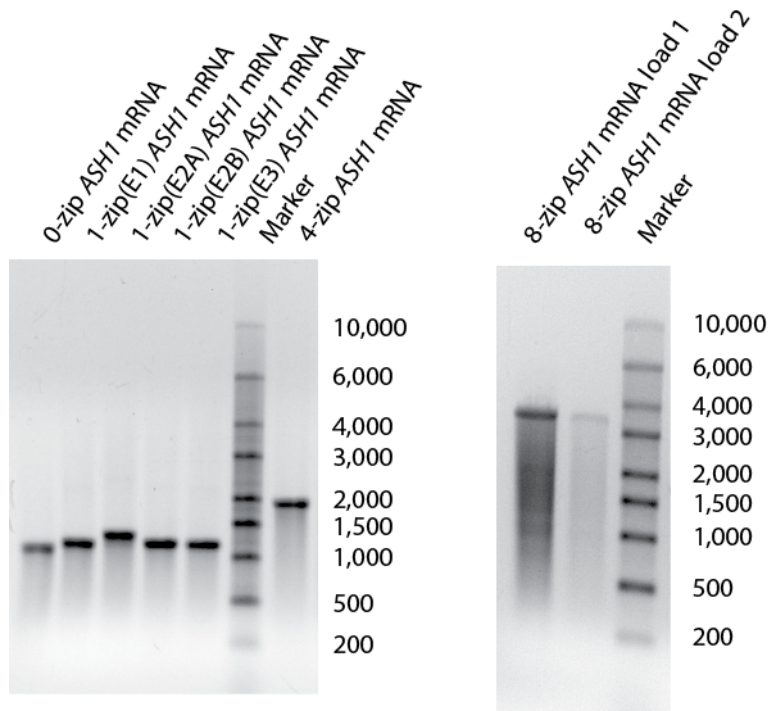


**Supplementary information for “Single molecule reconstitution of mRNA transport by a class V myosin”**

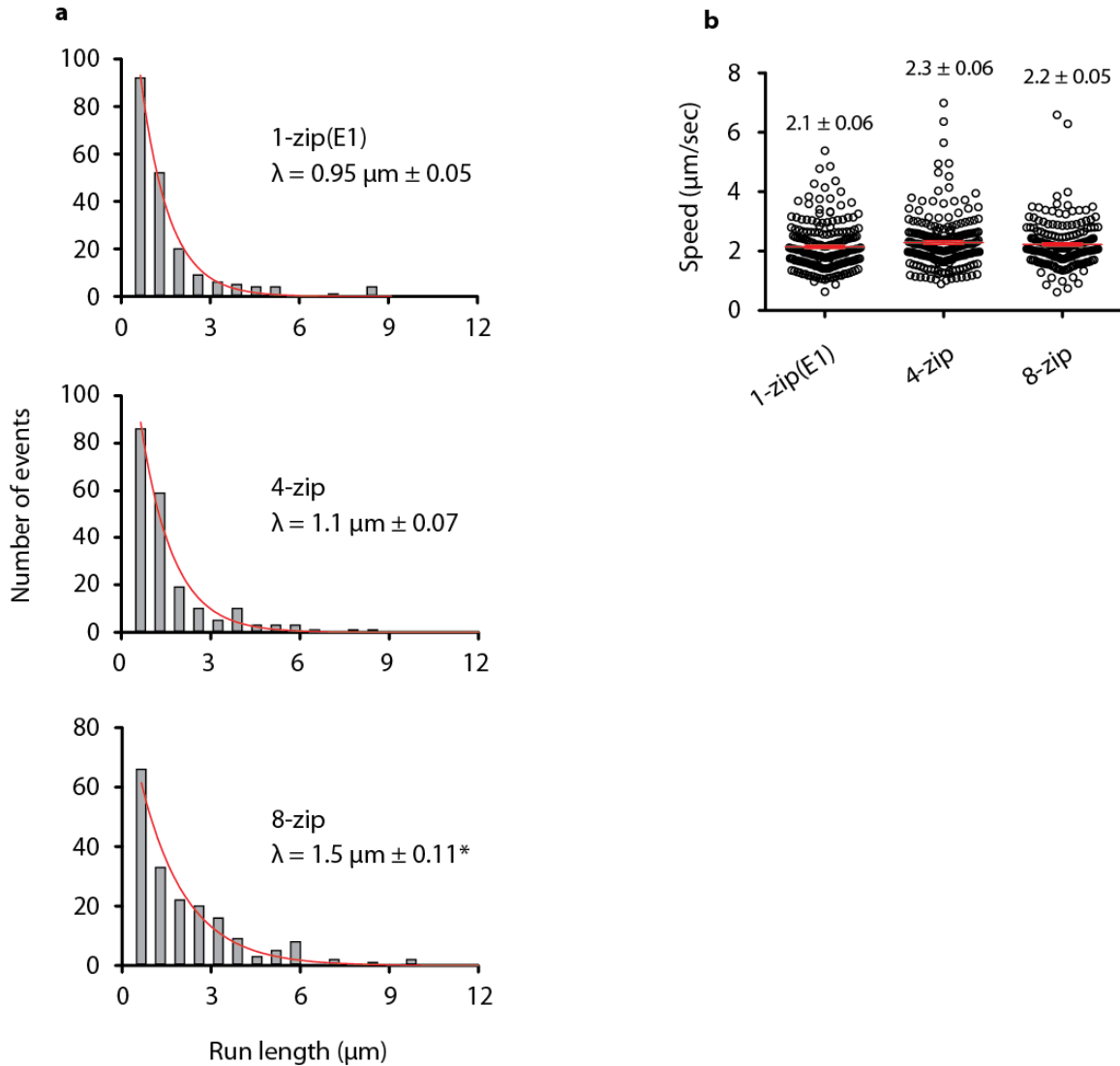
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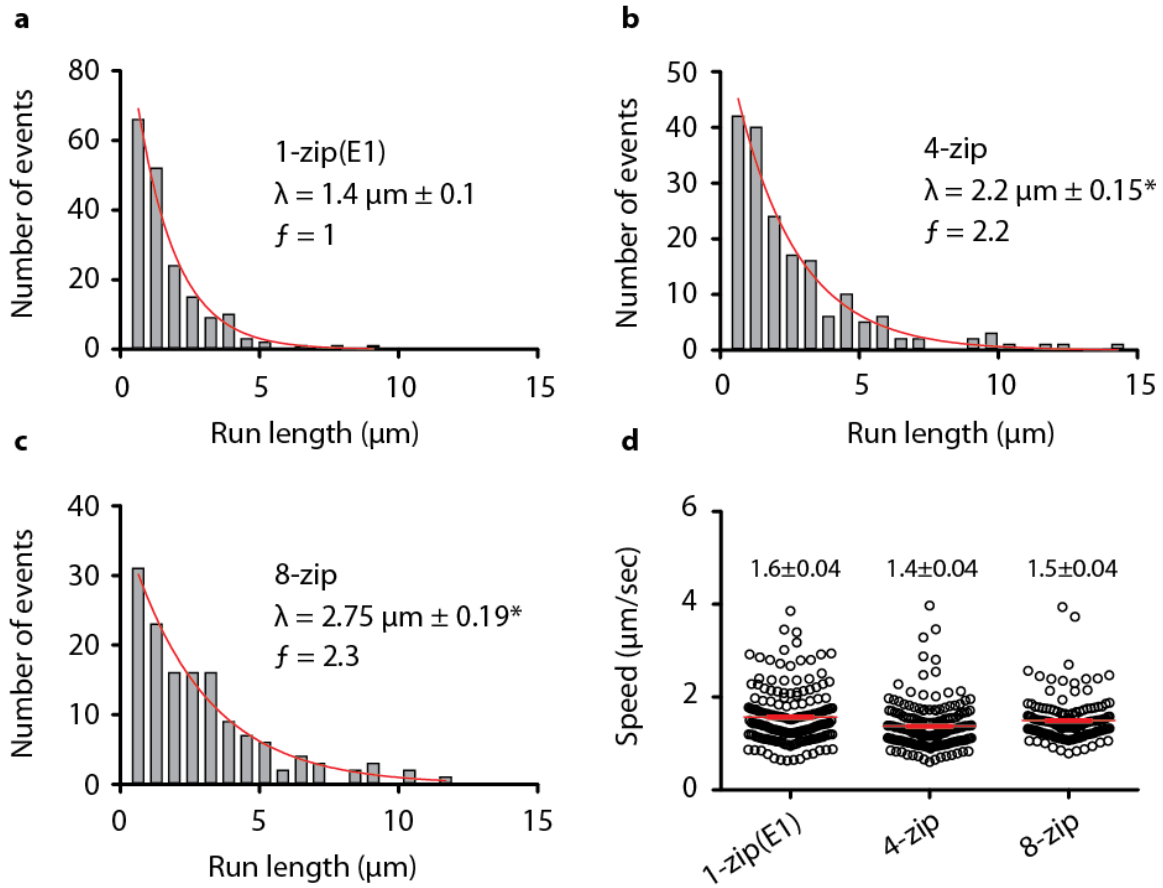
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**Supplementary Figure 1** Gel electrophoresis showing the relative sizes of Alexa-488 labeled *ASH1* mRNA constructs following *in vitro* synthesis. For the 8-zip *ASH1* mRNA construct, two loading amounts of mRNA are shown.



**Supplementary Figure 2** Characteristic run length and speed of Myo4p mRNPs containing *ASH1* mRNA with the indicated number of zipcodes. **(a)** Run length histograms of Myo4p mRNPs from a representative experiment comparing *ASH1* mRNA sequences containing, 1(E1), 4 or 8 zipcodes. The 1-zip(E1) data are from a different data set than that shown in Table 1. Asterisk (\*) indicates run lengths that are significantly different from the 1-zip(E1) and the 4-zip construct with  $P < 0.001$  (Kolmogorov-Smirnov Test). **(b)** Speed distributions of Myo4p motile mRNPs. The mean speed is indicated with a red line. Error is in s.e.m. Conditions: 140 mM KCl, pH 7.4, 1 mM MgATP



**Supplementary Figure 3** Characteristic run length and speed of labeled Myo4p mRNPs in the presence of excess unlabeled motor and *ASH1* mRNA to promote zipcode occupancy. Run length histograms of Myo4p mRNPs containing **(a)** 1(E1), **(b)** 4 and **(c)** 8 zipcodes. Reactions included 0.35 nM labeled *ASH1* mRNA, 10 nM unlabeled *ASH1* mRNA, and 250 nM Myo4p. Asterisk (\*) indicates run lengths that are significantly different from the 1-zip(E1) *ASH1* construct with  $P < 0.001$  (Kolmogorov-Smirnov Test). The run frequency (number of runs per  $\mu\text{M}$  Myo4p per  $\mu\text{m}$  actin per sec) with 1-zip(E1) is normalized to one. **(d)** Speed distributions of Myo4p motile mRNPs in the presence of excess unlabeled Myo4p and *ASH1* mRNA. The mean speed is indicated with a red line. Error is in s.e.m. Conditions: 140 mM KCl, pH 7.4, 1 mM MgATP

**Supplementary Table 1. Sequences constituting the four native *ASH1* mRNA zipcodes**

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E1 zipcode  
T<sub>618</sub>ATCAAGACTATGTTAAAATACGCGAAGAAGTGGCTCATTTC AAGCCATTAAGTA  
TACCCA ACTTAACTAATAATCAAATAAT<sub>702</sub>

E2A zipcode  
A<sub>1102</sub>AATTGCTTGCGAATAGAGACATTCTATCGAACAATTCCAAATCTAATGTAAGGA  
AACCATCTAAGAACAAAATCTCAAAGCAA<sub>1185</sub>

E2B zipcode  
T<sub>1258</sub>CTGCATCTTCATCTCCATCTCCCTCCACACCGACGAAAAGTGGCAAGATGAGA  
TCAAGATCATCCTCACCTGTGCGTCCCAAG<sub>1341</sub>

E3 zipcode  
T<sub>1765</sub>GATACATGGATAACTGAATCTCTTTCAACTAATAAGAGACATTATCACGAAACA  
ATTGTACATTT<sub>1830</sub>

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**Supplementary Table 2. Nucleotide sequences present or base pair changes made to ablate zipcode elements for each *ASH1* mRNA construct**

<i>ASH1</i> Construct	<i>ASH1</i> Sequence
0-zip	1-618, 703-1104
1-zip	1-1104
2-zip(E2A,E2B)	1-617, 703-1590
3-zip(E1,E2A,E2B)	1-1590
4-zip	1-1837
8-zip	4-zip concatenated (1-1827, 382-1837)
2-zip(E1,E3)	C <sub>641</sub> GA → TAA and C <sub>1813</sub> GA → TGA

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## SUPPLEMENTARY MOVIE LEGENDS

**Supplementary Movie 1** No movement of mRNPs containing an *ASH1* mRNA construct without zipcode elements on yeast actin–tropomyosin tracks. mRNA, green. Actin, red. Conditions: 140 mM KCl, 1mM MgATP. Movie time, 17 s. Image width, 7  $\mu$ m.

**Supplementary Movie 2** Movement of mRNPs containing the native four zipcode *ASH1* mRNA construct on yeast actin–tropomyosin tracks. mRNA, green. Actin, red. Conditions: 140 mM KCl, 1mM MgATP. Movie time, 17 s. Image width, 7  $\mu$ m.

**Supplementary Movie 3** Movement of mRNPs containing an *ASH1* mRNA construct with a single (E1) zipcode element on yeast actin–tropomyosin tracks. Run frequency is 1/3 that of native *ASH1*. mRNA, green. Actin, red. Conditions: 140 mM KCl, 1mM MgATP. Movie time, 17 s. Image width, 7  $\mu$ m.

**Supplementary Movie 4** Movement of mRNPs containing an *ASH1* mRNA construct with eight zipcode elements on yeast actin–tropomyosin tracks. Run frequency is 2-fold higher than with the native *ASH1*. mRNA, green. Actin, red. Conditions: 140 mM KCl, 1mM MgATP. Movie time, 17 s. Image width, 7  $\mu$ m.