

Sirtuin 3: A Mitochondrial Watchdog Protein

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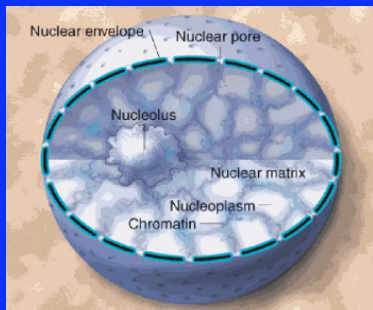


Vanderbilt Medical School

The Human Sirtuins

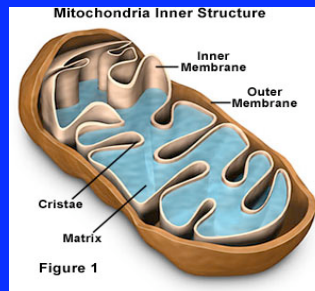
Nuclear

- **Sirt1**
- Sirt6
- Sirt7



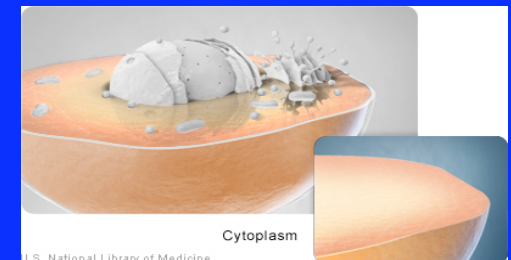
Mitochondrial

- **Sirt3**
- Sirt4
- Sirt5



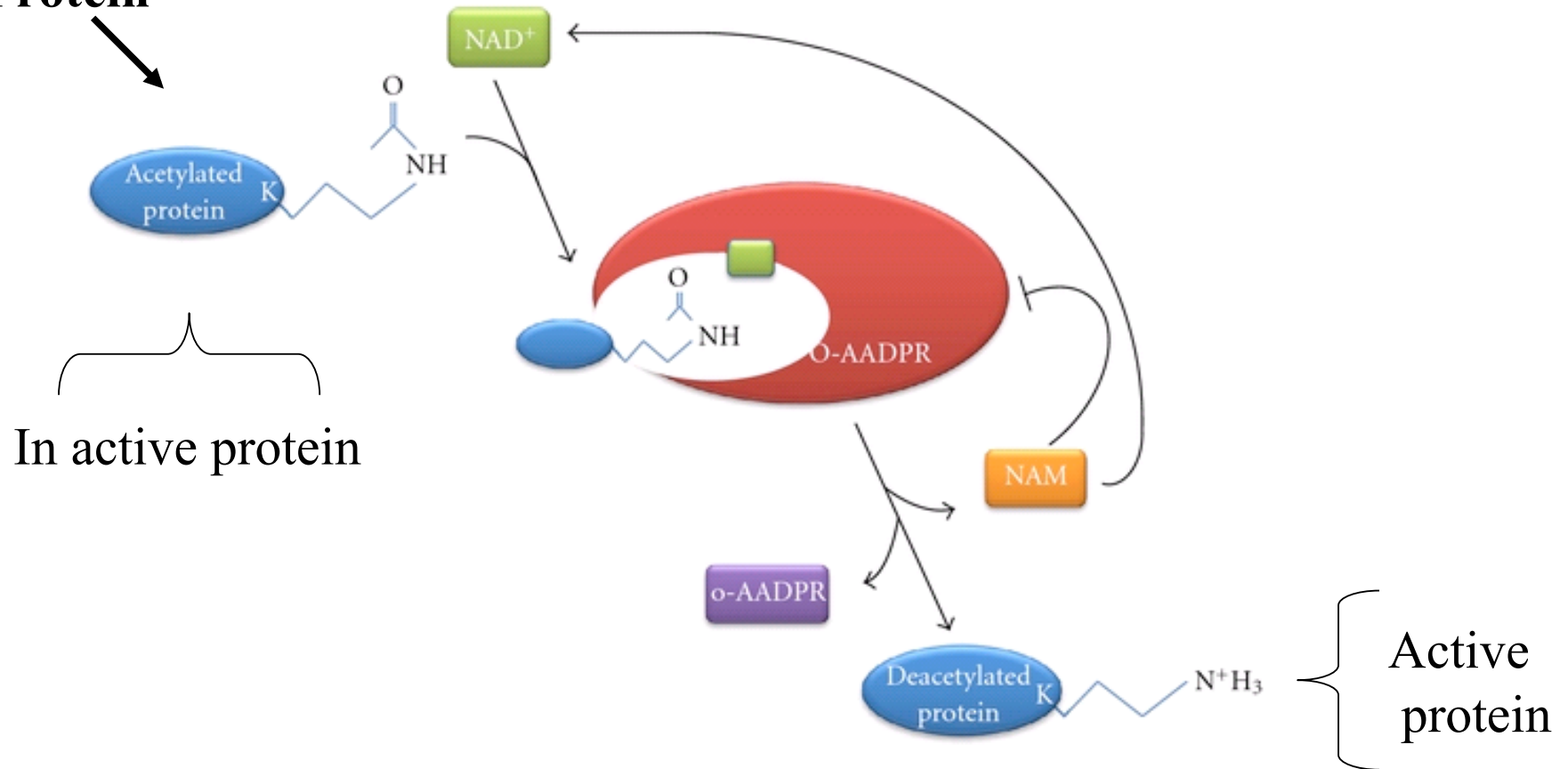
Cytoplasmic

- **Sirt2**



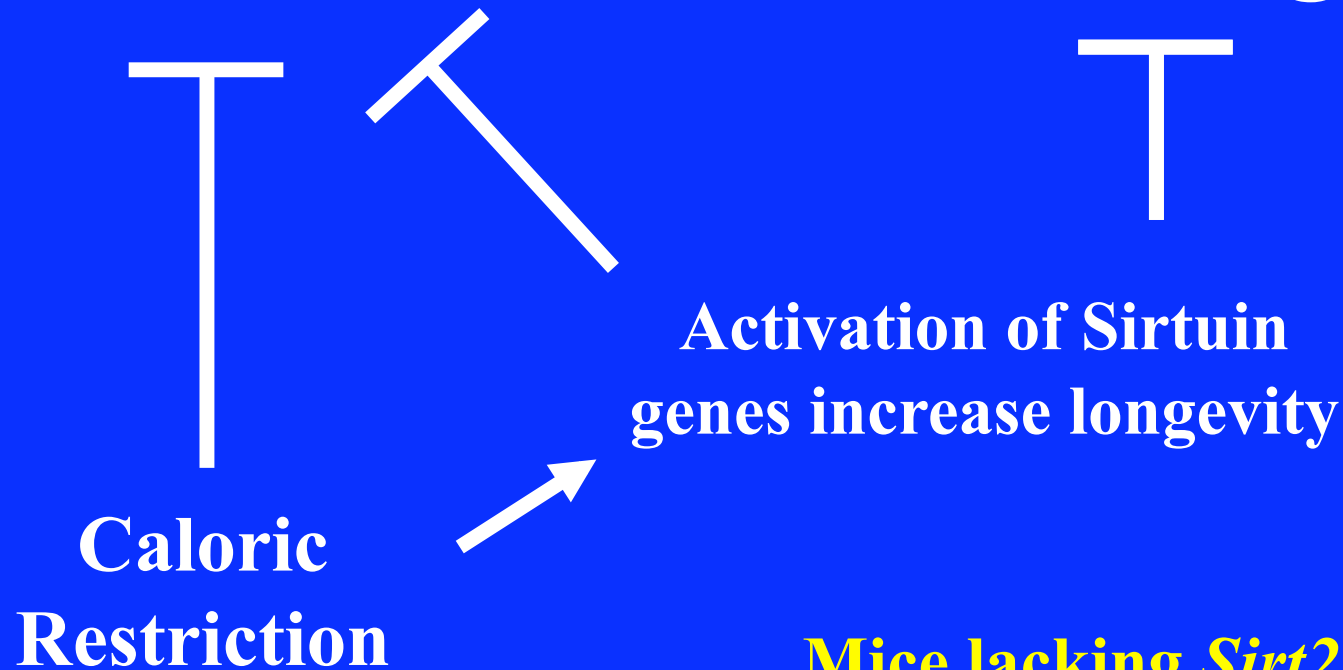
Protein Deacetylation as a Post-Translation Protein Modification

Non-Histone Protein



Rational for Sirtuins as Tumor Suppressors

Decreases aging → ? Carcinogenesis

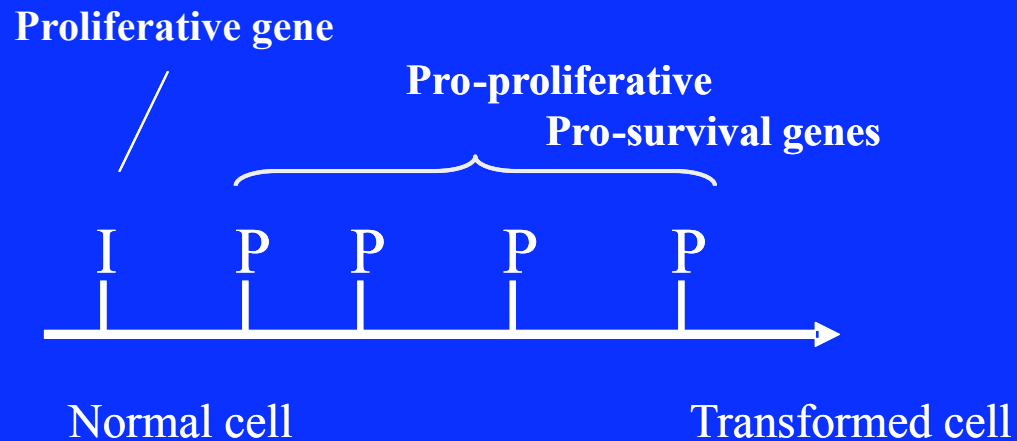
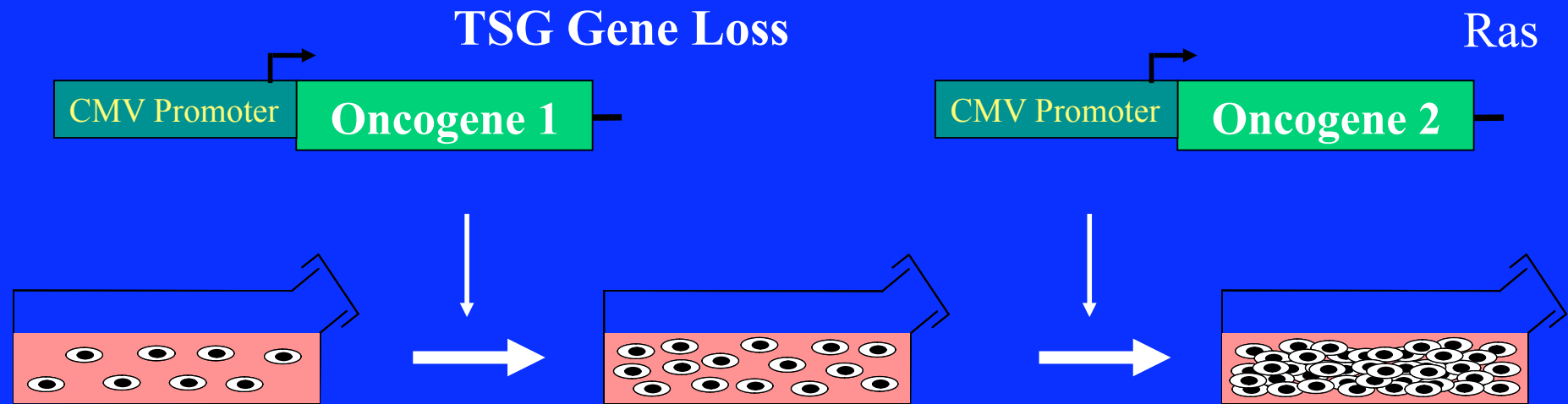


Mice lacking *Sirt2* and *Sirt3* were constructed to determine if they prevent cancer / TSGs?

Tumor Suppressor Gene

- Loss of function results in an *in vitro* tumor permissive cellular phenotype (two hit tissue culture immortalization).
- Genetic knockout in mice results in the formation of murine tumors.
- There is a loss of function or decrease in protein levels in human malignancies and this matches human samples.

Two Gene Transformation Model for MEFs



Sirt3^{-/-} MEFs are immortalized by a Single Oncogene

TABLE 1. Immortalization of Sirt3^{-/-} MEFs only requires a single oncogene

	Control	Myc	Ras	Myc/Ras
MEF Sirt3 ^{+/+}	None	None	None	Immort
MEF Sirt3 ^{-/-}	None	Immort	Immort	Immort

None , no MEF immortalization.

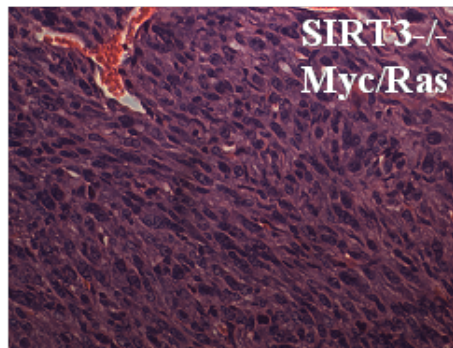
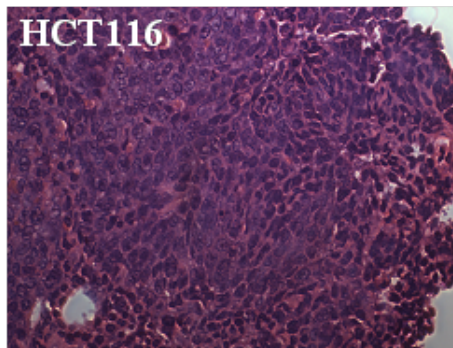
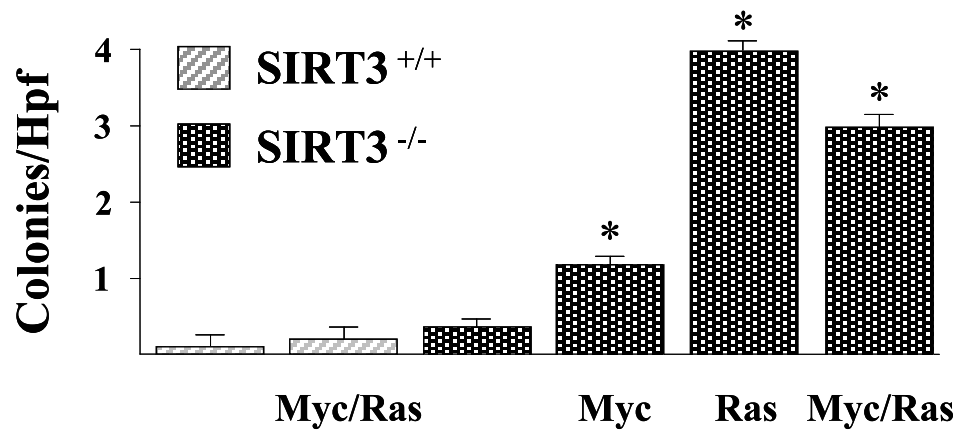
Immort, immortalization.

Sirt3^{+/+} Myc/Ras cells

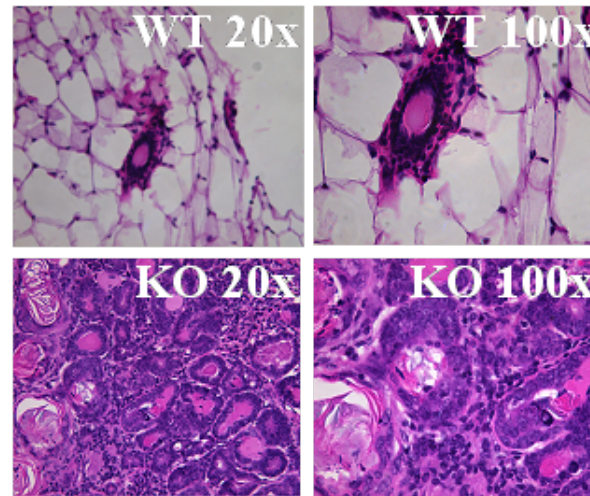
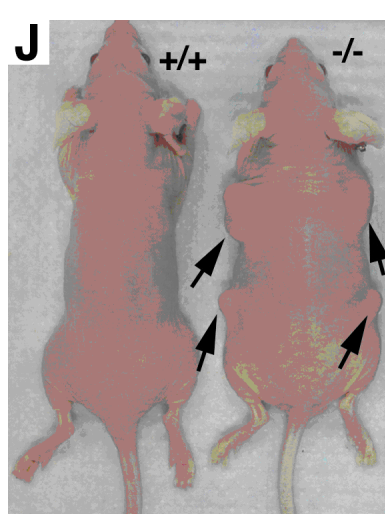
Sirt3^{-/-} Myc/Ras cells

Kim et al, 2010 *Cancer Cell*

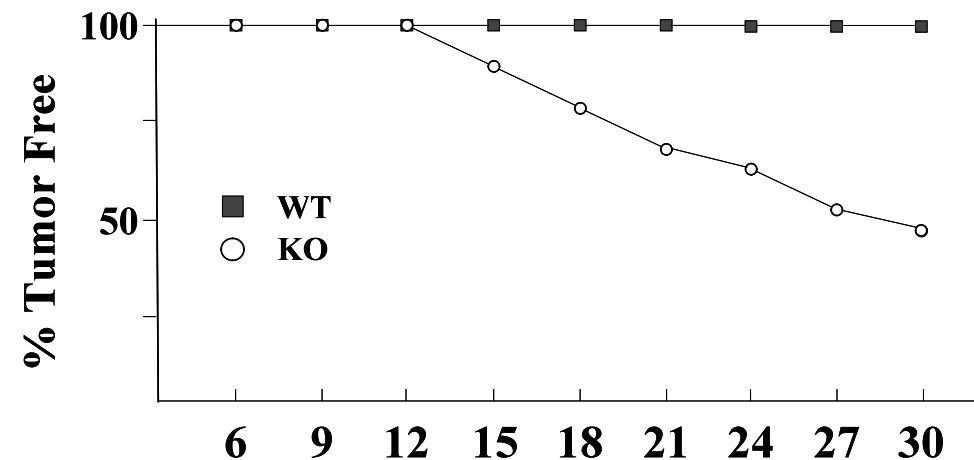
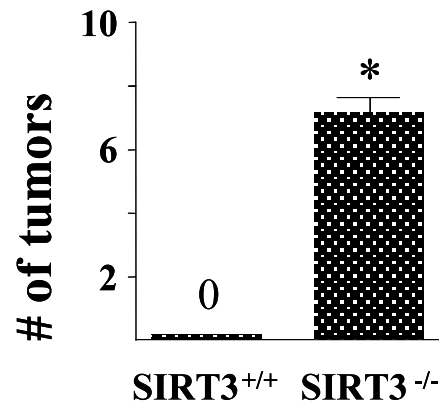
In Vitro Transformation Sirt3^{-/-} MEFs by a Single Oncogene



Mammary Carcinogenesis in the *Sirt3* knockout mice

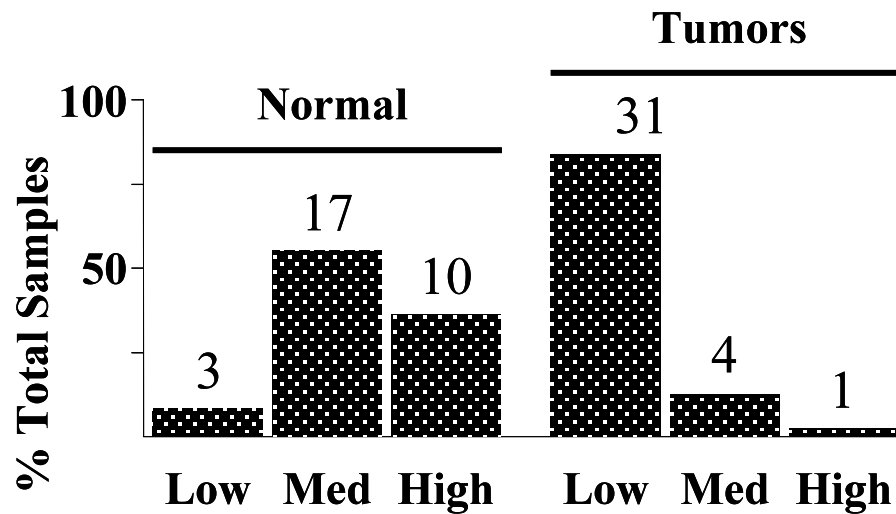


	<u>ER</u>	<u>PR</u>
Tumor #1	++	++
Tumor #2	+	+
Tumor #3	+	+
Tumor #4	+	+
Tumor #5	++	++
Tumor #6	+	+
Tumor #7	++	+

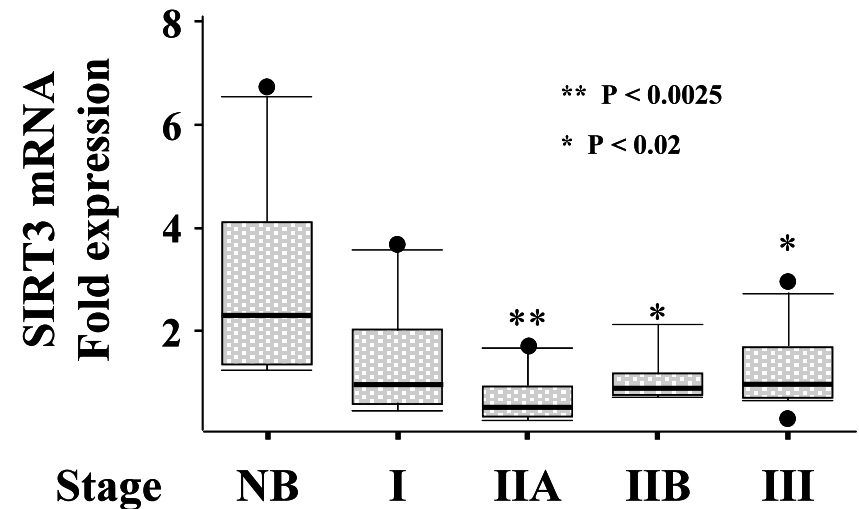


SIRT3 is Decreased in Human Breast Cancers

Tissue Array (IHC)

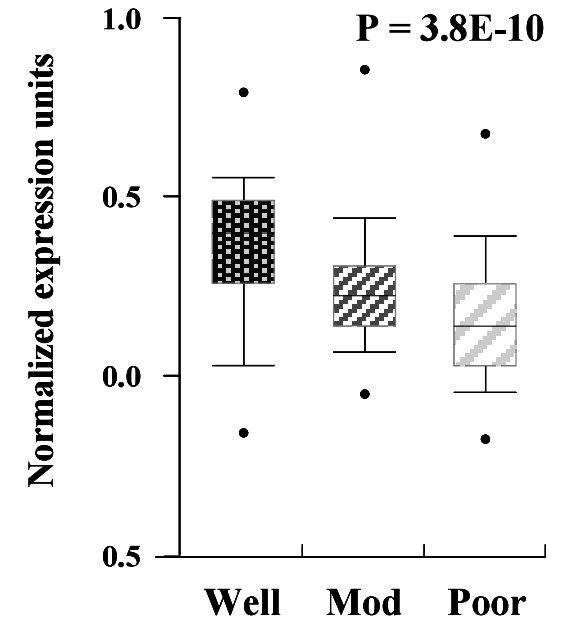
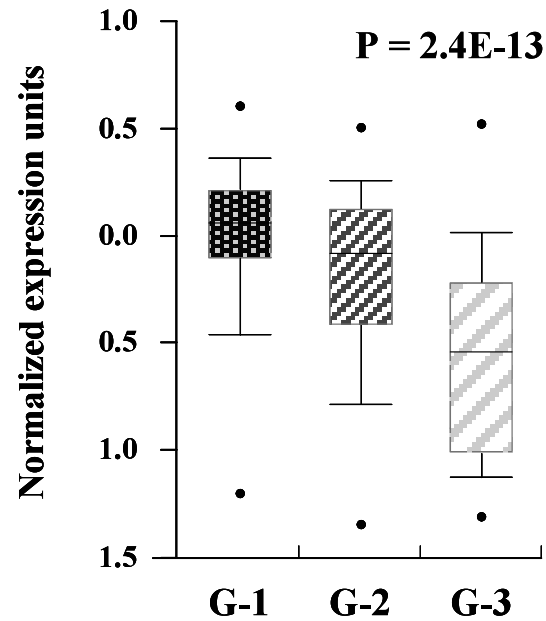
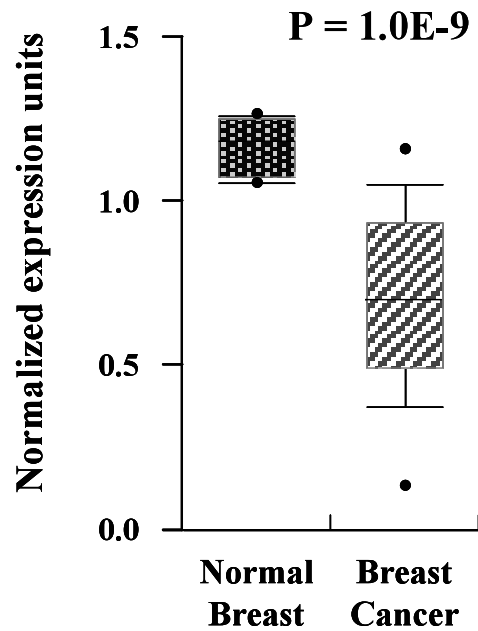


RNA Array (RT-PCR)



Kim et al, 2010 *Cancer Cell*

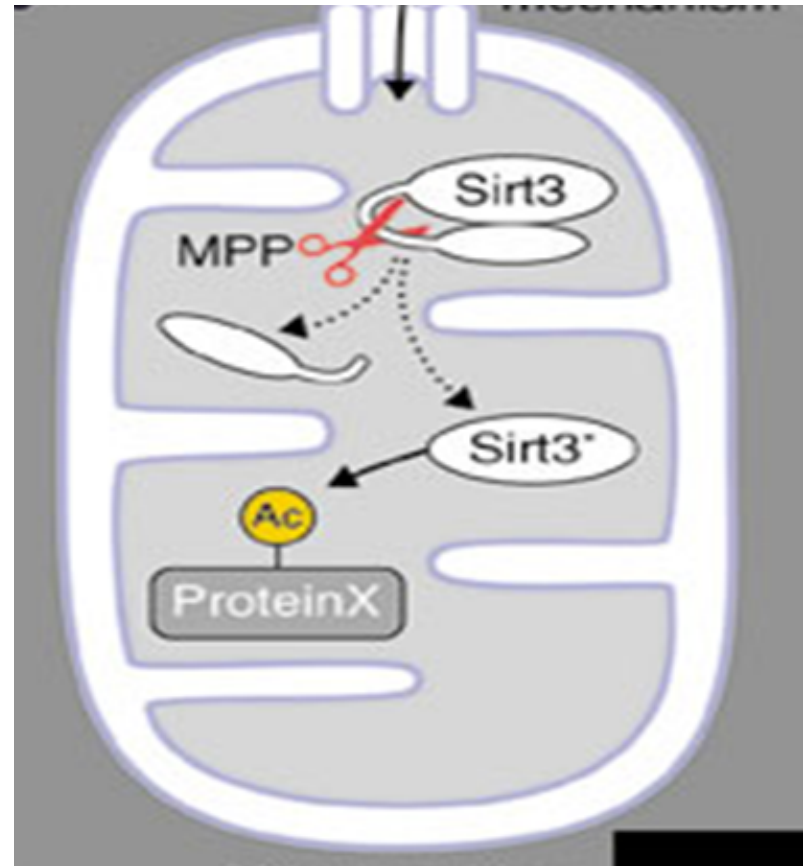
***SIRT3* is Decreased in Human Breast Cancers**



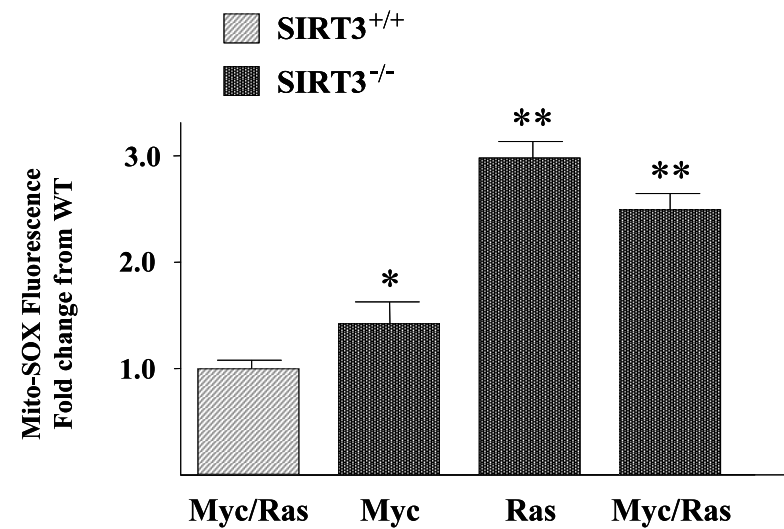
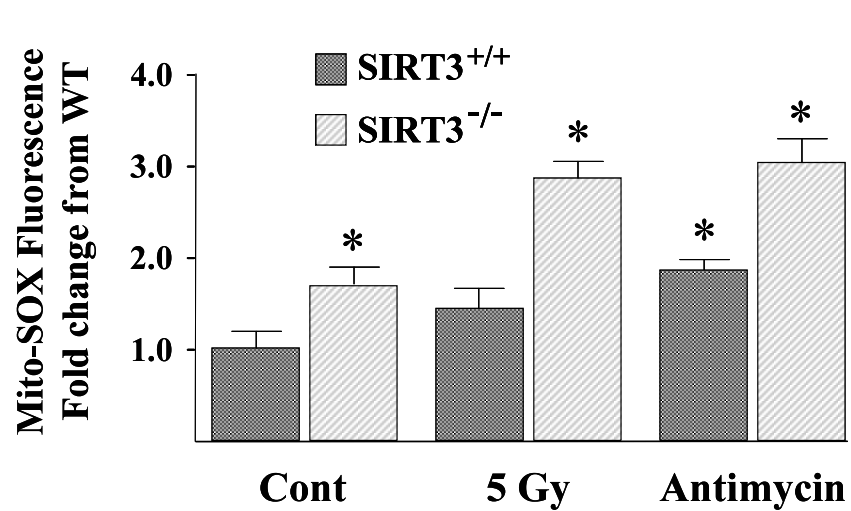
Oncomine, UMich

Sirt3 is a mitochondrial tumor suppressor but...

- Mechanism?
- Is it a sensing protein?
- What are the targets of Sirt3 ?
- Or what dysregulated proteins play a role in the Sirt3^{-/-} tumor permissive?

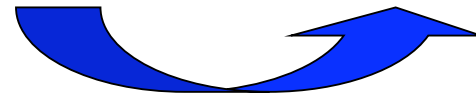
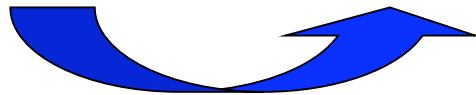


Transformed *Sirt3* KO MEFs exhibit mt Superoxide



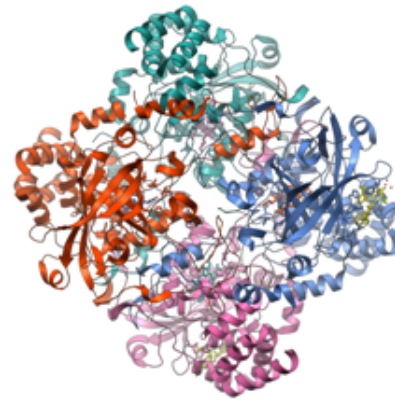
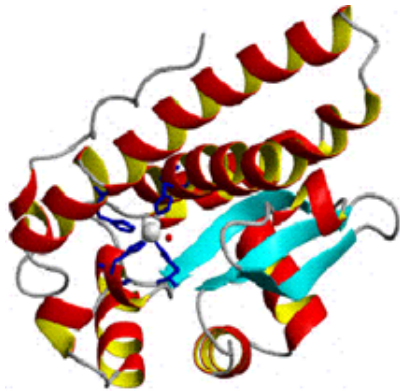
Kim et al, 2010 *Cancer Cell*

Primary Mitochondrial O₂⁻ Detoxification Pathway



MnSOD

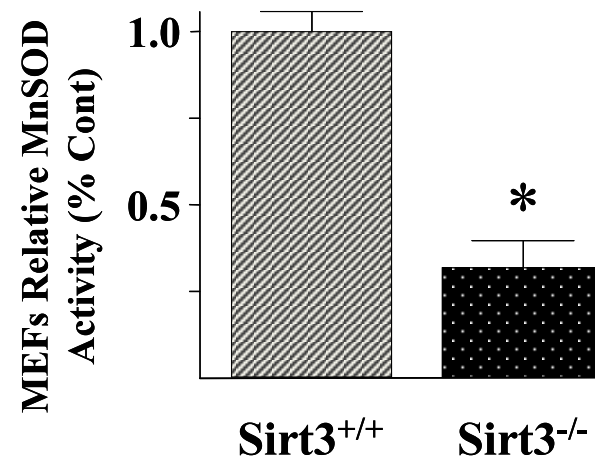
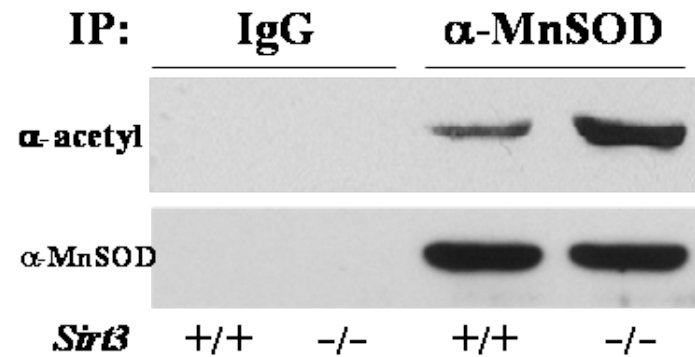
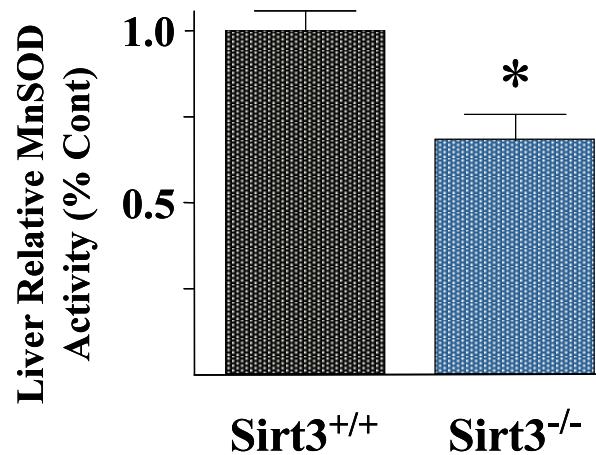
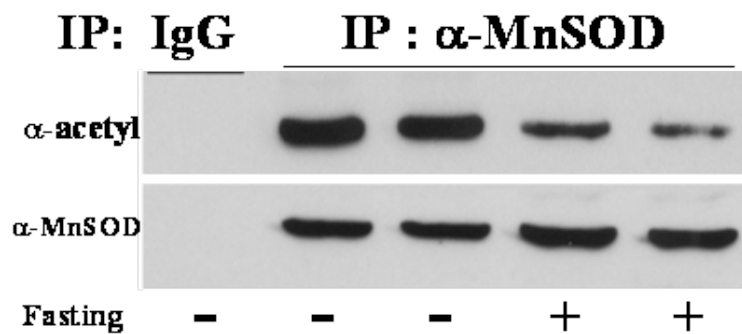
Catalase



Criteria for Potential Sirt3 physiological Target

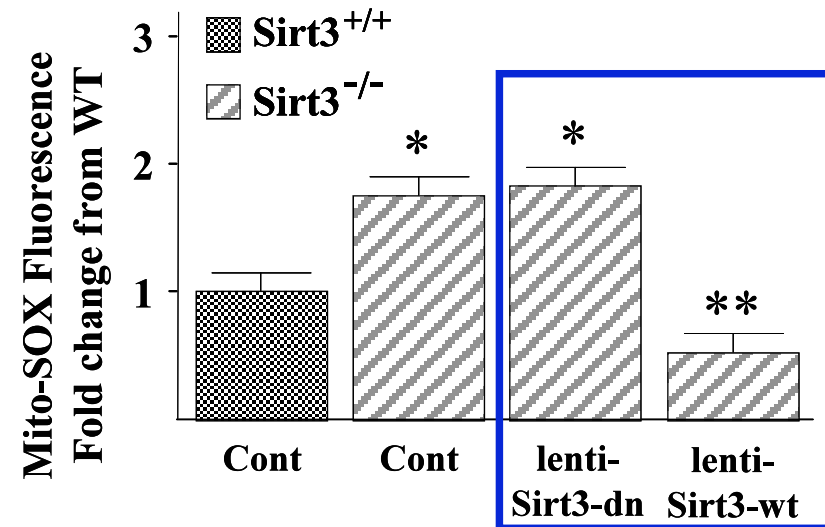
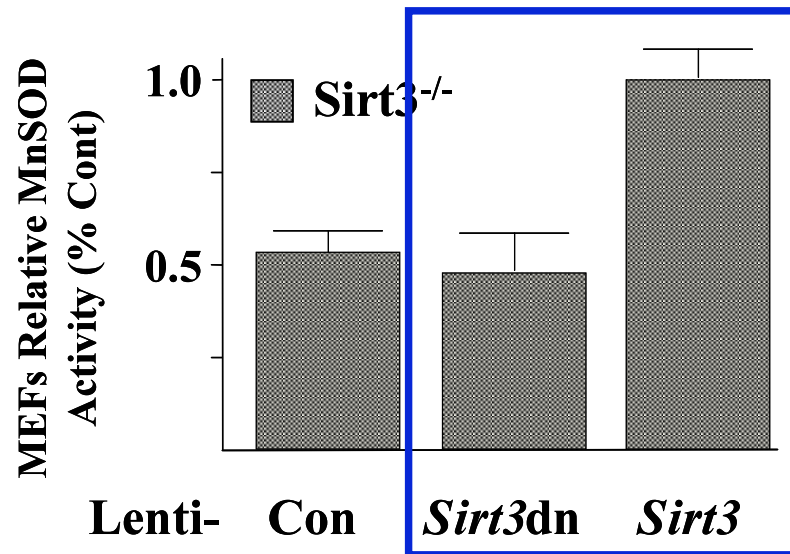
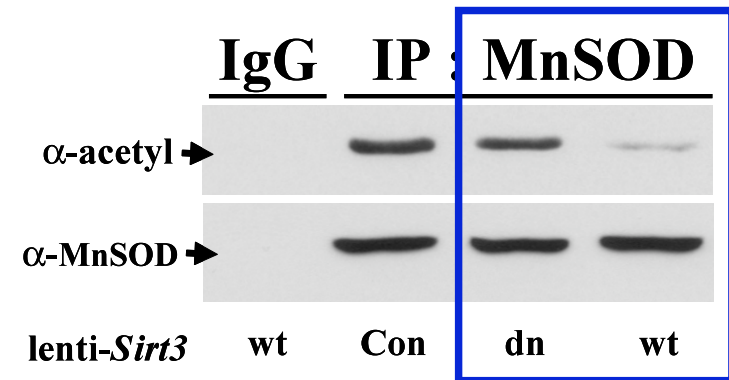
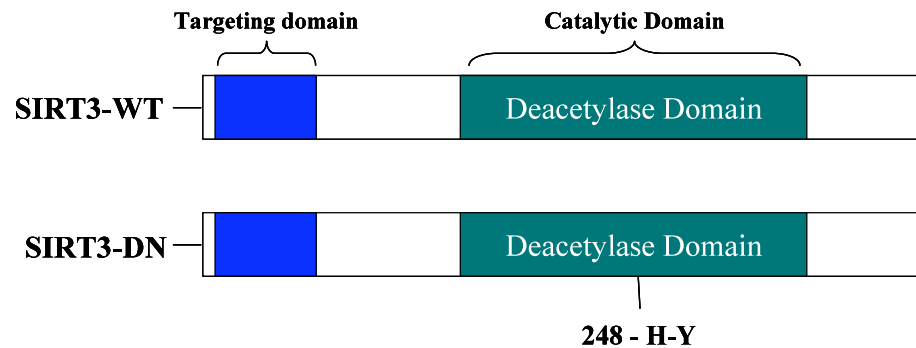
- A protein that contain at least one reversible acetyl lysine that is altered by either caloric restriction, feasting, or other type of stress.
- A Protein is hyperacetylated in the *Sirt3* knockout livers or MEFs.
- A protein contains at least one lysine that is deacetylated by Sirt3 both in vitro and in vivo.
- The reversible acetyl lysine is evolutionary through out multiple species including less complex species.
- Acetylation of the target lysine regulates enzymatic activity.

MnSOD contains a reversible lysine

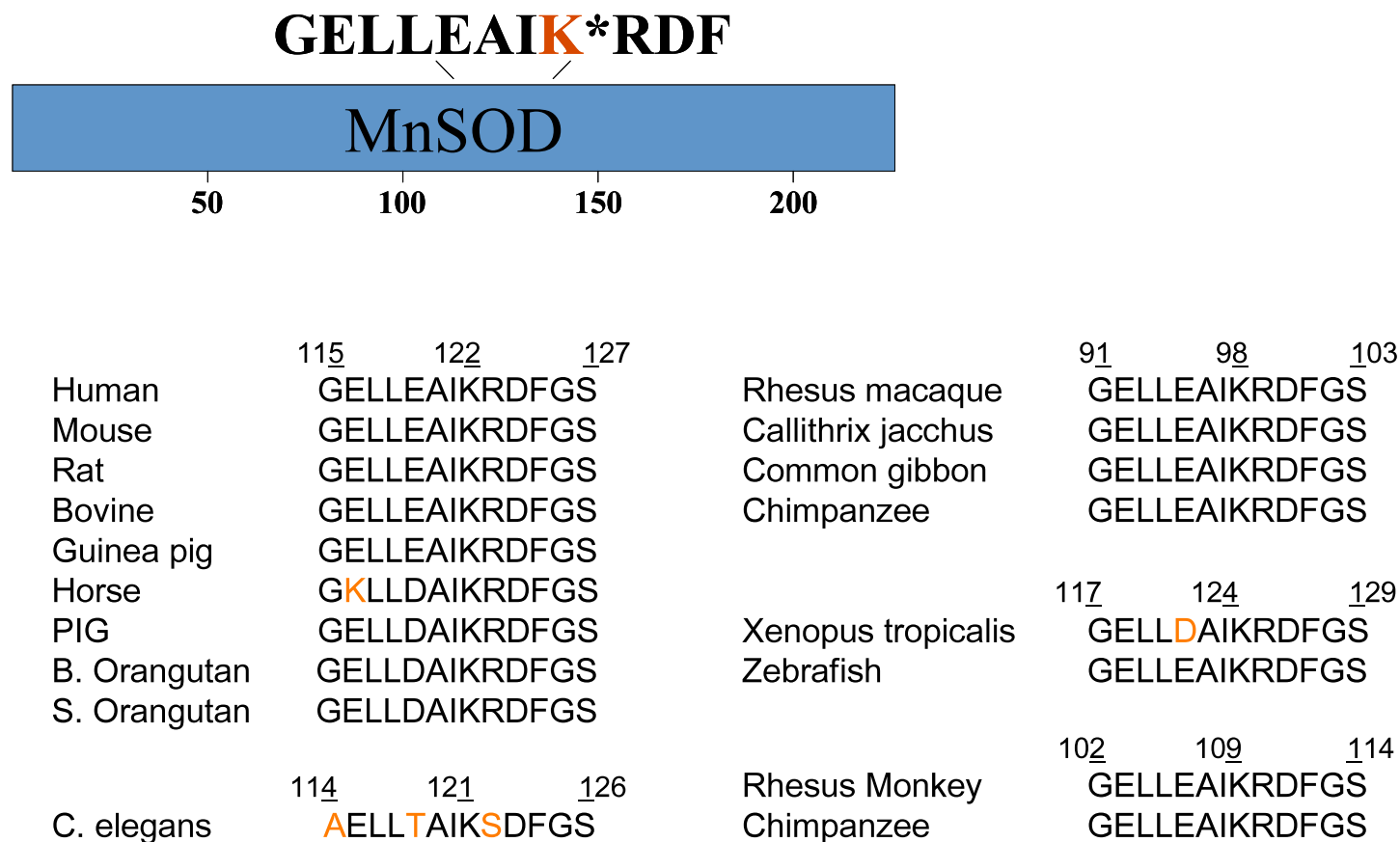


Tao et al., 2010, *Molecular Cell*

MnSOD's reversible is deacetylated by Sirt3



MnSOD K122 is an evolutionarily conserved reversible lysine

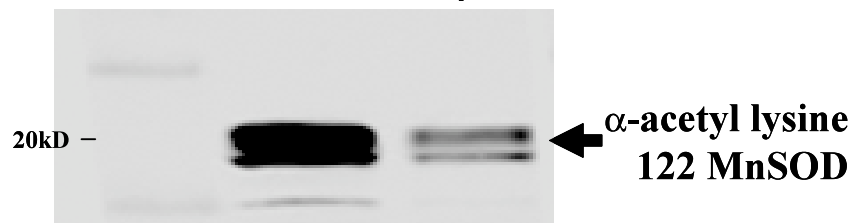


Tao et al., 2010, *Molecular Cell*

MnSOD K122 is Deacetylated by Sirt3 in vitro and in vivo

In vitro

TSA	+	+
MnSOD	+	+
SIRT3	+	+
NAD ⁺	-	+



In vivo

α -K122
MnSOD

α -MnSOD

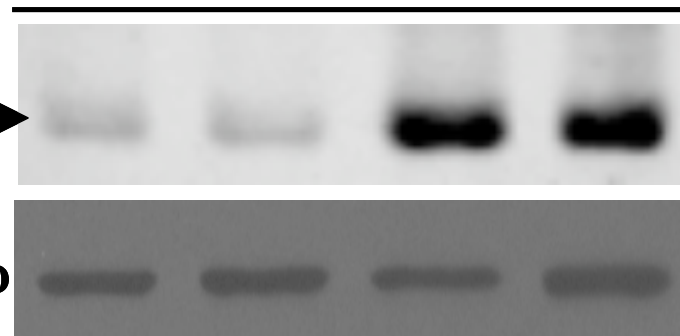
Sirt3

+/+

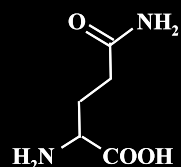
+/+

-/-

-/-



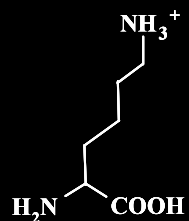
Wild-type



Lysine (Lys, K)

lenti-MnSOD^{K122}

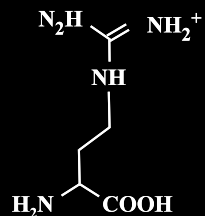
Acetylated



Glutamine (Gln, Q)

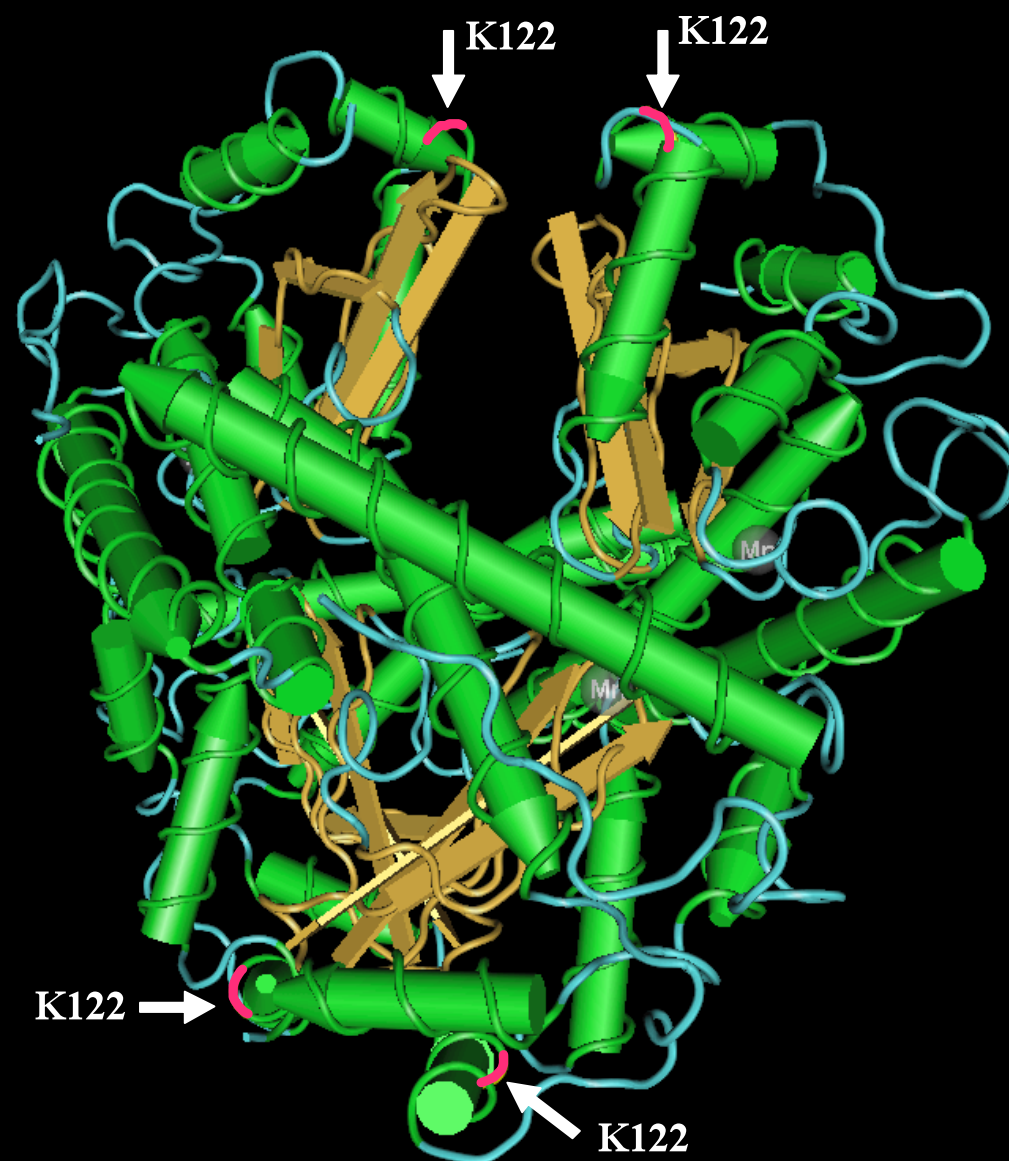
lenti-MnSOD^{K122-Q}

De-acetylated



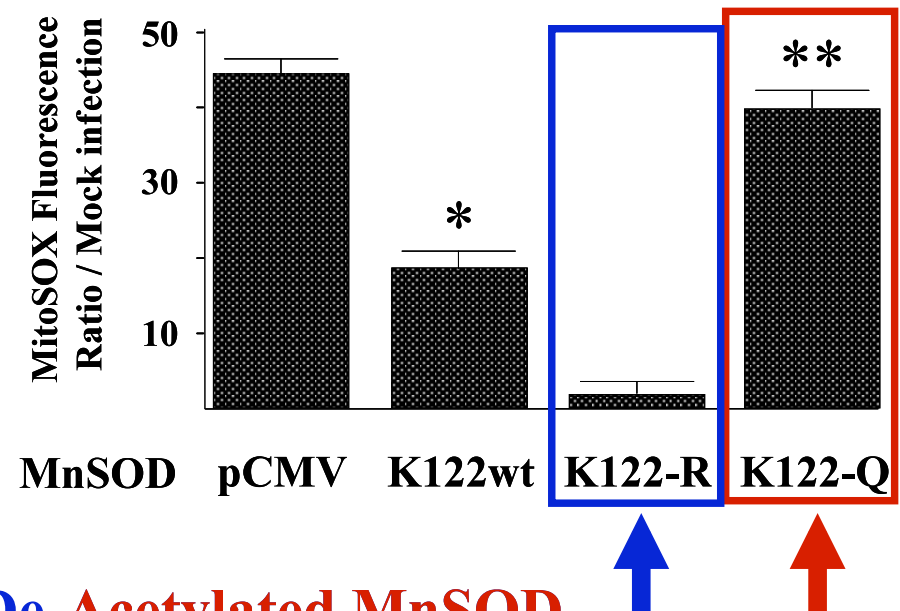
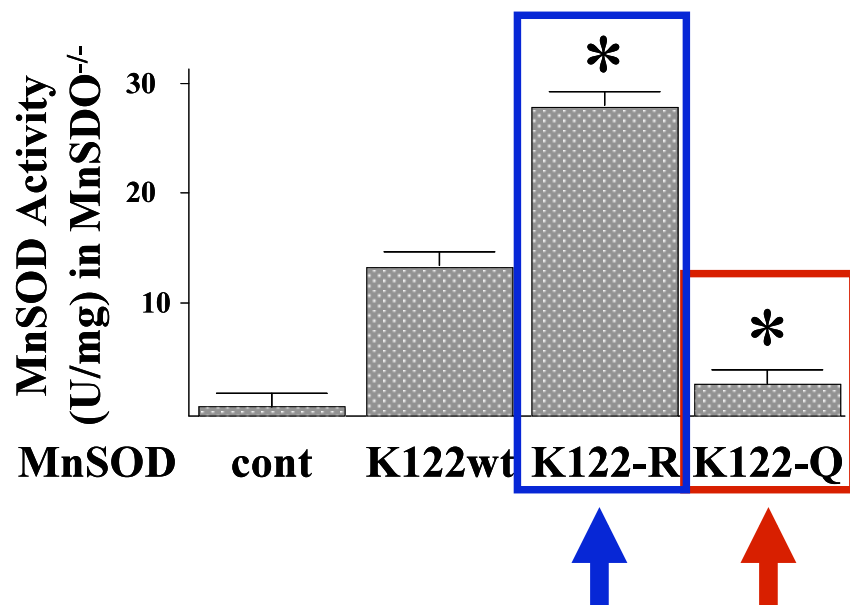
Arginine (Arg, R)

Lenti-MnSOD^{K122-R}



MnSOD^{K122} acetylation status directs dismutase activity

MnSOD^{-/-} MEFs



De-Acetylated MnSOD

Tao et al., 2010, *Molecular Cell*

MnSODK122-R prevents *in vitro* Immortalization

TABLE 1. MnSOD prevents Immortalization of SIRT3^{-/-} MEFs by a single oncogene

MEFs	Control	Myc	Ras	Myc/Ras
SIRT3 ^{+/+}	None	None	None	Immort
SIRT3 ^{-/-}	None	Immort	Immort	Immort
SIRT3 ^{-/-} + lenti-MnSOD ^{K122-Q}	None	Immort	Immort	Immort
SIRT3 ^{-/-} + lenti-MnSOD ^{K122-R}	None	None	None	Immortt

None , no MEF immortalization.

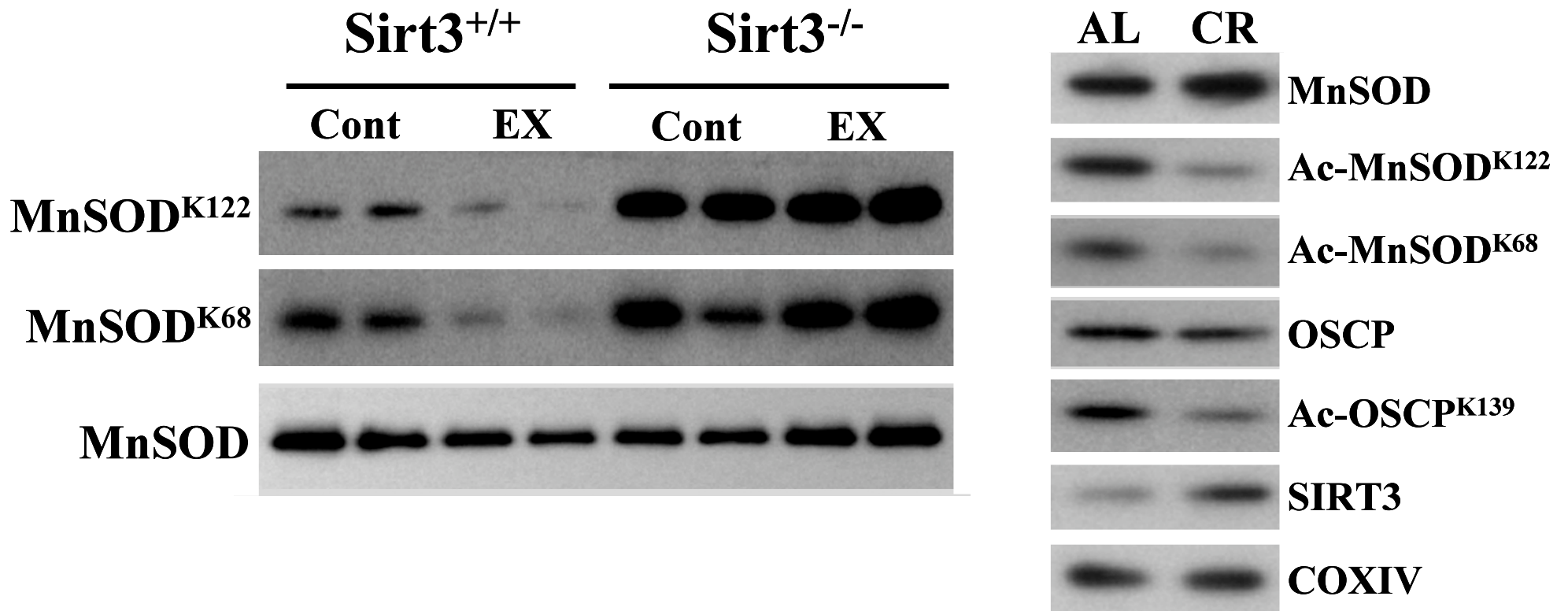
Immort, immortalization.

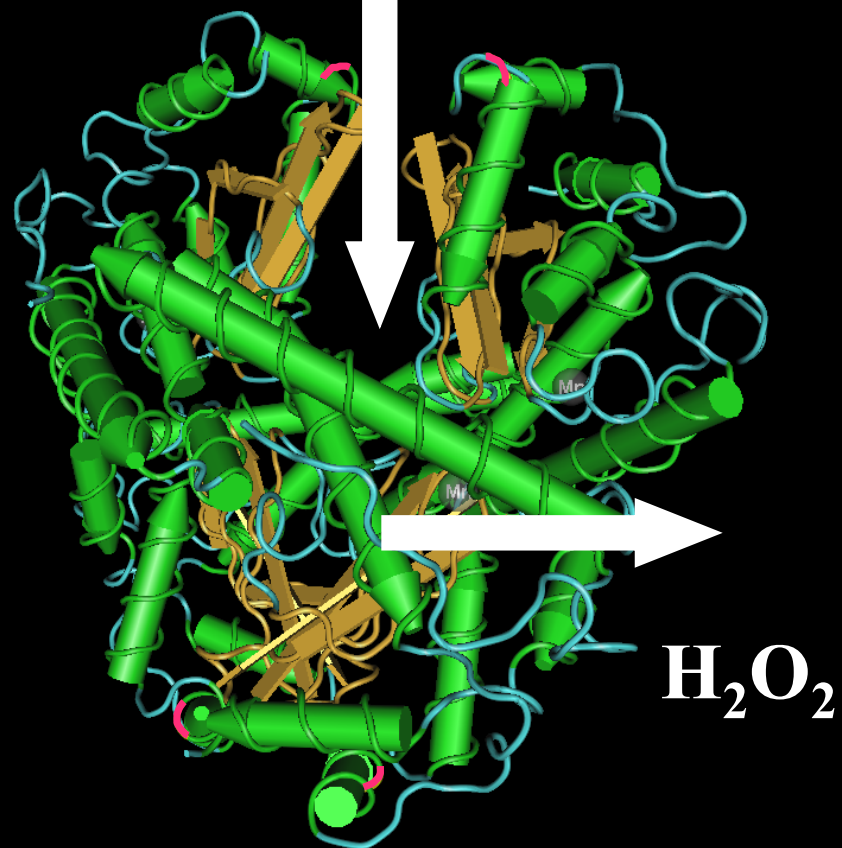
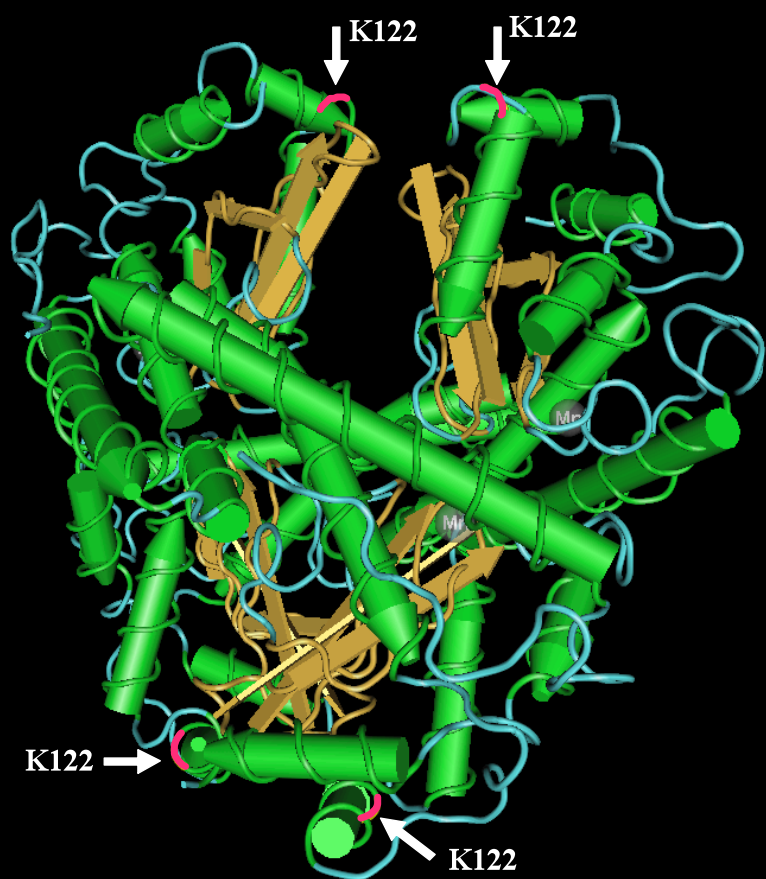
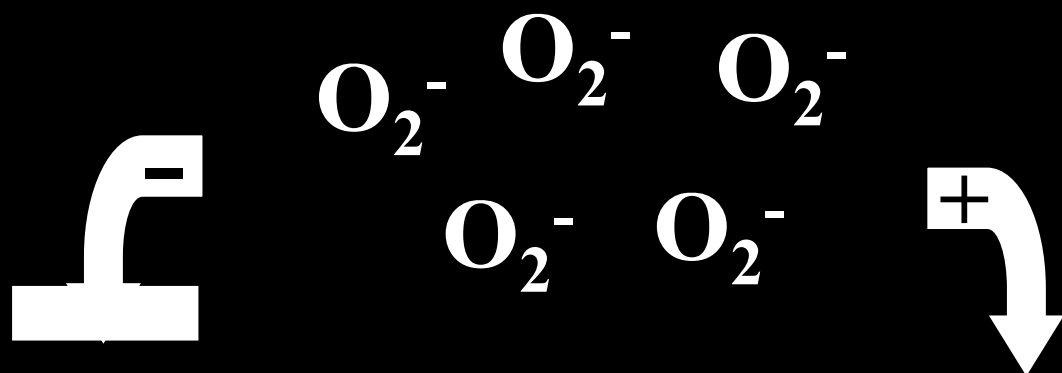
lenti-MnSOD 10 MOI.

Immortalization experiments were done in triplicate.

Tao et al., 2010, *Molecular Cell*

MnSOD De-Acetylation Responds to Exercise and CR

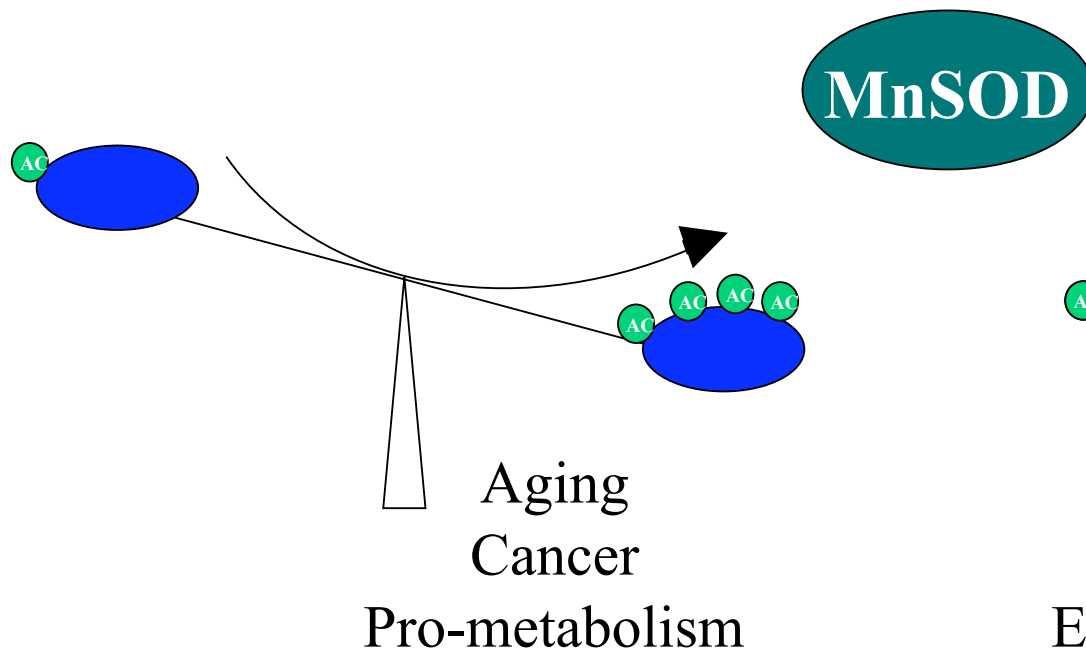




How does MnSOD fit into this model??

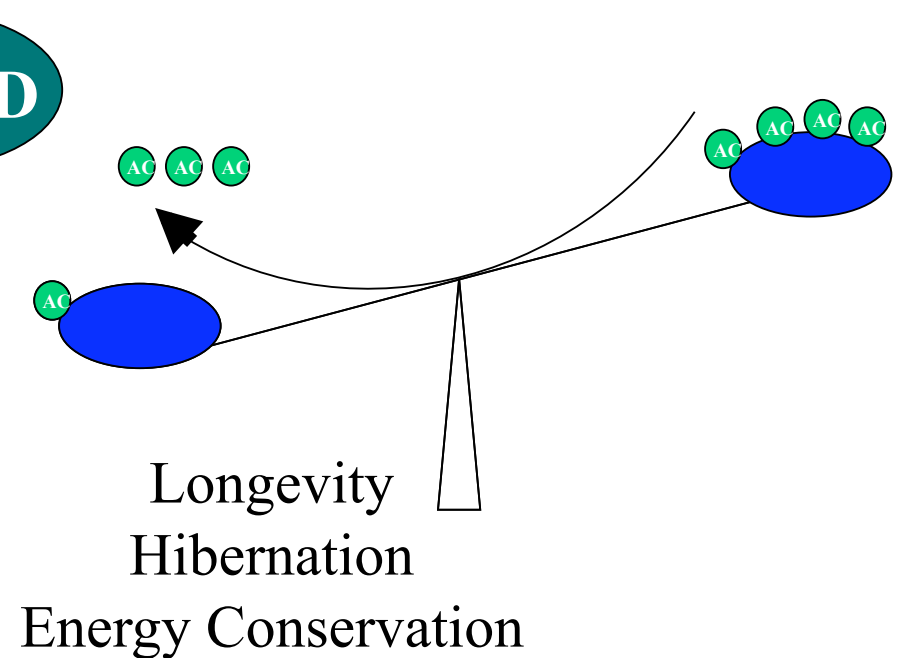
Feasting Metabolic State

ATP and Oxidative damage



Fasting Metabolic State

Repair of Oxidative damage



The Gius Lab

