

Kennedy's Disease Research at the Lim Lab

- Signaling pathway modulating Androgen Receptor post-translational modifications and its clearance as well as their contribution to KD

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Translational Neuroscience

Neurodegenerative Diseases

successful therapies?

pre-clinical trials

drug discovery

candidate targets for therapeutic intervention

SBMA

gene identification

disease models

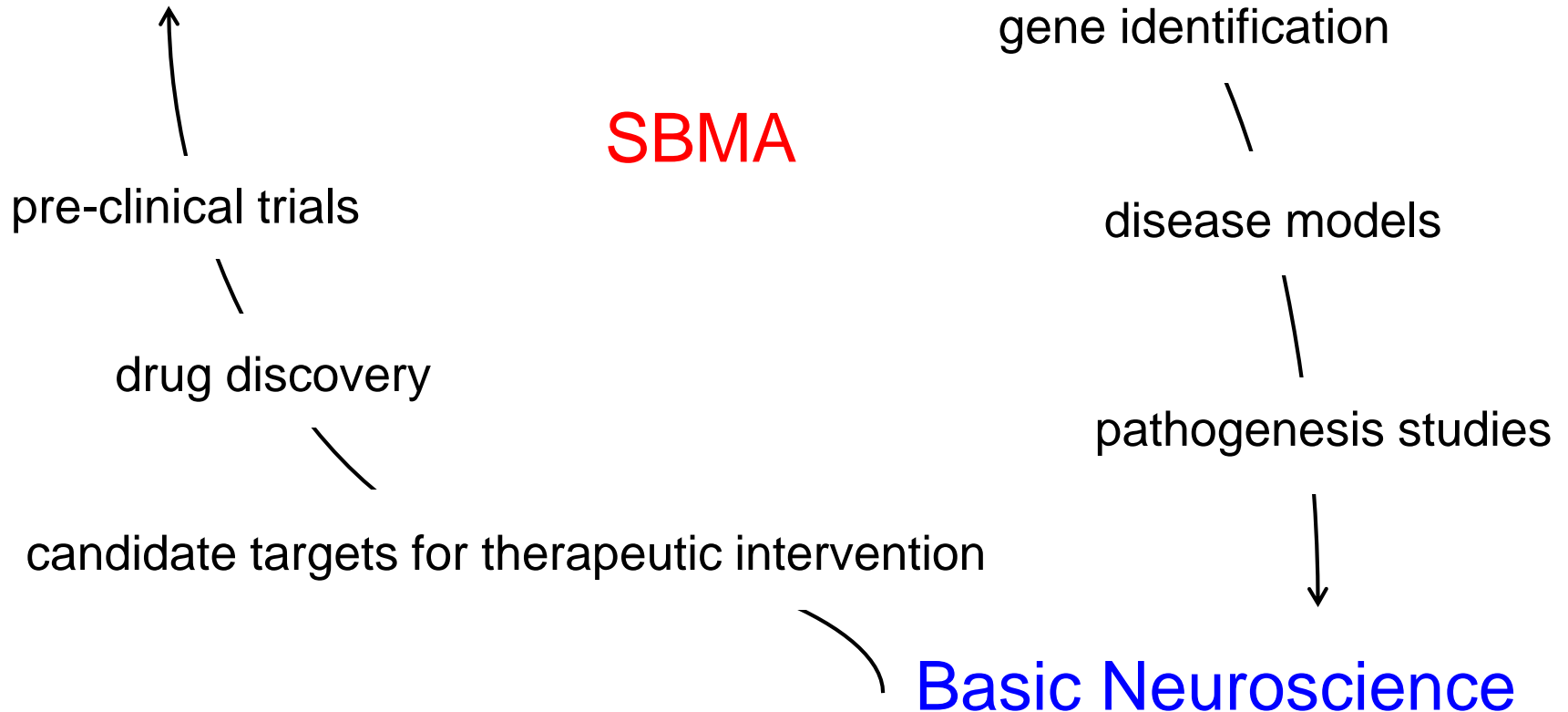
pathogenesis studies

Basic Neuroscience

Translational Neuroscience

Neurodegenerative Diseases

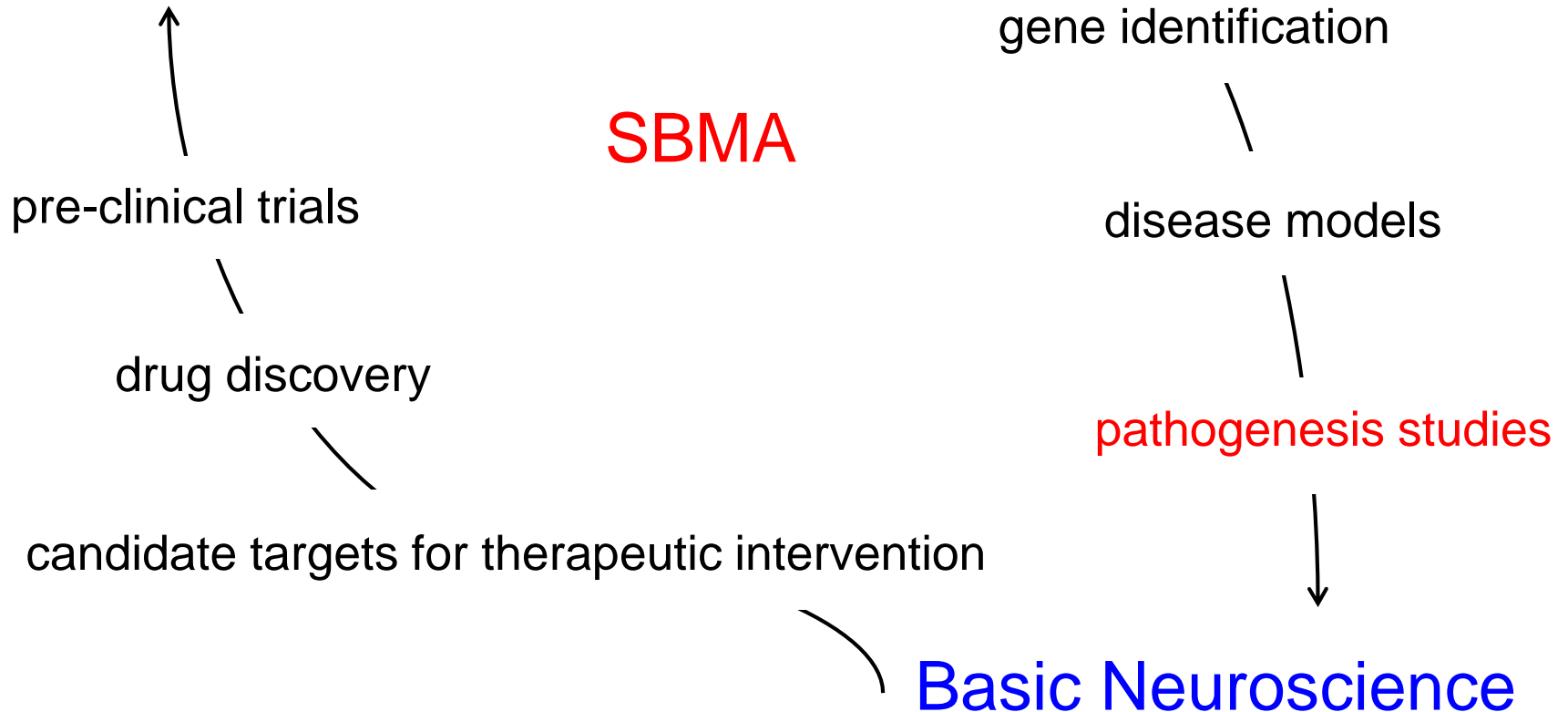
successful therapies?



Translational Neuroscience

Neurodegenerative Diseases

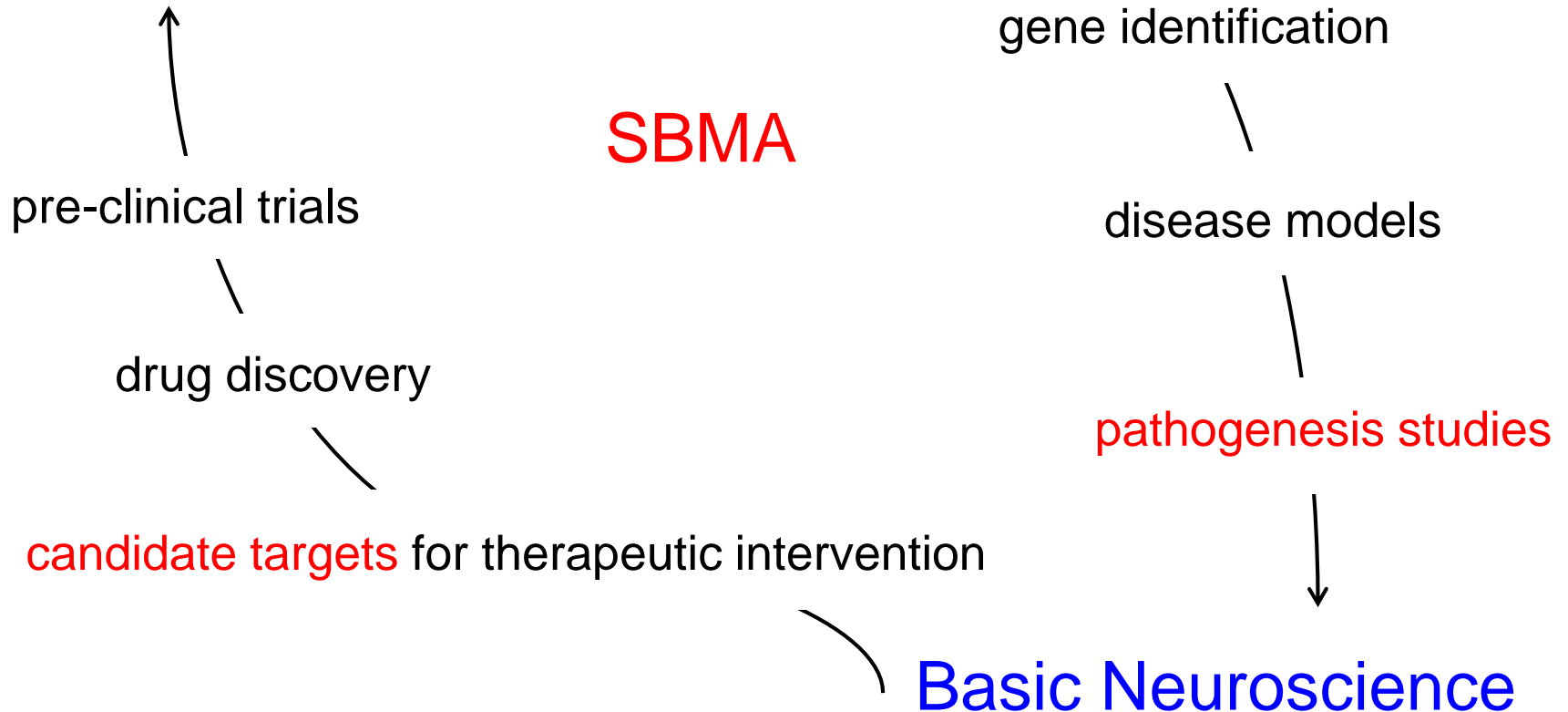
successful therapies?



Translational Neuroscience

Neurodegenerative Diseases

successful therapies?



Are there any good **candidate targets** for therapeutic intervention for SBMA?

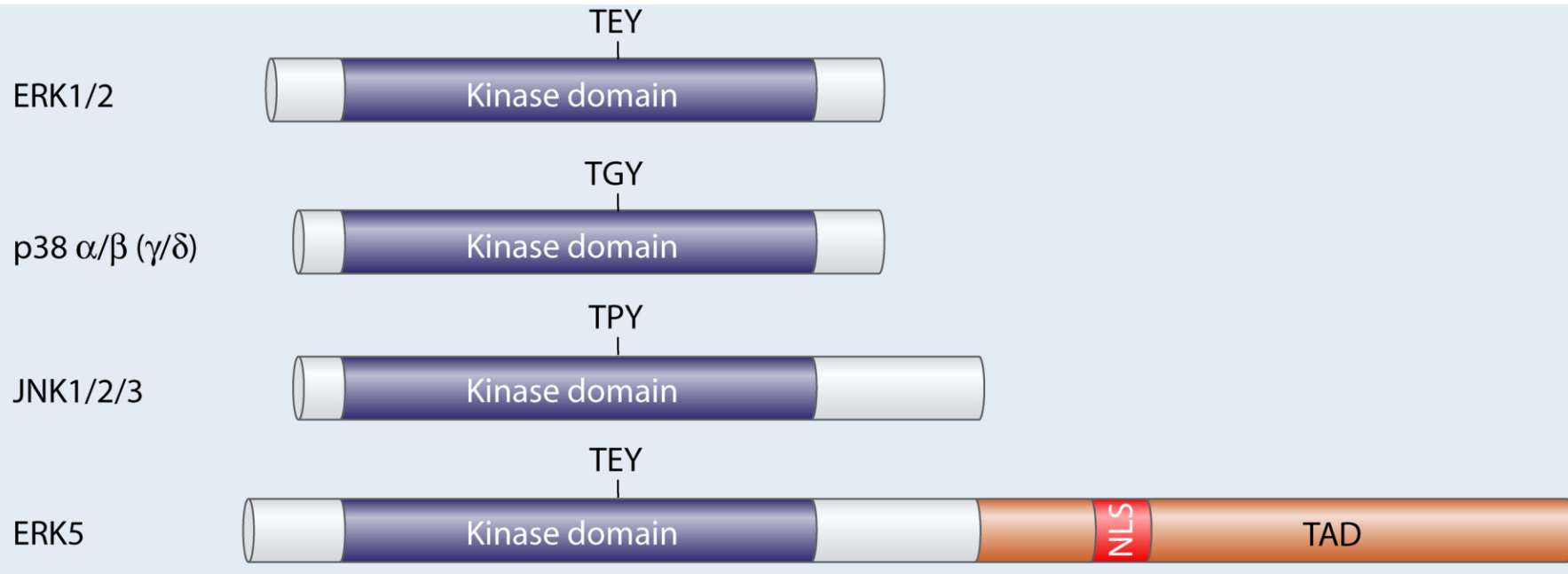
Are there any good **candidate targets** for therapeutic intervention for SBMA?

Nemo-Like Kinase (NLK)

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- Conserved MAPK-like serine/threonine protein kinase

Conventional MAPKs



Atypical MAPKs



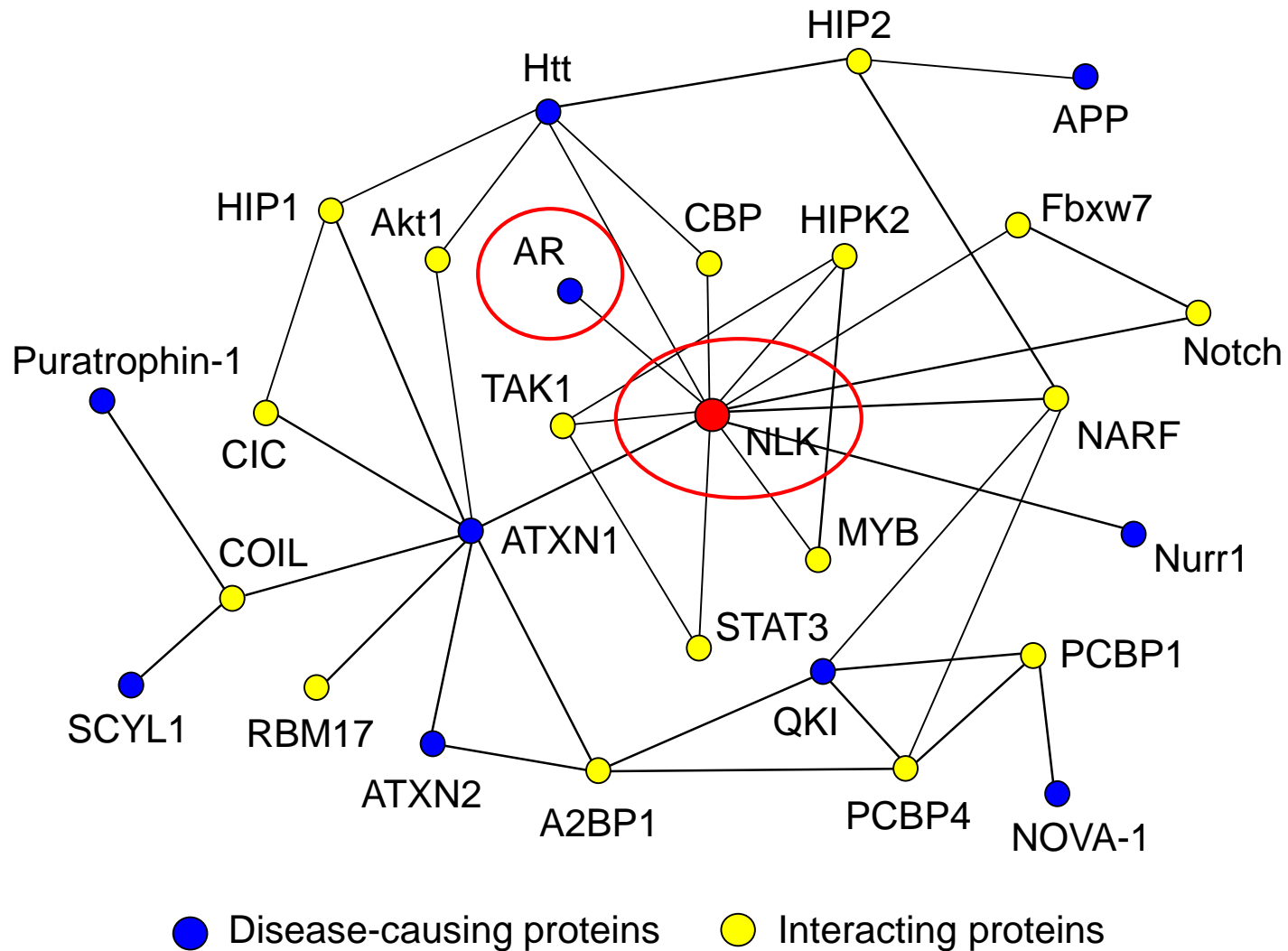
Nemo-Like Kinase (NLK)

- Conserved MAPK-like serine/threonine protein kinase
- Linked to several cell signaling pathways and processes (Wnt, TGF- β , Notch, etc.)

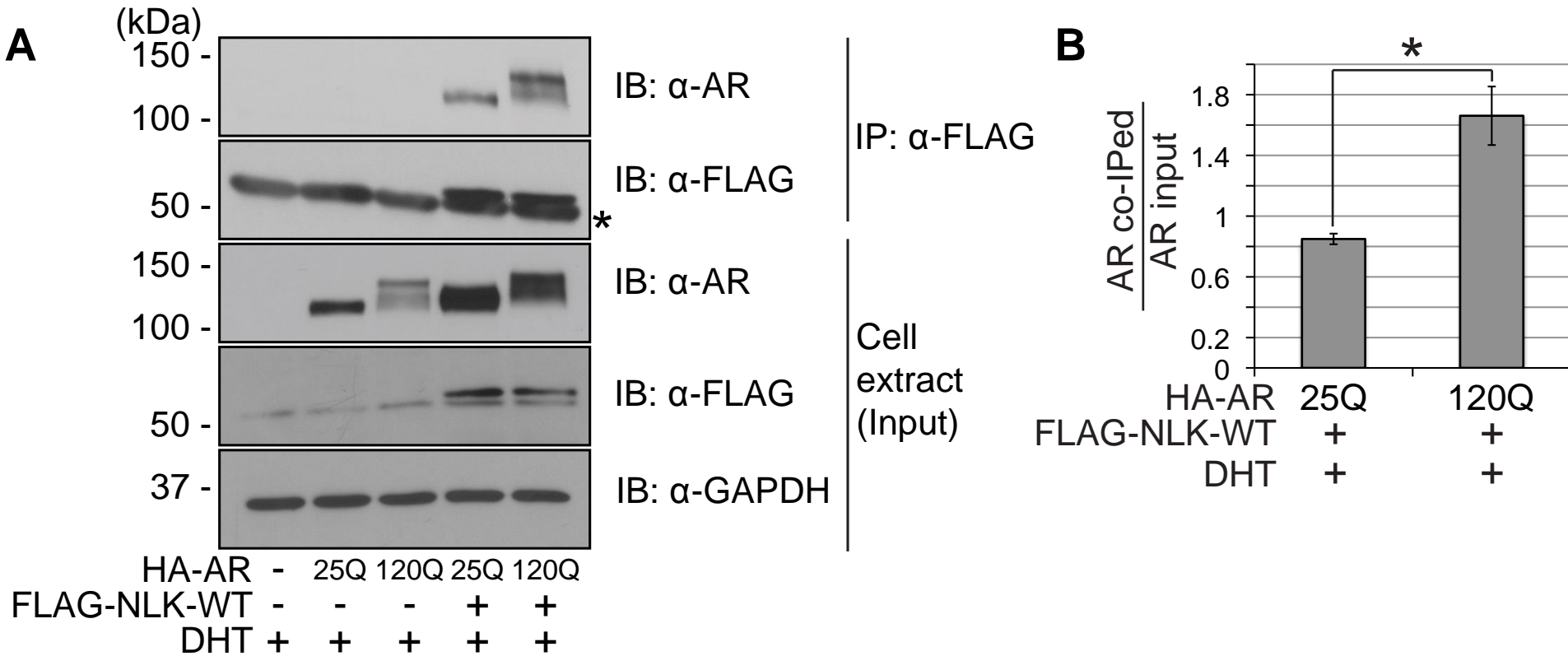
Nemo-Like Kinase (NLK)

- Conserved MAPK-like serine/threonine protein kinase
- Linked to several cell signaling pathways and processes (Wnt, TGF- β , Notch, etc.)
- Interacts with many disease-associated proteins

Nemo-Like Kinase as a potential modifier of many neurodegenerative diseases

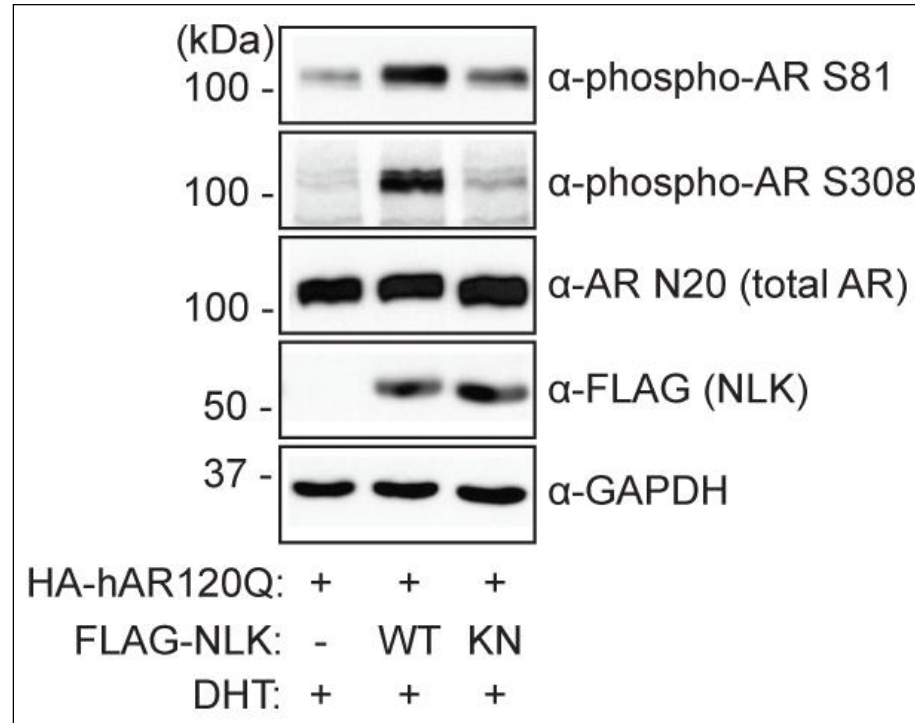


NLK interacts with both wild-type and polyQ-expanded mutant AR

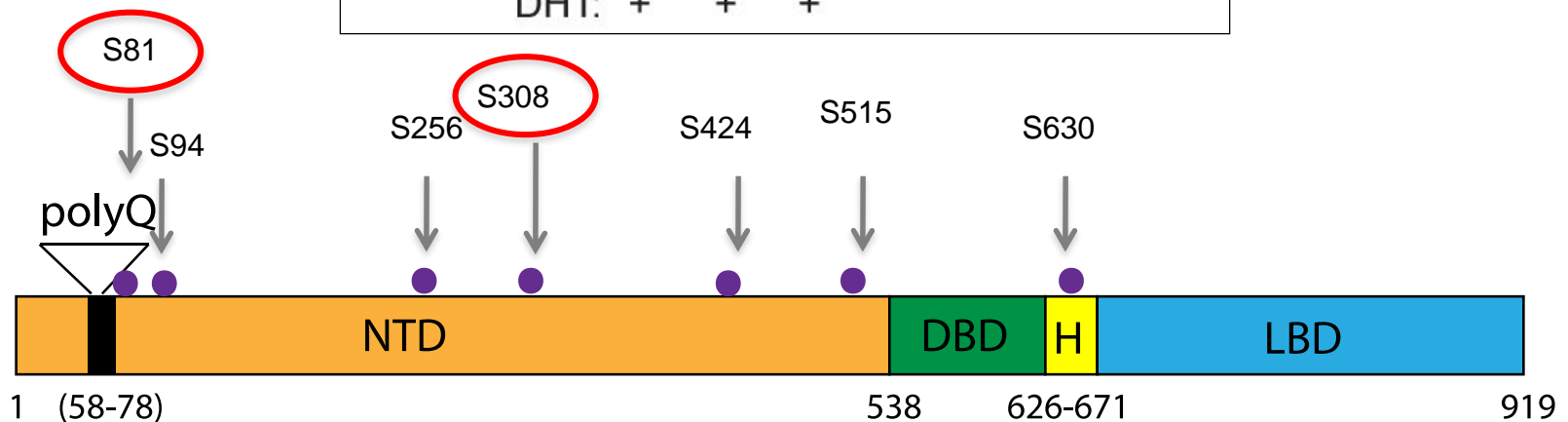


NSC-34 motor neuron cells

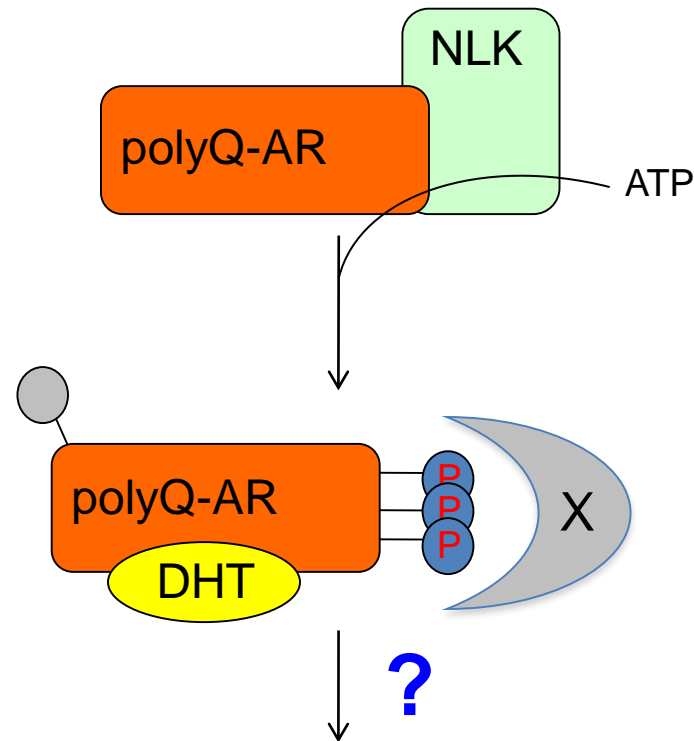
NLK influences the phosphorylation status of AR in cell culture



NSC-34
motor neuron cells



A potential model for the role of NLK in SBMA pathogenesis



SBMA phenotypes

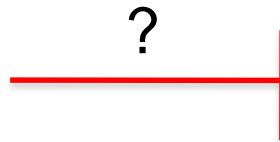
- Aggregation
- Cytotoxicity
- Neuromuscular degeneration

Can NLK modulate SBMA pathogenesis?

Altering *Nlk* expression

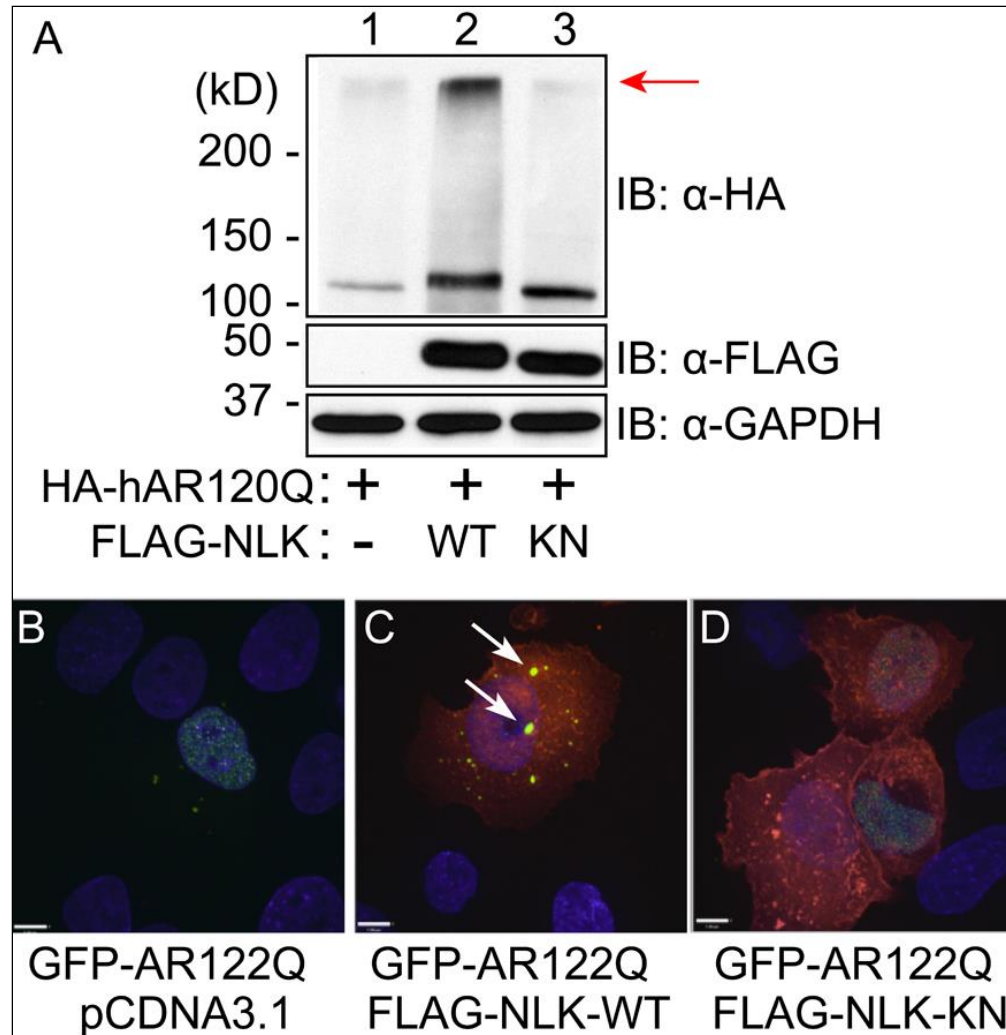
or

Modulating NLK activity



SBMA pathogenesis

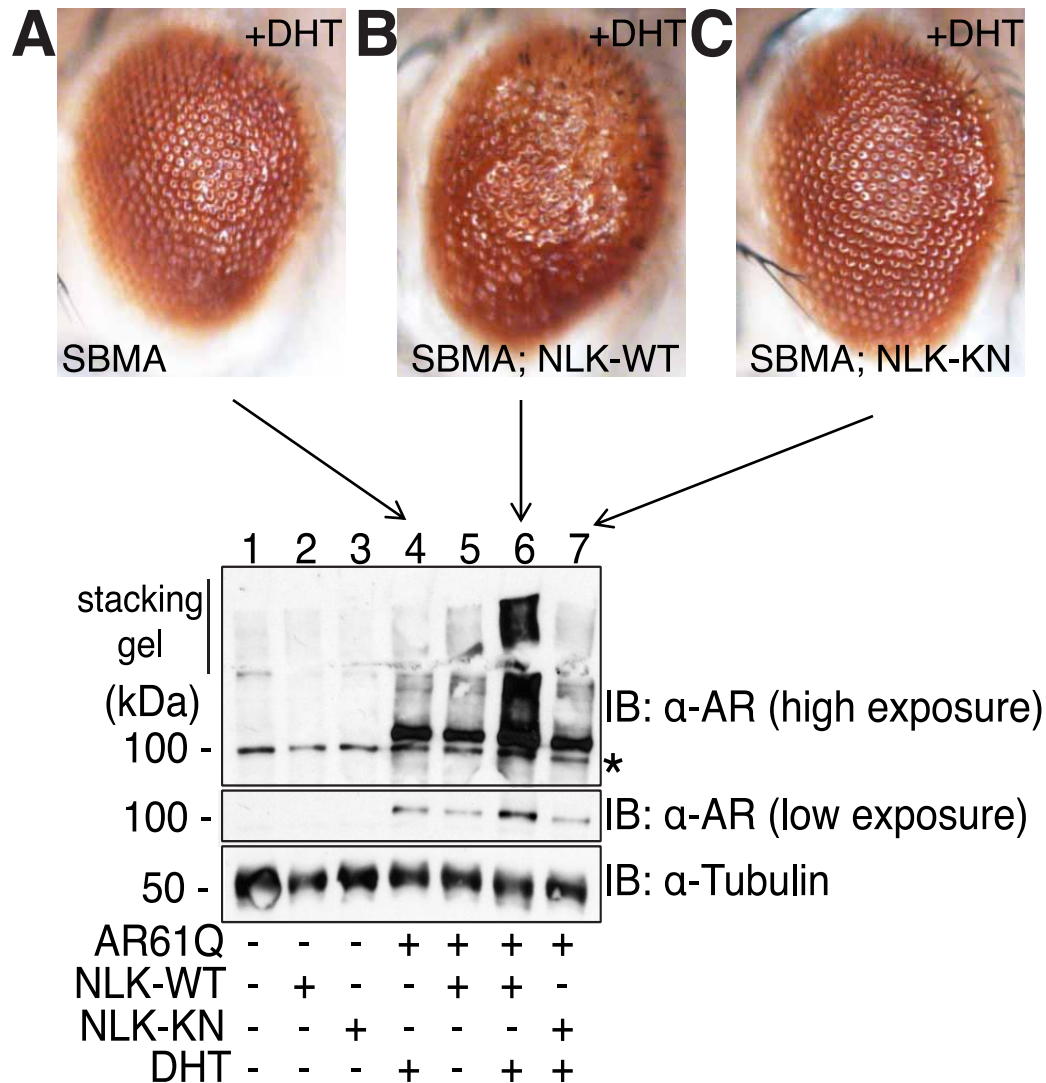
NLK promotes polyQ-expanded mutant AR phenotypes in cell culture system



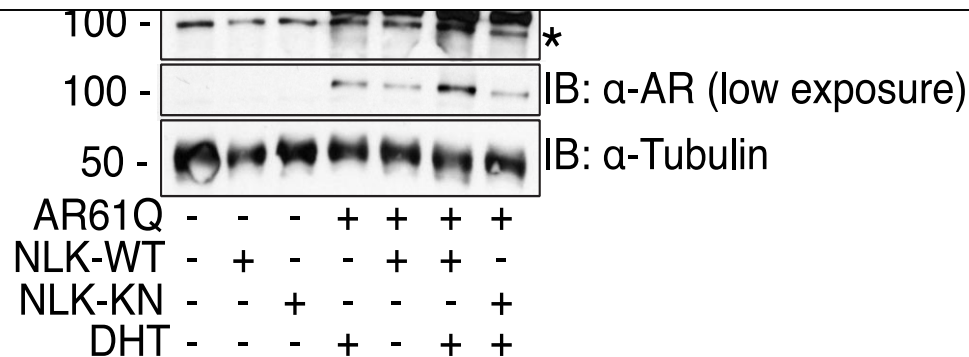
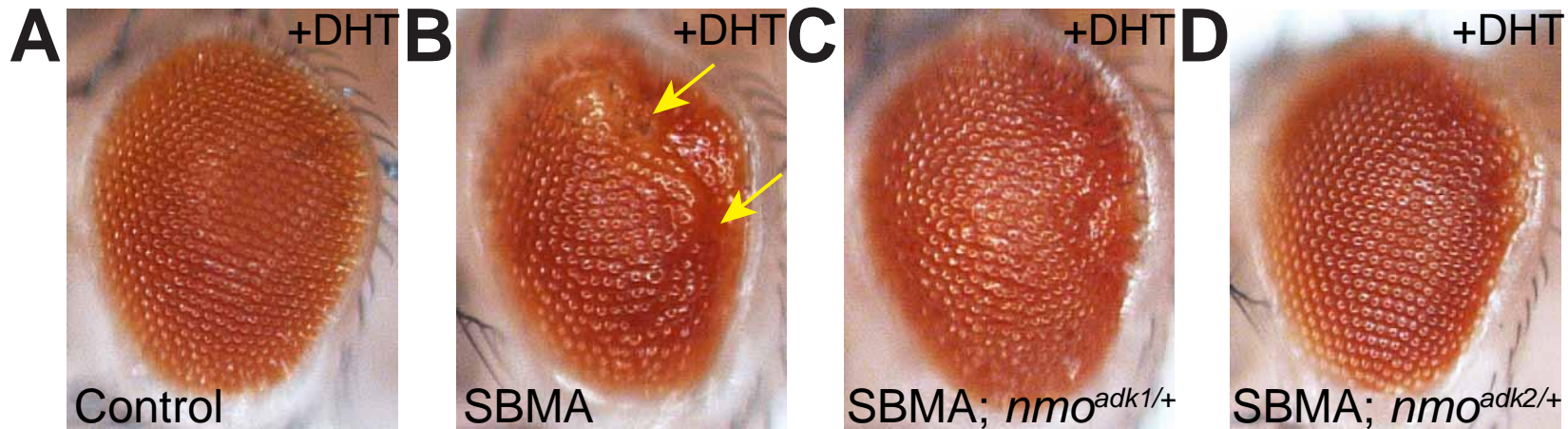
KN: Kinase Activity
Defective

GFP (AR)
A-FLAG (NLK)
DAPI (nuclei)

NLK modulates polyQ-expanded mutant AR toxicity in a *Drosophila* model of SBMA

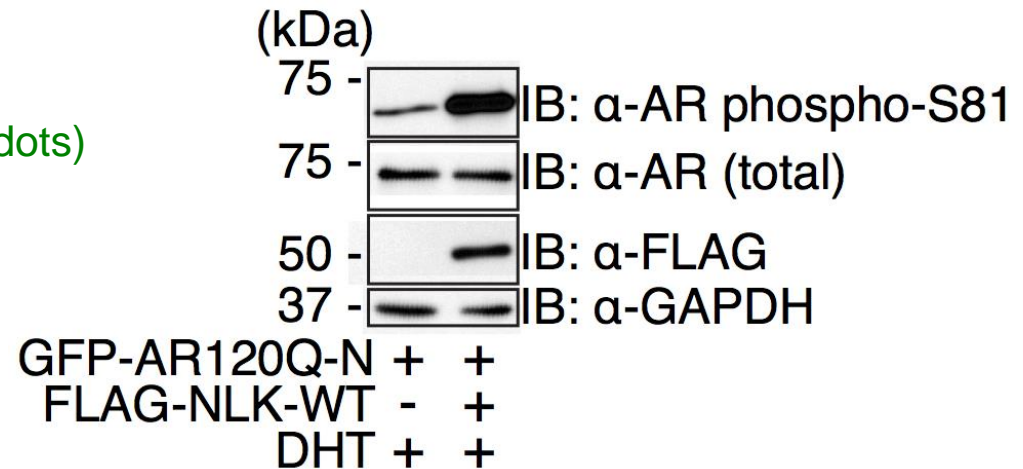
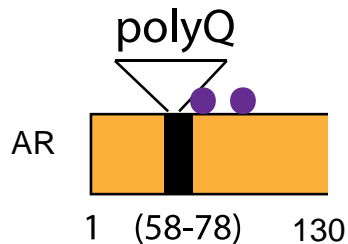


NLK modulates polyQ-expanded mutant AR toxicity in a *Drosophila* model of SBMA

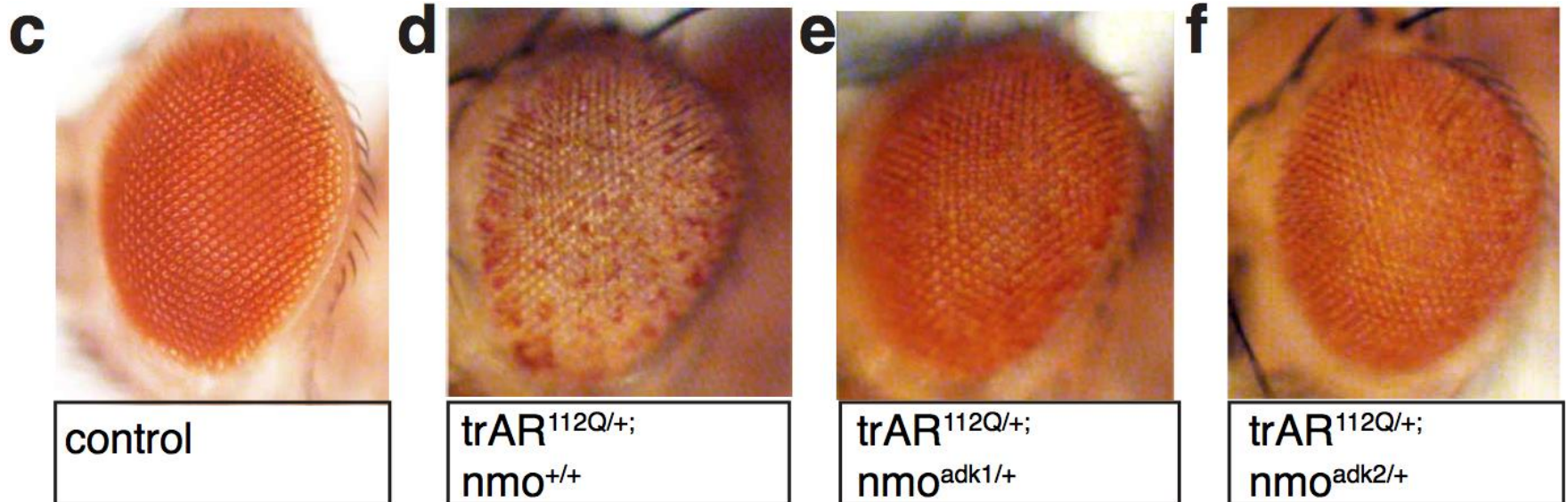


Reduced expression of NLK suppresses the toxicity induced by a mutant AR fragment

- trAR112Q: N-terminal 130 amino acids contains polyQ region contains S81 and S94 (purple dots)



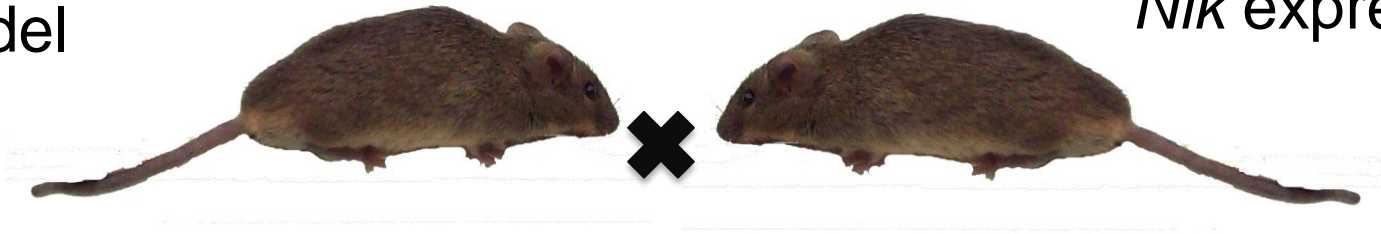
Todd et al., *in revision*



Can loss of *Nlk* affect SBMA-related phenotypes *in vivo*?

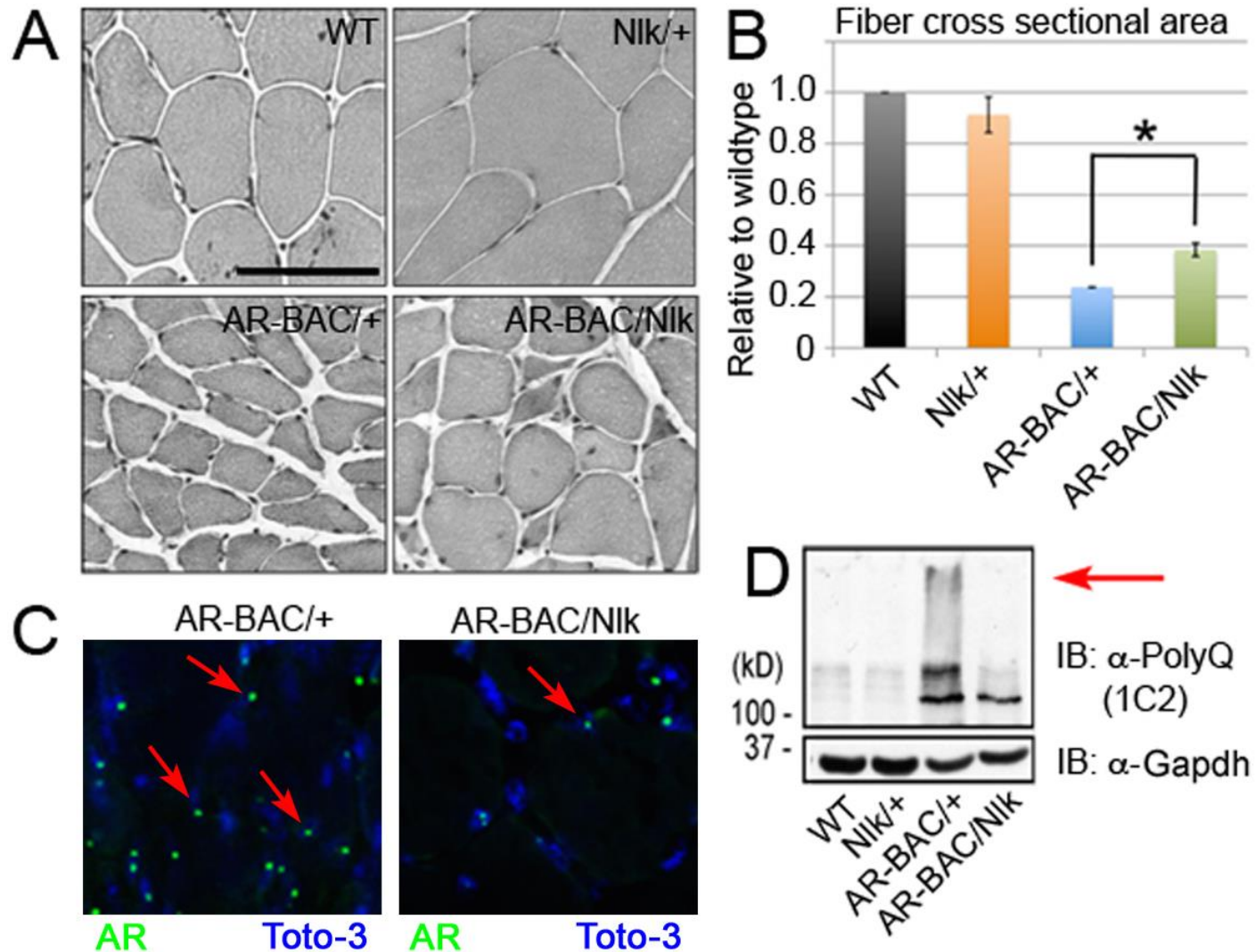
SBMA mouse
model

Mice with reduced
Nlk expression

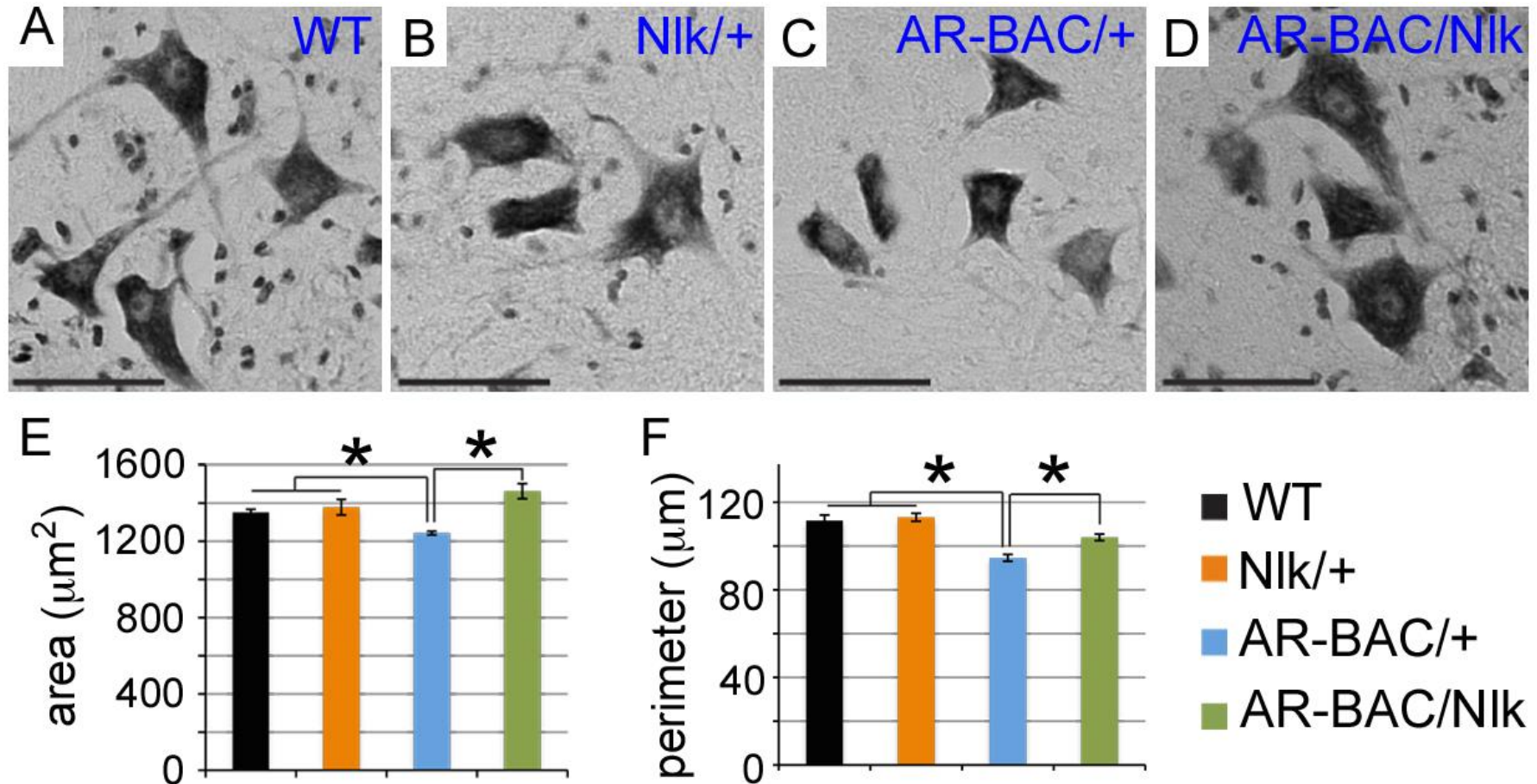


Compare the
male progeny

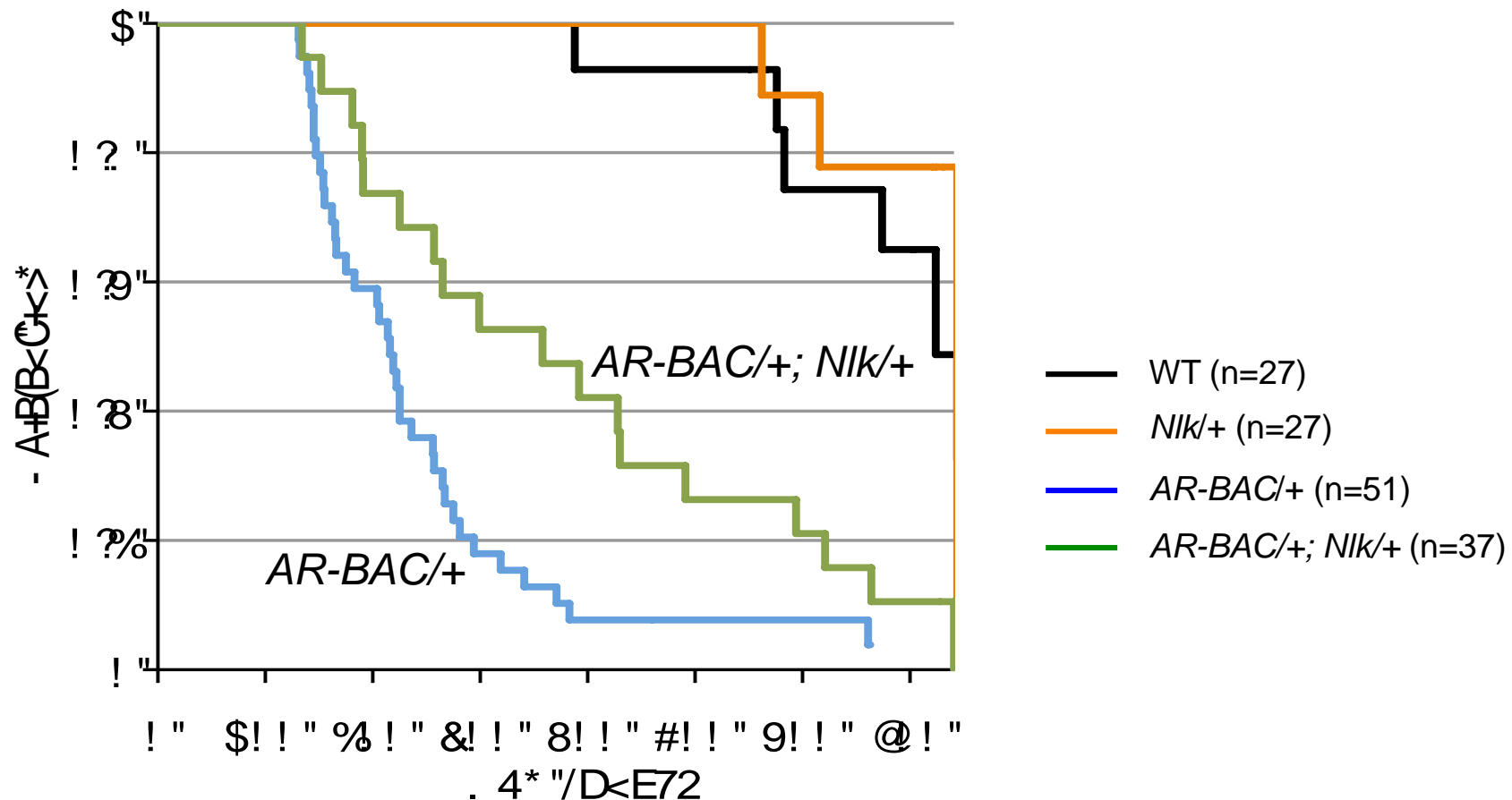
A fifty percent reduction in NLK partially rescues SBMA pathology in mice



Loss of one copy of *Nlk* improves the pathogenic change in motor neuronal soma size in SBMA mice



Loss of one copy of *Nlk* significantly extends overall lifespan of AR-BAC mice



Kaplan Meier, *log rank* test, $p = 0.00107$

Interim Summary

- NLK interacts with, and phosphorylates, polyQ-expanded mutant AR.
- Increased NLK expression promotes SBMA phenotypes in cell culture and *Drosophila* in a kinase activity-dependent manner.
- Decreased expression of *Nlk* partially, but significantly, suppresses SBMA-related phenotypes in *Drosophila* and mice.

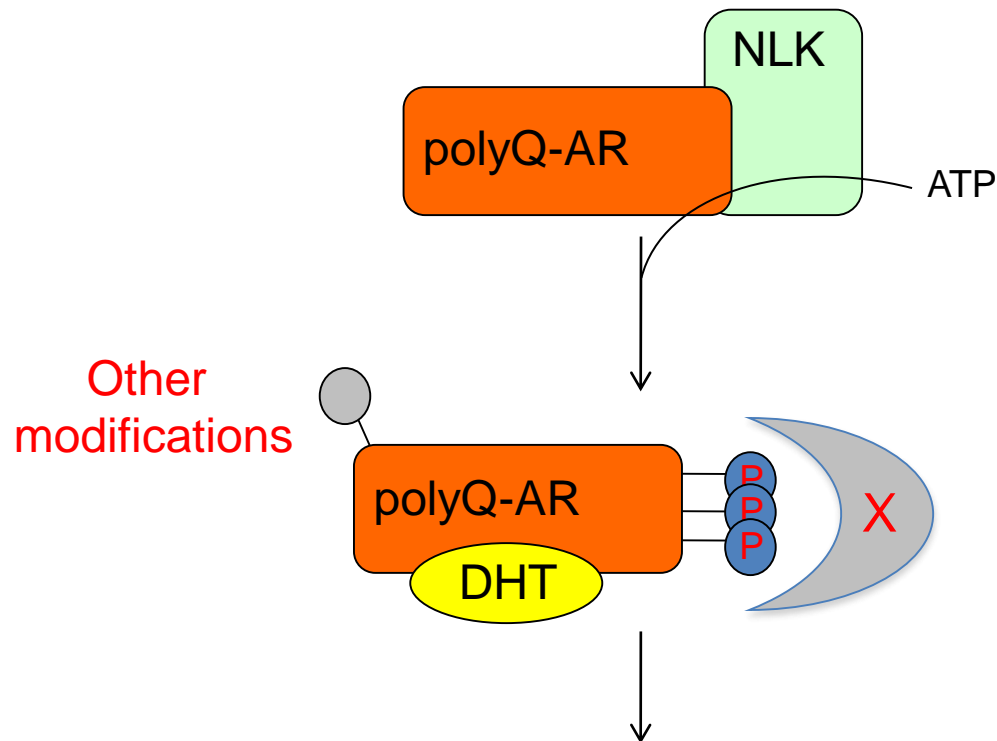
Next questions are ...

1. What is the **molecular mechanism** for the NLK-mediated effects on SBMA?
2. Whether **pharmacological inhibition** of NLK could suppress SBMA phenotypes?

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1. What is the **molecular mechanism** for the NLK-mediated effects on SBMA?
2. Whether pharmacological inhibition of NLK could suppress SBMA phenotypes?

A potential model for the role of NLK in SBMA pathogenesis

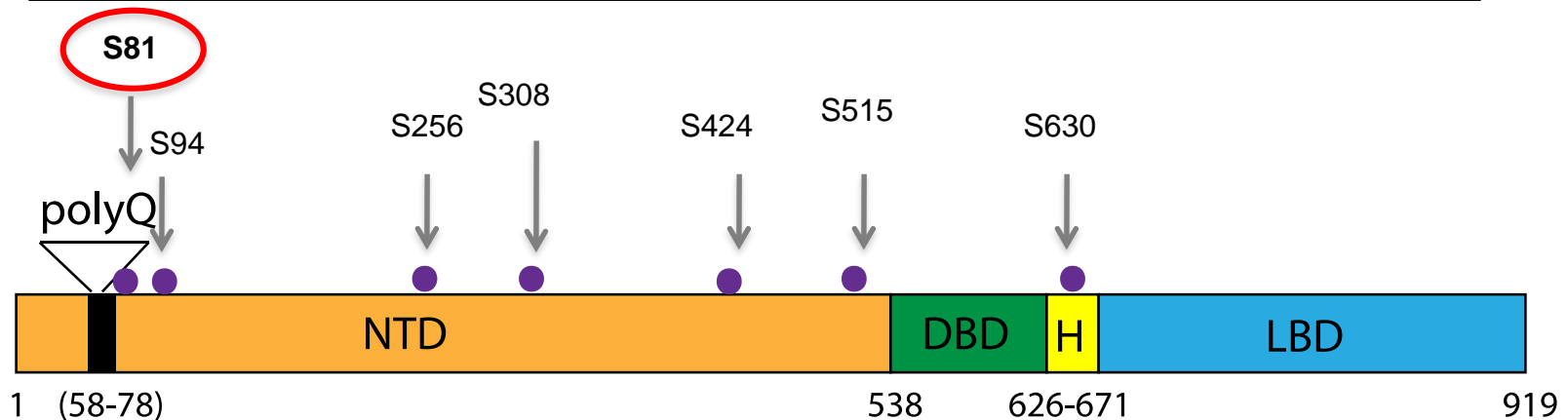
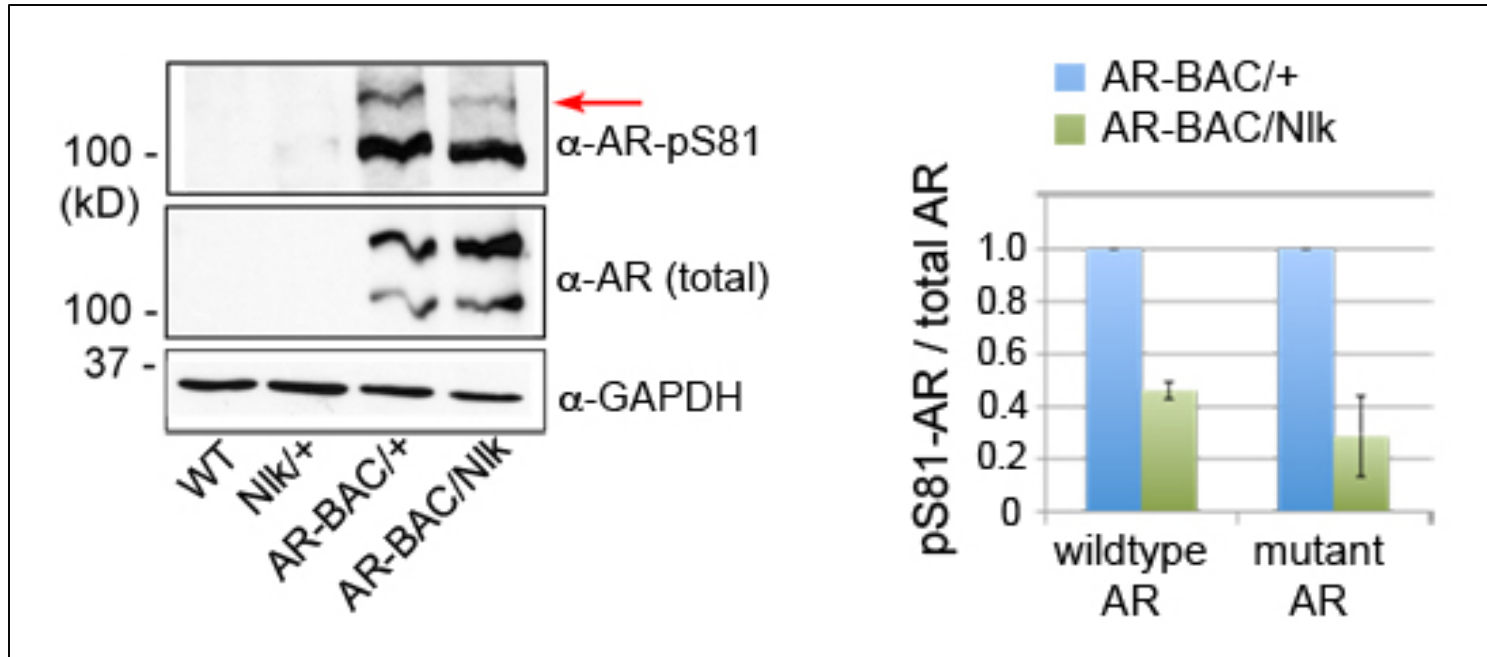


SBMA phenotypes

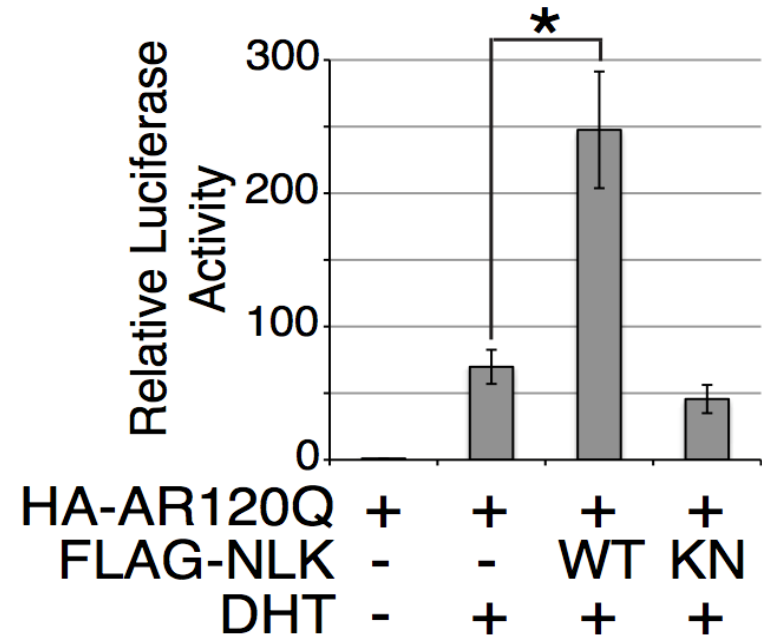
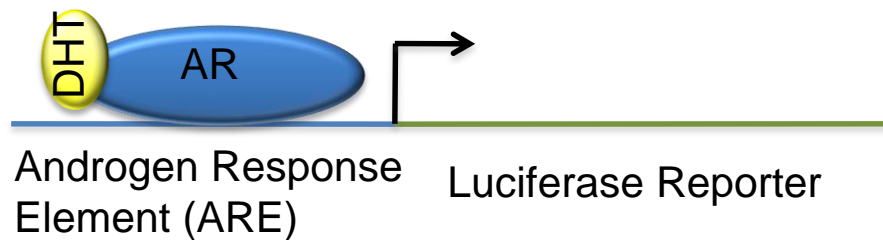
- Aggregation
- Cytotoxicity
- Neuromuscular degeneration

NLK influences the phosphorylation status of AR in mice

AR-BAC mouse muscle

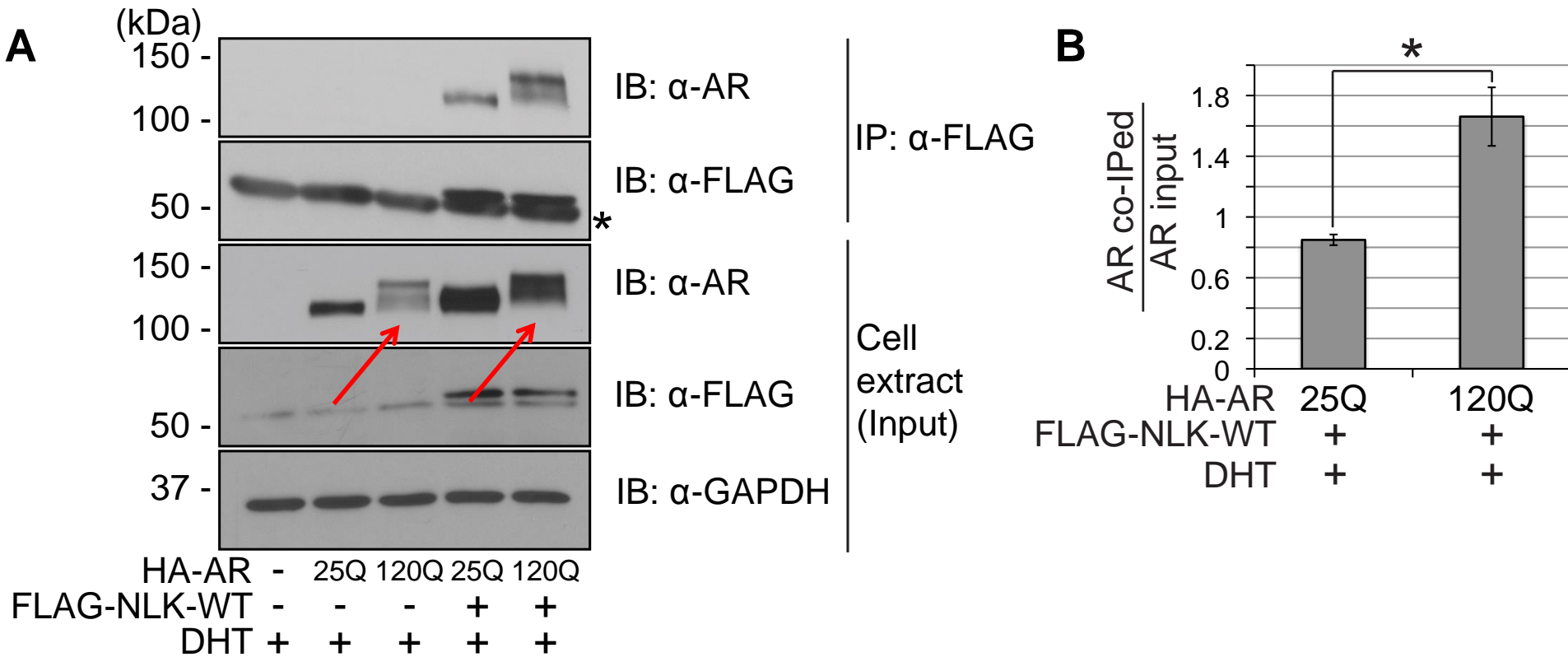


NLK increases AR-dependent gene transcription in a kinase activity-dependent manner



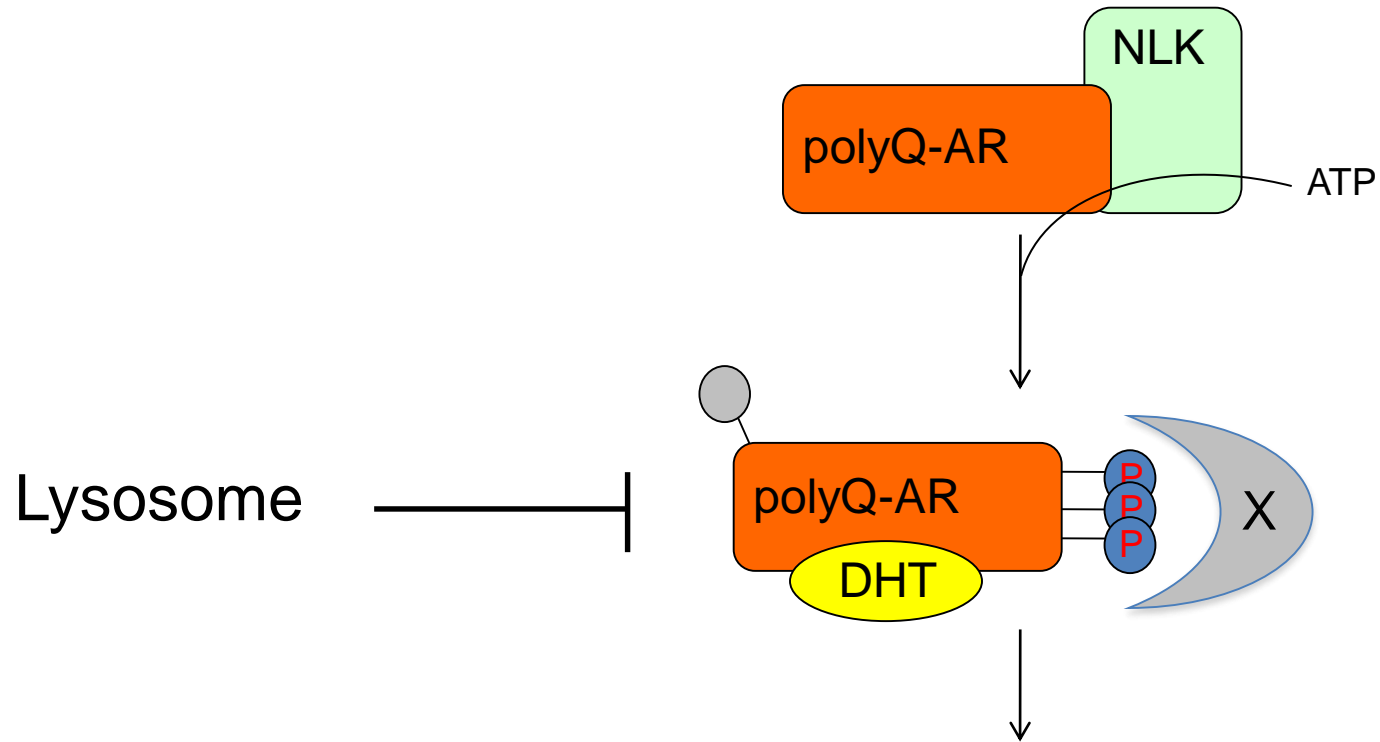
NSC-34 cells

Increased NLK elevates AR protein expression



NSC-34 motor neuron cells

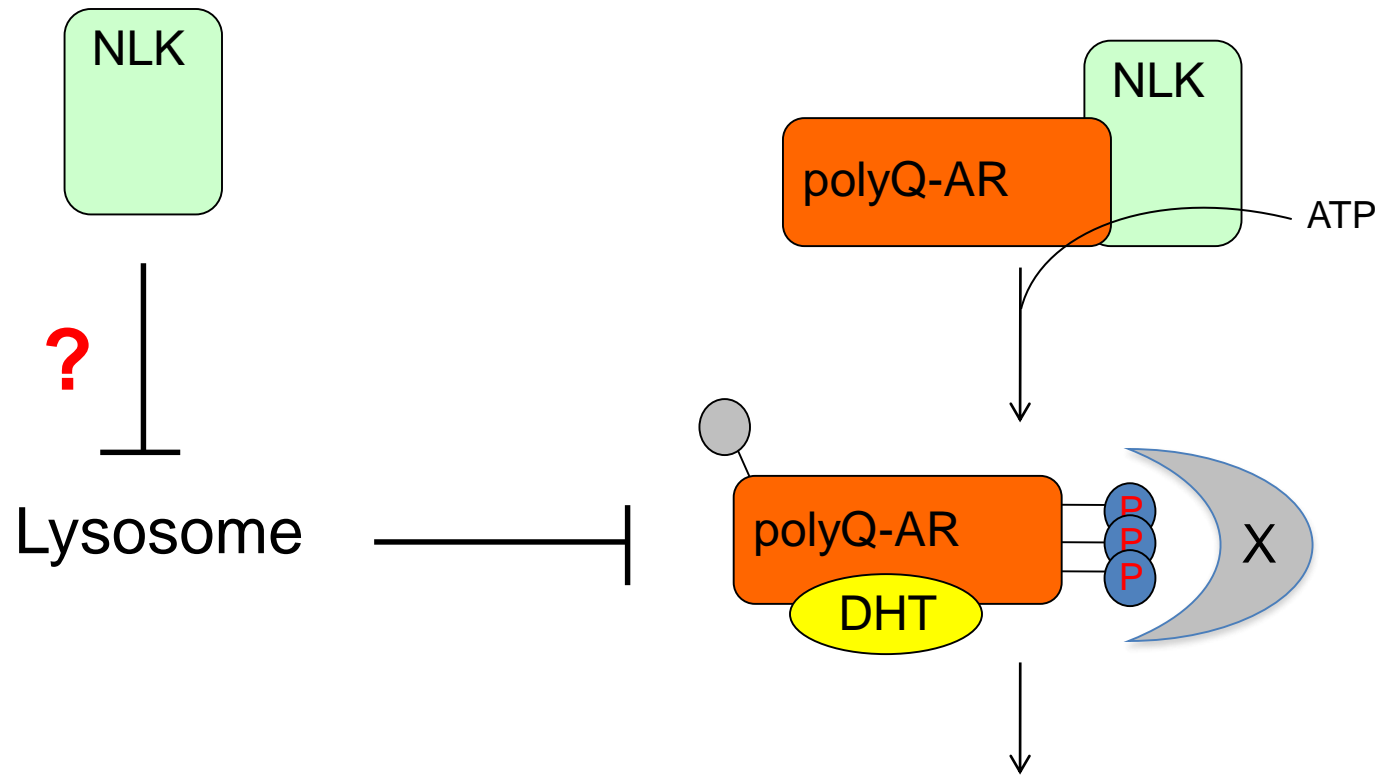
AR is degraded by the autophagy-lysosomal pathway



SBMA phenotypes

- Aggregation
- Cytotoxicity
- Neuromuscular degeneration

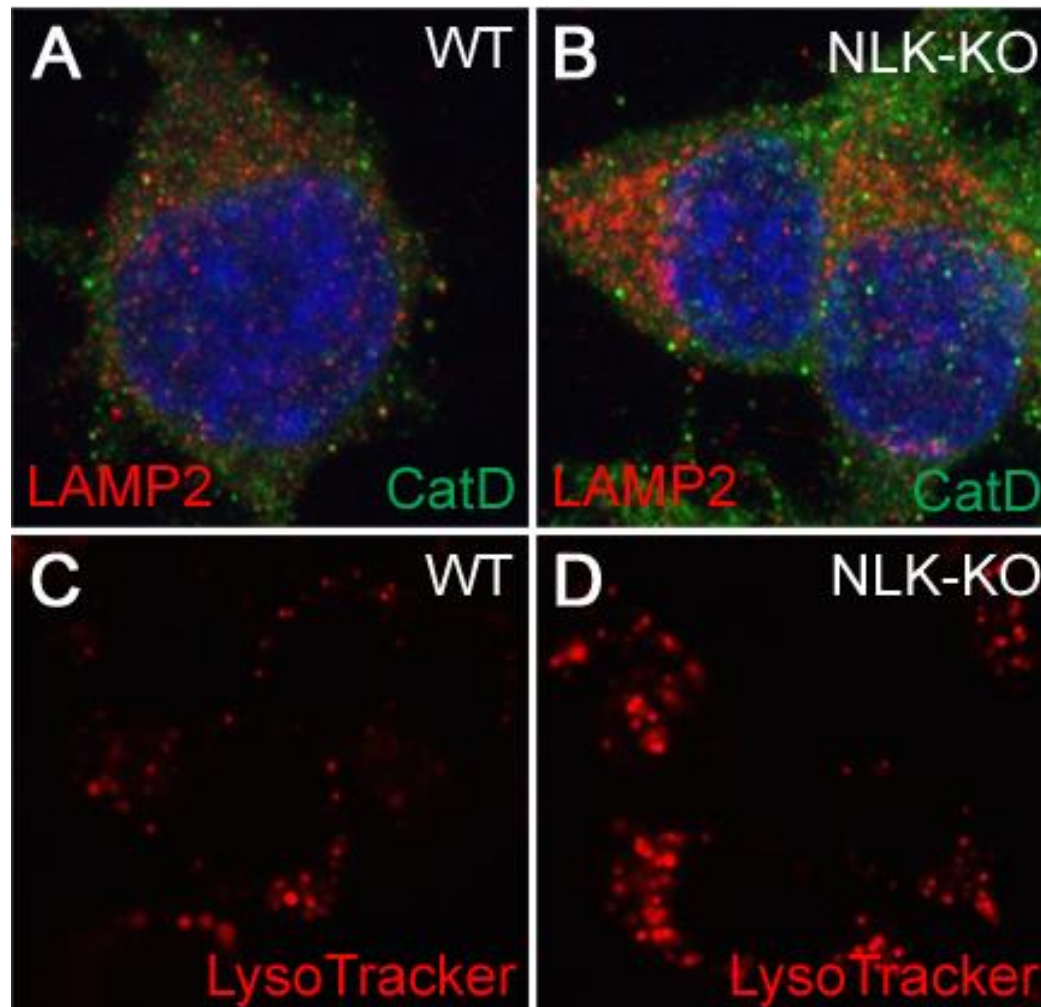
Can NLK modulate SBMA via regulating the autophagy-lysosomal pathway?



SBMA phenotypes

- Aggregation
- Cytotoxicity
- Neuromuscular degeneration

Loss of NLK increases lysosome number/size

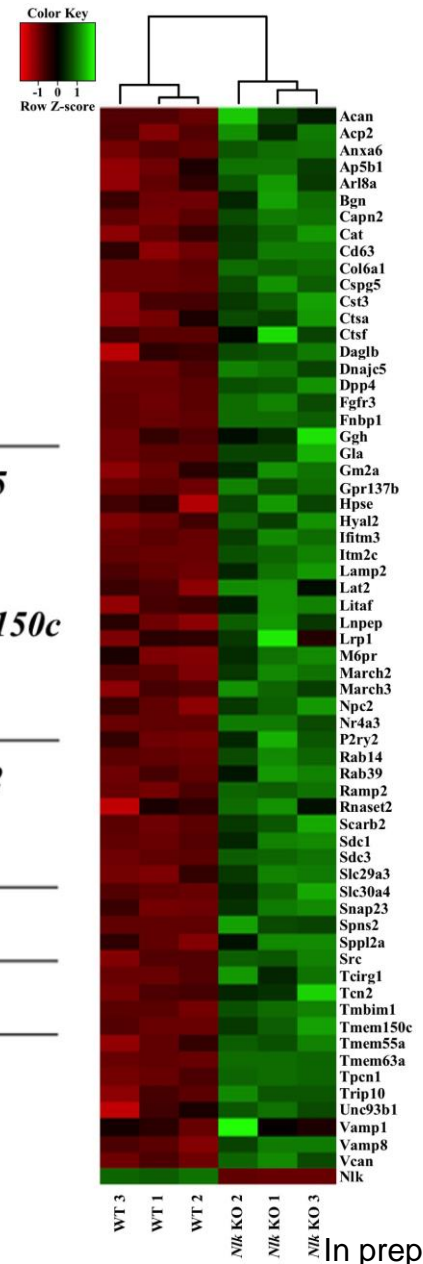


N2a cells

Loss of *Nlk* leads to up-regulation of a subset of lysosomal genes

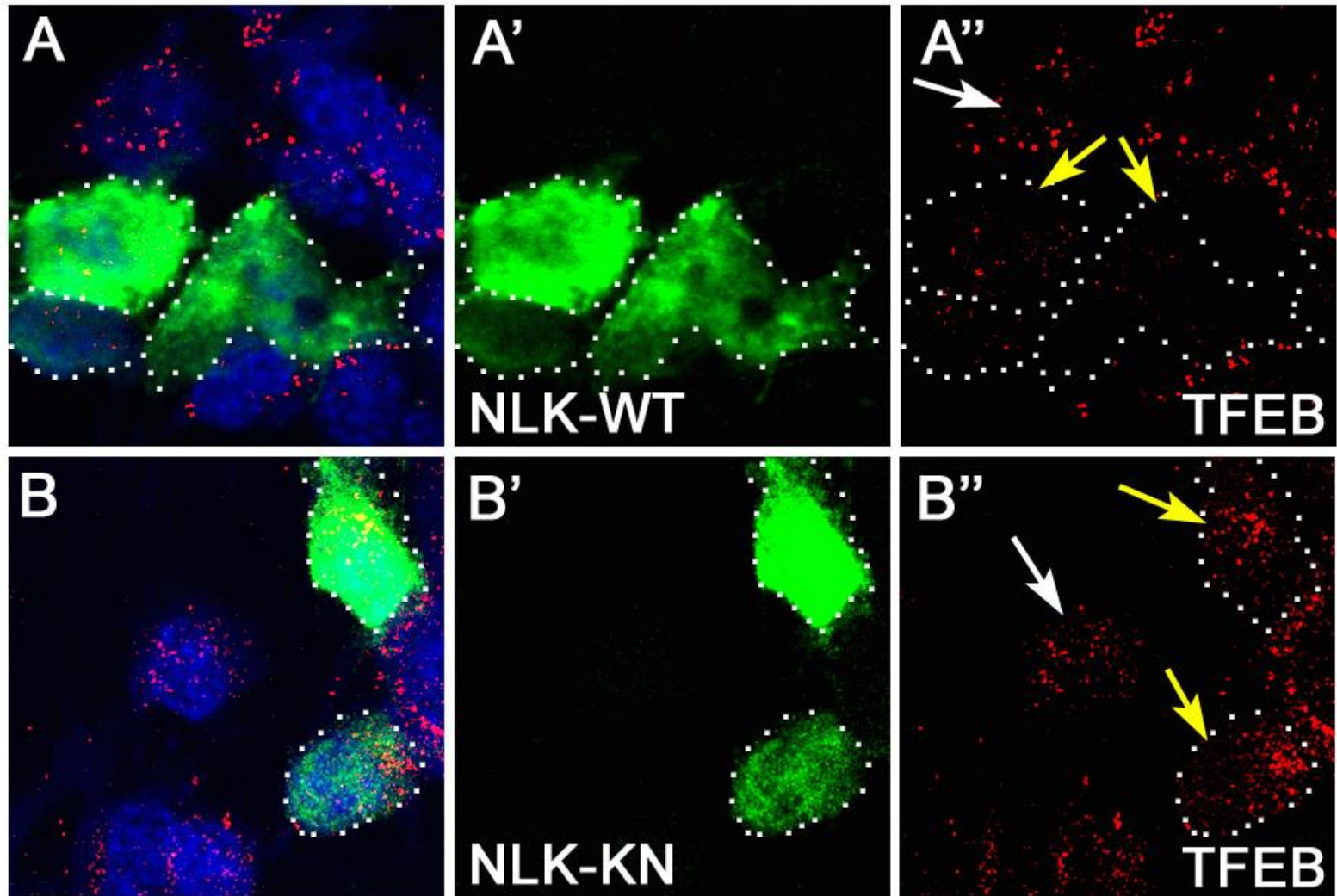
- RNA sequencing from WT and *Nlk* KO N2a cells
- The location or function of lysosomal genes based on Gene Ontology, significantly up-regulated in *Nlk* KO cells

Lysosome Membrane	<i>Acp2</i>	<i>Anxa6</i>	<i>Ap5b1</i>	<i>Cd63</i>	<i>Col6a1</i>	<i>Daglb</i>	<i>Dnajc5</i>
	<i>Dpp4</i>	<i>Dpr137b</i>	<i>Hpse</i>	<i>Litaf</i>	<i>Lnpep</i>	<i>Lrp1</i>	<i>M6pr</i>
	<i>March2</i>	<i>Rab14</i>	<i>Spns2</i>	<i>Sppl2a</i>	<i>Tcirg1</i>	<i>Tmbim1</i>	<i>Tmem150c</i>
	<i>Tmem55a</i>	<i>Tmem63a</i>	<i>Tpcn1</i>	<i>Vamp1</i>	<i>Vamp8</i>		
Lysosome Lumen	<i>Acan</i>	<i>Bgn</i>	<i>Cspg5</i>	<i>Gla</i>	<i>Gm2a</i>	<i>Rnaset2</i>	<i>Scarb2</i>
	<i>Sdc1</i>	<i>Sdc3</i>	<i>Tcn2</i>	<i>Vcan</i>			
Enzymes	<i>Cat</i>	<i>Ctsa</i>	<i>Ctsf</i>	<i>Hyal2</i>			
Transporters	<i>Slc29a3</i>	<i>Slc30a4</i>					



In preparation

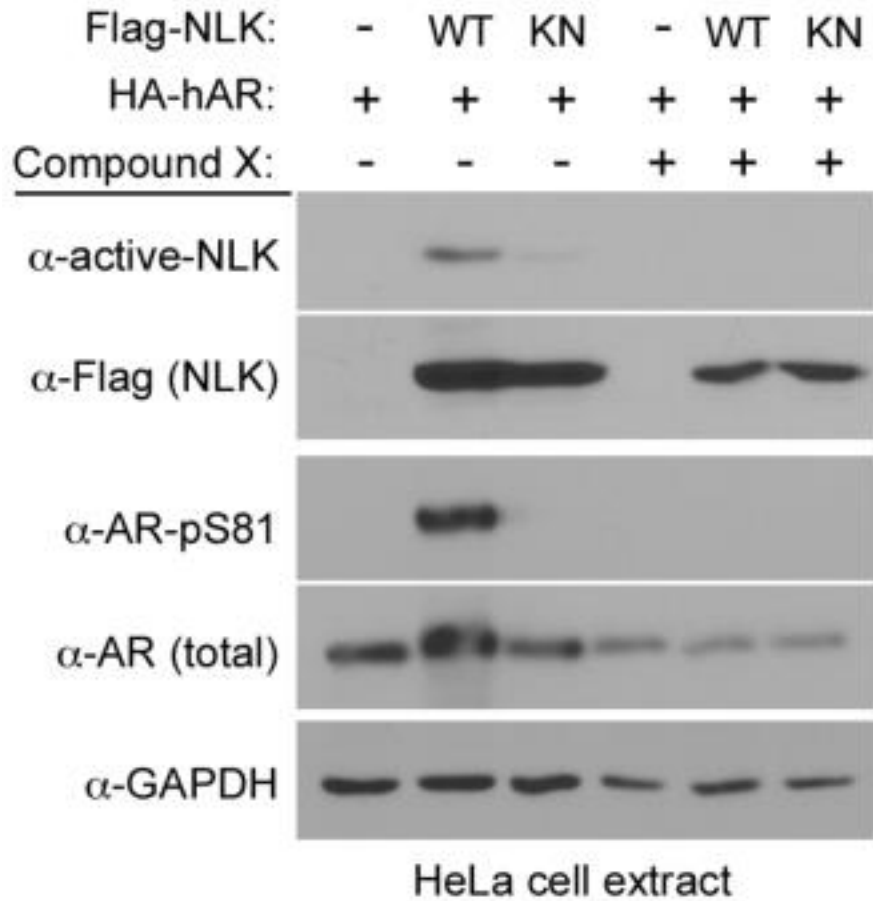
NLK decreases expression of TFEB, a master regulator of autophagy-lysosome biogenesis



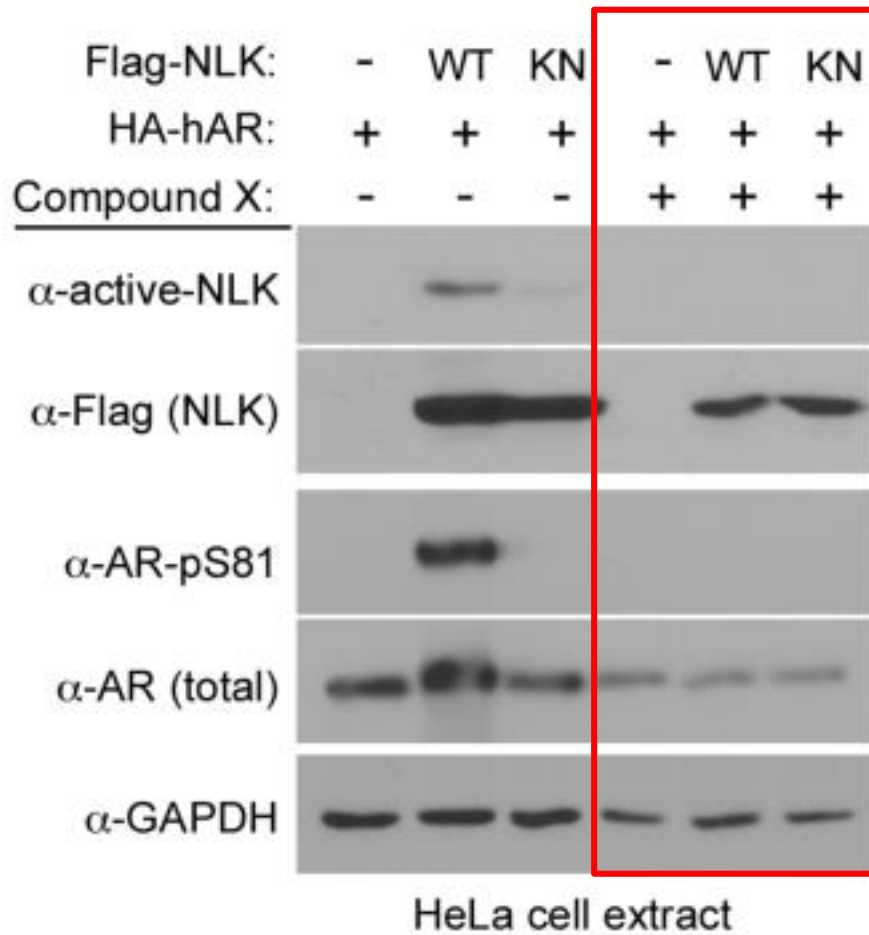
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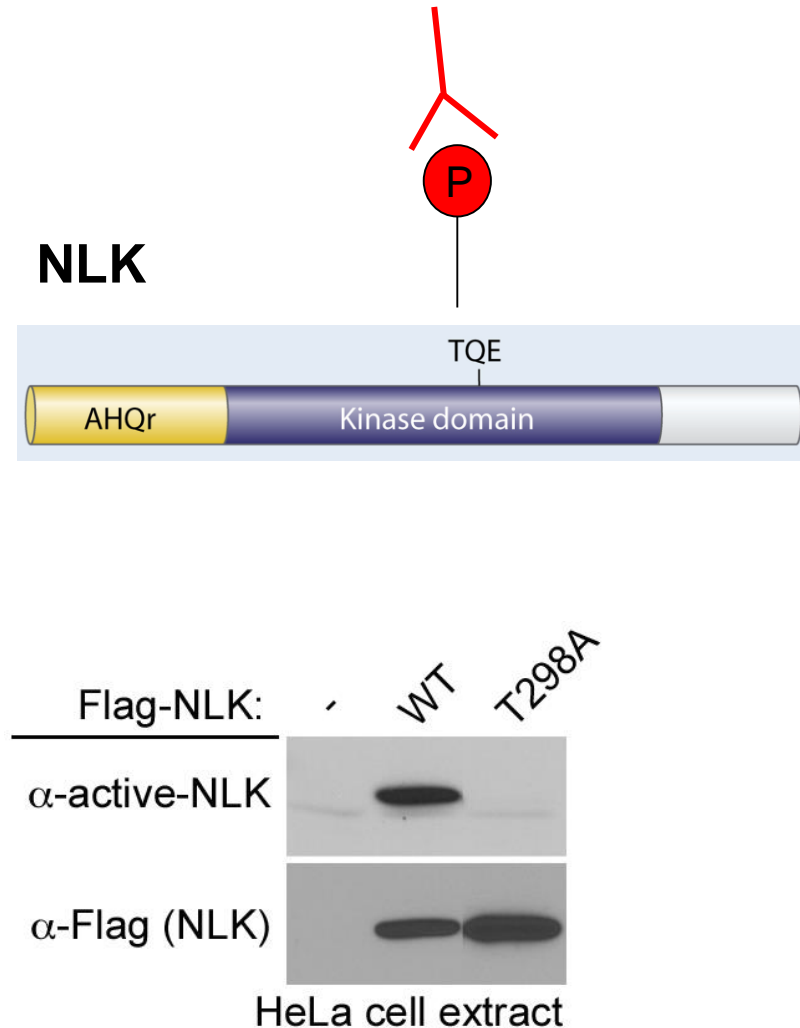
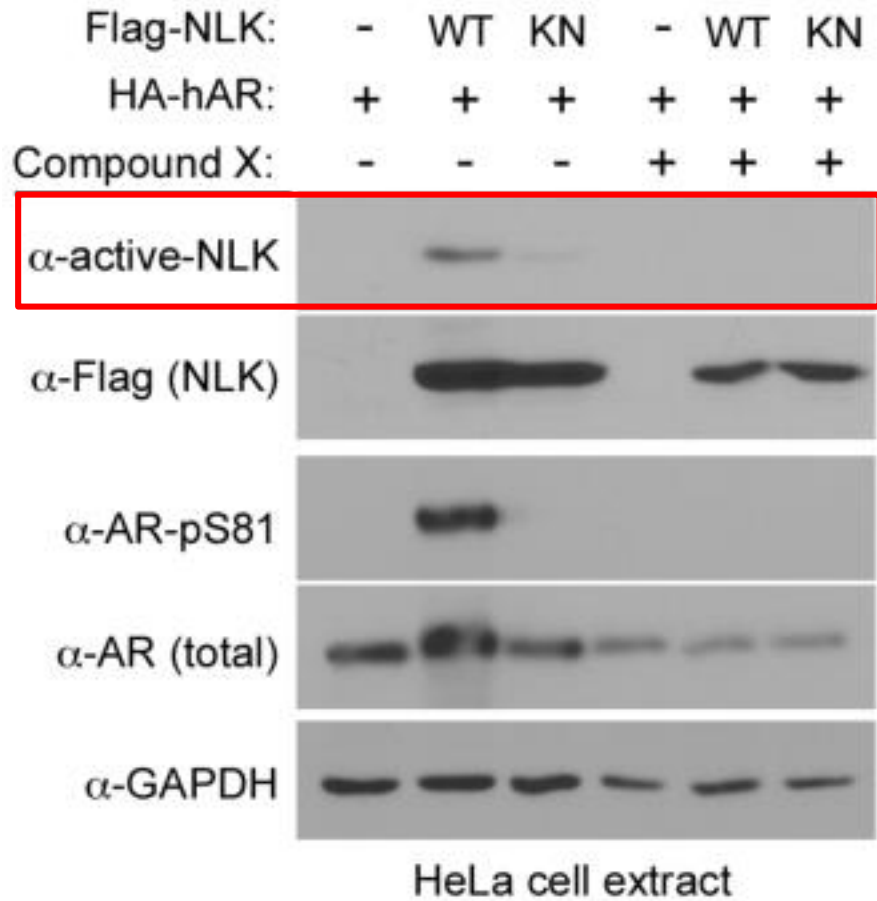
SB23080 can inhibit NLK activity in cell culture



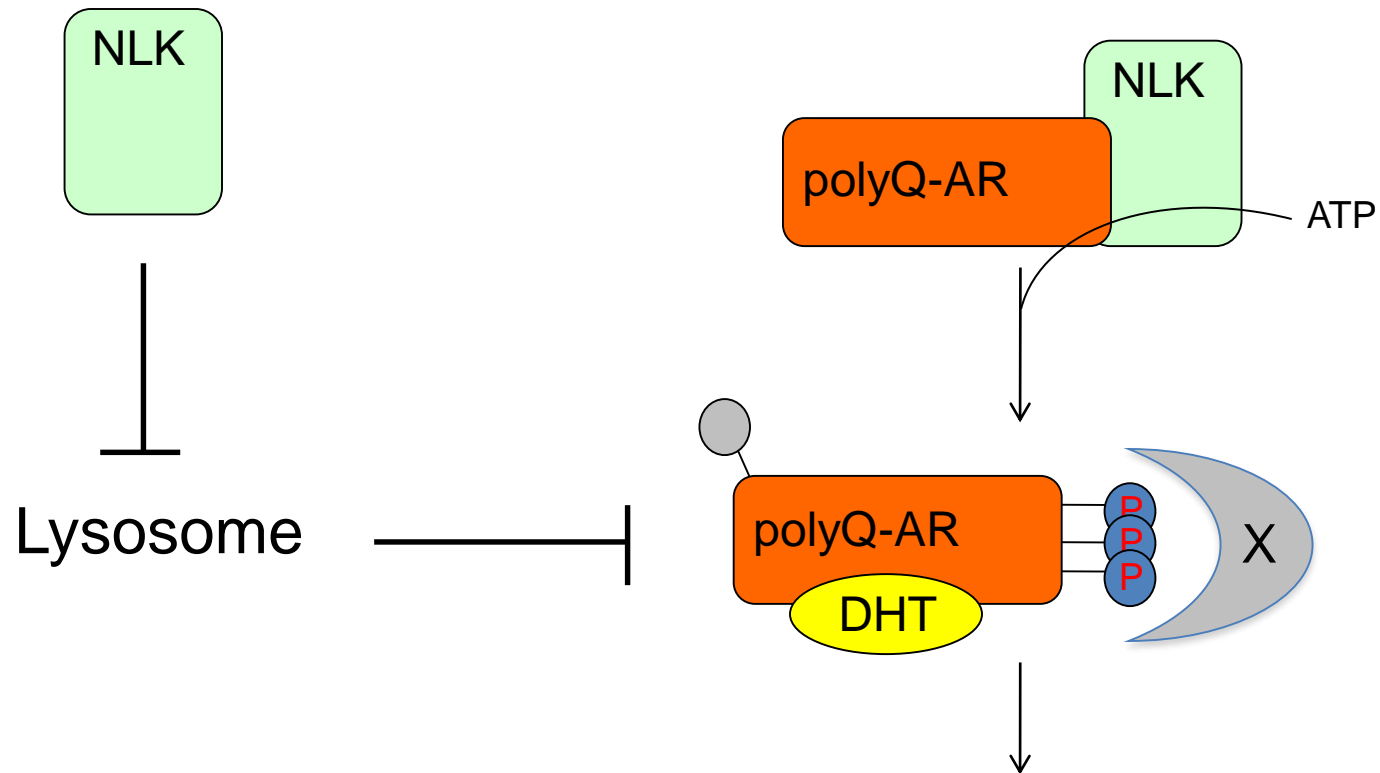
SB23080 can inhibit NLK activity in cell culture



SB23080 can inhibit NLK activity in cell culture



Working model for the role of NLK in SBMA pathogenesis



SBMA phenotypes

- Aggregation
- Cytotoxicity
- Neuromuscular degeneration

Summary

- NLK promotes SBMA phenotypes in a kinase activity-dependent manner.
- Reducing NLK expression is beneficial in SBMA.
- NLK interacts with, and phosphorylates, polyQ-expanded mutant AR.
- NLK can regulate expression levels of AR proteins by controlling the autophagy-lysosomal pathway.

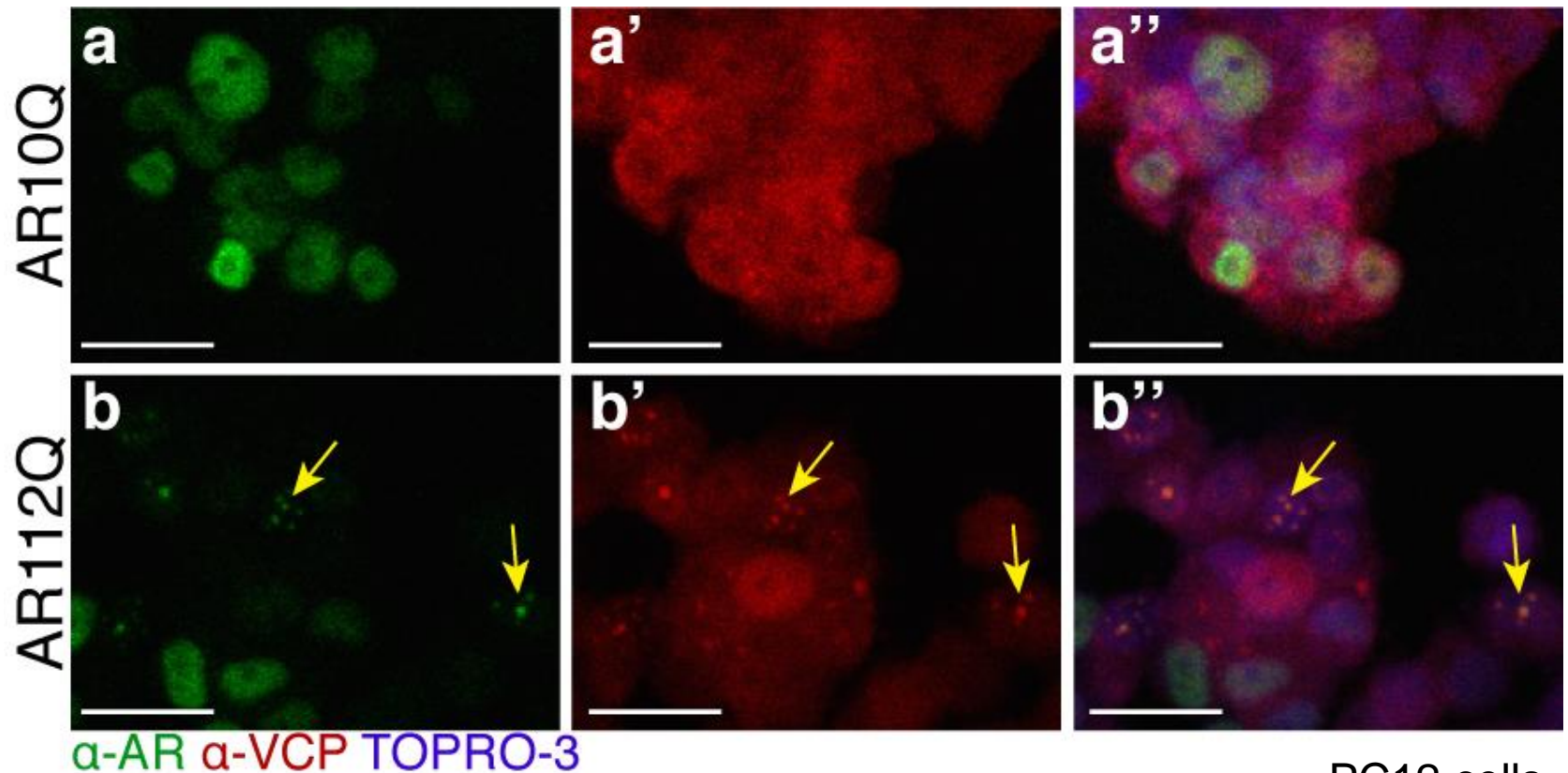
The role of VCP in Kennedy's Disease

- Supported by 2016 KDA Research Grant

VCP

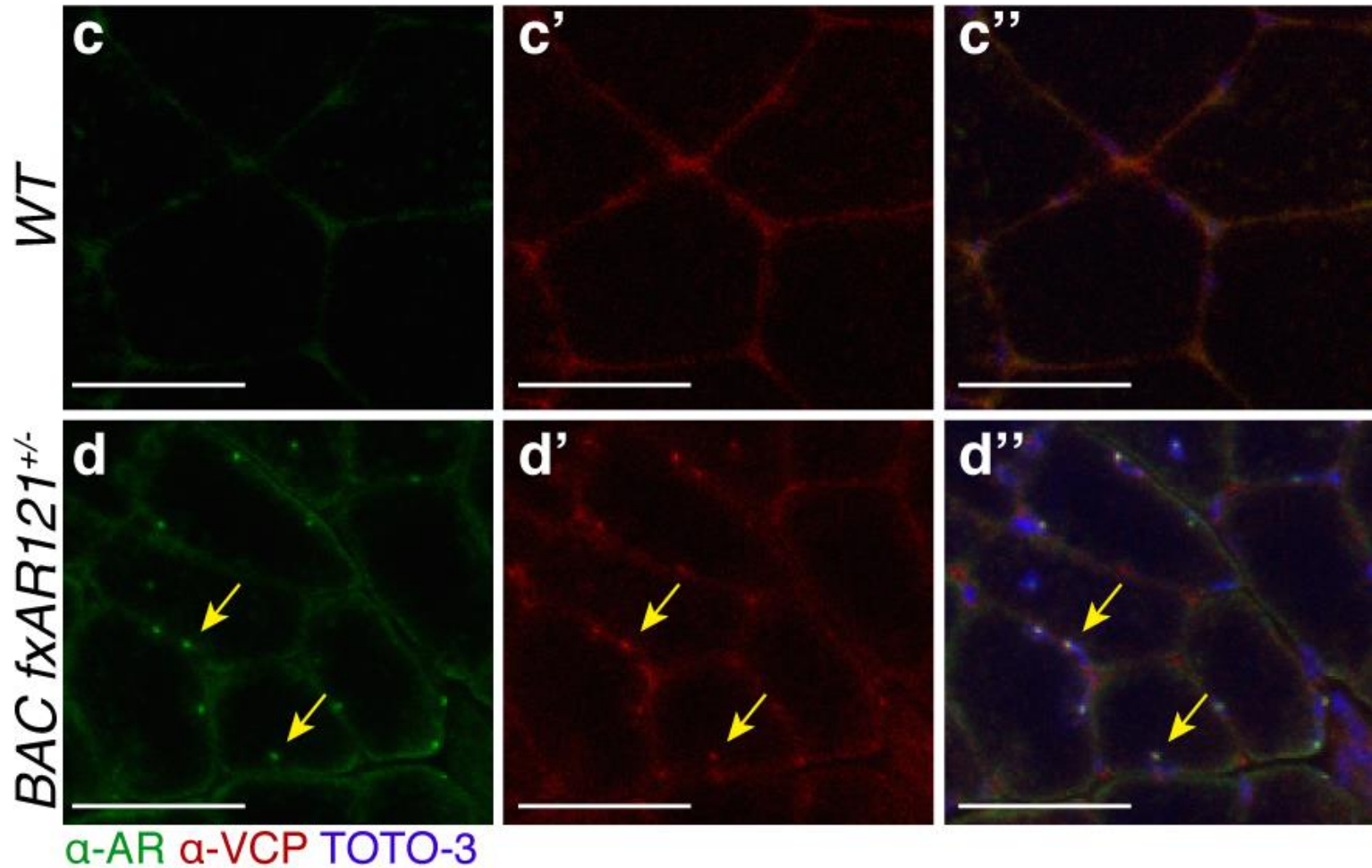
- Valosin-containing protein, also named p97 or cdc48.
- ATPase associated with other activities (AAA+) protein family.
- Implicated in multiple cellular processes
 - cell cycle regulation, DNA replication, organelle biogenesis, and [protein degradation](#), etc.
- Missense mutations in VCP cause IBMPFD (Inclusion Body Myopathy with Paget Disease of Bone and/or Frontotemporal Dementia).
=> Multisystem degenerative disorder with three variably penetrant phenotypes.
 - Inclusion Body Myopathy (IBM)
 - Paget's Disease of the Bone (PDB)
 - Frontotemporal Dementia (FTD)
 - other phenotypes: cardiomyopathy, cataracts and neuropathy

VCP co-localizes with mutant AR in cultured cells

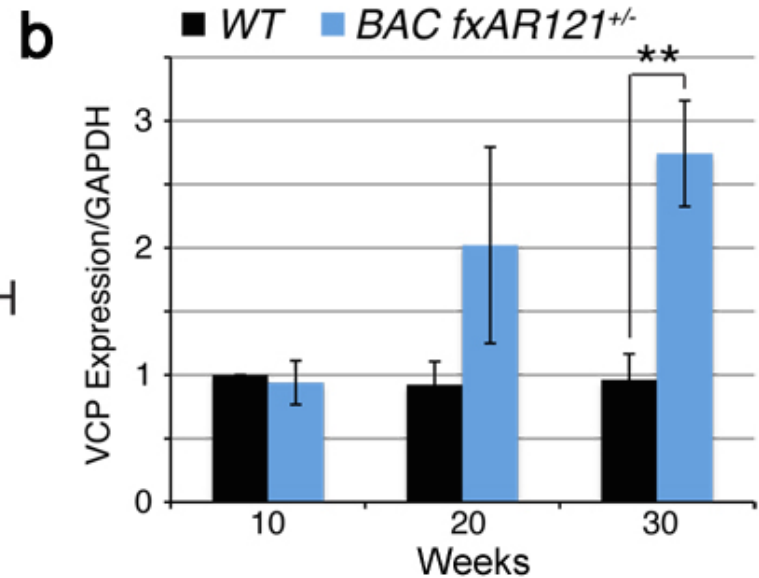
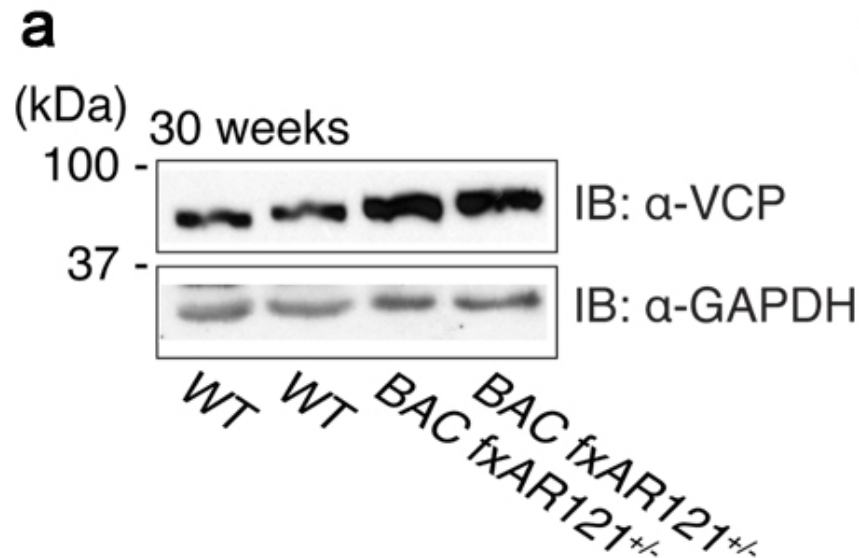


PC12 cells
+ DHT

VCP co-localizes with mutant AR in SBMA mouse skeletal muscle



VCP expression is increased in SBMA mouse skeletal muscle at symptomatic stages



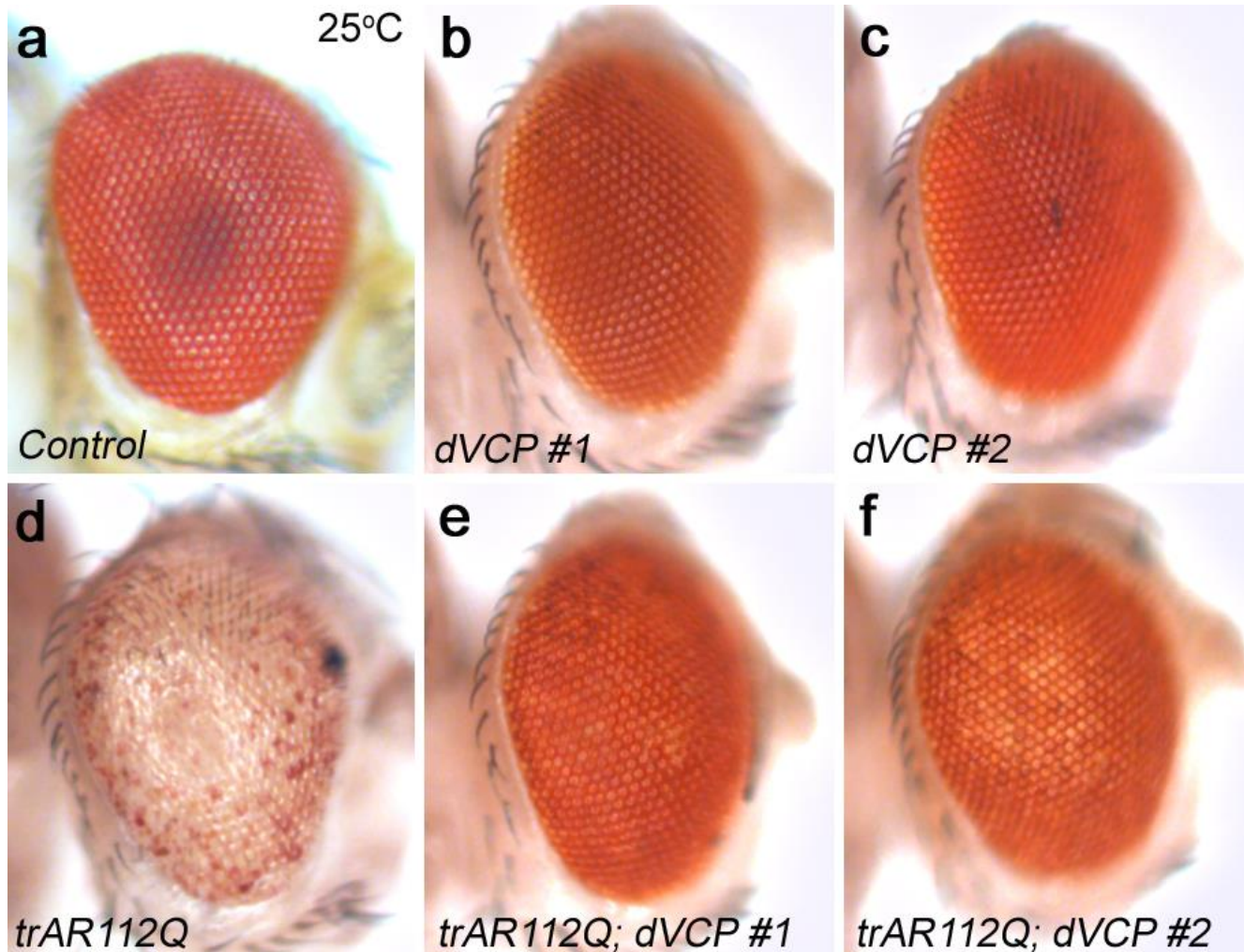
For 10 weeks, n = 2 vs. 3

For 20 weeks, n = 4 vs. 5

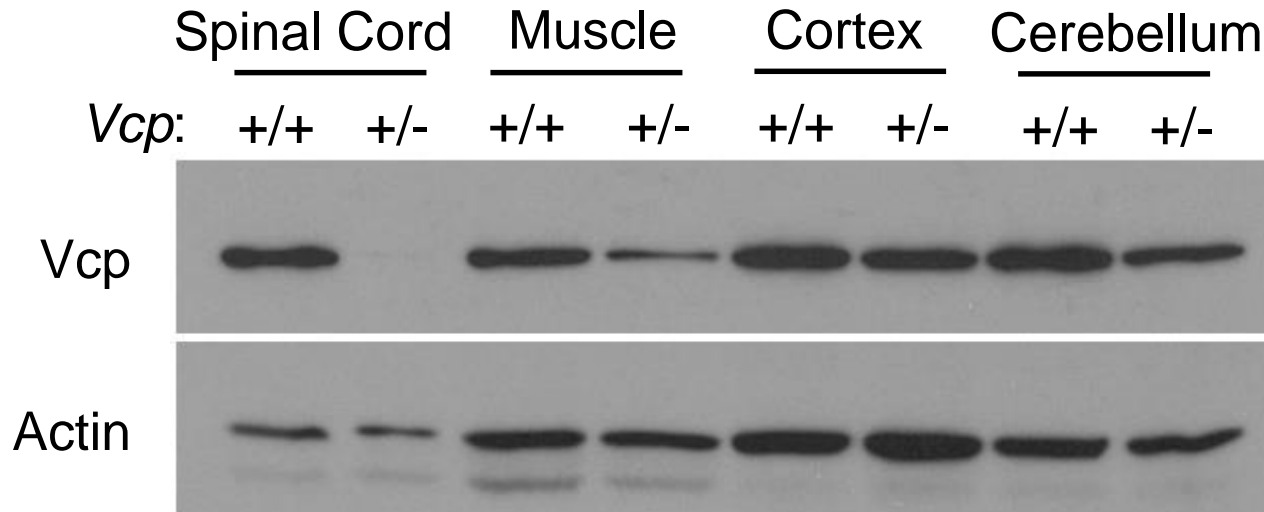
For 30 weeks, n = 7 vs. 8

**p < 0.005 (t-test).

Increased expression of VCP suppresses the toxicity induced by a mutant AR fragment in *Drosophila*



Generation of *Vcp* knockout mice



> Backcrossed over 10 generations onto pure C57BL/6J and 129S6/SvEv backgrounds.

Future VCP Questions in SBMA

- Whether, and how, VCP modulates the pathogenesis of SBMA?
 1. Can altered expression of VCP modulate mutant AR phenotype and toxicity in SBMA?
 2. Is increased VCP expression in SBMA mice beneficial or detrimental?
 3. What mechanisms underlie the function of VCP in SBMA?
 4. Is AAA+ ATPase enzymatic activity in VCP important for modulating SBMA phenotypes?

Translational Neuroscience

Neurodegenerative Diseases

successful therapies?

Kennedy's Disease

pre-clinical trials

drug discovery

gene identification

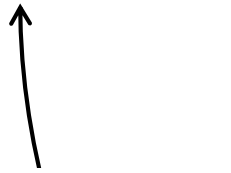
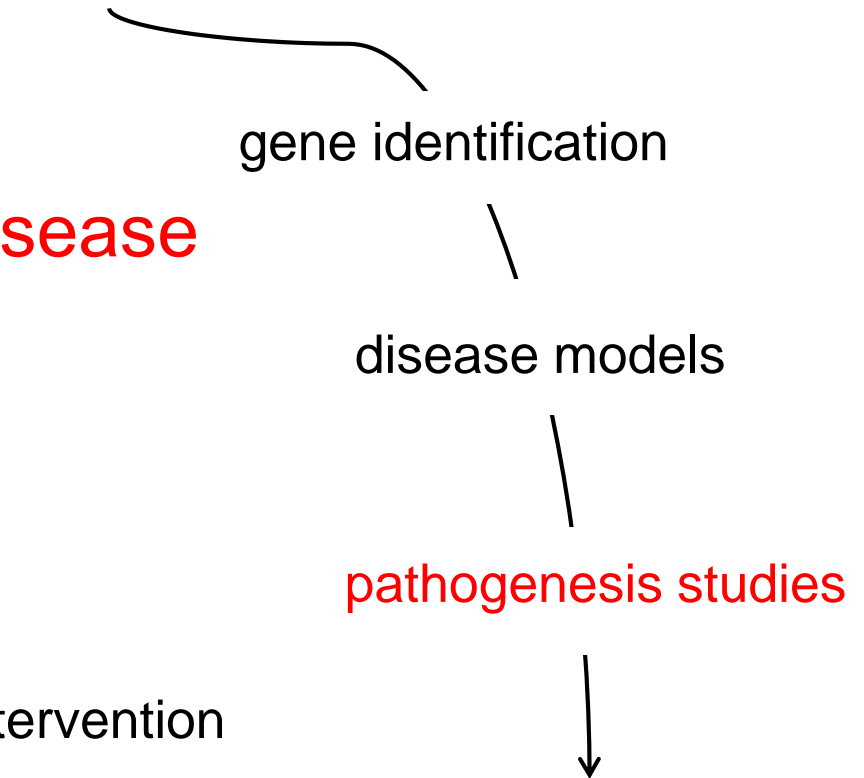
disease models

pathogenesis studies

candidate targets for therapeutic intervention

NLK, VCP &

Basic Neuroscience



Acknowledgements

- Lab members:
 - Hiroshi Kokubu
 - Terri Driessen
 - Tingting Dong
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 - Cleo Smeets
 - Xiaowen Yu

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 - Diane E. Merry, Thomas Jefferson University
 - J. Paul Taylor, St. Jude Children's Research Hospital / HHMI

- Funding: NIH, Kennedy's Disease Association