Supplemental Materials

Roles of *Porphyromonas gulae* proteases in bacterial and host cell biology

Alam Saki Urmi, Hiroaki Inaba, Ryota Nomura, Sho Yoshida, Naoya Ohara, Fumitoshi Asai, Kazuhiko Nakano, Michiyo Matsumoto-Nakano

Supplemental Experimental Procedures

Hemagglutination assay

Hemagglutination assay procedures were performed as follows. *P. gulae* D040, D044, D049, D066, and D77 strains were centrifuged, then washed and resuspended in phosphate-buffered saline (PBS) at an optical density of 0.25 at 600 nm. Serial two-fold dilutions of the bacterial suspension in PBS were prepared; 100 µl aliquot of each dilution mixed with an equal volume of 2.5% mouse erythrocytes. The suspension was incubated in a round-bottom microtiter plate for 3 h.

Host protein cleavage assay

The recombinant proteins human γ-globulin, fibrinogen, and fibronectin (5 µg) were incubated with bacterial suspensions at 37°C for 1 h, then *P. gulae* ATCC 51700 cells were removed by centrifugation and the supernatants were collected. Protein samples were subjected to SDS-PAGE and transferred electrophoretically to PVDF membrane. Staining of PVDF-bound proteins was performed with Coomassie Brilliant Blue R250.

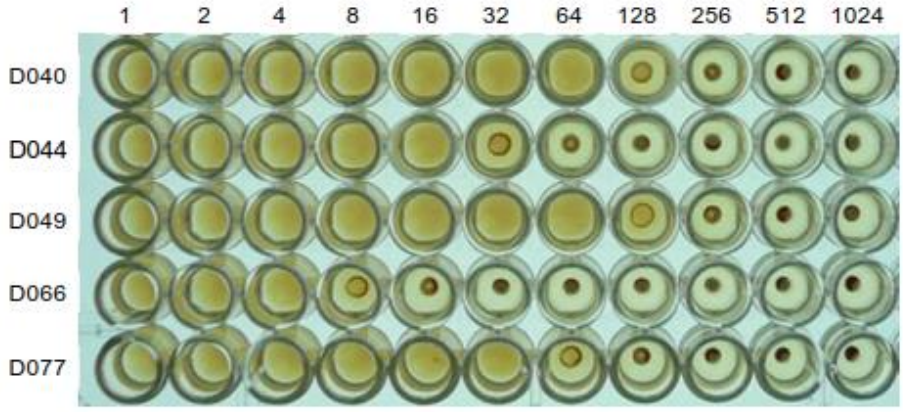

Figure S1. Hemagglutinating activity of *P. gulae* strains.

P. gulae strains D040, D044, D049, D066, and D077 were grown in TSB, then washed with and resuspended in PBS at an optical density at 600 nm of 0.25. Suspensions and its dilutions in 2-fold series were applied to the wells of a microtiter plate from left to right, then mixed with a mouse erythrocyte suspension and incubated at room temperature for 3 h. Data are representative of three independent experiments.

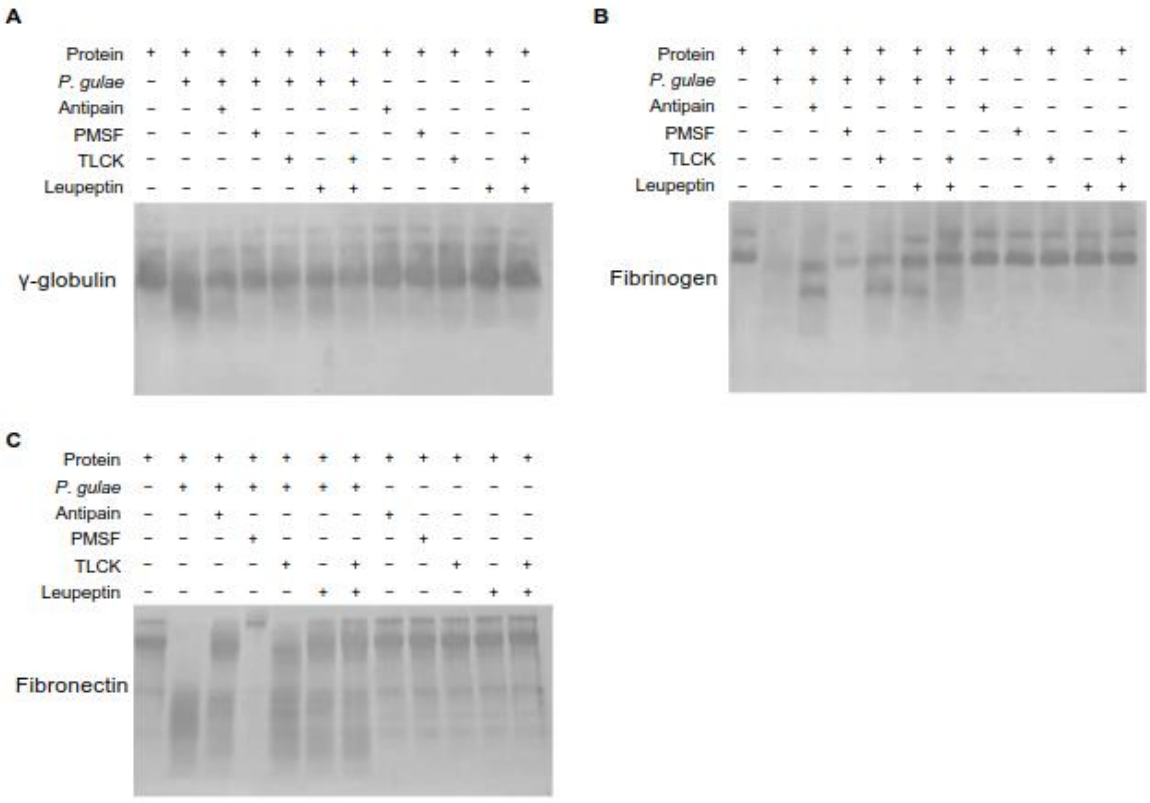

Figure S2. Degradation of various human proteins by *P. gulae* ATCC 51700.

The protease inhibitors antipain, PMSF, TLCK and leupeptin (100 μM) were added 2 h prior to addition of the bacterial suspension. The recombinant human proteins γ-globulin, fibrinogen, and fibronectin (5 μg) were incubated with *P. gulae* ATCC 51700 ($5x10^7$ cells) treated with/without each protease inhibitor at 37ºC for 1 h. Protein profile expressions were analyzed by SDS-PAGE. Following transfer to PVDF membranes, proteins were visualized by Coomassie brilliant blue R250 staining. Data are representative of three independent experiments.