

Phenamacril is a reversible and non-competitive inhibitor of *Fusarium* class I myosin

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Running title: *Inhibition of Fusarium class I myosin by phenamacril*

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List of supplementary information: Table S1, Figure S1, Figure S2, Figure S3, Figure S4, Figure S5

Table S1. Oligo DNA primers used to amplify the regions encoding *Fusarium* calmodulin and myosin class I motor domain constructs. Fg: *Fusarium graminearum*, Fa: *F. avenaceum*, Fs: *F. solani*, CaM: calmodulin, LIC: ligase independent cloning.

Primer ID	Sequence 5'-3'	Target locus	Protein
P1152	TTTCTCGAGATGGGCCGACTCACTTACTGAAG	FGSG_01891	FgCaM
P1153	GTTTGCTAGCTTATTTTTGCATCATAAGCTGG	FGSG_01891	FgCaM
P1125	GAAGGATCCATGGATTACAAGGATGACGACG ATAAGATGGGAATATCGAGACGCCCGAA	FGSG_01409	FgMyo1
P1126	CCCAAGCTTTTAGTGGTGGTGGTGGTGGTGTG AACCTGATCGCATGTGCTCCAGAGCGAA	FGSG_01409	FgMyo1
P1125	GAAGGATCCATGGATTACAAGGATGACGACG ATAAGATGGGAATATCGAGACGCCCGAA	FGSG_01409	FgMyo1 _{IQ2}
P1127	CCCAAGCTTTTAGTGGTGGTGGTGGTGGTGTG AACCTGAGTCACGAAGCTGGAGAAATTC	FGSG_01409	FgMyo1 _{IQ2}
P1130	GAAGGATCCATGGATTACAAGGATGACGACGA TAAGATGGGAATATCGAGACGCCCTAA	FAVG1_11042	FaMyo1 _{IQ2}
P1132	CCCAAGCTTTTAGTGGTGGTGGTGGTGGTGTGA ACCTGAGTCCCGGAGTTGCAGATACT	FAVG1_11042	FaMyo1 _{IQ2}
P1135	GAAGGATCCATGGATTACAAGGATGACGACG ATAAGATGGGAATATCAAGACGTCCCAA	NECHADRAFT_10 3022	FsMyo1 _{IQ2}
P1137	CCCAAGCTTTTAGTGGTGGTGGTGGTGGTGTG AACCTGAATCTCGAAGCTGCAAGTACTCG	NECHADRAFT_10 3022	FsMyo1 _{IQ2}
P1345	AAAACCTCTATTTTCAGGCCGACTCACTTACT GAAGAG	FGSG_01891	FgCaM _{LIC}
P1346	TATCCACCTTACTGTTATTTTTGCATCATAAG CTGG	FGSG_01891	FgCaM _{LIC}

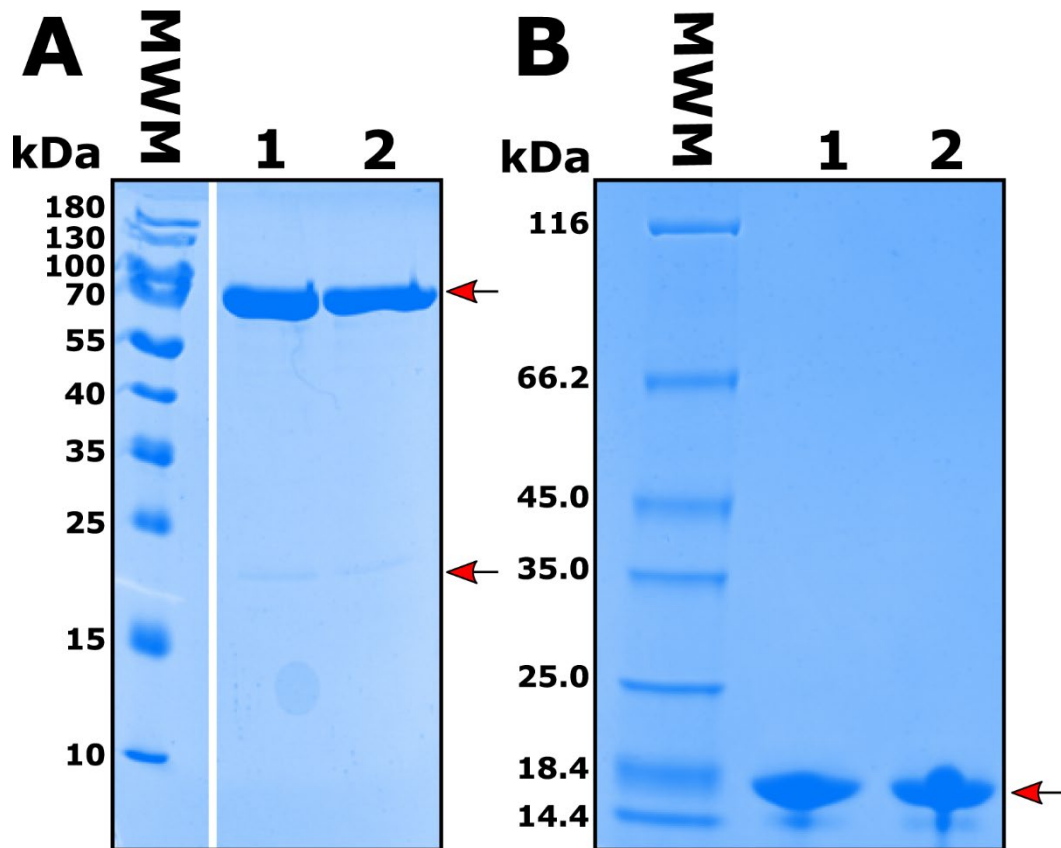


Figure S1. Coomassie-Brilliant Blue stained polyacrylamide gels showing two representative fractions resulting from the size-exclusion chromatographic purification of (A) FgMyo1_{IQ2} co-expressed with calmodulin from *F. graminearum* PH-1 in *Sf9* insect cells. White line indicates removed lanes. (B) *E. coli* BL21(DE3) produced calmodulin from *F. graminearum* PH-1 (FgCaM). Red arrows serve to highlight the presence of the relevant protein-bands.

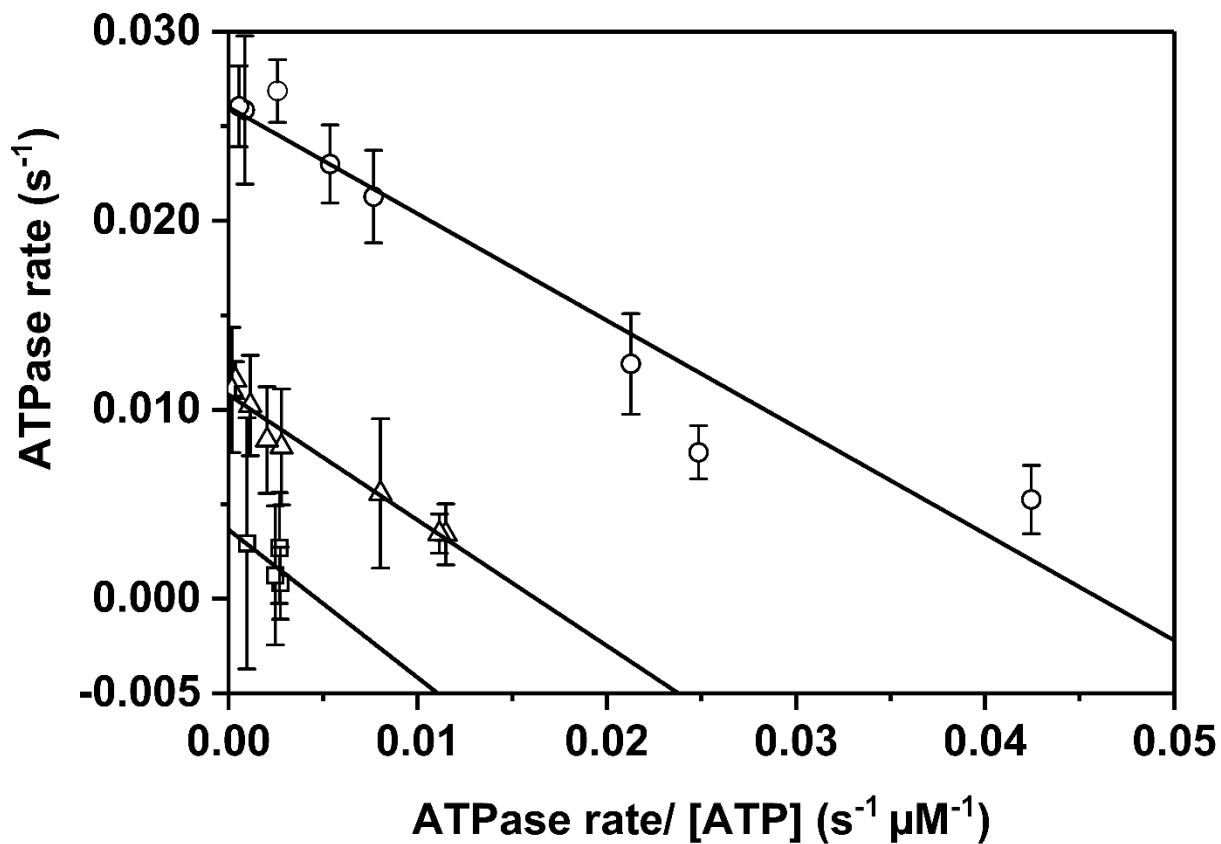


Figure S2. Eadie-Hofstee plots of Phenamacril inhibition of basal ATP turnover in the presence of Ca²⁺ by FgMyo1. A least-squares regression analysis was used to determine the Eadie-Hofstee linear correlations. Measurements were performed in the presence of 0 (○), 0.6 (Δ) and 10 μM (□) Phenamacril. Error bars represent standard deviations around the mean (n = 3).

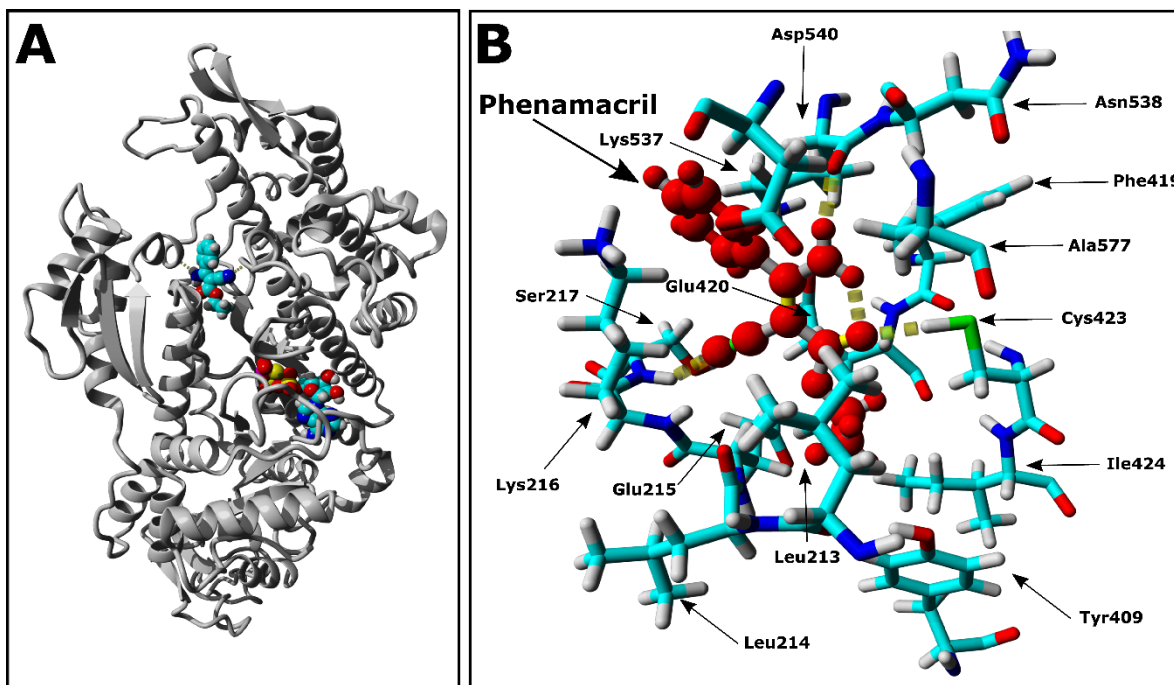


Figure S3. Phenamacril docks into the actin-binding groove of the *F. graminearum* class I myosin motor domain (A) Position of Phenamacril within the docked homology model of the *F. graminearum* class I myosin motor domain. For reference, the position of the nucleotide binding-pocket is indicated by the position of ADP-vanadate (B) Close-up view, highlighting the protein-ligand interactions between Phenamacril (shown as red ball-and-stick model) and amino acid residues from the motor domain (stick-model). Hydrogen-bonds are shown as dotted yellow lines.

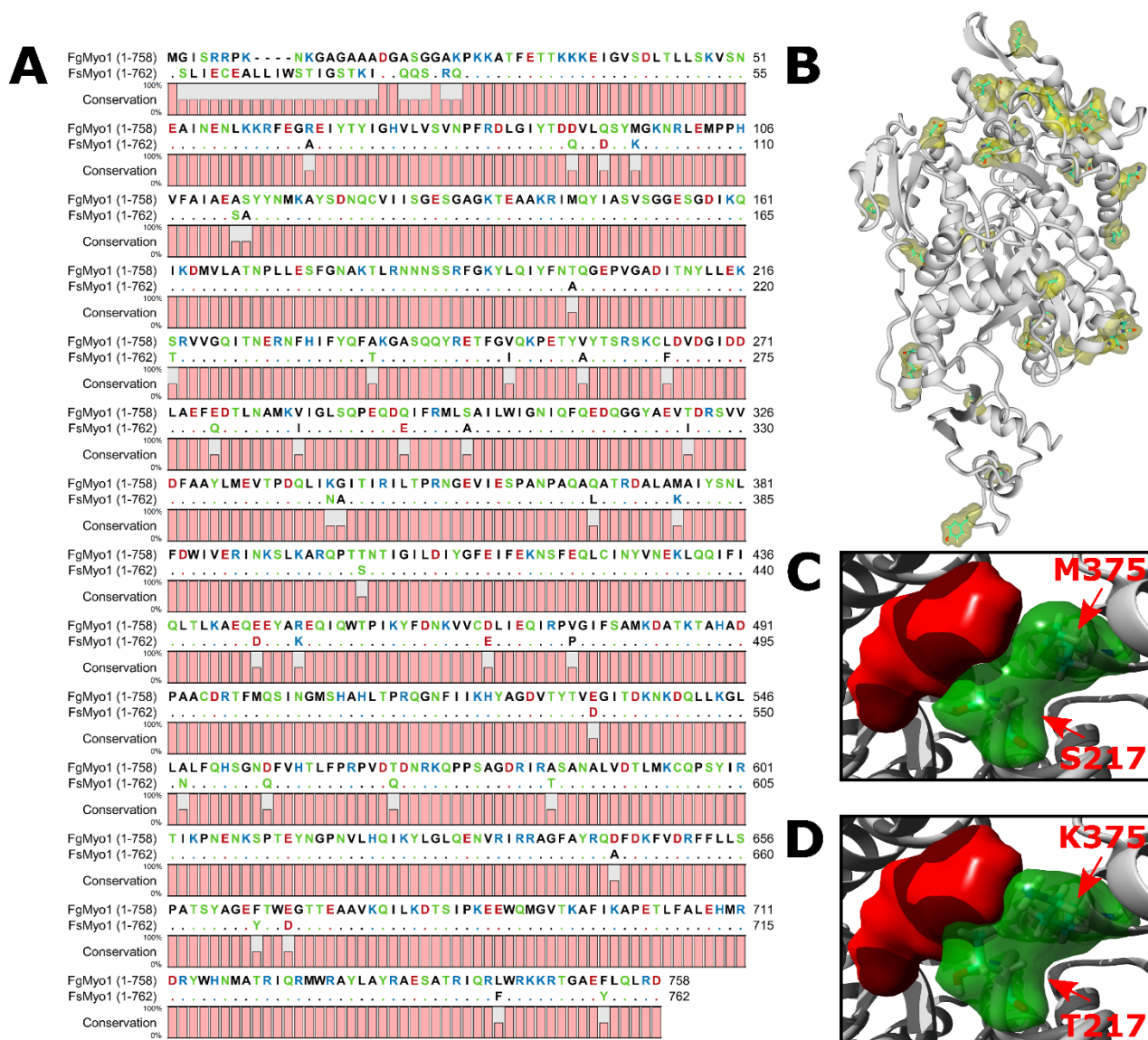


Figure S4. (A) Pairwise alignment of the class I myosin motor domains from *F. graminearum* PH-1 (FgMyo1), *F. solani* f. sp. *pisi* 77-14-4 (FsMyo1). Amino acids are colored by polarity, dots indicating residues that are conserved in comparison to the reference sequence of FgMyo1. (B) Pre-power stroke homology model of FsMyo1. The molecular surfaces highlighted in yellow (FsMyo1) correspond to the amino acid residues that differ from FgMyo1. (C) Surface-rendering of the Phenamacril docking-pose (red surface) and residues Met375 and S217 (green surfaces). (D) Substitutions S217T and M375K (both present in *F. solani*) in the model of FgMyo1 results in overlap of the volumes enclosed by the red (Phenamacril) and green surface areas.

Fg myosin 1 -----MGISRRPKNKGAGAAADGASGGAKPKKATFETTKK----- 35
 Fa myosin 1 -----P-----N.V----- 35
 Fs myosin 1 -----MSLIECEALLIWSTIGSTKI.QQS.RQ----- 39
 Dd myosin 1E -----MIP.T----- 5
 Dd myosin 1B -----MSKKVQ----- 6
 Dd myosin 2 MNPIDHRTSDYHKYLVKVKQSDSLFKLTVSDK.YIWNPPDKER.SYEC.EIVSETSDSF.F.TVDGQDRQVKKDDAN 78
 Hs myosin 1c -----MALQVELVPTGEIRVVHPPRPCKLALGSDGVRVTMESAL.AR----- 43
 Fg myosin 1 -----KEIGVSDLTLL-SKVSNEAINEENLKKRFEGREITYYIGHVLVSNPFRDLGIYDDVLQSYMGKNRLEMPPHV 107
 Fa myosin 1 -----A-----Q.E----- 107
 Fs myosin 1 -----A-----Q.D.K----- 111
 Dd myosin 1E -----AE.P.FV.-NQITEN.FI.TM.HKSDN.D.VI.T.KN.N.KESDIKAN.RYKY----- 77
 Dd myosin 1B -----AKQ.TD.VM.-P.EDE.C.YMDF.N.P.I.N.NNSGP.FIEAR.HAQ.V----- 78
 Dd myosin 2 QRNPI.FD.E.MSE.YLNEP.VFH.RV.YNQDL.S.LF.A.KRIP.QEMVDIFK.RR.N.VA.I 155
 Hs myosin 1c -----DRV.Q.FV.ENFT.EA.FI.RR.RENL.P.Y.Q.SRQHMERR.VSFY.V.L 116
 Fg myosin 1 FAIAEASYYNMKAYSNDQCVIISGESGAGKTEAAKRIMQYIASVSGGESGD-IKQIKDMVLATNPLLESFGNAKTLRN 184
 Fa myosin 1 -----A----- 184
 Fs myosin 1 -----SA----- 188
 Dd myosin 1E Y.L.NDA.RS.RQSQE-----S.K.FLTF.SNQ.PN-GER.SK.L.DS.A----- 154
 Dd myosin 1B YQL.SA.RA.NDQE-----L.G.VSAI.TEKVEYV.HVI.ES.A----- 154
 Dd myosin 2 .SDVA.RS.LDDRQ.SLL.T-----NT.KVI.L.A.RNQANGSGVLEQQI.QA.I.A.T----- 233
 Hs myosin 1c .V.DTV.RALRTERRD.A.M-----T.LL.FY.ETCP--APERGGAVR.RL.QS.V.A----- 192
 Fg myosin 1 NNSSRFGKYLQIYFNTQGEIPVGADITNYLLEKSRVVGQITNERNFHIFYQFAKASQYRETFGVQ-KPETYYVTSRS 261
 Fa myosin 1 -----Q.E----- 261
 Fs myosin 1 -----A-----T----- 265
 Dd myosin 1E D.MEMQ.AV.S.I.GK-----RTQG.S.ML.L.SKLNEL.LTPNAPA.E.LKK 232
 Dd myosin 1B FE.Q.DKA.D.GK.Y-----Y.NPG.LLA.A.EKRDYVLS.S.S.Y.LNQ 231
 Dd myosin 2 FIE.Q.SA.FIS.S.QS-----F.SET.Y.LLA.TAEKKALHLA.G.SFN.LNQ 310
 Hs myosin 1c D.MDVQ.DFK.A.GH.LS-----H.NHG.LLE.GEEETLRLR.LERN.QS.L.LVKG 270
 Fg myosin 1 KCLDVGIDDLAEFEDTLNAMKVIIGLSQPEQDQIFRMSALWIGNIQFQEDQGGYA-----EVTDRSVVDFAAYLME 334
 Fa myosin 1 -----A.D-----N-----K----- 334
 Fs myosin 1 -----F-----Q-----I-----E-----A-----I----- 338
 Dd myosin 1E G.F.ST.SG.KIIVK.ETL.KESD.NS.W.I.A.H.T.A.AAEQRTGTTTVK.S.TKSLAA.SCLK 310
 Dd myosin 1B Q.YT.N.VSDYAEVRQ.DT.TAQ.SD.I.IVACV.H.Y.I.DK.N-----AIY.PNALEL.SMLC 304
 Dd myosin 2 G.V.IK.VS.SE.KI.RQ.DIV.F.E.MS.K.IIAG.HL.K.EKGA.EG-----VLK.KTALNA.STVFG 383
 Hs myosin 1c Q.AK.SS.N.KSDWKVVRK.LT.DFTED.VEDLLSIVASV.HL.H.AANEESN-----Q.TENQLKYLTR.LS 343
 Fg myosin 1 VTPDQLIKGITIRILTPRN-----G-----EVIESPANPAQAQATRDALAMAIYSNLFDWIVERINKSL--KARQP--T 399
 Fa myosin 1 -----E.T-----S-----K----- 399
 Fs myosin 1 -----NA-----L-----K----- 403
 Dd myosin 1E TDQQS.SIALCY.SI STGV-----KRC.SV.MDCN.AYS-----K.L.ER.N.L.SK.TIINCTTEKG.--- 380
 Dd myosin 1B IDSAT.QNA.LF.VINTGGAGGA.NRRSTYV.Q.VE.NG-----RT.DRM.S.L.V.Q.S--YYKS.---Y 377
 Dd myosin 2 .N.SV.E.ALMEPRI LAGR-----DLVAQHL.VEKSSSS.VK.L.GR.L.L.KK.NV.C--QE.KA--- 447
 Hs myosin 1c EGST.REAL.H.KIIAKG-----ELL.L.LE.AYA-----K.V.RT.T.L.GK.R.ASKDVES.SWRS 413
 Fg myosin 1 TNTIGLDIYGFIEFKNSFEQLCINYNVEKQQIFIQLTLKAQEYAREQIQWTPIKY-FDNKVVCDLIEQIRPVG 476
 Fa myosin 1 -----V----- 476
 Fs myosin 1 S-----D.K-----E-----P----- 480
 Dd myosin 1E -V-----V.QN.N.FC.F.L.E.S.V.G.E.KN.E.-N.PI.E.-KK.I 454
 Dd myosin 1B Q.V-----F-----G.F.F.F.E-----V.G.K.E.N.QI-----GKS.P 454
 Dd myosin 2 -YF.V.S-----KV-----T-----F.NHHMF.L.LK.K.N.F.DFGL.SQATI.DGRQ.P 524
 Hs myosin 1c .TVL.L-----V.QH-----F.C-----L.E.S.EA.G.A.E.VQ.-N.II.V.-EKFK 489
 Fg myosin 1 IFSAMKDATKTAHADPAACDRTFMQSINGMSHAHLTPR-----QGNFIIKHYAGDVYTYVEGITDKNKD 540
 Fa myosin 1 -----S----- 540
 Fs myosin 1 -----D----- 544
 Dd myosin 1E LI.LLDE.CLI--AKST.Q.LD.CKQFKNPQLQSYVVSVDKDR--SIGDTC.RL-----D.R.FL 525
 Dd myosin 1B .LLD.ICS.L.QSTGT.QK.LEKMA.IYDG.HW.GM-----T.A.A.E.EA.FS 520
 Dd myosin 2 .LALLDEQSVF-----N.T.N.LITKL-----S.FSKKNAYEPR--FSKTE.GVT-----Q.M.EIQDWLE 590
 Hs myosin 1c .I.LLDEECLR--PGE.T.L.LEKLEDYVKH.PHFLTTHKLADQRTKSLGR.E.RLL.E.S.T.FL.N 564
 Fg myosin 1 QLLKGLLALFQHSGNDFVHLLFRRPVDTDNRKQPP--SAGDRIASANALVDTLMKQPSYIRTIKPNENKSPTEYN 615
 Fa myosin 1 -----S-----Q----- 615
 Fs myosin 1 -----N-----Q----- 619
 Dd myosin 1E T.FGD.ISSM.S.SDPL.QG-----PTRPE.SK.R.E--T.SQF.NAM.IT.LA.S.H.V.C.S.D.QAGVID 600
 Dd myosin 1B T.FFD.IEAI.C.KMP.LAS.NEDTGSLOK.R.T--T.FK.KT.GE.MKA.SQ.T.H.C-----T.KAKDWE 595
 Dd myosin 2 P.QQD.ELC.KD.SDNV.TK.ND.NIASRA.KGANFITVAAQYKEQLAS.MA.ETTN.HFV.C.I.NKQL.AKLE 668
 Hs myosin 1c L.FRN.KETMCS.K.PIMSQC.D.SELS.-K.R.E--TVATQFKM.LLQ.EIQSKE.A.V.C.DA.Q.GRFD 638
 Fg myosin 1 GPNVLHQIKYLGLENVRIIRAGFAYRQDFDKFVDRFFLLSPAT-SYAGEFTWEGTTEAAVKQILKDTSPKKEEWQMG 692
 Fa myosin 1 -----K-----E----- 692
 Fs myosin 1 -----A-----Y.D----- 696
 Dd myosin 1E EDR.R.VR.L.V.G.IEYTR.YN.YKM.CKK.WPS-----FN.AKQ.TEL.QQHN.D.IR 673
 Dd myosin 1B NSR.K.VQ.L.V.NT.VLK.YKK.SK.WGIW--K.DAIEGC.T.FQ.MNLEAGQ.L 671
 Dd myosin 2 DKV.D.LRCN.VL.GI.T.K.PN.IIYAD.K.YY.A.NV-PR--DAEDSQK.TDAV.HLN.DP.QYRF 740
 Hs myosin 1c EVLIR.V.L.L.V.RKYEA.LQ.YKS.C.E.WPT--A.RPQDG.AVLVRHLGYKP.YK 711
 Fg myosin 1 VTKAFIKAPETLFALEHMRDRYWHNMATRIRMWRAYLAYR-----AESATRIQR-----LWR 745
 Fa myosin 1 -----F----- 745
 Fs myosin 1 -----F----- 749
 Dd myosin 1E K.V.RN.T.YF.EK.ELEMPRIV.L.KT.G.R.RSKWNQ-RKA.IK.L-----FY 730
 Dd myosin 1B K.V.RH.V.L.EAL.KKDFDCTAK.KAF.N-----EAL.KKDFDCTAK.KAF.N-----K 709
 Dd myosin 2 I.I.FR.GQ.ARI.EA.EQRISEI.KA.AAT.GWI.RKVYKQAR.HTVAARIQQNLRAYIDFKSWPW.K 812
 Hs myosin 1c R.I.RF.K.T.DALEVRRQSL.K.AA.GFHWKQFLRVKR.IC.S-----W 769

Figure S5. Multiple sequence alignment of the motor domain constructs used in this study. Fg: *Fusarium graminearum*, Fa: *F. avenaceum*, Fs: *F. solani*, Dd: *Dictyostelium discoideum*, Hs: *Homo sapiens*. Positions of amino acid residues implicated in mutation-induced Phenamacril-resistance in *F. graminearum* are shown in red boxes. Amino acids substitutions are highlighted as red dots.