

Expanded View Figures

Figure EV1. Isolation and transcriptional profiling of qNSCs.

- A Dot plots depicting the expression of mRNA for neurogenic transcription factors *Sox11*, *Arx*, *Dlx1*, and *Dlx2* in prospectively isolated cellular populations from the SEZ.
- B Venn diagram depicting common neural stem enriched genes from three different publications.
- C Graph showing GO terms enriched in the set of 74 overlapping genes identified in (B). GO terms related to neurogenesis are shown in red and to the cell cycle in magenta.
- D Micrographs showing neural stem cells (hGFAP-GFP⁺; CD133⁺) isolated from the BrdU-treated hGFAP-eGFP animals and fixed 2 h after plating.
- E Table showing predicted microRNA-mRNA interactions and functional regulations.
- F Pie chart depicting predicted and published targets of miR-204 among priming factors following the expression pattern DE < qNSC < aNSC < NB.
- G Dot plot depicting the functional regulation of neurogenic priming factors by miR-204 in luciferase reporter assays.
- H Graph showing GO terms enriched in the set of 34 putative miR-204 targets. GO terms related to neurogenesis are shown in red and to the cell cycle in magenta.

Data information: Abbreviations: Dien., diencephalon; qNSC, quiescent neural stem cell; aNSC, activated neural stem cell; NB, neuroblast; DE, diencephalon astrocyte; WT, wild type; CTX, cortex; RMS, rostral migratory stream; SEZ, subependymal zone; OB, olfactory bulb; DE, diencephalon astrocyte. Scale bar (D) 10 μ m. Data are shown as mean \pm SEM; each symbol represents independent biological replicate; significance was tested using Kruskal–Wallis ANOVA; **P* value < 0.05, ***P* value < 0.01, ****P* value < 0.001.

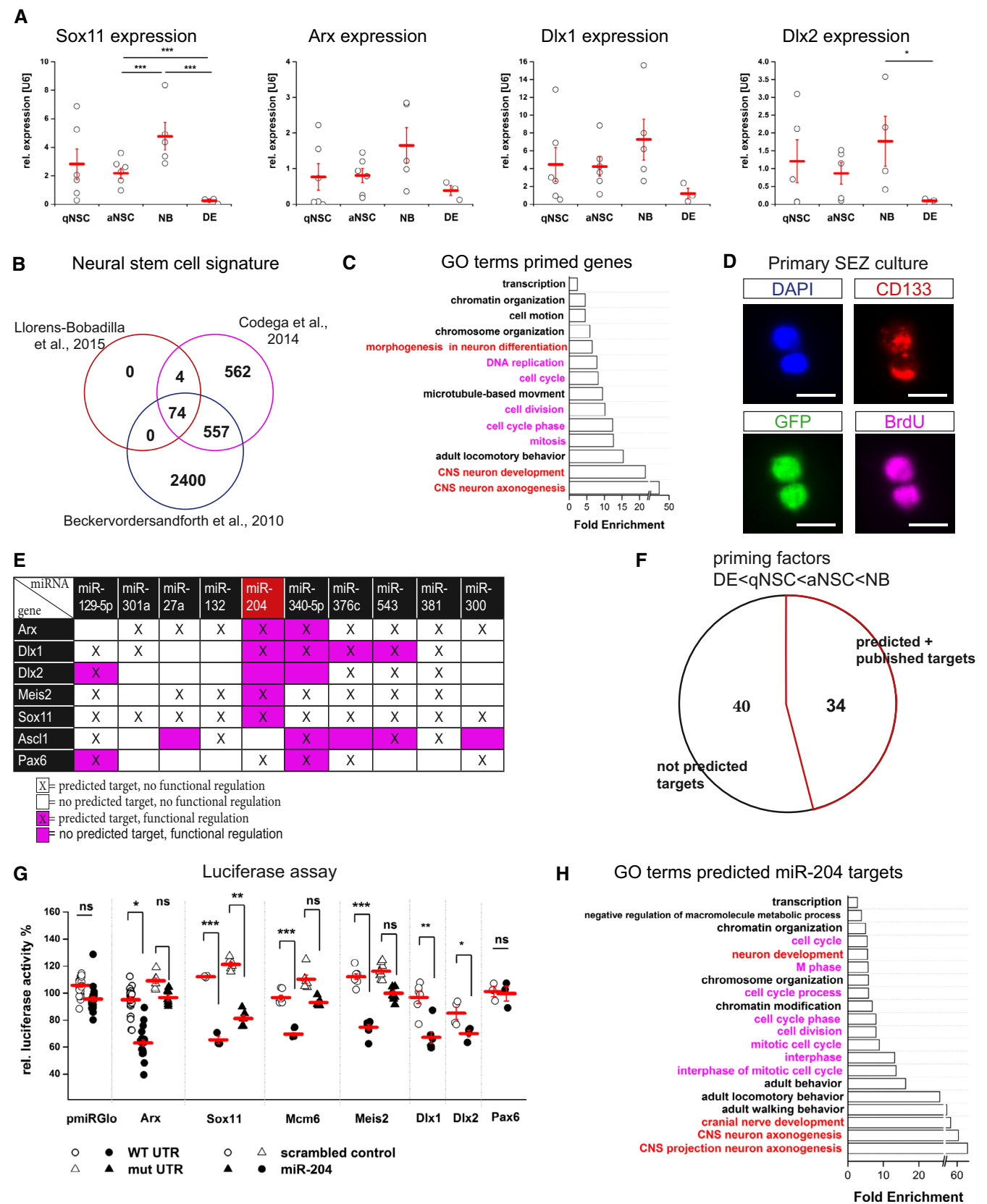


Figure EV1.

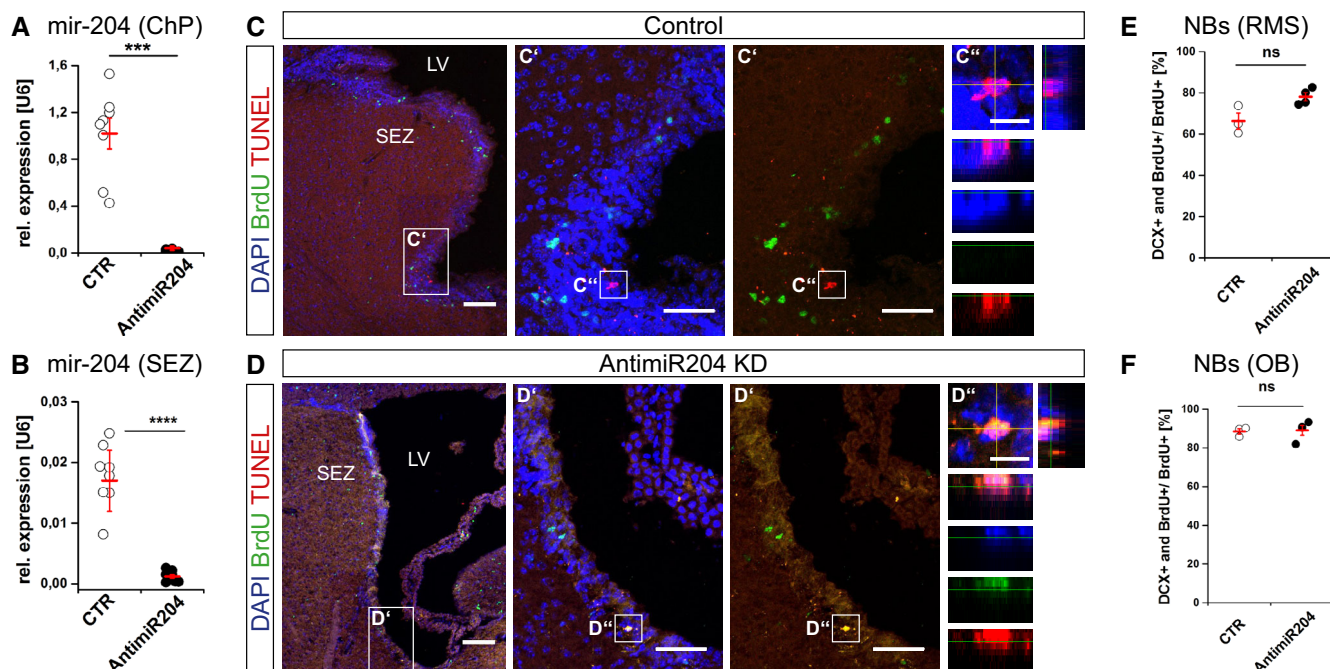


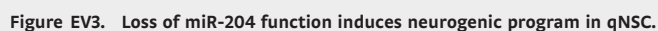
Figure EV2. miR-204 regulates SEZ neurogenesis.

A, B Dot plots showing miR-204 levels in ChP (A) and in the SEZ (B) 3 days after injection of aCSF or AntimiR204 into the lateral ventricle.

C, D Micrographs depicting the TUNEL staining in the SEZ 7 days after injection of aCSF (control, C) or AntimiR204 (D). Boxed areas correspond to the higher magnifications in the adjacent panels (C', C'', D', D''). Note that almost no BrdU⁺ TUNEL⁺ cells could be found in any of the analyzed conditions.

E, F Dot plots depicting the proportion of the neuroblasts (BrdU⁺ DCX⁺) from label-retaining cells in the RMS (E) and in the OB (F) 7 days after aCSF or AntimiR204 injection.

Data information: Abbreviations: ChP, choroid plexus; CTR, control; SEZ, subependymal zone; LV, lateral ventricle; NB, neuroblast; RMS, rostral migratory stream; OB, olfactory bulb. All images are full Z-projections of confocal Z-stack, except of C' and D'' representing orthogonal projection in the single plane. Data are shown as mean ± SEM; each single dot represents independent biological replicate; significance was tested using Kruskal–Wallis ANOVA; ****P* value < 0.001, *****P* value < 0.0001. Scale bars (C, D) 100 μm, (C', D') 50 μm, (C'', D'') 10 μm.



C, D Histogram showing GO terms biological processes (C) and cellular compartments (D) enriched (fold enrichment > 2, *P*-values are indicated on the bars) in the set of genes up-regulated after miR-204 inhibition as shown in (A).

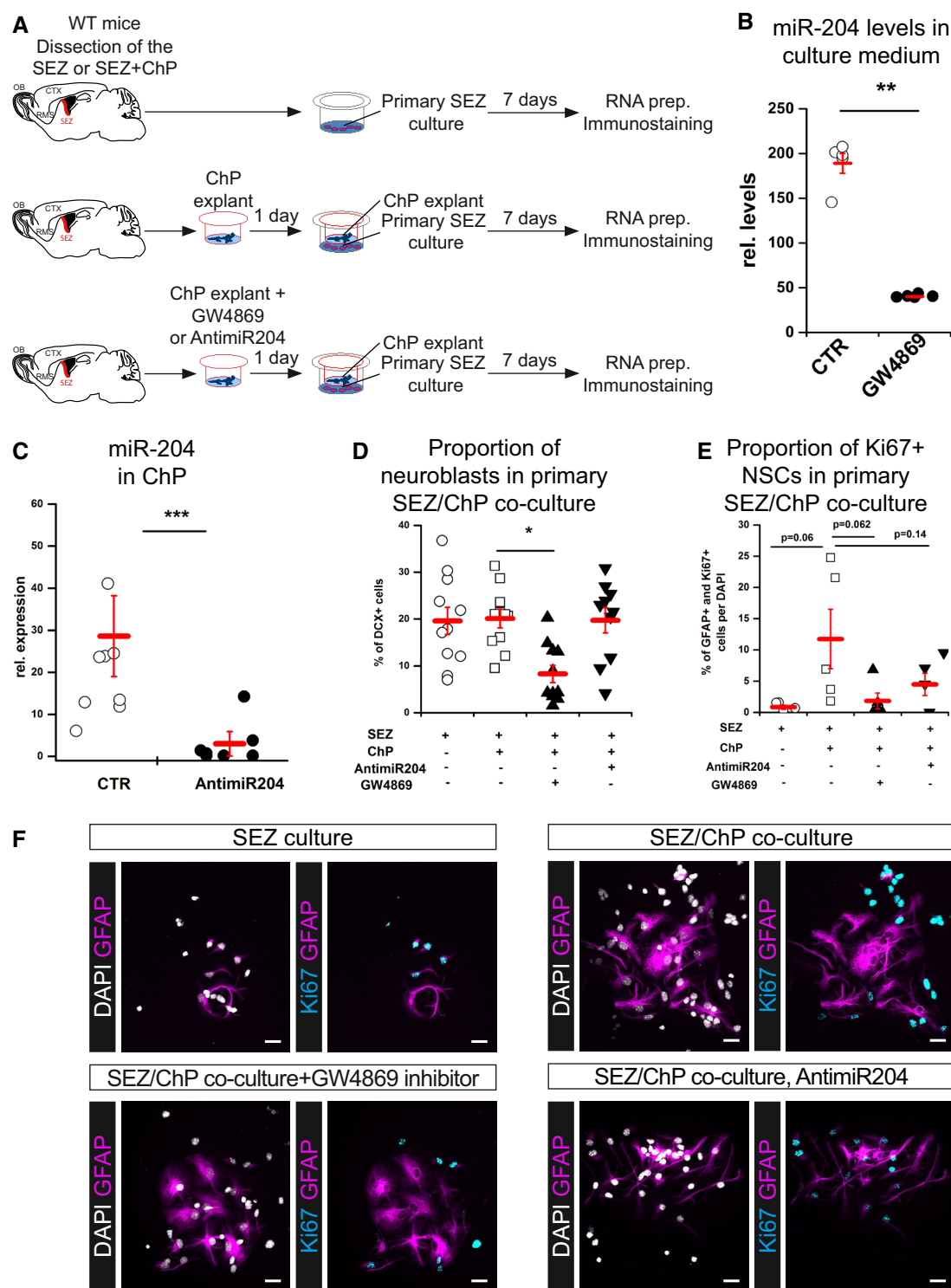


Figure EV4.

Figure EV4. ChP-specific inhibition of miR-204 or exosomal inhibition in the ChP reduces miR-204 levels in the culture medium.

- A Schematic representation of the experimental setup for primary SEZ/ChP co-culture.
- B Dot plot representing RT-qPCR analysis of the miR-204 levels in the medium after 24-h incubation of ChP with GW4869 inhibitor or DMSO control.
- C Dot plot showing miR-204 levels in the ChP used for the co-culture 1 day after incubation with or without AntimiR204.
- D Dot plot showing the proportion of DCX-positive cells out of DAPI⁺ cells in primary SEZ/ChP co-culture without treatment or treated with GW4869 inhibitor or AntimiR204.
- E Dot plot depicting the proportion of proliferative (Ki67⁺) neural stem cells out of DAPI⁺ cells in primary SEZ/ChP co-culture without treatment or treated with GW4869 inhibitor or AntimiR204.
- F Micrographs showing Ki67⁺ GFAP⁺ cells within SEZ cultures after co-culturing with control-treated, GW4869 inhibitor-treated, or AntimiR204-treated ChP 7 days after plating.

Data information: Abbreviations: WT, wild type; SEZ, subependymal zone; ChP, choroid plexus; CTX, cortex; RMS, rostral migratory stream; OB, olfactory bulb; CTR, control; NSC, neural stem cell; AntimiR, antagomir. Data are shown as mean \pm SEM; each symbol represents independent biological replicate; significance was tested by non-parametric Kruskal–Wallis ANOVA; **P* value < 0.05, ***P* value < 0.01, ****P* value < 0.001. Scale bars (F) 20 μ m.

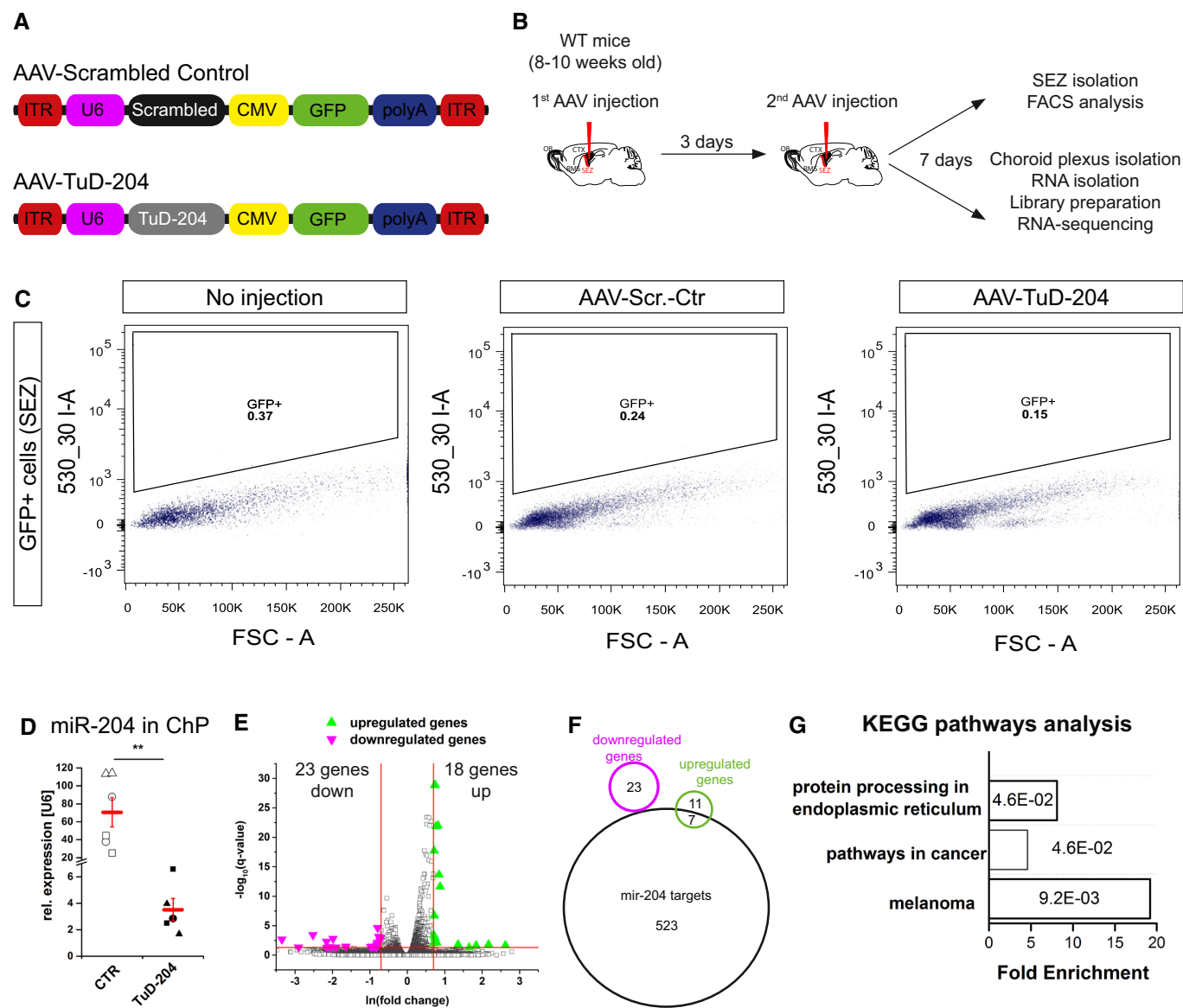


Figure EV5. The miR-204 loss-of-function effect on the ChP transcriptome.

- A Scheme showing the constructs for AAVs serotype 5 encoding for either a scrambled control or miR-204 specific tough decoys (TuD-204).
- B Schematic representation of the experimental setup to address the specificity of the AAVs serotype 5 and the effect of loss of miR-204 function in the ChP.
- C FACS analysis of the cells isolated from the SEZ 7 dpi after injection of AAV5 encoding for scrambled control and miR-204-specific TuD. Note that almost no GFP⁺ cells could be detected.
- D Dot plot depicting the levels of miR-204 in ChP 7 days after second ventricular injection of AAV5 encoding for miR-204-specific TuD compared to scrambled control. Same symbols represent ChP isolated from left and right hemisphere.
- E Volcano plot illustrating regulated transcripts after TuD-204 injection in the ChP (fold change > 2 and q-value < 0.05 are depicted with red line).
- F Venn diagram depicting overlap of regulated genes shown in (E) and predicted miR-204 targets.
- G Histogram showing KEGG pathways enrichment (fold enrichment > 2, P-values are indicated on the bars) of the regulated genes shown in (F).

Data information: Abbreviations: WT, wild type; CTX, cortex; RMS, rostral migratory stream; OB, olfactory bulb; SEZ, subependymal zone; FSC, forward scatter; Ctr, control; ChP, choroid plexus. Data are shown as mean \pm SEM; each symbol represents independent biological replicate; significance was tested by non-parametric Kruskal–Wallis ANOVA; **P value < 0.01.