STUDIES OF G-PROTEIN-COUPLED RECEPTORS

Scientific Background on the Nobel Prize in Chemistry 2012

Robert J.Lefkowitz

Brian K.Kobilka

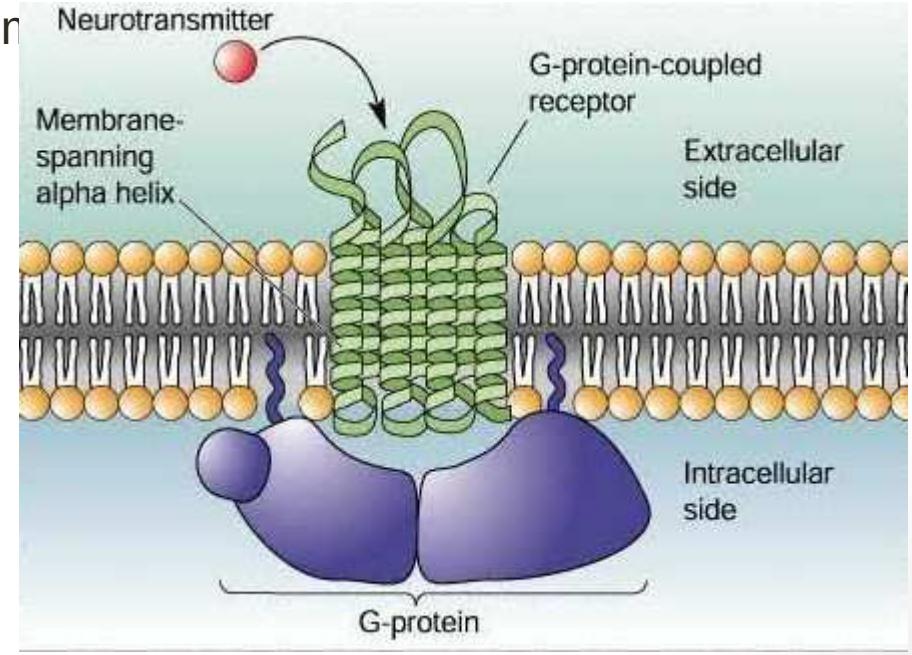
The Nobel Prize in Chemistry 2012 is awarded to Brian Kobilka and Robert J.Lefkowitz
for studies of G-protein-coupled receptors.

The name GPCR refers to a common mode of receptor signalling via GTP-binding proteins on the inside of the cell.

Because their polypeptide chain passes seven times through the plasma membrane, the GPCRs are also called seven-transmembrane (7TM) receptors.

They mediate a wide range of physiological signals from the outside of the cell.

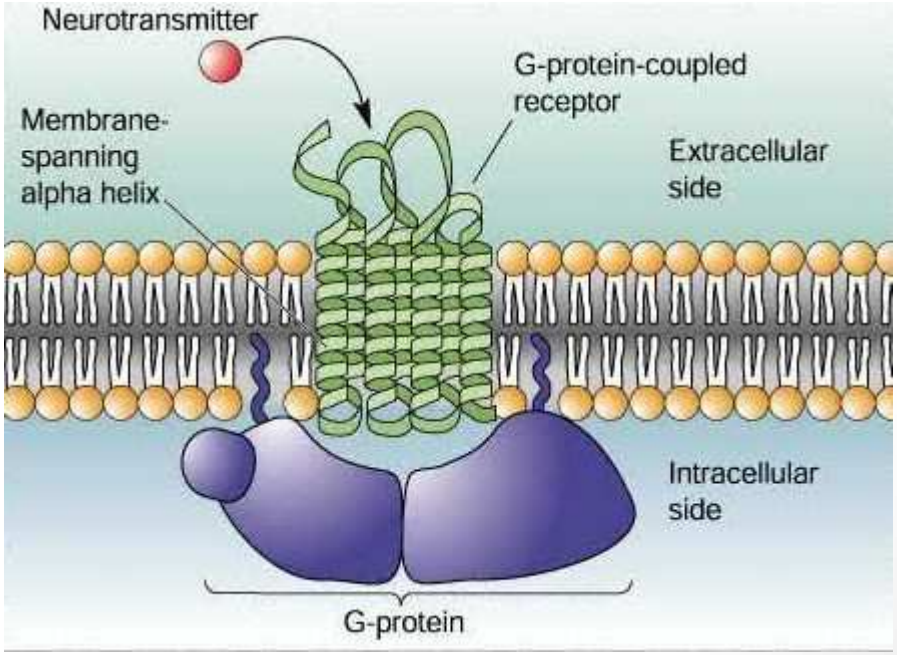
The human genome encodes thousands of G protein-coupled receptors, about 350 of which detect horr
endogenous ligands.



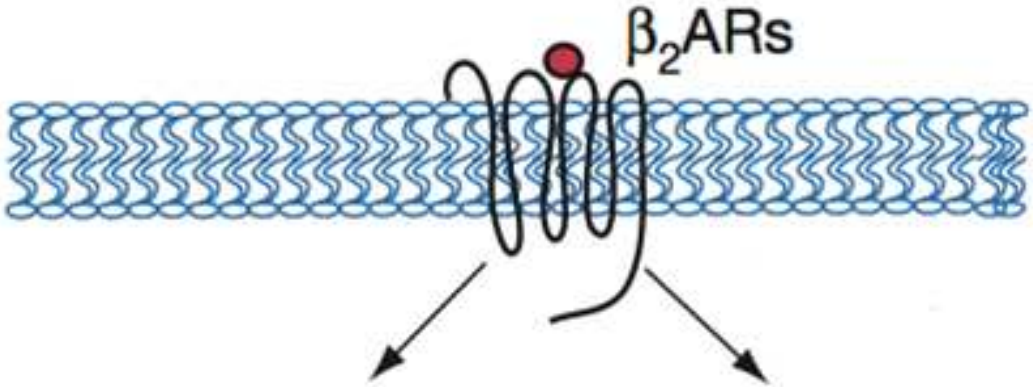
In 1980, a general mechanism for receptor activation, the so-called ternary complex model, was proposed by Lefkowitz and his coworkers.

An important aspect of the signalling mechanism is that the ligand does not pass through the membrane: the signal is transferred to the inside of the cell by conformational changes in the receptor protein.

The signalling pathway is selected based on ligand identity, and the same 7TM receptor may be involved in both G-protein–dependent and G-protein–independent signalling.



A stress response pathway regulates DNA damage through b2-adrenoreceptors and b-arrestin-1



Gs-dependent signalling → **Gs/PKA**

Gs-independent signalling → **PI3K/AKT**

Lefkowitz, Nature 2011

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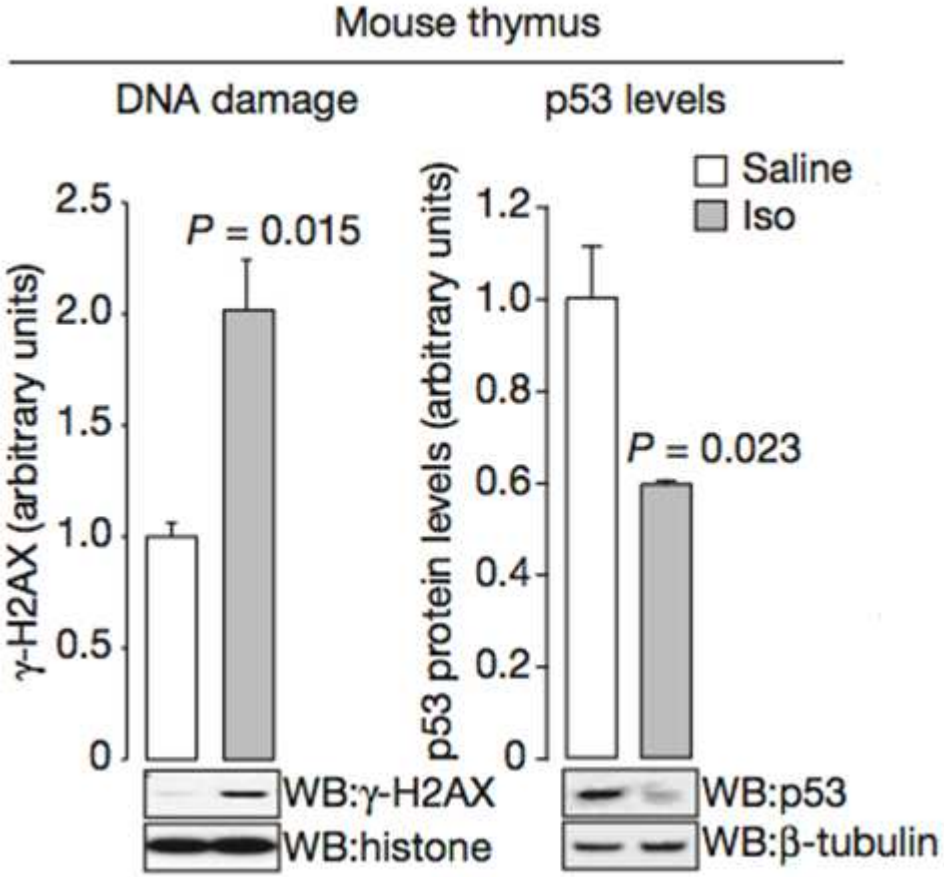
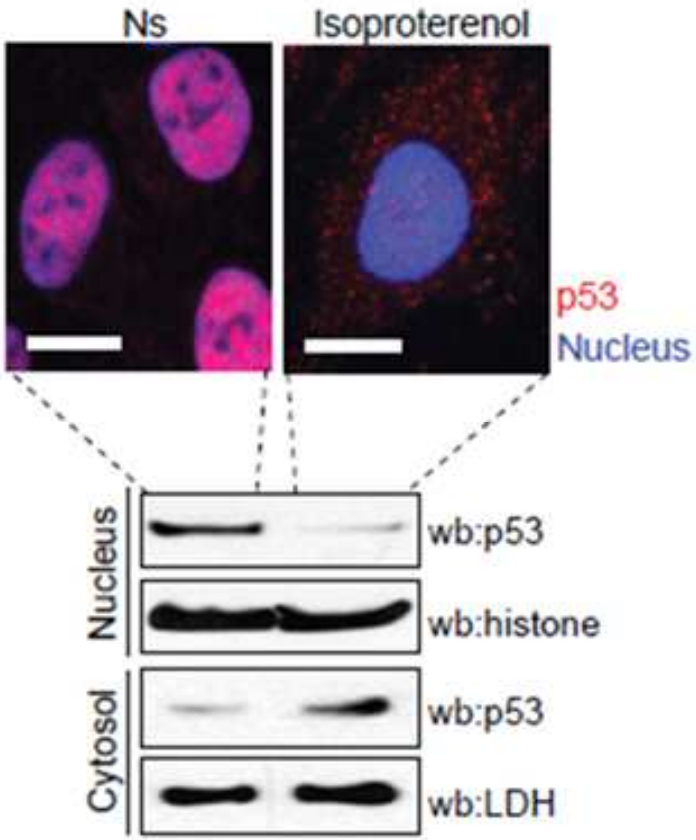
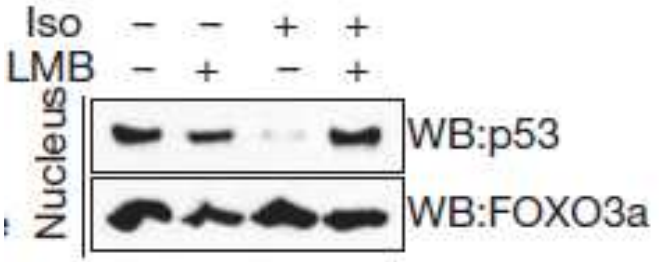


Iso: Isoproterenol, syntetic analogue of adrenaline

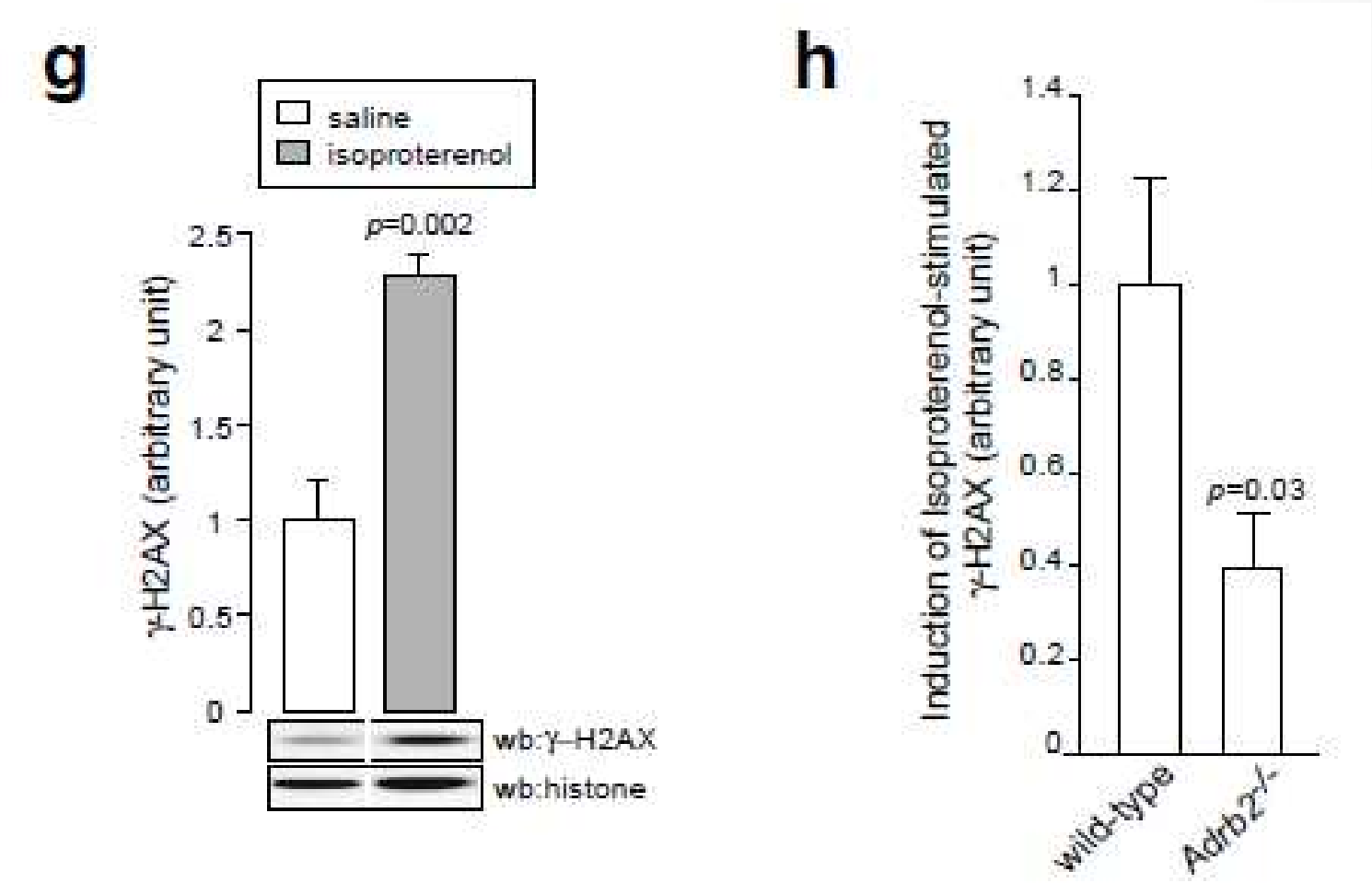
Treatment with isoproterenol leads to accumulation of DNA damage and decreased p53 levels in mouse thymus.



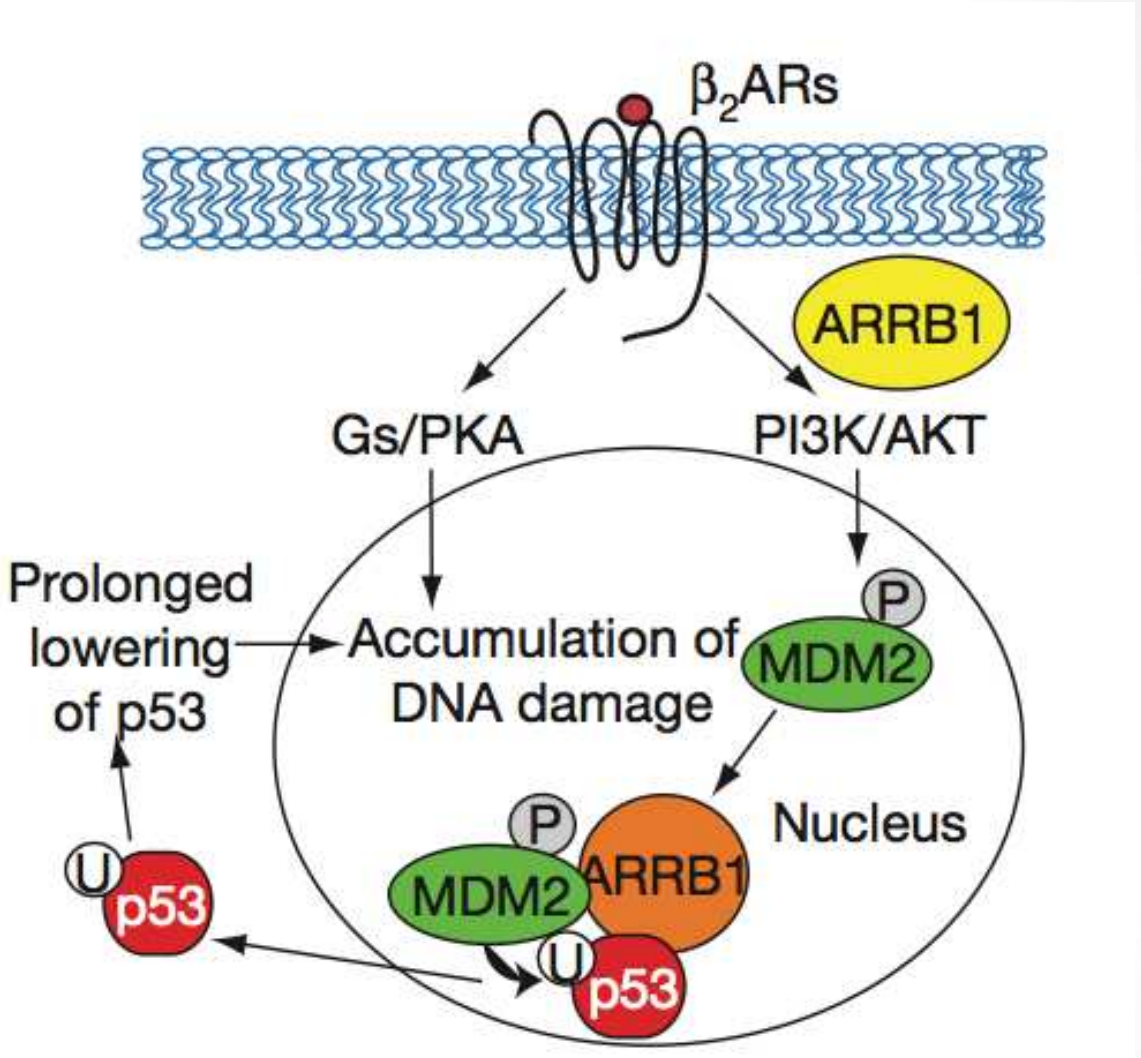


LMB: Leptomycin B, nuclear export inhibitor

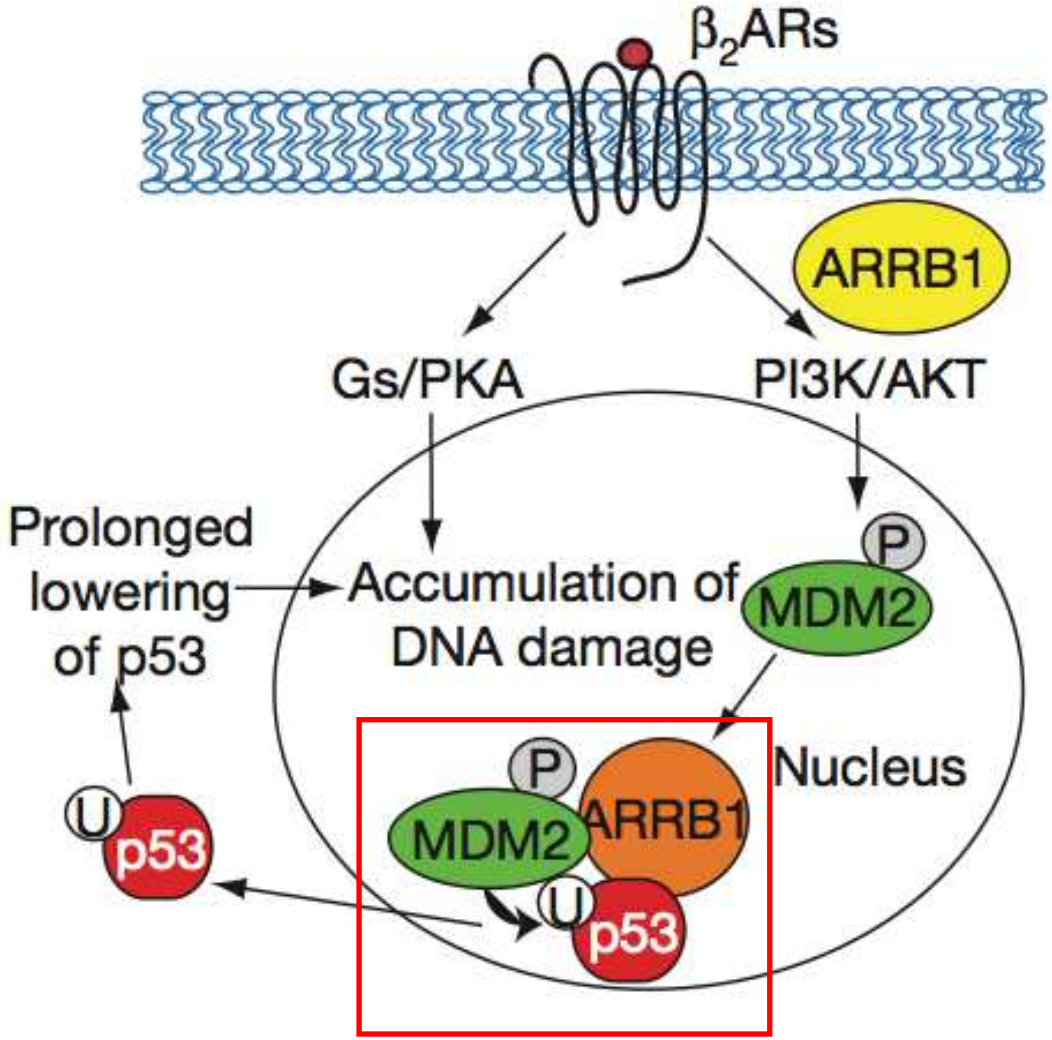
Isoproterenol stimulation leads to p53 translocation from the nucleus to the cytosol. Cells were immunostained with an anti-p53 antibody and examined by confocal microscopy. Leptomycin B pretreatment reverses isoproterenol-induced nuclear export of p53.



Stimulation of the beta2 adrenoreceptors results in the nuclear export and degradation of p53 in a specific manner.



The E3 ligase MDM2 has been shown to have an important role in the regulation of p53 nuclear export and degradation.



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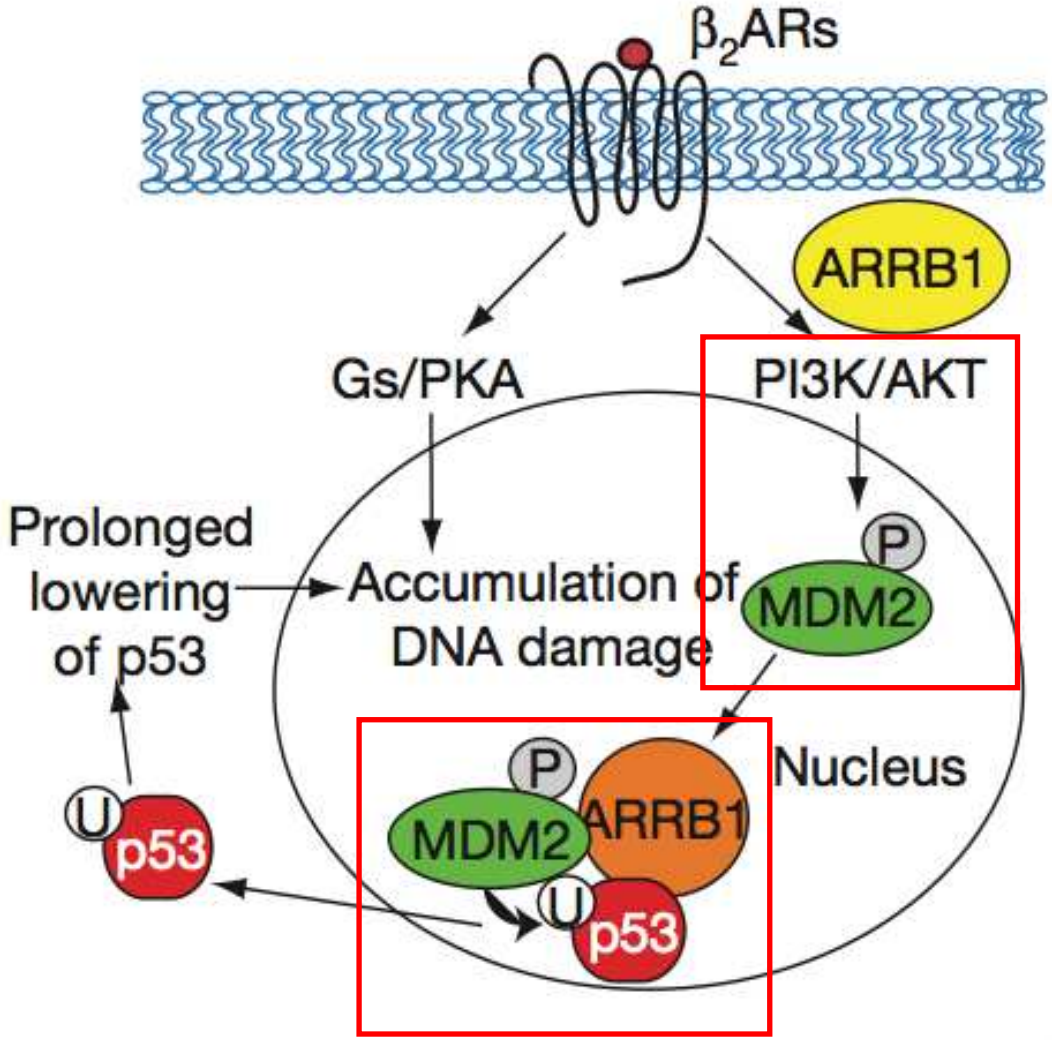
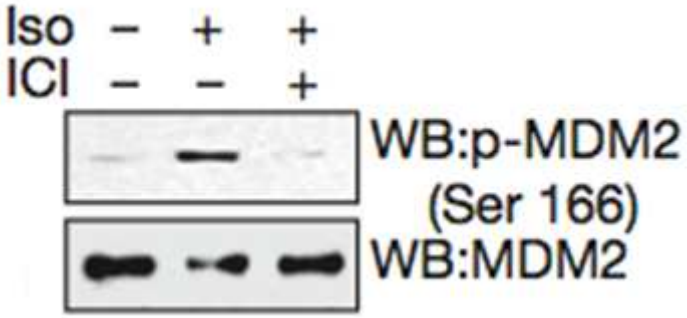
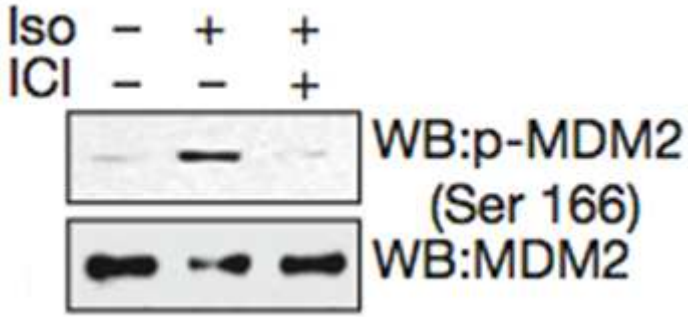


Before MDM2-mediated ubiquitination of p53 the PI3K/AKT cascade phosphorylates MDM2, activating its E3 ligase function.



Isoproterenol stimulation leads to MDM2 phosphorylation at Ser 166, an AKT phosphorylation site, and the effect is antagonized by an adrenoreceptor antagonist.



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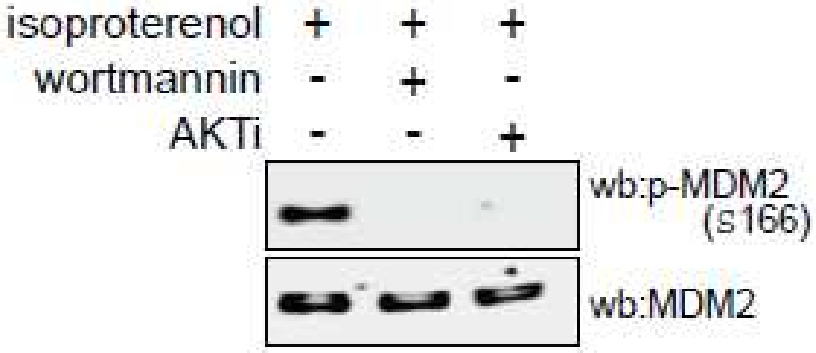
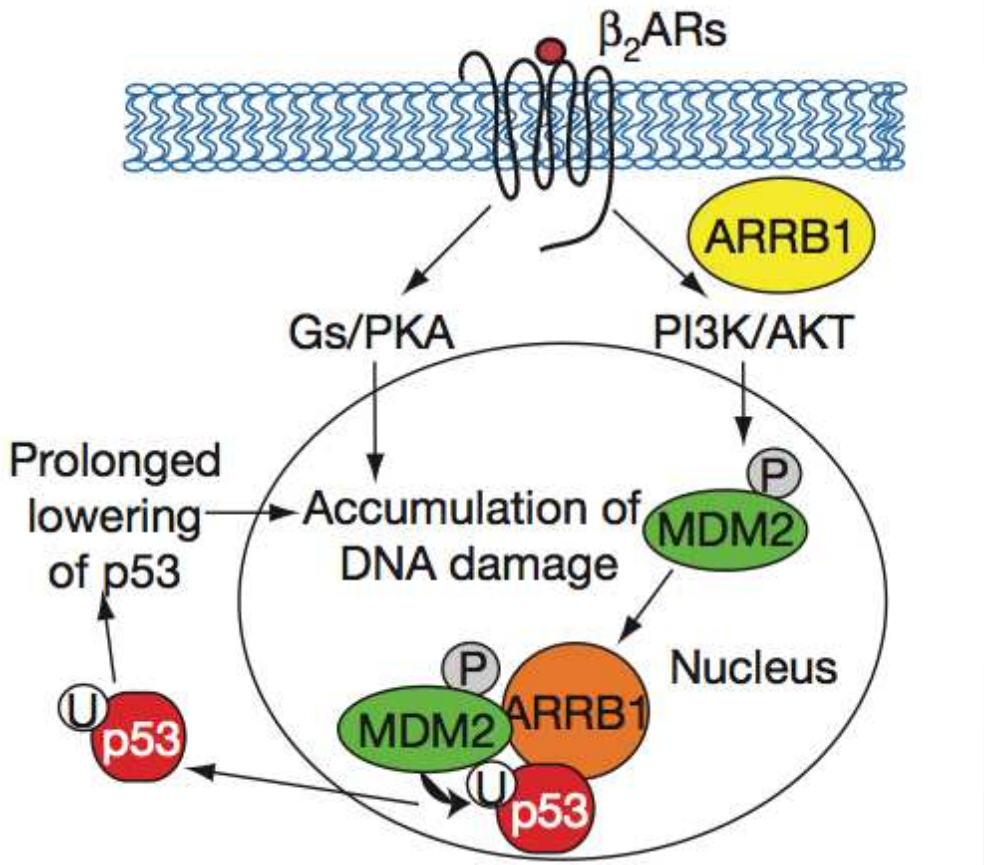


Inhibition of the PI3K/AKT cascade abolishes isoproterenol-stimulated MDM2 phosphorylation at S166.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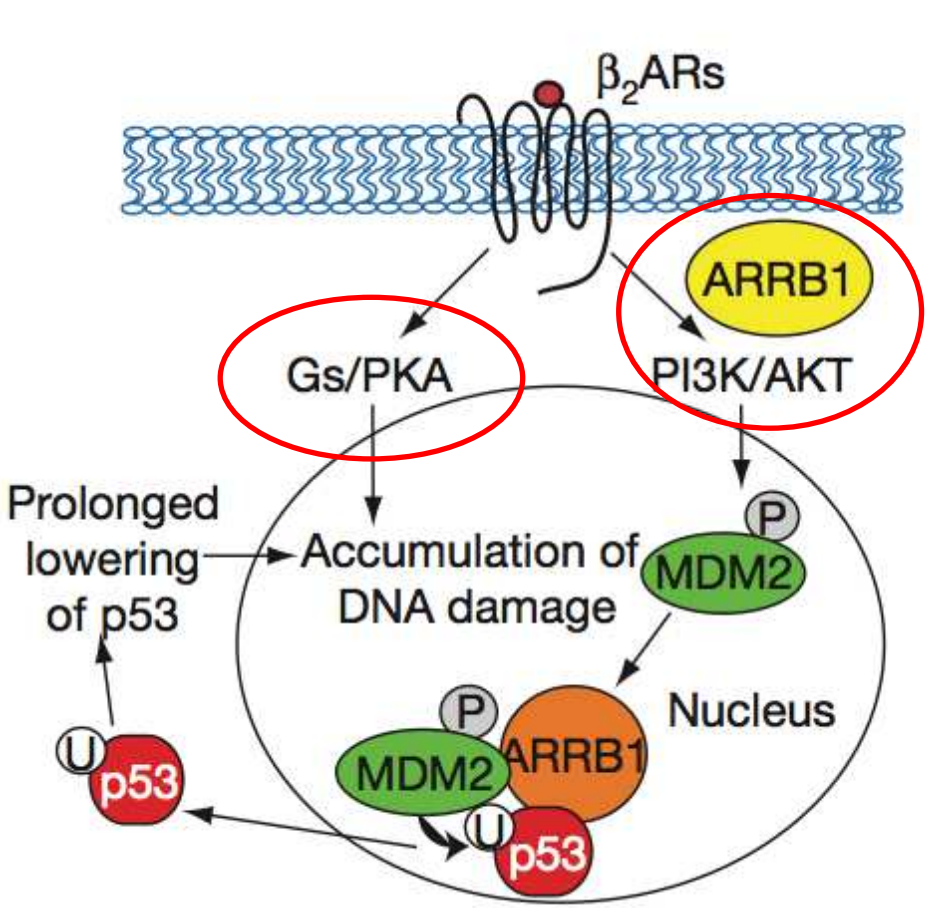
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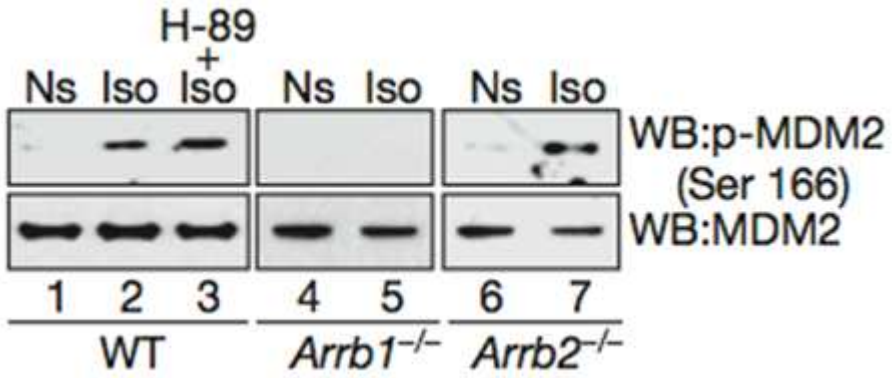
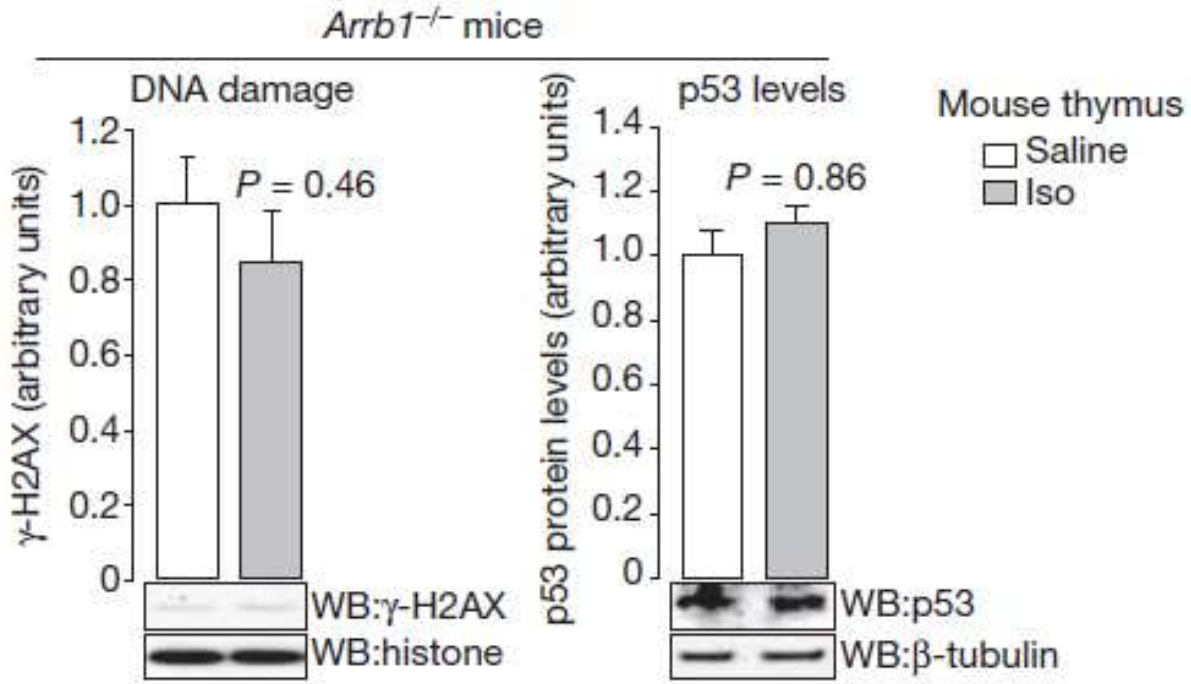


- In wild type MEFs, H-89 does not inhibit isoproterenol-stimulated MDM2 phosphorylation.
- The isoproterenol effect is abrogated in Arrb1 -/-, but not in Arrb2-/-, MEFs.
- A Gs-independent, ARRB1-dependent signalling pathway regulates the activation state of MDM2 through the PI3K/AKT cascade.

ARRB1 facilitates catecholamine-induced p53 degradation by MDM2.



Levels of p53 in isoproterenol-infused Arrb1 -/- mice remain constant and there is no accumulation of DNA damage.

Conclusions:

- Beta2 adrenoreceptors are involved in a stress response pathway which regulates DNA damage in cells.
- Adrenergic catecholamines, acting through a beta-arrestin-mediated pathway, trigger DNA damage and suppress p53 levels both in vitro and in vivo.
- Catecholamine-induced lowering of p53 levels may lead to increased survival of cells containing DNA damage, owing to an impaired DNA damage checkpoint and repair cascade. This would then facilitate accumulation ofDNA damage.
- Another prominent cascade leading to DNA damage is the generation of reactive oxygen species through Gs-PKA signalling.
- G-protein-mediated and ARRB1-mediated pathways may sinergistically affect the accumulation of isoproterenol-induced DNA damage, with effects on genomic integrity.

Bibliography:

Makoto R.Hara et al. - A stress response pathway regulates DNA damage through b2-adrenoreceptors and b-arrestin-1. Nature-2011

Kobilka-The structure and function of G protein-coupled receptor-Nature Review 2009