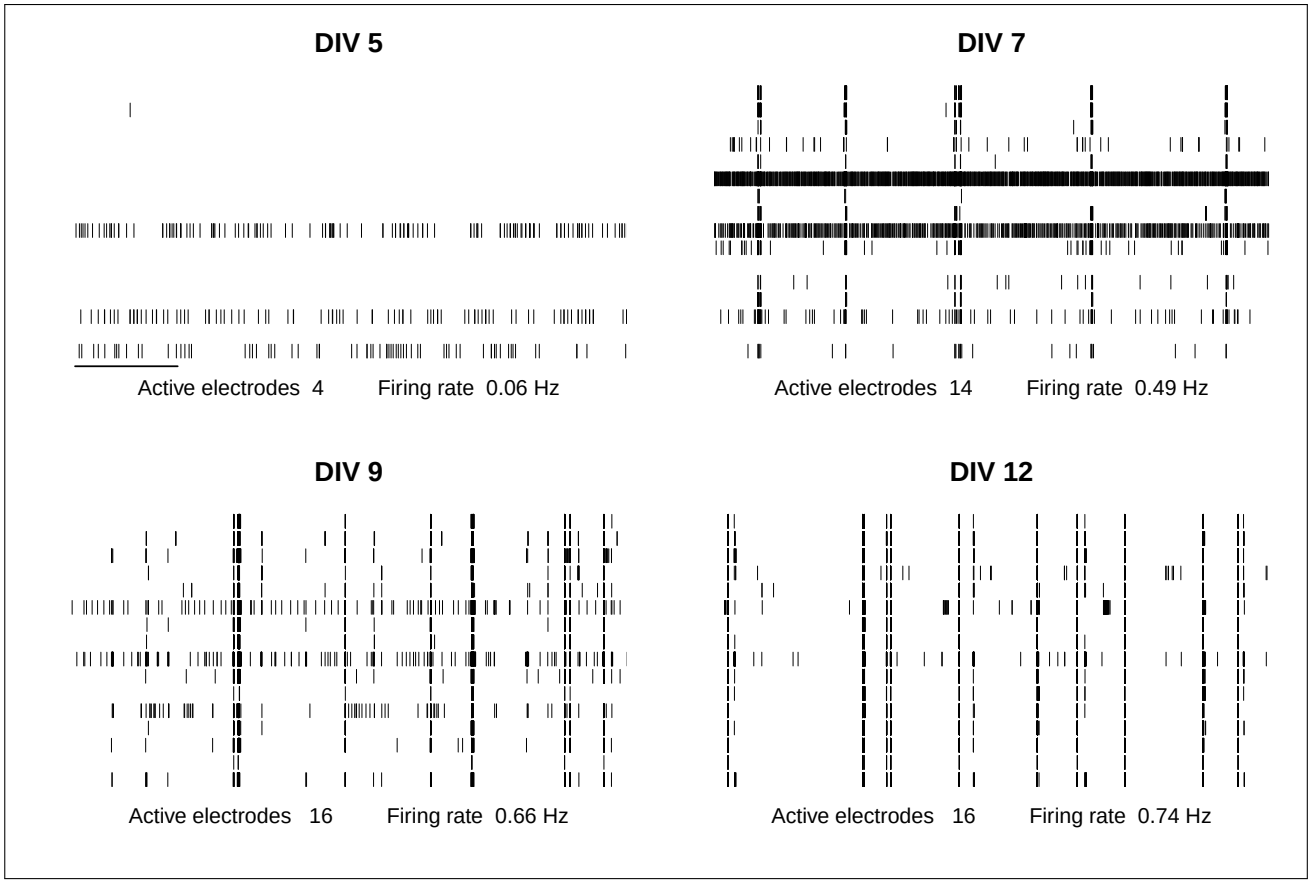

Figure 1: Example raster plots from the same well over the four developmental ages (days in vitro, DIV) studied. Each row represents the spike train from one electrode. The scale bar for all raster plots is 60s.

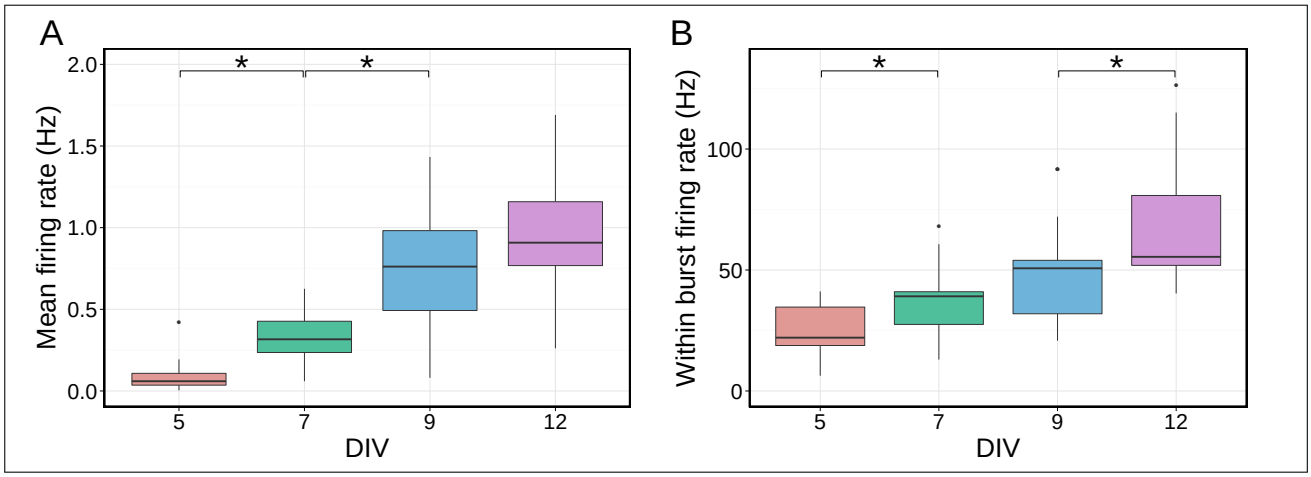

Figure 2: Mean firing rate (A) and within burst firing rate (B) increase with development. Box plots showing median and interquartile range are shown for n=16 plates. Stars represent significant difference in median values between pairs of consecutive ages at the 0.05 level. P-values obtained from a Mann-Whitney test, corrected for multiple comparisons using the false discovery rate method.

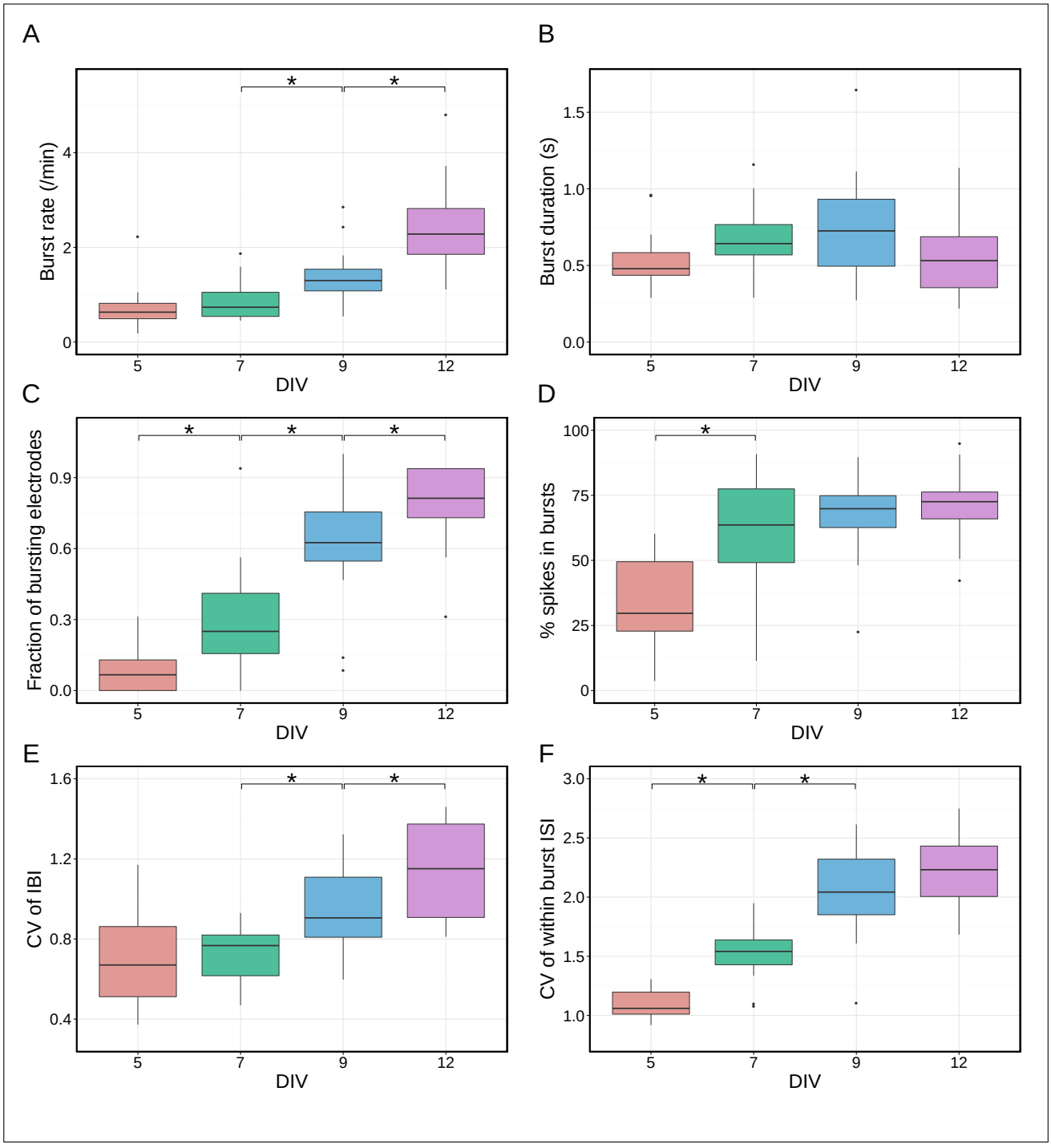

Figure 3: Bursting properties of spontaneous activity mature with development. Each panel shows box plots of one burst feature across development. A: Bursts per minute. B: Burst duration. C: Fraction of bursting electrodes. D: Percentage of spikes in bursts. E: Coefficient of variation (CV) of interburst interval (IBI). F: CV of within burst interspike interval (ISI). Stars represent significant difference in median values between pairs of consecutive ages at the 0.05 level. P-values obtained from a Mann-Whitney test, corrected for multiple comparisons using the false discovery rate method.

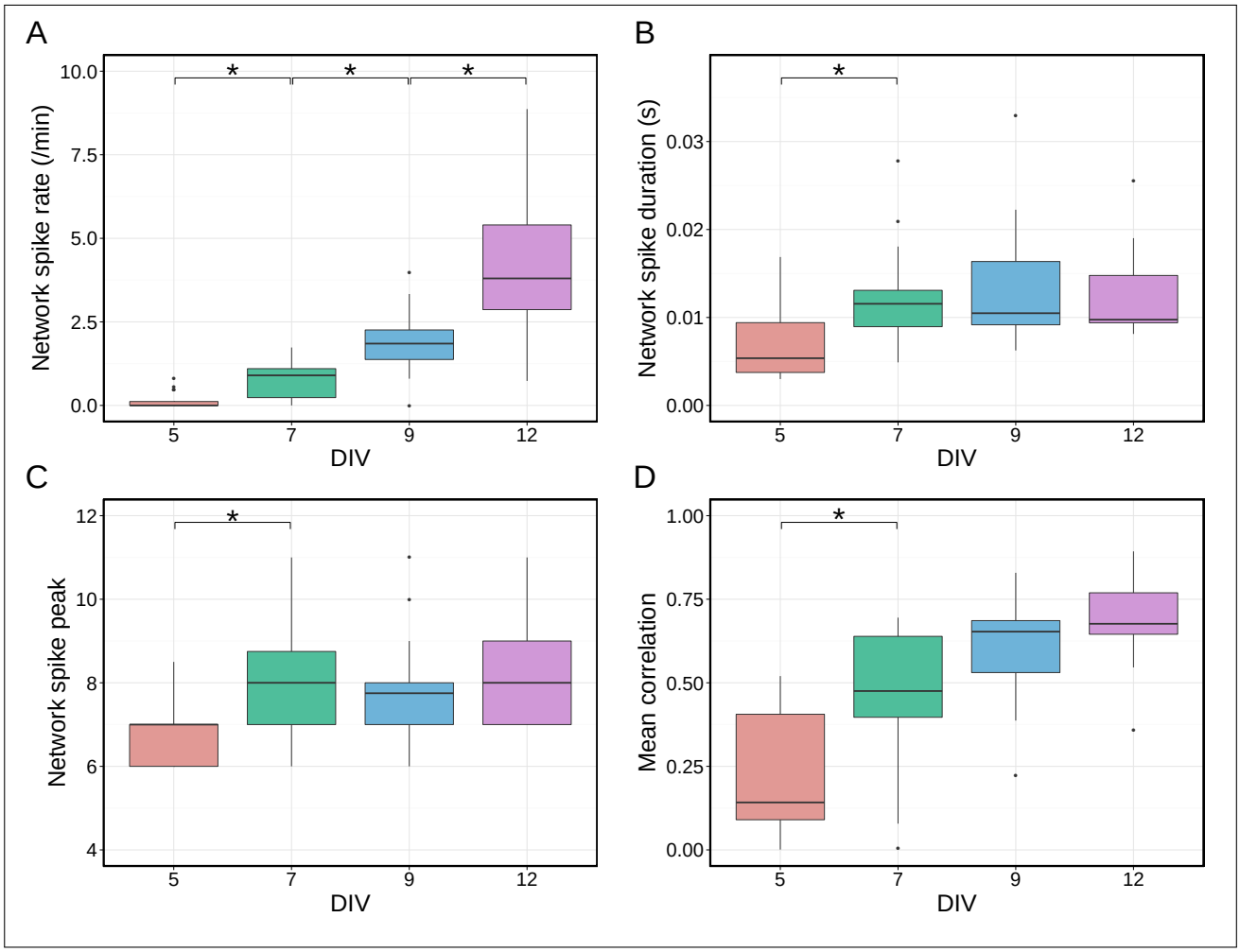

Figure 4: Network properties of spontaneous activity assessed across development. A: Network spike rate. B: Network spike duration. C: Network spike peak. D: Mean pairwise correlation. Stars represent significant difference in median values between pairs of consecutive ages at the 0.05 level. P-values obtained from a Mann-Whitney test, corrected for multiple comparisons using the false discovery rate method.

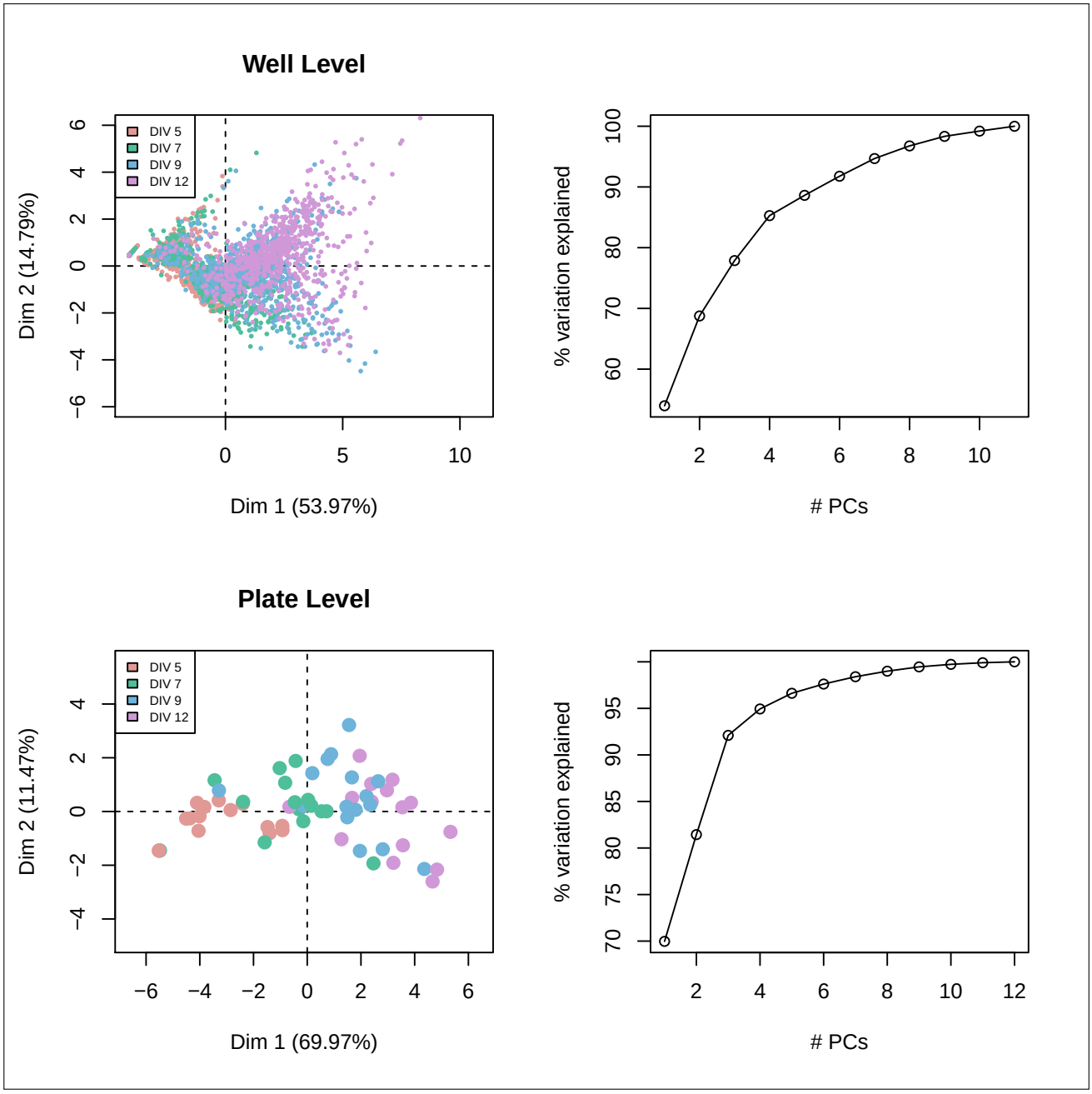

Figure 5: [Top left] Well-level PCA projection of 12-dimensional feature vectors onto PC dimension 1 (x-axis) and 2 (y-axis). Each dot represents a well, colored by day in vitro (DIV) of recording. Rough ordering from youngest (red, DIV 5) to oldest (purple, DIV 12) wells is apparent in change of colors along the positive direction . (Top right) Scree plot displays % variance explained by the number of PC dimensions. [Bottom left] Plate-level PCA projection of plate averages onto PC dimension 1 (x-axis) and 2 (y-axis). As in top, rough ordering of observations by DIV is apparent in the red to purple transition along the x-axis. (Bottom right) Scree plot of plate-level PCA. Compared to the well-level PCA scree plot, a larger amount of variation is captured in the first two PC dimensions indicating that well averaging reduces variability.

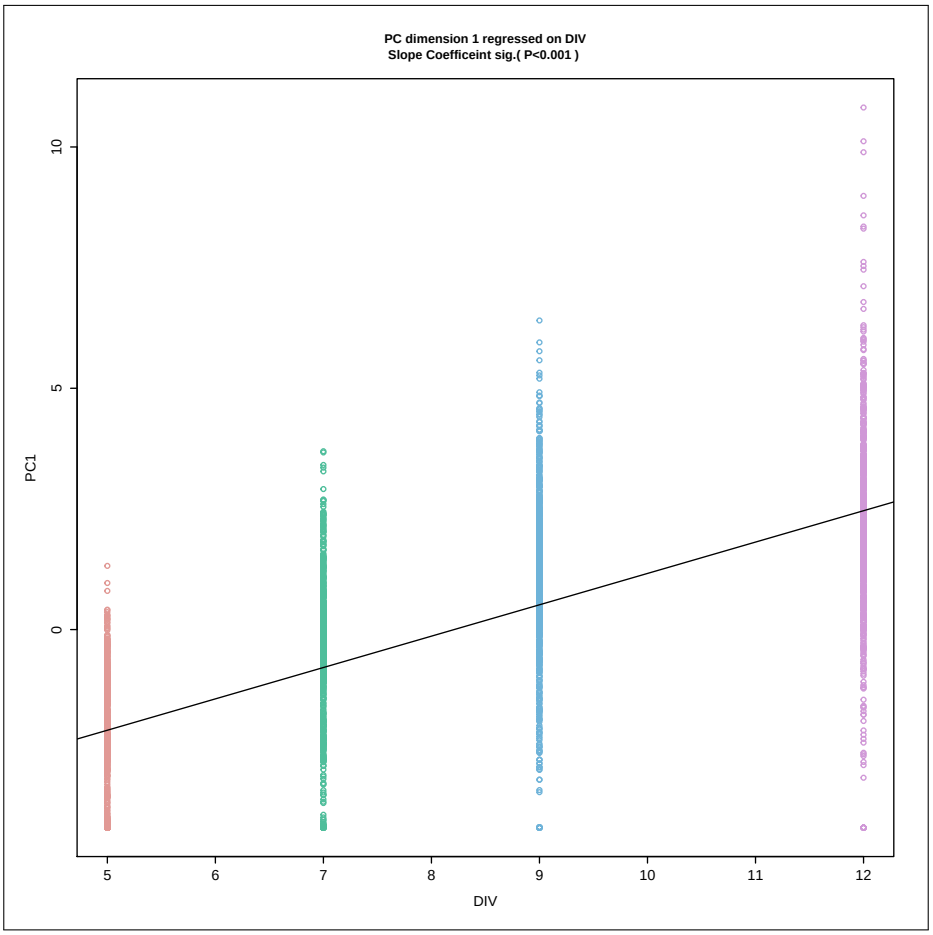

Figure 6: Scatter plot of projection of well level data onto first principle component (PC1), colored by DIV. Black line shows PC1 regressed on DIV, with slope coefficient p-value<0.001. PC1 increases significantly with increasing age of culture indicating.

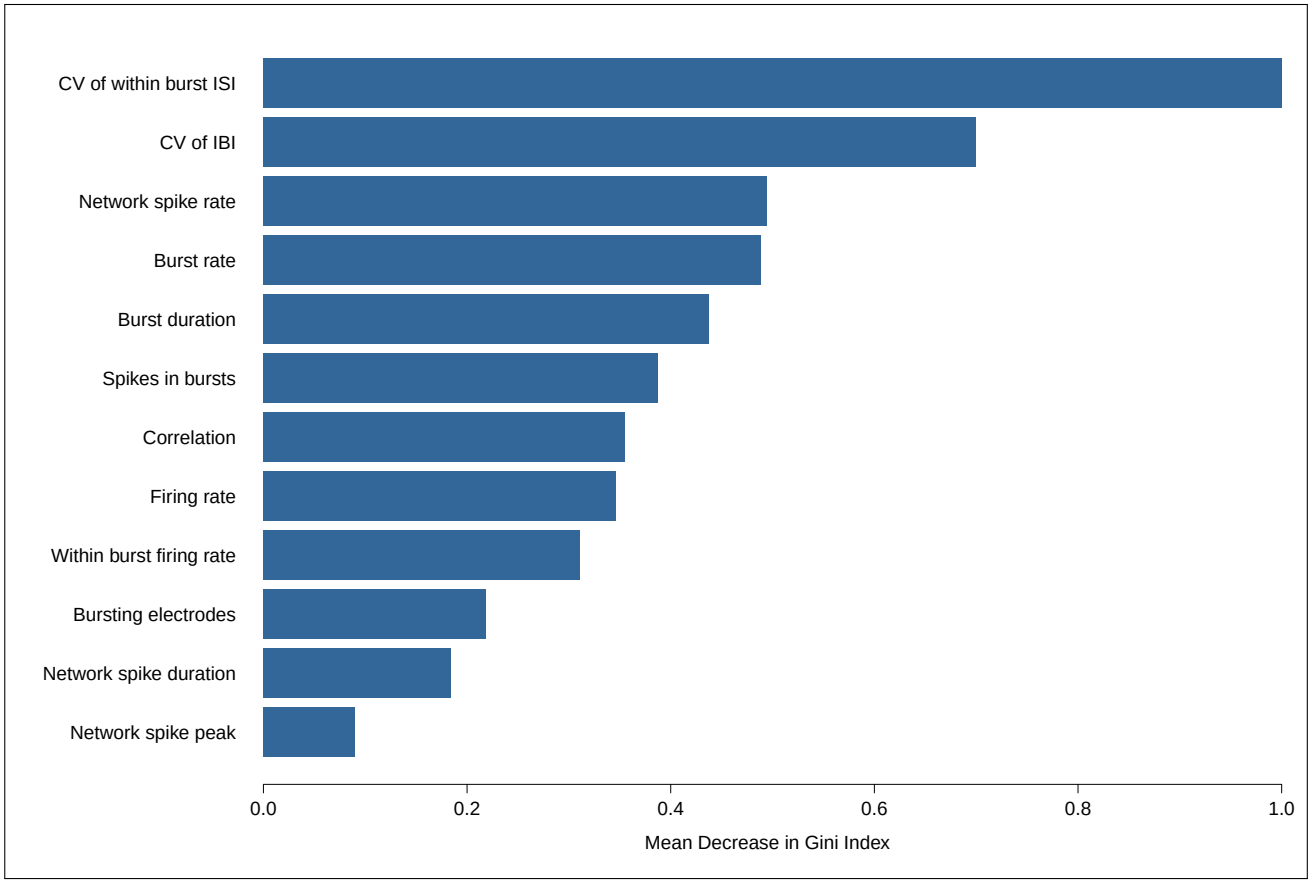

Figure 7: Average importance of features in driving classification, relative to the most important feature.

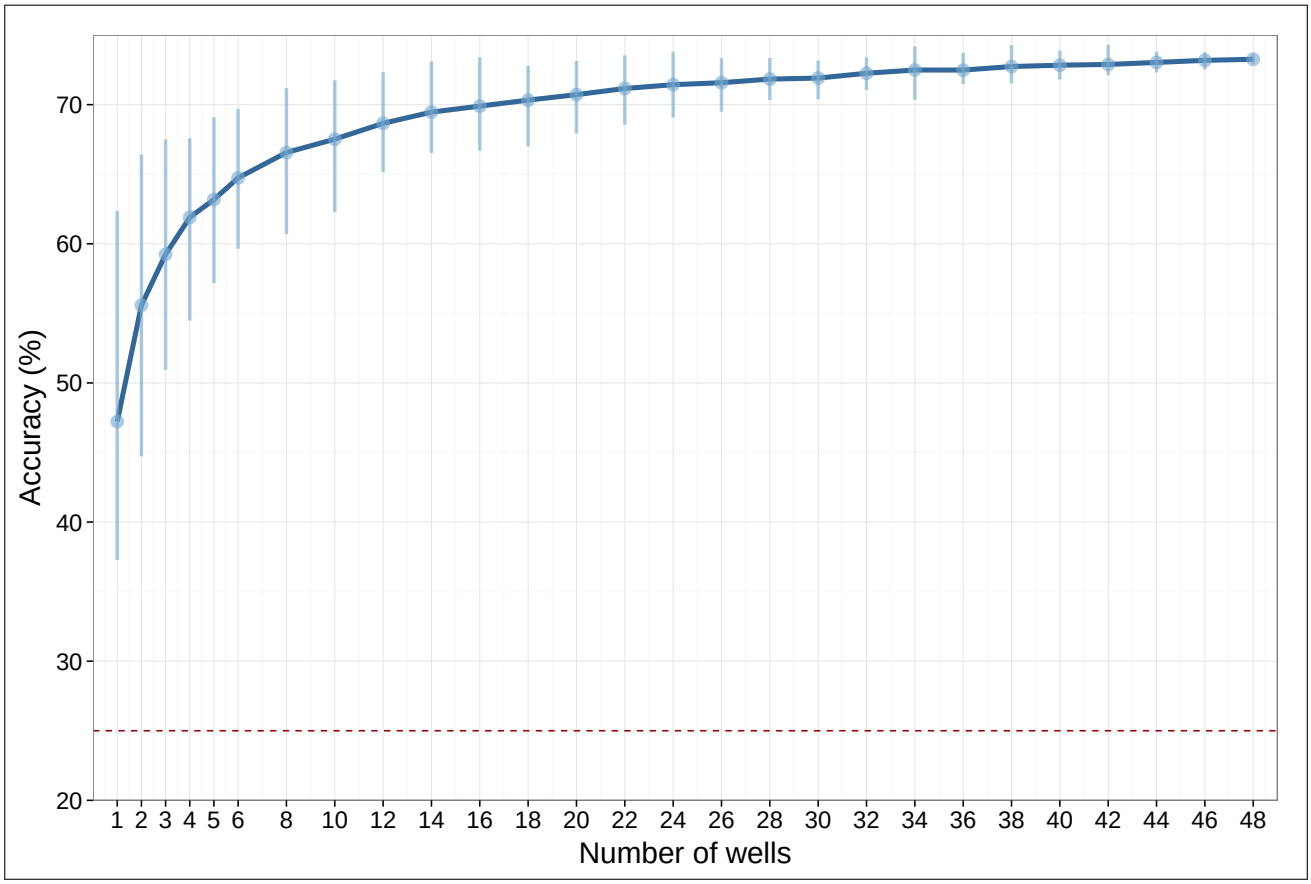

Figure 8: Accuracy of predicting the age of each well by sampling $n \leq 48$ wells on each plate. Dark blue line shows the mean accuracy, while the vertical lines show the minimum and maximum accuracy over 100 trials with random choices of wells. For $n = 48$, the error bars indicate the small variability in classification due to the partioning of data into train/test sets. The red dotted line indicates baseline level of performance (25%) for a classifier.

Feature	Description
Mean firing Rate	The mean firing rate (MFR) on each electrode was calculated. The well value was the median value of all active electrodes.
Burst rate	The number of bursts per minute on an electrode was calculated. The well value was the median value from all electrodes that exhibited bursting behaviour.
Burst duration	The mean duration of all bursts on an electrode over the recording period was calculated. The well value was the median value from all electrodes that exhibited bursting behaviour.
Fraction of bursting electrodes	An electrode was classified as bursting if the burst rate on the electrode was at least one per minute. The well value was the number of electrodes classified as bursting as a fraction of the total number of active electrodes on the well.
Within burst firing rate	The electrode value was the mean firing rate within all bursts on the electrode. The well value was the median value from all electrodes that exhibited bursting behaviour.
Percentage of spikes in bursts	The number of spikes on an electrode classified as being within bursts divided by the total number of spikes on the electrode. The well value was the median value from all electrodes that exhibited bursting behaviour.
CV of IBI	The ratio of the standard deviation to the mean of the length of all interburst intervals (IBI) on an electrode. The well value was the median value from all electrodes that exhibited bursting behaviour.
CV of within burst ISIs	The ratio of the standard deviation to the mean of the length of all interspike intervals (ISI) within bursts on an electrode. The well value was the median value from all electrodes that exhibited bursting behaviour.
Network spike rate	The well value was the number of network spikes on the well per minute of the recording period (See Methods section for definition of a Network Spike).
Network spike duration	The duration of a network spike was defined as the length of time during which the number of active electrodes on the well exceeded the threshold value (5). The well value was taken as the median duration of all network spikes on the well during the recording period.
Network spike peak	The maximum number of active electrodes during each network spike. The well value was taken as the median peak value of all network spikes on the well during the recording period.
Mean correlation	The correlation between every pairwise combination of electrodes on a well was calculated using the spike time tiling coefficient (Cutts & Eglen, 2015) with $\Delta t = 50ms$. (See Methods section for definition). The well value was the mean of the pairwise correlations between all distinct electrodes on the well.

Table 1: Features used in our analysis and a brief description of how they were calculated.

Feature	Accuracy %
CV of within burst ISI	49.2
CV of IBI	58.3
Network spike rate	62.0
Burst rate	65.0
Burst duration	66.0
% Spikes in bursts	68.3
Correlation	69.5
Firing rate	71.4
Within burst firing rate	72.7
Bursting electrodes	73.0
Network spike duration	73.5
Network spike peak	73.4

Table 2: Classifier performance at predicting the age of arrays. Features are listed in decreasing order of importance, and the value in each row $n = 1 \ldots 12$ is the mean percentage of correct classifications using the top n features. For example, row 4 shows that the classifier was 65.0% accurate at predicting age using the top four features.

Feature	**Accuracy %**					
	5 vs 7	5 vs 9	5 vs 12	7 vs 9	7 vs 12	9 vs 12
CV of within burst ISI	75.0	87.5	91.8	69.9	78.1	57.4
CV of IBI	77.4	89.5	93.6	76.8	85.5	64.7
Network spike rate	79.3	90.3	95.5	79.6	88.0	68.7
Burst rate	79.3	90.3	95.3	81.0	88.4	72.8
Burst duration	79.7	90.8	95.3	81.0	88.6	74.0
% Spikes in bursts	81.6	91.3	95.6	81.5	90.4	76.4
Correlation	82.2	92.1	95.6	82.3	90.9	77.1
Firing rate	82.3	91.7	95.7	82.3	90.9	80.2
Within burst firing rate	84.2	92.7	96.2	82.4	91.3	81.2
Bursting electrodes	83.6	92.5	96.2	82.6	91.8	81.5
Network spike duration	83.7	92.9	96.8	82.9	92.8	82.0
Network spike peak	82.2	92.5	96.8	82.6	93.0	82.1

Table 3: Classifier performance at predicting the age of arrays for each pairwise combination of ages (days in vitro). Features are listed in decreasing order of importance, and the value in each row $n = 1 \ldots 12$ is the mean percentage of correct classifications using the top n features, as described in Table 2.