Supplementary Data

Design, synthesis and biological evaluation of novel antagonist compounds of Toll-like receptors 7, 8 and 9

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One Table (Table S1) and Twelve Figures (Figs. S1-S12) available.

Table S1. IC_{50} of antagonist compounds for inhibition of TLR7-, 8- and 9-mediated NF-κB activation in HEK293 cells

Antagonist #	IC_{50}, μg/ml antagonist		
	TLR7	TLR8	TLR9
1	0.01	0.021	0.368
2	0.005	0.005	0.229
3	0.004	0.021	0.174

Figure S1

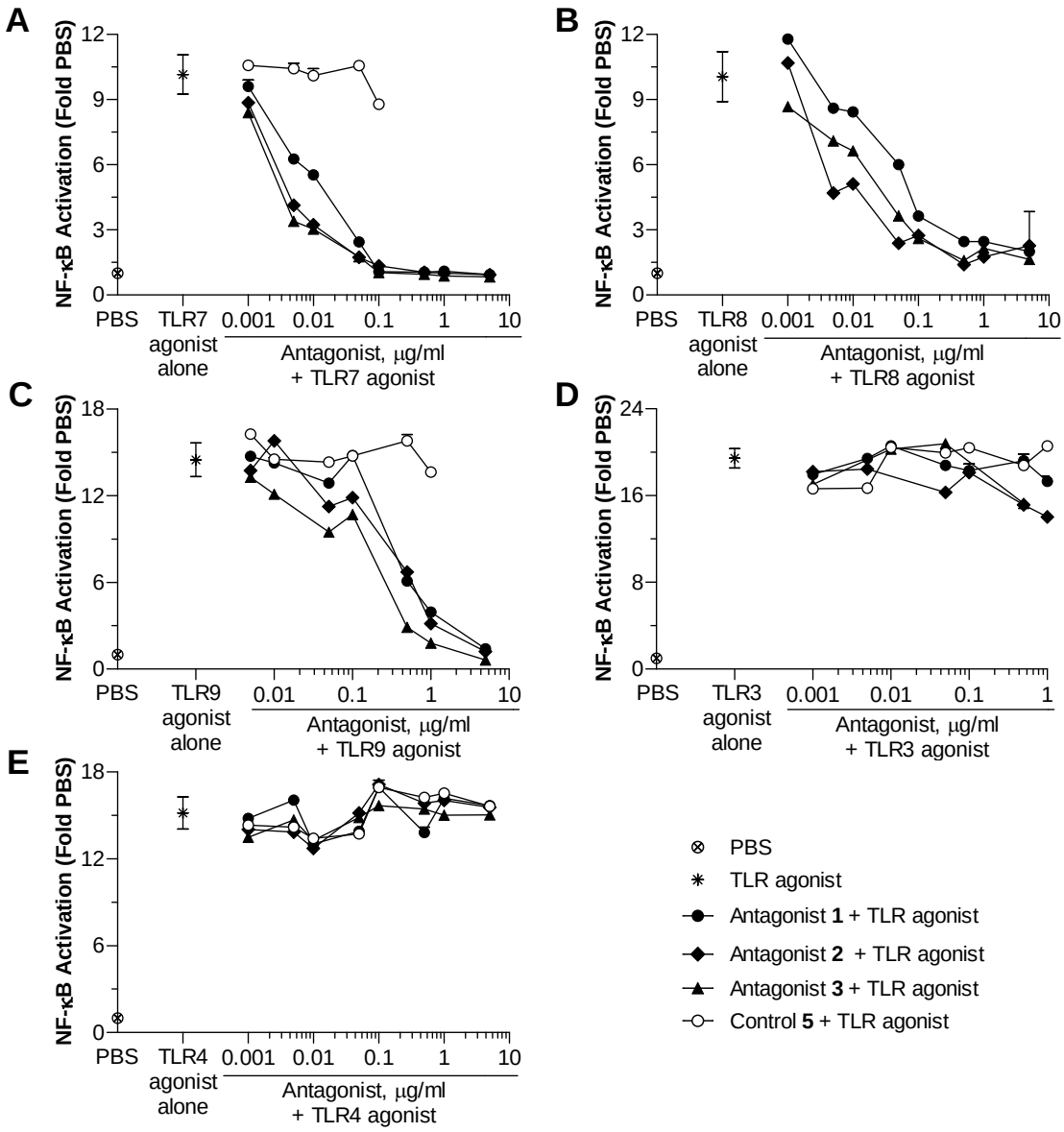

Figure S1. Antagonists do not show NF-κB activation in HEK293 cells expressing A) human TLR7, B) human TLR8, C) mouse TLR9, D) human TLR3 and E) human TLR4. Experiments were carried out as described in Figure 1 legend and Materials and Methods Section in the main text.

Figure S2

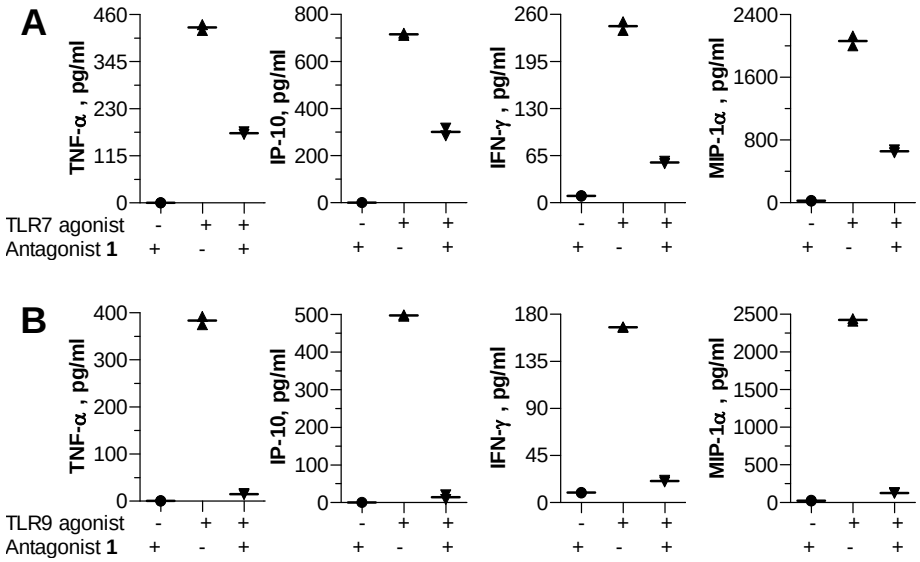

Figure S2. Inhibition of **A**) TLR7 and **B**) TLR9 agonist-induced cytokines by antagonist **1** in mouse spleen cell cultures. Spleen cells were incubated with TLR7 agonist (50 μg/ml), TLR9 agonist (1 μg/ml) or TLR4 agonist (1 μg/ml) in the absence or presence of 10 μg/ml concentration of antagonist **1** for 24 hr. Cell culture supernatants were assessed for cytokine levels by Luminex multiplex assay. Each data point represents one well and black bar represents mean and the data are representative of three independent experiments. IL-12 and IL-6 are shown in Figure 2 in the main text.

Figure S3

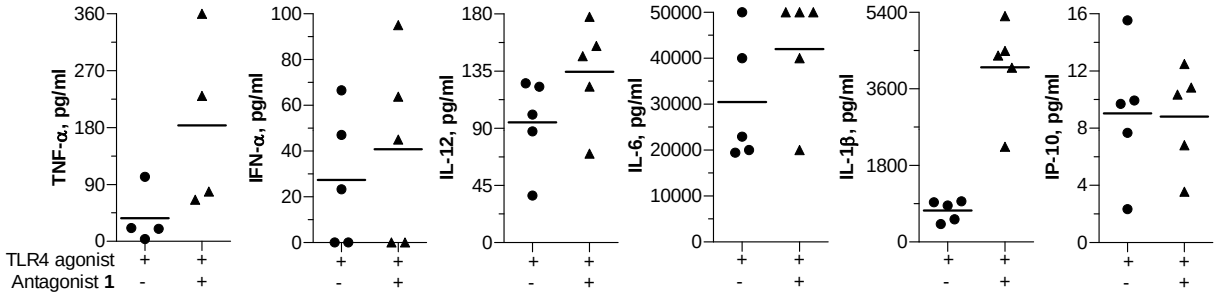

Figure S3. Effect of antagonist **1** on TLR4 agonist-induced cytokines in human PBMCs. Human PBMCs from healthy volunteer blood were isolated and cultured with 0.1 μg/ml TLR4 agonist in the absence or presence of 10 μg/ml antagonist **1** for 24 hr. Supernatants were analyzed for cytokine levels by Luminex multiplex assay. Each data point represents one donor and black line indicates mean of all donors.

Figure S4

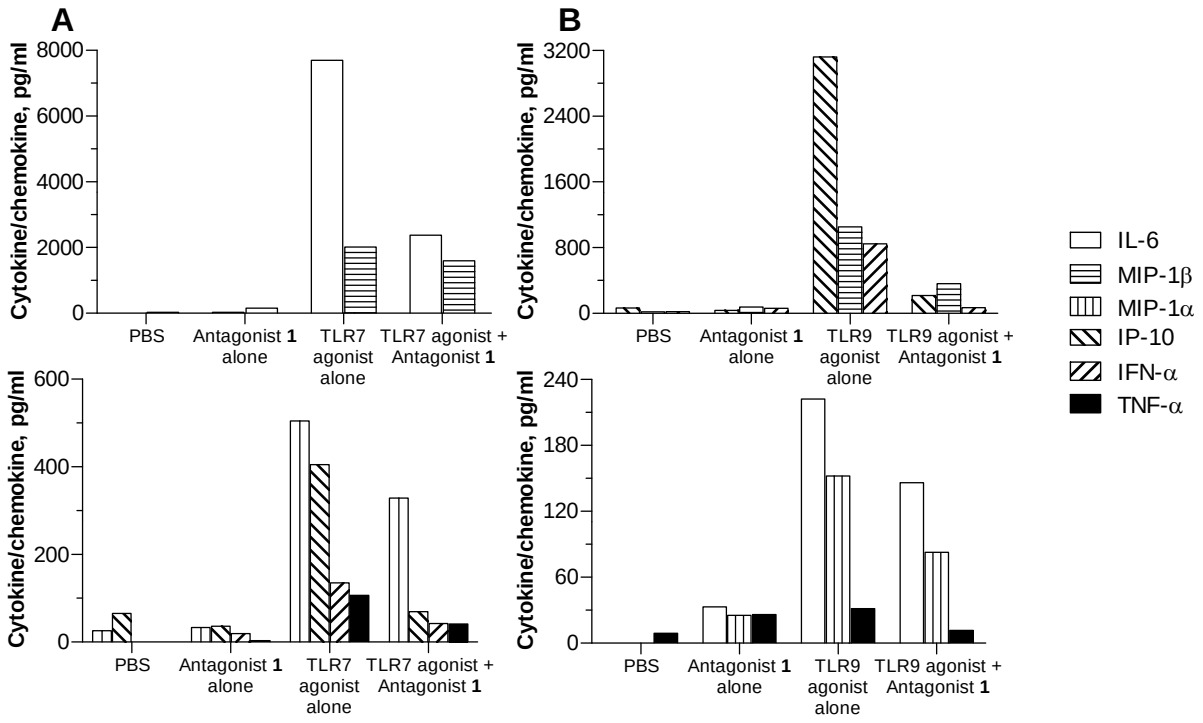

Figure S4. Antagonist compound **1** inhibits **A**) TLR7-, and **B**) TLR9-mediated cytokine induction in PBMCs obtained from lupus patient blood. PBMCs from lupus patient blood were isolated and cultured with 50 μg/ml TLR7 agonist or 3 μg/ml TLR9 agonist in the absence or presence of 10 μg/ml antagonist compound **1** for 24 hr. Supernatants were analyzed for cytokine levels by Luminex multiplex assay. Data shown are for one representative donor of three individual donors studied.

Figure S5

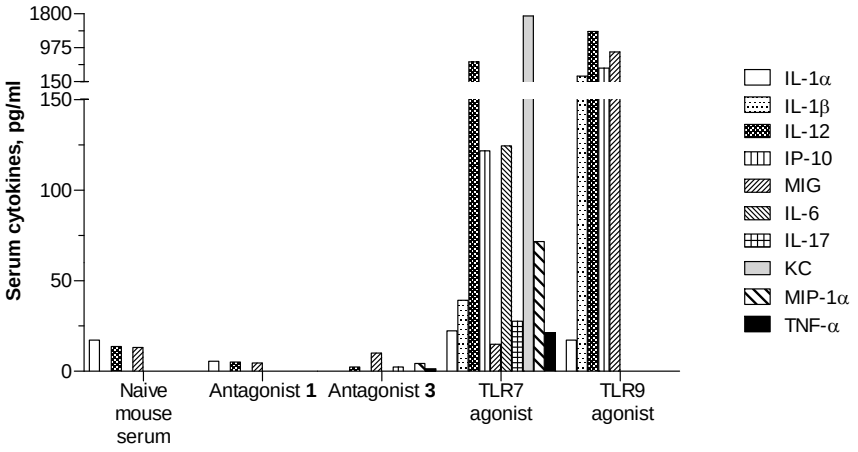

Figure S5. Antagonists do not induce cytokines in mice. C57BL/6 mice (N = 3) were injected s.c. with a dose of 15 mg/kg of antagonist **1** or **3**, 10 mg/kg TLR7 agonist or 0.25 mg/kg TLR9 agonist. Two hours post oligo administration, blood was collected and serum cytokines were determined by multiplex analysis. Naïve mouse serum was used as control. Data shown are representative of three independent experiments.

Figure S6

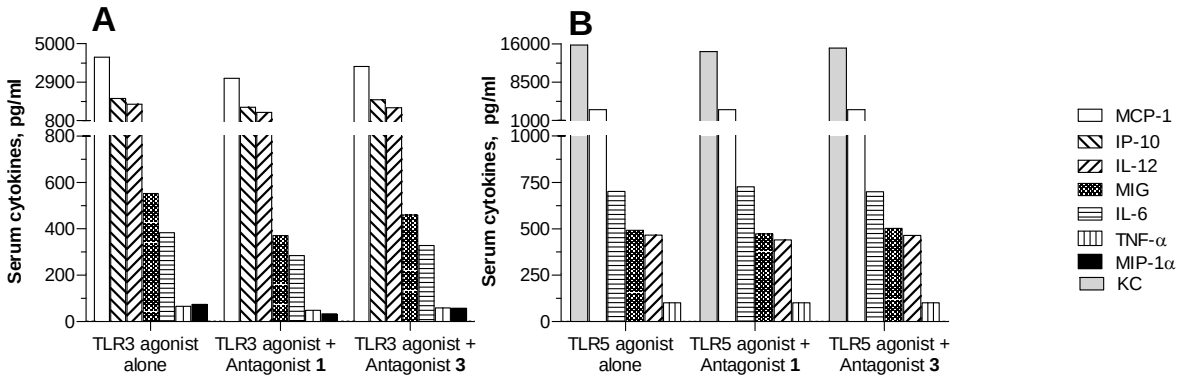

Figure S6. Antagonist compounds have minimal effect on **A**) TLR3- and **B**) TLR5-mediated cytokine induction in mice. C57BL/6 mice (N = 3) were s.c. injected with 5 mg/kg antagonist compound **1** or **3**, 2h hr later an agonist of TLR3 (25 mg/kg) or TLR5 (0.25 mg/kg) was s.c. injected. Blood was drawn 2 hr post TLR agonist administration and serum cytokines were measured by luminex multiplex assay. Data shown are representative of two independent experiments.

Figure S7

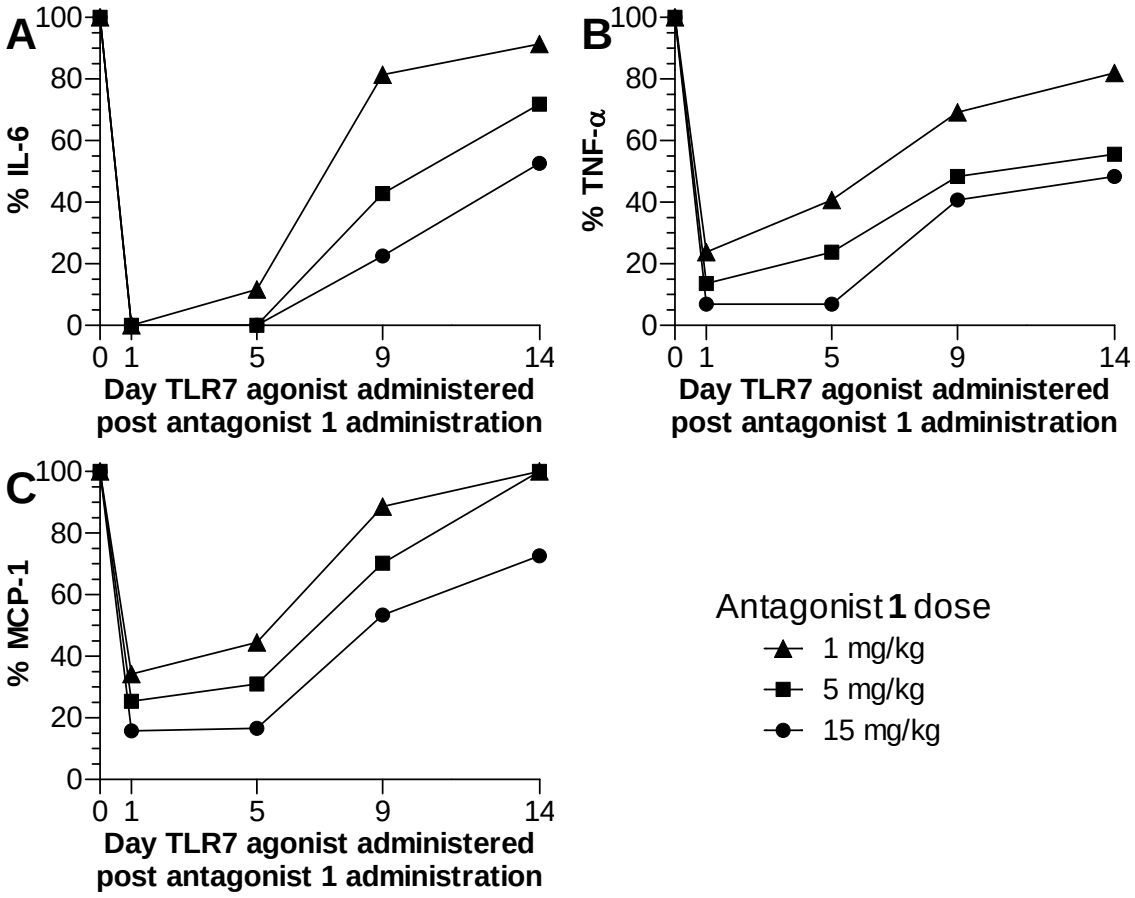

Figure S7. Effect of dose of antagonist **1** on TLR7 agonist induced **A**) IL-6, **B**) TNF-α and **C**) MCP-1 and duration of antagonist activity in mice. C57BL/6 mice (N = 3) were injected s.c. with 1, 5 or 15 mg/kg antagonist **1**. After 1, 5, 9, or 14 days of antagonist administration an agonist of TLR7 (10 mg/kg) was injected s.c. Blood was drawn 2 hr post TLR7 agonist administration at each time point and serum cytokines were measured by luminex multiplex assay. Data shown are representative of two independent experiments.

Figure S8

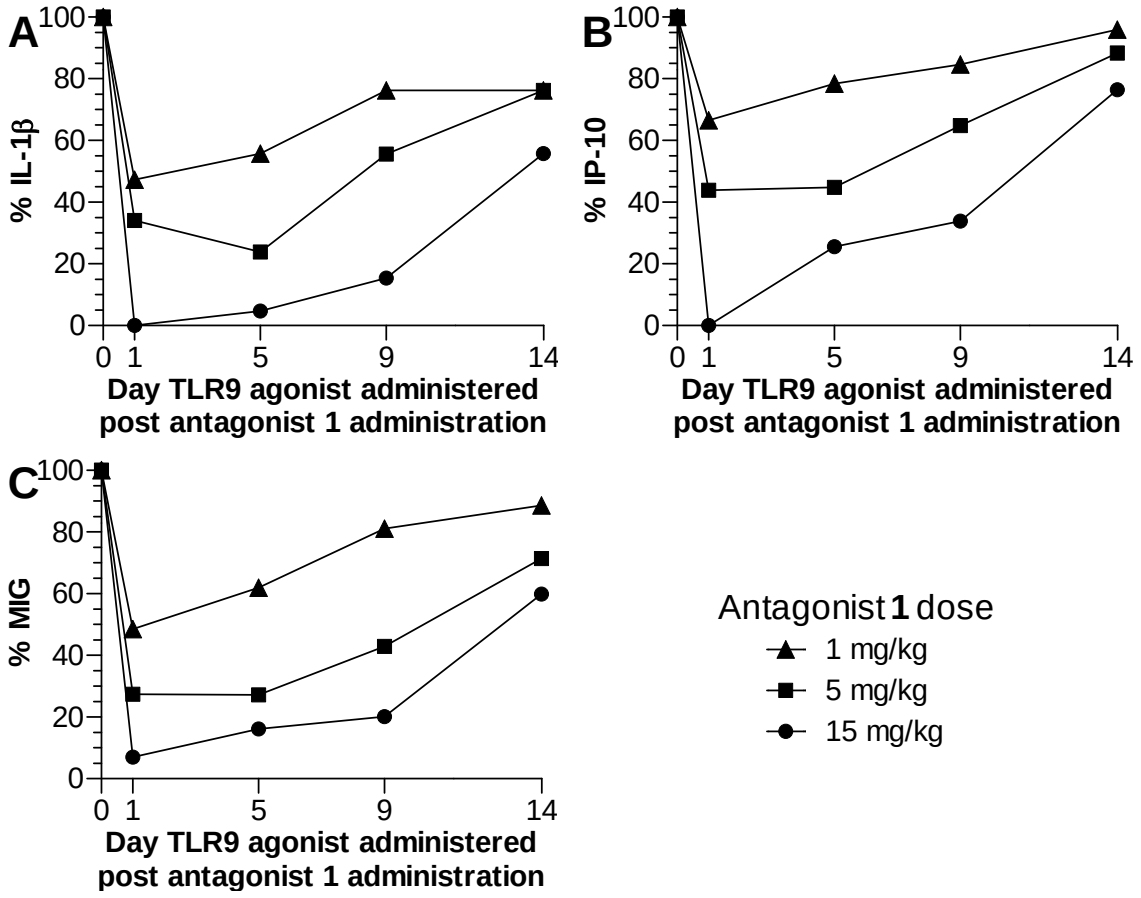

Figure S8. Effect of dose of antagonist **1** on TLR9 agonist induced **A**) IL-1β, **B**) IP-10 and **C**) MIG and duration of antagonist activity in mice. C57BL/6 mice (N = 3) were injected s.c. with 1, 5 or 15 mg/kg antagonist **1**. After 1, 5, 9, or 14 days of antagonist administration, TLR9 agonist (0.25 mg/kg) was injected s.c. Blood was drawn 2 hr post TLR9 agonist administration at each time point and serum cytokines were measured by Luminex multiplex assay. Data shown are representative of two independent experiments.

Figure S9

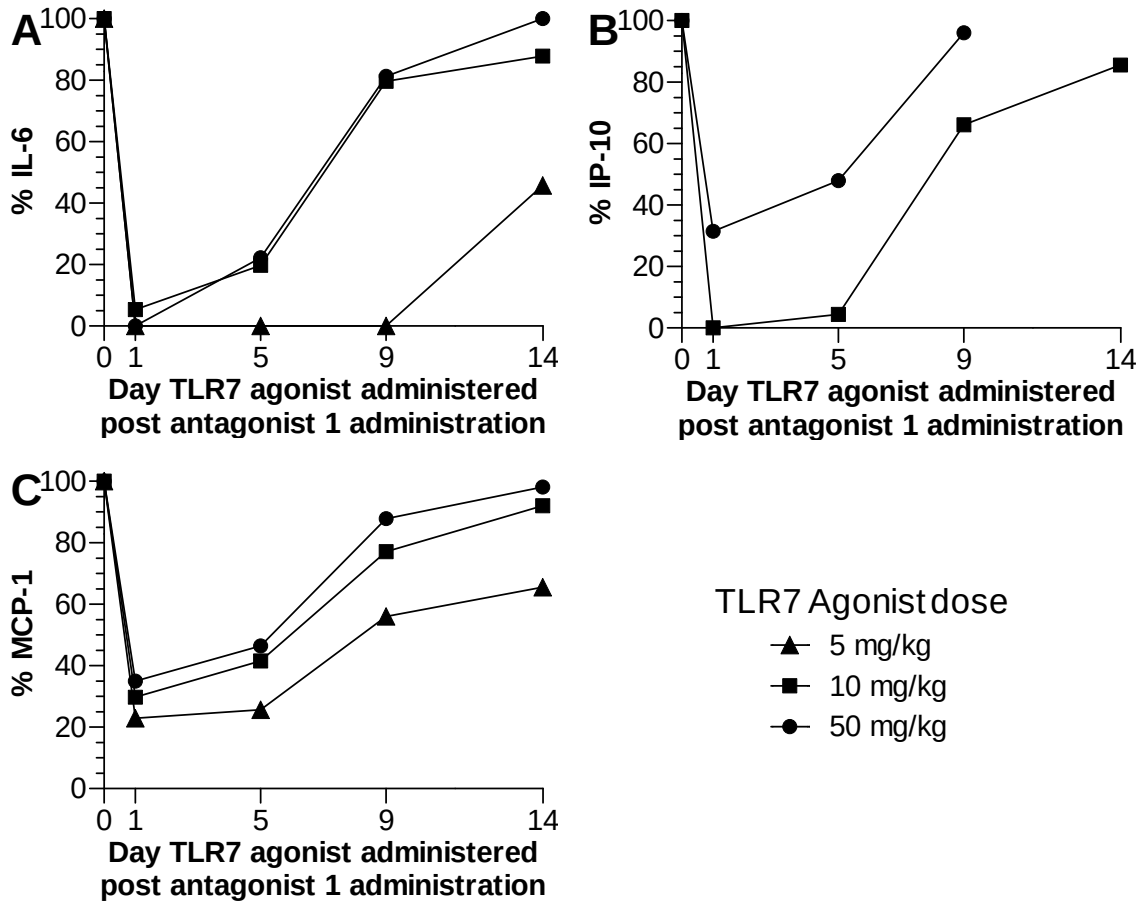

Figure S9. Effect of TLR7 agonist dose on the extent and duration of **A**) IL-6, **B**) IP-10 and **C**) MCP-1 inhibition by antagonist **1** in mice. C57BL/6 mice (N = 3) were injected s.c. with 5 mg/kg antagonist **1**. After 1, 5, 9, or 14 days of antagonist administration, an agonist of TLR7 (5, 10 or 50 mg/kg) was injected s.c. Blood was drawn 2 hr post TLR7 agonist administration at each time point and serum cytokines were measured by luminex multiplex assay. Data shown are representative of two independent experiments.

Figure S10

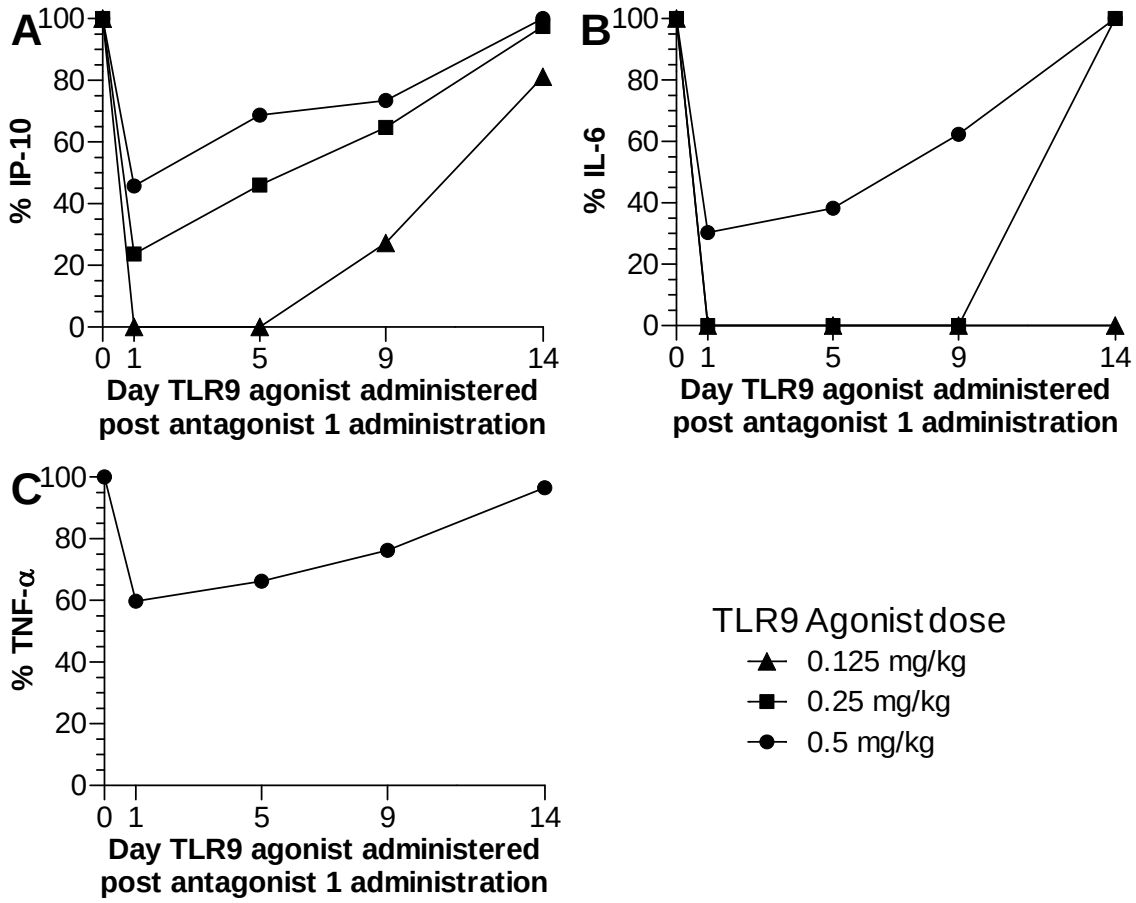

Figure S10. Effect of TLR9 agonist dose on the extent and duration of inhibition of **A**) IP-10, **B**) IL-6 and **C**) TNF-α by antagonist **1** in mice. C57BL/6 mice (N = 3) were injected s.c. with 5 mg/kg antagonist **1**. After 1, 5, 9, or 14 days of antagonist administration, TLR9 agonist (0. 125, 0.25 or 0.5 mg/kg) was injected s.c. Blood was drawn 2 hr post TLR9 agonist administration at each time point and serum cytokines were measured by luminex multiplex assay. Data shown are representative of two independent experiments.

Figure S11

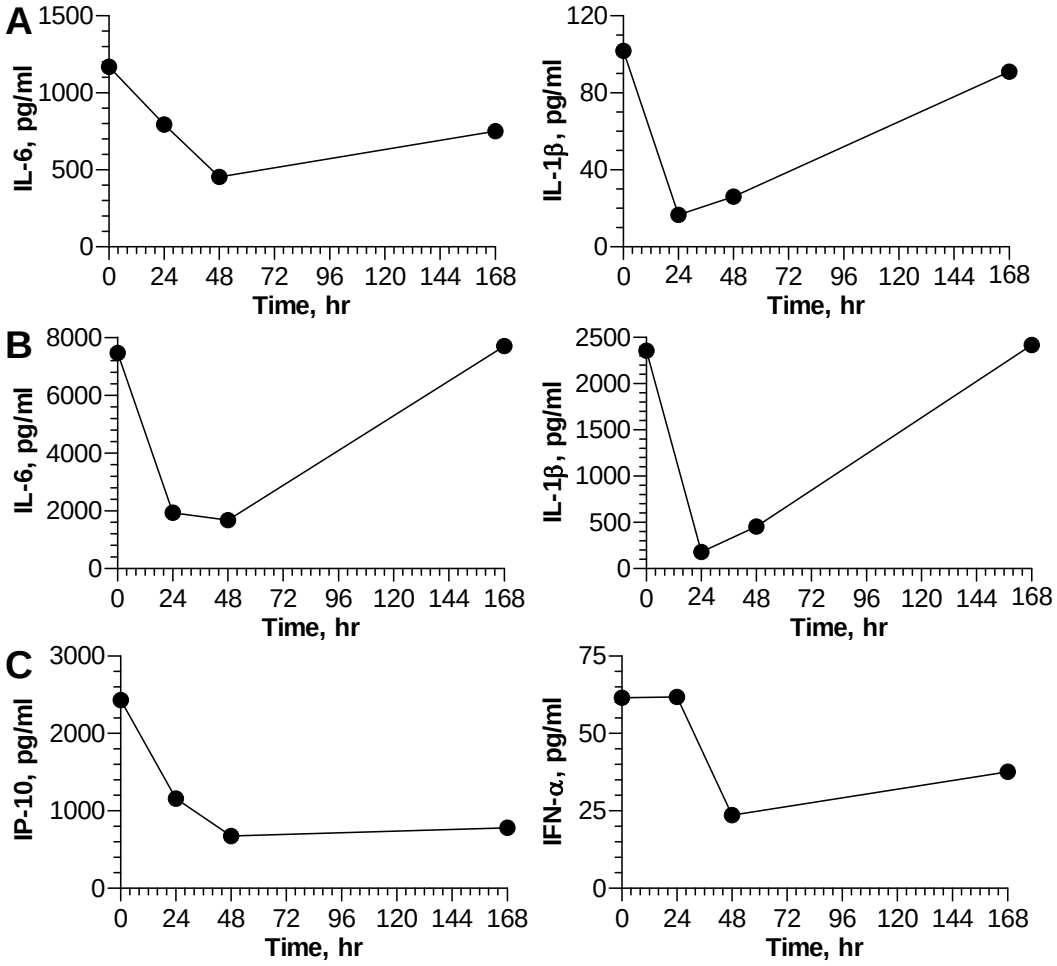

Figure S11. Duration of inhibition of cytokines in PBMCs of non-human primates treated with antagonist **1** in response to **A**) TLR7, **B**) TLR8 and **C**) TLR9 agonist stimulation. Cynomolgus monkeys (N = 4) were injected s.c. with 1.5 mg/kg antagonist **1** and collected blood at pre-dose and various post-dose time points. PMBCs were isolated and stimulated for 24 with TLR7 (50 μg/ml), TLR8 (50 μg/ml), and TLR9 (3 μg/ml) agonists. Cell culture supernatants were analyzed for cytokine levels using a multiplex assay. Each individual data point indicates mean of four animals.

Figure S12

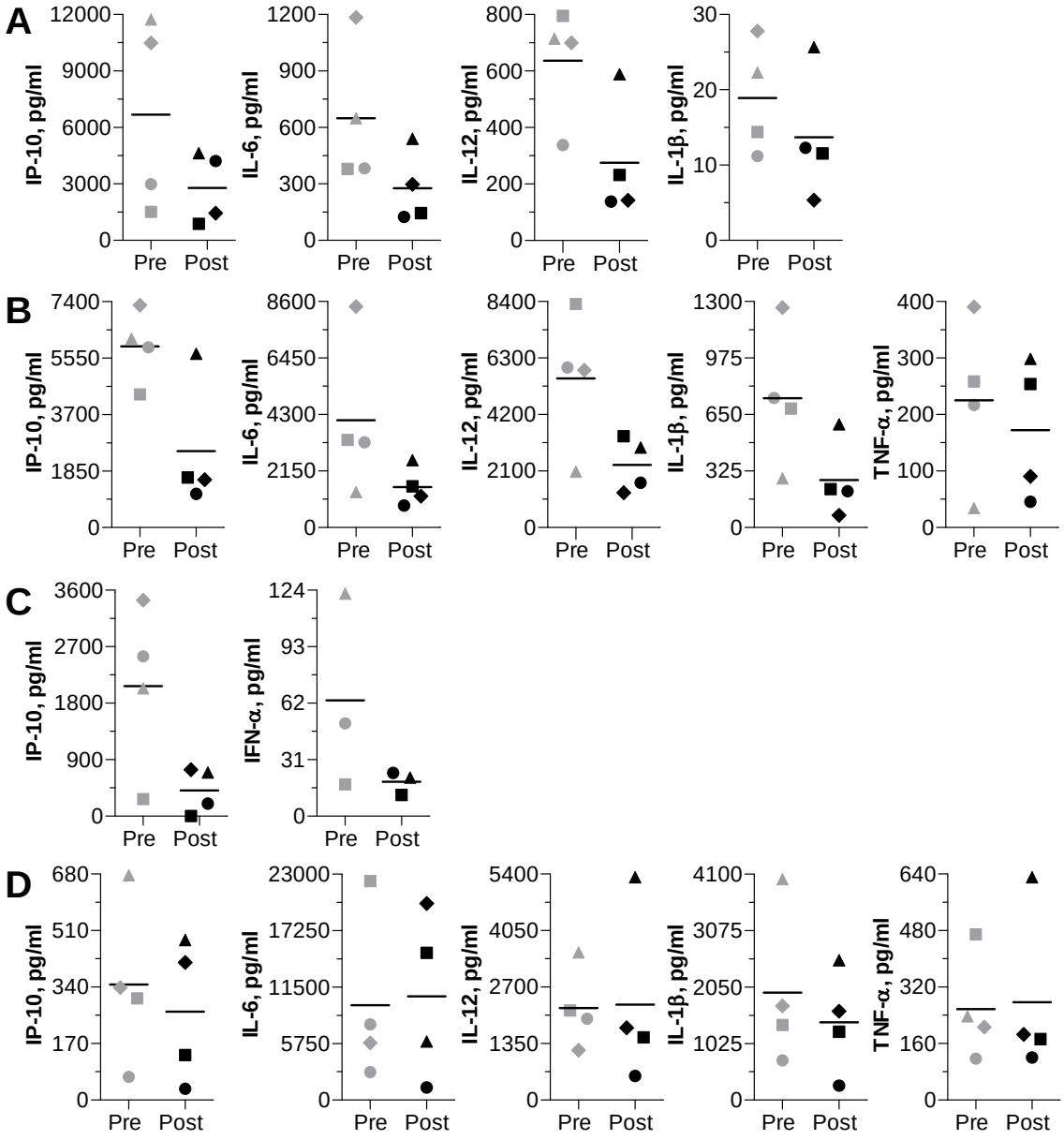

Figure S12. Antagonist compound **3**-treated non-human primate PBMCs show reduced cytokine induction in response to **A**) TLR7, **B**) TLR8 and **C**) TLR9, but not **D**) TLR4, agonist stimulation. Cynomolgus monkeys (N = 4) were s.c. injected with 1.5 mg/kg

antagonist compound **3** and collected blood at pre-dose and various post-dose time points. PMBCs were isolated and stimulated for 24 with TLR7 (50 μg/ml), TLR8 (50 μg/ml), TLR9 (3 μg/ml) and TLR4 (0.1 μg/ml) agonists. Cell culture supernatants were analyzed for cytokine levels using a multiplex assay. Pre and Post indicate pre-dose and 48 hr post-dose time points. Each individual data point indicates data for one animal and black line indicates mean of all animals.