HHS Public Access

Author manuscript

Nature. Author manuscript; available in PMC 2012 July 12.

Published in final edited form as:

Nature. ; 481(7380): 195–198. doi:10.1038/nature10712.

Ars2 maintains neural stem cell identity via direct transcriptional activation of *Sox2*

Celia Andreu-Agullo[1,3], **Thomas Maurin**[1], **Craig B. Thompson**[2], and **Eric C. Lai**[1,3]

[1]Sloan-Kettering Institute, Department of Developmental Biology, 1275 York Ave, Box 252, New York, NY 10065

[2]Sloan-Kettering Institute, Department of Cancer Biology and Genetics

SUMMARY

Fundamental questions concern the transcriptional networks that control the identity and self-renewal of neural stem cells (NSCs), a specialized subset of astroglial cells endowed with stem properties and neurogenic capacity. We observed that the zinc finger protein Ars2 is expressed by adult NSCs from the subventricular zone (SVZ). Selective knockdown of *Ars2* in GFAP$^+$ cells within the adult SVZ depleted NSC number and their neurogenic capacity. These phenotypes were recapitulated in the postnatal SVZ of *hGFAP-Cre::Ars2$^{fl/fl}$* conditional knockouts, but were more severe. *Ex vivo* assays showed that Ars2 was necessary and sufficient to promote NSC self-renewal, by positively regulating the expression of *Sox2*. Although plant[1–3] and animal[4,5] orthologs of Ars2 are known for their conserved roles in microRNA biogenesis, we unexpectedly observed that Ars2 retained capacity to promote self-renewal in *Drosha* and *Dicer* knockout NSCs. Instead, chromatin immunoprecipitation revealed that Ars2 bound a specific region within the 6kb NSC enhancer of *Sox2*. This association was RNA-independent, and the bound region was required for Ars2-mediated activation of *Sox2*. We used gel-shift analysis to confirm direct interaction, and to refine the region bound by Ars2 to a specific conserved DNA sequence. The importance of *Sox2* as a critical downstream effector was shown by its ability to restore the self-renewal and multipotency defects of *Ars2* knockout NSCs. Altogether, we reveal Ars2 as a novel transcription factor that controls the multipotent progenitor state of NSCs via direct activation of the pluripotency factor *Sox2*.

Stem cells reside in most mammalian tissues throughout adult life, and contribute to normal homeostasis and repair after injury[6]. They are defined by their capacity to both self-renew and differentiate, thus perpetuating themselves whilst generating more committed daughter cells. Two major stem cell niches exist in the adult brain, within the hippocampus and the subventricular zone (SVZ). Relatively quiescent neural stem cells (NSCs) give rise to actively proliferating transit-amplifying progenitors (TAPs), which generate

---

[3]Authors for correspondence: andreuac@mskcc.orglaie@mskcc.org tel: (212) 639-5578 fax: (212) 717-3604.

Author contribution

C.A.-A. performed and designed all the experiments, T.M. performed *in vivo* lentivirus injections, and C.B.T provided reagents. C.A.-A and E.C.L conceived the project, interpreted the results and wrote the manuscript.

oligodendrocytes destined to the corpus callosum[7] and neuroblasts (NBs) that migrate rostrally and differentiate into local interneurons in the olfactory bulb (OB)[9,10]. Much remains to be understood about the mechanisms and factors that control NSC self-renewal and multipotency[13].

Mammalian Ars2 was reported as essential for cell proliferation, to be downregulated in quiescent cells, and required for accumulation of several miRNAs implicated in cellular transformation[4]. Unexpectedly, we observed that Ars2 expression in the adult SVZ did not correlate with proliferation, since 95±2% of Ars2$^+$ cells lacked the proliferative marker Ki67. Moreover, Ars2 was present in only 7±2% of Mash1$^+$ TAPs (Supplementary Figure 1b) and was absent from Doublecortin$^+$ (DCX) NBs (Figure 1a); these comprise the most highly proliferative cells in the SVZ. Ars2 was also absent from GFAP$^+$ Nestin$^-$ Sox2$^-$ astroglial cells and S100β$^+$ mature astrocytes (Supplementary Figure 1). Instead, Ars2 was expressed by niche astrocytes, ependymal cells and by GFAP$^+$ CD133$^+$ stem cells[14] (Supplementary Figure 1). A hallmark of NSCs is their quiescence, reflected by their ability to retain S-phase labels such as 5-chlorodeoxyuridine (CldU) for extended periods (i.e., label retaining cells, LRCs)[15,16]. We observed expression of Ars2 in 87±3% of LRCs marked one month earlier (Figure 1a), demonstrating presence of Ars2 in this slow dividing population *in vivo*.

To assay roles of Ars2 in NSCs *in vivo*, we used shRNAs that suppressed endogenous Ars2 (Supplementary Figure 2c). We packaged these into GFP-expressing Mokola lentivirus, which specifically transduces astroglial cells[17] (Supplementary Figure 3). We injected these into the adult SVZ and sacrificed mice 48 hours, 5 days or 1 month later (Figure 2c). At 48 hours post-infection, shArs2-GFP$^+$ cells exhibited 80% reduction in *Ars2* mRNA relative to shControl cells (Figure 2d). Apoptosis was unaffected by shArs2, and the number of GFP$^+$ Ki67$^+$ cells and levels of *CyclinD1* or *CyclinE* transcripts were also unchanged (Supplementary Figures 3, 4b, 5b). However, 5 days post-infection, shArs2 SVZs exhibited 50% reduction in the number and the proliferation rate of the GFAP$^+$ Nestin$^+$ NSCs (Figure 1e, f), still without change in apoptosis (Supplementary Figure 5b). Loss of NSC potential has been linked to an increase in mature astrocytes[18–20]. Accordingly, we observed a 50% increase in the number of GFP$^+$ S100β$^+$ cells (Figure 1g).

To assess LRCs, we injected shRNA-infected mice with CldU and sacrificed one month later. Strikingly, we observed ~50% decrease in transduced LRCs in shArs2 SVZs (Figure 1h), suggesting that Ars2 maintains the NSC pool. If true, this is expected to have downstream consequences on neurogenesis. Indeed, 5 days post-infection, we observed a decrease in DCX$^+$ NBs (Supplementary Figure 6). LRCs also label post-mitotic cells that incorporated CldU just prior to cell cycle exit (such as differentiated cells and newborn OB interneurons). One month post-infection, the population of shArs2-GFP$^+$, newly formed CldU$^+$ OB interneurons was strongly reduced (Figure 1i-k).

We performed additional analysis using neurospheres derived from shRNA-infected SVZ. Long-term self-renewal assays revealed that depletion of Ars2 rapidly extinguished neurosphere cultures, indicating a defect in self-renewing divisions (Figure 1m). This defect was fully restored by an shRNA-resistant form of Ars2 (Figure 1m and Supplementary

Figure 2d). Reciprocally, *in vivo* overexpression of Ars2 in wildtype mice increased neurosphere formation (Figure 1n). Multipotency of Ars2-deficient neurospheres was also affected, since the frequency of clones that generated ßIII-tubulin$^+$ neurons and O4$^+$ oligodendrocytes was decreased in favor of unipotent GFAP$^+$ clones (Figure 1o). We conclude that Ars2 is required to maintain NSCs in a self-renewing and multipotent state.

We sought to confirm these shRNA results by breeding the conditional knockout allele of *Ars2* (*Ars2$^{fl/fl}$*) with *hGFAP-Cre*[21]. *hGFAP-Cre::Ars2$^{fl/fl}$* (i.e. *Ars2$^{\Delta/\Delta}$*) mice (Supplementary Figure 7d) were born at the expected Mendelian ratios relative to wild-type and *hGFAP-Cre::Ars2$^{fl/+}$* littermates (used as controls). However, by postnatal day (P)15, *Ars2$^{\Delta/\Delta}$* mice showed progressive growth retardation, hydrocephalus and ataxia, resulting in death between P20 and P25. Further analysis of P15 *Ars2$^{\Delta/\Delta}$* mice revealed enlarged ventricles and smaller olfactory bulbs (Figure 2a, b), suggestive of a requirement of Ars2 during postnatal neurogenesis. The expression pattern of Ars2 in P15 wild-type SVZ was analogous to the adult SVZ (Supplementary Figure 7a,b), and analysis of the conditional knockout confirmed essentially complete absence of Ars2 in the SVZ (Supplementary Figure 7c). Importantly, the number of NSCs (marked by expression of Nestin, Sox2, Lex, and GFAP) was reduced by 80% in *Ars2$^{\Delta/\Delta}$*SVZ, and their proliferation rate decreased 2-fold (Figure 2c,d,f). This was not due to cell death as assessed by Caspase 3 staining (Supplementary Figure 8a,b). Conversely, we observed profound astrogliosis in *Ars2$^{\Delta/\Delta}$*, as assessed by GFAP and S100ß staining (Figure 2e,f).

As Ars2 is expressed in niche astrocytes and ependymal cells, in addition to NSCs, we wished to demonstrate an autonomous function of Ars2 in NSCs. We co-injected GFP$^+$ and Split-Cre plasmids that specifically drive excision in GFAP$^+$ CD133$^+$ NSCs[14] into the SVZ of P0-1 *Ars2$^{fl/fl}$* pups, and introduced them using electroporation. Five days later, we isolated GFP$^+$ cells and plated for self-renewal assay. GFAP$^+$ CD133$^+$ NSCs deleted for *Ars2* were strongly compromised for neurosphere generation, demonstrating a cell-autonomous requirement of Ars2 in this population (Figure 2g and Supplementary Figure 9). Consistent with decreased NSC number, *Ars2$^{\Delta/\Delta}$* mice exhibited ~2.5-fold fewer DCX$^+$ NBs (Figure 2h), although their proliferation rate was not affected (data not shown). In the OB, the frequency of Tyrosine Hydroxylase$^+$ (TH), Calbindin$^+$ (CB) and Calretinin$^+$ (CR) interneurons per glomerulus were also deeply reduced in *Ars2$^{\Delta/\Delta}$* (Figure 2i,j and Supplementary Figure 10). In summary, the severe defects of postnatal SVZ deleted for *Ars2* solidified its requirement to maintain NSC identity.

Since Ars2 functions in miRNA biogenesis[2–5], we tested if the ability of Ars2 to promote NSC self-renewal was mediated by miRNAs. This was not the case, since overexpression of Ars2 in *Dicer$^{\Delta/\Delta}$* and *Drosha$^{\Delta/\Delta}$* cells increased neurosphere yield (Figure 3a,b). We sought further insight by examining transcription factors known to have substantial roles in NSC self-renewal, including Hes1, Hes5 and Sox2[22–24]. *Sox2*, but not *Hes1* mRNA, was significantly reduced 48 hr after *Ars2* knockdown *in vivo* (Figure 3c). One month post-infection, *in vivo* knockdown of *Ars2* decreased *Hes1* and *Hes5* mRNA levels by ~30%, but resulted in a more substantial 70% reduction in *Sox2* (Figure 3d). Reciprocally, *Sox2* increased by ~60% in neurospheres overexpressing Ars2 (Figure 3e). These effects appeared to be transcriptional in nature, since Ars2 activated a 6kb *Sox2-luc* reporter containing cis-

regulatory sequences responsible for *Sox2* expression within adult neurogenic zones[25] (Figure 3f). This was not simply due to the cancelling effects of overexpressing any self-renewal gene, because *in vitro* overexpression of Sox2 (Figure 3g), but not Hes5 (Figure 3h) rescued the Ars2-dependent loss in self-renewal capacity of shArs2 cells. Reciprocally, *in vitro* knockdown of *Sox2* (Supplementary Figure 11a) caused rapid depletion of self-renewing neurospheres[26] (Figure 3i) and compromised multipotency (Supplementary Figure 11b) similar to the effects of *Ars2* knockdown (Figure 1m,o). Altogether, these data suggested Sox2 as a critical downstream effector of Ars2 in NSCs.

To evaluate whether the ability of Ars2 to activate *Sox2* expression might reflect a transcriptional role for this nuclear protein, we performed chromatin immunoprecipitation (ChIP) of Ars2 in NSCs, querying across the 6kb *Sox2* promoter and the *Sox2* transcription unit. Interestingly, Ars2 associated not only with its 5' UTR and 3' UTR, but was highly enriched in region 8 (−2 to −2.5 kb) of the *Sox2* promoter (Figure 4a); we validated this binding pattern using an independent antibody (Supplementary Figure 12a). RNase treatment of chromatin samples eliminated UTR-associated Ars2 ChIP signals, consistent with this reflecting CBC-mediated association with capped transcripts[4]. However, binding of Ars2 to promoter region 8 was maintained, suggesting here more direct association of Ars2 with chromatin (Figure 4b). No binding was found to the *Sox2* coding region (Figure 4a), or to the promoters of *Hes1*, *Hes5*[27], *K14*, and *Myod1* (Supplementary Figure 12b-e). Chromatin association of Ars2 was cell type-dependent, since Ars2 did not bind the *Sox2* enhancer in NIH3T3 cells (Figure 4a), which express high levels of Ars2 (Supplementary Figure 2a).

Simple binding of Ars2 to the *Sox2* enhancer might not necessarily be of functional consequence. We prepared two deletions of the 6kb *Sox2-luc* reporter, removing the Ars2-bound region 8 or a control region. Loss of region 8 strongly reduced *Sox2-luc* expression relative to the control deletion, while reciprocally, ectopic Ars2 activated the control deletion but not the version lacking the Ars2 binding site (Figure 4c). Therefore, Ars2 activates *Sox2* via promoter region 8. We then incubated NSC nuclear extract with a series of overlapping 90 bp radiolabeled probes covering the ~500 bp of *Sox2* region 8, and observed a specific gel-shift of subregion #8-3 (Figure 4d). This band was completely super-shifted by inclusion of Ars2 antibody, but not Myc antibody (Figure 4d). We narrowed down the Ars2 binding site, which revealed that Ars2 bound specifically to the central portion of *Sox2* region #8-3 (Figure 4d). This reflected direct DNA binding activity of Ars2, since *in vitro* translated Ars2 recapitulated specific binding to *Sox2* probe #8-3b (Figure 4d). This identified a sequence that is highly constrained across mammalian genomes (Figure 4e and Supplementary Figure 13).

To determine whether Sox2 mediates Ars2 function *in vivo*, we electroporated *Sox2* expression construct into $Ars2^{\Delta/\Delta}$ postnatalSVZ. Strikingly, Sox2 rescued the self-renewal and multipotency defects of *Ars2* knockout cells (Figure 4f-j). In contrast, NSCs derived from $Ars2^{\Delta/\Delta}$ SVZ electroporated with CyclinD1 lacked self-renewal capacity (Figure 4f) and multipotency (Figure 4g,k). This confirmed that *Ars2* knockout cells cannot be rescued by driving proliferation. Instead, Ars2 confers NSC identity as a self-renewing cell type by activating *Sox2*.

A central goal of stem cell biology is to understand the molecular mechanisms that regulate stem cell self-renewal and multipotency. We showed that Ars2 is specifically expressed by NSCs and not by TAPs and NBs, and that it maintains the self-renewal and multipotency capacity of postnatal and adult NSCs. In this setting, Ars2 is not required for cell viability, but is instead essential for maintaining core NSC properties. *Ars2* depletion or knockout decreased the NSC pool, decreased neurogenesis and strongly increased non-neurogenic astrocytes. We assigned a new molecular function for the conserved RNA factor Ars2 as a sequence-specific DNA binding protein, and a critical direct activator of *Sox2* during *in vivo* NSC self-renewal and multipotency. More generally, in light of excitement surrounding the role of Sox2 as a core pluripotency factor in ES and iPS cells, Ars2 may conceivably regulate stem cell self-renewal in these settings as well. This possibility is bolstered by the early embryonic arrest of *Ars2* knockout mice[28], which bears substantial resemblance to the *Sox2* knockout[29]. These connections deserve future investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Xin Lu, M, Götz, Angie Rizzino, P.M. LLedo, P. Charneau, M. Segura, P.L. Howard and S. Olejniczak for reagents. We are grateful to K. Hadjantonakis, A. Ferrer-Vaquer, J. Zhang, and Y. Ganat for assistance. U. Ruthishauser, V. Tabar and the Molecular Cytology Core Facility at MSKCC graciously shared equipment. S. R. Ferron, H. Mira, A. Joyner, H. Duan, Q. Dai, I. Farinas, and S. Shi for provided critical comments. Work in E.C.L.'s group was supported by the Burroughs Wellcome Fund, the Starr Cancer Consortium (I3-A139) and the NIH (R01-GM083300). C.A.A is a recipient of an EMBO Long-Term Fellowship (ALTF 718-2008).

References

1. Laubinger S, et al. Dual roles of the nuclear cap-binding complex and SERRATE in pre-mRNA splicing and microRNA processing in Arabidopsis thaliana. Proc Natl Acad Sci U S A. 2008; 105:8795–8800. [PubMed: 18550839]
2. Lobbes D, Rallapalli G, Schmidt DD, Martin C, Clarke J. SERRATE: a new player on the plant microRNA scene. EMBO Rep. 2006; 7:1052–1058. [PubMed: 16977334]
3. Yang L, Liu Z, Lu F, Dong A, Huang H. SERRATE is a novel nuclear regulator in primary microRNA processing in Arabidopsis. Plant J. 2006; 47:841–850. [PubMed: 16889646]
4. Gruber JJ, et al. Ars2 links the nuclear cap-binding complex to RNA interference and cell proliferation. Cell. 2009; 138:328–339. [PubMed: 19632182]
5. Sabin LR, et al. Ars2 regulates both miRNA- and siRNA- dependent silencing and suppresses RNA virus infection in Drosophila. Cell. 2009; 138:340–351. [PubMed: 19632183]
6. Fuchs E, Tumbar T, Guasch G. Socializing with the neighbors: stem cells and their niche. Cell. 2004; 116:769–778. [PubMed: 15035980]
7. Menn B, et al. Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci. 2006; 26:7907–7918. [PubMed: 16870736]
8. Whitman MC, Greer CA. Synaptic integration of adult-generated olfactory bulb granule cells: basal axodendritic centrifugal input precedes apical dendrodendritic local circuits. J Neurosci. 2007; 27:9951–9961. [PubMed: 17855609]
9. Kriegstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci. 2009; 32:149–184. [PubMed: 19555289]
10. Lledo PM, Merkle FT, Alvarez-Buylla A. Origin and function of olfactory bulb interneuron diversity. Trends Neurosci. 2008; 31:392–400. [PubMed: 18603310]

11. Doetsch F, Caille I, Lim DA, Garcia-Verdugo JM, Alvarez-Buylla A. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell. 1999; 97:703–716. [PubMed: 10380923]
12. Morshead CM, Garcia AD, Sofroniew MV, van Der Kooy D. The ablation of glial fibrillary acidic protein-positive cells from the adult central nervous system results in the loss of forebrain neural stem cells but not retinal stem cells. Eur J Neurosci. 2003; 18:76–84. [PubMed: 12859339]
13. Shi Y, Sun G, Zhao C, Stewart R. Neural stem cell self-renewal. Crit Rev Oncol Hematol. 2008; 65:43–53. [PubMed: 17644000]
14. Beckervordersandforth R, et al. In vivo fate mapping and expression analysis reveals molecular hallmarks of prospectively isolated adult neural stem cells. Cell Stem Cell. 2010; 7:744–758. [PubMed: 21112568]
15. Potten CS. Stem cells in gastrointestinal epithelium: numbers, characteristics and death. Philos Trans R Soc Lond B Biol Sci. 1998; 353:821–830. [PubMed: 9684279]
16. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell. 1990; 61:1329–1337. [PubMed: 2364430]
17. Alonso M, et al. Turning astrocytes from the rostral migratory stream into neurons: a role for the olfactory sensory organ. J Neurosci. 2008; 28:11089–11102. [PubMed: 18945916]
18. Raponi E, et al. S100B expression defines a state in which GFAP-expressing cells lose their neural stem cell potential and acquire a more mature developmental stage. Glia. 2007; 55:165–177. [PubMed: 17078026]
19. Shi Y, et al. Expression and function of orphan nuclear receptor TLX in adult neural stem cells. Nature. 2004; 427:78–83. [PubMed: 14702088]
20. Zencak D, et al. Bmi1 loss produces an increase in astroglial cells and a decrease in neural stem cell population and proliferation. J Neurosci. 2005; 25:5774–5783. [PubMed: 15958744]
21. Zhuo L, et al. hGFAP-cre transgenic mice for manipulation of glial and neuronal function in vivo. Genesis. 2001; 31:85–94. [PubMed: 11668683]
22. Ohtsuka T, Sakamoto M, Guillemot F, Kageyama R. Roles of the basic helix-loop-helix genes Hes1 and Hes5 in expansion of neural stem cells of the developing brain. J Biol Chem. 2001; 276:30467–30474. [PubMed: 11399758]
23. Hitoshi S, et al. Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev. 2002; 16:846–858. [PubMed: 11937492]
24. Ferri AL, et al. Sox2 deficiency causes neurodegeneration and impaired neurogenesis in the adult mouse brain. Development. 2004; 131:3805–3819. [PubMed: 15240551]
25. Suh H, et al. In vivo fate analysis reveals the multipotent and self-renewal capacities of Sox2+ neural stem cells in the adult hippocampus. Cell Stem Cell. 2007; 1:515–528. [PubMed: 18371391]
26. Favaro R, et al. Hippocampal development and neural stem cell maintenance require Sox2-dependent regulation of Shh. Nat Neurosci. 2009; 12:1248–1256. [PubMed: 19734891]
27. Ohtsuka T, et al. Visualization of embryonic neural stem cells using Hes promoters in transgenic mice. Mol Cell Neurosci. 2006; 31:109–122. [PubMed: 16214363]
28. Wilson MD, et al. ARS2 is a conserved eukaryotic gene essential for early mammalian development. Mol Cell Biol. 2008; 28:1503–1514. [PubMed: 18086880]
29. Avilion AA, et al. Multipotent cell lineages in early mouse development depend on SOX2 function. Genes Dev. 2003; 17:126–140. [PubMed: 12514105]
30. Andreu-Agullo C, Morante-Redolat JM, Delgado AC, Farinas I. Vascular niche factor PEDF modulates Notch-dependent stemness in the adult subependymal zone. Nat Neurosci. 2009; 12:1514–1523. [PubMed: 19898467]

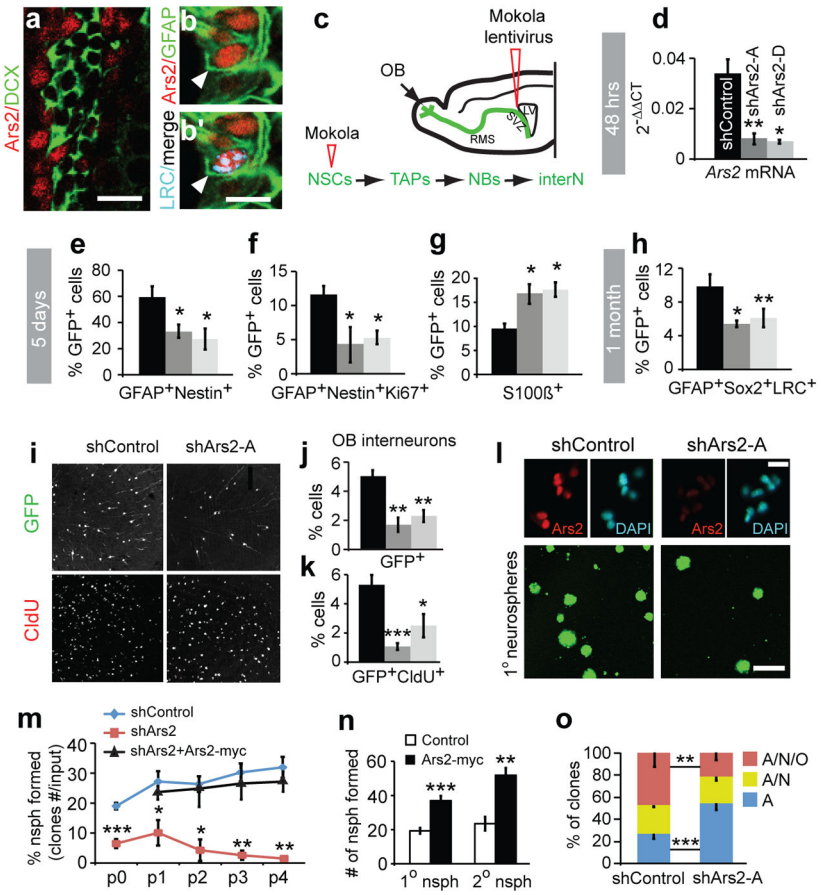

Figure 1. Ars2 maintains neural stem cells in the adult SVZ

In the adult SVZ, Ars2 (red) is not expressed by DCX$^+$ (green) neuroblasts (a), but colocalizes with GFAP$^+$ (green), CldU-retaining (blue) neural stem cells (NSCs) (b, b'). (c) Experimental protocol. (d) Validation of *Ars2* knockdown (n=3 animals per condition). (e) *Ars2* knockdown SVZs exhibited reduced GFAP$^+$ Nestin$^+$ NSCs, reduced NSC proliferation (f), and increased S100ß+ mature astrocytes (g); n=3 animals per condition. (h) *Ars2* knockdown reduced label-retaining cells (LRCs) (n=3–7 animals per condition). (i) OB sections stained for GFP (green), CldU (red) and DAPI (blue). (j) Percentage of GFP$^+$ cells per OB slice (n=7–11 animals per condition). (k) Reduced newborn neurons after *Ars2* knockdown (n=7-11 animals per condition). (l) Immunocytochemistry for Ars2 (red) and DAPI (aqua) (top) and GFP$^+$ transduced primary neurospheres (bottom). (m) Longterm self-renewal assay (n= 4 cultures per condition). (n) *In vivo* Ars2 overexpression increased neurosphere formation (n= 4 cultures per condition). (o) Ars2 deficiency reduced NSC multipotency (n= 4 cultures per condition). *, $P<0.05$, **, $P<0.01$, ***, $P<0.005$. Errors bars show s.e.m. Table S2 provides details of numbers of scored cells. Scale bars: a, b, 10 μm; j, 100 μm; m, 200 μm.

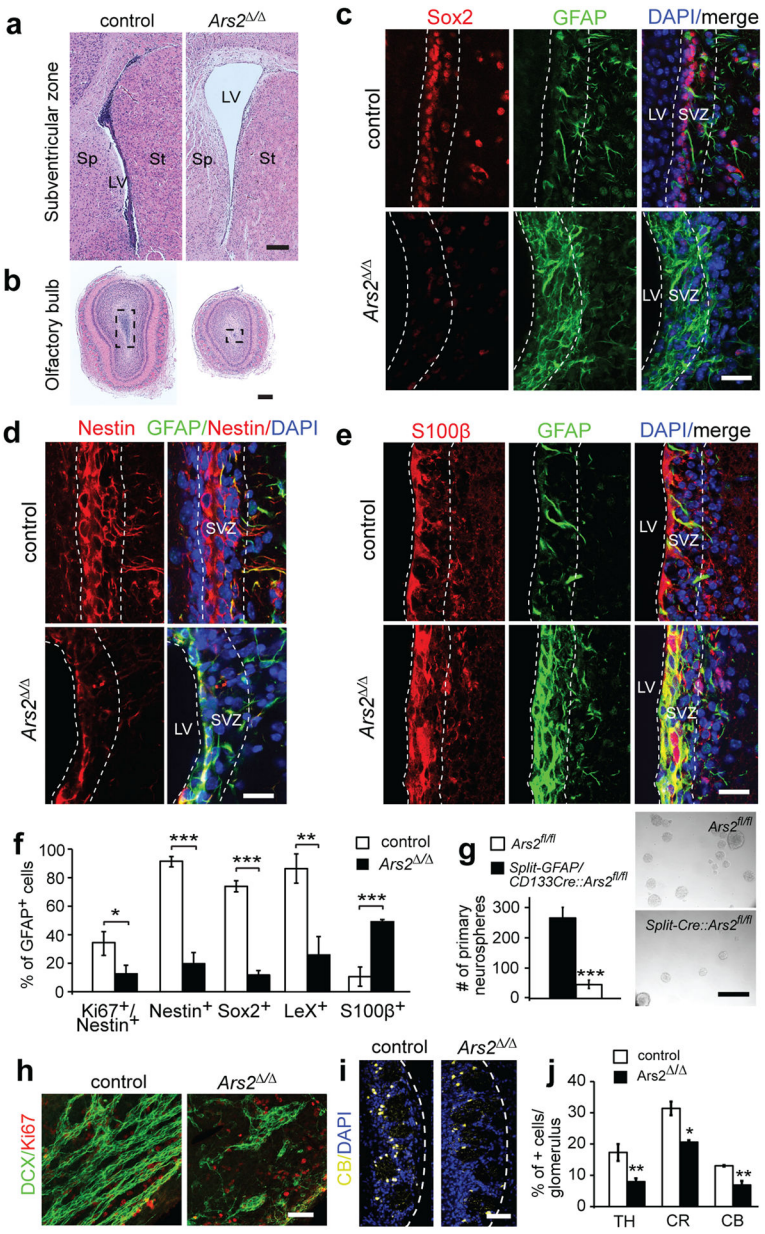

Figure 2. Ars2 regulates postnatal neurogenesis

(a, b) Coronal sections from P15 SVZ and OB of control and *Ars2*$^{\Delta/\Delta}$ mice stained with hematoxylin-eosin. SVZ deletion of *Ars2* depleted NSCs and induced astrogliosis, as evidenced by loss of Sox2^{+} (red) and accumulation of GFAP^{+} (green) cells (c), loss of Nestin^{+} (red) cells (d), and increased S100ß$^{+}$ (red) cells (e); DAPI is in blue. (f) Reduced number and proliferation of NSCs and elevation of mature astrocytes in *Ars2*$^{\Delta/\Delta}$ mice. (g) Strong reduction of primary neurospheres from *GFAP/CD133-Cre::Ars2*$^{fl/fl}$ electroporated pups. (h) Wholemount immunostaining for DCX (green) and Ki67 (red). (i) OB coronal sections immunostained for CB (green) and DAPI (blue). (j) Quantification of interneuron reduction in control and *Ars2*$^{\Delta/\Delta}$ mice. *, $P<0.05$, **, $P<0.01$, ***, $P<0.005$. Error bars

show s.e.m from 6 animals per genotype/condition. Scale bars: a, b, 100 μm; c, d, e, 30 μm; f, g, 50 μm.

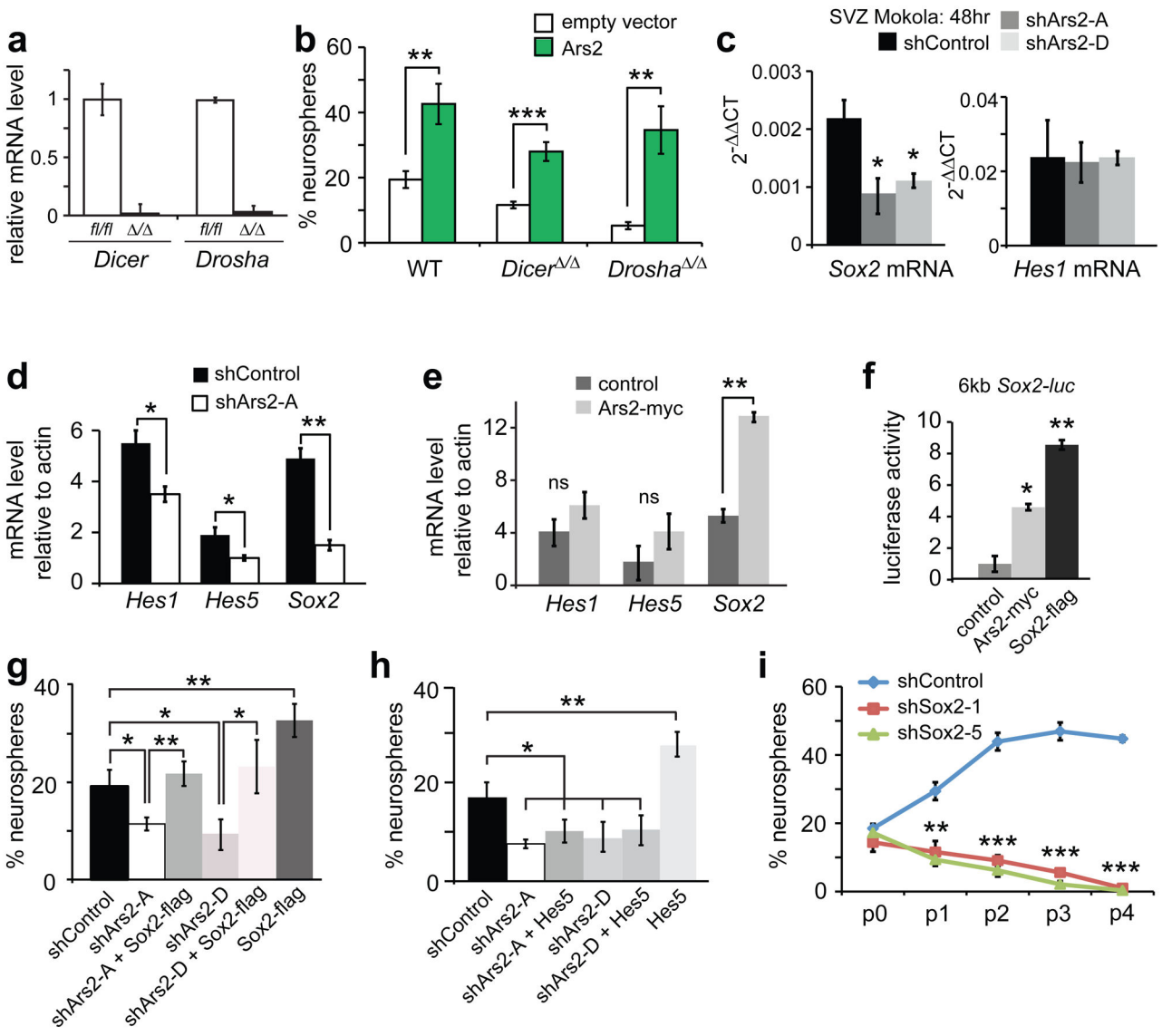

Figure 3. Ars2 acts independently of miRNA pathway to promote NSC self-renewal via *Sox2*

(a) Absence of *Dicer* and *Drosha* in floxed NSC cultures following Cre treatment (n=2 experiments per condition). (b) Ectopic Ars2 promoted NSC self-renewal in *Dicer* and *Drosha*-deleted NSCs (n=3 experiments per condition). (c) qPCR measurements from purified GFP$^+$ cells 48 h post-infection (n=3). (d) qPCR measurements of self-renewal genes from primary infected GFP$^+$ NSCs (n=3). (e) Overexpression of Ars2 increased *Sox2* mRNA (n=3). (f) Both Ars2 and Sox2 activate a *Sox2* transcriptional reporter (n=3). (g,h) Percentage of neurospheres formed from NSCs infected with the indicated constructs (n=3). (i) Longterm self-renewal assay shows that Sox2 is required for NSC self-renewing divisions (n=4). *, $P<0.05$, **, $P<0.01$, ***, $P<0.005$. Error bars show s.e.m; ns, not significant.

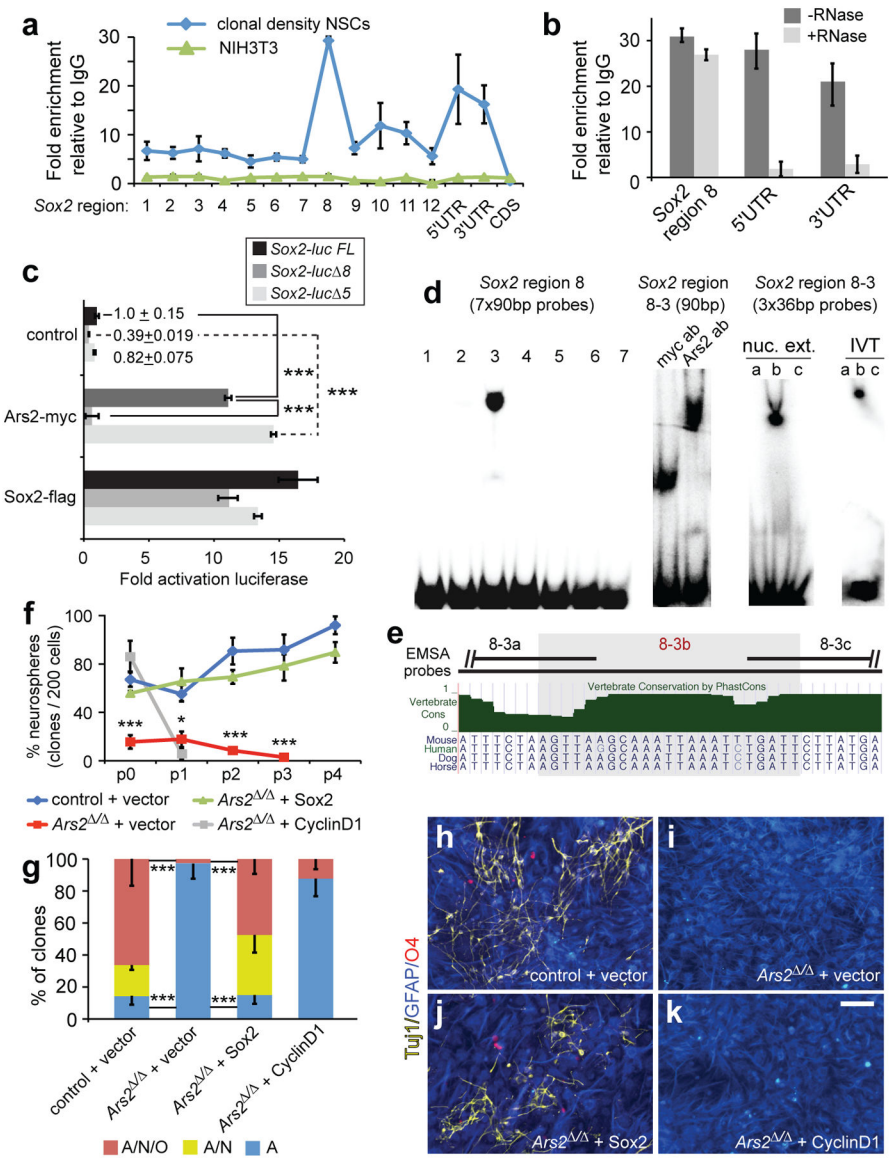

Figure 4. Ars2 directly activates transcription of *Sox2* to mediate NSC self-renewal and multipotency

ChIP of Ars2 to the *Sox2* locus (n=4 independent experiments). (b) Ars2 binding to *Sox2* promoter region 8 is RNA independent (n=2). (c) Ars2 requires region 8 to activate *Sox2–luc* (n=4). (d) Gel-shifts of the Ars2 binding site in the *Sox2* promoter using nuclear extract or in vitro-translated (IVT) Ars2. (e) Conservation of the Ars2-binding site in *Sox2*. (f) Long-term self-renewal assay from NSCs isolated from control and $Ars2^{\Delta/\Delta}$ mice electroporated *in vivo* with the indicated constructs (n=6 animals per condition). (g) $Ars2^{\Delta/\Delta}$ defect in multipotency can be rescued by *in vivo* expression of Sox2 (n=6 animals per condition). (h-k) Differentiated NSC colonies stained for GFAP (blue), Tuj1 (yellow), and O4 (pink). *, $P<0.05$, ***, $P<0.005$. Error bars show s.e.m.