Supplementary Information

Metabolomics profiling to investigate nanomaterial toxicity *in vitro* and *in vivo*

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Supplementary Table S1: Overview of NM cytotoxicity in RLE-6TN cells reproduced from (Karkossa *et al.*, 2019)

IC50 and IC25 values in [µg/cm2] for 24h and 48h were given. Abbreviations NR: not reached; NA: not assessed

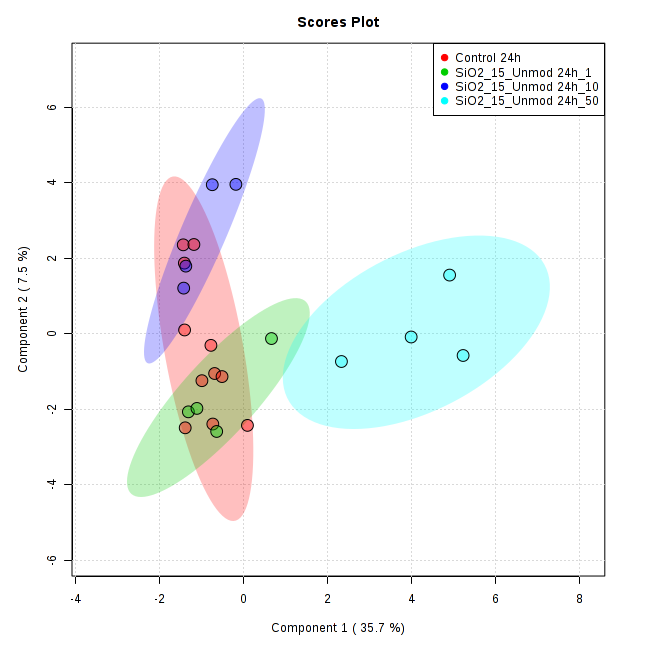
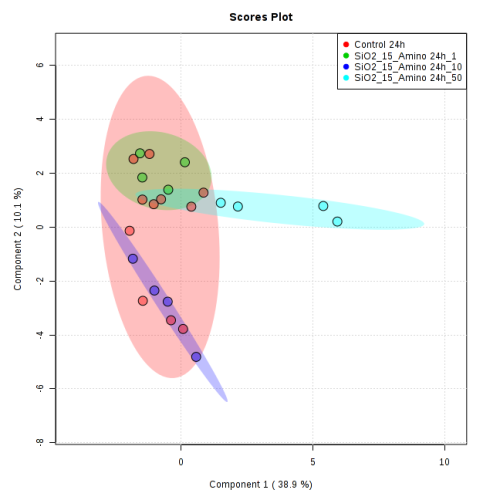
|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | NM | IC50  24h | IC50  48h | IC25  24h | IC25  48h | Overall  Cytotoxicity |
| RLE-6TN | SiO2\_15\_Unmod | NR | NR | NR | NR | None |
| SiO2\_15\_Amino | NR | NR | NR | NR | None |
| SiO2\_40 | NR | NR | 37 | NR | Weak |
| SiO2\_7 | NR | NR | 38 | 56 | Weak |
| TiO2 NM-105 | 7 | 7 | 1 | 1 | Strong |

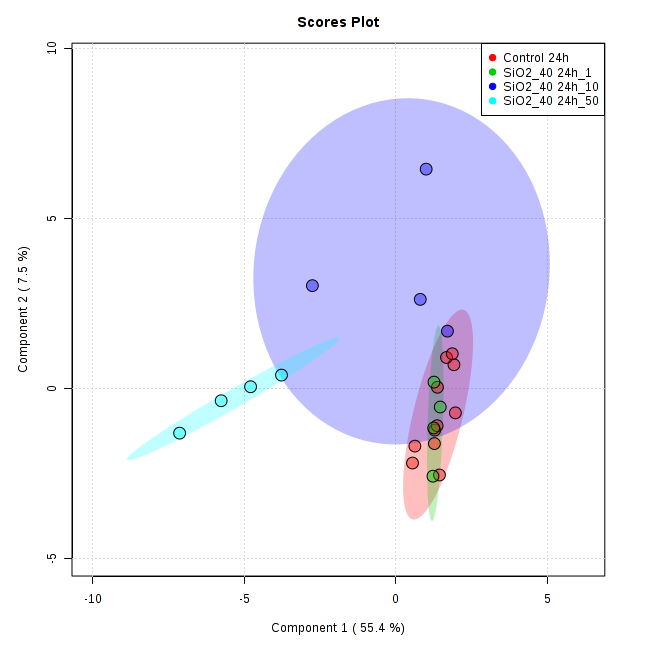
Table S2: Overview of NM cytotoxicity in NR8383 cells (reproduced from (Bannuscher *et al.*, 2019, including additional data for SiO2\_15\_Amino from the same experiment series)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **NM** | **Dose** | **LDH [% of PC]** | | |
|  | [µg/cm2] | **Mean** |  | **SD** |
| **SiO2\_15\_Unmod** | 0 | 21.67 | ± | 2.37 |
| 2.5 | 28.36 | ± | 1.12 |
| 5 | 44.93 | ± | 8.97\* |
| 10 | 93.51 | ± | 19.75\* |
| **SiO2\_15\_Amino** | 0 | 21.67 | ± | 2.37 |
| 2.5 | 25.34 | ± | 2.90 |
| 5 | 26.87 | ± | 1.75 |
| 10 | 59.65 | ± | 6.74\* |
| **SiO2\_7** | 0 | 55 | ± | 1.53 |
| 2.5 | 41.25 | ± | 16.35 |
| 5 | 91.23 | ± | 16.07\* |
| 10 | 95.97 | ± | 15.11\* |
| **SiO2\_40** | 0 | 55 | ± | 1.53 |
| 2.5 | 22.01 | ± | 5.04 |
| 5 | 23.91 | ± | 3.56 |
| 10 | 71.37 | ± | 19.11\* |

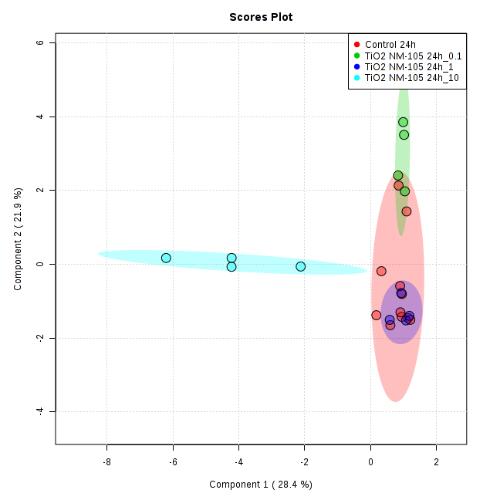
Supplementary Table S3: Parameters for MPPD calculations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **TiO2 NM-105** | | **SiO2\_7** | |
| **Aerosol concentration [mg/m3]** | Exposure | Recovery | Exposure | Recovery |
| 2.55  4.92  14.98  41.97 | 2.47  3.41  11.57 | 0.74  2.58  5.024 | 0.97  1.41  5.298 |
| **MV [m3/min]** | 0.0002142 | | | |
| **Density [g/cm3]** | 3.89 | | 2.65 | |
| **GSD** | 1.78 | | | |
| **t [min]** | 1800 | | | |
| **Df (TB+Alv)** | Exposure | Recovery | Exposure | Recovery |
| 0.1066  0.1109  0.1139  0.0881 | 0.101  0.1242  0.1103 | 0.127  0.098  0.1028 | 0.1216  0.1268  0.1209 |

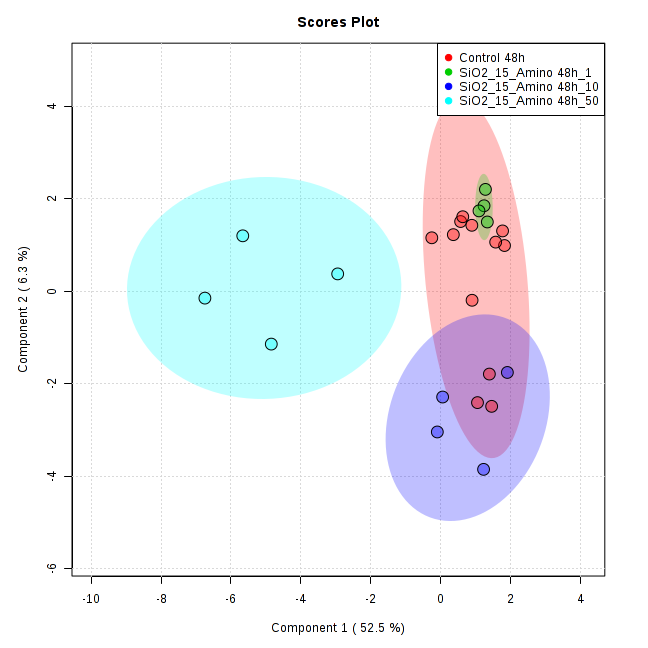
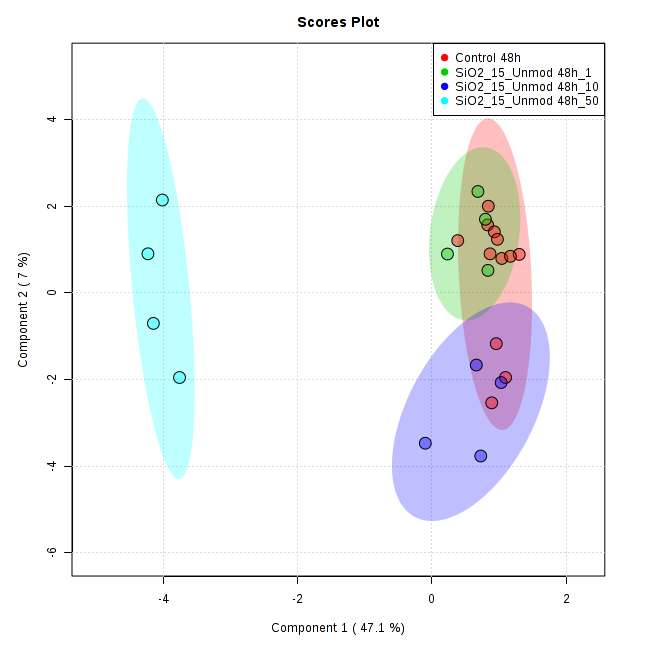


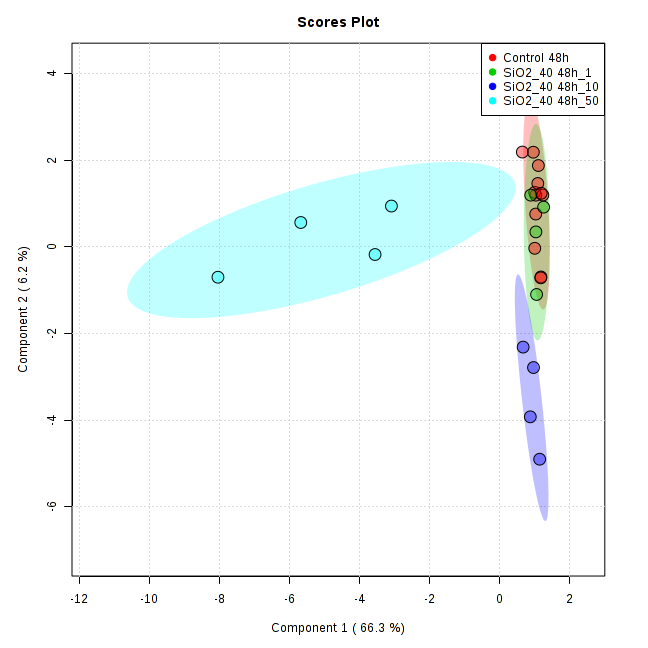
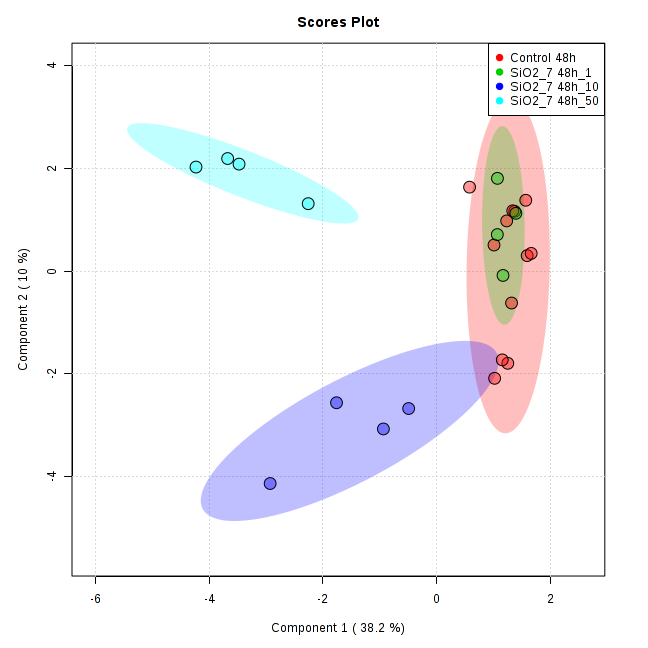
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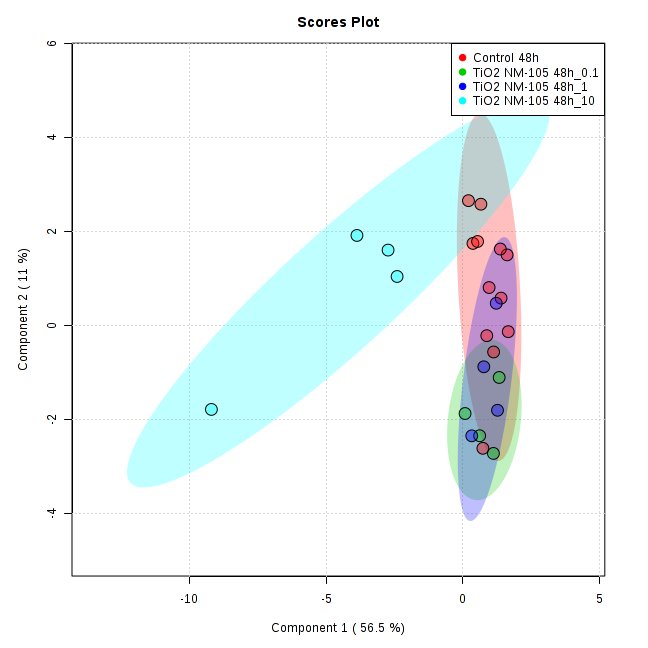
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**Figure S1 sPLS-DA of metabolomics data in RLE-6TN epithelial cells after 24h of NM exposure.**







**Figure S2 sPLS-DA of metabolomics data in RLE-6TN epithelial cells after 48h of NM exposure.**

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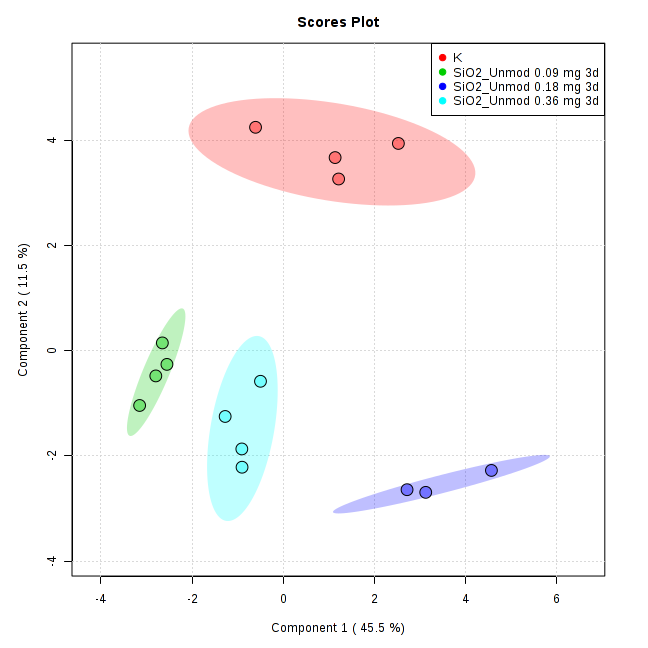
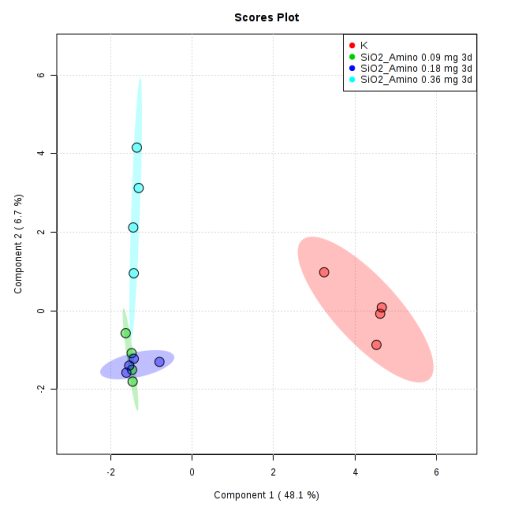
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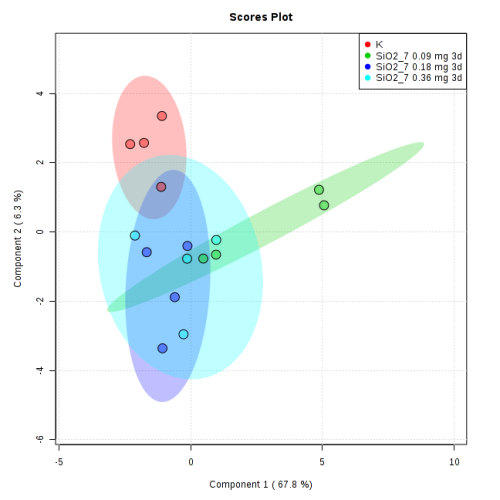
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Figure S3 sPLS-DA of metabolomics data in NR8383 Macrophages after 24h of NM exposure.

**Figure S4 Number of significantly changed metabolites *in vitro* in RLE-6TN epithelial cells (A) and NR8383 Macrophages (B).** Blue indicates decreased and red increased amount of metabolite compared to untreated controls.





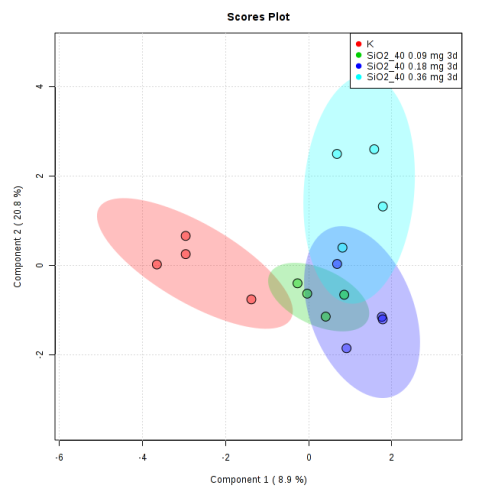
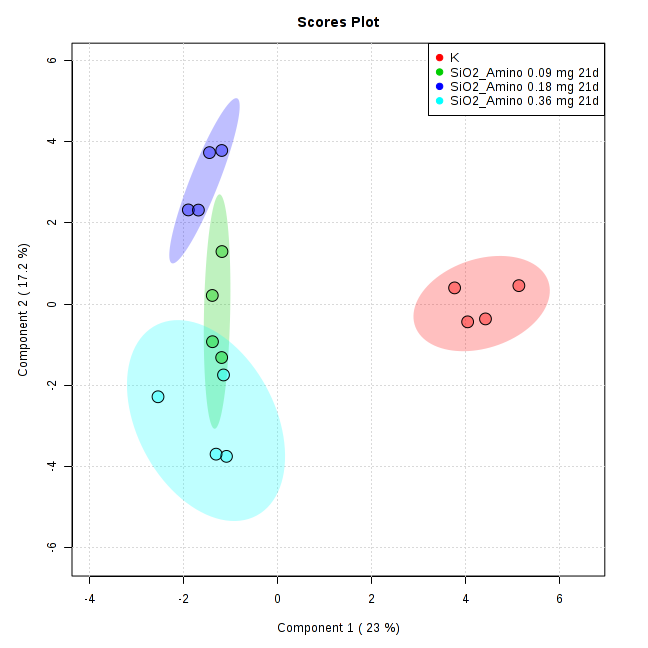
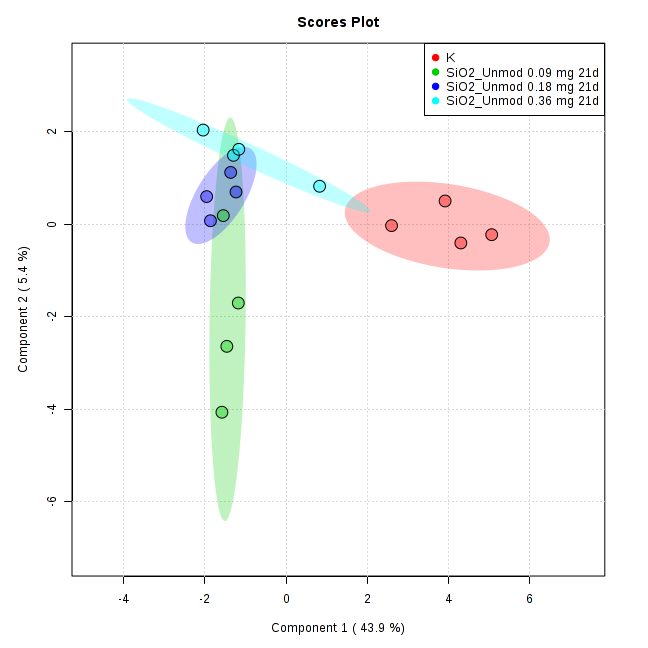


Figure S5 sPLS-DA of metabolomics data of instillation of NMs in exposure group.



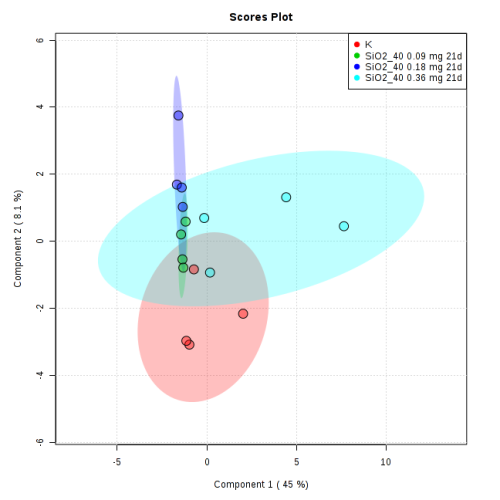
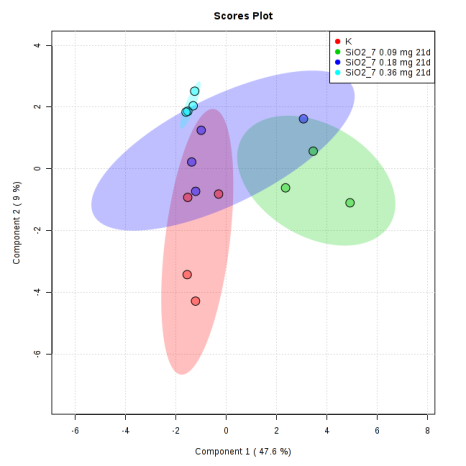


Figure S6 sPLS-DA of metabolomics data of instillation of NMs in recovery group.

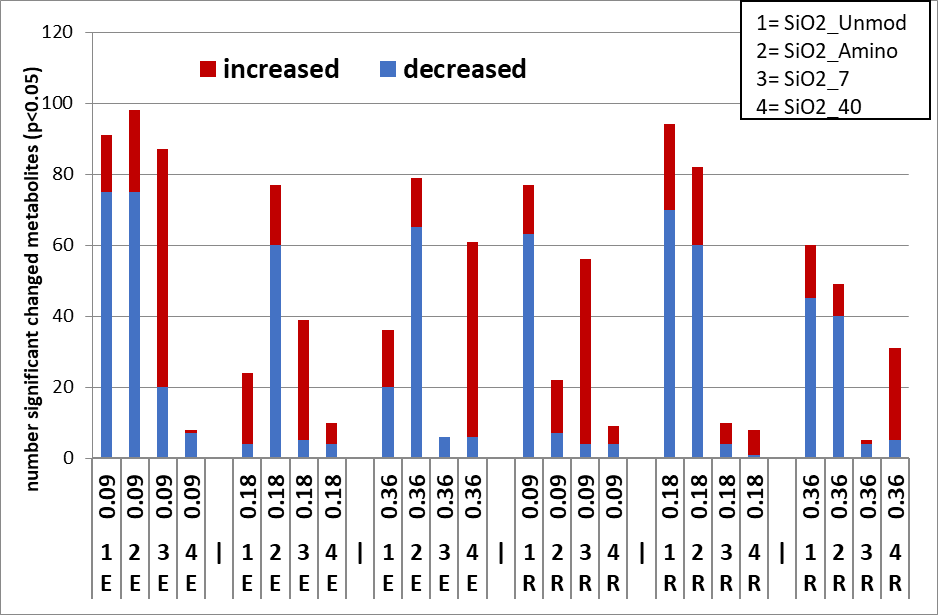
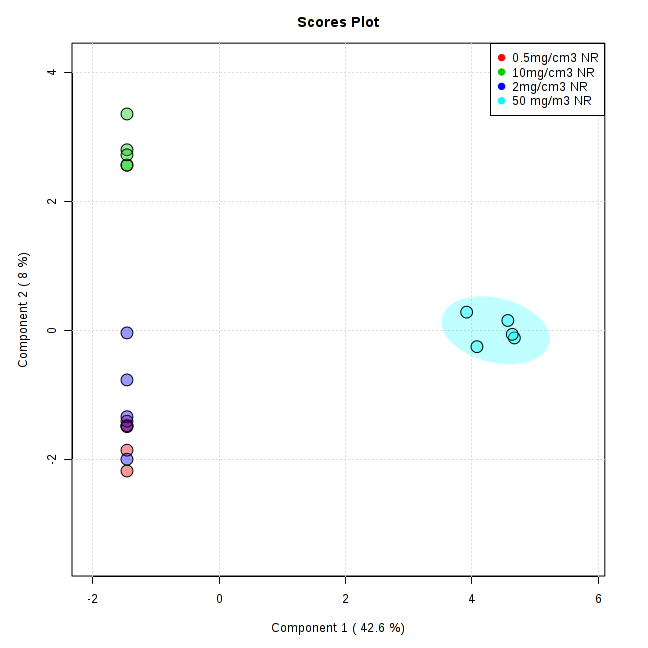
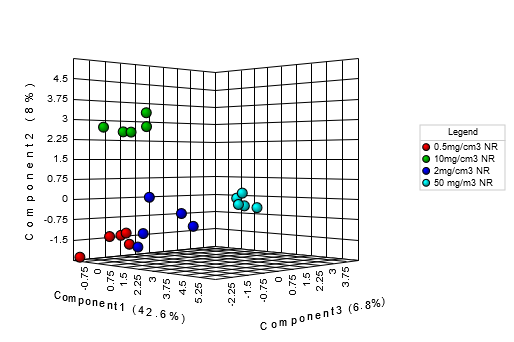
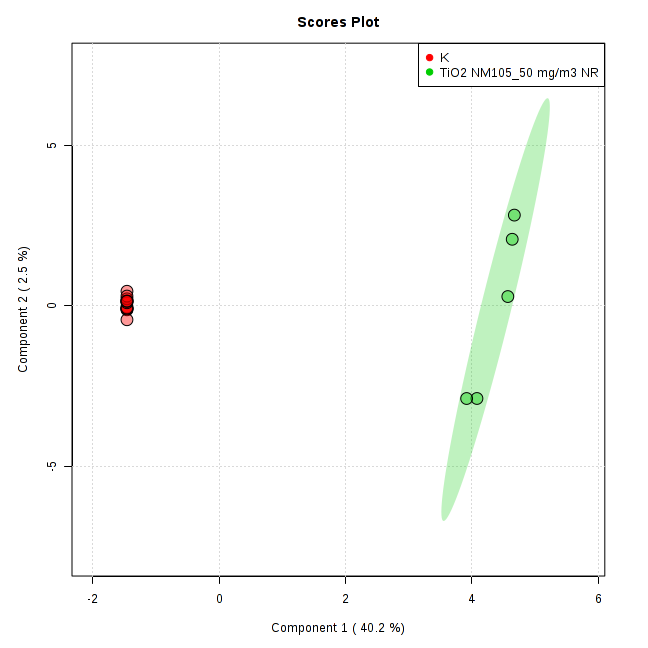
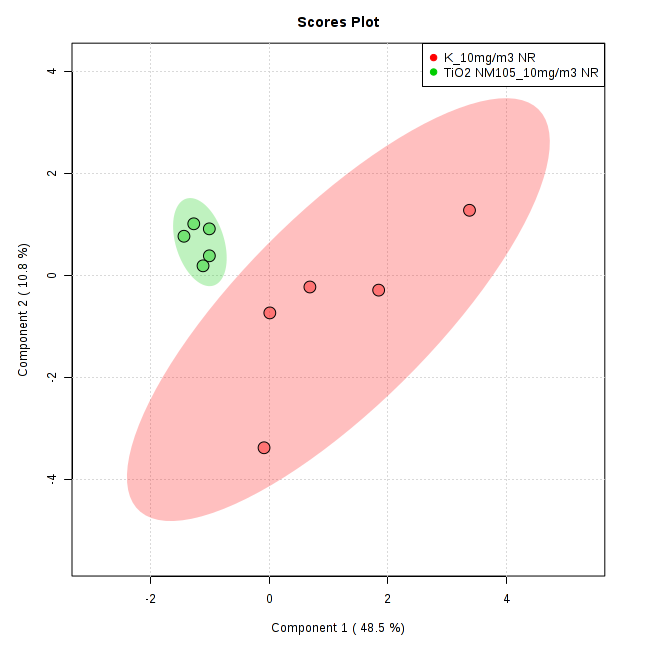
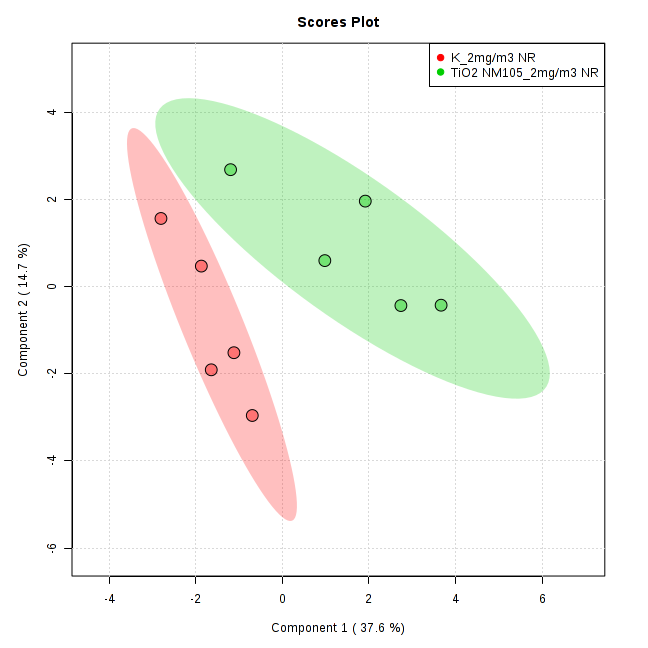
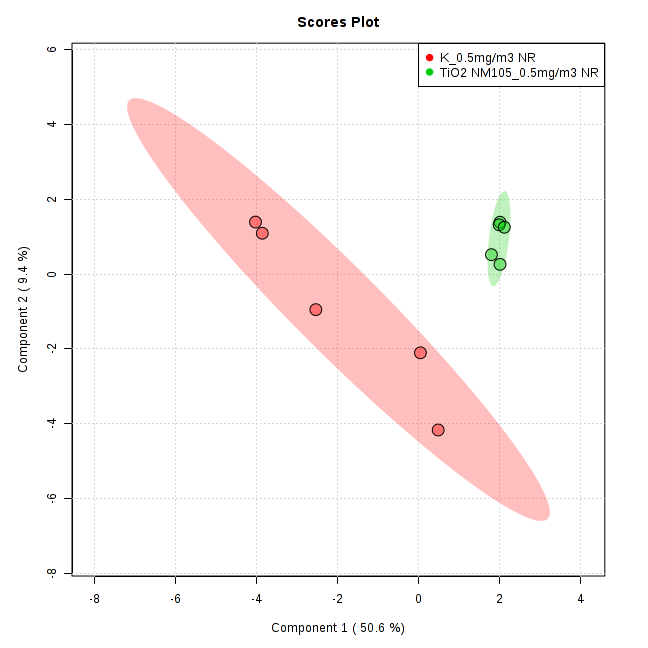
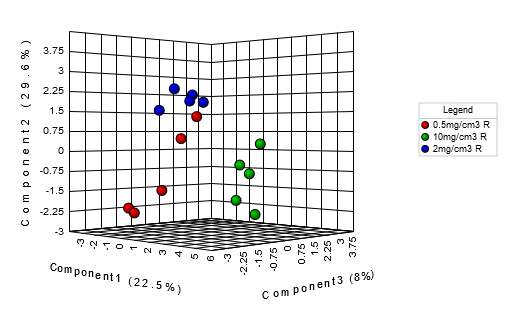


Figure S7 Number of significantly changed metabolites vs. vehicle treated controls secondary to *in vivo* instillations. Blue indicates decreased and red increased amount of metabolites compared to untreated controls.



Exposure



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Automatisch generierte Beschreibung

Recovery

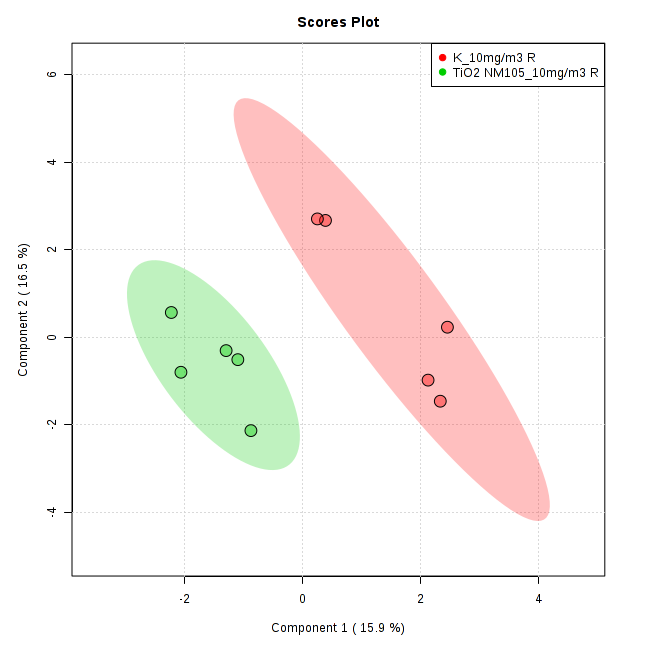
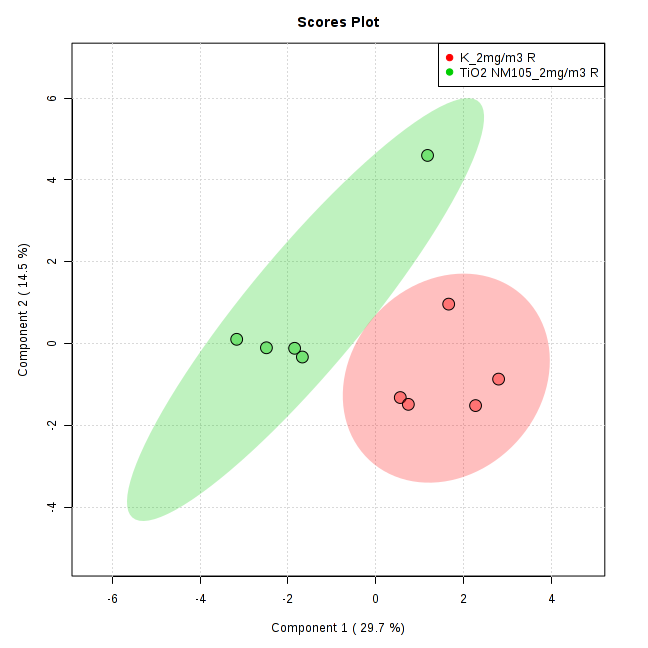
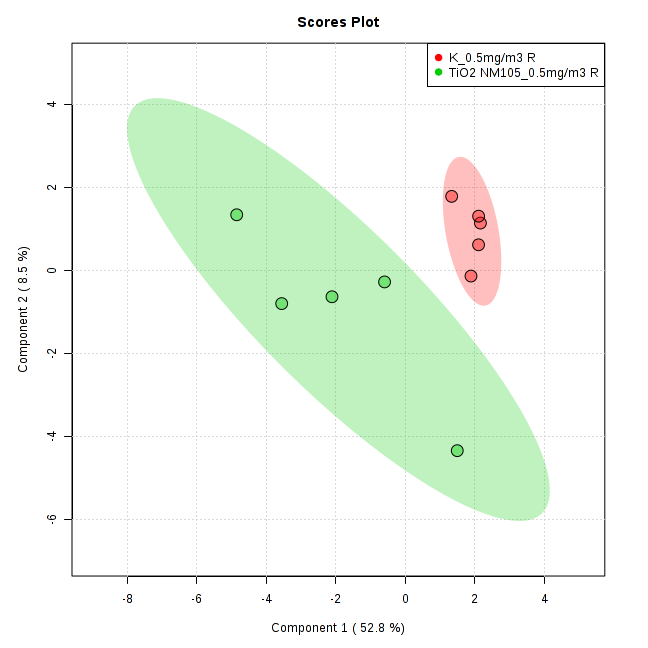
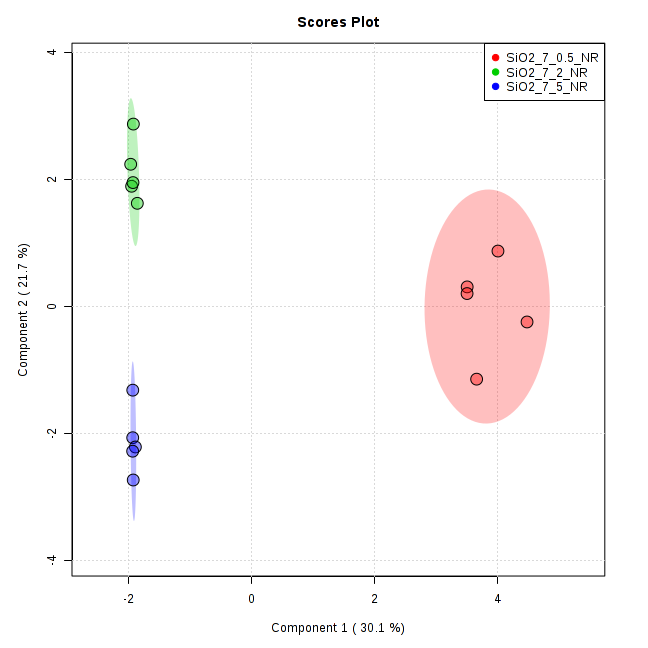
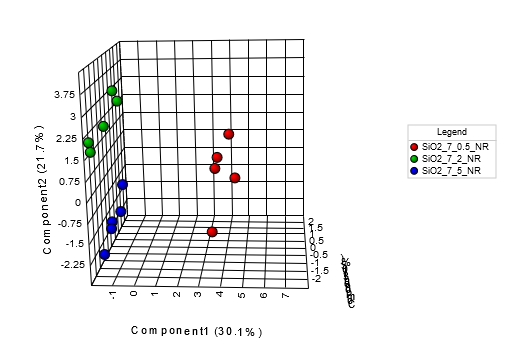
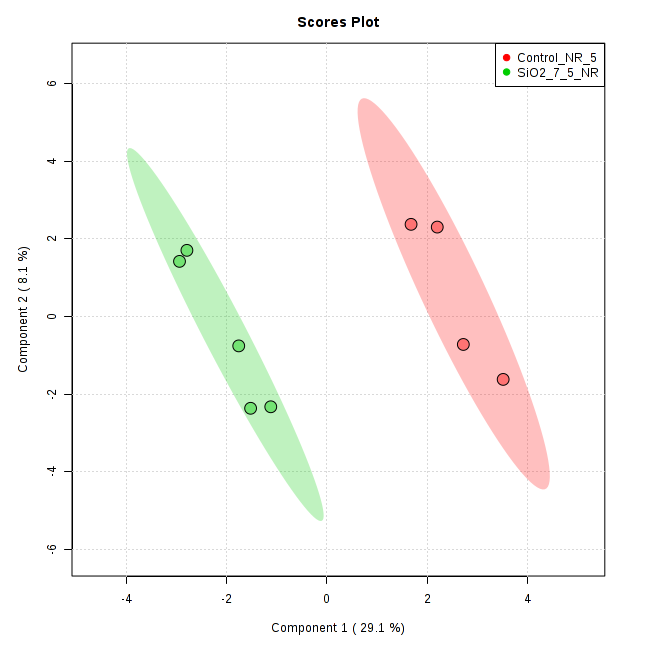
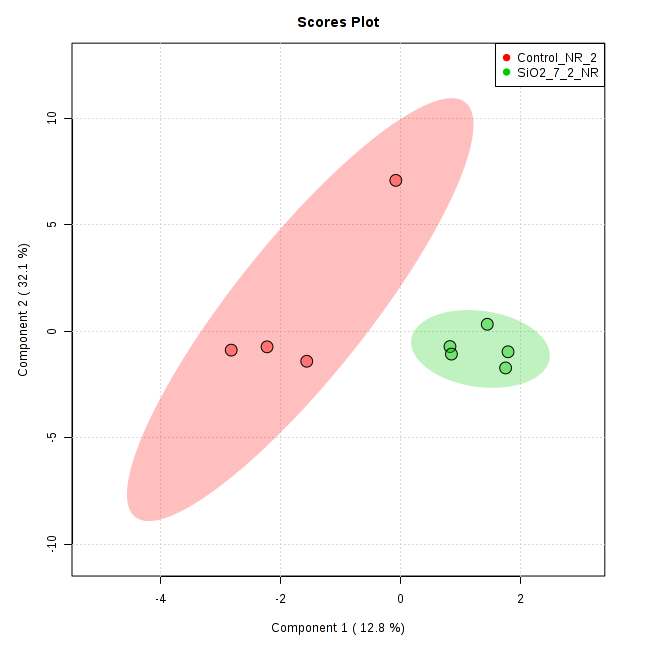
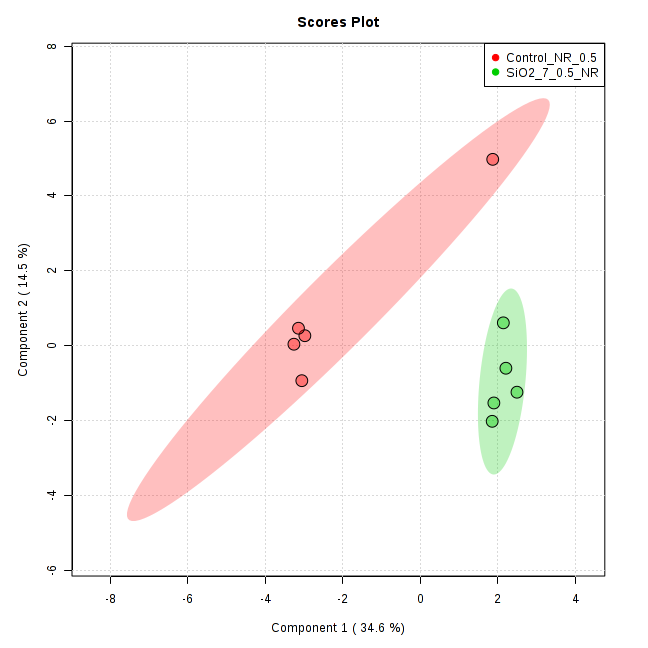
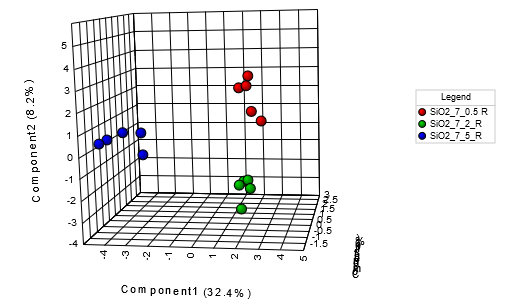


Figure S8 sPLS-DA (3D right, 2D left) of metabolomics data of STIS of TiO2 NM-105 in exposure and recovery groups.



Exposure



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Automatisch generierte Beschreibung

Recovery

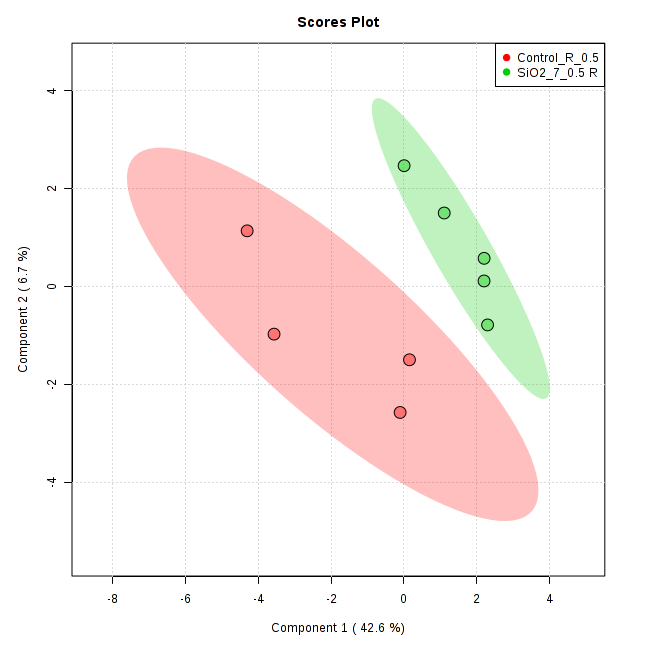
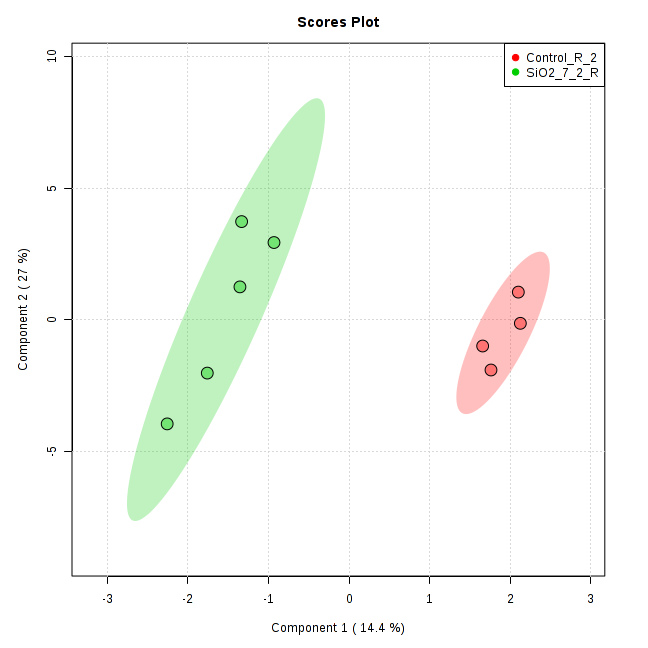
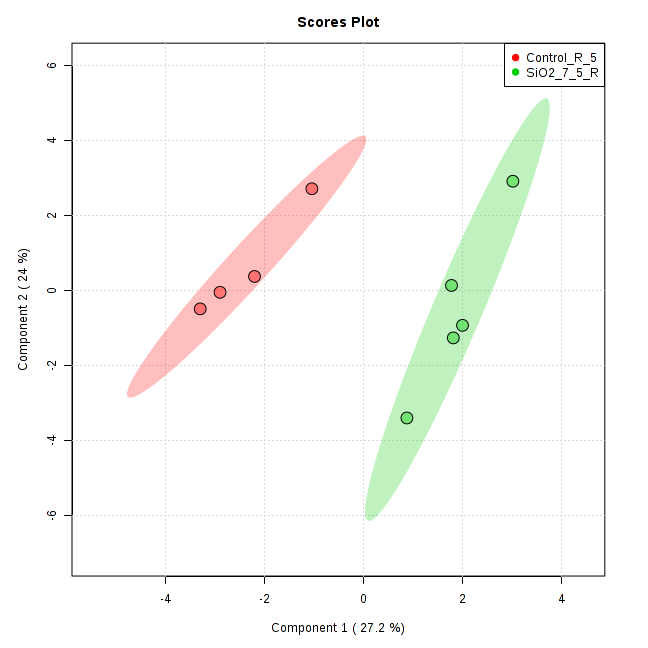
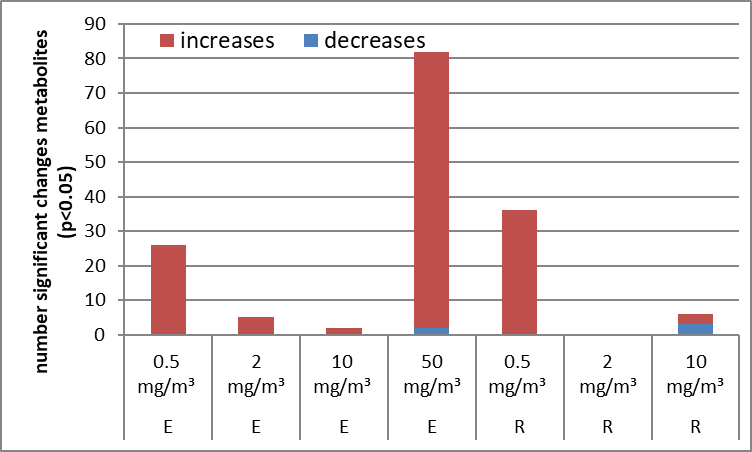
  

Figure S 9 sPLS-DA (3D left, 2D right) of metabolomics data of STIS of SiO2\_7 in exposure and recovery groups.



**TiO2 NM-105**

Figure S10 Number of significant changed metabolites of *in vivo* STIS.

Blue indicates decreased and red increased amount of metabolites compared to untreated controls.

Table S4: Overview feature (Metabolite) selection based on sPLS-DA of *in vitro* data.

|  |  |  |  |
| --- | --- | --- | --- |
| NM | 24h | | 48h |
| **RLE-6TN**  **Epithelial Cells** | **NR8383 Macrophages** | **RLE-6TN**  **Epithelial Cells** |
| SiO2\_15\_Unmod | Putrescine  Gly  LysoPCaC18:0  LysoPCaC18:2  LysoPCaC16:0  Creatinine  Spermidine  Spermine  Lys  Phe  SMC26:1 | SMOHC16:1  SMOH14:1  PCaeC44:3  SMC16:0  SMC18:0  PCaeC44:4  Asp  Glu  Spermidine  Putrescine  Thr  Gly  Trp  PCaeC44:5 | PCaeC40:1  Gly  PCaeC38:4  PCaeC38:1  Spermine  Lys  Asp  Spermidine  C12:1  C3  Creatinine |
| SiO2\_15\_Amino | Thr  PCaaC42:4  PCaeC40:1  PCaeC40:5  Spermidine  Spermine  Lys  LysoPCaC16:0  Phe  SMC20:2  Gly | PCaeC44:5  LysoPCaC16:0  PCaaC36:4  LysoPCaC17:0  Orn  Putrescine  Kynurenine  Ser  Thr  Gln  Asp | Thr  SMC18:1  SMC24:1  LysoPCaC16:1  SMC18:0  Gln  LysoPCaC17:0  PCaaC24:0  Spermidine  Spermine |
| SiO2\_7 | C16,  LysoPCaC18:0  PCaaC40:6  Cit  Pro  Trp  Tyr  Ile  Asn  His  Creatinine  Lys  SM C20:2  SM C24:1  PC ae C30:2 | Gln  Ala  Asp  Glu  Pro  Asn  Trp  SM C22:3  Taurine  LysoPCaC24:0  LysoPCaC26:0  PCaaC24:0  PCaeC44:6  Orn  Spermine  Gly  Cit | Gly  Pro  Putrescine  SMC18:1  Lys  LysoPCaC16:0  Spermine  lysoPCaC26:1  Ala  PCaaC40:2  Glu  SMC26:0  LysoPCaC28:1  LysoPCaC18:0 |
| SiO2\_40 | PCaeC42:2,  LysoPCaC18:0  LysoPCaC17:0  PCaeC40:1  LysoPCaC18:2  Pro  Spermine  Spermidine  Orn  SMC26:1  Lys  C2 | Spermidine  PCaeC44:3  PCaeC44:4  LysoPCaC20:3  ADMA  Ser  Spermine  Lyso PCaC16:0  LysoPCaC17:0  Orn  His  Asp  Kynurenine | PCaeC42:3  PCaaC42:4  LysoPCaC17:0  SMC16:0  Spermidine  Spermine  Orn  Ala  Taurine  SMC22:3  Arg  Thr |
| TiO2 NM-105 | Putrescine,  PCaaC42:1,  Cit  PCaeC40:1,  PCaeC30:1,  LysoPCaC28:0  PCaaC24:0  C3  lysoPCaC18:2  lysoPCaC16:0  lysoPCaC18:0  Taurine  SMOHC24:1 | PCaaC36:0,  PCaeC36:5,  PCaeC40:6,  His,  Trp,  Asp,  Val,  Phe,  Lys,  Tyr,  Met  Kynurenine  Glu | Putrescine  Spermine  PCaaC42:1  PCaeC34:2  SMOHC24:1  LysoPCaC20:4  LysoPCaC16:1  LysoPCaC18:0  C16  C3  SMC26:0  Cit |

**Table S5 Overview feature (Metabolite) selection based on sPLS-DA of *in vivo* instillation data.**

Metabolites were selected of highest components containing highest loadings. Results of exposure and recovery groups were regarded of *in vivo* instillation studies.

|  |  |  |
| --- | --- | --- |
| Name | *In vivo* instillation | |
| **Exposure** | **Recovery** |
| SiO2\_15\_Unmod | LysoPCaC16:0  LysoPCaC20:4  LysoPCaC18:0  LysoPCaC17:0  C4-OH Pro  Total DMA  Taurine  C5OH  C8  Leu  Phe  PCaeC44:3  C18:2  Thr  Creatinine  C4  PCaaC30:2 | Total DMA  PCaaC34:2  PCaaC36:2  PCaaC38:3  PCaaC34:1  Kynurenine  Trp  PCaeC44:5  PCaeC30:1  Spermidine  Ser  LysoPCaC16:1  LysoPCaC17:0  Glu  C0  Phe |
| SiO2\_15\_Amino | Dopamine  C4-OH Pro  PCaaC42:2  PCaaC34:4  PCaaC40:3  Spermidine  Gly  Ala  Asn  Met  C7-DC  PCaaC42:1  Pro  C6  C16 | C16-OH  TotalDMA  C10  Taurine  C4-OH -Pro  Spermidine  Putrescine  Met-SO  LysoPCaC16:1  LysoPCaC20:4  LysoPCaC18:2  C16  Ser  Spermine  LysoPCaC17:0  Tyr |
| SiO2\_7 | Asn  Asp  LysoPCaC16:1  C5-OH  Kynurenine  C8  Histamine  C10  C3-DC  C6  Spermidine  Trp  Arg  Putrescine | PCaaC36:3  SMC26:1  PCaaC34:3  PCaaC34:2  His  Creatinine  Ser  C2  t4-OH-Pro  Asp  LysoPCaC18:0  C10  LysoPCaC20:4  C0  Spermine |
| SiO2\_40 | Alpha-AAA  Spermidine  PCaaC42:1  Asp  LysoPCaC16:1  C10  C6  C0  Thr  Trp  Ser  Pro  C3-DC  Gly  Kynurenine | PCaaC40:3  PCaaC42:1  PCaeC34:0  SMOHC24:1  SMC18:1  C10  C5-OH  Glu  PCaeC44:3  t4-OH-Pro  Spermidine  Val  Histamine  C3-DC  LysoPCaC17:0  Met  Carnosine  lysoPCaC16:1 |

**Table S6 Overview feature (Metabolite) selection based on sPLS-DA of *in vivo* STIS data.**

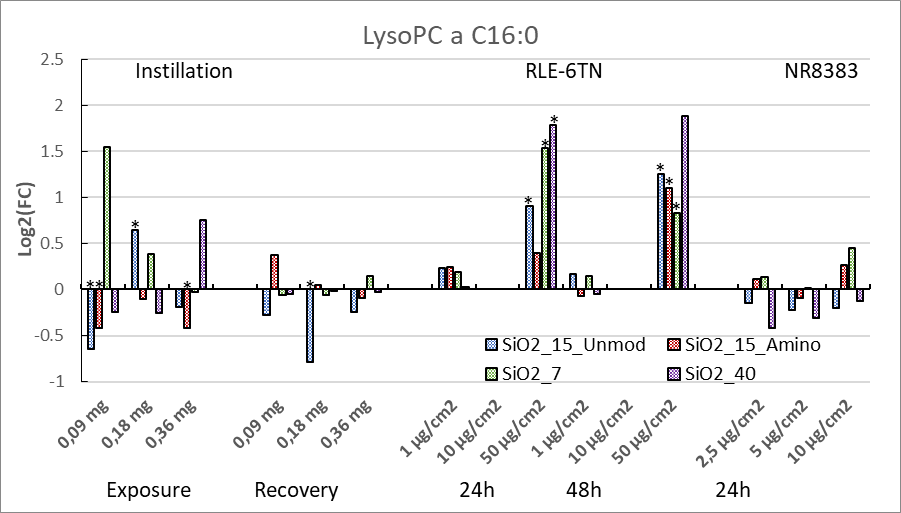
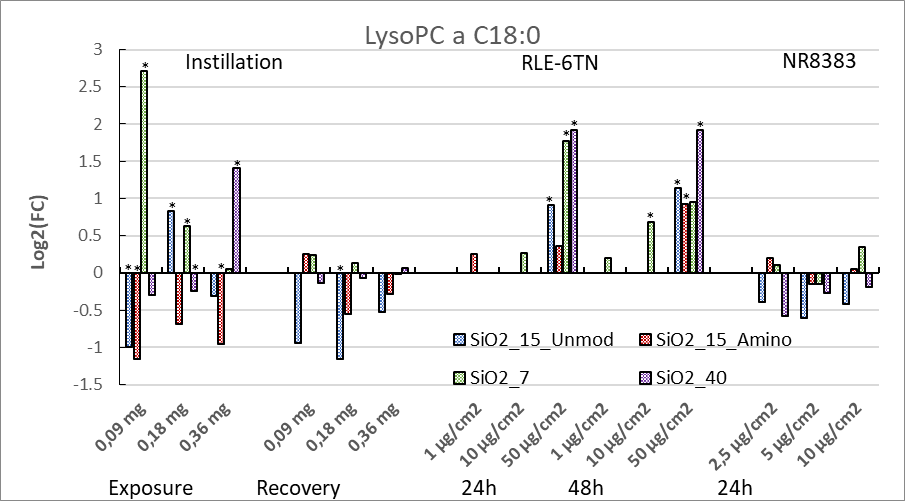
Metabolites were selected of highest components containing highest loadings. Results of exposure and recovery groups were regarded of *in vivo* STIS.

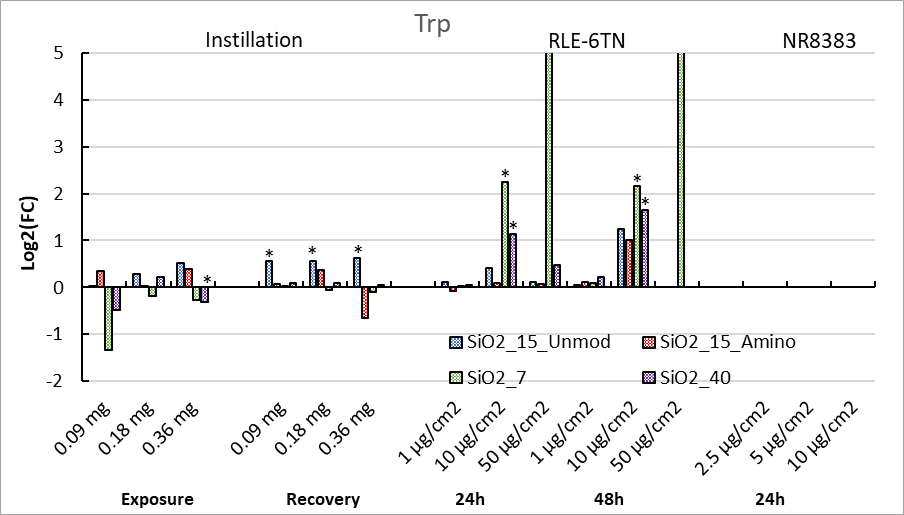
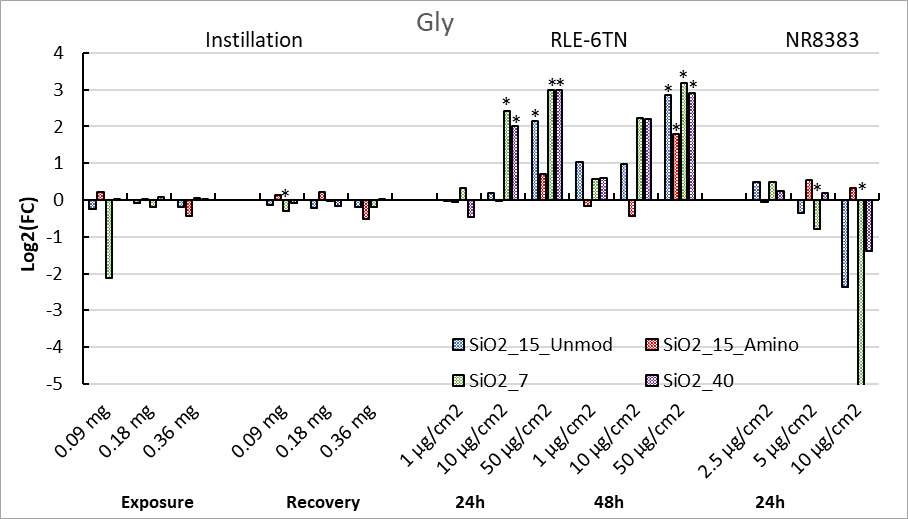
|  |  |  |
| --- | --- | --- |
| Name | *In vivo* STIS | |
| **Exposure** | **Recovery** |
| SiO2\_7 | C12  C16  C14  C5:1-DC  C18  C16:1-OH  C18:2  PCaaC36:3  SMC26:1  PCaaC34:2  PCaaC40:3  t4-OH Pro  Asp  ADMA  lysoPCaC18:0  C10  Ac-Orn  Spermine  lysoPCaC20:4  lysoPCaC18:2 | C16:2-OH  C5:1-DC  C16:1-OH  C18  C16-OH  C14:1  C14:2  Asn  Creatinine  PCaeC38:1  SMOHC24:1  PCaeC36:2  lysoPCaC20:4  lysoPCaC17:0  Spermine  Met  Histamine |
| TiO2 NM-105 | C5-OH  C8  C6  C16-OH  SMOHC22:2  C6:1  Ac-Orn  Spermidine  SMC20:2  His  C2  Kynurenine  Histamine  Val  C5  Orn  Gly | C6:1  Gln  Putrescine  Gly  SMOHC22:2  lysoPCaC16:0  lysoPCaC18:2  Spermine  SMC26:0  Glu  Creatinine  C0  Met  Val  Arg  Orn  Ile  Leu  C3  C3-DC |

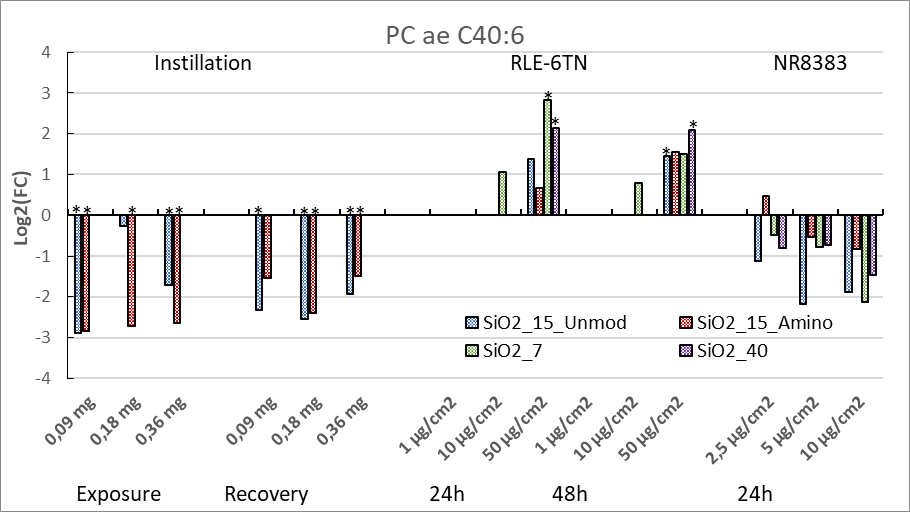
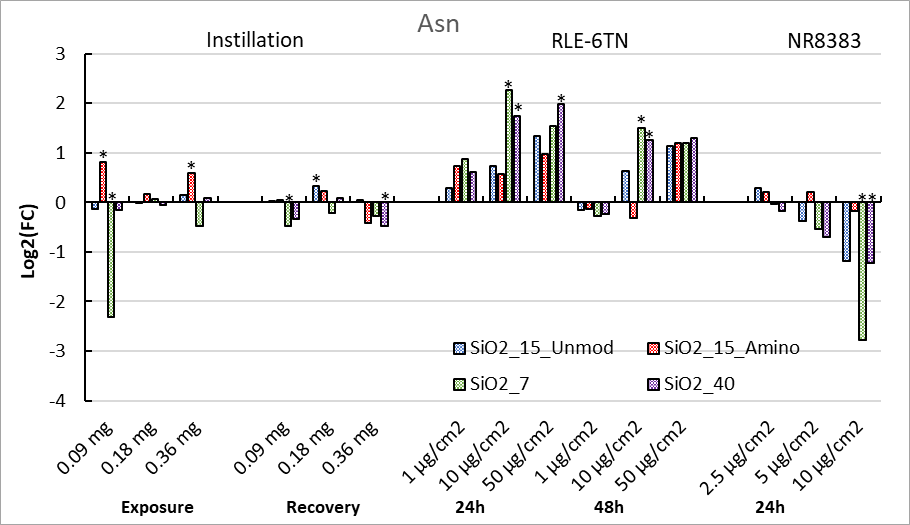
Table S7 Overview feature (Metabolite) selection based on sPLS-DA of *in vivo* instillation data.

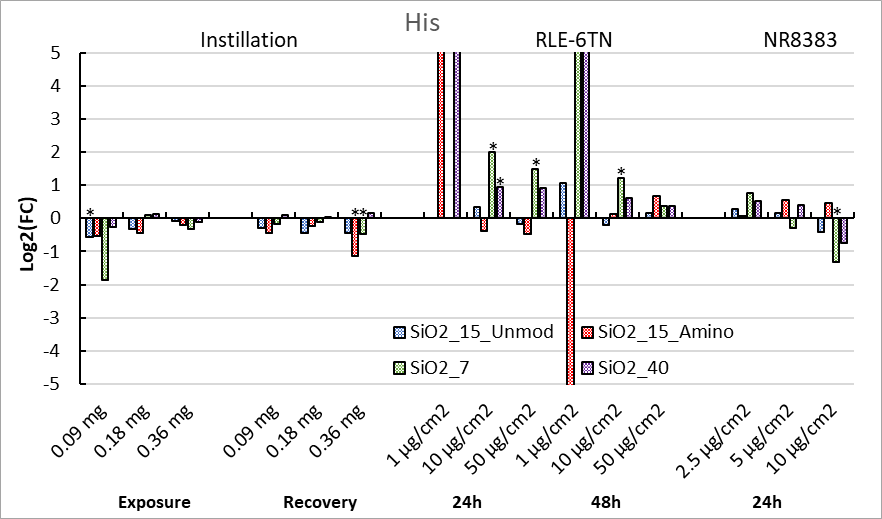
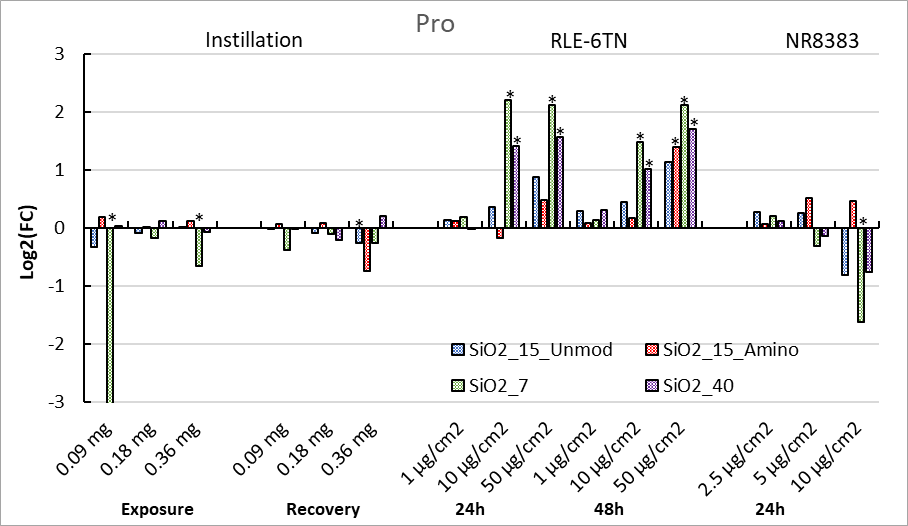
Metabolites were selected of highest components containing high loadings. Results of exposure and recovery groups were regarded of *in vivo* instillation studies.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Name | *In vivo* instillation | | *In vivo* STIS | |
| **Exposure** | **Recovery** | **Exposure** | **Recovery** |
| SiO2\_15\_Unmod | LysoPCaC16:0  LysoPCaC18:0  C4-OH Pro  Taurine  C5OH  C8  C18:2 | Total DMA  PCaaC34:2  PCaaC36:2  Trp  Spermidine  Ser  LysoPCaC16:1  C0 | NA | |
| SiO2\_15\_Amino | C4-OH Pro  PCaaC42:2  PCaaC40:3  Spermidine  Ala  Asn  C7-DC | C16-OH  C4-OH -Pro  Spermidine  Putrescine  Met-SO  LysoPCaC16:1  LysoPCaC18:2  Ser | NA | |
| SiO2\_7 | Asn  Asp  LysoPCaC16:1  C5-OH  Histamine  Spermidine  Arg  Putrescine | PCaaC36:3  His  Ser  C2  t4-OH-Pro  Asp  LysoPCaC18:0  Spermine | C12  C18:2  PCaaC36:3  SMC26:1  t4-OH Pro  Asp  lysoPCaC18:0  Spermine | C18  C16-OH  Asn  SMOHC24:1  PCaeC36:2  lysoPCaC17:0  Spermine  Histamine |
| SiO2\_40 | Spermidine  PCaaC42:1  Asp  LysoPCaC16:1  C6  C0  Ser  Gly | PCaaC40:3  SMC18:1  C10  C5-OH  t4-OH-Pro  Spermidine  Histamine  lysoPCaC16:1 | NA | |
| TiO2 NM-105 | NA | | SMOHC22:2  C6:1  Spermidine  His  C2  Histamine  C5  Gly | C6:1  Gln  Putrescine  Gly  lysoPCaC16:0  lysoPCaC18:2  Spermine  C0 |

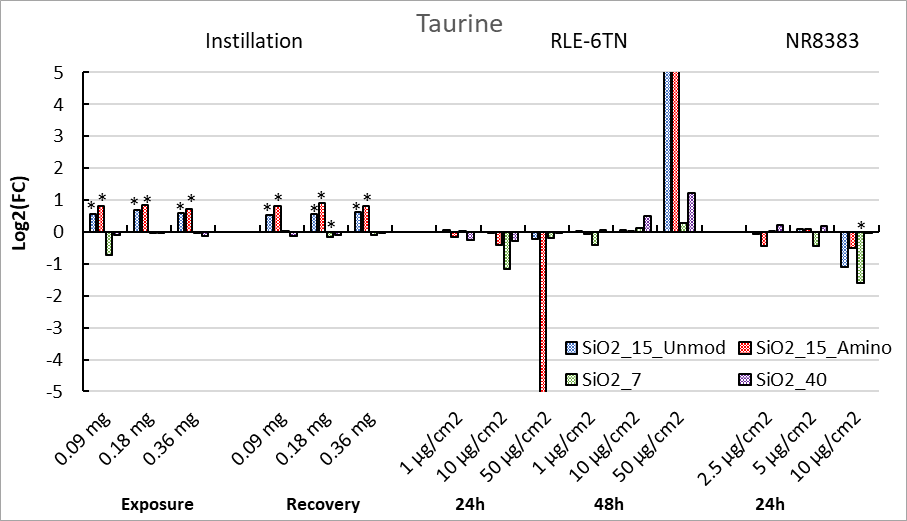
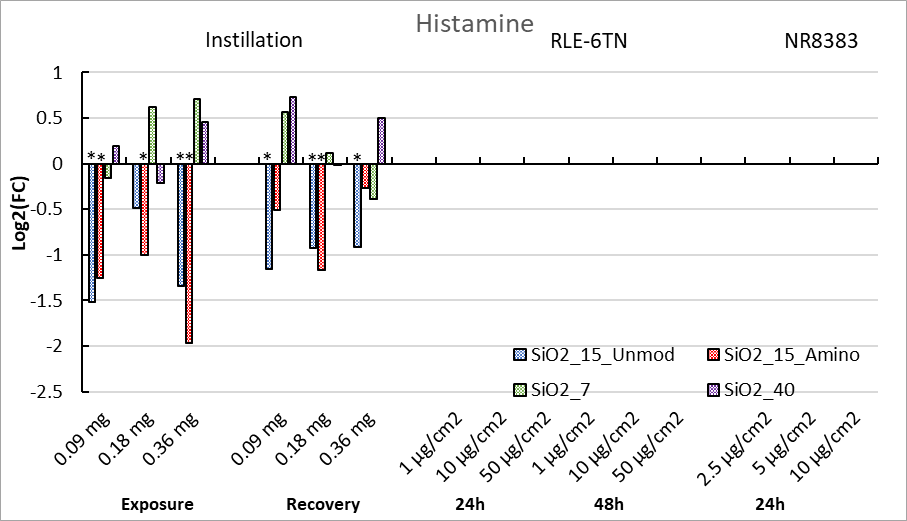
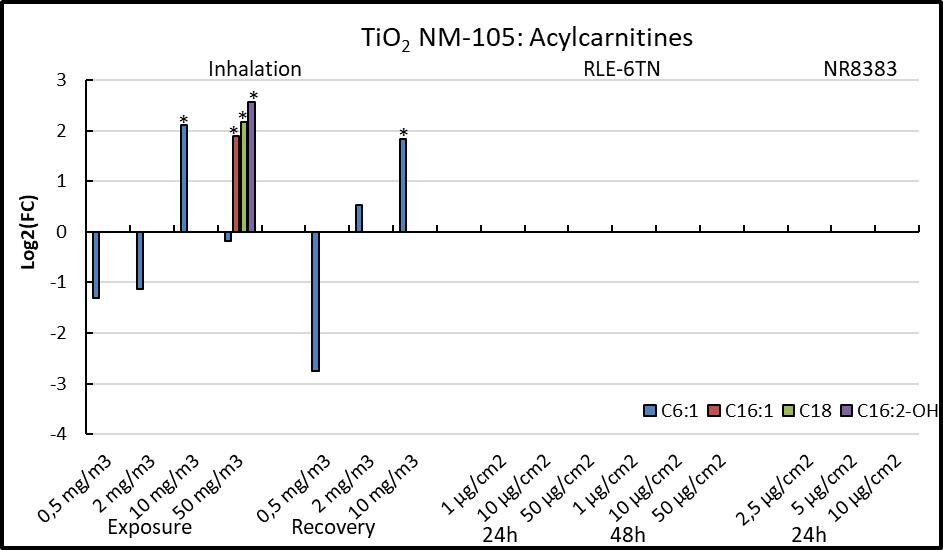
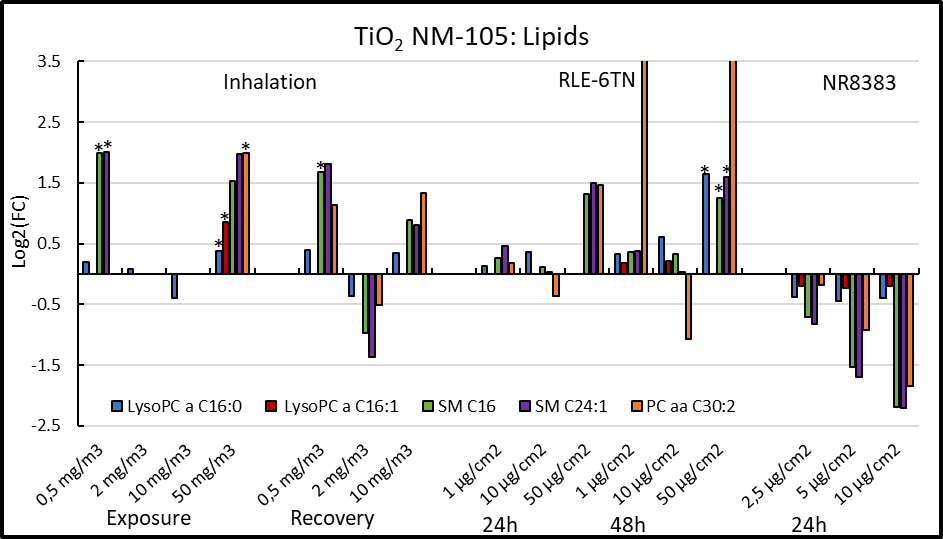
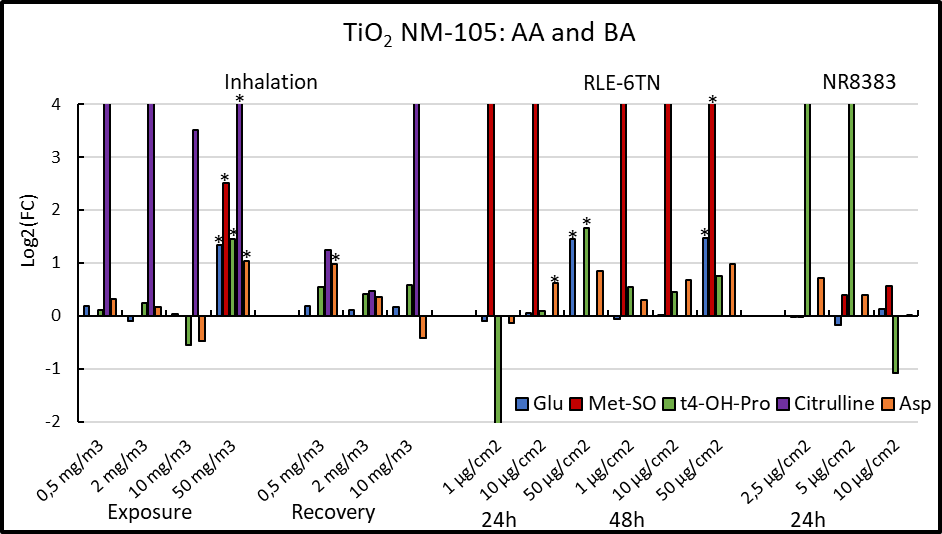
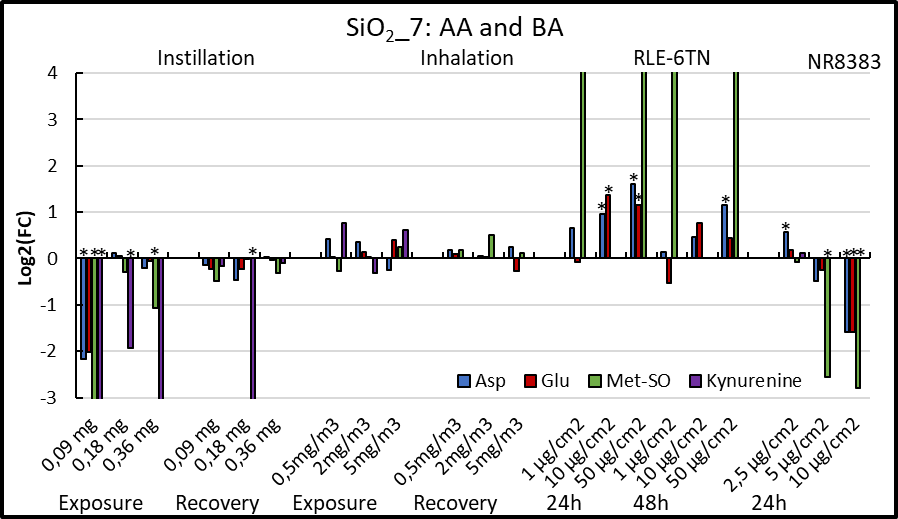
 

Figure S11. Metabolite changes following *in vitro* treatments and *in vivo* instillation exposures of silica NMs. Values are expressed as log2 values of the fold change vs. control (FC).

Supplementary Metabolite Tendencies





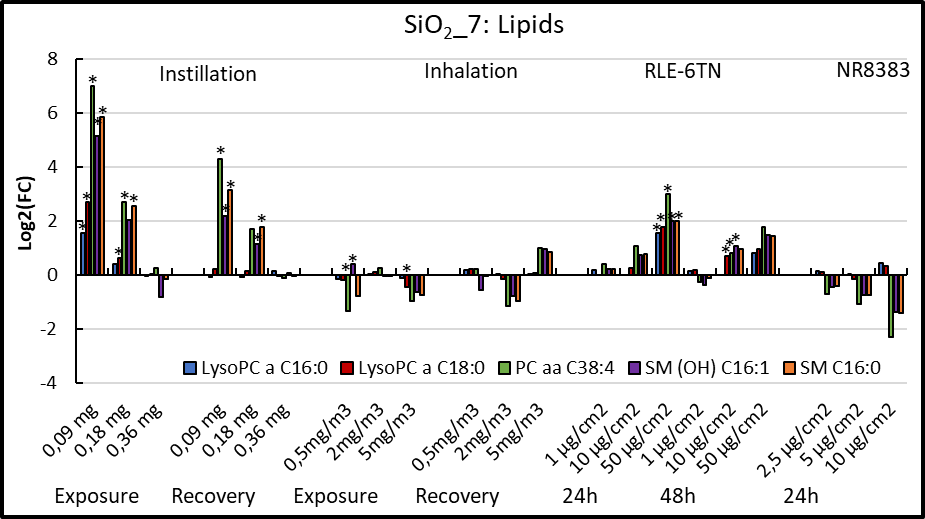


Figure S12. Metabolite tendencies comparing *in vitro* treatments and *in vivo* STIS for TiO2 NM-105 and SiO2\_7. Values are expressed as log2 values of the fold change vs. control (FC).

**References**

Bannuscher, A., Karkossa, I., Buhs, S., Nollau, P., Kettler, K., Balas, M., Dinischiotu, A., Hellack, B., Wiemann, M., Luch, A., Von Bergen, M., Haase, A. & Schubert, K., 2019. A multi-omics approach reveals mechanisms of nanomaterial toxicity and structure–activity relationships in alveolar macrophages. *Nanotoxicology***,** 1-15.

Karkossa, I., Bannuscher, A., Hellack, B., Bahl, A., Buhs, S., Nollau, P., Luch, A., Schubert, K., Von Bergen, M. & Haase, A., 2019. An in-depth multi-omics analysis in RLE-6TN rat alveolar epithelial cells allows for nanomaterial categorization. *Particle and Fibre Toxicology,* 16**,** 38.