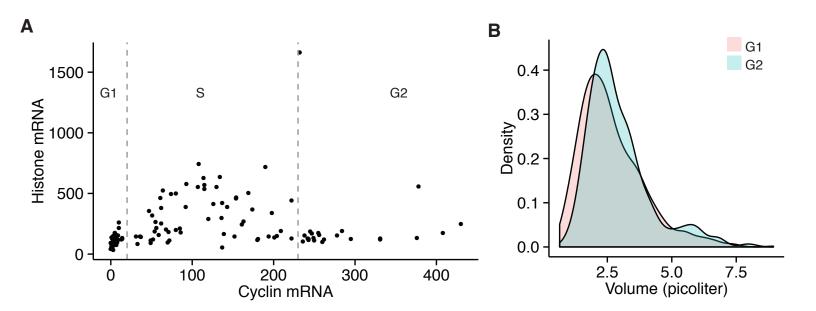
## **Supplementary Figure 6**



## Supplementary Fig. 6. Cell cycle characterization.

A. We simultaneously measured *CCNA2* and *HIST1H4E* mRNA by RNA FISH to precisely determine cell cycle position. Each data point is a single cell measurement. *CCNA2* is highly expressed in S and G2, but not G1. *HIST1H4E* is highly expressed only in S phase. Cells with low *CCNA2* and *HIST1H4E* are in G1 (cutoff = 20 *CCNA2* mRNA), cells with mid-range *CCNA2* and high *HIST1H4E* are in S, and cells with high *CCNA2* and low *HIST1H4E* are in G2 (cutoff = 230 *CCNA2* mRNA). We determined thresholds for all samples using this method. Data shown are from one of four biological replicates.

B. Volume distributions in G1 and G2. We determine cell cycle position using CCNA2. We note that G2 cells are larger than G1 cells, but only 10% larger on average, possibly due to non-linearities in growth over the course of the cell cycle. n = 841 cells in G1, 191 cells in G2.