Supplementary Material

Direct Administration of Nerve-Specific Contrast to Improve Nerve Sparing Radical Prostatectomy

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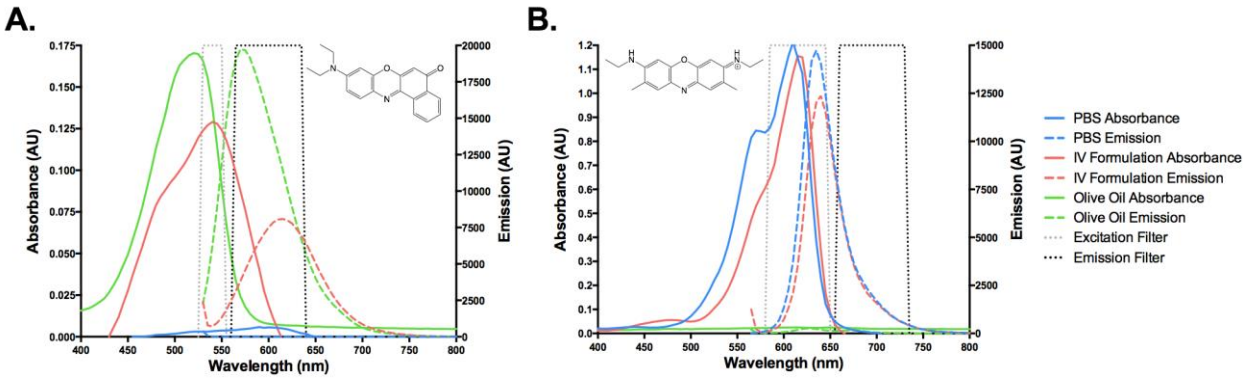


Figure S1: *Fluorescence spectra and structures of Oxazine 4 and Nile Red fluorophores.* **(A)** Absorbance and emission spectra for 10 μM **(A)** Nile Red and **(B)** Oxazine 4 in PBS, co-solvent formulation, or olive oil with overlaid excitation and emission filter spectra used for *in vivo* imaging. The fluorophores' chemical structures are inlaid into each graph.

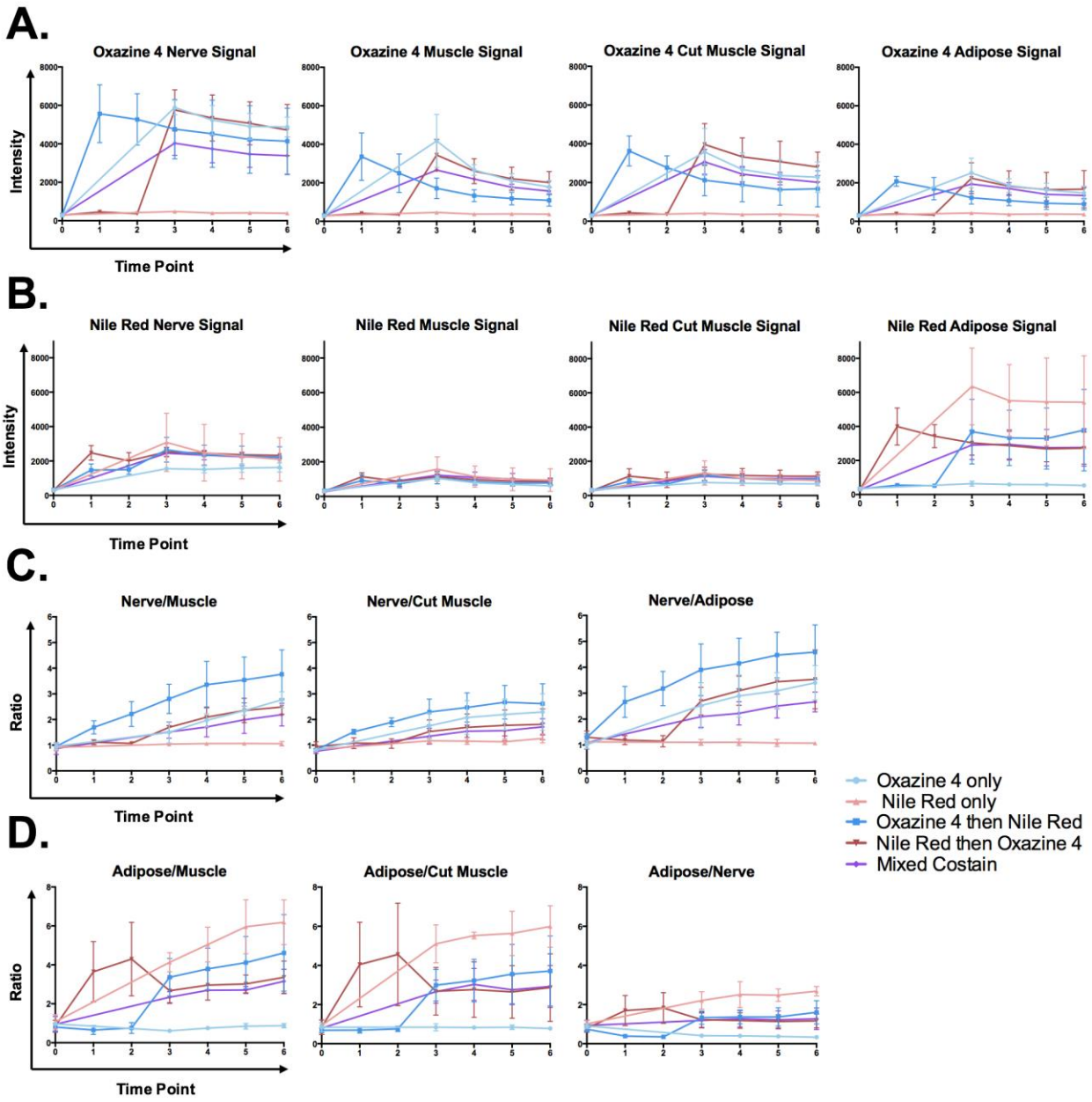
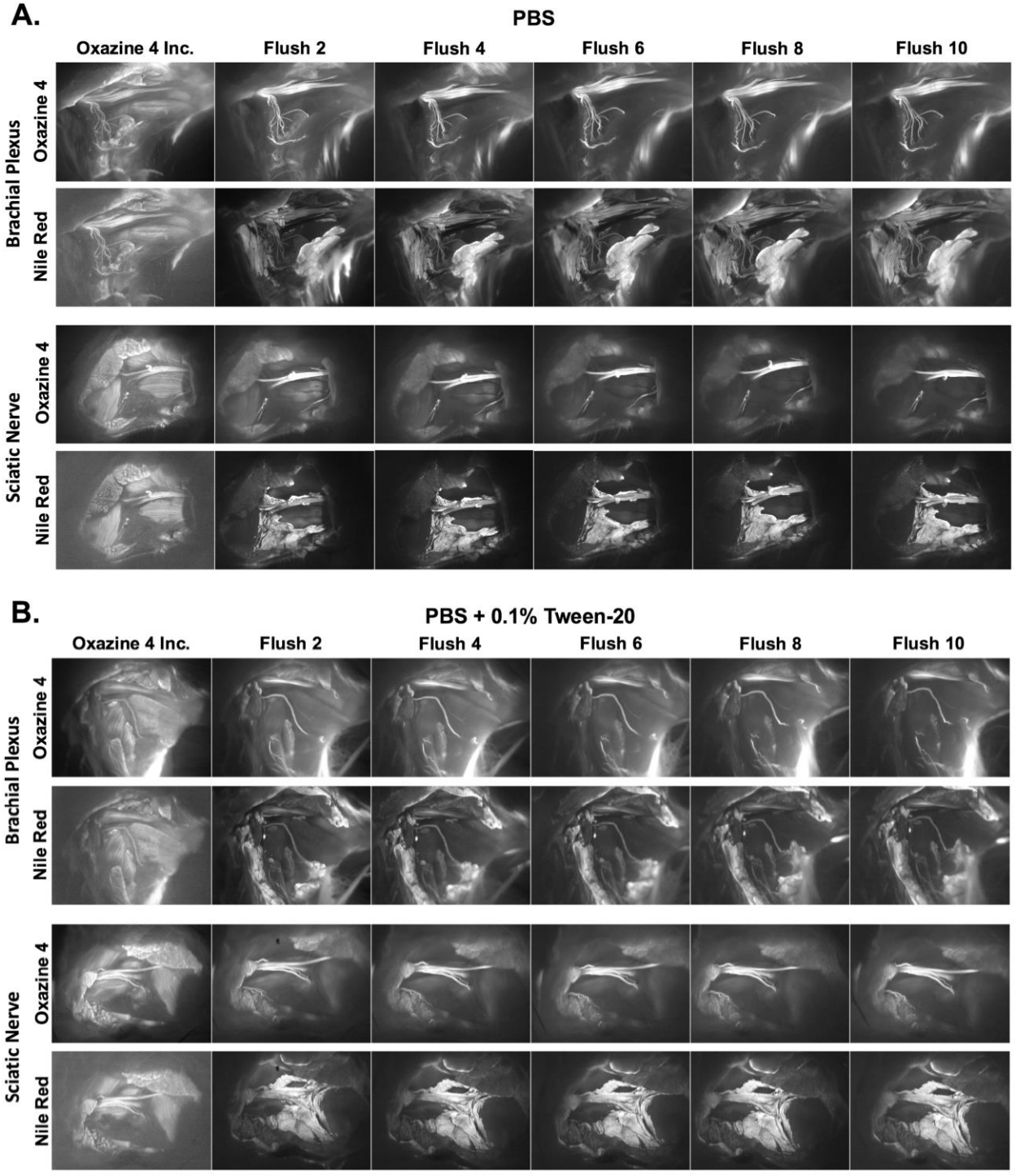


Figure S2: *Fluorophore cross-talk between Oxazine 4 and Nile Red.* Fluorescence signal intensities for each major visible tissue type and nerve to background tissue ratios were determined using region of interest analysis on images collected in the **(A)** Oxazine 4 fluorescence channel or **(B)** Nile Red fluorescence channel. The **(C)** nerve or **(D)** adipose to background tissue ratios were calculated from the intensity values determined through region of interest analysis. All data was determined from images collected for n=3 nerve sites at several time points: 0 = prior to the initial Oxazine 4 incubation, 1 = following the Oxazine 4 incubation, 2 = following the first blank co-solvent formulation wash, 3 = following the Nile Red incubation, 4 = following the second blank co-solvent formulation wash, 5 = following the PBS + 0.1% Triton X-100 wash, 6 = following the PBS + 0.1% Tween-20 wash. Time point 0 was considered the baseline fluorescence intensity for each tissue type. The data was determined from images collected at either 1 s exposure time for the time 0 point images or 2.5 ms exposure time for all other time points. All data in presented as mean +/- standard deviation.


A.
PBS
Oxazine 4 Inc.
Flush 2
Flush 4
Flush 6
Flush 8
Flush 10
Brachial Plexus
Oxazine 4
Nile Red
Sciatic Nerve
Oxazine 4
Nile Red
B.
PBS + 0.1% Tween-20
Oxazine 4 Inc.
Flush 2
Flush 4
Flush 6
Flush 8
Flush 10
Brachial Plexus
Oxazine 4
Nile Red
Sciatic Nerve
Oxazine 4
Nile Red

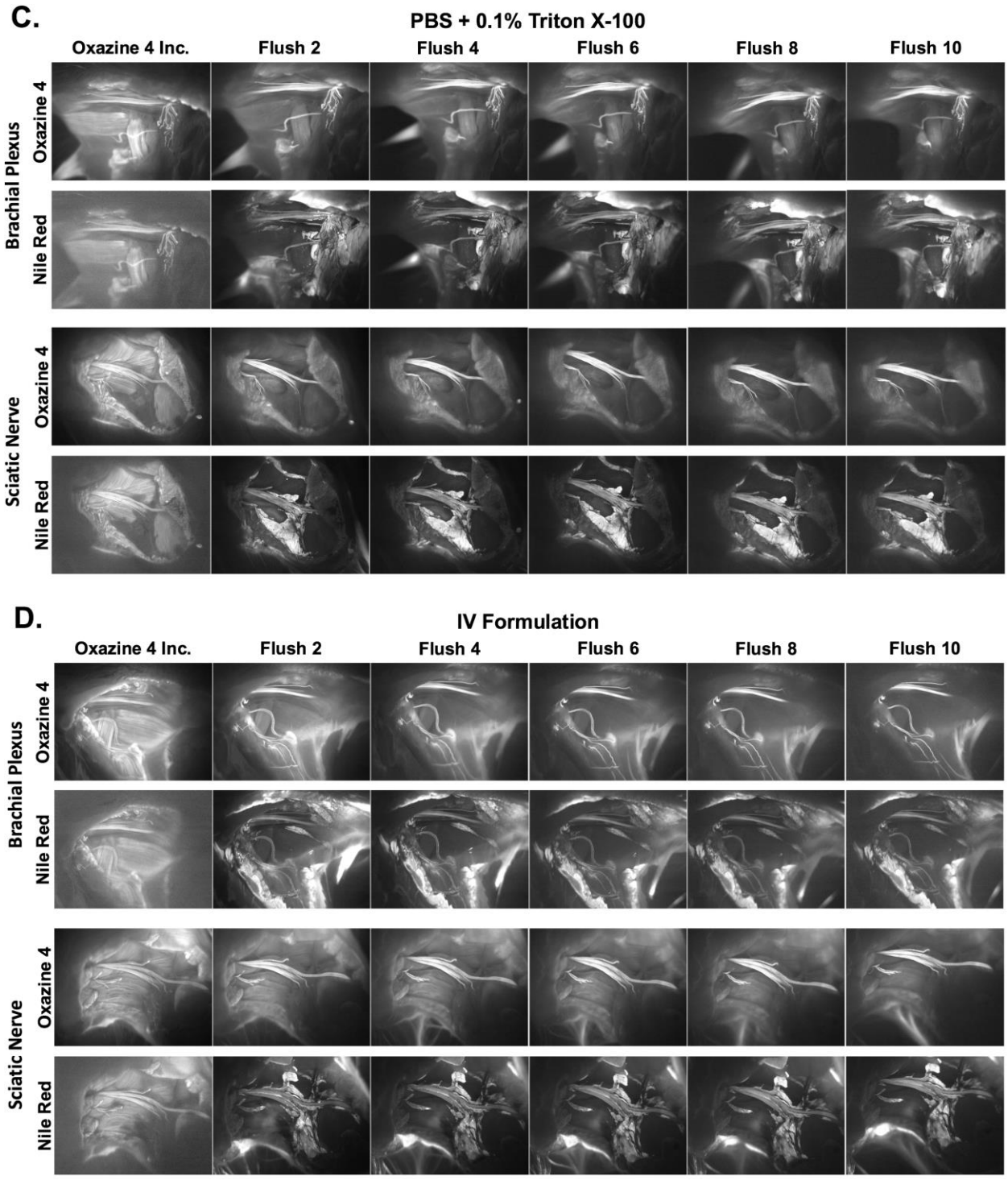


Figure S3: *Washing solution composition comparison.* Representative fluorescence images for nerve sites washed with **(A)** PBS, **(B)** PBS + 0.1% Tween-20, **(C)** PBS + 0.1% Triton X-100, or **(D)** blank co-solvent formulation in a series of 10 flushes are shown. All images are representative of data collected for n=3 nerve sites following completion of each fluorophore incubation and flush step. All images were collected at 2.5 ms exposure time.

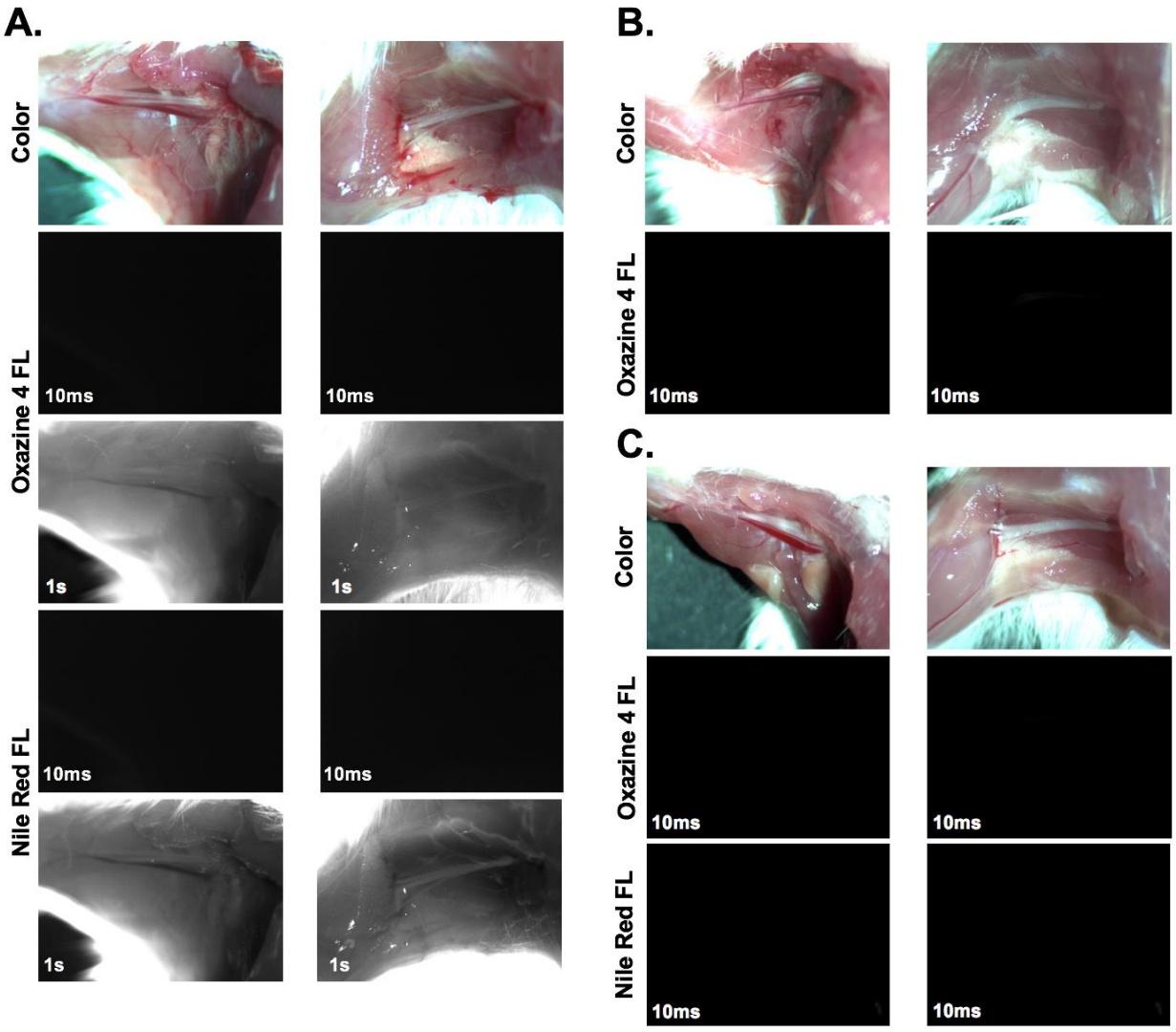


Figure S4: *Murine control images and video rate systemic administration images.* Representative fluorescence images for **(A)** unstained nerve sites used to calculate control nerve to background tissue ratios, **(B)** nerve sites stained by systemic administration of Oxazine 4 alone taken at 10 ms exposure time, and (**C)** nerve sites stained by systemic administration of Oxazine 4 and Nile Red taken at 10 ms exposure times. All images are representative of data collected for n=3 nerve sites following completion of the staining procedure.

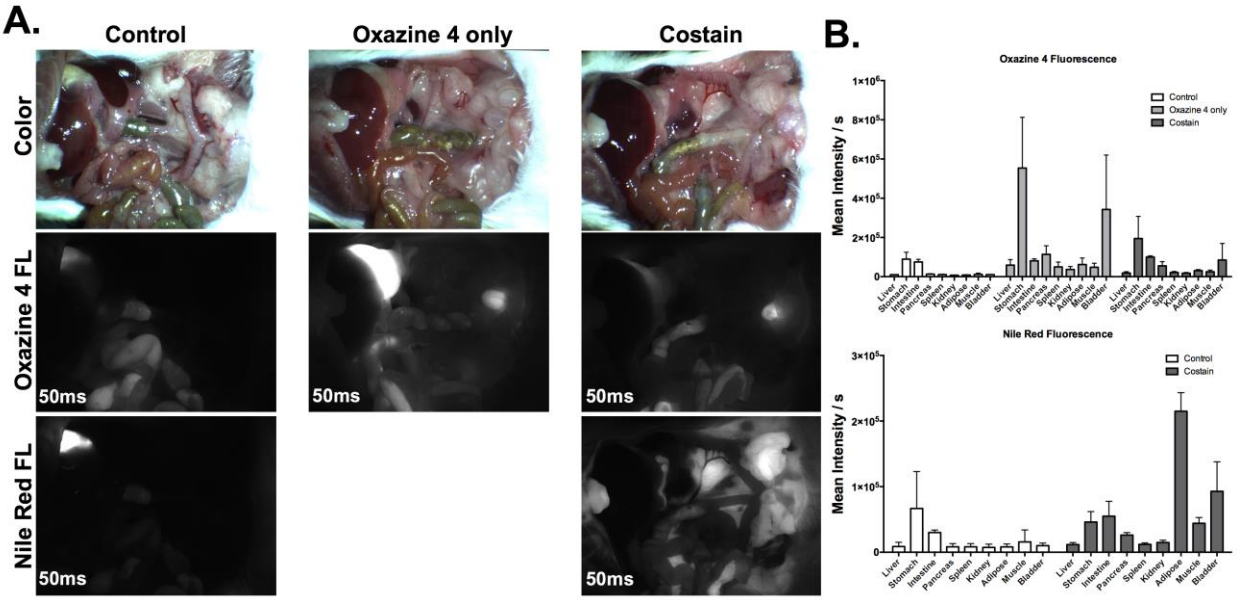


Figure S5: *Fluorophore peritoneal biodistribution following systemic administration.* **(A)** Representative color and fluorescence images for control blank co-solvent, Oxazine 4 only, and Oxazine 4 with Nile Red co-stain systemically administered mice. **(B)** Fluorophore biodistribution was quantified using region of interest analysis. The mean intensities normalized to exposure time (Mean Intensity/second (s)) as shown. All images were collected at 50 ms exposure time and are displayed with equal contrast and brightness. The data is presented as mean +/- standard deviation.