

ZEB1 activity in stem cells: The role in neuroinflammatory processes of multiple sclerosis (MS)

Elham Poonaki 1,2, Ulf Dietrich Kahlert 3, Ali Gorji 2* and Sven G. Meuth 1*

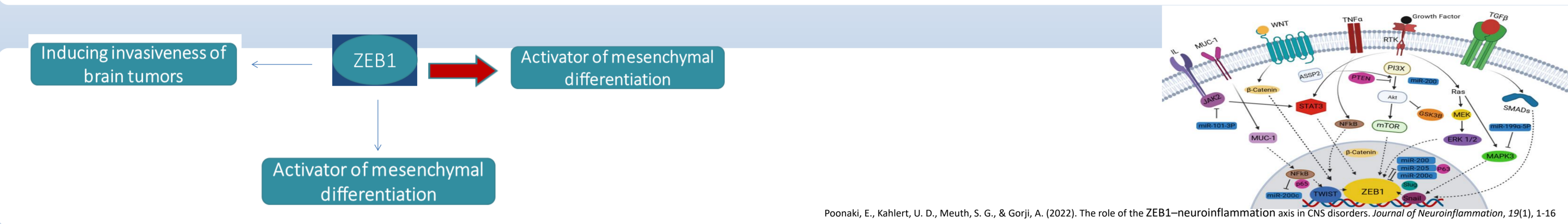
1 Department of Neurology, Faculty of Medicine, Heinrich-Heine-University, Düsseldorf, Germany.

2 Epilepsy Research Center, Department of Neurosurgery, Westfälische Wilhelms-Universität Münster

3 Molecular and Experimental Surgery, Faculty of Medicine, University Clinic for General-, Visceral-, Vascular- and Transplantation Surgery, Otto-Von-Guericke-University,

Purpose

Stem cell therapy is one of the main remedies for curing some chronic neurological diseases such as multiple sclerosis (MS). However, how to overcome nerve cell transplant failure after treatment remains controversial. The key role of zinc finger E-box binding homeobox 1 (ZEB1) plays a well-known role in both inflammatory signaling and regulation of epithelial-mesenchymal transition (EMT). Since neuroinflammation is one of the main hypotheses for the failure of stem cell transplantation in MS, this work examined the inflammatory response of neural stem cells (NSCs) by downregulating the ZEB1 gene. First, we aim to evaluate ZEB1 expression in neural stem cells (NSCs) isolated from the subventricular zone (SVZ) of rat brains and differentiated into mature myelinating oligodendrocytes (OLs) using our standard methodology. We then downregulated the ZEB1 gene in neural stem cells and examined its effect on migration and inflammation.



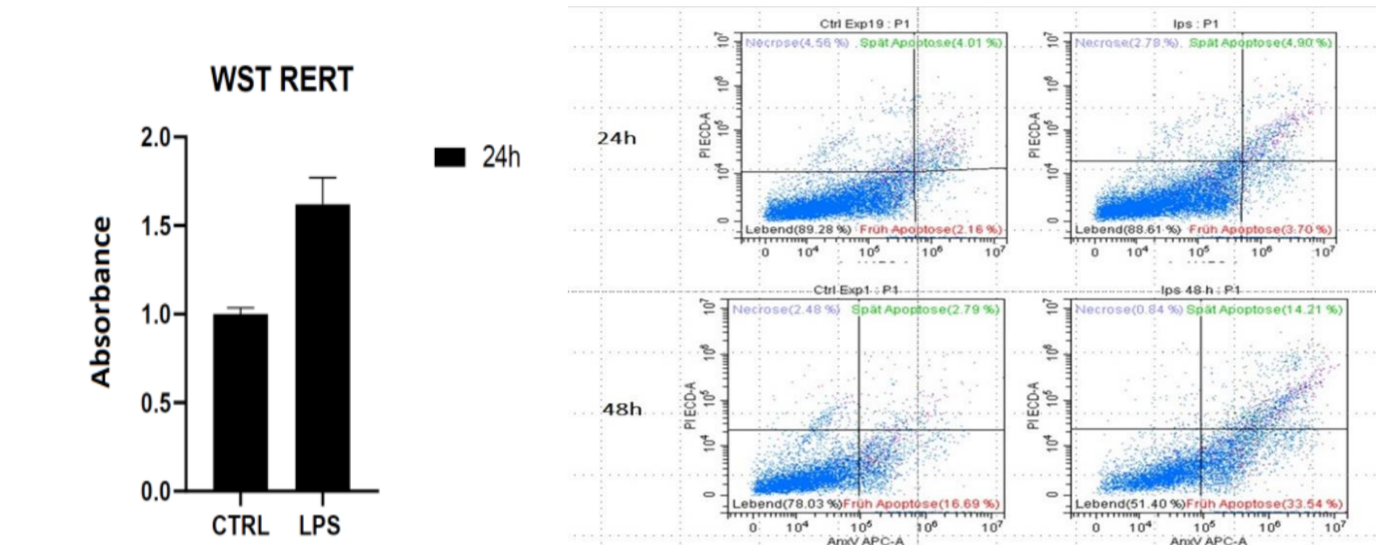
Poonaki, E., Kahlert, U. D., Meuth, S. G., & Gorji, A. (2022). The role of the ZEB1–neuroinflammation axis in CNS disorders. *Journal of Neuroinflammation*, 19(1), 1-16

Methods

The extraction and characterization of NSCs with nestin and SOX2 as the main markers of NSCs and their differentiation into oligodendrocytes with their specific markers such as O4, SOX10 and MOG as well as the assessment of ZEB1 expression were carried out. Subsequently, after transfection of siRNA onto stem cells, neuroinflammation and migration of stem cells were examined using immunocytochemistry (ICC), qPCR, wound healing, ROS, and colony formation in a simulated inflammatory state using LPS soup from brain microglia.

Treatment with LPS (100ng/ml)

WST did not show significant toxicity after 24 hours with LPS soup treatment extracted from macroglia. Evaluation of Annexin PI after 24h and 48h treatment of NSCs with LPS. The graph also indicates significant movements showing inflammation in terms of early and late apoptosis.

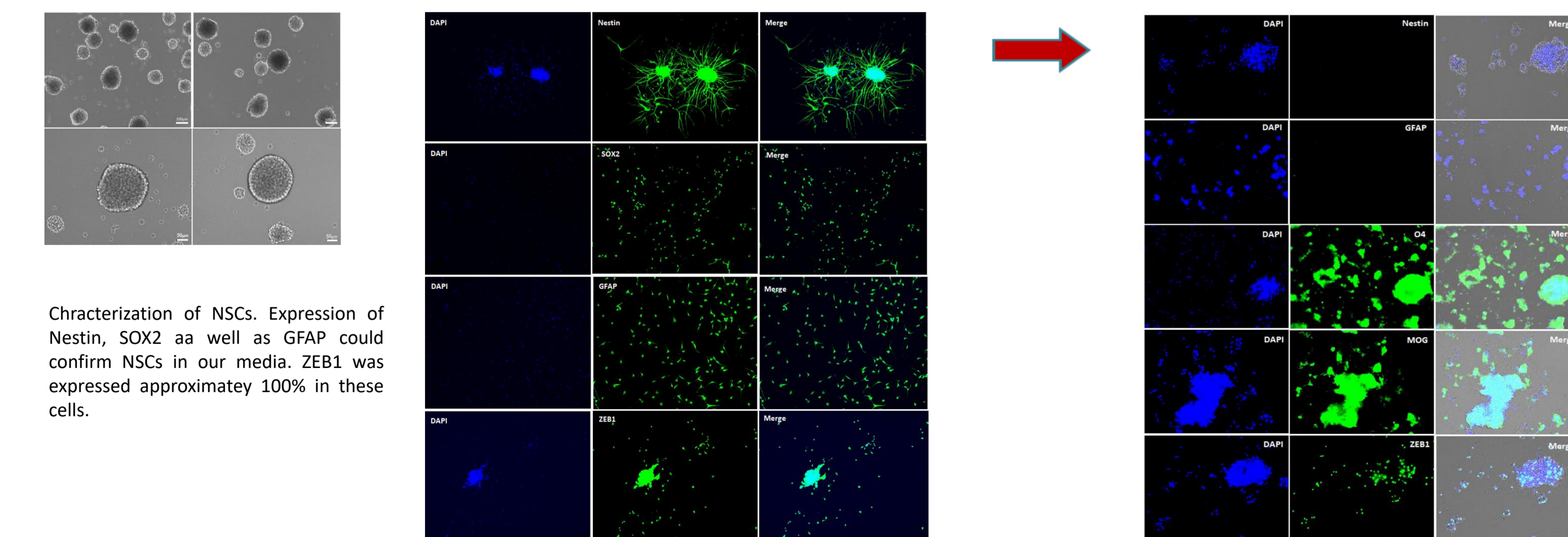


Results

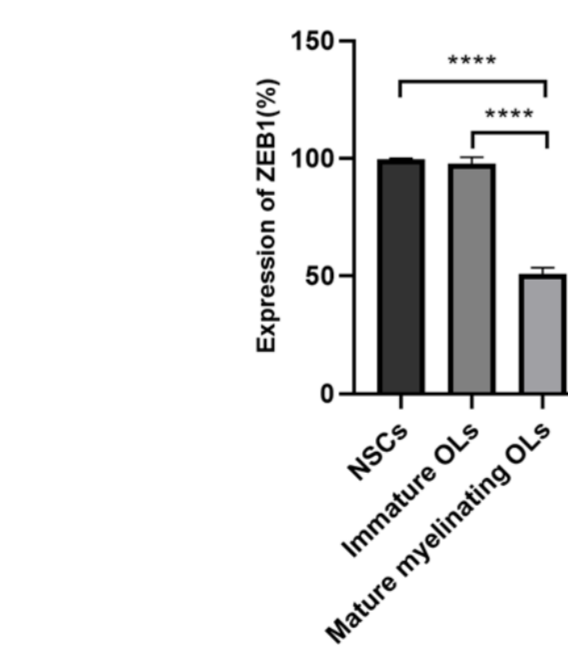
Differentiation of NSCs to Mature myelinating Oligodendrocytes(OLs) and evaluation ZEB1 expression

NSCs → OPCs → Premyelinating Oligodendrocyte → Mature Myelinated Oligodendrocyte

Markers: Nestin, SOX2, SOX10, O4, O4, MOG

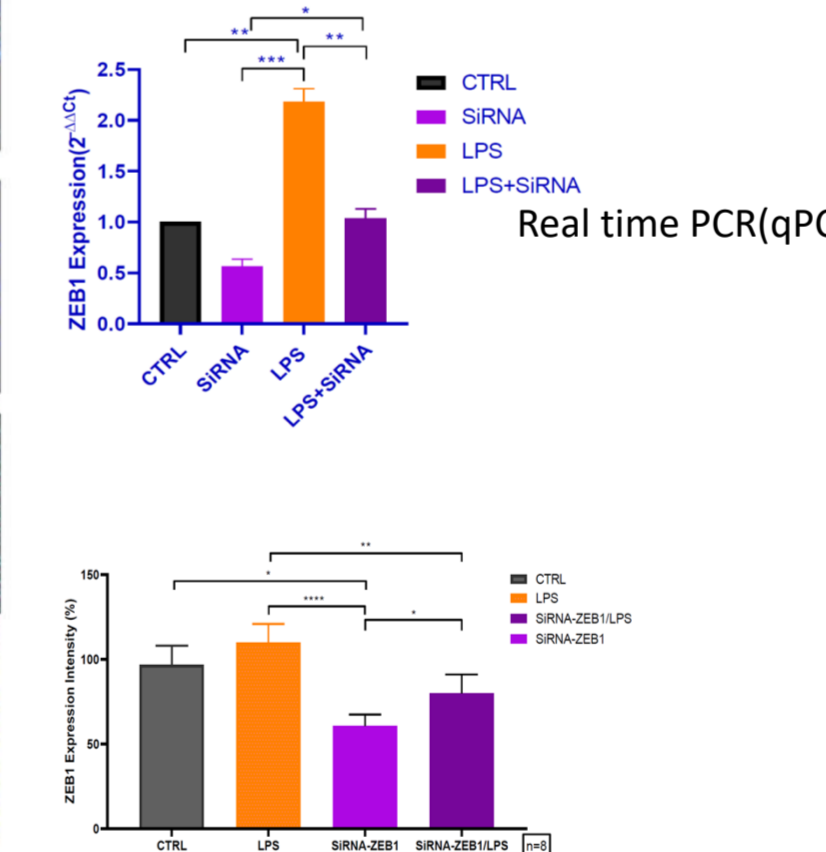
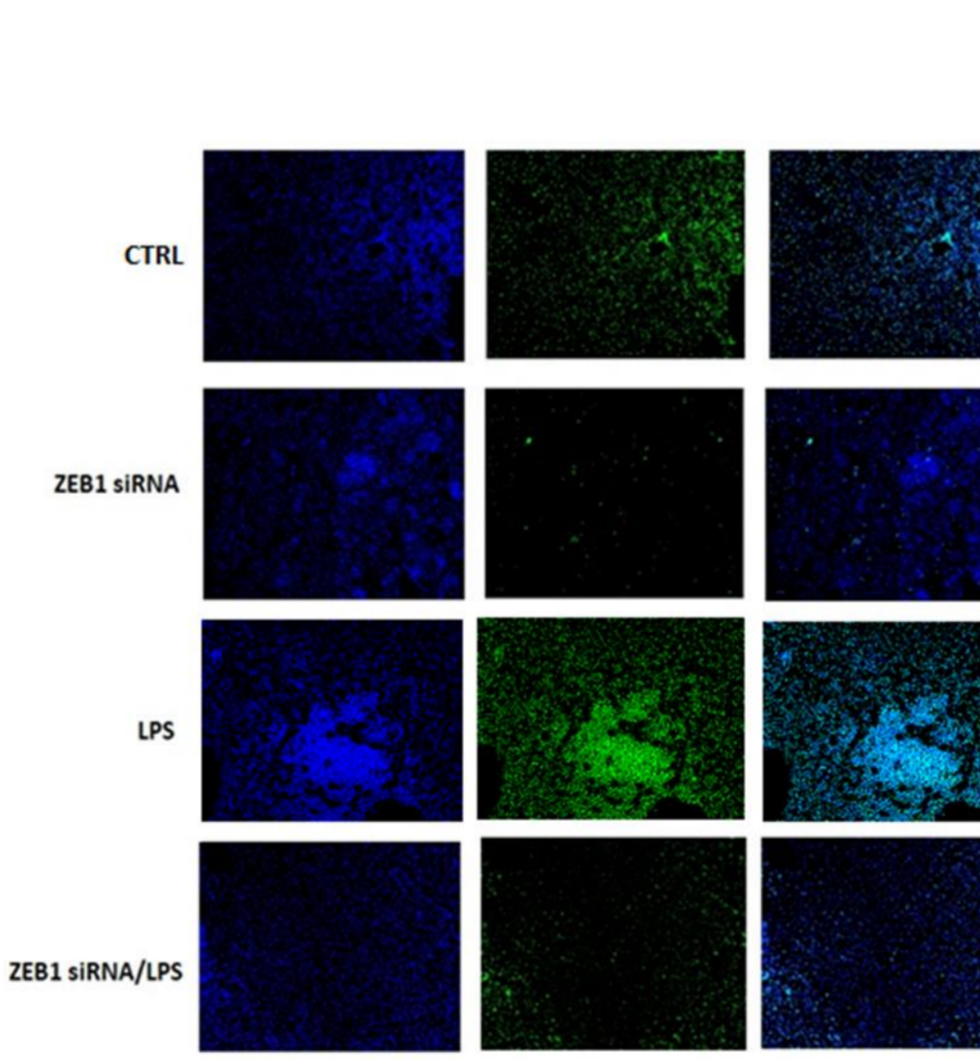


Characterization of NSCs. Expression of Nestin, SOX2 as well as GFAP could confirm NSCs in our media. ZEB1 was expressed approximately 100% in these cells.

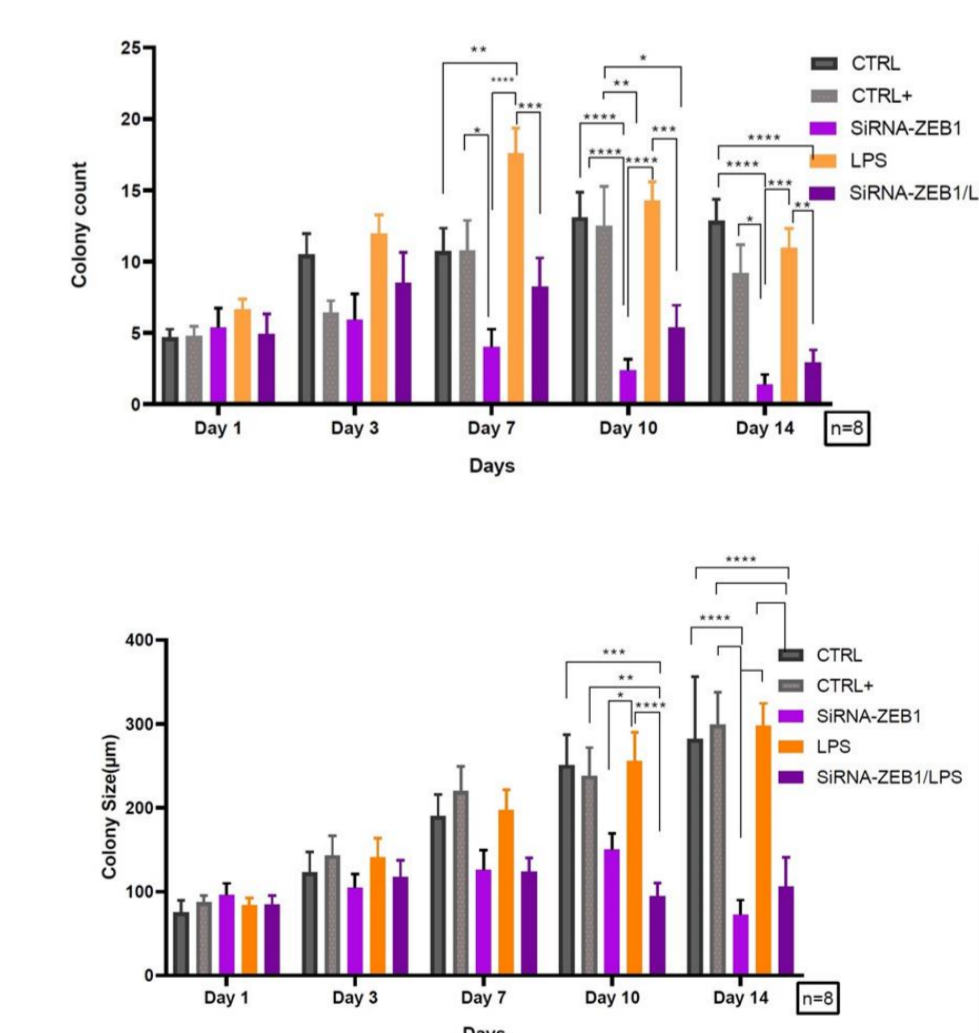
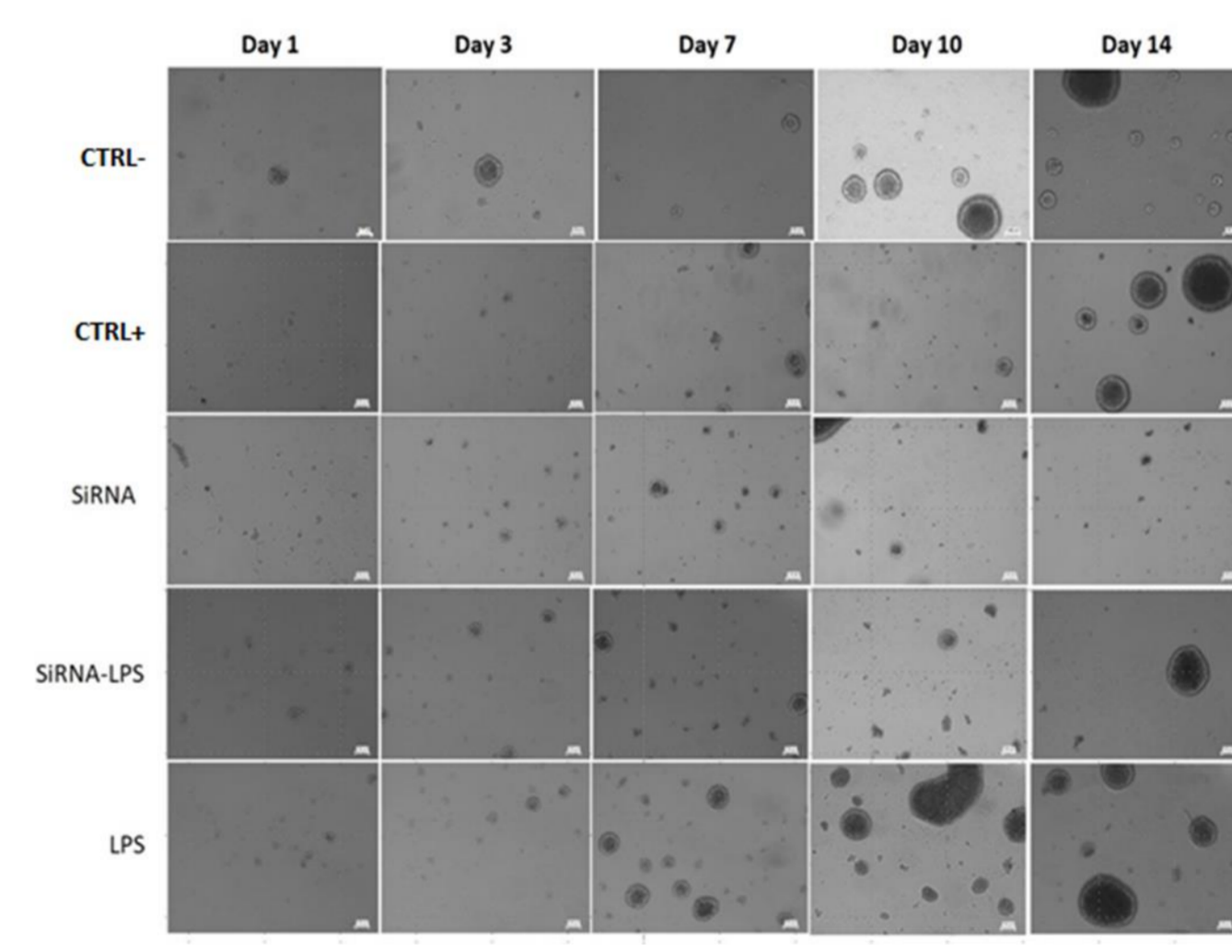


Characterization of mature myelinating oligodendrocytes (OLs). From up to down: Expression of Nestin, GFAP, O4, MOG and ZEB1.

Immunocytochemistry and qPCR after modulating ZEB1 in NSCs



Colony formation Assay



Conclusion

ZEB1 was expressed at all stages of transformation of NSCs into premyelinating oligodendrocytes. However, the expression of ZEB1 decreases after differentiation into mature myelinating oligodendrocytes. In summary, the results showed that silencing of the ZEB1 gene significantly affected the expression of neuroinflammatory cytokines, colony formation and migration in neural stem cells. In summary, although targeting ZEB1 may be useful for neural stem cell localization, further research is needed to fully understand the role of ZEB1.

Reactive Oxygen Species (ROS) and wound healing assay

