Supplementary Information:

Structural elucidation of recombinant *Trichomonas vaginalis* 20S proteasome bound to covalent inhibitors

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a

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                1         10        20        30        40        50        60        70
Tv_alfa_1  ...MSSGADRYLTVFSAEGRLWQVEYSFKAVKQAEVTAVAVKSKNAVCVAVQKKVSDKLIDPSTVTHMYRITDNVGACLV
Tv_alfa_2  ....MGDSDFSLTTFSSGGKLNQIESALKAVSLG.GQCVGVKAKNGAVIACESKPSSPLVEKVTNLKVQKINDNVGIVYS
Tv_alfa_3  ...MTYRYDAGTTTFSSDGRILQVEYAIQSINQA.GTAIGVQFTNGVVLAAEKKNTGRLVDYLFPEKMAKIDGHIVTAVA
Tv_alfa_4  ....MSDYTRSITRFSPDGRLFQIDHAHAAVQRG.TTVVATRSKDMIVIAVEKTAVAKLQDPHTFSKICSLDKHVMCAFA
Tv_alfa_5  MFNSGSEYDRNVNTFSPDGRLLQVEYAIEAVKLG.SSAVAILCPEGVIFAVEKRLSSQLLIASSVEKVYAIDDHVGVVMA
Tv_alfa_6  ..MFRSKYDENATTFSPEGRILQVENAMKAVQQG.MPTVGLKSKTHAVIA..GVMHSPSEFSSHQPKIFKIDQHIGVAIS
Tv_alfa_7  MSGAGSGYDFNPITFSPDGRQFQVEYATKAVEKD.SLALGVKCKDGILLAAEKNLTSTLLTPGGNPRIFWINDSIACATI

                80        90        100       110       120       130       140       150
Tv_alfa_1  GLPSDVNFIVMLLRSFANNFEYKQGFSIPVSILAQMLSE.......RHQLESQLVYVRPSAVSAILFGLDGPSDSFALYK
Tv_alfa_2  GVNTDFHVILKSLRKASIKYSLRLGVEMPTREVVKHA........AHKMQYYTQIGGVRPFGVSLLIIGWEEL..GPTLWQ
Tv_alfa_3  GLTADANTLVDLMRTSAQKYLKTYDEQMPVEQLVRMVCDE.......KHSYTQYGGLRPYGVSFLIAGYDRHK.GCQLYL
Tv_alfa_4  GLHADARRLIQSGQRQCQSHRLTYEDPISIENIARYIATL.......QLKNTQSGGARPYGVSTLICGFDDMTSQPHIYE
Tv_alfa_5  GLAADGRTMVEHMRVEAQNHRFSFDEPIGIKAVTQSVCDLALAFGEGRRKKGDGQMSRPFGTALLVAGIENG..KCHLFH
Tv_alfa_6  GLTADGRGLCKFLRNECLHHTFCFGTEIRVADLADTV.......ALQSQKKTSKVGKRPYGVGLLMIGAGVD..GPRLFE
Tv_alfa_7  GHRPDCYSIVEQSRNRAETFTSNFGIKITVPQLASEVSQ.......QFHLAHYYQAYRPFGCTVIFASYKDD....ALYA

                160       170       180       190       200       210       220
Tv_alfa_1  IEPSGYSNGFRAVACGVKEIEAMSALEKKMEDFETPEATAEFTLSTLQTVCGVDFEAQDVEVSLLTR....DNSKFSKLP
Tv_alfa_2  VDPSCTFWAWKATALGKRSDGSRTFLERRYSEDQSVDDAIHTAISTLKEGFDGQL....TAELIEIG.VVDETRKFRTLS
Tv_alfa_3  TDPSCNFGGWKATAIGENNQTAQSILKSQYKDNMTATEAMDLTVKVLCKTLDSTSLSADKLEFAVLQFREEYGPKVRILT
Tv_alfa_4  TLPSCTYAEWKARTIGRHDQTVMEYLEKHYKDDMTDEEAQKLAIGALLEVVENGSKN...LEVAYMK....RGGTMEIMA
Tv_alfa_5  TDPSCTYTECRARAIGGSEGAEALLRDLYKDGMTLHEAEDLALSTLRQVIQEKLNENNVEVACARV.S...TGKFEIYT
Tv_alfa_6  TCPSCQHWEYNAQAIGRRAQAAKTYLETNLNEFPDC.TRDQLIRHALRALNDCKSRESDSLEAIALG.VVGIDEPFTILE
Tv_alfa_7  IEPSCAFYGYFASCFGKNSNLARAELQKTEWKNITVREAVPEVARIIKSLHESQFKKWEIEMF.WLC.EETNGRPQKVPE

                230       240
Tv_alfa_1  NDKVNEILHAVAEKD........
Tv_alfa_2  TAEIRDFLTEV............
Tv_alfa_3  TSEVDTLMKRYEETIKKSAEEKE
Tv_alfa_4  EEVLDALIESTKAK.........
Tv_alfa_5  SEQRQEIVARLPPPIIPE.....
Tv_alfa_6  GPELQKYID..............
Tv_alfa_7  DVFQSRFVNENPQN.........
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b

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                                                                1         10        20        30
Tv_beta_1  ..........................................MSEYQFP.KESMGTTLLAIQCTDGVVMASDSRTSSGSFI
Tv_beta_2  ....................MEAGLGFDFSNYARNK...SLEPKLGKPQLLSTGTTIAAAIFDGGVVLGADTRATAGPIV
Tv_beta_3  ..........................................MSD....ISTYNGSCVLAMAGDHCVAIASDRRLGVNMLT
Tv_beta_4  ..................................................MLSIVGLQGPDWVLIAADSSVSSSIIC
Tv_beta_5  MQSLYLKPRDEVESEETTKALEPAHVPDPCQFVKNHISLSYTNEPGKM.AAVHGTTTLSFIYNGGIVVAVDSRATGGQFI
Tv_beta_6  ....................ME.......GEFRENK.......KGQWSPYEMHGGTAIGICGDDYVVIGADTRLSVDYSI
Tv_beta_7  ..........................................MQVITASGAIVAAKYDGGILLASDLSITYGSMF

                40        50        60        70        80        90        100       110
Tv_beta_1  PNRATNKITEIQPKIFAARCGNAADTQFLARAVKNYLNALNITRENTDDSTILVASNVIRSLI...VRYRQY.LSAGVIV
Tv_beta_2  AVKDEMKLHYISDNIWVCGAGIAADNDNINAVISAKLRLFQMNTG..LQPRVDQCTNILASRL...FQYMGY.IQAALIV
Tv_beta_3  VSKDFKRIFQINDRIYLGLAGLATDVLTVREQLRFDVNLLELREE..RPIDPKKFMNLVKSTLY.EKRFSPF..FVTPVI
Tv_beta_4  MSENYDRIAQLDDRHALAMSGETGDCLQLSEYLQGNVALYKFRNGVE..LSSDALAHFIRHTMAKAVRKSP..YEVNMLL
Tv_beta_5  FSQTVMKILPLAPNMIGTMAGGAADCQYWLRNLSRLIQLHKFRYQ..QPLTVAAASKILVNEL...YRYKGYNLSIGSMI
Tv_beta_6  DSRHKARIFKMNSNCMISATGFDGDIDAFITRMRSILL..NYENQHFHEMSVESVARCVSNTLY.SKRFFPY..YINILV
Tv_beta_7  RHNNVSHFVEVAPNIIIGASGEFADFQTLIEVIKSVILQQQCKHN.GEYLTASEVHNYIKRYMYQCRSNMK.PLSCKVIV

                120       130       140       150                 160       170       180
Tv_beta_1  GGWDS..AGPQVYSIEVSGMA.IKKKIASNGSGSTYIQAYIDQNYR.........EDMTMEEATKFAIAAVTGAIIRDGS
Tv_beta_2  GGIDF..QGPQVYQVAPHGSF.SKQPFIAQGSGSLAAISVLENRWH.........NKMNEHDCMEMVADAIYAGITNDLG
Tv_beta_3  AGLLPETNEPYLAASDSIGAFAFPKDFAVAGTCEESLYGICESAWR.........PNMNPDELFECTAKCLIAAVERDSI
Tv_beta_4  SGYDG...KPHLYFMDYLGTL.QSIPYGAQGYCQYFVMSVFDKHYK.........EGLTLEDGKELMKLALNQIKQRFTV
Tv_beta_5  CGYDN..TGPHIFYIDNHGSR.IAGKRFSVGSGSTHAYGVLDTCYR.........EDMTKEEACELGRRAIYHATYRDSG
Tv_beta_6  GGINSEG.KGKLYGYDPVGTI.EDLHYDSNGSGSSLAAPLLDSAFGTIHHNTRPFPAVSLQDAKNIVRDAICSVTERDIY
Tv_beta_7  AGINPDG.SKFLACTDPYGAS.WESDHIGTGFGKYLQGLQIADV..........VNGSFDDVKKGITEVFRAVNARNTT

                190       200       210
Tv_beta_1  SGGVVNIVQINADGAKRMTVRPAQQPFN.YDIVKG.....................................................
Tv_beta_2  SGSHVNLCVIKRENPEDKQSKVIYTFYKDYRVPHENDRNFRLEPQINNIDVEVIKTTERPLTLPDVHLEILDDAPA
Tv_beta_3  SGWGGIVYIITQDKVIIKEIKTRMD.....................................................
Tv_beta_4  APHGFIVKLVDKNGITKIDLE.........................................................
Tv_beta_5  SGGRVSVVHITQNGVEWIDKTDVFDMHD.FSKTTF.........................................
Tv_beta_6  TGDALQLCVFTKDGFAQEEFPLPRH.....................................................
Tv_beta_7  ANGKIEFITVTPQGINHLAPEQID...PNWEVVEGTWDQ.....................................
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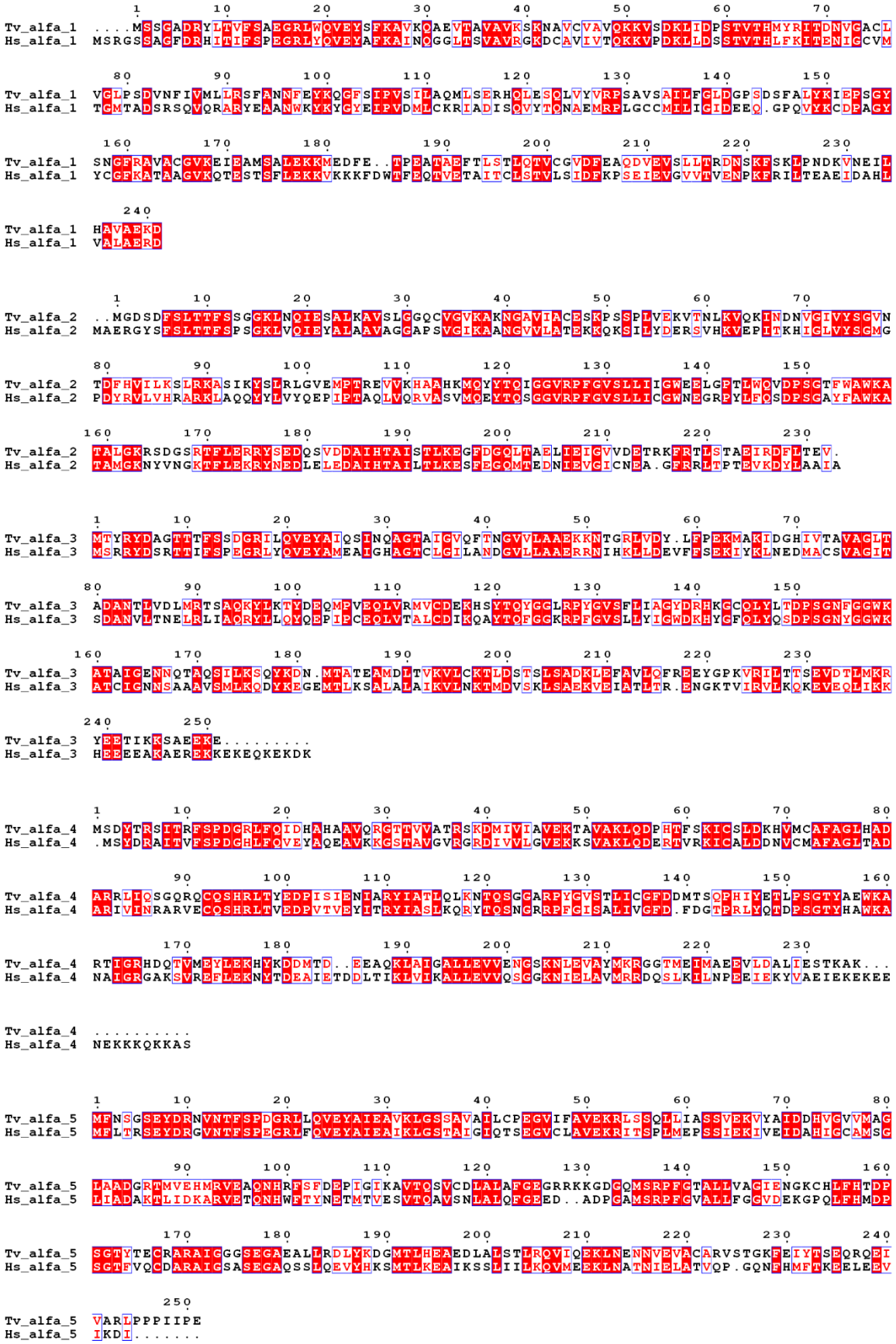
c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Hs_alfa_1 MSRGSSAGFDRHITIFSPEGRLYQVEYAFKAINQGGLTSVAVRGKDCAVIVTQKKVPDKLLDSSTVTHLFKITENIGCVM
Tv_alfa_1 VGLPSDVNFIVMLLRSFANNFEYKQGFSIPVSILAQMLSERHQLESQLVYVRPSAVSAILFGLDGPSDSFALYKIEPSGY
Hs_alfa_1 TGMTADSRSQVQRARYEAANWKYKYGYEIPVDMLCKRIADISQVYTQNAEMRPLGCCMILIGIDEEQ.GPQVYKCDPAGY
Tv_alfa_1 SNGFRAVACGVKEIEAMSALEKKMEDFE..TPEATAEFTLSTLQTVCGVDFEAQDVEVSLLTRDNSKESKLPNDKVNEIL
Hs_alfa_1 YCGFKATAAGVKQTESTSFLEKKVKKKFDWTFEQTVETAITCLSTVLSIDFKPSEIEVGVVTVENPKERILTEAEIDAHL
Tv_alfa_1 HAVAEKD
Hs_alfa_1 VALAERD
Tv_alfa_2 ..MGDSDFSLTTFSSGGKLNQIESALKAVSLCGQCVGVKAKNGAVIACESKPSSPLVEKVTNLKVQKTNDNVGIVYSGVN
Hs_alfa_2 MAERGYSFSLTTFSPSGKLVQIEYALAAVAGGAPSVGIKAANGVVLATEKKQKSILYDERSVHKVEPITKHIGLVYSGMG
Tv_alfa_2 TDFHVILKSLRKASIKYSLRLGVEMPTREVVKHAAHKMQYYTQIGGVRPFGVSLLIIGWEELGPTLWQVDPSGTFWAWKA
Hs_alfa_2 PDYRVLVHRARKLAQQYYLVYQEPIPTAQLVQRVASVMQEYTQSGGVRPFGVSLLICGWNEGRPYLFQSDPSGAYFAWKA
Tv_alfa_2 TALGKRSDGSRTFLERRYSEDQSVDDAIHTAISTLKEGFDGQLTAELIEIGVVDETRKFRTLSTAEIRDFLTEV.
Hs_alfa_2 TAMGKNYVNGKTFLEKRYNEDLELEDAIHTAILTLKESFEGQMTEDNIEVGICNEA.GFRRLTPTEVKDYLAAIA
Tv_alfa_3 MTYRYDAGTTTFSSDGRILQVEYAIQSINQAGTAIGVQFTNGVVLAAEKKNTGRLVDY.LFPEKMAKIDGHIVTAVAGLT
Hs_alfa_3 MSRRYDSRTTIFSPEGRLYQVEYAMEAIGHAGTCLGILANDGVLLAAERRNIHKLLDEVFFSEKIYKLNEDMACSVAGIT
Tv_alfa_3 ADANTLVDLMRTSAQKYIKTYDEQMPVEQLVRMVCDEKHSYTQYGGLRPYGVSFLIAGYDRHKGCQLYLTDPSGNFGGWK
Hs_alfa_3 SDANVLTNELRLIAQRYLLQYQEPIPCEQLVTALCDIKQAYTQFGGKRPFGVSLLYIGWDKHYGFQLYQSDPSGNYGGWK
Tv_alfa_3 ATAIGENNQTAQSILKSQYKDN.MTATEAMDLTVKVLCKTLDSTSLSADKLEFAVLQFREEYGPKVRILTTSEVDTLMKR
Hs_alfa_3 ATCIGNNSAAAVSMLKQDYKEGEMTLKSALALAIKVLNKTMDVSKLSAEKVEIATLTR.ENGKTVIRVLKQKEVEQLIKK
Tv_alfa_3 YEETIKKSAEEKE.........
Hs_alfa_3 HEEEEAKAERERKKEKEQKEKDK
Tv_alfa_4 MSDVTRSITRFSPDGRLFQIDHAHAAVQRGTTVVATRSKDMIVIAVEKTAVAKLQDPHTFSKICSLDKHVMCAFAGLHAD
Hs_alfa_4 .MSVDRAITVFSPDGHLFQVEYAQEAVKKGSTAVGVRGRDIVVLGVEKKSVAKLQDERTVRKICALDDNVCMAFAGLTAD
Tv_alfa_4 ARRLIQSGQRQCQSHRLTYEDPISIENIARYIATLQLKNTQSGGARPYGVSTLICGFDDMTSQPHIYETLPSGTYAEWKA
Hs_alfa_4 ARIVINRARVECQSHRLTVEDPVTVEYITRYIASLKQRYTQSNGRRPFGISALIVGFD.FDGTPRLYQTDPSGTYHAWKA
Tv_alfa_4 RTIGRHDQTVMEYLEKHYKDDMTD..EEAQKLAIGALLEVVENGSKNLEVAYMKRGGTMEIMAEEVLDALIESTKAK...
Hs_alfa_4 NAIGRGAKSVREFLEKNYTDEAIETDDLTIKLVIKALLEVVQSGGKNIELAVMRRDQSLKILNPEEIEKYVAEIEKEKEE
Tv_alfa_4 ....
Hs_alfa_4 NEKKKQKKAS
Tv_alfa_5 MFNSGSEYDRNVNTFSPDGRLLQVEYAIEAVKLGSSAVAILCPEGVIFAVEKRLSSQLLIASSVEKVYAIDDHVGVVMAG
Hs_alfa_5 MFLTRSEYDRGVNTFSPEGRLFQVEYAIEAIKLGSTAIGIQTSEGVCLAVEKRITSPLMEPSSIEKIVEIDAHIGCAMSG
Tv_alfa_5 LAADGRTMVEHMRVEAQNHRFSFDEPIGIKAVTQSVCDLALAFGEGRRKKGDGQMSRPFGTALLVAGIENGKCHLFHTDP
Hs_alfa_5 LIADAKTLIDKARVETQNHWFTYNETMTVESVTQAVSNLALQFGEED..ADPGAMSRPFGVALLFGGVDEKGPQLFHMDP
Tv_alfa_5 SGTYTECRARAIGGGSEGAEALLRDLYKDGMTLHEAEDLALSTLRQVIQEKLNENNVEVACARVSTGKFEIYTSEQRQEI
Hs_alfa_5 SGTFVQCDARAIGSASEGAQSSLQEVYHKSMTLKEAIKSSLIILKQVMEEKLNATNIELATVQP.GQNFHMFTKEELEEV
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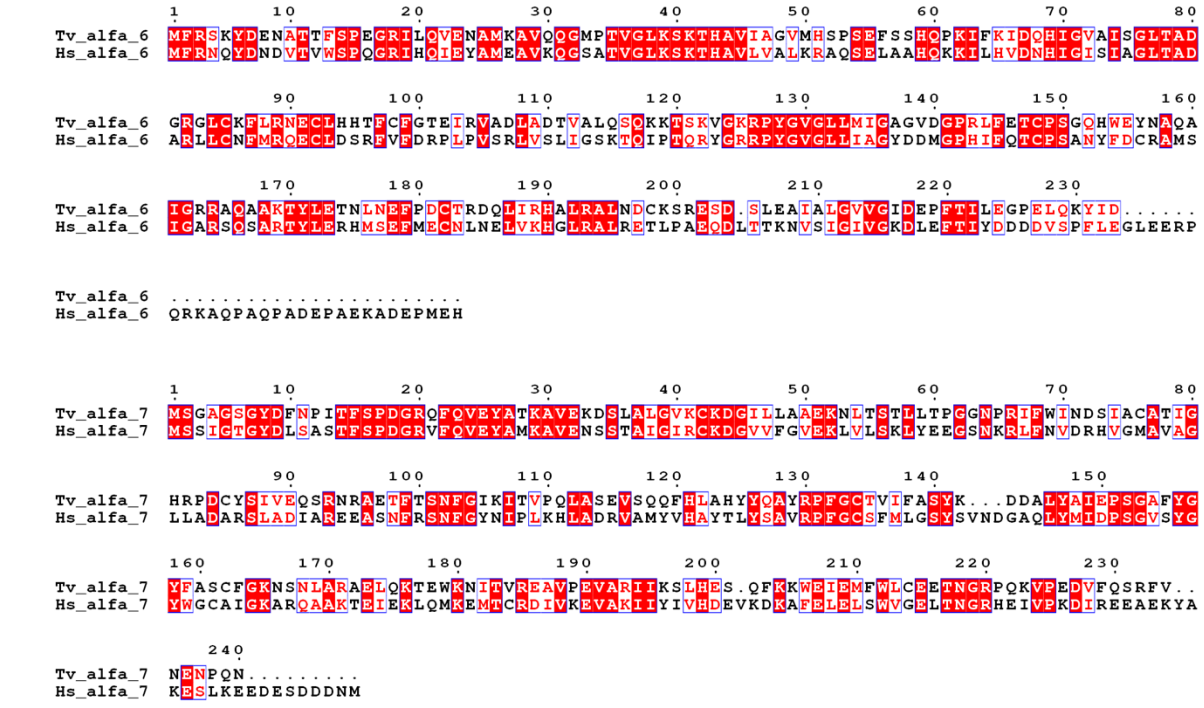
Hs_alfa_5 IKDI.......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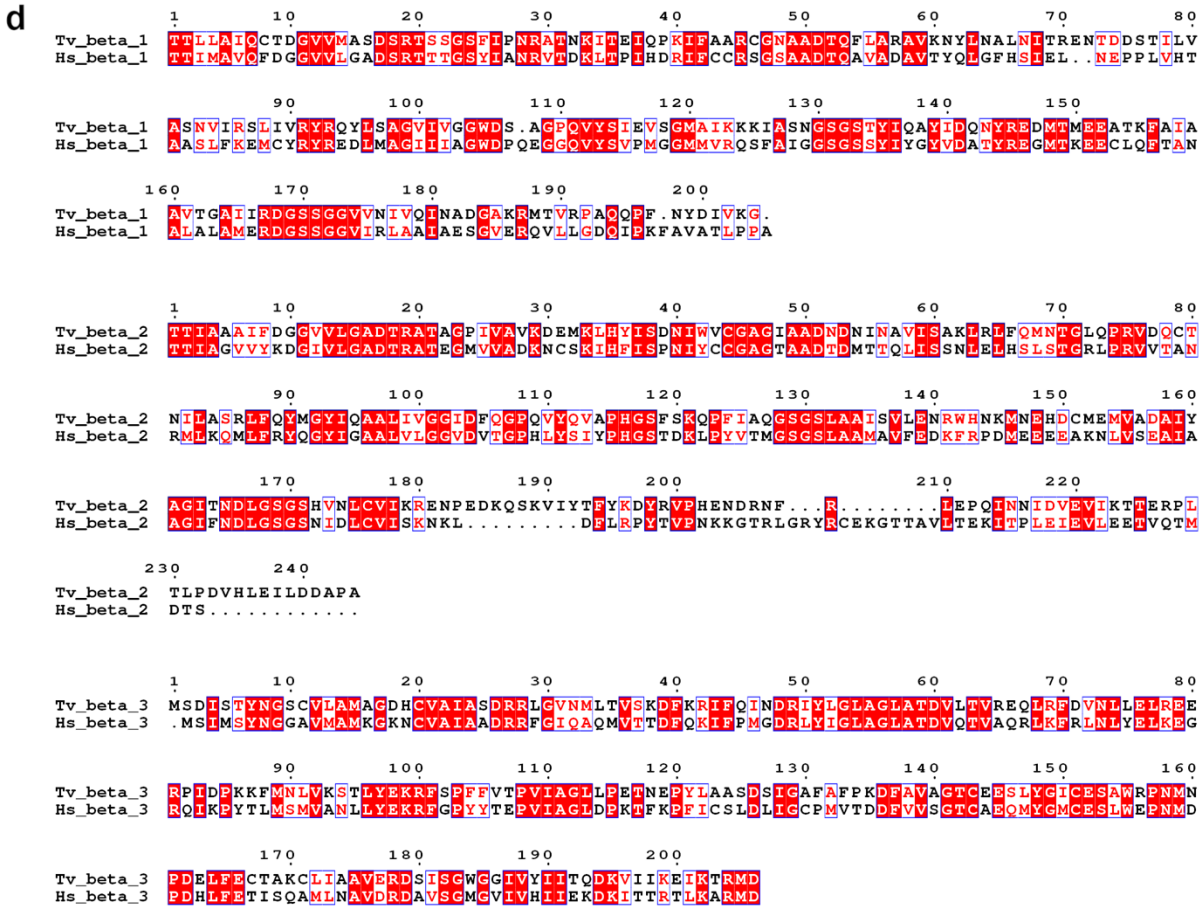
Hs_alfa_6 MFRNQYDNDVTVWSPQGRIHQIEYAMEAVKQGSATVGLKSKTHAVLVALKRAQSELAAHQKKILHVDNHIGISIAGLTAD
Tv_alfa_6 GRGLCKFLRNECLHHTPCFGTEIRVADLADTVALQSQKKTSKVGKRPYGVGLLMIGAGVDGPRLFETCPSGQHWEYNAQA
Hs_alfa_6 ARLLCNFMRQECLDSRPVFDRPLPVSRLVSLIGSKTQIPTQRYGRRPYGVGLLIAGYDDMGPHIFQTCPSANYFDCRAMS
Tv_alfa_6 IGRRAQAAKTYLETNLNEFPDCTRDQLIRHALRALNDCKSRESD.SLEAIALGVVGIDEPFTILEGPELQKYID......
Hs_alfa_6 IGARSQSARTYLERHMSEFMECNLNELVKHGLRALRETLPAEQDLTTKNVSIGIVGKDLEFTIYDDDVSPFLEGLEERP
Tv_alfa_6 ....
Hs_alfa_6 QRKAQPAQPADEPAEKADEPMEH
Tv_alfa_7 MSGAGSGYDFNPITFSPDGRQFQVEYATKAVEKDSLALGVKCKDGILLAAEKNLTSTLLTPGGNPRIFWINDSIACATIG
Hs_alfa_7 MSSIGTGYDLSASTFSPDGRVFQVEYAMKAVENSSTAIGIRCKDGVVFGVEKLVLSKLYEEGSNKRLFNVDRHVGMAVAG
Tv_alfa_7 HRPDCYSIVEQSRNRAETFTSNFGIKITVPQLASEVSQQFHLAHYQAYRPFGCTVIFASYK...DDALYAIEPSGAFYG
Hs_alfa_7 LLADARSLADIAREEASNFRSNFGYNIPLKHLADRVAMYVHAYTLYSAVRPFGCSFMLGSYSVNDGAQLYMIDPSGVSYG
Tv_alfa_7 YFASCFGKNSNLARAELQKTEWKNITVREAVPEVARIIKSLHESQFKKWEIEMFWLCEETNGRPQKVPEDVFQSRFV..
Hs_alfa_7 YWGCAIGKARQAAKTEIEKLQMKEMTCRDIVKEVAKIIYIVHDEVKDKAFELELSWVGELTNGRHEIVPKDIREEAEKYA
Tv_alfa_7 NENPQN.........
Hs_alfa_7 KESLKEEDESDDDNM

d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Tv_beta_1 ASNVIRSLIVRYRQYLSAGVIVGGWDSAGPQVYSIEVSGMAIKKKIASNGSGSTYIQAYIDQNYREDMTMEEATKFAIA
Hs_beta_1 AASLFKEMCYRYREDLMAGIIIAGWDPQEGGQVYSVPMGGMMVRQSFAIGGSGSSYIYGYVDATYREGMTKEECLQFTAN
Tv_beta_1 AVTGAIIRDGSSGGVVNIVQINADGAKRMTVRPAQQPF.NYDIVKG.
Hs_beta_1 ALALAMERDGSSGGVIRLAAIAESGVERQVLLGDQIPKFAVATLPPA
Tv_beta_2 TTIAAAIFDGGVVLGADTRATAGPIVAVKDEMKLHYISDNIWVCGAGIAADNDNINAVISAKLRLFQMNTGLQPRVDQCT
Hs_beta_2 TTIAGVVYKDGIVLGADTRATEGMVVADKNCSKIHFISPNIYCCGAGTAADTDMTTQLISSNLELHSLSTGRLPRVVTAN
Tv_beta_2 NIIASRLFQYMGYIQAALIVGGIDFQGPQVVQVAPHGSFSKQPFIAQGSGSLAAISVLENRWHNKMNEHDCMEMVADAIY
Hs_beta_2 RMLKQMLFRYQGYIGAALVLGGVDVTGPHLYSIYPHGSTDKLPYVTMGSGSLAAMAVFEDKFRPDMEEEEAKNLVSEAIA
Tv_beta_2 AGITNDLGSGSHVNLCVIKRENPEDKQSKVIYTFYKDYRVPHENDRNF...R.........LEPQINNIDVEVIKTTERPL
Hs_beta_2 AGIFNDLGSGSNIDLCVISKNKL.........DFLRPYTVENKKGTRLGRYRCEKGTTAVLTEKITPLEIEVLEETVQTM
Tv_beta_2 TLPDVHLEILDDAPA
Hs_beta_2 DTS............
Tv_beta_3 MSDISTYNGSCVLAMAGDHCVAIASDRRLGVNMLTVSKDFKRIFQINDRIYLGLAGLATDVLTVREQLRFDVNLLELREE
Hs_beta_3 .MSIMSYNGGAVMAMKGKNCVAIAADRRFGIQAQMVTTDFQKIFPMGDRLYIGLAGLATDVQTVAQRIKFRLNLYELKEG
Tv_beta_3 RPIDPKKFMNLVKSTLYEKRFSPFFVTPVIAGLLPETNEPYLAASDSIGAFAFPKDFAVAGTCEESLYGICESAWRPNMN
Hs_beta_3 RQIKPYTLMSMVANLLYEKRFGPYYTEPVIAGLDPKTFKPFICSLDLIGCPMVTDDFVVSGTCAEQMYGMCESLWEPNMD
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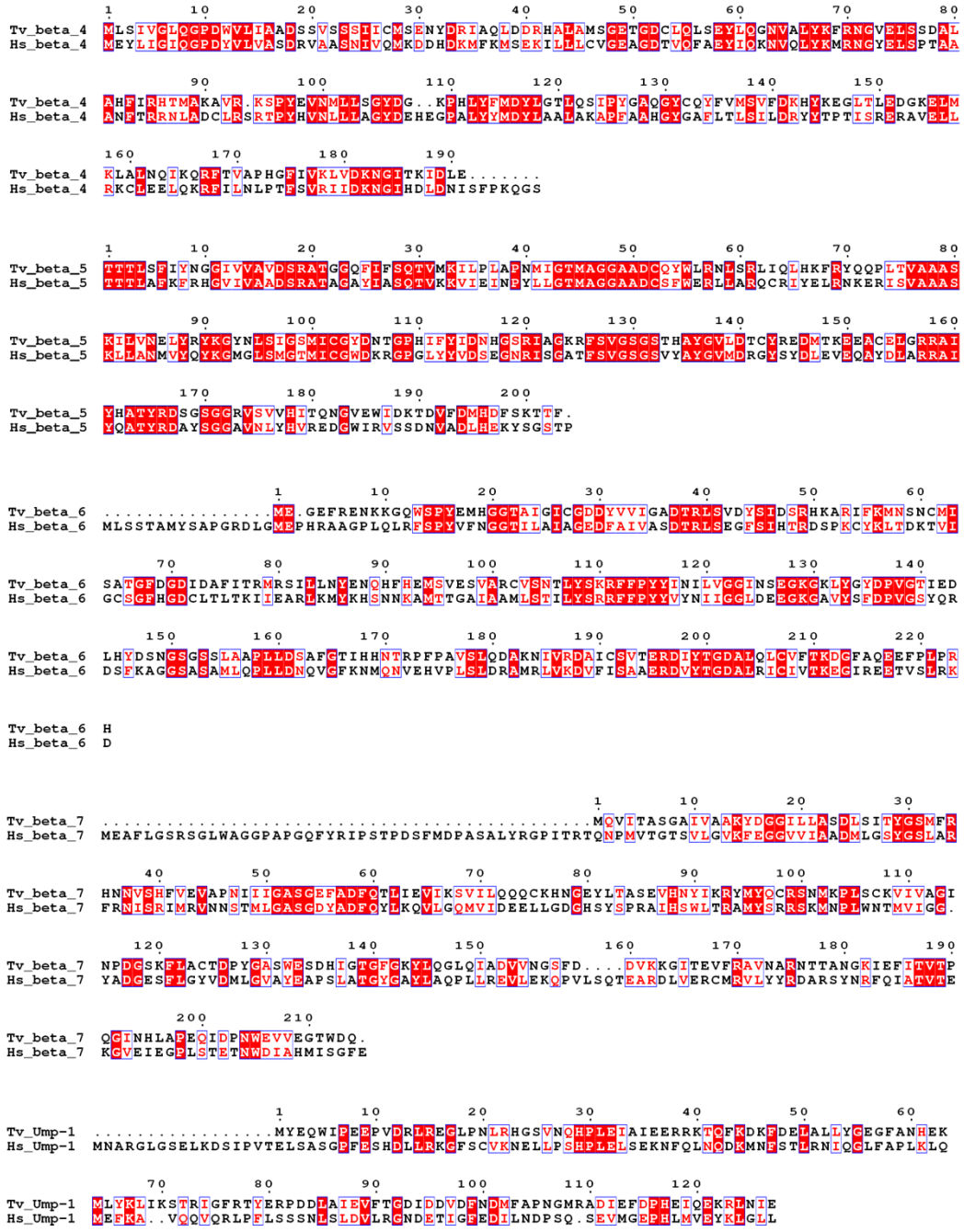
Tv_beta_4
Hs_beta_4
Tv_beta_5
Hs_beta_5
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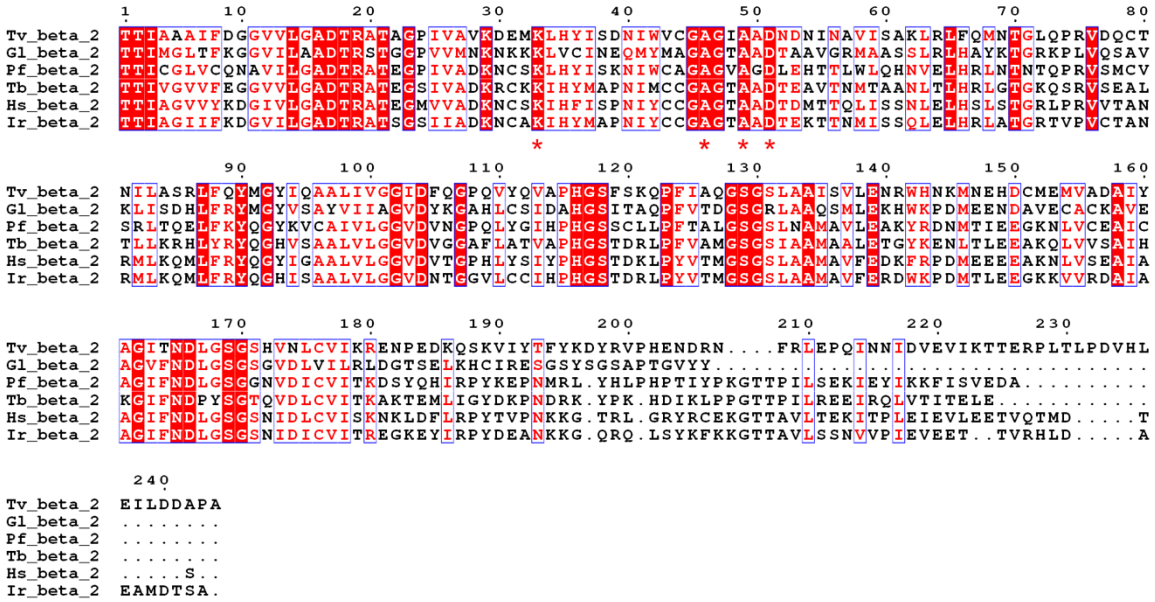
Hs_beta_6
Tv_beta_7
Hs_beta_7

e

Tv_Ump-1
Hs_Ump-1

f

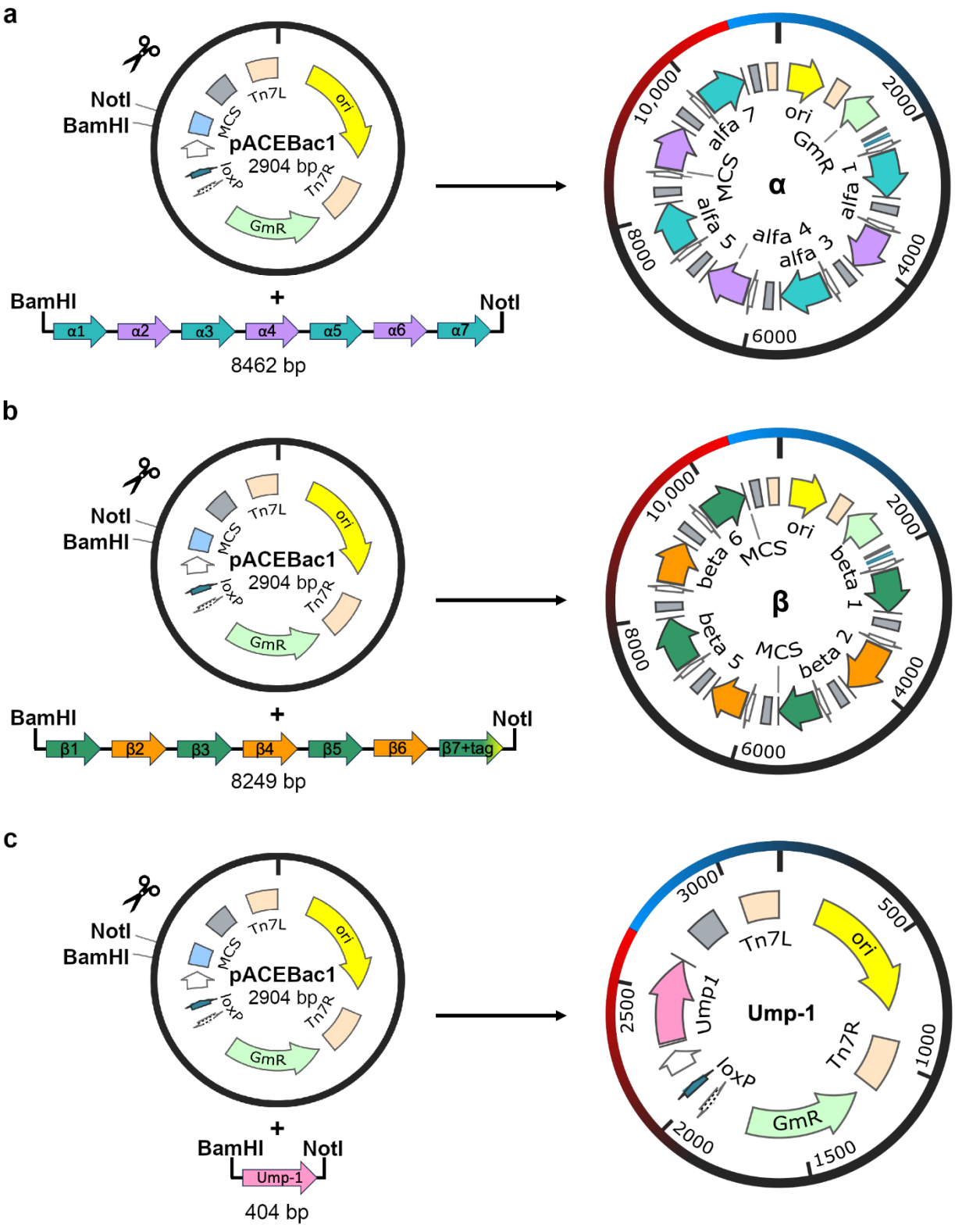


g

Subunit	*Tv* Uniprot ID	*Hs* Uniprot ID
α1	A2F568	P60900
α2	A2FJV7	P25787
α3	A2FT79	P25789
α4	A2DTN3	O14818
α5	A2FCM7	P28066
α6	A2E1I9	P25786
α7	A2D8G5	P25788
β1	A2E7Z2	P28072
β2	A2F2T6	Q99436
β3	A2F3H9	P49720
β4	A2F8W4	P49721
β5	A2DD57	P28074
β6	A2F716	P20618
β7	A2F3X4	P28070
Ump-1	A2FJW0	Q9Y244

Supplementary Figure 1. Sequence alignment of *Tv*20S and human 20S proteasomes and Ump-1 chaperone.

Panel **a)** represents multiple sequence alignments of all seven α subunits (α1-α7) and **b)** all seven β subunits (β1-β7) from *Tv*20S, with β1, β2 and β5 shown as proenzymes, and **c)** & **d)** aligned individually with their human α & β subunits (*Hs*20S) counterparts. Conserved residues are boxed, and identical residues are highlighted in red. Conservation patterns and variations between species are illustrated. **e)** Sequence alignment of *Tv* Ump-1 and with *Hs* Ump-1, highlighting conserved regions. **f)** Sequence alignment of mature β2 subunit from *T. vaginalis (Tv), Giardia lamblia (Gl), Plasmodium falciparum (Pf), Trypanosoma brucei (Tb), Homo sapiens (Hs),* and *Ixodes ricinus (Ir)*. The asterisk denotes highly conserved residues Lys33 and a loop consisting of Ala46, Ala49, and Asn52. The aligned sequences were visualized using ESP3 software according to Robert, X. and Gouet, P. (2014).[1] **g)** Uniprot accession codes of each gene used.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α1
α2
α3
α4
α5
α6
α7
NotI
8462 bp
α
ori
GmR
alfa 1
alfa 3
alfa 4
alfa 5
alfa 7
MCS
2000
4000
6000
8000
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b
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Tn7L
ori
MCS
loxP
Tn7R
GmR
BamHI
β1
β2
β3
β4
β5
β6
β7+tag
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8249 bp
β
ori
MCS
beta 1
beta 2
beta 5
beta 6
2000
4000
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loxP
Tn7R
GmR
BamHI
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ori
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2000
2500
3000

d Insert BamHI_α1_α2_α3_α4_α5_α6_α7_ NotI *- sequences encoding Kozak sequence, start and stop codon, gene,* ***polyhedrin promoter****, SV40 polyA tail and twin-strep tag.are highlighted.*

ggatccGCCACCATGAGCAGCGGTGCAGATCGTTATCTGACCGTTTTTAGTGCCGAAGGTCGTCTGTGGCAGGTTGAATATAGTTTTAA
AGCAGTTAAACAGGCCGAAGTTACCGCAGTTGCAGTTAAAAGCAAAAATGCAGTTTGTGTTGCCGTGCAGAAAAAGGTTAGCGATAAAC
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ATTGTTATGCTGCTGCGTAGCTTTGCCAACAACTTTGAATATAAACAGGGCTTTAGCATCCCGGTTTCAATTCTGGCACAGATGCTGAG
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GCTGTGCGAAGAAACCAATGGTCGTCCGCAGAAAGTGCCGGAAGATGTTTTTCAGAGCCGTTTTGTTAATGAAAACCCGCAGAAT**TAA***g*
cggccgc

Insert* BamHI_β1_β2_β3_β4_β5_β6_β7+tag_ NotI** *- sequences encoding Kozak sequence, start and stop codon, gene,* ***polyhedrin promoter*, SV40 polyA tail and twin-strep tag.are highlighted.*

ggatccGCCACCATGAGCGAATATCAGTTTCCGAAAGAAAGCATGGGTACAACCCTGCTGGCAATTCAGTGTACCGATGGTGTTGTTAT
GGCAAGCGATAGCCGTACCAGCAGCGGTAGCTTTATTCCGAATCGTGCAACCAACAAAATTACCGAAATTCAGCCGAAAATCTTTGCAG
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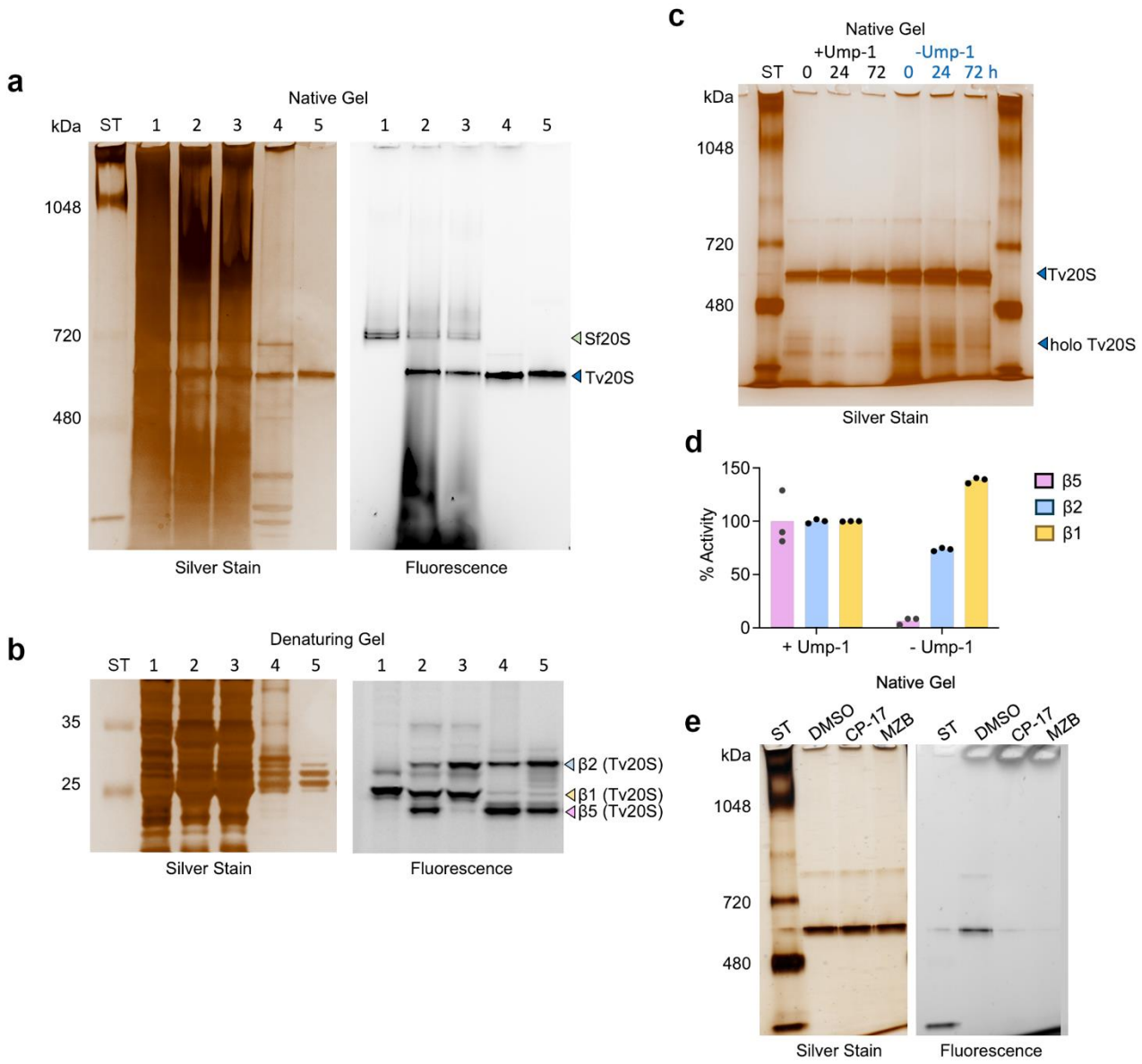
CCGGTTTTGGTAAATATCTGCAGGGTCTGCAGATTGCCGATGTTGTTAATGGTAGCTTTGATGATGTGAAAAAGGGCATCACCGAAGTT
TTTCGTGCAGTTAATGCCCGTAATACCACCGCAAATGGTAAAATCGAATTTATCACCGTTACACCGCAGGGCATTAATCATCTGGCACC
GGAACAAATTGATCCGAATTGGGAAGTTGTTGAAGGCACCTGGGATCAG**AGCGCTTGGAGCCACCCGCAGTTCGAAAAAGGTGGAGGTT**
CTGGCGGTGGATCGGGAGGTTCAGCGTGGAGCCACCCGCAGTTCGAGAAATAA*gcggccgc*

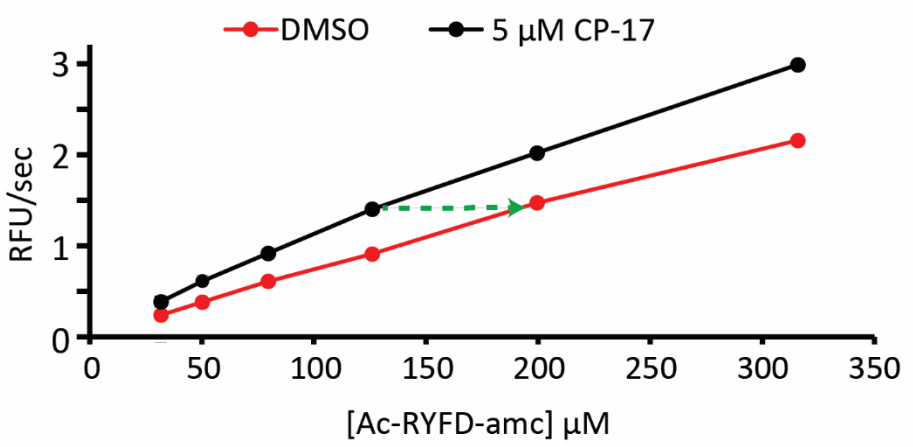
Insert BamHI_Ump-1_NotI

*ggatcc*GCCACCATGTACGAACAGTGGATTCCGGAAGAACCGGTTGATCGTCTGCGTGAAGGTCTGCCGAATCTGCGTCATGGTAGCGT
TAATCAGCATCCGCTGGAAATTGCCATTGAAGAACGTCGTAAAACCCAGTTCAAAGACAAATTTGATGAACTGGCACTGCTGTATGGTG
AAGGTTTTGCAAATCATGAGAAGATGCTGTACAAACTGATTAAAAGCACCCGTATTGGTTTTCGCACCTATGAACGTCCGGATGATCTG
GCAATTGAAGTTTTTACCGGTGATATCGATGATGTGGATTTCAACGATATGTTTGCACCGAATGGTATGCGTGCCGATATTGAATTTGA
TCCGCATGAAATCCAAGAGAAACGCCTGAATATTGAA**TAA***gcggccgc*

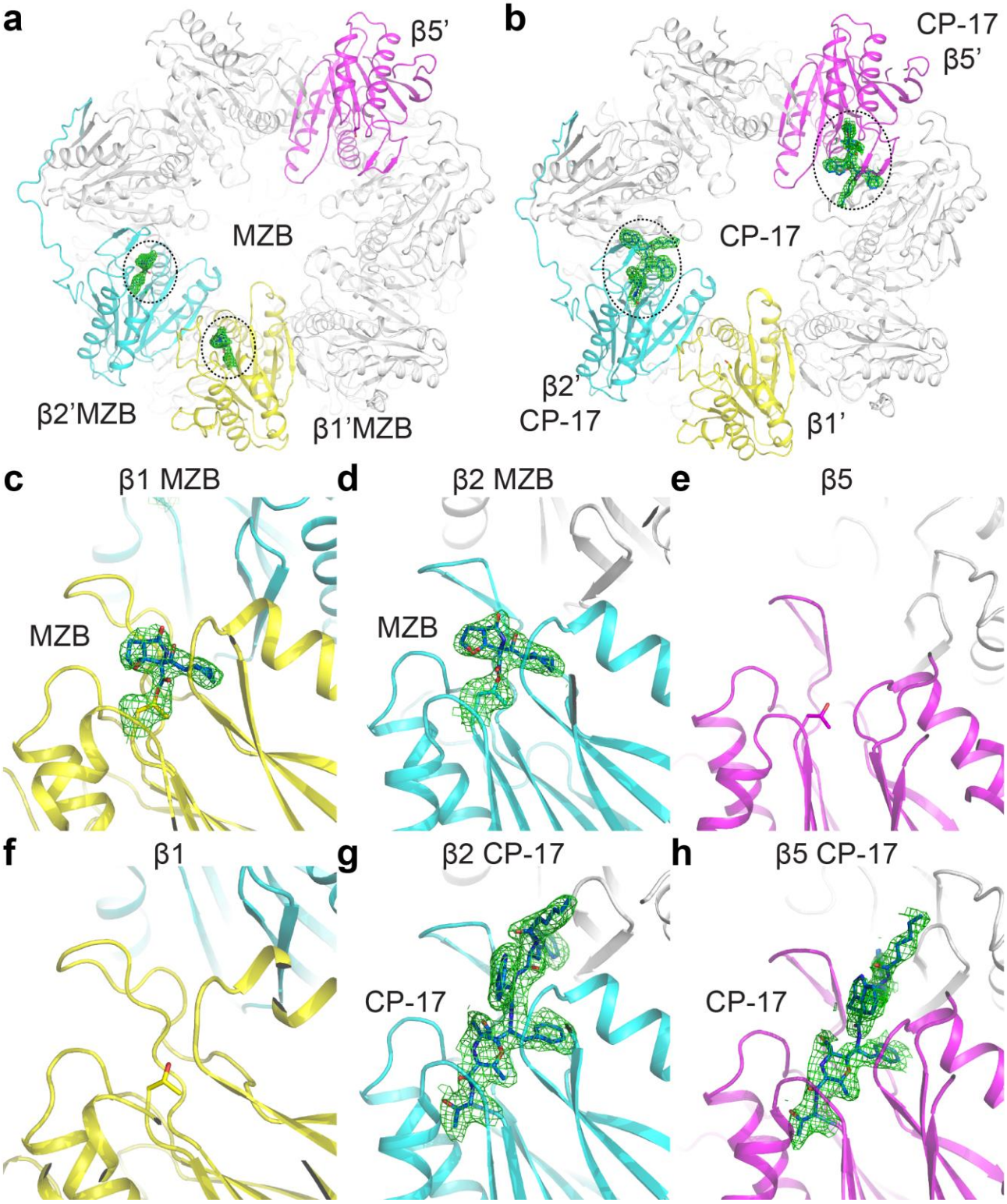
Supplementary Figure 2. Cloning plasmids and proteasome gene sequences. a-c) Schematic representation of the cloning of the three plasmids used to generate recombinant baculoviruses. The figure was created using SnapGene® (snapgene.com). **d)** The inserted DNA sequences correspond to the seven *Tv*20S α subunits (BamHI_α1_α2_α3_α4_α5_α6_α7_NotI), seven Tv20S β subunits (BamHI_β1_β2_β3_β4_β5_β6_β7+tag _NotI) and *Tv*20S Ump-1 (BamHI_Ump-1_NotI). The accession numbers for each subunit are listed in Supplementary Table 3. Sequences are labelled as follow: *restriction enzyme cleavage site*, Kozak sequence, **start** and **stop** codon, gene, **polyhedrin promoter**, SV40 polyA tail, twin-strep tag.



Supplementary Figure 3. Confirmation of recombinant Ump-1 function and 20S stability. Panel **a)** shows a native protein gel demonstrating Me4BodipyFL-Ahx3Leu3VS probe labelling of the proteasome. The left panel is a silver-stained gel, while the right panel is a fluorescent scan of the same gel at 470 nm excitation and 530 nm emission. Panel **b)** displays a denaturing gel with the same protein samples as panel a lane 1 represents Sf9 cell lysate, lane 2 represents Sf9 cell lysate infected with alpha, beta, and Ump-1 baculovirus, lane 3 represents Sf9 cell lysate infected with alpha and beta baculovirus, lane 4 represents native *Tv*20S, and lane 5 represents purified r*Tv*20S. Panel **c)** shows the time-dependent assembly of the 20S proteasome in the presence and absence of Ump-1, while panel **d)** shows the enzymatic activity of r*Tv*20S in the presence and absence of Ump-1. Assays were performed in technical replicates (n=3). **e)** Silver-stained gel showing the mass of r*Tv*20S is unaltered in the presence of inhibitors while the fluorescent gel shows that reduced labelling of the active sites. All gels were repeated independently two times with similar results. 'ST' stands for the molecular weight ladder.



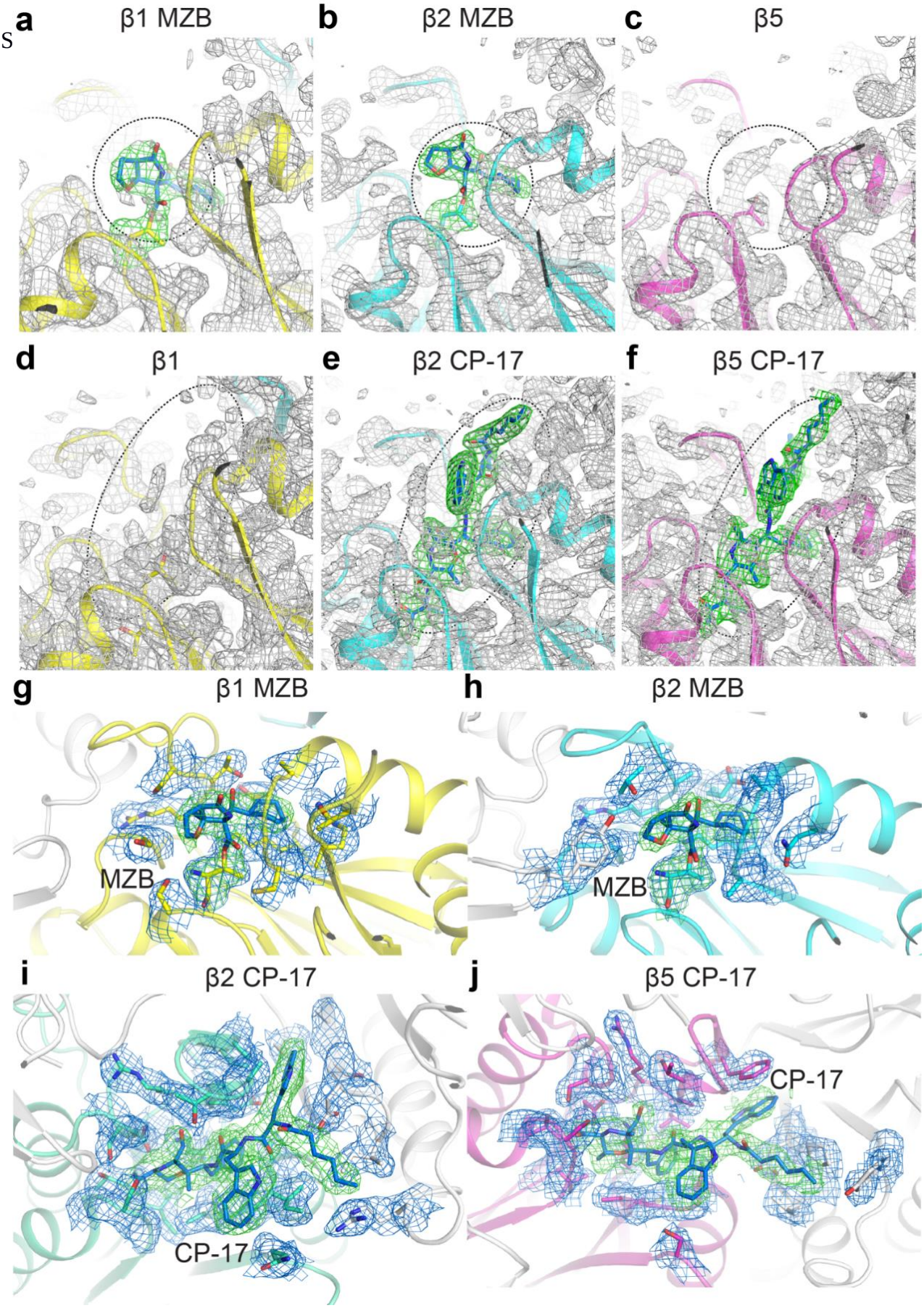
Supplementary Figure 4. Activation of β1 in the presence of CP-17. r*Tv*20S was preincubated with 5 µM of CP-17 to inactivate β5 only and then assayed with Ac-RYFD-amc. Activity was compared to a vehicle control. Assays were performed in triplicate, with each dot representing the mean of 3 technical replicates. The green arrow shows an example of the apparent increase in substrate concentration (from 126 µM to 200 µM) within the proteasome core in the presence of CP-17.



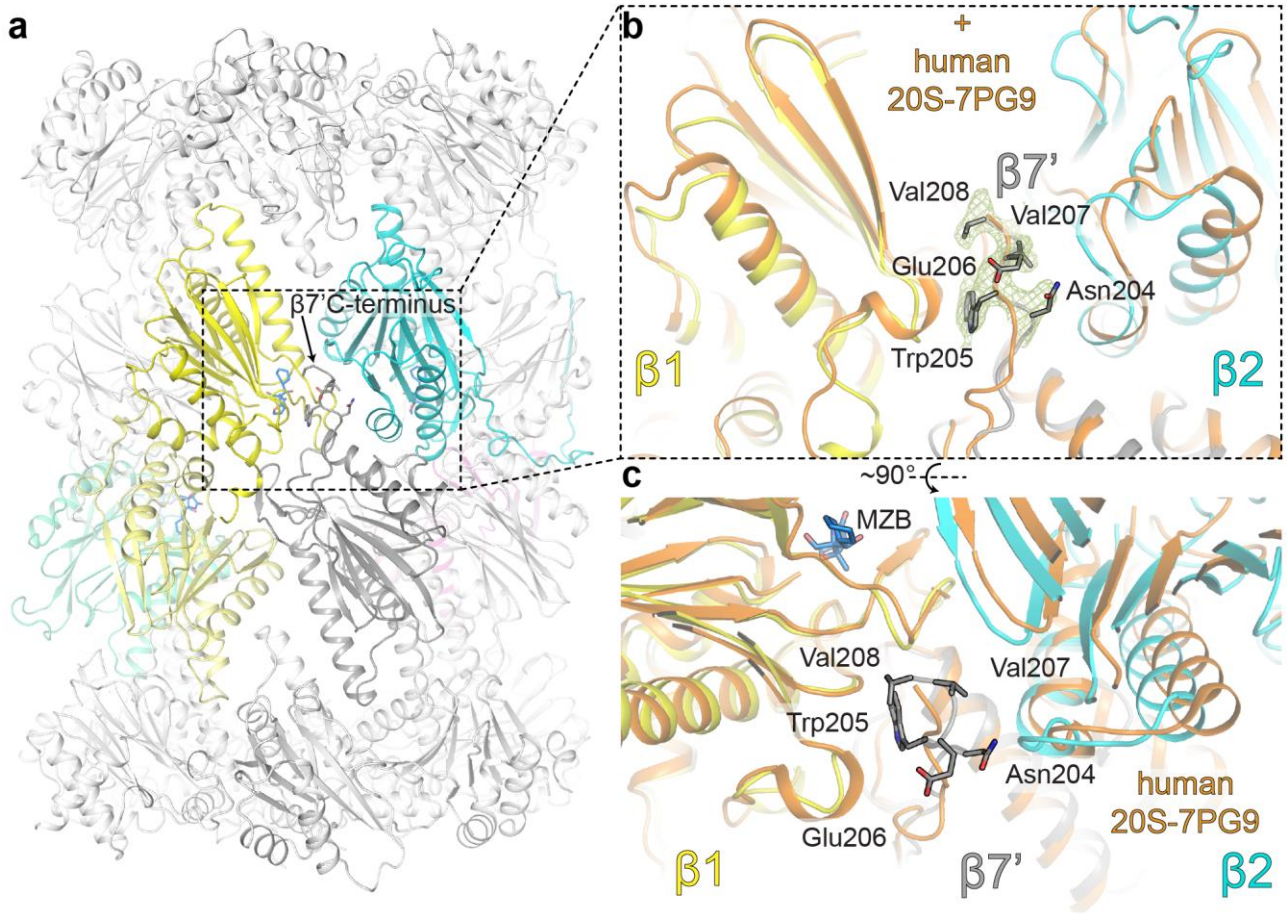
Supplementary Figure 5. Structural comparison of cryo-EM maps of the inhibitor molecules MZB and CP-17 covalently bound in the active sites of *Tv*20S

The top views of both *Tv*20S structures with **a)** MZB and **b)** CP-17 inhibitors (both shown as blue sticks). Cryo-EM Maps are carved around inhibitors for clarity as green mesh at sigma 4. Detailed views on the active sites are shown as cartoon and sticks for inhibitors. MZB (panels **c)**, **d)** and **e**) and CP-17 (panels **f**, **g** and) are displayed with active sites in order: β1 (panels **c**&f shown in yellow), β2 (panels **d) & g),** shown in cyan), and β (panel **e) & h)**, shown in magenta).

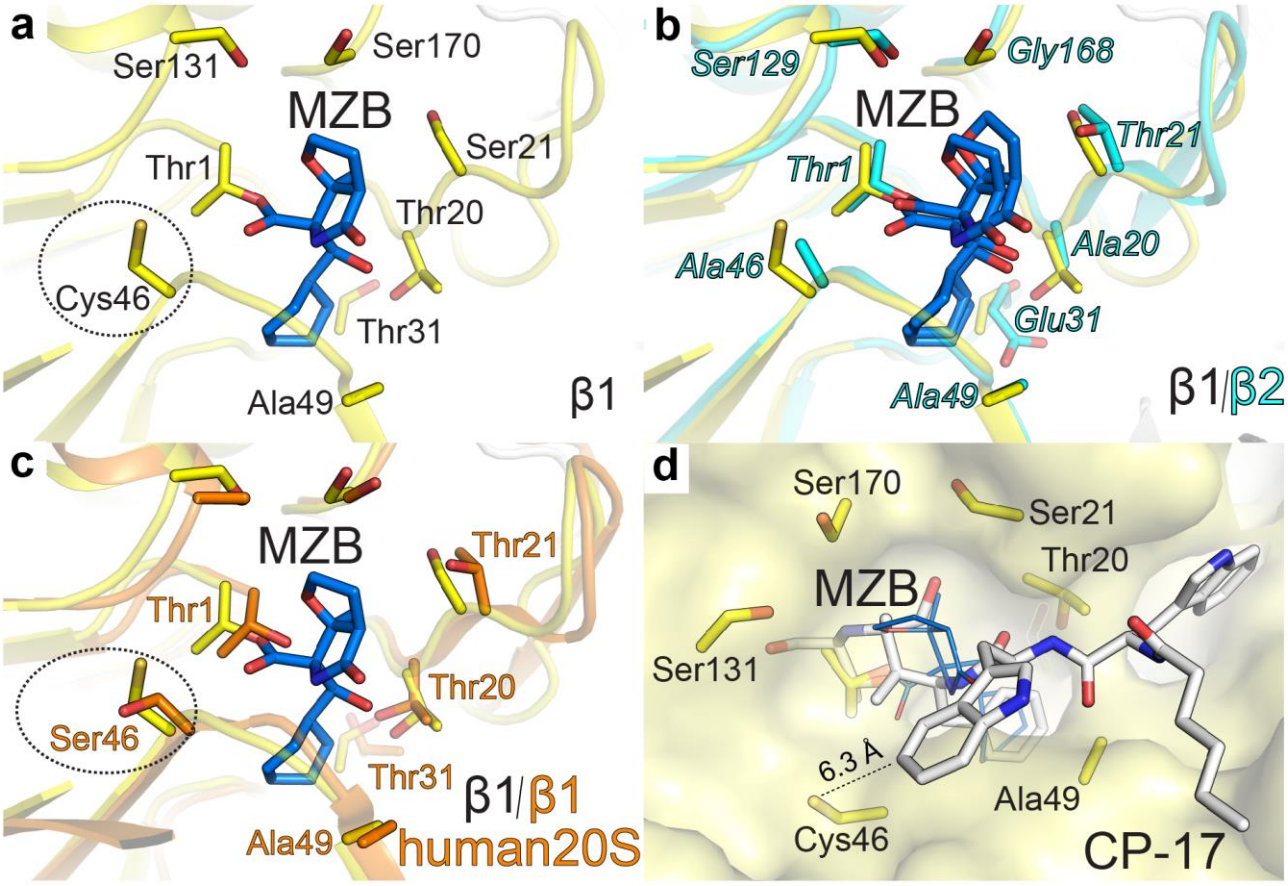
S



Supplementary Figure 6. Cryo-EM maps in all three active sites for both *Tv*-20S structures
Comparison of cryo-EM maps for both *Tv*20S structures with MZB (**a**,**b**,**c**,**g**,**h**) and CP-17 (**d**,**e**,**f**,**i**,**j**) inhibitors. Maps are shown as grey mesh at sigma 4 and density around inhibitors is depicted in green. Active sites are displayed in order: β1 (panels **a)** & **d)** are shown in yellow), β2 (panels **b)** & **e)**, shown in cyan), and β (panel **c)** & **f)**, shown in magenta. Panels **g) - j)** additionally show the density around *Tv20S* residues that form the binding site, and ligand, shown simultaneously as sticks and also cryo-EM maps at the same sigma level. The map around *Tv20S* residues is depicted in blue.

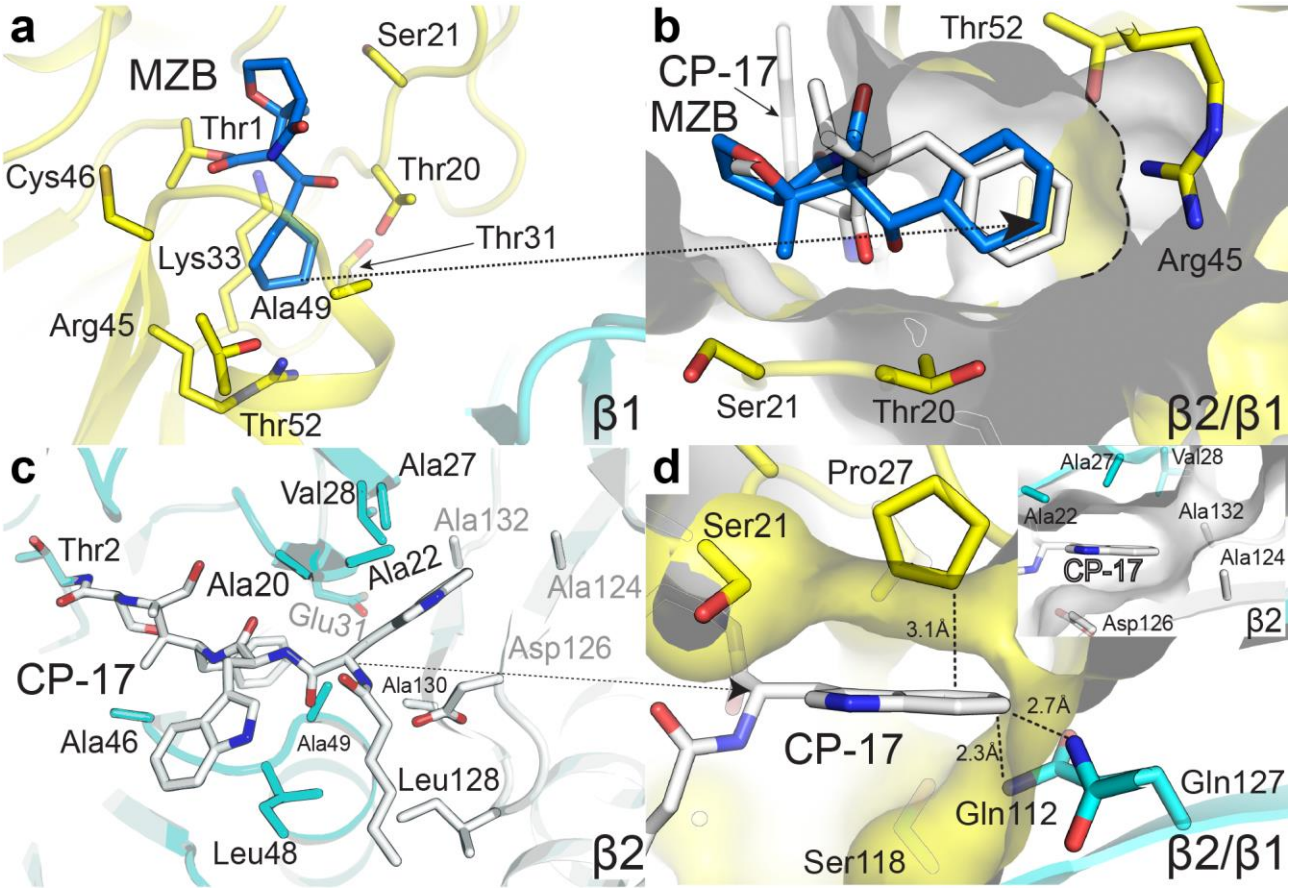


Supplementary Figure 7. C-terminal end being properly inserted between the β1 and β2. a) *Tv*20S with MZB cartoon representation where β1 is shown in yellow, β2 cyan and β7' grey with its the C-terminus marked by arrow. Panels **b)** shows local cryoEM map (green mesh) and **c)** show detailed view with an overlay with superimposed human 20S proteasome (PDB ID = 7PG9) shown in orange. The residues of β7' Asn204-Val208 of *Tv*20S shown as grey sticks inserted between β1 and β2 chains.



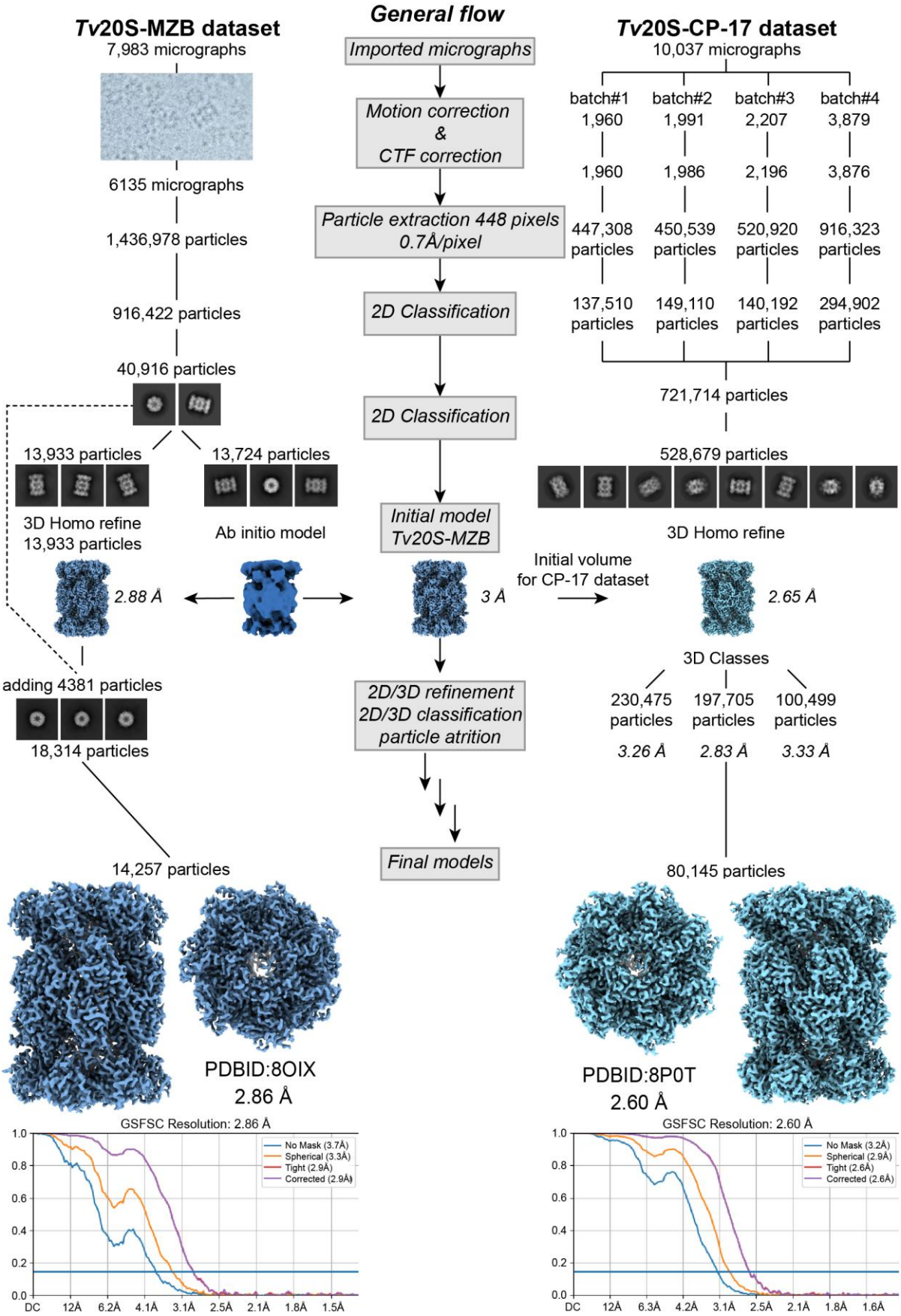
Supplementary Figure 8. Prospects for developing inhibitors that target Cys46 of the β1 subunit of *Tv*20S proteasome.

a) The active site of the β1 subunit in the *Tv*20S-MZB proteasome structure is highlighted in yellow, while MZB is shown as blue sticks. This region includes neighbouring residues, including Cys46. **b)** The structural overlay of β1 and β2 (cyan sticks) subunits of *Tv*20S-MZB proteasome. **c)** An overlay comparison is made between the β1 active site of *Tv*20S-MZB (shown in yellow, with MZB represented as blue sticks) and the human 20S structure, PDB ID = 7PG9 (shown in orange). **d)** Another overlay is presented, showcasing only the CP-17 inhibitor (depicted in white) of the β2 subunit onto the β1 active site of *Tv*20S-MZB (represented in yellow, with MZB shown as blue lines).



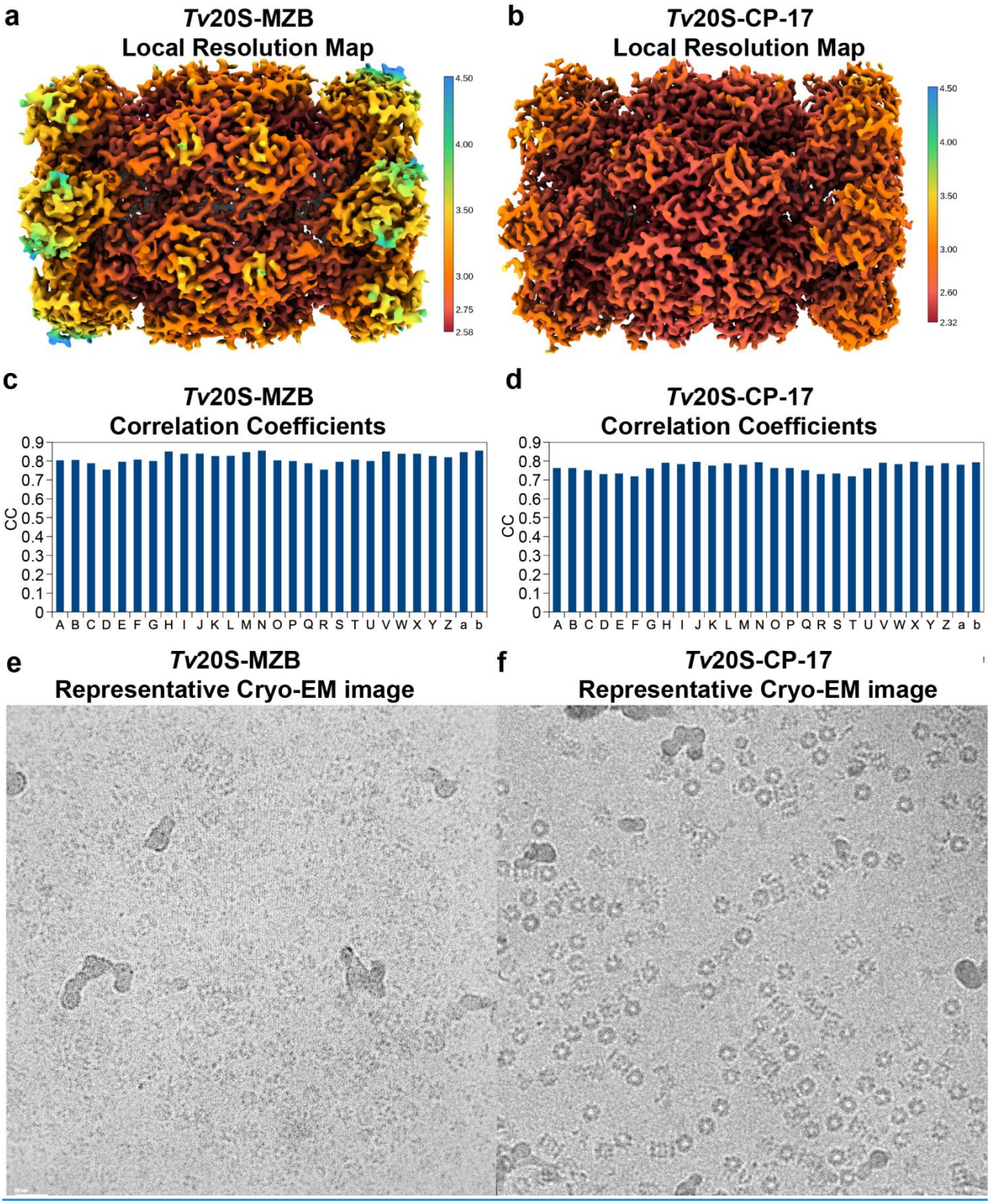
Supplementary Figure 9. Model of CP-17 "virtual" clashes within the *Tv*20S proteasome β1 active site pocket

a) The β1 active site of *Tv*20S-MZB structure (shown in yellow, MZB in blue) with neighbouring residues including Cys46. **b)** The surface of β1 with MZB (yellow and blue sticks) and β2 with and overlay of surface and CP-17 inhibitor (white and white sticks) from the β2 of *Tv*20S-CP-17 proteasome. CP-17 is shown as white sticks for clarity. **c)** Detail of β2 with CP-17 with highlighted residues mainly surrounding indole rings. **d)** The inset of the figure shows surface of β2 S3 pocket of *Tv*20S-CP-17 proteasome. The main panel shows surface of β1 site with CP-17 (white) modelled by superimposition of β2 and β1 subunits. The β1 pocket is lined with much bulkier residues such as Pro27 and polar residues Ser118, Gln112 and Gln127. Unlike in the β2 pocket lined with smaller and more hydrophobic residues Ala (22,27,124,126,132) and Val28.

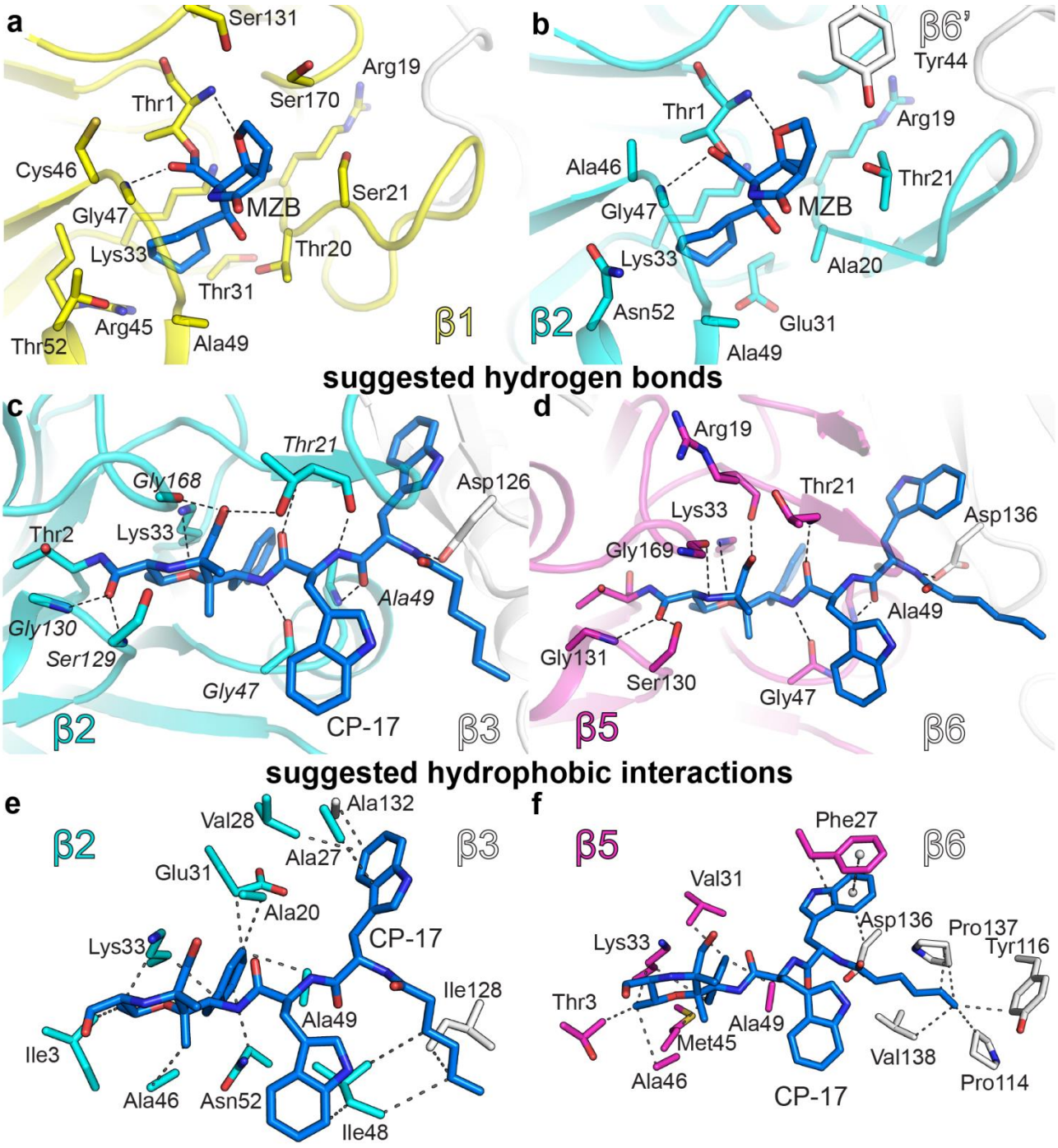


Supplementary Figure 10. Cryo-EM workflow of data processing. This image processing workflow was employed to reconstruct the *Tv*20S structures in cryo-EM. Both datasets were acquired at Titan Krios with the Falcon 4i detector under identical conditions using the same setup (refer to Supplementary Table 1 for details). The *ab initio model* served as the starting point for 3D homogeneous refinement (Homogeneous Refinement) to enhance the quality of the maps. Multiple iterations, including 3D classification and 2D classification, were

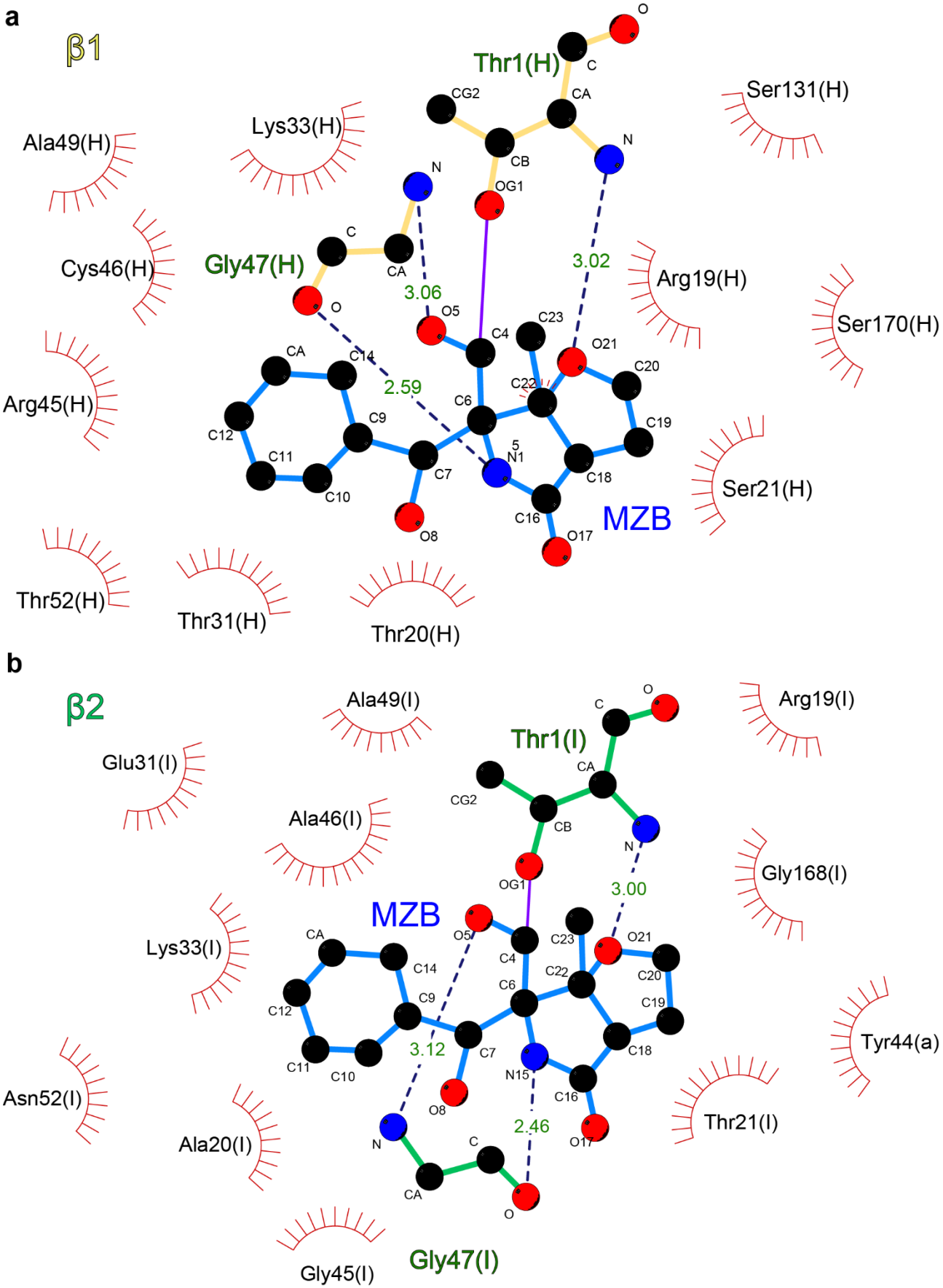
carried out in several rounds to eliminate unwanted particles, refine the resolution, and improve the maps. The unsharpened maps of the final reconstruction and the gold-standard Fourier Shell Correlation (FSC) curve using different masks are shown. The figures of the maps were generated by ChimeraX[2]. The resolution in italics corresponds to a particular Fourier Shell Correlation Cryosparc and was used to navigate the process of data analysis. The final resolution was estimated by Fourier Shell Correlation job in Cryosparc[3] when a tight mask was applied and was estimated to be 2.86 Å for 8IOX and 2.60 Å for 8P0T. An example of the *Tv*20S-MZB route of processing is as follows: 1,436,978 particles were extracted from 6135 processed images. After several rounds of 2D classification to sort out unwanted classes, only 40,916 particles remained. These were then reclassified with independent 2D classifications for the top views and the side views. Separately, classes with 13,724 particles of both views were used for an ab initio model. This initial cryo-EM map was used as a starting model for homogeneous reconstruction, where 13,933 side view particles were used. Next, 4381 particles corresponding to the top views were added in the next round. For the final model, additional particles were removed during several rounds of homogeneous reconstruction and 3D classifications. Note: clear top views of the full proteasome were assumed to be the particles with the highest contrast. Representative Cryo-EM images are shown in subsequent Supplementary Figure 11.



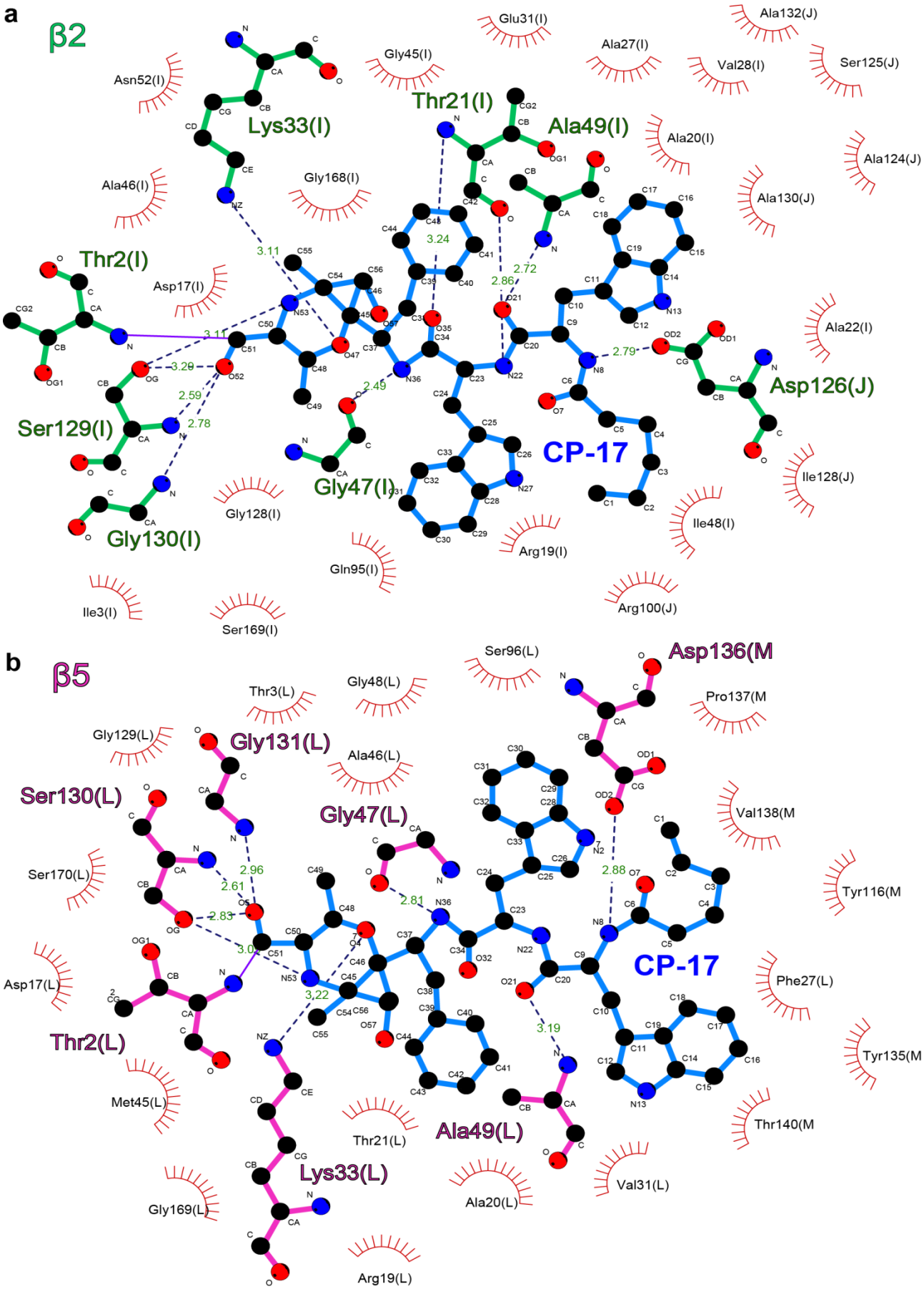
Supplementary Figure 11. Cryo-EM maps coloured according to the calculated local resolution and example of Cryo-EM images. The estimate of local resolution for both *Tv*20S structures **a)** 8OIX and **b)** 8P0T are compared. **c**&**d** show average map correlation coefficient for each chain. Source data are provided as a Source Data file Figures.xlsx. Panels **e**&**f** demonstrate the particle density and quality of Cryo-EM images for both datasets.



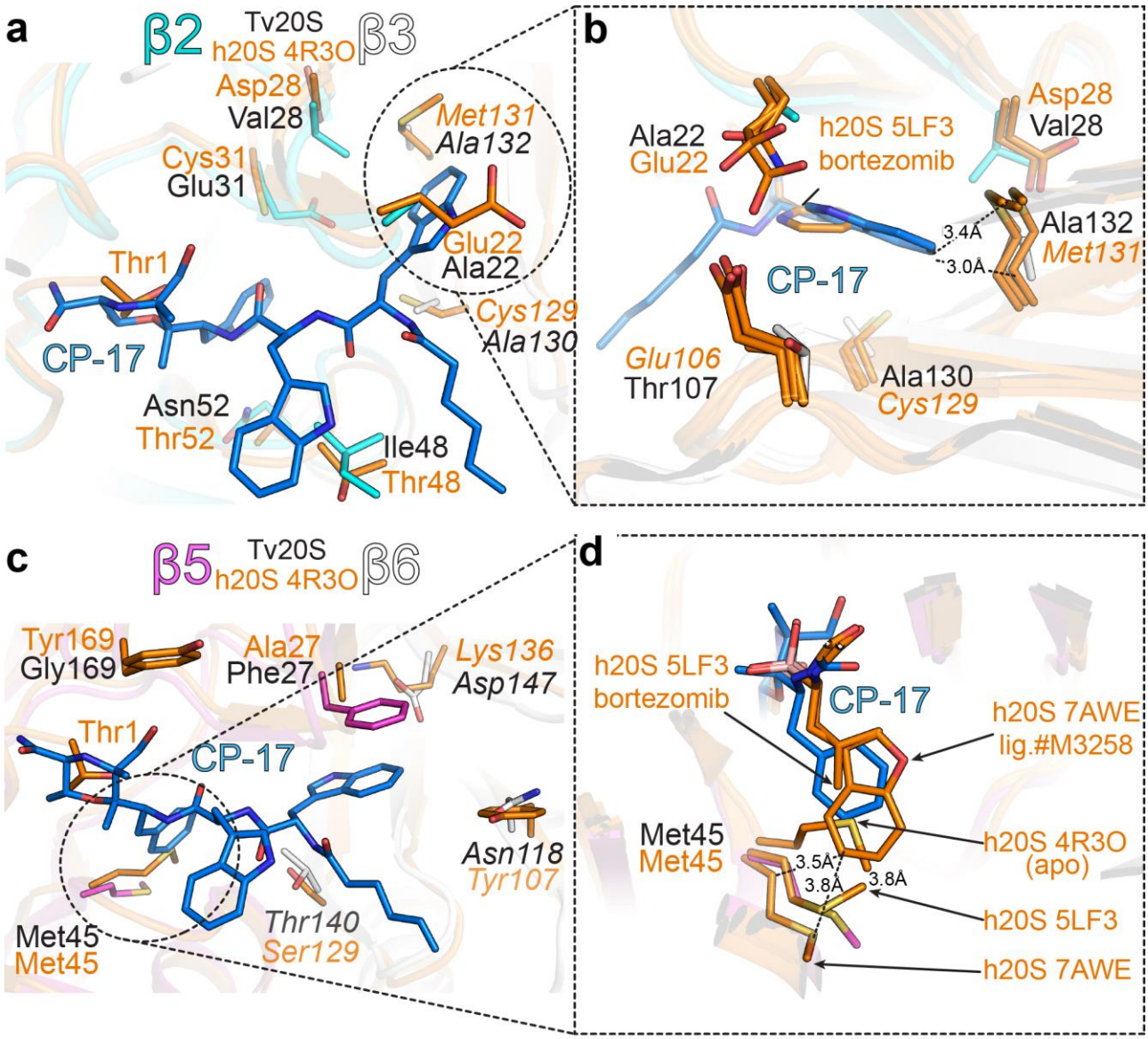
Supplementary Figure 12. Detailed interactions between small molecular inhibitors and active site pockets of *Tv*20S. The interactions between amino acid residues surrounding the active sites of *Tv*20S with the inhibitors; **a) & b)** MZB and **c)-f)** CP-17 with highlighted residues shown as sticks suggest interactions with inhibitors. Dashed lines represent hydrogen bridges in panels **a)-d)** and "hydrophobic" interactions are shown in panels **e) & f)**.



Supplementary Figure 13. MZB 2D representation of interactions between small molecular inhibitor MZB and *Tv*20S active site. a) & **b)** highlight residues in close contact with inhibitor in β1 and β2 active sites. Dashed lines represent hydrogen bridges, and "fan-like structures" demonstrate possible hydrophobic interaction.



Supplementary Figure 14. The lig plot CP-17 2D representation of interactions between molecular inhibitor CP-17 and the active site of *Tv*20S. a) & **b)** highlight residues in close contact with inhibitor in β2 and β5 active sites. Dashed lines represent hydrogen bridges, and "fan-like structures" demonstrate possible hydrophobic interaction.



Supplementary Figure 15. The main differences of *Tv*20S and potential clashes CP-17 in human 20S proteasome β2 and β5 active site pockets. Structural superimposition of *Tv*20S-CP-17 (8P0T) with human 20S (PDBID = 4R3O, and 5LF3 with 7AWE in right hand panels). **a**) The rotamer of human 20S (PDB ID = 4R3O) Met131 (β3) is relatively close and bulky compared to Ala of *Tv*20S, **b**) another view to the β2 active site besides 4R3O structure with the overlay of additional two structures 5LF3, 7AWE shows potential clash between CP-17 and Met131. Additionally, the Glu106 (β3) and Glu22 (β2) of human 20S suggest additional site of steric hindrance for CP-17 Panels **c**) & **d**) show similar contact of possible clash yet the rotamers in different structures such as 7AWE show deflection from the possible clash. Human 20S proteasome is shown in orange.

Supplementary Table 1. Structural alignment of 20S proteasome *Tv*20-CP1 7ZYJ with leishmania and human 20S proteasomes.

The table represents RMSD values for alignments of Cα of individual chains *Tv*20-CP17 with *Leishmania tarentolae* 20S proteasome and human proteasome:

	RMSD *Leismania*7ZYJ	Chain ID	RMSD **Human 7PG9**		RMSD *Leismania* 7ZYJ	Chain ID	RMSD **Human 7PG9**
α1	1.252	A	**1.153**	β1	0.975	H	**0.976**
α2	1.219	B	**1.031**	β2	0.807	I	**0.989**
α3	0.985	C	**1.260**	β3	0.886	J	**0.898**
α4	1.048	D	**1.028**	β4	0.960	K	**1.289**
α5	1.252	E	**1.099**	β5	0.870	L	**1.010**
α6	1.116	F	**1.144**	β6	1.228	M	**1.050**
α7	1.121	G	**1.205**	β7	1.178	N	**1.489**

Overall RMSD values:
20S proteasome *Tv*20-CP17 with *Leishmania tarentolae* 20S proteasome **RMSD = 2.729**
20S proteasome *Tv*20-CP17 with human 20S proteasome **RMSD = 2.541**
The average RMSD values:
for all chains of *Leishmania tarentolae* 20S proteasome RMSD = 1.064
20S proteasome *Tv*20-CP17 with human 20S proteasome RMSD = 1.116*
(RMSD values were calculated for *Tv*20-CP17 (8P0T as a fix model) and human crystal structure (7PG9) for Cα atoms in the Pymol [The PyMOL Molecular Graphics System, Version 2.5.5 Schrödinger, LLC] using the command:align)

Supplementary Table 2. Cryo-EM statistics for data collection, refinement, and validation.

Deposited Cryo-EM structure	**Tv20S-MZB PDBID: 8OIX**	**Tv20S-CP-17 PDBID: 8P0T**
Microscope	Titan Krios	Titan Krios
Detector	Falcon 4i	Falcon 4i
Magnification (nominal)	165,000x	165,000x
Voltage (kV)	300	300
Spherical aberration	2.7 mm	2.7 mm
Total electron dose (e^-)	40	40
Defocus range (μm)	(-2.4)–(-0.9)	(-2.4)–(-0.9)
Exposure (s)	2.25	2.25
Pixel size (Å)	0.75	0.75
Number of Micrographs	7,983	10,037
Final number of particles	14,257	80,145
Map resolution (Å) [FSC threshold]	2.86 [$FSC_{0.143}$]	2.60 [$FSC_{0.143}$]
Refinement		
Initial model used (PDBID)	7ZYJ	8OIX
Symmetry during reconstruction	C2	C2
Validation		
MolProbity score	1.62	1.67
Clashscore, all-atom	11.17	11.90
Rotamer outliers	0.93 %	1.21 %
Ramachandran plot		
Favoured	97.73 %	97.92 %
Outliers	0.00 %	0.00 %
RMSD bonds		
Lenght (Å)	0.004	0.004
Angles (°)	0.611	0.659
Model vs. Data		
Ligands (no.)	4 (MZB)	4 (CP-17)
CC (mask/box/ligand)	0.87 / 0.68 / 0.80	0.84 / 0.64 / 0.77
B-factor protein (min/max/mean)	3.38 / 99.85 / 40.13	0.00 / 78.57 / 29.79
B-factor ligand (min/max/mean)	42.42 / 57.82 / 49.23	12.71 / 39.10 / 24.94
Resolution Estimates (Å)		
FSC model unmasked (0/0.143/0.5)	2.7 / 2.7 / 3.1	2.5 / 2.5 / 2.8

Refinment and model vs data values were calculated using Phenix 1.20.1-4487

Supplementary references

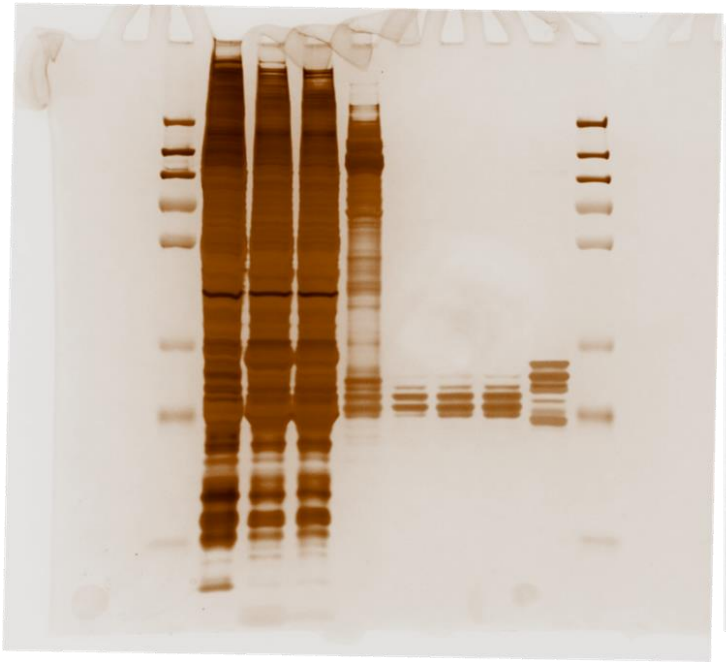
1 Robert, X. & Gouet, P. Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res* **42**, W320-324 (2014). https://doi.org:10.1093/nar/gku316

2 Meng, E. C. *et al.* UCSF ChimeraX: Tools for structure building and analysis. *Protein science : a publication of the Protein Society* **32**, e4792 (2023). https://doi.org:10.1002/pro.4792

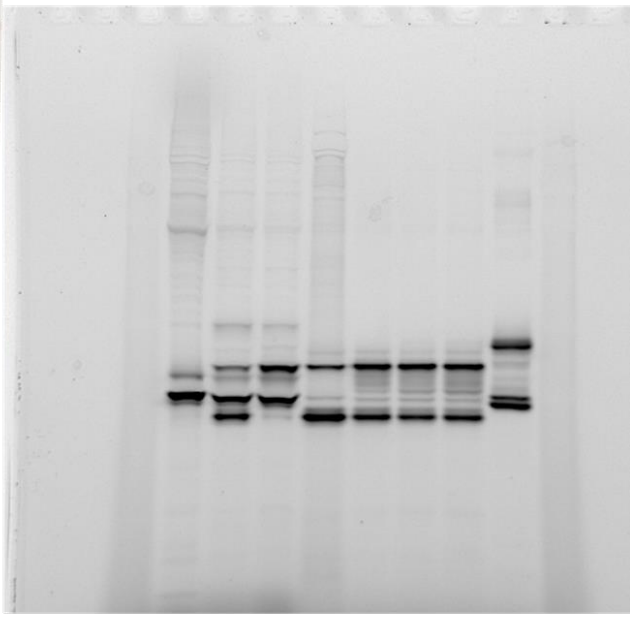
3 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nature methods* **14**, 290-296 (2017). https://doi.org:10.1038/nmeth.4169

Uncropped gels for Supplementary Figure 3a, b

denaturing gel - silver stained



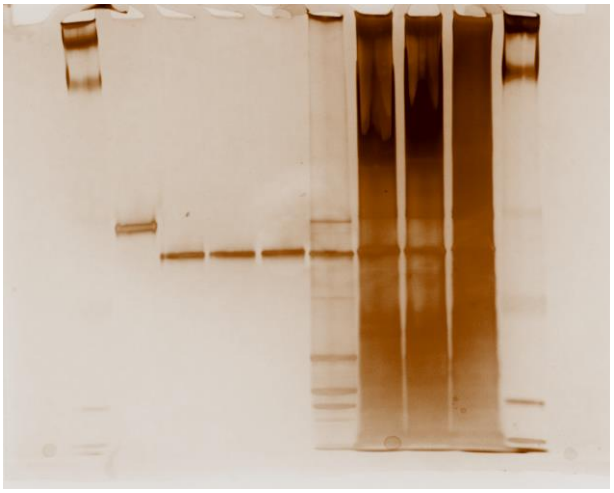
fluorescence

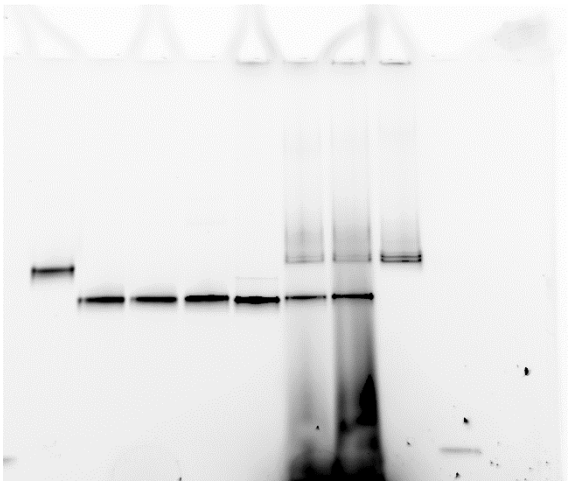


1. Sf lysate non-infected cells
2. alpha, beta, ump-1 coexpression
3. alpha, beta coexpression
4. 6. native Tv20S

5.-7. rTv20S

8. c20S



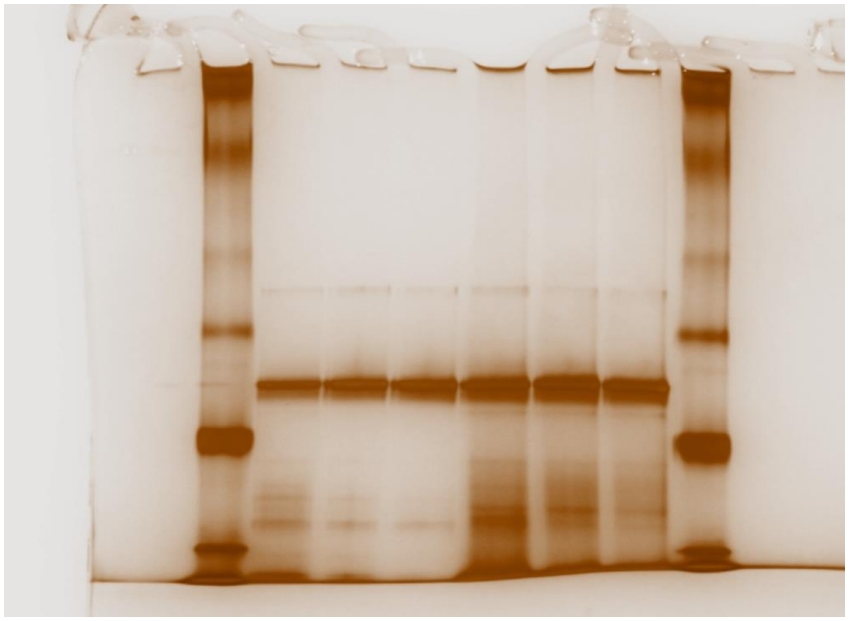


1. Sf lysate non-infected cells
2. alpha, beta, ump-1 coexpression
3. alpha, beta coexpression
4. 6. native Tv20S

5.-7. rTv20S

8. c20S

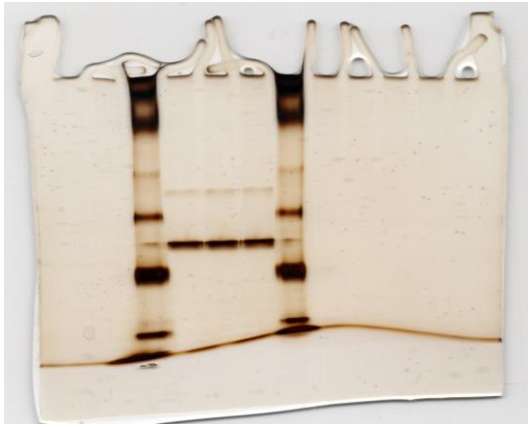
Uncropped gel for Supplementary Figure 3c



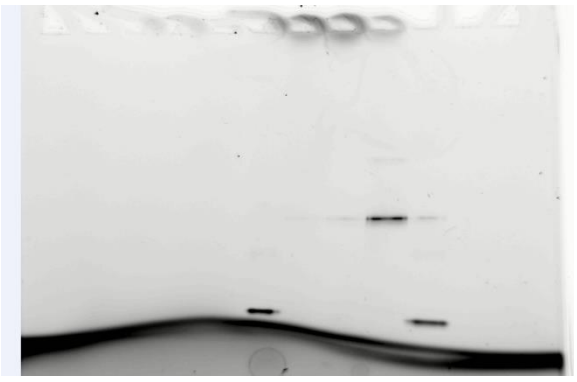
1) rTv20S + Ump time 0
2) rTv20S + Ump time 24 h
3) rTv20S + Ump time 72 h
4) rTv20S-Ump time 0
5) rTv20S - Ump time 24 h
6) rTv20S - Ump time 72 h

Uncropped gels for Supplementary Figure 3e

Native silver stain



Fluorescence



1) rTv20S+DMSO
2) rTv20S+CP17
3) rTv20S+MZB