# Subventricular zone stem cells are heterogeneous wi

**SUMMARY** (149 words)

**We determined the embryonic origins of adult forebrain subventricular zone (SVZ) stem cells by Cre-lox fate mapping in transgenic mice. We found that all parts of the telencephalic neuroepithelium, including the medial and lateral ganglionic eminences (MGE and LGE) and the cerebral cortex, contribute multipotent, self-renewing stem cells to the adult SVZ. Descendants of the embryonic LGE and cortex settle in ventral and dorsal aspects of the dorsolateral SVZ, respectively. Both populations contribute new (BrdU-labeled) Tyrosine Hydroxylase- and Calretinin-positive interneurons to the adult olfactory bulb. However, Calbindin-positive interneurons in the olfactory glomeruli were generated exclusively by LGE-derived stem cells. Thus, different SVZ stem cells have different embryonic origins, colonize different parts of the SVZ and generate different neuronal progeny - suggesting that some aspects of embryonic patterning are preserved in the adult SVZ. This could have important implications for the design of endogenous stem cell-based therapies in the future.**

## METHODS

### *Transgenic mice*

The generation and genotyping of *Nkx2.1-Cre*, *Gsh2-Cre*, *Emx1-Cre*, *Dbx1-Cre* and *Emx1-CreER*$^{T2}$

hours. The blocking solution was replaced with 10 ml of blocking solution containing rabbit anti-GFP (1:6000, Abcam) and placed on a shaking platform overnight at 4°C. Matrices were washed in PBS during the day and stained with AlexaFluor 488-conjugated goat anti-rabbit IgG (1:1000, Invitrogen) overnight at 4°C. Matrices were again washed in PBS for four hours at room temperature before colony size was measured using a gridded Petri dish (Stem Cell Technologies). The neural stem cell colonies were characterized as ≥ 2mm in diameter (Brent Reynolds unpublished data; Bull and Bartlett, 2005) and scored as GFP-positive or -negative under a fluorescence dissecting microscope.

***Immunocytochemistry***

For GFP immunolabeling, sections were washed with PBS and permeabilized with blocking solution containing Triton-X100 and NGS (see previous paragraph) before incubating overnight with rabbit anti-GFP (1:6000) or rat anti-GFP IgG2a (1:1000, Nacalai Tesque Inc.). Sections were washed three times with PBS before adding AlexaFluor 488-conjugated anti-rabbit IgG or AlexaFluor 488-conjugated anti-rat IgG (1:1000; Invitrogen) in blocking solution. Sections were treated with Hoescht 33258 ($1:10^4$; Sigma) to detect cell nuclei and washed with PBS. Floating sections (30 μm) were transferred onto glass slides and mounted using Fluoromount (DAKO). Sections were co-stained with either CY3-conjugated mouse anti-GFAP (1:3000; Sigma); rabbit anti-GFAP (1:500; Dako); mouse

being located near the ventral extremity of the lateral ventricle (**Fig. 1E, J**). In occasional sections cells were also observed at the ventral edge of the dorso-lateral corner of the SVZ (**Fig. 1I**). The majority of cells lining the lateral wall of the lateral ventricle, including the dorso-lateral SVZ, were derived from the *Gsh2* domain (**Fig. 1F, K, L**). Together with the previous result, this indicates that most of the lateral wall cells are descendants of the LGE, as previously thought (see Introduction). However, we also observed a significant population of GFP-labeled SVZ cells in the *Emx1-Cre/R26-GFP* mice. These were found in the roof of the lateral ventricle and in the dorso-lateral corner of the SVZ (**Fig. 1G, M**)

The *Emx1*-derived SVZ cells were found mainly in the dorsal half of the dorso-lateral corner of the SVZ (**Fig. 1M**), suggesting that they were genuine descendants of the embryonic cortical VZ, not *Gsh2*-derived cells that had up-regulated *Emx1* postnatally and consequently recombined de novo in the adult. We tested this directly by inducing transient recombination in *Emx1-CreER$^{T2}$/R26-YFP* mice with a single dose of Tamoxifen at E10.5 and following the subsequent fates of labeled cells in the adult brain. The distribution of GFP-positive cells within the SVZ of pulse-labeled *Emx1-CreER$^{T2}$/ R26-YFP* mice (**Fig. 1N**) was similar to the constitutive *Emx1-Cre/R26-YFP* mice (**Fig. 1M**). These data demonstrate that cells from the embryonic cortex do indeed give rise to the dorsal and dorso-lateral edge of the adult SVZ. Therefore we conclude that the adult SVZ is comprised of cells derived predominantly from the VZ of the embryonic LGE and cortex (**Fig. 1O**), and that the location of these cells potentially defines germinal domains in the SVZ similar to those known to exist developmentally.

***Multipotent, self-renewing SVZ stem cells are descended from both the LGE and cortex***

To determine whether SVZ cells derived from both *Gsh2*- and *Emx1*-expressing embryonic territories continue to divide in the adult SVZ, as would be expected for stem or progenitor cells, we asked whether they can incorporate BrdU in vivo. Adult *Cre/ R26-GFP* mice were given a dose of BrdU every 6 hours for 24 hours and analyzed three hours after the final dose. Double immunolabeling for GFP and BrdU detected small numbers of *Nkx2.1* (MGE)-derived cells in the lateral wall of the SVZ that were BrdU-positive (**Fig. 2A, B**). The majority of BrdU-positive cells in the SVZ were derived from *Gsh2*-expressing territories (**Fig. 2C, D**), so most proliferative SVZ cells have their origins within the LGE. A significant number of BrdU-labeled cells in the dorso-lateral corner of the SVZ were *Emx1*-derived (**Fig. 2E, F**), confirming that the embryonic cortex also contributes proliferative cells to the adult SVZ. These could be either multipotent stem cells or neural progenitor cells with more restricted potential.

To investigate the stem and/or progenitor cell nature of the *Emx1*- and *Gsh2*-derived SVZ cells we turned to cell culture assays. The adult SVZ was dissected from *Cre/R26-GFP* mice and dissociated to generate neurosphere cultures (see Methods). Since each neurosphere was derived from a single proliferating cell in the starting culture, each sphere was either entirely positive or entirely negative for GFP (**Fig. 3A**). The proportions of GFP-positive and GFP-negative neurospheres were determined for each of our Cre lines. The majority of neurosphere-forming cells in the SVZ were of striatal origin; ~70% of all neurospheres were GFP-positive in cultures from *Gsh2-Cre/R26-GFP* mice (**Fig. 3B**). By comparison, *Emx1*-derived SVZ cells accounted for ~20% of the neurosphere-forming activity (**Fig. 3B**). Some *Nkx2.1*-derived neurospheres were detected but their contribution was small, in keeping with the low contribution of the *Nkx2.1*-expressing VZ to the adult SVZ (see above). All SVZ neurosphere-forming activity could be accounted for by the combination of *Gsh2*-derived (LGE plus MGE) and *Emx1/Dbx1*-derived (cortex plus cortico-striatal sulcus) SVZ cells (**Fig. 3B**)**.** These data provide clear evidence that the neurosphere-forming SVZ cells are heterogeneous in origin.

Neurospheres derived from *Gsh2-Cre*, *Emx1-Cre* and *Emx1/Dbx1-Cre* mice could be serially passaged more than seven times while remaining GFP-positive, confirming the presence of self-renewing stem cells (data not shown). Moreover, cultures from each of these Cre mice contained GFP-positive neurospheres that were able to generate GFAP-positive astrocytes, O4-positive oligodendrocytes and βIII-tubulin-positive neurons when transferred to differentiation conditions, confirming their multipotency in vitro (see Methods) (**Fig. 3C**). However, the *Nkx2.1*-derived neurospheres were less neurogenic than the others, because a smaller proportion of GFP-positive neurospheres generated βIII-tubulin-positive neurons (**Fig. 3D**).

To quantify the proportions of true SVZ stem cells descended from different regions of the embryonic VZ, we dissociated SVZ cells from *R26-GFP* reporter mice carrying *Nkx2.1-Cre*, *Gsh2-Cre*, *Emx1-Cre* or *Emx1/Dbx1-Cre* and plated them in a mitogen-containing collagen matrix. Neural progenitor cells with limited self-renewing capacity formed small colonies and stopped proliferating after 1-2 weeks, whereas self-renewing stem cells continued to proliferate for more than 3 weeks, forming colonies ≥ 2mm in diameter. Stem cell colonies that were GFP-positive (**Fig. 3E, F**) and others that were GFP-negative (**Fig. 3G, H**) were identified in all cultures after three weeks (**Fig. 3I**). The proportions of self-renewing SVZ stem cells contributed from each embryonic territory, as judged by this collagen matrix

(**Fig. 3B, I**). This suggests that LGE-derived adult SVZ cells might contribute a greater proportion of

***LGE-and cortex-derived adult SVZ stem cells have different properties***

*Gsh2*- and *Emx1*-derived cells were found in all layers of the olfactory bulb, although there was a relatively higher contribution of *Emx1*-derived cells to the glomerular layer than the granule cell layer (**Fig. 5K**

*CreER*$^{T2}$*/ R26-YFP* transgenic line.  Tamoxifen was administered at E1

## DISCUSSION

Hydroxylase (dopaminergic sub-population) and are all generated or regenerated continuously during adulthood (Kosaka et al., 1995;Dellovade et al., 1998;Hack et al., 2005;Kohwi et al., 2005). Adult-born Calretinin-positive interneurons are also found in the granule neuron layer (see **Fig. 6**). Our data confirm that these neuronal subtypes continue to be generated during adulthood. However when we examined the turnover of interneurons expressing Parvalbumin, a third calcium-binding protein of the EF-hand family, we found that these neurons, located in the external plexiform layer (EPL), are not turned over significantly during adulthood. In this sense they resemble projection neurons such as mitral cells but differ from most olfactory interneurons. Parvalbumin-positive neurons are also present in the human olfactory bulb and these were reported

despite their being less neurogenic overall than th

### *Cell fate determinants in the SVZ and RMS*

We have taken advantage of the spatially restricted

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## FIGURE LEGENDS

**Figure 1** All regions of the telencephalic neuroepithelium contribute to the adult SVZ. Coronal sections (30 μm) of adult (P50) mouse brains were stained with rabbit anti-GFP (green) and Hoescht 33258 to visualize cell nuclei (blue). GFP-positive cells were detected by immuno-labeling in sections from *R26-GFP* reporters carrying *Nkx2.1-Cre* (**A, E**), *Gsh2-Cre* (**B, F**), *Emx1-Cre* (**C, G**) or *Emx1/Dbx1-Cre* (**D, H**) transgenes. Each region of the embryonic neuroepithelium contributed preferentially to distinct regions of the adult SVZ. Images (I-M) are higher-magnification confocal images of the areas boxed in panels (E-H). (**I, J**) *Nkx2.1-Cre/R26-GFP* brain. Groups of GFP-positive cells were occasionally detected at the ventral edge of the dorso-lateral corner of the SVZ (**I**), as well as the ventral region of the lateral wall (**J**). (**K, L**) *Gsh2-Cre/R26-GFP* brain. Many GFP-positive cells were found in the lateral wall (**K**) and the dorso-lateral corner (**L**) of the SVZ. (**M**) *Emx1-Cre/R26-GFP* brain. Significant numbers of GFP-labeled cells are present, mainly in the dorsal half of the dorso-lateral corner of the SVZ. (**N**) Adult (P50) *Emx1-CreER$^{T2}$/ R26-YFP* brain, following Tamoxifen induction at E10.5. The distribution of GFP-labeled cells was found to be similar to the *Emx1-*

**Figure 7:** LGE- and cortex-derived SVZ stem cells generate different sub-populations of olfactory interneurons in the adult. BrdU was administered to adult (seven week old) *R26-GFP* reporter mice carrying either *Gsh2-Cre* or *Emx1-Cre* transgenes. Four weeks later (~P80), coronal sections of olfactory bulbs were triple-immunolabeled for BrdU (blue), GFP (green) and either Parvalbumin (**A, B**), Tyrosine Hydroxylase (TH) (**C, D**), Calretinin (Crt) (**E, F**) or Calbindin (Cb) (**G, H**) (red). Cells indicated by arrows in the compressed confocal series are shown at high magnification in the insets (single confocal scans). The proportions of adult-born interneurons of each genotype are presented graphically in (**I**). *Gsh2*- and *Emx1*-derived stem cells generate different proportions of TH-, Crt- and Cb-positive neurons - the most striking example being Cb-positive neurons which were exclusively *Gsh2*

stem cells in the SVZ generate neuroblasts that migrate via the RMS into the olfactory bulb and differentiate as olfactory interneurons. Scale bars: 50 µm.

**Supplementary Figure S1:** Emx1-derived cells in the adult SVZ do not express Gsh2. Coronal sections through the SVZ of an adult *Emx1-Cre/R26-YFP* transgenic mouse were immuno-labeled with anti-Gsh2 (red), anti-GFP (green) and counter-stained with Hoechst 33258 (blue). (**A, B**) Scattered Gsh2-positive nuclei (arrow) were detected, but never in YFP-positive cells (**C**), suggesting that there are distinct Emx1-expressing and Gsh2-expressing cell populations in the adult SVZ. A single confocal scan (~1 µm) is shown. Scale bar: 20 µm.

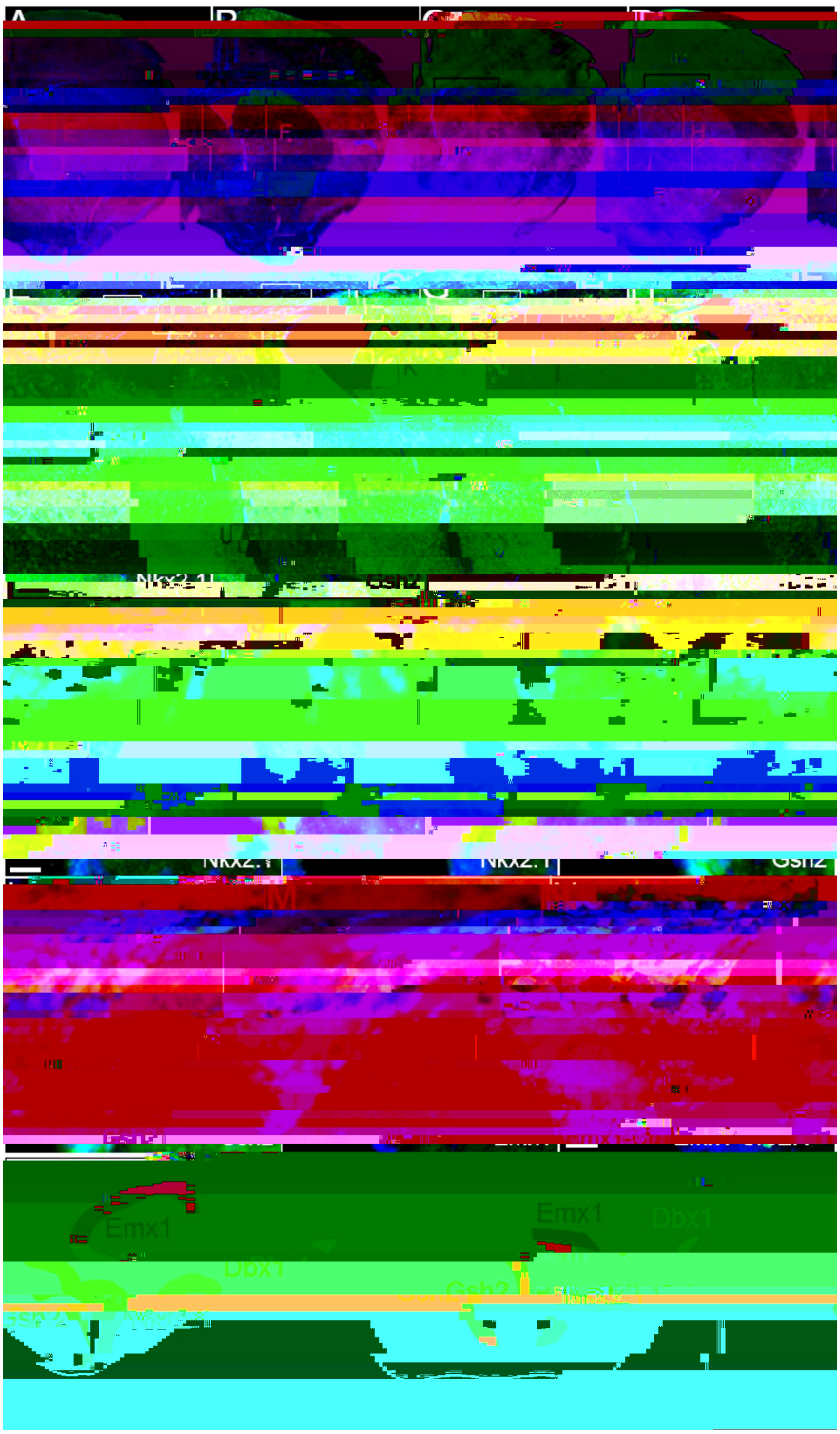



**FIGURE 1**

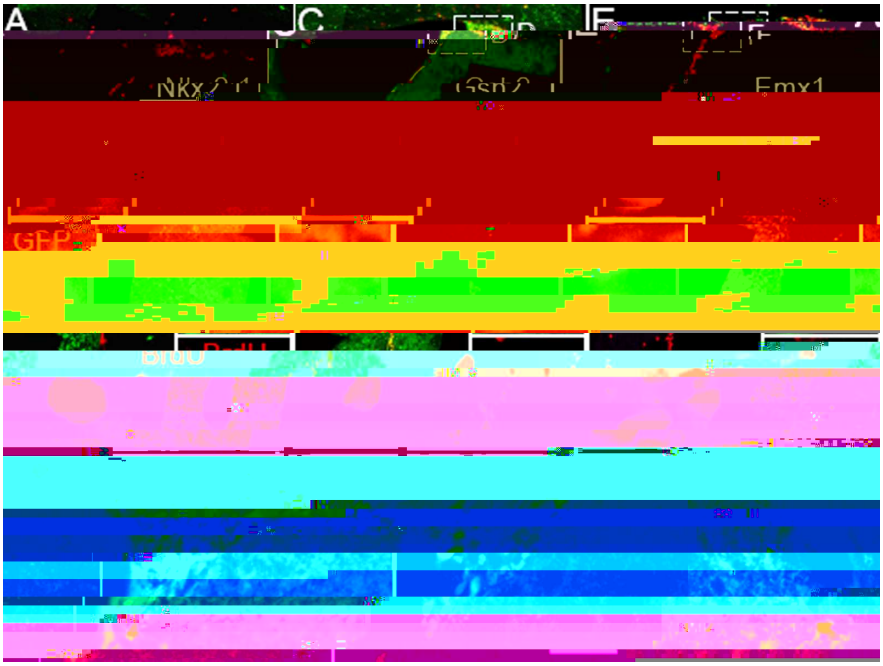
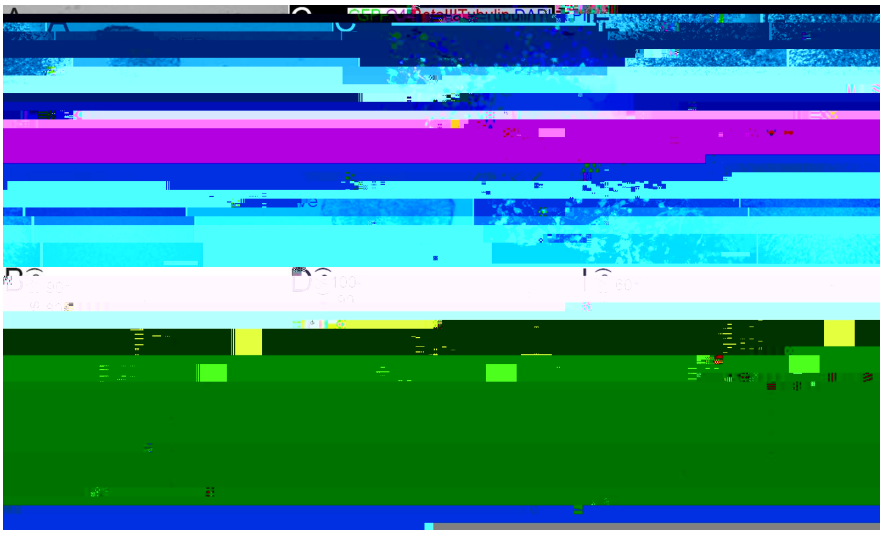



**FIGURE 2**



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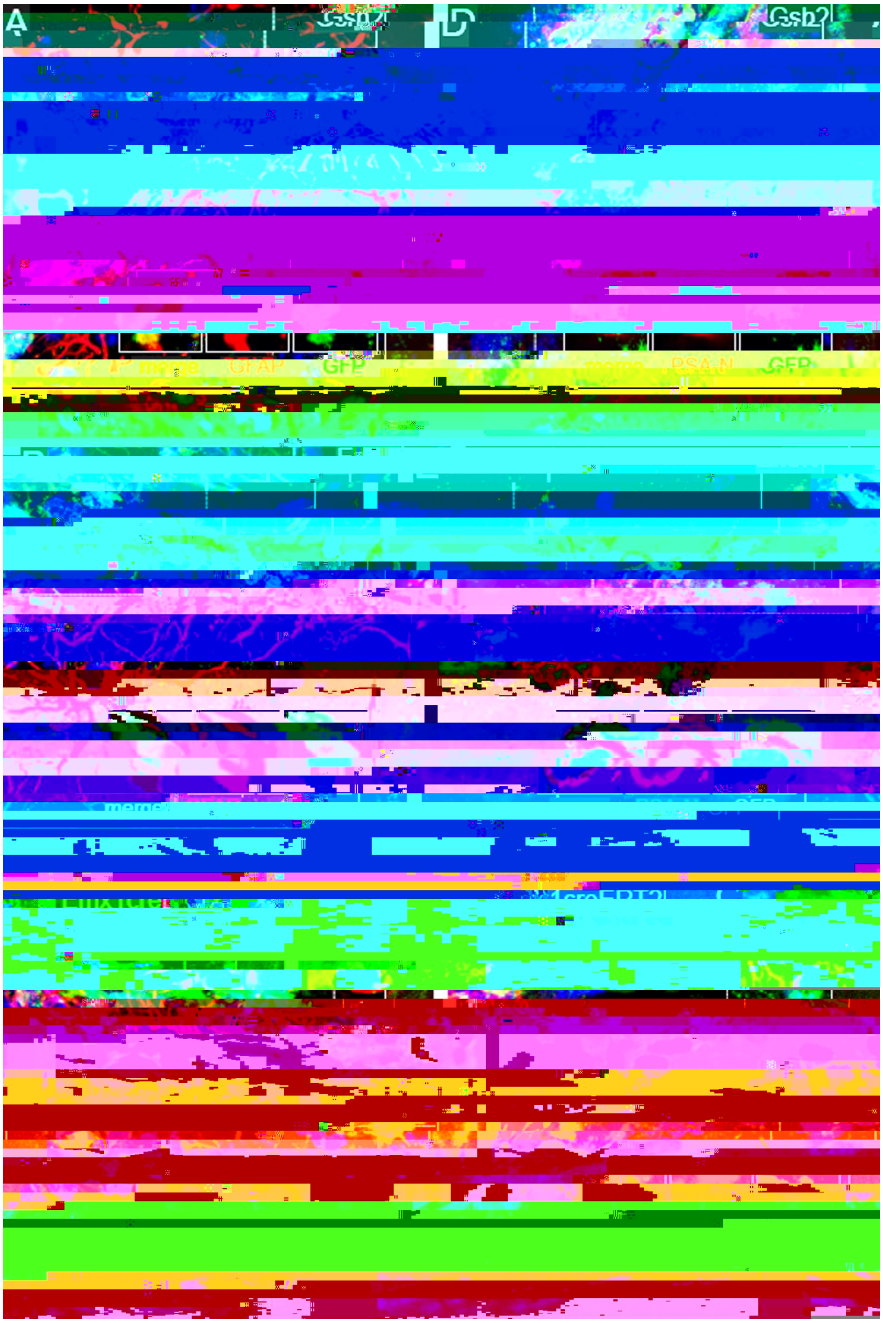

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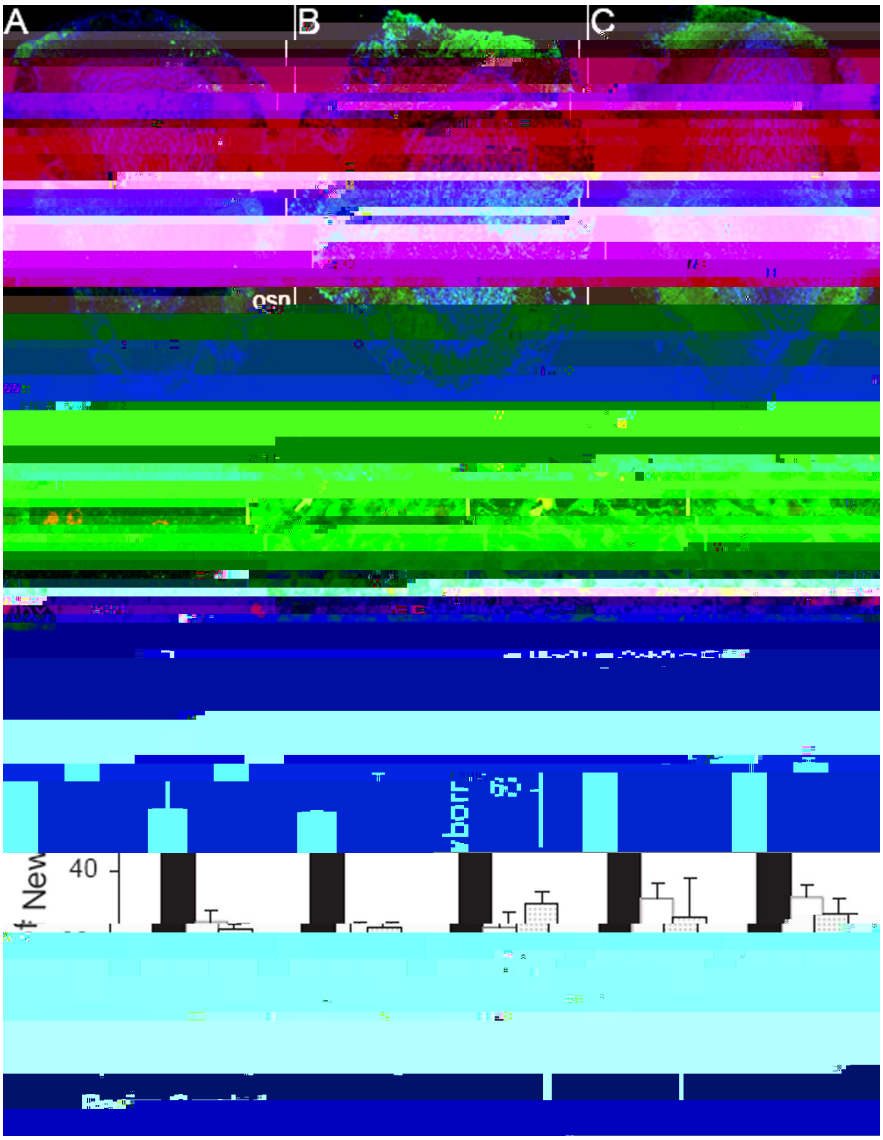


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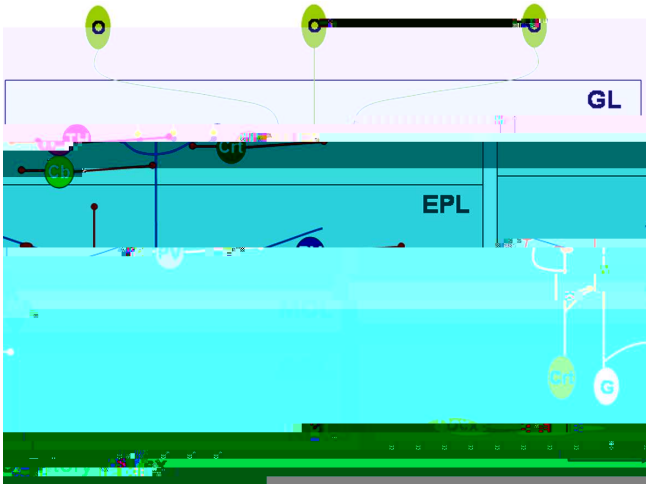



**FIGURE 6**

**FIGURE 7**

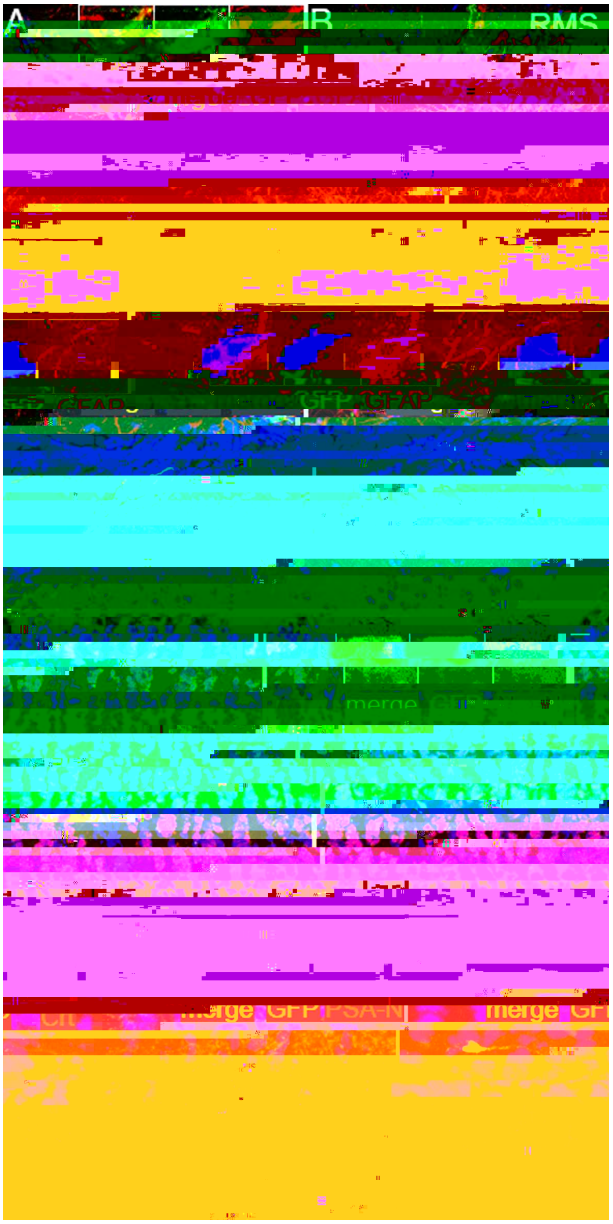
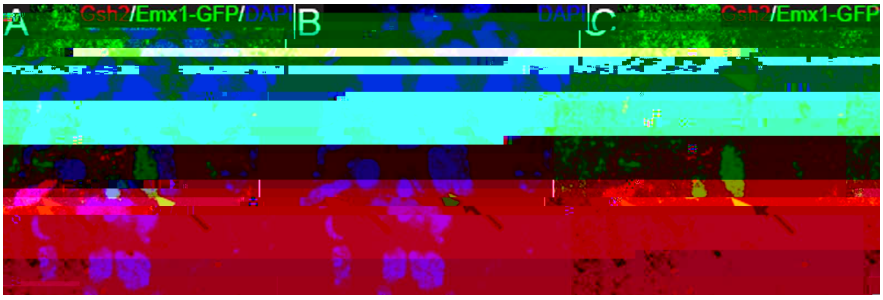



**FIGURE 8**




**Supplementary FIGURE 1**