





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 \pm 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), I κ B- α , IKK- α/β , or Actin. The data are representative of three independent experiments with similar results.

FIGURE S2. rGRA7-mediated NF- κ B 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 μ g/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 μ g/ml)-induce NF- κ B 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 I κ B- 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 \pm SD of three experiments **(D)**.

FIGURE S3. rGRA7-mediated IRAK4 associates with TRAF6. RAW264.7 cells were stimulated with rGRA7 (5 µg/ml) for the indicated times, followed by IP with αIRAK4 and IB with αTRAF6. WCLs were used for IB with αIRAK4, αTRAF6 or αActin. 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)
<i>TNF-α</i>	gtcaacctctctctgccat	ccaaagtagacctgcccaga	188
<i>IL-6</i>	agtctgatccagttcctgc	aagctgcgcagaatgagatg	150
<i>IL-1β</i>	ggagaatgacctgagcacct	ggaggtggagagcttcagt	185
<i>IL-12p40</i>	cagagggggacaacaaggagt	tccacctgccgagaattctt	219
<i>IL-12p35</i>	gccttcaccactcccaaac	atggtaaacaggcctccact	153
<i>TLR11</i>	ggcagaggctccatagttac	ccgtctctcagttgctcac	116
<i>TLR12</i>	cgcttatgtccaggacaaga	gaggagaggcaagccaatta	144
<i>β-actin</i>	cgagcgtggctacagctca	aggaagaggatgcggcagtg	122
Human	5' - 3' Forward primer	5' - 3' Reverse primer	Product (bp)
<i>TLR1</i>	aactctgctgatcgtcacca	ccagaaagaatcgtgccac	214
<i>TLR2</i>	aagggcagctcaggatcttt	agactgccaggggaagaaaa	197
<i>TLR4</i>	acctccccttcaaccaag	ggctctgatatgccccatct	150
<i>TLR5</i>	ctgactcgttctctgggggtt	cccggaactttgtgactgtg	156
<i>TLR6</i>	acatgattctgcctgggtga	tcgtaatggcaccactcact	195
<i>TLR10</i>	ccacacatgctttcccga	ccaagttccaaggtgtg	193
<i>β-actin</i>	ggtggcttttaggatggcaag	actggaacggtgaaggtgacag	161