Molecular Mechanisms Underlying Ascl1-Mediated Astrocyte-to-Neuron Conversion

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SUMMARY

Direct neuronal reprogramming potentially provides valuable sources for cell-based therapies. Proneural gene Ascl1 converts astrocytes into induced neuronal (IN) cells efficiently both in vitro and in vivo. However, the underlying mechanisms are largely unknown. By combining RNA sequencing and chromatin immunoprecipitation followed by high-throughput sequencing, we found that the expression of 1,501 genes was markedly changed during the early stages of Ascl1-induced astrocyte-to-neuron conversion and that the regulatory regions of 107 differentially expressed genes were directly bound by ASCL1. Among Ascl1’s direct targets, Klf10 regulates the neurogenesis of IN cells at the early stage, Myt1 and Myt1l are critical for the electrophysiological maturation of IN cells, and Neurod4 and Chd7 are required for the efficient conversion of astrocytes into neurons. Together, this study provides more insights into understanding the molecular mechanisms underlying Ascl1-mediated astrocyte-to-neuron conversion and will be of value for the application of direct neuronal reprogramming.

INTRODUCTION

The central nervous system of mammals has a limited capacity to regenerate when neurons are injured or lost in traumatic or neurodegenerative diseases. Together with stem cell-derived neuronal products, direct neuronal reprogramming offers promising alternatives to achieve neuronal repair (Colasante et al., 2019; Masserodetti et al., 2016; Tsunemoto et al., 2015; Xu et al., 2015). Mounting evidence shows that non-neuronal cells such as fibroblasts and astrocytes can be directly converted into neurons and a number of neuronal subtypes (Addis et al., 2011; Berninger et al., 2007; Caiazzo et al., 2011; Colasante et al., 2015; Guo et al., 2014; Heinrich et al., 2010; Heins et al., 2002; Kim et al., 2011; Li et al., 2019; Liu et al., 2013, 2015; Niu et al., 2013; Pflügerer et al., 2011; Son et al., 2011; Torper et al., 2013; Vadodaria et al., 2016; Vierbuchen et al., 2010; Xu et al., 2016). However, the molecular mechanisms underlying direct neuronal reprogramming remain poorly understood.

The past several years have seen much progress in our understanding of how fibroblasts are converted into neurons. It has been reported a hierarchical mechanism operates in the direct reprogramming of fibroblasts into neurons mediated by the transcription factors (TFs) Ascl1, Bm2, and Myt1l. Ascl1 acts as an “on-target” pioneer factor by occupying most cognate genomic sites in both the opened and closed chromatin in fibroblasts (Wapinski et al., 2013). Further study showed that Ascl1 opens closed chromatin at its target sites within 12 h, and induces rapid chromatin remodeling and nucleosome phasing that precedes neuronal maturation in direct reprogramming of fibroblasts to neurons (Wapinski et al., 2017). By performing single-cell RNA sequencing (RNA-seq), Treutlein et al. (2016) revealed that the direct reprogramming of fibroblasts to neurons contains two stages: the initiation stage when Ascl1 induces neuronal and myocyte fates and the maturation stage when Bm2 and Myt1l promote reprogrammed fibroblasts to permanently acquire neuronal identity. Mall et al. (2017) further showed that the TF Myt1l...
represses multiple somatic cell lineage programs to establish and maintain neuronal identity. Besides TFs, polypyrimidine tract-binding proteins, microRNAs, and epigenetic regulators also actively participate in the conversion of fibroblasts into neurons (He et al., 2018; Hu et al., 2018; Lee et al., 2018; Lu and Yoo, 2018; Luo et al., 2019; Zhang et al., 2016).

During direct reprogramming of astrocytes into neurons, it has been reported that Neurog2 and Ascl1 rapidly induced distinct transcriptional programs with only a small subset of target genes in common at 24 h after induction, including Insm1, NeuroD4, Prox1, and Sox11. Among these downstream TFs, only NeuroD4 is sufficient to induce a small fraction of neuronal cells (1%–3%) from cerebral cortex astrocytes at postnatal day 6–7 (P6–7), and together with Insm1 they induced a glutamatergic neuronal phenotype (Masserdotti et al., 2015). However, how the downstream factors of proneural genes contribute to the astrocyte-to-neuron conversion remains largely unknown. Here, we combined RNA-seq, chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq), and analysis of the TF regulation network to dissect the mechanisms underlying Ascl1-induced astrocyte-to-neuron conversion. We found that Ascl1 induced rapid and global transcriptional changes by directly binding to its target genes. Among these direct target genes, the TFs Klf10, Myt1, and Neurod4, and chromatin remodeling factor Chd7 played important roles in the direct reprogramming of astrocytes into neurons.

RESULTS

Ascl1 Induces Rapid and Global Transcriptional Changes

To investigate the molecular mechanisms underlying Ascl1-induced astrocyte-to-neuron conversion, we infected the cultured dorsal midbrain astrocytes with FUGW-Ascl1 or control viruses and performed the assays within 10 days post infection (DPI) (Figure 1A). We assessed the purity of the starter astrocytes by performing immunostaining. The results showed that the majority of the cells were stained positive for GFAP (83.3% ± 1.6%, n = 3 independent experiments) while few cells were stained positive for TUJ1 (0.8% ± 0.2%, n = 3 independent experiments) and NG2 (0.5% ± 0.2%, n = 3 independent experiments). The TUJ1+ cells did not exhibit neuronal morphology. Notably, in Marius Wernig’s work on direct conversion of fibroblasts to functional neurons by defined factors (Vierbuchen et al., 2010), it has been reported that rare TUJ1-positive cells with fibroblast-like morphology exist in starting mouse embryonic fibroblasts (MEFs), indicating weak expression of TUJ1 in non-neuronal cells. Conversely,
SOX2 (a neural stem cell marker)-positive cells could hardly be detected in the astrocyte cultures (data not shown). At 10 DPI when infected with the control lentivirus FUGW expressing GFP only, the astrocytes maintained the glial morphology and did not express the neuronal marker TUJ1. In contrast, most of the astrocytes infected with FUGW-Ascl1 adopted a neuronal fate and expressed TUJ1 (67.2% ± 5.1%, n = 3 independent experiments, 5701 GFP+ cells counted) and exhibited characteristic neuronal morphology (Figures 1B and 1C). We then examined the expression of the neuronal markers TuJ1, Map2, and NeuN by performing quantitative RT-PCR (qRT-PCR) at various time points during the direct reprogramming. We found that the expression of neuronal markers was markedly increased at 2 DPI and reached peak levels at 5 DPI (Figure 1D).

To reveal the genome-wide transcriptional changes during astrocyte-to-neuron conversion, we performed RNA-seq assays at 2 DPI and 5 DPI with five groups of samples including day0-A (astrocytes control without virus infection at day 0), day2-Ctrl, and day5-Ctrl (astrocytes infected with FUGW at day 2 and day 5), day2-Ascl1 and day5-Ascl1 (astrocytes infected with FUGW-Ascl1 at day 2 and day 5). The results showed that the two biological replicates in each group correlated very well (Figure 2A).

Correlation analysis confirmed that the normalized RNA-seq tag counts of these genes were consistent with their expression levels that were detected by qRT-PCR (Figure 2B). Principal component analysis (PCA) separated the Ascl1-infected samples from controls in the principal component 1 (PC1) dimension and revealed that day2-Ascl1 was an intermediate state across the direct reprogramming (Figures 2C, S1A, and S1B). We found that the expression of 1,501 genes was markedly changed (fold change >1.5, p < 0.05) in FUGW-Ascl1-infected astrocytes compared with that in control astrocytes during direct reprogramming, and these differentially expressed genes (DEGs) could be clustered into five groups by their expression levels at different time points. The expression of genes in group 1 was upregulated only at day 2 (G1), the genes in group 2 were upregulated at day 2 and their expression was maintained at day 5 (G2), group 3 was composed of genes whose expression was sequentially upregulated from day 2 to day 5 (G3), and group 4 consisted of genes upregulated only at day 5 (G4), whereas the genes in group 5 were downregulated at both day 2 and day 5 (G5) (Figures 2D and 2E). Furthermore, functional enrichment analysis revealed that the genes in each group were associated with distinct biological processes (Figure 2D). The list of 1,501 DEGs can be found in Table S1. We have compared the results with previous works (Masserdotti et al., 2015; Wapinski et al., 2013), and the comparisons can be found in Tables S2-S4 and Figure S2.

Genome-wide Binding Sites of ASCL1 in Astrocytes
To search for the genome-wide binding sites of ASCL1 in astrocytes, we performed ChIP-seq for ASCL1 in astrocytes at day 2 after virus infection. The results showed that 955 ASCL1-binding peaks were identified with at least 3-fold enrichment (Figure 3A), and the ASCL1 bound sites were mainly located in the distal regions of target genes (Figure 3B and Table S5). The genes with enriched binding peaks within 50 kb upstream or downstream of transcriptional start sites (TSSs) were defined as ASCL1 direct binding targets. We found that 696 genes were directly bound by ASCL1 (Table S6). De novo motif discovery analysis was performed to identify ASCL1 binding motifs within its binding regions. Among the top-ranking motifs, the canonical E-box motif CANNTG, which is associated with ASCL1 binding in fibroblasts (Wapinski et al., 2013) and neural stem cells (Raposo et al., 2015), was highly enriched across the binding sites (Figure 3C). Combining the results of ChIP-seq with RNA-seq, we found that 107 of 1,501 DEGs were directly bound by ASCL1 during direct reprogramming (Figure 3D and Table S7).

We applied the same criteria to analyze the ChIP-seq results, which were collected in fibroblasts reprogrammed for 2 days (Wapinski et al., 2013), and identified 12,321 ASCL1-binding peaks and 6,115 possible binding targets. Among these possible binding targets, 2,644 genes were distributed on either PC1 or PC2 dimension, which classified the direct neuronal reprogramming process to distinct stages. We defined the 2,644 genes as ASCL1 direct downstream targets in this system. Notably, 92 genes out of the 107 ASCL1 direct downstream targets in astrocytes induced for 2 days (this study) were also identified as ASCL1 downstream targets in fibroblasts reprogrammed for 2 days (Wapinski et al., 2013) (Table S7).

To unravel the connection of ASCL1 and the downstream targets, we analyzed the Connection Specificity Index (CSI) of TFs in five DEG groups and generated a TF co-expression network (Ascl1 negatively correlated TF group was removed in Figure S1C). We connected Ascl1 and its direct downstream targets through deep yellow arrows. Being closer to ASCL1, the target gene displayed more significant differential expression, and we made the arrow bold. We then reconnected and reclustered the TFs based on the detailed CSI coefficient, and the correlation between every two TFs was shown (Figure S1C). TFs Klf10, Myt1, and Neurod4 were chosen as representative DEGs of day2 upregulated only (G1), day2-day5 upregulated sequentially (G3), and day5 upregulated only (G4) to investigate what roles they might play during the Ascl1-induced astrocyte-to-neuron conversion (Figures 3E-3G and S1C). Furthermore, among the 1,501 DEGs identified during Ascl1-induced astrocyte-to-neuron conversion, 61 epigenetic factors were differentially expressed (fold change >1.5, p < 0.05),...
and an ATP-dependent chromatin remodeler chromo-helicase-DNA-binding protein 7 (Chd7) was the most significant DEG. Meanwhile, the expression profile of Chd7 highly correlated with that of Ascl1 (Figure S3). Thus, we also studied the function of the epigenetic factor Chd7 (Figure 3H). We have confirmed the binding of ASCL1 on the regulatory regions of Klf10, Myt1, Neurod4, and Chd7 by ChIP-PCR at 2 DPI and the expression of these genes at 2 DPI and 5 DPI by qRT-PCR (Figures 3I and 3J).

**Klf10 Regulates Neuritogenesis and the Electrophysiological Properties of Induced Neuronal Cells**

The expression of TF Klf10 was upregulated only at day 2 during direct reprogramming (Figure 3J). To investigate whether Klf10 plays critical roles in initiating the astrocyte-to-neuron conversion, we designed specific short hairpin RNAs (shRNAs) against Klf10 (Figure S4A) to reduce its expression. A previous work reported that phosphomutant ASCL1 can...
enhance neuronal induction activity in *Xenopus* embryos and improve neuronal transdifferentiation efficiency (Ali et al., 2014). Therefore, we mutated all six serine-proline sites (SP) to alanine-proline (SA) in ASCL1 to generate S-A ASCL1 (SAA) and used SAA as a substitute for ASCL1 to induce astrocyte-to-neuron conversion. Astrocytes were co-infected with FUW-SAA-IRES-tdTomato and pLKD-shKlf10-GFP or pLKD-shCtrl-GFP lentiviruses. We found that the morphology of SAA virus-infected astrocytes was transformed quicker upon Klf10 knockdown compared with that of control (data not shown). The cellular phenotypes of SAA and shRNA virus co-infected cells (tdTomato+GFP+) were analyzed at 10 DPI (Figures 4A and 4B). Notably, more TUJ1-positive neuronal cells were generated (86.6% ± 4.4% versus 66.5 ± 4.2, n = 4, 1,207–1,370 tdTomato+GFP+ cells counted), and the total neurite length (TUJ1+) was increased (391.3 ± 23.08 μm versus 215.1 ± 23.53 μm, n = 3, 61–70 tdTomato+GFP+ cells measured) upon Klf10 knockdown (Figures 4C–4E). Moreover, the dendrite complexity was also enhanced upon Klf10 knockdown, as measured by increased total dendritic branch length (MAP2+) (1.54 ± 0.07 versus 1.00 ± 0.07, n = 3, 60 tdTomato+GFP+ cells measured) and total dendritic branch tip number (MAP2+) (1.36 ± 0.08 versus 1.00 ± 0.07, n = 3, 60 tdTomato+GFP+ cells measured) (Figures 4C, 4F, and 4G).

To evaluate whether early Klf10 expression controls other aspects of neuronal differentiation, we performed electrophysiological recording and qRT-PCR. The recording results showed that upon Klf10 knockdown, the membrane properties of induced neuronal (iN) cells, such as...
membrane capacitance (Cm), input resistance (Rin), and resting membrane potential (RMP), remained largely unchanged (data not shown). Conversely, the height of action potential (AP) was markedly reduced (Figure 4H). As we reported previously, we categorized IN cells into four groups based on their current and voltage response patterns: non-active cells (“non-active”), cells exhibiting inward current without AP (“inward”), single AP (“sAP”), and multiple APs (“mAPs”) (Liu et al., 2015). When Klf10 was knocked down, the percentage of IN cells firing mAPs was markedly reduced whereas the percentage of non-active cells and IN cells firing sAP increased (Figure 4I). Meanwhile, the qRT-PCR results showed that the mRNA expression of synaptic proteins such as Synapsin I and Homer1 was not markedly changed when Klf10 was knocked down. Interestingly, the mRNA expression of sodium channel gene Scn1a but not sodium channel genes Scn2b and Scn8a increased when Klf10 was knocked down (data not shown). These results indicate that early Klf10 expression controls the neuritogenesis and the electrophysiological properties of IN cells.

Myt1 and Myt1l Are Critical for the Electrophysiological Maturation of IN Cells

A recent study reported that pan-neuron-specific TF Myt1-like (Myt1l) exerts a pro-neuronal function by direct repression of many different somatic lineage programs except the neuronal program during direct reprogramming of...
fibroblasts to neurons (Mall et al., 2017). Besides Myt1, there are two other Myt family members, Myt1 and Myt3 (also known as St18) (Yee and Yu, 1998). Interestingly, during astrocyte-to-neuron conversion, the expression of Myt1 was increased enormously both at day 2 and day 5, whereas the expression of Myt1 and Myt3 was upregulated only at day 5 by 116-fold and 26-fold, respectively (Figure S4B). We investigated their functions by knocking down Myt1, Myt1l, or Myt3 individually or Myt1 and Myt1l simultaneously (Figure S4C). The results showed that individual knockdown of Myt1, Myt1l, or Myt3 did not affect the morphology and re-programming efficiency of iN cells (data not shown). Interestingly, double knockdown of Myt1 and Myt1l resulted in iN cells with thicker neurites and stretched-out morphology (Figures 5A and 5B), although the number of induced TUJ1-positive cells remained largely unchanged compared with that of the control (70.1% ± 2.9% versus 56.4% ± 5.85%, n = 3, 1,020–1,036 tdTomato+ GFP+ cells counted). We also performed immunostaining with antibodies of more mature markers such as MAP2 and SYNAPSIN I. The results showed that compared with the scramble control, iN cells induced by FUW-SAA-IRES-tdTomato together with pLKD-shMyt1/1L-

Figure 5. Knockdown of Myt1 and Myt1l Inhibits the Electrophysiological Maturation of iN Cells
(A and B) TUJ1 immunostaining of cells co-infected with FUW-SAA-IRES-tdTomato and shMyt1/1L (B) or shCtrl (A) at 10 DPI.
(C and D) MAP2 immunostaining of cells co-infected with FUW-SAA-IRES-tdTomato and shMyt1/1L (D) or shCtrl (C) at 14 DPI.
(E) Differential interference contrast image of whole-cell recording from an iN cell (green and red fluorescence) at 22 DPI.
(F) Representations of single action potentials (sAP) or multiple action potentials (mAPs) generated by iN cells from control group when recorded in current-clamp mode.
(G) Percentages of iN cells with four different degrees of membrane excitability (non-active, inward, sAP, or mAPs) when co-infected with FUW-SAA-IRES-tdTomato and shRNA against Myt1 and Myt1l or control shRNA.
(H–J) Cm (H), Rin (I), and RMP (J) of iN cells when both Myt1 and Myt1l were knocked down.
Data are presented as mean ± SEM, n = 3 independent experiments. *p < 0.05, **p < 0.01. Scale bars, 100 μm. See also Figure S4 and Table S9.
GFP expressed MAP2 (Figures 5C and 5D) and SYNAPSIN 1 (data not shown) as well.

We further examined the electrophysiological properties of iN cells by performing whole-cell recording upon Myt1 and/or Myt1l knockdown. FUW-SAA-IRES-tdTomato and pLKD-shMyt1/shMyt1l-GFP lentivirus co-infected cells (tdTomato+ GFP+) were recorded at 22 DPI (Figure 5E). Knockdown of Myt1 or Myt1l individually resulted in a decreased percentage of iN cells firing mAPs, increased percentage of iN cells firing sAP, and generation of non-active iN cells compared with that in cells infected with only FUW-SAA-IRES-tdTomato (Figure 5D). More notably, upon knockdown of both Myt1 and Myt1l, the majority of iN cells were non-active (Figures 5F and 5G). Moreover, upon Myt1 and Myt1l double knockdown, the iN cells had lower Cm (shMyt1/1L 10.6 ± 0.9 pF, shCtrl 14.6 ± 1.0 pF, n = 45), tended to have higher Rin (shMyt1/1L 3575 ± 458.4 MΩ, shCtrl 2278 ± 526.8 MΩ, n = 45) and had more negative RMP (shMyt1/1L -86.9 ± 4.1 mV, shCtrl -69.4 ± 2.4 mV, n = 45) compared with that of control (Figures S5H–S5J). Taking these results together, Myt1 and Myt1l were critical for the electrophysiological maturation of iN cells.

Neurod4 Can Partially Substitute Ascl1 to Induce iN Cells

Neurod4 is a TF that contributes to the neuronal differentiation program (Guillemtot, 2007). During astrocyte-to-neuron conversion, the expression of Neurod4 was markedly increased (Figure 3J). Consistent with a previous report (Masserdotti et al., 2015), the conversion efficiency of astrocytes into neurons markedly decreased upon Neurod4 knockdown (33.4% ± 4.6% versus 66.9% ± 5.9%, n = 3, 876–1,062 tdTomato+ GFP+ cells counted) (Figures S5A and 6A–6C). To explore whether the downstream TFs of Neurod4, Ascl1, and Neurod4 could induce a genuine astrocyte-to-neuron switch and are in line with the observation that Neurod4 is sufficient to induce a genuine astrocyte-to-neuron switch and are in line with the observation that Neuroid is a chromatin remodeling factor, to be markedly increased during the reprogramming process of astrocytes into neurons (Figure 3J). Therefore, we characterized the function of Chd7 in Asc1l-induced astrocyte-to-neuron conversion by shRNA-mediated knockdown (Figure S5B). The results showed that the conversion efficiency markedly decreased upon Chd7 knockdown (24.7% ± 2.3%, n = 3, 786 tdTomato+GFP+ cells counted) compared with that of the control (59.0% ± 3.7%, n = 3, 2,516 tdTomato+GFP+ cells counted) (Figures 7A–7C, 5S, and 5D). To investigate how Chd7 affects the reprogramming, we constructed a network of Chd7 correlated TFs based on their expression correlation CSI (CSI > 0.8) (Figure 7D). The TFs included those that were upregulated at day 2 and day 5 (in yellow), upregulated continuously at day 2 and day 5 (in blue), upregulated only at day 5 (in brown), and downregulated at both day 2 and day 5 (in green) (Figure 7D). In line with the CSI analysis, qRT-PCR results showed that the expression of Chd7 positively correlated genes was markedly reduced upon Chd7 knockdown (Figure 7E). Interestingly, these decreased genes, such as Sox11, Myt1, Ebf3, Neurod4, and Zfp821, are related to neuronal development and differentiation, which may explain why knockdown of Chd7 reduced the efficiency of direct reprogramming. These results indicate that besides neurogenic TFs, epigenetic regulator Chd7 is also indispensable for the astrocyte-to-neuron conversion. Combining the results of Klf10, Myt1, Myt1l, Neurod4, and Chd7 during Asc1l-mediated astrocyte-to-neuron conversion, we found that different downstream targets of Asc1l may play distinct roles: Klf10 was transiently expressed at 2 DPI, and its early expression controlled the neuritogenesis and the electrophysiological properties of iN cells; the expression of Myt1 and Chd7 was continually increased at 2 DPI and 5 DPI, and they were positive driving forces for iN cells to proceed with the reprogramming...
process and acquire mature neuronal properties; the expression of *Neurod4* was upregulated largely at 5 DPI and promoted the maturation of iN cells (Figure 7F).

**DISCUSSION**

In this study we found that *Ascl1* induced rapid and global transcriptional changes at 2 DPI and 5 DPI by performing RNA-seq during astrocyte-to-neuron conversion. Meanwhile, ChIP-seq performed at 2 DPI showed that the regulatory regions of 696 genes were directly bound by ASCL1. Combining the results of RNA-seq and ChIP-seq, we found that ASCL1 directly bound to the regulatory regions of 107 DEGs. Among these downstream genes of ASCL1, we found that the early expression of *Klf10* controlled the neuritogenesis and the electrophysiological properties of iN cells, *Myt1* and Myt family member *Myt1l*.
were critical for the electrophysiological maturation of iN cells, and *Neurod4* and *Chd7* were required for the efficient conversion of astrocytes to neurons.

The similarity between this study and that of Wapinski et al. (2013) is that 2 days after *Ascl1* induction, both systems entered intermediate stages (Figures S2A and S2B) and showed similar transcriptome identities (Figure S2C). Genes upregulated on day 2, such as TFs *Sox11*, *Hes5*, and *Hes6*, were mostly related to neuronal differentiation and relevant biological processes. Notably, *Klf10* was upregulated in both systems as well (Figure S2C). PC1 dimension reflected the neuronal maturation procedure (Figure S2A). Meanwhile, hierarchical clustering analysis revealed that Day5_Ascl1_1/2 clustered closer to BAM_22d of Wapinski et al. (2013) (Figure S2B), indicating a faster reprogramming process of our system. However, in PC2, our samples (Day5_Ascl1_1/2) showed differences compared with BAM_13d and BAM_22d (Figure S2D), indicating that induction time plays important roles in direct reprogramming.

Comparative analysis showed the correlation between our system and the reprogramming method applied in Masserdotti et al., 2015 (Figure S2E) as well. Pearson correlation coefficient assays with RNA-seq and microarray data showed that, at 4 h after ASCL1ERT2 activation, there was no significant change in transcriptome, corroborating with the fact that there were only a few DEGs at 4 h (data not shown). At 24 h after ASCL1ERT2 activation, astrocytes...
were transformed into another stage, similar to our intermediate state (day2-Ascl1). At 48 h after ASCL1ERT2 activation, the astrocytes had a tendency toward later reprogramming stages (day2-Ascl1 to day5-Ascl1) (Figure S2E). Notably, poor reprogramming was observed in the sample 2 Ascl1-48h-2 (Figure S2E).

Moreover, we found the expression of 630 overlapping genes was markedly changed during Ascl1-mediated conversion of astrocytes and MEFs into neurons, and the regulatory regions of 629 genes were bound by ASCL1 in both astrocytes and MEFs. For example, the expression of Klf10 and Sox11 were augmented during Ascl1-mediated conversion and their regulatory regions were bound by ASCL1 in both astrocytes and MEFs (data not shown). Wapinski et al. (2013) identified Zfp238 as a key downstream target of Ascl1 that can partially substitute for Ascl1 during direct reprogramming of fibroblasts into neurons. However, in this study we found that the regulatory regions of Zfp238 were not bound by ASCL1 and that Ascl1 largely could not induce the expression of Zfp238 in astrocytes (data not shown). Instead, we found that another ASCL1 downstream target Neurod4 could convert astrocytes into TUJ1-positive neuronal cells, which is consistent with a previous study (Masserdotti et al., 2015). In MEFs, Neurod4 alone was incapable of eliciting neuronal conversion, while together with Ins1 they generated iN cells at 14 DPI (Masserdotti et al., 2015). The direct binding of ASCL1 to large amounts of overlapping genes in astrocytes and fibroblasts reveals a highly similar occupancy of ASCL1 even in distantly related cell types. Conversely, a modest fraction of overlapping genes was differentially expressed during the conversion of astrocytes and fibroblasts to neurons, demonstrating that Ascl1 regulates the expression of downstream genes in cell context-dependent fashion. Furthermore, it has been reported that Ascl1 converts Müller glia cells from the retina and astrocytes from the neocortex and cerebellum into different neuronal subtypes (Chouchane et al., 2017; Guimaraes et al., 2018; Pollak et al., 2013). In Table S4, Pvalb was upregulated in iN cells generated from neocortex astrocytes but not in iN cells generated from dorsal midbrain astrocytes (this study), indicating different neuronal subtypes. This warrants further studies to reveal the identities of iN cells converted from astrocytes that are located in different regions.

It is unknown whether Klf10 plays physiological functions in the nervous system (Subramaniam et al., 2010). In this study, we found that the expression of Klf10 was upregulated at day 2 but downregulated at day 5 during direct reprogramming of astrocytes into neurons. Interestingly, upon Klf10 knockdown, more TUJ1-positive neuronal cells were generated with longer neurite length and more complex dendrites but with reduced electrophysiological activity. Moreover, the expression of Klf10 was also upregulated during direct reprogramming of fibroblasts to neurons at day 2 (Treutlein et al., 2016), and we found that the efficiency of fibroblast-to-neuron conversion was increased upon Klf10 knockdown as well (data not shown). These results show that Klf10 may play a similar role during the direct reprogramming from different donor cells at the early stages.

Although Ascl1 alone is sufficient to convert MEFs into iN cells in optimized culture conditions, endogenous Myt1l is induced during the conversion and exogenous Myt1l considerably increases the efficiency of conversion and the functional maturation of the iN cells (Chanda et al., 2014; Vierbuchen et al., 2010). We have previously shown that Ascl1 alone is sufficient to convert midbrain astrocytes into functional, synapse-forming neurons in vitro (Liu et al., 2015). In this study, we found that the expression of Myt1l was increased more remarkably compared with that of Myt1l and Myt3 during Ascl1-induced astrocyte-to-neuron conversion. Meanwhile, ASCL1 directly bound to the regulatory region of Myt1l. This is corroborated by the observation that Myt1l is a direct target of ASCL1 at the onset of neuronal differentiation (Vasconcelos et al., 2016). However, knockdown of Myt1l, Myt1l, or Myt3 individually did not affect the conversion efficiency of astrocytes into iN cells induced by Ascl1 (data not shown). Moreover, double knockdown of Myt1l and Myt1l largely did not affect the conversion efficiency of astrocytes into iN cells, although these cells had thicker neurites, stretched-out morphology, and inactive electrophysiological properties. This warrants investigating further whether the Myt1 family members play similar roles also for the iN cells converted from fibroblasts to obtain functional neuronal properties.

Wapinski et al. (2013) found that Ascl1 acts as a pioneer factor at neurogenic loci marked by a close chromatin state to direct conversion of fibroblasts into neurons, and this is in agreement with the observation that Ascl1 coordinately regulates gene expression and the chromatin landscape during neurogenesis (Raposo et al., 2015). Enhanced chromatin accessibility has also been observed in TF Neurog2, microRNAs, and CRISPR/cas9-mediated fibroblast-to-neuron conversion (Abernathy et al., 2017; Black et al., 2016; Smith et al., 2016). Moreover, epigenetic factor ten-eleven translocation 3 (Tet3), which regulates DNA demethylation, and a histone H3 lysine 4 methylase KMT2B contribute to the direct conversion of fibroblast into functional neurons (Barbagiavanni et al., 2018; Zhang et al., 2016). Conversely, incomplete MyoD-induced transdifferentiation of human fibroblasts is associated with chromatin remodeling deficiencies (Mandhar et al., 2017). It has been reported that the chromatin remodeling factor Chd7 regulates adult neurogenesis via activation of SoxC TFs and is...
indispensable for normal cerebellar development (Feng et al., 2013, 2017). Here, we found the efficiency of astrocyte-to-neuron conversion to be markedly decreased upon Chd7 knockdown. Moreover, CSI results showed that Chd7 is required for expression of the genes Sox11, Myt1, Ebf3, Neurod4, and Zfp821 that are related to neuronal development and differentiation. It will be of interest to investigate how Chd7 is recruited to specific targets and remodels the chromatin structure. Indeed one way to improve direct neuronal reprogramming is to understand the possible barriers in the context of a higher-order chromatin landscape (Gascon et al., 2017; Guo and Morris, 2017; Ninkovic and Gotz, 2018; Riemens et al., 2018).

**EXPERIMENTAL PROCEDURES**

**Astrocyte Culture**

Astrocytes were cultured as previously described with some modifications (McCarthy and de Vellis, 1980).

**Viral Production**

Lentiviruses were produced from HEK293FT cells that were transiently transfected with lentiviral, viral envelope-typing, and VSVG-pseudo-typing plasmids (Tiscornia et al., 2006).

**Immunostaining**

Immunostaining on cultured cells was performed essentially as previously described except that the primary antibodies were incubated for overnight (Vierbuchen et al., 2010).

**RNA Sequencing**

Total RNA was isolated with TRIzol reagent. Libraries were prepared, and sequencing was performed in 100-bp paired-end format on the Illumina HiSeq 2000 system.

**Chromatin Immunoprecipitation Followed by Sequencing**

ChIP-seq was carried out in astrocytes 2 days after FUGW-Ascl1 infection as described previously (Jin et al., 2009). Approximately 9–12 × 10^6 cells were used for each ChIP-seq experiment, and sequencing reads were generated on HiSeq 2000 Illumina platforms.

**Gene Knockdown**

To acutely knock down Klf10, Myt1, Neurod4, and Chd7, we cloned one or two shRNAs specifically against targeting sequence and one control scramble shRNA sequence into the lentiviral vector pLKD (OBiO Technology [Shanghai]). The shRNA sequences can be found in Table S9.

**Electrophysiological Recording**

Whole-cell voltage-clamp or current-clamp recording was performed as described previously (Lu et al., 2007).

**Data and Code Availability**

The accession number for the RNA-seq data is GEO: GSE132674. The accession number for the ChIP-seq data is GEO: GSE132671.

**SUPPLEMENTAL INFORMATION**

Supplemental Information can be found online at https://doi.org/10.1016/j.stemcr.2021.01.006.

**AUTHOR CONTRIBUTIONS**

L.C., N.J., and Z.R. designed the project. Z.R. and S.L. performed the experiments. R.W. and L.M. analyzed the RNA-seq and ChIP-seq. S.H. and Y.S. conducted electrophysiological experiments. J.Y. carried out the lentivirus production. Z.R. and L.C. wrote the manuscript.

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Supplemental Information

Molecular Mechanisms Underlying Ascl1-Mediated Astrocyte-to-Neuron Conversion

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Figure S1. Identification of transcriptional changes induced by Ascl1 during astrocyte-to-neuron conversion. Related to Figure 2 and Figure 3.

(A) Heatmaps of top 100 positive and negative genes with highest quantitative correlation in the first principal component (PC1).

(B) Heatmaps of top 100 positive and negative genes with highest quantitative correlation in the second principal component (PC2).

(C) Connection Specificity Index (CSI) network of Ascl1 downstream transcription factors. Gene expression correlation coefficient-derived CSIs are calculated based on the RNA-seq expression value.
Figure S2. Comparison of direct reprogramming of astrocytes and fibroblasts into neurons. Related to Figure 2.

(A) Principal component analysis of RNA samples from this study and Wapinski et al. (2013).
(B) Unsupervised hierarchical clustering analysis of RNA samples from this study and Wapinski et al. (2013).
(C-D) Heatmaps show the expression of differentially expressed genes during the direct reprogramming processes across different time points of this study and Wapinski et al. (2013). Top gene ontology (GO) terms and transcription factors (TFs) are shown on the right side.
(E) Comparative analysis of RNA-seq data from this study and microarray data from Masserdotti et al. (2015) during the direct reprogramming processes across different time points.
Figure S3. Expression of epigenetic factors during Ascl1-mediated direct reprogramming.

Related to Figure 2.

(A) Volcano Plot shows the 1,501 DEGs during direct reprogramming. Plotted genes have \( P < 0.05 \) and fold change of > 1.5. The four genes that we studied were labeled in red dots.

(B) Volcano Plot shows the 61 differentially expressed epigenetic factors during direct reprogramming. Plotted genes have \( P < 0.05 \) and fold change of > 1.5. The top 10 epigenetic factors with highest fold changes were labeled.

(C) Bar plot shows the fold changes of top 10 epigenetic factors. Chd7 was labeled in red.

(D) Bar plot shows the expression correlation coefficient between Ascl1 and top 10 epigenetic factors during direct reprogramming. Chd7 was labeled in red.
Figure S4. Validation of specific shRNAs. Related to Figure 4 and Figure 5.

(A) qRT-PCR results showing the knockdown efficiency of specific shRNAs against Klf10 in Neuro2a cell line. The effect of each shRNA against Klf10 was compared with that of shCtrl. shKlf10-2 was selected for further experiments.

(B) The expression of Myt family members during direct reprogramming of astrocytes into neurons at early stages 2 DPI and 5 DPI.

(C) qRT-PCR results showing the knockdown efficiency of specific shRNAs against Myt1 family members in Neuro2a cell line.

(D) Percentages of iN cells with four different degrees of membrane excitability (non-active, inward, sAP or mAPs) when co-infected with SAA and control shRNA or specific shRNAs against Myt1 or Myt1l. Data are presented as mean ± SEM, n = 3 independent experiments. *p < 0.05, **p < 0.01.
Figure S5. Validation of specific shRNAs. Related to Figure 6 and Figure 7.
(A-B) qRT-PCR results showing the knockdown efficiency of specific shRNAs against Neurod4 (Nd4) and Chd7 in Neuro2a cell line. The shRNAs marked in blue were selected for further experiments.
(C) The representative images of cells co-infected with SAA and shCtrl or shChd7 at 10 DPI.
(D) Efficiency of conversion of astrocytes into neurons upon Chd7 knockdown.
Data are presented as mean ± SEM, n = 3 independent experiments. *p < 0.05, **p < 0.01. Scale bars: 100 μm.
Table S2 Comparison of gene expression during direct reprogramming of fibroblasts (Cell 2013_48h) and astrocytes (this study) into neurons. Related to Figure 2.

To compare the RNA-seq results with previous works, we downloaded the RNA-seq data from a study published by Wapinski et al. (2013). Using the criteria applied in this study (fold change > 1.5, p < 0.05), we identified 1,049 genes up-regulated after Ascl1 induced reprogramming of fibroblasts for 2 days. It is noteworthy that quite a number of genes upregulated in reprogramming of fibroblast for 2 days were upregulated in astrocyte-to-neuron conversion for 2 days in this study. The top 50 genes are shown below.

| Gene      | Fold change | Gene      | Fold change | Gene      | Fold change |
|-----------|-------------|-----------|-------------|-----------|-------------|
| Tnnt2     | 97.09       | Miat      | 66.64       | Stmn4     | 27.40       |
| R3hdml    | 82.64       | Gal       | 56.56       | Chgb      | 22.14       |
| Gm9994    | 41.49       | Tubb3     | 20.79       | Hes5      | 21.43       |
| Gal3st2   | 38.31       | Dll3      | 17.33       | Snap25    | 18.76       |
| Tnnc1     | 38.17       | Htr3a     | 14.31       | Celf3     | 18.48       |
| Fabp7     | 37.31       | Crtac1    | 11.15       | Stmn3     | 17.02       |
| Pgam2     | 32.15       | Sox11     | 9.97        | Ina       | 15.38       |
| Bmp7      | 27.70       | Snca      | 9.24        | Myt1      | 13.60       |
| Parm1     | 25.71       | Mchr1     | 8.40        | Hrk       | 12.24       |
| Prph      | 25.58       | Homer2    | 8.34        | Igf2      | 12.21       |
| Septin 4  | 22.62       | Cdk5r1    | 8.21        | Scrt2     | 11.77       |
| Nkd1      | 20.75       | Tnr       | 7.31        | Gm12238   | 11.66       |
| Tnnt1     | 20.16       | Hes6      | 7.24        | Elavl3    | 11.52       |
| Tnnt3     | 19.65       | Fam57b    | 6.78        | Dcc       | 11.45       |
| Gm7325    | 19.57       | Ncald     | 6.34        | Celsr3    | 11.29       |
| Tnni1     | 18.98       | Ass1      | 6.03        | 150016L03Rik | 11.14 |
| Shisa2    | 16.47       | Scx       | 5.38        | Actl6b    | 10.70       |
| Dynl1l    | 16.00       | Slc29a4   | 5.36        | 5430421N21Rik | 9.43 |
| Atoh8     | 15.87       | Chrb1     | 5.22        | Atcay     | 8.85        |
| Des       | 15.75       | Sox8      | 5.18        | Septin3   | 8.64        |
| Pvalb     | 15.50       | Gna14     | 5.16        | Elavl4    | 8.52        |
| Gm6086    | 15.15       | Ube2q1l   | 4.75        | Reln      | 8.45        |
| Kcnm3     | 14.66       | 2610109H07Rik | 4.75 | Rbfox3    | 8.31        |
| Tspan18   | 14.20       | Gabbr2    | 4.72        | Nisr2     | 8.08        |
| Prune2    | 13.59       | Chfa2I3   | 4.60        | Brsk2Mir3104 | 8.06 |
| Dlx3      | 13.39       | Gng2      | 4.56        | Spock1    | 7.68        |
| Arsi      | 12.89       | Chst11    | 4.51        | Caen4     | 7.62        |
| Prex1     | 12.63       | Gnrb3     | 4.47        | Pak7      | 7.60        |
| Cox8b     | 12.52       | Enox1     | 4.38        | Chga      | 7.60        |
| Setc      | 12.42       | Cdon      | 4.31        | Gprin1    | 7.31        |
| Gene  | Log2FC | Gene  | Log2FC | Gene  | Log2FC |
|-------|--------|-------|--------|-------|--------|
| Kcne1l| 12.39  | D930015E06Rik | 4.17  | Cpxl1 | 7.11  |
| Cdkn1c| 12.30  | Tmem229b | 3.98  | Grik2 | 6.94  |
| Acta1 | 11.92  | Rarres1 | 3.97  | Sema5a | 6.86 |
| Rbm24 | 11.44  | Klf10  | 3.97  | 270009003Rik | 6.83 |
| Filip1 | 11.39 | Phf16 | 3.87  | Caen2 | 6.75  |
| Adamtsl2 | 11.33 | Zfp238 | 3.71  | Neurl1a | 6.60 |
| Plac1 | 11.04  | 2310040G24Rik | 3.66  | 6330407J23Rik | 6.48 |
| Heyl | 10.15  | Pkib | 3.62  | Celf4 | 6.46  |
| Rgs16 | 9.94   | Map3kl | 3.55  | Srrm4 | 6.44  |
| Pdlim3 | 9.91   | Cacna2d2 | 3.47  | Jgbp1 | 6.41  |
| Lzt51 | 9.85   | Smoc1  | 3.33  | Dlgap3 | 6.40 |
| Rasgef1b | 9.73   | Srpk3 | 3.32  | Dscam1 | 6.35 |
| Ankrd1 | 9.65   | Chn2   | 3.29  | Mgat5b | 6.34 |
| Gpr56 | 9.00   | Satb1  | 3.23  | 9330159F19Rik | 6.26 |
| Gdf10 | 8.98   | Opn3   | 3.18  | Atp2b2 | 6.21 |
| Ccdc88c | 8.84 | Nos1  | 3.16  | Ebf3 | 6.14 |
| Gl0s2 | 8.76   | Svil   | 3.15  | Gdap1 | 6.12 |
| Wnt4 | 8.63   | Rorc   | 3.13  | Sgc2  | 6.06 |
| Susd5 | 8.61   | Stxbp1 | 3.12  | 3380026J04Rik | 6.02 |
| 1810020D 17Rik | 8.59 | Col18a1 | 3.11  | Chrb2 | 5.79 |
Table S3 Comparison of gene expression during direct reprogramming of fibroblasts (Cell 2013_13d & 22d) and astrocytes (this study) into neurons. Related to Figure 2.

To compare the RNA-seq results with previous works, we downloaded the RNA-seq data from a study published by Wapinski et al. (2013). Using the criteria applied in this study (fold change > 1.5, p < 0.05), we identified 1,726 genes up-regulated after Ascl1 induced fibroblast-to-neuron conversion for 13 days and 22 days. It is noteworthy that many genes upregulated in reprogramming of fibroblast to neurons for 13 and 22 days were upregulated in astrocyte-to-neuron conversion for 5 days in this study. The top 50 genes are shown below.

| Gene     | Fold change | Gene     | Fold change | Gene     | Fold change |
|----------|-------------|----------|-------------|----------|-------------|
| Scg3     | 89.29       | Miat     | 66.64       | Gal      | 56.56       |
| Nts      | 72.99       | Stmn4    | 27.40       | Hex5     | 21.43       |
| Klk1     | 62.11       | Chgb     | 22.14       | Igf2     | 12.21       |
| Snbg11   | 34.72       | Tubb3    | 20.79       | Srt2     | 11.77       |
| Rtn1     | 31.15       | Snap25   | 18.76       | Gm12238  | 11.66       |
| Ppshr2   | 22.73       | Celf3    | 18.48       | 1500016L03Rik | 11.14   |
| Gng3     | 21.41       | Dll3     | 17.33       | Sox11    | 9.97        |
| Mapt     | 21.32       | Stmn3    | 17.02       | 5430421N21RikKrt81Krt83Krt85 | 9.43 |
| Clec3b   | 20.88       | Ina      | 15.38       | Mchr1    | 8.40        |
| Lyz2     | 20.20       | Htr3a    | 14.31       | Homer2   | 8.34        |
| Fstl5    | 18.45       | Myt1     | 13.60       | Rbfox3   | 8.31        |
| Rnf208   | 18.28       | Hrk      | 12.24       | Ntsr2    | 8.08        |
| Scg5     | 17.67       | Elav3    | 11.52       | Brsk2Mir3104 | 8.06   |
| Car3     | 17.51       | Dec      | 11.45       | Tnr      | 7.31        |
| Cadm3    | 16.98       | Celsr3   | 11.29       | Hex6     | 7.24        |
| Camk2b   | 16.98       | Crtac1   | 11.15       | Sema5a   | 6.86        |
| Vat1l    | 15.77       | Act6b    | 10.70       | 2700090003Rik | 6.83 |
| Gc       | 15.58       | Snca     | 9.24        | Igfbp1   | 6.41        |
| Disp2    | 15.43       | Atcay    | 8.85        | Dscam1l  | 6.35        |
| Crmp1    | 15.36       | Septin3  | 8.64        | Ebf3     | 6.14        |
| Vgf      | 14.75       | Elavl4   | 8.52        | Ass1     | 6.03        |
| Emd2     | 14.35       | Reln     | 8.45        | Sez6l    | 5.52        |
| Syt1     | 14.06       | Cdk5r1   | 8.21        | Gm2694   | 5.51        |
| Vstn2a   | 13.21       | Spock1   | 7.68        | Slc44a5  | 5.45        |
| Nrnx2    | 13.19       | Caeng4   | 7.62        | Scx      | 5.38        |
| Mt3      | 13.18       | Chga     | 7.60        | Panx1    | 5.36        |
| Brsk2    | 13.14       | Pak7     | 7.60        | Gsx1     | 5.31        |
| Pou3f2   | 12.94       | Gprin1   | 7.31        | Chrb1    | 5.22        |
| Gap43    | 12.82       | Cplx1    | 7.11        | Sox8     | 5.18        |
| Syngr3   | 12.69       | Grik2    | 6.94        | Gna14    | 5.16        |
| Tmem130  | 12.64       | Fam57b   | 6.78        | Mycn     | 5.09        |
| Atp6v0e2 | 12.48       | Caeng2   | 6.75        | Cdh22    | 4.93        |
| Uchl1    | 12.38       | Neurl1a  | 6.60        | Stmn1    | 4.90        |
| Sult4a1  | 12.33       | 6330407J23Rik | 6.48   | Chbln1   | 4.89       |
| Gene    | Log2 fold change | Gene    | Log2 fold change | Gene    | Log2 fold change |
|---------|------------------|---------|------------------|---------|------------------|
| Gabrb3  | 12.06            | Celf4   | 6.46             | Mex3b   | 4.76             |
| Lpl     | 12.00            | Srrm4   | 6.44             | Lhx1    | 4.76             |
| Nefh    | 11.86            | Dlgap3  | 6.40             | 2610109H07Rik | 4.75 |
| Dgkk    | 11.81            | Mgat5b  | 6.34             | Scn5a   | 4.66             |
| Asnl    | 11.70            | Ncald   | 6.34             | C530008M17Rik | 4.57 |
| Cd200   | 11.67            | 9330159F19Rik | 6.26 | Gng2   | 4.56             |
| Cadps   | 11.38            | Atp2b2  | 6.21             | Chst11  | 4.51             |
| Snurf   | 10.91            | Gdap1   | 6.12             | Gad1    | 4.51             |
| Cnih2   | 10.86            | Scg2    | 6.06             | Gnb3    | 4.47             |
| Acta1   | 10.76            | Mapk8ip2 | 6.02  | Tbc1d16 | 4.40             |
| Mtap7d2 | 10.71            | Chrnb2  | 5.79             | Enox1   | 4.38             |
| Snrpn   | 10.70            | Ly6h    | 5.77             | Bcan    | 4.37             |
| Apoe    | 10.62            | Chd7    | 5.76             | Ppp1r17 | 4.35             |
| Cyfip2  | 10.60            | Nol4    | 5.65             | Cdon    | 4.31             |
| Rell2   | 10.57            | Bex2    | 5.63             | Meg3Mir1906-2Mir770 | 4.27 |
| Cntn1   | 10.47            | 2410066E13Rik | 5.61 | Cck    | 4.20             |
Table S4 Comparison of gene expression during direct reprogramming of astrocytes into neurons ((Cell Stem Cell 2015_48h) and this study). Related to Figure 2.

To compare the RNA-seq results with previous works, we downloaded the transcriptome data in Ascl1 induced reprogramming of astrocytes from the postnatal cerebral cortex for 2 days (Masserdotti et al., 2015). Since the transcriptome data collected from microarray analysis is less sensitive than that collected from RNA-Seq, we used the criteria (fold change > 1.5, p < 0.01) to find the comparable differentially expressed genes (DEGs). We identified 370 DEGs at 2 days after Ascl1 induction (246 genes up-regulated and 124 genes down-regulated). Among the up-regulated genes, 82 genes were similarly up-regulated in our system and the top 50 genes are shown below.

| Up-regulated in Cell Stem Cell 2015_48h only | Up-regulated both in Cell Stem Cell 2015_48h and this study | Up-regulated in this study only |
|---------------------------------------------|-------------------------------------------------------------|---------------------------------|
| Gene                         | Fold change | Gene                         | Fold change | Gene                         | Fold change |
| Lzt5l                        | 12.77       | Map                          | 66.64       | Celf3                        | 18.48       |
| Cdkn1c                       | 10.35       | Gal                          | 56.56       | Hrk                          | 12.24       |
| Nefm                         | 10.32       | Stmn4                        | 27.40       | Igf2                         | 12.21       |
| Nefl                         | 8.60        | Chgb                         | 22.14       | Gm12238                      | 11.66       |
| A930017K11 Rik               | 5.34        | Hes5                         | 21.43       | Dcc                          | 11.45       |
| Bbd11                        | 5.16        | Tubb3                        | 20.79       | 1500016L03Rik                | 11.14       |
| Dcx                          | 4.63        | Snap25                       | 18.76       | Actl6b                       | 10.70       |
| Ohip2a                       | 4.39        | Dll3                         | 17.33       | 5430421N21Rik                | 9.43        |
| Lhx3                         | 3.91        | Stmn3                        | 17.02       | Reln                         | 8.45        |
| Crmp1                        | 3.89        | Ina                          | 15.38       | Mchr1                        | 8.40        |
| Trp53i11                      | 3.67       | Htr3a                        | 14.31       | Ntsr2                        | 8.08        |
| Pvalb                        | 3.55        | Myt1                         | 13.60       | Brsk2Mir3104                 | 8.06        |
| Nt5dc2                        | 3.49        | Scr2                         | 11.77       | Cacng4                       | 7.62        |
| Rbm38                        | 3.48        | Elavl3                       | 11.52       | Tnr                          | 7.31        |
| Tex14                        | 3.40        | Celsr3                       | 11.29       | Gprin1                       | 7.31        |
| Dleu7                        | 3.14        | Crtac1                       | 11.15       | Cpxl1                        | 7.11        |
| 1810041L15 Rik               | 3.13        | Sox11                        | 9.97        | Sema5a                       | 6.86        |
| Dgkk                         | 3.02        | Snca                         | 9.24        | 2700090003Rik                | 6.83        |
| Asic1                        | 3.00        | Acay                         | 8.85        | Caeng2                       | 6.75        |
| Bmp7                         | 2.94        | Septin3                      | 8.64        | Neurl1a                      | 6.60        |
| Lmo1                         | 2.90        | Elavl4                       | 8.52        | 6330407J23Rik                | 6.48        |
| A930009L07 Rik               | 2.79        | Homer2                       | 8.34        | Igbpl1                       | 6.41        |
| Sh3kbp1                      | 2.69        | Rhfox3                       | 8.31        | Dlgap3                       | 6.40        |
| Tmnt2                        | 2.69        | Cdk5r1                       | 8.21        | DccamI                       | 6.35        |
| Plxmd1                       | 2.62        | Spock1                       | 7.68        | Ncald                        | 6.34        |
| Grpr                         | 2.61        | Chga                         | 7.60        | Atp2b2                       | 6.21        |
| Nap1l2                       | 2.58        | Pak7                         | 7.60        | Ebf3                         | 6.14        |
| Plk3                         | 2.57        | Hes6                         | 7.24        | Ass1                         | 6.03        |
| Dlx2                         | 2.53        | Grik2                        | 6.94        | Mapk8ip2                     | 6.02        |
| Pak3                         | 2.51        | Fam57b                       | 6.78        | Chrnb2                       | 5.79        |
| Mfng                         | 2.50        | Celf4                        | 6.46        | Ly6h                         | 5.77        |
| Gene  | FC  |
|-------|-----|
| Kcnq4 | 2.49|
| Shisa2| 2.48|
| Eomes | 2.47|
| Jade3 | 2.43|
| Ralgds| 2.42|
| Eya2  | 2.41|
| Shf   | 2.39|
| Parm1 | 2.38|
| Gse1  | 2.30|
| Pcp4  | 2.28|
| Prmt8 | 2.28|
| Sstr2 | 2.24|
| Ifi205| 2.20|
| Tnem178b| 2.19|
| Tac2  | 2.18|
| Rph3a | 2.18|
| Megf11| 2.17|
| Fgfbp3| 2.17|
| Arc   | 2.15|

| Gene  | FC  |
|-------|-----|
| Srrm4 | 6.44|
| Mgat3b| 6.34|
| 9330159F19Rik | 6.26|
| Gdap1| 6.12|
| Scg2  | 6.06|
| Nol4  | 5.65|
| Tic9b | 5.60|
| Rab3c | 5.54|
| Sez6l | 5.52|
| Kif5c | 5.50|
| Slc29a4| 5.36|
| Gna14 | 5.16|
| Mycn  | 5.09|
| Sez6l2| 4.99|
| Cd2h2 | 4.93|
| Mex3b | 4.76|
| Lhx1  | 4.76|
| Ube2qtl1 | 4.75|
| Spns2 | 4.62|

| Gene  | FC  |
|-------|-----|
| Chd7  | 5.76|
| Bex2  | 5.63|
| 2410066E13Rik | 5.61|
| Scrt1 | 5.58|
| Add2  | 5.56|
| Gria2 | 5.54|
| Gm2694| 5.51|
| Scl44a5| 5.45|
| Scx   | 5.38|
| Mtap2 | 5.36|
| Panx1 | 5.36|
| Gsx1  | 5.31|
| Gdap1I1| 5.23|
| Chrnb1| 5.22|
| Sox8  | 5.18|
| BC005764| 5.15|
| Bsn   | 5.15|
| Xkr7  | 5.13|
| Grin1 | 5.11|
| Septin4  | 6030407003Rik | Ap1b1 | C1qtnf1 | Cntnap4 | Ensa  |
| Septin7  | 6430562015Rik | Ap3b2 | C230024C17Rik | Col16a1 | Ephb5 |
| Septin11 | 6430706222Rik | Apol8 | C230079003Rik | Col18a1 | Ephb7 |
| Selenof  | 7530416G11Rik | Agrp9 | Cables1 | Col23a1 | Ephb2 |
| 0610007P14Rik | 8030423J24Rik | Arc | Cacna2d3 | Coro1c | Ephx4 |
| 1110028F11Rik | 8430422H06Rik | Arhgap12 | Calml4 | Coro2b | Erbb4 |
| 1110038F14Rik | 9030204H09Rik | Arhgap26 | Camtal | Cpa1 | Erdr1 |
| 1500015O10Rik | 9930704L06Rik | Arhgap29 | Cant1 | Cplx3 | Ergic1 |
| 1600002D24Rik | A330074K22Rik | Arid1b | Capn12 | Cpv1 | Erh  |
| 1700066H21Rik | A330093E20Rik | Arid3a | Capn8 | Cradl | Eva1a |
| 1700034G11Rik | A330072M11Rik | Arsi | Ccd60 | Cyp2r1 | Fam110a |
| 1700066B17Rik | A330023P12Rik | Asc2 | Cdc68l | D630003M21Rik | Fam167a |
| 1810041L15Rik | A330031A15Rik | Ass1 | Ccnk | D730050B12Rik | Fam167b |
| 201003K11Rik | Atab | Atg10 | Ccr6 | D8Etra82e | Fam174a |
| 2010111O10Rik | Abcc1 | Atf2 | Ccser2 | Dbx2 | Fam175b |
| 2210409D07Rik | Abhd10 | Atoh8 | Cdc42ep1 | Dgki | Fam185a |
| 231001H17Rik | Acpp | Atp1b3 | Cda7l | Dgkz | Fam49a |
| 2310015A10Rik | Acrs6 | Atp2a3 | Cdh20 | Dhx9 | Fam53b |
| 231002B05Rik | Actn1 | Atp5l | Cdk2ap1 | Dip2c | Fbxw7 |
| 2310043L19Rik | Acvr1 | Atp8a2 | Cdk3-ps | Disc1 | Figfr4 |
| 2810006K23Rik | Adam12 | Atxn10 | Cdk5r1 | Dleu7 | Fhit |
| 2810049E08Rik | Adams14 | Atxn2 | Cdk5rap2 | Dlg5 | Filip1l |
| 2900011O08Rik | Admats17 | B3gnt5 | Cdkal1 | Dlap1 | Fkbp1 |
| 3110039J08Rik | Adcy2 | B4galnt5 | Cebp | Dil1 | Flnb |
| 4631405J19Rik | Adcyap1r1 | Bag1 | Ccrl2 | Dil2c | Flt1 |
| 4930401O10Rik | Adm | Bag5 | Celsr1 | Dmpk | Fnnl2 |
| 4930447J18Rik | Adora1 | Ba2b | Cer1 | Dnajh5 | Foxn3 |
| 4930455H04Rik | Adbkb2 | Bc065040 | Cerk | Dnajc5b | Foxo6 |
| 4930459C07Rik | Afgp1 | Bc065402 | Chad5 | Dock1 | Fra10ac1 |
| 4930474M22Rik | Aff3 | Bcl2l1 | Chad7 | Dpp6 | Fzd7 |
| 4930567K20Rik | Agmat | Bcl7a | Chn2 | D pysl4 | Gab1 |
| 4930584F22Rik | Aida | Bcr | Chrac1 | Dyps15 | Gal |
| 4930596J21Rik | Aif1l | Bfsp2 | Chrm4 | Dsn1 | Gapdh |
| 4931440F15Rik | Aim1 | Birc3 | Chrn4 | Dyn1c2 | Gas6 |
| 4933417E11Rik | Akap2 | Bptf | Chrm4 | Ednrb | Gldp5 |
| 4933426M11Rik | Akap6 | Brap | Chxt1 | Efcb5 | Gli3 |
| 4933427D06Rik | Akt2 | Brnnp2 | Cited4 | Efhd2 | Gm10007 |
| 4933433G14Rik | Amer3 | Brsk2 | Cldn14 | Ehd2 | Gm10432 |
| 5031425E22Rik | Ampd3 | Bbd11 | Clip3 | Elf3 | Gm10494 |
| 5430421N21Rik | Angpl2 | Bbbd6 | Cmpk2 | Efln1 | Gm12669 |
| 5730435O14Rik | Ank2 | Btg2 | Cntk6 | Elk3 | Gm12695 |
| 5930412G12Rik | Ano6 | C1qa | Cnot7 | Engase | Gm13152 |
| Gm14461 | Ifo1  | Lpin1 | Mir7072 | Odc1  | Polr1a | Rnf19b |
| Gm15941 | Iffo2 | Lrig1  | Mir7218 | Offj2 | Ppib   | Rnf216 |
| Gm16039 | If27  | Lrp1l  | Mir7243 | Olfn1 | Ppp4r2 | Rnf217 |
| Gm16386 | Igdc4 | Lrp8   | Mir8095 | Olfn2 | Prdlm10 | Rp9    |
| Gm19897 | Igsf2l| Lrc30  | Mir9-f | Olfn2b | Prkar1a | Rpa1   |
| Gm4285  | Il15ra| Lhbp4  | Mkln1os | Olig1 | Prkdhp | Rph3al |
| Gm527   | Il2rb | Lyph6  | Mrkn1  | Opalin | Prr18   | Rpl29  |
| Gm5294  | Impdh1| Lyz14os| Morn1  | Opclm | Prr5   | Rps6ka2 |
| Gm6994  | Inppl1| Mab213 | Mroh2a | Oprl1  | Prss33  | Rps6kc1 |
| Gm7616  | Iqsec1| Mad111 | Mrps28 | Osm   | Prss52  | Rptoros |
| Gm9159  | Irf2hp2| Map3k14 | Msntd1 | Ostc   | Psmh7   | Rrp7a  |
| Gm9899  | Iga9  | Map6   | Mst2   | Pah    | Psmc6   | Rtn4r11 |
| Gna12   | Jade1 | Mapk8ip1| Msra   | Pde1b  | Ptgfrn  | Rfyl1  |
| Gpc5    | Jade3 | Mapkapk2| Mtr1b  | Pde2a  | Ptk2    | Sae1   |
| Gpr114  | Jag1  | Mapre1 | Mtss1  | Pde4b  | Ptp4a2  | Scarb1 |
| Gpr156  | Jard2 | Marveld3| Mxra7  | Pdgfa  | Prprd   | Sdc3   |
| Gpr52   | Kat6b | Mast4  | Myo3b  | Pdlim4 | Ptpnrn  | Sec22c |
| Gpr56   | Kazn  | Mbp    | Myo5c  | Pdpn   | Ptprs   | Sec61b |
| Gprc5d  | Kcnh2 | Mc2r   | Myt1   | Peps   | Pvalb   | Sema5a |
| Gprin3  | Kenk13| Mejfd2 | Myzap  | Pex19  | Pvr     | Sema6d |
| Gpx2    | Konk2 | Mcm2   | Nab2   | Pga2   | Pwwp2b  | Sefgef |
| Grhl2   | Kcnq1 | Mcur1  | Nat10  | Phl12  | Qpct    | Serping |
| Grik4   | Kcnq4 | Mdjic  | Nav2   | Phld1b | Qprt    | Sertad3 |
| Grin2b  | Kcd14 | Me3    | Nbl1   | Phld2  | Qsox1   | Sestd1 |
| Grin3a  | Kdm4d | Med31  | Neald  | Piezo2 | R74862  | Sefdt5 |
| Grin7   | Kdm6b | Metap1d| Nck2   | Pigv   | Rab6b   | Sh3f1  |
| Grm8    | Kdm7a | Mgat5  | Nckap5 | Pigz   | Rabgap11| Shb    |
| Gsx11   | Kj20b | Miat   | Ncor2  | Pip4k2a| Rad51b  | Sii1   |
| Gf3a    | Kj26b | Micalc | Ndrg1  | Ptpnmm | Ratgds  | Sim2   |
| Gucy1b2 | Kiil  | Mif4gd | Ndafa12| Phkd1  | Ratgps1 | Sipa11 |
| H2afy3  | Kl10  | Mir128-2| Neh1   | Pkig   | Ranbp9  | Scl14a2 |
| H6pd    | Kl13  | Mir182  | Nenf   | Pkm    | Rasal2  | Scl30a4 |
| Has2    | Khl21 | Mir1902 | Neurod4| Pikp1  | Rassf3  | Scl35b1 |
| Hdac11  | Khl25 | Mir1931 | Nkbia  | Pla2g2c| Rbfox1  | Scl35e1 |
| Hdac7   | L2hgdh| Mir1969 | Ngfy1  | Plac8ll| Rbms3   | Scl38a1 |
| Herpud2 | Lif   | Mir343  | Nkd1   | Plce1  | Rbpfj   | Scl38a4 |
| Hexim2  | Limal | Mir3473f| Noc4l  | Plcgl  | Reep1   | Scl39a11|
| Hey1    | Linep1| Mir374c | No4l   | Plekha1| Rere    | Scl51b  |
| Hgd     | Ling01| Mir5133 | Notch1 | Plekha7| Rgr     | Scl7a5  |
| Hibadl  | Lnf1  | Mir6364 | Nphp4  | Plekhg5| Rgs12   | Scl8a1  |
| Hip1    | Lmnb2 | Mir6368 | Nron   | Plet1  | Rgs3    | Scl9a3  |
| Hipk2   | Lmo1  | Mir6401 | Nsmce2 | Pik3   | Rhbd13  | Sli1    |
| Hivep3  | Lmo2  | Mir6409 | Ntsr2  | Plxdc2 | Rimbp3  | Sli3    |
| Hook2   | Lmod2 | Mir6904 | Nudt18 | Plxna2 | Ritt2   | Smad5   |
| Iba57   | LOC101056043 | Mir7041 | Numb | Plxna2 | Rnaseh2b | Smm3    |
| Idh2    | Lphn3 | Mir705  | Nxxn   | Pold3  | Rnf122  | Sna1    |
| Snca | Sstr5 | Tek | Tmsb10 | Tsc1 | Vmn1r195 | Wscd1 |
|------|-------|-----|--------|------|----------|-------|
| Snld1 | St3gal2 | Tgbi | Tnfsf13b | Tstd2 | Vmn2r6 | Zc3h12c |
| Snora19 | Stk33 | Tgif1 | Tnkh | Tc28 | Vps37b | Zfand5 |
| Snora3 | Stx6 | Thbs2 | Tnr | Tc8 | Vps41 | Zfhx3 |
| Surpe | Stx8 | Thbs4 | Tns3 | Tll3 | Vsm4 | Zfp423 |
| Snta1 | Sun1 | Them6 | Top1 | Tll5 | Vwa2 | Zfp536 |
| Sntg1 | Sv2b | Thpo | Toc3 | Tulp4 | Vwa8 | Zfp609 |
| Snx29 | Svil | Tmem177 | Traf3ip2 | Tswg1 | Wdr1 | Zfp648 |
| Soxs3 | Synpo | Tmem178b | Traf4 | Ubf | Wdr27 | Zfp664 |
| Sorl1 | Tapt1 | Tmem191c | Traf6 | Ubxn8 | Wdr34 | Zfp827 |
| Sox11 | Tas1r1 | Tmem201 | Trim29 | Unc5a | Wdr70 | Zfp930 |
| Spon1 | Tcf12 | Tmem236 | Trit1 | Upp2 | Wdr95 | Zfp940 |
| Spon2 | Tct11 | Tmem255b | Trmt5 | Usp12 | Wnt5a | Znrf2 |
| Srp9 | Tead1 | Tmem44 | Trmt61a | Ust | Wnt7b | Zpld1 |
| Sspn | Tecr | Tmie | Tspan14 | Yash1 | Wnt9a |
Table S7 Direct downstream targets of ASCL1 during direct reprogramming of astrocyte into neurons. The genes in bold were shared in Wapinski et al. Cell 2013 and this study. Related to Figure 2 and Figure 3.

| Gene  | Day2 Up-regulated only | Day2-Day5 Up-regulated in order | Day5 Up-regulated Only | Down-regulated Both |
|-------|------------------------|-------------------------------|-----------------------|---------------------|
| Adam12 | A330093E20Rik           | Lpin1                         | Ap3b2                 | 5031425E22Rik       | 1500015O10Rik       |
| Adams14 | A930003A15Rik           | Lrp11                         | Cdk5r1                | Adrbk2              | Aim1                |
| Ass1   | Ank2                   | Mapkapk2                    | Chd7                  | Akap6               | Chrab4              |
| Atp2a3 | Arid1b                 | Micalcl                      | Lmo2                  | Atp8a2              | Glis3               |
| Chst11 | Cdk2ap1                | Phldb2                       | Miat                  | Cacna2d3            | Kazn                |
| Col18a1 | Cerk                   | Pcg1                         | Myt1                  | Camta1              | Neb1                |
| Dock1  | Coro1c                 | Plxna2                       | Ncald                 | Cecr2               | Plce1               |
| Exosc9 | Ctf                 | Ptprs                        | Pde1b                 | Chn2                | Plekha7             |
| Gal    | Ctnnb1                 | Pwpp2b                       | Snca                  | Chrm4               | Prkcdbp             |
| Jag1   | Dhx9                  | Rbpj                         | Sox11                 | Cntnap4             | Sema6d              |
| Klf10  | Dip2c                 | Rere                         | Unc5a                 | Dpys5               | Snta1               |
| Lrig1  | Ephb2                 | Rnaseh2b                     | Erbb4                 | Synpo               |                     |
| Mcm2   | Ephx4                 | Sestd1                       | Kcnh2                 |                     |                     |
| Olig1  | Foxn3                 | Shb                           | Neurod4               |                     |                     |
| Plekha1 | Foxo6                | S1c8a1                       | Ntsr2                 |                     |                     |
| Sema5a | Frail10ac1             | Svil                          | Prr18                 |                     |                     |
| Tcf12  | Gm527                 | Traf4                         | Rab6b                 |                     |                     |
| Thbs2  | Hdac11                | Traf6                         | Reep1                 |                     |                     |
| Tnr    | Hipk2                 | Tsscl                         | Sx29                  |                     |                     |
| Wnt7b  | Hook2                 | Tulp4                         | Zfp423                |                     |                     |
| Inpp11 | Usp12                 |                               |                       |                     |                     |
| Lima1  | Zfand5                |                               |                       |                     |                     |
| Gene   | Forward Primer  | Reverse primer          |
|--------|-----------------|-------------------------|
| Gapdh  | AGGTCGGGTGTAAGCGATTTTG | TGTAGACCATGATGTTGAGGTCATA |
| Tuj1   | TAGACCCAGCGCGCACTAT  | GTTCCAGGTCCAAATCTCACC   |
| Map2   | GCCAGCCTCACAGAAACAACAG | AAGGTCTTGGAGGGAAGAAGAAC |
| NeuN   | GTAGAGGGAGGAAAATTGAGG | GTGGGGTATGGGGAAACCTGG   |
| Klf10  | ATGCTCAACTTGGGCTTCTT  | CGCTTACCGGCCTTGAAGC     |
| Chd7   | GACCCAGGGATGATGAGTTCTT | ATGGGGTTCACGGGTTTTC    |
| Sox11  | CACAACCGCACTCTTCAA  | GGGTCCGTCTGGGCTTTTGG    |
| Myt1   | ATGATGACCCGGTGGGAAATTAGG | GGACACTTGGATTTCACGGCTTCT |
| Myt1l  | TGGTCACGTCAGGCGGAATAATA | TGCAAAATGTTTTTCGCTGGGG |
| Myt3   | TGATTGGCTCTATGGGCGAAGAG | CCCATGTCTGGGCTTGAAGT    |
| Neurod4 | AGCTGGTCAACACACAATCTCT | GTTCGCCAGCATTCACATAAGAGC |
| Ebf3   | CAGGCCATCGTGTATGAAGG | GTGTCTCGTTTCTATTGCCACA  |
| Zfp821 | CTCTGCCAGCTAGACTGTGGG | GCAGTTGGACCGCTGATCT    |
| Klf7   | TCCACGACACCGGCTACTT  | GGGAGCAAGCAAGGGGCTCTA  |
| Nhlh1  | GCTTTGGGAGCTACAGGAGGAGG | CTTTAAGTGAGGGACTTGGGCTAT |
| Runx2  | TTCAACGATCTGAGTATTTGTGGG | GGATGAGGAATGCGCCTA     |
| Phx1   | ACTGCTGTTCTGACAGAGTCTC | CTCTGCGGTTCTGCTGGGAC   |
| Cdk2b  | CCGTGCCAACCTTACCCAGA | CAGATACCCCGTGAATGCTCAG  |
| Col24a1 | TTCACTGCTTCAAACACCCCAAGG | CCATCCTGAATCTTTCAGTCTCAT |
| Thx4   | TCCCCAGCTACAAGGGTAAAAATGT | ACCATCCATTGTTGTCACAGAAGA |
| Myh3   | AAAAGGCCATCAGTACGC | CAGCTCCTGCATCCGTGTCTC   |
| Myo18b | TCTCCGCCTCCTTGTCTTCTT | TGCTGGGATCTACTTCTGG     |
Table S9. shRNA sequences. Related to Figures 4, 5, 6, and 7.

| Name   | shRNA sequence                  |
|--------|---------------------------------|
| shKlf10-1 | GCGACTGGAAGTCTCTATTCA          |
| shKlf10-2 | GGGTCAATCTGACTGATCA         |
| shKlf10-3 | GCGCTGCATATGACTTTTG         |
| shMyt1/1l | GGTCATTGAAGTCAAGTCA         |
| shMyt1    | GCCCAGAGCTACATAGCTCTAA       |
| shMyt1l    | GGGCATTGAATAGCAACA          |
| shMyt3    | GCAGCAGTATCCAGTCTTTAA       |
| shNeurod4-1 | GCCTCAACCATTAAAGAGT    |
| shNeurod4-2 | GCTCGCCTTGAAAGATTC        |
| shNeurod4-3 | CTGGCAAGGAACACTACATCT   |
| shChd7-1   | CCTCCTGCTGAGCTGAGAAGAATAT |
| shChd7-2   | CAGGCAGCTATTGACAGATTCTCCA  |
| shChd7-3   | TGACAGGTGAGTCTCTACTGTGTTA  |
| shChd7-4   | GCCAGCGCGTCGGACCATTTC      |
| shChd7-5   | GCTCCAGACTGGACCGATATA      |
| shCtrl     | AGACGCACGACAACGGCATAT      |
SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Astrocyte Culture
Astrocytes were cultured as previously described with some modifications (McCarthy and de Vellis, 1980). Briefly, the dorsal midbrain astrocytes from P5–P7 mice were dissected and dissociated with 0.25% trypsin for 15 min. The tissue was pipetted up and down gently for further dissociation and then seeded in 25 cm$^2$ flask for expansion with medium containing DMEM/F-12 (Invitrogen), 10% fetal bovine serum (Invitrogen), penicillin/streptomycin (Invitrogen), and supplemented with B27 (Invitrogen), 10 ng/ml epidermal growth factor (EGF), and 10 ng/ml fibroblast growth factor 2 (FGF2). After 7–9 days, the oligodendrocytes were shaken off to obtain the astrocyte culture and reseeded in 60 mm dishes. All animal procedures are approved by the Animal Care Committee at the Institute of Neuroscience, CAS center for Excellence in Brain Science and Intelligence Technology, Chinese Academy of Sciences (Reference NA-010-2016).

Viral Production and Transduction
Lentiviruses were produced from HEK293FT cells that were transiently transfected with lentiviral, viral envelope-, and VSVG-pseudo-typing plasmids (Tiscornia et al., 2006). Viral particles were concentrated from supernatant by ultracentrifugation at 25,000 g for 2 hours. Viral particles were tittered by clonal analysis after transduction of 293FT cells. Viral titers were typically $10^8$–$10^9$ infectious particles/ml. Lentiviral transduction of astrocytes was performed 2–4 hours after seeding cells on poly-D-lysine-coated 24-well plate at a density of 70,000 cells per well. Twenty-four hours after transduction, the medium was replaced completely by a medium consisting of DMEM/F-12, B27 supplement, and penicillin/streptomycin. Then the cells were allowed for direct reprogramming, from 6 days post-infection (DPI) onward, brain-derived neurotrophic factor (20 ng/ml; PeproTech) were added to the medium every 3 day by changing 50% of the medium.

Immunostaining
Immunostaining on cultured cells was performed essentially as previously described except the primary antibodies were incubated for overnight (Vierbuchen et al., 2010). The primary antibodies were used as follows: rabbit anti-GFAP (1:1000; Z0334; DAKO), mouse anti-S100β (1:1000; S2532; Sigma), rabbit anti-NG2 (1:200; AB5320; Millipore), mouse anti-O4 (1:500; MAB345; Millipore), rabbit anti-Olig2 (1:500; AB9610; Millipore), rabbit anti-IBAI (1:500; 019-19741; Wako), mouse anti-CNPase (1:500; ab6319; Abcam), mouse anti-Ascl1 (1:200;556604; BD Biosciences), mouse anti-Tuj1 (1:500; MMS-435P; Covance), mouse anti-Map2 (1:500; M4403; Sigma), rabbit anti-GFP (1:1000; A6455; Invitrogen), chicken anti-GFP (A10262; 1:1000; Invitrogen), rabbit anti-DsRed (1:500; 632496; Clontech). Cy3- and Cy5-conjugated secondary antibodies were obtained from Jackson ImmunoResearch. AlexaFluor-350-, Alexa Fluor-488-, and Alexa Fluor-546-conjugated secondary antibodies were obtained from Invitrogen.

Quantitative RT-PCR
Total RNA was extracted with Trizol reagent (Invitrogen) according to the manufacturer’s instructions. The cDNA was synthesized using PrimeScript™ RT reagent kit with gDNA eraser (Takara). Quantitative RT-PCR was performed on LightCycler480 (Roche) using JumpStart Ready Mix (Sigma) and the Eva Green (Biotium). Data analysis was performed with the DDCt method. The primers for
quantitative RT-PCR can be found in Table S8.

RNA Sequencing and Data Processing
Total RNA was isolated with Trizol reagent. Libraries were prepared, and sequencing was performed in 100-bp paired-end format on the Illumina HiSeq 2000. The quality of the reads was evaluated using the FASTQC tool. Raw reads were mapped to mm10 version of mouse genome using TopHat version 2.0.4 program (Trapnell et al., 2009). We calculated fragment per kilobase per million reads (FPKM) as expression level using Cufflinks version 2.0.2 with default parameters (Trapnell et al., 2010). Genes with the FPKM > 1.0 in at least one sample across all samples were retained for further analysis. Then the expression levels were transformed to logarithmic space by using the $\log_2$ (FPKM + 1). Differentially expressed genes (DEG) between clusters were identified using RankProd (Hong et al., 2006) with P value < 0.05 and fold change > 1.5. DEG heatmaps were generated using Cluster 3.0 and visualized using Java TreeView software (Saldanha, 2004). PCA analysis was performed using FactoMineR package in R (http://www.r-project.org).

Connection Specificity Index (CSI).
CSI of the TF groups were calculated. CSI employs the PCC (Pearson Correlation Coefficient) as a first-level association index to rank the similarity between nodes, and then uses a constant of 0.8 to define the boundary of interaction-profile similarity. Finally, we generated a TF co-expression network based on the CSI (CSI > 0.8) coefficient matrix (Fuxman Bass et al., 2013).

Functional Enrichment Analysis
Functional enrichment of gene sets with different expression patterns was performed using the Database for Annotation, Visualization and Integrated Discovery version 6.8 (DAVID v6.8) (Huang da et al., 2009).

Chromatin Immunoprecipitation Followed by Sequencing
ChIP-seq was carried out in astrocytes 2 days after FUGW-Ascl1 infection. Approximately 9-12x10⁶ cells were used for each ChIP-seq experiment. Cells were cross-linked in 1% formaldehyde for 10 min, lysed and sonicated to generate DNA fragments with an average size of 300 bp (Jin et al., 2009). About 5 ng IP DNA and input DNA measured by Agilent Technologies 2100 Bioanalyzer were used to construct DNA library by using ChIP-Seq Sample Prep Kit (Illumina). Enriched DNA sequencing was performed on Illumina HiSeq 2000. Immunoprecipitations were performed with mouse anti-Ascl1 (Pharmining 556604) or control LEAF-purified mouse IgG1 (Cambridge Biosciences). Sequencing reads were generated on HiSeq 2000 Illumina platforms. The primers for ChIP-PCR were as follows: Klf10, 5′-AGCTCTTCTCTGCCTCTCC-3′ and 5′-CAGACACAGGGTTCAGGTTT-3′; Chd7, 5′-TTGGAAGCAGTTTGTGCACTG-3′ and 5′-GGCTCCATTTCAGCATATA-3′; Sox11, 5′-TTCTGCAGCTGCCTCCTC-3′ and 5′-ATGCAAGGTGCCGCAAGT-3′; Myt1, 5′-CCAGGCAGGGGACTACATAA-3′ and 5′-GATAGGCCACAGAGCACTGG-3′; and Neurod4, 5′-CCCTCCATTCCTCTCTC-3′ and 5′-TGGCAGTCCAACAGATT-3′.

ChIP-seq Data Analysis
Raw reads were mapped to mm10 version of mouse genome using bowtie2 (version 2.3.1) (Langmead and Salzberg, 2012), and then peaks were called using HOMER (version 4.2) (Heinz et al., 2010) with
default parameters. The enrichment tags were visualized using Integrative Genomics Viewer (IGV, version 2.4).

**Gene Knockdown**

To acutely knockdown *Klf10*, *Myt1*, *Neurod4*, and *Chd7*, one or two shRNA specifically against targeting sequence and one control scramble shRNA sequence were cloned into the lentiviral vector *pLKD* (OBIOTechnology (Shanghai) Corp., Ltd.) using AgeI and EcoR1. The shRNA sequences can be found in Table S9.

**Image Acquisition and Morphological Analysis**

Z-stack images at 1 μm interval were acquired on a Nikon TiE-A1 plus scanning confocal microscope with a 20X Neofluor objective (N.A. = 1.3, for quantitative morphological analysis). Total dendritic branch length (TDBL) and total dendritic branch tip number (TDBTN) were measured and counted using ImageJ. At 8 DPI, iN cells have not yet developed dendritic spines, so protrusions longer than 3 μm were considered dendritic branch tips (Bian et al., 2015).

**Electrophysiological Recording**

Whole-cell voltage- or current-clamp recording was performed as described previously (Lu et al., 2007), with some modifications. Whole-cell recordings were made from GFP⁺ and tdTomato⁺ cells with borosilicate glass micropipettes filled with an internal solution containing the following (in mM): 130 K-gluconate, 20 KCl, 10 HEPES, 0.2 EGTA, 4 Mg2ATP, 0.3 Na2GTP, and 10 Na2-phosphocreatine, at pH 7.3 (290–310 mOsm). The pipette resistance was in the range of 2.0–4.0 MΩ. To evoke currents, step voltages (500 ms, 10 mV step) from -110 to 60 mV were applied in the voltage-clamp mode. To evoke membrane potential deflections, step currents (500 ms duration) were injected in the current-clamp mode. For separating spontaneous EPSCs (sEPSCs) and sIPSCs, cells were voltage clamped to -66 and 0 mV, respectively, using the Cs⁺-based internal solution that contained the following (in mM): 125 Cs⁺-gluconate, 5 tetraethylammonium-Cl, 2 CsCl, 1 EGTA, 10 HEPES, 4 Mg-ATP, 0.3 GTP, 10 phosphocreatine, and 3 QX-314, pH 7.2. Electrical signals were amplified and filtered at 2–10 kHz (low pass) with Axon MultiClamp 700B (Molecular Devices), digitized at 20–100 kHz (Digidata 1322A; Molecular Devices), and acquired by a computer with the pClamp 9.2 (Molecular Devices). The data analysis was done with the Clampfit and a custom program in MATLAB (MathWorks). All chemicals were from Sigma or Tocris Bioscience.

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