Supplementary Figure S1

Materials and Methods
C57BL/6 (CLEA Japan Inc, Tokyo, Japan) mice, at 7 to 9 weeks of age were used in all experiments. The procedures used on all animals conformed to the guideline of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mice were anesthetized by intraperitoneal injection of 4% chloral hydrate (10 ml/kg), and the corneas were anesthetized by topical oxybuprocaine hydrochloride solution (Benoxy 0.4% solution; Santen, Osaka, Japan). The center of the cornea was demarcated by a 2 mm diameter biopsy punch (Kai industries, Seki, Japan), and the corneal epithelia cells in this area were removed with a rust ring remover (Handy Micro Motor; Inami, Tokyo, Japan). After the wound, 5 μl of 0.5mM Methylene blue (MB) or PBS was applied to each cornea. Then mice in the experimental group were irradiated with light from a halogen lamp at an intensity of 6mW/cm² for 10 minutes, while the controls were left in the dark for 10 minutes. Thus, the mice were then distributed into four groups (Light+MB+, Light+MB-, Light-MB+, and Light-MB-). Experiments were performed with three animals per experimental group. Mice were euthanized one week after treatment, the eyes were enucleated and fixed in 4% paraformaldehyde or methanol at 4°C overnight. They were then embedded in paraffin and 5 μm sections were cut through the central cornea and stained with hematoxylin and eosin. The sections were examined and photographed with a CCD camera (DP-50; Olympus) attached to a microscope (BX-50; Olympus).
HE staining of C57BL/6 mouse corneas treated with PDT with abrasions but no infection. (A) Light-MB-, (B) Light+MB-, (C) Light-MB+, and (D) Light+MB+. Light microscopy showed that none of the treatments (B), (C), or (D) induced corneal damage, such as corneal thinning or epithelial defects, compared to (A). Magnification, ×400.