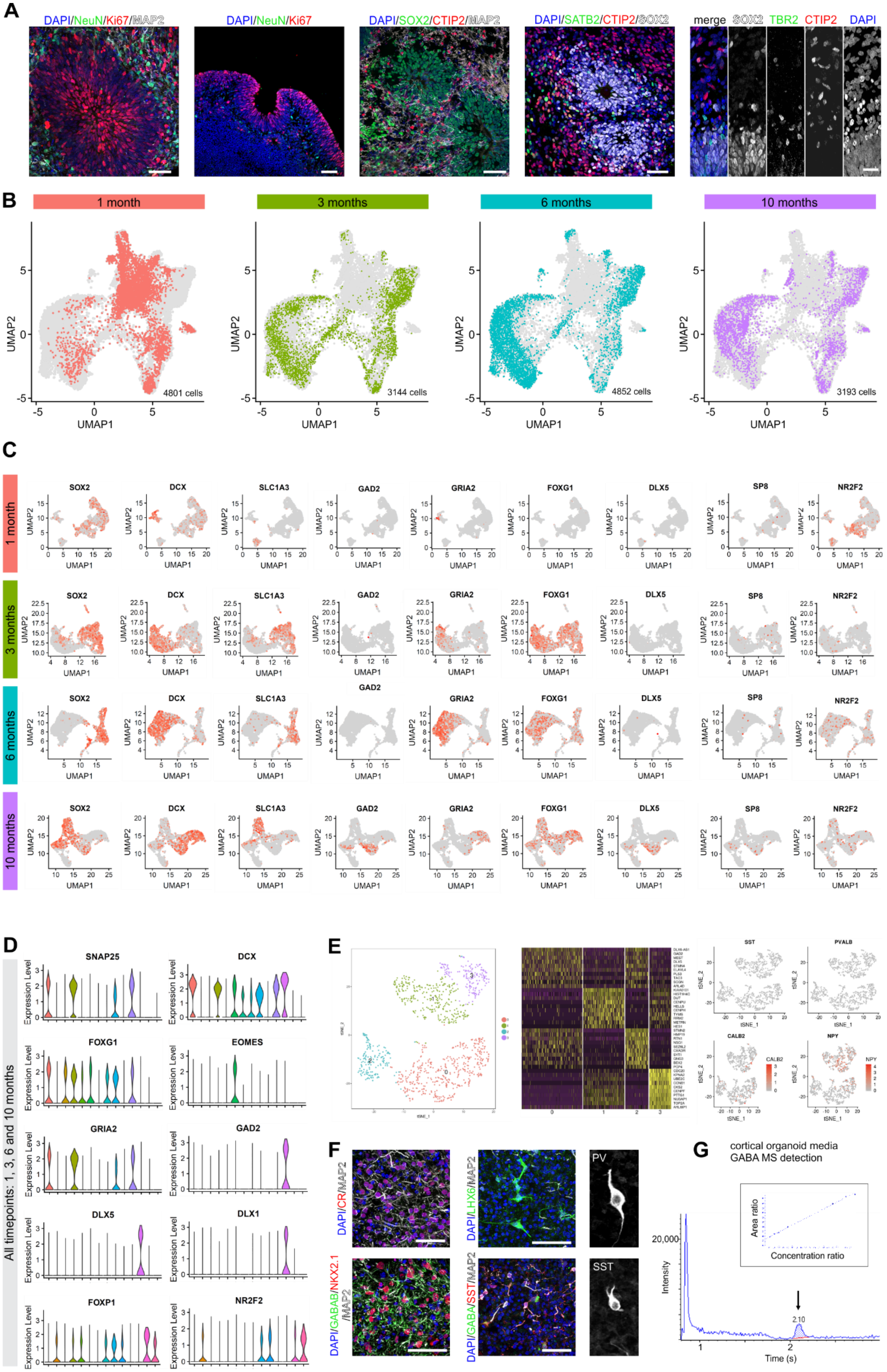

Figure S1. Cellular and molecular characterization of human cortical organoids. Related to Figure 1. (A)

Organoids are composed of a proliferative region surrounded by intermediate progenitor cells, cortical neurons. Scale bar, 50 μm. (B) UMAP plots highlighting time point specific cells. (C) UMAP plots for individual time point showing cell-type specific marker expression levels (D) Violin plots of marker gene expression across all clusters. (E) Subcluster analysis of GAD2 population from 10-month cortical organoids. (F) GABAergic neuronal markers expression of 10-month cortical organoids. (G) Detection of GABA neurotransmitter in the culture media using mass spectrometry. The average GABA concentration in the media was 0.028 ± 0.014 μM.

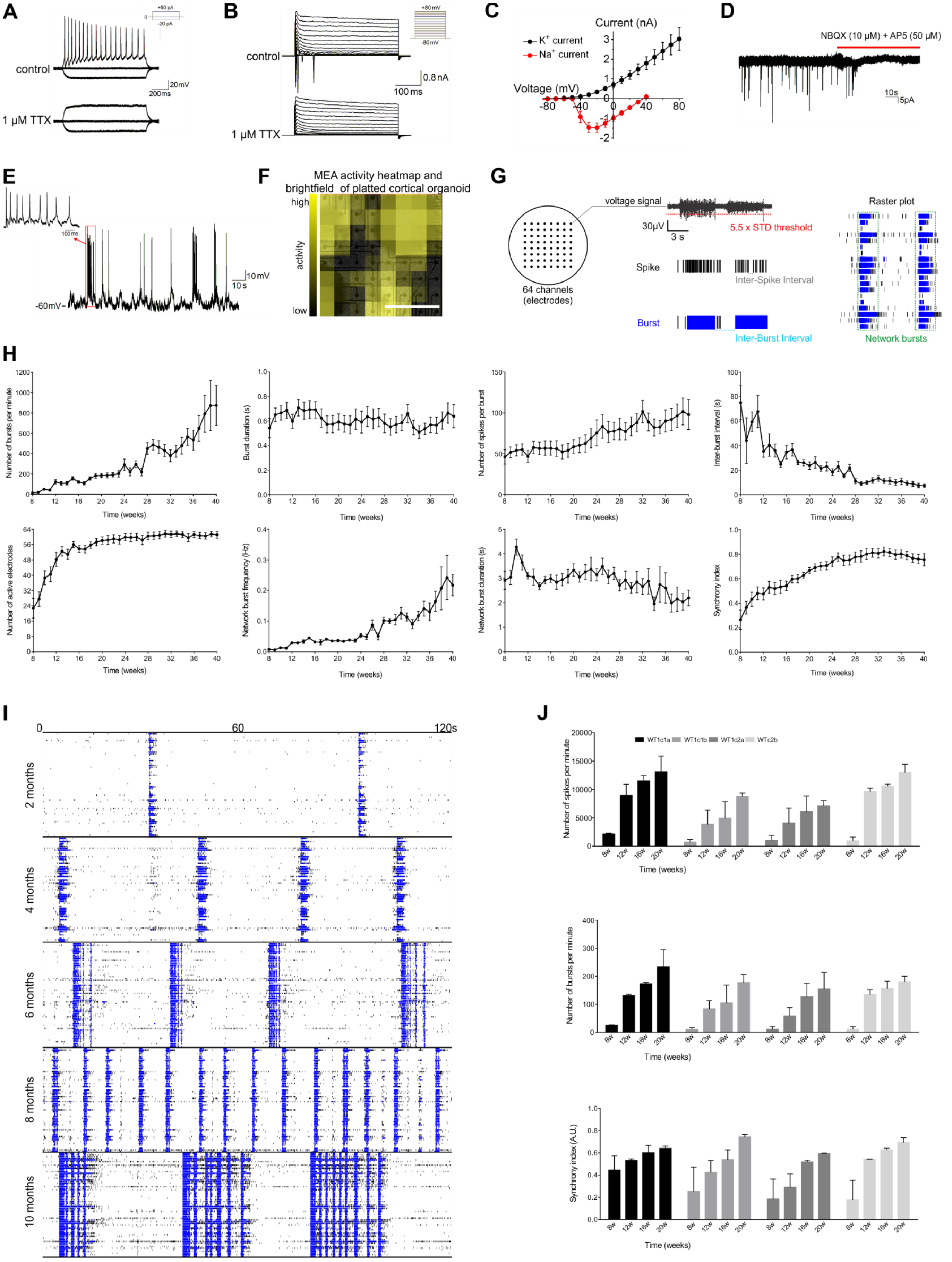

Figure S2. Long-term cortical organoid network activity. Related to Figure 2. (A) Electrophysiological characteristics of 6-month human iPSC-derived cortical organoids. Whole-cell current-clamp recording of a representative neuron from 6-month cortical organoids showing repetitive action potential firing in response to 50 pA current injection from a resting membrane potential of -63 mV. Application of 1 µM TTX abolished the firing (lower panel). (B) Whole-cell voltage-clamp recording in the same neuron showing voltage-gated K^+ current and TTX-sensitive Na^+ current, elicited from a holding potential of -80mV to the indicated voltages. (C) Plot of the peak current sizes of K^+ channels and Na^+ channels as a function of voltage determined from neurons of 6-month cortical organoids (peak INa size = -1466.86 ± 575.18 pA. Peak IK size = 3031.79 ± 1405.19 pA. *n = 6* neurons). (D) Voltage-clamp recording at -60 mV exhibiting spontaneous excitatory postsynaptic currents (sEPSCs) in another representative neuron (frequency of sEPSCs = 0.25 ± 0.10 Hz; *n = 5* neurons. Amplitude of sEPSCs = -19.92 ± 5.90 pA; *n = 5* neurons; we observed sEPSCs in 84% of the tested neurons). Application of NBQX and AP5 fully inhibited the sEPCS. (E) Representative traces showing that a human iPSC-derived neuron displays spontaneous AP firing (AP firing frequency = 13.67 ± 1.11 Hz; *n = 6* neurons). The data are shown as mean ± s.e.m. (F) Representative activity heatmap and bright-field image of cortical organoids on the MEA plate. (G) Schematic representation of the electrical activity features analyzed from the MEA recordings. Each bar represents a spike; and a spike cluster (in blue) represents a burst. Bursts occurring at the same time in different channels characterize a network burst. The synchrony index is based on the cross-correlogram and represents a measure of similarity between two spike trains. (H) Temporal evolution of network activity characterized by different parameters. (I) Raster plots illustrating the development of network activity. (J) Consistent and reproducible development of electrical activity in cortical organoids over time. The data are shown as mean ± s.e.m (*n = 8*, independent experiments performed in duplicates using two clones of an iPSC line).

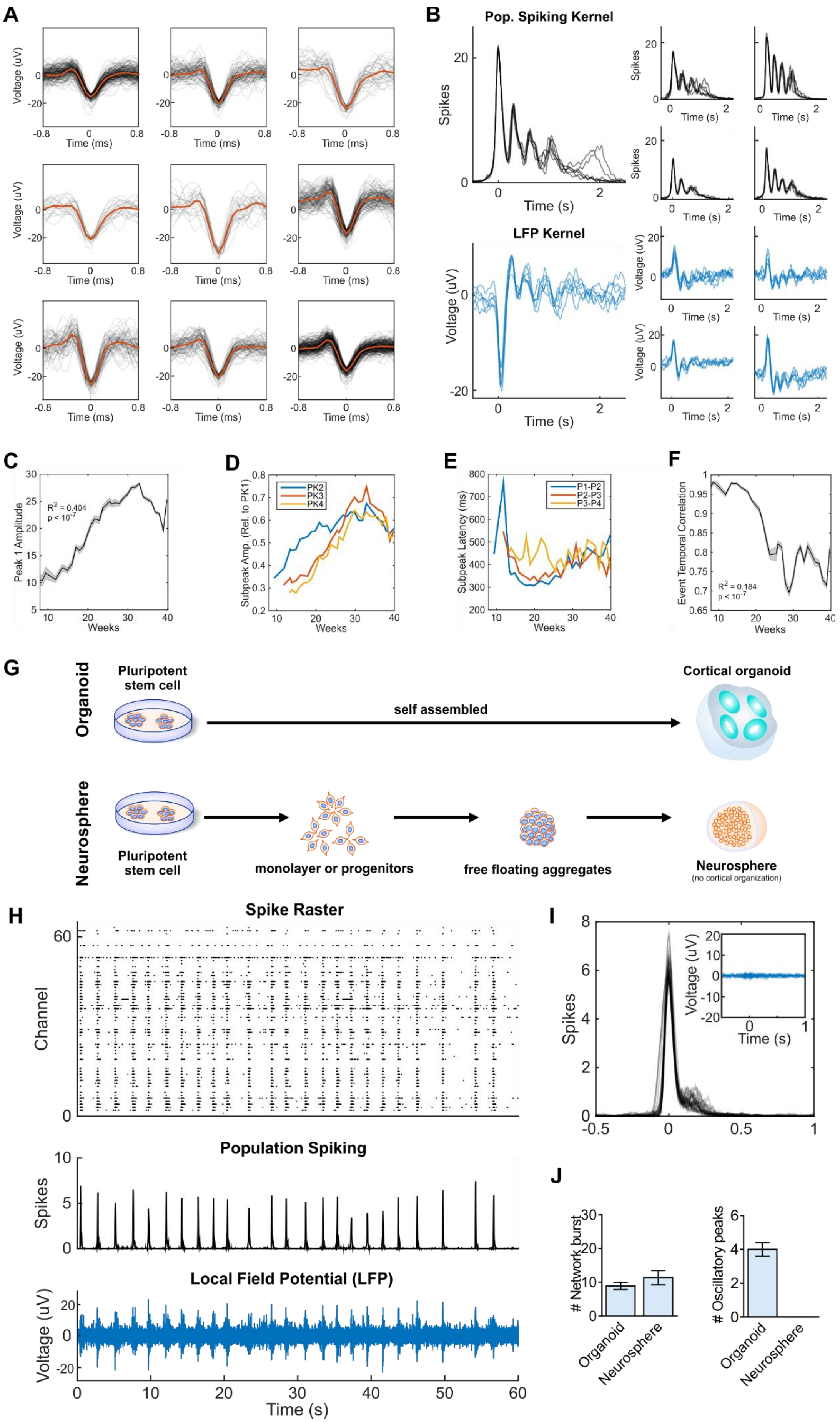

Figure S3. Extended characterization of cortical organoid network electrophysiology. Related to Figure

2. (A) Spikes detected on 9 channels. Black traces represent single spikes, blue and red traces represent the average of positive and negative spikes, respectively. Spike trains are not sorted for their polarity in the subsequent analyses, as total population spiking is the main feature of interest. (B) Representative oscillatory network events. Each overlapping trace represents a single occurrence of an event recorded on the same channel. LFP polarity of events differs between channels due to the spatial configuration of cells around the electrode. (C) Event onset peak (Peak 1) increases in amplitude until 30 weeks, while (D) subpeak amplitude continues to increase (for the 2nd-4th peak) throughout development. (E) Subsequent peaks occur with a consistent latency of ~400 ms after the previous peak, particularly for Peak 3 and 4. (F) Temporal similarity of network events during the 3-s window is high at early time points, but decreases with development, acquiring more variable dynamics within an event. The data showed in C and F are presented as mean ± s.e.m., linear (C, F) model regression. (G) Comparison of the protocol for neurosphere and cortical organoid generation. (H) Network-wide giant depolarizing potentials occur in neurosphere at a similar rate to those found in organoids recordings, and visible perturbations are observed in the LFP trace. However, the network recruitment in neurospheres is lower with significantly shorter events. Coherent low-frequency depolarizations are observed in filtered LFP events, but with much lower amplitude when scaled to the same range as those recorded from organoids (I, J).

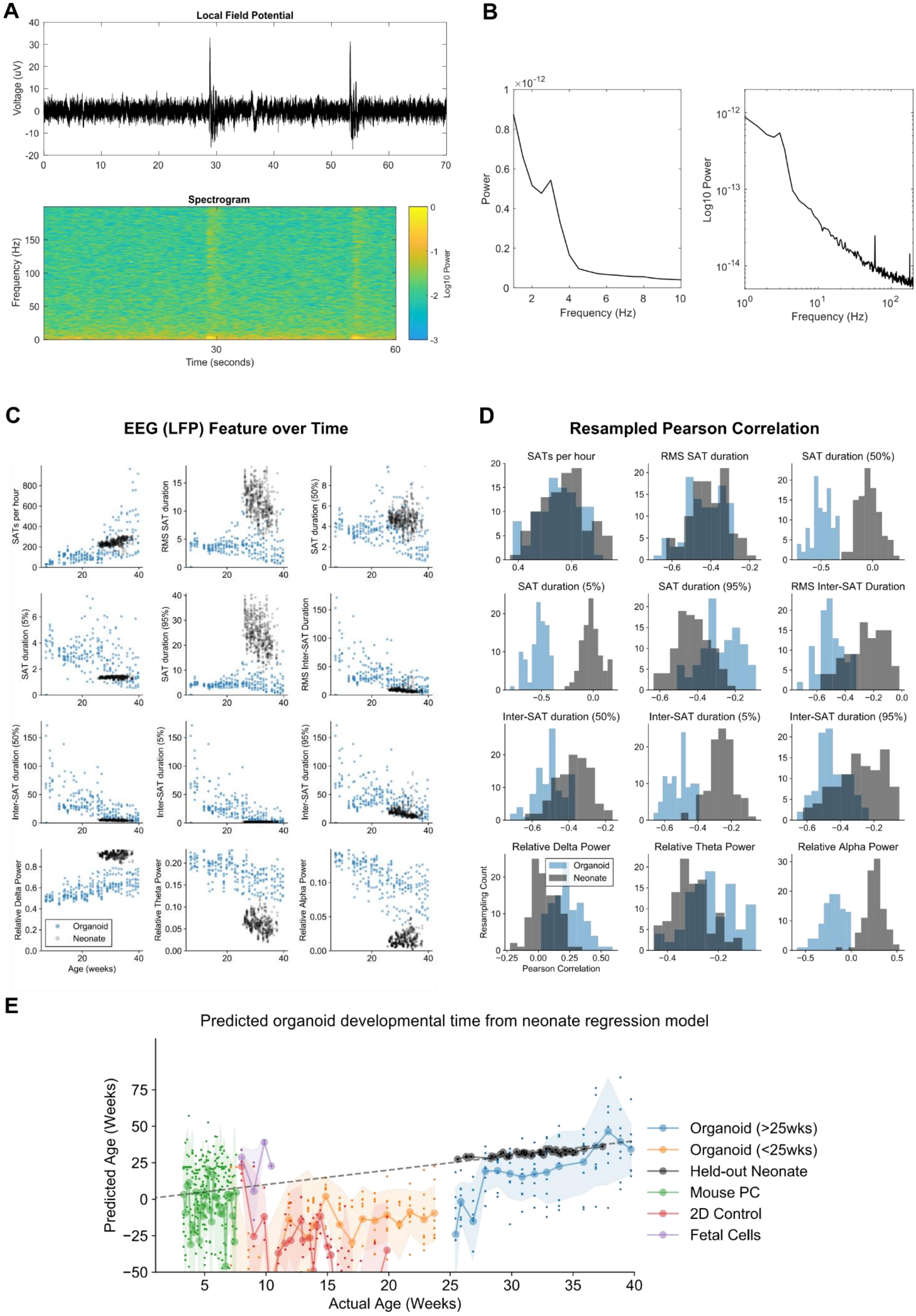

Figure S4. Network activity in cortical organoids and oscillatory features in the developing human brain.

Related to Figure 4. (A, B) Time-frequency and spectral representation of time series data from a 6-month cortical organoid, demonstrating oscillatory phenomenon. Spectrogram (A) of organoid LFP shows bursts of activity localized at low frequencies, as well as 100Hz and beyond, while power spectral density (PSD, B) displays canonical *1/f* power law decay and a narrow oscillatory peak at 3 Hz. (C) Comparison of preterm neonate EEG and cortical organoid features over time. For included EEG features, see Table S2. (D) Distributions of resampled Pearson correlation coefficients between feature and age for preterm neonate and organoid. (E) Model-predicted developmental time (y-axis, age in weeks) follows actual weeks-in-culture (x-axis) for organoids (orange and blue), as well as true age of held-out preterm neonate data points (black), excluding spectral features. Dashed line represents unity, signifying perfect prediction. Large circles on solid lines and shaded regions denote mean ± std of prediction, respectively, while dots indicate per-sample prediction ($n = 8$ for organoids at all time points). The unnormalized feature weights are: Constant: 53.93093; SATs per hour: 0.05791; RMS SAT duration: 0.17439; SAT duration (50%): 0.46857; SAT duration (5%): -1.59115; SAT duration (95%): -0.17140; RMS Inter-SAT Duration: 1.01745; Inter-SAT duration (50%): -1.67926; Inter-SAT duration (5%): 0.00000; Inter-SAT duration (95%): -0.24631; Relative Delta Power: -31.94628; Relative Theta Power: -39.72896; and Relative Alpha Power: 30.92235.

SUPPLEMENTAL TABLES

Supplemental Table 1. Top expressed genes of each cell cluster. Related to Figure 1.

cluster	gene	avg_logFC	pct.1	pct.2	p_val_adj
GABAergic Neurons	DLX5	1.17944	0.403	0.362	0.47966
GABAergic Neurons	DLX6-AS1	0.97634	0.75	0.396	5.59E-81
GABAergic Neurons	SEZ6L2	0.96254	0.945	0.713	2.58E-22
GABAergic Neurons	SYT1	0.91593	0.947	0.814	2.55E-20
GABAergic Neurons	CHCHD2	0.84772	0.468	0.45	0.01172
GABAergic Neurons	HMP19	0.84097	0.734	0.755	0.17341
GABAergic Neurons	ARL4D	0.82671	0.413	0.485	1
GABAergic Neurons	INSM1	0.82003	0.813	0.444	2.87E-57
GABAergic Neurons	DLX2	0.81130	0.361	0.336	2.48E-06
GABAergic Neurons	SCG3	0.78232	0.761	0.732	0.03532
GABAergic Neurons	RTN3	0.78167	0.958	0.907	1.17E-12
GABAergic Neurons	NSG1	0.76102	0.876	0.742	3.63E-08
GABAergic Neurons	DCX	0.75629	0.782	0.736	1.97E-27
GABAergic Neurons	TERF2IP	0.74991	0.95	0.851	4.66E-28
GABAergic Neurons	PAFAH1B3	0.74128	0.937	0.853	9.39E-21
GABAergic Neurons	YWHAQ	0.74116	0.971	0.921	2.55E-53
GABAergic Neurons	DAAM1	0.73920	0.913	0.765	5.32E-10
GABAergic Neurons	TAGLN3	0.72672	0.905	0.774	6.58E-05
GABAergic Neurons	TTC3	0.71927	0.976	0.909	8.55E-16
GABAergic Neurons	STMN2	0.69866	0.905	0.892	8.26E-52
GABAergic Neurons	TAC3	0.69627	0.342	0.404	1.99E-12
GABAergic Neurons	CD24	0.67659	0.95	0.808	6.11E-12
GABAergic Neurons	TXNIP	0.67286	0.939	0.832	1.04E-05
GABAergic Neurons	STMN4	0.66186	0.934	0.885	0.04858
GABAergic Neurons	NREP	0.64996	0.918	0.802	0.00264
GABAergic Neurons	RAB3A	0.63717	0.937	0.771	1.01E-12
GABAergic Neurons	SCGN	0.63426	0.216	0.15	1.62E-28
GABAergic Neurons	SVBP	0.63364	0.932	0.717	1.74E-06
GABAergic Neurons	BEX1	0.62955	0.821	0.835	1.69E-37
GABAergic Neurons	DSTN	0.62593	0.955	0.894	2.21E-24
GABAergic Neurons	GAD1	0.61455	0.679	0.33	1.30E-41
GABAergic Neurons	PROX1	0.61165	0.121	0.332	4.76E-93
GABAergic Neurons	HN1	0.60568	0.963	0.936	6.77E-94
GABAergic Neurons	DCLK1	0.60078	0.368	0.657	2.00E-22
Glutamatergic Neurons	NEUROD6	1.49931	0.9	0.771	0
Glutamatergic Neurons	BHLHE22	1.46368	0.905	0.793	0
Glutamatergic Neurons	STMN2	1.43649	0.99	0.834	0
Glutamatergic Neurons	GRIA2	1.39439	0.829	0.782	0
Glutamatergic Neurons	NEUROD2	1.35791	0.893	0.827	0
Glutamatergic Neurons	SNAP25	1.24934	0.803	0.764	0
Glutamatergic Neurons	TTC9B	1.20497	0.811	0.771	0
Glutamatergic Neurons	SYT4	1.11807	0.762	0.803	0
Glutamatergic Neurons	SNCA	1.10911	0.829	0.643	0
Glutamatergic Neurons	HMP19	1.10021	0.757	0.753	0
Glutamatergic Neurons	LY6H	1.09133	0.778	0.548	0
Glutamatergic Neurons	RAB3A	1.08320	0.857	0.726	0
Glutamatergic Neurons	INA	1.08214	0.777	0.777	0
Glutamatergic Neurons	GAP43	1.06736	0.943	0.731	0
Glutamatergic Neurons	HPCA	1.03546	0.73	0.624	0
Glutamatergic Neurons	CXADR	1.03219	0.862	0.651	0
Glutamatergic Neurons	TSPAN13	1.02606	0.809	0.57	0
Glutamatergic Neurons	CD24	1.01863	0.91	0.752	0
Glutamatergic Neurons	SYT1	1.00629	0.891	0.773	0
Glutamatergic Neurons	MAPT	1.00501	0.737	0.688	0
Glutamatergic Neurons	DCX	1.00326	0.865	0.661	0
Glutamatergic Neurons	RTN1	0.98969	0.935	0.718	0
Glutamatergic Neurons	NSG1	0.96682	0.796	0.716	0
Glutamatergic Neurons	SCG3	0.95190	0.726	0.736	0
Glutamatergic Neurons	CELF4	0.94679	0.671	0.721	1.75E-274
Glutamatergic Neurons	CRMP1	0.91715	0.878	0.727	0
Glutamatergic Neurons	LMO3	0.90245	0.712	0.805	1.51E-267
Glutamatergic Neurons	NELL2	0.90035	0.749	0.643	0
Glia	KIAA0101	1.09206	0.638	0.486	2.16E-187

Glia	TTYH1	1.07238	0.948	0.689	0.00E+00
Glia	SLC1A3	1.04241	0.855	0.621	0.00E+00
Glia	MT2A	1.00780	0.89	0.624	0.00E+00
Glia	SFRP1	1.00450	0.938	0.684	0.00E+00
Glia	SOX2	1.00279	0.946	0.643	0.00E+00
Glia	HES1	0.99522	0.832	0.59	0.00E+00
Glia	ID4	0.92316	0.912	0.687	0.00E+00
Glia	CLU	0.91605	0.978	0.732	0.00E+00
Glia	PEA15	0.89375	0.917	0.62	0.00E+00
Glia	HOPX	0.86840	0.818	0.622	0.00E+00
Glia	PMP2	0.78593	0.775	0.572	5.97E-304
Glia	METRN	0.76712	0.798	0.583	0.00E+00
Glia	ZFP36L1	0.74666	0.73	0.539	3.48E-258
Glia	PTN	0.74622	0.996	0.894	0.00E+00
Glia	IFI44L	0.74479	0.79	0.595	0.00E+00
Glia	CDO1	0.74388	0.869	0.631	0.00E+00
Glia	VIM	0.73690	0.993	0.858	0.00E+00
Glia	CENPH	0.71893	0.588	0.58	8.18E-45
Glia	HSPB1	0.71396	0.904	0.624	0.00E+00
Glia	PDLIM3	0.71318	0.807	0.62	0.00E+00
Glia	FGFBP3	0.68433	0.797	0.632	0.00E+00
Glia	FAM107A	0.68371	0.748	0.593	1.17E-226
Glia	C8orf4	0.68118	0.668	0.564	2.34E-135
Glia	B2M	0.67571	0.897	0.569	0.00E+00
Glia	PON2	0.67504	0.671	0.426	2.30E-204
Glia	STXBP6	0.66039	0.732	0.638	1.59E-177
Glia	SOX3	0.65821	0.76	0.435	0.00E+00
Glia	HMGB2	0.65603	0.824	0.572	0.00E+00
Glia	QKI	0.64190	0.775	0.611	1.01E-217
Glia	SRI	0.64014	0.899	0.654	0.00E+00
Glia	PHGDH	0.63803	0.766	0.615	1.70E-216
Glia	APOE	0.61466	0.78	0.575	6.86E-190
Glia	FOS	0.60483	0.777	0.642	7.93E-168
Glia	PSAT1	0.60446	0.788	0.598	1.05E-253
Glia	C1orf61	0.60285	0.976	0.863	0.00E+00
Glia	DBI	0.60169	0.932	0.781	0.00E+00
Intermediate Progenitors	EOMES	1.14946	0.697	0.545	1.50E-123
Intermediate Progenitors	TAC3	1.12454	0.535	0.392	3.21E-41
Intermediate Progenitors	NHLH1	1.08712	0.766	0.53	4.67E-254
Intermediate Progenitors	GADD45G	1.00439	0.794	0.619	5.82E-155
Intermediate Progenitors	ELAVL2	0.88637	0.828	0.724	1.16E-130
Intermediate Progenitors	NNAT	0.88553	0.911	0.841	8.74E-188
Intermediate Progenitors	RND3	0.64990	0.54	0.47	3.81E-21
Intermediate Progenitors	NEUROG1	0.62298	0.59	0.585	1.87E-10
Intermediate Progenitors	GDAP1L1	0.62253	0.757	0.671	1.08E-57
Intermediate Progenitors	TAGLN3	0.60150	0.856	0.77	1.68E-98
Early Progenitors	IGFBP7	0.89242	0.559	0.394	1.49E-30
Early Progenitors	TTR	0.84753	0.844	0.49	0
Early Progenitors	S100B	0.74367	0.833	0.481	0
Early Progenitors	SPARCL1	0.61008	0.928	0.434	0
Early Progenitors	TPPP3	0.60842	0.539	0.293	5.00E-23
Early Progenitors	CA2	0.58266289	0.614	0.403	4.70E-69
Early Progenitors	RBP1	0.56764771	0.879	0.438	0
Early Progenitors	TRPM3	0.56440266	0.768	0.324	0
Early Progenitors	CD9	0.54907859	0.743	0.263	0
Early Progenitors	ID3	0.54768285	0.889	0.558	0
Early Progenitors	CXCL14	0.53064106	0.567	0.402	1.01E-10
Early Progenitors	ID1	0.51512222	0.809	0.495	0
Mitotic Cells	UBE2C	2.08768	0.999	0.38	0
Mitotic Cells	TOP2A	1.92072	0.988	0.456	0
Mitotic Cells	CDC20	1.88666	0.969	0.294	0
Mitotic Cells	CENPF	1.83910	0.993	0.513	0
Mitotic Cells	NUSAP1	1.79161	0.985	0.531	0
Mitotic Cells	PTTG1	1.77245	1	0.532	0
Mitotic Cells	CCNB2	1.73661	0.956	0.298	0
Mitotic Cells	CCNB1	1.71891	0.939	0.362	0
Mitotic Cells	CDK1	1.68391	0.981	0.507	0
Mitotic Cells	ASPM	1.59134	0.939	0.436	0
Mitotic Cells	BIRC5	1.58211	0.963	0.37	0

Mitotic Cells	TPX2	1.55923	0.94	0.524	0
Mitotic Cells	PLK1	1.51556	0.88	0.386	2.04E-293
Mitotic Cells	MAD2L1	1.51083	0.967	0.555	0
Mitotic Cells	FAM64A	1.51073	0.91	0.313	0
Mitotic Cells	CKS2	1.50084	0.997	0.645	0
Mitotic Cells	PBK	1.47827	0.917	0.414	0
Mitotic Cells	CCNA2	1.46832	0.92	0.536	0
Mitotic Cells	GTSE1	1.44523	0.899	0.306	0
Mitotic Cells	AURKA	1.43015	0.876	0.399	1.86E-293
Mitotic Cells	CDKN3	1.40121	0.859	0.321	1.75E-264
Mitotic Cells	AURKB	1.39178	0.901	0.299	0
Mitotic Cells	NUF2	1.38631	0.898	0.478	0
Mitotic Cells	KPNA2	1.38186	0.982	0.578	0
Mitotic Cells	SMC4	1.37867	0.955	0.493	0
Mitotic Cells	HMGB2	1.36699	1	0.612	0
Mitotic Cells	CDCA3	1.36522	0.854	0.425	5.74E-253
Mitotic Cells	PSRC1	1.34100	0.914	0.501	2.10E-296
Mitotic Cells	CENPA	1.32905	0.869	0.291	0
Mitotic Cells	CKS1B	1.32614	0.971	0.525	0
Other	LGALS1	1.34401	0.97	0.479	1.62E-80
Other	IGF2	1.26378	0.955	0.402	7.22E-142
Other	COL3A1	1.25618	0.973	0.439	1.48E-136
Other	RBP1	1.14081	0.967	0.531	1.32E-123
Other	MGP	1.00395	0.602	0.45	5.94E-05
Other	DCN	0.95928	0.554	0.484	0.56345
Other	IFITM3	0.91015	0.708	0.362	5.14E-36
Other	COL1A1	0.89389	0.545	0.363	1
Other	SPARC	0.83734	0.916	0.663	2.93E-66
Other	APOE	0.81812	0.88	0.617	7.53E-38
Other	ANXA1	0.80045	0.867	0.335	6.95E-109

Supplemental Table 2. Electrophysiological features in preterm neonatal EEG dataset and analogous features computed in organoid LFP. Related to Figure 4.

Neonatal EEG features	Computed organoid LFP features
Envelope (50%)	None
Envelope (5%)	None
Envelope (95%)	None
rEEG (50%)	None
rEEG (5%)	None
rEEG (95%)	None
SATs per hour	Network Events per hour
RMS SAT duration	RMS network event duration
SAT duration (50%)	Network event duration (50%)
SAT duration (5%)	Network event duration (5%)
SAT duration (95%)	Network event duration (95%)
RMS Inter-SAT Duration	RMS Inter-event Duration
Inter-SAT duration (50%)	Inter-event duration (50%)
Inter-SAT duration (5%)	Inter-event duration (5%)
Inter-SAT duration (95%)	Inter-event duration (95%)
Temporal Theta Power	None
Activation Synchrony Index	None
Interhemispheric Correlation	None
Total Spectral Power	None
Relative Delta Power	Relative Delta Power
Relative Theta Power	Relative Theta Power
Relative Alpha Power	Relative Alpha Power
Relative Beta Power	Relative Beta Power

Shaded cells indicate features used in the development time prediction model.