



**SUPPLEMENTARY FIG. S4.** Error associated with percent beacon expression remained relatively constant within a given well (~5%). This indicates that the variability across multiple images for a single sample did not change over time. The error associated with percent beacon expression among samples was small for the first 5 days (2–4%) but increased to 8–10% at later time points. This decrease in uniformity indicates that samples diverged over time, with some samples exhibiting higher levels of *ALPL* signal than others compared with the first few days. Another possible explanation, or contributing factor, was poorer recognition of true versus false signal by the image analysis algorithms as samples became more complex. Signals could be clearly identified early on, but more background noise accumulated due to multiple beacon treatments, cellular proliferation, and matrix deposition.