A new subtype of progenitor cell in the mouse embryonic neocortex

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Supplementary Figures 1-6:

Supplementary Movies 1-9:

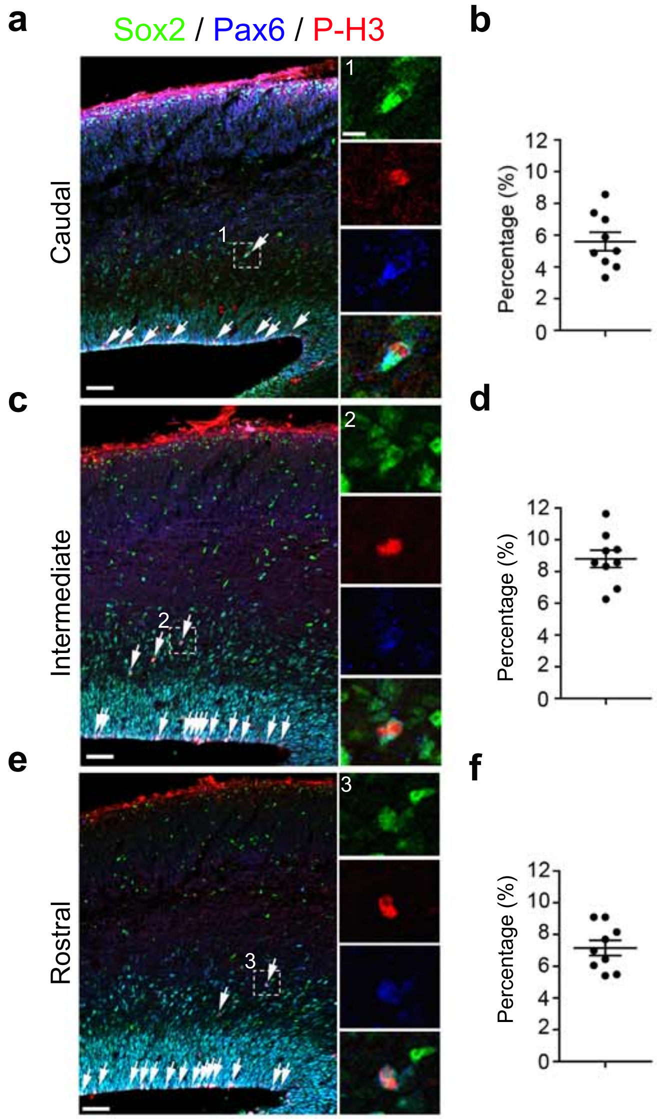
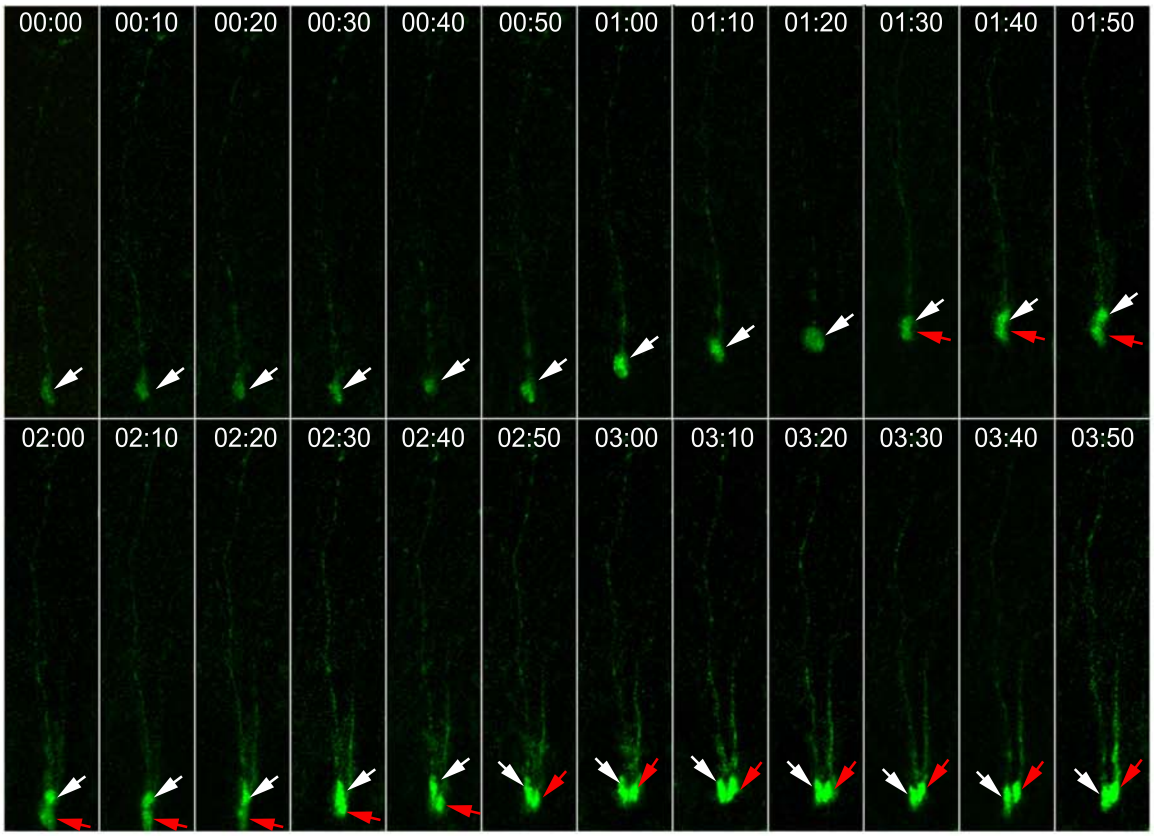
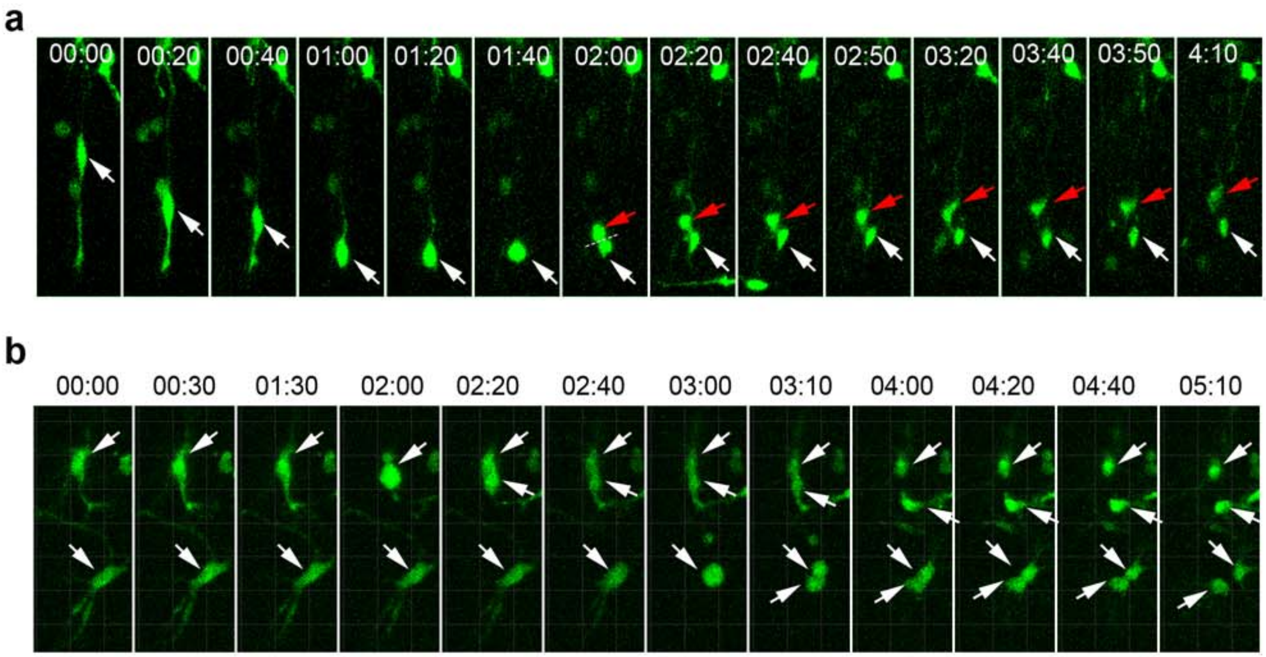


Figure 1. oRG cells in the developing mouse neocortex. (a) Labelling of RG and oRG-like cells with Adeno-GFP. Note the oRG-like cell (box 1) that has a long basal process (open arrowhead) but no apical process. High magnification images are shown to the right (1 and 2). Scale bars: 50 μm and 15 μm. (b) Representative oRG-like cell (arrow), open arrowheads indicate the basal process. Scale bars: 25 μm. (c) Phosphovimentin (P-Vim, green) labels oRG cells in mitosis. The basal process has varicosities characteristic of M-phase oRG cells. The oRG-like cells co-stain with radial glial progenitor markers Pax6 (blue) and Sox2 (red). Arrows indicate triple positive oRG-like cells; open arrowheads indicate the basal process. Scale bars: 50 μm and 10 μm. (d) Quantification of the percentage of mitotic, basal process bearing oRG-like cells identified by P-Vim+, Pax6+, and Sox2+ immunostaining in the VZ (92.95 ± 5.90%) and superficial SVZ (7.05 ± 3.70%) (Total 78 cells from six animals). (e) Phosphovimentin+ (green) oRG cells at E16.5 co-stain for Sox2 (red) but are Tbr2- (blue, an intermediate progenitor marker). High magnification images of a representative outlined cell are shown to the right. Arrows indicate oRG-like cells co-stained for P-Vim (green) and Sox2 (red), open arrowheads indicate the basal process. Scale bars: 50 μm and 10 μm. (f) Quantification of the percentage of oRG-like cells identified by P-Vim+/Sox2+/Tbr2+ (0 %) or P-Vim+/Sox2+/Tbr2- (100%) in the superficial SVZ (Total 46 cells from six animals).

Wang and Kriegstein Supplenmentary Figure 1

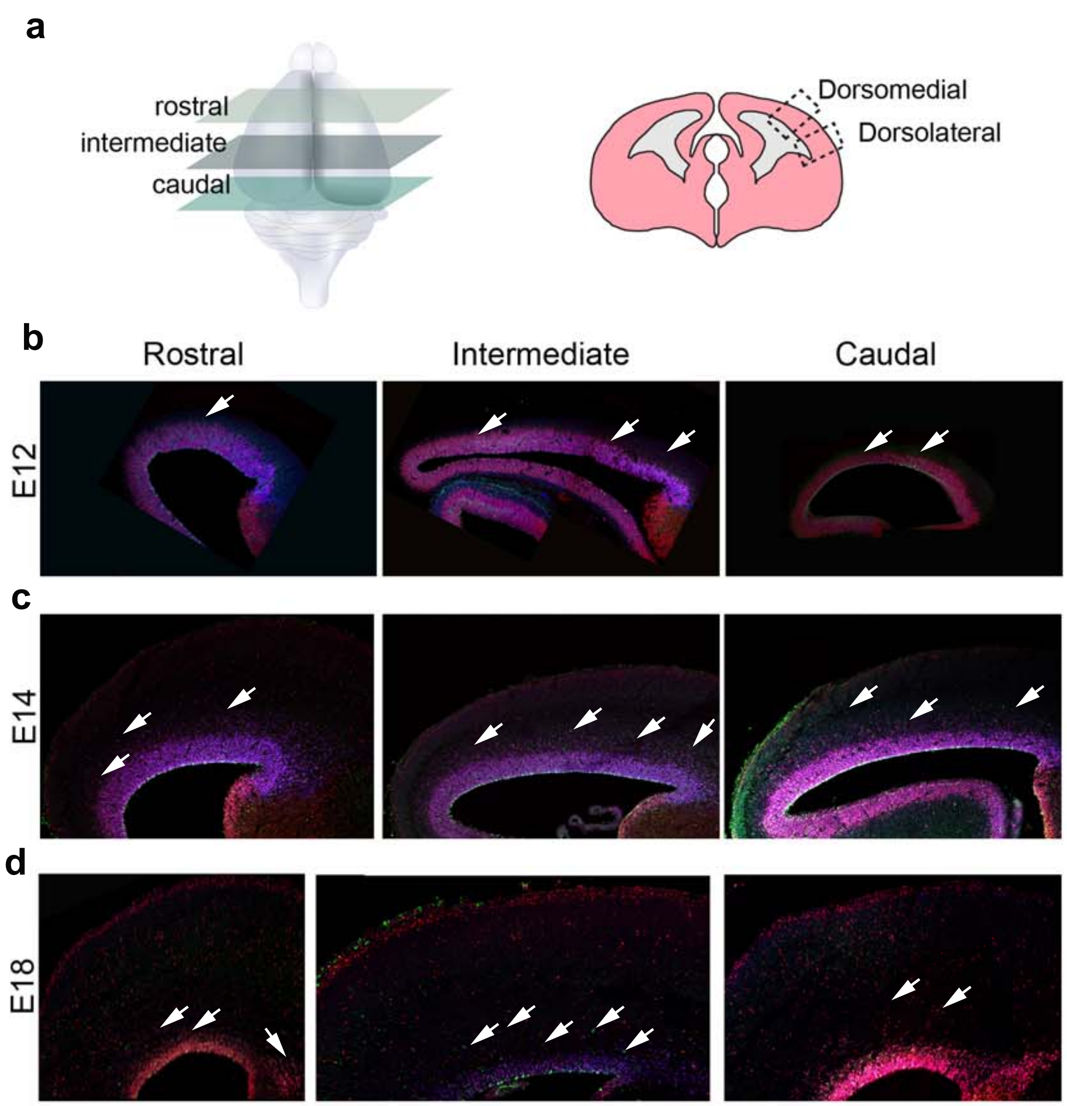


Supplementary Figure 2. Very few oRG cells undergo symmetric division in the developing neocortex. Time-lapse imaging showing symmetric oRG cell division. Arrows (white) indicate the mother oRG cells, arrows (yellow and red) indicate daughter oRG cells. Scale bar: 20 μm.



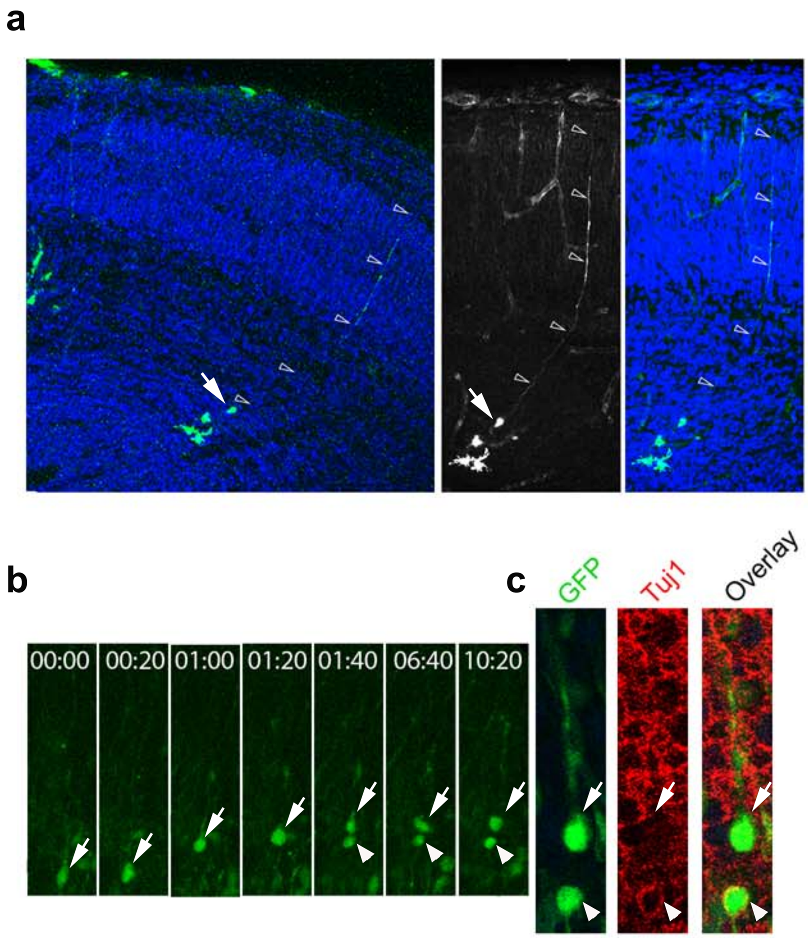
Supplementary Figure 3. Distinct behaviours of mitotic RG cells and IP cells. (a) Time-lapse sequence of an RG cell division. The RG cells move toward the apical surface during G2 to undergo mitosis at the ventricular lumen (see supplementary Movie 2). White arrows indicate RG cells and red arrows indicate daughter cells. (b) An IP cell divides in situ without nuclear translocation (see supplementary Movie 3). White arrows indicate IP cells and yellow arrows indicate daughter cells. Scale bar: a-b, 20 μm and 10 μm.

Wang and Kriegstein Supplementary Figure 3



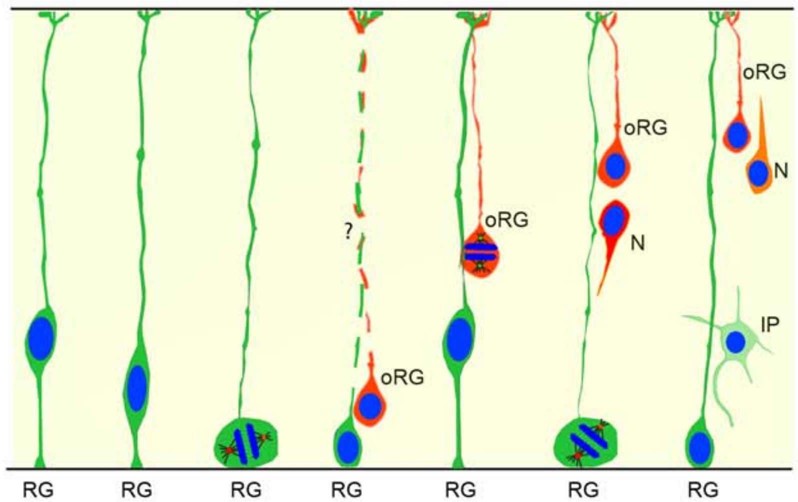
Supplementary Figure 4. Distribution pattern of oRG cells in developing mouse neocortex. (a) Schematic diagram of the rostro-caudal axis and dorsomedial and dorsolateral cell locations. (b-d) Distribution of the Pax6+/sox2+/P-Vim+ cells at E12 (b), E14 (c) and E18 (d). Arrows indicate Pax6+/sox2+/P-Vim+ cells located in the superficial SVZ. Scale bar: 200 μm.

Wang and Kriegstein Supplementary Figure 4



Supplementary Figure 5. oRG cells generate neurons. (a) Retrograde labelled oRG cell (arrows) with a basal process (open arrowhead) contacting the pial surface counter-stained with DAPI (blue). Scale bar: 100 µm. (b) Time-lapse of an oRG cell division. White arrows indicate the oRG cell; yellow arrows indicate the basal daughter cell, and arrowheads (yellow) indicate the apical daughter cell. Scale bar: 15⊠m (c) An oRG cell generates a daughter neuron (Tuj+, yellow arrowhead). An Adeno-GFP labelled cell (green) was time-lapse imaged at 10m intervals 2d after infection and immunostained for Tuj1, a neuronal marker (red) after 12h more in culture. Scale bar: 10 ⊠m.

Wang and Kriegstein Supplenmentary Figure 5



Supplementary Figure 6. A model illustrating that RG cells can divide at the VZ surface to generate self-renewed RG cells and daughter oRGs. Daughter oRG cells translocate and asymmetrically divide to self-renew and generate daughter neuron. N: neurons and IP: intermediate progenitors.

Wang and Kriegstein Supplenmentary Figure 6

Supplementary Movie 1. This movie shows an example of mitotic somal translocation and oRG cell division.

An oRG cell undergoes mitotic somal translocation and divides. The upper cell inherits the basal fiber whereas the lower cell rapidly extends a prominent process towards the ventricle. Images were acquired every 10m and the play rate is seven frames per second.

Supplementary Movie 2. This movie shows interkinetic nuclear migration (INM) of RG cells in mouse neocortex.

An RG cell undergoes INM and divides at the VZ surface. Images were acquired every 10m and the play rate is seven frames per second.

Supplementary Movie 3. This movie shows an example of two IP cell divisions *in situ*.

Two IP cells divide *in situ* without nuclear translocation. Images were acquired every 10m and the play rate is seven frames per second.

Supplementary Movie 4. This movie shows an oRG cell that divides and generates a daughter neuron.

An oRG cell undergoes mitotic somal translocation and divides. The apical daughter cell acquires neuronal morphology 40h after division, and extends a leading process toward the pia. After acquiring a short trailing process, the bipolar daughter cell migrates rapidly to the cortical plate. Images were acquired every 30m and the play rate is seven frames per second.

Supplementary Movie 5. This movie shows an example of an asymmetric oRG cell division that yields a daughter oRG cell and a daughter neuron.

Cell fate was revealed by immunostaining with anti-Pax6 (progenitor marker) and anti-NeuN (neuronal marker) after 12h further culture following time-lapse imaging.

Supplementary Movie 6. This movie shows an example of oRG cells originating from RG cells.

Two days after *in-utero* intra-ventricular retrovirus infection at E11.5, a GFP-labelled RG cell was monitored at 15m intervals. Asymmetric division of the RG cell generates a self-renewed RG cell, which undergoes a second division. Another daughter cell undergoes mitotic somal translocation before mitosis, a defining feature of oRG cell behaviour. Images were acquired every 15m and the play rate is seven frames per second.

Supplementary Movie 7. This movie shows centrosome behaviour in mitotic oRG cells.

The centrosome is revealed by DsRedex-Centrin1. Frames were acquired every 10m and the play rate is seven frames per second.

Supplementary Movie 8. This movie shows centrosome behaviour in mitotic RG cells.

The centrosome is revealed by DsRedex-Centrin1. Frames were acquired every 10m and the play rate is seven frames per second.

Supplementary Movie 9. This movie shows centrosome behaviour in mitotic IP cells.

The centrosome is revealed by DsRedex-Centrin1. Frames were acquired every 10m and the play rate is seven frames per second.

Appendix Movie 1. This movie shows interkinetic nuclear migration (INM) of group RG cells in mouse neocortex.

Group of RG cell undergoes INM and divides at the VZ surface. Images were acquired every 10m and the play rate is seven frames per second.

Appendix Movie 2. This movie shows no oRG cells mitosis after purposefully severing the apical processes of radial glial cells.

We used a scalpel to remove the apical surface of the VZ in order to severe the apical processes of radial glial cells. This process disrupted INM and division of RG cells at the VZ surface and does not produce oRG-like cell mitosis. Images were acquired every 10m and the play rate is seven frames per second.