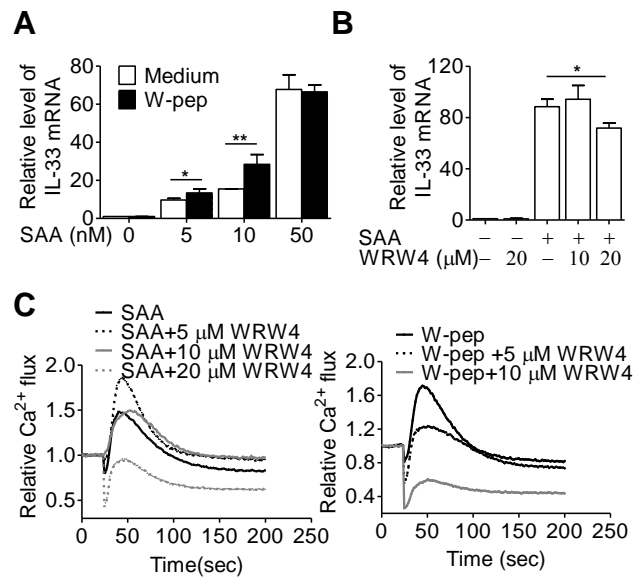


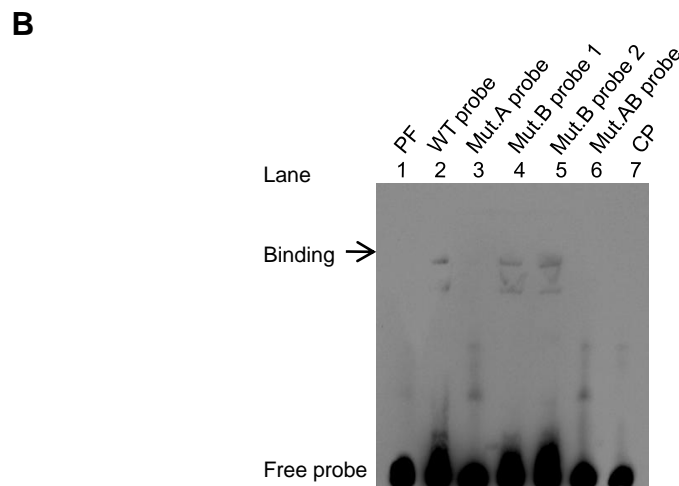
Supporting Information Figure 1, IL-33 protein in culture medium and caspase-3 activity in SAA-activated monocytes. (A) THP-1 cells were stimulated with 0.05 μ M SAA for the indicated times. IL-33 in the culture medium was measured by ELISA. The results are representative of three independent experiments. (B) THP-1 cells were stimulated with 0.05 μ M SAA for the indicated times. Caspase-3 protein activity was detected by Western blotting with a specific anti-caspase-3 antibody. Data are representative of three samples pooled from three independent experiments and densitometry values (means + SEM) are shown (B).



Supporting Information Figure 2, Effect of FPR2 agonist and antagonist on SAA-induced IL-33 expression. (A) THP-1 cells were stimulated for 8 h with WKYMVm (W-pep, 500 nM), together with different concentrations of SAA (1 nM to 50 nM). Relative changes in IL-33 mRNA were detected by quantitative real-time PCR. (B) WRW4 at indicated concentrations was applied to THP-1 cells 1 h before SAA (0.05 μ M) stimulation for 8 h. Data shown are means + SEM of six samples pooled from three independent stimulation experiments. * $p < 0.05$, ** $p < 0.01$, one-way ANOVA followed by Bonferroni post-testing. (C) THP-1 cells were pretreated with or without WRW4 at the indicated concentrations for 1 h and then stimulated with 0.1 μ M SAA and W-pep (0.1 μ M), respectively. The changes in intracellular calcium were analyzed. Data were acquired by SoftMax Pro 6 (Molecular Devices) and a typical set of calcium traces from one representative experiment (total of 3) is shown.

A

		<u>IRF7 #1</u>	<u>IRF7 #2</u>	
WT	probe:	-277	AACAGAATTTCAAATGGACAGTGCTGAAGTTGAAGTTAGGA	-235
Mut.A	probe:	-277	AACAT <u>TGCG</u> TTCAAATGGACAGTGCTGAAGTTGAAGTTAGGA	-235
Mut.B	probe:	-277	AACAGAATTTCAAATGGACAGTGCT <u>TCGATT</u> GAAGTTAGGA	-235
Mut.AB	probe:	-277	AACAT <u>TGCG</u> TTCAAATGGACAGTGCT <u>TCGATT</u> GAAGTTAGGA	-235



Supporting Information Figure 3, IRF7 binding to IRF7 #1 site in IL-33 promoter in vitro. (A) Probe sequence and the mutations introduced. WT probe, -277/-235 region of IL-33 promoter with both IRF7 #1 and IRF7 #2 binding sites; Mut.A probe, mutations in IRF7 #1 sites (underlined); Mut.B probe, mutations in IRF7 #2 (underlined); Mut.AB probe, mutations in both IRF7 #1 and IRF7 #2 binding sites (underlined). (B) EMSA with WT or mutant probes of the human IL-33 promoter was performed on HEK-293T cell nuclear protein extracts after overexpression of HA-tagged IRF7. Approximately 50 fmol probe were used in Lane 1-6 except for Lane 5 (100 fmol). PF, protein free; CP, cold competitor probe. Data are representative of three experiments.

Supporting Information Table 1: Primers used for plasmid construction and RT-PCR.

Primer Name	Forward Primer (5'-3')	Reverse Primer (5'-3')
IL-33 pro (-1050 to +49)	AGTTGAGCTCCCCAAGCCTGGTCA GCCA	GCGACTCGAGTTTCTTCCCTGAAGA GCT
IL-33 pro (-662 to +49)	GCGAGAGCTCATTACTTCATCCCTTT AT	GCGACTCGAGTTTCTTCCCTGAAGA GCT
IL-33 pro (-520 to +49)	ATTAGAGCTCATCTTCTGCGAGATT	GCGACTCGAGTTTCTTCCCTGAAGA GCT
IL-33 pro (-460 to +49)	ATATGAGCTCCCCGTCAGATATGTT	GCGACTCGAGTTTCTTCCCTGAAGA GCT
IL-33 pro (-300 to +49)	TATTGAGCTCGCCAAAAGTGAATAT	GCGACTCGAGTTTCTTCCCTGAAGA GCT
IL-33 pro (-160 to +49)	ATTAGAGCTCCAGATGGAGGGAGG ACGC	GCGACTCGAGTTTCTTCCCTGAAGA GCT
Human IRF7 (FL)	ATAGGATCCATGGCCTTGGCTCCTG AG	TATCTCGAGCTAGGCGGGCTGCTC CAG
IL-33-pro Mut. A	ATATAAATGGCAACATGCGTTCAAAA TGGACAGT	ACTGTCCATTTTGAACGCATGTTGC CATTTATAT
IL-33-pro Mut. B	AAAATGGACAGTGCTTCGATTGAAG TTTAGGAGT	ACTCCTAAACTTCAATCGAAGCACT GTCCATTTT
Human IL-33	TGAATCAGGTGACGGTGTT	TGGTCTGGCAGTGGTTTT
Human IRF7	AGCCGTACCTGTCACCCTCC	CGCAGCAGTTCCTCCGTGT
Human GAPDH	TCAAGAAGGTGGTGAAGCA	AAGGTGGAGGAGTGGGT
Mouse IL-33	TTCTTCGTCCTTCACA	CATCTTTCTCCTCCACT
Mouse GAPDH	CCTTCCGTGTTCCCTACCC	CAACCTGGTCCTCAGTGTAG
Chip Primer for RT-PCR	GGACAGTGCTGAAGTTGAAGTTTA	CGTCCTCCCTCCATCTGAATATTT