

Supplemental Method

MTT cell viability assay

The viability of NCI-H295A cells were detected using MTT assay kit purchased from Cayman Chemical Company (Ann Arbor, Michigan) following the manufacturer's instruction. In brief, NCI-H295A cells were treated with nicotine at different doses (0, 1, 10, and 100 μ M) for 7 days. At the end of incubation, cell cultures were incubated with 20 μ l of 5 mg/ml MTT solution for 4 hours at 37°C in a humidified, 5 % CO₂ atmosphere. The medium was aspirated, and the precipitated formazan was solubilized with 200 μ l DMSO. The absorbance at 570 nm was measured and used to calculate the relative ratio of cell viability.

Supplemental Results

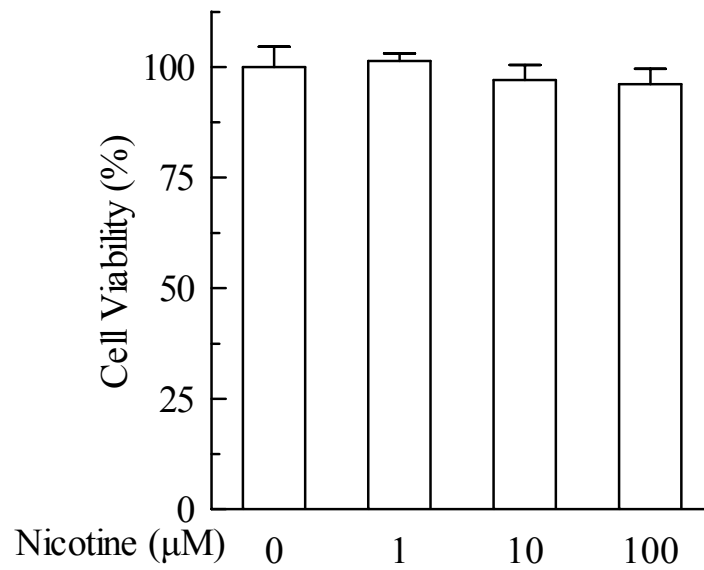


Fig S1. **Effect of nicotine on the viability of NCI-H295A cells by MTT assay.** NCI-H295A cells were treated with nicotine at different doses (0, 1, 10, and 100 µM) for 7 days. The absorbance at 570 nm was measured and used to calculate the relative ratio of cell viability. Data are expressed as mean \pm S.E.M. (n=7).

ATGGTCTCTA CTGCCTGGTA AACACCCCTGG CTCACTCTCG CGAGA CCGTG GTTCTCAAAG TGTAGTGTGT AGTCCACACA
 ACACCTGCAT TGCAACCACT GGGTATTTAT TTATTTATTT ATTTAATTTA TTTATTTATG ACCGAGTCTC ACTCTGTCCG
 CCAGGATGGA GTGCAGTGGC ACCGATCTTGG CTTACTGCAA CCTCTGCCTC CTGGGTTCAA GTGATTCTCA TGCCTCAGCC
 TCCCGAGTAG CTGGGACTAC AGGTGCCTGC CACATCACCC CGCTAATTTT TTGTATTTT AGTAGAGATG AGGTTTCACC
 ATGTTGGCCA TGCTGGTCTC GAA CCGCTGA CCTCAAGTGA TCTGCCACC TCCGCTCCC AAAGTGCTGG GGTTACAGGC
 STAAGCCACC GCGCCTGGCC AAGGGAAGTT TTTTCTTTT CTTTTTTTCT TTTTCTTTT CTTTTCCTC TTTTTTTTTT
 TTTTTTTTTT TTTTTTTTTT TTTTTTAACA CAGGTTTCTG AGCCTCAATT CCAGATCAGC TGAGCCTGGA GTTTCTGAAG
 ACAAGGGCTA GAAATCTGCA CTTTAAAGTC TTGAAAACCA CTGTGTGCCT TCATCTAAGC TGCCCTGCT TCTCTCCCCT
 CCATCCCTCG CCTGGCCCTG TCCTCCCTAC TCTCCCCTGC ACCCTCCCC CGCCCCAAGCT CCCCACAAAC GGCCAAAGCA
 GCAGTGTGAG GCAATCGCTC TATCCTTGAC CCCTTCCTTT GCACAGTGAG TGATGGCGTT TTTATCTCCT GATGATGATG
 CACAGCCTT AC CGGGGGAC ATTTAAGACG CAGAACACCA GGTCCAGGCT GCAGCTCGG GACTCAGAGG CGAAGCTTGA
 GGGGCTCAGG AAGGACGAAG AACCACCCTT GAGAGAAGAG GCAGCAGCAG CGGCGGCAGC AGCAGCGGCA GCGACCCAC
 CACTGCCACA TTTGCCAGGA AACATGCTG CTAGCGACAT TCAAGCTGTG CGCTGGGAGC TCCTACAGAC ACATGCGCAA
 CATGAAGGGT GAGCGCTGCG GGAAGGAGGC GATGAGGGGT TGGCCAGCTC TCAGCGGATG AGGCTCAGGC CACCCAATTC
 TGATCCTAGT TGTGCTCTT ACTGGGTGAA CCTGGGCAAG TTTCTTCCCT TCTTGAATCT CAGTTTTCCC CTGGAAGAA

Fig S2. **Human StAR gene proximal promoter sequence and CpG map.** Pink highlighted, nt -377; Cyan, transcription initiation site; Red, translation start codon; green, CpG dinucleotides. Two subregions from nucleotides labeled in blue (forward primer sequence) to nucleotides labeled in red (reverse primer sequence) indicate nt -719 to -280 and nt -9 to +402, respectively.



Fig S3. Bioinformatics analysis of human StAR promoter region. MacVector, software for gene analysis, and some online web services such as PROMO, TFBIND, TFSEARCH and Match were utilized for prediction the potential binding site of transcription factors. The result showed that several gene consensus sequences, including a glucagon-G3 promoter consensus (CGCCTGA), were predicted as potential binding site of transcription factors. The nucleotide sequence CGCCTGA labeled in yellow indicates the glucagon-G3 promoter consensus, tentative Pax6 binding motif.