDISSSNHHLARVN | 0.5±0.2 |
| 31 | RIPQHQLQQQYLSQQQQYAQQQQQHPQPQSQQPQSQQSPQSQKQGNNVAAQQYARVN | 1.8±0.6 |
| 32 | RIPNGWQIISSSSGATPTSKEQSGSSTNGSNGSESSKNRTVSGGQYVVAAAPNARVN | 2.1±0.3 |
| 33 | RIPYMYQNQFPGYSYPGMFDSQGYAYGQQYQQLAQNNAQTSGNANQYNFQQGYARVN | 0.14±0.04 |
| 34 | RIPASTISSAYLSSRRSSGISPCFSSRRSSEASQAEGRPQNVSVADSYDPISTARVN | 0.33±0.01 |
| 35 | RIPAPAVSPLIGNGTQSNNTCSLGGPMTLLPGRSDLSGVDVTMLNMLNRRDSSARVN | 0.3±0.2 |
| 36 | RIPSGLELPLTDGGSIGDLSAIDETPIMDSTISTATTALALQARRNPAGTKWMARVN | 0.26±0.07 |
| 37 | RIPYYQTQNGQEQQSPNQGVAQHSEDSQQKQSQQQQQQQPQGQPQPEVQMQNGARVN | 4.28±0.01 |
| 38 | RIPVNGVRASDSLLTYSPDDLAENLNLDDGWNCDDDVDVADLPIVLRAMVNIGARVN | 0.22±0.06 |
| 39 | RIPNGGGAFSQARSSSTGSSSSTGGGGQESQPSPLALLAATCSRIESPNENSNARVN | 0.14±0.01 |
| 40 | RIPLPPVLVPRHSEYNPQHSLLAQFRNLGQNEPHMPLNATFPDSFQQPHSHPFARVN | 0.15±0.05 |
| 41 | RIPQYQQLAQNNAQTSGNANQYNFQQGYGQAGANTAAANLTSAAAAAAASPATARVN | 0.27±0.07 |
| 42 | RIPGQAGANTAAANLTSAAAAAAASPATAHAQPQQQQPYGGSFMPYYAHFYQQARVN | 0.28±0.09 |
| 43 | RIPGQNEPHMPLNATFPDSFQQPHSHPFPQSPNSSYPNSPGSSSSTYPHSPTSARVN | 0.7±0.2 |
| 44 | RIPNSQGPSQSGGTGELDLTATQLSQGANGWQIISSSSGATPTSKEQSGSSTNARVN | 4.89±0.01 |
| 45 | RIPPQSPNSSYPNSPGSSSSTYPHSPTSSDPGSPFQMPADTPPPAYLPPEDPMARVN | 0.9±0.7 |
| 46 | RIPSDPGSPFQMPADTPPPAYLPPEDPMAQDGSQPMDTNMTNMTAPTLPAEINARVN | 0.5±0.2 |
| 47 | NSGSHNFTTQQRKR | 3.1±0.2 |
| 48 | NSGSEDKKAGDEMKELREEIERLKLELSHKKDQETPNEDFKNELGG | 5.3±0.4 |
| 49 | NSGSQKVPSATNSKTTKSKANINNKSKKRGTNLVNKNSNSTPRQKKSQRYVSNLQRS | 2.7±0.2 |
| 50 | NSGSCSFENSNSTSIPSPASSSQSHTPMRNMSSLSDNSVFSRNMEQSSPITPSMGGETLMGG | 0.85±0.03 |
| 51 | NSNTEADSS | 4.9±0.4 |
| 52 | NSGSHKIRRMLSKELQDHRKVMNDVANKDVGEPSSEKLELNAEYTGKQFEHGG | 2.6±0.4 |
| 53 | NSGSTIQMRYGHSLPEARALNIEDCDLDRATNDFYLEPLIERDLLAHYDHQIVMDVRMVNLGG | 3±2 |
| 54 | NSGSKQLAHEEHINNDGDNDDENSNNIESSPLKQGHHHPKGQADDNNEGPDEEESTKEVPKPGGG | 2.3±0.9 |
| 55 | NSGSLRKRRQQQLSSNSTDNSLHPNSGQTPRASDSQRRHKKRNTTNLDRLRQEREENSLEMDCTQSGG | 1.0±0.6 |
| 56 | NSGSANVDSQSNNKHDGKDDDATNNNDGQDNNTNNDHNNNSNINNNNVGSHGISSHSPSSIRDTGG | 2.6±0.4 |
| 57 | NSGSLLSRRHGSATAKQRA | 2.7±0.4 |
| 58 | NSGSSQSINSANNIQSSDSDLVQHFESLAQEIRHHKKYKQNNSKQRKILKKIQDLKQTPPEATLGG | 0.26±0.02 |
| 59 | NSGSSPEQQVISEENDAKKLEQSALNSEASEDSEAMDEESKALKAAAEKADAPIDTNKMD | 1.9±0.9 |
| 60 | NSGSAKHQEISSAGTSSNTTKNVNNNKNDSNDDNNGNNNNDASNLMESVLDKTSSHSPSSIRDT | 2.0±0.2 |
| 61 | NSGSEQNKTT | 2.73±0.09 |
| 62 | NSGSEGIVTRLNETFNPEIQALPPLREIISGTSETHSSNNPFEIHSSNIDSELRNRFDYSEEEMDEDDDVDVFAGG | 2.8±0.1 |
| 63 | NSGSTSSLNEITPSKNRVTSACNSERRTTSQEANNLEGYHSCAQE | 3.0±0.2 |
| 64 | NSGSNTTSENIE | 3.0±0.1 |
| 65 | NSGSMKEAQKQLEEVQEQLTEYESQNLKLKKKLEATKTENSELQSTIVTLNTELENLKKNKKAQKKYS | 2.6±0.2 |
| 66 | NSGSLTVEIKEETSKD | 4.2±0.2 |
| 67 | NSGSCEGPSHGGLPGG  | 3.2±0.1 |
| 68 | NSGSFRNPDSAFSANAKRGSKLVALRRINMEHIQQSRDNKQY  | 0.82±0.05 |
| 69 | NSGSQQILSRQQSLGSAFGHSPPLIHPAPTFPTQRPIPGIPTVLGG | 0.17±0.02 |
| 70 | NSGSADQKTTGKDIGGAAVSSMSGCPVMHESSSSSPPSSECP  | 2.8±0.1 |
| 71 | NSGSHGSLPHVAEPSVPYRGTVFAMDPRNGYMEPHYHPPHLFPAGG  | 0.35±0.07 |
| 72 | NSGSRVTSMTQLNHHGRSPTSSPGNESSALLLTHSWVNKTSLHSVEADSS  | 0.93±0.09 |
| 73 | NSGSNRFHRGKKSVERIRKFQNRQKNAKIKASDDAISKKSTSVNVSDGKIKRRDKKVSAGRTTVVVENTKGG | 1.4±0.1 |
| 74 | NSGSSHDDVANGIVPKHVVNVQNPPKQEVFEKIPSPEFNSNNEKELVQRKGSANEKLHQELGEKQPASGG  | 4.2±0.2 |
| 75 | NSGSHLDLVKERPRFERSLEDLLKENATLAIELTKEITVSKRSSGEEKNDSETKGTHVERRRVPFLRDLLVKKRTTLKPRVPTLKRRRVPFLRDLLVKKRTTLKPRVPTLKRRRVPFLSLQSLPERLPEADSS | 0.22±0.02 |
| 76 | NSGSSWKDVQQEQEI  | 4.2±0.2 |
| 77 | NSGSTDGTGDDHDGAPLSSSPSFGQQNDNSNWTCLKQGCF  | 1.8±0.2 |
| 78 | NSGSERAKANLRAI  | 1.9±0.3 |
| 79 | NSGSYHLQNDYSDAKTIVDTETEENLSTPLNLSTLNWKPQSWVLVCLTWVTTKPTNCLSTKLSTKRDLTLEPEELRLKKKTSVTL | 0.18±0.01 |
| 80 | NSGSTEDITVQEPVPLPEDAPEDAEPQFKEVTKTIKKDVLGMTAKTFALNPVELNDLIEKENELRNQDKLVAGG | 0.48±0.04 |
| 81 | NSGSTPWKVYHRNLERKVGPIEERS | 1.4±0.1 |
| 82 | NSGSMKRHVSSSFNNKVPLIKASSSNNSATSSPSIANSQLKSLENGG | 3.3±0.3 |
| 83 | NFNSHNVYITADKQKNGIKANFKIRHNVEDGGVQLADHYQQNTPIGDGPVLLPDNHYLSYQSKLSKDPNEKRDHMVLLEFVTAAGITHGMDELYRIRRLELINVT | 2.6±0.2 |
| 84 | NSGSLPPTMGKKTLLSLLPLLKTD | 0.5±0.1 |
| 85 | NSGSQYGLEAARSGGPSFGPGGPGGAGGAGGFPGGAGGFSGGHAFSNEDAFNIFSQFFGGSSPFGGPDDSVSLSLLTHLVVVLVWVVCQEADSS | 0.7±0.1 |
| 86 | NSGSPMEQIQKKQQELRQARQRMFRHER  | 1.6±0.2 |
| 87 | NSGSMRCLAPRPAGFYLFEPQGFSQCATELGPLEGGYLELLNSDADPLCLYHFYDQMDLAGEEEIELYS EPDTDTINCDQFSRLLCDMEGDEETREAYANIAEGG | 1.0±0.1 |
| 88 | NSGSEFISLSPPHEALDYHFGLEEGEGIRDLFDCDFGDLTHWTSEADSS | 4.6±0.2 |
| 89 | NSGSELFQDLSQLQETWLAEAQVPDNDEQFVPDYQAESLVWEADSS | 2.0±0.1 |
| 90 | NSGSFVFTYPEADSFPPGGAGGAGVSQVVLVVSLVVTLSLTKTLSTSSLNSSVVLLHSVVLTTLVSLSLLTHLVVVLVWVVCQEADSS | 0.91±0.08 |
| 91 | NSGSMEVAPEQPRWMAHPAVLNAQHPDSHHPGLAHNYMEPAQLLPPDEVDVFFNHLDSQGNPYYANPAHARARVGG | 0.19±0.02 |
| 92 | NSGSSSPSMRPDVSSPPSSSSTATTGPPPKLCLVCSDEASGCHYGG  | 1.5±0.2 |
| 93 | NSGSEFKLELVEKLFAEDTEAKNPFSTQDTDLDLEMLAPYIPMDDDFQLRSFDQLSPLESSSASPESASPQSTVGG  | 1.0±0.1 |
| 94 | NSGSDIGLSLQRVFTDLKNMDATWLDSLLTPVRLPSIQAIPCAPGG  | 0.14±0.01 |
| 95 | NSGSLPQQATYFPPSPPSSEPGSPDRQAEMLQNLTPPPSYAATIASKLAIHNPNLPTTLPGG  | 0.34±0.04 |
| 96 | NSGSMDFFRVVENQQPPATMPLNVSFTNRNYDLDYDSVQPYFYCDEEENFYQQQQQSELQPPAPSEDIWKKFELLPTPPLSPSRRSGLCSPSYVAVTPFSLRGDNDGGGGSFSTADQLEMVTELLGGDMVNQGG | 0.37±0.06 |
| 97 | NSGSIESLQELLREQVENYYSLPGQSCSEPTSPTSNCSDGMPECGG | 3.4±0.4 |
| 98 | NSGSMEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLGG  | 0.81±0.05 |
| 99 | NSGSDVSKLGEEEDEISPLSHDNFQYESEENGNPSPPIYKKSGELVKSSLKRRSKSLPITPKSIFNKTGSKGG | 0.73±0.02 |

**Table S3.** Yeast Strains.

|  |  |  |
| --- | --- | --- |
| **strain** | **genotype** | **source** |
| BY4741 | *MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* | (1) |
| *pdr5∆* | BY4741 with *pdr5::kanMX4* | (2) |
| MAHQ1 | *pdr5∆* with *uba1-204*  | this study |
| YYS40 | *MAT***a** *RPN11-3* × *FLAG-HIS3 ade2 can1 his3 leu2 trp1 ura3 ssd1* | (3) |
| *S. pombe* | *h+ his+ ade6-M216 leu1-32 ura4-D18 arg+* | (4) |

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