

**Figure 4-figure supplement 5.** DNA binding by PhoP or OmpR is not acid-sensitive. (**A**) PALM-Brightfield overlay images of PhoP-PAmCherry grown in MgM media at acid pH (left panel) or neutral pH (right panel). Scale bar = 2 µm. **(B**) Boxplot shows the quantification of the number of PhoP molecules in individual cells. The number of molecules/µm2 was calculated by normalizing the total number of localizations within individual cells to its corresponding cell area (µm2). The total number of cells analyzed for PhoP-PAmCherry at pH 5.6 = 122, at pH 7.2 = 140 cells. Statistical analysis was performed by using an unpaired two-tailed *t*-test (Microsoft Excel). \*\*\*\* Denotes p<0.0001. (**C**) The fraction of bound and free PhoP-PAmCherry molecules obtained from Spt-PALM during growth in MgM media at acid or neutral pH. The bound fraction (*F1*) does not change significantly in the two different growth conditions. (**D**) PALM-Brightfield overlay images of OmpR-PAmCherry when grown in MgM media in acid pH (left panel) or neutral pH (right panel). Scale bar = 2 µm. (**E**) Boxplot quantifies the number of OmpR molecules in individual cells. The total number of cells analyzed for OmpR-PAmCherry at pH 5.6 = 75, at pH 7.2 = 75 cells. Statistical analysis was performed by using an unpaired two-tailed *t*-test (Microsoft Excel). \*\*\*\* Denotes p<0.0001. (**F**) The fraction of bound and free OmpR-PAmCherry molecules obtained from Spt-PALM during growth in MgM media in acid or neutral pH. The bound fraction (*F1*) was higher in acid (21.9 ± 1.4) compared to neutral pH (16.4 ± 1.0), indicating a 5% increase in the number of OmpR molecules bound to DNA in acid pH.