Supplementary Information

Probing impaired neurogenesis in human brain organoids exposed to alcohol

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Figure S1. Representative bright field images of brain organoids with ethanol exposure from day 12 to 21. Brain organoids exhibits decreased growth in the presence of EtOH. Scale bars: 1 mm.



Figure S2. Validation of the RNA-seq results by quantitative real-time PCR (qPCR). The expression profiles of 5 UP and 1 DOWN genes were analyzed by qPCR to validate the RNA-seq results. The expression value was normalized to the Actin B expression level. Values are represented as average mRNA expression \pm SD bars, n = 3 replicates. *, *p*-value<0.05, **, *p*-value <0.01.



Figure S3. Bright field images tracing of neurite projected from brain slices in the presence or absence of EtOH for

96 hrs. Highly abundant neurites were projected after brain slices were attached to the plate. Scale bars: 200 μ m.

Rank	Up-Gene	log2Ratio	Rank	Down-Gene	log2Ratio
1	HBA1	9.75656	1	GSX2	-6.22882
2	HBE1	3.56098	2	RAX	-3.86701
3	MSTN	3.42325	3	VSX2	-3.69758
4	HBG2	3.26342	4	SCGN	-3.59108
5	SNCG	3.10385	5	FZD5	-3.53464
6	SMIM11A	2.93299	6	ALDH1A3	-3.18304
7	RSPO2	2.78513	7	BEGAIN	-3.18049
8	PPP1R17	2.65948	8	SIX6	-3.09814
9	TLX3	2.58538	9	SIX3	-2.79664
10	PCDH15	2.54825	10	DLK1	-2.60958
11	LYPD1	2.45462	11	ME3	-2.49226
12	SCRG1	2.40541	12	FEZF1	-2.39958
13	GPR39	2.40242	13	LAMP5	-2.25808
14	BARHL1	2.37470	14	FAM69C	-2.25194
15	OLFM3	2.36109	15	BLACAT1	-2.19851
16	NTRK1	2.30289	16	FZD8	-2.06637
17	POU4F1	2.29528	17	SOX21	-2.04633
18	CBLN1	2.28060	18	PRSS23	-2.03345
19	SYT4	2.27793	19	SHISA2	-1.97649
20	NEUROD1	2.26758	20	ATP6V1B1	-1.95088

 Table S1. Significantly altered genes in brain organoids exposed to ethanol.