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Supplemental Material

Maternal Exposure of BALB/c Mice to Indoor NO₂ and Allergic Asthma Syndrome in Offspring at Adulthood with Evaluation of DNA Methylation Associated Th2 Polarization

Huifeng Yue, Wei Yan, Xiaotong Ji, Rui Gao, Juan Ma, Ziyu Rao, Guangke Li, and Nan Sang

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Figure S1. Gating strategy for the identification of macrophage, eosinophils, neutrophils, lymphocytes, Th2 (CD4⁺ IL-4⁺) and CD4⁺ cells. Abbreviations: Mac, macrophages; Eos, eosinophils; Neu, neutrophils; Lym, lymphocytes.

Figure S2. (A) Effects of maternal NO₂ exposure on eosinophils numbers and type 2 cytokine IL-4 level in the lung of female and male offspring from PND1. (B) Effects of maternal NO₂ exposure on eosinophils numbers and IL-4 level in the lung of female and male offspring from PND42. The values are expressed as the mean ± SE. * $p < 0.05$ compared with air group, respectively.

Figure S3. (A) Effects of maternal NO₂ exposure on AHR of female (A1) and male (A2) offspring from different treatment groups. (B) Effects of maternal NO₂ exposure on eosinophils numbers in the lung of female (B1) and male (B2) offspring from different treatment groups. (C) Effects of maternal NO₂ exposure on OVA-specific IgE in the serum of female (C1) and male (C2) offspring from different treatment groups. (D) Effects of maternal NO₂ exposure on type 2 cytokine IL-4 expression in the lung of female (D1) and male (D2) offspring from different treatment groups. The values are expressed as the mean ± SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with saline groups, respectively. # $p < 0.05$, ## $p < 0.01$ compared with Al(OH)₃ groups, respectively. † $p < 0.05$ compared with AOO group.

Figure S4. Effect of maternal NO₂ exposure on methylation status of the *IL4* promoters (A) and *IL13* promoters (B) in the lungs of the offspring at PND 42. Five individual clones from 3 mice and a total of 15 clones from each treatment were sequenced. Each row represents an individual clone of the promoter. A total of 5 CpG sites on region 1 and 10 CpG sites on region 2 of *IL4* and 36 CpG sites on region 1 and 6 CpG sites on region 2 of *IL13* were analyzed. Each circle represents a CpG site within the promoter. White circles represent unmethylated CpGs, and black circles represent methylated CpGs. Met % and Met ‰, average percent of total CpG methylation. The data are expressed as the mean ± standard error. S1, S2 and S3 refer to sample 1, sample 2 and sample 3, respectively.