

**Supplementary Information for**

**Exogenous *N*-acyl-homoserine lactone-based quorum sensing regulation benefits**

***Nitrosomonas europaea* resistance to CeO<sub>2</sub> nanoparticle acute stress**

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**The followings are included as supporting information for this paper:**

number of pages: 11

number of figures: 5

number of tables: 2

### **Text S1**

The influent cultivation medium was chiefly contained 10 mM of  $(\text{NH}_4)_2\text{SO}_4$ , 0.8 mM of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 mM of  $\text{K}_2\text{HPO}_4$ , 0.1 mM of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 10 mM of 3-[4-(2-Hydroxyethyl)-1-piperazine] propanesulfonic acid, 2.4  $\mu\text{M}$  EDTA- $\text{Fe}^{3+}$ , 1  $\mu\text{M}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.9  $\mu\text{M}$  of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.4  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.3  $\mu\text{M}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.02  $\mu\text{M}$   $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ .

### **Text S2**

The culture suspensions (100 mL) were centrifuged at 9,000 r/min for 10 min, The supernatant was filtered through 0.22  $\mu\text{m}$  membrane and was extracted by liquid-liquid extraction with equal volume acidified ethyl acetate. The solvent extracts were evaporated using a rotary evaporator (ASONE, Japan) at 37 °C, and reconstituted in 1 mL ethyl acetate as AHLs in the supernatant, stored at -20 °C.

Extraction of AHLs in *N. europaea* was described below. Biomass achieved after centrifugation was homogenized in 5 mL acidified ethyl acetate and then completely disrupted by an ultrasonic cell disruption system (Xinzhi, Ningbo, China) at 200 W for 15 min (in ice-bath at 3 sec on and 3 sec off). The mixture was subsequently centrifuged at 10,000 r/min for 10 min, and the supernatant was evaporated with nitrogen gas through a pressured gas blowing concentrator (Ruicheng, Hangzhou, China). The residues were reconstituted in 1 mL of methanol for concentration and stored at -20 °C.

AHLs were detected by a high-performance liquid chromatography. An Agilent 1260 Infinity HPLC system with a ZORBAX Eclipse XDB-C18 column (1.8  $\mu\text{m}$  particle size, 50 × 2.1 mm) (Agilent Technologies, USA) was used for AHL analysis. Briefly, 20  $\mu\text{L}$  solutions were applied to the column and eluted with a linear gradient of methanol in water (5 ~ 95 %) over a 15 min period at a flow rate of 0.3 mL/min.

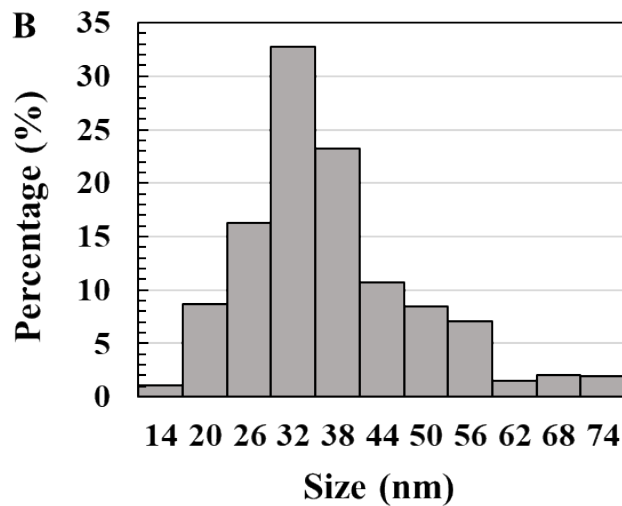
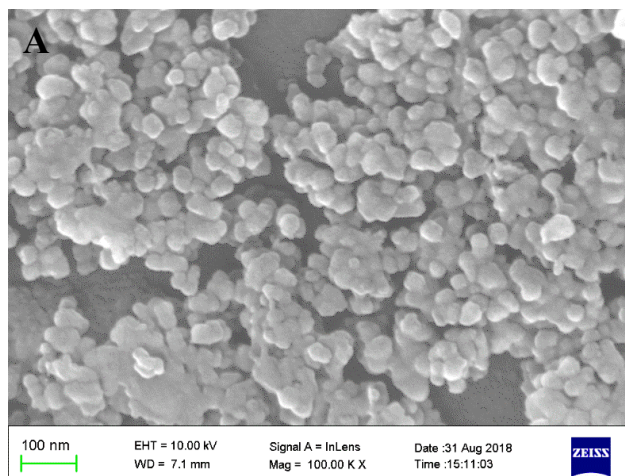
### **Text S3**

The ligated products were amplified with PCR according to the following program: initial denaturation at 95 °C for 10 min; denaturation at 95 °C for 10 s, annealing at 60 °C for 60 s (40 cycles).

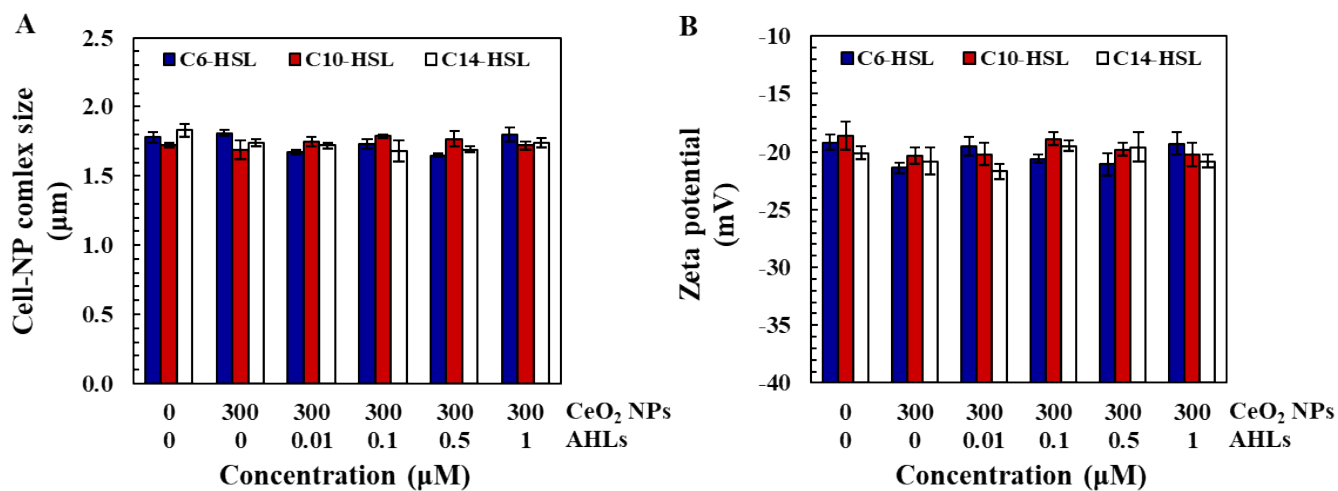
### **Text S4**

The sequence reads consisting of at least 85% bases were progressively trimmed at the 5'-ends until a quality value  $\geq 20$  was kept. Downstream analyses were performed using the generated clean reads of no shorter than 25 bp. The clean reads of each sequenced strains were aligned to the *N. europaea* reference genome (NC\_004757.1) using Bowtie (version 2.2.5). The strand-specific coverage for each gene was calculated using RSeQC (version 2.3.6), and to evaluate the differential *t* in triplicate bacterial cell cultures. CummeRbund package in R (<http://compbio.mit.edu/cummeRbund/>) were used to conduct the statistical analyses and visualization. Gene transcription was calculated as transcripts per million reads (TPM) by RSEM (<http://deweylab.github.io/RSEM/>). Changes in expression values were calculated between *N. europaea* control, 50 mg/L CeO<sub>2</sub> NP-treated *N. europaea*, and C<sub>6</sub>-HSL and CeO<sub>2</sub> NP-simultaneous treated *N. europaea* by determining the log<sub>2</sub> transformed (FC) of the averaged TPM values of triplicate experiments. Genes with  $|FC| \geq 1$  and *p*-value of  $< 0.05$  were labeled genes with highly differentially expression.

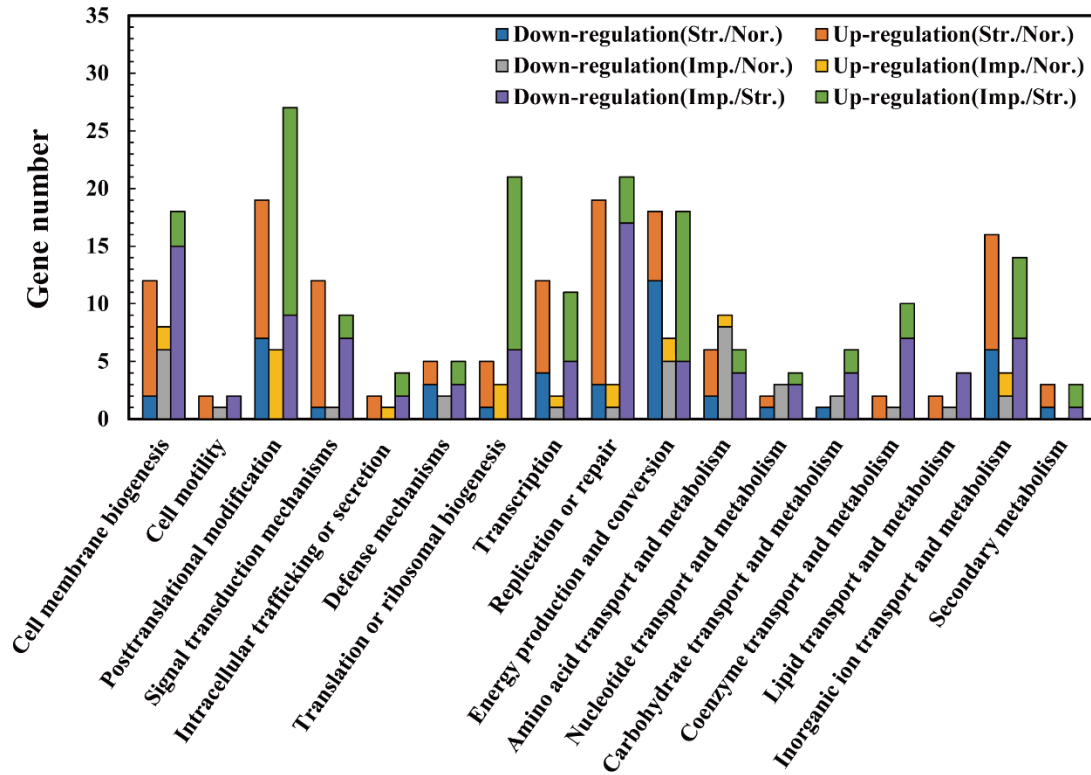
**Fig. S1** SEM image of CeO<sub>2</sub> NPs (A) and their size distribution (B) (based on measurements of 100 random particles).



**Fig. S2** Effects of C<sub>6</sub>-HSL, C<sub>10</sub>-HSL, or C<sub>14</sub>-HSL on particle size (A) and zeta potential (B) of the cell-NP complex.

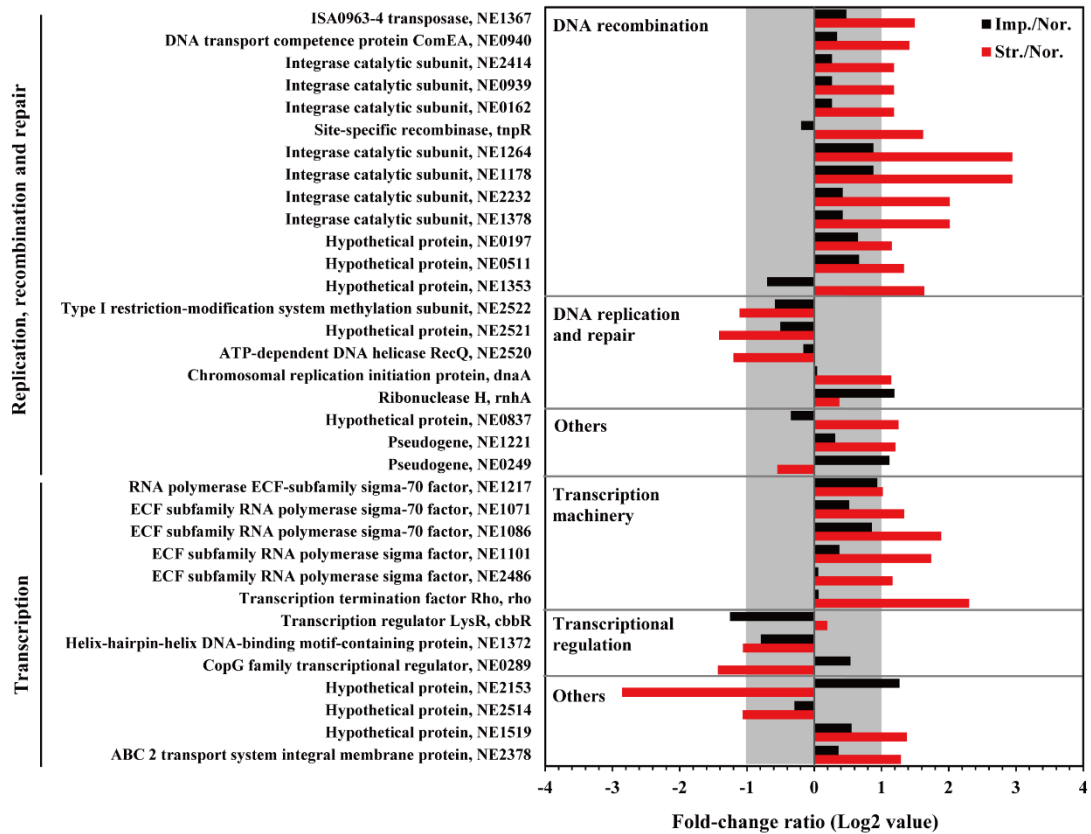


**Fig. S3** Clusters of orthologous group (COG) assignments of differentially expressed genes of *N. europaea* after 6-h exposure to 50 mg/L CeO<sub>2</sub> NPs (Str. / Nor.) and to both NPs and C<sub>6</sub>-HSL (Imp. / Nor.), respectively, compared with the unexposed normal cells, or compared with the exposed cells (Imp. / Str.).



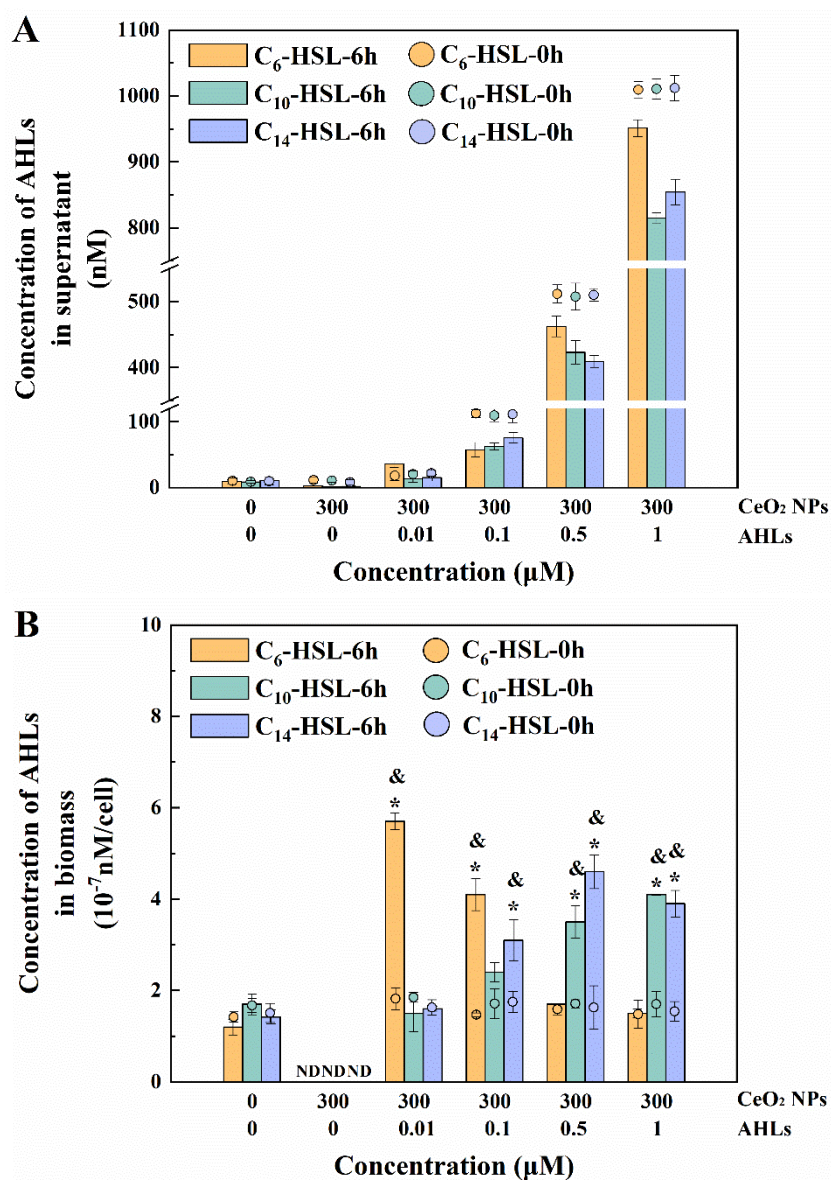
**Fig. S4** Differentially expressed functional genes related to DNA replication, recombination and repair and transcription after 6-h CeO<sub>2</sub> NP exposure or NPs / C<sub>6</sub>-HSL mixed exposure.

Note: the unexposed normal cells are the reference ( $p \leq 0.05$ , and  $|FC| \geq 1.0$ ).



**Fig. S5** Concentrations of AHLs in supernatant (A) and biomass (B).

Note: ‘\*’ and ‘&’ indicate significant differences ( $p \leq 0.05$ ) for test samples when compared to normal cells and NP-treated cells without AHL addition, respectively. ND: not detected.





**Table S1** The oligonucleotide primers for selected genes used in qRT-PCR quantification.

<b>Target Gene</b>	<b>Primer Sequence</b>	<b>References</b>
16S rRNA	F:5' CGTGTCGTGAGATGTTGGGT 3' R:5' CGTGCTTTCTGAGATTGGC 3'	[1]
<i>amoC3</i>	F:5' GGGGCTTCGTTATCCTGG 3' R:5' AGAATGGCTCTGTCCTGCTT 3'	This study
<i>amoA1</i>	F:5' TGGCGACATACCTGTCACAT 3' R:5' ACAATGCATCTTTGGCTTCC 3'	[2]
NE0925	F:5' AGCGTCTTTATGTCCGTTTCAGC 3' R:5' GGCATCAGCACCGATTTGTTT 3'	[3]
<i>cyt_c552</i>	F:5' GTCGTTGGCCCTGCACT 3' R:5' ATACACCGCTGCTGCCG 3'	[4]
<i>rpsE</i>	F:5' TGGGACGCTTCACCACTCTGTAA 3' R:5' AAATCGCTCTCATTGGCCCT 3'	[4]

**Table S2** Functional genes related to posttranslational modification and chaperones with significant transcriptional responses to C<sub>6</sub>-HSL ( $p \leq 0.05$ , and  $|FC| \geq 1.0$ ).

Gene	Locus_tag	Product	FC (log <sub>2</sub> value)		
			Str./Nor.	Imp./Nor.	Imp./Str.
<b>Fe-S cluster assembly</b>					
	NE1452	Hypothetical protein	-3.18	-	3.93
	NE1451	hesB/yadR/yfhF family protein	-3.58	-	4.41
	NE1450	Cysteine desulfurase activator complex subunit SufB	-3.83	-	4.66
ycf16	NE1449	Iron-regulated ABC transporter ATPase subunit SufC	-3.26	-	3.69
	NE1448	Hypothetical protein	-3.12	-	3.40
	NE1445	Nitrogen-fixing protein NifU	-2.06	-	2.44
<b>Chaperone</b>					
	NE2074	HSP20 family protein	-	-	1.24
grpE	NE1950	Heat shock protein GrpE	-	1.03	1.11
dnaK	NE1949	Molecular chaperone DnaK	-	1.63	1.87
dnaJ	NE1948	Chaperone protein DnaJ	-	1.13	1.06
	NE1024	U7 family peptidase	-	-	1.02
	NE0970	Insulinase family protein	-	-	1.28
fkpA	NE0079	FKBP-type peptidyl-prolyl cis-trans isomerase	-	-	1.11
tig	NE0030	FKBP-type peptidyl-prolyl cis-trans isomerase	-	-	1.25
groEL	NE0028	Chaperonin GroEL	-	2.50	3.30
groES	NE0027	Co-chaperonin GroES	-1.00	2.34	3.36
	NE1897	M48 family peptidase	1.56	-	-1.19
<b>Oxidative stress</b>					
	NE2468	Hypothetical protein	1.18	-	-1.00
	NE1911	Glutaredoxin-related protein	1.75	-	-1.17
	NE1408	Sensory transduction histidine kinases	1.32	-	-1.21
nlaB	NE1184	Phospholipid and glycerol acyltransferase	1.11	-	-1.01
bcp	NE0772	Bacterioferritin comigratory protein	1.21	-	-1.47

### Cytochrome *c*. maturation

cycH	NE0771	Hypothetical protein	1.48	-	-1.52
ccmH	NE0770	Cytochrome <i>c</i> -type biogenesis protein CcmH	-	-	-1.67
ccmG	NE0769	periplasmic protein thiol: disulfide oxidoreductase	-	-	-1.17
ccmF	NE0768	Cytochrome <i>c</i> -type biogenesis protein CcmF	1.27	-	-1.02
ccmE	NE0767	Cytochrome <i>c</i> -type biogenesis protein CcmE	1.27	-	-1.01

### Others

NE1898	Hypothetical protein	2.27	-	-2.88
NE1285	Band 7 protein	-	1.07	1.22
NE1181	PemK-like protein	-	-	1.03
NE0295	Hypothetical protein	-	-	1.02

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### References

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