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Supplemental Information

Inhibition of *Gata4* and *Tbx5* by Nicotine-Mediated DNA Methylation in Myocardial Differentiation

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Supplemental Figures and Legends

Figure S1. Effect of nicotine on embryonic body formation and cell viability (Related to Figure 1).

To test the effect of nicotine on cardiac differentiation, embryonic bodies (EBs) developed from mESCs were treated with a serial dosage of nicotine from 0.01-10 μM during hanging drop culture and differentiation. EB formation and cell viability were analyzed. The results showed that nicotine has no effect on either EB formation or cell viability. Instead, MTT assay revealed that 10 μM nicotine treatment slightly increased cell numbers at day 3.

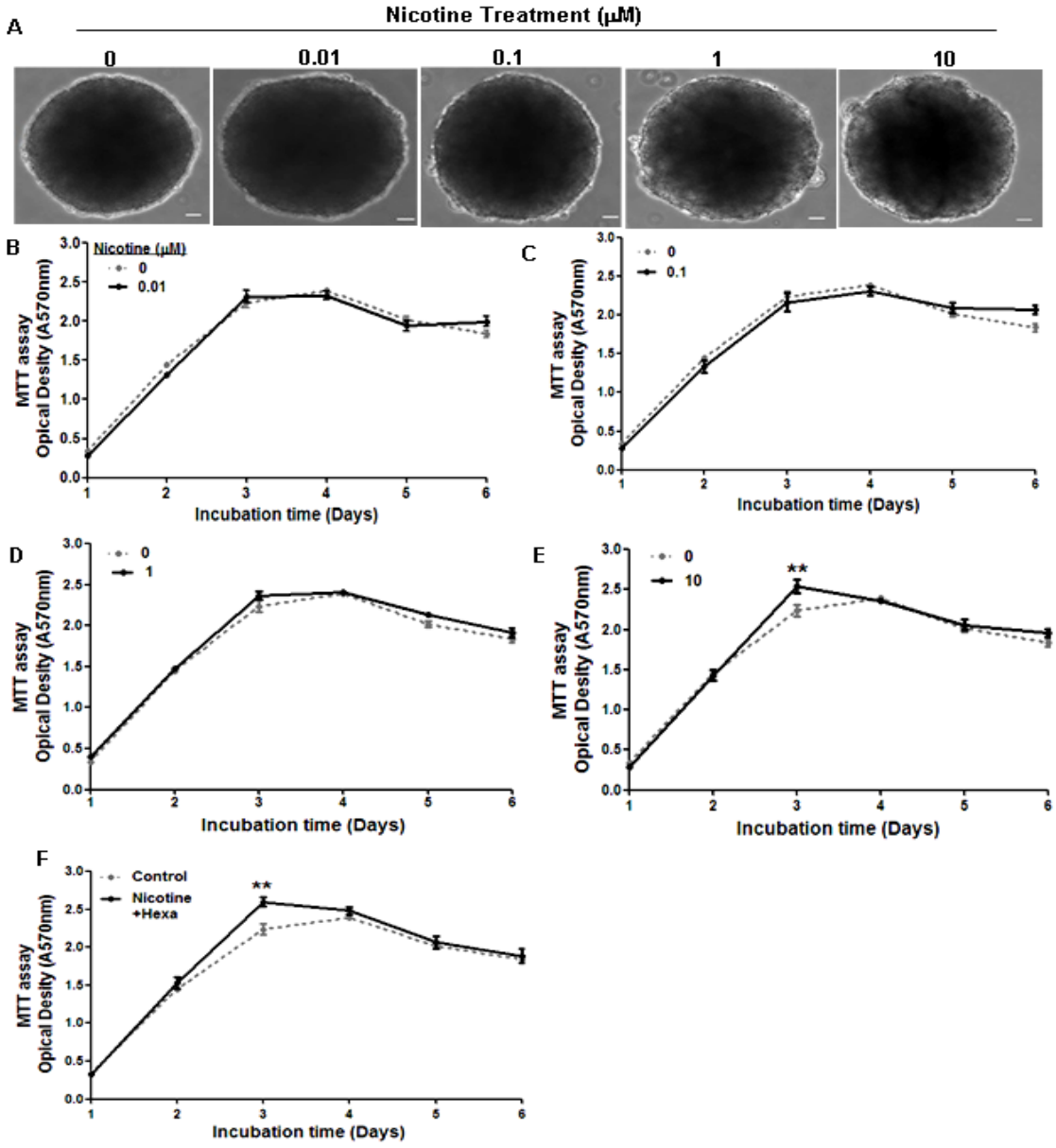

Figure S1. Effect of nicotine on embryonic body formation and cell viability.

(A) Morphology of EBs derived from mESCs was obtained directly after 4-day hanging drop culture with 0.01-10 μM nicotine. Scale bar represents 100 μm. mESCs were incubated for 6-days in differentiation medium with 0.01-10 μM nicotine alone (B-E) or in 10 μM nicotine combined with 10 μM Hexa (F). (B-F) MTT assays were performed for cell viability. Data represent the means±SEM of three independent experiments. ***P*<0.01.

Figure S2. nAChRs subtypes expressed in mESCs and mouse heart (Related to Figure 1).

RT-PCR was used to detected nAChRs subtypes expressed in mESCs and mouse heart. Several nAChRs subtypes including *a1*, *a2*, *a4*, *a6*, *a7*, *β1*, *β2*, and *β3* were found to express in both mESCs and mouse heart.

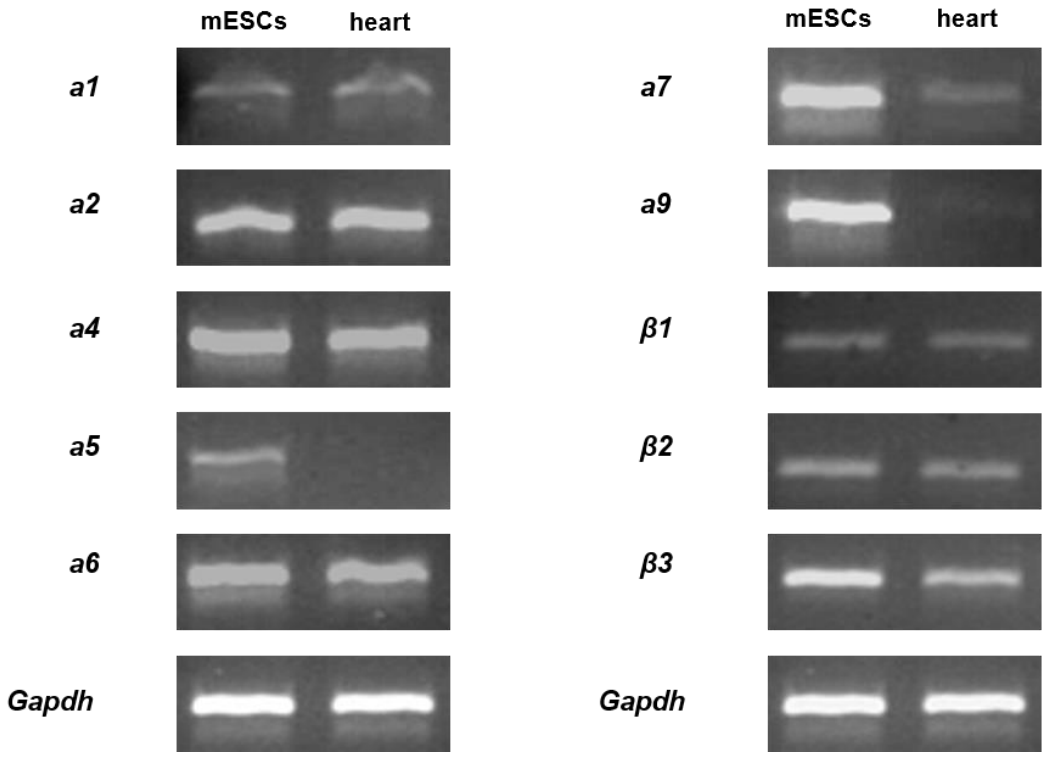

Figure S2. nAChRs subtypes expressed in mESCs and mouse heart.

nAChRs subtypes in mESCs and mouse heart were tested by RT-PCR. Three independent experiments.

Figure S3. The representative images of M-mode echocardiography (Related to Figure 7).

To determine the effect of nicotine on cardiac function, echocardiography(echo) was performed in the neonates at postnatal Day 1. M-mode echograms from nicotine-exposed groups with or without Hexa exposure showed similar patterns as those from control.

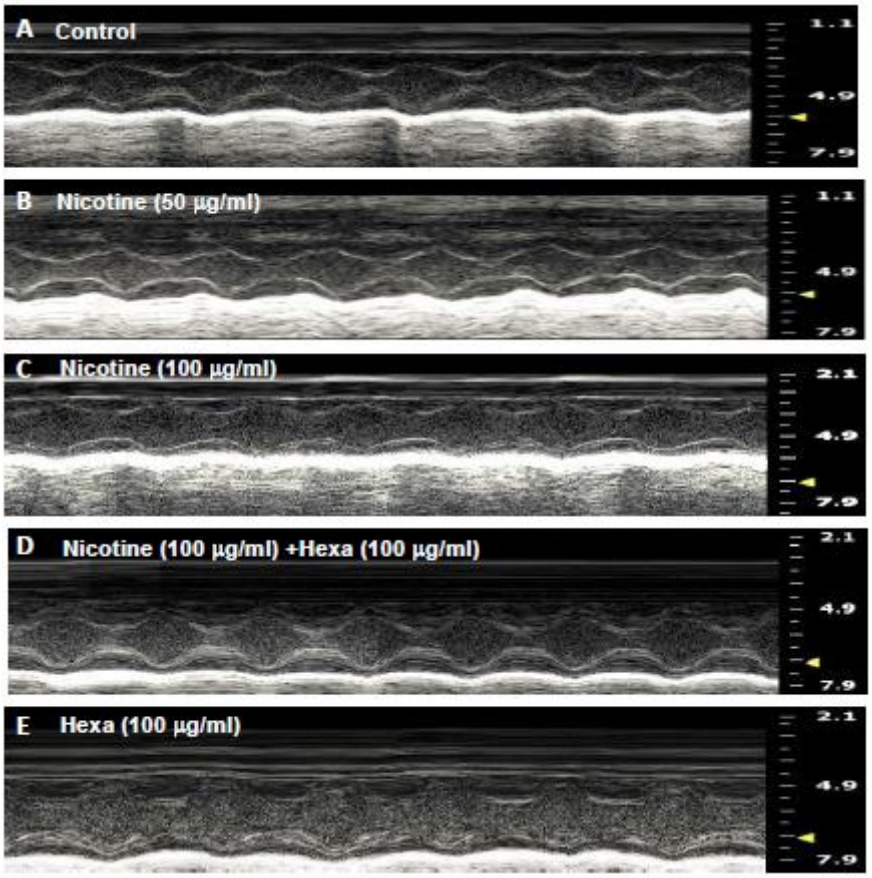

Figure S3. The representative images of M-mode echocardiography.

M-mode echocardiography was represented for control (A), 50 µg/ml (B) and 100 µg/ml (C) nicotine-exposed groups, and 100 µg/ml nicotine with 100 µg/ml Hexa (D), 100 µg/ml Hexa (E) treated group respectively.

Supplemental Tables

Table S1. The sequences of primer pairs for qPCR of mouse genes (Relative to Figure 1).

Gene	Primer sequence	Size (bp)
Oct4	Forward 5'-TATGCAAATCGGAGACCCTG-3' Reverse 5'-AAGCTGATTGGCGATGTGAG-3'	143
Nanog	Forward 5'-CAGCCCTGATTCTTCTACCAG-3' Reverse 5'-GATGCGTTCACCAGATAGCC-3'	384
Tbx5	Forward 5'-GGAGCCTGATTCCAAAGACA-3' Reverse 5'-TTCAGCCACAGTTCACGTTC-3'	153
Gata4	Forward 5'-GTTCCCAGGCCTCTTGCAATGCGG-3' Reverse 5'-AGTGGCATTGCTGGAGTTACCGCTG-3'	154
Gapdh	Forward 5'-ACCACAGTCCATGCCATCAC-3' Reverse 5'-CATGCCAGTGAGCTTCCCGT-3'	168

Table S2. The sequences of primer pairs for qPCR of rat genes (Relative to Figure 5).

Gene	Primer sequence	Size (bp)
Tbx5	Forward 5'-GGTCCGTAACTGGTAAAG-3' Reverse 5'-ATTTTCGTCTGCTTTCAC-3'	212
Gata4	Forward 5'-TGAGGGCGAGCCTGTTTGCAA-3' Reverse 5'-GCTGGTGGCGTTGCTGGAGT-3'	205
Gapdh	Forward 5'-CCATGGAGAAGGCTGGGG-3' Reverse 5'-CAAAGTTGTCATGGATGACC-3'	195

Table S3. The primer pairs of nAChRs subtypes for RT-PCR of mouse genes. (Relative to Figure 1 and Figure S2)

Gene	Primer sequence	Size (bp)
a1	Forward 5'-TGGAAGCACTGGGTGTTCTA-3' Reverse 5'-AACATATACTTCCCGATCAGG-3'	287
a2	Forward 5'-CTTCGGTGAAGGAAGATTGG-3' Reverse 5'-GGAGCCAACATGAGGGACAT-3'	155
a4	Forward 5'-GACTTTGCAGTCACCCACCT-3' Reverse 5'-CGGCTGTGCATGCTCACCAA-3'	197
a5	Forward 5'-TCATGCCGATAGGTACTTC-3' Reverse 5'-ATTGGCCCATTTATAAATAA-3'	262
a6	Forward 5'-CTTTGTCACGCTGTCCAT-3' Reverse 5'-GCCTCCTTTGTCTTGTCC-3'	159
a7	Forward 5'-ACAGTACTTCGCCAGCACCA-3' Reverse 5'-AAACCATGCACACCAATTCA-3'	145
a9	Forward 5'-CAATGCTCTGCGTCCAGTAG-3' Reverse 5'-ACACCAGATCGCTGGGAATC-3'	209
β1	Forward 5'-TCCTTAGTGTTGTGGTCCTC-3' Reverse 5'-TGTGGTTCAGGGAGTTGGTC-3'	151
β2	Forward 5'-GAGTGTGAGGGAGGATTGGA-3' Reverse 5'-TCGTGGCAGTGTAGTTCTGG-3'	134
β3	Forward 5'-AACACTGAGCTTAAAAGGAA-3' Reverse 5'-GCGGACACATTTCTGATAAC-3'	196
Gapdh	Forward 5'-ACCACAGTCCATGCCATCAC-3' Reverse 5'-CATGCCAGTGAGCTTCCCGT-3'	168

Table S4. Echocardiography of offspring hearts at postnatal day 1 (Relative to Figure 7). Details in excel file.

Supplemental Videos

Video of spontaneously beating EBs (Related to Figure 1).

Spontaneously beating EBs in control (A) and 10 μM nicotine-exposed group (B) at day 12 differentiation were recorded. As was shown in the videos, the area, intensity together with frequency of beating EBs were significantly reduced with 10 μM nicotine exposure.