

Supporting Information

In Vivo Cell Death Mediated by Synthetic Ion Channels

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Materials and Methods

Mammalian Cell Culture and Microscopy

HeLa cells were maintained in DMEM media supplemented with Glutamine, Penicillin, Streptomycin, and 10% FBS at 37°C and 5 % carbon dioxide. For imaging experiments, cells were plated on an 8-well Lab-Tek chambered cover glass (Nunc, Rochester, NY) and allowed to grow to approximately 80% confluence. Prior to imaging, the media was replaced with fresh DMEM media, and cells were treated with either **1**, **3**, or **4** (80 µM). Brightfield images were acquired at 5 sec intervals for a total of 20 mins using a Nikon Eclipse TE-2000U epifluorescence microscope (Nikon Corporation, Tokyo, Japan). All imaging experiments were performed in triplicate.

Animal Models and Protocol

All animal procedures were approved by the University of Notre Dame Animal Study Committee. Athymic nude mice (strain *nu/nu*, Taconic Inc., New York) were anesthetized (1.5% isoflurane inhalation) and injected intramuscularly in the rear leg muscle with either **2**, **3**, **4**, or **5** (8 mM in 50 µL ethanol). The same location on the opposite leg was injected with 50 µL of saline, which served as a vehicle control. Two hours after treatment, each mouse was intravenously injected via the tail vein with probe **6** (300 µM in 100 µL 1% DMSO:water).

Whole Animal Fluorescence Imaging

Athymic nude mice were anesthetized (1.5% isoflurane inhalation) and placed inside a Carestream Health In Vivo Multispectral Imaging Station FX (Carestream Health; Rochester, NY) configured for epi-illumination. The animals were irradiated with filtered light of wavelength 750 ± 10 nm, and an image of emission intensity at 830 ± 20

nm was collected by a CCD camera during a 30 s acquisition period (bin = 2 x 2, *f*-stop = 2.51, field of view = 120 mm). Mice were imaged 3, 6, 12 and 24 h after injection of probe **6**. After the 24 h time point, the animals were euthanized by cervical dislocation under anesthesia.

Image Analysis and Presentation

First, all images were exported as 16-bit tiff files (no scaling) using Carestream Health MI software. Images were then processed using *ImageJ 1.44*. The 16-bit tiff images were background subtracted using the rolling ball algorithm (radius = 1000 pixels) that is included in the software. The image stack was set to the “Fire” fluorescence intensity scale, which color codes the fluorescence counts contained in each pixel. At this point, region of interest (ROI) analysis was performed by selecting a circle ROI from the *ImageJ* tool bar, and drawing it to circumscribe the appropriate anatomical location of each mouse. The integrated densities of the target (T) and non-target (NT) were measured by *ImageJ* and recorded for each mouse. The T/NT ratios were then calculated, and statistical analysis was performed to acquire the average of each ratio (n=4) with the standard error of the mean (SEM). The resulting ROI values were plotted using *Graphpad Prism 4*.

Histology

Following humane euthanasia of mice, samples of skeletal muscle from injection sites were harvested, fixed for 24 h in 10% neutral buffered formalin, and placed in 70% ethanol before embedding in paraffin. The tissues were then sectioned at 4 μ m, stained with hematoxylin and eosin and examined by light microscopy for histological changes.

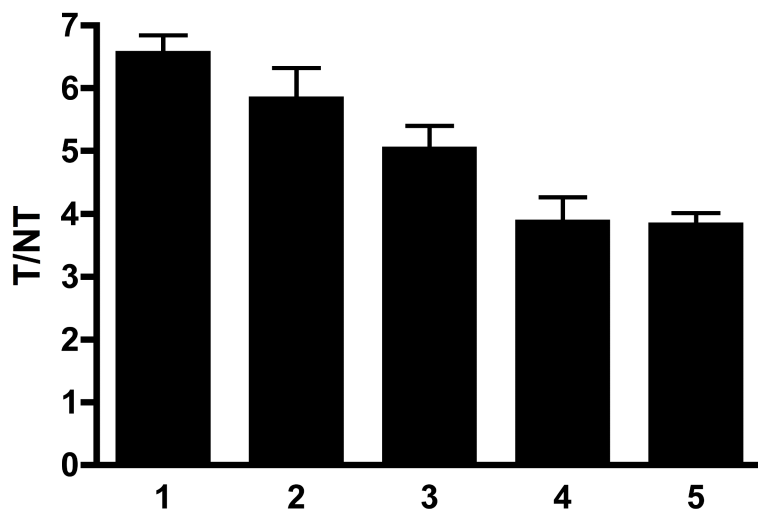


Figure S1. Target to non-target (T/NT) values of probe **6** in mice treated with **1-5**. Near-infrared fluorescent images were acquired 24 h post-injection of probe **6**. Regions of interests were drawn around the muscle treated with either **1-5** (T) and the muscle on the contralateral leg (NT) in each image and the integrated densities were measured. T/NT $\pm$ standard error of the mean. N = 4.

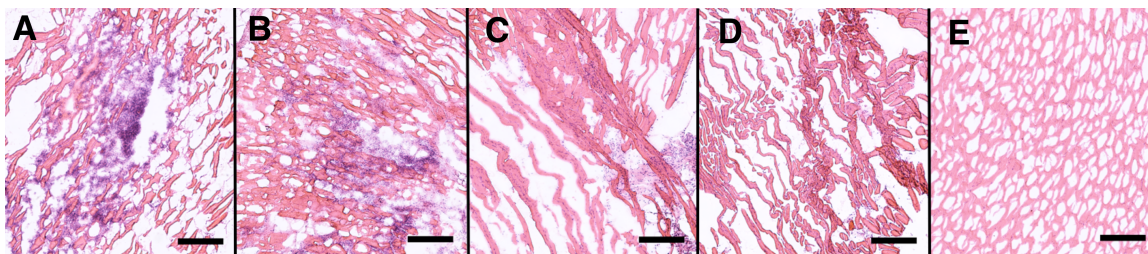


Figure S2. Representative H&E stained micrographs of muscle tissue treated with **2** (A), **3** (B), **4** (C), **5** (D), and saline (E). scale bar = 500 μ m.