Elevated-temperature-induced acceleration of PACT clearing process of mouse brain tissue

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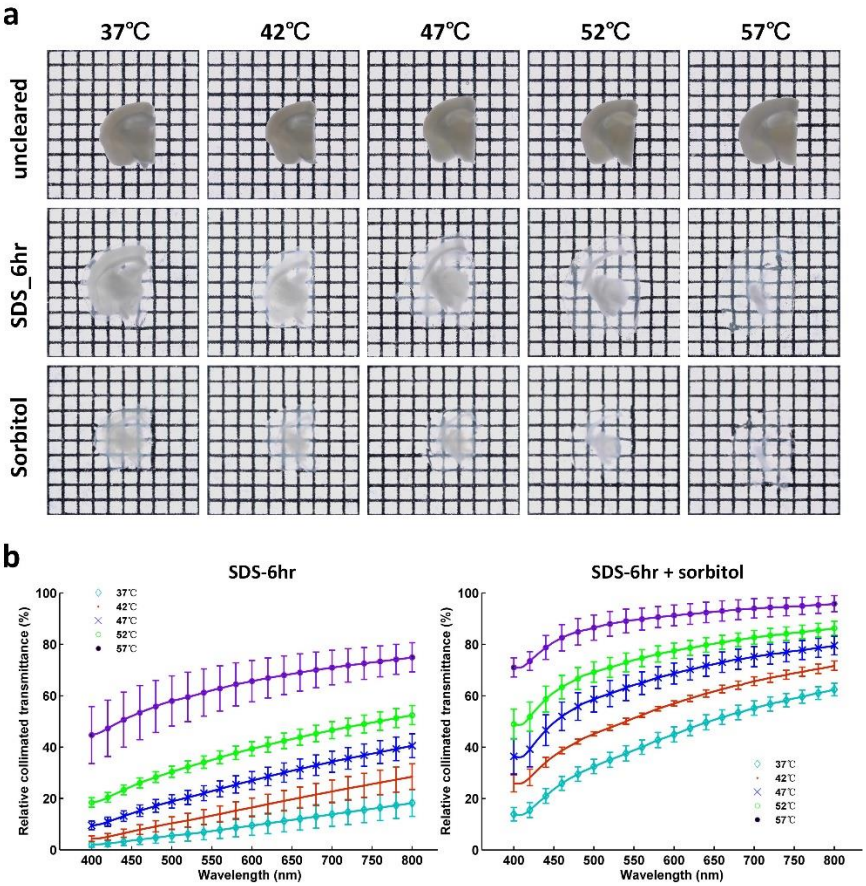
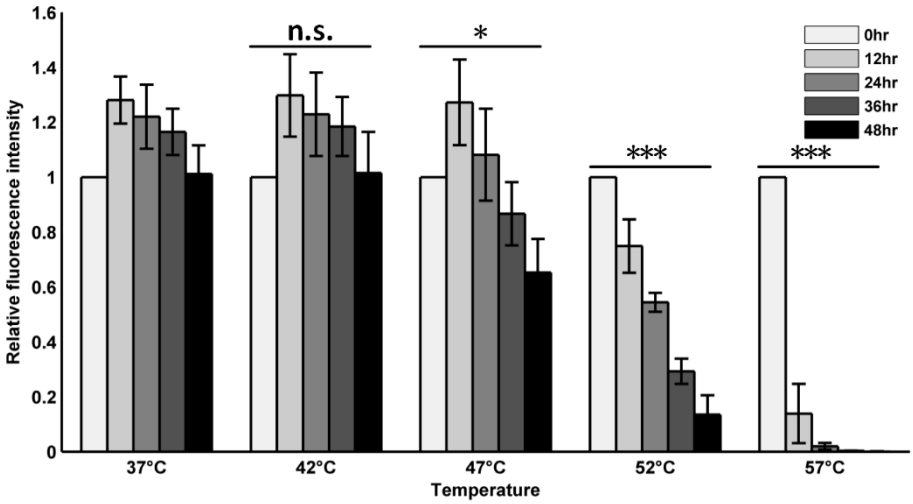
Supplementary Figure 1



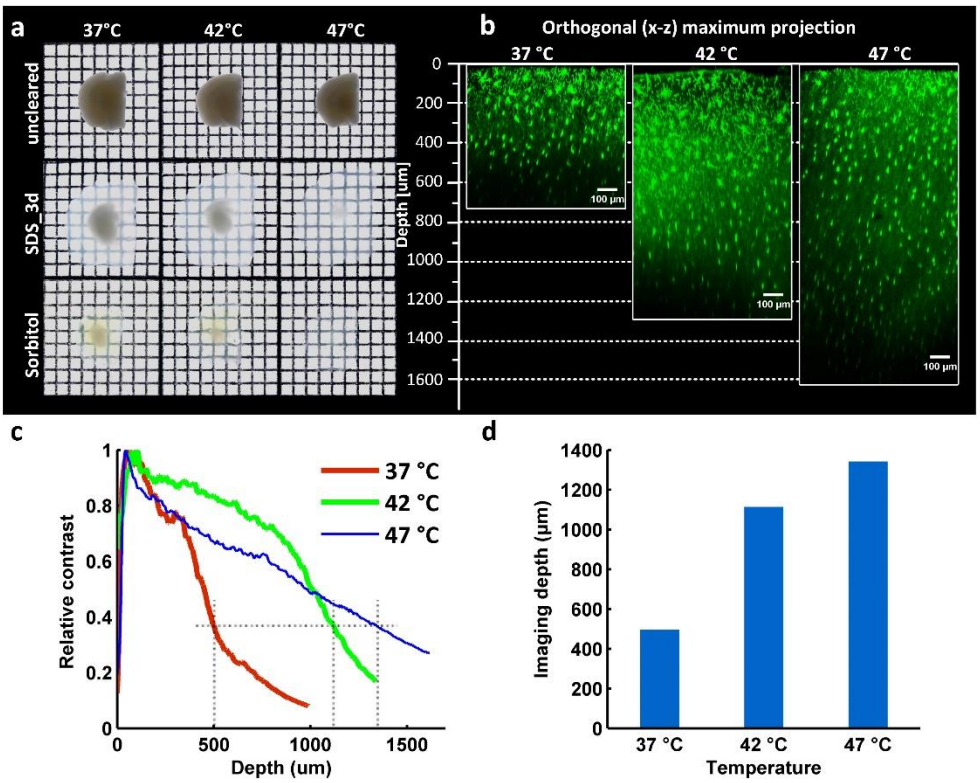
Supplementary Figure 1. (a) Bright-field images of 1-mm-thick brain sections cleared in SDS for 6 hours and further incubated in sorbitol solution. “uncleared” indicates the sample with only hydrogel-embedded and placed in PBS. “SDS-6hr” indicates 6-hours SDS clearing. “Sorbitol” indicates further incubation in sorbitol solution after 6-hours SDS clearing. **(b)** Relative collimated transmittance of brain blocks after 6-hours SDS clearing and further sorbitol incubation. “SDS-6hr” indicates the sample with 6-hours SDS clearing, “SDS-6hr + sorbitol” indicates the sample with further sorbitol incubation after 6-hours SDS clearing. Error bars denote standard deviations.

Supplementary Figure 2



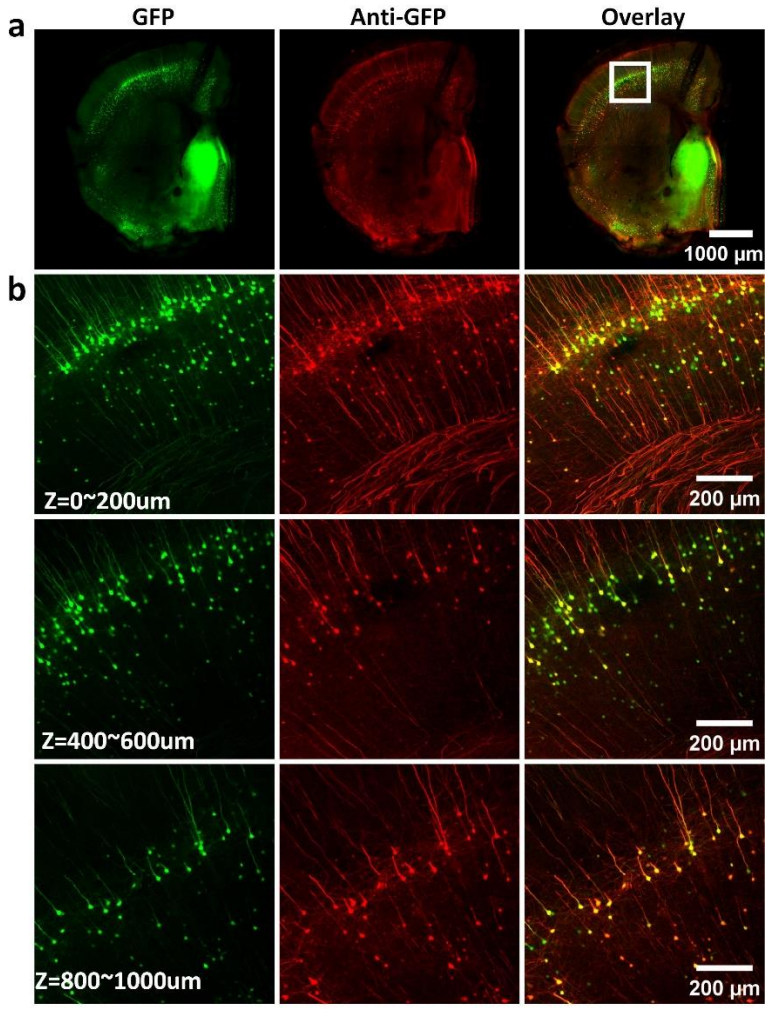
Supplementary Figure 2. Quantitative calculation of relative fluorescence intensity. Error bars denote standard errors. Statistic method: two-way ANOVA with mixed design. n.s., not significant; * $P<0.05$; *** $P<0.001$. The statistical analyses show that the difference of fluorescence change within 48 hours between 42 °C and 37 °C is not significant, while for 47 °C, the fluorescence change is significantly different from 37 °C. 52 °C and 57 °C present highly significant difference with 37 °C.

Supplementary Figure 3



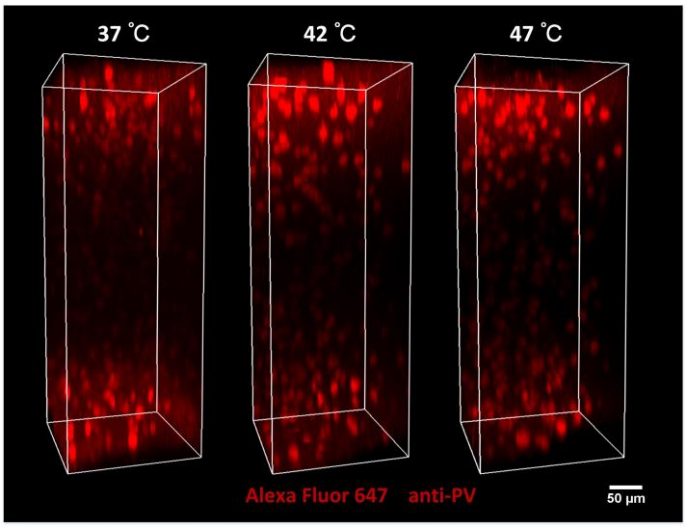
Supplementary Figure 3. To further investigate the effect of temperature on imaging depth of larger samples, 3-mm-thick brain blocks were cleared with SDS for 3 days followed by sorbitol incubation, then imaged with upright confocal fluorescence microscopy (A1RMP, Nikon, Japan) equipped with the 16×/0.8 water-immersion objective (W.D. 3.0 mm). **(a)** Transparency of the brain blocks after 3-days SDS clearing with 37 °C, 42 °C and 47 °C and sorbitol immersion. The opaque area of the brain blocks decreases with increasing temperature. **(b)** The 20-μm maximum projections of orthogonal (x-z) slices, taken from z-stack transverse images of the center region of cleared 3-mm-thick brain blocks. **(c-d)** Quantification of imaging depth. The imaging depth is 492 μm, 1120 μm and 1332 μm for three temperatures, respectively. For 42 °C and 47 °C, the value increases to 2.28 and 2.71 folds, respectively.

Supplementary Figure 4



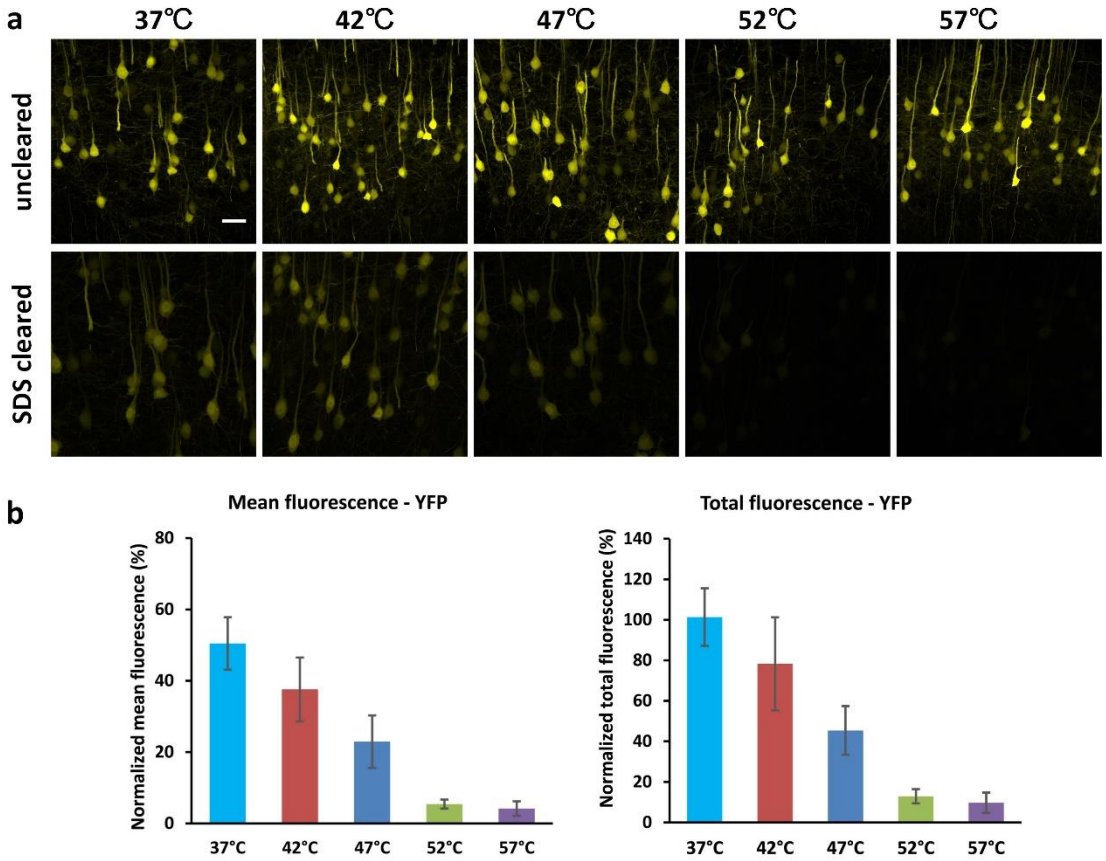
Supplementary Figure 4. Immunostaining for *Thy1*-GFP-M of 1-mm-thick mouse block after PACT clearing at 47 °C. **(a)** Single plane images of coronal block of GFP mouse brain immunostained for GFP. Scale bar, 1000 μm. **(b)** Maximum projections of the boxed region in the cortex in (a) at different depths (thickness: 200 μm). Scale bar, 200 μm. Left, endogenous GFP (green); middle, anti-GFP (red); right, overlay. The signals on the coronal surface and middle of the brain block overlap well, which indicates that moderately rising temperature (e.g. 47 °C) for PACT works well with subsequent immunostaining.

Supplementary Figure 5



Supplementary Figure 5. Immunostaining of 1-mm-thick brain slices. Cleared brain slices with PACT in different temperatures were immunostained for parvalbumin (Alexa Fluor 647 anti-PV), then incubated in sorbitol solution for 1 h and mounted for imaging.

Supplementary Figure 6



Supplementary Figure 6. YFP Fluorescence intensity of neurons before and after SDS clearing in gradient temperature. **(a)** Each image is the maximum projection of image stacks acquired with confocal microscope (10×/0.5 objective). Scale bar, 50 μm. **(b)** Quantitative calculation of relative fluorescence intensity. Error bars denote standard deviations.