SUPPORTING INFORMATION

Near-infrared II photobiomodulation augments nitric oxide bioavailability via phosphorylation of endothelial nitric oxide synthase

Shinya Yokomizo[1,2], Malte Roessing[3,4*], Atsuyo Morita[4*], Timo Kopp[3,4], Emiyu Ogawa[5], Wataru Katagiri[6], Susanne Feil[3], Paul L. Huang[4], Dmitriy N. Atochin[4#], and Satoshi Kashiwagi[1#]*

[1]*Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital, 149 13th Street, Charlestown, MA, 02129, USA*

[2]*Department of Radiological Science, Tokyo Metropolitan University, 7-2-10 Higashi-Ogu, Arakawa, Tokyo 116-8551, Japan*

[3]*Interfaculty Institute of Biochemistry (IFIB), University of Tübingen, Auf der Morgenstelle 34, Tübingen 72076, Germany*

[4]*Cardiovascular Research Center, Department of Medicine, Massachusetts General Hospital 149 13th Street, Charlestown, MA 02129, USA*

[5]*School of Allied Health Science, Kitasato University, 1-15-1 Kitasato Minami-ku Sagamihara, Kanagawa, Japan*

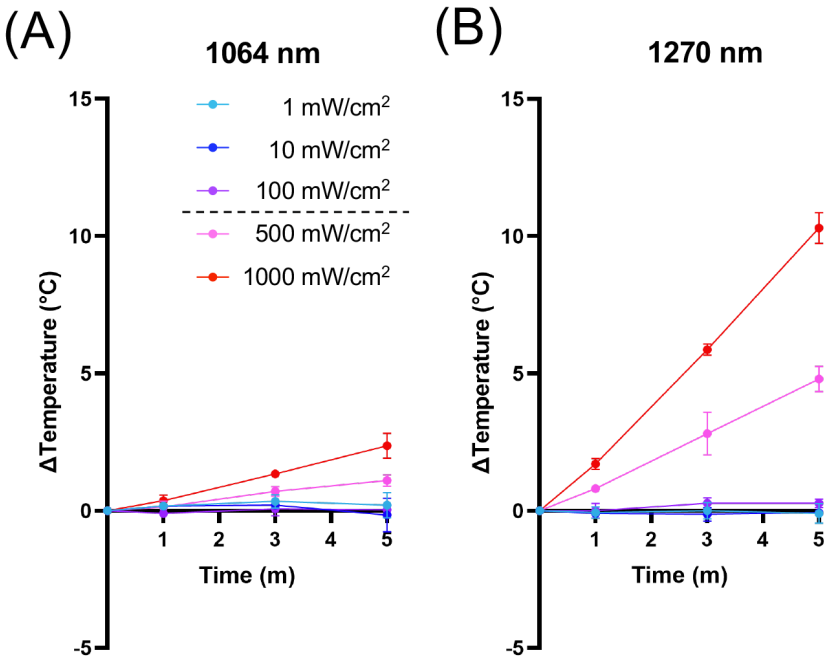
[6]*Graduate School of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan*

*These authors contributed equally.

[#]**Correspondence should be addressed:**
Dmitriy N. Atochin, MD, Ph.D.
Cardiovascular Research Center, Department of Medicine

Massachusetts General Hospital
149 13th Street, Charlestown, MA 02129, USA
Email: atochin@cvrc.mgh.harvard.edu
or
Satoshi Kashiwagi, MD, Ph.D.
Gordon Center for Medical Imaging, Department of Radiology
Massachusetts General Hospital
149 13th Street, Charlestown, MA 02129, USA
Tel: 617-726-6265; Email: skashiwagi@mgh.harvard.edu

TABLE OF CONTENTS



47

48 **Figure S1 Negligible heat generation in the irradiation system**

49 The cells were irradiated with 1064 or 1270 nm NIR laser on the system shown in Fig. 1 at an
50 irradiance of 1-1000 mW/cm^2 for 5 min. The temperature change in the irradiated area was
51 monitored using an infrared (IR) camera. Results were pooled from four independent experiments.
52 Note that the negligible temperature change was observed at up to 100 mW/cm^2, which was used
53 in this study, and a significant temperature increase was observed when we increased laser power
54 beyond 500 mW/cm^2, which was 5 times higher than the maximum dose.

55

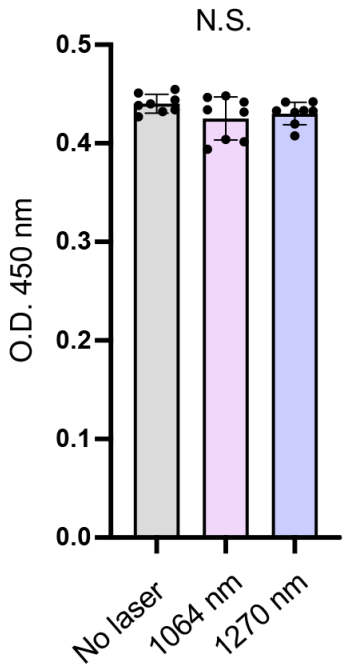


Figure S2 No endothelial proliferation induced by NIR-II exposure

The cell proliferation assay was treated with 1064 or 1270 nm laser at an irradiance of 10 mW/cm^2 for 5 min. Cell viability for 24 h after treatment was determined using a CCK-8 assay. Note that exposures of endothelial cells to 1064 nm at 10 mW/cm^2 had no appreciable changes in cell proliferation in this experimental setting. Results were pooled from three independent experiments. n = 8 cell preparations for each group. Error bars denote SEM.

Wavelength (nm)	Power (mW/cm^2)	Exposure time (min)	No. of counted cells (Ave ± SD)	No. of removed cells (Ave ± SD)	% of removed cells (Ave ± SD)
No laser	0	0	26.8 ± 5.8	2.6 ± 1	9.7 ± 3.2
1064	0.1	1	31.3 ± 3.6	3.8 ± 2	12 ± 6.3
		5	23.4 ± 5.1	1.6 ± 2.2	5.2 ± 6.1
	1	1	28 ± 3.1	2.6 ± 1.7	8.7 ± 5.5
		5	27.8 ± 1.6	1.6 ± 0.5	5.8 ± 2
	10	1	34.8 ± 2.4	3.6 ± 1	10.2 ± 2.6
		5	27.3 ± 3.2	2 ± 0.8	7.6 ± 3.7
	100	1	25.8 ± 2.6	2.2 ± 0.7	8.5 ± 2.7
		5	29 ± 6.1	2.6 ± 1.7	8.3 ± 3.9
1270	0.1	1	26.9 ± 4.2	3.3 ± 1.6	12.2 ± 4.5
		5	12.4 ± 4.2	0.6 ± 0.9	4.8 ± 6.5
	1	1	32 ± 4.8	3.8 ± 1.2	11.7 ± 2.6
		5	27.6 ± 4.8	2.8 ± 2	9.3 ± 6.3
	10	1	28 ± 3.9	1.6 ± 0.8	5.8 ± 2.9
		5	24.6 ± 5.1	2.8 ± 0.7	11.5 ± 2.8
	100	1	27.8 ± 5	2.2 ± 1.9	7.4 ± 6.2
		5	29.8 ± 3.7	2.8 ± 1.2	9.6 ± 4.2

Table S1 No acute cytotoxicity induced by NIR-II exposure

Cultured HUVECs were treated with 1064 or 1270 nm laser at an irradiance of 0.1-100 mW/cm^2 for 1-5 min. Inadequate cell images for the analysis, including those of cells in low viability, out of focus, and active division, were identified using a typical diameter of objects with nuclear counterstaining and excluded from the analysis on the CellProfiler. Note that no laser parameter showed an appreciable increase in the rate of excluded images compared to the no-laser control group, suggesting that low-power NIR-II exposure used in this study has minimal toxicity to endothelial cells. Results were pooled from two independent experiments. $n = 5$ cell preparations for each group.