



Supplementary Figure 1

Procedure for implanting spinal glass window. (a) Following an incision of ~1.5 cm with scissors from T12 to L2, resect the muscles immediately lateral of the transverse processes throughout the length of the incision. Scale bar, 2 mm. (b,c) Position stereotaxic suspension forks below the transverse processes at T12 and L2. Further resect the muscles between the transverse and lateral processes from T12 to L2, and resect the ventral muscles from the lateral processes of T12 and L2. (b) Implant modified staples below the transverse processes snug to the pedicles of the vertebral arches of T12 and L2. Scale bar, 2 mm. (c) Glue the implanted staples in place with small drops of cyanoacrylate placed at the entry point of each staple tip. Inset shows a side view of the implanted staples highlighting the correct angle and location of implantation. (d) Remove the animal from the 'fork' stereotaxic holder and glue all of the tissue between the lateral processes and skin with cyanoacrylate. Apply a layer of dental cement to the same regions and implant a modified paperclip within the dental cement. Scale bar, 2mm. (e) Once the dental cement is hardened, hold the animal in place with a clamp. Inset shows a side view of the implanted staples and clamp. Next, remove the remaining muscles over T13 and L1 with forceps, and perform a dorsal laminectomy from T13 to L1. (f) Shape the lateral processes of T13 and L1 to act as the main supporting points (arrowheads) for the implanted window. Take special care to ensure that the bones are shaped so that the window rests flat relative to the spinal cord, but does not touch or compress the spinal cord. Once the exposed dura mater is cleaned and dried, apply a line of Kwik-Sil along the midline for the entire length of the window (black outline). Scale bar, 2 mm. (g) Immediately following the application of Kwik-Sil, lower the window gently onto its vertebral supports and hold in place for ~60 seconds. Glue the window (black outline) in place with a generous layer of cyanoacrylate placed over all exposed tissues and along the edges of the window, but without contaminating the middle of the window. Scale bar, 2 mm. (h,i) Apply dental cement to all regions with cyanoacrylate. Dashed box in h outlines region shown in i. Dashed box in i outlines region shown in j. Scale bar, 2 mm (h), 1 mm (i). (j) Maximal projection of a two-photon image stack of axons and vasculature after injection with QDot-655. Scale bar, 200 μ m. Rostral is up and Caudal is down for a, b, d, and f-j.