UCI 趙 Chao Family Comprehensive Cancer Center

3D Spheroid DNA-Encoded Library Screening Technology:
Hit Finding on the STING Pathway

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BIDD Program Leader, CFCCC

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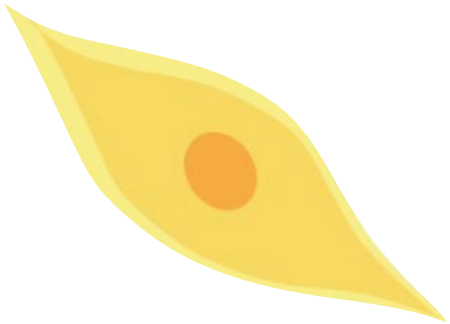
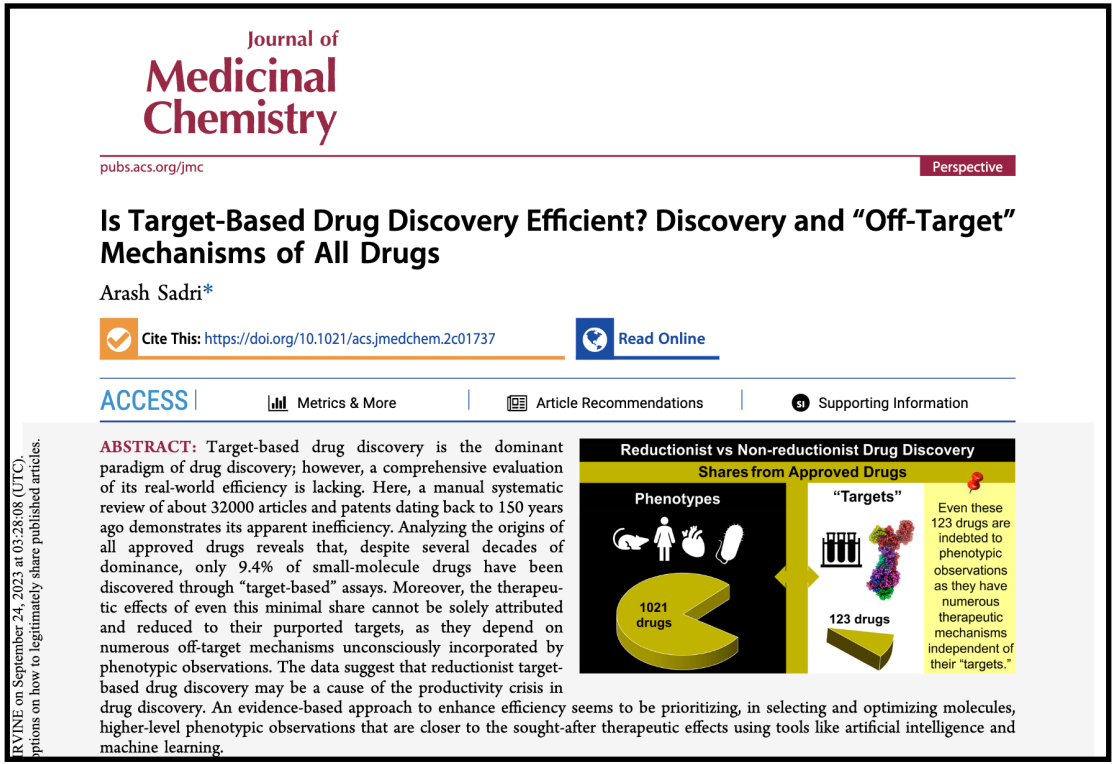
TARGET-BASED



CELL-BASED



Journal of Medicinal Chemistry

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Perspective

Is Target-Based Drug Discovery Efficient? Discovery and "Off-Target" Mechanisms of All Drugs

Arash Sadri*

Cite This: https://doi.org/10.1021/acs.jmedchem.2c01737

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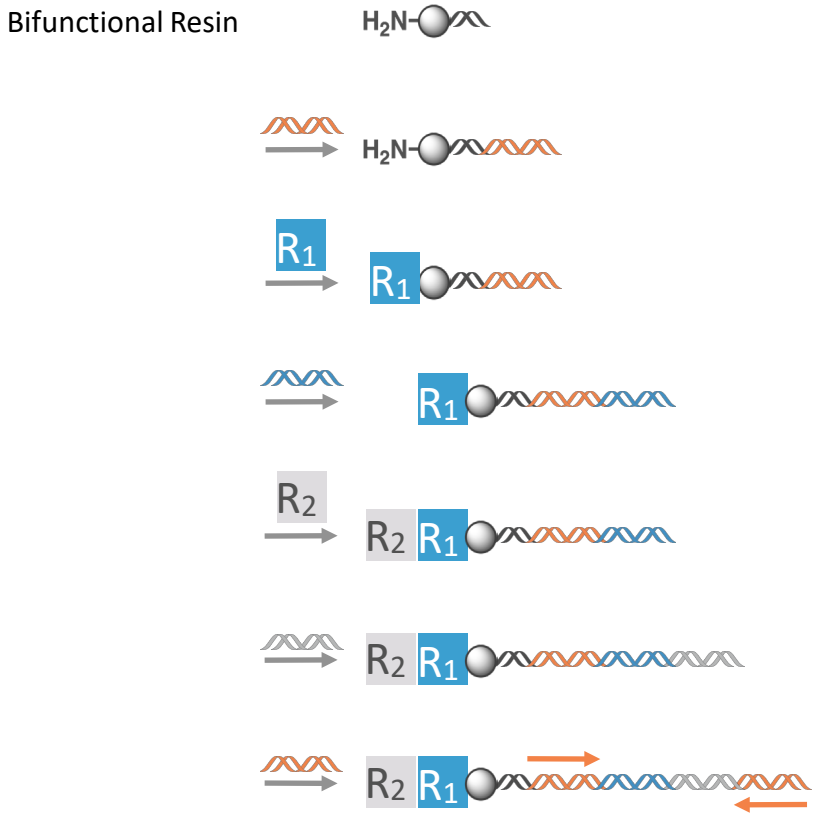
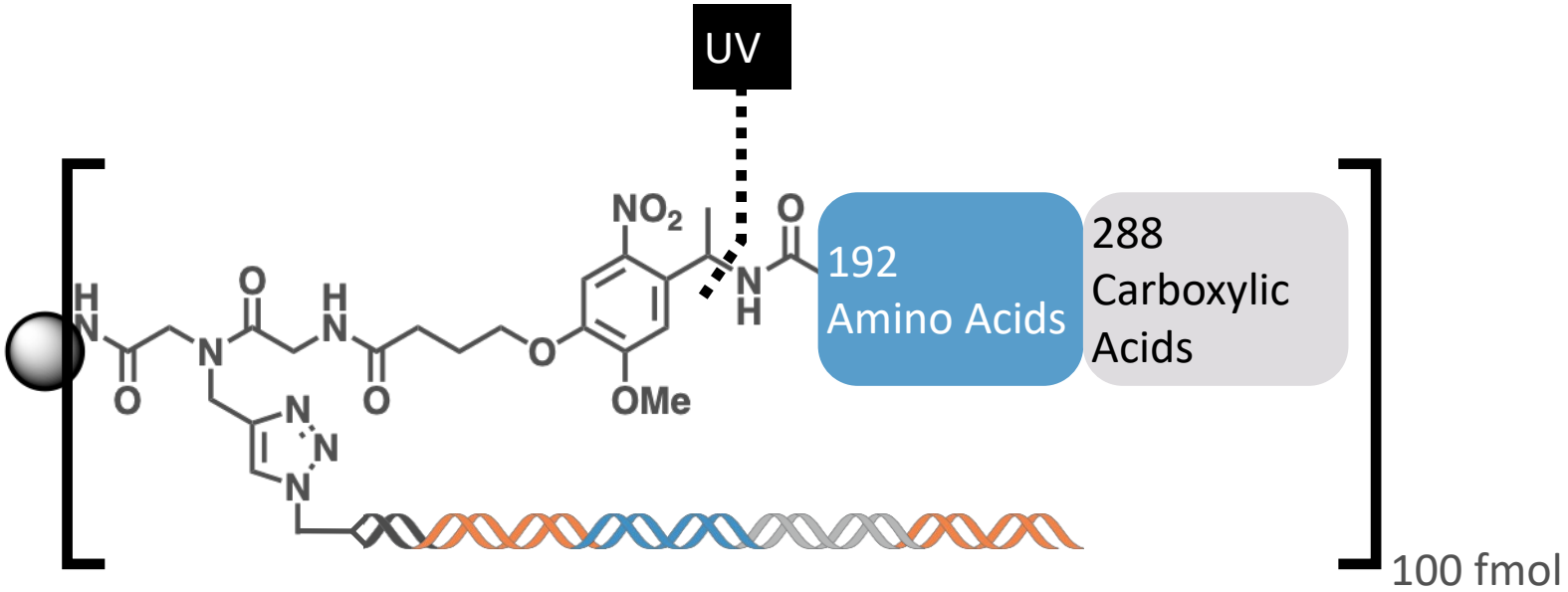
ABSTRACT: Target-based drug discovery is the dominant paradigm of drug discovery; however, a comprehensive evaluation of its real-world efficiency is lacking. Here, a manual systematic review of about 32000 articles and patents dating back to 150 years ago demonstrates its apparent inefficiency. Analyzing the origins of all approved drugs reveals that, despite several decades of dominance, only 9.4% of small-molecule drugs have been discovered through "target-based" assays. Moreover, the therapeutic effects of even this minimal share cannot be solely attributed and reduced to their purported targets, as they depend on numerous off-target mechanisms unconsciously incorporated by phenotypic observations. The data suggest that reductionist target-based drug discovery may be a cause of the productivity crisis in drug discovery. An evidence-based approach to enhance efficiency seems to be prioritizing, in selecting and optimizing molecules, higher-level phenotypic observations that are closer to the sought-after therapeutic effects using tools like artificial intelligence and machine learning.

Sadri, J. Med. Chem. (2023)

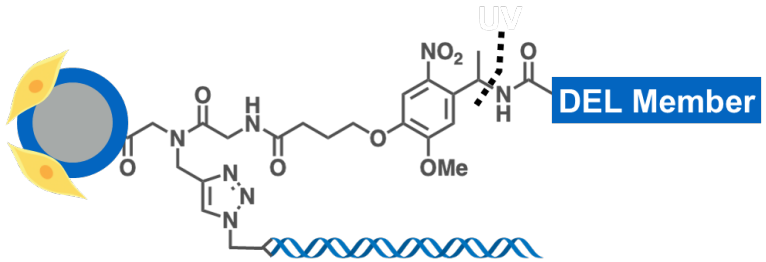
Solid-Phase DNA-Encoded Synthesis

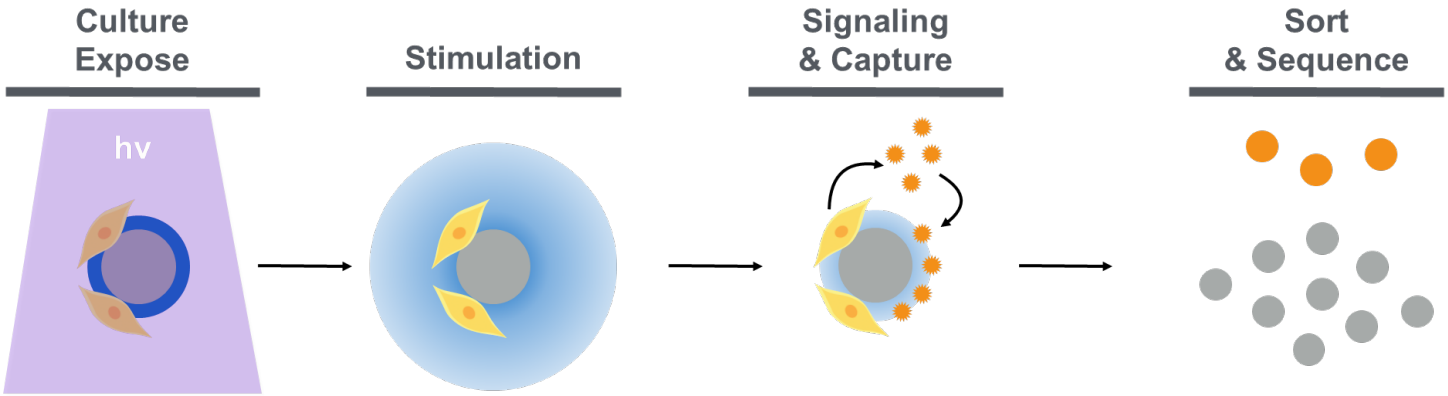


MacConnell et al., ACS Comb Sci. (2015)

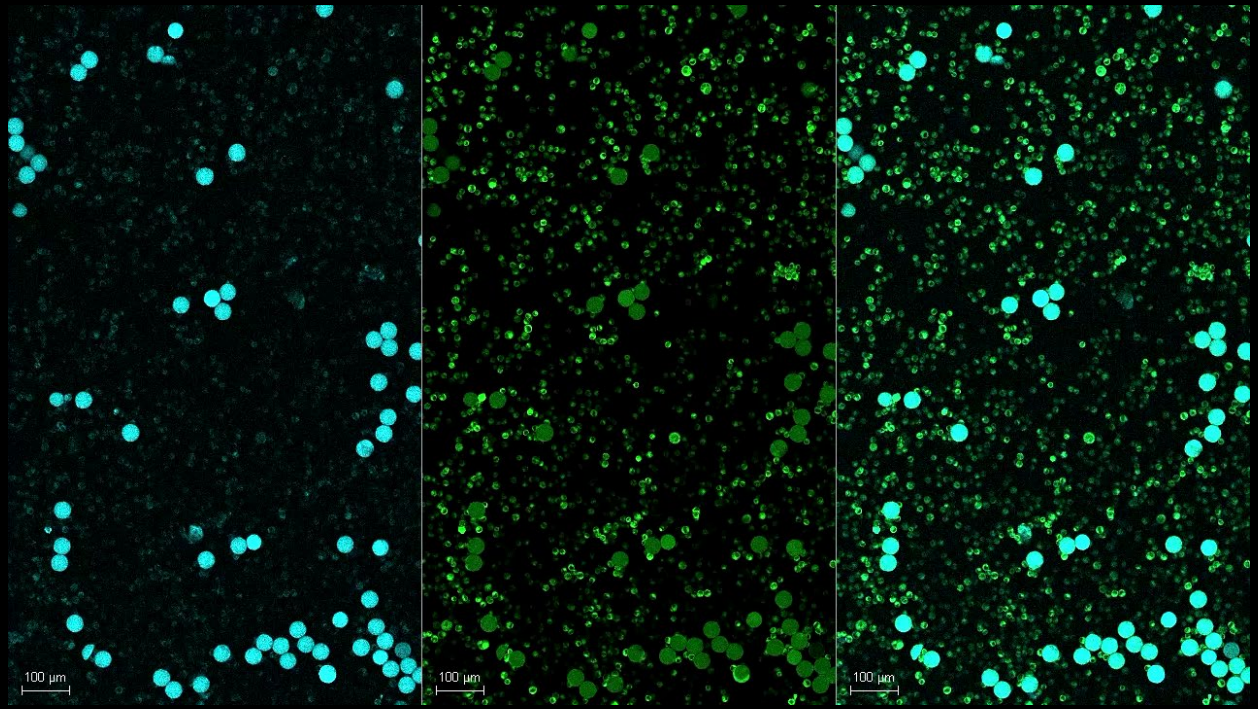


Cochrane et al., ACS Comb Sci. (2019)
Fitzgerald et al., ACS Med Chem Lett. (2023)


UV
NO2
O
N
H
DEL Member
O
N
H
O
O
N
N
N
OMe

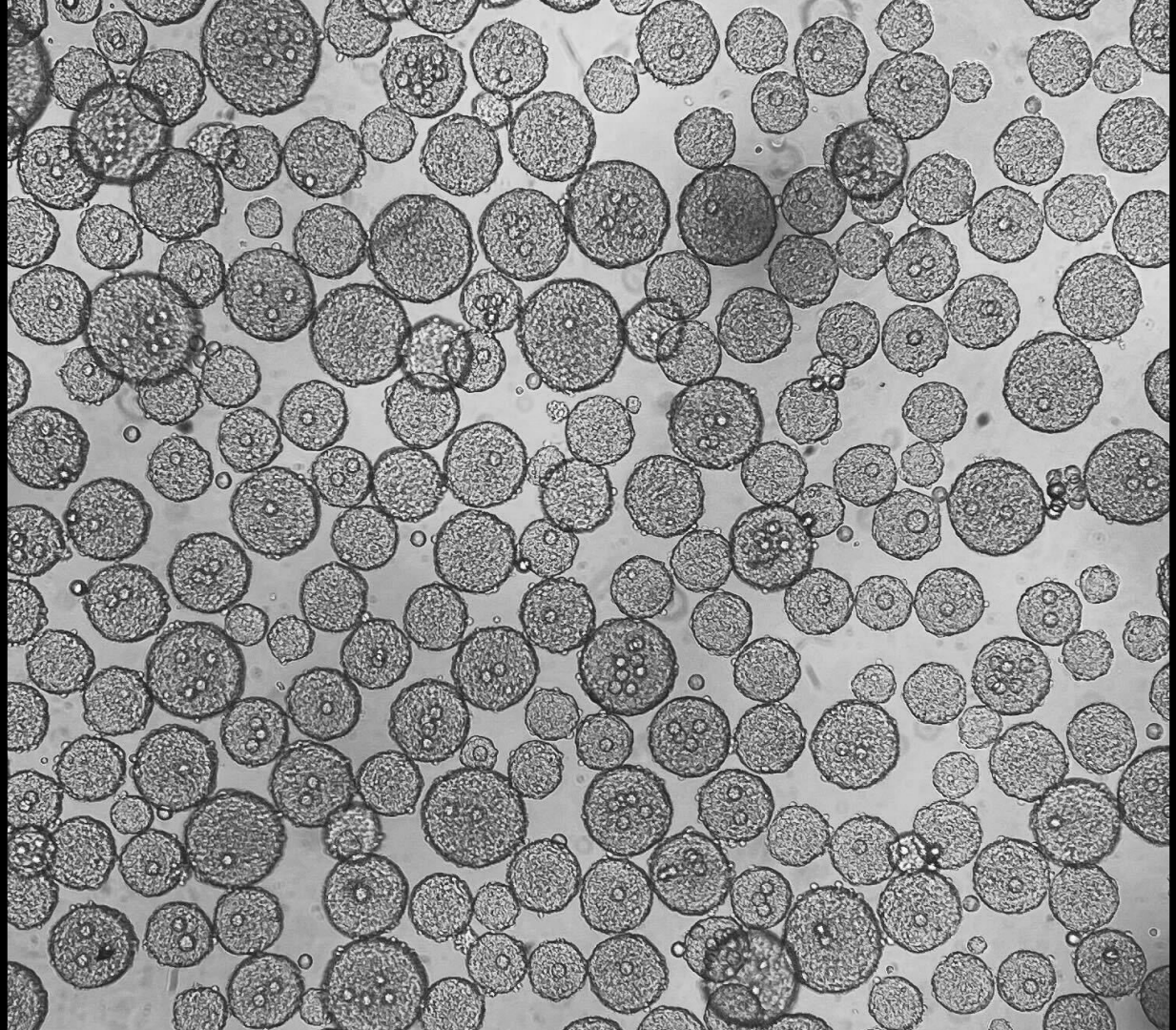

Culture
Expose
Stimulation
Signaling
& Capture
Sort
& Sequence
hv

HEK293T Bead Seeding Occurs Rapidly

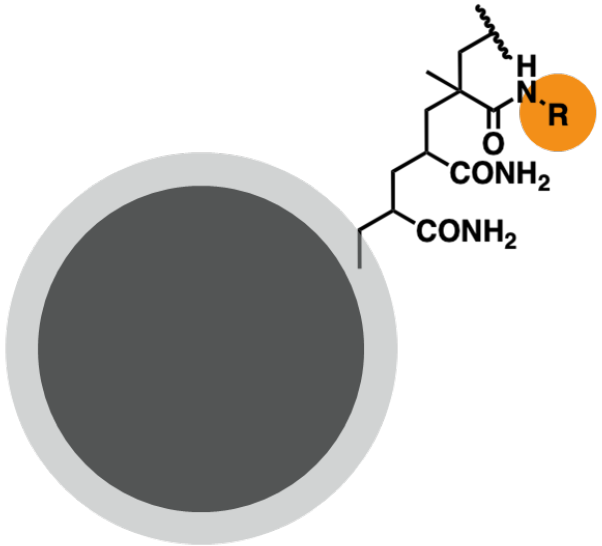


- Seeding over 17 h, 20 min / frame
- DEL beads in cyan (left), cells in green (cente), merged (right)

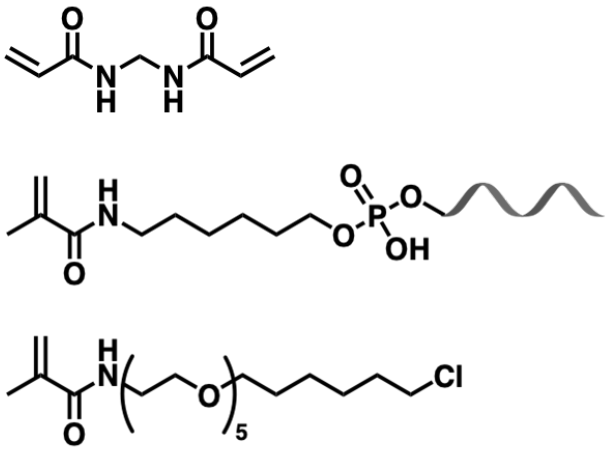
Callie Fredlender (unpublished)



Hydrogel Coat is a Multifunctional Signal Detection Scaffold



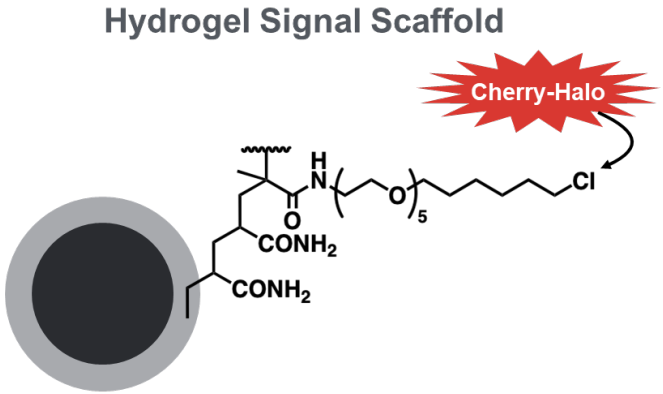
Hydrogel Shell
Signaling Scaffold

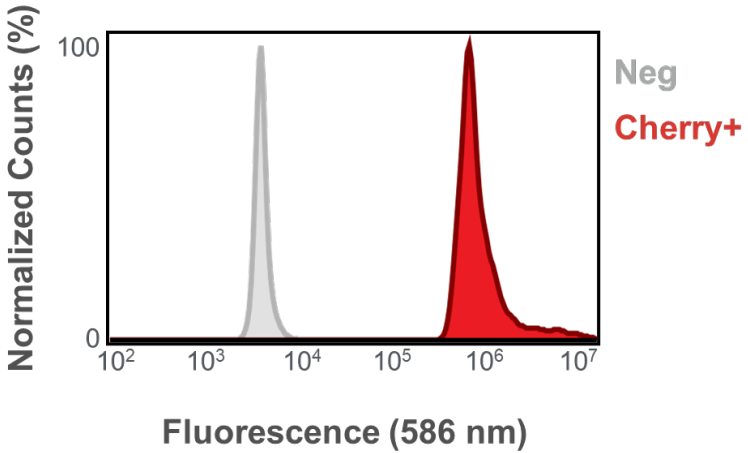


PLL, FN, other ECM

Fryer et al., ACS Cent Sci. (2022)
Cavett et al., ACS Cent Sci. (2023)

Co-polymerized Affinity Tag Ligands Capture Tagged Proteins

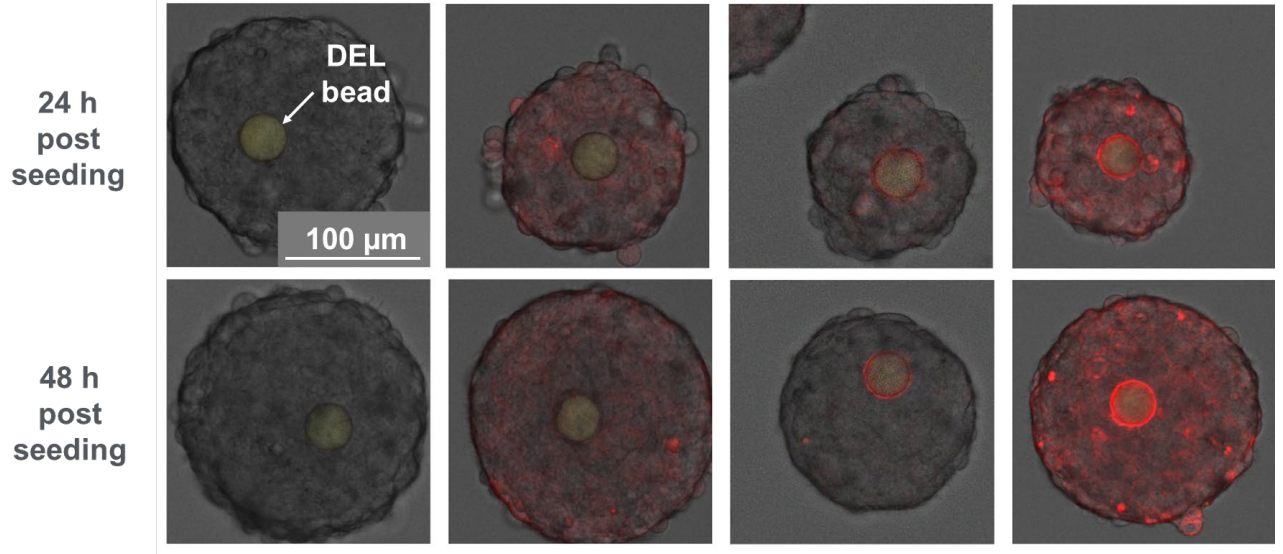




Callie Fredlender (unpublished)

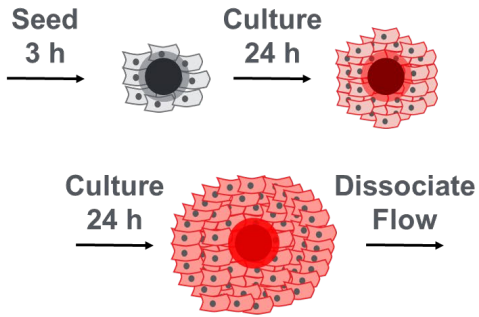
Fluorescent Protein Capture from HEK293T Spheroids (Constitutive Reporters)

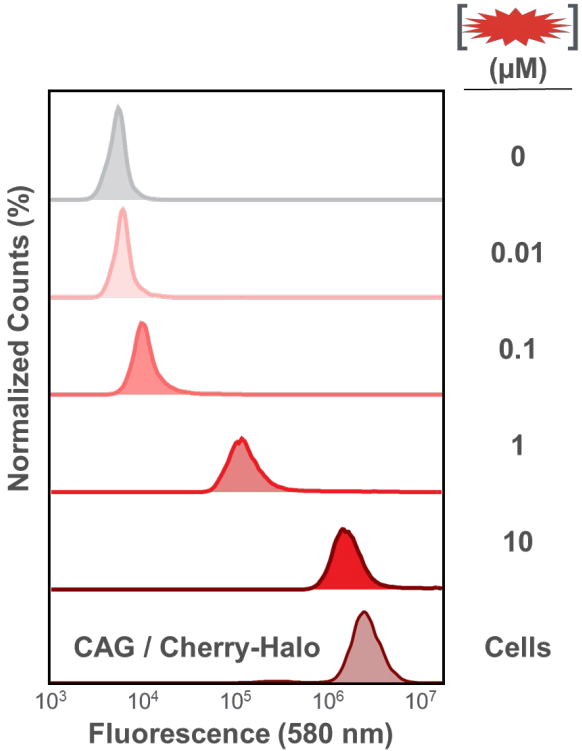
Reporter	—	mCherry-Halo	mCherry-Halo	mCherry-Halo
Secretion Tag	—	—	+	+
Promoter	—	PGK (low)	PGK (low)	CAG (high)



Callie Fredlender (unpublished)

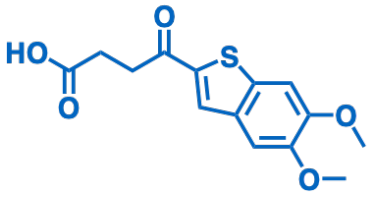
Constitutive Reporter Labels DEL Beads @ ~10 µM Equivalent Labeling Rxn



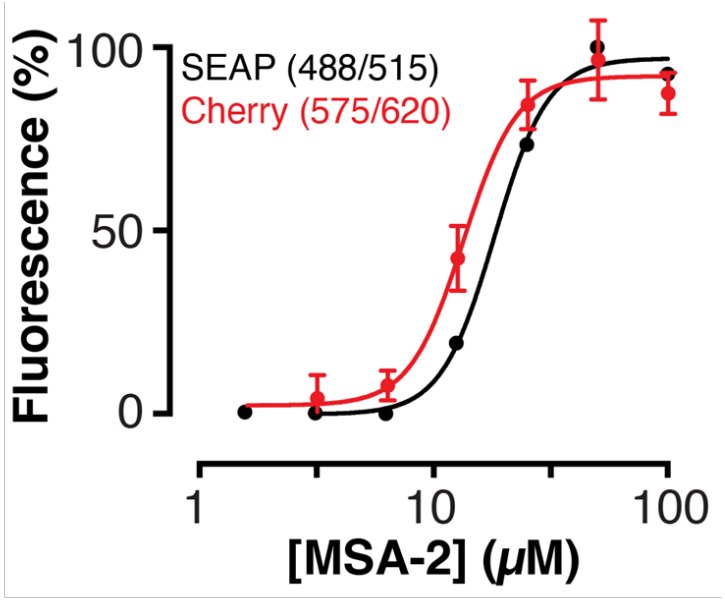


Callie Fredlender (unpublished)

Cherry-HaloTag STING Reporter Line Responds Similarly to Commercial Line

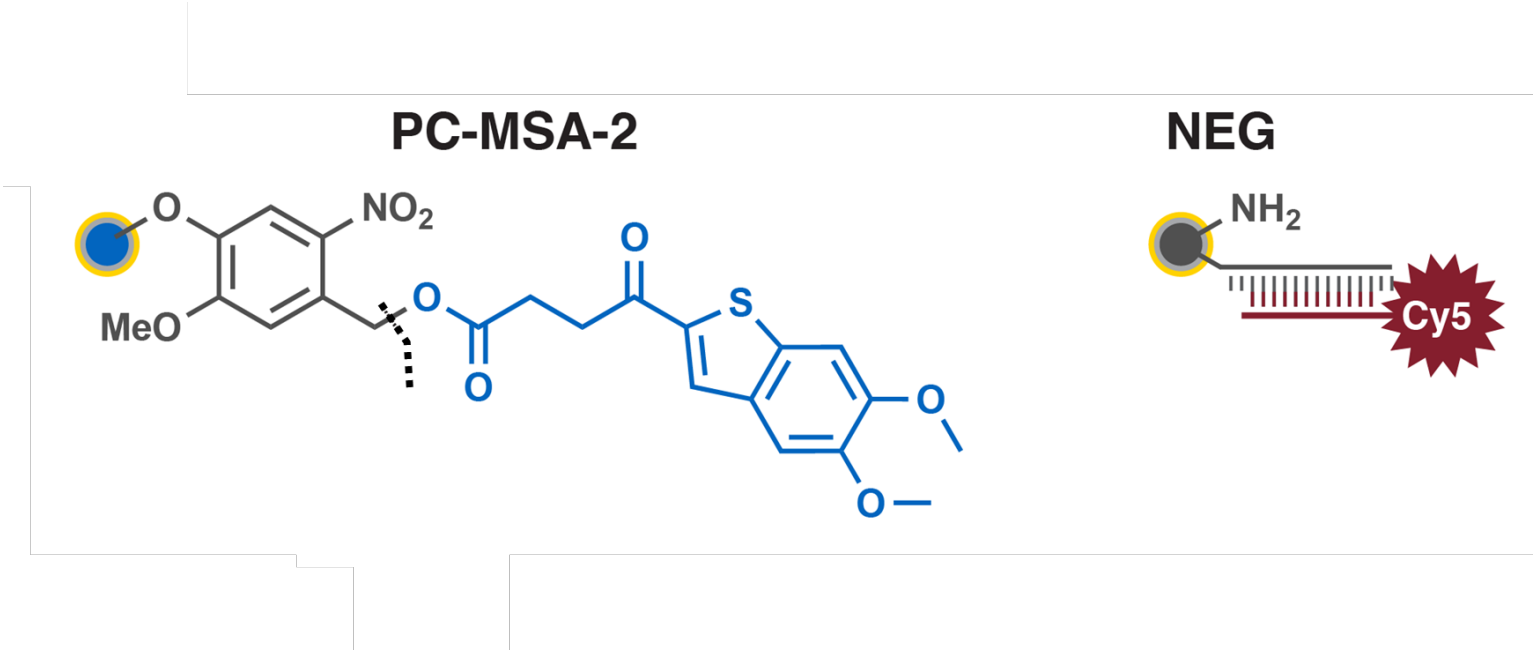


MSA-2



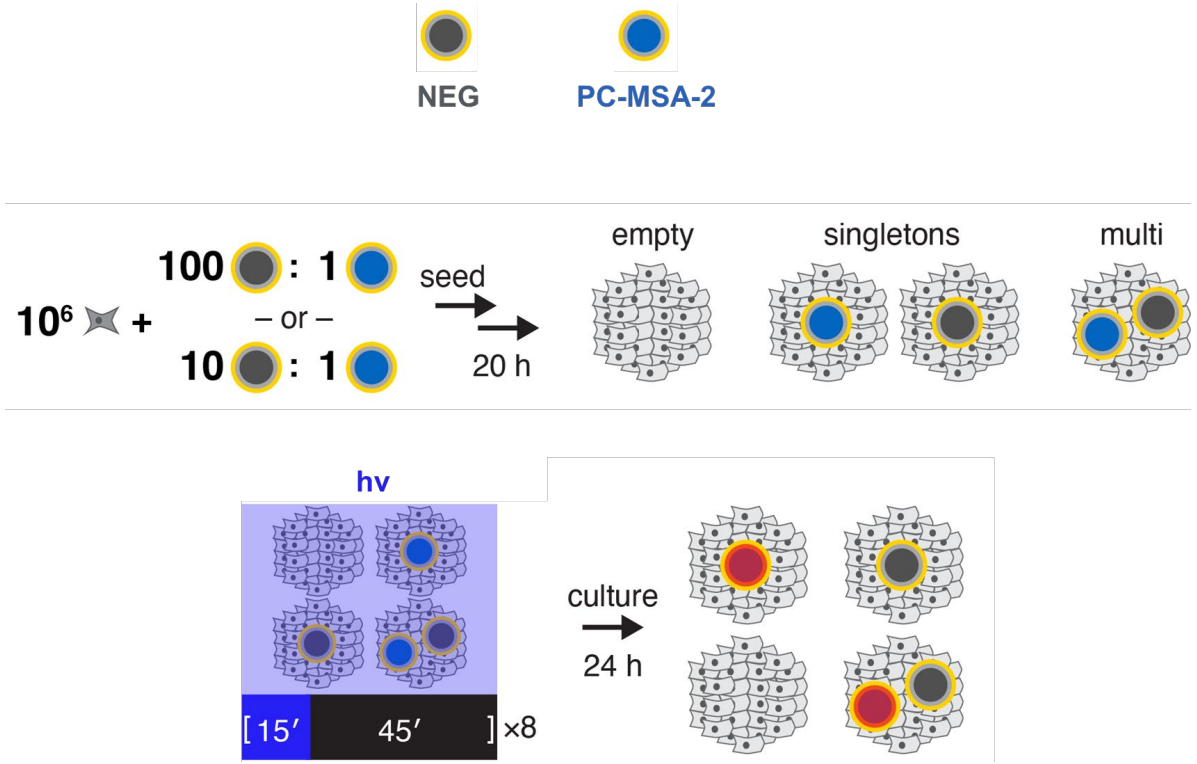
Pan et al. Science (2020)
Callie Fredlender (unpublished)

Control Bead Structure

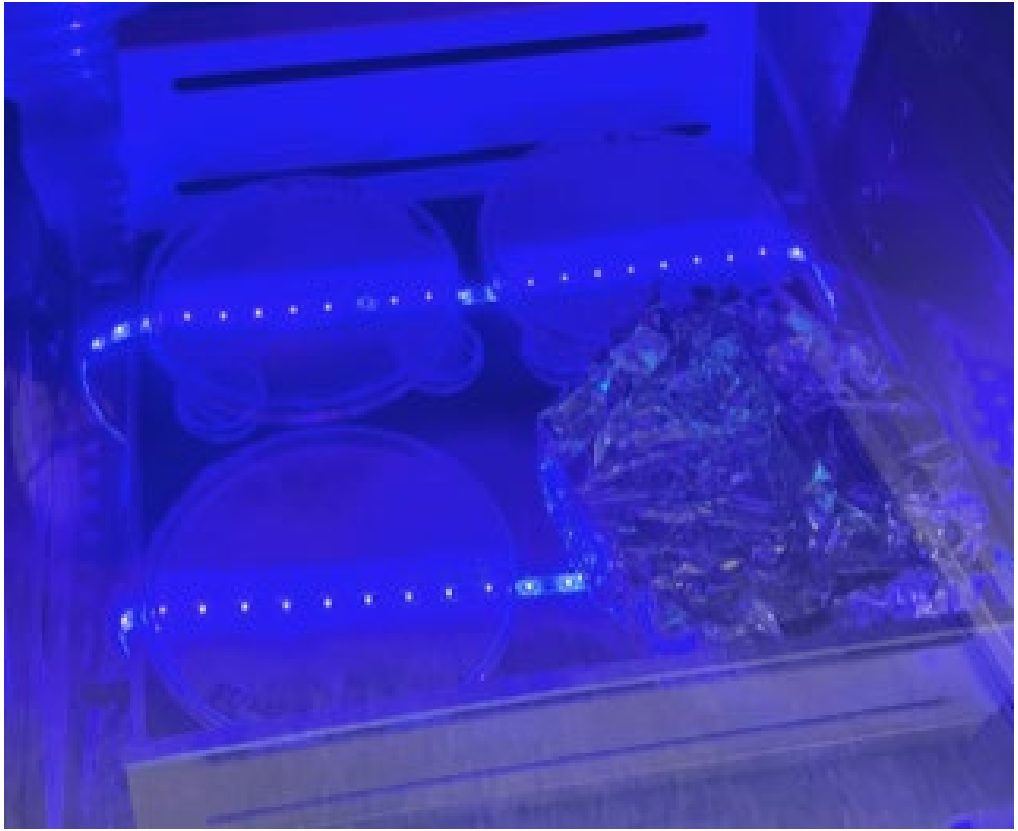


Pan et al. Science (2020)

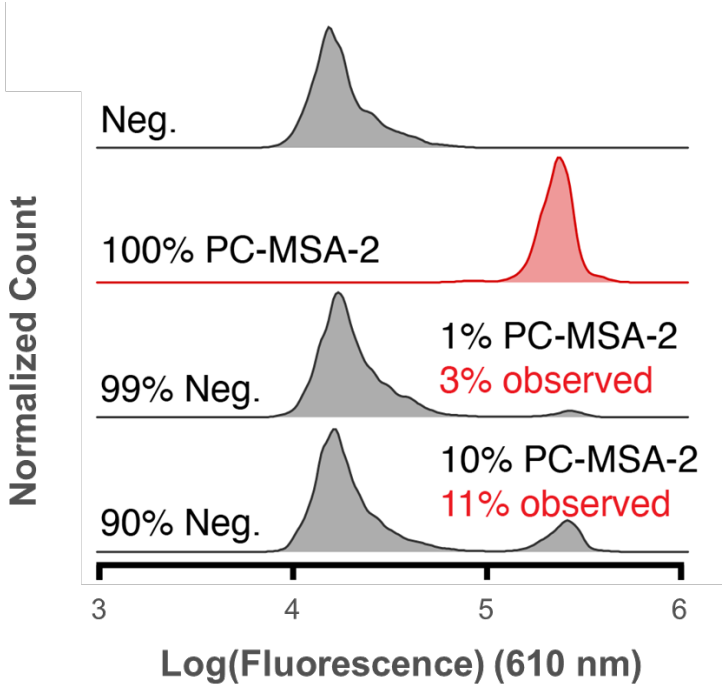
Control PC-MSA-2 Beads Used to Generate Mock Libraries (1 & 10% Hit Rates)



Party Light Photocleavage of Spheroid DEL Culture



Robust Separation Observed in Flow Cytometry of PC-MSA-2 Beads

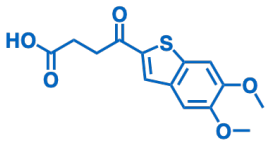


Callie Fredlender (unpublished)

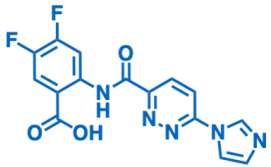
Mini-DEL Explores Structural Themes of Known STING Agonists

48 Amino Acids	72 Carboxylic Acids

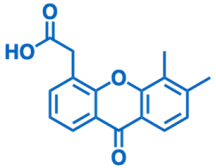
Diversity = 3,456 Compounds



MSA-2



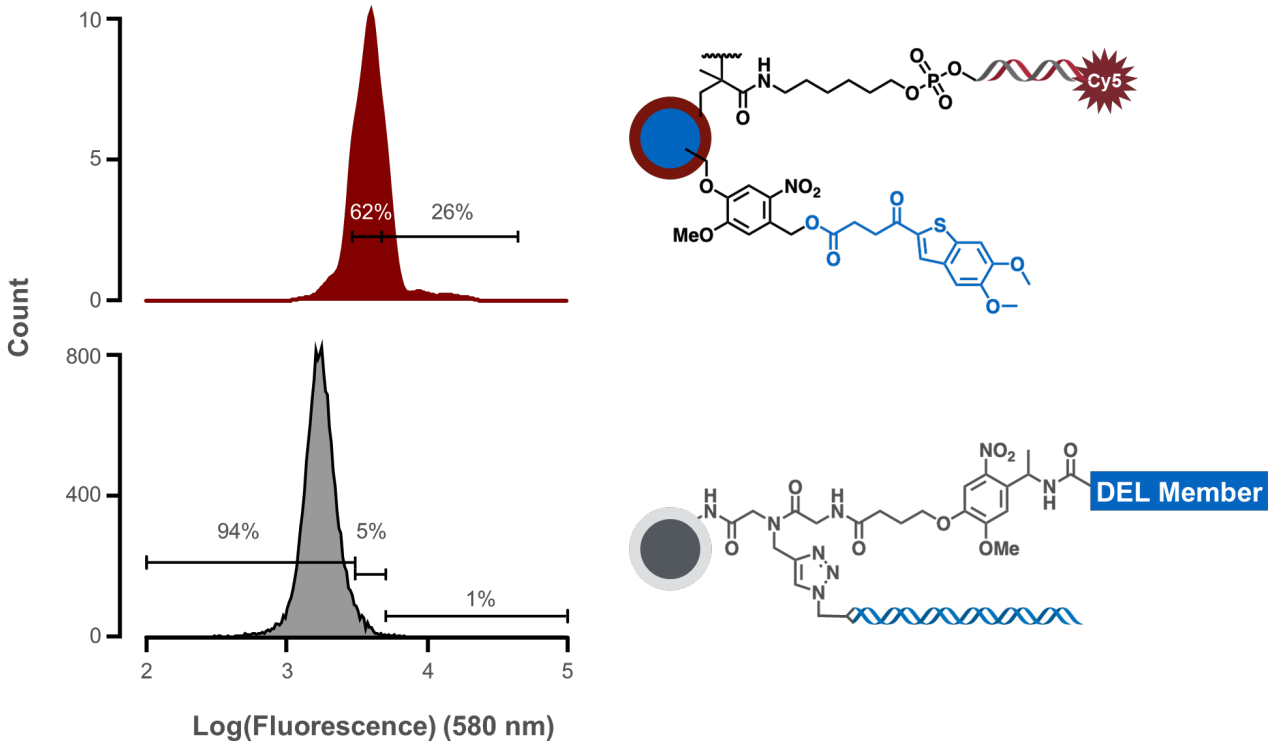
SR-717



DMXAA

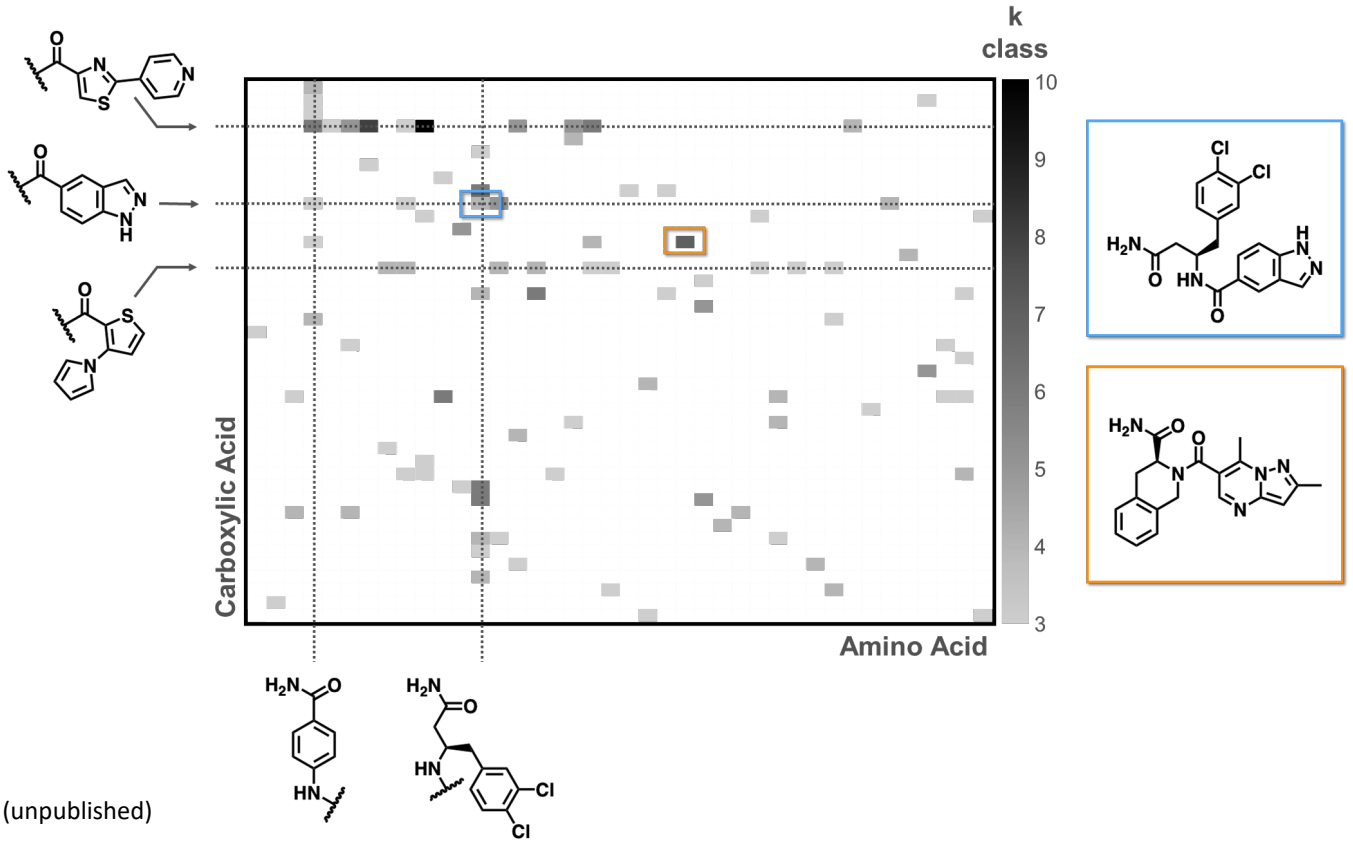
Pan et al. Science (2020)
Chin et al. Science (2020)

Cellular DEL Screens Isolate High Cherry Fluorescence Beads as Hits



Callie Fredlender (unpublished)

Cellular DEL Screening: Hit Structure Deconvolution



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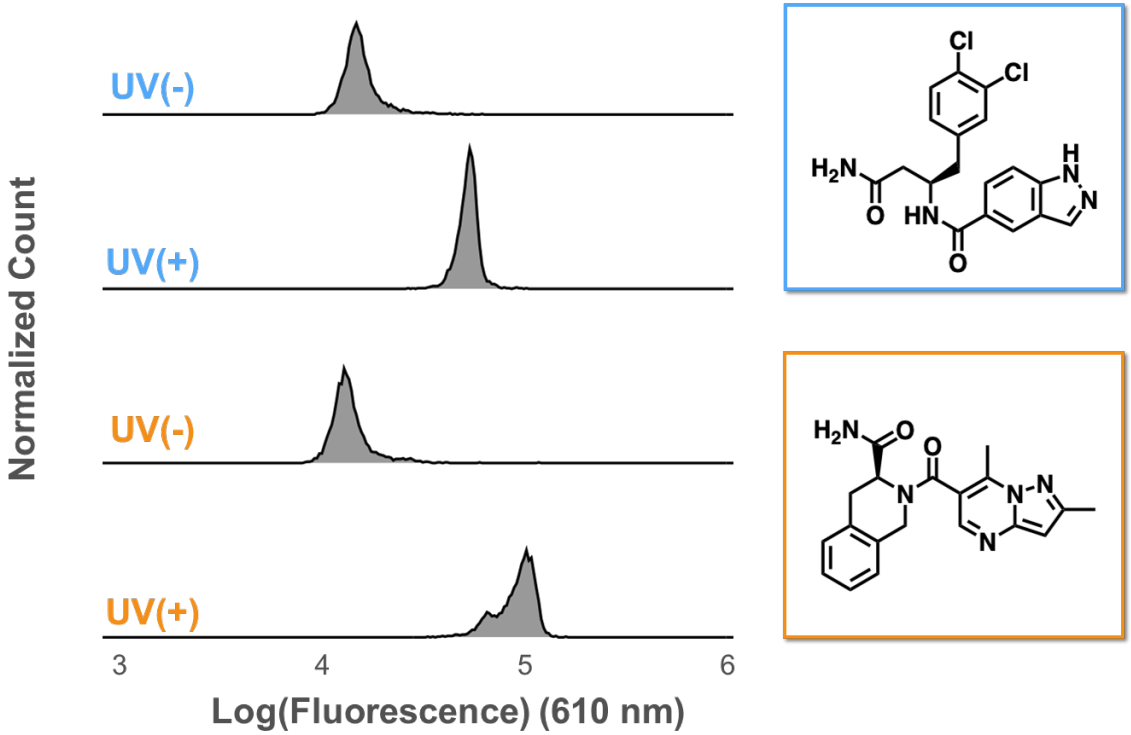
Two Selected Cellular DEL Screening Hits Validate in Spheroid Culture



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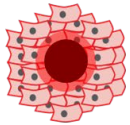
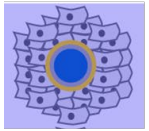
Cells form spheroids around DEL beads



On-bead secreted reporter capture is proximity-driven



Photocleavage liberates sufficient ligand to stimulate signaling

Labeled beads can be sorted and sequenced to find hits

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Anjali Dixit
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Callie Fredlender
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Sherry Huang
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Alix Chan (Genentech)
John Chaput (UCI)
Christian Cunningham (PeptiDream)
Matthew Disney (Scripps)
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Margot Paulick (Initial)
Jennifer Prescher (UCI)
Alex Satz (WuXi)
Robert Spitale (UCI)

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Thank You

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