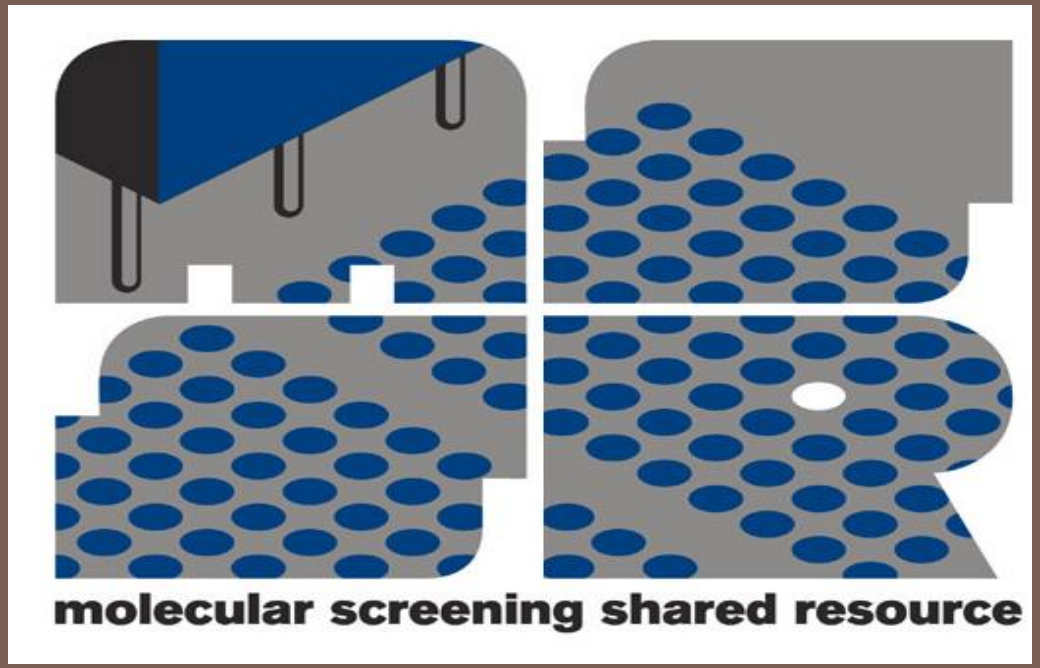


HIGH THROUGHPUT BIOLOGY

ROBERT DAMOISEAUX, PH.D.
PROFESSOR, MOLECULAR AND MEDICINAL PHARMACOLOGY
DIRECTOR, MOLECULAR SCREENING SHARE RESOURCE

Disclosure

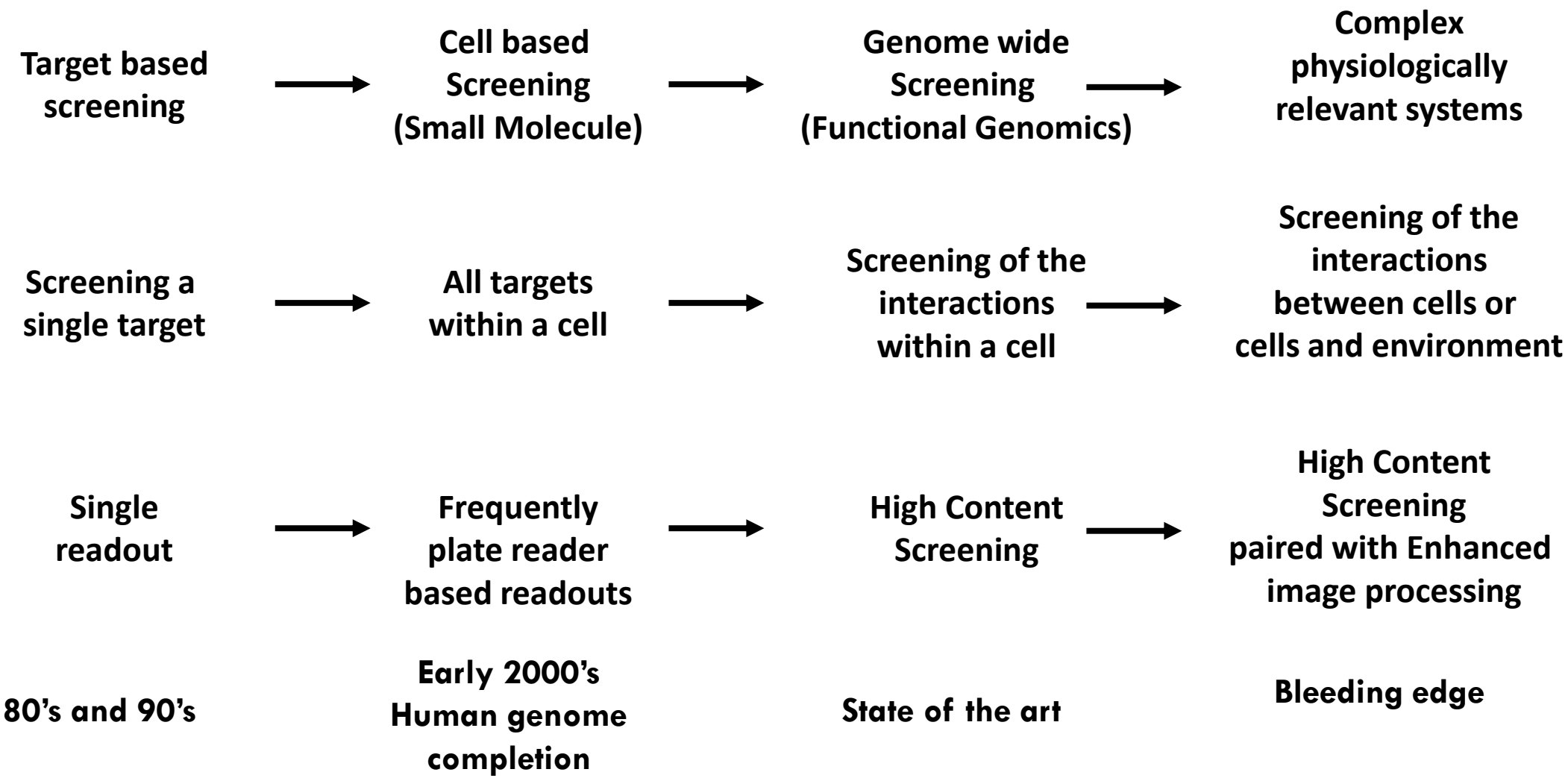
- I am co-founder of Forcyte Biotechnologies that aims at commercializing force-phenotyping for drug discovery.
- I am co-founder of Pharma15 which commercializes antibodies.
- I am co-founder of Enspire Bio that commercializes IP from the School of Medicine Metabolism theme.
- I am also consultant to Amgen and Panorama Medicine

Overview

- **A short introduction to the High Throughput Screening and the Drug Discovery efforts at the MSSR**
- **High Throughput Biology: Small molecule vs chemical genomics**
- **Making and assessing model systems with CRISPR (the good, the bad and the ugly)**

THE MOLECULAR SCREENING SHARED RESOURCE
AN OVERVIEW

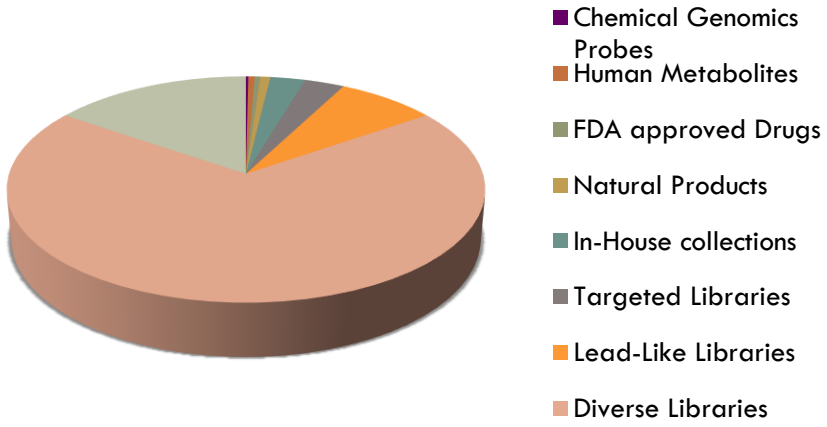
The Evolution of High Throughput Screening (HTS)



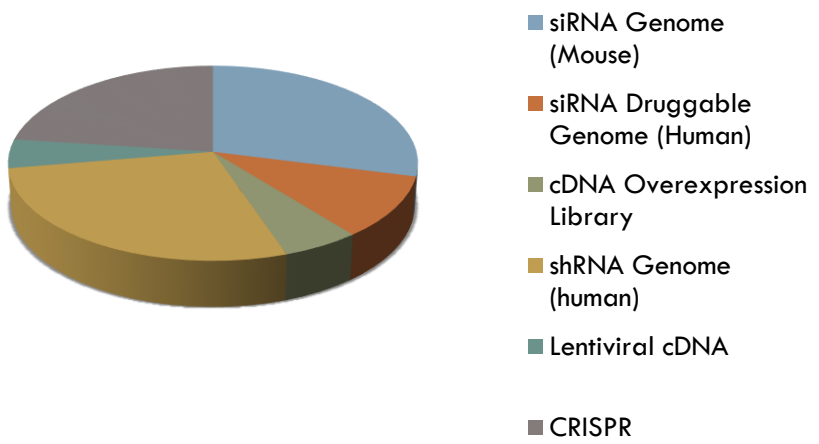
High Throughput Screening is the Main Focus of the MSSR

The MSSR is housed centrally at UCLA in the California NanoSystems Institute in 3600 sqf BSL-2+ approved space.

~ 300k small molecules



~ 291k functional genomics probes



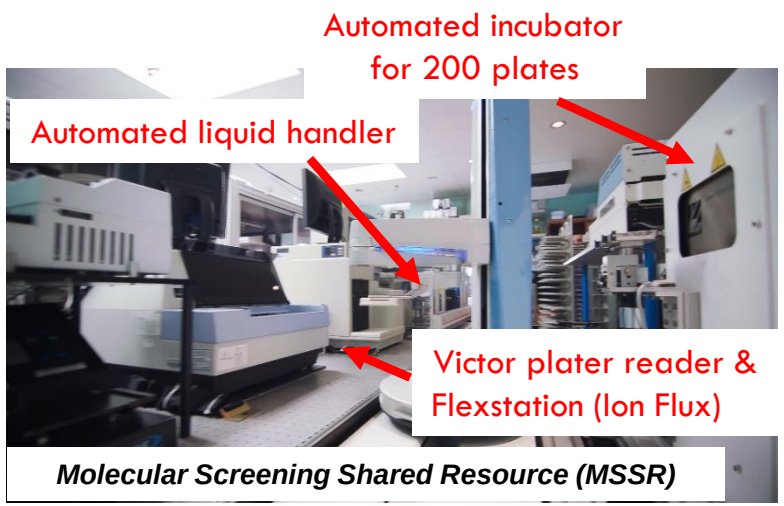
Genome wide CRISPR pool screening is available, a genome wide arrayed CRISPR set is available as well.

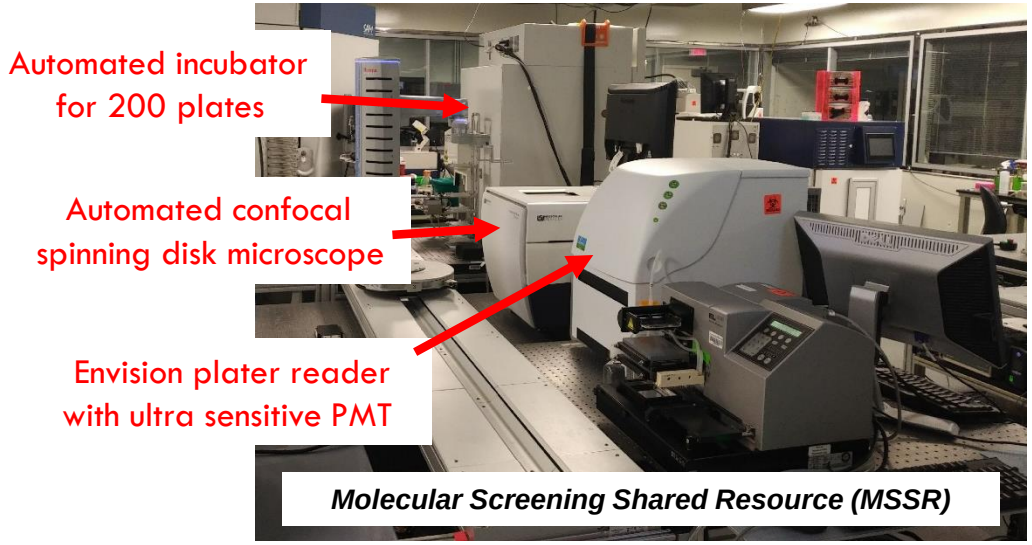
Success rate is ~90% for the discovery of tractable small molecule hits from HTS campaigns.

A Closer Look at Our Functional Genomics Libraries

- CRISPR: Human Genome: ~120k gRNA's covering the full genome
  - ~6 guides per target in a pCLIP-Dual lentiviral vector expressing GFP and two guides per vector (individual clones available)
- shRNA: Human genome: ~75k shRNA's covering the full genome
  - ~3 shRNA's per target in pGIPZ lentiviral vector expression GFP, (individual clones available)
  - Broken into sublibraries against high value targets (kinases, GPCRs etc…)
- Lentiviral cDNA: ½ human genome, ~12k constructs
  - One construct per target in a lentiviral pLEX304 expression vector with c-term tag, (individual clones available)
- Transfection based cDNA: MGC collection, ~ 16k constructs
  - One to two constructs per gene, mouse and human mixed set in pSPORT6, (individual clones available)
- siRNA: Full genome for mouse, druggable for mouse and human
  - 3 or 4 siRNA's per target, non-pooled for easy screening and follow up

The Tools of the Trade

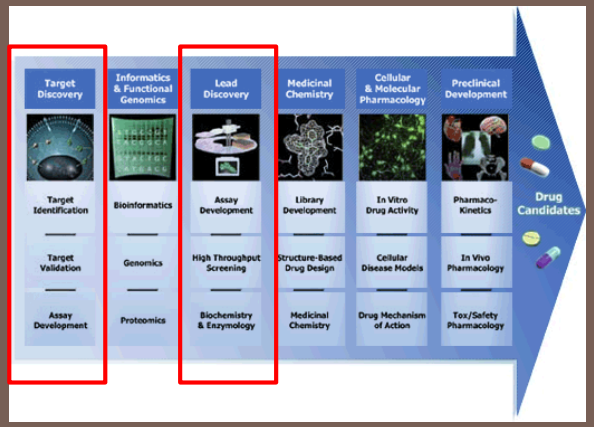




Large Particle Sorter
Sorts Everything from Zebrafish to Solid Support Chemistry Beads!

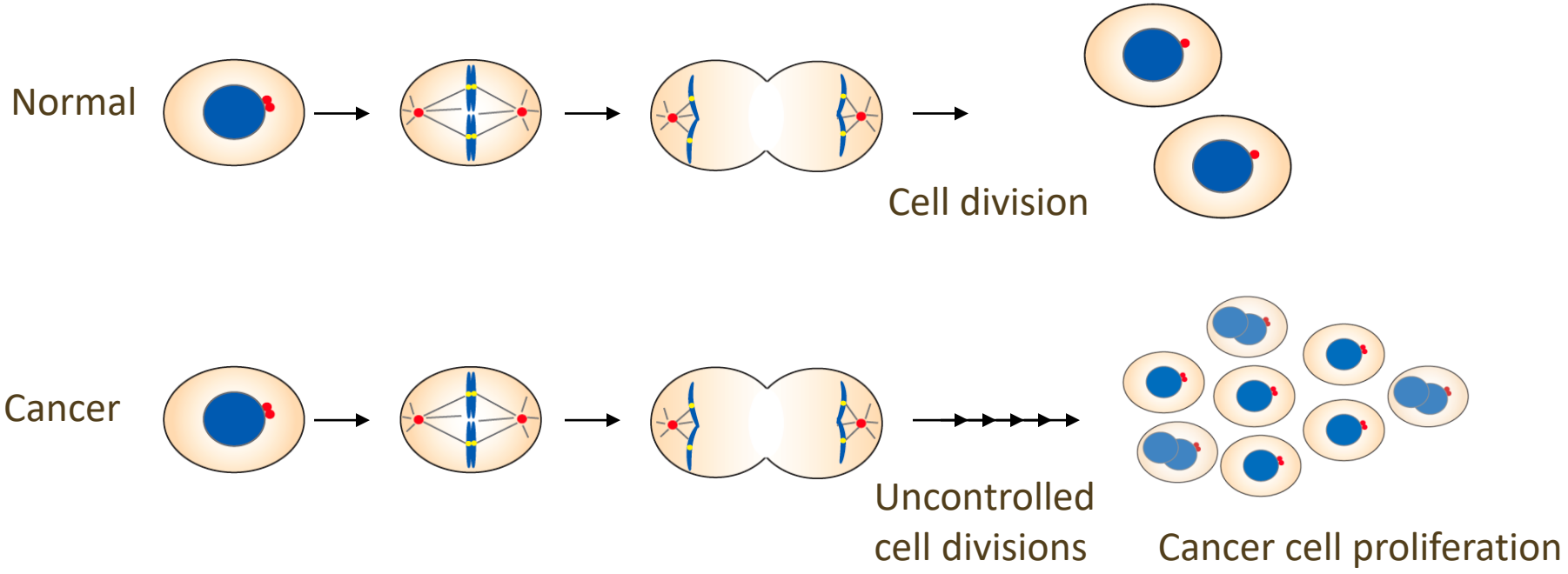
MSSR Affiliations at UCLA and a Few Facts

- The MSSR was leveraged for a total of >$250 million in grant funding since 2004.
- Since its inception in 2004, roughly 100 papers were published using the MSSR.
- Various compounds discovered at the MSSR are now in clinical trials (phase I and II).
- Operational funding is provided exclusively via grants and recharges.
- The CNSI provides funding for equipment via Garamendi sources to keep equipment a the leading edge.

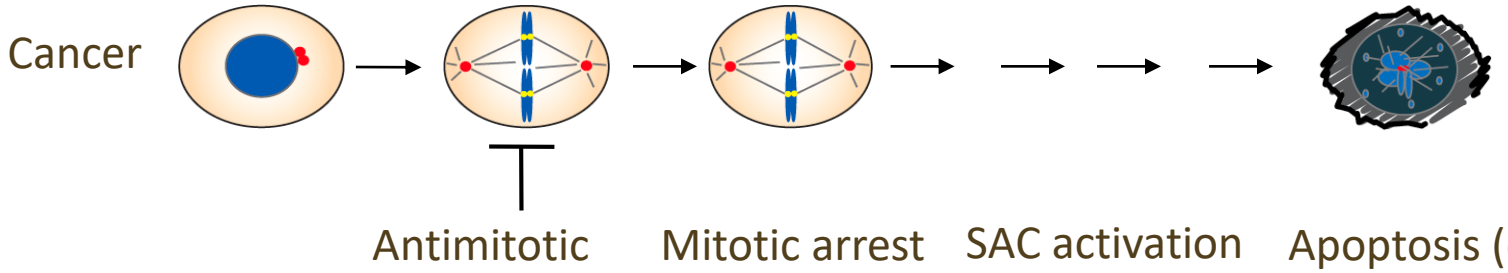


PART 2
SMALL MOLECULE SCREENING AND FUNCTIONAL GENOMICS TO FIND ANTI-CANCER DRUGS AND SPINDLE ASSEMBLY CHECKPOINT SUPPRESSORS

Inhibition of cell division in the treatment of cancer

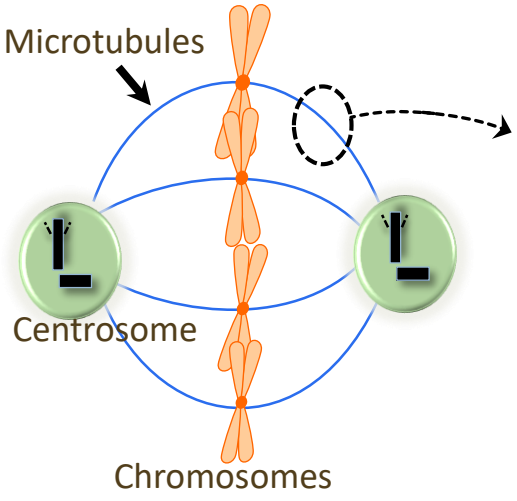


Strategy in the treatment of cancer: inhibit cell division

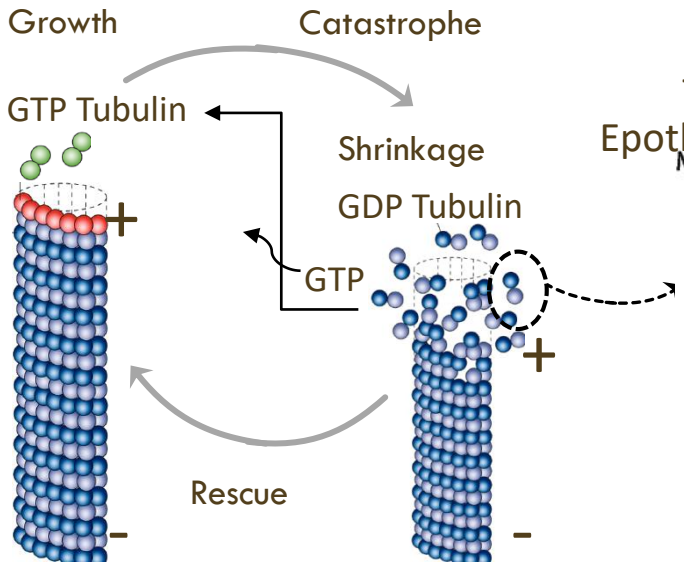


β-Tubulin is a prime target for eliciting M-phase arrest

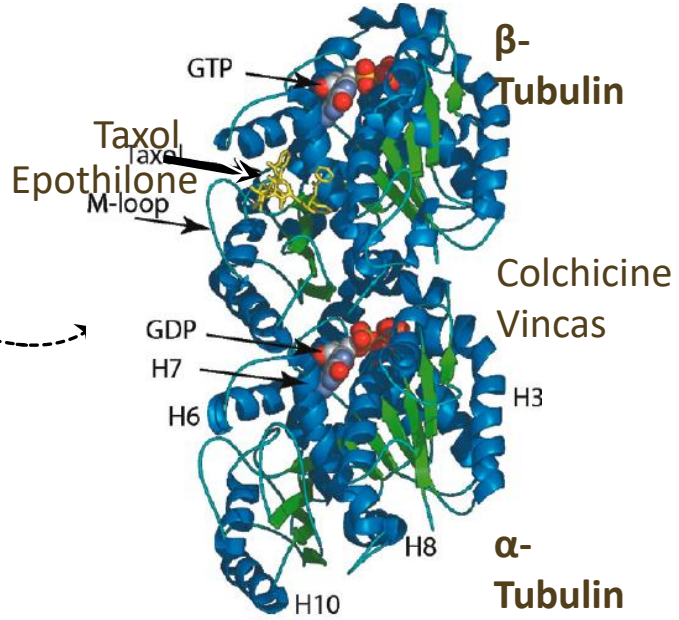
A. Mitotic Spindle



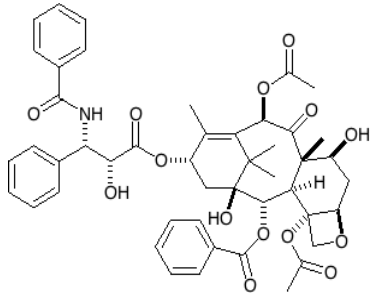
B. Microtubule Dynamic Instability



C. Tubulin Targeting Agents



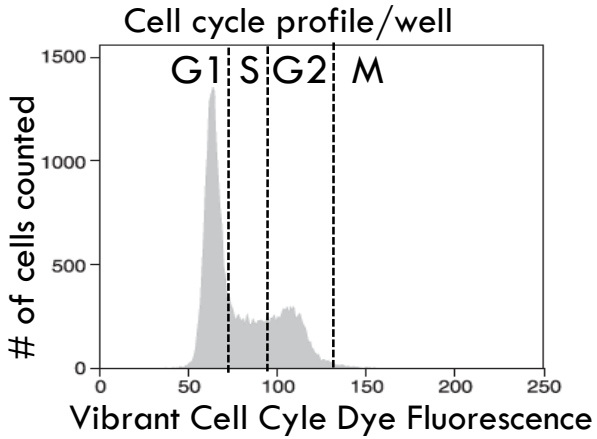
D. Taxol

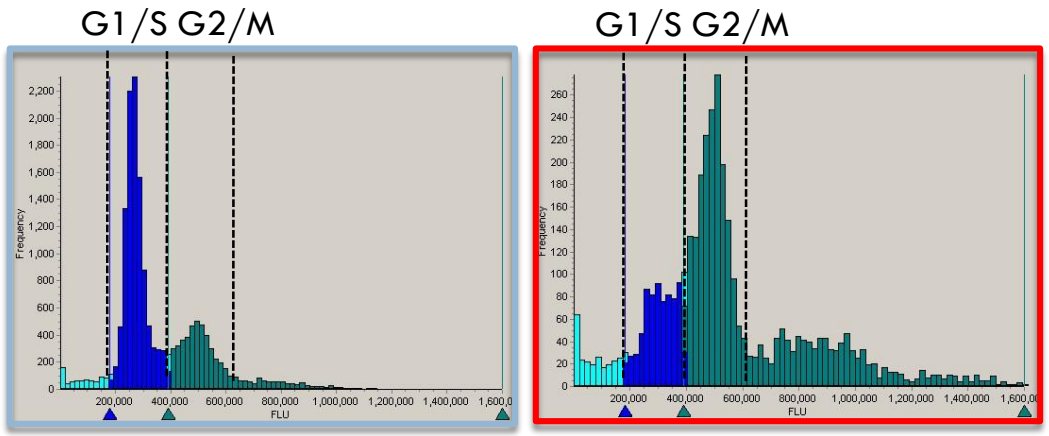


Limitations of Taxol

1. Synthesis/production
2. Poor solubility
3. Vehicle: Cremophor EL
4. Unwanted side-effects
5. Excluded from the brain by BBB and BTB
6. Resistance to Taxol
   a. βIII-Tubulin overexpression
   b. MDR drug efflux pumps
   c. Mutation of βI-Tubulin

Screening for SAC suppressors





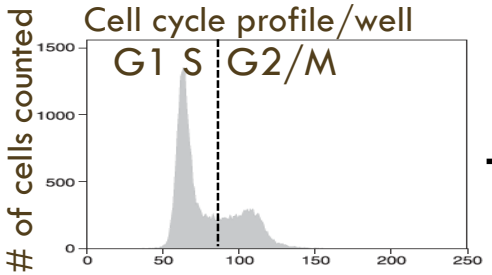
DMSO
Asynchronous

Taxol
G2/M arrest

Dose response, follow up and selection of lead compounds

1. Mitotic arrest

20 hr →



→ Acumen/ImageXpress

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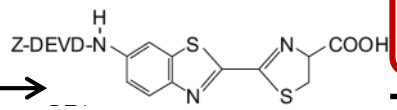
2. Apoptosis

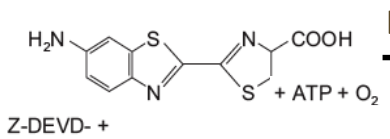
Caspase-Glo (Total caspase activity)

48/72 hr →



Z-DEVD-Luciferin

Active Caspase →



Luciferin

Luciferase
Mg^{2+}
→ Light

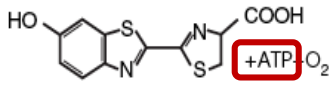
High-throughput luminometer

---

3. Cell Viability

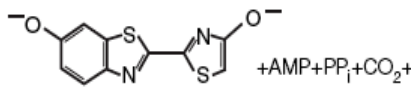
CellTiter-Glo (Total cell number)

48/72 hr →



Luciferin

Luciferase
Mg^2
+
ATP from live cells
→

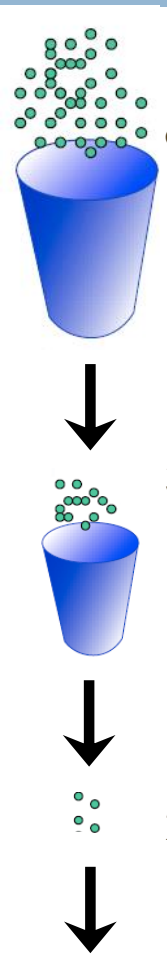


Oxyluciferin

→ Light

High-throughput luminometer

Primary Screening



90,000 drug-like molecules

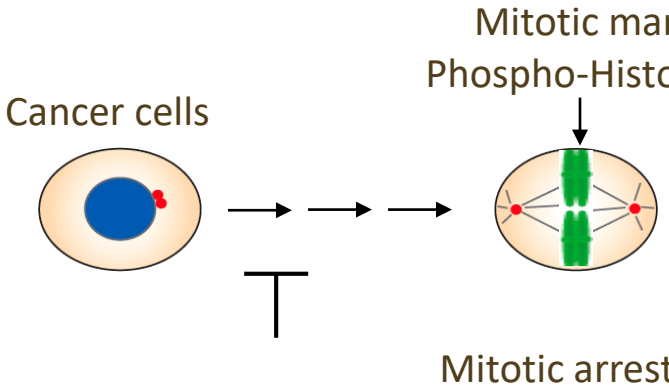
311 G2/M hits

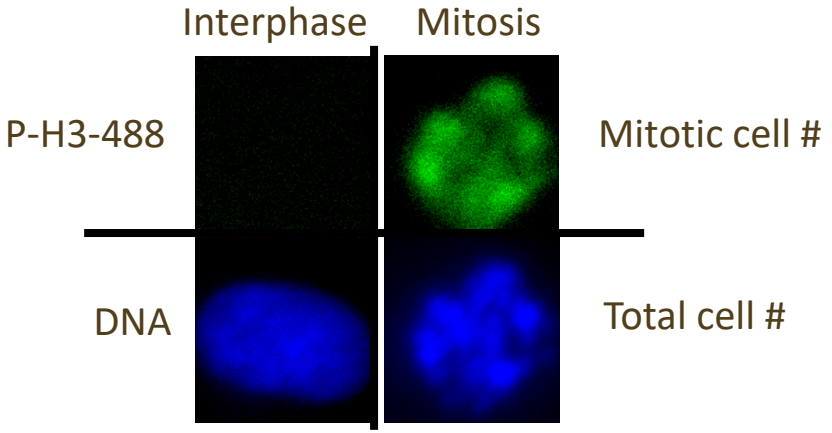
268 G2/M re-confirmed hits

Secondary screens and selection of lead compounds

About 0.3% hit rate and 86% reconfirmation rate.

Secondary screening and selection of compounds leading to M arrest (not G2)





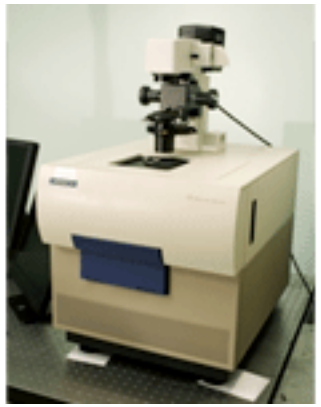


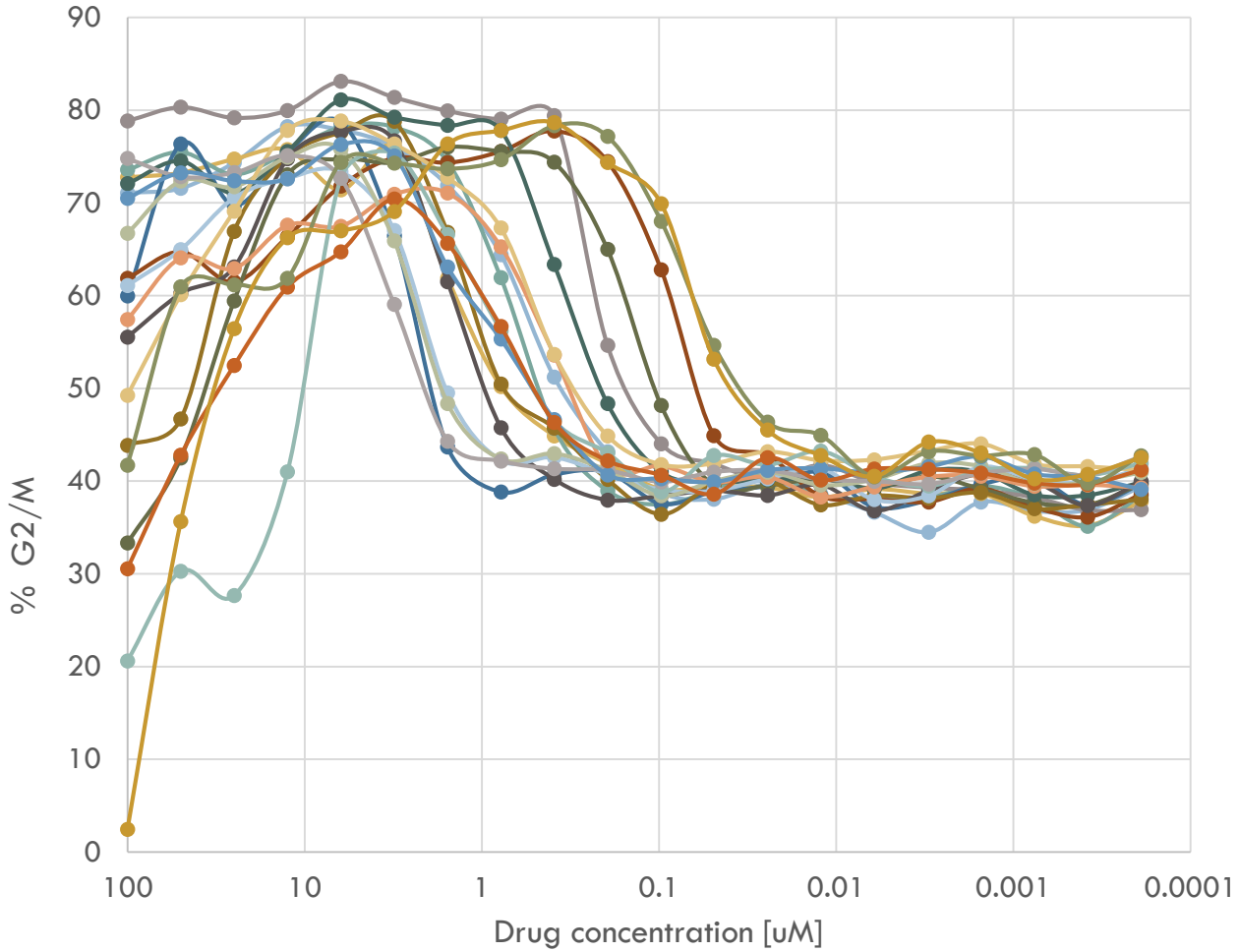
Image-based high content screening microscope with antibodies as orthogonal readout

Mitotic Index

$$MI = \frac{\text{Mitotic cell \#}}{\text{Total cell \#}}$$

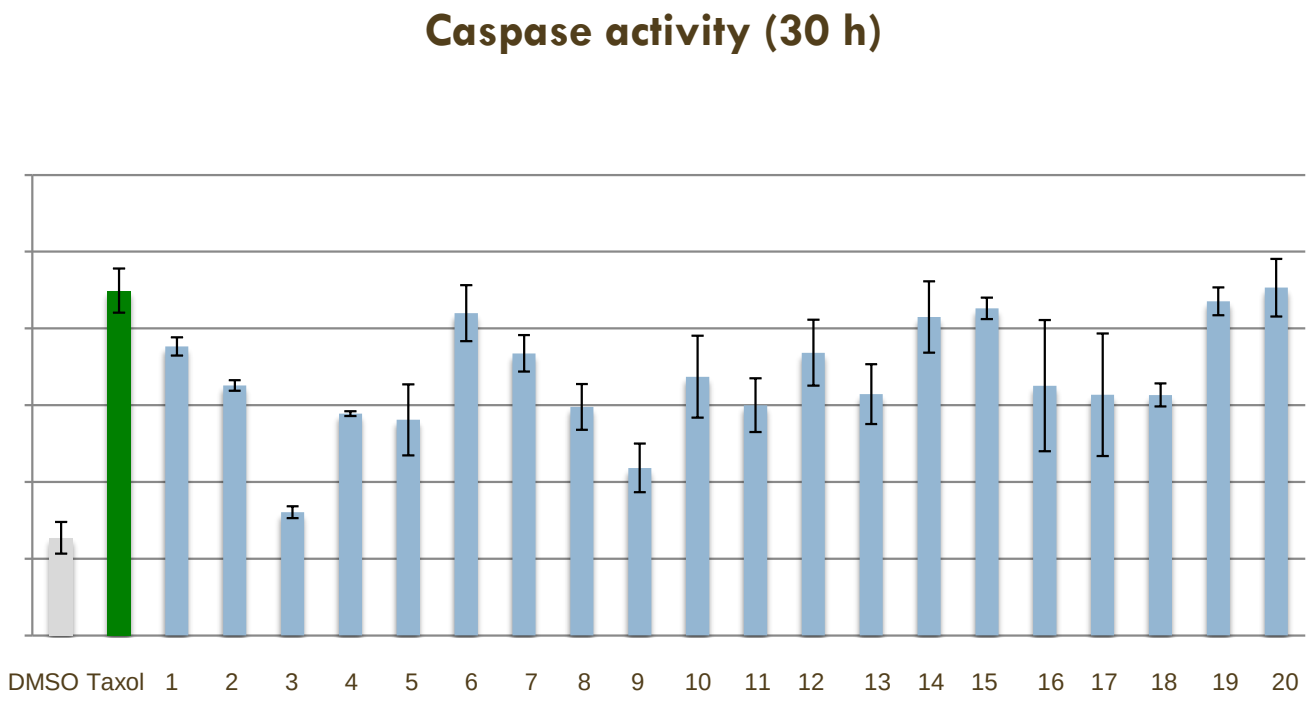
A good problem to have: 228 of 268 compounds were antimitotic.

Dose response and selection of compounds leading to M arrest (not G2) – Top 20



Many of our drugs are already nanomolar!

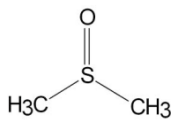
The Discovered Compounds Kill Cancer Cells via Apoptosis



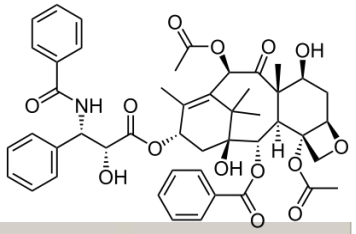
The Discovered Hits Cause M-Phase Arrest In Cancer Cells

A

DMSO

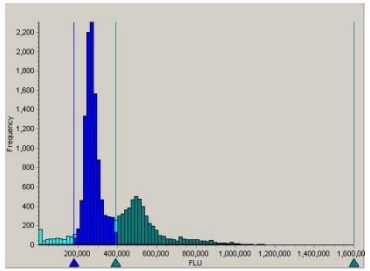


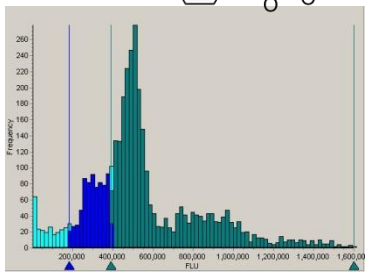
Taxol

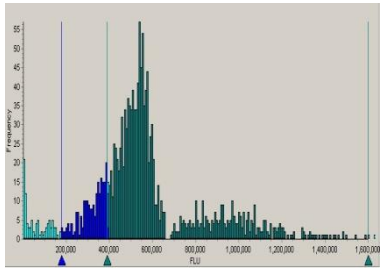


Compound-1

B







	DMSO	Taxol	Compound-1
G2/M(%)	39.28288955	75.92054719	79.96628667
G1/S (%)	56.37036864	18.32640469	13.78101667

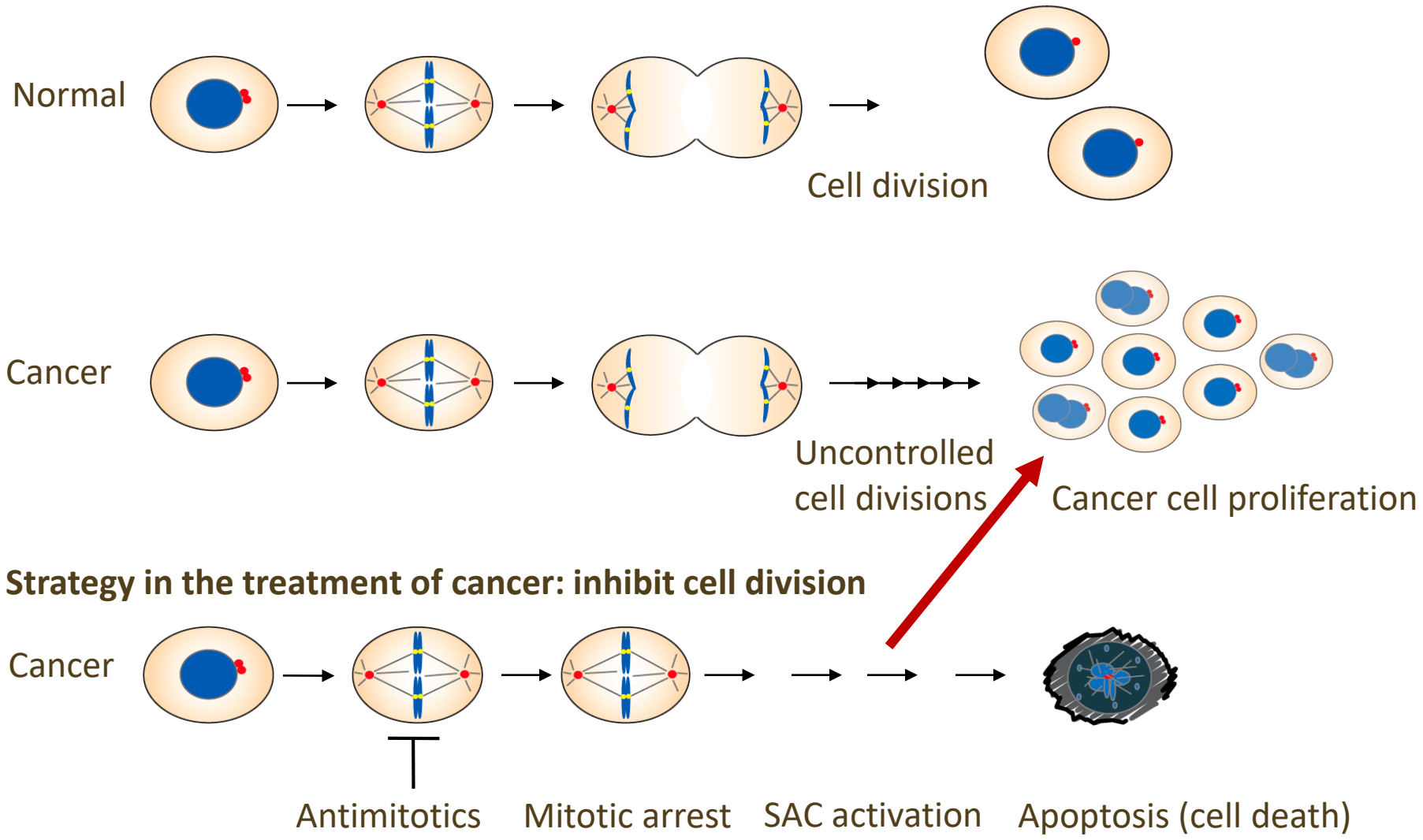
The Discovered Compounds Have Improved Propteries: In silico characterization of Compound-1

In-silico Chemical Properties Predictions	Ideal	Compound-1	Taxol	Docetaxel	Epothilone B
BBB permeability (Clark et al.)	yes	YES	no	no	no
Lipophilicity (logP)	~4	4.01	4.34	3.58	3.76
H-bond donors	<7	1	4	5	2
Polar surface area (PSA) Aº	40 – 90	82.7	189.9	215.6	97
Molecular weight	~400	308.4	853.9	807.9	507.7
Oral bioavailability (Veber Rules)	yes	YES	no	no	yes
Rotatable bonds	<10	2	15	14	2
Polar surface area (PSA) Aº	<140	82.7	189.9	215.6	97
Absorption/permeability (Lipinski Rules)	yes	YES	no	no	no
H-bond donors	<5	1	4	5	2
Molecular weight	<500	308.4	853.9	807.9	507.7
logP	<5	4.01	4.34	3.58	3.76
H-bond acceptors	<10	3	15	15	7
Solubility (logS)	> -5.7	-5.519	-8.43	-7.1	-3.45
Pgp efflux substrate	no	NO	yes	yes	yes
H-bond donors	<8	1	4	5	2
Molecular weight	<400	310.3	853.9	807.9	507.7

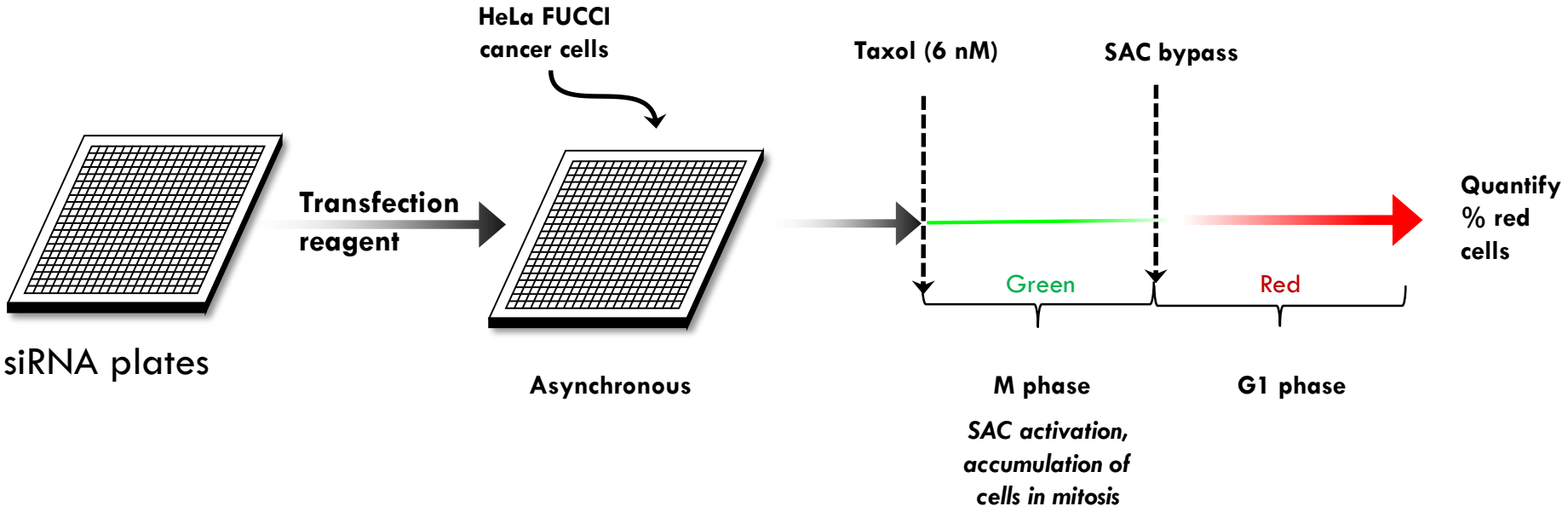
Xia et al. ACS Chem Biol. 2019

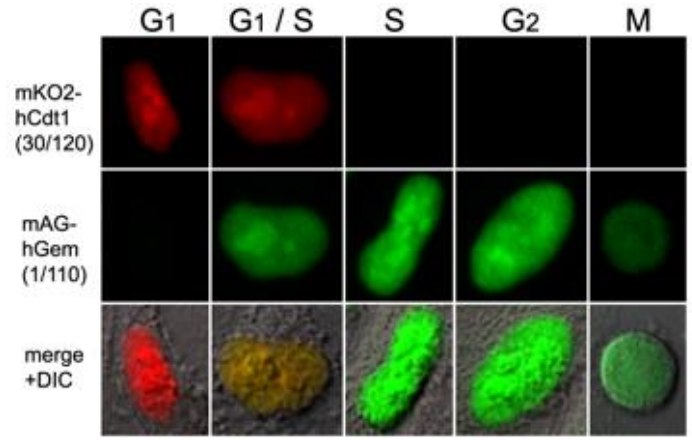
Another view at the same problem using high throughput biology (siRNA, and oldie but goodie…)

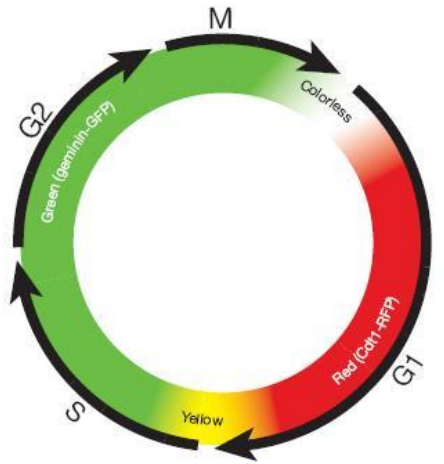
Inhibition of cell division in the treatment of cancer



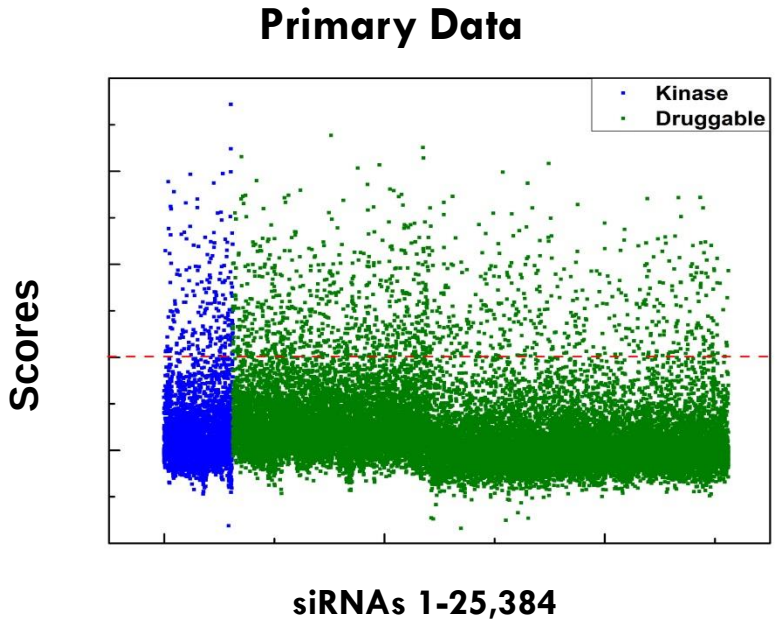
Screening for SAC suppressors (Primary)

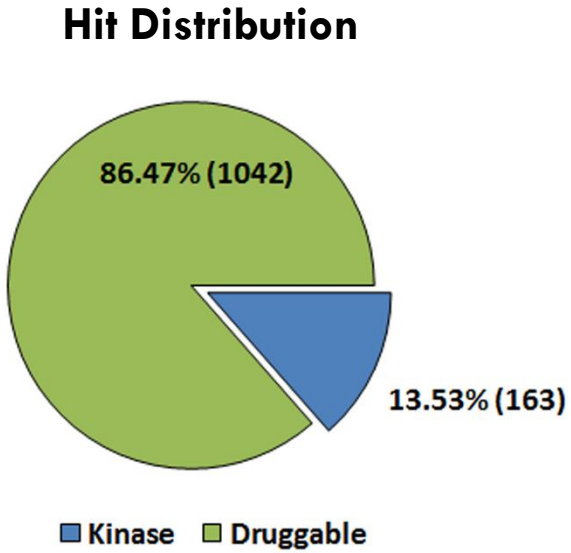


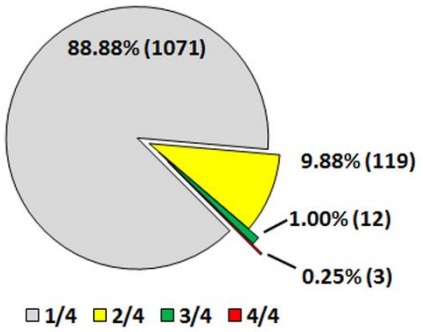




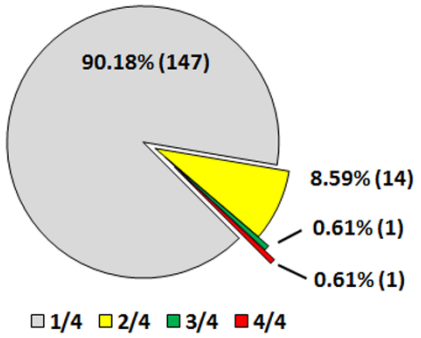
Overview of the hits



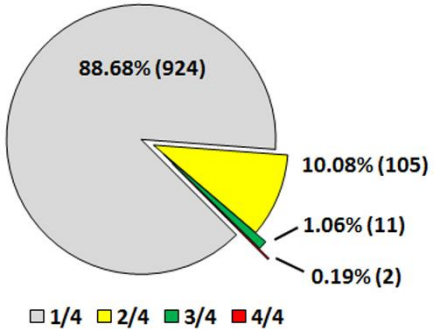




Kinome and Druggable

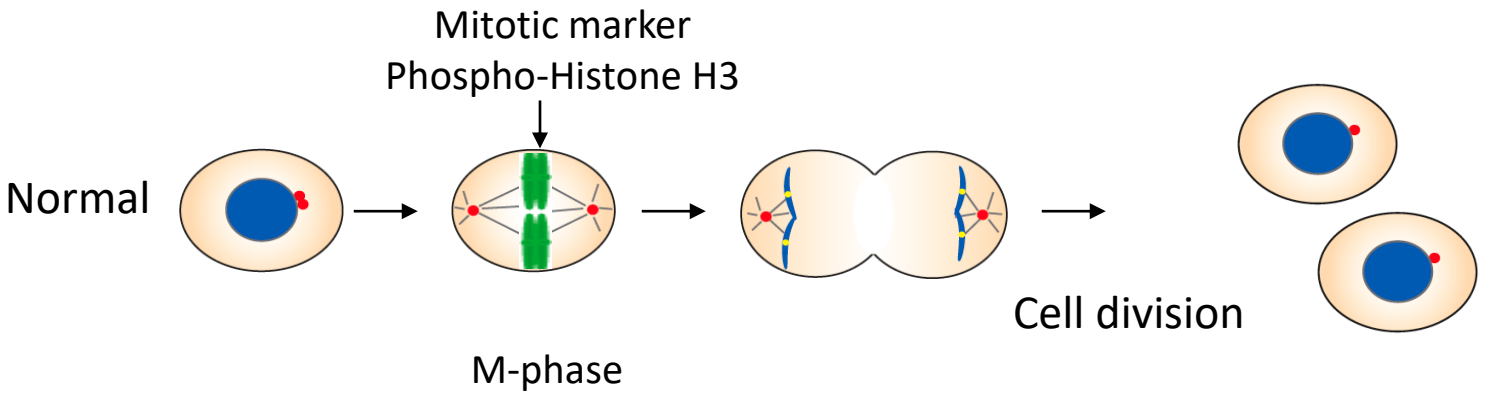


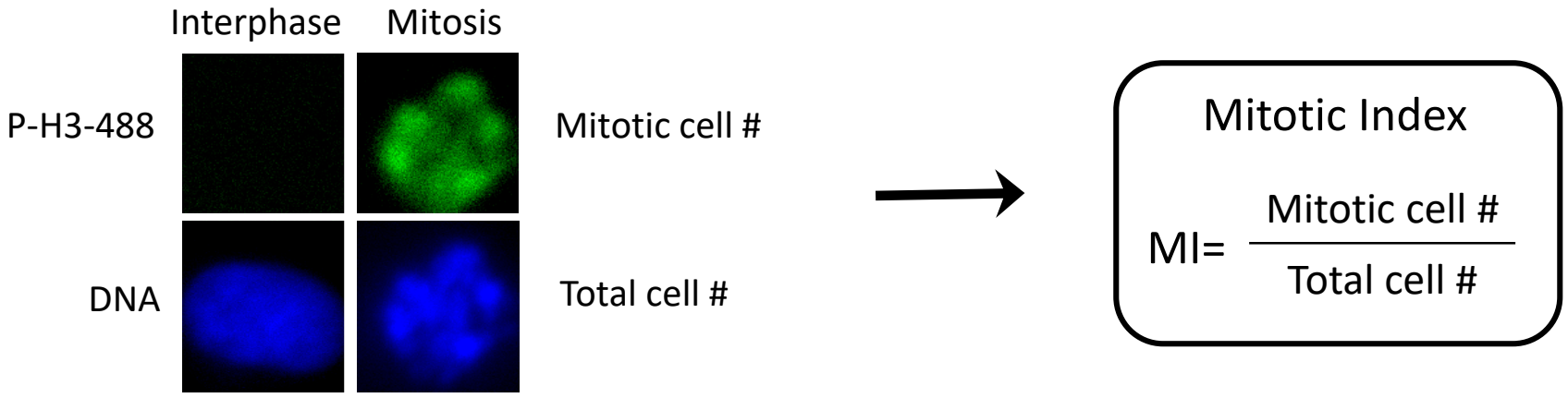
Kinome



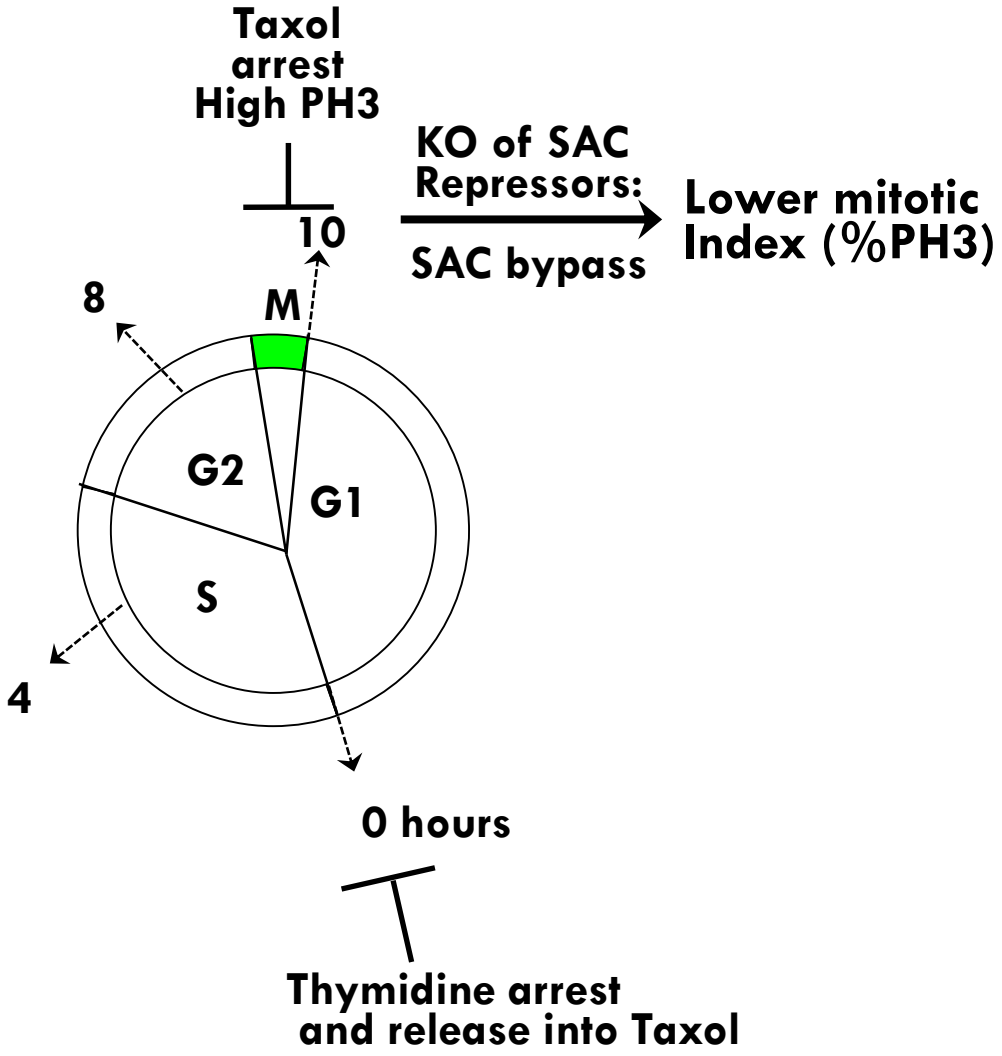
Druggable

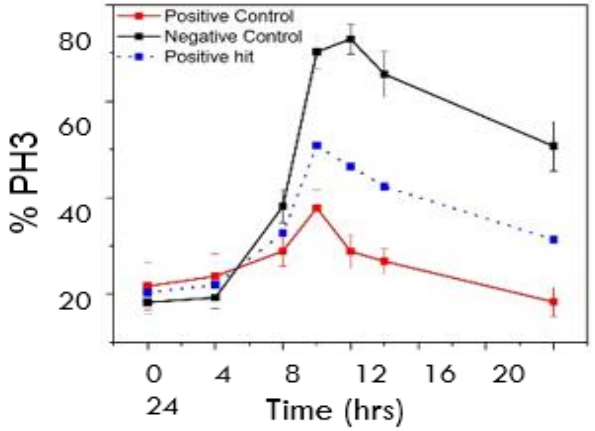
Screening for SAC suppressors (Secondary)



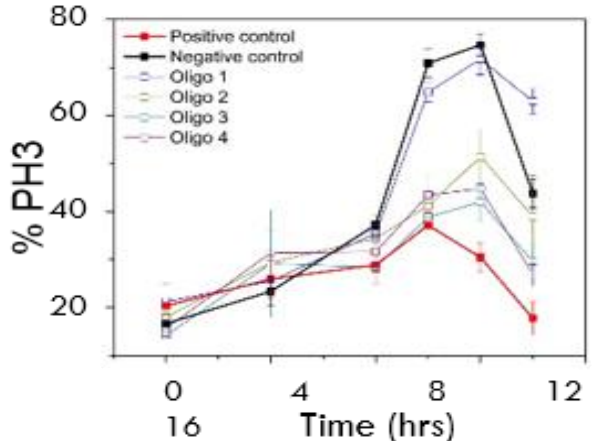


Kinetic follow up assay for SAC suppressors

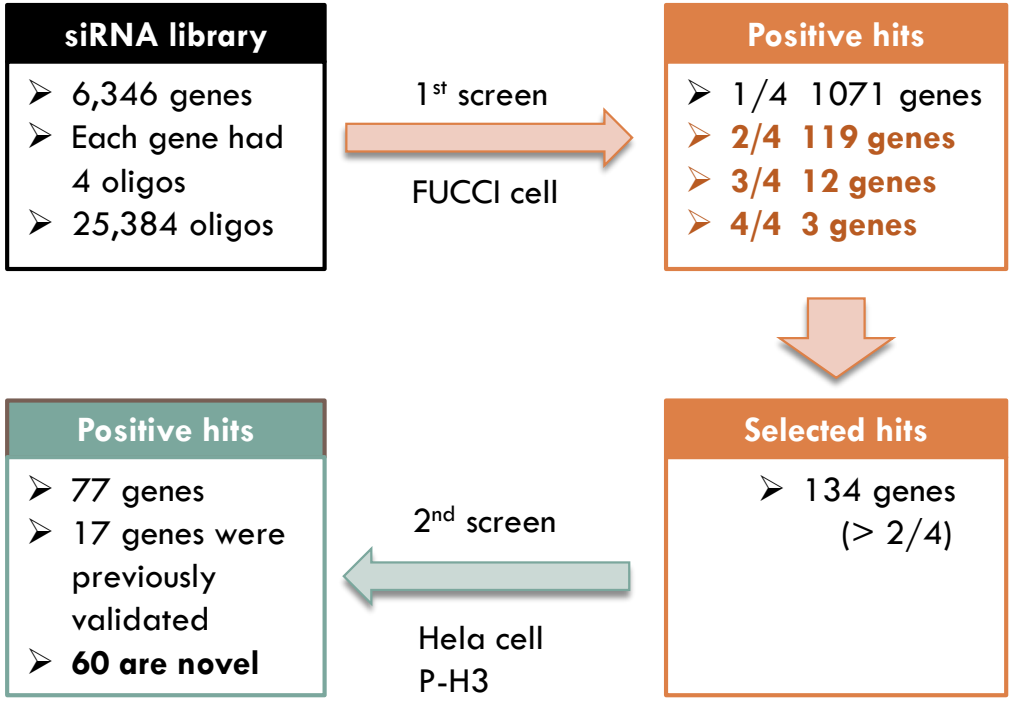




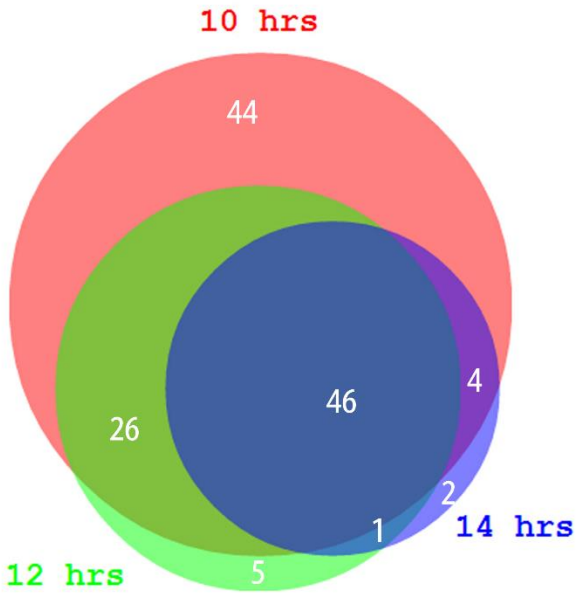
JAK1siRNAs

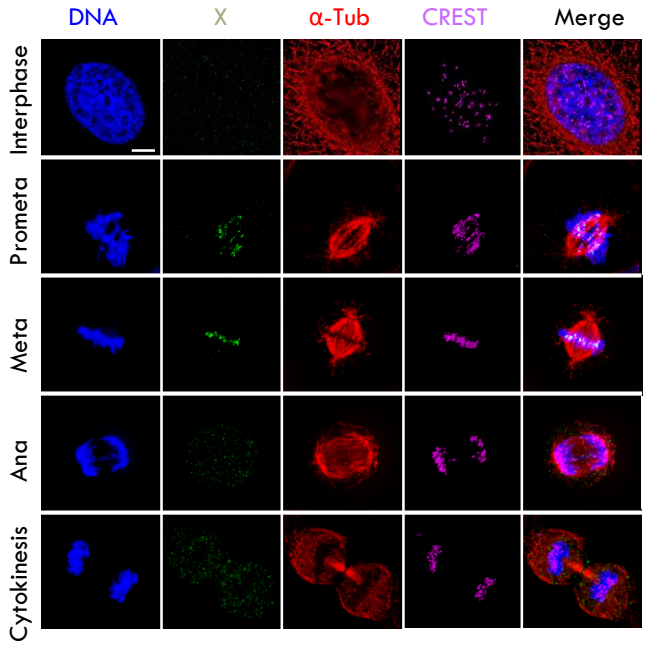


Results from the siRNA screen



Results from the follow up





Hit genes validated very well and can be grouped by their kinetic properties.
One particularly interesting gene (X) co-localizes with the centromeric region during prometaphase and was not know to be involved in this process.

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