

Supplemental Information

Maturation of dendritic cells (DCs) by NEPTT-treated cells

BC-1 mouse DCs were stimulated with Au nanoshell-treated B16 cells for 48 h prior to the analysis by flow cytometry (BD Biosciences FACSCan). Cells were washed 3 times in DPBS followed by blocking with Fc block (2 $\mu\text{g/ml}$ purified rat anti-mouse CD16/32) for 15 min on ice. Cells were then stained with 0.05 $\mu\text{g/ml}$ anti-mouse FITC-conjugated CD-86 and 0.05 $\mu\text{g/ml}$ anti-mouse PE conjugated MHCII for 30 minutes at 4°C. The cells were then washed 3 times prior to being re-dispersed in 200 μl of FACs buffer containing 7-AAD. The samples were analyzed within one hour. BC-1 cells treated with 1 ng/ml LPS were used as a positive control. LPS leads to the upregulation of both MHCII and CD86.

Supplementary Table 1. Estimated DAMP concentrations within a tumor or in blood. The measured concentration of the DAMPs ATP, ADP, and uric acid were converted to the amount per tumor cell. In case I, it was assumed that DAMPs remained within the tumor. A tumor of 5×10^6 cells and with 5 mm in diameter was used. In case II, it was assumed that DAMPs were dispersed into the blood stream. The blood volume was estimated for humans (4 liters in total) and mice (2 ml total).

DAMP	Measured Concentration	Cell Generation	Tumor	Blood Circulation (Human)	Blood Circulation (Mouse)
ATP	mM	mmol/cell	mM	mM	mM
100,000 NS/Cell	4.1E-04	2.0E-13	1.9E+00	2.5E-07	5.1E-04
75,000 NS/Cell	2.9E-04	1.4E-13	1.4E+00	1.8E-07	3.6E-04
50,000 NS/Cell	3.3E-04	1.7E-13	1.6E+00	2.1E-07	4.2E-04
25,000 NS/Cell	1.8E-04	8.9E-14	8.5E-01	1.1E-07	2.2E-04
Water Bath	9.8E-05	4.9E-14	4.7E-01	6.1E-08	1.2E-04
Freeze Thaw	7.6E-05	3.8E-14	3.6E-01	4.7E-08	9.5E-05
Control Cell	5.3E-05	2.6E-14	2.5E-01	3.3E-08	6.6E-05
Media	2.7E-05	1.3E-14	1.3E-01	1.7E-08	3.3E-05
ADP					
75,000 NS/Cell	7.9E-03	3.9E-12	3.8E+01	4.9E-06	9.8E-03
50,000 NS/Cell	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Uric Acid					
75,000 NS/Cell	5.4E-03	2.7E-12	2.6E+01	3.4E-06	3.3E-05
50,000 NS/Cell	7.6E-03	3.8E-12	3.6E+01	4.8E-06	3.3E-05

Supplementary Figure 2. Maturation of BC-1 DCs by NEPTT-treated B16 cells. The degree of BC-1 maturation was evaluated by measuring the surface expression of CD86 and MHCII by flow cytometry. (A) BC-1 DCs were stimulated with treated B16 cells at 1:1 ratio of B16 to BC-1 with the dosage of 100,000-25,000 nanoshells/cell. (B) BC-1 DCs were stimulated with treated B16 cells at ratios of B16 to BC-1 from 4:1 to 1:1 at a Au nanoshell dosage of 50,000 nanoshells/cell.

