1 **Robert-Genthon et al.,**

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3 **Supp Materials and Methods**

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5 **Proteomic analyses**

6 ***Protein digestion***

7 Proteins were stacked on the top of a 4-12% NuPAGE gel (Invitrogen) and stained with R-250
8 Coomassie blue. The gel bands were manually excised and cut in pieces before being washed
9 by 6 successive incubations of 15 min in 25 mM NH_4HCO_3 and in 25mM NH_4HCO_3 containing
10 50% (v/v) acetonitrile. Gel pieces were then dehydrated with 100 % acetonitrile and
11 incubated for 45 min at 53°C with 10 mM DTT in 25mM NH_4HCO_3 and for 35 min in the dark
12 with 55 mM iodoacetamide in 25mM NH_4HCO_3. Alkylation was stopped by adding 10 mM
13 DTT in 25mM NH_4HCO_3 and mixing for 10 min. Gel pieces were then washed again by
14 incubation in 25 mM NH_4HCO_3 before dehydration with 100% acetonitrile. 0.15 µg of
15 modified trypsin (Promega, sequencing grade) in 25 mM NH_4HCO_3 was added to the
16 dehydrated gel pieces for an overnight incubation at 37°C. Peptides were then extracted
17 from gel pieces in three 15 min sequential extraction steps in 30 µL of 50% acetonitrile, 30
18 µL of 5% formic acid and finally 30µL of 100% acetonitrile. The pooled supernatants were
19 then dried under vacuum. ***Nano-LC-MS/MS analyses.*** The dried extracted peptides were
20 resuspended in 5% acetonitrile and 0.1% trifluoroacetic acid and analyzed by online nanoLC-
21 MS/MS (Ultimate 3000, Dionex and LTQ-Orbitrap Velos pro, Thermo Fischer Scientific).
22 Peptides were sampled on a 300 µm x 5 mm PepMap C18 precolumn and separated on a 75
23 µm x 250 mm C18 column (PepMap, Dionex). The nanoLC method consisted in a 120-minute
24 gradient at a flow rate of 300 nl/min, ranging from 5% to 37% acetronitrile in 0.1% formic

25 acid during 114 min before reaching 72% for the last 6 minutes. MS and MS/MS data were
26 acquired using Xcalibur (Thermo Fischer Scientific). Spray voltage and heated capillary were
27 respectively set at 1.4 kV and 200°C. Survey full-scan MS spectra (m/z = 400–1600) were
28 acquired in the Orbitrap with a resolution of 60 000 after accumulation of 10^6 ions
29 (maximum filling time: 500 ms). The 20 most intense ions from the preview survey scan
30 delivered by the Orbitrap were fragmented by collision induced dissociation (collision energy
31 35%) in the LTQ after accumulation of 10^4 ions (maximum filling time: 100 ms).
32 ***Bioinformatics analyses.*** Data were processed automatically using Mascot Daemon software
33 (version 2.3.2, Matrix Science). Concomitant searches against PA01-UW strain protein
34 sequence databank (February 1, 2012 release from www.pseudomonas.com, 5572 entries),
35 classical contaminants database (67 sequences, homemade) and the corresponding reversed
36 databases were performed using Mascot (version 2.4). ESI-TRAP was chosen as the
37 instrument, trypsin/P as the enzyme and 2 missed cleavage allowed. Precursor and fragment
38 mass error tolerances were set respectively at 10 ppm and 0.6 Da. Peptide modifications
39 allowed during the search were: carbamidomethyl (C, fixed) acetyl (N-ter, variable),
40 oxidation (M, variable) and deamidation (NQ, variable). The IRMa software (version 1.31.1)
41 was used to filter the results: conservation of rank 1 peptides, peptide identification FDR <
42 1% (as calculated by employing the reverse database strategy), and minimum of 1 specific
43 peptide per identified protein group. The filtered results were uploaded into a relational
44 mass spectrometry identification database (MSIdb) and a homemade tool was used for the
45 compilation, grouping and comparison of the protein groups from the different samples.
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