
Supplementary information

**Structure of the human Mediator–RNA
polymerase II pre-initiation complex**

In the format provided by the
authors and unedited

MED6

MED6_H.s

1 10 20 30 40 50

MED6_H.s MA AV . DIRDN **LLG** **ISW** V D S S **WIPI** . . . **L** N S G S **VLD** **YFS** E R S N **PFY** D R T C **NNE** V V **KMQ** R L .
Med6_S.p MA SG APPSVD **LTS** **IQW** R M P E **WVQ** S M G G **L** R T E N **VLE** **YFS** Q . . S **PFY** S H K S **NNE** M L **KMQ** S Q F
Med6_S.p

α1 α2 α3

MED6_H.s

60 70 80 90 100

MED6_H.s . . . T **LE** H **L** N Q M V **GI** E Y I L L **HAQ** E **P** I **L** F I **I** R **KQ** R R Q S P A Q V **I** P L A D **Y** Y I I A G V **I** Y Q A
Med6_S.p N A L D **L** G D L N S Q **L** K R L T **GI** Q F V I I **H** E R P **P** F **L** W V **I** Q **KQ** N R L N E N E V **K** P L T V **Y** F V C N E N **I** Y M A
Med6_S.p

α4 β1 β2 β3 β4

MED6_H.s

110 120 130 140 150 160

MED6_H.s **P** D L G S V I N S **R** V **L** T A V H G I **Q** S A F D E A M S Y C R **Y** H **P** S K **G** Y W W H F K D H E E Q D K V R P K A K R K E E P
Med6_S.p **P** N A Y T L L A T R **M** L N A T Y C F **Q** K A L T K I E K F P Q **Y** N **P** Q E **G** Y T Y
Med6_S.p

α5 β5 β6

MED6_H.s

170 180 190 200 210 220

MED6_H.s S S I F Q R Q R V D A L L L D L R Q **K** F P P K F V Q L K P G E K P V **P** V D Q T **K** K E A . **E** P I P E T V K **P** E K E T T K
Med6_S.p P **K** L S N D N L E V D H S N T N E **P** A D E N **K** N Q S I **E** N A D Y S F S **P** E D F S V V R
Med6_S.p

α6

MED6_H.s

230 240

MED6_H.s N V Q **Q** T V . **S** A **K** G P **P** E K R M R L Q
Med6_S.p A F M **Q** S L H **S** S **K** E A P **P** D V K
Med6_S.p

α6

MED11

MED11_H.s

1 10 20 30 40 50

MED11_H.s **M** A T Y S . . . **L** A N E R L R A L E D I E R E **I** G A **I** L Q N A G T V **I** L E L S K E K T N **E** R L L D R Q A A
Med11_S.p **M** T N S D D D **L** F S E K S T S S D T Q Q V Q N I L E L **E** A K **I** P D **I** L S S A G K C **I** E A I Q L N N S L **E** D F . R K Y S K
Med11_S.p

α1 η1

MED11_H.s

60 70 80 90 100

MED11_H.s A **F** T A S **V** Q H V E A E **L** S A **Q** I R Y **L** T Q V A T G **Q** P H E G S S Y S S R K D C Q M A L K R V D **Y** A R L K **L** S D
Med11_S.p E **F** L E T **V** E F I S T G **L** R R **Q** A L E **L** E K A E V P **V** V S L **Q** P K K R **Y** A S T P L S N
Med11_S.p

α2 α3

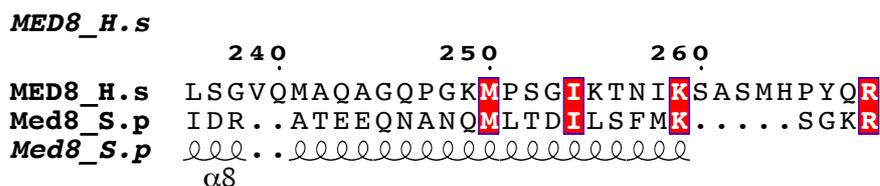
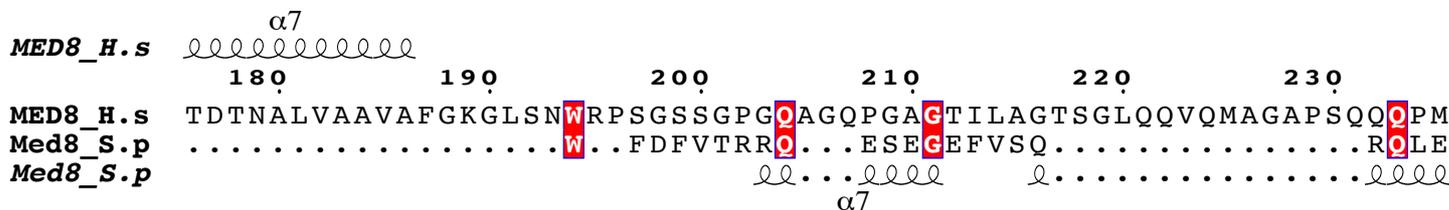
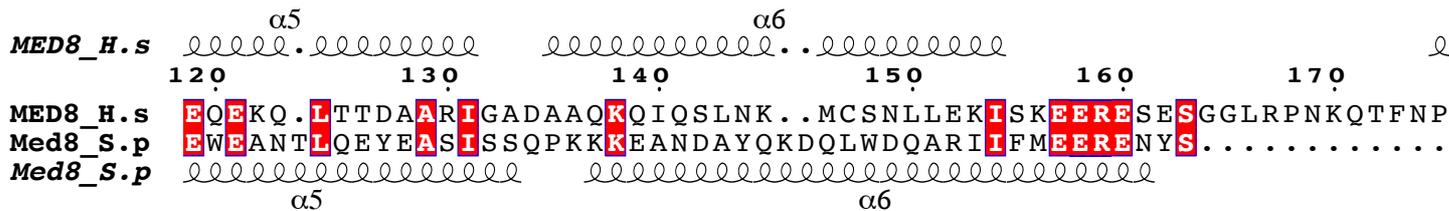
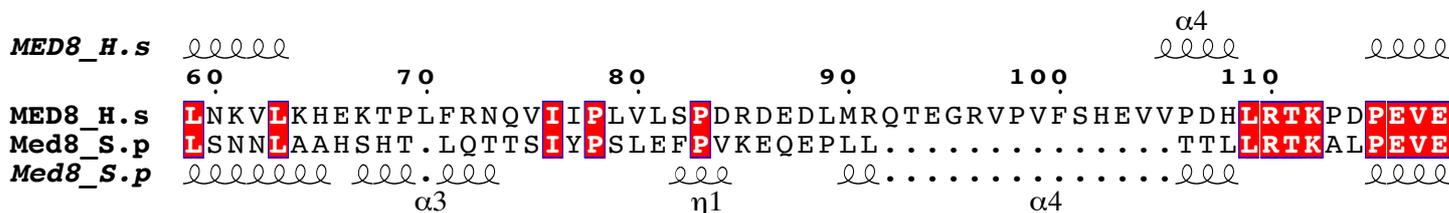
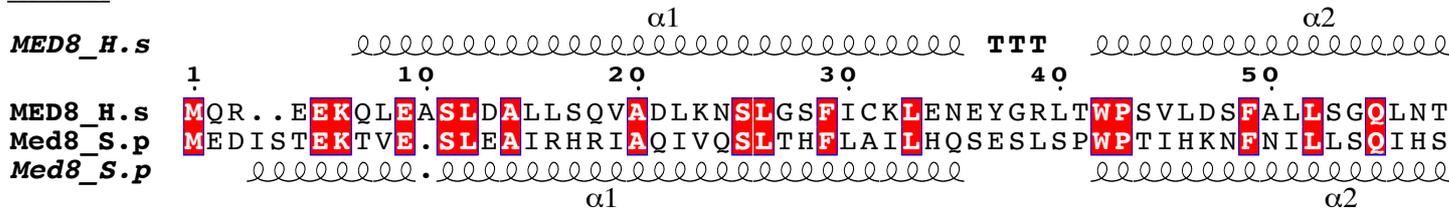
MED11_H.s

110

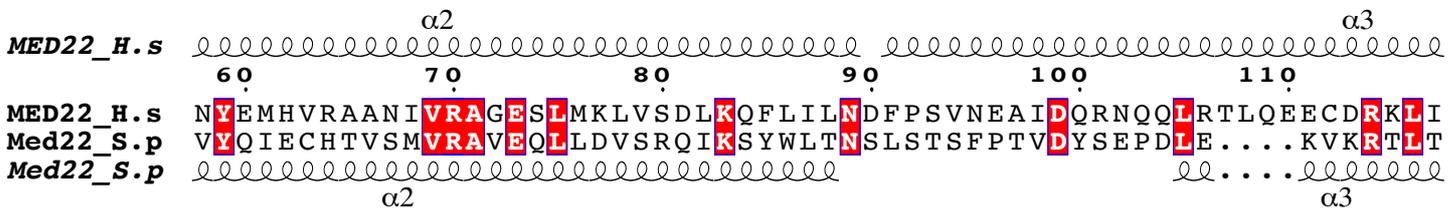
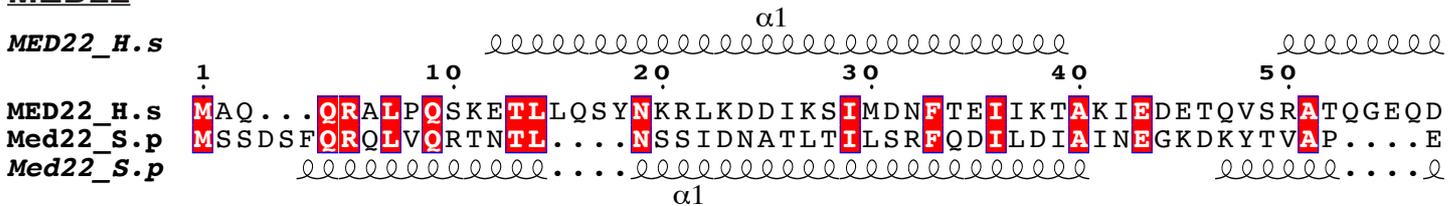
MED11_H.s V A R T C E Q M **L** E N
Med11_S.p L I F D Q S S K **L** M .
Med11_S.p

α3

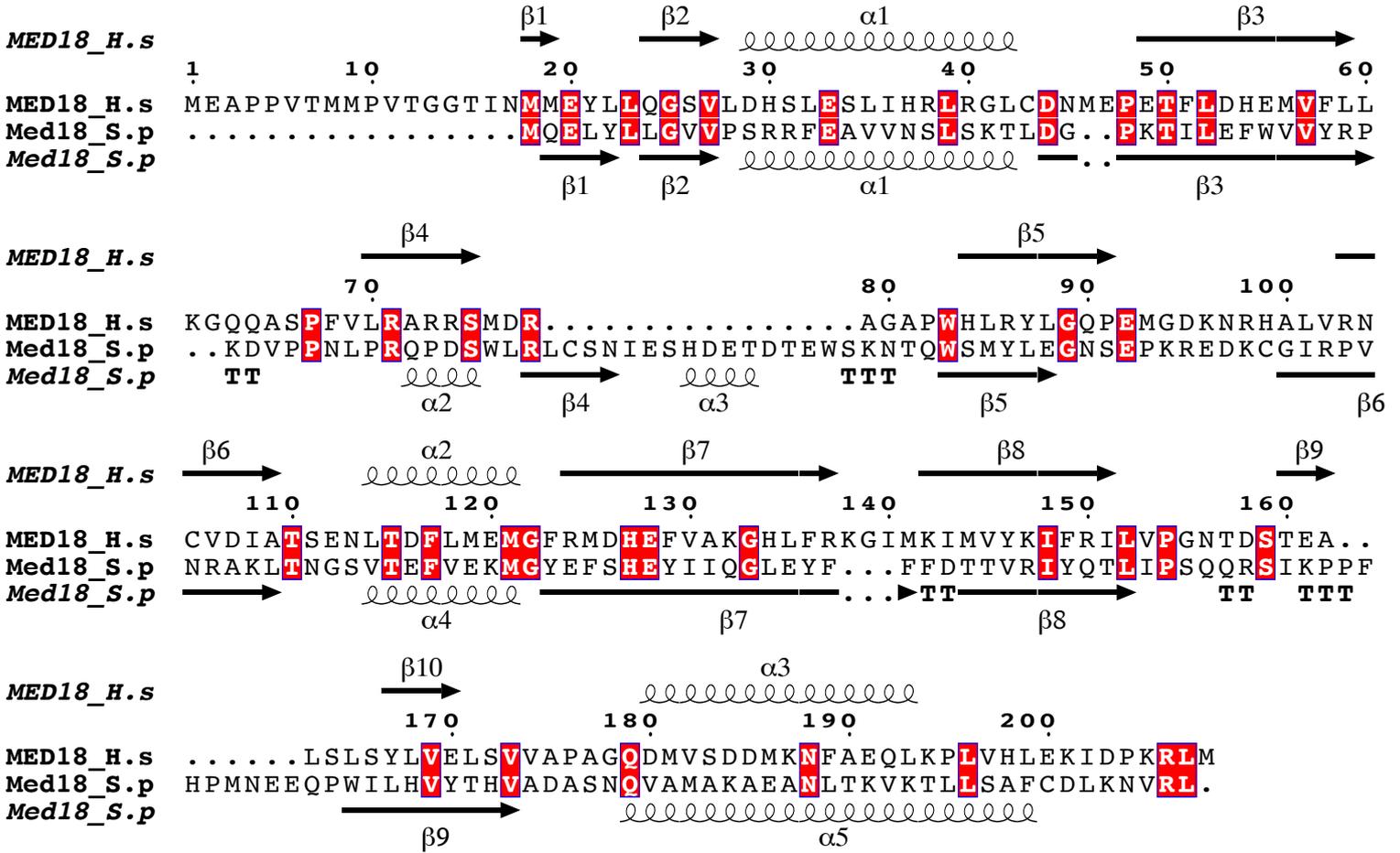
MED8



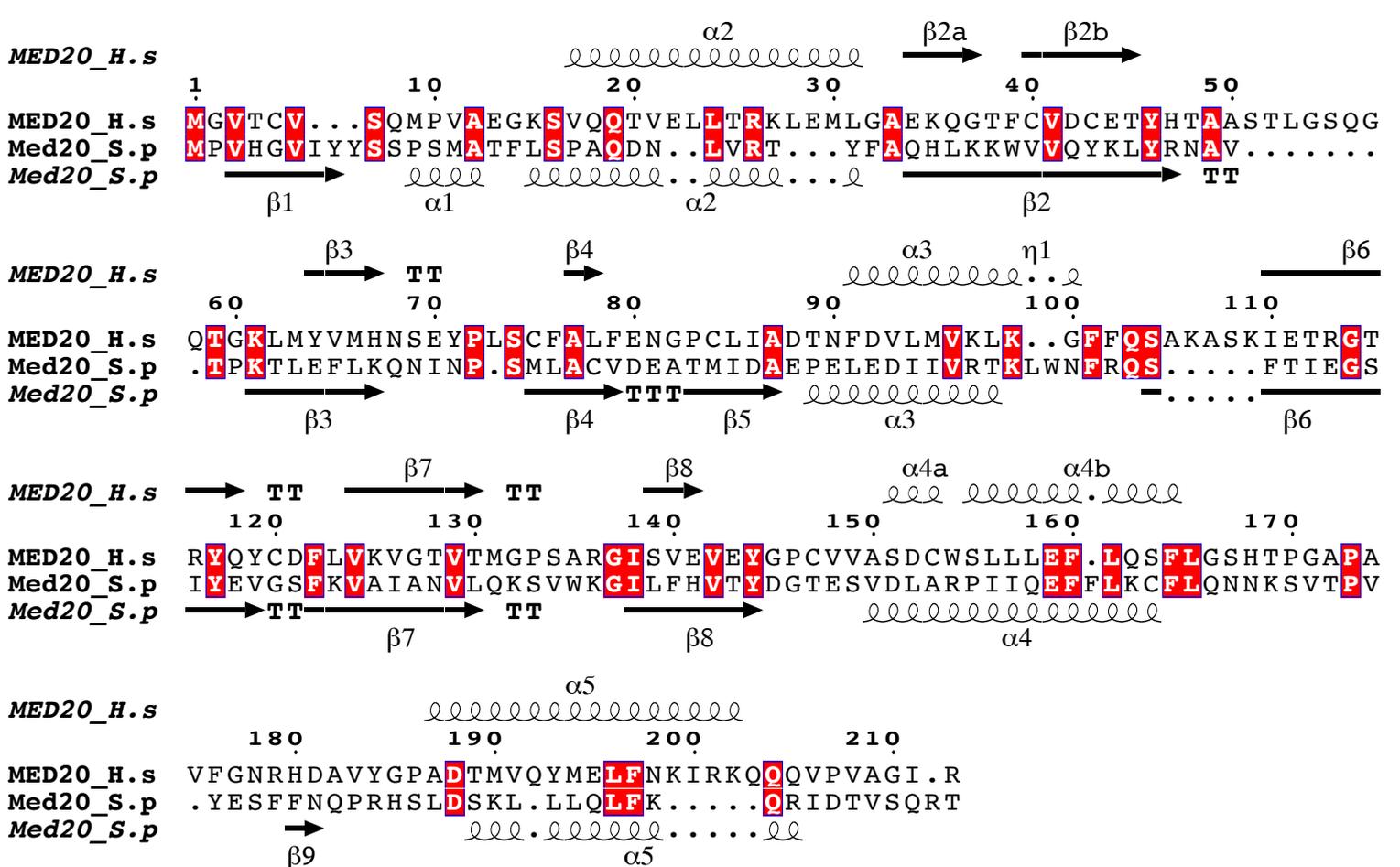
MED22



MED18



MED20



MED17

MED17_H.s

MED17_H.s 20 30 40 50 60
MSGVRAVRISIESACEKQVHEVGLDGTETYLPLPLSMSQNLARLAQRIDFSQGSSEEEEA
Med17_S.p MAEEANKDADISLSLSLSDPE.IIIGQNNF.....LENNLQQIFOKIIQERGPFRDLKEE
Med17_S.p TTTT
α1

MED17_H.s

MED17_H.s α2
AGTEGDAQEWPGAGSSADQDDEEGVVKFQPS.....LWPWDSVRNNLR
Med17_S.p DLQKELQKESIKDESSAKSSETENVLEFAT.LDSKRVNDTEVESMDSQAYKKELIEQIM
Med17_S.p TTTT
α2

MED17_H.s

MED17_H.s α3
SALTEMCVLYDVLSSIVRDKKF.....MTLDPVSQDALPPKQNPQTQL
Med17_S.p IAQTECSLALDMTSLLLSKFKENSIETISPFLLKSTVPPSSLQFSRSQPPESKESDATLAK
Med17_S.p TTTT
α3 η1 α4

MED17_H.s

MED17_H.s α4 α5
ISKKKSLAGAAQILLKGAERLTKSVTENQENKLQDFNSELRLRQH.WKLRKVGDK...
Med17_S.p CWKEKSLTSSCKFLFEAKERLTSVET.....EHYYTELVKVKEASWPLFNFSQGSNHL
Med17_S.p TTTT
α5 β1

MED17_H.s

MED17_H.s β1 β2
.....ILGDLSYRSAGSLFPHHGTFEVIKNTDLDDLKIPEDYCPLDVQIPSDLEGSAYI
Med17_S.p SVQYSCLGGISL.....GLGIRMKPESKSFVQS.....SLLYS.....QAAL
Med17_S.p TTTT
β2 β3 β4

MED17_H.s

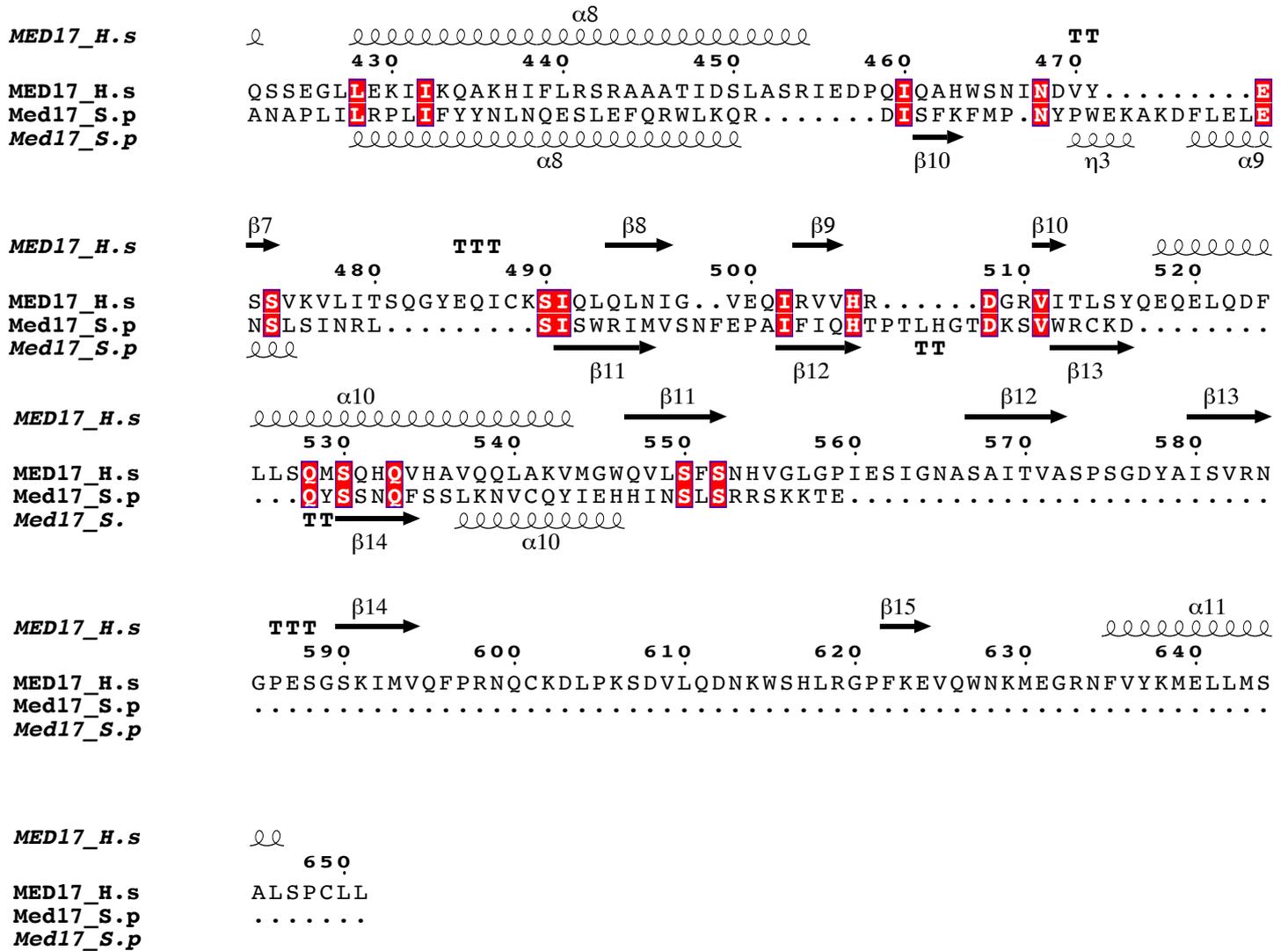
MED17_H.s β3 α6
KVSIQKQAPDIGDLGTVNLFKRPLPKSKPGSPHWQTKLEAAQNVLLCKEITFAQLSREAVQ
Med17_S.p KISILNKD...RDEIGSSTWSWPSQNC...NSVLLKDIYKLOEILFEMDIWNSLLQEAS
Med17_S.p TTTT
β5 β6 α6

MED17_H.s

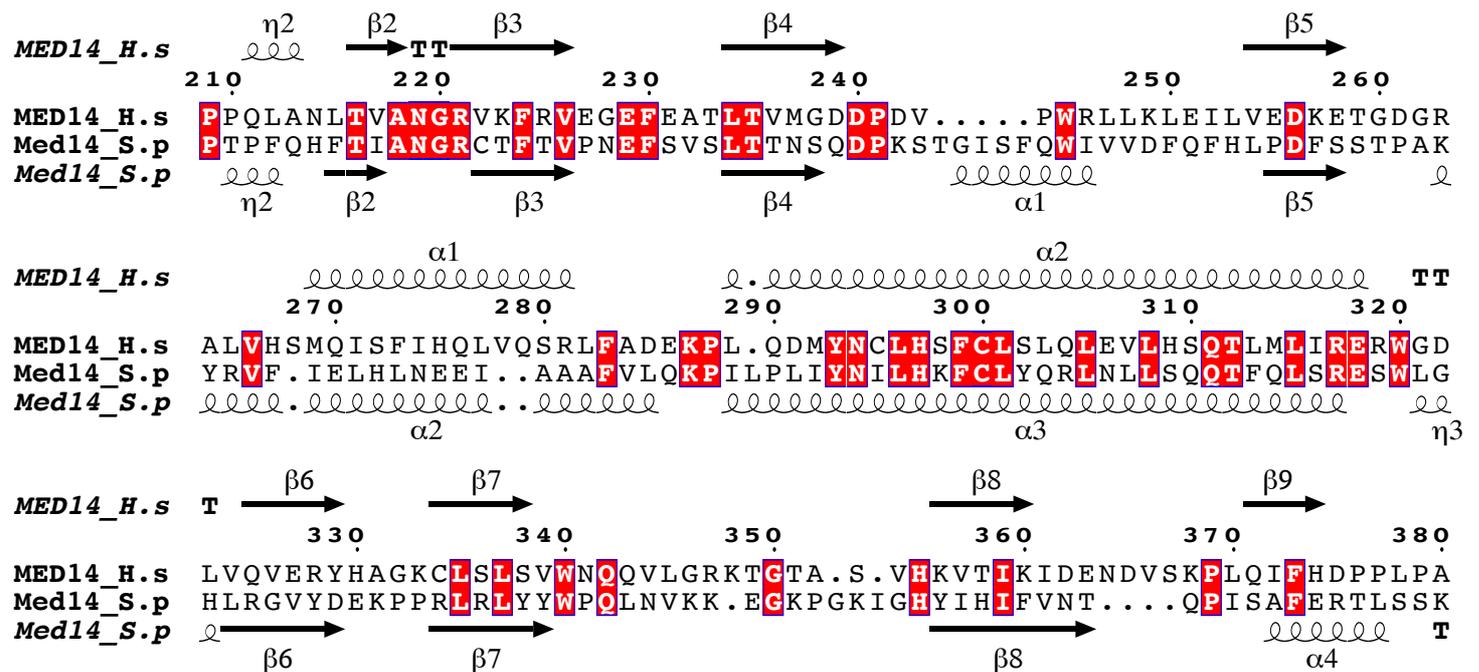
MED17_H.s β4 β5 T T β6
IKSQVPHIVVKNQIISQPFPSLQLSISLCHSSNDKKSQKFATEKQCPEDH.....
Med17_S.p CGNQ.GVNFTGDEILVPIISDDHVVRITLETSSKNTESGFTEDKKSNEDETSTNFVTIKQEK
Med17_S.p TTTT
η2 β7 β8 β9

MED17_H.s

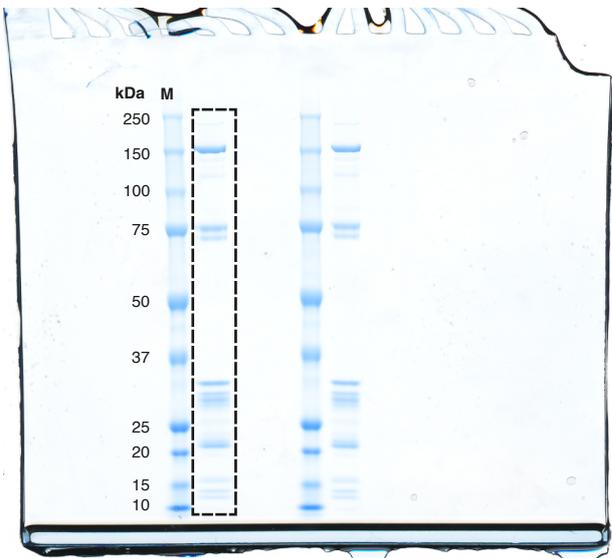
MED17_H.s α7 α7a α7b α7c
.....LYVLEHNLHLLIREFHKQTLSSIMPHPASAPFGHKRMLSGPQAFDKNEINSL
Med17_S.p ELLKCLCDTLNAIAHLLFLK.HCRKSDRRSQPELYMAID.....
Med17_S.p TTTT
α7



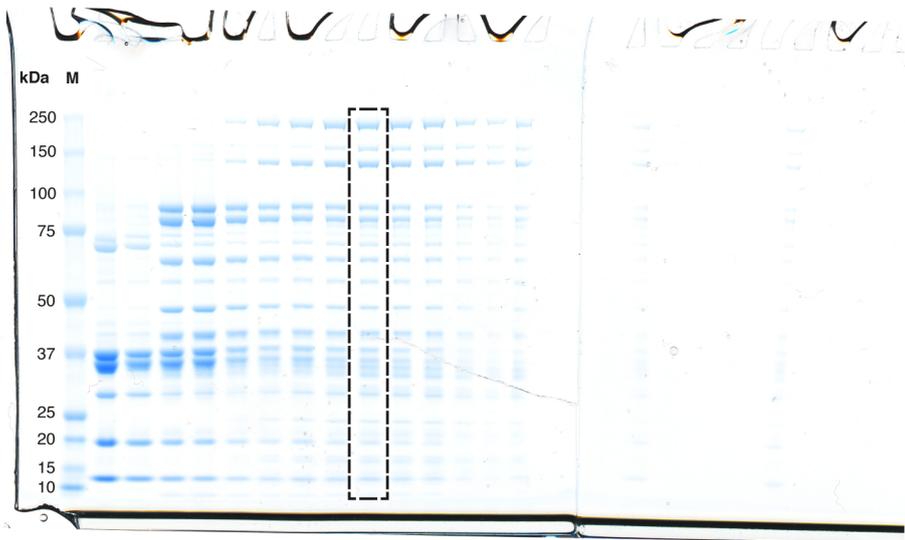
MED14



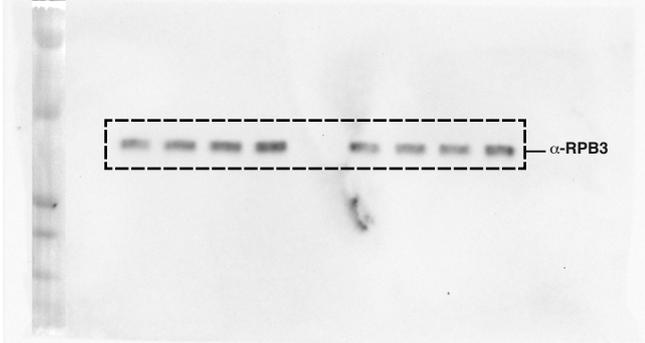
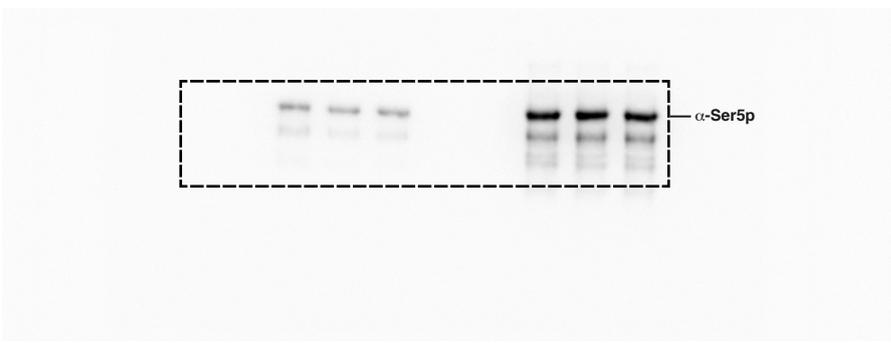
Extended data figure 1a



Extended data figure 1b



Extended data figure 1c



Supplementary Figure 2 | Raw unedited data of SDS-PAGE gels and western blots. Dashed boxes represent the regions cropped and used for interpretation