Stony Brook University

Novel observations on KAP1/TRIM28 in the context of the life cycle of Epstein-Barr Virus

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The switch from latency to lytic is paramount to the pathology of herpesviruses

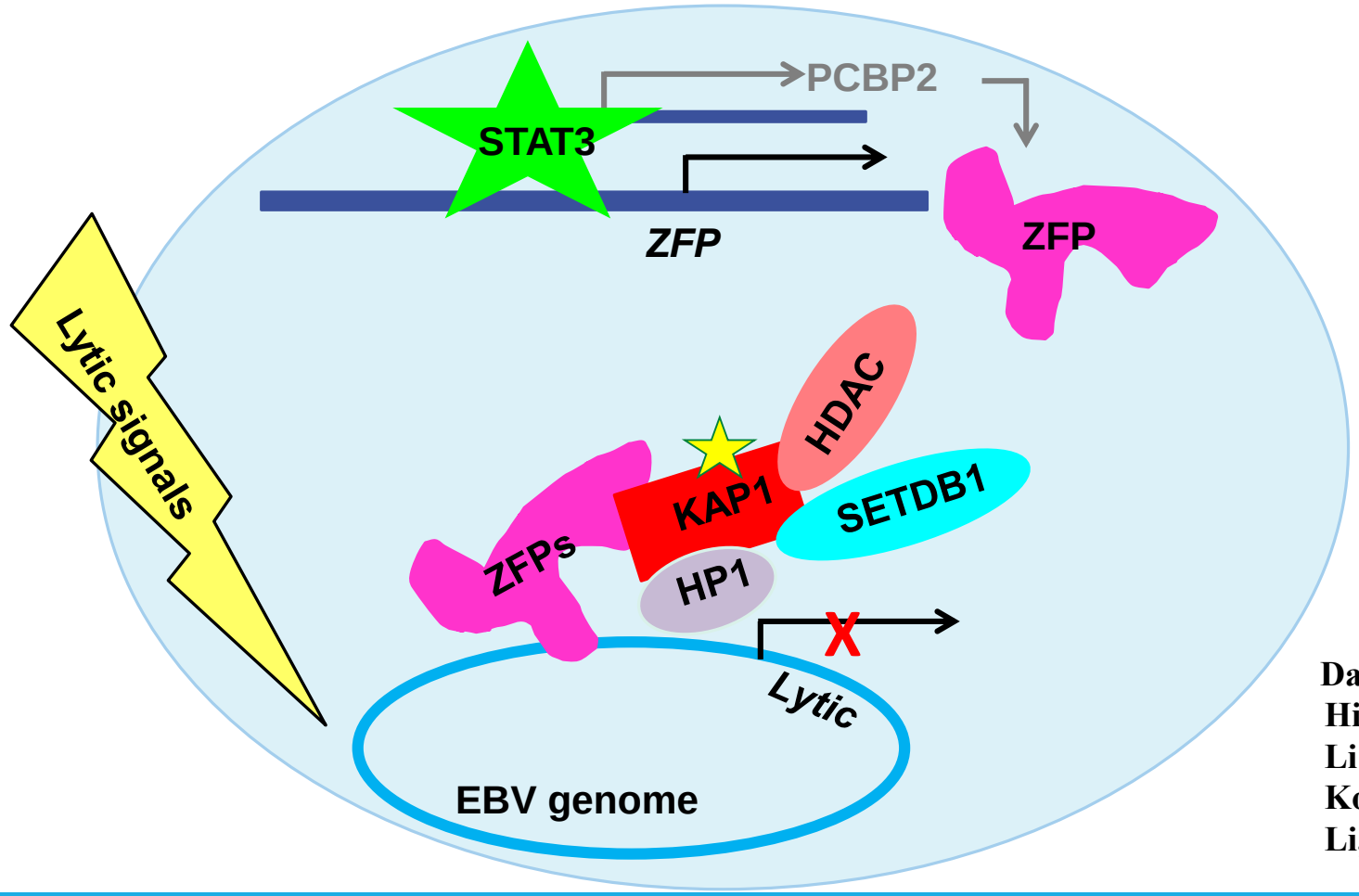
- Herpesvirus pathology is predominantly related to the lytic phase of life cycle

- Understanding cellular regulators of latency to lytic phase is key to discovering factors that are common across herpesvirus biology

-We discovered that STAT3 was causal to repressing the latent to lytic switch in EBV and KSHV, and later others found STAT3 was functioning similarly in HSV-1 as well. (Daigle et al, J. Virol, 2010) (Hill et al, J. Virol, 2013)(Koganti et al, J. Virol, 2015) (Li et al, J. Virol, 2015)(Du, PNAS 2013)

Cellular factors that regulate susceptibility to EBV lytic activation

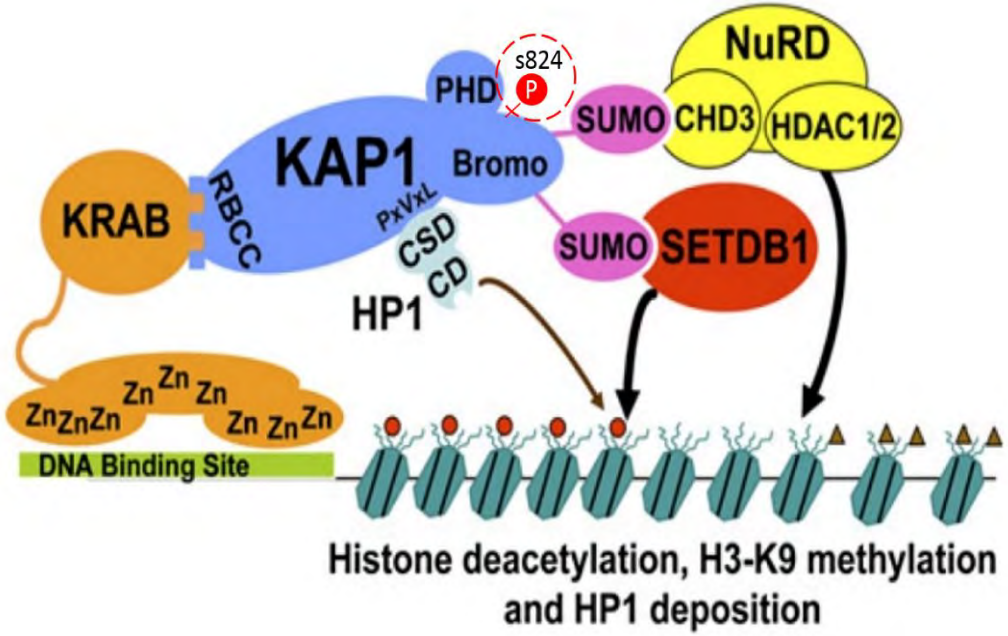
- STAT3
- PCBP2
- KRAB-ZFPs
- **KAP1**



Daigle et al., J. Virol, 2010
Hill et al., J. Virol, 2013
Li et al, J. Virol, 2015
Koganti, et al., J. Virol, 201
Li, et al., PLoS Path, 2017

Krüppel-associated Box (KRAB)-associated protein (KAP1)

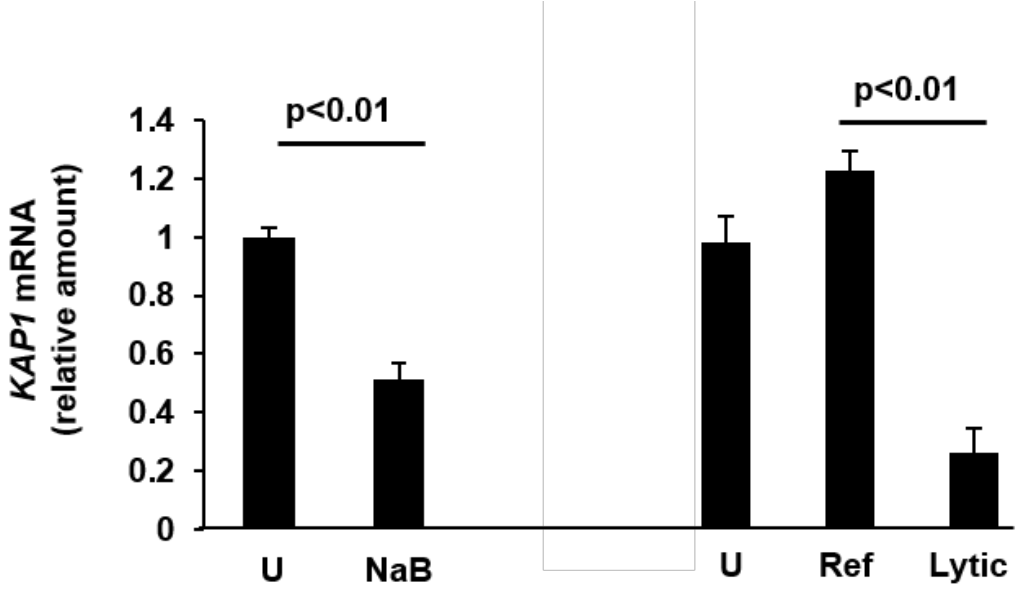
- Heterochromatin inducing co-repressor, localized by KRAB-ZFPs
- Plays vital role in promoting repair of DSBs within heterochromatin
- **Regulated via phosphorylation of S473/S824**

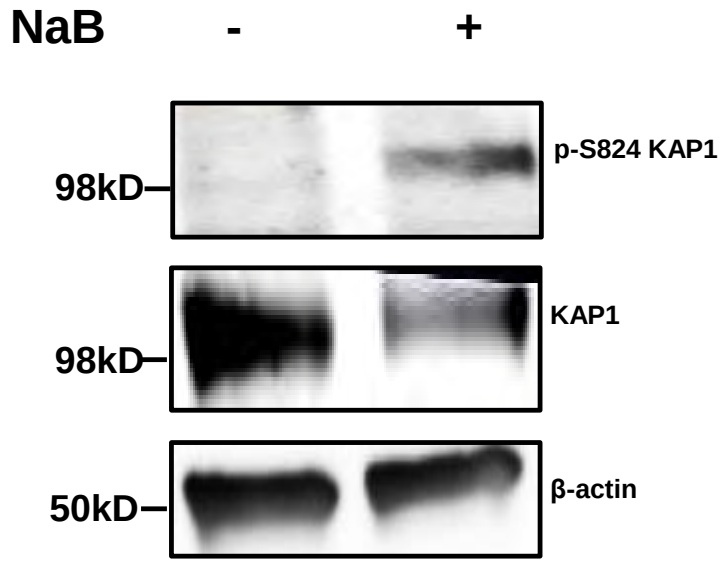


KAP1 in Herpesvirology

- STAT3 regulates KAP1 levels during KSHV lytic activation (Li et al, 2015 J Virology)
- KAP1 regulates EBV lytic susceptibility as well and is functionally repressed during lytic activation (Li et al, PLoS pathogens 2017)
- KAP1 is vital to latency establishment and regulates the latent to lytic switch for both KSHV and hCMV (Chang et al, 2009 Cancer Res) (Rauwel et al, 2015 Elife) (Gjyshil et al, 2015 J Virology)
- Common mechanism: phosphorylation of S824

KAP1 protein and RNA levels decrease during lytic activation



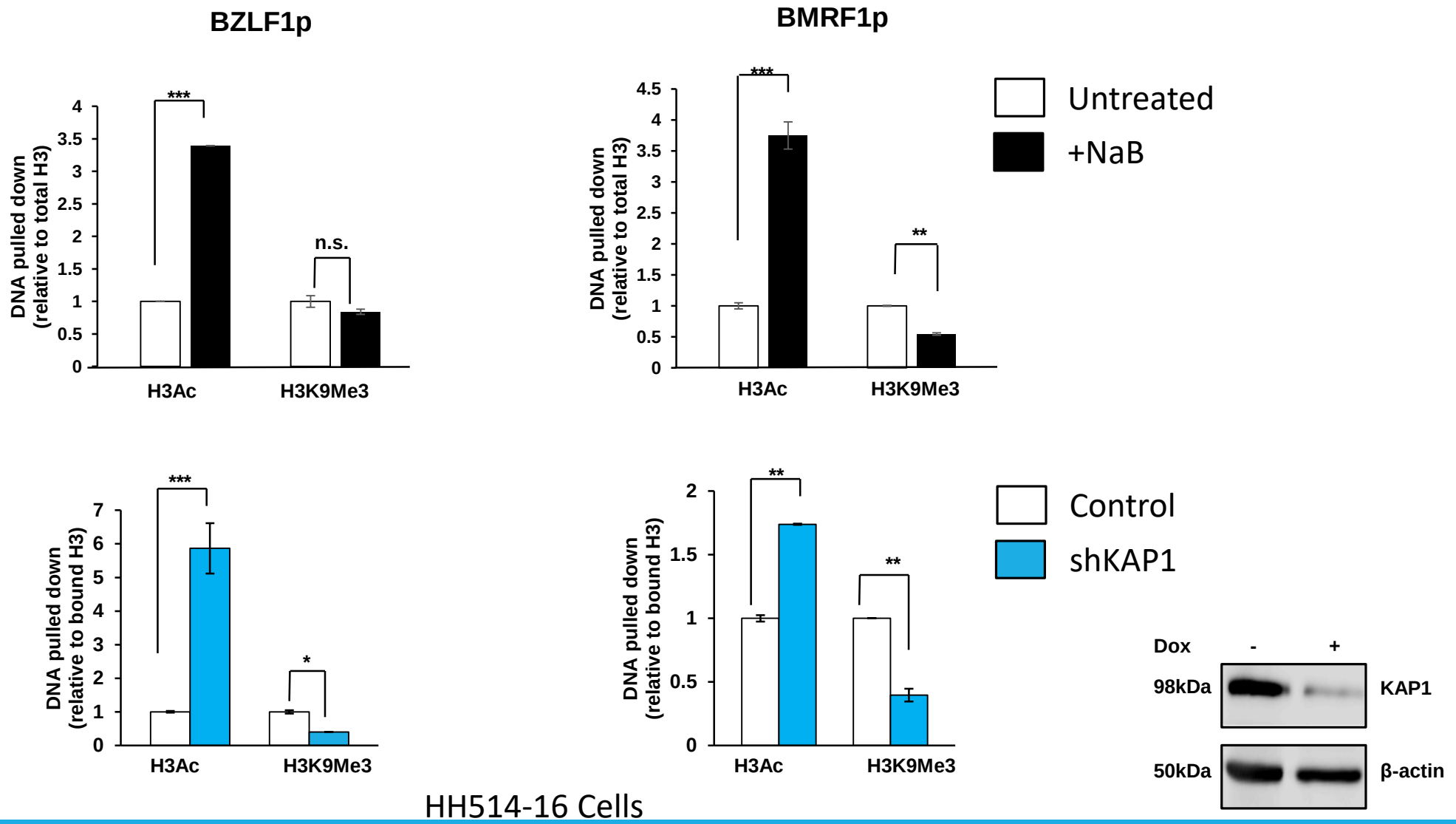


HH514-16 Cells

Koganti, Unpublished 2016

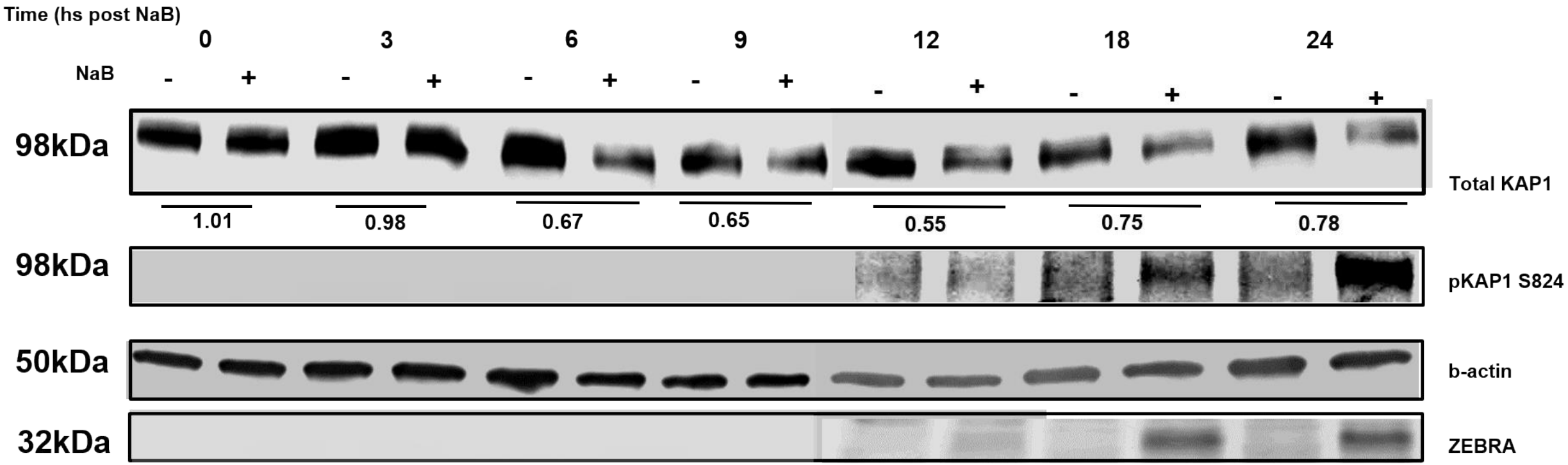
Burton, Unpublished 2016

KAP1 regulates heterochromatin markers surrounding *BZLF1* and *BMRF1*



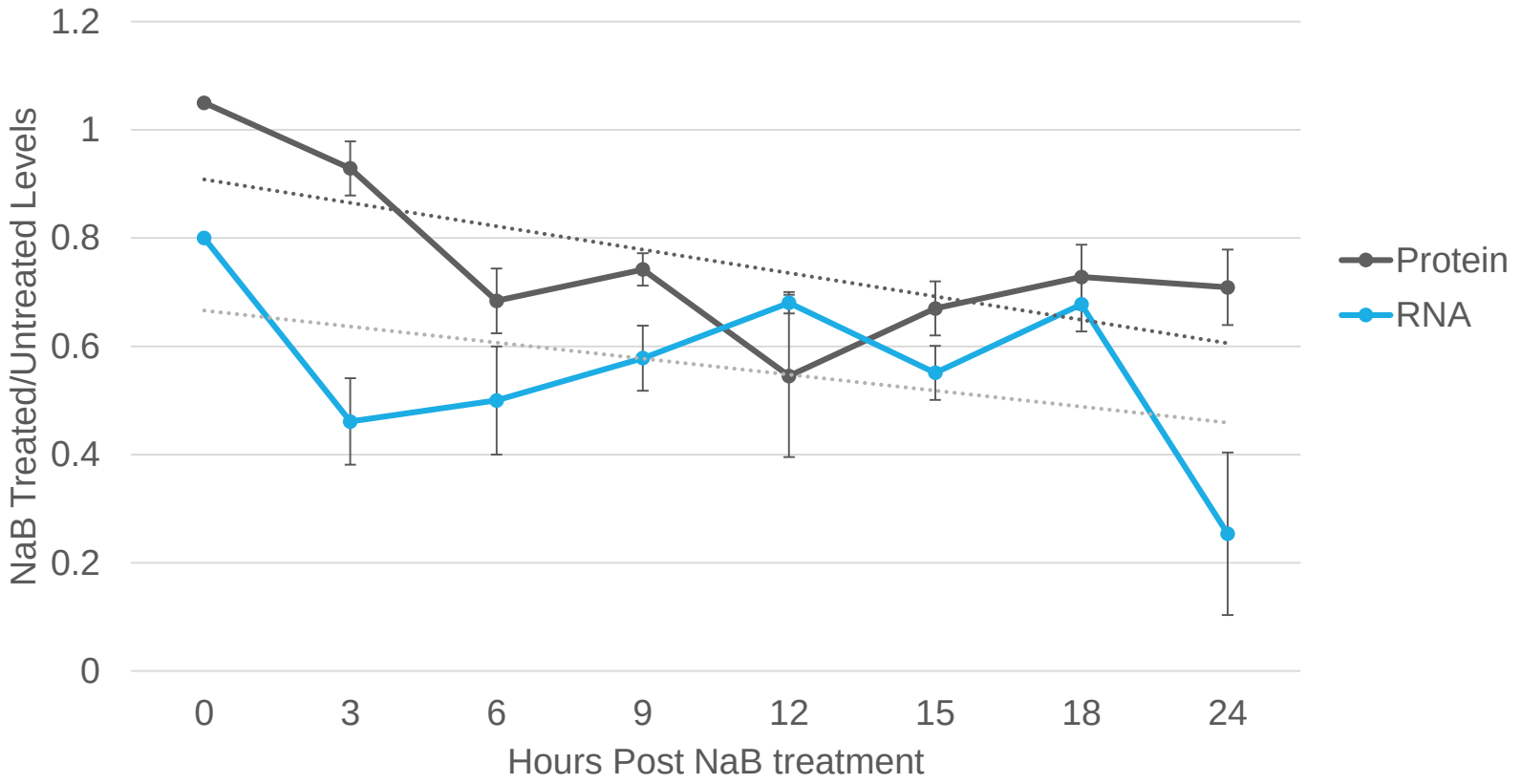
HH514-16 Cells

Total KAP1 protein levels decrease before KAP1 phosphorylation and lytic gene expression



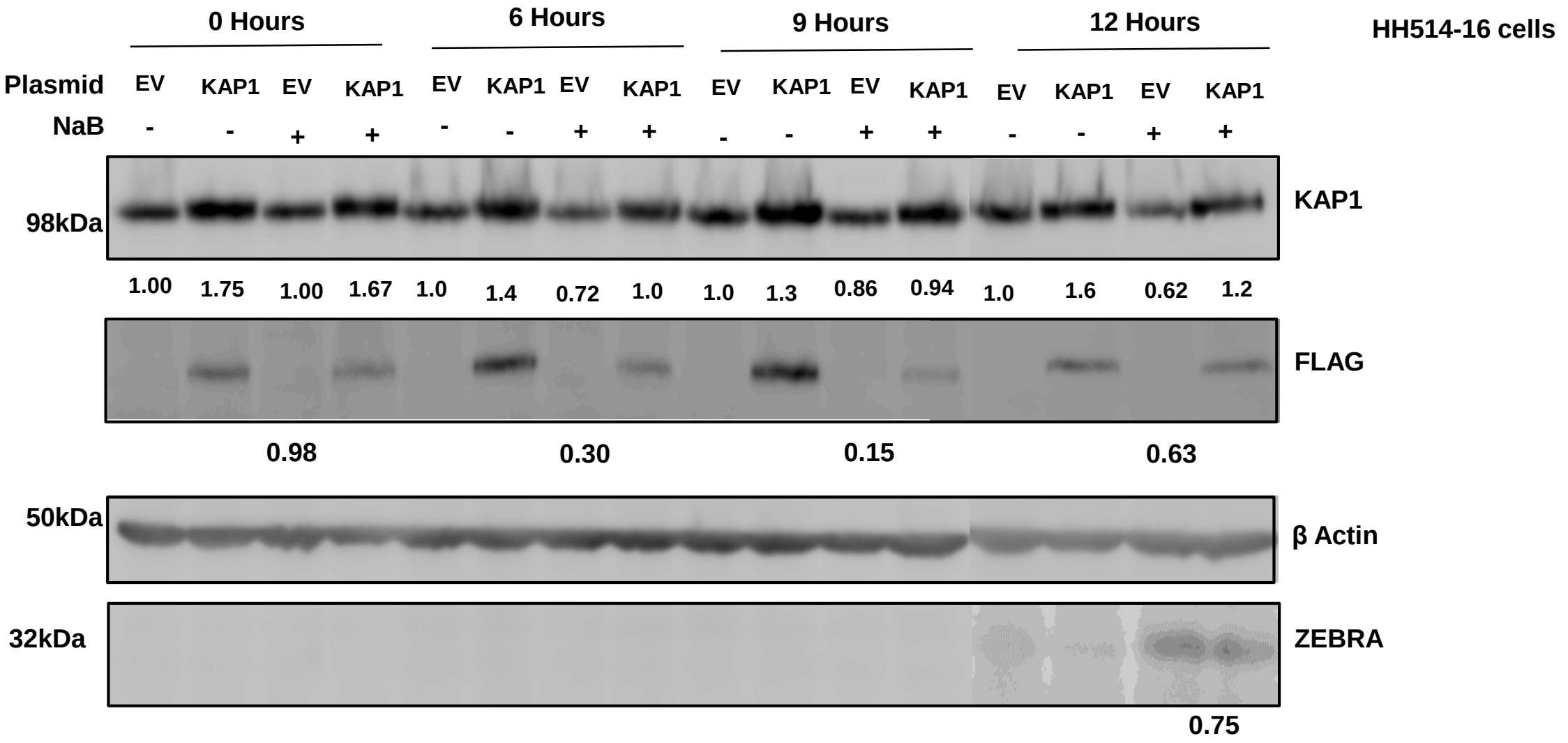
HH514-16 Cells

KAP1 transcript levels decrease along with protein levels during lytic activation

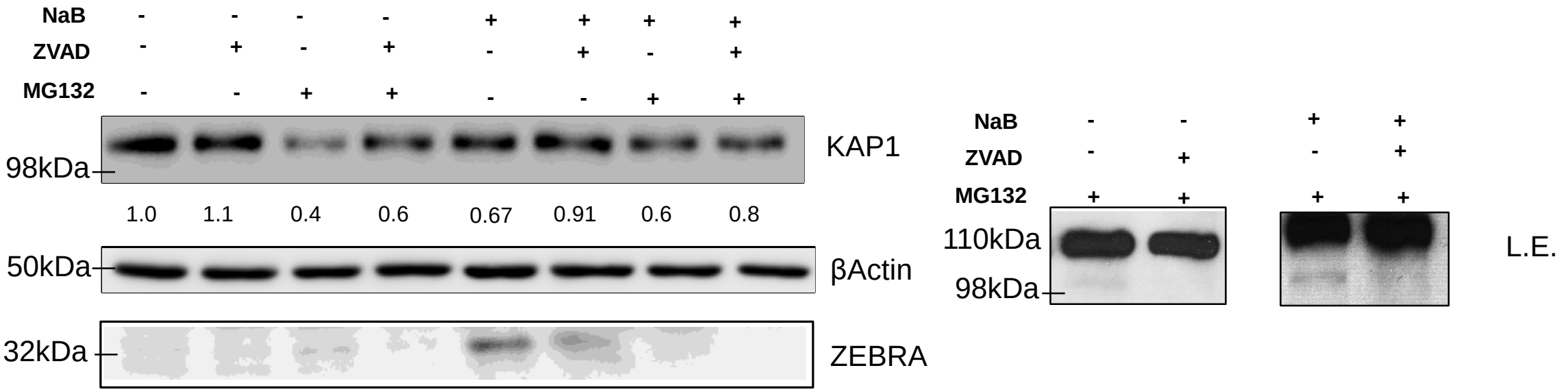


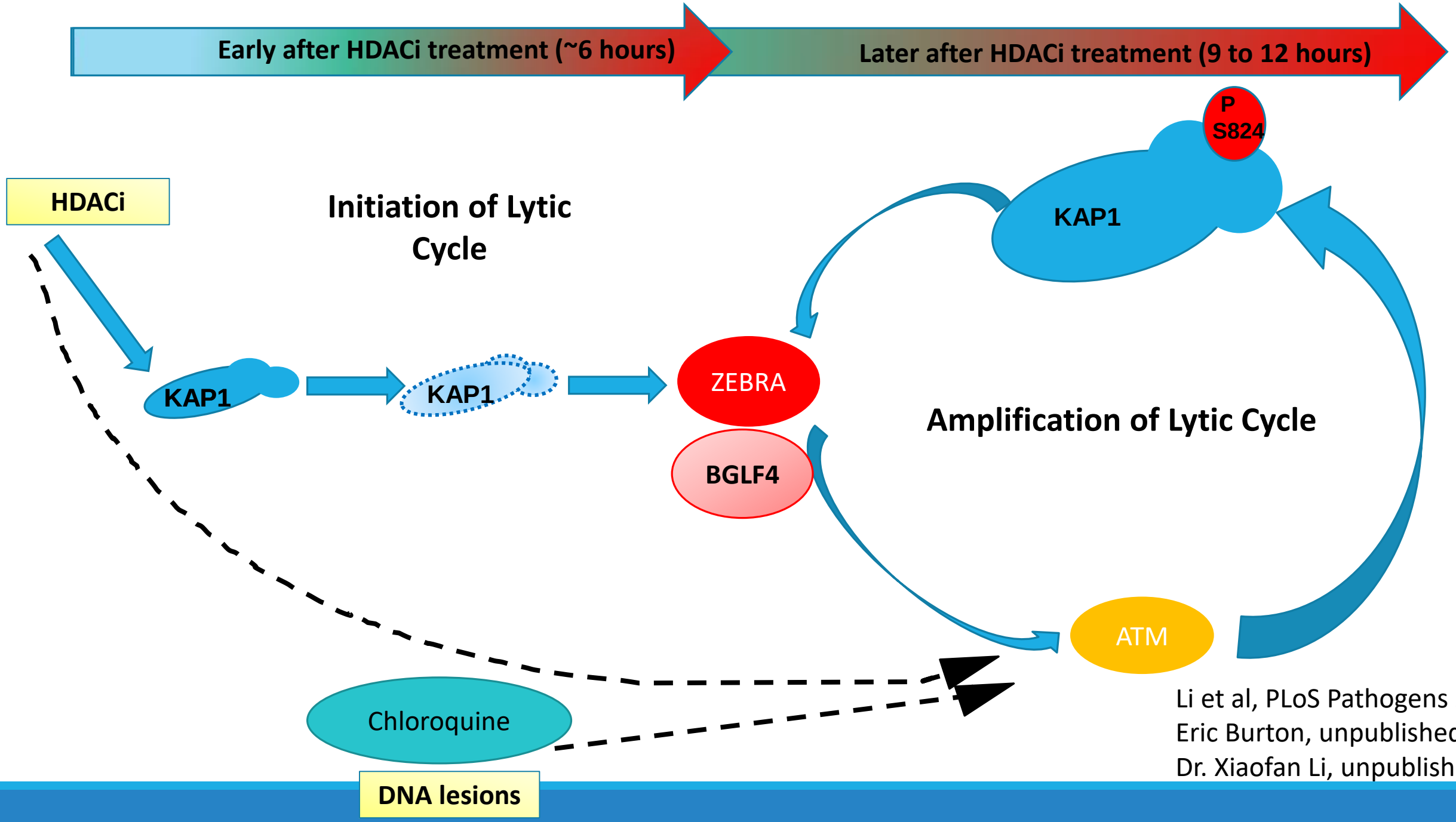
HH514-16 Cells

Overexpression of KAP1 rescues KAP1 after lytic stimulation



Caspases, but not the proteasome, are important in regulating KAP1 during lytic activation





Li et al, PLoS Pathogens 2017
Eric Burton, unpublished
Dr. Xiaofan Li, unpublished

Acknowledgments

Bhaduri-McIntosh Lab

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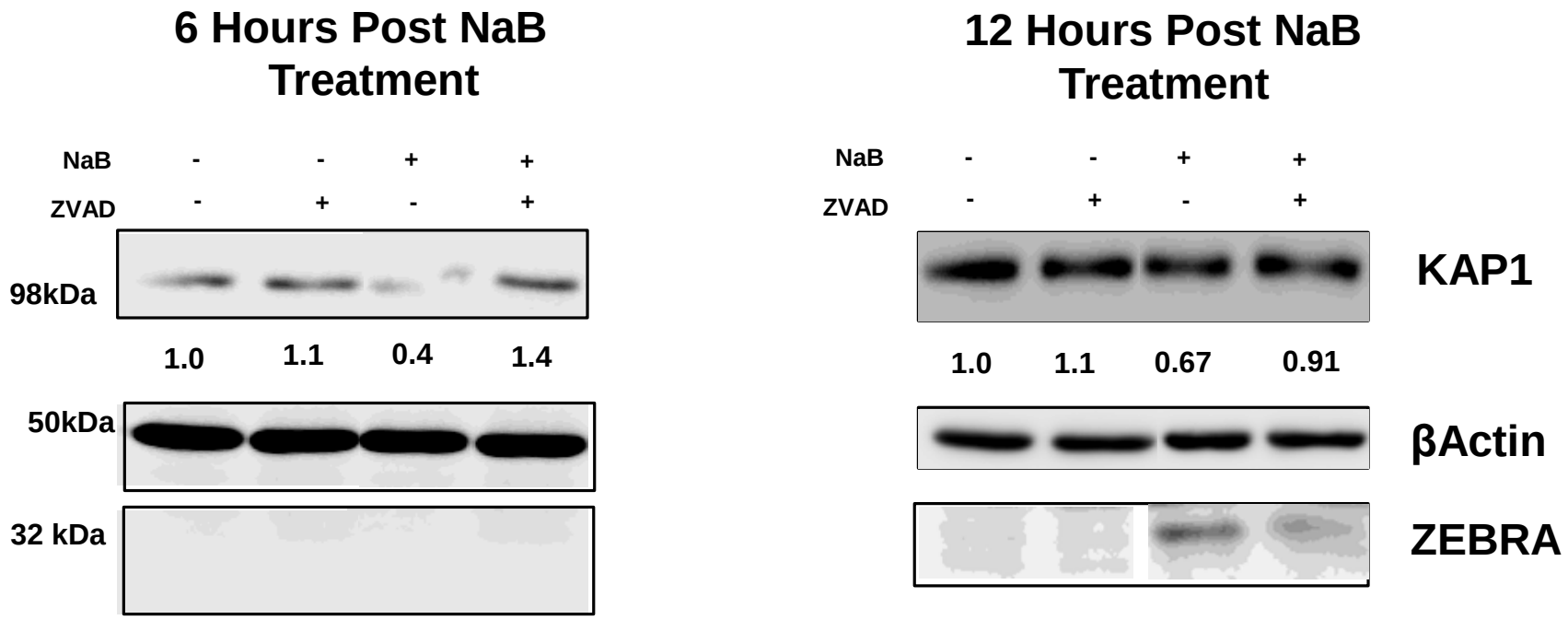
Molecular Genetics and Microbiology and Department

Stony Brook University

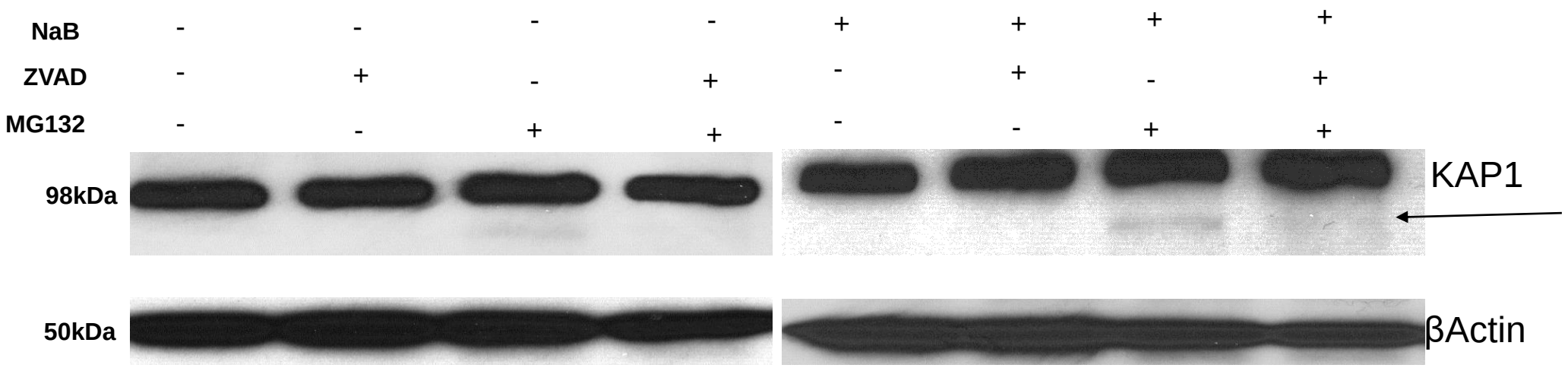
NIH MCBID

The Bhaduri-McIntosh Lab is moving to The University of Florida.
Looking for Post-doctoral researchers!

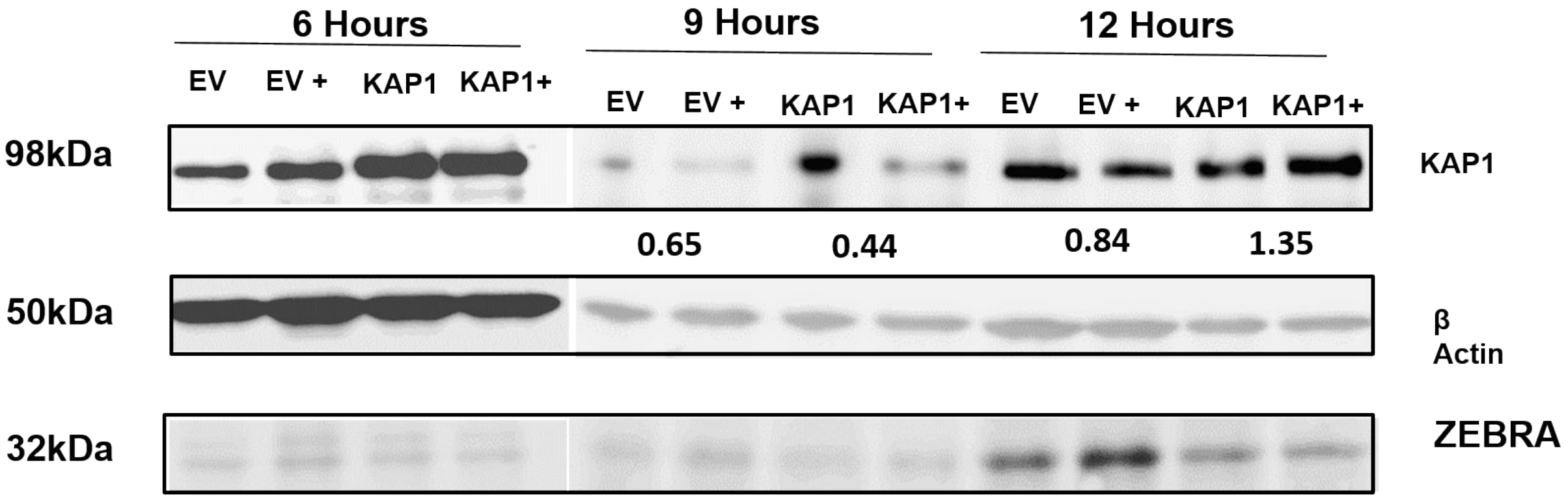
Pan-caspase inhibitor ZVAD-FMK is able to rescue KAP1 levels after lytic induction



KAP1 is cleaved early after NaB treatment via a Caspase-dependent mechanism

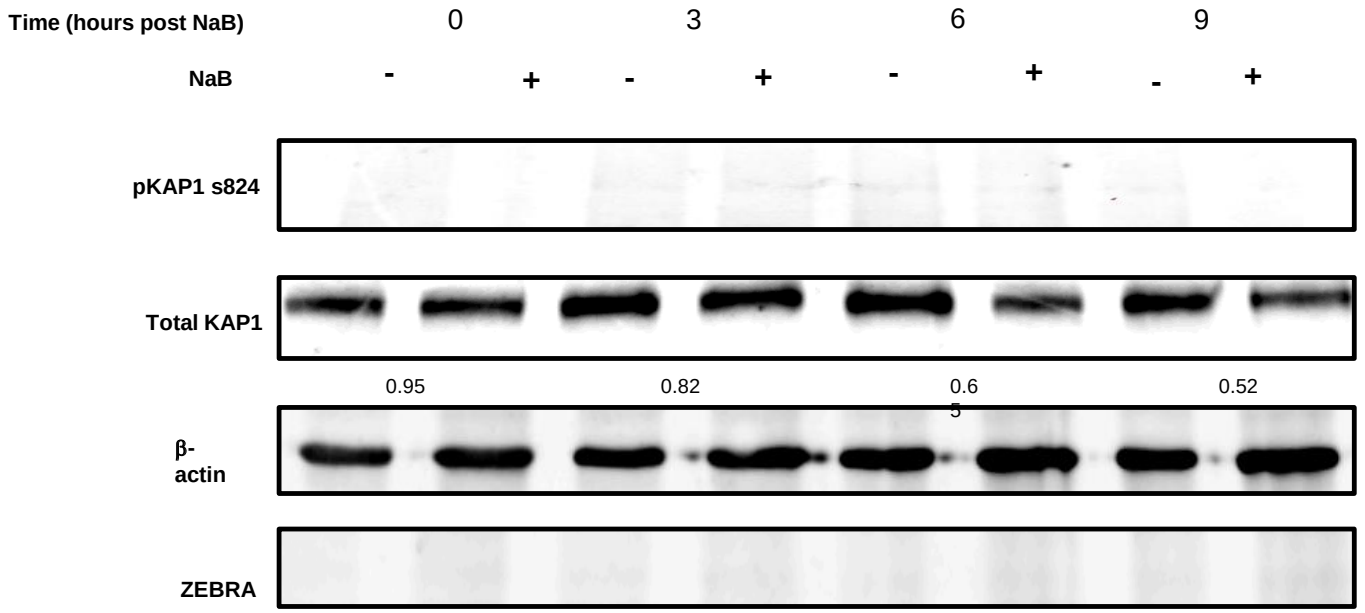


Overexpression of KAP1 cannot rescue KAP1 after lytic stimulation



BL Cells

Time course analysis of KAP1 protein and pKAP1 levels



Burton, Unpublished 2016

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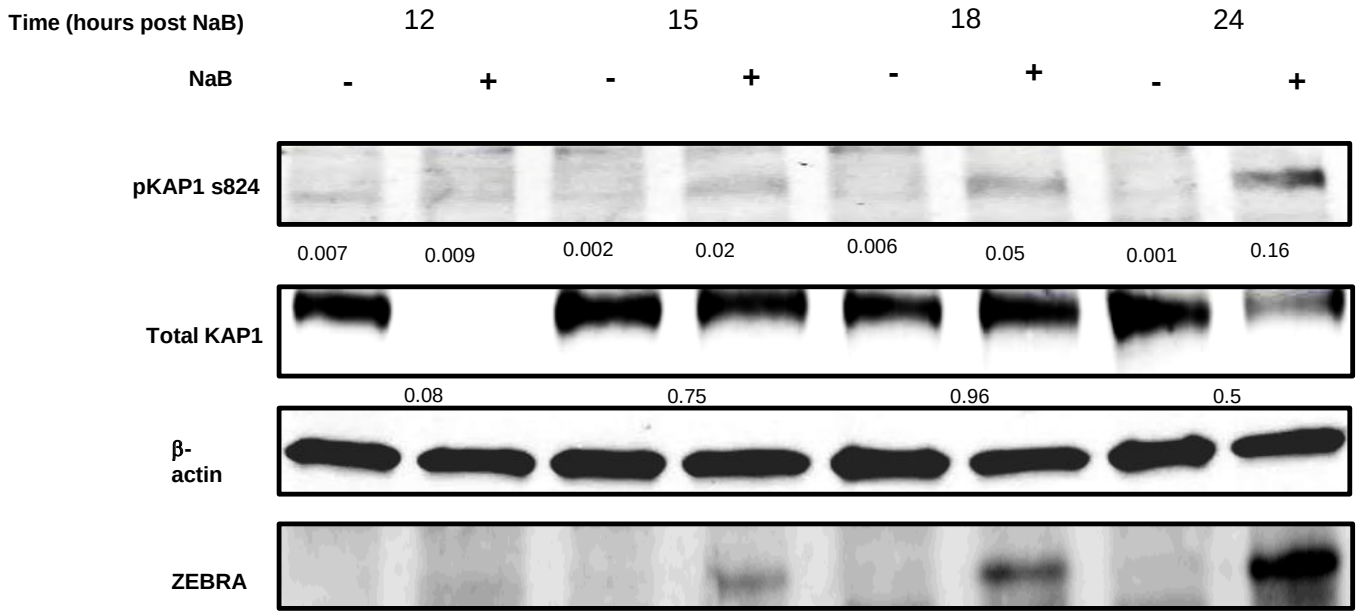
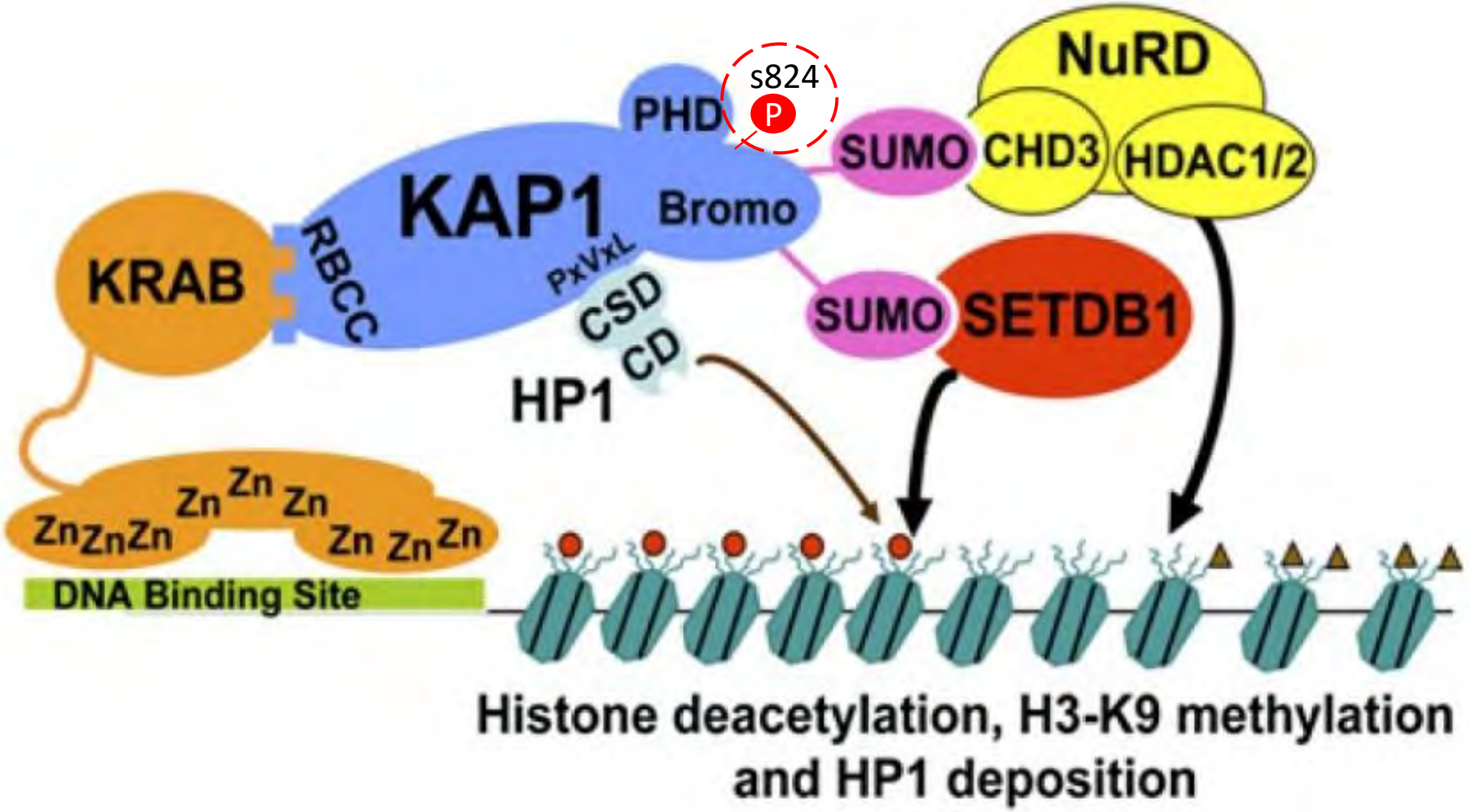
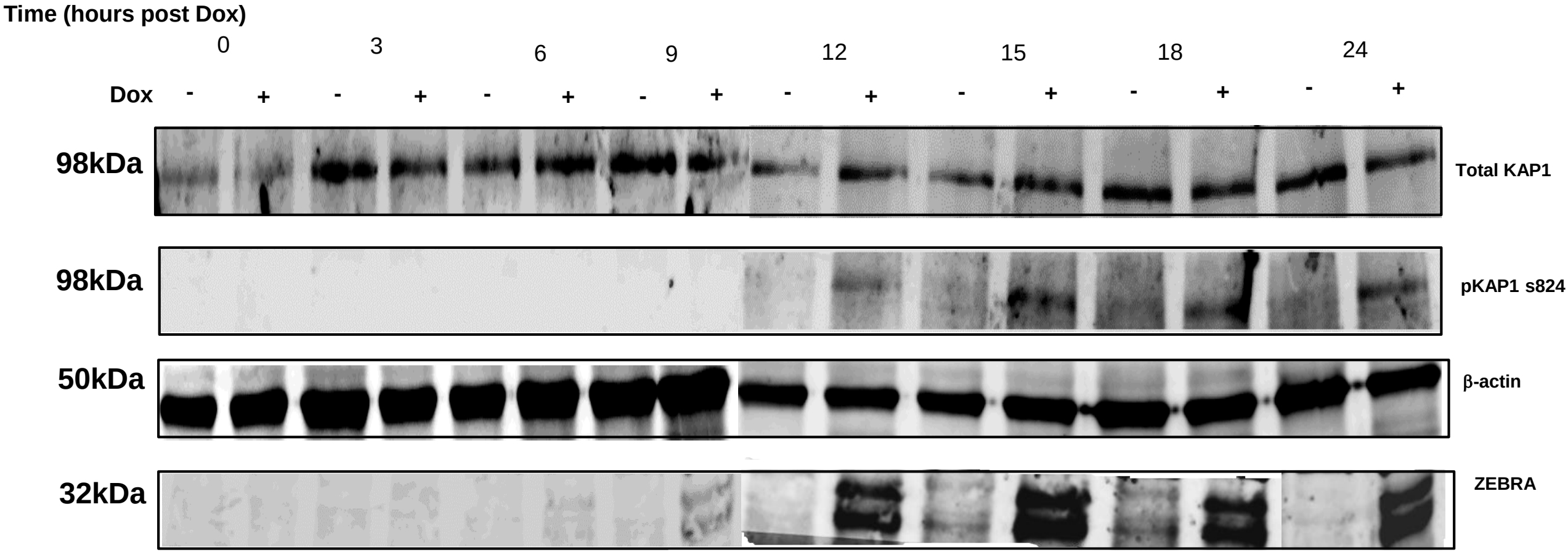


Analysis performed using Li-COR Image Studio Suite as a relative comparison between p KAP1/b-Actin ratios

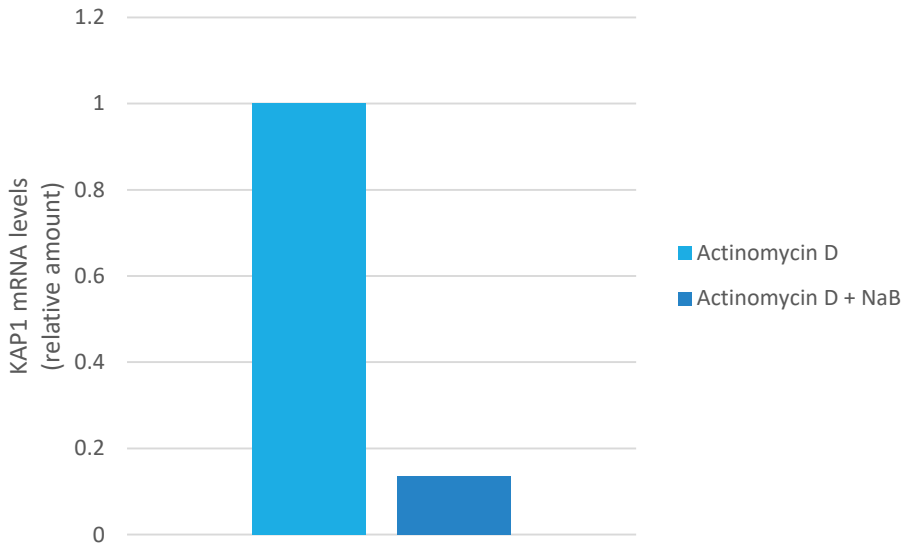
Burton, Unpublished 2016


NuRD
s824
P
PHD
SUMO
CHD3
HDAC1/2
KAP1
Bromo
KRAB
RBCC
PxVxL
CSD
SUMO
SETDB1
HP1
CD
Zn Zn Zn Zn Zn Zn Zn Zn Zn
DNA Binding Site
Histone deacetylation, H3-K9 methylation
and HP1 deposition

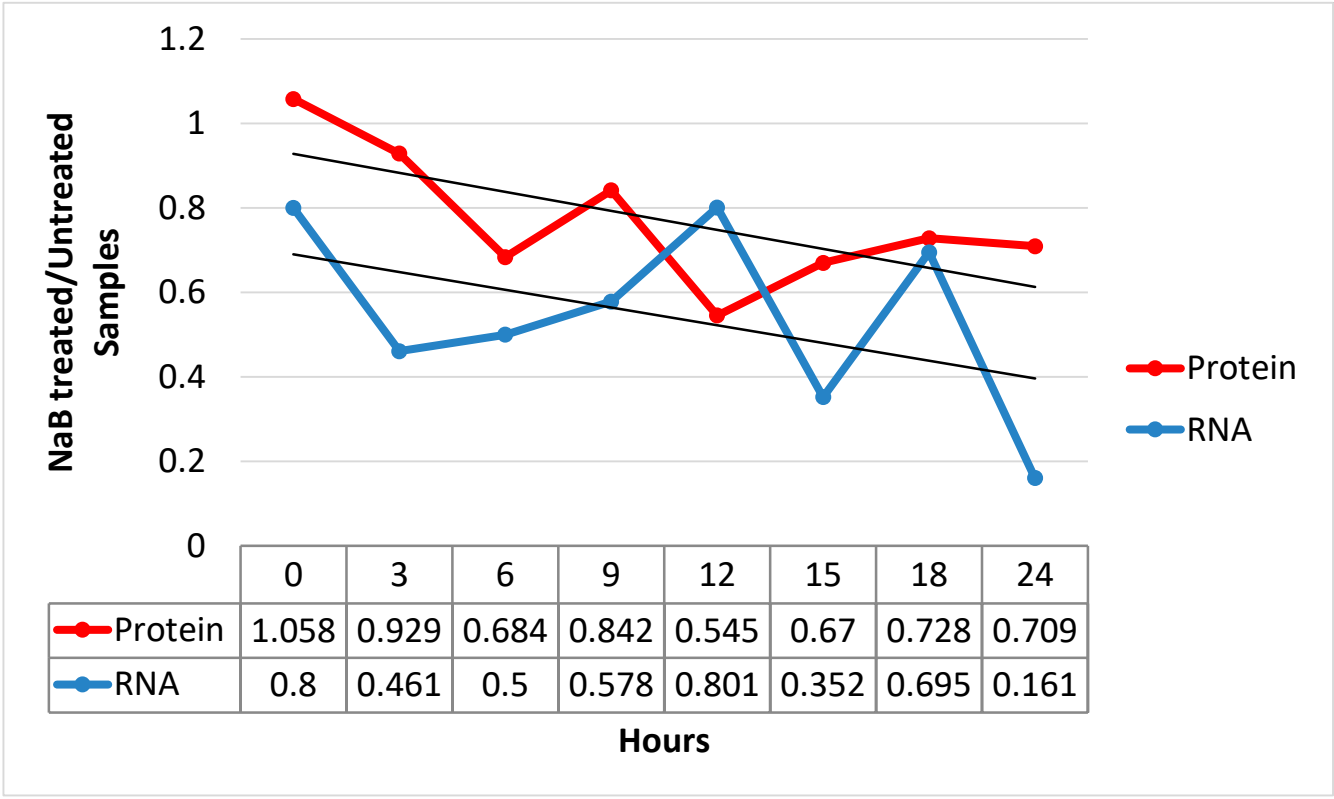
Reduced KAP1 levels are not observed when activating EBV lytic cycle via ZEBRA expression



KAP1 transcripts degrade more rapidly after lytic induction

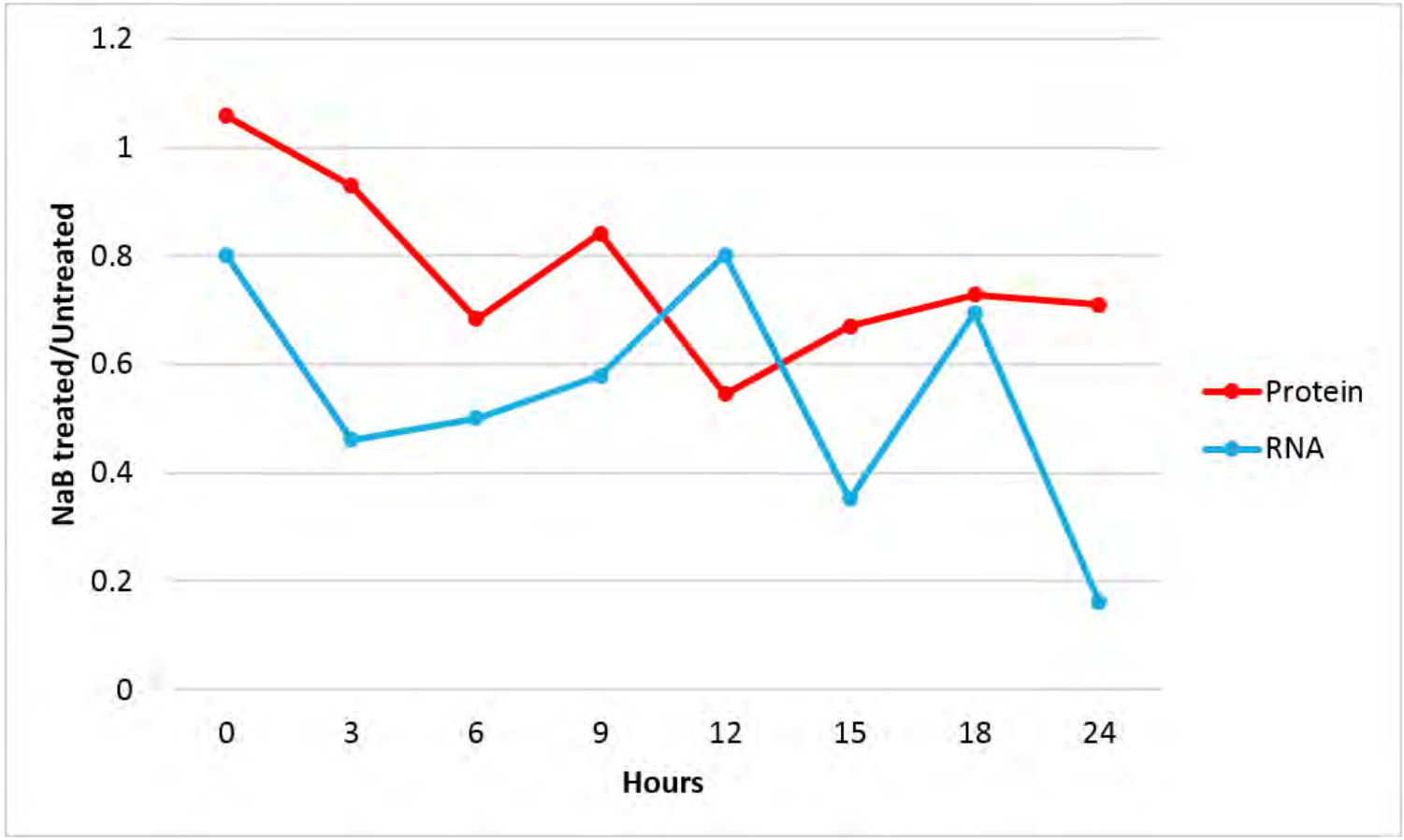


Timecourse analysis of KAP1 Protein and mRNA levels after NaB treatment



	0	3	6	9	12	15	18	24
Protein	1.058	0.929	0.684	0.842	0.545	0.67	0.728	0.709
RNA	0.8	0.461	0.5	0.578	0.801	0.352	0.695	0.161

KAP1 transcript levels decrease along with protein levels during lytic activation



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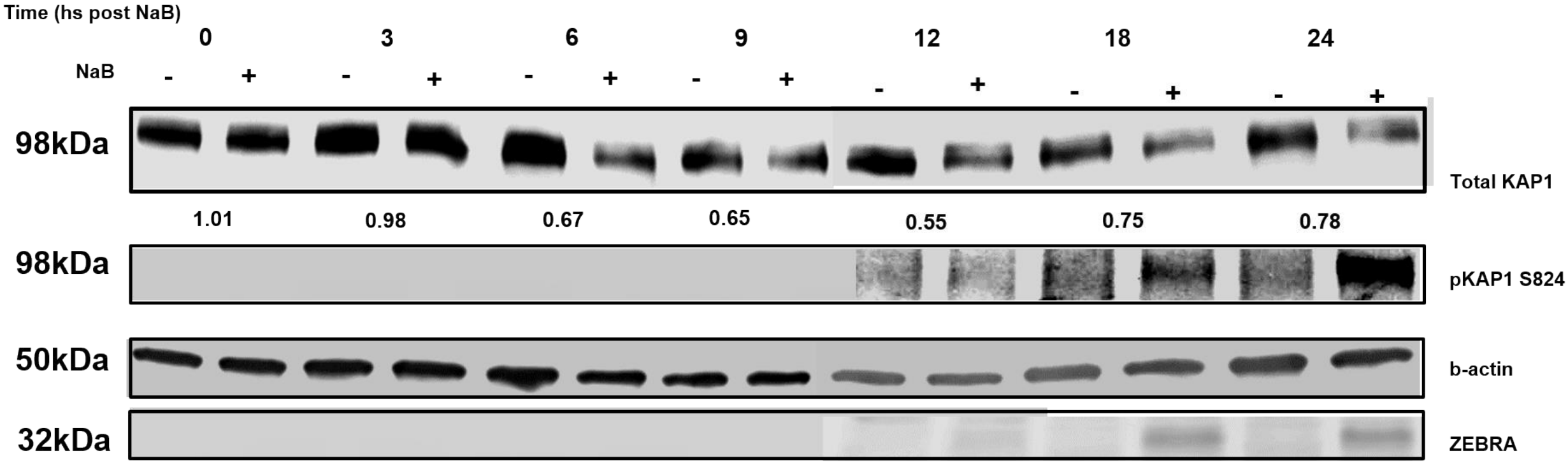

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