# Science Advances

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## Supplementary Materials for

## CryoEM structure of MxB reveals a novel oligomerization interface critical for HIV restriction

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- fig. S13. Comparison of MxB and MxA GTPase-BSE domains.

**Other Supplementary Material for this manuscript includes the following:**
(available at advances.sciencemag.org/cgi/content/full/3/9/e1701264/DC1)

- movie S1 (.mp4 format). Molecular dynamics flexible fitting of MxB into the cryoEM map.
- movie S2 (.mp4 format). MxB assembles into a helical array.
- movie S3 (.mp4 format). Sα1c helix kinks from linear to tubular array.
- movie S4 (.mp4 format). Formation of Interface 3 during helical assembly.

**table S1. Effects of MxB mutations at intermolecular interfaces 1, 3, and 4.**

| Interface | Mutation | Fold Effect on HIV-1 restriction* | Oligomerization[‡] | References |
|---|---|---|---|---|
| Putative Interface 1 crystal | I423D | < 1 × | ND | (15) |
| | I423D | ND | >250kDa[xl] | (19) |
| | I423D | ~ 1 × | >250kDa[xl] | (18) |
| | E424R | ~ 1 × | >250kDa[xl] | (19) |
| | K663D | < 1 × | ND | (15) |
| | K663D | ~ 0.8 | >250kDa[xl] | (19) |
| | M666D | < 1 × | ND | (15) |
| | M666D | ND | >250kDa[xl] | (19) |
| | M666D | < 1 × | >250kDa[xl] | (18) |
| | L669D | ~ 2 × | >250kDa[xl] | (19) |
| | I423D/K663D | ~ 1 × | dimer[gf] | (15) |
| | I423D/K663D/M666D | ND | dimer[gf] | (15) |
| Interface 1 cryoEM | **F420D** | ~ 1 × | oligomers[em] | This study |
| | **K693D** | ~ 1 × | ND | This study |
| | **F420D/K693D** | ~ 1 × | ND | This study |
| Interface 3 cryoEM | **E484K** | ~ 3 × | ND | This study |
| | **F495D** | ~ 3.5 × | No oligomers[em] | This study |
| | **R449D** | ~ 3 × | No oligomers[em] | This study |
| Interface 4 cryoEM | **E285K** | ~ 1 × | oligomers[em] | This study |
| | **W677D** | ~ 1 × | ND | This study |
| | **E285K/W677D** | ~ 1 × | ND | This study |
| Other mutations | Δ440-448(L1)/GSGGSG | ~ 2 × | ND | (11) |
| | Δ488-497(L2)/GSGGSG | ~ 3.5 × | ND | (11) |
| | Δ584-615(L4)/GAGAG | ~ 2 × | ND | (11) |
| | Δ580-608 (L4) | ~ 2 × | ~ 190kDa[xl] | (18) |
| | G439D | ~ 1 × | ND | (11) |
| | G439D | ~ 1 × | >250kDa[xl] | (18)[‡] |
| | R455A | ~ 2 × | ND | (11) |
| | R455D | ~ 5.5 × | ND | (11) |
| | R455D | ~ 5 × | No oligomers[xl] | (18)[‡] |
| | $^{487}$YRGK$^{490}$/AAAA | < 1 × | ~ 190kDa[xl] | (18)[‡] |
| | $^{487}$YRGK$^{490}$/AAAA | ~ 2 × | ND | (15) |
| | E681A | ~ 1 × | ND | (15) |
| | E681A | ~ 2 × | >250kDa[xl] | (18) |
| | R689A | ~ 2 × | ND | (15) |
| | R689A | ~ 2 × | >250kDa[xl] | (18) |

*Fold defect in HIV-1 restriction compared to the wild-type. 2x effect means 50% of wild-type antiviral activity. ‡Measurement of oligomerization by cross-linking (xl) or gel filtration (gf) or electron microscopy (em). ‡Mutations were thought to be at the hypothetical "*Interface 3*" (18) (fig. S8B). Mutations in bold are from this study and those in red have significantly reduced effect on anti-HIV-1 activity of MxB (cut off at < 3 fold effective compared to the wild-type). ND, not determined.

N-terminal Region (NTR)

Bα1  Gβ1  Gα1  Gβ2

| | | |
|---|---|---|
| MX2_HUMAN | 1 | MSKAHKPWPYRRRSQFSSRKYLKKEMNSFQQQPPPFGTVPPQMMFPPNWQGAEKDAAFLAKDFNFLTLNNQPPPGNRSQPRAMGPENNL |
| MX1_HUMAN | 1 | ...................................................MVVSEVDIAKADPAAASHPLLLNGDATVAQKNPGSVAENNL |
| MX1_MOUSE | 1 | ...........................................................................MDSVNNL |
| MX2_MOUSE | 1 | ...................................................MVLSTEENTGVDSVNLPSGETGLGEKDQESVNNL |
| MX2_PIG | 1 | MPKPRMSWPYQRHRQASSPPHPHKEMNFFPQQPLPLGAGPGQMTFPLNWQMGGKD...LTKGLNMLTLSPQQPGGKSGQQTSKGPENNL |
| MX_CHICKEN | 1 | ..MNNPWSNFSSAFGCPIQIPKQNSNVPPSLPVPVGVFGVPLRSGCSNQMAFCAPELTDRKPE.HEQKVSKRLNDREEDKDEAAACSL |
| MXA_ZEBRAFISH | 1 | ..................................................................MEKLSYTF |
| DYN1_HUMAN | 1 | ..................................................................MG |
| DYN2_HUMAN | 1 | ..................................................................MG |
| DYN3-2_HUMAN | 1 | ..................................................................MG |
| DNM1L_HUMAN | | ...................................................................... |
| DNM1_YEAST | | ...................................................................... |

Bα1  Gβ1  Gα1  Gβ2

| | | |
|---|---|---|
| MX2_HUMAN | 90 | YSQYEQKVRPCIDLIDSLRALGVEQDIALPAIAVIGDQSSGKSSVLEALSGVA.LPRGSGIVTRCPLVLKLKKQP............ |
| MX1_HUMAN | 42 | CSQYEEKVRPCIDLIDSLRALGVEQDIALPAIAVIGDQSSGKSSVLEALSGVA.LPRGSGIVTRCPLVLKLKKLVN............ |
| MX1_MOUSE | 8 | CRHYEEKVVRPCIDLIDTLRALGVEQDIALPAIAVIGDQSSGKSSVLEALSGVA.LPRGSGIVTRCPLVLKLRKLKE............ |
| MX2_MOUSE | 35 | CSQYEEKVVRPCIDLIDSLRALGVEQDIALPAIAVIGDQSSGKSSVLEALSGVA.LPRGSGIVTRCPLVLKLRKLNE............ |
| MX2_PIG | 87 | YSPFEEKVVRPCIDLIDSLRALGVEQDIALPAIAVIGDQSSGKSSVLEALSGVP.LPRGSGIIRCPLALRLKKKE............ |
| MX_CHICKEN | 86 | DNQYDRKIQFCIDLVDSDRKLDIGNDIMLPAIAVIGDRNSGKSSVLEALSGVA.LPRDKGVITRCPLELKLKKMTA............ |
| MXA_ZEBRAFISH | 9 | SQQYEEKIRFCIDTIDNIRSLGVEKDIALPAIAVIGDQSSGKSSVLEALSGVP.LPRGSGIVTRCPLELKMIRTKD............ |
| DYN1_HUMAN | 3 | NRGMEDLIPIVNRLQDAFSAIGQNADIDLPQIAVVGGQSAGKSSVLENFVGRDFLPRGSGIVTRRPLVLQLVNAT............ |
| DYN2_HUMAN | 3 | NRGMEELIPIVNKLQDAFSSIGQSCHIDLPQIAVVGGQSAGKSSVLENFVGRDFLPRGSGIVTRRPLILQLIFSK............ |
| DYN3-2_HUMAN | 3 | NREMEELIPIVNRLQDAFSALGQSCILELPQIAVVGGQSAGKSSVLENFVGRDFLPRGSGIVTRRPLVLQLVTSK............ |
| DNM1L_HUMAN | 1 | ...MEALIPVINKLQDVFNTVGADI.IQLPQIVVVGTQSSGKSSVLESLVGRDLLPRGTGIVTRRPLILQLVHVSQEDKRKTTGEENGV |
| DNM1_YEAST | 1 | MASLEDLIPTVNKLQDVMYDSGIDT.IDLPQIALVVGSQSSGKSSILEILVGRDFLPRGTGIVTRRPLVLQLNNISPNSPLIEEDDNSVN |

GβE1  GαE1  GβE2  Gβ3

| | | |
|---|---|---|
| MX2_HUMAN | 164 | ....................CEAWAGRISYRNTELELQDPGQVEKEIHKAQNVMAGNGRGISHELISLBITSPEVPDLTIID |
| MX1_HUMAN | 117 | ....................EDKWRGKVSYQDYEIEISDASEVEKEINKAQNAIAGEGMGISHELITLISSRDVPDLTLID |
| MX1_MOUSE | 83 | ....................GEEWRGKVSYDDIEVELSDPSEVEEAINKGQNFIACVGLGISDKLISLVSSPNVPDLTLID |
| MX2_MOUSE | 110 | ....................GEEWRGKVSYDDIEVELSDPSEVEEAINKGQNFIAGVGLGISDKLISLDVSSPNVPDLTLID |
| MX2_PIG | 161 | ....................CPHQGRISYRKVELQLQDPSQVEREIRKAQDAIAGSSGVGISHELISLIVTSPEVPDLTLID |
| MX_CHICKEN | 161 | ....................PQEWKGVIYYRNTEIQLQNASEVKKAIRKAQDIVAGTNGSISGELISLBIWSDVPDLTLID |
| MXA_ZEBRAFISH | 84 | ....................QDRHGRISYKTCEEDFDDPAEVEKKIRQAQDEMAGAGVGISEEIISLGITSADVPDLTLID |
| DYN1_HUMAN | 78 | ....................TEVAEFLHCKG..KKFTDFEEVRLEIEAETDRVTGTNKGISPVPINLVYSPHVLNLTLVD |
| DYN2_HUMAN | 78 | ....................TEHAEFLHCKS..KKFTDFDEVRLEIEAETDRVTGTNKGISPVPINLVYSPHVLNLTLID |
| DYN3-2_HUMAN | 78 | ....................AEVAEFLHCKG..KKFTDFDEVRLEIEAETDRVTGMNKGISSIPINLVYSPHVLNLTLID |
| DNM1L_HUMAN | 86 | E....................AEEWGKFLHTKN..KIYTDFDEIRQEIENETERISGNNKGVSPEPIHLKIFSPNVVNLTLVD |
| DNM1_YEAST | 89 | PHDEVTKISGFEAGTKPLEYRGKERNHADEWGEFLHIPG..KRFYDFDDIKREIENETARIAGKDKGISKIPINLRVFSPHVLNLTLVD |

Gα2  Gβ4  Gα3  Gβ5  Gα4

4    4 44

| | | |
|---|---|---|
| MX2_HUMAN | 226 | LPGITRVAVDNQPRDIGLQIKALIKKYIQRQQTINLVVVPCNVDIATTEALSMAHEVDPBGDRTIGILTKPDLMDRGTEKSVMNVVRNL |
| MX1_HUMAN | 179 | LPGITRVAVGNQPADIGYKIKLLIKKYIQRQETISLVVVPSNVDIATTEALSMAQEVDPBGDRTIGILTKPDLVDKGTEDKVVDVVRNL |
| MX1_MOUSE | 145 | LPGITRVAVGNQPADIGRQIKRLIKTYIQKQETINLVVVPSNVDIATTEALSMAQEVDPBGDRTIGILTKPDLVDRGTAEGKVLDVVRNL |
| MX2_MOUSE | 172 | LPGITRVAVGNQPADIGRQIKRLIKTYIQKQETINLVVVPSNVDIATTEALSMAQEVDPBGDRTIGILTKPDLVDRGTEDKVVDVVRNL |
| MX2_PIG | 222 | LPGITRVAVGNQPODIGLQIKALIRKYIQEQQTINLVVVPCNVDVEREIRKAQDAIAGSSGVGISHELISLIVTSPEVPDLTLID... |
| MX_CHICKEN | 223 | LPGIAREAVGNQPQDNGQQIKLLLKKYIGCKETIIVVVVPCNVDIATTEALRMAQEVDPTGERTLGVLTKPDLVNEGTEETVLKIIQNE |
| MXA_ZEBRAFISH | 146 | LPGIARVAVKGQPENIGDQIKRLIRKFVTRQETINLVVVPCNVDIATTEALQMAQAEDPDGERTLGILTKPDLVDGITSGTGVVDIVHNE |
| DYN1_HUMAN | 137 | LPGMTKVPVGDQPQDIEFQIRDMLMQFVTKENCLILAVSPANSDLANSDALKVAKEVDPQGQRTIGVITKLDLMDEGTD..ARDVLENK |
| DYN2_HUMAN | 137 | LPGITKVPVGDQPPDIEYQIKDMLLQFISRESSLILAVTPANMDLANSDALKLAKEVDPQGQRTIGVITKLDLMDEGTD..ARDVLENK |
| DYN3-2_HUMAN | 137 | LPGITKVPVGDQPPDIEYQIRPMIMQFITRENCLILAVTPANTDLANSDALKLAKEVDPQGLRTIGVITKLDLMDSGTD..ARDVLENK |
| DNM1L_HUMAN | 147 | LPGMTKVPVGDQPKDIELQIRELILFISNPNSILILAVTAANTDMATSEAKISREVDPDGRRTLAVITKLDLMDAGTD..AMDVLMGR |
| DNM1_YEAST | 176 | LPGITKVPIGEQPQDIEKQIKNLILDYIATPNCLILAVSPANVDLVTSESLKLAREVDPQGKRTIGVITKLDLMDSGTN..ALDILSGK |

Gβ6  GαE2  Gα5  Bα2

| | | |
|---|---|---|
| MX2_HUMAN | 315 | TYPLKKGYMIVKCRGQQEITNRLSLAEATKKEITFFQTHPYFRVLLEEGSATVPRLAERLTTELIMHIQKSLPLLEGQIRESHQKATEE |
| MX1_HUMAN | 268 | VEHLKKGYMIVKCRGQQEIQDQLSLSEALQREKIFFENHPYFRDLLEEGKATVPCLAEKLTSELITHICKSLPLLENQIKETHQRITEE |
| MX1_MOUSE | 234 | VVPLKKGYMIVKCRGQQDIQEQLSLIEAFQKEQVFFKDHSYFSILLEDGKATVPCLAERLTEELTSHICKSLPLLENQINSSHQSASEE |
| MX2_MOUSE | 261 | VVHLKKGYMIVKCRGQQDIQEQLSLTEALQNEQIFFKEHPYFRILLEEGKATVPCLAERLTAELTHCKSLPLLENQIKESHQSASEE |
| MX2_PIG | 311 | TVHLKKGYMIVKCRGQQEILNKISLAEATKREIAFFHSHPHFRILLEEGKATVPRLAERLTVELIGHISKSLPLLFSQIKEIHQSATEE |
| MX_CHICKEN | 312 | VPLRKGYMIVKCYGQMDFCNELSFTSAIQQEREFFETHKHFSILLDENKATIPHLANKLTDELVGRILKILPAIEKQVHDALQQAKKE |
| MXA_ZEBRAFISH | 235 | VIHLTKGYMIVKCRGQKEIMDQVTLNEATETESAFFKDHPHFSKLYEEGFAIIPKLAEKLTIEIVHHIQKSLPRLEEQIETKLAETQKE |
| DYN1_HUMAN | 224 | LLPLRRGYIGVVNRSQKDIDGKKDITAALAAERKFFLSHPSYRHLADRMGTPYLQKTLNQQLTNHIRDTLPGLRNKLQSQLLSIEKE |
| DYN2_HUMAN | 224 | LLPLRRGYIGVVNRSQKDIDGKKDIRAALAAERKFFLSHPAYRHMAD.RMGTPYLQKTLNQQLTNHIRESLPALRSKLQSQLLSLEKE |
| DYN3-2_HUMAN | 224 | LLPLRRGYVGVVNRSQKDIDGKKDIKAAMLAAERKFFLSHPAYRHIAD.RMGTPYLQKTLNQQLTNHIRDTLPNFRNKLQGQLLSIEHE |
| DNM1L_HUMAN | 234 | VIPVKLGIIGVVNRSQLDINNKKSVTDSIRDEYAPLQKK..YPSLAN..RNGTKYLARTLNRLLMHHIRDCLPELKTRINVLAAQYQSL |
| DNM1_YEAST | 263 | MYPLKLGFVGVVNRSQQDIQLNKTVEESLDREEDYFRKHPHPVYRTIST..KCGTRYLAKLLNQTLLSHIRDKLPDIKTKLNTLISQTEQE |

Figure S1 continue

Figure S1 continue

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                              Bα3

MX2_HUMAN     706  LCQFSSKEIH...............................................................
MX1_HUMAN     657  LAQFPG...................................................................
MX1_MOUSE     626  LAKFSD...................................................................
MX2_MOUSE     650  LAKFPG...................................................................
MX2_PIG       702  LSKFAQSLQG...............................................................
MX_CHICKEN    699  LVDFKSL..................................................................
MXA_ZEBRAFISH 629  LGHLLEVTFIDYCNILMQ.......................................................
DYN1_HUMAN    742  IGDINTTIVSTPMPPPVDDSWLQVQSVPAGRRSPTSSPTPQRR..APAVPPARPGS.RGPAPGPP....PAGSALG.GAPPVPSRPG..
DYN2_HUMAN    736  IGDISTSTVSTPVPPPVDDTWLQSAS.........SHSPTPQRRPVSSIHPPGRPPAVRGPTPGPPLIPVPVGAAASFSAPPIPSRPGPQ
DYN3-2_HUMAN  732  IGDISTATVSTPAPPPVDDSWIQHSR.....RSPPPSPTTQRRP.TLSAPLARPTSGRGPAPAIP....SPGPHS..GAPPVPFRPGPL
DNM1L_HUMAN   727  IAEIRETHLW...............................................................
DNM1_YEAST    753  ISNIL....................................................................

                     4  4
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MX2_HUMAN           ...............................................
MX1_HUMAN           ...............................................
MX1_MOUSE           ...............................................
MX2_MOUSE           ...............................................
MX2_PIG             ...............................................
MX_CHICKEN          ...............................................
MXA_ZEBRAFISH       ...............................................
DYN1_HUMAN    821   ....ASPDPFGPPPQVPSRPNR....APPGVPSRSGQASPSRPESPRP..PFDL.
DYN2_HUMAN    817   SVF.ANSDLFPAPPQIPSRPVRIPPGIPPGVPSRRPPAAPSRPTIIRPAEPSLLD
DYN3-2_HUMAN  809   PPFPSSSDSFGAPPQVPSRPIR....APPSVPSRRPPPSPTRPTIIRPLESSLLD
DNM1L_HUMAN         ...............................................
DNM1_YEAST          ...............................................
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**fig. S1. Sequence alignment of Mx and dynamin proteins.** Amino acid sequences of Mx and dynamin proteins were aligned using MUSCLE and the alignment visualized using ESPript3. The sequences include: MX2_HUMAN (Uniprot ID P20592), MX1_HUMAN (P20591), MX1_MOUSE (P09922), MX2_MOUSE (Q9WVP9), MX2_PIG (A7VK00), MX_CHICKEN (Q90597), MXA_ZEBRAFISH (Q8JH68), DYN1_HUMAN (Q05193), DYN2_HUMAN (P50570), DYN3-3_HUMAN (Q9UQ16), DNM1L_HUMAN (O00429) and DNM1_YEAST (P54861). Residues with a conservation of greater than 70% are color-coded (D, E in red; R, K, H in blue; N, Q, S, T in grey; L, I, V, F, Y, W, M, C in green; P, G in brown). Alpha-helices are shown as cylinders, while beta-sheets as arrows, with colors as in Figure 3C. The N-terminal region is represented as red dashed line and the unresolved regions in black dashed line. Residues involved in the interfaces (1, 3 and 4) are indicated on top of the alignment in bold (black in this study, red for MxB crystal contacts,4WHJ) or at the bottom underlined (for dynamin 3 tetramer, 5A3F) and italicized (for MxA crystal contacts, 3SZR).
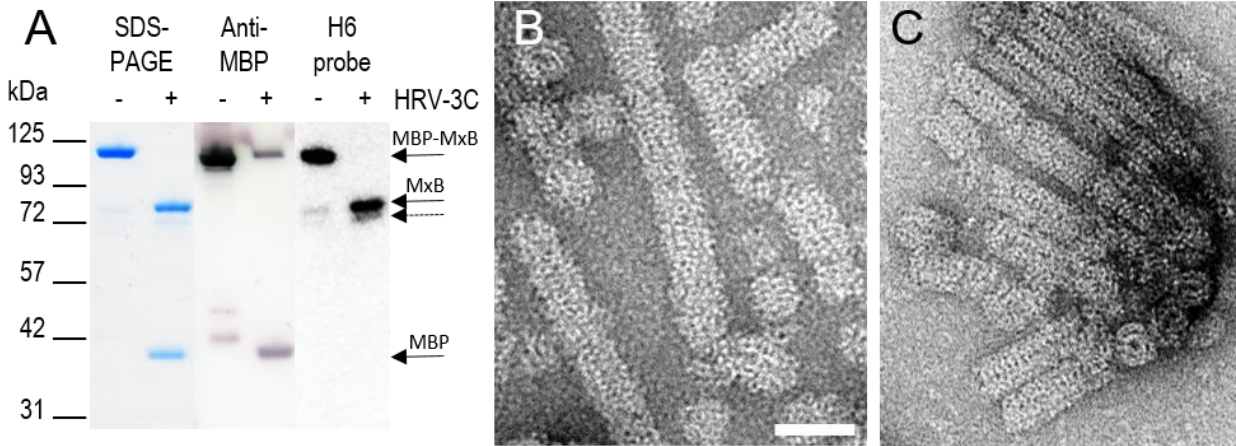
**fig. S2. MxB assembles into helical tubes with and without the MBP tag.** (**A**) Coomassie-stained SDS-PAGE gel and corresponding western blots, with the indicated primary antibody or probe. MBP-MxB-H6 was treated as indicated with HRV-3C protease at 150 mM NaCl. Proteins are indicated by arrows on the right. Dashed arrow points to a minor species of MxB with both MBP and the linker between MBP and MxB removed. (**B&C**) Negative stain EM images of samples in (A) without the protease (B) and with the protease (C). Removal of the MBP-tag resulted in ordered, but largely aggregated, MxB tubes. Scale bar, 50 nm.
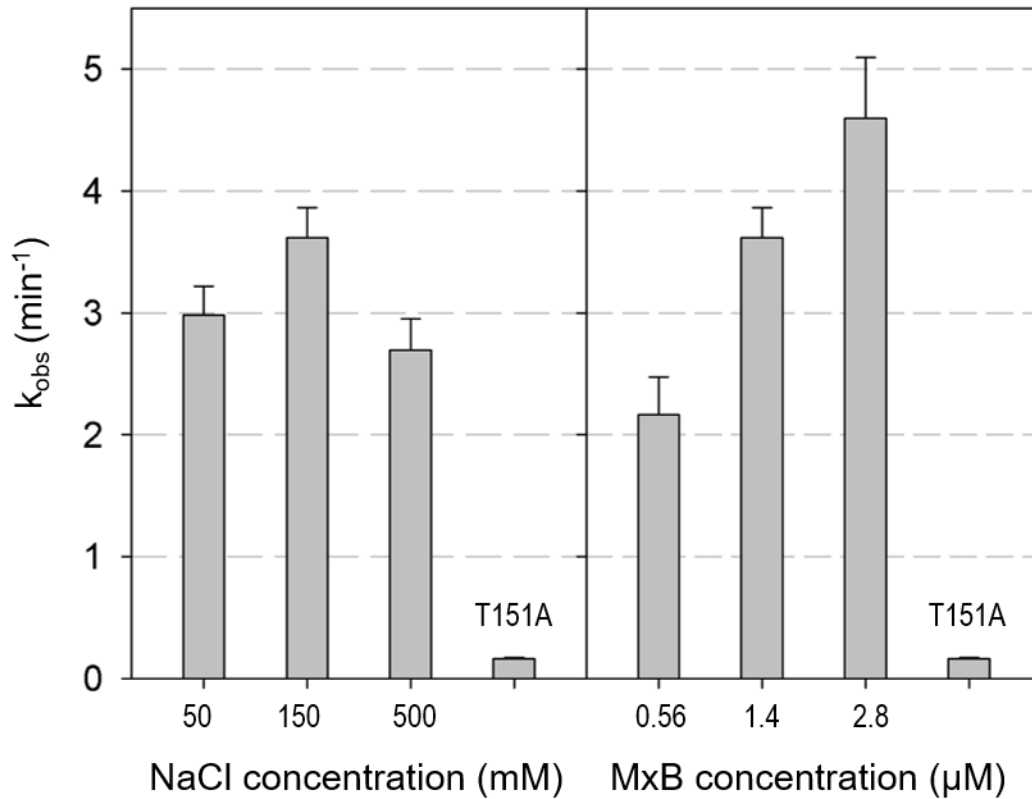
**fig. S3. GTPase activity of MBP-MxB at different protein and salt concentrations.**
GTPase activities on the left were measured at a constant MxB concentration of 1.4 µM, and on the right were measured at a constant NaCl concentration of 150 mM. GTPase activities of wt MxB were assayed using a continuous NADH-coupled assay at 1 mM GTP and 37ºC. Each gray bar represents the mean of three independent measurements while the error bars represent the 95% confidence interval. MxB T151A at 1.3 µM concentration and 150 mM NaCl is included as negative control.
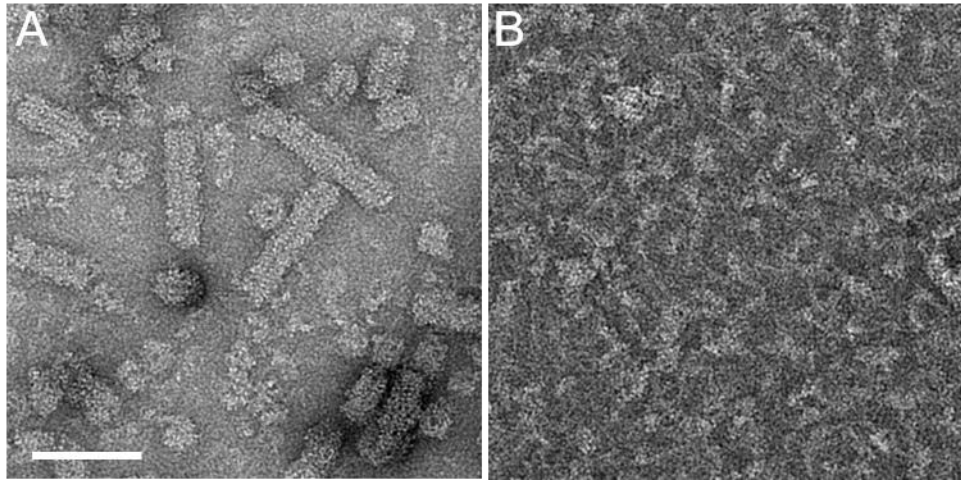
**fig. S4. MBP-MxB tubes in the presence of GTP without or with MgCl₂.** Negative-stain projection image of MBP-MxB helical assembly after incubating with assembly buffer for 2 hours in the presence of GTP without (**A**) and with MgCl$_2$ (**B**). Scale bar, 100 nm.
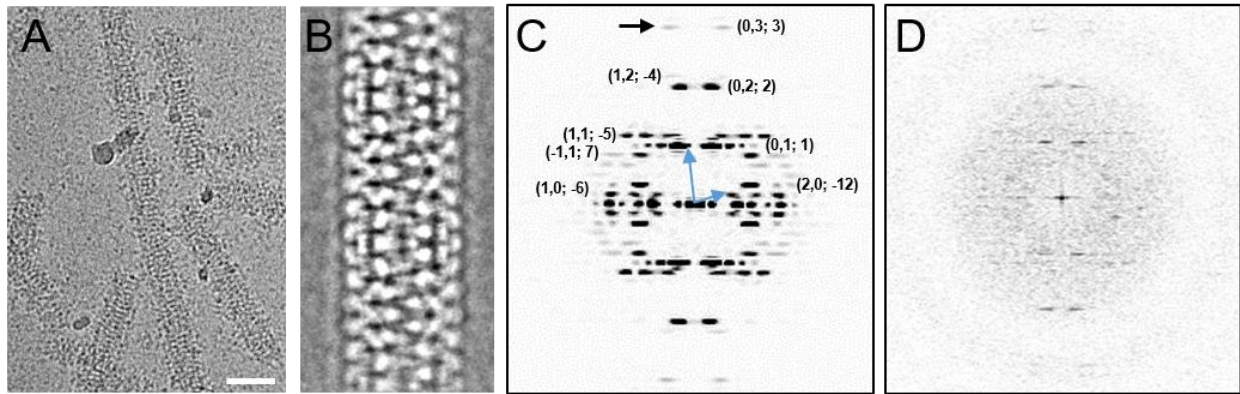
**fig. S5. CryoEM of the MBP-MxB assembly.** (**A**) A low-dose projection image of MBP-MxB tubular assembly. Scale bar, 50 nm. (**B**) A 2D class average of MxB tubes. (**C**) Fourier transform of the class average in B. The tube belongs to a 1-start helical family of (-6, 1). Helical indices are shown. Blue arrows point to the two vectors (1,0; -6) and (0,1;1) in helical indexing. Black arrow points to the layer-line at 16 Å resolution. (**D**) A Fourier transform of a raw image of MBP-MxB tube.
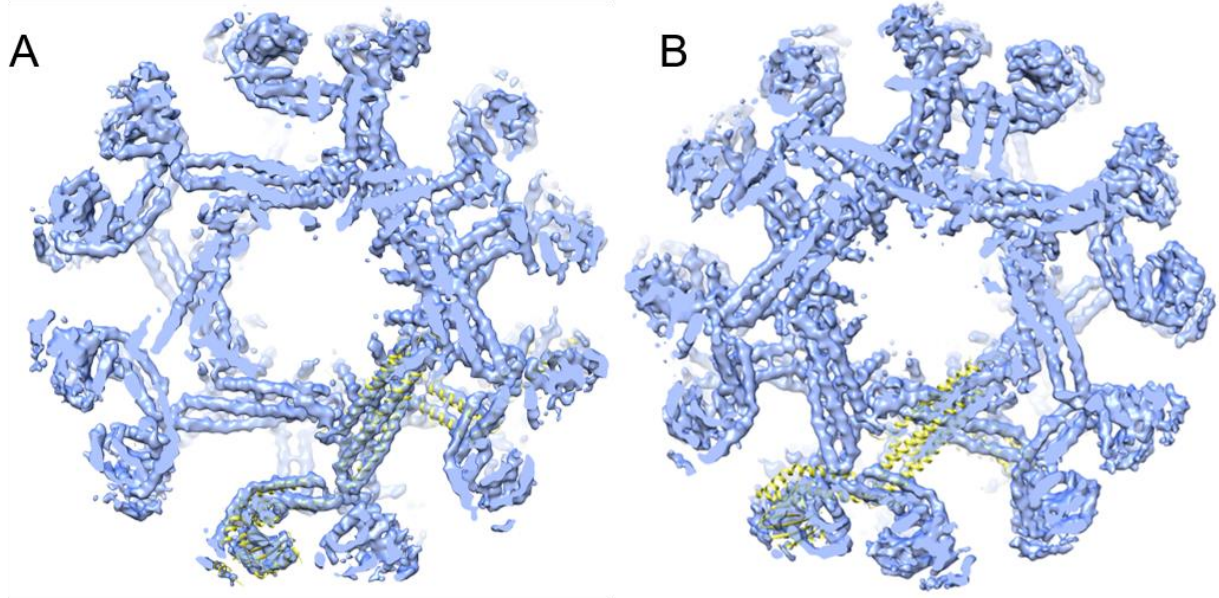
**fig. S6. Handedness of the MxB helical assembly map.** (**A**) Docking of the crystal structure of dimer MxB (PDB:4WHJ, yellow) into the right-handed MBP-MxB helical assembly map. (**B**) Docking of the crystal structure of dimer MxB into the left-handed map.
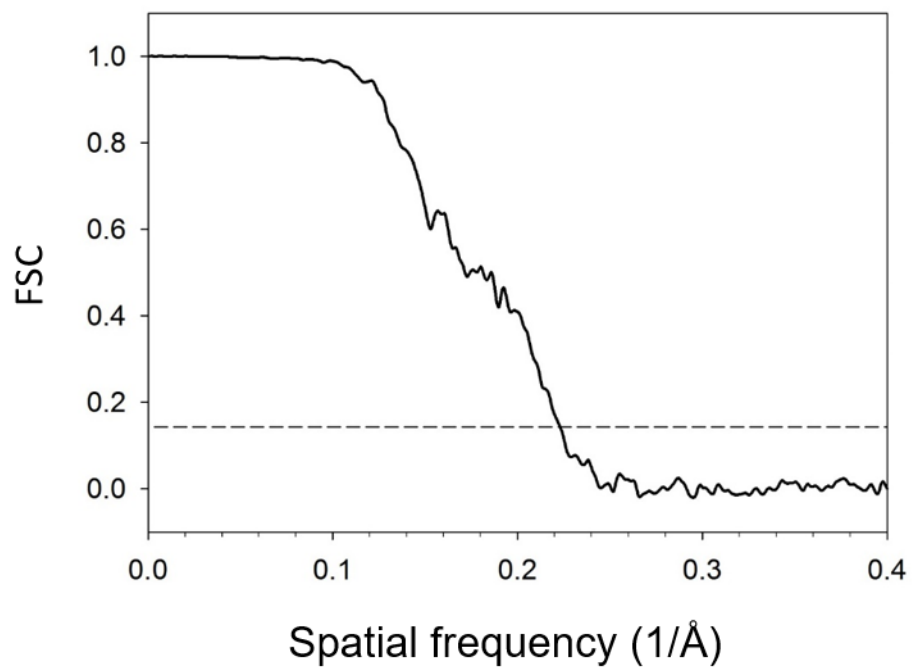
**fig. S7. Gold-standard Fourier shell correlation curve of the MBP-MxB density map.** The overall resolution is 4.6 Å using an FSC cut-off of 0.143.
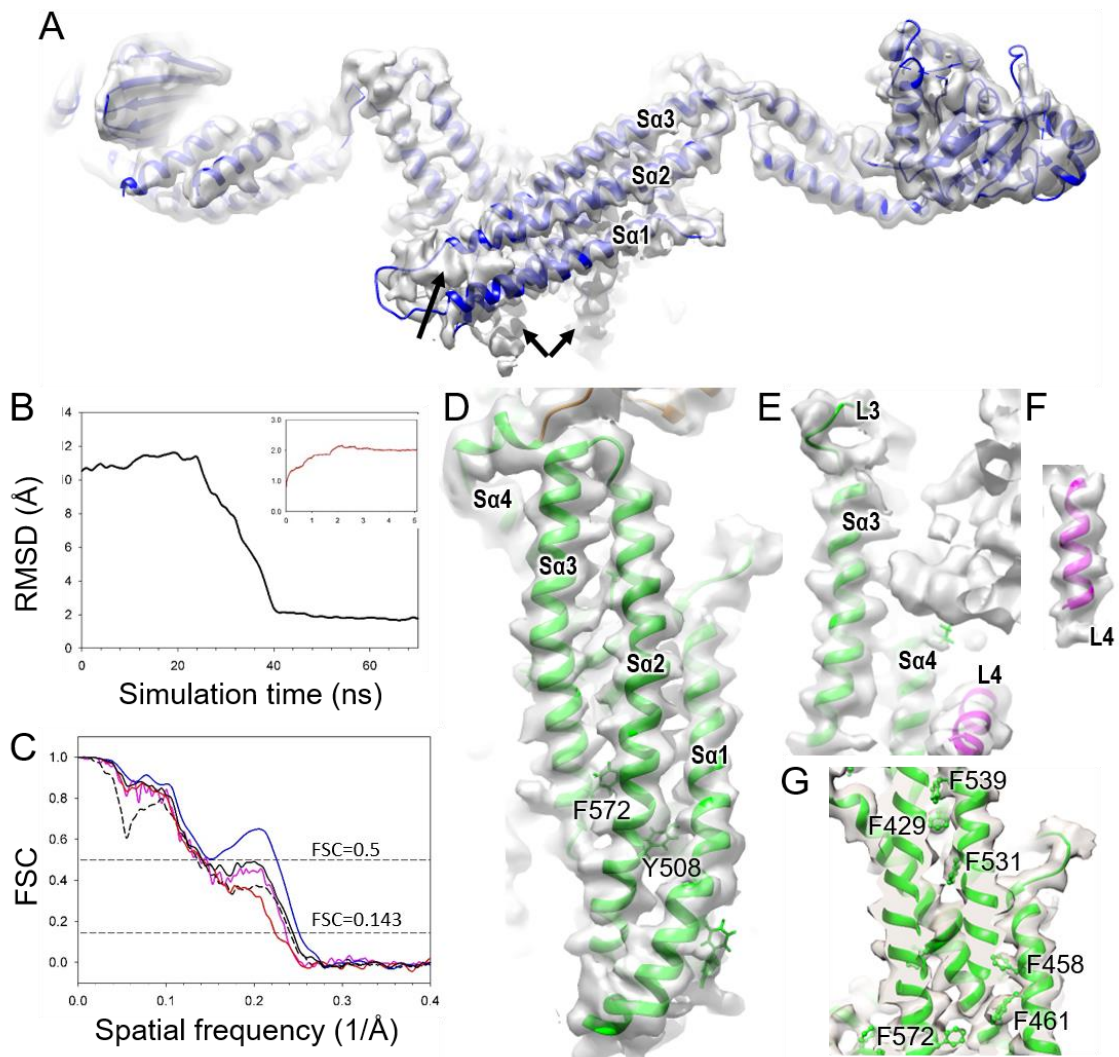
**fig. S8. MDFF and real-space refinement of the MxB helical assembly model.** (**A**) Rigid body docking of individual domains of the MxB crystal structure (4WHJ). Arrows point to densities that do not fit. (**B**) Time-evolution of the Cα-RMSD between the MDFF simulation and the initial MxB dimer model; the initial model was built based on the MxB crystal-structure. Inset: time-evolution of the Cα-RMSD for the refinement of the MDFF-derived tubular model; the MDFF model is refined by applying a series of gaussian-smoothed electron-densities with sigma decreasing from 5 to 0 Å in 1 Å steps. Note the difference in the scale. (**C**) FSC curves between the cryo-EM density map and the molecular models of MxB GTPase-BSE domain (red), Stalk domain (blue), dimer (black), tube (purple), and rigid body docking of individual domains of the crystal structure (dashed black). (**D** to **F**) Representative views of the fitting of the refined model into the density map: stalk helices (Sα1, Sα2 and Sα3, along with bulky amino acids Y508 and F572) (D), stalk helices (Sα3 and Sα4) and loops (L3, L4 helix) (E), and clear helical density in L4 (F). The extra unfitted density in (E) is from the other monomer.

(**G**) A clipped view shows densities of bulky amino acid side chains (F539, F429, F531, F458, F461 and F572).
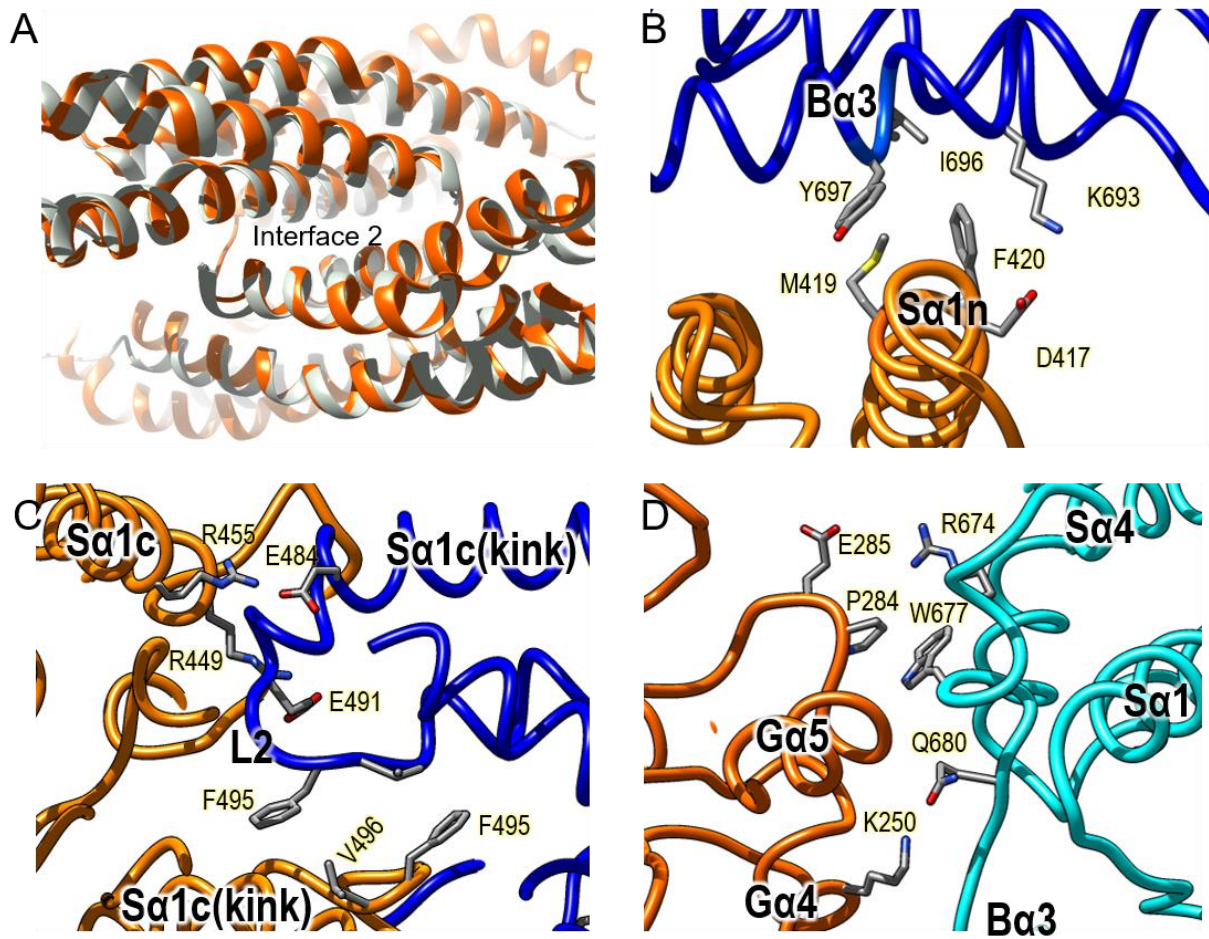
**fig. S9. Enlarged views of the intermolecular interfaces in the MxB assembly.** (**A**) Overlay of the MxB crystal dimer (grey) and the cryoEM dimer (orange) at the dimer interface, Interface 2. (**B** to **D**) Detailed view of the Interface 1 (B), Interface 3 (C) and Interface 4 (D). Specific residues at the interfaces are labeled.
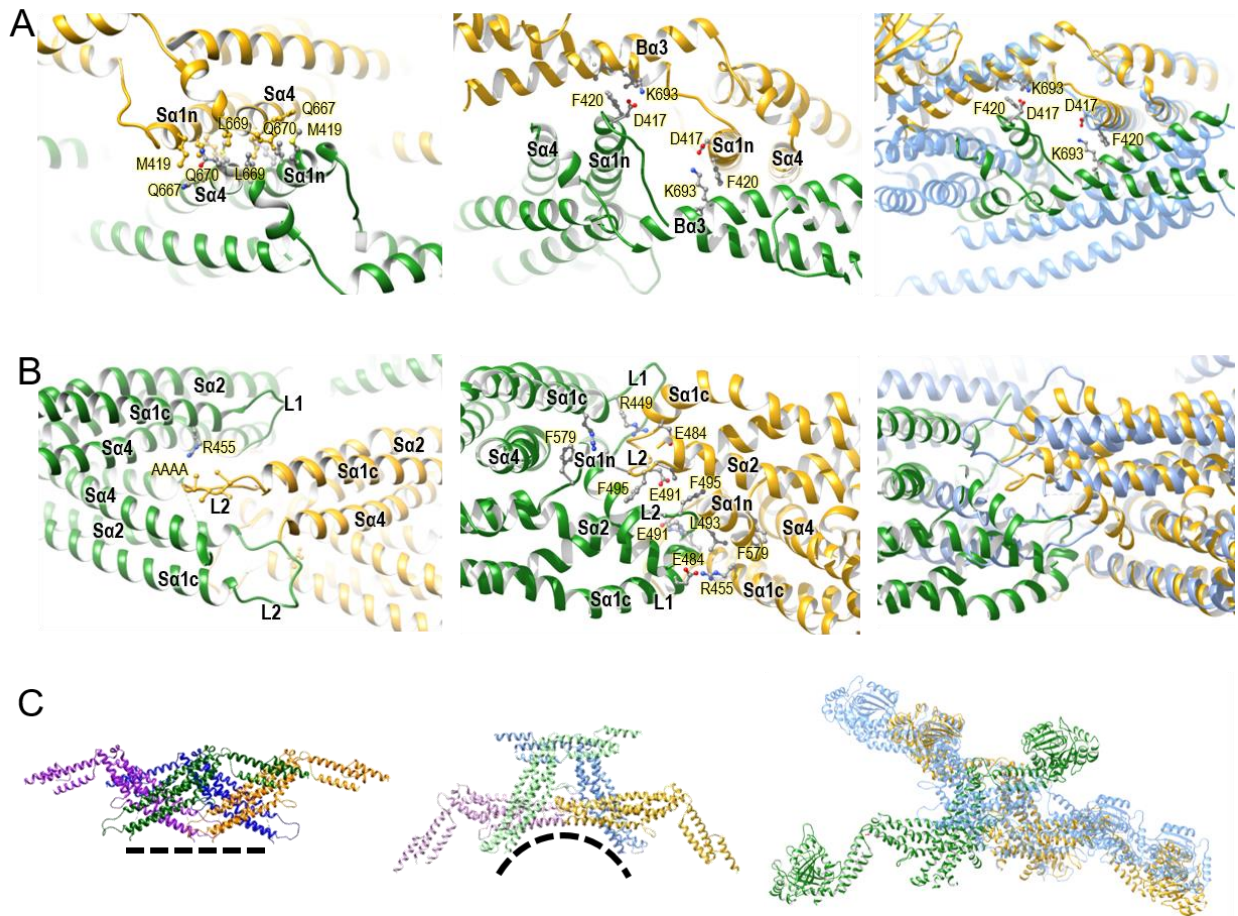
**fig. S10. Comparison of interfaces between cryoEM and crystal structures.** (**A**) Putative interface 1 at crystal contacts (4WHJ) (left), Interface 1 in the cryoEM assembly (middle) and overlay of the two interfaces (cryoEM gold/green, 4WHJ, light blue). The intermolecular interactions are entirely different. (**B**) Hypothetical Interface 3 (26) (no interaction) in the crystal structure (4WHJ) (left), extensive interactions at Interface 3 in the cryoEM assembly (middle), and overlay of the interfaces (cryoEM gold/green, 4WHJ, light blue). Amino acids involved in the interfaces are shown. Alpha helices and loops are labeled. (**C**) Connection of two adjacent MxB dimers in linear crystal arrays (left), cryoEM curved assembly (middle) with each monomer colored individually, excluding G domains for clarity, and overlay of crystal (light blue) and cryoEM (gold/green) (right), showing completely different dimer/dimer organization.
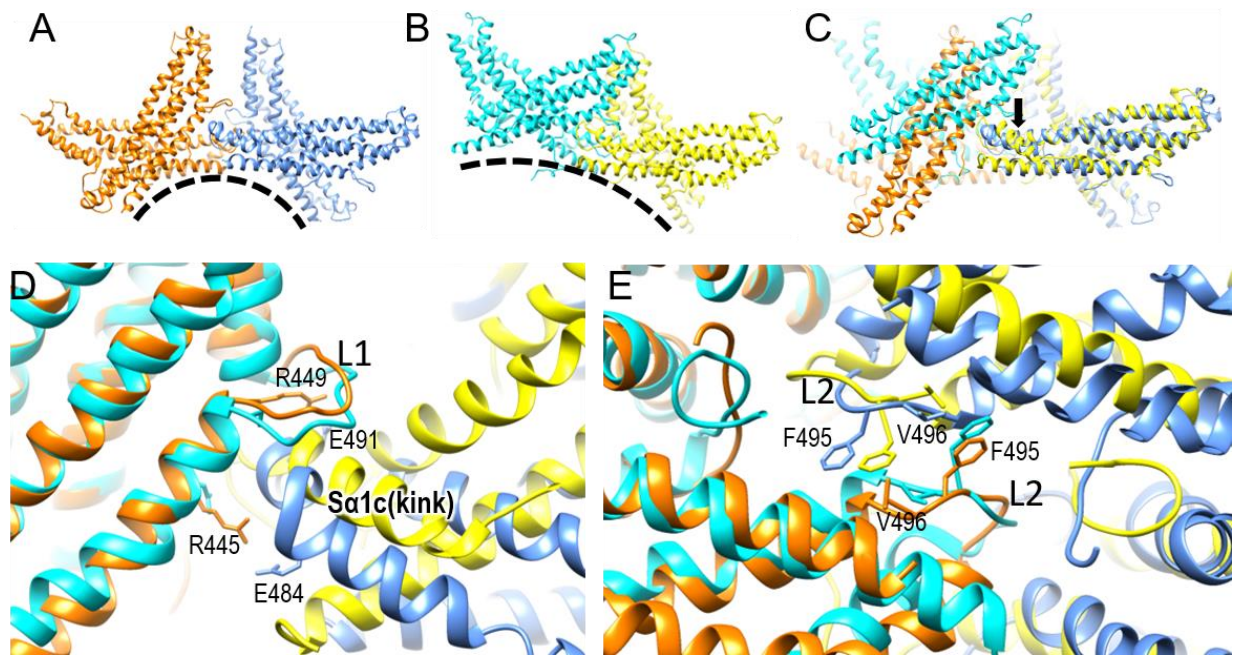
**fig. S11. Comparison of L1 and L2 contacts of MxB and dynamin 3.** (**A** to **B**) Tetramers of MxB (A, orange and blue) and dynamin 3 (5A3F, B, cyan and yellow) show different degrees of curvature (black dashed line). Only the stalk domains are shown for clarity. The dimers are individually colored. (**C**) The stalk tetramers were superposed using the blue (MxB) and yellow (dynamin 3) dimer pairs, which aligned with RMSD of 1.146 Å. The orange and cyan dimers clearly do not overlay well. (**D**) Superposition of MxB and dynamin 3 at Interface 3 at L1. Residues from MxB are shown. Differences between MxB and dynamin 3 are observed. (**E**) Superposition of MxB and dynamin 3 at Interface 3 residues in L2, wherein a similar interface is seen between MxB and dynamin 3. Residues from both MxB and dynamin 3 are shown.
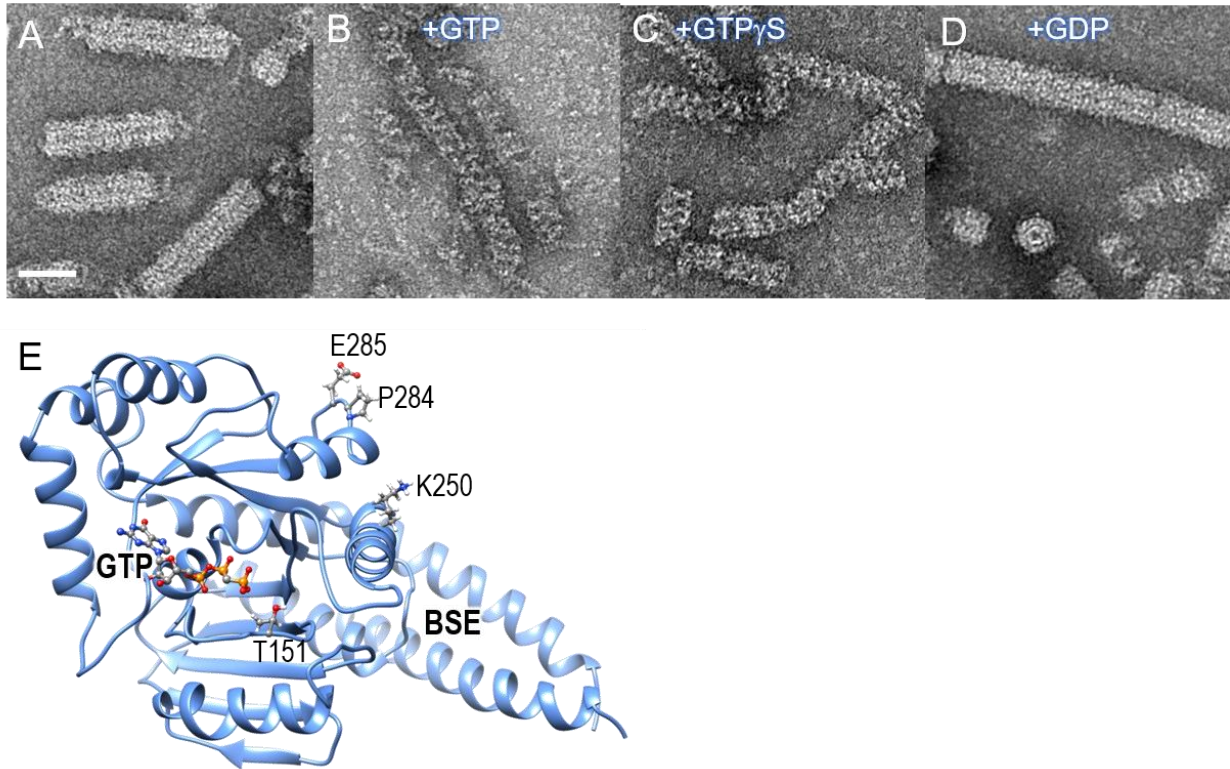
**fig. S12. Interface 4 mutations relative to GTP-binding site.** (**A** to **D**) MBP-MxB-T151A has been shown to bind GTPγS (*28*) but is unable to hydrolyze GTP. MBP-MxB-T151A self-assembled into ordered tubular structures at 150 mM NaCl (A) or in the presence of GDP (D), loosened tubes in the presence of GTP (B) or GTPγS. Scale bar, 50 nm. (**E**) GTPase domain of MxB is shown, with residues at Interface 4 (P284, E285 and K250) located at the opposite side of GTP binding site (T151) labeled.
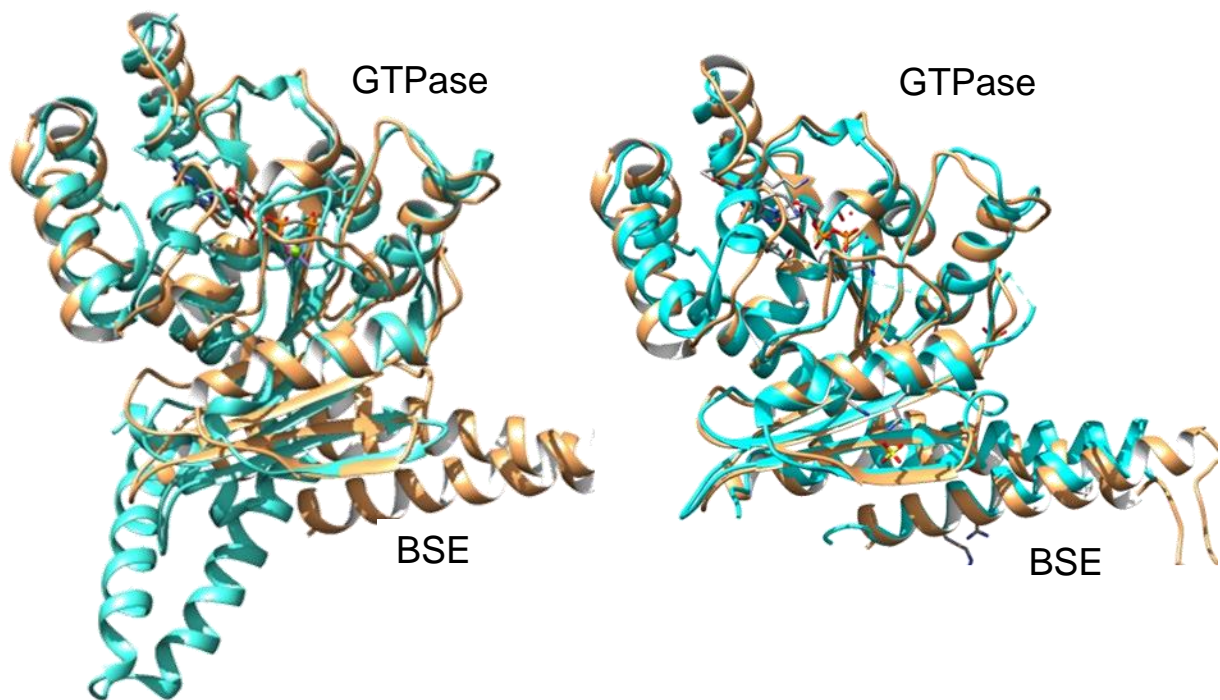
**fig. S13. Comparison of MxB and MxA GTPase-BSE domains.** MxB GTPase-BSE domains (orange) are overlaid with the corresponding domains in MxA (cyan) in GMP-PCP bound "open" state (4P4S, left) and MxA in GDP-bound "closed" state (4P4T, right).