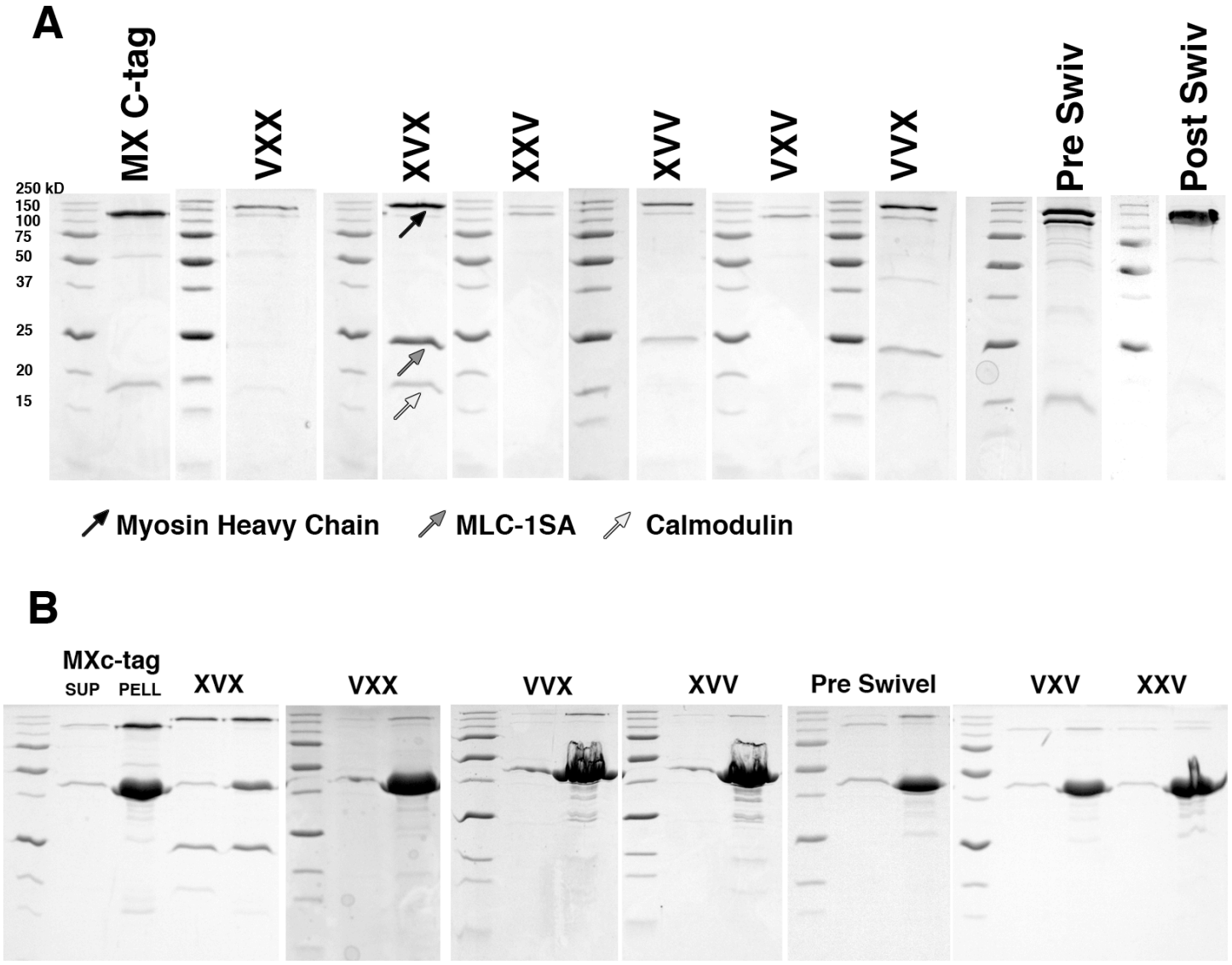

Figure S1: Preparation of myosin chimeras and swivel mutants. A) SDS-PAGE gels of purified motor constructs are shown. Marker lanes shown to the immediate left of each preparation are from the same gel, and are duplicated as needed. The arrows indicate the heavy chain, the myosin V specific light chain MLC1-SA and calmodulin. The molecular weights are: Myosin X C-tag -111.5 kD; Myosin X-GFP - 139.1; VXX–142.8; XVX–148.4; XXV–149.6; XVV–158.4; VXV–151.9; VVX–152.1; Pre Swivel–139.1 and Post Swivel–111.5. Note that the myosin X C-tag and Post Swivel constructs lack a C-terminal GFP, but instead contain the short C-tag sequence at the C-terminus. The myosin V specific light chain is present in all constructs with the myosin V lever arm, confirming their identity. For constructs obtained in high yield, calmodulin is also visible on the gel indicating the full complementation of IQ domains. B) Motor constructs bind to actin. Actin (at 10 μM) and motors were incubated for 15 minutes, and sedimented @ 95000 rpm for 15 minutes (Beckman TLA 100 rotor) in the absence of ATP. The supernatant and pellets were run on an SDS-PAGE gel. Note the enrichment of the motor band in the pellet.

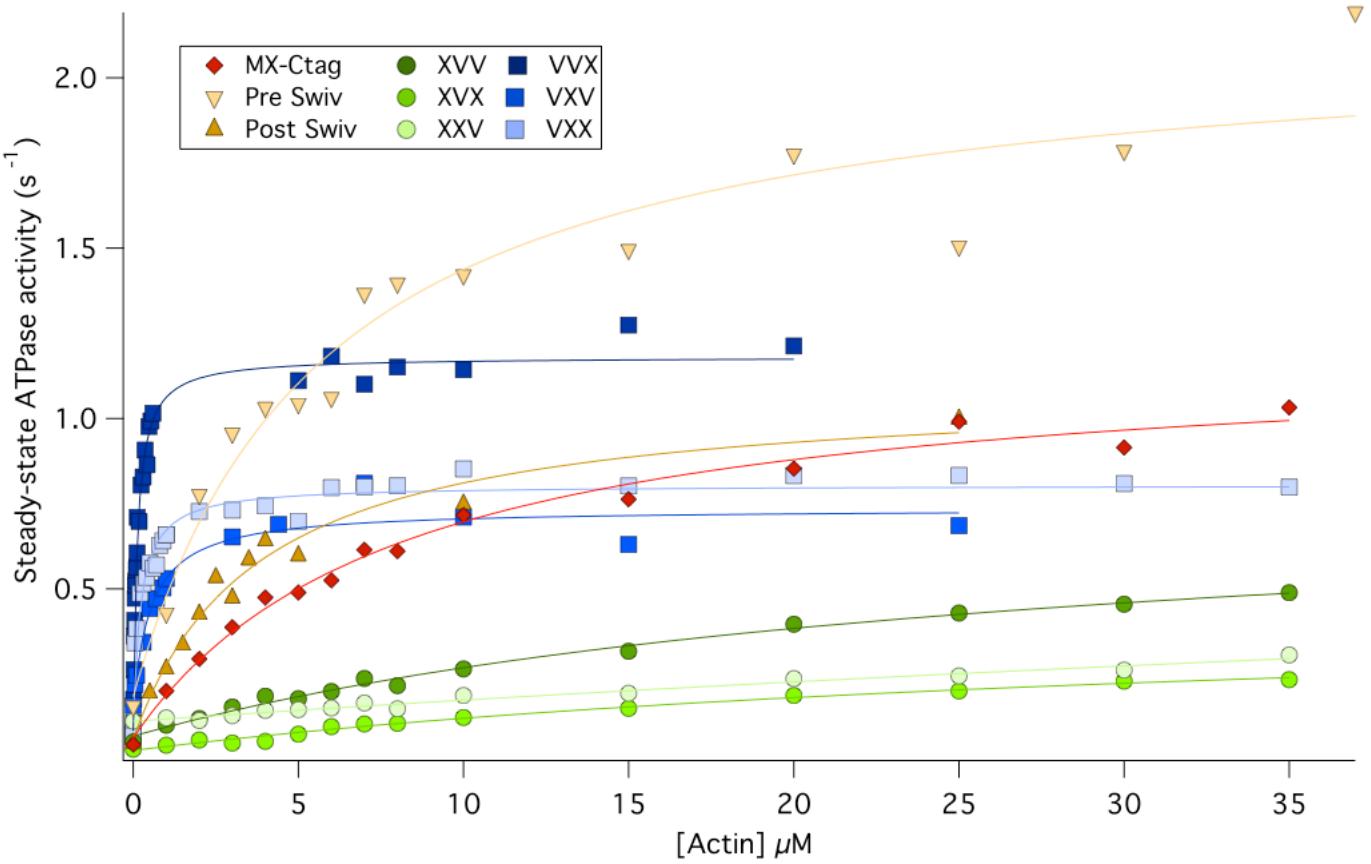

Figure S2: Actin activated ATPase measurements of motor constructs. The steady-state actin activated ATPase activity of the constructs was measured by monitoring Pi production using the EnzChek Phosphate Assay Kit [Molecular Probes]. The assay was performed as described by De La Cruz [Kinetic and equilibrium analysis of the myosin ATPase De La Cruz, E. M. & Ostap, M. *Methods in Enzymology* 2009;455:157-192]. Assays were performed in a buffer containing 10 mM imidazole•HCl pH 7.5, 20 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, 2 mM Mg•ATP, 30 μg/mL calmodulin, 200 μM MESG, 1 unit/mL PNP, and 100 nM motor protein. The motor concentration (as determined from gel densitometry with a myosin II as a known concentration standard) used was 100 nM. Assays were performed in 100 μL solutions in a 96 well plate with various f-actin concentrations in parallel. Actin was prepared by dialysis into 50 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 2 mM DTT, and 10 mM imidazole, pH 7.5. This polymerizes actin and removes free ATP. Shown is a plot of the steady-state rate of Phosphate production in units of [Pi] $[myosin]^{-1}$ s^{-1} vs. [actin]. The data was fit to the Briggs-Haldane steady-state equation: rate = $\upsilon_o + (k_{cat} \times [Actin])/(K_{ATPase} + [Actin])$. The fit constants are shown in Table S2 below. Note that the K_{ATPase} values group into two sets of values based on the identity of the motor head.

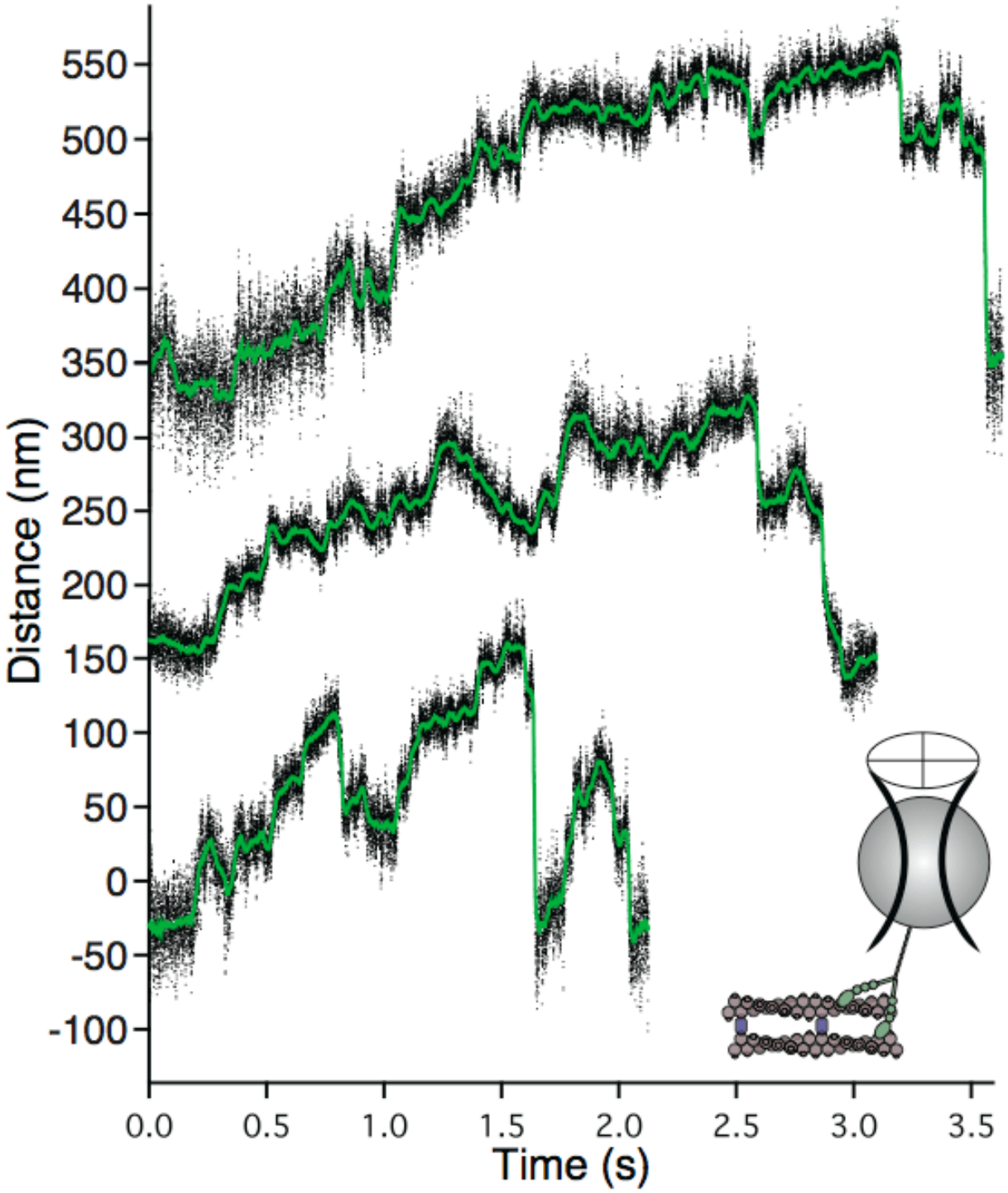

Figure S3: Single-bead optical trapping traces indicate complex stepping behavior of myosin X with a short (<36 nm) step-size, related to Fig. 5. A cartoon of the experimental setup is inset. Fascin-actin bundle is adhered to the coverslip surface and a polystyrene bead conjugated to motor is lowered to the track and allowed to interact, leading to processive motions detected on the position detector. Raw position data is in black and a 100 point median filter is in green. The motor is seen to take forward as well as backward steps as it finds its way along the bundle surface.

Motor Construct	Runlength on Fascin-actin (μm)	Runlength on Single-actin (μm)
Myosin X	0.63 ± 0.08 (n = 100)	0.17 ± 0.05 (n = 24)
Myosin V	0.57 ± 0.06 (n = 134)	0.66 ± 0.05 (n = 231)
VXX	0.49 ± 0.02 (n = 571)	0.29 ± 0.01 (n = 779)
XVX	0.21 ± 0.01 (n = 323)	n/d (two events)
XXV	No events	No events
XVV	0.31 ± 0.02 (n = 334)	0.27 ± 0.02 (n = 129)
VXV	No events	No events
VVX	0.76 ± 0.02 (n = 817)	0.45 ± 0.02 (n = 556)
Pre SAH	0.27 ± 0.01 (n = 728)	0.19 ± 0.01 (n = 566)
Post SAH	0.22 ± 0.03 (n = 96)	0.23 ± 0.01 (n = 204)

Table S1. Measured TIRF runlengths of all motor constructs on single filaments and bundles. Errors are ± SEM. See Figure 2 and Figure 4.

	Construct	k_{cat} (s^{-1})	K_{ATPase} (μM)
	Myosin X C-tag	1.15 ± 0.04	8.08 ± 1.08
	VVX	1.10 ± 0.02	0.10 ± 0.01
	VXV	0.64 ± 0.04	0.77 ± 0.18
	VXX	0.67 ± 0.03	0.24 ± 0.03
	XVV	0.74 ± 0.06	27.0 ± 4.77
	XVX	0.43 ± 0.05	33.7 ± 8.0
	XXV	0.68 ± 0.32	92.7 ± 59.8
	Pre Swivel	1.97 ± 0.15	5.7 ± 1.6
	Post Swivel	1.03 ± 0.06	3.7 ± 0.7

Table S2: Actin activated steady-state ATPase rate constants for all constructs. Values are obtained from the curves shown in Figure S2**.**