

Supplementary Material

Inflammation Regulation via an Agonist and Antagonists of $\alpha 7$ Nicotinic Acetylcholine Receptors in RAW264.7 Macrophages

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Table S1. Primers Table.

| nAChRs | NCBI number | 5'→3' | Tm(°C) | Length (bp) |
|-----------------|-------------|--|--------|-------------|
| <i>Chrna-3</i> | NM_110834 | F: CTACGACAAGGCAAAGATCG R: CCTCACAGCAGTTGTACTTG | 53 | 134 |
| <i>Chrna-4</i> | NM_11438 | F: CATCTCGGTGCTGCTTTCTC R: GGTGGTGTACATTGAGCACG | 55 | 164 |
| <i>Chrna-5</i> | NM_110835 | F: CACTCAGAGAGAAGAAGCCG R: CATCCGATCGAGAACTTGGG | 55 | 166 |
| <i>Chrna-6</i> | NM_11440 | F: GACTTCCAGGTCGAAGGCAA R: AGGTCGATTTCTGCCTTGTC | 55 | 179 |
| <i>Chrna-7</i> | NM_11441 | F: TGCAGTGGAAACATGTCTGAG R: AATGCCCAGATGCATTACCC | 54 | 153 |
| <i>Chrna-9</i> | NM_231252 | F: GCCGTGAAGAACGTCATCTC R: GCGGAGCGAGGAACGATATG | 55 | 142 |
| <i>Chrna-10</i> | NM_504186 | F: CAAGTGCCTTGAGACCAGTG R: GCCACACTAGACTGCTGGGA | 55 | 213 |
| <i>Chrn-2</i> | NM_11444 | F: GGTGACTGTACAGCTCATGG R: GCTTAGAAGGGAGTCGGACT | 55 | 167 |
| <i>Chrn-3</i> | NM_108043 | F: CAATGAAGTTCCGATCCTGG R: AAGCCTTCTCTCTGTTGCC | 53 | 148 |
| <i>Chrn-4</i> | NM_108015 | F: GGACCTATGACCACACAGAG R: AAGTCATAGGTCACGTCCAC | 56 | 157 |
| <i>Tnf-α</i> | Y00467 | F: GAACTGGCAGAAGAGGCAC R: GTAGACAGAAGAGCGTGGTG | 55 | 137 |
| <i>Il-1β</i> | NM_008361 | F: ACTTCGCAGCAGCACATC R: GCTCATGTCCTCATCCTGGA | 55 | 120 |
| <i>Il-6</i> | M24221 | F: TGTATGAACAACGATGATGCACTTG R: AGGTAGCTATGGTACTCCAGAAGAC | 55 | 147 |
| <i>β-actin</i> | NM_007393 | F: CTGTATTCCCCTCCATCGTGG R: TCTGGGTCATCTTTTCACGGT | 55 | 279 |
| <i>Gapdh</i> | DQ403054 | F: CATCACTGCCACCCAGAAG R: CGTTCAGCTCTGGGATGAC | 55 | 137 |

After finding the corresponding gene sequence on NCBI, the ORF region was found through EditSeq software, primers were designed in Snap Gene software, and blast was performed in NCBI to confirm the specificity of the primers.

Table S2. Statistical table of expression of nAChR subunits in RAW264.7

| Number | $\alpha 3$ | $\alpha 4$ | $\alpha 5$ | $\alpha 6$ | $\alpha 7$ | $\alpha 9$ | $\alpha 10$ | $\beta 2$ | $\beta 3$ | $\beta 4$ |
|--------|------------|------------|------------|------------|------------|------------|-------------|-----------|-----------|-----------|
| 1 | + | + | + | + | + | + | + | + | + | + |
| 2 | + | + | + | + | + | + | + | + | + | + |
| 3 | + | + | + | + | + | + | + | + | + | + |
| 4 | + | + | + | + | + | + | + | + | + | + |
| Total | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

Cultured four different batches of mouse monocyte macrophages RAW264.7 to determine the expression of nAChR subunits respectively.

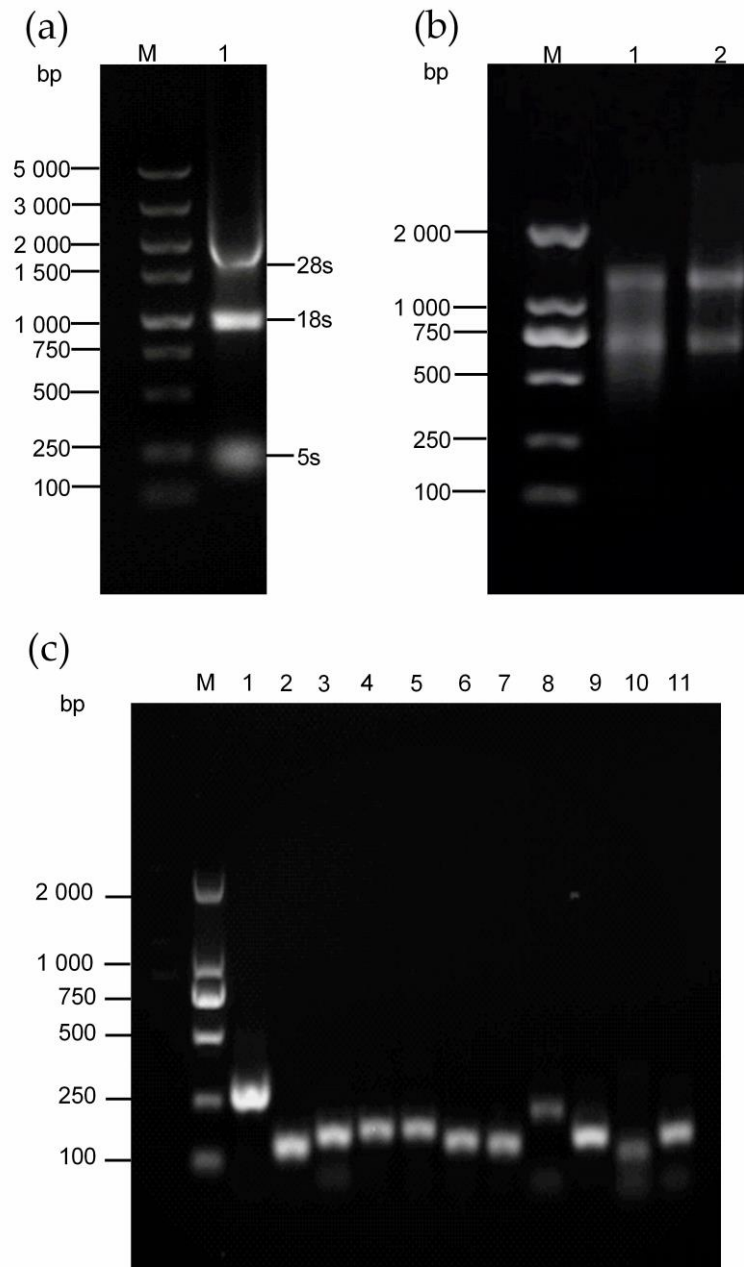


Figure S1. Agarose gel electrophoresis for identification of nAChR subunits. After the cells were cultured for 24 hours, the total RNA was extracted and reversed transcription into cDNA, and PCR amplification was performed using cDNA as a template to determine the expression of nAChR subunits in RAW264.7. **(a):** 0.8% Agarose gel electrophoresis analysis of RAW 264.7 total RNA, M: DL5 000 RNA Marker; **(b):** 1% Agarose gel electrophoresis analysis of RAW 264.7 cDNA, M: DL2 000 DNA Marker, 1: control 2: cDNA; **(c):** 1% garose gel electrophoresis analysis of RAW 264.7 PCR product M: DL2 000 DNA Marker , 1-11: *Gapdh*, $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 6$, $\alpha 7$, $\alpha 9$, $\alpha 10$, $\beta 2$, $\beta 3$, $\beta 4$.

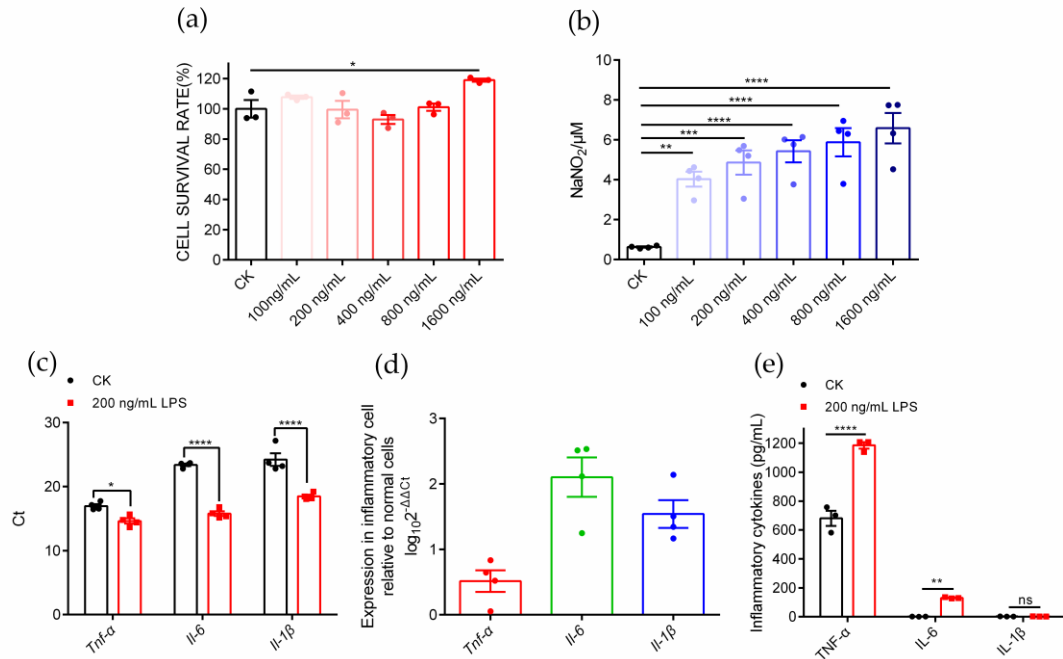


Figure S2. Construction of RAW264.7 inflammation model. After normal cells were cultured for 24 h, LPS (200 ng/mL) was administered to induce 12-16 h, and the inflammatory response was determined from the upregulation of NO level, proinflammatory cytokine(*Tnf-α*, *Il-6*, *Il-1β*) gene and protein levels. (a): CCK8 experiment is used to determine the appropriate induction concentration for a series of concentration gradients of LPS; (b): NO detection for a series of concentration gradients of LPS is used to determine the appropriate induction concentration; (c): Comparison of Ct values of inflammatory cytokines in normal cells and inflammatory cells; (d): Upregulation of the expression of proinflammatory cytokines mRNA in inflammatory cells compared with normal cells; (e): Upregulation of proinflammatory cytokines protein expression in inflammatory cells compared with normal cells. mRNA expression of proinflammatory cytokines were determined by using qPCR, Protein expression of proinflammatory cytokines were determined by ELISA kit. ns, not significant or $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$ compared to the control groups, by one-way ANOVA followed by the Dunnett's multiple comparisons test, the error bars in all figures indicate SEM($n=3-5$).