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_103022	$FsMyo1_{IQ2}$
P1137	CCCAAGCTTTTAGTGGTGGTGGTGGTGGTGTG AACCTGAATCTCGAAGCTGCAAGTACTCG	NECHADRAFT_103022	$FsMyo1_{IQ2}$
P1345	AAAACCTCTATTTTCAGGCCGACTCACTTACT GAAGAG	FGSG_01891	$FgCaM_{LIC}$
P1346	TATCCACCTTTACTGTTATTTTTGCATCATAAG CTGG	FGSG_01891	$FgCaM_{LIC}$

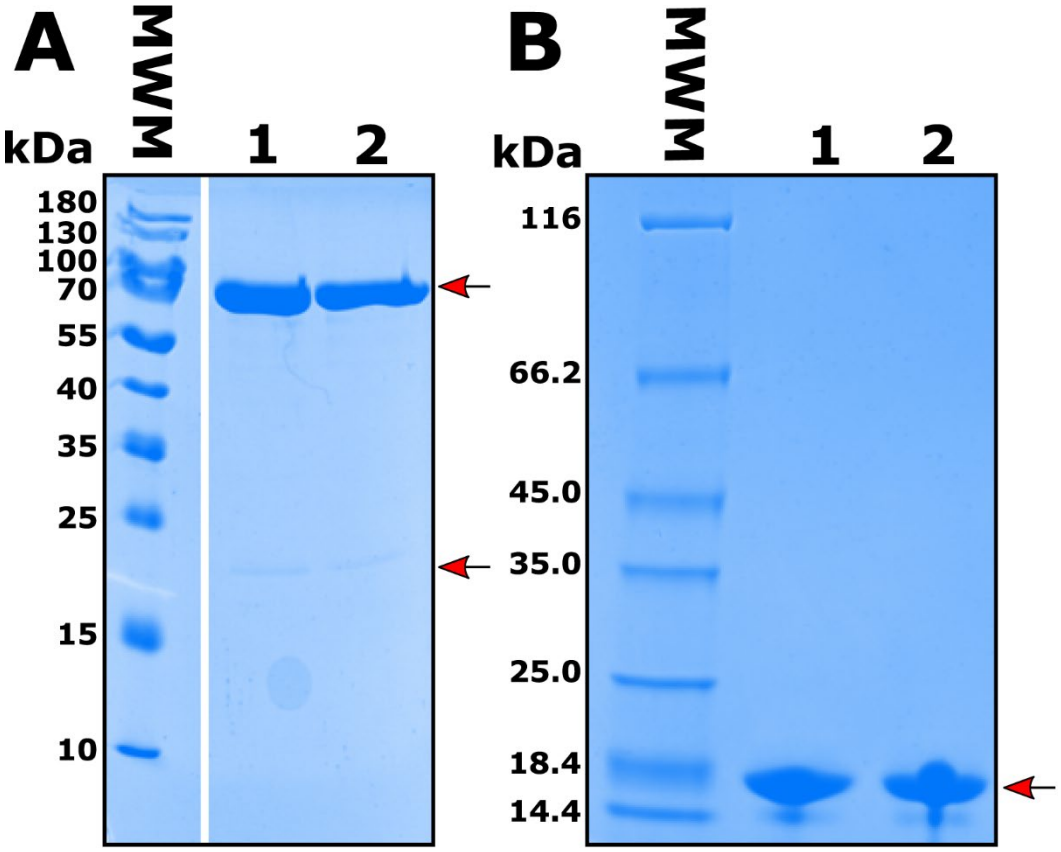

Figure S1. Coomassie-Brilliant Blue stained polyacrylamide gels showing two representative fractions resulting from the size-exclusion chromatographic purification of (**A**) FgMyo1$_{IQ2}$ co-expressed with calmodulin from *F. graminearum* PH-1 in *Sf*9 insect cells. White line indicates removed lanes. (**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.

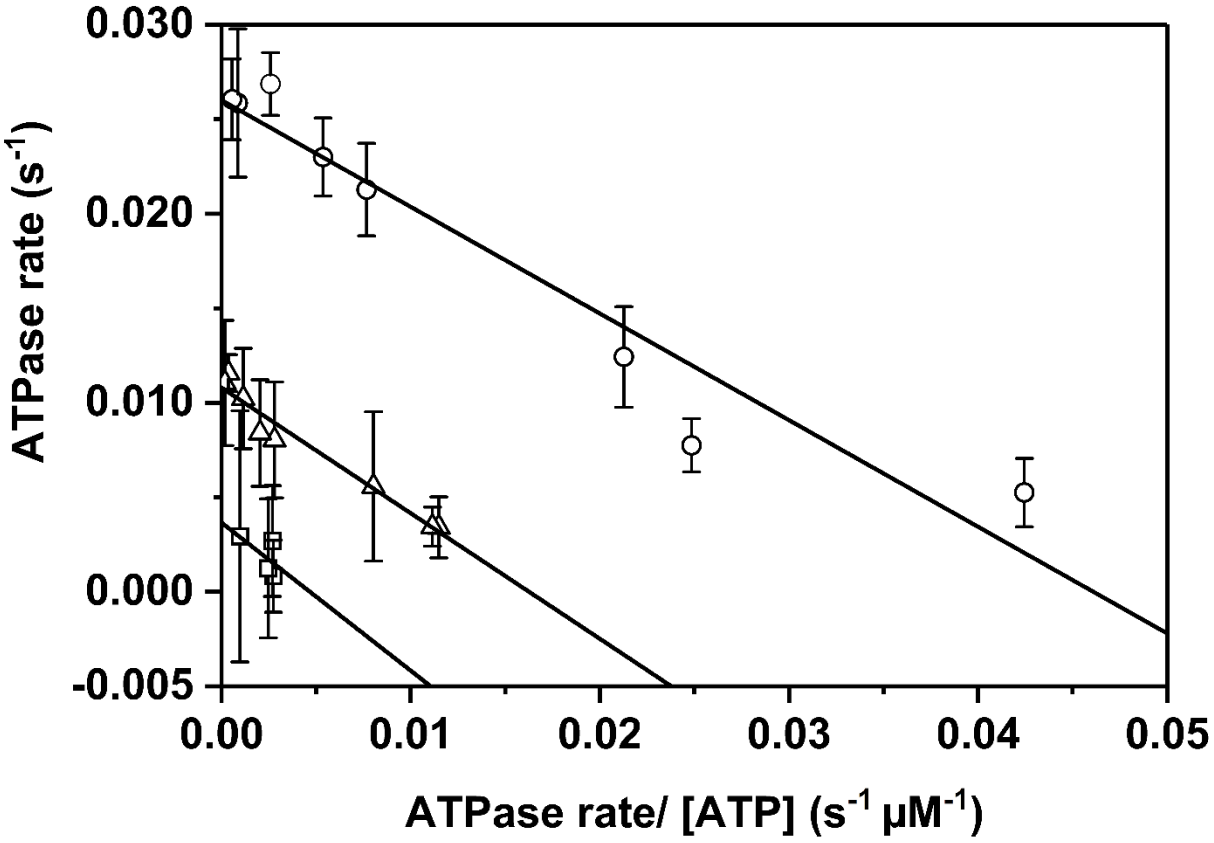

Figure S2. Eadie-Hofstee plots of Phenamacril inhibition of basal ATP turnover in the presence of Ca^{2+} by FgMyo1. A least-squares regression analysis was used to determine the Eadie-Hofstee linear correlations. Measurements were performed in the presence of 0 (○), 0.6 (Δ) and 10 µM (□) Phenamacril. Error bars represent standard deviations around the mean (n = 3).

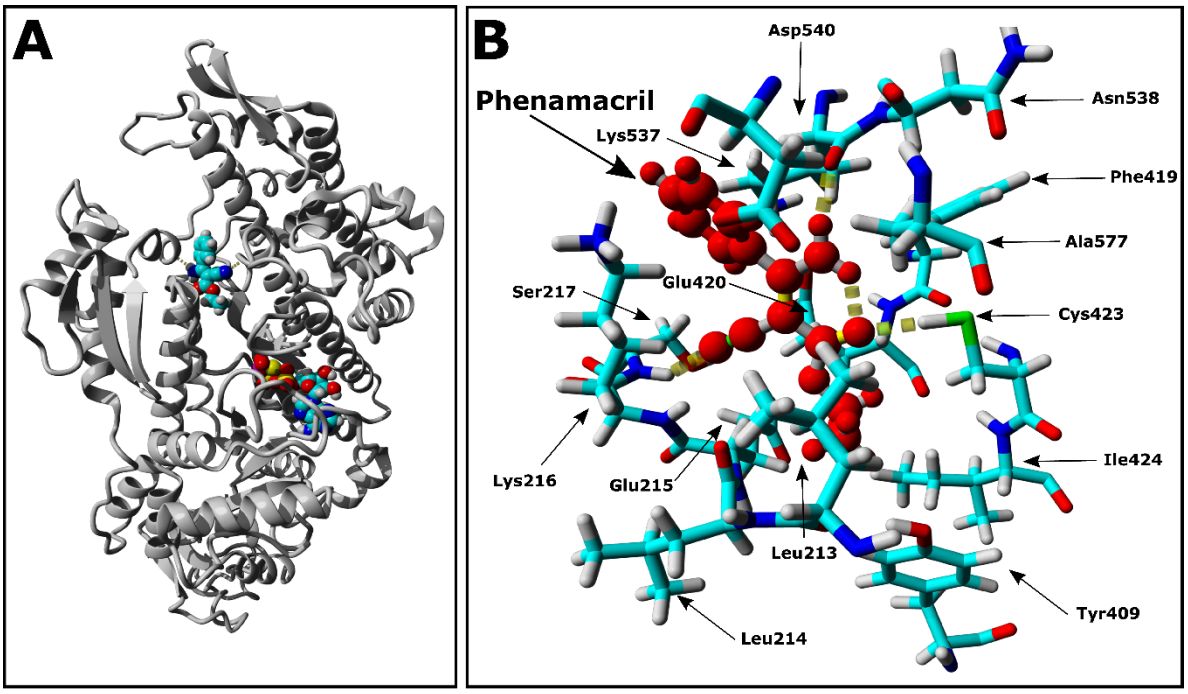

Figure S3. Phenamacril docks into the actin-binding groove of the *F. graminearum* class I myosin motor domain (**A**) Position of Phenamacril within the docked homology model of the *F. graminearum* class I myosin motor domain. For reference, the position of the nucleotide binding-pocket is indicated 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.

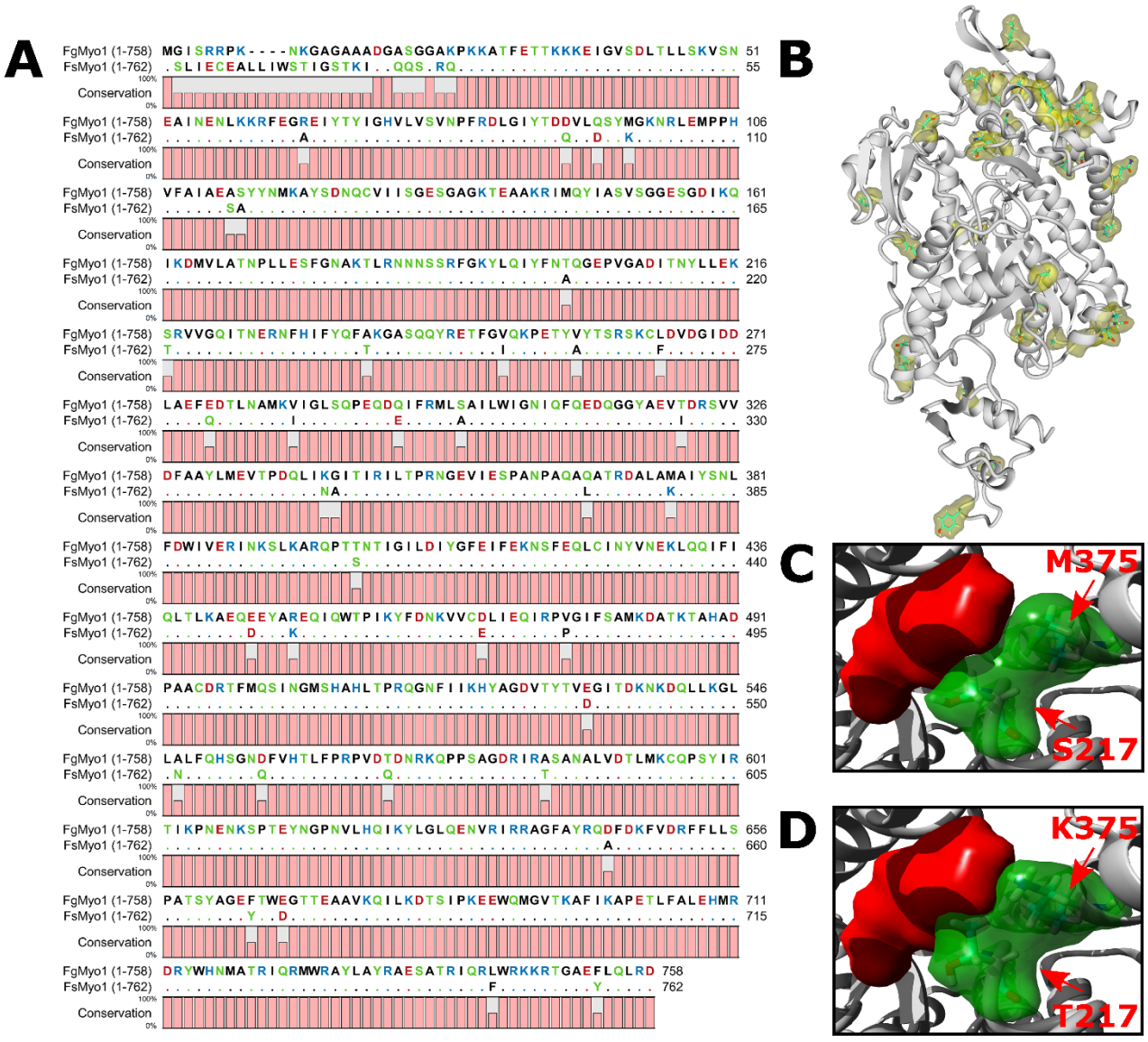

Figure S4. (**A**) Pairwise alignment of the class I myosin motor domains from *F. graminearum* PH-1 (FgMyo1), *F. solani* f. sp. *pisi* 77-14-4 (FsMyo1). Amino acids are colored by polarity, dots indicating residues that are conserved in comparison to the reference sequence of FgMyo1. (**B**) Pre-power 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.

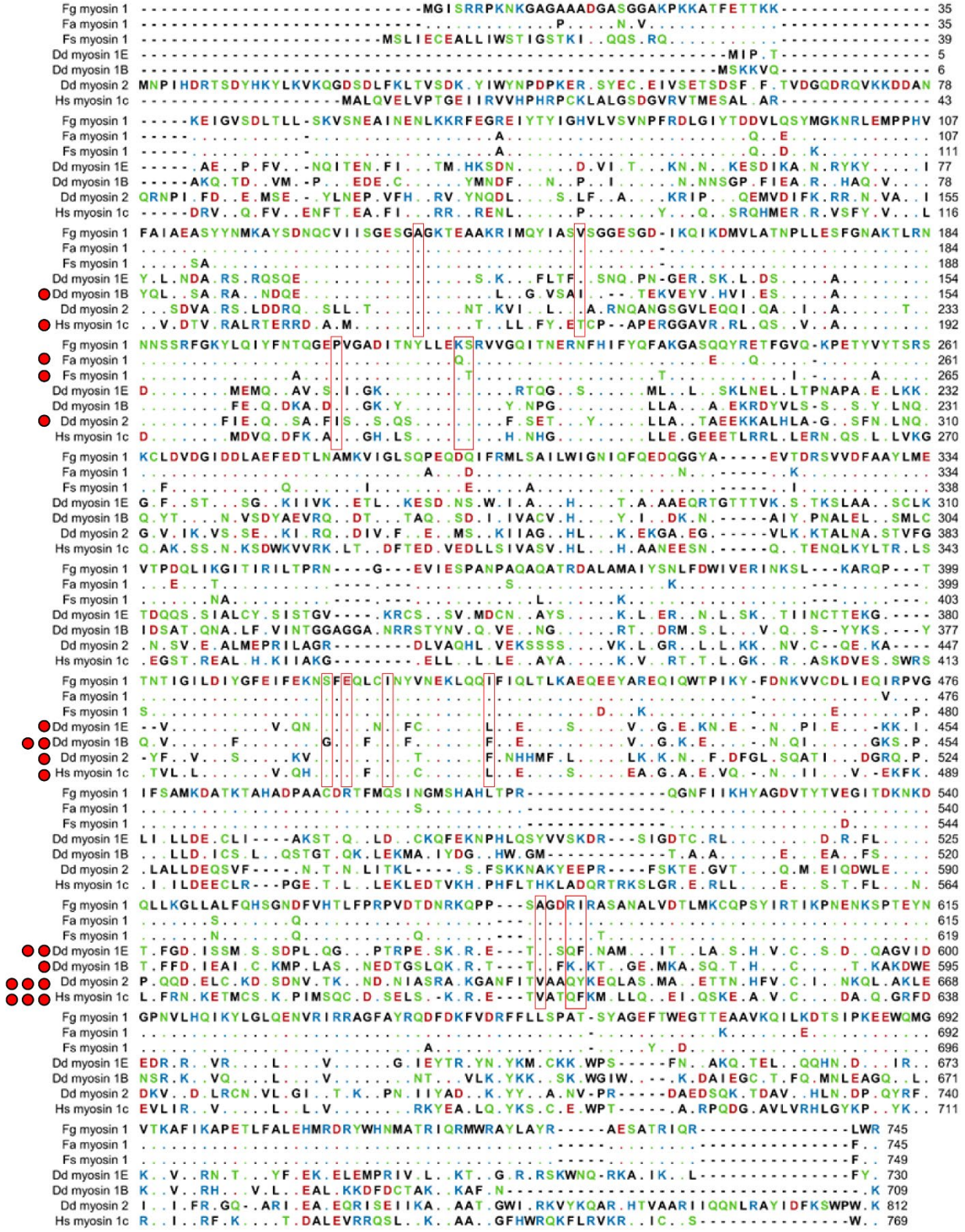
Fg myosin 1
Fa myosin 1
Fs myosin 1
Dd myosin 1E
Dd myosin 1B
Dd myosin 2
Hs myosin 1c

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.