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# **Supporting Material**

### Title: Single-Molecule Adhesion Forces and Attachment Lifetimes of Myosin-I

Authors: Serapion Pyrpassopoulos, Henry Shuman, and E Michael Ostap

# **Supplementary Tables**

Table S1: Number of contact cycles and interactions for ramp-force data.							
Loading Rate (pN/s)	# Cycles	# Single-Peak Events					
2%PtIns(4,5)P <sub>2</sub> & myo1c <sup>IQ-tail</sup>							
250 ± 29	8235	162 (2.0%)					
930 ± 120	38106	340 (0.9%)					
$1800 \pm 230$	67134	945 (1.4%)					
Lipid extraction							
1100 ± 130	5115	236 (4.6%)					

 Table S2:
 Number of contact cycles and interactions for constant force data

Uncorrected Data	Control Data
(2%PtIns(4,5)P <sub>2</sub> & myo1c <sup>IQ-tail</sup> )	(2%PtIns(4,5)P <sub>2</sub> & No myo1c <sup>IQ-tail</sup> )

Force (pN)	# Cycles	# Attachments	# Cycles	# Attachments	% Specific Attachments
0.3	6417	1172 (18%)	7023	574 (8.2%)	9.8%
0.9	5453	613 (11%)	3140	162 (5.2%)	5.8%
1.7	7231	635 (8.8%)	4005	26 (0.65%)	8.2%
2.5	19018	1735 (9.1%)	12716	117 (0.92%)	8.2%

### Supplementary Figure Legends

# Fig. S1

Diagram of experimental step-up used to measure the interaction of pedestal-attached myo1c<sup>IQ-tail</sup> with membrane-coated beads held in an optical trap. The trap position was oscillated, resulting in the compression of the trapped beads against myo1c<sup>IQ-tail</sup>-coated pedestals, followed by retraction. Adhesions forces displace the bead from the trap center during retraction, which is measured as a beam deflection at the back focal plane with a quadrant detector. Formation and subsequent rupture of bonds appeared as negative peaks in the data traces (Fig. 1). Myo1c<sup>IQ-tail</sup> molecules were site-specifically attached to pedestals via neutravidin-biotin linkages. Beads and protein molecules are not drawn to scale.

# Fig. S2

Frequency distributions of non-specific interactions per oscillation cycle for three control experiments at loading rate of 1840 pN/s: (white bars) 100% DOPC coated beads interacting with pedestals decorated with myo1c<sup>IQ-tail</sup>, (hatched bars) 2% PtdIns(4,5)P<sub>2</sub>-98%DOPC coated beads interacting with pedestals in the absence of myo1c<sup>IQ-tail</sup> and (grey bars) 2% PtdIns(4,5)P<sub>2</sub>-98%DOPC coated beads interacting with pedestals decorated with myo1c<sup>IQ-tail</sup> in the presence of 100  $\mu$ M InsP<sub>6</sub>.

### Fig. S3

Frequency distributions of attachment-durations under acquired under constant forces between beads coated with 2% PtdIns(4,5)P<sub>2</sub> - 98% DOPC and pedestals decorated with (•) myo1c<sup>IQ-tail</sup> or ( $\Delta$ ) in the absence of myo1c<sup>IQ-tail</sup>. Frequencies were calculated over the total number of contacts between the trapped bead and the pedestals. The constant pulling force is indicated in each plot.



Figure S1.



Figure S2.



Figure S3.