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Supplementary Materials

# Serum Lowers Bioactivity and Uptake of Synthetic Amorphous Silica by Alveolar Macrophages in a Particle Specific Manner

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## Calculation of Mean Cellular Particle Burden as used in Figure 8

To calculate the mean cellular burden under the conditions of the macrophage model the totally applied dose of particles per well of a 96-well plate is calculated from the particle concentration per mL multiplied by 0.2, divided by the initial number of cells in the vial, which is $3 \times 10^5$ cells/well und serum-free conditions. Provided that the material is taken up completely by the cells, the concentration range of 11.25, 22.5, 45 and 90 µg/mL, may then be converted to a mean cellular burden of 7.5, 15, 30 and 60 pg/cell, respectively. To obtain the cellular burden shown in Figure 8 (abscissa values) each mean cellular burden was multiplied by the percentage of ingested particles found for the lowest concentration only. Importantly, this concentration was non-toxic (10% FCS added) or nearly non-toxic (protein-free). A direct measurement of the particle load over the whole concentration range would have caused progressive cell lysis, counteracting the isolation of particle-laden cells and subsequent measurements of cell-associated silica. The calculation used here circumvents this problem but should be understood as an approximation.

**Table S1.** Hydrodynamic diameter of SAS in $H_2O$ and cell culture media.

| | | | Hydrodynamic Diameter [nm] | | | | |
|---|---|---|---|---|---|---|---|
| **Material** | **Fluid** | **% FCS** | **Mean ± SEM** | **Mode ± SEM** | **d10 ± SEM** | **d50 ± SEM** | **d90 ± SEM** |
| **AEROSIL® 380 F** | $H_2O$ | 0 | n.d. | n.d. | n.d. | n.d. | n.d. |
| | KRPG | 0 | 151.7 ± 0.6 | 104.0 ± 2.7 | 90.3 ± 1.5 | 135.7 ± 0.4 | 223.8 ± 3.2 |
| | KRPG | 10 | 163.2 ± 1.5 | 112.8 ± 1.3 | 99.5 ± 0.7 | 132.3 ± 1.7 | 256.7 ± 3.0 |
| | F-12K | 0 | 168.5 ± 2.3 | 134.2 ± 9.6 | 110.2 ± 2.2 | 154.3 ± 2.1 | 230.8 ± 7.3 |
| | F-12K | 10 | 436.3 ± 5.4 | 457.7 ± 30.3 | 247.6 ± 6.8 | 442.0 ± 9.6 | 593.3 ± 14.1 |
| **AEROSIL® OX50** | $H_2O$ | 0 | 184.0 ± 1.8 | 178.1 ± 7.2 | 122.5 ± 1.2 | 170.9 ± 2.4 | 247.2 ± 5.1 |
| | KRPG | 0 | 367.5 ± 12.4 | 365.5 ± 37.6 | 218.9 ± 14.8 | 363.2 ± 13.8 | 493.9 ± 5.7 |
| | KRPG | 10 | 214.8 ± 0.6 | 200.6 ± 12.8 | 140.8 ± 1.0 | 201.0 ± 3.9 | 287.5 ± 2.8 |
| | F-12K | 0 | 198.4 ± 0.6 | 159.7 ± 5.0 | 135.3 ± 0.7 | 182.8 ± 0.7 | 263.8 ± 1.3 |
| | F-12K | 10 | 217.8 ± 2.2 | 190.0 ± 8.0 | 145.9 ± 4.5 | 202.9 ± 1.2 | 290.6 ± 2.4 |
| **SIPERNAT® 50** | $H_2O$ | 0 | 238.7 ± 3.3 | 172.4 ± 2.3 | 147.2 ± 2.1 | 200.9 ± 4.1 | 373.3 ± 6.8 |
| | KRPG | 0 | 227.6 ± 5.5 | 174.9 ± 3.4 | 138.4 ± 3.5 | 190.0 ± 5.5 | 363.2 ± 11.4 |
| | KRPG | 10 | 248.0 ± 0.9 | 181.9 ± 7.3 | 157.0 ± 3.8 | 212.8 ± 2.2 | 378.7 ± 0.6 |
| | F-12K | 0 | 251.3 ± 0.7 | 164.7 ± 0.3 | 146.6 ± 1.2 | 217.5 ± 4.5 | 410.1 ± 12.7 |
| | F-12K | 10 | 258.2 ± 2.6 | 185.1 ± 6.8 | 154.4 ± 2.4 | 216.9 ± 4.3 | 419.6 ± 2.5 |
| **SIPERNAT® 160** | $H_2O$ | 0 | 212.5 ± 0.8 | 146.4 ± 3.4 | 127.6 ± 0.7 | 180.5 ± 2.1 | 325.8 ± 2.0 |
| | KRPG | 0 | 269.2 ± 11.1 | 188.1 ± 3.6 | 165.5 ± 6.1 | 241.3 ± 11.7 | 415.4 ± 18.0 |
| | KRPG | 10 | 276.9 ± 6.0 | 194.7 ± 10.0 | 165.5 ± 3.2 | 248.5 ± 2.9 | 421.1 ± 6.8 |
| | F-12K | 0 | 226.2 ± 5.3 | 173.7 ± 11.0 | 138.6 ± 1.8 | 194.6 ± 5.6 | 344.4 ± 14.6 |
| | F-12K | 10 | 235.6 ± 3.9 | 182.2 ± 5.5 | 141.0 ± 2.5 | 202.5 ± 3.7 | 356.7 ± 12.6 |

Footnote to Table S1. Values for d10, d50, d90 describe the cumulative particle size distribution at 10%, 50% and 90% of the maximum value. Particle concentration was 9 μg/mL in all measurements. n.d.: particles not detectable.

**Table S2.** Trypan Blue exclusion test and cell numbers of SAS-treated cells used for mass spectrometric determination of the cell-associated $SiO_2$ mass.

| Material | Effects of SAS Treatment | | | |
|---|---|---|---|---|
| | Serum-free | | 10 % Serum | |
| | cells/mL | % dead cells | cells/mL | % dead cells |
| Control Cells | 345,000 | 3.6 | 490,000 | 1.5 |
| SIPERNAT® 160 | 420,000 | 9.4 | 632,500 | 1.2 |
| SIPERNAT® 50 | 480,000 | 7.3 | 630,000 | 2.4 |
| AEROSIL® OX50 | 400,000 | 4.2 | 592,500 | 2.5 |
| AEROSIL® 380 F | 447,500 | 10.6 | 662,500 | 1.5 |

Footnote to Table S2. Values are means from values measured in duplicates.

**Table S3.** Numerical values from in vitro tests with the Alveolar Macrophage Model.

| | | LDH [% pos. Control] | | | | GLU [% pos. Control] | | | | $H_2O_2$ [µg/mL] | | | | TNFα [pg/mL] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Material | [µg/mL] | w/o FCS | | 10 % FCS | | w/o FCS | | 10 % FCS | | w/o FCS | | 10 % FCS | | w/o FCS | | 10 % FCS | |
| **AEROSIL® 380 F** | **0** | 13.4 ± 2.7 | | 14.5 ± 3.0 | | 1.7 ± 0.5 | | 1.7 ± 1.7 | | 0.9 ± 0.2 | | 0.8 ± 0.1 | | 14.3 ± 14.7 | | 16.7 ± 8.9 | |
| | **11.25** | 32.5 ± 2.9 | *** | 13.2 ± 2.3 | | 4.1 ± 0.7 | * | 1.6 ± 0.6 | | 1.2 ± 0.1 | | 0.8 ± 0.2 | | 42.5 ± 11.1 | | 13.1 ± 7.0 | |
| | **22.5** | 86.4 ± 3.2 | *** | 13.5 ± 1.9 | | 12.9 ± 1.1 | *** | 2.0 ± 0.5 | | 1.2 ± 0.1 | | 0.6 ± 0.5 | | 132.5 ± 17.2 | *** | 17.0 ± 7.3 | |
| | **45** | 103.9 ± 6.4 | *** | 16.8 ± 1.5 | | 18.8 ± 0.9 | *** | 3.1 ± 0.7 | | 1.4 ± 0.1 | | 0.9 ± 0.2 | | 140.1 ± 36.7 | *** | 20.0 ± 5.8 | |
| | **90** | 100.8 ± 5.1 | *** | 24.5 ± 2.8 | ** | 19.1 ± 0.7 | *** | 7.5 ± 0.9 | *** | 1.9 ± 0.2 | *** | 0.9 ± 0.2 | | 126.1 ± 44.7 | *** | 43.7 ± 5.0 | |
| **AEROSIL® OX50** | **0** | 13.4 ± 2.7 | | 14.5 ± 3.0 | | 1.7 ± 0.5 | | 1.7 ± 1.7 | | 0.9 ± 0.2 | | 0.8 ± 0.1 | | 14.3 ± 14.7 | | 16.7 ± 8.9 | |
| | **11.25** | 12.6 ± 2.3 | | 13.0 ± 2.2 | | 1.6 ± 0.4 | | 1.4 ± 0.6 | | 1.0 ± 0.2 | | 0.9 ± 0.1 | | 15.6 ± 13.5 | | 14.6 ± 6.1 | |
| | **22.5** | 17.9 ± 3.2 | | 14.6 ± 2.4 | | 2.2 ± 0.4 | | 1.3 ± 0.9 | | 1.2 ± 0.0 | | 1.0 ± 0.2 | | 20.3 ± 15.7 | | 13.0 ± 7.0 | |
| | **45** | 70.8 ± 9.5 | *** | 16.1 ± 2.6 | | 10.5 ± 1.6 | *** | 2.2 ± 1.2 | | 1.2 ± 0.1 | | 0.9 ± 0.3 | | 84.9 ± 22.6 | *** | 15.9 ± 4.6 | |
| | **90** | 100.4 ± 3.0 | *** | 25.2 ± 3.4 | * | 21.9 ± 1.9 | *** | 4.6 ± 1.0 | * | 1.7 ± 0.2 | *** | 0.9 ± 0.2 | | 67.8 ± 31.7 | ** | 24.4 ± 6.8 | |
| **SIPERNAT® 50** | **0** | 13.4 ± 2.7 | | 14.5 ± 3.0 | | 1.7 ± 0.5 | | 1.7 ± 1.7 | | 0.9 ± 0.2 | | 0.8 ± 0.1 | | 14.3 ± 14.7 | | 16.7 ± 8.9 | |
| | **11.25** | 14.9 ± 2.7 | | 14.8 ± 2.3 | | 1.6 ± 0.2 | | 1.7 ± 0.7 | | 1.2 ± 0.1 | | 0.5 ± 0.6 | | 15.8 ± 8.9 | | 17.9 ± 10.3 | |
| | **22.5** | 23.3 ± 4.4 | ** | 15.9 ± 2.3 | | 2.4 ± 0.6 | | 2.0 ± 1.0 | | 1.4 ± 0.1 | | 0.5 ± 0.7 | | 32.9 ± 19.9 | | 16.3 ± 5.4 | |
| | **45** | 64.9 ± 1.1 | *** | 18.5 ± 3.2 | | 7.9 ± 0.9 | *** | 2.8 ± 1.3 | | 1.8 ± 0.1 | * | 0.8 ± 0.3 | | 90.0 ± 36.8 | ** | 20.9 ± 10.6 | |
| | **90** | 91.4 ± 1.9 | *** | 23.9 ± 4.6 | ** | 15.5 ± 0.3 | *** | 4.5 ± 2.2 | * | 2.2 ± 0.2 | *** | 1.0 ± 0.2 | | 97.0 ± 51.1 | *** | 22.5 ± 10.7 | |
| **SIPERNAT® 160** | **0** | 13.4 ± 2.7 | | 14.5 ± 3.0 | | 1.7 ± 0.5 | | 1.7 ± 1.7 | | 0.9 ± 0.2 | | 0.8 ± 0.1 | | 14.3 ± 14.7 | | 16.7 ± 8.9 | |
| | **11.25** | 19.1 ± 2.1 | * | 14.3 ± 2.1 | | 1.8 ± 0.3 | | 1.3 ± 0.8 | | 1.0 ± 0.2 | | 0.6 ± 0.6 | | 19.9 ± 10.7 | | 14.6 ± 5.5 | |
| | **22.5** | 78.2 ± 1.0 | *** | 16.8 ± 2.0 | | 9.7 ± 0.5 | *** | 2.0 ± 0.8 | | 1.3 ± 0.1 | | 0.4 ± 0.9 | | 38.5 ± 15.5 | | 14.0 ± 5.0 | |
| | **45** | 102.5 ± 1.7 | *** | 24.7 ± 2.3 | *** | 18.5 ± 0.2 | *** | 3.3 ± 0.8 | | 1.6 ± 0.2 | | 0.6 ± 0.5 | | 77.0 ± 24.8 | ** | 20.7 ± 9.9 | |
| | **90** | 92.6 2.7 | *** | 50.3 3.1 | *** | 17.3 ± 1.4 | *** | 9.9 ± 2.1 | *** | 1.9 ± 0.2 | * | 0.8 ± 0.4 | | 82.5 ± 40.1 | *** | 30.3 ± 7.2 | |
| **Corundum** | **0** | 13.2 ± 2.3 | | 16.1 ± 2.9 | | 1.7 ± 0.4 | | 1.7 ± 0.4 | | 1.1 ± 0.3 | | 0.9 ± 0.1 | | 15.3 ± 11.9 | | 16.5 ± 6.8 | |
| | **22.5** | 13.6 ± 2.5 | | 12.9 ± 1.9 | | 2.1 ± 0.7 | | 1.5 ± 2.3 | | 1.1 ± 0.2 | | 0.9 ± 0.1 | | 11.6 ± 7.8 | | 12.8 ± 8.4 | |
| | **45** | 15.1 ± 3.4 | | 15.3 ± 1.9 | | 1.8 ± 0.6 | | 1.5 ± 2.1 | | 1.2 ± 0.2 | | 1.0 ± 0.1 | | 12.2 ± 7.1 | | 12.9 ± 7.4 | |
| | **90** | 18.0 ± 3.3 | | 17.2 ± 1.5 | | 2.0 ± 1.0 | | 2.2 ± 1.5 | | 1.1 ± 0.3 | | 1.0 ± 0.1 | | 12.9 ± 10.0 | | 14.3 ± 8.4 | |
| | **180** | 21.5 ± 2.9 | * | 18.9 ± 1.3 | | 2.6 ± 0.8 | | 3.4 ± 1.0 | | 1.7 ± 0.3 | ** | 1.2 ± 0.1 | | 15.2 ± 13.4 | | 11.9 ± 6.1 | |
| **Quartz DQ12** | **0** | 13.2 ± 2.3 | | 16.1 ± 2.9 | | 1.7 ± 0.4 | | 1.7 ± 0.4 | | 1.1 ± 0.3 | | 0.9 ± 0.1 | | 15.3 ± 11.9 | | 16.5 ± 6.8 | |
| | **22.5** | 12.3 ± 2.2 | | 13.3 ± 0.9 | | 1.8 ± 0.3 | | 2.3 ± 1.4 | | 1.1 ± 0.3 | | 0.9 ± 0.1 | | 12.8 ± 8.2 | | 14.2 ± 7.8 | |
| | **45** | 15.8 ± 3.1 | | 15.4 ± 1.3 | | 2.0 ± 0.6 | | 3.2 ± 1.9 | | 1.2 ± 0.3 | | 0.9 ± 0.1 | | 13.7 ± 10.6 | | 14.4 ± 4.1 | |
| | **90** | 34.6 ± 7.8 | *** | 28.9 ± 4.2 | *** | 4.4 ± 1.6 | | 7.2 ± 2.7 | ** | 0.9 ± 0.4 | | 1.0 ± 0.2 | | 38.8 ± 25.8 | | 34.6 ± 8.8 | |
| | **180** | 78.7 ± 9.9 | *** | 61.9 ± 5.6 | *** | 15.4 ± 4.2 | *** | 20.3 ± 3.5 | *** | 2.0 ± 0.3 | *** | 1.1 ± 0.2 | | 101.2 ± 35.9 | *** | 99.1 ± 16.8 | *** |
| **Zymosan** | **360** | - | | - | | - | | - | | 15.2 ± 0.4 | *** | 11.6 ± 0.7 | *** | - | | - | |
| **LPS** | **0.5** | - | | - | | - | | - | | - | | - | | 1153 ± 214 | *** | 2634 ± 55.4 | *** |

Footnote to Table S3. LDH: lactate dehydrogenase, GLU: glucuronidase, $H_2O_2$: hydrogene peroxide, TNF: tumor necrosis factor $\alpha$, LPS: lipopolysaccharide. * Level of significance is shown by asterisks with *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$.

**Table S4**. EC50 values for the release of LDH, GLU and TNF$\alpha$ calculated for cell-associated SAS.

| Particle Name | LDH | | GLU | | TNF$\alpha$ | |
|---|---|---|---|---|---|---|
| | **EC50 [pg/cell]** | **Goodness of Fit (R2)** | **EC50 [pg/ cell]** | **Goodness of Fit (R2)** | **EC50 [pg/cell]** | **Goodness of Fit (R2)** |
| SIPERNAT® 160 | 6.38 *(15.5)* | 0.992 | 7.87 *(19.4)* | 0.997 | 9.26 | 0.998 |
| SIPERNAT® 50 | 10.60 *(31.7)* | 0.998 | 13.61*(36.3)* | 0.993 | 7.86 | 1.000 |
| AEROSIL® OX50 | 28.00 *(22.7)* | 1.000 | 33.61*(36.5)* | 0.999 | 17.16 | 0.967 |
| AEROSIL® 380F | 2.03 *(13.2)* | 0.998 | 2.50 *(14.0)* | 1.000 | 1.67 | 0.993 |

Footnote to Table S4. Values were non-linear fittings from the curves shown in Figure 8 using GraphPad Prism 8 Software. Italic values in brackets are EC50 values previously determined in μg/mL [18].

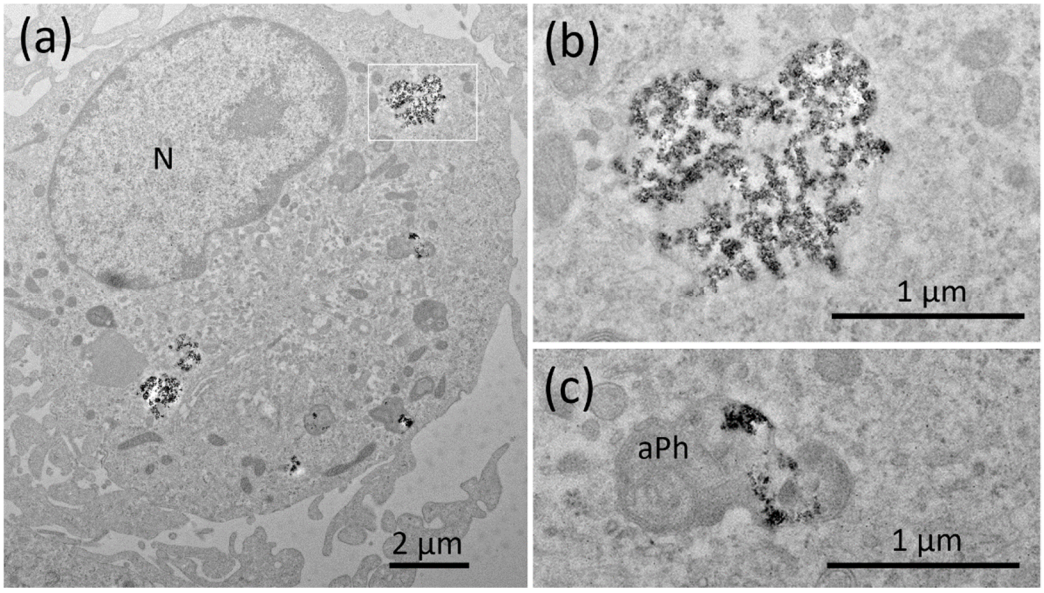


**Figure S1.** Electron microscopy of NR8383 cells laden with SIPERNAT® 50 in the absence of FCS for 16 h. (**a**) Overview of a cell with particle-containing phagosomes (large arrows), autophagosomes (aPh) and lysosomes; N: nucleus; the white box is enlarged in (**b**) and shows a particle-filled phagosome; (**c**) an autophagosomes with condensed particulates (aPh).

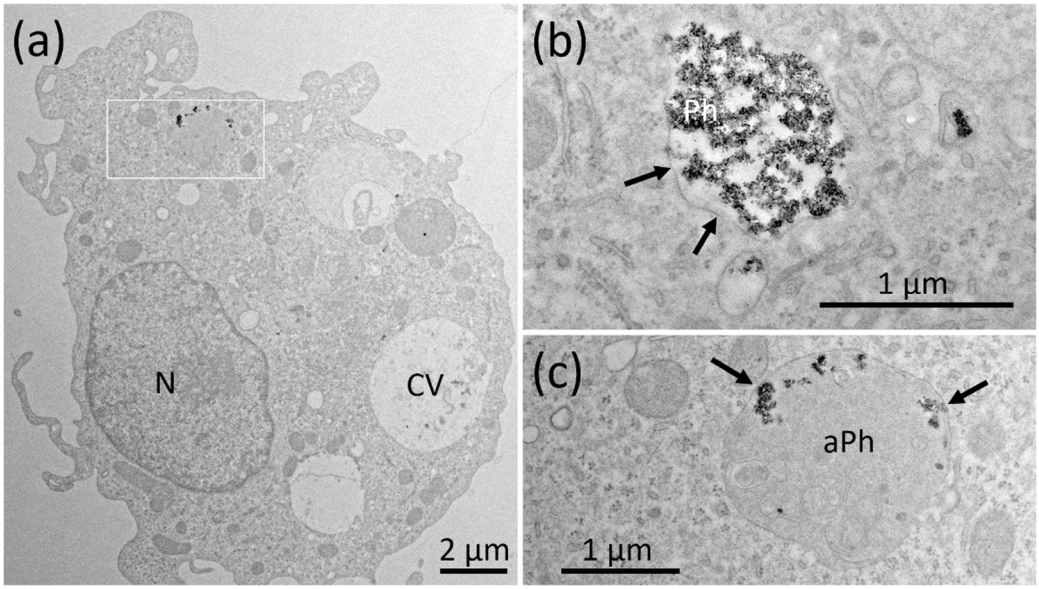


**Figure S2.** Electron microscopy of NR8383 cells laden with SIPERNAT® 50 in the presence of FCS for 16 h. (**a**) Overview of a cell with particle-containing autophagosome; CV: clear vesicle; N: nucleus. (**b**) particle-filled phagosome (Ph); the white box in (**a**) is enlarged in (**c**) and shows an autophagosome (aPh) with condense particulates. Arrows in (**b**) and (**c**) point to continuous membranes of the particle-containing vesicles.

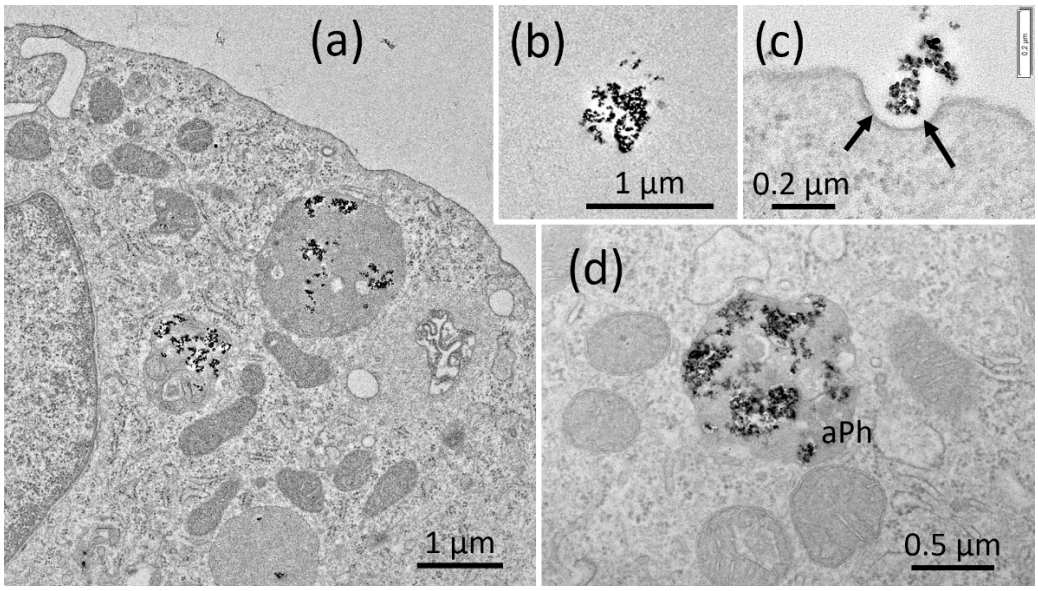


**Figure S3.** Electron microscopy of NR8383 cells laden with SIPERNAT® 160 in the absence of FCS for 16 h. (**a**) Section of a cell with particle-containing vesicles. Extracellular aggregates/agglomerates of SIPERNAT® 160 in the medium (**b**), and (**c**) close to the cell membrane during beginning endosome formation (large arrows). (**d**)Autophagosome (aPh) partly filled with particles aggregates/agglomerates.

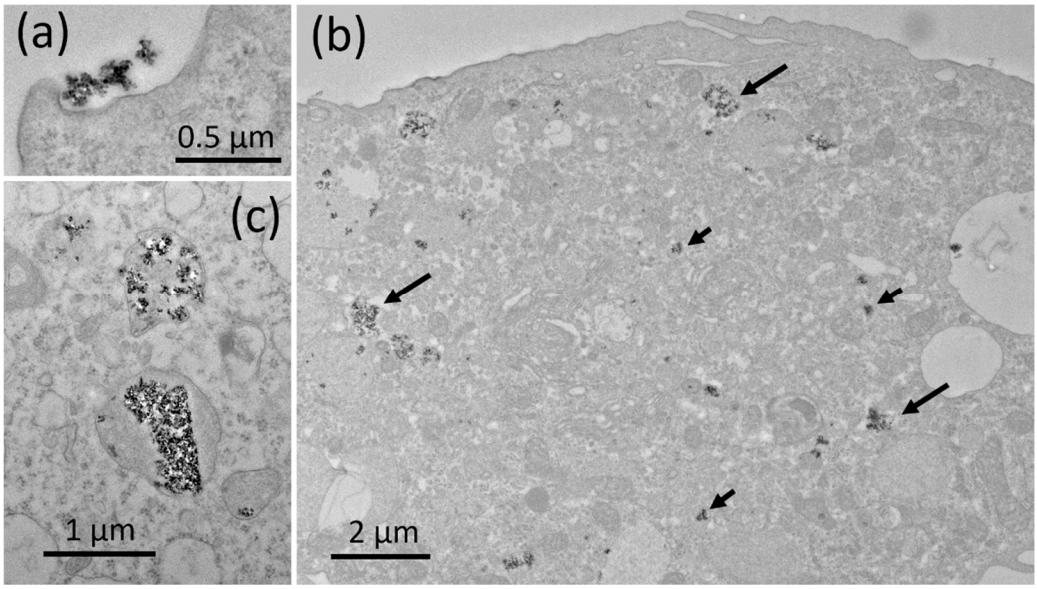


**Figure S4.** Electron microscopy of NR8383 cells laden with SIPERNAT® 160 in the presence of FCS for 16 h. (**a**) Extracellular aggregates/agglomerates of SIPERNAT® 160 close to the cell membrane during early endosome formation. (**b**) Section of a cell with particle-containing phagosomes (large arrows) and numerous small particle deposits in endosomes (small arrows), lysosomes and autophagosomes. (**c**) Aggregates/agglomerates of SIPERNAT® 160 lysosomes.