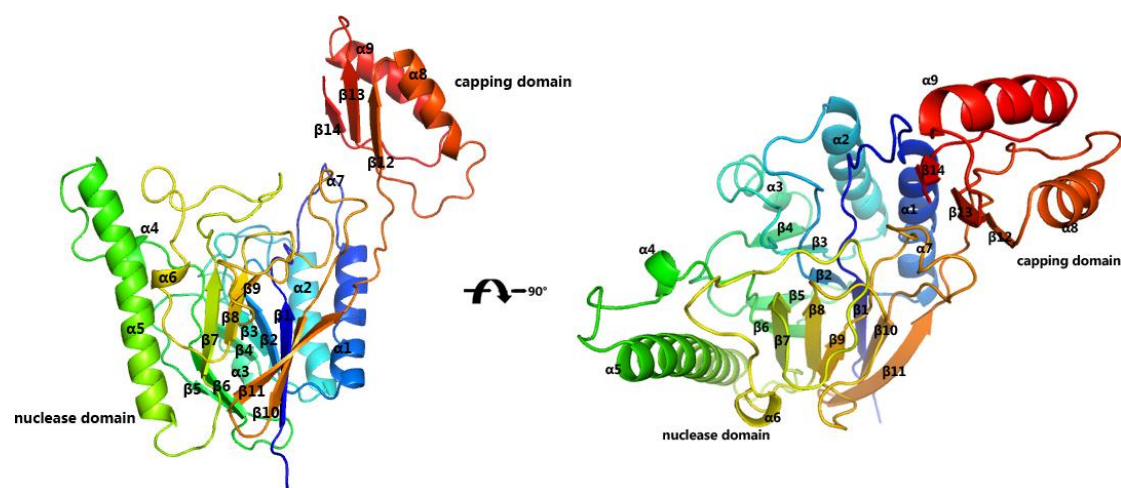
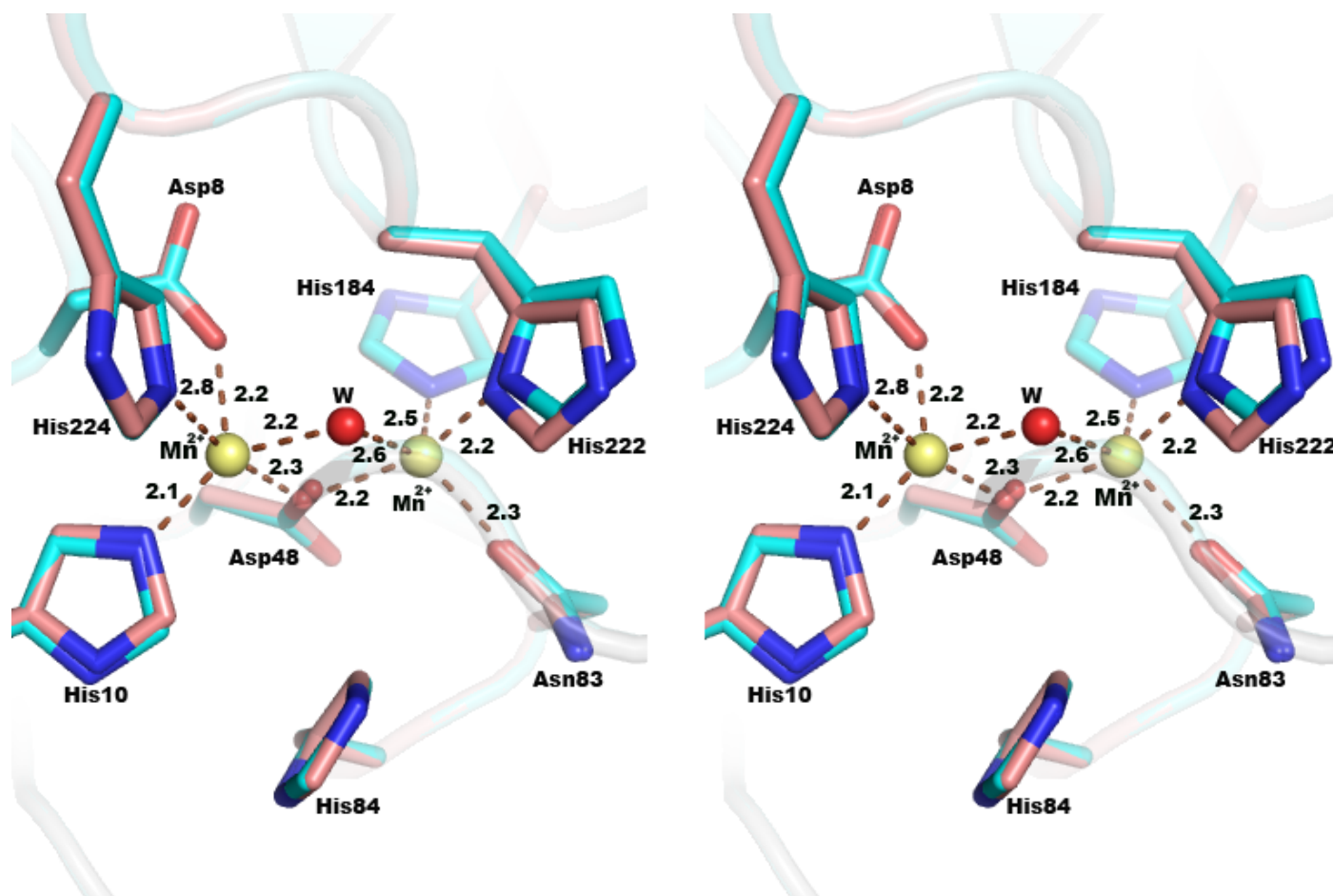


Supplementary Material



Supplementary Figure S1. Overall structure of SbcD monomer.



Supplementary Figure S2. Wall-eyed stereo presentation of the active site of SbcD and SbcD-Mn²⁺. SbcD and SbcD-Mn²⁺ are shown in brown and cyan, respectively. Oxygen atoms and nitrogen atoms are shown in red and blue, respectively. Yellow and red spheres represent Mn²⁺ and water molecule W, respectively. Interactions between atoms are depicted as brown short-dashed lines and interatomic distances are shown in Å.

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MjMre11 1 .....MMPVH*ADN*HL*CYRQYNL...DD*DEKDIYDSFKLC*IKK*ILE*IK*EVVLHS*GDL*FND.L
PfMre11 1 .....MKP*AHL*AD*IN*LCYE*QPHK...PQ*DEEF*AEAPKNA*LE*IAV*QEN*VD*FILL*AGDL*FHS.S
HuMre11 1 MSTADALDDENTFKIL*VAT*DI*NLC*PM*EKD...AV*GN*DTFV*TLDEI*LR*LAQ*ENE*VD*FILL*GDL*FHE.N
TmMre11 1 .....MINL*KELK*IL*HT*SD*WHL*CVTSWTSSRPVD*REEL*KKALDKV*VEE*AK*REVD*LILL*TGDL*LHSRN
SbcD 1 .....MRI*LHT*SD*WHL*CQNFYSK...S*REAE*HQAPLDWL*LET*AQTH*QV*DAI*IVA*GDF*EDT.G

MjMre11 55 RPPV*KAL*RIAMQA*FK*KLH*ENN.....IKV*IV*A*GN*HE*MPRR.
PfMre11 55 RPSFG*TLKKAIAL*LQ*IPKEHS.....IPV*FA*IE*GN*HD*ORTQRG
HuMre11 66 KPSRK*TLHTC*LEL*LR*KYCM*GDRFVQFEILSDQSVNFGFSKFPVNYQDGNLNI*SIV*PSI*HN*GD*DPTGA
TmMre11 65 NPSV*VALHDL*LDY*LK*RM*MTA.....FVV*VIL*GN*ED*DKGLK
SbcD 54 SP*PSY*ARTLYN*RFV*VNL*QCTG.....CHL*VVL*AG*NE*DSVATL

MjMre11 91 .....LGEES.....F*LALL*KDY*VK.....ILDG*KDV*IN*VNGEE*FICG*TTY
PfMre11 92 PSVLN*L.....LED*FGLVY.....VIGMR*KEK*VENE*YLTSE*RLGNG*EYL*VKG*VYKDL*EIGH*MKY
HuMre11 136 DALCALDILSCAGFVNHPGRSMGVKIDISFVLLQ*KGS*T*KIALYGL*GSIPDE*RLY*RMFVN*KKVTML*RPKE
TmMre11 101 .....LPGNFVT.....SI*SSD*ITF*VMS*PEPVD*VEAK*RG*QK*VRIL*PPFP*DESEAL*R
SbcD 91 NESRD.....IMAF*LNTTVVASAGHAP*QIL*PR*RDGT*FGAV*LCPI*FP*LR*PD*IT*SQAG*LNGIEK*QQH

MjMre11 129 HKKSKREEMLDKLN*FES.....EAK*NYKK*K*ILMLH*QG.....
PfMre11 146 MSSAWPEANKEILKRL*FR.....PTDN.....AIL*MLH*QG.....
HuMre11 206 DENSWFNL*FVIHQ*NRSKHG*STN*PIEQ*PLDD*FIDL*VIW*GHEHECK*IAPT*KNE*QQL*FYIS*QPGSS*VVTSLS
TmMre11 148 KNEGDFRFFLESRLN*KLY.....LAGY*AGIE*QGRE*IIIN*RAL*IPS*VVDY*AAL*GHT*HS*FRE
SbcD 153 LLAAITDY*YQQHYADACK.....LRGD*Q*PLPI*ATGHL*TTVG.....

MjMre11 161 .....INPY*IFLD.....YELE*HFPD*LPK*.FSY*YA*LGH*TH*KRI*L
PfMre11 176 .....VREV*SEARGEDY*FEIGL*GDL*LPEGY*LYAL*GHT*HKRY*E
HuMre11 276 PGEAVKKHVGLLRIGRKMNMHKIPLHTVRQFFMEDI*VLAN*HPDI*FPN*DPN*KV*TQAI*QSFC*LEK*TEML*E
TmMre11 106 .....LAGY*AGIE*QGRE*IIIN*RAL*IPS*VVDY*AAL*GHT*HS*FRE
SbcD 190 .....ASK*SDA*V*REDIYIG*TLDA*PPAQ*N*PPPADY*IAL*GHT*TH*RAQ*I

MjMre11 193 ERFNDGI.....LAYSG.....STEI*YRNEYED*.YKKEG*KGFYLV
PfMre11 213 TSYSGSP.....GVY*IVED*FKPRFVEIKVRPFID*VKIKG.....SEE*EIRKA*IKRLI*PLIP*KN*AV*VRNL*IGW
HuMre11 346 NAERERLGN*SHQ*PEKPLVRL*VDYSGGPEP*PSVLR*FSQ*KFVDRVAN*PKD*IIH*PPRH*EQKEKT*GEEIN*FG
TmMre11 223 IQKQFLT.....IYPG.....SLIRID*FGEEAD*.EKGAV*PVELK
SbcD 229 IGGMEHVR.....YCG.....SPI*PLSFDECGK.....SKYVHLV

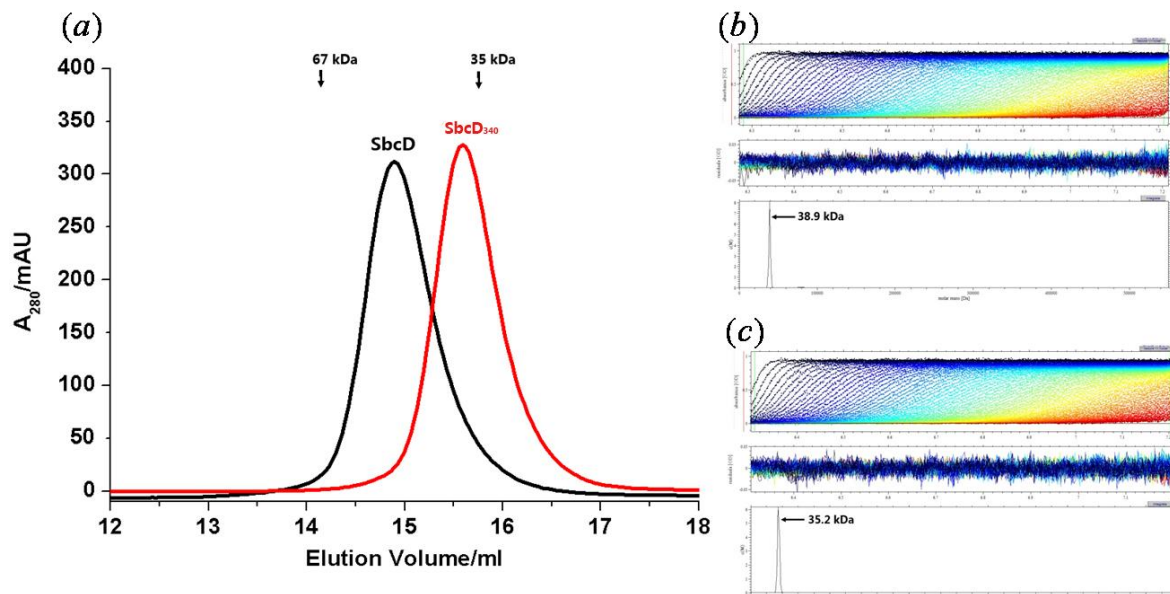
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PfMre11 249 YGVNK.....GPY*IVED*FKPRFVEIKVRPFID*VKIKG.....SEE*EIRKA*IKRLI*PLIP*KN*AV*VRNL*IGW
HuMre11 416 KLITKPS*EGTTL*RVED*LVKQYFQ*TA*EKNV*QLSLL*TERGMGEA*VQE*FVDK*EKDAIE*RLVKY*QLEKT*QRFL
TmMre11 256 RGEPP.....RYE*RIDAS*PLPLK*TLYYKKID*TSALKS.....IRD*FCRN*PPGY*VRVY*EED*SG*ILEP*LMG
SbcD 259 TF*SGN.....KLE*SVEN*LNVPV*TQPM*AVLKGD*LASIT....AQLE*QWRD*VSQE*PPVWLD*IEIT*DEY*LHD

MjMre11 285 KP*.WFD*TL*KDK*IL*IN*KAI*IVDD.....EPT*DMFD*NVD*TES*LN*IKELLV
PfMre11 309 RK*.FPDL*TEI*KEL*LNVEY*EKLID*TWRIKERTDEESGKIGLPSDFPTEFELKII*DILGE*KD*FDD*FDY*IIKLI
HuMre11 486 KERHIDA*LED*KID*EEVRR*PRE*TRQKNTNEEDDEVREAMTRARALRSQSEESAS*APSADD*LMS*IDLA*EQMA
TmMre11 316 EIDNLV*KIB*RKS*REIEE*VLRE.....SPEEP*KEE*LDK*LDY*PEL*FK
SbcD 320 IQRKIQAL*TE*SL*VEVLL*VRS.....REQRERV*LASQ*QRET*LSEL*SVVE*EVFN

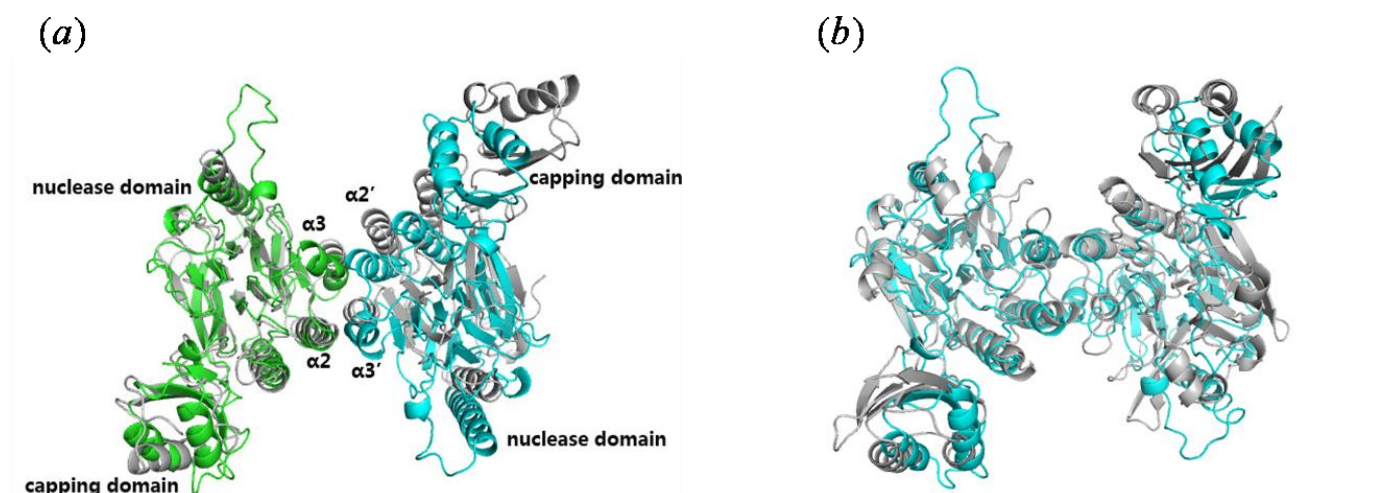
MjMre11 327 DYAN.....RQGI*DGDL*VL*SLYKALLNNEN*NKE*LLDEY*YNTK*FRG.....
PfMre11 378 TEG.....KVEE*EGPLE*EA*VKKVSE*EKGKT*VRQ*KIESIPKKKRG*TLDSWL
HuMre11 556 NDSDD*SISAATN*KGRGRGRGRGRGRGQNSAS*RGGSQ*RGRAD*TGLETSTR*GRNS*KTAV*SASRNMS*IIDAFK
TmMre11 357 EYLK.....KRE*ENHEK*LLKIL*DEL*LDDEVK*KEA.....
SbcD 368 RRLA.....LE*ELDESQ*QQL*QHL*PTTT*LHTL*AGEHEA.....

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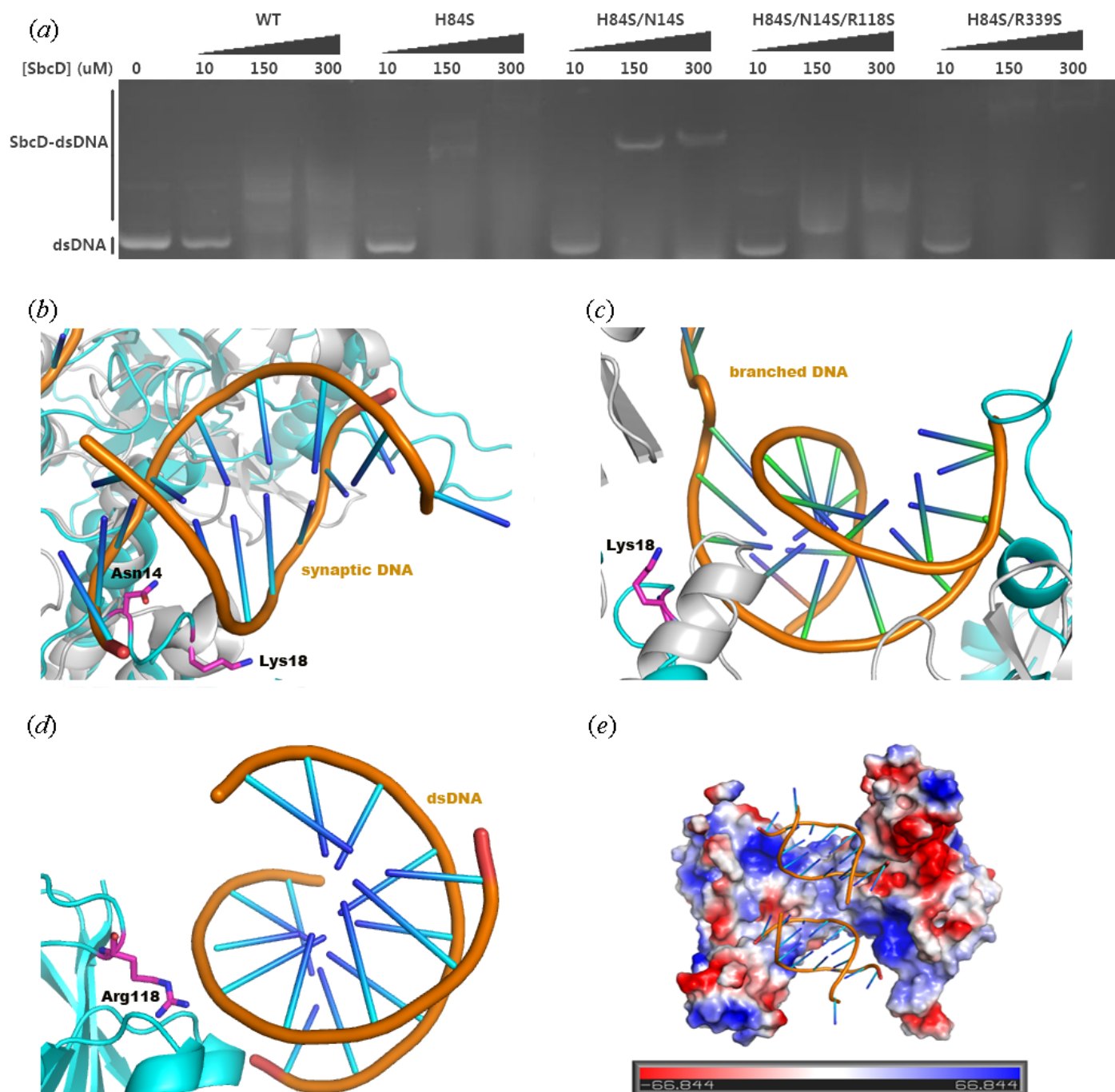
Supplementary Figure S3. Primary sequence alignment between *MjMre11*, *PfMre11*, *HuMre11*, *TmMre11* and *SbcD*.



Supplementary Figure S4. (a) Size-exclusion chromatography of SbcD and SbcD₃₄₀. SbcD and SbcD₃₄₀ proteins were applied to a 24 ml superdex200 column, respectively. (b) Analytical ultracentrifugation assay of SbcD. (c) Analytical ultracentrifugation assay of SbcD₃₄₀.

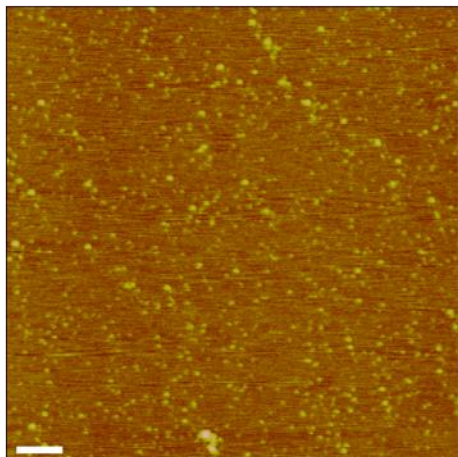


Supplementary Figure S5. Structural comparison of SbcD dimer with other Mre11 dimers. (a) Structural comparison of SbcD dimer (green and cyan) with *Tm*Mre11 dimer (gray) via one protomer (left). (b) Structural comparison of SbcD dimer (cyan) with *Pj*Mre11 dimer (gray) via one protomer (left).

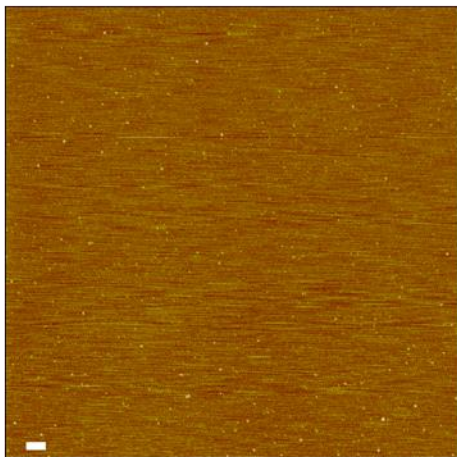


Supplementary Figure S6. (a) EMSA assays of wild-type SbcD and SbcD variants using dsDNA. (b) Superposition of SbcD with *Pf*Mre11-synaptic DNA. Synaptic DNA is shown by cartoon in brown. SbcD and *Pf*Mre11 are shown in cyan and gray, respectively. Asn14 and Lys18 of SbcD are shown by stick in purple. Oxygen atoms and nitrogen atoms are shown in red and blue, respectively. (c) Superposition of SbcD with *Pf*Mre11-branched DNA. Branched DNA is shown by cartoon in brown. SbcD and *Pf*Mre11 are shown in cyan and gray, respectively. Lys18 of SbcD is shown by stick in purple. Nitrogen atoms are shown in blue. (d) A diagram of interactions between dsDNA and Arg118 of SbcD. SbcD and dsDNA are shown by cartoon in cyan and brown, respectively. Arg118 of SbcD is shown by stick in purple. Oxygen atoms and nitrogen atoms are shown in red and blue, respectively. (e) dsDNA-binding model of SbcD dimer. Surface electrostatic potential map of SbcD dimer is shown.

(a)



(b)



Supplementary Figure S7. AFM analysis of SbcD and SbcD₃₄₀. *(a)* SbcD (1 ng/μl). *(b)* SbcD₃₄₀ (1 ng/μl). Scale bar=200 nm.