Supplementary Information

Inhibition of autophagy enhances the anticancer activity of silver nanoparticles

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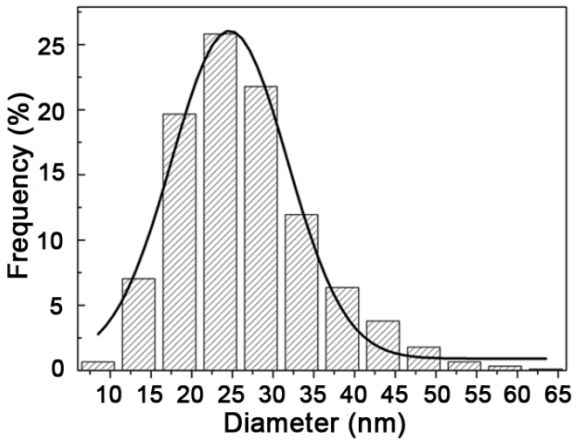

Figure S1. Size distribution of Ag NPs. Size distribution of Ag NPs was determined by counting all the nanoparticles in a TEM photograph.

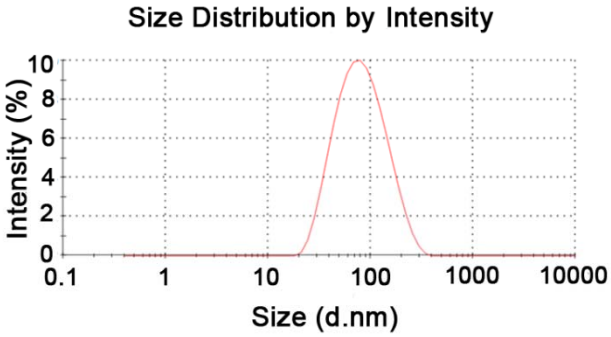

Figure S2. Hydrodynamic diameter of Ag NPs. Size distribution of Ag NPs was measured by dynamic light scattering (DLS).

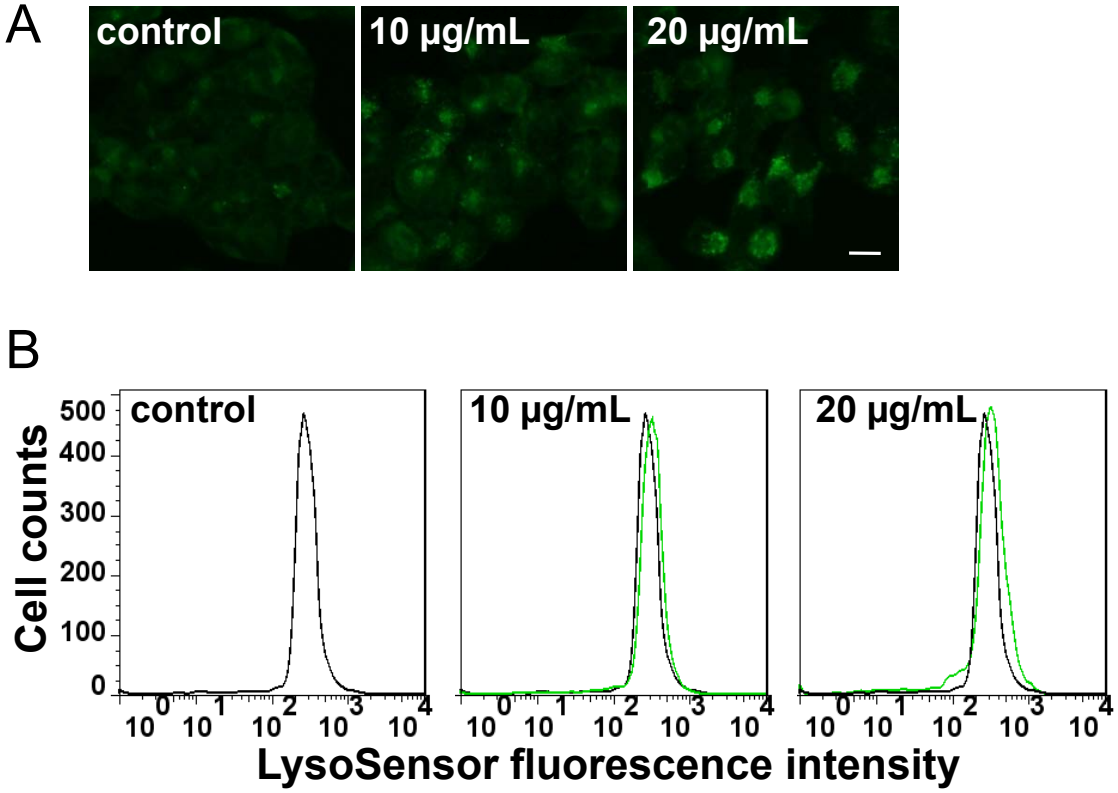
A
control
10 μg/mL
20 μg/mL
B
control
10 μg/mL
20 μg/mL
Cell counts
500
400
300
200
100
0
10 10 10 10 10
LysoSensor fluorescence intensity
C
control
10 μg/mL
20 μg/mL
D
Fluorescence Intensity per Cell (%)
200
160
120
80
40
0
***
***
0
10
20
Ag NPs (μg/mL)

Figure S3. Ag NPs did not disrupt lysosomal function. (A) Representative fluorescence of HeLa cells treated with PBS (control), 10 and 20 μg/mL Ag NPs for 24 h, then exposed to 1 μmol/L LysoSensor Green DND-189 for 30 min. The effect of Ag NPs treatment on lysosome acidity was shown as fluorescence intensity. (Scale bar, 20 μm.) (B) FACS analysis of lysosome pH in HeLa cells stained with LysoSensor Green DND-189 in samples cultured as in (A). (Black line: control cells; green line: Ag NPs treated cells.) (C) Representative fluorescence pictures of HeLa cells treated with Magic Red CTSB substrate for 30 min after PBS (control), 10 and 20 μg/mL Ag NPs treatment for 24 h. (Scale bar, 20 μm.) (D) Fluorescence intensity of the fluorescent molecules released by CTSB hydrolyzation of nonfluorescent substrate was quantified by Image J software. The samples cultured as in (C), at least 50 cells were analyzed for each treatment. Mean±SEM, n = 3. ***$P < 0.001$ comparing to the control group.

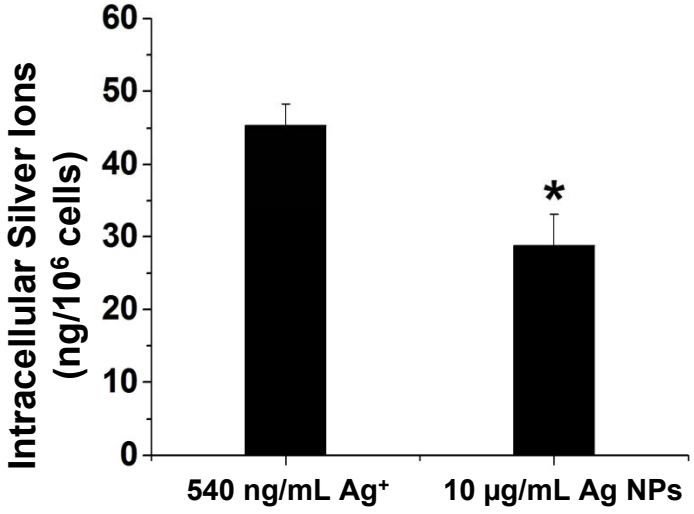

Figure S4. The cytosolic silver concentrations of treated cells. HeLa cells were treated with 10 μg/mL Ag NPs or 540 ng/mL Ag^+ for 24 h. And the silver concentration in cytoplasm was determined by ICP-MS. Mean±SEM, n = 3, *$P < 0.05$, **$P < 0.01$.

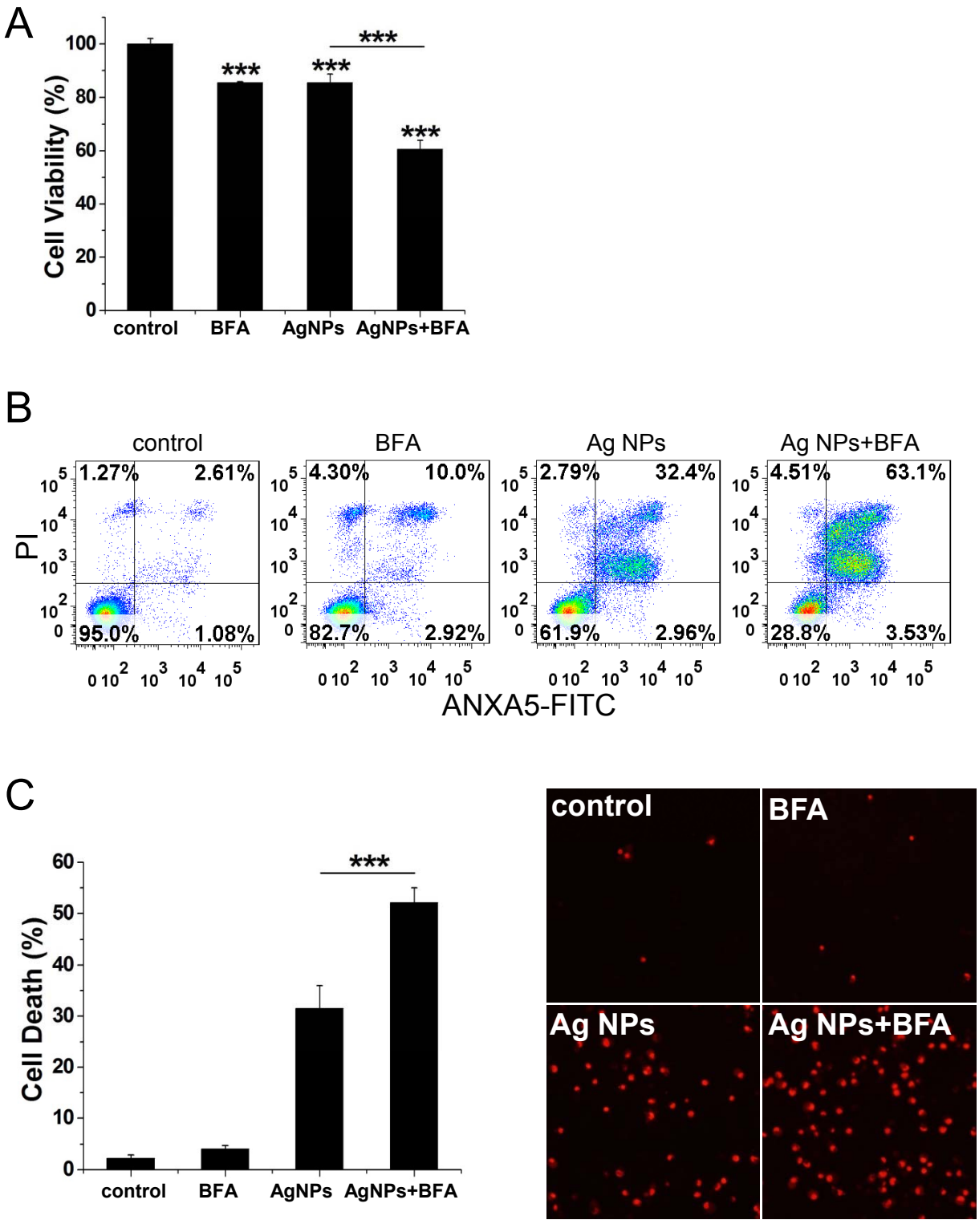
A
Cell Viability (%)
100
80
60
40
20
0
***
***
***
***
control
BFA
AgNPs
AgNPs+BFA
B
control
BFA
Ag NPs
Ag NPs+BFA
PI
1.27%
2.61%
95.0%
1.08%
4.30%
10.0%
82.7%
2.92%
2.79%
32.4%
61.9%
2.96%
4.51%
63.1%
28.8%
3.53%
ANXA5-FITC
C
Cell Death (%)
60
50
40
30
20
10
0
***
control
BFA
AgNPs
AgNPs+BFA
control
BFA
Ag NPs
Ag NPs+BFA

Figure S5. Inhibition of autophagy by bafilomycin A_1 (BFA) treatment enhanced cytotoxicity of Ag NPs in HeLa Cells. (A) MTT cytotoxicity assay of HeLa cells treated with PBS (control) or 10 μg/mL Ag NPs for 24 h in the presence or absence of 100 nM BFA. Mean±SEM, n = 5. ***$P < 0.001$ comparing to the control group. (B) ANXA5-FITC PI assay of HeLa cells treated with PBS (control) or 10 μg/mL Ag NPs for 24 h in the presence or absence of BFA. (C) Representative fluorescence pictures (the right panel) and the cell death rate of HeLa cells treated with PBS (control) or 10 μg/mL Ag NPs for 20 h in the presence or absence of BFA. Mean±SEM, n = 3. ***$P < 0.001$.

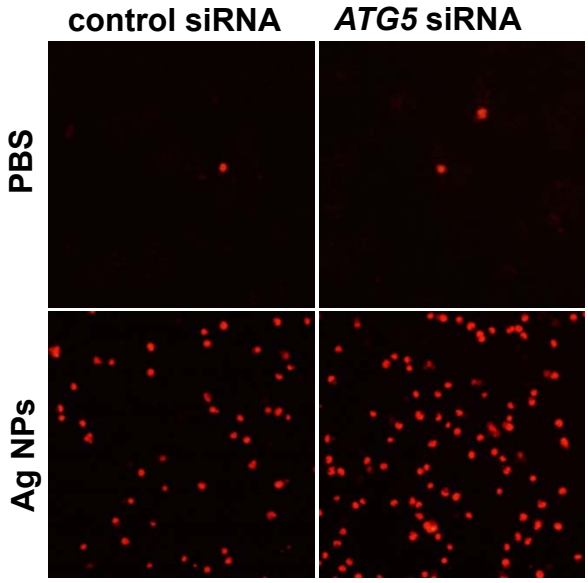

Figure S6. Representative fluorescence pictures of PI-stained HeLa cells treated with PBS (control) or 10 μg/mL Ag NPs for 20 h after transfection with *ATG5* siRNA or control siRNA for 48 h.

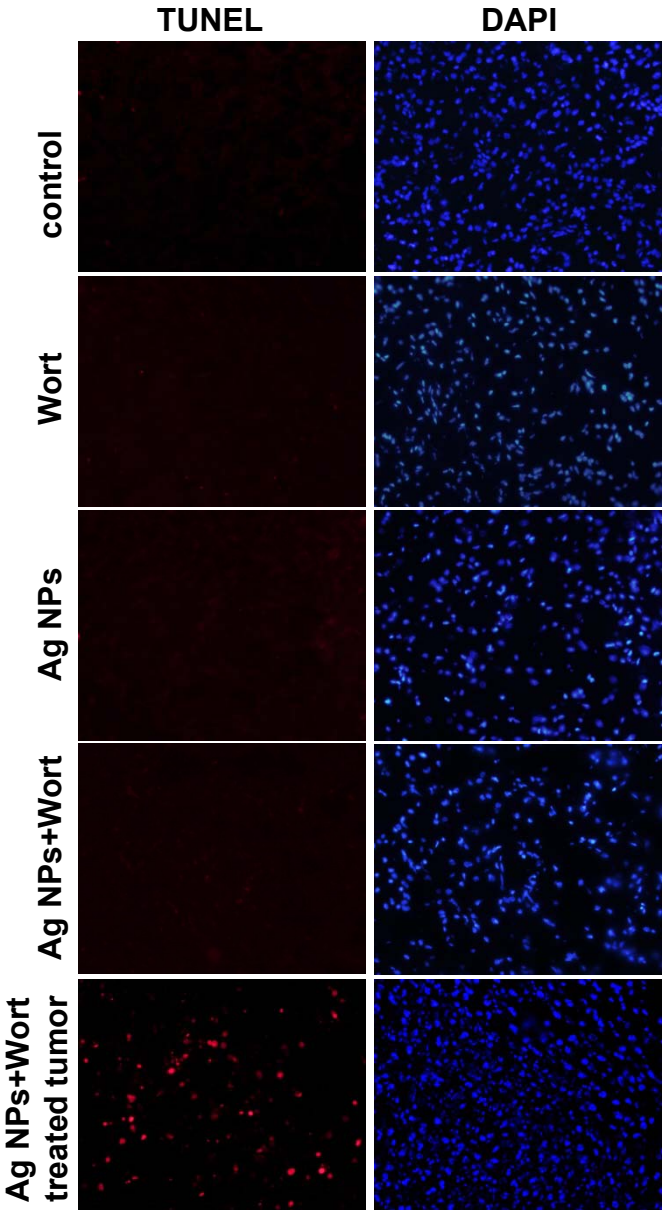

Figure S7. Minimal cell death in the endothelium adjacent to the tumor treated with Ag NPs. TUNEL staining (red) of sections from endothelium of adjacent tumors in the various groups was performed to show apoptotic cells. Nuclei were stained with DAPI (blue). A tumor section from an Ag NPs and wortmannin-cotreated mouse was used as positive control.

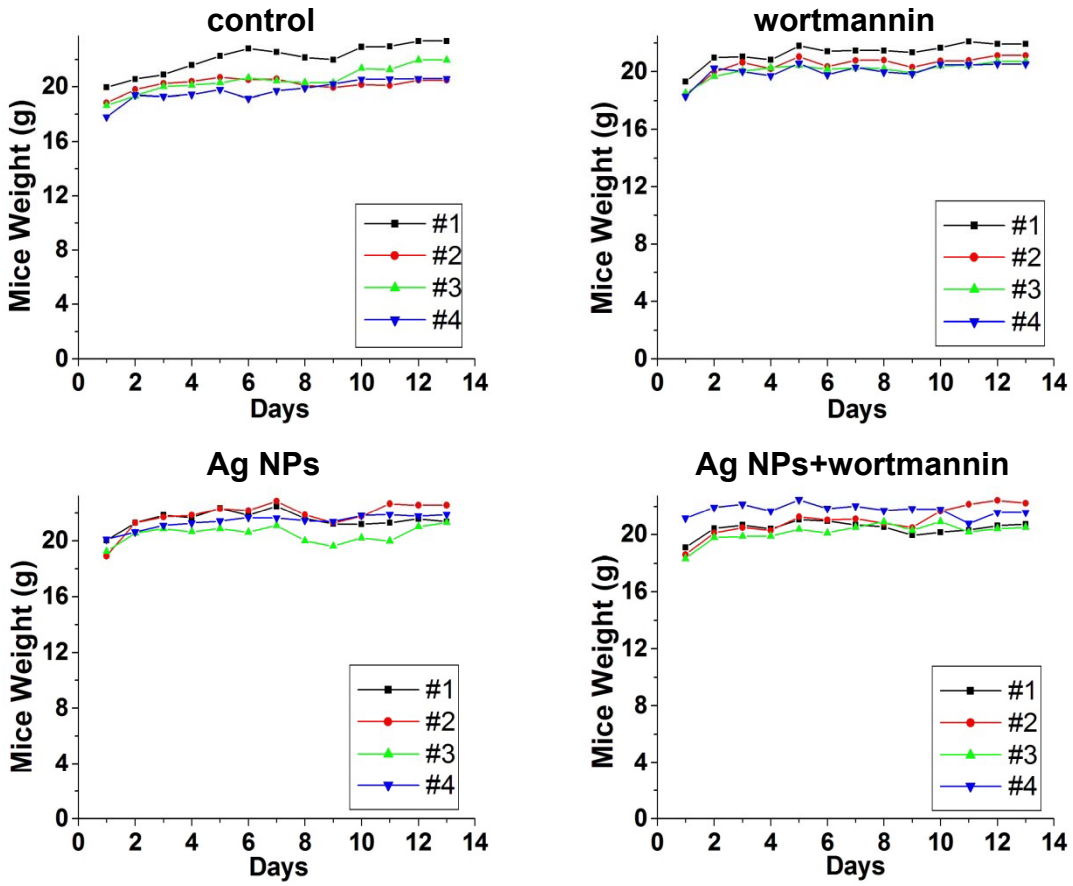

Figure S8. Body weight of mice treated with saline, wortmannin, Ag NPs or Ag NPs plus wortmannin. Mice in each group were weighed once daily, from the beginning of injections of the B16 melanoma cells to time of sacrifice.