Supplementary information

SOX2 and SOX9 expression in developing postnatal opossum (*Monodelphis domestica*) cortex

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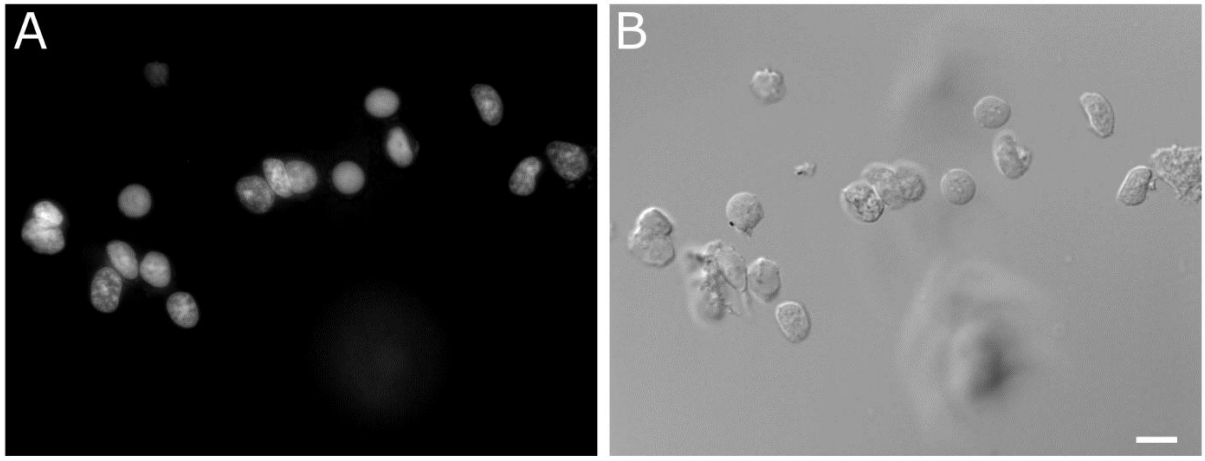

Figure S1. P6 cortex of *Monodelphis domestica* processed with isotropic fractionator method, mounted on a glass slide with coverslip. (A) Cell nuclei stained with Hoechst 33342, projection of 10 µm z-stack acquired with 0.25 µm steps displayed in grayscale. (B) Differential interference contrast (DIC) image of the same optical field confirms that all nuclei are efficiently stained following tissue fixation, homogenization and immunostaining. Images were acquired with 60x 1.42 NA oil immersion objective using Olympus IX83 fluorescent microscope equipped with DIC and fluorescence optics (see Methods). Scale bar, 10 µm.

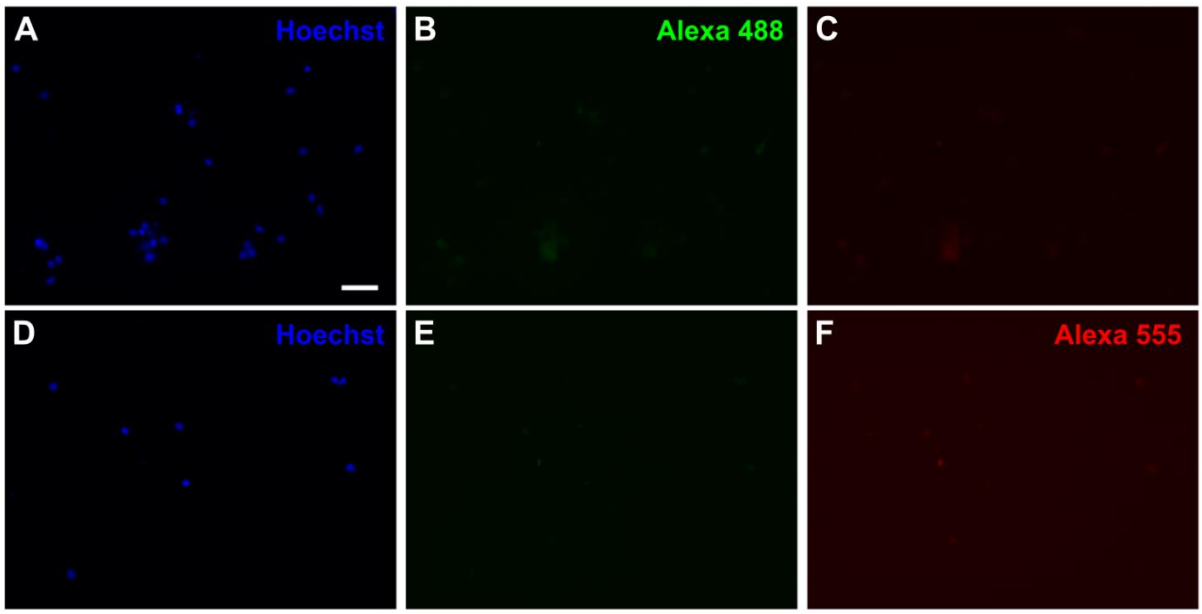

Figure S2. Isotropic fractionator control experiment with omitted primary antibodies. Evaluation of background staining of the secondary antibodies was performed as follows: P30 opossums were processed with isotropic fractionator method as described in Methods and instead of primary antibody, 1% (w/vol) BSA in PBS was used for the overnight incubation. The secondary antibodies goat anti-rabbit Alexa Fluor™ 488 (A-C) and goat anti-rabbit Alexa Fluor™ 555 (D-F) were used separately at the same concentration and incubation time. The same exposure time was kept during imaging. 20x and 0.5 NA objective was used. For every experiment three channels (Olympus fluorescence filter cubes U-FUNA, U-FBW and U-FGW) were acquired. Hoechst 33342-labelled nuclei (A and C) served as a reference for fluorescence signal of stained sample in both experiments. (A-C) projection of 28 stacks, (D-F) projection of 23 stacks with 1 μm step. Scale bar, 20 μm.

Table S1. Age, body weight and size of opossums at postnatal ages from P4 to P30, used in this work. At least 3 different pups from 3 different litters were used for each postnatal age.

Age	Body weight (g)	Body size (mm)
P4	0.19 ± 0.02	11.5 ± 1.73
P5	0.22 ± 0.02	12.6 ± 0.42
P6	0.25 ± 0.03	13.5 ± 0.71
P16	1.43 ± 0.04	31.20 ± 2.05
P17	1.63 ± 0.05	33.33 ± 1.15
P18	1.71 ± 0.11	33.77 ± 0.94
P30	5.02 ± 0.13	47.75 ± 3.49

Table S2. Absolute (total) number of cells in opossum cortex at different ages. A fraction of Hoechst 33342-stained homogenized nuclei of fixed cortices was loaded on hemocytometer and counted using Olympus IX73 inverted fluorescence microscope equipped with long working distance objective (20x, 0.45 NA), fluorescence optics and Olympus XM-10 CCD camera. Isotropic fractionator method was performed as described in Methods.

Age	Total cell number (in millions)
P4-6	5.20 ± 0.31
P16-18	12.72 ± 1.19
P30	21.80 ± 1.00