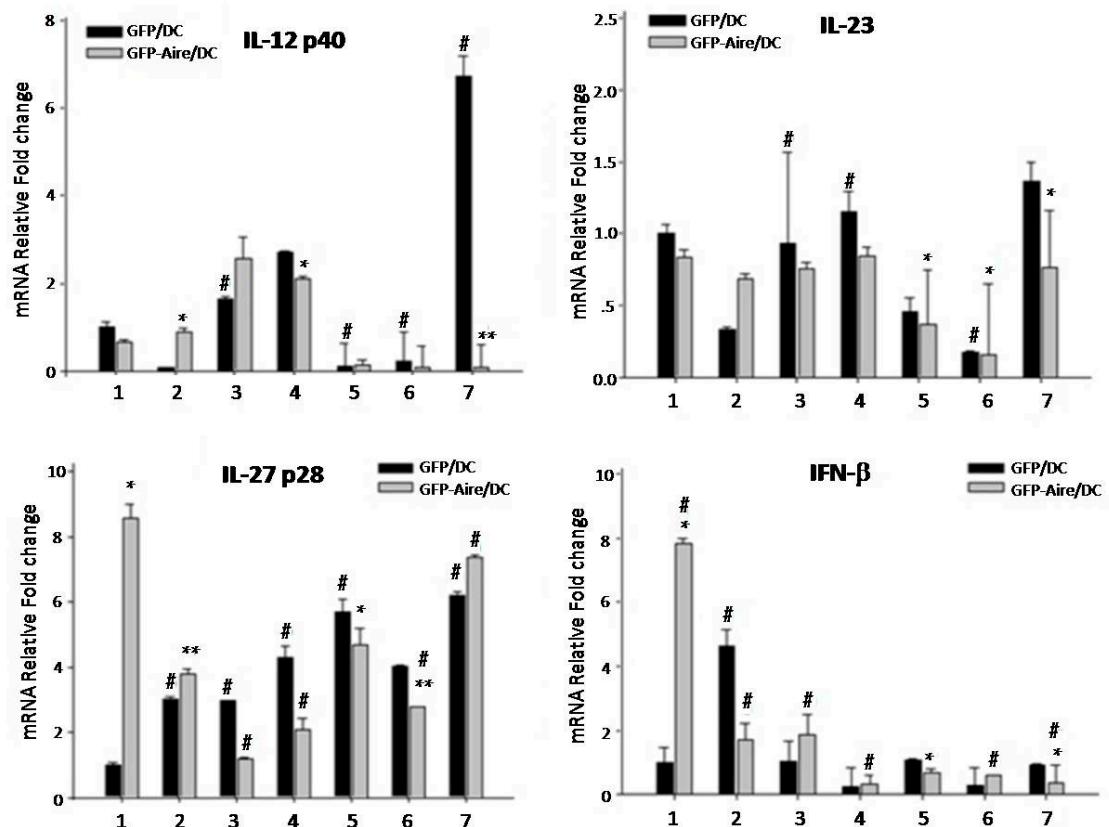
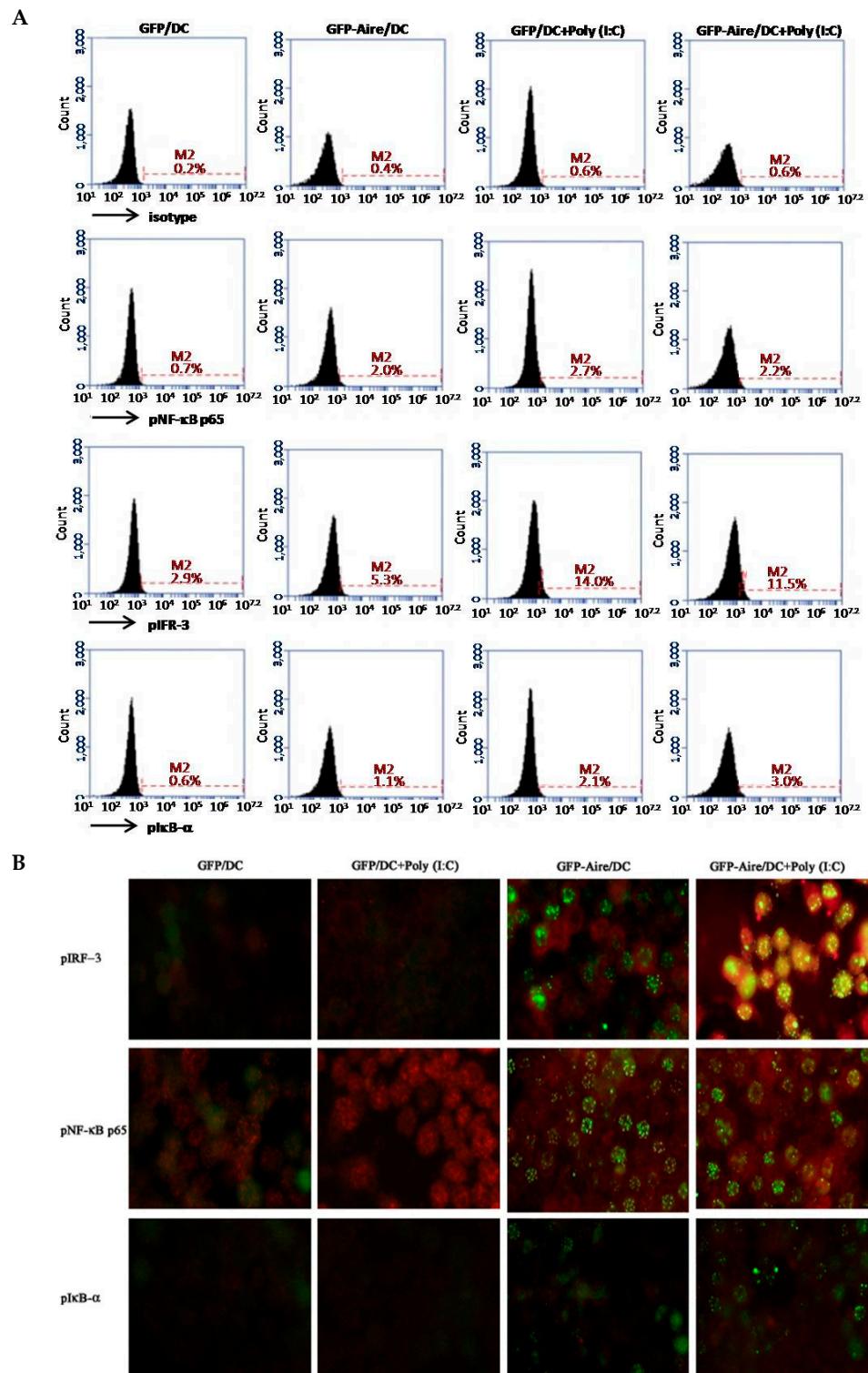


# Supplementary Material: Autoimmune Regulator Expression in DC2.4 Cells Regulates the NF-κB Signaling and Cytokine Expression of the Toll-Like Receptor 3 Pathway

Jitong Sun, Kunwei Niu, Haiying Fu, Haijun Li, Yi Li and Wei Yang



**Figure S1.** Effects of Aire on downstream gene expression in GFP-Aire/DC cells treated with a lower dose of Poly (I:C). The levels of IL-12 p40, IL-23, IL-27 p28, and IFN- $\beta$  transcript expression in GFP-Aire/DC and GFP/DC cells were detected by RT-qPCR. All qPCR data are shown as the gene expression relative to GAPDH expression and are depicted as the fold change relative to GFP/DC cells, which were normalized to 1. x-axis: (1): Control; (2,3): 3 h Poly I:C at 1 and 12.5 mg/mL, respectively; (4,5): 6 h Poly I:C at 1 and 12.5 mg/mL, respectively; (6,7): 12 h Poly I:C at 1 and 12.5 mg/mL, respectively. \*  $p < 0.05$ , \*\*  $p < 0.01$  compared with the GFP/DC control; #  $p < 0.05$  compared with the GFP-Aire/DC control.



**Figure S2.** (A) Immunofluorescent detection of NF- $\kappa$ B, IRF-3, and I $\kappa$ B- $\alpha$  (red) in GFP/DC and GFP-Aire/DC treated with Poly (I:C) via confocal microscopy. Original magnification: 200 $\times$ ; (B) Phospho-NF- $\kappa$ B, I $\kappa$ B- $\alpha$ , and IRF-3 expression were detected by FCM. The results are representative of at least three independent experiments.

**Table S1.** Oligonucleotide primer sequences.

Gene	Sequences	Size (bp)
<i>GAPDH</i>	S: 5'-GACTTCAACAGCAACTCCCCACTC-3' AS: 5'-TAGCCGTATTCAATTGTCTATACCAG-3'	107
<i>TLR1</i>	S: 5'-CTGAGGGTCCTGATAATGTCCTAC-3' AS: 5'-GATCACCTTAGCTCATTGTGGG-3'	114
<i>TLR2</i>	S: 5'-TTGCCTTACATCTTGAACTG-3' AS: 5'-ACTACGTCTGACTCCGAGGG-3'	96
<i>TLR3</i>	S: 5'-CAACGGTTCCCTCTCCTATCTC-3' AS: 5'-TTGCTTAGTAAATGCTCGCTTC-3'	139
<i>TLR4</i>	S: 5'-CTTCATTCAAGACCAAGCCTTC-3' AS: 5'-AACCGATGGACGTGTAAACCAG-3'	126
<i>TLR5</i>	S: 5'-TCTACAACATATCCACCGAAGACTG-3' AS: 5'-TTATGACTACAAGGGTGTGACGAG-3'	123
<i>TLR6</i>	S: 5'-ACAACATTCCCAGTAGATACCACA-3' AS: 5'-TCCTCATTGACTAAGGCTAACTTC-3'	148
<i>TLR7</i>	S: 5'-TGAGGGCATTCCCACAAACAC-3' AS: 5'-TCCAGATGGTCAGCCTACGG-3'	96
<i>TLR8</i>	S: 5'-TTCCTCACATTCTTACACACCTC-3' AS: 5'-GTGATAGATAAACCAAACATCCCAG-3'	87
<i>TLR9</i>	S: 5'-TCTGTCTTACTACACCGCTATTG-3' AS: 5'-AAACTACCCATTACAGCCAACC-3'	93
<i>IL-12p40</i>	S: 5'-ACTGGAACTACACAAGAACGAGAG-3' AS: 5'-CTTCAAGTCCTCATAGATGCTACCA-3'	108
<i>IL-23</i>	S: 5'-AAATAATGTGCCCGTATCCAGT-3' AS: 5'-TCCCCTTGAAGATGTCAGAGTC-3'	140
<i>IL-27p28</i>	S: 5'-CTCTGCTTCCTCGCTACCAC-3' AS: 5'-GGGGCAGCTTCTTCTTCT-3'	234
<i>IFN-β</i>	S: 5'-AAGAGTTACACTGCCTTGCCATC-3' AS: 5'-CACTGTCTGCTGGTGGAGTTCATC-3'	135
<i>MyD88</i>	S: 5'-TCCGGCAACTAGAACAGACAGACT-3' AS: 5'-GCGGCGACACCTTTCTCAAT-3'	119
<i>NF-κB</i>	S: 5'-GGACCTATGAGACCTCAAGAG-3' AS: 5'-CTACCCGAAACTCAACTTCTGT-3'	111
<i>TICAM</i>	S: 5'-GATGGAGAGGTCCACAAGGA-3' AS: 5'-GAGTGTAGCGTGGGGAGTGT-3'	109
<i>IRF-3</i>	S: 5'-AGCCTGCATCAAATCAACC-3' AS: 5'-CCACCTTCTGGCGAAGA-3'	180