Supplemental Material ESM_13

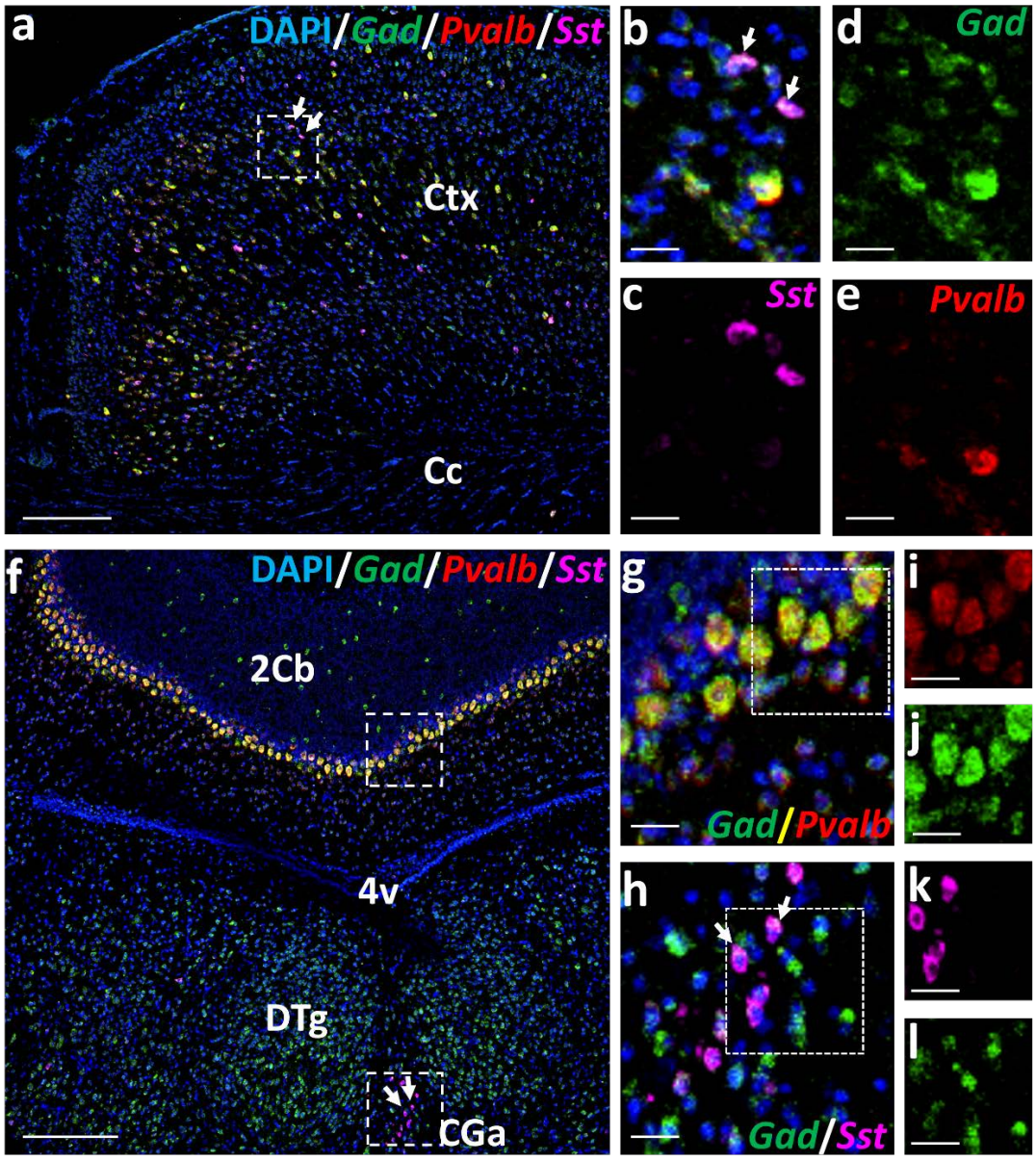

Fig. ESM_13_1. *Sst* probe specificity. Distinct labeling of *Sst* and *Pvalb* shown in the multiplexed HCR FISH images of fresh-frozen mouse cortex and brainstem (20 µm thin sections) validates the cell-type and anatomical specificity of the *Sst* probe-set. Fig. a and f show colocalization of *Sst* (white, marked by arrows) and *Pvalb* (yellow) in the mouse cortex and brainstem regions, respectively. Fig. b, g and h show zoomed-in view of the respective boxes. *Sst* (magenta, Fig. c and k) selectively colocalizes with a sub-population of GABAergic interneurons (Fig. d (faint green cells) and l). On the other hand, *Pvalb* (red, Fig. e and i) colocalizes with a different sub-population of GABA neurons (Fig. d (bright green cells) and j (Purkinje cells). 2Cb-2nd cerebellar lobule, 4v- 4th ventricle, Cc- corpus callosum, CGa- central gray, Ctx- cortex, DTg-dorsal tegmental nucleus. Scale bars: a) 200 µm, b-e) 20 µm, f) 180 µm, and g-l) 20 µm.

Supplemental Material ESM_13

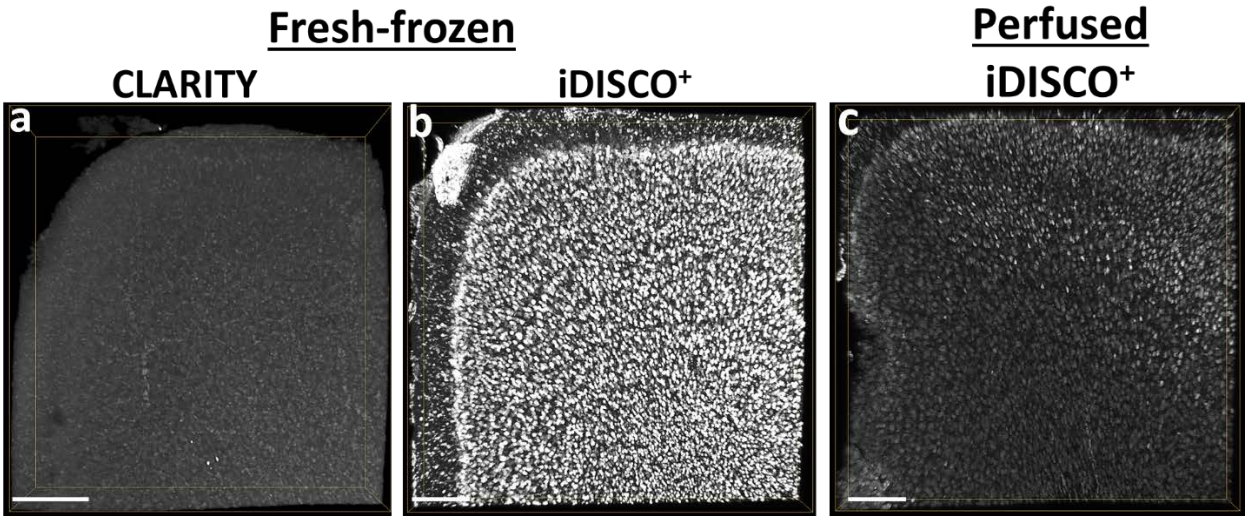

Fig. ESM_13_2. The effect of different clearing methods and fixation on the *in-situ* levels of total RNA. Thin fresh-frozen and perfused brain slices (1 mm) were stained with SYTO RNASelect green fluorescent cell dye after clearing the samples by following the manufacturer's instructions at 500 nm concentration. Qualitatively, the observed fluorescence signal was substantially brighter in the (b) fresh-frozen iDISCO$^+$ (n=3) than (c) Perfused iDISCO$^+$ (n=2) samples, whereas weak signal in (a) fresh-frozen CLARITY (n=3) slices might indicate its incompatibility with the dye. The signal difference between fresh-frozen and perfused tissue potentially indicates the presence of greater cross-linking in the perfused samples. Using a confocal microscope, we detected only a superficial staining up to 200 µm following overnight incubation which might be due to its extremely short life span. Since the proprietary mechanism is not known, we could not further optimize the procedure for SYTO RNASelect green fluorescent cell dye (emits strong bright green fluorescence upon binding to RNA, absorption/emission maxima ~490/530 nm, catalog number: S32703, Thermofisher Scientific). Scale bars- 200 µm.

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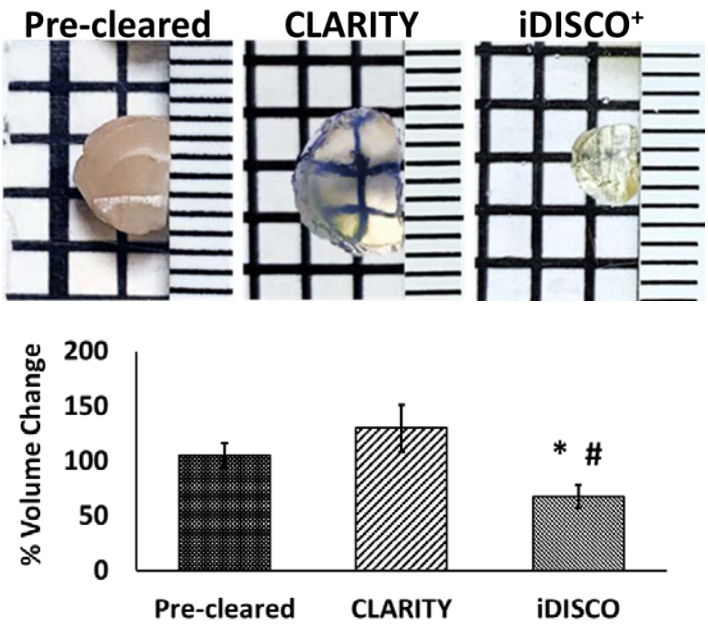

Fig. ESM_13_3. Effect of clearing methods on the tissue volume. Volume measurements (length x width x thickness) were obtained for (1) pre-cleared samples (n=6), (2) CLARITY samples after incubation in 88% histodenz (n=3), and (3) iDISCO$^+$ samples following incubation in DBE (n=3) and presented as percent volume change. iDISCO$^+$ samples showed significant shrinkage in comparison to the CLARITY (p=0.011) samples and those measured before processing (p=0.012). CLARITY samples did not show significant expansion (p=0.177) in comparison to the samples measured before processing. A mm-scale was placed next to the slices before photography. RI matched iDISCO$^+$ samples become superficially opaque after coming in contact with the air, transparency is restored after returning back into the DBE.

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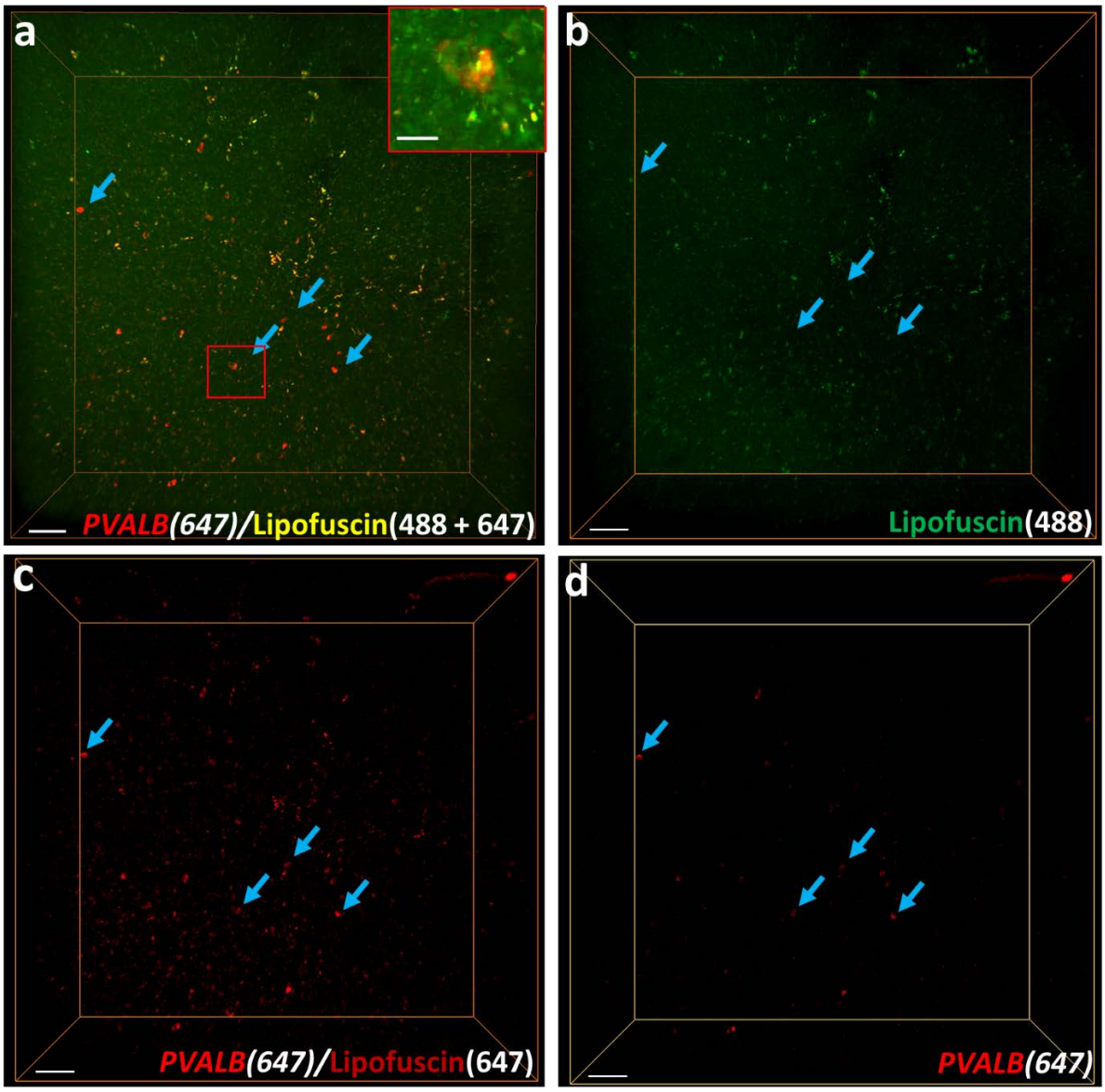

Fig. ESM_13_4. *PVALB* FISH in iDISCO^{+} cleared temporal cortex slice (600 µm) of postmortem human brain. (a) 3D volume rendered stack acquired on a confocal microscope shows *PVALB* FISH signal (AF-647) mixed with lipofuscin autofluorescence signal (488 + 647 nm lasers). Inset, zoomed in view a PVALB neuron. (b) Lipofuscin signal acquired using 488 nm laser. (c) PVALB FISH signal mixed with lipofuscin autofluorescence signal using 647 nm laser) (d) Extraction of *PVALB* specific signal through spectral subtraction using autofluorescence signal (d = c-b). Note that the spectral subtraction method using pixel gray-levels reduces the overall signal intensity of cells in fig. d in comparison to fig. c. Representative images belong to the control subject-2292, (age-55, RIN-6.6). Scale bars- 100 µm; Inset- 20 µm

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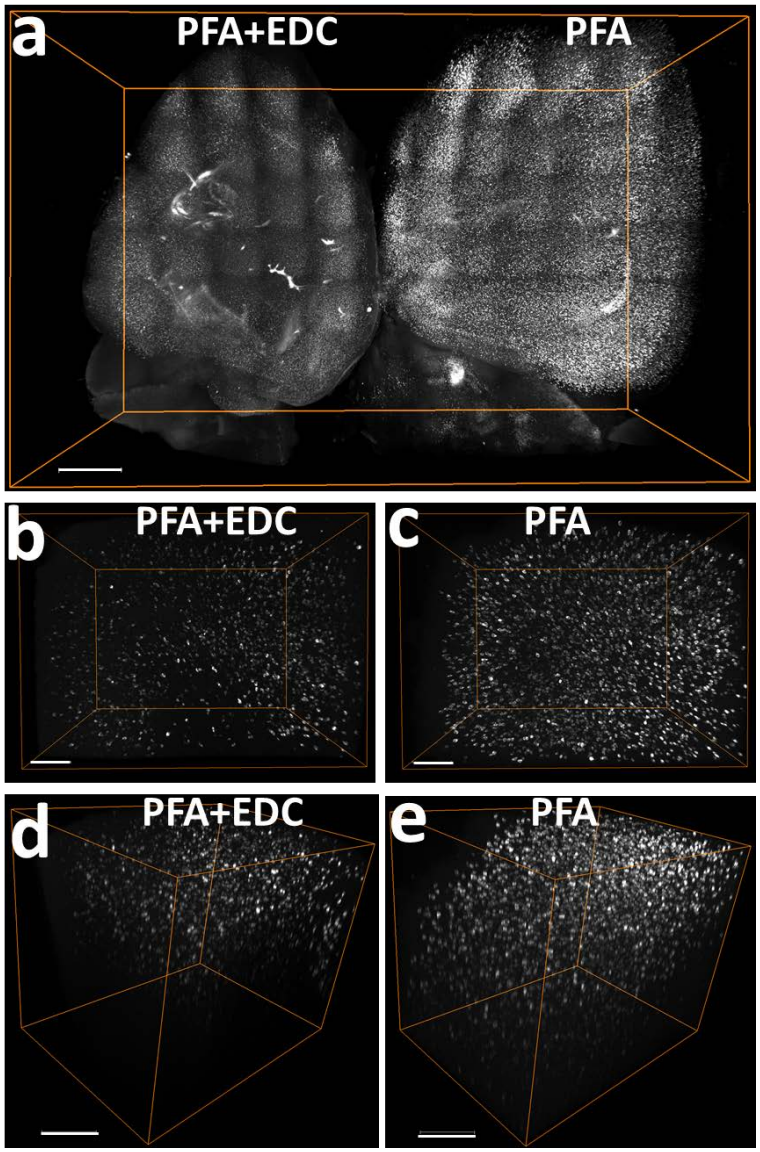

Fig. ESM_13_5. The effect of RNA cross-linker, EDC on *Sst* FISH signal. (a) iDISCO^{+} hemispheres from a single mouse brain processed and imaged together. 3D volume rendered left hemisphere (PFA + EDC fixed) shows weak FISH signal, higher autofluorescence (vasculature) and greater shrinkage in comparison to the right hemisphere (PFA fixed). Similar qualitative differences in the signal quality was seen for CLARITY samples, (b, d) PFA/Acrylamide + EDC and (c, e) PFA/Acrylamide fixed cortical samples cleared using CLARITY method. (b-c) *xy*-view; (d-e) *xz*-view. Scale bars- (a) 1000 µm, (b-e) 200 µm.

Supplemental Material ESM_13

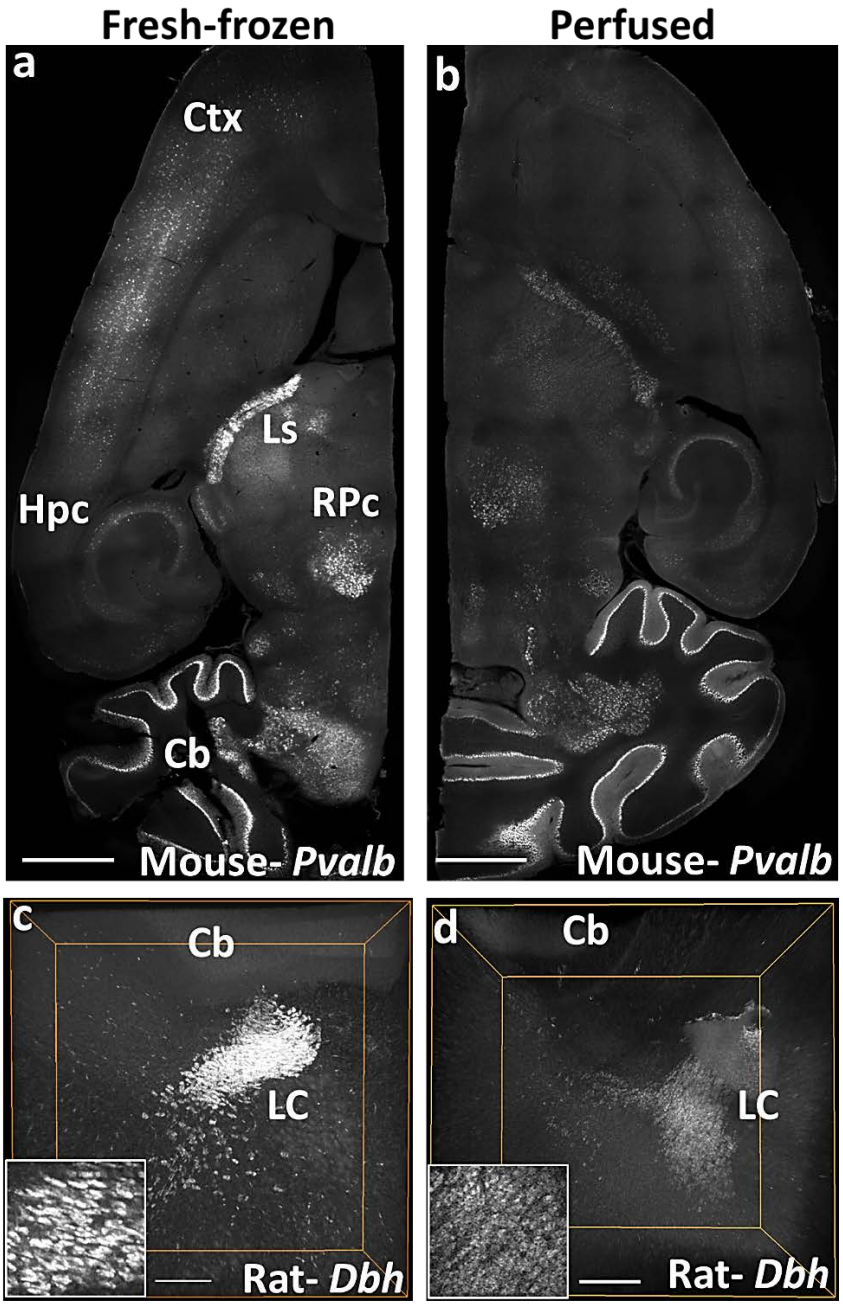

Fig. ESM_13_6. The effect of immersion-fixation (fresh-frozen) and perfusion fixation on the mouse *Pvalb* and rat *Dbh* FISH signal in brain tissues cleared using iDISCO^{+} method. Representative z-plane of dorsoventrally acquired image volume showing greater *Pvalb* FISH signal intensity in the fresh-frozen (a) compared to the perfusion fixed (b) mouse hemisphere. Volume rendered images of *Dbh* labeling in the rat locus coeruleus (LC) area of confocal acquired stacks showed similar difference in the signal intensity between fresh-frozen (c) and perfused (d) tissues. Inset, zoomed-in view of a z-plane. Ctx- Cortex, Ls- Lateral septal nucleus, Hpc- ventral hippocampus, RPc- Red nucleus, parvicellular part, Cb- cerebellum. Scale bars- (a-b) 1000 µm; (c-d) 200 µm.

Supplemental Material ESM_13

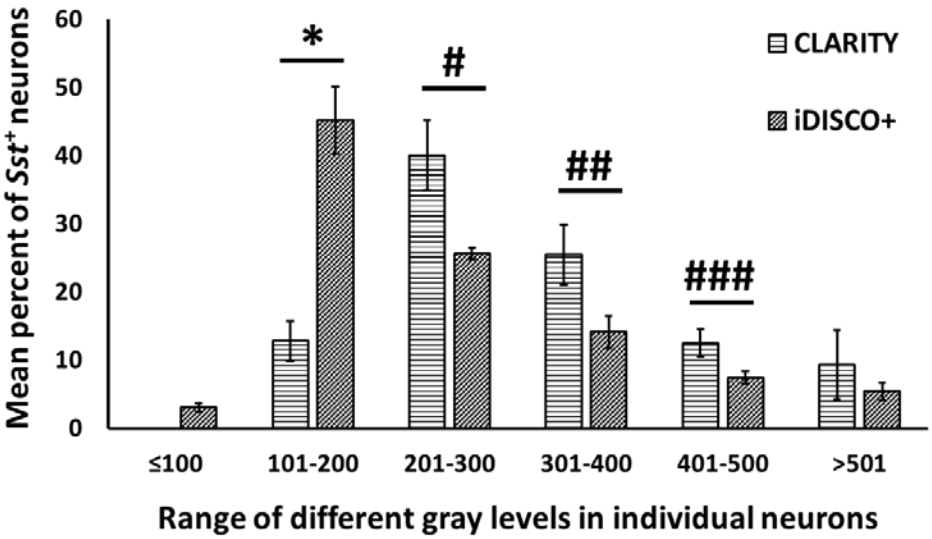

Fig. ESM_13_7. Effect of clearing methods on the range of different gray-levels in individual neurons. Histogram shows binned range of different gray-levels (*x-axis*) and mean percent of *Sst*$^{+}$ neurons (*y-axis*). CLARITY samples had significantly more neurons with higher range of gray-levels (201-300: 40%, 301-400:25%, and 401-500:12%) compared to the iDISCO samples (25.6%, 14%, and 7%, respectively). On the other hand, 45% of neurons in the iDISCO samples had the lowest range of 101-200, which was significantly higher in comparison to the CLARITY samples with only 13% neurons showing range of 101-200. Observation of fewer neurons below the range of 100 was mainly due to the selected cutoff thresholding value in order to minimize the inclusion of poorly fragmented cells. Overall, the result suggests that CLARITY samples (cells have more pixel coverage, Fig. 1d) provide greater possibility of representing the individual cell associated intensities in form of wide range of gray-levels whereas the shrinkage-induced lower pixel coverage in the iDISCO^{+} samples, significantly reduces the range of gray-levels within the cells (2-tailed T-test, *: $t(8)=-11.724$, $p<0.001$; #: $t(8)=6.984$, $p<0.001$; ##: $t(8)=5.414$, $p=0.001$; ###: $t(8)=5.523$, $p=0.001$).