Supporting Information

Fig. S1. Separation of biologically active proteases in 18h LB and BHI culture cell-free supernatants by native PAGE. Post-run gel overlain on 1% milk LB agar showing proteolytic activity as indicated by clearing zones corresponding to protein bands of PA14 WT and *aprA*::Tn LB and BHI 18h culture cell-free supernatants.

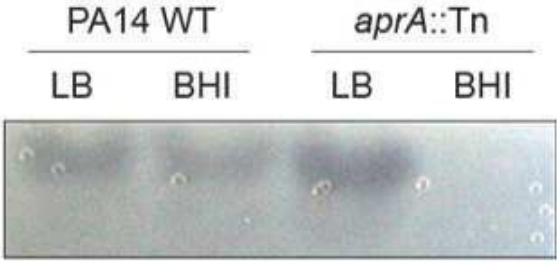

Fig. S2. SDS PAGE separation of *aprA*::Tn proteins. Comparison of whole supernatant samples (Sup) against native PAGE (NP) excised samples. For the latter, bands corresponding to proteolytic portions of the native gel from Figure S1 were excised and loaded onto the SDS PAGE gel. The 33kD band subjected to mass spectrometry analysis is indicated by an asterisk. An identical 33-kDa band was observed for PA14 WT in both LB and BHI Sup and NP samples (not shown).

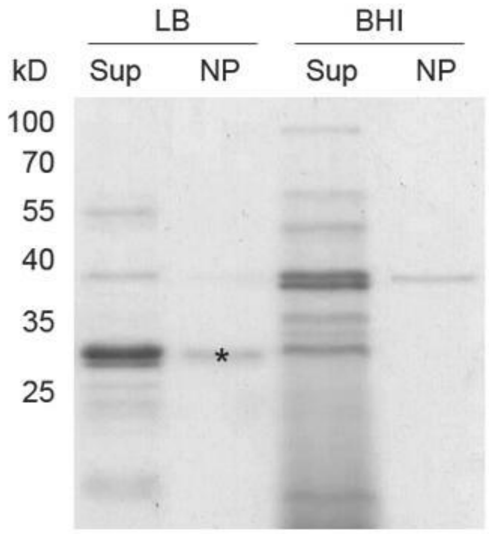

Table S1. Selected PA14 transposon mutants based on fold changes of induced IL-8 response in A549-Gluc cells

Locus name	Gene name	Gene product description	IL-8 fold difference to PA14 WT[a]
PA14_00760		hypothetical protein	0.628
PA14_02300		putative short-chain dehydrogenase	0.615
PA14_03900	*spuC*	putrescine aminotransferase	0.433
PA14_04820		putative transcriptional regulator, TetR family	0.615
PA14_05220		cystathionine beta-synthase	2.379
PA14_05450		conserved hypothetical protein	0.549
PA14_05560		putative ATP-dependent RNA helicase, DEAD box family	0.485
PA14_05910		putative bacterial periplasmic transport system	0.536
PA14_06830	*norB*	nitric-oxide reductase subunit B	0.484
PA14_06950		putative transcriptional regulator, LuxR family	0.521
PA14_07370		conserved hypothetical protein	0.601
PA14_07400		hypothetical protein	0.412
PA14_07740	*pdxA*	pyridoxal phosphate biosynthetic protein PdxA	0.358
PA14_07940	*trpE*	anthranilate synthetase component I	0.624
PA14_08500		putative integral membrane protein	0.536
PA14_10440		putative porin	0.497
PA14_11290		putative permease	0.549
PA14_11700		conserved hypothetical protein	0.733
PA14_11900		hypothetical protein	0.321
PA14_13090		probable 3-ketoacyl-CoA thiolase	0.484
PA14_14380		putative transmembrane component of ABC transporter	0.497
PA14_14420		hypothetical protein	0.321
PA14_15840		conserved hypothetical protein	0.497
PA14_16360		putative oxidase	0.456
PA14_16470		putative chemotaxis sensor/effector fusion protein	0.471
PA14_16780		putative cytochrome P450	0.769
PA14_16980		conserved hypothetical protein	0.503
PA14_18600		putative ABC transporter, ATP-binding protein	0.814
PA14_18720		putative outer membrane protein precursor, OmpA family	1.683
PA14_20380	*phnI*	putative phosphonate metabolism protein	0.796
PA14_20700		putative glycosyltransferase	1.379
PA14_20730	*flgM*	putative negative regulator of flagellin synthesis, FlgM	0.471
PA14_20960		putative isomerase	0.506
PA14_23470	*wbpM*	nucleotide sugar epimerase/dehydratase WbpM	0.501
PA14_24290	*gbt*	glycine betaine transmethylase	0.448
PA14_25220		conserved hypothetical protein	0.285
PA14_25390	*sth*	soluble pyridine nucleotide transhydrogenase	0.376
PA14_25830		conserved hypothetical protein	0.412
PA14_26230	*hisQ*	histidine transport system permease protein	2.071

PA14_26330		putative transcriptional regulator, AraC family	0.673
PA14_26670		putative biotin carboxylase/biotin carboxyl carrier protein	0.641
PA14_26760		putative transcriptional regulator	0.628
PA14_27070		putative membrane protein	0.615
PA14_27280		putative transcriptional regulator, LysR family	0.471
PA14_27770		putative ABC transporter, ATP-binding protein	0.43
PA14_29410		putative serine/threonine dehydratase	0.646
PA14_29940	*nuoG*	NADH dehydrogenase I chain G	0.412
PA14_30280	*trxB1*	thioredoxin reductase 1	0.656
PA14_31840		putative protease	0.825
PA14_31990	*czcB*	cobalt/zinc/cadmium efflux RND transporter, membrane fusion protein, CzcB family	0.484
PA14_32590		probable thiol:disulfide interchange protein	0.412
PA14_32890		hypothetical protein	2.191
PA14_33650	*pvdD*	pyoverdine synthetase D	0.045
PA14_33820	*pvdQ*	penicillin acylase-related protein	1.412
PA14_33890		putative oxidoreductase	0.726
PA14_35290	*gnd*	gluconate dehydrogenase	0.664
PA14_36870		putative short-chain dehydrogenase	1.188
PA14_37310		putative allophanate hydrolase subunit 2	0.654
PA14_37880		putative binding protein component of ABC transporter	0.521
PA14_38110		putative transporter, sodium-dicarboxylate symporte	0.358
PA14_41090	*mltD*	membrane-bound lytic murein transglycosylase D precursor	0.43
PA14_41390	*ppiB*	peptidyl-prolyl cis-trans isomerase B	0.412
PA14_41500		putative lyase	0.832
PA14_43380	*kdpB*	potassium-transporting ATPase, B chain	0.575
PA14_43920	*braB*	branched chain amino acid transporter	0.213
PA14_44420		putative ferredoxin	0.778
PA14_44960		putative phosphatase	0.673
PA14_45630	*fliA*	motility sigma factor FliA	0.321
PA14_45680	*flhA*	flagellar biosynthesis protein FlhA	0.592
PA14_45760	*fliQ*	flagellar biosynthetic protein FliQ	0.506
PA14_45770	*fliP*	flagellar biosynthetic protein FliP	0.121
PA14_46960	*ggt*	gamma-glutamyltranspeptidase precursor	0.412
PA14_47300	*phnW*	2-aminoethylphosphonate:pyruvate aminotransferase	0.376
PA14_49930		conserved hypothetical protein	0.471
PA14_50080	*fliJ*	flagellar protein FliJ	0.421
PA14_50130	*fliG*	flagellar motor switch protein FliG	0.355
PA14_50140	*fliF*	Flagella M-ring outer membrane protein precursor	0.547
PA14_50200	*fleS*	two-component sensor	0.556
PA14_50220	*fleQ*	transcriptional regulator FleQ	0.563
PA14_50340	*flgL*	flagellar hook-associated protein type 3 FlgL	2.179
PA14_50360	*flgK*	flagellar hook-associated protein 1 FlgK	1.717

PA14_50380	*flgJ*	flagellar protein FlgJ	0.394
PA14_50460	*flgD*	flagellar basal-body rod modification protein FlgD	1.429
PA14_50470	*flgC*	flagellar basal-body rod protein FlgC	0.484
PA14_50480	*flgB*	flagellar basal-body rod protein FlgB	0.295
PA14_50700		conserved hypothetical protein	0.542
PA14_51310		conserved hypothetical protein	0.521
PA14_51880	*oprD*	Basic amino acid, basic peptide and imipenem outer membrane porin OprD precursor	1.308
PA14_52050	*purN*	phosphoribosylaminoimidazole synthetase	0.305
PA14_52610		possible threonine aldolase	0.557
PA14_53000	*phhB*	pterin-4-alpha-carbinolamine dehydratase	0.503
PA14_54390	*mucD*	serine protease MucD precursor	0.43
PA14_56000		chemotactic transducer PctA	1.274
PA14_56620		putative transcriptional regulator	0.267
PA14_57100	*ampG*	putative permease	1.825
PA14_57950		putative ribosomal subunit interface protein	0.434
PA14_59240	*pilL2*	type IV B pilus protein	0.448
PA14_59840		Conserved hypothetical protein	0.556
PA14_61020		ankyrin-like protein	0.303
PA14_61950		hypothetical protein	0.628
PA14_62770	*nusA*	N utilization substance protein A	0.537
PA14_63250		putative acyl-CoA thiolase	0.466
PA14_63480		putative amino acid permease	0.358
PA14_63730		putative transporter	0.562
PA14_65170	*rpsR*	30S ribosomal protein S18	0.556
PA14_65320	*miaA*	delta 2-isopentenylpyrophosphate transferase	0.487
PA14_65350	*mutL*	DNA mismatch repair protein MutL	0.601
PA14_65770	*aspC*	aspartate transaminase	0.747
PA14_67540		conserved hypothetical protein	0.539
PA14_68900		putative iron ABC transporter, periplasmic iron-binding protein	0.654
PA14_69950		putative acetyl-CoA hydrolase/transferase	0.394
PA14_71650	*aspA*	aspartate ammonia-lyase	0.638
PA14_72510	*thrB*	homoserine kinase	0.588

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[a] Mean values of two biological replicas