

Title page

Increased Fetal Thymocytes Apoptosis Contributes to Prenatal Nicotine Exposure-induced Th1/Th2 Imbalance in Male Offspring Mice

**Ting Chen⁺, You-e Yan⁺, Sha Liu, Han-xiao Liu, Hui-yi Yan, Li-fang Hou, Wen Qu
& Jie Ping***

*Department of Pharmacology, Wuhan University School of Basic Medical Sciences,
Wuhan 430071, China.*

***Corresponding author: Jie Ping**

Address: 185, Donghu Road, Wuhan 430071, China.

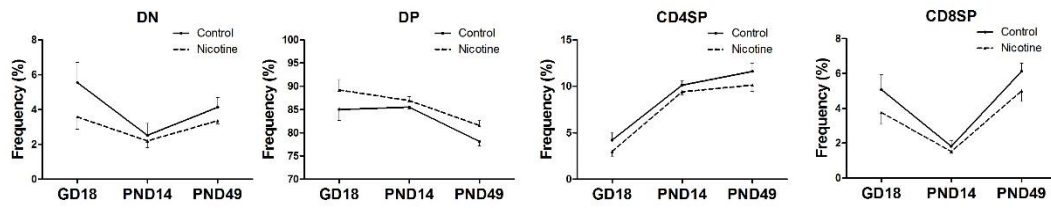
Tel.: +86 27 6875 8665.

Fax: +86 27 8733 1670.

E-mail: pingjie@whu.edu.cn

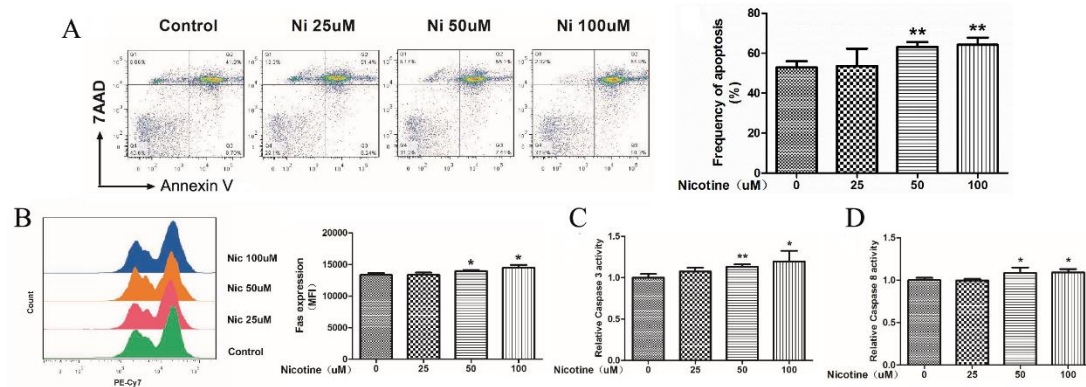
+These authors contributed equally to this work.

Supplementary Figure S1



Supplementary Figure S1. The thymocyte phenotypes in male offspring at different time points. DN, double negative cell; DP, double positive cell; SP, single positive cell; GD, gestational day; PND, postnatal day.

Supplementary Figure S2



Supplementary Figure S2. Effects of nicotine (25, 50 and 100 μ M) on cell apoptosis, Fas expression, caspase 3 and caspase 8 activity in primary thymus cells. Thymus cells were prepared from weaned BALB/c mice and cultured with different concentrations of nicotine for 48 h. A. Cell apoptosis was detected by flow cytometry; B. Protein expression of Fas on the surface of thymus cells; C and D: caspase 3 and caspase 8 activity in thymus cells. Mean \pm SD, n=6. * P <0.05, ** P <0.01 vs control.

Supplementary Table 1

Supplementary Table 1. Oligonucleotide primers and PCR conditions of mouse in quantitative real-time PCR.

Genes	Forward primer	Reverse primer	Product (bp)	Annealing
$\alpha 7nAChR$	CACATTCCACACCAACGTCTT	AAAAGGGAACCAGCGTACATC	106	60°C, 30 s
Bim	CCGGAGATACGGATTGCACAG	CAGCCTCGCGGTAATCATTG	97	60°C, 30 s
Caspase-3	CGTGGTTCATCCAGTCCCTTT	ATTCCGTTGCCACCTTCCT	102	60°C, 30 s
Caspase-8	AGGTACTCGGCCACAGGTTA	TGGGATGTAGTCCAAGCACA	137	60°C, 30 s
Fas	ATGCACACTCTGCGATGAAG	CAGTGTTACAGCCAGGAGA	120	60°C, 30 s
FasL	GCAGAAGGAACTGGCAGAAC	TTAAATGGGCCCACTCCTC	128	60°C, 30 s
GAPDH	AACTTTGGCATTGTGGAAGG	GGATGCAGGGATGATGTCT	132	60°C, 30 s
IFN- γ	CTCAAGTGGCATAGATGTGGAAG	GATGGCCTGATTGTCTTTCAAG	120	60°C, 30 s
IL-4	ATGGATGTGCCAAACGTCTT	AAGCACCTTGAAGCCCTAC	78	58°C, 30 s;