

Supplementary Materials

Development of an Antibody Delivery Method for Cancer Treatment by Combining Ultrasound with Therapeutic Antibody-Modified Nanobubbles Using Fc-Binding Polypeptide

Yusuke Yano ^{1,†}, Nobuhito Hamano ^{1,†}, Kenshin Haruta ¹, Tomomi Kobayashi ¹, Masahiro Sato ¹, Yamato Kikkawa ², Yoko Endo-Takahashi ¹, Rui Tada ¹, Ryo Suzuki ³, Kazuo Maruyama ⁴, Motoyoshi Nomizu ² and Yoichi Negishi ^{1,*}

¹ Department of Drug Delivery and Molecular Biopharmaceutics, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

² Department of Clinical Biochemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo 192-0392, Japan

³ Laboratory of Drug and Gene Delivery Research, Faculty of Pharma-Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan

⁴ Laboratory of Ultrasound Theranostics, Faculty of Pharma-Sciences, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan

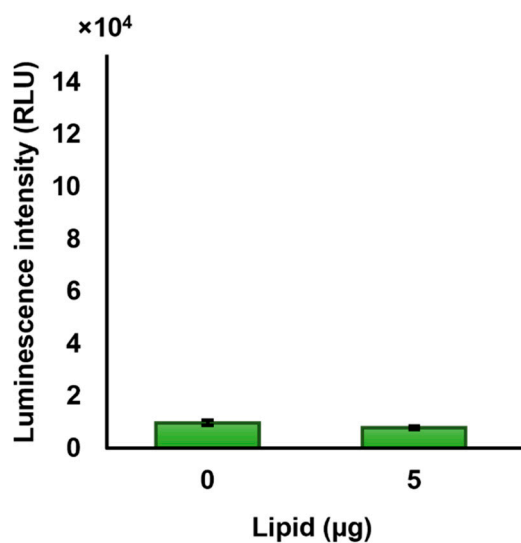
* Correspondence: negishi@toyaku.ac.jp; Tel.: +81-42-676-3183

† These authors contributed equally to this work.

Figure. S1 Experimental section

ADCC activity of PEG-NBs after TUS exposure

The preparation of PEG-NBs is same as the manuscript. SKOV3 (0.5×10^4 cells/ 100 μ L /well) were seeded onto a 96-well plate the day before observation and incubated overnight at 37°C in 5% CO₂. The next day, after the removal of the medium, 25 μ L of assay buffer (RPMI 1640 containing 4% (v/v) low IgG FBS) was added to each well, and PEG-NBs (5 μ g as a Lipid) which was exposed to TUS were added. Then, Jurkat effector cells were added to the wells at a density of 7.5×10^4 cells/ 25 μ L and incubated at 37°C in 5% CO₂ atmosphere. After 6 h, 75 μ L of Bio-Glo™ luciferase assay reagent was added to the wells and incubated at room temperature for 15 min. The resulting luminescence in the culture supernatants was measured using a Synergy HTX Multi-Mode Plate Reader (BioTek Instruments, Inc., Whiting, VT, USA). TUS condition is same as the manuscript.



Supplement Figure S1

Luminescence intensity of PEG-NBs exposed ultrasound

To evaluate the ADCC activity, luminescence intensity of Jurkat effector cells, expressing NFAT-RE-luc2, was measured by ADCC reporter bioassay against SKOV3 (T). SKOV3 cells were plated in a 96-well plate the before assay. On the day of assay, the samples and effector cells (E) were added to the plate (cell rate; E: T= 15: 1). After 6 h of incubation at 37°C/5% CO₂ condition, Bio-Glo™ luciferase assay reagent was added, and the luminescence intensity was determined using luminometer.