Fig. S1

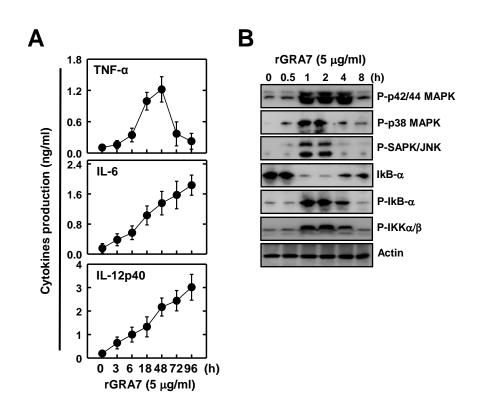
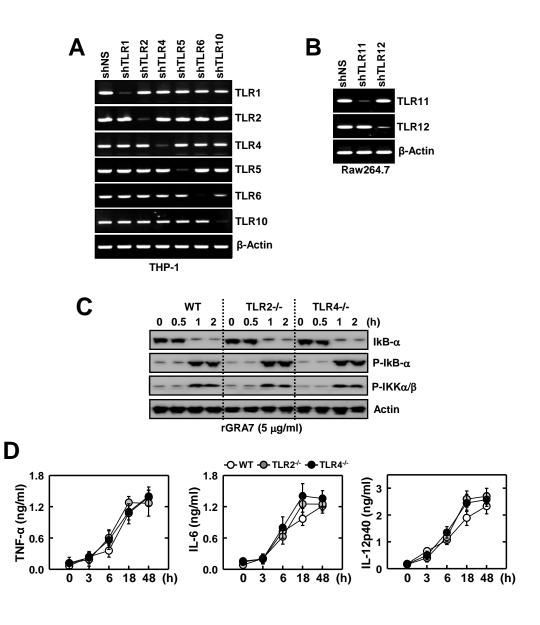
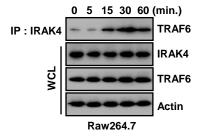
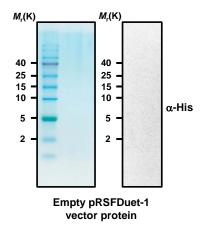


Fig. S2







Supplemental Figure Legends

FIGURE S1. rGRA7 induces MAPK activation and pro-inflammatory cytokine production in macrophages. BMDMs were stimulated with rGRA7 for the indicated lengths of time. (**A**) Culture supernatants were harvested and levels of TNF-α, IL-6, and IL-12p40 were measured by ELISA. Data shown are the means ± SD of three experiments. (**B**) Cells were harvested and then subjected to IB with the phosphorylated and total forms of MAPKs (p42/p44, p38, and SAPK/JNK), IkB-α, IKK-α/β, or Actin. The data are representative of three independent experiments with similar results.

FIGURE S2. rGRA7-mediated NF-κB activation and cytokines expression was independent on TLRs. (A and B) Semiquantitative RT-PCR was used to determine the efficiency of lentiviral transduction. THP-1 cells (A) or RAW264.7 cells (B) were transduced with lentivirus expressing nonspecific shRNA (sh-*NS*) or shRNA specific for *TLR1*, 2, 4, 5, 6, 10 (sh-*TLR1*, 2, 4, 5, 6, 10; A) or *TLR11*, 12 (sh-*TLR11*, 12; B) with polybrene (8 μg/mL). After 2 day, the cells were then harvested and subjected to semiquantitative RT-PCR for human TLR1, 2, 4, 5, 6, 10 and mouse TLR 11, 12. β-actin is as a loading control. (C and D) rGRA7 (5 μg/ml)-induce NF-κB activation (C) or cytokine production (D) in BMDMs from WT, TLR2-/-, and TLR4-/- mice for indicated times. (C) Cells were harvested and then subjected to IB with the phosphorylated and total forms of IkB-α, IKK-α/β or Actin. The data are representative of three independent experiments with similar results (A to C). Data shown are the means ± SD of three experiments (D).

FIGURE S3. rGRA7-mediated IRAK4 associates with TRAF6. RAW264.7 cells were stimulated with rGRA7 (5 μ g/ml) for the indicated times, followed by IP with α IRAK4 and IB with α TRAF6. WCLs were used for IB with α IRAK4, α TRAF6 or α Actin. The data are representative of three independent experiments with similar results.

FIGURE S4. Bacterially purified recombinant protein as a negative control. Bacterially purified 6xHis-pRSFDuet-1vector protein was analyzed by Coomassie blue staining (left) or immune Blot (IB) with αHis (right).

Online Supplemental Information

Supplementary Table 1. Human and murine primer pairs as used for RT-PCR detection in this study.

Murine	5' - 3' Forward primer	5' - 3' Reverse primer	Product (bp)
TNF-α	gtcaacctcctctctgccat	ccaaagtagacctgcccaga	188
IL-6	agtcctgatccagttcctgc	aagctgcgcagaatgagatg	150
IL-1β	ggagaatgacctgagcacct	ggaggtggagagctttcagt	185
IL-12p40	cagaggggacaacaaggagt	tccacctgccgagaattctt	219
IL-12p35	gccttcaccactcccaaaac	atggtaaacaggcctccact	153
TLR11	ggcagaggctccatagttac	ccgtctcttcagttgctcac	116
TLR12	cgcttatgtccaggacaaga	gaggagaggcaagccaatta	144
β-actin	cgagcgtggctacagcttca	aggaagaggatgcggcagtg	122
Human	5' - 3' Forward primer	5' - 3' Reverse primer	Product (bp)
TLR1	aactctgctgatcgtcacca	ccagaaagaatcgtgcccac	214
TLR2	aagggcagctcaggatcttt	agactgcccagggaagaaaa	197
TLR4	acctccccttctcaaccaag	ggctctgatatgccccatct	150
TLR5	ctgactcgttctctggggtt	cccggaactttgtgactgtg	156
TLR6	acatgattctgcctgggtga	tcgtaatggcaccactcact	195
TLR10	ccacacatgcttttcccgaa	ccaagtgttccaagggtgtg	193
β-actin	ggtggcttttaggatggcaag	actggaacggtgaaggtgacag	161