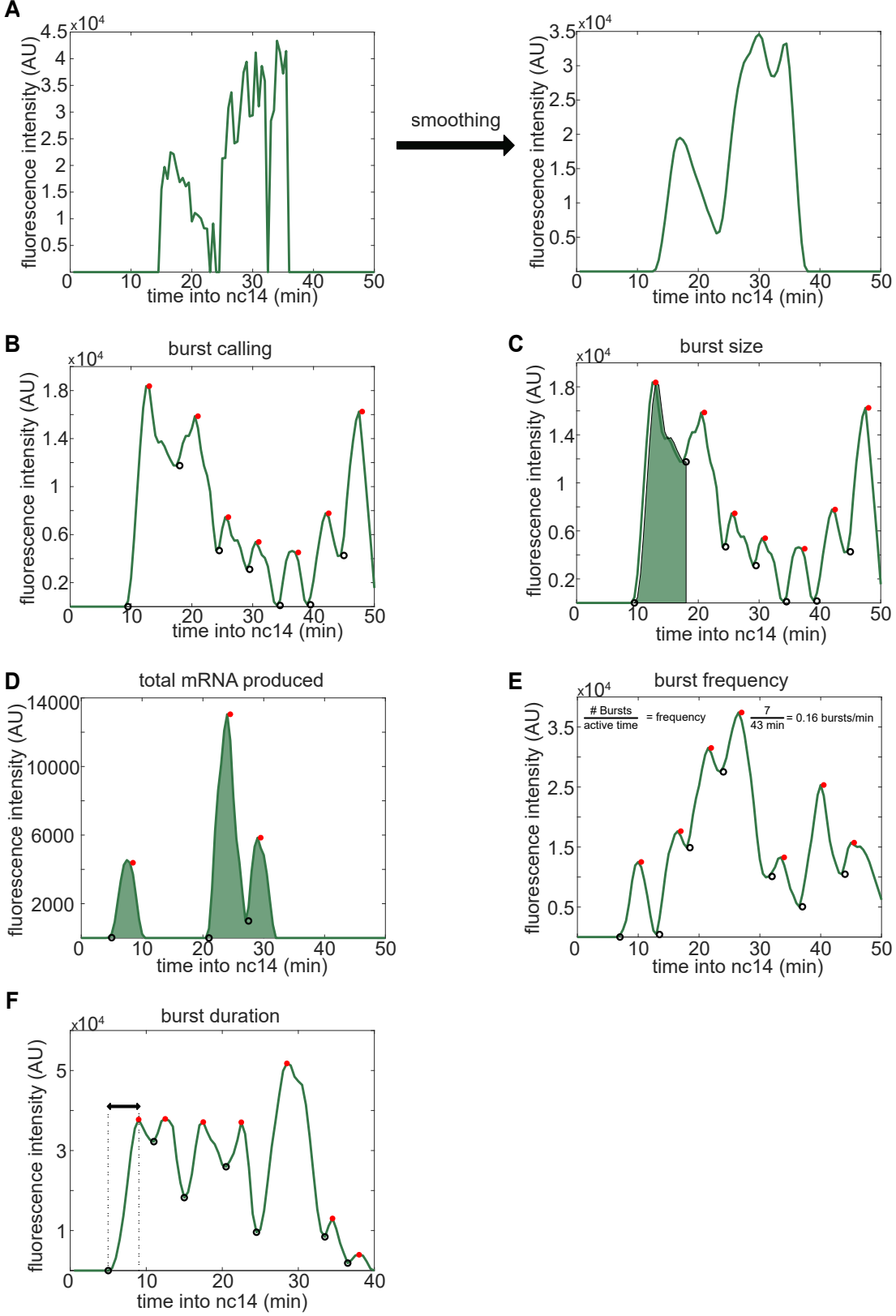

Figure 2 - figure supplement 4: Visual inspection of burst calling algorithm
To extract the bursting parameters examined (burst size, frequency, and duration), individual fluorescence traces were first smoothed using the LOWESS method with a span of 0.1. Our burst calling algorithm then determined the periods of promoter activity or inactivity based on the slope of the fluorescence trace. **A**. Representative example of smoothing of transcriptional traces. **B**. Representative fluorescence trace of a single spot across the time of nc14. Black open circles indicate time points where the promoter is switched to being called "on", red filled circles indicate time points where the promoter is switched to being called "off". **C**. Same trace as in A with shading representing the area under the curve used to calculate the size of the first burst. This area is calculated using the trapz function in MATLAB and is done for each burst, from the time point the promoter is called "on" until the next time it is called "on". D-F show additional representative fluorescence traces of single transcriptional spots across the time of nc14. **D**. A trace with shading representing the area under the entire curve during nc14 used to calculate the total amount of mRNA produced. This area is calculated using the trapz function in MATLAB and is done from the time the promoter is first called active until 50 minutes into nc14 or the movie ends, whichever comes first. **E**. Burst frequency is calculated by dividing the number of bursts that occur from the time the promoter is first called active until 50 minutes into nc14 or the movie ends, whichever comes first. **F**. Burst duration is defined as the amount of time between when the promoter is called active and it is next called inactive.