THE ROLE OF TIPIN-RPA INTERACTION IN THE DNA DAMAGE CHECKPOINT RESPONSE

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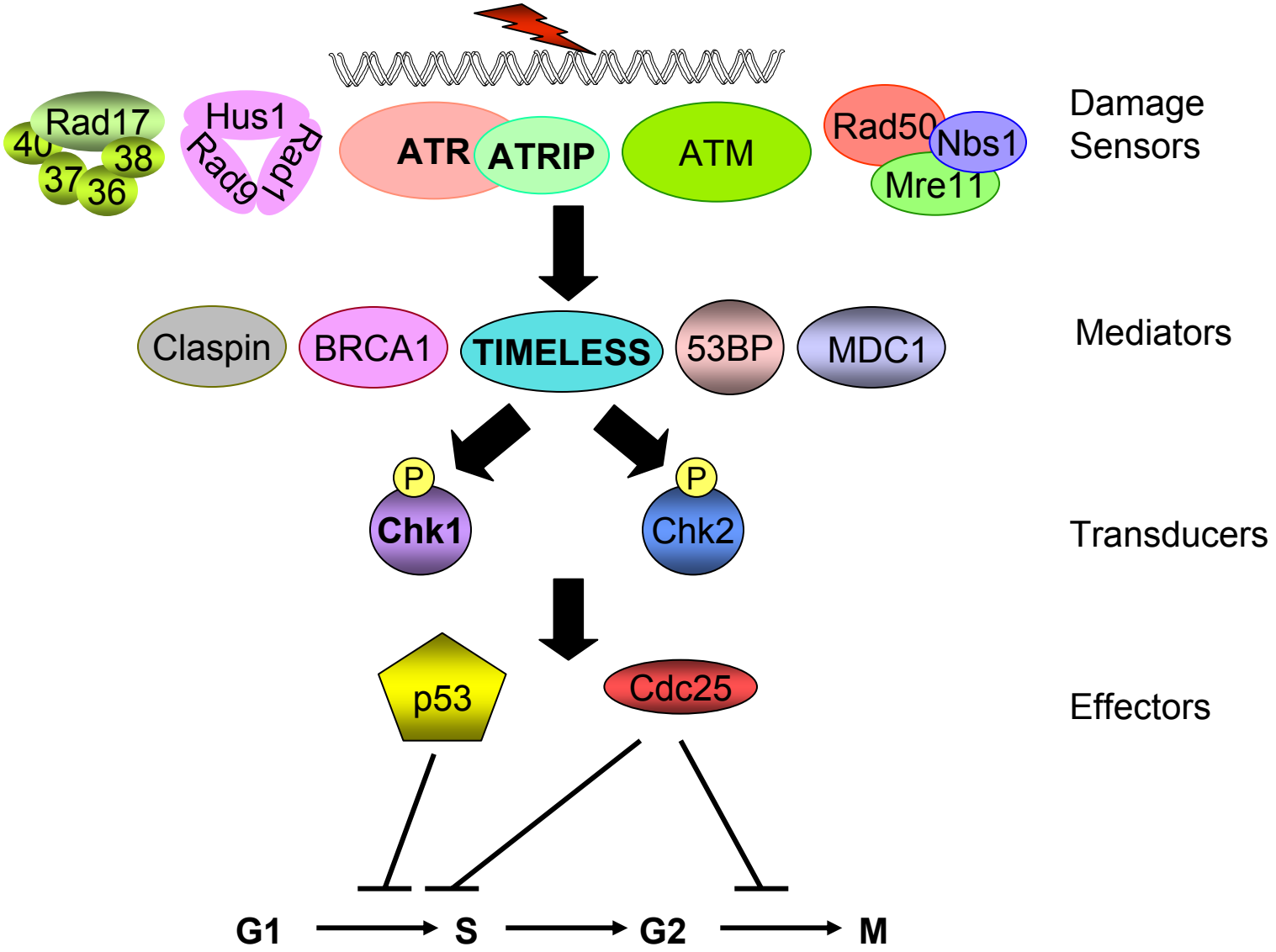
Signal Transduction Pathway Regulating the DNA Damage Checkpoint



Modified from Sancar, A., Lindsey-Boltz, L. A., Ünsal-Kaçmaz, K., and Stuart, L. (2004) *Annu. Rev. Biochem.* 73: 39-85

FPC: Fork Protection Complex

Swi1-Swi3 complex is required for survival of fork arrest and for activation of the replication checkpoint kinase Cds1.

Noguchi *et al.* (2004) *Mol Cell Biol*

Tof1-Csm3 complex interacts directly with MCM helicase during both replication fork progression and stalled DNA replication

Nedelcheva et al. (2005*) J Mol Biol*

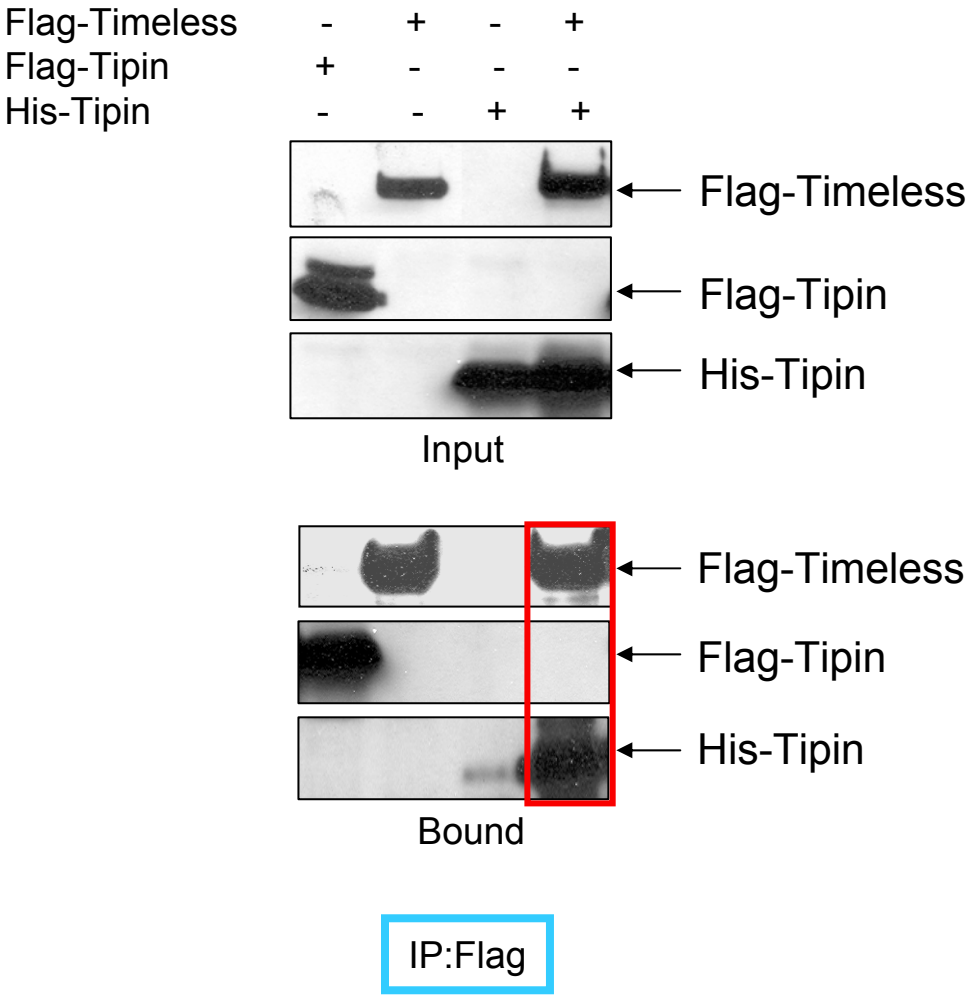
Mouse Tim-Tip transcripts are co-expressed in similar tissues during embryonic development and in adult brain.

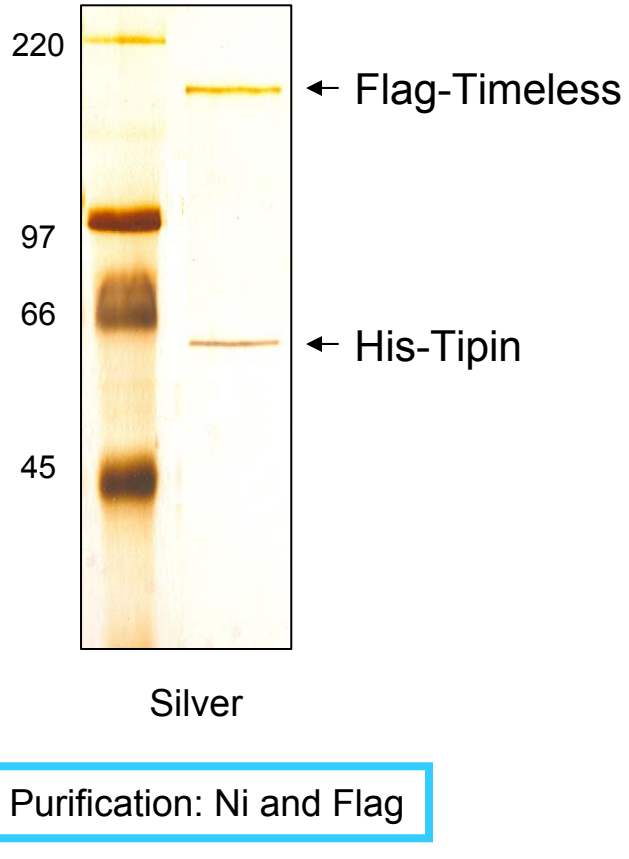
Gotter (2003) *J Mol Biol*

Timeless is required for activation of the replication checkpoint kinase Chk1

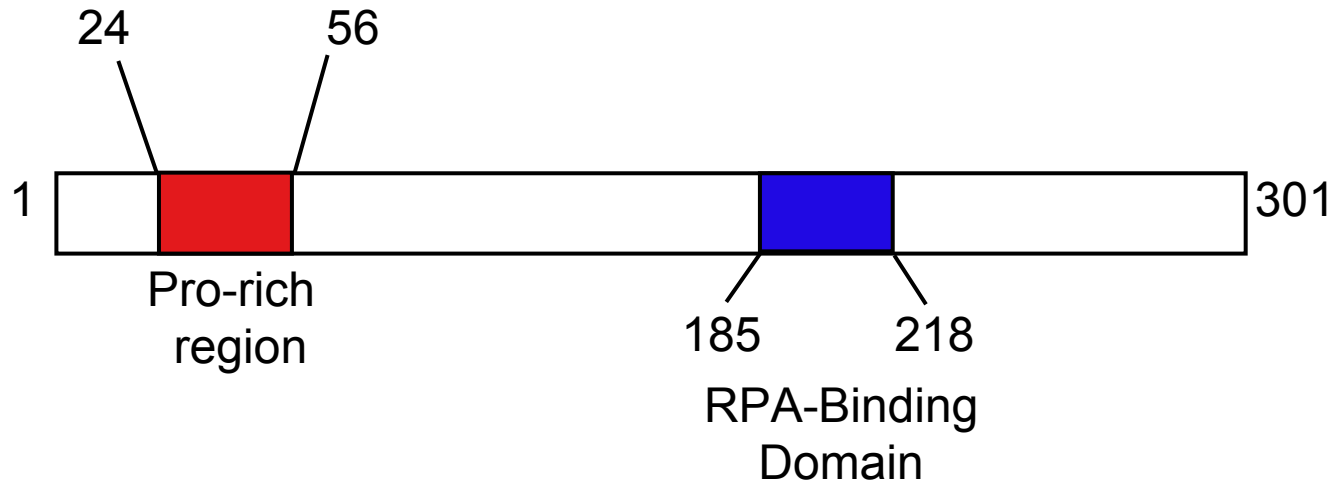
Unsal-Kacmaz et al. (2005) *Mol Cell Biol*

Timeless interacts with Tipin





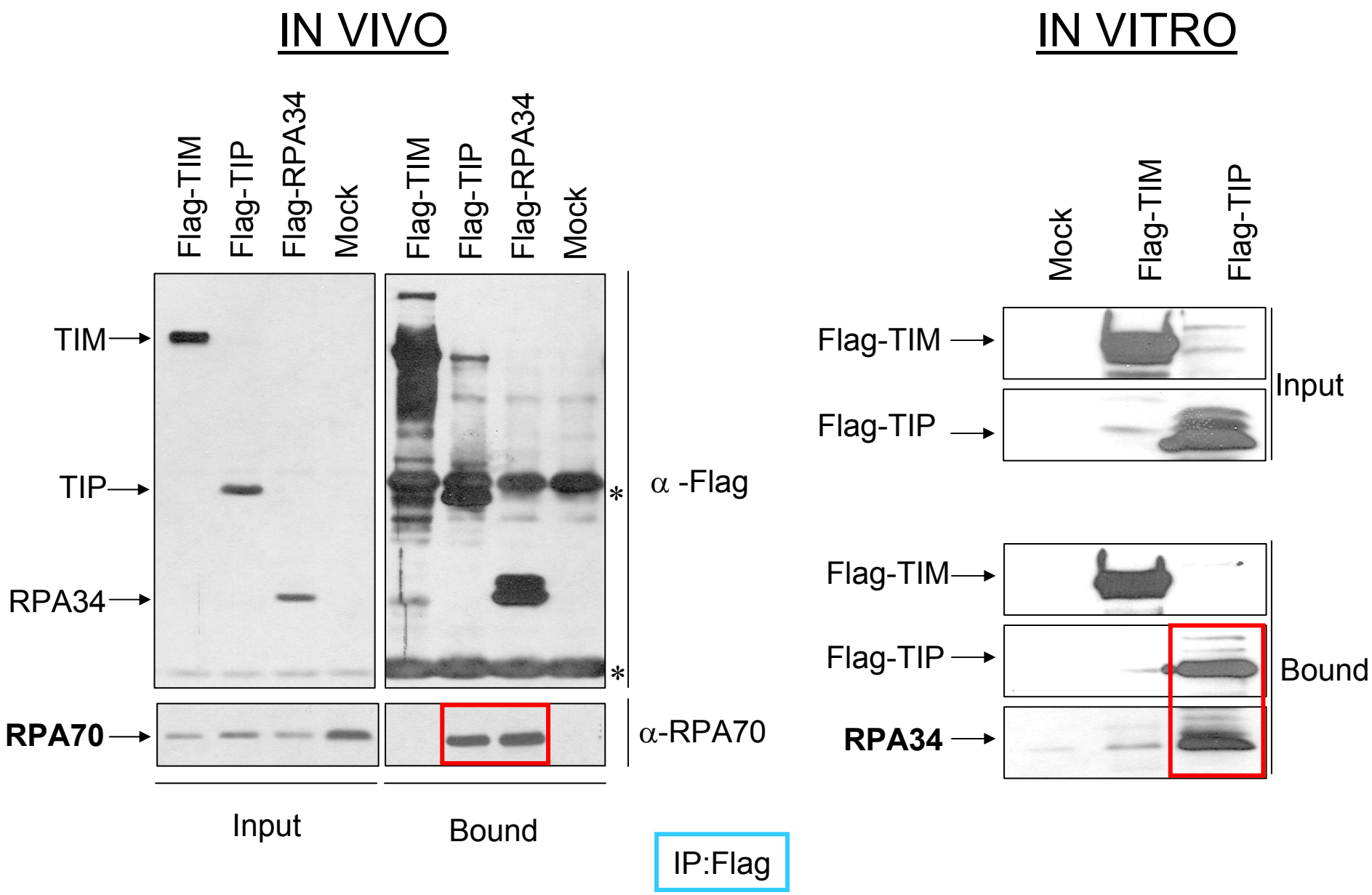
Tipin shows homology to RPA34 binding domain of XPA



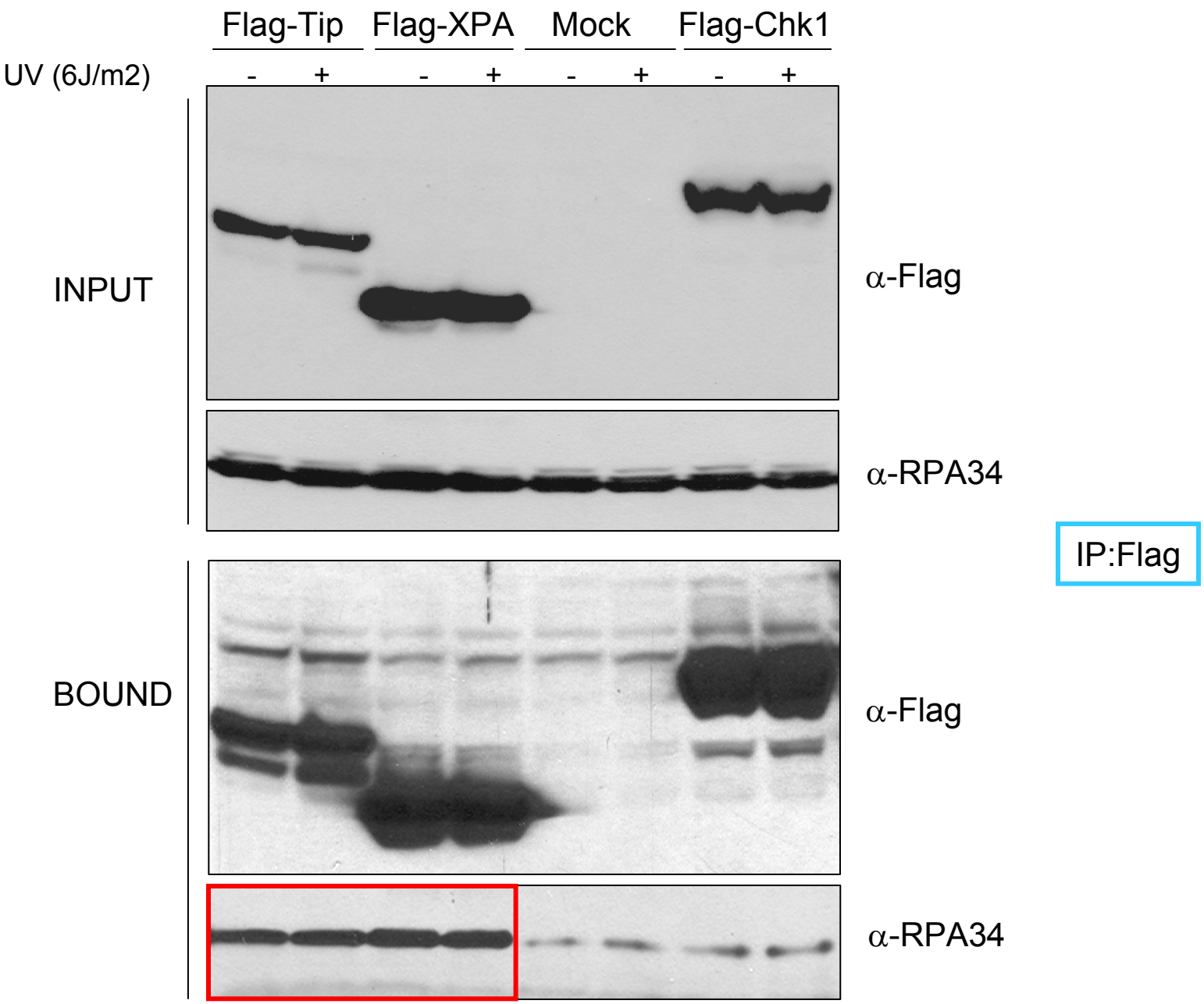
XPA 10
EAA**A**L**E**QPAE**L**PASVRAS**IERKKQRALMLRQARL**

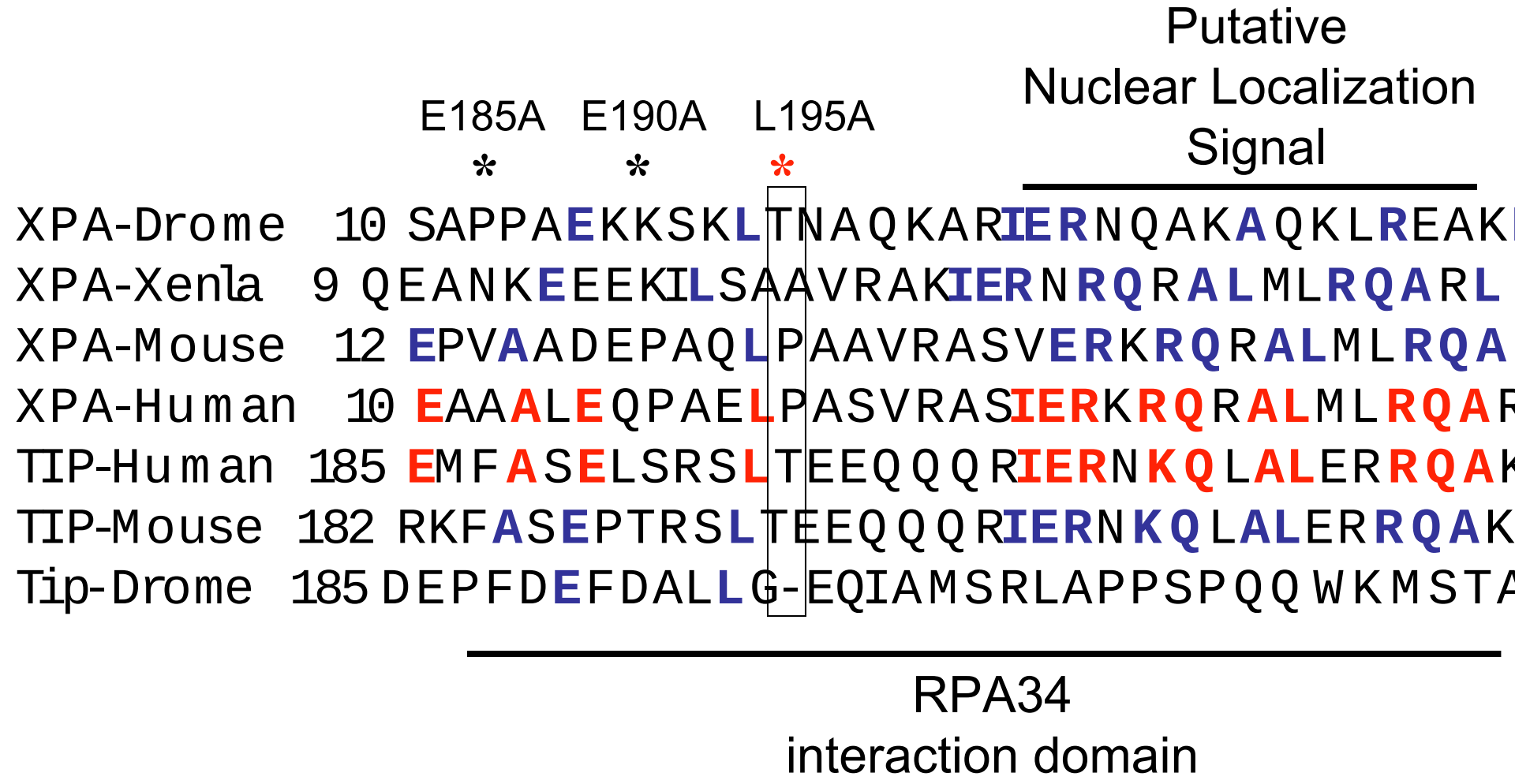
TIP 185
EMF**A**S**E**LSRS**L**TEEQQRR**IERNKQLALERRQAKL**

Tipin interacts with RPA

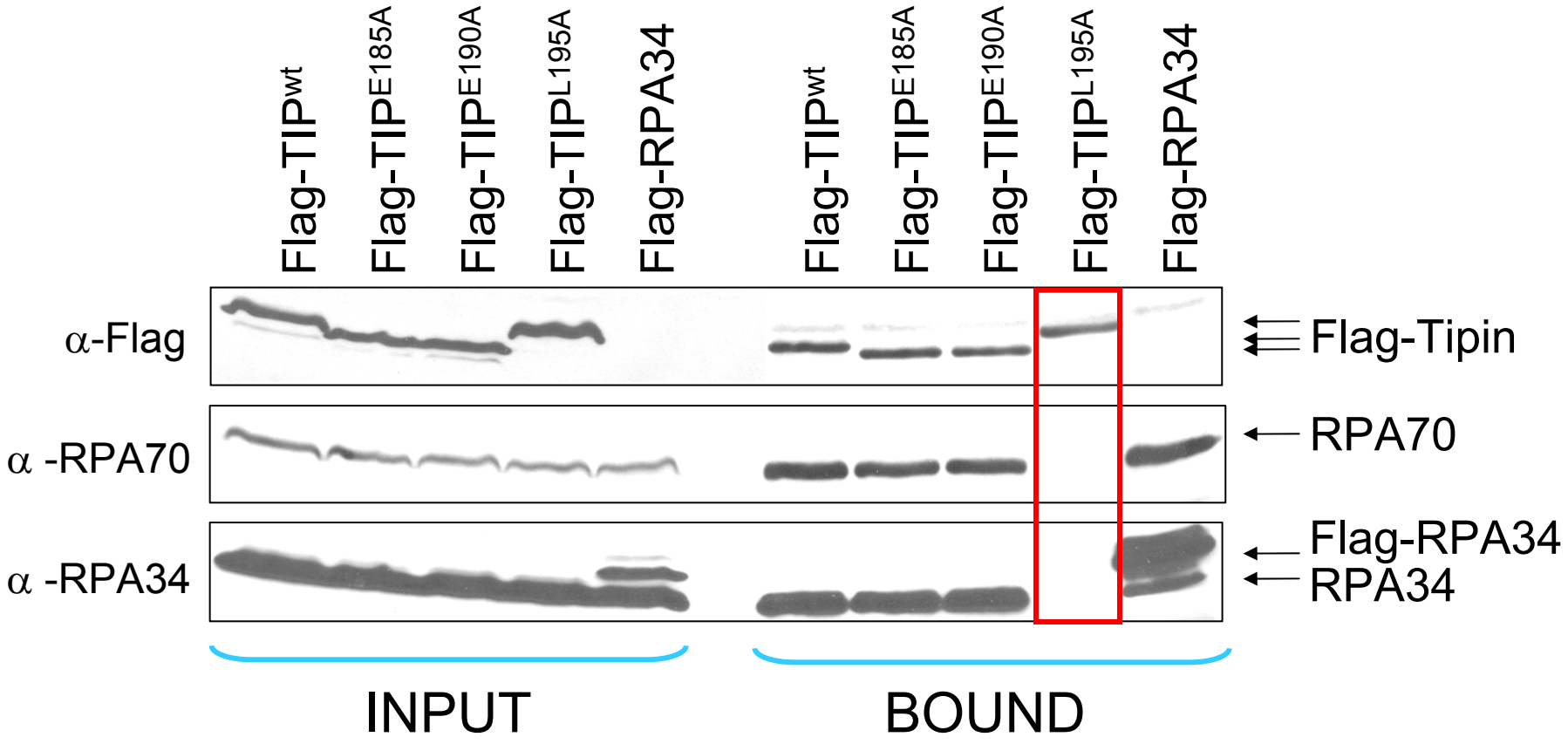


XPA-RPA and Tipin-RPA interactions are not damage induced

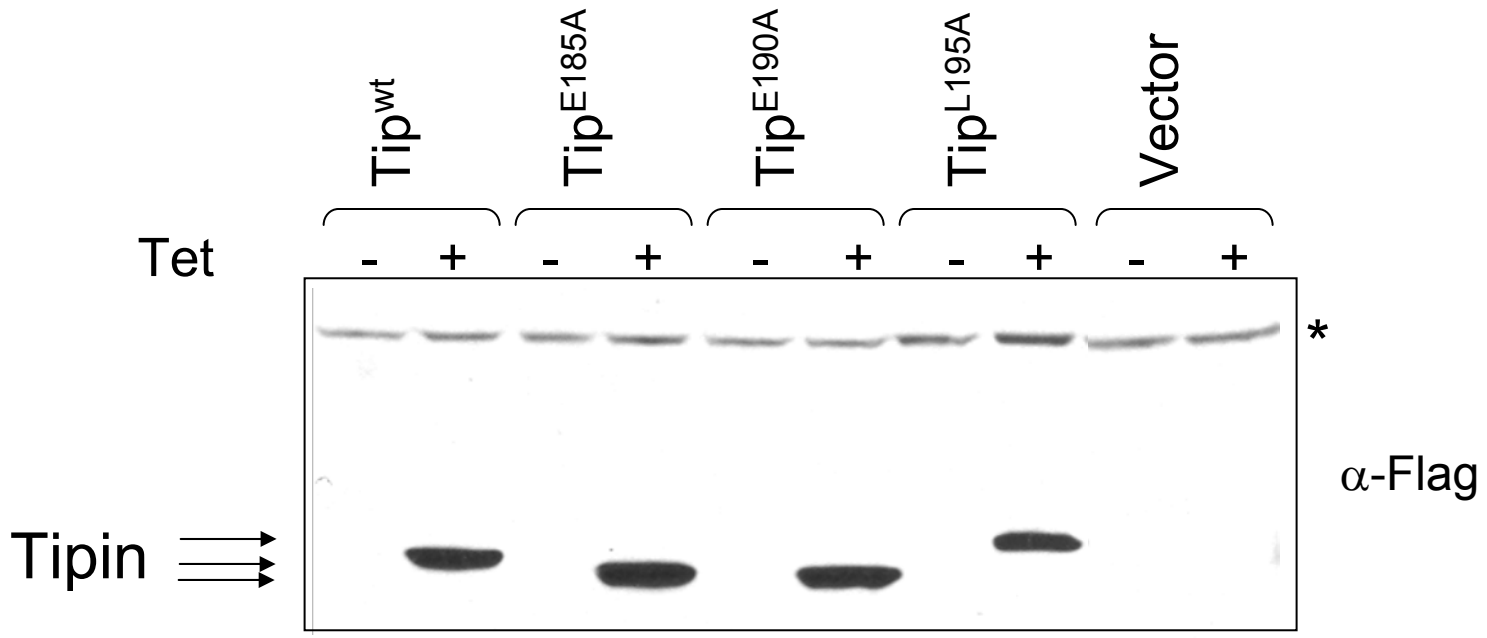



Putative
Nuclear Localization
Signal
E185A
E190A
L195A
XPA-Drome 10 SAPPAEKKSKLTNAQKARIERNQAKAQKLREAK
XPA-Xenla 9 QEANKEEEKILSAAVRAKIERNRQRALMLRQARL
XPA-Mouse 12 EPVAADEPAQLPAAVRASVERKRQRALMLRQA
XPA-Human 10 EAAALEQPAELPASVRASIERKRQRALMLRQAR
TIP-Human 185 EMFASELSRSLTEEQQQRIERNKQLALERRQAK
TIP-Mouse 182 RKFASEPTRSLTEEQQQRIERNKQLALERRQAK
Tip-Drome 185 DEPFDEFDALLG-EQIAMSRLAPPSPQQWKMSTA
RPA34
interaction domain

Tipin^{L195A} mutant is defective in binding to RPA *in vivo*

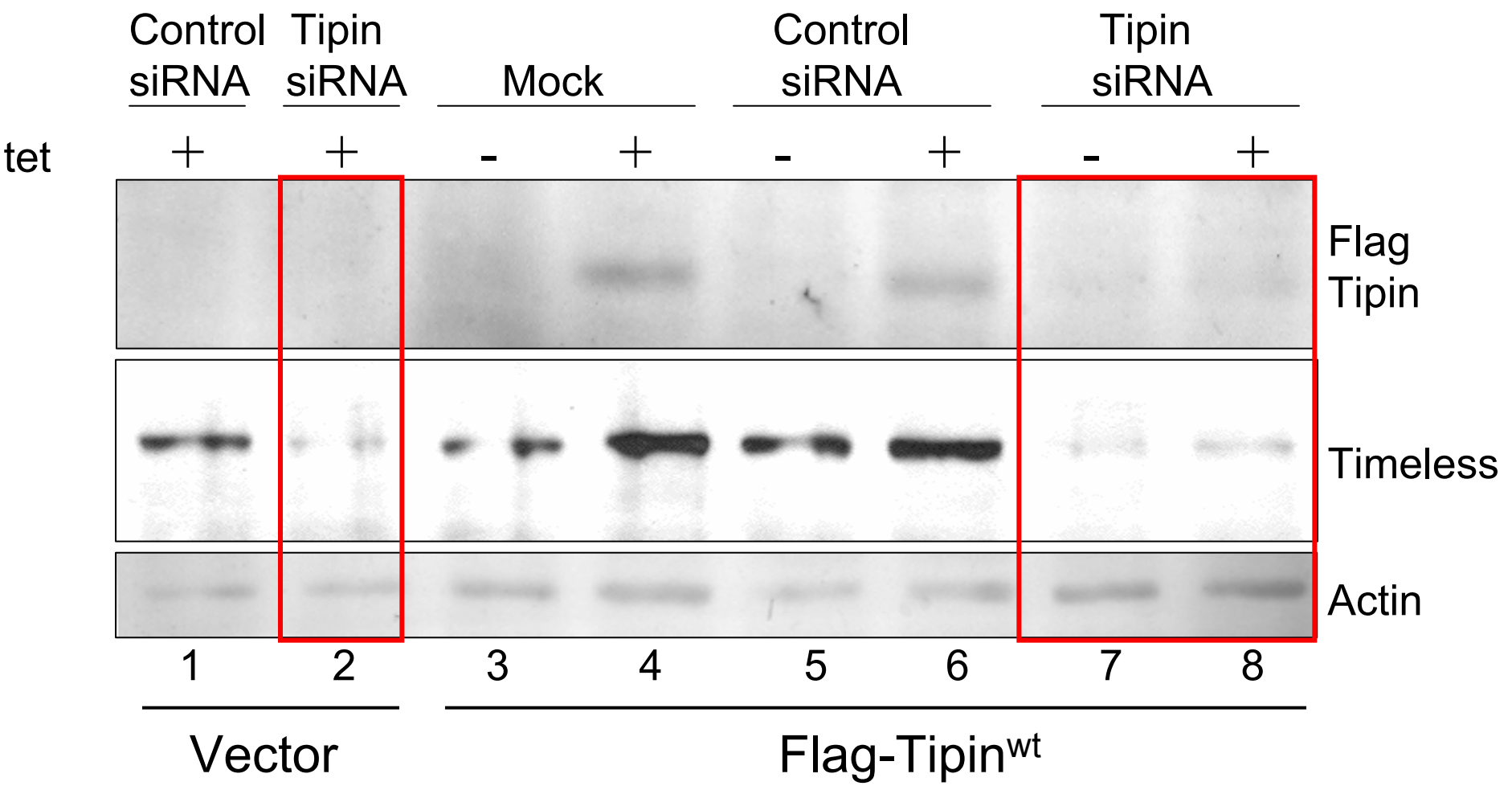


Generation of stable cell lines that can inducibly express Tipin

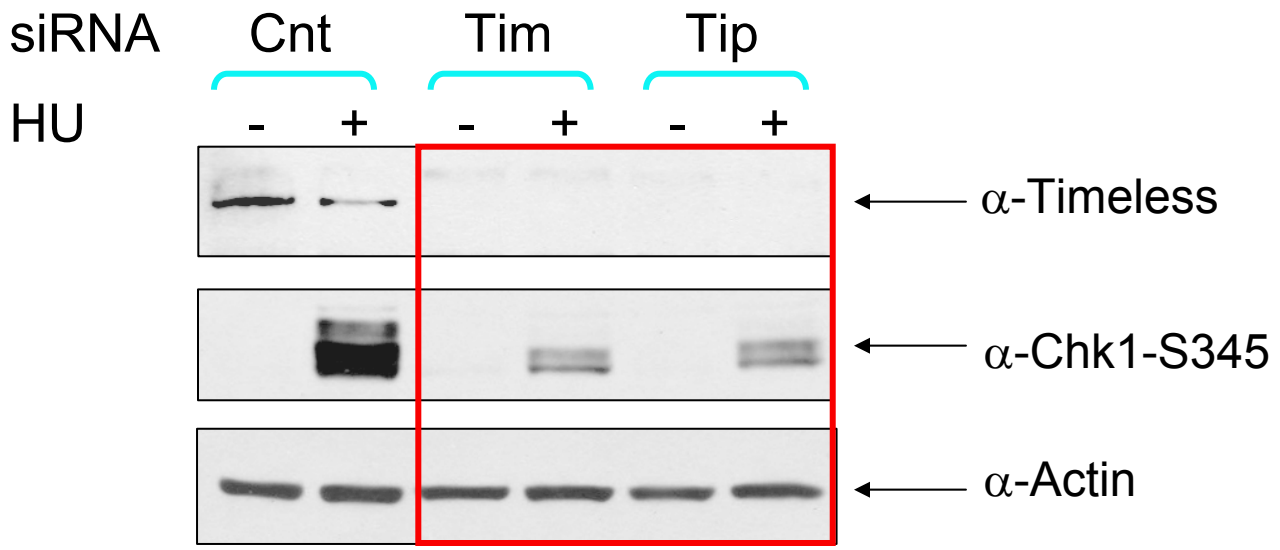


* Cross-reacting non-specific band

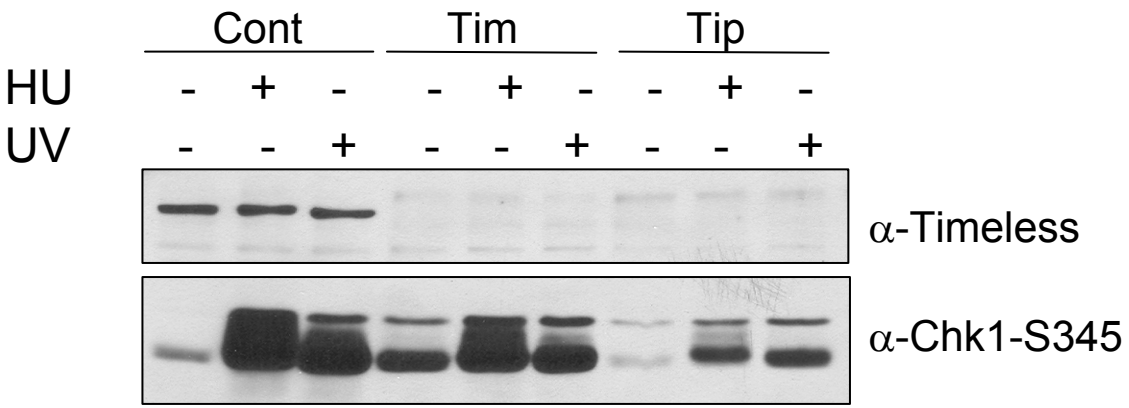
Tipin is required for Timeless stabilization

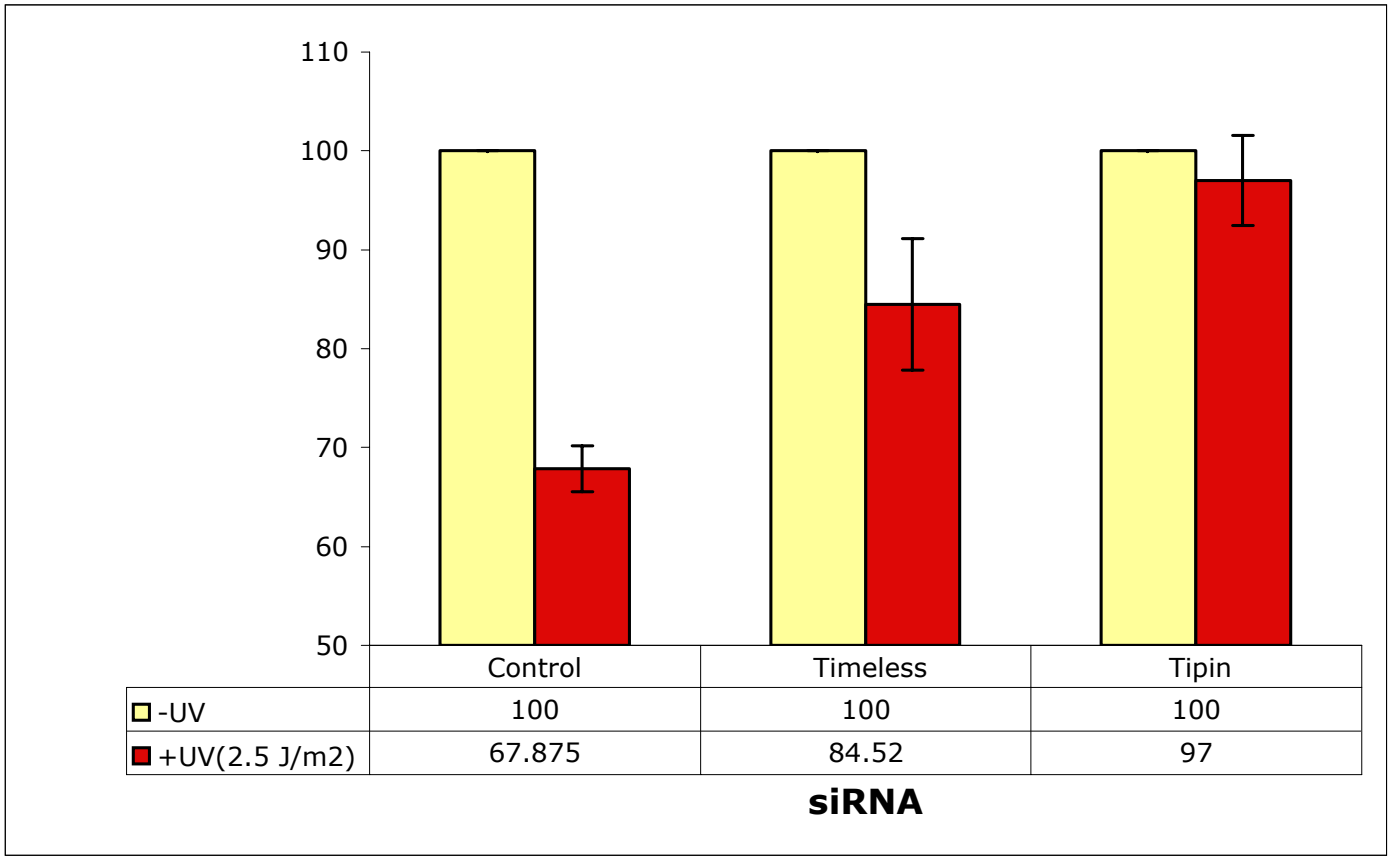


Tipin is required for Chk1 phosphorylation



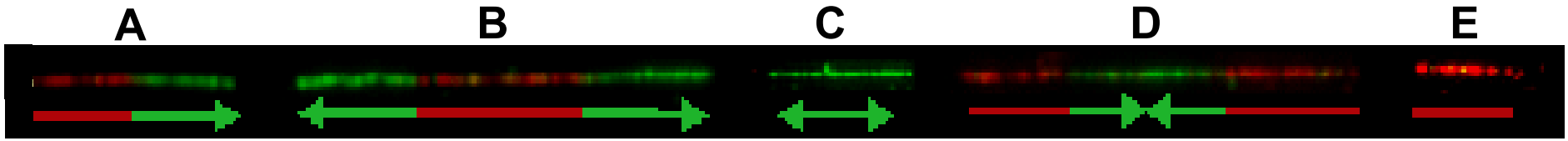
Radio-Resistant DNA Synthesis (RDS)

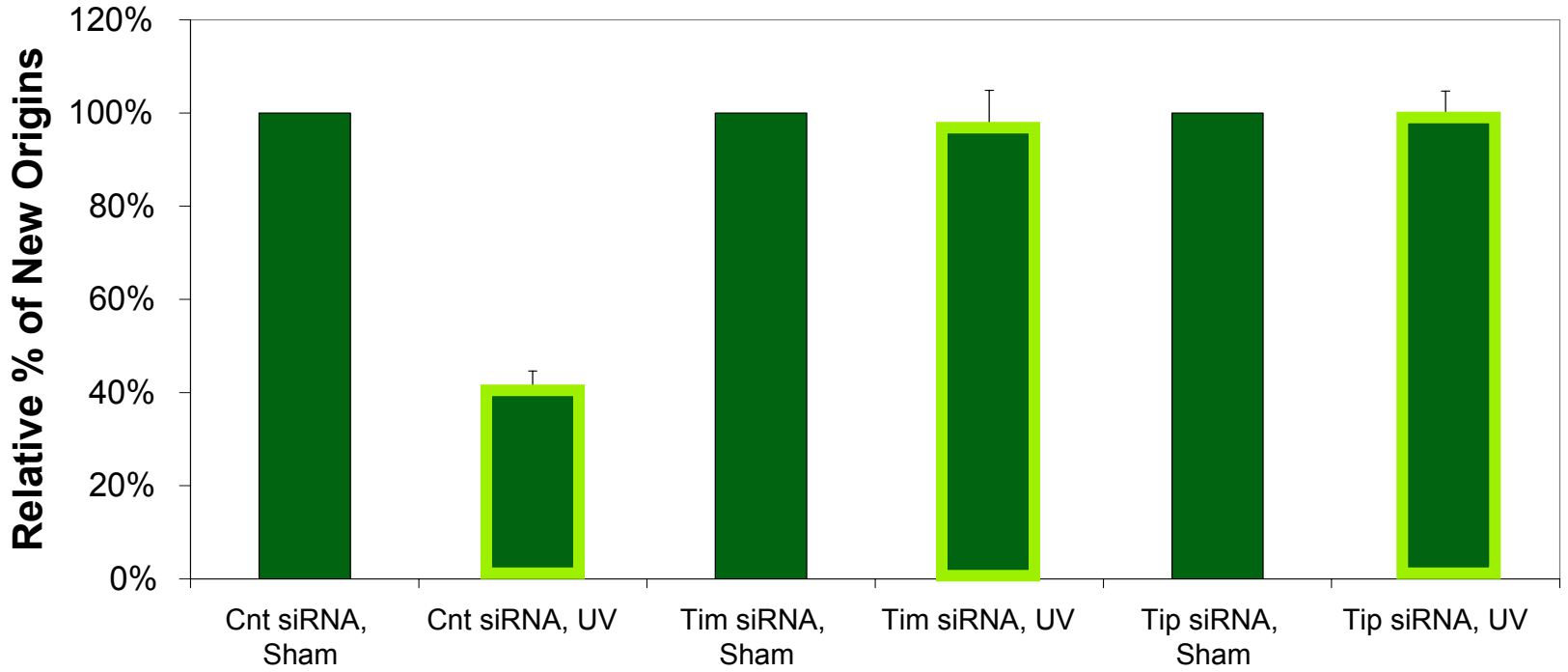




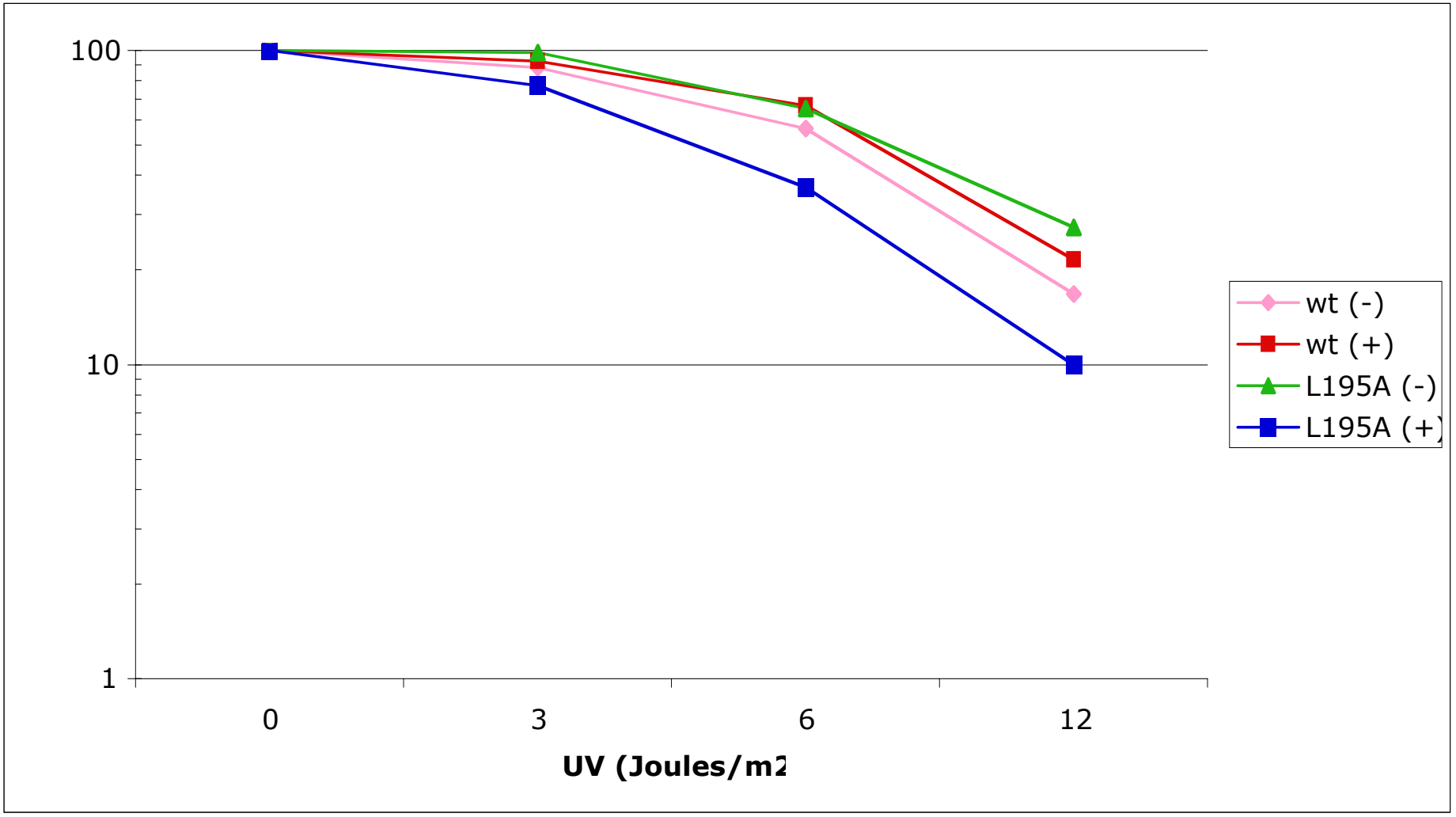
DNA Combing Analysis

Examples of an ongoing replication fork (A), new initiation during the IdU pulse (B), **new initiation during the CldU pulse (C),** termination during the CldU pulse (D), termination or fork stalling during the IdU pulse (E).

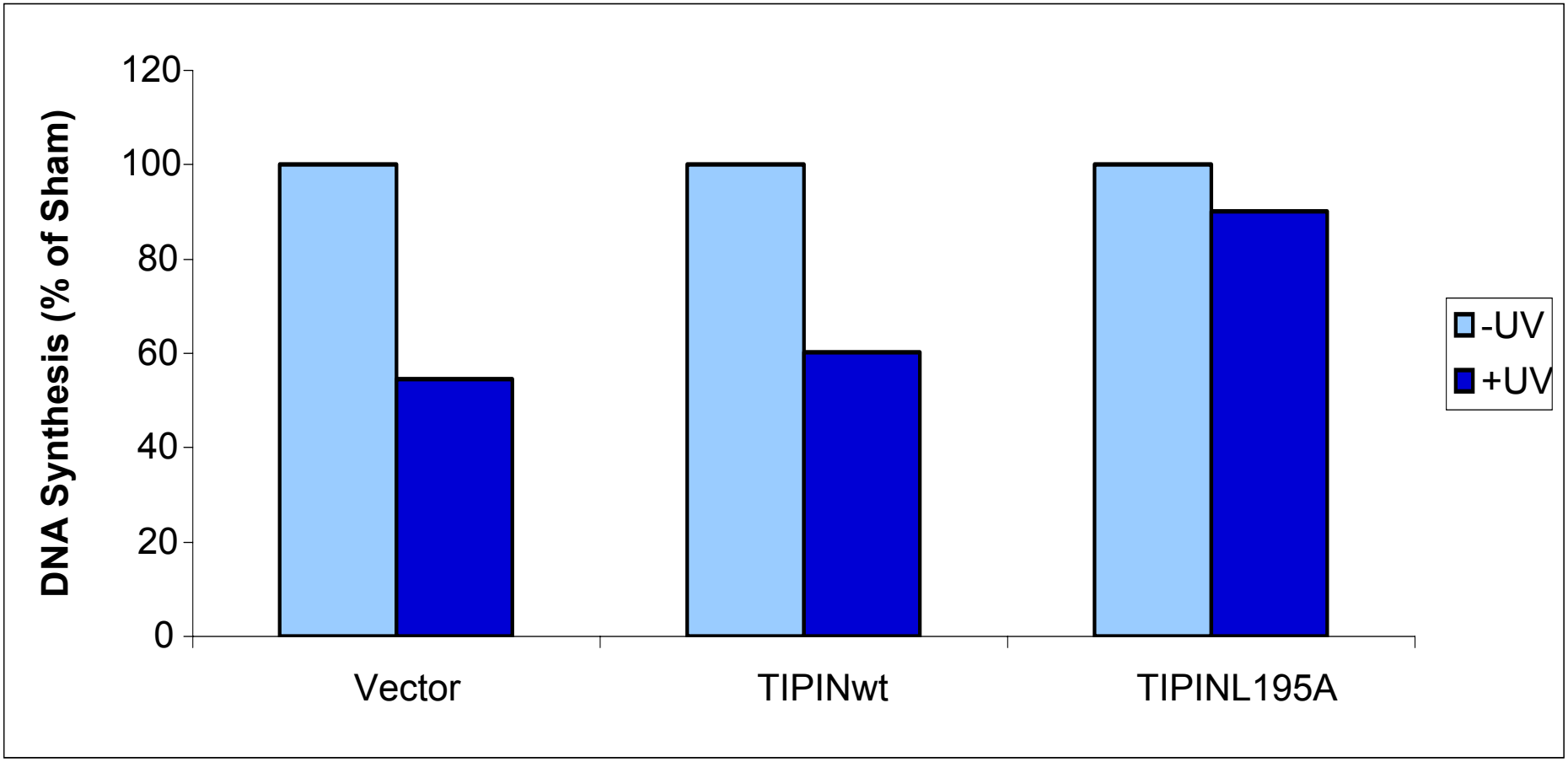




TipinL195A overexpression sensitizes cells to UV



RDS with cells overexpressing wt and mutant Tipin



Summary

- Human Timeless forms a complex with Tipin
  - Tipin is required for the stability of Timeless

- Tipin is a critical component of checkpoint machinery
  - Tipin is required for UV and HU-induced Chk1 phosphorylation
  - Tipin is required for intra-S checkpoint activation
    - suppression of replication inititation

- Tipin interacts with RPA
  - Tipin RPA interaction is mediated at L195 of Tipin
  - Tipin L195A acts as a dominant negative mutant
    - sensitizes the cells to UV
    - overrides the intra-S checkpoint

Acknowledgments

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