# Supplementary file 1: Tables

**Table S1**. Fold changes of gene expression in pleura and lung of mice exposed to Mitsui-7 (Mit-7) or NM-401. Statistical analysis was performed in R using means of linear regression, adjusted for total exposure time and sex (*n* = 19). High dose (HD), low dose (LD).

| **Pleura** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mit-7 LD | | | Mit-7 HD | | | NM-401 LD | | | NM-401 HD | | |
| | Mean | SD | p-value | Mean | SD | p-value | Mean | SD | p-value | Mean | SD | p-value |
| *Bcl-2* | 0.339 | 0.079 | 5.50E-04 | 0.422 | 0.099 | 8.00E-03 | 0.541 | 0.122 | 3.60E-02 | 0.809 | 0.185 | 4.30E-01 |
| *Ccl11* | 0.859 | 0.102 | 3.20E-01 | 0.717 | 0.090 | 5.00E-02 | 1.024 | 0.132 | 9.70E-01 | 0.983 | 0.122 | 8.40E-01 |
| *Ccl12* | 5.787 | 0.990 | 3.40E-10 | 18.238 | 3.481 | 5.10E-17 | 10.765 | 1.654 | 4.70E-16 | 17.354 | 2.996 | 6.50E-18 |
| *Ccl3* | 4.582 | 0.864 | 3.10E-07 | 5.828 | 1.029 | 3.50E-09 | 4.819 | 0.745 | 4.80E-09 | 10.448 | 1.836 | 2.60E-13 |
| *Cdh1* | 1.013 | 0.264 | 9.10E-01 | 0.994 | 0.273 | 8.70E-01 | 0.853 | 0.190 | 5.30E-01 | 1.216 | 0.288 | 6.70E-01 |
| *Col1a2* | 0.544 | 0.228 | 1.70E-01 | 1.265 | 0.502 | 8.40E-01 | 3.352 | 1.299 | 3.20E-02 | 1.771 | 0.602 | 3.40E-01 |
| *Cxcl2* | 3.646 | 0.718 | 9.30E-06 | 5.700 | 1.042 | 5.30E-09 | 5.105 | 0.799 | 1.60E-09 | 6.990 | 1.228 | 1.00E-10 |
| *Il1a* | 1.285 | 0.327 | 6.40E-01 | 1.202 | 0.324 | 8.00E-01 | 2.124 | 0.486 | 4.60E-02 | 1.521 | 0.366 | 3.40E-01 |
| *Il1b* | 1.673 | 0.595 | 3.80E-01 | 1.635 | 0.635 | 4.50E-01 | 2.890 | 0.840 | 2.70E-02 | 3.523 | 1.118 | 1.40E-02 |
| *Mmp13* | 0.940 | 0.210 | 6.20E-01 | 1.462 | 0.381 | 4.50E-01 | 1.637 | 0.349 | 2.10E-01 | 3.230 | 0.763 | 2.50E-03 |
| *Mmp2* | 1.124 | 0.124 | 4.80E-01 | 2.168 | 0.263 | 1.30E-05 | 1.967 | 0.202 | 1.60E-05 | 2.026 | 0.212 | 1.60E-05 |
| *Mmp8* | 2.986 | 0.592 | 7.90E-04 | 2.943 | 0.669 | 2.40E-03 | 3.071 | 0.581 | 3.30E-04 | 2.956 | 0.558 | 7.60E-04 |
| *Mmp9* | 1.071 | 0.304 | 9.20E-01 | 0.651 | 0.196 | 1.80E-01 | 1.364 | 0.379 | 5.80E-01 | 1.158 | 0.296 | 8.90E-01 |
| *Timp1* | 5.755 | 2.208 | 2.30E-03 | 8.203 | 3.191 | 3.40E-04 | 8.593 | 3.023 | 6.60E-05 | 14.397 | 4.521 | 1.00E-06 |
| *Timp4* | 0.154 | 0.069 | 7.90E-04 | 0.278 | 0.153 | 2.40E-02 | 0.329 | 0.119 | 1.80E-02 | 0.271 | 0.107 | 9.80E-03 |
| **Lung** | | | | | | | | | | | | |
| | Mit-7 LD | | | Mit-7 HD | | | NM-401 LD | | | NM-401 HD | | |
| | Mean | SD | p-value | Mean | SD | p-value | Mean | SD | p-value | Mean | SD | p-value |
| *Bcl-2* | 0.627 | 0.045 | 1.10E-05 | 0.870 | 0.066 | 1.80E-01 | 0.712 | 0.050 | 6.30E-04 | 0.835 | 0.056 | 6.50E-02 |
| *Ccl11* | 2.894 | 0.390 | 3.80E-05 | 2.419 | 0.298 | 5.90E-04 | 2.302 | 0.235 | 3.40E-04 | 2.344 | 0.263 | 6.90E-04 |
| *Ccl12* | 2.122 | 0.338 | 1.20E-03 | 4.122 | 0.694 | 4.30E-08 | 2.447 | 0.367 | 6.70E-05 | 3.191 | 0.474 | 7.70E-07 |
| *Ccl3* | 1.670 | 0.214 | 4.00E-02 | 1.782 | 0.262 | 2.70E-02 | 1.160 | 0.131 | 1.00E+00 | 1.786 | 0.203 | 1.00E-02 |
| *Cdh1* | 0.997 | 0.066 | 3.00E-01 | 0.961 | 0.066 | 1.60E-01 | 0.859 | 0.051 | 4.80E-03 | 0.917 | 0.056 | 4.70E-02 |
| *Cdkn2a* | 3.838 | 0.660 | 3.00E-06 | 4.064 | 0.698 | 1.10E-06 | 4.007 | 0.574 | 1.10E-07 | 4.632 | 0.767 | 8.50E-08 |
| *Col1a2* | 3.522 | 0.426 | 3.70E-11 | 5.779 | 0.744 | 1.20E-15 | 4.482 | 0.514 | 6.70E-15 | 6.012 | 0.658 | 6.00E-18 |
| *Cxcl2* | 2.065 | 0.284 | 3.50E-04 | 2.104 | 0.280 | 2.20E-04 | 1.489 | 0.174 | 2.80E-02 | 2.273 | 0.285 | 3.50E-05 |
| *Il10* | 4.508 | 0.936 | 8.30E-05 | 3.913 | 0.737 | 2.00E-04 | 4.294 | 0.803 | 6.50E-05 | 3.521 | 0.669 | 5.90E-04 |
| *Il1a* | 0.448 | 0.052 | 4.60E-06 | 0.515 | 0.063 | 1.90E-04 | 0.588 | 0.065 | 1.00E-03 | 0.413 | 0.045 | 3.40E-07 |
| *Il1b* | 1.061 | 0.178 | 9.70E-01 | 2.356 | 0.427 | 1.50E-03 | 1.327 | 0.210 | 3.20E-01 | 1.653 | 0.262 | 5.40E-02 |
| *Mmp13* | 0.987 | 0.148 | 8.80E-01 | 1.847 | 0.259 | 1.30E-03 | 0.596 | 0.084 | 1.40E-02 | 1.231 | 0.154 | 1.70E-01 |
| *Mmp2* | 2.110 | 0.155 | 1.70E-06 | 3.115 | 0.246 | 2.30E-12 | 2.423 | 0.174 | 3.40E-09 | 2.672 | 0.200 | 4.00E-10 |
| *Mmp8* | 1.807 | 0.347 | 3.80E-02 | 2.135 | 0.449 | 1.20E-02 | 1.571 | 0.267 | 8.50E-02 | 2.011 | 0.355 | 1.20E-02 |
| *Mmp9* | 0.952 | 0.140 | 7.80E-01 | 1.623 | 0.251 | 2.80E-02 | 1.140 | 0.164 | 5.40E-01 | 1.462 | 0.202 | 6.40E-02 |
| *Timp1* | 3.023 | 0.500 | 7.10E-06 | 4.455 | 0.721 | 5.80E-09 | 3.800 | 0.554 | 1.10E-08 | 3.666 | 0.528 | 3.80E-08 |
| *Timp4* | 1.101 | 0.135 | 6.60E-01 | 1.253 | 0.162 | 2.50E-01 | 1.237 | 0.143 | 2.30E-01 | 1.069 | 0.123 | 7.80E-01 |

**Table S2**. Fold change values of exposed HBEC-3KT cells. Complete list of gene expression presented as mean of fold change of exposed cells after 4 (W4), 8 (W8) and 13 (W13) weeks of exposure to NM-401 nanomaterials to the controls (*n* = 2). High dose (HD), low dose (LD).

| | W4_LD | | W4_HD | | W8_LD | | W8_HD | | W13_LD | | W13_HD | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| *CASP3* | 1.656 | 0.025 | 1.796 | 0.256 | 1.640 | 0.084 | 1.816 | 0.069 | 1.223 | 0.025 | 1.256 | 0.074 |
| *CYCS* | 0.564 | 0.020 | 0.670 | 0.179 | 0.367 | 0.092 | 0.426 | 0.082 | 0.352 | 0.055 | 0.322 | 0.013 |
| *DIABLO* | 1.213 | 0.030 | 1.261 | 0.022 | 1.315 | 0.024 | 1.549 | 0.066 | 0.903 | 0.050 | 0.969 | 0.010 |
| *FADD* | 1.202 | 0.420 | 1.252 | 0.246 | 1.196 | 0.233 | 1.198 | 0.097 | 0.872 | 0.428 | 0.780 | 0.279 |
| *FAS* | 1.076 | 0.197 | 1.125 | 0.191 | 1.735 | 0.376 | 2.157 | 0.726 | 1.184 | 0.430 | 1.273 | 0.248 |
| *DRAM1* | 9.516 | 1.356 | 12.001 | 1.109 | 8.968 | 2.483 | 12.486 | 2.092 | 5.183 | 1.011 | 5.127 | 0.254 |
| *BCL-2* | 1.229 | 0.246 | 1.341 | 0.551 | 1.114 | 0.130 | 1.509 | 0.243 | 0.755 | 0.053 | 0.677 | 0.100 |
| *AKT1* | 1.864 | 0.065 | 1.992 | 0.418 | 1.415 | 0.104 | 1.427 | 0.172 | 1.280 | 0.041 | 1.149 | 0.123 |
| *NPC1* | 2.130 | 0.031 | 2.549 | 0.279 | 1.853 | 0.041 | 1.956 | 0.182 | 1.427 | 0.195 | 1.310 | 0.058 |
| *SKI* | 1.352 | 0.096 | 1.449 | 0.158 | 1.140 | 0.145 | 1.222 | 0.233 | 1.123 | 0.112 | 1.052 | 0.153 |
| *BAX* | 2.797 | 0.282 | 2.980 | 0.316 | 3.201 | 0.167 | 3.466 | 0.702 | 3.279 | 0.089 | 3.264 | 0.248 |
| *ING1* | 1.878 | 0.027 | 2.200 | 0.145 | 1.896 | 0.098 | 2.074 | 0.267 | 1.448 | 0.095 | 1.338 | 0.162 |
| *TP53* | 1.945 | 0.155 | 2.090 | 0.646 | 1.391 | 0.109 | 1.360 | 0.092 | 1.270 | 0.034 | 1.092 | 0.039 |
| *TNF* | 10.986 | 1.400 | 18.453 | 1.190 | 6.599 | 2.240 | 8.880 | 0.019 | 6.763 | 1.871 | 9.128 | 4.308 |
| *MYC* | 0.988 | 0.157 | 1.093 | 0.113 | 1.004 | 0.018 | 1.000 | 0.004 | 0.575 | 0.108 | 0.620 | 0.046 |
| *HDAC4* | 1.427 | 0.039 | 1.342 | 0.049 | 1.597 | 0.395 | 1.102 | 0.101 | 0.734 | 0.040 | 0.654 | 0.010 |
| *NEK2* | 0.553 | 0.070 | 0.793 | 0.335 | 0.791 | 0.408 | 0.757 | 0.006 | 0.340 | 0.104 | 0.394 | 0.280 |
| *CDKN2A* | 3.066 | 0.406 | 3.390 | 0.127 | 8.077 | 0.425 | 9.491 | 0.810 | 8.423 | 0.521 | 9.372 | 2.004 |
| *CDKN1B* | 1.850 | 0.069 | 2.015 | 0.246 | 1.559 | 0.096 | 1.681 | 0.020 | 1.408 | 0.064 | 1.420 | 0.115 |
| *CDKN1A* | 1.959 | 0.249 | 2.064 | 0.021 | 2.993 | 0.248 | 3.517 | 0.142 | 2.473 | 0.222 | 3.019 | 0.266 |
| *ATM* | 1.193 | 0.086 | 1.097 | 0.022 | 0.953 | 0.125 | 1.091 | 0.059 | 1.072 | 0.045 | 0.934 | 0.071 |
| *ATR* | 1.564 | 0.049 | 1.498 | 0.035 | 1.195 | 0.025 | 1.236 | 0.054 | 0.779 | 0.078 | 0.791 | 0.043 |
| *CHEK1* | 1.706 | 0.051 | 2.129 | 0.740 | 1.462 | 0.016 | 1.693 | 0.312 | 0.912 | 0.035 | 0.790 | 0.018 |
| *CHEK2* | 1.430 | 0.104 | 1.558 | 0.402 | 1.617 | 0.119 | 1.666 | 0.024 | 1.033 | 0.087 | 0.920 | 0.064 |
| *RAD1* | 1.878 | 0.169 | 2.131 | 0.207 | 1.984 | 0.149 | 2.211 | 0.047 | 0.979 | 0.005 | 1.112 | 0.025 |
| *RAD17* | 0.814 | 0.025 | 0.765 | 0.057 | 0.875 | 0.056 | 0.958 | 0.105 | 0.577 | 0.108 | 0.669 | 0.057 |
| *DNMT1* | 2.254 | 0.309 | 2.647 | 1.017 | 2.012 | 0.410 | 2.150 | 0.514 | 1.333 | 0.013 | 1.082 | 0.130 |
| *DNMT3A* | 1.605 | 0.045 | 1.619 | 0.065 | 1.373 | 0.125 | 1.387 | 0.045 | 1.470 | 0.203 | 1.370 | 0.284 |
| *DNMT3B* | 0.534 | 0.095 | 0.706 | 0.030 | 0.747 | 0.175 | 0.902 | 0.302 | 0.515 | 0.067 | 0.501 | 0.015 |
| *ALKBH1* | 1.763 | 0.076 | 2.129 | 0.124 | 1.759 | 0.028 | 2.004 | 0.383 | 1.017 | 0.167 | 1.023 | 0.213 |
| *ALKBH5* | 1.472 | 0.083 | 1.543 | 0.278 | 1.095 | 0.014 | 1.176 | 0.278 | 0.894 | 0.003 | 0.814 | 0.016 |
| *ERCC2* | 2.409 | 0.016 | 2.883 | 0.749 | 1.489 | 0.272 | 1.426 | 0.290 | 1.593 | 0.039 | 1.280 | 0.018 |
| *NEIL3* | 0.989 | 0.140 | 1.579 | 0.958 | 0.732 | 0.138 | 0.701 | 0.246 | 0.401 | 0.056 | 0.317 | 0.109 |
| *XPA* | 0.566 | 0.031 | 0.683 | 0.067 | 0.632 | 0.209 | 0.706 | 0.282 | 0.370 | 0.092 | 0.419 | 0.086 |
| *OGG1* | 0.909 | 0.099 | 0.936 | 0.169 | 0.953 | 0.083 | 0.973 | 0.032 | 0.658 | 0.101 | 0.690 | 0.119 |
| *STAT6* | 0.848 | 0.081 | 0.837 | 0.042 | 0.845 | 0.034 | 0.904 | 0.003 | 0.740 | 0.073 | 0.834 | 0.133 |
| *NFE2L2* | 0.642 | 0.150 | 0.649 | 0.118 | 0.945 | 0.378 | 1.099 | 0.426 | 0.563 | 0.234 | 0.658 | 0.226 |
| *CAT* | 0.618 | 0.029 | 0.746 | 0.121 | 0.986 | 0.040 | 1.044 | 0.045 | 0.834 | 0.031 | 0.806 | 0.012 |
| *NOX1* | 0.995 | 0.120 | 0.956 | 0.238 | 0.865 | 0.031 | 0.995 | 0.073 | 0.779 | 0.058 | 0.848 | 0.013 |
| *SOD2* | 11.641 | 0.548 | 12.359 | 0.710 | 12.690 | 0.160 | 14.439 | 0.960 | 6.459 | 1.533 | 7.002 | 1.085 |
| *IL8* | 2430.368 | 118.413 | 4027.346 | 823.579 | 1638.320 | 579.911 | 2255.515 | 374.169 | 172.115 | 23.580 | 183.506 | 51.592 |
| *TERC* | 1.189 | 0.548 | 0.952 | 0.179 | 0.291 | 0.020 | 0.270 | 0.044 | 0.296 | 0.030 | 0.316 | 0.058 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *TERT* | 5.083 | 2.438 | 3.978 | 0.993 | 5.495 | 0.946 | 4.749 | 0.723 | 7.858 | 1.022 | 4.770 | 0.358 |
| *CDH1* | 0.777 | 0.116 | 2.053 | 0.205 | 1.030 | 0.084 | 1.038 | 0.145 | 0.977 | 0.219 | 0.726 | 0.219 |
| *CXCL2* | 30.350 | 2.254 | 45.743 | 21.130 | 22.427 | 5.791 | 30.354 | 1.655 | 3.002 | 0.214 | 5.999 | 3.023 |
| *MMP2* | 1.783 | 0.000 | 10.823 | 3.423 | 2.887 | 0.679 | 4.848 | 4.381 | 6.803 | 3.770 | 10.820 | 6.620 |
| *CCL3* | 3.954 | 1.037 | 4.822 | 1.689 | 2.048 | 1.001 | 10.529 | 9.193 | 1.236 | 0.258 | 9.646 | 10.942 |

## Supplementary file 1: Figures

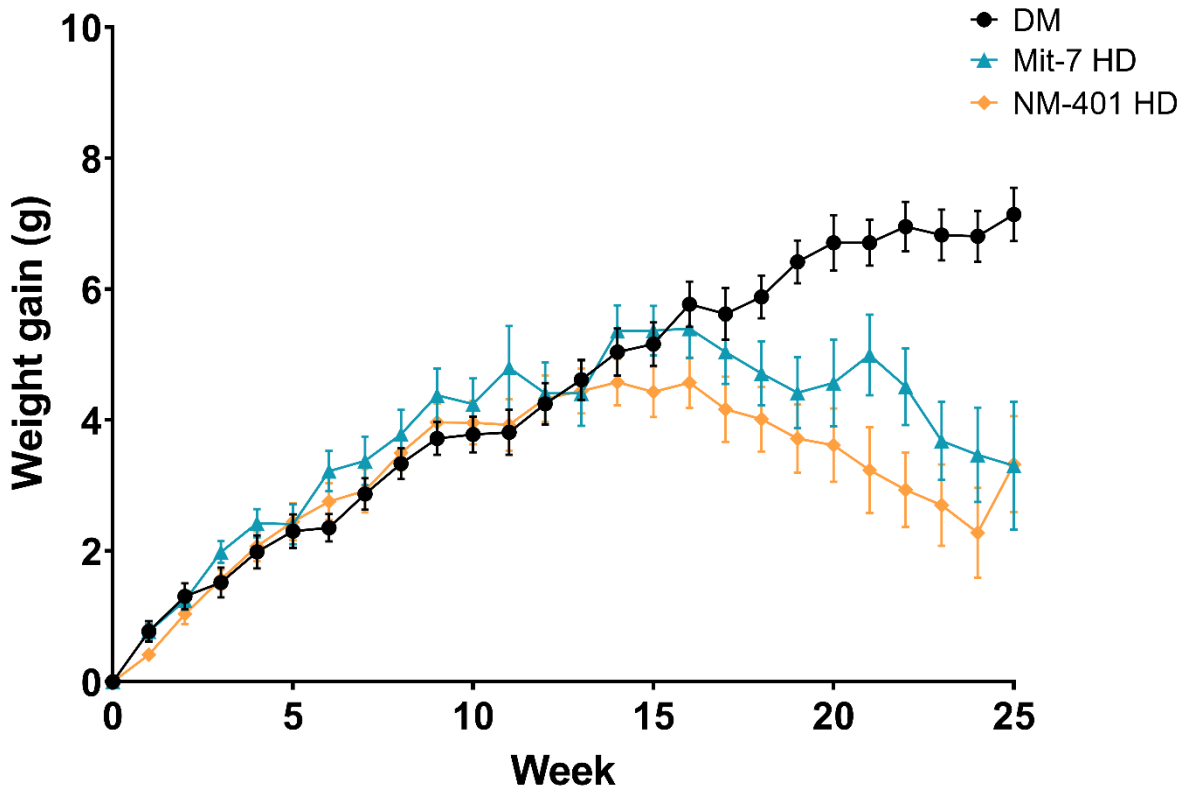


**Figure S1**. Body weight gain of animals after intrapleural injection of MWCNT. Body weight normalized to each animal at time 0**.** High dose (HD), Mitsui-7 (Mit-7). Values are mean ± SEM (*n* = 19).

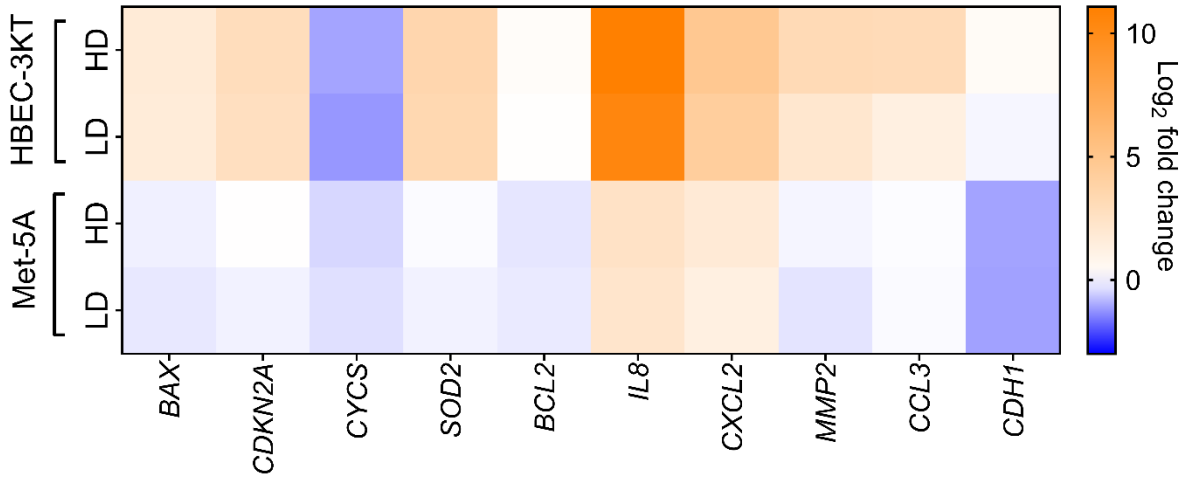


**Figure S2.** Heatmap illustrating selected genes involved in fibrosis and inflammation**.** Selected genes were run following NM-401 exposure in HBEC-3KT and Met-5A cells. Data presented as mean values of merged data from all weeks for low dose (LD): 0.96 µg/cm$^2$ and high dose (HD): 1.92 µg/cm$^2$ (*n* = 6).

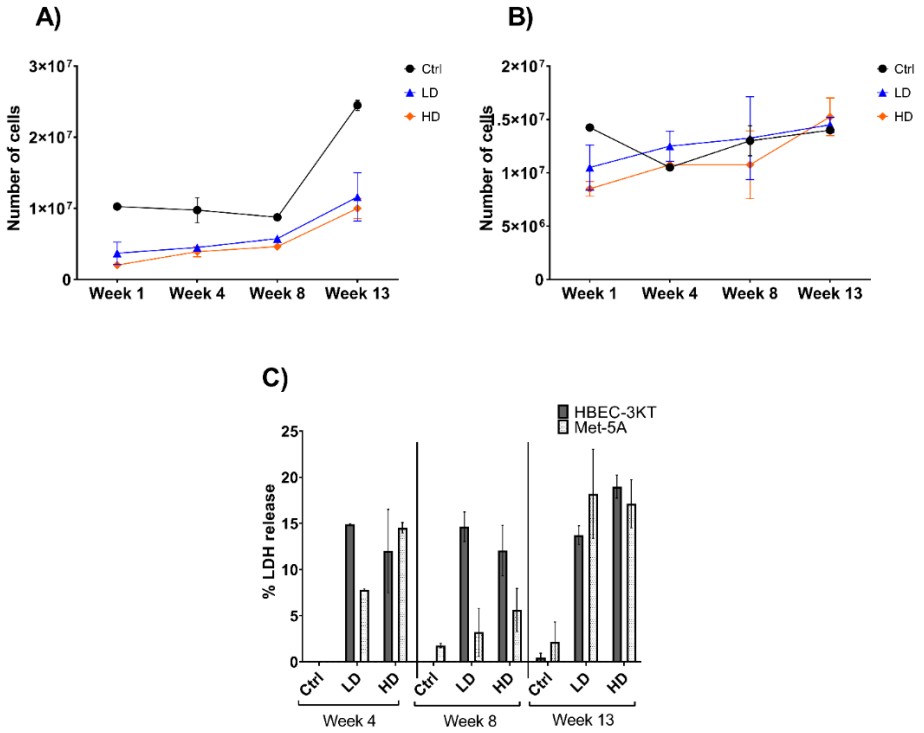


**Figure S3.** Cell proliferation and LDH release of exposed cells to NM-401. Cell count for HBEC-3KT cells (A) and Met-5A cells (B) for control and exposed cells assessed by trypan blue assay presented after 1, 4, 8 and 13 weeks of exposure. c) LDH assay was assessed after 4, 8 and 13 weeks of exposure. Control (Ctrl), low dose (LD): 0.96 µg/cm$^2$ and high dose (HD): 1.92 µg/cm$^2$. Data indicates mean±SEM (*n* = 2).

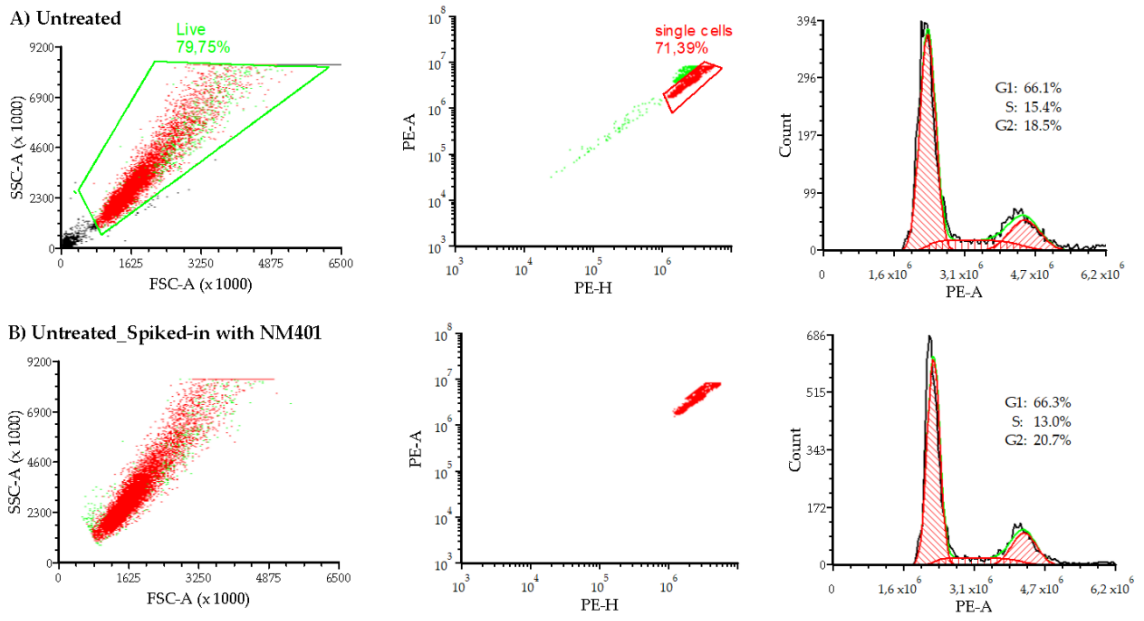


**Figure S4. Assessment NM-401 nanoparticle interference with cell cycle analysis using flow cytometry.** Representative scatter plots and histogram of untreated cells (A) and untreated cells spiked with high-dose of NM-401 (B).

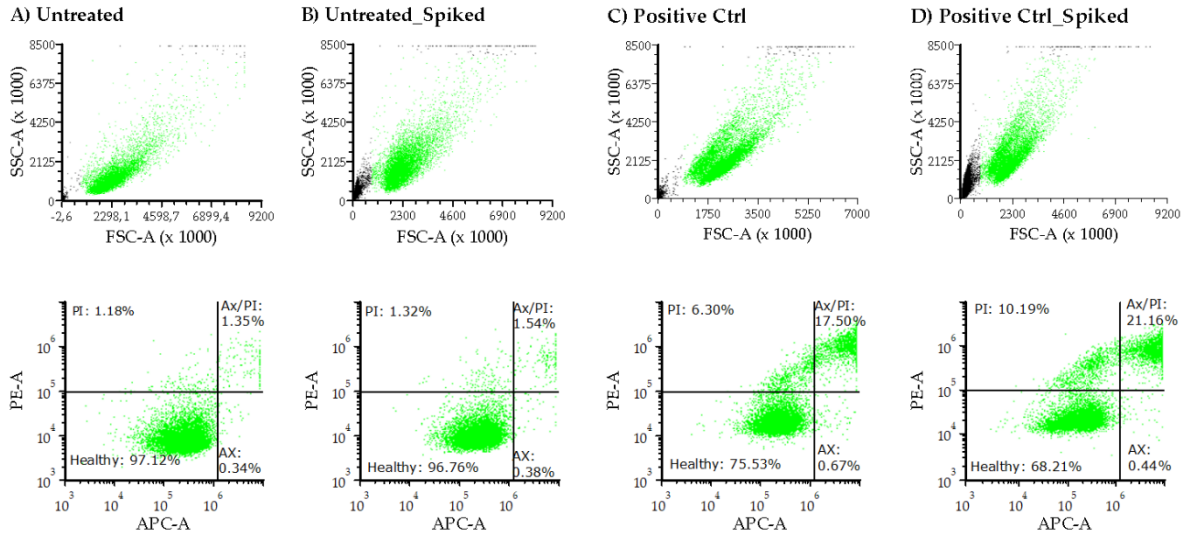


**Figure S5. Assessment NM-401 nanoparticle interference with the analysis of apoptosis by flow cytometry.** Representative scatter plots of untreated cells (A), untreated cells spiked with high-dose of NM-401 (B), positive control (Ctrl) i.e., cells heat shocked at 56˚C for 5 min (C) and positive control spiked with high-dose (HD = 1.92 µg/cm$^2$) of NM-401 (D). PI: propidium iodide and AX: Annexin-V.