PPAR GAMMA REGULATES TUMOR-SPECIFIC REPRESSION OF MnSOD EXPRESSION: TOWARD TARGETED “OXIDATION THERAPY” IN ESTROGEN-INDEPENDENT BREAST CANCER

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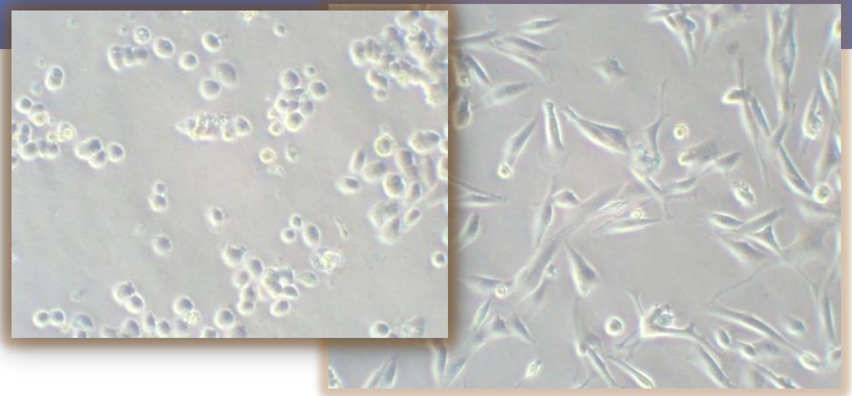
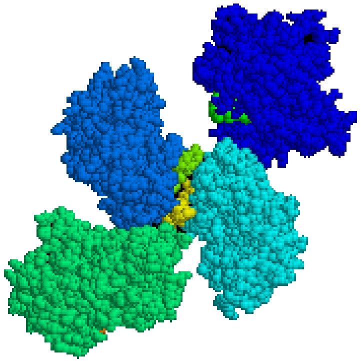
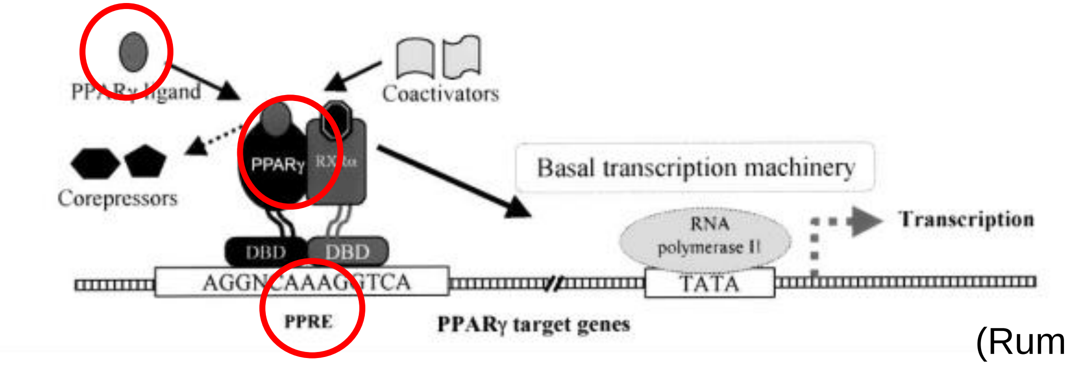
Breast Cancer



- Most common cancer in women worldwide, constitutes 16% of all female cancers
- About 1.3 million women will be diagnosed annually and estimated 15% death (American Cancer Society)
- Most common malignancy among Singaporean women, accounting for 29.7% of all female cancers (Jara-Lazaro et al., 2010)
- Most death are caused by metastases of breast cancer to other organs in the body; bone, lungs, liver and brain
- Poor prognosis and statistics show that the 10-year survival rate of metastatic breast cancer is only 10% with optimal treatment (Merck, 2008)
- A class of anticancer drugs: activators of PPARs (Elstner et al., 2002)

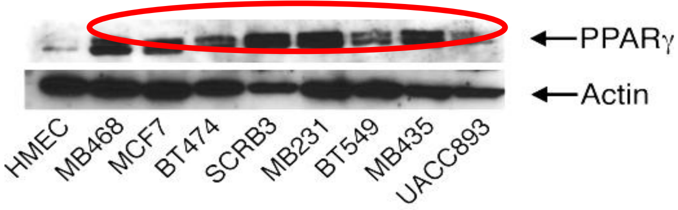
Peroxisome Proliferator- activated Receptor gamma (PPARγ) and Cancer





(Rumi et al., 2004)

- Among the three PPAR isoforms, PPARγ activation appears to play an important role in diverse physiological events
- Ligands: 15d-PGJ$_2$, synthetic glitazones
- Tumor breast cells express higher than normal levels of PPARγ (Elstner et. al., 1998; Zaytseva et al., 2008; Kumar et al., 2009)

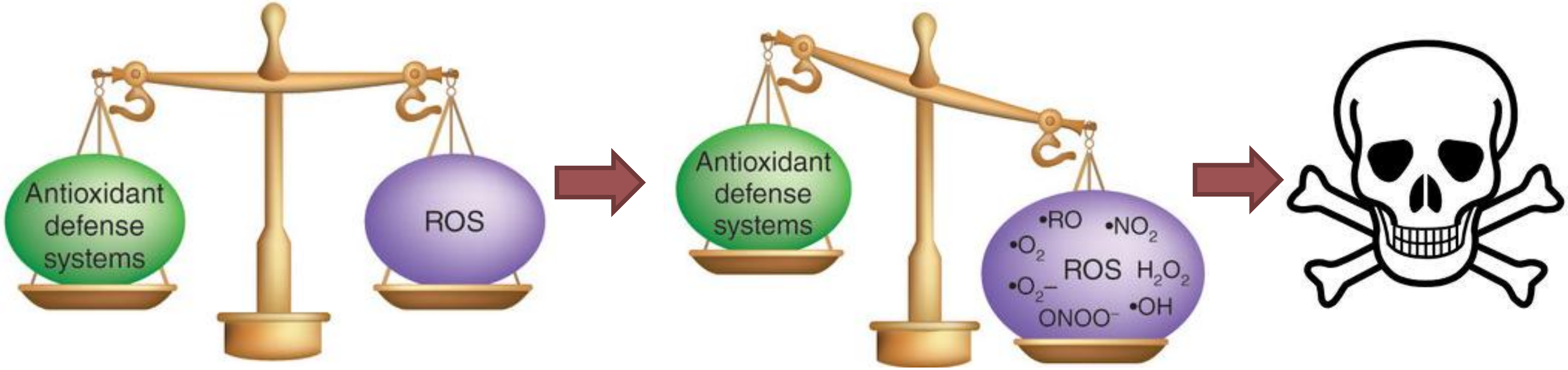


(Zaytseva et al., 2008)

- Ligand activation of PPARγ has been shown to inhibit proliferation and induce apoptosis in several human tumor cell types
- Mechanism of cell death unknown

ROS in Chemotherapy

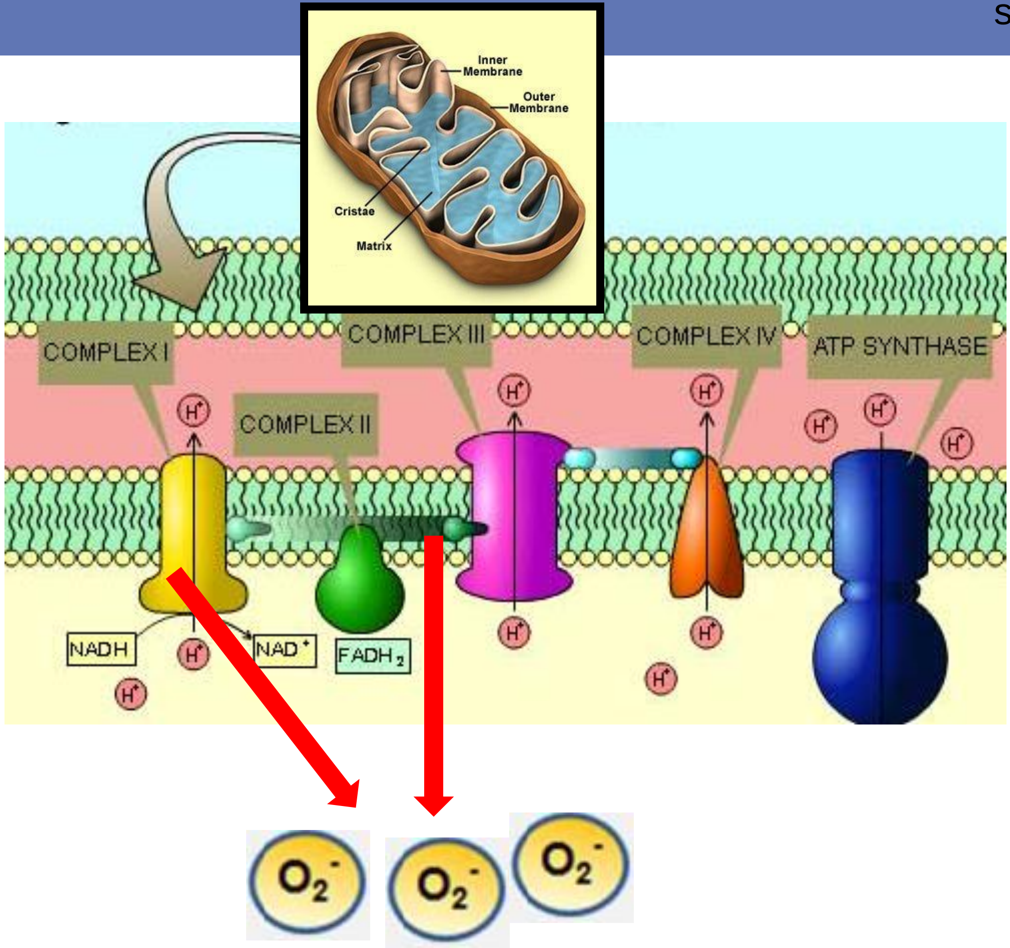
- Intricate balance of ROS required for survival (Tomaselli et al., 2010)



- Excess ROS -> cell death
- Antioxidants: e.g. SOD, catalase, glutathione, metal ion chelators
- ROS has been widely utilized in chemotherapy -> inducing cell death in cancer cells (Akram et al., 2006, Kumar et al., 2007; Ozben, 2007, Low et al., 2010)

PPARγ and ROS production

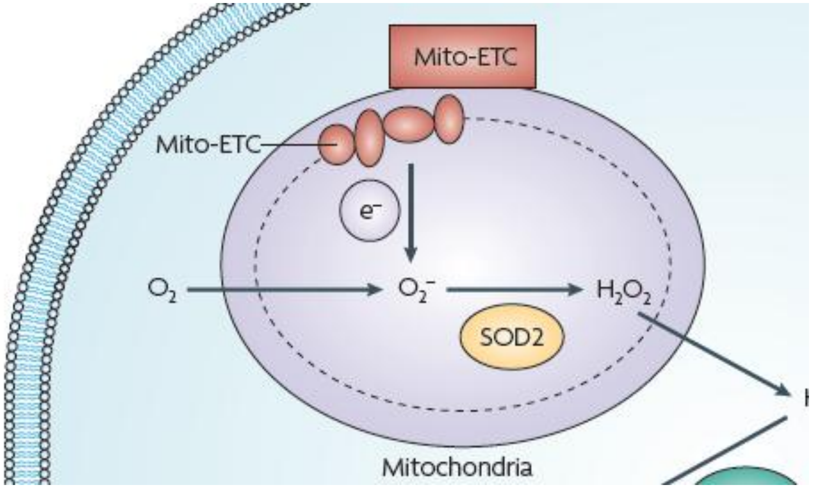
	Ligand	**Concentration**	**Type of ROS**	**Probe**	**Suggested mechanism**	**Cell type**	**Source**
1)	15d-PGJ_2	2.5μM	H_2O_2, $ONOO^-$, ·OH, $O_2^{\cdot -}$	Carboxy-H_2DCFDA; MitoSOX Red	Not reported	B lymphocytes	Ray DM et al, The Journal of Immunology, 2006, 177: 5068–5076.
2)	15d-PGJ_2	5 – 20μM	H_2O_2, $ONOO^-$, ·OH	Carboxy-H_2DCFDA	NADPH activation	Leukemic cells, colorectal cancer cells	SS et al, Clin Cancer Res 2009;15(17) September 1, 2009
3)	15d-PGJ_2, PGD_2, Rosiglitazone, Ciglitazone, Troglitazone	8μM	H_2O_2, $ONOO^-$, ·OH	Carboxy-H_2DCFDA	Nucleophilic addition reactions with thiols	Leukemic cells	Y.-C. Chen et al., Biochimica et Biophysica Acta 1743 (2005) 291–304
4)	Ciglitazone	10μM	H_2O_2, $ONOO^-$, ·OH	Carboxy-H_2DCFDA	Not reported	Renal cells	C.H. Kwon et al. / Toxicology 257 (2009) 1–9
5)	Ciglitazone	20μM	H_2O_2, $ONOO^-$, ·OH	Carboxy-H_2DCFDA	Mitochondrial depolarization	Glioma cells	Dong WK et al., Neurochem Res (2008) 33:551–561
7)	15d-PGJ_2	1 – 30μM	H_2O_2, $ONOO^-$, ·OH	Carboxy-H_2DCFDA	Disruption of mitochondrial membrane potential	Osteoblastic cells	S.J. Lee et al. / Toxicology 248 (2008) 121–129
8)	15d-PGJ_2	1 – 10μM	·OH, $O_2^{\cdot -}$	Carboxy-H_2DCFDA, Lucigenin	Xanthine oxidase	Lymphocytes	A´ lvarez-Maqueda M et al., The Journal of Bio Chem. Vol. 279, No. 21, Issue of May 21, pp. 21929–21937, 2004
9)	Troglitazone, Ciglitazone	10 – 100μM	H_2O_2	Carboxy-H_2DCFDA	Inhibition of mitochondria complex I & II	Jurkat T cells	Soller M et al., Mol Pharmacol 71:1535–1544, 2007



What regulates mitochondrial superoxide levels in cells?

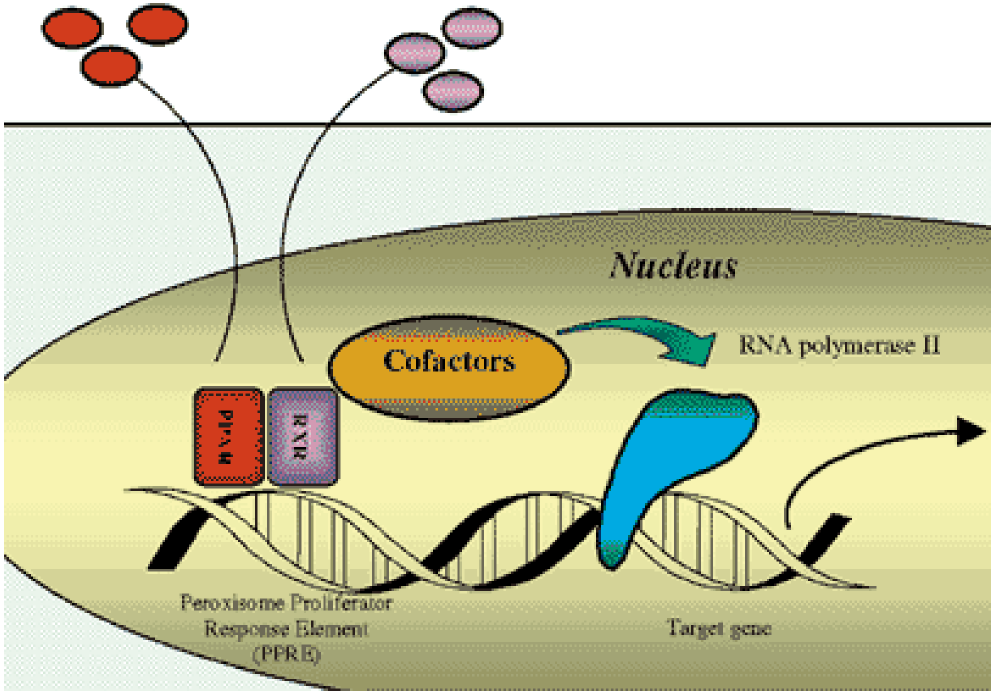
Manganese Superoxide Dismutase (MnSOD)

- Antioxidant enzyme found in mitochondria and peroxisomes



- Prime importance in maintaining cellular ROS balance
- ROS stress seems to render cancer cells more dependent on SODs to protect themselves (Huang et al., 2000)
- MnSOD KO mice die just after birth (Lebovit et al., 1996)
- Down-regulation of MnSOD in breast cancer cells lead to activation of mitochondrial-driven apoptotic processes (Murias et al., 2008)
- Mouse MnSOD is a PPARγ target gene (Ding et al., 2007)

Is human MnSOD a target gene of PPARγ**?**



hMnSOD
???

medscape.com

Putative PPRE sites in Human MnSOD Promoter

A

PPRE1

-2742 TGCAGAGGACATCCTGAGCTGGCTGGAGTAACTT<u>GGGACACAGGTCA</u>AT //

PPRE2

//-1673 ACTTG<u>AGGTCAGGCGTTCG</u>AGACCATCCTGACCAACATAGTGAAACCCCGT //

PPRE3

//-713 TCCTGTCCTGGAAT**<u>AGGTCCCAAGGTCG</u>**GCTTACTTGCAAAGCAAGGGTACGGCGCAAGA

-653 GTACTGAATACGGGTTGGAAGGGCGCTGGCTCTACCCTCAGCTCATAGGCCGGCTGGGCG

-593 GCGCTGACCAGCAGCTAGGCCCCGTCTTCCCTAGGAACGGCCACGGGGGCCCTGGGAGGG

-533 TATGAATGTCTTTTTGCAGTGAGGCCTCTGGACCCCGCGGCCCCCCGGCAGCGCAACCAA

-473 AACTCAGGGGCAGGCGCCGCAGCCGCCTAGTGCAGCCAGATCCCCGCCGGCACCCTCAGG

-413 GGCGGAGCCGGAGGCAGGGCCTTCGGGCCGTACCAACTCCACGGGGGCAGGGGCCGCCTC

-353 CCTTCGGCCGCGCGCCACTCAAGTACGGCAGACAGGCAGCGAGGTTGCCGAGGCCGAGGC

-293 TAGCCTGCAGCCTCCTTTCTCCCGTGCCCTGGGCGCGGGGTGTACGGCAAGCGCGGGCGG

-233 GCGGGACAGGCACGCAGGGCACCCCCGGGGTTGGGCGCGGCGGGCGCGGGGCGGGGCCCG

-173 CGGGGGGGGGGGCGGGGCGGCGGTGCCCTTGCGGCGCAGCTGGGGTCGCGGCCCTGCTC

-113 CCCGCGCTTTCTTAAGGCCCGCGGGCGGCGCAGGAGCGGCACTCGT**G**GCTGTGGTGGCTT

-53 CGGCAGCGGCTTCAGCAGATCGGCGGCATCAGCGGTAGCACCAGCACTAGCAGC**ATG**TTG

Sequence ID:
NCBI-GI: 67782305
NCBI-GeneID: 6648
Ensembl: ENSG00000112096

B

Target genes/consensus name	Binding efficiency invivo/invitro	Sequence	Strand
Human MnSOD			
Strong PPARgamma	0.42/0.49	GGGACACAGGTCA	+
Strong PPARgamma	-/0.89	**<u>AGGTCCCAAGGTCG</u>**	+

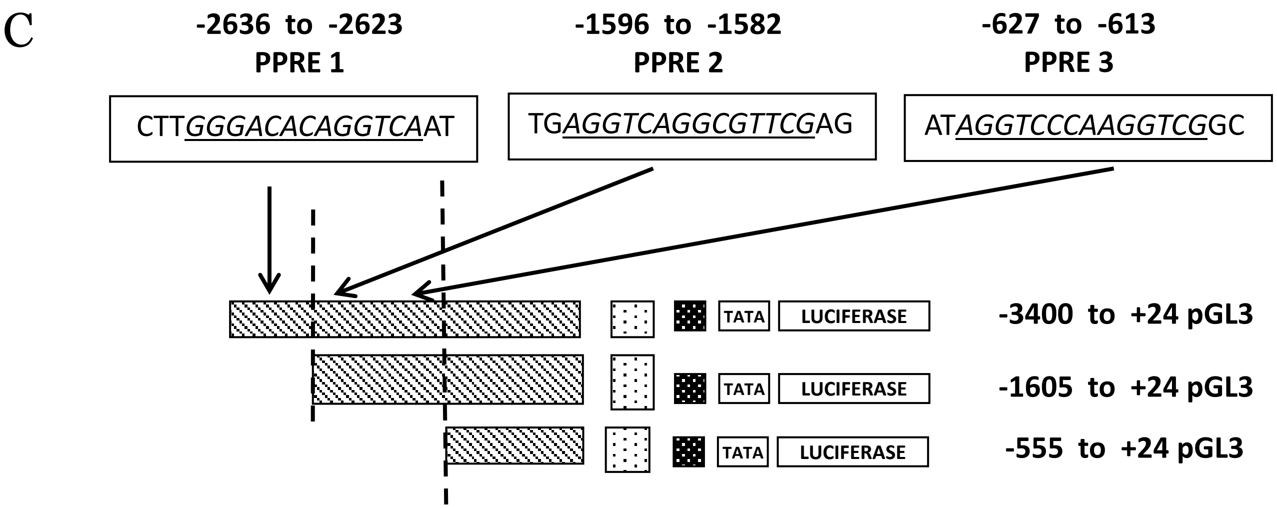
PPRE1

PPRE3

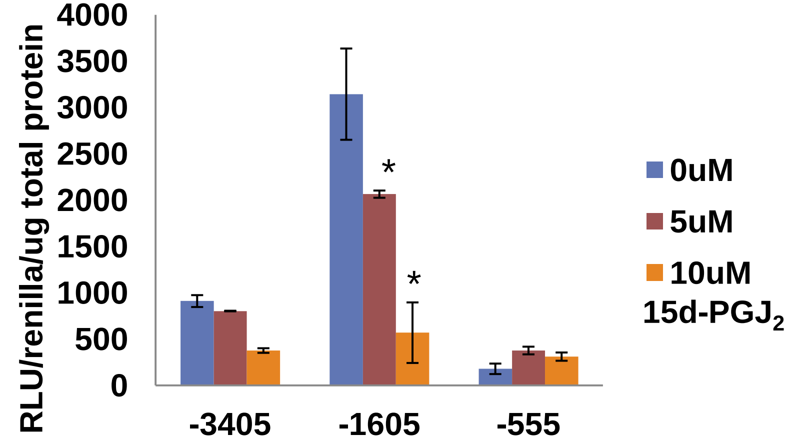
From PPRESearch: http://www.cellfate.org/PPRE

Gireedhar V; Kumar AP; Loo SY; Pervaiz S; Clement MV; and Sakharkar MK (2009) Computational identification and experimental validation of PPRE motifs in NHE1 and MnSOD genes of Human. BMC Genomics. 10(Suppl 3):S5.

(Gireedhar V et al., 2009)

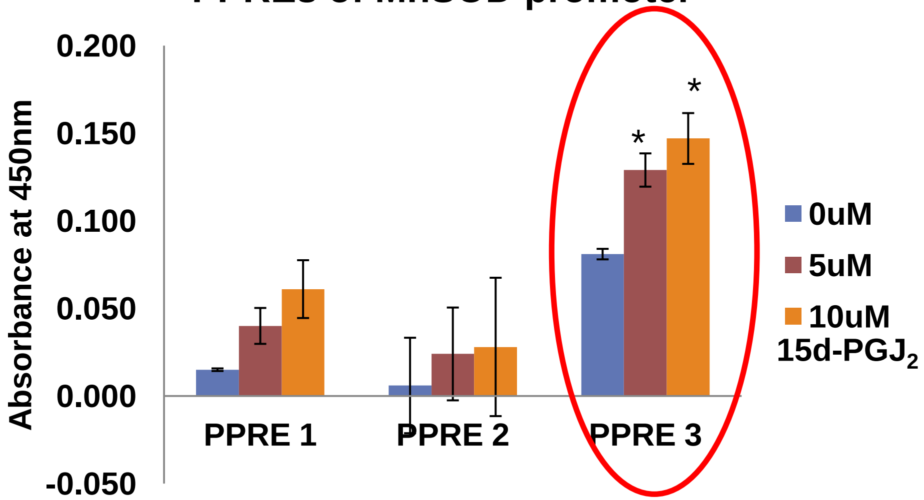


MnSOD promoter activity



Luciferase assay of MnSOD promoter activity in MDA-MB-231 transfected with MnSOD deletion contructs followed by 15d-PGJ$_2$ treatment.

DNA binding activity of PPARγ at PPREs of MnSOD promoter

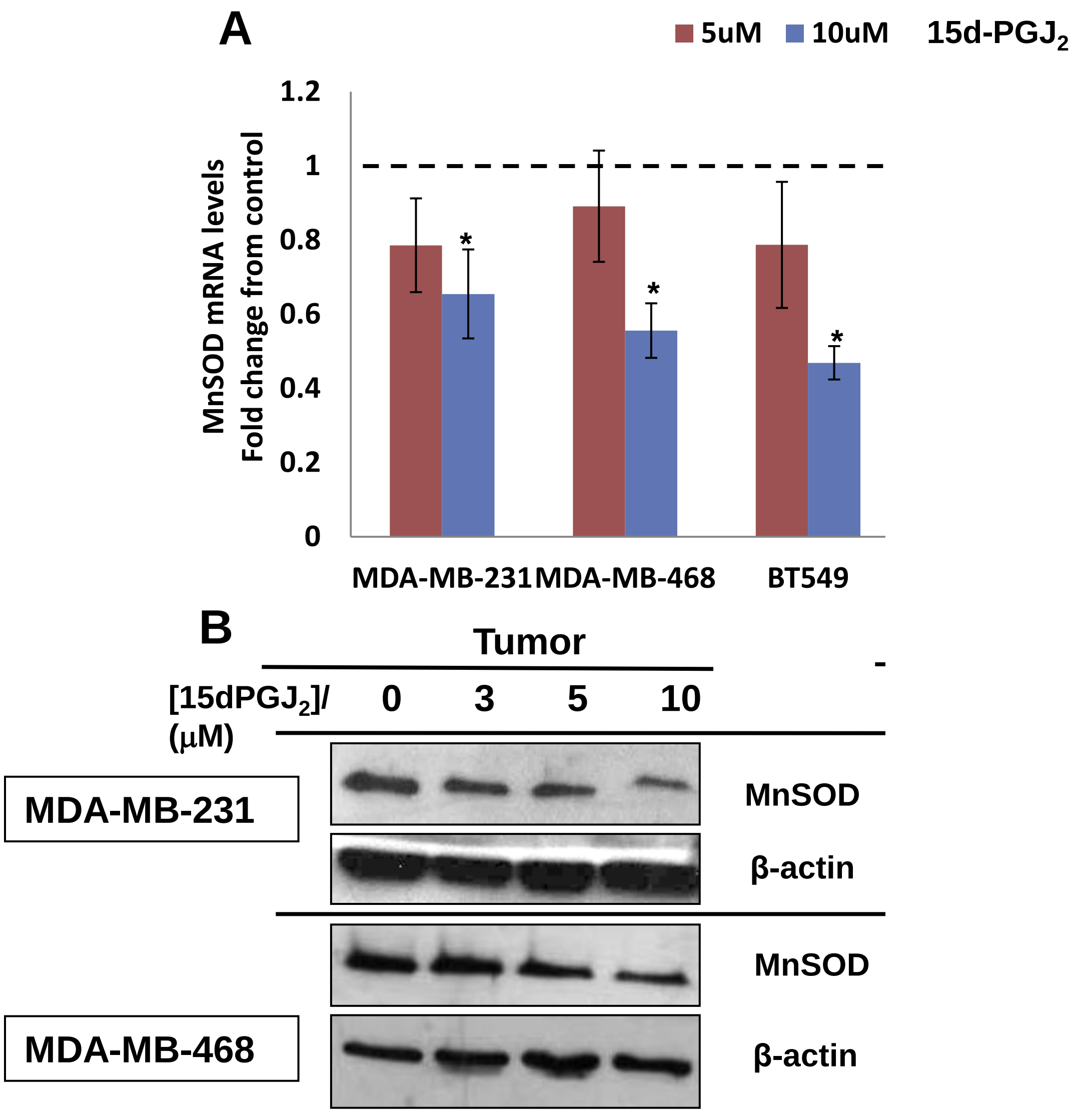


No-shift DNA binding assay of DNA binding activity of PPARγ at three PPREs of MnSOD promoter

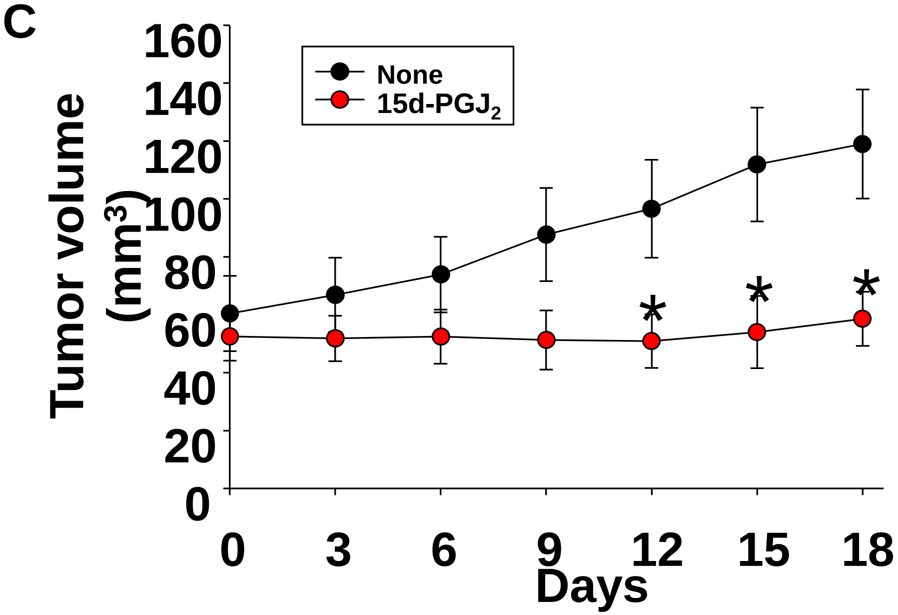
MnSOD is a target gene of PPARγ and PPRE3 is the bona fide binding site.

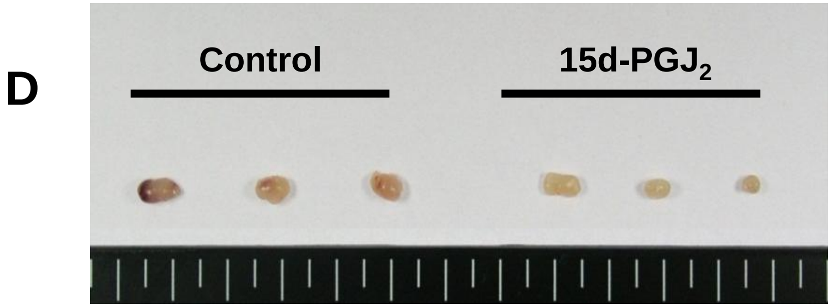
What is the effect of PPARγ activation on MnSOD levels**?**

PPARγ activation in vitro



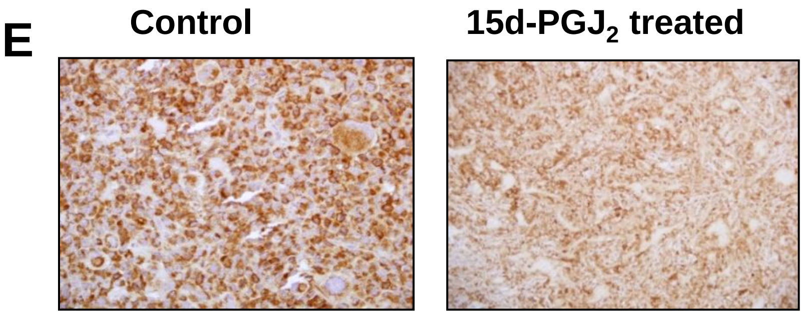
PPARγ activation in vivo





Weight (g)

Control			15d-PGJ$_2$		
1	2	3	1	2	3
0.077	0.06	0.065	0.037	0.03	0.014



PPARγ activation down-regulates MnSOD expression in vitro and in vivo.

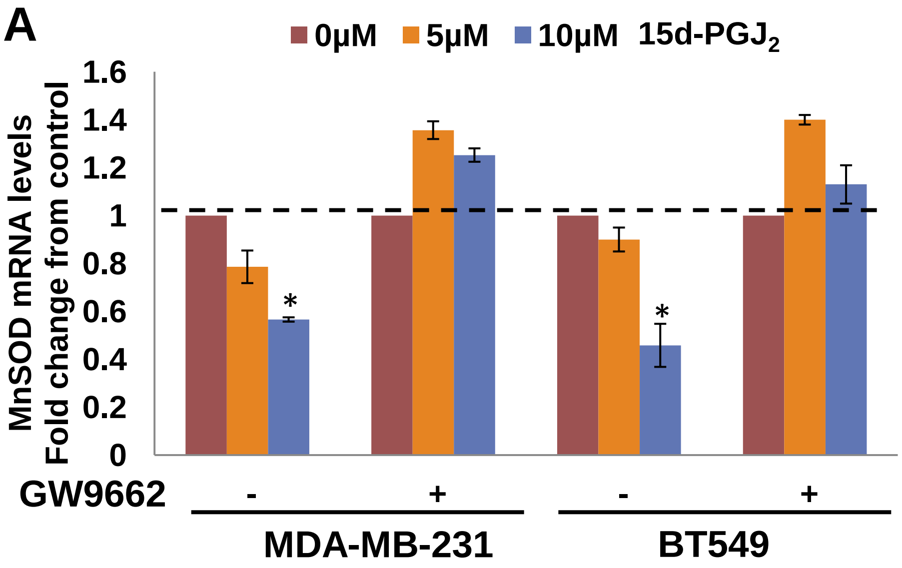
1) Human MnSOD is down-regulated by PPARγ activation

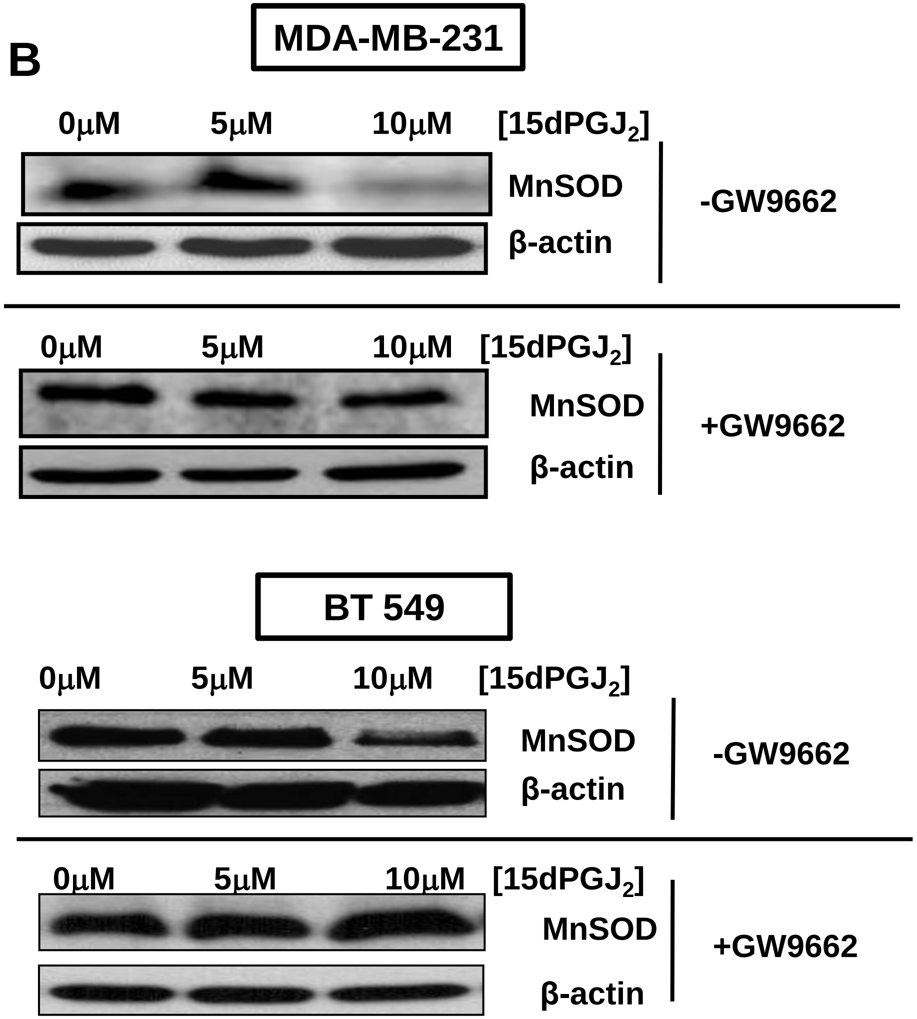
Is this effect PPARγ-dependent?

→ GW9662

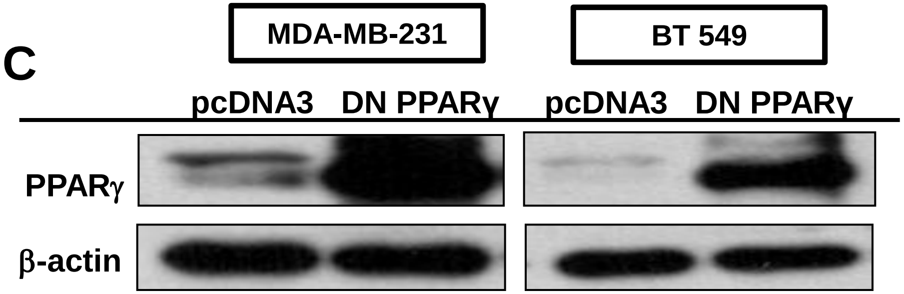
→ DN PPARγ

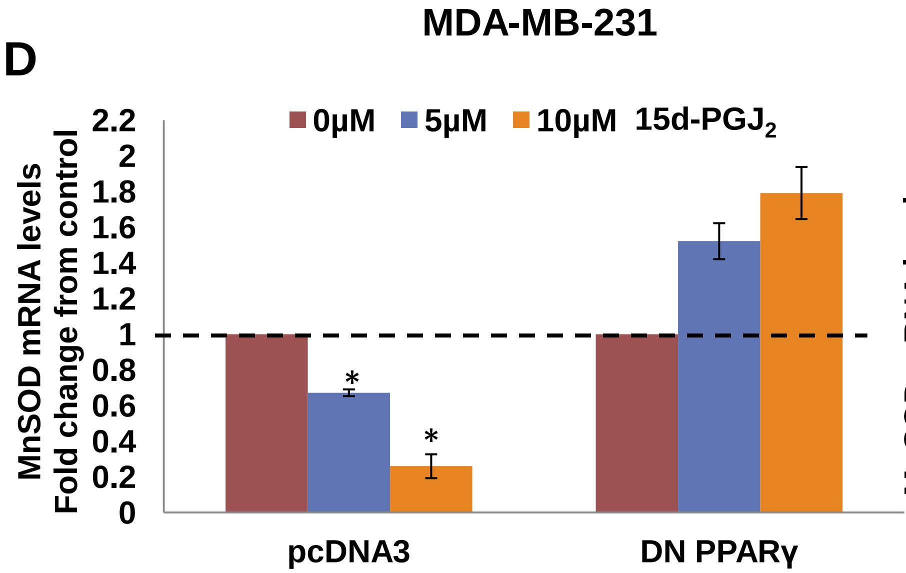
1) PPARγ inhibitor: GW9662

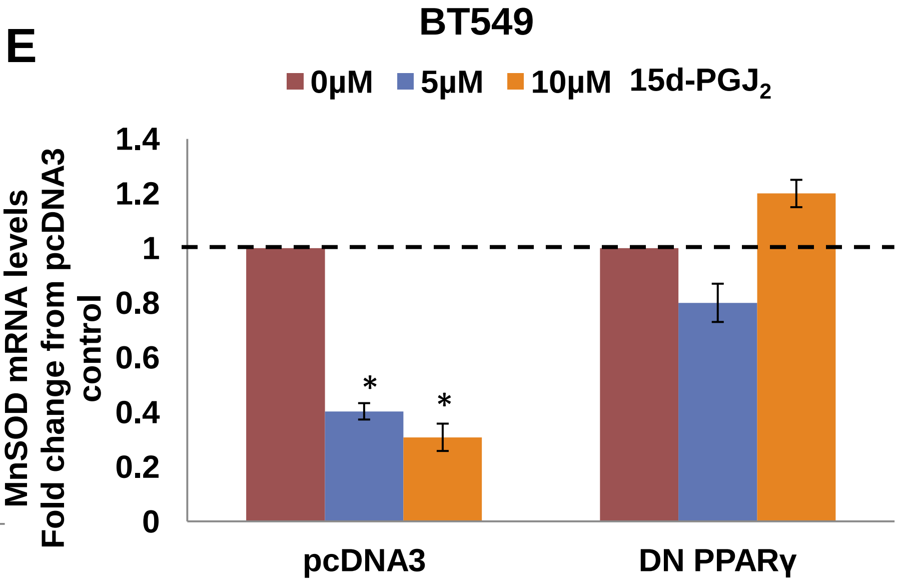




2) Transfection of dominant negative PPARγ





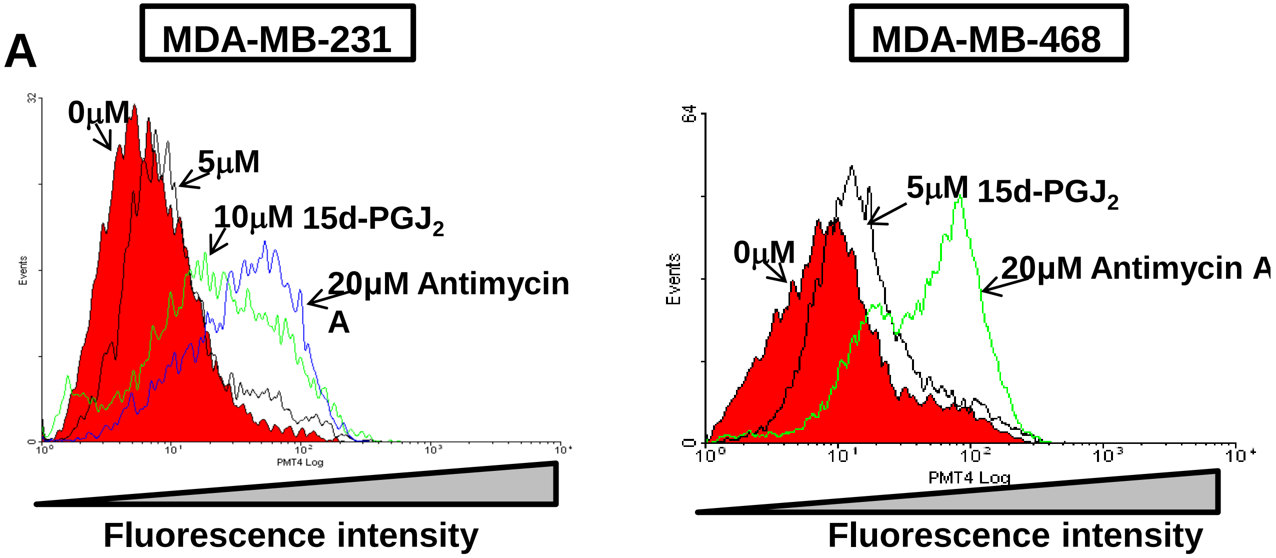


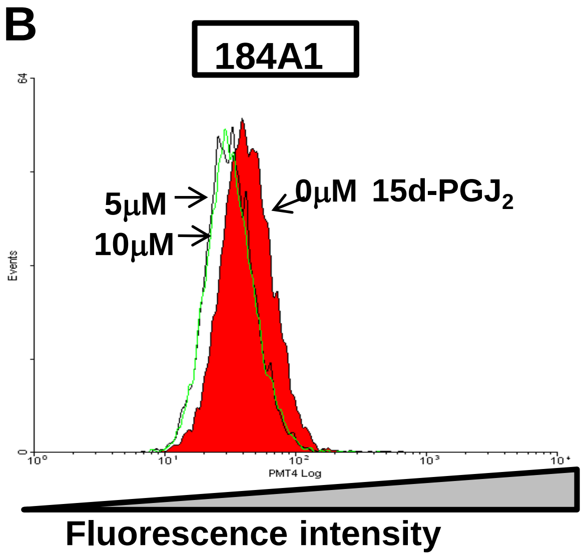
Down-regulation of MnSOD expression is PPARγ-dependent.

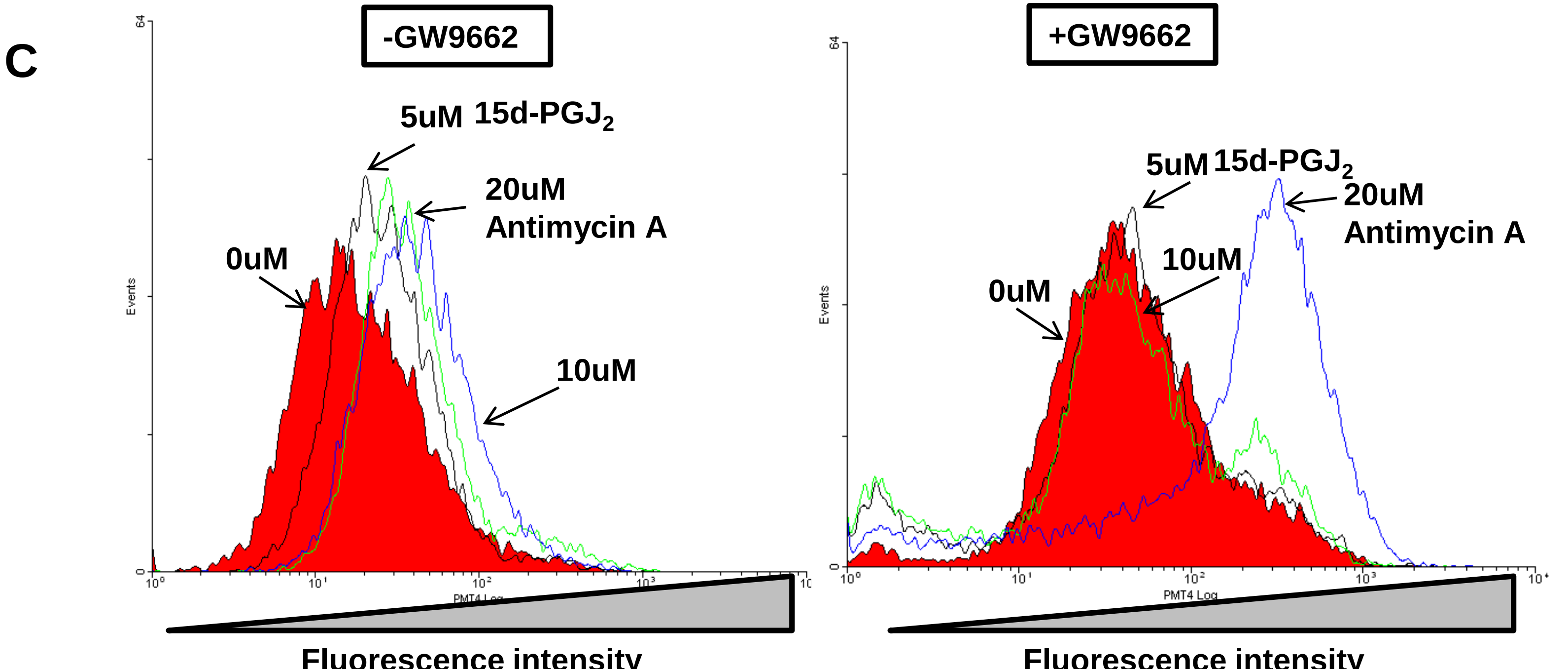
1) Human MnSOD is down-regulated by PPARγ activation
2) PPARγ-dependent

How does PPARγ activation affect intracellular ROS levels?

PPARγ-induced ROS Production



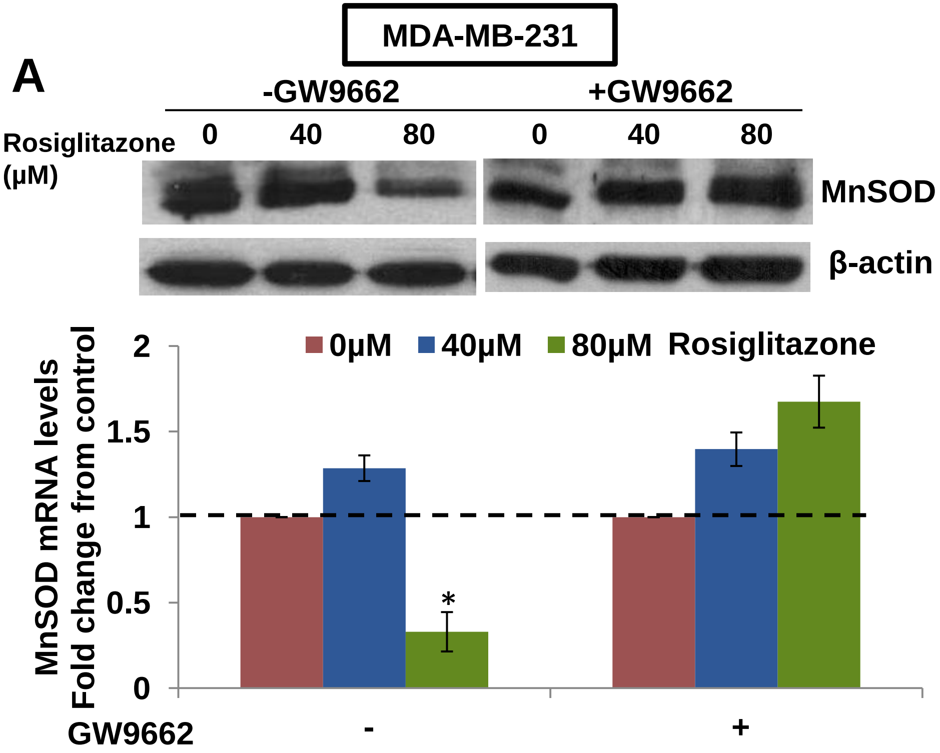


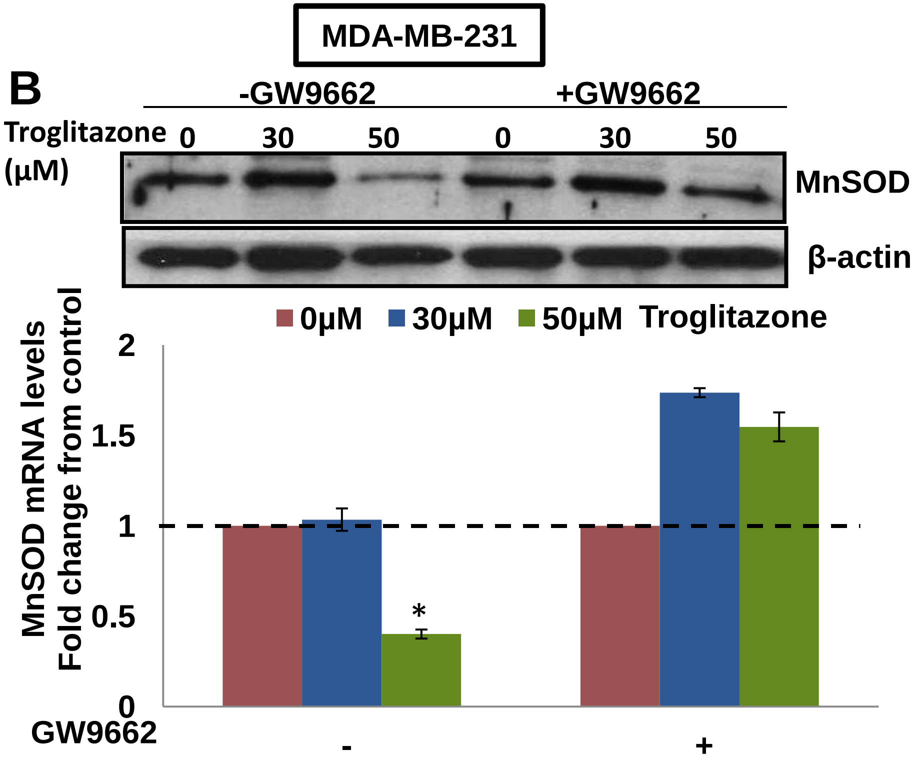


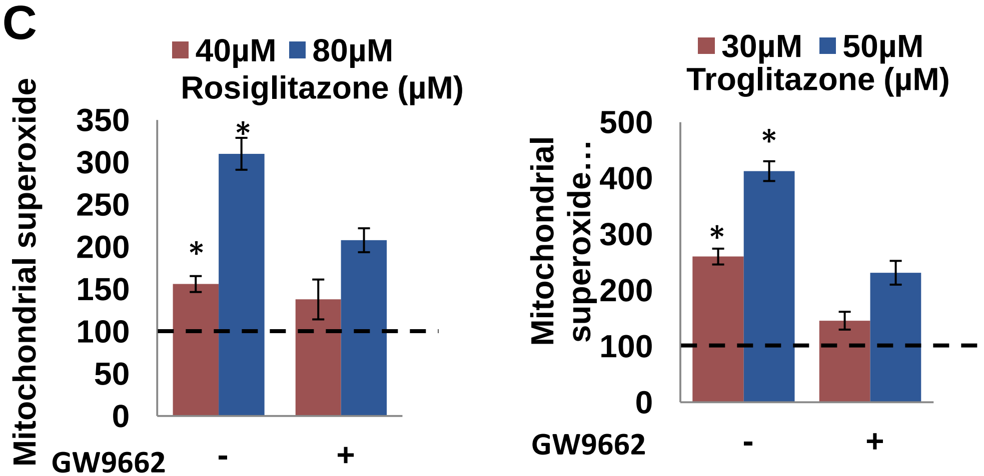
1) Human MnSOD is down-regulated by PPARγ activation
2) PPAR γ-dependent
3) Increase $0_2^{\cdot -}$ levels

Do synthetic glitazones have the same effect?

PPARγ Activation by Synthetic Glitazones







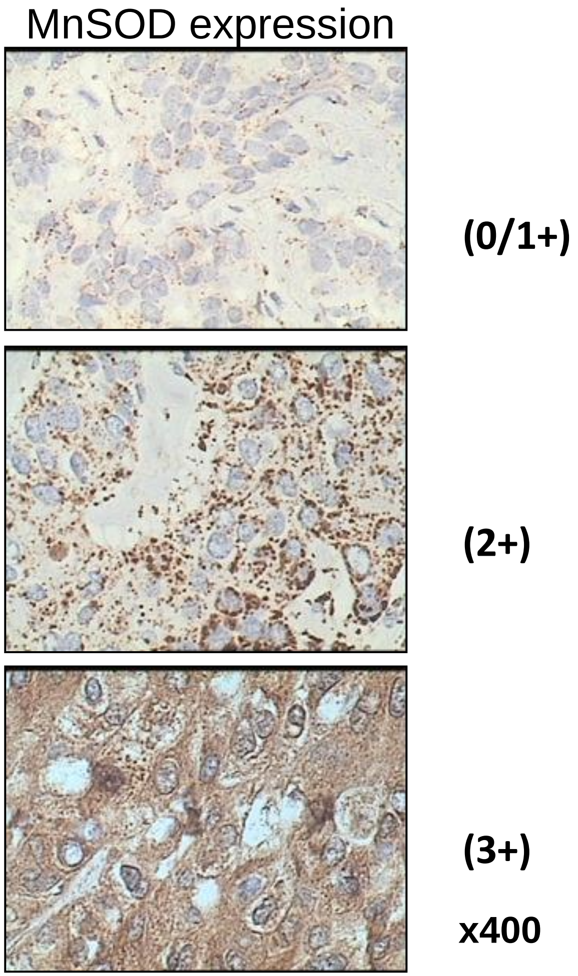
Cohort Study of Breast Cancer Patients

Group	Diabetes	Treatment for diabetes
I	Yes	Glitazones
II	Yes	Other anti-diabetics
III	No	NA

Effect of Glitazone Treatment in Breast Cancer Patients

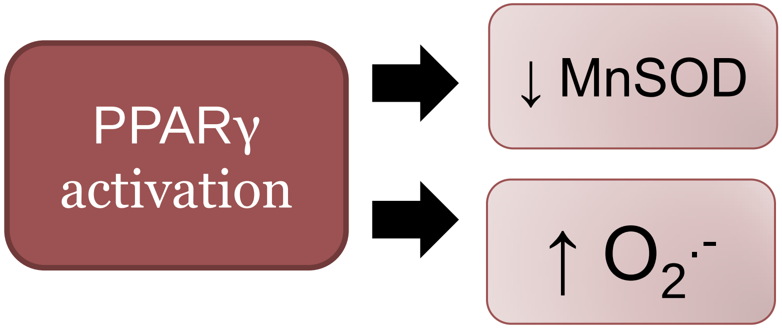
D

	MnSOD (IHC) Scoring	Tumour	Normal
	Breast cases		
Group I - On Glitazones	Case1	0	2+
	Case2	0	2+
	Case3	2+	NA
	Case4	0	2+
Group II - On other antidiabetics	Case5	2+	3+
	Case6	1+	2+
	Case7	2+	3+
	Case8	2+	2+
	Case9	2+	1+
	Case10	2+	2+
Group III - Non diabetics	Case11	2+	2+
	Case12	2+	2+
	Case13	3+	2+
	Case14	2+	2+
	Case15	2+	2+
	Case16	2+	2+



Synthetic glitazones achieve the same effects of down-regulating MnSOD in vitro and in vivo.

1) Human MnSOD is down-regulated by PPARγ activation
2) PPARγ-dependent
3) Increase $0_2^{\cdot -}$ levels



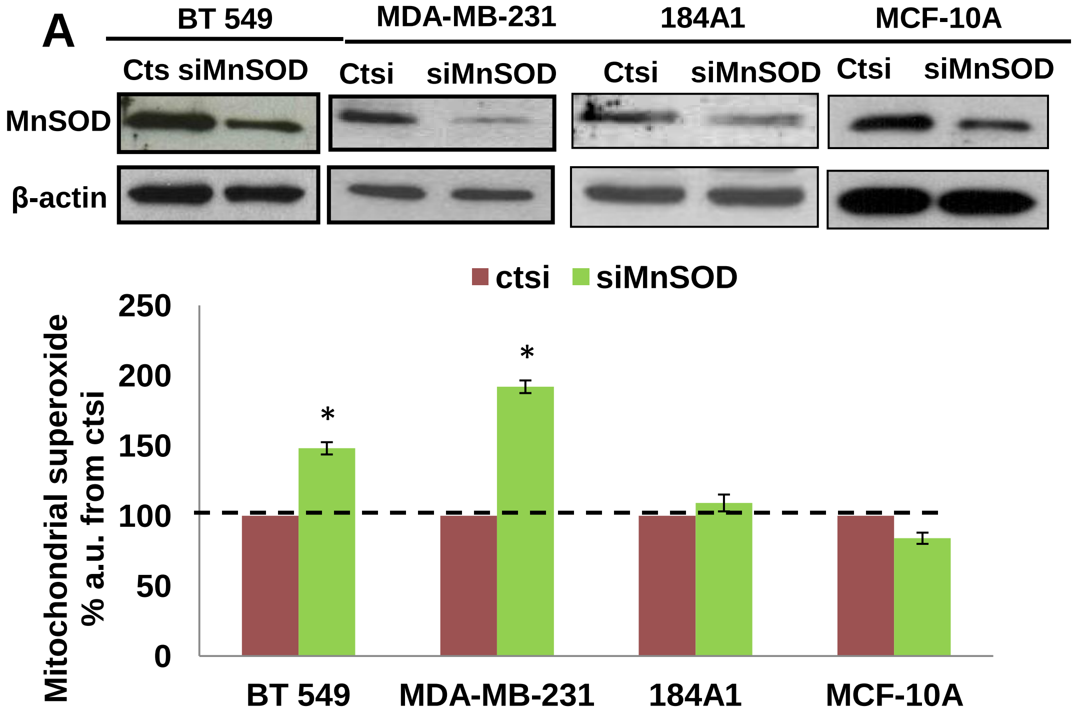
PPARγ activation

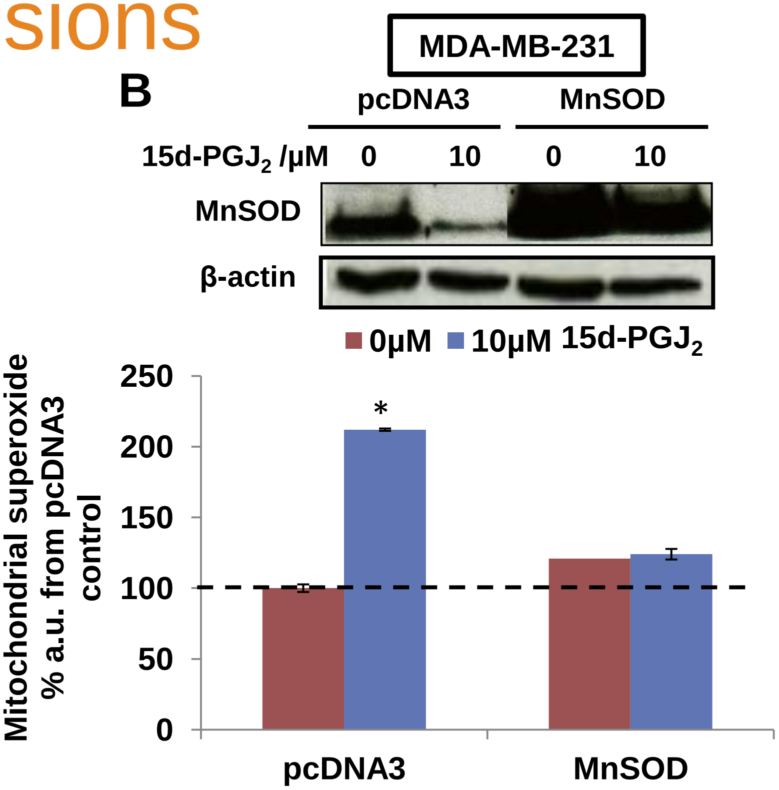
↓ MnSOD

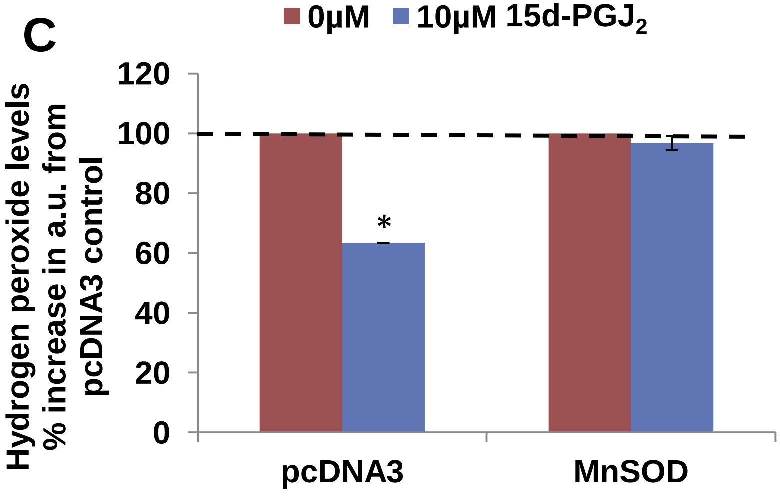
↑ $O_2^{\cdot -}$

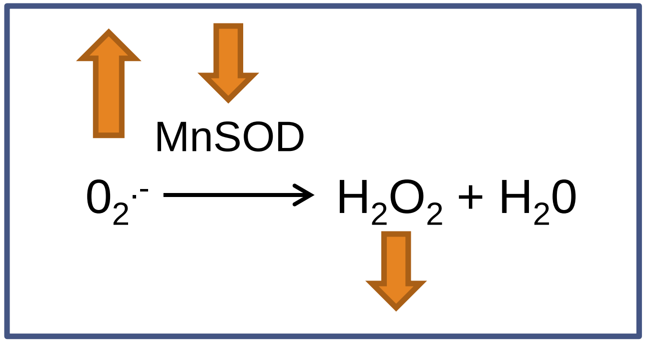
Can down-regulation of MnSOD account for increased ROS levels?

Changes in MnSOD expressions regulate ROS levels





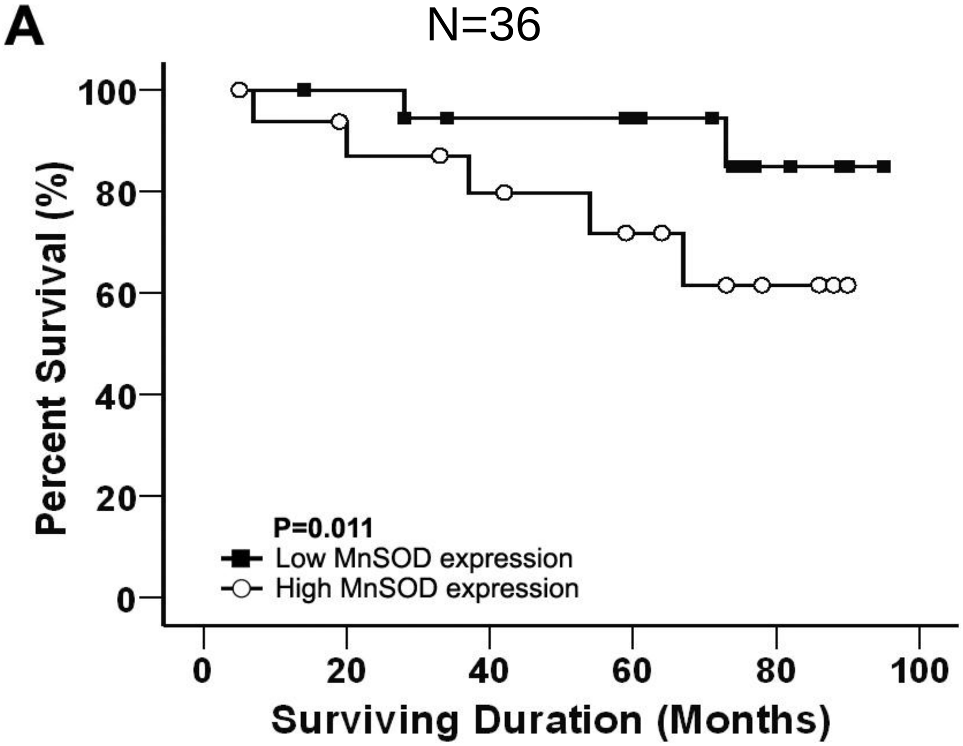


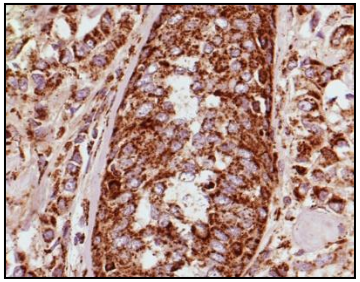


MnSOD

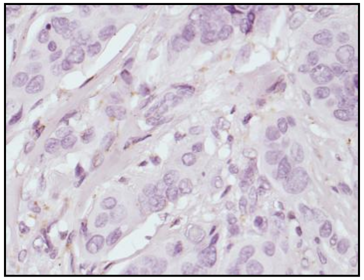
$0_2^{\cdot -} \longrightarrow H_2O_2 + H_20$

Kaplan-Meier curve showing survival differences of MnSOD expression in patients with stage 1 and 2 breast cancer



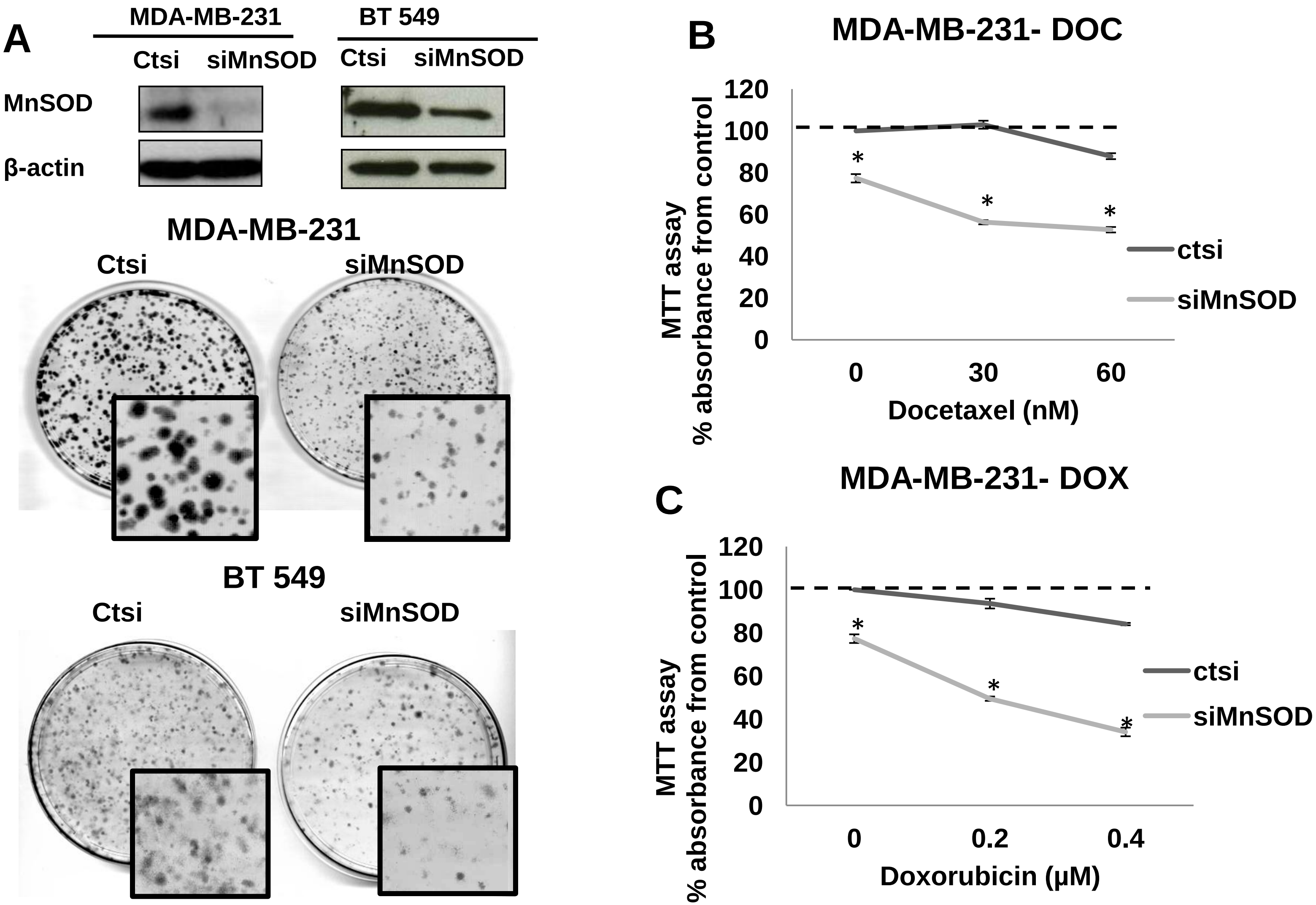


High MnSOD



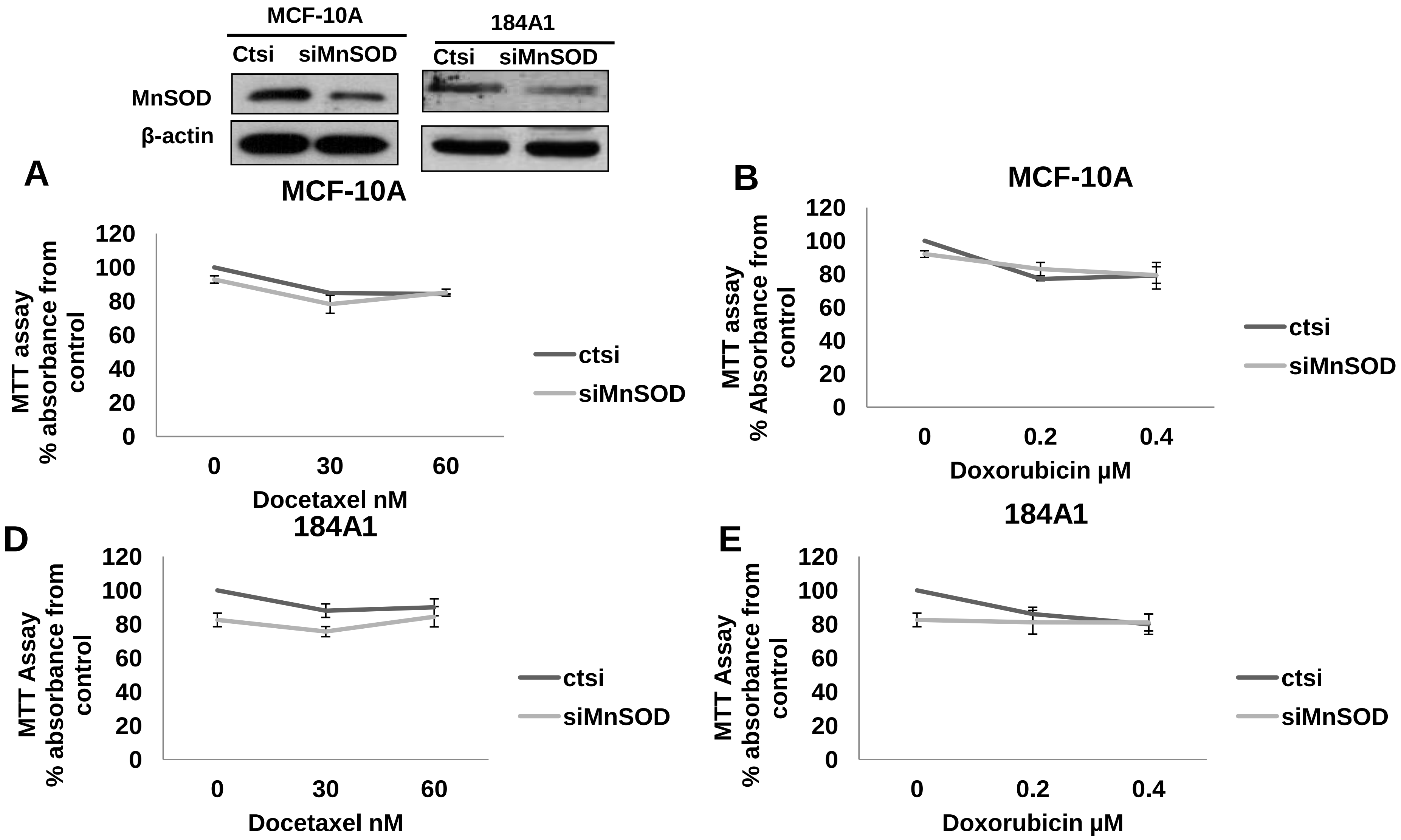
Low MnSOD

Sensitization in Breast Tumor Cells



Suppression of MnSOD increases chemosensitivity of breast tumor cells to anti-cancer drugs.

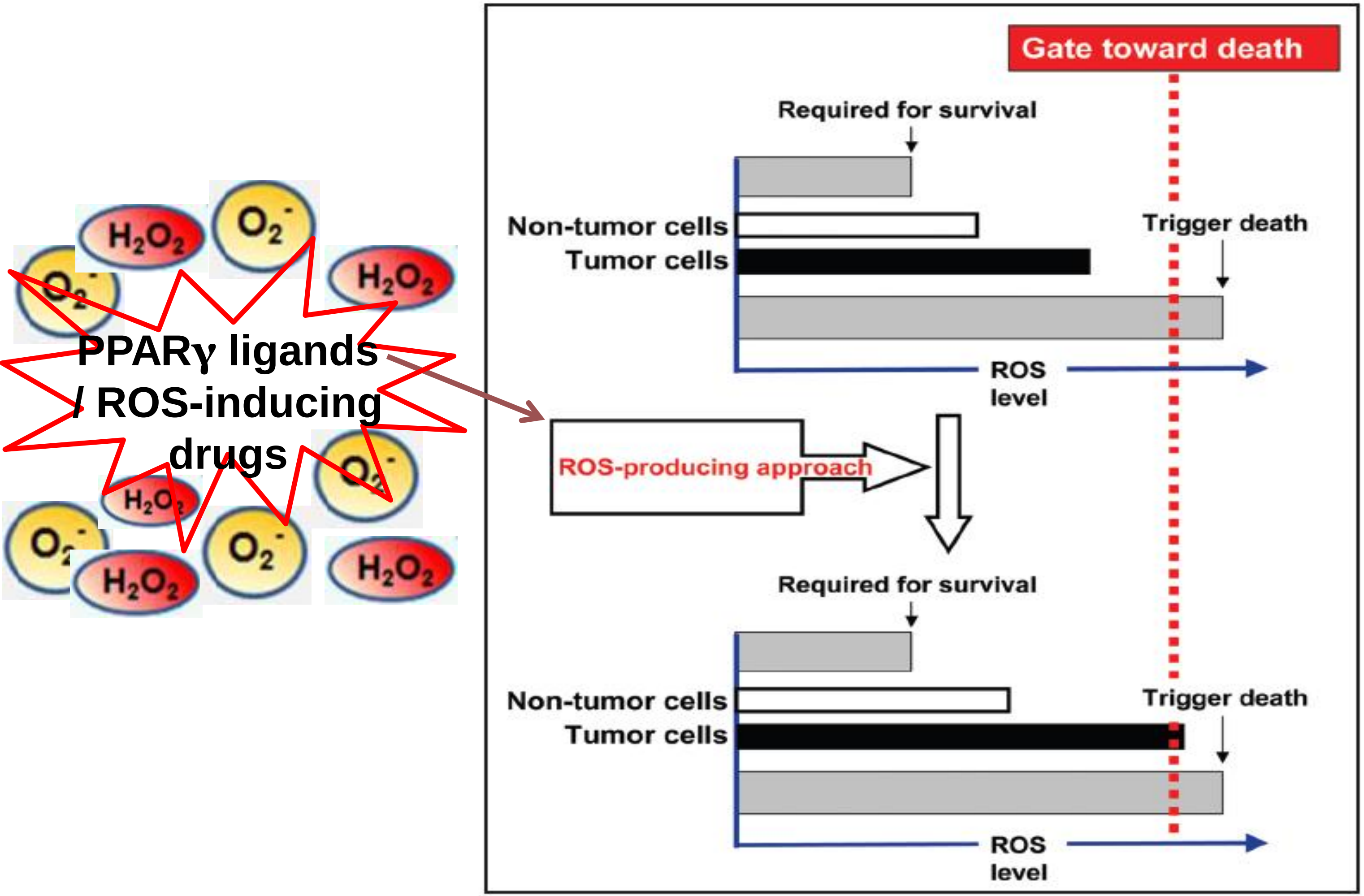
Normal Breast Epithelial Cells



Normal breast cells are not affected by suppression of MnSOD.

Oxidation Therapy

- Cancer cells are generally under reactive oxygen species (ROS) stress (Heliman et al., 2004; Zhou et al., 2003)

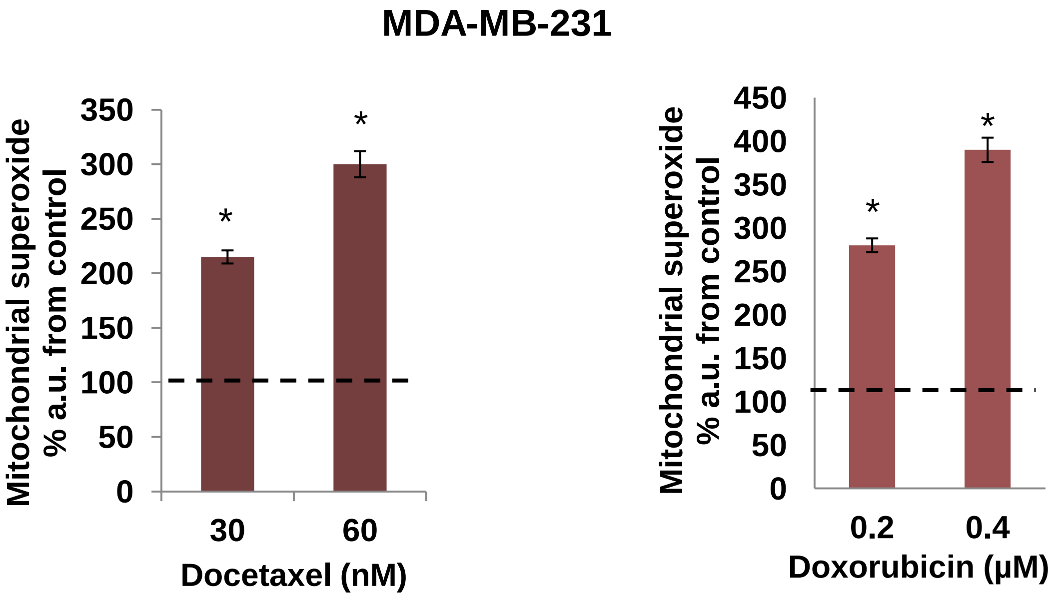


PPARγ ligands / ROS-inducing drugs

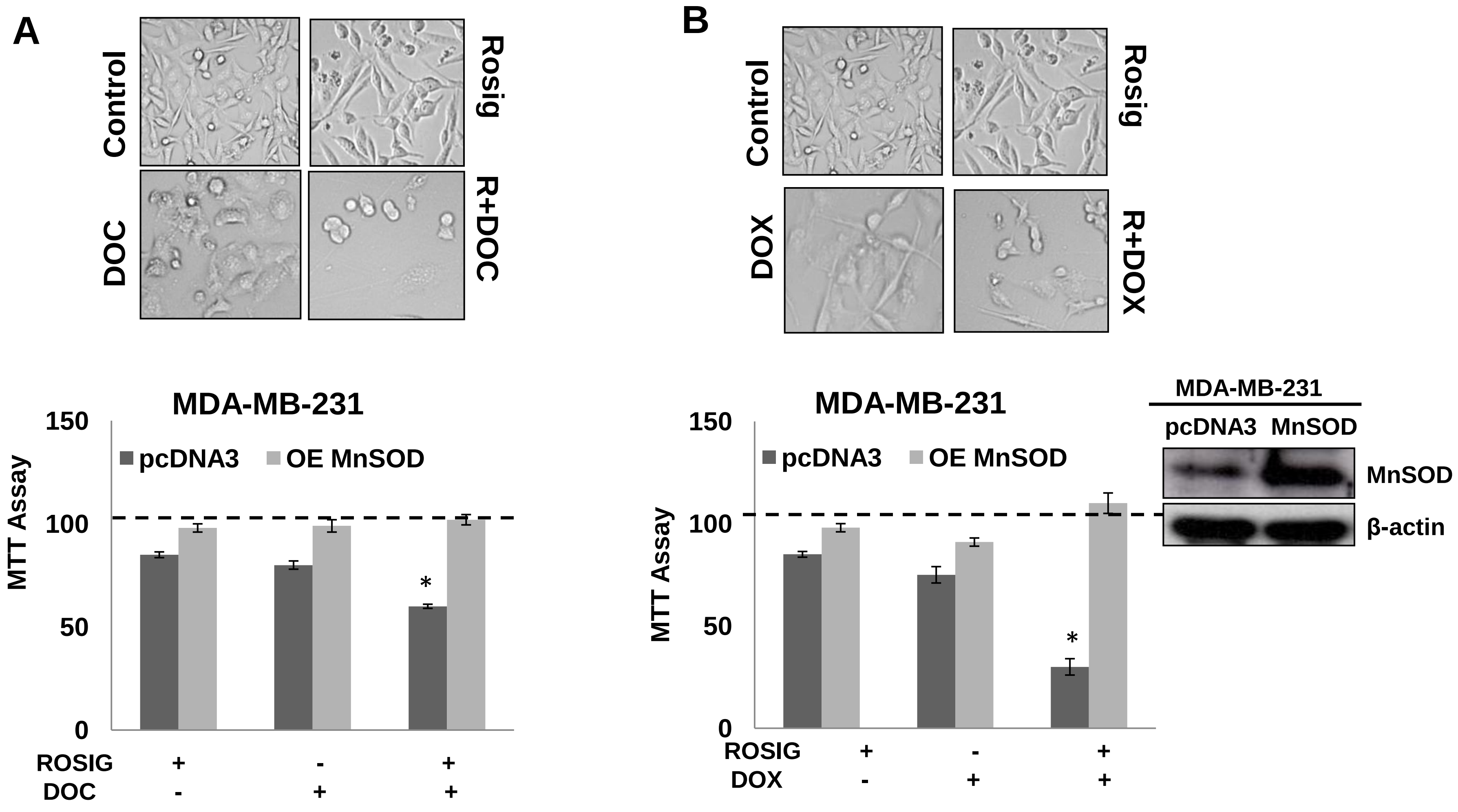
(Wang J and Yi J, 2008)

DOC and DOX: ROS-inducing anticancer drugs

- Reported to increase the level of intracellular ROS (Hur GC et al., 2003, Wang J et al., 2008)

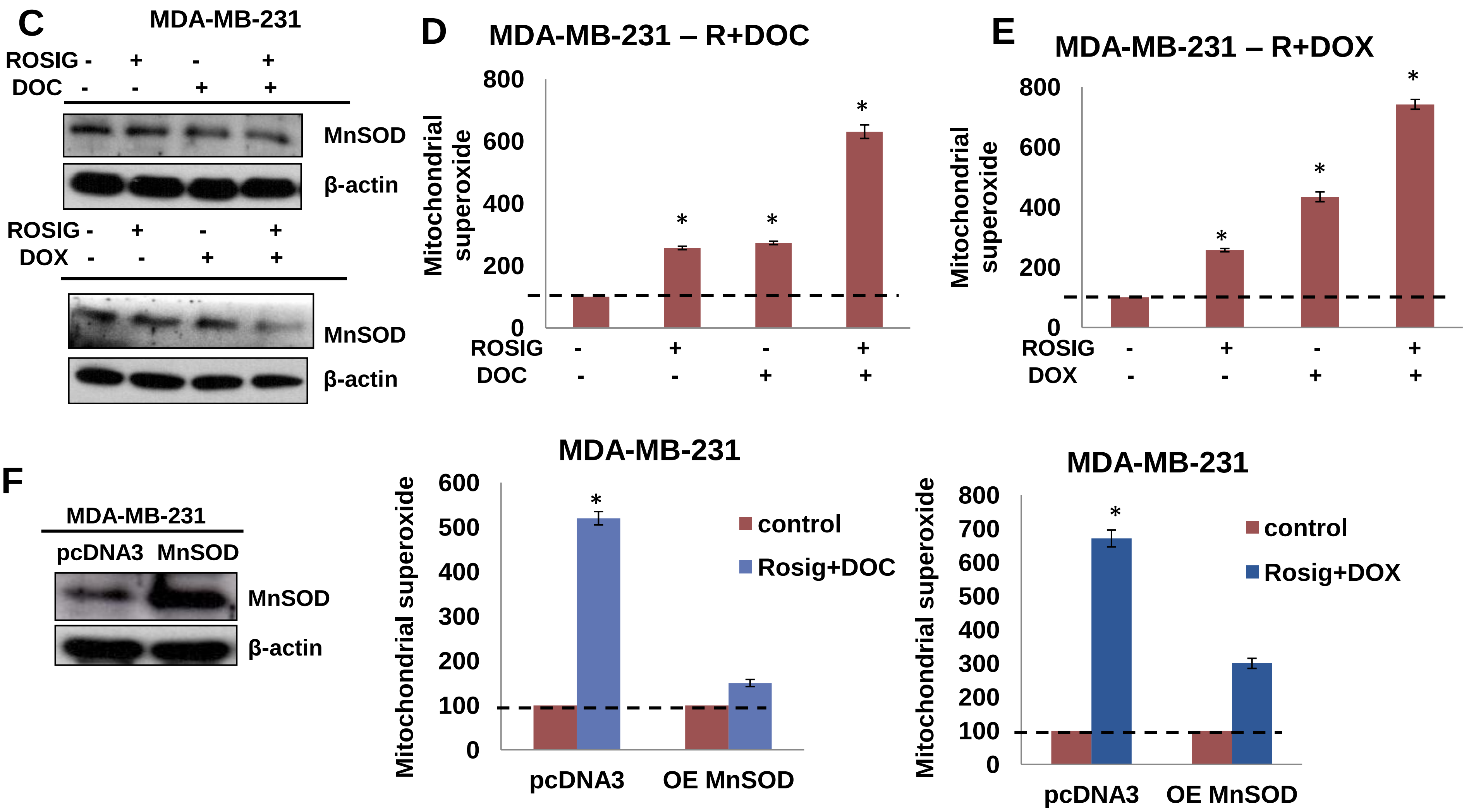


Sensitization in Breast Tumor Cells



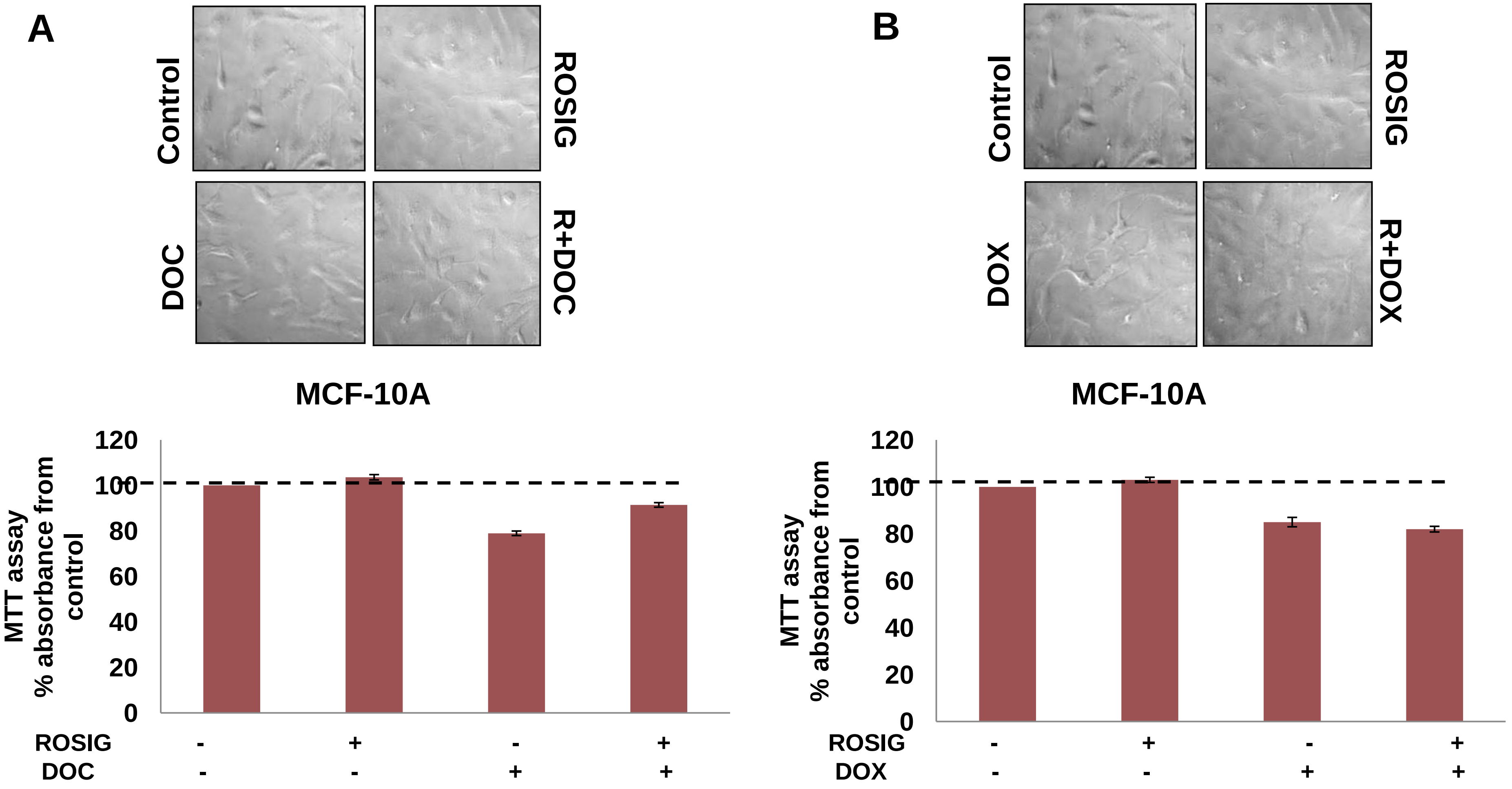
Combination treatment sensitizes breast cancer cells and sensitization can be blocked by overexpression of MnSOD.

Increased ROS Levels in Breast Tumor Cells



Combination treatment increases ROS levels in breast tumor cells and ROS increase can be blocked by overexpression of MnSOD.

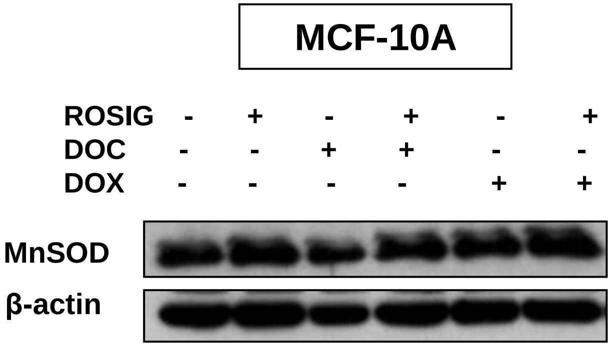
Cell Viability of Normal Breast Epithelial Cells



Combination treatment does not affect normal breast cells

ROS Levels in Normal Breast Epithelial Cells

D



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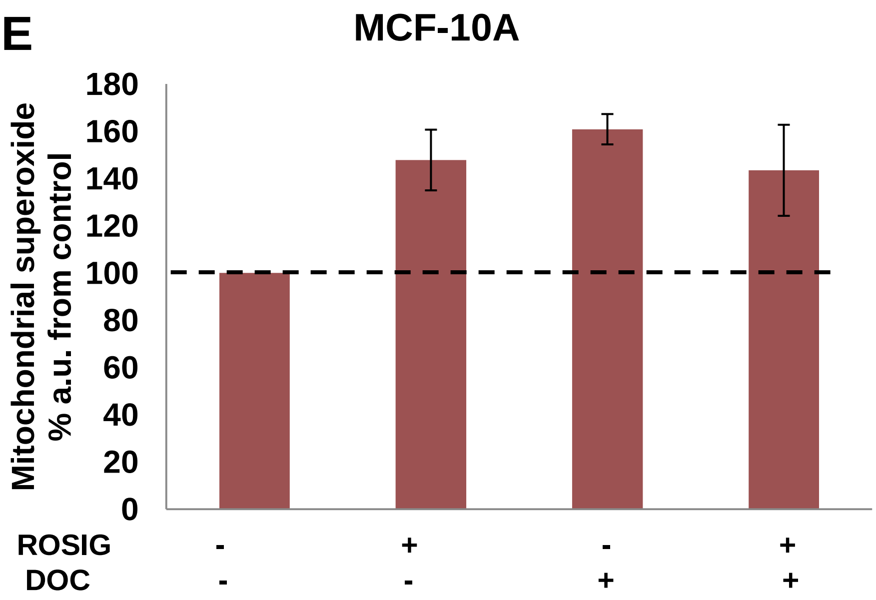

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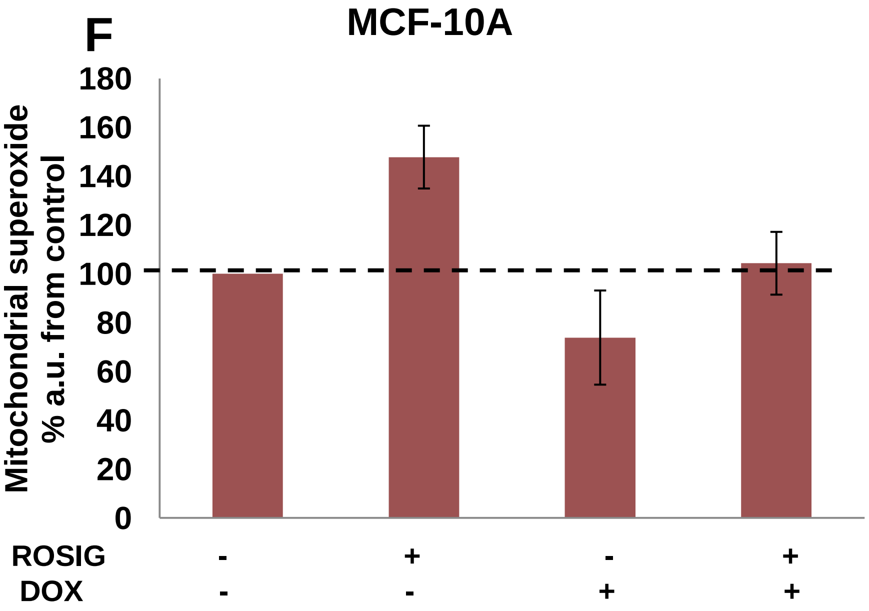
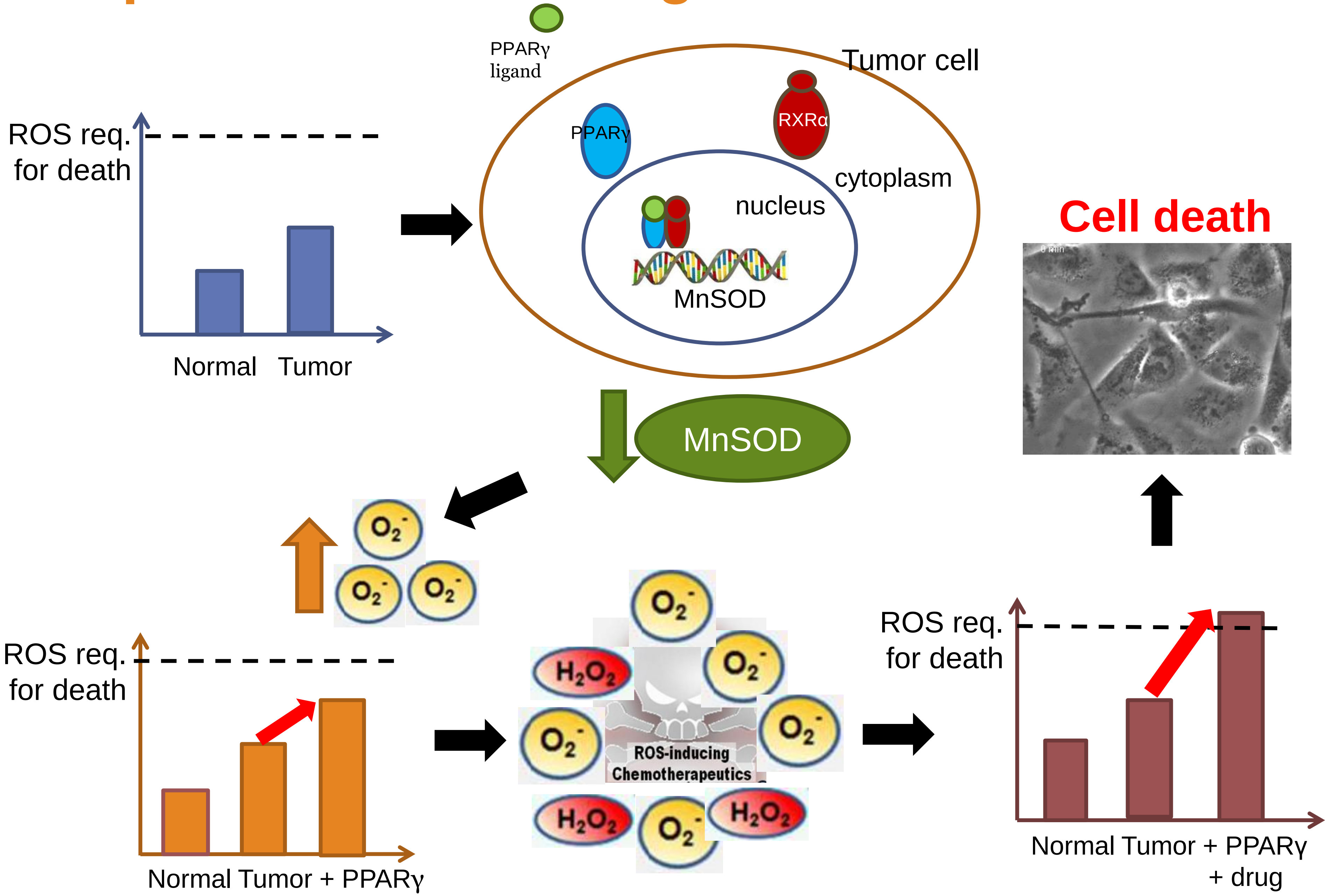


Combination treatment is specific to breast tumor cells

Proposed Treatment Regimen



- THE END -

Acknowledgements

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  - Ms. Diana Hay Hui Sin
  - Ms. Chen Luxi
  - Ms. Loo Ser Yue
  - Ms. Goh Jen Nee (Research Asst.)
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