**SUPPLEMENTARY FIGURES**

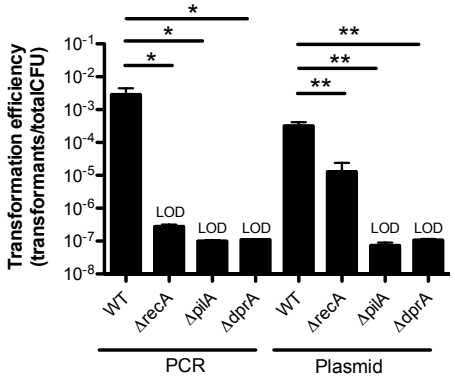


**Fig. S1** – *Uptake of plasmid DNA is independent of recombination*. Chitin-dependent transformation assay with the indicated strains using either linear PCR product or plasmid as tDNA. All data are shown as the mean ± SD and are the result of at least three independent biological replicates. * = $p<0.05$, ** = $p<0.01$, and LOD = limit of detection.

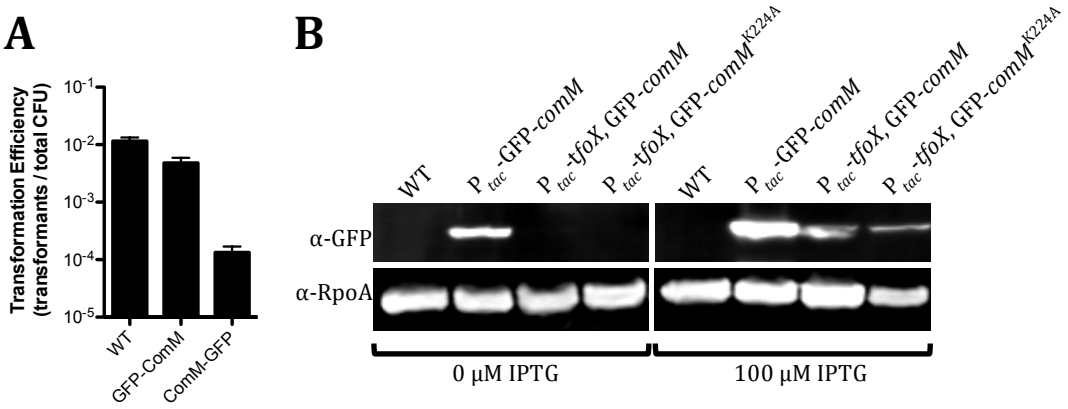


**Fig. S2** – *N-terminal ComM fusions are functional*. (**A**) Chitin-dependent transformation assay with the indicated strains using linear PCR product as the tDNA. (**B**) Representative western blot to detect GFP-ComM and RpoA (loading control) in the indicated strains grown in the presence or absence of 100 μM IPTG. Blot indicates that GFP-*comM* and GFP-*comM*$^{K224A}$ at the native locus are induced when TfoX is ectopically expressed to induce competence. Also, this blot indicates that the $P_{tac}$-GFP-*comM* construct is leaky and expressed in the absence of inducer. Data in **A** are shown as the mean ± SD and are the result of at least three independent biological replicates.

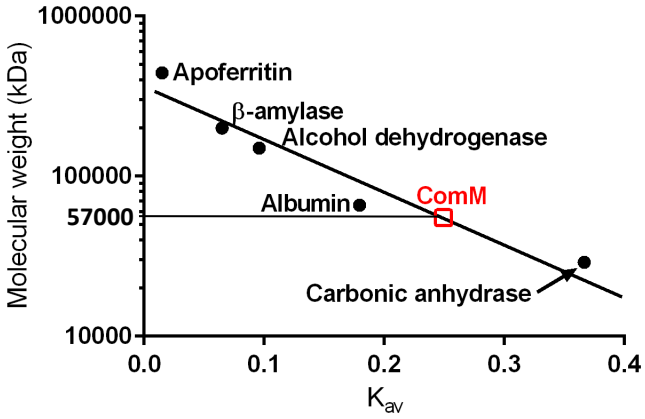


**Fig. S3** – *ComM is monomeric in soluble form.* Purified StrepII-ComM was analyzed by gel filtration and compared to a set of protein standards to determine size.

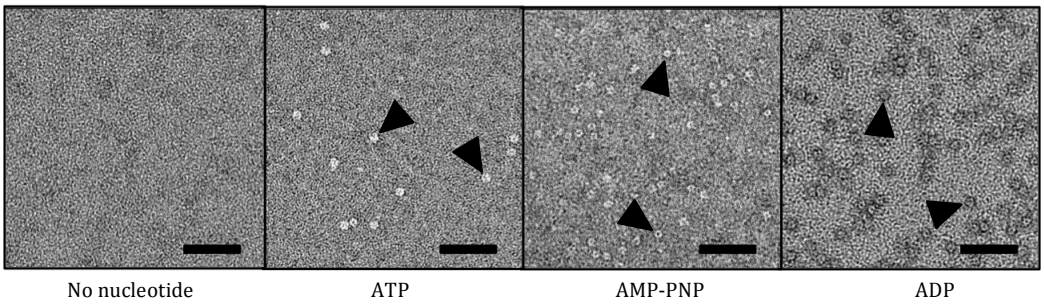


**Fig. S4** – *ComM oligomerizes in the presence of ADP and AMP-PNP.* Negative stain EM of purified ComM incubated with 5 μM ssDNA and 5mM of the indicated nucleotide or nucleotide analog. Representative ring-like densities in these samples are indicated by black arrows. Scale bar = 50 nm.

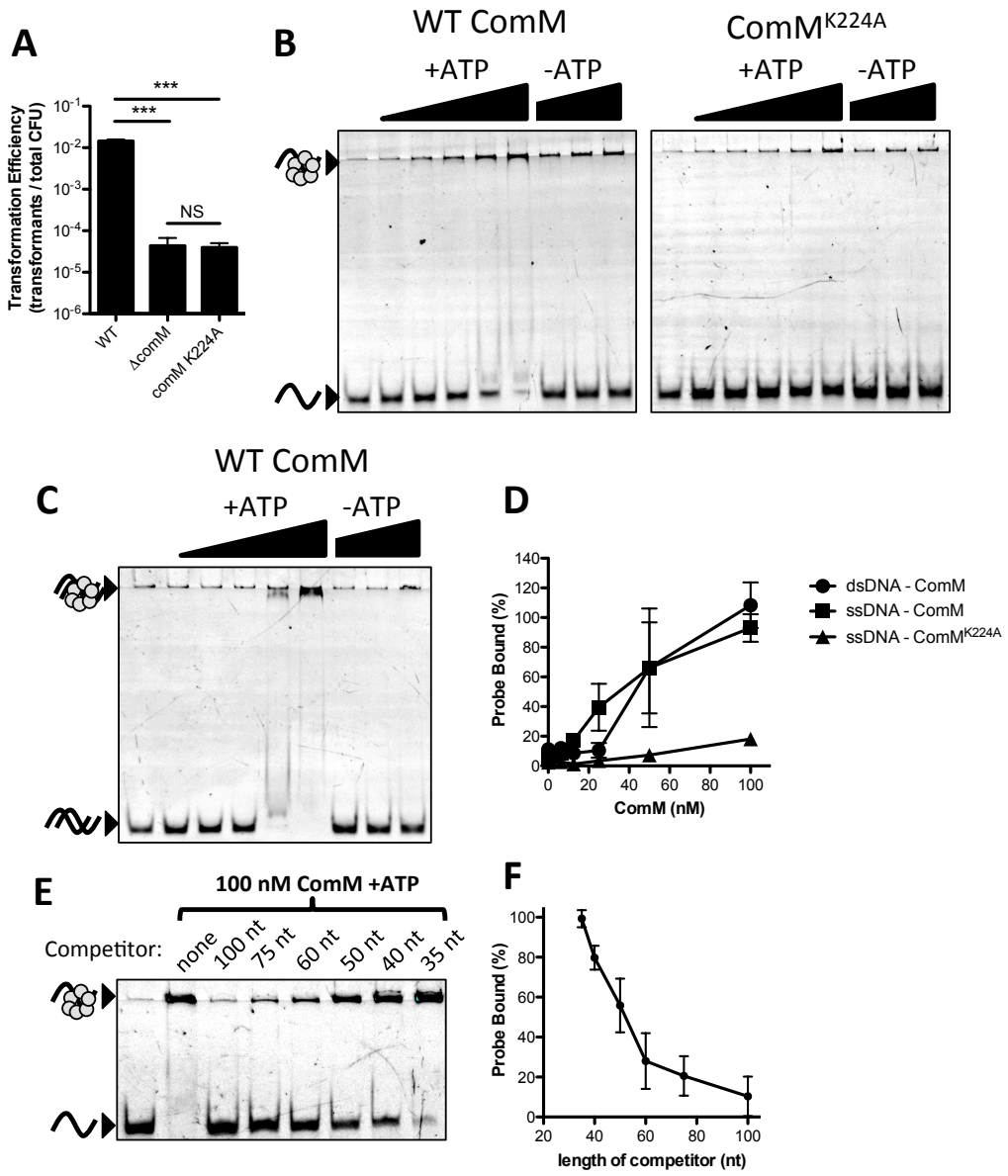


**Fig. S5** – *ComM binds ssDNA in the presence of ATP.* (**A**) Chitin-dependent transformation assay of the indicated strains using a linear PCR product as tDNA. Data are shown as the mean ± SD and are the result of at least three independent biological replicates. *** = $p<0.001$, NS = not significant. (**B**) EMSA with purified ComM and ComM$^{K224A}$ and a ssDNA probe. Protein concentrations (of the hexamer) used in the presence of ATP (+ATP) were 0, 6.25, 12.5, 25, 50, 100 nM, and in the absence of ATP (-ATP) were 25, 50, and 100 nM. Bound probe is retained in the well due to the large size of the DNA-bound oligomeric complex. (**C**) Representative EMSA with purified ComM and dsDNA probe. All reactions were performed in the presence of ATP and the protein concentrations (of hexamer) used were the same as in **B.** (**D**) Replicate EMSAs from **B** and **C** were quantified and plotted as indicated. (**E**) A representative EMSA where binding was competed with cold competitor DNA of the indicated length. The labeled probe was a 100 nt poly-dT oligo. The cold competitor was of the length indicated (also poly-dT) and was added at 100X molar excess to the labeled probe. (**F**) Replicate EMSAs from **E** were quantified and plotted as indicated. Data in **B**, **C**, and **E** are representative of at least three independent experiments.

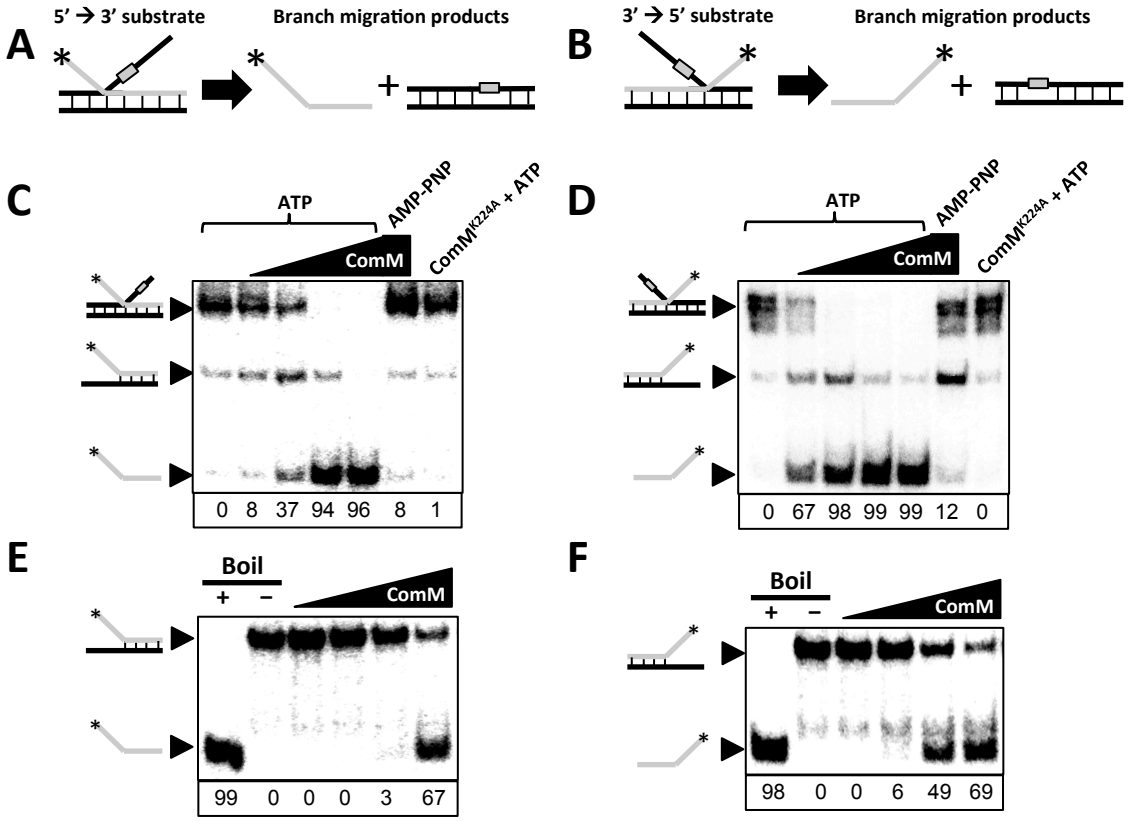


**Fig. S6** – *ComM exhibits branch migration activity on short substrates* in vitro. Schematics of the substrates used to test (**A**) 5'→3' and (**B**) 3'→5' branch migration activity. The gray box indicates a region on the substrate that is not homologous to the complementary strand. This was introduced to prevent spontaneous branch migration. The labeled strand has 60 bp of annealed sequence and a 30 nt tail (**C** and **D**) Representative branch migration assays using the substrates described in **A** and **B**, respectively**.** Substrates were incubated with 0, 25, 50, 100, or 200 nM WT ComM hexamer or 200 nM ComM$^{K224A}$ as indicated. Reactions were incubated with ATP or AMP-PNP as indicated. The % of final branch migration product generated at each concentration of ComM is indicated below each lane. (**E** and **F**) Representative helicase assays using forked substrates derived from the same oligos used to generate the three-stranded branch described in **A** and **B**, respectively. Substrates were incubated with 0, 25, 50, 100, or 200 nM WT ComM hexamer in the presence of ATP. The % of unwound product generated at each concentration of ComM is indicated below each lane. All data are representative of at least three independent experiments.


**Fig. S7** – *ComM is broadly conserved.* (**A**) Phylogenetic trees of species based on a concatenated alignment of 36 conserved protein sequences. Green text indicates species with an identifiable ComM homolog. (**B**) Phylogenetic tree of ComM alleles.

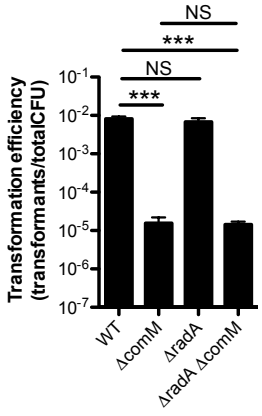


**Fig. S8** – *RadA is not required for natural transformation in* V. cholerae*.* All strains contain $P_{tac}$-*tfoX* mutations and were transformed via chitin-independent transformation assays using a linear PCR product as the tDNA. All data are shown as the mean ± SD and the result of 6 independent biological replicates. *** = $p<0.001$, NS = not significant.

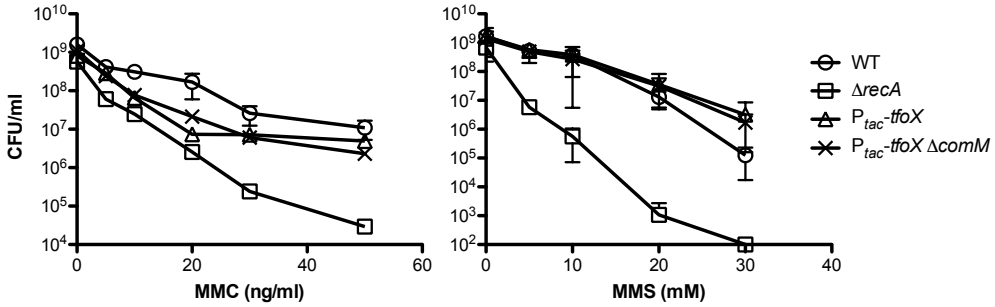


**Fig. S9** – *ComM is not required for DNA repair.* Strains were treated with increasing doses of the DNA damaging agent indicated on the X-axis and then plated for viability. All data are shown as the mean ± SD and are the result of at least three independent biological replicates.

**SUPPLEMENTARY TABLES**

**Table S1** – *Strains used in this study*

| Strain Name in Manuscript | Genotype and Antibiotic Resistances | Description | Reference / strain# |
|---|---|---|---|
| WT | $Sm^R$ | Wildtype *V. cholerae* O1 El Tor strain used throughout this study | (1) / (SAD030) |
| **Strains Used for Transformation Assays** | | | |
| Δ*comM* | ΔVC0032::$Spec^R$ | Replacement of VC0032 with a spectinomycin resistance cassette | This Study (SAD083) |
| $P_{tac}$-*tfoX* | VC1153 OE $Kan^R$ | OE KanR = a fragment of the Tn10 transposon from pDL1093, including the rrnB antiterminator, Ptac, LacI, and KanR. This fragment, and its use in overexpression of VC1153 (tfoX) is described in | (2) / (SAD061) |
| Δ*comM* Ptac-*tfoX* | VC0032::$Spec^R$, VC1153 OE $Kan^R$ | Replacement of VC0032 with $Spec^R$ cassette in a VC1153 OE $Kan^R$ parent strain | This Study (SAD066) |
| $P_{tac-}$ *comM* | $P_{tac}$-*comM* at *lacZ* locus $Spec^R$ | VC0032 fused with an IPTG inducible promoter at the lacZ locus | This Study (SAD1065) |
| Δ*recA* | Δ*recA::*$Spec^R$ | Replacement of VC0543 with $Spec^R$ cassette | This Study (SAD081) |
| Δ*pilA* | Δ*pilA*::$Spec^R$ | Replacement of VC2423 with $Spec^R$ cassette | This Study (SAD780) |
| Δ*dprA* | Δ*dprA::*$Spec^R$ | Replacement of VC0048 with $Spec^R$ | This Study (SAD079) |
| $P_{tac-}$ *comM*, Δ*comM* | $P_{tac}$-*comM* at *lacZ* locus $Spec^R$ , ΔVC0032::$Amp^R$ | VC0032 fused with an IPTG inducible promoter at the lacZ locus in a Δ*comM* parent strain | This Study (SAD1066) |
| Δ29bp ($Tm^S$) | ΔVC1807::TmR*Δ29bp, ΔmutS MuGENT edit, LPQEN KanR | ΔVC1807::TmR*Δ29bp, ΔmutS MuGENT edit, LPQEN KanR; NOT TmR resistant | This Study (TND0226/ SAD1321) |
| Δ29bp ($Tm^S$) Δ*comM* | ΔcomM::CarbR, (ΔVC1807::TmR*Δ29bp, ΔmutS MuGENT edit, LPQEN KanR) | VC0032 deletion in a $Tm^S$ parent strain | This Study (TND0229/ SAD1322) |
| Point mutant ($Tm^S$) | ΔmutS MuGENT edit, LPQEN::SpecR, ΔVC1807::TmR*TI | ΔmutS MuGENT edit, LPQEN::SpecR, ΔVC1807::TmR*TI; ΔVC1807::TmR*TI is the TmR cassette at VC1807, except it has a transition point mutation that introduces a premature stop codon. | This Study (TND0220/ SAD1323) |
| Point mutant ($Tm^S$), Δ*comM* | ΔmutS MUGENT edit, ΔcomM::CarbR, LPQEN::KanR, ΔVC1807::TmR*TI | ΔmutS MUGENT edit, ΔcomM::CarbR, LPQEN::KanR, ΔVC1807::TmR*TI; ΔVC1807::TmR*TI is the TmR cassette at VC1807, except it has a transition point mutation that introduces a premature stop codon. | This Study (TND0221/ SAD1324) |
| *comM*$^{K224A}$ | LPQEN::$Kan^R$, *comM*$^{K224A}$ | K to A residue substitution disrupts ATP binding. FRT Kan cassette following the LPQEN amino acid sequence in *lacZ* (VC2338) used for | This Study (SAD1026) |

| | | | |
|---|---|---|---|
| | | selection during co-transformation. | |
| *gfp-comM* | *gfp-comM* | N-terminal GFP-comM at native locus | This Study (SAD924) |
| *comM-gfp* | *comM-gfp* | C-terminal comM-GFP at native locus | This Study (SAD925) |
| parent (ADP1) | *ΔACIAD1551::*$P_{tac}$*-lacZ, ΔmutS::SpecR* | lacZ introduced into a defunct transposase (neutral gene = ACIAD1551), mutS deleted and replaced with $Spec^R$ (ACIAD1500) | This Study (TND0137/ SAD1325) |
| *ΔcomM* (ADP1) | ΔACIAD1551::$P_{tac}$*-lacZ, ΔmutS::SpecR, ΔcomM* | lacZ introduced into a defunct transposase (neutral gene = ACIAD1551), mutS deleted and replaced with $Spec^R$ (ACIAD1500), comM in-frame mutation (ACIAD0242) | This Study (TND0149/ SAD1326) |
| Δ180 (ADP1) | *ΔmutS::*$Kan^R$ | MutS deleted and replaced with a $Kan^R$ cassette | This Study (SAD742) |
| Δ180 *ΔcomM* (ADP1) | *ΔmutS::*$Kan^R$*, ΔcomM* | MutS deleted and replaced with a $Kan^R$ cassette, ΔcomM in-frame | This Study (TND0144/ SAD1327) |
| Point mutant (ADP1) | ACIAD1551::*Spec*$^R$ (point mutant), *ΔmutS::*$Kan^R$ | SpecR cassette in ACIAD1551 is inactivated with a point mutation. MutS deleted and replaced with $Kan^R$ | This Study (TND0150/ SAD1328) |
| Point mutant, Δ*comM* (ADP1) | ACIAD1551::*Spec*$^R$ (point mutant), *ΔmutS::*$Kan^R$*, ΔcomM* | SpecR cassette in ACIAD1551 is inactivated with a point mutation. mutS was deleted and replaced with $Kan^R$ and comM was deleted in-frame | This Study (TND0164/ SAD1329) |
| pBAD18 Kan | pBAD18 Kan | TG1 E. coli strain used to purifiy a replicating plasmid with a $Kan^R$ cassette | This Study (SAD233) |
| $P_{tac}$*-tfoX,* Δ*radA* | VC1153 OE $Kan^R$, *radA*::*Spec*$^R$ | *radA* (VC2343) deleted and replaced with $Spec^R$ in a TfoX overexpressing background. | This Study (TMN0135/ SAD1813) |
| $P_{tac}$*-tfoX*, Δ*radA,* Δ*comM* | VC1153 OE $Kan^R$, *radA*::*Spec*$^R$, *comM*::*Carb*$^R$ | *comM* (VC0032) deleted and replaced with $Carb^R$ in a TfoX overexpressing, *radA* deletion background. | This Study (TMN0136/ SAD1814) |
| **Strains used in ComM Induction** | | | |
| $P_{tac}$*-gfp* | Ptac-GFPmut3 *Spec*$^R$ at the *lacZ* locus | Replaced lacZ gene in SAD030 with a fragment from the transposon vector pDL1098 which encodes LacI, SpecR and has a Ptac promoter. Cloned GFPmut3 downstream of Ptac promoter | This Study (SAD559) |
| $P_{tac}$*-gfp-comM* | Ptac N-terminal *gfp-comM* at *lacZ Spec*$^R$*, ΔcomM Kan* | VC0032 deletion strain containing an N-terminally GFP tagged comM at the lacZ locus under the control of Ptac | This Study (SAD921) |
| *gfp-comM* | N-terminal GFP-comM at the native locus | N-terminally GFP tagged comM was cloned into SAD030 at the native locus | This Study (SAD924) |
| $P_{tac}$*-tfoX, gfp-comM* | VC1153 OE $Kan^R$, N-terminally GFP-comM at the native locus | Amplified VC1153 OE Kan from SAD061 and cloned fragment into SAD924 | This Study (TMN0140 / SAD1320) |

| | | | |
|---|---|---|---|
| $P_{tac}$-*tfoX, gfp-comM*$^{K224A}$ | VC1153 OE *Kan*$^{R}$, N-terminal *gfp-comM*$^{K224A}$ at the native locus, *Spec*$^{R}$ | Amplified *comM*$^{K224A}$ mutation from SAD1026 and cloned fragment into SAD1320 | This Study (TMN0148 / SAD1545) |
| **Strains Used for Protein Purification** | | | |
| ComM | ComM cloned into *Amp*$^{R}$ StrepII expression vector | ComM N terminally tagged with 4x StrepII | This Study (pMB486 / SAD1330) |
| ComM$^{K224A}$ | ComM$^{K224A}$ cloned into Amp$^{R}$ StrepII expression vector | ComM$^{K224A}$ N terminally tagged with 4x StrepII | This Study (pMB488 / SAD1331) |
| Rosetta 2 (DE3) | | *E. coli* expression strain | (pMB131/ SAD1332) |

**Table S2**- *Primers used in this study-*

| Primer Name | Primer Sequence (5' to 3')* | Description |
|---|---|---|
| **Mutant constructs** | | |
| ABD855 | CATGAATCACTTTGGCATGAGG | Δ*comM* F1 |
| ABD856 | gtcgacggatccccggaatCATTGCTTCCCTTAGTATTTGATC | Δ*comM* R1 |
| ABD857 | gaagcagctccagcctacaTAGTACTCTGACCTGCAGAGTTC | Δ*comM* F2 |
| ABD858 | AAATTCCAGAAAAACCACGTC | Δ*comM* R2 |
| BBC749 | CCGTGAAGCGAGCATGGTcgcACCCGTCCCCGGAG | *comM*$^{K224A}$ R1 |
| BBC750 | CTCCGGGGACGGGTgcgACCATGCTCGCTTCACGG | *comM*$^{K224A}$ F2 |
| ABD812 | AAATGGAGTTTGATCGCATTGGC | Δ*recA* F1 |
| ABD921 | gtcgacggatccccggaatCATTACTCTCTCCGGATAGTCACTC | Δ*recA* R1 |
| ABD922 | gaagcagctccagcctacaTAATCGGCAGGCTGAATGCAAAG | Δ*recA* F2 |
| ABD815 | TGATCAGCGTTTGGAATACGTCG | Δ*recA* R2 |
| BBC401 | ACCAGCAAAGCTAATAAAATCGAG | Δ*pilA* F1 |
| BBC402 | gtcgacggatccccggaatGAGCATATGCCTTGCTACACAAG | Δ*pilA* R1 |
| BBC403 | gaagcagctccagcctacaACTGCAGGTGCAACAATTAACTAA | Δ*pilA* F2 |
| BBC404 | CGCCATACTAACCCAATACACTC | Δ*pilA* R2 |
| ABD820 | CGCTCTTATCTGCTTGGATAATGG | Δ*dprA* F1 |
| ABD998 | gtcgacggatccccggaatCATTAACTGGCATCATCAACC | Δ*dprA* R1 |
| ABD999 | gaagcagctccagcctacaTAGCTATGATGATGGATATTTTGATG | Δ*dprA* F2 |
| ABD823 | TGAAGTACAAGGCCAGTTACTGG | Δ*dprA* R2 |
| BBC907 | AAAGAGCAGTTGTCGCTAGAC | Δ*radA* F1 |
| BBC908 | gtcgacggatccccggaatCAATCCTCGAACTTGCTCTCAC | Δ*radA* R1 |
| BBC909 | gaagcagctccagcctacaTAATGGGTAGTTGGTTTTGAAC | Δ*radA* F2 |
| BBC910 | ATGAAGAAATCTTAGTCCGCAG | Δ*radA* R2 |
| ABD824 | TTTAGCCCCATTGGCGAACTGGG | ΔmutS F1 |
| ABD825 | GAGTATCTTTGACGTATTGGATCtcatattatactaCATAATCTTATGTC GCTGCTTATC | ΔmutS R1 |
| ABD826 | GATAAGCAGCGACATAAGATTATGtagtataatatgaGATCCAATACGT CAAAGATACTC | ΔmutS F2 |
| ABD360 | AGATCTTGCCTGATGACGCTTTACTC | ΔmutS R2 |
| BBC717 | AAATAGATTTGGTGACTTTACCTCC | VC1807::Ab$^{R}$ F1 |
| ABD340 | gtcgacggatccccggaatACGTTTCATTAGTCACCTCTATTGTTAACTTG TTC | VC1807::Ab$^{R}$ R1 |
| ABD341 | gaagcagctccagcctacaTAGTCGAAAATAAAAAAAAGAGGCTCGCCTC | VC1807::Ab$^{R}$ F2 |
| BBC718 | CTTTACGCCTGATTGTCTACAC | VC1807::Ab$^{R}$ R2 |

| | | |
|---|---|---|
| ABD332 | GGCTGAACGTGGTTGTCGAAAATGAC | lacZ F1 |
| ABD263 | gtcgacggatccccggaatAACTGATCCAATTTTTCAGCGCATATTTTGG | lacZ LPQEN::Ab$^R$ R1 |
| ABD262 | gaagcagctccagcctacaTGCCGCAGGAAAACCGCCCCCTaATC | lacZ LPQEN::Ab$^R$ F2 |
| ABD256 | CCCAAATACGGCAACTTGGCG | lacZ R2 |
| ABD269 | gaagcagctccagcctacaAATTGTGTAAACGTTTCCACAATTTAAATAGAGG | Spec$^R$ upstream of lacZ R1 |
| ABD268 | gtcgacggatccccggaatGGTGAGTGGTTCACAGAATCGGTG | Spec$^R$ upstream of lacZ F2 |
| ABD495 | AAAAAAATCTTCAATCGCGAGTATCGGCTaGCGGTAGAGATACACATCGCGAAAGATGCC | lacZ 820bp linked R1 |
| ABD494 | TCTTTCGCGATGTGTATCTCTACCGCtAGCCGATACTCGCGATTGAAGATTTTTTTATCC | lacZ 820bp linked F2 |
| ABD329 | GAACATGGGGTGTACGGCAGTGCCATTaAACGATGTGCGGGTTTTGCCAATCTTG | lacZ 245bp linked R1 |
| ABD328 | CAAGATTGGCAAAACCCGCACATCGTTtAATGGCACTGCCGTACACCCCATGTTC | lacZ 245bp linked F2 |
| BBC1157 | GTAAAACTTGAACGTGTTACGAATTGATTCAAAAGTCTTGCGTC | Tm$^R$ Δ29bp R1 |
| BBC1158 | GACGCAAGACTTTTGAATCAATTCGTAACACGTTCAAGTTTTAC | Tm$^R$ Δ29bp F2 |
| BBC747 | GTCCAACCAACAGCCATTGGTTtTAGGTAATAGCTTTAAACAGGAGC | Tm$^R$ Point mutation |
| BBC748 | GCTCCTGTTTAAAGCTATTACCTAaAACCAATGGCTGTTGGTTGGAC | Tm$^R$ Point mutation |
| BBC498 | GGGTAACGCCAGGGTTTTCtCAGTCACGACGTTGTAAAAC | SpecR point mutant R1 |
| BBC499 | GTTTTACAACGTCGTGACTGGtAAAACCCTGGCGTTACCC | SpecR point mutant F2 |
| BBC280 | TCCACCACTTCCACCtGCGACGTTCTGCGCACTGAGC | GFP-ComM R1 |
| BBC351 | GTTCTTCTCCTTTACGCATTACGTTACCTCCTTTTGATCAAAAAGCCTTCAGC | ComM-GFP R1 |
| BBC352 | GCaGGTGGAGCAGGTGGAGGACTTGCGATCATTCATAGC | ComM-GFP F2 |
| ABD688 | CCACTGTTGCGCAGTTGAATACC | tfoX OE Kan F1 |
| ABD691 | ATGATGTCAAACCATGAACCCGG | tfoX OE Kan R2 |
| BBC331 | CAATTTCACACAGGATCCCGGGAGGAGGTAACGTAATGGGACTTGCGATCATTC | $P_{tac}$-comM F |
| BBC365 | tgtaggctggagctgcttcCTAGACGTTCTGCGCACTGAG | $P_{tac}$-comM R |
| DOG0140 | GTTGCTGCATTTGTTCGATCTG | ΔcomM (ADP1) F1 |
| DOG0141 | gtcgacggatccccggaatCATACTATTATTGTTCCATTATGGTGC | ΔcomM (ADP1) R1 |
| DOG0142 | gaagcagctccagcctacaTATCGCAGTGAACATAGCTAAAA | ΔcomM (ADP1) F2 |
| DOG0143 | ATCAGTGGTTGGGAAGGTG | ΔcomM (ADP1) R2 |
| **Inserts for cloning** | | |
| MB1225 | CGGGATCCATGGGACTTGCGATCATTCATAGCCG | *comM* ORF F1 |
| MB1214 | CGATCGATCTCGAGCTAGACGTTCTGCGCACTGAGC | *comM* ORF R1 |
| MB1215 | TATTGTTTCTCGGCCCTCCGGGGACGGGTGCGACCATGCTCGCTTCACGGCTGTGCGATT | *comM$^{K224A}$* F1 |
| MB1216 | AATCGCACAGCCGTGAAGCGAGCATGGTCGCACCCGTCCCCGGAGGGCCGAGAAACAATA | *comM$^{K224A}$* R1 |
| **Oligomerization assays/Negative stain EM** | | |
| ABD363 | CGTTAAATGAAATTAATACGACTCACTATAGGGAGAGAGGTTTGCTCTGTTTGAGAAGCC | ssDNA substrate for oligomerization |
| **EMSA probes** | | |
| BBC742 | ATTCCGGGGATCCGTCGACCTGCAGTTCAGAAGCAGCTCCAGCCTACA | EMSA binding probe (ssDNA, dsDNA) |
| BBC743 | TGTAGGCTGGAGCTGCTTCTGAACTGCAGGTCGACGGATCCCCGGAAT | EMSA binding probe (dsDNA) |

| | | |
|---|---|---|
| MB1040 | TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T | 100 bp poly-dT ssDNA probe |
| MB1039 | TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT | 75 bp poly-dT ssDNA probe |
| MB1038 | TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT | 60 bp poly-dT ssDNA probe |
| M551 | TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TT | 50 bp poly-dT ssDNA probe |
| MB1037 | TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T | 40 bp poly-dT ssDNA probe |
| MB1140 | TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TT | 35 bp poly-dT ssDNA probe |
| **Helicase substrates** | | |
| MB1167 | TTTTTTTTTTTTTTTTTTTTTTTTTTGTGTCACTCACATAGCGTTC | Poly-dT 5’ tail |
| MB1168 | GAACGCTATGTGAGTGACACTTTTTTTTTTTTTTTTTTTTTTTTT | Poly-dT 3’ tail |
| MB1510 | TTTTTTTTTTTTTTTTTTTTTTT/iInvdT/GTGTCACTCACATAGCGTTC | Poly-dT inverted 5’ tail |
| MB1511 | GAACGCTATGTGAGTGACAC/iInvdT/TTTTTTTTTTTTTTTTTTTTTTT | Poly-dT inverted 3’ tail |
| **Short three-stranded branch migration substrates** | | |
| BBC1916 | TTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTGAATCTGGTGCTGTAGGTCAACATGTTGTAAATATGCAGCTAAAG | 5’ to 3’ bottom strand |
| BBC1915 | TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCACAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAA | 5’ to 3’ top strand with polyDT tail (also for forked helicase substrate) |
| BBC1917 | CTTTAGCTGCATATTTACAACATGTTGACCTACAGCAAAGAATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAA | 5’ to 3’ top strand for 3’ branch |
| BBC1913 | CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAA | 3’ to 5’ bottom strand |
| BBC1912 | TTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTGAATCTGGTGCTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT | 3’ to 5’ top strand with polyDT tail (also used for forked helicase substrate |
| BBC1914 | TTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTATAAAATCTGGTGCTGTAGGTCAACATGTTGTAAATATGCAGCTAAAG | 3’ to 5’ top strand for 5’ branch |

*Lowercase letters indicate overlap sequences for SOE PCRs or mutated nucleotides when generating point mutations

**SUPPLEMENTARY METHODS**

*Protein expression and purification*

The *comM* open reading frame was PCR-amplified from *V. cholerae* genomic DNA using oligonucleotides MB1225 (CGGGATCCATGGGACTTGCGATCATTCATAGCCG) and MB1214 (CGATCGATCTCGAGCTAGACGTTCTGCGCACTGAGC), digested with *Bam*HI and *Xho*I, and ligated into the same sites in plasmid pMB131 to generate pMB486. This cloning added an N-terminal 4x Strep-tag II to the translated protein. The expression plasmid encoding the ATPase- and helicase-dead *comM-K224A* allele (pMB488) was created site-directed mutagenesis of pMB486 with oligonucleotides MB1215 (TATTGTTTCTCGGCCCTCCGGGGACGGGTGCGACCATGCTCGCTTCACGGCTGTGCGATT) and MB1216 (AATCGCACAGCCGTGAAGCGAGCATGGTCGCACCCGTCCCCGGAGGGCCGAGAAACAATA) and verified by DNA sequencing (ACGT, Inc.). Expression plasmids were transformed into Rosetta 2(DE3) pLysS cells and selected for at 37°C on LB medium supplemented with 100 µg/mL ampicillin and 34 µg/mL chloramphenicol. Fresh transformants were used to inoculate one or more 5-mL LB cultures supplemented with antibiotics and incubated at 30°C for ~6 h with aeration. These starter cultures were then diluted 1:100 in ZYP-5052 autoinduction medium containing 1x trace metals mix (3), 100 µg/mL ampicillin, and 34 µg/mL chloramphenicol and incubated at 22°C with agitation to $OD_{600}$ >3 (15-18 h). Cells were harvested by centrifugation for 10 min at 5,500 x g and 4°C. Cell pellets were weighed and frozen at -80°C prior to lysis or for long-term storage.

Frozen cell pellets were thawed at room temperature by stirring in 4 mL/g cell pellet resuspension buffer (25 mM Na-HEPES (pH 7.5), 5% (v/v) glycerol, 300 mM NaOAc, 5 mM MgOAc, and 0.05% Tween-20) supplemented with 1x protease inhibitor cocktail (Sigma), and 20 µg/mL DNase I. Cells were lysed by six passed through a Cell Cracker operated at >1000 psi. All subsequent steps were performed at 4°C. The soluble fraction was clarified by centrifugation for 30 min at 33,000 x g followed by filtering the supernatant through a 0.22-µm membrane. This mixture was then applied to a Strep-Tactin Sepharose column (IBA) pre-equilibrated in resuspension buffer using an ÄKTA Pure (GE Healthcare Life Sciences). The column was washed with 20 column volumes (CVs) of resuspension buffer, 10 CVs of resuspension buffer supplemented with 5 mM ATP, and 10 CVs of resuspension buffer. Protein was eluted with 15 CVs of resuspension buffer supplemented with 2.5 mM desthiobiotin (IBA). Column fractions were examined on 8% SDS-PAGE gels run at 20 V/cm and stained with Coomassie Brilliant Blue R-250 (BioRad). Peak fractions were pooled, concentrated with Amicon Ultra-4 30K centrifugal filters, and loaded onto a HiPrep 16/60 Sephacryl S-200 HR column (GE Healthcare Life Sciences) pre-equilibrated in gel filtration buffer (25 mM Na-HEPES (pH 7.5), 5% glycerol, 300 mM NaCl, 5 mM $MgCl_2$, and 0.05% Tween-20). The protein was eluted with 1.5 CVs gel filtration buffer, and fractions were analyzed by SDS-PAGE as above. Peak fractions were pooled, snap-frozen with liquid nitrogen, and stored at -80°C.

The *Saccharomyces cerevisiae* Pif1 helicase was overexpressed In Rosetta cells from plasmid pMB330 as described for ComM above. Pif1 purification was likewise identical, except the protein from the Strep-Tactin column was polished by Ni-affinity chromatography instead of size exclusion. Briefly, the pooled peak fractions were applied to a His60 Ni Superflow (Clontech) gravity column, washed with 10 CVs resuspension buffer supplemented with 25 mM imidazole (pH 8), and eluted with 4.5 CVs of a step gradient of resuspension buffer containing 100 mM, 250, and 500 mM imidazole (pH 8). Peak fractions were pooled, buffer exchanged into storage buffer (4), snap-frozen

with liquid nitrogen, and stored at -80°C. The *Mycobacterium smegmatis* SftH was purified exactly as previously described (5).

ComM preps were tested for nuclease activity by incubating a labeled ssDNA probe with 100 nM of each protein prep in resuspension buffer for 1 hour at 37°C. Samples were then deproteinated with 1X stop load buffer and separated by native PAGE to assess degradation of the probe. All ComM protein preps used lacked detectable nuclease activity.

*Blue native PAGE*

Oligomerization of ComM protein was assayed using Blue Native PAGE electrophoresis. 2.5 μM purified ComM was incubated for 30 min at room temperature in reaction buffer [10 mM Tris-HCl pH7.5, 20mM KCl, 1mM DTT, 10% Glycerol] with 5 mM ATP and/or 5 nM ssDNA (oligo ABD363) where indicated. 1 μL 20x sample buffer [5% Coomassie G-250, 0.5 M aminocaproic acid pH 7] was added to each reaction and samples were run on 4-16% Native PAGE gels [gel buffer = 0.5 M aminocaproic acid pH 7.0, 0.05 M Bis-Tris pH 7.0]. The cathode buffer was composed of 50 mM Tricine, 15 mM Bis-Tris pH 7.0, 0.02% Coomassie G-250, while the anode buffer was composed of 50 mM Bis-Tris pH 7.0. Samples were run at 150 V for 30 min, then 200 V for 45 min.

*Negative stain electron microscopy*

The nominal magnification for the images is 60,000x, which is equivalent to 1.8 Å per pixel at the final image. Initial image processing, particle boxing, and CTF determination were performed using EMAN2 (6). A phase-flipped particle dataset was then imported into Relion (7) for 2D classification. Classes showing noisy images were discarded at this stage. As we observed clear six-fold symmetry from the classes, the subsequent processing imposed C6 symmetry. The remaining "good" classes were used to generate the initial models using e2initialmodel.py. The 3D classification was carried out using the initial model that was low-pass filtered to 40 Å to eliminate the possible effect from the model bias. Three 3D classes were obtained; the highest population (46%) of the classes was subjected to further structure refinement in Relion. Approximately 32,958 particles were used to generate the final 3D reconstruction. The reported resolution is ~13.8 Å using gold-standard Fourier shell correlation at a 0.143 cutoff; however, it is an over-estimated value because of the use of negative stain. The structure is rendered using UCSF Chimera (8).

*Helicase Assays*

Fork substrates for helicase assays were made by 5'-end labelling oligonucleotides (**Table S2**) with T4 polynucleotide kinase (T4 PNK; NEB) and γ[$^{32}$P]-ATP. Labelled oligonucleotides were separated from free label using illustra ProbeQuant G-50 micro columns (GE Healthcare) following the manufacturer's instructions. Oligonucleotides were annealed by incubation with an equimolar amount of partially complementary oligonucleotides overnight at 37°C in annealing buffer (20 mM Tris-HCl [pH 8], 4% glycerol, 0.1 mM EDTA, 40 μg/mL BSA, 10 mM DTT, and 10 mM MgOAc) (9).

The DNA fork that allows for 5'-3' and 3'-5' activity was made by annealing oligonucelotides MB1167 with MB1168. The DNA fork that only allows for 5'-3' helicase activity was made by

annealing MB1167 / MB1511. The DNA fork that only allows for 3'-5' helicase activity was made by annealing MB1168 / MB1510. DNA unwinding was assessed by incubating the indicated concentrations of helicase with 5 mM ATP and 0.1 nM radiolabelled fork in resuspension buffer. Reactions were incubated at 37°C for 30 min and stopped with the addition of 1x Stop-Load dye (5% glycerol, 20 mM EDTA, 0.05% SDS, and 0.25% bromophenol blue) supplemented with 400 µg/mL Proteinase K followed by a 10-min incubation at 37°C. Unwound DNA was then separated on 8% 19:1 acrylamide:bis-acrylamide gels in TBE buffer at 10 V/cm. Gels were dried under vacuum and imaged using a Typhoon 9210 Variable Mode Imager. DNA binding was quantified using ImageQuant 5.2 software.

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