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Supplementary information

Human brain organoid-on-a-chip to model prenatal nicotine exposure

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The authors have no conflicts of interest to declare.

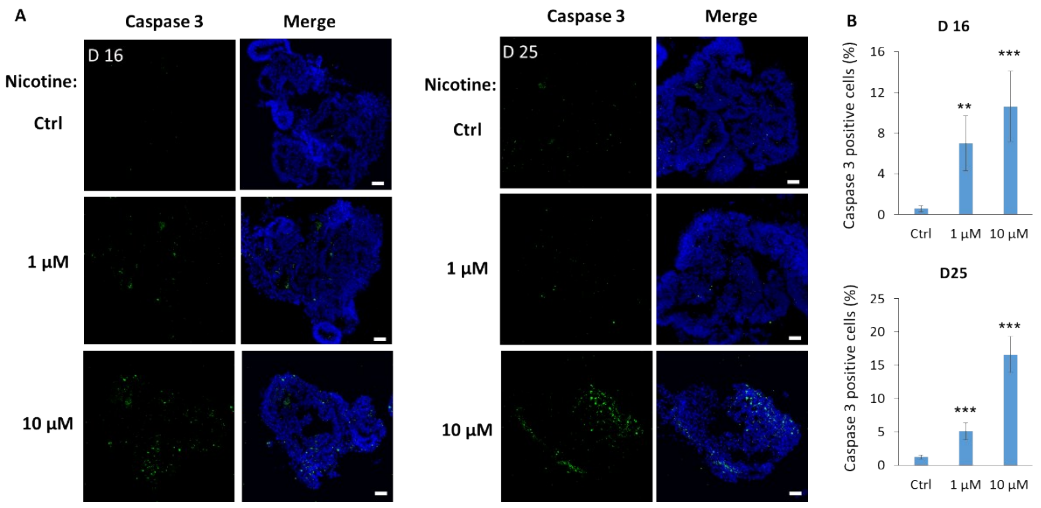

Fig. S1 Assessment of the cell apoptosis in brain organoids after nicotine exposure under different conditions. (A) The apoptosis cells were identified by immunohistochemical staining for active Caspase 3 in brain organoids treated with and without nicotine (1 μM and 10 μM) on day 16 and day 25. DAPI marks nuclei (blue). Scale bars, 50 μm. (B) The percentage of Caspase 3 positive cells were quantified by Image Pro Plus 6.0 software. The data represent means of 6 replicates ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$, Student's *t*-test.

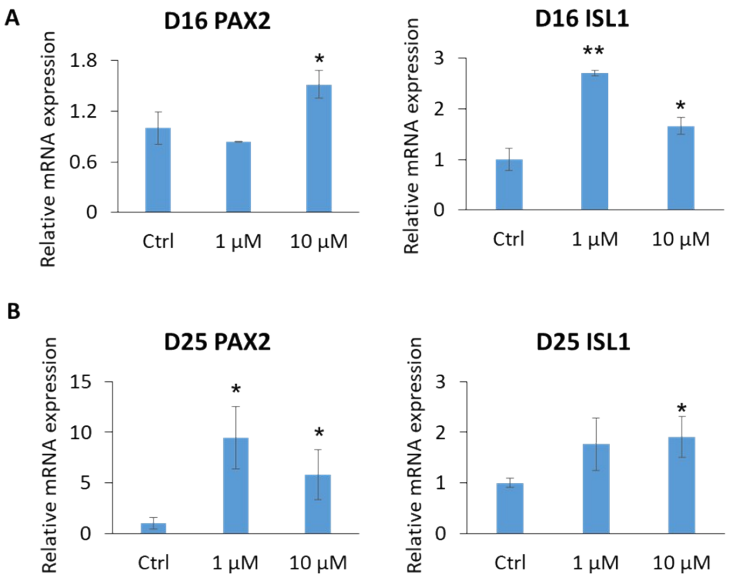

Fig. S2 Examination of the expression of hindbrain markers (PAX2, ISL1) in nicotine-exposed brain organoids by real-time PCR. (A-B) Expression of PAX2 and ISL1 in brain organoids in the presence or absence of nicotine on day 16 (A) and day

25 (B). The data shown are fold changes of genes expression in brain organoids with nicotine treatment versus that without nicotine treatment. The β-actin expression was used for normalization. Data are mean ± SD. Student's *t*-test, *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.