

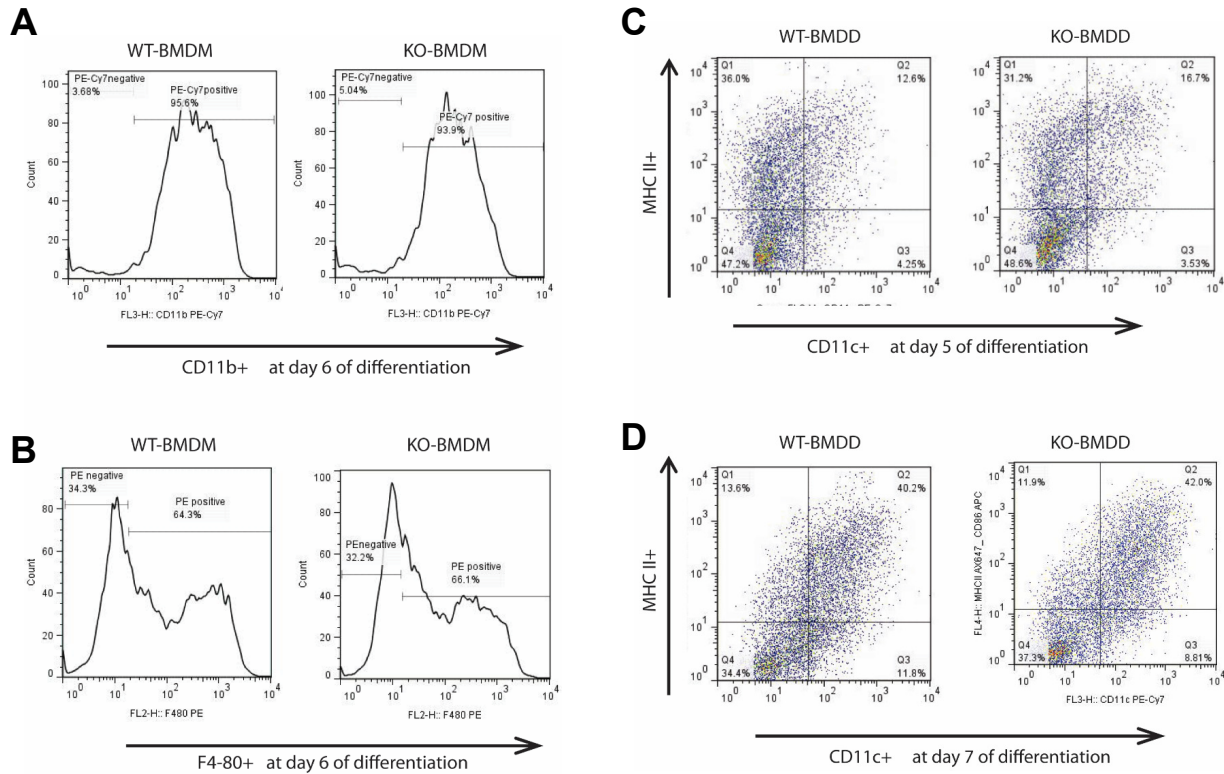
p204 is Required for Canonical Lipopolysaccharide-Induced TLR4 Signaling in Mice

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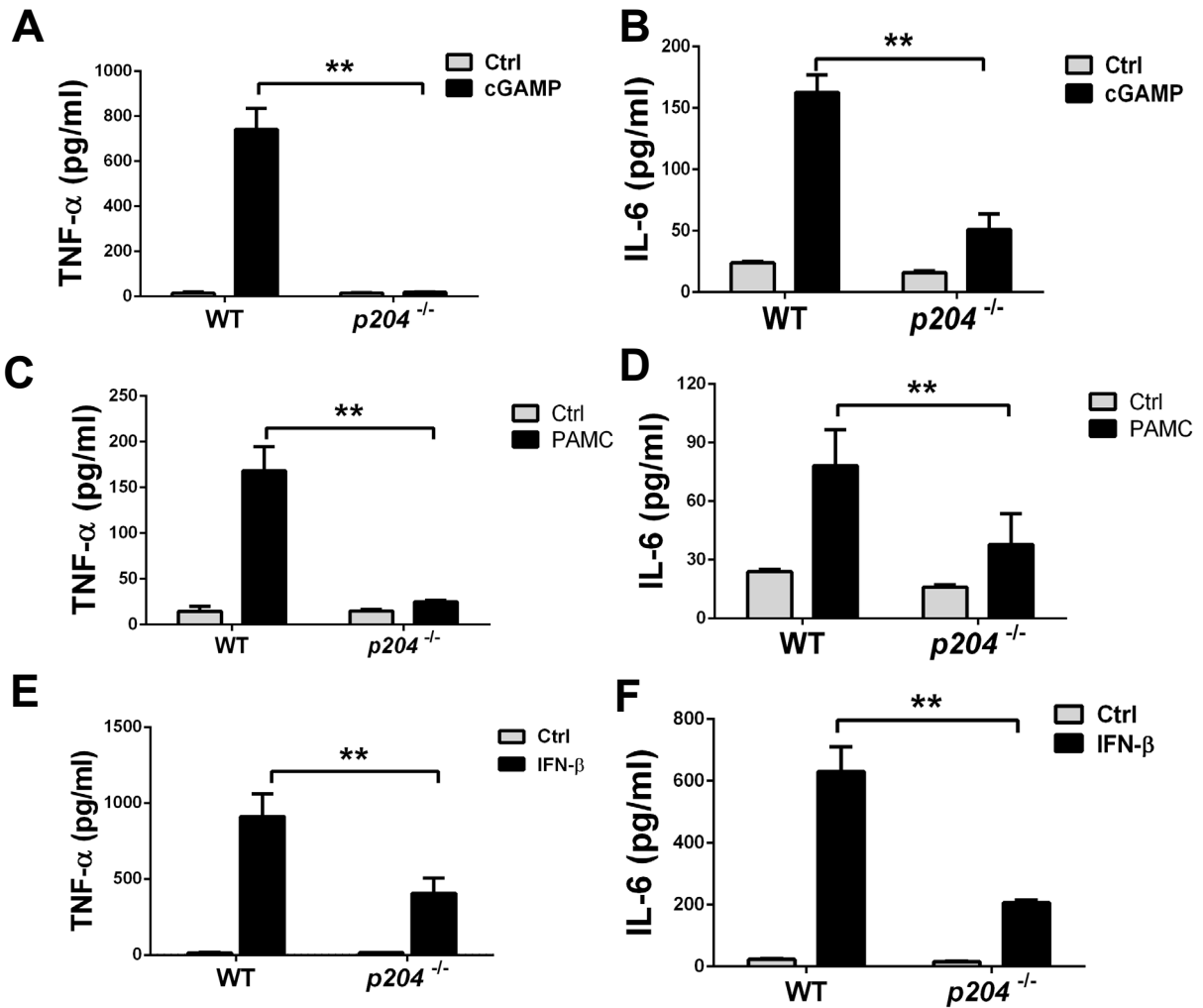
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Figs. S1 to S4
Table S1



Supplementary Fig. 1. p204 does not affect the differentiations of bone marrow derived macrophages (BMDMs) and dendritic cells (BMDDs). Bone marrow cells were isolated from WT and *p204*^{-/-} (KO) mice, and differentiated into BMDMs and BMDDs in vitro. The population of BMDMs was stained with two macrophage markers, CD11b (A) and F4-80 (B). The population of BMDDs was stained with dendritic cells markers, CD11c⁺, at two time points of differentiation (C-D).



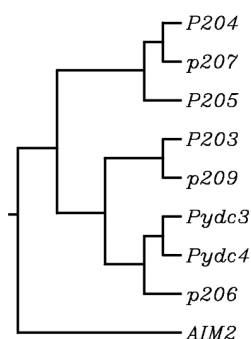
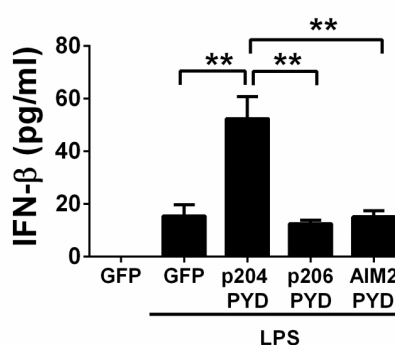
Supplementary Fig. 2. BMDMs from *p204*^{-/-} mice show defected response to the productions of pro-inflammatory cytokines, TNF and IL-6, triggered by cGAMP, PAMC and IFN- β . BMDMs from WT and *p204*^{-/-} mice were treated with cGAMP (50 nM) (A-B), PAMC (Pam₃Cys, 100ng/ml) (C-D), and IFN- β (10ng/ml) for 24 hours, and the levels TNF α (A, C, E) and IL-6 (B, D, F) in culture medium were measured by ELISA. ** stands for $p < 0.01$.

A

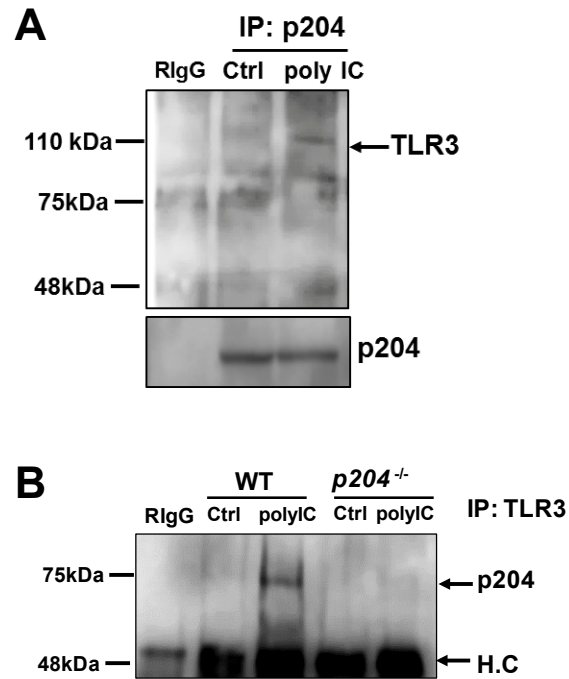
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P204 -----HYFSLFKSLLARDLNLERDNQEYTTIQIANMMEEKFPADSGLGKLI AFCEEVPALRKRAEILK-----
p207 MVKEYKRIVLLKGLCEINKHHFSLFKSLLARDLSLERDNQEKYSTIQIANMMEEKFKPDAGLGELIEFCEKVPALRKRAEILKKERSE
P205 -----HQFNLFKSLMVKDLNLEEDNQEKYTTTFQIANMMVKKFPADAGLDRLLNFCEVPTLRKRAEILK-----
P203 -----YQFRTVKSLRRLKELKLTCKMQEDYDRIQADWMEKFPKADAGLDKLIKVCEHIKDLKDLAKKLG-----
p209 -----HDFRMVKSLLSKELKLN-RMQDQYDRVKIADLMEDKFPKADAGVDQLIKLYKQIPGLGDIANKLK-----
Pydc3 -----LLSGLEYMNDYNFRALKSLLNHDLKLTKNMQDDYDRIKIADLMEEKFPEDAGLSKLEI VCEDIPELAARVD-----
Pydc4 -----LLSGLEYMNDYNFRALKSLLNHDLKLTKNMQDDYDRINIADLMEEKFPEDAGLSKLEI VCEDIPELAARVD-----
p206 -----YNFRALKSLLNHDLKLTKNMQDDYDRIKIADLMEEKFPEDAGLSKLEI VCEDIPELADHV DILR-----
AIM2 -----EELDRFKFPLSDEFNIATGKLHTANRIQVATLMIQNAGAVSAVMKTI RIFQKLN YMLLAKRLQ-----
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B**C**

Supplementary Fig. 3. Sequence analysis of Pyrin domain of murine p200 family members. (A) The sequence of the Pyrin domain of p200 family members was obtained from GenBank, and aligned with Clustal Omega software. The unique three positive charged amino acids are marked in red. (B) Phylogenetic Tree of murine p200 family members. (C) Pyrin domains of p206 and AIM2 fail to restore the response of *p204*-deficient cells to LPS. GFP vector, or Pyrin domain from p204, p206 and AIM2 was transiently transfected into *p204*-deficient Raw cells. 24 hours after transfection, the cells were treated with LPS (100ng/ml) for 24 hours. The levels of IFN- β in the cell culture medium was measured by ELISA.



Supplementary Fig. 4 p204 binds to TLR3. (A) BMDMs from WT mice were treated with poly I:C (20 μ g/ml) for 2 hour. Antibodies against p204 were used for IP and the immunoprecipitated complexes were probed with anti-TLR3 antibodies. (B) BMDMs from WT and *p204* KO mice were treated with poly I:C (20 μ g/ml) for 2 hour, and anti-TLR3 antibodies were used for IP, and immunoprecipitated complexes were probed with anti-p204 antibodies.

Table 1. Sequences of PCR primers used in this study

Targets		Sequences (5' to 3')
p204-1	For	CTT CAG TCC ACG TAC CAA GAG C
	Rev	GCT AGA GCA AGA TAC CAA CAC TCG
p204-2	For	GGG AGA TTG TGA AGC CAT AGA GC
	Rev	AAG GGT TAT TGA ATA TGA TCG GA
IFN- β	For	CAG CTG AAT GGA AAG ATC AAC C
	Rev	GCT GGA GAA ATT GTT TCT GAA G
IFN- α	For	ATG AGA AGT TCC CAA ATG GC
	Rev	CTC CAC TTG GTG GTT TGC TA
IL-6	For	AGT CCG GAG AGG AGA CTT CA
	Rev	ATT TCC ACG ATT TCC CAG AG
IL-1 β	For	TGT AAT GAA AGA CGG CAC ACC
	Rev	TCT TCT TTG GGT ATT GCT TGG
GAPDH	For	AGA ACA TCA TCC CTG CAT CC
	Rev	AGT TGC TGT TGA AGT CGC