

L6 – Engineering Transcriptional Responses with a Chemical Probe

February 27, 2020



D. melanogaster

13,600



C. elegans

19,500



Homo sapiens

21,000



Oryza sativa

45,000



Zea mays

50,000

alternative
splicing

post-translational
processing



>100,000 proteins of unknown structure or function
How do these parts give rise to organismal complexity?



D. melanogaster

13,600

450



C. elegans

19,500

750



Homo sapiens

21,000

2,600



Oryza sativa

45,000

2,000



Zea mays

50,000

760

alternative
splicing

post-translational
processing



>100,000 proteins of unknown structure or function
How do these parts give rise to organismal complexity?

transcription factors

of eukaryotic cells

1 Activator proteins bind to pieces of DNA called enhancers. Their binding causes the DNA to bend, bringing them near a gene promoter, even though they may be thousands of base pairs away.

Enhancers

Activator proteins

Other transcription factor proteins

2 Other transcription factor proteins join the activator proteins, forming a protein complex which binds to the gene promoter.

Gene

Promoter

3 This protein complex makes it easier for RNA polymerase to attach to the promoter and start transcribing a gene.

RNA polymerase

note

This diagram simplifies the DNA greatly—promoters, enhancers, and insulators can be dozens or even hundreds of base pairs long.

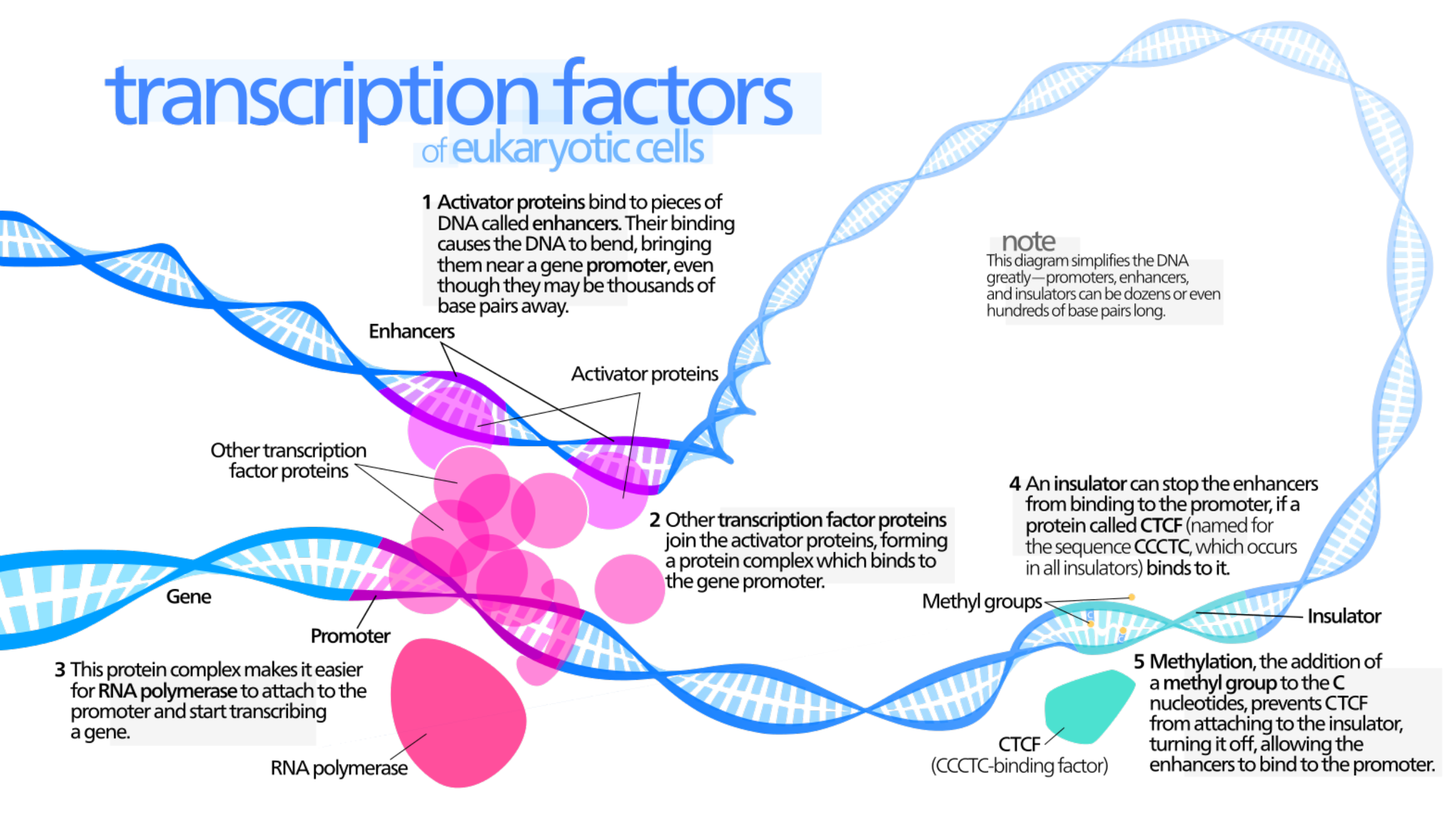
4 An insulator can stop the enhancers from binding to the promoter, if a protein called CTCF (named for the sequence CCCTC, which occurs in all insulators) binds to it.

Methyl groups

Insulator

5 Methylation, the addition of a methyl group to the C nucleotides, prevents CTCF from attaching to the insulator, turning it off, allowing the enhancers to bind to the promoter.

CTCF
(CCCTC-binding factor)



Transcriptional
protein complex 1



Individual factors



Transcriptional
protein complex 2



Transcriptional
protein complex 3



Transcriptional
protein complex 1
Transcriptional program 1



Individual factors
Transcriptional program 4

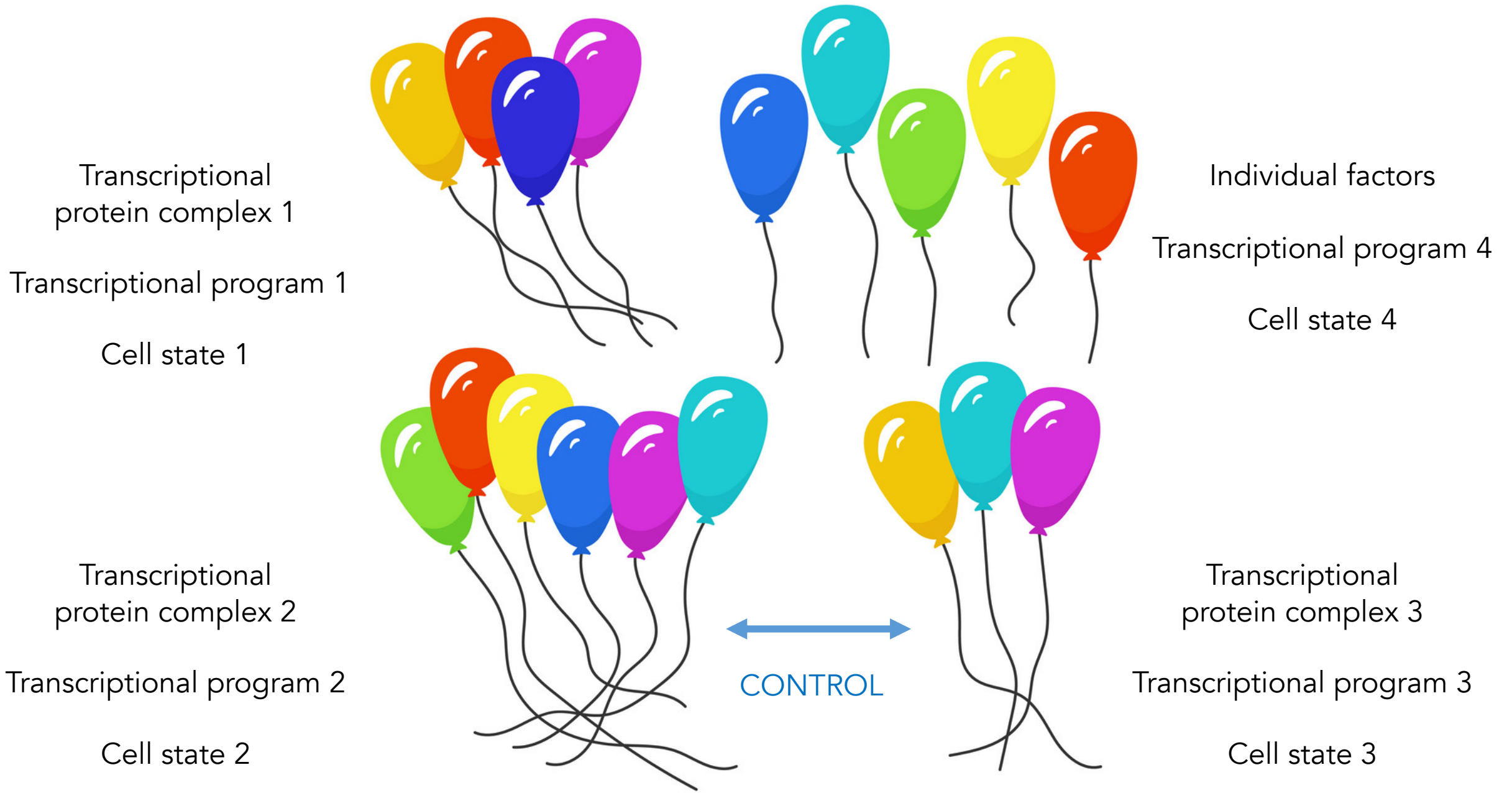


Transcriptional
protein complex 2
Transcriptional program 2



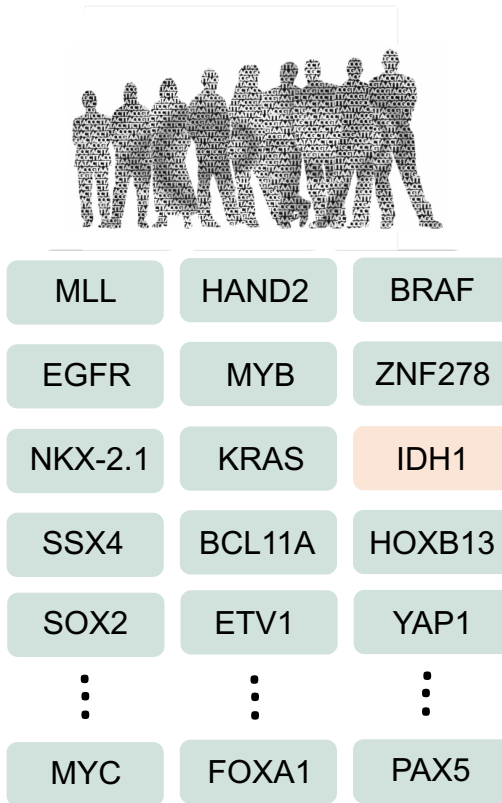
Transcriptional
protein complex 3
Transcriptional program 3



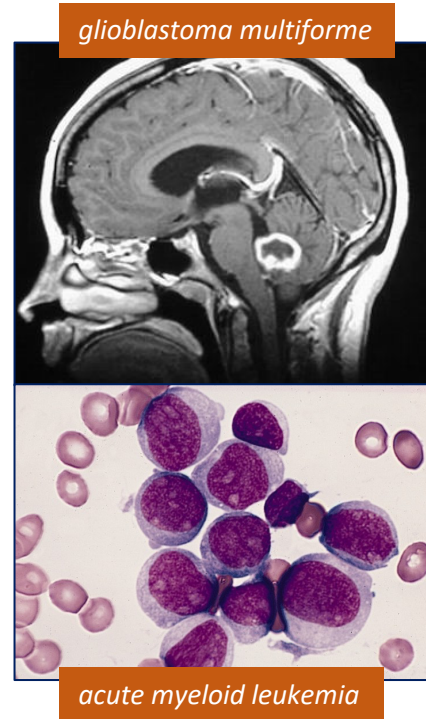


Therapeutically-driven probe discovery

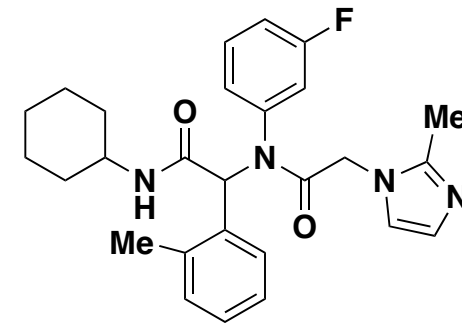
target cause of disease revealed by human genetics



patient samples
reveal list of
disease genes



test impact of disease
genes in a physiologic
settings



discover molecules
that reverse impact of
disease genes

Transcription factors

implicated in a broad spectrum of disease

<i>AVGR8</i>	central corneal thickness	<i>MEIS1</i>	restless leg syndrome
<i>BCL11A</i>	β -hemoglobin disorders	<i>MLXIPL</i>	coronary artery disease
<i>CAMTA1</i>	episodic memory deficit	<i>NFATC2</i>	T1D
<i>ELF1</i>	systemic lupus erythematosus	<i>NOTCH2</i>	T2D
<i>ETS1</i>	systemic lupus erythematosus	<i>PBX4</i>	coronary artery disease
<i>GATA3</i>	periodontitis	<i>PPARG</i>	T2D
<i>GTF2H1</i>	amyloidosis	<i>RELA</i>	rheumatoid arthritis
<i>HHEX-IDE</i>	T2D	<i>RFX4</i>	Parkinson's disease
<i>HIF2A</i>	RCC	<i>SP7</i>	BMD
<i>HNF1B</i>	T2D	<i>STAT3</i>	various AI disorders and cancers
<i>HPB1</i>	osteoarthritis	<i>STAT4</i>	systemic lupus erythematosus
<i>IRF5</i>	various AI disorders	<i>TCF4</i>	schizophrenia, corneal dystrophy
<i>IRF8</i>	MS	<i>TCF7L2</i>	T2D
<i>LBXCOR1</i>	restless leg syndrome	<i>THAP1</i>	early-onset torsion dystonia
<i>MAF</i>	early-onset obesity	<i>ZNF469</i>	central corneal thickness
<i>MECP2</i>	autism	<i>ZNF804A</i>	schizophrenia

...

Transcription factors

misregulation in cancer

amplified TF cancer genes

<i>JUN</i>	sarcoma
<i>LMO1</i>	T-ALL, neuroblastoma
<i>MITF</i>	melanoma
<i>MYC</i>	various cancers
<i>MYCL1</i>	small cell lung
<i>MYCN</i>	neuroblastoma
<i>NKX2-1</i>	follicular lymphoma
<i>REL</i>	Hodgkin lymphoma
<i>SOX2</i>	NSCLC, esophageal SCC

germline mutated TF cancer genes

<i>HNF1</i>	HCC, hepatic adenoma
<i>LMO1</i>	neuroblastoma
<i>PHOX2B</i>	neuroblastoma
<i>RB1</i>	various cancers
<i>SMAD4</i>	gastrointestinal polyps
<i>SMARCB1</i>	malignant rhabdoid
<i>SUFU</i>	medulloblastoma
<i>TP53</i>	various cancers
<i>WT1</i>	Wilms tumor

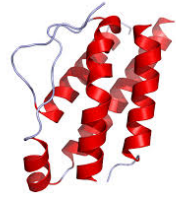
TF cancer genes with frameshift mutations

<i>ARID1A</i>	clear cell ovarian carcinoma, RCC
<i>ASXL1</i>	MDS, CMML
<i>ATRX</i>	pancreatic neuroendocrine
<i>CEBPA</i>	AML, MDS
<i>CREBBP</i>	ALL, AML, DLBCL, B-NHL
<i>DAXX</i>	pancreatic neuroendocrine
<i>EP300</i>	various cancers
<i>GATA1</i>	megakaryoblastic leukemia
<i>GATA3</i>	breast
<i>HNF1</i>	HCC, hepatic adenoma
<i>HRPT2</i>	parathyroid adenoma
<i>NOTCH2</i>	marginal zone lymphoma, DLBCL
<i>PBRM1</i>	breast, clear cell renal carcinoma
<i>PHOX2B</i>	neuroblastoma
<i>PRDM1</i>	DLBCL
<i>RB1</i>	various cancers
<i>SMAD4</i>	gastrointestinal polyps
<i>SMARCA4</i>	NSCLC
<i>SMARCB1</i>	malignant rhabdoid
<i>SUFU</i>	medulloblastoma
<i>TP53</i>	various cancers
<i>WT1</i>	Wilms tumor

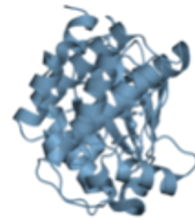
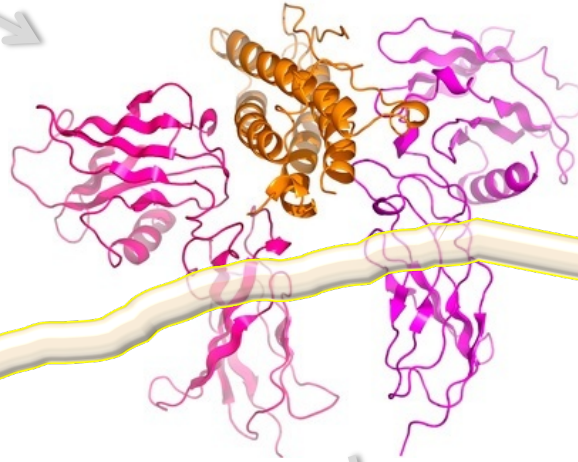
somatically mutated TF cancer genes

AFF4	ALL	IRF4	MM	POU2AF1	NHL
ARNT	AML	JAZF1	endometrial stromal tumors	POU5F1	sarcoma
ATF1	melanoma, AFH	JUN	sarcoma	PPARG	follicular thyroid
BTG1	BCLL	KLF6	prostate, glioma	PRDM1	DLBCL
CBFB	AML	LAF4	ALL	PRDM16	MDS, AML
CDX2	AML	LMO1	T-ALL, neuroblastoma	RARA	APL
CEBPA	AML, MDS	LMO2	T-ALL	RB1	various cancers
CIC	soft tissue sarcoma	LPP	lipoma, leukemia	REL	Hodgkin lymphoma
CIITA	PMBL, Hodgkin lymphoma	LYL1	T-ALL	RUNX1	AML, pre B-ALL
CREB1	clear cell sarcoma	MAFB	MM	RUNXBP2	AML
CREBBP	ALL, AML, DLBCL, B-NHL	MAML2	salivary gland	SMAD4	colorectal, pancreatic
CRTC3	salivary gland mucoepidermoid	MDS1	MDS, AML	SMARCA4	NSCLC
DUX4	soft tissue sarcoma	MDS2	MDS	SMARCB1	malignant rhabdoid
EBF1	lipoma	MECT1	salivary gland	SOX2	NSCLC, esophageal SCC
ELF4	AML	MHC2TA	head-neck squamous cell, renal	SS18	synovial sarcoma
ELK4	prostate	MITF	melanoma	SS18L1	synovial sarcoma
ELKS	papillary thyroid	MKL1	AML	SSX1	synovial sarcoma
EP300	various cancers	MLF1	AML	SSX2	synovial sarcoma
ERG	AML, Ewing sarcoma, prostate	MLL1	ALL	SSX4	synovial sarcoma
ETV1	Ewing sarcoma, prostate	MLL2	ALL, colorectal	SUFU	synovial sarcoma
ETV4	Ewing sarcoma, prostate	MLL3	ALL, breast cancers	SUZ2	medullablastoma
ETV5	prostate	MLL4	AML	TAF15	endometrial stromal tumors
ETV6	various cancers	MLL6	AML	TAL1	ALL, EMC
EV11	AML, CML	MLL7	ALL	TAL2	lymphoblastic leukemia
EWSR1	Ewing sarcoma, ALL	MYB	ALL	TCEA1	T-ALL
FEV	Ewing sarcoma	MYC	adenoid cystic sarcoma	TCF12	salivary adenoma
FLI1	Ewing sarcoma	MYCL1	various cancers	TCF3	EMC
FOXL2	ovarian	MYCN	small cell lung	TFE3	pre B-ALL
FOXO1A	alveolar rhabdomyosarcomas	NCOA1	neuroblastoma	TFEB	renal, alveolar soft sarcoma
FOXO3A	AL	NCOA2	alveolar rhabdomyosarcoma	TFPT	renal (child epithelioid)
FOXP1	ALL	NCOA4	AML	THRAP3	pre B-ALL
GATA1	megakaryoblastic leukemia	NFIB	papillary thyroid	TIF1	aneurysmal bone cysts
GATA2	AML	NFKB2	lipoma, ACC	TLX1	APL
GATA3	breast	NKX2-1	B-NHL	TLX3	T-ALL
HLF	ALL	NOTCH1	NSCLC	TP53	T-ALL
HLXB9	AML	NOTCH2	T-ALL	TRIM27	various cancers
HMGA1	various cancers	NR4F3	DLBCL, marginal zone lymphoma	TRIM33	papillary thyroid
HMGA2	various cancers	NRF2	EMC	TSHR	papillary thyroid
HOXA11	CML	OLIG2	NSCLC, HNSCC	WT1	toxic thyroid adenoma
HOXA13	AML	PAX3	T-ALL	ZNF145	Wilm tumor
HOXA9	AML	PAX5	DLBCL, marginal zone lymphoma	ZNF198	APL
HOXC11	AML	PAX7	EMC	ZNF278	MPD, NHL
HOXC13	AML	PAX8	NSCLC, HNSCC	ZNF331	Ewing sarcoma
HOXD11	AML	PBX1	T-ALL	ZNF384	follicular thyroid adenoma
HOXD13	AML	PHOX2B	alveolar rhabdomyosarcoma	ZNF521	ALL
HNF1	HCC	PLAG1	NHL	ZNF9	ALL
HRPT2	parathyroid adenoma	PMX1	alveolar rhabdomyosarcoma	ZNFN1A1	aneurysmal bone cysts
IKZF1	ALL		follicular thyroid		ALL, DLBCL
			pre B-ALL		
			neuroblastoma		
			salivary adenoma		
			AML1		

extracellular factors



membrane receptors



intracellular signaling proteins

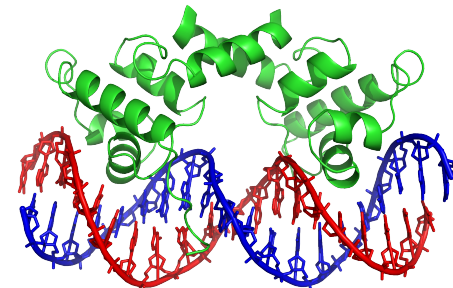


cellular response



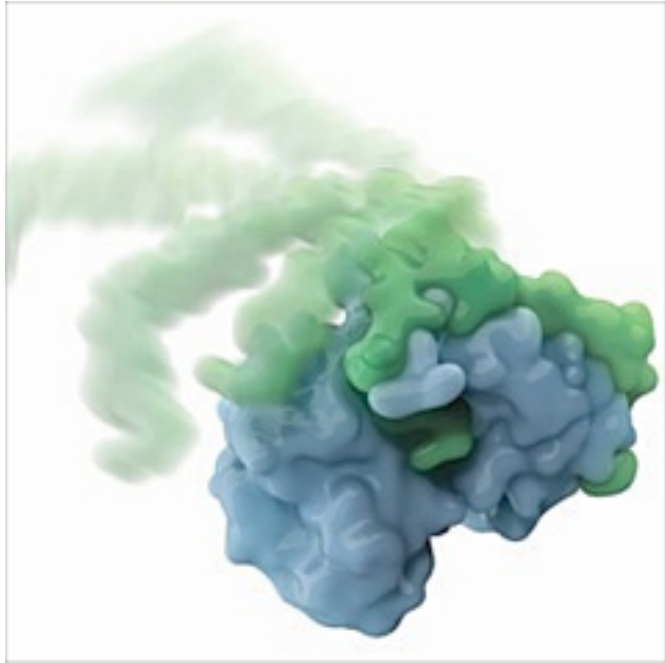
transcriptional regulators

more cogent target?



A complex task?

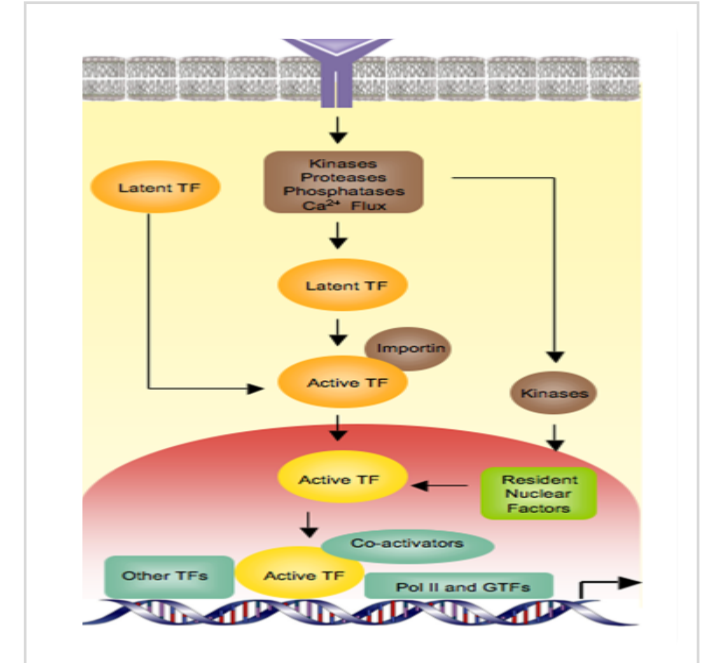
transcription factors are the prototype of an 'undruggable' target



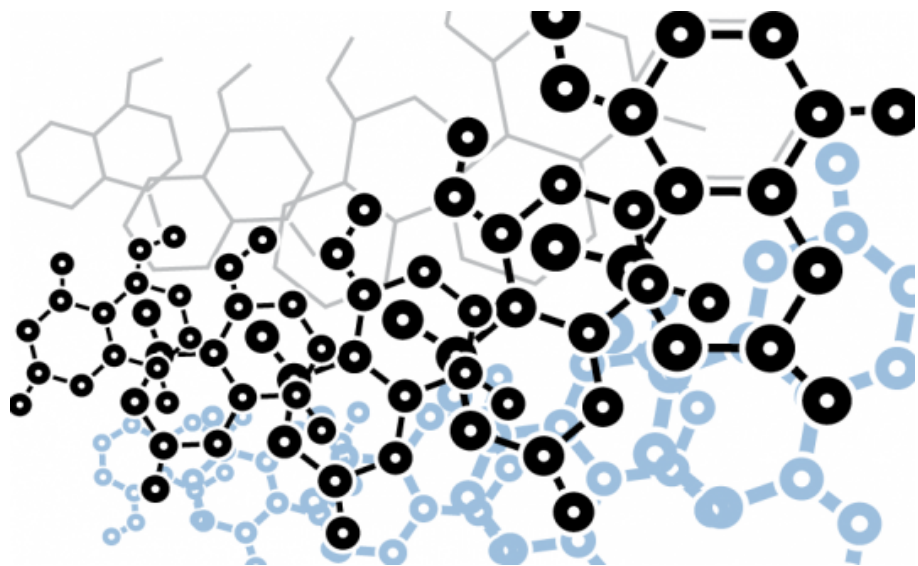
disordered when isolated
from binding partners



DNA-binding domains
lack obvious pockets



transit to reach resident
nuclear factors

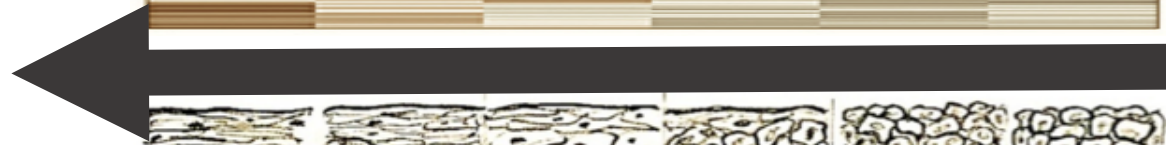


Can we build general and systematic platforms for developing **chemical probes** for transcriptional regulators?

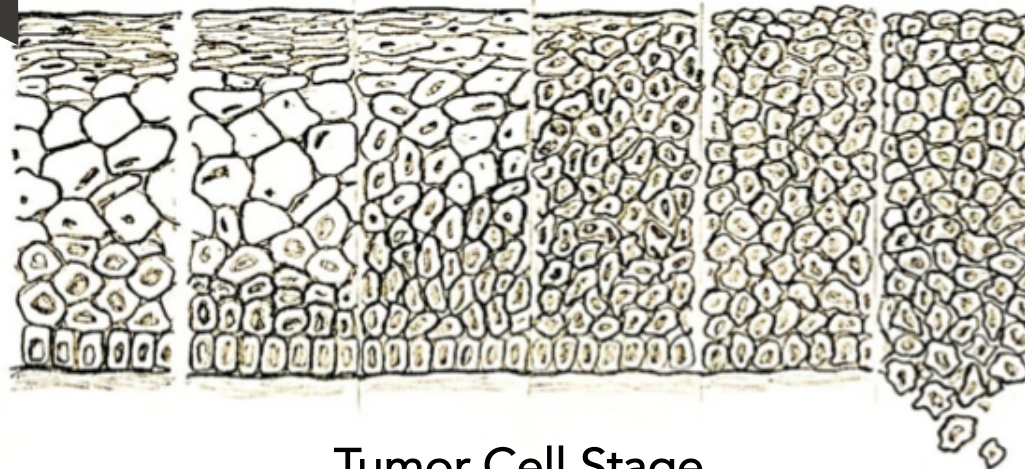
Transcription Profile



Pre-Cancerous



Metastatic Tumors



Tumor Cell Stage

Can we tune dysregulated gene expression programs
and impact cell state?

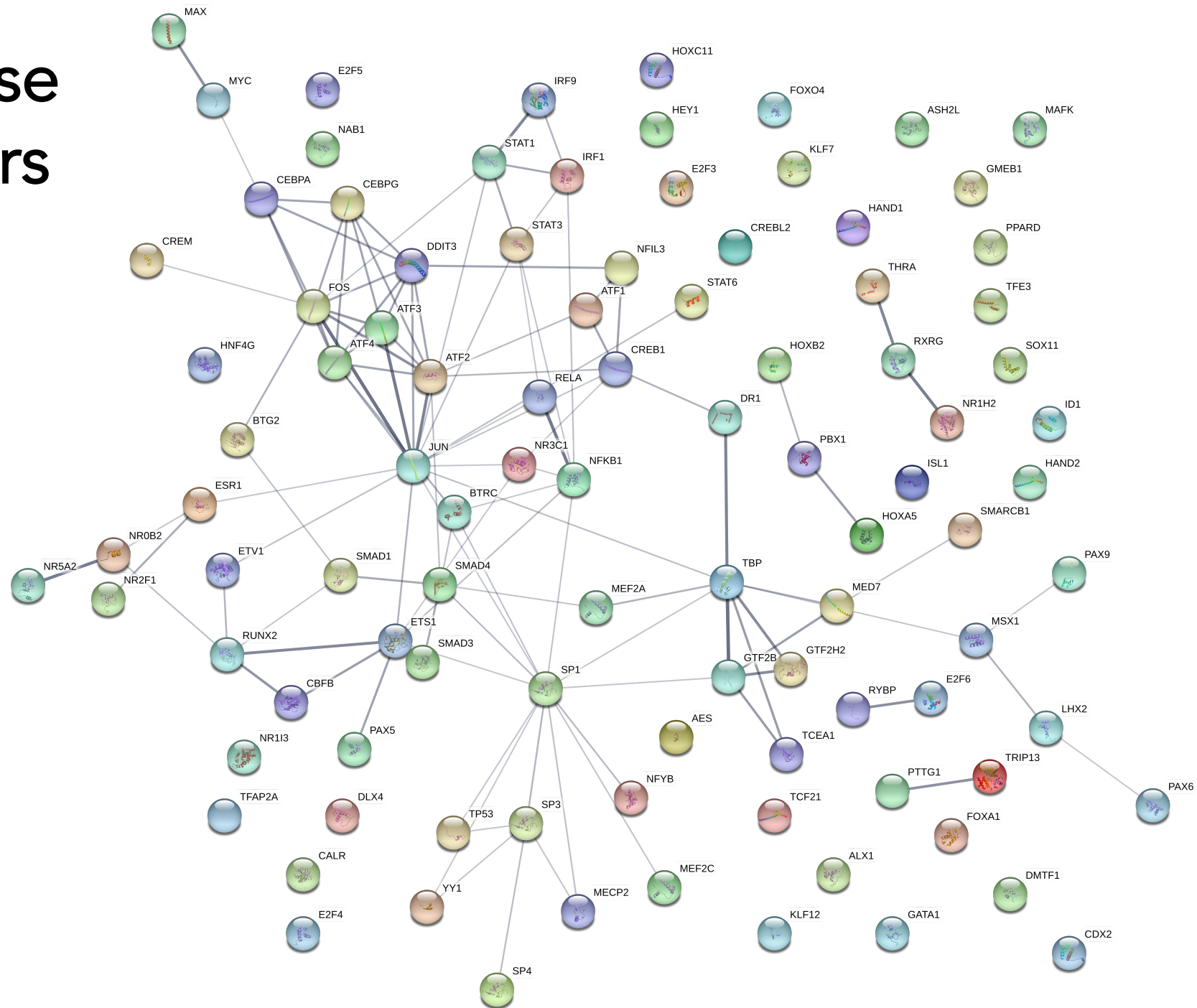
Profiling 100 diverse transcription factors

commercially available
purified, His-tagged

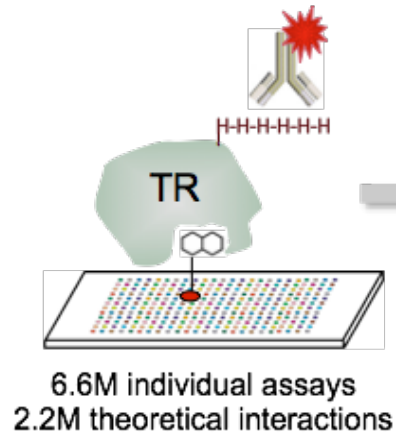
145 InterPro domains
e.g. bZip, Znf_C2H2, Fbox, Ets, etc.

>500 GO terms
e.g. nuclear, chromatin remodeling,
basal transcription, etc.

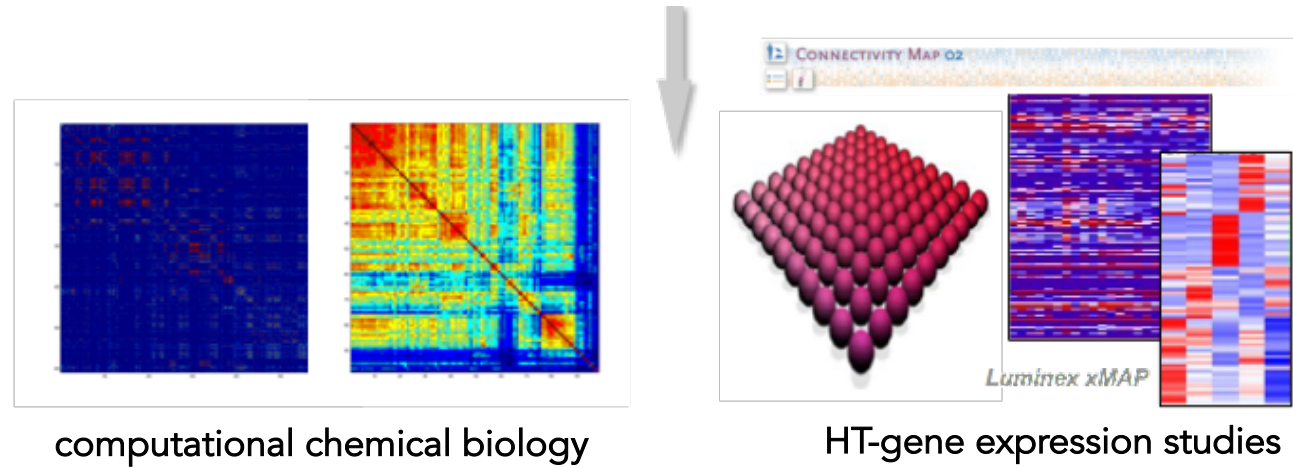
>100 KEGG pathways
e.g. Wnt signaling, chronic myeloid
leukemia, circadian entrainment, etc.



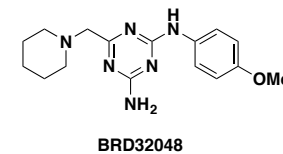
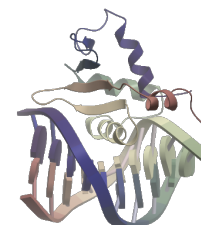
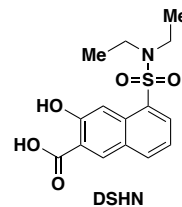
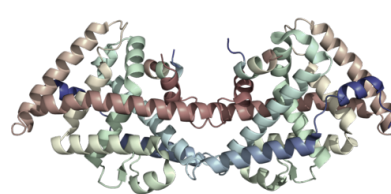
'100 Transcription Factor' SMM Screen



Clemons et al., PNAS 107, 18787-18792, 2010
Clemons et al., PNAS 108, 6817-6822, 2011



Chemical stabilizer of Small Heterodimer Partner
Yang et al., Mol Cancer Ther, 15, 2294-2301, 2016



Chemical inhibitor of ETV1
Pop et al., Mol Cancer Ther 13, 1492-1502, 2014

characterize and optimize probes for individual TFs

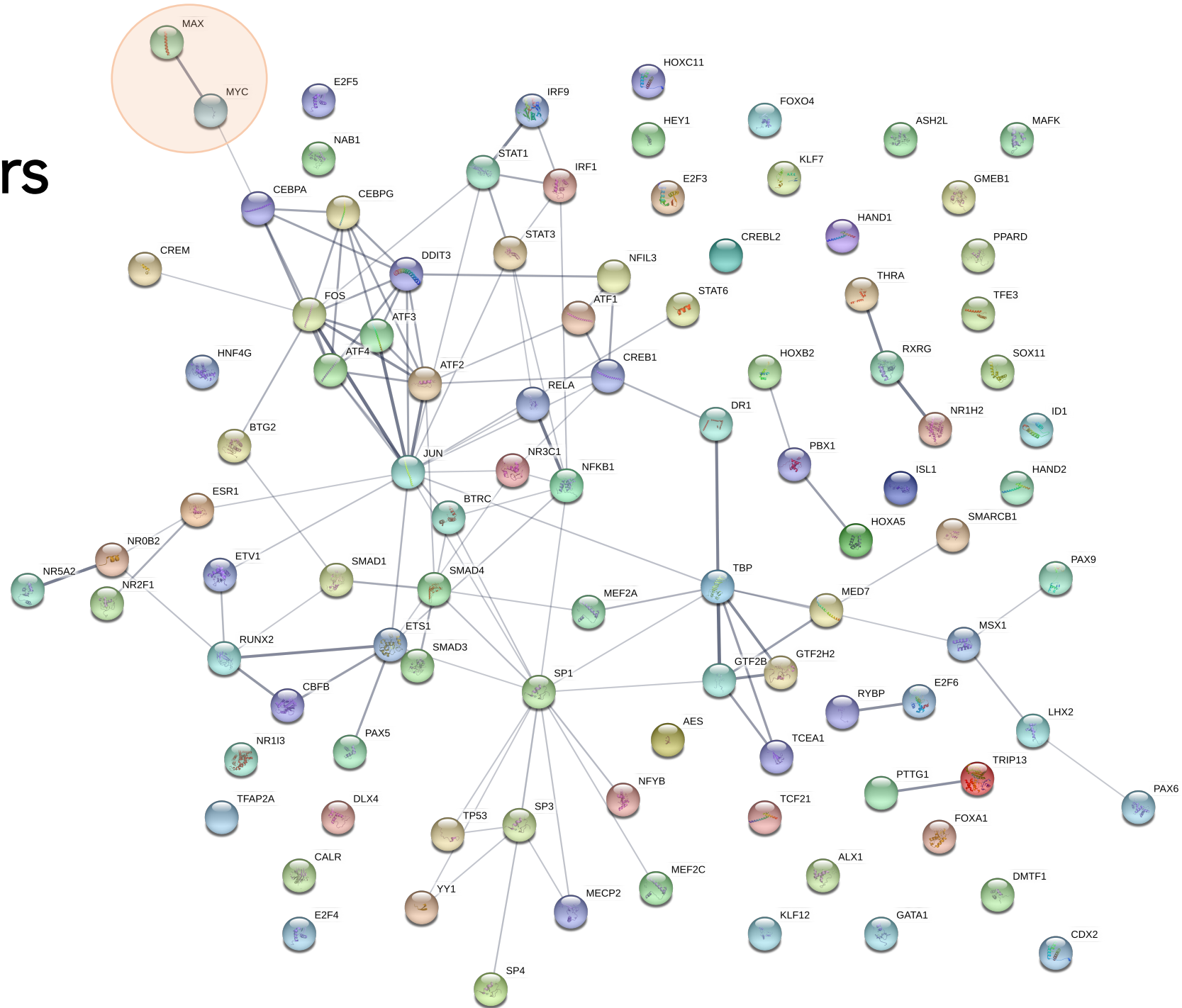
100 diverse transcription factors

commercially available
purified, His-tagged

145 InterPro domains
e.g. bZip, Znf_C2H2, Fbox, Ets, etc.

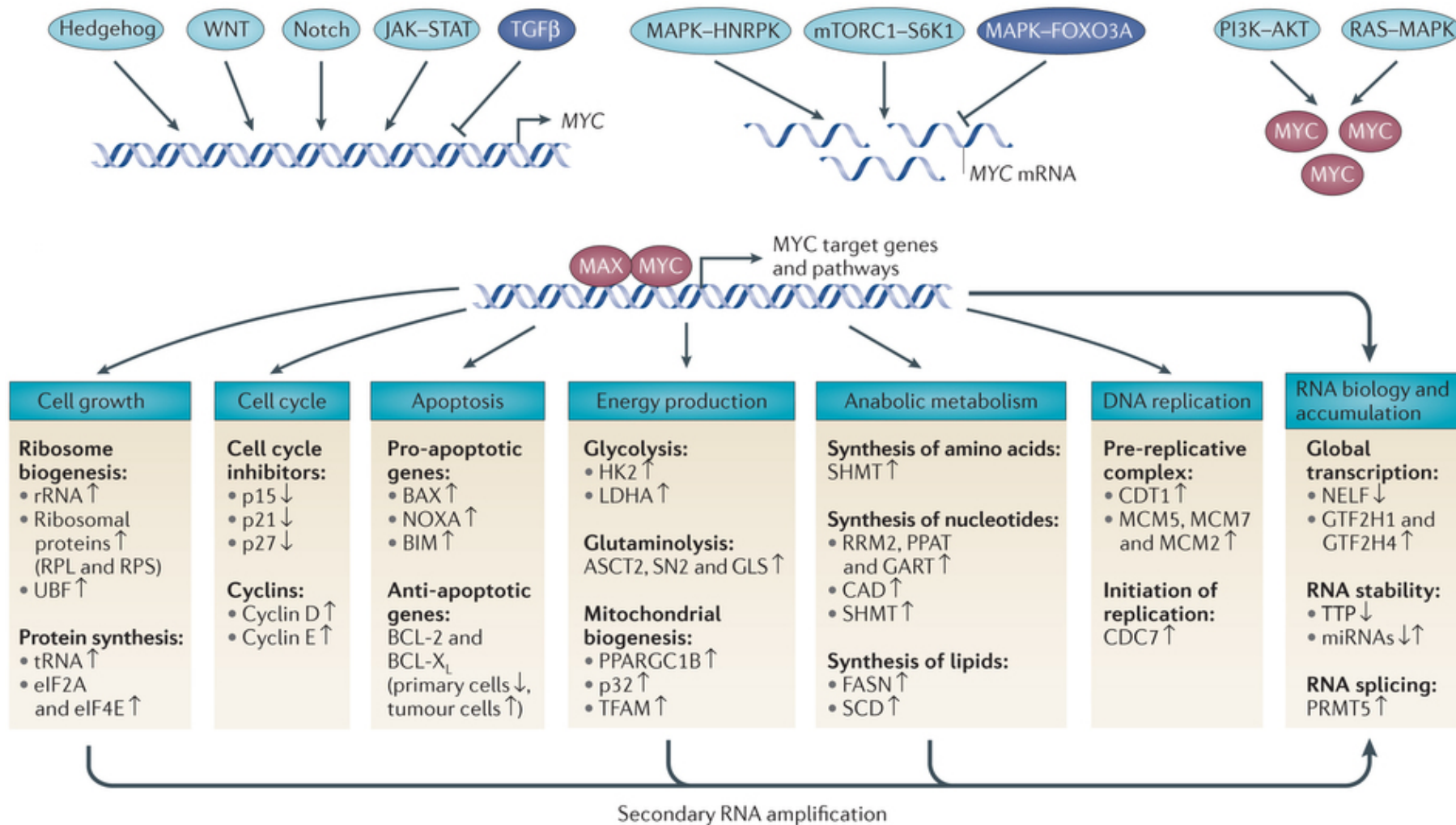
>500 GO terms
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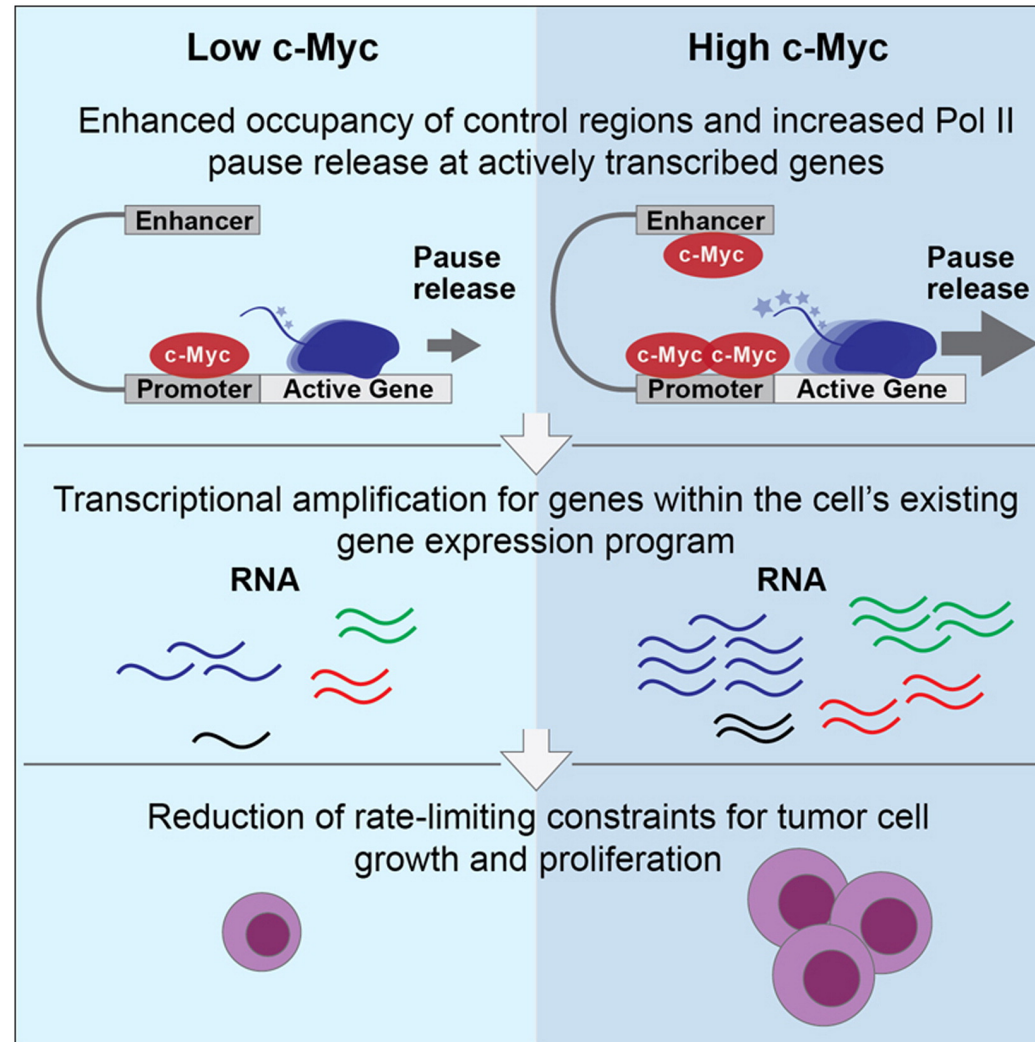
MYC family of transcription factors

master regulators of broad cellular processes



c-Myc

accumulates in promoter regions and amplifies transcription when overexpressed in cancer



MYC expression in haploinsufficient mice

amelioration of age-associated phenotypes

Article

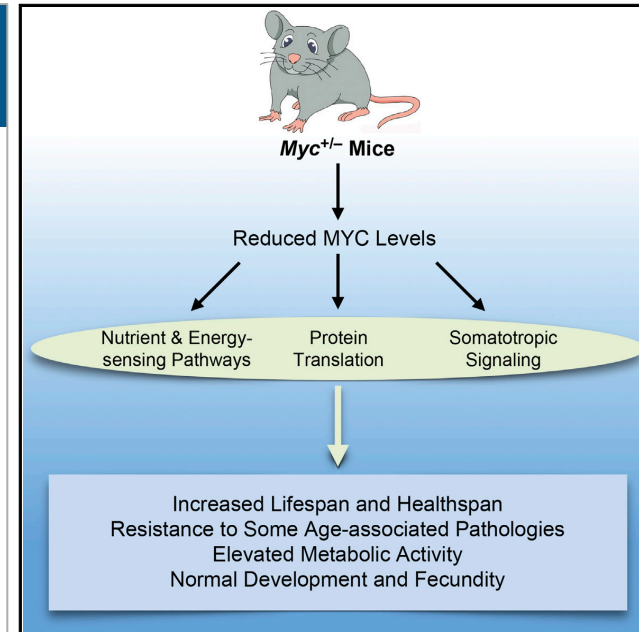
Hofmann et al., Cell, 160, 477-488 (2015)

Cell

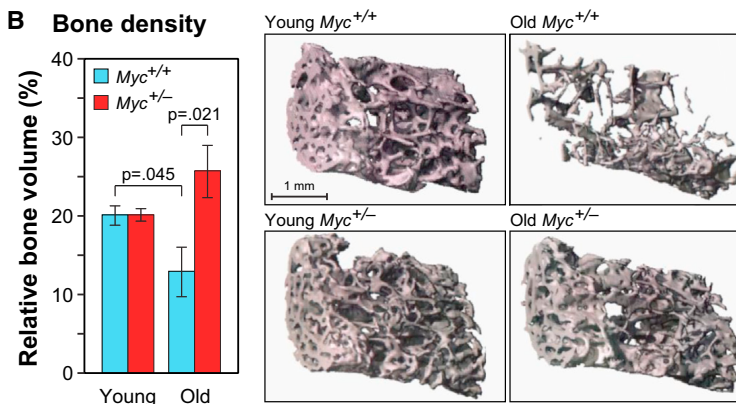
Reduced Expression of MYC Increases Longevity and Enhances Healthspan

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 Xiayi 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,*}

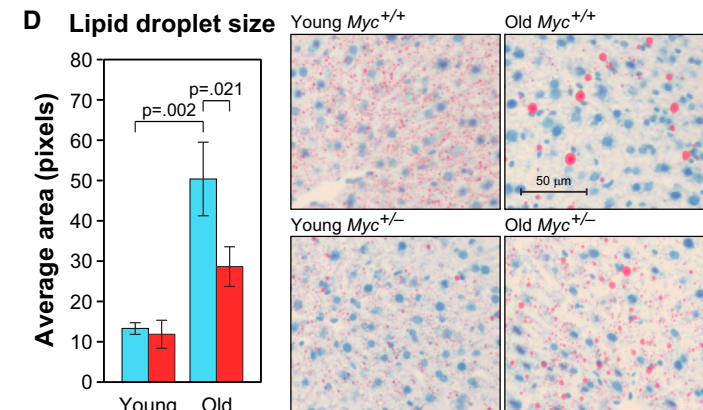
¹Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI 02912, USA
²Department of Cellular and Structural Biology, Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA
³Translational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



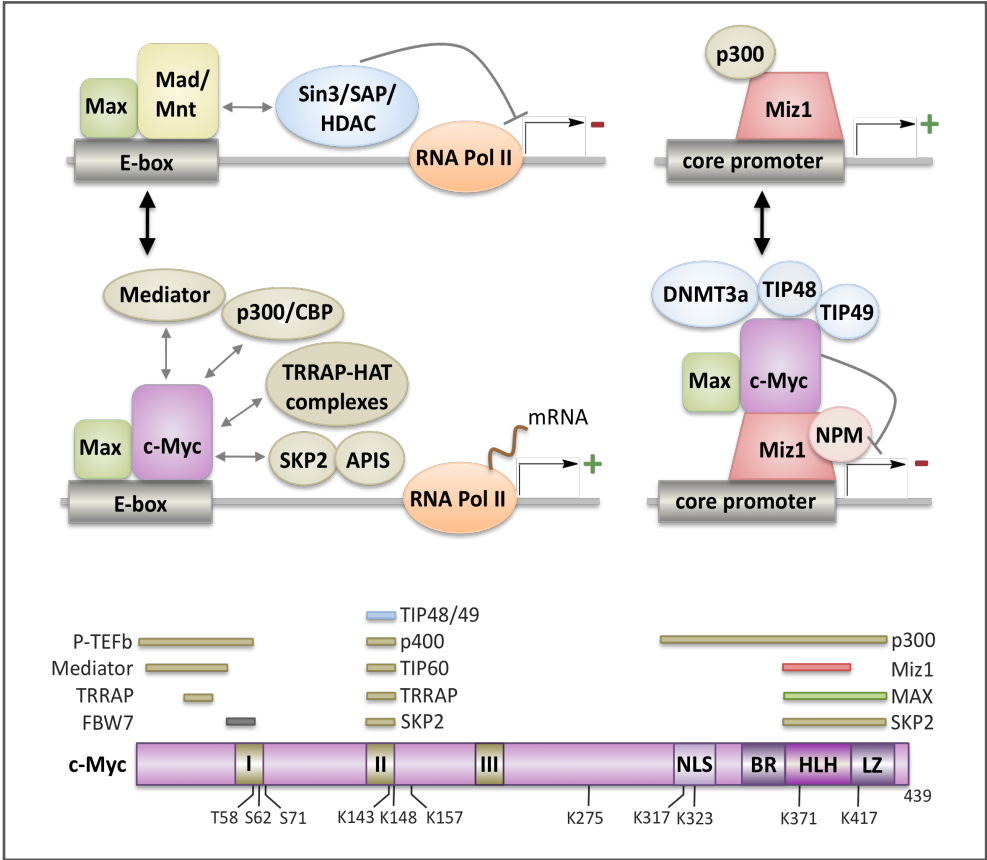
B Bone density



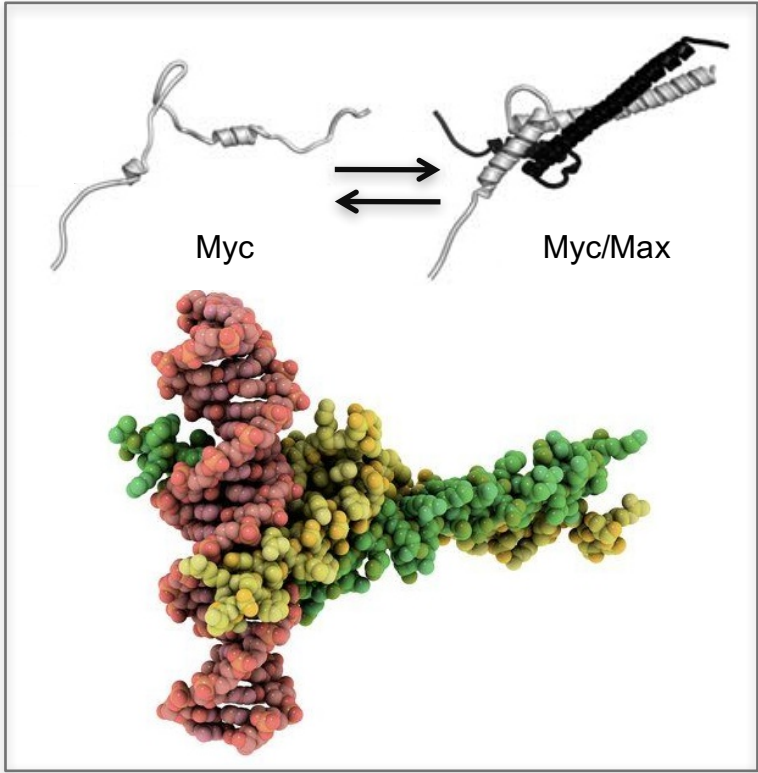
D Lipid droplet size



An obstinate therapeutic target

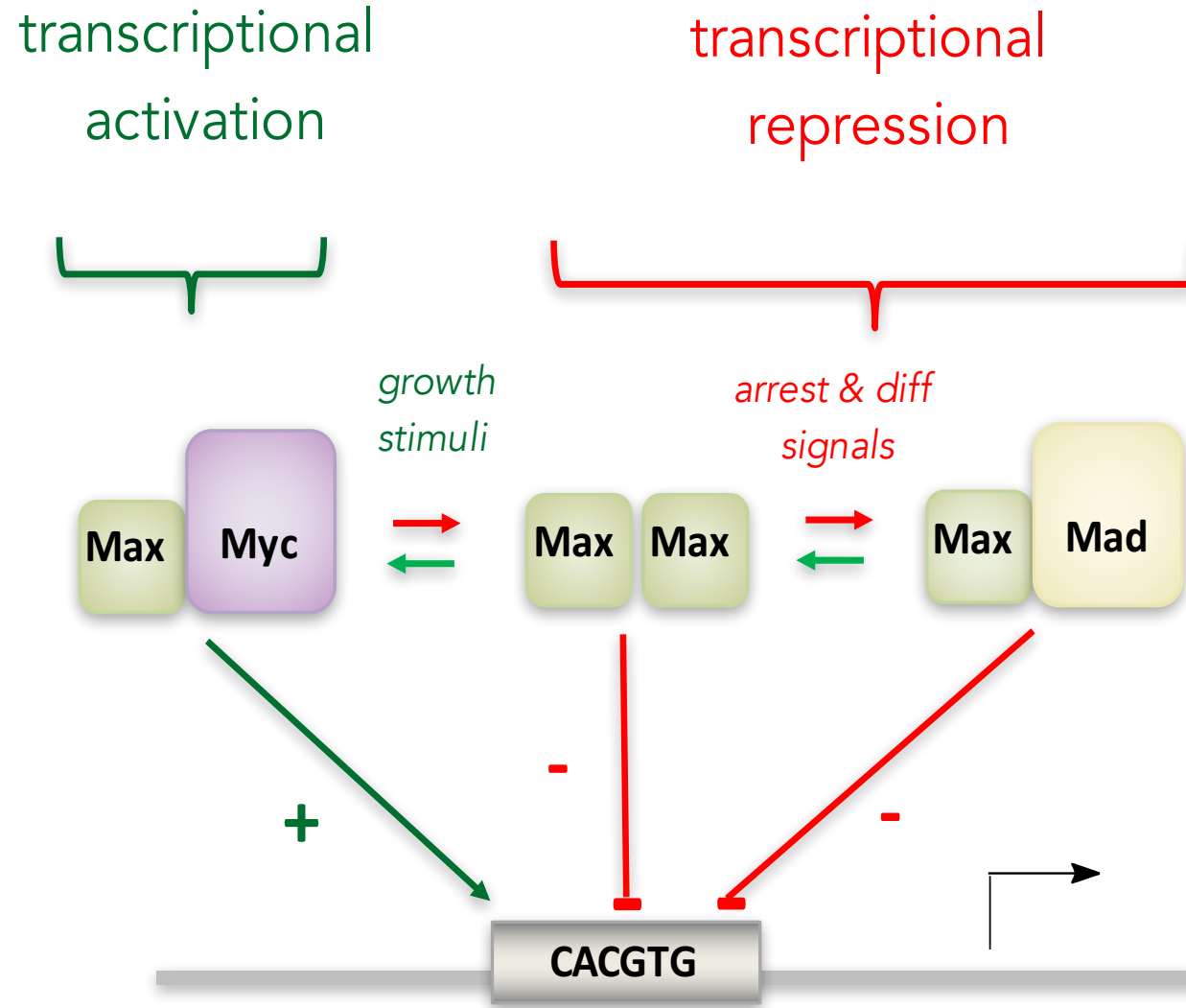


many protein-protein interactions

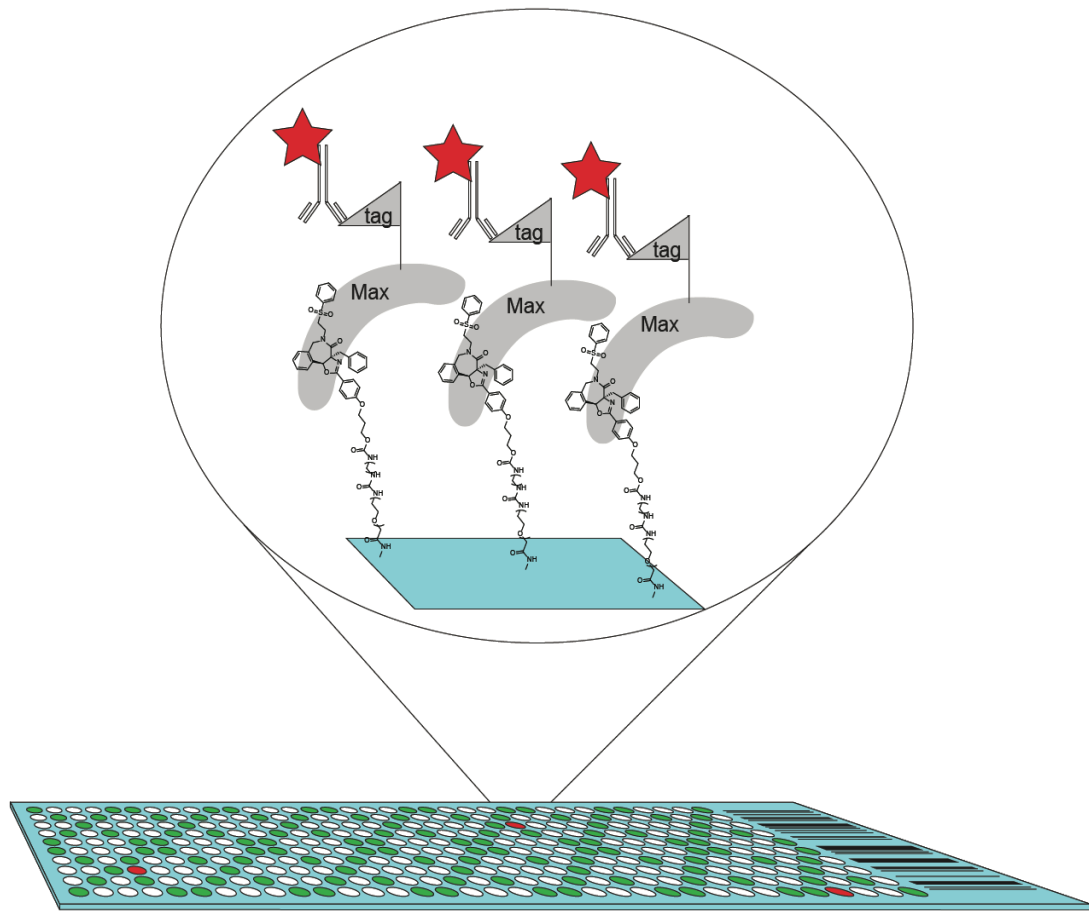


unstructured domains
no traditional binding pockets
large buried interface

Max as a target: heterodimer/homodimer dynamics

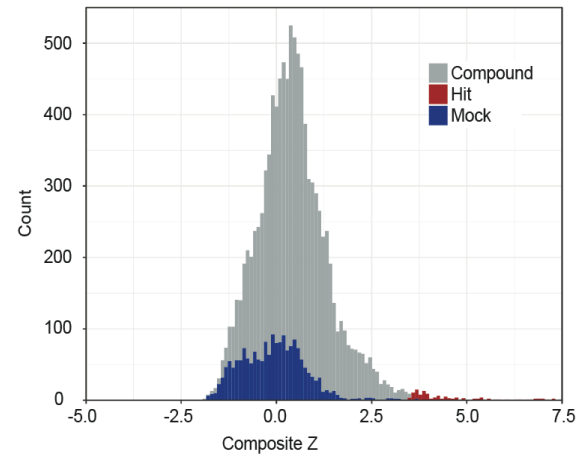


SMM screens: purified Max transcription factor

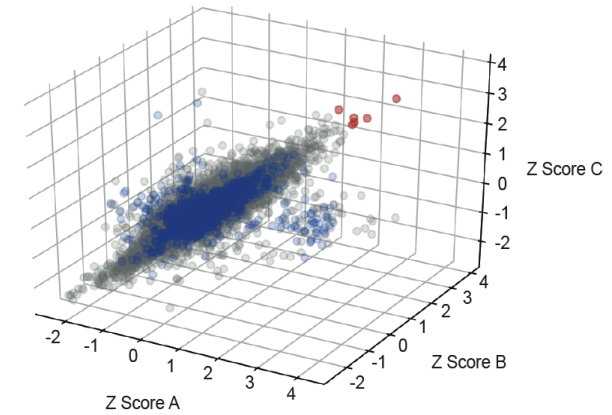
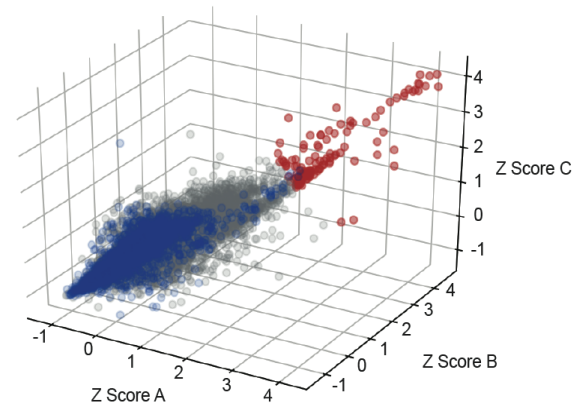
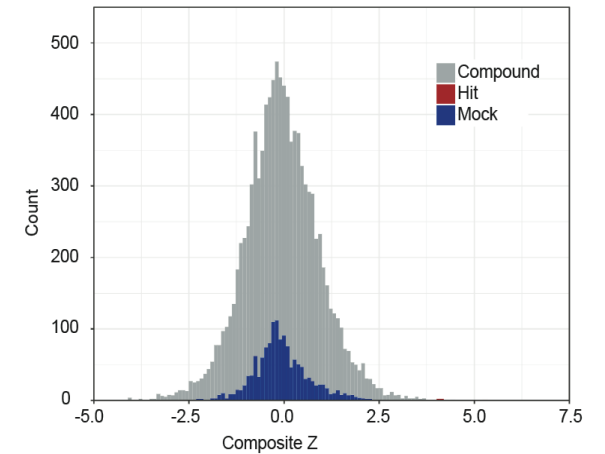


>45k compounds screened

synthetic compound collection

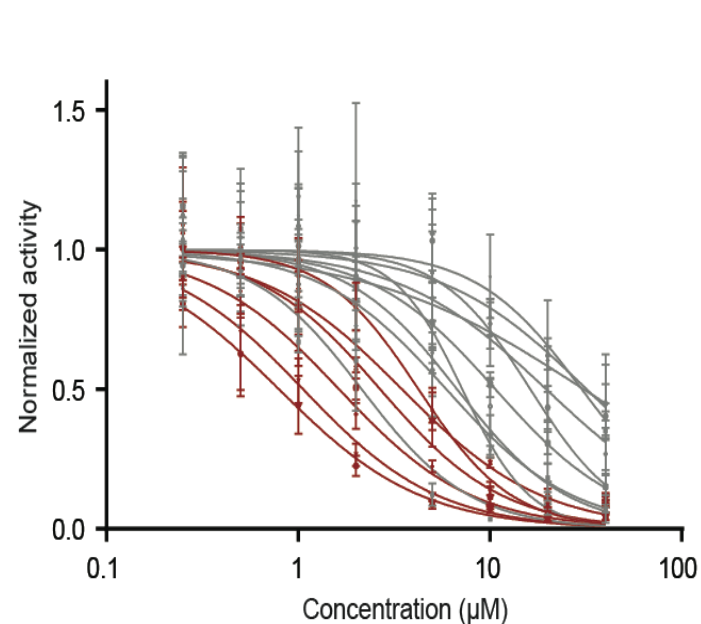
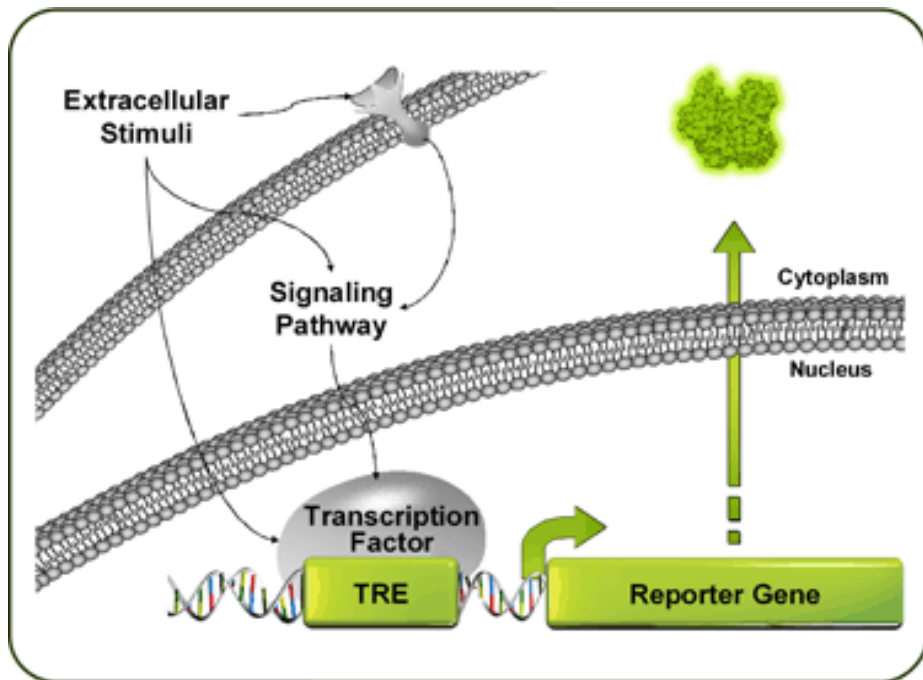


natural products



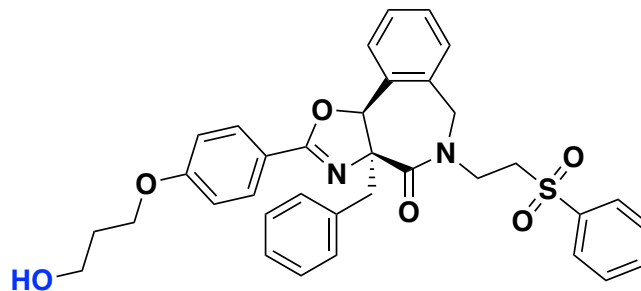
117 assay positives

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



- BRD-K47764688-001-01-8
- BRD-K86761848-001-01-3
- BRD-K37194137-001-02-5
- BRD-K19261677-001-01-2
- BRD-K11302628-001-01-2
- BRD-K00944562-001-01-2
- BRD-K89131691-001-01-4
- BRD-K90970053-001-01-4
- BRD-K05452595-001-01-2
- BRD-K77877933-001-01-4
- BRD-K94173926-001-01-6
- BRD-K39984193-001-01-0
- BRD-K90849765-001-01-9
- BRD-K89156937-001-01-5
- BRD-K16290432-001-01-0
- BRD-K55071917-001-01-0

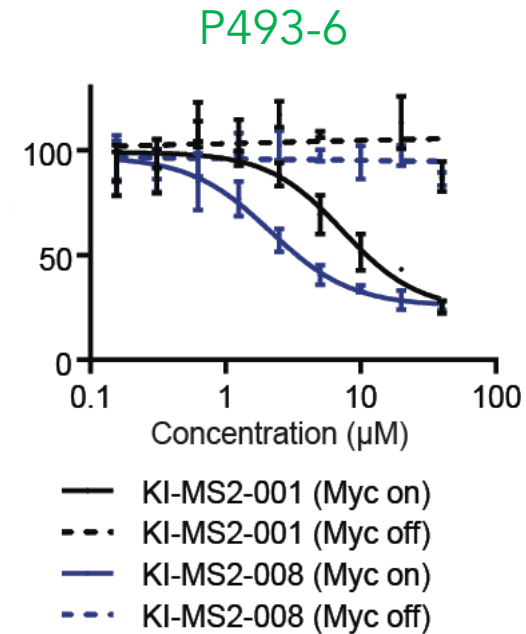
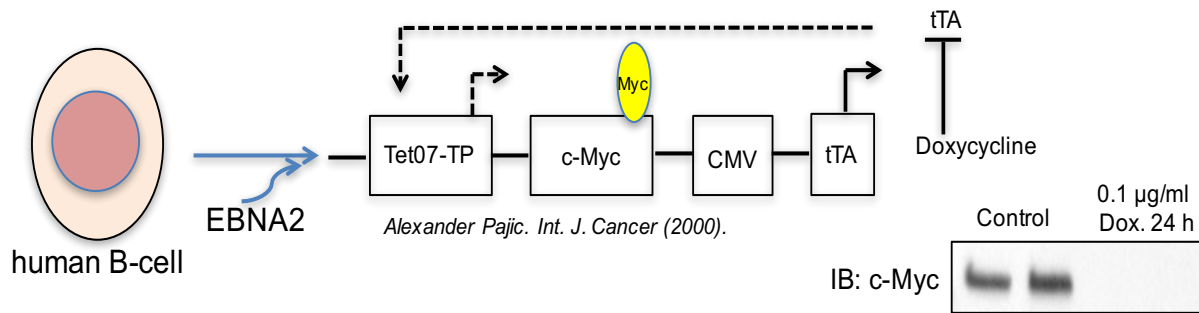
'KI-MS2'



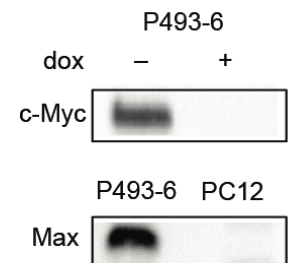
$\text{IC}_{50} = 1.06 \mu\text{M}$
MW = 610.73
cLogP = 5.15

Cell viability assays: Are Myc or Max required?

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

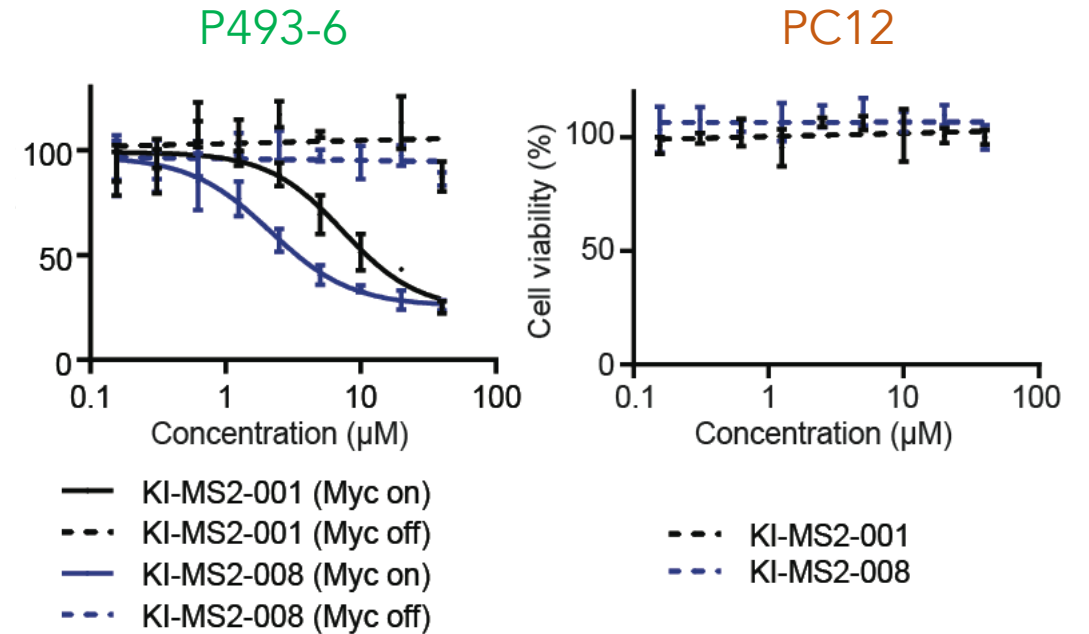
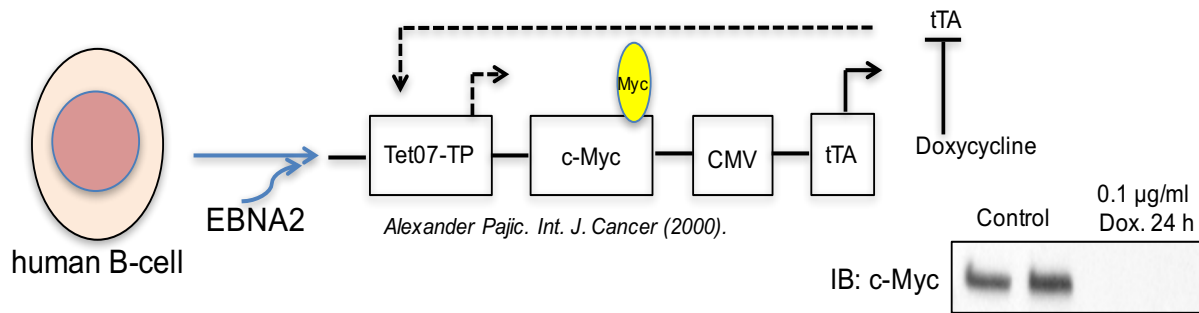


	KI-MS2-001	KI-MS2-008
Myc reporter	1.98 μM	1.28 μM
P493-6 Myc on	7.36 μM	2.15 μM
P493-6 Myc off	>50 μM	>50 μM



Cell viability assays: Are Myc or Max required?

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



Max-deficient PC12 pheochromocytoma cells

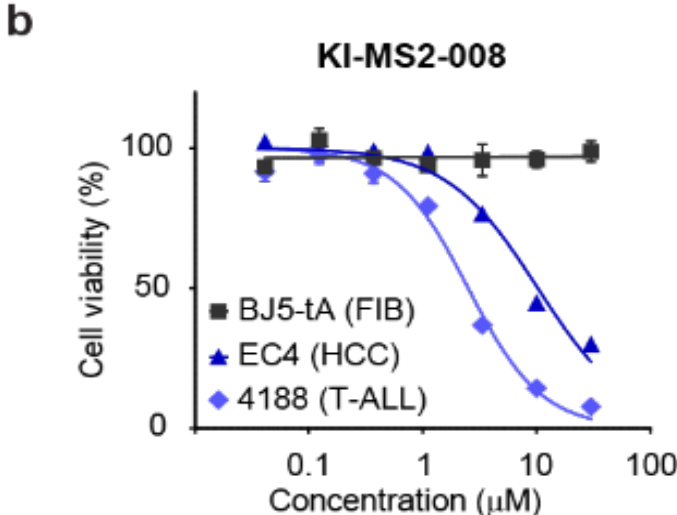
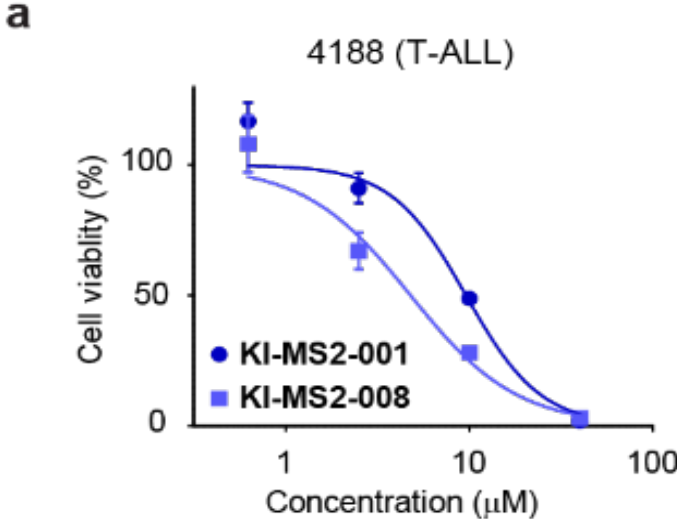
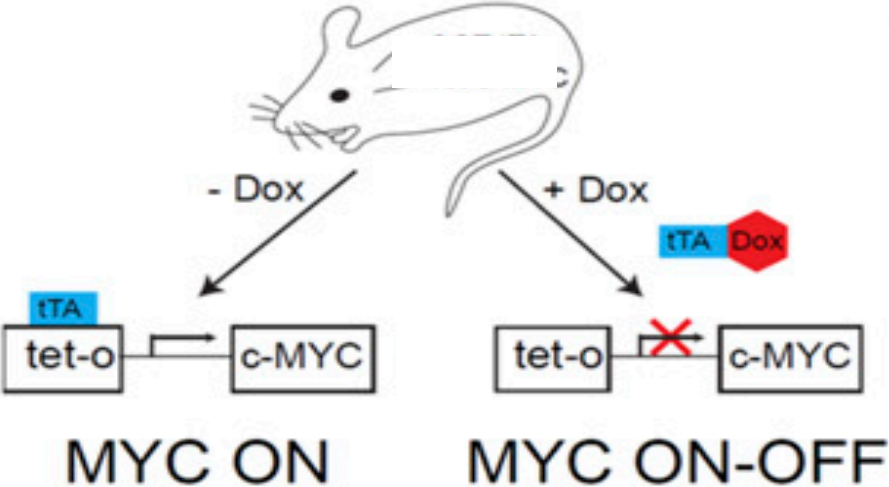


	KI-MS2-001	KI-MS2-008	
Myc reporter	1.98 µM	1.28 µM	P493-6 dox - + c-Myc
P493-6 Myc on	7.36 µM	2.15 µM	
P493-6 Myc off	>50 µM	>50 µM	P493-6 PC12 Max
PC12	>50 µM	>50 µM	

Conditional cellular models of MYC expression

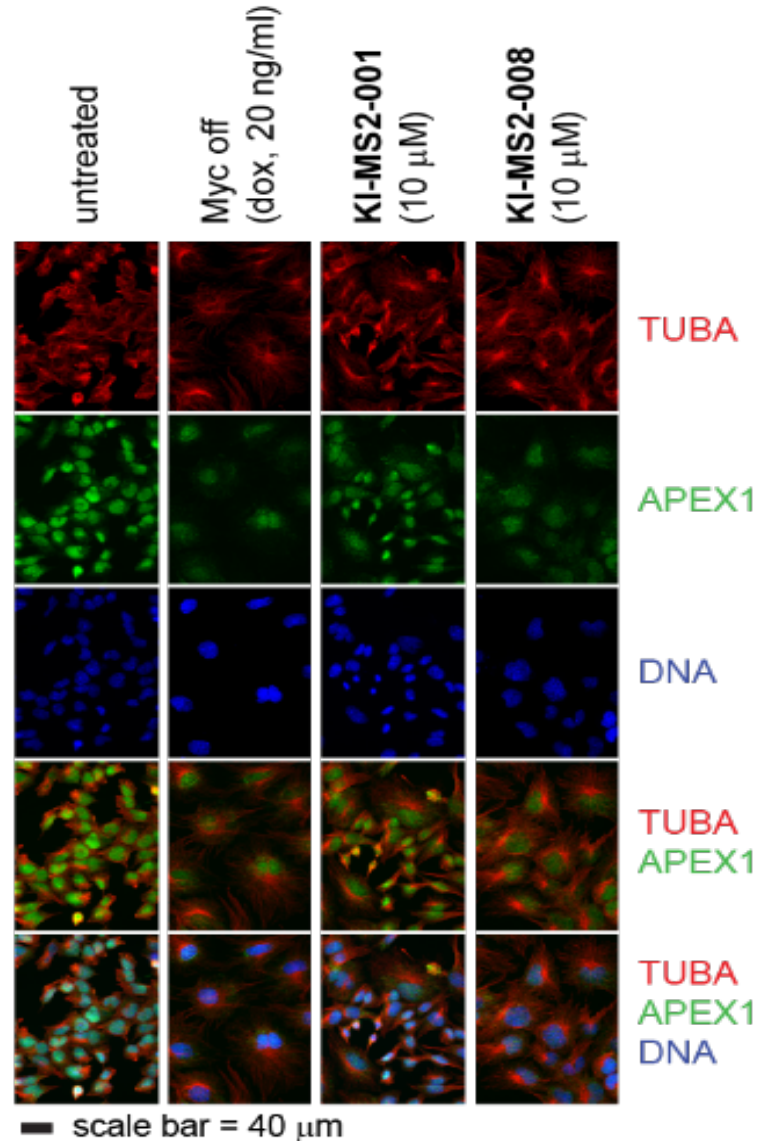
Myc 'on/off' mouse models:

- lymphoma
- HCC
- RCC
- osteosarcoma

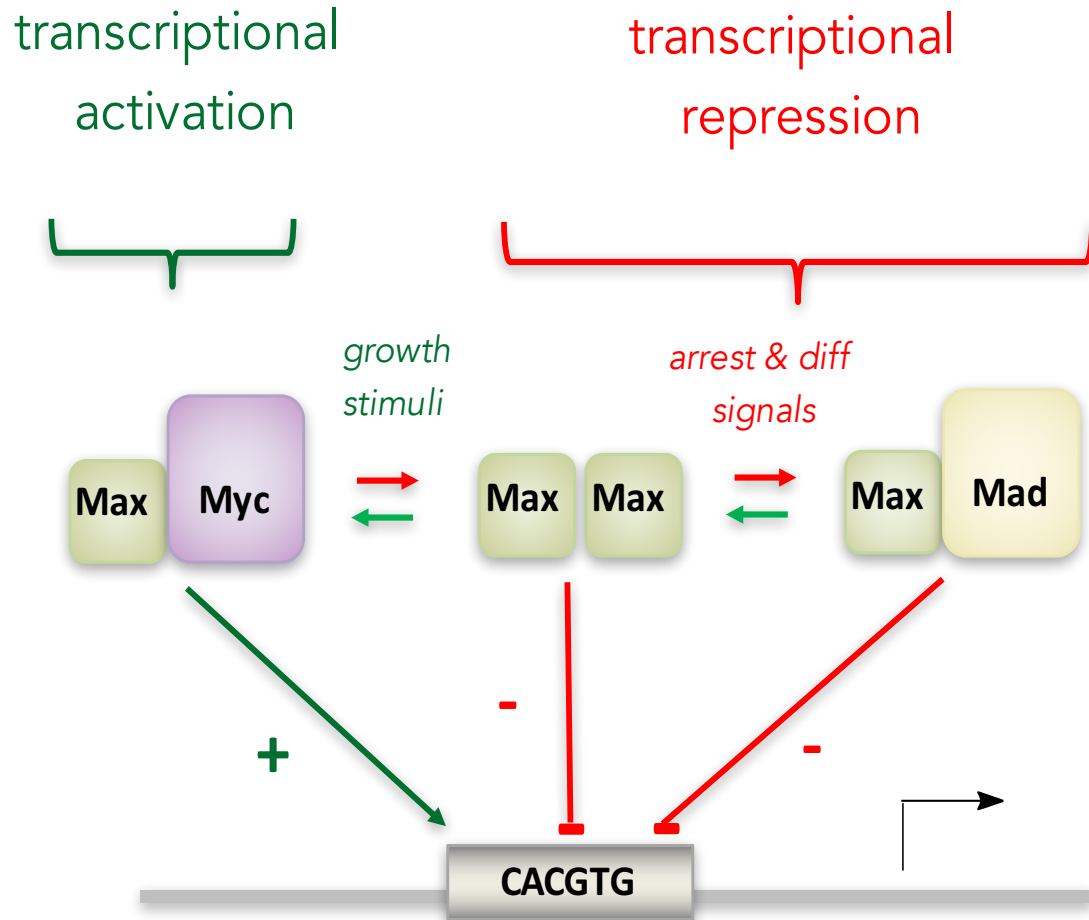


Imaging of biomarkers: conditional vs. chemical modulation

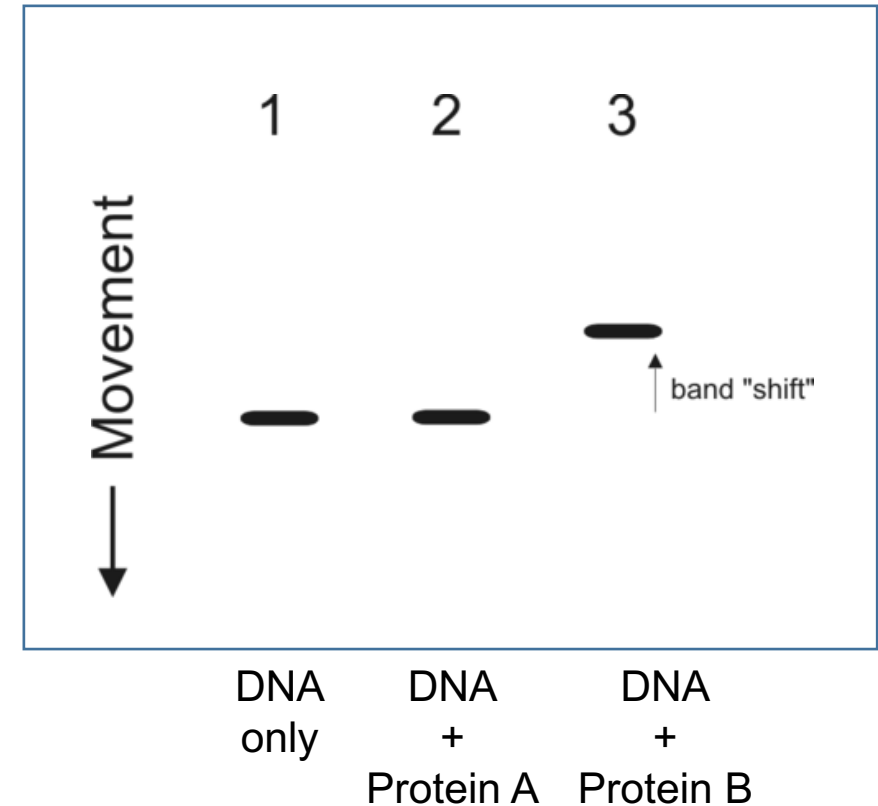
modulating Myc in an engineered osteosarcoma model



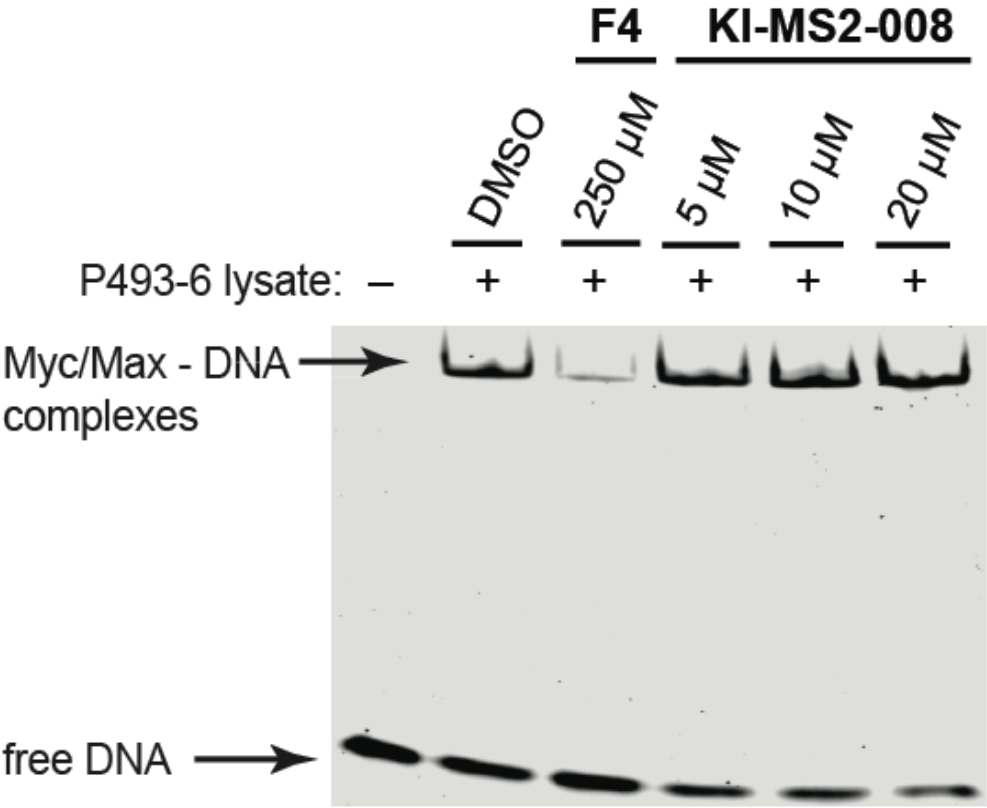
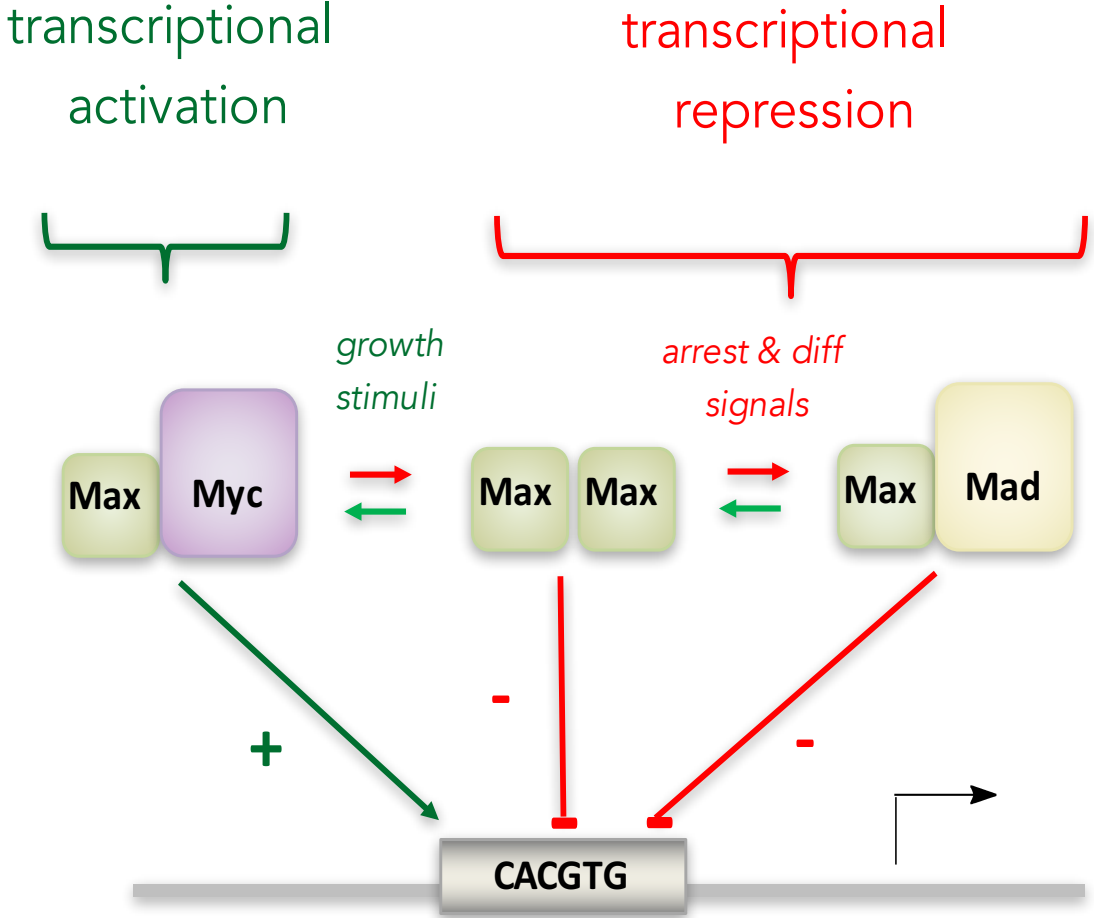
Does the probe antagonize the Myc/Max heterodimer?



Electrophoretic Mobility Shift Assay (EMSA)
aka Gel Shift Assay

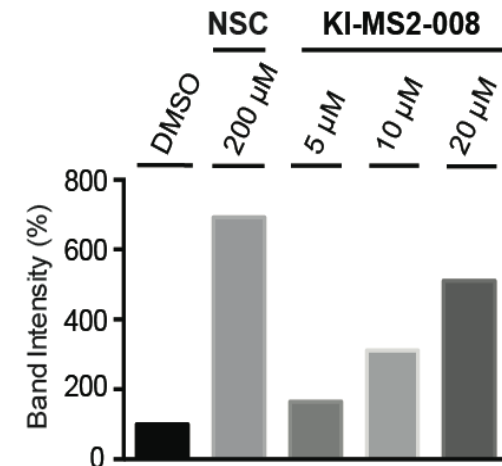
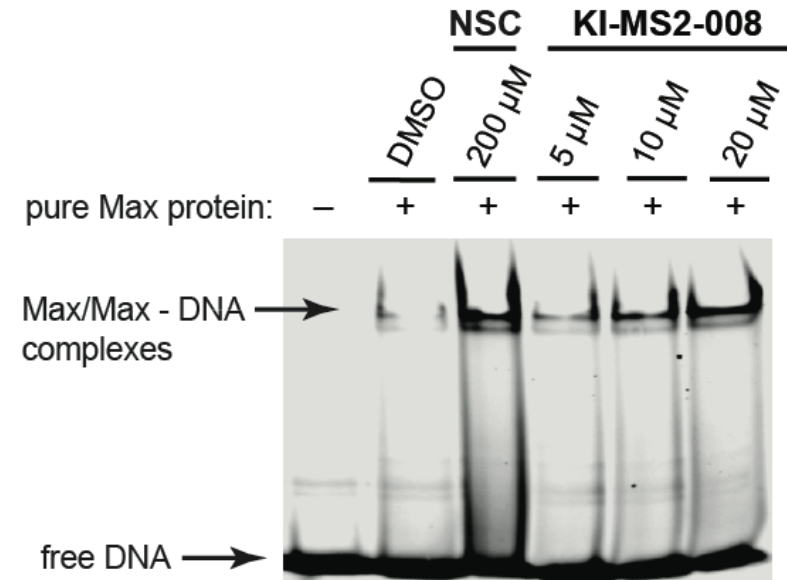
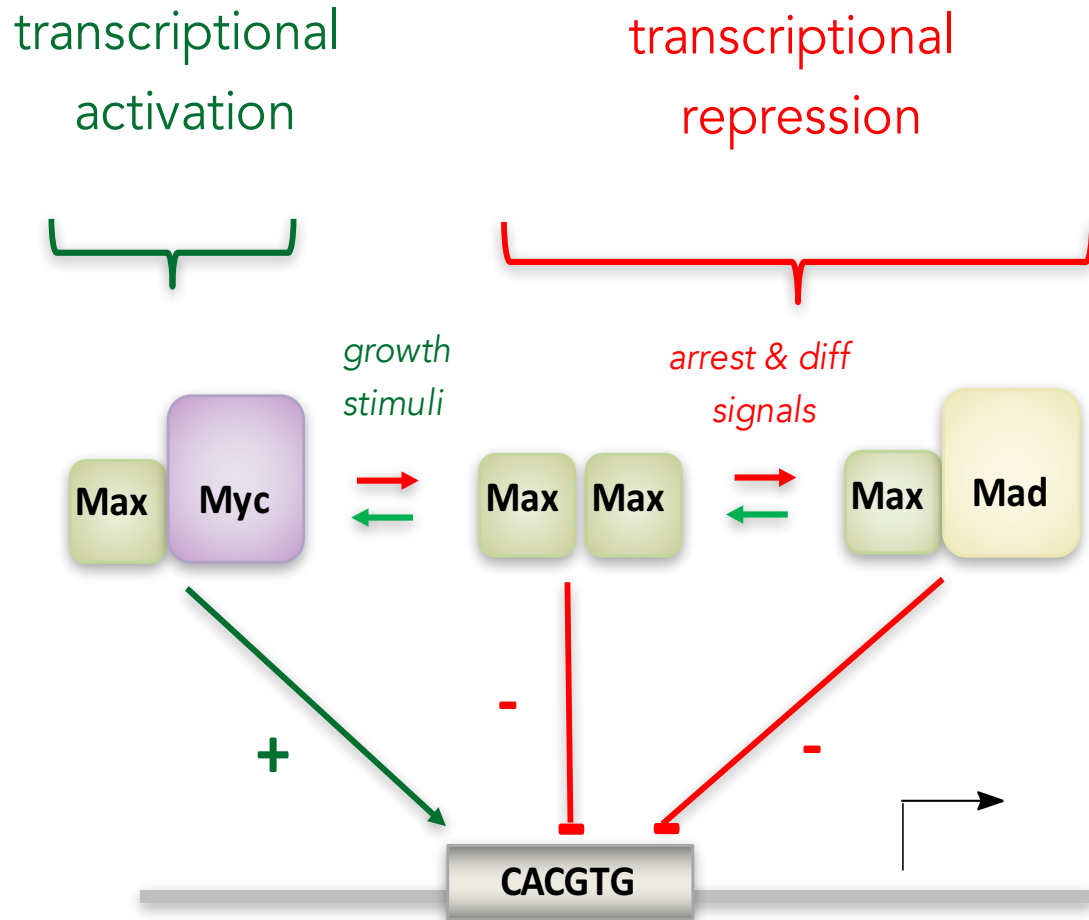


Does the probe antagonize the Myc/Max heterodimer?

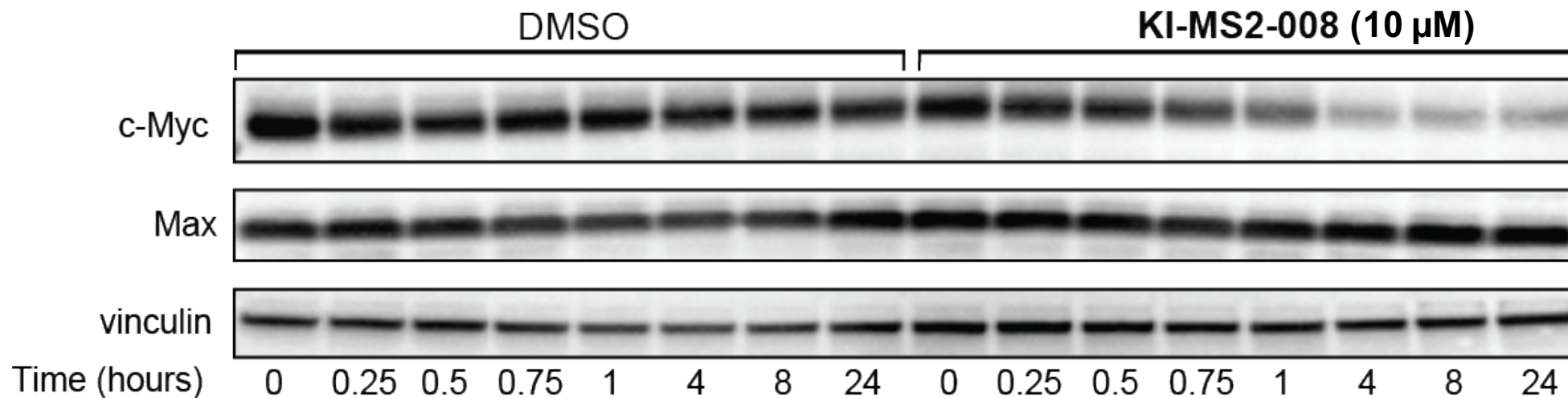


EMSA

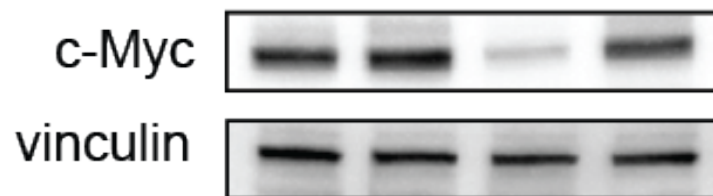
Does the probe stabilize the Max/Max homodimer?



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

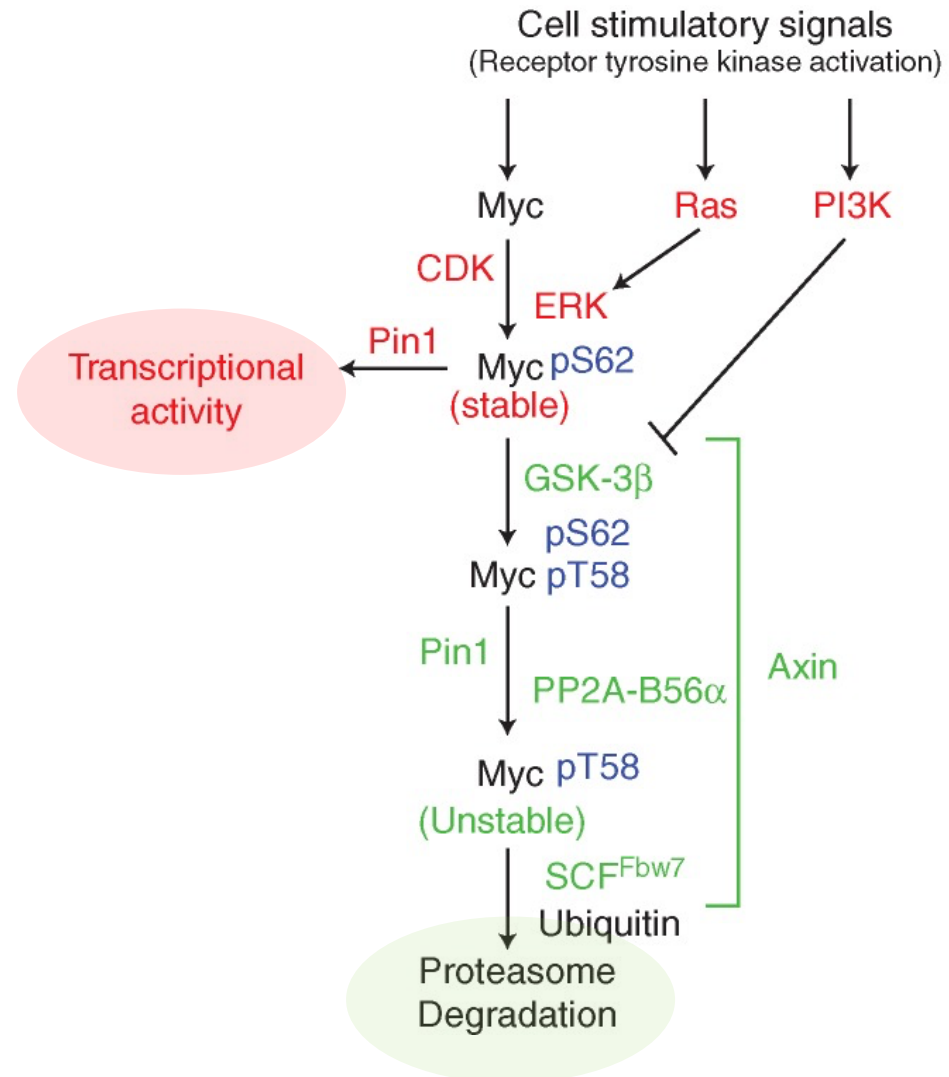


KI-MS2-008	-	-	+	+
MG132	-	+	-	+



rescue experiment with
10 μM proteasome inhibitor
MG132

Myc protein stability is regulated by the ubiquitin-proteasome system

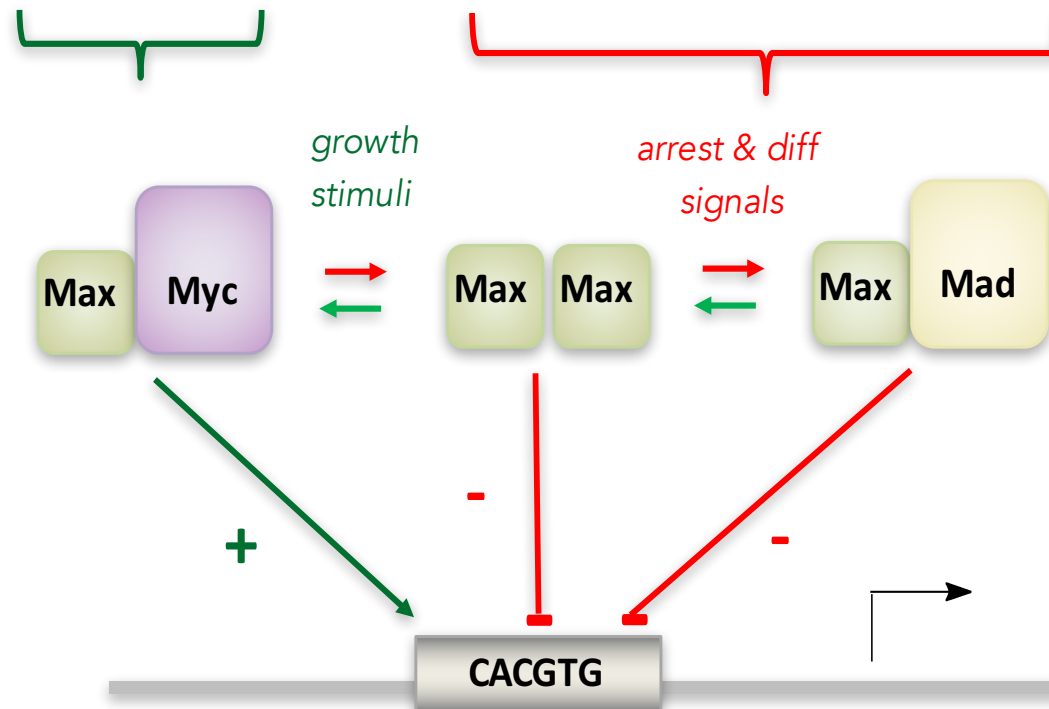


KI-MS2-008

mixed mechanism inhibitor?

transcriptional
activation

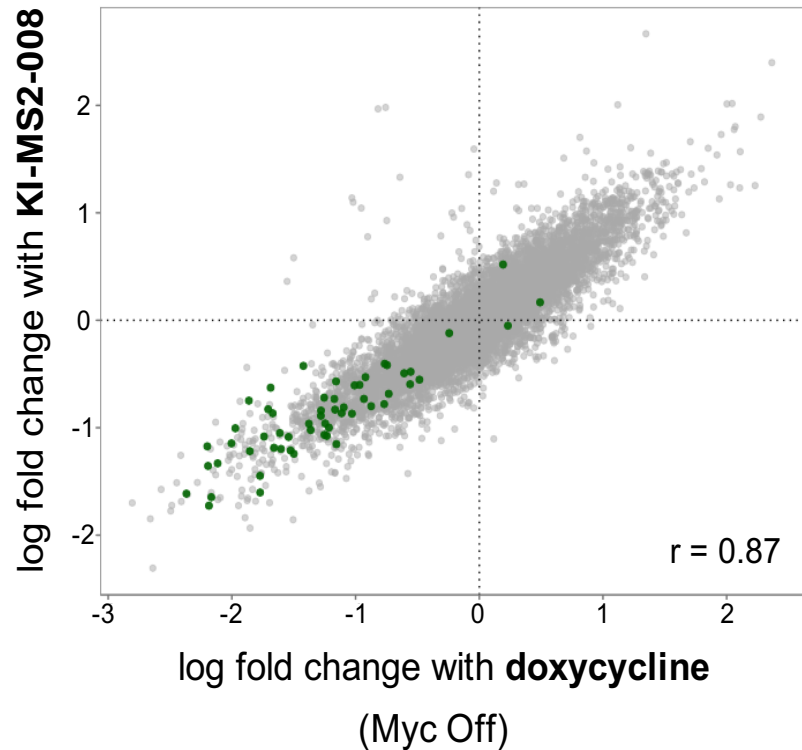
transcriptional
repression



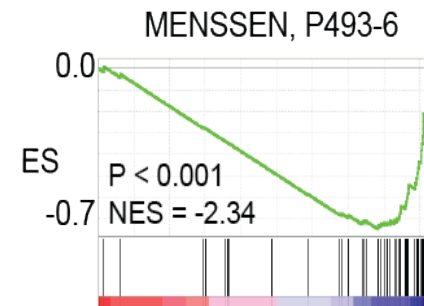
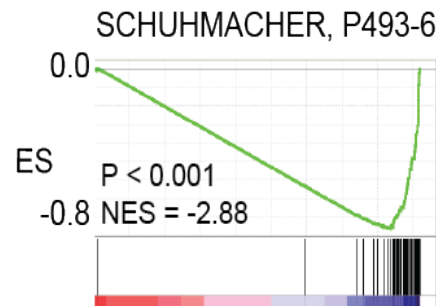
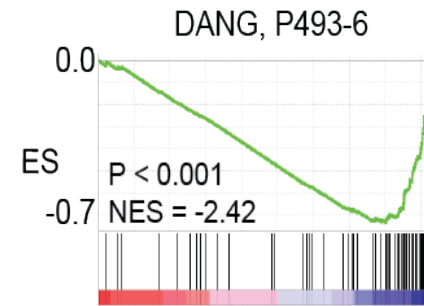
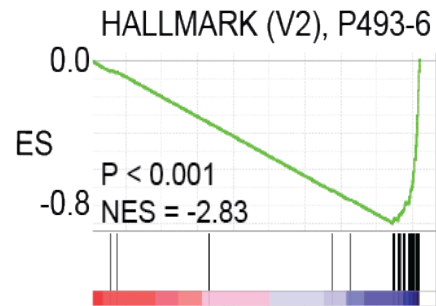
Gene expression profiling: KI-MS2-008 mimics MYC inactivation

Gene Set Enrichment Analysis reveals an enrichment of Myc target genes

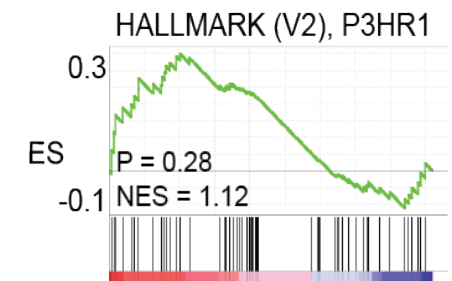
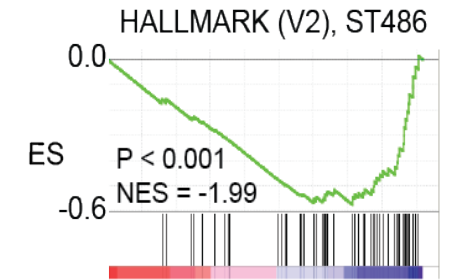
Hallmark V2 MYC-dependent genes in green



sensitive
P493-6



sensitive
Burkitt's lymphoma

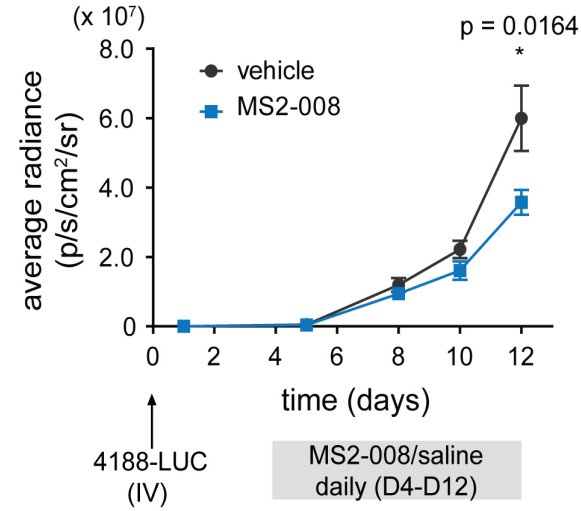
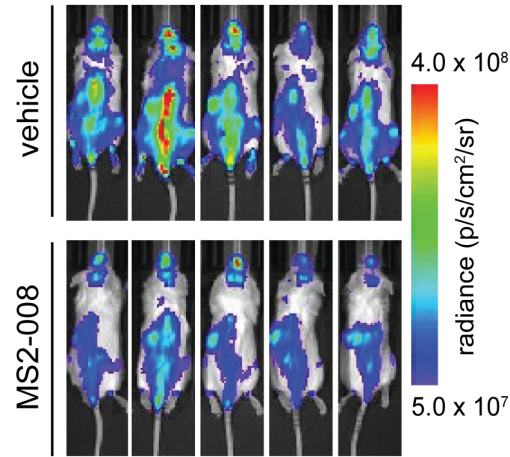


insensitive
Burkitt's lymphoma

You will learn more about this method you will learn more about in module 2

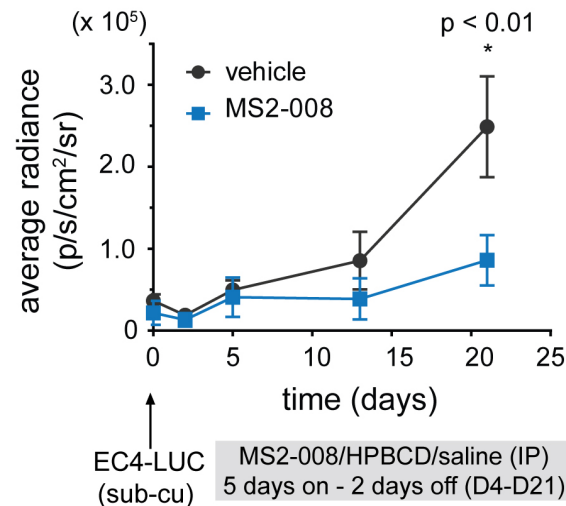
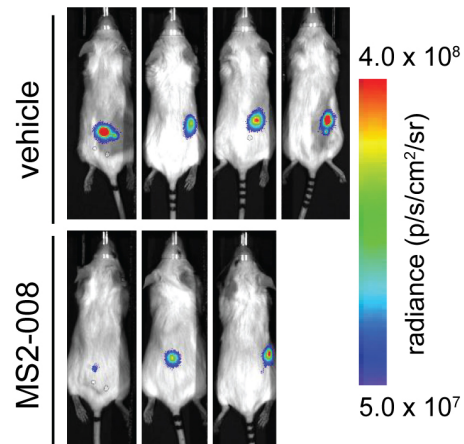
In vivo studies: KI-MS2-008 modulates tumor volume in Myc-dependent mouse models of cancer

T-cell acute lymphoblastic leukemia
blood cancer



0.06 mg/kg
daily IV administration

hepatocellular carcinoma
solid tumor

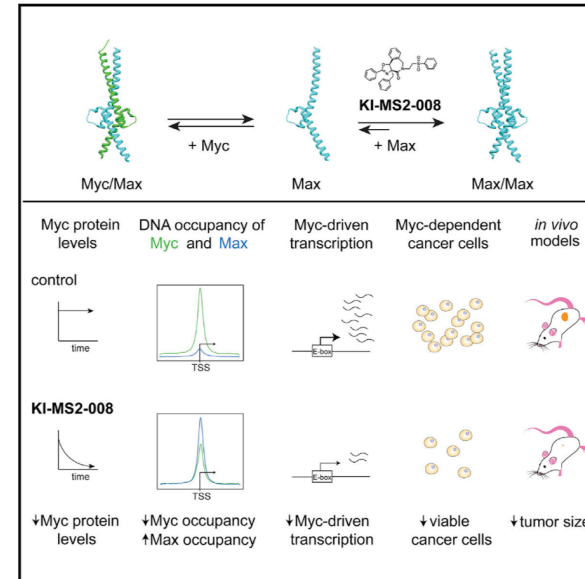


0.24 mg/kg
subcutaneous administration
5d on/2d off cycles

Cell Chemical Biology

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

Graphical Abstract



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

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.

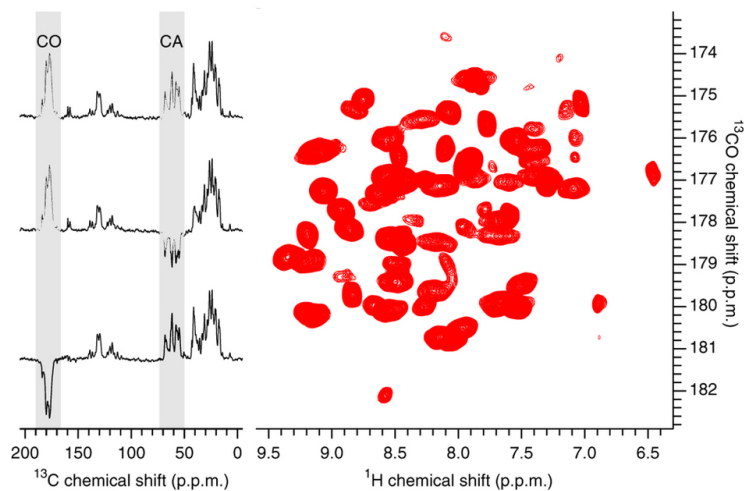
**Publication Date
is March 14, 2019**

Current directions

optimize potency and solubility, PK/PD-guided medicinal chemistry



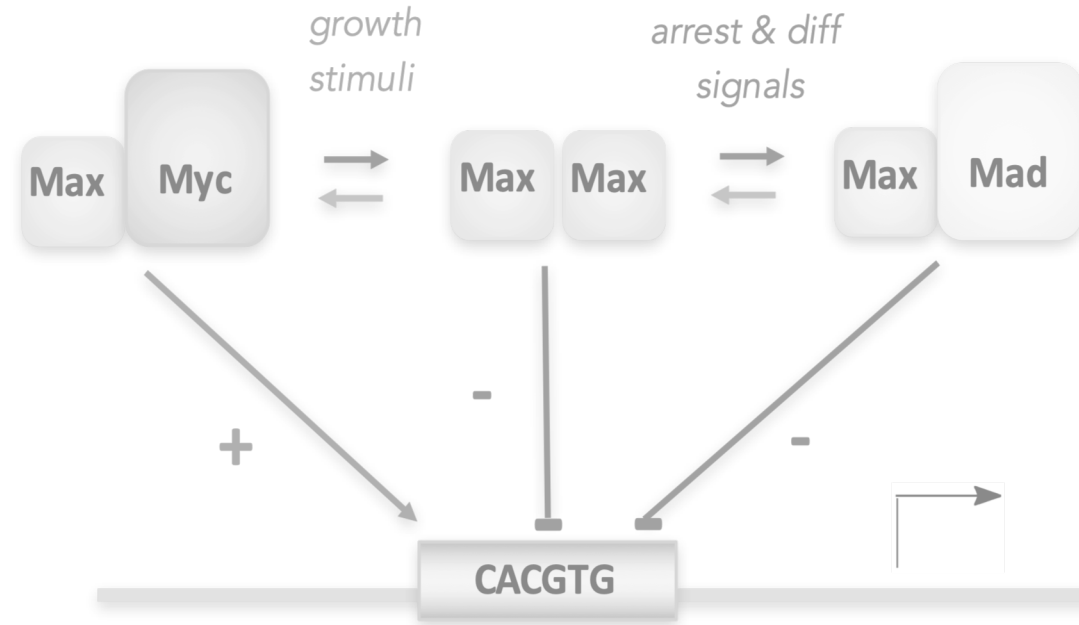
structural biology



additional tumor models
same tumor models + new readouts



stabilizing repressive states vs. inhibiting activating states?



stabilizing vs. inhibiting PPIs?

Our path to finding ligands - lectures

2/5/20	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
2/11/20	Lecture 2	Our protein target: TDP-43
2/13/20	Lecture 3	Small molecule microarrays
2/18/20	No Lecture	
2/20/20	Lecture 4	Quantitative evaluation of protein-ligand interactions
2/25/20	Lecture 5	A ligand discovery vignette: sonic hedgehog
2/27/20	Lecture 6	Engineering transcriptional responses with a small molecule
3/3/20	Lecture 7	Wrap up discussion: suggestions for how to report your findings