



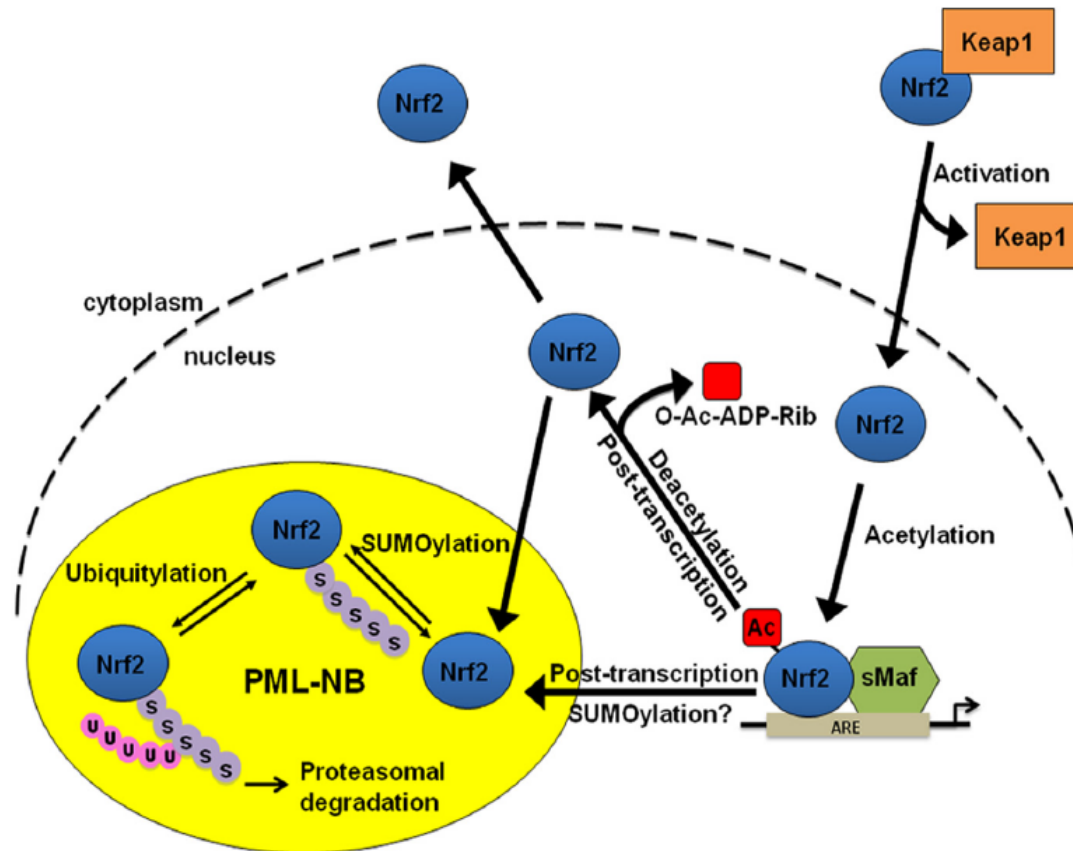
PML-RAR α interferes with Nrf2 function in Acute Promyelocytic Leukemia Cells

Nelida I. Noguera, Gianfranco Catalano, Serena Travaglini, Cristina Banella, Maria Teresa Voso, Francesco Lo Coco

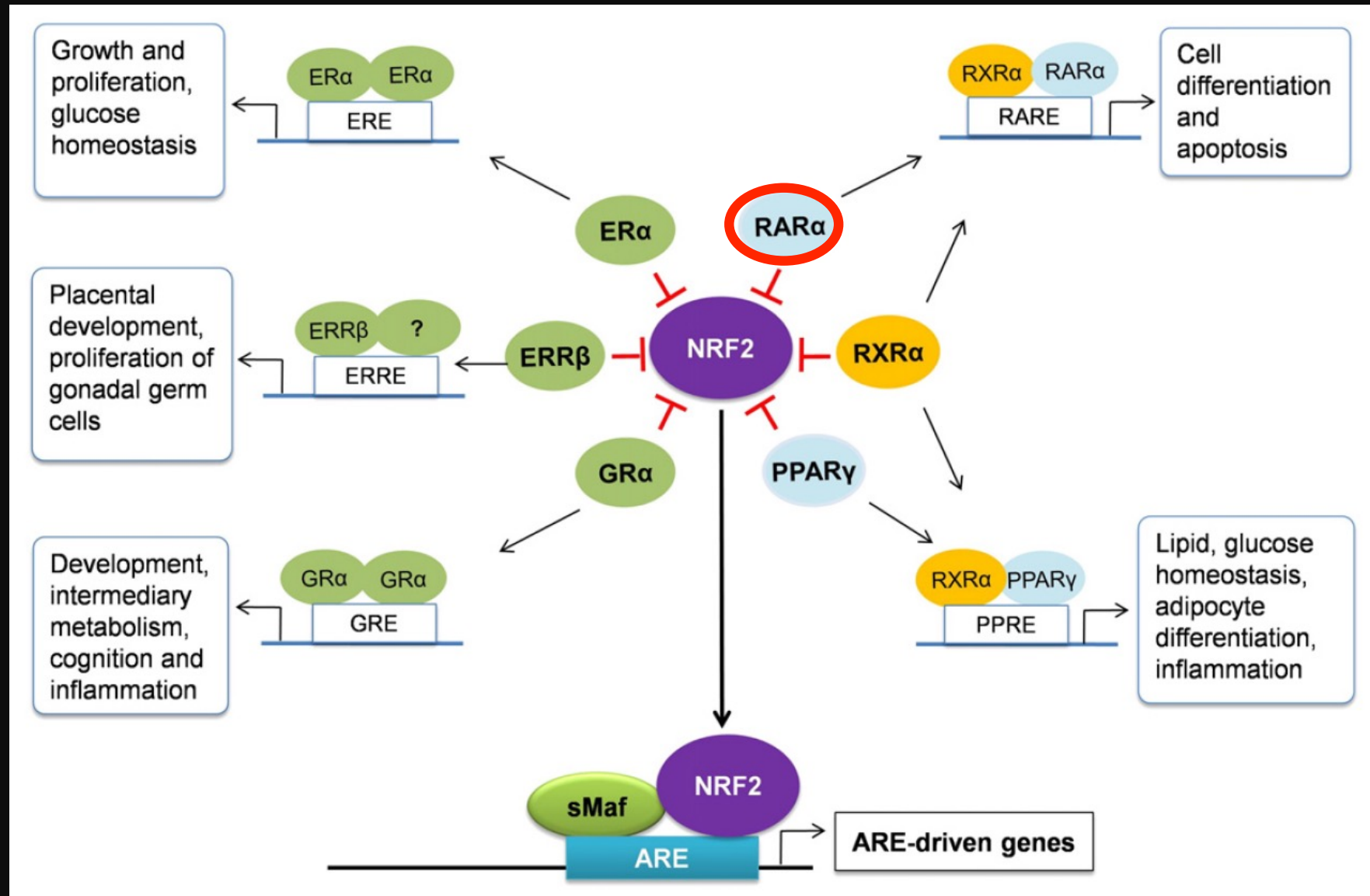
Keap1-Nrf2 regulation System

- ❖ NRF2 is a transcription factor, involved in the cellular response to oxidative stress.**
- ❖ In normal conditions, it is maintained at low levels via Keap-1 binding, constitutive ubiquitylation and proteasomal degradation.**
- ❖ Electrophiles and oxidants inhibit NRF2 degradation, enabling its accumulation in the nucleus and initiates a genetic program to allow cellular adaptation to stress**

Sumoylation and Nuclear degradation of NRF2



Inhibition of NRF2 by nuclear receptors



STUDY AIMS

- ❖ APL blasts are more sensitive to treatment with oxidants than AML blasts
- ❖ We hypothesized that NRF2 function is impaired in APL

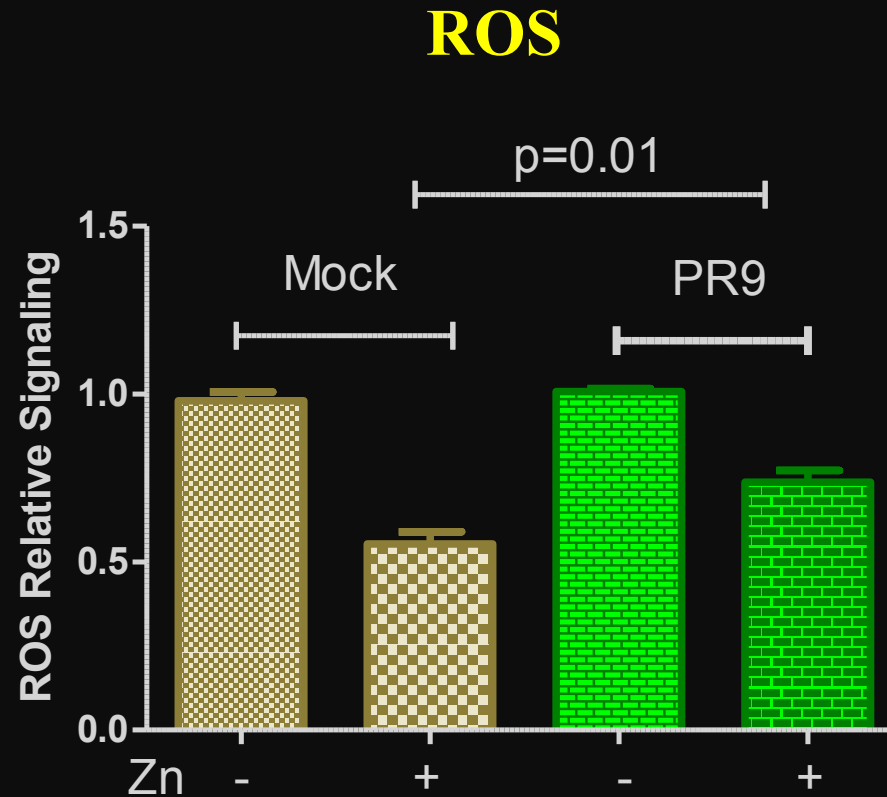
METHODS

- ❖ NRF2 expression in primary APL and AML blasts by Q-RT-PCR and WB
- ❖ The effects of PML-RARa on NRF2 expression and its transcriptional activity, by Q-RT-PCR and CHIP analysis
- ❖ Expression of its principal regulator Keap-1 by WB
- ❖ The effects of PML-RARa expression on NRF2 localization, by confocal microscopy and nuclear / cytoplasmic fractionation
- ❖ NRF2 half life in presence of PML-RARa

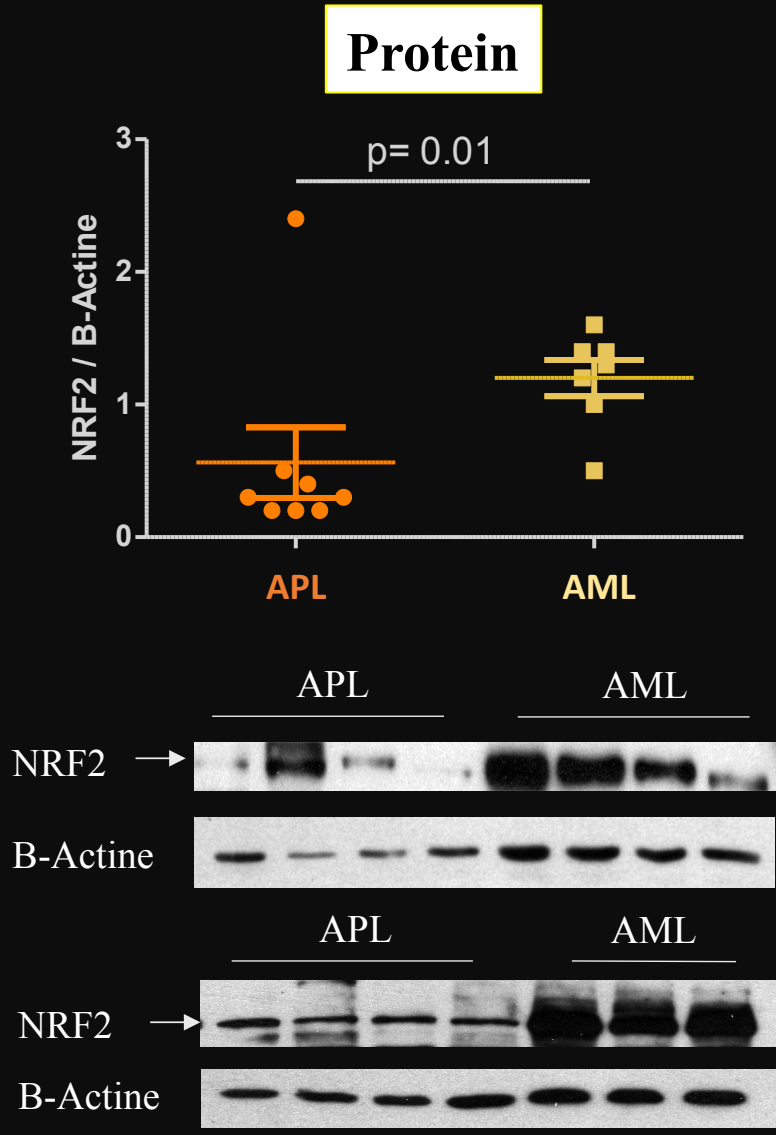
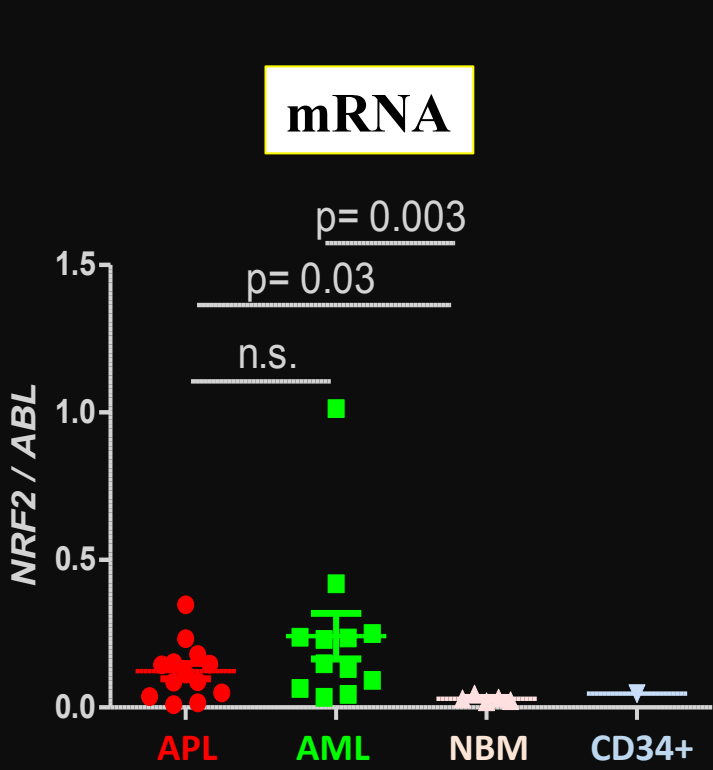
RESULTS

PML-RARa expression impairs cellular response to ROS Production

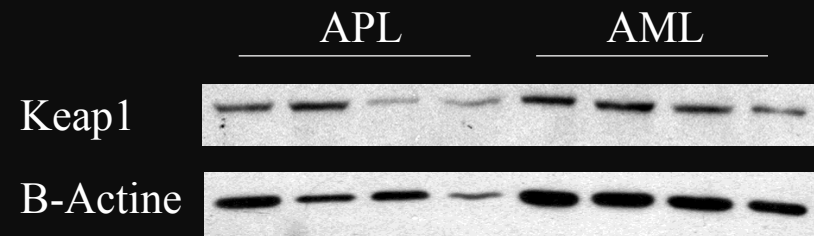
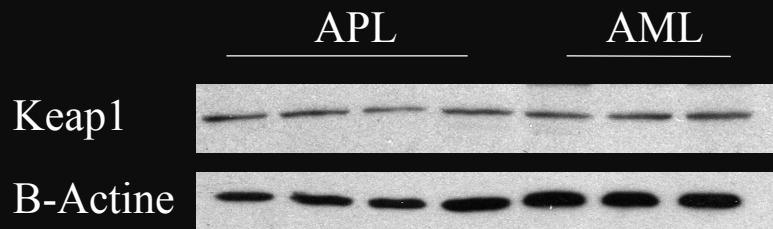
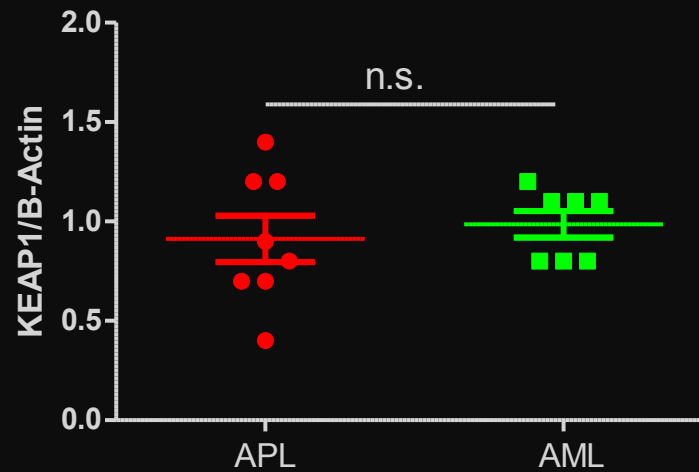
- ❖ PR9 is a PML/RARa Zn-inducible cell-line
- ❖ Addition of Zn induces NRF2 expression



NRF2 protein level is lower in APL compared to other AML samples

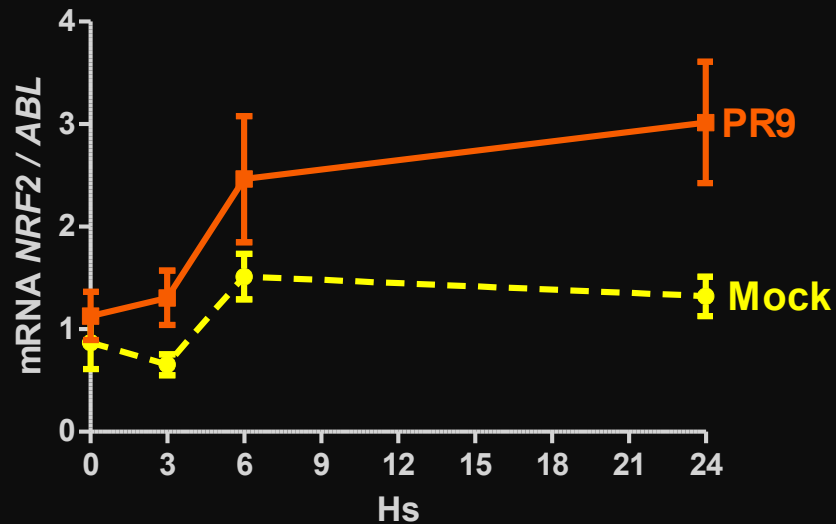


Keap-1 is expressed at similar levels in APL and AML patients' samples

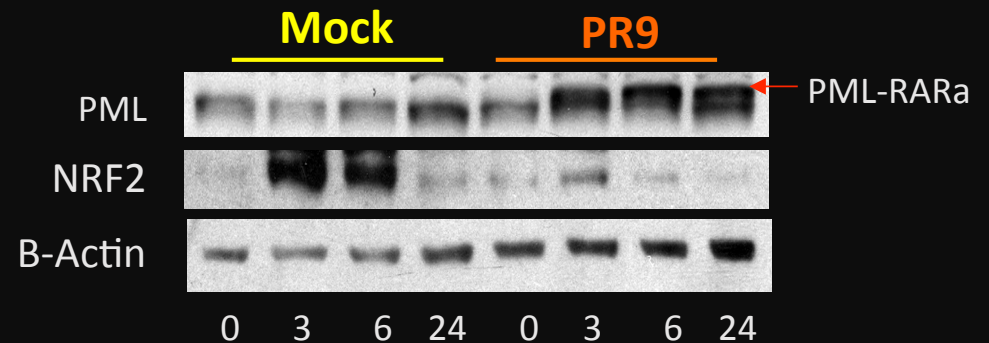
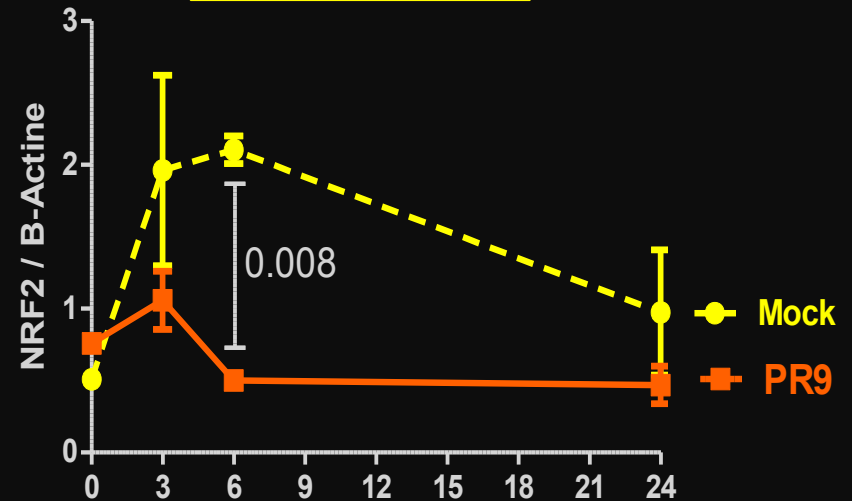


In PR9 cells, expression of PML-RARa inhibits the Zn-related increase of NRF2 protein

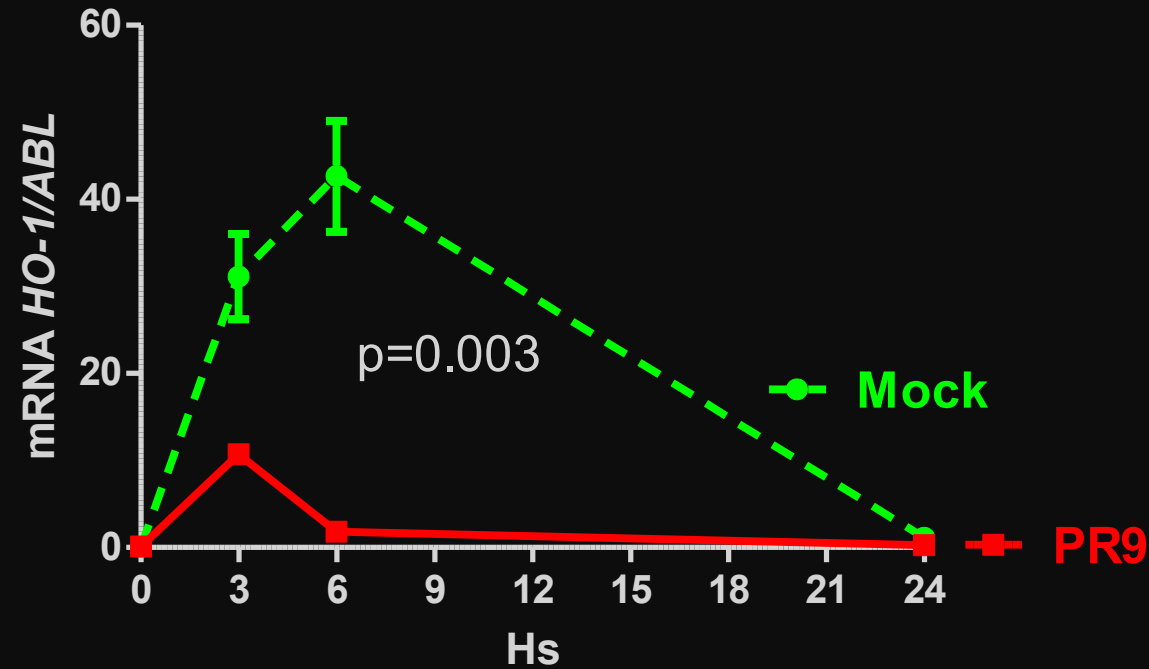
NRF2 mRNA



NRF2 Protein

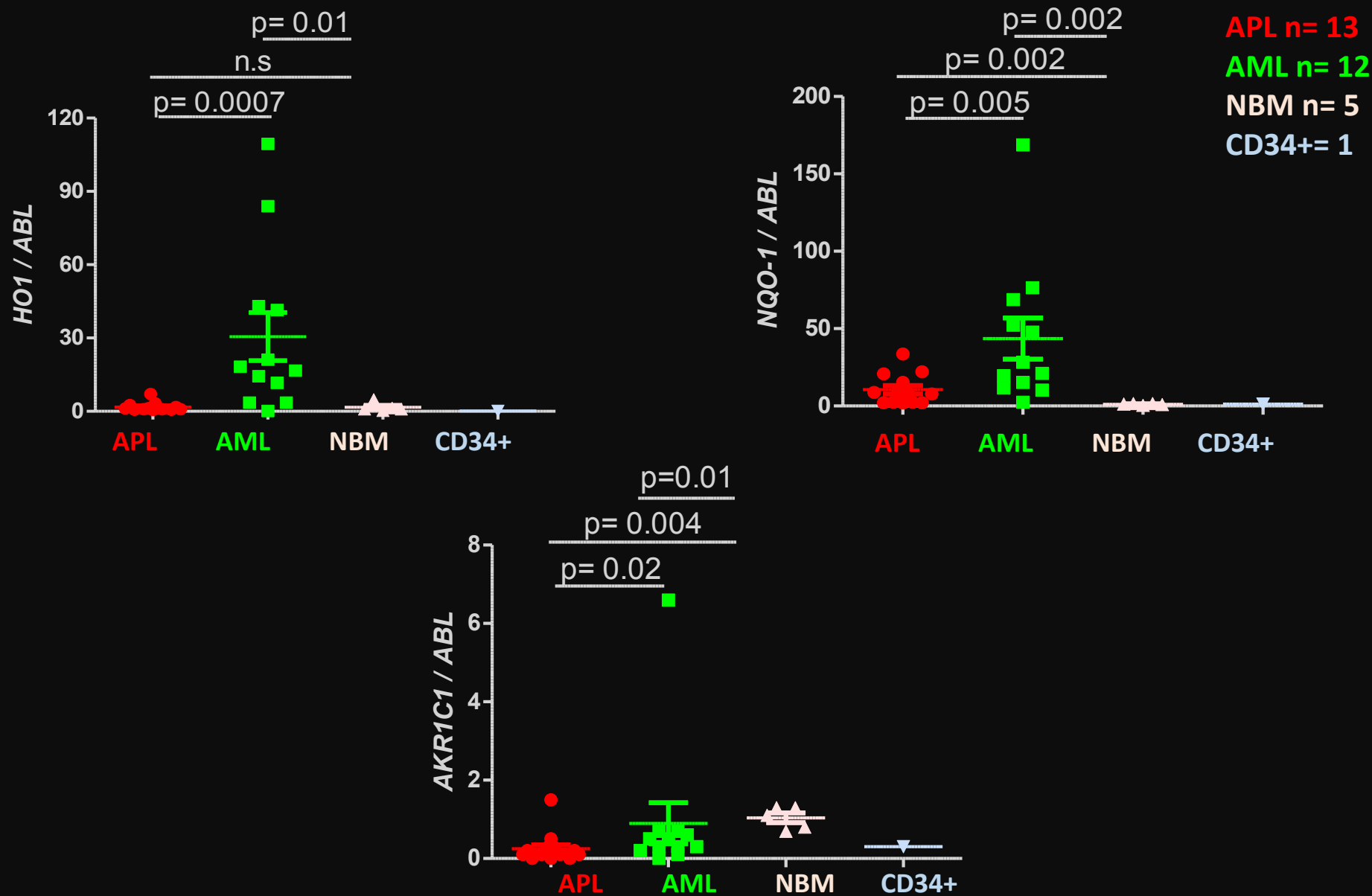


❖ HO-1 is a NRF2 target gene

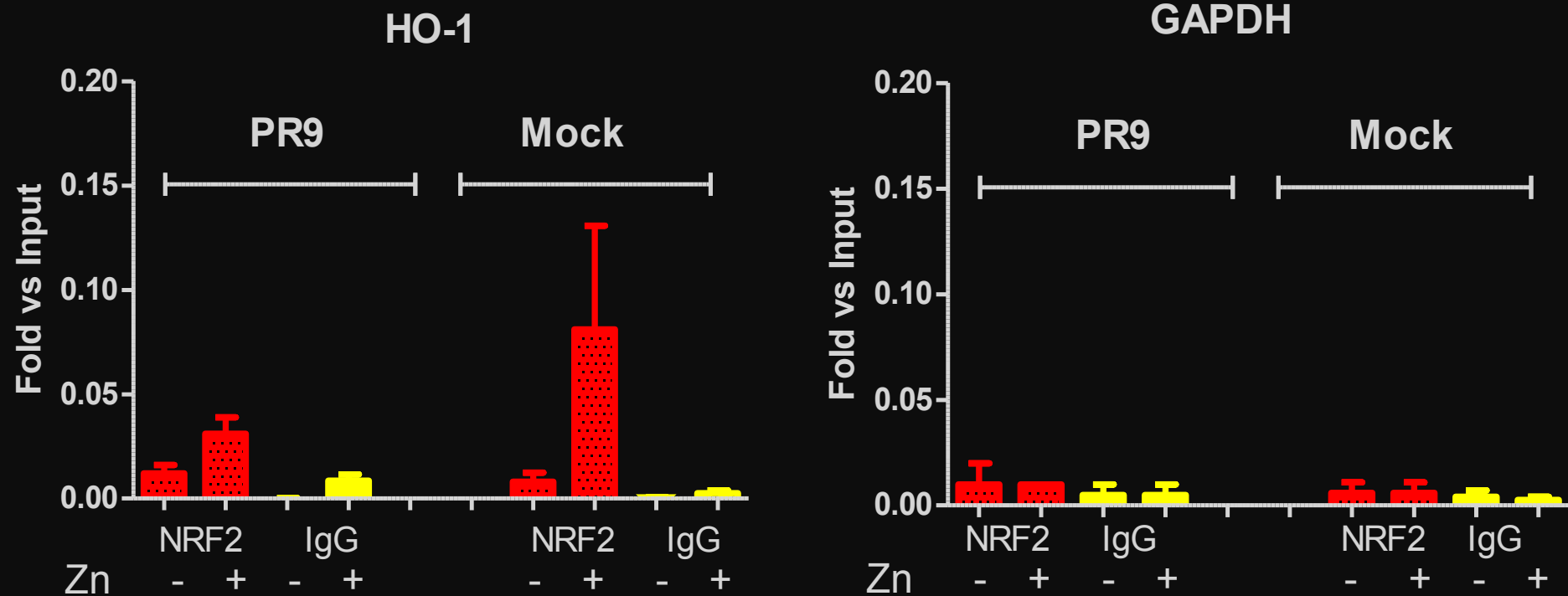


PR9 + Zn: PML-RARa induction impairs HO-1 upregulation

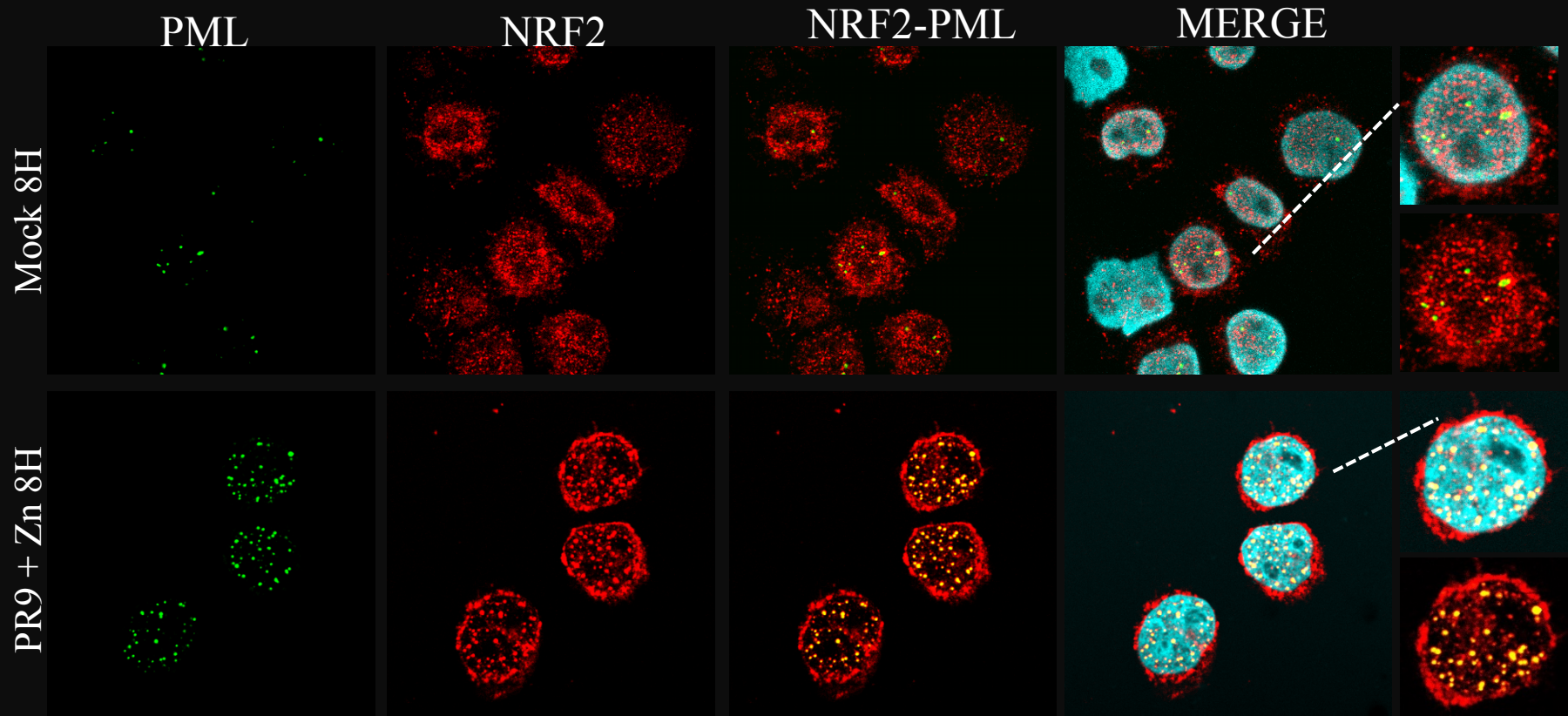
Expression of NRF2 target genes in primary blasts from APL and AML samples



PML-RAR α inhibits NRF2 binding to HO-1 promoter (CHIP: PR9 + Zn)

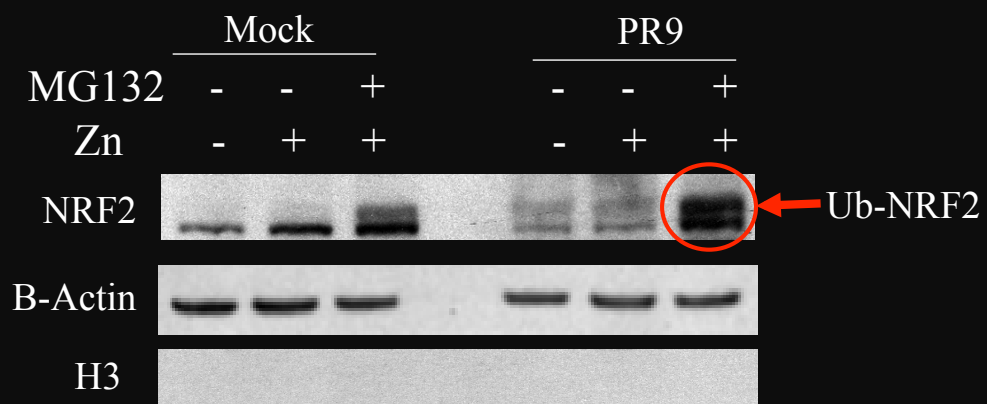


NRF2 is delocalized to the cytoplasm in the presence of PML-RARa

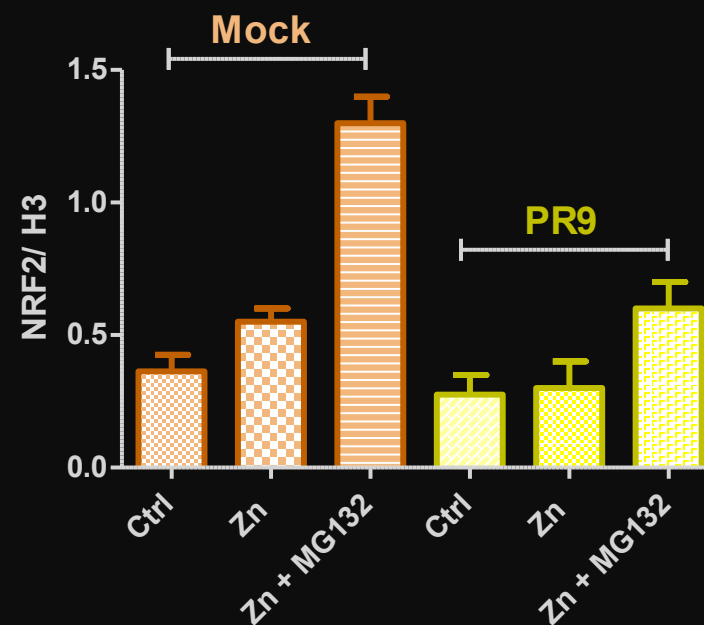
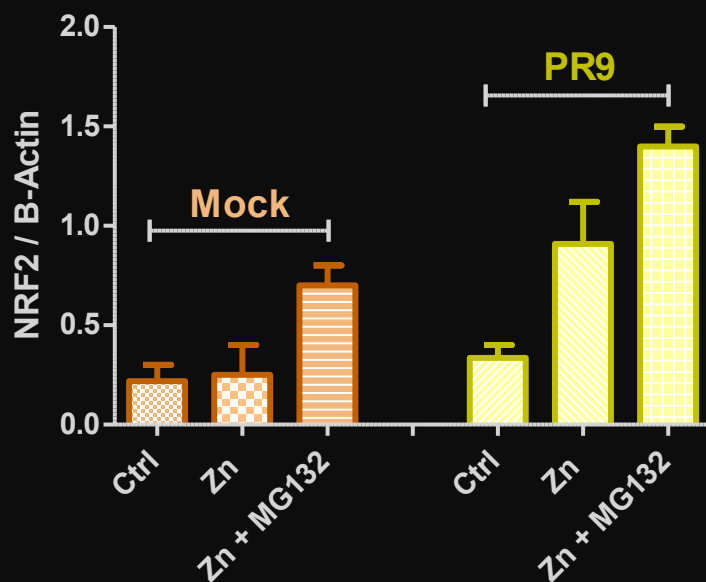
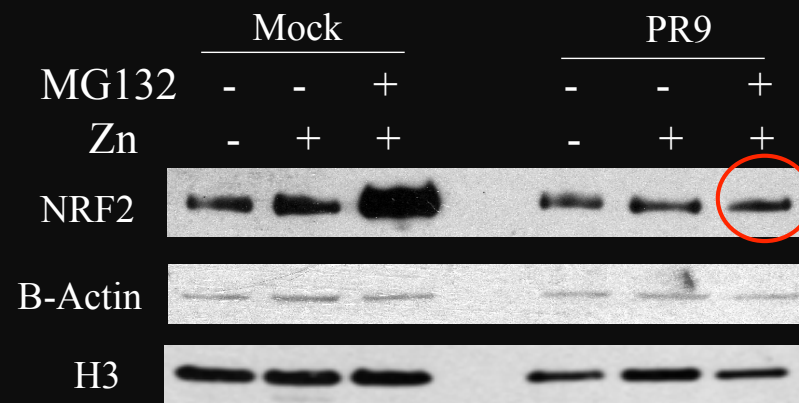


PML-RAR α induces NRF2 degradation in the cytoplasm, but not in the nucleus

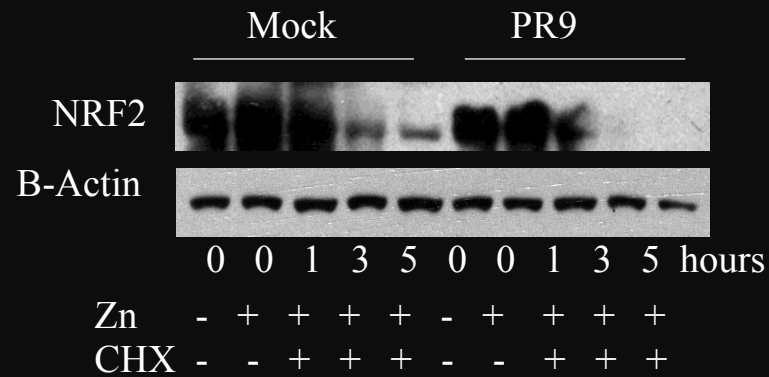
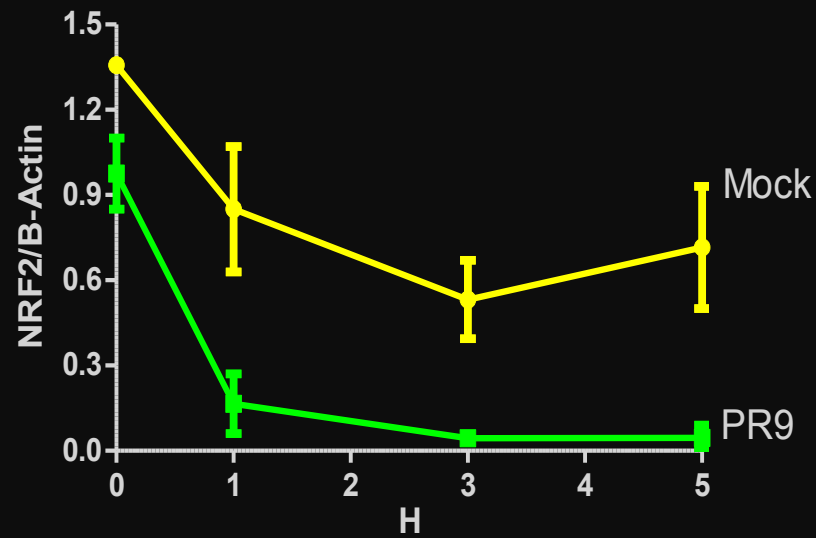
Cytoplasm



Nucleus



PML-RARa reduces NRF2 Half Life



Conclusions

- ✓ NRF2 protein is significantly downregulated in APL compared with other AML
- ✓ PML-RARa inhibits NRF2 transcriptional activity.
- ✓ PML-RARa protein induces translocation of NRF2 to the cytoplasm, and increases its proteosomal degradation, reducing its half-life
- ✓ In APL, impairment of Nrf2 is associated to deregulation of redox cell metabolism, and probably sensitizes cells to the cytotoxic effects of oxidants

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