Supplementary Information

Supplementary Figure 1

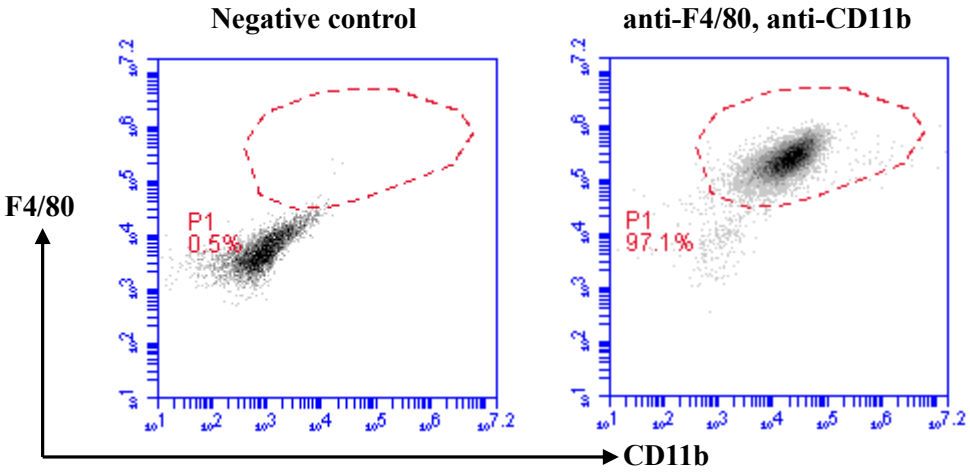


Figure S1. The purity of murine PEMs. Murine primary PEMs were stained with anti-CD11b-FITC and anti-F4/80-APC, followed by FACS analysis. The data are shown as a representative result of three independent experiments.

Supplementary Figure 2

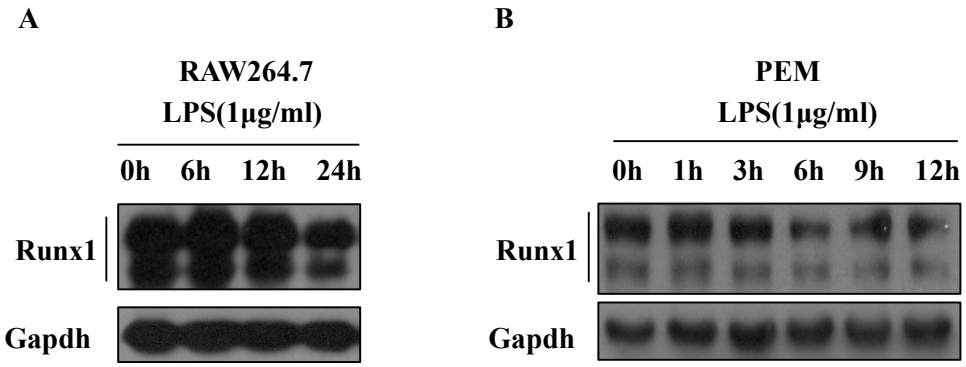


Figure S2. The protein levels of Runx1 in LPS-stimulated RAW264.7 cells and PEMs. The protein levels of Runx1 in LPS-stimulated RAW264.7 cells (A) and PEMs (B) were detected by immunoblotting. The results are shown as a representative result of three independent experiments.

Supplementary Figure 3

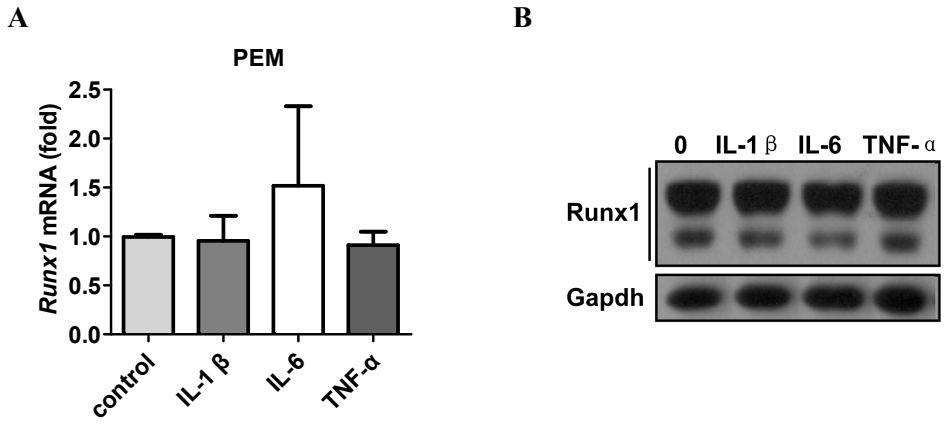


Figure S3. The expression levels of Runx1 in PEMs after stimulation with cytokines. (A)The Runx1 mRNA levels were checked in 10 ng/ml IL-1β, 100 ng/ml IL-6 or 10 ng/ml TNF-α-stimulated PEMs after 6h. The data are shown as the means ± SEM of three independent experiments. (B) The Runx1 protein levels were checked in IL-1β, IL-6 and TNF-α-stimulated PEMs after 24h. The data are shown as a representative result of three independent experiments.

Supplementary Figure 4

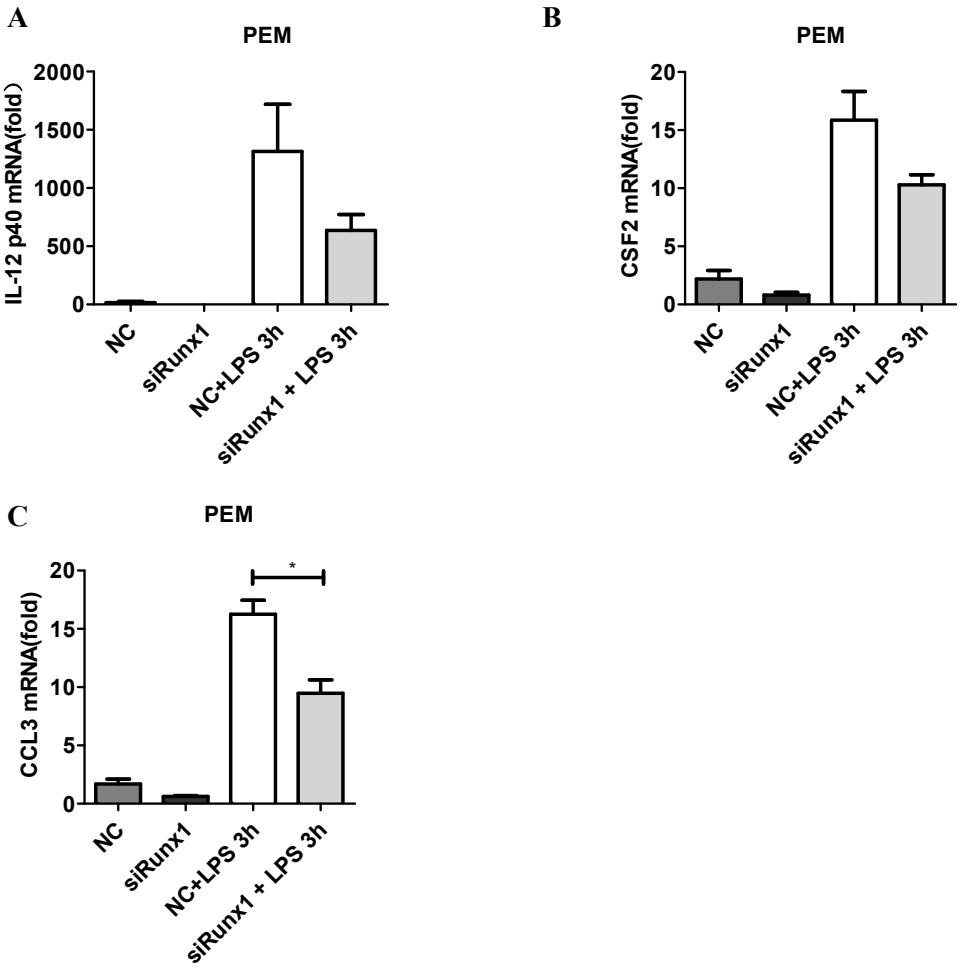


Figure S4. The mRNA expression levels of LPS-dependent genes in PEMs after Runx1 siRNA transfection. The mRNA expression levels of IL-12p40 (A), CSF2 (B), and CCL3 (C) were detected by RT-qPCR in resting or LPS stimulated siRunx1-transfected PEMs. The data are shown as the means ± SEM of three independent experiments. * P <0.05.

Supplementary Figure 5

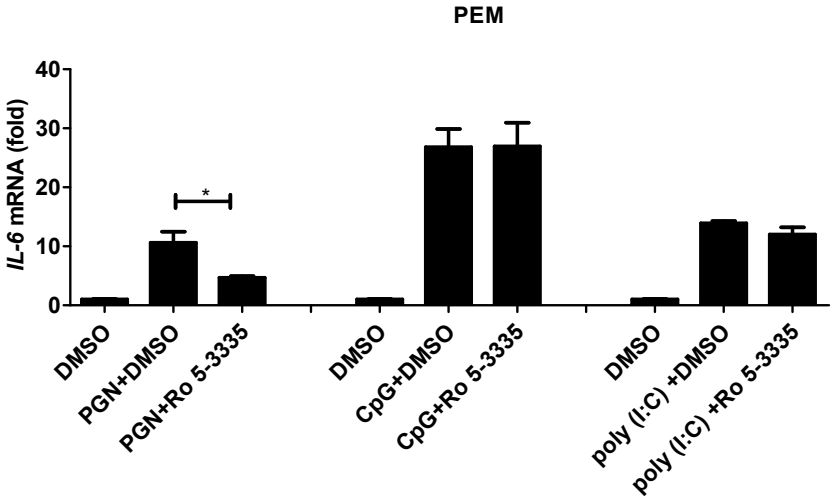


Figure S5. The RUNX1 inhibitor Ro 5-3335 reduced IL-6 mRNA levels in PGN-treated PEMs. PEMs were pretreated with DMSO or 50 μM Ro5-3335, then stimulated with 10 μg/ml PGN (TLR2 agonist), 1 μM CpG (TLR9 agonist) and 10 μg/ml poly (I:C) (TLR3 agonist) for 6h. The IL-6 mRNA expression levels were detected by RT-qPCR. The data are shown as the means ± SD of a representative experiment. * P <0.05.

Supplementary Figure 6

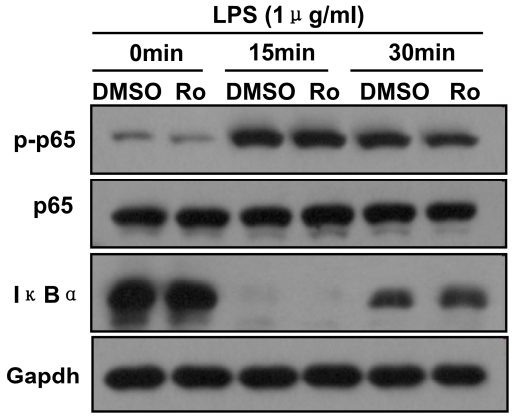


Figure S6. The RUNX1 inhibitor Ro 5-3335 does not affect p65 phosphorylation and IκBα degradation in LPS-stimulated PEMs. PEMs were pretreated with DMSO or 50 μM

Ro5-3335, then stimulated with 1 μg/ml LPS for 15min and 30min. The p65 phosphorylation and IκBα degradation were detected by immunoblotting. The data are shown as a representative result of three independent experiments.

Supplementary Figure 7

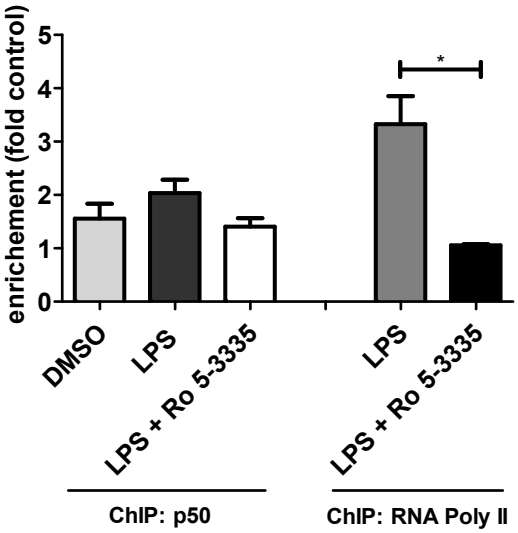
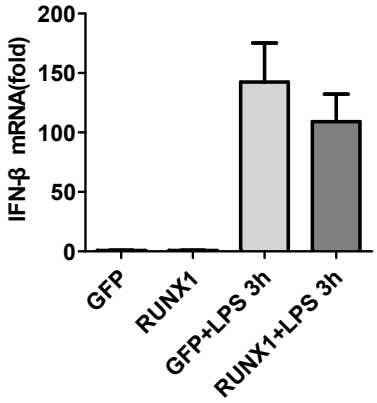


Figure S7. The effect of RUNX1 on the recruitment of p50 to the *IL-6* promoter. RAW 264.7 cells were stimulated with 100 ng/ml LPS for 5h with or without the treatment of 50 μM Ro 5-3335. ChIP assays were followed by qPCR to examine the amount of p50 and RNA polymerase II in the *IL-6* promoter. Data are shown as the means ± SEM of two independent experiments.

Supplementary Figure 8

A



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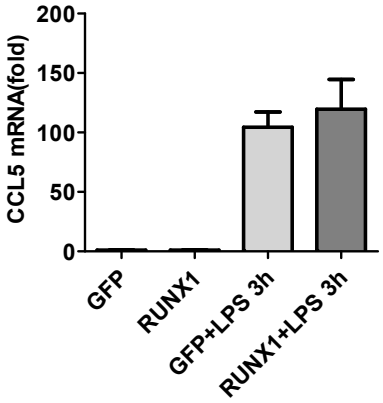


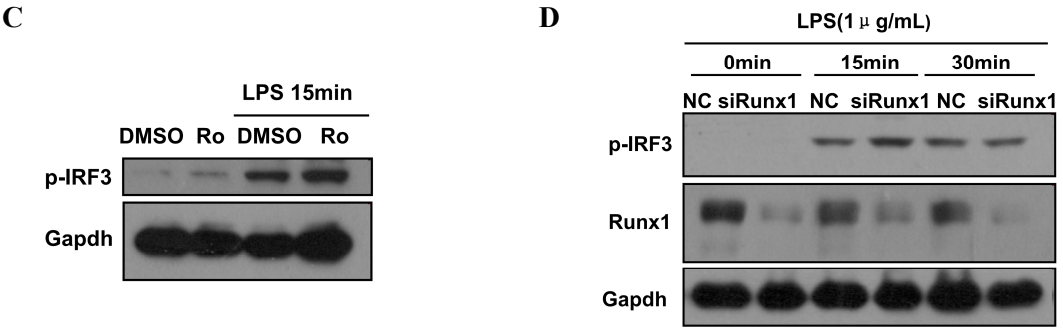
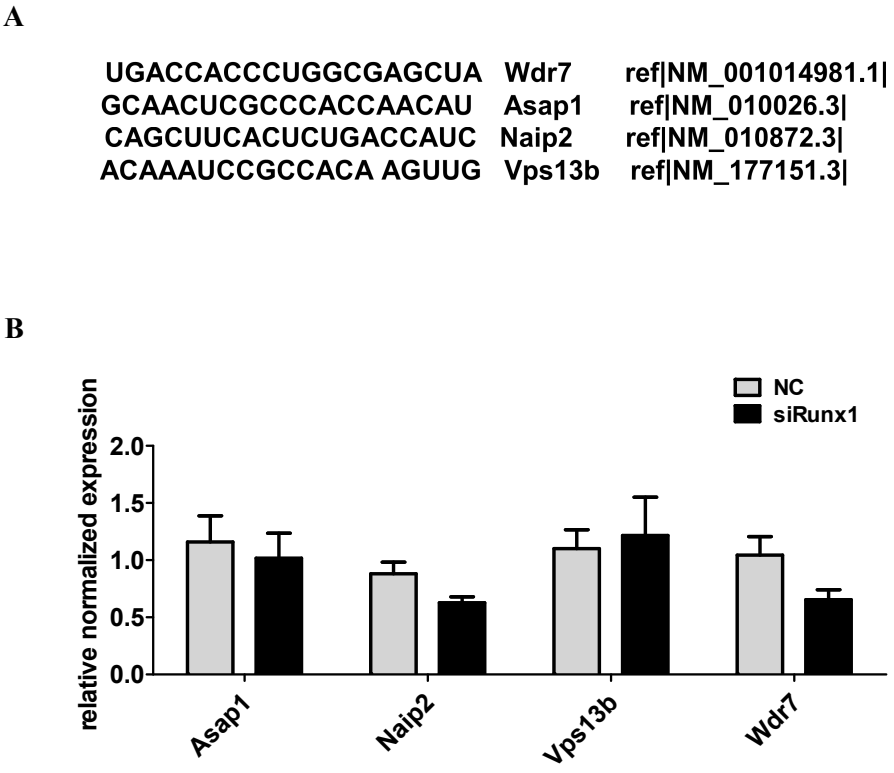


Figure S8. The effect of RUNX1 on TRIF-dependent cytokines and IRF3 phosphorylation. The stable transfected RAW264.7 cells overexpressing GFP or RUNX1 were stimulated with LPS for 3h, mRNA expression levels of IFN-β (A) and CCL5 (B) were detected by RT-qPCR. The data are shown as the means ± SEM of three independent experiments. The IRF3 phosphorylation was detected in LPS-stimulated PEMs, which were pretreated with Ro5-3335 (C) or transfected with Runx1 siRNA (D). The data are shown as a representative result of three independent experiments.

Supplementary Figure 9



C

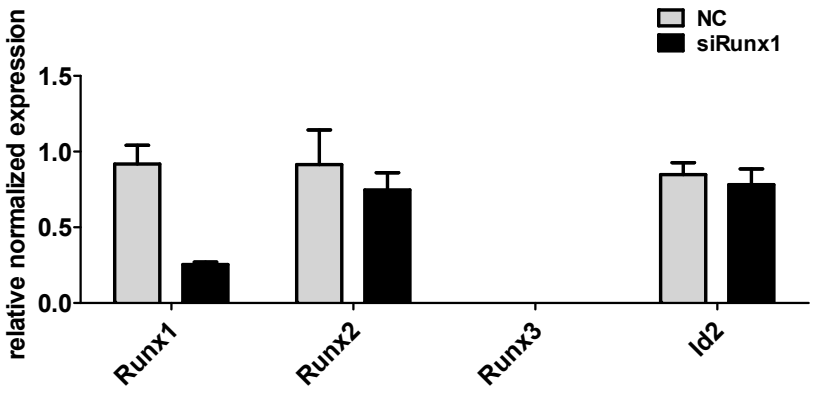


Figure S9. The specificity of Runx1 siRNA. According to the PubMed BLAST database, the four genes which might be the off-target genes and are highly expressed in macrophages, including Asap1, Naip2, Vps13b and Wdr7. Their sequences were listed (A) and the mRNA expression levels were detected in PEMs after transfection with the nonspecific siRNA or Runx1 siRNA (B). We also checked the expression levels of the Runx family members, including Runx2 and Runx3, as well as Id2 that plays important roles in myeloid cell differentiation. And the relative Runx1 mRNA levels were also shown (C). The data are shown as the means ± SEM of three independent experiments.

Supplementary Table 1. The primers used to clone target genes.

Gene	Forward primer	Reverse primer
R1-453-HA	CCGCTCGAGCCACCATGGTGTACCCATACGACGTCCCAGACTACGCTATGCGTATCCCCGTAGATGCC	CGGAATTCTCAGTAGGGCCTCCACACGGC
R1-242-HA	CCGCTCGAGCCACCATGGTGTACCCATACGACGTCCCAGACTACGCTATGCGTATCCCCGTAGATGCC	CGGAATTCTCAATCCTGCATCTGACTCTGAGG
R243-453-HA	CCGCTCGAGCCACCATGGTGTACCCATACGACGTCCCAGACTACGCTATGACAAGGCAGATCCAACCATCC	CGGAATTCTCAGTAGGGCCTCCACACGGC
R50-178-HA	CCGCTCGAGCCACCATGGTGTACCCATACGACGTCCCAGACTACGCTATGAGCATGGTGGAGGTGCTGGCC	CGGAATTCTCATCTTCGAGGTTCTCGGGGCCC
p50-FLAG	CCGCTCGAGCCACCATGGATTACAAGGATGACGACGATAAGATGGCAGAAGATGATCCATAT	CGGAATTCCTAGGTTCCATGCTTCATCCCAGC
P105-FLAG	CCGCTCGAGCCACCATGGATTACAAGGATGACGACGATAAGATGGCAGAAGATGATCCATAT	CGGAATTCCTAAATTTTGCCTTCTAGAGG

Supplementary Table 2. siGENOME mouse Runx1 siRNA –SMARTpool.

Number	target sequence
1.	UGACCACCCUGGCGAGCUA
2.	GCAACUCGCCCACCAACAU
3.	CAGCUUCACUCUGACCAUC
4.	ACAAAUCCGCCACAAGUUG

Supplementary Table 3. Negative control scrambled siRNA

Number	target sequence
1.	UUCUCCGAACGUGUCACGU

Supplementary Table 4. The primers used for Real-time qPCR.

Gene	Forward primer	Reverse primer	products size (bp)
Mouse *IL-1β*	CTGGTACATCAGCACCTCAC	AGAAACAGTCCAGCCCATAC	124
Mouse *IL-6*	TGTATGAACAACGATGATGCACTT	ACTCTGGCTTTGTCTTTCTTGTTATCT	197
Mouse *IL-12p40*	TGGTTTGCCATCGTTTTGCTG	ACAGGTGAGGTTCACTGTTTCT	123
Mouse *TNF-α*	AGTGACAAGCCTGTAGCCC	GAGGTTGACTTTCTCCTGGTAT	252
Mouse *CCL3*	CCCAGCCAGGTGTCATTT	GCATTCAGTTCCAGGTCAGT	103
Mouse *CCL5*	GACACCACTCCCTGCTGCTT	CACTTGGCGGTTCCTTCG	132
Mouse *IFN-β*	TCACCTACAGGGCGGACTTC	TCTCTGCTCGGACCACCATC	345
Mouse *CSF2*	CCTGGGCATTGTGGTCTACAG	AGAAAGGTTTTAAGGCTGTCTATG	360
Mouse *Runx1*	TGGCAGGCAACGATGAAAAC	CGCTCGGAAAAGGACAAACTC	274
Mouse *Runx2*	TTCAACGATCTGAGATTTGTGGG	GGATGAGGAATGCGCCCTA	221
Mouse *Runx3*	GACTCCTTCCCCAACTATACACC	CGCTGTTCTCGCCCATCTT	124
Mouse *Asap1*	CTATGGAATCGGATGCCGGAC	CGTGGTGGGCGAGTTGTAG	81
Mouse *Naip2*	AGCTTGGTGTCTGTTCTCTGT	GCGGAAAGTAGCTTTGGTGTAG	180
Mouse *Vps13b*	GTTCTGCAAGTGAATCCGGTT	GGTCCAGGTGATGGCAAAGA	171
Mouse *Wdr7*	CCAACAGCAACGAACCTCTTA	GGCAGGCACAATAATGATGCTT	104
Mouse *β-actin*	CAGCTGAGAGGGAAATCGTG	CGTTGCCAATAGTGATGACC	150
Human *IL-6*	GGATTCAATGAGGAGACTTGC	GTTGGGTCAGGGGTGGTTAT	197
Human *RUNX1*	TGAGCTGAGAAATGCTACCGC	ACTTCGACCGACAAACCTGAG	76
Human *GAPDH*	CCCTCAACGACCACTTTGTCA	TTCCTCTTGTGCTCTTGCTGG	144

Supplementary Table 5. The primers used for qPCR-coupled ChIP analysis.

Gene	Forward primer	Reverse primer	products size (bp)
Mouse *IL-16*	TCCCATCAAGACATGCTCAA	CTAGGAAGGGGAAAGTGTGCT	113