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Determining the Role of Sam68 in T-antigen Cellular Expression

Sean Miller

Elizabethtown College, millers3@etown.edu

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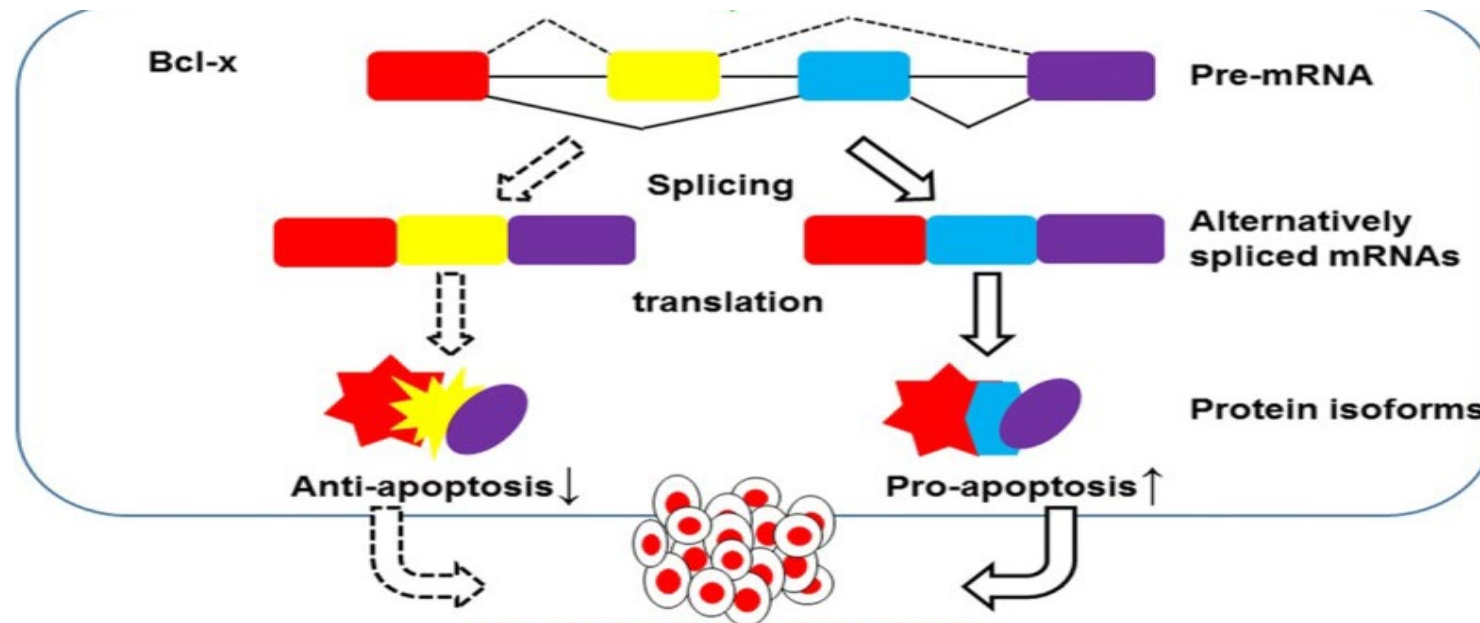
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Determining the Role of Sam68 in T-antigen Cellular Transformation

Sean Miller

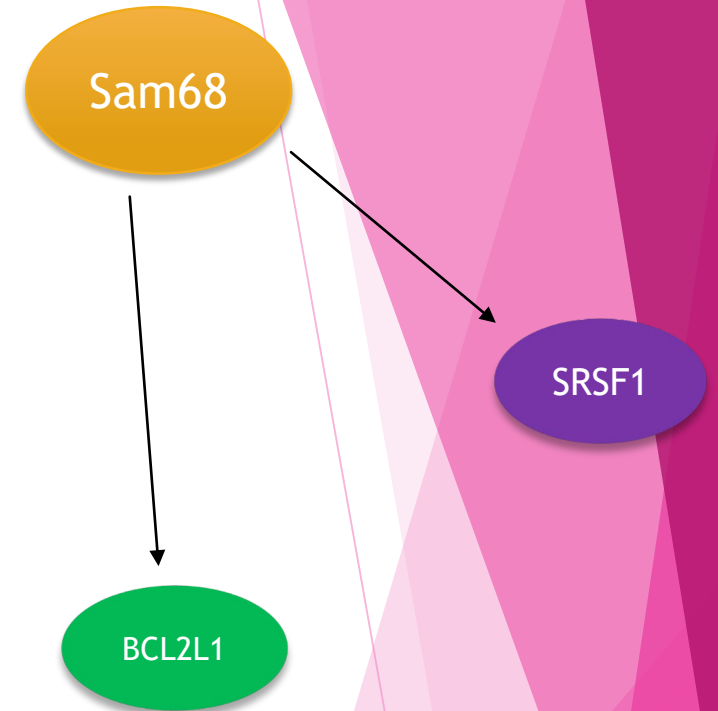
Alternative Splicing

- Process of creating multiple, unique gene products from one gene
- Splicing of pre-mRNA, inclusion and exclusion of different exons
- Formation of different protein isoforms
 - Isoforms carry out unique functions
 - Allows for protein diversity (~20k genes, yet millions of unique proteins)



What is Sam68?

- Sam68 is one of many proteins that carries out the process of alternative splicing
- Splices pre-mRNA of many genes involved in cell growth, cell cycle regulation, and apoptosis
 - BCL2L1, SRSF1, and many more
- Has been shown to physically associate with many proteins playing roles in many other cellular processes



SRSF1

- ▶ Involved in AS and RNA metabolism
- ▶ Alternatively splices the RNA transcripts of BIN, BIM, and MCL1
 - ▶ Proteins heavily involved in apoptosis
- ▶ Overexpressed in different cancer types
 - ▶ Overexpression has been shown to cause the formation of anti-apoptotic isoforms of the above proteins
- ▶ Sam68 can splice to a NMD isoform or the functional isoform

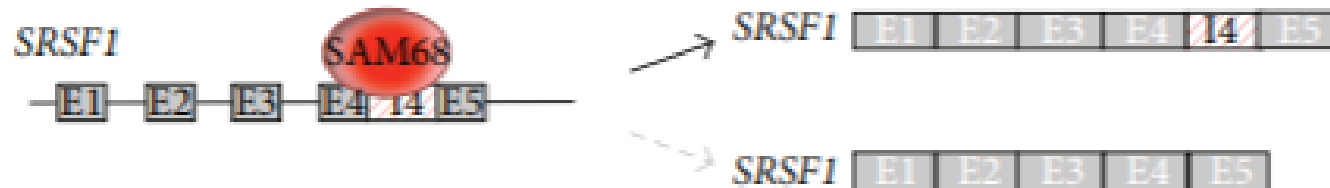
Sam68

SRSF1

BIN

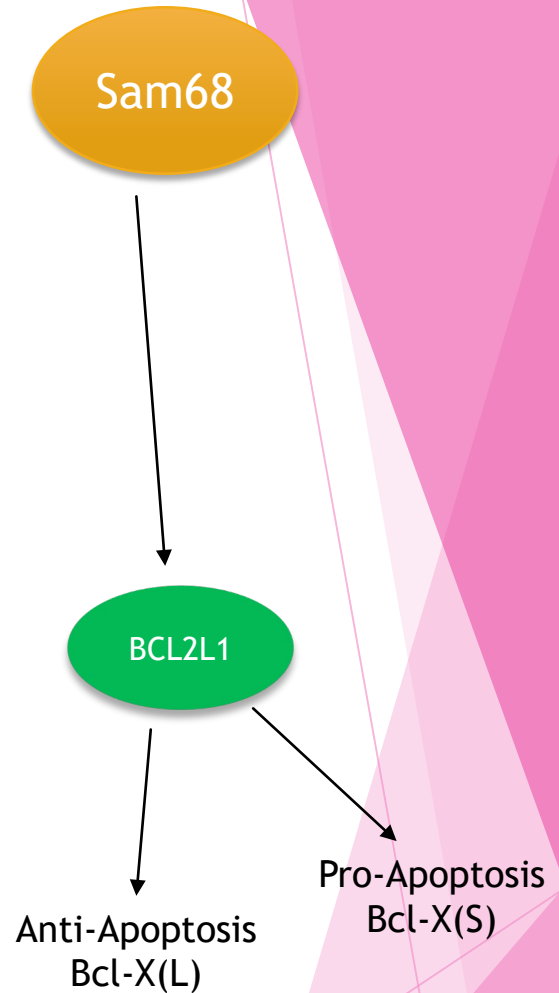
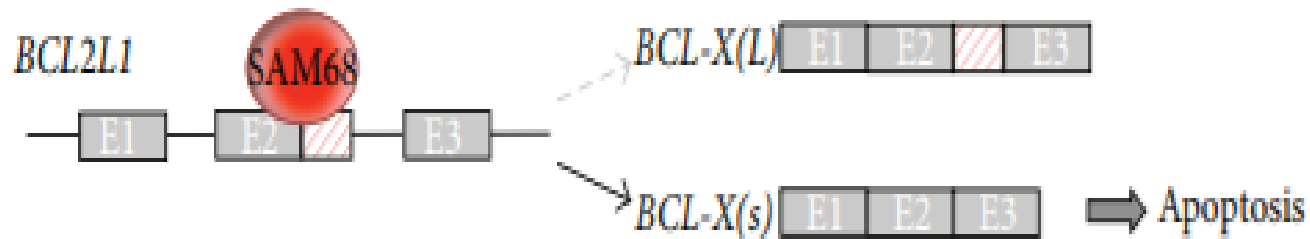
BIM

MCL1



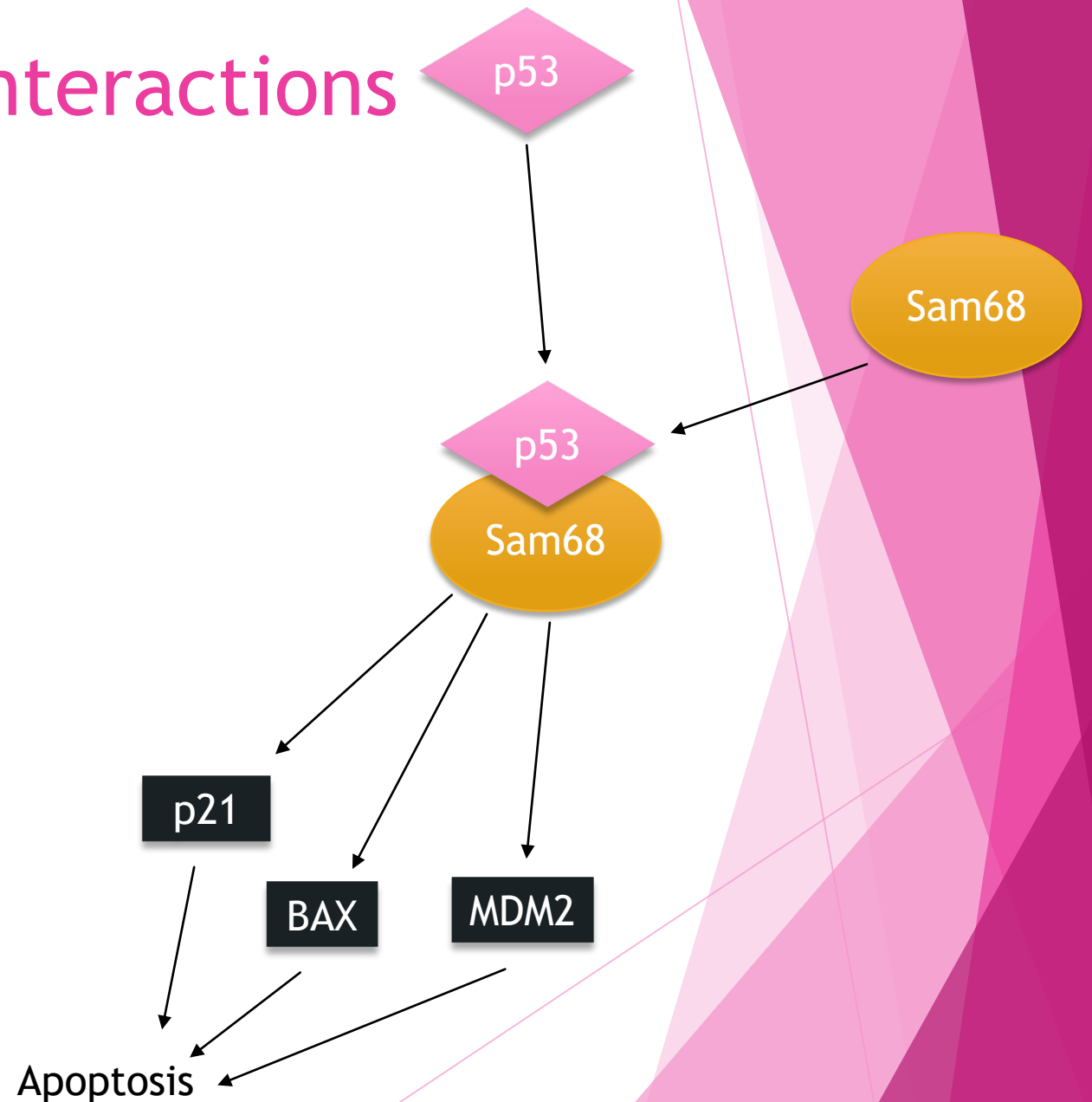
BCL2L1

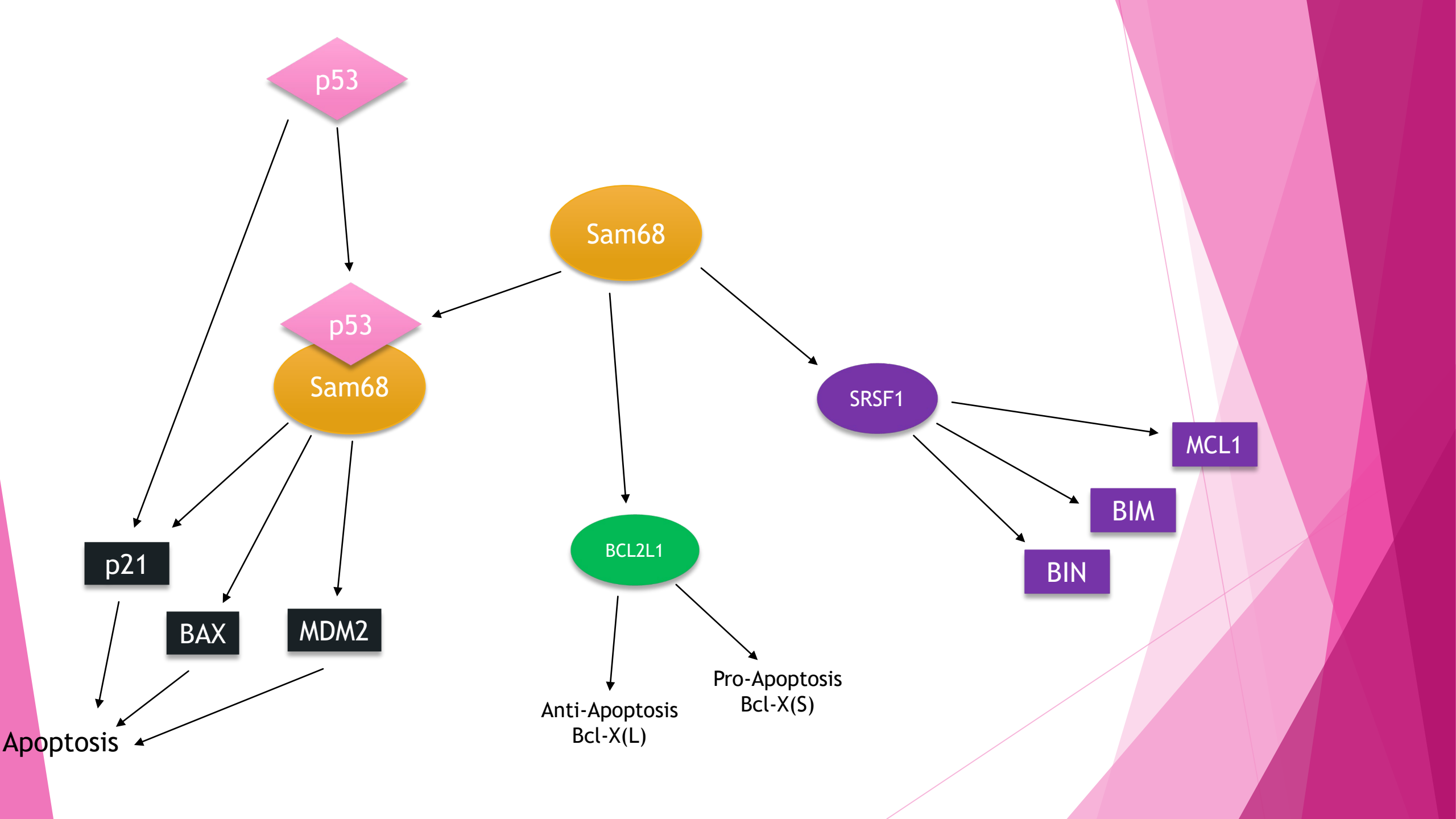
- ▶ Heavily involved in apoptosis
- ▶ BCL2L1 is spliced into two major isoforms: Bcl-X(L) and BCL-X(S)
 - ▶ BCL-X(L) sequesters pro-apoptotic proteins
 - ▶ BCL-X(S) cannot sequester pro-apoptotic proteins, thus allowing for apoptosis



Sam68 Protein-Protein Interactions

- ▶ Sam68 also plays a role in many cellular functions via protein-protein interactions
- ▶ Ex: Sam68 has been shown to play a role in transcription regulation
 - ▶ Sam68 physically interacts with p53, a pro-apoptotic transcription factor
 - ▶ Sam68-p53 complex increases transcription of p53-transcribed genes
 - ▶ p53 transcribes pro-apoptotic genes in response to DNA damage



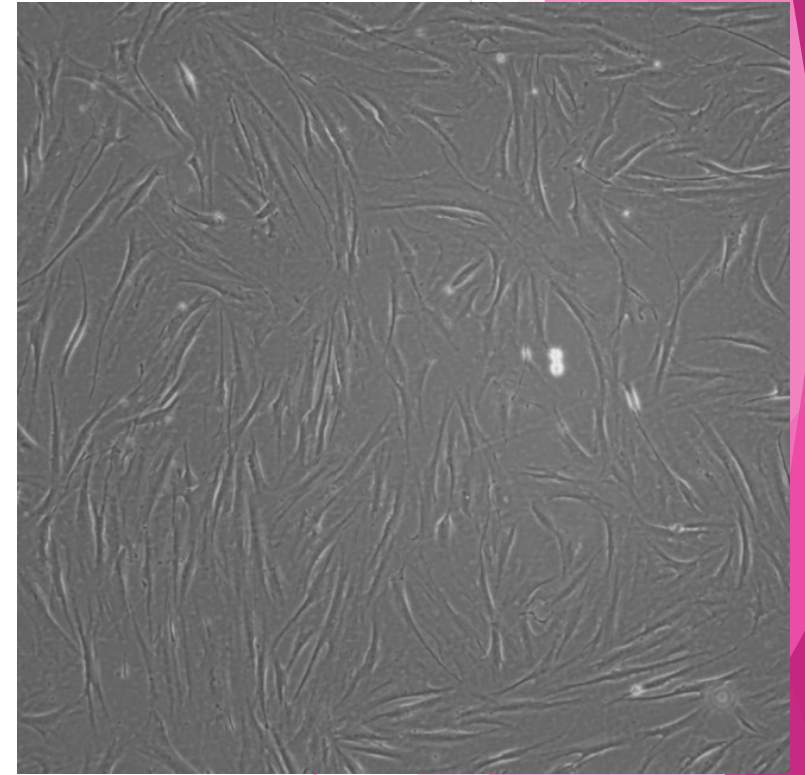


Simian Virus 40 Large T-antigen as a Model

- ▶ Oncogenic viral protein
- ▶ Interferes with many cell cycle regulators resulting in tumorigenesis
 - ▶ Goal is to deregulate the cell cycle
- ▶ > 12% of human cancers are caused by oncogenic viruses
 - ▶ Human papillomavirus, Epstein-Barr virus, hepatitis B and C, etc.

Human Diploid Fibroblast Cells

- ▶ Immortalized by telomerase
 - ▶ No limit on amount of cell divisions
- ▶ HDF(Tert) cells are WT and do not express T-antigen
- ▶ HDF(Tert) + T Clone cells have been transformed to express T-antigen
 - ▶ Clones: T-antigen implements into the genome at different locations
 - ▶ HDF(Tert) cells stably transfected with exogenous T-antigen



Experimental Question:

What is Sam68's role in T-antigen transformation?
How much is Sam68 expressed in T-antigen expressing cells, and how does this impact Sam68's ability to splice RNA and its protein interactions?

Experimental Procedures

Western Blotting

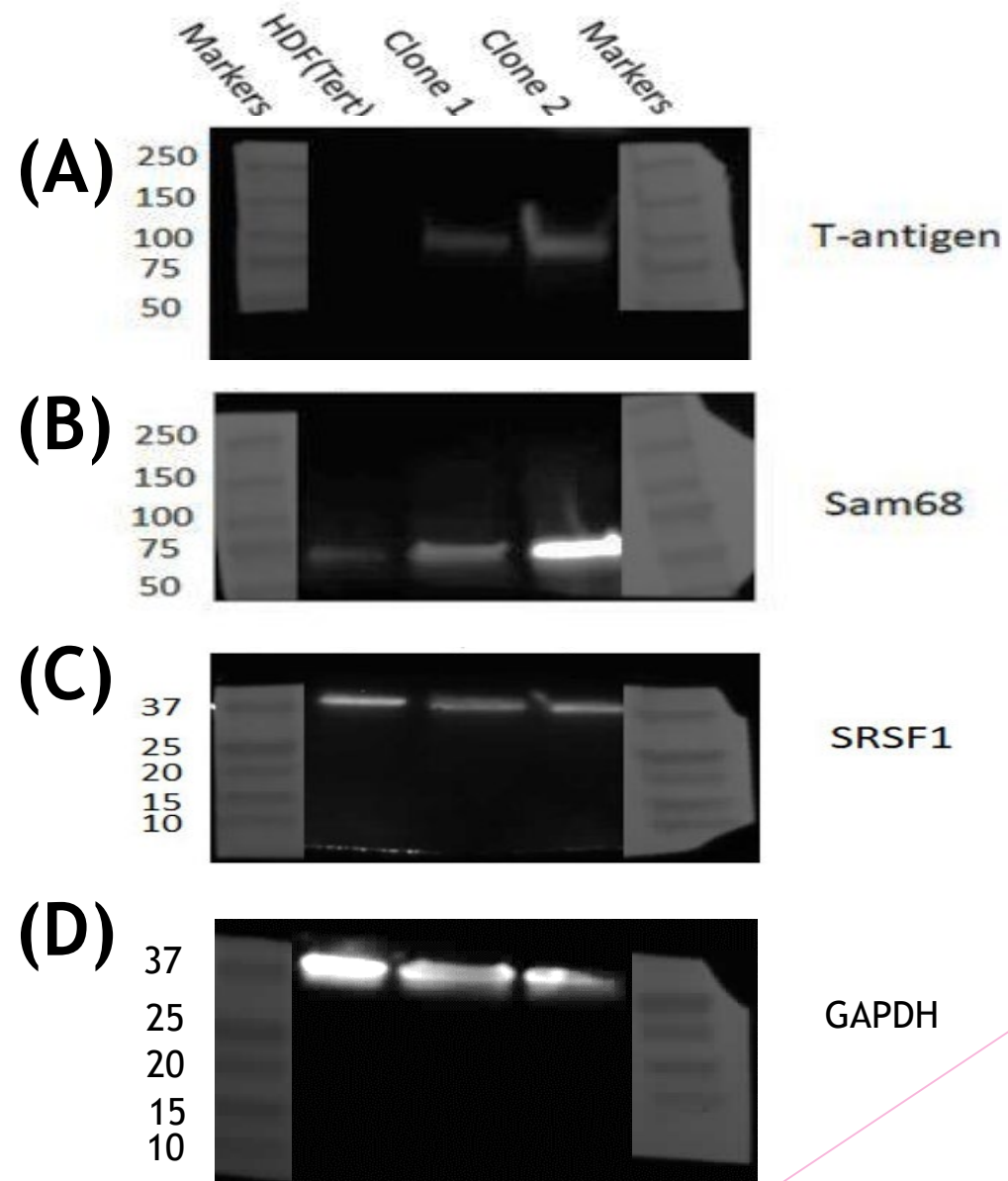
Immunofluorescence

PCR

Co-Immunoprecipitation

Western Blot

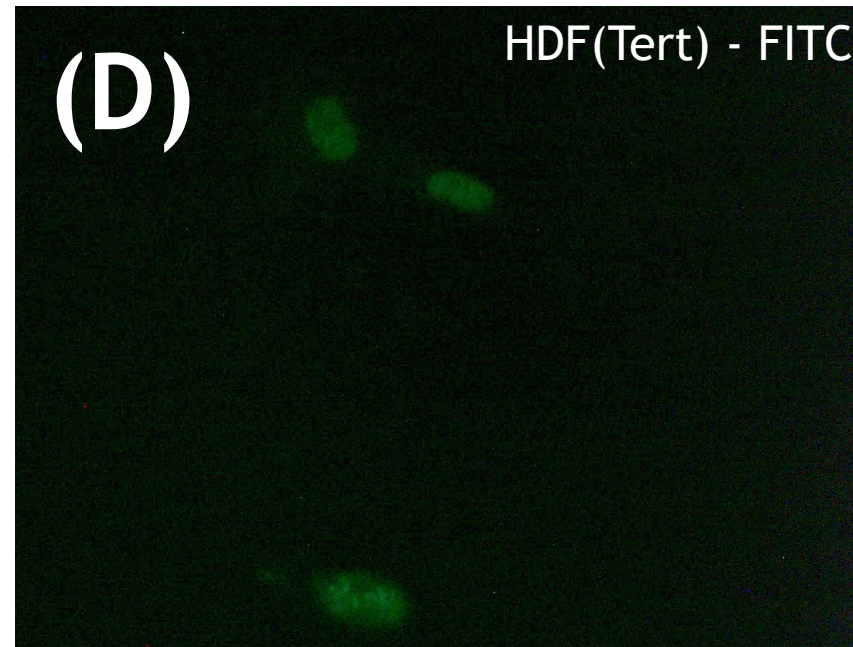
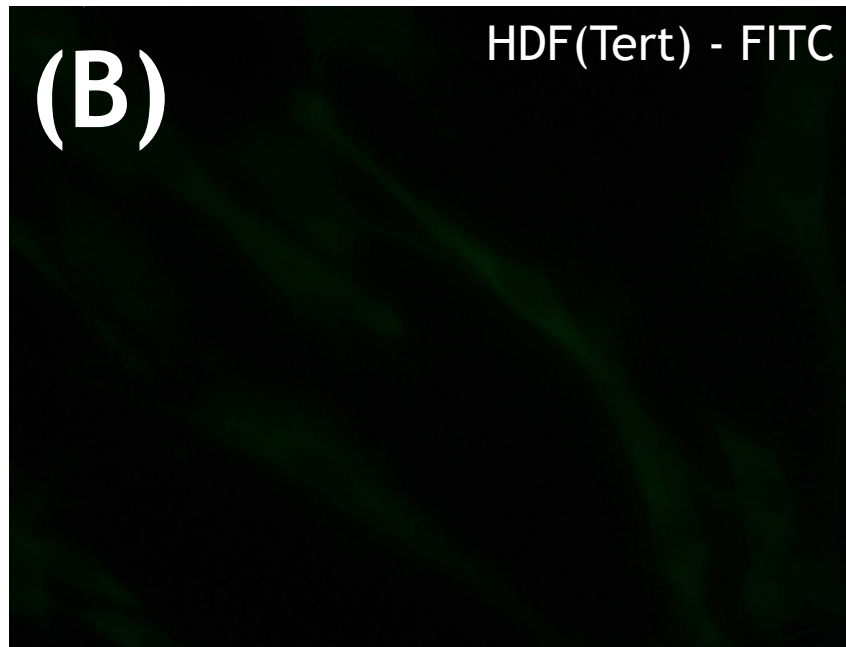
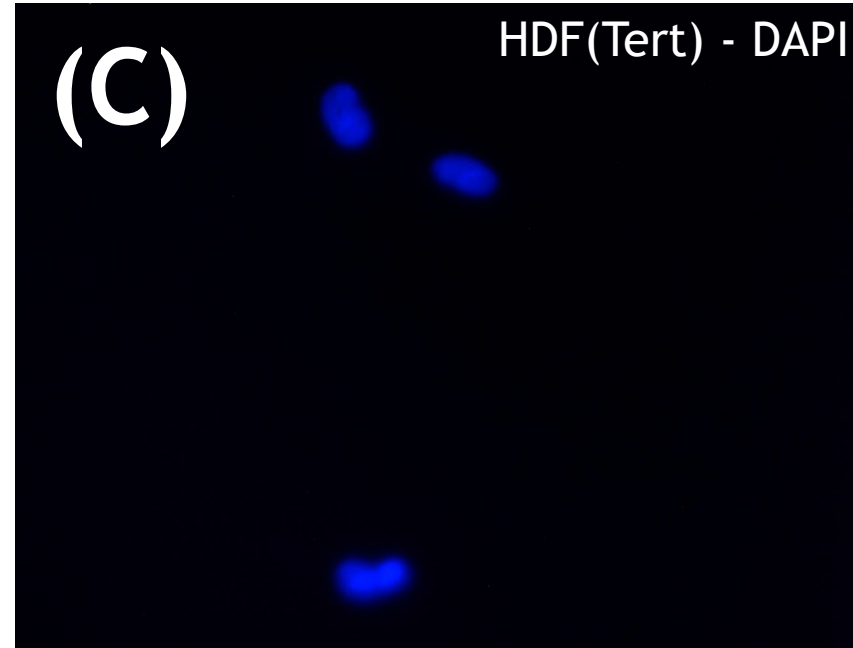
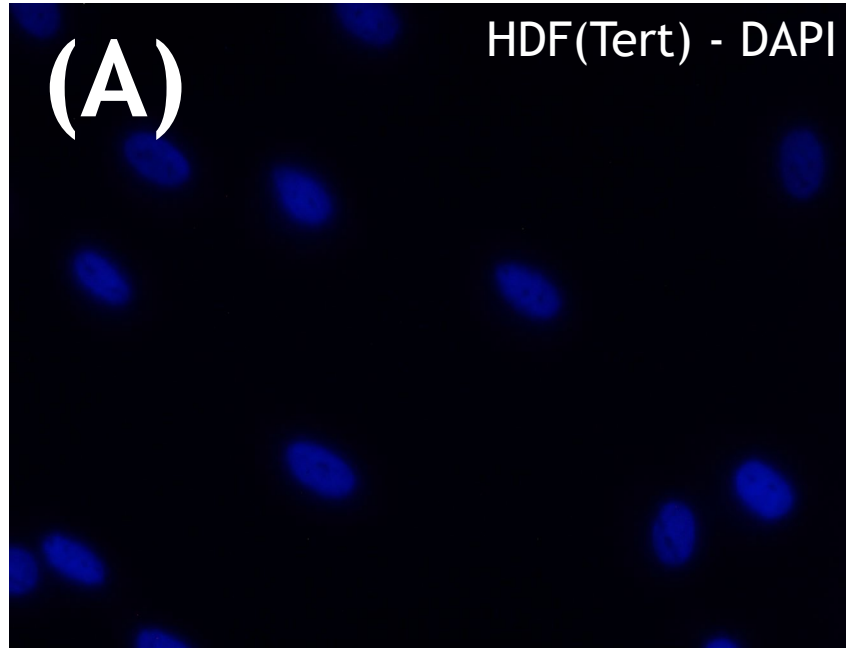
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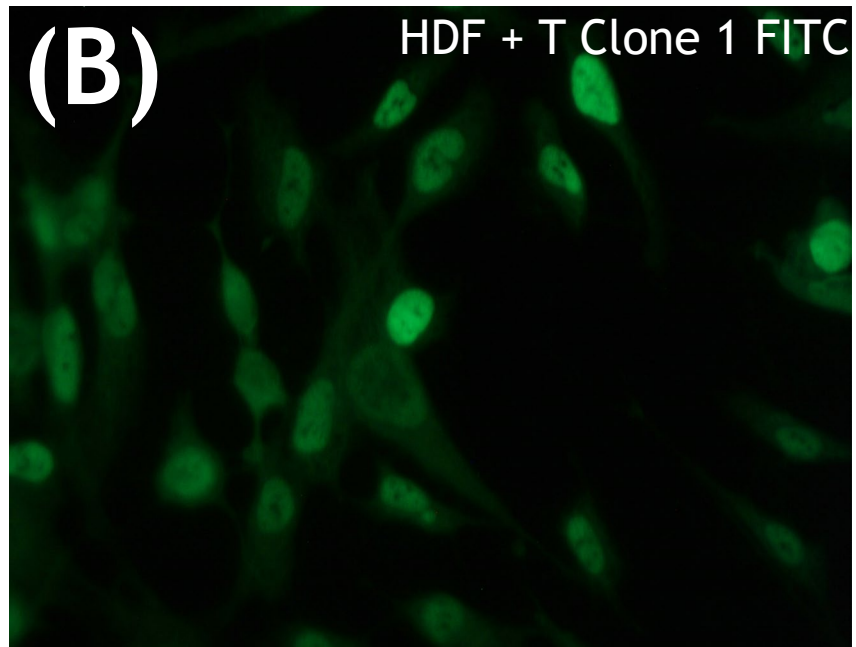
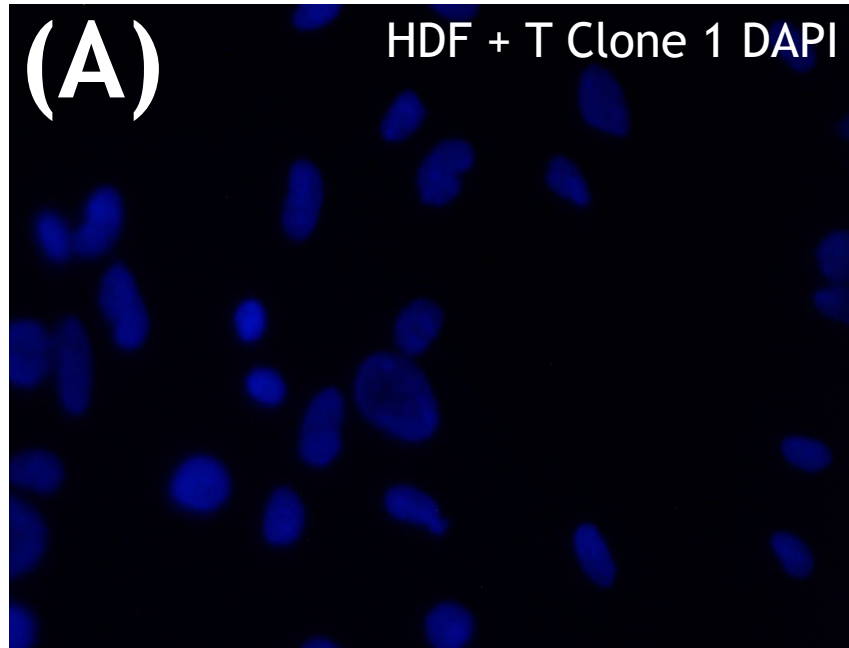
T-antigen

Sam68

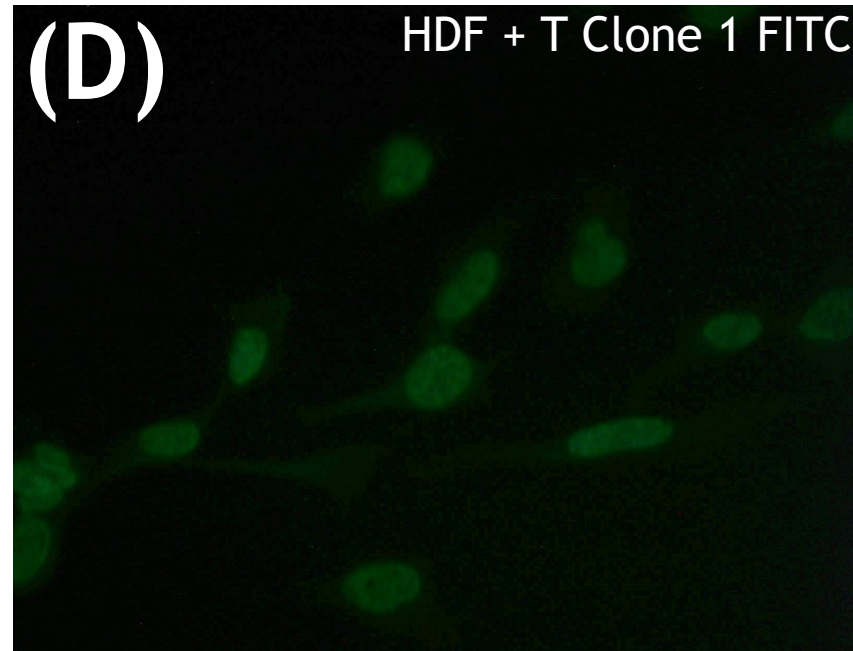
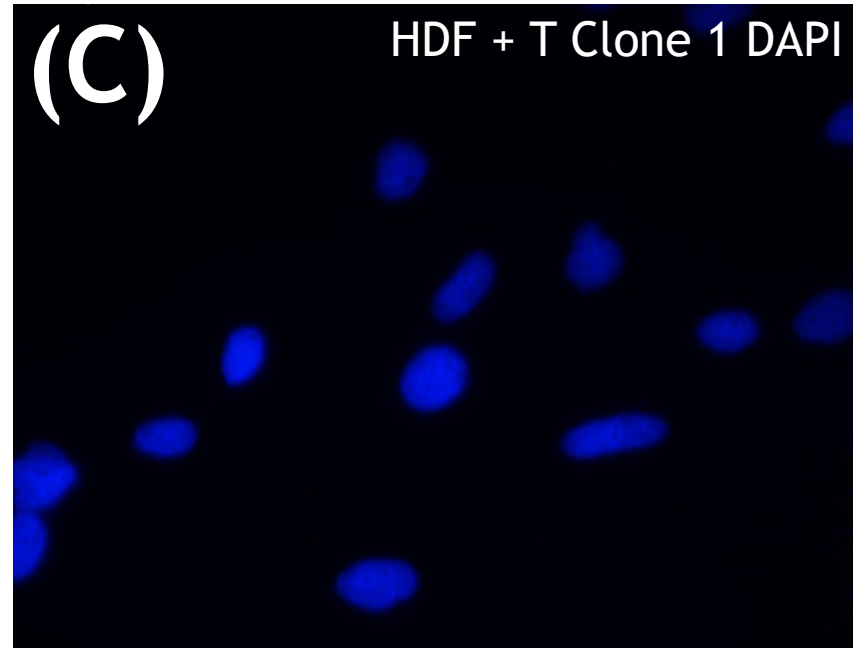
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T-antigen



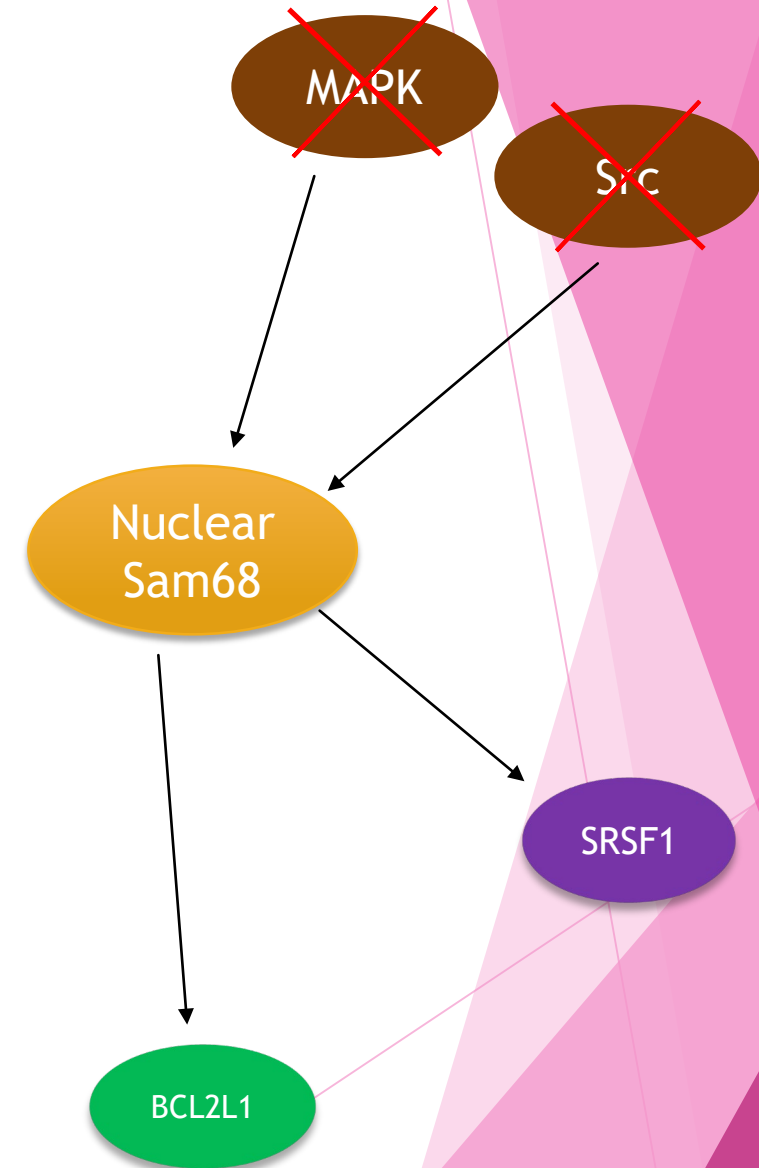
Sam68



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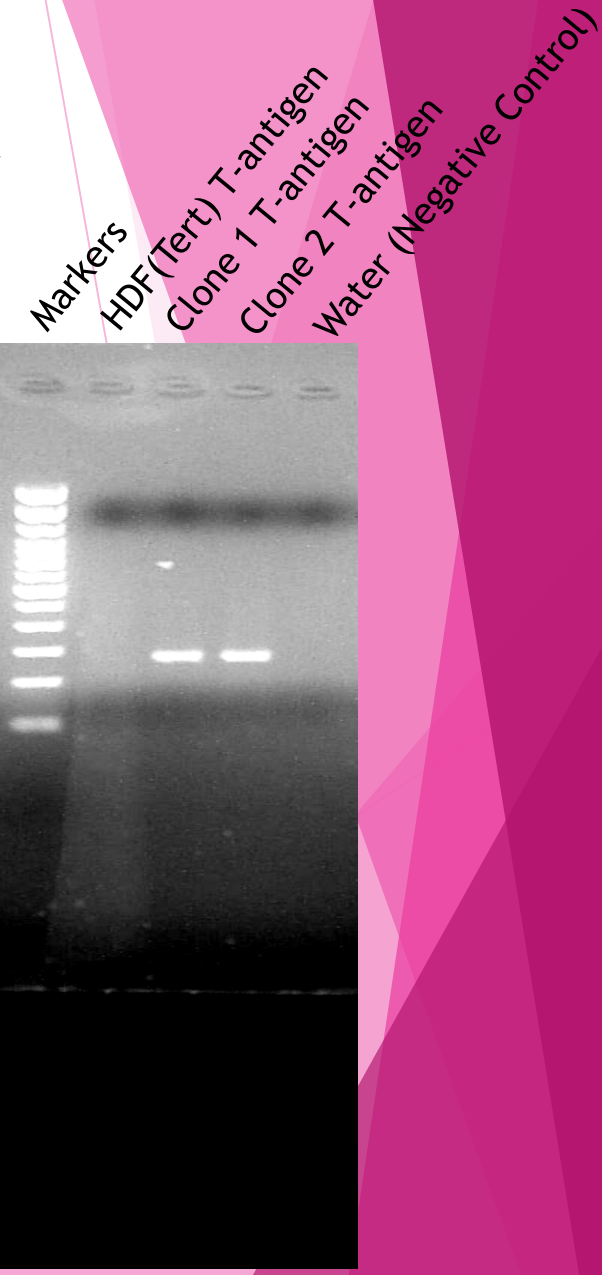
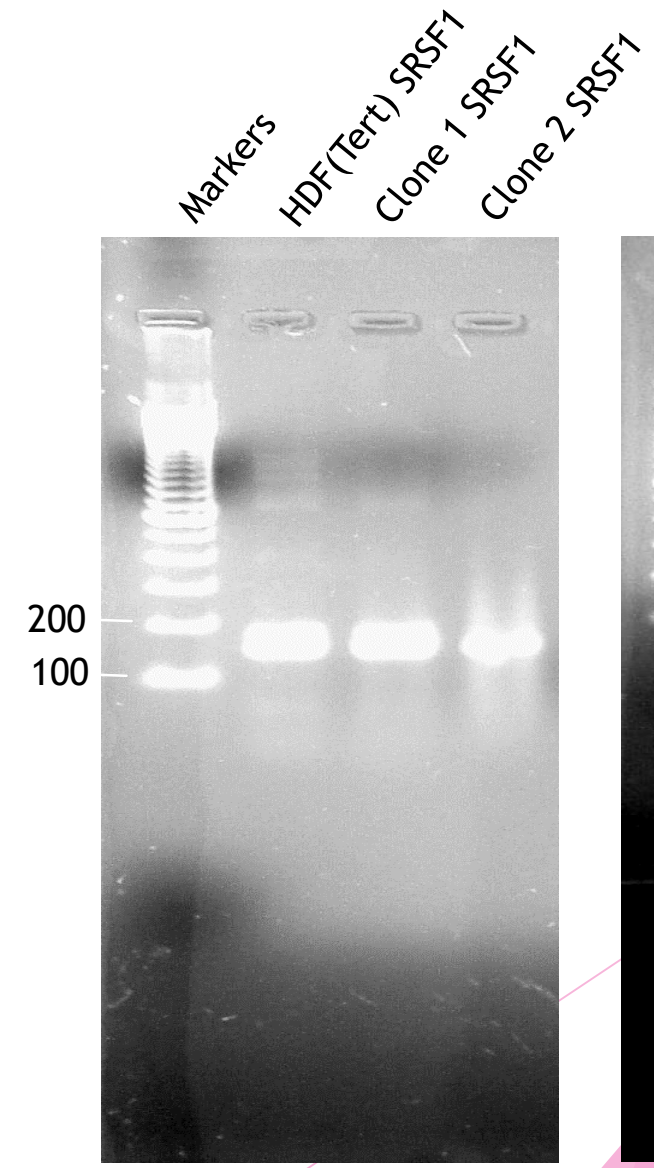
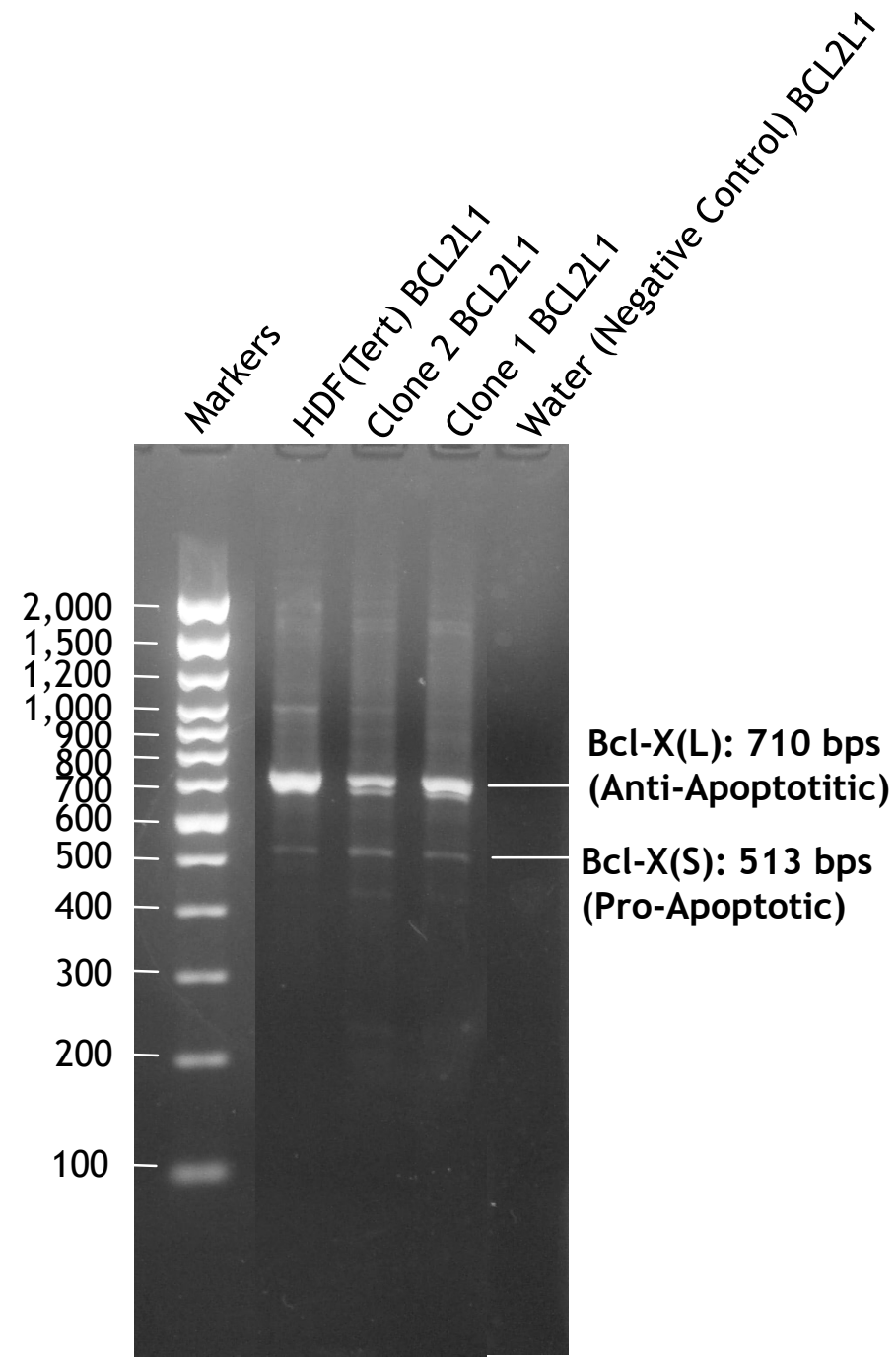
Functions/Targets of Sam68

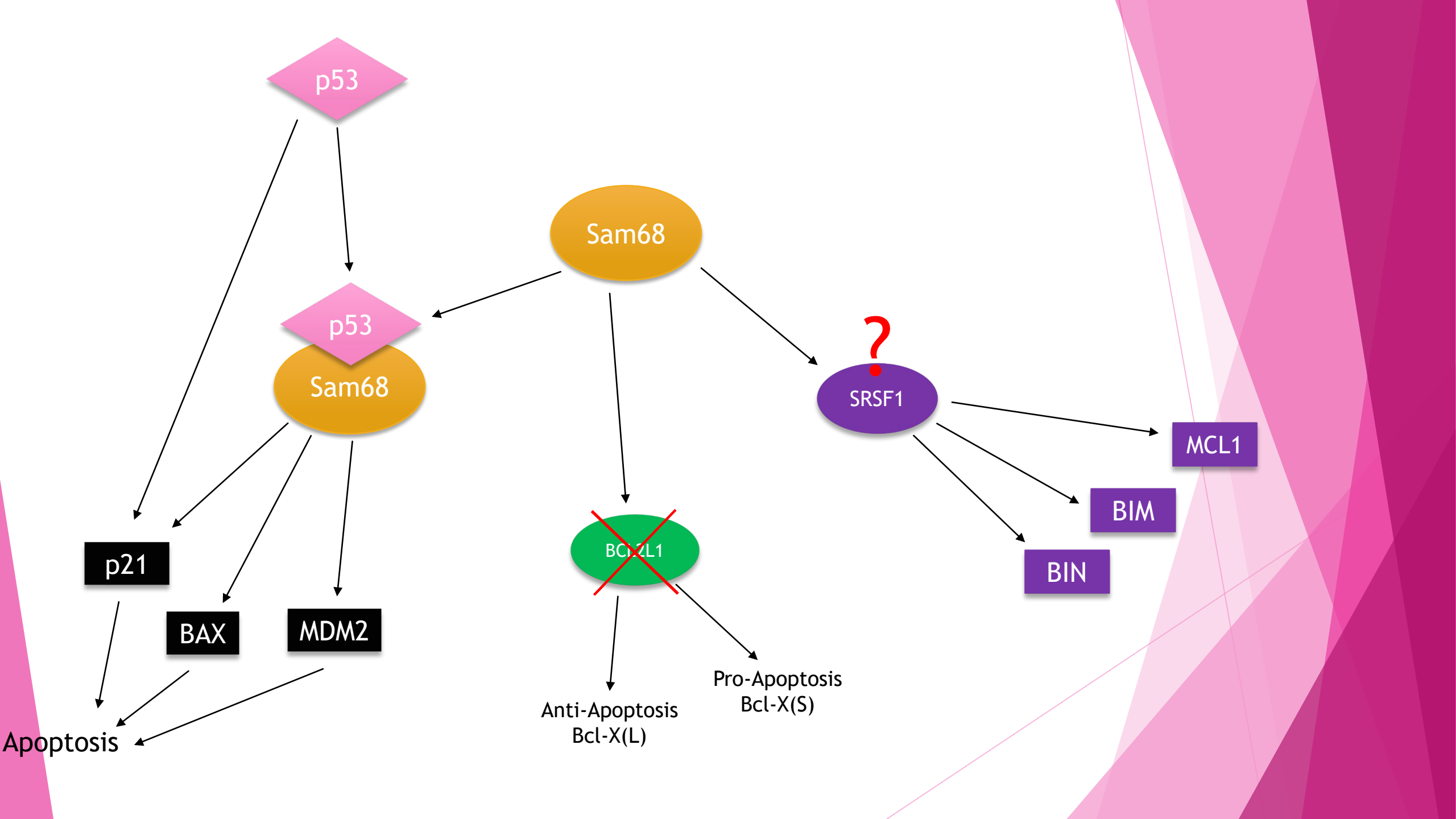
- ▶ SRSF1 concentrations do not seem to be significantly different across cell lines
 - ▶ Past lab data shows slight increase in SRSF1 in transformed cells
- ▶ Examination of other Sam68 mRNA targets
 - ▶ What isoforms of BCL2L1 does Sam68 overexpression favor?
 - ▶ Are there different isoforms of SRSF1 being expressed?
- ▶ Because Sam68 seems mostly nuclear in our cells, this rules out cytoplasmic functions of Sam68
 - ▶ Cytoplasmic Sam68 is involved in signal transduction



PCR

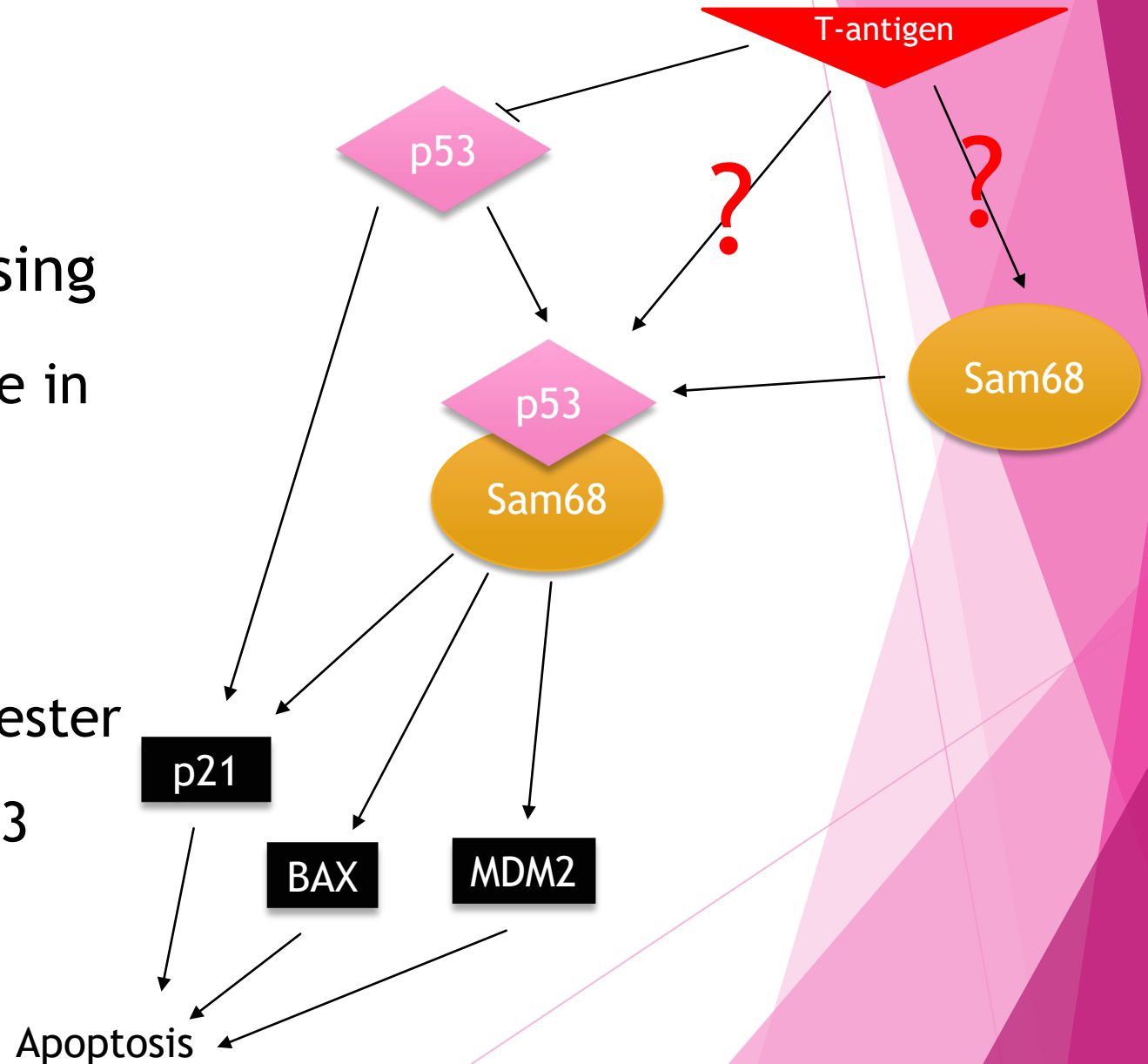
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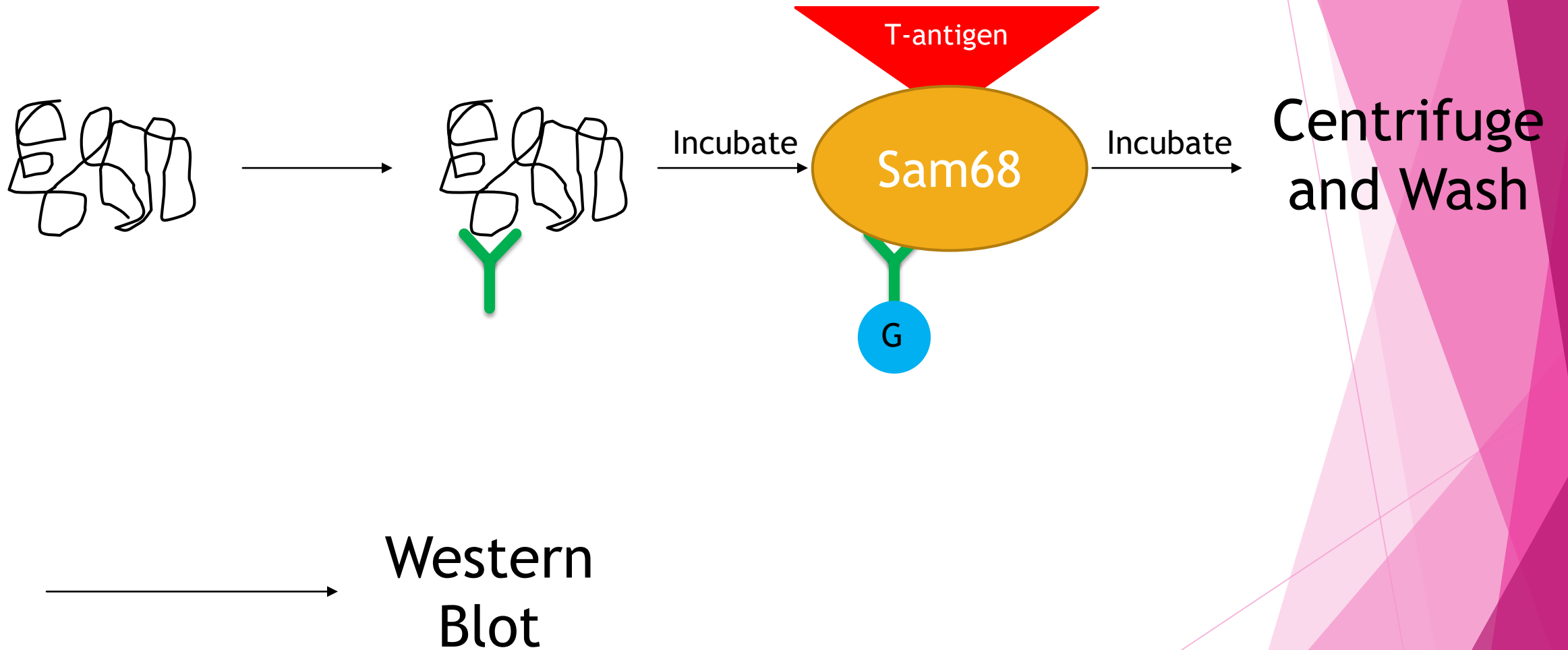


T-antigen Targeting of Regulatory Proteins

- p53 is found in higher concentrations in cells expressing T-antigen
 - Binds to p53 (normally unstable in health cell), stabilizing it and keeping it present in the cell
- Sam68 is also found in higher concentrations in T-antigen-expressing cells
 - Could T-antigen bind and sequester Sam68 in a similar way to p53?
 - Possible T-antigen/ Sam68/ p53 complex?

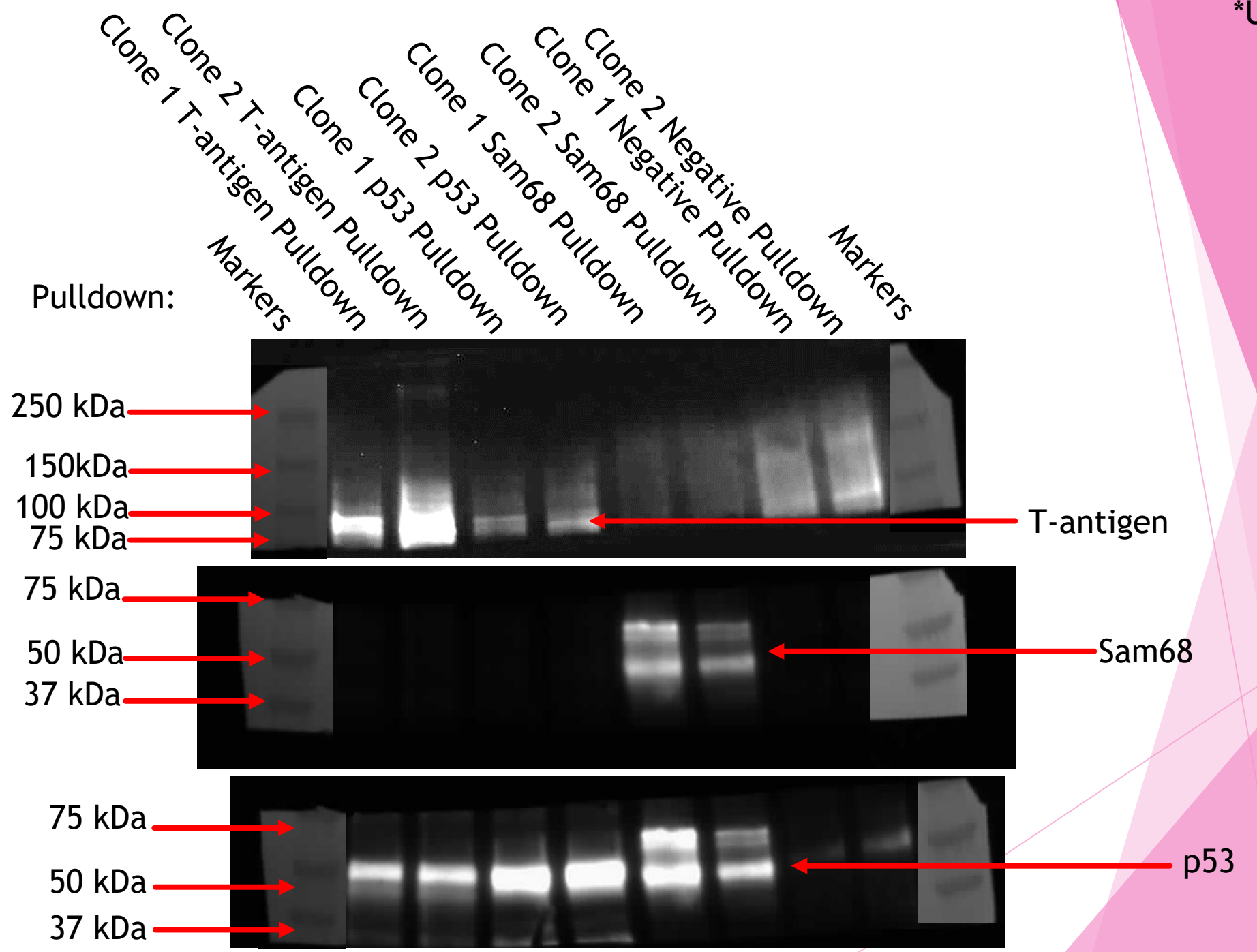


Co-Immunoprecipitation



Co-IP

*Unpublished Data



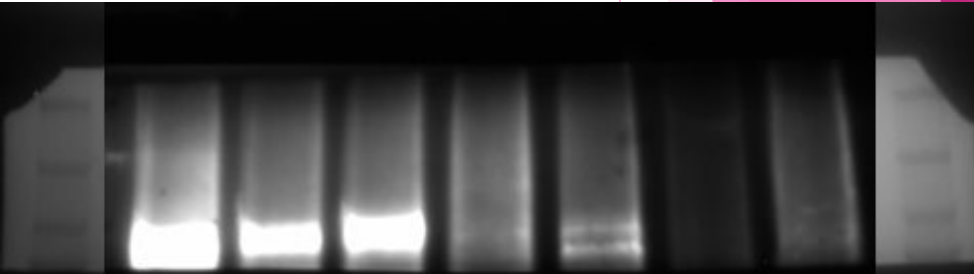
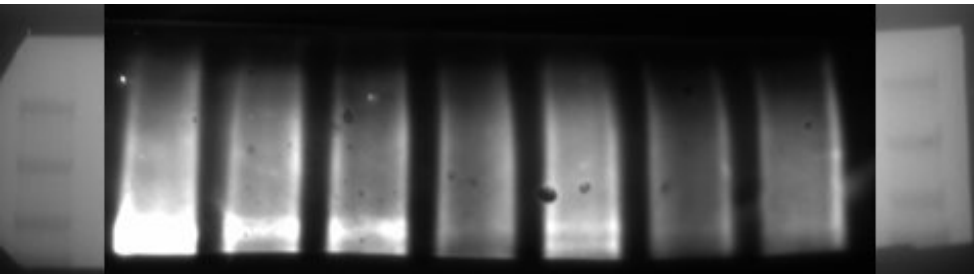
Blots probed using Anti-Sam68

Pulldown:

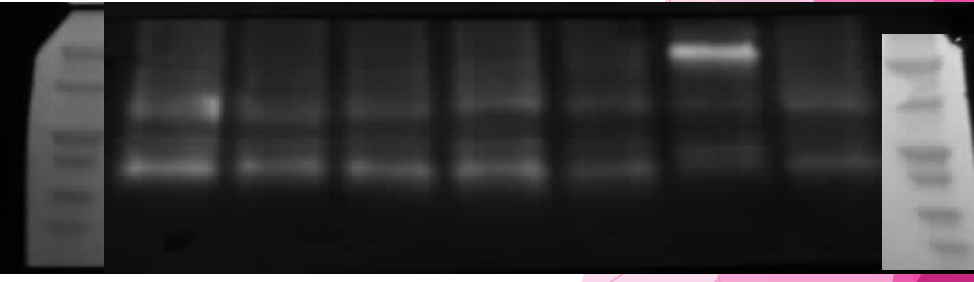
Anti-Tag 901
Anti-Tag 902
Anti-Tag 416
Anti-p53 DO-1
Anti-p53 246
Anti-Sam68
Negative Control

Anti-Tag 901
Anti-Tag 902
Anti-Tag 416
Anti-p53 DO-1
Anti-p53 246
Anti-Sam68
Negative Control

T-antigen



Sam68



Clone 1 Lysate

Clone 2 Lysate

Review of the Data

- ▶ Sam68 splicing in T-antigen-expressing vs. WT HDF(Tert) cells
 - ▶ SRSF1 is not being expressed in significantly different levels
 - ▶ BCL2L1 is not being alternatively spliced in a significantly different way
- ▶ Sam68 does not appear to interact with T-antigen at a detectable amount
 - ▶ Co-IP will need to be repeated

Future Experiments

- ▶ Examine isoforms of other Sam68 pre-mRNA targets
 - ▶ Cyclin D1, mTOR, Survivin
- ▶ Shifting view in our lab that Sam68 may be so highly expressed due to protein-protein interactions
 - ▶ Sam68 is an alternative splicing protein but also plays role in cellular functions via protein-protein interactions
 - ▶ No difference in Sam68-spliced transcripts noted in our lab, so it seems more likely Sam68's protein interactions are altered

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