Supporting Information

A micro/nano composite for combination treatment of melanoma lung metastasis

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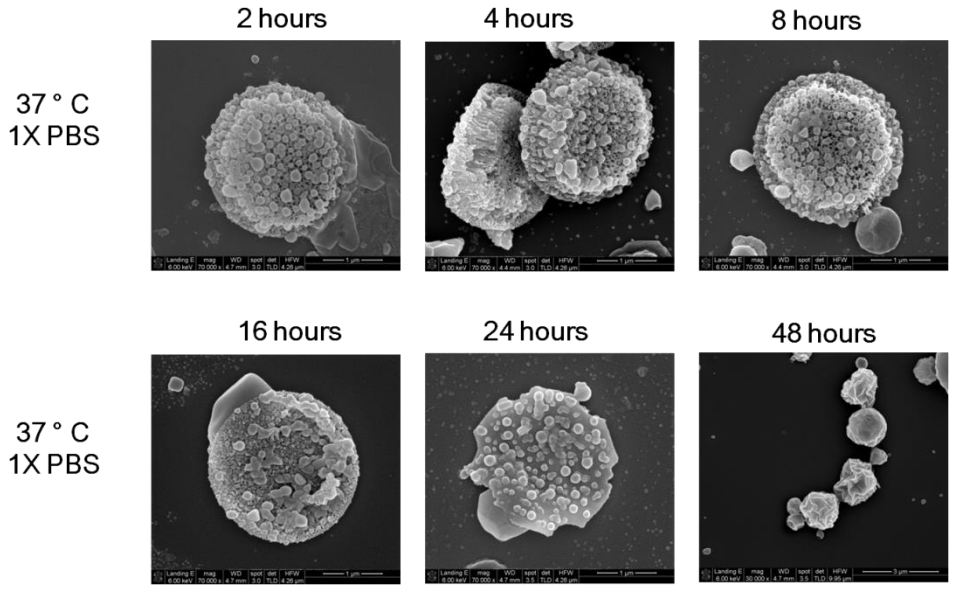

Figure S1. Biodegradation of the micro/nano composite (MNC) in phosphate buffered saline (PBS) at 37 °C.

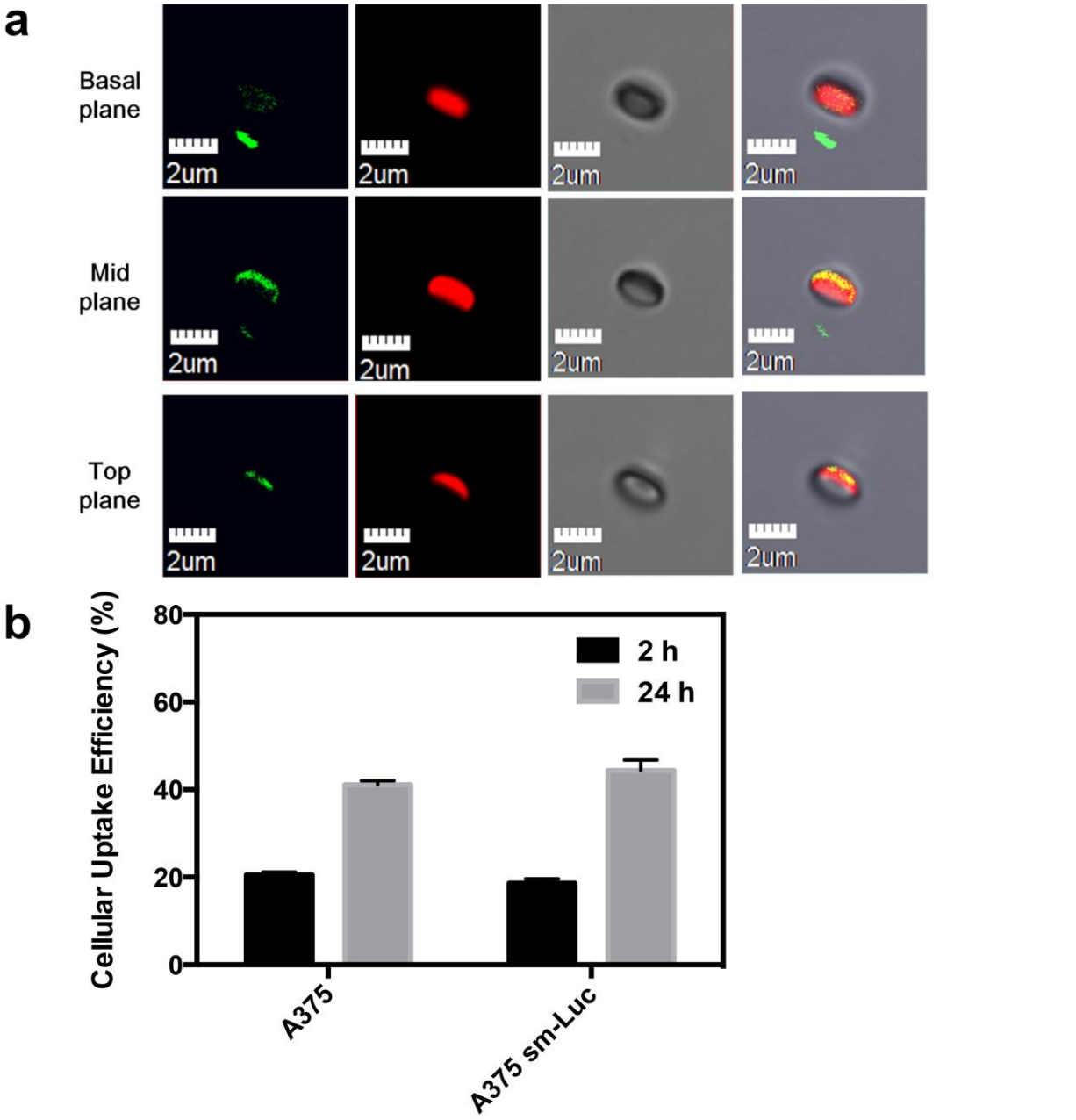

Figure S2. a) Confocal laser scanning microscopy images of a MNC positioned on its side. Columns from left to right show: coumarin 6 (green)-loaded poly(lactide-co-glycolide) (PLGA)-polyethylene glycol (PEG) nanoparticles in the FITC channel, AF555-labeled small interfering RNA (siRNA, red)-loaded liposomes in the TRITC channel, the MNC in the bright field channel, and the MNC in the FITC, TRITC, and bright field channels. b) Normalized cellular uptake efficiency of the MNC in A375 cells and A375 sm-Luc cells after 2 h and 24 h.

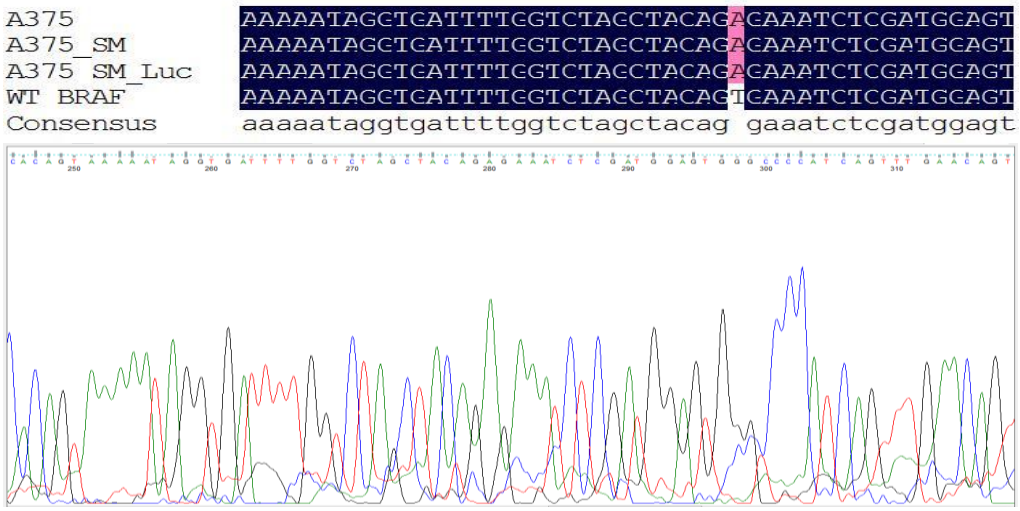

Figure S3. DNA sequence analysis of A375 cells (A375), highly metastatic A375 cells (A375SM) and A375SM cells with luciferase expression (A375SM-Luc). The data reveals the presence of the BRAF V600E mutation in the cell lines. WT, wild type.

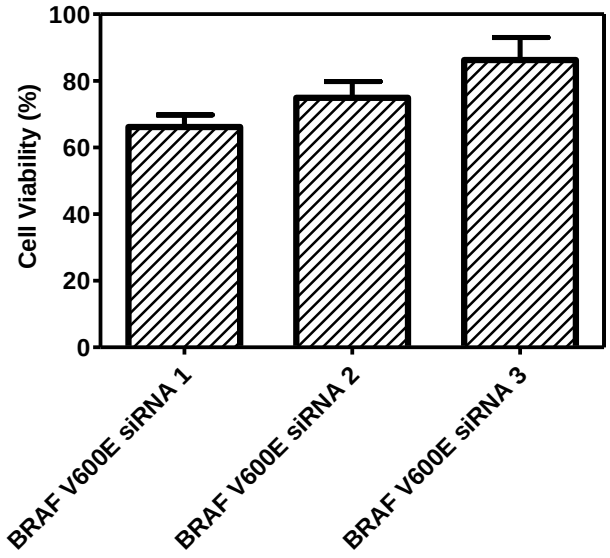

Figure S4. Cell viability of A375 melanoma cells treated with various BRAF V600E siRNAs (100 nM) for 72 h. The commercial transfection reagent INTERFERin was used for transfection. Results are presented as the mean ± SD of six replicates.

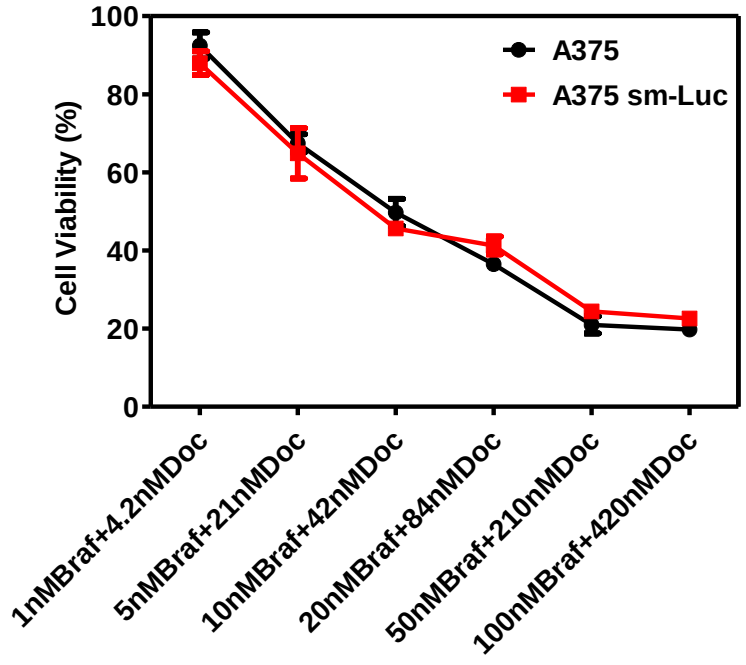

Figure S5. Cell viability of A375 cells and A375SM-Luc cells after treatment with MNCs containing various concentrations of BRAF siRNA (Braf) and docetaxel (Doc) for 72 h. Results are presented as mean ± SD of six replicates.

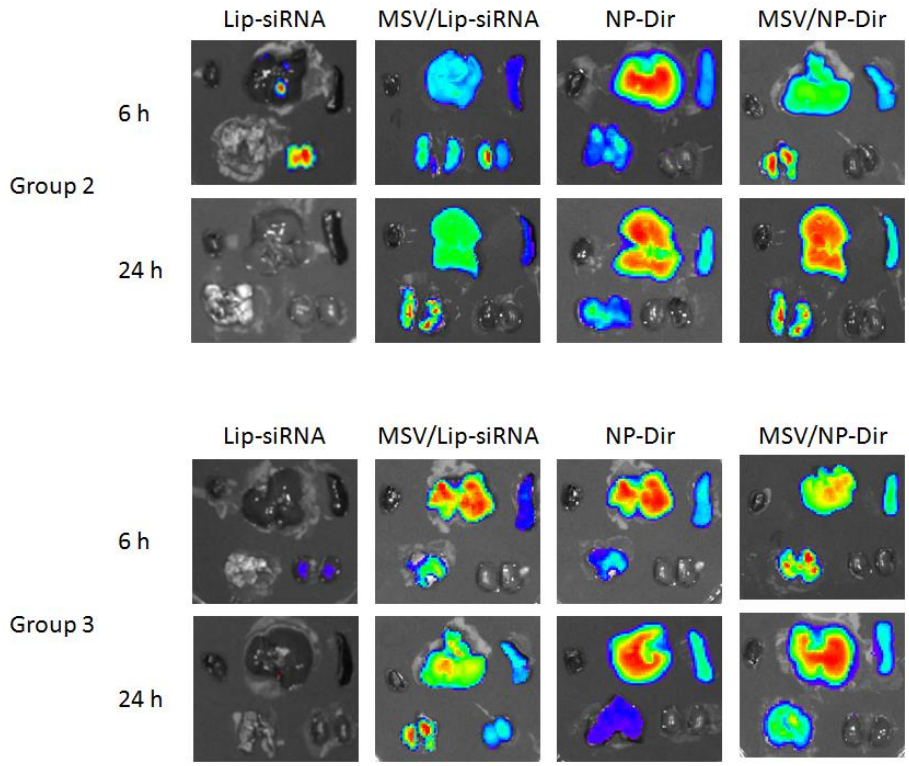

Figure S6. Biodistribution of particles in nude mice bearing A375SM-Luc melanoma lung metastasis. Top lane (left to right): heart, liver, and spleen. Bottom lane (left to right): lungs and kidneys.

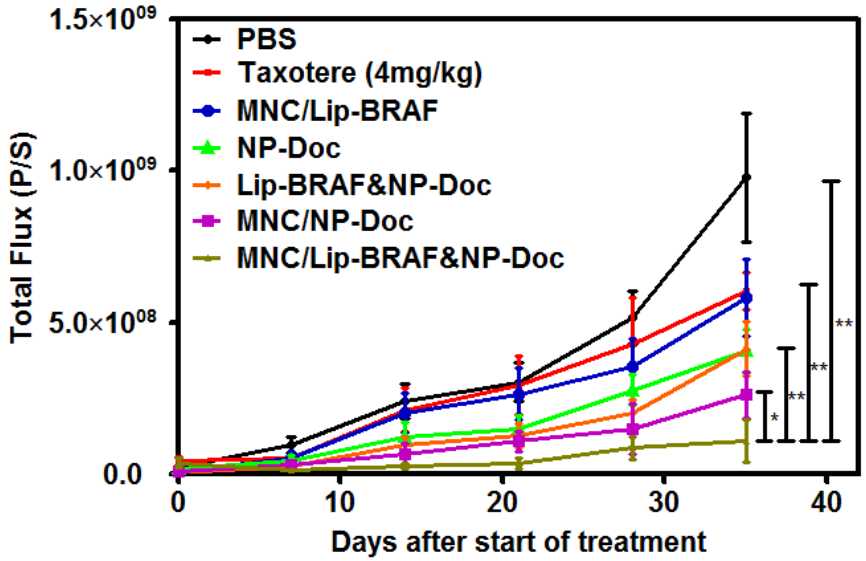

Figure S7. Anticancer activity of the MNC in nude mice bearing A375SM-Luc melanoma lung metastases. Treatment was administered intravenously once a week for four weeks (docetaxel: 4 mg/kg, BRAF siRNA: 1 mg/kg). Therapeutic efficacy was assessed with bioluminescent imaging. Results are presented as mean ± SD (n=5). *, $p<0.5$; **, $p<0.01$ (T-test). Lip, liposome.

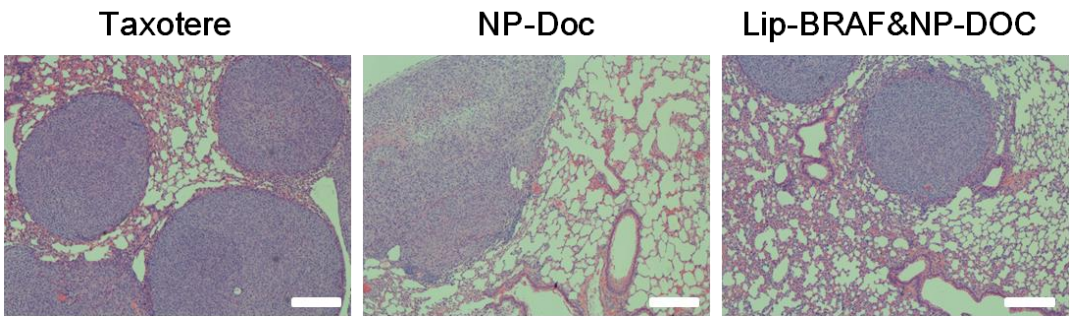

Figure S8. Histological images of the lungs 35 days after treatment initiation. Treatment was administered intravenously once a week for four weeks (docetaxel: 4 mg/kg, BRAF siRNA: 1 mg/kg). Tissues were stained with hemaetoxylin and eosin. Scare bar, 200 μm.

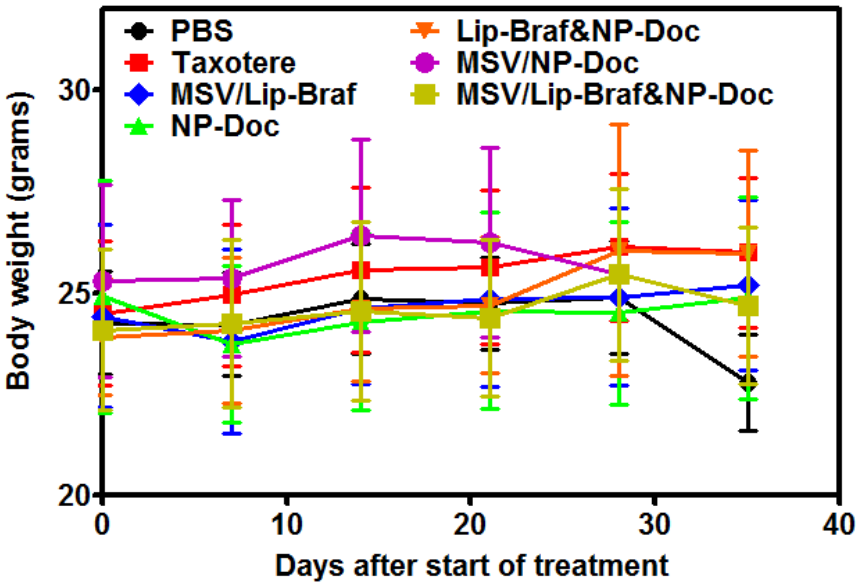

Figure S9. Mouse body weights. Day 0 represents treatment initiation. Treatment was administered intravenously once a week for four weeks (docetaxel: 4 mg/kg, BRAF siRNA: 1 mg/kg). Results are presented as mean ± SD (n=8).

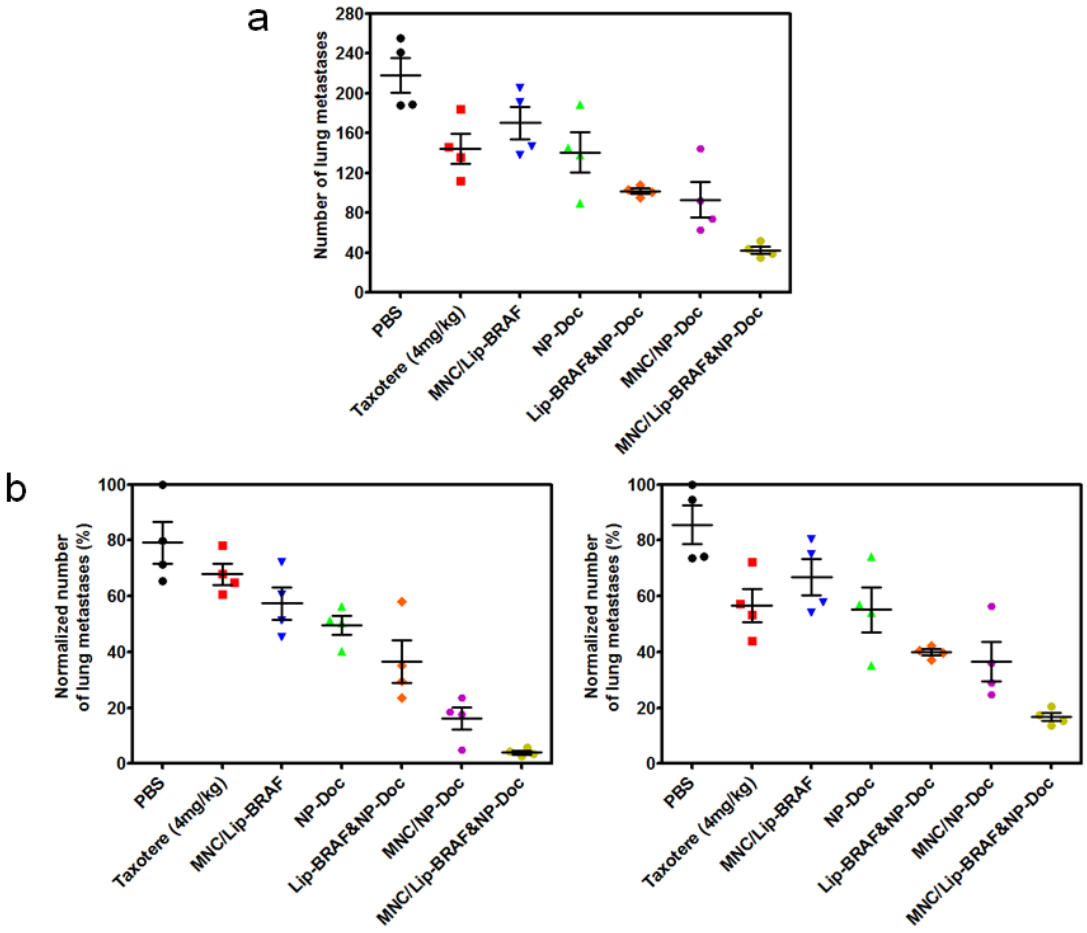

Figure S10. a) Number of pulmonary surface metastases 22 days after treatment initiation. Cancer cells were injected into six-week-old mice (10^6 cells/mouse). Data is presented as the mean ± SD (n=4). b) Normalized number of lung metastasis. Cancer cells were injected into ten-week old mice (left graph) and six-week-old mice (right graph) (10^6 cells/mouse). In all cases, treatment was initiated two weeks after cancer cell injection.

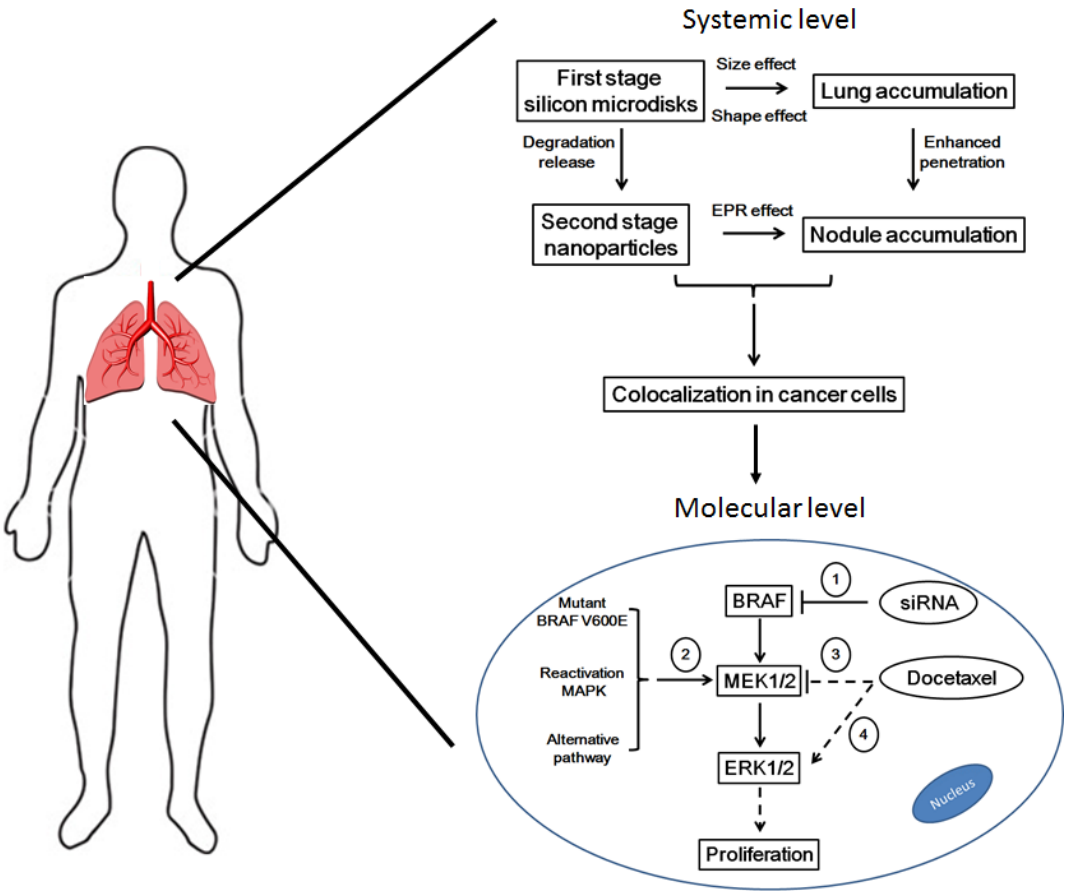

Figure S11. Proposed mechanisms for therapeutic synergy. In the *in vitro* diagram, numbers represent: 1) inhibition of BRAF by siRNA, 2) possible mechanisms of resistance to BRAF inhibition, 3) inhibition of mitogen-activated protein kinase kinase 1/2 (MEK1/2) by docetaxel, and 4) mechanism of resistance to docetaxel. EPR, enhanced permeability and retention; ERK, extracellular-signal-regulated kinase; MAPK, mitogen activated kinase.

Table S1. BRAF V600E siRNA sequences

Name	siRNA Sequence
BRAF V600E siRNA 1 (selected)	GCUACAGAGAAAUCUCGAU [1]
BRAF V600E siRNA 2	AACAGUCUACAAGGGAAAGUG [2]
BRAF V600E siRNA 3	GCTACAGAGAAATCTCGAT [3]

Experimental Section

Confocal microscopy: The micro/nano composite (MNC) containing coumarin 6-loaded poly(lactide-co-glycolide) (PLGA)-polyethylene glycol (PEG) nanoparticles and AF555-labeled small interfering RNA (siRNA)-loaded liposomes was suspended in water, dropped on a 4-well cover glass chamber, and sealed for confocal microscopy imaging.

Cellular uptake: A375 cells and A375 sm-Luc cells were seeded in black 96-well plates (Costar, IL, USA) at a density of 5×10^3 cells/well (0.1 ml) and cultured to 80% confluence. The cells were then exposed to coumarin 6-loaded MNCs at a concentration of 0.1 billion particles/mL. After 2 h or 24 h, the particle suspension was removed and the wells were washed three times with 0.1 mL PBS. Subsequently, the cells were lysed with 50 µL of 0.5% Triton X-100 in 0.2 N NaOH solution and a microplate reader (Genios, Tecan, Switzerland) was used to measure fluorescence intensity (ex 430 nm / em 485 nm). Fluorescence intensity values were normalized to those of wells containing the original MNC suspension.

In vitro anticancer activity: A375 cells and A375SM luciferase expressing (A375SM-Luc) cells were seeded in 96-well plates (Costar, IL, USA) at a density of 3×10^3 cells/well (0.1 ml). After 12 h, cells were exposed to treatment groups and cell viability was measured after 72 hours using the Cell Counting Kit-8 (CCK-8).

Animal weight measurements: Treatment of mice bearing A375SM melanoma lung metastases was initiated two weeks after cancer cell injection. Mice received weekly intravenous injections of particles (BRAF siRNA: 1 mg/kg; docetaxel: 4 mg/kg) for four weeks. Animal weights were recorded once a week.

DNA sequence analysis: The genomic DNA of A357 cells, A375SM cells, and A375 SM-Luc cells was isolated using the QIAamp DNA Mini Kit (catalog number: 51304, Qiagen) according to the manufacturer's instructions. Polymerase chain reaction (PCR) was performed to confirm the presence of the BRAF V600E mutation. The following primers were used for the reaction: primers: 5'-GCATCTCACCTCATCCTAACAC-3' and 5'-CTAGTAACTCAGCAGCATCTCA-3'. Amplification was carried out as follows: 95 °C for 10 min, 30 cycles of 94 °C for 15 s, 58°C for 30 s, 72 °C for 30 s, and 72 °C for 5 min. The PCR products were purified with MinElute PCR purification kit (catalog number: 28004, Qiagen) and sequenced using the following primer: 5'-GCATCTCACCTCATCCTAACAC-3'.

References

[1] S. R. Hingorani, M. A. Jacobetz, G. P. Robertson, M. Herlyn, D. A. Tuveson, *Cancer Res.* **2003**, *63*, 5198.

[2] C. Wellbrock, R. Marais, *J. Cell Biol*. **2005**, *170*, 703.

[3] H. Sumimoto, F. Imabayashi, T. Iwata, Y. Kawakami, *J. Exp. Med*. **2006**, *203*, 1651.