**Supporting information**

1,2-naphthoquinone enhances IFN-γ-induced MHC-I expression in dendritic cells, thereby inducing CD8 T cell activation

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**Table of contents**

**1. Supplemental methods**

**2. Supplemental Figures**

**1. Materials and methods**

**Mice**

Male B10.BR mice (5-8 weeks old) and male C57BL/6 mice (5-8 weeks old) were obtained from Japan SLC (Hamamatsu, Japan) and housed in an animal facility at Okayama University. This study was approved by the Committee on Animal Experiments, Okayama University (OKU-2022127, OKU-2023354).

**Preparation of BMDC**

BMDCs were prepared as previously reported [1]. Briefly, bone marrow cells were collected from the tibia and femur of mice. The cells were cultured in RPMI-1640 medium containing 10% FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 50 µM β-mercaptoethanol in the presence of 10 ng/mL mouse GM-CSF at 37 °C in 5% CO2 for 7–8 days.

**Cell culture**

B16F10 cells, a mouse melanoma cell line, was obtained from RIKEN BRC. B16F10 cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 50 µM 2-mercaptoethanol at 37°C in the presence of 5% CO2.

**Reference**

[1] T. Inamoto, K. Furuta, C. Han, M. Uneme, T. Kano, K. Ishikawa, C. Kaito, Short-chain fatty acids stimulate dendrite elongation in dendritic cells by inhibiting histone deacetylase, FEBS J 290 (2023) 5794-5810. 10.1111/febs.16945.

**2. Figure Legends**

**Figure S1. Effects of 1,2-NQ and 1,4-NQ on the viability of DC2.4 cells**

DC2.4 cells were stimulated for 24 hours with 1,2-NQ (from left to right: 0, 0.1, 0.5, 1, 5, 10, and 20 µM), 1,4-NQ (0, 0.1, 0.5, 1, 5, 10, and 20 µM), and p-BQ (0, 0.1, 0.5, 1, 5, 10, 20 µM). Cell viability was assessed by measuring the cells that had taken up propidium iodide as dead cells using flow cytometry (n=3, from three independent experiments). Statistical differences between the groups were determined using Dunnett’s multiple comparison test. \* p<0.05, \*\* p<0.01, and \*\*\* p<0.001.

**Figure S2. Effects of 1,2-NQ on the cell surface expression of MHC-I**

DC2.4 cells were incubated with IFN-γ (20 ng/mL) in the presence of 1,2-NQ (0, 1, 3, 10 µM) for 24 h at 37°C. The cell surface expression of MHC-I was analyzed using flow cytometry. The graph presents the relative mean fluorescence intensity (0 µM= 1). The. values are expressed as the average ± SEM (n=3, from three independent experiments). Statistical differences between the groups were determined using Dunnett’s multiple comparison test, comparing each treatment group to the 1,2-NQ(+), IFN-γ(+) treatment. \* p<0.05, \*\* p<0.01, and \*\*\* p<0.001.

**Figure S3. Effects of 1,2-NQ on the cell surface expression of MHC-I and MHC-II on BMDCs**

BMDCs prepared from B10.BR mice were incubated with (+) or without (-) IFN-γ (20 ng/mL) in the presence of 1,2-NQ (10 µM) for 24 h at 37°C. The cell surface expression of MHC-I (A), MHC-II (B) was analyzed using flow cytometry. BMDCs prepared from C57BL6 mice were incubated with (+) or without (-) IFN-γ (20 ng/mL) in the presence of 1,2-NQ (10 µM) and OVA (1 mg/mL) for 24 h at 37°C. The cell surface expression of MHC-I bound OVA peptide (SIINFEKL) was analyzed with an anti-H-2Kb bound to SIINFEKL (clone 25-D1.16) using flow cytometry (C). The graphs present the relative mean fluorescence intensity (no stimulation = 1). values are expressed as the average ± SEM (n=3, from three independent experiments). Statistical differences between the groups were determined using Tukey’s multiple comparison test. \* p<0.05, \*\* p<0.01, and \*\*\* p<0.001.

**Figure S4. Effects of 1,2-NQ on the cell surface expression of MHC-I on B16F10 cells**

B16F10 cells were incubated with (+) or without (-) IFN-γ (10 ng/mL) in the presence of 1,2-NQ (0, 1, 3 µM) for 24 h at 37°C. The cell surface expression of MHC-I was analyzed using flow cytometry. The graphs present the relative mean fluorescence intensity (no stimulation = 1). values are expressed as the average ± SEM (n=3, from three independent experiments). Statistical differences between the groups were determined using Dunnett’s multiple comparison test. \*\* p<0.01, and \*\*\* p<0.001.

**Figure S5. Effects of 1,2-NQ on the mRNA expression of regulation factors of MHC in BMDCs**

BMDCs cells were incubated with (+) or without (-) IFN-γ (20 ng/mL) in the presence of 1,2-NQ (10 µM) for 24 h at 37°C. The mRNA expression levels of NLRC5 (A), CIITA (B), and GAPDH were analyzed using qPCR. Data are expressed as the average ± SEM (n=3-4, from three or four independent experiments). Statistical differences between the groups were determined using Dunnett’s multiple comparison test. \* p<0.05, \*\* p<0.01, and \*\*\* p<0.001.