

Supplementary Information

**The monomeric but not homotrimeric spike protein of SARS-CoV-2
activates TLR4 signaling**

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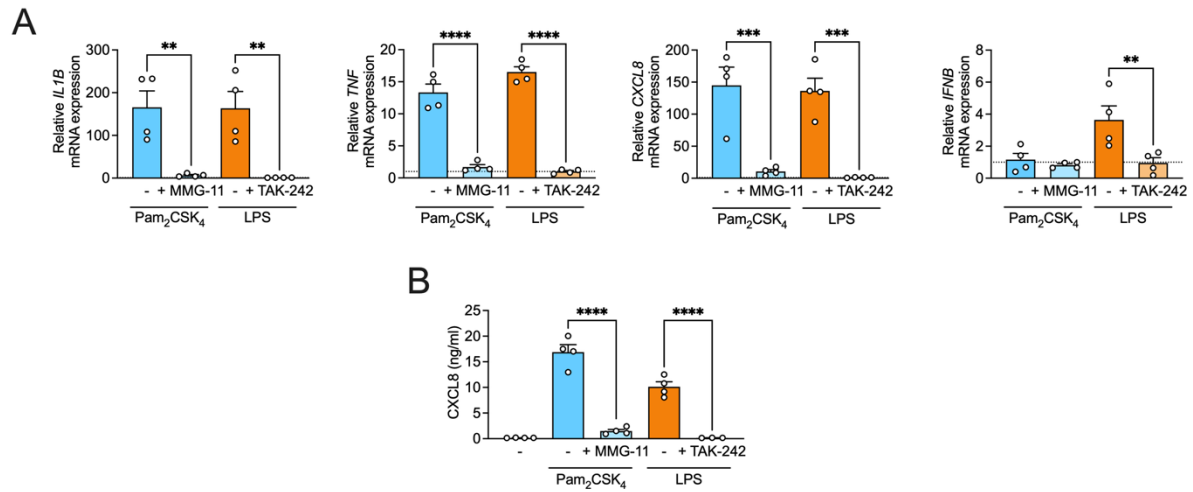
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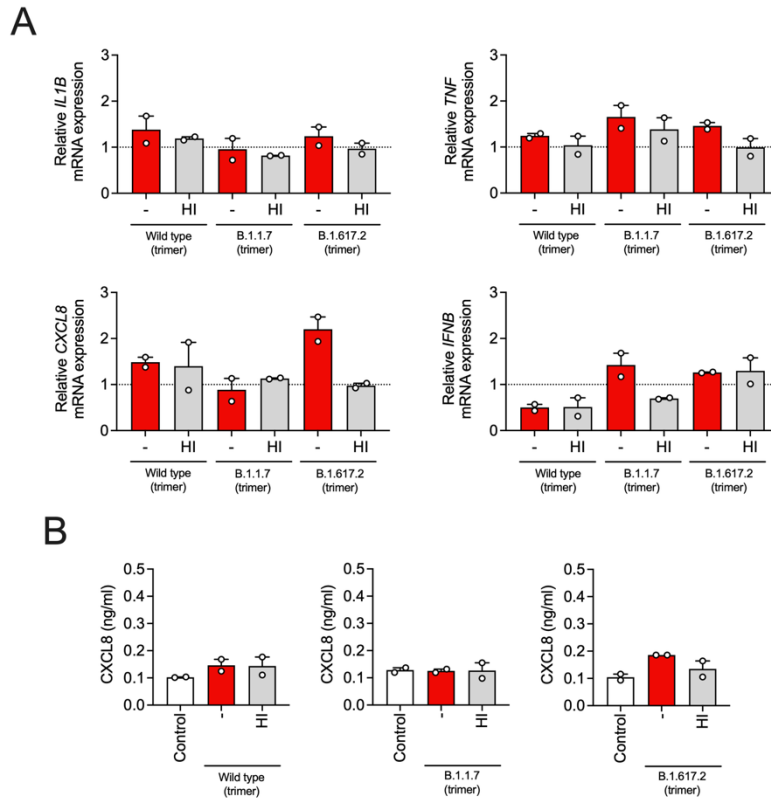
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Supplementary Figures 1 and 2

Supplementary figures



Supplementary Fig. 1. (A, B) THP-1 macrophages were preincubated with or without the TLR2 antagonist MMG-11 (50 μ M) or the TLR4 antagonist TAK-242 (2.8 μ M) for 1 h and subsequently stimulated with Pam₂CSK₄ (1 ng/ml) or LPS (10 ng/ml) for 4 h. **(A)** Gene expression of *IL1B*, *TNF*, *CXCL8*, and *IFNB* were normalized to the housekeeping gene *GAPDH* (control assigned as 1.0, dotted line). **(B)** Cell culture supernatants were analyzed by ELISA for CXCL8. Mean + SEM ($n = 4$). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ANOVA followed by Bonferroni posttest.



Supplementary Fig. 2. (A, B) THP-1 macrophages were stimulated with trimeric recombinant SARS-CoV-2 spike protein wild type, B.1.1.7 (alpha variant), or B.1.617.2 (delta variant), or the corresponding heat-inactivated proteins (HI) (each at a concentration of 133 nM) for 4 h. **(A)** Gene expression values of *IL1B*, *TNF*, *CXCL8*, and *IFNB* were normalized to the housekeeping gene *GAPDH* (control assigned as 1.0, dotted line). **(B)** Cell culture supernatants were analyzed by ELISA for CXCL8. Mean + SEM ($n = 2$).