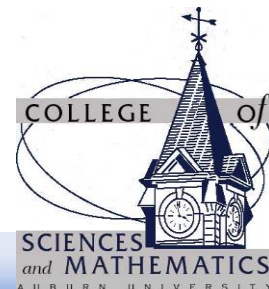




Behavioral Effects of SQSTM1/p62 Overexpression in Mice: Support for a Mitochondrial Role in Depression and Anxiety

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ALZHEIMER'S DISEASE

Quick Facts about Alzheimer's Disease and Dementia:

2015 Worldwide Cases of Dementia

47.5 MILLION
6th

2050 Worldwide Cases of Dementia

Leading cause of death in the United States
150 MILLION



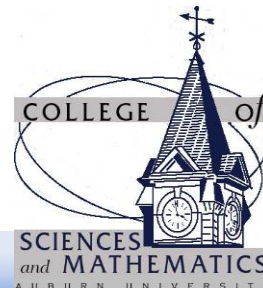
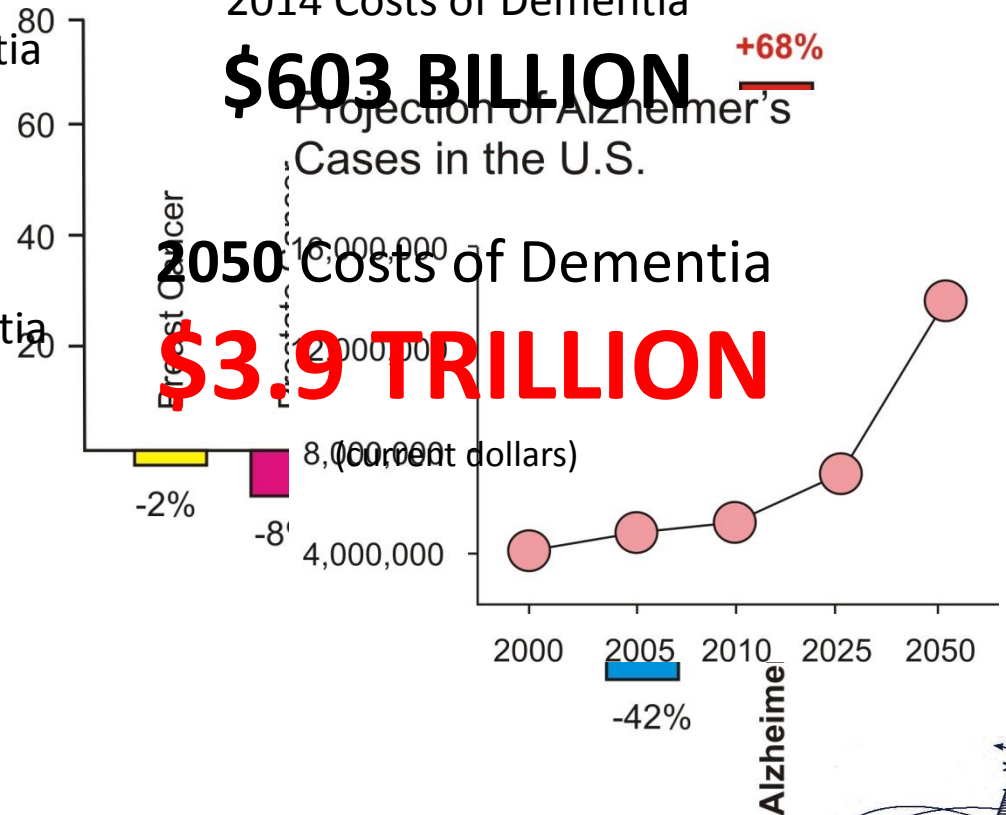
World Health Organization

alz.org | alzheimer's association

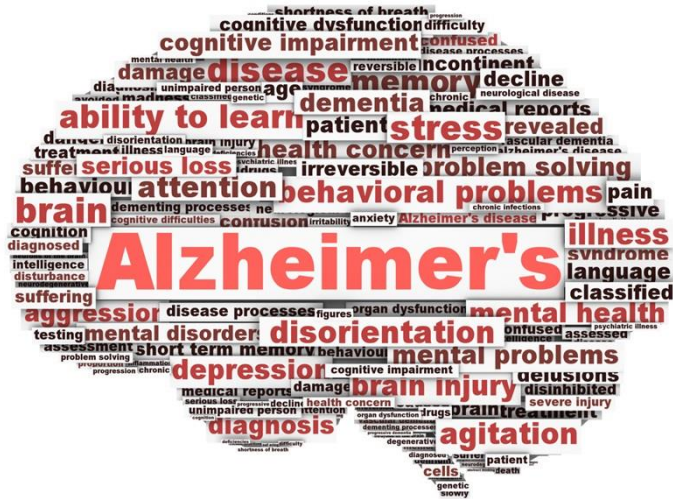
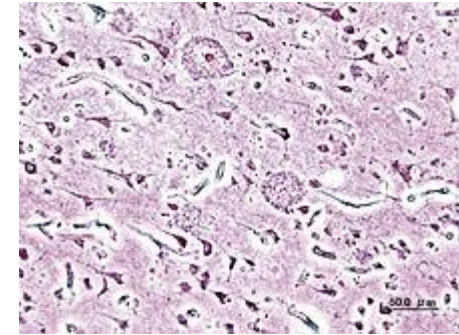
2014 Costs of Dementia
\$603 BILLION +68%

Projection of Alzheimer's Cases in the U.S.

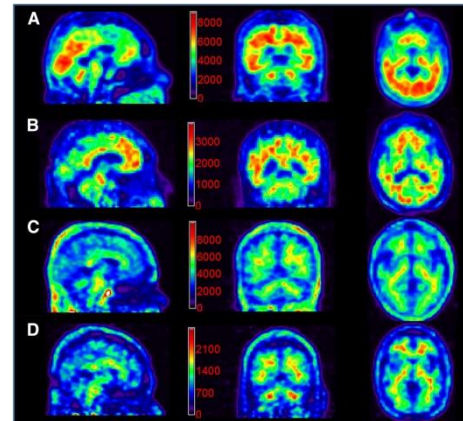
2050 Costs of Dementia
\$3.9 TRILLION



Histochemical Amyloid plaques

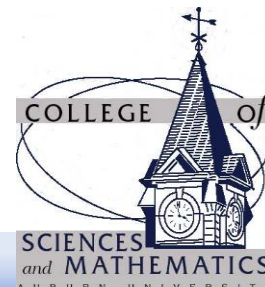


Imaging/PET Scans

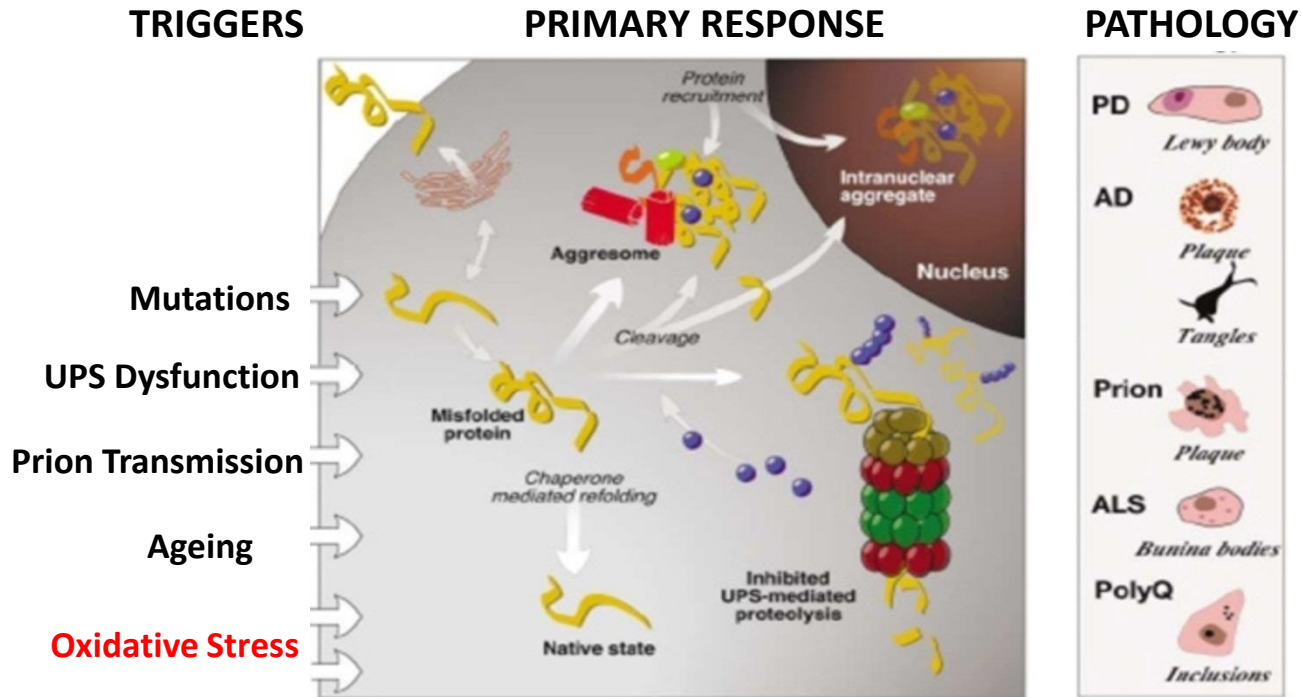


A **biomarker**, or **biological marker**, generally refers to a measured characteristic which may be used as an indicator of some biological state or condition.

Biological/Biochemical

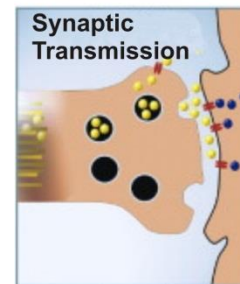
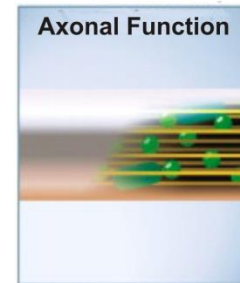
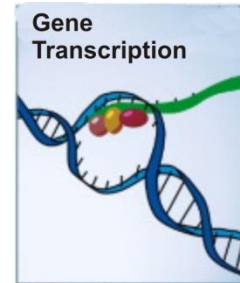


Pathogenesis of Neurodegeneration



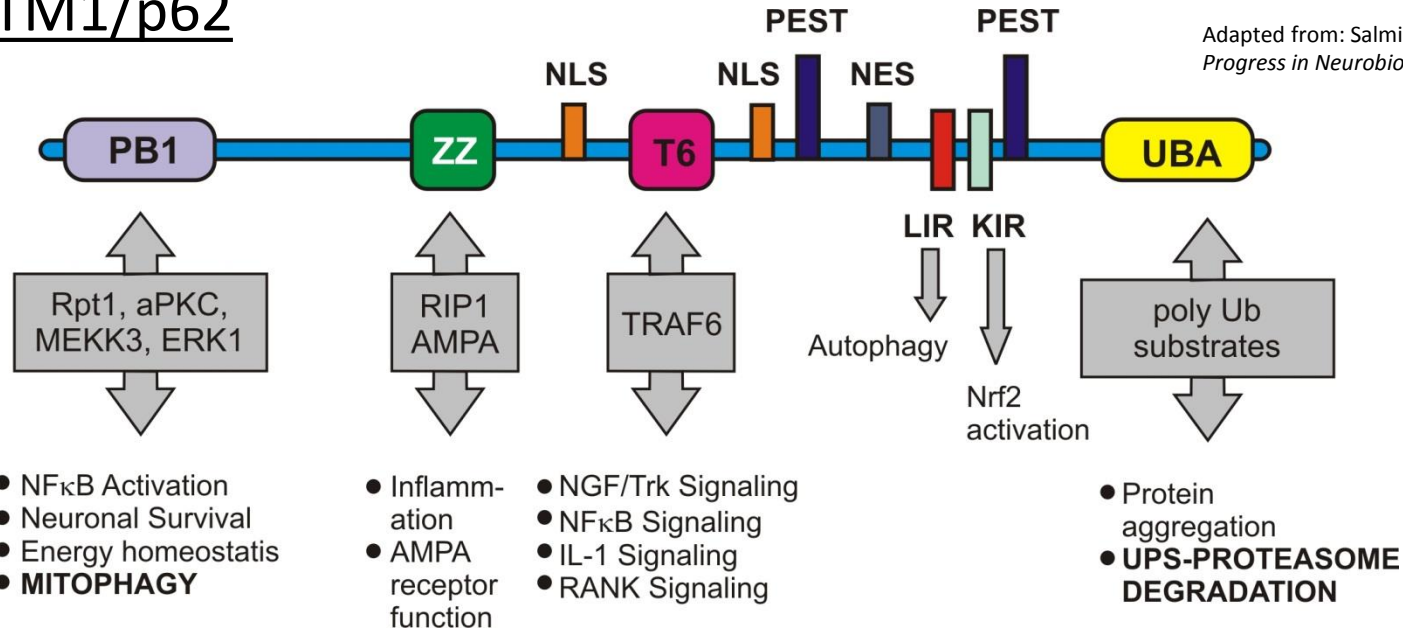
Ciechanover and Brundin, 2003 Neuron 40:427

SQSTM1/p62 is a component of ALL aggregates/inclusions



SQSTM1/p62

Adapted from: Salminen, et.al. 2012.
Progress in Neurobiology 96:87-95



Neurodegenerative Pathologies

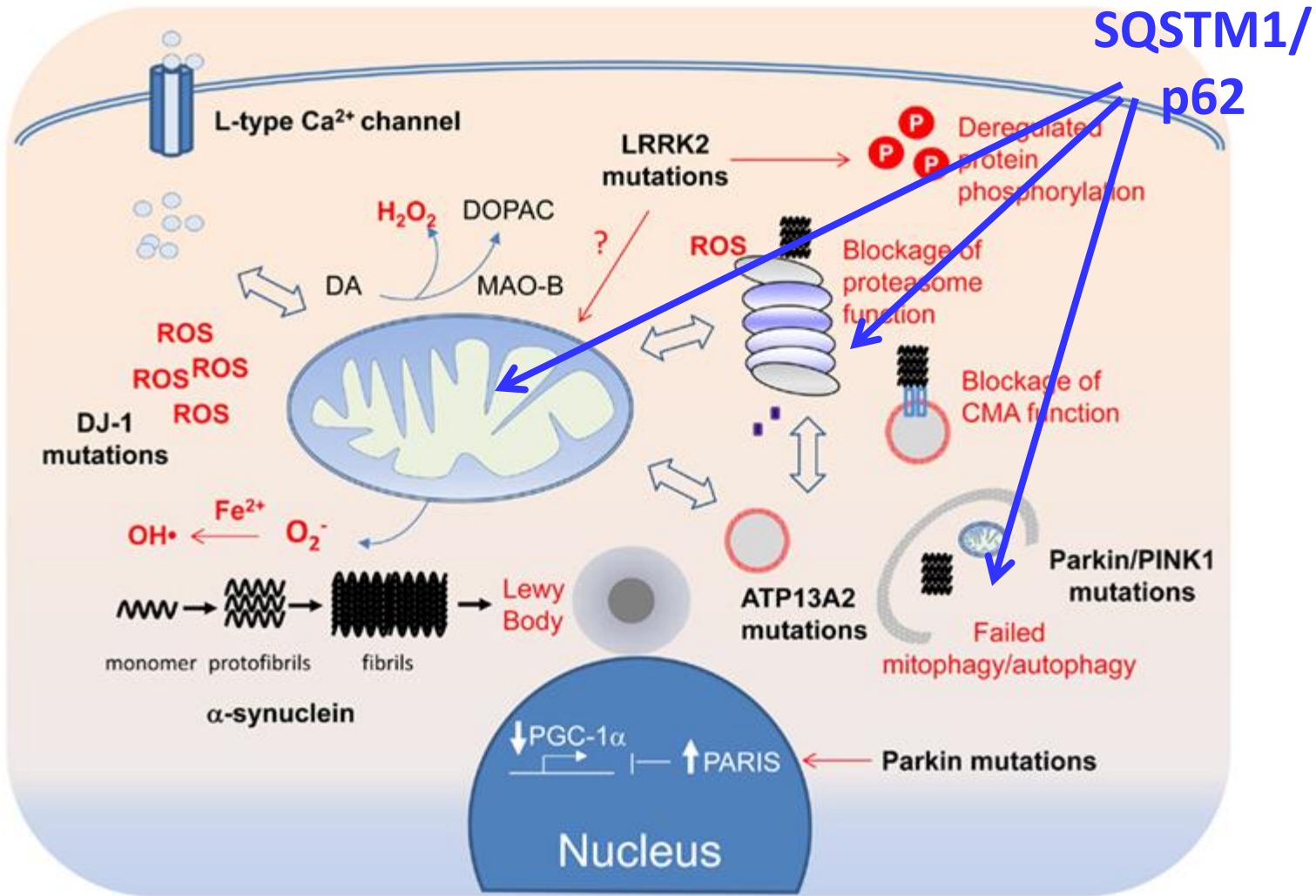
component of specific pathological protein aggregates

Ubiquitin – Proteasome Degradation

removal of misfolded or damaged proteins

Mitophagy/Autophagy

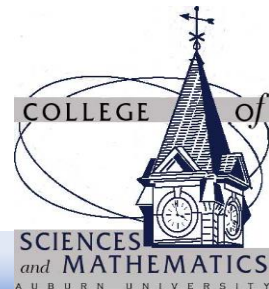
removal of defective mitochondria and proteins



Kah-Leong Lim and Cheng-Wu Zhang Front. Neurol., 08 April 2013

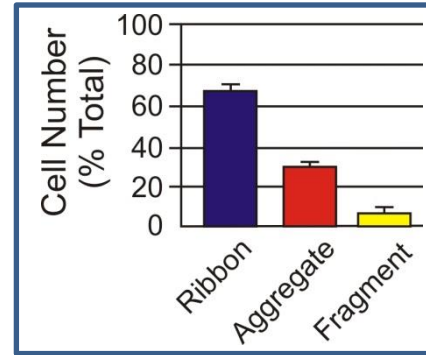
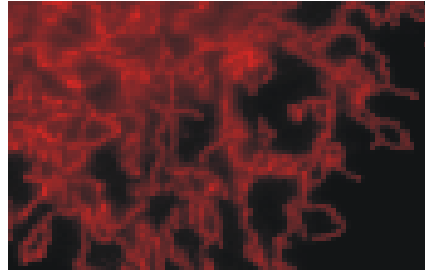
p62 shows the potential to be used as a biomarker for Neurodegenerative Disease.

**Examine the relationship between SQSTM1/p62
and mitochondrial functionality under physiological
conditions**

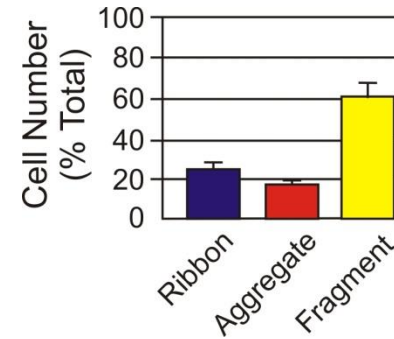
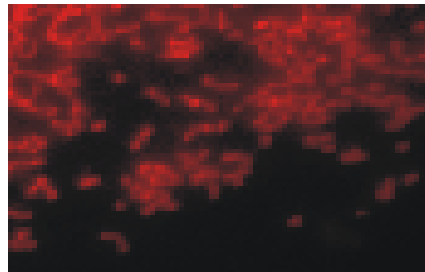


p62 Regulation of Mitochondrial Morphology

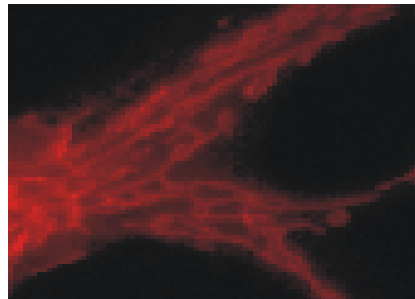
WT MEF
Cells



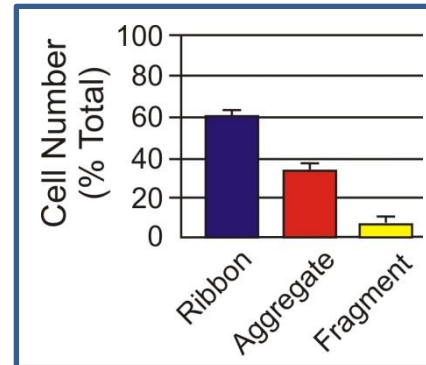
KO MEF
Cells



KO MEF
Cells
+ myc-p62

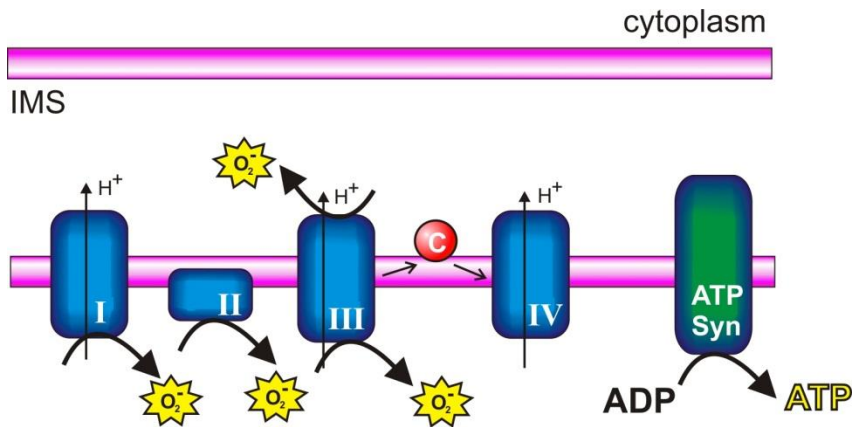


Mitochondria stained with MitoTracker Red

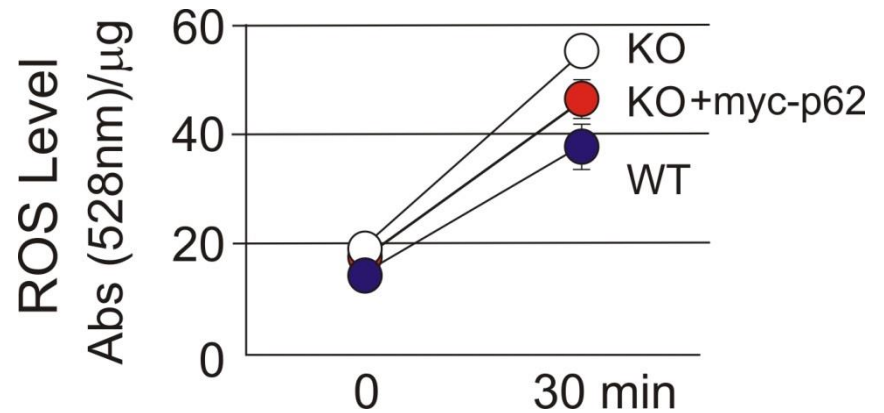
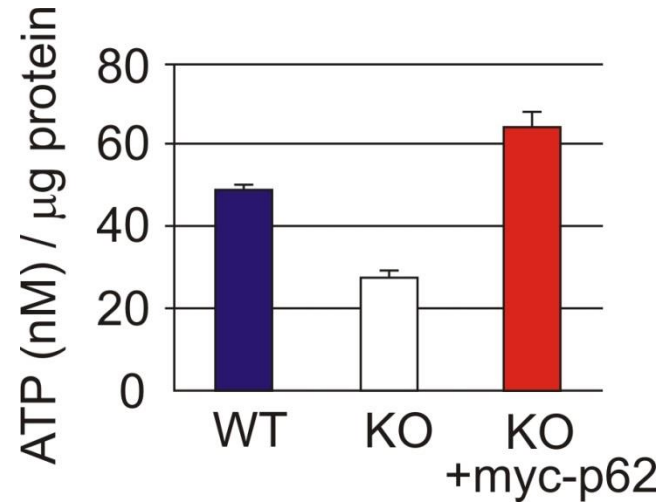


p62 plays a role in regulating mitochondrial morphology

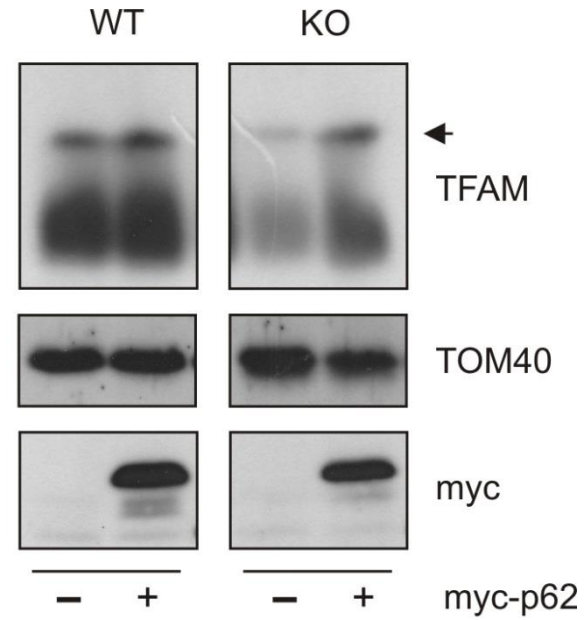
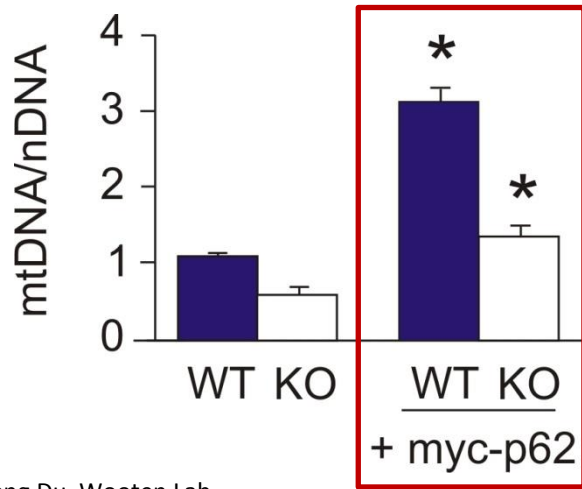
p62 and Mitochondrial Functionality



p62 not only regulates morphology but also affects mitochondrial metabolism and energy production

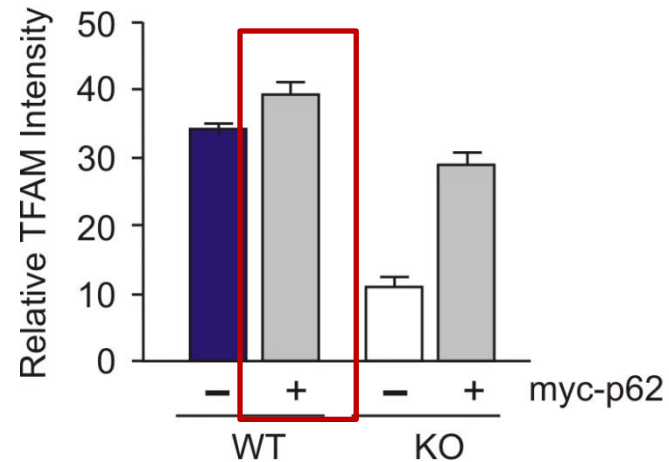


p62 and the Mitochondrial Genome



Dr. Yifeng Du, Wooten Lab

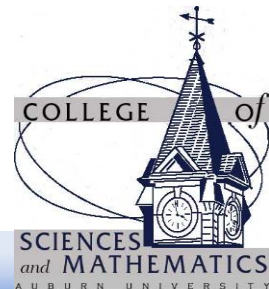
p62 protects mitochondrial genome integrity by a TFAM import related process.



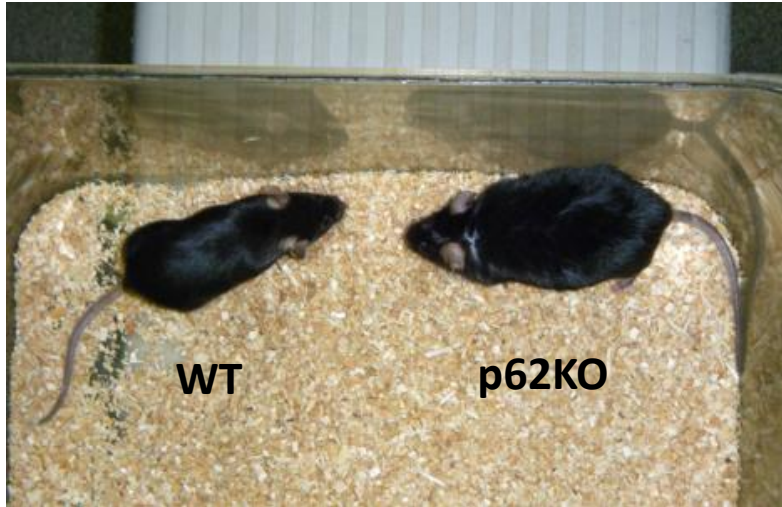
- p62 plays an active role in affecting mitochondrial morphology and functionality.
- Reintroduction of p62 to a null-background restores mitochondrial function.
- **Overexpression of p62 improves mitochondrial functionality above what is seen in WT.**

Overexpress SQSTM1/p62 in a mouse model.

- examine its effects on mitochondrial dynamics
- mitochondrial relationship to mouse behavior
*anxiolytic behaviors and
learning and memory*



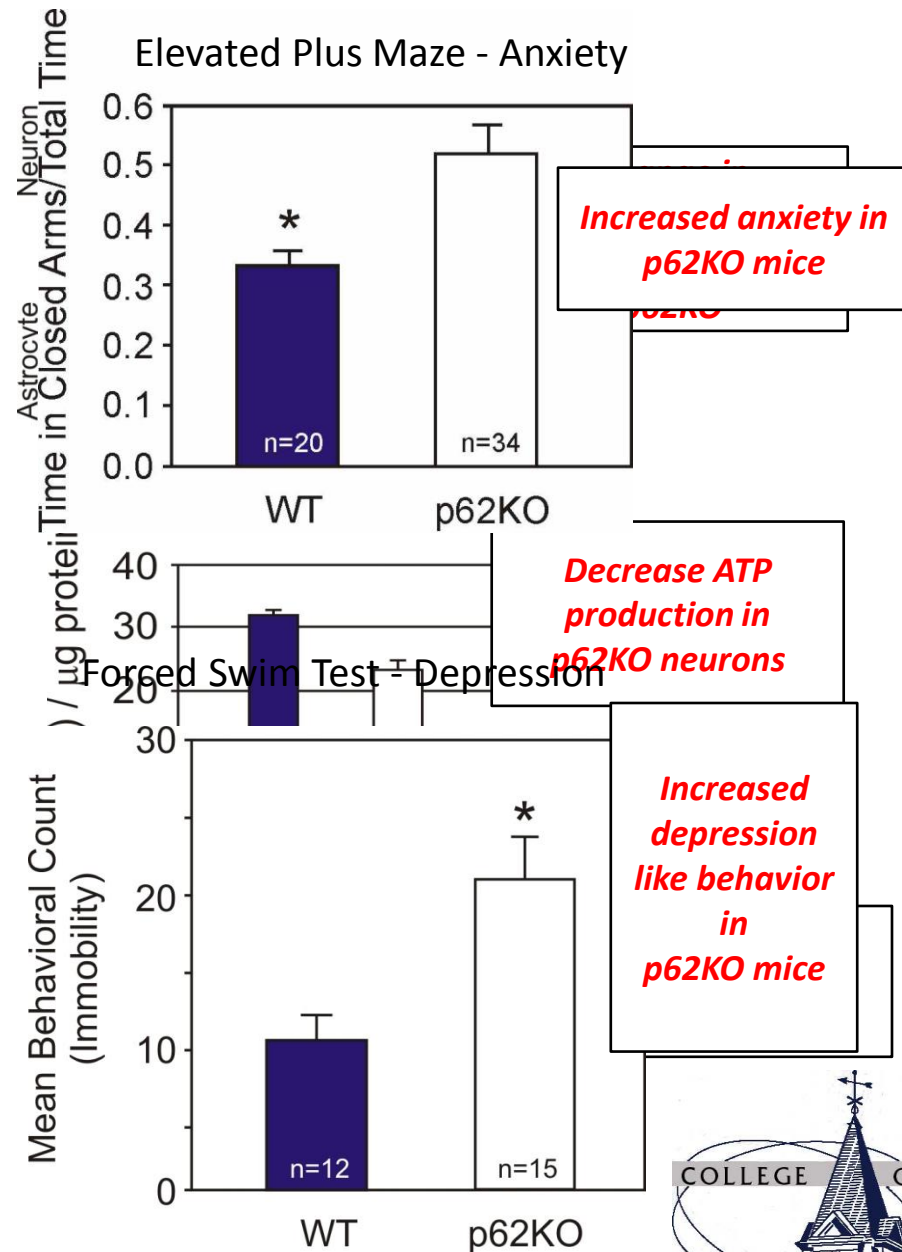
Mitochondrial Function and Behavior Patterns in p62KO Mice



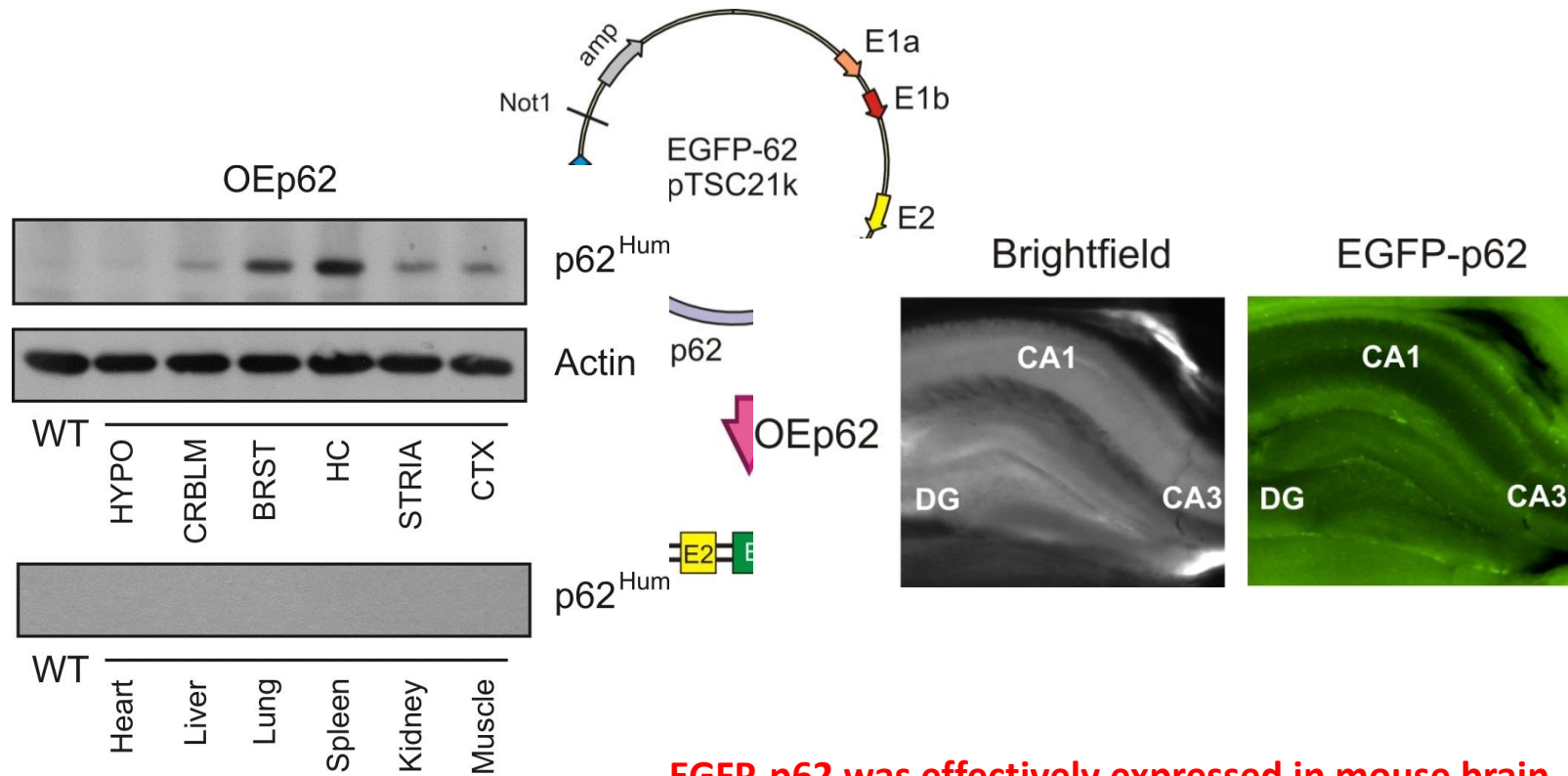
p62KO mice exhibit significant levels of mitochondrial dysfunction.

Loss of p62 results in behavior patterns similar to those seen in Alzheimer's Disease.

Hippocampal Neurons

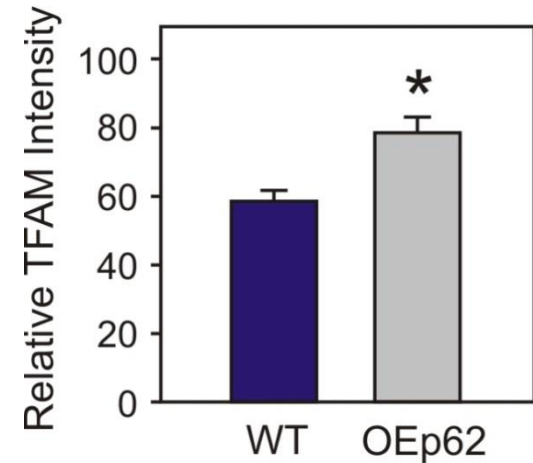
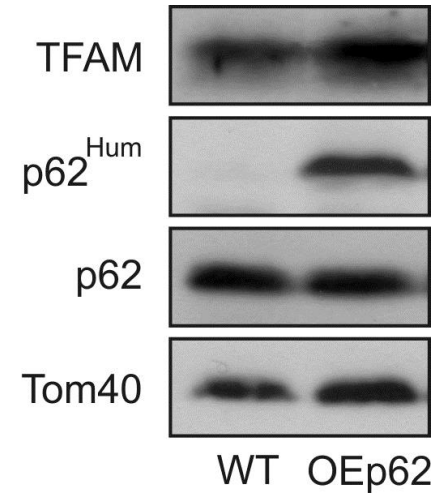
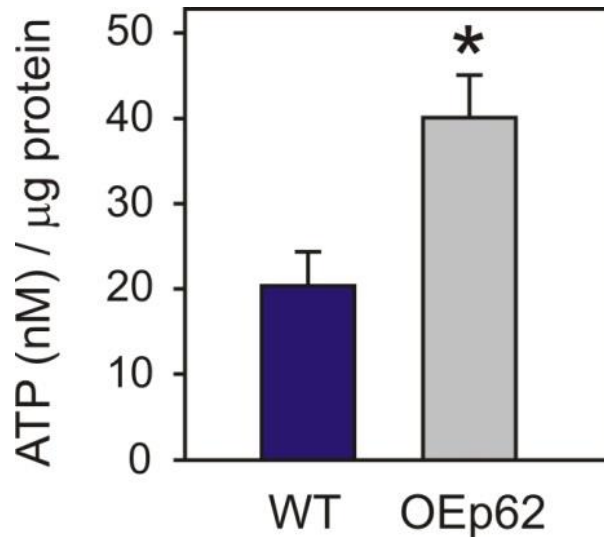
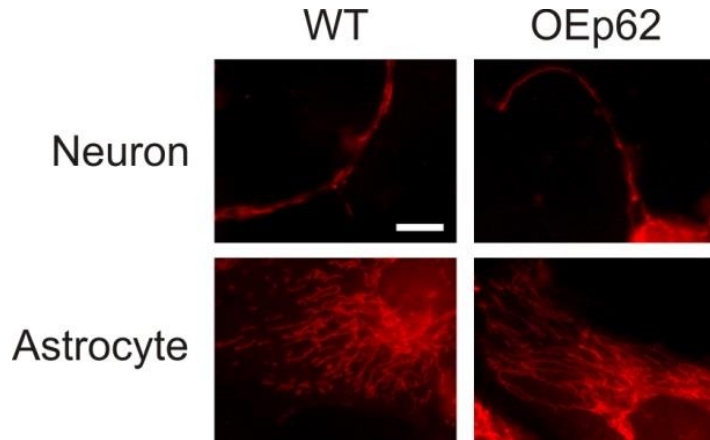


Expression of EGFP-p62 in Mouse Tissue



EGFP-p62 was effectively expressed in mouse brain.

Mitochondrial Metabolism in OEp62 in Hippocampus

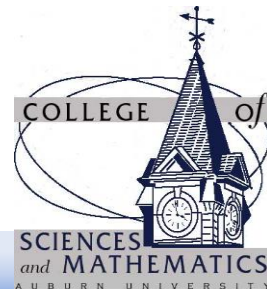


p62 overexpression affects mitochondrial morphology and function.

Mitochondrial Functionality

- SQSTM1/p62 was effectively expressed in the brain, predominantly in the hippocampus, generating an overexpressing mouse model.
- Mitochondrial structure and metabolism improved in the presence of excess SQSTM1/p62.

Does improved mitochondrial function correlate with positive changes in behaviors associated with neurodegenerative disease??



Behaviors Associated with Alzheimer's Disease

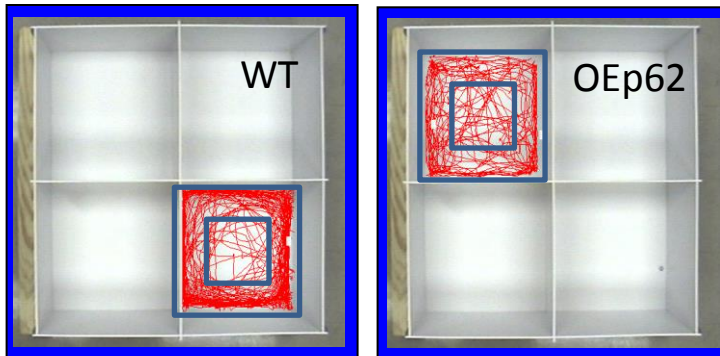
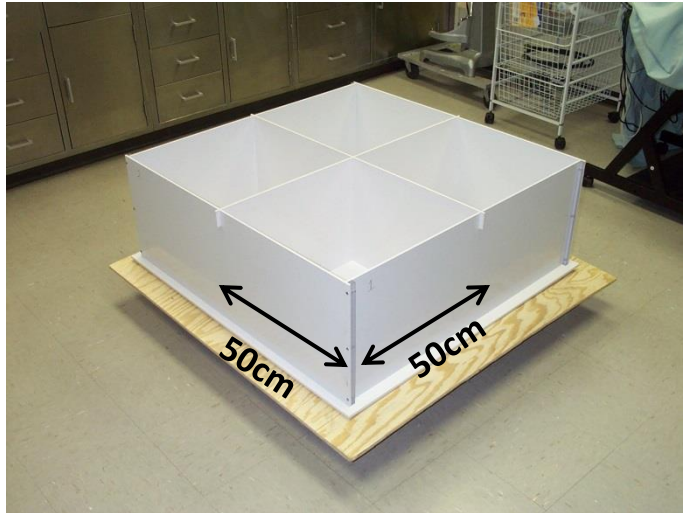
Anxiety



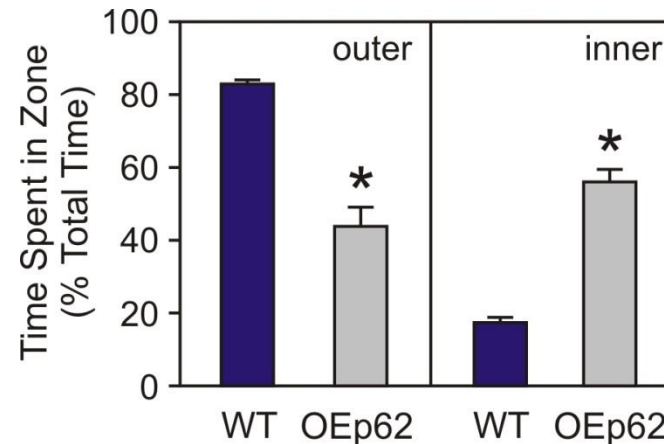
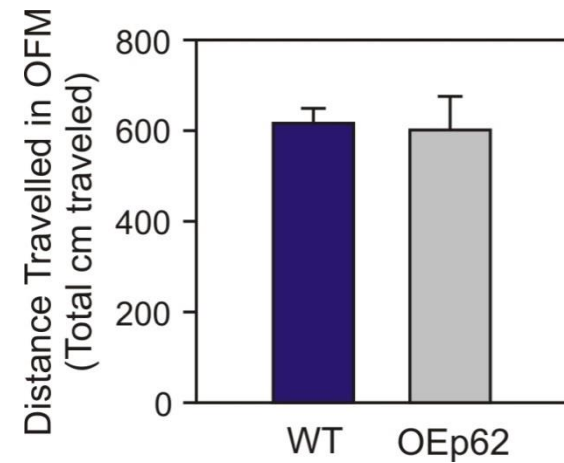
Affective Spectrum Disorders

Open Field Maze

Used to measure general locomotor activity and anxiety



measure activity for 10 minutes



Distance traveled in the OFM was the same for overexpressing mice compared to WT.

However, overexpressing mice spent more time in the inner area of the maze reflecting decreased anxiogenic behavior.

Forced Swim Test

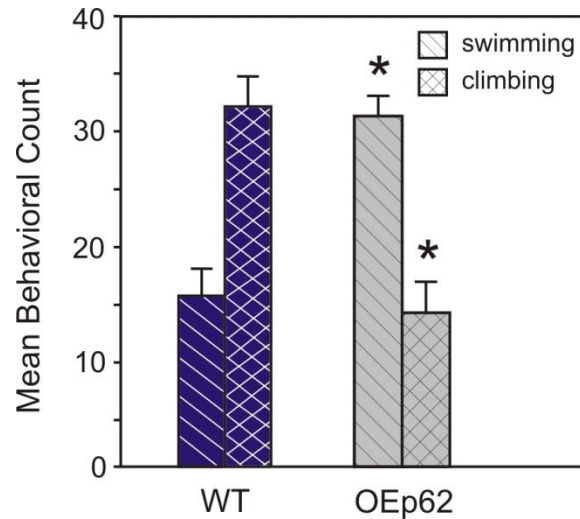
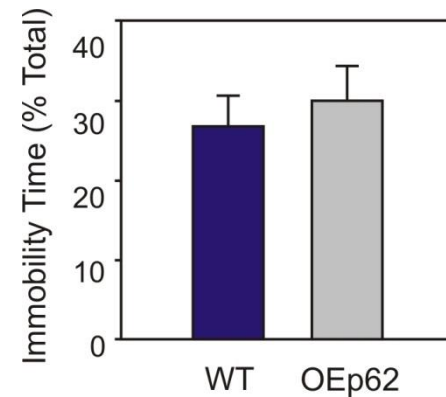
Modification of the Porsolt Swim test used to measure despair and depression.



measure activity for 6 minutes

record behavior every 5 seconds

1. **Immobile** – floating with no or limited movement
2. **Swimming** – active movement around beaker
3. **Climbing** – actively trying to escape by climbing walls of beaker

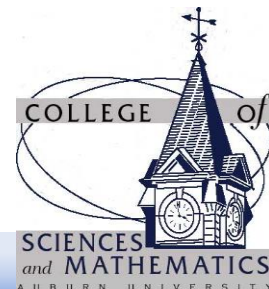


There is no measurable difference in immobility time between genotypes.

However, recorded behaviors during the test did show significant improvement in depression like behaviors.

Affective Spectrum Disorder Behaviors

- Improvement in anxiety related behaviors.
- Distinguishing specific behaviors during the FST show overexpressing mice exhibit decreased depression/despair.
- **Overall improvement in affective spectrum disorder behavior patterns in SQSTM1/p62 overexpressing mice.**

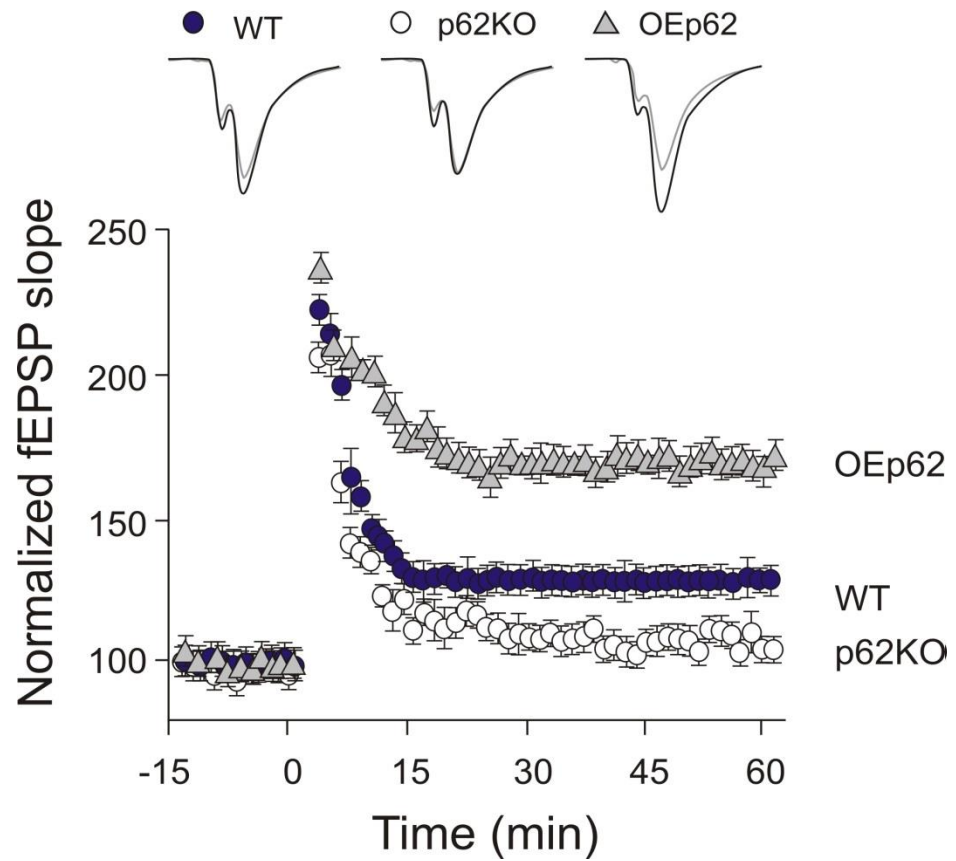
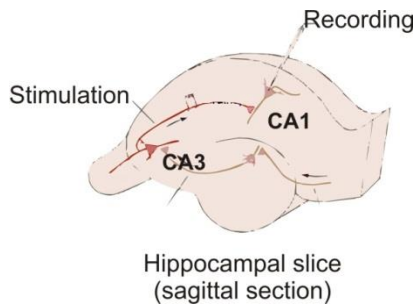


Learning and Memory



Long Term Potentiation

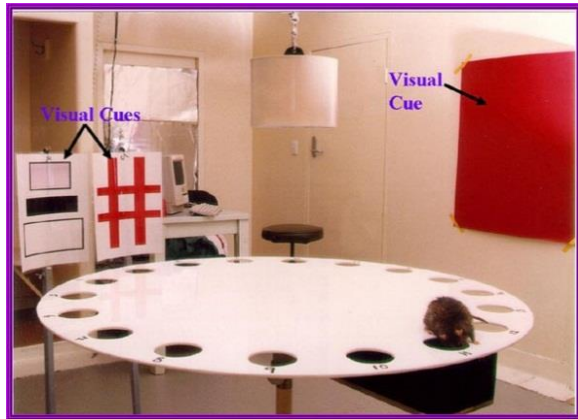
The long term enhancement of signal transmission between neurons.



Overexpressing mice show improved LTP compared to WT.

Barnes Maze

Noninvasive test for hippocampal dependent spatial learning and memory

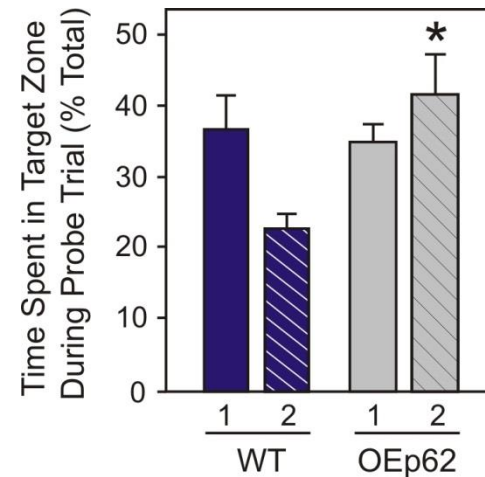
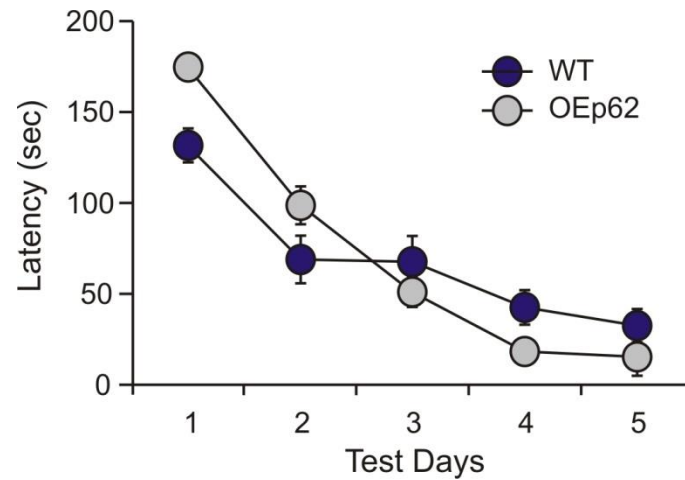


ADAPTATION (Day 1) – 4 trials/day; max – 3 min

SPATIAL ACQUISITION (Day 2-5) – 4 trials/day;
Max – 3 min

PROBE TRIAL 1 (short term memory) (Day 5) –
2 hours post last acquisition trial; 90 second
observation

PROBE TRIAL 2 (long term memory) (Day 12) –
90 second observation

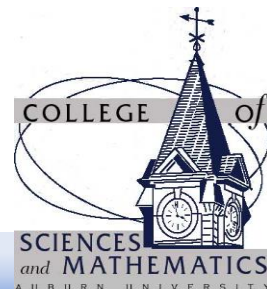


Overexpressing mice showed slight improvement to latency in hidden box search times.

No difference in short term memory was observed, however, long term memory was improved in overexpressing mice.

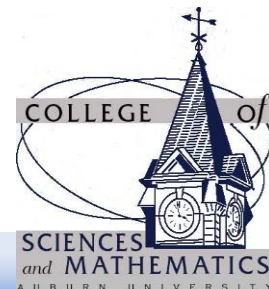
Learning and Memory

- Overexpression of SQSTM1/p62 shows enhanced LTP compared to WT.
- Spatial learning is slightly improved with overexpression of SQSTM1/p62.
- Spatial long term memory is strengthened with overexpression of SQSTM1/p62.
- **Overall, learning and memory are improved with overexpression of SQSTM1/p62 in the brain.**

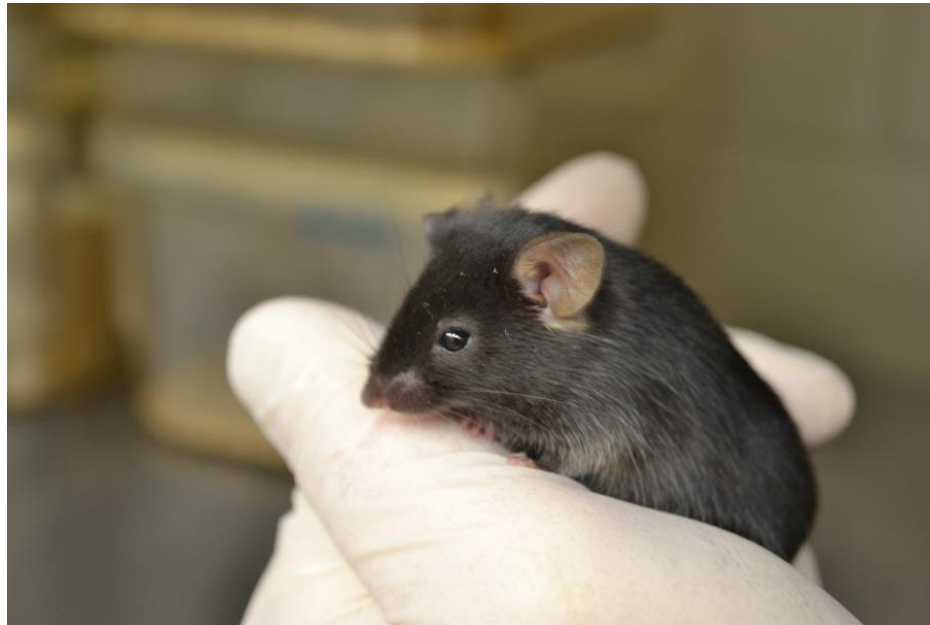


Conclusions

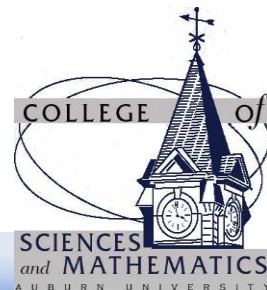
- SQSTM1/p62 levels affect mitochondrial functionality as well as behavior patterns associated with neurodegenerative diseases.
- SQSTM1/p62 appears to be a prime candidate for a protein that changes mitochondrial functionality and also affects behavior making it a potential *biomarker for neurodegenerative diseases*.
- SQSTM1/p62 could be a *novel target for potential drug discovery* to treat anxiety and affective spectrum disorders as well as, improve cognitive function associated with neurodegenerative diseases.



Generation of p62OE mice



C57BL/6-Tg(Thy1-SQSTM1)02MCWo/J (Stock# 27258)



Wish to Thank:

Wooten Lab Members:

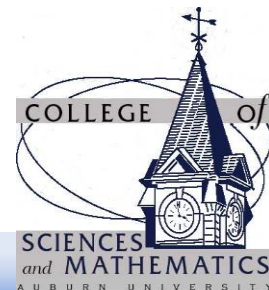
Michael C. Wooten
Yifeng Du
Luis Calderilla Barbosa
Jin Yan
Denise Landers

Others:

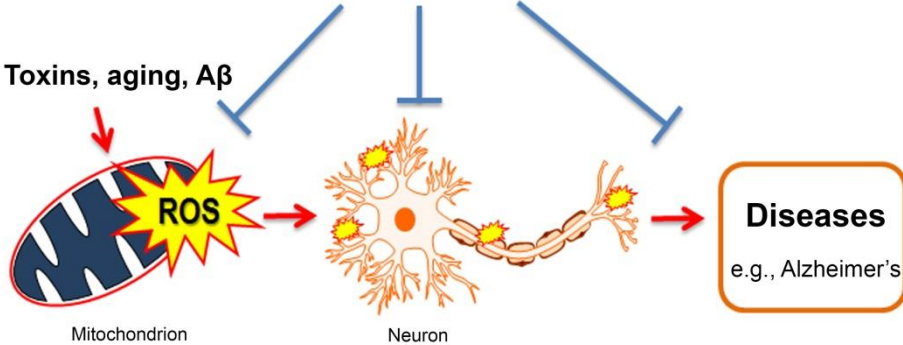
Jorge Moscat
Maria-Theresa Diaz-Meco

Mike Irwin
Carl Pinkert

Vishnu Suppiramaniam
Kodeeswaran Parameshwaran



Identifying novel targets of intervention



Qitao Ran, UT Health Science Center