

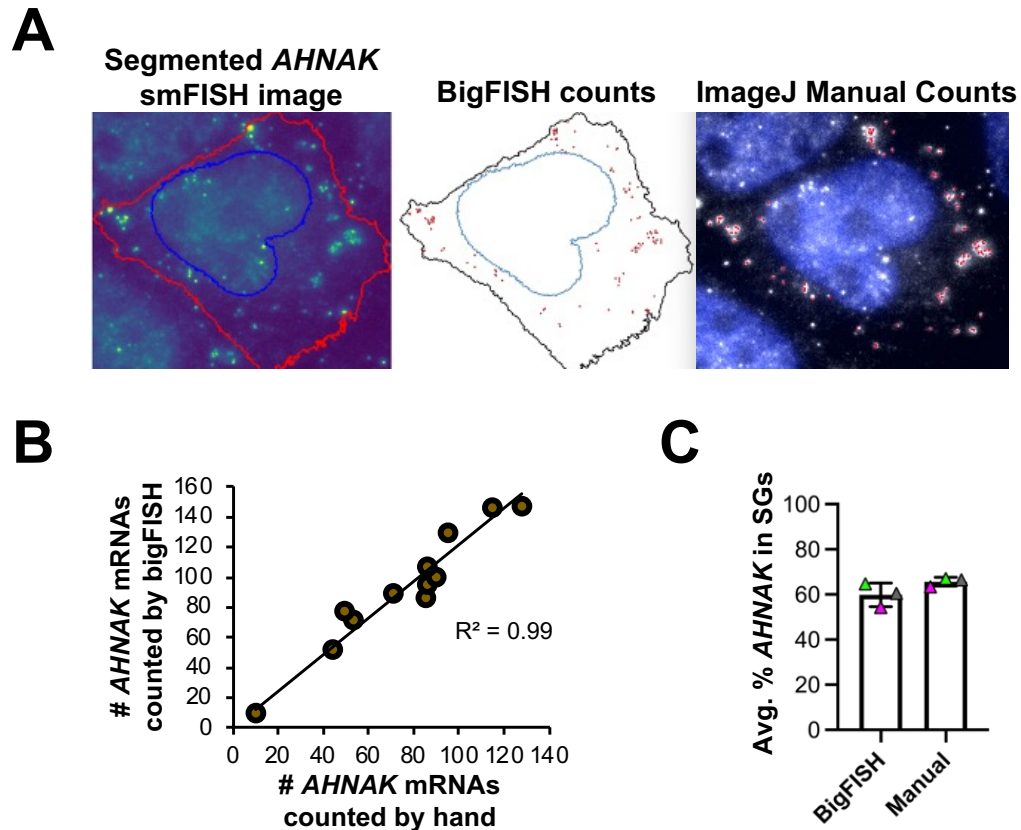


Figure S1. Validation of automated smFISH spot counting with BigFISH. smFISH with probes to *AHNAK* was performed on arsenite-treated U-2 OS cells expressing GFP-G3BP1. A) Example image demonstrating RNA spot detection by the BigFISH pipeline with automated segmentation of the nucleus (blue line) and cytoplasm (red line) compared to counting by hand with the ImageJ cell counter tool. B) Correlation between the number of *AHNAK* molecules per cell from $n=12$ cells from 3 independent replicates determined by BigFISH versus counting by hand with the ImageJ cell counter tool. The R^2 for a linear regression with y-intercept set to 0 is shown. C) The average $\pm$ s.e.m. percent *AHNAK* mRNA in stress granules from $n=3$ replicates (4 cells counted per replicate) were determined using the BigFISH pipeline or manually using the ImageJ cell counter tool.