Crystallization of human SUMO1 via fusion protein crystallization

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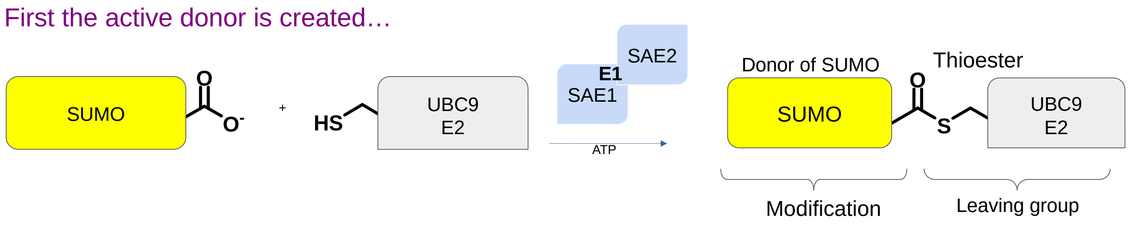
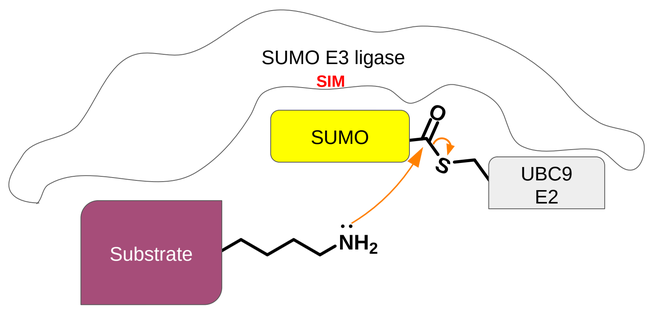
SUMO biology

First the active donor is created…

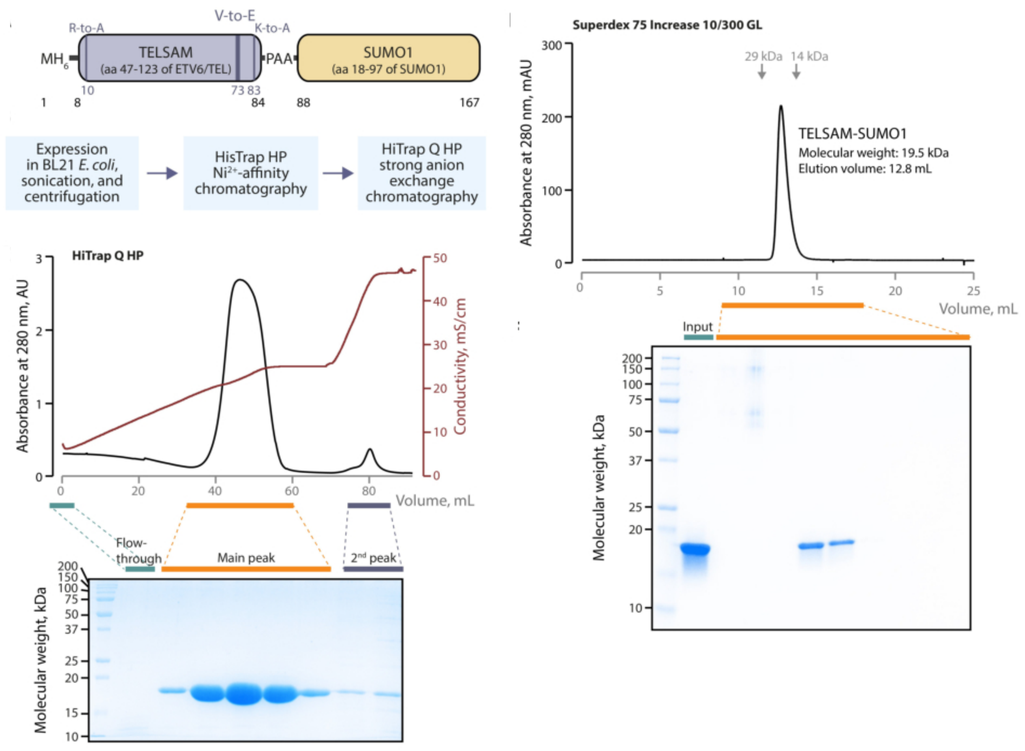


… then the donor reacts with the substrate



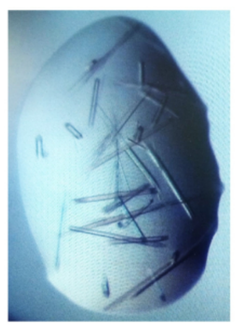
SUMOylation is an essential post-translational modification system in eukaryotes wherein, SUMO1, a protein modifier, interacts noncovalently with SUMO interacting motifs (SIMs). Interestingly, SUMO1 has been shown to be crystallized in complexes but never alone.

TELSAM-SUMO1 production and purification

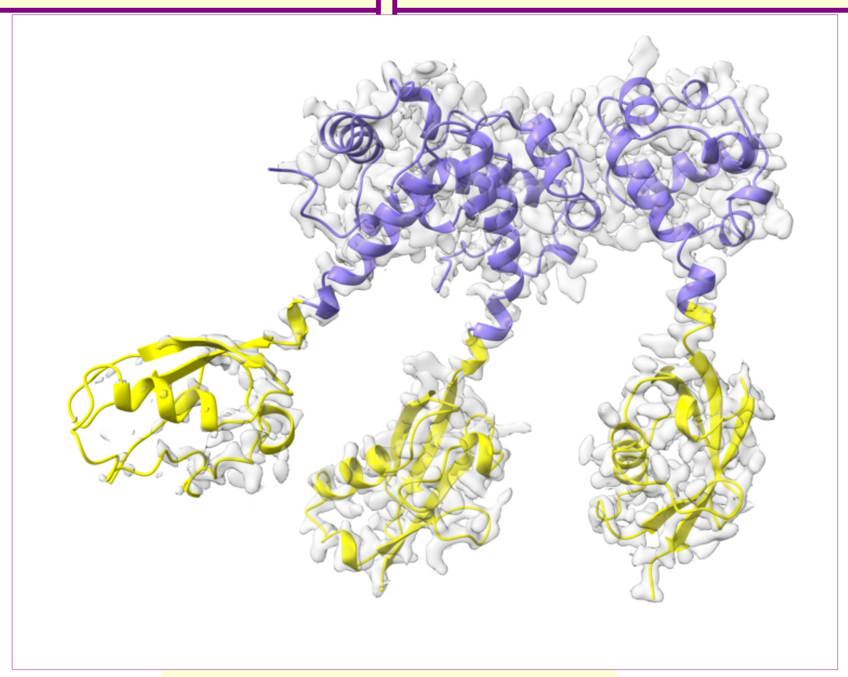


Simple purification yields highly pure TELSAM-SUMO1.

TELSAM-SUMO1 crystal lattice

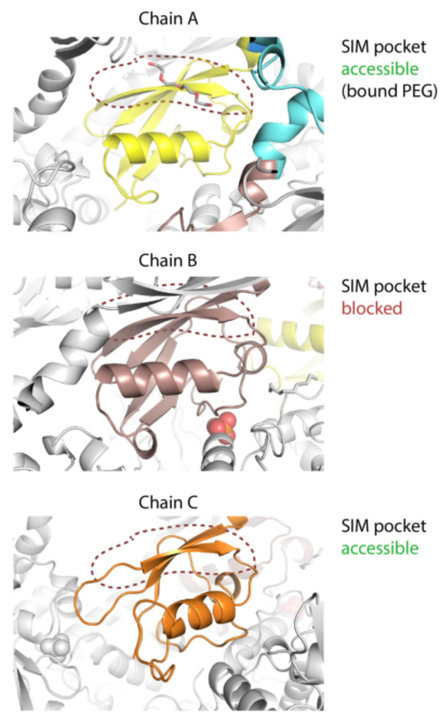


Crystals belong to the $P2_12_12_1$ space group.



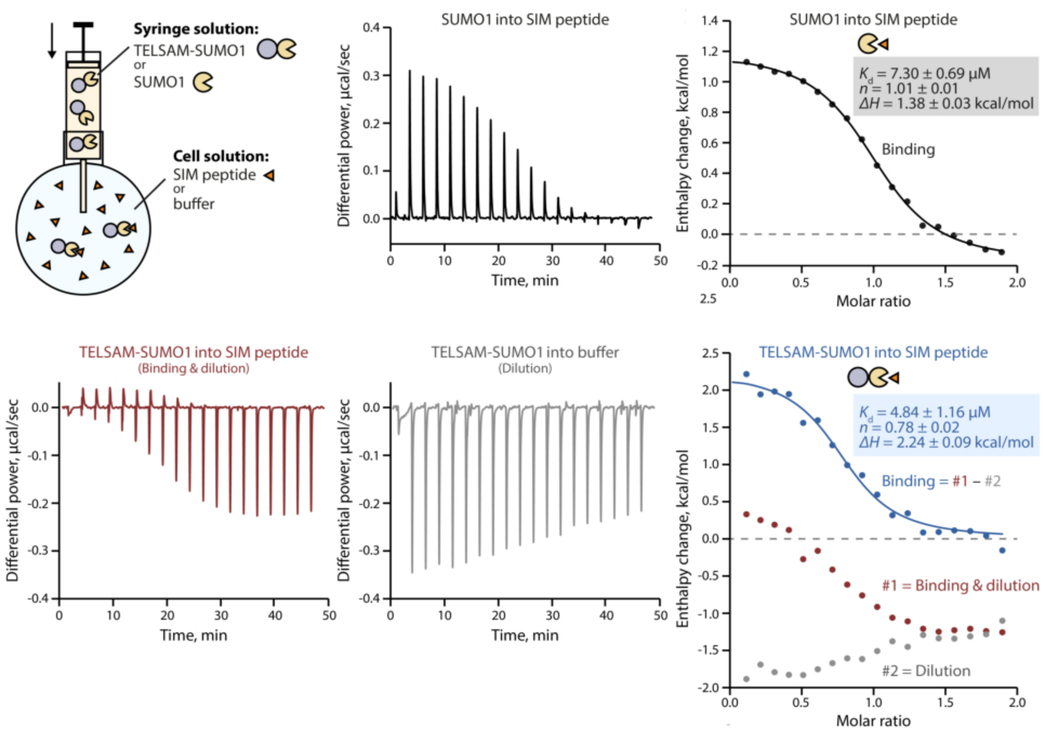
PDB ID: 9Q9E
Resolution: 2.05 Å
2Fo-Fc: 1.0 σ
(clipped to the region within 2 Å of the refined model)

SIM binding regions present in the TELSAM-SUMO1 structure



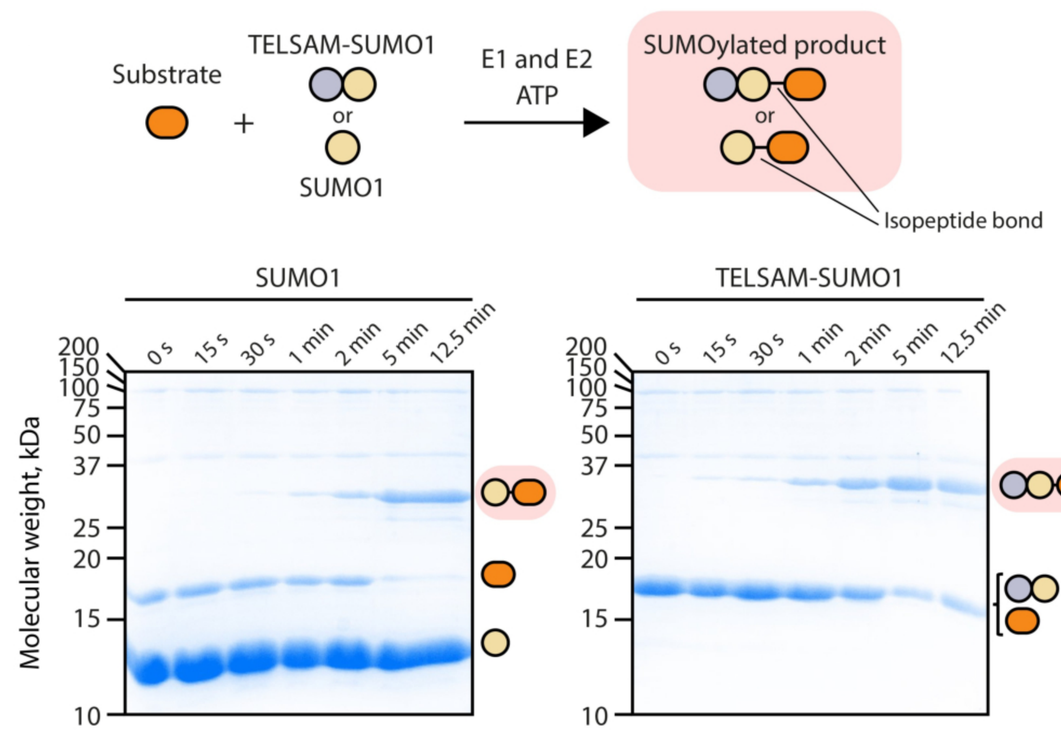
TELSAM-SUMO1 structure shows space for SUMO interacting motif (SIM) binding.

Isothermal titration calorimetry analysis of SIM binding



TELSAM-SUMO1 and SUMO1 bind a SIM peptide with comparable affinity.

SUMOylation assay



TELSAM-SUMO1 is efficiently conjugated to substrates *via* the SUMOylation cascade

Perspectives

- Co-crystallisation and soaking with SIM peptides to elucidate SUMO:SIM binding mechanisms
- Strategy can be used with SUMO paralogues (SUMO2, SUMO3)
- Co-crystallisation of SUMO proteins with SIMs in the presence of post-translational modifications (e.g., phosphorylation, acetylation)

Acknowledgments

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Also, we thank every person who contributes and makes Research phenomenal.

Effect of an SDHI pesticide mixture on the proliferation of murine neural stem cells *in vitro*

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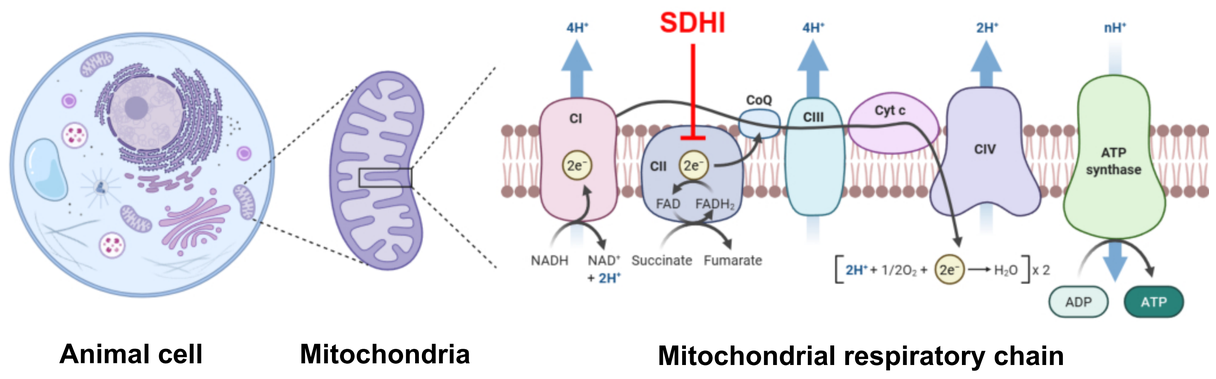
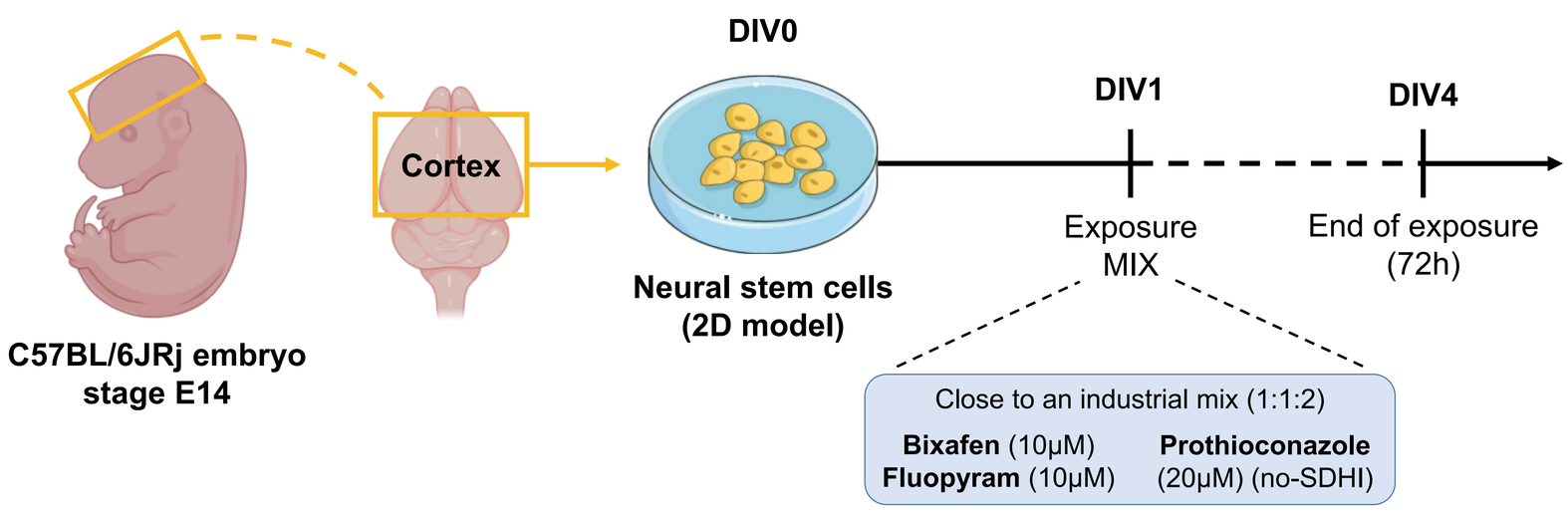
INTRODUCTION

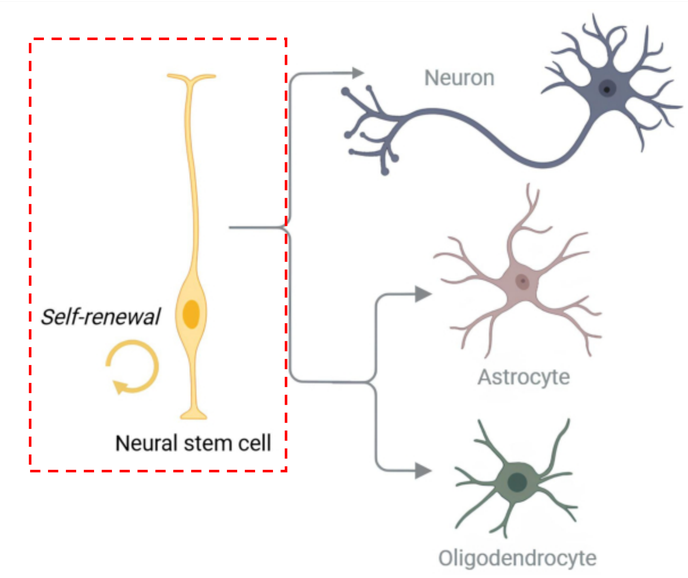
Succinate dehydrogenase inhibitors (SDHIs) are fungicides widely used in agriculture, for the storage of fruits, vegetables, and seeds, as well as in home gardening. Highly stable, they can persist in the environment for several months. Their mode of action targets succinate dehydrogenase (SDH, complex II of the mitochondrial respiratory chain), which is highly conserved from yeast to humans. Several studies have shown that SDHIs lack specificity and can alter mitochondrial activity in non-target species. Since mitochondrial function is crucial for neurogenesis, the potential neurotoxic effects of these compounds remain largely unexplored. We therefore aim to investigate the impact of these fungicides on brain homeostasis, starting with a 2D model mainly composed of neural stem cells.



Animal cell **Mitochondria** **Mitochondrial respiratory chain**

METHODS





RESULTS

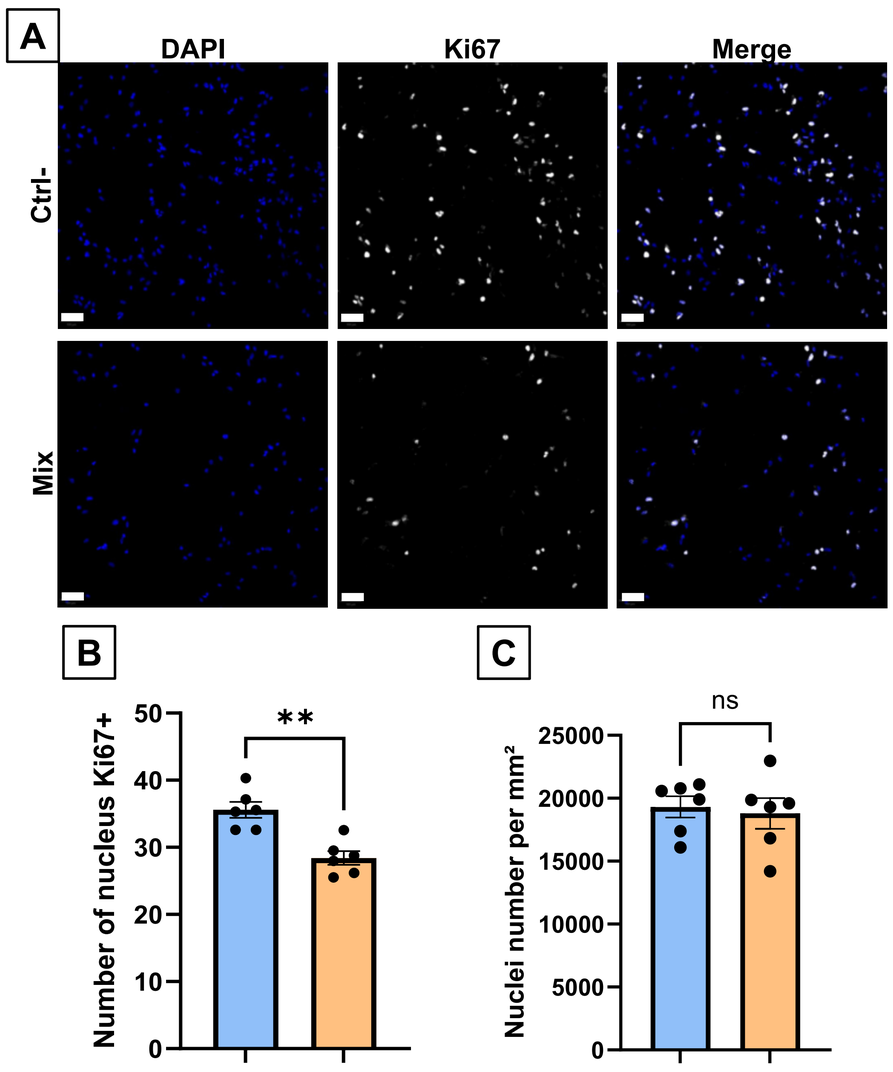


Figure 1: 72-hour exposure to the mix reduces proliferation status. Decreased nuclear Ki67 levels (A–B), despite no change in total nuclei density per mm² (C). *n* = 6 per condition. Scale bar = 100 µm. Mean ± SEM. Mann-Whitney Test. ***p*<0.005

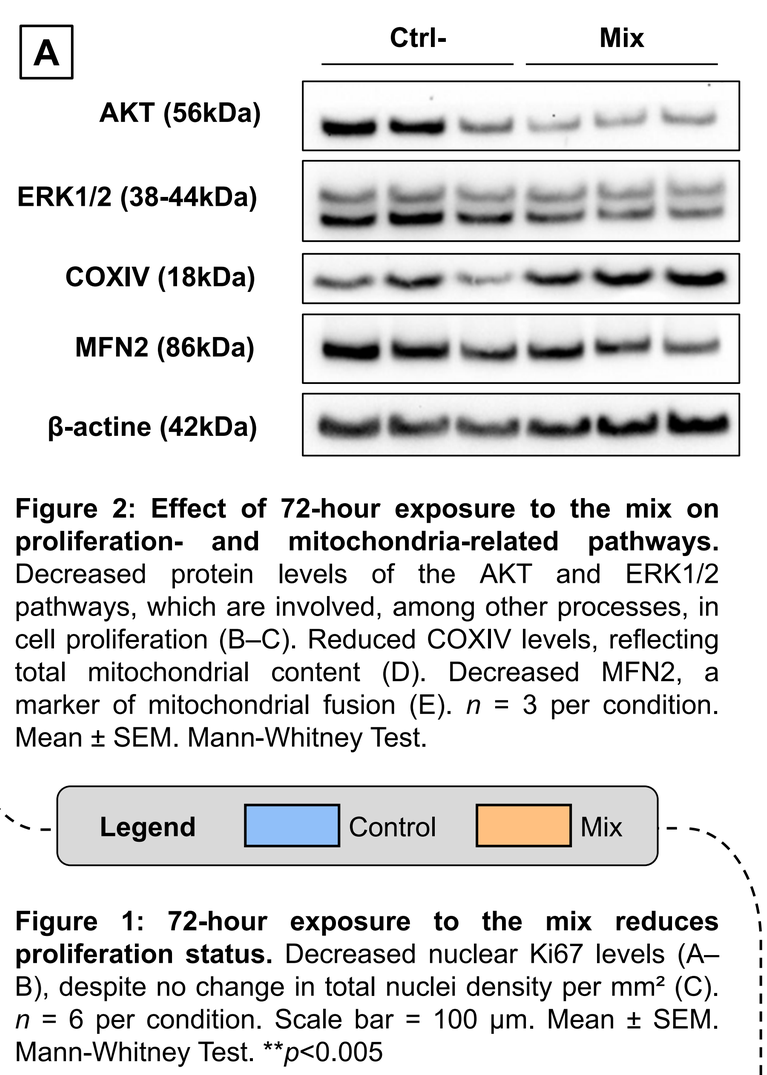


Figure 2: Effect of 72-hour exposure to the mix on proliferation- and mitochondria-related pathways. Decreased protein levels of the AKT and ERK1/2 pathways, which are involved, among other processes, in cell proliferation (B–C). Reduced COXIV levels, reflecting total mitochondrial content (D). Decreased MFN2, a marker of mitochondrial fusion (E). *n* = 3 per condition. Mean ± SEM. Mann-Whitney Test.

CONCLUSION AND PERSPECTIVES

- This pesticide mixture disrupts the proliferative state as well as the mitochondria of mouse neural stem cells, potentially affecting neurogenesis and brain development in the long term.
- The effects on proliferation may result from decreased cell division and/or premature differentiation.
- Further analyses (SOX2, Notch1, Shh, Wnt) are needed to clarify the underlying mechanisms.
- It would also be important to assess the impact under different glucose concentrations (4.5 g/L used here).
- The effects on a differentiated model (3D mouse neurospheroids) remain to be investigated (Figure 3).

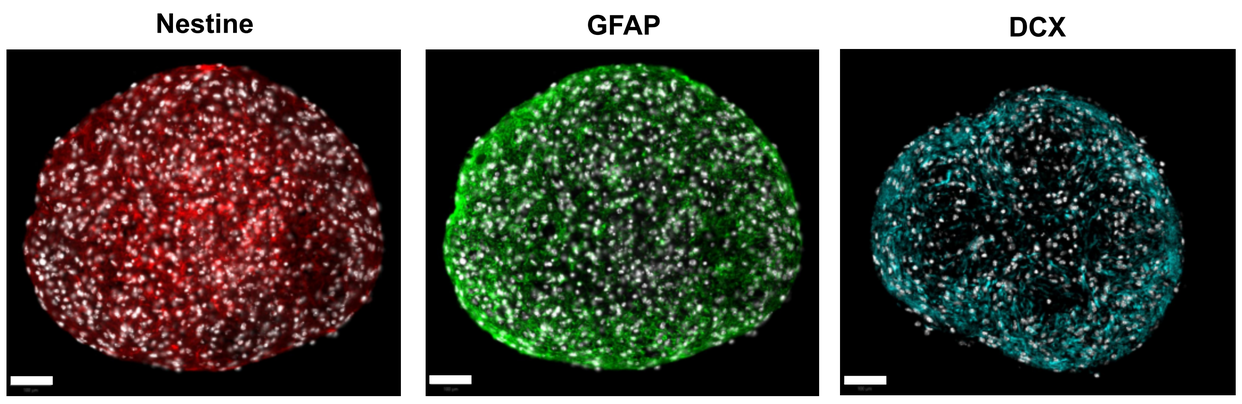


Figure 3: Characterization of 5-week-old mouse neurospheroids by cryosection. Immunocytochemistry of neural stem cells (Nestin, red), astrocytes (GFAP, green), and immature neurons (DCX, blue). Scale bar = 100 µm.

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