Alternative Models (Part III): Yeast

Special series on Laboratory animal science
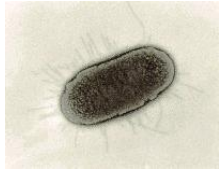
Asvin Lakkaraju

01.11.2016

Outline

Introduction

Paper I: Yeast model to discover mode of action of a drug

Paper 2: Yeast and Drosophila genetics to decipher TDP43 toxicity

Paper 3: Combination of Yeast and human synthetic lethality screen to identify new therapies for cancer
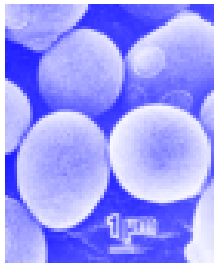
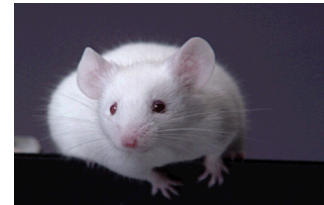# Some commonly used model organisms

 Escherichia coli

 Budding yeast — *Saccharomyces cerevisiae*

 Round worm — *Caenorhabditis elegans*

 Fruit fly — *Drosophila melanogaster*

 Zebrafish — *Danio rerio*

 Mouse — *Mus musculus*
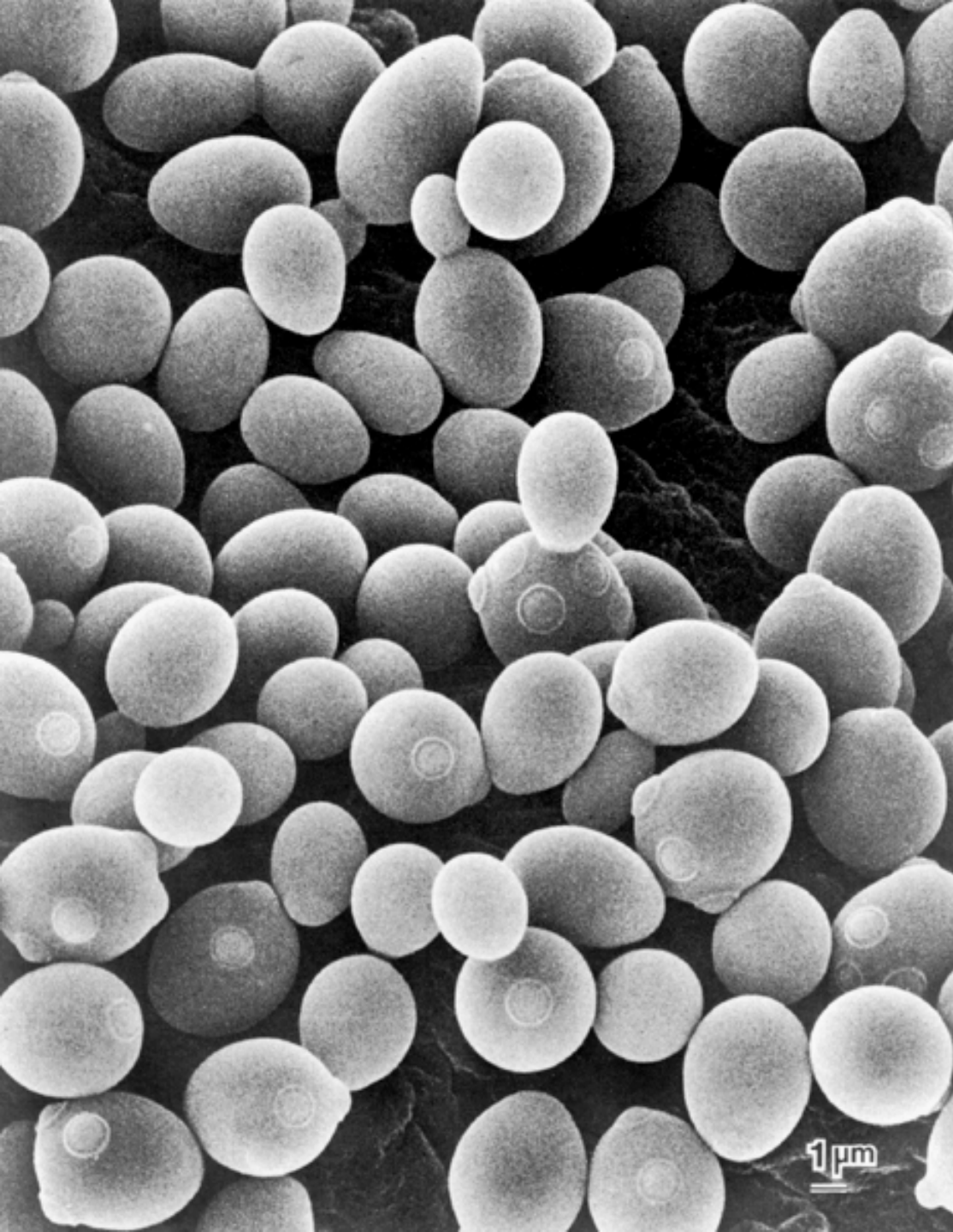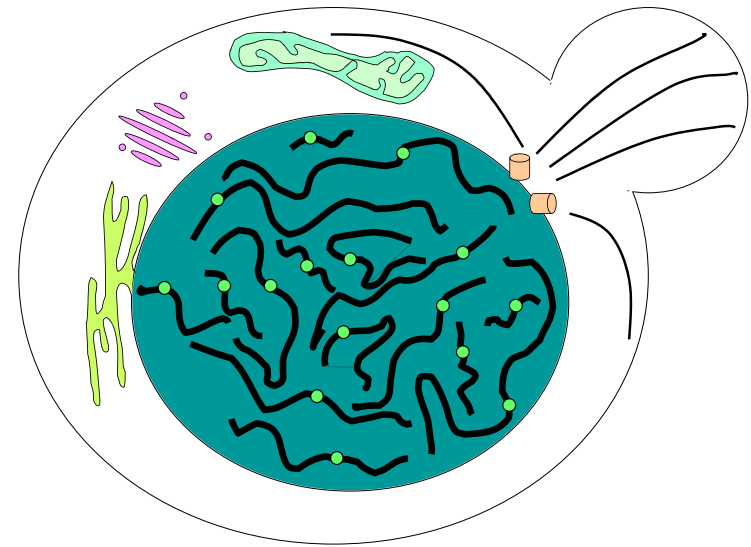
 Thale cress — *Arabidopsis thaliana*

Baker's yeast = budding yeast
=

Saccharomyces cerevisiae

- Yeast is a eukaryote
- 16 chromosomes
- ~6000 genes
- Very few introns

1μm

Few facts about yeasts

- Can appear in haploid or diploid forms.

- Can grow on different types of carbon mediums.

- Can use respiration or fermentation as their preferred carbon consuming method.

- Have a relatively high reproduction rate.

The first official written definition about yeast: 1775 by Samuel Johnson in the Dictionary for English language.

. . the ferment put into drink to make it work; and into bread, to lighten and swell it.
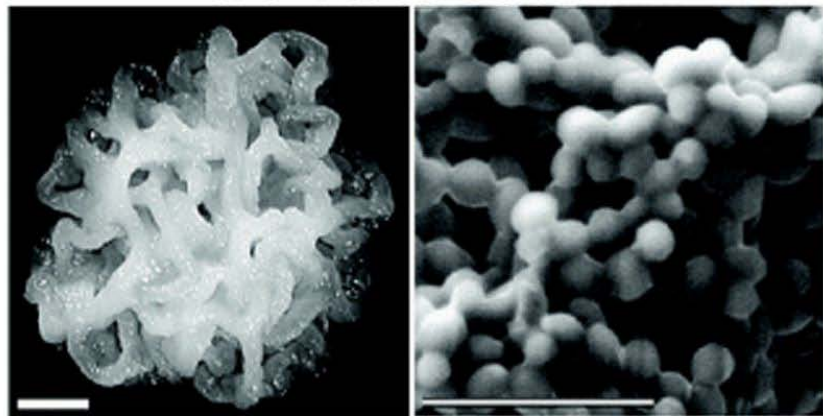
For next 150 years was mainly described in fermentation processes.

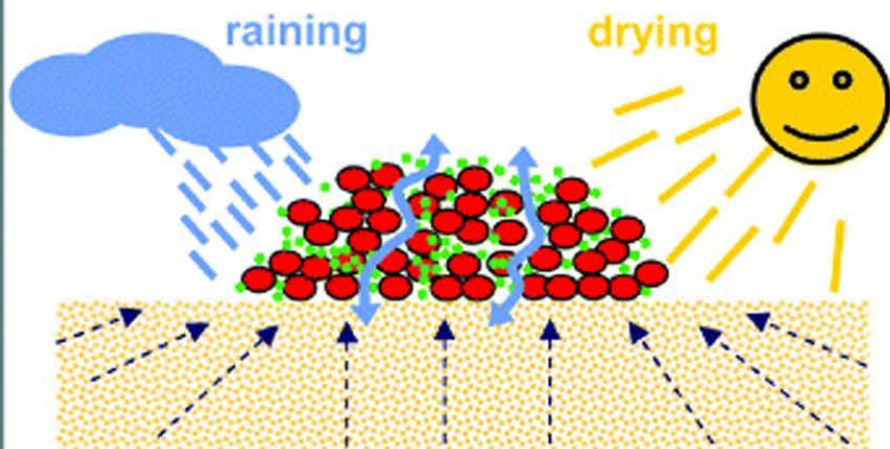IN 1930s, Winge and Lindegren began work on yeast for the first time as an experimental organism.

In 1996, S.*cerevesiae* became the first eukaryote to be completely sequenced.
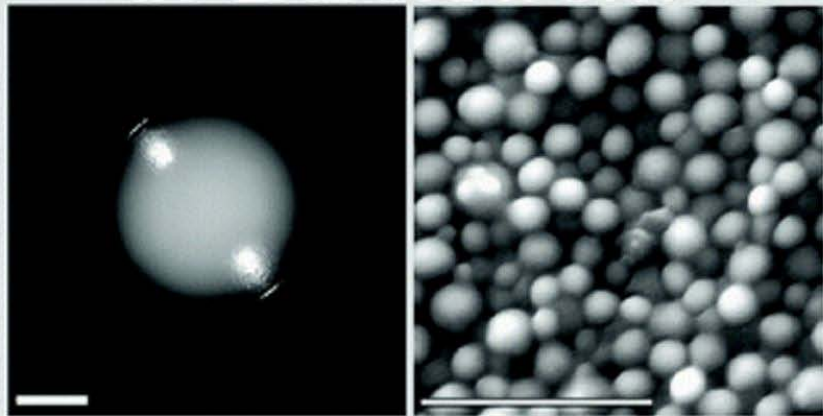
IN NATURE

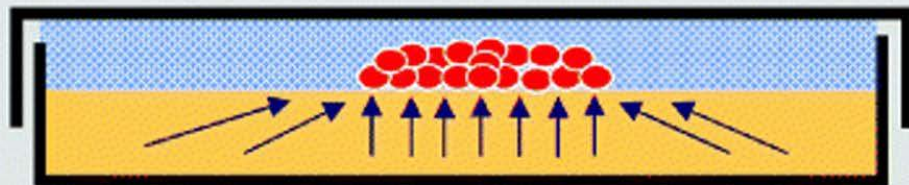IN LABORATORY

Temperature and humidity fluctuate

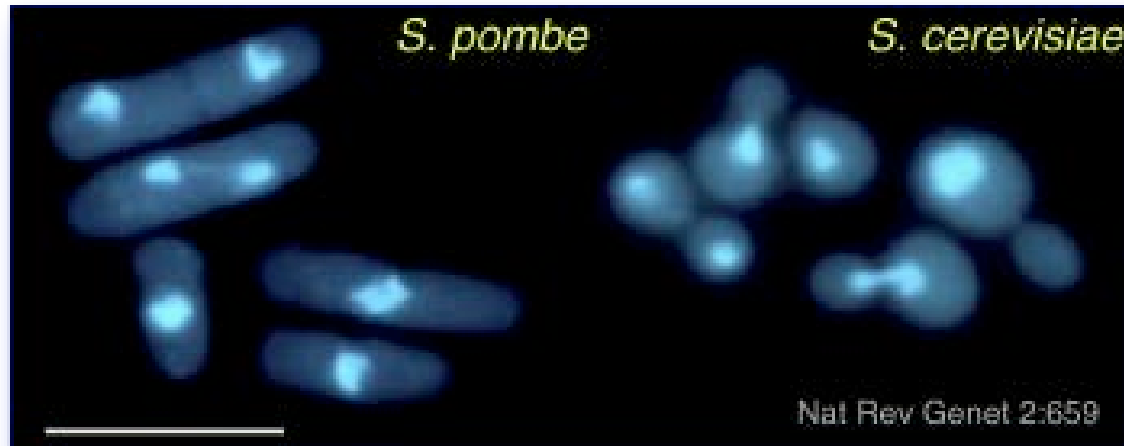raining   drying

Limited concentration of nutrients

Relatively stable humidity and standard temperature

High initial concentration of nutrients

DOMESTICATION

Types of yeast used in the lab

- *S. cerevisiae* has approximately 5,600 open reading frames; *S. pombe* has approximately 4,970 open reading frames.
- Despite similar gene numbers, *S. cerevisiae* has only about 250 introns, while *S. pombe* has nearly 5,000.
- *S. cerevisiae* has 16 chromosomes, *S. pombe* has 3.
- *S. cerevisiae* is often diploid while *S. pombe* is usually haploid.
- *S. pombe* has a shelterin-like telomere complex while *S. cerevisiae* does not.[13]
- Both species share genes with higher eukaryotes that they do not share with each other. *S. pombe* has RNAi machinery genes like those in vertebrates, while this is missing from *S. cerevisiae*. *S. cerevisiae* also has greatly simplified heterochromatin compared to *S. pombe*.[14]
- Conversely, *S. cerevisiae* has well-developed peroxisomes, while *S. pombe* does not.
- *S. cerevisiae* has small point centromere of 125 bp, and sequence-defined replication origins of about the same size. On the converse, *S. pombe* has large, repetitive centromeres (40–100 kb) more similar to mammalian centromeres, and degenerate replication origins of at least 1kb.
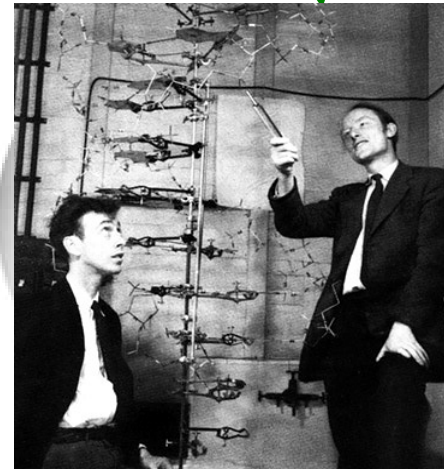
Source: Wikipedia

yeast cell

human cell
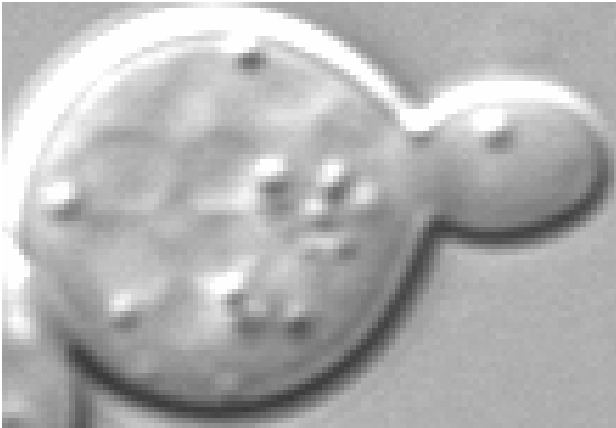
Golgi app.

Nucleus

Vacuole
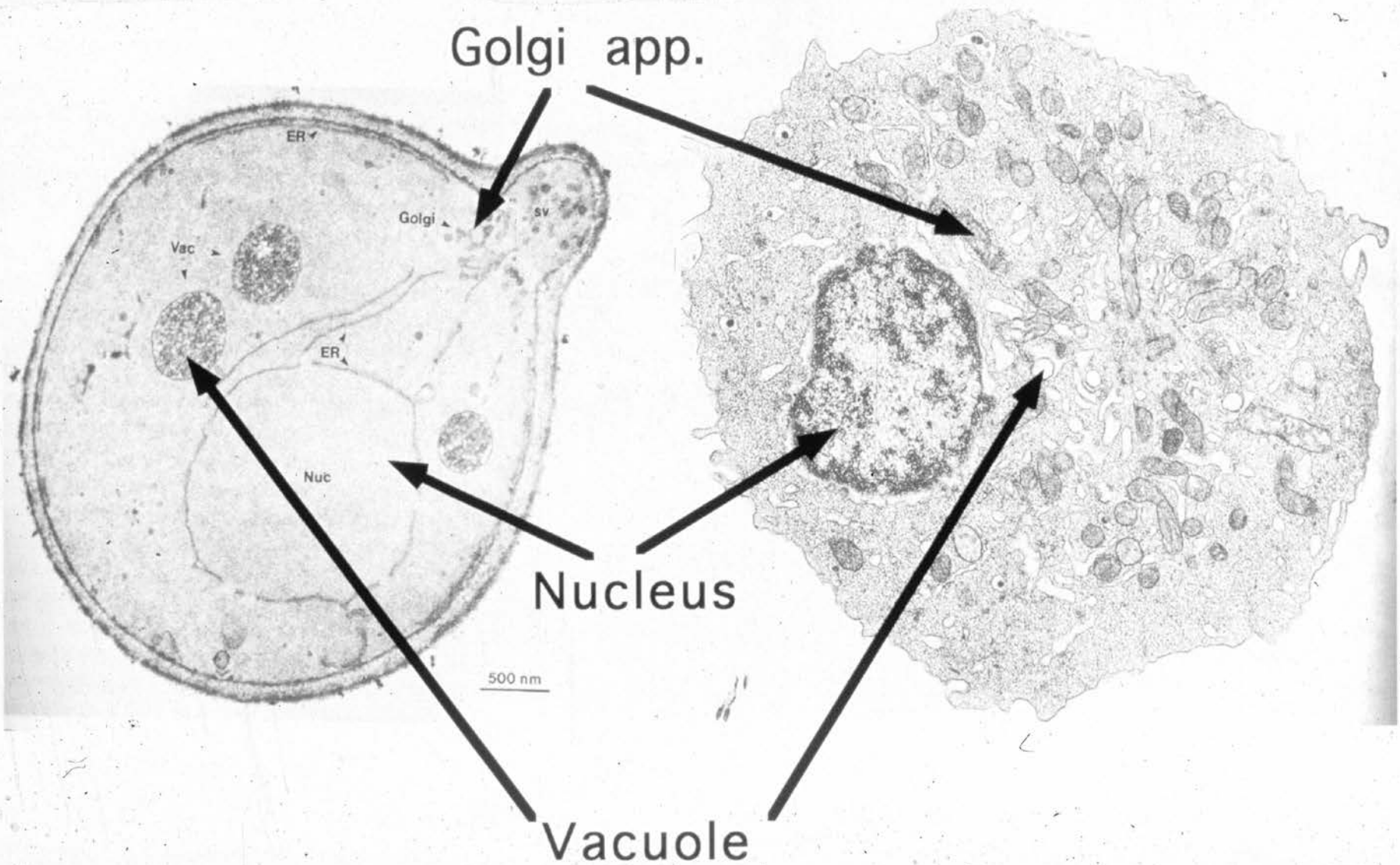
Yeast vs. Human

~50% of yeast genes
have at least one
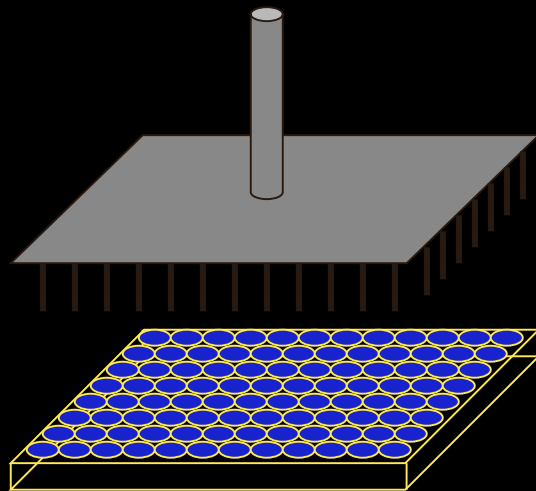similar human gene

~50% of human genes
have at least one
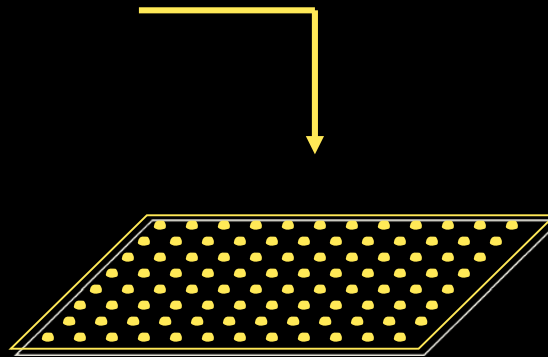similar yeast gene

Human vs. Yeast
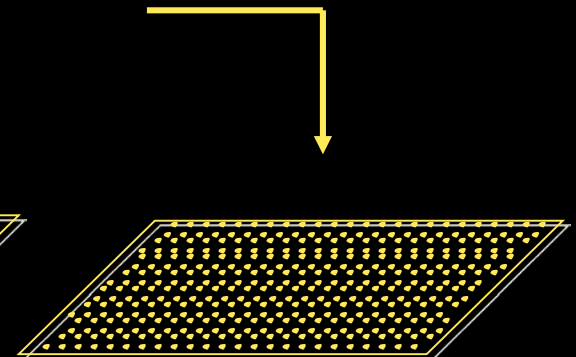
S. cerevisiae Genome Deletion Project

- "Complete" set of yeast nonessential deletion mutants

- ~4,700 haploid strains
- ~4,700 homozygous diploid strains
  nonessential genes deleted with kanMX = fifty 96 well plate
- ~5,800 heterozygous diploid strains
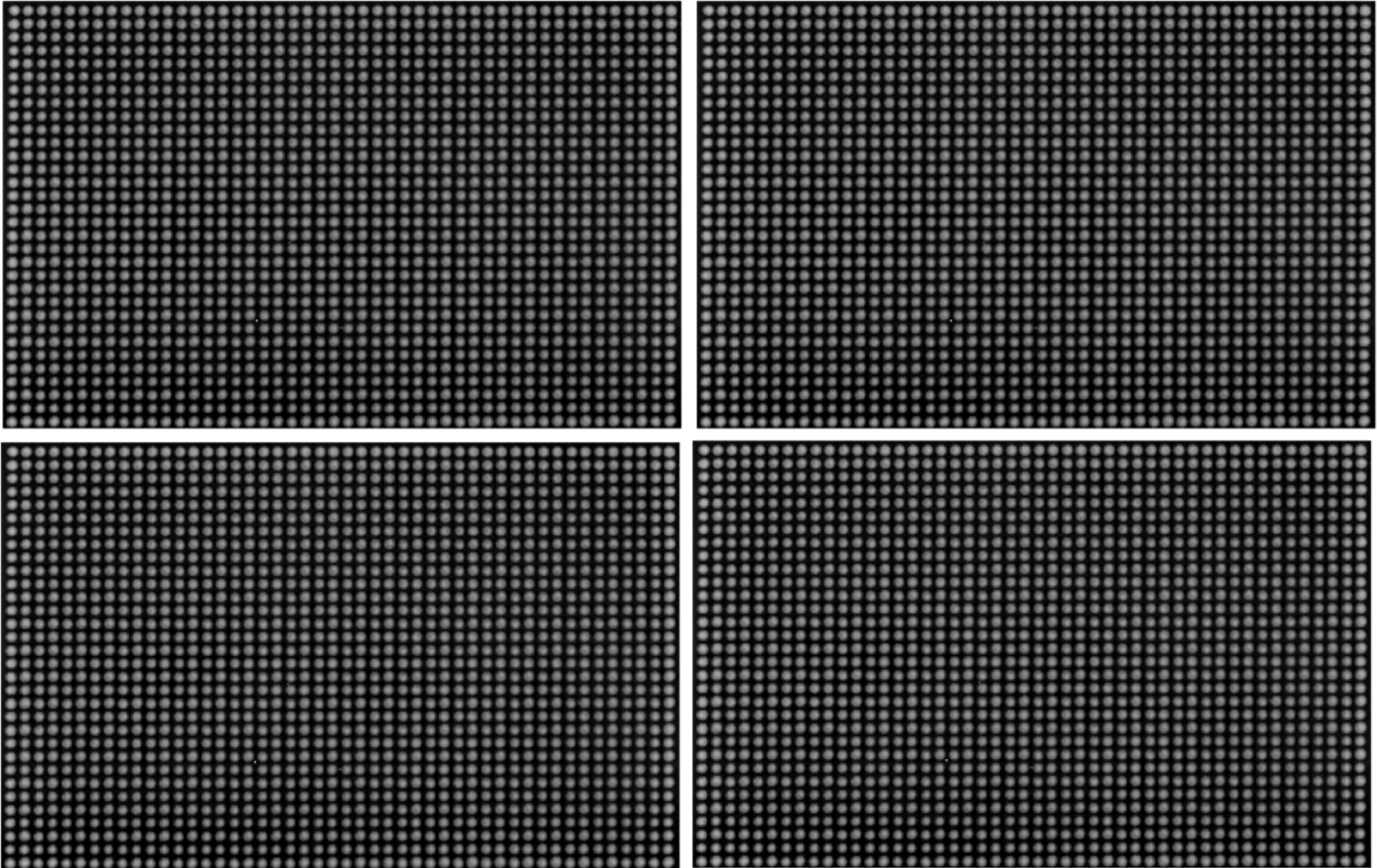
96 well plate
frozen glycerol stock

pin 96 strains
onto G418 plates

condense 4
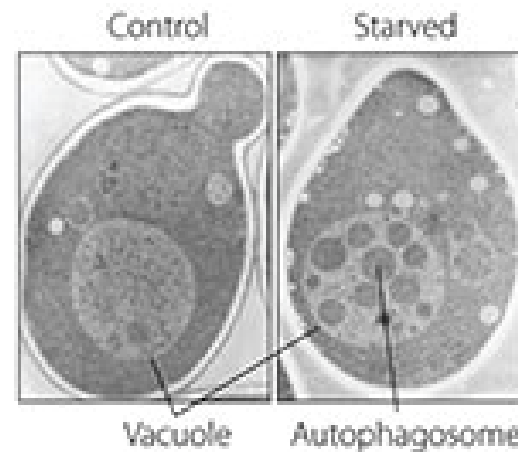plates onto 1
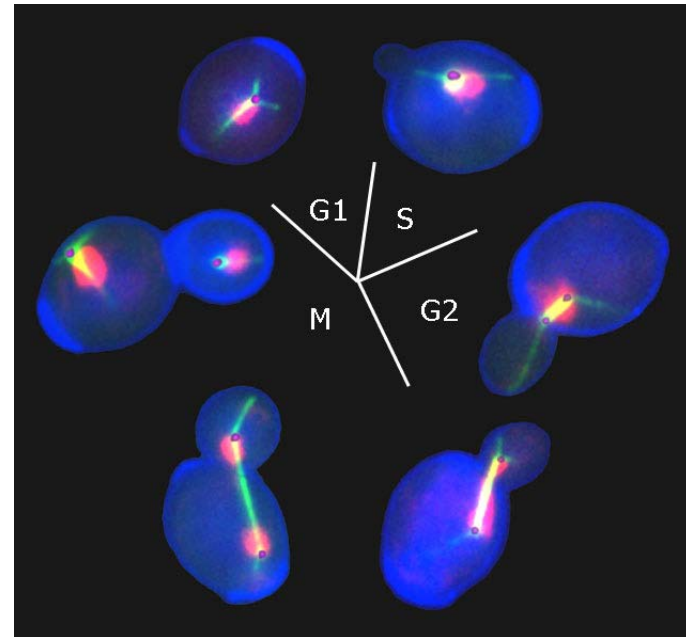
The yeast gene knockout collection

Order any knock out clone from : ATCC, Invitrogen, Open Biosystems and EUROSCARF .

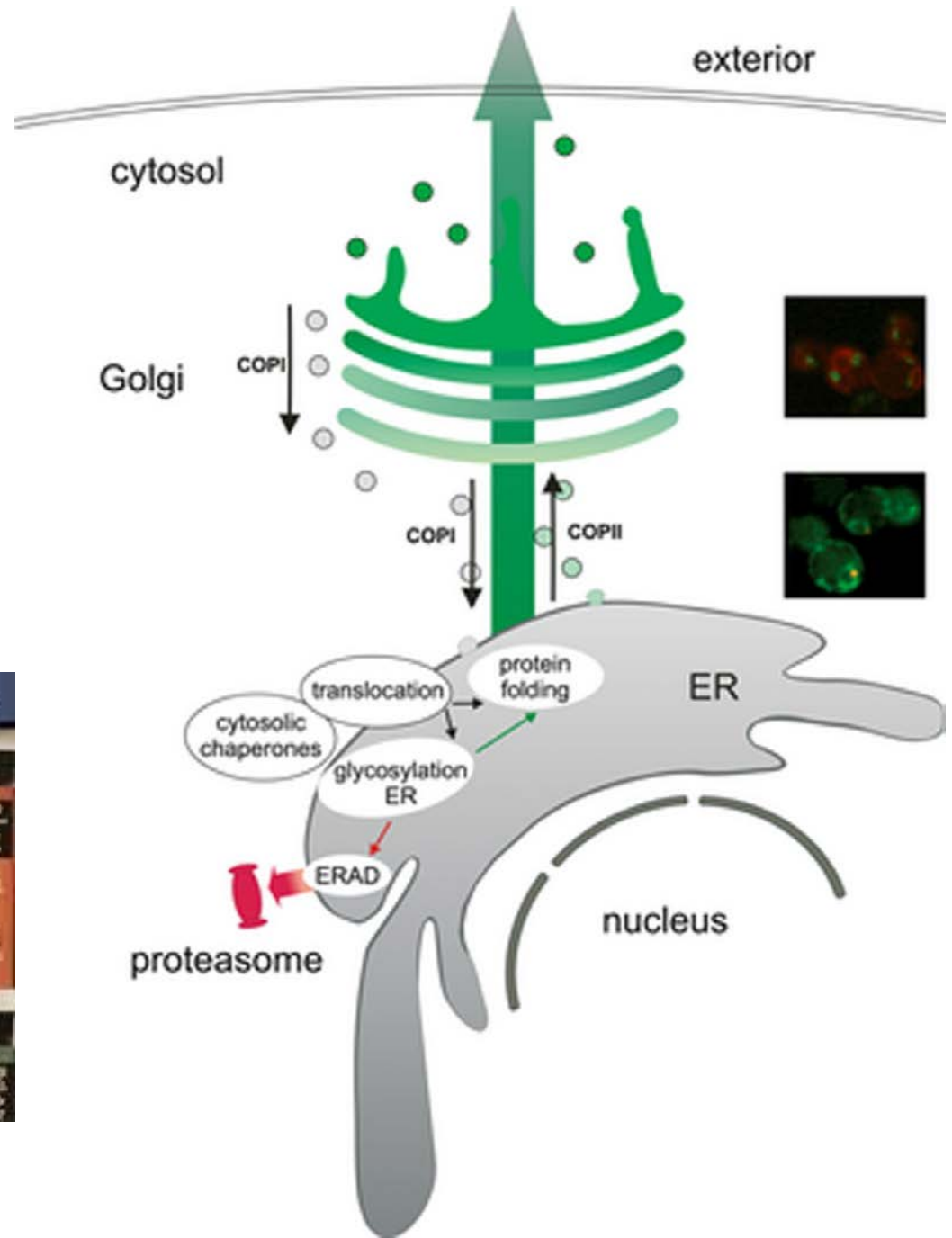Currently more than 20,000 strains are avilable

Yeast genetics: Chromatin and cellular functions

Leland Hartwell, Paul Nurse and Tim Hunt were awarded Noble prize for their discovery of Cyclins and check points during cell division

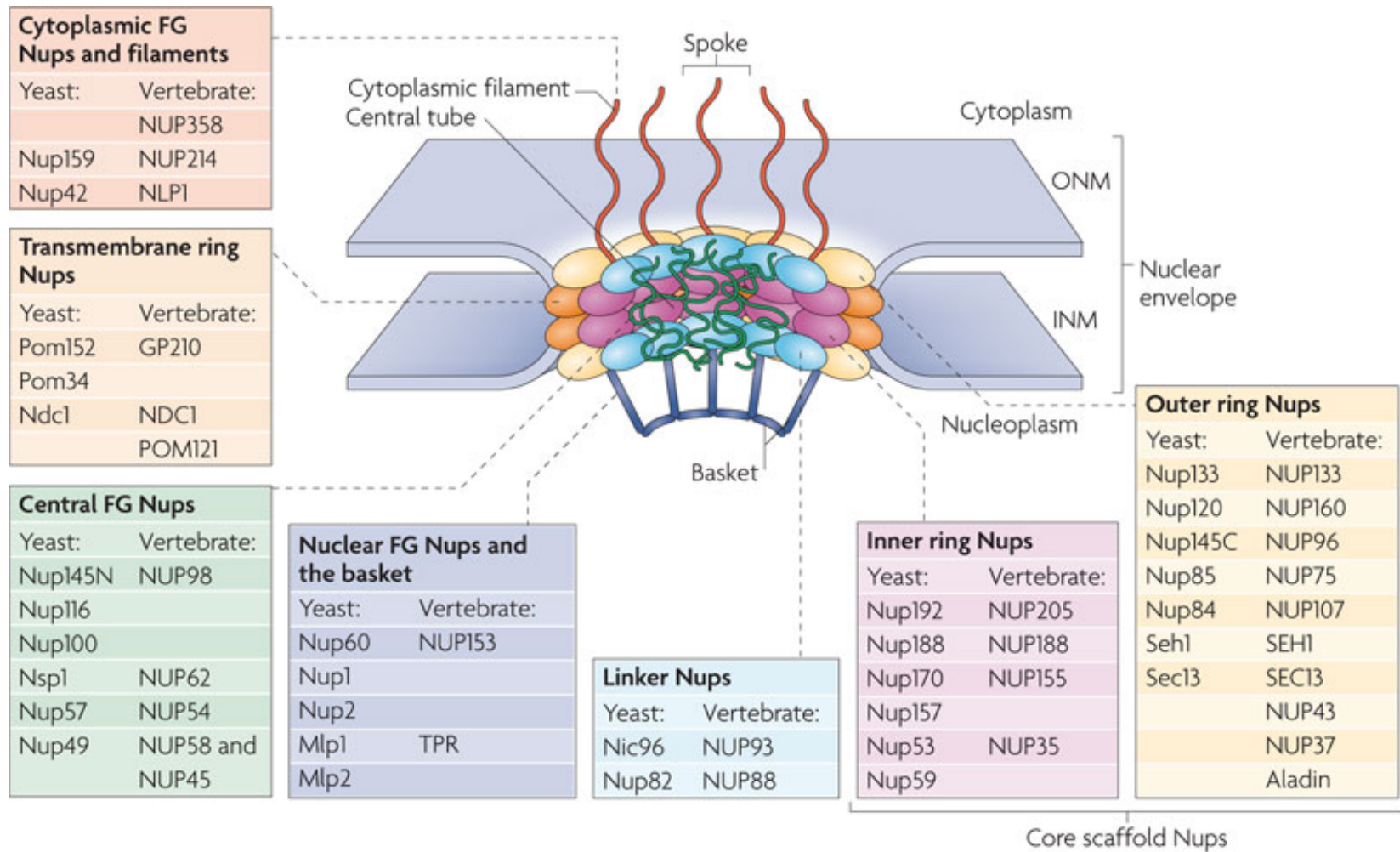# Secretory Pathway

Randy Schekmann: Noble prize

Nuclear Pore complex

Nature Reviews | Molecular Cell Biology
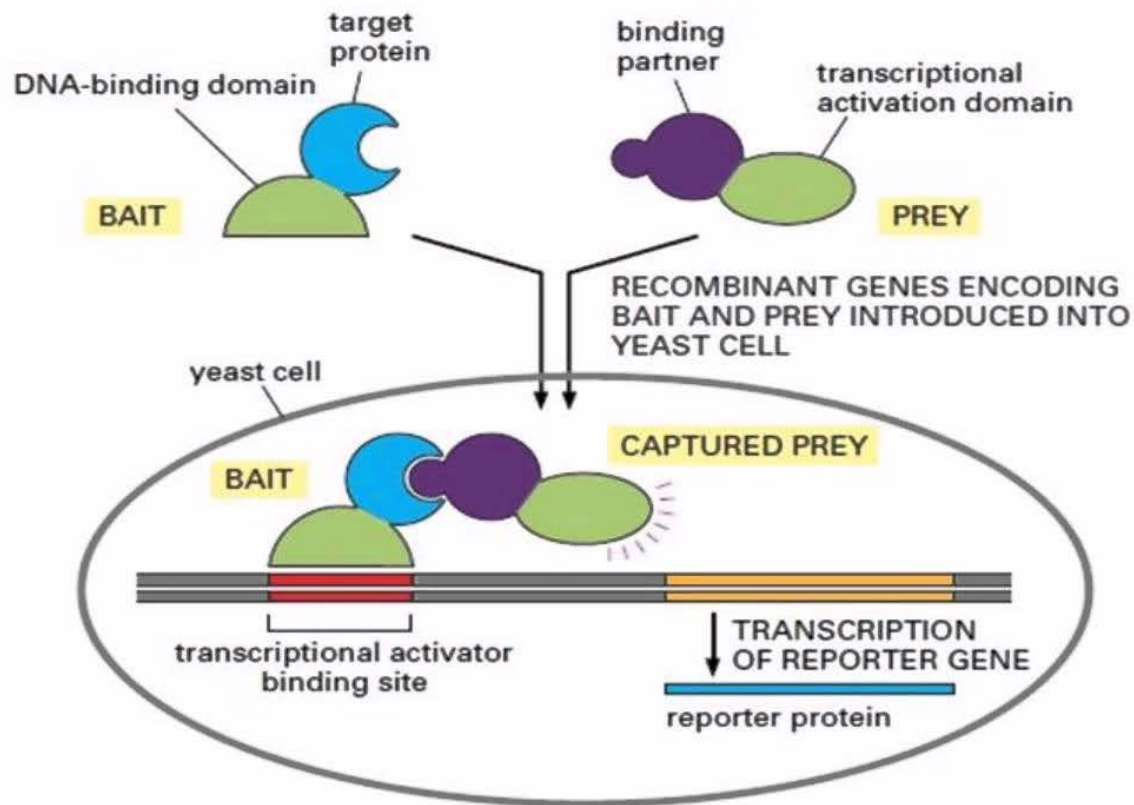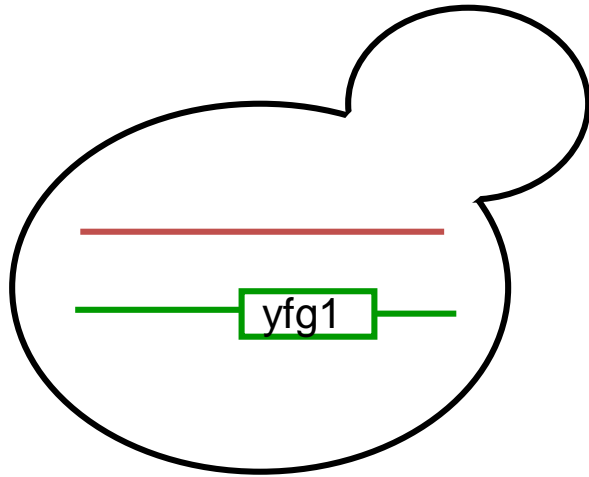
Yeast Prions
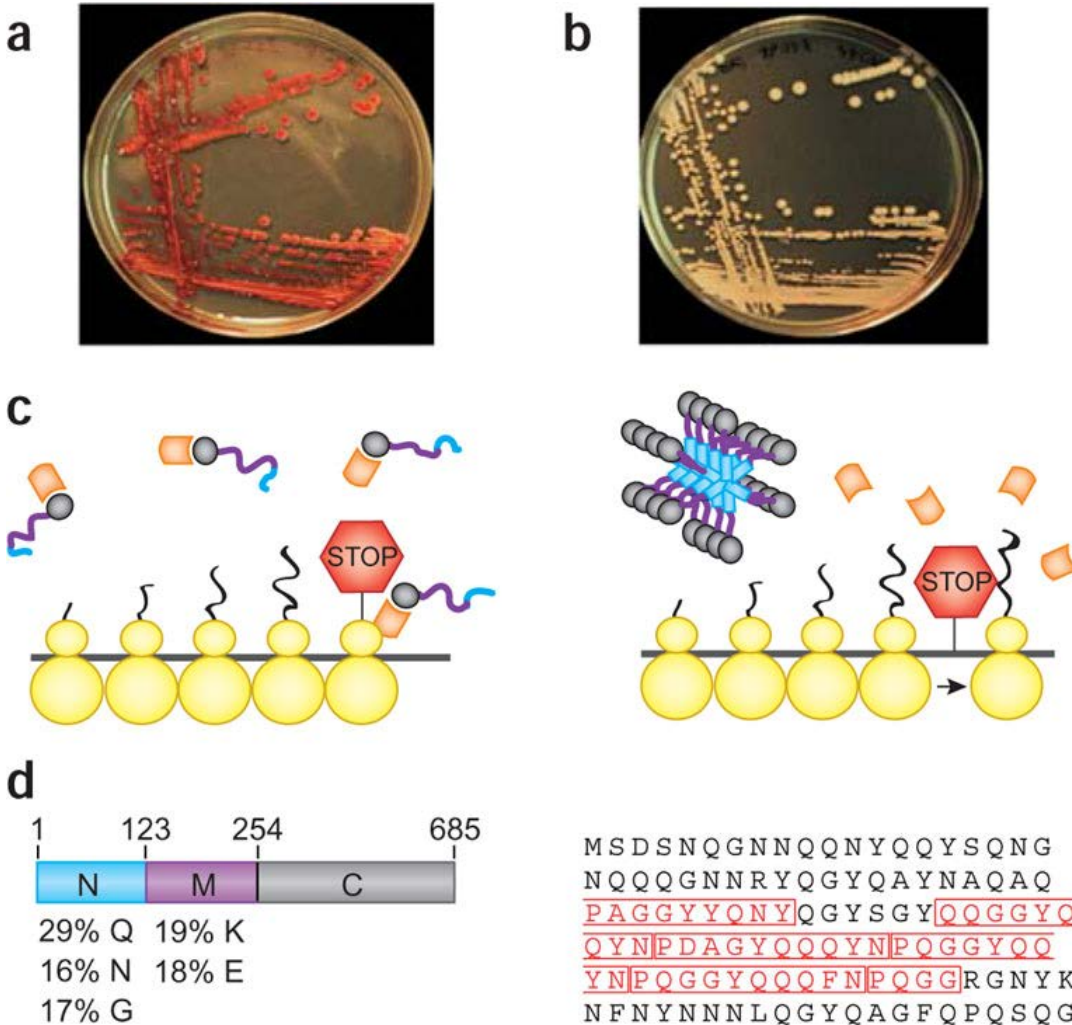
Yeast Two Hybrid System

Yeast Two Hybrid System

www.technologyinscience.blogspot.com

Synthetic Lethality

Viable
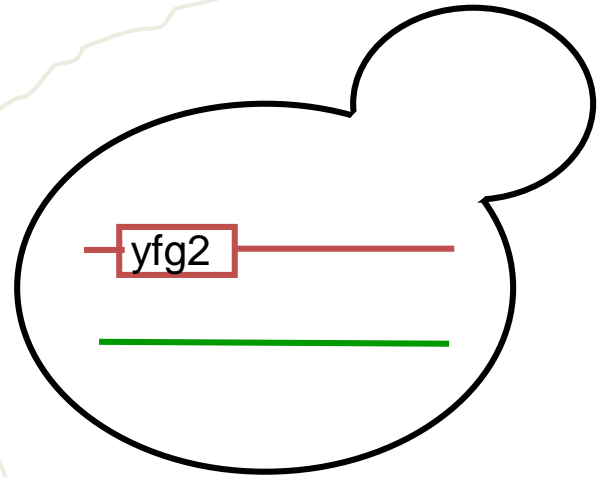
Viable
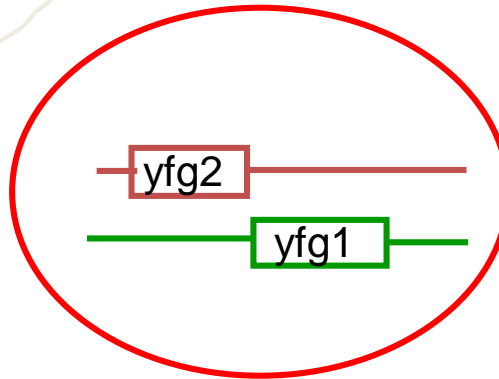
Yfg2 = Drug target

Yfg1 = CIN mutant

Normal

Tumor

Dead

Yeast Reveal a "Druggable" Rsp5/Nedd4 Network that Ameliorates α-Synuclein Toxicity in Neurons

Daniel F. Tardiff,[1] Nathan T. Jui,[2] Vikram Khurana,[1,3] Mitali A. Tambe,[4] Michelle L. Thompson,[5]* Chee Yeun Chung,[1] Hari B. Kamadurai,[6] Hyoung Tae Kim,[7] Alex K. Lancaster,[1]† Kim A. Caldwell,[5] Guy A. Caldwell,[5] Jean-Christophe Rochet,[4] Stephen L. Buchwald,[2] Susan Lindquist[1,8]‡

Aim: To identify **MOA** of a new drug discovered.

Parkinson's Disease

- loss of dopamine neurons
- Lewy bodies: aggregated aSynuclein
- aggregation: necessary for pathogenicity
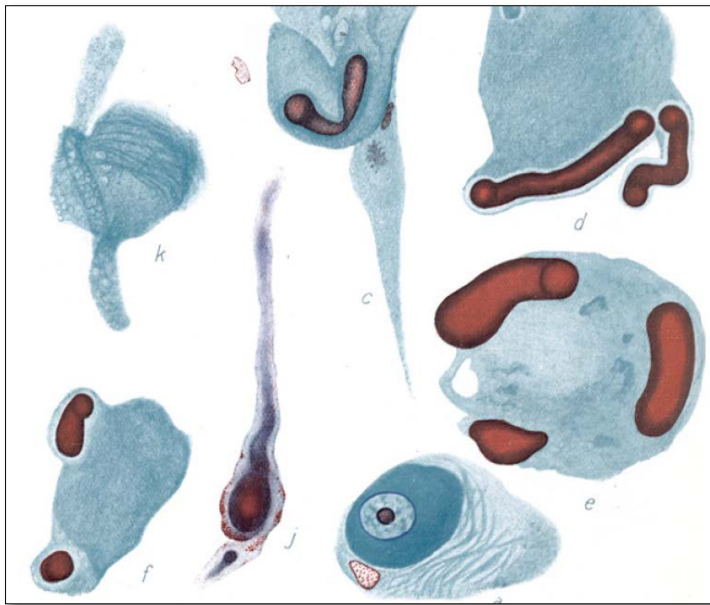
(Springer-Verlag, Berlin, 1923)
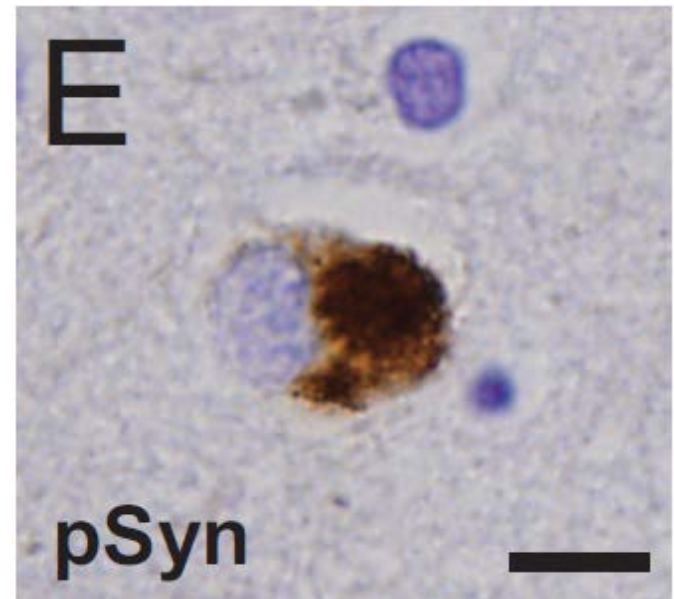
(Luk et al., 2009)

Alpha synuclein in Yeast

A

kD
68-
52-
40-
28-

WT A53T A30P   WT A53T A30P

one copy   2 μ

B

| | WT | A53T | A30P |

one copy

WT αSyn   A53T   A30P

uninduced

induced
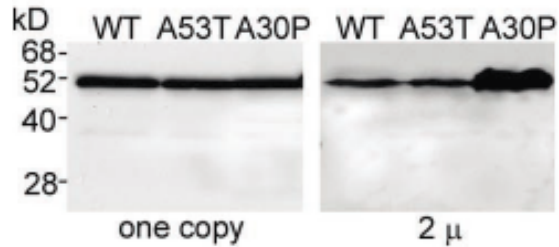
Alpha Syn is membrane associated.

Two copies inhibited growth
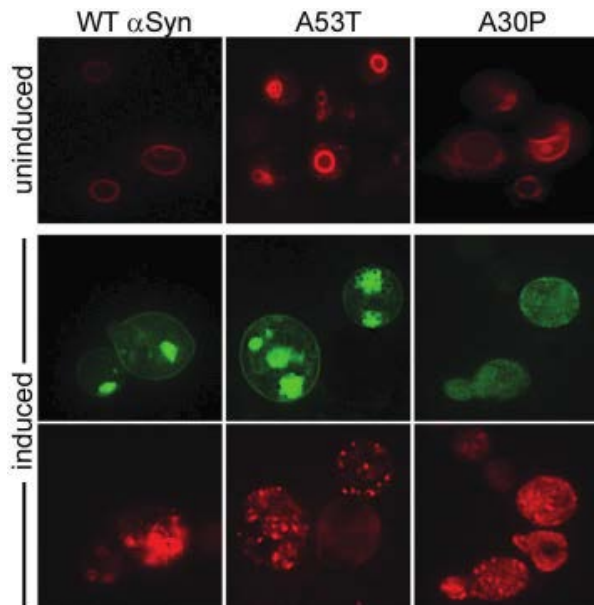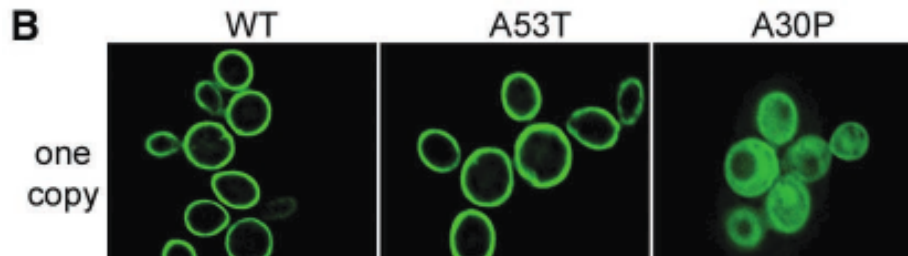
Overexpression of alpha syn alters the vesicular
Pools: Defects in endocytosis.

N-aryl benzimadazole (NAB) rescues alpha syn toxicity

NAB were discovered in a
screen to identify rescuers of
TDP43 toxicity.

Nematode and human models: Validating NAB

Dopaminergic neurons

cortical neuronal cultures
From PD patient

Cell line models: Validating NAB

Rat primary cortical cultures
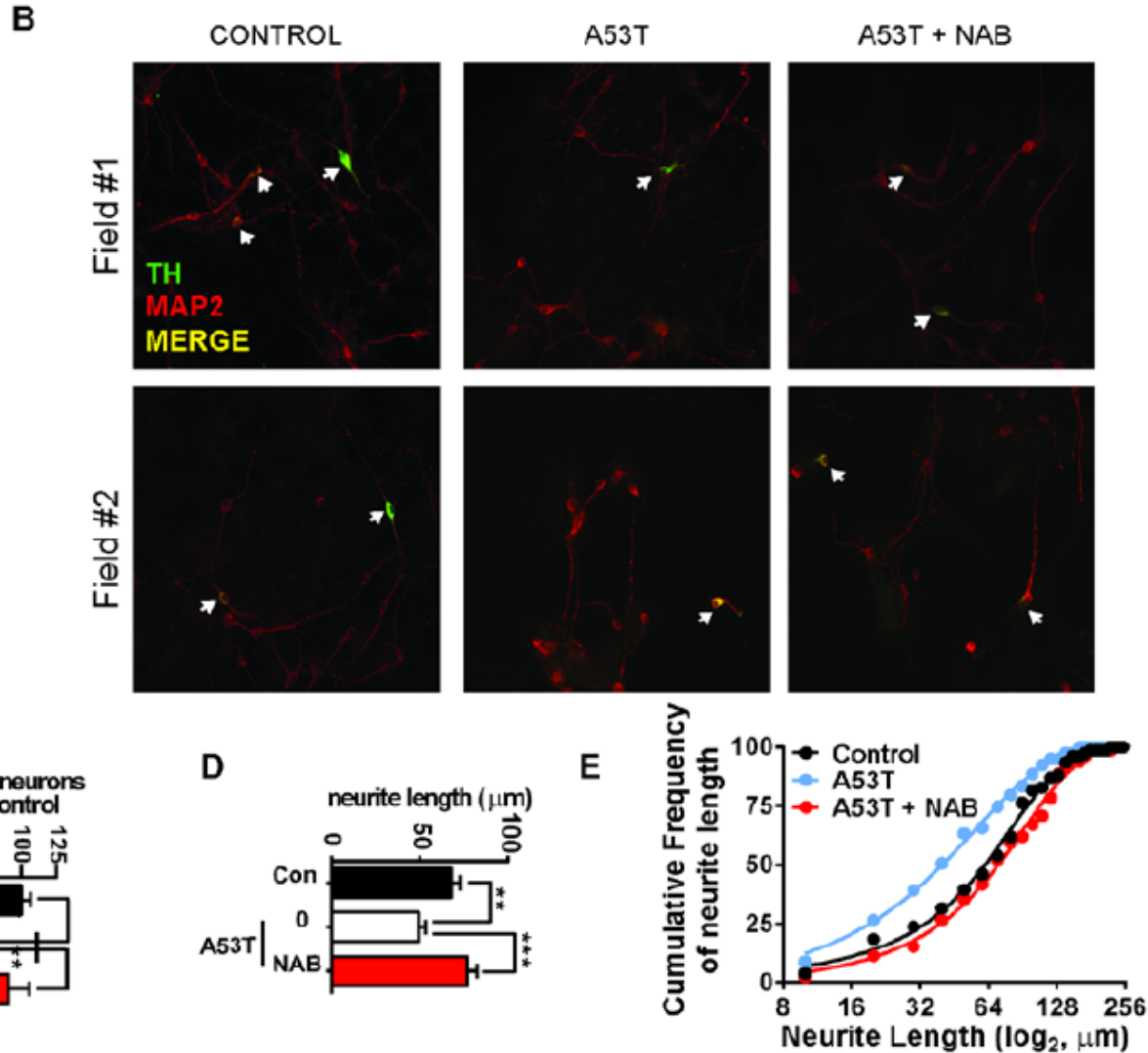
Synthesis of analogs of NAB

A

Active NAB Analogs

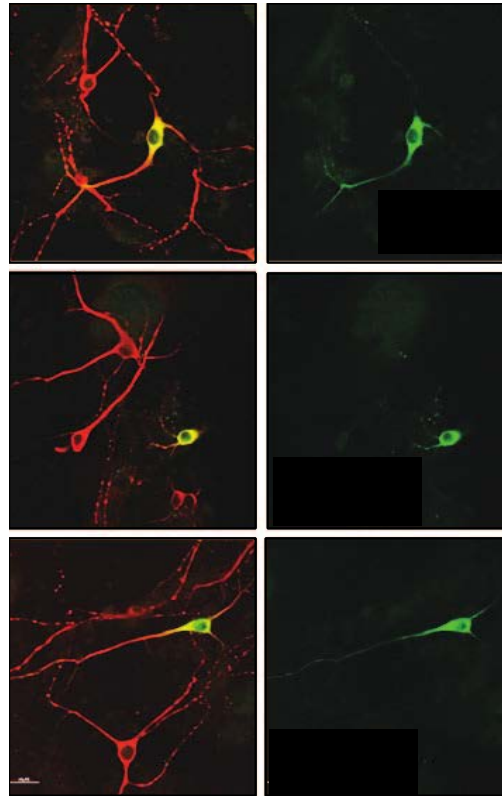DMSO | NAB1 (10 μM) | NAB2 (2.5 μM) | NAB3 (15 μM)

Inactive NAB Analogs

NAB22 (20 μM) | NAB26 (20 μM) | NAB29 (20 μM)

NAB27 (20 μM) | NAB25 (20 μM) | NAB28 (20 μM)
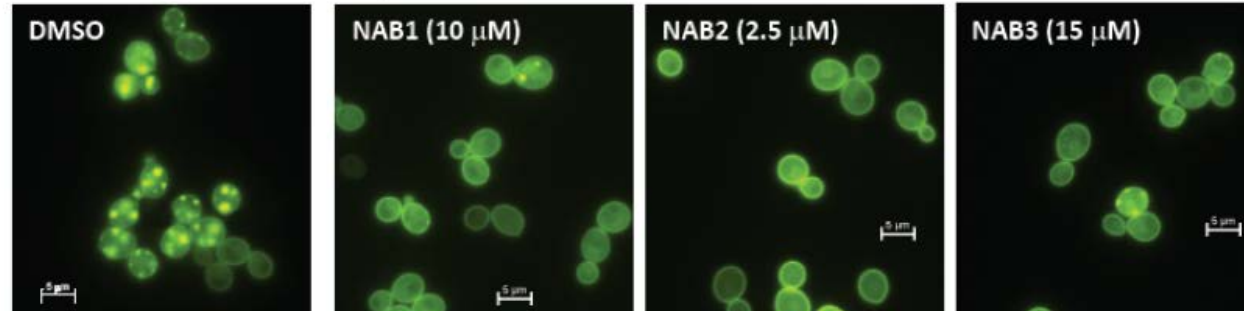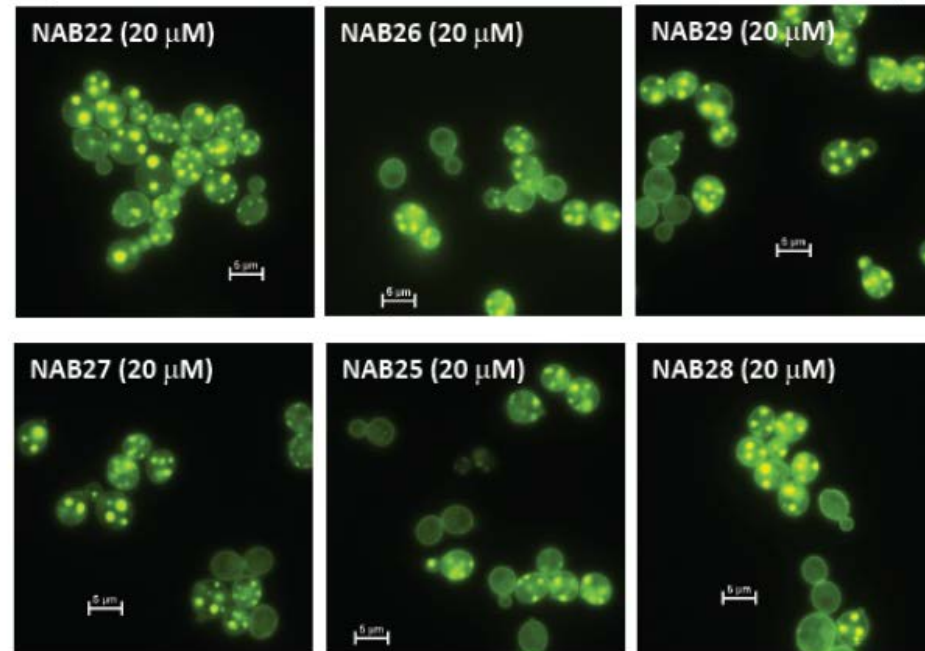
NAB not only rescued the phenotype but also
Inhibited cell growth at high concentration
without affecting the Viability.

A

WT growth inhibition IC_{40} (μM)

$\rho = .7461$

NAB1

NAB2

α-syn efficacy EC_{40} (μM)

B

NAB2 (μM)
0   36
21.6   60

Viable Cells/well

Time (hours)
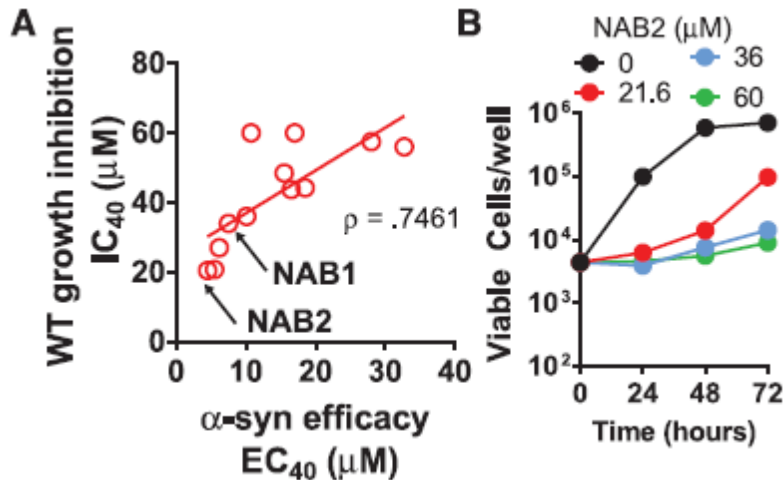
What is the mode of action of NAB2?

What is the target of NAB?

Genetic screens to identify
Mutants that allowed the growth
At high conc
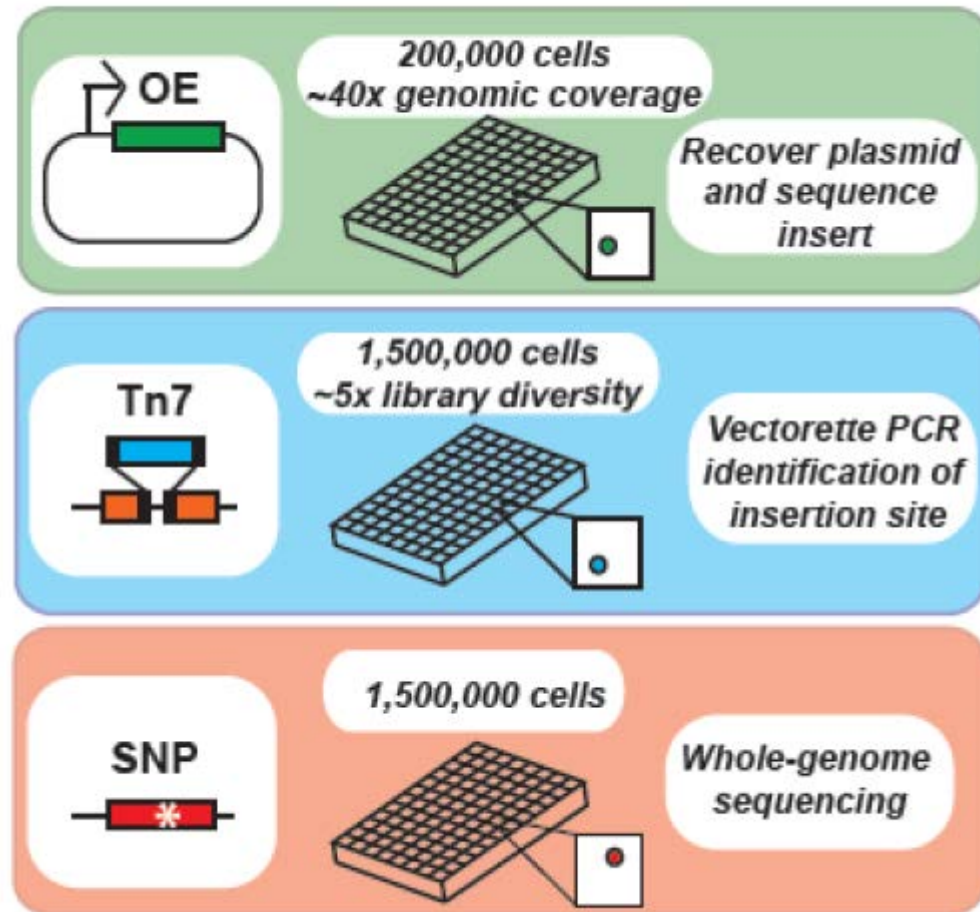
A small set of hits were recovered and all these hits formed a highly connected network
Of genes.

Hits formed a part of E3 Ub ligase complex

Deletion of individual genes

No effect on NAB2
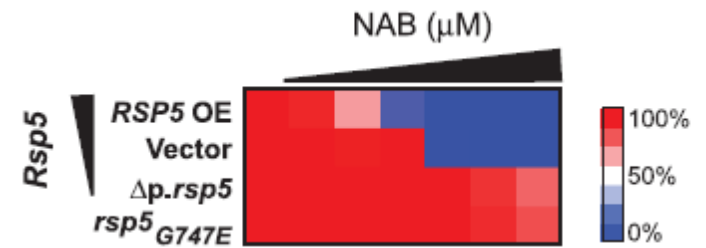
NAB2 promotes Rsp5 mediated functions

Rsp5 regulates the
Membrane traffic of diverse
Membrane proteins

NAB2 promotes Rsp5 dependent endocytosis of MUP1

NAB2 rescues Cpy trafficking defets in the context of alpha syn toxicity

W

NAB2 rescues phenotypes associated with alpha syn toxicity
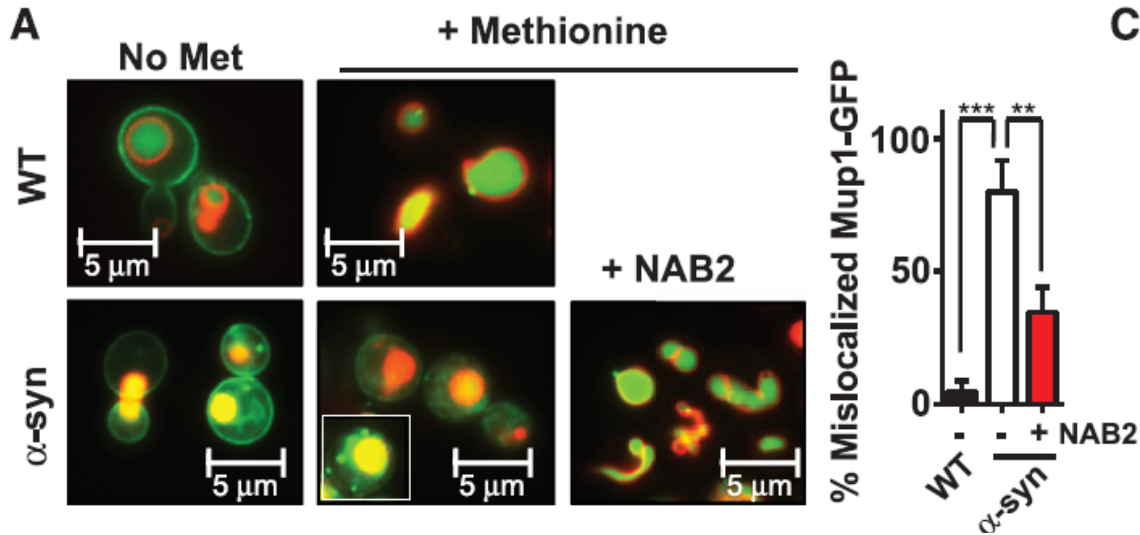
Alpha syn impedes methionine dependent endocytosis of MUP1.

Red: FM1, markes vacuoles
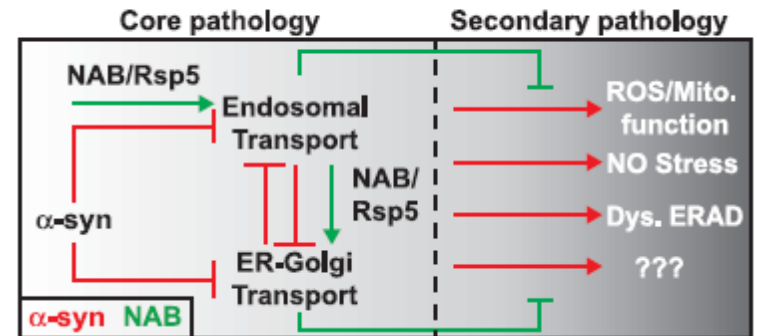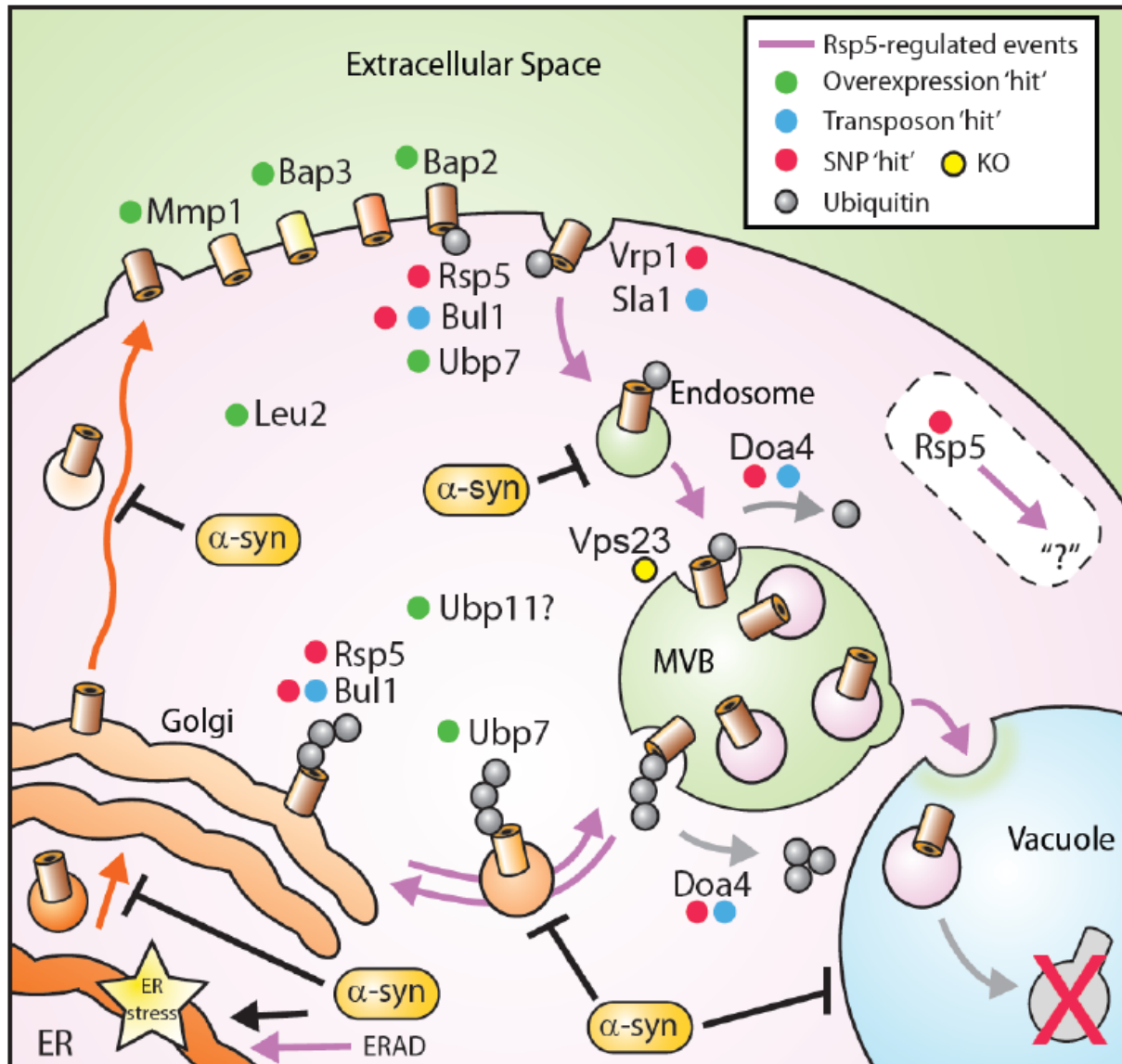
Model

Therapeutic modulation of eIF2α phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models

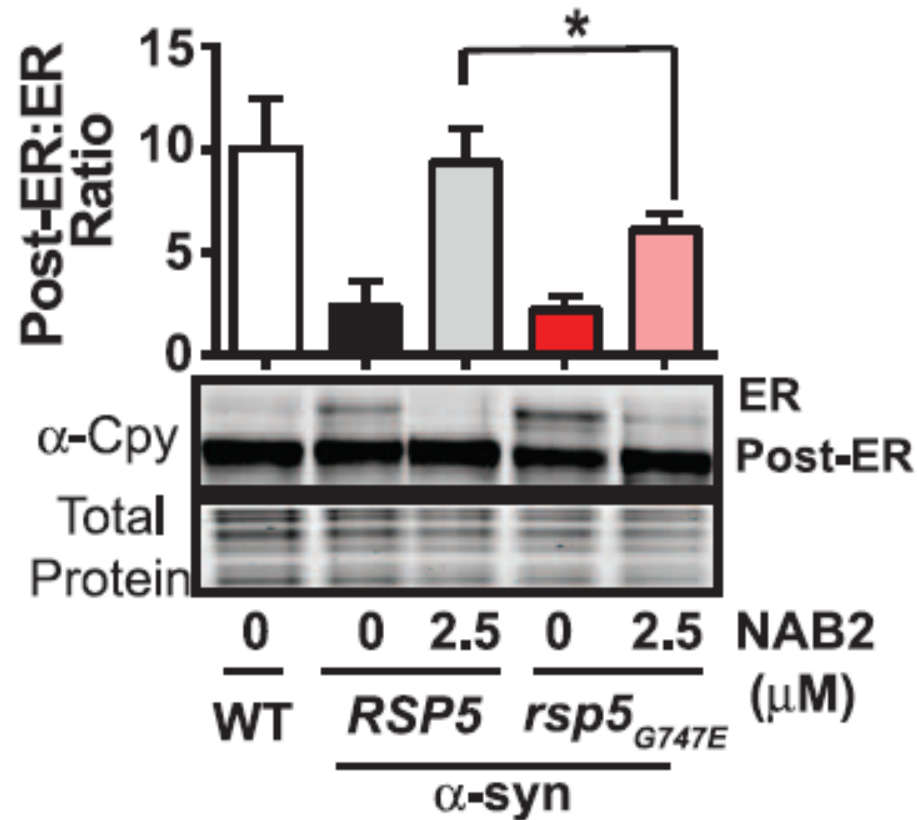Hyung-Jun Kim[1,5], Alya R Raphael[2], Eva S LaDow[3], Leeanne McGurk[1], Ross A Weber[1], John Q Trojanowski[4], Virginia M-Y Lee[4], Steven Finkbeiner[3], Aaron D Gitler[2] & Nancy M Bonini[1]

Aim: Identify components crictical to TDP43 toxicity
and validate them in different model systems

Yeast   Drosophila
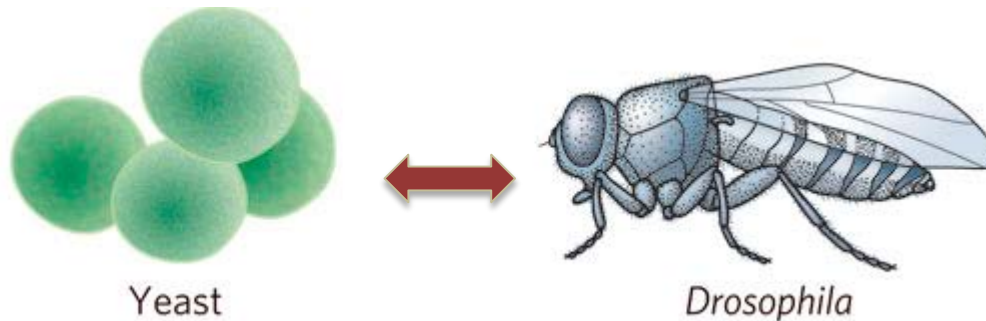
Motor neurons of the spinal cord transmit to muscle the received commands from the brain to the movement

Amyotrophic Lateral Sclerosis
(degeneration of motor neurons)

Normal

Nerve cell

ALS

Affected nerve cell

Muscles contract allowing voluntary movements of the body

Voluntary muscles no longer receive commands from the brain and atrophy
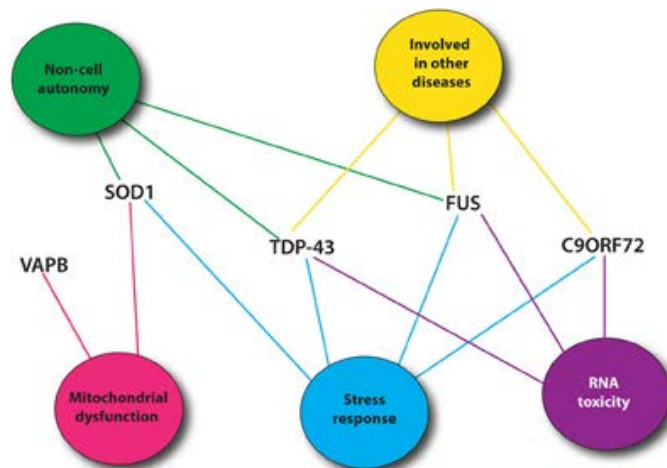
Non-cell autonomy

Involved in other diseases

SOD1

FUS

VAPB

TDP-43

C9ORF72

Mitochondrial dysfunction

Stress response

RNA toxicity

Yeast model system for TDP43 toxicity

Overexpression of TDP43 inhibits growth

Screen to identify genes modifying toxicity of TDP43
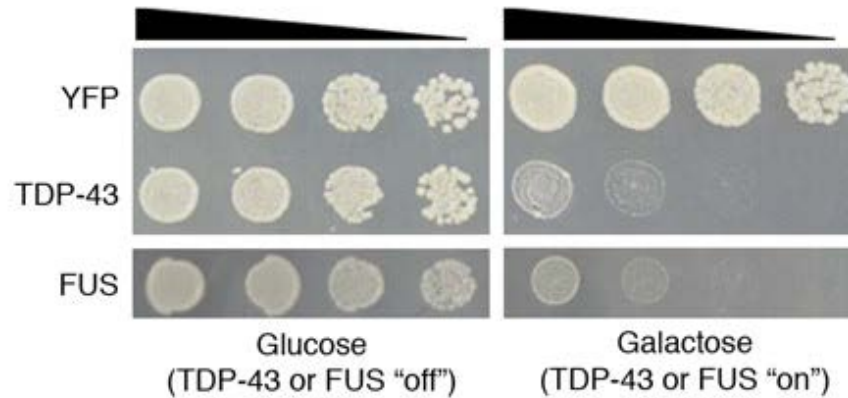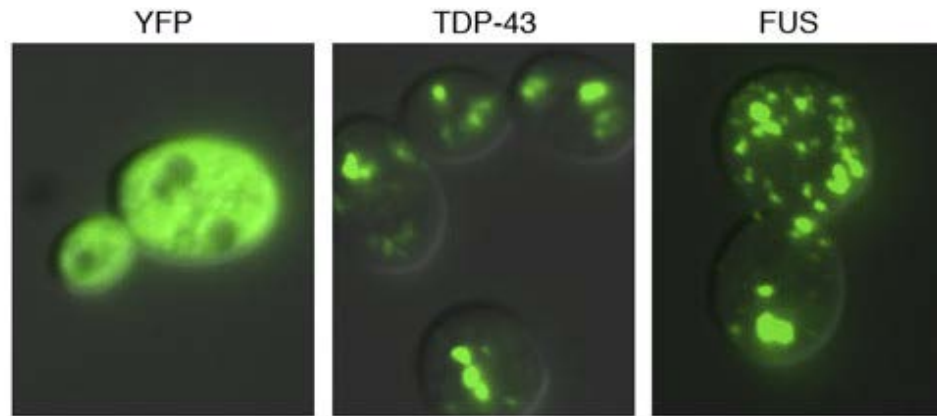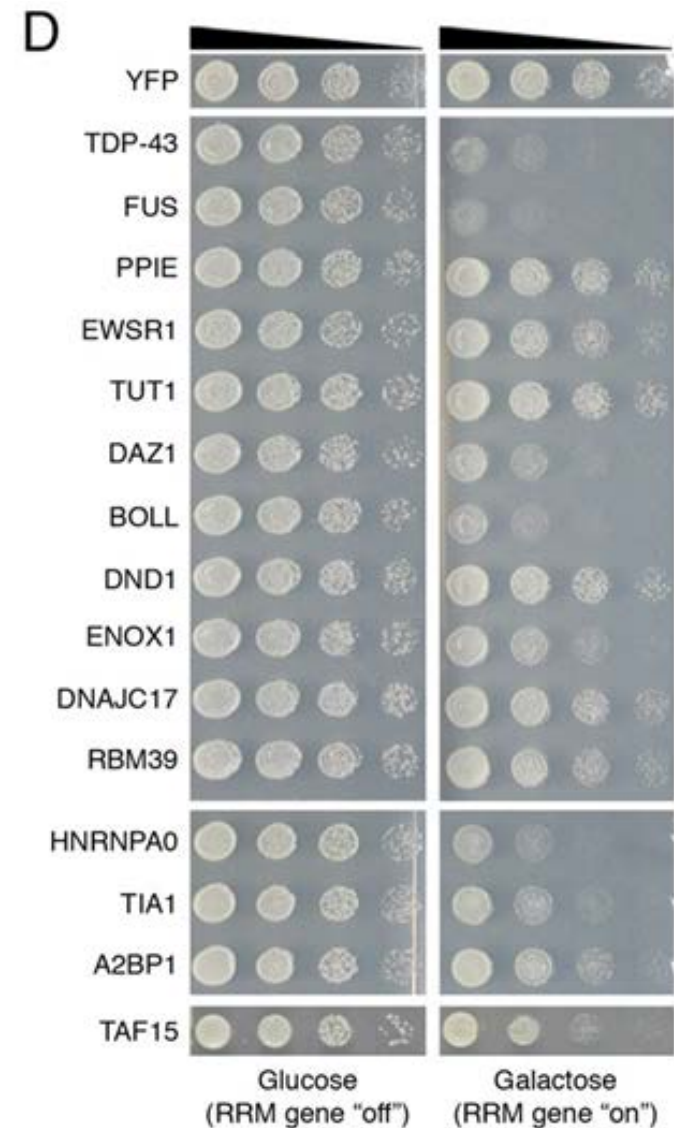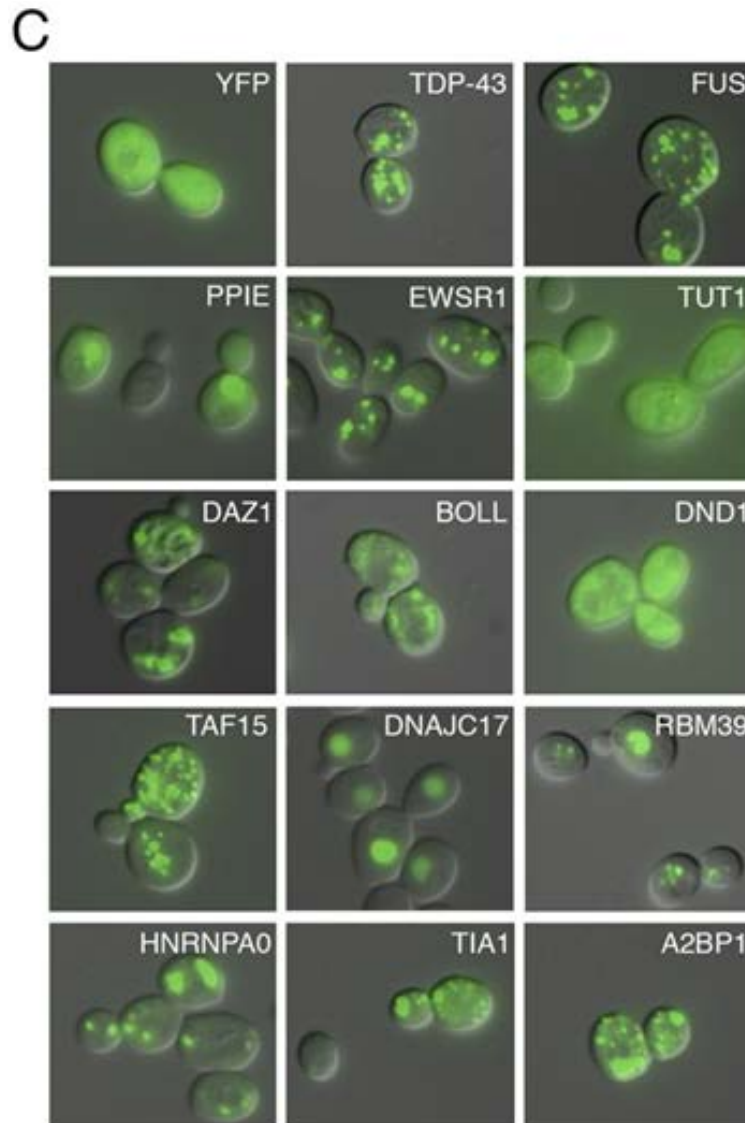
Screen reveals suppresors and enhancers of toxicity

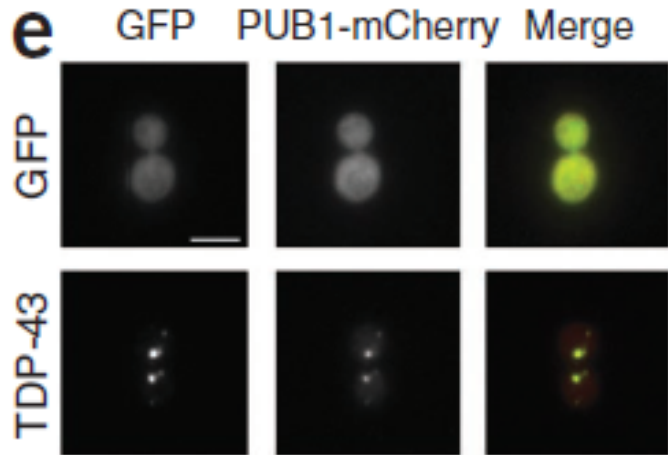**Table 1 Genes that suppress or enhance TDP-43 toxicity in yeast when overexpressed**

Effect	Gene	Human homolog of encoded protein	Description
Suppressor	ADY3	CENPE	Protein wall formation
Suppressor	BFR1		Component of mRNP complexes associated with polyribosomes
Suppressor	CYC8		Transcription corepressor; part of complex that recruits SWI/SNF and SAGA complexes to promoters
Suppressor	FMP48	STK36	Protein of unknown function
Suppressor	HSP104		Heat shock protein chaperone
Suppressor	ICS2		Protein of unknown function
Suppressor	NNK1	DCLK1	Protein kinase; interacts with TORC1, Ure2p and Gdh2p
Suppressor	PGM1	PGM1	Phosphoglucomutase
Suppressor	RDR1		Transcriptional repressor
Suppressor	RIM15	STK38	Glucose-repressible protein kinase
Suppressor	TIS1	ZNF36/TTP	mRNA-binding protein; component of stress granules
Suppressor	VTS1	SAM4B/Smaug	RNA-binding protein containing a SAM domain; component of P granules
Suppressor	XRS2		Protein required for DNA repair
Enhancer	CDC6	CDC6	Essential ATP-binding protein required for DNA replication
Enhancer	DIP5	SLC7A7	Dicarboxylic amino acid permease
Enhancer	HRP1	Musashi-1 and Musashi-2	RNA-binding protein; component of stress granules
Enhancer	KEL1	RAB9	Protein required for proper cell fusion and cell morphology
Enhancer	KEM1	XRN1	5'-3' exonuclease component of P bodies
Enhancer	KIN3	NEK2	Serine/threonine protein kinase
Enhancer	MEC1	ATR	Genome integrity checkpoint protein
Enhancer	MSA1	Mucin17	Involved in regulation of timing of G1-specific gene transcription and cell cycle initiation
Enhancer	MSN5	XPO5	Karyopherin involved in nuclear import and export
Enhancer	MTH1		Negative regulator of the glucose-sensing signal transduction pathway
Enhancer	PBP1	Ataxin-2	Interacts with Pab1p; component of stress granules
Enhancer	PBP2	PCBP1, PCBP2, PCBP3 and PCBP4	RNA-binding protein
Enhancer	PCL6		Pho85p cyclin of the Pho80p subfamily
Enhancer	PIB2	WDFY3	Protein-binding phosphatidylinositol 3-phosphate
Enhancer	RGA2	ARHGAP15	GTPase-activating protein for Cdc42p
Enhancer	ROM2	NET1	GDP/GTP exchange protein (GEP) for Rho1p and Rho2p
Enhancer	SAK1	CAMKK1	Upstream kinase for the SNF1 complex
Enhancer	SFG1		Putative transcription factor
Enhancer	SLF1	LARP1	RNA-binding protein that associates with polysome
Enhancer	SLG1		Sensor-transducer of the stress-activated PKC-MPK1 kinase pathway
Enhancer	SOL1	PGLS	Protein with possible role in tRNA transport
Enhancer	SRO9	LARP2	RNA-binding protein that associates with translating ribosomes
Enhancer	TSC11	RICTOR	Subunit of TORC2
Enhancer	UBP7	USP21	Ubiquitin-specific protease
Enhancer	VHS1	MARK2	Cytoplasmic serine/threonine kinase
Enhancer	YCK2	CSNK1G2	Casein kinase 1 isoform
Enhancer	YHR131C		Putative protein of unknown function

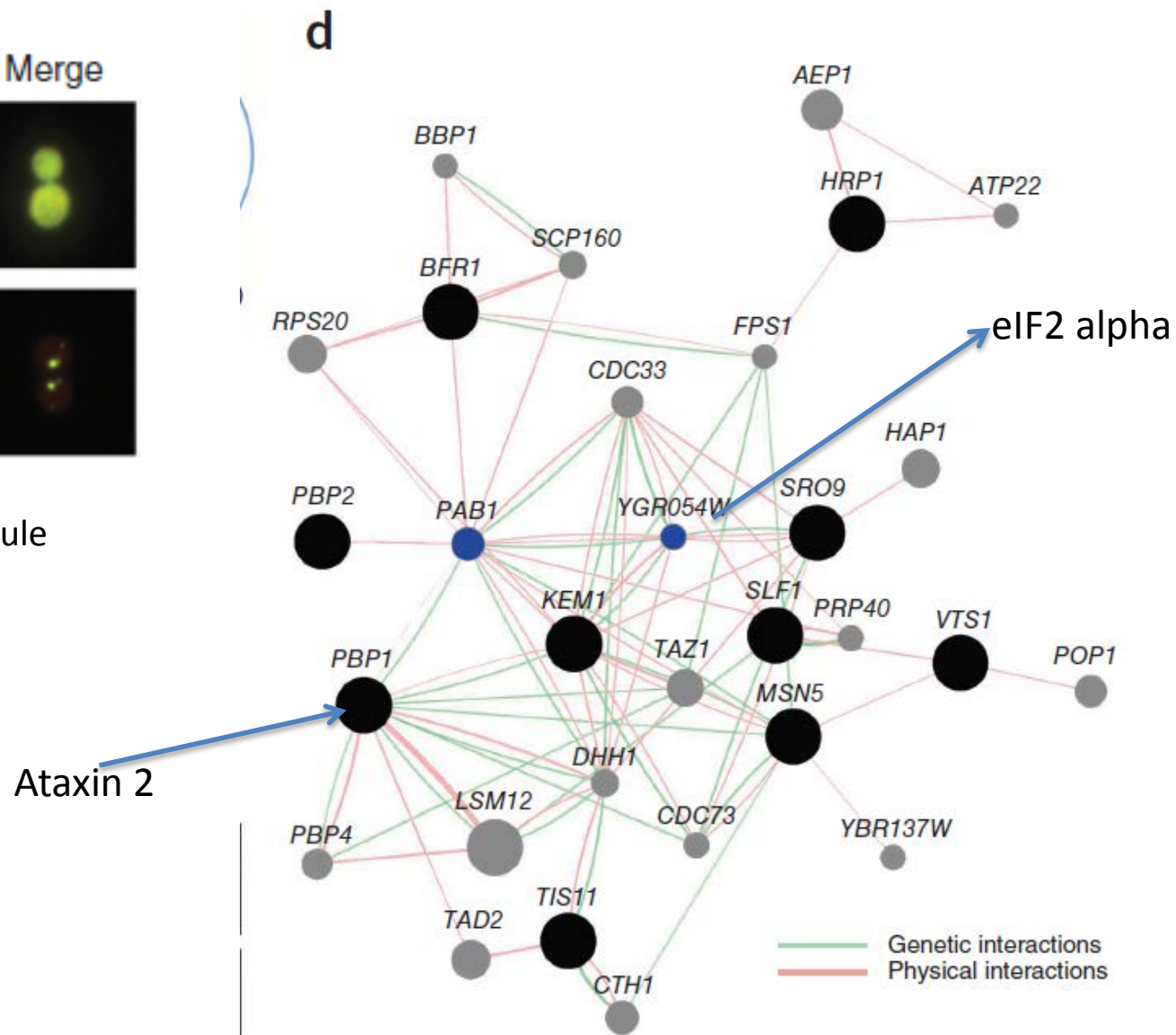Descriptive information is from the *Saccharomyces* Genome Database (see URLs).
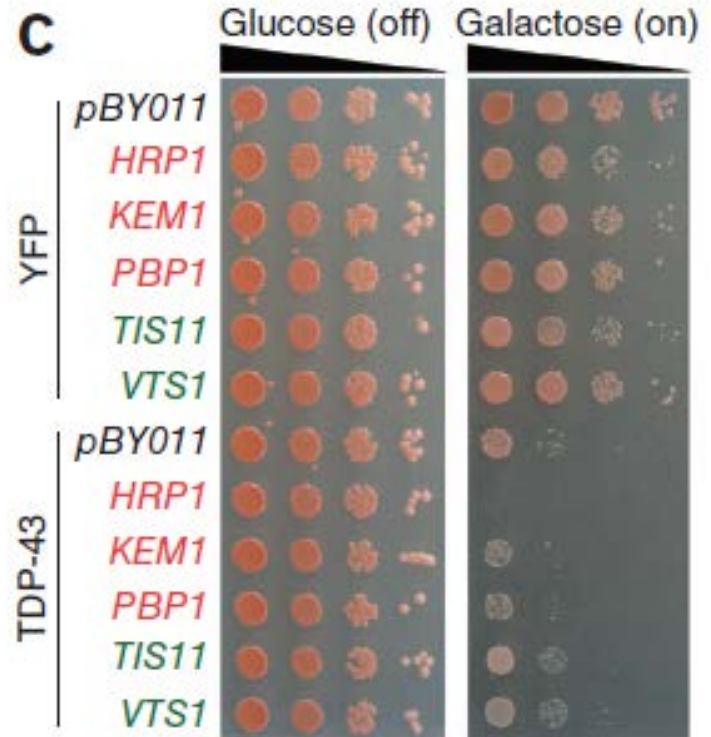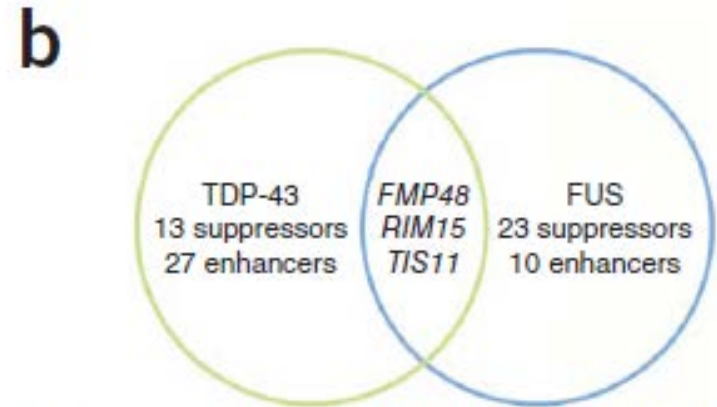
Genes associated with stress granules
And RNA metabolism were identified.

13 suppressors and 27 enhancers

e GFP PUB1-mCherry Merge

GFP

TDP-43

TDP43 colocalizes with stress granule
Marker PUB1

d

AEP1
BBP1
HRP1
ATP22
SCP160
BFR1
RPS20
FPS1
CDC33
HAP1
PBP2
PAB1
YGR054W
SRO9
eIF2 alpha
KEM1
SLF1
PRP40
VTS1
PBP1
TAZ1
POP1
MSN5
Ataxin 2
DHH1
CDC73
YBR137W
PBP4
LSM12
TAD2
TIS11
CTH1

Genetic interactions
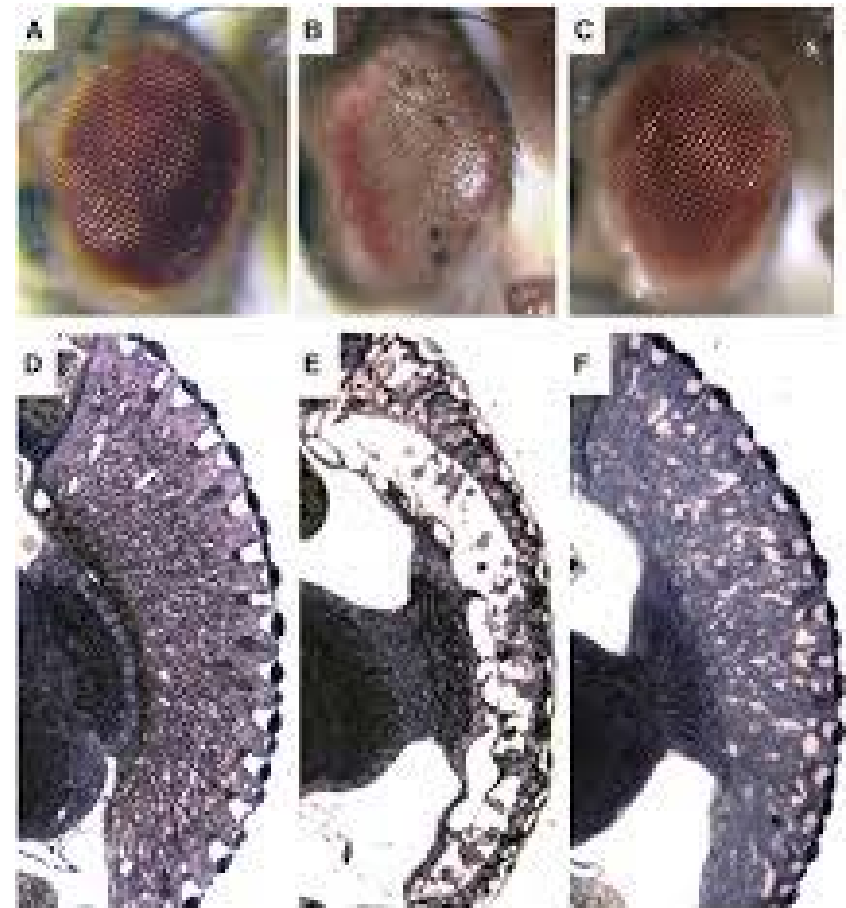Physical interactions

Validate the discovery in yeast in another model:
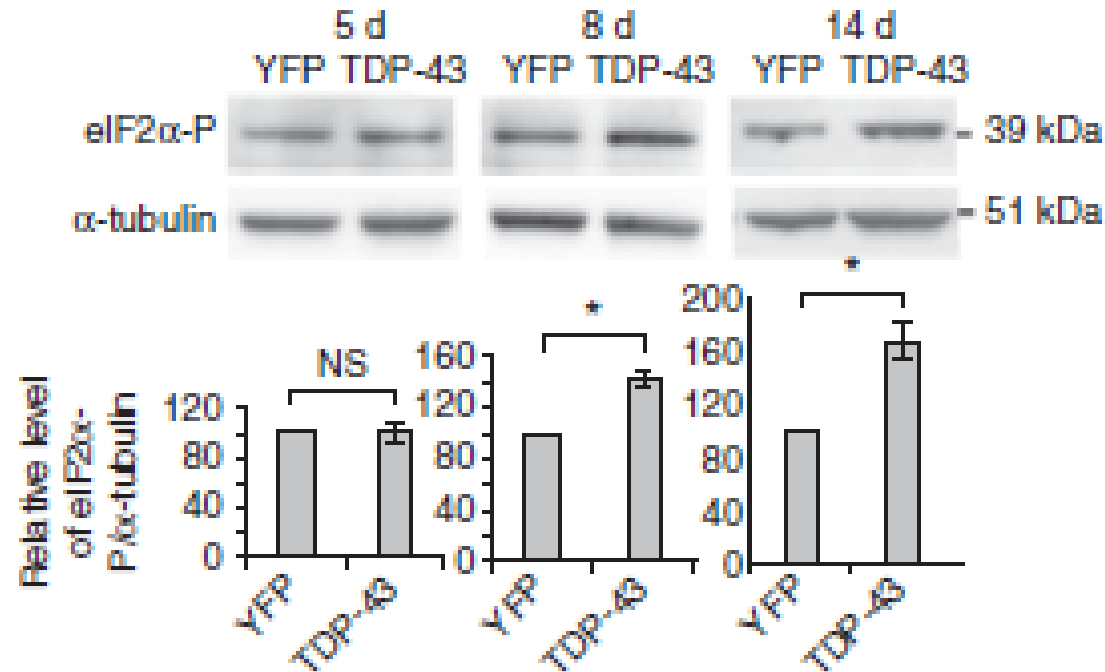
Drosophila was used

Retina expressing TDP43

Monitor the toxicity

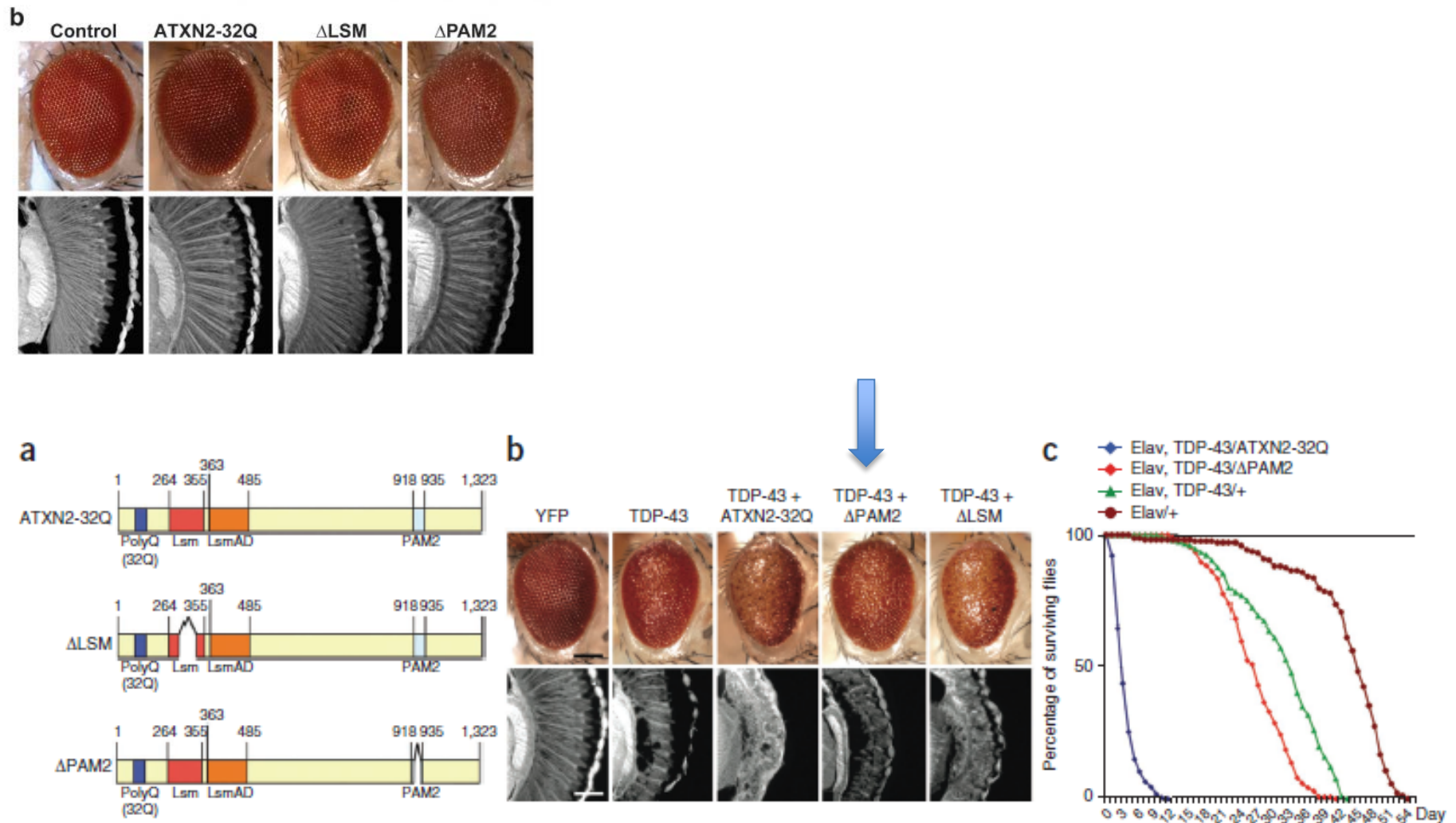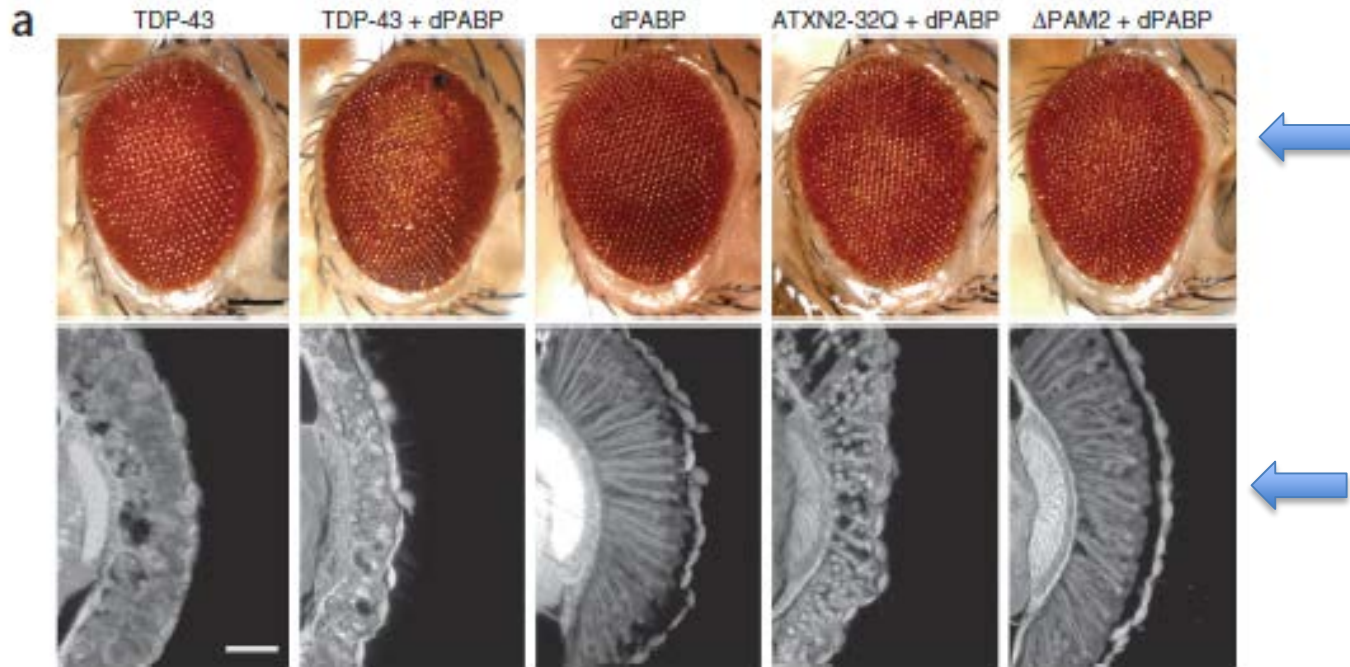# Drosophila model for TDP43 toxicity: eIF2 alpha is phosphorylated

TDP43 can be inducibly expressed in the flies under elav GS /UAS driver.

Ataxin-2 and TDP43 interaction

Ataxin 2 regulates the assembly and function of stress granules and Poly Q expansion are a risk for ALS
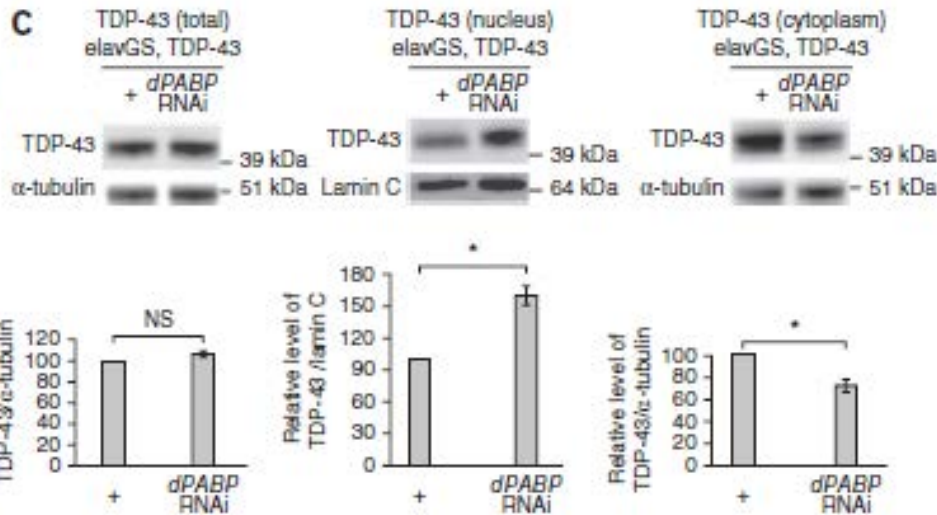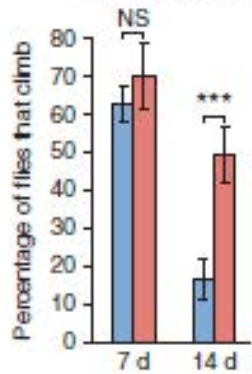
What happens to PABP that binds to PAM

a TDP-43 | TDP-43 + dPABP | dPABP | ATXN2-32Q + dPABP | ΔPAM2 + dPABP

b ■ elavGS, TDP-43/UAS-YFP ■ elavGS, TDP-43/dPABP RNAi

Percentage of flies that climb — NS (7 d), *** (14 d)

c TDP-43 (total) elavGS, TDP-43 / + dPABP RNAi
TDP-43 — 39 kDa, α-tubulin — 51 kDa

TDP-43 (nucleus) elavGS, TDP-43 / + dPABP RNAi
TDP-43 — 39 kDa, Lamin C — 64 kDa

TDP-43 (cytoplasm) elavGS, TDP-43 / + dPABP RNAi
TDP-43 — 39 kDa, α-tubulin — 51 kDa

Relative level of TDP-43/α-tubulin — NS (+, dPABP RNAi)
Relative level of TDP-43/lamin C — * (+, dPABP RNAi)
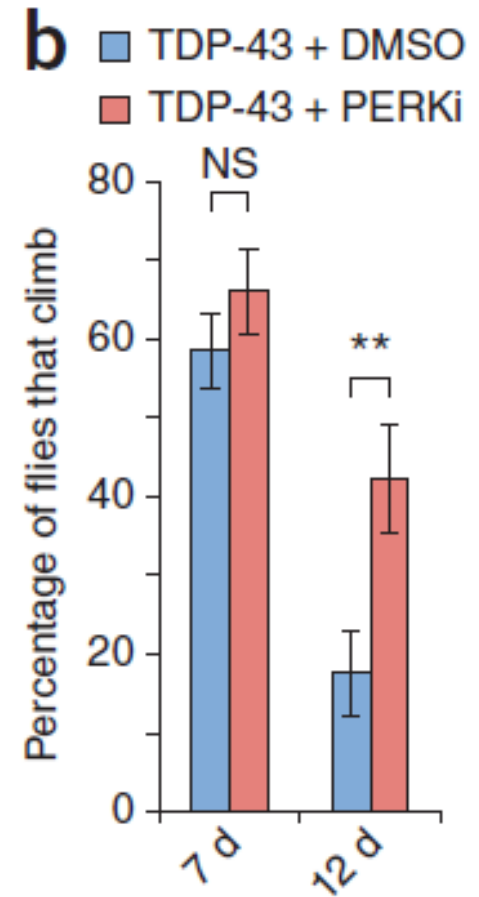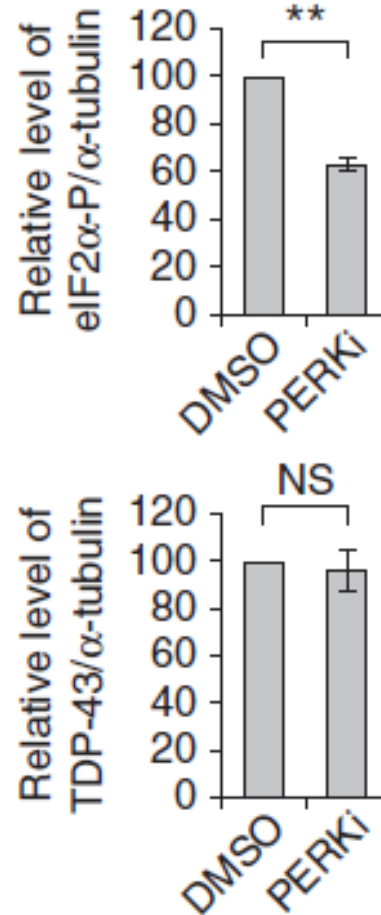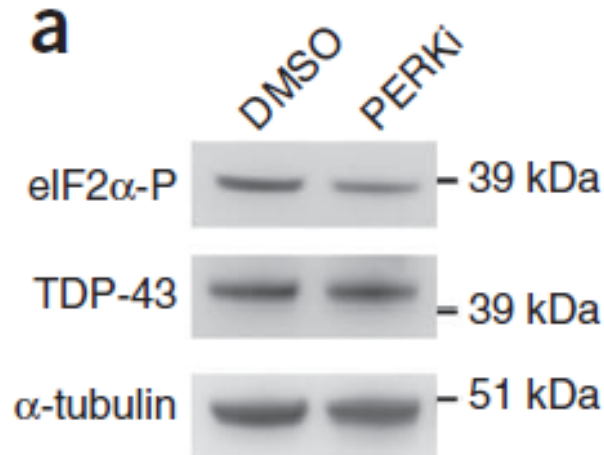Relative level of TDP-43/α-tubulin — * (+, dPABP RNAi)

RNAi of PABP1

PABP is mislocalized in motor neurons from tissues of human ALS patients
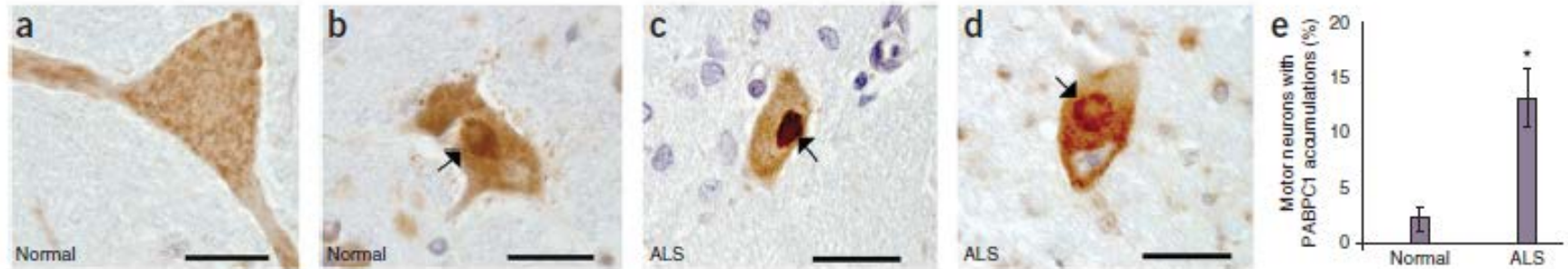
How does this relate to TDP43 toxicity?

Pharmacological rescue of TDP43 toxicity

RNAi against ER stress
inducing components

Pharmacological rescue of TDP43 toxicity

RNAi against ER stress inducing components and stress granules components

GADD34 RNAi: promotes ER stress
PEK RNAi: Inhibits ER stress
Rox8 RNAi: Inhibits stress granule formation

Summary

1) TDP43 toxicity modulator screen analysis reveals a role of eIF2 alpha in regulating toxicity.

2) TDP43 toxicity is enhanced by the genes associated with the formation and maintenance Of stress granules.

3) Could modulators of ER stress or stress granules be a better therapeutic option?

A Network of Conserved Synthetic Lethal Interactions for Exploration of Precision Cancer Therapy

Rohith Srivas,[1,2,9,11] John Paul Shen,[1,3,9,10] Chih Cheng Yang,[4] Su Ming Sun,[5] Jianfeng Li,[6,7] Andrew M. Gross,[8] James Jensen,[8] Katherine Licon,[1,2] Ana Bojorquez-Gomez,[2] Kristin Klepper,[1] Justin Huang,[8] Daniel Pekin,[1] Jia L. Xu,[1] Huwate Yeerna,[1] Vignesh Sivaganesh,[1] Leonie Kollenstart,[5] Haico van Attikum,[5] Pedro Aza-Blanc,[4] Robert W. Sobol,[6,7] and Trey Ideker[1,2,3,9,*]

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[8]Bioinformatics and Systems Biology Program, University of California, San Diego, La Jolla, CA 92093, USA
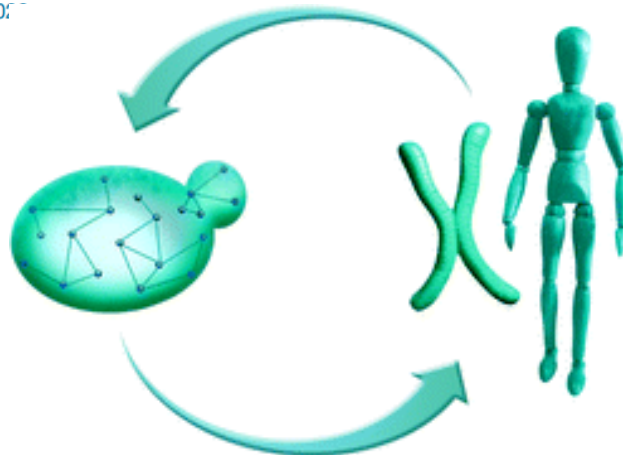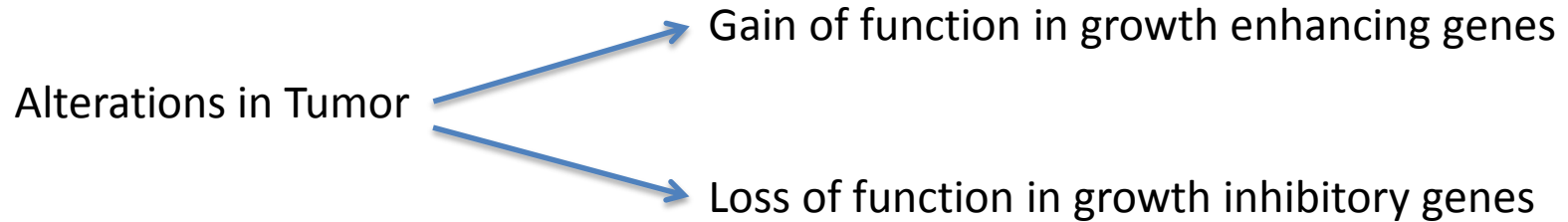[9]The Cancer Cell Map Initiative
[10]Co-first author
[11]Present address: Department of Genetics, Stanford University School of Medicine, Stanford, CA 94034, USA
*Correspondence: tideker@ucsd.edu
http://dx.doi.org/10.1016/j.molcel.2016.06.0

Cancer therapy

Alterations in Tumor → Gain of function in growth enhancing genes

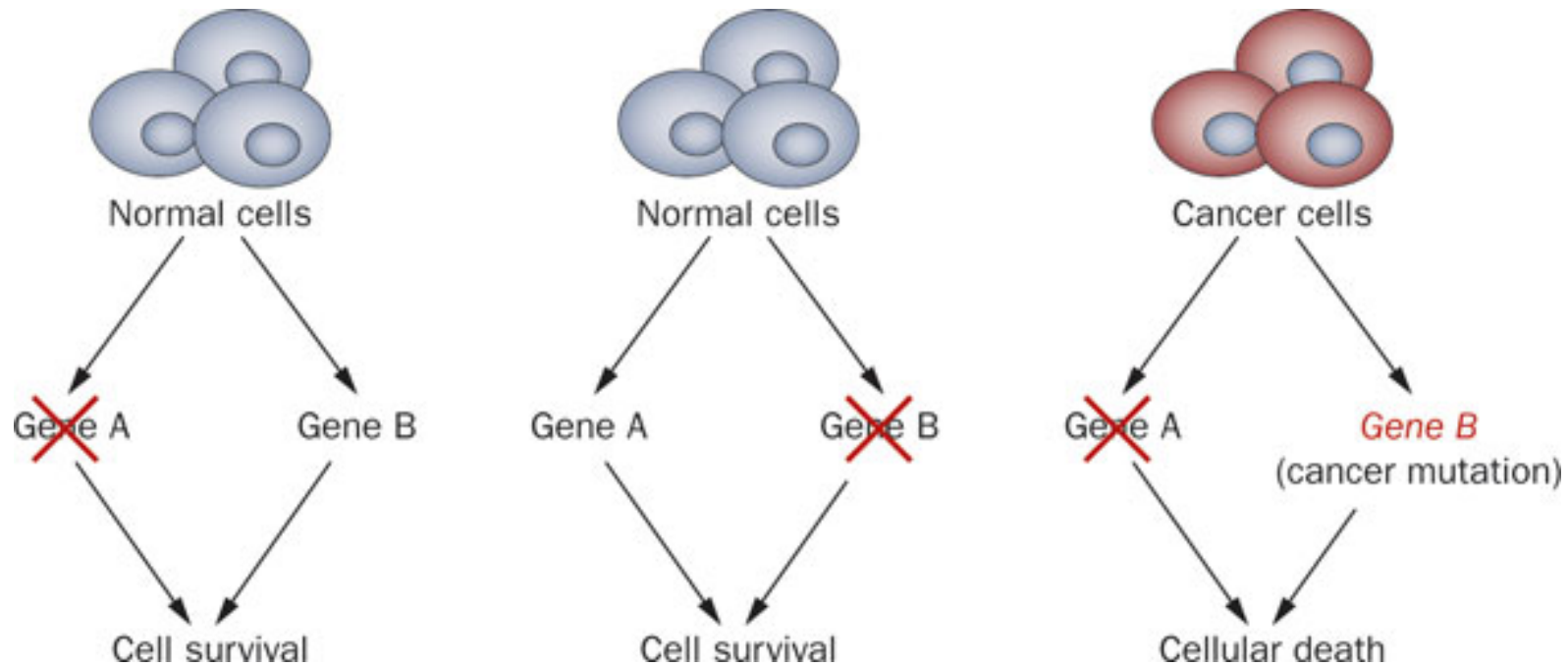Alterations in Tumor → Loss of function in growth inhibitory genes

Currently not possible to restre the function of mutated Tumor supressor genes (TSG).

Selective lethality in Tumor

↓

Synthetic lethal interactions

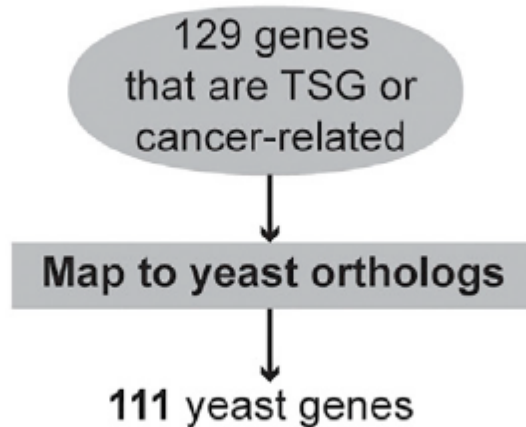# Synthetic lethality as a source for Cancer therapy

So far this approach has not yielded to success:

1) Population of tumor genomes analyzed to detect pairs that are co mutated
2) Directed combinatorial disruption in an unbiased way human genome
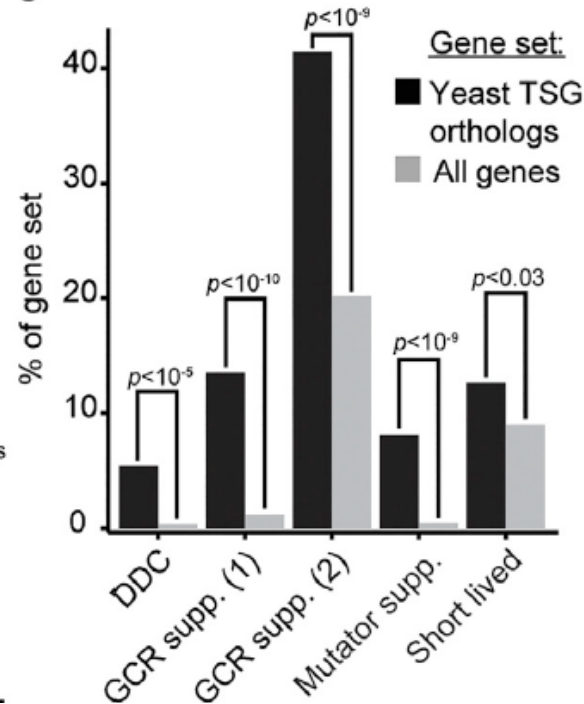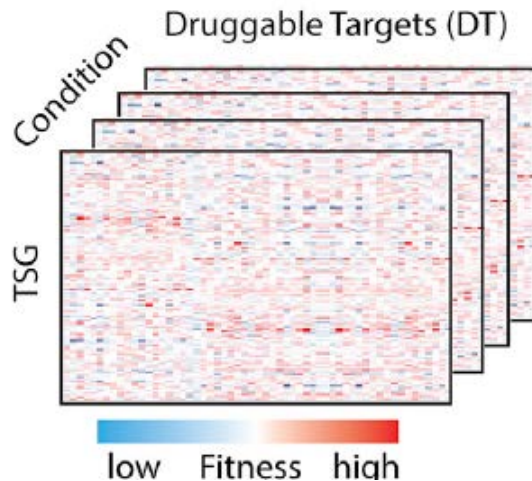
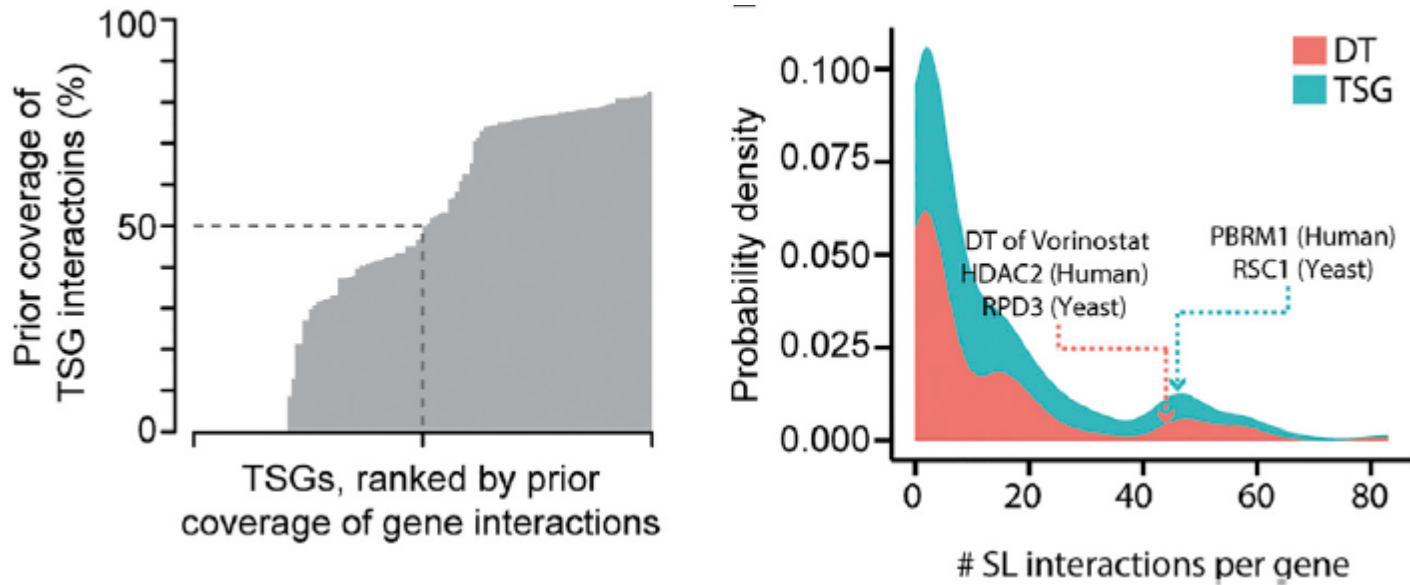# A novel strategy
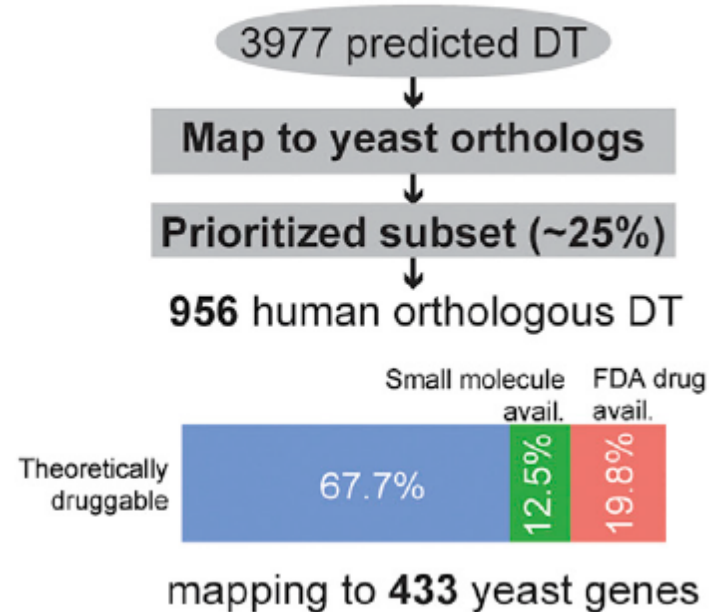
Libraries and Yeast screen
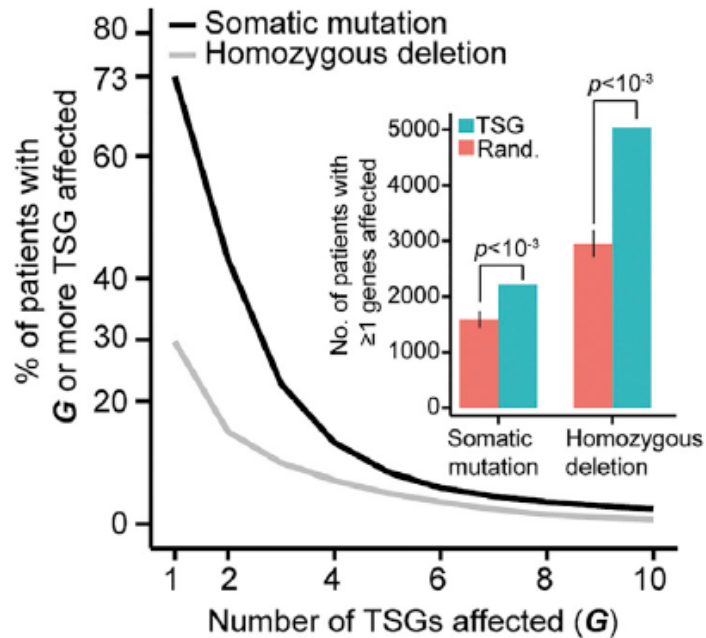
DDC: DNA damage checkpoints

GCR: gross chromosomal suppresors

A total of 169000 gene-gene interactions Were tested.

Read out: Survival

Yeast screen: results

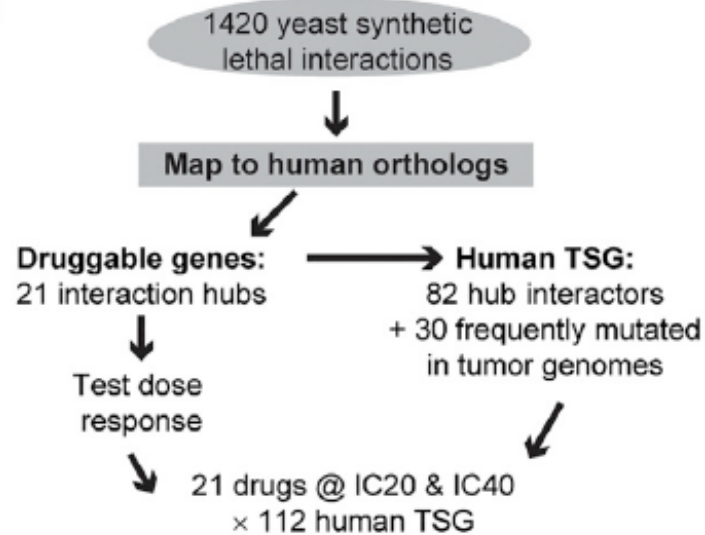A total of 1420 synthetic lethal interactions were identified. Map to human genome

From the yeast screen 21 drugs that had highest rate of synthetic lethals were chosen.

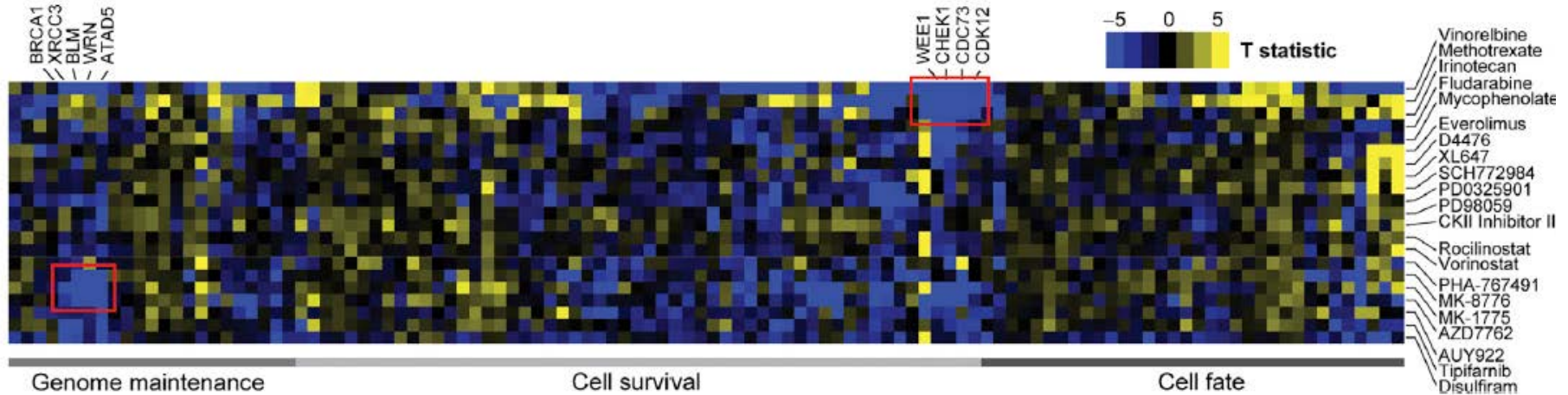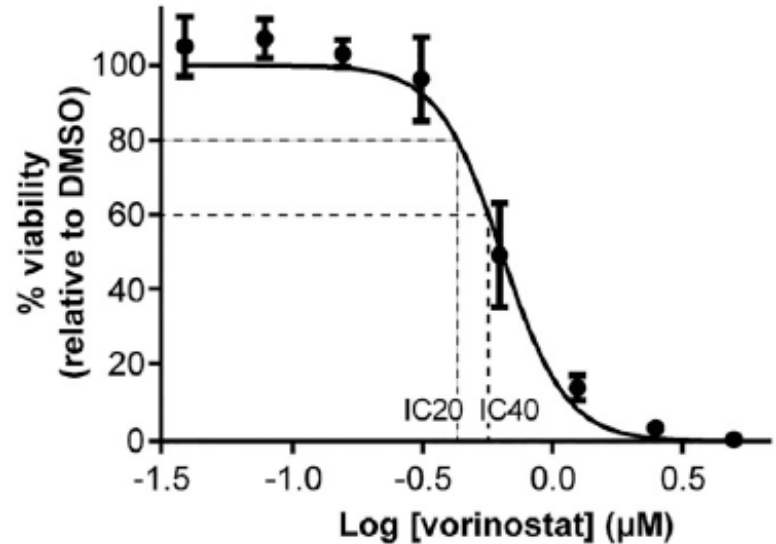Dose response curves were established for all the 21 drugs.

82 TSG were implicated with the 21 drugs.
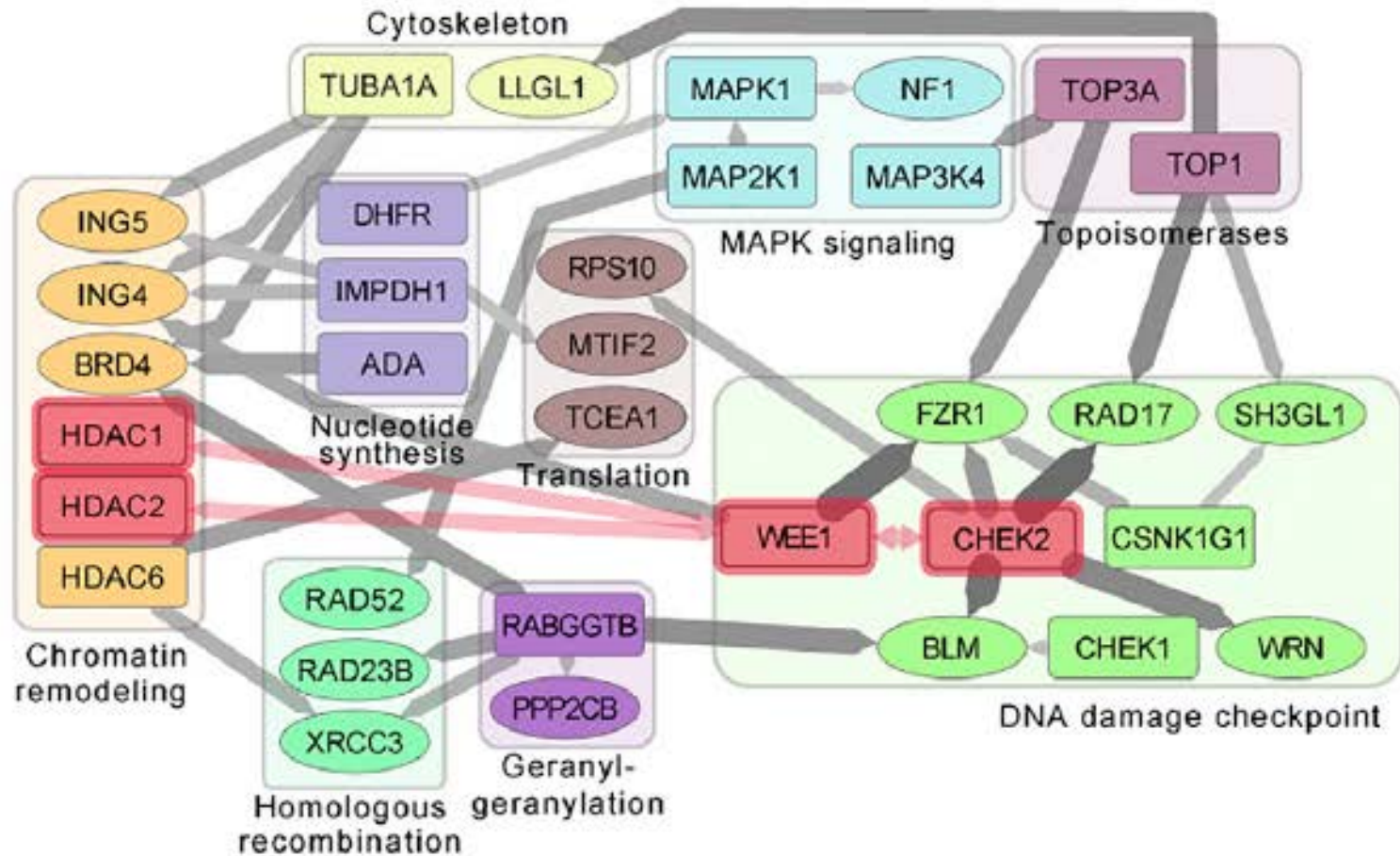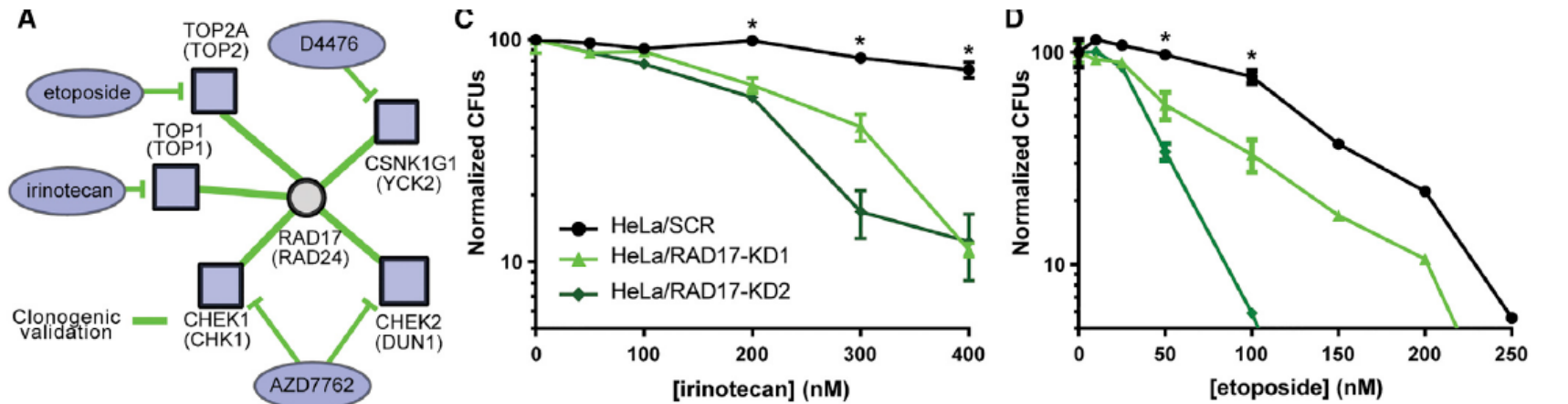
Screen in HeLa cells

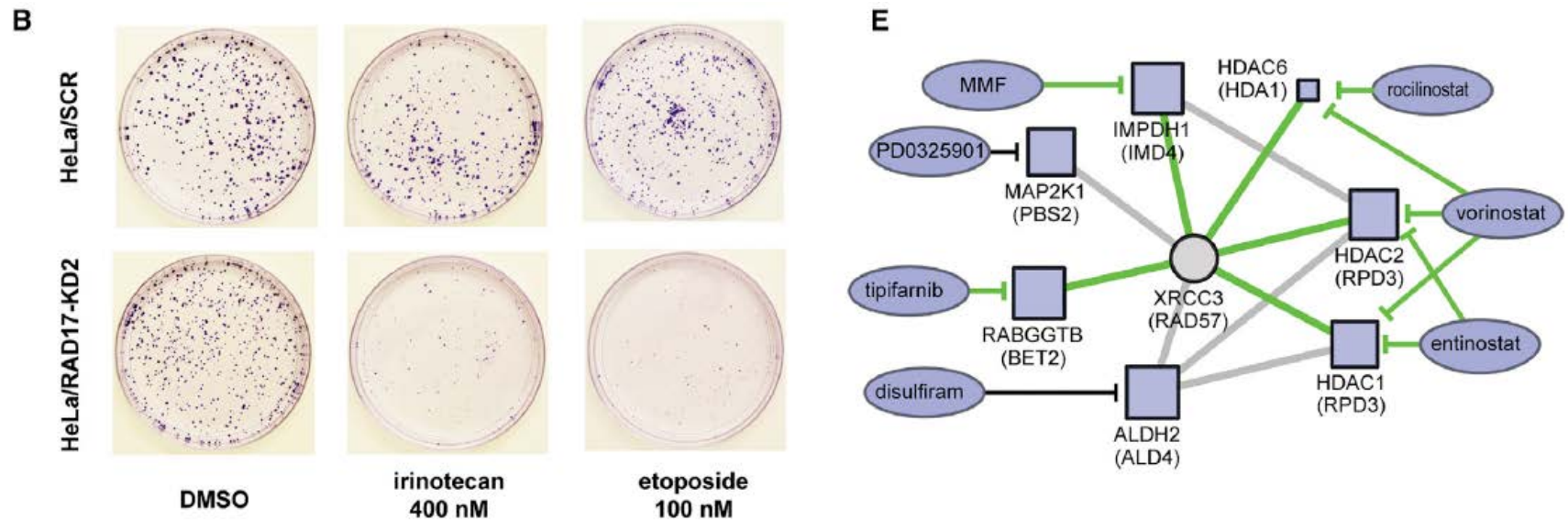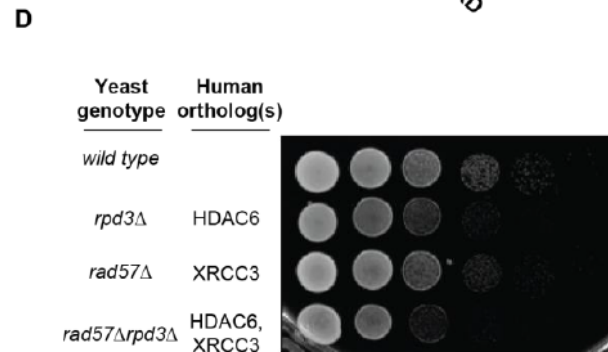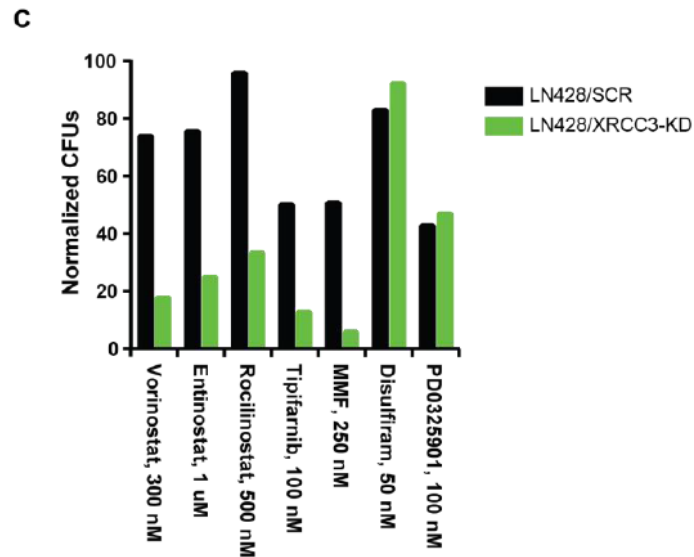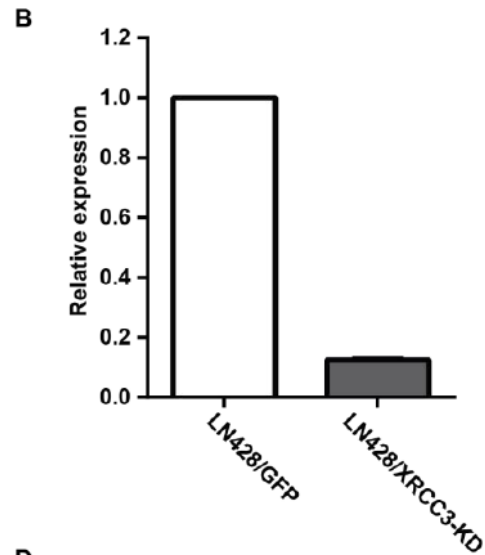A total of 127 SL interactions were identified

Screen in HeLa cells: Results

Screen in HeLa cells: Validation

A

TOP2A (TOP2) — etoposide
D4476
CSNK1G1 (YCK2)
TOP1 (TOP1) — irinotecan
RAD17 (RAD24)
Clonogenic validation
CHEK1 (CHK1)
CHEK2 (DUN1)
AZD7762

C

Normalized CFUs vs [irinotecan] (nM)

- HeLa/SCR
- HeLa/RAD17-KD1
- HeLa/RAD17-KD2

D

Normalized CFUs vs [etoposide] (nM)

RAD17 is involved in 5% of prostrate and ovarian cancers (deletion)

B

HeLa/SCR / HeLa/RAD17-KD2

DMSO — irinotecan 400 nM — etoposide 100 nM

E

MMF — IMPDH1 (IMD4)
HDAC6 (HDA1) — rocilinostat
PD0325901 — MAP2K1 (PBS2)
XRCC3 (RAD57)
HDAC2 (RPD3) — vorinostat
tipifarnib — RABGGTB (BET2)
entinostat
disulfiram — ALDH2 (ALD4)
HDAC1 (RPD3)

Screen in HeLa cells: Validation
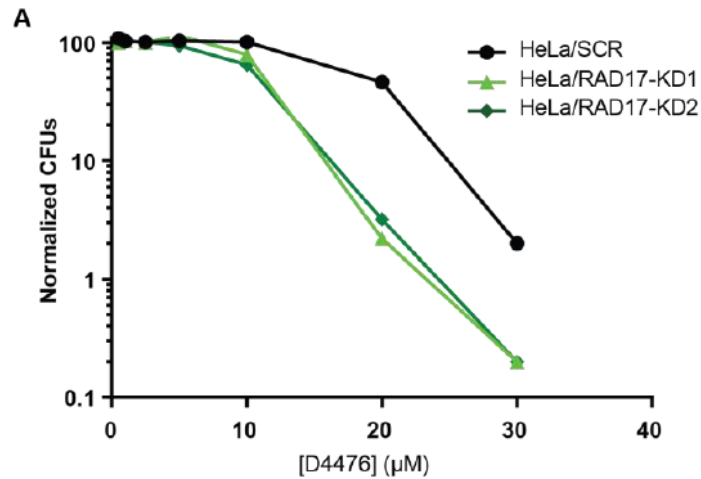
Clinical application
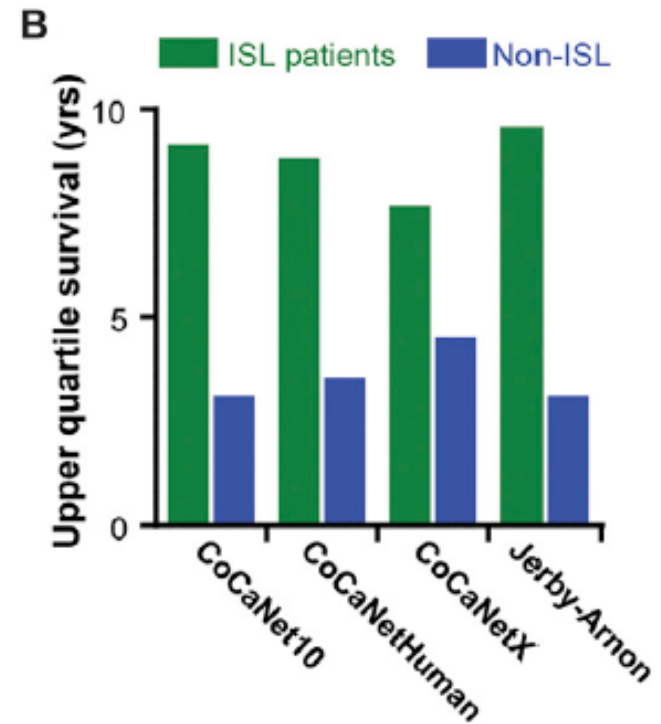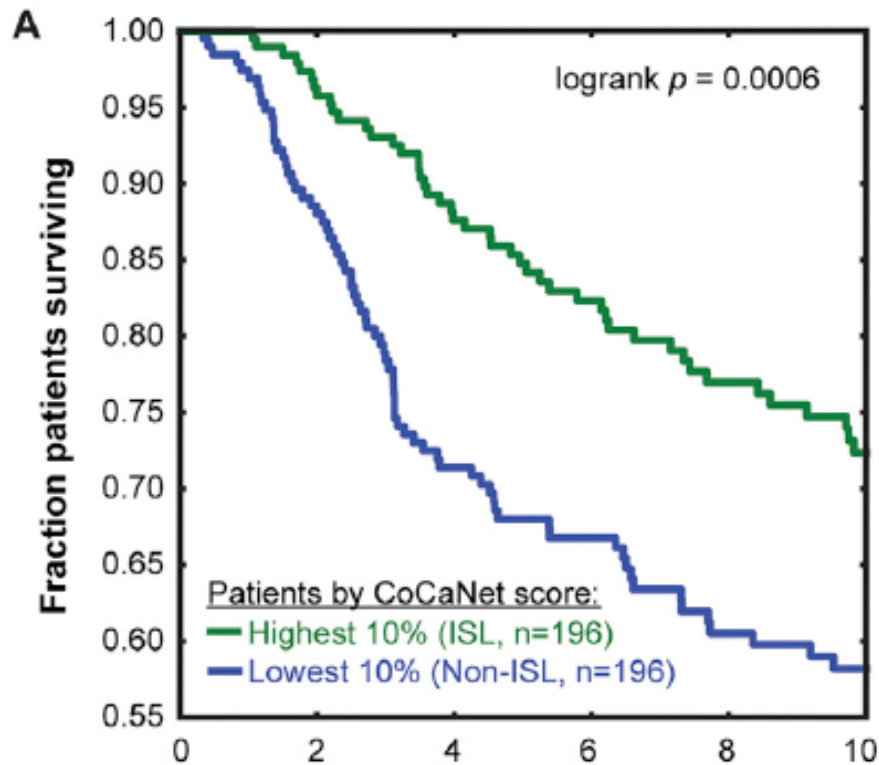
Summary

A novel strategy to find new targets for cancer therapy.

Synthetic lethality screens between TSG and DT reveals combinatorial targets.