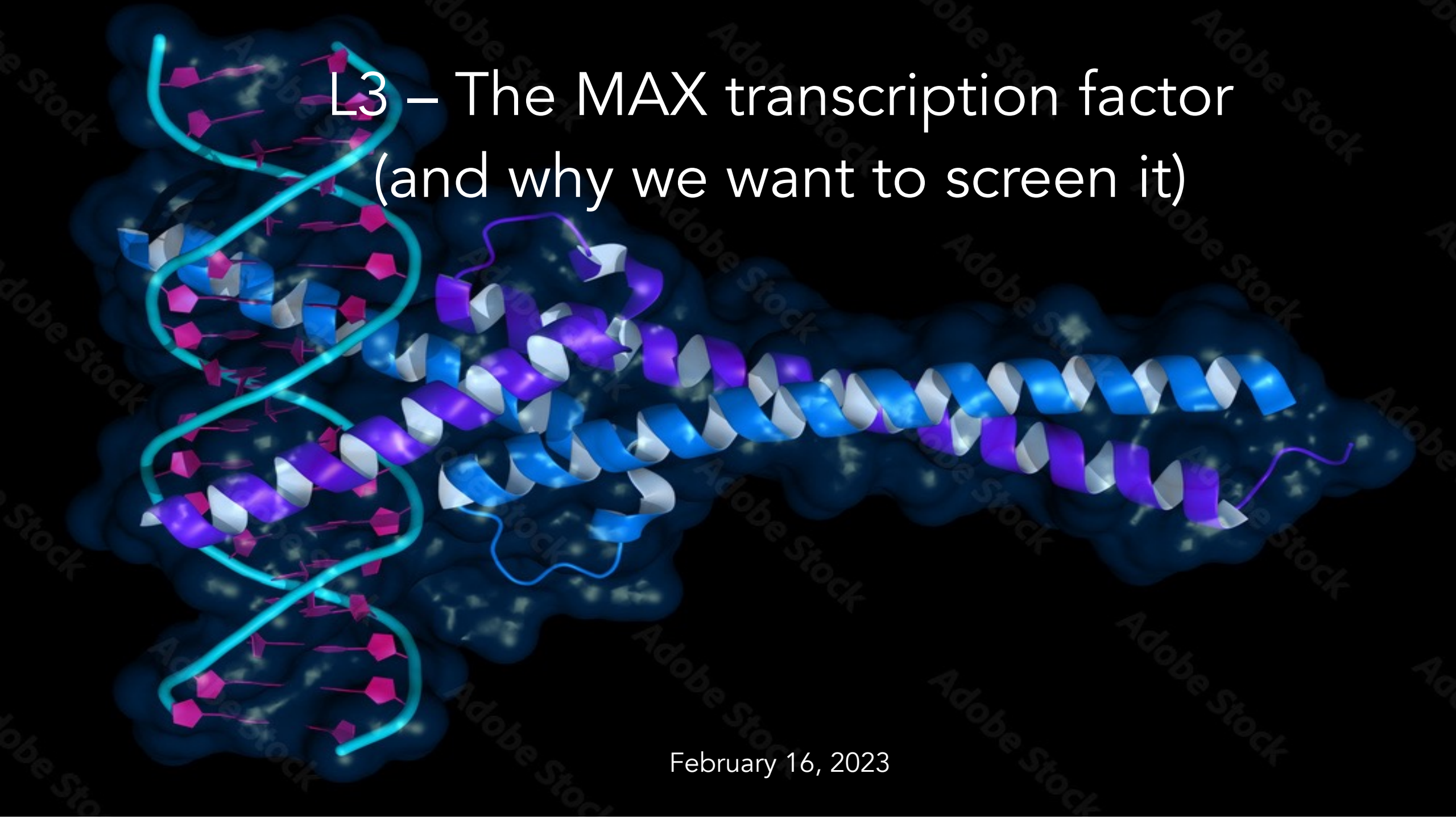
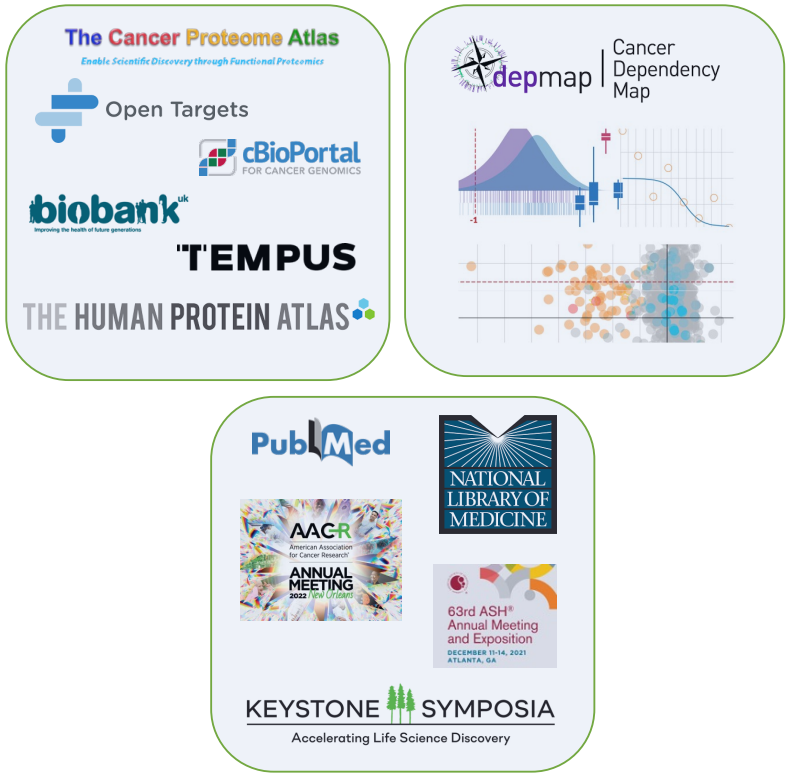
L3 – The MAX transcription factor (and why we want to screen it)



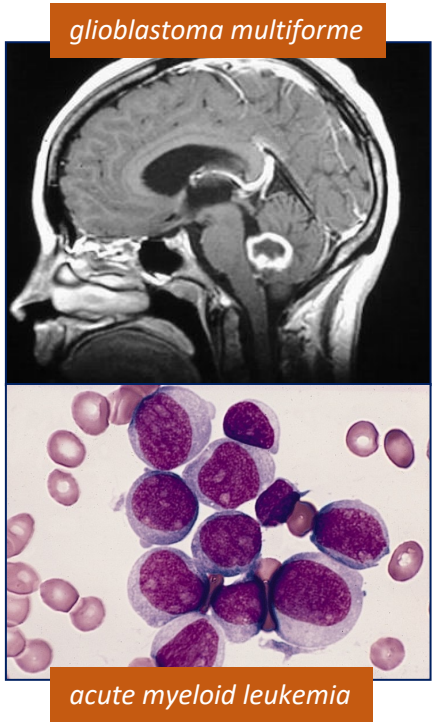
February 16, 2023

From L1 (again): Therapeutically-driven probe discovery

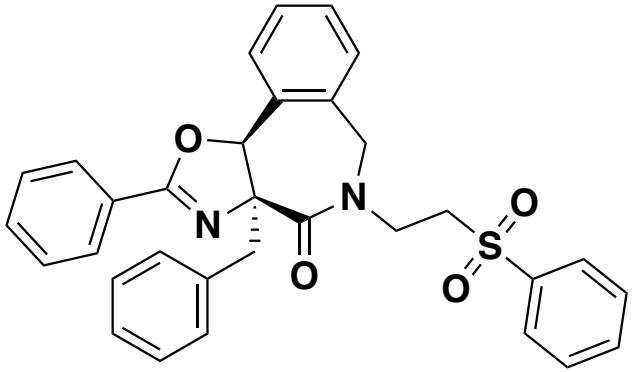
assess tractability of emerging target candidates



cell lines and patient samples
reveal list of disease genes



test impact of disease
genes in a physiologic settings

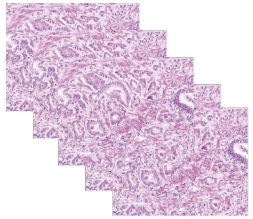


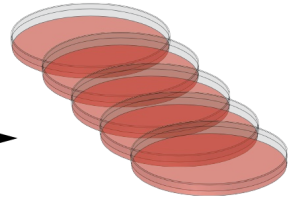
chemical probe

discover molecules that reverse
impact of disease genes

The Cancer Dependency Map

interrogation of viability effects in cancer cell lines to map genetic dependencies



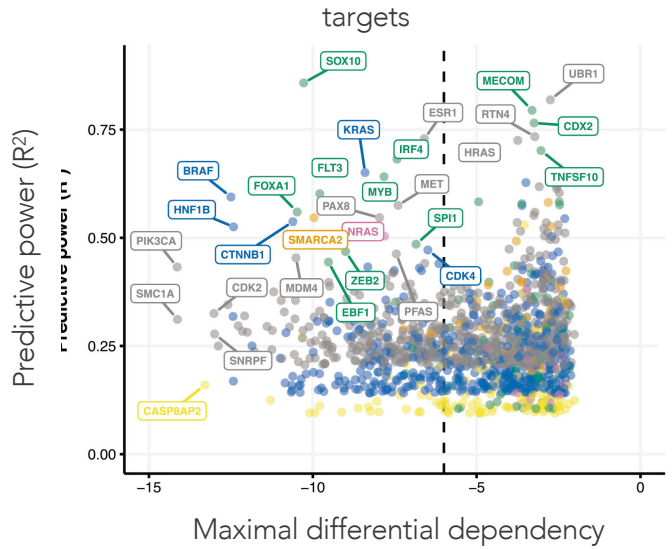


patient models (cell lines/organoids) representing many tumors

molecular and genetic information

dependency map Consortium

genetic dependencies and drug sensitivities



map of cancer dependencies

Discovery of potential TARGETS for therapeutic discovery

Discovery of PREDICTORS of patient response

Pre-publication DATA RELEASES to enable the scientific community at depmap.org

As of 4/21/22:

>2,000 cancer models 3913 genetic dependency screens 33 drug panels in sensitivity screens

Tsherniak et al., Cell, 170 (3): 564-576 (2017)
McDonald et al., Cell, 170 (3): 577-592 (2017)
Vazquez & Boehm, Mol Sys Bio, 16 (7): e9757 (2020)
Dharia et al., Nat Genet, 53 (4): 529-538 (2021)

example query: multiple myeloma

Dependencies enriched in Multiple Myeloma

Show/Hide Columns or disappear

Type	Gene/Compound		Dataset	T-Statistic	P-Value
gene	IRF4	*	CRISPR (DepMap 21Q4 Public+Score, Chronos)	-24	1.17E-101
gene	PRDM1	*	CRISPR (DepMap 21Q4 Public+Score, Chronos)	-23.2	3.48E-96
gene	PRDM1	*	CRISPR (DepMap 21Q4 Public, Chronos)	-23.5	6.94E-96
gene	IRF4	*	CRISPR (DepMap 21Q4 Public, Chronos)	-22.4	2.5E-89
gene	IRF4	*	CRISPR (Project Score, Chronos)	-24.4	1.14E-74
gene	IRF4	*	RNAi (Achilles+DRIVE+Marcotte, DEMETER2)	-20.4	7.31E-73
gene	PIM2		CRISPR (DepMap 21Q4 Public+Score, Chronos)	-19.2	9.45E-71
gene	POU2AF1	*	CRISPR (DepMap 21Q4 Public+Score, Chronos)	-18.6	8.99E-67
gene	PIM2		CRISPR (DepMap 21Q4 Public, Chronos)	-18.8	1.16E-66
gene	IRF4	*	CRISPR (Project Score, CERES)	-22.2	1.43E-66
gene	NFKB1	*	CRISPR (DepMap 21Q4 Public, Chronos)	-18.6	6.92E-66
gene	POU2AF1	*	CRISPR (DepMap 21Q4 Public, Chronos)	-18.6	1.56E-65
gene	MEF2C	*	CRISPR (DepMap 21Q4 Public+Score, Chronos)	-18.1	1.11E-63
gene	NFKB1	*	CRISPR (DepMap 21Q4 Public+Score, Chronos)	-17.8	2.44E-62
gene	HERPUD1		CRISPR (DepMap 21Q4 Public+Score, Chronos)	-17.8	5.97E-62
gene	IRF4	*	RNAi (DRIVE, DEMETER2)	-19.3	2.52E-58
gene	HERPUD1		CRISPR (DepMap 21Q4 Public, Chronos)	-16.8	3.31E-55
gene	SMAD7		CRISPR (DepMap 21Q4 Public+Score, Chronos)	-16.6	5.65E-55
gene	TCF3	*	CRISPR (DepMap 21Q4 Public+Score, Chronos)	-15.9	4.48E-51
gene	TCF3	*	CRISPR (DepMap 21Q4 Public, Chronos)	-16	4.96E-51

Previous Page 1 of 235 20 rows Next

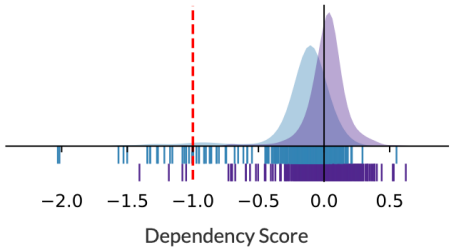
IRF4 interferon regulatory factor 4

example query: IRF4

Overview | Dependency | Characterization | Description

Dependent Cell Lines

CRISPR: 42/558 STRONGLY SELECTIVE
RNAi: 11/711 STRONGLY SELECTIVE



Dependency Score: Outcome from DEMETER2 or CERES. A lower score means that a gene is more likely to be dependent in a given cell line. A score of 0 is equivalent to a gene that is not essential whereas a score of -1 corresponds to the median of all common essential genes.

Strongly Selective: A gene whose dependency is at least 100 times more likely to have been sampled from a skewed distribution than a normal distribution (i.e. skewed-LRT value > 100).

View more

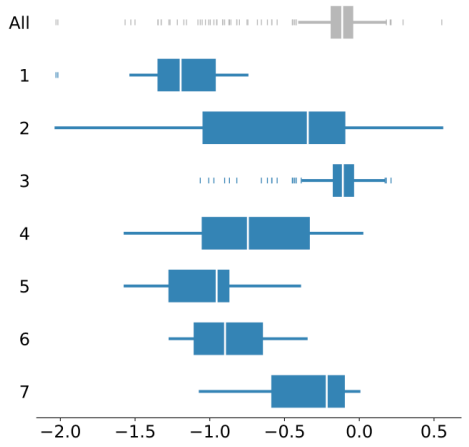
The protein encoded by this gene belongs to the IRF (interferon regulatory factor) family of transcription factors, characterized by an unique tryptophan pentad repeat DNA-binding domain. The IRFs are
View more

Search external sites for IRF4

- PubMed (996 entries)
- GeneCards
- GTEx
- NCBI

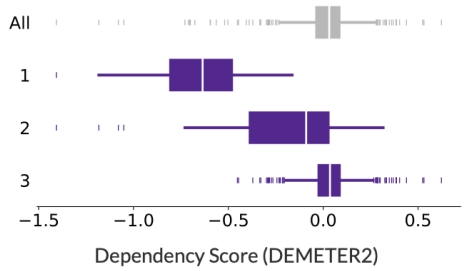
Enriched Lineages

CRISPR



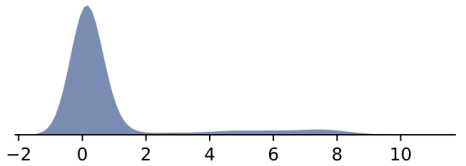
1. Multiple Myeloma (4.4e-79)
2. Haematopoietic And Lymphoid (3.4e-39)
3. Solid (4.5e-38)
4. Lymphoma (4.0e-15)
5. T-cell lymphoma Other (9.5e-12)
6. ALCL (9.9e-07)
7. Melanoma (1.3e-04)

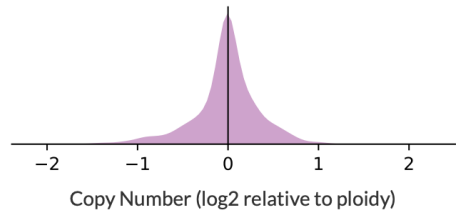
RNAi



1. Multiple Myeloma (1.3e-72)
2. Haematopoietic And Lymphoid (3.3e-28)
3. Solid (6.5e-21)

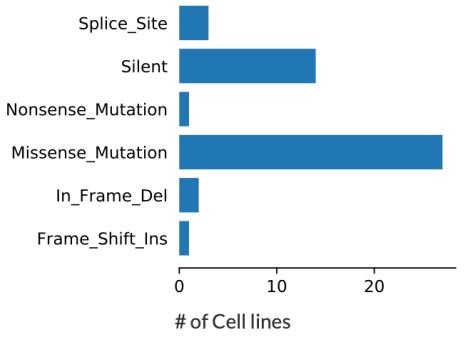
Expression & CN





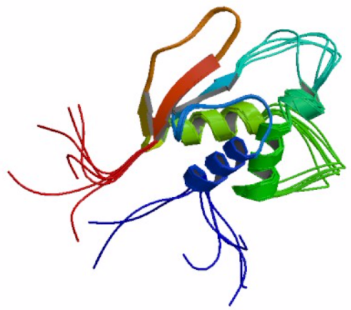
View more

Mutations



View more

Target Tractability



Bioactive Compounds	No
Druggable Structure	No
Druggable by Ligand Based Assessment	No
Enzyme	No

Original and additional data on *CanSAR*

View mor

Top Co-dependencies

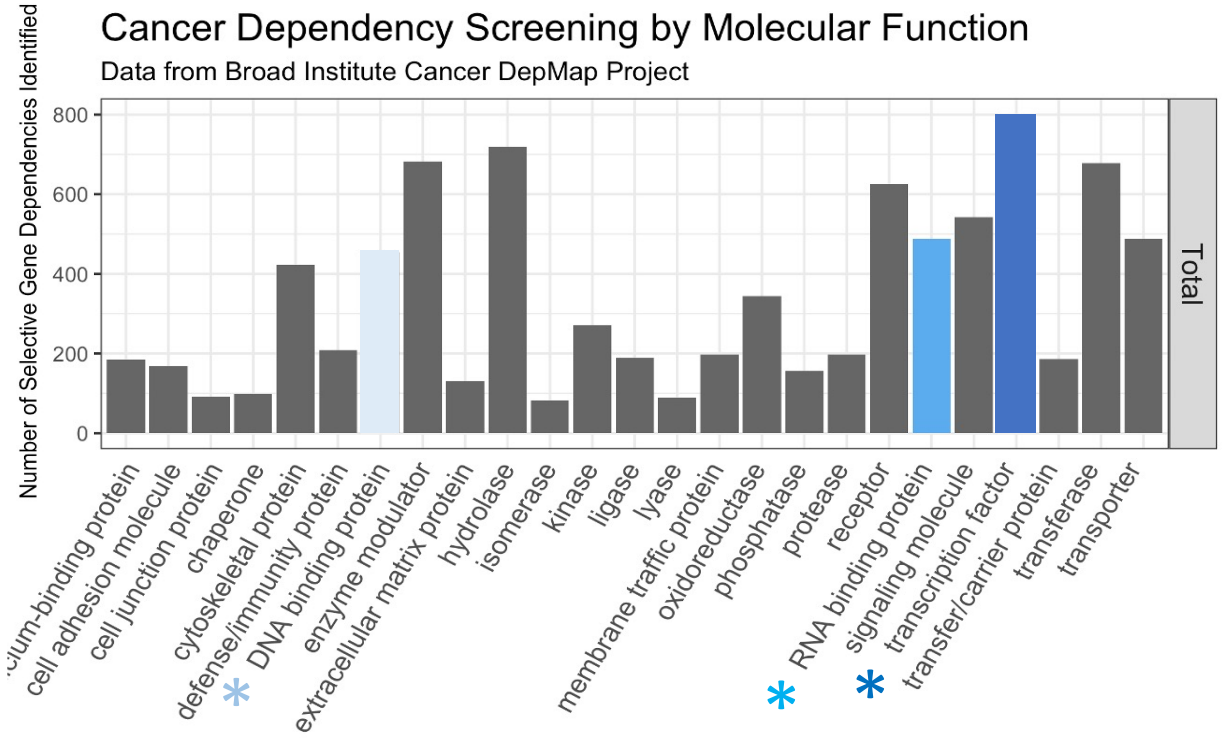
CRISPR (Avana) Public 19Q1

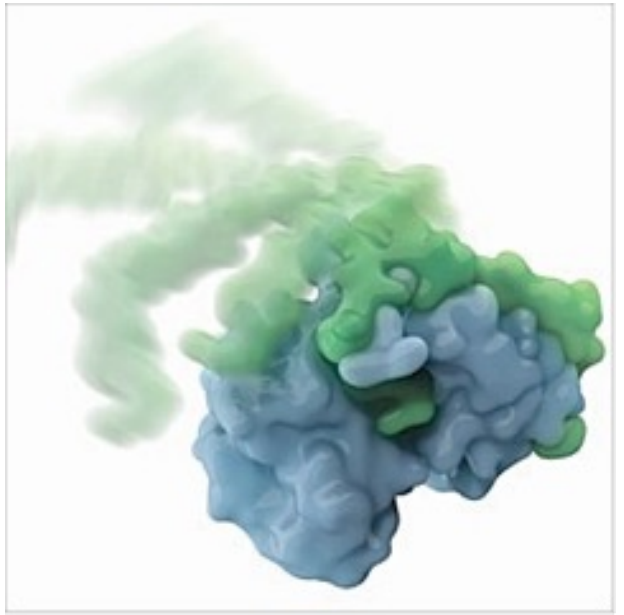
	Gene	Pearson correlation
Plot	PRDM1	0.58
Plot	POU2AF1	0.57
Plot	NFKB1	0.50
Plot	MEF2C	0.47
Plot	IKBKB	0.45

Combined RNAi (Broad, Novartis, Marcotte)

	Gene	Pearson correlation
Plot	NFKB1	0.35
Plot	ARMCX6	0.33
Plot	TLNRD1	0.32
Plot	SP2	-0.31
Plot	MYSM1	0.31

depmap





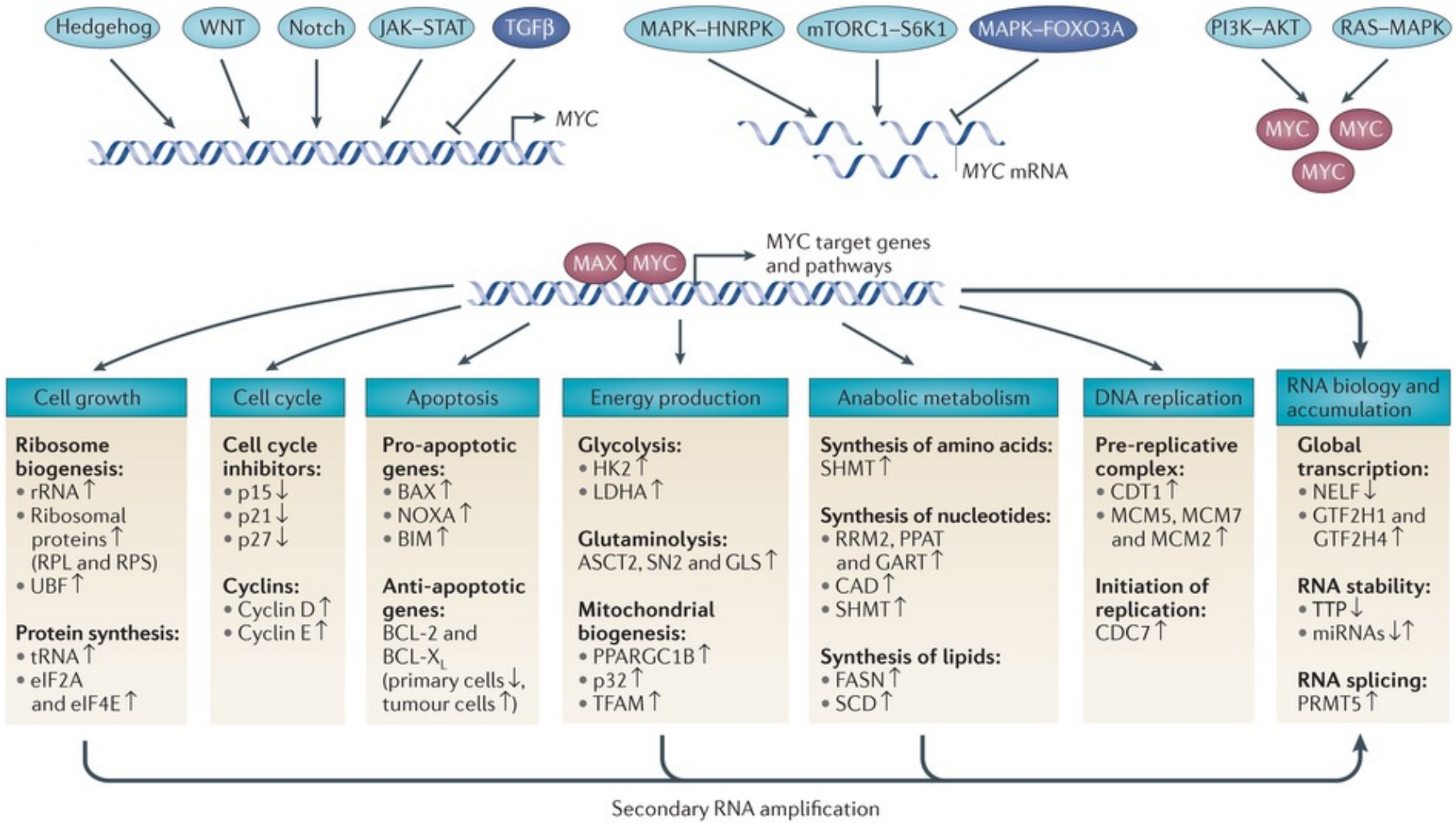
36 out of the top 80 dependencies are TFs

Henley & Koehler, *Nature Rev Drug Disc*, 20: 669-688 (2021)

https://depmap.org/portal/depmap/

MYC family of transcription factors

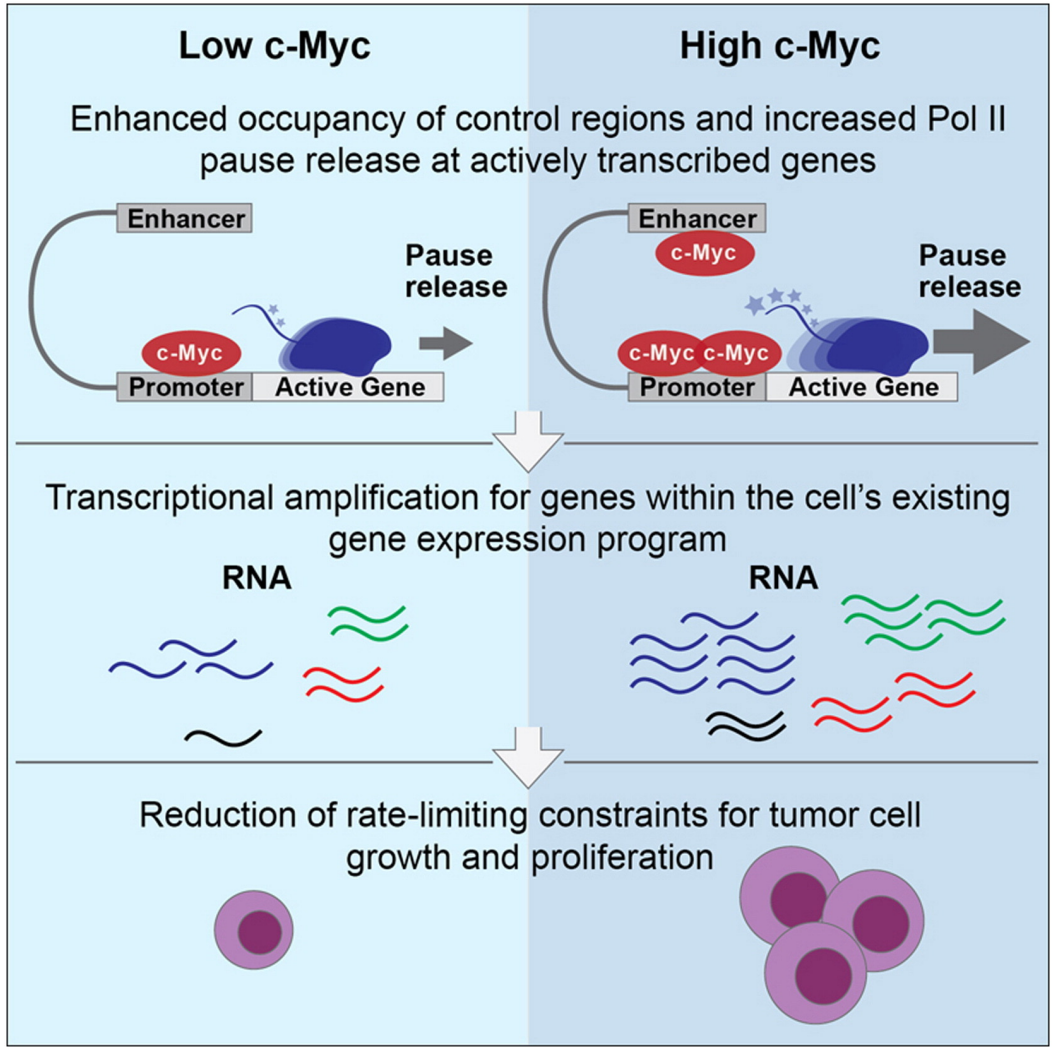
'master regulators' of broad cellular processes



Nature Reviews | Cancer

MYC

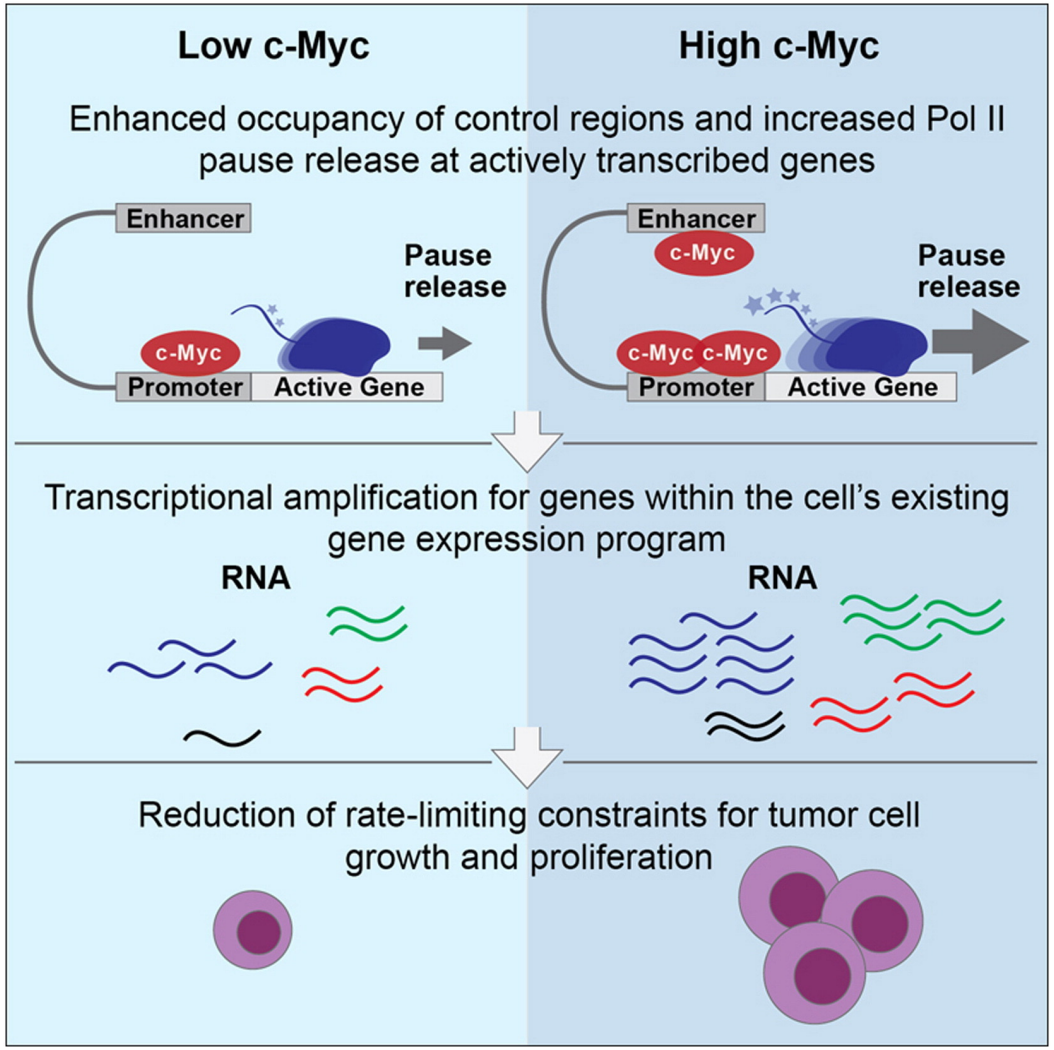
accumulates in promoter regions and amplifies transcription when overexpressed in cancer



Lin et al., Cell, 151, 56-67 (2012); Nie et al, Cell 151, 68-79 (2012)

MYC

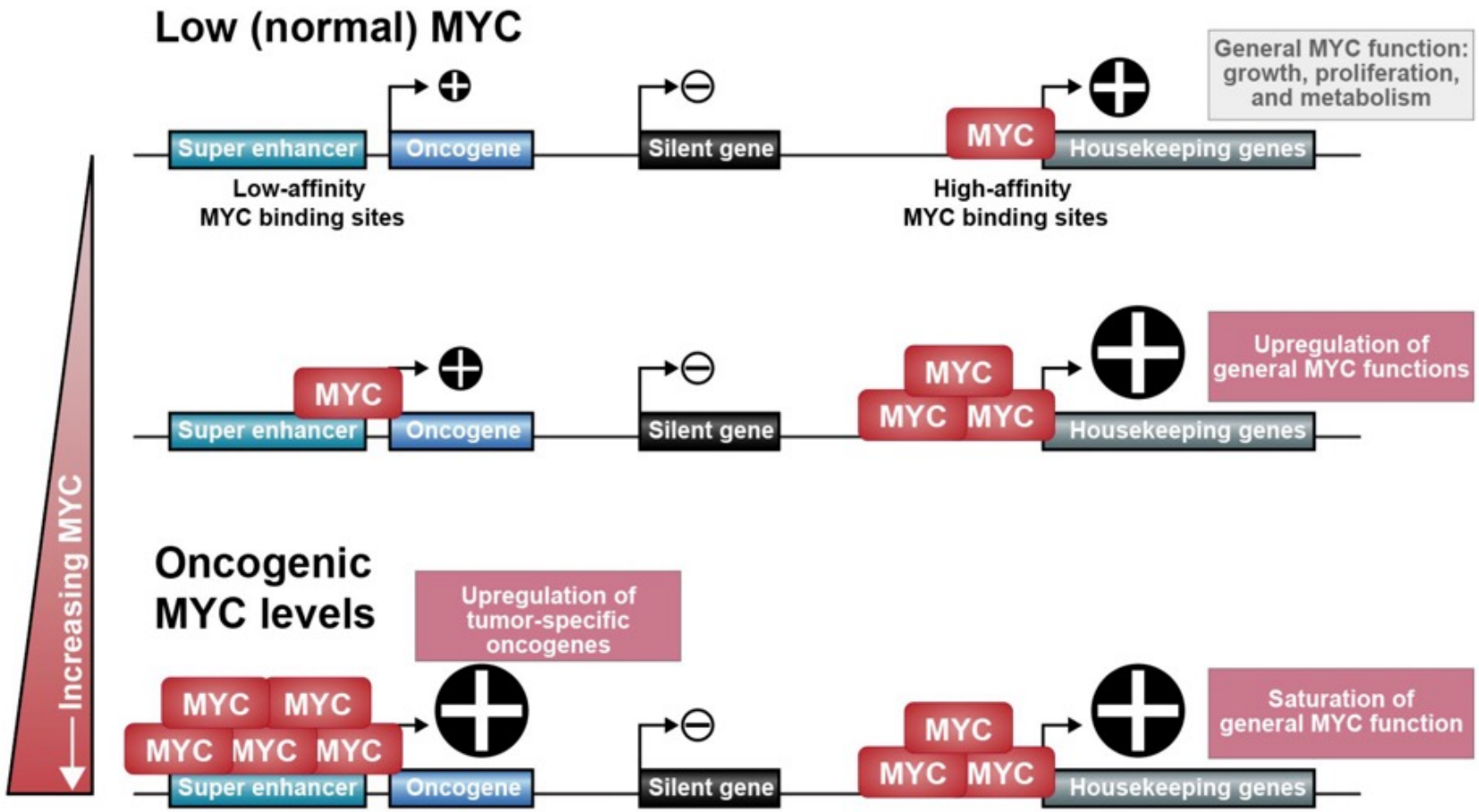
accumulates in promoter regions and amplifies transcription when overexpressed in cancer



'silver bullet' drug

Lin et al., Cell, 151, 56-67 (2012); Nie et al, Cell 151, 68-79 (2012)

Cancers dysregulate MYC by increasing its expression



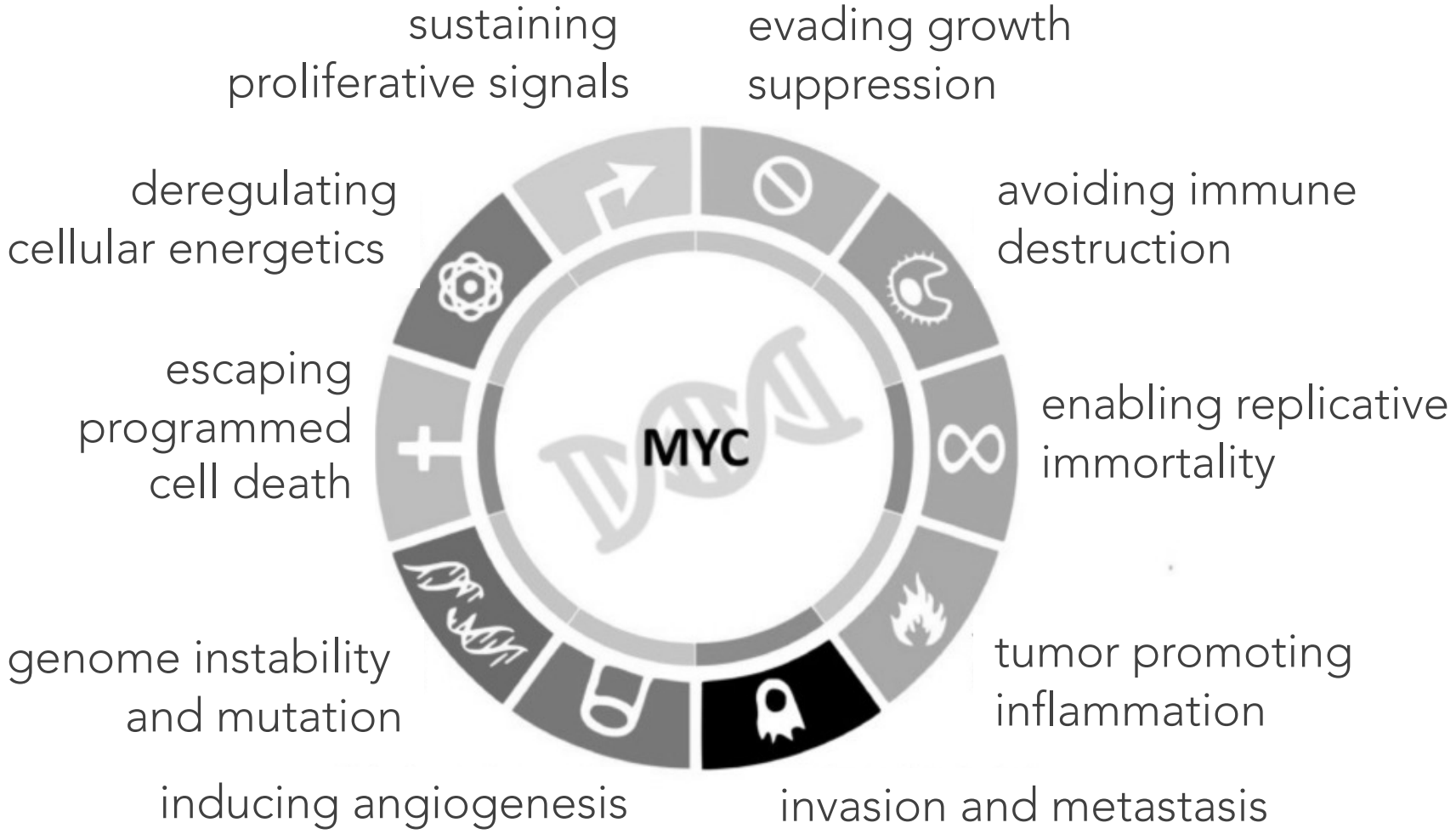
in typical cells, steady state *MYC* levels regulate general housekeeping functions

MYC can be transiently upregulated in typical cells (e.g. during wound healing)

tumor cells need persistently upregulated *MYC* at super physiologic levels to drive tumor-specific oncogenes

Adapted from Wolf et al., *Trends Cell Biol*, 25, 241-248 (2015)

Oncogenic levels of MYC regulate all hallmarks of cancer



Adapted from Llombart and Mansour, *eBioMedicine*, 75 (2022)

MYC expression in haploinsufficient mice

amelioration of age-associated phenotypes

Article

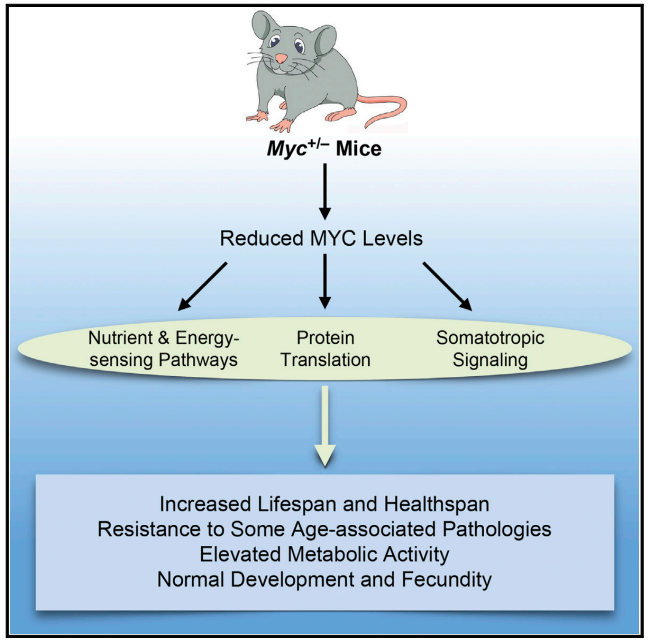
Hofmann et al., Cell, 160, 477-488 (2015)

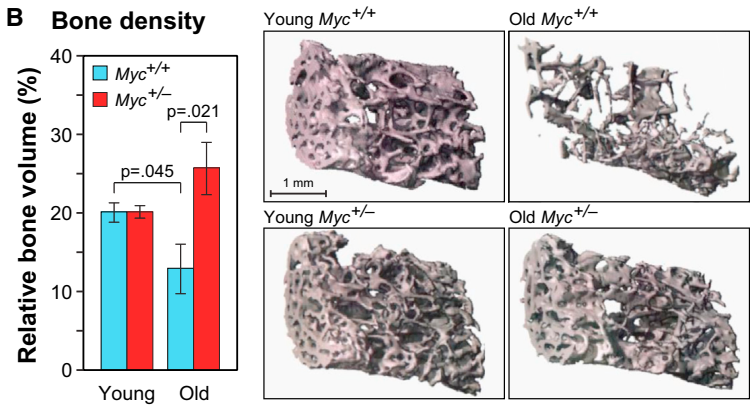
Cell

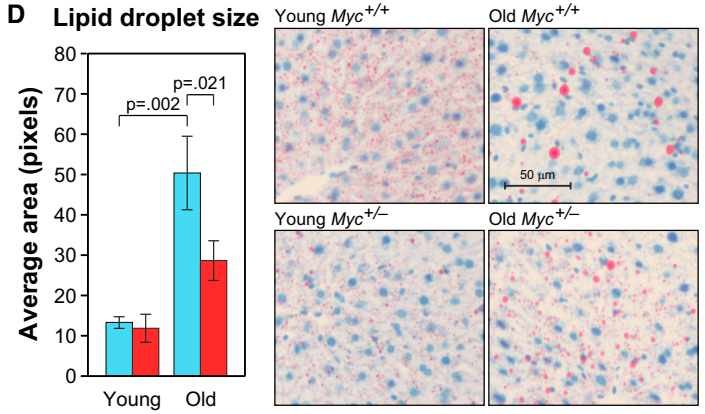
Reduced Expression of MYC Increases Longevity and Enhances Healthspan

Jeffrey W. Hofmann,[1,7] Xiaoai Zhao,[1,7] Marco De Cecco,[1] Abigail L. Peterson,[1] Luca Pagliaroli,[1] Jayameenakshi Manivannan,[1] Gene B. Hubbard,[2] Yuji Ikeno,[2] Yongqing Zhang,[3] Bin Feng,[4] Xiaxi Li,[5] Thomas Serre,[5] Wenbo Qi,[2] Holly Van Remmen,[2] Richard A. Miller,[6] Kevin G. Bath,[5] Rafael de Cabo,[3] Haiyan Xu,[4] Nicola Neretti,[1] and John M. Sedivy[1,*]

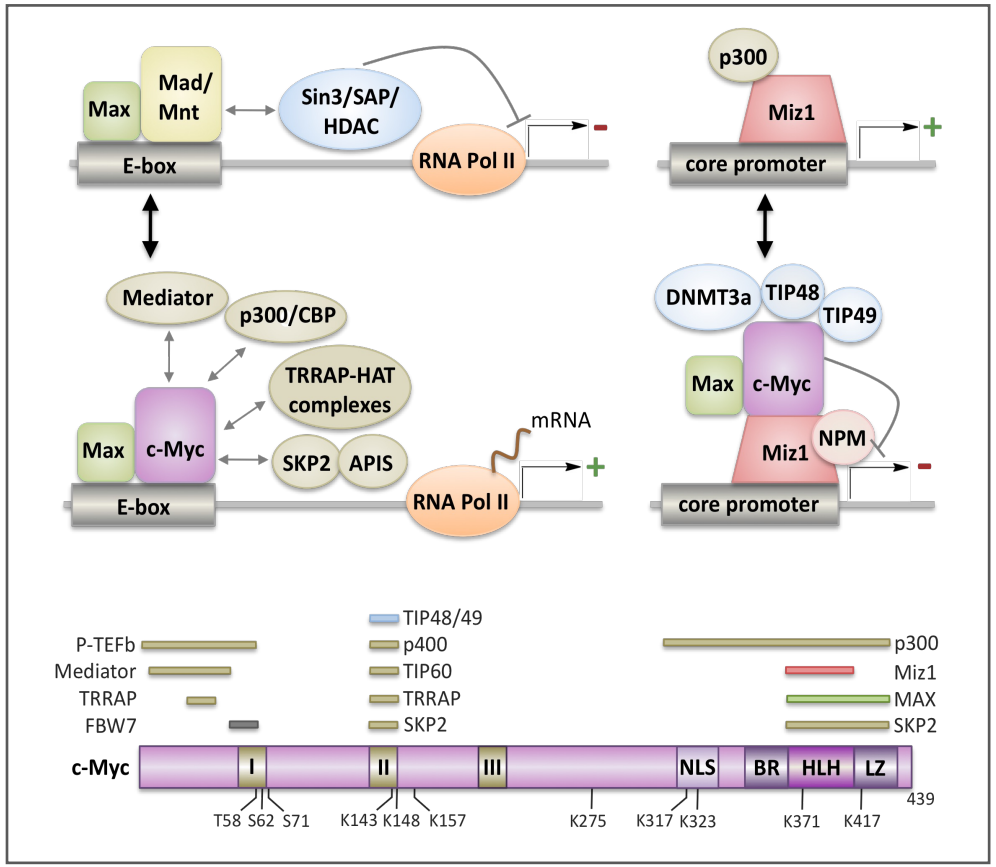
[1]Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI 02912, USA
[2]Department of Cellular and Structural Biology, Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA
[3]Translational Gerontology Branch, National Institute on Aging, 251 Bayview Boulevard, Suite 100, Baltimore, MD 21224, USA
[4]Hallett Center for Diabetes and Endocrinology, Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI 02903, USA
[5]Department of Cognitive, Linguistic, and Psychological Sciences, Brown University, Providence, RI 02912, USA
[6]Department of Pathology and Geriatrics Center, University of Michigan, Ann Arbor, MI 48109, USA
[7]Co-first author
*Correspondence: john_sedivy@brown.edu
http://dx.doi.org/10.1016/j.cell.2014.12.016



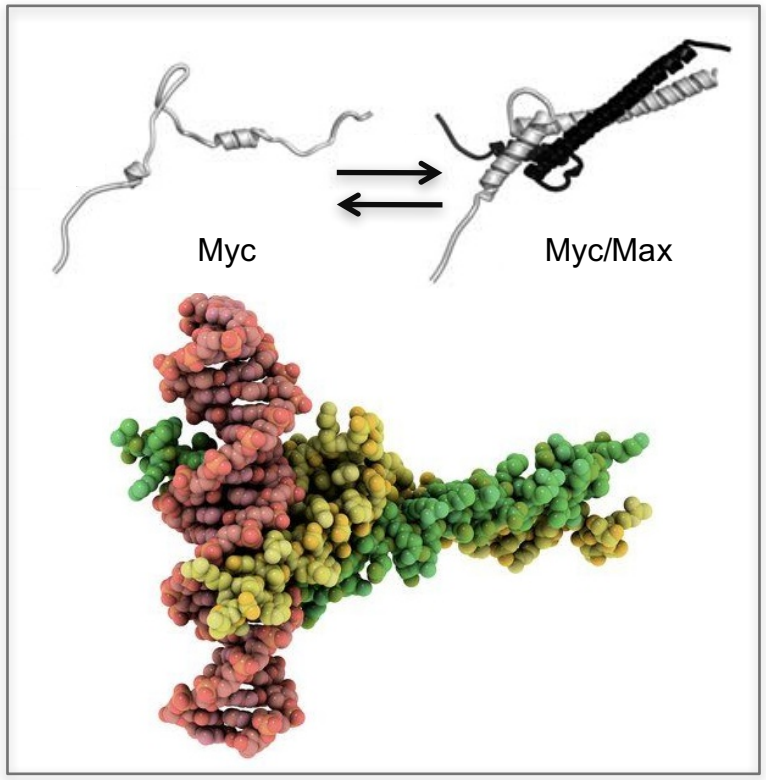




MYC is an obstinate therapeutic target



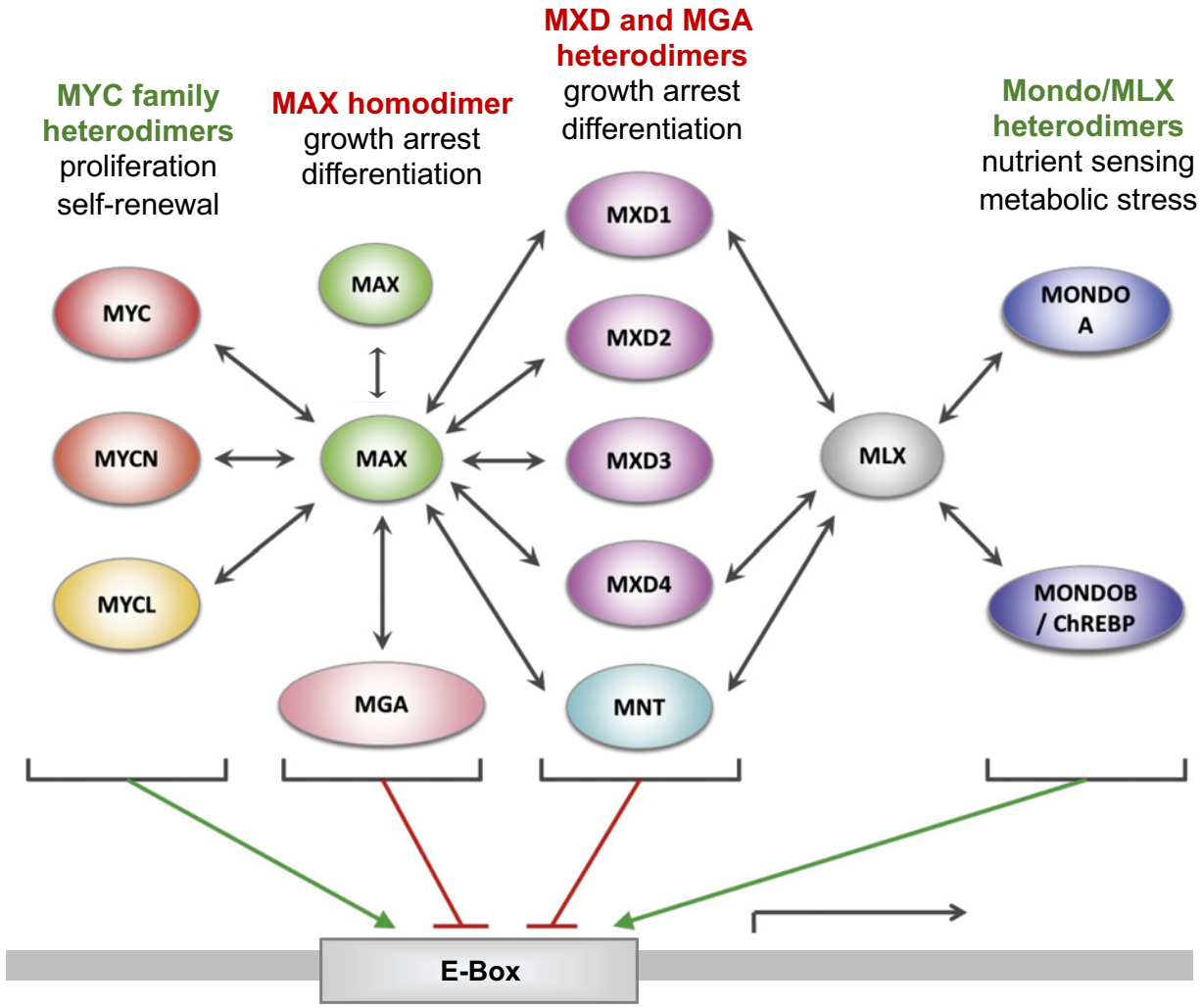
many protein-protein interactions



unstructured domains
no traditional binding pockets
large buried interface

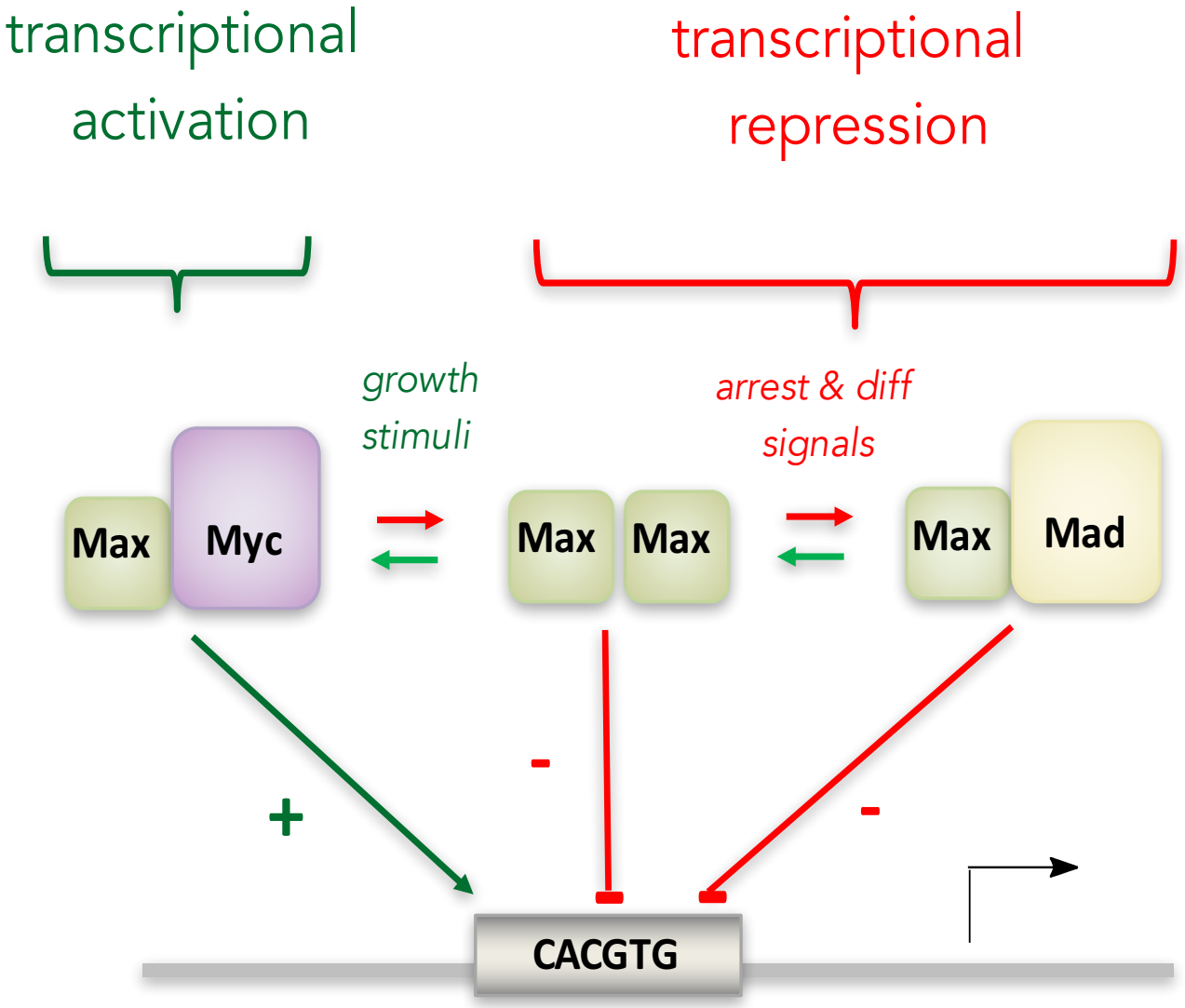
Myc/Max/Mxd Network

alternative paths to modulating amplified Myc-driven transcription in cancer



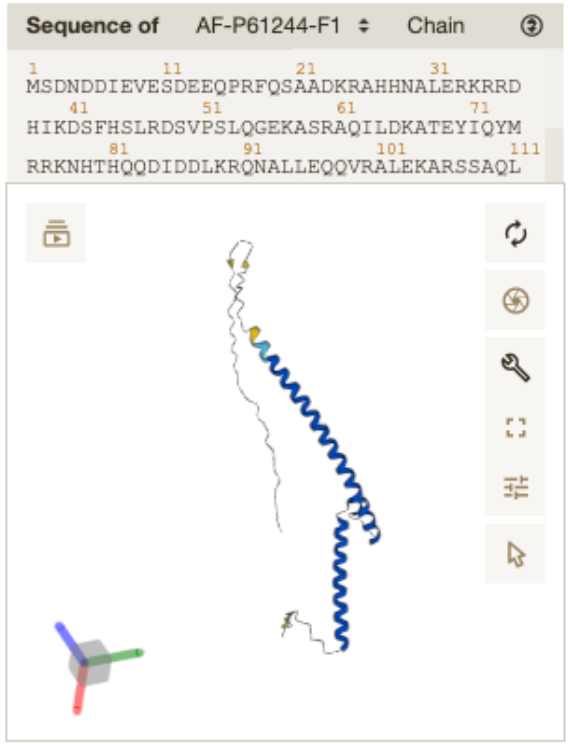
Adapted from Diolaiti, McFerrin, Carroll, Eisenmann, *Biochimica et Biophysica Acta* 1849, 484-500 (2015)

MAX as a target: alter heterodimer/homodimer dynamics



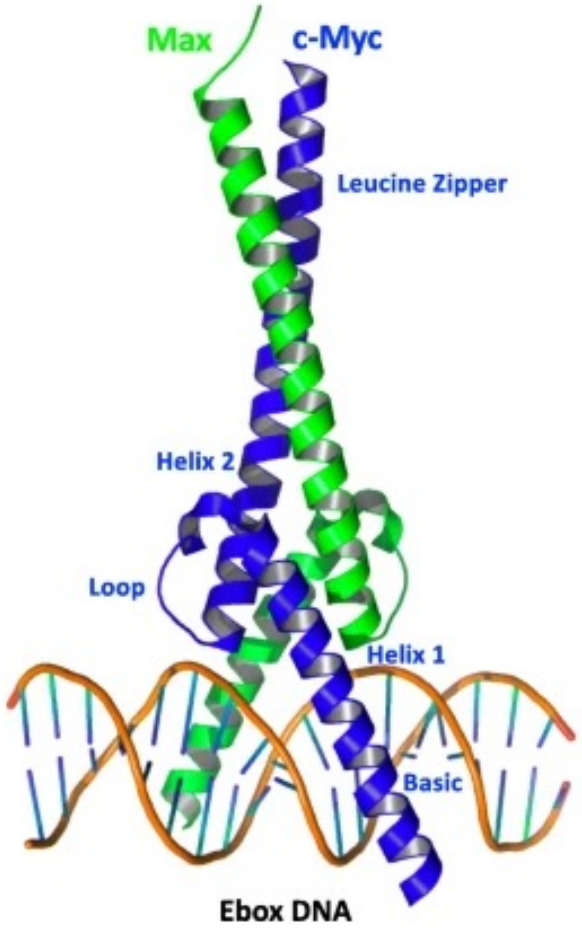
Diolaiti, McFerrin, Carroll, Eisenmann, *Biochimica et Biophysica Acta* 1849, 484-500 (2015)

MAX: Myc-Associated factor X

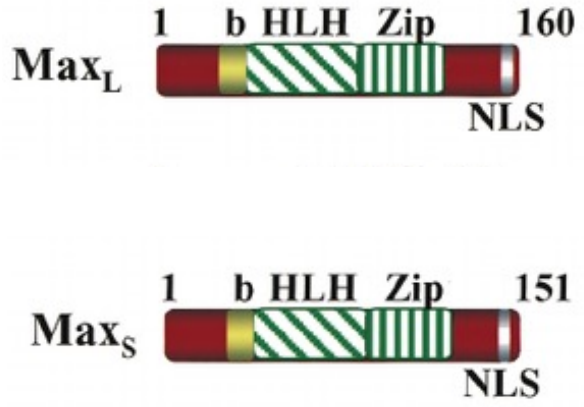


Bioactive Compounds	Yes
Druggable Structure	No
Druggable by Ligand Based Assessment	No
Enzyme	No

AlphaFold (predicted)



crystal structure with Myc
basic helix loop helix leucine zipper
bHLH-LZ

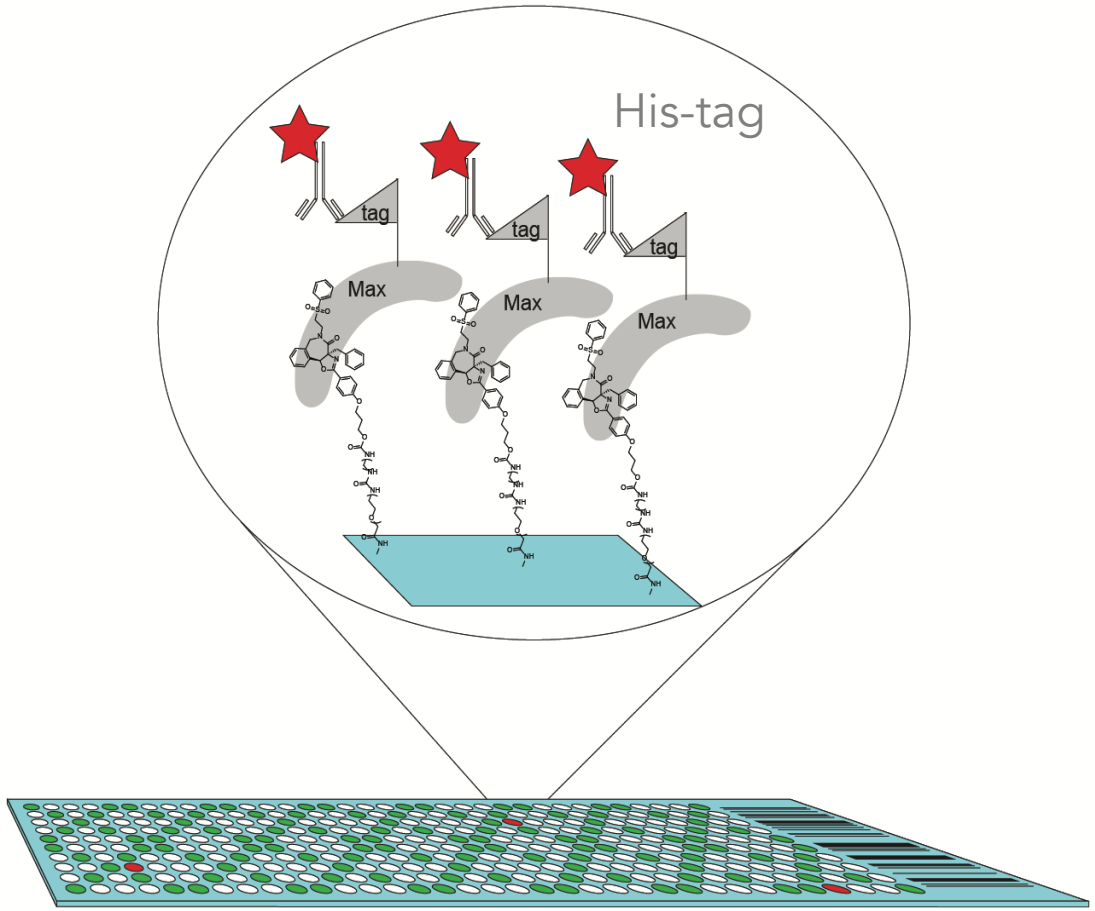


splice variants

MAX: Myc-Associated factor X

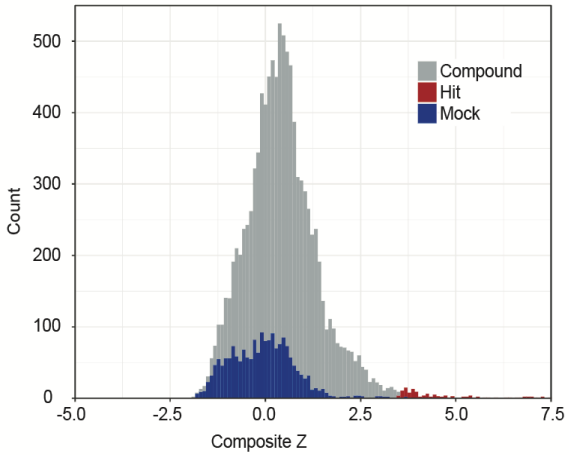
Cellular localization:	primarily nuclear
Tissue specificity:	high levels in brain, heart, lung low levels in liver, kidney, skeletal muscle
Post-translational mod:	acetylation (localization) phosphorylation (stability)
Diseases:	mutated in pheochromocytoma mutated in small cell lung cancers
	potential tumor suppressor role in ‘neuroendocrine’ tumors, which are tumors that form from cells that release hormones into the blood in response to signals from the nervous system

SMM screens: purified Max transcription factor

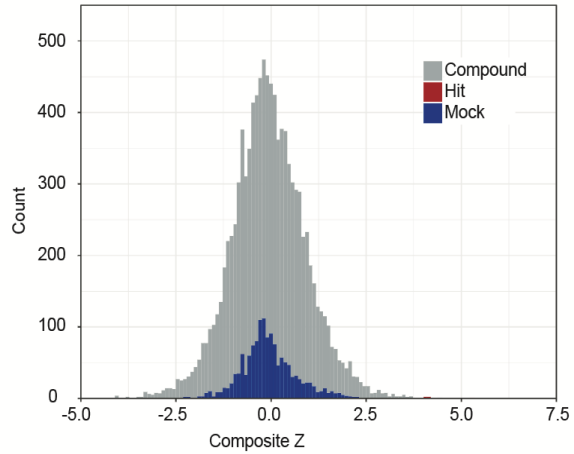


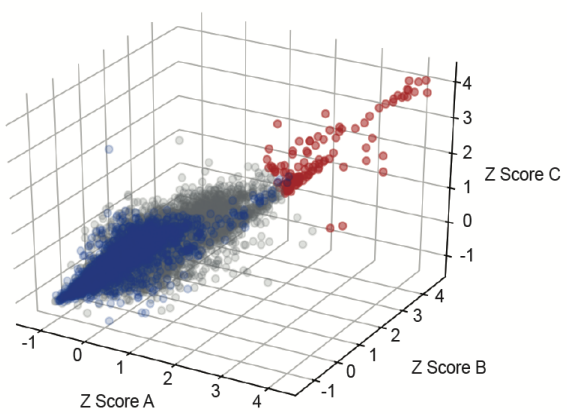
>45k compounds screened

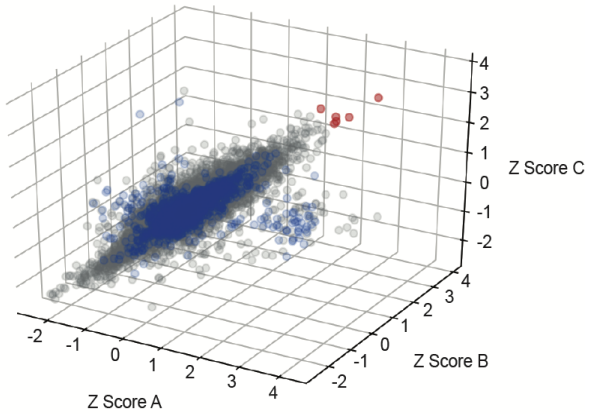
synthetic compound collection



natural products

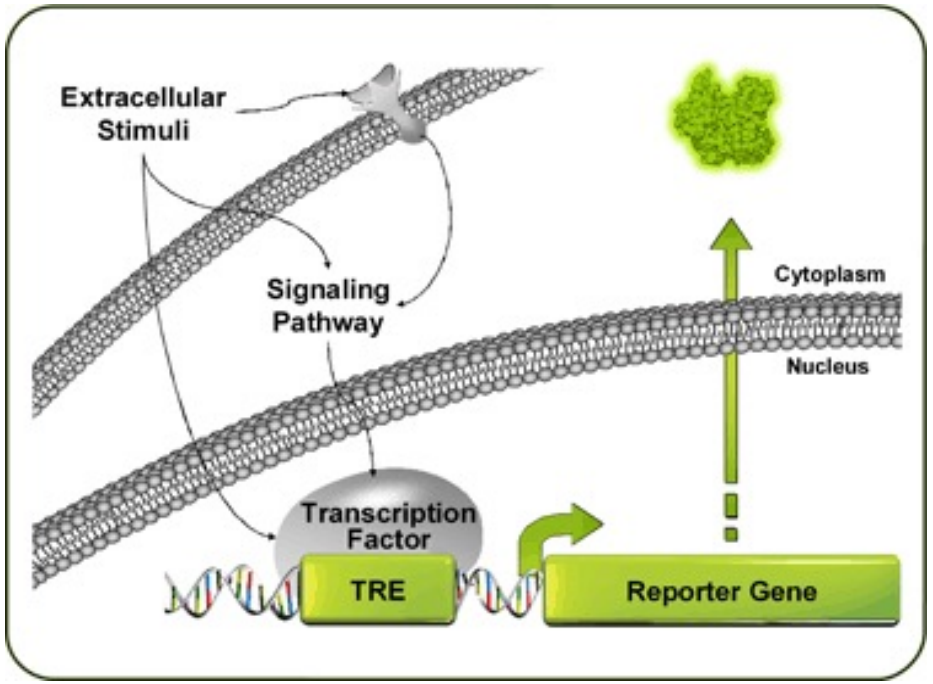


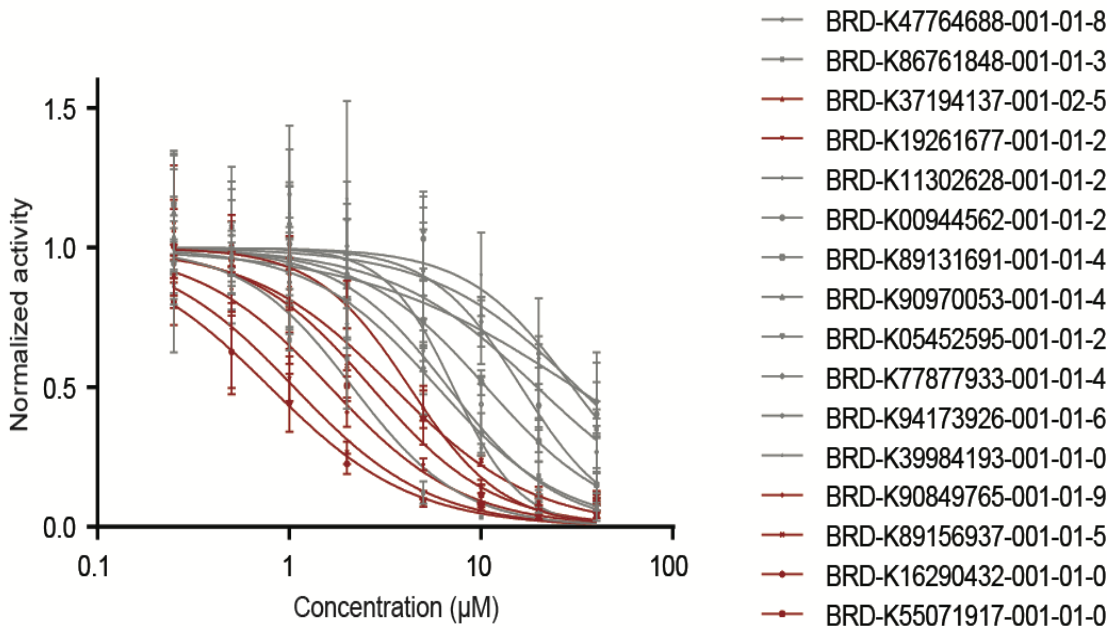




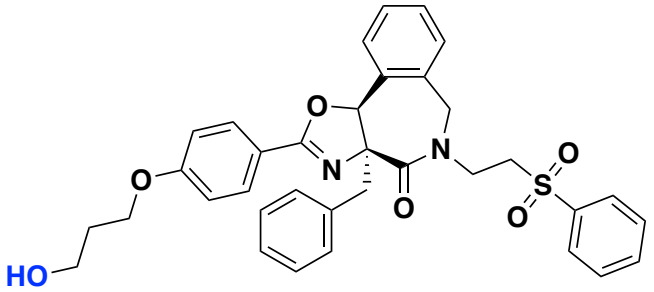
117 assay positives

Reporter gene assays: putative Max binders modulate Myc-driven transcription





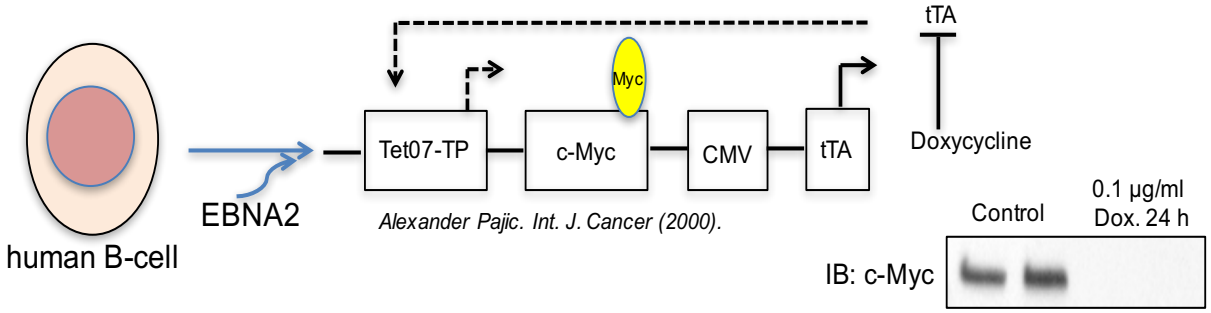
'KI-MS2'



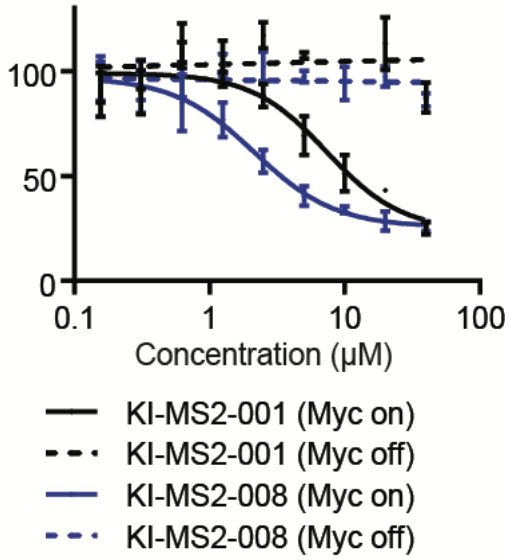
IC_{50} = 1.06 μM
MW = 610.73
cLogP = 5.15

Cell viability assays: Are Myc or Max required?

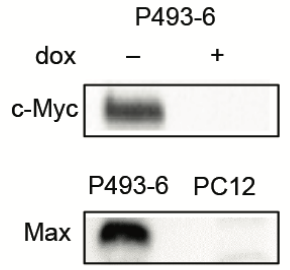
P493-6
Doxycycline-repressible cells for MYC 'on/off' studies



P493-6

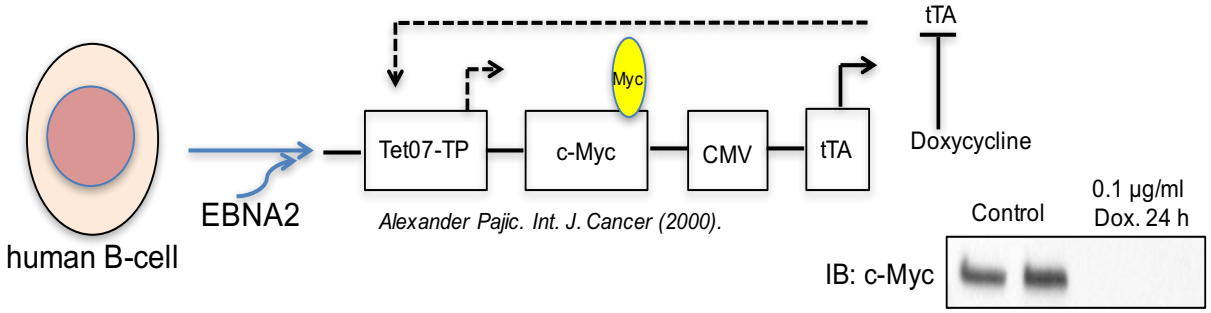


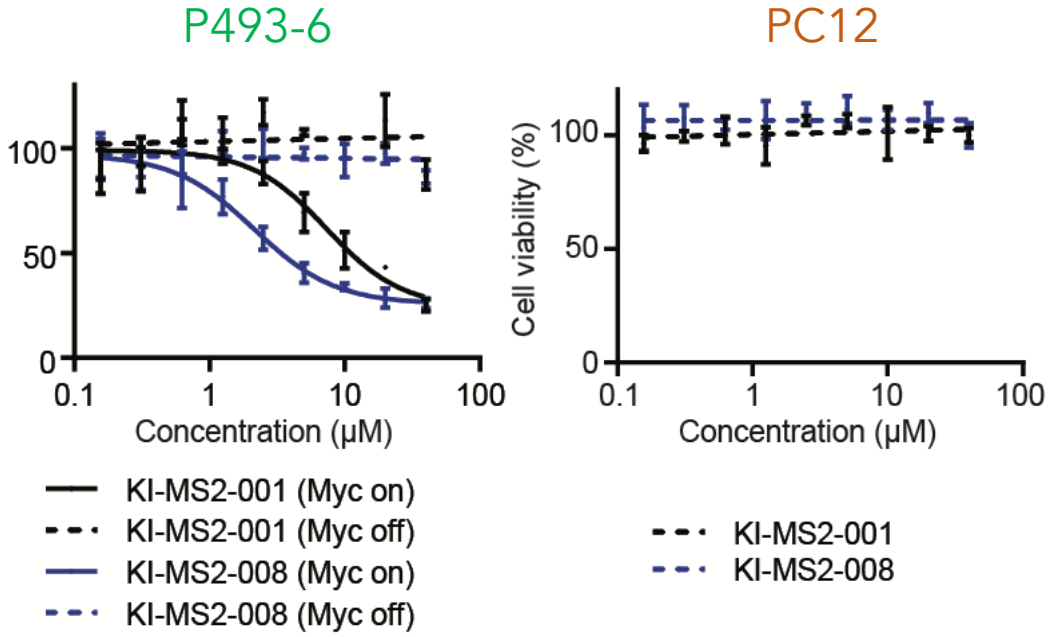
	KI-MS2-001	KI-MS2-008
Myc reporter	1.98 µM	1.28 µM
P493-6 Myc on	7.36 µM	2.15 µM
P493-6 Myc off	>50 µM	>50 µM



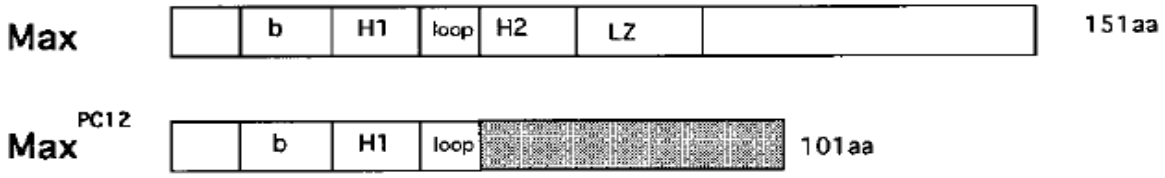
Cell viability assays: Are Myc or Max required?

P493-6
Doxycycline-repressible cells for MYC 'on/off' studies

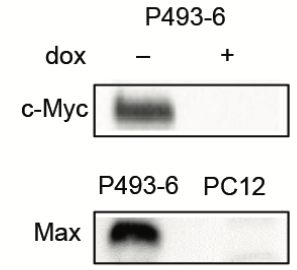




Max-deficient PC12 pheochromocytoma cells



	KI-MS2-001	KI-MS2-008
Myc reporter	1.98 µM	1.28 µM
P493-6 Myc on	7.36 µM	2.15 µM
P493-6 Myc off	>50 µM	>50 µM
PC12	>50 µM	>50 µM



Conditional cellular models of *MYC* expression

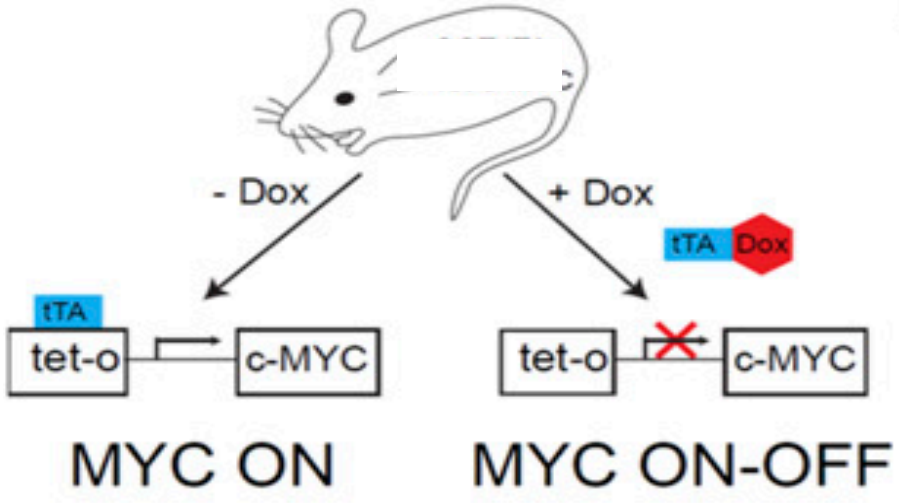
Myc 'on/off' mouse models:

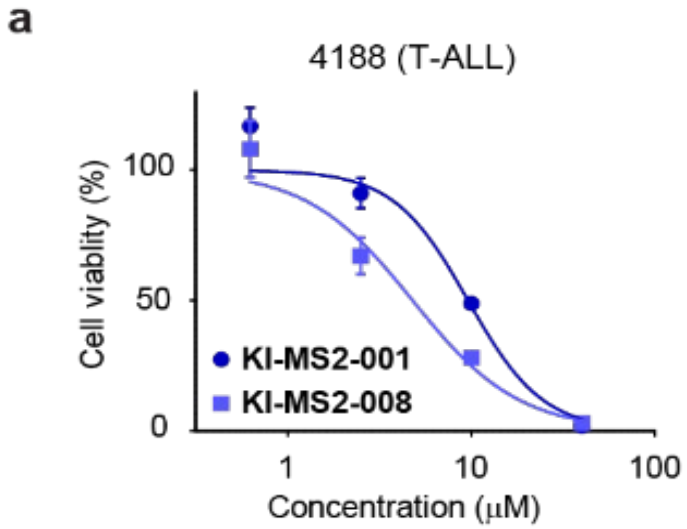
lymphoma

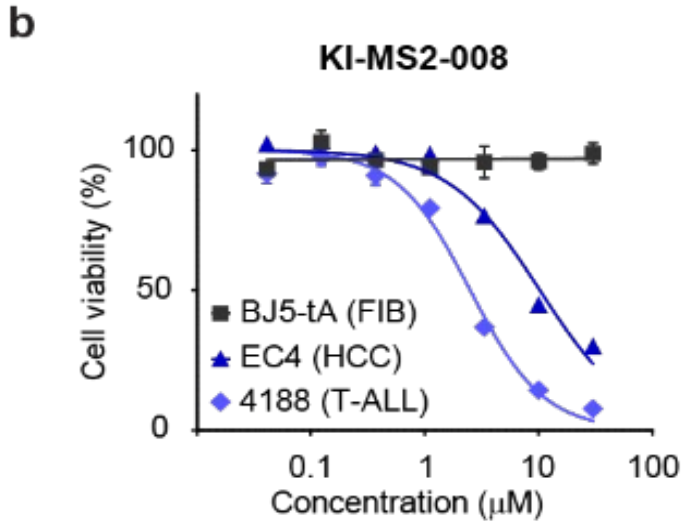
HCC

RCC

osteosarcoma



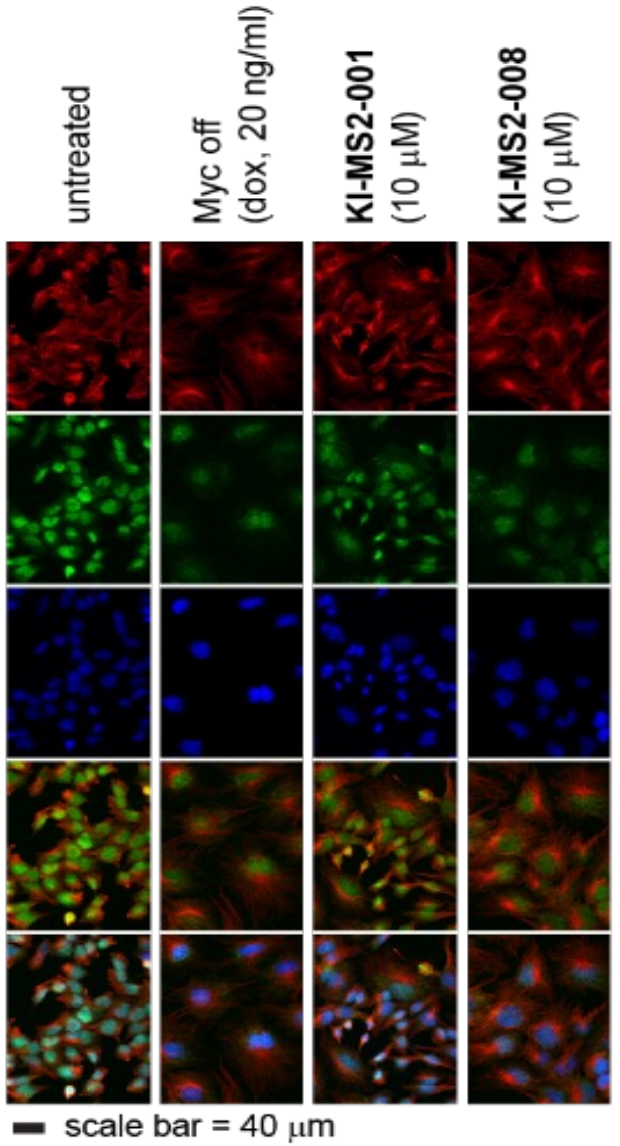




Anja Deutzmann, Felsher Lab Stanford

Imaging of biomarkers: conditional vs. chemical modulation

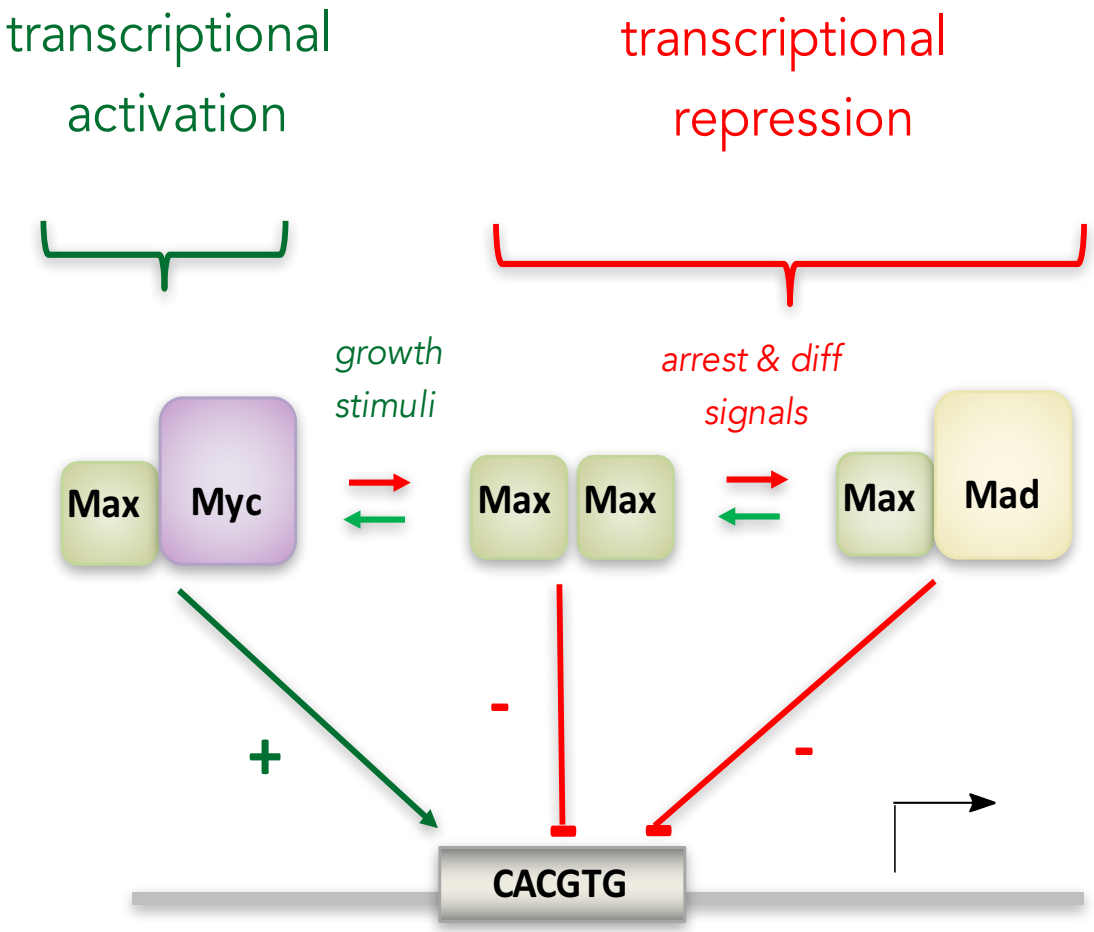
modulating Myc in an engineered osteosarcoma model



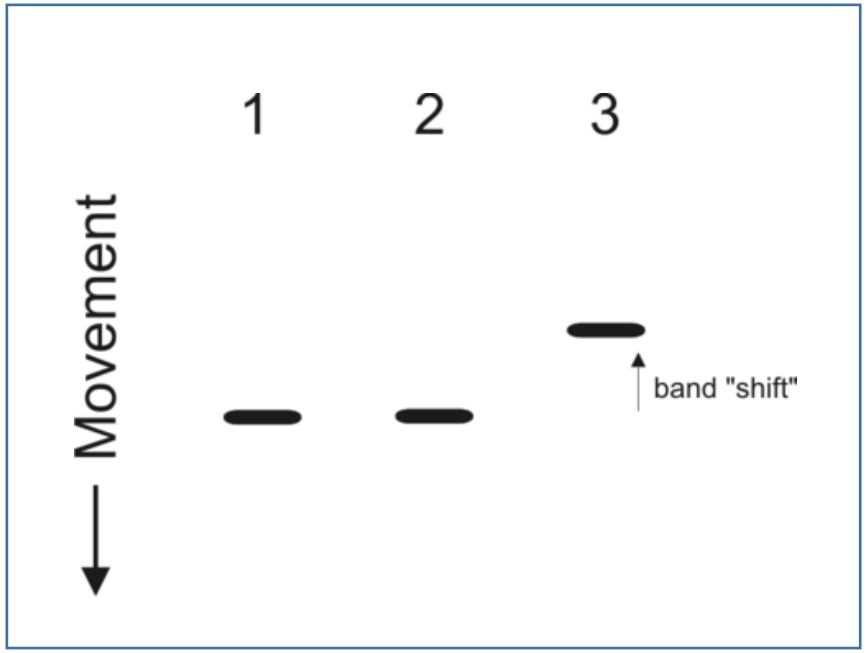
'MYC OFF marker'

Anja Deutzmann, Felsher Lab Stanford

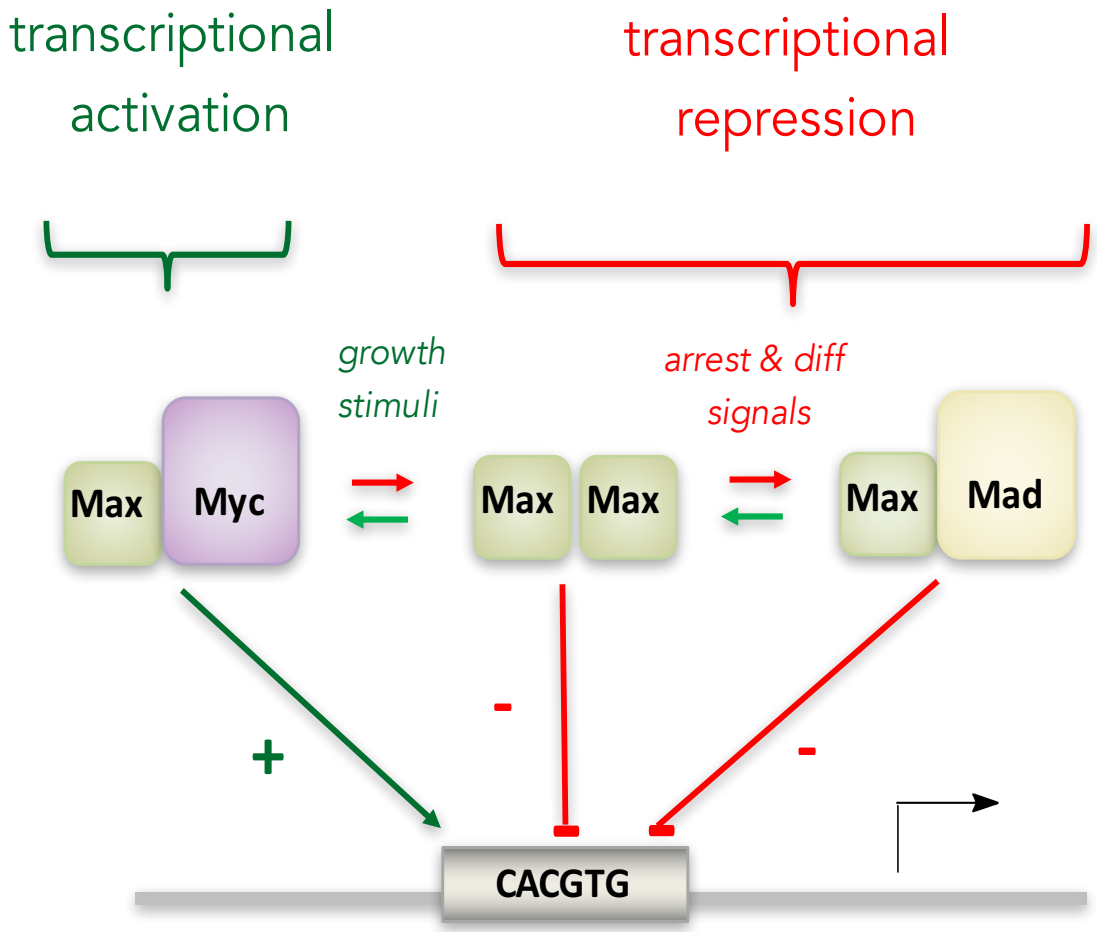
Does the probe antagonize the Myc/Max heterodimer?

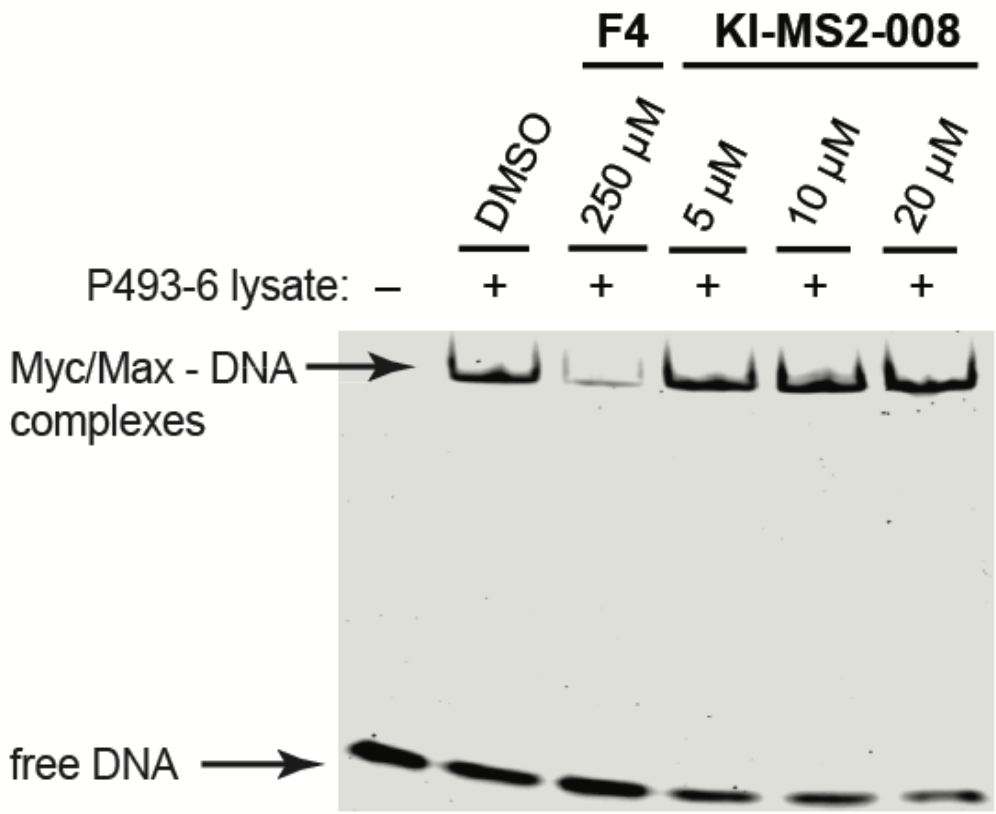


Electrophoretic Mobility Shift Assay (EMSA)
aka Gel Shift Assay



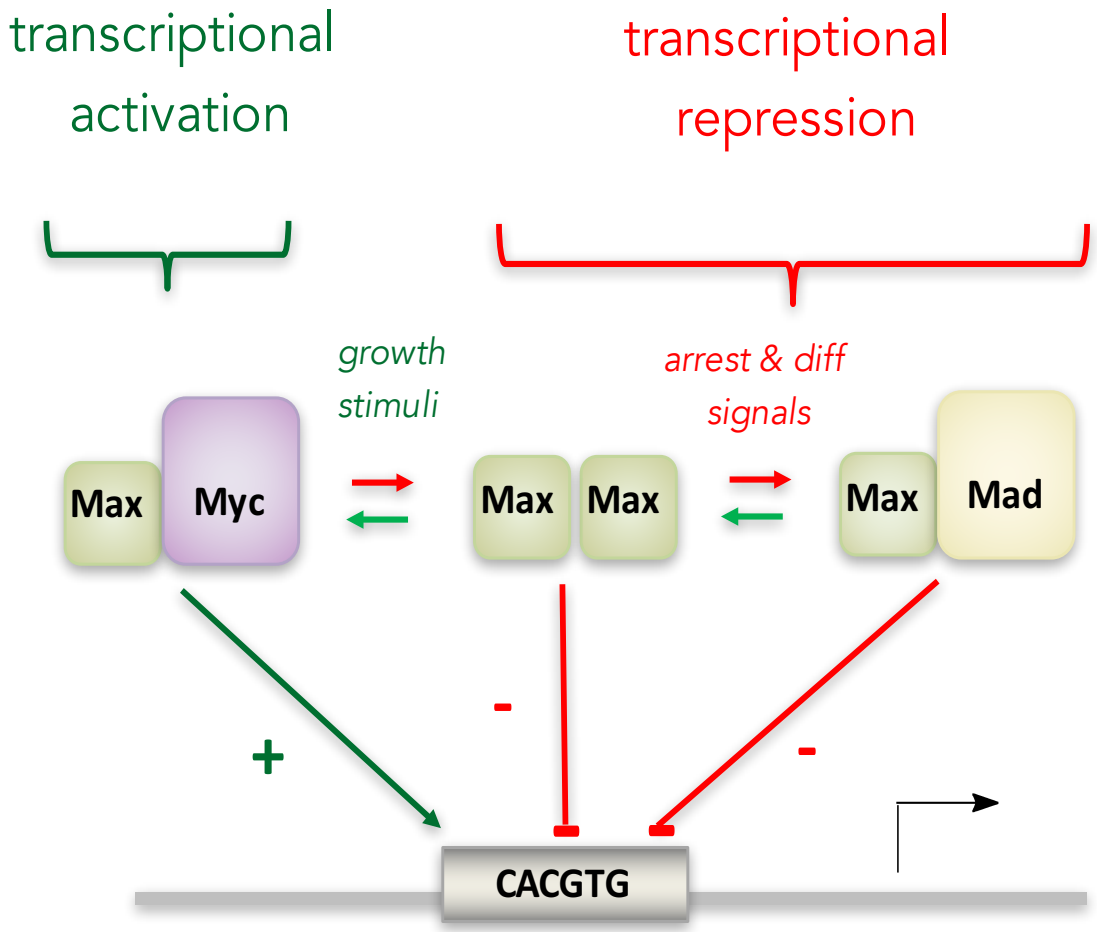
Does the probe antagonize the Myc/Max heterodimer?

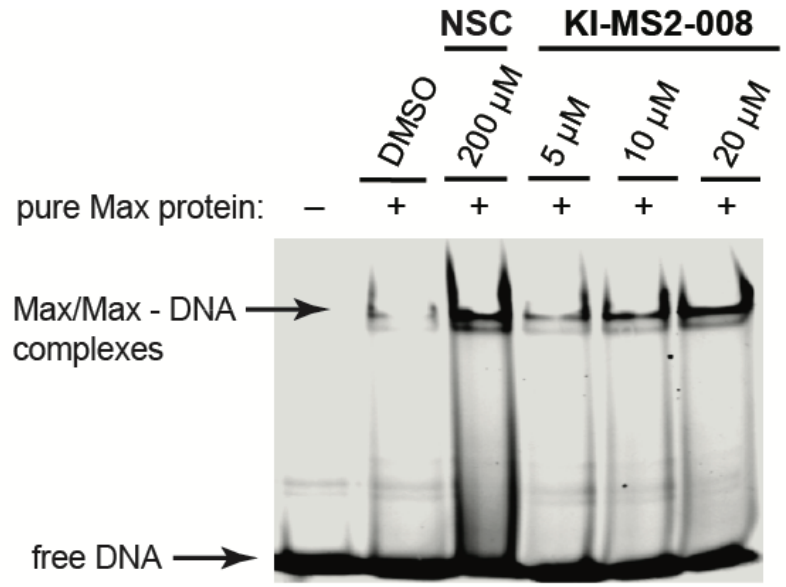


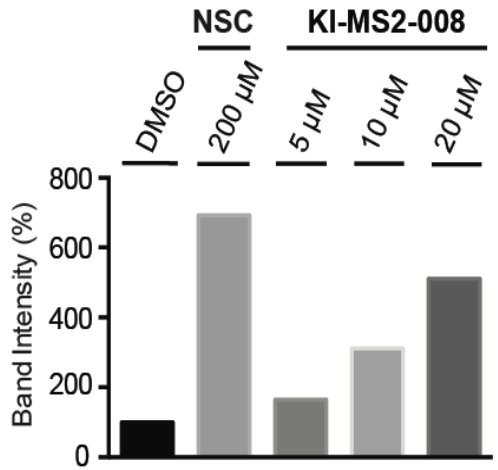


EMSA

Does the probe stabilize the Max/Max homodimer?



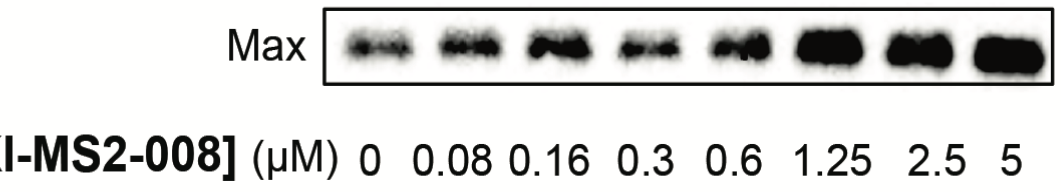


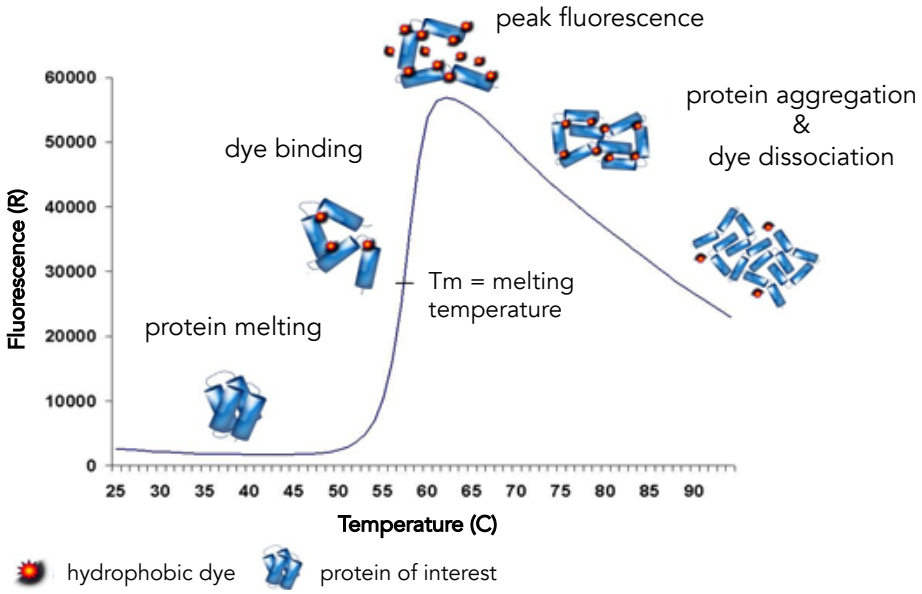


Does the probe engage Max in cells?

dose-dependent cellular thermal shift assays (CETSA) in live cells

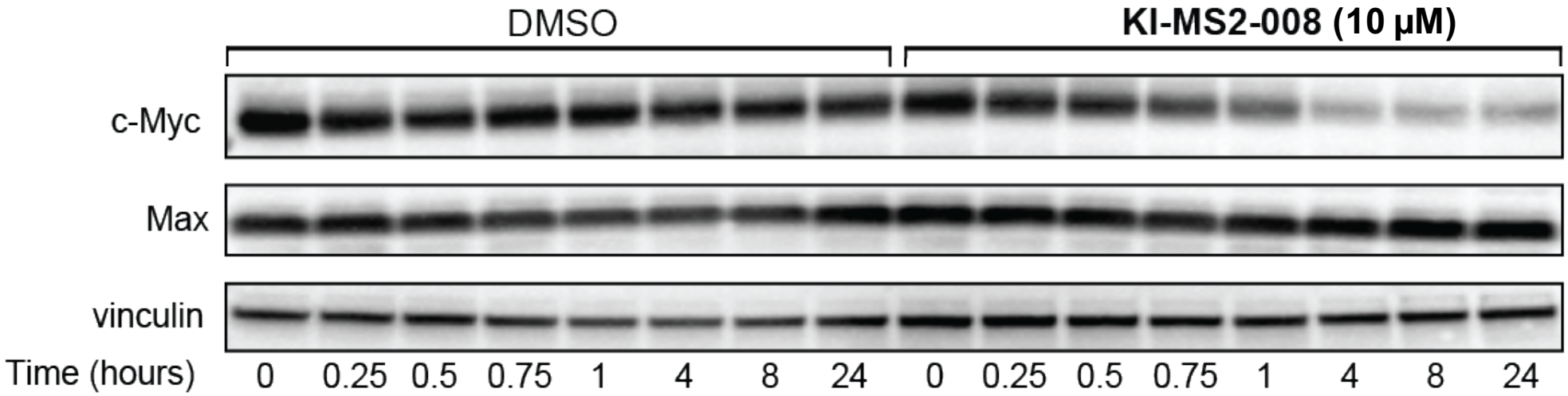
Dose-response
1 hour treatment with compound
60 °C

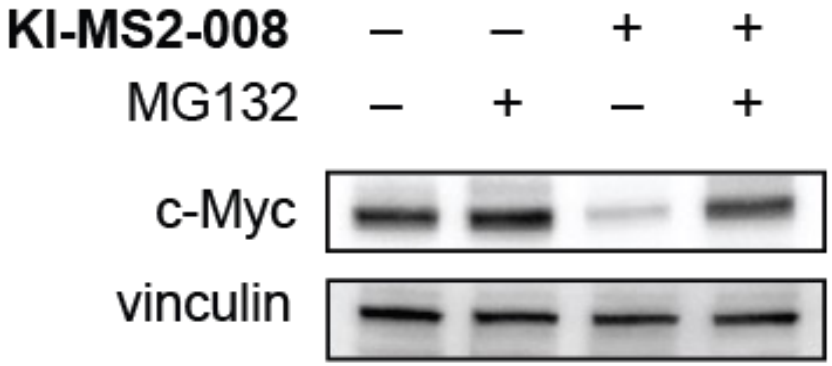




Struntz et al., Cell Chem Biol, 26, 711-723 (2019)

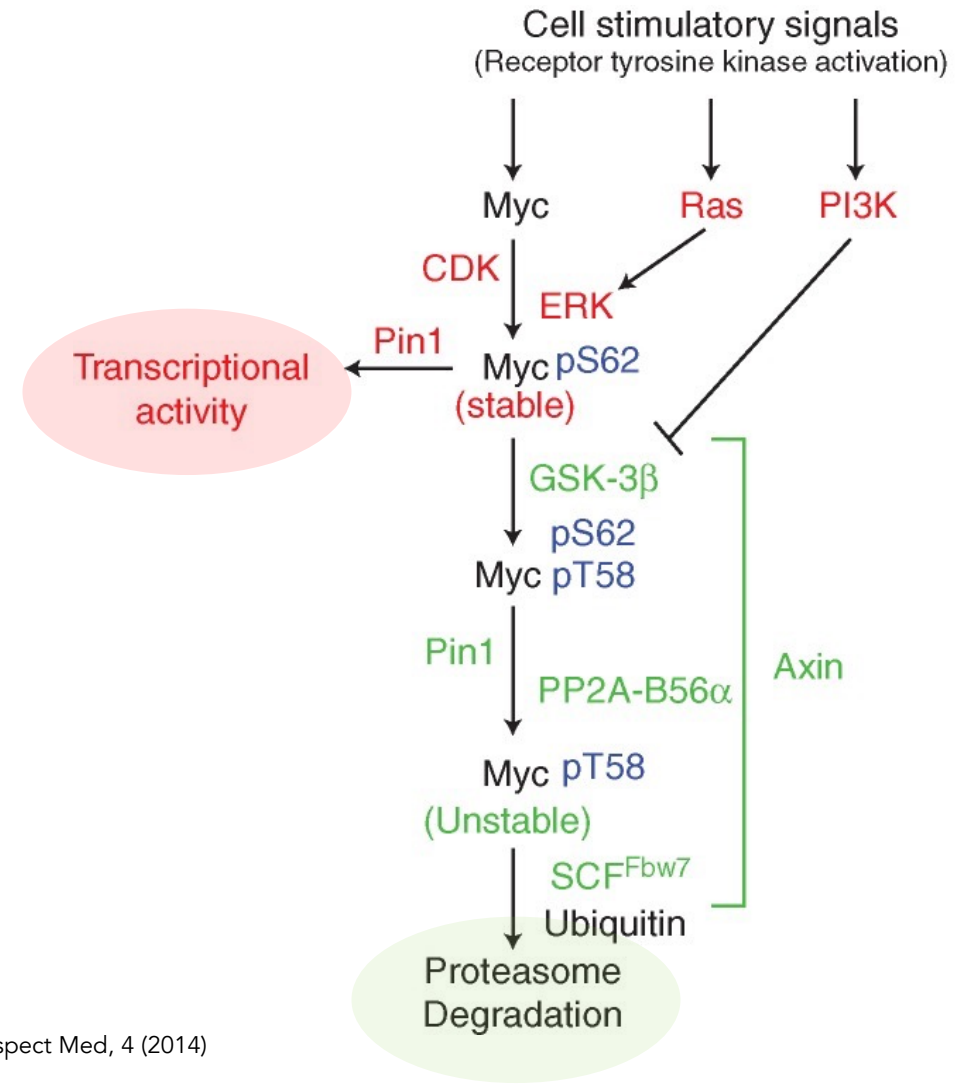
Western blots: KI-MS2-008 alters Myc protein levels

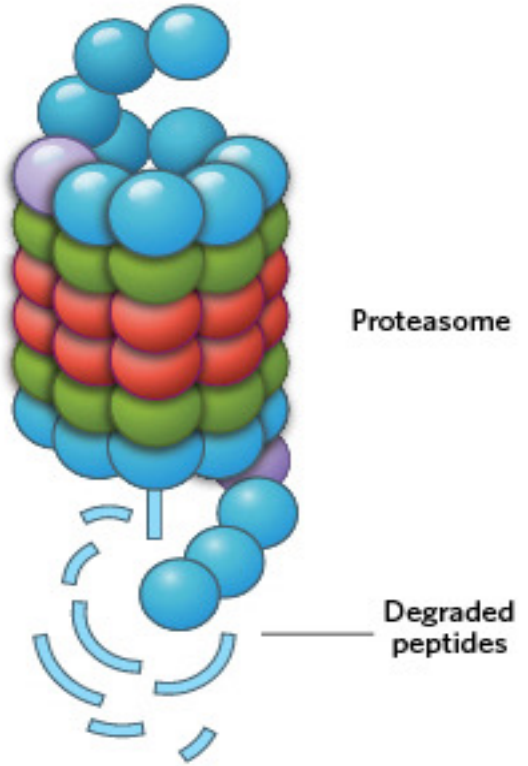




rescue experiment with
10 μM proteasome inhibitor
MG132

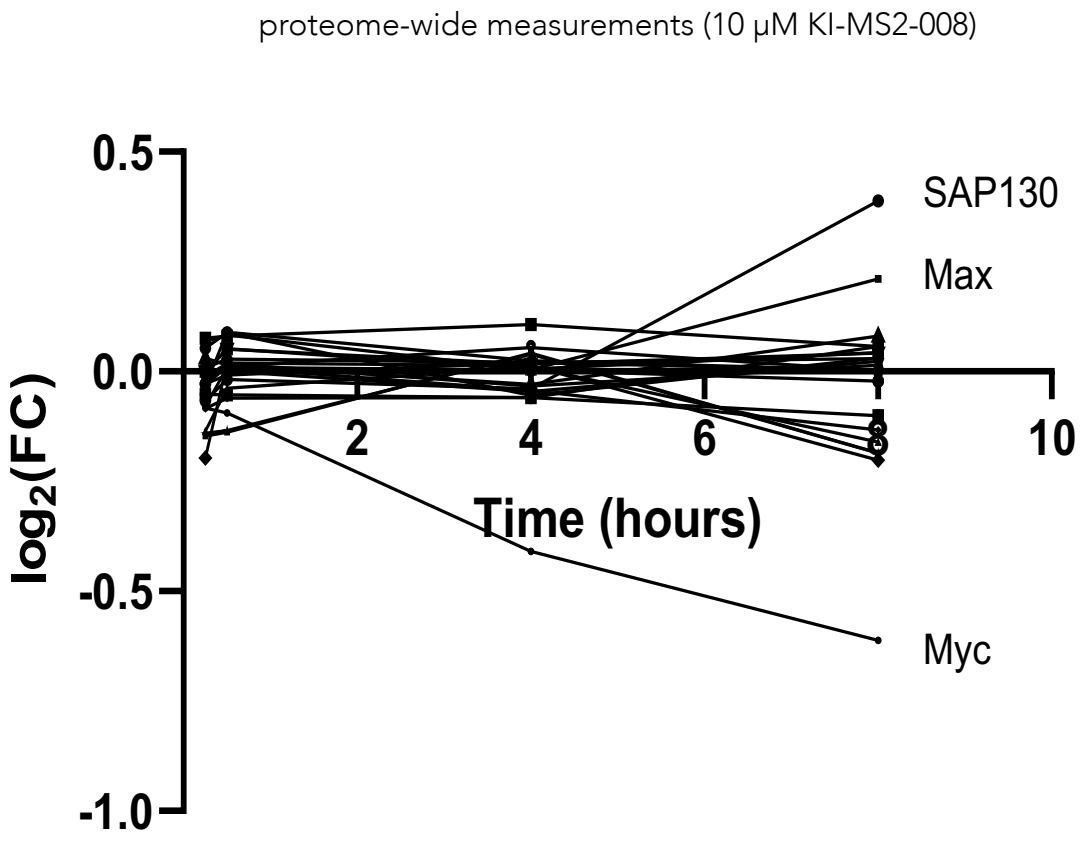
Myc protein stability is regulated by the ubiquitin-proteasome system





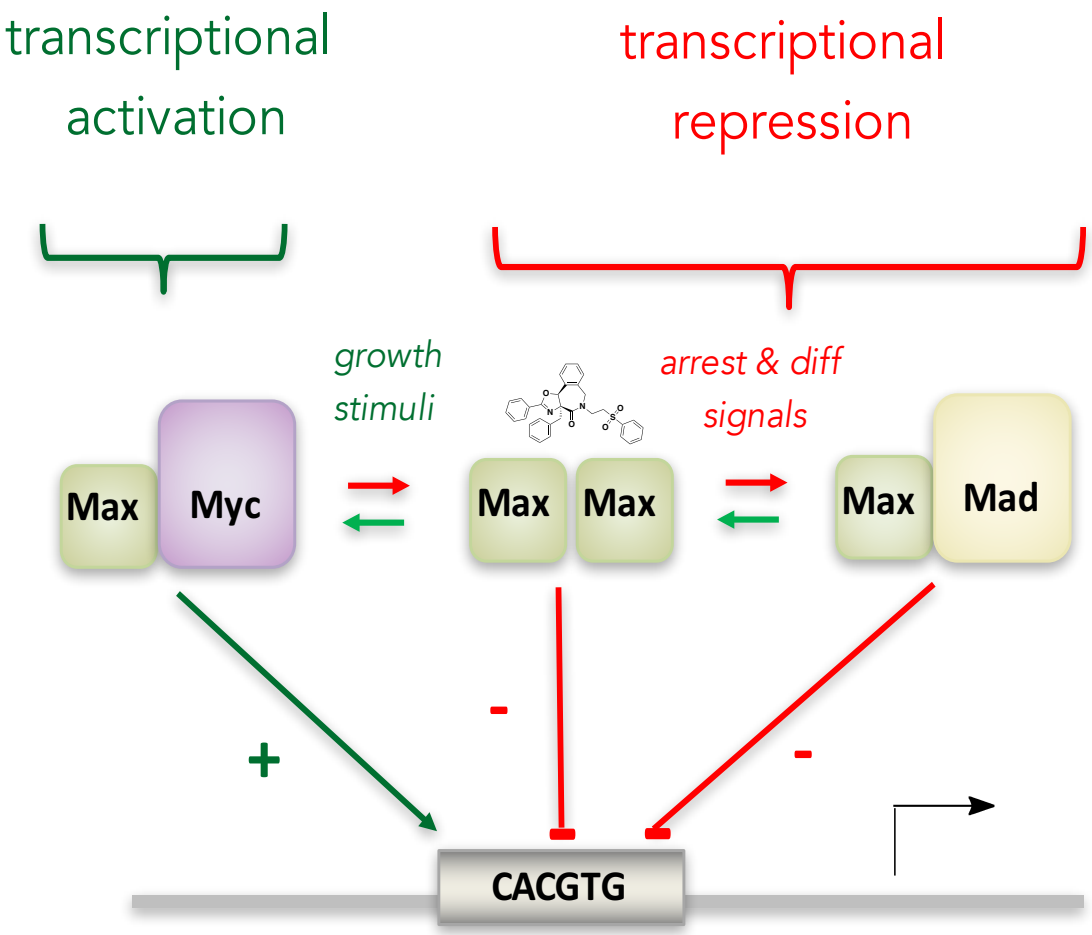
Farrell & Sears, Cold Spring Harb Perspect Med, 4 (2014)

Proteomics by mass spec: KI-MS2-008 decreases MYC



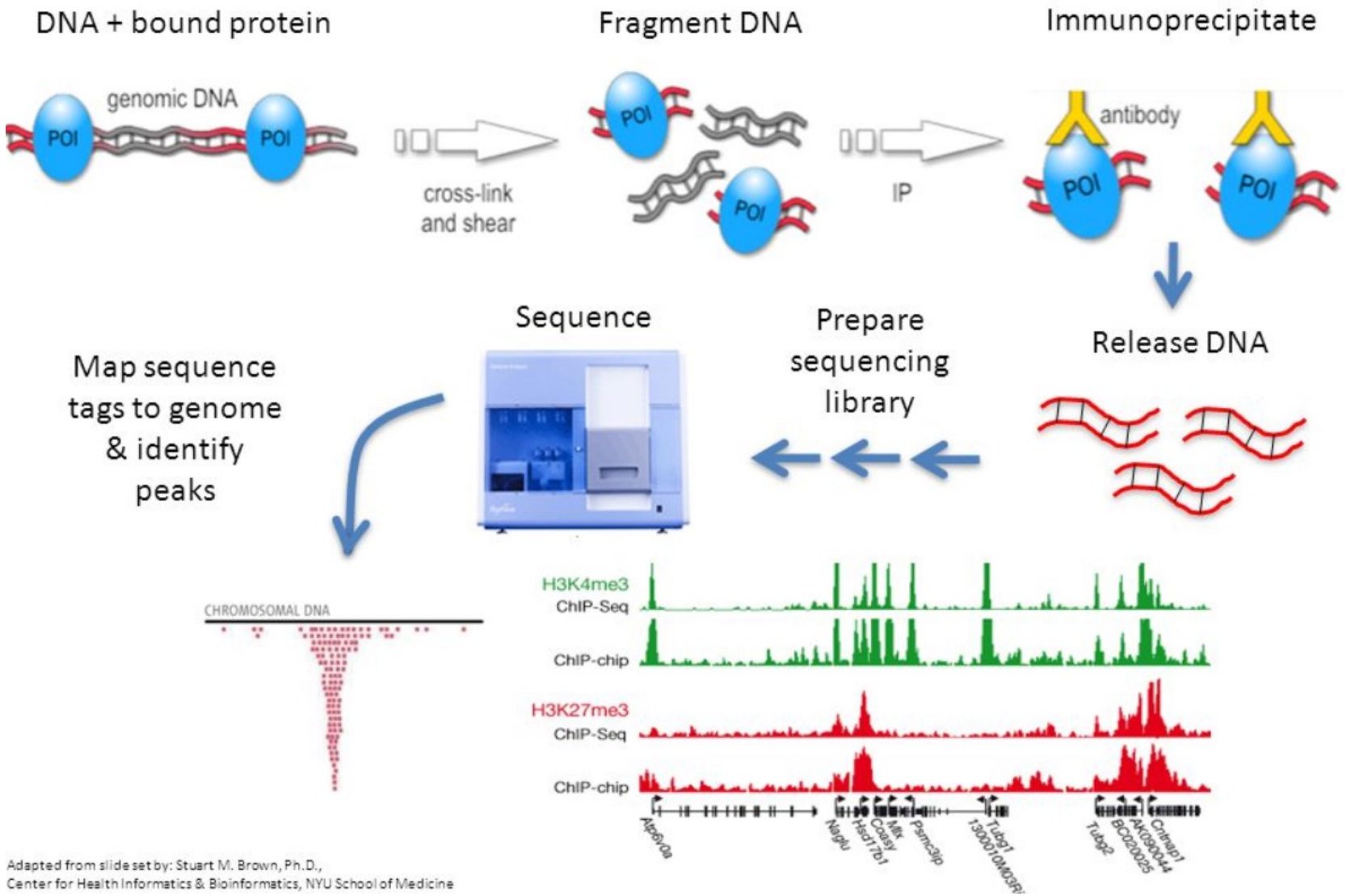
KI-MS2-008 – a mixed mechanism probe?

competition for DNA binding + destabilization of MYC



CHIP-Seq or CHromatin ImmunoPrecipitation coupled to Sequencing

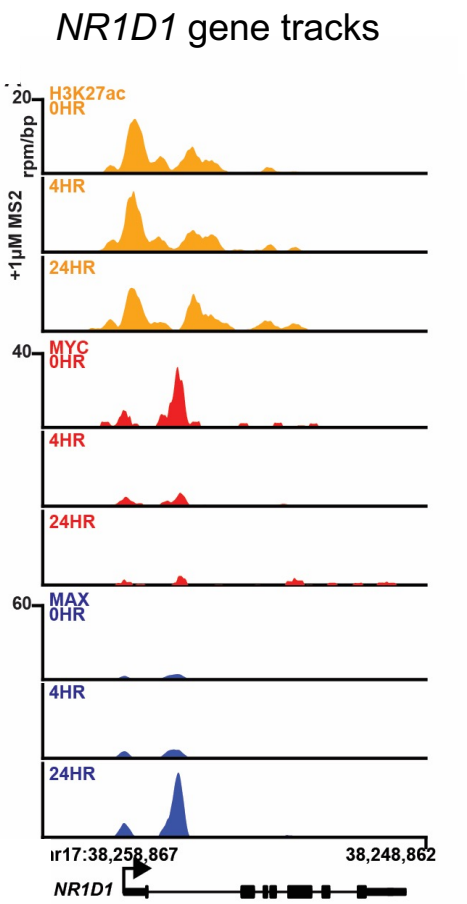
is a protein bound to a piece of DNA or not?

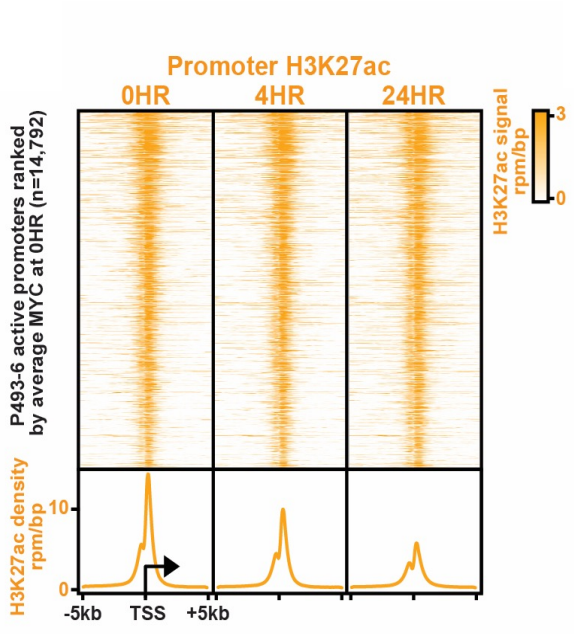


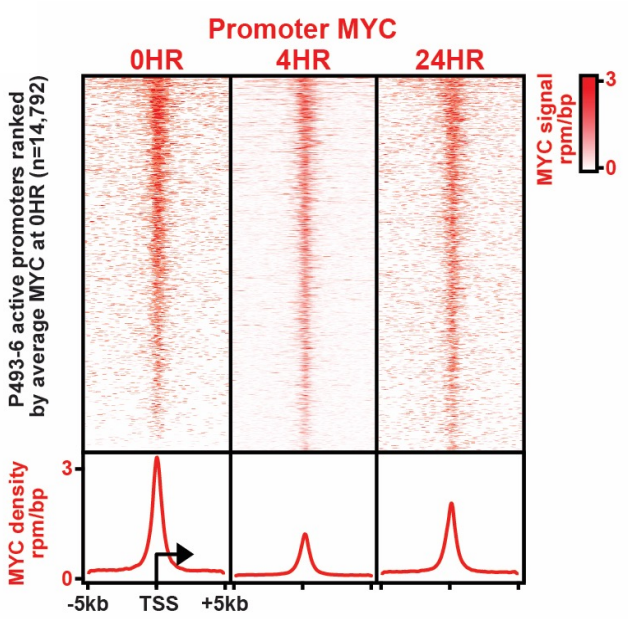
Adapted from slide set by: Stuart M. Brown, Ph.D.,
Center for Health Informatics & Bioinformatics, NYU School of Medicine

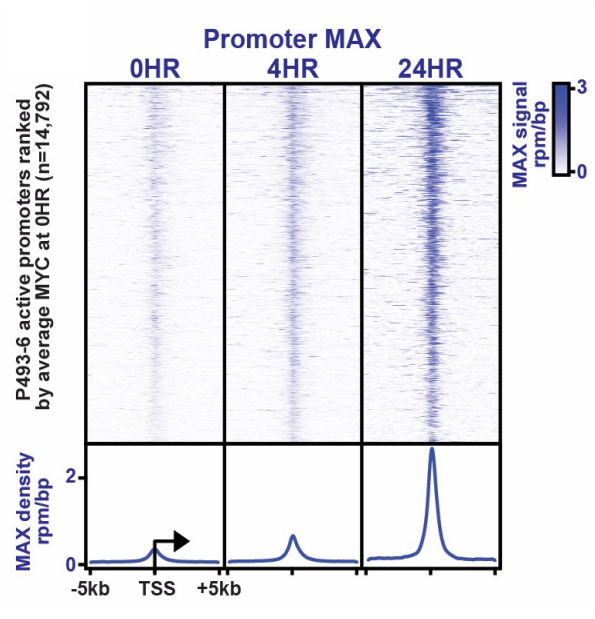
CHIP-Seq

KI-MS2-008 perturbs binding of Myc and Max at promoters of *MYC*-occupied genes









heatmaps of **H3K27ac** , **c-Myc** and **Max** levels at transcription start site regions - 0, 4, and 24 hr of 1 μM **KI-MS2-008** treatment

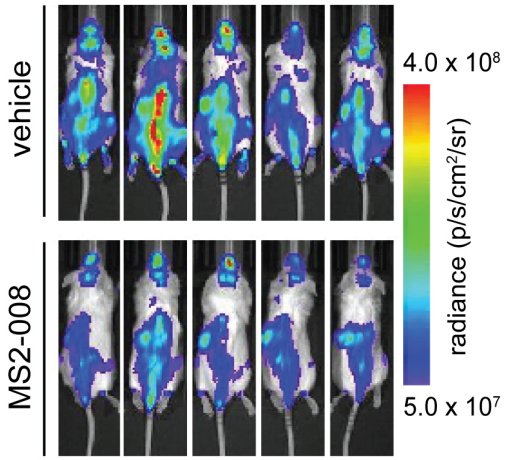
Baylor College of Medicine

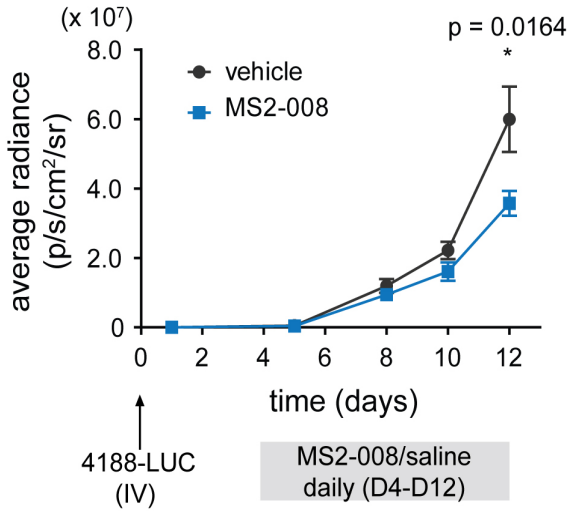
Lin Lab

Struntz et al., *Cell Chem Biol*, 26, 711-723 (2019)

In vivo studies: KI-MS2-008 modulates tumor volume in Myc-dependent mouse models of cancer

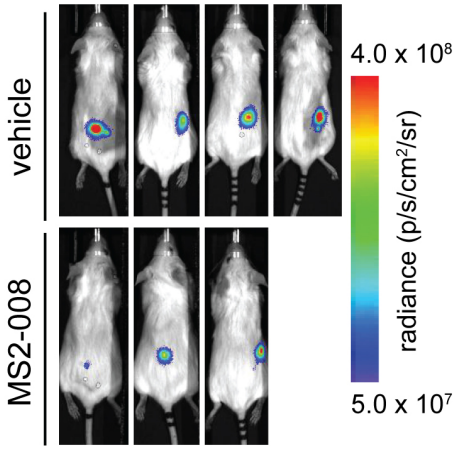
T-cell acute lymphoblastic leukemia
blood cancer

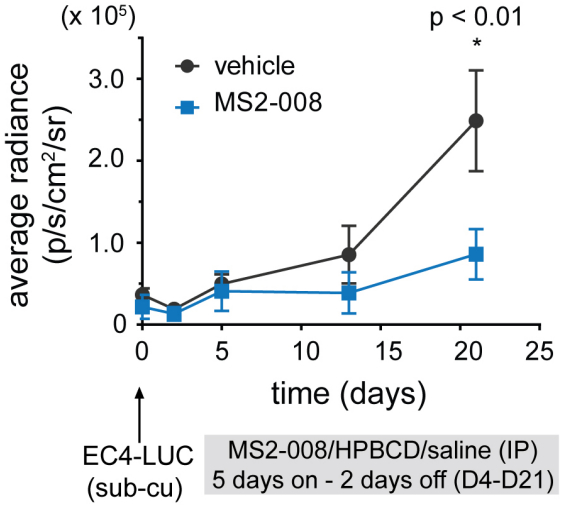




0.06 mg/kg
daily IV administration

hepatocellular carcinoma
solid tumor



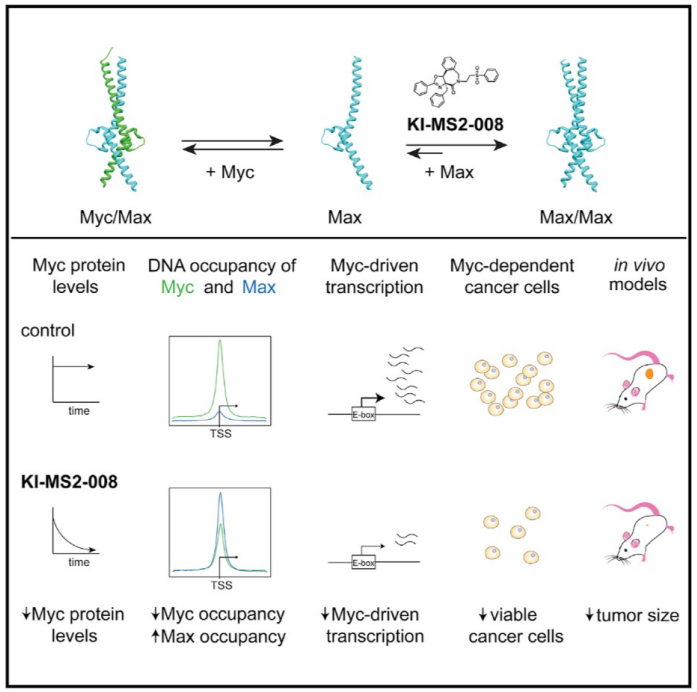


0.24 mg/kg
subcutaneous administration
5d on/2d off cycles

Cell Chemical Biology

Stabilization of the Max Homodimer with a Small Molecule Attenuates Myc-Driven Transcription

Graphical Abstract



Authors

Nicholas B. Struntz, Andrew Chen, Anja Deutzmann, ..., Charles Y. Lin, Dean W. Felsher, Angela N. Koehler

Correspondence

koehler@mit.edu

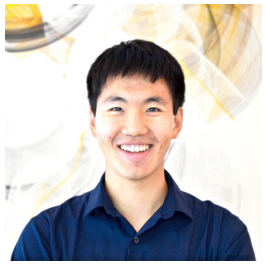
In Brief

Myc/Max-mediated transcription is deregulated in most of human cancers. Struntz et al. discovered a small molecule that stabilizes the Max homodimer and attenuates Myc-driven transcription with efficacy in cellular and murine cancer models. This discovery reinforces an alternative Myc-targeting strategy and could inform development of compounds to treat Myc-dependent cancers.

Highlights

- KI-MS2-008 is a Max-binding small molecule that attenuates Myc-driven transcription
- The compound stabilizes the Max homodimer
- Effects on DNA occupancy and the transcriptome resemble loss of Myc
- Treatment with KI-MS2-008 exhibits efficacy in cellular and murine cancer models


Francisco Caballero


Andrew Chen


Brice Curtin


Helen Evans


David Freeman


Dylan Neel


Marius Pop


Eric Stefan

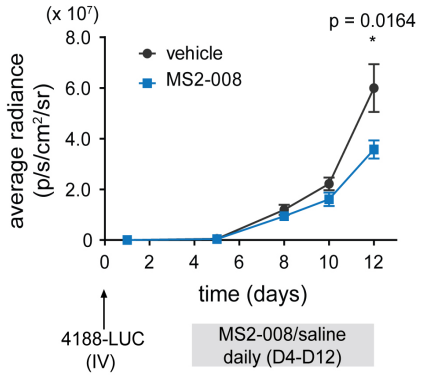

Nick Struntz


Yulong Su


Thijs Wildschut

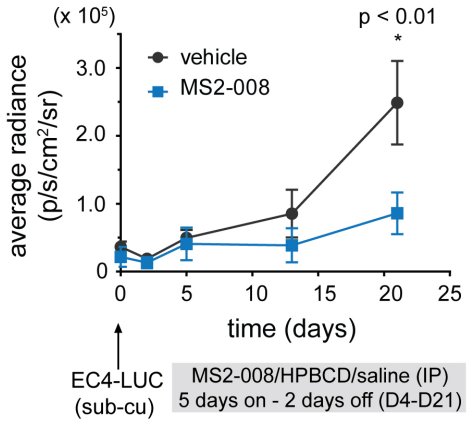

Rob Wilson

T-ALL



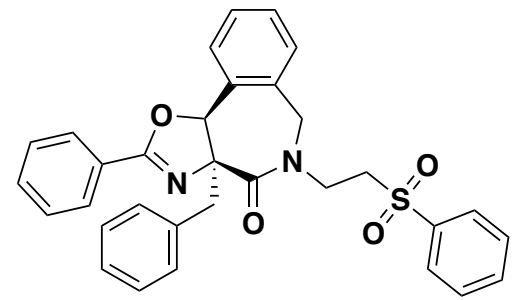
0.06 mg/kg
daily IV administration

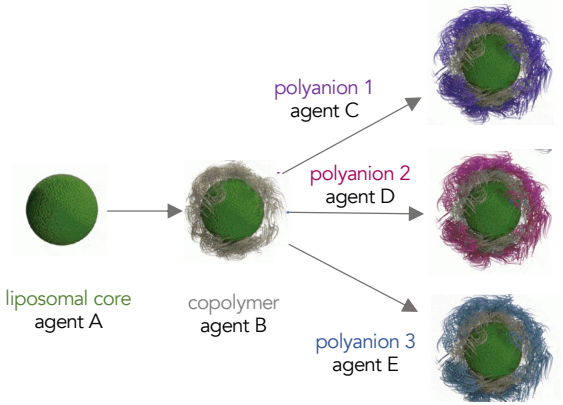
HCC



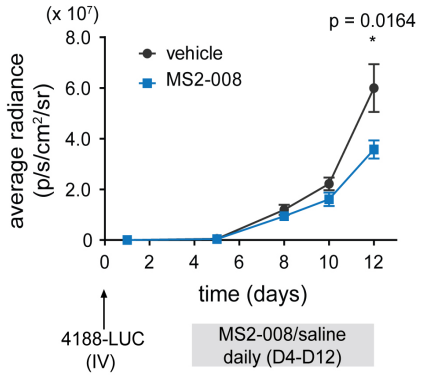
0.24 mg/kg , subcutaneous administration
5d on/2d off cycles

LEUKEMIA & LYMPHOMA SOCIETY®



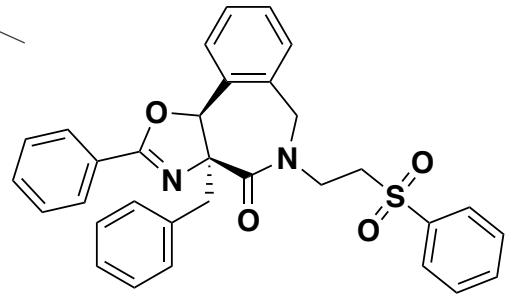


T-ALL



0.06 mg/kg
daily IV administration

LEUKEMIA & LYMPHOMA SOCIETY®



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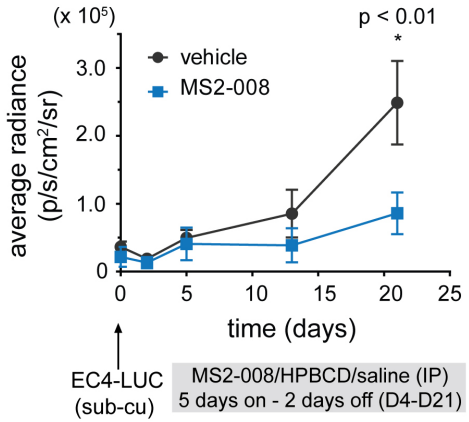
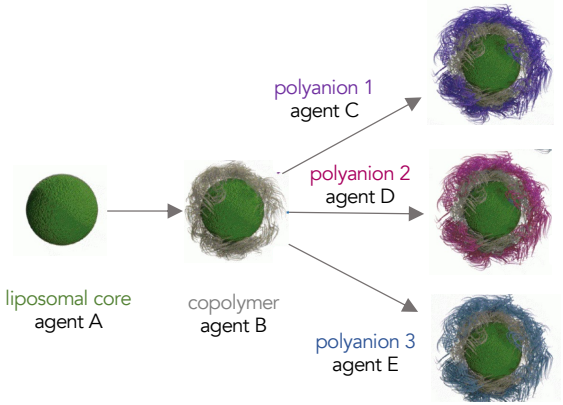


0.24 mg/kg , subcutaneous administration
5d on/2d off cycles



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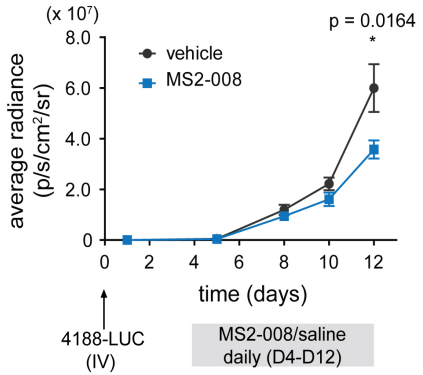

0.06 mg/kg
daily IV administration

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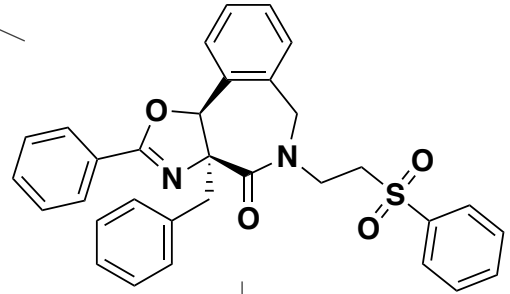

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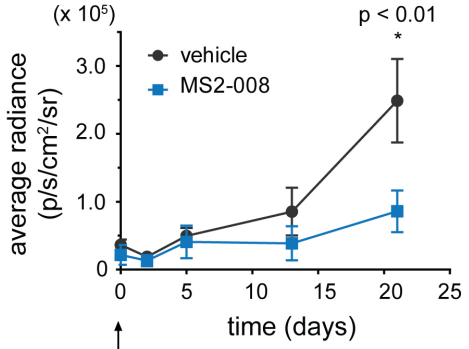
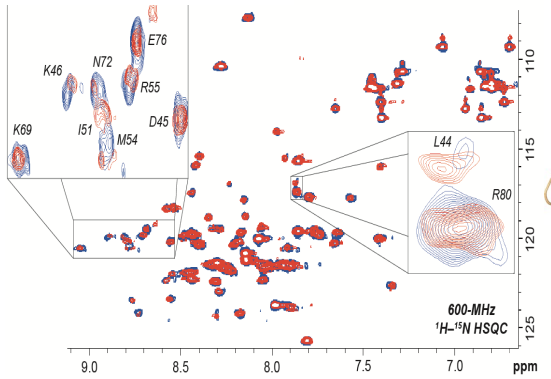
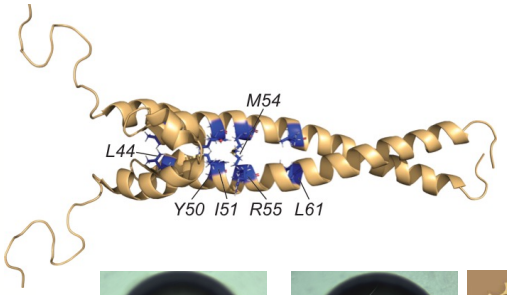
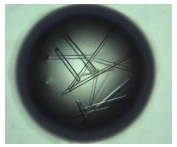


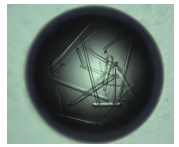
0.24 mg/kg , subcutaneous administration
5d on/2d off cycles

HSQC with ^{15}N-labeled Max

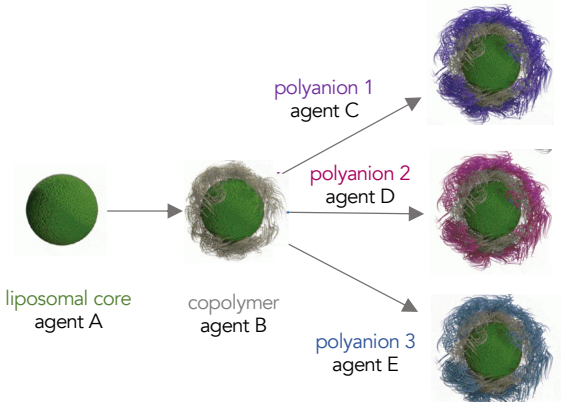


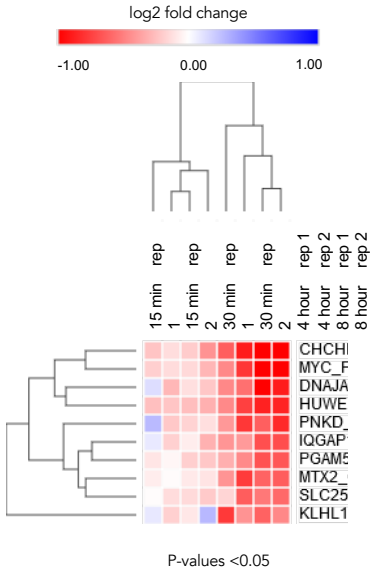


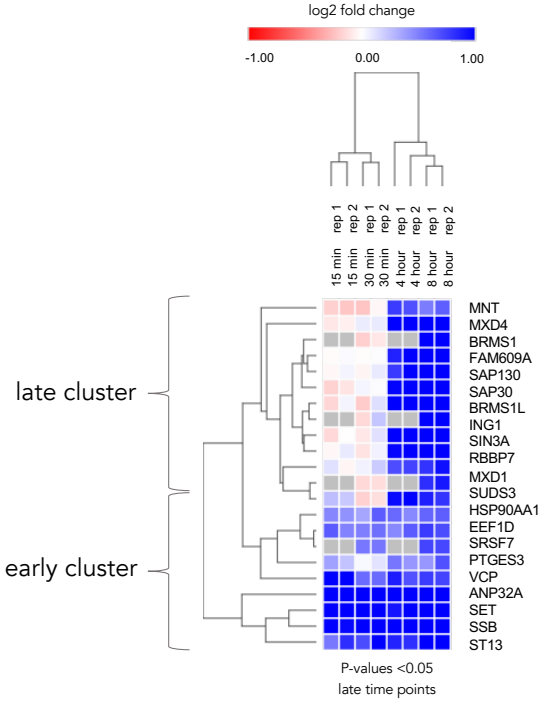




NSF CAREER Award







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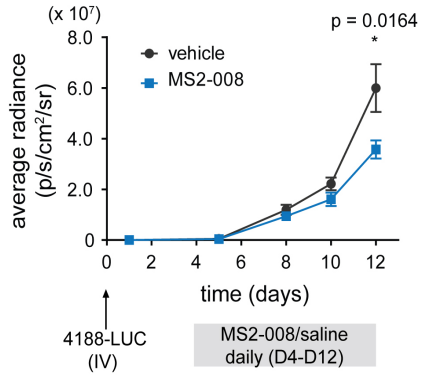

0.06 mg/kg
daily IV administration

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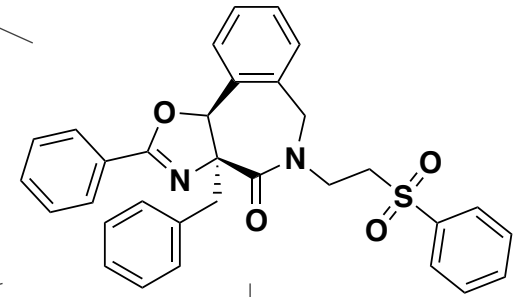

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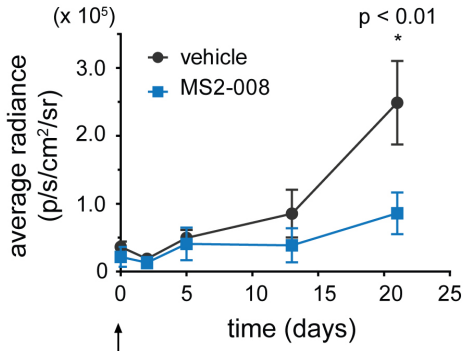
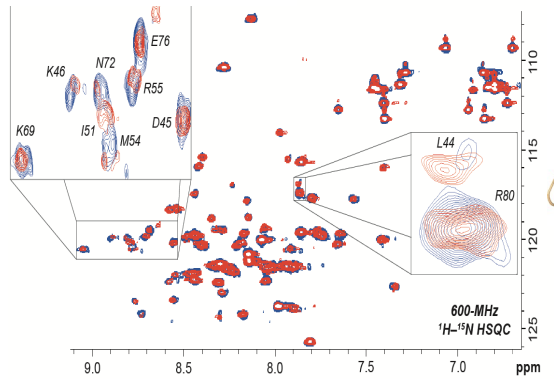
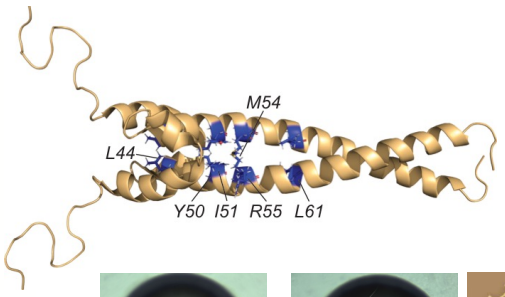
HCC

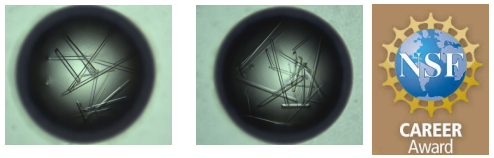


0.24 mg/kg , subcutaneous administration
5d on/2d off cycles

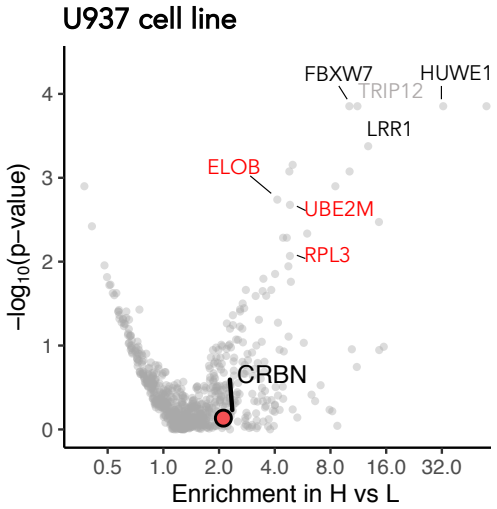
HSQC with ^{15}N-labeled Max

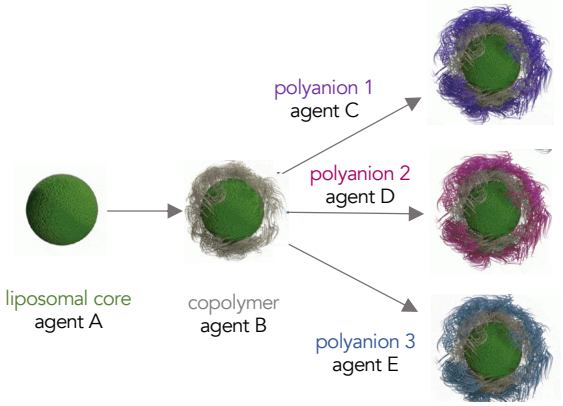


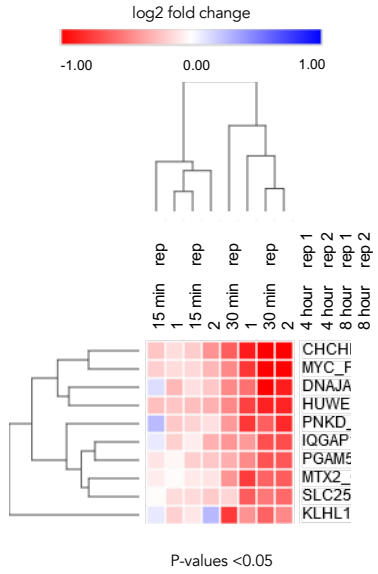


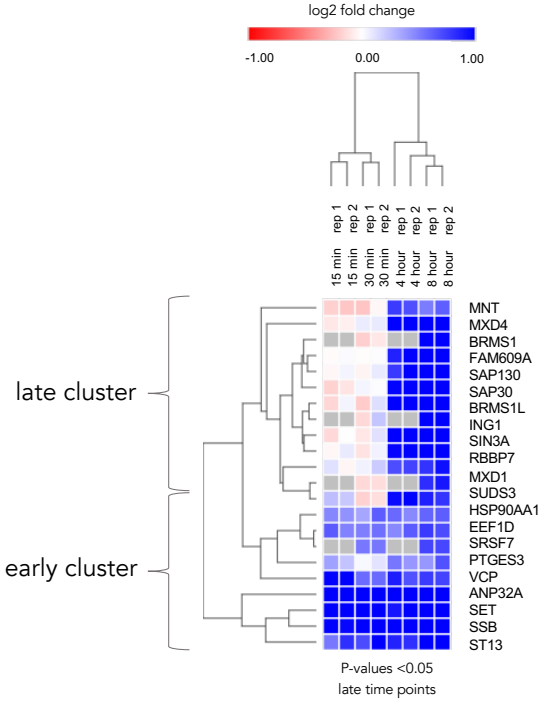


U937 cell line









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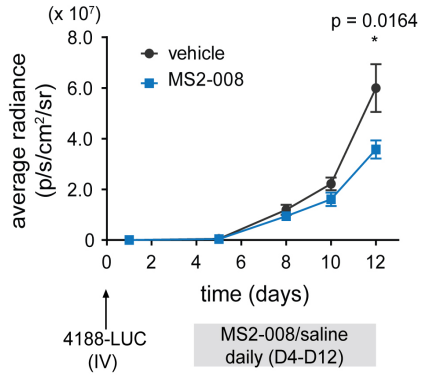

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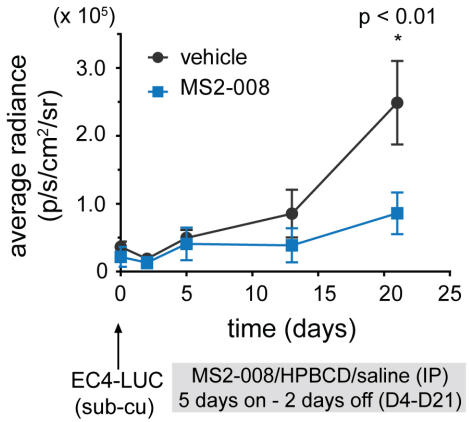

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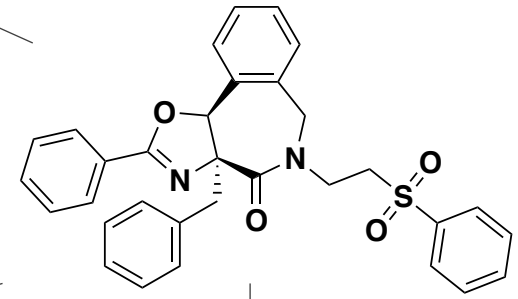
LEUKEMIA & LYMPHOMA SOCIETY®



LIMITED SOLUBILITY

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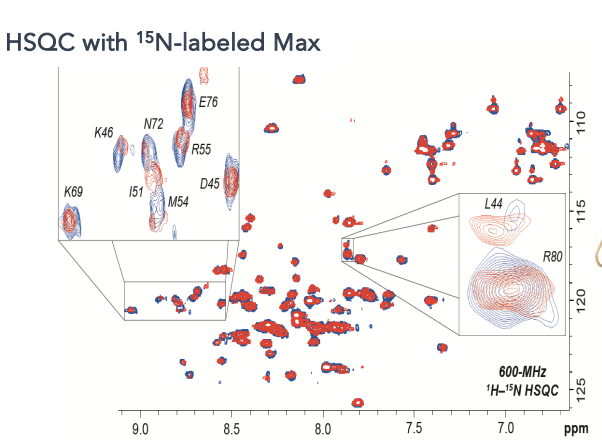
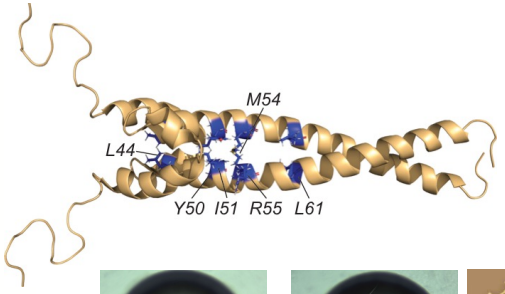
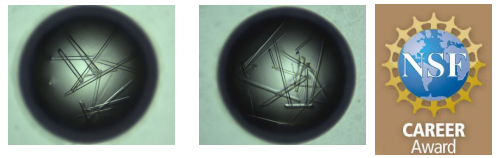
HSQC with ^{15}N-labeled Max







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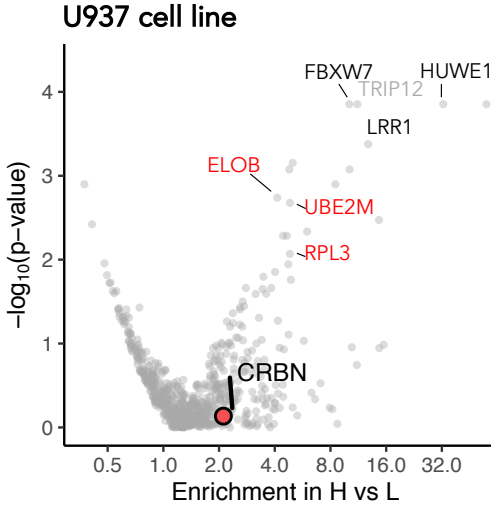

For future drug hunters – Lipinski's Rule of 5

1997 - is a rule of thumb to evaluate 'drug-likeness' or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans (Chris Lipinski, Pfizer)

MW < 500 Da
CLogP < 5
H-bond donor < 5
H-bond acceptor < 10

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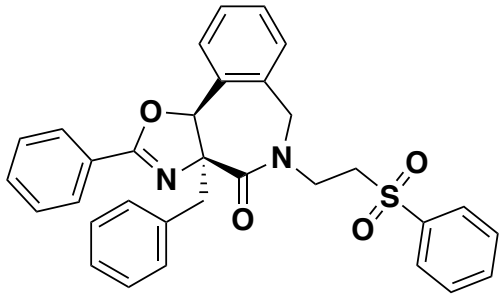
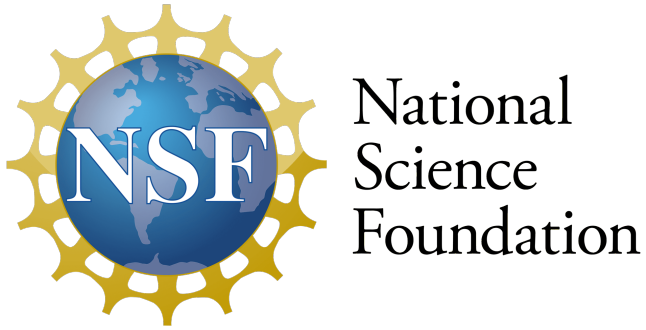


MW = 536.65
ClogP = 6.38
H-bond donor = 0
H-bond acceptor = 4

Can we screen MAX against a new chemical library and find compounds with better physicochemical properties?

Can we find MAX binders with different modes of action?



Upcoming Lectures

2/9/23	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
2/14/23	Lecture 2	Small Molecule Microarray (SMM) technique
2/16/23	Lecture 3	Our protein target – MAX
2/21/23	No Lecture	
2/23/23	Lecture 4	Quantitative evaluation of protein-ligand interactions
2/28/23	Lecture 5	An SMM ligand discovery vignette for sonic hedgehog
3/2/23	Lecture 6	KB-0742: A Phase 2 clinical candidate discovered by SMMs
3/7/23	Lecture 7	Wrap up discussion for Mod 1 experiments and report