SCUOLA NORMALE SUPERIORE

CNR NANO PISA NEST

Second Year PhD Exam

External Supervisor: Dr. Marco Cecchini

Internal Supervisor: Prof. Gian Michele Ratto

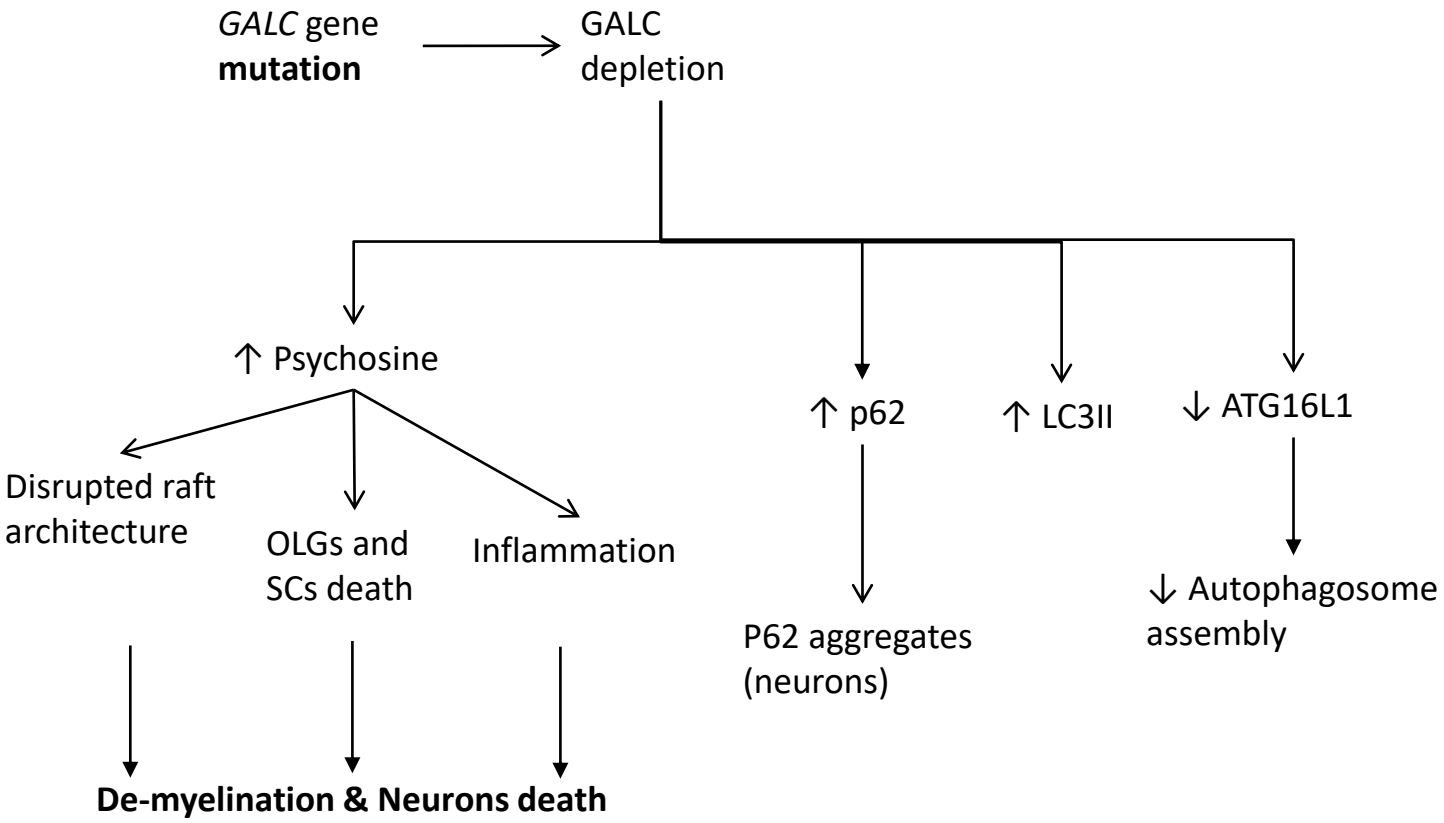
Roberta Mezzena

Pisa, 20/10/2020

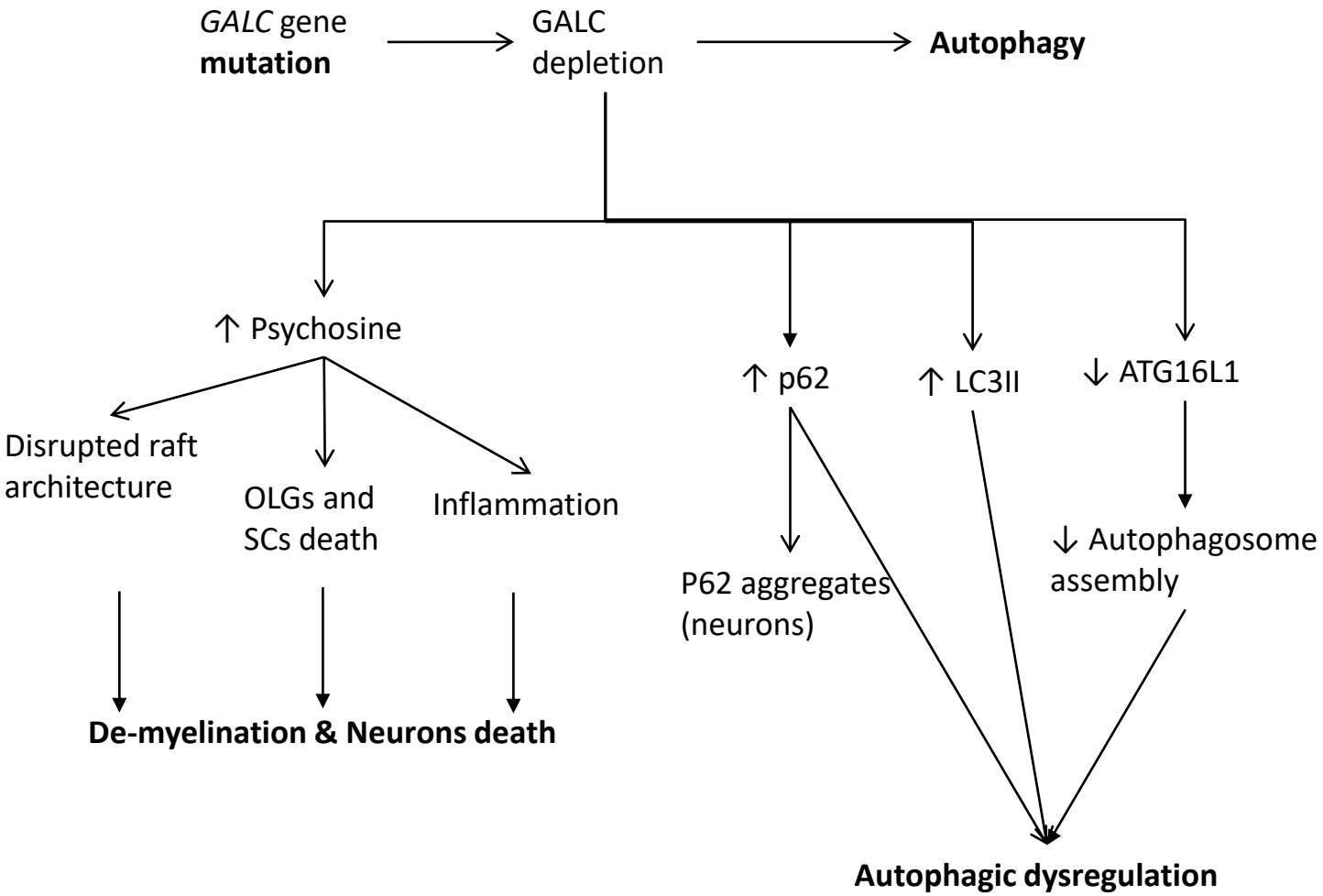
National Enterprise for nanoScience and nanoTechnology

NEST

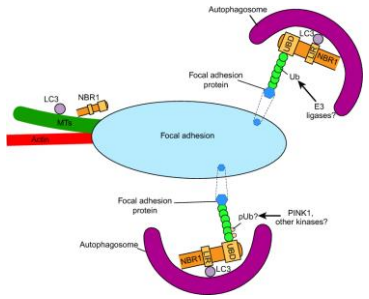
Krabbe Disease



Krabbe Disease



Krabbe Disease



GALC gene **mutation** → GALC depletion → **Autophagy** ↔ NBR1*

↑ Psychosine
- Disrupted raft architecture
- OLGs and SCs death
- Inflammation

→ **De-myelination & Neurons death**

↑ p62 → P62 aggregates (neurons)

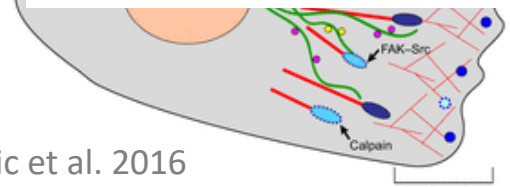
↑ LC3II

↓ ATG16L1 → ↓ Autophagosome assembly

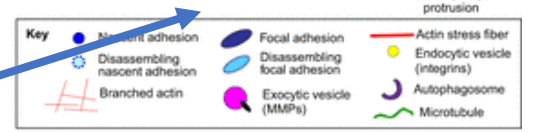
Autophagic dysregulation

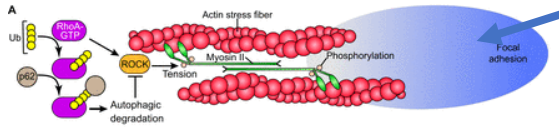
Turn-over focal adhesion → Migration

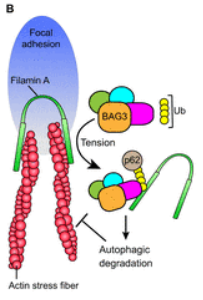
Turn-over focal adhesion → Mechanotransduction

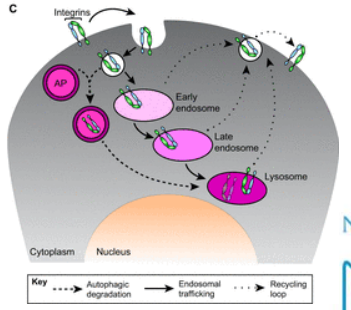


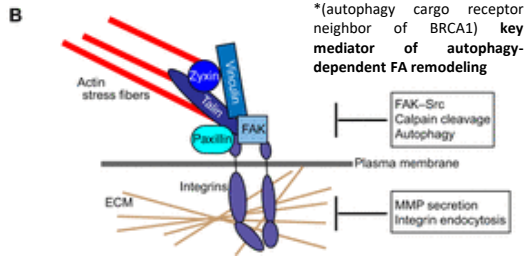
Kenific et al. 2016











*(autophagy cargo receptor neighbor of BRCA1) **key mediator of autophagy-dependent FA remodeling**

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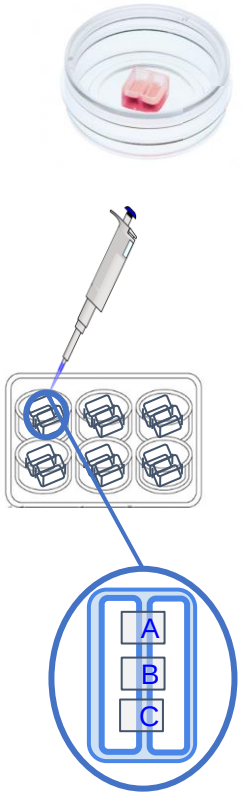
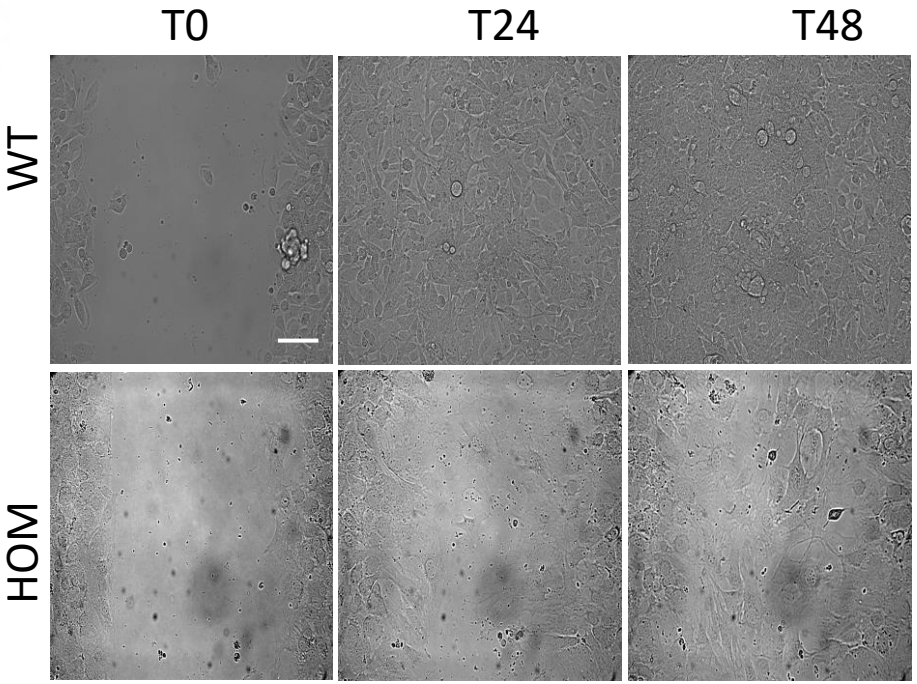
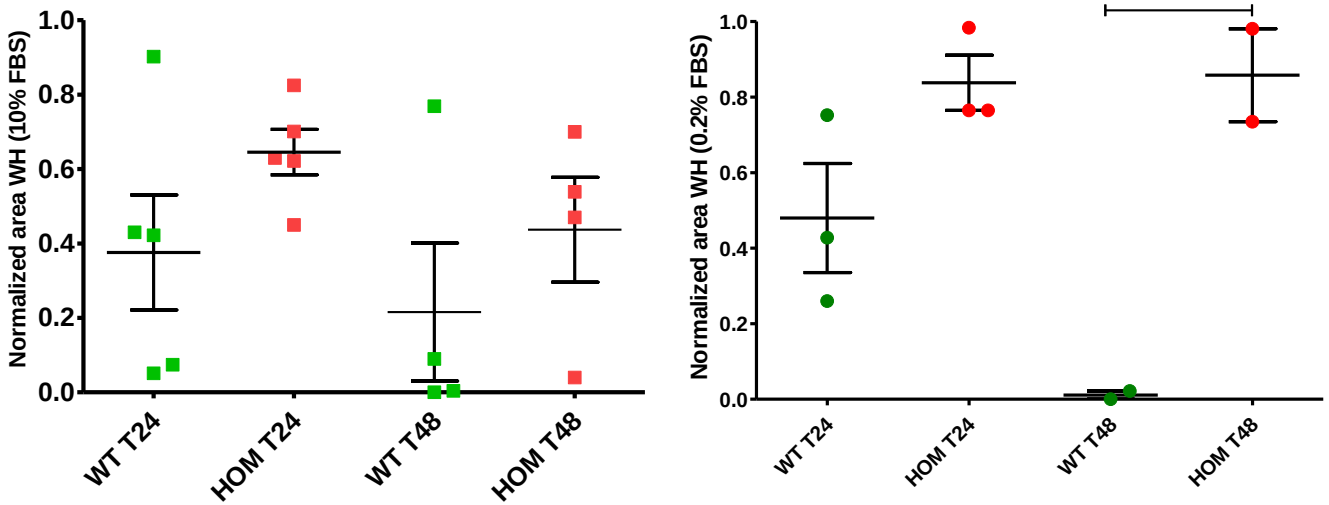
Collective migration KD fibroblasts



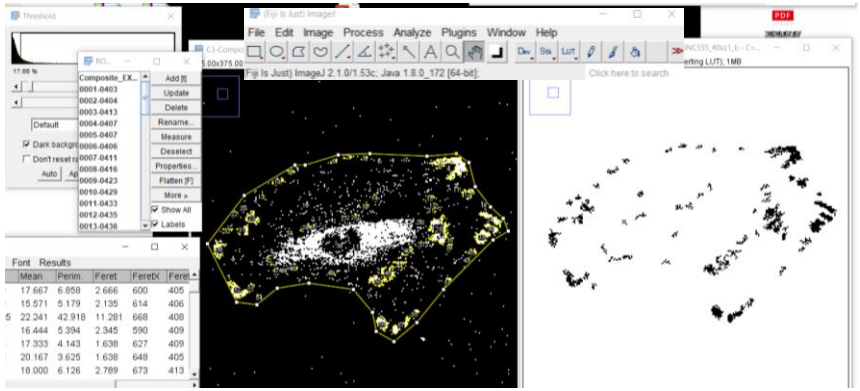


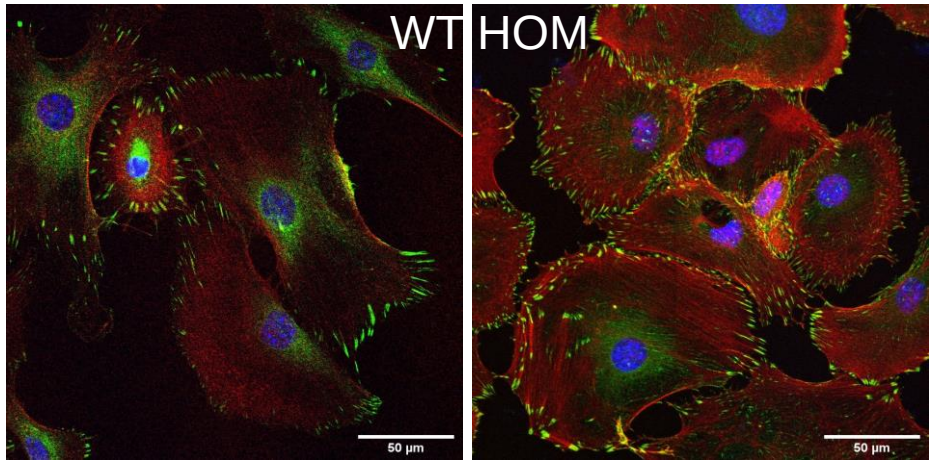


At least 2 area for each experiments were used, *t*-test

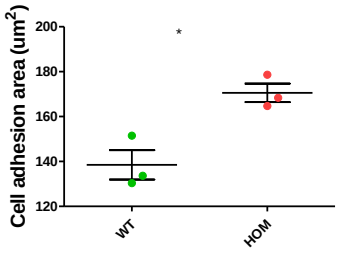
HOM fibroblasts tend to close the gap less

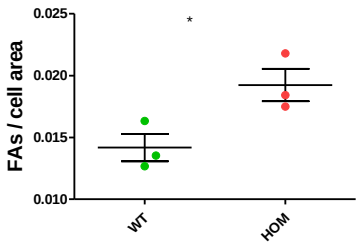
Focal Adhesion KD fibroblast

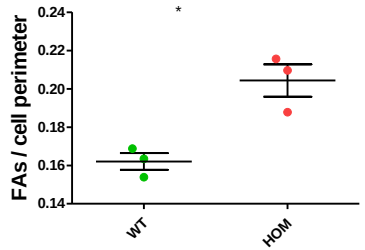


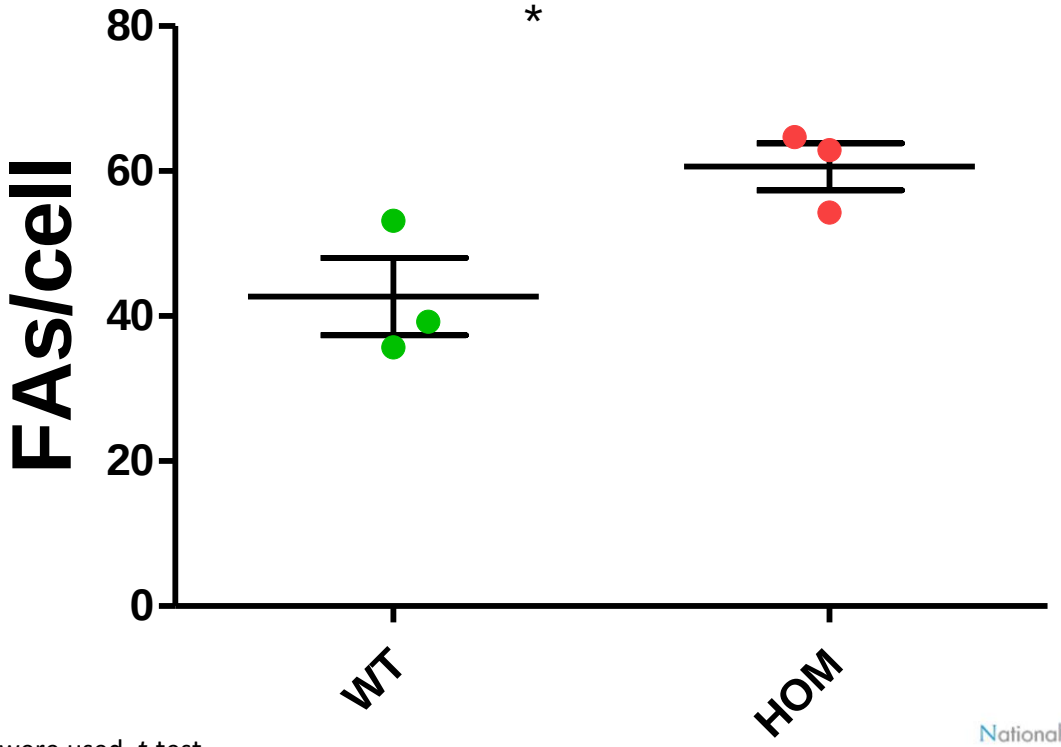


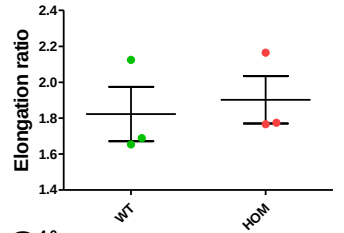
WT fibroblasts do, respect to HOM, less FAs

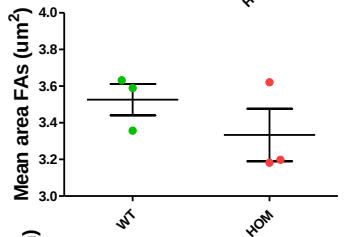


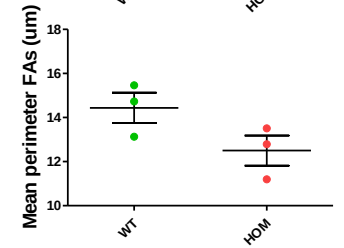






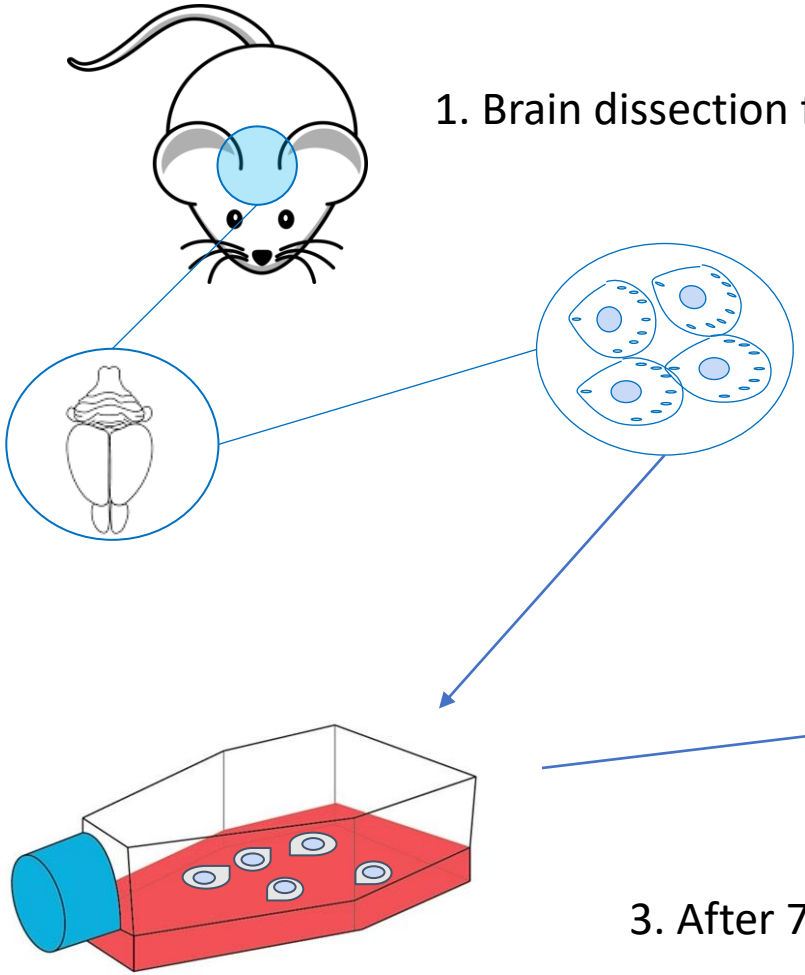






At least 15 cells for each exp were used, *t*-test

Glial KD cells

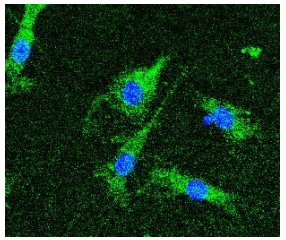


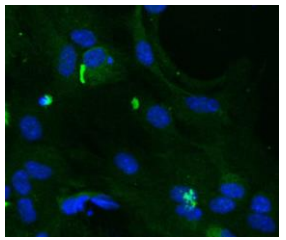
1. Brain dissection from P0-P3 newborn mouse

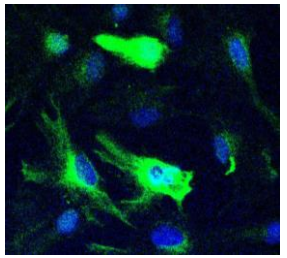
2. The tissue is finely shredded and plated in a flask (T25)

3. After 7-10 days cells are confluent.

4. Three cell layers have formed. From top to bottom we have:
   1. Microglia
   2. Oligodendrocytes
   3. Astrocytes







5. With a shaker (or manually) the three layers are divided and the each cell-type plated according to its needs.

Angelman Syndrome

IOPscience

Nanotechnology

PAPER

Study of adhesion and migration dynamics in ubiquitin E3A ligase (UBE3A)-silenced SYSH5Y neuroblastoma cells by micro-structured surfaces

R Mezzena[1], C Masciullo[1], S Antonini[1], F Cremisi[2], M Scheffner[3], M Cecchini[1] and I Tonazzini[1,4]

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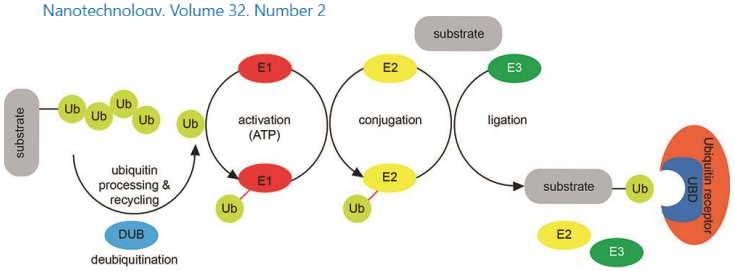
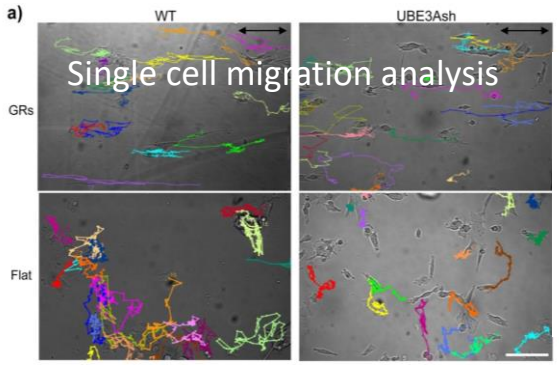
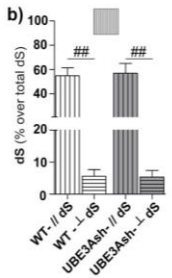
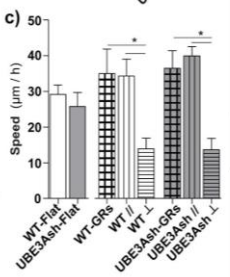
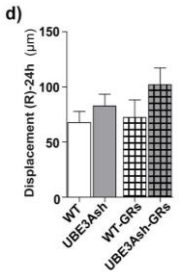




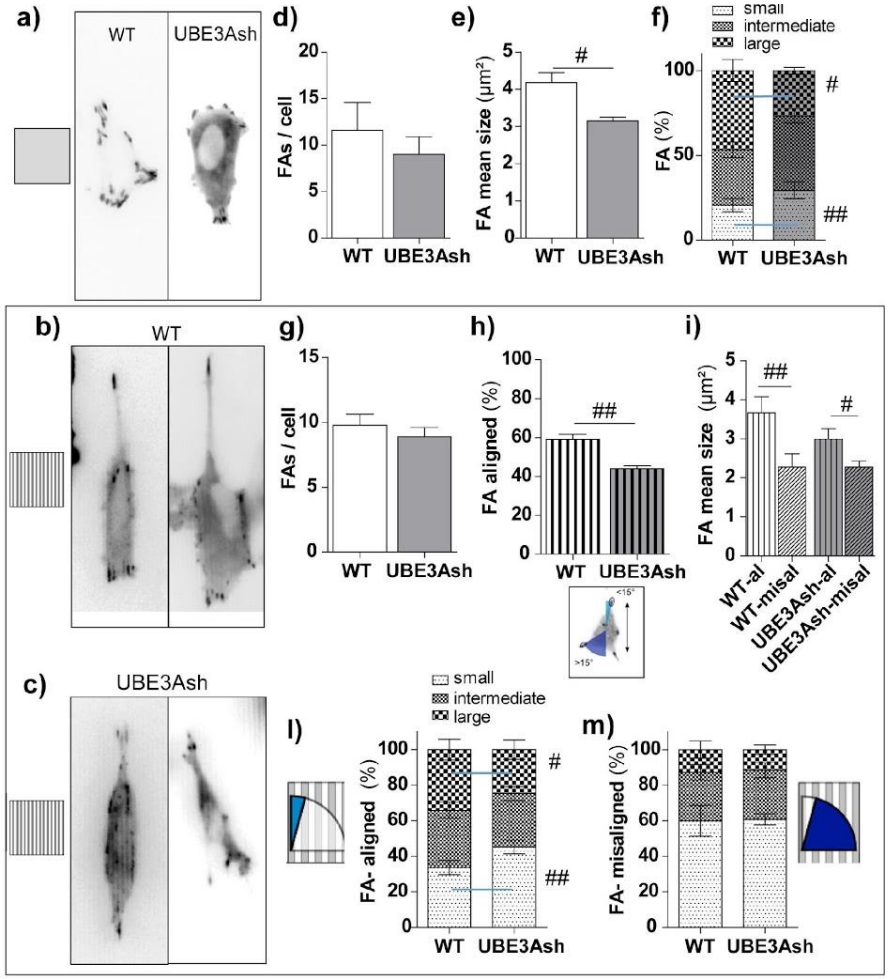
Single cell migration analysis



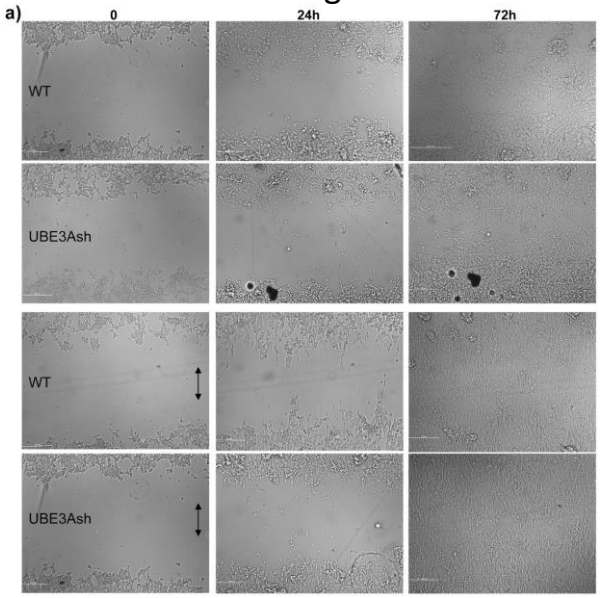




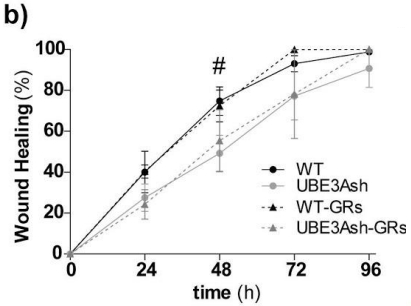
Impact of the loss of UBE3A on FA assembly and spatial distribution in SHs neuronal cells.



SHs collective migration



Wound closure



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Peripheral Nerve Injuries

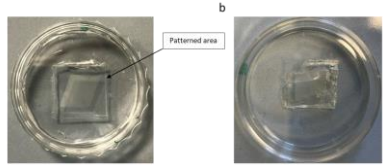
Solvent casting patterned chitosan (CS) film for periferal nerve regeneration:

1. O/N stirring of CS solution → **homogeneous solutions**.
2. Deposition of CS solution on patterned mold and evaporation of the solvent at RT → **less rigid and brittle films**.
3. Neutralization for 30' with 0.5% NaOH solution → **polymer mesh less stressed** and a more **cell-friendly substrate** than the previous one.

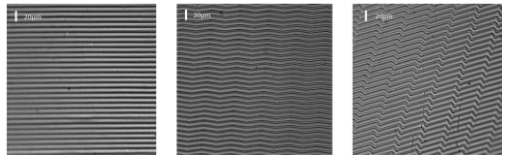
→ **Time cutting** from 11 hours to 1 hour and 30 minutes.

→ Completely **out of the CR**, making it repeatable also in other lab.

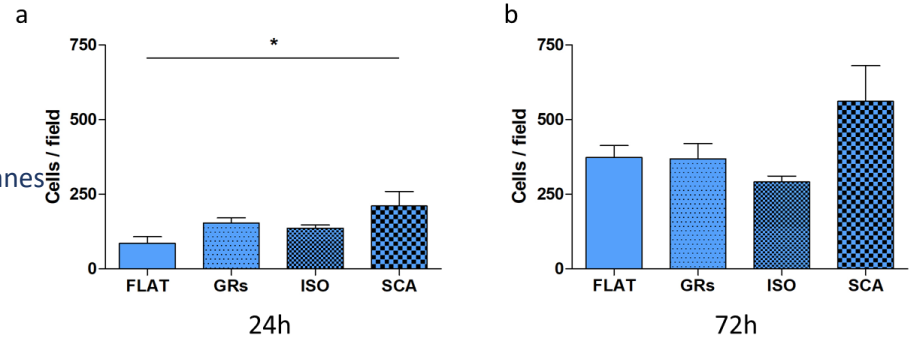
PDMS intermediate mold and chitosan film



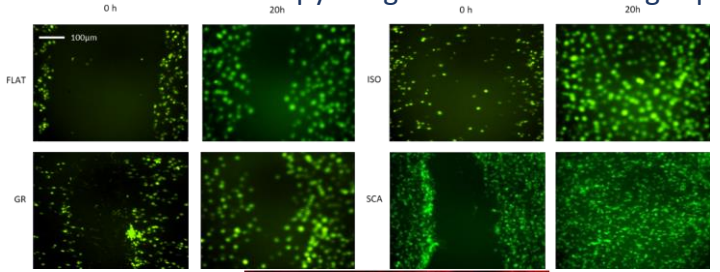
Bright filed microscopy of the micro-structured chitosan membranes



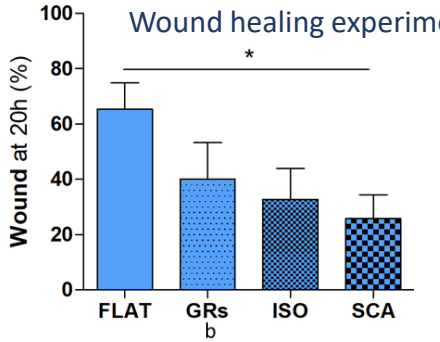
Proliferation rate of RT4 Schwann cells



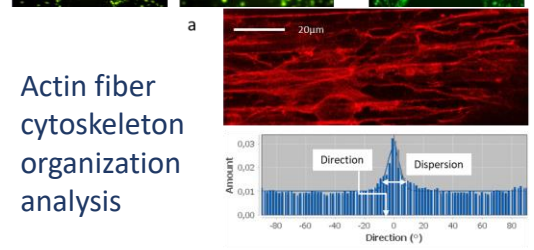
Fluorescence microscopy images of wound healing experiments



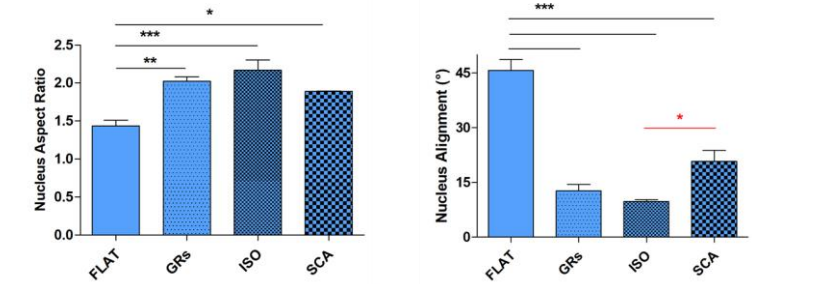
Wound healing experiments



Actin fiber cytoskeleton organization analysis



Nuclear morphological analysis.



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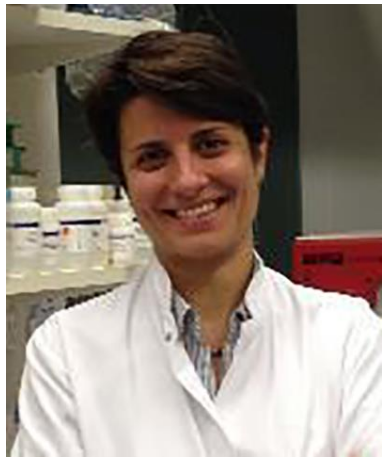
NeuroSens group

Mentors

Dr. Marco Cecchini



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