Supporting Information



Fig. S1. Conventional staining of lipid (a) and protein (b) using amphioxus sections. Lipid was stained with Oil Red O, and protein is stained with Hg – bromophenol blue. Scale bars = $200 \mu m$.



Fig. S2 The stimulated Raman scattering microscopy setup. A high repetition rate picosecond laser is used to provide Stokes beam at 1064 nm. The second harmonic (532 nm) is employed to drive the optical parameter oscillator (OPO) to generate the pump beam. EOM: electro-optical modulator, PBS: polarizing beamsplitter, DL: delay line, DM: dichroic mirror, GM: galvo mirror, O: objective, S: sample, C: condenser, M: flat mirror, L: lens, F: filter, PD: photodiode



Fig. S3. Collagen fibers in notochordal sheath. (a) Transmission confocal image of transverse section of amphioxus notochord. *np*: notochordal plate; *ns*: notochordal sheath; *nt*: neural tube. (b) SHG microscopy image of collagen fibers in notochordal sheath. Scale bars = 50μ m. (c) The zoomed image of transverse section of amphioxus notochord. (d) High resolution SHG microscopy image of collagen fibres in notochordal sheath. Scale bars = 5μ m.



Fig. S4. SRS and CARS images of living zebrafish larvae at 2950 cm⁻¹. SRS image has better contrast and CARS image shows artifacts from the non-resonant background of water. Scale bar = $50 \mu m$.