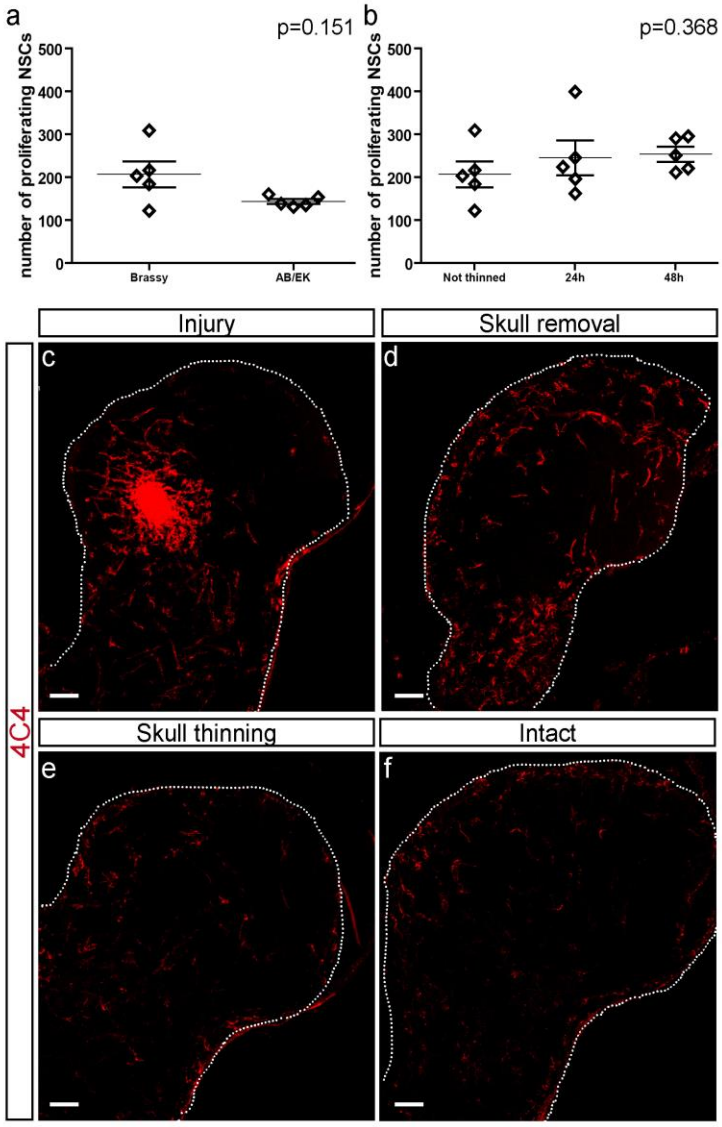
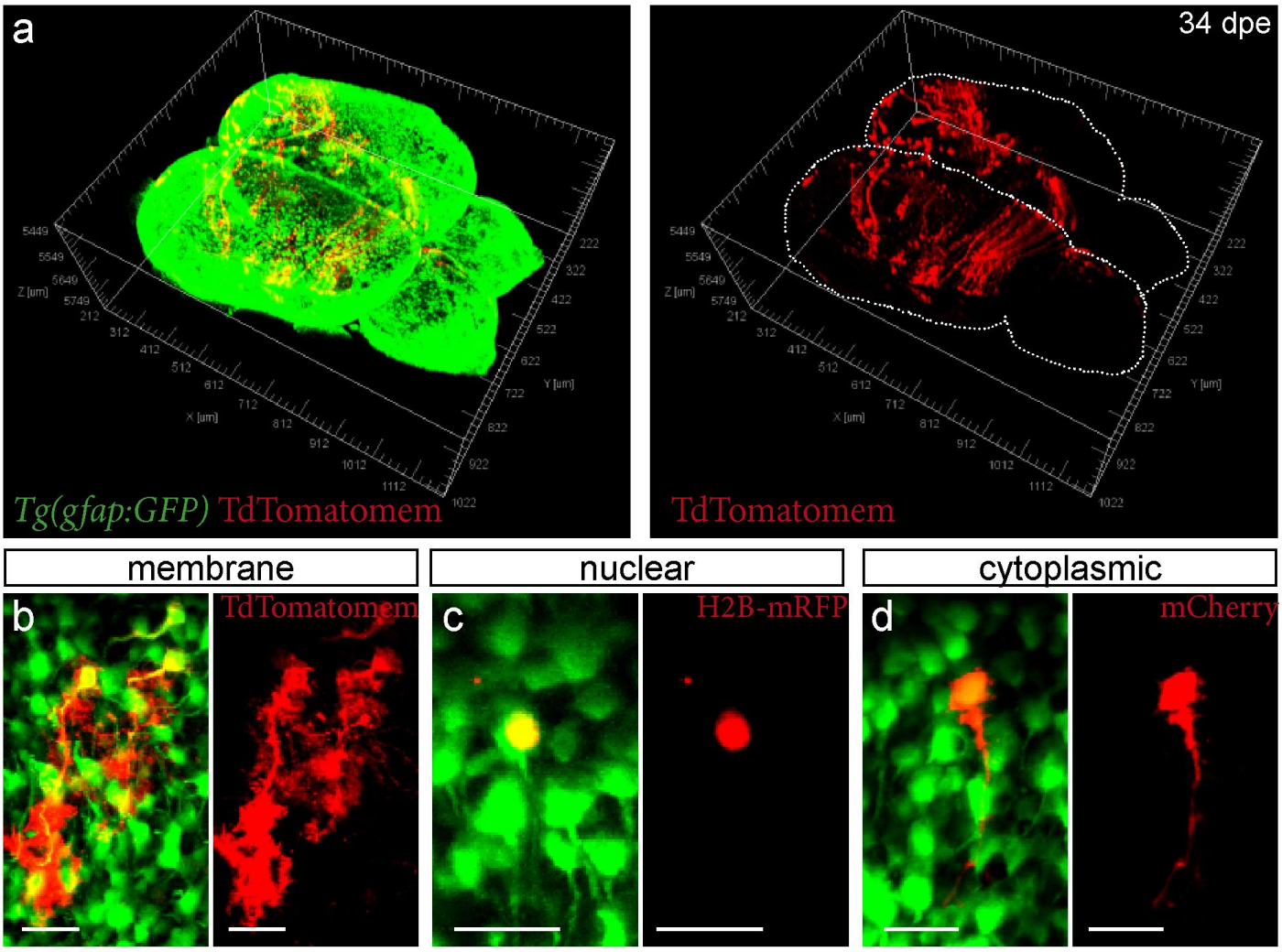


Supplementary Figure 1

Effect of the animal genetic background and the skull thinning procedure on aNSCs proliferation and microglia reaction.

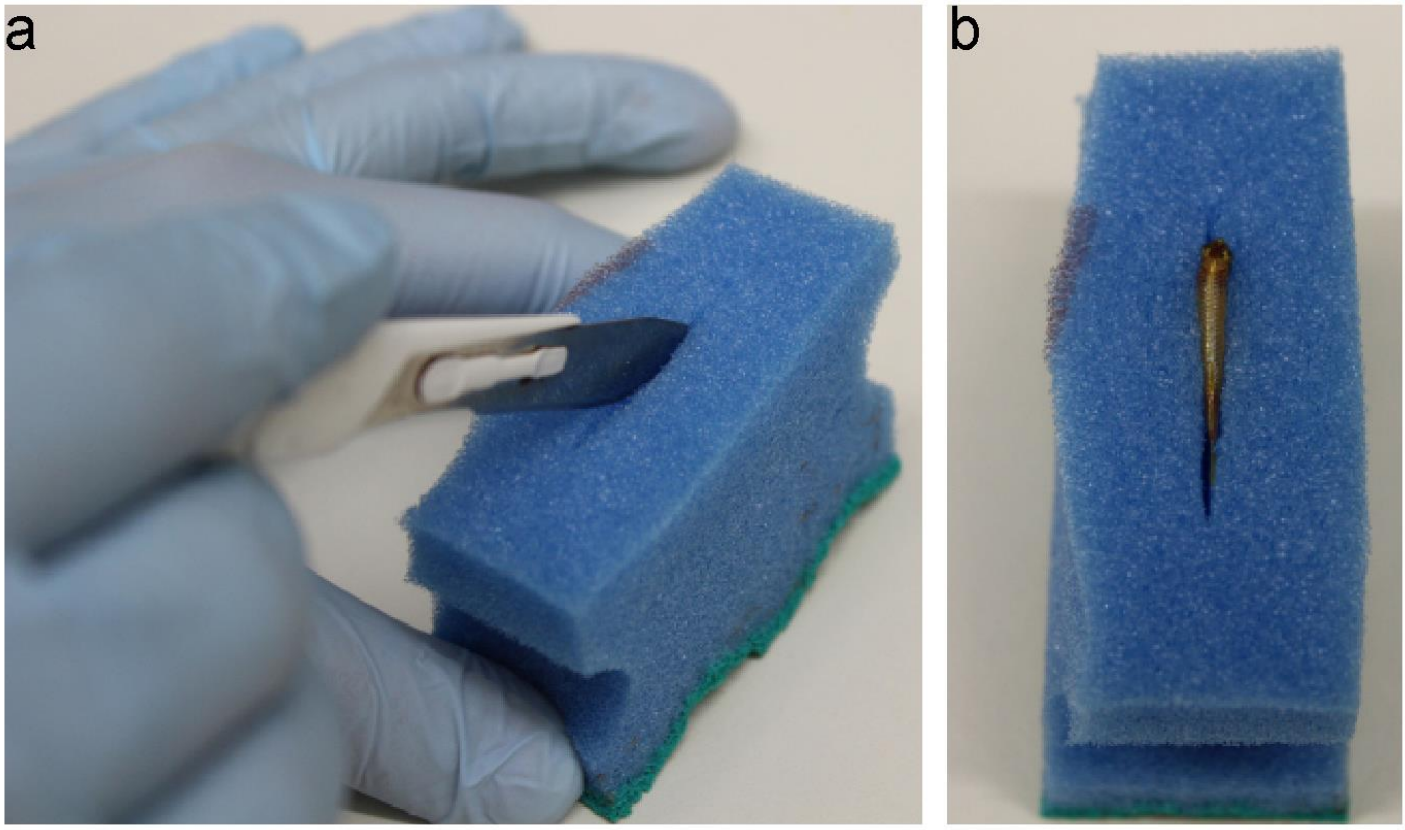
(a) Quantification by fluorescence activated cell sorting (FACS) of the number of proliferating NSCs (*gfap*:GFP+ and PCNA+), performed according to Barbosa et al 2015, in fish with the *brassy* or the wild type AB/EK background. Data are shown as mean ± SEM with symbols representing single animals. Mann-Whitney test was used for the statistical evaluation (n=5). **(b)** Quantification of the number of proliferating NSCs in fish of the *brassy* background without skull thinning procedure and 24h or 48h after thinning the skull. Data are shown as mean ± SEM with symbols representing single animals. Kruskal–Wallis multiple comparison test was used for the statistical evaluation (n=5). **(c)** to **(f)** Coronal hemi-sections of the zebrafish telencephalon, stained for 4C4, which labels microglia cells, 19h after injury (as described in Baumgart et al) (c), removal of the skull (d) or thinning of the skull (e) in the area above the telencephalon. Note the strong reaction of microglia after injury (c) and skull removal (d), compared to the situation after thinning of the skull (e) and in the intact brain (f). All the pictures were acquired using the same laser settings, for an appropriate comparison of 4C4 levels. Scale bars 50µm. Dotted line outlines telencephalic hemisphere in (c) to (f).



Supplementary Figure 2

Immunostaining in the whole brain using the BABB clearing method.

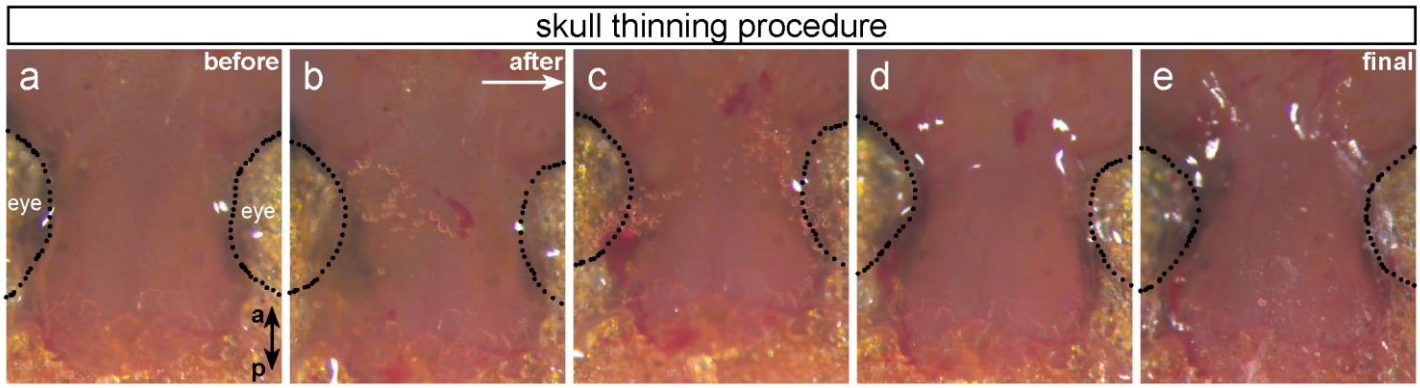
(**a**) Example of a whole brain in the Tg(gfap:GFP) line, dissected, stained (see antibodies used in Table 2) and cleared after electroporation with TdTomatomem plasmid. Note the high amount of electroporated cells at the ventricle with a radial morphology typical of aNSCs in this system. (**b**) to (**d**) Examples of three types of plasmid used, in which the red fluorescent proteins are localized at the plasma membrane (**b**), nucleus (**c**) or the cytoplasm (**d**) of the cells. Abbreviation: dpe-days post electroporation. Scale bars b-d 20µm.



Supplementary Figure 3

Preparation of a sponge to hold the fish for injection and electroporation procedures.

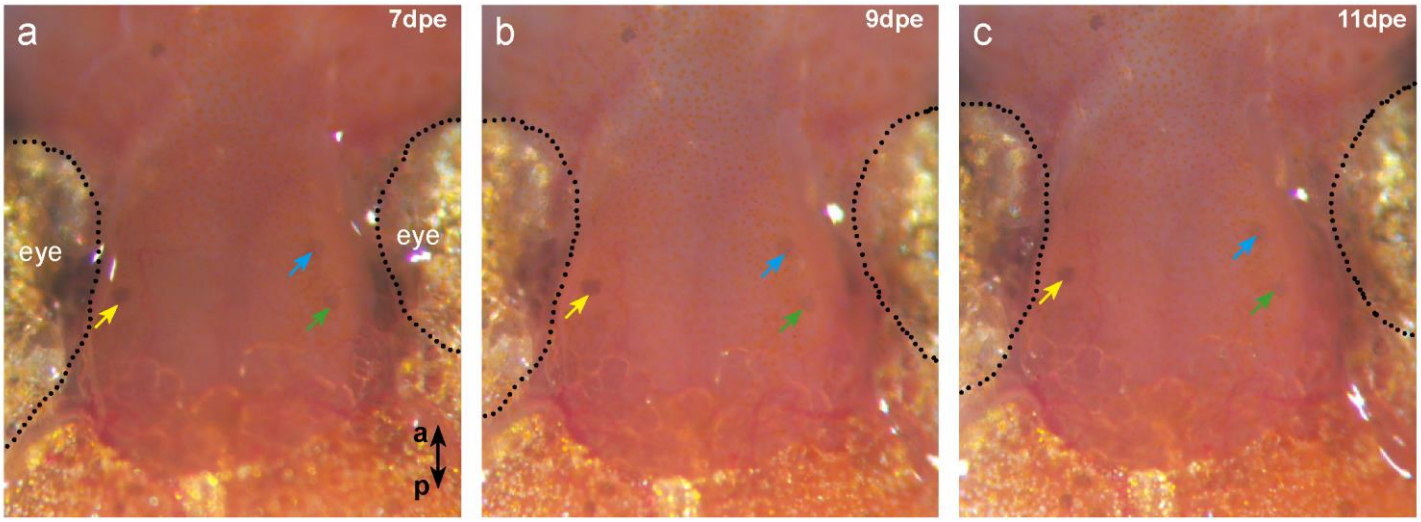
(**a**) Using a normal sponge (the ones made for dish washing) make a longitudinal cut with a scalpel. (**b**) Place the fish within the cut.



Supplementary Figure 4

Skull thinning procedure.

(**a**)-(**e**) Dorsal view of the telencephalon before (**a**), at different steps during (**b**-**d**) the skull thinning procedure and after the procedure (**e**). Note that the skull is gradually more exposed, and the surface above the brain turns from a shiny skin (**a**) to a dull skull (**e**). Do not drill for more than 1min. Abbreviations: a-anterior, p-posterior.



Supplementary Figure 5

Orientation of the fish in different imaging sessions using pigment cells as landmarks.

(**a**)-(**c**) Dorsal view of the telencephalon at different imaging time-points, in which three pigments are visible and are stable throughout time (each colored arrow marks the same pigment over the different time-points). Abbreviations: a-anterior, p-posterior, dpe-days post electroporation.