Dear Editor,

Neural progenitor cells (NPCs) have proven potential to facilitate mechanistic studies of neurological disorders in vitro, as well as the discovery of new medicines. In addition, NPCs have been proposed as promising cell sources for cell replacement therapy of neurological diseases (Liu et al., 2011). For these areas of study, experimental animals are indispensable models. Among the possible animal species, pigs are advantageous compared to rodents because of their physiological and anatomical similarities to humans (Lind et al., 2007). Despite the shown advantages of porcine models in different fields, their applications are significantly restricted due to the limited access of porcine cells, including NPCs. To date, encouraging breakthroughs have been made in obtaining NPCs from a series of species by different methods, including primary cell isolation from tissues, differentiation from pluripotent stem cells, and direct reprogramming from other somatic cells (Vierbuchen et al., 2010; Giorgetti et al., 2012; Lujan et al., 2012; Thier et al., 2012; Zhang et al., 2013).

Here, we report the successful generation of induced porcine NPCs (ipNPCs) from porcine fetal fibroblasts (PFFs) (Fig. S1A, upper panel). Using our method, functional ipNPCs can be readily obtained via direct cell reprogramming without going through a pluripotent state. We show that ipNPCs retain the ability for long-term culture and efficient neural differentiation in vitro. Moreover, ipNPCs could effectively integrate into the local neural network after cell transplantation in vivo.

In order to initiate the direct cell reprogramming, we sought to prime PFFs using non-integrative episomal vectors expressing reprogramming factors (Oct4, Sox2, Klf4, Lin28, and L-Myc) (Li et al., 2011) and then subjected the cells to human embryonic stem cell-amenable culture conditions (Liu et al., 2012b). For these areas of study, experimental animals are indispensable models. Among the possible animal species, pigs are advantageous compared to rodents because of their physiological and anatomical similarities to humans (Lind et al., 2007). Despite the shown advantages of porcine models in different fields, their applications are significantly restricted due to the limited access of porcine cells, including NPCs. To date, encouraging breakthroughs have been made in obtaining NPCs from a series of species by different methods, including primary cell isolation from tissues, differentiation from pluripotent stem cells, and direct reprogramming from other somatic cells (Vierbuchen et al., 2010; Giorgetti et al., 2012; Lujan et al., 2012; Thier et al., 2012; Zhang et al., 2013).

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Next, in order to assess the neural differentiation potency of ipNPCs, in vitro spontaneous neural differentiation was performed. After three weeks of culturing in spontaneous neural differentiation medium (NDM), the cell bodies of most cells were clustered and long neurites protruded.

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Generation of induced neural progenitor cells from porcine fibroblasts

**A** DNA, **B** Nestin, **C** NCAM, Nestin, Pax6

**D** DNA, **E** Tuj1, GFAP, MBP

**F** Current (pA) vs. Time (ms)

**G** DNA, GFP, DNA/GFP
Immunofluorescence staining showed that the majority of differentiated cells were Tuj1 positive neurons while GFAP positive glial cells were also present (Fig. 1D). Correspondingly, the mRNA levels of Tuj1 and GFAP significantly increased after ipNPCs spontaneous differentiation (Fig. 1E). In addition, up-regulation of myelin basic protein (MBP), which is enriched in oligodendrocytes, was also observed by qPCR (Fig. 1E). In addition, up-regulation of myelin basic protein (MBP), which is enriched in oligodendrocytes, was also observed by qPCR (Fig. 1E). In addition, up-regulation of myelin basic protein (MBP), which is enriched in oligodendrocytes, was also observed by qPCR (Fig. 1E). In addition, up-regulation of myelin basic protein (MBP), which is enriched in oligodendrocytes, was also observed by qPCR (Fig. 1E). 

Finally, we explored the neural differentiation potency of ipNPCs in vivo. The ipNPCs were labeled with GFP by lentiviral vectors, and then transplanted into the dentate gyrus (DG) of NOD/SCID mice. Four weeks after transplantation, brains of recipient mice were sectioned and analyzed. We found that most GFP-labeled cells were localized in the DG region, indicating a robust survival of ipNPCs in vivo (Fig. 1G). We further observed GFP positive neurons with complex branching morphology that were present at neighboring zones of the DG region (Fig. 1G), which suggested that ipNPCs were able to effectively integrate into the local neural network after transplantation. No teratoma formation was observed in any mouse brains examined, which further supports the safety of ipNPCs in vivo. (Data not shown)

In summary, we report here a new strategy to obtain integration-free functional porcine neural progenitor cells by direct reprogramming of porcine fetal fibroblasts in vitro. For the first time, porcine neural progenitor cells were directly generated from somatic cells, and functionally characterized both in vitro and in vivo. Considering the importance of pigs as a model species, a sufficient supply of functional porcine neural progenitor cells are of great interest in translational medicine studies of neuroscience. However, the difficulties to establish porcine pluripotent stem cells including embryonic stem cells and integration-free iPSCs limit the production of porcine NPCs through traditional cell differentiation approaches (Wu et al., 2009; Rasmussen et al., 2011; Liu et al., 2012c; Fan et al., 2013). Therefore, how to obtain porcine NPCs directly from the somatic cells is attracting a lot of attention in the field. Similar to many other direct reprogramming methods, our strategy bypassed obstacles in establishing porcine pluripotent stem cells. Moreover, our method provided a robust and efficient way of generating porcine NPCs with low risk of tumor formation. To our knowledge, this is the first attempt to direct reprogram somatic cells into neural progenitor cells using the porcine species. As a promising species of model animals, the ipNPCs generated in our study may provide an exciting tool to bridge the present gaps in neuroscience studies between rodents and humans.
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All institutional and national guidelines for the care and use of laboratory animals were followed.

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