Thank you for providing the reviewer's comments on our manuscript entitled "Using Transcriptional Factors for In vitro Direct Reprogramming of Neuron". We thanked the reviewers for their helpful comments. Please find our response to each specific comment highlighted in yellow below. Modifications within the revised manuscript are indicated as tracked changes.

Reviewer #1: This is a nice written review paper about current models used for the reprogramming of neurons. Authors have clearly described the basic mechanisms of neural lineage regulation and transcription factors required for the different neural specifications. Review is well written, and can be easily understood by general reader. The method of computational predictions seems to be a promising approach for the neuron reprogramming. As suggestion for further improvement, I propose that Author provide a small paragraph about clinical perspectives of neuron reprogramming and challenges for clinical application of this method.

Thank you for your helpful suggestion. As request, in the revised manuscript we added a new section to discuss the clinical challenges and application for neuron reprogramming. The following are added (Part 5, page 10):

5. Clinical perspectives of *in vitro* neuronal reprogramming and challenges for clinical application

Allogeneic transplantation represents a promising cell therapy approach to replace neurons lost by injury or neurodegenerative disease, such as Parkinson's disease (84). However, there are two major challenges related to transplantation: 1) the shortage of donor tissue for transplantation, and 2) immuno-rejection issues of the grafted tissue. Development of stem cell and cell reprogramming technology to generate patient-specific cells *in vitro* would be critical to overcome these hurdles. Cell therapies strategy using pluripotent stem cells have been extensively highlighted previously (85–87). In comparison, notably direct reprogramming bypasses the pluripotent stem cell state, thus this is potentially a faster and more cost-effective approach to generate neurons *in vitro*, with less tumorigenic risks compared to pluripotent stem cell strategy. Moreover, there is also the exciting opportunity to combine with gene therapy to correct disease-causing mutation(s) in the cells *in vitro*, prior to transplantation to patients to treat hereditary neurodegenerative diseases.

To facilitate clinical translation of direct reprogramming technology, it is critical to develop robust reprogramming protocol to generate target cells with high purity and efficiency. Optimization of transcription factors for direct reprogramming, as well as improved method for gene delivery would be key to improving the reprogramming efficiency. Flow cytometry or magnetic-activated cell sorting can be used to enrich the purity of the target cell type prior to transplantation. For clinical applications, cells derived by direct reprogramming should be produced under good manufacturing practices (GMP) conditions. To ensure the quality of the reprogrammed cells, it is important that the derived cells are extensively characterised for marker expression and functional studies, and screened to ensure the derived cells have a normal karyotype. In the latter case, the use of non-integrative methods for direct reprogramming is desirable, such as Sendai viruses or episomal vectors.

Reviewer #2: This review is worth publishing because of the importance of the aim: transcription factors that promote direct reprogramming of specific neuronal subtypes with particular focus on glutamatergic, GABAergic, dopaminergic, sensory and retinal neurons. Furthermore, we will discuss the potential. Furthermore, the manuscript is written well. Accept

Thank you for your feedback. We are pleased to hear your positive comments.

Reviewer #3: The MS by El Wazan and Collaborators is well written and conveys useful and timely informations. I would suggest to add some figures or diagrams to better illustrate some of the key issues described, e.g. specification vs. differentiation factors, novel computational approaches, ecc.

Thank you for your helpful suggestion. As suggested, in the revised manuscript we added a new figure (Figure 1) that summarizes the transcription factor combinations used for direct reprogramming into specific neuron subtypes in human and mouse. In this figure we highlighted the combination of specification and differentiation factors used, as well as the starting cell type used for previous direct reprogramming experiments.

Editor-in-chief:

EIC Notes: To enhance the clarity of the manuscript, the authors need to address the following two issues: (1) The authors need to specify what they mean in different color illustrations, including blue, green, yellow, purple, grey, and brown. If no meaning in color, they should have stated so – the color-coded illustration is of distraction. Their statement: "Figure 1 Transcription factor combination used for in vitro direct reprogramming to specific neuron subtypes, including sensory neurons, GABAergic neurons, glutamatergic neurons, dopaminergic neurons, photoreceptors, and retinal ganglion cells. Top and bottom panel illustrate direct reprogramming in human and mouse cells, respectively. The numbers indicate the starting cell type for reprogramming: (1) Fibroblasts; (2) Müller glia; (3) iris cells; (4) astrocytes. The specification factors neurogenin 2 and achaete-scute homolog are depicted with bigger circles to highlight its importance in neural fate induction. NGN2: Neurogenin 2; ASC Y: Achaete-scute homolog; RGC: Retinal ganglion cells." (2) Figure 1, as the current stand, is confusing about the mouse and human origin. They should create two panels: one for mouse and the other for human. A flow chart should be used to illustrate the input cells to the destiny cell types.

Response:

We thank the editor for this constructive feedback. We have remade Figure 1 to address these comments (figure below). In particular:

1. The new figure does not use different colour boxes to avoid confusion.
2. We have separate the studies done on human and mouse as 2 different figures as requested (figure 1A and 1B). Also we have remade the figure as flow chart to show starting and target cell types for reprogramming.

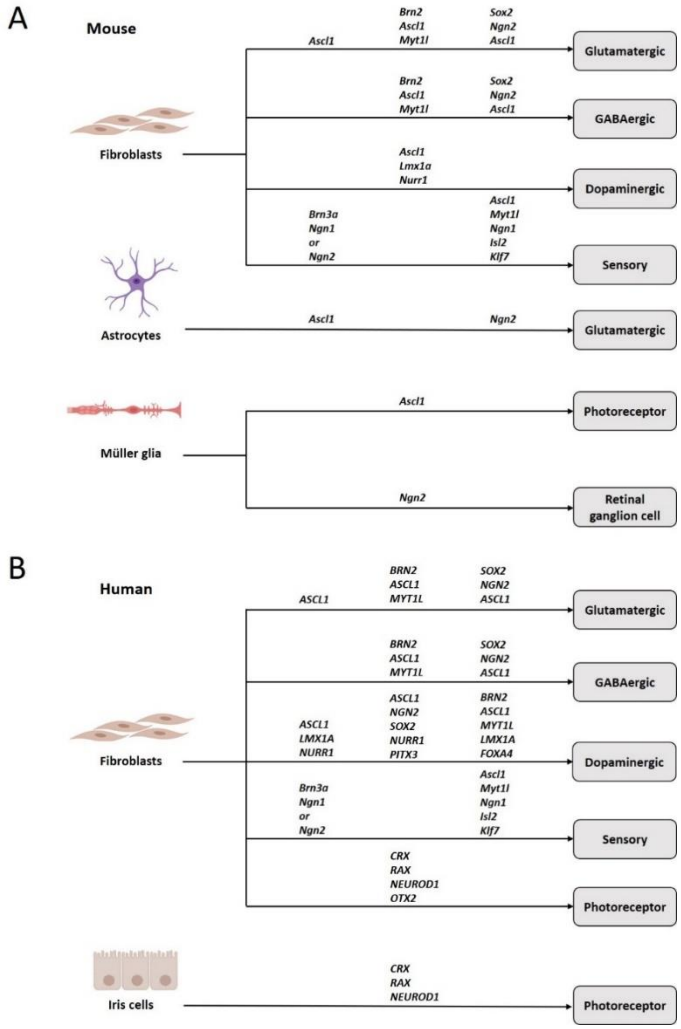

Figure 1: Transcription factor combination used for in vitro direct reprogramming to specific neuron subtypes in A) mouse and B) human, including sensory neurons, GABAergic neurons, glutamatergic neurons, dopaminergic neurons, photoreceptors and retinal ganglion cells.