Cell Reports

Supplemental Information Inventory

Formation of the Cortical Subventricular Zone Requires MDGA1-mediated Aggregation of Basal Progenitors

Perez-Garcia and O'Leary

The revised manuscript (Perez-Garcia and O'Leary) contains 7 "Supplemental Figures" complementing the data presented in the main text. It also contains "Supplemental Experimental Procedures" and "Supplemental References" sections.

Supplemental Figures

This section complements the data described in the main figures.

Figure S1 contains a detailed description of the expression of MDGA1 in the forebrain, in particular the rostral migratory stream (RMS) and the olfactory bulb (OB). Progenitors for the RMS and OB derive from the cortical subventricular zone (SVZ), which is the focus of our study. This figure complements Figure 1 that shows the expression of MDGA1 in the cortical SVZ.

Figure S2 contains several panels illustrating the expression pattern of MDGA1 during cortical development, with special emphasis in the cortical SVZ/VZ and in the upper layer neurons. This figure complements Figures 1 and 2 showing the expression of MDGA1 in vivo (immunofluorescence, electron microcopy) and in vitro (transfection assays).

Figure S3 contains two main panels showing the biochemical characterization of MDGA1 as a plasma membrane protein, and the complementary expression of MDGA1 and Connexin43 in the SVZ at P0. This figure complements Figures 1-3 showing MDGA1 expression in discrete domains in the basal progenitor cell membranes in the

SVZ, its confirmation in vitro in transfection assays, and its co-localization in vitro and in vivo (E16.5) with the gap junction protein Connexin43.

Figure S4 shows the gene targeting and generation of the MDGA1^{flox} allele. Proof of principles confirming the presence of the allele by southern blot and the effectiveness of the conditional deletion at the transcript and protein levels are also shown. This figure is the base of all the experiments done with the conditional knockout (cKO) in Figures 4-7.

Figure S5 shows 1) the reduction in thickness in the SVZ at P0 and 2) a confirmation of the absence of ectopic Tbr2+ cells in the cortical plate when we delete MDGA1 from early postmitotic neurons using the Nex-Cre driver. This figure complements the data showed in Figures 4-5.

Figure S6 shows that the ectopic Tbr2+ cells are a non-proliferative cell type and that they presumably die by apoptosis. This figure complements the data presented in Figures 4-7 illustrating the presence of the ectopic Tbr2+ cells in the cKO, their lack of neuronal differentiation and their failure to generate a proportion of cortical layer neurons.

Figure S7 is a summary of our major findings and a functional model, including: the expression of MDGA1 and its cellular localization in discrete domains in the cell membrane of Basal Progenitors (BPs) in the SVZ (Figure 1-2); its interaction with the gap junction protein Connexin43 in the SVZ (Figure 3); and the consequences of the deletion of MDGA1 from BPs (Figures 4-7). This figure also represents our model summarizing the role of MDGA1 in BPs, its function in maintain the integrity of the SVZ,

and the consequences for the SVZ and the cortical layers when MDGA1 is deleted from BPs in the cKO.

Supplemental Experimental Procedures

This section complements the main techniques described in the main text.

The *Animals* section complements the experimental procedures in the main text. It describes the procedures to anesthetize and perfuse the animals, and includes a statement indicating that all animals have been treated according to the guidelines of the National Institutes of Health and the Institutional Animal Care Use and Committee of The Salk Institute.

The *Immunohistochemistry* section describes the Nissl and Immunofluorescence protocols with a detailed description of the secondary antibodies used for immunofluorescence, and complements the histological techniques described in the Experimental Procedures section in the main text.

The *Electron Microscopy* section describes in detail the methodology used to prepare the samples for ultrastructure analysis using a Transmission Electron Microscopy.

The *Proliferation Assays section* describes the methodology used for BrdU injection and detection of both BrdU and PH3.

The *Cell Counting and Statistics* section describes the number of individuals and the software used in our statistics studies, with emphasis in the description of the protocol used to measure and counting in brain sections.

The *Cell Culture* section describes the in vitro procedures described in the main text, summarizing the protocol used for immunolabeling 293 cells transfected with Myc-MDGA1 or MDGA1, and the antibodies used in those assays.

The *Biochemistry* section is a compendium of the biochemical techniques used in the main text.

The *Enrichment Differential Centrifugation Assay* explains in detail the protocol used to isolate the microsome and soluble fractions from P7 wild type (WT) brains and the antibody used to specifically detect MDGA1 in the microsome fraction, as described in Figure S3.

The *Immunoprecipitation Assay* section describes in detail the protocol used to co-immunoprecipitate MDGA1 and Connexin43 in WT brain samples and in 293 cells transfected with Myc-MDGA1. The procedure for immunodetection in the blots and the antibodies used are also described, and the results are shown in Figure 3.

The *Phosphatidylinositol-Specific Phospholipase C Assay* section described in detail the standard protocol used to release MDGA1 from plasma membranes of WT brains samples by cleavage through its GPI anchor domain, as well as the procedure used to detect the presence of MDGA1 in the blot, and the results are shown in Supplemental Figure S3.

The *Gene targeting and generation of the floxed MDGA1 mice* section is a detailed explanation of the procedures used to generate the MDGA1^{Flox} allele used in the present study.

Supplemental References

This section summarizes additional references cited exclusively in the Supplemental Information.