Turning astrocytes from the rostral migratory stream into neurons: A role for the olfactory sensory organ

M. Alonso, I. Ortega-Pérez, M.S. Grubb, J.P. Bourgeois, P. Charneau[2] and P.M. Lledo

Supplementary figure legends

Supplementary figure 1. Ara-C treatment eliminates neuroblasts in the SVZ and the RMS. A: Animals were sacrificed for analysis immediately after the Ara-C treatment. **B: Quantification of PSA-NCAM staining. The mean gray value was quantified in each selected region by adding the gray values of all the pixels divided by the number of pixels (ImageJ software). This value was subtracted from background immunolabeling intensity, as measured in a separate area of tissue located 50 μm from the SVZ and RMS. TOTO staining was used to draw up the boundaries of RMS and SVZ regions. Note that more than 90% of PSA-NCAM staining disappears after Ara-C treatment in both SVZ and the RMS regions. Moreover, no distinct neuroblasts can be detected (PSA-NCAM$^+$ cells) after treatment. Mean values for each animal were compared. Comparison between treatments was performed by Bonferroni posttest after two-way ANOVA (n=2 per group). **$P < 0.01$. C:** Confocal micrographs showing PSA-NCAM staining (red). Slices were counterstained with TOTO to visualize nuclei (blue). Images represent the maximal projections of merged channels. Scale bars: 50 μm. IHC, immunohistochemistry; SVZ, subventricular zone; RMS, rostral migratory steam; OB, olfactory bulb; LV, lateral ventricle.

Supplementary figure 2. Lentiviral vectors do not transduce neuroblasts or neurons when injected directly into the OB. A: The Mokola vector was injected into the OB of Ara-C treated mice. Animals were killed for analysis at 1 or 2 d.p.i. **B-C:** Confocal micrographs showing infected cells double-stained with GFP (green) and GFAP **(B**, red). Note the absence of NeuN$^+$ infected cells (**C**, red). Images represent the maximal projections of each individual channel (left and middle), and the orthogonal analysis of a single optical plane (right). **D:** Quantitative analysis of double labeled cells present at the injection site in the OB. Data are expressed as mean ± S.E.M. of the percentages of double-labeled cells with respect to the total amount of GFP-expressing cells (from 2 mice). The total numbers of GFP$^+$ cells

analyzed are indicated in brackets. Scale bars: 20 μm. IHC, immunohistochemistry; SVZ, subventricular zone; RMS, rostral migratory steam; OB, olfactory bulb; LV, lateral ventricle; inj site, injection site; ND, non detected.

Supplementary figure 3. GFP-transduced cells in the SVZ generate newborn neurons in the adult OB. A: The Mokola vector was injected **into** the SVZ of Ara-C treated mice. Animals were sacrificed for analysis at 21 d.p.i. **B-D:** Confocal micrographs showing GFP-expressing cells (green) double-stained with the neuronal marker NeuN (**B**, red) in the GCL. This result verifies that cells infected in the SVZ using the same lentiviral vector are also able to generate *in vivo* neurons in the OB. Astrocytes double-stained with GFP (green) and $GFAP^+$ (**C**, red) are still present at the injection site, at 21 d.p.i. Neuroblasts double-stained with GFP (green) and PSA-NCAM (**D**, red) are also found in the RMS and in the OB, at 21 d.p.i., suggesting continuous neuronal production by transduced cells. Images represent the maximal projections of each individual channel (left and middle), and orthogonal analysis in a single optical plane (right). **E:** Bar diagrams representing the percentage of double labeled cells (yellow) as compared to total number of GFP-expressing cells (green + yellow) in the analyzed regions, from 2 mice (total number of GFP^+ cells analyzed: 156, 310 and 95 for the double labeling with NeuN, GFAP and PSA-NCAM, respectively). Scale bars: 20 μm. IHC, immunohistochemistry; SVZ, subventricular zone; RMS, rostral migratory steam; OB, olfactory bulb; LV, lateral ventricle; GCL, granular cell layer; inj site, injection site.

Supplementary figure 4. Ara-C treatment eliminates transit-amplifying type-C cells in the SVZ and the RMS. A: Animals were sacrificed for analysis immediately after the Ara-C treatment. **B:** Confocal micrographs showing Mash1 staining (red). Note the complete elimination of type-C cells ($Mash1^+$) after the Ara-C treatment in the SVZ and the RMS elbow. Slices were counterstained with TOTO to visualize nuclei (blue). Images represent the maximal projections of merged channels. Scale bar: 50 μm. IHC, immunohistochemistry; SVZ, subventricular zone; RMS, rostral migratory steam; OB, olfactory bulb; LV, lateral ventricle.

Supplementary figure 5. RMS-newborn periglomerular cells become fully integrated neurons in the adult OB. A: The Mokola vector was injected into the RMS elbow of Ara-C treated mice. Animals were sacrificed for analysis at 60 d.p.i. Electrophysiological whole-cell recordings from RMS-generated periglomerular cells (PGCs) were

performed in the glomerular layer (GL). B: Example of voltage-dependent Na^+ current traces elicited by increasing depolarizing voltage-steps (successive jumps of 10 mV from a holding potential of -110 mV) in RMS-generated PGCs. C: Examples of traces of outward sIPSCs recorded from PGCs in standard conditions (*std*) or in the presence of the GABA antagonist gabazine (*Gz*, 10 µM). Neurons were held at 0 mV, the reversal potential for glutamatergic events. D: Examples of traces of inward sEPSCs recorded from PGCs in standard conditions (*std*) or in the presence of the AMPA antagonist NBQX (*NBQX*, 10 µM). Neurons were held at -70 mV, the reversal potential for GABAergic events. E and F: Expression of different proteins by RMS-generated PGCs was evaluated using double-labeling immunohistochemistry at 90 d.p.i. Confocal micrographs showing GFP-expressing PGCs (green) in the GL, double-stained with tyrosine hydroxylase (TH in red; E) and calretinin (CalR in red; F). Images represent the maximal projections of each individual channel (right), and the orthogonal analysis in a single optical plane (left). Scale bars: 10 µm. SVZ, subventricular zone; RMS, rostral migratory steam; OB, olfactory bulb; LV, lateral ventricle, IHC, immunohistochemistry.