# SCF-mediated degradation of p100: Mechanisms and relevance in multiple myeloma

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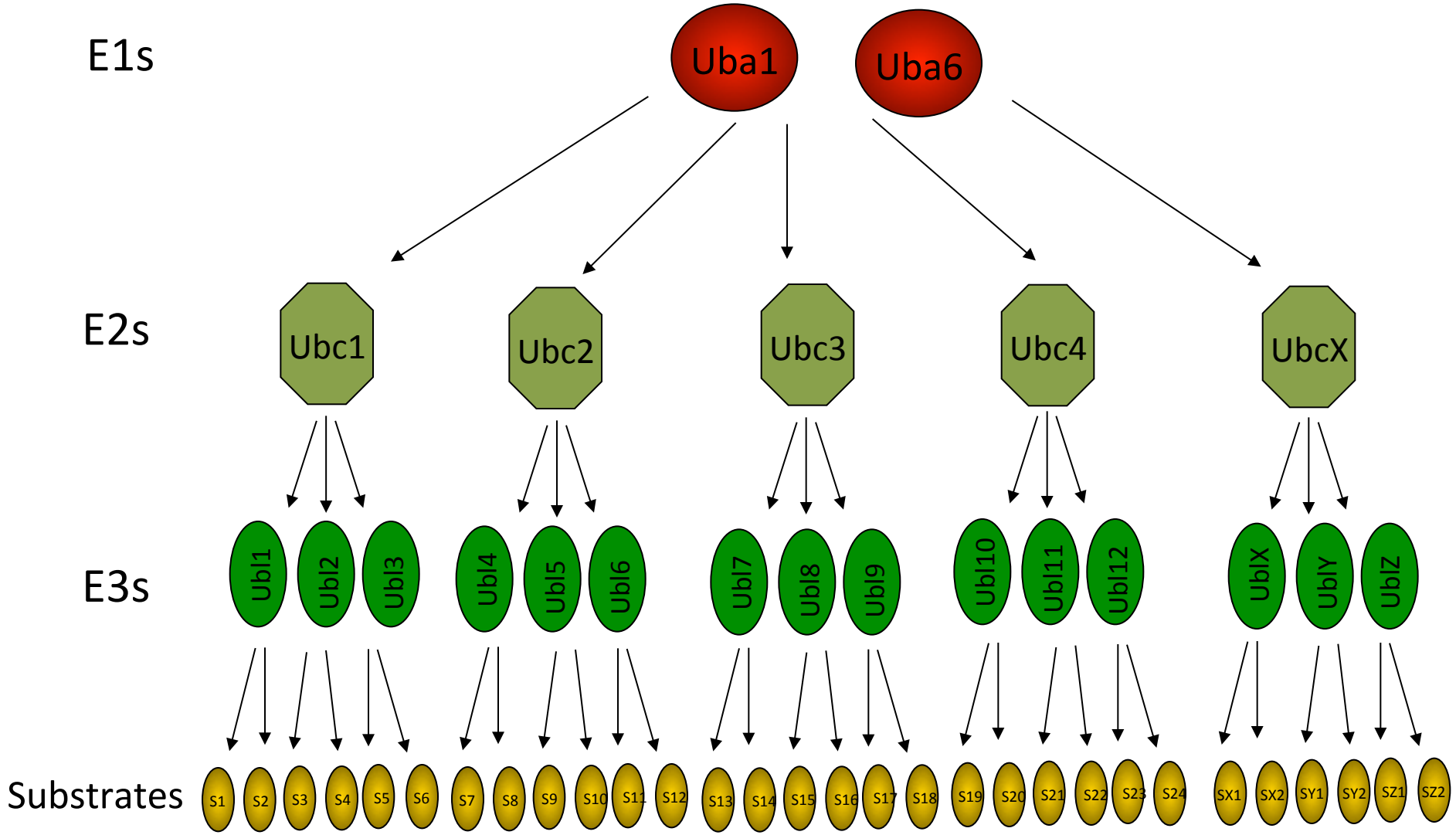
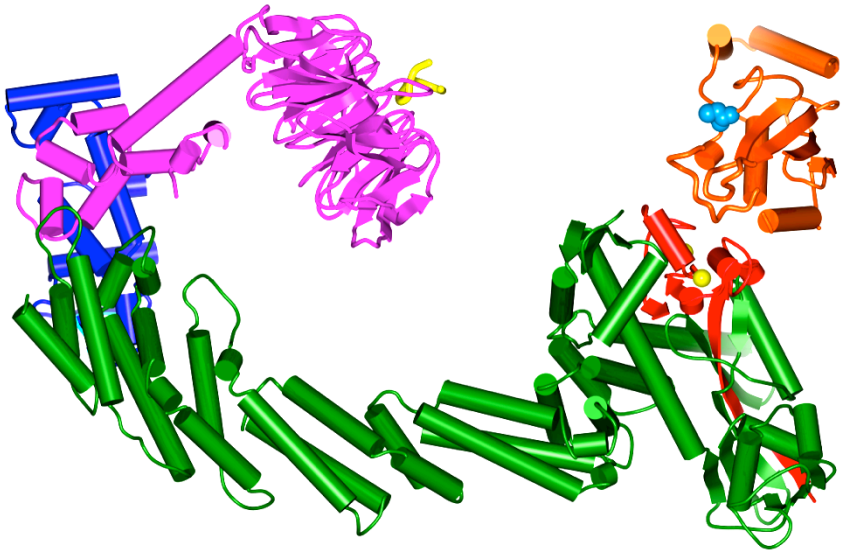
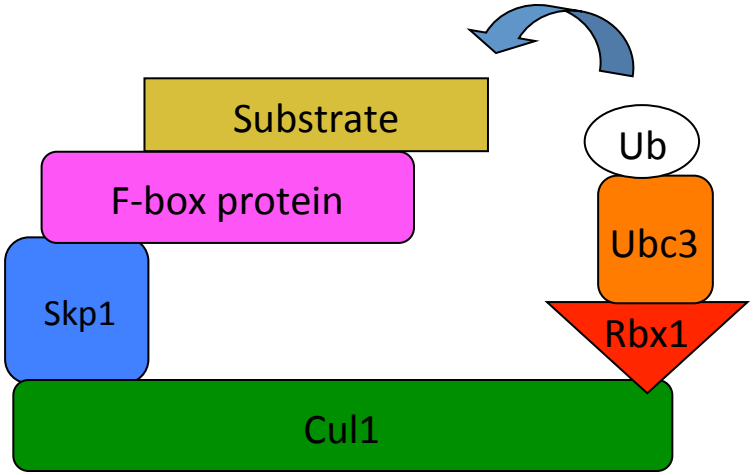
# The hierarchy of the ubiquitin system



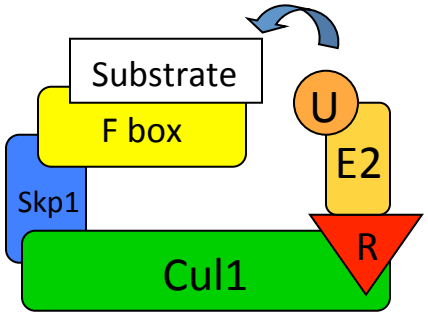
# The SCF ubiquitin ligase complex



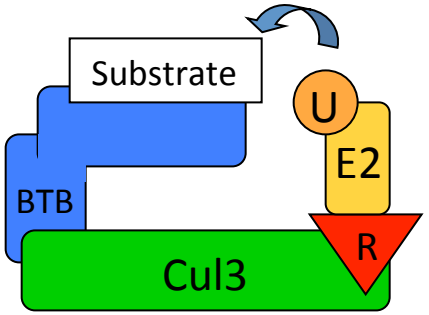



Wu et al., *Molecular Cell* 2003

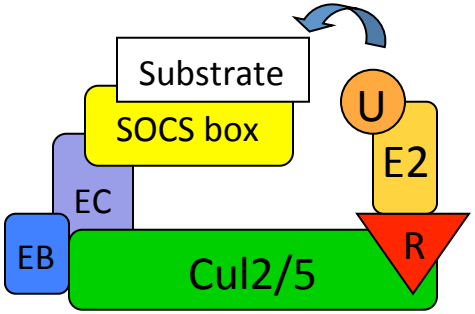
# Four major families of Cullin-RING ubiquitin Ligase complexes (CRLs) (> 200 different enzymes with different substrate receptors)



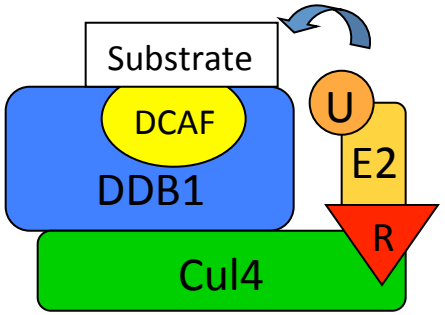

69 F-box proteins = 69 SCF ligases




~100 BTB proteins = ~100 CRL3 ligases




~50 SOCS-box proteins = ~50 CRL2/5 ligases

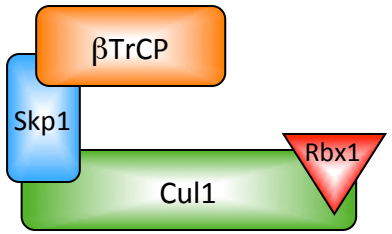



~30 DCAF proteins = ~30 CRL4 ligases

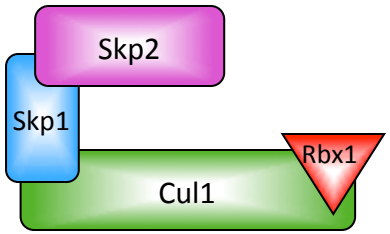
SCF and the SCF-like ligase APC/C control a variety of key cellular functions, including gene transcription, protein synthesis, DNA damage responses, chromosome stability, centrosome duplication, dNTP synthesis, apoptosis, circadian clock oscillation, *etc.*

Substrates

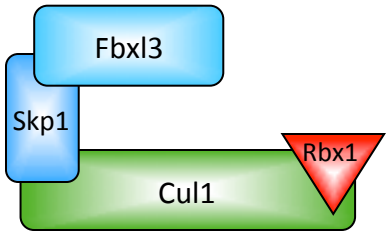
β-catenin, IkBα/β/ε
Emi1, ATF4
Cdc25A, , BimEL
Claspin, Wee1
Per1/2, REST
PDCD4, Bora
DEPTOR



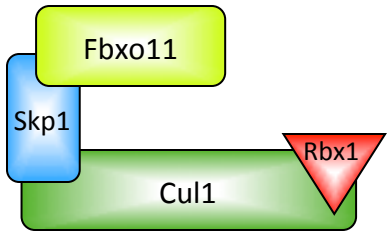

p27, p21
p57, p130
Foxo1, Tob1




CRY1, CRY2

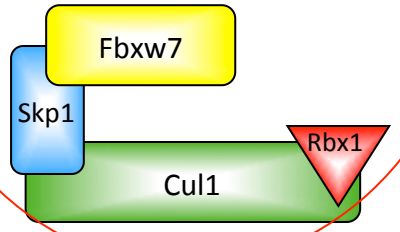



BCL6

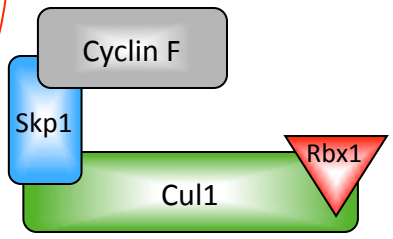



Substrates

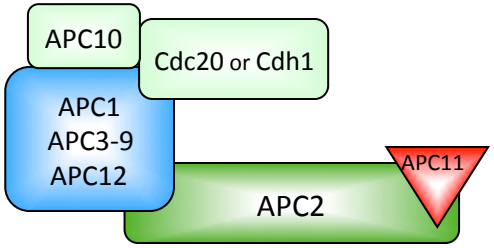
NFκB2,
Notch1/4, Mcl1
Cyclin E, Jun
c-Myc, Srbp1
Presenilin




RRM2,
CP110, Nusap1

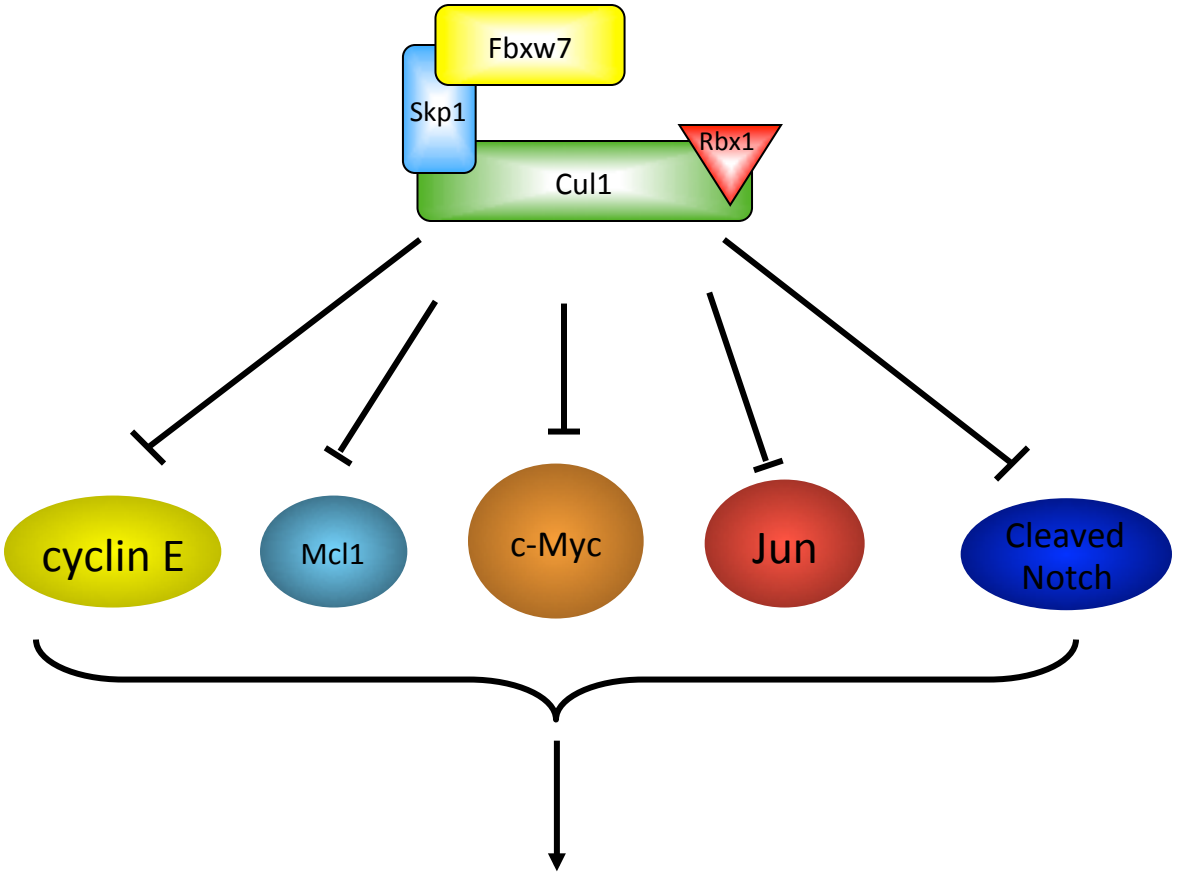



Cyclin A, Cyclin B,
Securin, Geminin,
Plk1, Tpx2, Cdc6
Cdc25A, Aurora A
Skp2, p21, Claspin,
Ubc10, Tk1, Tmk1
Cks1, Nek2




*Red color denotes the contributions from work in our laboratory*

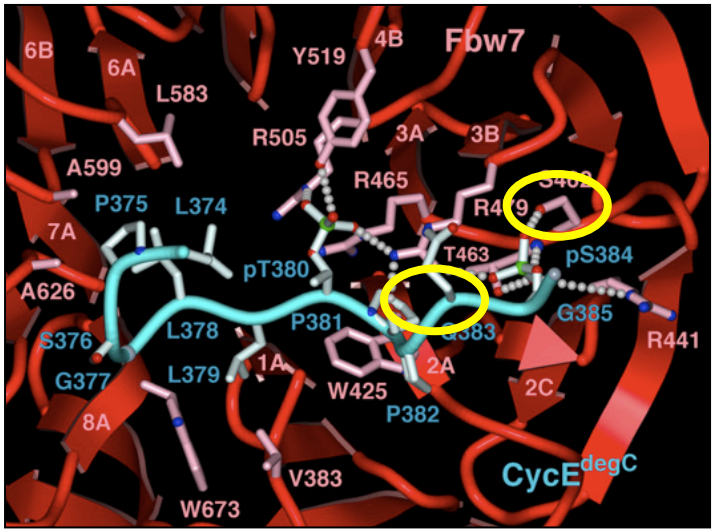
# The best characterized substrates of Fbxw7



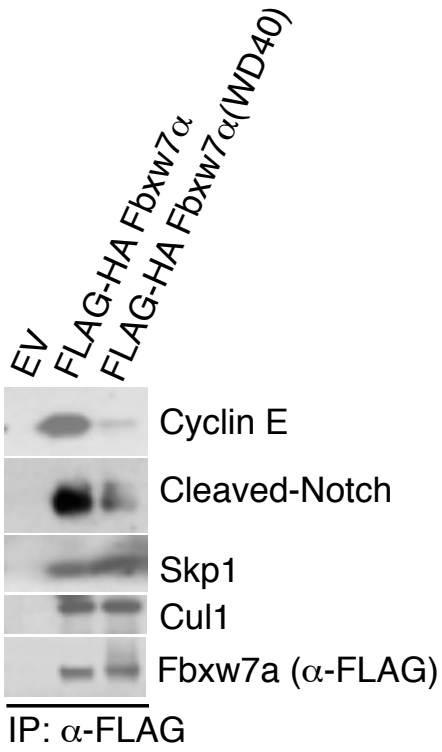

A novel function of Fbxw7 as a pro-survival gene in diseases with elevated NF-κB activity

# Differential purification strategy: Utilizing an Fbxw7α mutant deficient in substrate binding

Red= Fbxw7 Blue = Cyclin E

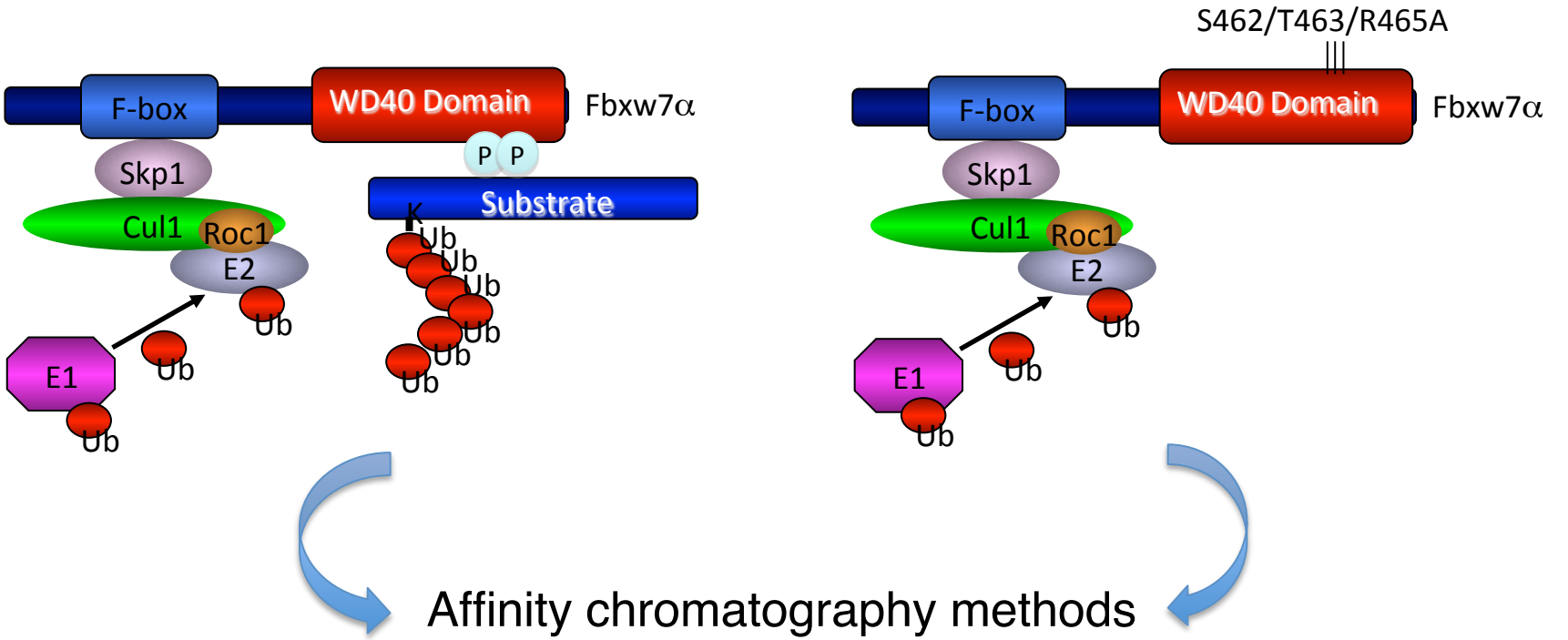



Hao et al. *Molecular Cell* 2007




- Mutation of these residues to Ala [Fbxw7 (WD40)] impairs binding to known substrates
- S462/T463/R465 are critical residues on Fbxw7 for binding to substrates

# Differential purification strategy: Utilizing an Fbxw7α mutant deficient in substrate binding




Affinity chromatography methods

Identification of proteins LC/MS Orbitrap

Peptide Subtraction (WT - WD40mut)

Validation of novel substrates

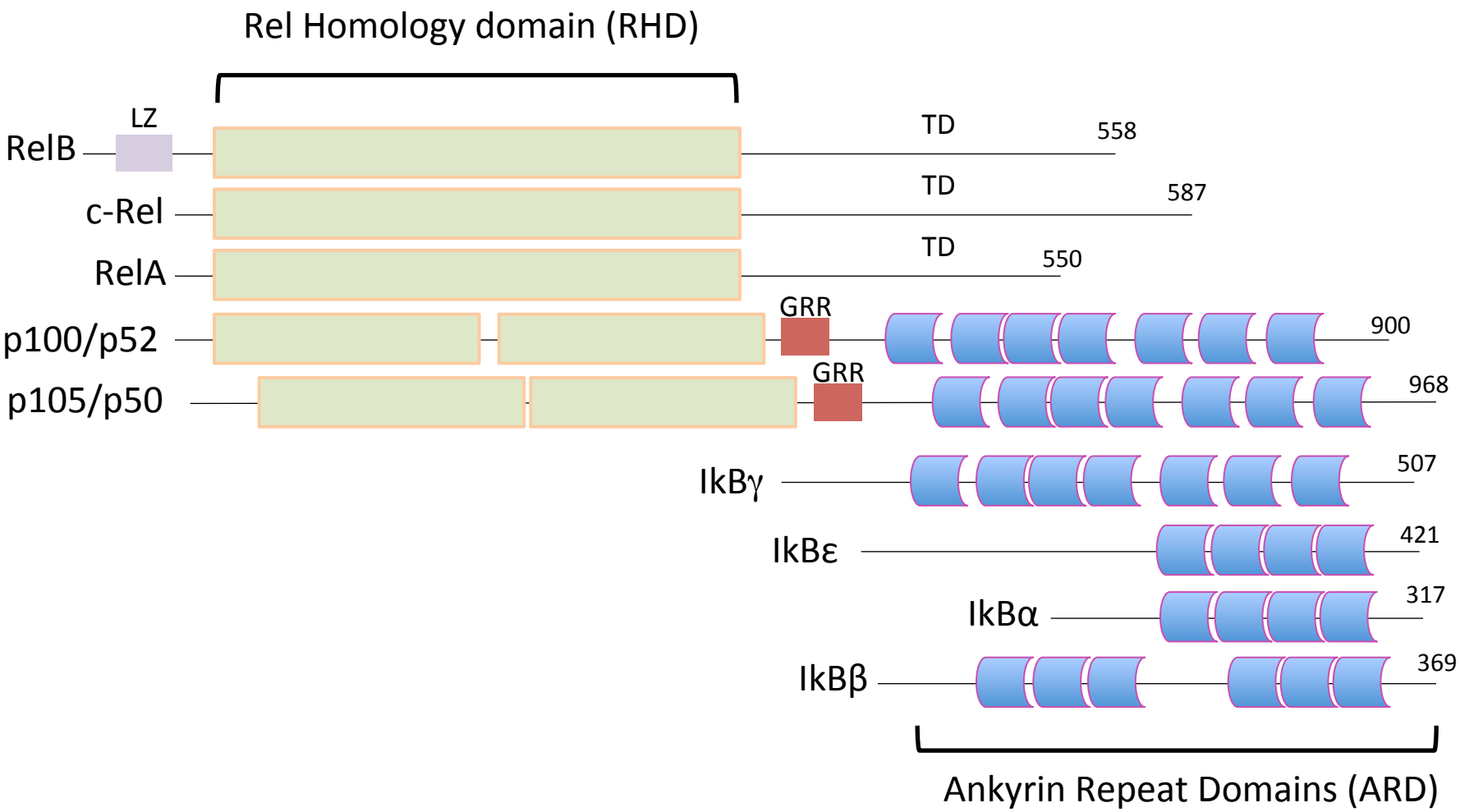
# Differential purification identifies p100 as a putative Fbxw7 substrate

| | WT | WD40 |
|---|---|---|
| SCF Accessory Factors (*e,g.,* Skp1,Cul1, Rbx1,Ubc3) | + | + |
| Substrates (*e.g.,* Myc, Notch) | + | - |
| Chaperones/Other Regulators, Adapters etc. | + | + |

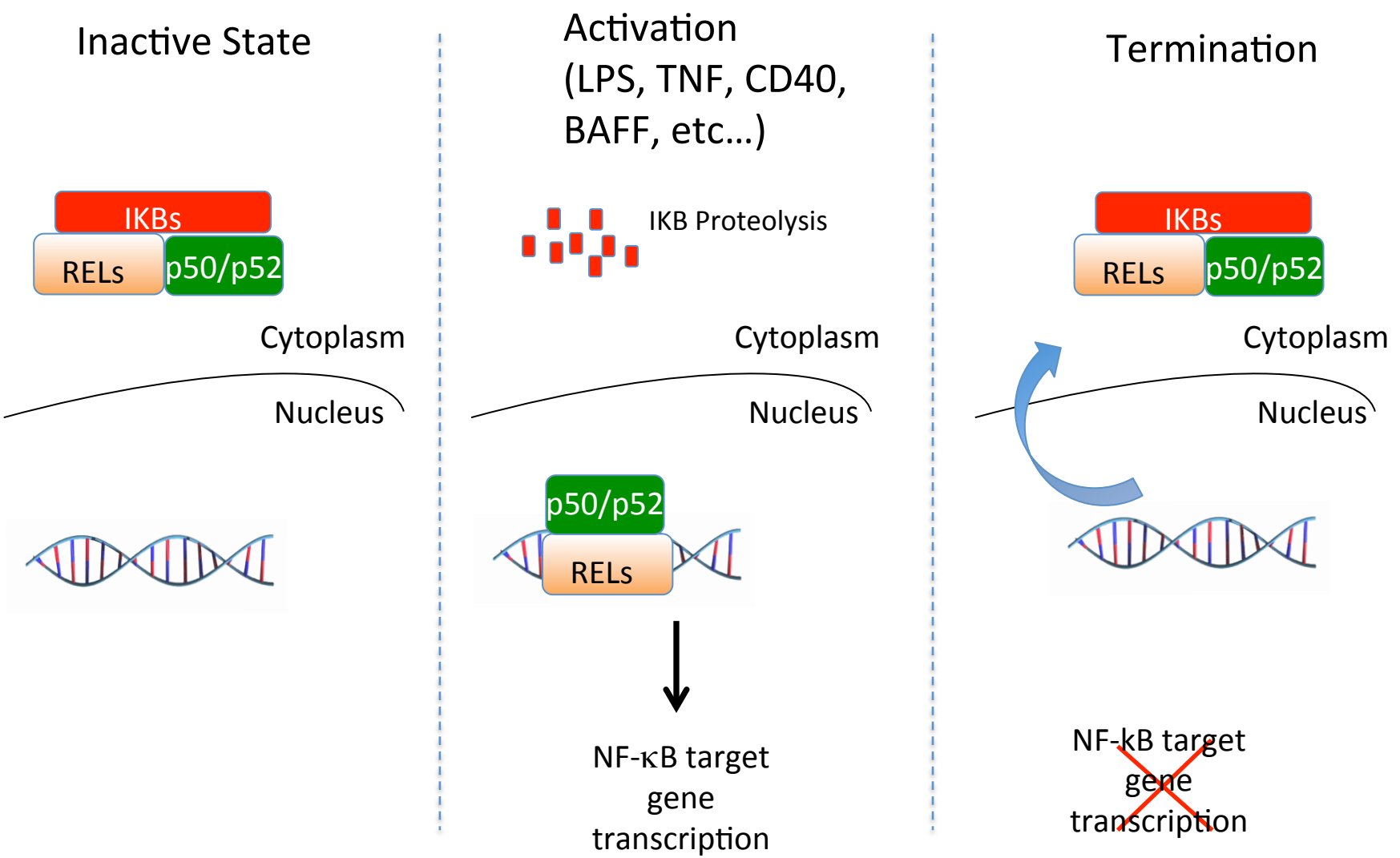
*Normalized Spectral Abundance (Mass Spectrometry)*

| Protein | FBXW7 single IP | FBXW7-WD40 mutant single IP | FBXW7 Double IP | FBXW7-WD40 mutant Double IP |
|---|---|---|---|---|
| Fbxw7 | 0.0443 | 0.0415 | 0.0889 | 0.0902 |
| Skp1 | 0.0428 | 0.052 | 0.0842 | 0.0596 |
| Cul1 | 0.0006 | 0.0015 | 0.0088 | 0.0069 |
| **p100** | 0.0002 | 0 | 0.0005 | 0 |

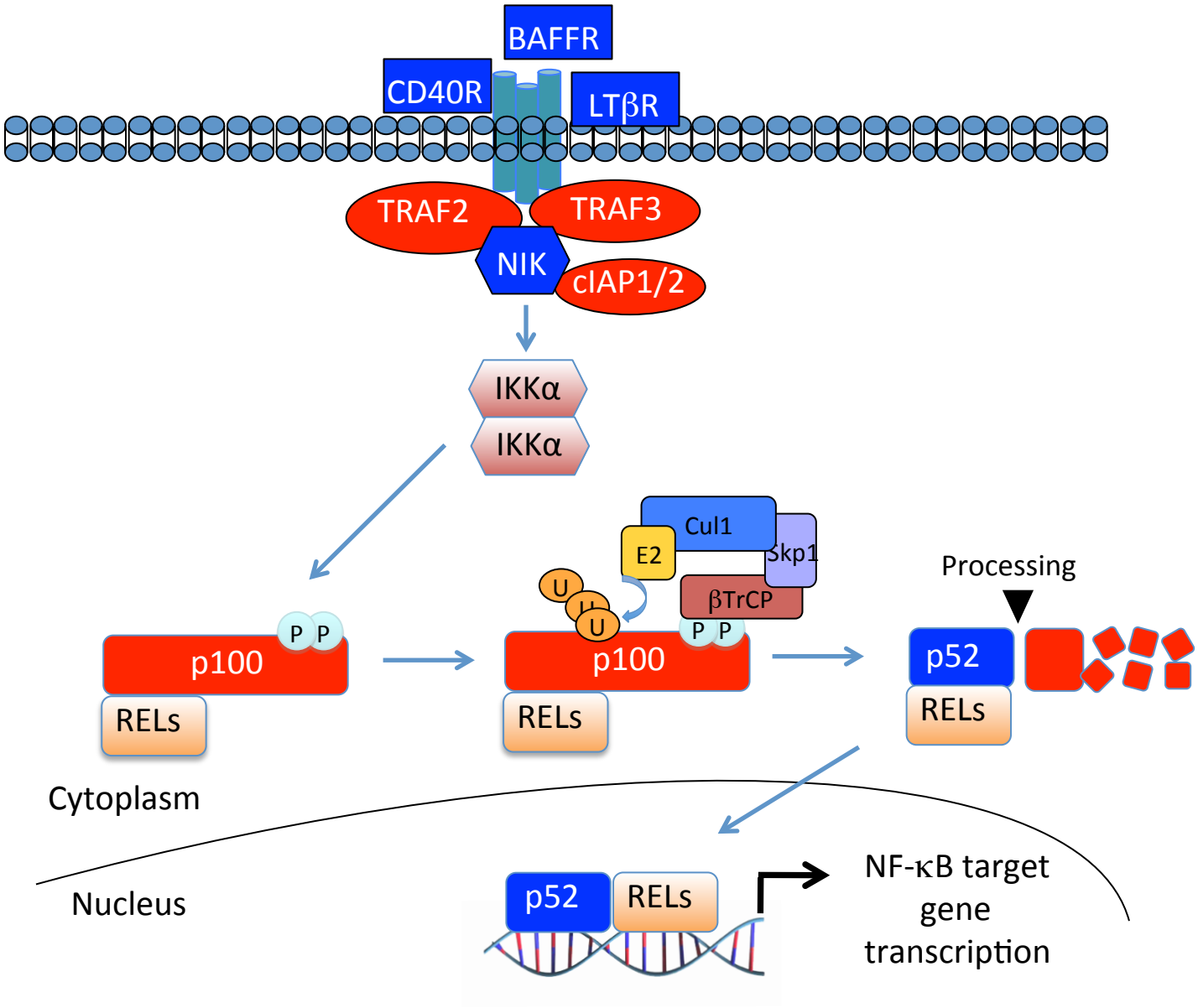
# The NF-κB Family of proteins



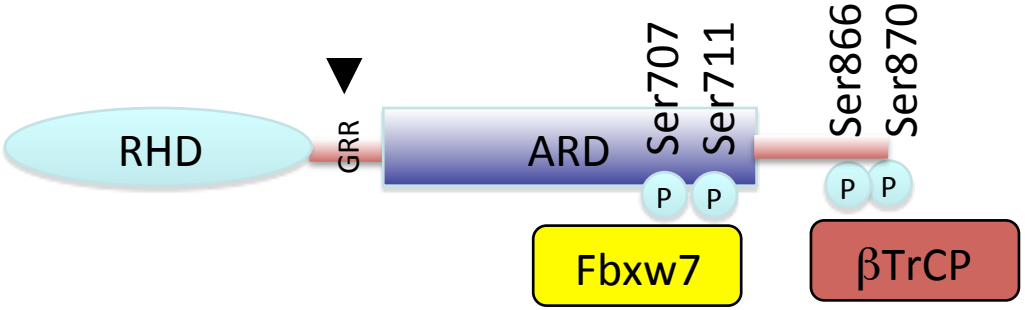
# Molecular mechanism of NF-κB activation and inhibition

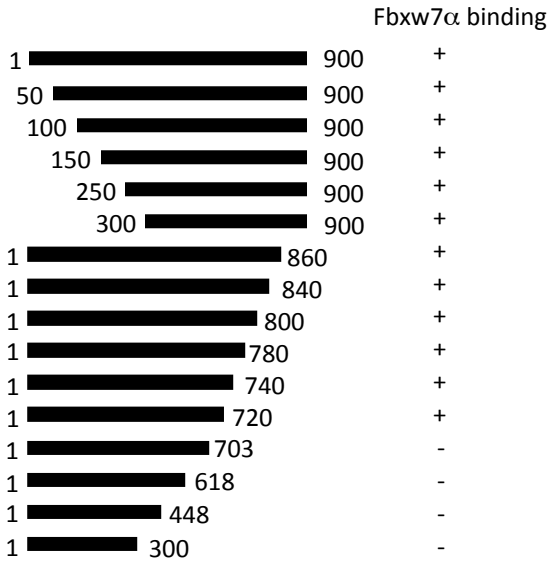


# The non-canonical pathway of NF-κB

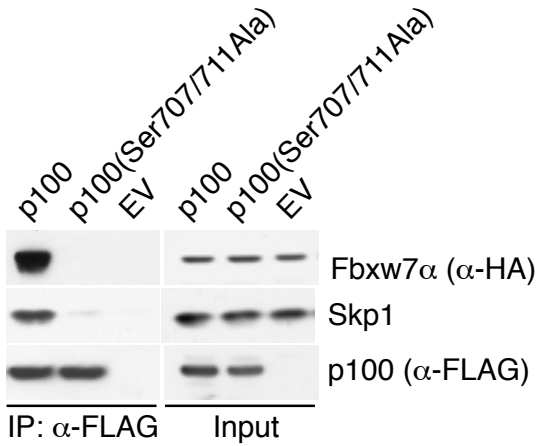


# p100 binds Fbxw7α through Ser707 and Ser711

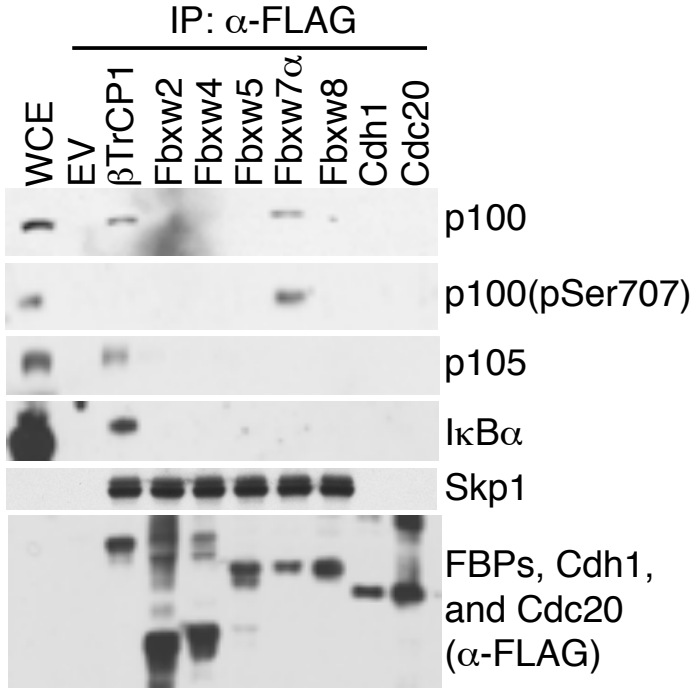


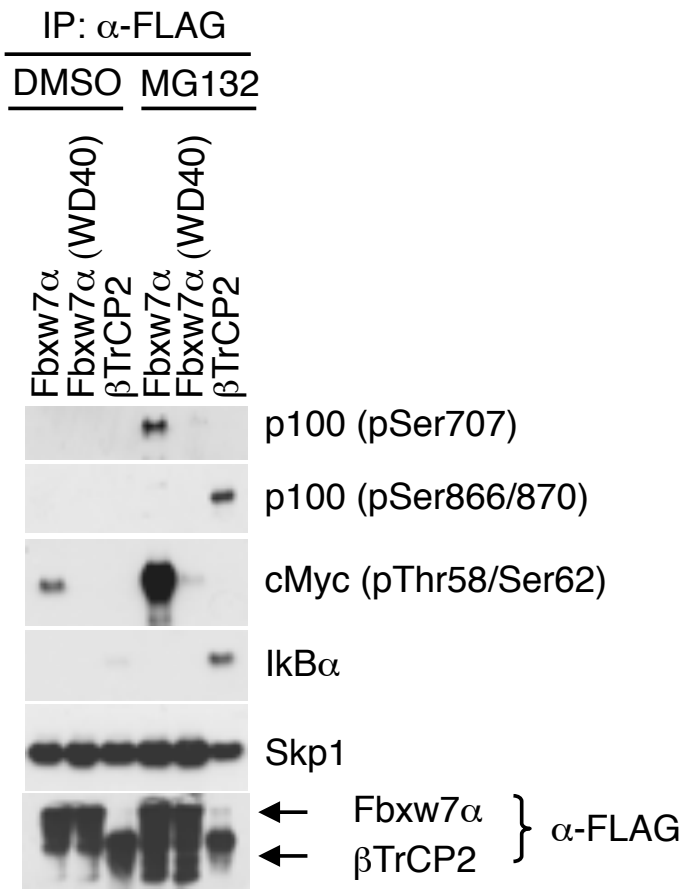

| | |
|---|---|
| p100 | LPSPPTSDS |
| CyclinE$_1$ | LLTPPQSGK |
| CyclinE$_2$ | IPTPDKEDD |
| MYC | LPTPPLSPS |
| JUN | GETPPLSPI |
| Notch1 | FLTPSPESP |
| SREBP1 | TLTPPPSDA |



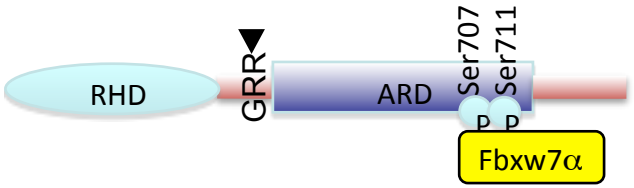
# Fbxw7α binds p100 phosphorylated on Ser707



# Fbxw7α and βTrCP bind two different phospho-forms of p100

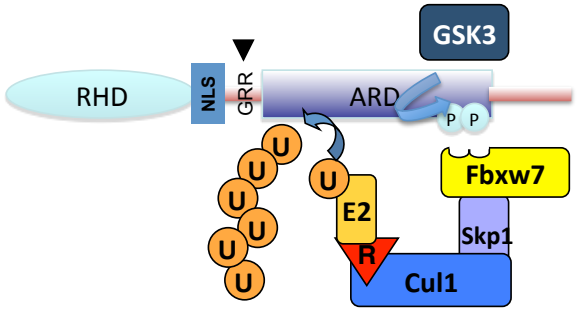


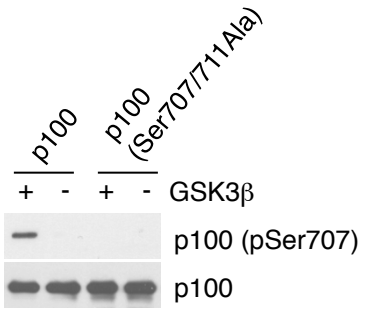

Fbxw7-mediated degradation



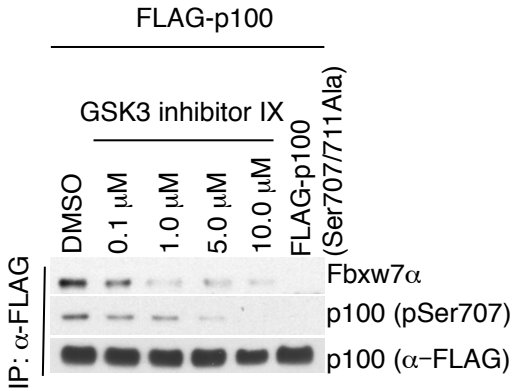

βTrCP-mediated processing

# GSK3 phosphorylates the p100 degron

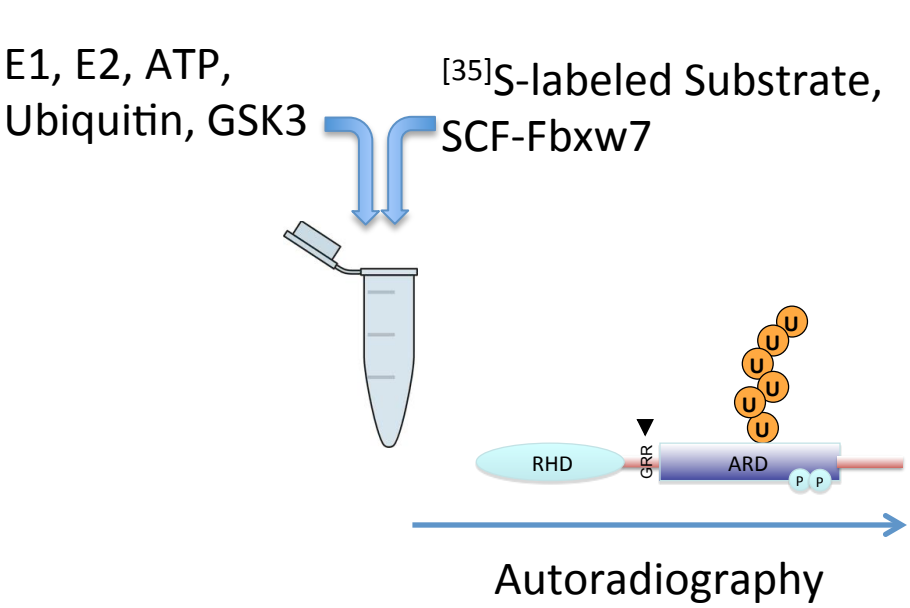


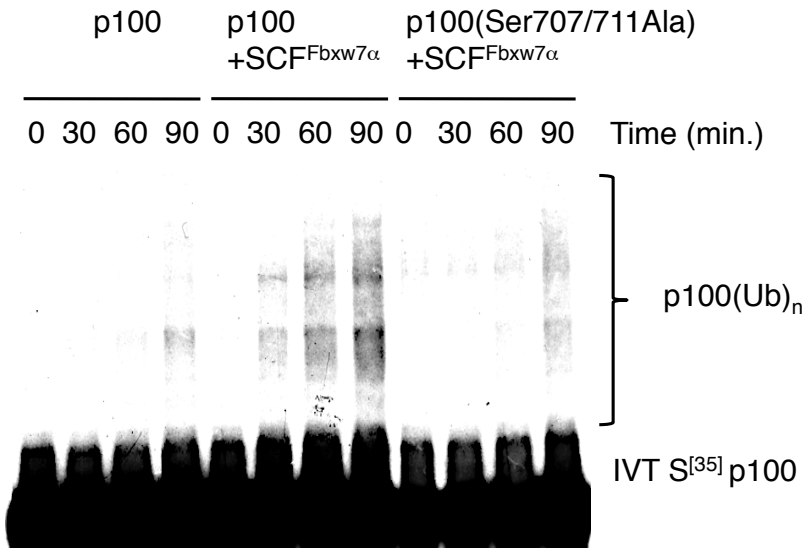





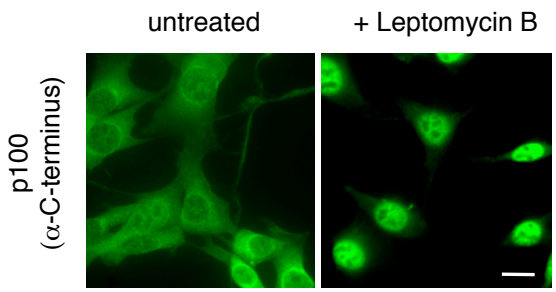


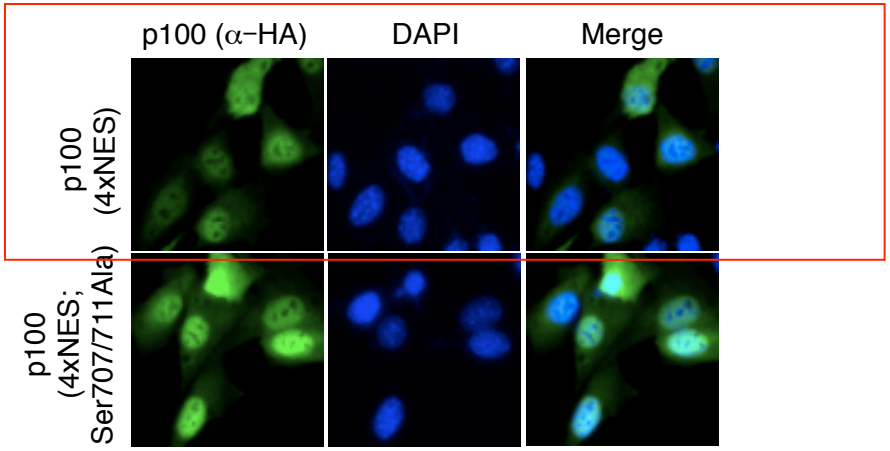
# p100 is ubiquitylated *in vitro* in an $SCF^{Fbxw7\alpha}$-dependent manner



# p100 shuttles between the nucleus and cytoplasm



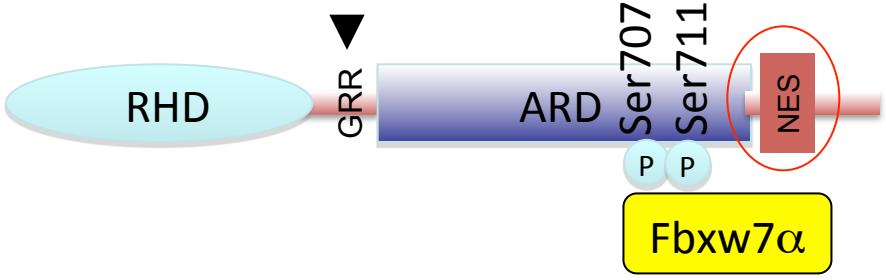




ΦxxxΦxxΦxΦ
LLEALSDMGL
AAEALSDAGA

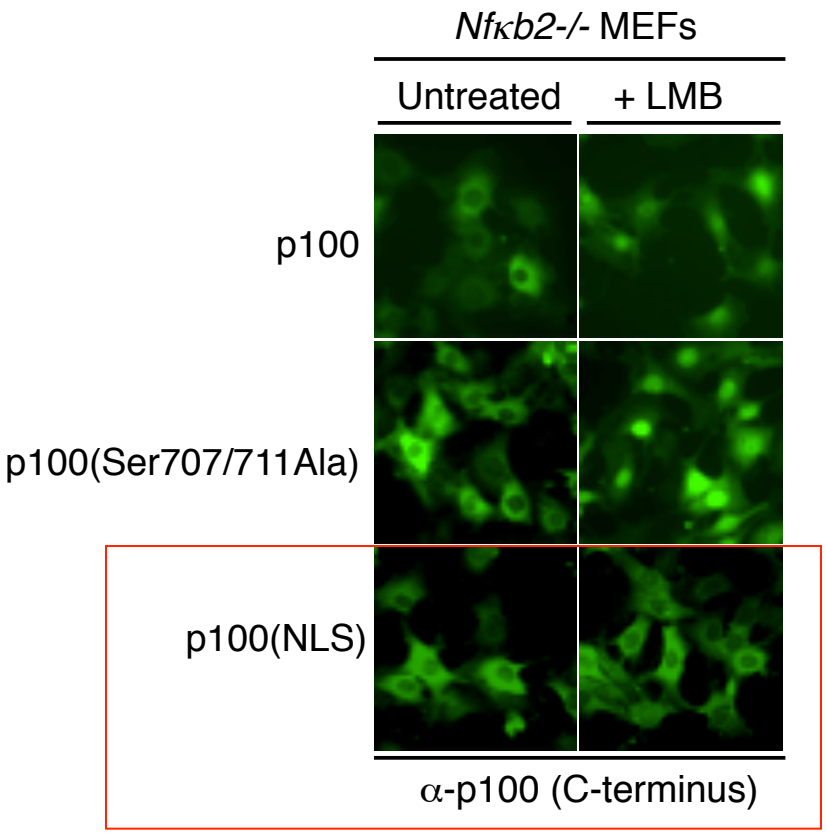
PKI NES consensus
p100 (aa 831-840)
p100 (aa 831-840; 4xNES)

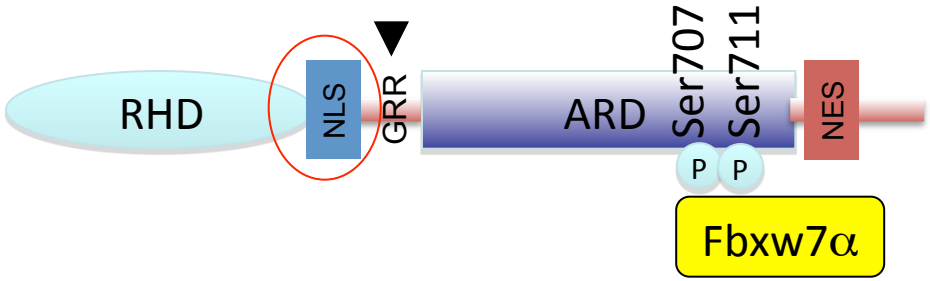
Φ = Hydrophobic Amino Acids
(Leu, Ile, Val, Met, Phe)

x = small/polar/charged amino acids

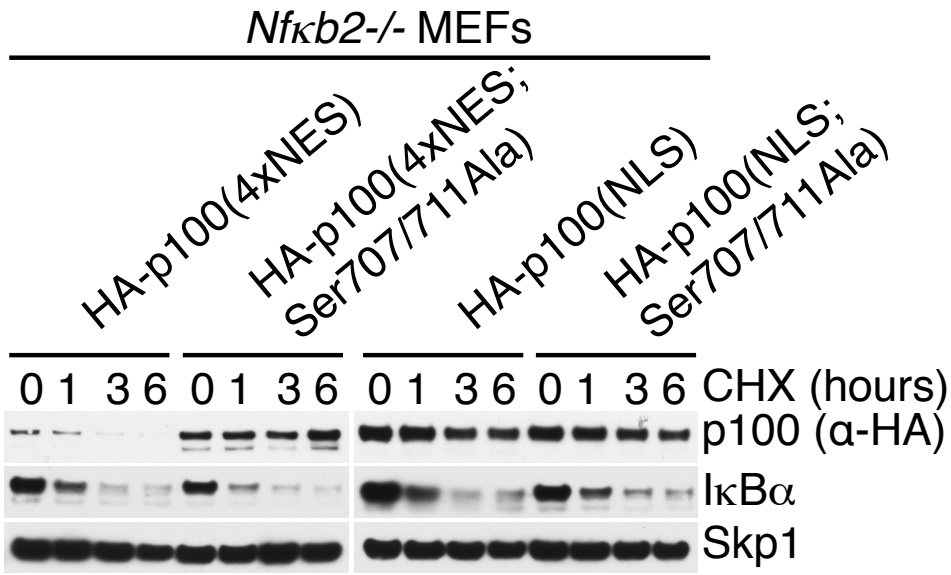


# p100 contains an NLS required for import into the nucleus

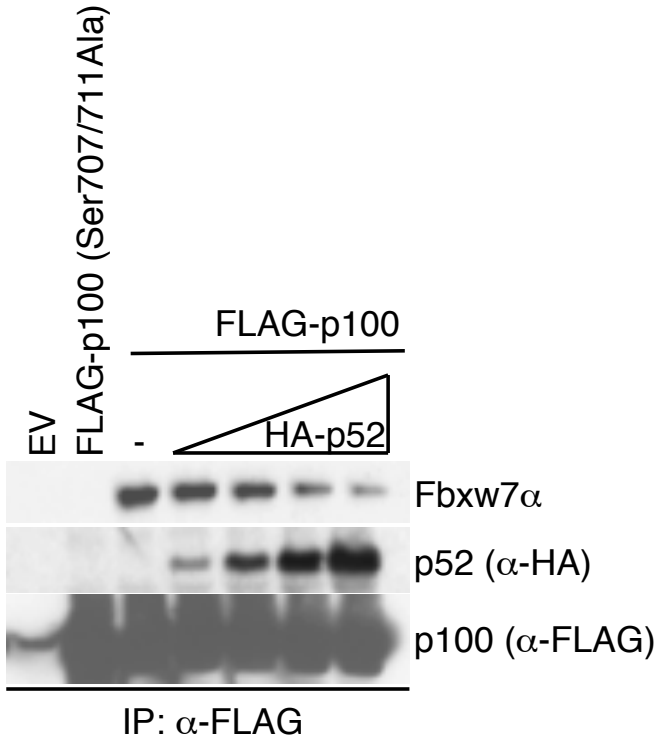


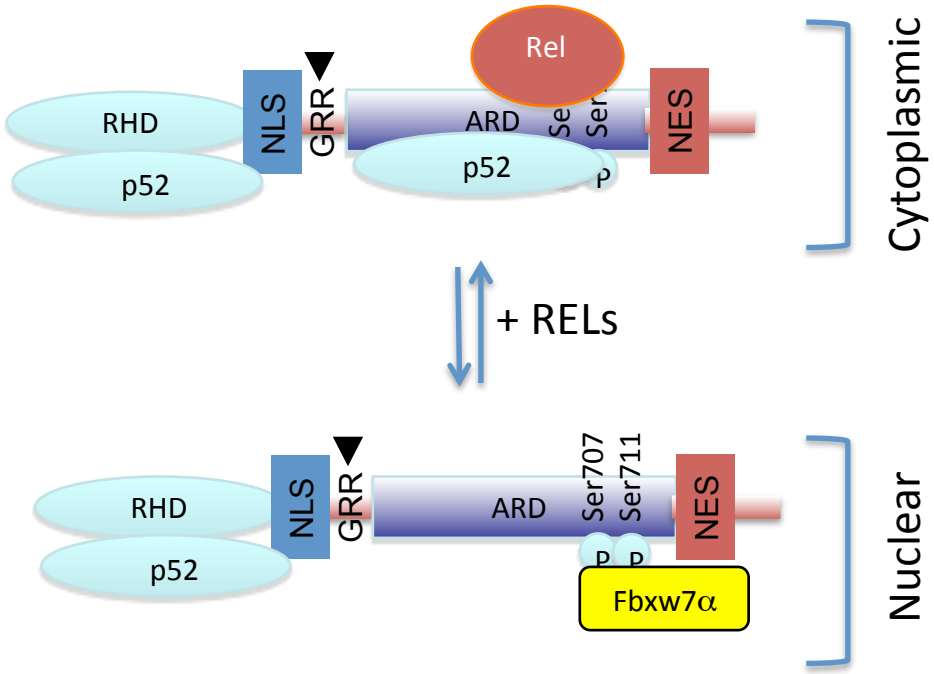



# Fbxw7α targets only nuclear p100 for protein degradation



# p52 competes with Fbxw7α for binding to the ARD of p100



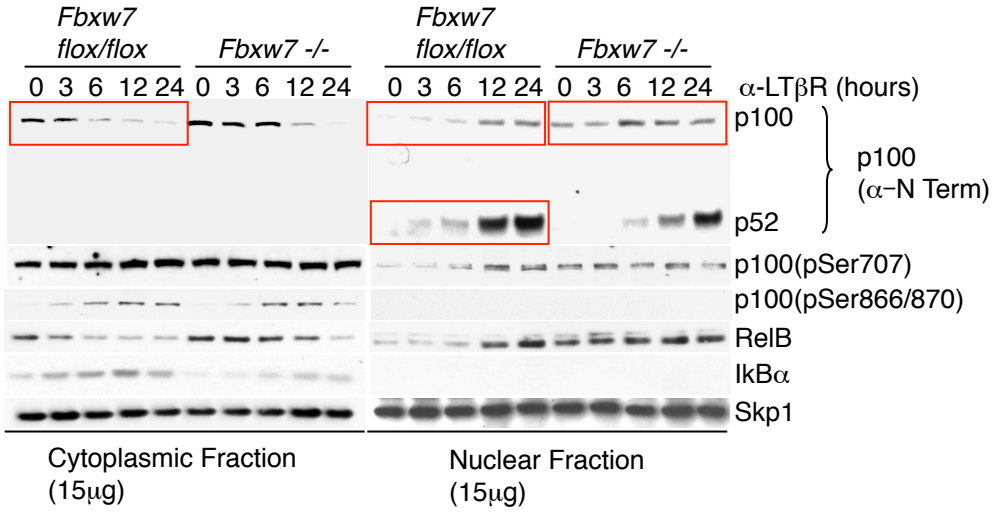



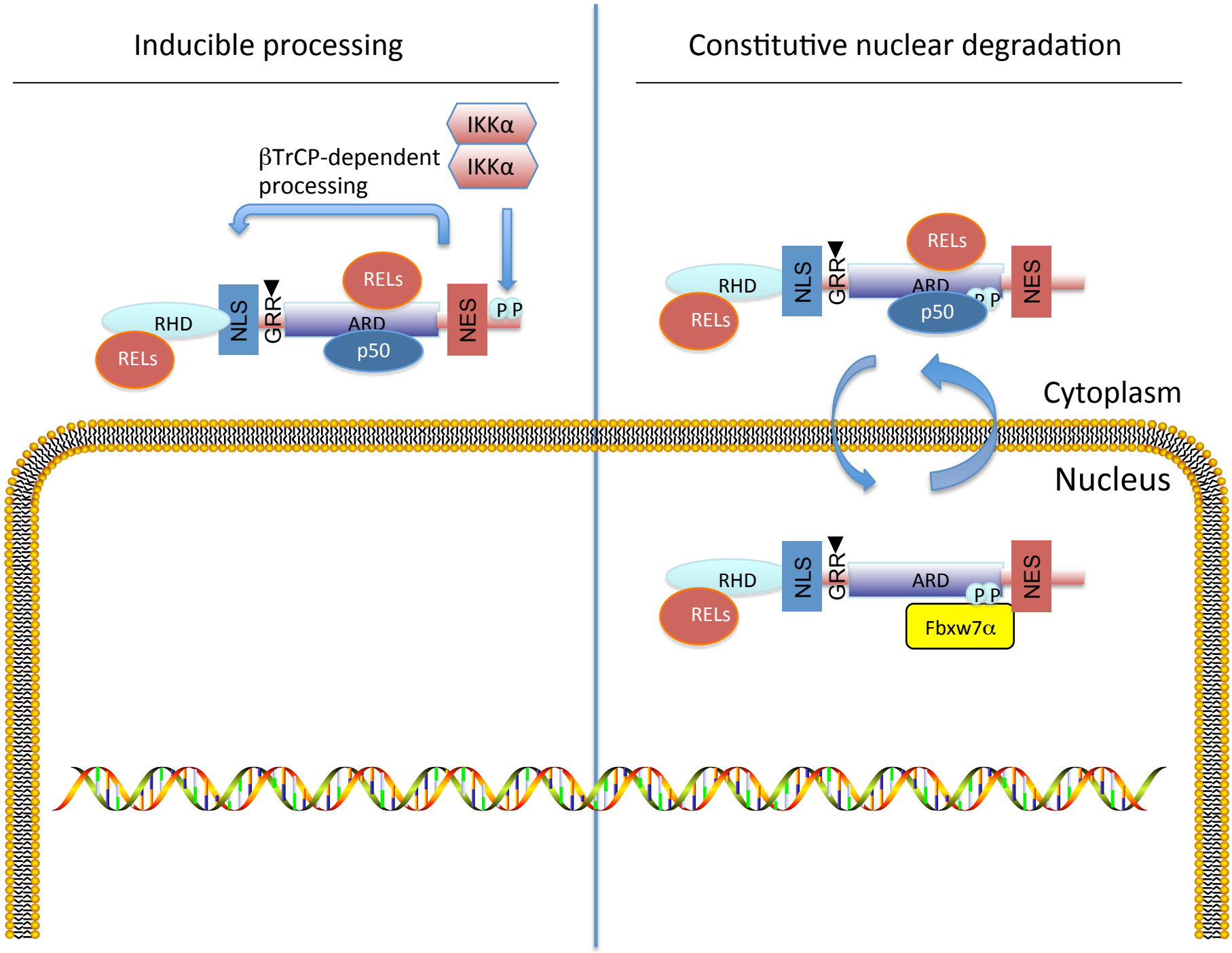
# Conclusions #1

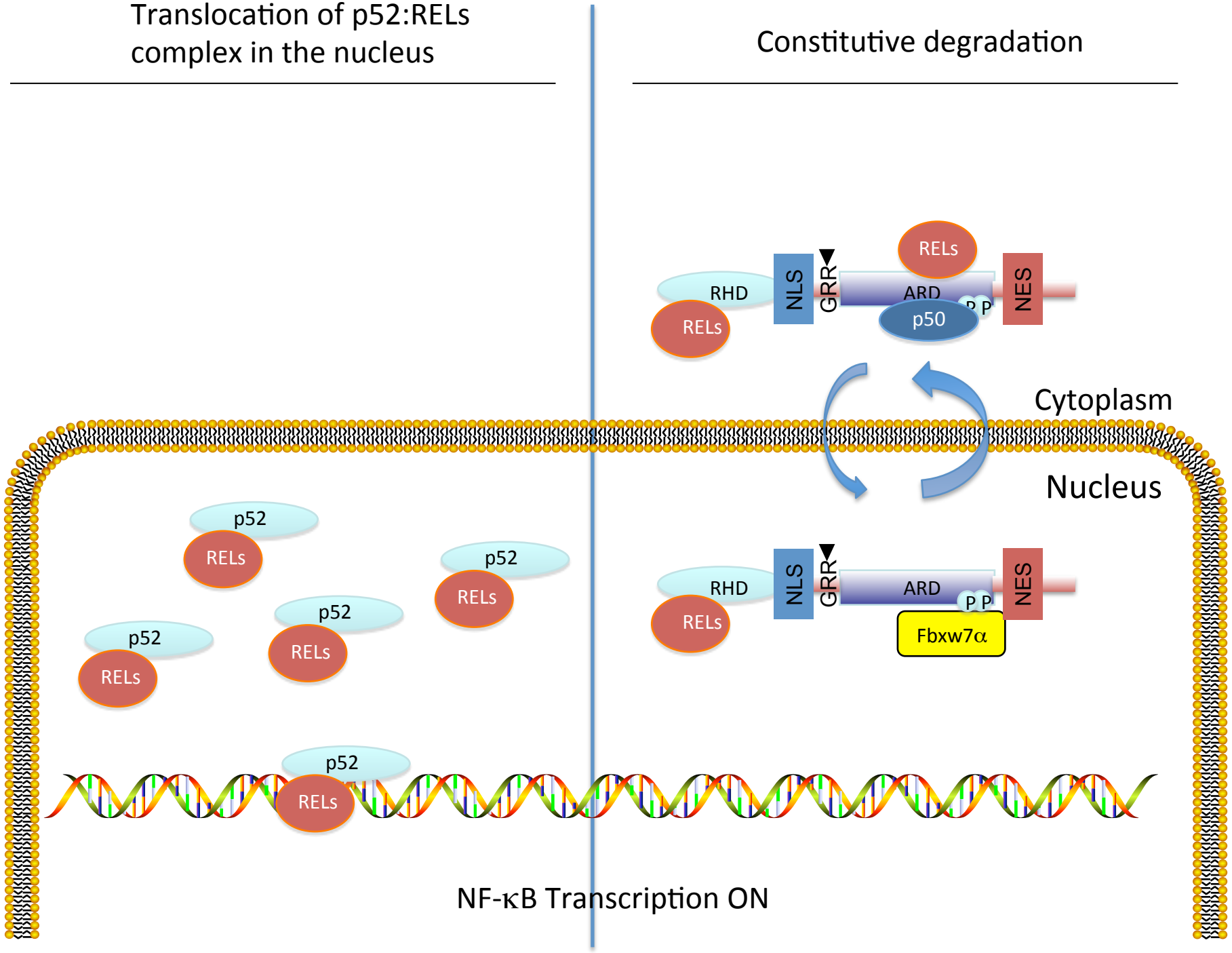
a) *Shuttling of p100 requires an intact NLS (nuclear localization signal) and NES (nuclear export signal).*

b*) The majority of p100 is cytoplasmic since the nuclear pool is constitutively targeted for protein degradation by Fbxw7/GSK3. Accordingly, the p100-cytoplasmic pool is insensitive to Fbxw7-mediated degradation.*

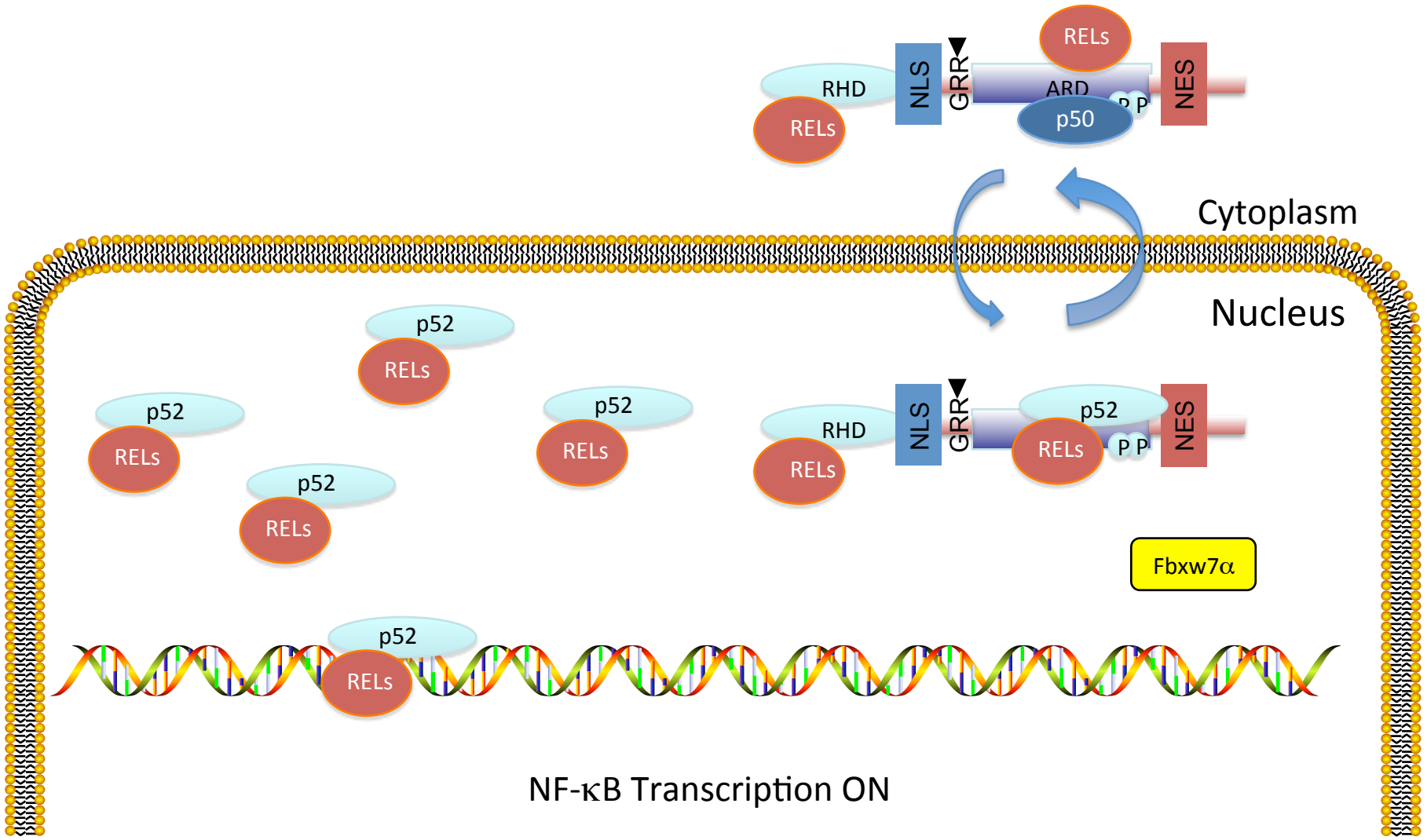
# Constitutive degradation of p100 in the nucleus via Fbxw7α is inhibited upon NF-κB activation



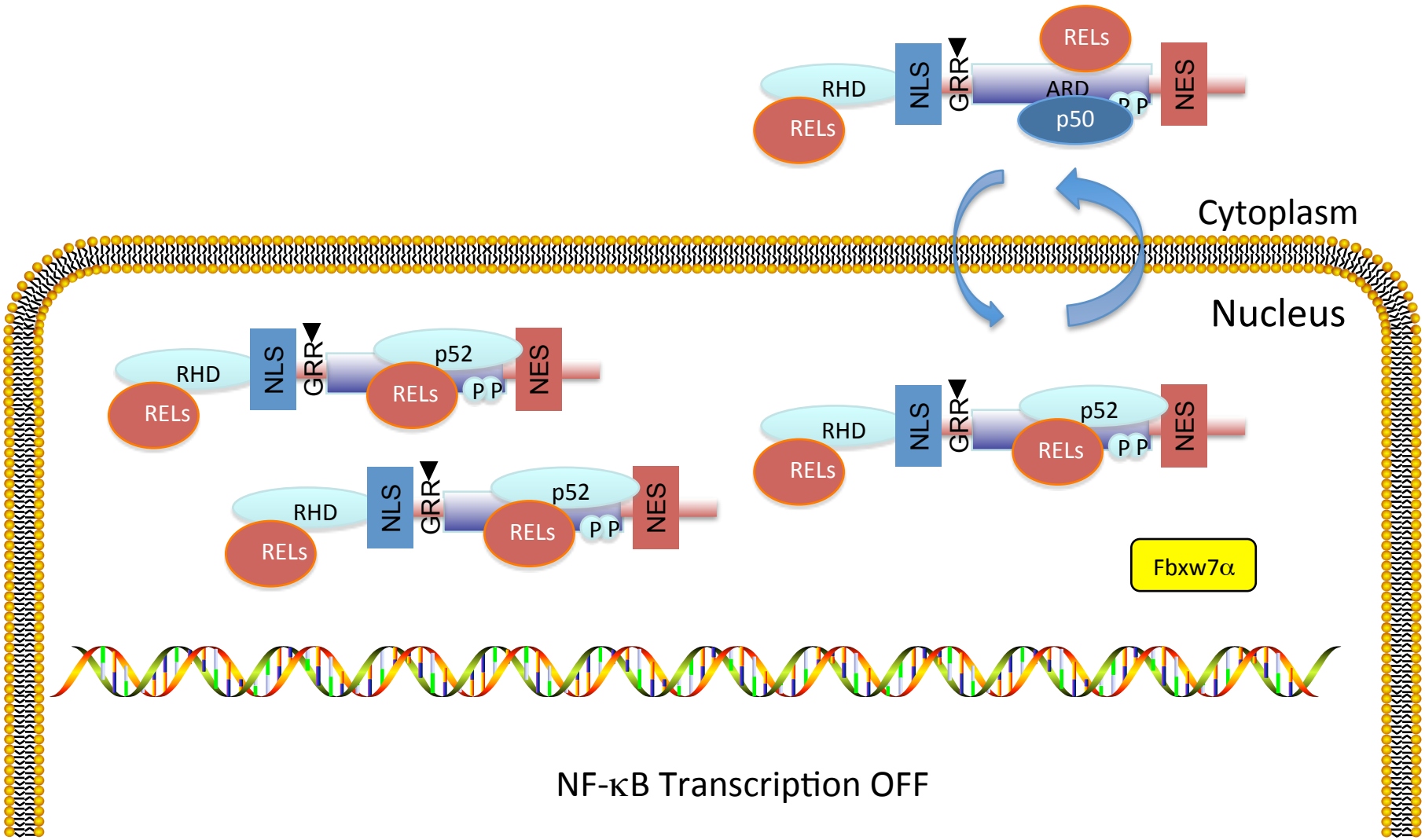

Inducible processing
Constitutive nuclear degradation
IKKα
IKKα
βTrCP-dependent
processing
RELs
RHD
RELs
NLS
GRR
ARD
p50
NES
P P
RELs
RHD
RELs
NLS
GRR
ARD
p50
P P
NES
Cytoplasm
Nucleus
RHD
RELs
NLS
GRR
ARD
P P
NES
Fbxw7α


Translocation of p52:RELs complex in the nucleus
Constitutive degradation
RELs
RHD
NLS
GRR
ARD
NES
P P
p50
RELs
Cytoplasm
Nucleus
p52
RELs
p52
RELs
p52
RELs
p52
RELs
RHD
NLS
GRR
ARD
NES
P P
RELs
Fbxw7α
p52
RELs
NF-κB Transcription ON

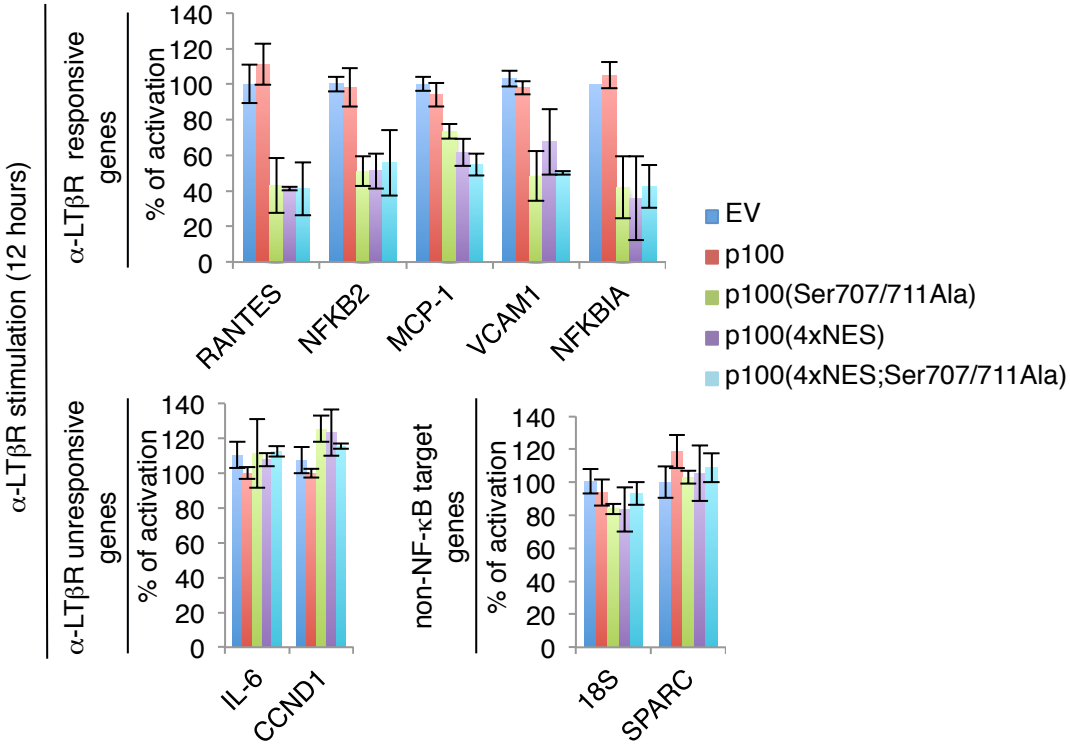
# p52-RELs competition with Fbxw7 for binding to p100



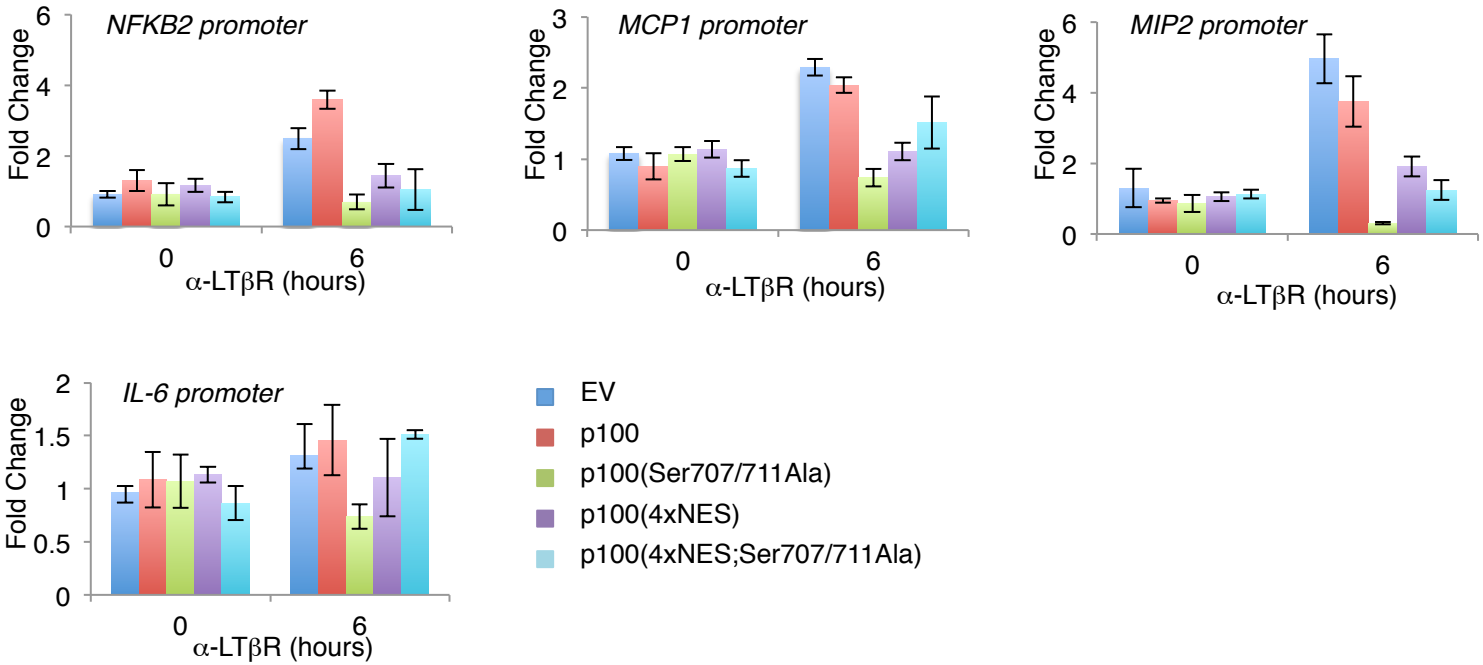
# p100 stabilization and termination of NF-κB signaling



# Stabilization of p100 in the nucleus inhibits NF-κB target gene transcription following LTβR activation



# Stabilization of p100 in the nucleus inhibits RelB binding at LTβR-responsive gene promoters



# Conclusions #2

*The molecular mechanisms controlling levels of p100 in the nucleus allow the initiation and termination of the NF-κB transcription program.*

# *FBXW7* mutations are absent in B-cell derived tumors

- Mutation of FBXW7 gene have been found in many cancers, including: T-ALL, breast cancers, cholangiocarcinoma, gastric adenocarcinoma, and head and neck squamous carcinoma

Thompson, B. J. *et al.*, *J Exp Med* 2007
Rajagopalan, H. *et al., Nature* 2004
O'Neil, J. *et al*., *J Exp Med* 2007
Stransky, N. *et al.*, *Science*, 2011

- Sequenced exons 9 and 10 of twenty-four multiple myeloma cell lines.
- No mutations found.

-Sequencing of *FBXW7* in primary B-cell tumors (from the literature):

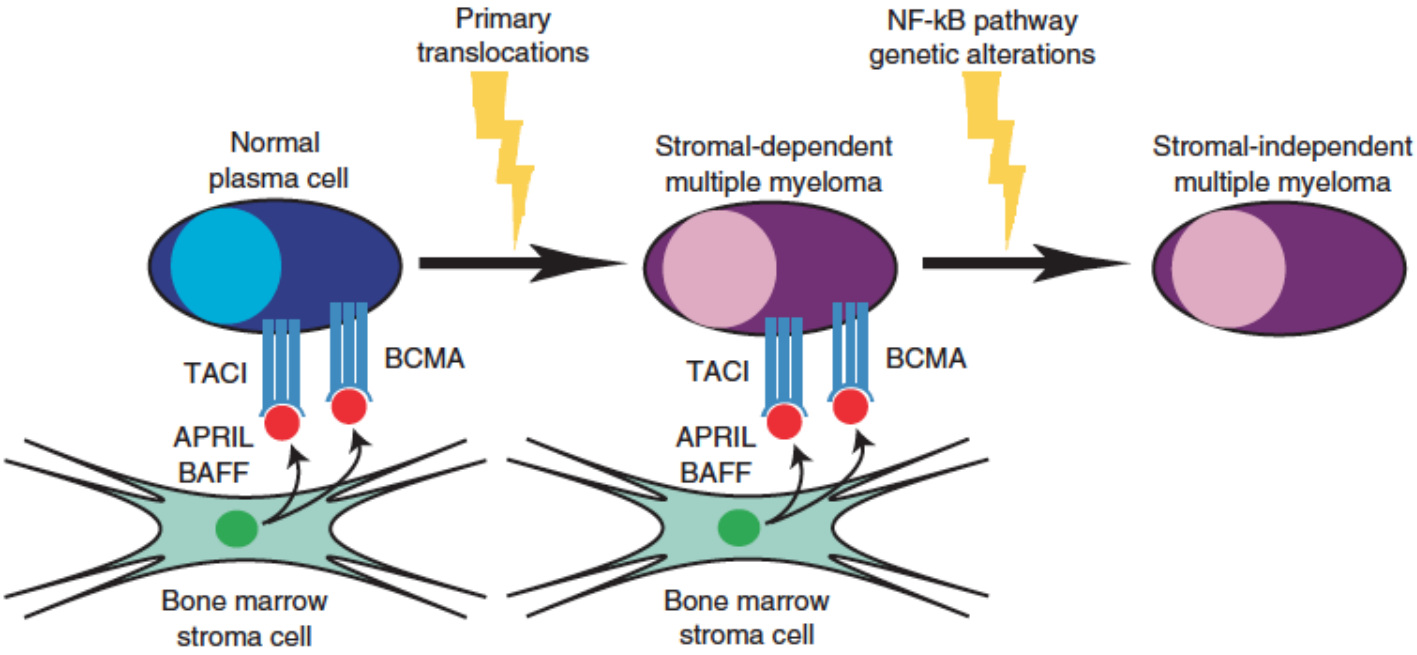
Study #1
0/20 B-ALL
0/20 B-CLL

Akhoondi et al. *Cancer Res*. 2007.

Study #2
0/92 non-Hodgkins lymphoma

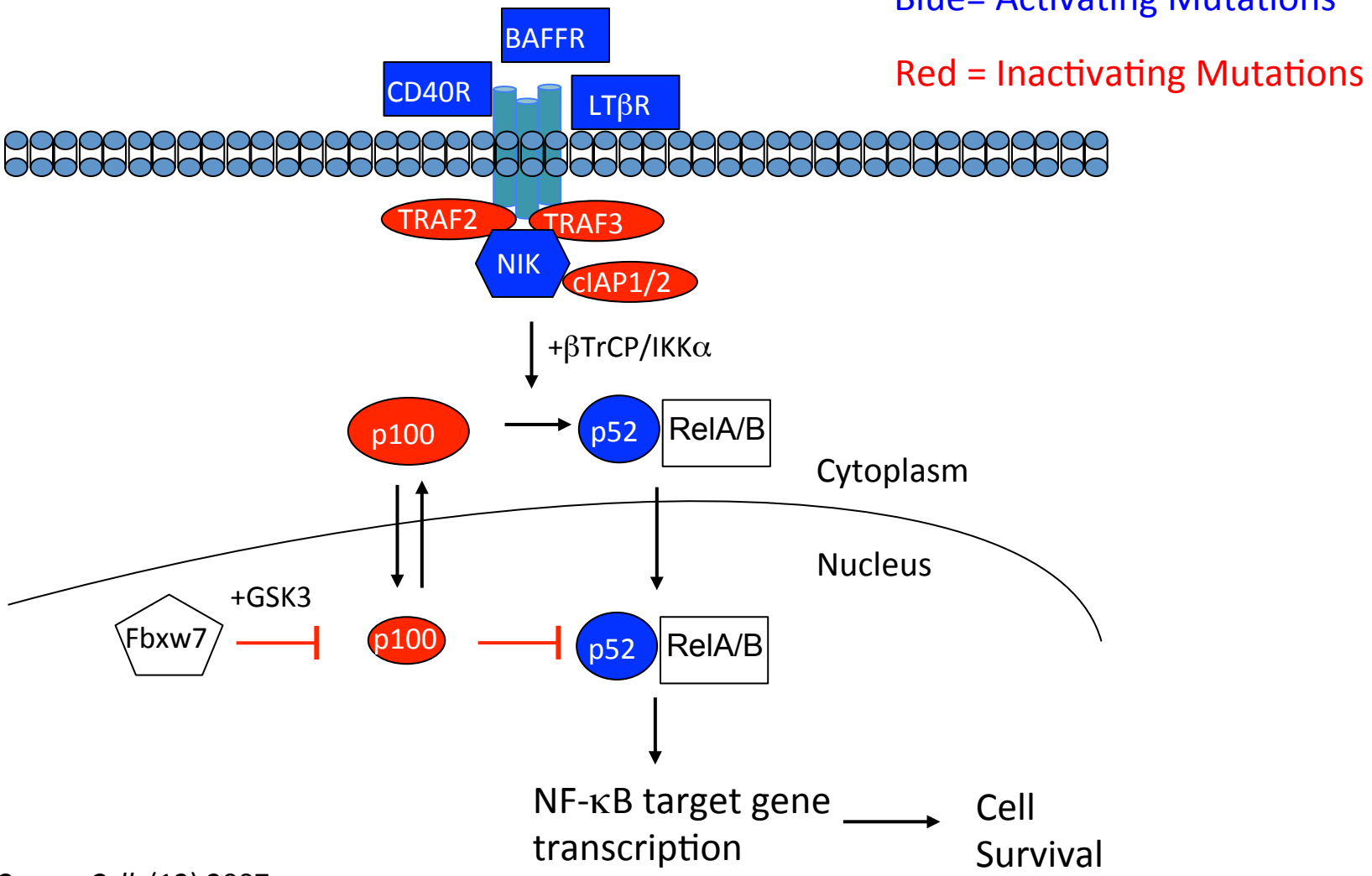
Song et al. *Leuk, Res*. 2008.

# Relevance of NF-κB signaling in stromal-independent multiple myeloma




Louis M. Staudt, *CSH Perspect in Biol*., 2010

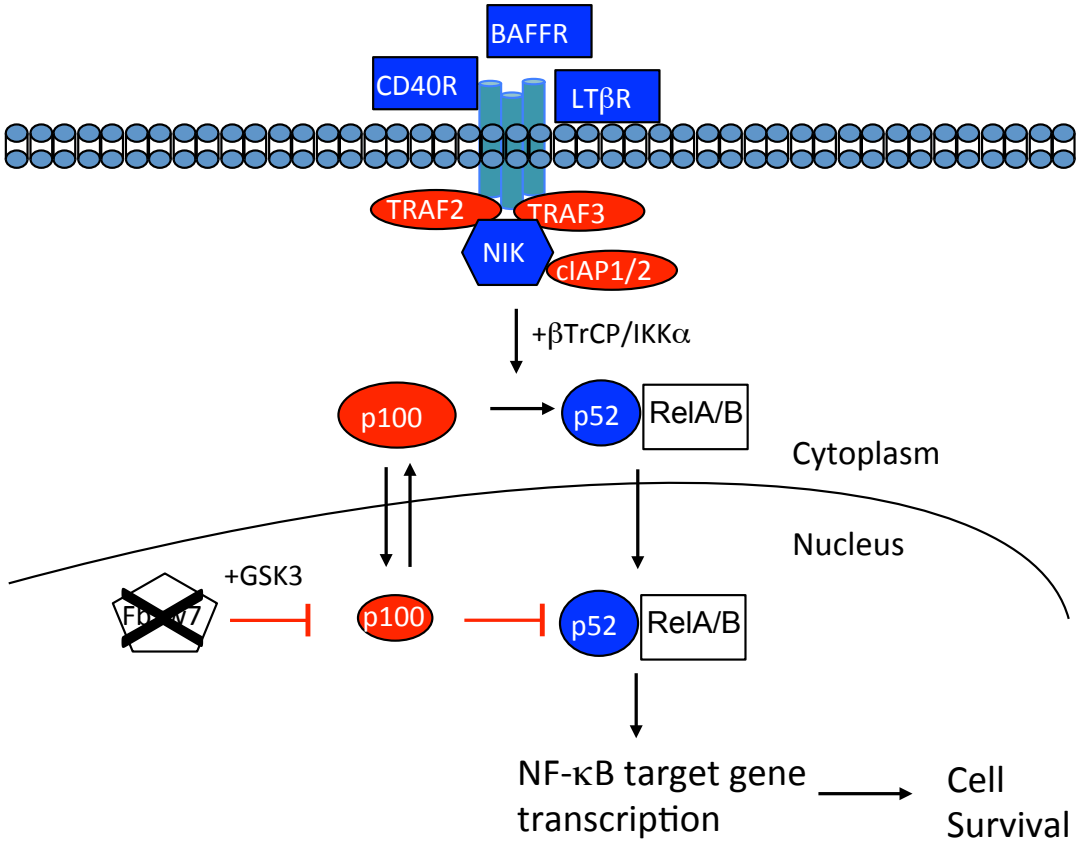
# Mutations activate non-canonical NF-κB signaling in multiple myeloma

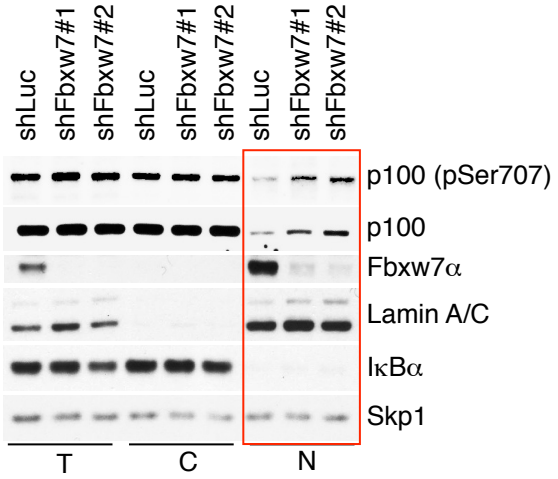


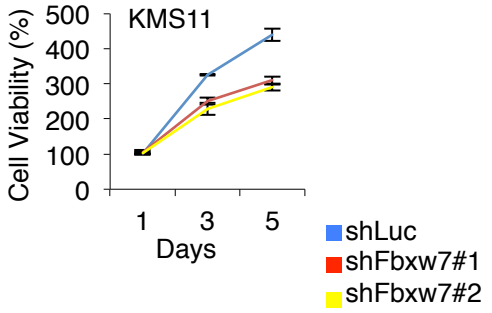

Keats et. al. *Cancer Cell.* (12) 2007.

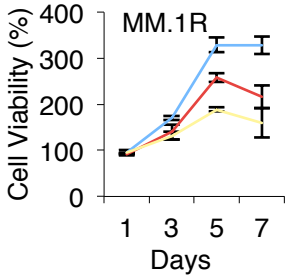
Annunziata et. al. *Cancer Cell*. (12) 2007.

# Depletion of Fbxw7 inhibits multiple myeloma cell growth

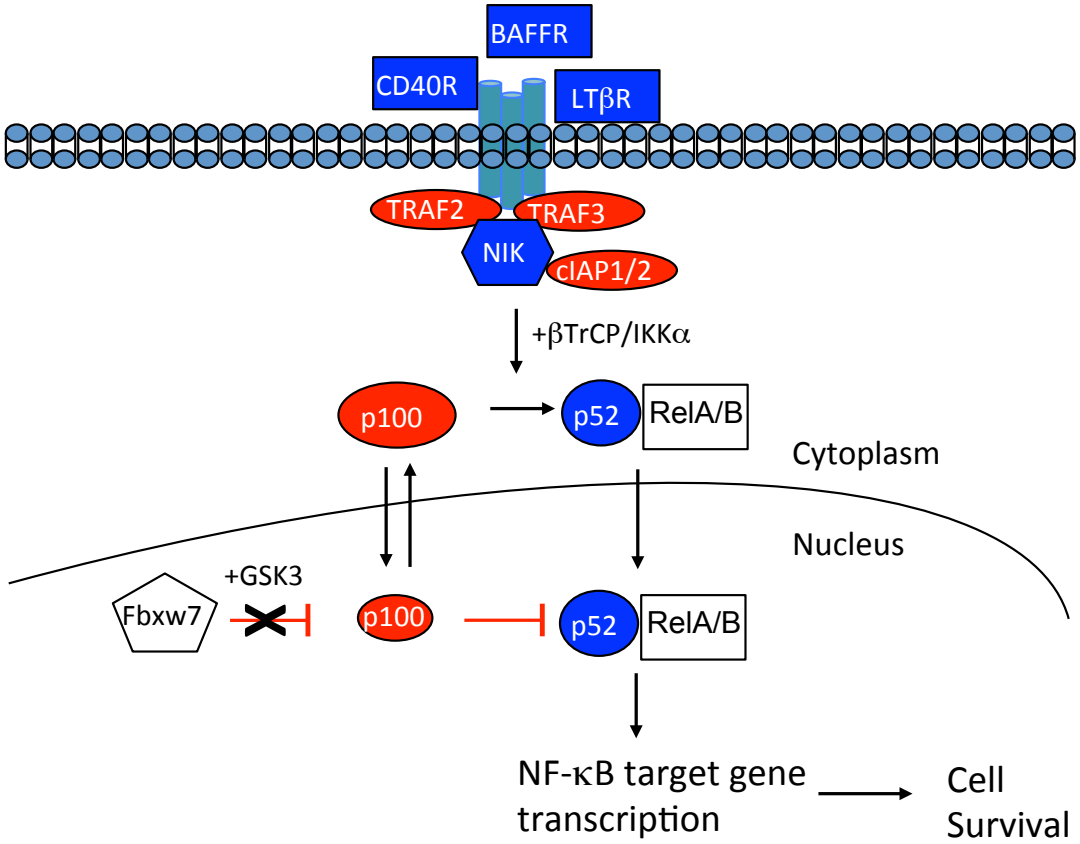


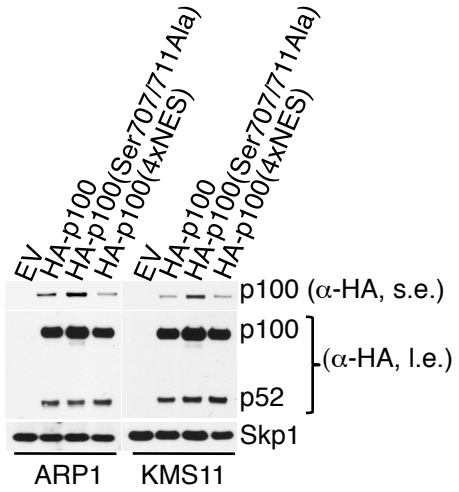



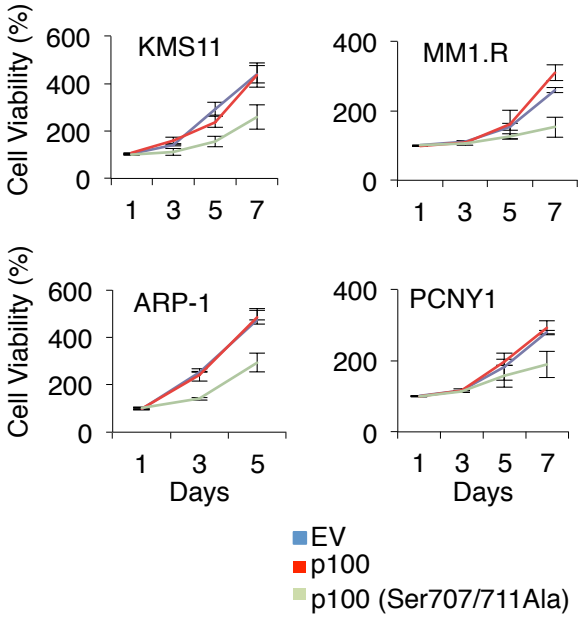



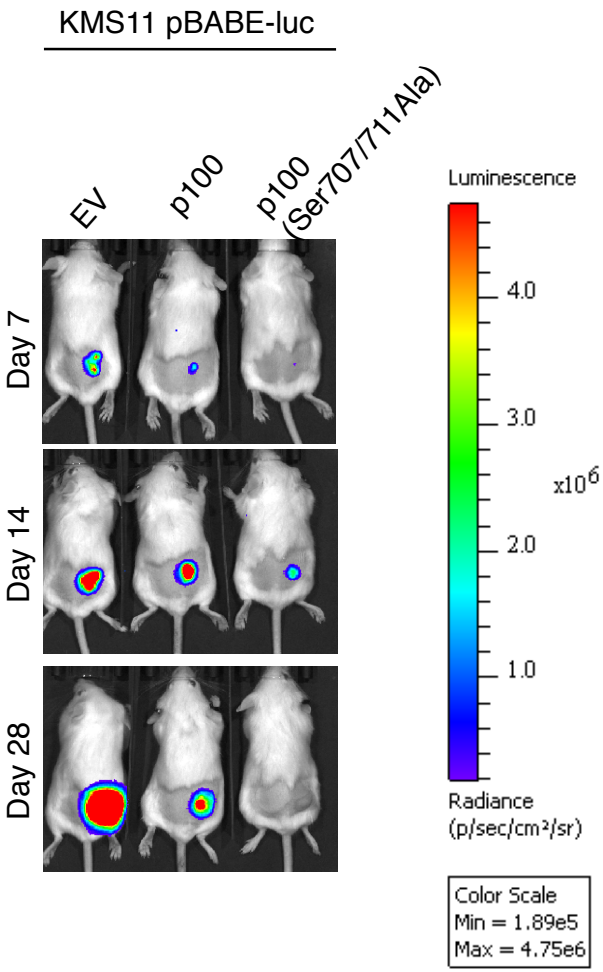
# Expression of a p100 mutant resistant to Fbxw7−dependent degradation inhibits multiple myeloma cell growth

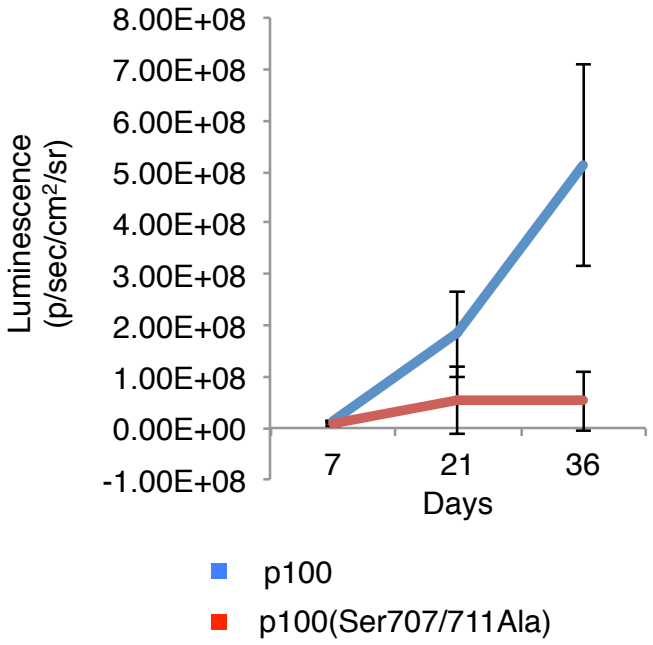


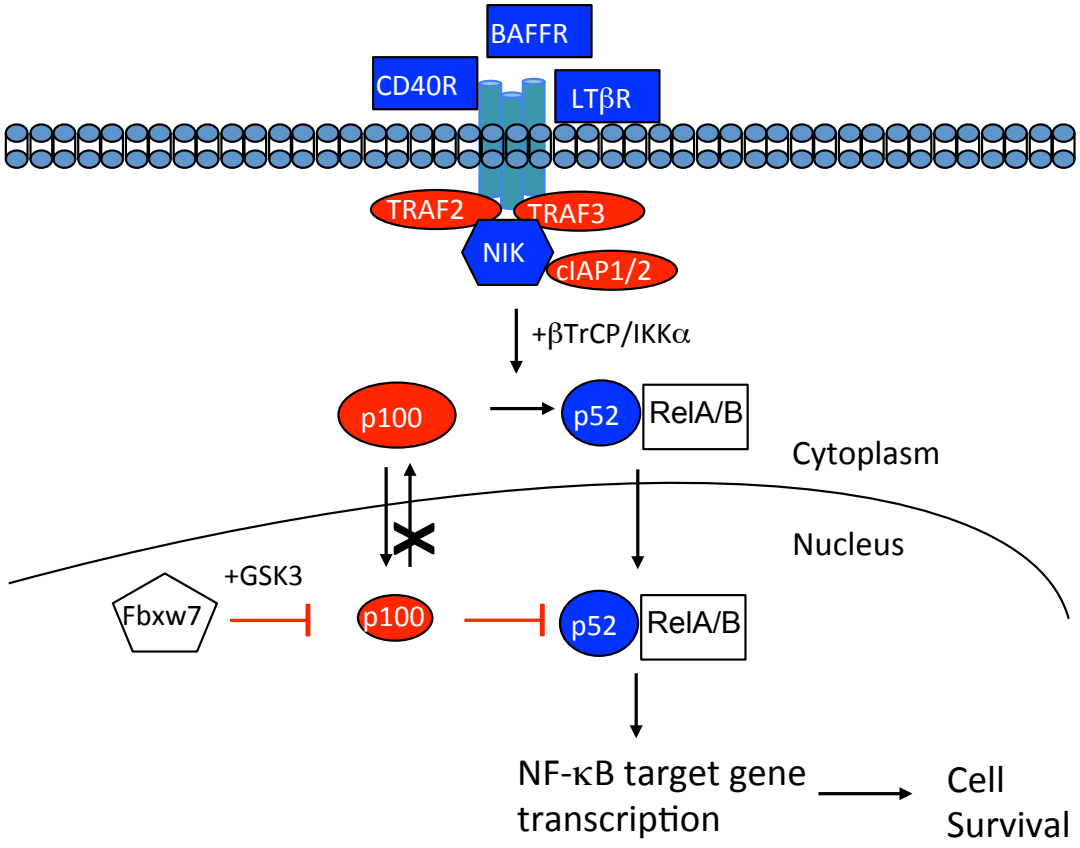



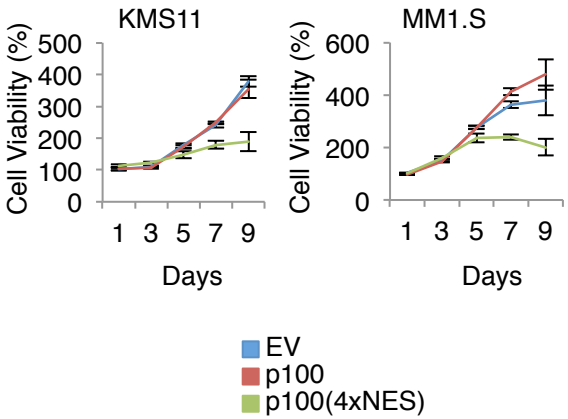
# Expression of stable p100 inhibits the growth of myeloma cells xenotransplanted into SCID mice



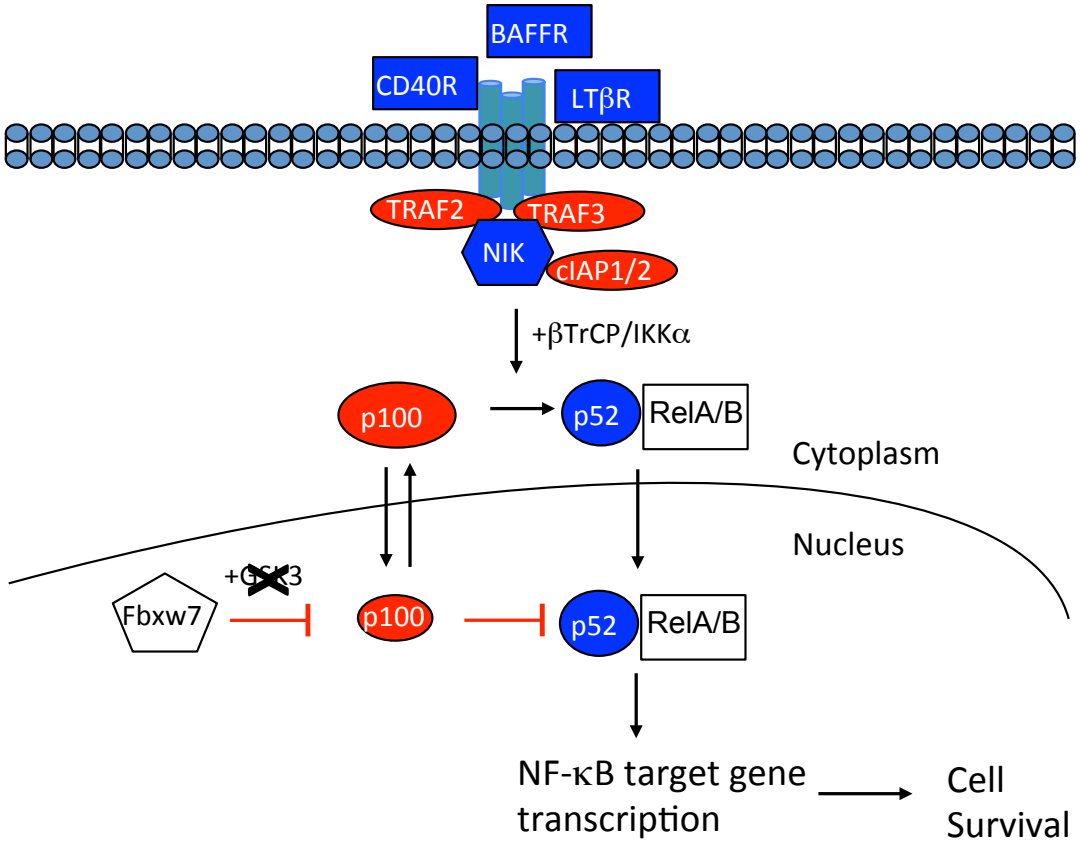



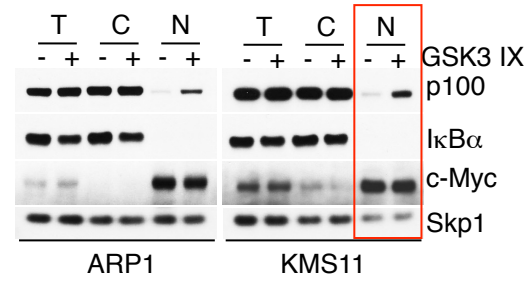
# Forced localization of p100 in the nucleus results in decreased growth of myeloma cells

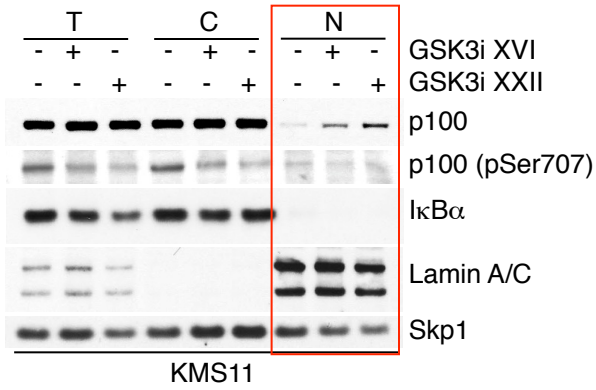


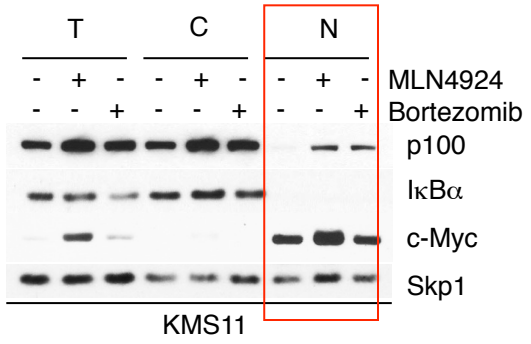



# Chemical inhibition of the proteasome, Cullin-RING ligases, or GSK3 leads to p100 accumulation in the nucleus

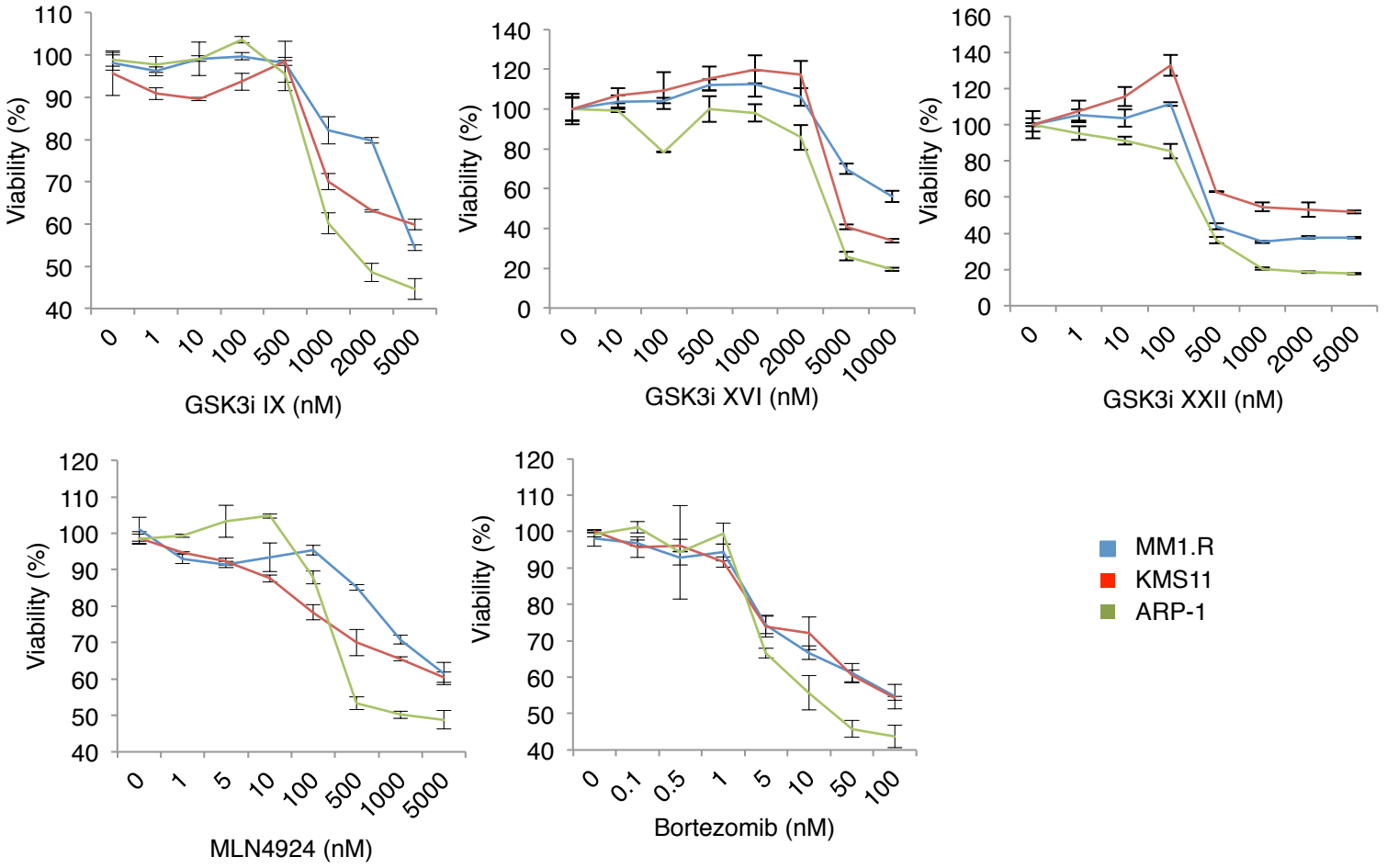


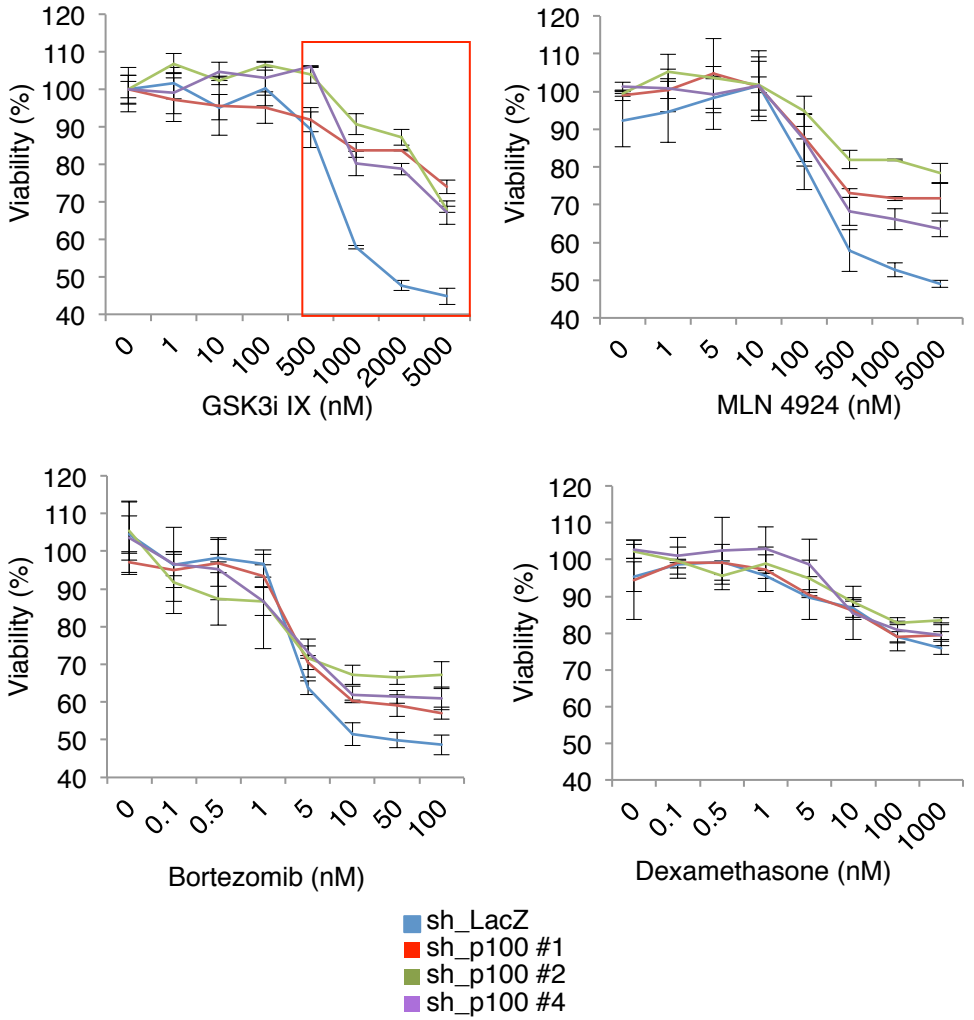



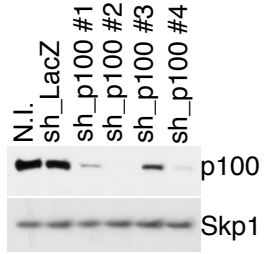



# Chemical inhibition of the proteasome, Cullin-RING ligases, or GSK3 is toxic to multiple myeloma cells



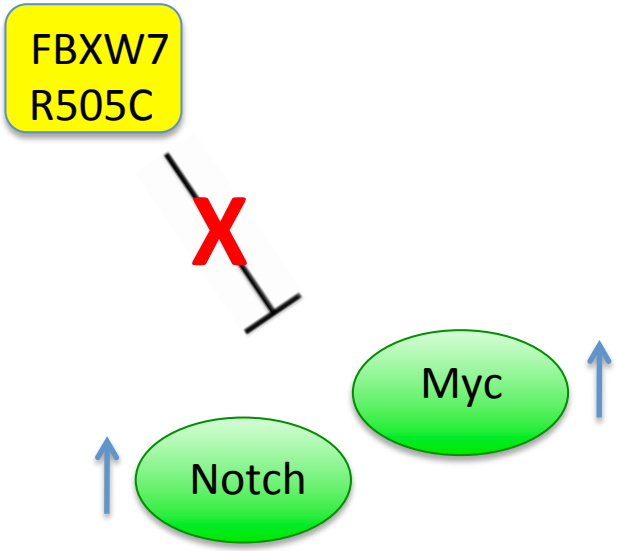
# GSK3 toxicity is partially dependent on p100



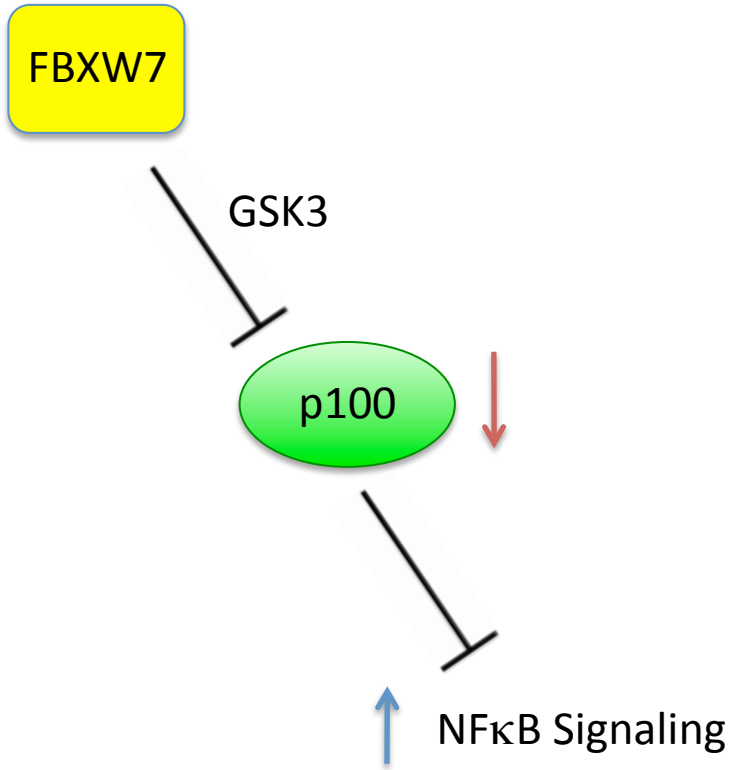



*FBXW7* may function as an oncogene or tumor suppressor depending on the genetic background of the cancer

*T-ALL*
*CLL*
*Colorectal Carcinoma*
*Cholangiocarcinoma*
*Gastric Carcinoma*
*Endometrial Carcinoma*
*Ovarian Carcinoma*




*Multiple Myeloma*
*Diffuse large B-cell lymphoma*



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