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Suppression of AKT/mTOR pathway and activation of mitophagy by melatonin via mitocondrial regulation in head and neck cancer

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Head and Neck Squamous Cell Carcinoma

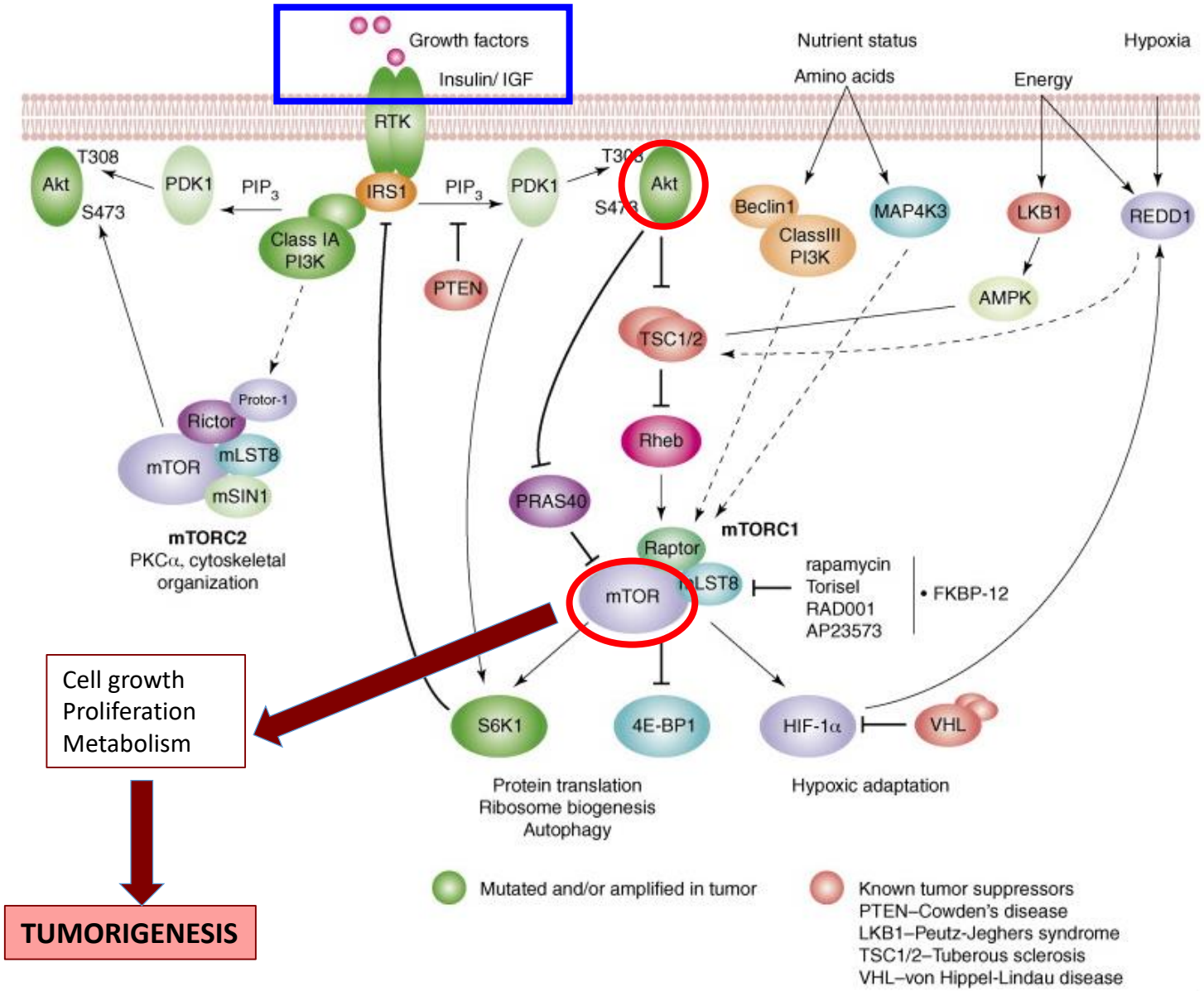
It is necessary to explore molecular mechanisms to find more effective therapeutic strategies

Liebe

The mTOR pathway plays an important role in HNSCC progression, metastasis and resistance to therapy

Resistance to therapy

mTOR pathway

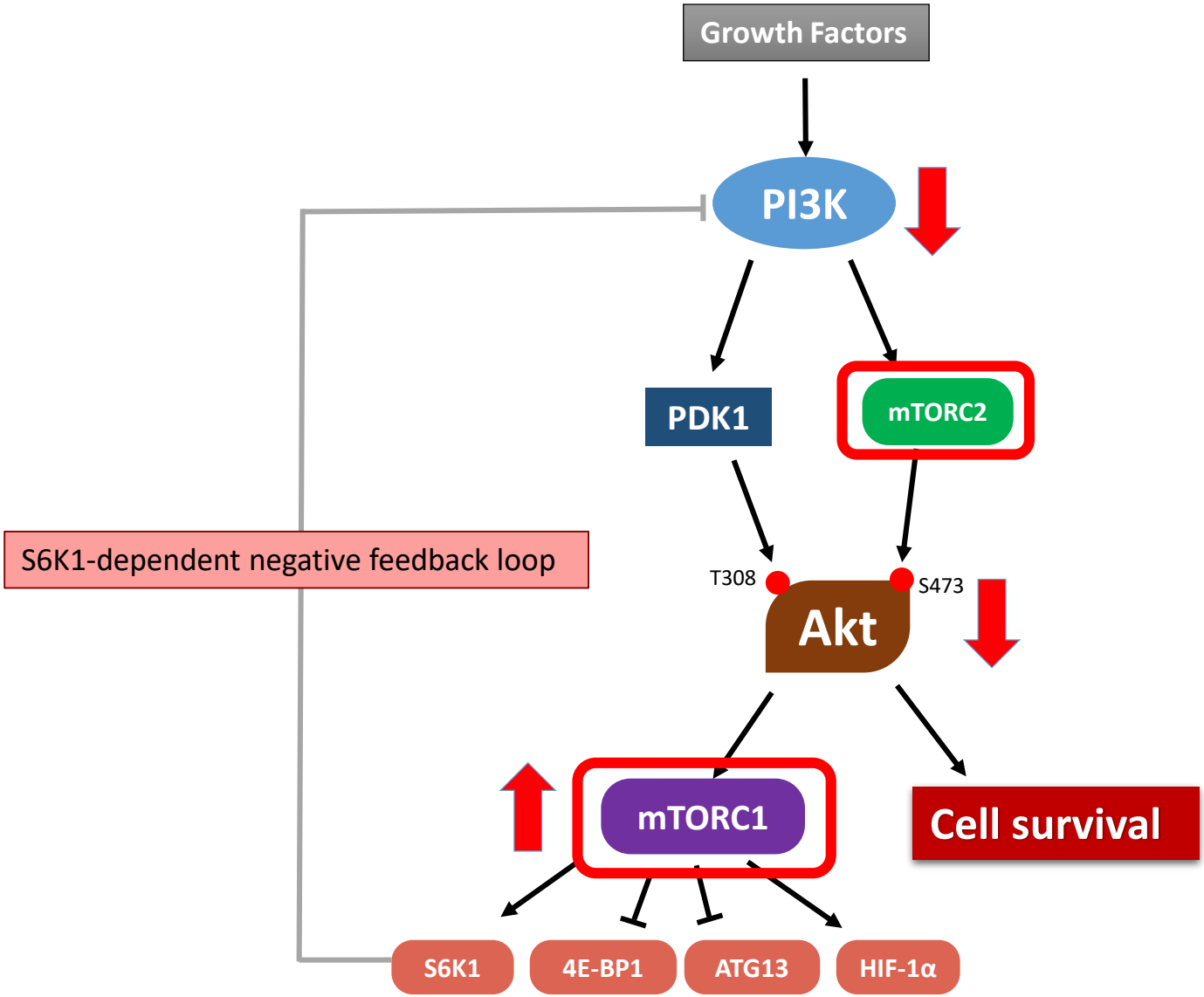


Cell growth
Proliferation
Metabolism

TUMORIGENESIS

Chiang, GG,Trends in Molecular Medicine , 2007

NEGATIVE FEEDBACK REGULATION OF mTOR SIGNALING



mTORC1: an anti-tumor target

Translation
Protein synthesis

Angioge

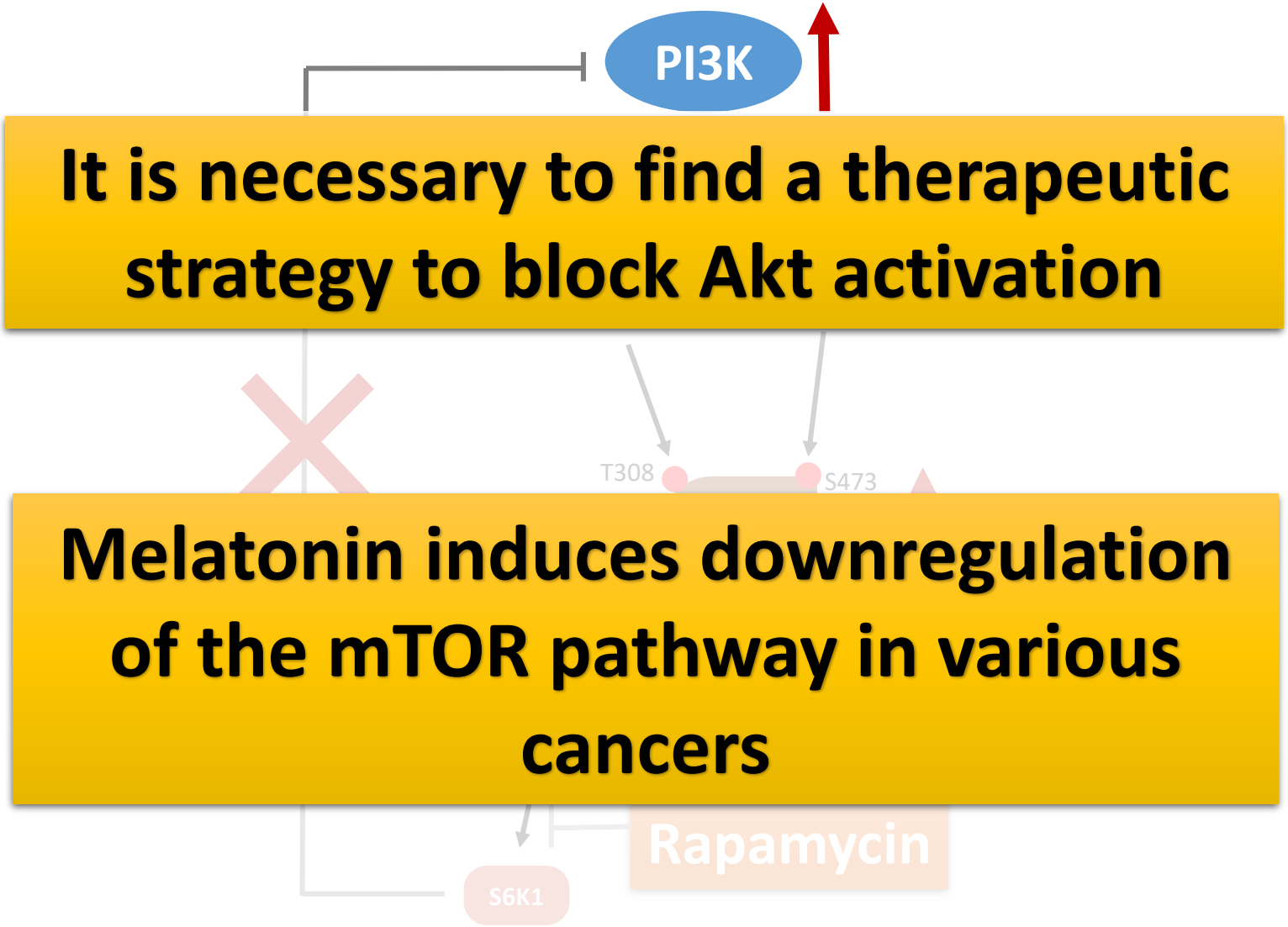
utophagy

Many cancer types show resistance to rapamycin treatment

RAPAMYCIN

Lipid synthesis

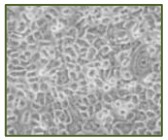
Rapamycin shortage



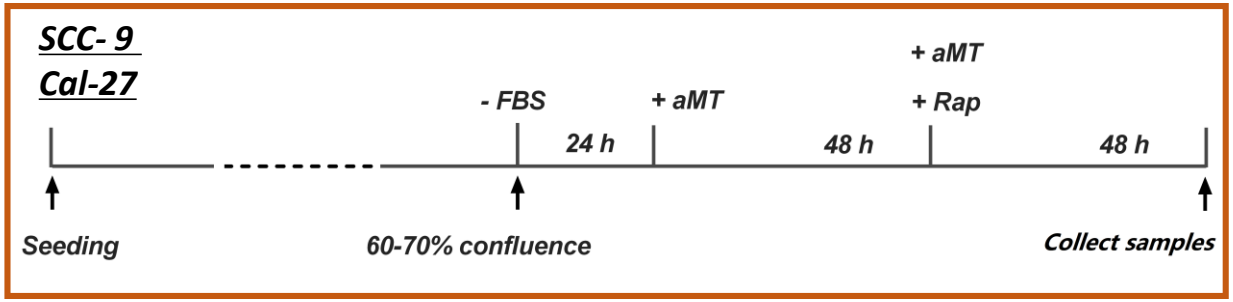
It is necessary to find a therapeutic strategy to block Akt activation

Melatonin induces downregulation of the mTOR pathway in various cancers

Experiment design

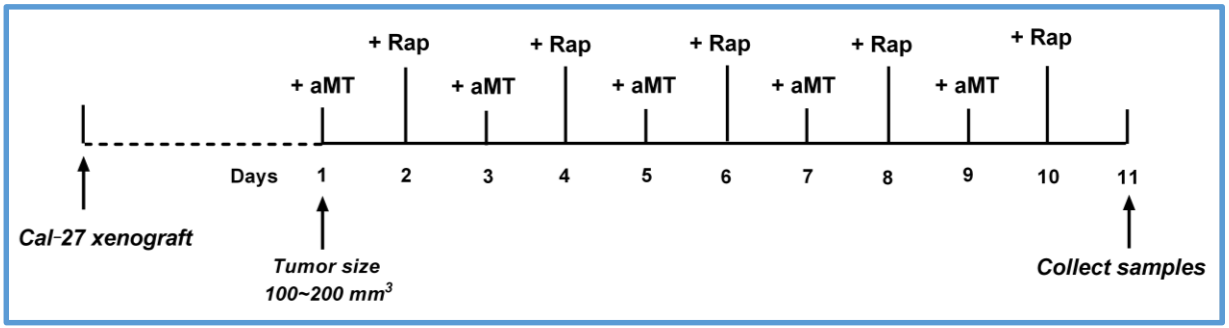


In vitro studies



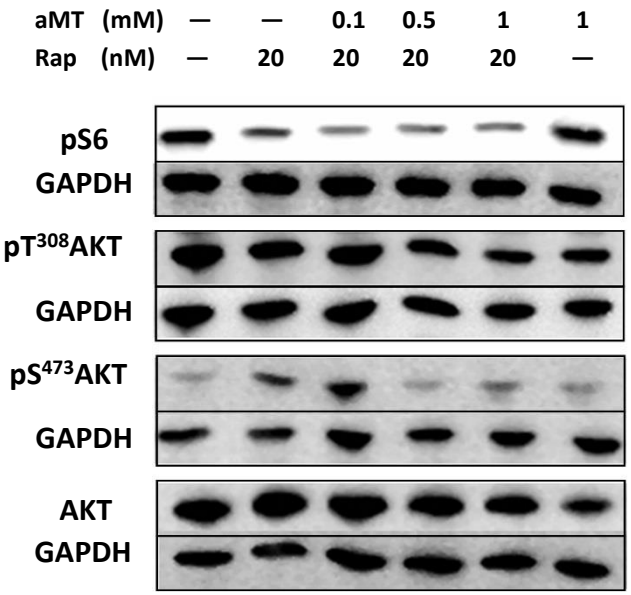
- Control
- Rap 20 nM
- Rap 20 nM+aMT 0.1 mM
- Rap 20 nM+aMT 0.5 mM
- Rap 20 nM+aMT 1 mM
- aMT 1 mM

In vivo studies

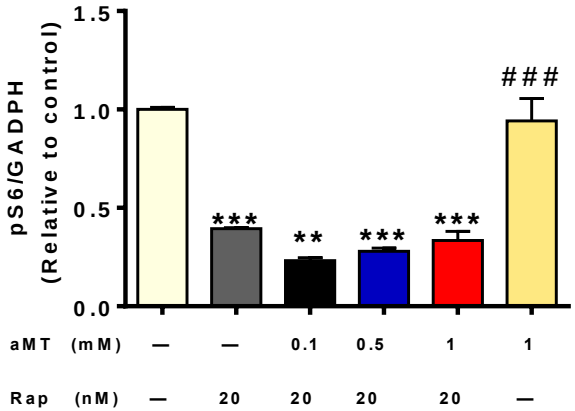


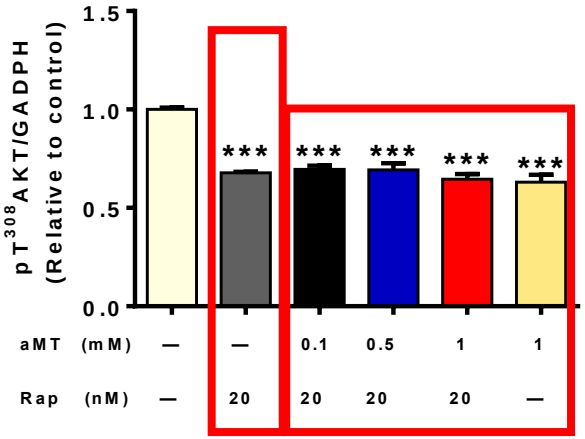
- Control
- Rap 1 mg/kg Intraperitoneally
- Rap + aMT 300 mg/kg Subcutaneously

mTOR pathway

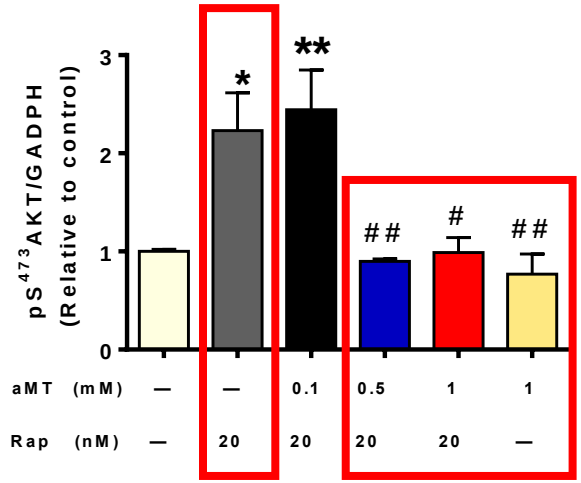


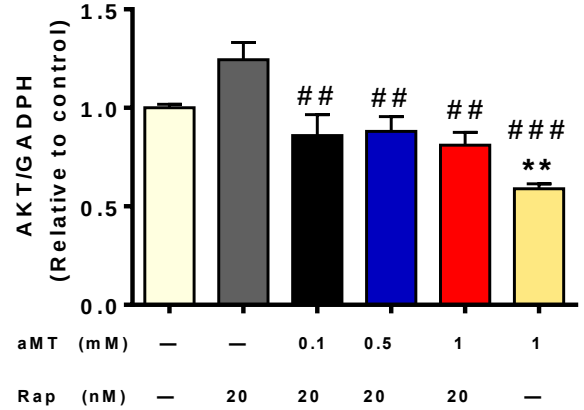
mTORC1



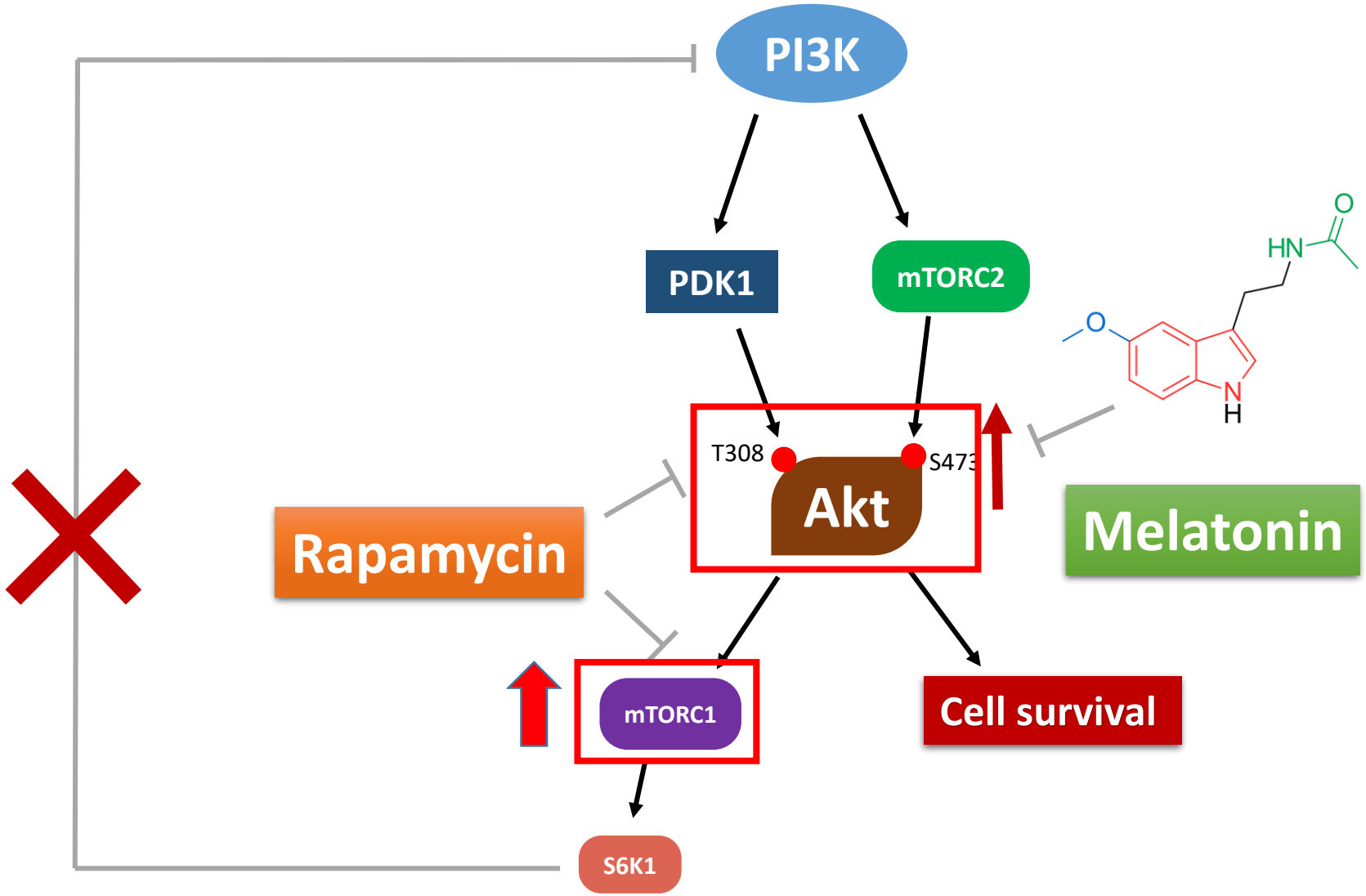


mTORC2





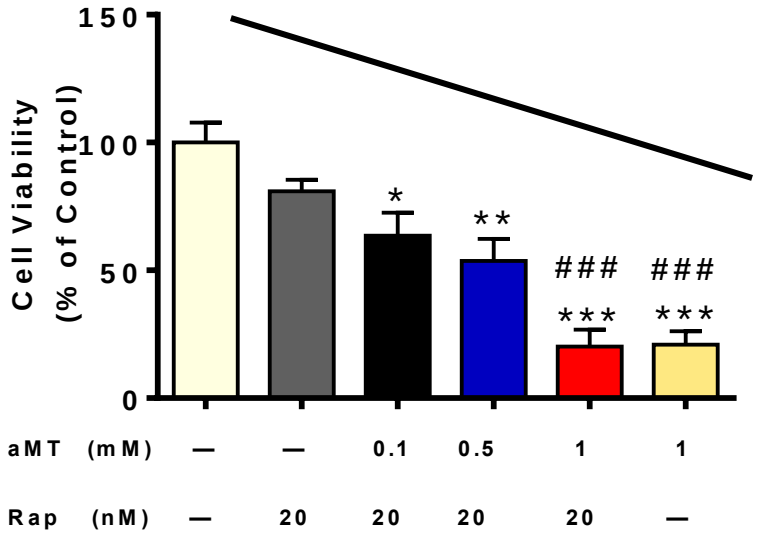
Melatonin inhibits the Akt activation



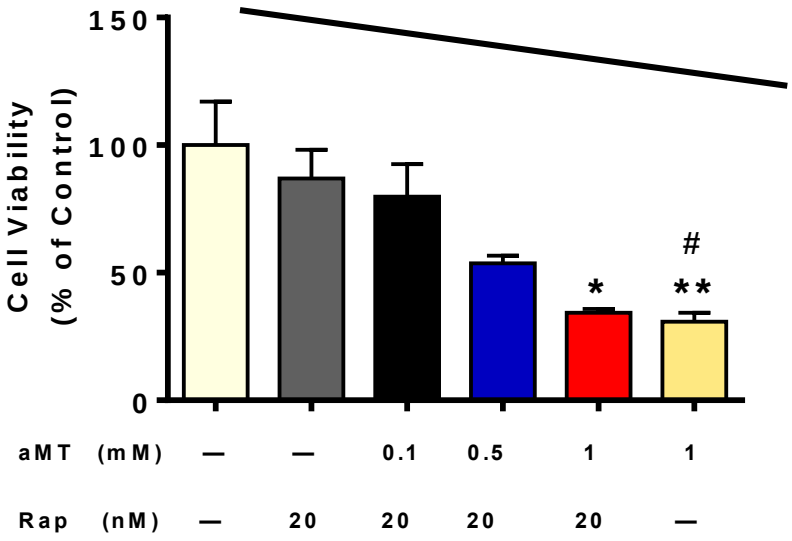
Cell viability

Melatonin enhances the effects of rapamycin

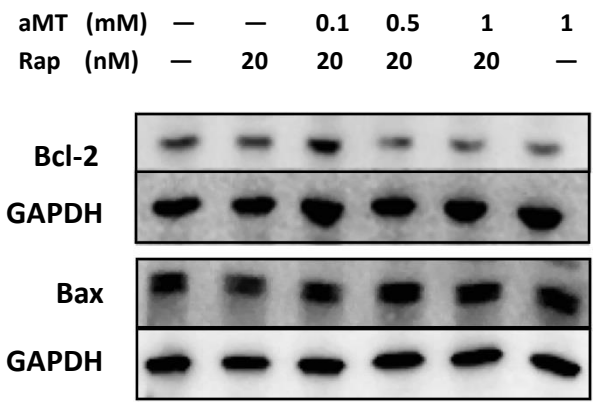
Cal-27

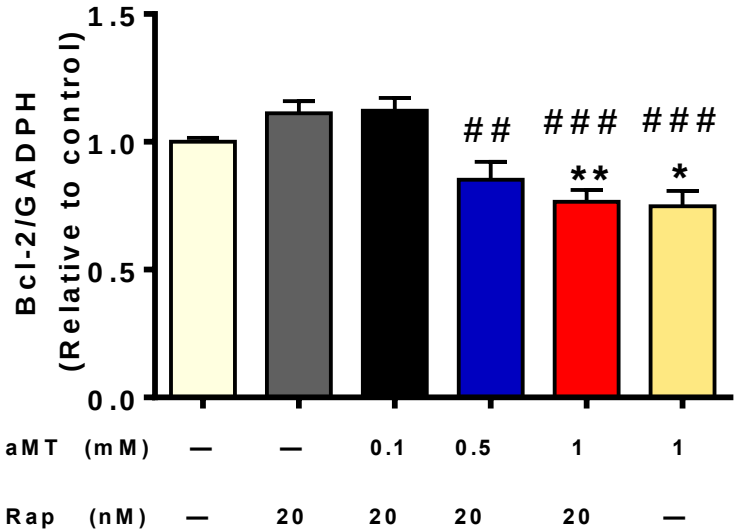


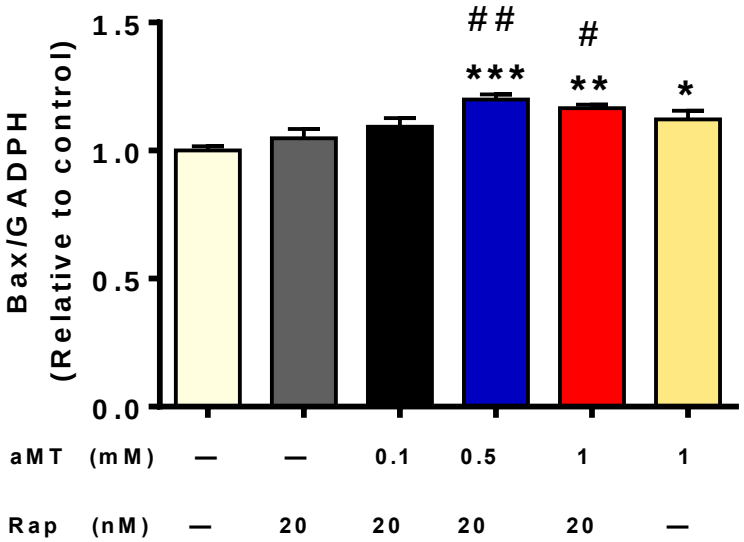
SCC-9

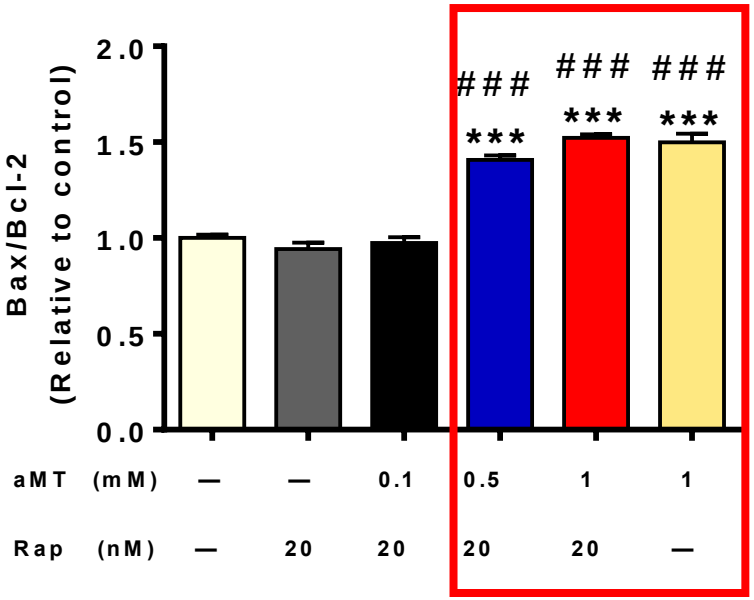


Apoptosis


aMT (mM) — — 0.1 0.5 1 1
Rap (nM) — 20 20 20 20 —
Bcl-2
GAPDH
Bax
GAPDH


Bcl-2/GADPH
(Relative to control)
1.5
1.0
0.5
0.0
##
###
***
*
aMT (mM) — — 0.1 0.5 1 1
Rap (nM) — 20 20 20 20 —


Bax/GADPH
(Relative to control)
1.5
1.0
0.5
0.0
##
#
***
**
*
aMT (mM) — — 0.1 0.5 1 1
Rap (nM) — 20 20 20 20 —


Bax/Bcl-2
(Relative to control)
2.0
1.5
1.0
0.5
0.0
###
***
aMT (mM) — — 0.1 0.5 1 1
Rap (nM) — 20 20 20 20 —

Mitochondria are the main target of melatonin

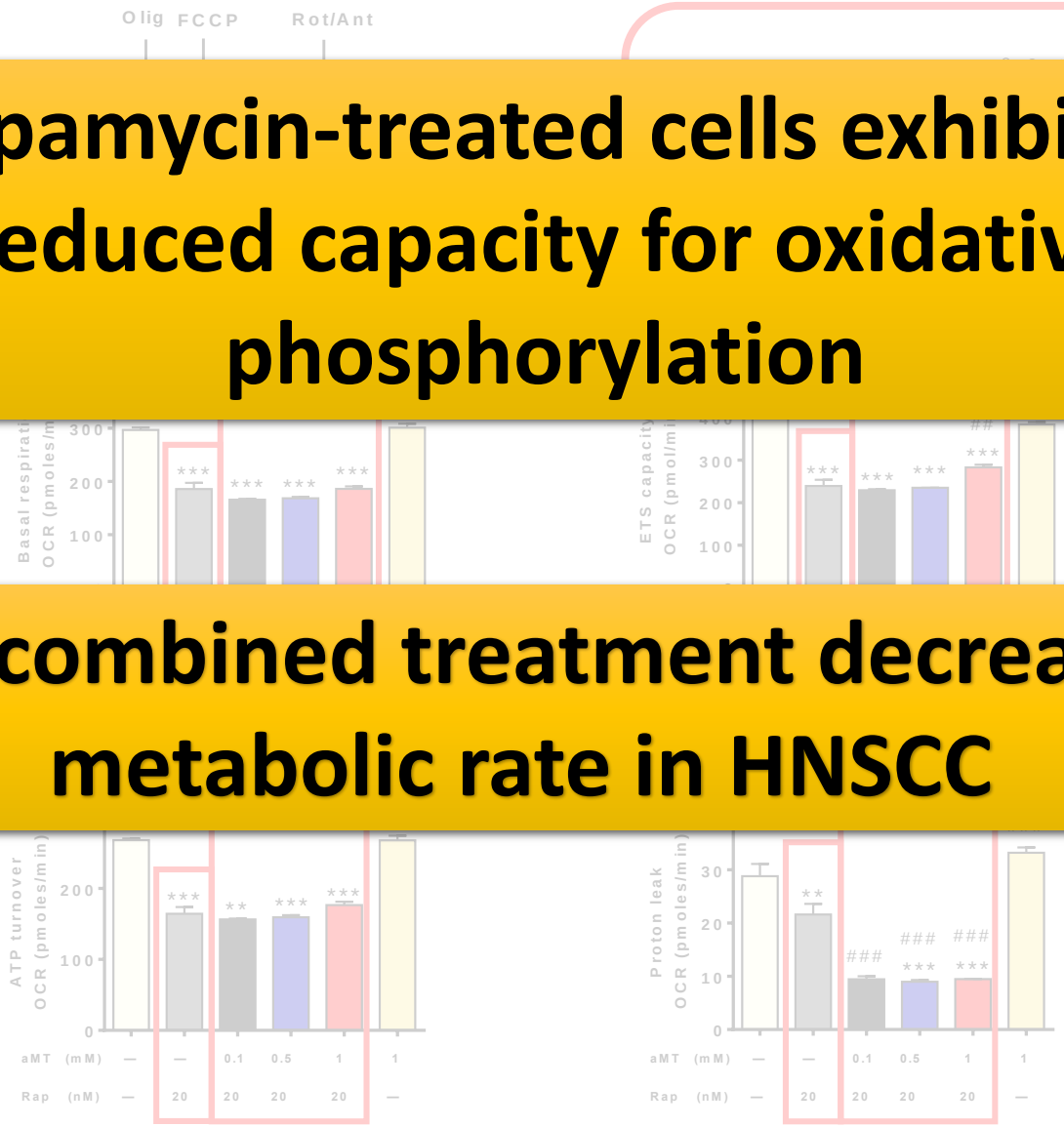
↓

We explored the possibility that melatonin enhances the effects of rapamycin through mitochondrial pathway

Mitochondrial respiration in HNSCC

Rapamycin-treated cells exhibited reduced capacity for oxidative phosphorylation

The combined treatment decreased metabolic rate in HNSCC



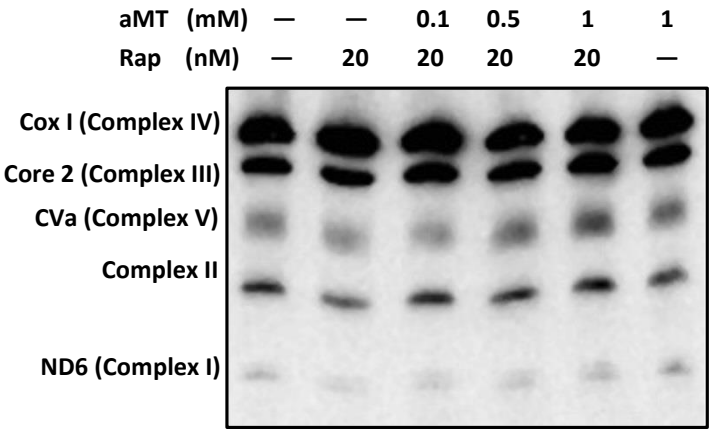
Melatonin enhances the effects of rapamycin, in terms of decreasing the number of mitochondria

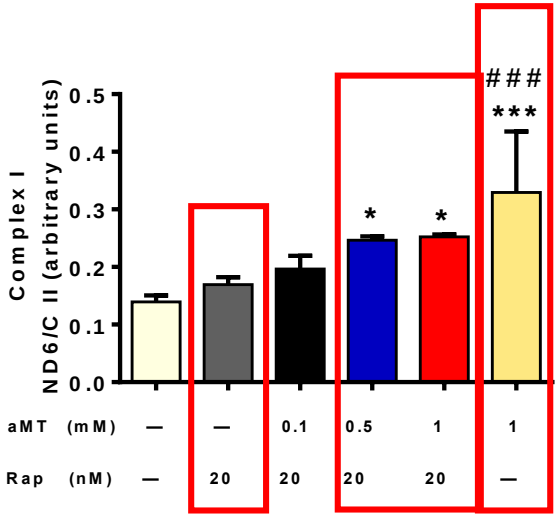
or

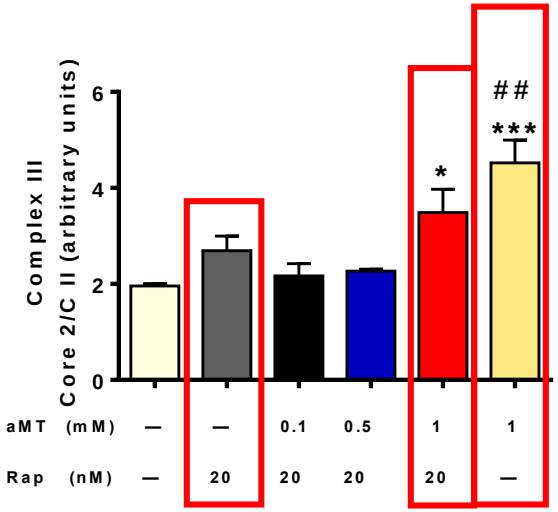
the number of functional mitochondria.

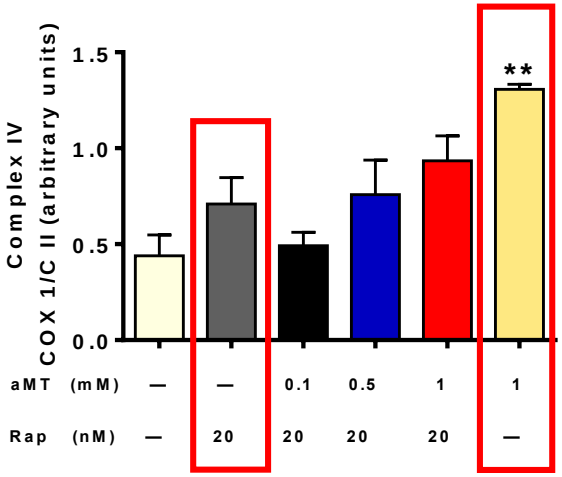
To confirm this hypothesis, we next examined OXPHOS, mitochondrial mass, and mtDNA.

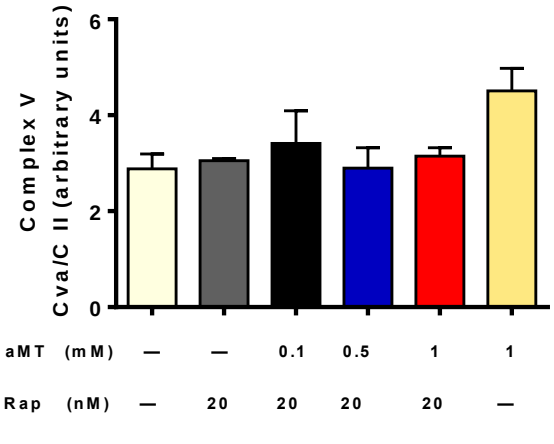
Mitochondrial OXPHOS expression in HNSCC



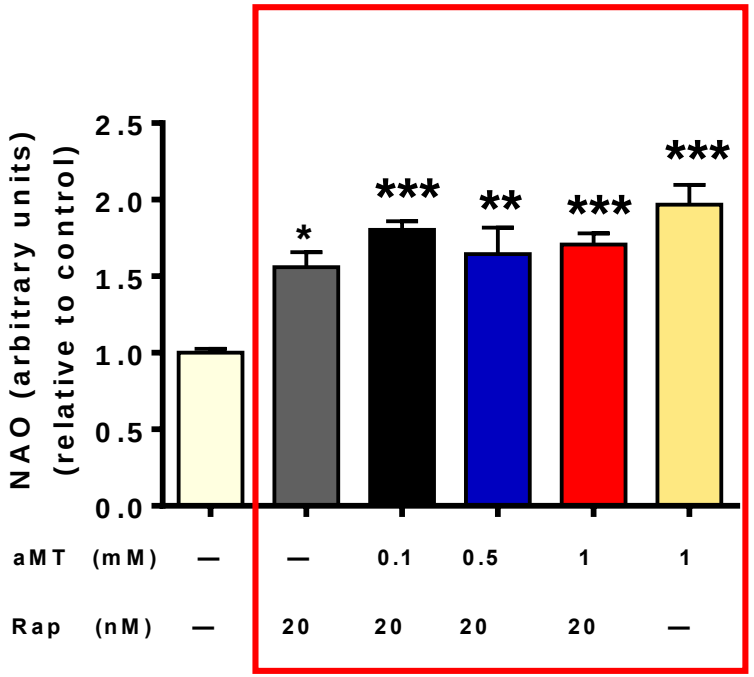


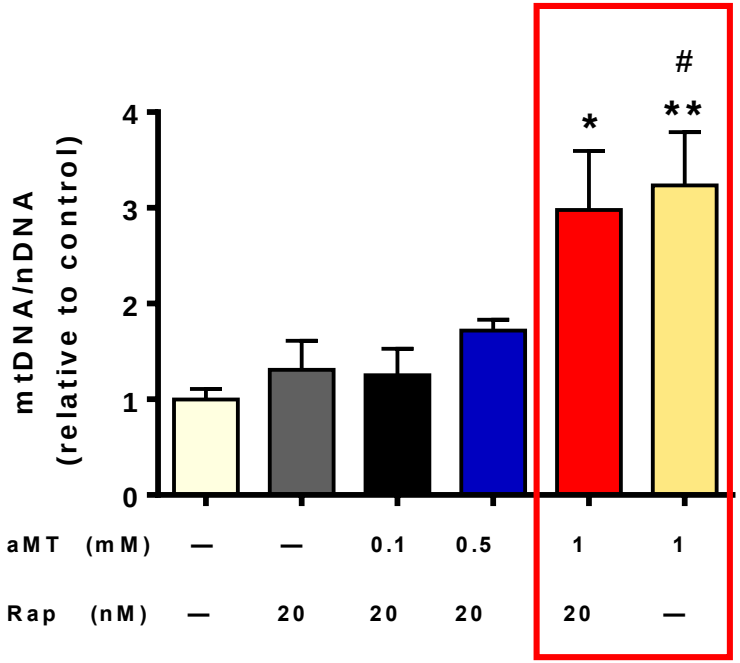






Mitochondrial mass and mtDNA





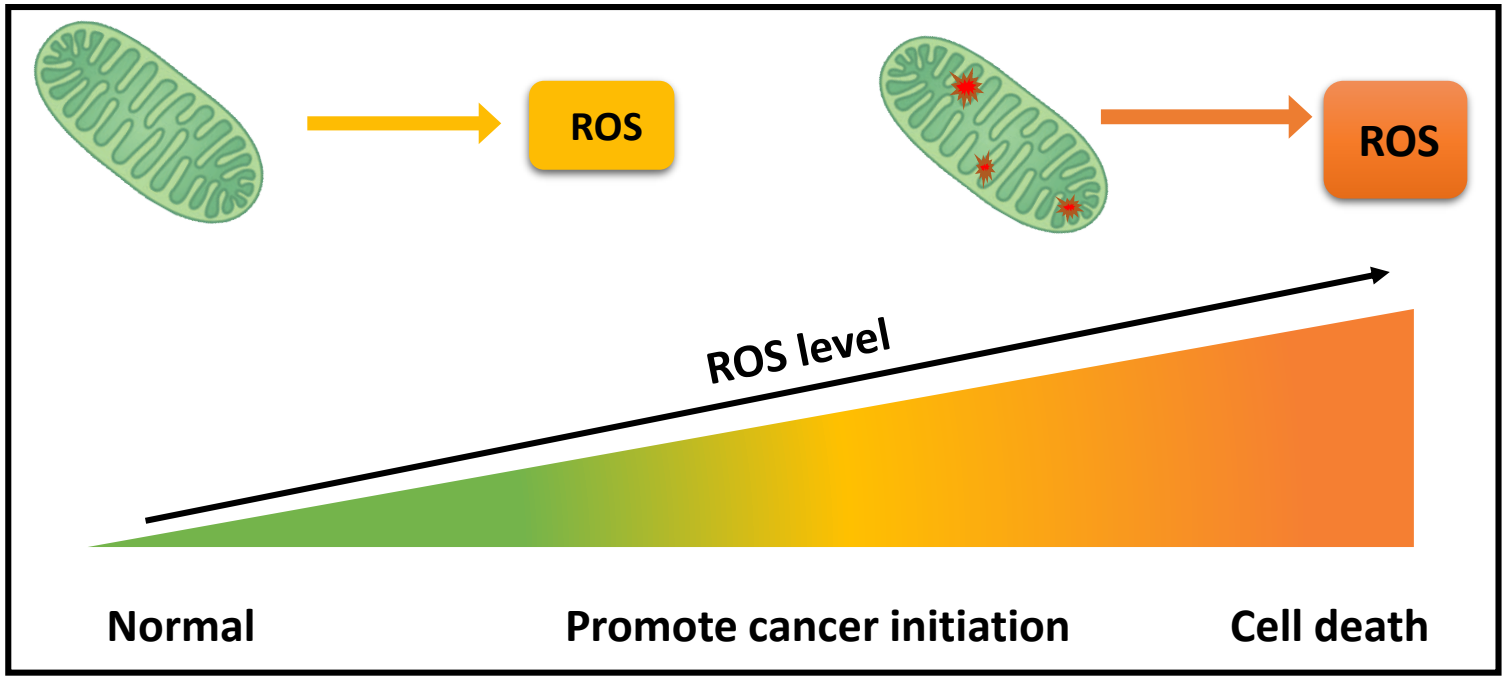
Mitochondrial complexes

Mitochondrial mass

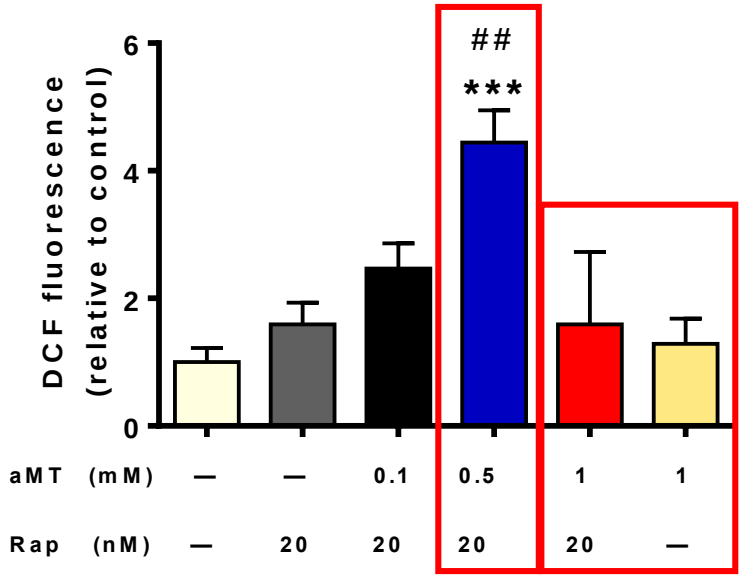
Mitochondrial DNA

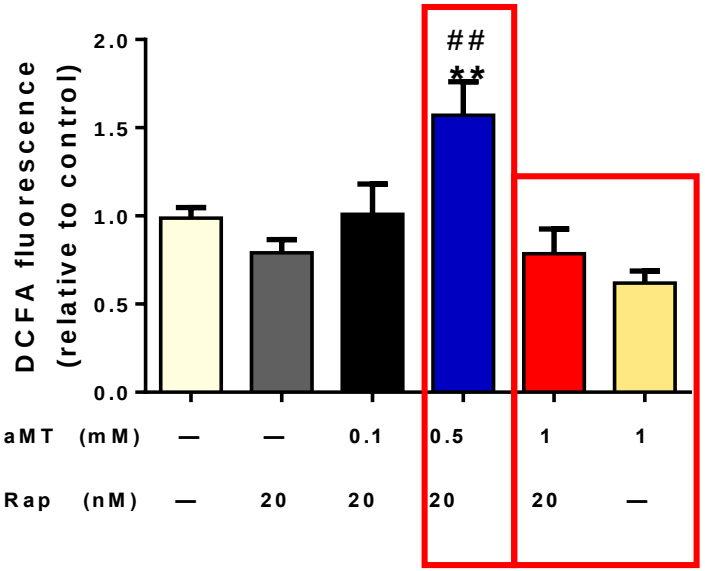
Mitochondrial respiration

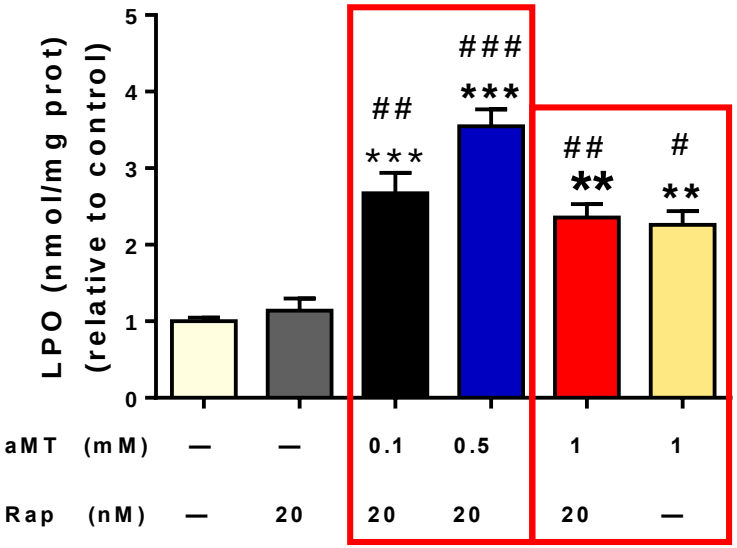
We hypothesized that melatonin enhances the cytotoxic effects of rapamycin by augmenting the number of dysfunctional mitochondria.

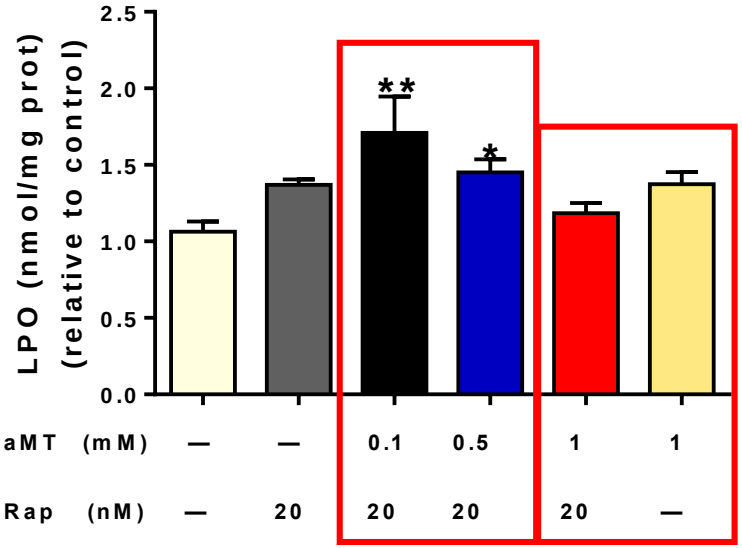


ROS and LPO levels

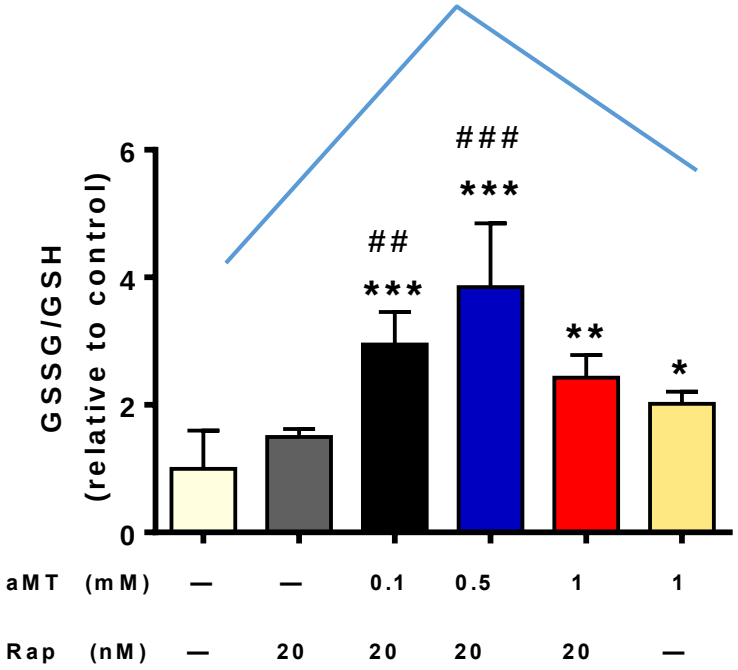


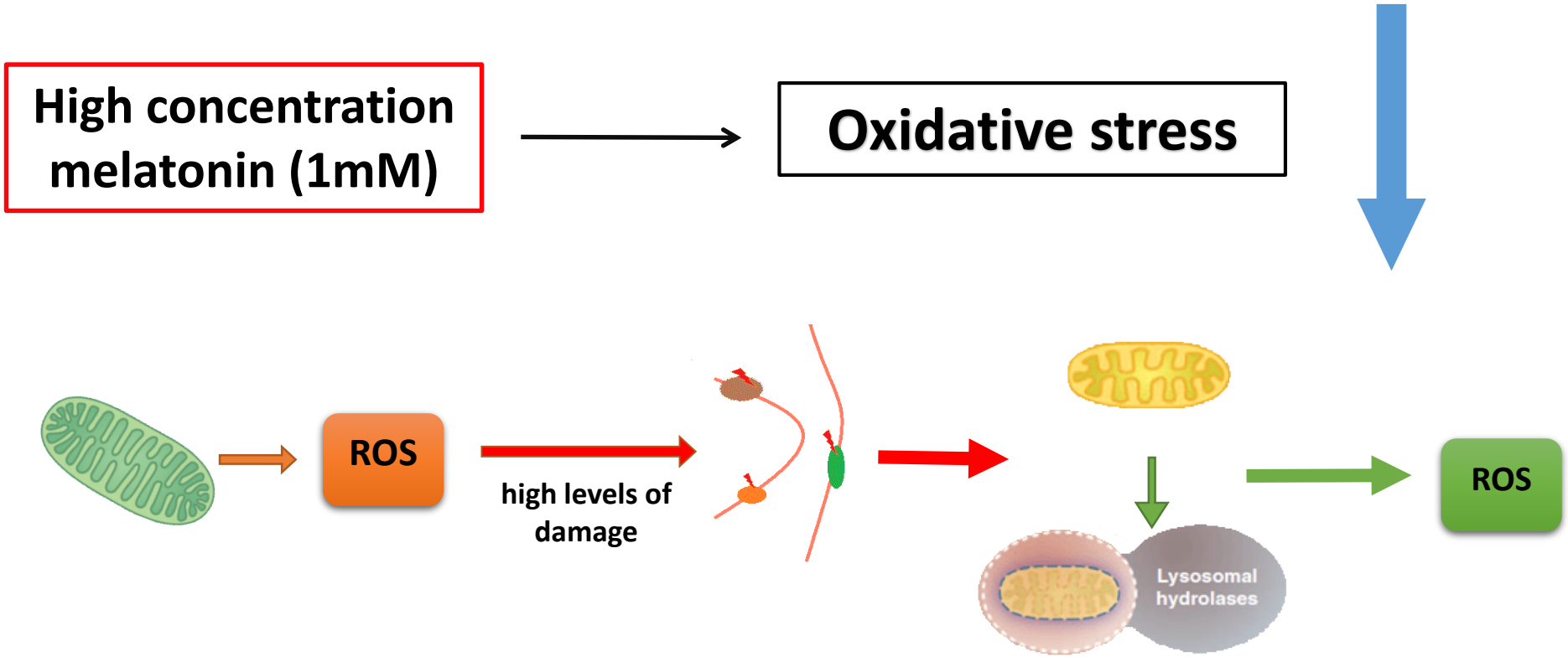






GSSG/GSH ratio

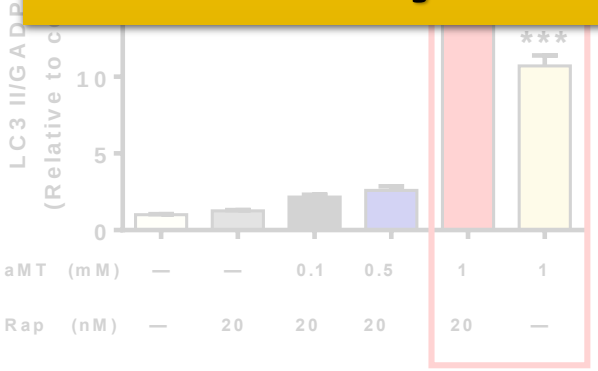


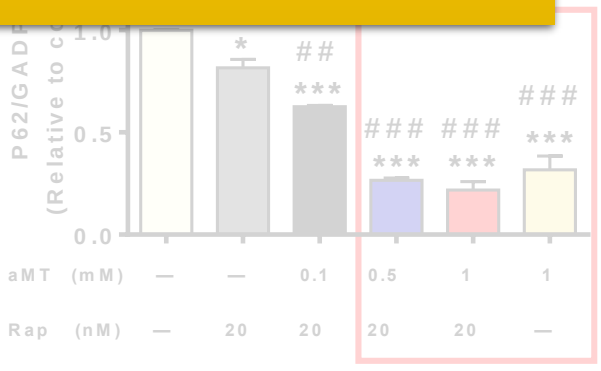


We suposed that at high concentration of melatonin, ROS accumulation under respiratory conditions may have resulted in mitochondrial protein degradation and even mitophagy

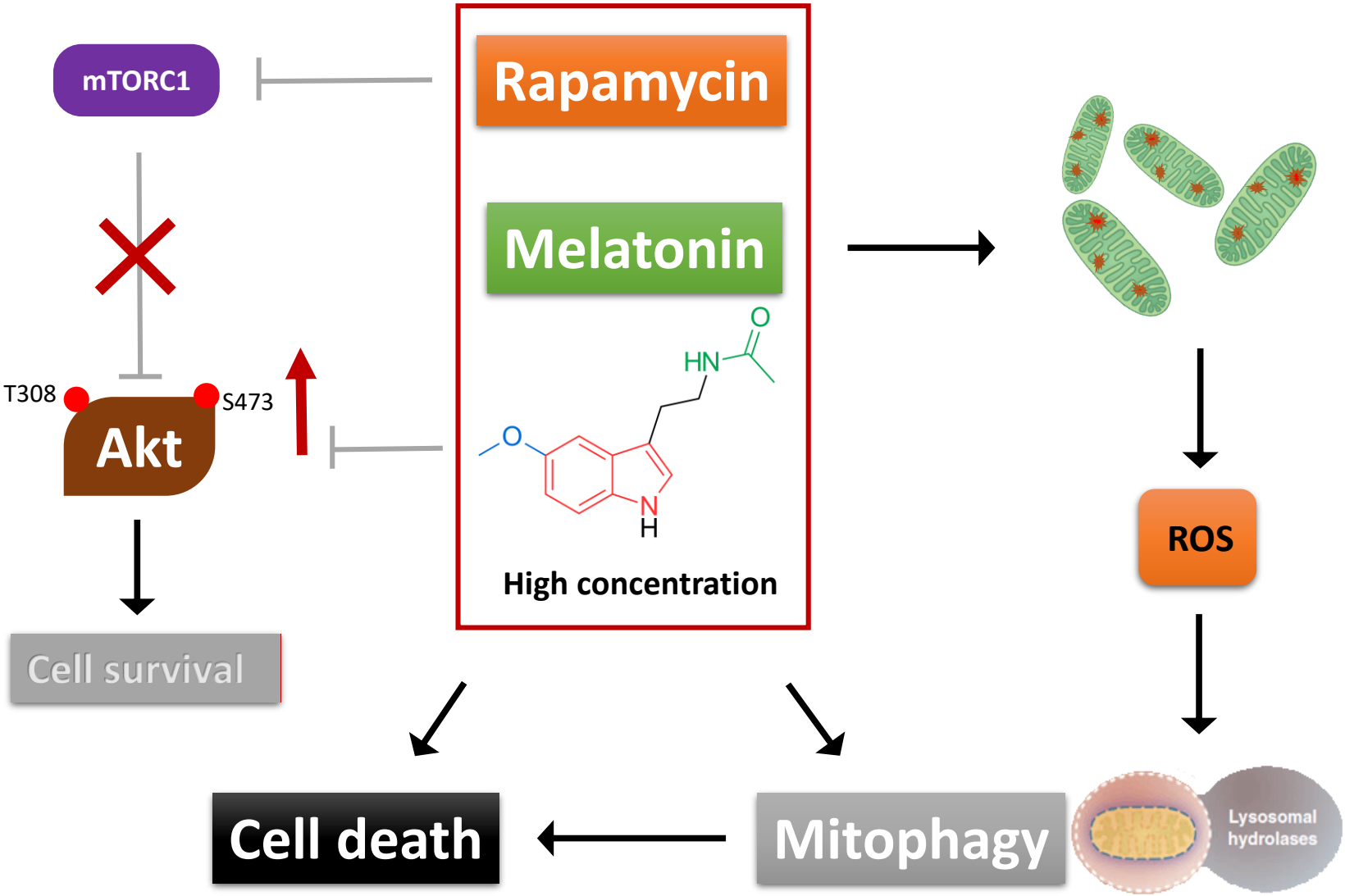
Mitophagy

These results indicated that high concentrations of melatonin contribute to induce mitophagy and to eliminate dysfunctional mitochondria





Synergistic effect of melatonin and rapamycin



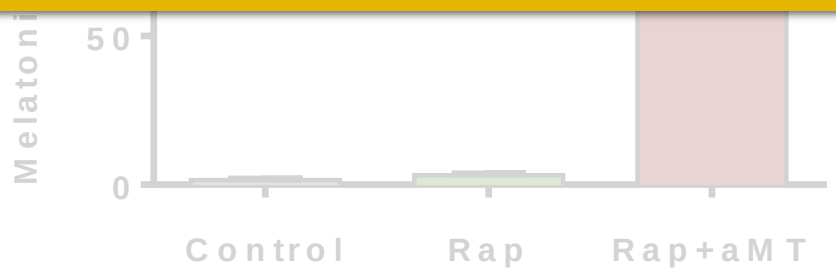
Xenograft mice tumor

These results were in contrast to the expectation that the combined treatment would be more effective based on our *in vitro* results

Melatonin level

Tumor tissue

We thought that melatonin didn´t reach the tumor at a sufficient concentration to inhibit tumor growth



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ORIGINAL ARTICLE

WILEY Journal of Pineal Research

Combination of melatonin and rapamycin for head and neck cancer therapy: Suppression of AKT/mTOR pathway activation, and activation of mitophagy and apoptosis via mitochondrial function regulation

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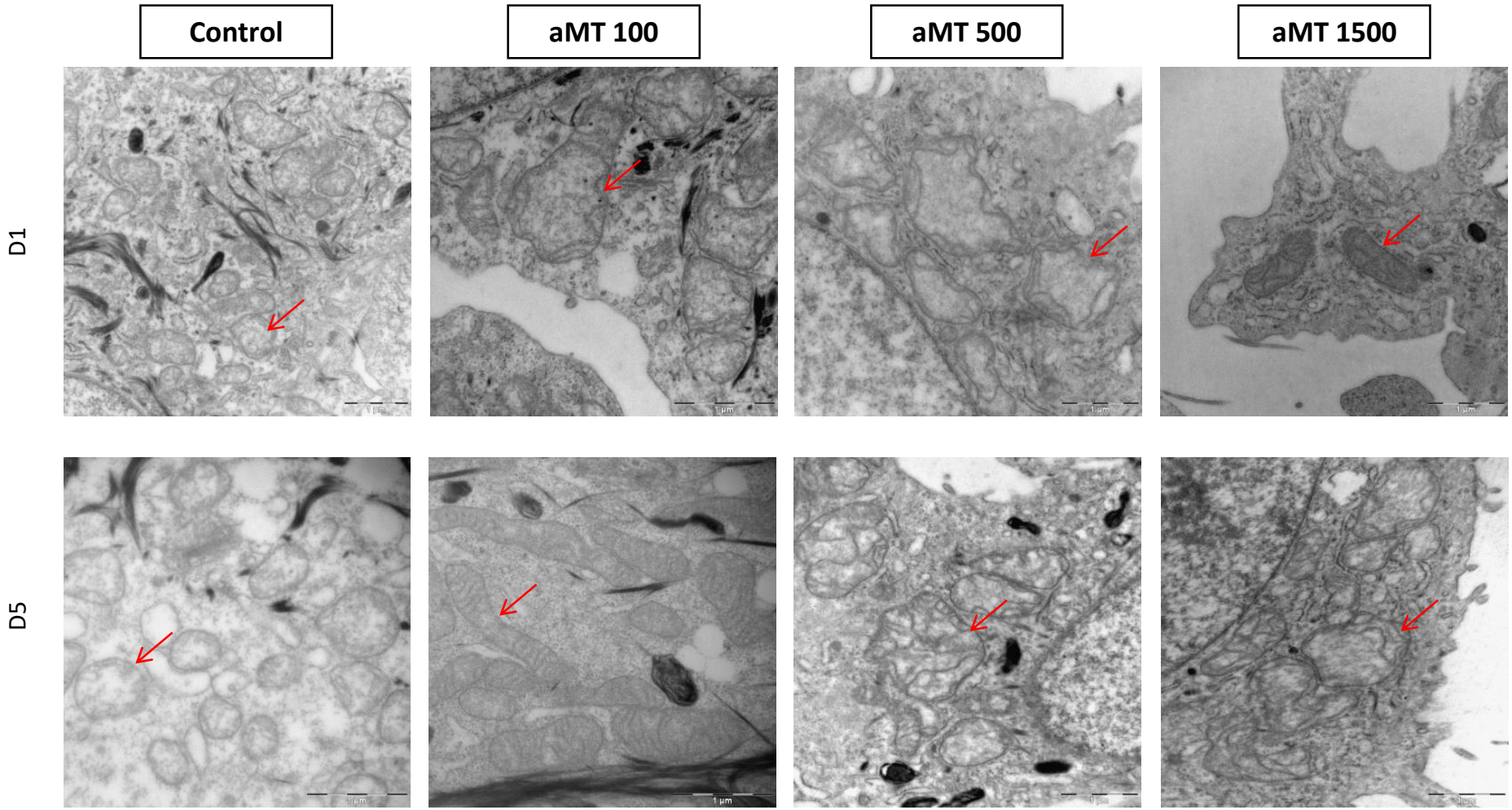
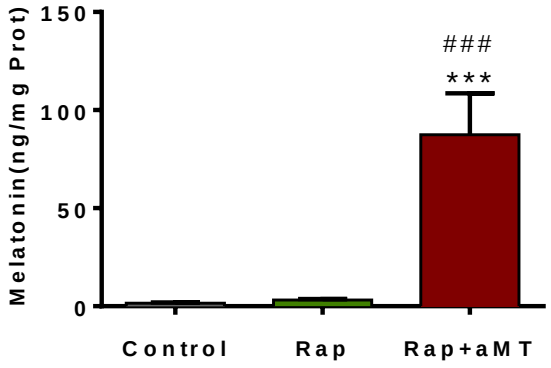
Abstract

Head and neck squamous cell carcinoma (HNSCC) clearly involves activation of the Akt mammalian target of rapamycin (mTOR) signalling pathway. However, the effectiveness of treatment with the mTOR inhibitor rapamycin is often limited by chemoresistance. Melatonin suppresses neoplastic growth via different mechanisms in a variety of tumours. In this study, we aimed to elucidate the effects of melatonin on rapamycin-induced HNSCC cell death and to identify potential cross-talk pathways. We analysed the dose-dependent effects of melatonin in rapamycin-treated HNSCC cell lines (Cal-27 and SCC-9). These cells were treated with 0.1, 0.5 or 1 mmol/L melatonin combined with 20 nM rapamycin. We further examined the potential synergistic effects of melatonin with rapamycin in Cal-27 xenograft mice. Relationships between inhibition of the mTOR pathway, reactive oxygen species (ROS), and apoptosis and mitophagy reportedly increased the cytotoxic effects of rapamycin in HNSCC. Our results demonstrated that combined treatment with rapamycin and melatonin blocked the negative feedback loop from the specific downstream effector of mTOR activation S6K1 to Akt signalling, which decreased cell viability, proliferation and clonogenic capacity. Interestingly, combined treatment with rapamycin and melatonin-induced changes in mitochondrial function, which were associated with increased ROS production, increasing apoptosis and mitophagy.

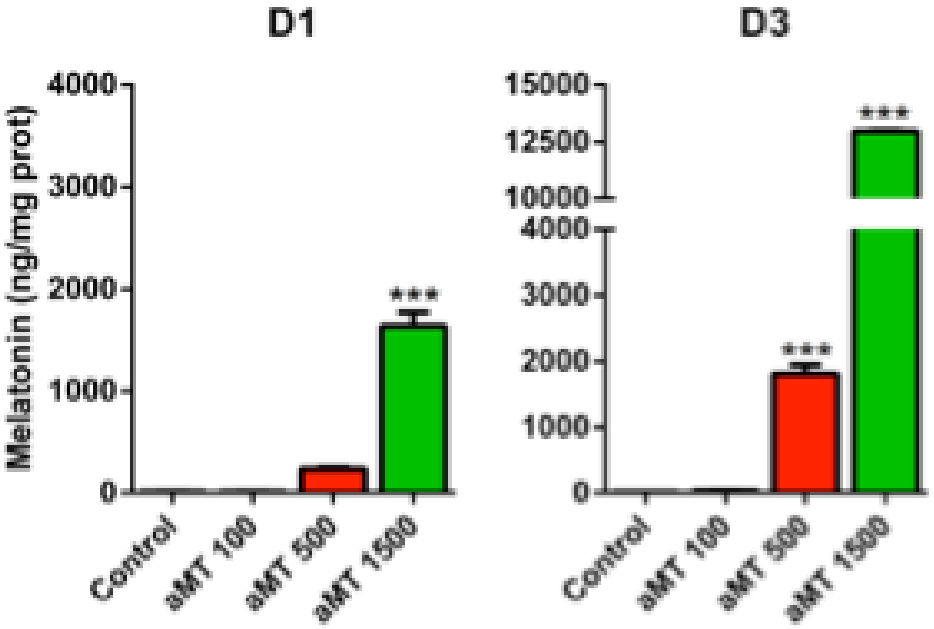
Thank you very much


Control
aMT 100
aMT 500
aMT 1500
D1
D5

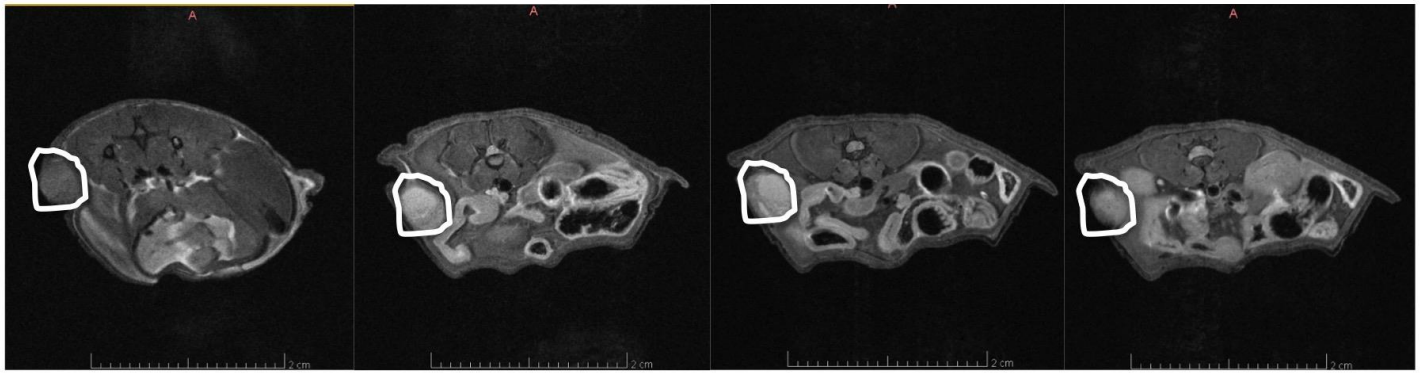
Tumor



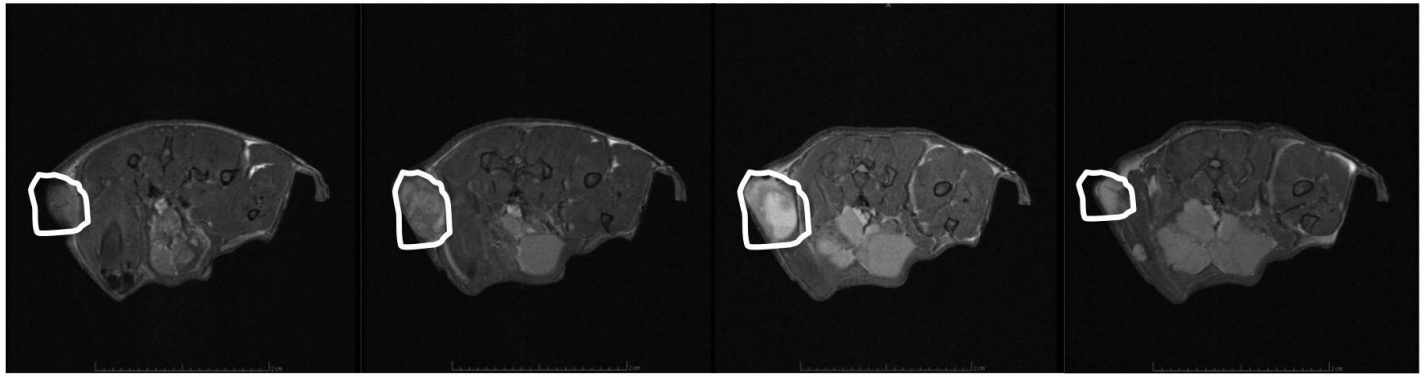
CAL-27 culture



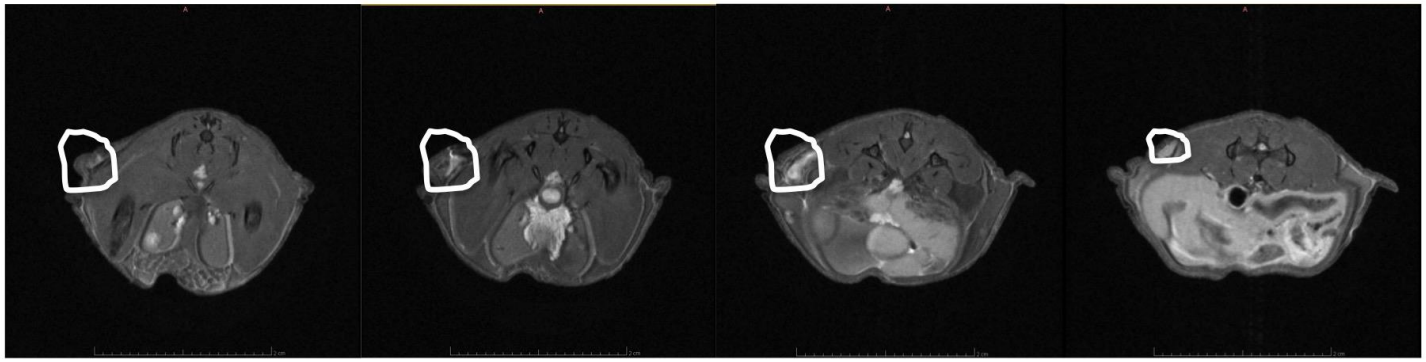
Control 21 días



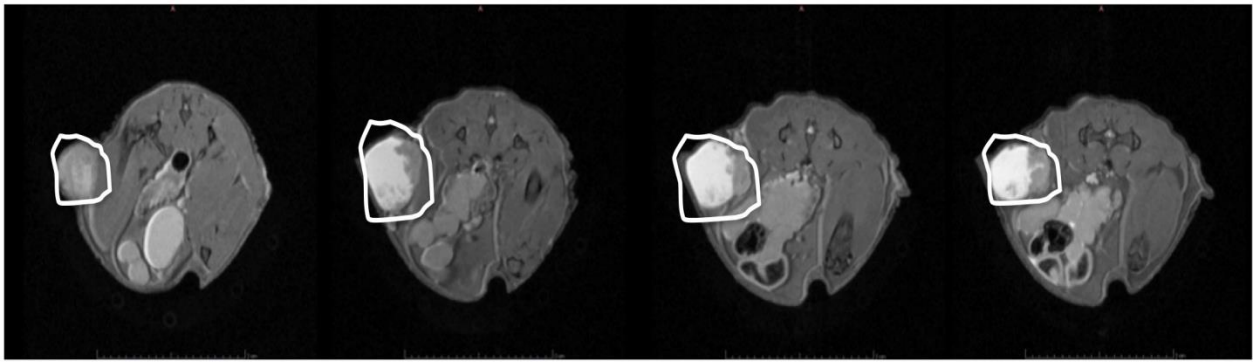
aMT sc 21 días



aMT intratumoral 21 días



Control 28 días



aMT intratumoral 28 días