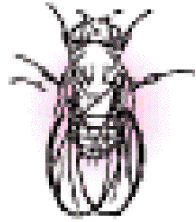


L6 – Engineering Transcriptional Responses with a Chemical Probe

March 1, 2018



D. melanogaster

13,600



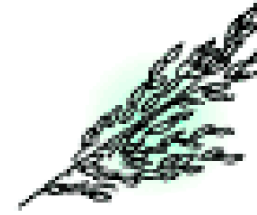
C. elegans

19,500



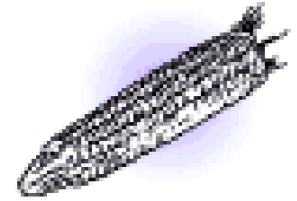
Homo sapiens

21,000



Oryza sativa

45,000



Zea mays

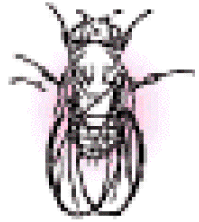
50,000

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

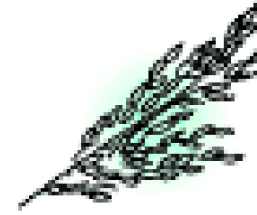
750



Homo sapiens

21,000

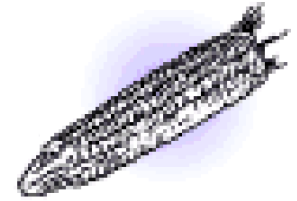
2,600



Oryza sativa

45,000

2,000



Zea mays

50,000

760

alternative
splicing

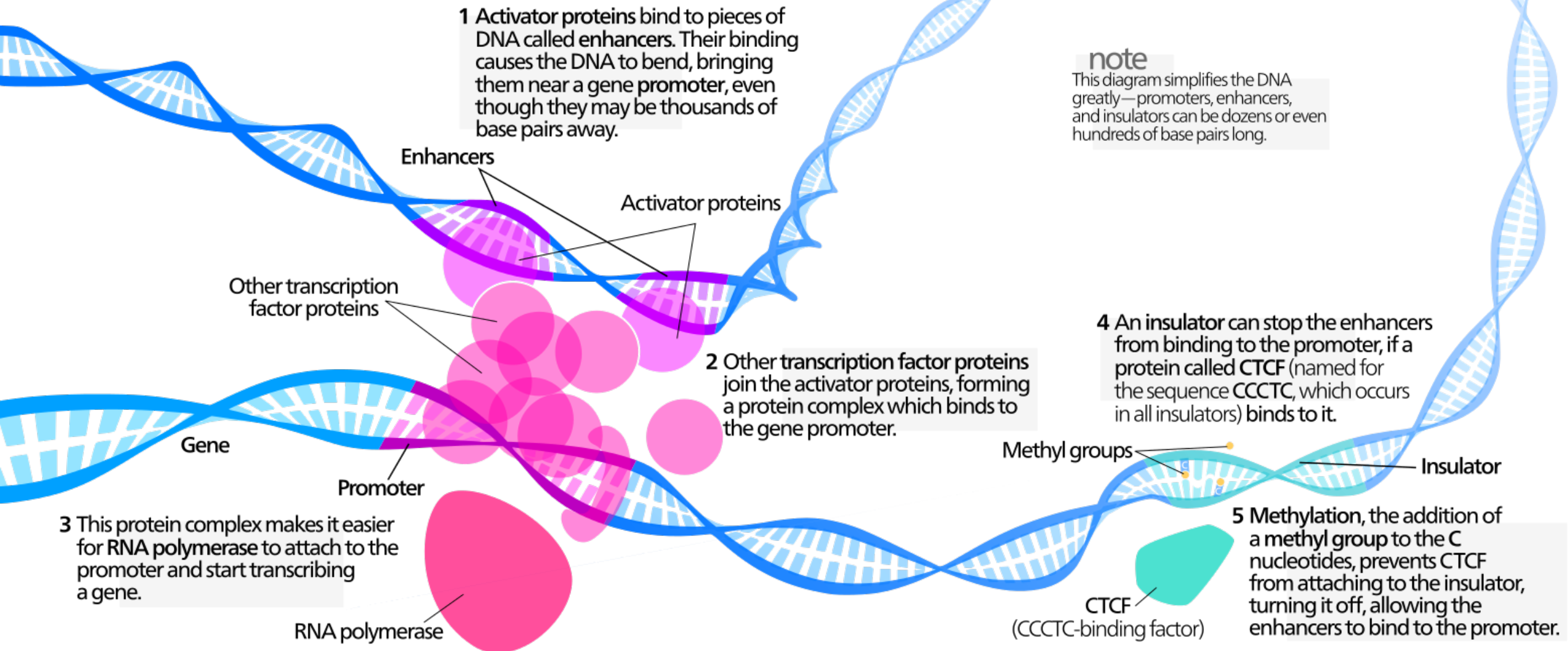
post-translational
processing



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

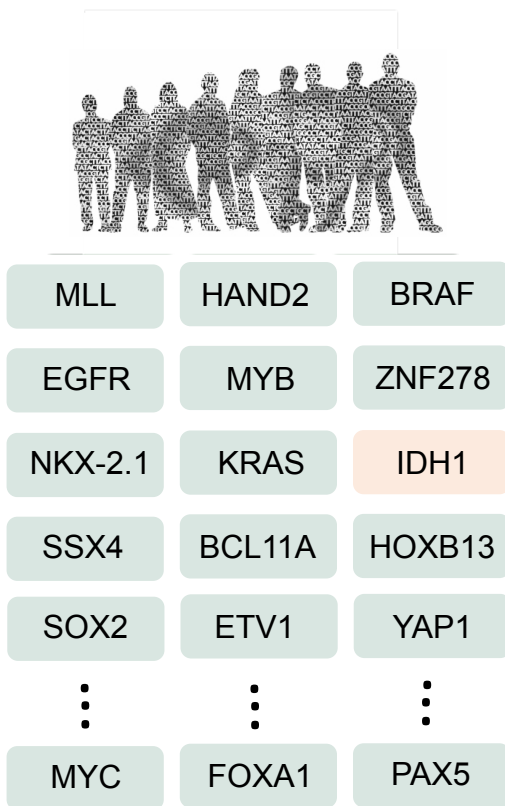
transcription factors

of eukaryotic cells

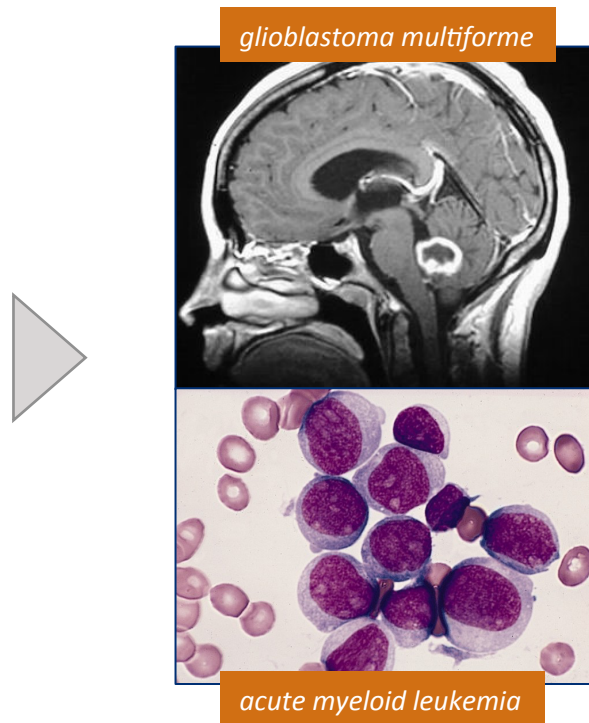


Therapeutically-driven probe discovery

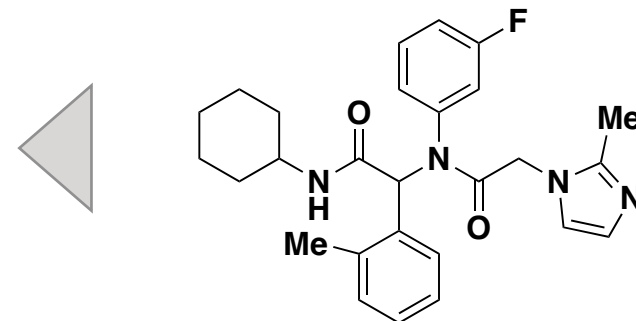
target cause of disease revealed by human genetics



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

AVGR8
BCL11A
CAMTA1
ELF1
ETS1
GATA3
GTF2H1
HHEX-IDE
HIF2A
HNF1B
HPB1
IRF5
IRF8
LBXCOR1
MAF
MECP2

central corneal thickness
β-hemoglobin disorders
episodic memory deficit
systemic lupus erythematosus
systemic lupus erythematosus
periodontitis
amyloidosis
T2D
RCC
T2D
osteoarthritis
various AI disorders
MS
restless leg syndrome
early-onset obesity
autism

MEIS1
MLXIPL
NFATC2
NOTCH2
PBX4
PPARG
RELA
RFX4
SP7
STAT3
STAT4
TCF4
TCF7L2
THAP1
ZNF469
ZNF804A

restless leg syndrome
coronary artery disease
T1D
T2D
coronary artery disease
T2D
rheumatoid arthritis
Parkinson's disease
BMD
various AI disorders and cancers
systemic lupus erythematosus
schizophrenia, corneal dystrophy
T2D
early-onset torsion dystonia
central corneal thickness
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

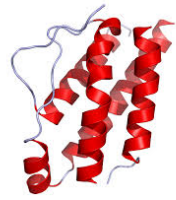
TF cancer genes with frameshift mutations

<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

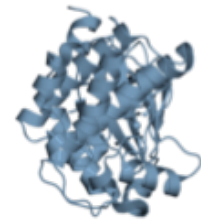
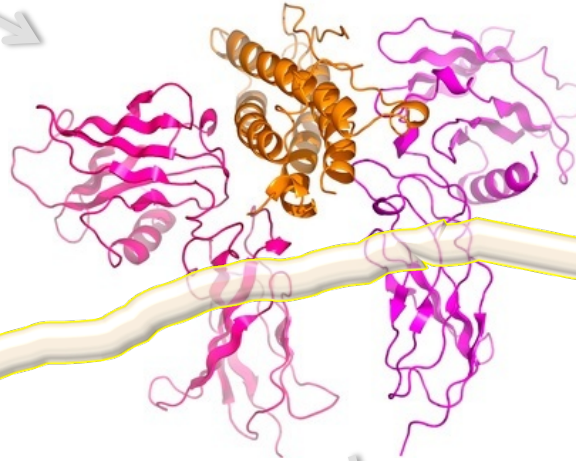
somatically mutated TF cancer genes

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	MLLT1	ALL	SSX4	synovial sarcoma
ETV1	Ewing sarcoma, prostate	MLLT10	ALL, colorectal	SUFU	medullablastoma
ETV4	Ewing sarcoma, prostate	MLLT2	ALL, breast cancers	SUZ2	endometrial stromal tumors
ETV5	prostate	MLLT3	AML	TAF15	ALL, EMC
ETV6	various cancers	MLLT4	AML	TAL1	lymphoblastic leukemia
EVI1	AML, CML	MLLT6	ALL	TAL2	T-ALL
EWSR1	Ewing sarcoma, ALL	MLLT7	ALL	TCEA1	salivary adenoma
FEV	Ewing sarcoma	MYB	adenoid cystic sarcoma	TCF12	EMC
FLI1	Ewing sarcoma	MYC	various cancers	TCF3	pre B-ALL
FOXL2	ovarian	MYCL1	small cell lung	TFE3	renal, alveolar soft sarcoma
FOXO1A	alveolar rhabdomyosarcomas	MYCN	neuroblastoma	TFEB	renal (child epithelioid)
FOXO3A	AL	NCOA1	alveolar rhabdomyosarcoma	TFPT	pre B-ALL
FOXP1	ALL	NCOA2	AML	THRAP3	aneurysmal bone cysts
GATA1	megakaryoblastic leukemia	NCOA4	papillary thyroid	TIF1	APL
GATA2	AML	NFIB	lipoma, ACC	TLX1	T-ALL
GATA3	breast	NFKB2	B-NHL	TLX3	T-ALL
HLF	ALL	NKX2-1	NSCLC	TP53	various cancers
HLXB9	AML	NOTCH1	T-ALL	TRIM27	papillary thyroid
HMGA1	various cancers	NOTCH2	DLBCL, marginal zone lymphoma	TRIM33	papillary thyroid
HMGA2	various cancers	NR4F3	EMC	TSHR	toxic thyroid adenoma
HOXA11	CML	NRF2	NSCLC, HNSCC	WT1	Wilm tumor
HOXA13	AML	OLIG2	T-ALL	ZNF145	APL
HOXA9	AML	PAX3	alveolar rhabdomyosarcoma	ZNF198	MPD, NHL
HOXC11	AML	PAX5	NHL	ZNF278	Ewing sarcoma
HOXC13	AML	PAX7	alveolar rhabdomyosarcoma	ZNF331	follicular thyroid adenoma
HOXD11	AML	PAX8	follicular thyroid	ZNF384	ALL
HOXD13	AML	PBX1	pre B-ALL	ZNF521	ALL
HNF1	HCC	PHOX2B	neuroblastoma	ZNF9	aneurysmal bone cysts
HRPT2	parathyroid adenoma	PLAG1	salivary adenoma	ZNFN1A1	\ALL, DLBCL
IKZF1	ALL	PMX1	AML1		

extracellular
factors



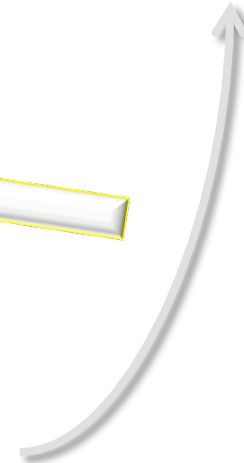
membrane
receptors



intracellular
signaling
proteins

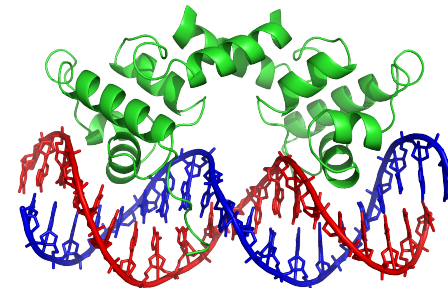


cellular
response



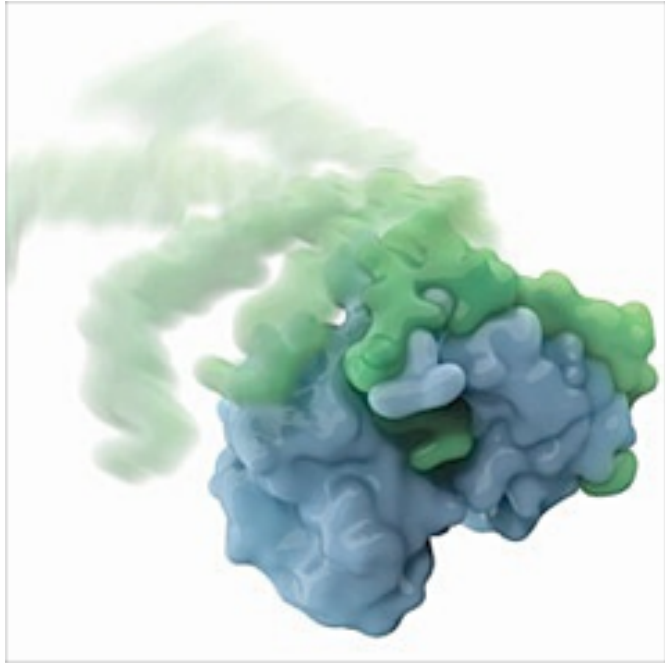
transcriptional regulators

more cogent target?



A complex task?

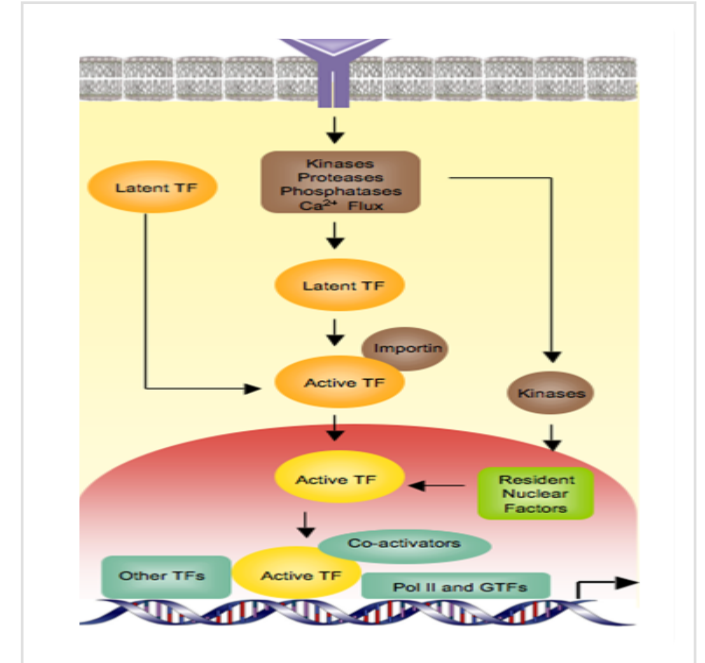
transcription factors are the prototype of an '*undruggable*' target



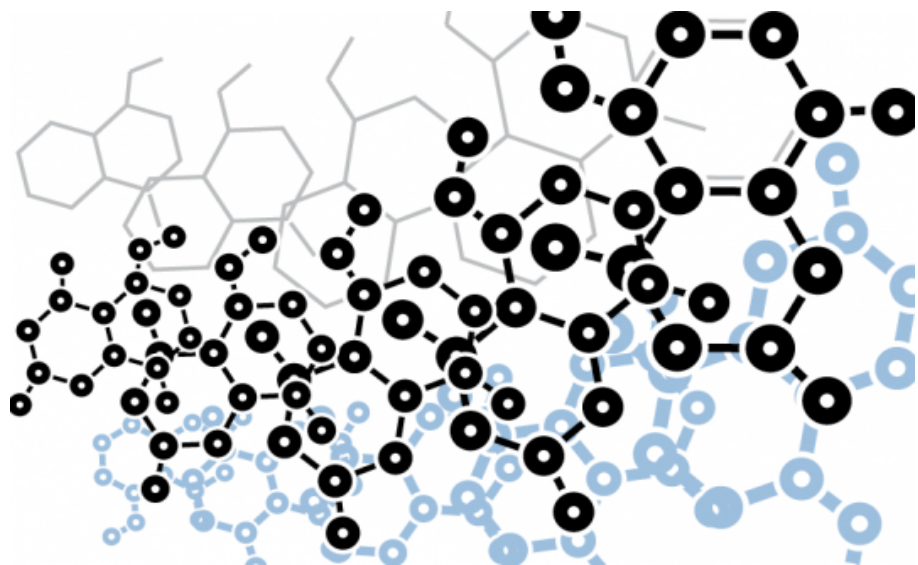
disordered when isolated
from binding partners



DNA-binding domains
lack obvious pockets

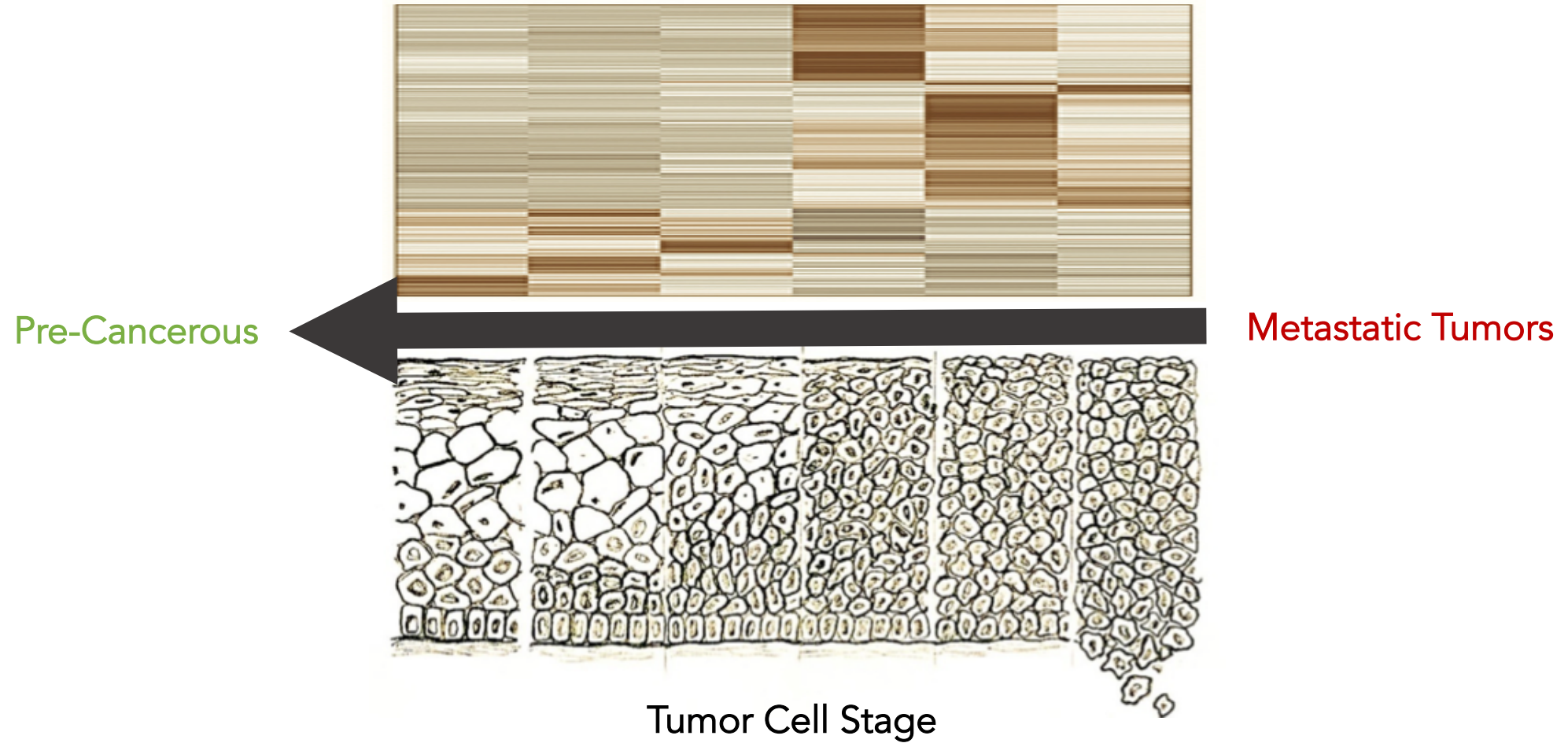


transit to reach resident
nuclear factors



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

Transcription Profile



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

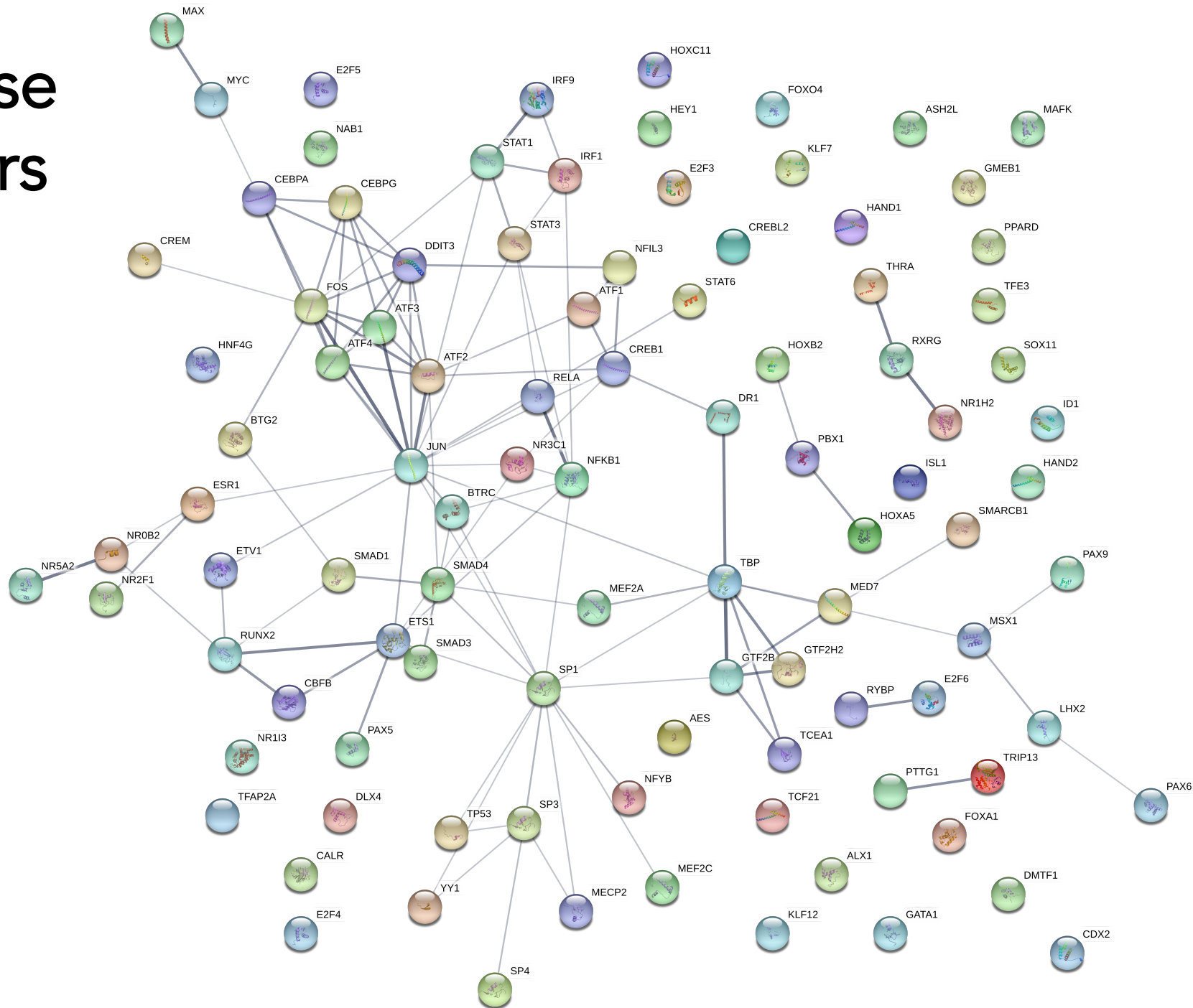
Profiling 100 diverse transcription factors

commercially available
purified, His-tagged

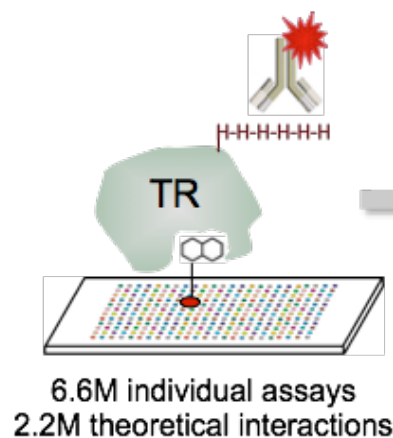
145 InterPro domains
e.g. bZip, Znf_C2H2, Fbox, Ets, etc.

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

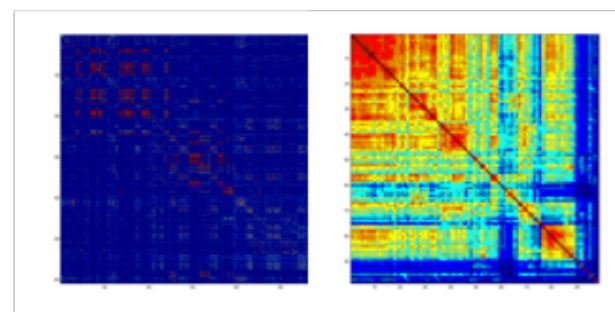
>100 KEGG pathways
e.g. Wnt signaling, chronic myeloid
leukemia, circadian entrainment, etc.



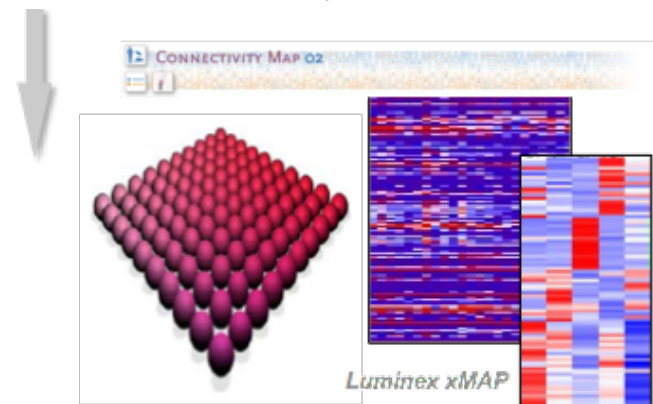
'100 Transcription Factor' SMM Screen



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

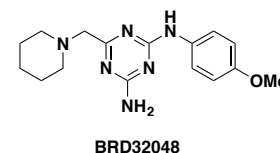
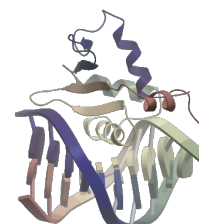
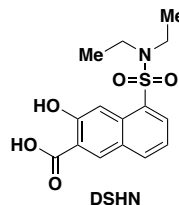
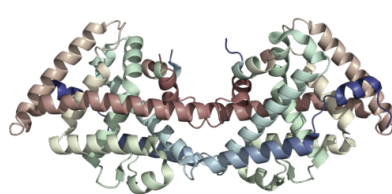


computational chemical biology



HT-gene expression studies

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

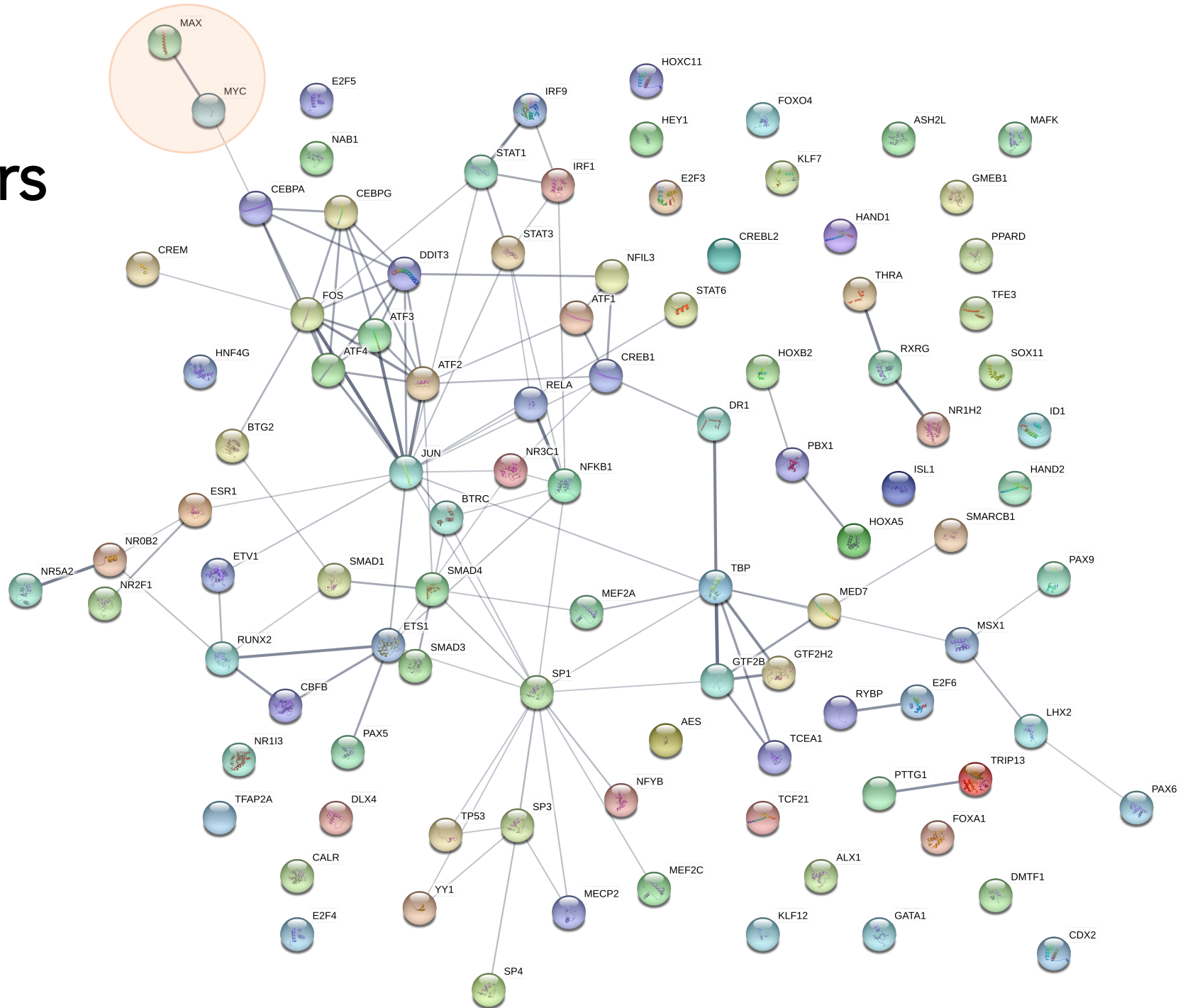
100 diverse transcription factors

commercially available
purified, His-tagged

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

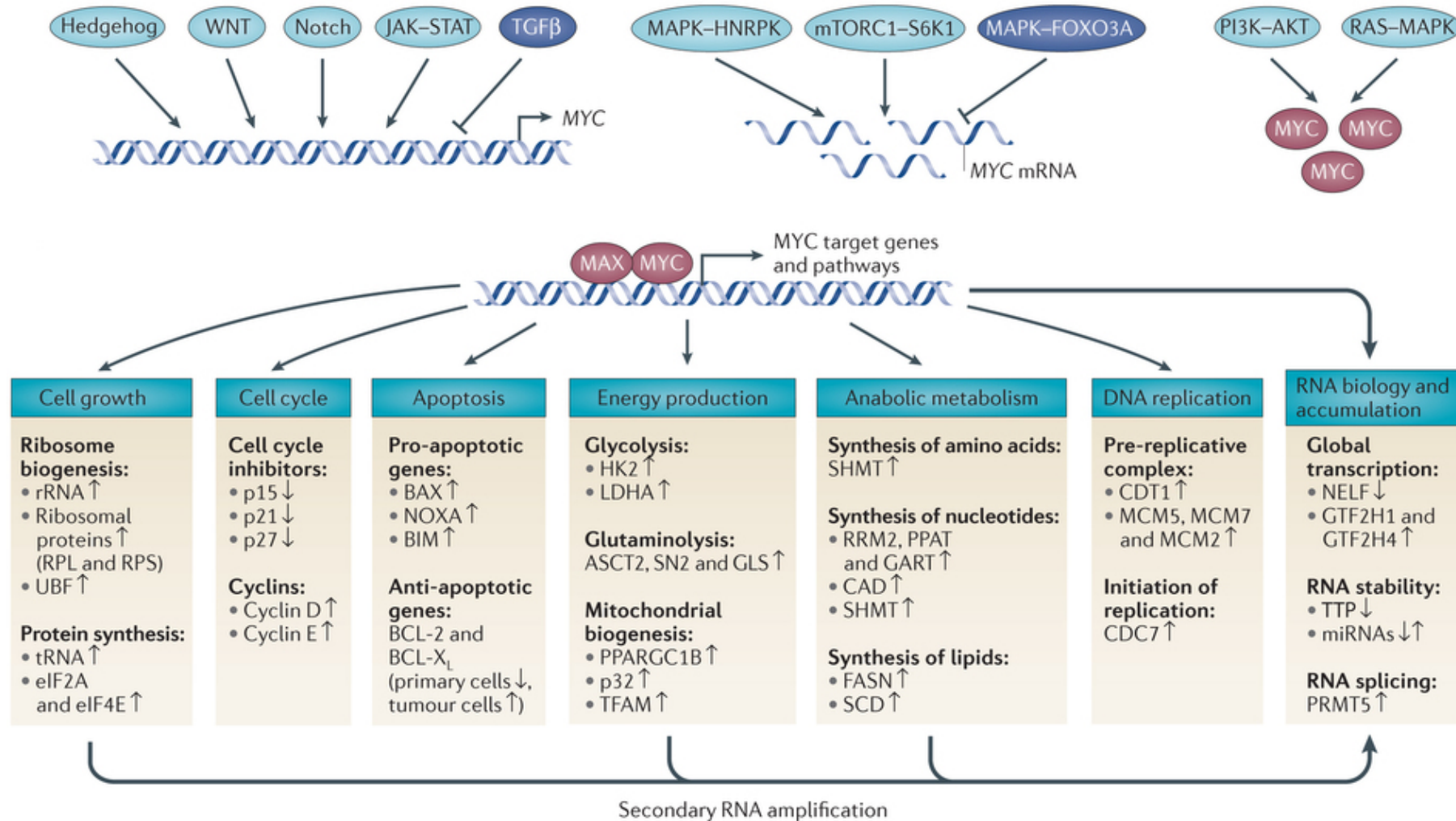
>500 GO terms
e.g. nuclear, chromatin remodeling,
basal transcription, etc.

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e.g. Wnt signaling, chronic myeloid
leukemia, circadian entrainment, etc.



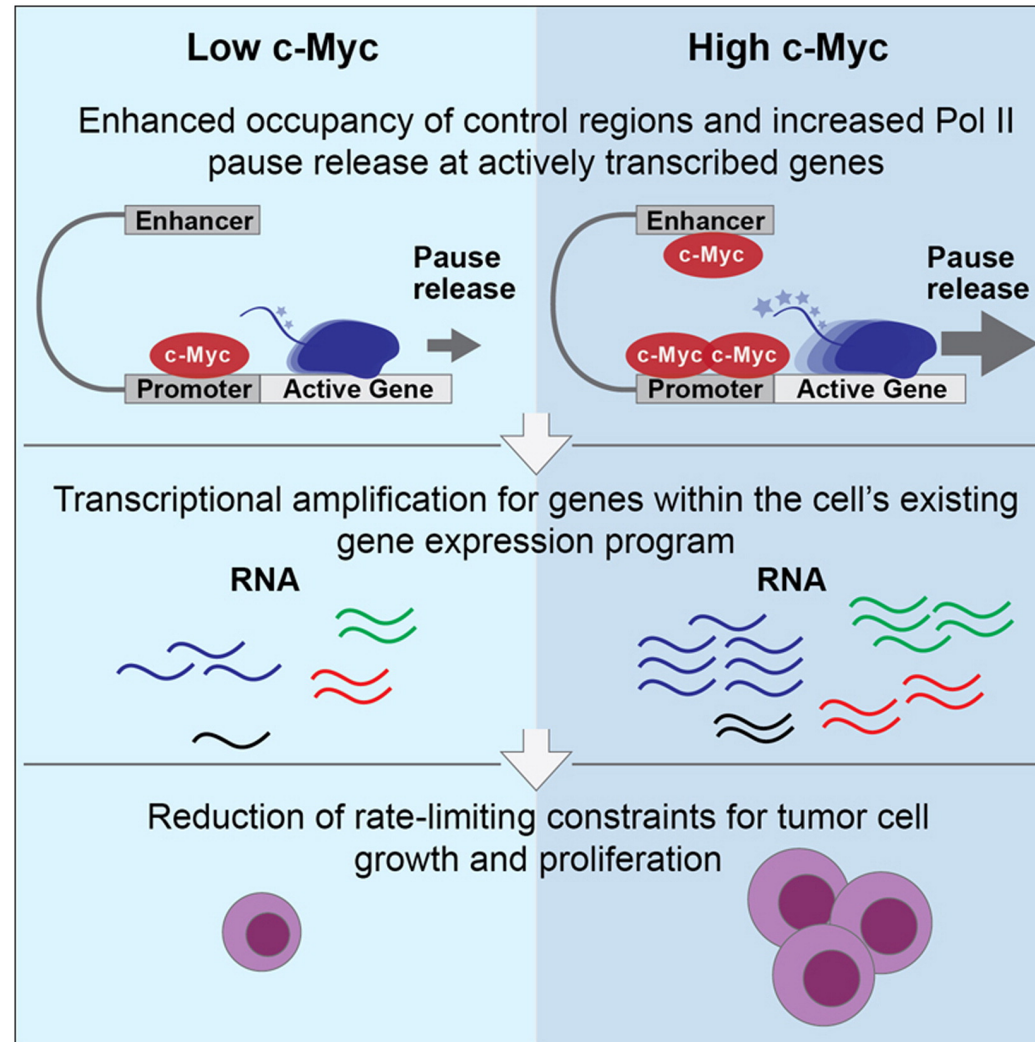
MYC family of transcription factors

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} Xiaoli 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 Xiaoli 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

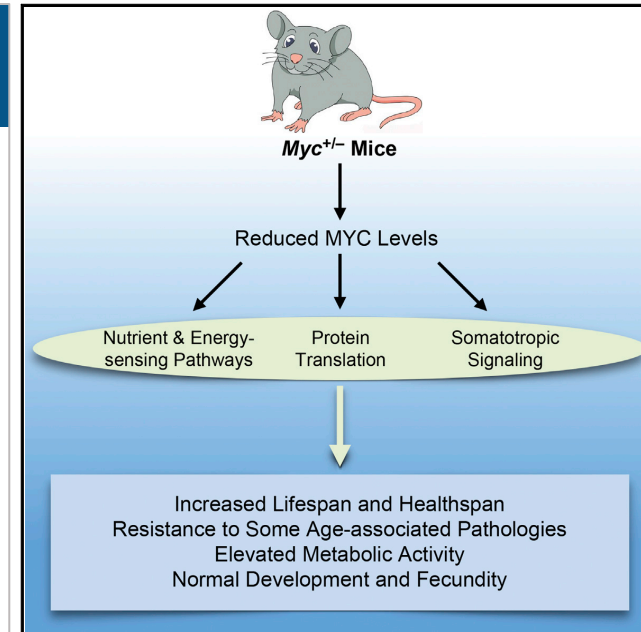
⁴Hallett Center for Diabetes and Endocrinology, Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, RI 02903, USA

⁵Department of Cognitive, Linguistic, and Psychological Sciences, Brown University, Providence, RI 02912, USA

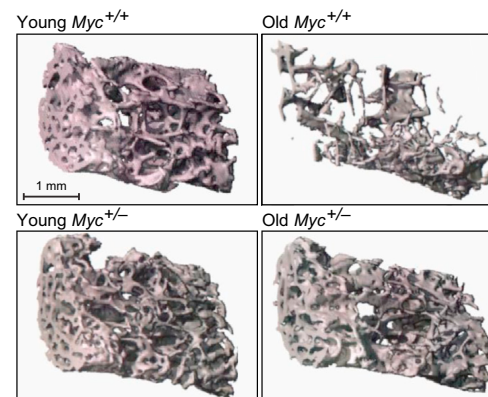
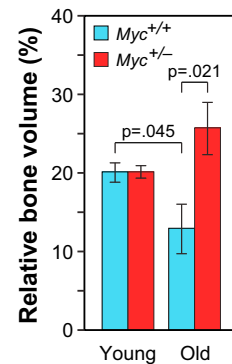
⁶Department of Pathology and Geriatrics Center, University of Michigan, Ann Arbor, MI 48109, USA

⁷Co-first author

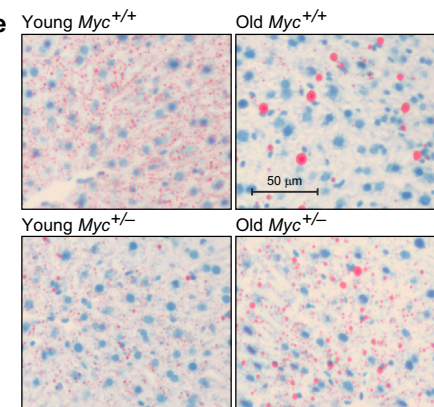
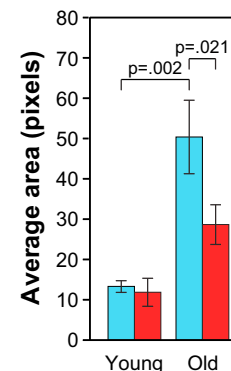
*Correspondence: john_sedivy@brown.edu
<http://dx.doi.org/10.1016/j.cell.2014.12.016>



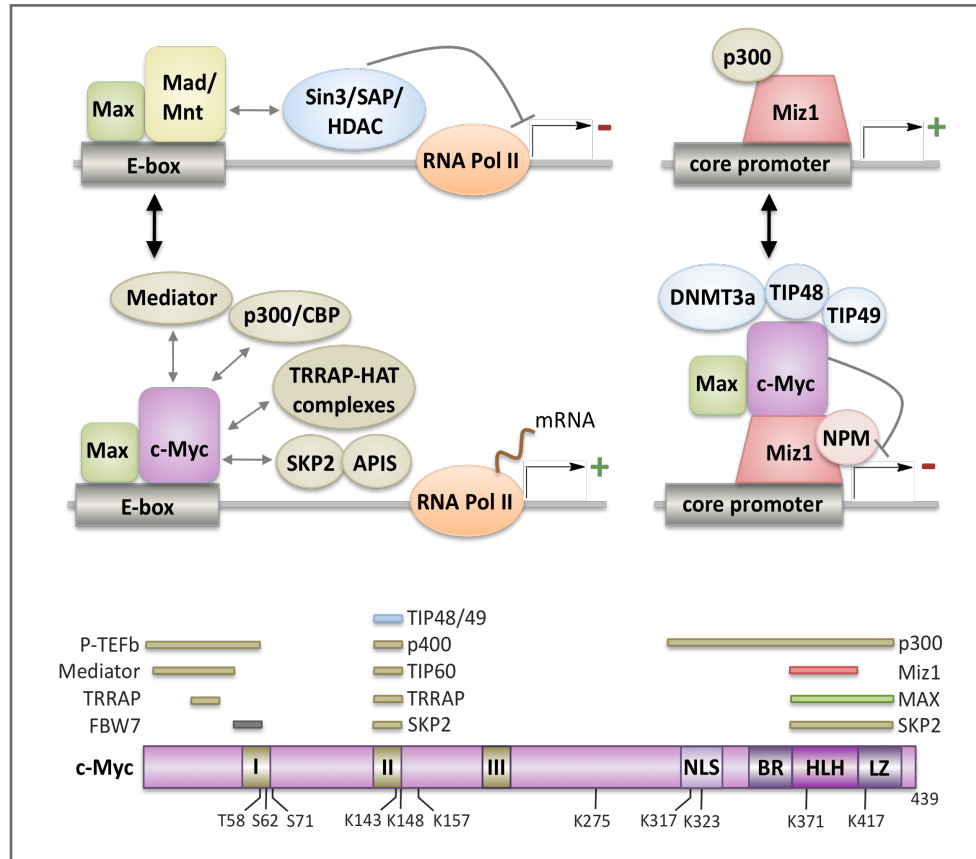
B Bone density



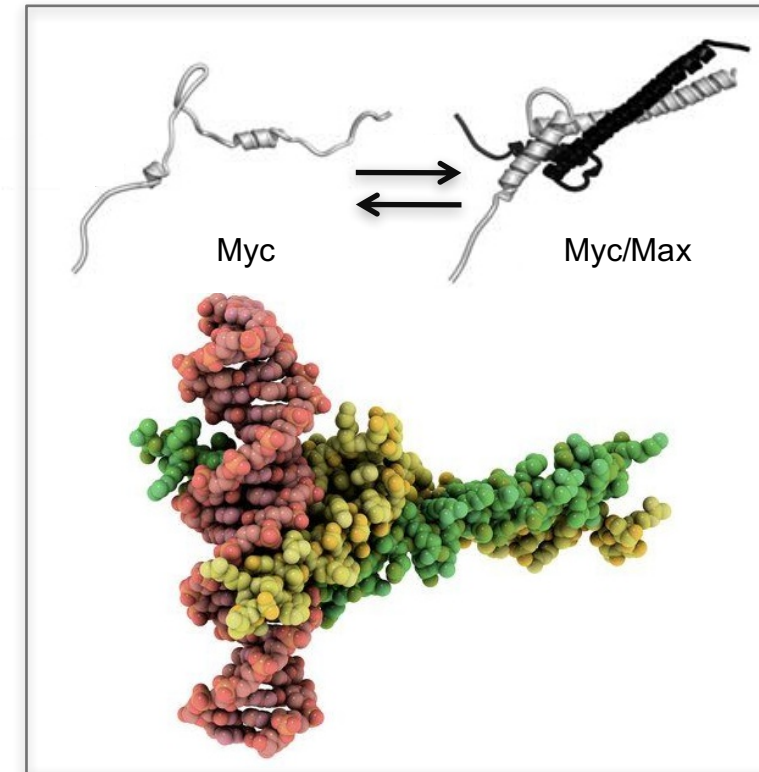
D Lipid droplet size



An obstinate therapeutic target

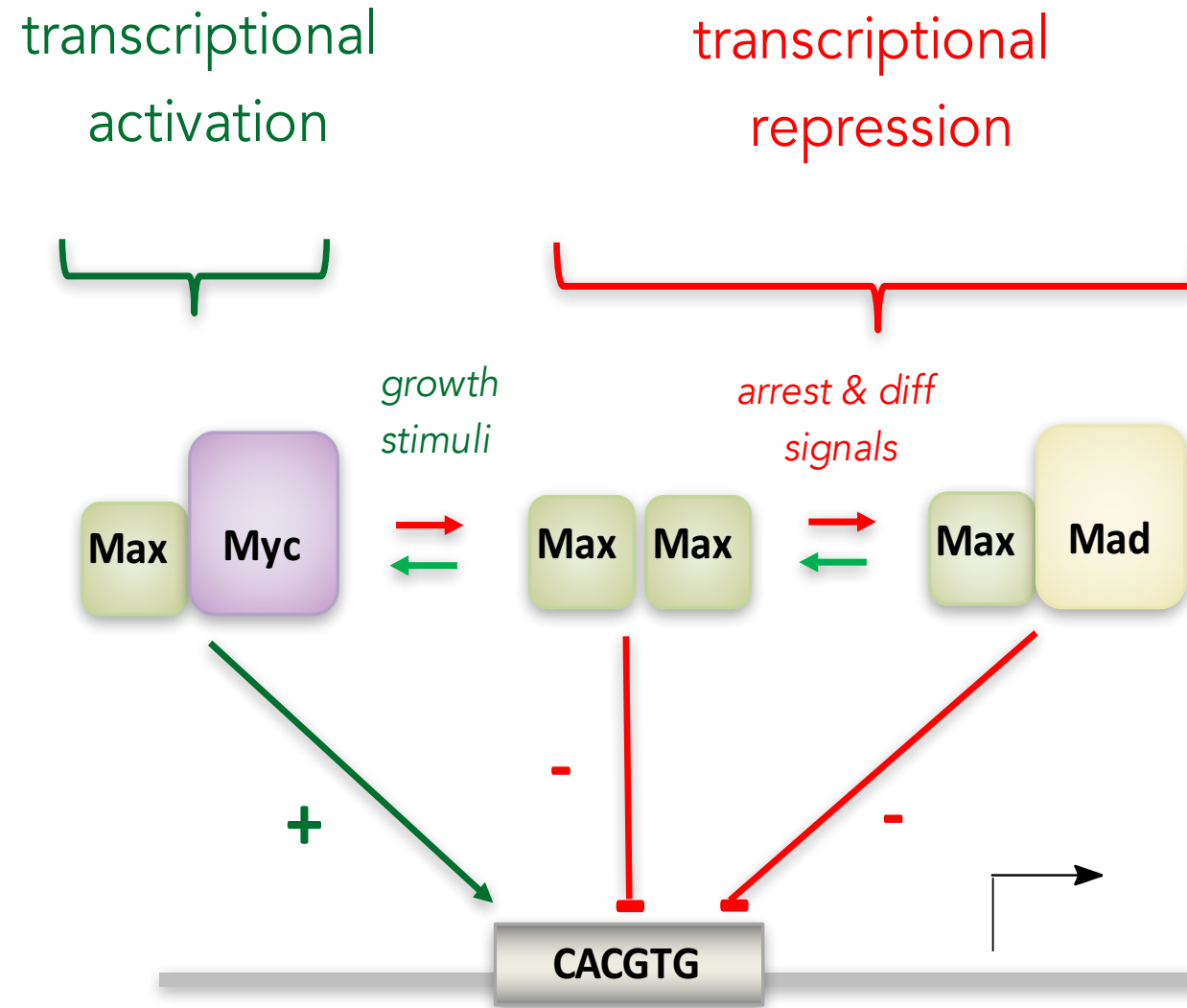


many protein-protein interactions

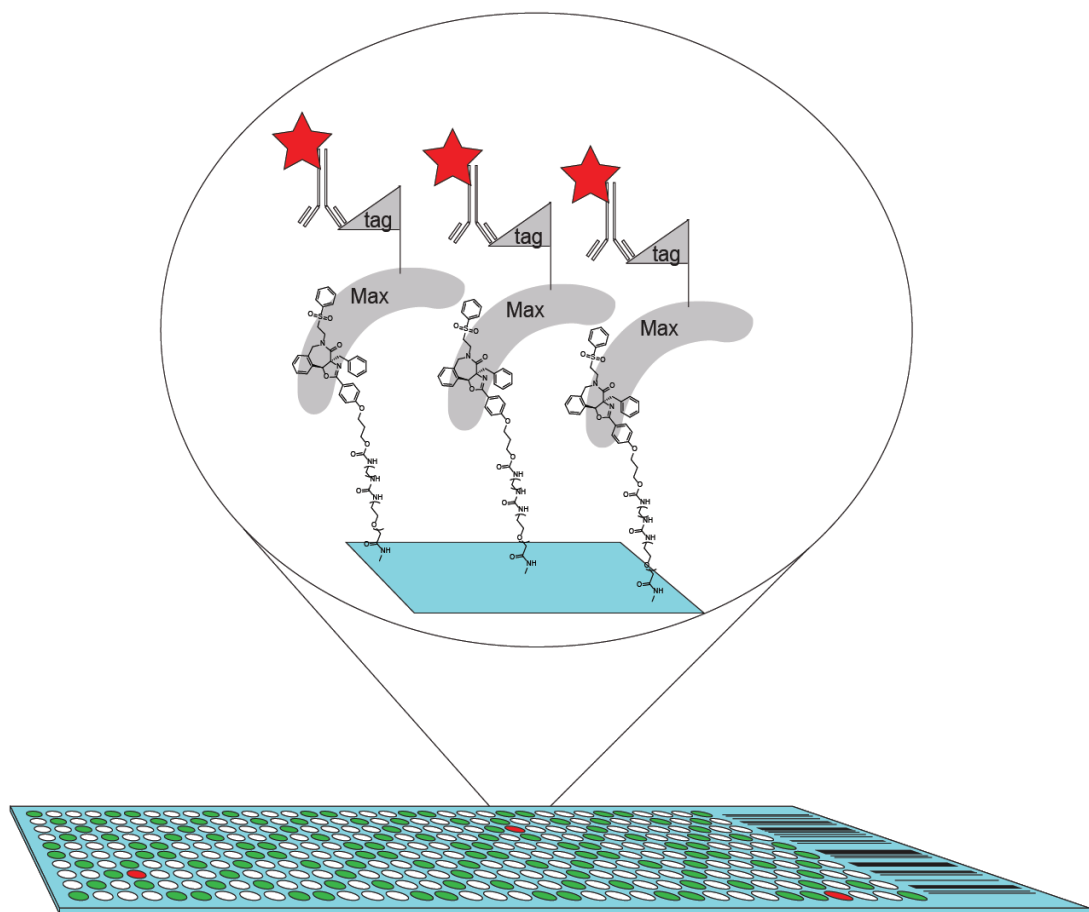


unstructured domains
no traditional binding pockets
large buried interface

Max as a target: heterodimer/homodimer dynamics

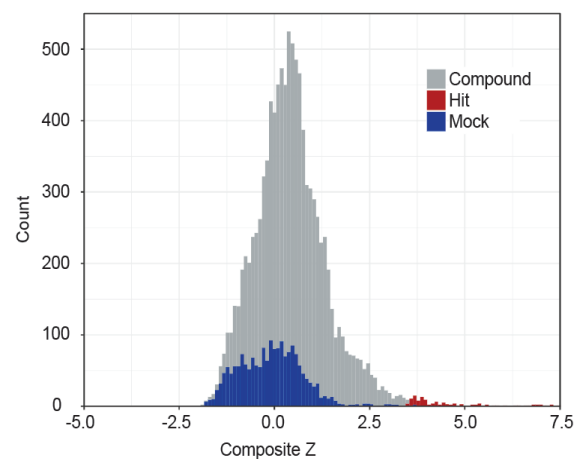


SMM screens: purified Max transcription factor

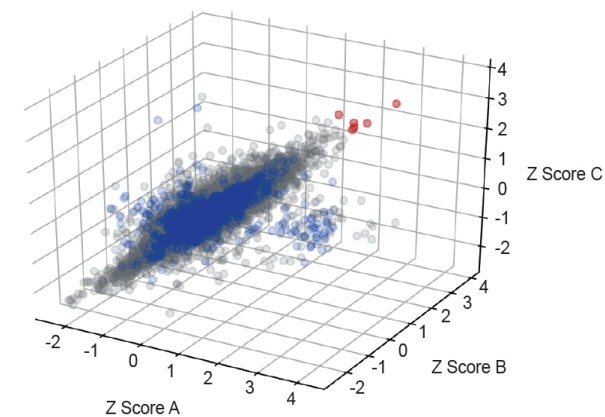
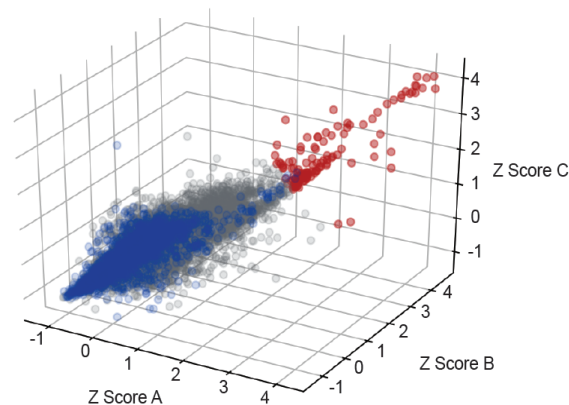
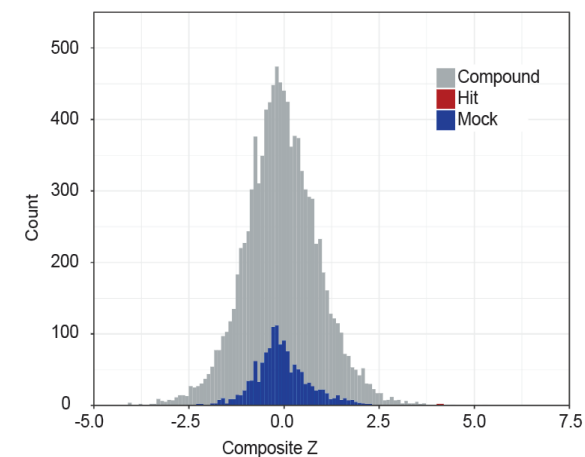


>45k compounds screened

synthetic compound collection

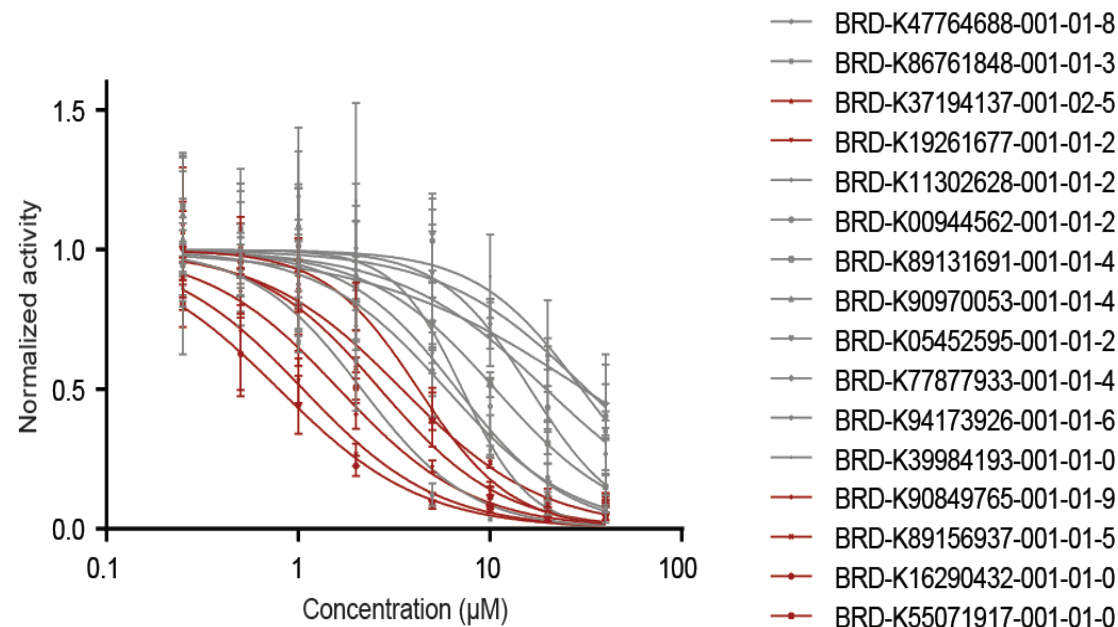
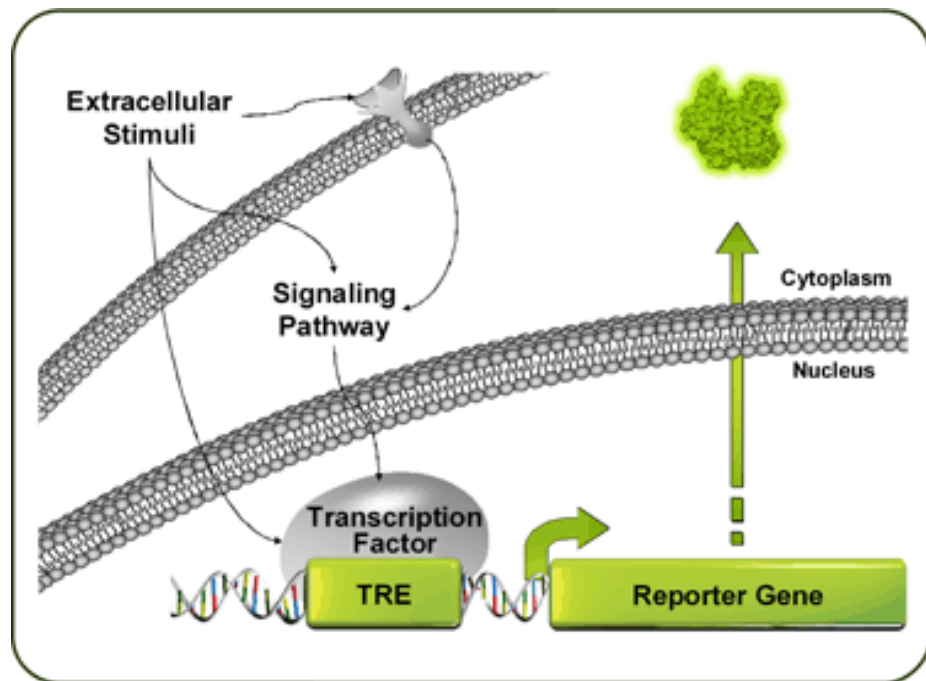


natural products

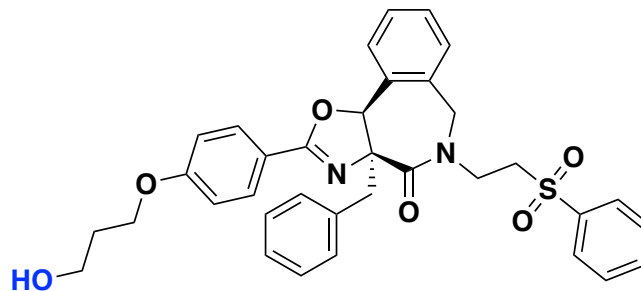


117 assay positives

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



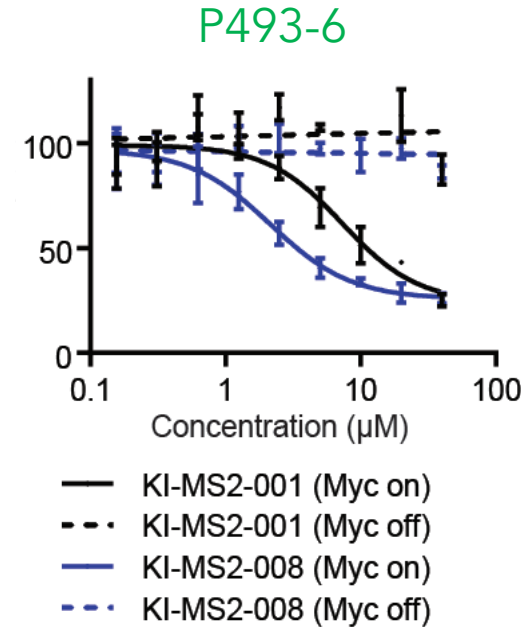
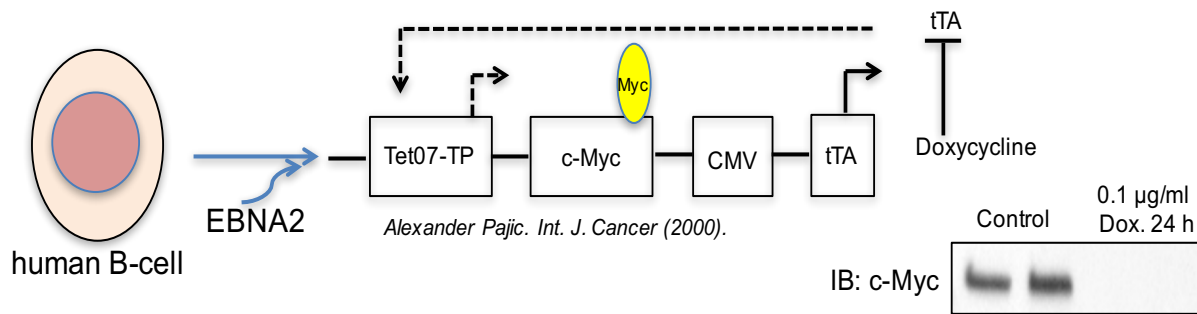
'KI-MS2'



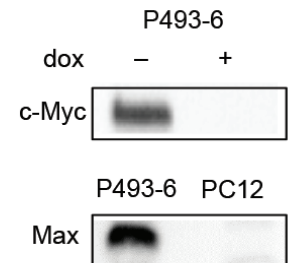
$IC_{50} = 1.06 \mu M$
MW = 610.73
cLogP = 5.15

Cell viability assays: Are Myc or Max required?

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

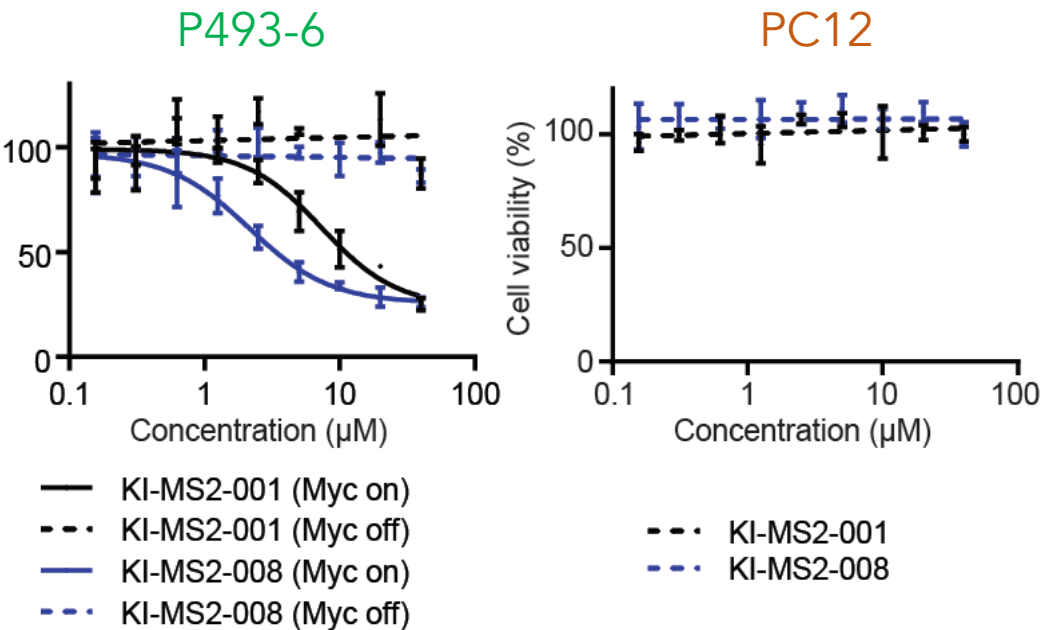
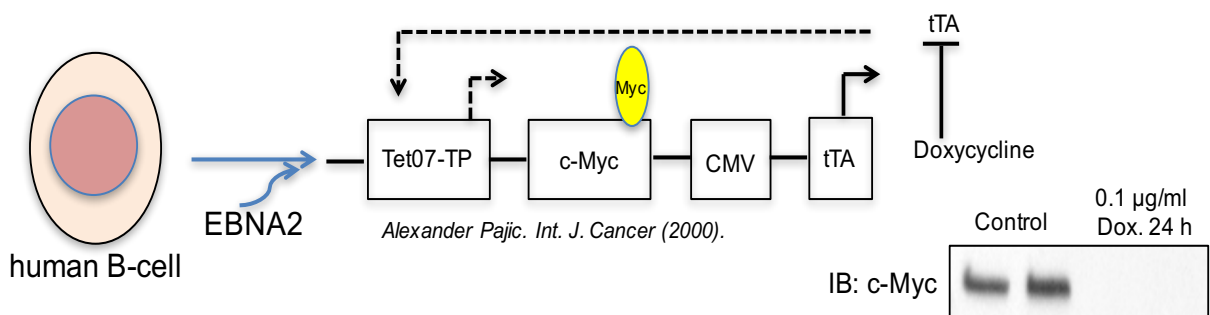


	KI-MS2-001	KI-MS2-008
Myc reporter	1.98 µM	1.28 µM
P493-6 Myc on	7.36 µM	2.15 µM
P493-6 Myc off	>50 µM	>50 µM



Cell viability assays: Are Myc or Max required?

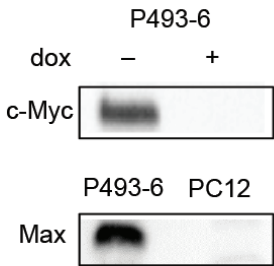
P493-6 Dox-repressible cells for MYC 'on/off' studies



Max-deficient PC12 pheochromocytoma cells



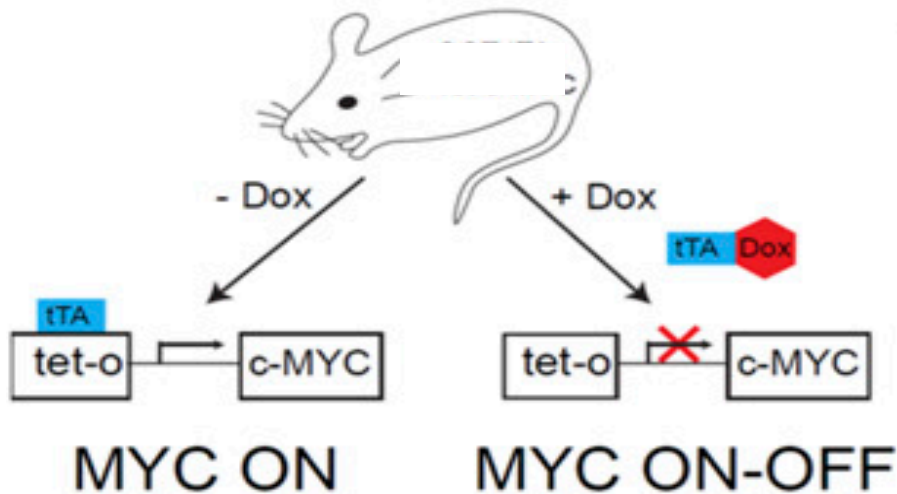
	KI-MS2-001	KI-MS2-008
Myc reporter	1.98 µM	1.28 µM
P493-6 Myc on	7.36 µM	2.15 µM
P493-6 Myc off	>50 µM	>50 µM
PC12	>50 µM	>50 µM



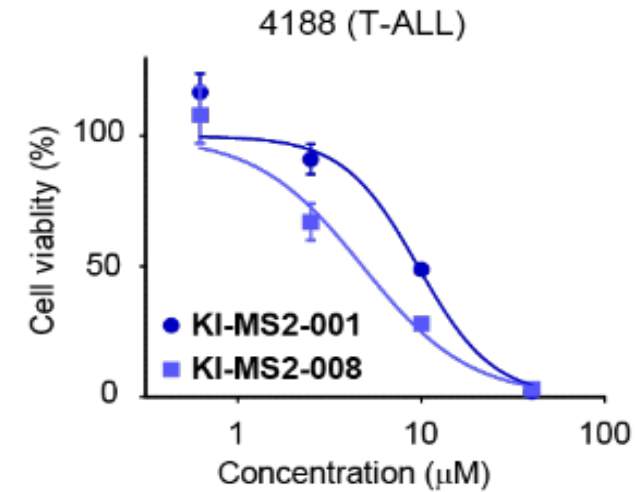
Conditional cellular models of *MYC* expression

Myc 'on/off' mouse models:

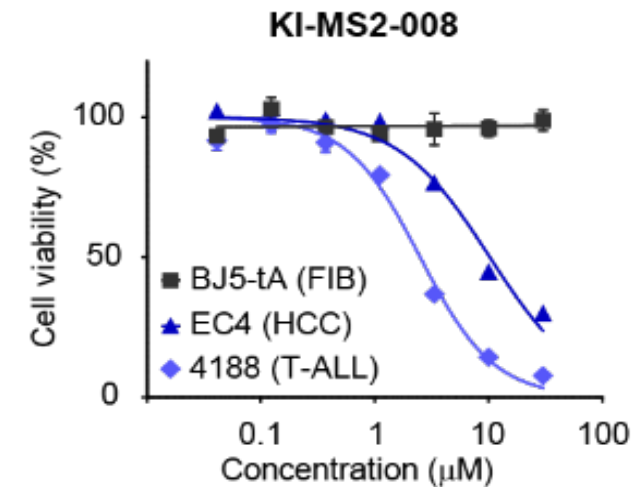
lymphoma
HCC
RCC
osteosarcoma



a

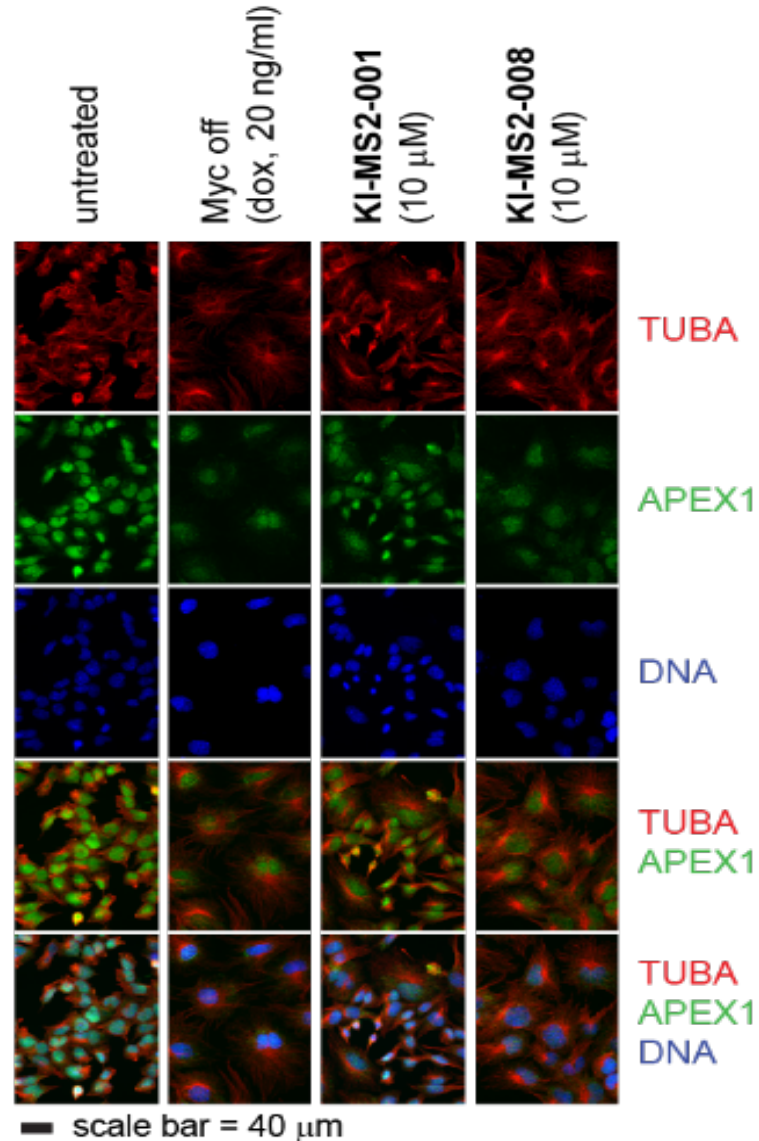


b



Imaging of biomarkers: conditional vs. chemical modulation

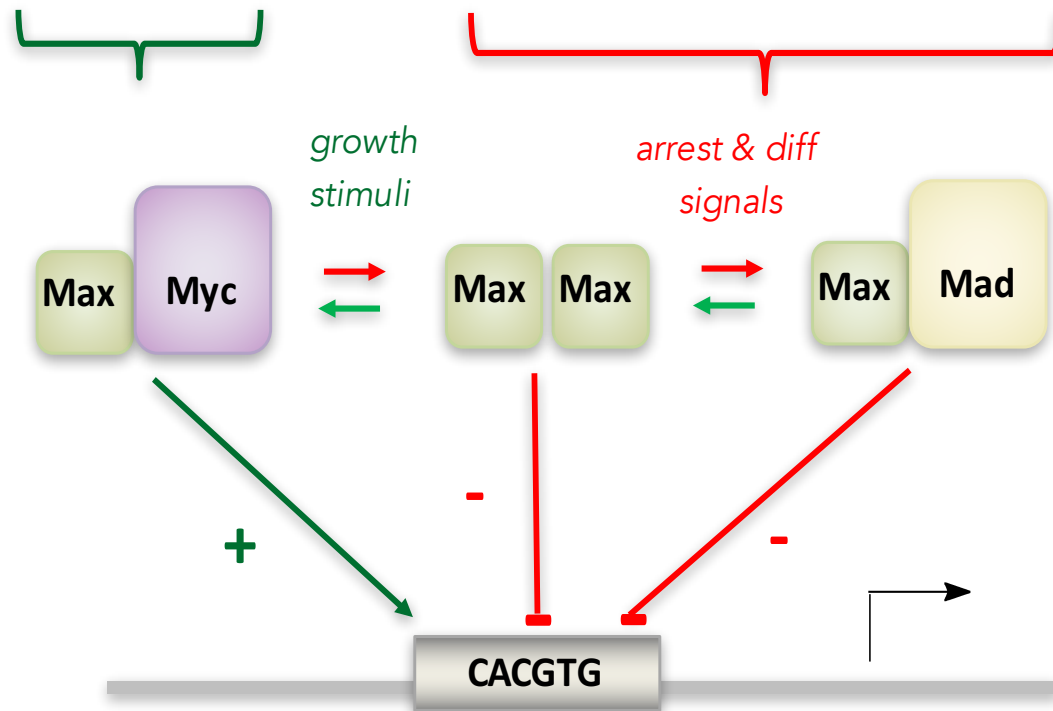
modulating Myc in an engineered osteosarcoma model



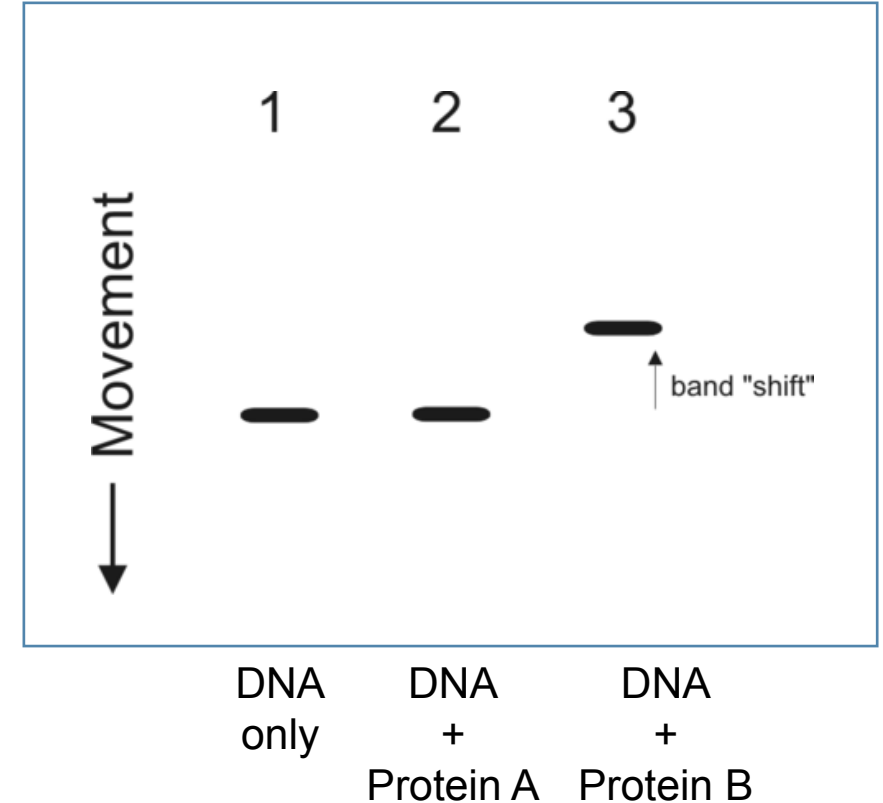
Does the probe antagonize the Myc/Max heterodimer?

transcriptional
activation

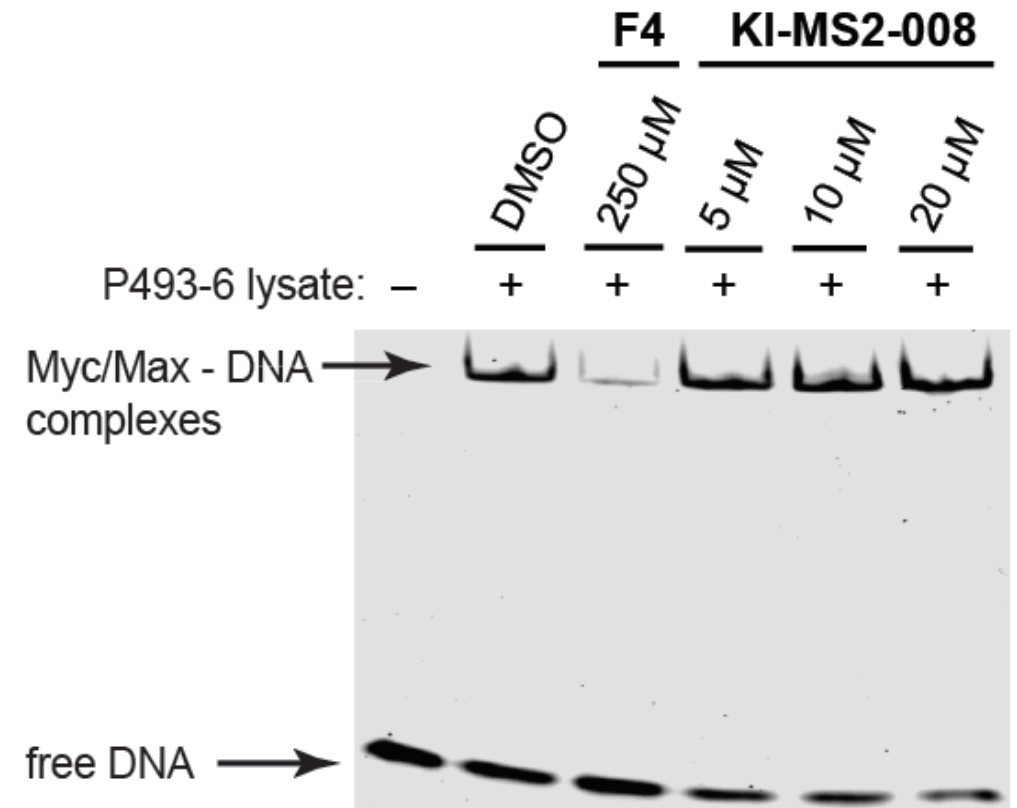
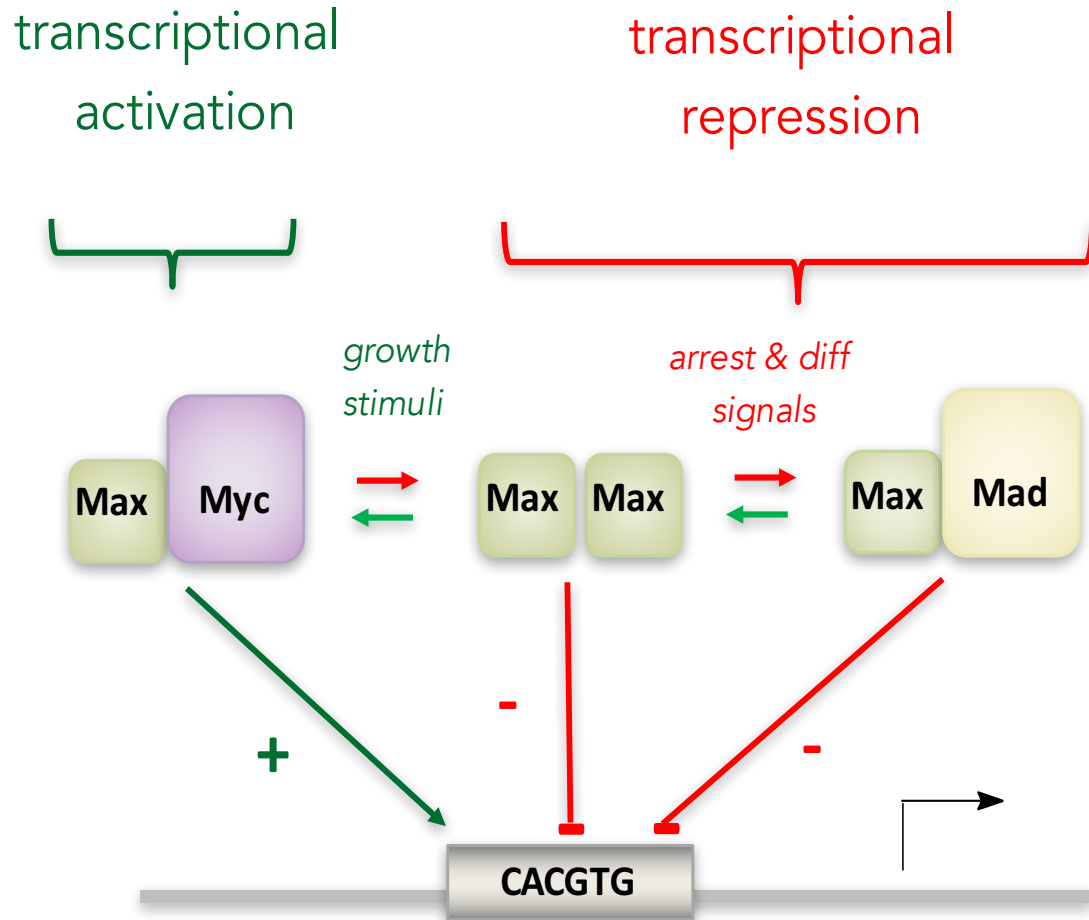
transcriptional
repression



Electrophoretic Mobility Shift Assay (EMSA)
aka Gel Shift Assay



Does the probe antagonize the Myc/Max heterodimer?

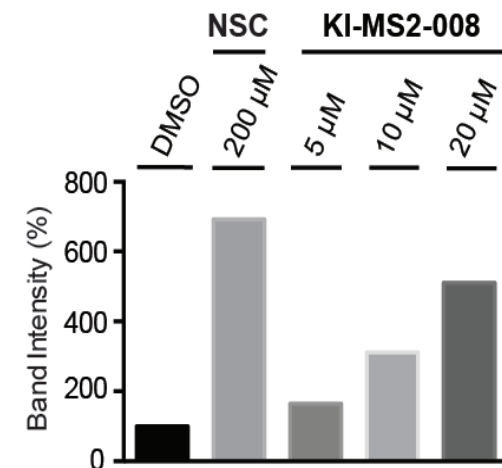
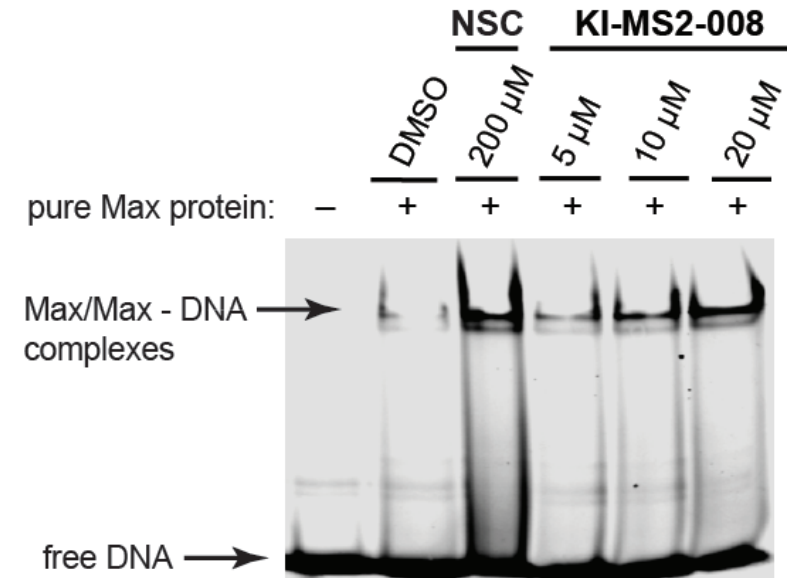
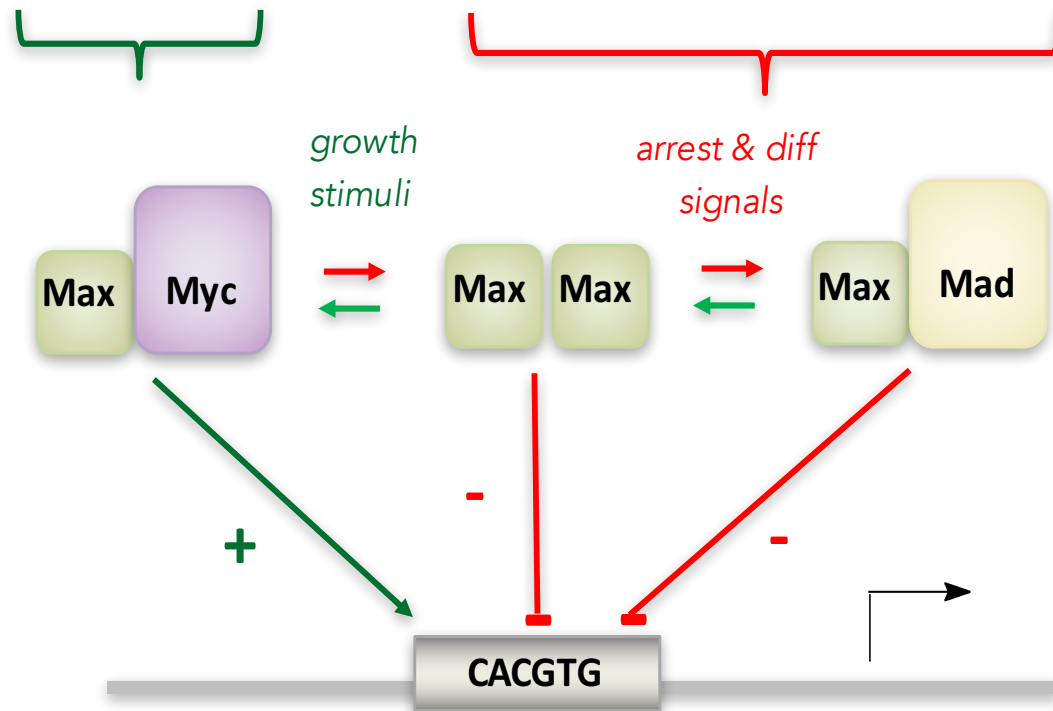


EMSA

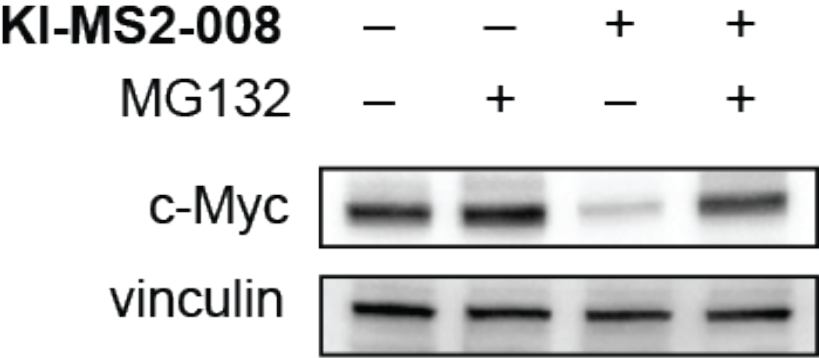
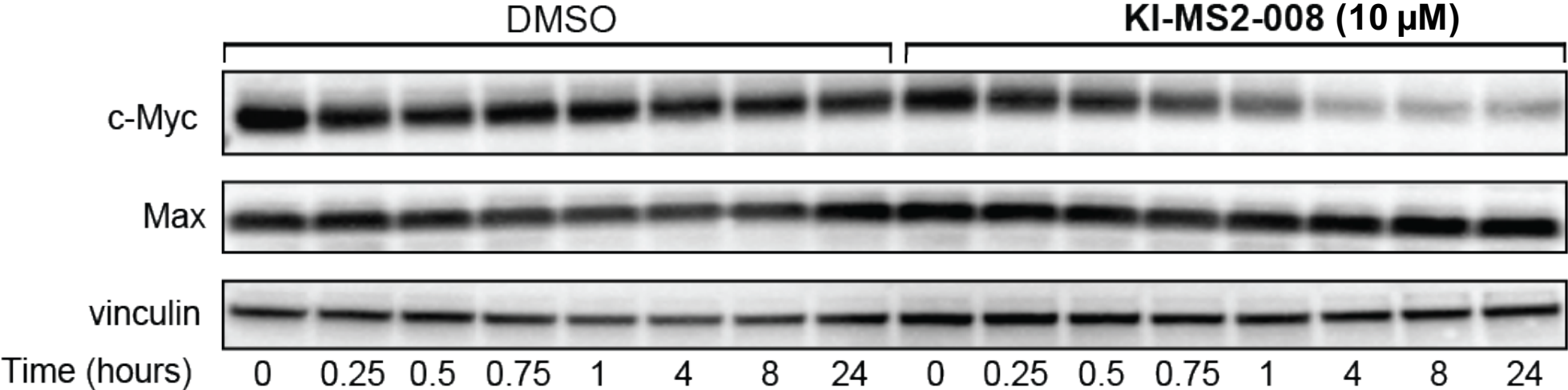
Does the probe stabilize the Max/Max homodimer?

transcriptional
activation

transcriptional
repression

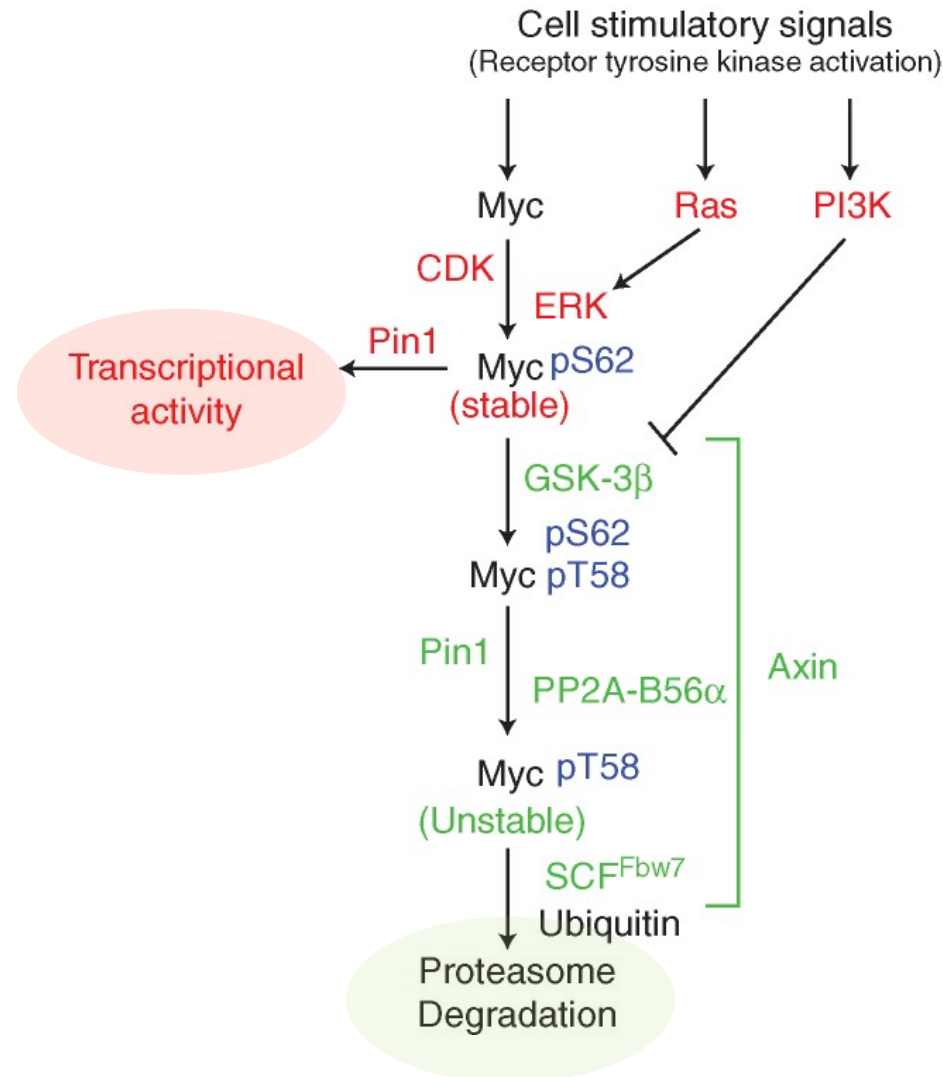


Western blots: KI-MS2-008 alters Myc protein levels



rescue experiment with
10 μ M proteasome inhibitor
MG132

Myc protein stability is regulated by the ubiquitin-proteasome system

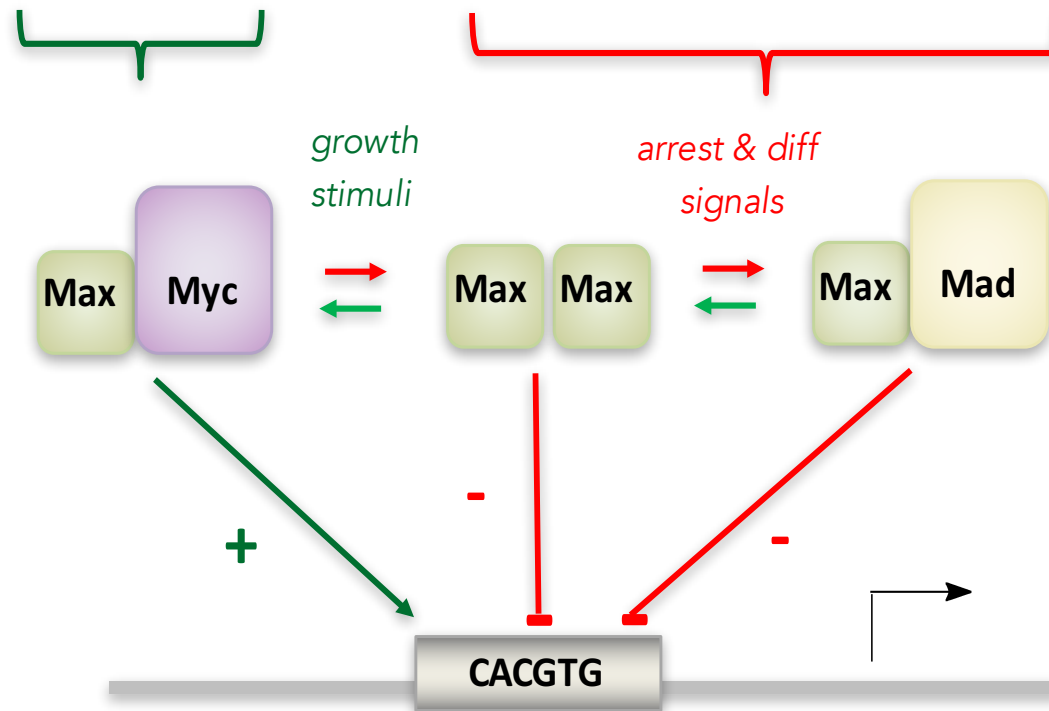


KI-MS2-008

mixed mechanism inhibitor?

transcriptional
activation

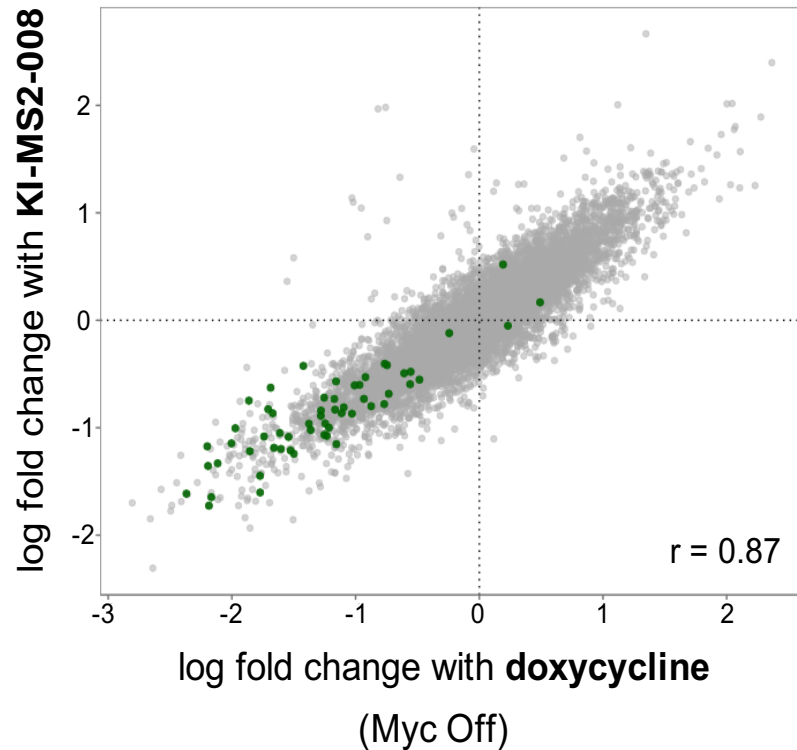
transcriptional
repression



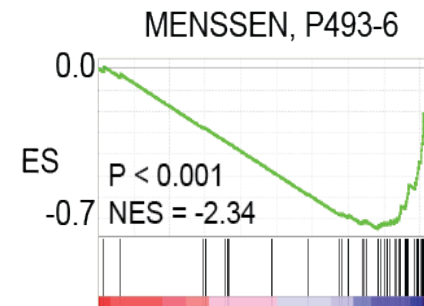
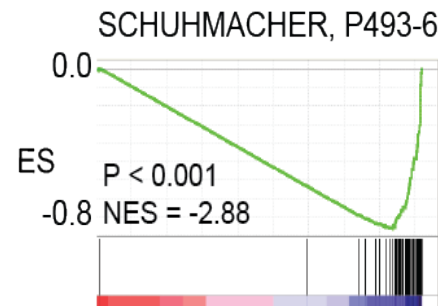
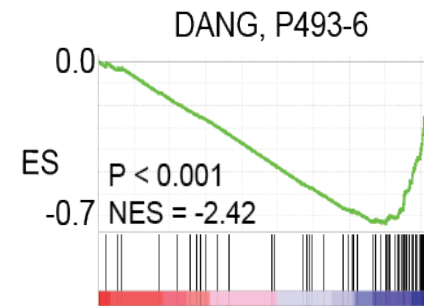
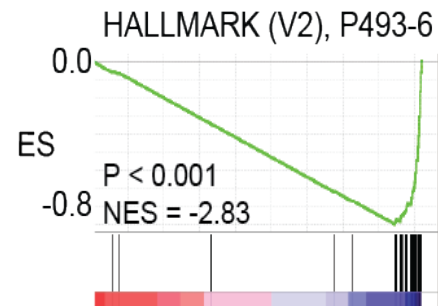
Gene expression profiling: KI-MS2-008 mimics *MYC* inactivation

Gene Set Enrichment Analysis reveals an enrichment of Myc target genes

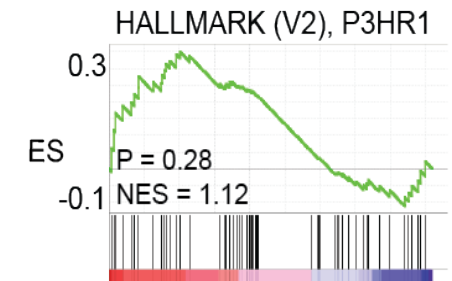
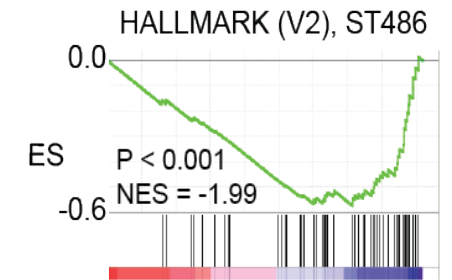
Hallmark V2 MYC-dependent genes in green



sensitive
P493-6



sensitive
Burkitt's lymphoma

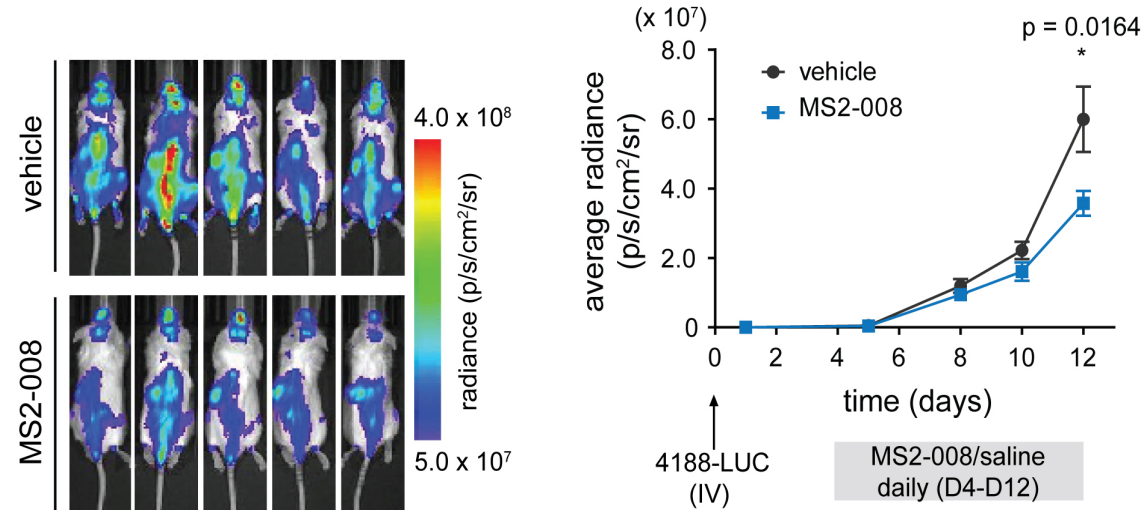


insensitive
Burkitt's lymphoma

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

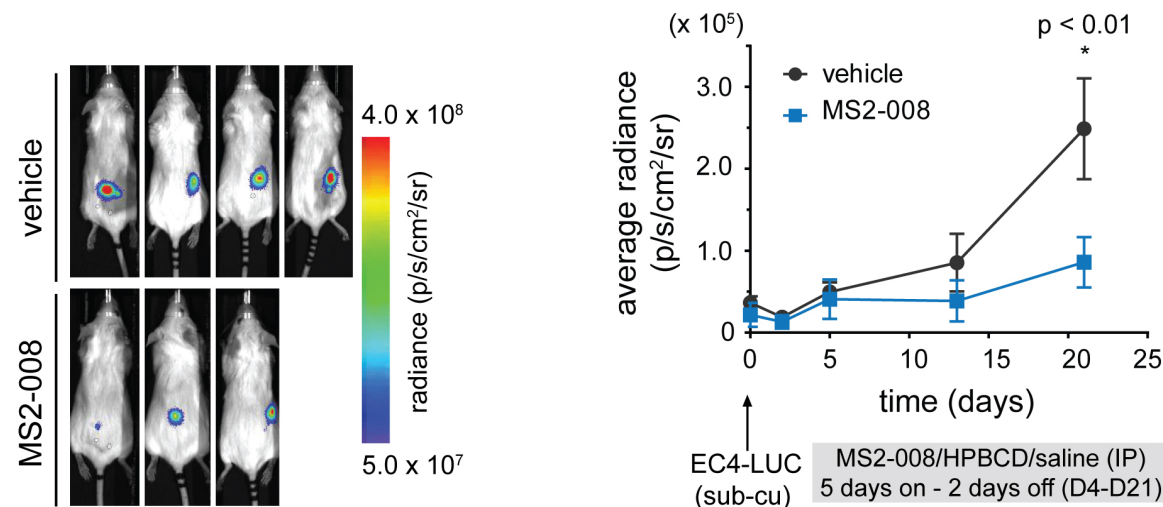
In vivo studies: KI-MS2-008 modulates tumor volume in Myc-dependent mouse models of cancer

T-cell acute
lymphoblastic
leukemia
blood cancer



0.06 mg/kg
daily IV administration

hepatocellular
carcinoma
solid tumor



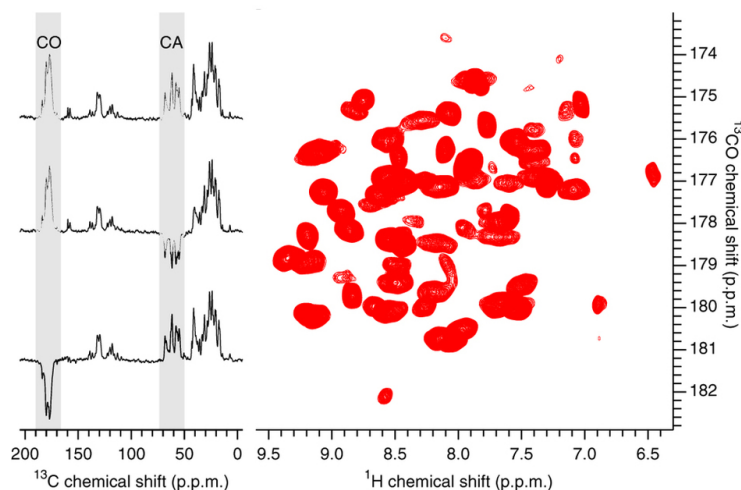
0.24 mg/kg
subcutaneous administration
5d on/2d off cycles

Current directions

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



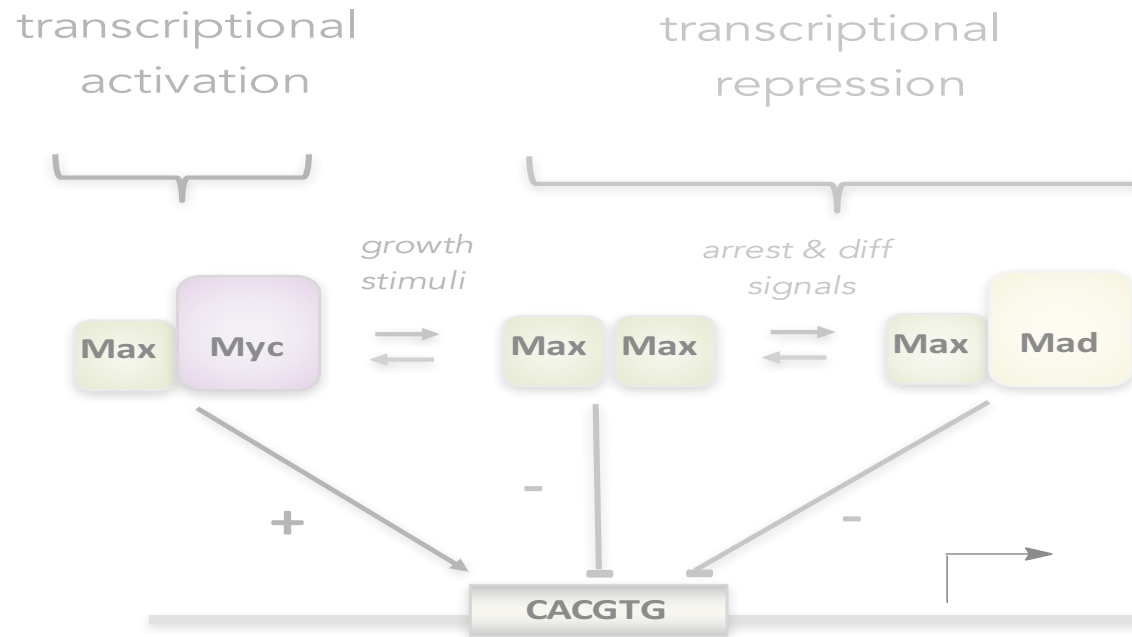
structural biology



additional tumor models
same tumor models + new readouts



stabilizing repressive states vs. inhibiting activating states?



stabilizing vs. inhibiting PPIs?

Our path to evaluate ligands - lectures

2/8/17	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
2/13/17	Lecture 2	For the love of proteins: FKBP12 and immunophilins
2/15/17	Lecture 3	Small-molecule microarrays
2/21/17	No Lecture	
2/22/17	Lecture 4	Quantitative evaluation of protein-ligand interactions
2/27/17	Lecture 5	A ligand discovery vignette: sonic hedgehog
3/1/17	Lecture 6	Engineering transcriptional responses with a small molecule
3/7/17	Lecture 7	Wrap up discussion: suggestions for how to report your findings