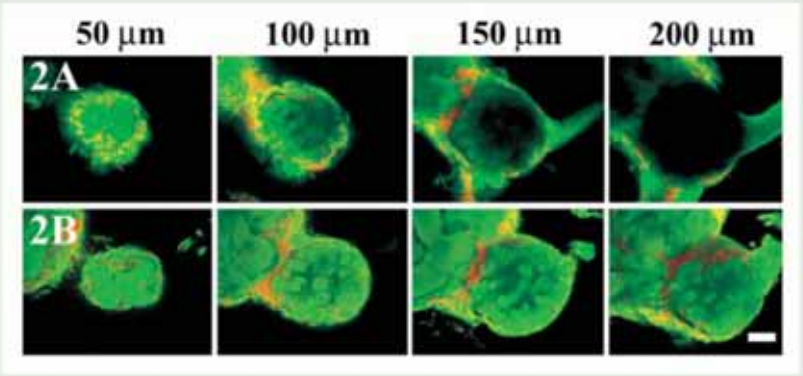
FocusClear 澄清液

適用於軟的生物組織：使顯微觀察之樣品變透明

上圖為500 um厚度的腦組織樣品
1A: 置於生理食鹽水中, 呈現不透明狀
1B~1C: 置於 FocusClear 澄清液中處理, 樣品逐漸變成透明

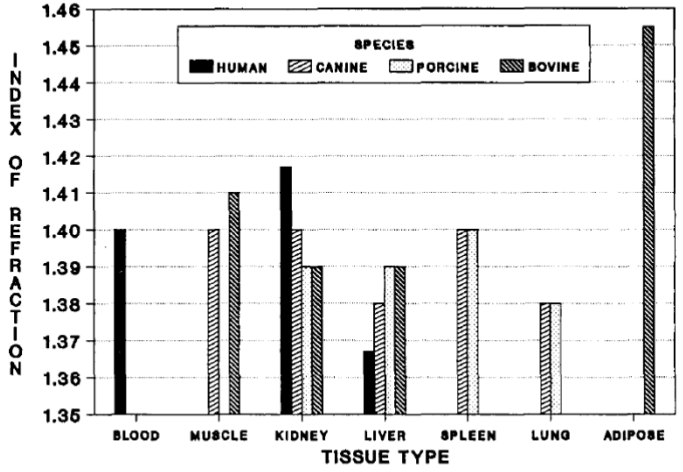
讓您的螢光顯微觀察更清晰; 更深入



2A: 一般之螢光顯微觀察
2B: 樣品經過FocusClear澄清液處理後所得之清晰的螢光顯微影像,
同時可觀察樣品更深層之影像 (可達600 um)

Refraction Index Matching

Refraction Index
Air: 1.00029
Water: 1.333
Fused silica: 1.458
Glycerol: 1.4729
Scale: 1.38
Urea: 1.48-1.49



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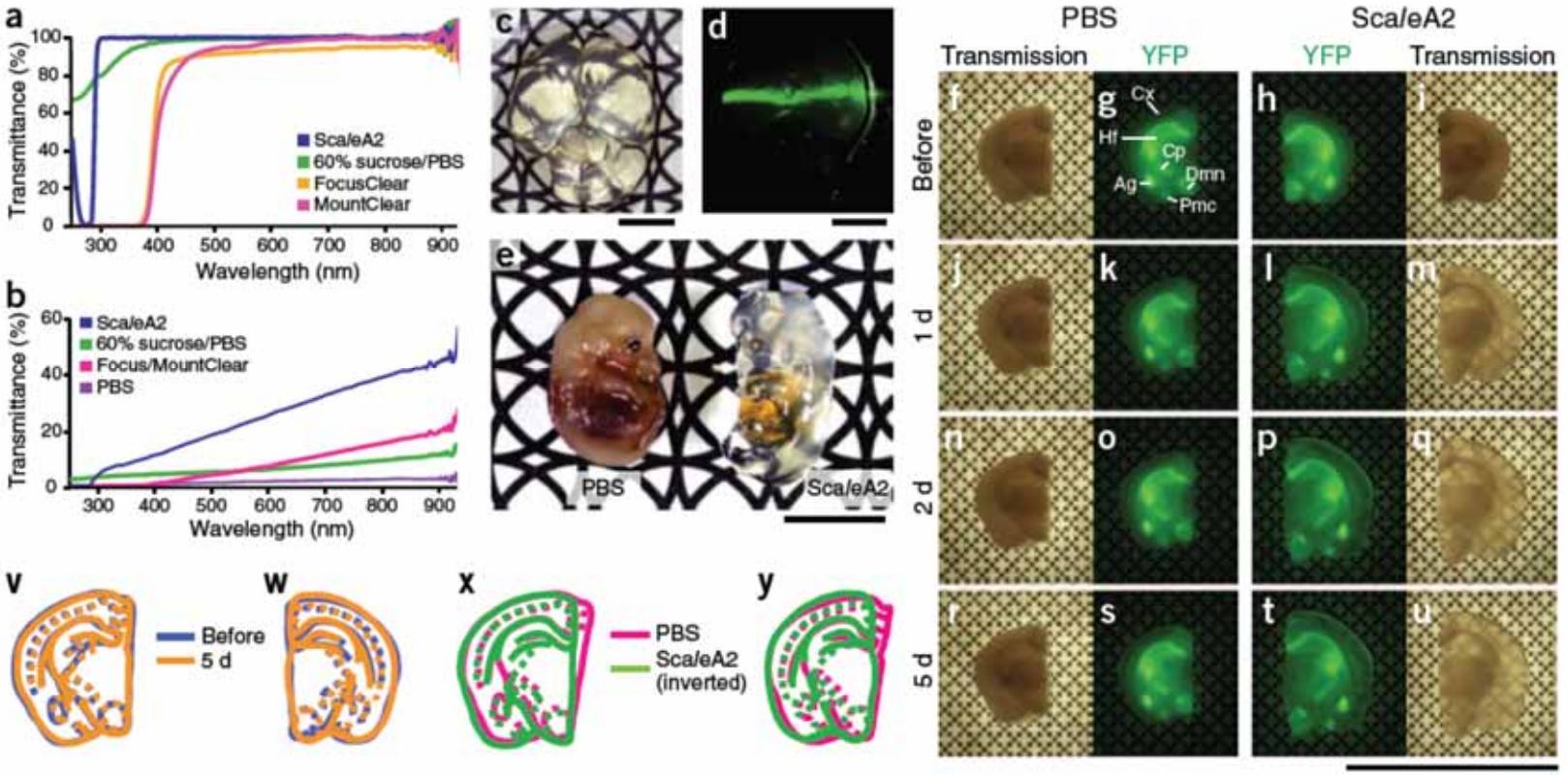
Sca*l*e: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain

Hiroshi Hama[1], Hiroshi Kurokawa[1,2], Hiroyuki Kawano[1,3], Ryoko Ando[1], Tomomi Shimogori[1], Hisayori Noda[1,4], Kiyoko Fukami[2], Asako Sakaue-Sawano[1,3] & Atsushi Miyawaki[1,3]



ity for fluorescence imaging is retained. It was reported that wild-type *Aequorea* green fluorescent protein (GFP) is sensitive to 8 M urea at acidic pH but not at neutral or alkaline pH[19]. We verified that the fluorescence of enhanced GFP (EGFP)

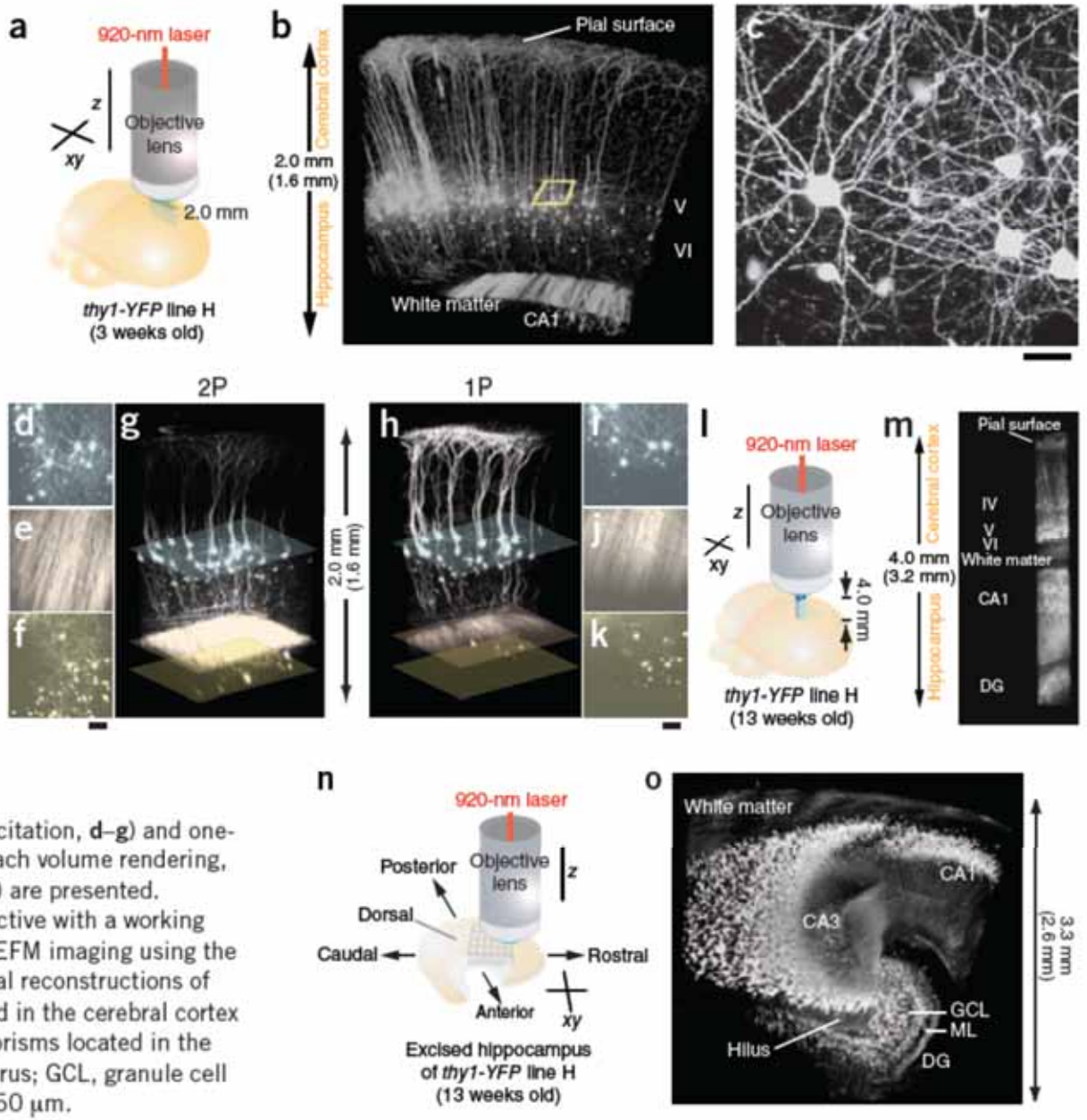


Figure 3 Three-dimensional reconstructions of YFP-expressing neurons in Sca*l*eA2-treated brain samples of YFP-H mice. The actual imaging depth is shown in parentheses. Unsectioned brains (**a**–**m**) and an excised hippocampus (**n**,**o**) were imaged. (**a**–**c**) TPEFM imaging using a 25× objective (XLPLN25XWMP, numerical aperture (NA) = 1.05, working distance = 2.0 mm). The experimental setup for TPEFM imaging using the commercially available objective is shown in **a**. A three-dimensional reconstruction of YFP-expressing neurons in 16 (8 × 2) quadratic prisms located in the cerebral cortex and hippocampus is shown in **b**. A high-magnification *xy* image at a depth of 0.9 mm (a yellow box in **b**) is shown in **c**. (**d**–**k**) Three-dimensional reconstruction of YFP-expressing neurons in a quadratic prism located in the cerebral cortex. The same brain region was imaged using a 20× objective (W-PlanApochromat, NA = 1.0, working distance = 2.0 mm) and taking both two-photon (920-nm excitation, **d**–**g**) and one-photon (514-nm excitation, **h**–**k**) approaches. For each volume rendering, three *xy* images at different *z* positions (**d**–**f** and **i**–**k**) are presented. (**l**–**o**) TPEFM imaging using a custom-designed objective with a working distance of 4.0 mm. The experimental setup for TPEFM imaging using the objective lens is shown in **l** and **n**. Three-dimensional reconstructions of YFP-expressing neurons in a quadratic prism located in the cerebral cortex and hippocampus (**m**) and in 24 (4 × 6) quadratic prisms located in the excised hippocampus (**o**) are shown. DG, dentate gyrus; GCL, granule cell layer; ML, molecular layer. All scale bars represent 50 μm.

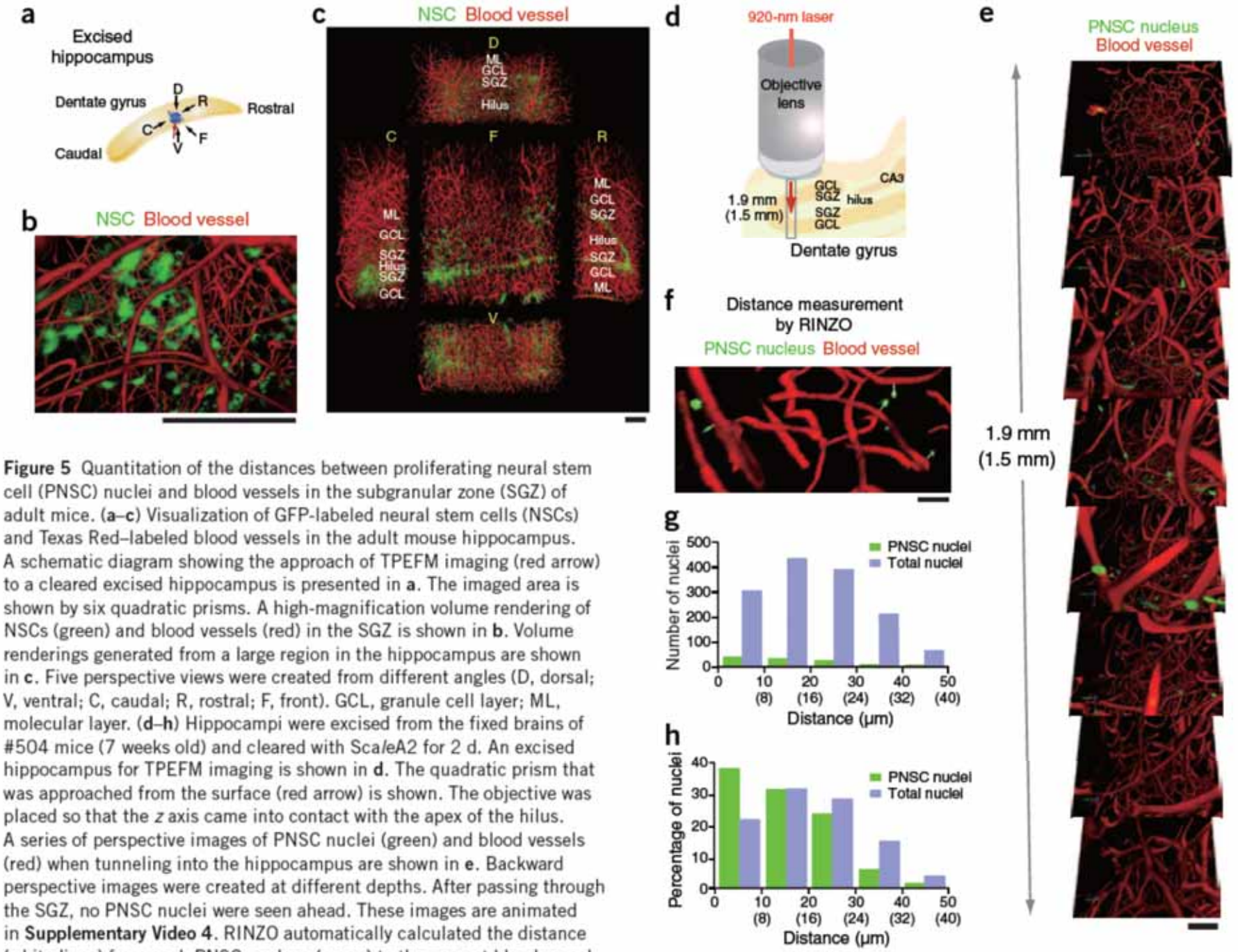
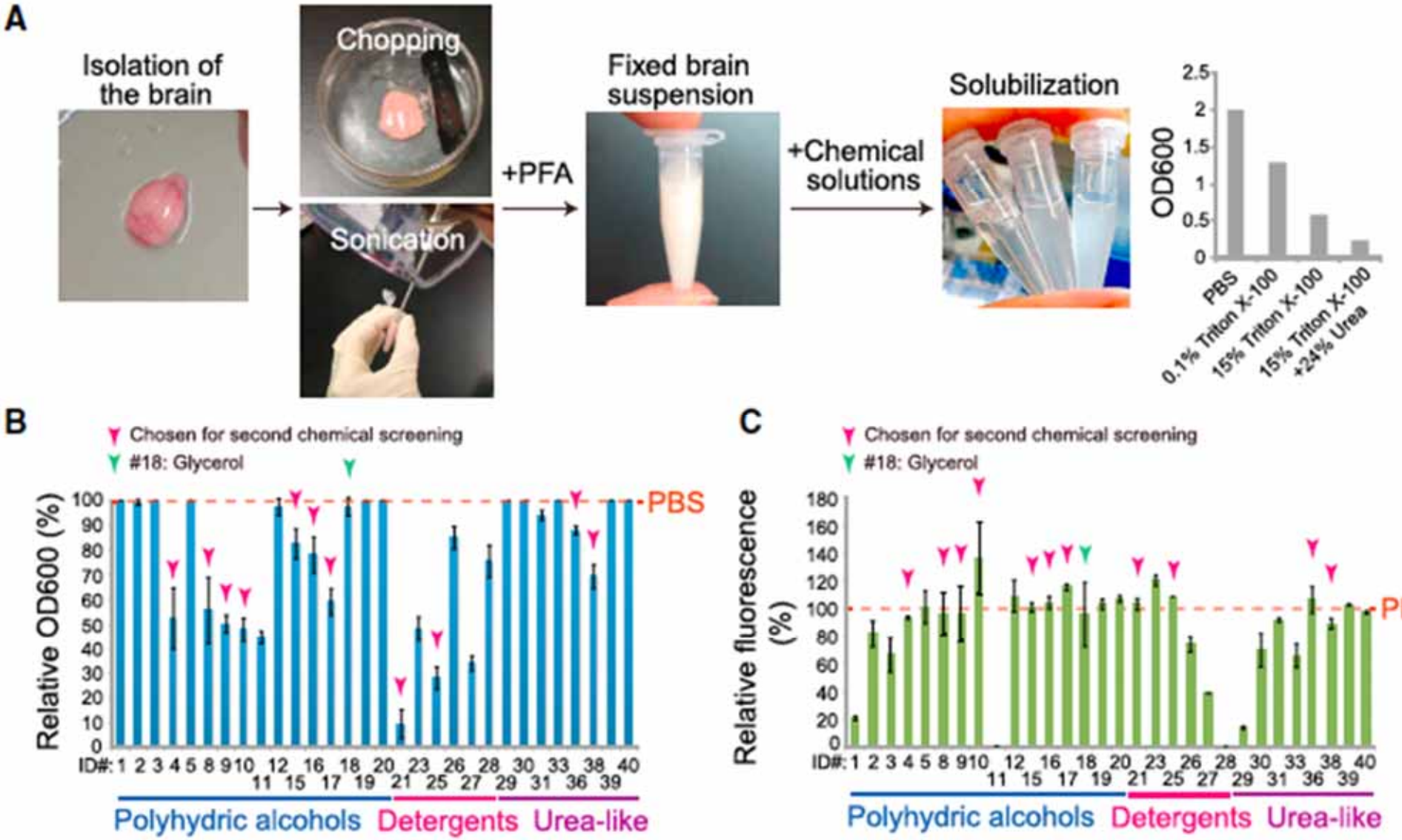
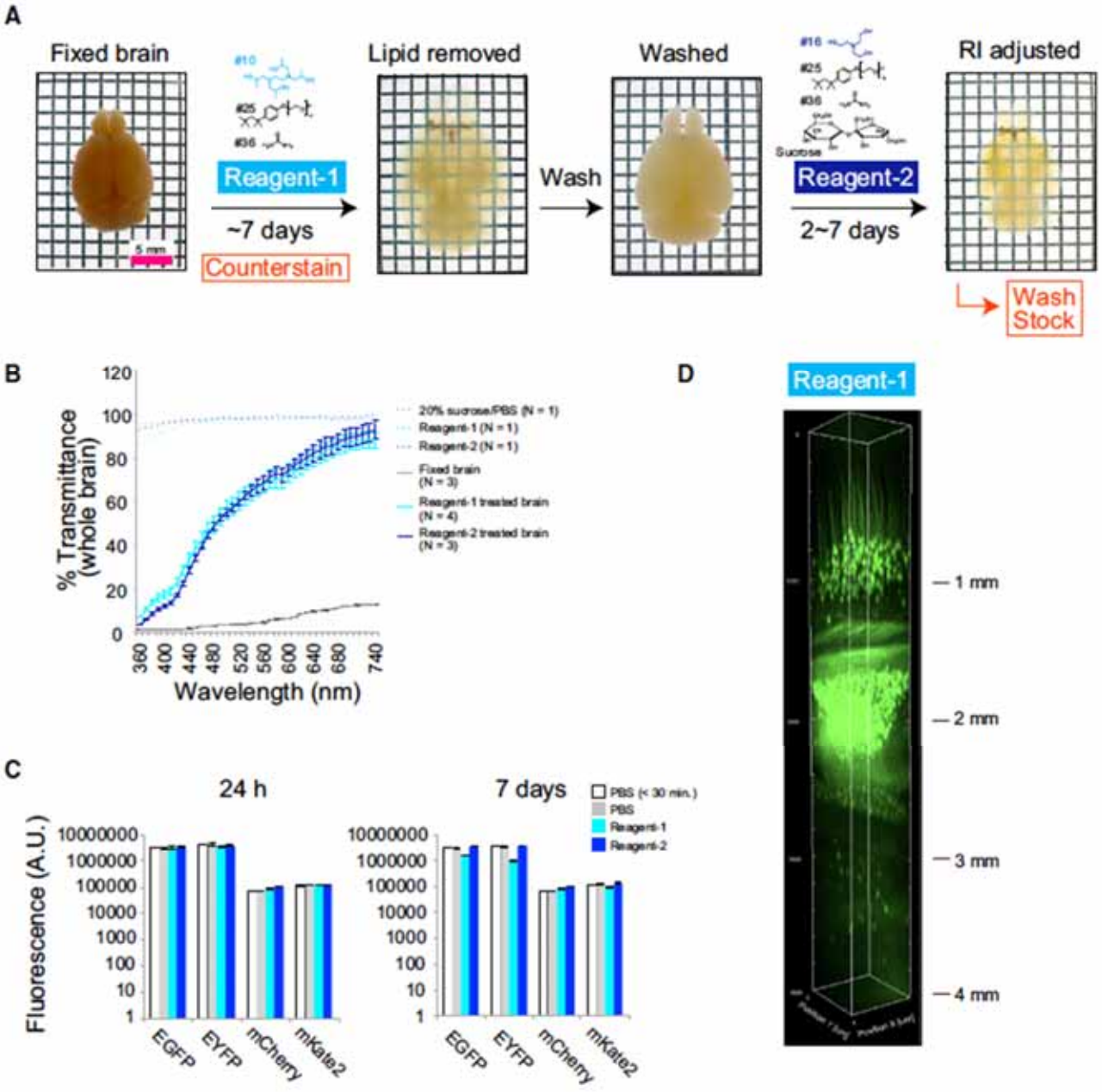
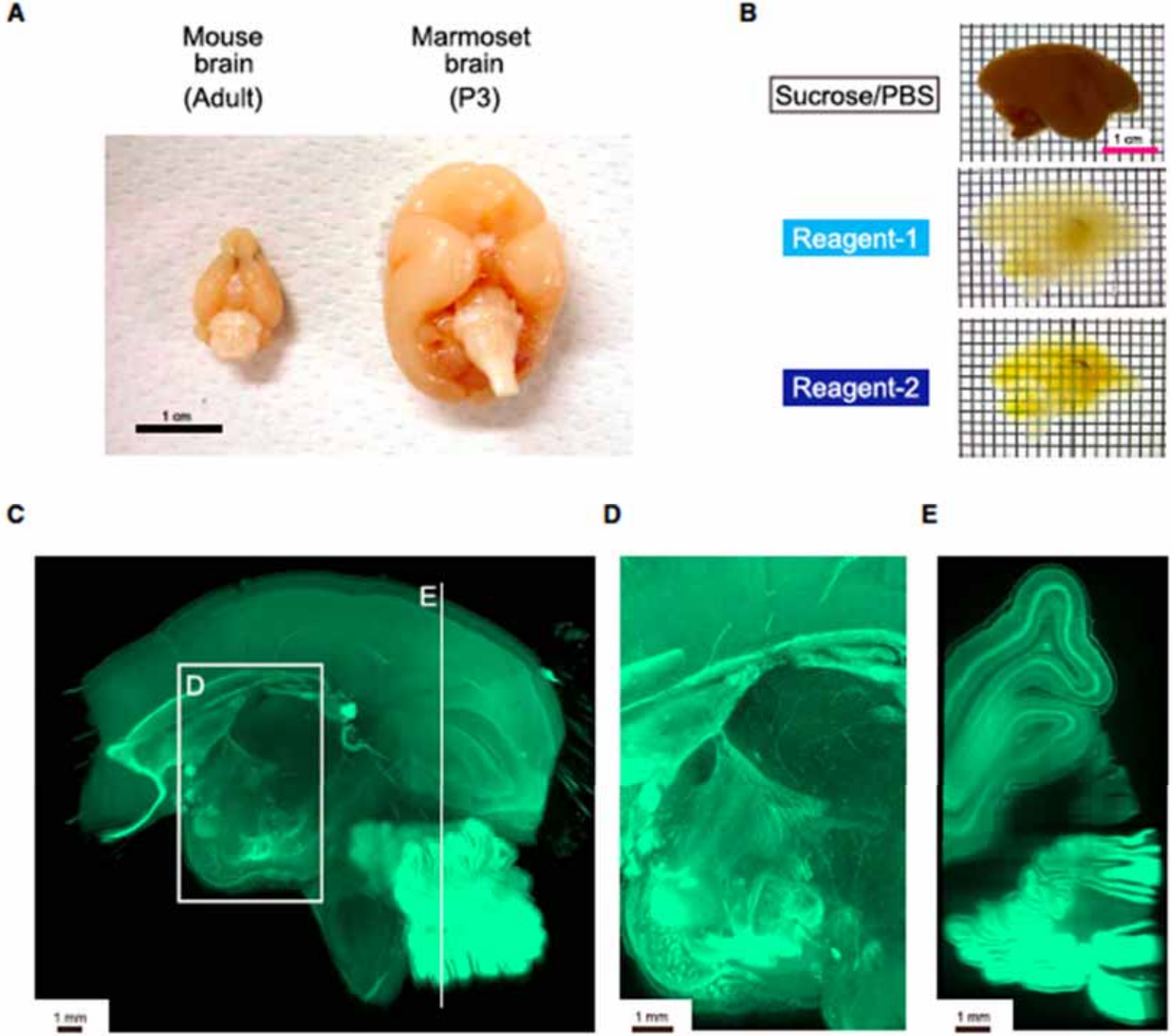


Figure 5 Quantitation of the distances between proliferating neural stem cell (PNSC) nuclei and blood vessels in the subgranular zone (SGZ) of adult mice. (**a**–**c**) Visualization of GFP-labeled neural stem cells (NSCs) and Texas Red–labeled blood vessels in the adult mouse hippocampus. A schematic diagram showing the approach of TPEFM imaging (red arrow) to a cleared excised hippocampus is presented in **a**. The imaged area is shown by six quadratic prisms. A high-magnification volume rendering of NSCs (green) and blood vessels (red) in the SGZ is shown in **b**. Volume renderings generated from a large region in the hippocampus are shown in **c**. Five perspective views were created from different angles (D, dorsal; V, ventral; C, caudal; R, rostral; F, front). GCL, granule cell layer; ML, molecular layer. (**d**–**h**) Hippocampi were excised from the fixed brains of #504 mice (7 weeks old) and cleared with Sca*l*eA2 for 2 d. An excised hippocampus for TPEFM imaging is shown in **d**. The quadratic prism that was approached from the surface (red arrow) is shown. The objective was placed so that the *z* axis came into contact with the apex of the hilus. A series of perspective images of PNSC nuclei (green) and blood vessels (red) when tunneling into the hippocampus are shown in **e**. Backward perspective images were created at different depths. After passing through the SGZ, no PNSC nuclei were seen ahead. These images are animated in **Supplementary Video 4**. RINZO automatically calculated the distance (white lines) from each PNSC nucleus (green) to the nearest blood vessel (red) surface (**f**). Histograms show the distribution of distances to blood vessels for all SGZ cell nuclei (violet), and for PNSC nuclei (green). Cell numbers (**g**) or their frequencies (**h**) were plotted. The real distance is shown in parentheses. Scale bars represent 500 μm (**b**,**c**) and 20 μm (**e**,**f**).


A
Isolation of the brain
Chopping
Sonication
+PFA
Fixed brain suspension
+Chemical solutions
Solubilization
OD600
PBS
0.1% Triton X-100
15% Triton X-100
15% Triton X-100 +24% Urea
B
Chosen for second chemical screening
#18: Glycerol
Relative OD600 (%)
PBS
ID#: 1 2 3 4 5 8 9 10 11 12 15 16 17 18 19 20 21 23 25 26 27 28 29 30 31 33 36 38 39 40
Polyhydric alcohols
Detergents
Urea-like
C
Chosen for second chemical screening
#18: Glycerol
Relative fluorescence (%)
PBS
ID#: 1 2 3 4 5 8 9 10 11 12 15 16 17 18 19 20 21 23 25 26 27 28 29 30 31 33 36 38 39 40
Polyhydric alcohols
Detergents
Urea-like


A
Fixed brain
5 mm
#10
#25
#36
Reagent-1
~7 days
Counterstain
Lipid removed
Wash
Washed
#16
#25
#36
Sucrose
Reagent-2
2~7 days
RI adjusted
Wash
Stock
B
% Transmittance (whole brain)
120
100
80
60
40
20
0
360
400
440
480
520
560
600
640
680
740
Wavelength (nm)
20% sucrose/PBS (N = 1)
Reagent-1 (N = 1)
Reagent-2 (N = 1)
Fixed brain (N = 3)
Reagent-1 treated brain (N = 4)
Reagent-2 treated brain (N = 3)
C
24 h
7 days
PBS (< 30 min.)
PBS
Reagent-1
Reagent-2
Fluorescence (A.U.)
10000000
1000000
100000
10000
1000
100
10
1
EGFP
EYFP
mCherry
mKate2
D
Reagent-1
1 mm
2 mm
3 mm
4 mm

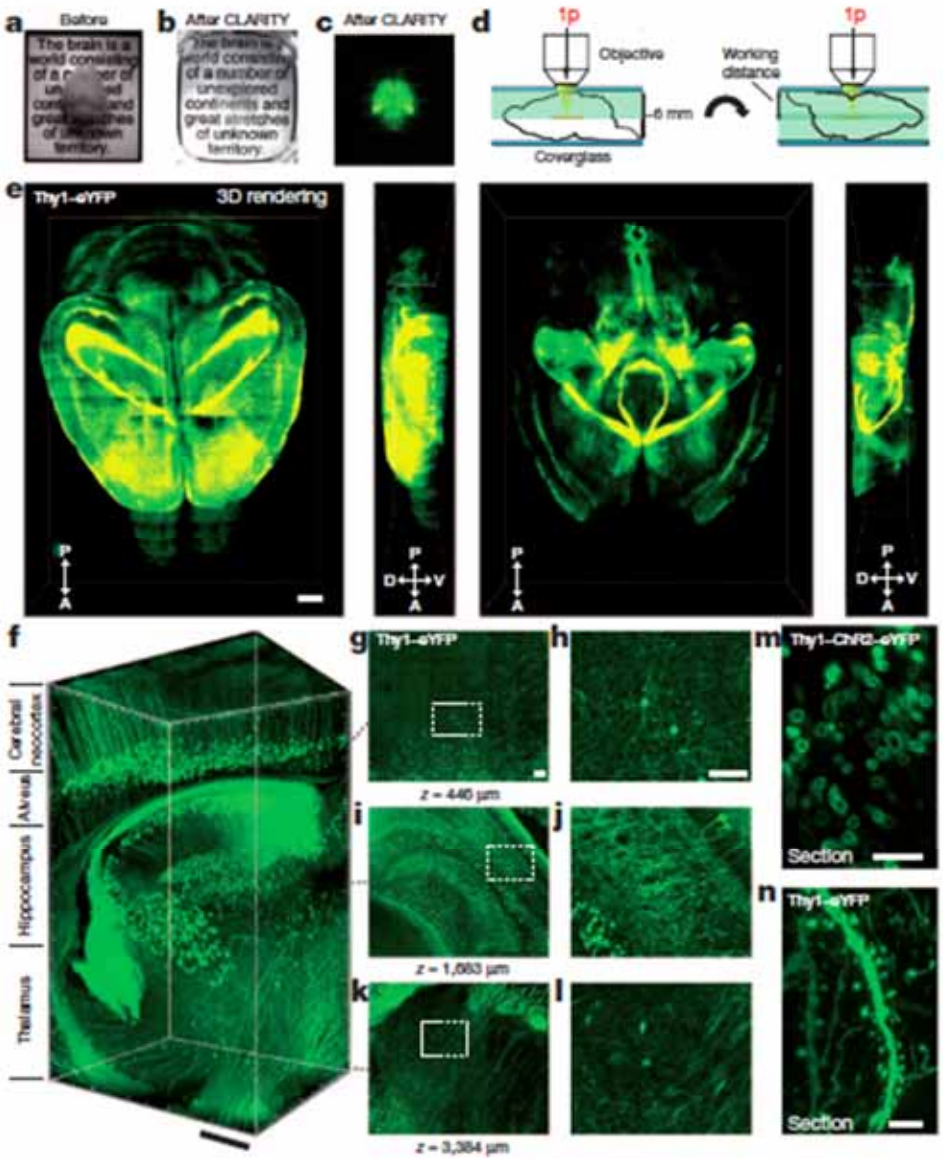

A
Mouse brain (Adult)
Marmoset brain (P3)
1 cm
B
Sucrose/PBS
Reagent-1
Reagent-2
1 cm
C
D
E
1 mm
D
1 mm
E
1 mm

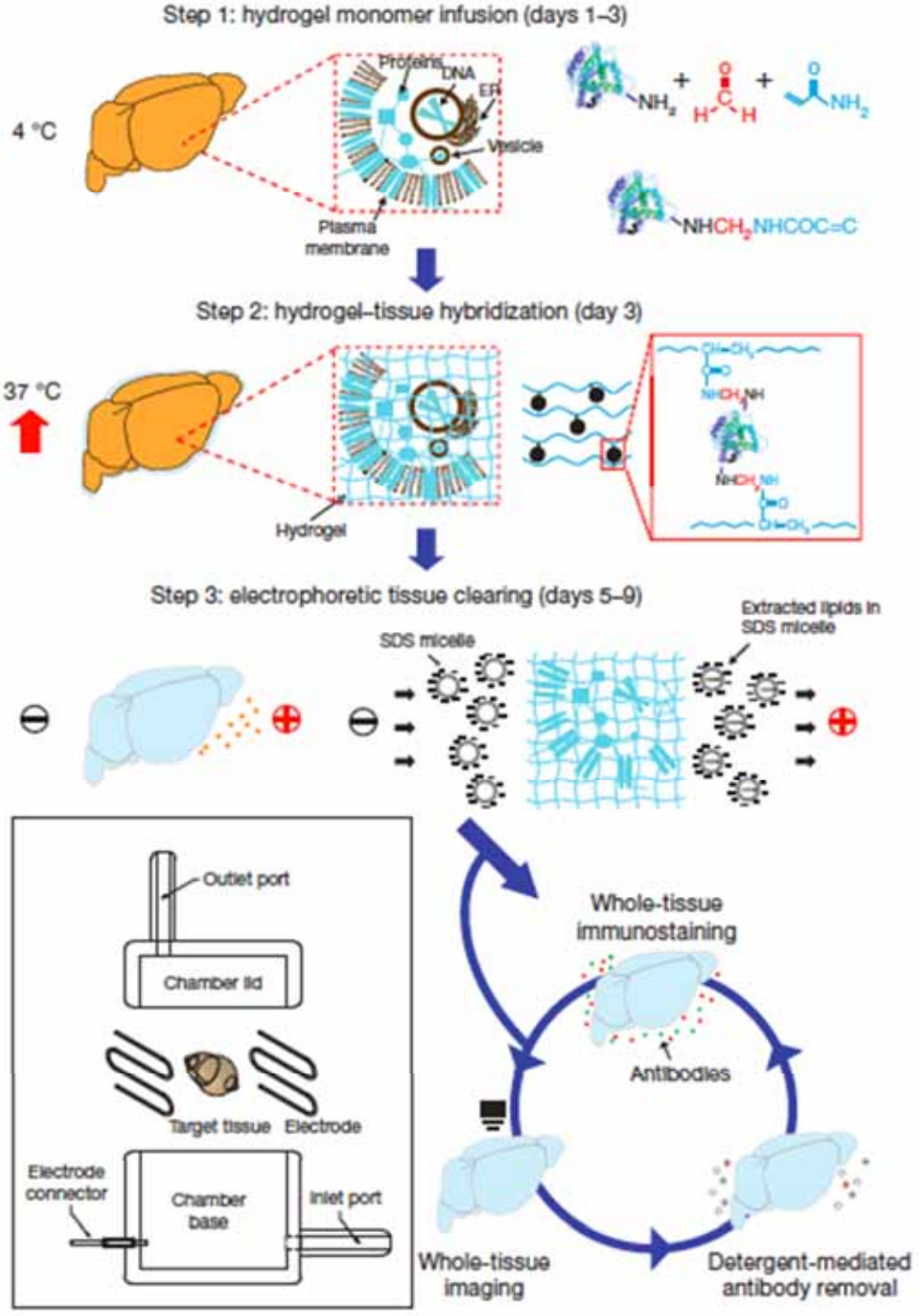
ARTICLE

doi:10.1038/nature12107

Structural and molecular interrogation of intact biological systems

Kwanghun Chung[1,2], Jenelle Wallace[1], Sung-Yon Kim[1], Sandhiya Kalyanasundaram[2], Aaron S. Andalman[1,2], Thomas J. Davidson[1,2], Julie J. Mirzabekov[1], Kelly A. Zalocusky[1,2], Joanna Mattis[1], Aleksandra K. Denisin[1], Sally Pak[1], Hannah Bernstein[1], Charu Ramakrishnan[1], Logan Grosenick[3], Viviana Gradinaru[2] & Karl Deisseroth[1,2,3,4]





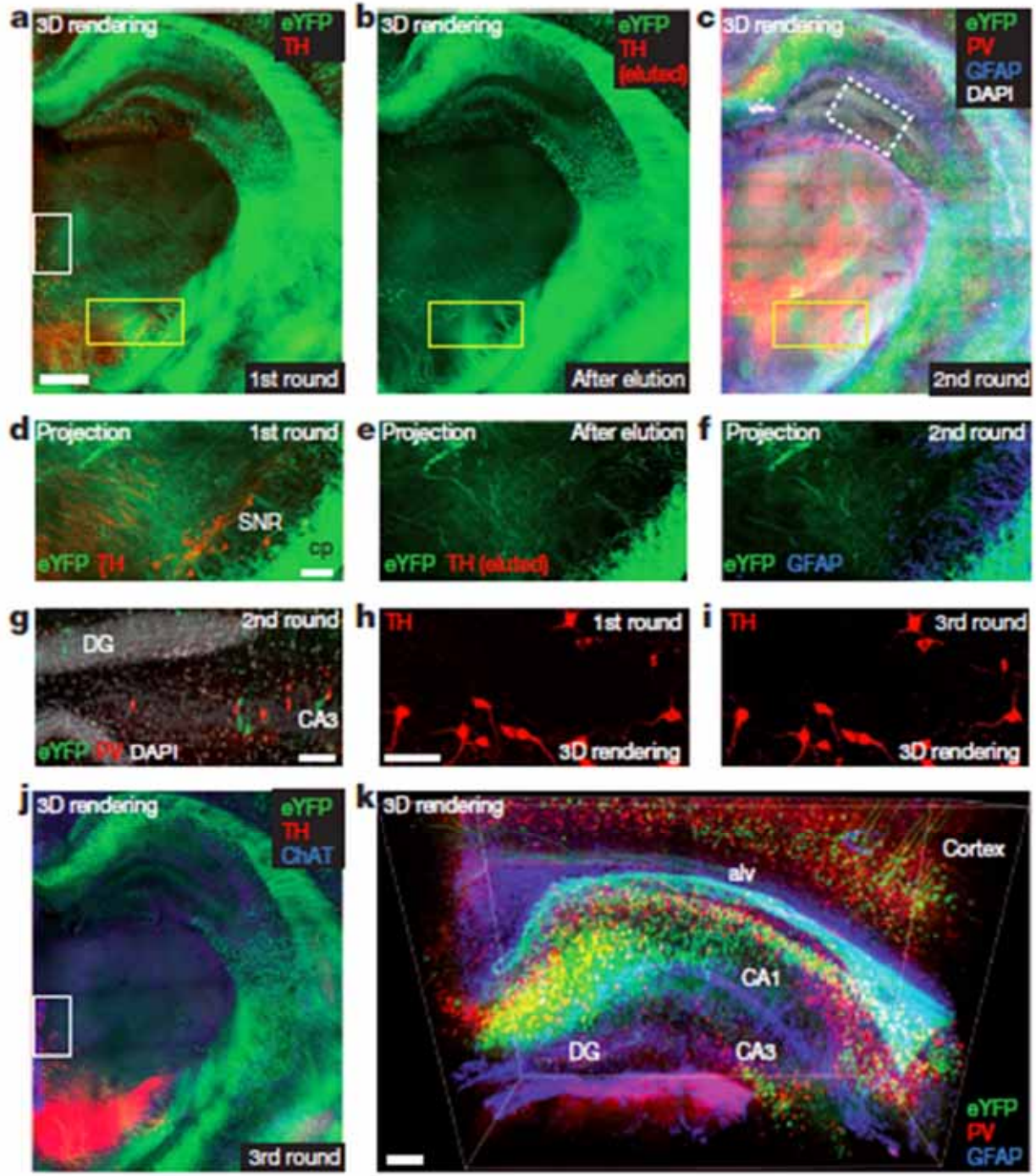
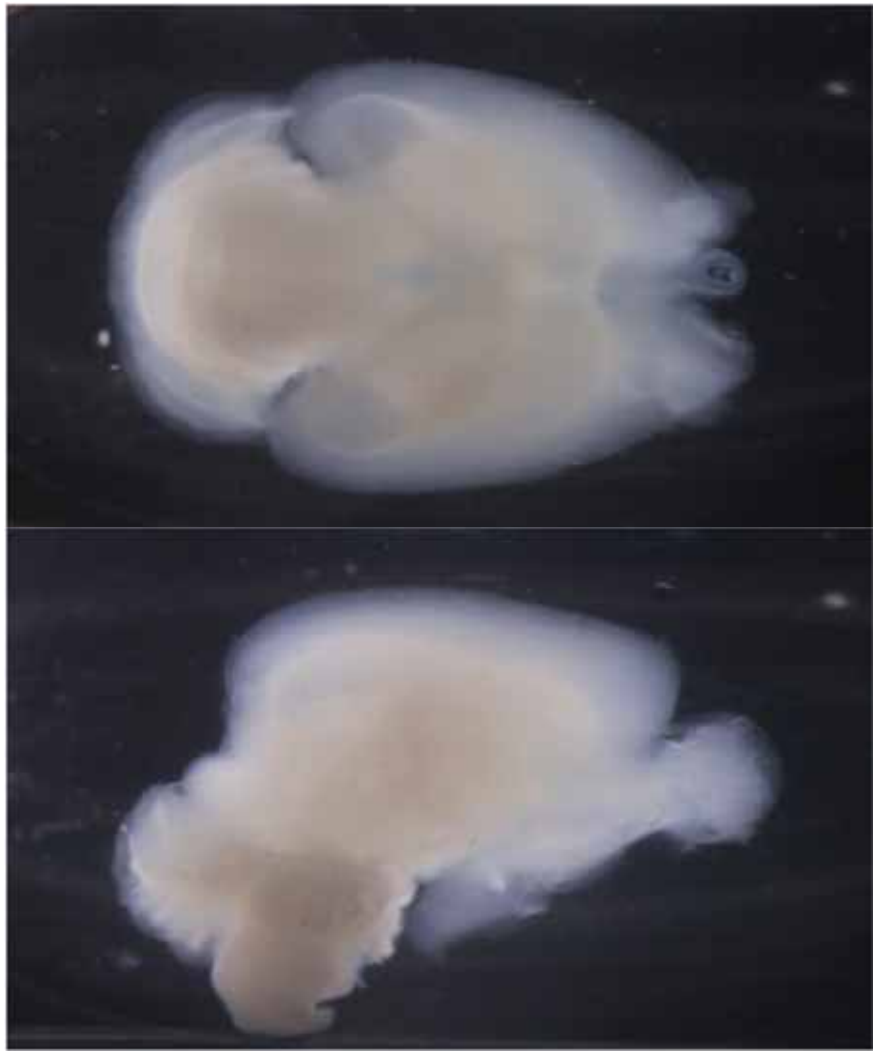
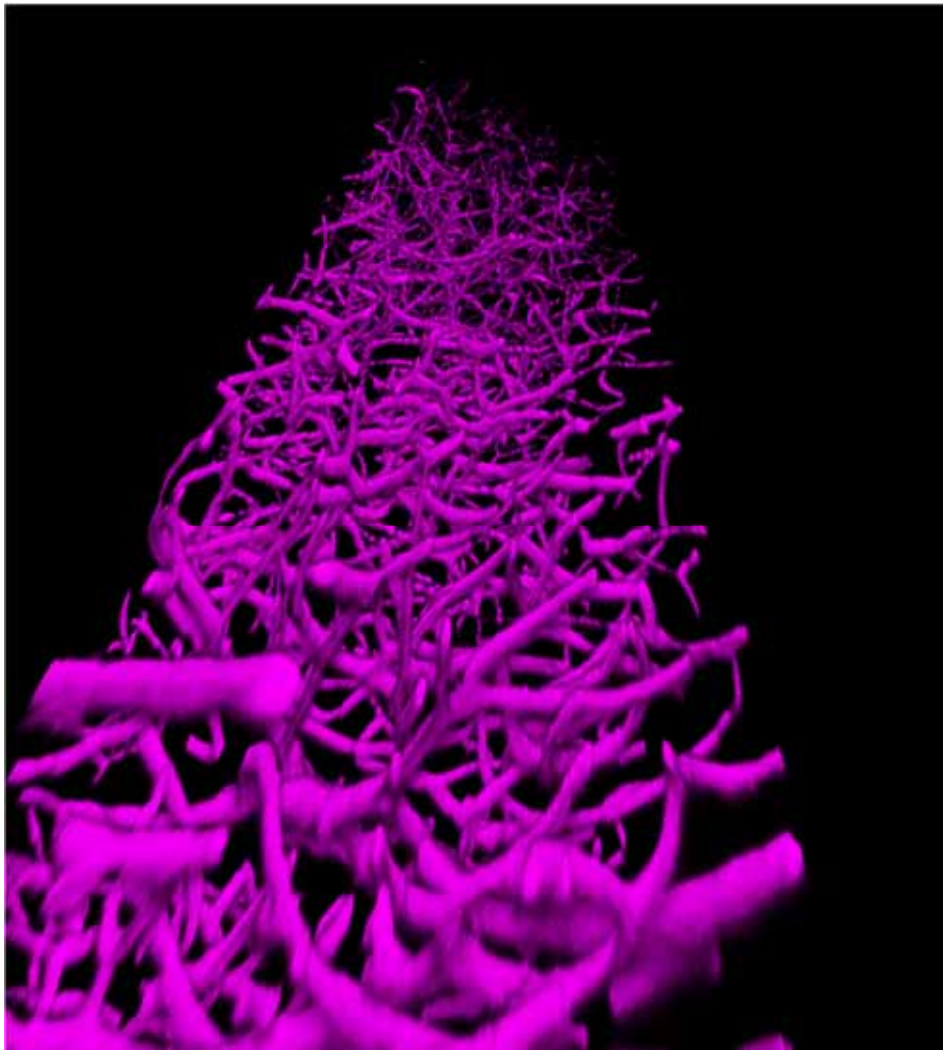
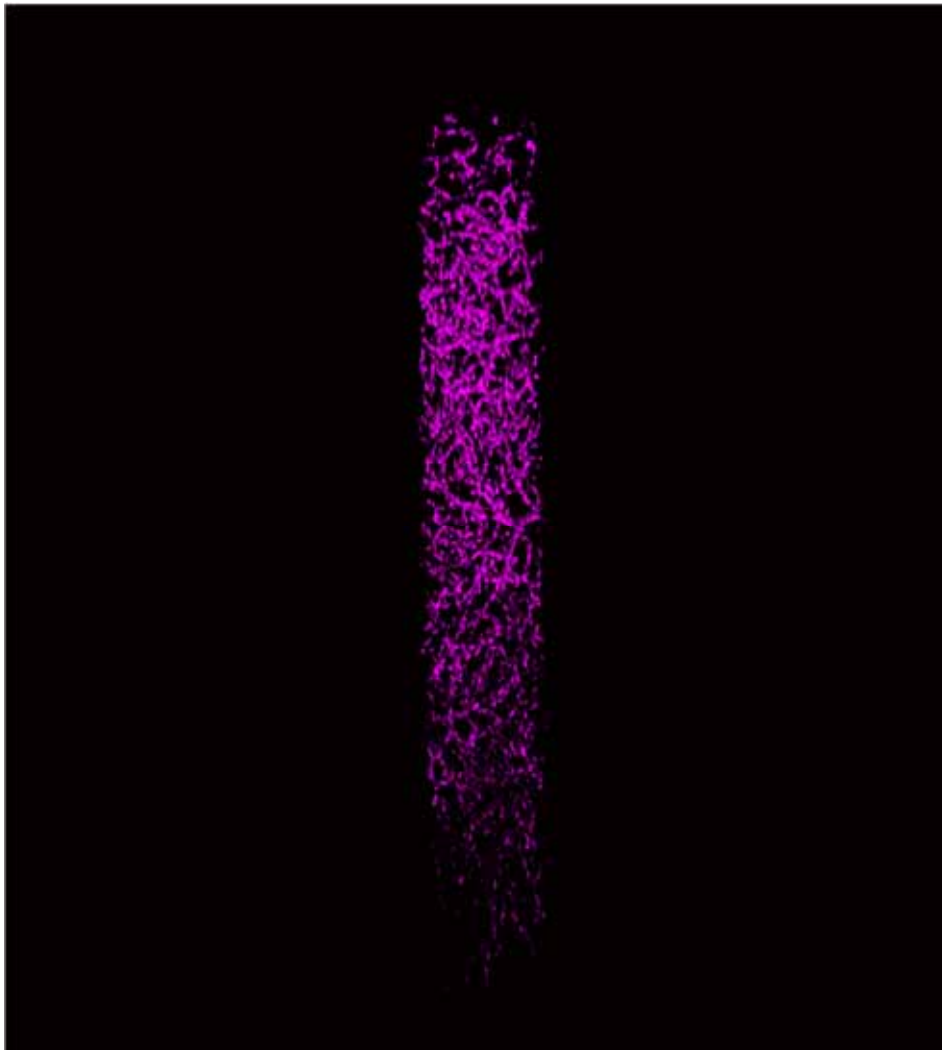
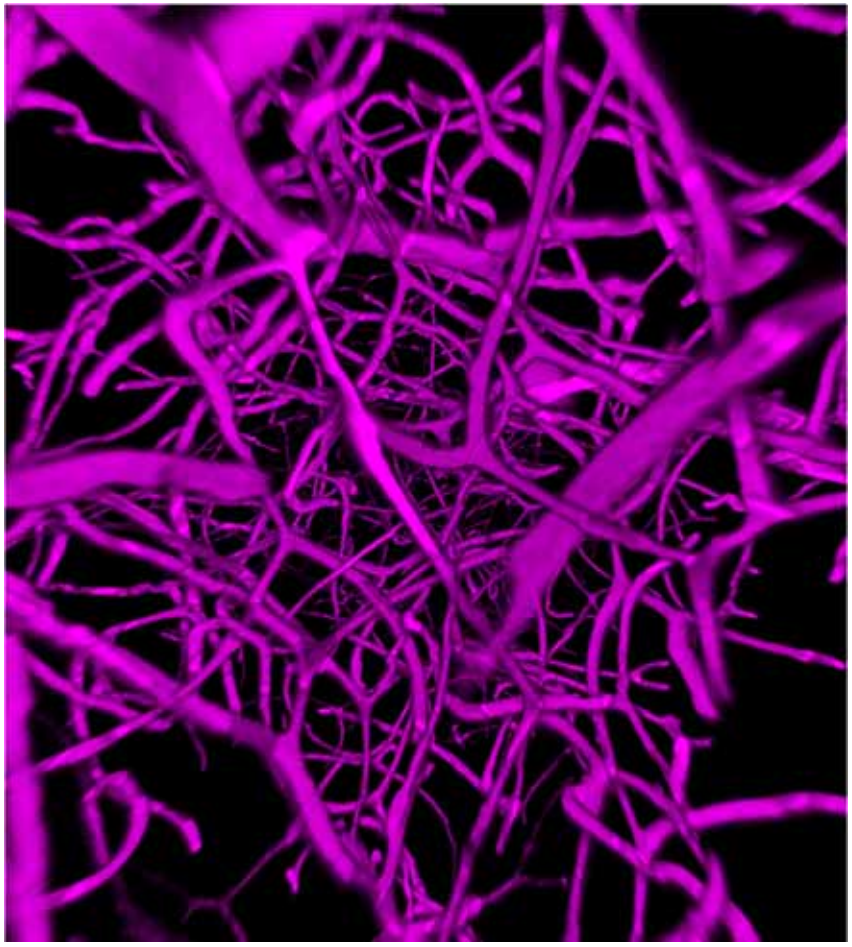


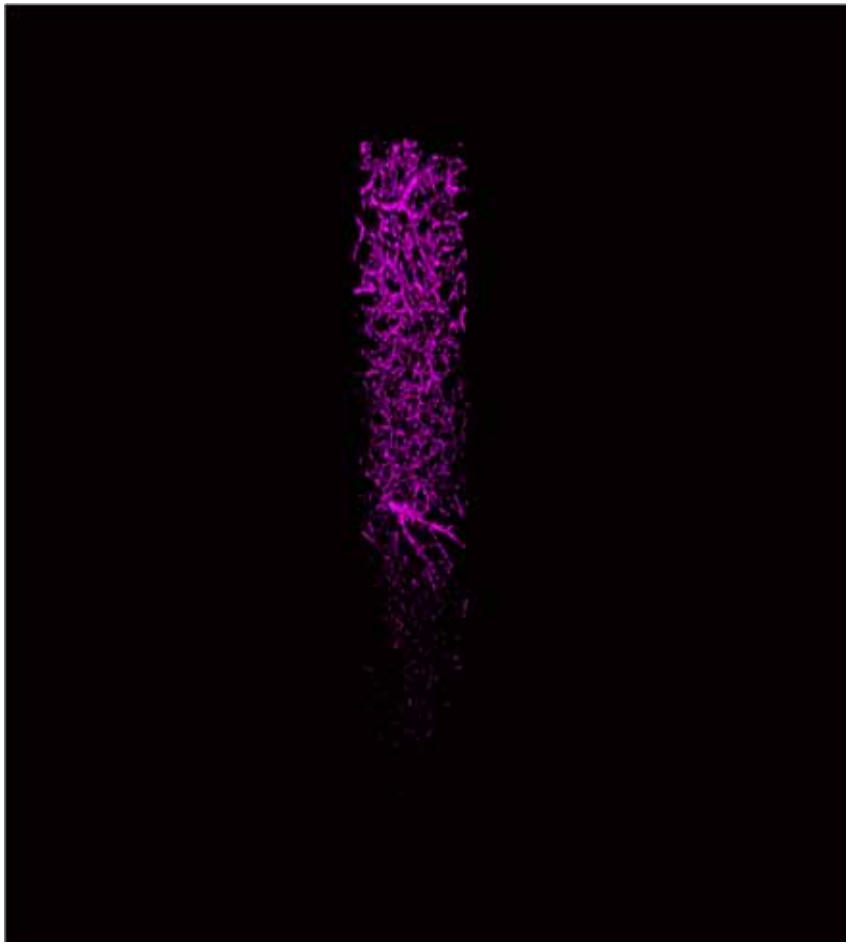
Figure 4 | Multi-round molecular phenotyping of intact tissue. a, First



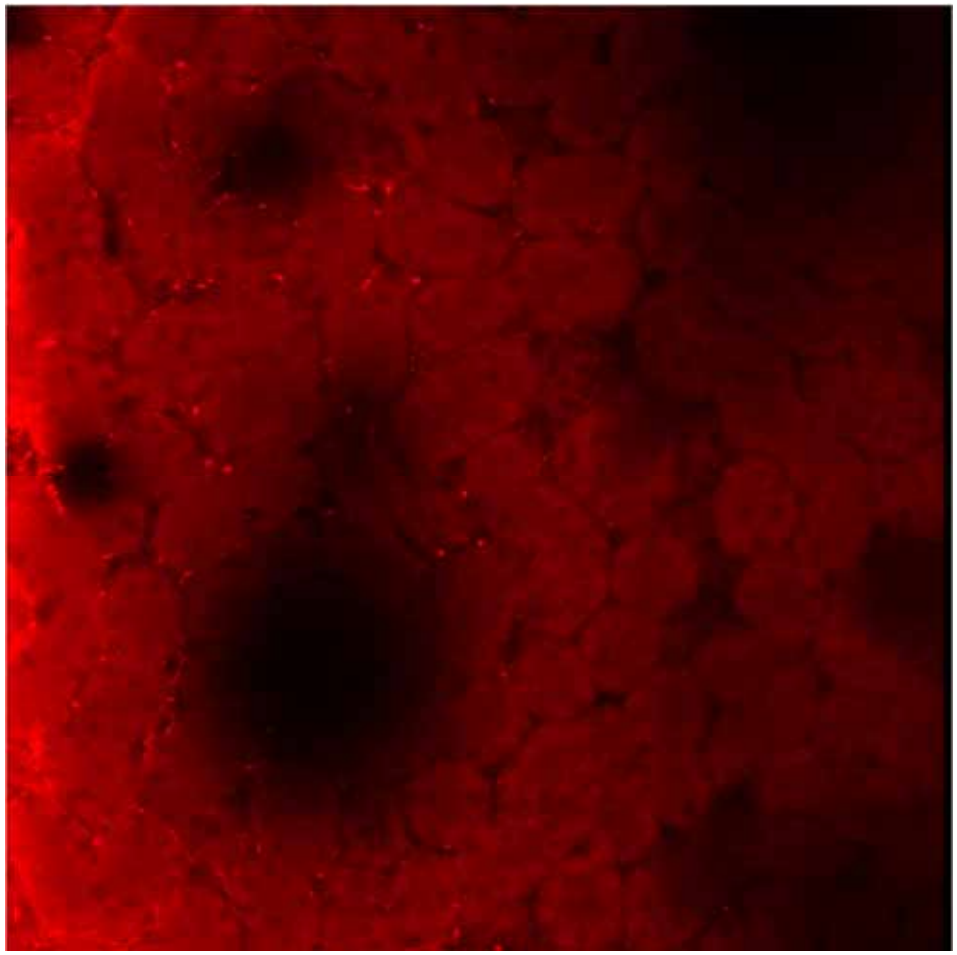


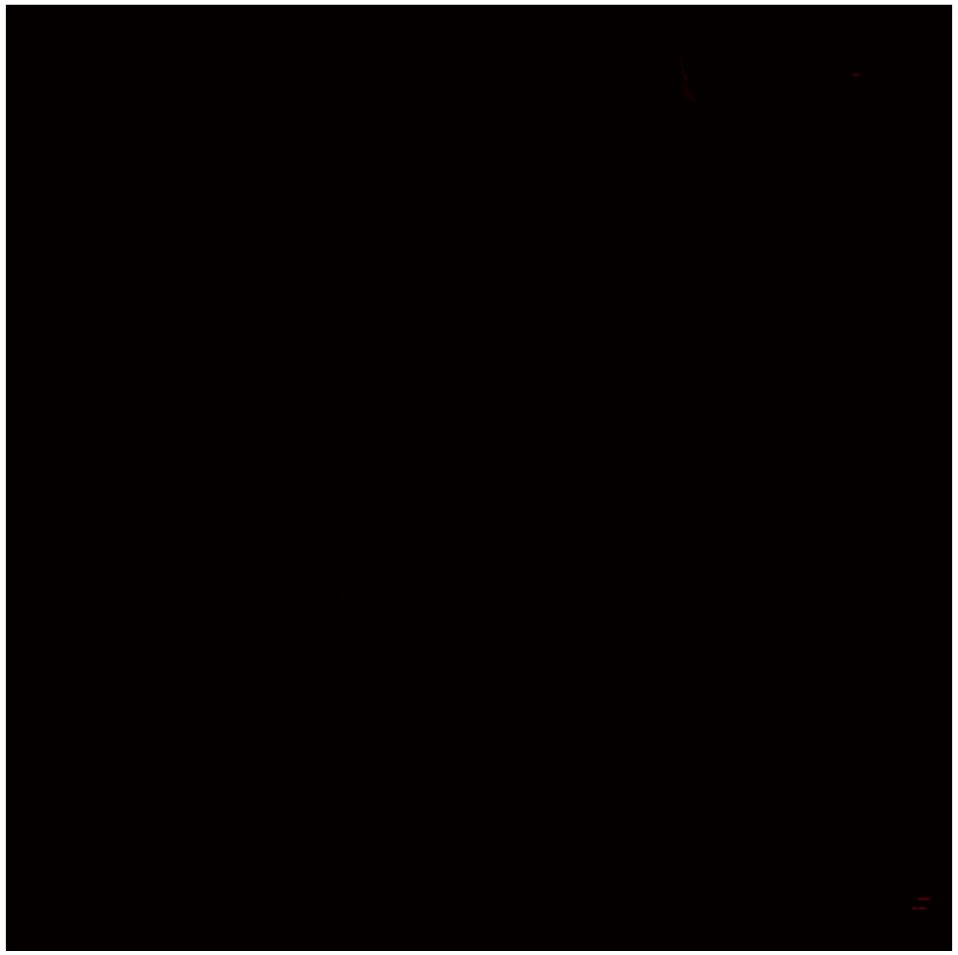






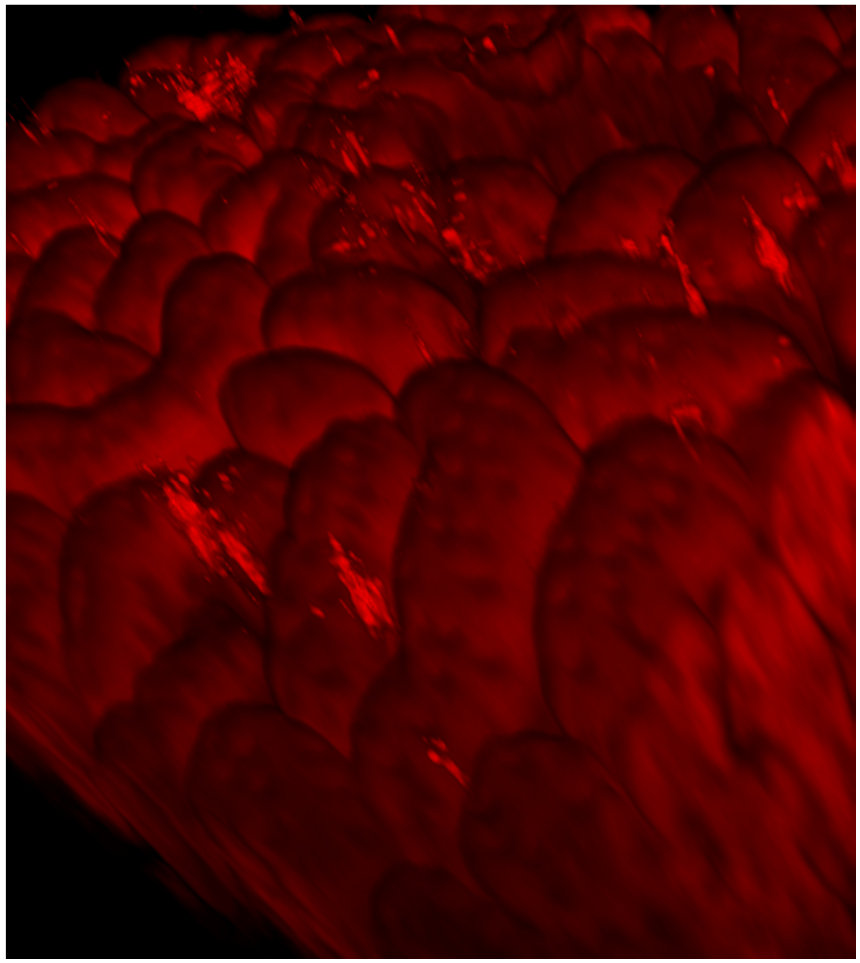
Mouse Kidney (Normal)

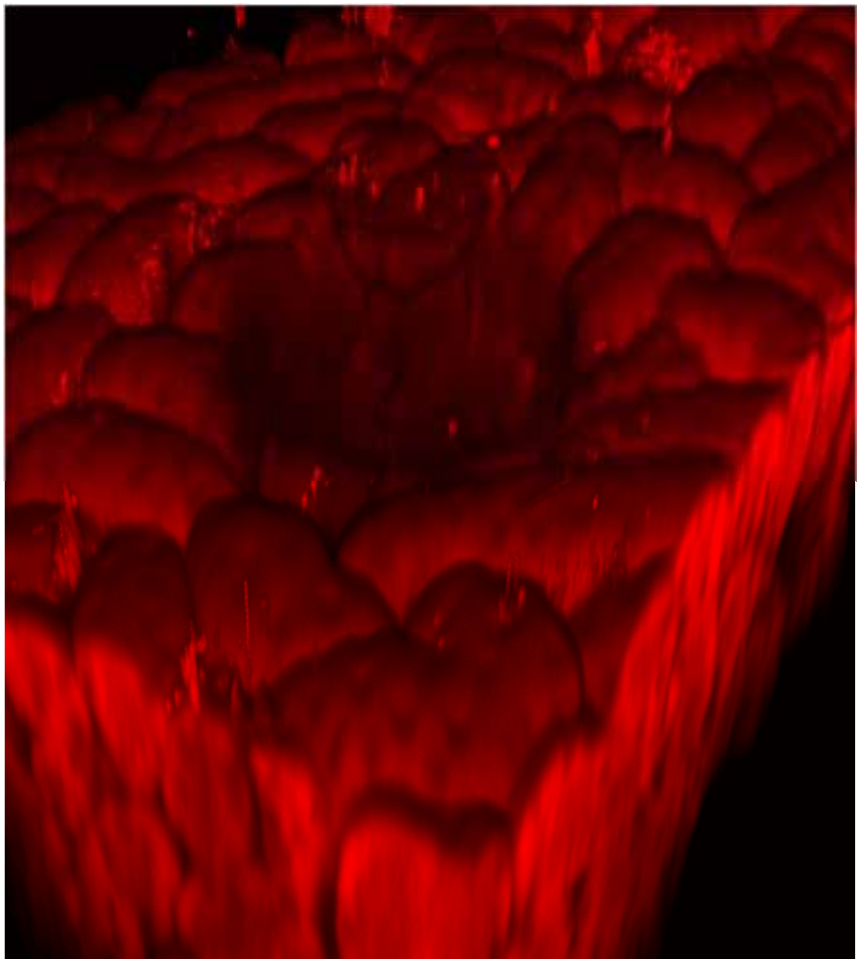




Red: two photon

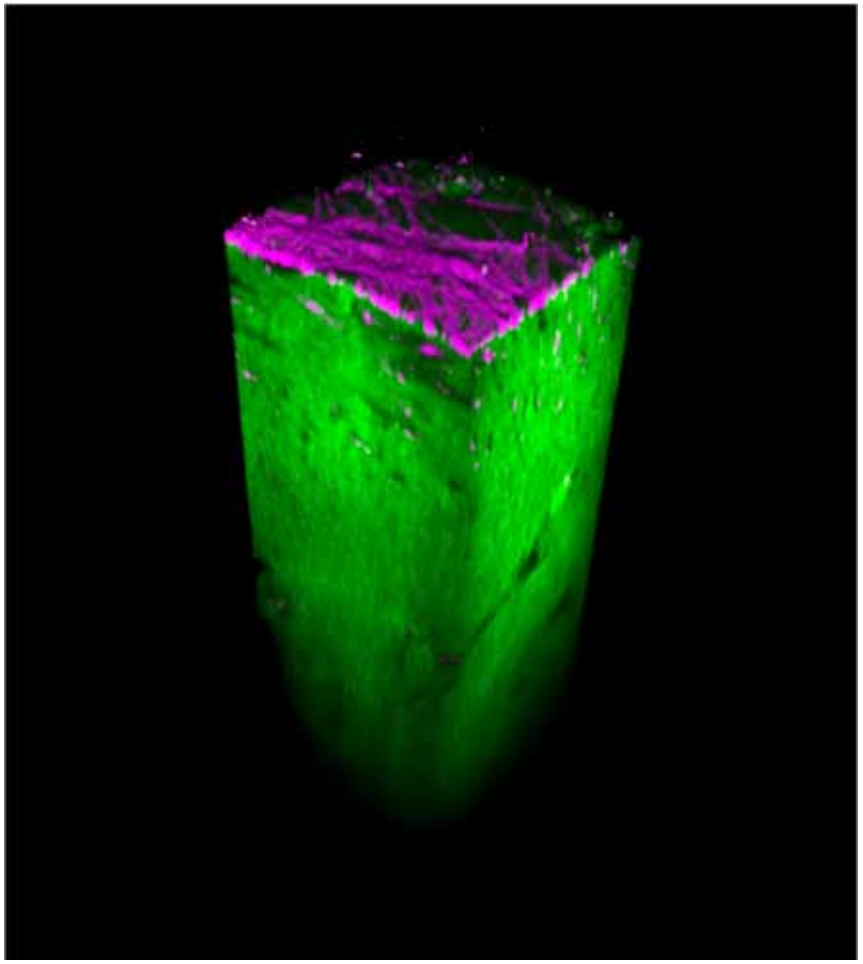
Mouse Kidney (Normal)

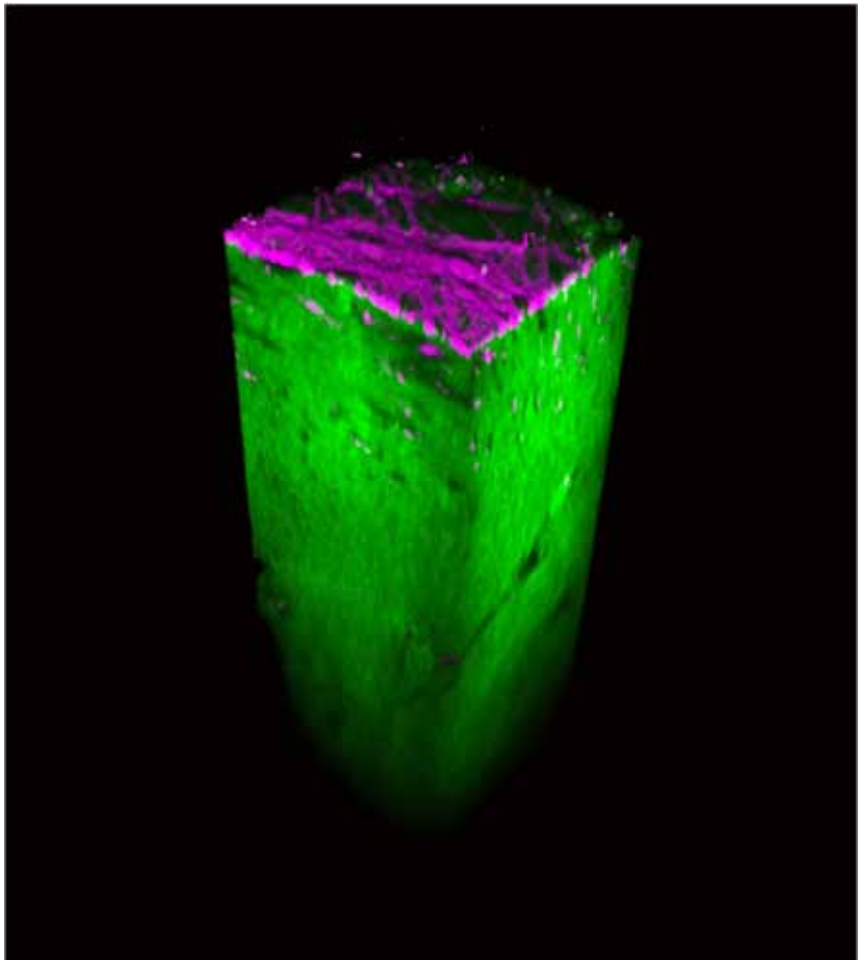




Red: two photon

Mouse heart

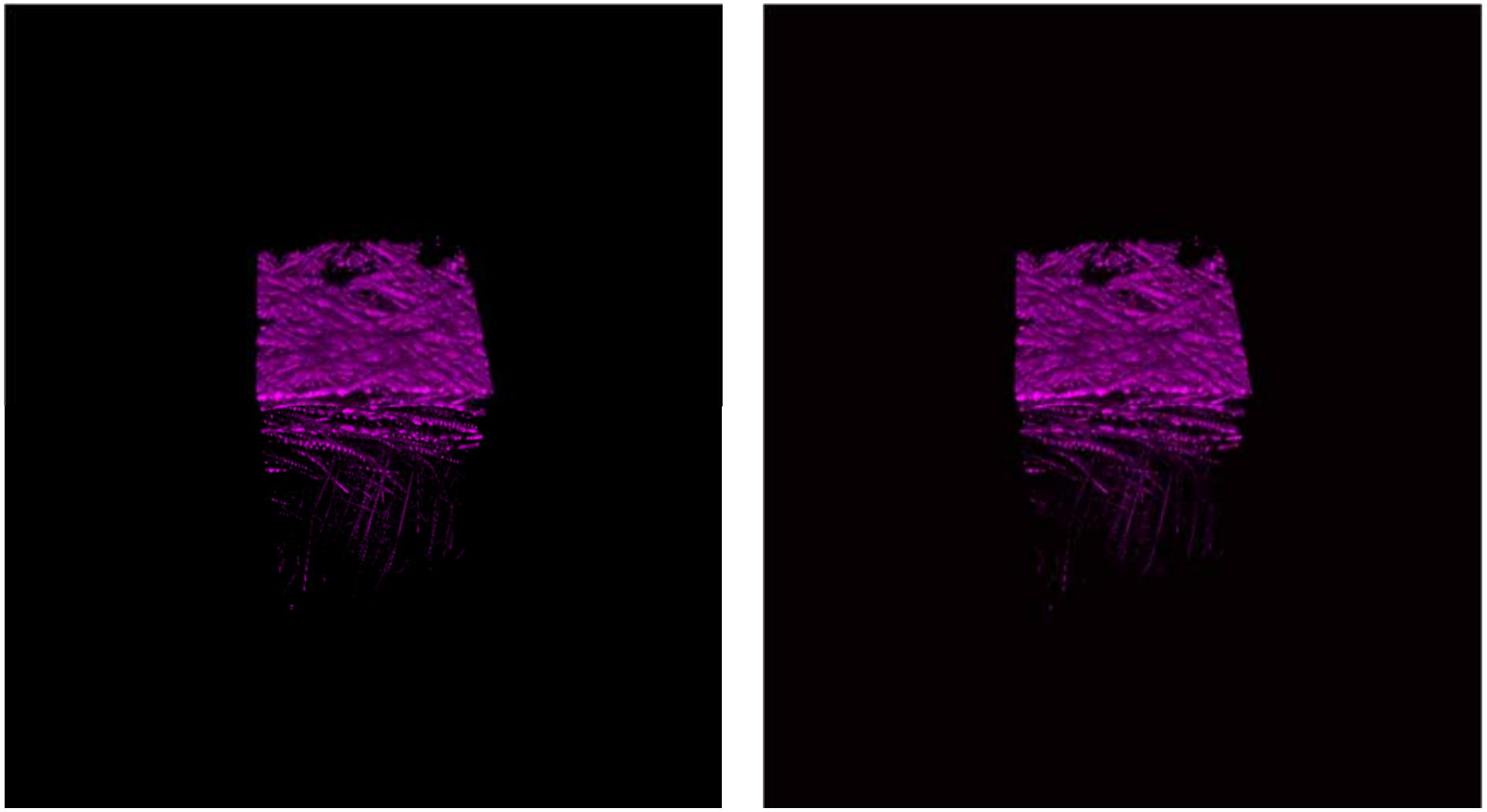




Green: two photon

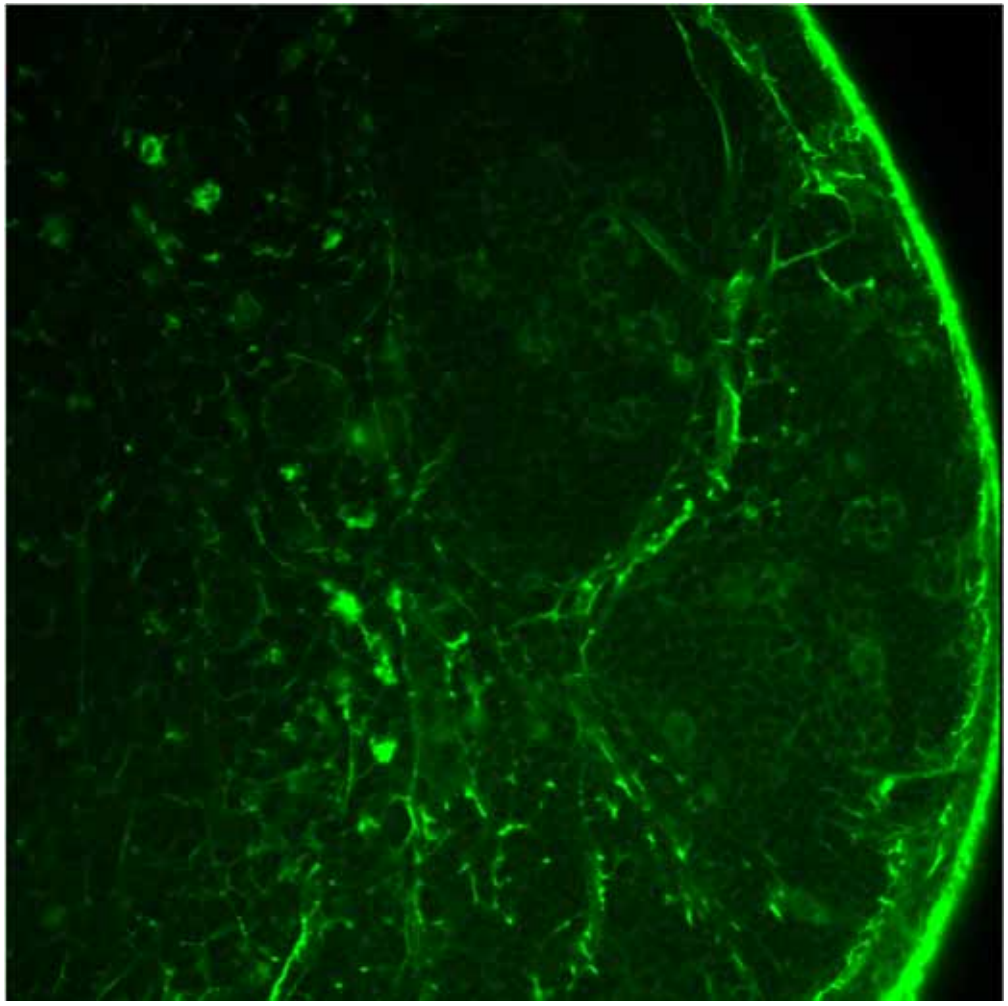
Magenta: SHG

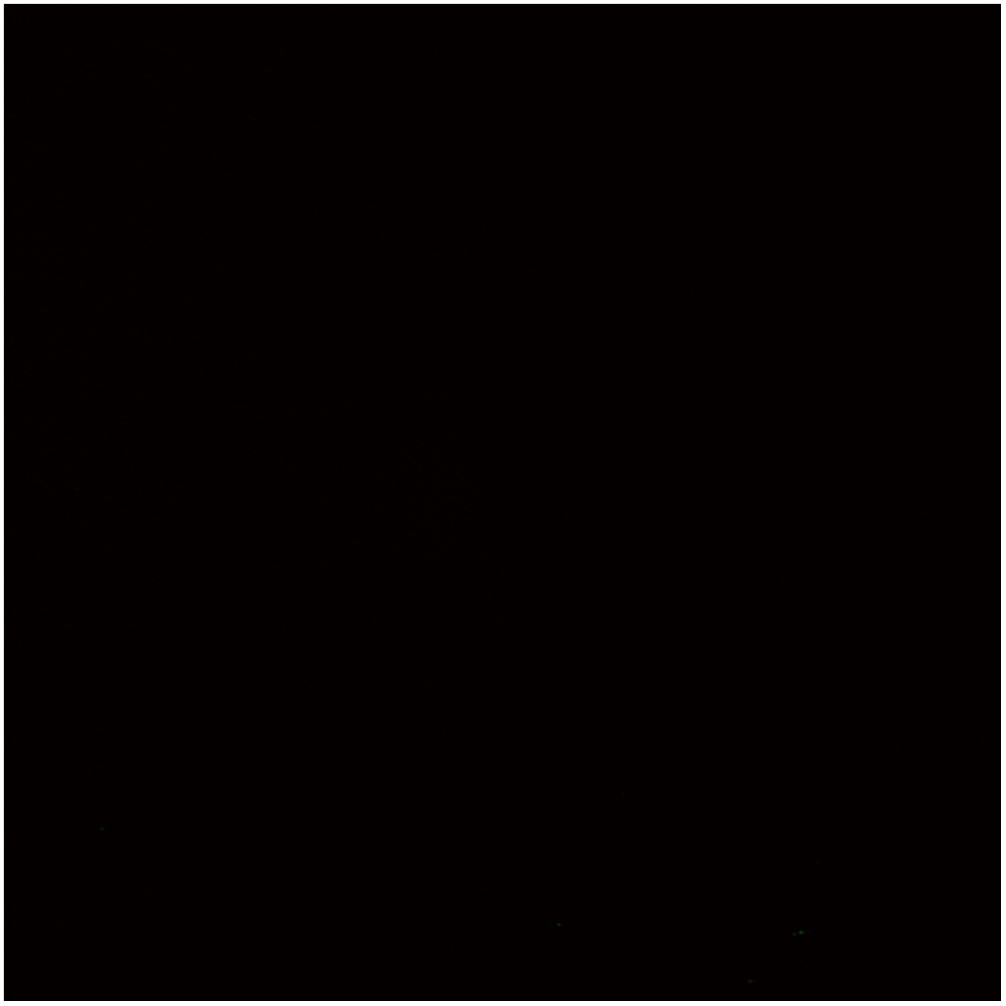
Mouse heart



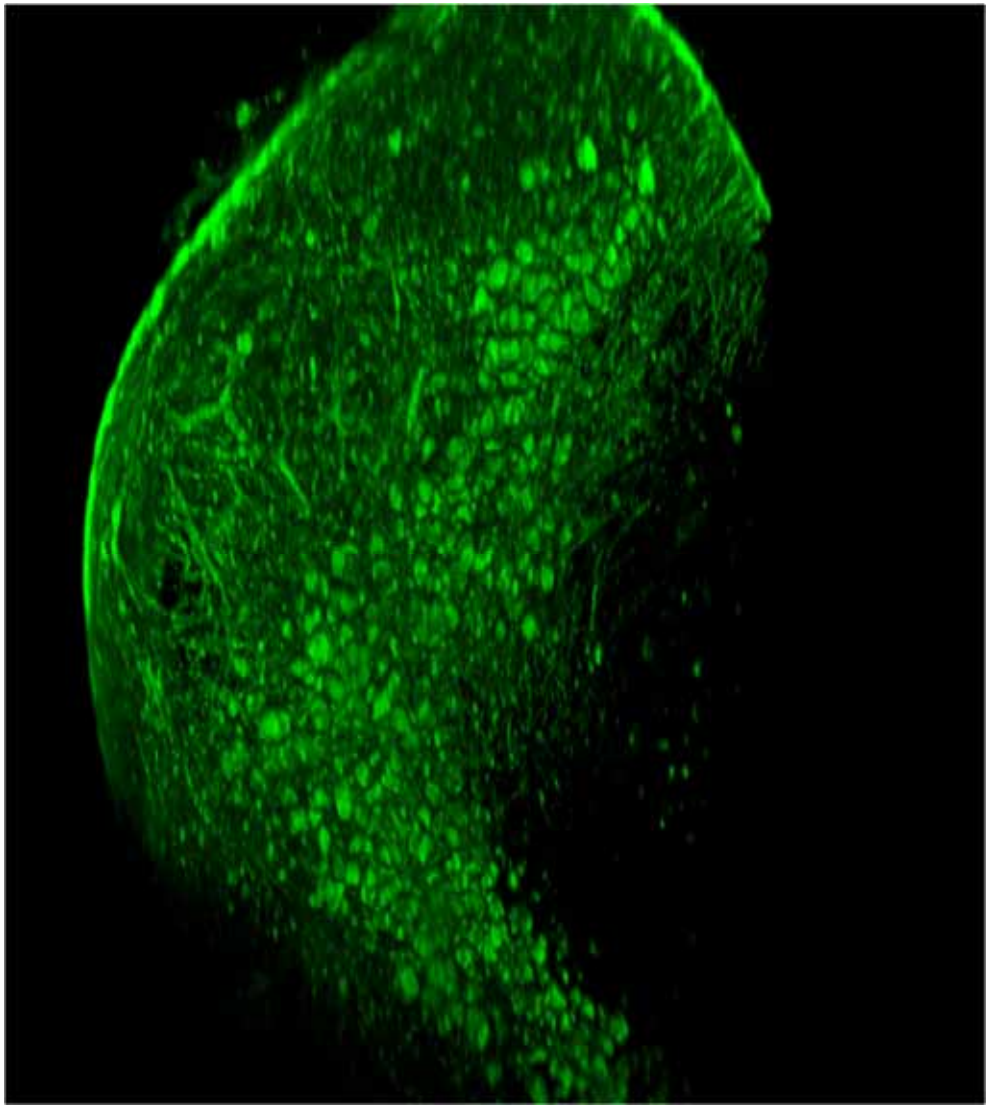
Magenta: SHG

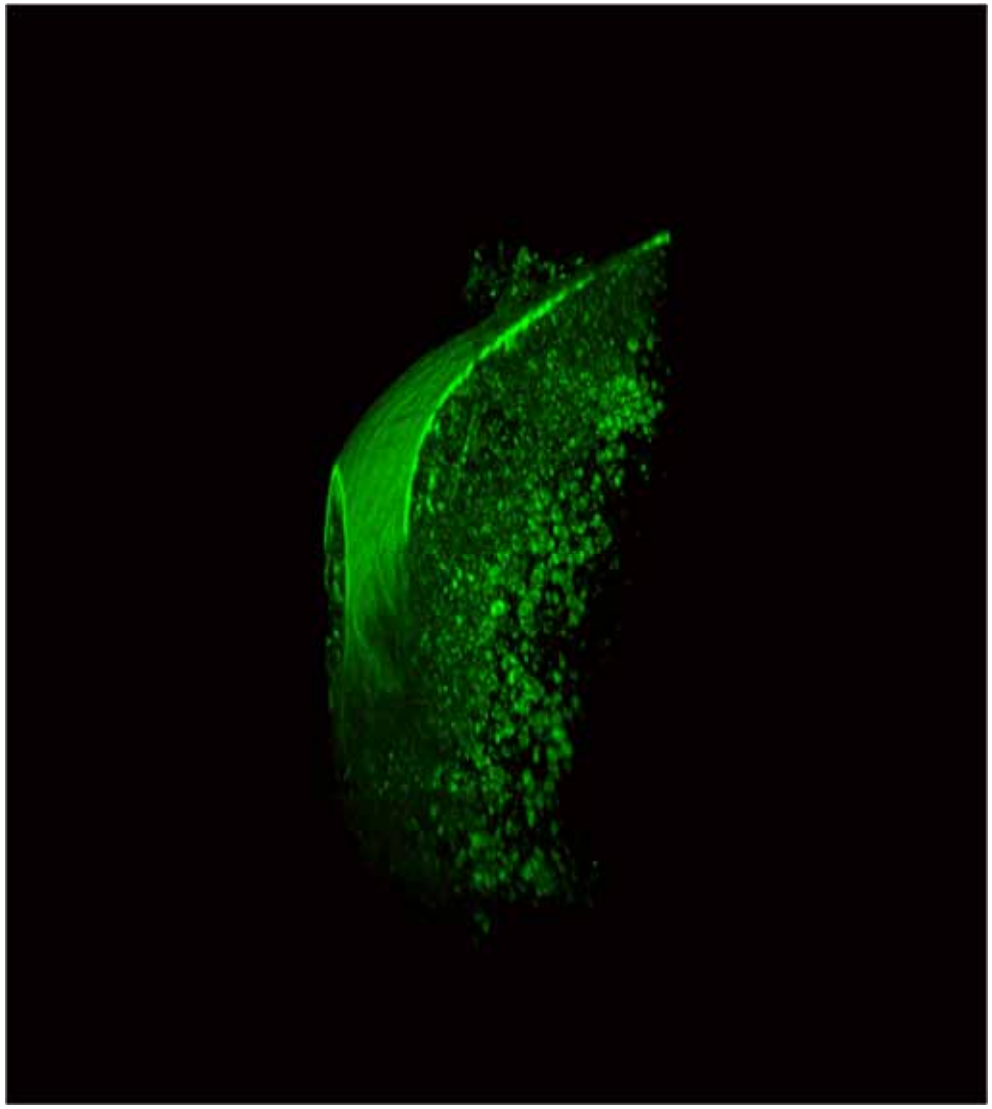
Lymph node (intestines)





Lymph node (intestines)





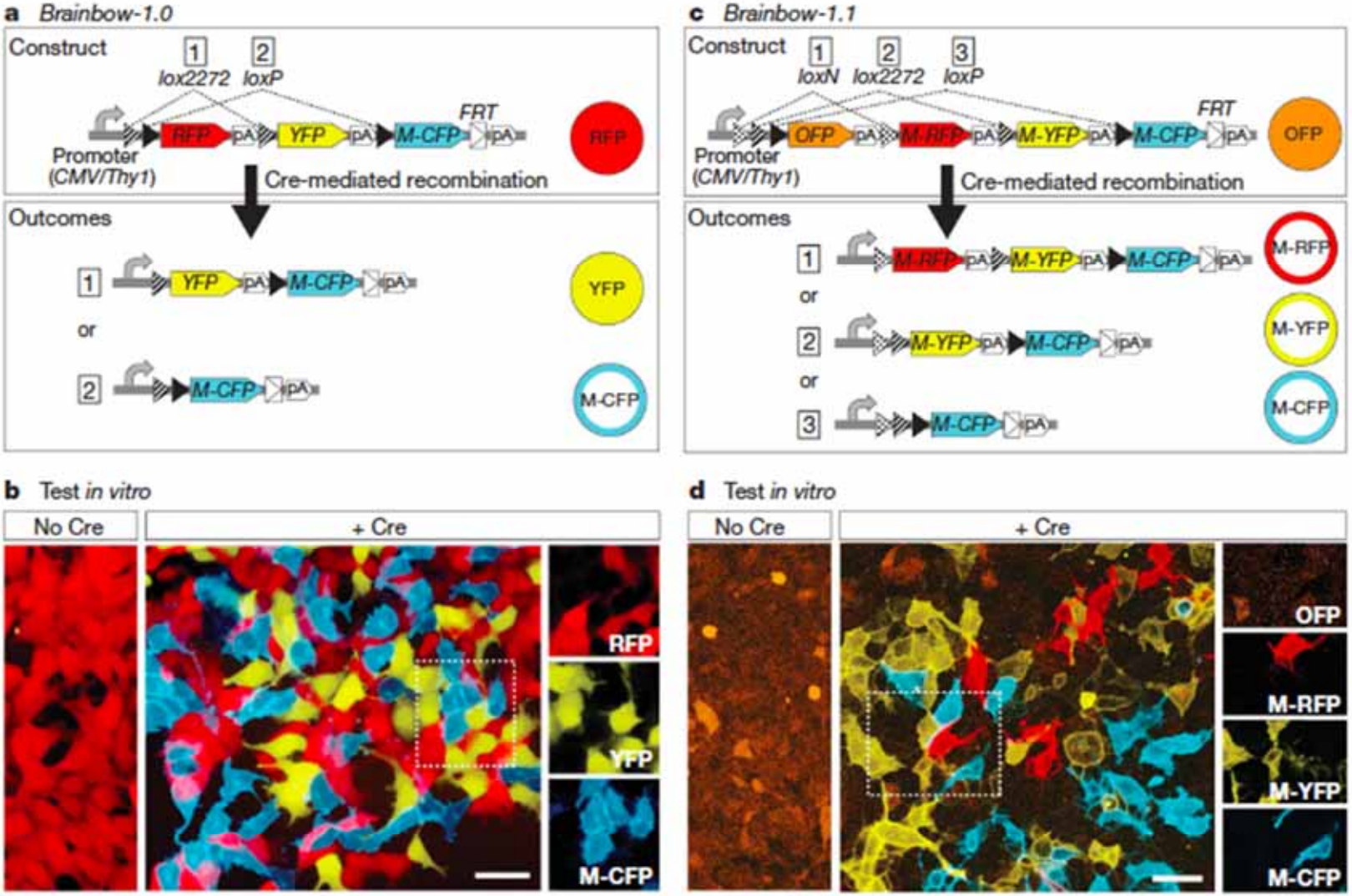
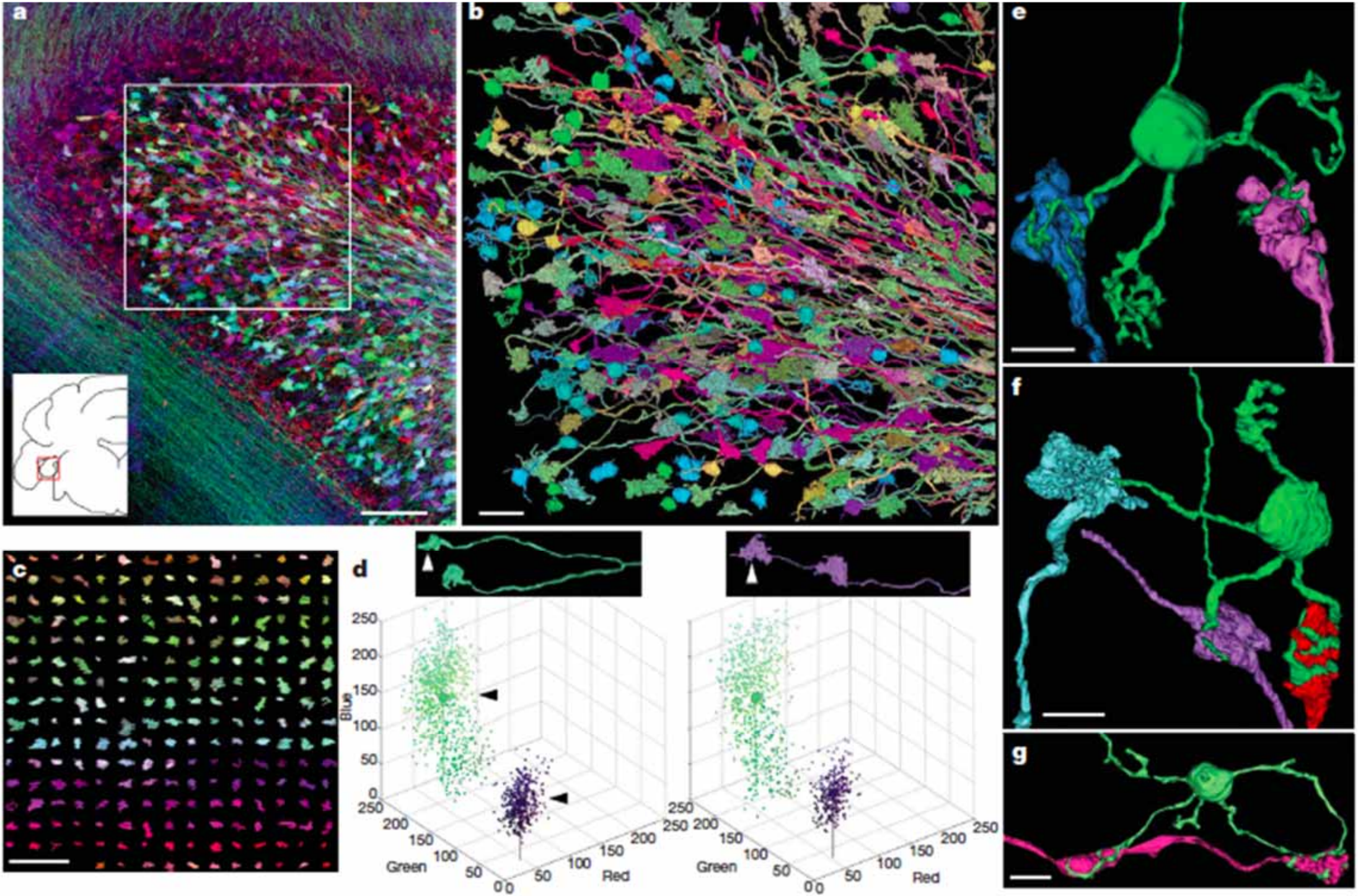
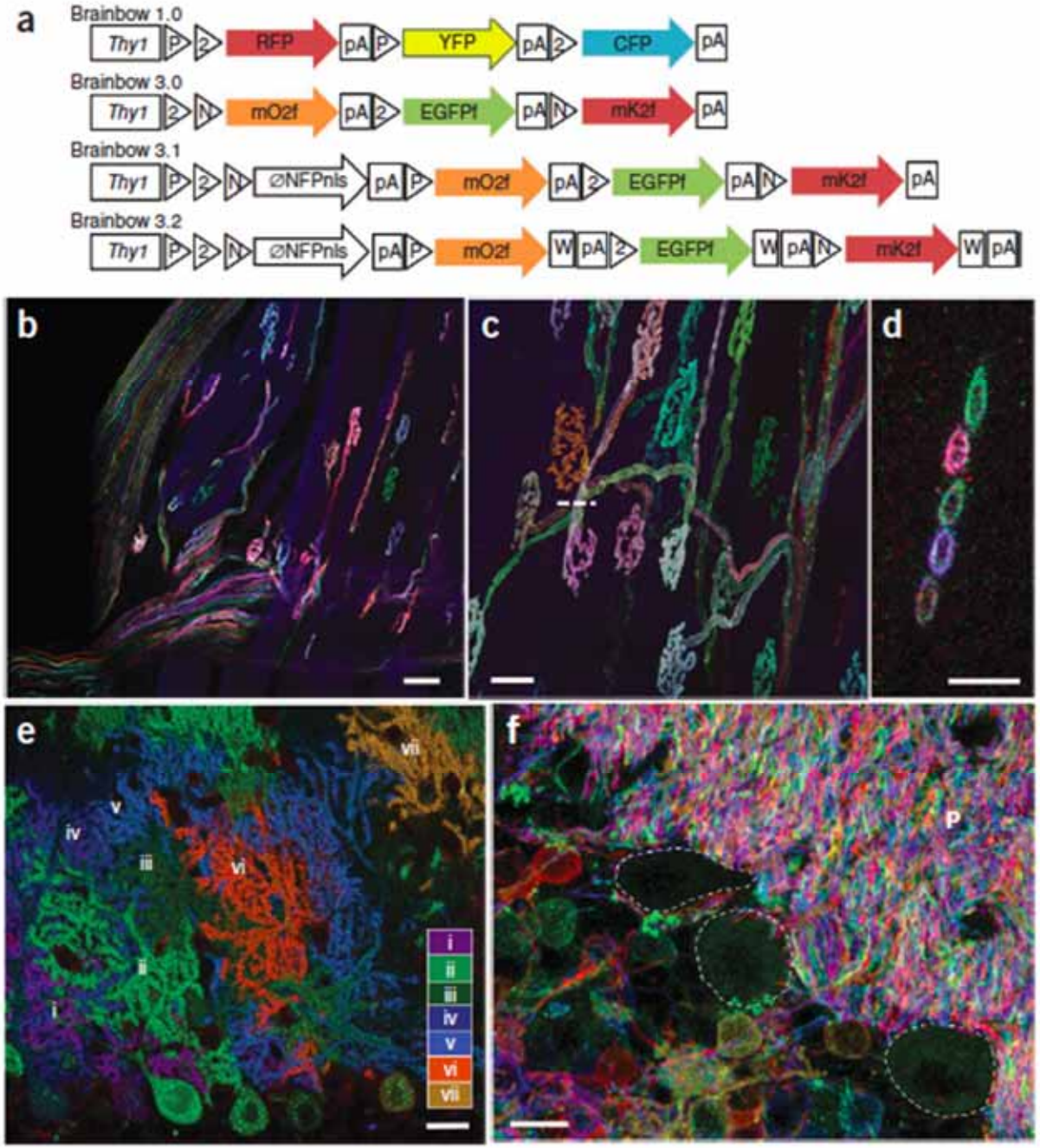


Figure 1 | *Brainbow-1*: stochastic recombination using incompatible *lox* variants. **a**, In *Brainbow-1.0*, incompatible sets of *lox* sites alternate: Cre chooses between excision events 1 or 2. Before Cre action, only the gene following the promoter is expressed (RFP). Recombination switches expression to either YFP (1) or M-CFP (2). **b**, HEK cells stably transfected with *CMV-Brainbow-1.0* express RFP. On transient transfection with Cre, these cells randomly switch to YFP or M-CFP expression. **c**, In *Brainbow-1.1*, a third set of incompatible *lox* sites (*loxN*) is added, creating three recombination possibilities (1, 2 or 3), switching OFP expression to RFP, YFP or CFP expression. **d**, Cells stably transfected with *Brainbow-1.1* express OFP. Cre recombination leads to expression of M-RFP, M-YFP or M-CFP. pA, polyadenylation signal; M-XFP, membrane-tethered XFP. *FRT* site allows reduction of transgene arrays (Fig. 4d). Scale bar, 50 μm.


a
b
c
d
e
f
g
Blue
Green
Red
0
50
100
150
200
250


a
Brainbow 1.0
Thy1
P
2
RFP
pA
P
YFP
pA
2
CFP
pA
Brainbow 3.0
Thy1
2
N
mO2f
pA
2
EGFPf
pA
N
mK2f
pA
Brainbow 3.1
Thy1
P
2
N
∅NFPnls
pA
P
mO2f
pA
2
EGFPf
pA
N
mK2f
pA
Brainbow 3.2
Thy1
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2
N
∅NFPnls
pA
P
mO2f
W
pA
2
EGFPf
W
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i
ii
iii
iv
v
vi
vii
f
P

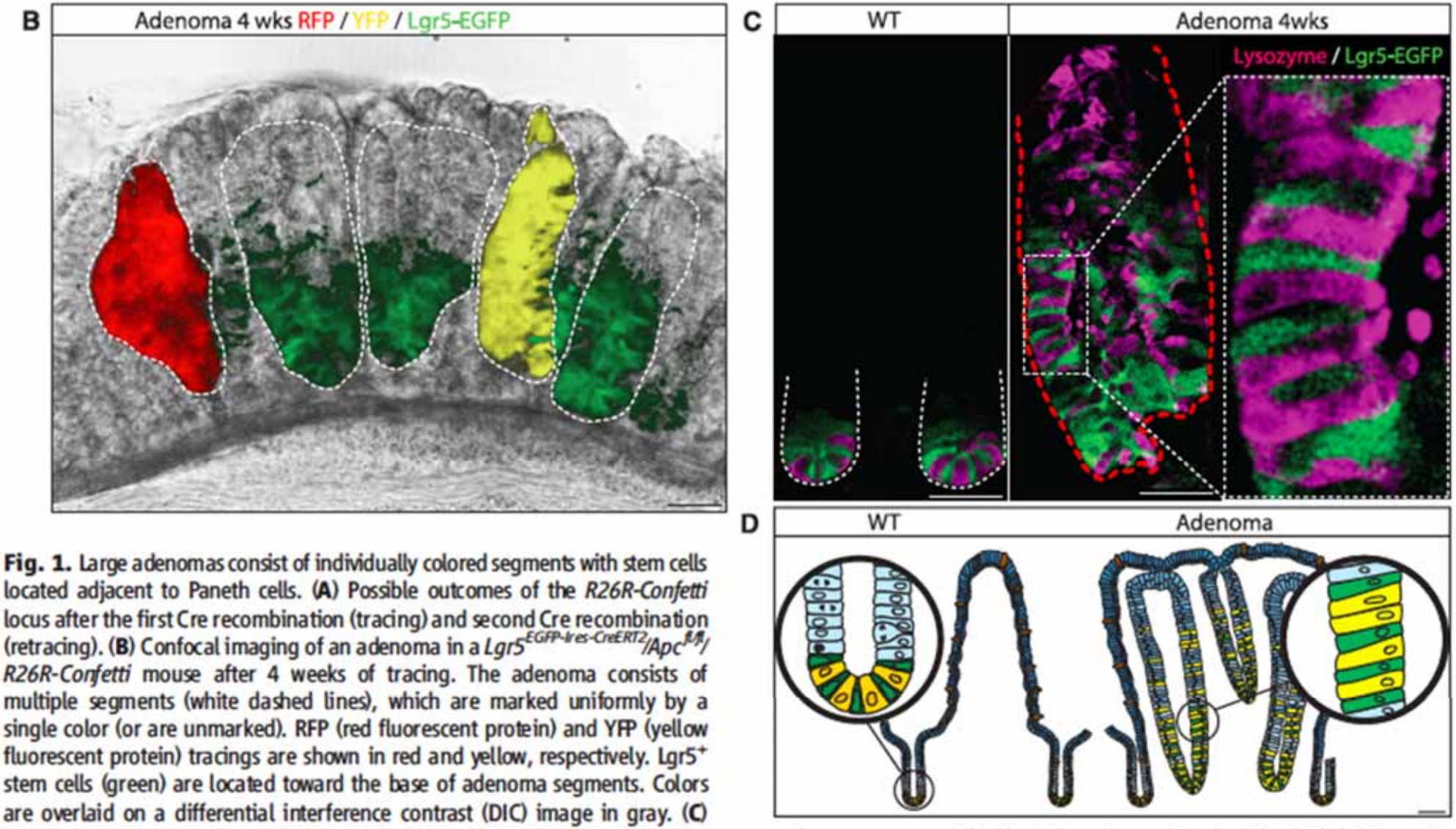

Fig. 1. Large adenomas consist of individually colored segments with stem cells located adjacent to Paneth cells. (**A**) Possible outcomes of the *R26R-Confetti* locus after the first Cre recombination (tracing) and second Cre recombination (retracing). (**B**) Confocal imaging of an adenoma in a *Lgr5*$^{EGFP\text{-}Ires\text{-}CreERT2}$/*Apc*$^{fl/fl}$/*R26R-Confetti* mouse after 4 weeks of tracing. The adenoma consists of multiple segments (white dashed lines), which are marked uniformly by a single color (or are unmarked). RFP (red fluorescent protein) and YFP (yellow fluorescent protein) tracings are shown in red and yellow, respectively. Lgr5^{+} stem cells (green) are located toward the base of adenoma segments. Colors are overlaid on a differential interference contrast (DIC) image in gray. (**C**) Distribution of stem cells marked by Lgr5-GFP and Paneth cells, marked by lysozyme (purple) at the base of wild-type (WT) crypts (left). (Right) Lgr5^{+} stem cells (green) are located adjacent to Paneth cells (purple) toward the base of an adenoma segment (indicated by the red dashed line). (**D**) Schematic representation of the intermingled stem cells and Paneth cells in wild-type crypts (left) and adenomas (right). Scale bars indicate 50 μm.