ASSESSMENT OF SATELLITE PROGENITOR CELL DIFFERENTIATION IN HD SKELETAL MUSCLE *IN VITRO*

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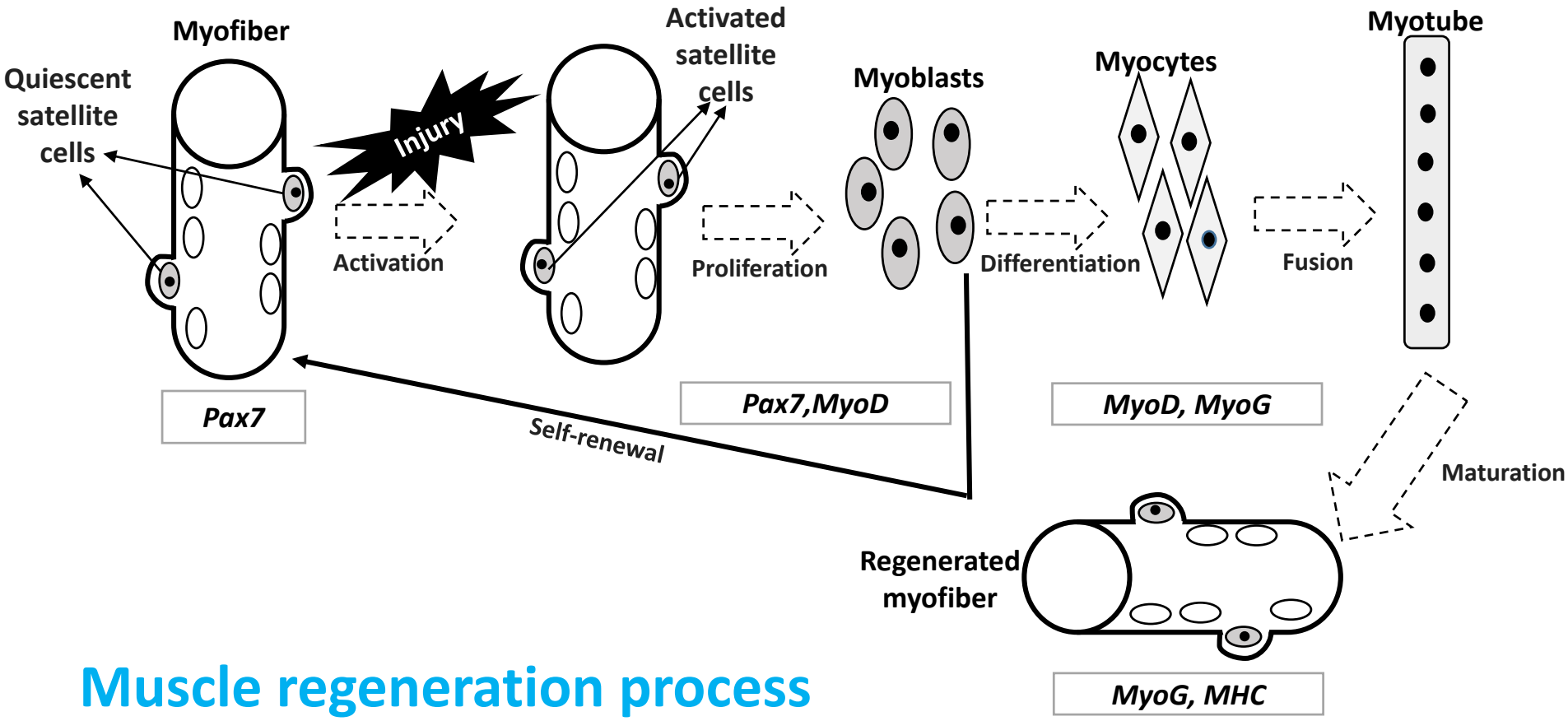
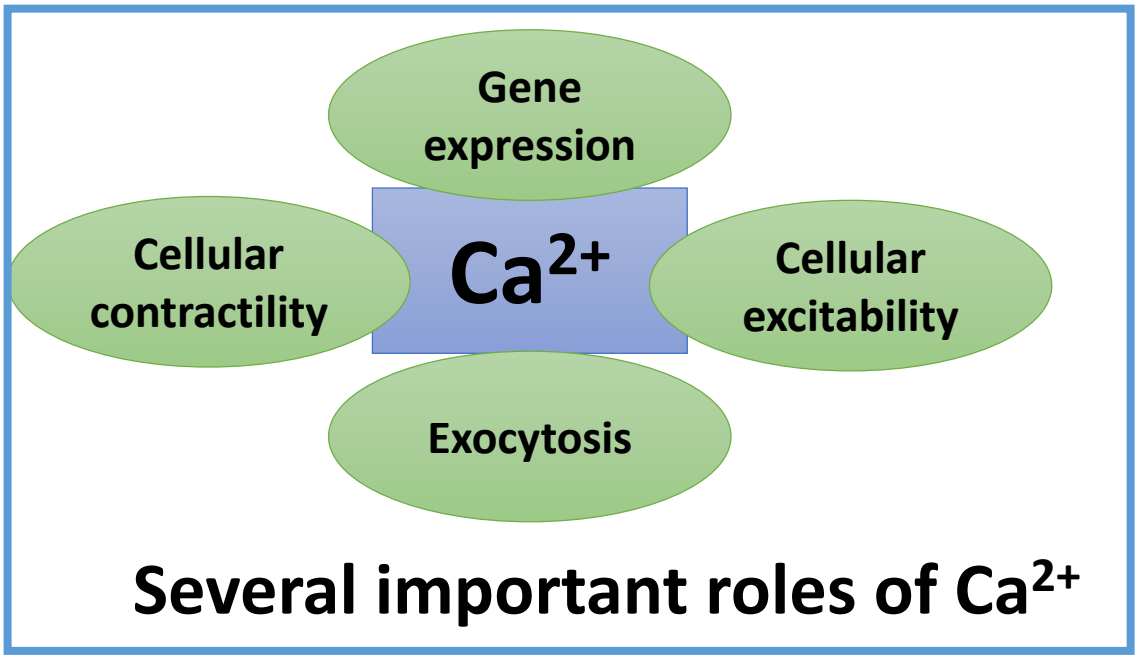
Background

Satellite progenitors and skeletal muscle



Muscle regeneration process

Intracellular Calcium and HD pathology



Several important roles of Ca^{2+}

Skeletal muscle pathology in HD:

- mHtt inclusion bodies
- Muscle wasting
- Reduced muscle strength
- Change in fiber type

HD affected neuron

Ca^{2+} homeostasis ↓

Does Ca^{2+} homeostasis contribute to this?

Giacomello, M. et al., Biofactors (2011)
Tang, T. S. *et al.*, Proc Natl Acad Sci U S A(2005)
Magnusson-Lind, A. *et al.*, J Huntingtons Dis(2014)

Ghrelin- A hunger hormone

- Regulate energy metabolism
- Improve brain function and neuronal survival
- Improve HD skeletal muscle morphology
- Induce Ca^{2+} mobilization


SCIENTIFIC REPORTS

OPEN

Ghrelin rescues skeletal muscle catabolic profile in the R6/2 mouse model of Huntington's disease

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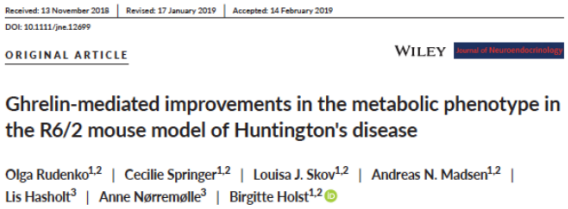
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ORIGINAL ARTICLE

WILEY Journal of Neuroendocrinology

Ghrelin-mediated improvements in the metabolic phenotype in the R6/2 mouse model of Huntington's disease

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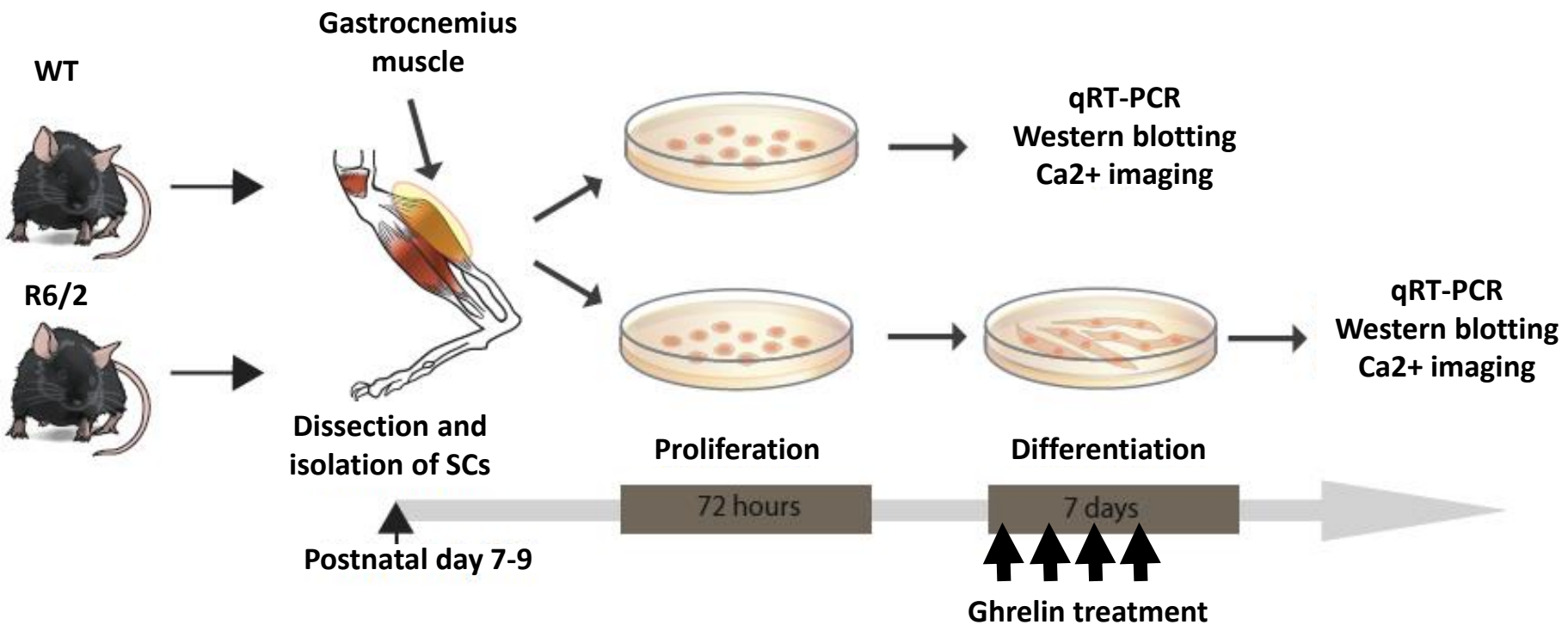
We aim to-

- **Establish an novel Ca^{2+} imaging analysis**
- **Characterize intracellular Ca^{2+} in R6/2 mouse muscle and HD patient myoblasts**
- **Assess the ameliorating effect of ghrelin on Ca^{2+} dynamics in myofibers**

Methods

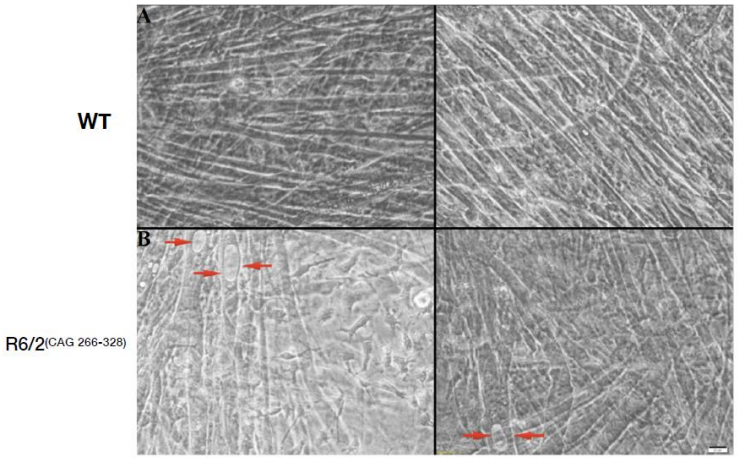
Satellite cell (SC) isolation



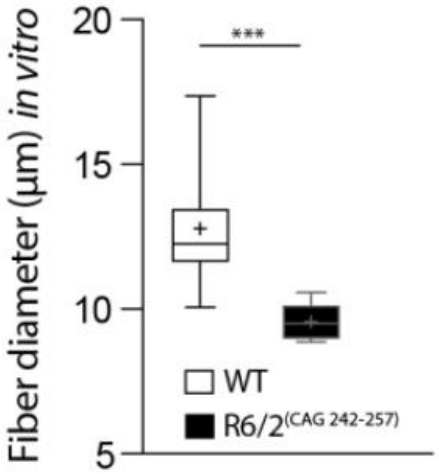
Results

R6/2 mice exhibit reduced myofiber diameter and MyoD expression level

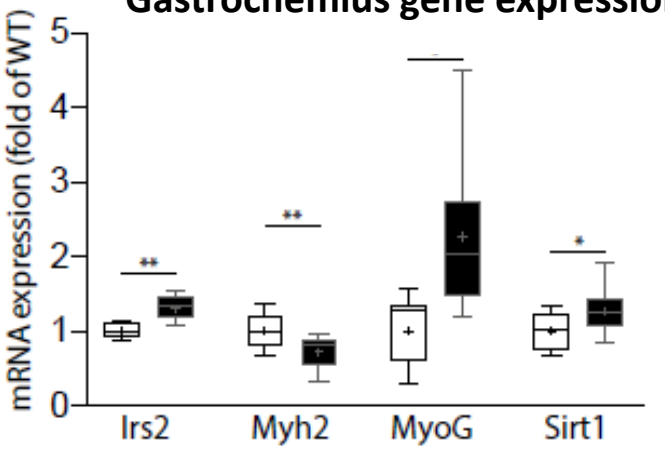
8th day of differentiation



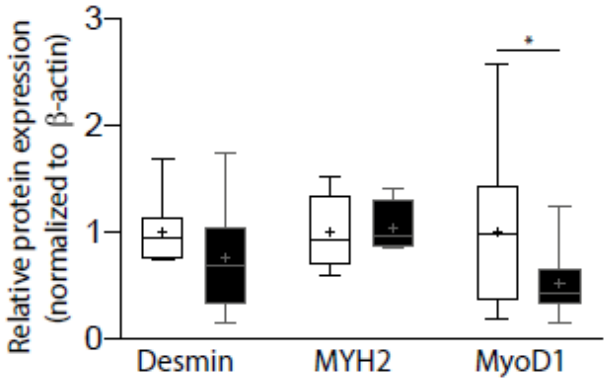
Gastrocnemius



Gastrocnemius gene expression

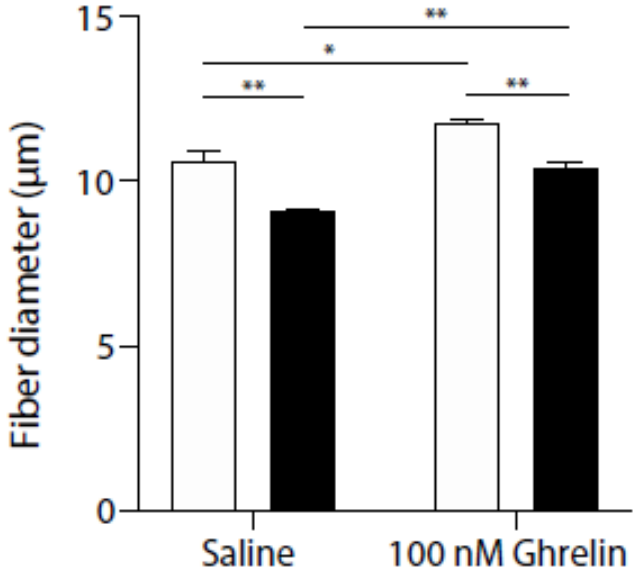


Gastrocnemius protein level

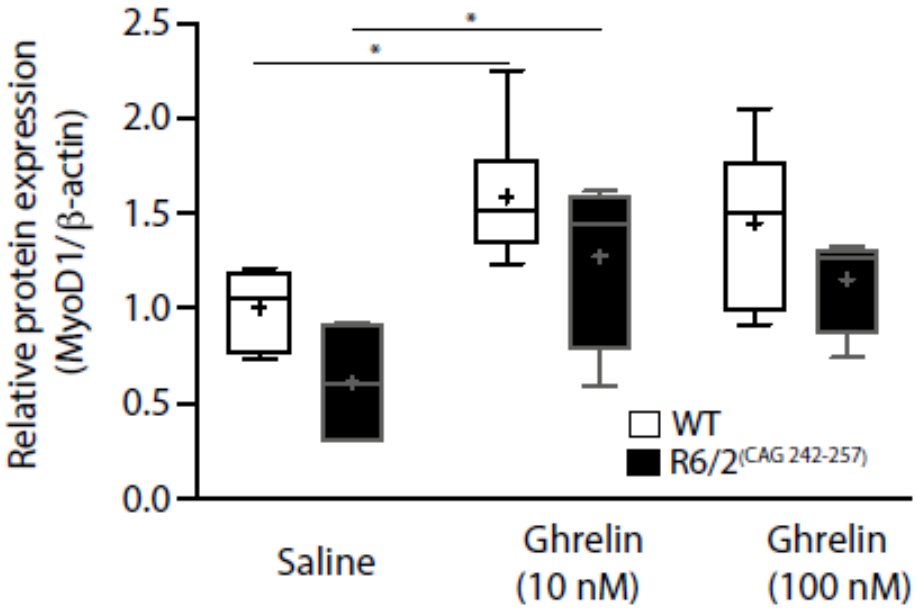


Ghrelin treatment ameliorates fiber size and MyoD expression level

Ghrelin treatment *in vitro*



MyoD1 protein levels *in vitro*



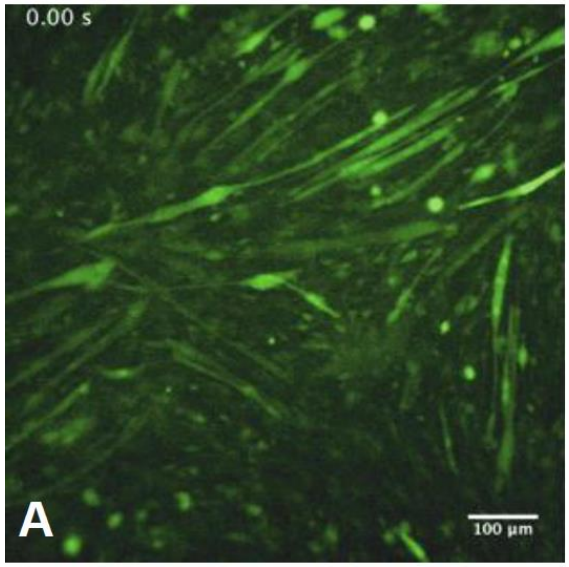
Summary

From our preliminary data, R6/2 mice exhibit:

- Reduced myofiber diameter compared to their WT littermates
- Altered gastrocnemius gene expression
- Reduced myoD expression level

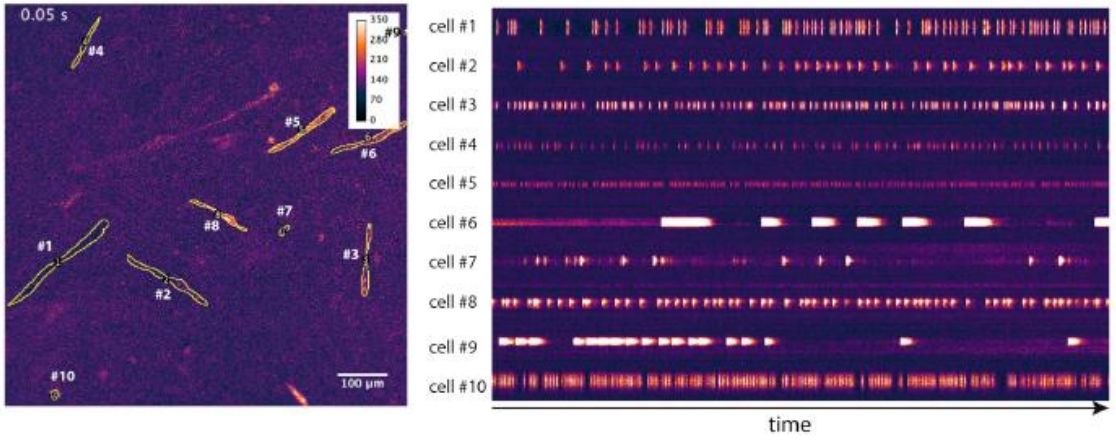
Treatment with Ghrelin may delay muscle atrophy by increasing myofiber diameter and improving MyoD expression level

Ongoing- Ca2+ imaging *in vitro*



The myofibers from WT mice are imaged using Fluo-4 AM calcium indicator at 7th day of differentiation

Cell calcium pattern in time domain



This can be correlated with shape descriptors:

	Area [um]	Perim.[um]	Circ.	Feret [um]	FeretX [um]	FeretY [um]	MinFeret	AR	Round	Solidity
Cell #1	3114.004	549.345	0.13	256.609	6.217	595.295	24.619	15.439	0.065	0.611
Cell #2	1631.199	399.26	0.144	187.162	239.361	494.265	23.568	12.282	0.081	0.564
Cell #3	975.995	311.625	0.126	142.131	707.204	575.089	14.376	14.343	0.07	0.632
Cell #4	659.521	241.142	0.143	114.902	129.006	124.344	11.175	13.434	0.074	0.662
Cell #5	1304.548	343.889	0.139	155.693	520.688	290.653	15.791	14.026	0.071	0.618
Cell #6	1362.528	335.123	0.152	158.782	645.032	293.762	18.789	11.387	0.088	0.619
Cell #7	198.098	62.379	0.64	25.492	544.003	455.408	10.991	2.543	0.393	0.863
Cell #8	645.026	268.862	0.112	127.272	363.705	387.019	15.646	10.803	0.093	0.474
Cell #9	91.802	41.249	0.678	15.543	784.918	62.172	9.891	1.653	0.605	0.835
Cell #10	253.662	61.091	0.854	20.206	91.703	780.255	18.079	1.035	0.966	0.913