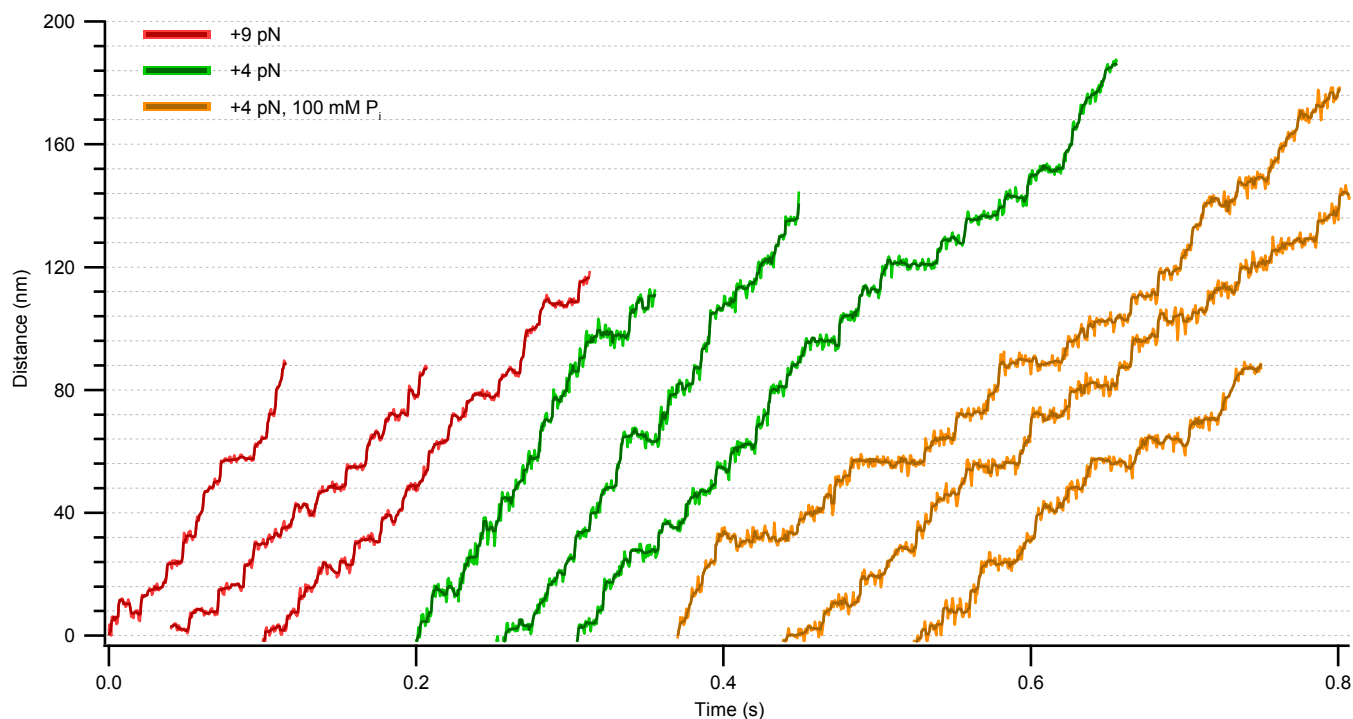
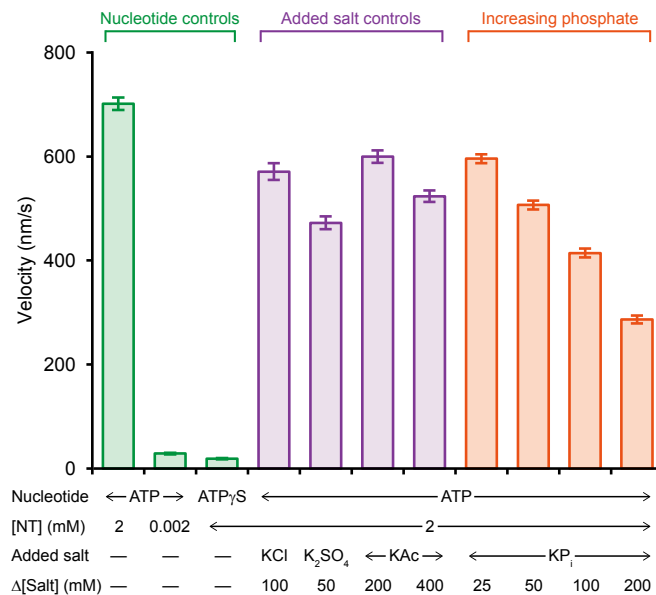


# Supporting Information

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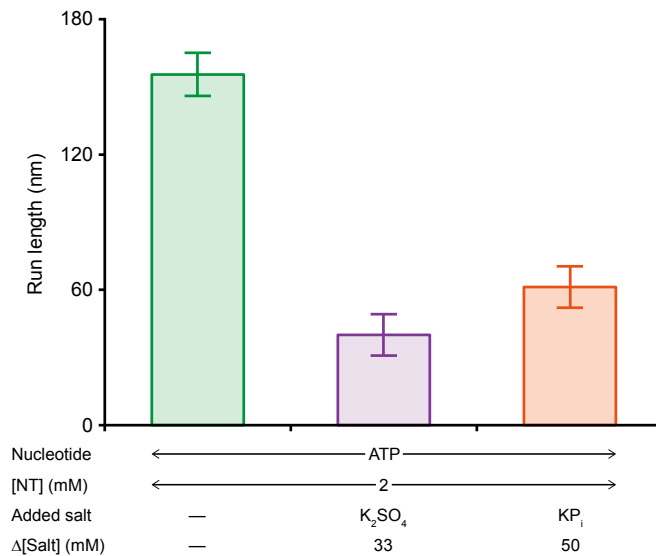


**Fig. S1.** Kinesin stepping traces under assisting loads. Representative single-molecule records of kinesin stepping measured under loads of +9 pN (red), +4 pN (green), and +4 pN in the presence of 100 mM added P<sub>i</sub> (orange). For each condition, dark-colored, median-filtered records (computed over a seven-data point sliding window) are superimposed upon light-colored, unfiltered data. Three records are displayed for each of the experimental conditions; gridlines are spaced at 8-nm intervals. Kinesin molecules take ~8-nm steps when subjected to assisting loads, as well as in the presence of high P<sub>i</sub> concentrations.



**Fig. S2.** Effect of salt on kinesin velocity. Single-molecule kinesin velocities (mean  $\pm$  SE;  $n = 93$ – $345$ ) measured under a +4-pN assisting load in the presence of nucleotide (NT) analogs (green), salt controls (purple), and added P<sub>i</sub> (orange), at the concentrations indicated. The addition of salt to the motility assay decreased kinesin velocity ( $P < 0.0001$ ;  $t$  test). The velocity drop in the presence of added P<sub>i</sub> ( $P < 0.0001$ ;  $t$  test) is consistent with previous data showing that P<sub>i</sub> competitively inhibits ATP binding (1) and that the rates affected are distinct from those involved in dissociation from the MT.

1. Schief WR, Clark RH, Crevenna AH, Howard J (2004) Inhibition of kinesin motility by ADP and phosphate supports a hand-over-hand mechanism. *Proc Natl Acad Sci USA* 101(5): 1183–1188.



**Fig. S3.** Added salt decreases run lengths under hindering load. Run lengths (mean  $\pm$  SE;  $n = 38$ – $588$ ) measured under a  $-4$ -pN hindering load in the presence of no added salt (green), added potassium sulfate (purple), or added potassium phosphate (orange), at the concentrations indicated. Run lengths in this force regime decreased dramatically in the presence of added salts (purple, orange). The addition of sulfate or phosphate resulted in a similar reduction of run length that was statistically significant relative to the baseline run length ( $P \leq 0.01$ ;  $t$  test).