Supplemental Materials

Molecular Biology of the Cell

Yoo et al.

Supplemental Material

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Dm_CapuCT  FHRTNTMRKSAVNPPKPMRPLYWTRIVTSAPPAPRPPSVANSTDSTENSGSSPDEPPAANGADAPPTAPPATKEIWTEIEETPLD----NI---DEFTELFSRQAIAPVSK---------681
mFmn1      -------RKPAIEPSCPMKPLYWTRIQINDKSQ------------------------------------DAAPTLWDSLEEPHIR----DT---SEFEYLFSKDTTQQKKKP--------1044
hFMN1      -------RKPAIEPSCPMKPLYWTRIQISDRSQ------------------------------------NATPTLWDSLEEPDIR----DP---SEFEYLFSKDTTQQKKKP--------1033
mFmn2      -------RKQLIEPCRPMKPLYWTRIQLHSKRD------------------------------------SSPSLIWEKIEEPSI-----DC---HEFEELFSKTAVKERKKP--------1199
hFMN2      -------RKQPIEPCRPMKPLYWTRIQLHSKRD------------------------------------SSTSLIWEKIEEPSI-----DC---HEFEELFSKTAVKERKKP--------1343
mFHOD1-------DGPRHPTKRKTVKLFWRELKLTGGPG------------------------------CSRSRFGPCPTLWWASLEPVSVD-----T---ARLEHLFESRAKDVLPTK-------714
YsBni1     -------FEKYPRPHKKLKQLHWEKLDCT------------------------------------------DNSIWGTGKAEKFADDLYEKGVLADLEKAFAAREIKSL-----------1407
mDia1      -------PKKVYKPEVQLRRPNWSKFVAEDLS---------------------------------------QDCFWTKVKEDRFE-NNELF---AKLTLAFSAQTKTSKAKK----D-QE816
mDia2      -------QKKLYKPDIPMKRINWSKIEPKELS---------------------------------------ENCVWLKLKEEKYE-NADLF---AKLALTFPSQMKGQRNTE-------A687
mDia3      -------PKKEFKPEISMRRLNWLKIGPNEMS---------------------------------------ENCFWIKVNENKYE-NRDLL---CKLENTFCCQEKEKRNTN----D-F- 678
mINF2     -------HRRVNPPTLRMKKLNWQKLPSNVAR--------------------------------------ERNSMWATLGSPCTAAVEPDF---SSIEQLFSFPTAK-------------647
mDAAM1     -------KKNIPQPTNALKSFNWSKLPENKLD----------------------------------------GTVWTEIDDTKVF-KILDL---EDLERTFSAYQRQQEFFVNNSKQKEA668
hFMNL1     -------AKKPIQTKFRMPLLNWVALKPSQIT----------------------------------------GTVFTELNDEKVL-QELDM---SDFEEQFKTKSQGPSLDLSALKSKAA 700
mDelphilin -------HRRSETSHMSVKRLRWEQVENS------------------------------------------EGTIWGQLGEDSDYDKLSDMVKYLDLELHFGTQKPPK------------870

Dm_CapuCT  -----PKELKVKRAKSIKVLDPERSRNVGIIWRSLH-VPSSEIEHAIYHID-TSVVSLEALQHMS-------------NIQATEDELQRIKEAA------GGDIPLDHPEQFLLDI--SL773
mFmn1      --LSEAYEKKNKVKKIIKLLDGKRSQTVGILISSLH-LEMKDIQQAIFTVD-DSVVDLETLAALY-------------ENRAQEDELTKIRKYYETSK-EEDLKLLDKPEQFLHEL--AQ1144
hFMN1      --LSETYEKKNKVKKIIKLLDGKRSQTVGILISSLH-LEMKDIQQAIFNVD-DSVVDLETLAALY-------------ENRAQEDELVKIRKYYETSK-EEELKLLDKPEQFLHEL--AQ1444
mFmn2      --ISDTISK-TKAKQVVKLLSNKRSQAVGILMSSLH-LDMKDIQHAVVNLD-NSVVDLETLQALY-------------ENRAQSDELEKIEKHSRSSKDKENAKSLDKPEQFLYEL--SL1299
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mFHOD1------K-AGEGRRTMTVVLDPKRSNAINIGLTTLP--PVHVIKAALLNFD-EFAVSKDGIEKLL-------------TMMPTEEERQKIEEAQ----LANPDVPLGPAENFLMTL--AS805
YsBni1     ------ASKRKEDLQKITFLSRDISQQFGINLHMYSSLSVADLVKKILNCDRDFLQTPSVVEFLSKSEIIEVSVNLARNYAPYSTDWEGVRNLEDAKPPEKDPNDLQRADQIYLQLMVNL1521
mDia1      GGEEKKSV-QKKKVKELKVLDSKTAQNLSIFLGSFR-MPYQEIKNVILEVN-EAVLTESMIQNLI-------------KQMPEPEQLKML---SE---LKEEYDDLAESEQFGVVM--GT912
mDia2      AEENRSGP-PKKKVKELRILDTKTAQNLSIFLGSYR-MPYEEIKNIILEVN-EEMLSEALIQNLV-------------KYLPDQNALREL---AQ---LKSEYDDLCEPEQFGVVM--ST783
mDia3      ---DEKKV-IKKRMKELKFLDPKIAQNLSIFLSSFR-VPYEKIRTMILEVD-ETQLSESMIQNLI-------------KHLPDEEQLKSL---SQ---FRSDYNSLCEPEQFAVVM--SN771
mINF2     PKEPSAAP-ARKEPKEVTFLDSKKSLNLNIFLKQFK-CSNEEVTSMIQAGD-TSKFDVEVLKQLL-------------KLLPEKHEIENL---RA---FTEERAKLSNADQFYVLL--LD743
mDAAM1     DAIDDTLS-SKLKVKELSVIDGRRAQNCNILLSRLK-LSNDEIKRAILTMDEQEDLPKDMLEQLL-------------KFVPEKSDIDLL---EE---HKHELDRMAKADRFLFEM--SR765
hFMNL1     QK----------APSKATLIEANRAKNLAITLRKGN-LGAERICQAIEAY-DLQALGLDFLELLM-------------RFLPTEYERSLITRFER---EQRPMEELSEEDRFMLCF--SR790
mDelphilin---PVPGPEPFRKKEVVEILSHKKAYNTSILLAHLK-LTPGELRQVLMSME-PRRLEPAHLAQLL-------------LFAPDADEEQRY---QA---FREAPGRLSEPDQFVLQM--LS964

Dm_CapuCT  ISMASERISCIVFQAEFEESVTLLFRKLETVSQLSQQLIESEDLKLVFSIILTLGNYMNGGNRQRGQADGFNLDILGKLKDVKS-KESHTTLLHFIVRTYIAQRRKE-------------879
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YsBni1     ESYWGSRMRALTVVTSYEREYNELLAKLRKVDKAVSALQESDNLRNVFNVILAVGNFMNDTSK---QAQGFKLSTLQRLTFIKD-TTNSMTFLNYVEKIVRLNYPSFND-----------1626
mDia1      VPRLRPRLNAILFKLQFSEQVENIKPEIVSVTAACEELRKSENFSSLLELTLLVGNYMNAGSRN-AGAFGFNISFLCKLRDTKS-ADQKMTLLHFLAELCENDHPEVLK-----------1019
mDia2      VKMLRPRLTSILFKLTFEEHVNNIKPSIIAVTLACEELKKSESFKRLLELILLVGNYMNSGSRN-AQSLGFKINFLCKIKDTKS-ADQKSTLLHFLAEICDEKYRDILK-----------890
mDia3      VKRLRPRLSAILFKLQFEEQVNNIKPDIMAVSTACEEIKKSKGFSKLLELVLLMGNYMNAGSRN-AQTFGFDLSSLCKLKDTKS-ADQKTTLLHFLVDVCEEKHADILH-----------878
mINF2     IPCYPLRVECMMLCEGTAIVLDMVRPKAQLVLTACESLLTSQRLPVFCQLILKIGNFLNYGSHT-GDADGFKISTLLKLTETKS-QQSRVTLLHHVLEEVEKSHPDLLQ-----------850
mDAAM1     INHYQQRLQSLYFKKKFAERVAEVKPKVEAIRSGSEEVFRSRALKQLLEVVLAFGNYMNKGQRG--NAYGFKISSLNKIADTKSSIDKNITLLHYLITIVENKYPKVLN-----------872
hFMNL1     IPRLPERMTTLTFLGNFPDTAQLLMPQLNAIIAASMSIKSSDKLRQILEIVLAFGNYMNSSKRG--AAYGFRLQSLDALLEMKS-TDRKQTLLHYLVKVIAEKYPQLTG-----------896
mDelphilinVPEYKTRLRSLHFQATLQEKTEEIRGSLECLRQASLELKNSRKLAKILEFVLAMGNYLNDGQPKTNKTTGFKINFLTELNSTKT-VDGKSTFLHILAKSLSQHFPELLG-----------1072

Dm_CapuCT  ----------------------GVHPLEIRLPIPEPADVERAAQMDFEEVQQQIFDLNKKFLGCKRTTAKVL--AASRPEIMEPFKSKMEEFVEGADKSMAKLHQSLDECRDLFLETMRF975
mFmn1      LFSLYFHIAVHPQRKSGLELKQEAGTDKSVFPLPEPQDFFLASQVKFEDLLKDLRKLKRQLEASEQQMKLVC--KESPREYLQPFKDKLEEFFKKAKKEHKMEESHLENAQKSFETTVGY1381
hFMN1      ----------------------EAGTEKSVFPLPEPQDFFLASQVKFEDLIKDLRKLKRQLEASEKQMVVVC--KESPKEYLQPFKDKLEEFFQKAKKEHKMEESHLENAQKSFETTVRY1334
mFmn2      ----------------------DAGKEQCVFPLAEPQELFQASQMKFEDFQKDLRKLKKDLKACEAEAGKVY--QVSSAEHMQPFKENMEQFISQAKIDQESQEAALTETHKCFLETTAY1500
hFMN2      ----------------------DAGKEQCLFPLPEPQDLFQASQMKFEDFQKDLRKLKKDLKACEVEAGKVY--QVSSKEHMQPFKENMEQFIIQAKIDQEAEENSLTETHKCFLETTAY1645
mFHOD1-------------------------------LYSEIPALTRCAKVDFEQLTENLGQLECRSQAAEDSLRSL-----AKHELSPALRARLTHFLAQCTRRVAMLRVVHRRVCNRFHAFLLY992
YsBni1     -------------------------------FLSELEPVLDVVKVSIEQLVNDCKDFSQSIVNVERSVEIGNLSDSSKFHPLDKVLIKTLPVLPEARKKGDLLEDEVKLTIMEFESLMHT1715
mDia1      -------------------------------FPDELAHVEKASRVSAENLQKSLDQMKKQIADVERDVQNF----PAATDEKDKFVEKMTSFVKDAQEQYNKLRMMHSNMETLYKELGDY1104
mDia2      -------------------------------FPDELEHVESAGKVSAQILKSNLVAMEQSILHLEKNIKNF----PPAESHHDKFVEKMMSFTQNAREQYDKLSTMHSNMLKLYESLGEY975
mDia3      -------------------------------FVDDLAHLDKASRVSVEMLEKNVKQMGRQLQQLEKNLETF----PPPEDLHDKFVIKMSSFVISANEQYEKLSTLLGSMTQLYQSIMGY962
mINF2     -------------------------------LSRDLEPPSQAAGINVEIIHSEASANLKKLLEAERKVSAS---IP---EVQKQYAERLQASI-EASQE---LDKVFDAIEQKKLELADY929
mDAAM1     -------------------------------LSEELRDIPQAAKVNMTELDKEISTLRSGLKAVETELEYQ---KSQPPQPGDKFVSVVSQFITLASFSFSDVEDLLAEAKELFTKAVKH958
hFMNL1     -------------------------------FHSDLHFLDKAGSVSLDSVLADVRSLQRGLELTQREFVRQ---DD---------CMVLKEFLRANSPTMDKLLADSKTAQEAFESVVEY973
mDelphilin-------------------------------FAQDLPTVPLAAKVNQRALTGDLADLHDTVSEIQVACQS------MAPSSEDRFAVVMASFLETAQPALRALDGLQREAMEELGKALAF1155

Dm_CapuCT  YHFSPKACTLTLAQCTPDQFFEYWTNFTNDFKDIWKKEITSLLNELMKKSKQAQIESRRN1035
mFmn1      FGMKPKTGE---KEVTPSYVFMVWFEFCSDFKTIWKRESKNISKERLKMAQASVSKL---    1435
hFMN1      FGMKPKSGE---KEITPSYVFMVWYEFCSDFKTIWKRESKNISKERLKMAQESVSKL---    1388
mFmn2      YFMKPKLGE---KEVSPNVFFSVWHEFSSDFKDAWKKENKLILQERVKEAEEVCRQK---1554
hFMN2      FFMKPKLGE---KEVSPNAFFSIWHEFSSDFKDFWKKENKLLLQERVKEAEEVCRQK---    1698
mFHOD1LGYTPQAA----RDVRIMQFCHTLREFALEYRTCRERVLQQQQKRATYRE--------RN    1040
YsBni1     YGEDSGDK------FAKISFFKKFADFINEYKKAQAQNLAAEEEERLYIKHKKIVEE---    1767
mDia1      FVFDPKK-------LSVEEFFMDLHNFRNMFLQAVKENQK---------RRETEEKMRRA    1149
mDia2      FIFDPNT-------VNMEEFFGDLNTFRTLFLEALKENHK---------RKEMEEKSRRA    1019
mDia3      YAVDMKK-------VSVEEFFNDLNNFRTSFMLALKENIK---------KREAAEKEKRA    1007
mINF2     LCEDPQQ-------LSLEDTFSTMKTFRDLFTRALKENKD--------RKEQMAKAERRK    974
mDAAM1     FGEEAGK-------IQPDEFFGIFDQFLQAVAEAKQENEN--------MRKRKEEEERRA    1003
hFMNL1     FGENPKT-------TSPGLFFSLFSRFIKAYKKAEQEVEQ--------WKKEAAAQEAGA    1018
mDelphilinFGEDSKA-------TTSEAFFGIFSEFMSKFERALSDLQA--------GDGP-----RSS    1195
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Figure S1. **Sequence conservation between different formins.** Sequence alignment of several formins. Sequences were acquired from Uniprot (*Drosophila*Capu, Q24120; mouse Fmn1, Q05860; mouse Fmn2, Q9JL04; human FMN1, Q68DA7; human FMN2, Q9NZ56; mouse Dia1, O08808; mouse Dia2, O70566; mouse Dia3, Q9Z207; mouse INF2, Q0GNC1; mouse Daam1, Q8BPM0; mouse FHOD1, Q6P9Q4; human FMNL1, O95466; mouse Delphilin, Q0QWG9; *S. cerevisiae* Bni1p, P41832) and aligned using ClustalW(Larkin *et al.*, 2007). Mutated residues are highlighted in their respective colors: K586 (blue), P597 (green), D662 (red), I751 (orange), L768 (turquoise), D854 (olive), H977 (purple). Identity matches are highlighted in the same color, while similar residues are shown in a colored font. Deleted residues in CapuCTΔloop are highlighted in black. The two conserved tryptophan residues are bolded and highlighted in gray.

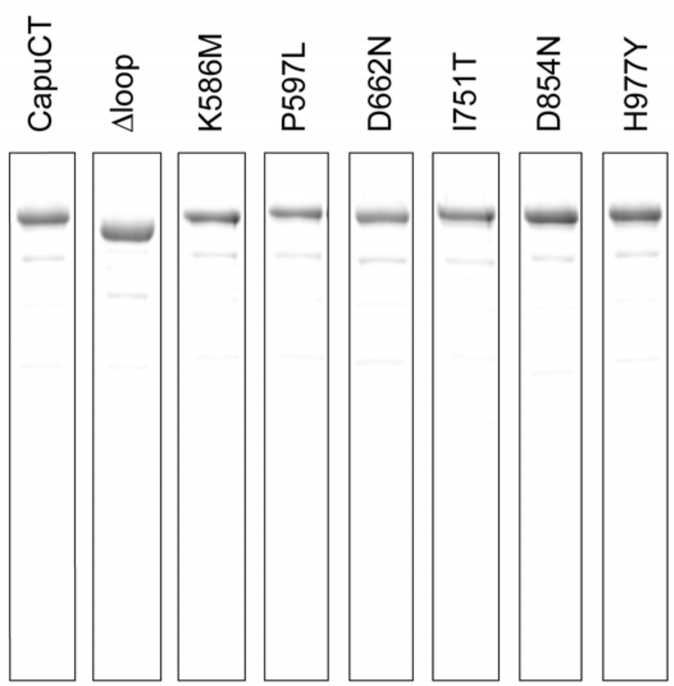

Figure S2. Purified CapuCT constructs used in this study. Approximately 2 ug of each purified protein was run on a polyacrylamide gel and stained for total protein with Sypro Red. For all samples, CapuCT is the most abundant protein.

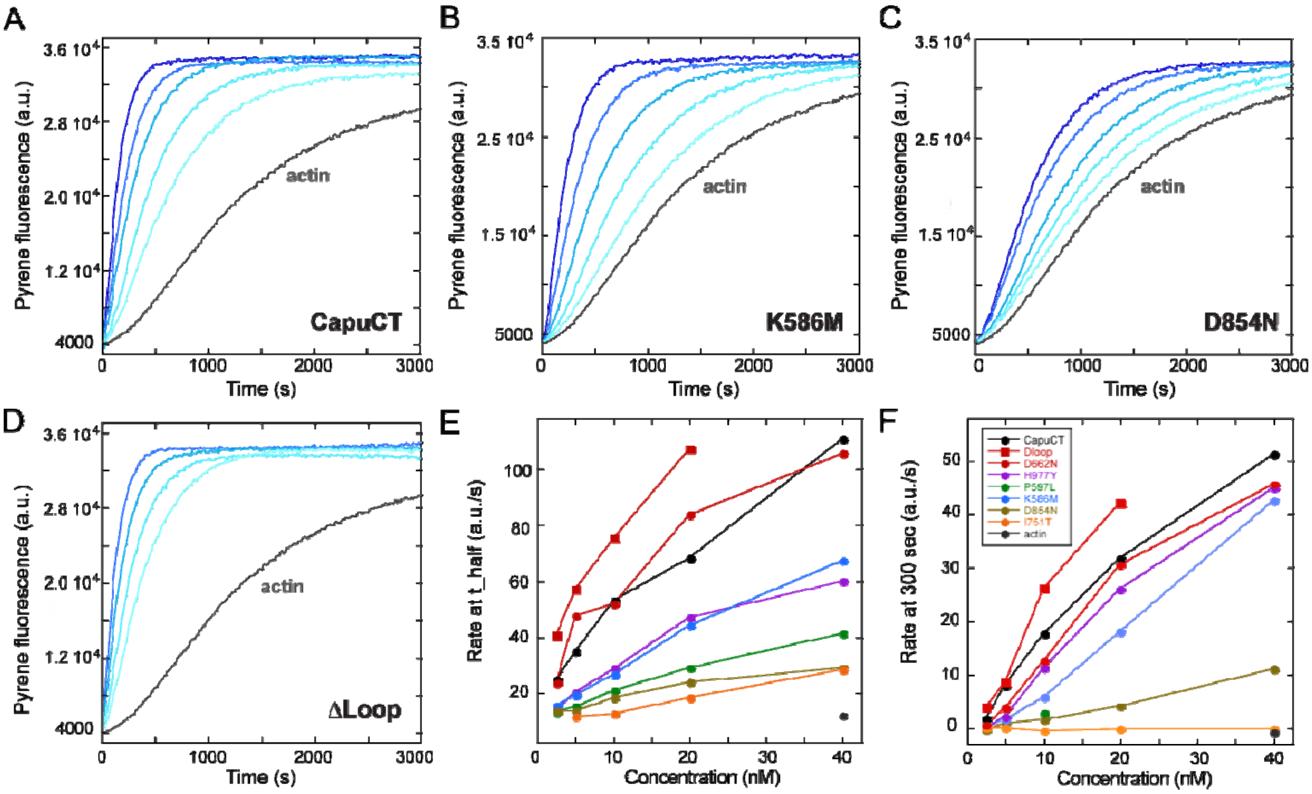

Figure S3. Bulk actin assembly activity of CapuCT constructs over a range of concentrations. CapuCT mutants were tested for their actin-assembly activity both in the absence (A-E) and the presence (F) of *S. pombe*profilin over a range of concentrations.(A-D) Four sets of representative traces showing the dose-dependent activity of CapuCT and selected mutants. The concentrations used are, from dark blue to light blue, 40 nM, 20 nM, 10 nM, 5nM, and 2.5 nMCapuCT.(E) The actin polymerization rate (slope) at $t_{1/2}$ in the absence of profilin. (F) The actin polymerization rate (slope) at 300 s in the presence of profilin. The legend for panels (E) and (F) is shown in as an inset in panel (F).

Supplementary References

Larkin, M. A. *et al.* (2007). Clustal W and Clustal X version 2.0. Bioinformatics *23*, 2947–2948.