

# **FBXO25 Suppress Cell Proliferation through Inhibition of ERK Phosphorylation**

## **Introduction**

Upon mitogen stimulation, ELK1 is phosphorylated by mitogen activated protein kinase (ERK) and recruited into the serum response factor complex, regulating the transcription of immediate early genes, such as *cfos*, *egr1*, and *mcl1* by interacting with their serum response element DNA regulatory sites and leading to cell proliferation. Recently, we showed that the E3 ligase SCF1 (FBXO25) complex mediates ubiquitination and degradation of ELK1. FBXO25 inhibited the activation of two ELK1 target genes in response to PMA.

## **Objectives**

We examine the role of FBXO25 in functional processes regulated by ELK1, such as cell proliferation and apoptosis. We also investigate the association of FBXO25 with other ELK1 signaling pathway components in response to mitogens.

## **Material and Methods**

Cell doubling, DNA synthesis and apoptosis: Stable cells Flp.FBXO25 or Flp.FBXO25 Fbox mutant were induced or not with tetracycline. To determine cell doubling the Flp cells were counted every 2 days during 8 days in Neubauer chamber. To measure DNA synthesis, Flp cells were exposed to BrdU for 2 h. Apoptosis was assayed using the Apo.BrdU TUNEL and apoptotic cells were detected by flow cytometry. Human Phospho kinase Array Analysis: To evaluate the phosphorylation state of 43 different kinases substrates Flp cells lysates (FBXO25 induced or not) were incubated with a preblotted membrane array of the Human Phospho-Kinase Array, processed for immunoblotting and subjected to densitometric scanning for spot quantification.

## **Discussion and Results**

Here, we show that the FBXO25 overexpression decreased cell proliferation by its ability to deregulate ERK1.2 activity in a manner dependent on its intact F-box domain. The most intriguing discovery is that ERK1.2 dephosphorylation occurred without preceding down-regulation of mitogen-activated protein kinase (MAPK) kinase (MEK).

## **Conclusion**

Taken together FBXO25 functions as a negative regulator of MAPK signaling via inactivation of ERK1.2 and suppression of cell proliferation.