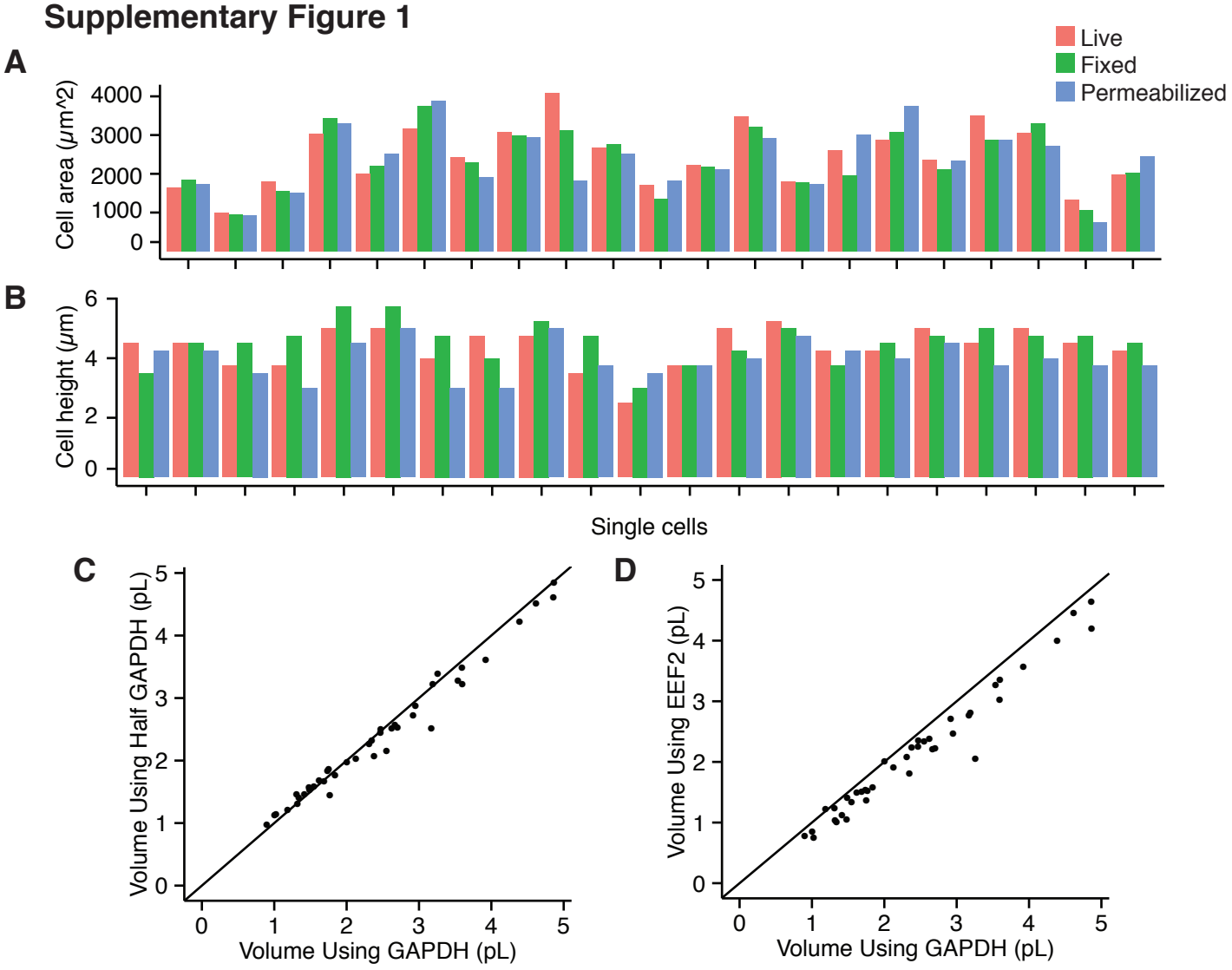
Supplementary Figure 1



Supplementary Fig. 1. Controls for volume calculation.
A and B. We monitored cells on the microscope throughout the process of fixation. We took measurements of the same cells live, after fixing in 4% formaldehyde for 10 minutes, and after permeabilizing in ethanol for 30 minutes.
A. We measured the areas of the cells through brightfield images.
B. We measured the height of the cells by coating the cells with fluorescent beads.
These measurements indicate that the cells remain roughly the same size throughout the fixation and permeabilization process.
C. To demonstrate the robustness of the volume calculation algorithm, we calculated volume for the same cells using all the *GAPDH* mRNA spot coordinates as detected by RNA FISH, or using only half of the points, chosen randomly. Both methods result in approximately the same volume, suggesting that the number of points we use is sufficient to calculate the volume accurately.
D. We calculated volume using a different gene, *EEF2*. On average, *EEF2* has an abundance that is less than half that of *GAPDH* (mean EEF2 = 1079 mRNA/cell, mean *GAPDH* = 2673 mRNA/cell). Volume calculated using *EEF2* is systematically lower than that calculated using *GAPDH*, but the values are similar.
Black lines indicate a fit with intercept = 0 and slope = 1.