Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information

Bismuth drugs tackle *Porphyromonas gingivalis* and attune cytokine response in human cells

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Supplementary materials and methods

Determination of minimal inhibitory concentrations

MIC determination was followed the broth dilution protocol described with some modification for *P. gingivalis* in sTSB[1]. Initial CFU (inocula) of *P. gingivalis* ATCC 33277 was 1×10^6 CFU/ml. Drugs (RBC or CBS) and metronidazole were 2-fold diluted with sterile distill water. Optical density at 600 nm was determined on a SpectraMax M2 microplate reader (Molecular Device). Although the media in wells appeared clear and transparent when higher concentration (> 50 μM) of RBC or CBS present in *P. gingivalis* culture, there were a thin layer of precipitation of possible bismuth-attached bacterial debris at the bottom of wells, which slightly increased the value of OD_{600} and decreased the calculated percentage of inhibition but did not affect the MICs. There was no black precipitation in wells for sterile control. For convenience, we used the following formula to estimate the inhibitory rate of *P. gingivalis*, $I(\%) = [1 - (OD - OD_{SC})/(OD_{GC} - OD_{SC})] \times 100$, where $I(\%)$ is the inhibitory percentage, OD_{GC} is the average OD_{600} of growth controls (without drugs or antibiotics), OD_{SC} is the average of OD_{600} of sterility controls (without bacteria). For comparison and controlling, MIC assays were also performed using Bi^{3+}-TRACER and its apo-counterpart, NTA-AC[2-4], ranitidine (neutralized with citric acid as control of RBC) and citrate (neutralized with sodium hydroxide as control of CBS). Same procedures were performed on *Actinomyces* spp. and *Streptococcus* spp.

It is noted that the increase of inocula leads to higher MICs.[1] We also used the inocula of 1×10^8 and 4×10^9 CFU/ml to define the concentration of both bismuth drugs to be used for other experiments, in which *P. gingivalis* should not be completely killed. Minimal bactericidal concentration was also determined by spotting 10 μl aliquots of culture from each well onto blood agar plates and anaerobically incubated for 5 days.

To fully understand the viability of drug-treated *P. gingivalis*, BacTiter-Glo™ Microbial Cell Viability Assay (Promega) and LIVE/DEAD® BacLight™ Bacterial Viability Kit (Thermo Fisher) were used, and the corresponding luminescence and fluorescence were recorded on the SpectraMax M2, respectively.

Time course study of bismuth drugs-treated *P. gingivalis*

Mid-log *P. gingivalis* (OD660 = 0.6, 4×10^9 CFU/ml) was treated with RBC and CBS, at 25, 50 and 100 μM respectively, and anaerobically incubated at 37 °C. Untreated *P. gingivalis* served as controls. At different time points, the bacterial culture was collected, and the density by OD_{600} and viability by BacTiter-Glo™ Microbial Cell Viability Assay were recorded using the SpectraMax M2 microplate reader with 100 μl of culture.

Determination of intracellular bismuth contents of *P. gingivalis*

The culture of *P. gingivalis* at steady state was subcultured with 1:20 dilution and incubated for about 16 h anaerobically at 37 °C until OD_{600} reaching 0.6, determined on a DU 730 UV/Vis Spectrophotometer (Beckman Coulter). *P. gingivalis* culture was divided into to two tubes, one for control and the other for Bi treatment by adding 25 μM RBC. Both tubes of *P. gingivalis* were further cultured for 24 h anaerobically at 37 °C. *P. gingivalis* cells were pelleted by centrifugation at 5,000 *g*, 4 °C for 10 min. The pellets were further washed with phosphate-buffered saline (PBS) twice and with water once. The pellets were lyophilized by using a FreeZone 4.5 Liter Benchtop Freeze Dry System (LABCONCO). The dry cell pellets were weighted and digested in concentrated nitric acid at 60 °C overnight and diluted in 1% nitric acid before subjected for determination of metal contents on an Agilent 7500a inductively coupled plasma mass spectrometer (ICP-MS).

Fluorescence confocal microscopy

At the mid-log phase, 50 μM Bi^{3+}-TRACER was added into PBS-washed *P. gingivalis* cell suspension (OD_{660} = 0.3) and incubated at 37 °C anaerobically for 30 min in the dark. The cells were collected and washed with cold PBS for three times. Reagents of LIVE/DEAD *Bac*Light Bacterial Viability Kit were added into bacterial suspension before subjected to confocal imaging under a Carl Zeiss LSM710 Inverted Confocal Microscope with a Plan-Apochromat 63×/1.40 Oil Ph3 M27 oil-immersion objective, excited with a 405-nm laser (for Bi^{3+}-TRACER), a 488-nm laser (for SYTO 9) and a 543-nm laser (for propidium iodide). All samples were observed at the same settings.

Gene cloning, expression and purification of recombinant proteins

Genomic DNA of *P. gingivalis* 33277 was extracted using Wizard Genomic DNA Purification Kit. Genes were amplified using Phusion High-Fidelity DNA Polymerase

with corresponding primer pairs (Table S2). Amplified gene fragments were digested by corresponding restriction enzymes (NEB) and inserted into pET-28a(+) (Novagen). The resulting plasmids were transformed into *E. coli* BL21(DE3) for over-expression.

Protein overexpression in 1 L Luria-Bertani (LB) broth medium was induced with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG, USB) at 25 °C for 16 hr. Cell pellets were lysed in 25 mM Tris-HCl with 500 mM NaCl and 25 mM imidazole, pH7.4 supplemented with cOmplete™, EDTA-free Protease Inhibitor Cocktail by sonication. Each recombinant protein was purified from lysate using HiTrap Chelating HP 5 mL column (GE Healthcare Life Sciences) with 25 mM Tris-HCl with 500 mM NaCl, 25 mM to 500 mM imidazole gradient, pH 7.4. His-tag was cleaved by thrombin (from bovine plasma, Sigma-Aldrich). Tag-removed proteins were finally treated with 10 mM dithiothreitol (DTT) and 10 mM ethylenediaminetetraacetic acid (EDTA) and purified using HiLoad 16/600 Superdex 200 pg (GE Life Sciences) in 20 mM Tris-HCl 150 mM NaCl, pH 7.4.

Exceptionally, the plasmid (pET28-RgpA-CD) for expressing the catalytic domain (228-720 aa, CD) of gingipain R1 was transformed into *E. coli* BL21(DE3) plysS, and RgpA-CD was overexpressed in terrific broth (TB) [24 g/L yeast extract, 20 g/L tryptone (Difco), 0.4% glycerol, 17 mM KH_2PO_4 and 72 mM K_2HPO_4] rather than LB. RpgA-CD-expressed *E. coli* cell pellets were lysed in 25 mM Tris-HCl with 500 mM NaCl, pH 7.4 supplemented with 1.5 mM 4,4'-dithiopyridine disulfide (Sigma-Aldrich) and purified with 25 mM Tris-HCl with 500 mM NaCl, 25 mM to 500 mM imidazole gradient, pH 7.4, using a HiTrap Chelating HP 5 mL column (GE Healthcare Life Sciences). Next, fractions with Rgp activities were pooled together and dialyzed against 25 mM Tris-HCl, pH7.4, and then purified with 0-1M NaCl gradient using a HiTrap Q HP 5 mL column (GE Healthcare Life Sciences). Rgp-active fractions were pooled together and concentrated using Amicon Ultra-15 Centrifugal Filter Units (MWCO 10 kDa, Merck Millipore), and subsequently purified using HiLoad 16/600 Superdex 200 pg (GE Life Sciences) in 20 mM Bis-Tris-HCl 150 mM NaCl, pH 6.8. An ÄKTA FPLC system (GE Life Sciences) was used for protein purification.

Reconstitution of recombinant superoxide dismutase (SOD) and activity

Apo-SOD and metal-reconstituted SOD proteins were prepared from purified recombinant *P. gingivalis* SOD, following the previous protocols[5]. Briefly, purified

SOD was treated with 10 mM Tris-HCl, pH 3.2, containing 3 M guanidine hydrochloride (GdnHCl, Thermo Fisher) and 20 mM 8-hydroxyquinoline (Sigma Aldrich) for 30 min, followed by buffer exchange into 10 mM Tris-HCl, pH 7.8 with 3 M GdnHCl using HiTrap Desalting column (GE Healthcare Life Sciences) and then into 10 mM Tris-HCl, pH 7.8, which produced apo-SOD. Metal reconstitution was performed by incubating the apo-SOD with 5 mM $(NH_4)_2Fe(SO_4)_2$ (for Fe-SOD), 5 mM $MnSO_4$ (for Mn-SOD) or 5 mM RBC (for Bi-SOD) in 20 mM Tris-HCl, pH 7.8 containing 7 M GdnHCl. The protein solutions were exchanged into 40 mM Tris-HCl, pH 7.8 with 2 mM corresponding metal salts or complexes using the desalting column, followed by incubation in 0.5 mM EDTA for 6 h. The final metal-reconstituted SODs were desalted into 10 mM Tris-HCl, pH7.8. SOD activity was determined using the SOD Assay Kit with either 1 μM purified metal-reconstituted recombinant SOD proteins or 1 mg/mL *P. gingivalis* lysate.

Thioredoxin (PGN_0033) activity

Purified thioredoxin (PGN_0033, 1 mg/ml) was either treated with or without 5 mM RBC at 4 °C overnight and RBC was removed by buffer exchange using Amicon Ultra-0.5 Centrifugal Filter Units (MWCO 3 kDa, Merck Millipore). Thioredoxin activity was determined using Thioredoxin Activity Fluorescent Assay Kit with either 10 μg/mL purified recombinant thioredoxin or 1 mg/mL of *P. gingivalis* lysate.

Gingipain activity

The catalytic domain (228-720 aa) of gingipain RgpA (20 μM RgpA-CD) and *P. gingivalis* lysates (1mg/ml, overnight culture) were used for bismuth (RBC) inhibition assay. No addition of protease inhibitors was made in *P. gingivalis* lysates. RgpA-CD was treated with either 1 or 5 mM RBC, and the lysates were treated with 5 mM RBC for 5 h at room temperature. R-gingipain activities were determined according to the established protocol[6], using 20 μl of above-mentioned RgpA-CD solution or lysates with L-BAPNA as the substrate. The binding of Bi was examined by UV-vis titration using bismuth (III) nitrilotriacetate (Bi-NTA). Purified recombinant RgpA-CD was firstly treated with 10 mM DTT overnight and desalted into 10 mM HEPES buffer 100 mM NaCl, pH 7.0. Aliquots of stock solution of Bi-NTA or EDTA were then titrated into 20 μM RgpA-CD solution and UV-vis absorption spectra were recorded in the

range from 250 to 600 nm using a Varian Cary 50 spectrophotometer with a 1-cm quartz cuvette at room temperature.

ATPase activity

The ATPase activity was determined by monitoring inorganic phosphate released continuously by enzymes using EnzChek® Phosphate Assay Kit. Briefly, reaction mixture (200 µl) containing enzymes but without the substrate ATP was prepared in each well of 96-well plates and incubated at 22 °C for 10 min. Then add final concentration of 200 µM ATP into each well to start the reaction and immediately read absorbance at 360 nm on a SpectraMax M2 microplate reader (Molecular Device) at 37 °C. The initial velocity was calculated by fitting the initial linear data.

Selection of qPCR housekeeping gene for *P. gingivalis*

Four commonest housekeeping genes were selected for evaluating the stableness of expression as a reference, including 16S rRNA (PGN_r0001), DNA-directed RNA polymerase beta subunit (*rpoB*, PGN_1571), DNA gyrase B subunit (*gyrB*, PGN_0413) and glucokinase (*glk*, PGN_0380) using a Python package, eleven 0.1.1, which implements the GeNorm multi-gene RT-qPCR normalization algorithm[7], showing that *gyrB* and *glk* were the two equally best reference. However, it was found that the *glk* gene has been disrupted by an insertion though the transcription is not affected[8]. Therefore, to avoid troubles, *gyrB* gene was chosen as a reference to normalize the mRNA expression levels throughout the study.

Hemin-agarose pull-down assay

Hemin-binding proteins in *P. gingivalis* lysates were analyzed using hemin-agarose binding pull-down assay. *P. gingivalis* were cultured in liquid media either with or without the supplement of 5.0 µg/mL hemin and treated with or without 25 µM RBC. The bacteria were lysed using B-PER. For each sample, 200 µl of hemin-agarose (Sigma Aldrich) was prewashed with 1 mL of 25 mM Tris-HCl pH 7.4, 100 mM NaCl for three times (10,000 *g*, 5 min). Bacterial lysate (500 µl and diluted in the above buffer to 2 mg/ml) was incubated with hemin-agarose at 37 °C for 3 h and then the supernatant was transferred to another tube. The hemin-agarose was washed with the same buffer for three times to remove non-specific binding proteins and bound proteins were eluted

with SDS-PAGE loading buffer. As suggested by Sigma Aldrich, Sepharose 4B (Sigma Aldrich) was used as the negative control for hemin-agarose.

P. gingivalis growth on bismuth blood agar plates

Bismuth blood agar plates were prepared by adding appropriate concentration of bismuth drugs (25 and 100 μM) into liquified blood agar before pouring onto plates. 10 μl of *P. gingivalis* in PBS (1×10^8 CFU/ml) were spotted onto the blood agar plates with or without bismuth drugs. The plates were further incubated anaerobically at 37 °C for four days prior to examination. The same procedures were also performed with *A. gerencseriae* and *A. israelii*.

Hemagglutination assay

Both *P. gingivalis* cells and cultured media were subjected to hemagglutination assay. Overnight cultured *P. gingivalis* cells and media were collected. The bacteria were washed with PBS for three times and suspended in PBS at OD_{600} of 1.3. The media were centrifuged at 13,000 *g* for 5 min to remove remnant bacterial cells. Fresh sheep red blood cell suspension (Cedarlane) was washed with PBS for three times by centrifugation at 1,500 rpm for 5 min. Both bacterial suspension and media were diluted in a two-fold series with PBS. For hemagglutination, 100 μl of sample suspension were mixed with equal volume of 1% sheep red blood cells suspension in a V-bottom 96-well plate and incubated at room temperature for 3h.

UV-vis spectroscopy

All UV-vis spectra were collected with a Varian Cary 50 spectrophotometer using a 1-cm quartz cuvette at room temperature. Hemin stock solution was prepared by dissolving 0.1 g hemin (Sigma-Aldrich) in water by adding 2 mL of 1 M NaOH. Solution of 10 μM hemin was prepared in 10 mM HEPES buffer 100 mM NaCl, pH 7.0. Aliquots of stock solution of Bi-NTA or EDTA were then titrated into hemin solution, and UV-vis absorption spectra were recorded in the range from 250 to 700 nm. Difference spectra were obtained by subtracting hemin spectrum from titrating spectra.

The binding of bismuth was examined by UV-vis titration using bismuth (III) nitrilotriacetate (Bi-NTA). Purified recombinant RgpA-CD was firstly treated with 10 mM DTT overnight and desalted into 10 mM HEPES buffer 100 mM NaCl, pH 7.0.

Aliquots of stock solution of Bi-NTA or EDTA were then titrated into 20 μM RgpA-CD solution and UV-vis absorption spectra were recorded in the range from 250 to 600 nm using a Varian Cary 50 spectrophotometer with a 1-cm quartz cuvette at room temperature.

Cell viability and cytotoxicity

Cell Counting Kit-8 (Sigma) was used to evaluate the cell viability, while Pierce LDH Cytotoxicity Assay Kit (Themo Fisher) was used to evaluate the cytotoxicity.

STRING analysis and enrichment for Bi^{3+}-associated proteins

Correctly identified proteins were considered when at least both Protein Score Confidence Interval (C.I. %) and Ion Score C.I. % are larger 95 and either Protein Score or Ion Score is larger than 100. The protein-protein interaction (PPI) among identified proteins were analyzed using STRING[9] and GO (Gene Ontology)[10] and KEGG (Kyoto Encyclopedia of Genes and Genomes)[11] enrichment. The interaction score cutoff of 0.5 and the Markov Cluster (MCL) Inflation parameter of 3 were used for primary clustering. The PPI network was visualized using Cytoscape 3.6.1 software[12].

qPCR data analysis

For qPCR of *P. gingivalis* RNA, relative standard curve quantitation was used. For RNA of human cells, comparative C_T ($\Delta\Delta C_T$) quantitation was used.

RNA-seq data analysis

Raw sequencing reads were filtered out low quality reads (1, reads with adaptors; 2, reads with more than 10% unknown bases; 3, reads with over 50% low quality bases whose sequencing quality < 5). Clean reads were mapped to *P. gingivalis* ATCC 33277 reference using Bowtie2 (reference genes)[13] and HISAT (for reference genomes)[14]. Genes expression levels were quantified in the units of Fragments Per Kilobase of transcript per Million mapped reads (FPKM) by RSEM[15].

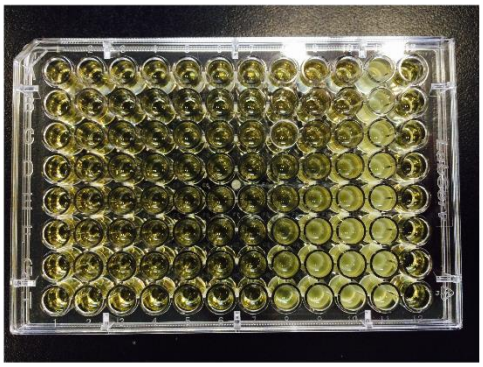
Based on the algorithm following the Poisson distribution, genes in *P. gingivalis* with and without (PgRBC and Pg, or treatment and control groups) at different time points were screened to identify differentially expressed genes (DEGs) and tested with significance. The *P*-value were corrected using Bonferonni test[16] and a False Discovery

Rate (FDR) procedure was performed to screen off type I and II errors[17]. DEGs were considered as those with FDR ≤ 0.001 and more than 2-fold change, i.e., $\log_2$Ratio ≥ 1.

For GO enrichment, all DEGs were firstly mapped to GO terms in the database (http://www.geneontology.org/), calculating gene numbers for every term, then uses hypergeometric test to find significantly enriched GO terms in the input list of DEGs. The calculated *P*-values were undertaken Bonferroni Correction[16], using corrected *P*-value ≤ 0.05 as a threshold. GO terms fulfilling the criteria were defined as significantly enriched GO terms in DEGs. For KEGG enrichment, all DEGs were mapped to KEGG pathway in the database (http://www.kegg.jp/).

Supplementary figures:

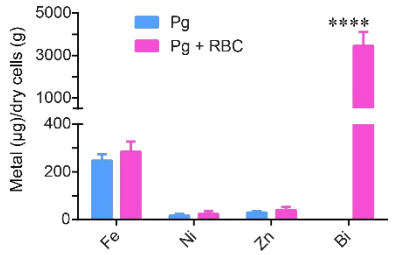
A



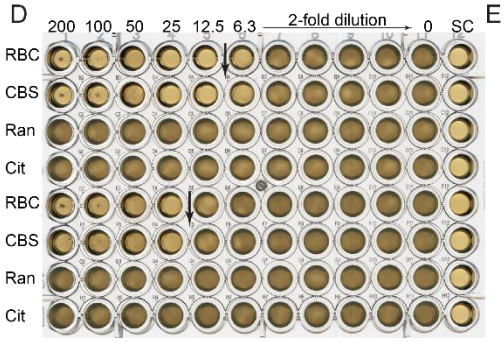
B

RBC \ MTZ	4	2	1	0.5	0.25	0.13	0.063	0.031	0.016	0
100	91.7	95.1	93.5	94.4	94.6	94.6	93.7	90.6	92.1	93.6
50	94.0	97.4	98.2	98.0	97.8	96.5	93.8	83.2	90.0	85.5
25	92.3	96.2	96.7	96.1	93.1	87.9	83.4	83.2	82.3	71.8
13	96.4	96.2	95.9	95.5	94.7	94.1	94.4	91.0	82.0	53.5
6.3	97.9	98.2	98.3	98.1	98.0	97.9	97.5	84.4	58.4	37.5
3.1	98.7	98.8	99.1	98.9	99.0	98.9	97.5	80.7	42.1	21.2
1.6	99.1	99.2	99.3	99.3	99.4	99.0	97.3	69.4	27.6	13.3
0	99.2	99.4	99.5	99.4	99.4	99.3	97.0	57.3	8.9	-1.9

C



D



E

	μM	200	100	50	25	12.5	6.3	3.1	1.6	0.78	0.39
10^6 CFU/ml	RBC	98.6	98.5	94.1	91.3	95.4	59.8	39.3	31.1	21.4	10.3
	CBS	97.7	97.3	90.7	90.7	92.8	61.3	27.5	33.5	13.0	11.4
	Ran	4.1	7.5	-2.7	-20.6	3.1	6.3	5.9	2.6	-10.6	-3.3
	Cit	-14.5	-0.5	4.2	-1.3	9.9	7.9	6.8	7.1	-23.3	6.4
10^8 CFU/ml	RBC	78.0	73.8	72.5	80.3	52.6	6.3	-1.9	13.8	22.3	26.4
	CBS	74.0	68.9	64.3	75.8	36.5	7.1	25.1	26.1	16.2	19.1
	Ran	25.2	19.1	24.2	25.7	-3.1	15.4	8.2	26.0	8.4	17.4
	Cit	14.7	17.1	19.2	-11.0	-15.2	5.9	9.8	15.8	24.2	12.1

Fig. S1 Bismuth drugs inhibited growth of *P. gingivalis*. (**A**) A representative 96-well plate of *P. gingivalis* culture (initially 10^6 CFU/ml) after 48 h treated by ranitidine bismuth citrate (RBC) and metronidazole (MTZ) at different concentrations. At higher concentrations, dark particles of bismuth on or in *P. gingivalis* cells or debris could be observed leading to the increase in absorbance reading and abnormality of inhibition ratio though the culture was visually clear void of apparent bacteria. (**B**) One representative inhibition ratio (%) for a 96-well plate of *P. gingivalis* culture (initially 10^6 bacteria/ml) after 48 h treated by RBC 4 and metronidazole (μg/ml) at series of concentrations. Blue lines indicate the borders for MIC_{50} of either RBC or metronidazole alone. The green box indicates the MIC_{50} when combined use of RBC and metronidazole. (**C**) *P. gingivalis* cells absorb significant amount of bismuth after treatment with 25 μM ranitidine bismuth citrate (RBC) for 24 h. Two-way ANOVA, n = 3, ****$P < 0.0001$. (**D** and **E**) A representative MICs of RBC, CBS and corresponding control compounds for inocula of 10^8 (upper four rows) and 10^6 CFU/ml (lower four rows), respectively. Ranitidine (citric acid-neutralized) is the control compound for RBC, and citrate (NaOH-neutralized) is the control compound for CBS. No inhibitory effects were observed for negative control compounds. One representative plate photo and table of inhibition ratio (%) were shown. SC: sterility controls. MICs were determined by both visual and OD_{600} reading, and the arrows indicate the MICs.

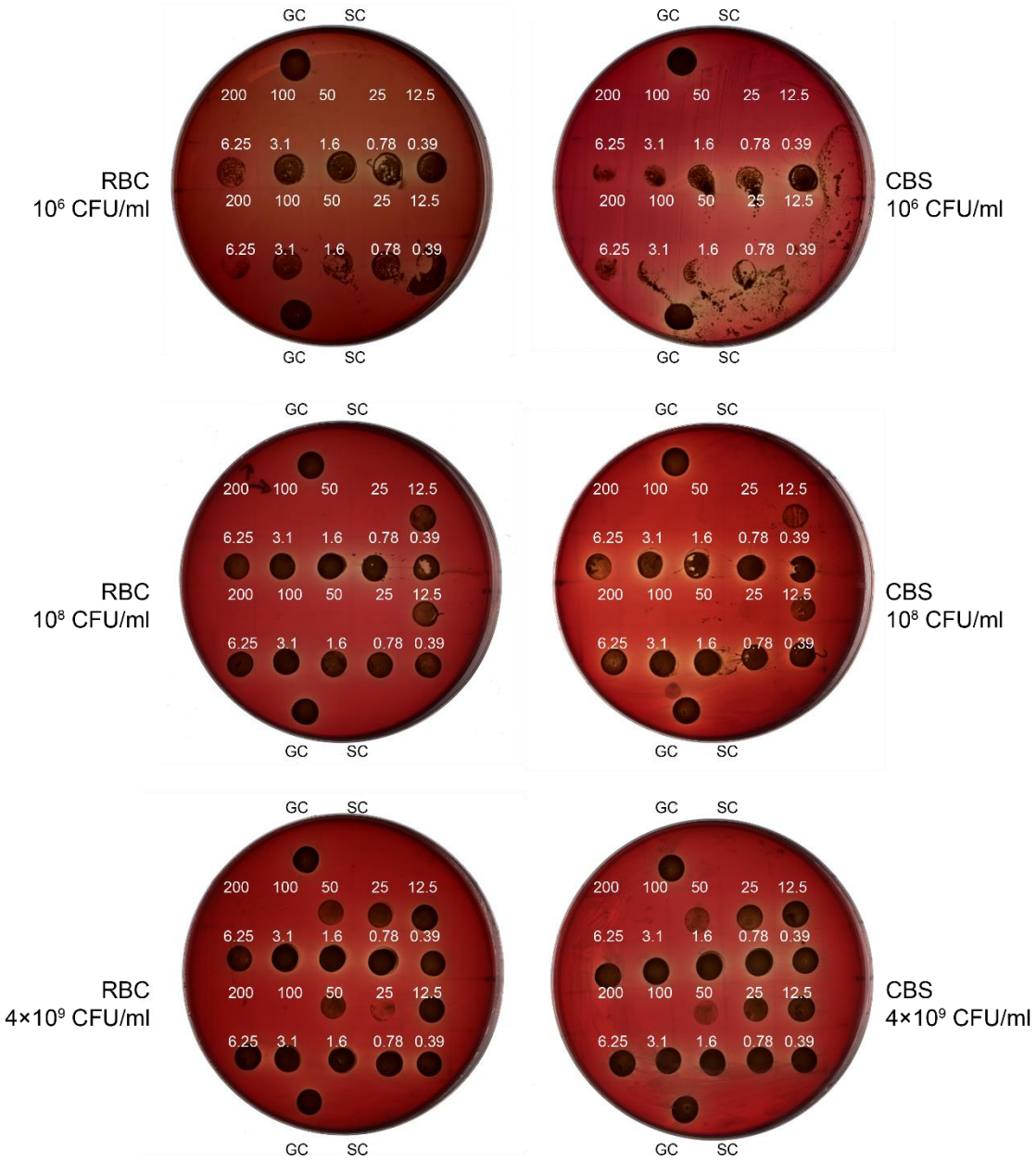


Fig. S2 The MBCs of bismuth drugs against *P. gingivalis.* 10 μl aliquots of *P. gingivalis* culture from each well of 48-h 96-well plates for MIC determination were spotted onto blood agar plates and incubated for 5 days. Both RBC and CBS were serially diluted in 2 folds from 200 – 0.39 μM (shown on the plates). GC: growth controls; SC: sterility controls. The inocula of *P. gingivalis* are 1×10^6, 1×10^8 and 4×10^9 CFU/ml, respectively. On each plate, duplicates were spotted on the upper and lower halves of the plate, respectively. For inocula of 10^6, MBCs of RBC and CBS are 12.5 μM; for inocula of 10^8, MBCs of RBC and CBS are 25 μM; and for the inocula of 4×10^9 (OD_{660}: 0.6), the MBCs of RBC and CBS are 100 μM.

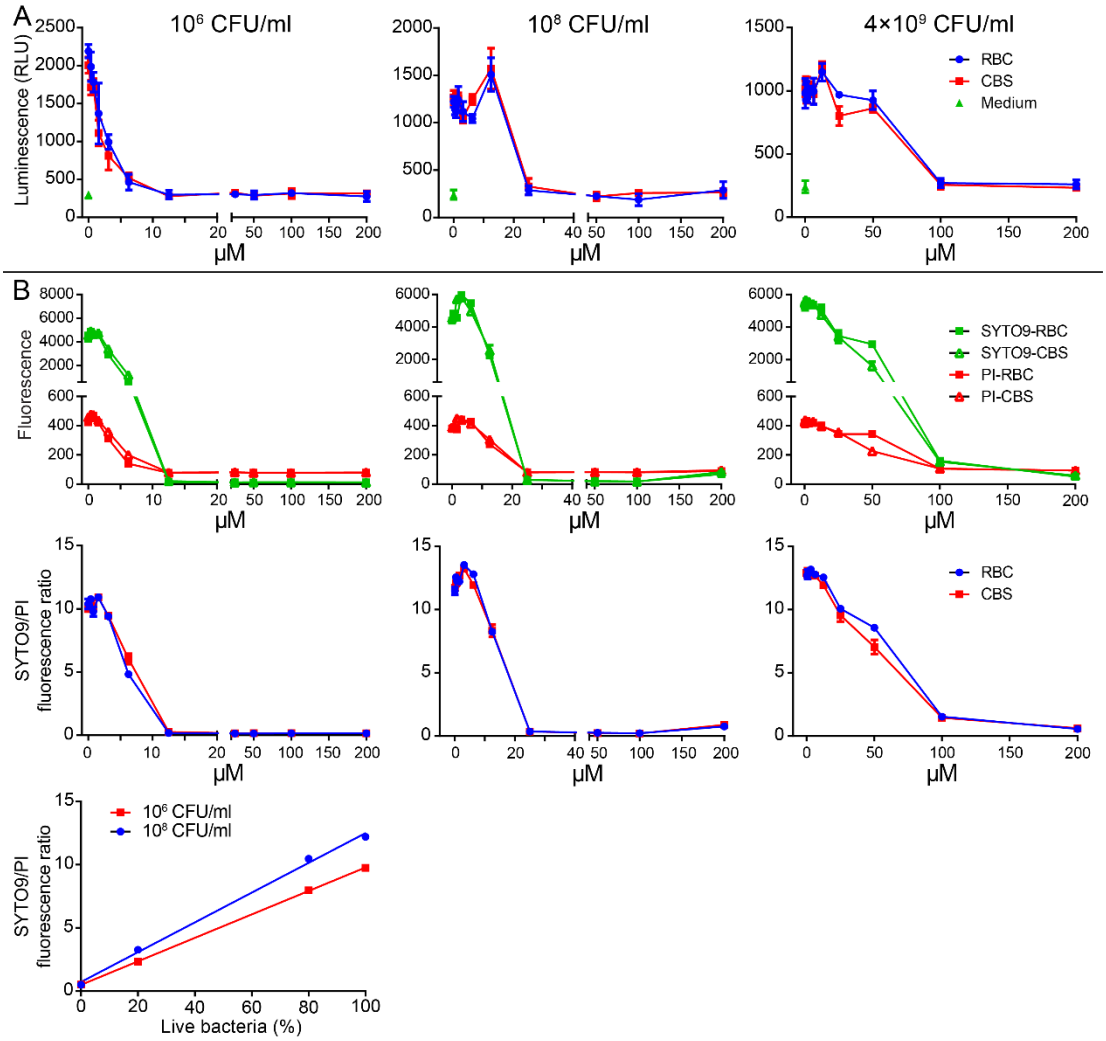


Fig. S3 Effects of bismuth drugs on viability of *P. gingivalis*. (**A**) 100 μl aliquots of *P. gingivalis* culture from each well of the same plates for MICs in Fig. S2 were assayed using BacTiter-Glo™ Microbial Cell Viability Assay, which is based on quantitation of the ATP present. For the inocula of 1×10^6 CFU/ml, both RBC and CBS totally killed *P. gingivalis* at 12.5 μM; for inocula of 1×10^8, both drugs did at 25 μM; and for the inocula of 4×10^9 (OD_{660}: 0.6) both drugs did at 100 μM. (**B**) Aliquots of *P. gingivalis* culture from each well of the same plates were washed with 0.85% NaCl and combinedly assayed using LIVE/DEAD® BacLight™ Bacterial Viability Kit. Untreated *P. gingivalis* (GC) was considered as live bacterial sample, and 70% isopropanol-treated GC was considered as dead bacterial samples. By combining live and dead bacterial samples, the standard curve for live bacteria percentage was plot against the corresponding SYTO9/PI fluorescence ratio. For the inocula of 1×10^6 CFU/ml, both RBC and CBS totally killed *P. gingivalis* at 12.5 μM; for inocula of 1×10^8, both drugs did at 25 μM; and for the inocula of 4×10^9 (OD_{660}: 0.6) both drugs did at 100 μM.

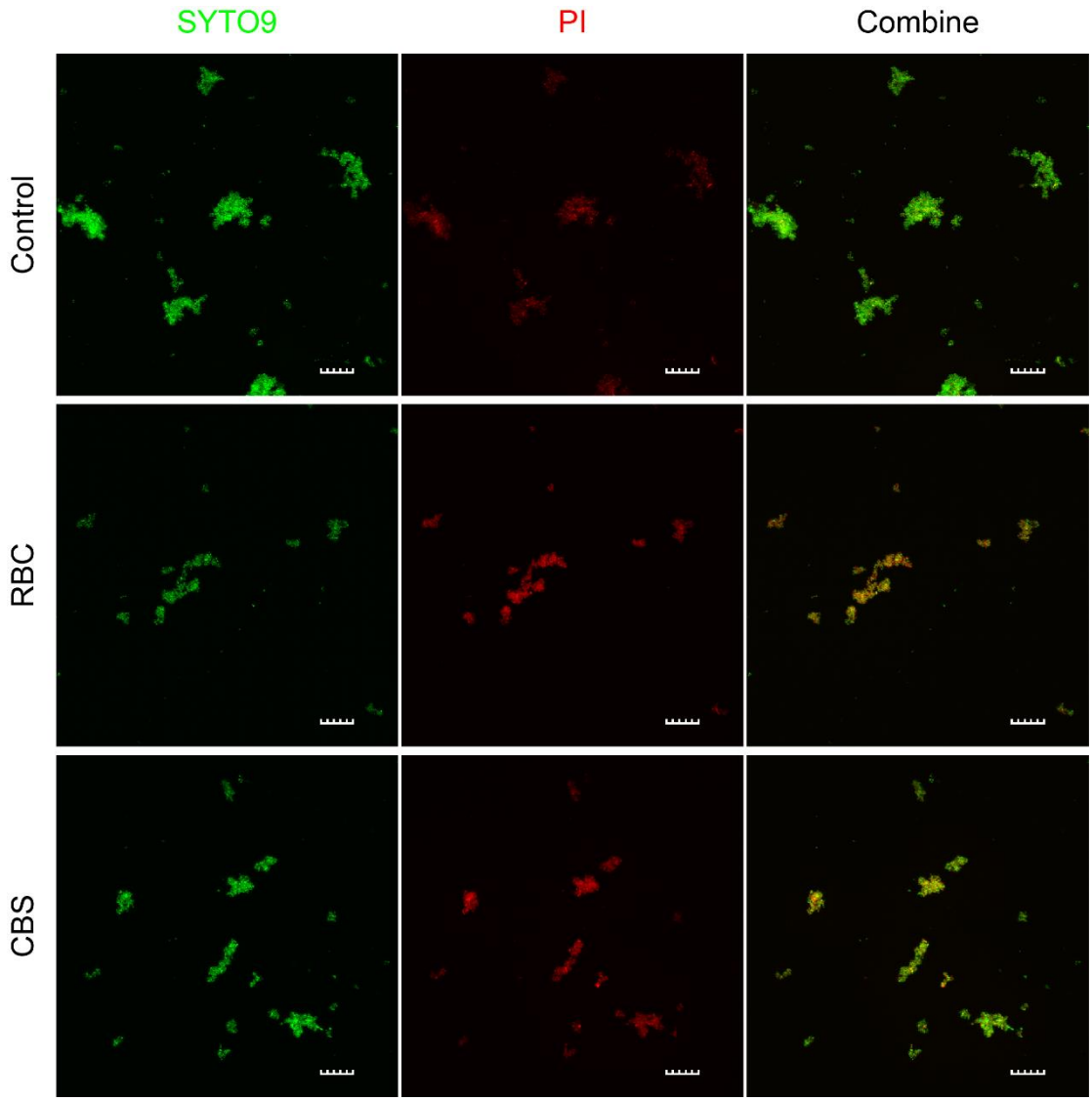


Fig. S4 Live/Dead staining of *P. gingivalis*. 10 μl aliquots of STO9/PI-stained *P. gingivalis* (inocula 1×10^8 CFU/ml) from the wells of GC and 12.5 μM-Bi-treated were mounted onto a Confocal Laser Scanning Biological Microscope FLUOVIEW FV1000 (Olympus) for imaging. Scale bar: 20 μm.

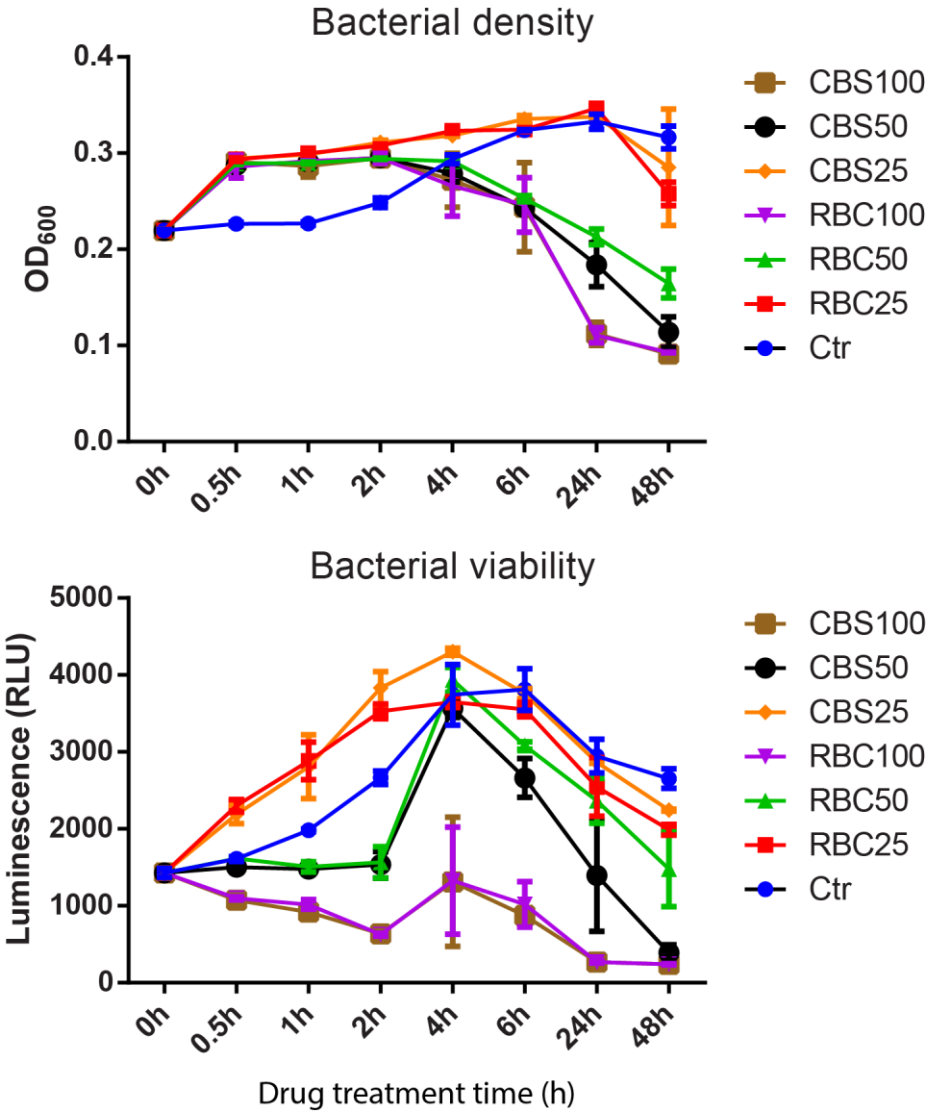


Fig. S5 Time-dependent effects of bismuth drugs on the bacterial density and viability of *P. gingivalis*. Mid-log *P. gingivalis* was treated with 25, 50 and 100 μM of RBC or CBS and anaerobically incubated at 37 °C. At each time point, 100 μl of *P. gingivalis* culture was collected for OD_{600} reading (microplate) and BacTiter-Glo™ Microbial Cell Viability Assay. Two-way ANOVA, n = 3. The OD_{600} reading (microplate) of 0.23 and 0.33 is approximately equivalent to the OD_{660} reading (1-cm cuvette) of 0.6 and 1.0, respectively.

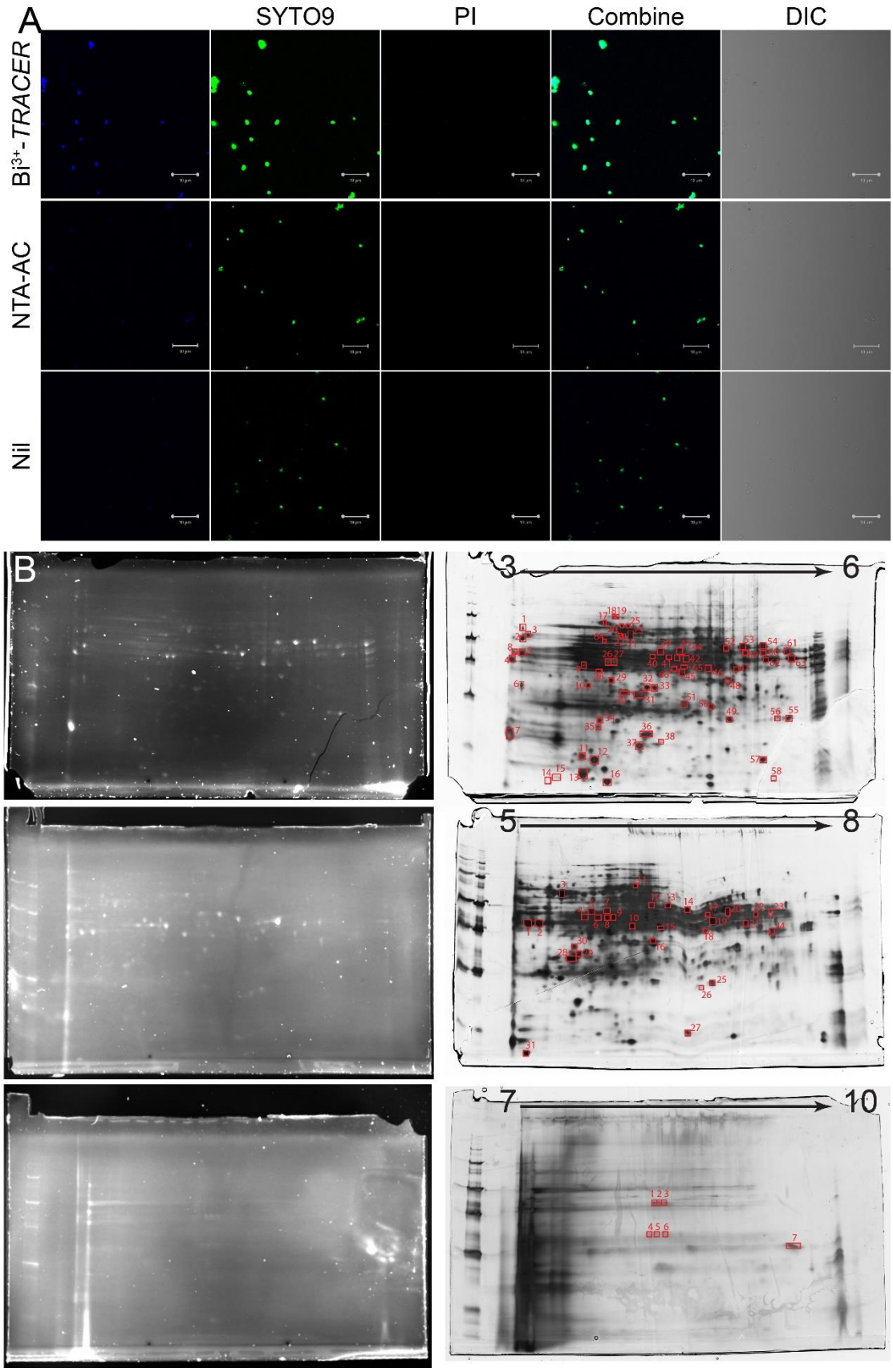


Fig. S6 Identification of bismuth-associated proteins in *P. gingivalis* by the fluorescence-based probe. (**A**) Bi^{3+}-TRACER lit up *P. gingivalis* cells by detecting endogenous bismuth-associated proteins. Leftist column was the blue fluorescence at 405 nm for the probe. The

viability of bacterial cells was examined by staining with Syto 9 (live) and propidium iodide (PI, dead). Scale = 10 μm. NTA-AC is the negative control compound for the probe, and Nil represents no addition of any compound. (**B**) Bi^{3+}-TRACER-based fluorescence 2D gels (Left) and silver-stained the same gels (Right). Three pH ranges of IEF were used, i.e. pH 3-6, pH 5-8 and pH 7-10. Lit-up spots (totally 106 spots) were excised from silver stained gels and subjected for peptide mass fingerprinting (PMF).

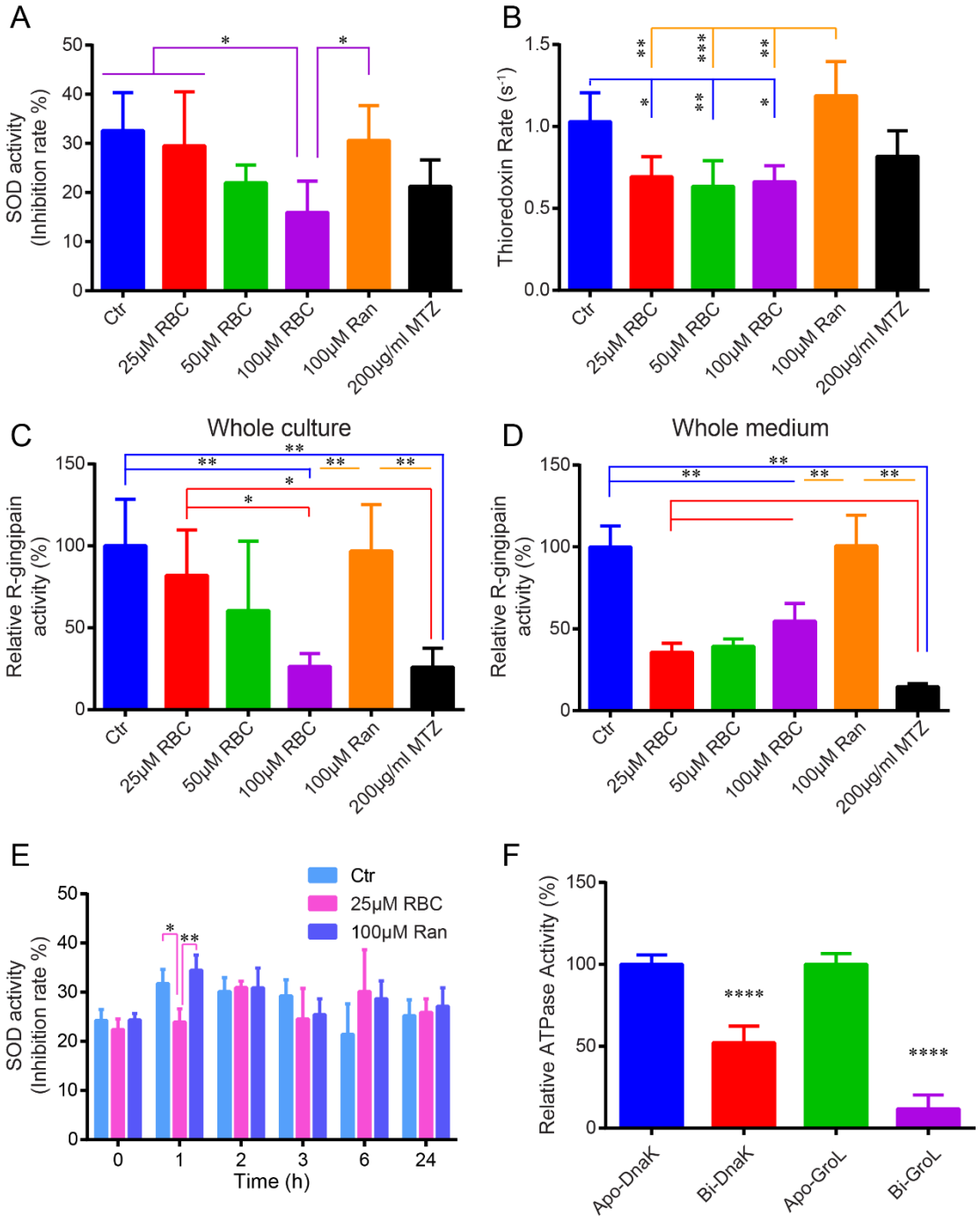


Fig. S7 Bismuth perturbed activity of selective proteins depending on concentration. (**A** and **B**) SOD and thioredoxin activities of *P. gingivalis* lysates (0.5 mg/ml) after the 24-hour treatment with 25, 50 or 100 μM RBC, 100 μM ranitidine (Ran) or 200 μg/mL metronidazole (MTZ). One-way ANOVA, n=3. (**C** and **D**) Activity of arginine-gingipains.10 μl of whole *P. gingivalis* culture (including bacteria and medium) and whole cultured medium with same treatment. One-way ANOVA, n=3. (**E**) Time-dependent SOD activity of *P. gingivalis* treated with 25 μM RBC. Two-way ANOVA, n=3, 0.5 mg/mL *P. gingivalis* lysates. (**F**) ATPase activities of 5 μM recombinant DnaK and GroL, apo and Bi-bound forms. Student's *t*-test, n=3. **P* < 0.5; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

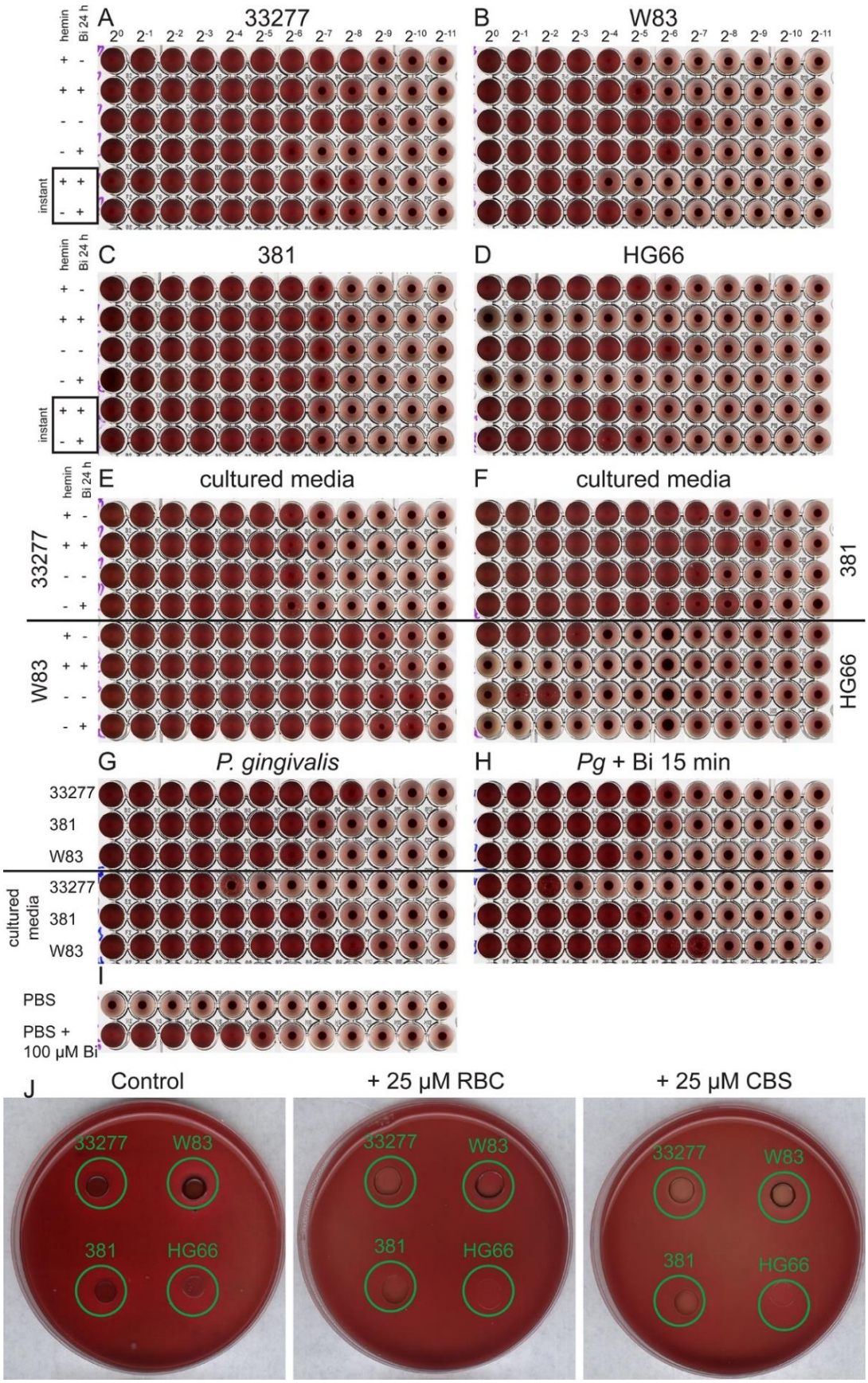

A
33277
B
W83
C
381
D
HG66
E
cultured media
F
cultured media
G
P. gingivalis
H
Pg + Bi 15 min
I
J
Control
+ 25 μM RBC
+ 25 μM CBS
hemin
Bi 24 h
instant
33277
381
W83
HG66
cultured media
PBS
PBS + 100 μM Bi

Fig. S8. Bismuth perturbed the hemagglutination activity and black pigmentation of *P. gingivalis.* Four strains of *P. gingvialis*, ATCC 33277 (**A**), W83 (**B**), 381 (**C**) and HG66 (**D**), were cultured in supplemented TSB with or without 5.0 μg/mL hemin and with or without treatment of 25 μM RBC for 24 h. Both cultured *P. gingivalis* cells and media were used in hemagglutination (HA) assay. Bismuth could inhibit the HA activity of ATCC 33277 and HG66 cells (**A** & **D** upper 4 rows), but not evidently the one of W83 and 381 cells (B & C upper 4 rows). However, both instant (**A**-**D** lower 2 rows) and 15-min (**G** & **H** upper 3 rows) treatment of 100 μM RBC could reduce the HA activity obviously and. 24-h Bi-treatment had complicated effects on HA activity in cultured media (**E** & **F**) while direct 15-min treatment of 100 μM Bi diminish the HA (**G** & **H** lower 3 rows). (**I**) PBS and PBS with RBC. (**J**) By spotting 10 μl of *P. gingivalis* suspension in PBS (OD_{660} > 1) on blood agar plates without or with 25 μM RBC or CBS. The growth of all four strains was compromised and the black pigmentation was perturbed on bismuth-containing plates, appearing light brownish color rather than dark black. On plates with 100 μM RBC or CBS, no growth was observed (not shown).

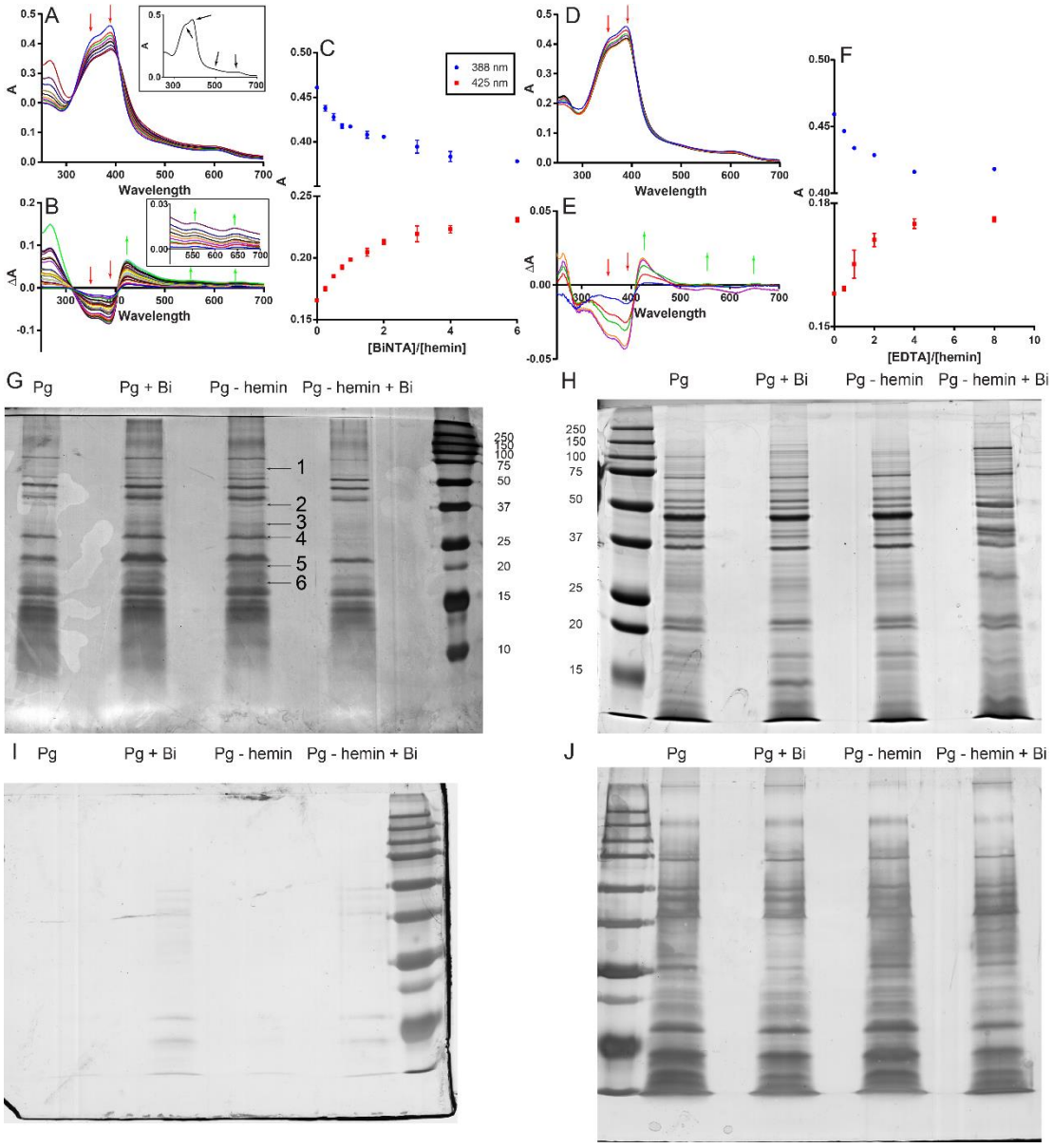


Fig. S9. Bismuth perturbed hemin and hemin-binding proteins. (**A**) Spectra of 10 μM hemin upon titrated with Bi-NTA up to 6 molar equivalents in 10 mM HEPES buffer 100mM NaCl, pH 7.0. Red arrows showed the decrease of absorbance of peaks at 360 and 388 nm. Inset: Spectra of 10 μM hemin. Arrows indicate the characteristic peaks of hemin. (**B**) Difference spectra of 10 μM hemin upon titrated with Bi-NTA up to 6 molar equivalents in 10 mM HEPES buffer 100mM NaCl, pH 7.0. Red arrows showed the decrease of intensity of peaks at 360 and 388 nm. Green arrows showed the increase of intensity of new peaks at 425, 555 and 641 nm. Inset: Zoomed-in difference spectra from 500-700 nm. (**C**) The plot of absorbance at 388 and 425 nm vs. molar ratio of Bi-NTA to hemin, respectively. (**D**) Spectra of 10 μM hemin upon titrated with EDTA up to 10 molar equivalents in 10 mM HEPES buffer 100mM NaCl, pH 7.0. Red arrows showed the decrease of absorbance of peaks at 360 and 388 nm. (**E**) Difference spectra of 10 μM hemin upon titrated with EDTA up to 10 molar equivalents in 10 mM HEPES

buffer 100mM NaCl, pH 7.0. Red arrows showed the decrease of intensity of peaks at 360 and 388 nm. Green arrows showed the increase of intensity of new peaks at 425, 555 and 641 nm. (**F**) The plot of absorbance at 388 and 425 nm vs. molar ratio of Bi-NTA to hemin, respectively. (**G**) Hemin-binding proteins eluted from hemin-agarose. (**H**) Unbound proteins in *P. gingivalis* lysates after hemin-agarose binding. The protein profiles (both hemin-binding and hemin-unbound) for *P. gingivalis* in hemin-lacking medium with bismuth treatment was apparently different with the others. The results of identification of the marked six bands were listed in Table S7. *P. gingivalis* was cultured in supplemented TSB with or without 5.0 μg/mL hemin and with or without treatment of 25 μM RBC for 24 h. Using Sepharose 4B as the negative control for hemin-agarose resin, there was no apparent resin-bound protein bands visible (**I**), and the patterns of unbound proteins for the four samples were similar (**J**).

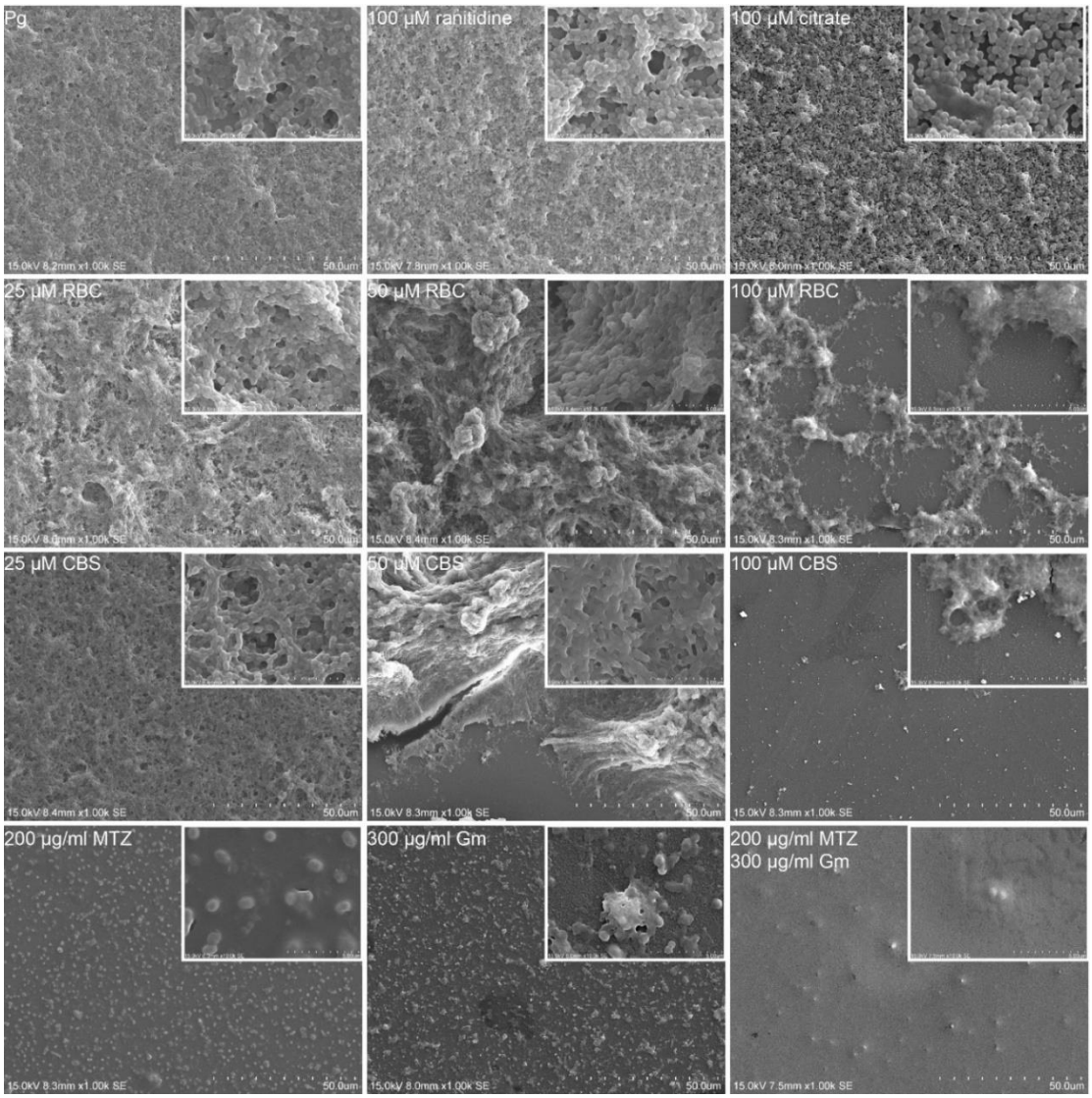


Fig. S10. Bismuth prevented the formation of *P. gingivalis* biofilms. 1×10^7 CFU of *P. gingivalis* in sTSB were seeded into each well of 12-well plates immediate treatment with drugs, antibiotics or control compounds (RBC, CBS, ranitidine, citrate, metronidazole and gentamicin). Fixed biofilm samples were examined using SEM. Magnification: × 1,000; insets: × 10,000. Scale bars: 50 μm; insets: 5 μm.

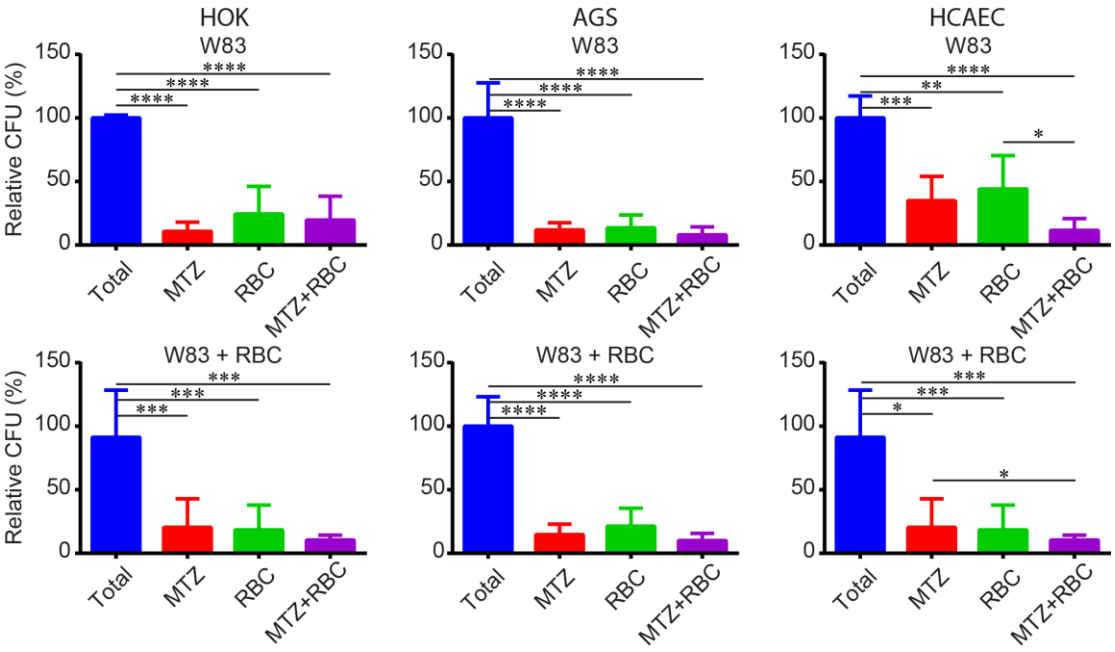


Fig. S11. Bismuth suppressed the internalized *P. gingivalis* W83 in human cells. *P. gingivalis* W83 infected HOK, AGS and HCAEC cells at multiplicity of infection (MOI) of 100 in the absence (upper) or presence (lower) of 50 μM RBC for 90 min. Infected cells were further treated with either 200 μg/mL metronidazole (as internalized *P. gingivalis*) or 50 μM RBC or both for 60 min. CFUs were normalized to the average CFU of internalized *P. gingivalis*. Three independent repeats were performed. One-way ANOVA, n = 3, **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001.

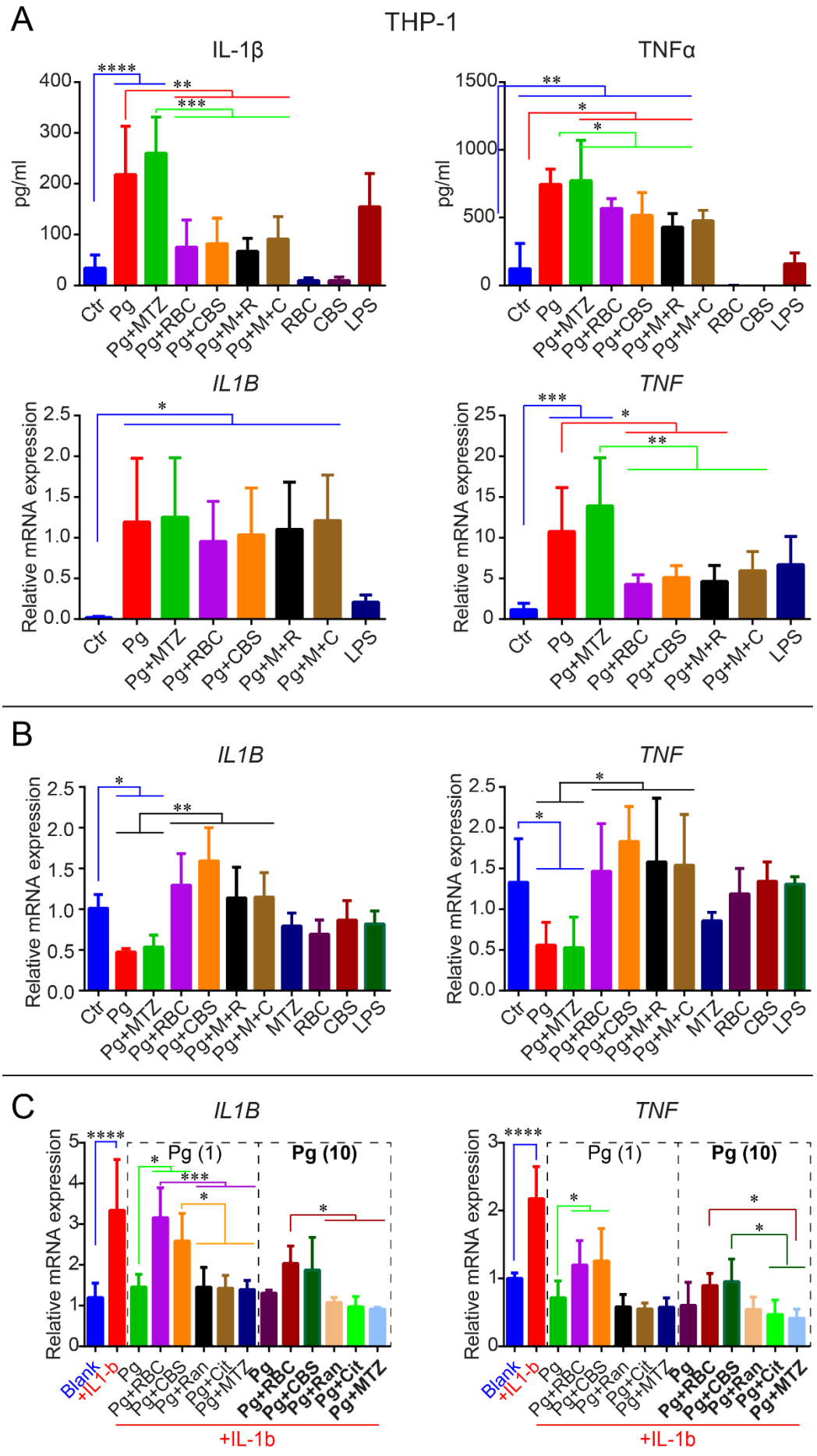

A
THP-1
IL-1β
pg/ml
TNFα
pg/ml
IL1B
Relative mRNA expression
TNF
Relative mRNA expression
Ctr
Pg
Pg+MTZ
Pg+RBC
Pg+CBS
Pg+M+R
Pg+M+C
RBC
CBS
LPS
B
IL1B
Relative mRNA expression
TNF
Relative mRNA expression
MTZ
C
IL1B
Relative mRNA expression
TNF
Relative mRNA expression
Pg (1)
Pg (10)
Blank
+IL1-b
Pg+Ran
Pg+Cit
+IL-1b

Fig. S12. Bismuth counteracted IL-1β and TNFα responses of THP-1 and HGEPs to *P. gingivalis*. (**A**) ELISA and mRNA levels of IL-1β and TNFα in *P. gingivalis*-infected THP-1 macrophage cells (MOI=100) with 200 μg/ml metronidazole (Pg+MTZ), 50 μM RBC or CBS, or combined (Pg+M+R or Pg+M+C). As controls, cells were directly treated with 50 μM RBC, CBS or 5 μg/mL *P. gingivalis* LPS. (**B**) mRNA levels of *IL1B* and *TNF* genes of HGEP cells of similar experiments. (**C**) mRNA levels of *IL1B* and *TNF* of IL-1β-treated (1ng/ml) HGEPs infected with *P. gingivalis* (MOI=100) treated with 50 μM RBC, CBS, Ran or Cit or 200 μg/ml MTZ. One-way ANOVA, n > 3, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

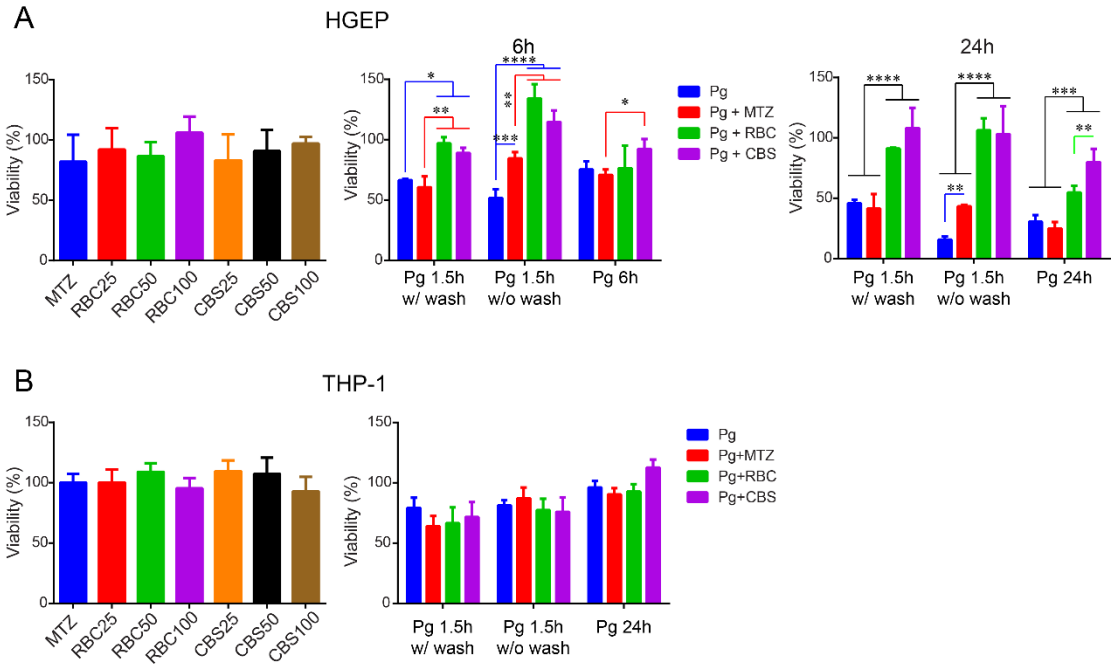


Fig. S13. Cell viability determined by CCK-8. (**A**) HGEPs (2×10^4 cells/well) treated with different compounds or challenged with *P. gingivalis* (MOI=100). MTZ: 200 μg/ml metronidazole; RBC25, RBC50 and RBC100: 25, 50 and 100 μM RBC; CBS25, CBS50 and CBS100: 25, 50 and 100 μM CBS. Pg 1.5h w/ wash: challenge cells with *P. gingivalis* for 1.5 h, remove the bacteria and wash cells with HBSS; Pg 1.5h w/o wash: same procedure without washing cells with HBSS; Pg 6h or Pg 24h: challenge cells with *P. gingivalis* for 6 h or 24h. Drugs were added at 1.5 h. (**B**) Macrophage THP-1 cells (4×10^4 cells/well) treated with different compounds or challenged with *P. gingivalis* (MOI=100). Two-way ANOVA, n > 3, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

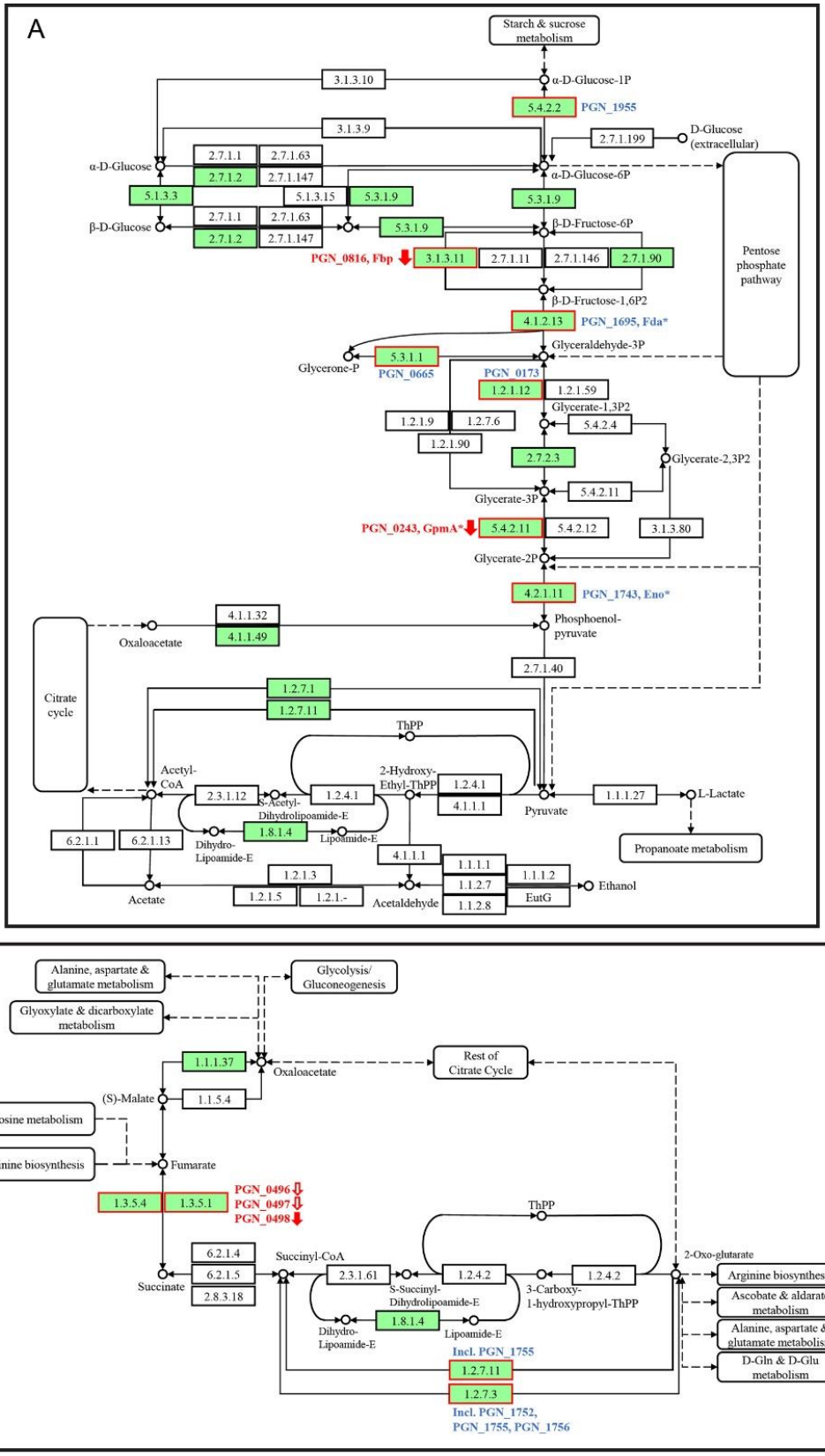


Fig. S14. The effects of bismuth on Glycolysis/Gluconeogenesis Pathway (KEGG: pgn00010) and Respiratory Chain (KEGG: pgn00020) of *P. gingivalis*. The diagrams were redrawn according to *P. gingivalis* ATCC 33277-specific pathways retrieved from KEGG

(copyrighted by Kanehisa Laboratories). *P. gingivalis* ATCC 33277-specific enzymes were filled with green while those from reference pathway were not filled. KEGG-enriched enzymes in this study (Bi-associated proteins, DEGs or significantly regulated proteins) from three different experiments were circled in red boxes. Asterisks indicate Bi association. Red downward arrows indicate more than 1.5-fold down-regulation in protein level. Protein names were colored in red for down-regulation and blue for non-evident regulation. Some irrelevant components in the original pathway map were omitted for clarity. (**A**) Glycolysis/Gluconeogenesis pathway. (**B**) Respiratory chain. Since *P. gingivalis* is a strictly anaerobic bacterium, it does not rely on Citrate cycle, and its respiratory chain has not yet been fully characterized. It has been implicated that *P. gingivalis* use fumarate respiration[18]. Therefore, parts of original Citrate cycle pathway map were omitted. Succinate dehydrogenase/fumarate reductase (PGN_0496-0498) is a key component in the respiratory chain.

Supplementary tables:

Table S1. Primers used in this study

For Cloning		
Primer Name	**Primer Sequence (5'-3')**	**Source**
PgSodA-for(NdeI)	GGAATTCCATATGACTCACGAACTCATTTCC	This study
PgSodA-rev(XhoI)	CCGCTCGAGTTAATACCGAGATTCTACAATATC	This study
PGN0033-for(NdeI)	GGAATTCCATATGGCACTGCAAATTACAGATG	This study
PGN0033-rev(XhoI)	CCGCTCGAGTTAAAACAAGGCATCCAATTCTT	This study
RgpA-CD-f(NdeI)	GGAATTCCATATGTACACACCGGTAGAGGAAAAA	This study
RgpA-CD720-r(XhoI)	CCGCTCGAGTTAGCTGCGAAGAAGTTCGGGG	This study
PgGroL-for(NdeI)	GGAATTCCATATGGCAAAAGAAATCAAATTCG	This study
PgGroL-rev(XhoI)	CCGCTCGAGTTACATCATGCCGCCCATTC	This study
PgDnaK-for(NdeI)	GGAATTCCATATGGGAAAAATCATTGGAATTGA	This study
PgDnaK-rev(XhoI)	CCGCTCGAGTTATTTCACTTCCTCGAAGTCT	This study
For qPCR		
16S-F	TGTTACAATGGGAGGGACAAAGGG	Ref. 19
16S-R	TTACTAGCGAATCCAGCTTCACGG	Ref. 19
glk-F	TCGGTGTAGGTGCTCCCAAT	This study
glk-R	AGCATTTGGGCGAAGGGTAT	This study
gyrB-F	AGTGGGCGTTTCTTGTGTGAA	This study
gyrB-R	CAGCTGAACTCCTGCATATGGA	This study
rpoB-F	GCGTTATTGTTTCCCAATTGC	This study
rpoB-R	AATGGTATGATTCGCGCTGAA	This study
SodA-F	TGGCTCCTGTTATCAGCAAAGA	This study
SodA-R	AATTCCGTGCCGATGATGAG	This study
PGN0033-F	CTGAAGGCAAGCCGATGGTA	This study
PGN0033-R	GATAGCGCGTCCTTCATATTCC	This study
rgpA-F	TCTTTGGCGGTTTCAGACACT	This study
rgpA-R	GGAGGGTGCAATCAGGACAT	This study
kgp-F	ACACCTGTTGTTCGCGTGAA	This study
kgp-R	AGAGGGTTGATGTGGCATGAG	This study
rgpB-F	TCCCCTACGTGTACGGACAGA	This study
rgpB-R	CATCACGCAGGATGAAAGGA	This study
IL6-F	GGAGACTTGCCTGGTGAAAATC	Ref. 20
IL6-R	GGGTCAGGGGTGGTTATTGC	Ref. 20
IL8-F	GACATACTCCAAACCTTTCCACC	Ref. 20
IL8-R	AACTTCTCCACAACCCTCTGC	Ref. 20
IL1B-F		Ref. 21
IL1B-R		Ref. 21
TNF-F	GAGGCCAAGCCCTGGTATG	PrimerBank (#25952110c2) Ref. 22
TNF-R	CGGGCCGATTGATCTCAGC	PrimerBank (#25952110c2) Ref. 22
ACTB-F	CATGTACGTTGCTATCCAGGC	PrimerBank (#4501885a1) Ref. 22

ACTB-R	CTCCTTAATGTCACGCACGAT	PrimerBank (#4501885a1) Ref. 22

Table S2. Antibodies used in this study

Antibody Name	**Antibody Source**
Rabbit polyclonal anti-PgSOD	This paper
Rabbit polyclonal anti-PGN0033	This paper
Rabbit anti-Porphyromonas gingivalis Gingipain R1	MyBioSource
Mouse monoclonal anti-GAPDH (clone GA1R)	Thermo Fisher
Goat polyclonal Anti-Rabbit IgG H&L, HRP-Conjugated	Abcam
Horse anti-mouse IgG, HRP-linked	Cell Signaling

Table S3. Peptide mass fingerprinting identification results of bismuth-associated proteins in *P. gingivalis* and GO and KEGG enrichment

The names of corresponding spots on Bi-TRACER fluorescence 2D gels (Fig. S2B) are listed in Sample Name. The full list of identification is in (**A**). Correctly identified proteins were considered when at least both Protein Score Confidence Interval (C.I. %) and Ion Score C.I. % are larger 95 and either Protein Score or Ion Score is larger than 100. Details for gingipains and hemagglutinins are in (**B**). GO and KEGG enrichment were performed using STRING and listed in (**C**). FDR cutoff is less than 0.05.

(A) Full list of identification

Plate Position	Sample Name	Protein Name	Pg 33277 Accession No.	Gene ID	MW
H14	Pg5/8-1	tetratricopeptide repeat protein, TPR-1	WP_012457845.1	PGN_0876	45886
H15	Pg5/8-2	tetratricopeptide repeat protein, TPR-1	WP_012457845.1	PGN_0876	45886
H16	Pg5/8-3	chaperonin, GroL	WP_012458280.1	PGN_1452	58210.3
H17	Pg5/8-4	translation elongation factor Tu, Tuf #			43575.1
H18	Pg5/8-5	tetratricopeptide repeat protein, TPR-2	WP_012458333.1	PGN_1513	51301.2
H19	Pg5/8-6	phosphopyruvate hydratase/Enolase, Eno	WP_012458500.1	PGN_1743	46049.4
H20	Pg5/8-7	tetratricopeptide repeat protein, TPR-2	WP_012458333.1	PGN_1513	51301.2
H21	Pg5/8-8	phosphopyruvate hydratase/Enolase, Eno	WP_012458500.1	PGN_1743	46049.4
I14	Pg5/8-9	translation elongation factor Tu, Tuf	WP_012458382.1	PGN_1578	43744.2
I15	Pg5/8-10	Major fimbrium subunit, FimA	WP_012457306.1	PGN_0180	41568.9
I16	Pg5/8-11	Minor fimbrium subunit, Mfa1	WP_012457396.1	PGN_0287	60862.5
I17	Pg5/8-12	50S ribosomal protein L14 #			13203.3
C9	Pg 5/8-13	lysine-specific cysteine proteinase; Kgp	WP_012458488.1	PGN_1728	188228.1
C10	Pg 5/8-14	lysine-specific cysteine proteinase; Kgp	WP_012458488.1	PGN_1728	188228.1
C11	Pg 5/8-15-1	aminomethyltransferase; glycine cleavage system protein T, GcvT ^	WP_005874565.1	PGN_0550	40438
C12	Pg 5/8-15-2	aminomethyltransferase; glycine cleavage system protein T, GcvT	WP_005874565.1	PGN_0550	40438
C13	Pg 5/8-16	class I fructose-bisphosphate aldolase, Fda	WP_012458465.1	PGN_1695	33051.9
C14	Pg 5/8-17	glutamate dehydrogenase, Gdh	WP_010956255.1	PGN_1367	49623.4
C15	Pg 5/8-18	phosphoserine transaminase #			40331.7
C16	Pg 5/8-19	NADP oxidoreductase; NAD(P)(+) transhydrogenase (Re/Si-specific) subunit alpha, PntA	WP_004585530.1	PGN_1120	41517.4
D9	Pg 5/8-20	glutamate dehydrogenase, Gdh	WP_010956255.1	PGN_1367	49623.4

D10	Pg 5/8-21	phosphoserine aminotransferase, SerC	WP_005874020.1	PGN_0612	40319.6
D11	Pg 5/8-22	OmpA family protein; outer membrane protein 40, OmpA-like	WP_012457732.1	PGN_0728	42578.2
D12	Pg 5/8-23	serine hydroxymethyltransferase, GlyA	WP_004583439.1	PGN_0038	46814.8
D13	Pg 5/8-24	malate dehydrogenase, Mdh	WP_004583610.1	PGN_1880	36422.6
D14	Pg 5/8-25	superoxide dismutase, SodA	WP_004585361.1	PGN_0564	21487.9
D15	Pg 5/8-26	peptidoglycan domain protein	WP_005874170.1	PGN_1670	21914.6
D16	Pg 5/8-27				
E9	Pg 7/10-1	OmpA family protein; outer membrane protein 41; immunoreactive 43kD antigen, OmpA_C-like	WP_012457733.1	PGN_0729	43477.2
E10	Pg 7/10-2	OmpA family protein; outer membrane protein 41; immunoreactive 43kD antigen, OmpA_C-like	WP_012457733.1	PGN_0729	43477.2
E11	Pg 7/10-3	OmpA family protein; outer membrane protein 41; immunoreactive 43kD antigen, OmpA_C-like	WP_012457733.1	PGN_0729	43477.2
E12	Pg 7/10-4	OmpA family protein; outer membrane protein 41; immunoreactive 43kD antigen, OmpA_C-like	WP_012457733.1	PGN_0729	43477.2
E13	Pg 7/10-5	OmpA family protein; outer membrane protein 41; immunoreactive 43kD antigen, OmpA_C-like	WP_012457733.1	PGN_0729	43477.2
E14	Pg 7/10-6	OmpA family protein; outer membrane protein 41; immunoreactive 43kD antigen, OmpA_C-like	WP_012457733.1	PGN_0729	43477.2
E15	Pg 7/10-7	glutamate dehydrogenase, Gdh	WP_010956255.1	PGN_1367	49623.4
E16	Pg 3/6-1	hypothetical protein HMPREF1988_02050, partial; Kgp; RgpA; cleaved adhesin domain protein [#]		PGN_1728 ; PGN_1970	
F9	Pg 3/6-2	arginine-specific thiol protease, RgpA	WP_043876339.1	PGN_1970	186817.8
F10	Pg 3/6-3	peptidase C25, partial [^]	WP_043876339.1	PGN_1970	186817.8
F11	Pg 3/6-4	arginine-specific thiol protease, RgpA	WP_043876339.1	PGN_1970	186817.8
F12	Pg 3/6-5	arginine-specific thiol protease, RgpA	WP_043876339.1	PGN_1970	186817.8
F13	Pg 3/6-6	Hemagglutinin A (HagA)	WP_012458492.1	PGN_1733	186817.8
F14	Pg 3/6-7	arginine-specific thiol protease, RgpA [^]	WP_043876339.1	PGN_1970	186817.8
F15	Pg 3/6-8	hemagglutinin/protease [#]			
F16	Pg 3/6-9	hypothetical protein, PorV	WP_004583425.1	PGN_0023	43239.6
G9	Pg 3/6-10	electron transport complex, RnfABCDGE type, G subunit; RnfG [^]	WP_004584790.1	PGN_1656	
G10	Pg 3/6-11	arginine-specific thiol protease, RgpA	WP_043876339.1	PGN_1970	186817.8
G11	Pg 3/6-13	50S ribosomal protein L7/L12, RplL	WP_010956001.1	PGN_1572	12735.8
G12	Pg 3/6-14	hemagglutinin; peptidase C25, partial [#]			
G13	Pg 3/6-15	30S ribosomal protein S19; RpsS [^]	WP_010956447.1	PGN_1864	9902.3
G14	Pg 3/6-16	thiol reductase thioredoxin	WP_004583434.1	PGN_0033	11539
G15	Pg 3/6-17	molecular chaperone, DnaK	WP_012457876.1	PGN_0916	69172.5

G16	Pg 3/6-18	peptidase M13; Endopeptidase, PepO ^	WP_012457383.1	PGN_0271	79063.9
H9	Pg 3/6-19	peptidase M13; Endopeptidase, PepO ^	WP_012457383.1	PGN_0271	79063.9
H10	Pg 3/6-20	chaperonin, GroL	WP_012458280.1	PGN_1452	58214.2
H11	Pg 3/6-21	chaperonin, GroL	WP_012458280.1	PGN_1452	58214.2
H12	Pg 3/6-22	peptidase family C25, ig-like domain protein, partial #			
H13	Pg 3/6-23	chaperonin, GroL	WP_012458280.1	PGN_1452	58214.2
H14	Pg 3/6-24	arginine-specific cysteine proteinase, RgpB ^	WP_012458292.1	PGN_1466	81278.2
J3	Pg3/6-25	arginine-specific cysteine proteinase, RgpB ^	WP_012458292.1	PGN_1466	81278.2
J4	Pg3/6-26	tetratricopeptide repeat protein, TPR-1	WP_012457845.1	PGN_0876	45886
J5	Pg3/6-27	tetratricopeptide repeat protein, TPR-1	WP_012457845.1	PGN_0876	45886
J6	Pg3/6-28	T9SS C-terminal target domain-containing protein ^	WP_012457704.1	PGN_0693	45011.8
J7	Pg3/6-29	GumN protein #	WP_012457322.1	PGN_0200	33587.3
J8	Pg3/6-30	electron transfer flavoprotein subunit beta, FixA	WP_005873659.1	PGN_1173	28703.8
J9	Pg3/6-31	electron transfer flavoprotein subunit beta, FixA	WP_005873659.1	PGN_1173	28703.8
J10	Pg3/6-32	hypothetical protein; fimbrial assembly protein; Mfa4	WP_012457398.1	PGN_0290	37144.1
K3	Pg3/6-34	indolepyruvate oxidoreductase subunit beta, IorB	WP_004585221.1	PGN_0709	21067
K4	Pg3/6-35	NAD dependent epimerase/dehydratase; UDP-glucose 4-epimerase; NAD-dependent nucleotide-diphosphate-sugar epimerase; WcaG	WP_004584504.1	PGN_1370	35306.7
K5	Pg3/6-36	2-Cys peroxiredoxin; lipid hydroperoxide peroxidase, Tpx	WP_018964814.1	PGN_0388	18129.4
K6	Pg3/6-37	NA starvation/stationary phase protection protein #	WP_012458706.1	PGN_2037	17908.3
K7	Pg3/6-38	outer membrane protein, OmpH ^	WP_012457407.1	PGN_0300	
K8	Pg3/6-39	tetratricopeptide repeat protein, TPR-2	WP_012458333.1	PGN_1513	51301.2
K9	Pg5/8-28	electron transfer flavoprotein subunit, FixA	WP_005873659.1	PGN_1173	28703.8
K10	Pg5/8-29	fimbrial assembly protein, Mfa4	WP_012457398.1	PGN_0290	37144.1
L3	Pg5/8-30	peptidylprolyl isomerase; peptidyl-prolyl cis-trans isomerase, FKBP-type, FkpA	WP_004585257.1	PGN_0743	27971.6
L4	Pg5/8-31	thiol reductase thioredoxin	WP_004583434.1	PGN_0033	11539
J12	Pg3/6-40	translation elongation factor Tu, Tuf	WP_012458382.1	PGN_1578	43744.2
J13	Pg3/6-41	translation elongation factor Tu, Tuf ^	WP_012458382.1	PGN_1578	43744.2
J14	Pg3/6-42	arginine-specific thiol protease, RgpA	WP_043876339.1	PGN_1970	186817.8
J15	Pg3/6-43	tetratricopeptide repeat protein, TPR-2	WP_012458333.1	PGN_1513	51301.2
J16	Pg3/6-44	phosphopyruvate hydratase/Enolase, Eno	WP_012458500.1	PGN_1743	46049.4
J17	Pg3/6-45	phosphate acetyltransferase, EutD	WP_004584444.1	PGN_1179	35976.1

J18	Pg3/6-46	fimbrilin; FimA type I fimbrilin, FimA	WP_012457306.1	PGN_0180	41568.9
J19	Pg3/6-47	glycine cleavage system protein T, GcvT	WP_005874565.1	PGN_0550	40438
K12	Pg3/6-48	class I fructose-bisphosphate aldolase, Fda	WP_012458465.1	PGN_1695	33080
K13	Pg3/6-49	ribosome-recycling factor, Frr	WP_012458570.1	PGN_1832	20785
K14	Pg3/6-50	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase, GpmA	WP_005875265.1	PGN_0243	28832.9
K15	Pg3/6-52	lysine-specific cysteine proteinase, partial; Kgp	WP_012458488.1	PGN_1728	188228.1
K16	Pg3/6-53	lysine-specific cysteine proteinase, partial; Kgp	WP_012458488.1	PGN_1728	188228.1
K17	Pg3/6-54	lysine-specific cysteine proteinase, partial; Kgp	WP_012458488.1	PGN_1728	188228.1
K18	Pg3/6-55	superoxide dismutase; SodA	WP_004585361.1	PGN_0564	21487.9
K19	Pg3/6-56	peptidoglycan domain protein	WP_005874170.1	PGN_1670	21914.6
L12	Pg3/6-57	NAD dependent epimerase/dehydratase; UDP-glucose 4-epimerase; NAD-dependent nucleotide-diphosphate-sugar epimerase; WcaG	WP_004584504.1	PGN_1370	35306.7
L13	Pg3/6-58	SusC/RagA family TonB-linked outer membrane protein; receptor antigen A, RagA ^	WP_080504438.1	PGN_0293	112329
L14	Pg3/6-59	glutamate dehydrogenase, Gdh	WP_010956255.1	PGN_1367	49623.4
L15	Pg3/6-60	glutamate dehydrogenase, Gdh	WP_010956255.1	PGN_1367	49623.4
L16	Pg3/6-61	glutamate dehydrogenase, Gdh	WP_010956255.1	PGN_1367	49623.4
L17	Pg3/6-62	NAD(P) transhydrogenase subunit alpha, PntA	WP_043890130.1	PGN_1120	41482.4
L18	Pg3/6-63	NAD(P) transhydrogenase subunit alpha, PntA	WP_043890130.1	PGN_1120	41482.4
L19	Pg3/6-64	DNA recombination protein RmuC #			
M12	Pg3/6-65	Fimbrillin #			
M13	Pg3/6-66	aspartate-semialdehyde dehydrogenase, Asd ^	WP_012457650.1	PGN_0618	37480.9
M14	Pg3/6-69	hypothetical protein #			
M15	Pg3/6-70	outer membrane protein 40 #			
M16	Pg3/6-71	glutamate dehydrogenase, partial #			
M17	Pg3/6-72	hypothetical protein #			

Note: #: Not correctly identified, ^ : Identified, either Protein Score or Ion Score is larger than 100.

(B) Details for gingipains and hemagglutinins

Plate Position	Sample Name	Protein Name	Pg 33277 Accession No.	Gene ID	MW	Region	Domain	Region MW

C9	Pg 5/8-13	lysine-specific cysteine proteinase; Kgp	WP_012458488.1	PGN_1728	188228.1	229-700	catalytic	51825.97
C10	Pg 5/8-14	lysine-specific cysteine proteinase; Kgp	WP_012458488.1	PGN_1728	188228.1	229-700	catalytic	51825.97
E16	Pg 3/6-1	Kgp, RgpA ^		PGN_1728; PGN_1970		978-1145; 996-1162	RgpA HA1 adhesin; Kgp39 adhesin	
F9	Pg 3/6-2	Kgp, RgpA ^		PGN_1728; PGN_1970			HA1-3; Kgp39-HA2	
F10	Pg 3/6-3	arginine-specific thiol protease; RgpA ^	WP_043876339.1	PGN_1970		550-1386		
F11	Pg 3/6-4	arginine-specific thiol protease; RgpA	WP_043876339.1	PGN_1970	186817.8			
F12	Pg 3/6-5	arginine-specific thiol protease; RgpA	WP_043876339.1	PGN_1970			CD,HA1-4	
F13	Pg 3/6-6	Hemagglutinin A (HagA)	WP_012458492.1	PGN_1733	285018.1			
F14	Pg 3/6-7	RgpA; Kgp; hemagglutinin ^						
F15	Pg 3/6-8	hemagglutinin/protease #						
G10	Pg 3/6-11	arginine-specific thiol protease; RgpA	WP_043876339.1	PGN_1970	188228.1	819-1414	HA1-3	64313.02
G12	Pg 3/6-14	hemagglutinin; peptidase C25, partial #						
H12	Pg 3/6-22	peptidase family C25, ig-like domain protein, partial #						
H14	Pg 3/6-24	arginine-specific cysteine proteinase, RgpB	WP_012458292.1	PGN_1466	81278.2			
J3	Pg3/6-25	arginine-specific cysteine proteinase, RgpB	WP_012458292.1	PGN_1466	81278.2			
J14	Pg3/6-42	arginine-specific thiol protease; RgpA	WP_043876339.1	PGN_1970	186817.8	36-678	Propeptide, catalytic	71241.92
K15	Pg3/6-52	lysine-specific cysteine proteinase; Kgp	WP_012458488.1	PGN_1728	188228.1	229-700	catalytic	51825.97
K16	Pg3/6-53	lysine-specific cysteine proteinase; Kgp	WP_012458488.1	PGN_1728	188228.1	120-700	Propeptide, catalytic	64129.95
K17	Pg3/6-54	lysine-specific cysteine proteinase; Kgp	WP_012458488.1	PGN_1728	188228.1	120-700	Propeptide, catalytic	64129.95

(C) GO and KEGG enrichment of identified bismuth-associating proteins in *P. gingivalis*

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
GO.0008150	**biological process**	17	3.69E-05	PGN_0038 (glyA), PGN_0180 (fimA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_0916 (dnaK), PGN_1367 (gdh), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1695 (fda), PGN_1728 (kgp), PGN_1733 (hagA), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS), PGN_1970 (rgpA)

GO.0044238	primary metabolic process	16	3.69E-05	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_0916 (dnaK), PGN_1367 (gdh), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1695 (fda), PGN_1728 (kgp), PGN_1733 (hagA), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS), PGN_1970 (rgpA)
GO.0071704	organic substance metabolic process	16	5.94E-05	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_0916 (dnaK), PGN_1367 (gdh), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1695 (fda), PGN_1728 (kgp), PGN_1733 (hagA), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS), PGN_1970 (rgpA)
GO.0009405	pathogenesis [&]	4	0.000289	PGN_0180 (fimA), PGN_1728 (kgp), PGN_1733 (hagA), PGN_1970 (rgpA)
GO.0019752	carboxylic acid metabolic process	7	0.00111	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_1367 (gdh), PGN_1695 (fda), PGN_1743 (eno)
GO.1901564	organonitrogen compound metabolic process	11	0.00119	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_1367 (gdh), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1695 (fda), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS)
GO.0006096	glycolytic process	3	0.00172	PGN_0243 (gpmA), PGN_1695 (fda), PGN_1743 (eno)
GO.0009069	serine family amino acid metabolic process	3	0.00172	PGN_0038 (glyA), PGN_0550 (gcvT), PGN_0612 (serC)
GO.0019538	protein metabolic process	9	0.00172	PGN_0916 (dnaK), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1728 (kgp), PGN_1733 (hagA), PGN_1832 (frr), PGN_1864 (rpsS), PGN_1970 (rgpA)
GO.0044237	cellular metabolic process	13	0.00172	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (SerC), PGN_0916 (dnaK), PGN_1367 (gdh), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1695 (fda), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS)
GO.0044712	single-organism catabolic process	4	0.00172	PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_1695 (fda), PGN_1743 (eno)
GO.0072524	pyridine-containing compound metabolic process	4	0.00172	PGN_0243 (gpmA), PGN_0612 (serC), PGN_1695 (fda), PGN_1743 (eno)
GO.1901575	organic substance catabolic process	4	0.00268	PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_1695 (fda), PGN_1743 (eno)
GO.0006508	proteolysis [&]	3	0.00645	PGN_1728 (kgp), PGN_1733 (hagA), PGN_1970 (rgpA)
GO.0006563	L-serine metabolic process	2	0.00645	PGN_0038 (glyA), PGN_0612 (serC)
GO.0044763	single-organism cellular process	8	0.0104	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_1367 (gdh), PGN_1695 (fda), PGN_1743 (eno), PGN_1832 (frr)
GO.0006732	coenzyme metabolic process	4	0.0128	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_1695 (fda), PGN_1743 (eno)

GO.0006544	glycine metabolic process	2	0.0144	PGN_0038 (glyA), PGN_0550 (gcvT)
GO.0009070	serine family amino acid biosynthetic process	2	0.0144	PGN_0038 (glyA), PGN_0612 (serC)
GO.0006520	cellular amino acid metabolic process	4	0.0179	PGN_0038 (glyA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_1367 (gdh)
GO.0034641	cellular nitrogen compound metabolic process	9	0.0217	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0612 (serC), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1695 (fda), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS)
GO.0044267	cellular protein metabolic process	6	0.0465	PGN_0916 (dnaK), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1832 (frr), PGN_1864 (rpsS)
GO.0003674	**molecular function**	16	0.000179	PGN_0038 (glyA), PGN_0180 (fimA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_0916 (dnaK), PGN_1367 (gdh), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1695 (fda), PGN_1728 (kgp), PGN_1733 (hagA), PGN_1743 (eno), PGN_1864 (rpsS), PGN_1970 (rgpA)
GO.0004197	cysteine-type endopeptidase activity &	3	0.00139	PGN_1728 (kgp), PGN_1733 (hagA), PGN_1970 (rgpA)
GO.0003824	catalytic activity	11	0.00307	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_1367 (gdh), PGN_1578 (tuf), PGN_1695 (fda), PGN_1728 (kgp), PGN_1733 (hagA), PGN_1743 (eno), PGN_1970 (rgpA)
GO.0008483	transaminase activity	2	0.0161	PGN_0550 (gcvT), PGN_0612 (serC)
GO.0030170	pyridoxal phosphate binding	2	0.0161	PGN_0038 (glyA), PGN_0612 (serC)
GO.0005575	**cellular component**	12	0.00078	PGN_0038 (glyA), PGN_0180 (fimA), PGN_0612 (serC), PGN_1367 (gdh), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1728 (kgp), PGN_1733 (hagA), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS)
GO.0005576	extracellular region	3	0.000363	PGN_1728 (kgp), PGN_1733 (hagA), PGN_1743 (eno)
GO.0005615	extracellular space	2	0.00379	PGN_1728 (kgp), PGN_1733 (hagA)
GO.0009986	cell surface	2	0.00379	PGN_1367 (gdh), PGN_1743 (eno)
GO.0005623	cell	10	0.00454	PGN_0038 (glyA), PGN_0180 (fimA), PGN_0612 (serC), PGN_1367 (gdh), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS)
GO.0044464	cell part	10	0.00454	PGN_0038 (glyA), PGN_0180 (fimA), PGN_0612 (serC), PGN_1367 (gdh), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS)

GO.0005622	intracellular	8	0.0245	PGN_0038 (glyA), PGN_0612 (serC), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS)
GO.0005737	cytoplasm	8	0.0245	PGN_0038 (glyA), PGN_0612 (serC), PGN_1452 (groL), PGN_1572 (rplL), PGN_1578 (tuf), PGN_1743 (eno), PGN_1832 (frr), PGN_1864 (rpsS)
KEGG				
00680	Methane metabolism	7	1.00E-07	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0612 (serC), PGN_1179 (eutD), PGN_1695 (fda), PGN_1743 (eno), PGN_1880 (mdh)
01200	Carbon metabolism	8	0.000245	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_1179 (eutD), PGN_1695 (fda), PGN_1743 (eno), PGN_1880 (mdh)
00260	Glycine, serine and threonine metabolism	5	0.000359	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0550 (gcvT), PGN_0612 (serC), PGN_0618 (asd)
01120	Microbial metabolism in diverse environments	8	0.000585	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0612 (serC), PGN_0618 (asd), PGN_1179 (eutD), PGN_1695 (fda), PGN_1743 (eno), PGN_1880 (mdh)
01230	Biosynthesis of amino acids	6	0.00171	PGN_0038 (glyA), PGN_0243 (gpmA), PGN_0612 (serC), PGN_0618 (asd), PGN_1695 (fda), PGN_1743 (eno)
03018	RNA degradation	3	0.0111	PGN_0916 (dnaK), PGN_1452 (groL), PGN_1743 (eno)
00010	Glycolysis / Gluconeogenesis	3	0.0217	PGN_0243 (gpmA), PGN_1695 (fda), PGN_1743 (eno)

Significant enrichment result (FDR < 0.05)

[&]: Interestingly enriched categories are in orange

Gene names were obtained from NCBI. Old locus tags were used, as "PGN_xxxx".

Table S4. Summary of DEGs of short- and long-term, up- and down-regulation in *P. gingivalis* at each timepoint after RBC treatment and their GO and KEGG enrichments

Definition: The DEGs found at 6h were considered long-term regulated, otherwise, short-term if present at two timepoints, and they were listed in the (**A**) and (**B**), respectively, and both new and old NCBI locus tags were shown. (**C** and **D**) The enrichment of DEGs at each timepoint. Threshold of significance for both GO and KEGG enrichment is P-value ≤ 0.05, but we also included those with P-value less than 0.06. It should be noticed that due to the GO database, two categories were incorrectly annotated, i.e., proteolysis and peptidase activity, both of which only contained six transposase genes and should be excluded. The values of log2(Y/X) were shown within parentheses.

(A) Short-termly regulated DEGs

Up-regulated genes		
Gene symbol	**Old symbol**	**Description**
PGN_RS00045	PGN_0008	ATP-dependent Clp protease ClpC
PGN_RS00160	PGN_0033	thiol reductase thioredoxin
PGN_RS00190	PGN_0041	molecular chaperone HtpG
PGN_RS00195	PGN_0042	phosphatidate cytidylyltransferase
PGN_RS00200	PGN_0043	cell division protein FtsH; ATP-dependent zinc metalloprotease FtsH
PGN_RS00245	PGN_0053	hypothetical protein
PGN_RS00250	PGN_0054	hypothetical protein
PGN_RS00255	PGN_0055	lysozyme
PGN_RS00260	PGN_0056	conjugal transfer protein
PGN_RS00505 #	PGN_0108	pseudo
PGN_RS01370	PGN_0285	FAD-dependent pyridine nucleotide-disulfide oxidoreductase
PGN_RS01505	PGN_0314	formate transporter
PGN_RS01715	PGN_0359	Fe-S cluster assembly protein SufD; ABC transporter permease protein
PGN_RS01765	PGN_0371	4'-phosphopantetheinyl transferase
PGN_RS01770	PGN_0373	thiol reductase thioredoxin
PGN_RS01845	PGN_0388	2-Cys peroxiredoxin
PGN_RS02340	PGN_0490	MATE family efflux transporter; DNA-damage-inducible protein F
PGN_RS02350	PGN_0492	copper-translocating P-type ATPase

PGN_RS02355		hypothetical protein
PGN_RS02670	PGN_0564	superoxide dismutase [Mn/Fe]
PGN_RS02880	PGN_0604	ferritin
PGN_RS03140	PGN_0660	peroxiredoxin; alkyl hydroperoxide reductase C subunit
PGN_RS03145	PGN_0661	alkyl hydroperoxide reductase subunit F
PGN_RS03435	PGN_0720	ABC transporter permease
PGN_RS03440	PGN_0721	phosphonate ABC transporter ATP-binding protein
PGN_RS03445	PGN_0722	hypothetical protein
PGN_RS03530	PGN_0741	TonB-dependent receptor
PGN_RS04020	PGN_0842	IS5/IS1182 family transposase
PGN_RS04365	PGN_0916	molecular chaperone DnaK
PGN_RS04370		mobilization protein
PGN_RS04375	PGN_0917	tyrosine recombinase
PGN_RS04380		hypothetical protein, discontinued
PGN_RS04475	PGN_0936	glycerate dehydrogenase
PGN_RS04650 #	PGN_0971	IS5/IS1182 family transposase
PGN_RS05030	PGN_1049	alkaline phosphatase
PGN_RS05040		hypothetical protein, discontinued
PGN_RS05320	PGN_1111	formate--tetrahydrofolate ligase
PGN_RS05355	PGN_1118	IS5/IS1182 family transposase
PGN_RS05570 #	PGN_1161	IS5/IS1182 family transposase
PGN_RS05800	PGN_1206	bifunctional methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase
PGN_RS05810	PGN_1208	ATP-dependent chaperone ClpB
PGN_RS05870	PGN_1221	cobalamin adenosyltransferase
PGN_RS05920	PGN_1232	thioredoxin-disulfide reductase
PGN_RS06005	PGN_1253	hypothetical protein
PGN_RS06120 #	PGN_1280	pseudo
PGN_RS06125 #	PGN_1281	pseudo
PGN_RS06145	PGN_1285	conjugal transfer protein
PGN_RS06150	PGN_1286	lysozyme
PGN_RS06155	PGN_1287	hypothetical protein
PGN_RS06160	PGN_1288	hypothetical protein

PGN_RS06250	PGN_1308	DNA-binding protein; iron dependent repressor; FeoA
PGN_RS06255	PGN_1309	ferrous iron transporter B; FeoB
PGN_RS06930	PGN_1451	molecular chaperone GroES
PGN_RS06935	PGN_1452	molecular chaperone GroEL
PGN_RS07285	PGN_1526	hypothetical protein
PGN_RS07290	PGN_1527	SAM-dependent methyltransferase
PGN_RS07295	PGN_1528	SAM-dependent methyltransferase
PGN_RS07375	PGN_1547	DUF2807 domain-containing protein
PGN_RS08155	PGN_1715	nucleotide exchange factor GrpE
PGN_RS08160	PGN_1716	molecular chaperone DnaJ
PGN_RS08165	PGN_1717	hypothetical protein
PGN_RS08170	PGN_1718	UDP-2,3-diacylglucosamine hydrolase
PGN_RS08175	PGN_1719	RNase III inhibitor
PGN_RS08220	PGN_1729	acetyltransferase
PGN_RS08225	PGN_1730	hypothetical protein
PGN_RS08525	PGN_1797	membrane protein
PGN_RS08530	PGN_1798	methyltransferase UbiE
PGN_RS09040 ^	PGN_1906	hypothetical protein; Hemagglutinin protein HagC
PGN_RS09240	PGN_1951	hypothetical protein
PGN_RS09245	PGN_1953	TonB-dependent receptor
PGN_RS09640	PGN_2037	DNA starvation/stationary phase protection protein
PGN_RS09825	PGN_2071	2-dehydropantoate 2-reductase
Down-regulated genes		
Gene symbol	**Old symbol**	**Description**
PGN_RS00840		pseudo
PGN_RS00845	PGN_0183	major fimbrial subunit protein (FimA); Major fimbrium subunit FimC
PGN_RS00850	PGN_0184	hypothetical protein; Major fimbrium tip subunit FimD
PGN_RS00855	PGN_0185	hypothetical protein; Major fimbrium tip subunit FimE
PGN_RS00860 #	PGN_0186	hypothetical protein
PGN_RS01450	PGN_0302	rubrerythrin
PGN_RS01455 #		hypothetical protein

PGN_RS01890	PGN_0395	hypothetical protein
PGN_RS02365	PGN_0496	succinate dehydrogenase/fumarate reductase cytochrome b subunit
PGN_RS02370	PGN_0497	succinate dehydrogenase/Fumarate reductase flavoprotein subunit
PGN_RS02740	PGN_0577	IS5/IS1182 family transposase
PGN_RS02930	PGN_0614	histidinol-phosphate aminotransferase
PGN_RS03705	PGN_0777	glycosyl transferase
PGN_RS04045 ^	PGN_0846	hypothetical protein
PGN_RS04275	PGN_0895	4Fe-4S ferredoxin
PGN_RS05095	PGN_1063	transposase
PGN_RS05715	PGN_1191	IS5/IS1182 family transposase, discontinued
PGN_RS06495		hypothetical protein
PGN_RS06725	PGN_1407	histidinol-phosphate aminotransferase
PGN_RS07125	PGN_1494	coproporphyrinogen III oxidase
PGN_RS07225	PGN_1515	type III pantothenate kinase
PGN_RS08715		pseudo, discontinued
PGN_RS09535	PGN_2014	RND transporter MFP subunit

^: FPKMs for both treated and untreated samples were less than 10. #: FPKMs either both treated or untreated samples were less than 50, the gene were colored in orange. Both were underlined and not considered for final interpretation.

(B) Long-termly regulated DEGs

Up-regulated genes		
Gene symbol	**Old symbol**	**Description**
PGN_RS01045 #	PGN_0218	IS5/IS1182 family transposase
PGN_RS02170 #	PGN_0454	IS982 family transposase
PGN_RS02300	PGN_0481	hypothetical protein
PGN_RS02645	PGN_0557	TonB-dependent receptor (hemin utilization receptor, HmuR)
PGN_RS02650	PGN_0558	hypothetical protein (HmuY)
PGN_RS04510	PGN_0945	TetR family transcriptional regulator
PGN_RS04515	PGN_0946	membrane protein

PGN_RS04520	PGN_0947	hypothetical protein
PGN_RS04525	PGN_0948	hypothetical protein
PGN_RS04530	PGN_0949	ABC transporter ATP-binding protein
PGN_RS04535	PGN_0950	ABC transporter ATP-binding protein
PGN_RS04540		hypothetical protein
PGN_RS05165 #	PGN_1077	IS5/IS1182 family transposase
PGN_RS06370	PGN_1334	hypothetical protein
PGN_RS06375	PGN_1335	TonB-dependent receptor
PGN_RS06380	PGN_1336	DUF4876 domain-containing protein
PGN_RS07040	PGN_1476	T9SS C-terminal target domain-containing protein
PGN_RS08495	PGN_1790	hypothetical protein
PGN_RS08500	PGN_1791	flavodoxin
PGN_RS09080		hypothetical protein
PGN_RS09085	PGN_1916	ABC transporter ATP-binding protein
PGN_RS09090	PGN_1917	ABC transporter ATP-binding protein
PGN_RS09095	PGN_1918	hypothetical protein
PGN_RS09100	PGN_1919	hypothetical protein
PGN_RS09105	PGN_1920	membrane protein
PGN_RS09110	PGN_1921	TetR family transcriptional regulator
Down-regulated genes		
Gene symbol	**Old symbol**	**Description**
PGN_RS01370	PGN_0285	FAD-dependent pyridine nucleotide-disulfide oxidoreductase
PGN_RS02125 #	PGN_0442	IS982 family transposase
PGN_RS02195 ^	PGN_0459	IS982 family transposase
PGN_RS04945	PGN_1032	hypothetical protein
PGN_RS02785 #	PGN_0585	IS982 family transposase
PGN_RS03075	PGN_0644	IS5/IS1182 family transposase
PGN_RS03770 ^	PGN_0790	IS982 family transposase
PGN_RS04120 #	PGN_0864	IS982 family transposase
PGN_RS05110 #	PGN_1066	IS982 family transposase

(C) GO Enrichment

30 min				
GO Term	**Cluster Freq.**	**Genome Freq.**	**Corrected P-value**	**DEGs List**
Biological Process				
homeostatic process	9 out of 139 genes, 6.5%	14 out of 980 genes, 1.4%	0.00569	PGN_RS00160 (2.4), PGN_RS01845 (2.8), PGN_RS02880 (2.2), PGN_RS03145 (1.8), PGN_RS03580 (-1.1), PGN_RS06255 (2.8), PGN_RS07030 (-1.1), PGN_RS09520 (-1.0), PGN_RS09640 (2.2)
cofactor biosynthetic process	17 out of 139 genes, 12.2%	46 out of 980 genes, 4.7%	0.01948	PGN_RS01705 (2.5), PGN_RS01715 (2.4), PGN_RS02545 (-1.1), PGN_RS02550 (-1.0), PGN_RS02690 (2.0), PGN_RS03025 (-1.1), PGN_RS03190 (-1.0), PGN_RS03230 (-1.1), PGN_RS05045 (2.2), PGN_RS05320 (3.1), PGN_RS05425 (-1.1), PGN_RS05800 (2.5), PGN_RS07225 (-1.2), PGN_RS08655 (-1.2), PGN_RS08660 (-1.2), PGN_RS09780 (-1.0), PGN_RS09825 (2.8)
coenzyme biosynthetic process	15 out of 139 genes, 10.8%	39 out of 980 genes, 4.0%	0.03202	PGN_RS02545 (-1.1), PGN_RS02550 (-1.0), PGN_RS02690 (2.0), PGN_RS03025 (-1.1), PGN_RS03190 (-1.0), PGN_RS03230 (-1.1), PGN_RS05045 (2.2), PGN_RS05320 (3.1), PGN_RS05425 (1.1), PGN_RS05800 (2.5), PGN_RS07225 (-1.2), PGN_RS08655 (-1.2), PGN_RS08660 (-1.2), PGN_RS09780 (-1.0), PGN_RS09825 (2.8)
cellular homeostasis	6 out of 139 genes, 4.3%	8 out of 980 genes, 0.8%	0.04725	PGN_RS00160 (2.4), PGN_RS01845 (2.8), PGN_RS02880 (2.2), PGN_RS03145 (1.8), PGN_RS03580 (-1.1), PGN_RS09640 (2.2)
1h				
Biological Process				
transposition, DNA-mediated	21 out of 92 genes, 22.8%	57 out of 980 genes, 5.8%	6.49E-07	PGN_RS00490 (1.5), PGN_RS00505 (3.3), PGN_RS00985 (-2.1), PGN_RS02740 (-1.3), PGN_RS02795 (-1.4), PGN_RS03075 (-9.1), PGN_RS03570 (-1.1), PGN_RS03980 (6.6), PGN_RS04020 (8.3), PGN_RS04650 (7.4), PGN_RS04825 (-2.4), PGN_RS05165 (2.1), PGN_RS05355 (3.8), PGN_RS05570 (1.1), PGN_RS05650 (-1.2), PGN_RS05715 (-1.3), PGN_RS05850 (-1.1), PGN_RS06105 (1.5), PGN_RS06120 (3.3), PGN_RS08715 (-1.4), PGN_RS09010 (6.5)
transposition	21 out of 92 genes, 22.8%	57 out of 980 genes, 5.8%	6.49E-07	PGN_RS00490 (1.5), PGN_RS00505 (3.3), PGN_RS00985 (-2.1), PGN_RS02740 (-1.3), PGN_RS02795 (-1.4), PGN_RS03075 (-9.1), PGN_RS03570 (-1.1), PGN_RS03980 (6.6), PGN_RS04020 (8.3), PGN_RS04650 (7.4), PGN_RS04825 (-2.4), PGN_RS05165 (2.1), PGN_RS05355 (3.8), PGN_RS05570 (1.1), PGN_RS05650 (-1.2), PGN_RS05715 (-1.3), PGN_RS05850 (-1.1), PGN_RS06105 (1.5), PGN_RS06120 (3.3), PGN_RS08715 (-1.4), PGN_RS09010 (6.5)
DNA recombination	22 out of 92 genes, 23.9%	73 out of 980 genes, 7.4%	1.92E-05	PGN_RS00490 (1.5), PGN_RS00505 (3.3), PGN_RS00985 (-2.1), PGN_RS02740 (-1.3), PGN_RS02795 (-1.4), PGN_RS03075 (-9.1), PGN_RS03570 (-1.1),

				PGN_RS03980 (6.6), PGN_RS04020 (8.3), PGN_RS04375(1.5), PGN_RS04650 (7.4), PGN_RS04825 (-2.4), PGN_RS05165 (2.1), PGN_RS05355 (3.8), PGN_RS05570 (1.1), PGN_RS05650 (-1.2), PGN_RS05715 (-1.3), PGN_RS05850 (-1.1), PGN_RS06105 (1.5), PGN_RS06120 (3.3), PGN_RS08715 (-1.4), PGN_RS09010 (6.5)
response to oxidative stress	5 out of 92 genes, 5.4%	6 out of 980 genes, 0.6%	0.00586	PGN_RS01450 (-2.1), PGN_RS02670 (1.5), PGN_RS03140 (1.2), PGN_RS03145 (1.4), PGN_RS05920 (1.1)
DNA metabolic process	26 out of 92 genes, 28.3%	130 out of 980 genes, 13.3%	0.00722	PGN_RS00490 (1.5), PGN_RS00505 (3.3), PGN_RS00985 (-2.1), PGN_RS02740 (-1.3), PGN_RS02795 (-1.4), PGN_RS03075 (-9.1), PGN_RS03570 (-1.1), PGN_RS03980 (6.6), PGN_RS04020 (8.3), PGN_RS04375 (1.5), PGN_RS04650 (7.4), PGN_RS04705 (-1.1), PGN_RS04825 (-2.4), PGN_RS05095 (-1.0), PGN_RS05165 (2.1), PGN_RS05355 (3.8), PGN_RS05570 (1.1), PGN_RS05650 (-1.2), PGN_RS05715 (-1.3), PGN_RS05850 (-1.1), PGN_RS05895 (1.6), PGN_RS06105 (1.5), PGN_RS06120 (3.3), PGN_RS08160 (2.4), PGN_RS08715 (-1.4), PGN_RS09010 (6.5)
oxidation-reduction process	26 out of 92 genes, 28.3%	130 out of 980 genes, 13.3%	0.00722	PGN_RS00160 (1.4), PGN_RS01450 (-2.1), PGN_RS01845 (2.4), PGN_RS02370 (-1.1), PGN_RS02375 (-1.1), PGN_RS02670 (1.5), PGN_RS02880 (2.5), PGN_RS03140 (1.2), PGN_RS03145 (1.4), PGN_RS04475 (1.6), PGN_RS04995 (1.8), PGN_RS05125 (-1.1), PGN_RS05800 (1.4), PGN_RS05895 (1.6), PGN_RS05920 (1.1), PGN_RS07875 (-1.6), PGN_RS07880 (-1.3), PGN_RS07885 (-1.2), PGN_RS07890 (-1.5), PGN_RS07895 (-1.4), PGN_RS08325 (-1.5), PGN_RS08340 (-1.3), PGN_RS08345 (-1.4), PGN_RS08500 (2.2), PGN_RS09640 (1.3), PGN_RS09825 (1.7)
cellular homeostasis	5 out of 92 genes, 5.4%	8 out of 980 genes, 0.8%	0.04688	PGN_RS00160 (1.4), PGN_RS01845 (2.4), PGN_RS02880 (2.5), PGN_RS03145 (1.4), PGN_RS09640 (1.3)
Molecular Function				
transposase activity	21 out of 100 genes, 21.0%	56 out of 1098 genes, 5.1%	1.87E-07	PGN_RS00490 (1.5), PGN_RS00505 (3.3), PGN_RS00985 (-2.1), PGN_RS02740 (-1.3), PGN_RS02795 (-1.4), PGN_RS03075 (-9.1), PGN_RS03570 (-1.1), PGN_RS03980 (6.6), PGN_RS04020 (8.3), PGN_RS04650 (7.4), PGN_RS04825 (-2.4), PGN_RS05165 (2.1), PGN_RS05355 (3.8), PGN_RS05570 (1.1), PGN_RS05650 (-1.2), PGN_RS05715 (-1.3), PGN_RS05850 (-1.1), PGN_RS06105 (1.5), PGN_RS06120 (3.3), PGN_RS08715 (-1.4), PGN_RS09010 (6.5)
electron carrier activity	6 out of 100 genes, 6.0%	13 out of 1098 genes, 1.2%	0.0514	PGN_RS02375 (-1.1), PGN_RS03145 (1.4), PGN_RS04275 (-2.9), PGN_RS07885 (-1.2), PGN_RS07895 (-1.4), PGN_RS08500 (2.2)
3h				
Molecular Function				
hydrolase activity, acting on acid anhydrides,	4 out of 24 genes, 16.7%	15 out of 1098 genes, 1.4%	0.00941	PGN_RS04530 (2.4), PGN_RS04535 (2.3), PGN_RS09085 (2.3), PGN_RS09090 (2.4)

catalyzing transmembrane movement of substances				
ATPase activity, coupled to transmembrane movement of substances	4 out of 24 genes, 16.7%	15 out of 1098 genes, 1.4%	0.00941	PGN_RS04530 (2.4), PGN_RS04535 (2.3), PGN_RS09085 (2.3), PGN_RS09090 (2.4)
ATPase activity, coupled to movement of substances	4 out of 24 genes, 16.7%	15 out of 1098 genes, 1.4%	0.00941	PGN_RS04530 (2.4), PGN_RS04535 (2.3), PGN_RS09085 (2.3), PGN_RS09090 (2.4)
primary active transmembrane transporter activity	4 out of 24 genes, 16.7%	18 out of 1098 genes, 1.6%	0.0202	PGN_RS04530 (2.4), PGN_RS04535 (2.3), PGN_RS09085 (2.3), PGN_RS09090 (2.4)
P-P-bond-hydrolysis-driven transmembrane transporter activity	4 out of 24 genes, 16.7%	18 out of 1098 genes, 1.6%	0.0202	PGN_RS04530 (2.4), PGN_RS04535 (2.3), PGN_RS09085 (2.3), PGN_RS09090 (2.4)
6h				
Biological Process				
proteolysis	6 out of 18 genes, 33.3%	69 out of 980 genes, 7.0%	0.04175	PGN_RS02195 (-5.5), PGN_RS02785 (-1.3), PGN_RS03770 (-4.3), PGN_RS04120 (-1.3), PGN_RS05110 (-4.5), PGN_RS06805 (-5.5)
Molecular Function				
hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	4 out of 19 genes, 21.1%	15 out of 1098 genes, 1.4%	0.00295	PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS09085 (1.4), PGN_RS09090 (1.6)
ATPase activity, coupled to transmembrane movement of substances	4 out of 19 genes, 21.1%	15 out of 1098 genes, 1.4%	0.00295	PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS09085 (1.4), PGN_RS09090 (1.6)
ATPase activity, coupled to movement of substances	4 out of 19 genes, 21.1%	15 out of 1098 genes, 1.4%	0.00295	PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS09085 (1.4), PGN_RS09090 (1.6)
primary active transmembrane transporter activity	4 out of 19 genes, 21.1%	18 out of 1098 genes, 1.6%	0.00641	PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS09085 (1.4), PGN_RS09090 (1.6)
P-P-bond-hydrolysis-driven transmembrane transporter activity	4 out of 19 genes, 21.1%	18 out of 1098 genes, 1.6%	0.00641	PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS09085 (1.4), PGN_RS09090 (1.6)

peptidase activity	6 out of 19 genes, 31.6%	67 out of 1098 genes, 6.1%	0.02267	PGN_RS02195 (-5.5), PGN_RS02785 (-1.3), PGN_RS03770 (-4.3), PGN_RS04120 (-1.3), PGN_RS05110 (-4.5), PGN_RS06805 (-5.5)
active transmembrane transporter activity	4 out of 19 genes, 21.1%	29 out of 1098 genes, 2.6%	0.04404	PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS09085 (1.4), PGN_RS09090 (1.6)
ATPase activity, coupled	4 out of 19 genes, 21.1%	29 out of 1098 genes, 2.6%	0.04404	PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS09085 (1.4), PGN_RS09090 (1.6)
Cellular Component				
membrane	10 out of 10 genes, 100.0%	356 out of 621 genes, 57.3%	0.01451	PGN_RS02645 (1.3), PGN_RS04515 (1.4), PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS04540 (1.1), PGN_RS04945 (-1.1), PGN_RS09080 (1.1), PGN_RS09085 (1.4), PGN_RS09090 (1.6), PGN_RS09105 (1.4)
integral component of membrane	9 out of 10 genes, 90.0%	321 out of 621 genes, 51.7%	0.05398	PGN_RS02645 (1.3), PGN_RS04515 (1.4), PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS04540 (1.1), PGN_RS04945 (-1.1), PGN_RS09080 (1.1), PGN_RS09085 (1.4), PGN_RS09090 (1.6), PGN_RS09105 (1.4)
intrinsic component of membrane	9 out of 10 genes, 90.0%	322 out of 621 genes, 51.9%	0.05538	PGN_RS02645 (1.3), PGN_RS04515 (1.4), PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS04540 (1.1), PGN_RS04945 (-1.1), PGN_RS09080 (1.1), PGN_RS09085 (1.4), PGN_RS09090 (1.6), PGN_RS09105 (1.4)
membrane part	9 out of 10 genes, 90.0%	324 out of 621 genes, 52.2%	0.05828	PGN_RS02645 (1.3), PGN_RS04515 (1.4), PGN_RS04530 (1.6), PGN_RS04535 (1.4), PGN_RS04540 (1.1), PGN_RS04945 (-1.1), PGN_RS09080 (1.1), PGN_RS09085 (1.4), PGN_RS09090 (1.6), PGN_RS09105 (1.4)

(D) KEGG enrichment

30 min								
Pathway	DEGs genes with pathway annotation (178)	All genes with pathway annotation (1277)	Pvalue	Qvalue	Pathway ID	Level 1	Level 2	DEGs List
Pantothenate and CoA biosynthesis	9 (5.06%)	26 (2.04%)	0.00601543	0.5353733	ko00770 /pgn00770	Metabolism	Metabolism of cofactors and vitamins	PGN_RS01765 (2.2), PGN_RS02550 (-1.0), PGN_RS03025 (-1.1), PGN_RS04365 (2.9), PGN_RS05320 (3.1), PGN_RS05425 (-1.1), PGN_RS05800 (2.5), PGN_RS07225 (-1.2), PGN_RS09825 (2.8)

Legionellosis	2 (1.12%)	3 (0.23%)	0.05266794	0.8522605	ko05134	Human Diseases	Infectious diseases: Bacterial	PGN_RS02370 (-1.0), PGN_RS06935 (2.4)
Vibrio cholerae pathogenic cycle	2 (1.12%)	3 (0.23%)	0.05266794	0.8522605	ko05111	Human Diseases	Infectious diseases: Bacterial	PGN_RS05780 (-1.1), PGN_RS07030 (-1.1)
Base excision repair	4 (2.25%)	11 (0.86%)	0.05444762	0.8522605	ko03410 /pgn03410	Genetic Information Processing	Replication and repair	PGN_RS01635 (-1.3), PGN_RS01745 (-1.1), PGN_RS04965 (-1.1), PGN_RS06935 (2.4)
1h								
Pathway	DEGs genes with pathway annotation (114)	All genes with pathway annotation (1277)	Pvalue	Qvalue	Pathway ID	Level 1	Level 2	
Carbon fixation pathways in prokaryotes	8 (7.02%)	22 (1.72%)	0.000349205	0.0230475	ko00720	Metabolism	Energy metabolism	PGN_RS02365 (-1.3), PGN_RS02370 (-1.1), PGN_RS02375 (-1.1), PGN_RS05320 (2.6), PGN_RS05800 (1.4), PGN_RS08325 (-1.5), PGN_RS08340 (-1.3), PGN_RS08345 (-1.4)
Citrate cycle (TCA cycle)	6 (5.26%)	22 (1.72%)	0.01007254	0.3052066	ko00020 /pgn00020	Metabolism	Carbohydrate metabolism	PGN_RS02365 (-1.3), PGN_RS02370(-1.1), PGN_RS02375 (-1.1), PGN_RS08325 (-1.5), PGN_RS08340 (-1.3), PGN_RS08345 (-1.4)
Legionellosis	2 (1.75%)	3 (0.23%)	0.02232825	0.3052066	ko05134	Human Diseases	Infectious diseases: Bacterial	PGN_RS02370 (-1.1), PGN_RS06935 (3.1)
Pantothenate and CoA biosynthesis	6 (5.26%)	26 (2.04%)	0.02312171	0.3052066	ko00770 /pgn00770	Metabolism	Metabolism of cofactors and vitamins	PGN_RS01765 (1.0), PGN_RS04365 (3.6), PGN_RS05320 (2.6), PGN_RS05800 (1.4), PGN_RS07225, (-1.0), PGN_RS09825 (1.7)

Butanoate metabolism	6 (5.26%)	26 (2.04%)	0.02312171	0.3052066	ko00650 /pgn00650	Metabolism	Carbohydrate metabolism	PGN_RS02365 (-1.3), PGN_RS02370 (-1.1), PGN_RS02375 (-1.1), PGN_RS05800 (1.4), PGN_RS05930 (-1.1), PGN_RS08340 (-1.3)
Tuberculosis	2 (1.75%)	4 (0.31%)	0.04206016	0.4626618	ko05152	Human Diseases	Infectious diseases: Bacterial	PGN_RS04365 (3.6), PGN_RS06935 (3.1)
3h								
Pathway	DEGs genes with pathway annotation (23)	All genes with pathway annotation (1277)	Pvalue	Qvalue	Pathway ID	Level 1	Level 2	
Ubiquinone and other terpenoid-quinone biosynthesis	2 (8.7%)	9 (0.7%)	0.01034971	0.06209826	ko00130 /pgn00130	Metabolism	Metabolism of cofactors and vitamins	PGN_RS06090 (2.7), PGN_RS08530 (1.4)
6h								
Pathway	DEGs genes with pathway annotation (17)	All genes with pathway annotation (1277)	Pvalue	Qvalue	Pathway ID	Level 1	Level 2	
Bacterial invasion of epithelial cells	1 (5.88%)	4 (0.31%)	0.05225607	0.1045121	ko05100	Human Diseases	Infectious diseases: Bacterial	PGN_RS04075 (1.1)

Table S5. LFQ results of significantly validly detected protein/protein group of *P. gingivalis* upon RBC treatment

Valid detection means at least two of non-zero LFQ intensity of each protein/protein group in all samples. Significance for both Student's t-test and FDR was marked with a '+'. Highly (100%, i.e. 2-fold, change) up- and down-regulated proteins/protein groups are shaded in grey. Evidently (50% change) up- and down-regulated proteins/protein groups are marked '#'.

Student's T-test Significant	FDR Significant	log2 change	Protein IDs	Gene name	NCBI locus	Old NCBI locus	Protein full name	Cluster
+		-1.53600883	B2RK10	rprY	PGN_RS05690	PGN_1186	DNA-binding response regulator	
+		-1.35672442	B2RK43	PGN_1219	PGN_RS05860	PGN_1219	rRNA pseudouridine synthase	
+		-1.12758255	B2RK11	NrnA	PGN_RS05695	PGN_1187	bifunctional oligoribonuclease/PAP phosphatase NrnA	
+		-0.88798205	B2RJU6	PGN_1122	PGN_RS05375	PGN_1122	NADP transhydrogenase subunit beta #	B
+		-0.8222847	B2RGN4	PGN_0010	PGN_RS00055	PGN_0010	L-threonine-O-3-phosphate decarboxylase #	
+		-0.7969087	B2RIZ0	fbp	PGN_RS03900	PGN_0816	fructose-1,6-bisphosphatase class 3#	B
+		-0.79556402	B2RIT0	PGN_0756	PGN_RS03600	PGN_0756	peptidase S9 #	
+		-0.7803491	P0C940; B2RH54; A0PA80; Q93R80; Q51822	fimA	PGN_RS00835	PGN_0180	FimA type I fimbrilin #	B
+		-0.75072988	B2RHQ1	aspA	PGN_RS01790	PGN_0377	aspartate ammonia-lyase #	
+		-0.68361028	B2RJF0	purC	PGN_RS04680	PGN_0976	Phosphoribosylaminoimidazole-succinocarboxamide synthase #	B
+		-0.65891838	B2RHB7	gpmA	PGN_RS01170	PGN_0243	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase #	
+	+	-0.63132222	B2RIQ1	PGN_0727	PGN_RS03470	PGN_0727	4-hydroxybutyryl-CoA dehydratase #	B
+		-0.59389559	B2RJX3	ptpA	PGN_RS05510	PGN_1149	prolyl tripeptidyl peptidase #	B
+		-0.58773994	B2RIM4	PGN_0700	PGN_RS03340	PGN_0700	glycosyl hydrolase family 109	
+		-0.57545598	B2RJZ6	PGN_1172	PGN_RS05625	PGN_1172	acyl-CoA dehydrogenase	
+		-0.55507469	B2RJR8	PGN_1094	PGN_RS05240	PGN_1094	glycine dehydrogenase	
+		-0.5319519	B2RKP2	PGN_1418	PGN_RS06770	PGN_1418	pyruvate:ferredoxin (flavodoxin) oxidoreductase	B
+		-0.52978198	B2RM79	PGN_1955	PGN_RS09255	PGN_1955	phosphoglucomutase	

+		-0.50110181	B2RH63	fabH	PGN_RS00875	PGN_0189	3-oxoacyl-ACP synthase III	
+		-0.48857435	B2RIZ3	leuS	PGN_RS03915	PGN_0819	leucine-tRNA ligase	
+		-0.48362223	B2RKG5	PGN_1341	PGN_RS06400	PGN_1341	acetyl-CoA hydrolase	
+		-0.43403308	B2RJY8	PGN_1164	PGN_RS05585	PGN_1164	3-keto-5-aminohexanoate cleavage protein	B
+		-0.42922211	B2RLW5	rpoA	PGN_RS08730	PGN_1841	DNA-directed RNA polymerase subunit alpha	
+		-0.41106923	B2RK42	asnS	PGN_RS05855	PGN_1218	asparagine-tRNA ligase	
+		-0.40684636	B2RII9	tpiA	PGN_RS03165	PGN_0665	triose-phosphate isomerase	
+		-0.3786513	B2RJZ8	PGN_1174	PGN_RS05635	PGN_1174	electron transfer flavoprotein subunit alpha	
+		-0.28867531	B2RGX4	lysA	PGN_RS00470 ; PGN_RS06085	PGN_0100 ; PGN_1272	diaminopimelate decarboxylase	
+		-0.27774938	B2RLS4	hutU	PGN_RS08535	PGN_1800	urocanate hydratase	B
+		-0.24324226	B2RLR0	PGN_1786	PGN_RS08475	PGN_1786	DNA polymerase III subunit beta	
+		-0.22271093	B2RIP7	PGN_0723	PGN_RS03450	PGN_0723	succinate-semialdehyde dehydrogenase	B
+		-0.17847188	B2RJU0	PGN_1116	PGN_RS05345	PGN_1116	aminotransferase	
+		0.24453227	B2RLH6	PGN_1702	PGN_RS08095	PGN_1702	protein translocase subunit SecDF	
+		0.44878832	B2RJY2	PGN_1158	PGN_RS05555	PGN_1158	phosphorylase	
+		0.48650106	B2RJN1; Q7WRG4	recA	PGN_RS05070	PGN_1057	DNA recombination/repair protein RecA	
+		0.52819951	B2RLR5	PGN_1791	PGN_RS08500	PGN_1791	flavodoxin	A3
+		0.54462941	B2RJL1	PGN_1037	PGN_RS04970	PGN_1037	hypothetical protein	
+		0.58484014	B2RH47	PGN_0173	PGN_RS00805	PGN_0173	type I glyceraldehyde-3-phosphate	A1
+		0.60395749	B2RGS8		PGN_RS00250	PGN_0054	hypothetical protein [#]	A1
+		0.60690053	B2RKG2	PGN_1338	PGN_RS06390	PGN_1338	pyruvate, phosphate dikinase [#]	
+		0.61185964	B2RLZ4; Q9RHH4	fusA	PGN_RS08870	PGN_1870	elongation factor G [#]	A2
+		0.73578389	B2RJ49	gyrA	PGN_RS04175	PGN_0875	DNA gyrase subunit A [#]	A4
+		0.79422569	B2RJC3		PGN_RS04530	PGN_0949	ABC transporter ATP-binding protein [#]	A4
+		0.91021601	B2RHL9	PGN_0345	PGN_RS01645	PGN_0345	hypothetical protein [#]	
+		0.9708964	B2RJ90	dnaK	PGN_RS04365	PGN_0916	molecular chaperone DnaK [#]	A3
+		1.02133687	B2RKS6	groL	PGN_RS06935	PGN_1452	molecular chaperone GroEL	A3
+		1.02934519	B2RL63	rpsI	PGN_RS07575	PGN_1589	30S ribosomal protein S9	

+		1.0421505	B2RM15	rpmB	PGN_RS08965	PGN_1891	50S ribosomal protein L28	
+		1.11122894	B2RMB8	PGN_1995	PGN_RS09440	PGN_1995	hypothetical protein	A4
+		1.11420695	B2RLW8	rpsM	PGN_RS08745	PGN_1844	30S ribosomal protein S13	A2
+		1.14236323	B2RI82	hmuY	PGN_RS02650	PGN_0558	hypothetical protein; heme-binding protein HmuY	A4
+		1.23391628	B2RK50	PGN_1226	PGN_RS05895	PGN_1226	ribonucleoside-diphosphate reductase, adenosylcobalamin-dependent	A1
+		1.34590054	B2RLW4	rplQ	PGN_RS08725	PGN_1840	50S ribosomal protein L17	A1
+		1.34729449	B2RLZ6	rpsL	PGN_RS08880	PGN_1872	30S ribosomal protein S12	A2
+		1.61257299	B2RL07	PGN_1533	PGN_RS07315	PGN_1533	carbonic anhydrase	A3
+		1.75603104	B2RGS7		PGN_RS00245	PGN_0053	hypothetical protein	A1
+		2.15881348	B2RHA9	PGN_0235	PGN_RS01130	PGN_0235	DNA-binding protein HU	A2
+		NaN	B2RGR5	htpG	PGN_RS00190	PGN_0041	molecular chaperone HtpG	A1
+		NaN	B2RI22	PGN_0498	PGN_RS02375	PGN_0498	succinate dehydrogenase/fumarate reductase iron-sulfur subunit	B
+	+	NaN	B2RIW5	PGN_0791	PGN_RS03775	PGN_0791	trigger factor	A3
+	+	NaN	B2RJC0		PGN_RS04515 ; PGN_RS09105	PGN_0946 ; PGN_1920	membrane protein	A3
+	+	NaN	B2RJY7	PGN_1163	PGN_RS05580	PGN_1163	hypothetical protein	A3
+		NaN	B2RKG0	PGN_1336	PGN_RS06380	PGN_1336	DUF4876 domain-containing protein	
+	+	NaN	B2RKP0	PGN_1416	PGN_RS06765	PGN_1416	peptidase; lysyl endopeptidasePepK	B
+	+	NaN	B2RLD0	rnfG	PGN_RS07885	PGN_1656	electron transporter RnfG	B
+		NaN	B2RLY2	rplN	PGN_RS08810	PGN_1858	50S ribosomal protein L14	A3
+	+	NaN	B2RM02	PGN_1878	PGN_RS08910	PGN_1878	alpha/beta hydrolase	B

Table S6. GO and KEGG enrichments of significantly regulated proteins of *P. gingivalis* upon RBC treatment

A total of 66 significantly regulated proteins (two sample t-test, $P < 0.05$) were subjected to STRING for GO and KEGG enrichment as shown in (**A**), and 31 of up-regulated and 35 down-regulated proteins were also enriched separately as shown in (**B** and **C**). NCBI old locus tags were used.

(A) Enrichment of a total of 66 significantly regulated proteins

Biological Process (GO)

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
GO.0008150	**biological process**	19	0.00144	PGN_0041 (htpG), PGN_0180 (fimA), PGN_0189 (fabH), PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0816 (fbp), PGN_0819 (leuS), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1149 (ptpA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0019538	protein metabolic process	13	0.000994	PGN_0041 (htpG), PGN_0819 (leuS), PGN_0916 (dnaK), PGN_1149 (ptpA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0044238	primary metabolic process	18	0.000994	PGN_0041 (htpG), PGN_0189 (fabH), PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0816 (fbp), PGN_0819 (leuS), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1149 (ptpA), PGN_1218 (asnS), PGN_1452 ((groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0044267	cellular protein metabolic process	12	0.00107	PGN_0041 (htpG), PGN_0819 (leuS), PGN_0916 (dnaK), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)

GO.0071704	organic substance metabolic process	18	0.00169	PGN_0041 (htpG), PGN_0189 (fabH), PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0816 (fbp), PGN_0819 (leuS), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1149 (ptpA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0044237	cellular metabolic process	17	0.00316	PGN_0041 (htpG), PGN_0189 (fabH), PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0816 (fbp), PGN_0819 (leuS), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0043170	macromolecule metabolic process	14	0.00324	PGN_0041 (htpG), PGN_0819 (leuS), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1149 (ptpA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0006094	gluconeogenesis	3	0.00591	PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0816 (fbp)
GO.0044260	cellular macromolecule metabolic process	13	0.00591	PGN_0041 (htpG), PGN_0819 (leuS), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0006412	translation	9	0.00963	PGN_0819 (leuS), PGN_1218 (asnS), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.1901576	organic substance biosynthetic process	13	0.00963	PGN_0819 (leuS), PGN_1218 (asnS), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0006457	protein folding	3	0.0282	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1452 (groL)
GO.1901564	organonitrogen compound metabolic process	11	0.0354	PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0819 (leuS), PGN_1218 (asnS), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)

GO.0034641	cellular nitrogen compound metabolic process	12	0.0418	PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0819 (leuS), PGN_1057 (recA), PGN_1218 (asnS), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0010467	gene expression	9	0.0435	PGN_0819 (leuS), PGN_1218 (asnS), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)

Cellular Component (GO)

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
GO.0005575	**cellular component**	16	0.00049	PGN_0041 (htpG), PGN_0180 (fimA), PGN_0189 (fabH), PGN_0665 (tpiA), PGN_0819 (leuS), PGN_1057 (recA), PGN_1149 (ptpA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0005622	intracellular	14	0.00049	PGN_0041 (htpG), PGN_0189 (fabH), PGN_0665 (tpiA), PGN_0819 (leuS), PGN_1057 (recA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0005623	cell	15	0.00049	PGN_0041 (htpG), PGN_0180 (fimA), PGN_0189 (fabH), PGN_0665 (tpiA), PGN_0819 (leuS), PGN_1057 (recA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0005737	cytoplasm	14	0.00049	PGN_0041 (htpG), PGN_0189 (fabH), PGN_0665 (tpiA), PGN_0819 (leuS), PGN_1057 (recA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0044464	cell part	15	0.00049	PGN_0041 (htpG), PGN_0180 (fimA), PGN_0189 (fabH), PGN_0665 (tpiA), PGN_0819 (leuS), PGN_1057 (recA), PGN_1218 (asnS), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)

GO.0005840	ribosome	6	0.0136	PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1872 (rpsL), PGN_1891 (rpmB)

KEGG

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
01200	Carbon metabolism	11	2.90E-05	PGN_0173, PGN_0243 (gpmA), PGN_0498, PGN_0665 (tpiA), PGN_0723, PGN_0727, PGN_0816 (fbp), PGN_1094, PGN_1172, PGN_1338, PGN_1418
01120	Microbial metabolism in diverse environments	11	0.000126	PGN_0100, PGN_0173, PGN_0243 (gpmA), PGN_0498, PGN_0665 (tpiA), PGN_0723, PGN_0727, PGN_0816 (fbp), PGN_1338, PGN_1418, PGN_1955
00010	Glycolysis / Gluconeogenesis	5	0.000771	PGN_0173, PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0816 (fbp), PGN_1955
01100	Metabolic pathways	22	0.00151	PGN_0100, PGN_0173, PGN_0189 (fabH), PGN_0243 (gpmA), PGN_0377, PGN_0498, PGN_0665 (tpiA), PGN_0723, PGN_0727, PGN_0816 (fbp), PGN_0976 (purC), PGN_1094, PGN_1122 (rpoA), PGN_1158, PGN_1172, PGN_1226, PGN_1338, PGN_1418, PGN_1786, PGN_1800 (hutU), PGN_1841 (rpoA), PGN_1955
00650	Butanoate metabolism	4	0.00848	PGN_0498, PGN_0723, PGN_0727, PGN_1172

(B) Enrichment of significantly up-regulated proteins

Biological Process (GO)

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
GO.0044267	cellular protein metabolic process	10	7.04E-05	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)

GO.0044260	cellular macromolecule metabolic process	11	0.000162	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0044238	primary metabolic process	11	0.00251	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0006412	translation	7	0.00326	PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0006457	protein folding	3	0.00437	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1452 (groL)
GO.0010467	gene expression	7	0.00974	PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0034641	cellular nitrogen compound metabolic process	8	0.0311	PGN_1057 (recA), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)

Molecular Function (GO)

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
GO.0003674	**molecular function**	11	0.00875	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0003735	structural constituent of ribosome	6	0.00875	PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0097159	organic cyclic compound binding	8	0.0149	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1452 (groL), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL)
GO.1901363	heterocyclic compound binding	8	0.0149	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1452 (groL), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL)
GO.0032550	purine ribonucleoside binding	5	0.0481	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1452 (groL), PGN_1870 (fusA)

GO.0032555	purine ribonucleotide binding	5	0.0481	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1452 (groL), PGN_1870 (fusA)
GO.0035639	purine ribonucleoside triphosphate binding	5	0.0481	PGN_0041 (htpG), PGN_0916 (dnaK), PGN_1057 (recA), PGN_1452 (groL), PGN_1870 (fusA)
GO.0003676	nucleic acid binding	5	0.0495	PGN_1057 (recA), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL)

Cellular Component (GO)

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
GO.0005622	intracellular	10	0.000203	PGN_0041 (htpG), PGN_1057 (recA), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0005623	cell	10	0.000203	PGN_0041 (htpG), PGN_1057 (recA), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0005737	cytoplasm	10	0.000203	PGN_0041 (htpG), PGN_1057 (recA), PGN_1452 (groL), PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1870 (fusA), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0005840	ribosome	6	0.000203	PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1872 (rpsL), PGN_1891 (rpmB)
GO.0044391	ribosomal subunit	2	0.0424	PGN_1858 (rplN), PGN_1872 (rpsL)

KEGG

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
03010	Ribosome	6	0.00814	PGN_1589 (rpsI), PGN_1840 (rplQ), PGN_1844 (rpsM), PGN_1858 (rplN), PGN_1872 (rpsL), PGN_1891 (rpmB)

(C) Enrichment of significantly up-regulated proteins

Biological Process (GO)

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
GO.0006094	gluconeogenesis	3	0.00299	PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0816 (fbp)
GO.0019752	carboxylic acid metabolic process	5	0.0417	PGN_0189 (fabH), PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0819 (leuS), PGN_1218 (asnS)

KEGG

#Pathway ID	Pathway description	Observed gene count	FDR	Matching proteins in network (Gene ID/Name)
01200	Carbon metabolism	9	7.45E-06	PGN_0243 (gpmA), PGN_0498, PGN_0665 (tpiA), PGN_0723, PGN_0727, PGN_0816 (fbp), PGN_1094, PGN_1172, PGN_1418
01100	Metabolic pathways	18	9.28E-06	PGN_0100, PGN_0189, PGN_0243 (gpmA), PGN_0377, PGN_0498, PGN_0665 (tpiA), PGN_0723, PGN_0727, PGN_0816 (fbp), PGN_0976 (purC), PGN_1094, PGN_1122, PGN_1172, PGN_1418, PGN_1786, PGN_1800 (hutU), PGN_1841 (rpoA), PGN_1955
01120	Microbial metabolism in diverse environments	9	1.56E-05	PGN_0100, PGN_0243 (gpmA), PGN_0498, PGN_0665 (tpiA), PGN_0723, PGN_0727, PGN_0816 (fbp), PGN_1418, PGN_1955
00010	Glycolysis / Gluconeogenesis	4	0.000687	PGN_0243 (gpmA), PGN_0665 (tpiA), PGN_0816 (fbp), PGN_1955
00650	Butanoate metabolism	4	0.000687	PGN_0498, PGN_0723, PGN_0727, PGN_1172
01110	Biosynthesis of secondary metabolites	8	0.00413	PGN_0100, PGN_0243 (gpmA), PGN_0498, PGN_0665 (tpiA), PGN_0816 (fbp), PGN_0976 (purC), PGN_1172, PGN_1955

Table S7. Peptide mass fingerprinting identification results of hemin-agarose pull-down assay for *P. gingivalis*

Sample Name	Protein Name	Pg 33277 Accession No.	Gene ID	MW
1[a]	lysine-specific cysteine proteinase; Kgp	WP_012458488.1	PGN_1728	188228
2	OmpA family protein; outer membrane protein 41; immunoreactive 43kD antigen, OmpA_C-like	WP_012457733.1	PGN_0729	43477.2
3[b]	arginine-specific thiol protease, RgpA	WP_043876339.1	PGN_1970	186818
4	OmpA family protein; outer membrane protein 41; immunoreactive 43kD antigen, OmpA_C-like	WP_012457733.1	PGN_0729	43477.2
5	PorT family protein	WP_021662358.1	PGN_1744	22053.2
6	hypothetical protein; starch binding outer membrane protein SusD	WP_012457401.1	PGN_0294	57151.6

P. gingivalis were cultured in liquid media either with or without the supplement of 5.0 μg/mL hemin and treated with or without 25 μM RBC. Equal amounts of lysates were loaded onto the hemin-agarose.

[a], Can also be gingipain RgpA or hemagglutinin HagA.

[b], Can also be hemagglutinin HagA

Supplementary references

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