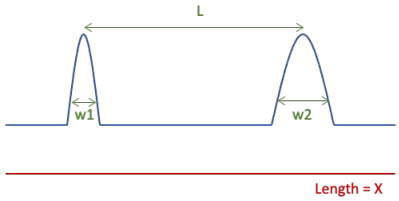
Supplementary Materials
Molecular Biology of the Cell
Fang *et al*.

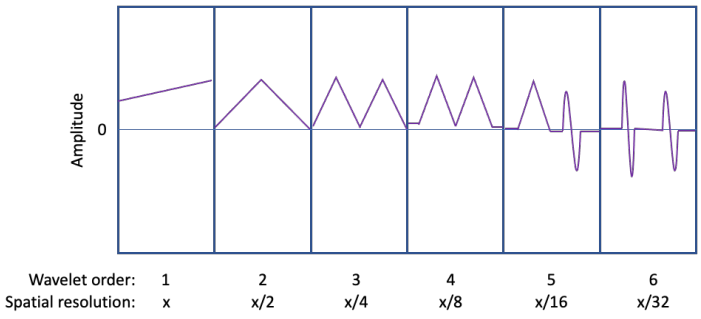
Supplemental Method: Wavelet Analysis

Disclaimer: We will attempt here to explain the essence of the wavelet method in non-mathematical terminology. That is a fraught enterprise, and we apologize in advance for the inevitable oversimplifications and inaccuracies. For an excellent conceptual introduction to wavelets we suggest https://towardsdatascience.com/the-wavelet-transform-e9cfa85d7b34

Imagine a simple, 1-dimensional distribution, like the one below, with a total length X. It has two peaks, one that has a width of w1 and another with a width of w2, which are separated from each other by a distance L.



Now let us consider wavelet analysis of this distribution. The point of this process will be to query the distribution at a series of spatial resolutions, from the highest resolution (querying short spatial distances) down to low resolution (where the entire distribution looks like a single element). The goal is to ask, for each size range, whether there are features in the original distribution, or spacings of features, that are in the size range defined by that wavelet order. We start by selecting a simple waveform that is used as a mathematical filter to query the distribution. Different waveforms can be used for this purpose depending on the nature of the distribution we want to analyze; here we have used a common form called a Daubechies 4 wavelet. The period of the filtering waveform is much smaller than the length X of the target distribution and it acts, in essence, as a kind of edge-detector. The wavelet is applied locally (by matrix multiplication) to the values of a groups of points at one end of the distribution, then stepped laterally to an overlapping group of points, then to the next, and to the next, etc., in the manner of a sliding window. At each point it will be multiplied by the values of the distribution to be analyzed. When the process is finished, it produces a filtered form of the original distribution that has half the resolution of the starting distribution. That filtered form is then subjected to the same procedure to produce the next lower order, and then repeated again, each time reducing the resolution by a factor of 2, until we reach the point that the entire distribution acts as a single entity.



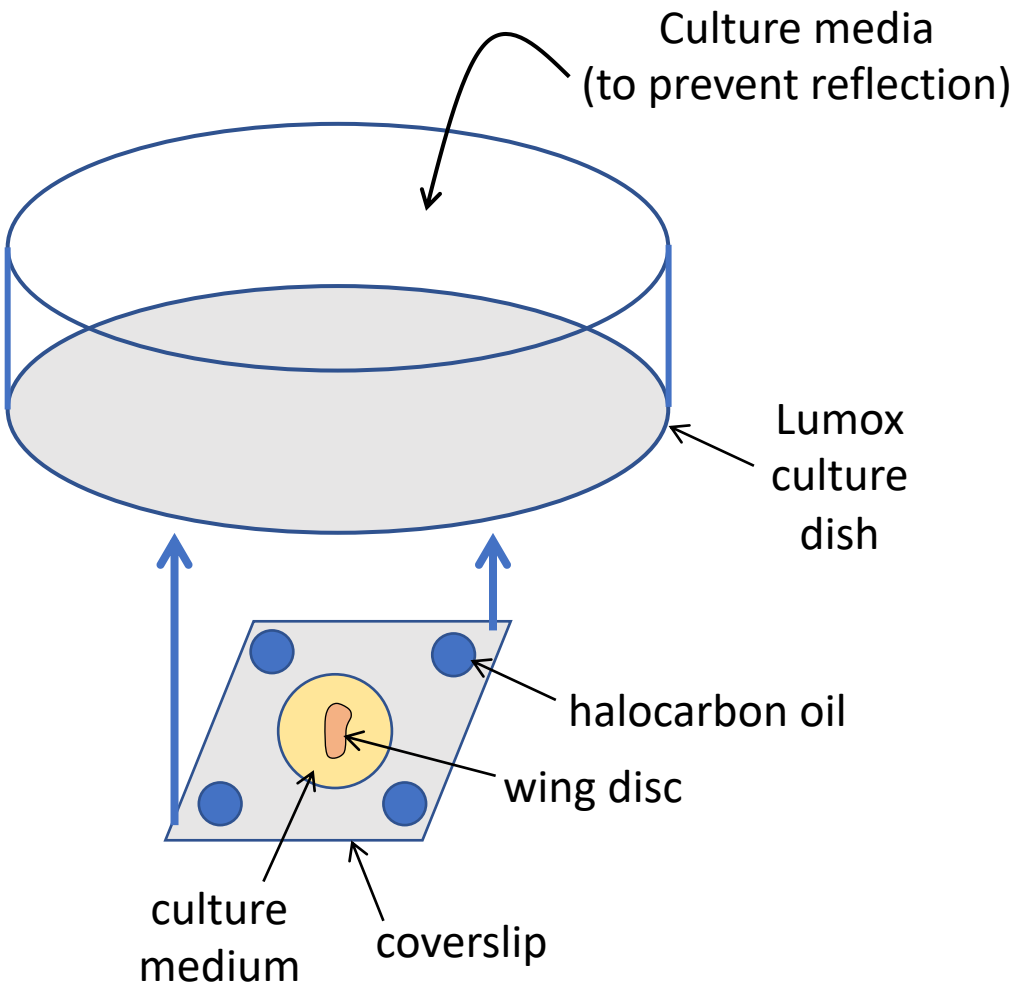
To think about what this does, it is easiest to start from the lowest resolution data and work our way to high resolution. In the example shown here, the 1st and 2nd orders simply show there is a signal somewhere in the distribution. The resolution is too low to tell more. In the 3rd order, the resolution is sufficient to show that two peaks are present but not to give detailed information about either one (ie., the resolution is better than length L, but is not high enough to dissect the properties of elements as small as w1 or w2). Once we get to 5th order, the resolution of the process is sufficient to distinguish the leading from the trailing edge of the broader peak (w2), so we see a more complex waveform for w2; ie., we can infer the approximate width of the peak. It is not, however, sufficient to detect the fine structure of w1. At 6th order, the analysis now has sufficient resolution to infer the widths of both peaks, w1 as well as w2. If there had been even finer detail to the structure of w1 (or w2), then it would have been necessary to take the analysis to yet higher orders to resolve that.

So the wavelet analysis allows us to measure the distribution of information in a starting curve: is the signal homogeneous or clumpy, and to the degree that it is clumpy, how wide are those clumps and how are they distributed along the length of the curve. In our actin analysis we are not using it to measure individual features of a single curve, but instead we average over all the timepoints for each trajectory, and over all the cells of a given genotype. Comparing the amplitudes obtained by that averaging across orders and across genotypes allows us to quantify how much each spatial scale is contributing to the global distribution of actin under different conditions.

In this paper, we use the wavelet method (rather than Fourier analysis, for example, which can also quantify spatial contributions to a distribution) because it is extremely effective for non-periodic curves with non-repeating, local features. This is particularly true if there are sharp discontinuities in the signal, as we have in an actin distribution in an axon. But in the context of the current paper, the key point is that it provides a rigorous way to define and quantify the way actin is distributed, globally, along an axon and to compare the distributions that form in different genotypes.

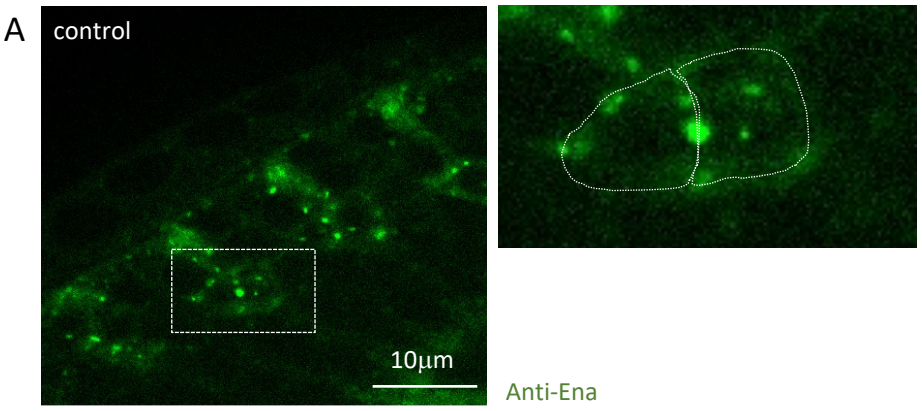
Supplemental Figure 1

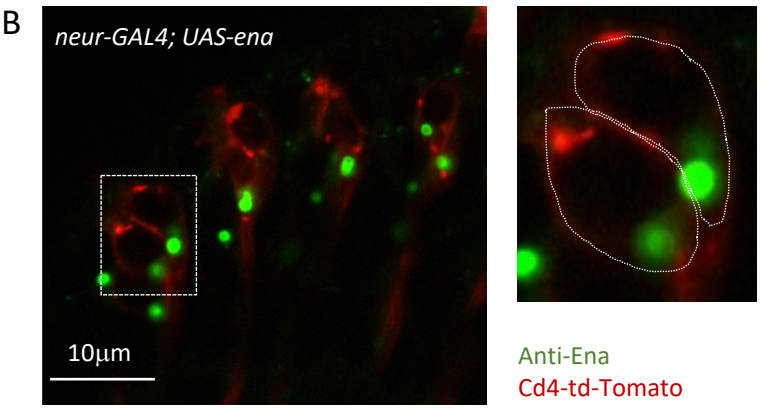
Schematic of imaging setup

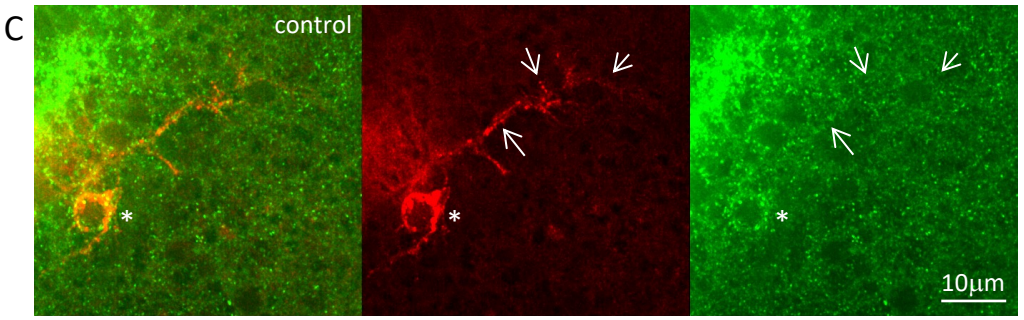


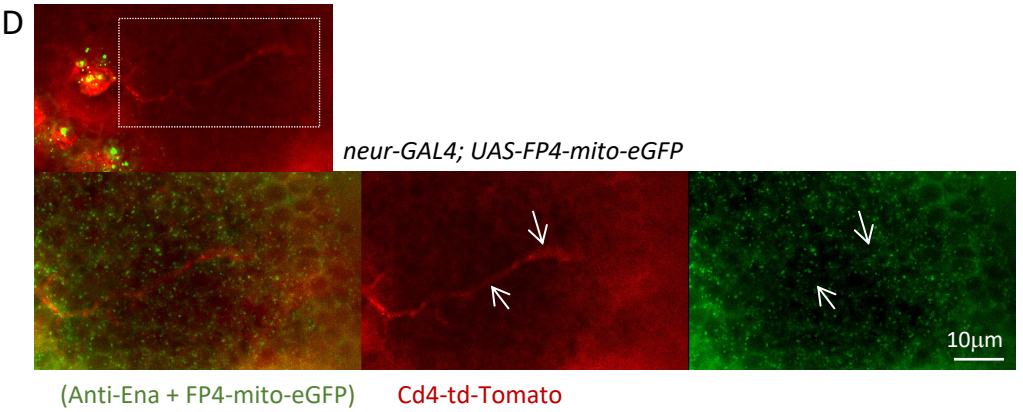
(Image from beneath)

Supplemental Figure 2



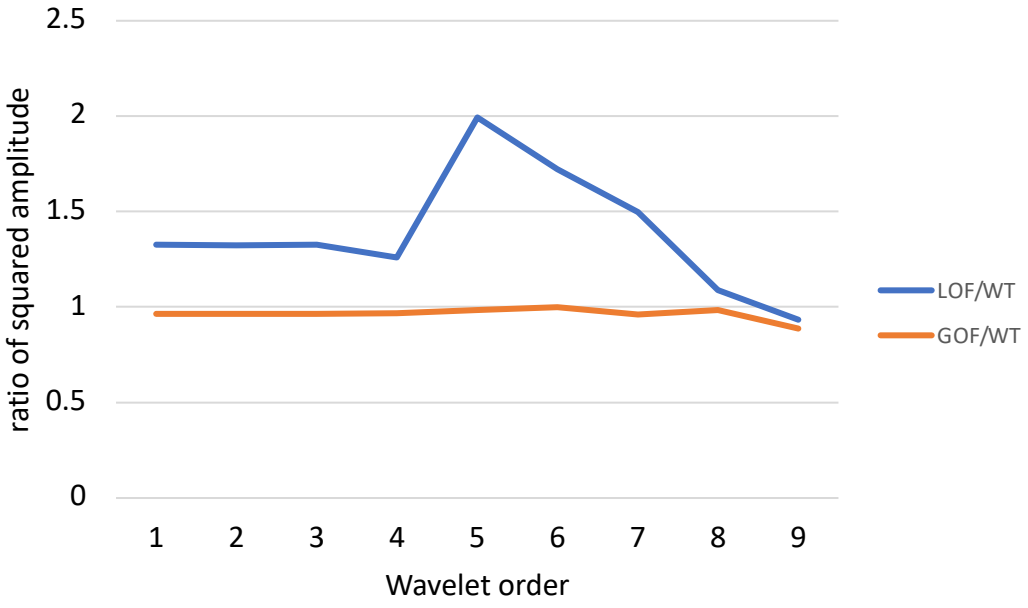






Supplemental Figure 3

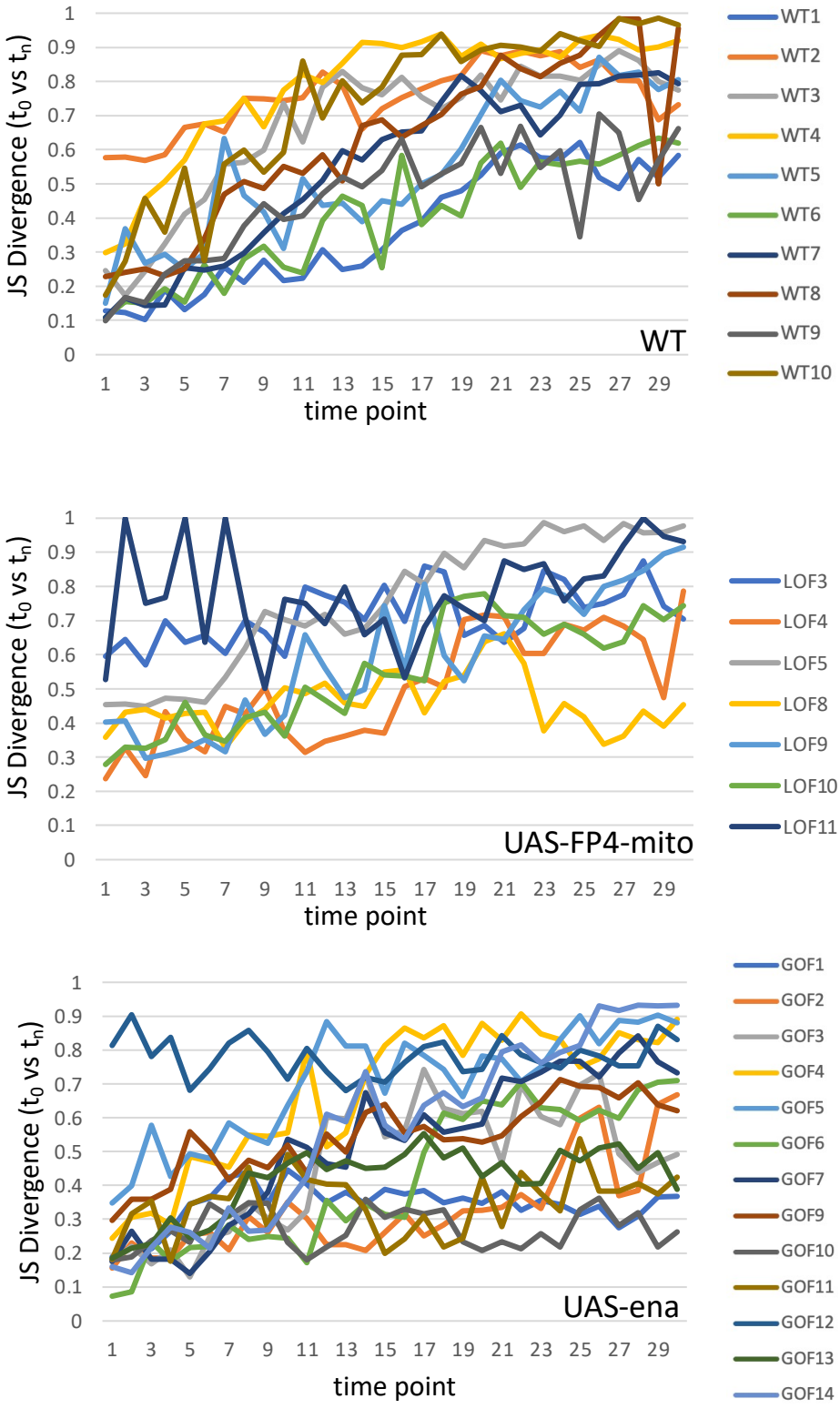
A



B

Wavelet order	Contributing spatial range
1	> 102.4 μm
2	> 51.2 μm
3	25.6 - 102.4 μm
4	12.8 - 51.2 μm
5	6.4 - 25.6 μm
6	3.2 – 12.8 μm
7	1.6 - 6.4 μm
8	0.8 - 3.2 μm
9	0.4 - 1.6 μm

Supplemental Figure 4



Supplemental Figure 5

A

Correlation (Kendal tau)

	Filopodial number	Filopodial length	Average filopodial order	Length of filopodial zone	Length of actin peak	Motion of actin peak	Offset: actin *vs* filopodia
Filopodial number						**Legend:** WT / UAS-FP4-mito / UAS-Ena	
Filopodial length	0.78 0.36 0.76						
Average filopodial order	0.50 -0.24 0.34	0.47 0.16 0.43					
Length of filopodial zone	-0.12 -0.05 -0.01	-0.11 0.04 -0.05	-0.23 -0.13 -0.10				
Length of actin peak	0.04 -0.16 -0.10	0.02 -0.14 -0.09	-0.03 -0.10 0.01	0.41 0.33 0.07			
Motion of actin peak	0.003 -0.02 -0.01	-0.01 0.04 -0.01	0 0.01 -0.003	-0.004 0.03 -0.03	-0.03 -0.03 -0.09		
Offset: actin *vs* filopodia	-0.15 -0.10 -0.05	-0.16 0.05 -0.06	-0.09 0.05 -0.09	0.05 0.05 -0.10	0.18 0.12 0.14	-0.18 -0.18 -0.16	

B

p-value

	Filopodial number	Filopodial length	Average filopodial order	Length of filopodial zone	Length of actin peak	Motion of actin peak	Offset: actin *vs* filopodia
Filopodial number						**Legend:** WT / UAS-FP4-mito / UAS-Ena	
Filopodial length	5.5E-88 6.8E-20 9.5E-109						
Average filopodial order	3.7E-37 2.9E-9 1.8E-23	1.3E-33 6.3E-5 2.2E-36					
Length of filopodial zone	0.001 0.17 0.88	0.006 0.36 0.11	1.7E-9 7.5E-4 0.003				
Length of actin peak	0.33 5.8E-5 0.004	0.63 3.5E-4 0.01	0.42 0.01 0.72	8.7E-26 1.0E-17 0.05			
Motion of actin peak	0.94 0.68 0.81	0.89 0.28 0.77	0.99 0.80 0.93	0.93 0.50 0.41	0.50 0.50 0.01		
Offset: actin *vs* filopodia	1.2E-4 0.01 0.11	5.9E-5 0.19 0.07	0.02 0.16 0.01	0.21 0.24 0.01	5.3E-6 0.001 3.5E-5	3.8E-6 1.8E-6 3.41E-6	

Critical value for BH FDR < 5%: **WT: 0.0063** **FP4-mito: 0.0019** **UAS-Ena: 0.004**

Supplemental Figure 6