INVENTORY OF SUPPLEMENTAL MATERIAL

Hierarchical reactivation of transcription during mitosis-to-G1 transition by Brn2 and Ascl1 in neural stem cells

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Supplemental data

This section is comprised of 12 Supplemental Figures and 11 Supplemental Tables. They are included in the document entitled Supplemental Material. Their content is described below, in order of citation in the manuscript.

Figure S1 is related to Figure 1, and describes Brn2 and Ascl1 expression in NS cells by immuno-staining.

Figure S2 is related to Figure 1, and describes cross-reactivity of the commercial Brn2 antibody used in this study, with Brn1.

Figure S3 is related to Figure 1, and describes the characterization of the Ascl1 NLS, using live-cell imaging analysis.

Figure S4 is related to Figure 1, and demonstrates the functionality of Ascl1-eGFP in transcriptional and differentiation assays.

Figure S5 is related to Figure 4, and provides raw data for calculating MCE values displayed on Figure 4, together with live-cell imaging analysis of additional POU3F TFs.

Figure S6 is related to Figure 5, and describes a second biological replicate of Brn2 ChIP-seq experiment comparing interphase and mitotic samples.

Figure S7 is related to Figure 5, and describes phosphorylation of Brn1/2 during mitosis, and FRAP data for phospho-mimetic Brn2 mutant (S362D).

Figure S8 is related to Figure 6, and provides live-cell imaging analysis of Ascl1 derivatives fused to NLSs.

Figure S9 is related to Figure 7, and provides examples of early reactivation of Nestin during mitotic exit in Ascl1 positive, and Ascl1 negative, NS cells.

Figure S10 is related to Figure 7, and contains smRNA-FISH data for Nestin gene in NS cells treated with transcriptional inhibitor triptolide.

Figure S11 is related to Figure 7, and contains smRNA-FISH data for Nestin and Dll1 gene in NS cells, using probes with alternative fluorophores.

Figure S12 is related to Figure 7, and contains smRNA-FISH data for Fabp7 in NS cells.

Supplemental Experimental Procedures

This section contains:

-Detailed information on immunolabeling on embryo sections and NS cells

-Detailed information on transcriptional and differentiation assays.

-Detailed information on Fluorescence recovery after photobleaching (FRAP).

-Detailed description on the generation of DN-Brn2 expressing NS cells.

Supplemental Tables

Table S1, describing PCR primers using in subcloning protocols when generating various expression vectors used in this study.

Table S2, describing primers used in site-directed mutagenesis when generating various expression vectors used in this study.

Table S3, describing sequences of oligonucleotides used in NLS constructs.

Table S4, describing primers used in Gibson assembly for generation of vectors used in CRISPR/Cas9 genome editing.

Table S5, describing sequences of oligonucleotides used in generation of tetracysteine-tag constructs.

Table S6, describing sequences of Dll1 exon smRNA-FISH probes.

Table S7, describing sequences of Dll1 intron smRNA-FISH probes.

Table S8, describing sequences of Nestin exon smRNA-FISH probes.

Table S9, describing sequences of Nestin intron smRNA-FISH probes.

Table S10, describing sequences of Fabp7 exon smRNA-FISH probes.

Table S11, describing sequences of Fabp7 intron smRNA-FISH probes.