2022 RESEARCH AND CLINICAL CARE MEETING

Diminished motor neuron activity driven by abnormal astrocytic GLAST glutamate transporter in spinal muscular atrophy is not fully restored after lentiviral SMN delivery

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Cell Biology, Neurobiology and Anatomy (Ebert lab)
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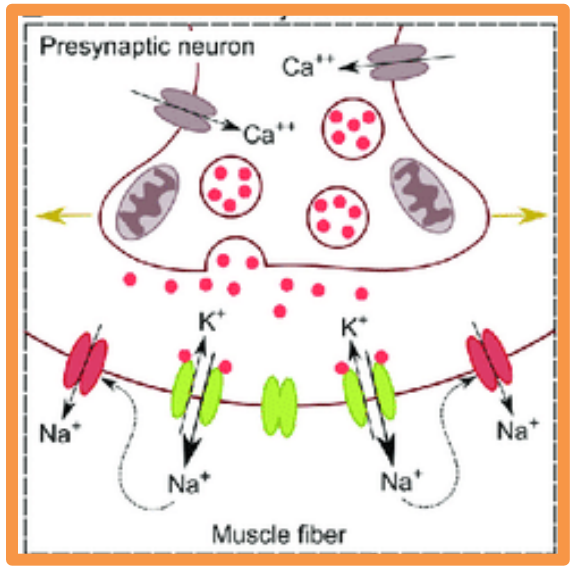
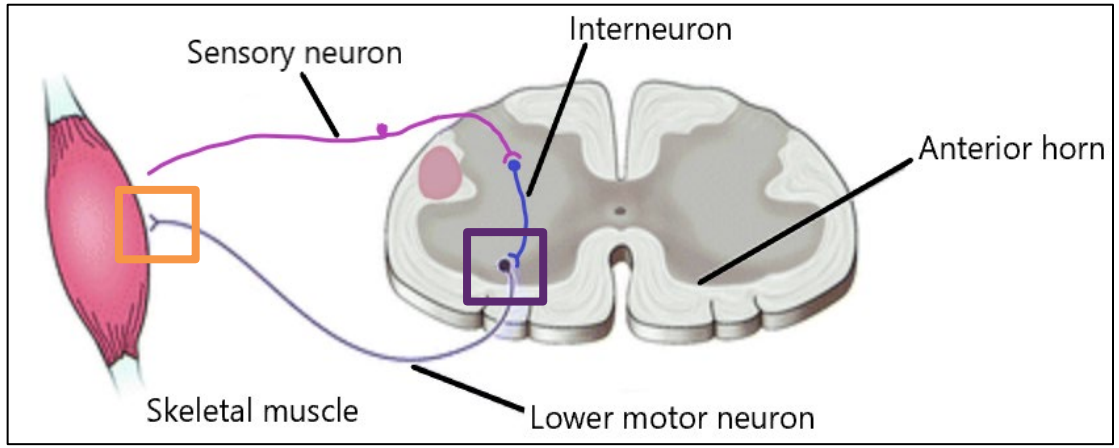
Contribution of other cell types to SMA motor neuron dysfunction?

Neuromuscular junction



- Cholinergic
- ↓ synaptic vesicle release
- ↓ NMJ endplate maturation (AChR clusters)
- Later in disease progression

e.g. monosynaptic reflex arc



Motor neuron cell autonomous defect?

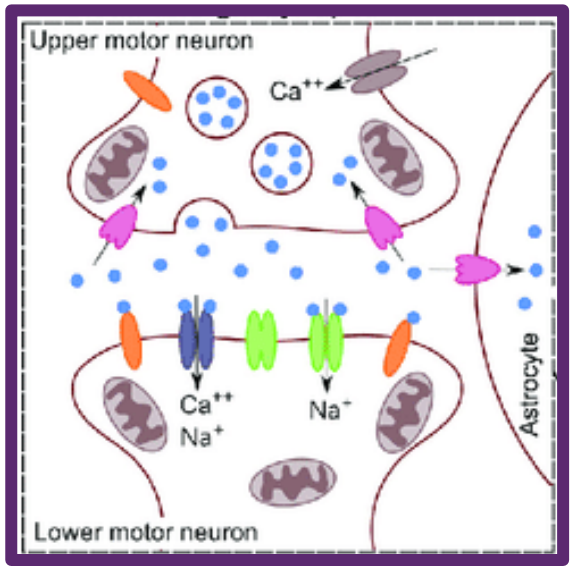
↓

Contribution of other cell types to motor neuron dysfunction?

↓

Glial cells?

Central afferent synapse

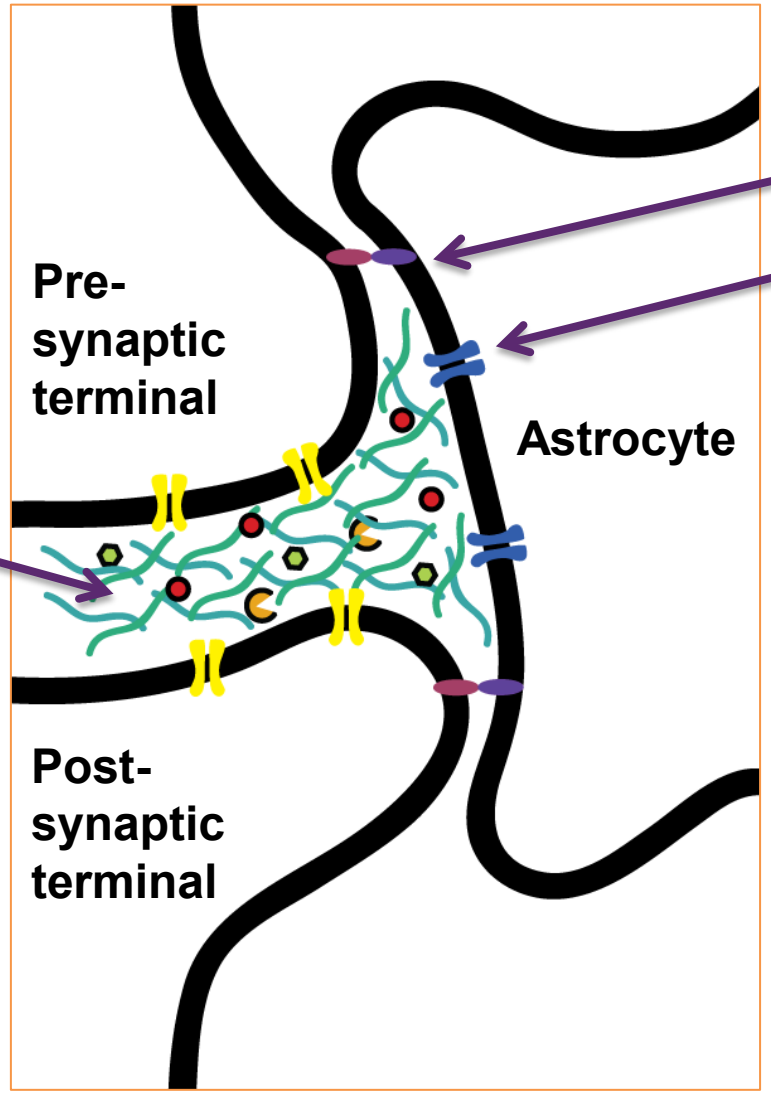


- Glutamatergic
- ↓ in excitatory glutamatergic neurotransmission
- Motor neuron hyperexcitability, but decreased post- synaptic action potentials
- Early in disease progression

Schematic adapted from Hawley et al., 2017

Astrocyte neuron-communication

- Astrocytes are key regulators of synapse formation and function
  - Excitatory
  - Glutamatergic
- Secreted factors into the synaptic cleft
  - Thrombospondin
  - Tenascin
  - SPARC
  - Gliotransmitters
    - Glutamate
    - ATP
    - D-serine
  - microRNAs

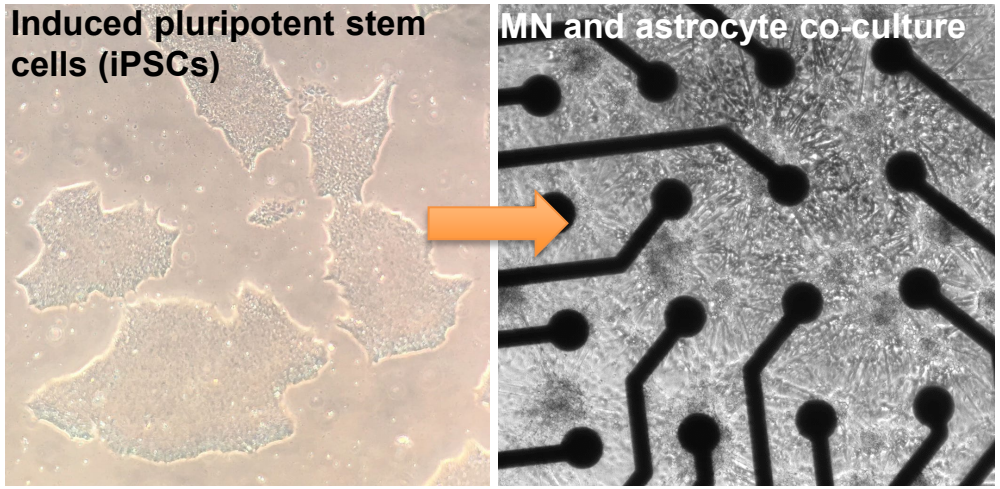


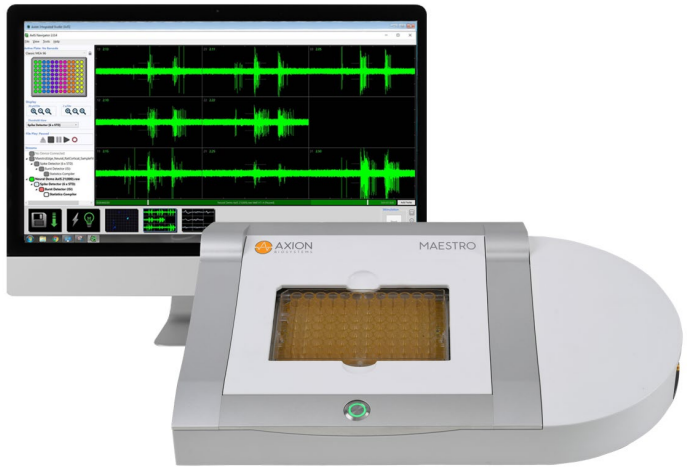
- Cell surface proteins
  - Cell adhesion molecules
    - Neuroligin
    - Neurexin
    - Cadherins
    - Ephrins
    - Protocadherins
  - Ion channels
    - $K_{ir}4.1$, Na+ K+ ATPases
  - Neurotransmitter receptors
    - Metabotropic glutamate receptors (mGluR, P2Y2)
  - Neurotransmitter transporters
    - Glutamate (GLT-1 and GLAST)

SMA astrocyte defects correlated to the synapse

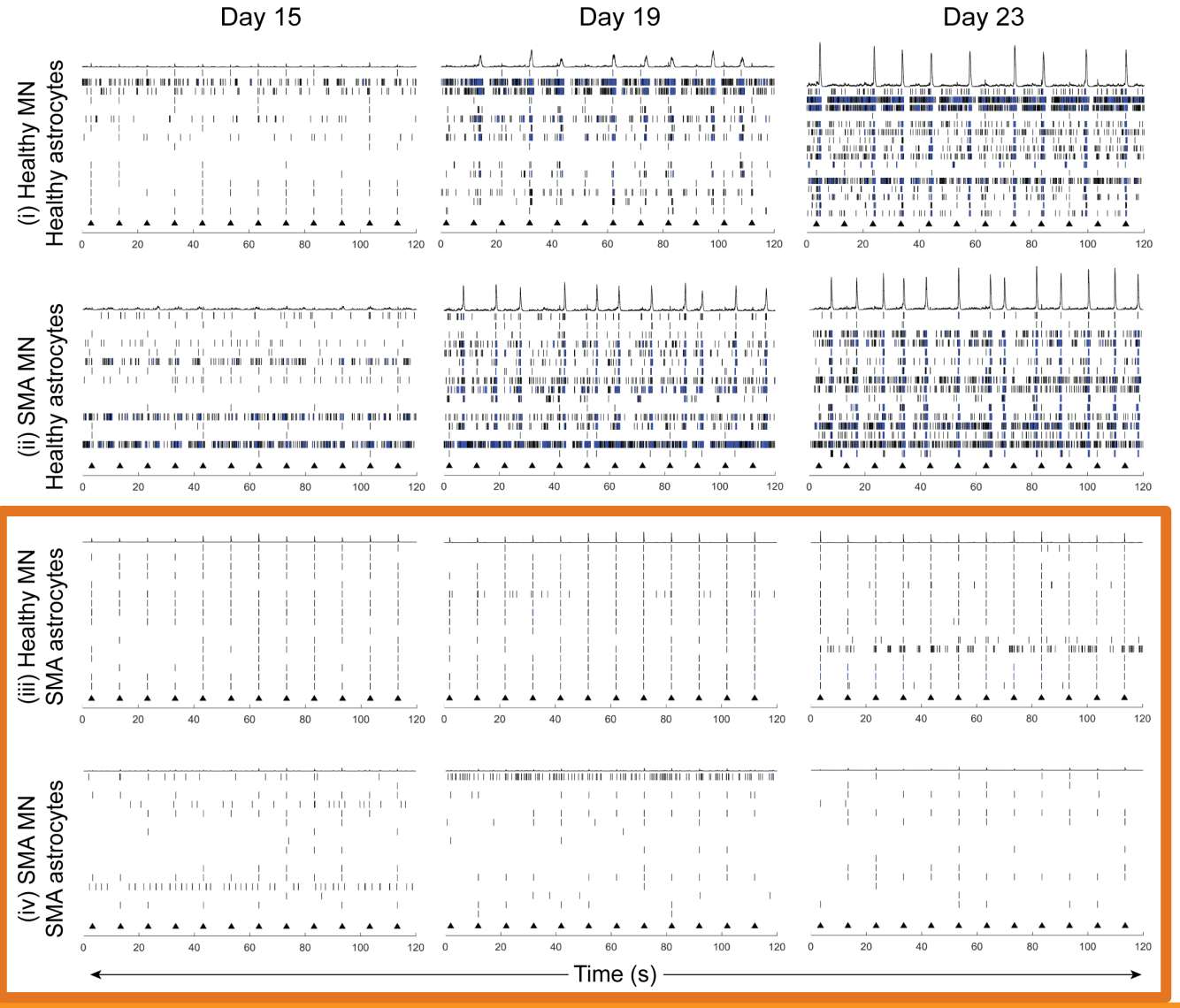
- Mouse studies
  - Synapse formation and activity is significantly reduced in SMA astrocyte motor neuron direct contact co-cultures (Zhou et al., 2016)
    - Potential candidate: Ephrin B2 reduction (axon guidance and synapse development)
  - Reduced inward rectifier potassium channel $K_{ir}4.1$ and glutamate transporter EAAT1 in SMA mouse spinal cords. Leads to reduced potassium and glutamate uptake in mouse SMN siRNA astrocytes (Leo et al., 2022)
- Human induced pluripotent stem cell (iPSC)-derived cultures
  - Astrocyte-mediated miR-146a may target extracellular matrix proteins within perineuronal nets to decrease motor neuron activity (Welby et al., 2021)
  - Human iPSC-derived astrocytes show increased basal calcium levels and minimal calcium response through ATP stimulation despite presence of purinergic receptors (McGivern et al., 2013)

SMA astrocytes diminish motor neuron activity (evoked)





Microelectrode array (Axion Biosystems)



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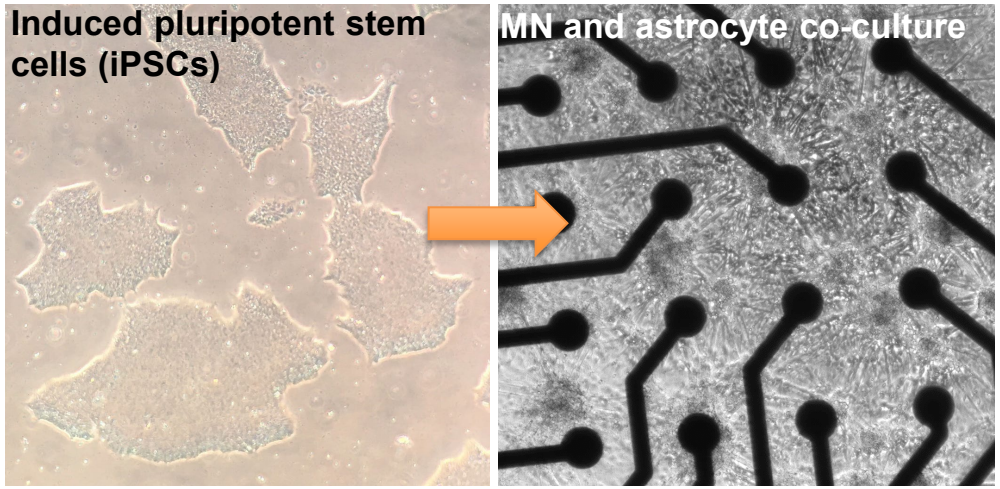

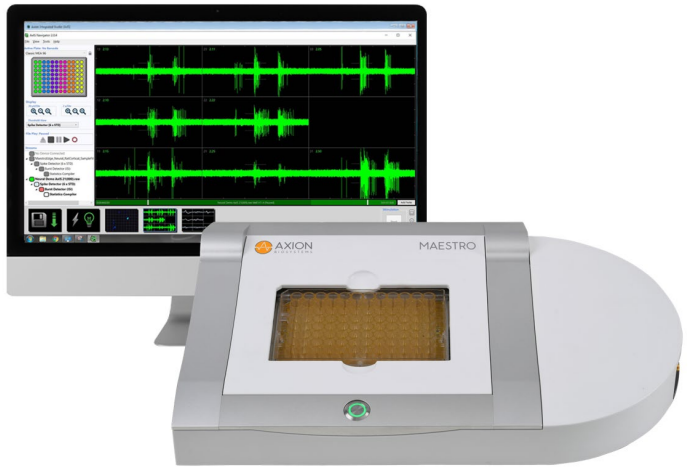

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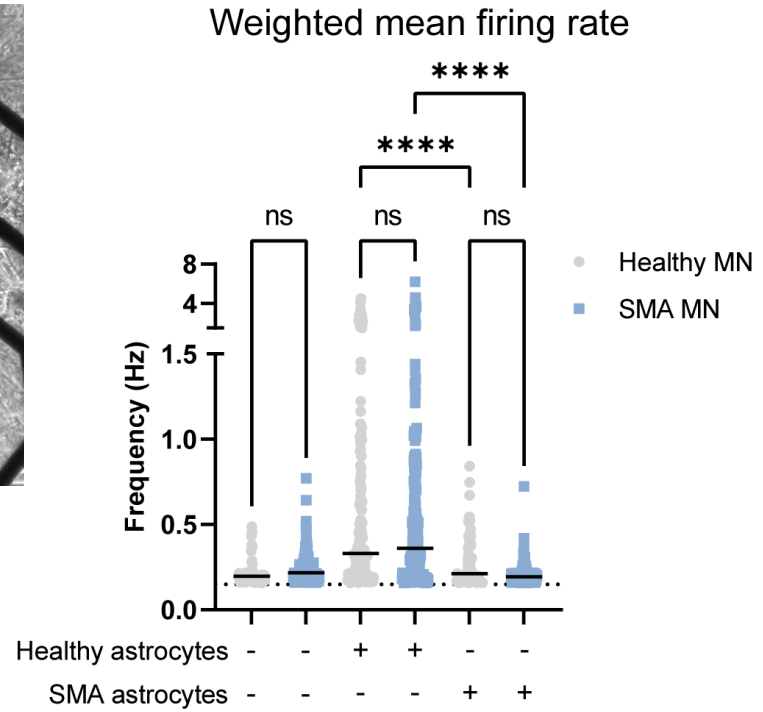
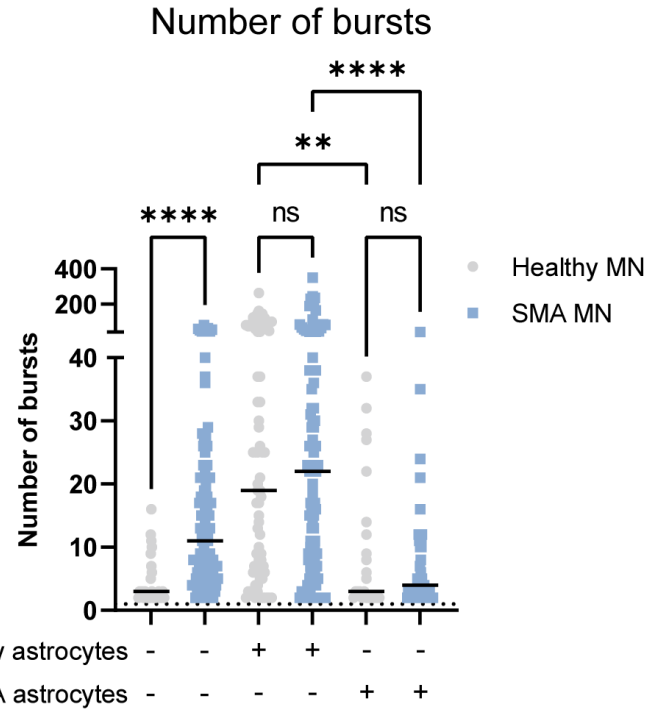
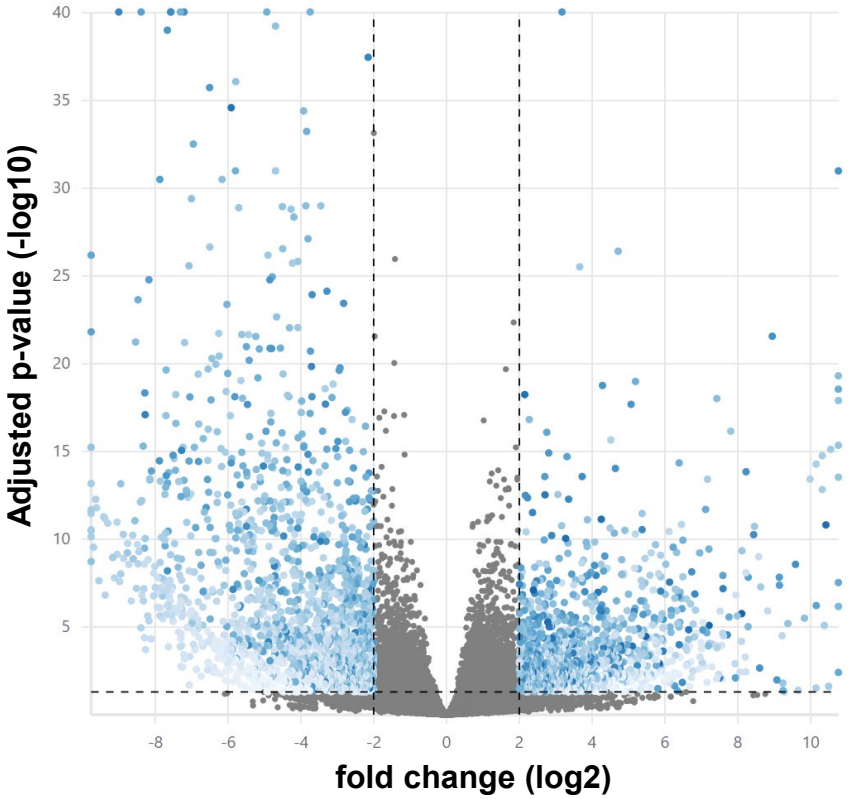


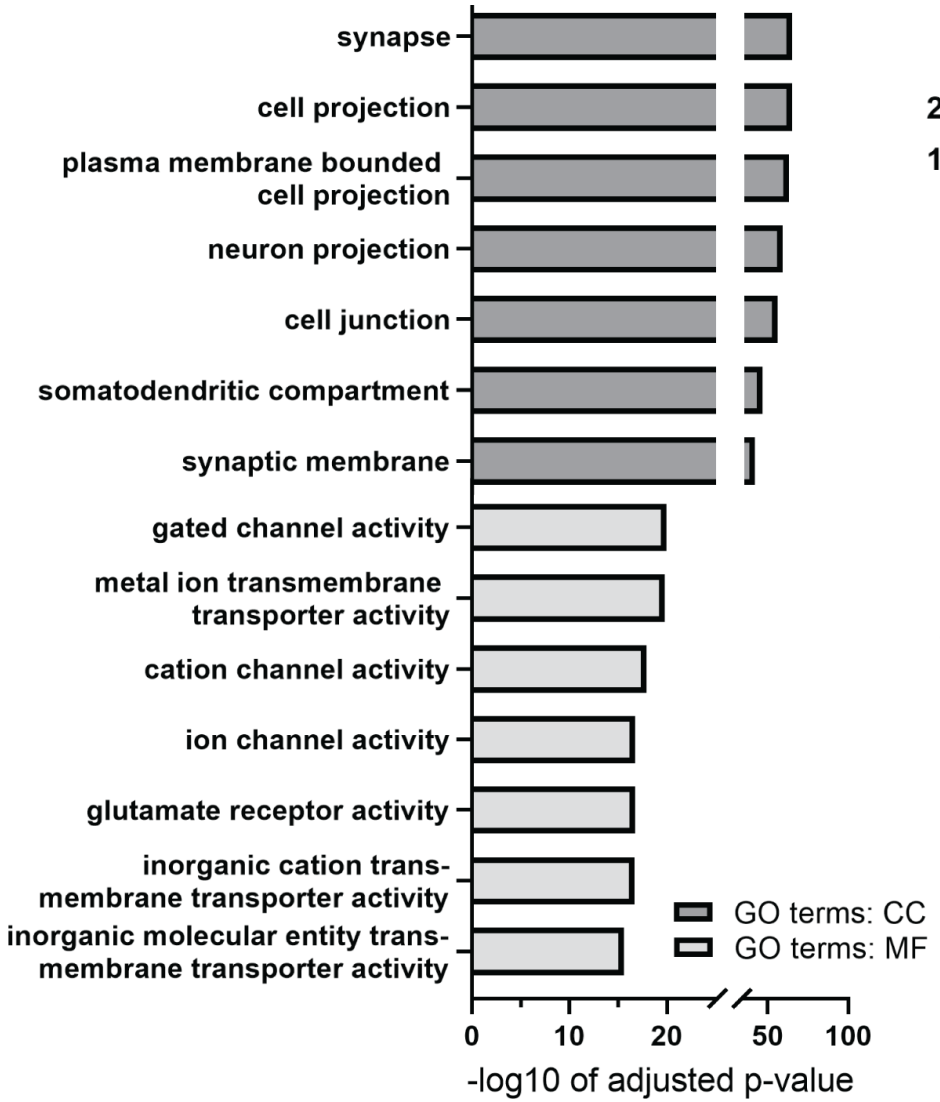


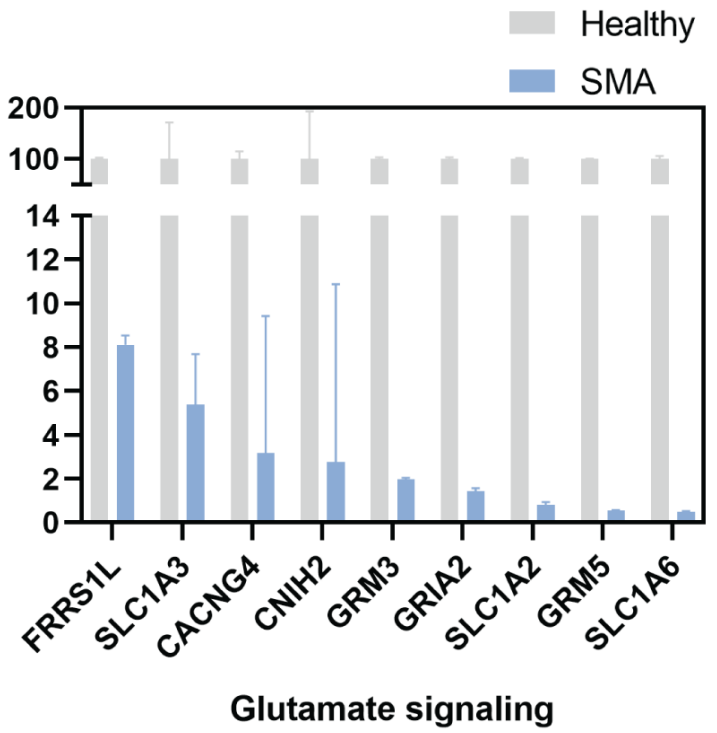
- SMA patient iPSC-derived astrocytes diminish motor neuron activity in direct contact co-cultures
- Healthy derived astrocytes normalize abnormal burst activity in patient motor neuron cultures

Astrocyte cell surface protein candidates (RNA seq data)



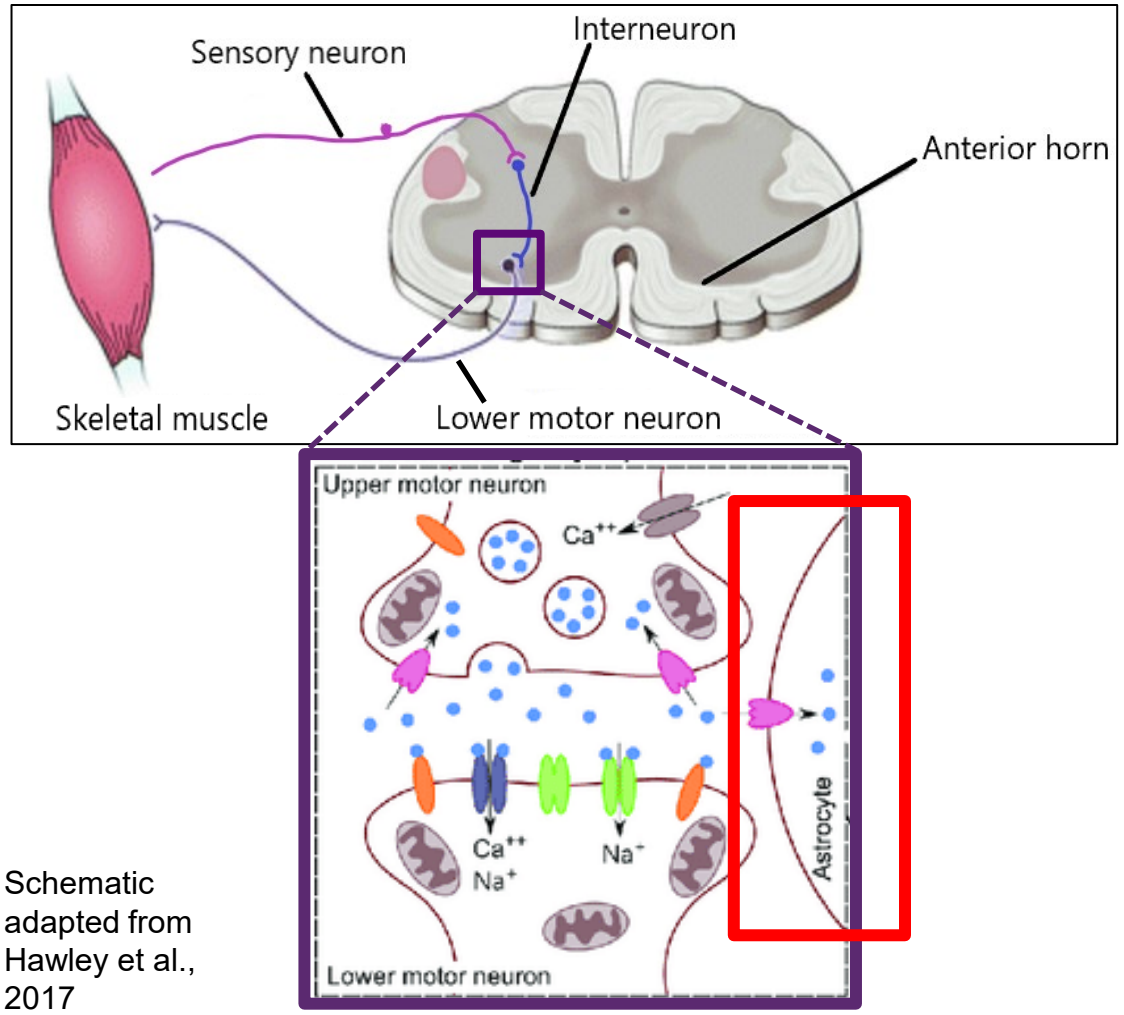
- 2995 significantly differentially expressed genes
  - 1221 upregulated
  - 1734 downregulated





- Downregulated genes in patient-derived astrocytes:
  - Ion buffering
  - Synaptic integrity
  - Glutamate regulation

Disrupted astrocytic glutamate neuromodulation contributes towards central afferent synapse dysfunction?



Schematic adapted from Hawley et al., 2017

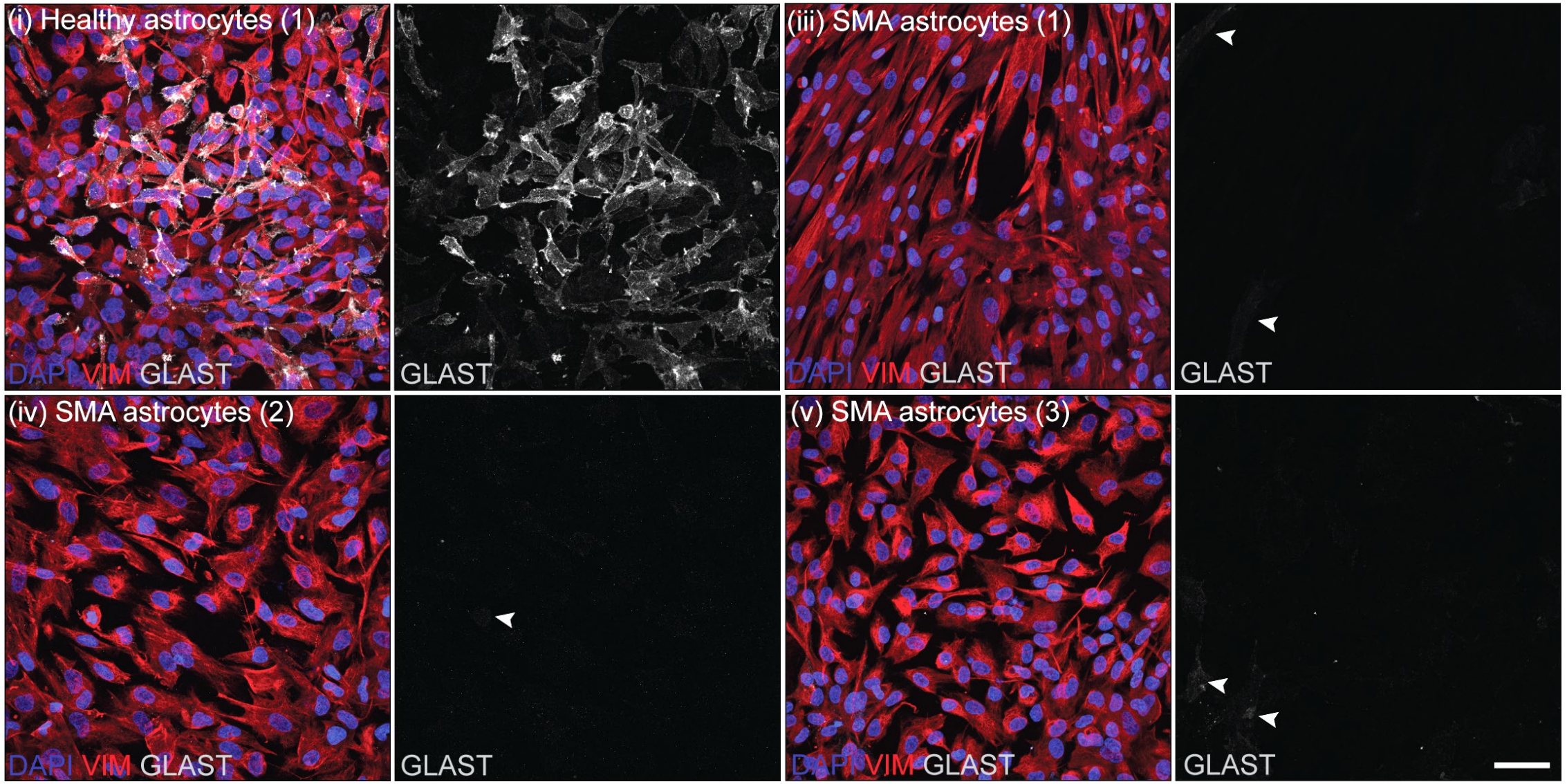
Hypothesis

SMA astrocytes contribute to motor neuron dysfunction due to the lack important synaptic-related cell surface glycoproteins

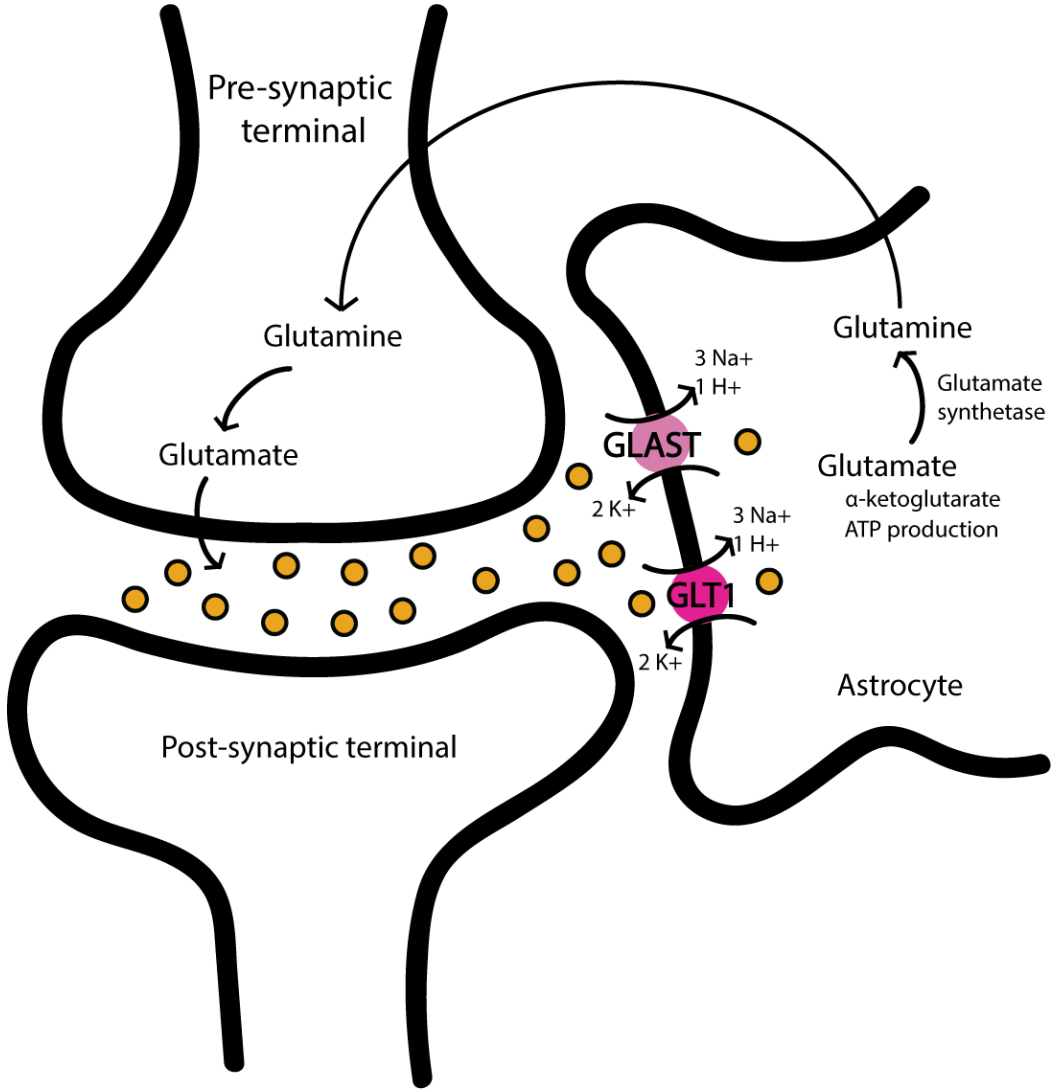
Strategy

1. Differences in cell surface transcripts/proteins between healthy and SMA astrocytes (RNA seq/cell surface capture data)
2. Candidate of interest validation
3. Impact on motor neurons (microelectrode array approach)
4. Mechanism: SMN dependent/independent?

Significant reduction of GLAST in SMA patient-derived astrocytes

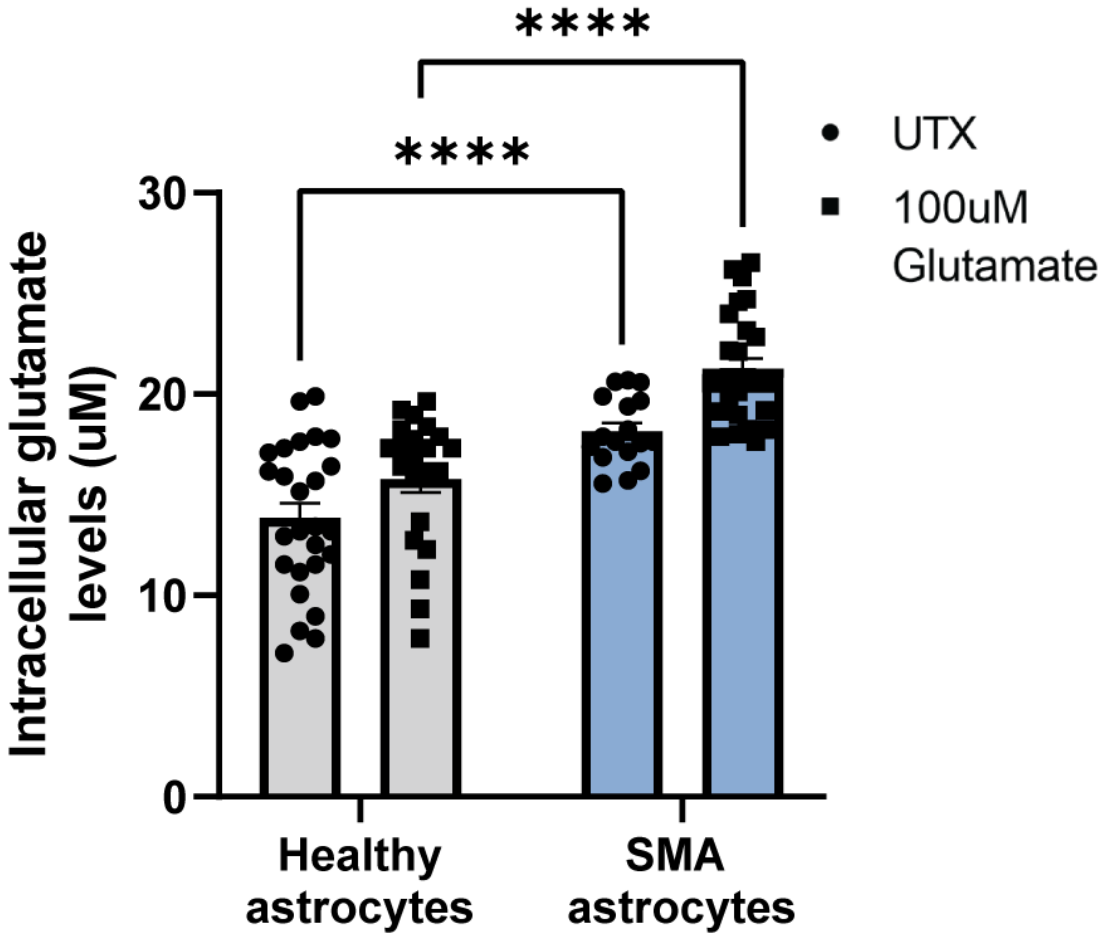


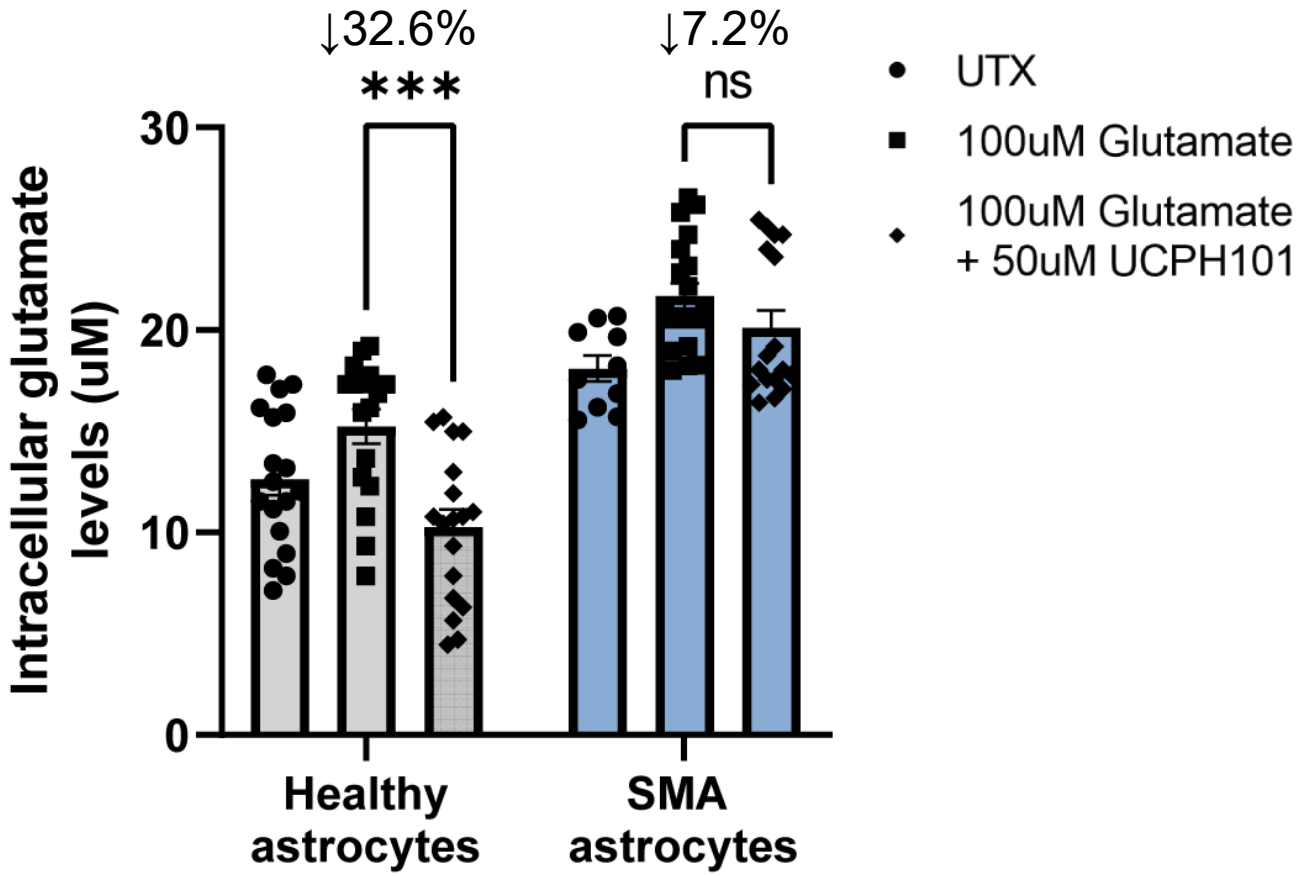
Astrocyte specific glutamate transporters: GLAST and GLT-1



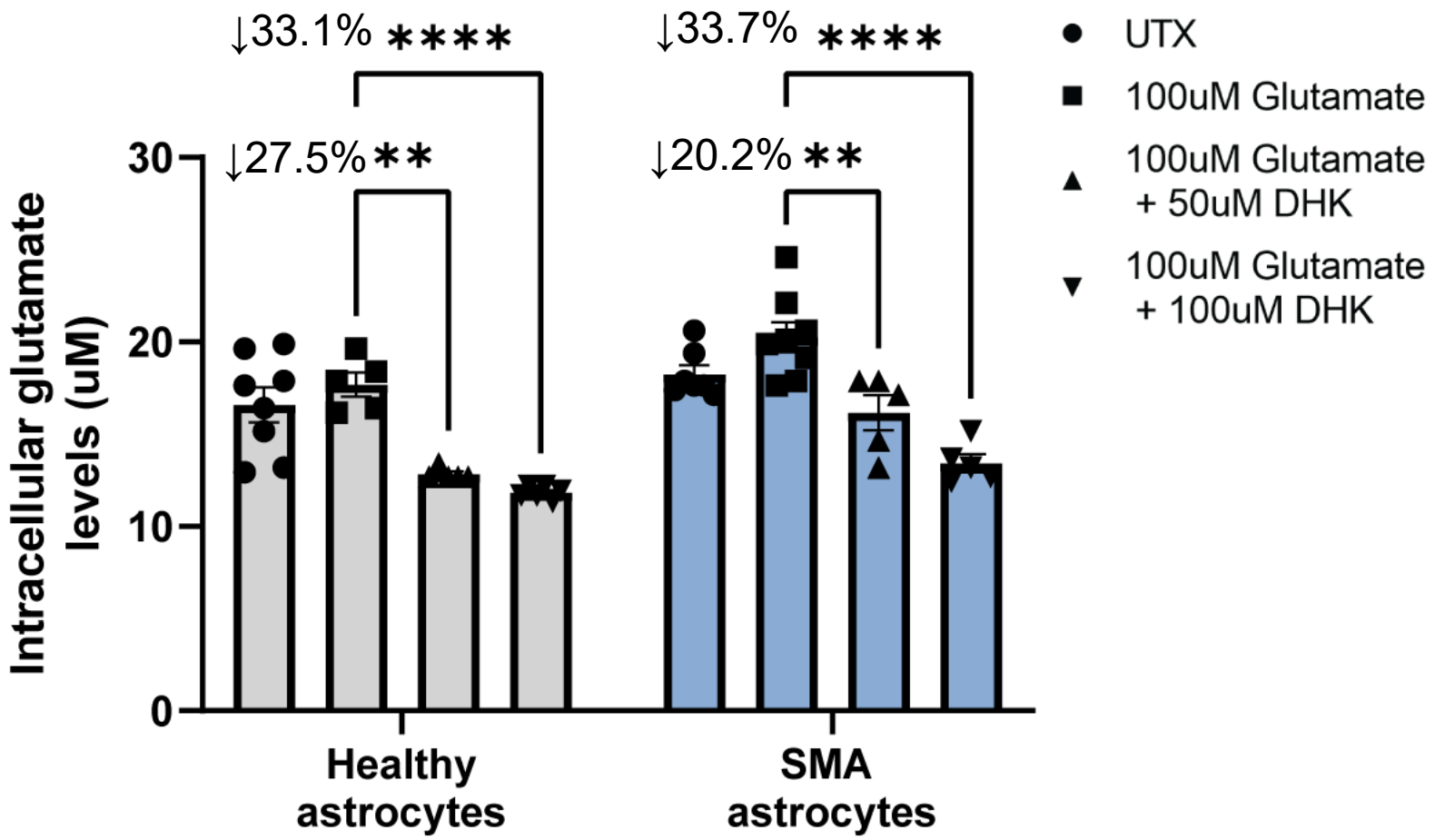
- **Responsible for at least 80% of synaptic glutamate clearance and recycling**
- **Uptake of glutamate against concentration gradient**
  - Na+ dependent glutamate uptake
  - Similar glutamate affinity for both transporters
- **Regulated by:**
  - Neurons (direct contact and secreted factors)
  - Synaptic activity (e.g. glutamate)
    - Coupled to Na+/K+ ATPases and mGluR
  - Extensive stimuli that affect trafficking of EAAT to and from intracellular pools and lipid rafts (caveolae)

Specific GLAST inhibition fails to decrease intracellular glutamate in SMA patient-derived astrocytes

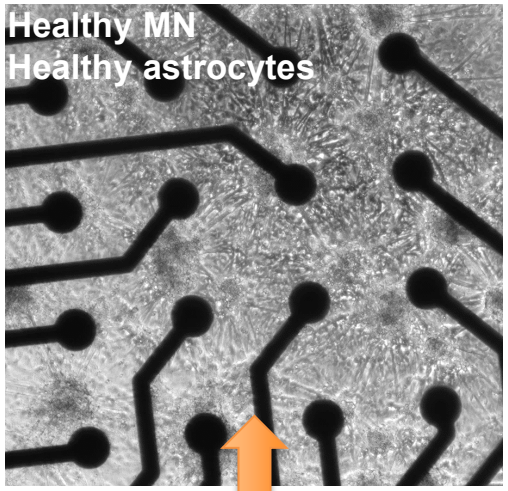




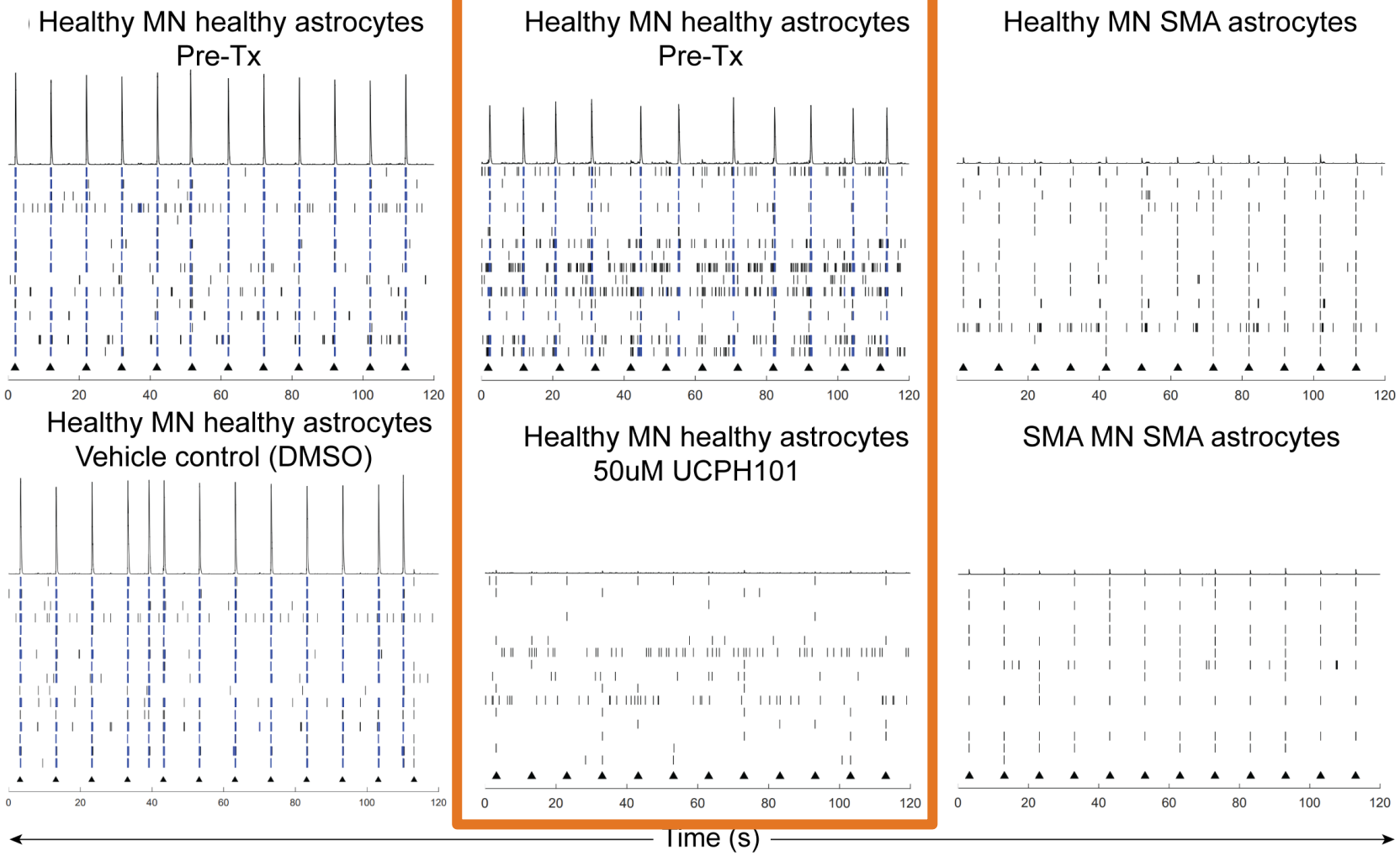
Specific GLT-1 inhibition decreases intracellular glutamate in healthy and SMA patient-derived astrocytes



GLAST inhibition mimics diminished neuron activity from SMA astrocyte co-cultures



UCPH101
(selective GLAST inhibitor)



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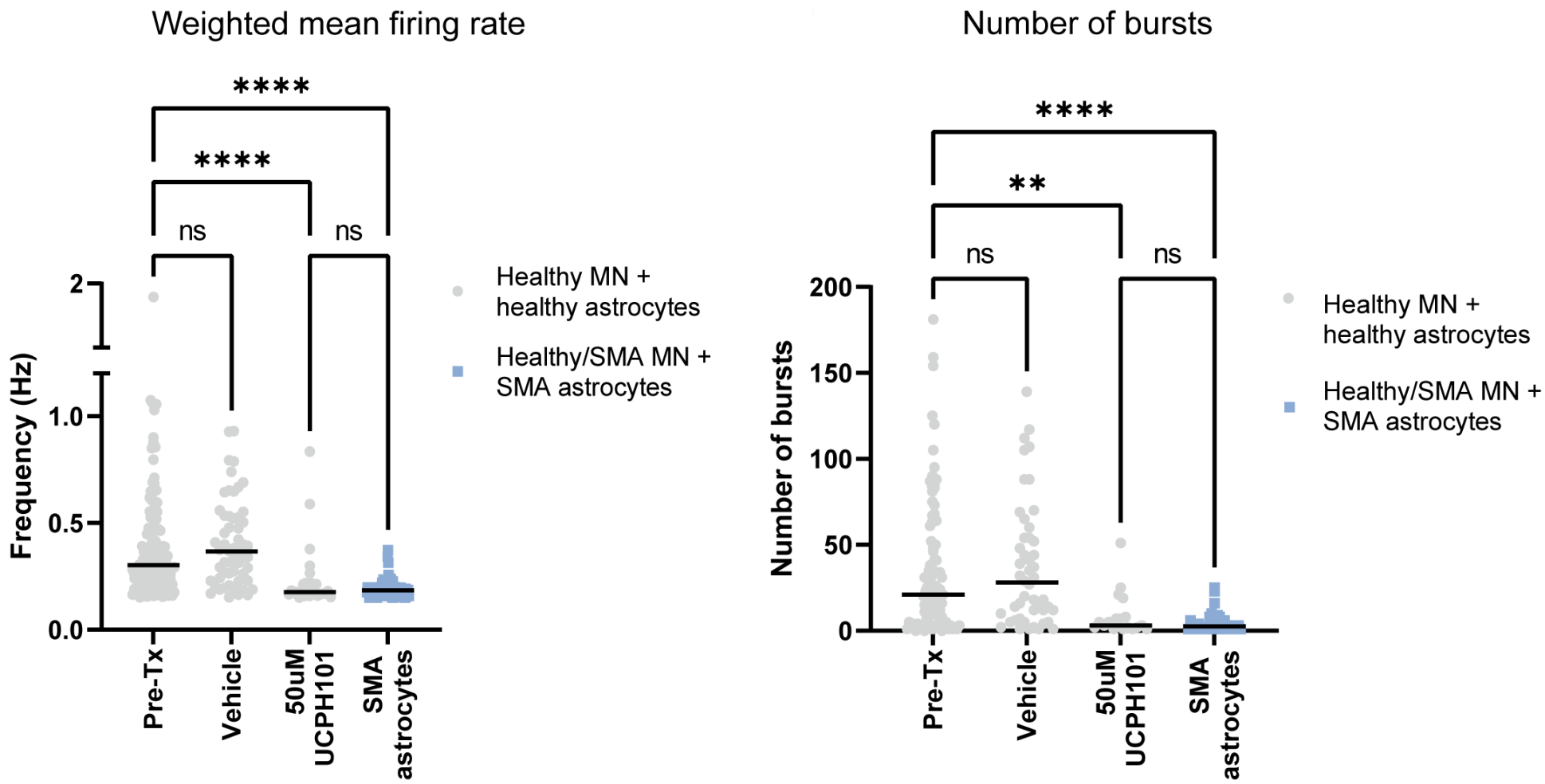
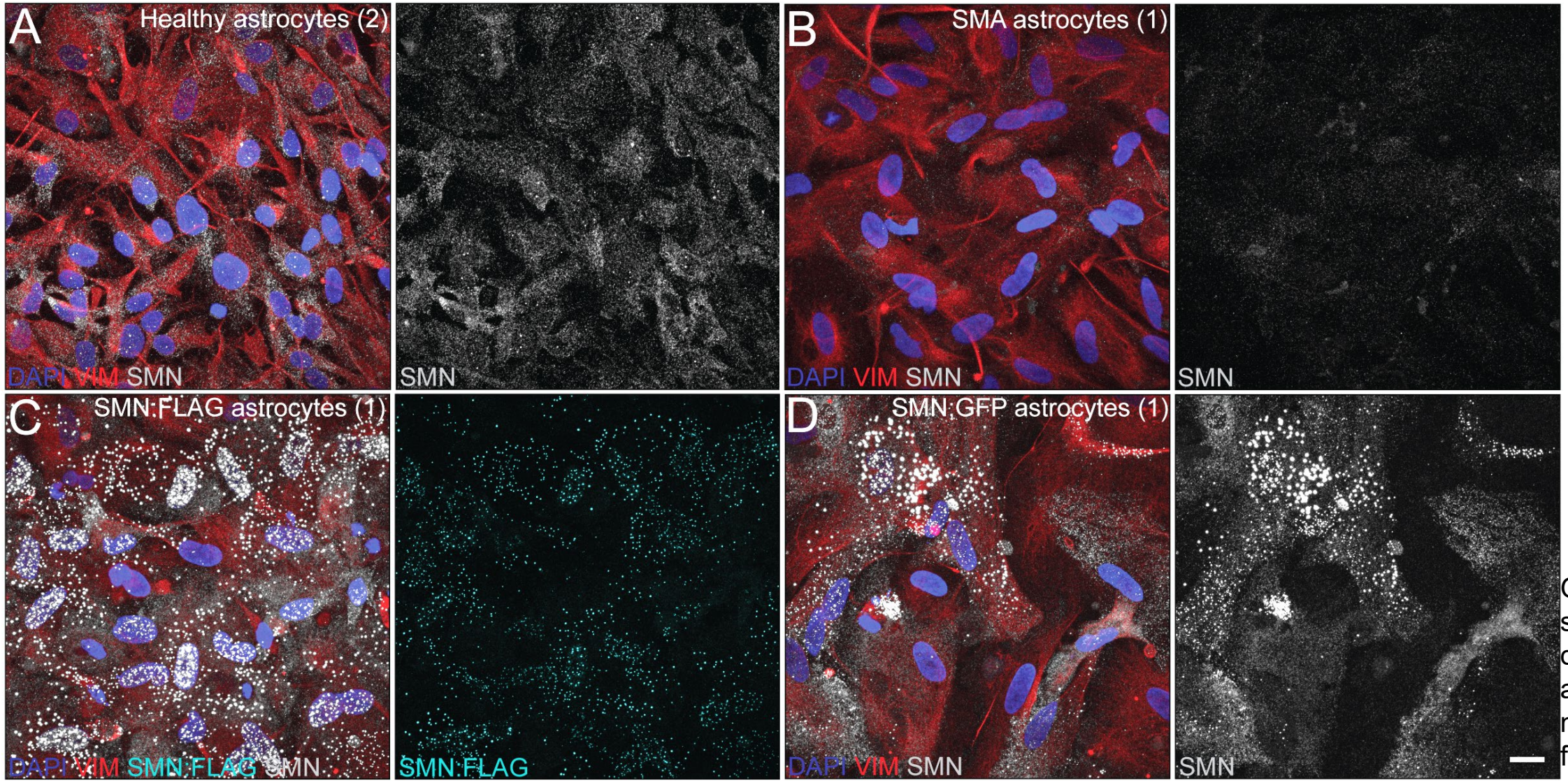
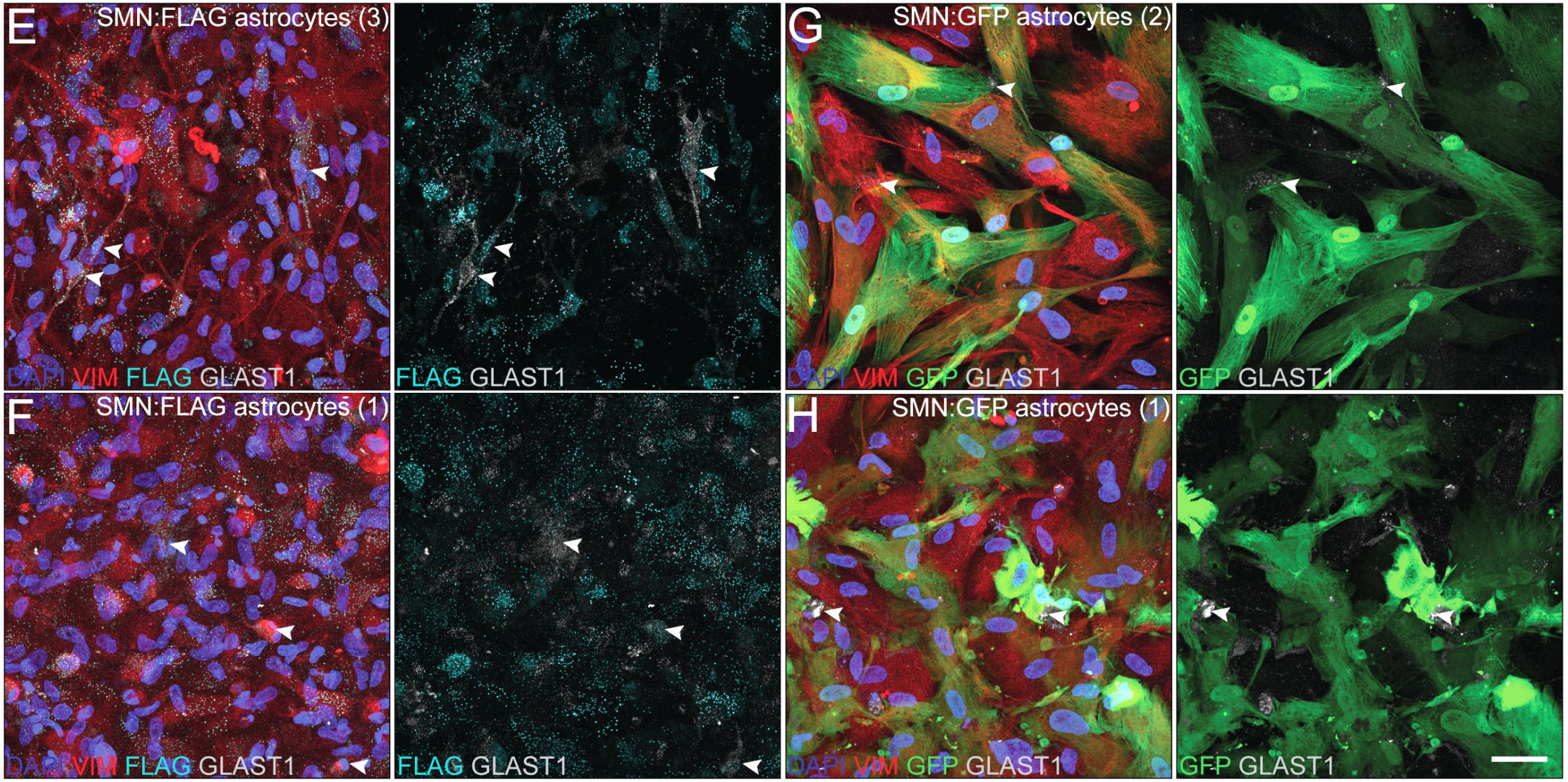


Lentiviral mediated delivery of SMN into SMA patient astrocytes



GFP signal lost due to acetone/ methanol fixation

Partial restoration of GLAST phenotype after SMN re-expression



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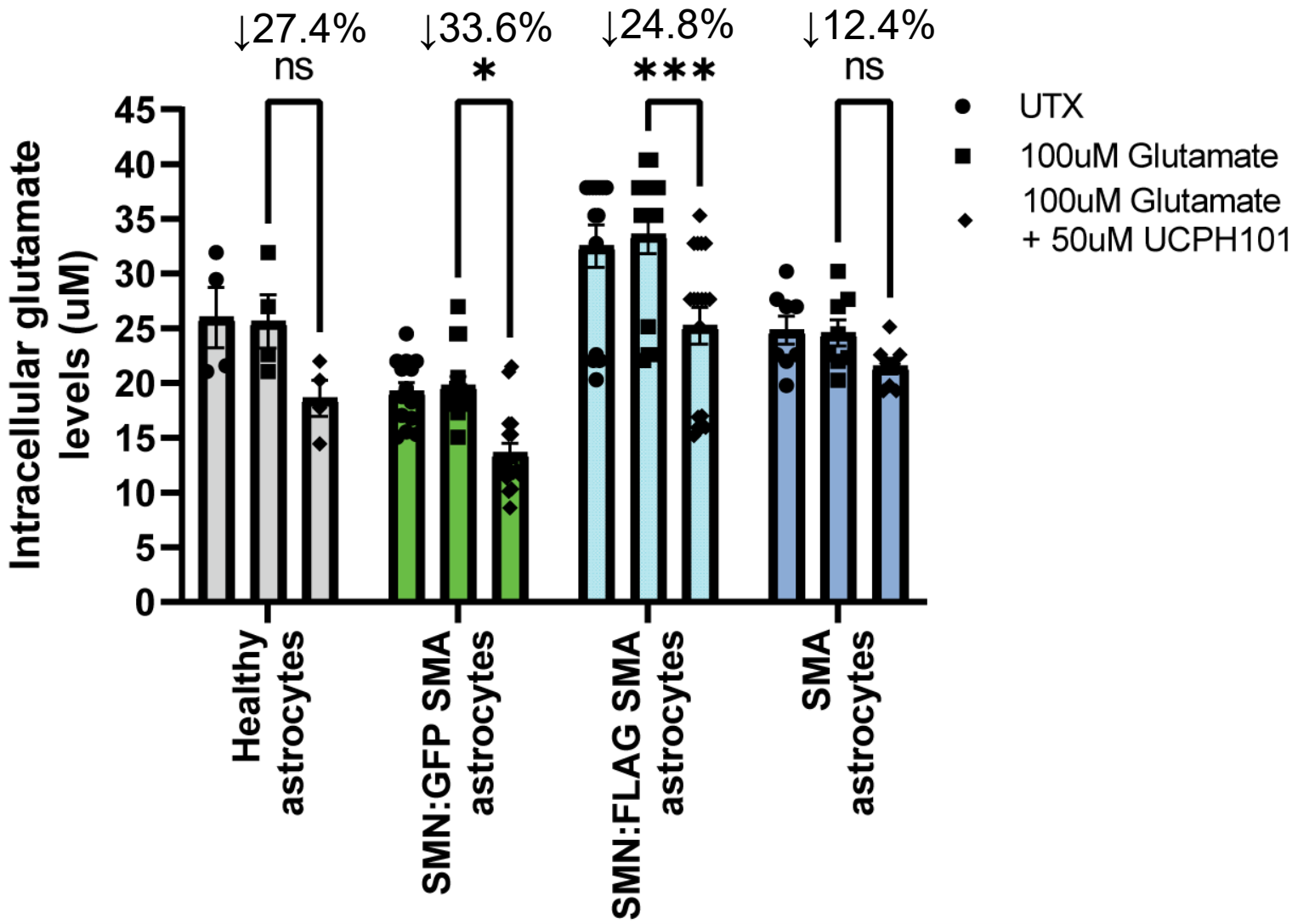
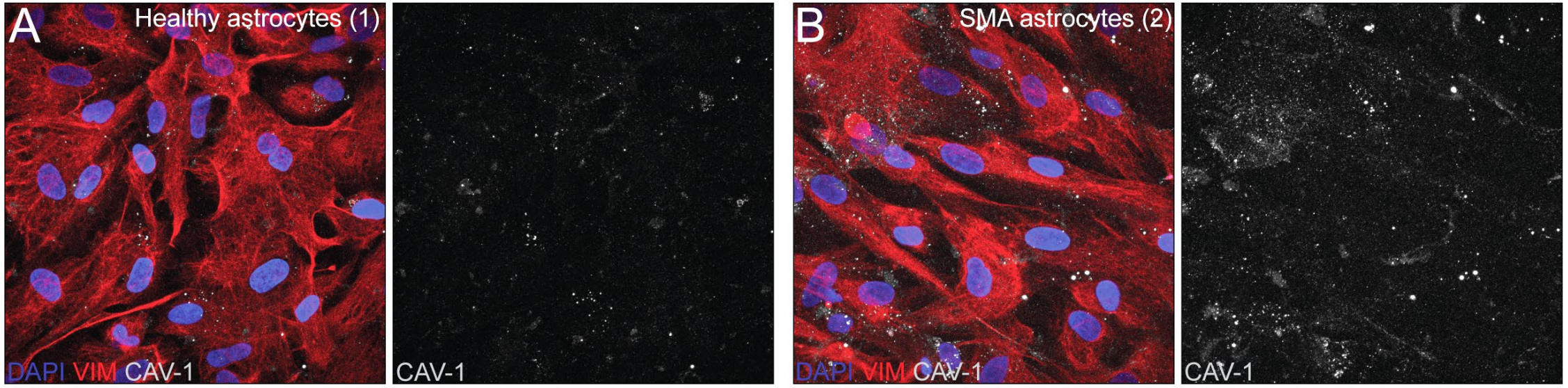


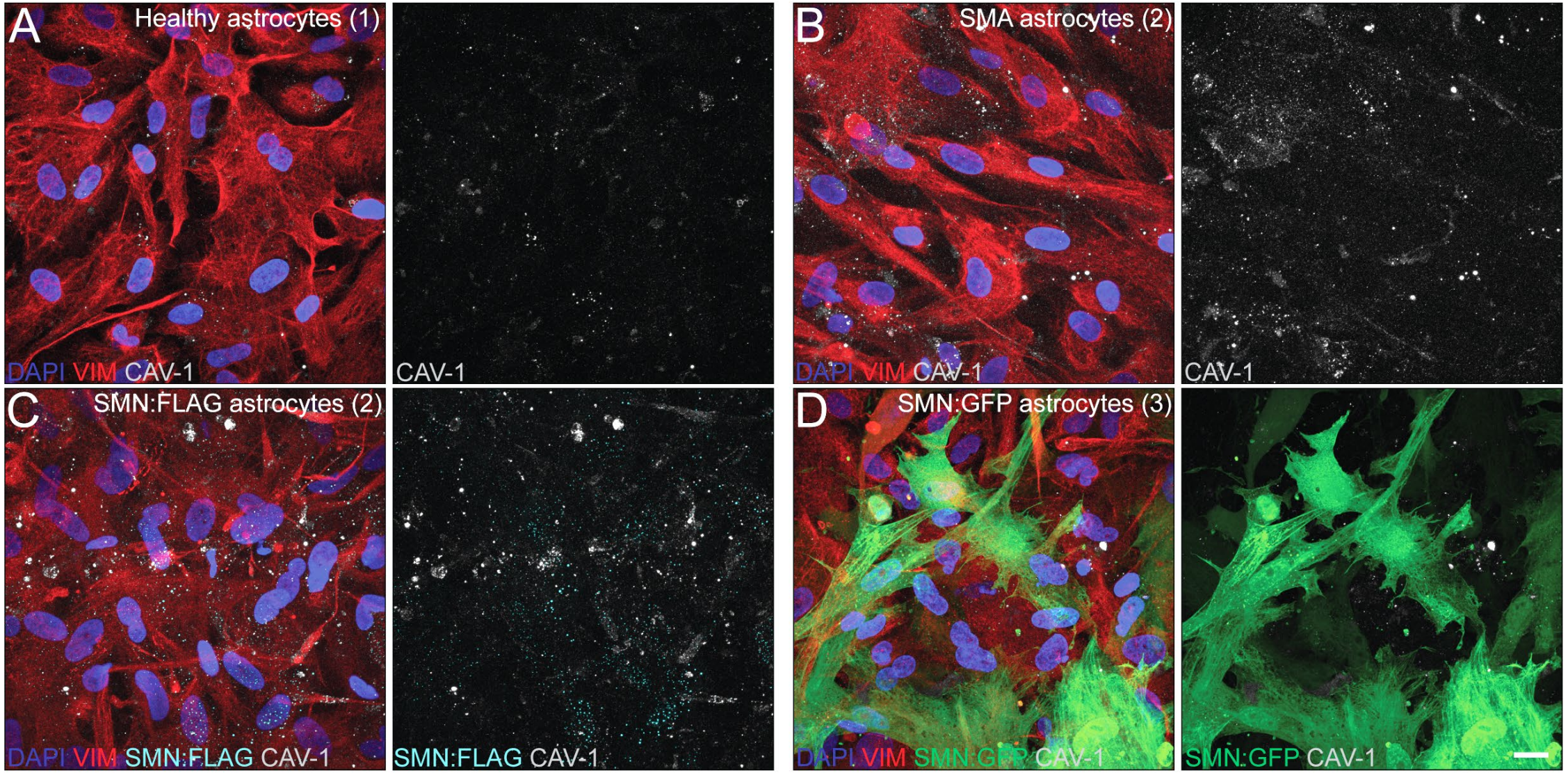
Abnormally upregulated caveolin-1 levels in patient astrocytes



Caveolin-1 (CAV-1)

- Scaffold protein required for the formation of caveolae (lipid raft endocytosis)
- Directly interacts with SMN to facilitate local protein translation (Gabanella et al., 2016)
- DJ-1 deficiency impairs caveolin-1 levels, lipid raft endocytosis and glutamate transporter expression and glutamate uptake in Parkinson's disease astrocytes (Kim et al., 2016)

Partial restoration of CAV-1 phenotype after SMN re-expression



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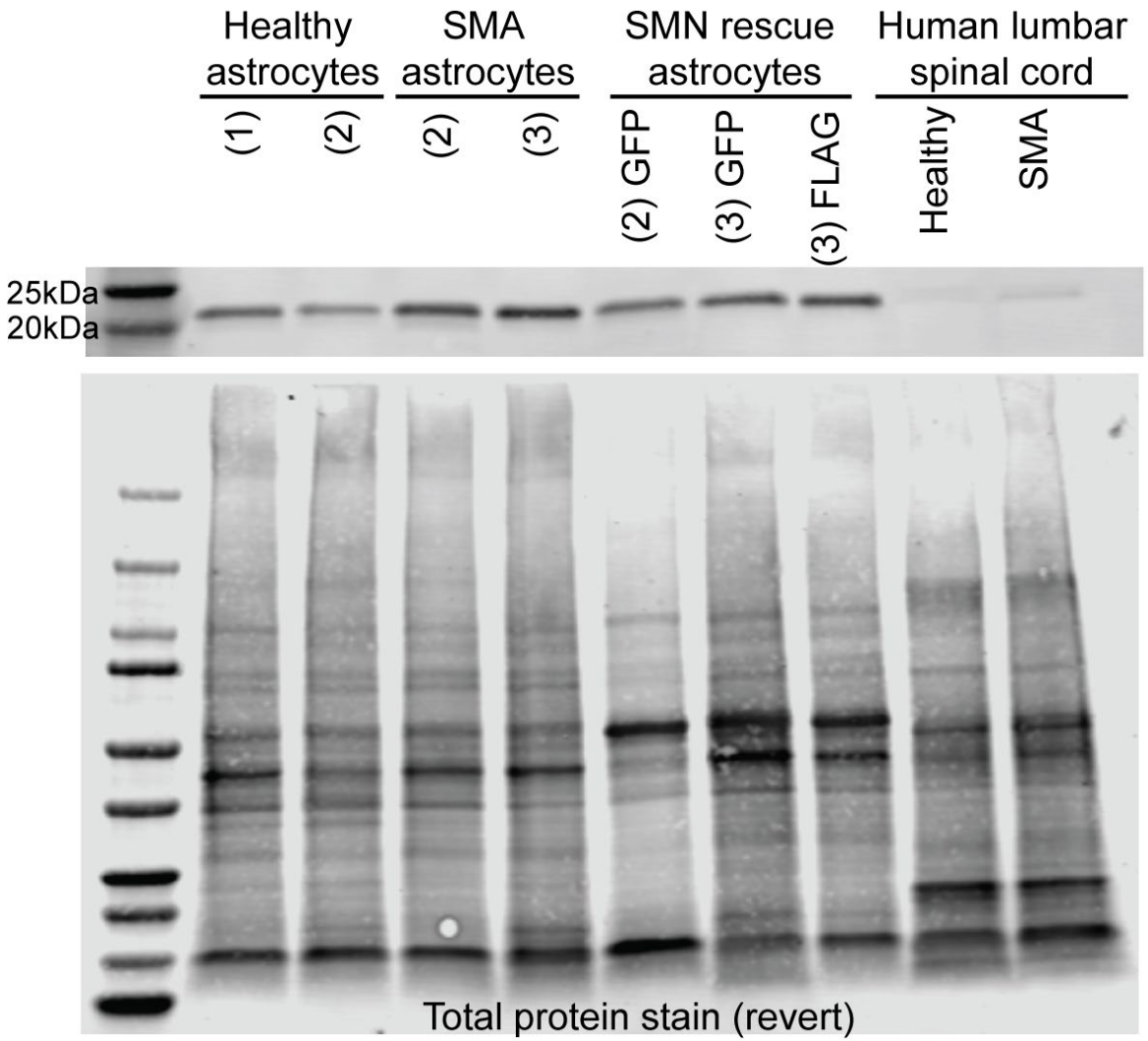
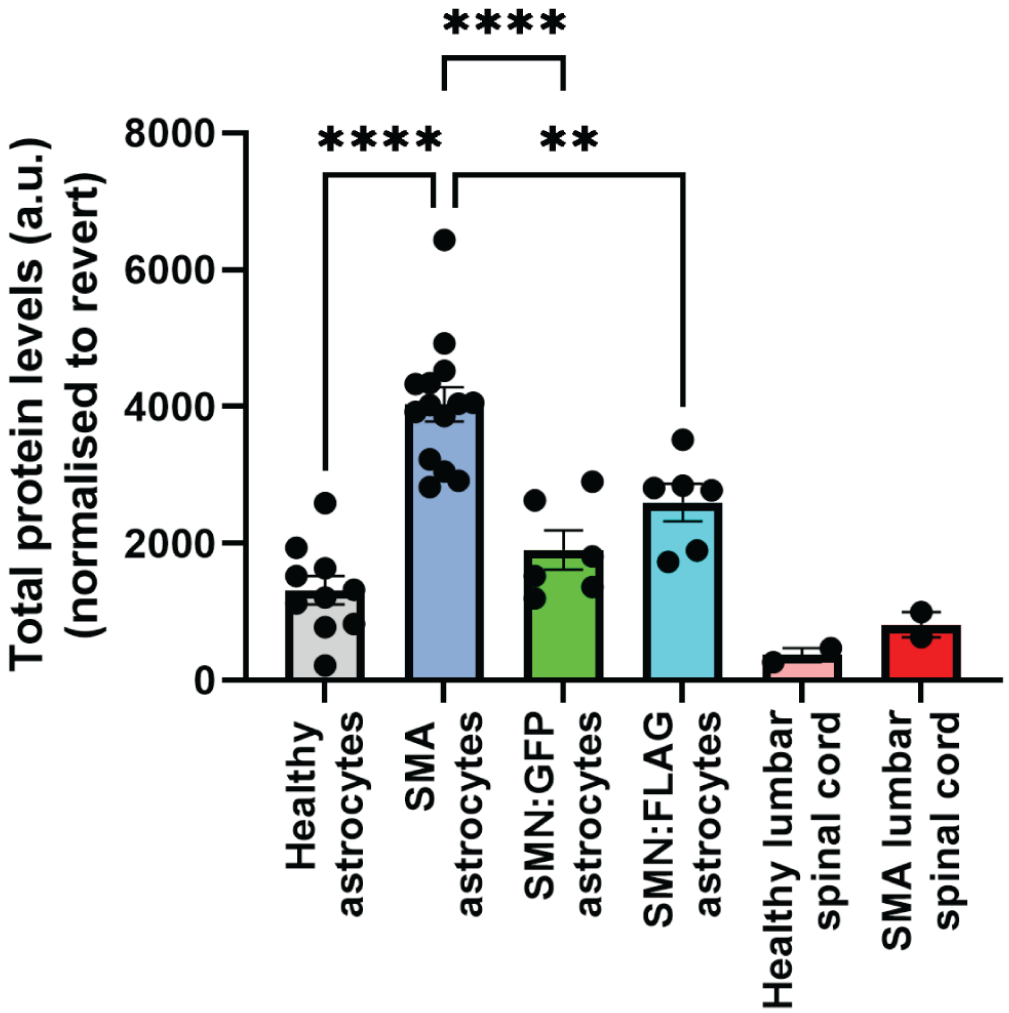
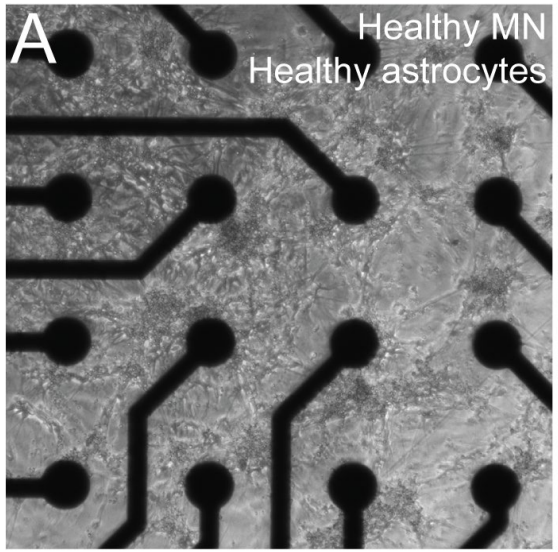
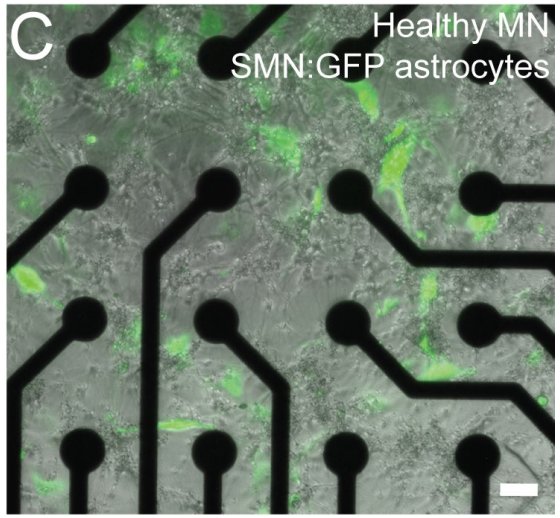


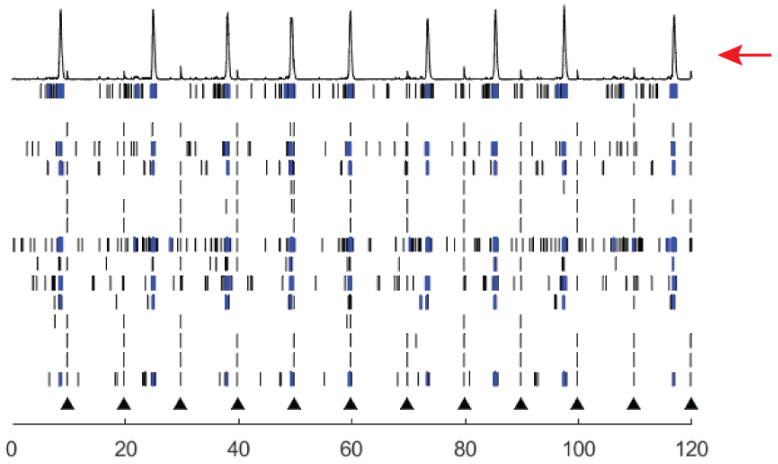


Minimal MN activity in SMN:FLAG/GFP patient-derived astrocytes

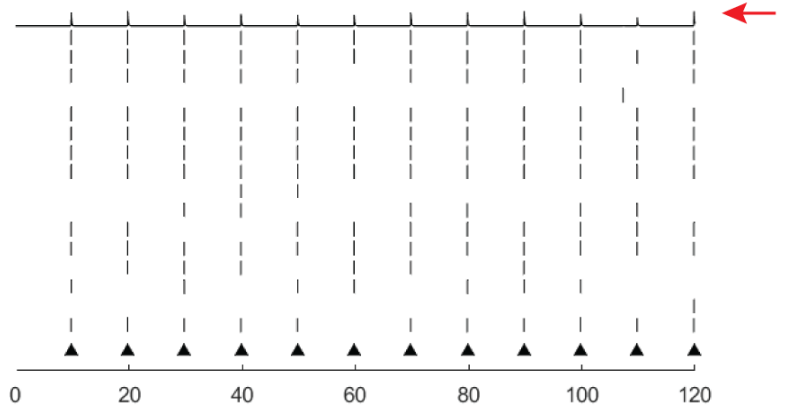




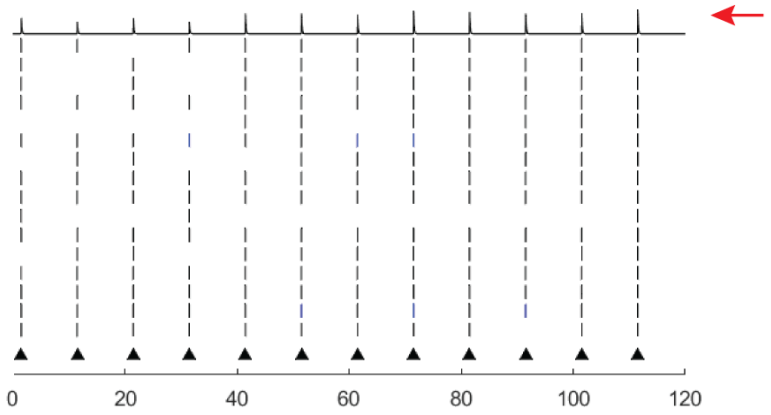
Healthy MN healthy astrocytes



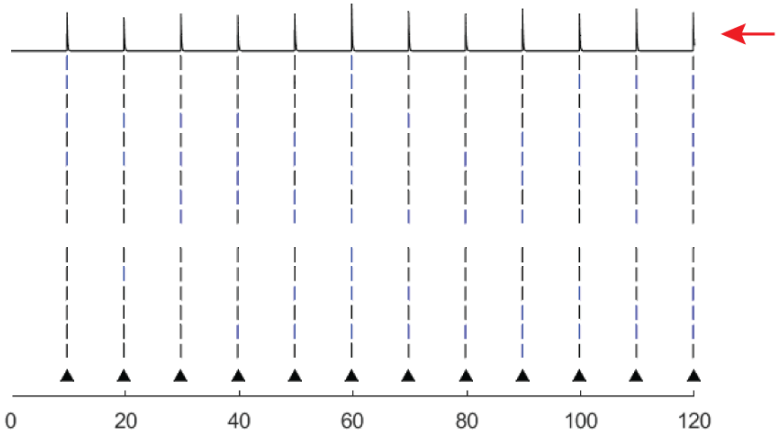
SMA MN SMA astrocytes



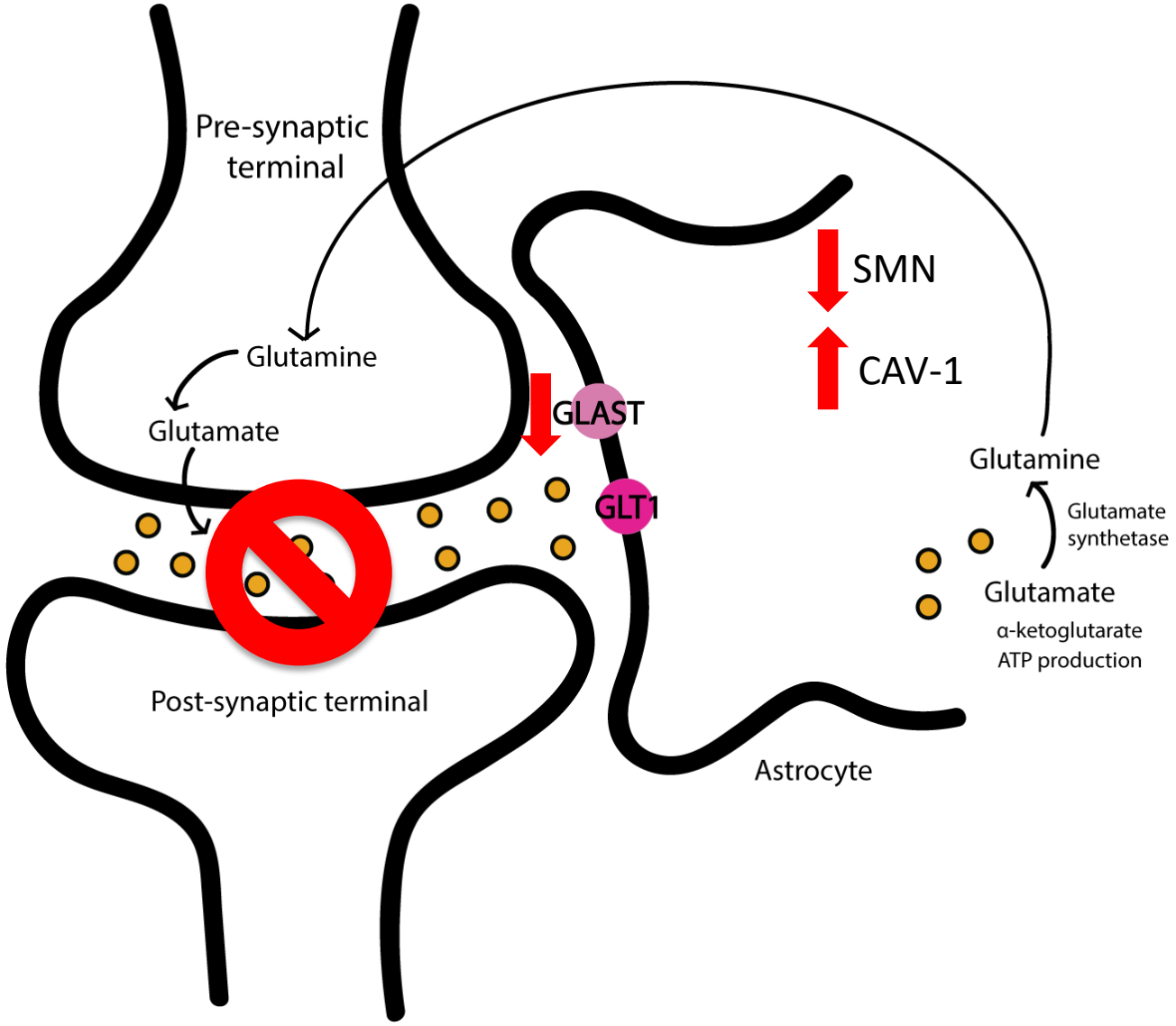
Healthy MN SMN:FLAG astrocytes



Healthy MN SMN:GFP astrocytes

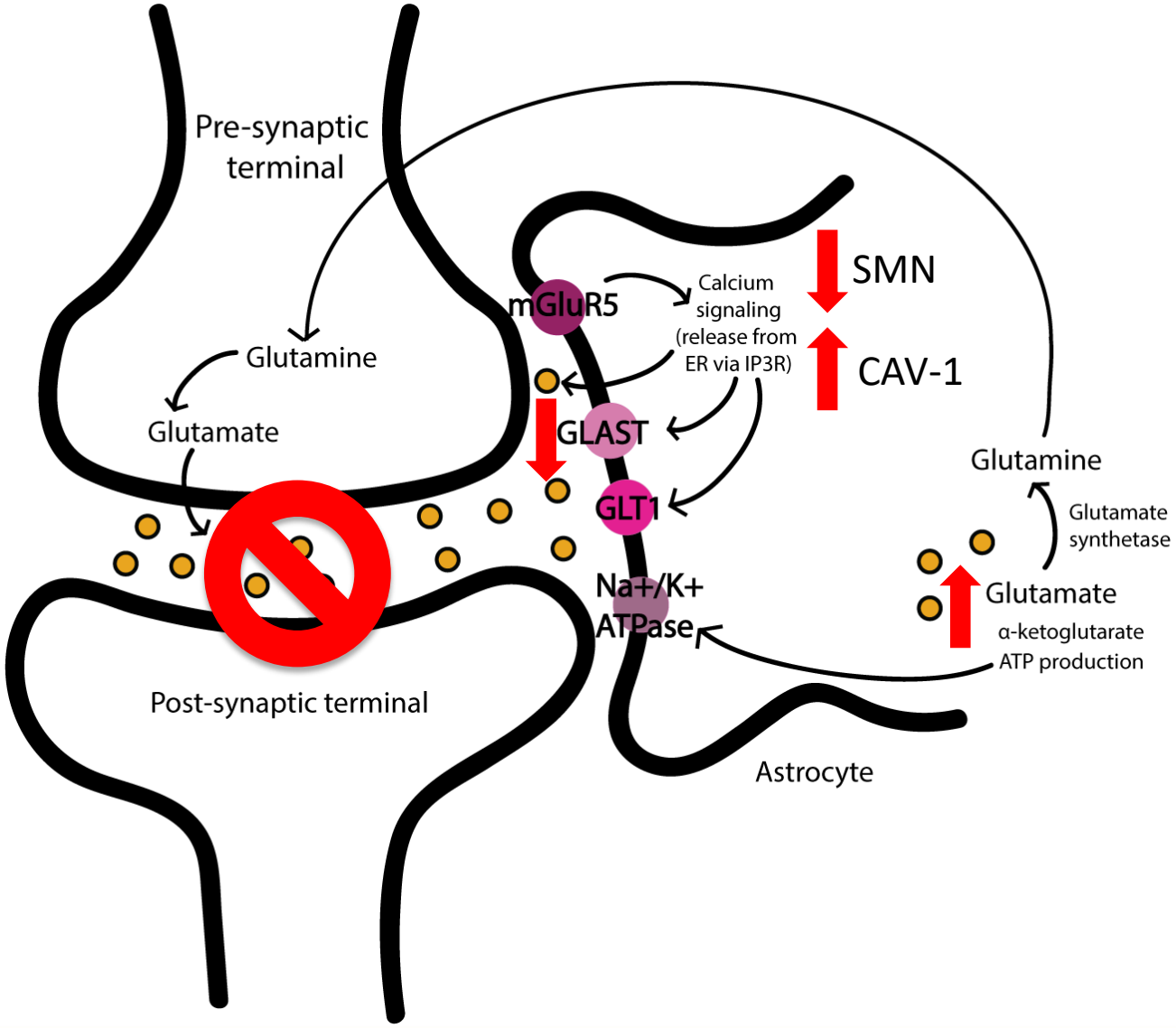


Conclusions and future directions



- SMA patient-derived astrocytes
  - directly impede motor neuron activity
  - reduced GLAST levels
  - Increased CAV-1 levels
- Disease mechanism involving SMN-CAV-1 regulation of GLAST
  - Impaired local protein translation and turnover of plasma membrane protein-> disrupted glutamate neurotransmission?
- SMN-dependent/associated mechanism?
  - Further SMN modulation needed?
  - Reactivity-> insensitive to SMN restoration?

Conclusions and future directions



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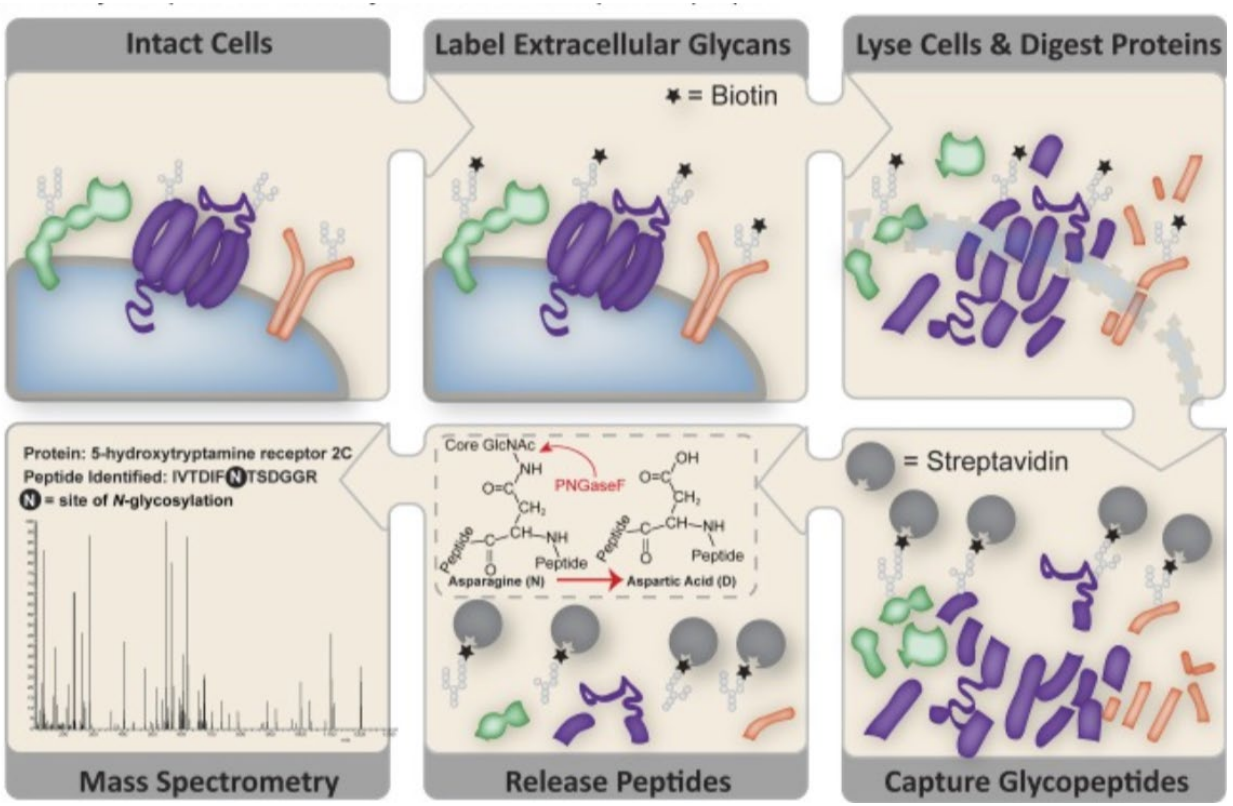
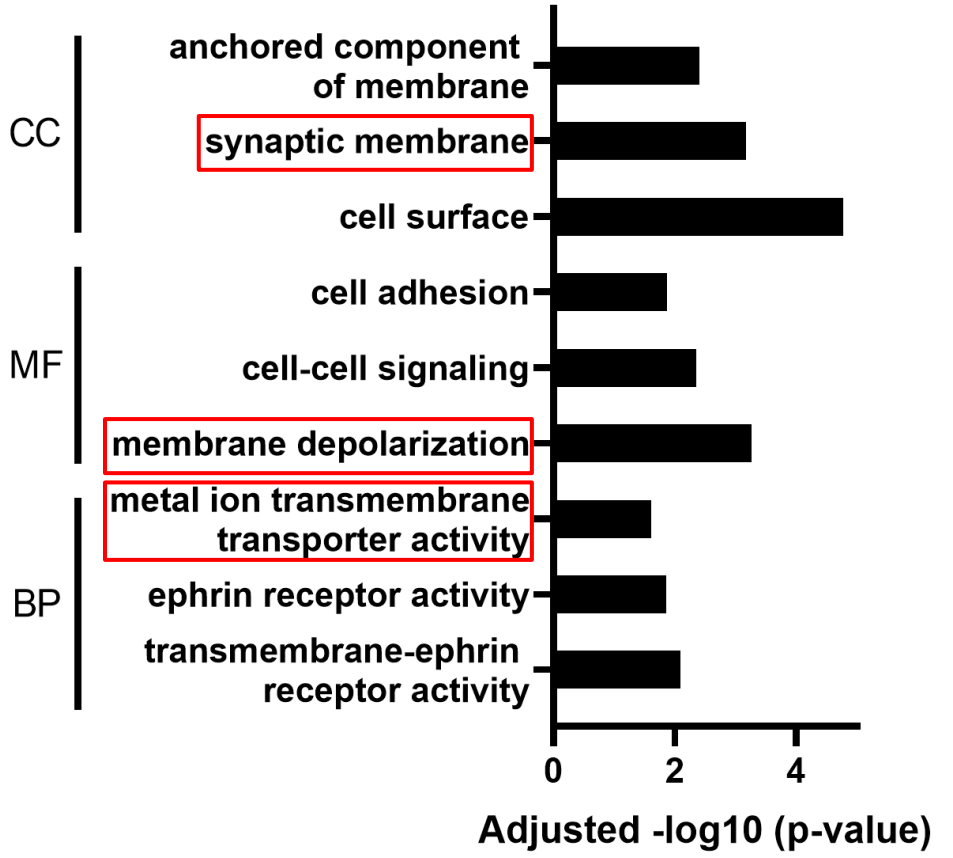
- SMN-dependent/associated mechanism?
  - Further SMN modulation needed?
  - Reactivity-> insensitive to SMN restoration?
- Other astrocytic defects linked glutamate neurotransmission?
  - Related to GLAST regulation (mGluR, Na+/K+ ATPases, calcium signaling)
  - Increased glutamate levels
    - Disrupted glutamate-glutamine cycle
  - Impaired synaptogenesis (ephrins)

Microscale cell surface capture mass spectrometry: enrichment of synapse-related glycoproteins in healthy-derived astrocytes



Collaboration with Dr. Rebekah Gundry (University of Nebraska Medical Center)

Enriched in healthy astrocytes only
(59 unique cell surface glycoproteins)



Acknowledgments

Ebert laboratory, MCW

- Allison Ebert, PhD
- John Whitinger IV, MD (Child Neurology resident)
- Ben O'Brien Hokanson (graduate student)
- Reilly Allison (graduate student)
- Jake Adelman (graduate student)

cure SMA Make today a breakthrough.

Audrey Lewis Young Investigator Award (2020)

NIH National Institute of Neurological Disorders and Stroke

Collaborations

uCSC mass spectrometry
- Rebekah Gundry, PhD
- Linda Luecke Berg, PhD

SMN:GFP/FLAG plasmids
- Xue Jun Li, PhD (University of Illinois-Chicago)
- Christian Lorson, PhD (University of Missouri)

Lentivirus production
- Viral Vector Core Facility (MCW/ Versiti BRI)

Microelectrode array system
- Cardiovascular Center (MCW)

mRNA sequencing
- Sridhar Rao, MD, PhD (MCW/Versiti BRI)

Human samples

Human iPSC lines (2 healthy individuals and 3 SMA patients)

Human spinal cord tissue
- University of Maryland Brain and Tissue Bank
- NIH NeuroBioBank

Questions/Info

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