

SUPPLEMENTARY INFORMATION

for

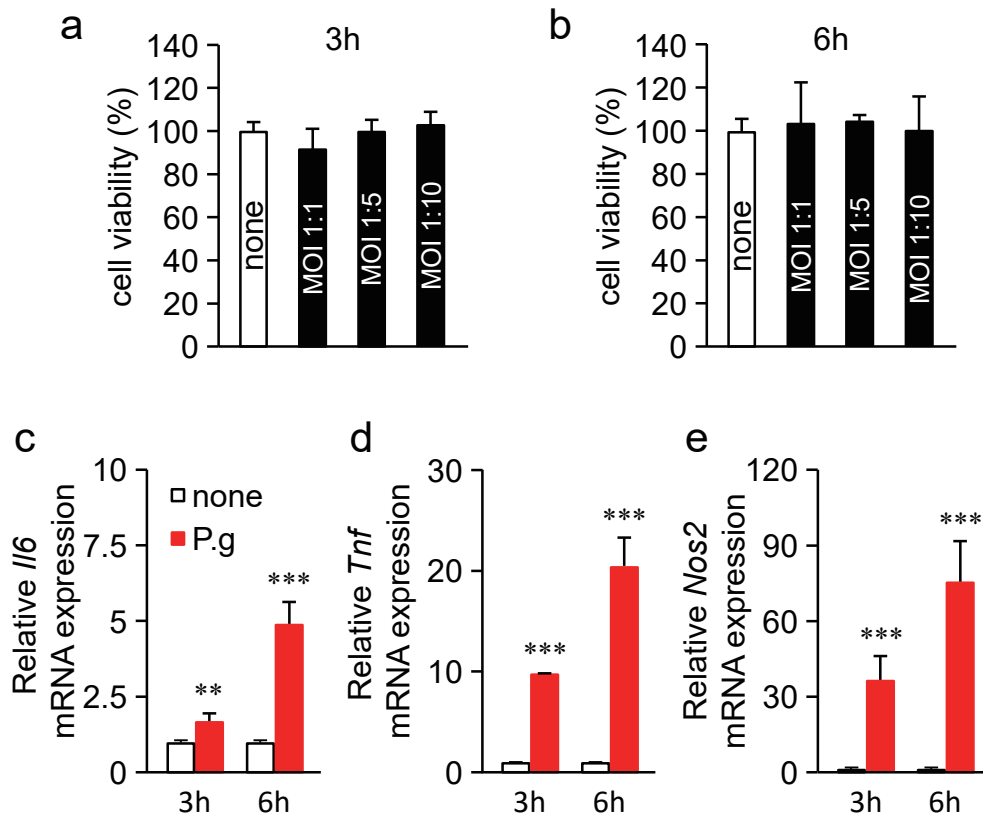
Diurnal dynamic behavior of microglia in response to infected bacteria through the
UDP-P2Y₆ receptor system

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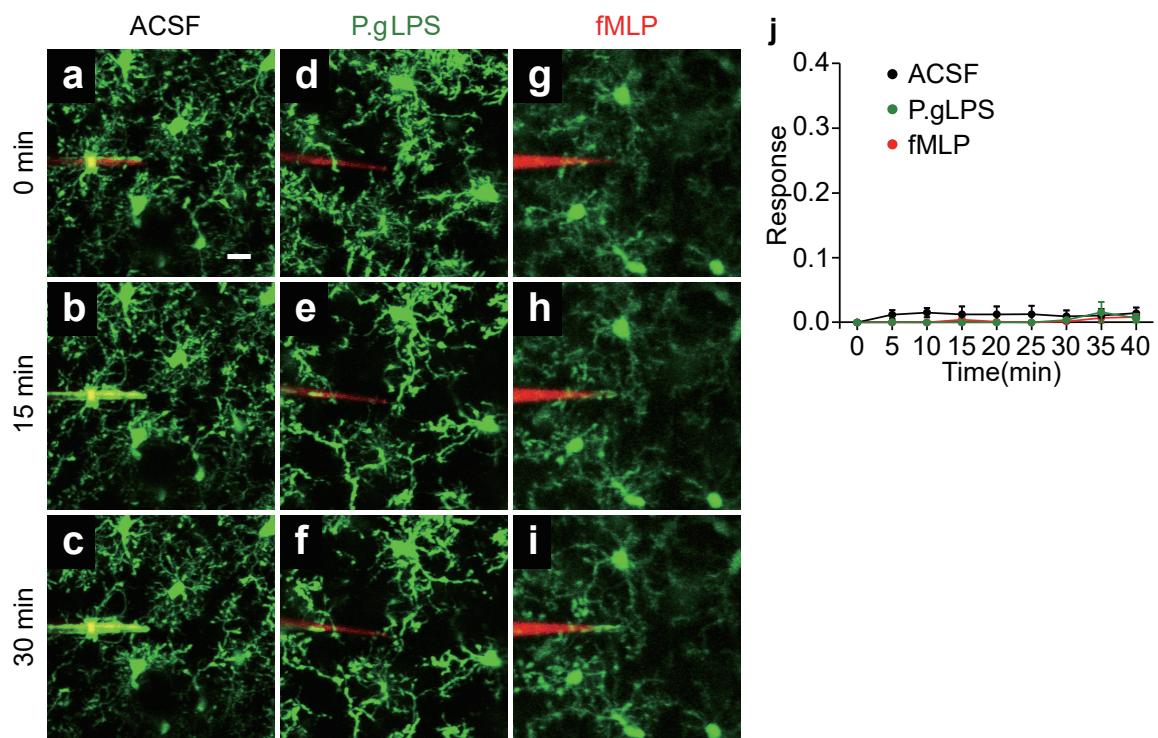
Supplementary information includes:

Supplementary Figures S1-S5

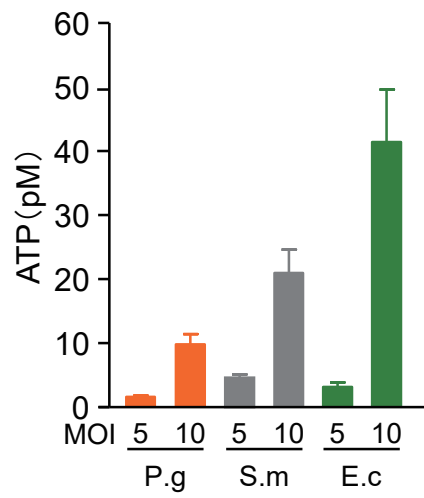
Supplementary Video S1-S4



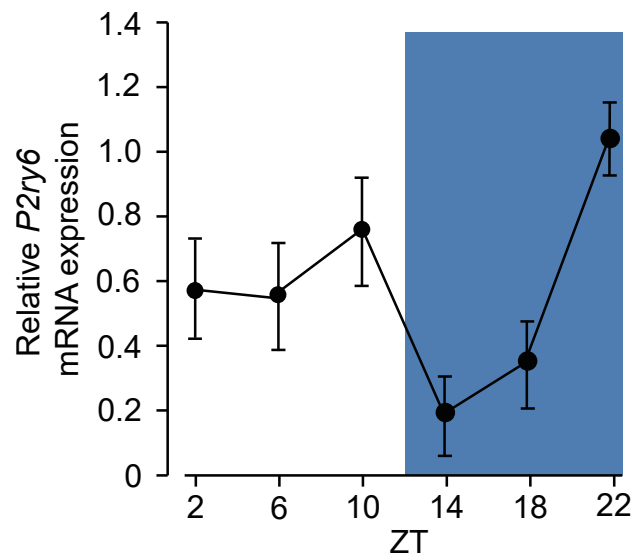
Supplementary Figure S1. Effects on the cell viability and the mRNA expression of proinflammatory-related molecules in MG6 cells following the infection with *P. gingivalis*. **(a,b)** Effects of *P. gingivalis* on cell viability in MG6 after infection with *P. gingivalis* at various MOIs; 1:1, 1:5, 1:10 for 3h **(a)** or 6h **(b)**. The data are presented as the mean \pm S.E.M. ($n = 3$, each). A one-way ANOVA with Dunnett's test as compared with non; MOI 1:1, $p = 0.2911$; 1:5, $p = 0.9999$; 1:10, $p = 0.8629$ (3h), 1:1, $p = 0.9767$; 1:5, $p = 0.9508$; 1:10, $p = 0.9999$ (6h). **(c-e)** The mRNA expression of proinflammatory-related molecules; **(c)** *Il6*, **(d)** *Tnf* **(e)** *Nos2* in MG6 cells after infection with *P. gingivalis* for 3h or 6h at MOI 1:5. The data are presented as the mean \pm S.E.M. ($n = 3$, each). A two-way repeated measure ANOVA with Sidak's test (non versus *P. gingivalis*); 3h: ** $p = 0.0192$; 6h: *** $p = 0.0001$ (c), 3h: *** $p = 0.0001$, 6h: *** $p = 0.0001$ **(d)**, 3h: *** $p = 0.0001$, 6h: *** $p = 0.0001$ **(e)**.



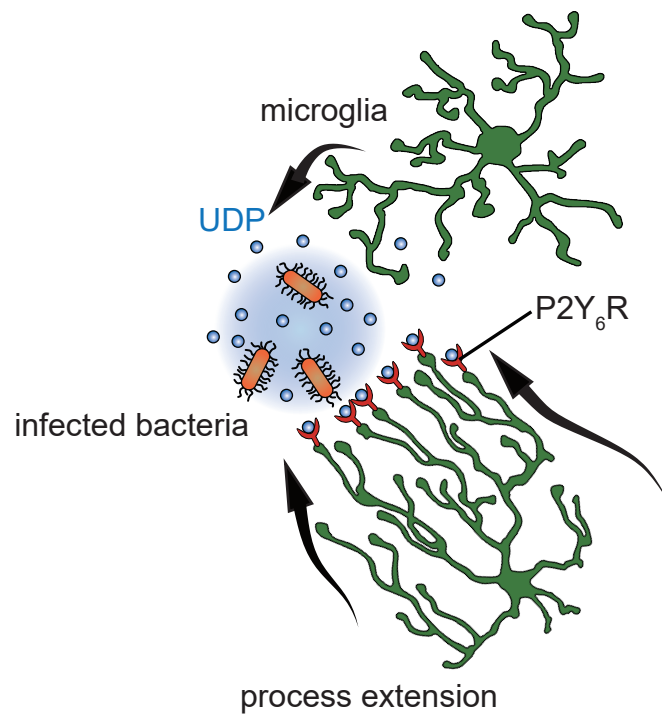
Supplementary Figure S2. *P.g* LPS and fMLP did not induce microglial response to the focal injection area. (a-c) ACSF, (d-f) 100 $\mu\text{g ml}^{-1}$ *P.g* LPS, (g-i) 10 μM fMLP (j) The kinetics of the mean fluorescent change of microglial response to ACSF, *P.g* LPS and fMLP. Scale bar: 10 μm . The data are presented as the mean \pm S.E.M. (N = 3 mice, n = 3-5 each). Two-way repeated measure ANOVA with Dunnett's test; from 10min to 40min: $p = 0.3078$, $p = 0.4363$, $p = 0.4475$, $p = 0.4212$, $p = 0.8556$, $p = 0.8570$, $p = 0.7224$ for ACSF (control) vs *P.g* LPS, $p = 0.4115$, $p = 0.2542$, $p = 0.6601$, $p = 0.4595$, $p = 0.3636$, $p = 0.6978$, $p = 0.9232$, $p = 0.8096$ for ACSF vs fMLP.



Supplementary Figure S3. MG6 cells released slightly few amount of ATP in response to various bacteria. The data are presented as the mean \pm S.E.M. (n = 3 each). MOI 1:5, 1:10, P. g; *P. gingivalis*, S.m; *S. mitis*, E.c; *E. coli*.



Supplementary Figure S4. Possible associations with the circadian variation of the microglial *P2ry6* expression. The circadian oscillations of the expression of *P2ry6* gene in cortical microglia in mice housed under the LD condition. The data are presented as the mean \pm S.E.M. (N=3 mice, n=3 each). One way Repeated measures ANOVA with a post hoc Tukey's test; ZT14 vs ZT10: $p = 0.0039$, ZT14 vs ZT22: $p = 0.001$, ZT22 vs ZT2: $p = 0.0140$, ZT22 vs ZT6: $p = 0.0125$, ZT22 vs ZT14: $p = 0.001$, ZT22 vs ZT18: $p = 0.0007$.



Supplementary Figure S5. A schematic diagram of microglial process extension to the infected bacteria through the UDP-P2Y₆R system. Infected bacteria induce microglial process extension by secretion of UDP from microglia around the infection site .