

The roles of iron regulatory proteins 1 and 2 in neuronal differentiation and iron-mediated cell death

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Abstract

Iron Regulatory Proteins 1 and 2 (IRP1 and IRP2) are key regulators of cellular iron levels. Iron is essential for proper brain development and function but can lead to cellular damage if not properly regulated. In order to study the effects of reduced expression of IRP1 and IRP2 on neuronal health and neurodegeneration, we are using mouse neurons that have been transfected with shRNA to specifically knockdown IRP1 or IRP2. Mouse neurons are well-studied and share many key cellular pathways with human neurons, making them an appropriate model to study the effects of IRP1 and IRP2 knockdown. We will begin to investigate the effect of the knockdowns on the mouse neurons through proliferation assays and differentiation assays. These experiments will reveal how the knockdown of IRP1 and IRP2 affect neuronal growth, maturation, and development compared to healthy control cells. Understanding these processes is incredibly important for humans as iron dysregulation can lead to neurodegenerative diseases such as Alzheimer's.

Approach

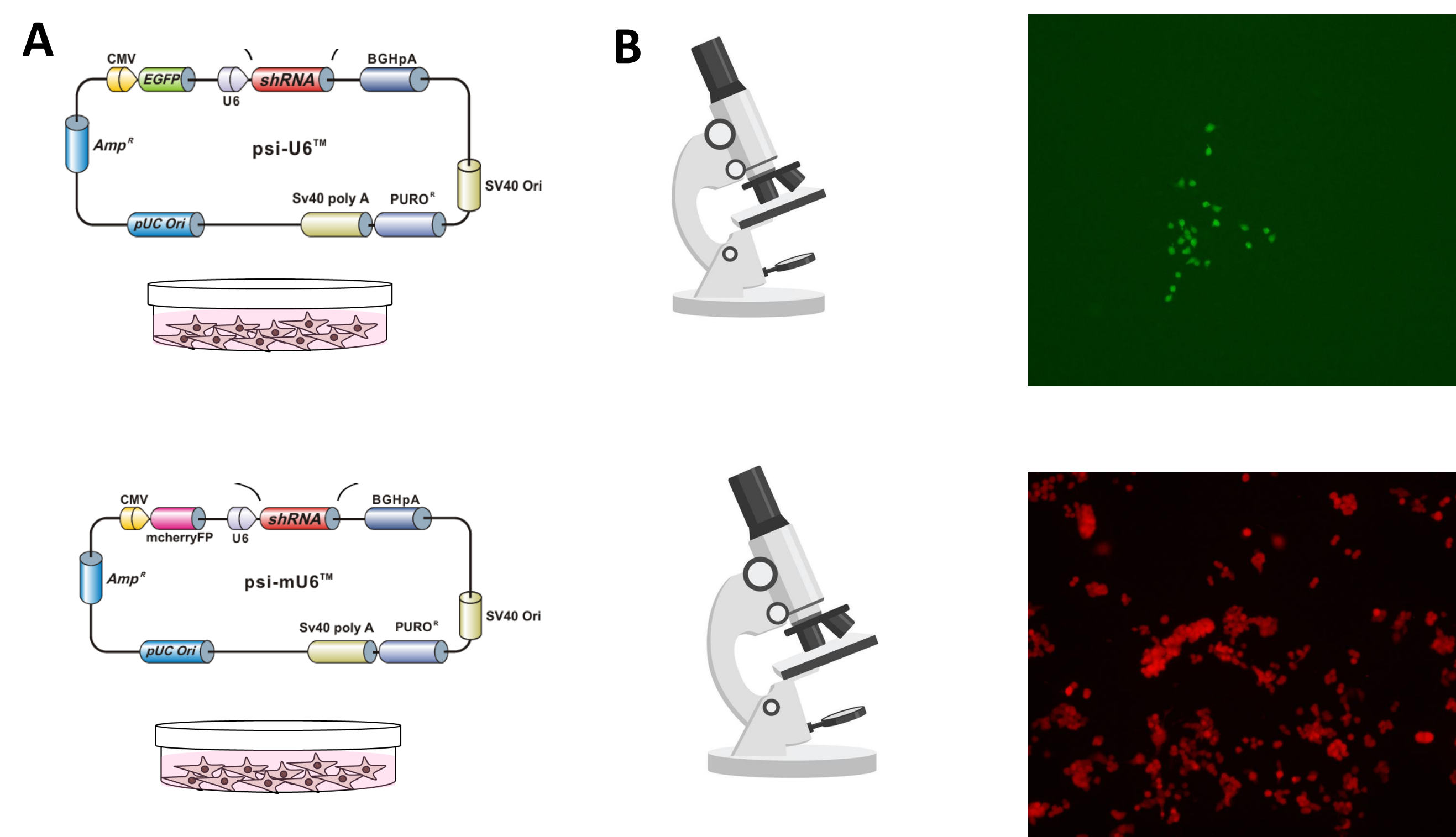


Figure 1. Experimental design for plasmid transfection and validation. (A) Two cell lines were generated by transfecting N2A cells with plasmids containing shRNA targeting IRP1 (green fluorescent reporter) and IRP2 (red fluorescent reporter), each carrying a puromycin resistance gene. Cells were cultured in DMEM supplemented with puromycin to maintain selection. (B) Following transfection, cells were viewed under a fluorescent microscope to confirm successful plasmid uptake.

Validation of model

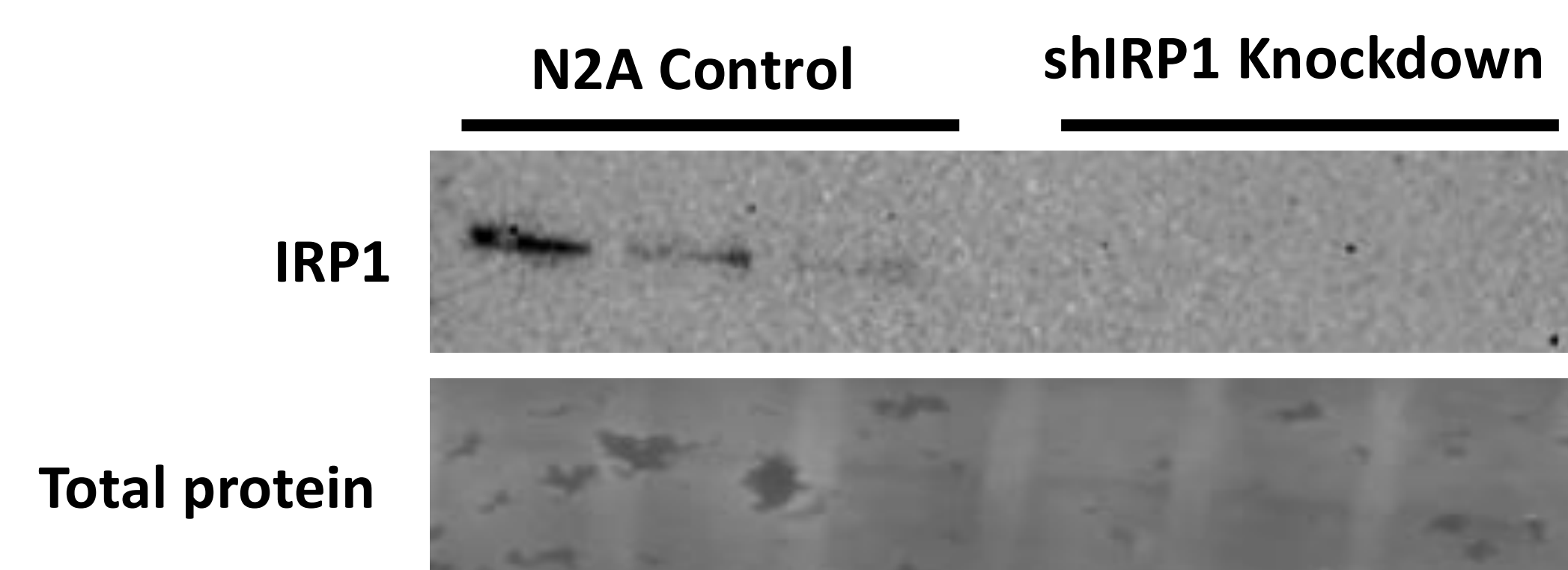


Figure 2. Western blot analysis confirming shRNA mediated knockdown of IRP1 protein levels in the shIRP1 knockdown cells compared to the control cells.

Results

IRP1 knockdown influences cell morphology

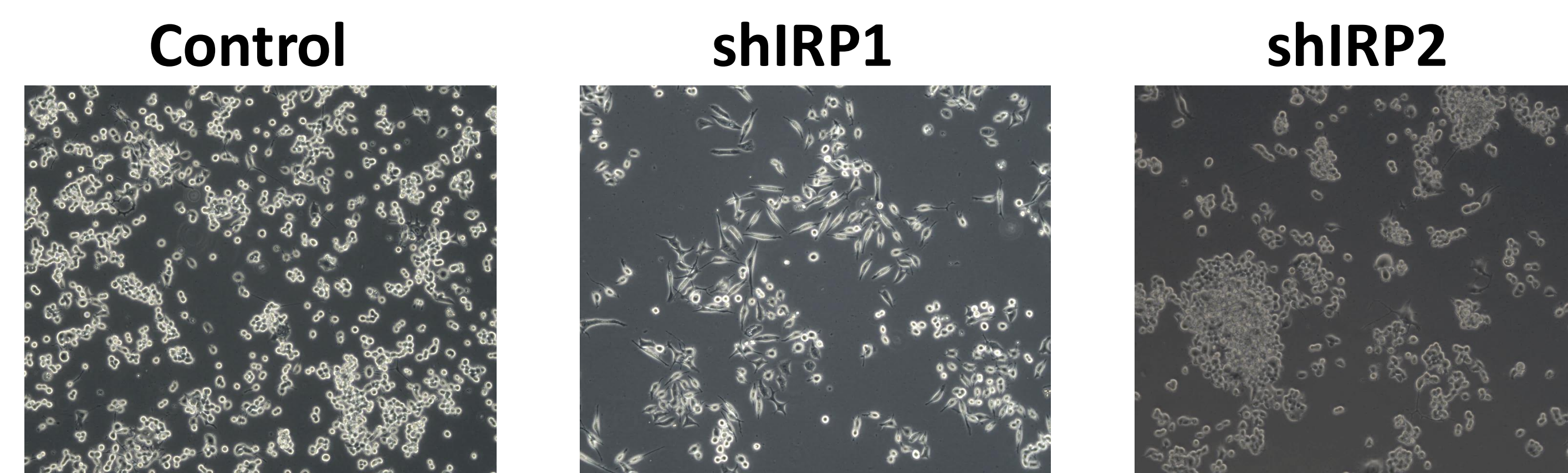


Figure 3. Microscopic images of control, shIRP1, and shIRP2 N2A cells. Knockdown of IRP1 led to observable differences in cell morphology compared to control cells. Images were taken at 10x magnification.

IRP1 knockdown increases cell proliferation

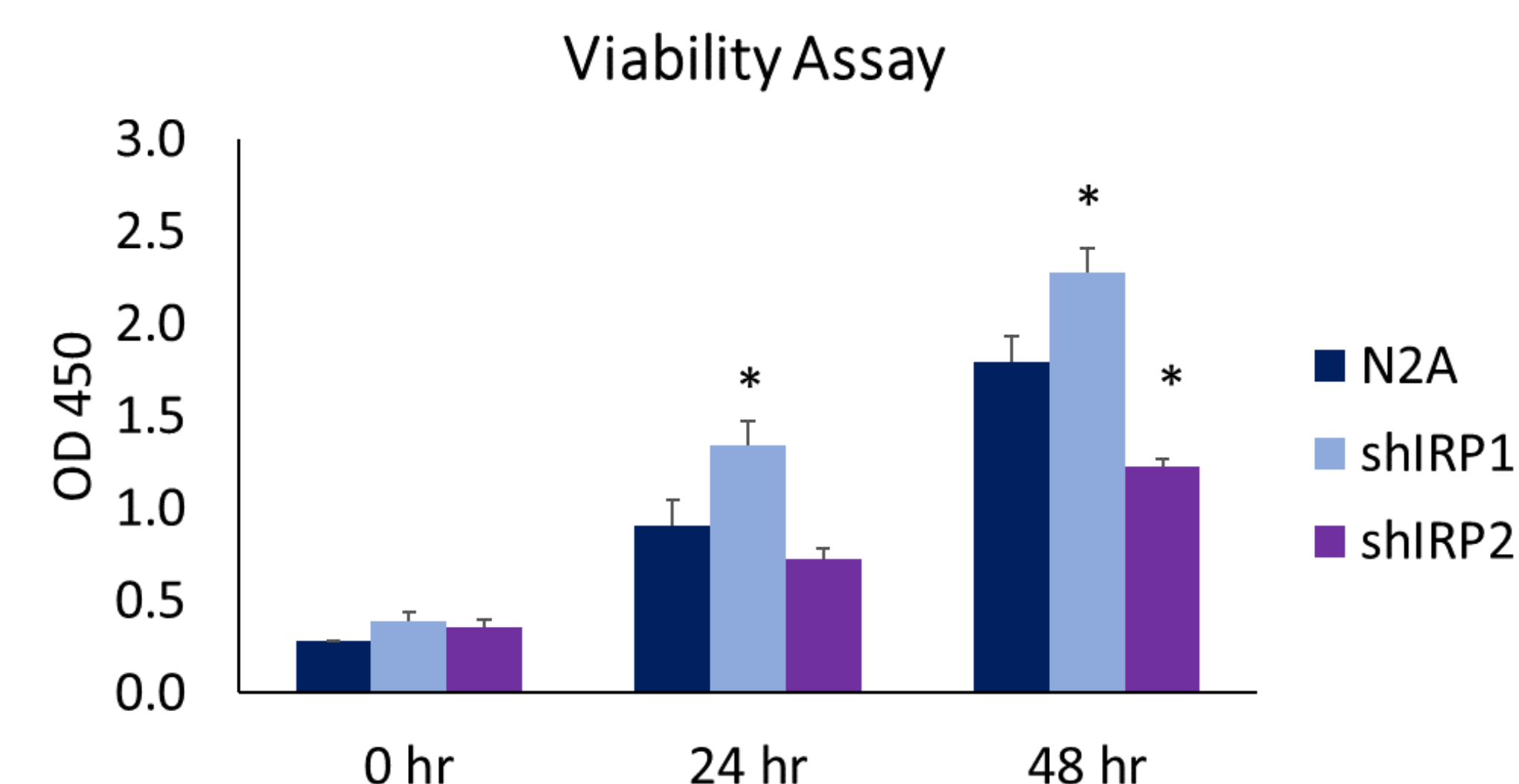


Figure 4. Cell viability was measured at 0, 24, and 48 hours in control N2A cells, shIRP1, and shIRP2 knockdown cells using a CCK-8 assay (OD 450). shIRP1 cells showed increased proliferation, while shIRP2 cells exhibited reduced proliferation compared to control cells. *Indicates statistical difference from respective control, $p < 0.05$.

IRP2 knockdown enhances the expression of neuronal markers

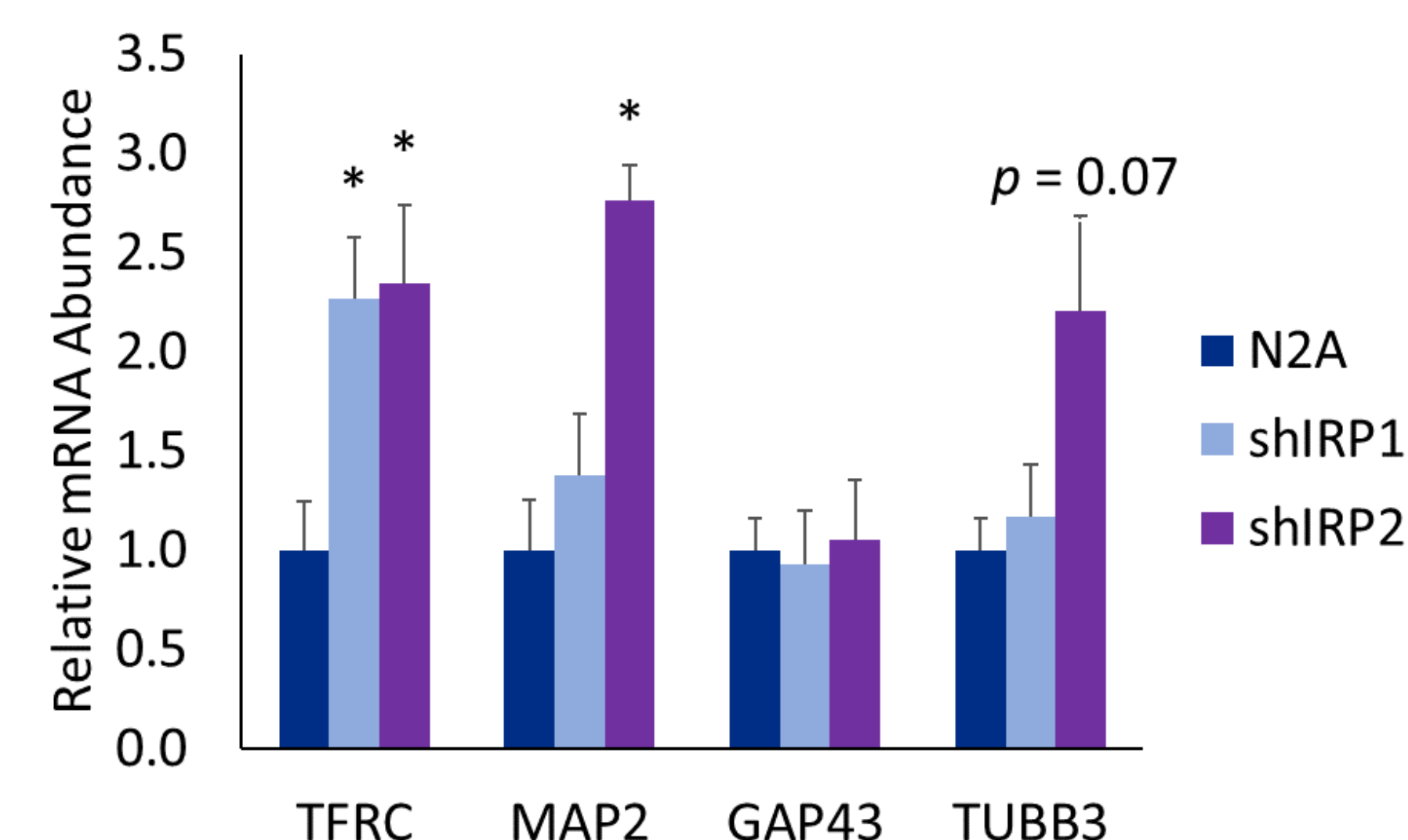
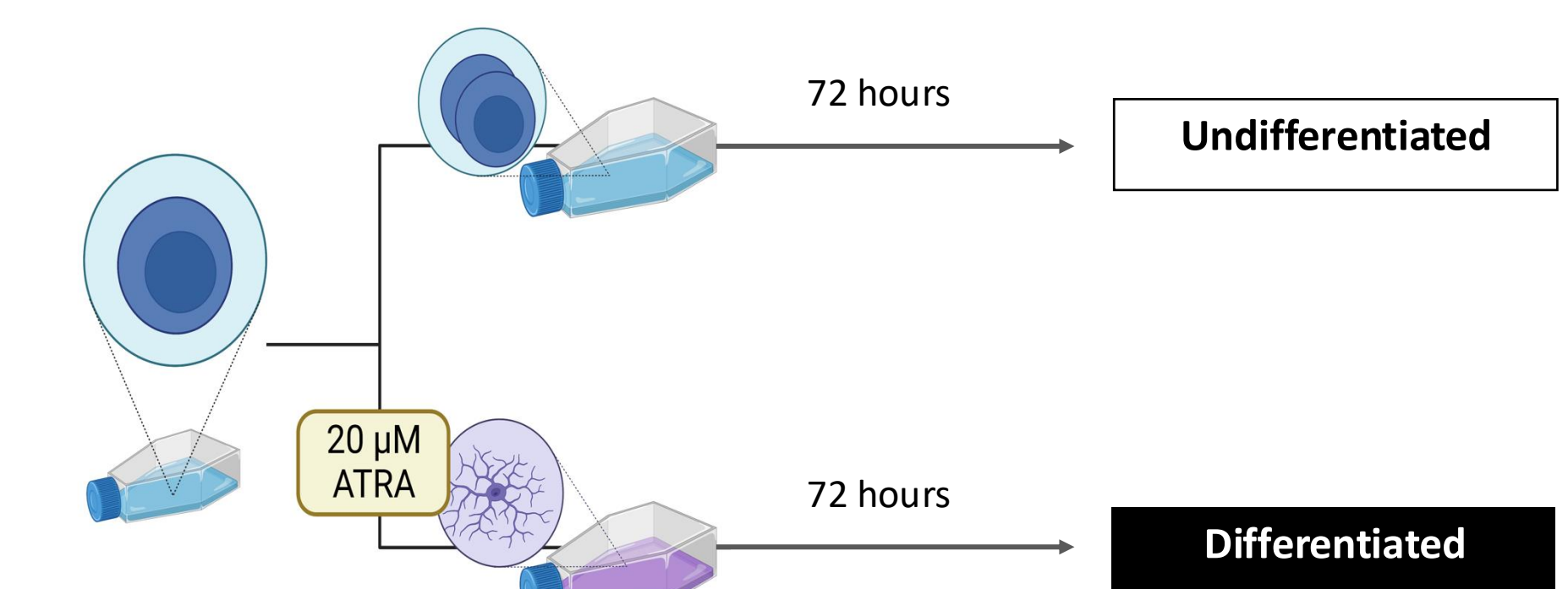


Figure 5. Relative mRNA abundance of neuronal marker genes TFRC, MAP2, GAP43, and TUBB3 was measured in control cells, shIRP1 cells, and shIRP2 cells. Knockdown of IRP1 and 2 significantly increased the expression of TFRC, while only knockdown of IRP2 increased MAP2 expression. *Indicates statistical difference from respective control, $p < 0.05$.

Conclusions

- Knockdown of iron regulatory proteins 1 and 2 alters neuronal cell morphology
- IRP1 knockdown impacted cell proliferation to a greater extent than IRP2 knockdown
- IRP2 and IRP1 knockdown significantly enhanced expression of Transferrin Receptor 1 (TFRC) that mediates iron uptake into cells
- IRP2 knockdown significantly increased Microtubule-Associated Protein 2 (MAP2) expression important for dendrite stabilization and neuronal morphology
- IRP2 knockdown strongly influenced an increase in Beta-III Tubulin (TUBB3) expression important in microtubule formation in neurons
- Expression of Growth-Associated Protein 43 (GAP43) was not altered by knockdown of IRP1 or IRP2

Future Directions



- Assess how IRP1 and IRP2 knockdown influence neuronal differentiation
- Assess oxidative stress and ROS levels to explore downstream effects of disrupted iron homeostasis
- Investigate if IRP2 knockdown alters APP expression in differentiated cells
- Measure A β aggregation to determine if irregular iron uptake promotes amyloid plaque formation
- Examine neuronal viability and function in response to differences in iron availability

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