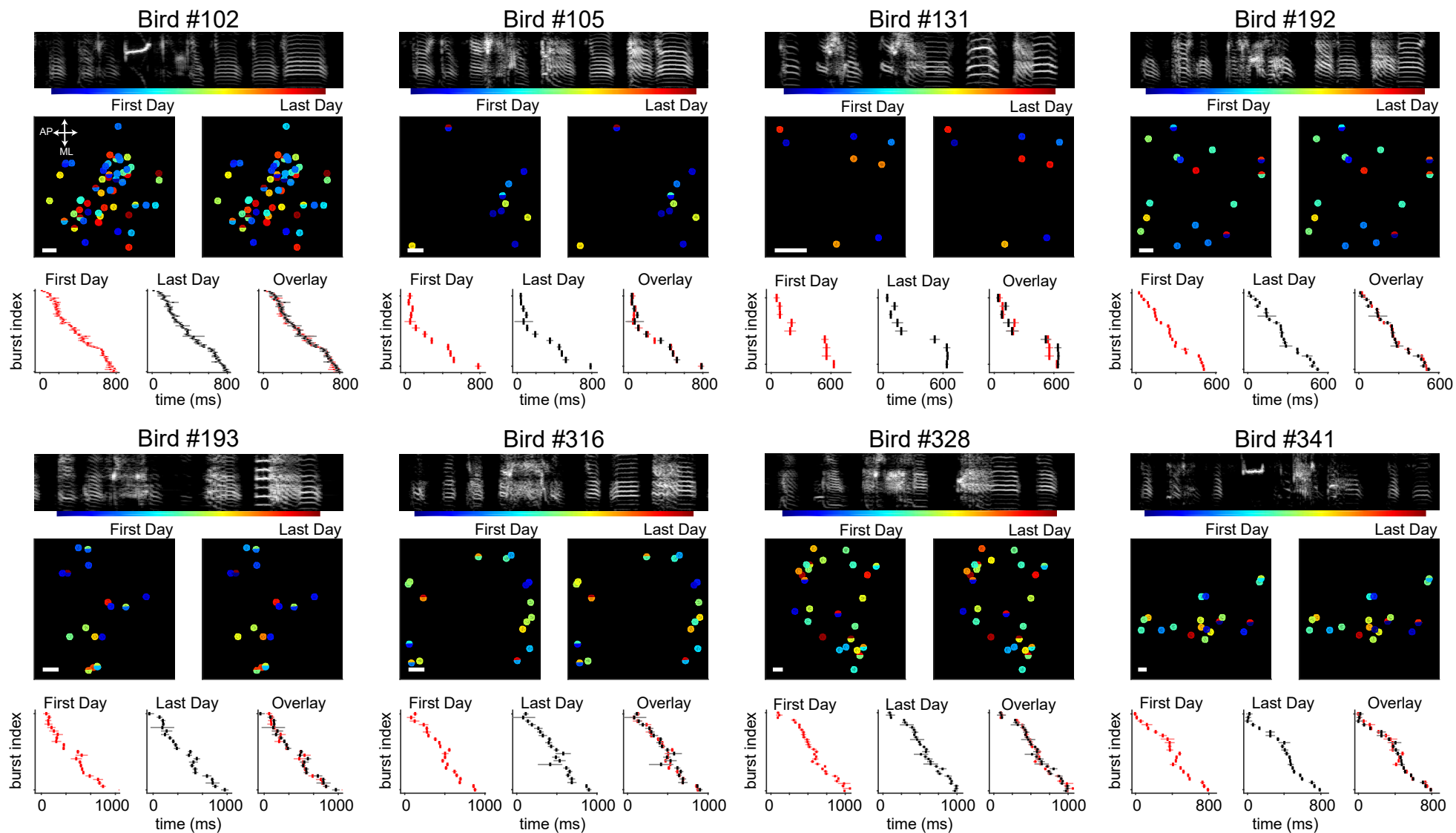


**Figure S1. Longitudinal Changes in Burst Number (Related to Figure 2)**

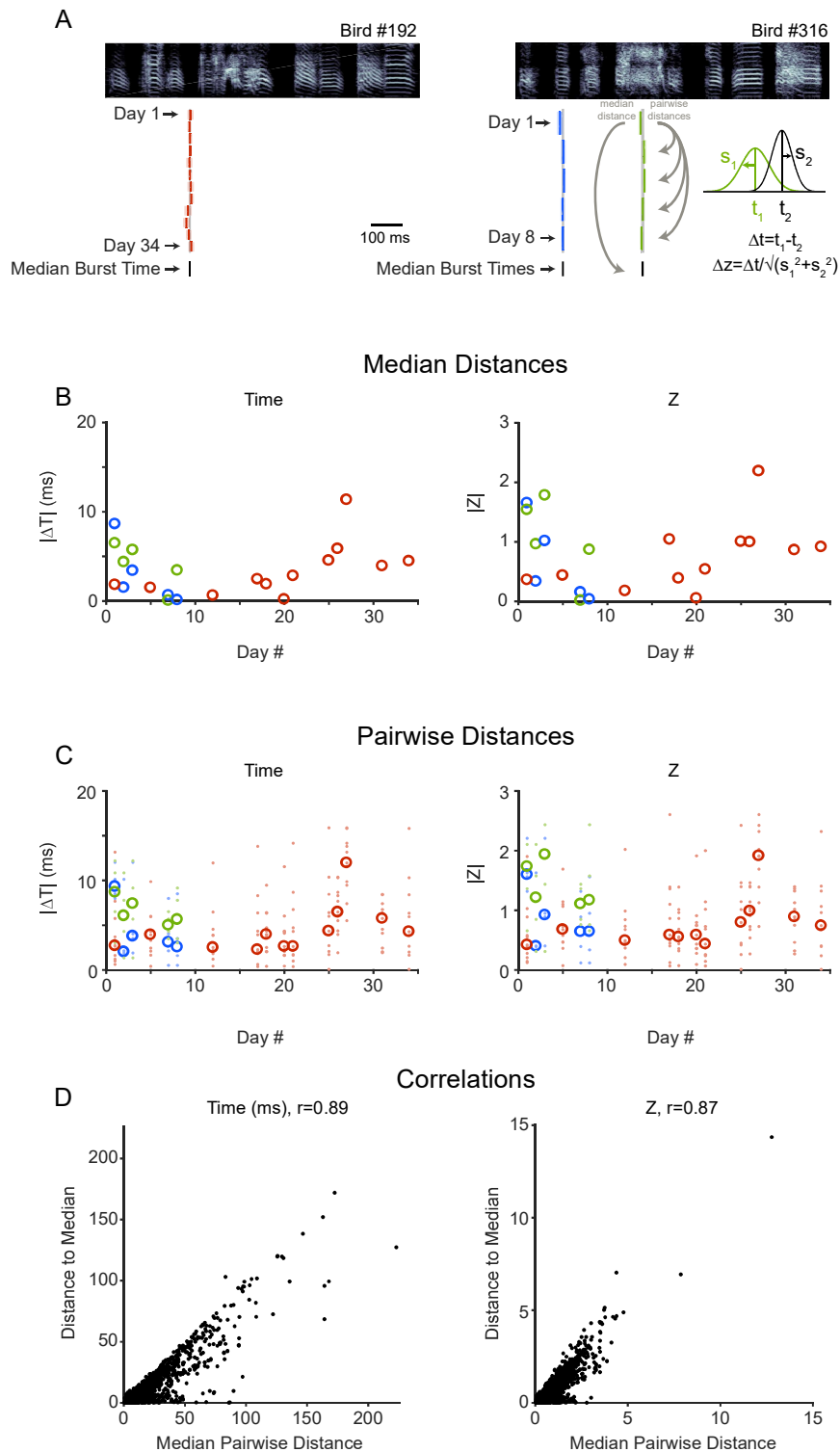
(A) Normalized fluorescence traces from two imaging sessions for an example cell. The red arrow indicates that the burst event present on Day 6 is missing on Day 21.

(B) Timeline of all 12 cells out of a population of 333 imaged neurons in our data set that changed their number of song-related bursting events. Circles indicate the number of bursts observed on a specific day relative to the first day the cell was imaged. Filled circles indicate those shown in (A).



**Figure S2. Population Data of Burst Timing for All Imaged Neurons (Related to Figure 2)**

For each bird in our data set, we present the tuning for all neurons on their first and last observations. Respective sonograms are at the top of each panel. The color of each cell represents its burst time relative to song (middle). Cells that burst more than once during song are displayed with more than one color. Scale bars: 50 microns. At the bottom of each panel, we show each burst event for the first day, the last day, and an overlay of the two. Vertical lines indicate burst times and horizontal lines represent relative uncertainty ( $\pm 2\sigma$ ). Events are sorted based on the median time across all observations.



### Figure S3. Assignment of Distance Scores (Related to Figure 2)

(A) Burst times of the two example cells from Figures 2A and 2B. Vertical lines indicate estimated burst times for each respective day, with colored shading representing the burst time uncertainty ( $\pm 2\sigma$ ). Uncertainty in the median burst time is shown in gray.

Inset: For each pair of bursts (e.g., green and black distributions), the burst time ( $t$ ) and uncertainty ( $s$ ) are used to calculate the temporal ( $\Delta t$ ) and normalized ( $\Delta z$ ) distance using the demonstrated formula.

(B) The **median** distances for each burst were defined as the distance in time (left) or Z (right) relative to the median burst time. Colors match those of (A and B).

(C) The **pairwise** distance score for each burst was defined as the average (circles) of comparisons to observations on all other days (dots). Colors match those of (A and B).

(D) Correlations between median and pairwise distance metrics for changes in time (left) and Z (right) for all bursts ( $n=1,869$ ). Title indicates correlation value.