Supporting Information

Oguchi *et al.* 0800564105

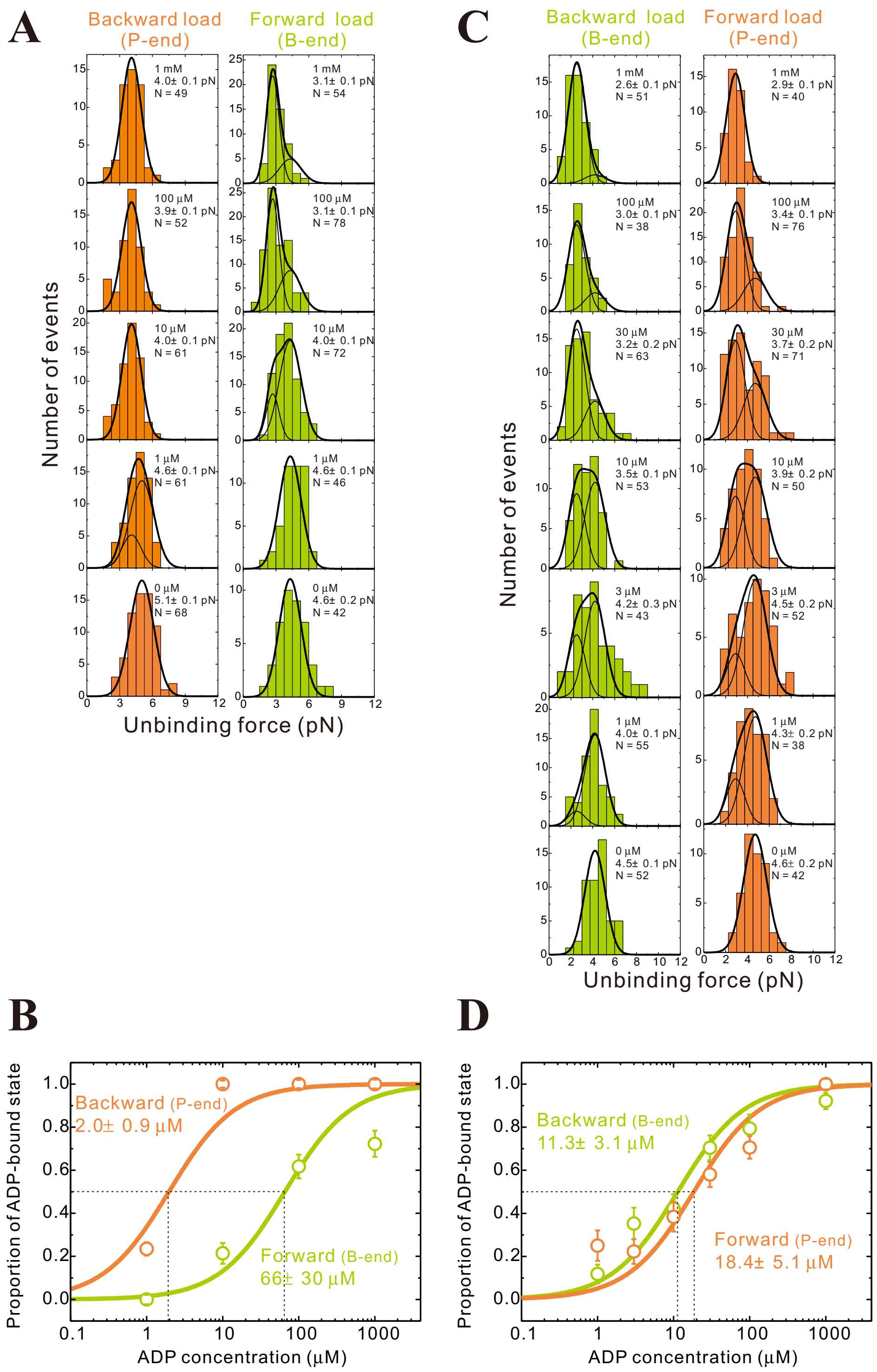


Fig. S1. The distributions of the unbinding forces with 0.75 pN bins for myosin V (*A*) and myosin VI (*C*) and ADP affinities for myosin V (*B*) and myosin VI (*D*).

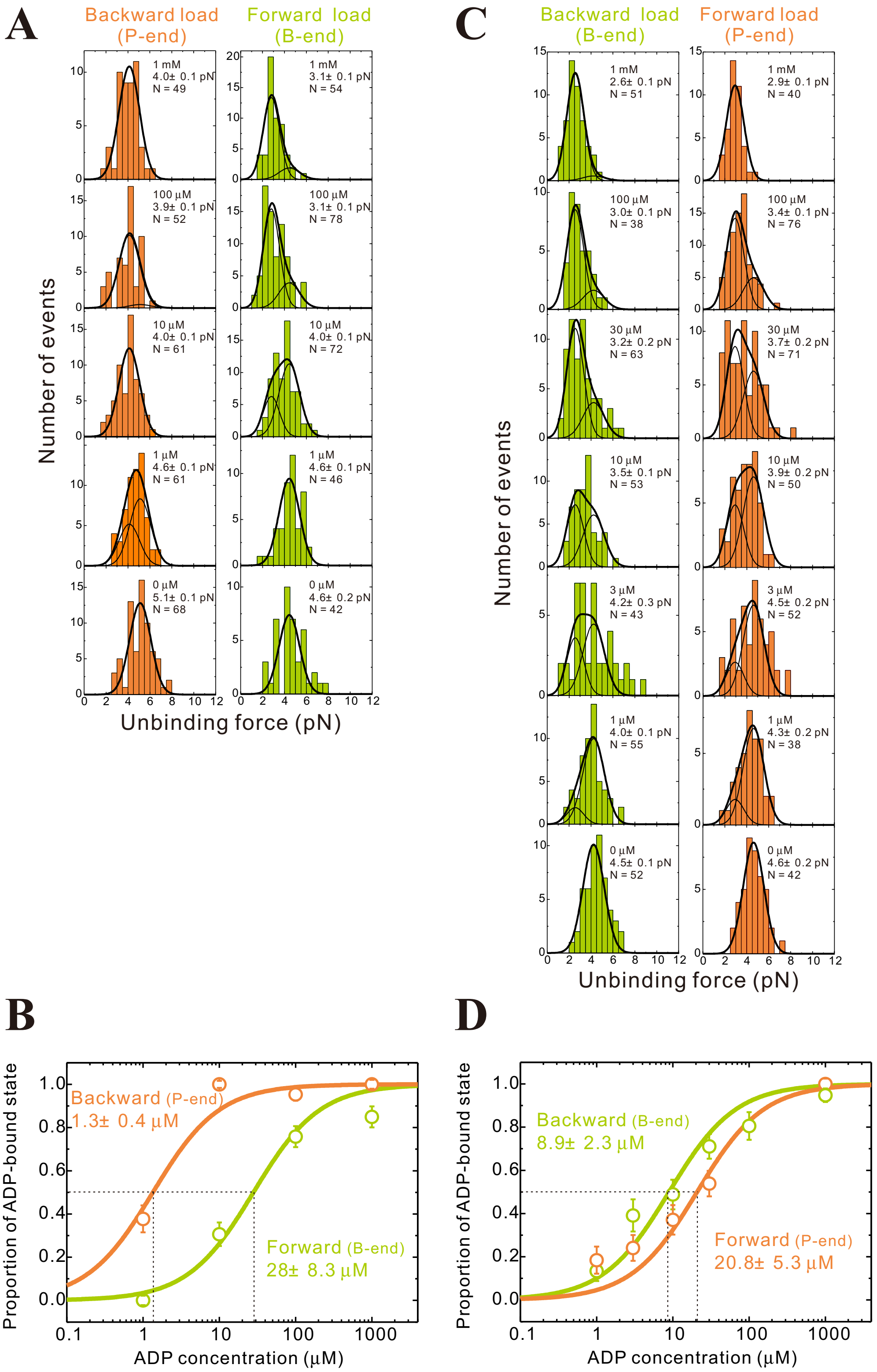


Fig. S2. The distributions of the unbinding forces with 0.5 pN bins for myosin V (*A*) and myosin VI (*C*), ADP affinities for myosin V (*B*) and myosin VI (*D*).

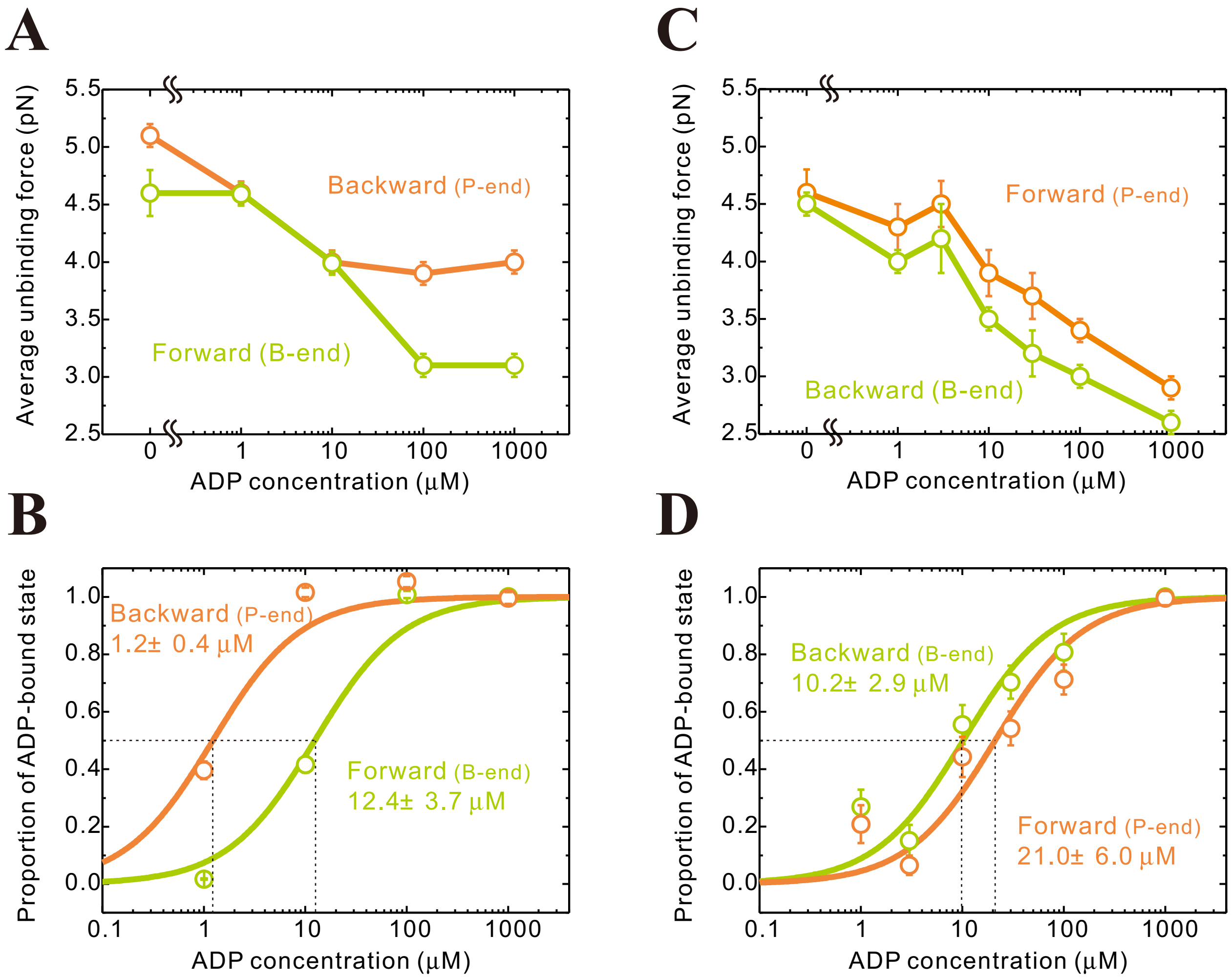


Fig. S3. Analysis using the average unbinding forces for myosin V (*A* and *B*) and myosin VI (*C* and *D*). To estimate the apparent ADP affinity without fitting the unbinding force distributions with the double Gaussians, the population of the ADP state, p_{ADP}, was determined from the values of average forces at each [ADP] as $p_{\mathrm{ADP}} = (\overline{F_{\phi}} - \bar{F})/(\overline{F_{\phi}} - \overline{F_{\mathrm{ADP}}})$, where $\overline{F_{\phi}}$ and $\overline{F_{\mathrm{ADP}}}$ denote the average unbinding force for the distributions at 0 and 1 mM ADP, respectively, and $\bar{F}$ is the average unbinding force for any given distribution.

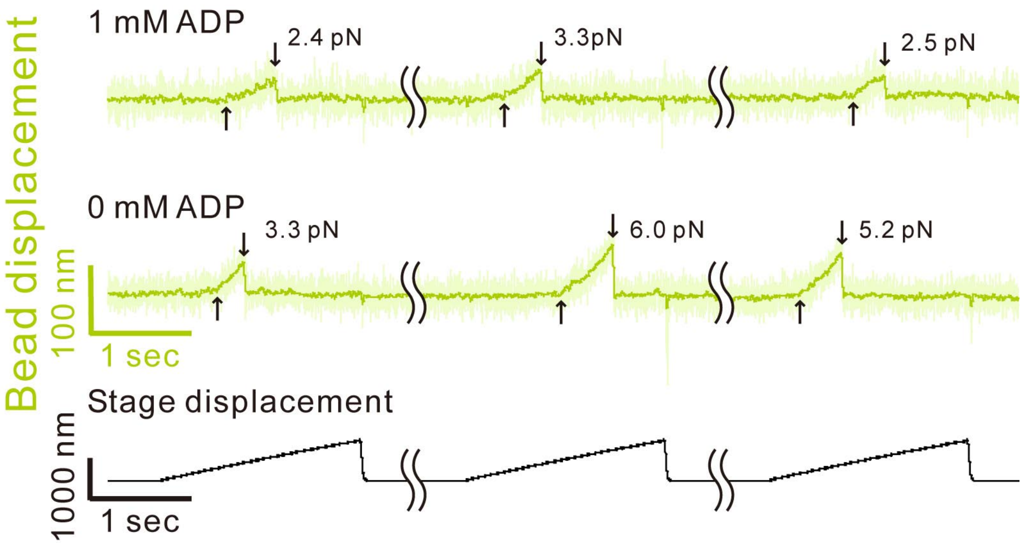


Fig. S4. Typical traces showing bead movement during pulling–unbinding of individual single-headed myosin VI–actin complex under backward (barbed-end directed) load. Traces at each ADP concentration (1 and 0 mM) were obtained with the same bead; the futile stage displacements, during which no binding was detected, and waiting time (5–10 sec between consecutive stage displacements) are not shown. The moment at which the external load began to be imposed on the actomyosin bond, and the moment of the bond rupture are indicated by the upward and downward arrows, respectively.

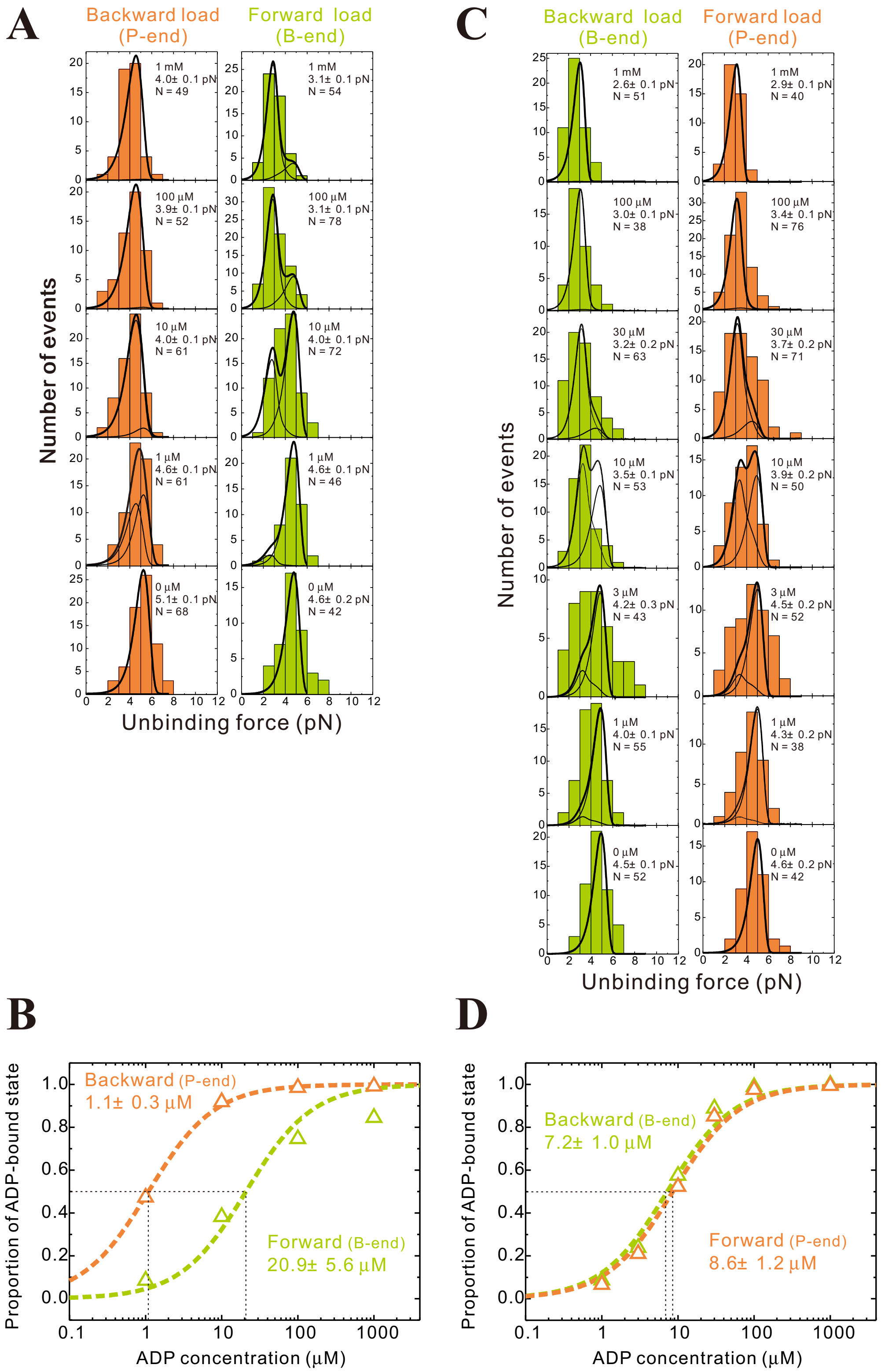


Fig. S5. The distributions of the unbinding forces (*A* and *C*) and ADP affinities (*B* and *D*) modeled with the obtained characteristic distances for myosin V (*A* and *B*) and myosin VI (*C* and *D*).

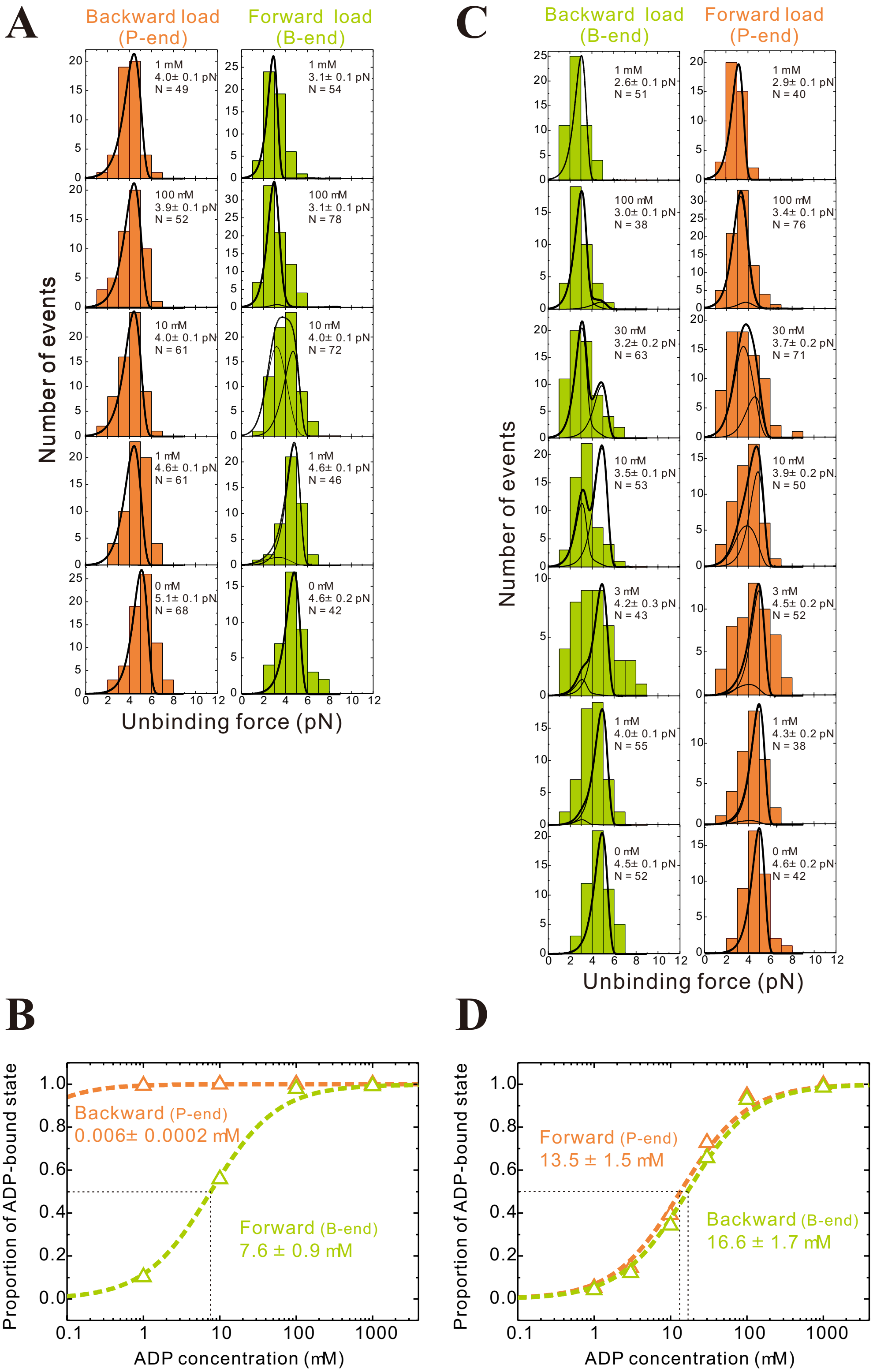


Fig. S6. The unbinding force distributions fitted with the model, in which d^{-}_{ADP} and d^{+}_{ADP} are constrained to be symmetrical, for myosin V (*A*) and myosin VI (*C*), and the estimated, based on this model, ADP affinities, determined from the population of the ADP peak, for myosin V (*B*) and myosin VI (*D*).

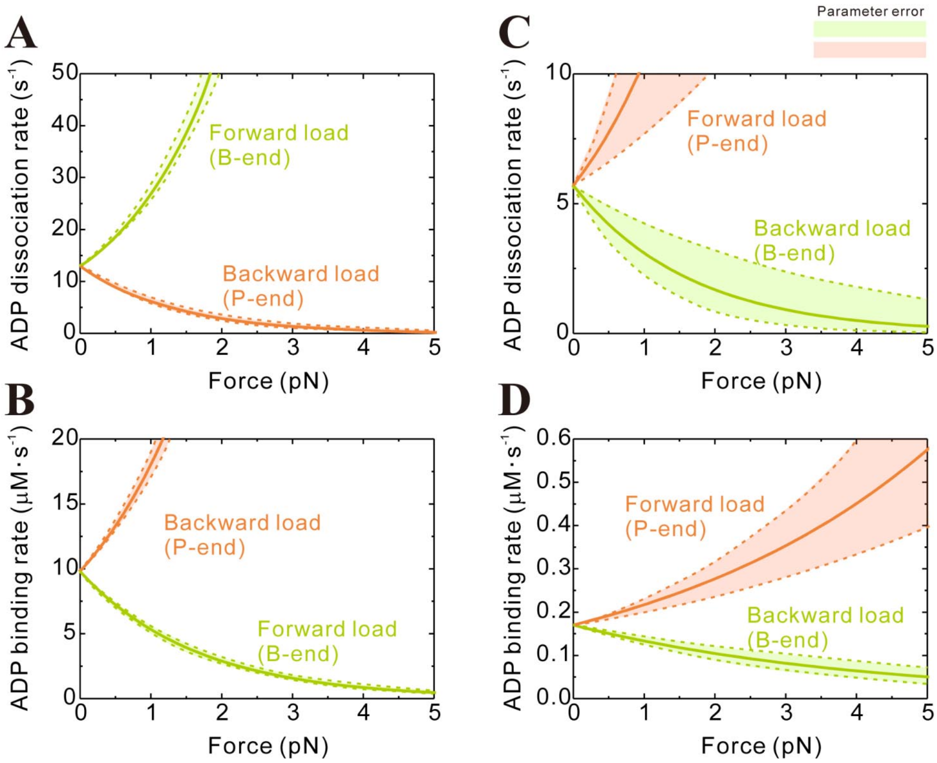


Fig. S7. Load-induced modulation of ADP dissociation and binding rates in myosins V and VI, determined by model analysis assuming d^{-}_{ADP} and d^{+}_{ADP} to be symmetrical. Dependence of the ADP dissociation (*A* and *C*) and binding (*B* and *D*) rates on load is plotted for myosins V (*A* and *B*) and VI (*C* and *D*). In all panels data obtained under barbed (B)- and pointed (P)- end directed loading are green and orange, respectively.

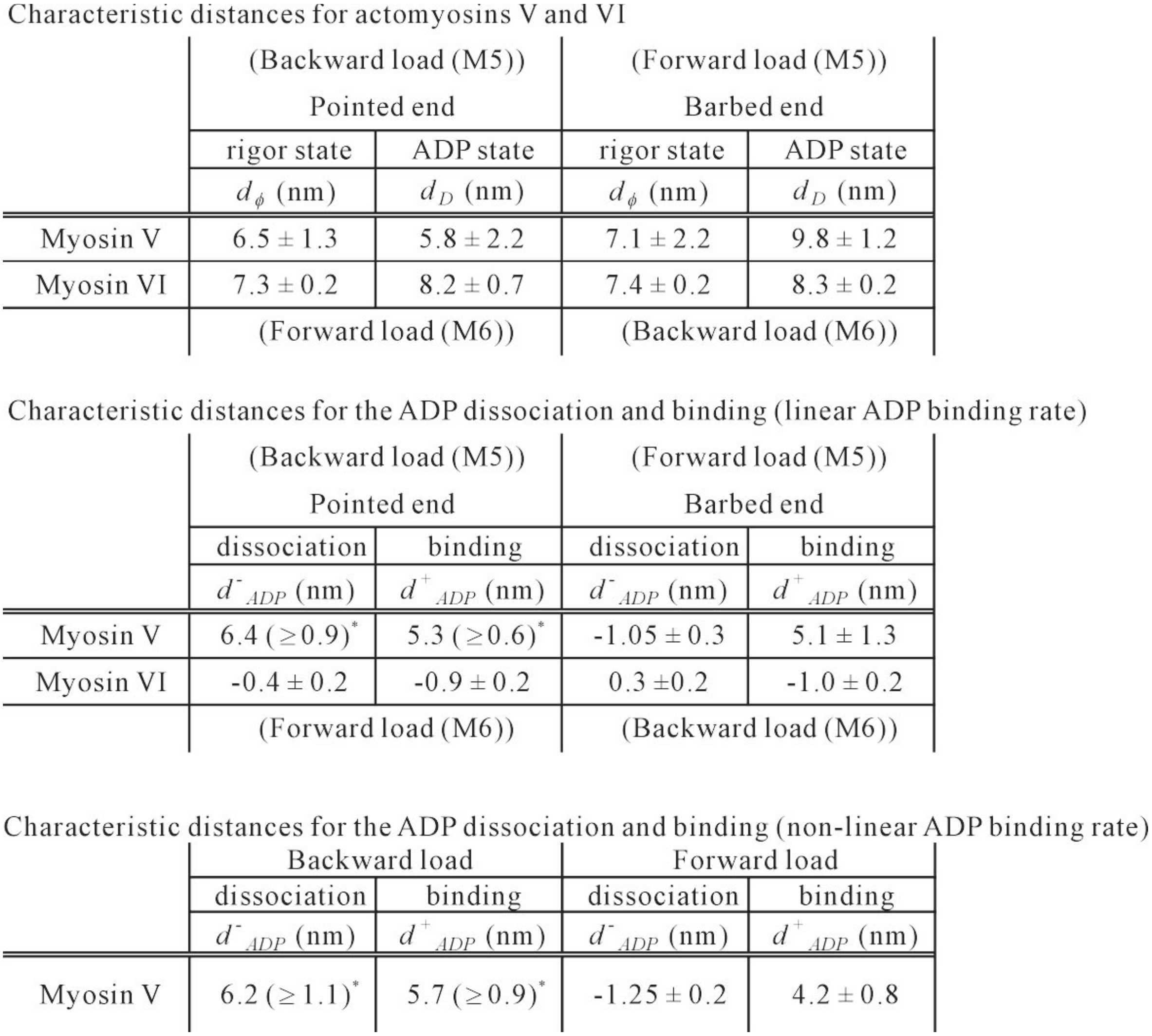
Table S1. Kinetic values under no load used as the initial parameters in the model analysis

Value	Myosin V	Myosin VI (T406A)
τ_{ϕ}, s	142.3 (1)	250 (2)
τ_{D}, s	25.6 (1)	14.3 (2)
$K_{d,0}$, μM	1.2*	34 (3)
$k^{-}_{ADP,0}$, s^{-1}	13*	5.7 (3)
$k^{+}_{ADP,0}$, $\mu M^{-1} \cdot s^{-1}$	9.8*	0.17 (3)

*Values determined in this study. Measurements were performed as described in ref. 3.

1. Hannemann DE, Cao W, Olivares AO, Robblee JP, De La Cruz EM (2005) Magnesium, ADP, and actin binding linkage of myosin V: Evidence for multiple myosin V-ADP and actomyosin V-ADP states. *Biochemistry* 44:8826–8840.
2. De La Cruz EM, Ostap EM, Sweeney HL (2001) Kinetic mechanism and regulation of myosin VI. *J Biol Chem* 276:32373–32381.
3. Robblee JP, Cao W, Henn A, Hannemann DE, De La Cruz EM (2005) Thermodynamics of nucleotide binding to actomyosin V and VI: a positive heat capacity change accompanies strong ADP binding. *Biochemistry* 44:10238–10249.

Table S2. Characteristic distances for actomyosins V and VI and ADP dissociation and binding obtained from the model analysis



Characteristic distances for actomyosins V and VI

	(Backward load (M5)) Pointed end		(Forward load (M5)) Barbed end	
	rigor state	ADP state	rigor state	ADP state
	d_ϕ (nm)	d_D (nm)	d_ϕ (nm)	d_D (nm)
Myosin V	6.5 ± 1.3	5.8 ± 2.2	7.1 ± 2.2	9.8 ± 1.2
Myosin VI	7.3 ± 0.2	8.2 ± 0.7	7.4 ± 0.2	8.3 ± 0.2
	(Forward load (M6))		(Backward load (M6))	

Characteristic distances for the ADP dissociation and binding (linear ADP binding rate)

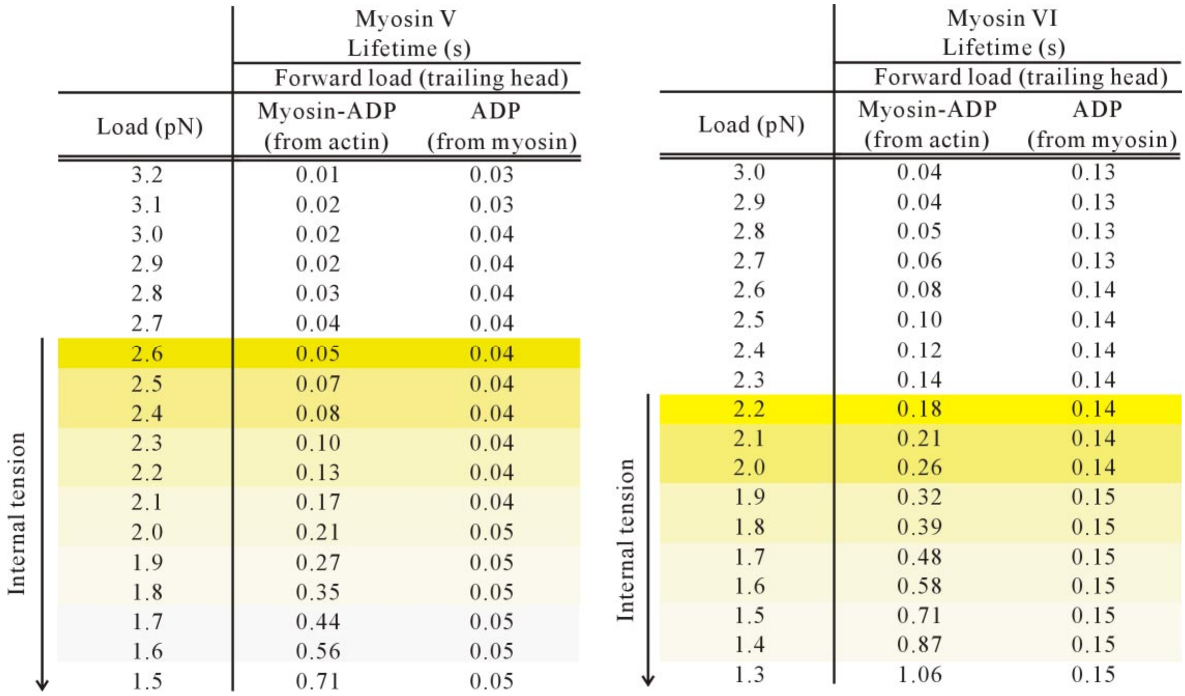
	(Backward load (M5)) Pointed end		(Forward load (M5)) Barbed end	
	dissociation	binding	dissociation	binding
	d^-_{ADP} (nm)	d^+_{ADP} (nm)	d^-_{ADP} (nm)	d^+_{ADP} (nm)
Myosin V	6.4 (≥0.9)*	5.3 (≥0.6)*	-1.05 ± 0.3	5.1 ± 1.3
Myosin VI	-0.4 ± 0.2	-0.9 ± 0.2	0.3 ±0.2	-1.0 ± 0.2
	(Forward load (M6))		(Backward load (M6))	

Characteristic distances for the ADP dissociation and binding (non-linear ADP binding rate)

	Backward load		Forward load	
	dissociation	binding	dissociation	binding
	d^-_{ADP} (nm)	d^+_{ADP} (nm)	d^-_{ADP} (nm)	d^+_{ADP} (nm)
Myosin V	6.2 (≥1.1)*	5.7 (≥0.9)*	-1.25 ± 0.2	4.2 ± 0.8

The values in parentheses denote the lower limit.

Table S3. Estimation of the intramolecular load in myosins V and VI from the lifetimes of myosin–ADP bound to actin, and ADP bound to myosin, at various loads



	Myosin V Lifetime (s)	
	Forward load (trailing head)	
Load (pN)	Myosin-ADP (from actin)	ADP (from myosin)
3.2	0.01	0.03
3.1	0.02	0.03
3.0	0.02	0.04
2.9	0.02	0.04
2.8	0.03	0.04
2.7	0.04	0.04
2.6	0.05	0.04
2.5	0.07	0.04
2.4	0.08	0.04
2.3	0.10	0.04
2.2	0.13	0.04
2.1	0.17	0.04
2.0	0.21	0.05
1.9	0.27	0.05
1.8	0.35	0.05
1.7	0.44	0.05
1.6	0.56	0.05
1.5	0.71	0.05

	Myosin VI Lifetime (s)	
	Forward load (trailing head)	
Load (pN)	Myosin-ADP (from actin)	ADP (from myosin)
3.0	0.04	0.13
2.9	0.04	0.13
2.8	0.05	0.13
2.7	0.06	0.13
2.6	0.08	0.14
2.5	0.10	0.14
2.4	0.12	0.14
2.3	0.14	0.14
2.2	0.18	0.14
2.1	0.21	0.14
2.0	0.26	0.14
1.9	0.32	0.15
1.8	0.39	0.15
1.7	0.48	0.15
1.6	0.58	0.15
1.5	0.71	0.15
1.4	0.87	0.15
1.3	1.06	0.15

Table S4. Characteristic distances for actomyosins V and VI and ADP dissociation and binding obtained from the model analysis, in which d^{-}_{ADP} and d^{+}_{ADP} are constrained to be symmetrical

Characteristic distances for the ADP dissociation and binding (linear ADP binding rate)

	(Backward load (M5)) Pointed end		(Forward load (M5)) Barbed end	
	dissociation	binding	dissociation	binding
	d^{-}_{ADP} (nm)	d^{+}_{ADP} (nm)	d^{-}_{ADP} (nm)	d^{+}_{ADP} (nm)
Myosin V	3.0 ± 0.2	-2.5 ± 0.2	-3.0 ±0.2	2.5± 0.2
Myosin VI	-2.5 ±1.3	-1.0 ± 0.3	2.5 ±1.3	1.0 ± 0.3
	(Forward load (M6))		(Backward load (M6))	

Characteristic distances for the ADP dissociation and binding (non-linear ADP binding rate)

	Backward load		Forward load	
	dissociation	binding	dissociation	binding
	d^{-}_{ADP} (nm)	d^{+}_{ADP} (nm)	d^{-}_{ADP} (nm)	d^{+}_{ADP} (nm)
Myosin V	4.5 ± 0.4	-1.0 ± 0.3	-4.5±0.4	1.0± 0.3