Supplemental information

Whole-brain optical access in a small adult

vertebrate with two- and three-photon microscopy

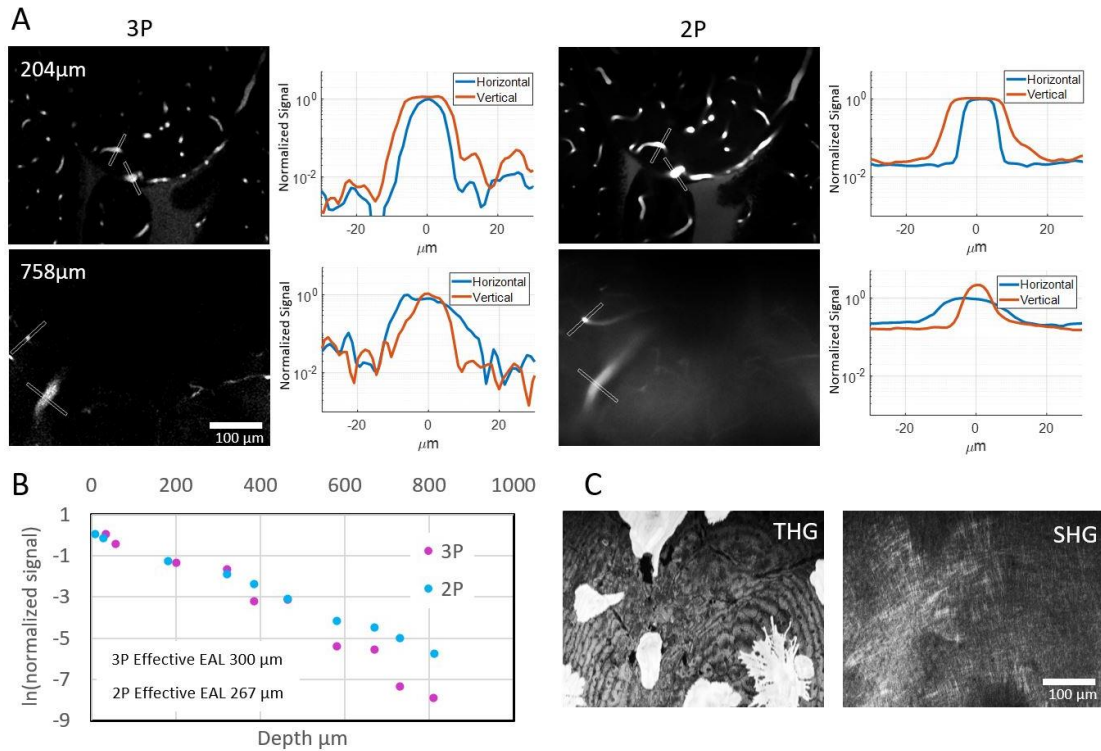Najva Akbari, Rose L. Tatarsky, Kristine E. Kolkman, Joseph R. Fetcho, Andrew H. Bass, and Chris Xu

Figure S1. Characterization of 2PM and 3PM images through the deepest part (midbrain) of the adult *D. dracula* brain, related to the STAR methods.

Excitation wavelengths of 920 nm at 80 MHz repetition rate and 1280 nm at 333 kHz repetition rate were used for 2PM and 3PM, respectively. To image the deepest part of the brain average power levels of 213 mW and 18 mW were used for 2PM and 3PM, respectively. A) Signal to background ratio comparison of horizontal and vertical blood vessels for 2PM and 3PM images at two depths inside the brain. In each line profile plot, the values are normalized to the maximum brightness of the horizontal blood vessel. B) Characterization of effective attenuation length inside the brain for 2PM and 3PM excitation wavelengths as described in the methods section. C) Maximum projection of THG (left) and SHG (right) of all frames containing the skin. Signals are generated by 1280 nm excitation. Pigments produce bright signal in the THG channel.
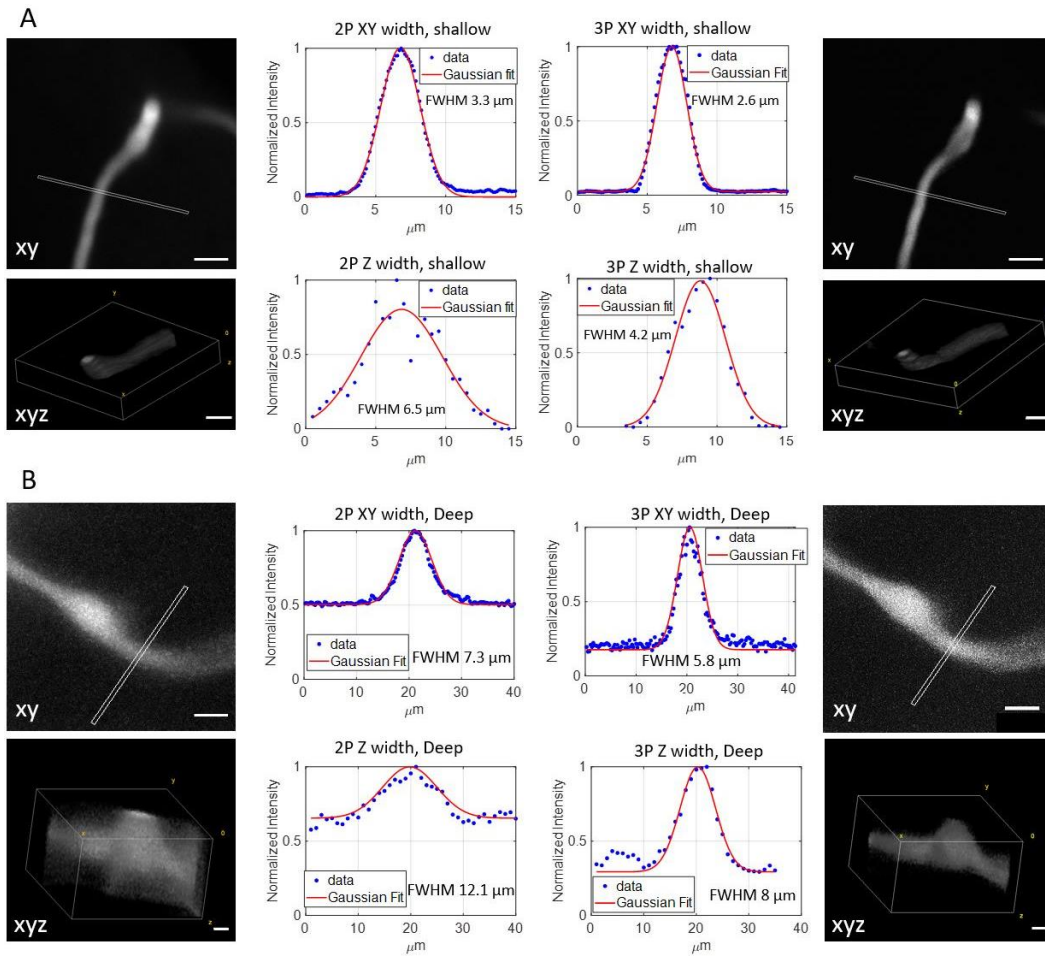
Figure S2. Lateral and axial width measurements of a small blood vessel, related to Figure 4.

Blood vessel is labeled with fluorescein (3PM, right panels) and Alexa Fluor 680 (2PM, left panels) and a 3D stack is collected in shallow (A) and deep (B) regions with 1280nm excitation.
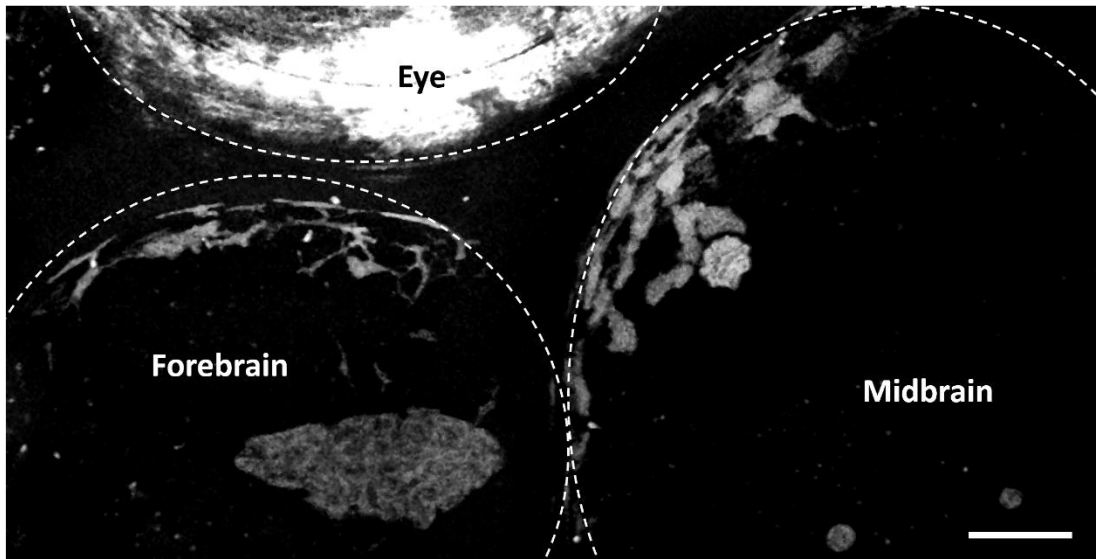
Figure S3. Maximum projection of autofluorescence images collected through the brain, related to the STAR methods.

The eye, forebrain, and midbrain are outlined with dashed white lines. Images were collected without any dye injections administered to the fish. Scale bar indicates 100 μm.
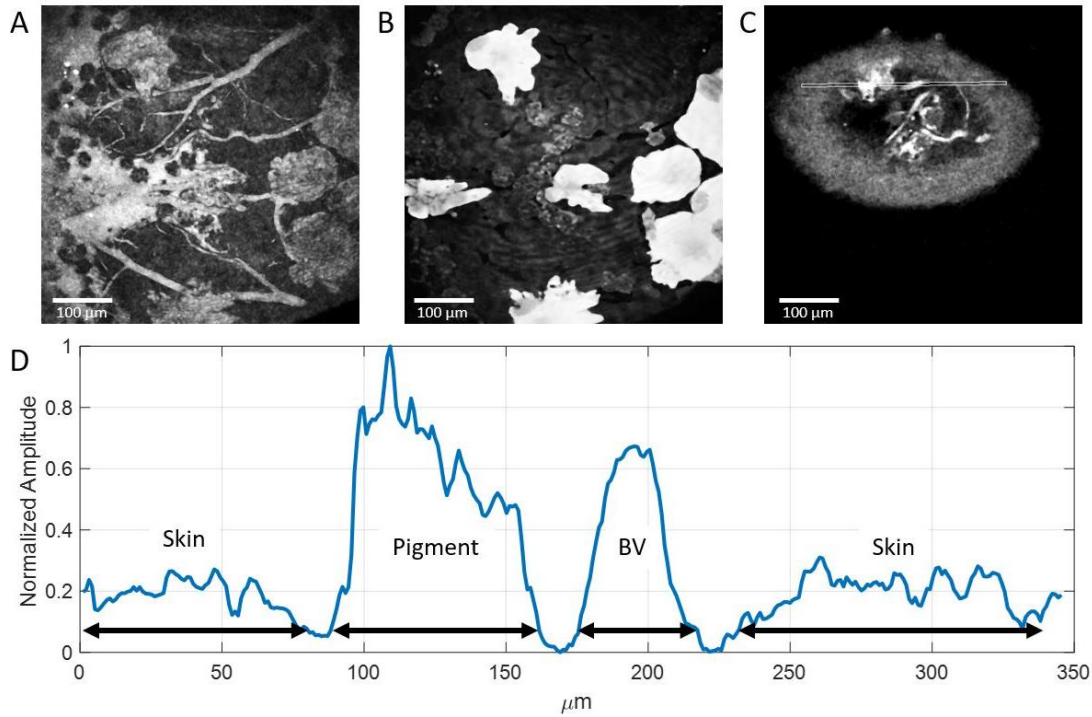
Figure S4. Characterization of pigment and skin autofluorescence, related to the STAR methods.

All images are obtained with 1280 nm excitation. Vasculatures contain dextran-coupled Fluorescein. A) Maximum projection of all fluorescence images containing the skin. B) Maximum projection of all THG images containing the skin. C) A single frame containing fluorescence signal from skin (autofluorescence), pigment (autofluorescence), and blood vessels (BV, fluorescein). A 5-pixel-wide line is drawn over a region containing pigment, skin, and blood vessels. D) Normalized intensity of the line depicted in part C. Regions corresponding to pigment, skin, and blood vessel are marked with black arrowed lines.