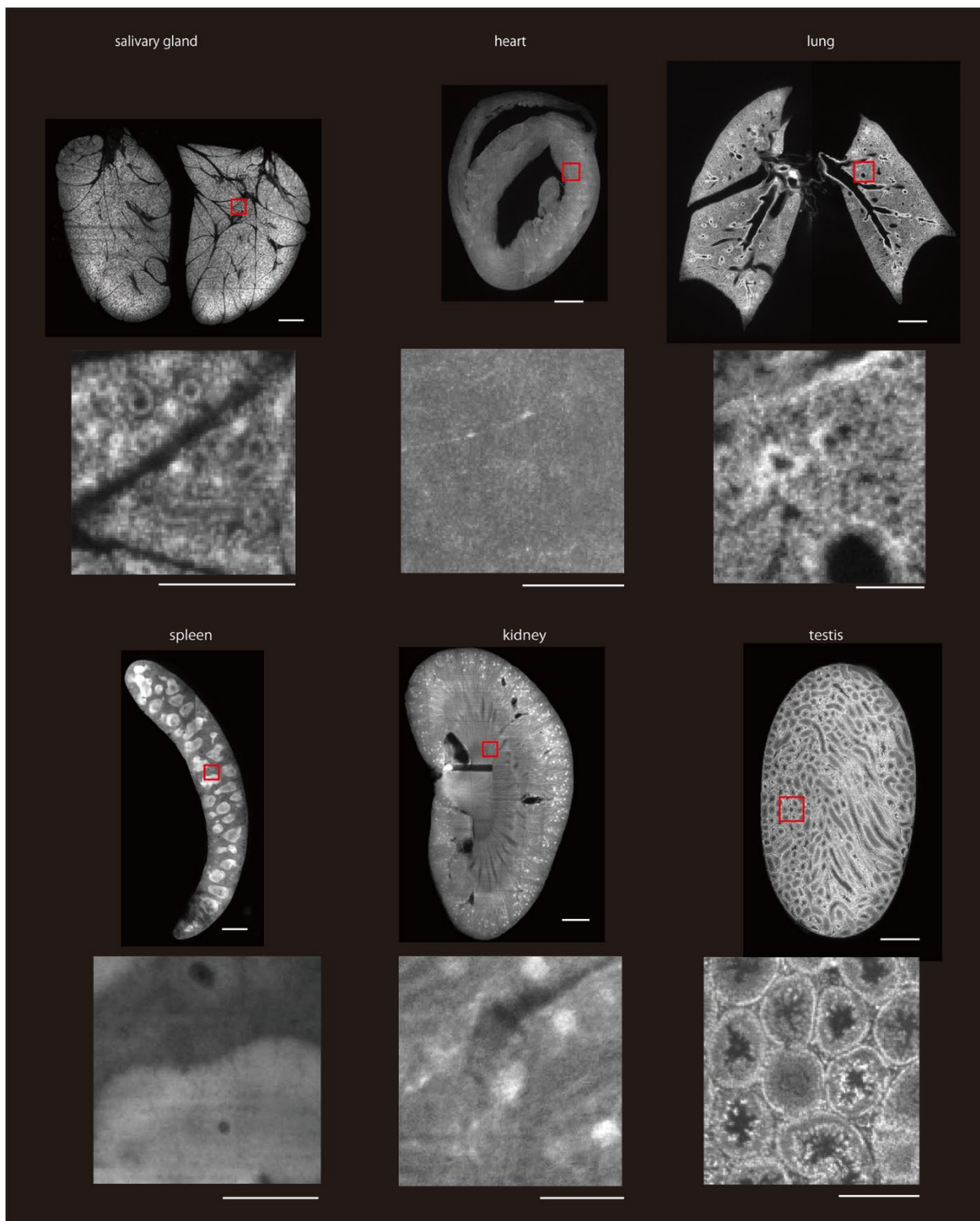


# A multiwell plate approach to increase the sample throughput during tissue clearing

In the format provided by the  
authors and unedited

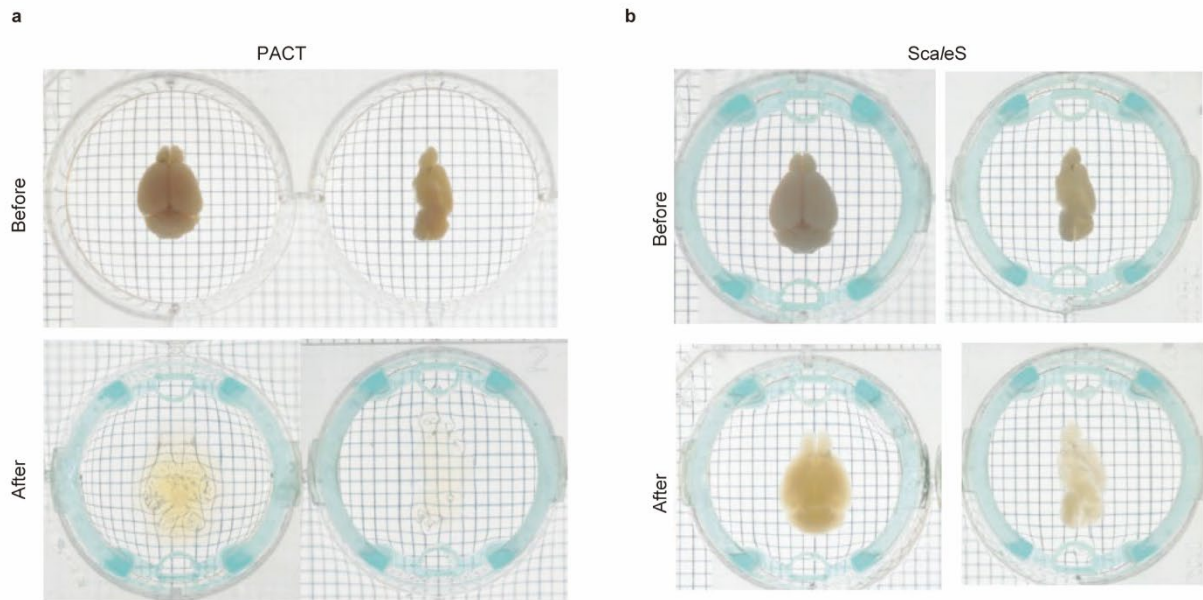
## Supplementary Information



# Supplementary Figure 1

Nuclear stained images of various mouse organs

Representative images for salivary glands, heart, lungs, spleen, kidney and testis stained with propidium iodide. All organs were imaged by light-sheet microscopy. (scale bar 2 mm and 500  $\mu$ m)



## Supplementary Figure 2

### Tissue clearing for other passive clearing method using multiwell plate

We performed other passive tissue clearing methods using the tissue clearing insert device on the whole mouse brain and 2mm thick slices in accordance with previous reports. grid size 2 mm

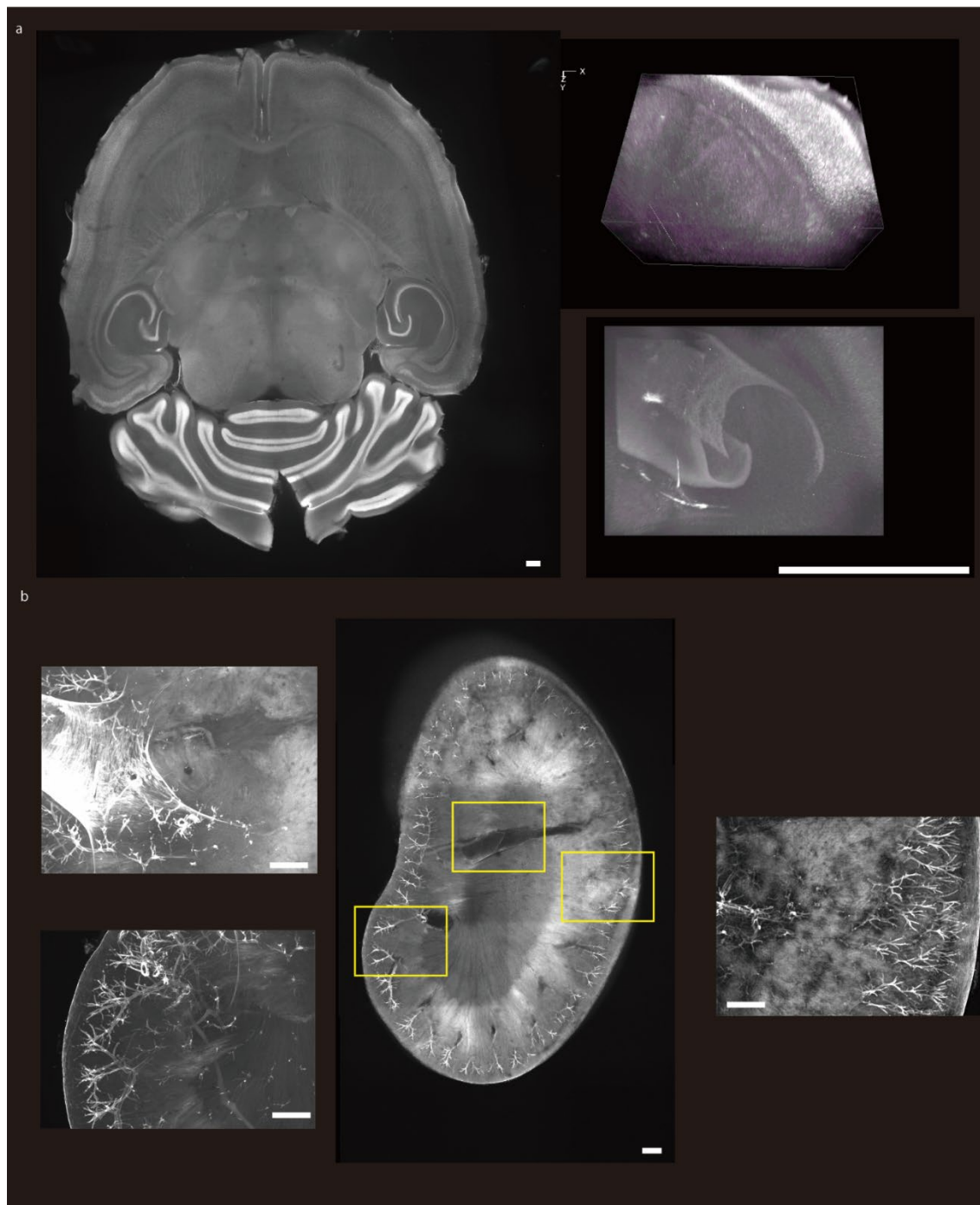
#### Procedure of PACT

1. Fix with 4% PFA in PBS at 4°C for one day.
2. incubate in A4P0 (4% acrylamide in PBS) supplemented with 0.25% VA-044 at 4°C overnight.
3. degas with nitrogen for 1-5 min.
4. incubate for 2-3 hour at 37°C to initiate tissue-hydrogel hybridization.
5. remove excess hydrogel via PBS washes.
6. incubate in a 6well plate with tissue clearing insert devices containing 8% SDS in PBS at 37°C with shaking for more than 5 days.
7. incubate in imaging media (RIMS : 40 g Histodenz™ in 30mL of 0.02M PB with 0.1% tween-20 with 0.01% sodium azide, pH to 7.5 with NaOH) at r.t. for 3 days.

#### Procedure of ScaleS

1. Fix with 4% PFA in PBS at 4°C for one day.
2. incubate in ScaleS0 solution (20% sorbitol, 5% glycerol, 1 mM methyl-β-cyclodextrin, 1 mM γ-cyclodextrin and 3% DMSO, excluding 1% N-acetyl-L-hydroxyproline) at 37°C for 12 hours (one day for the whole brain).
3. incubate in ScaleS1 (20% sorbitol, 10% glycerol, 4M urea and 0.2% Triton X-100) at 37°C for 12 hours (one day for the whole brain).
4. incubate in ScaleS2 (27% sorbitol, 2.7M urea, 0.1% Triton X-100 and 8.3% DMSO) at 37°C for 12 hours (one day for the whole brain).
5. incubate in ScaleS3 (36.4% sorbitol, 2.7M urea and 9.1% DMSO) at 37°C for one day.
6. wash with PBS at 37°C for 12 hours (one day for the whole brain).
7. incubate in ScaleS4 (40% sorbitol, 10% glycerol, 4M urea, 0.2% Triton X-100 and 15% DMSO) at 37°C for 12 hours (one day for the whole brain).

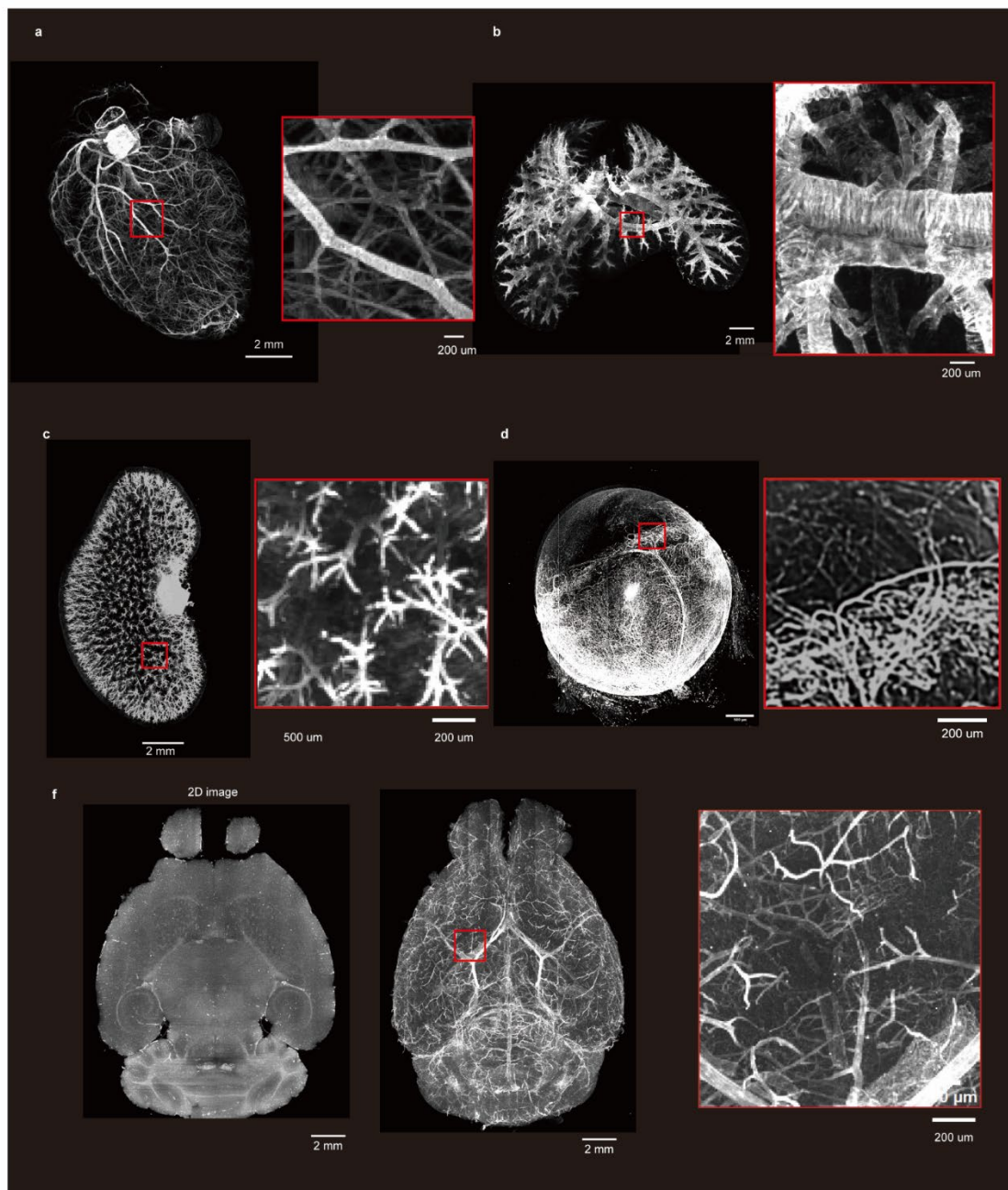
These procedures are not optimized for each method, so the solution immersion time, temperature, etc. must be optimized for higher performance.



### Supplementary Figure3

Volumetric images using structured illumination microscopy on the 6-well multiwell plate

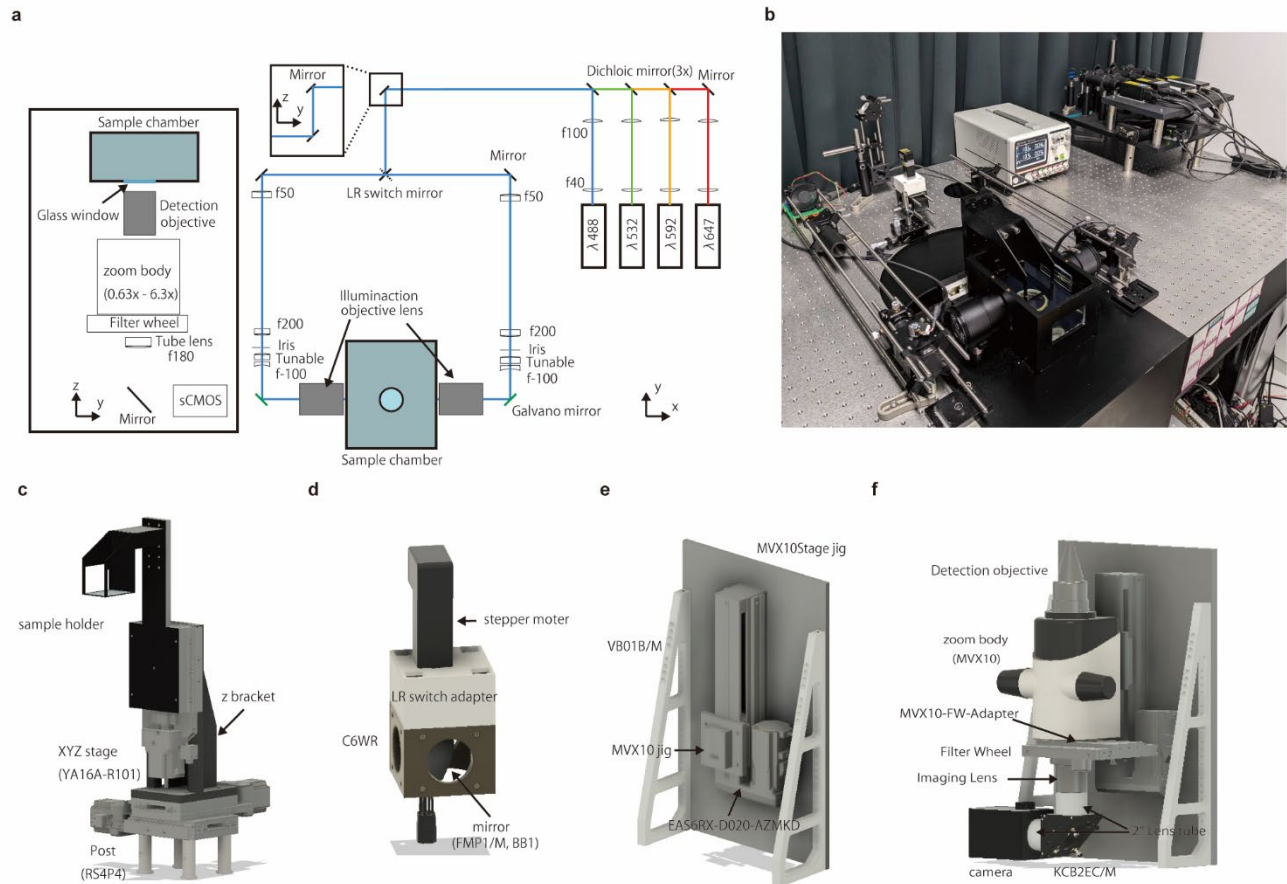
(a) Volumetric imaging for mouse half brain stained with anti-NeuN antibody. Captured up to depth of 2000  $\mu\text{m}$ . Tissue clearing was performed whole brain using tissue clearing insert device and immunostaining were performed with CUBIC-HV. (b) Volumetric imaging for half mouse kidney stained with anti-alpha-SMA antibody. Observed from the cutting surface and captured up to depth of 2000  $\mu\text{m}$ . (scale bar 500  $\mu\text{m}$ )



#### Supplementary Figure 4

##### Volume rendered images for mouse organs stained with anti-alpha-SMA

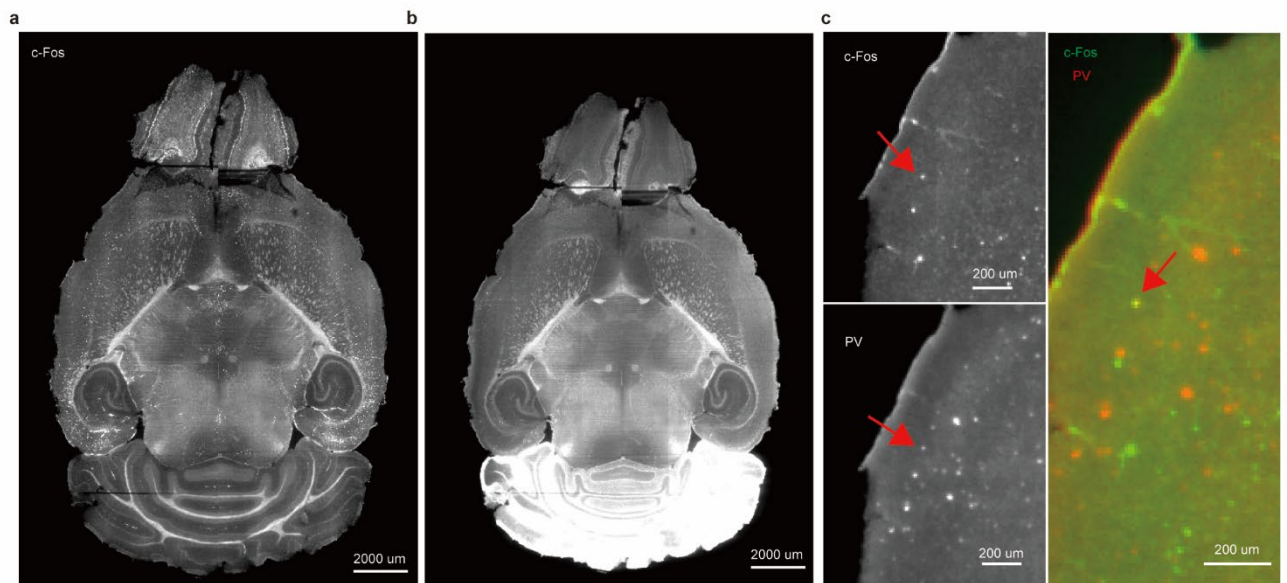
Volume rendered images for mouse organs stained with anti-alpha-SMA antibody with CUBIC-HV and imaged by light-sheet microscopy. (a)Heart (b) Lung (c) Kidney (d) Eye. (scale bar (a-c)2 mm, (d) 500  $\mu$ m). (e) 2D and volume rendered image for mouse organs stained with anti-CD31 antibody. Volume rendered images of heart, lung, kidney and brain were reconstructed using the 3D projection function of ImageJ for raw images. Volume rendered image of eye was processed by subtract background function of ImageJ and enhanced vessel signals.



## Supplementary Figure 5

### Custom made light-sheet microscopy

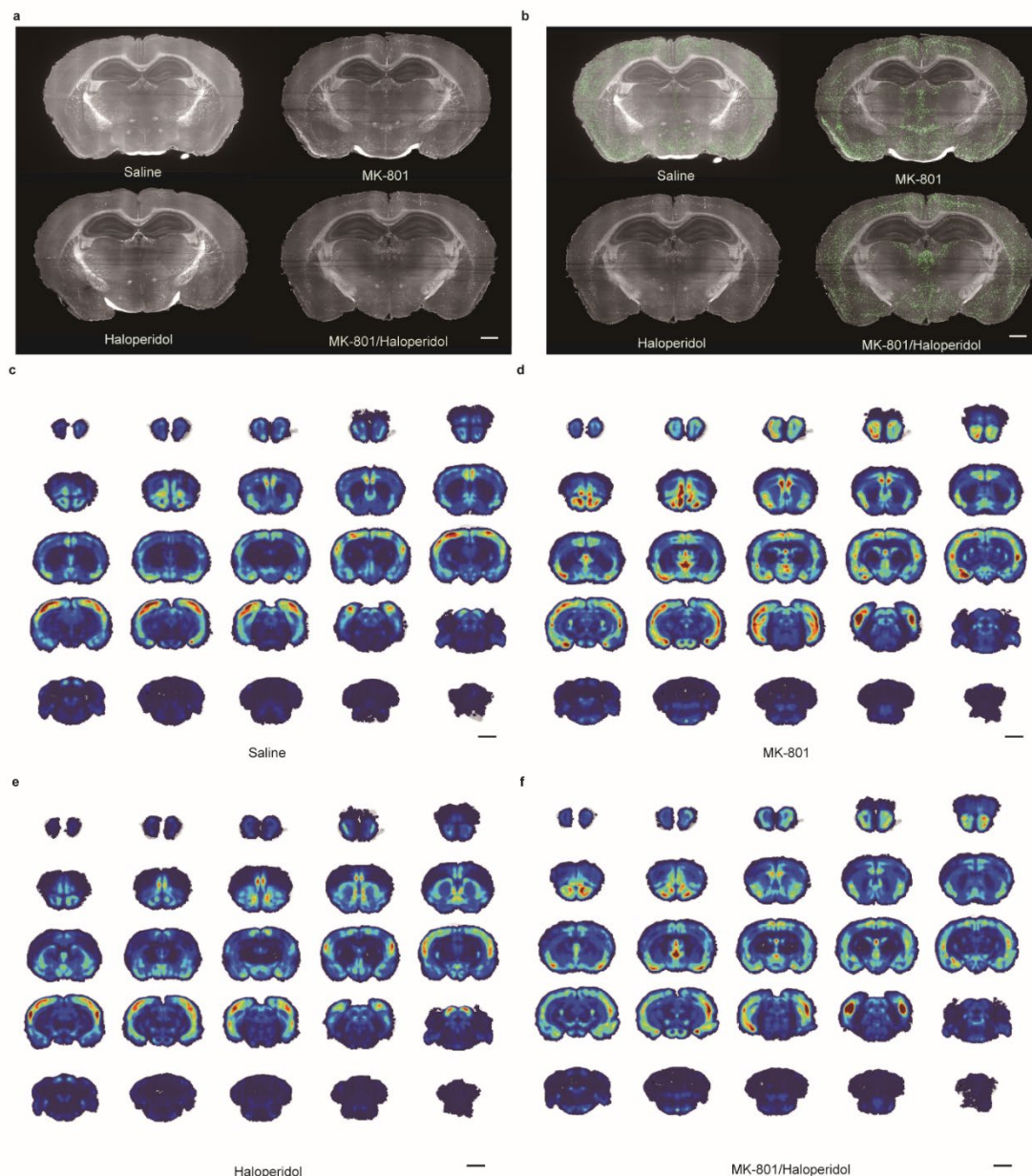
(a) 2D drawing the light-sheet microscopy. (b) Picture of the light-sheet microscopy. (c) 3D CAD image for sample holder and xyz stage. Sample holder and z bracket were made of aluminum, anodized black, and were custom ordered. The sample holder can hold a conventional large slide glass (76mm×52mm), which can be used to image the gel-embedded sample. Non-gel-embedded samples are also possible. (d) 3D CAD image for LR switch mirror device. LR switch adapter was created by the 3D printer. (e) 3D CAD image of the jigs and focus control stage. MVX10 stage jig and MVX10 jig were made of aluminum and were custom ordered. (f) 3D CAD image for imaging unit. All custom made parts of the 2D drawings (pdf) or 3D CAD data (stl files) for custom-made parts are available from the Supplementary Data.



### Supplementary Figure 6

Representative images for c-Fos and PV double staining mouse brain.

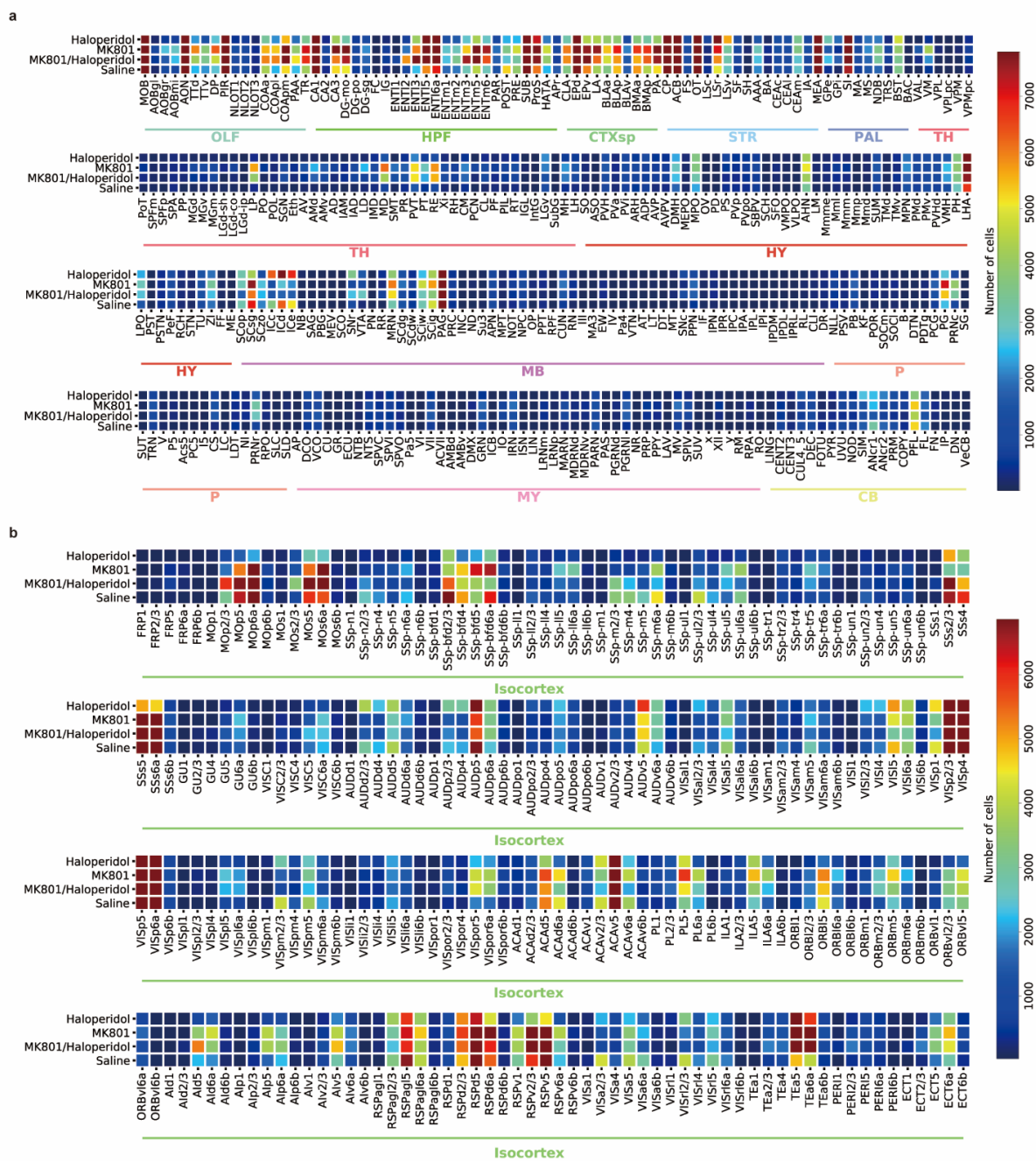
(a) c-Fos image and (b) PV image at 4150  $\mu\text{m}$  depth from the top of the brain. (c) Magnified and overlapped images. Arrows indicate c-Fos and PV double positive cells.



### Supplementary Figure7

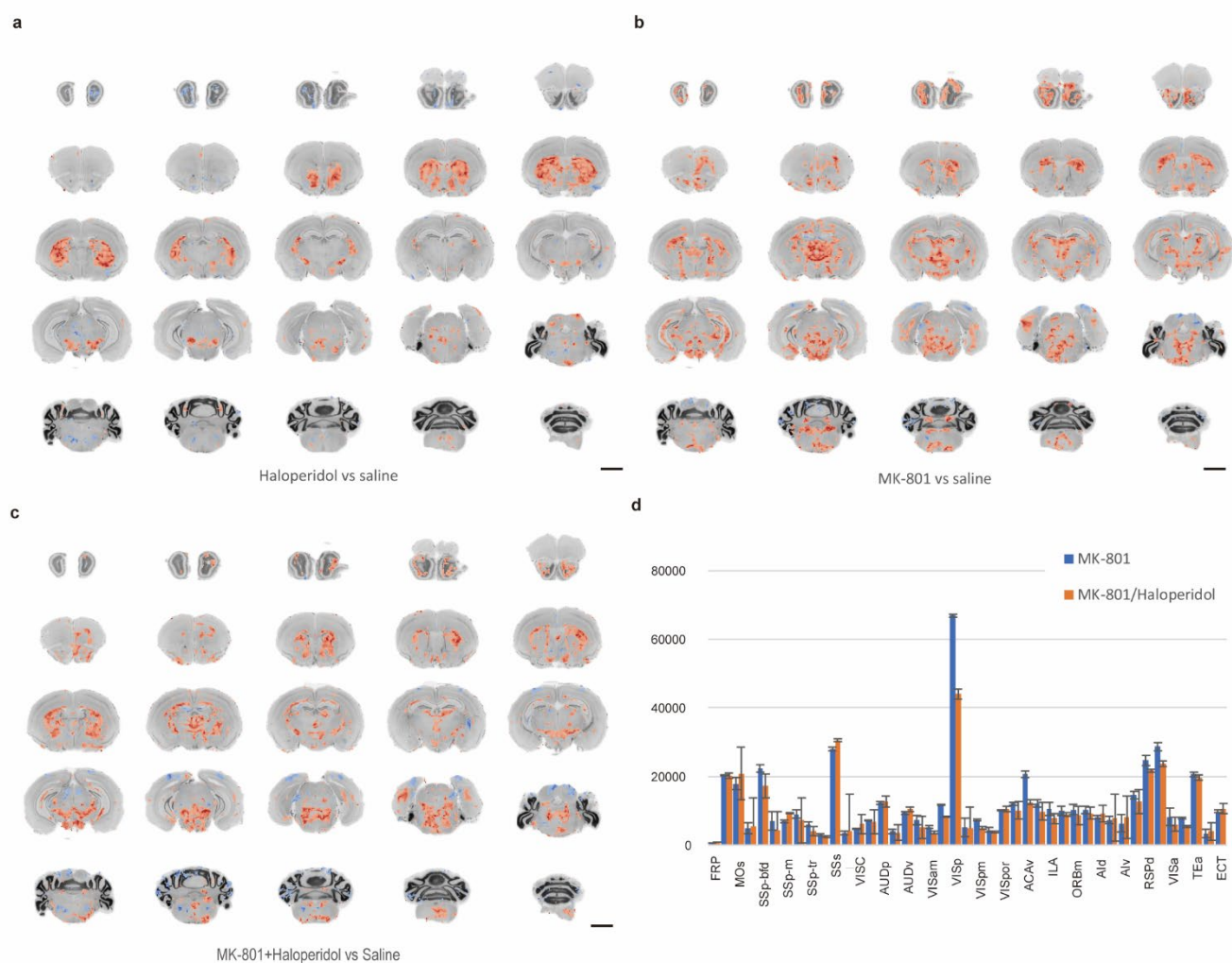
Spatial heat map of c-Fos positive cell

Spatial heat map of c-Fos positive cell numbers for each group. (a) Saline (b) MK-801 (c) Haloperidol (d) MK-801 and haloperidol administered samples, respectively (each n = 3). Figures were generated with CUBIC-Cloud. (scale bar 5 mm). Scale bars were illustrated after exporting the png files. Scales were calculated from the original CUBIC-Atlas.



### Supplementary Figure 8

Heat map of c-Fos positive cell numbers at each brain region for each group (n = 3).



## Supplementary Figure9

Spatial p-value map of the c-Fos positive cell

(a-c) Spatial p-value map of the c-Fos positive cell numbers for each group (n = 3). Figures were generated with CUBIC-Cloud. (scale bar 5 mm). (d) Bar graph of the c-Fos positive cell numbers for each isocortex region (n=3). Scale bars were illustrated after exporting the png files. Scales were calculated from the original CUBIC-Atlas. Bar graph was created by Microsoft Excel using downloaded csv file data from CUBIC-Cloud.

