Table S1

(1) P values in Figure 1B

	SMIFH2	pAB
Non-treated	< 0.0001	< 0.0001

(2) P values in Figure 1D

	30μM SMIFH2	50μM SMIFH2	100μM SMIFH2	200μM SMIFH2	100μM pAB
Non-treated	0.0030	< 0.0001	< 0.0001	< 0.0001	< 0.0001
100μM pAB	< 0.0001	< 0.0001	0.0003	0.0107	

(3) P values in Figure 2G

	12.5μM SMIFH2	25μM SMIFH2	50μM SMIFH2
Non-treated	0.0002	< 0.0001	< 0.0001

(4) P values in Figure 2H

	12.5μM SMIFH2	25μM SMIFH2	50μM SMIFH2	100μM SMIFH2	100μM SMIFH2	100μM pAB
Non-treated	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
100μM pAB	< 0.0001	< 0.0001	0.2153	0.0009	0.7841	

Table S2

	0 µM SMIFH2	100 µM SMIFH2
Time, min.	**% phosphorylated**	**% phosphorylated**
0.5	50.8	55.4
1.5	71.2	70.4
4.5	82.1	81.2

Table S2: SMIFH2 has no effect on myosin light chain kinase. Nonmuscle myosin 2A was incubated with myosin light chain kinase in the presence or absence of 100 µM SMIFH2 for the indicated times. The extent of phosphorylation was quantified by mass spectrometry (Apffel et al., 1995; Taggart et al., 2000).

Table S3

Myosin	**IC_{50} (µM SMIFH2)**
Human non-muscle Myo2A	**50**
Rabbit skeletal muscle Myo2	**30**
Drosophila Myo7A	**30**
Bovine Myo10	**15**
Drosophila Myo5	**2**

Table S3: Recapitulation of IC_{50} for the various myosin types we probed in this work