Supplemental Informatioon

cCMP and cUMP occur *in vivo*

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Table S1. Basal cNMP concentrations in Balb/c mouse organs

Organ	cAMP (pmol/mg protein)	cGMP (pmol/mg protein)	cCMP (pmol/mg protein)
Heart	3.329 ± 0.705	0.031 ± 0.019	0.021 ± 0.023
Lung	4.746 ± 1.256	0.134 ± 0.096	0.371 ± 0.162
Pancreas	3.651 ± 1.280	0.535 ± 0.764	4.751 ± 2.827
Liver	2.811 ± 1.070	0	0.146 ± 0.106
Spleen	3.259 ± 0.818	0	6.296 ± 4.425
Kidney	3.145 ± 0.971	0.026 ± 0.032	0.114 ± 0.086
Bladder	5.036 ± 2.941	0.462 ± 0.640	0.109 ± 0.141
Brain	11.610 ± 3.903	0.116 ± 0.058	0.035 ± 0.035
Thymus	4.845 ± 1.339	0.043 ± 0.075	0.443 ± 0.276
FRS	3.270 ± 0.749	0	3.944 ± 1.477
Testis	4.719 ± 2.681	0.087 ± 0.086	0.053 ± 0.014

cNMP concentrations were determined using 50 – 200 mg organ tissues from 7 male and 7 female Balb/c mice (age, 8-10 weeks). Samples were processed for HPLC-MS/MS analysis as described in Materials and Methods. Data shown in Fig. 1 were analyzed for column statistics. The Table shows mean ± SD values for the concentrations of cAMP, cGMP and cCMP in mouse organs. Data for cUMP are not included because values were below LLOQ. It should be noted that even the small organ cNMP values reported in the table could be unequivocally measured by our HPLC-MS/MS system.

Table S2. Identification of cCMP in various organs from Balb/c mice via HPLC-MS/TOF

Sample	m/z (precursor ion)	m/z (main fragment ion)
cCMP standard	306.0478	112.0516
Pancreas	306.0485 ± 0.0008 (Δ: 2.1 ppm)	112.0512 ± 0.0009 (Δ: 2.9 ppm)
Spleen	306.0490 ± 0.0015 (Δ: 3.9 ppm)	112.0512 ± 0.0008 (Δ: 3.2 ppm)
FRS	306.0487 ± 0.0001 (Δ: 2.9 ppm)	112.0503 ± 0.0006 (Δ: 11.6 ppm)

cNMPs were determined using 50 – 200 mg organ tissues from female Balb/c mice (age, 8-10 weeks). Samples were processed for HPLC-MS/TOF analysis as described in Materials and Methods. m/z ratios of precursor and main fragment ions are shown in comparison to the data obtained with authentic cCMP standard. Data shown are the means ± SD of three independent experiments.

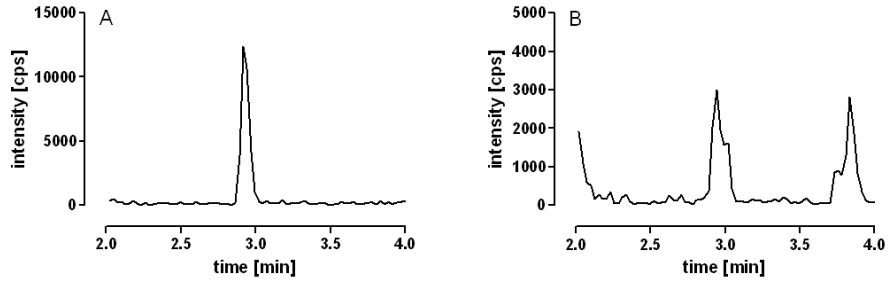

Fig. S1. Quenching of cUMP MS signals by organ matrix. A cUMP sample (15 nM) was dissolved in buffer and analysed by HPLC-MS/MS. The cUMP peak elutes at a retention time of 2.9 min (**A**). The same cUMP sample (15 nM) was dissolved in Balb/c mouse lung extract and subjected to HPLC-MS/MS. The cUMP becomes broader, and signal intensity decreases by about four-fold. In addition, peaks unrelated to cUMP and at later elution times (3.1 and 3.8 min) show up (**B**). These peaks are probably due to matrix components. Representative chromatograms are shown. Similar results were obtained in three independent experiments.

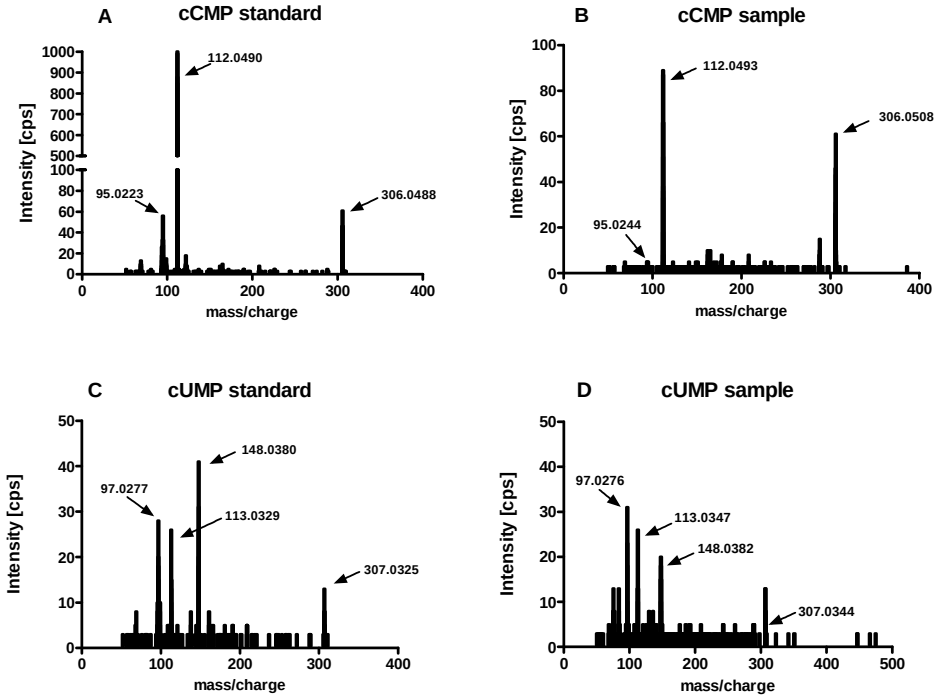

Fig. S2. Identification of cCMP and cUMP in lung tissue from C57/BL6 mice *via* HPLC-MS/TOF. Fragment spectra of cCMP and cUMP were recorded using 50 mg of lung tissue from female C57/BL6 mice infected with recombinant *P. aeruginosa* strain PA103Δ*exoU*e*xoT::Tc* pUCP*exoY* for 4 h and then processed for HPLC-MS/TOF analysis. Representative values of results based on two experiments are given. The instrument standard deviation is ± 1-5 mDa. The highest numbers in each panel represent the masses of the native cNMP, the lower numbers the masses of the corresponding cNMP fragments. Note that in panel **A**, the y-axis is interrupted because the signal of the cCMP fragment with a mass of 112.0490 Da is much larger than of the other fragments. It should also be noted that matrix effects in cCMP and cUMP organ samples (panels **B** and **D**) differentially affected signal intensity as compared to the corresponding control samples with cCMP and cUMP standard (panels **A** and **C**). The critical information of this Figure is not reflected in absolute signal intensity depicted on the y-axis but rather in positioning of the peak on the x-axis, reflecting cNMP (fragment) mass. The absolute magnitude of signals also varies among panels (compare **A** with **B**, and **C** with **D**) because the amount of cNMP applied in each sample was different.