Natural Product-inspired Macrocyclic Toolbox for "Undruggable Targets": *Our Wnt Journey*

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Citation:
E. A. Zerhouni, Turning the Titanic. *Sci. Transl. Med.* **6**, 221ed2 (2014).

DRUG DISCOVERY

Turning the Titanic

AT THE END OF THE 20TH CENTURY, BIG PHARMA AND ITS CUSTOMERS EXPERIENCED heady days. Translation of medicines such as cholesterol-lowering agents, HIV protease inhibitors, and the first molecularly targeted cancer drugs improved lives and enriched the pharmaceutical industry. The recipe for success appeared obvious: Tweeze apart biological pathways in model systems, and pinpoint molecular targets likely to be pivotal in a disease process. Use this information to develop high-throughput assays to screen for drug candidates. Test promising lead compounds in animal models of disease, and optimize the winners by using medicinal chemistry. Demonstrate safety and efficacy in clinical trials in order to satisfy the approval requirements of regulators, and deploy in the marketplace to benefit patients.

Expensive? Yes. But for a time, the formula was successful often enough to make medical and financial sense. More recently, costly failures in late-stage clinical trials have stalled the Titanic, and these leaks in the translational pipeline have produced a biomedical innovation gap: Most newly marketed drugs are close relatives of already approved, rather than first-in-class, entities (*1*).

Underestimated the complexity of human biology!

Vol 437|22 September 2005 nature

NEWS & VIEWS FEATURE

PHARMACEUTICALS

A new grammar for drug discovery

Mark C. Fishman and Jeffery A. Porter

To realize the potential of the genome for identifying candidate drugs we must move beyond individual genes and proteins. The signalling pathways in cells provide the right level for such analyses.

Mark Fishman
President
NIBR, 2002-2016

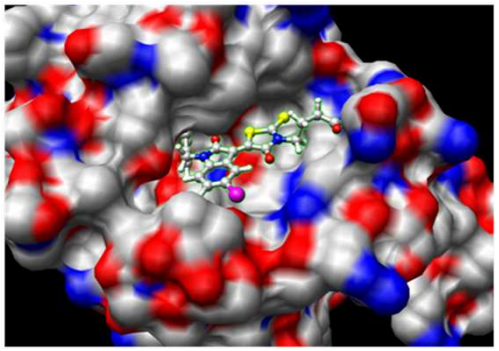
…Our current understanding of molecular pathways is insufficient as a platform for effective pharmaceutical discovery…

…Several biotechnology companies have focused on the known elements of a few key pathways to target them with new medicines. But for the genome to be translated into medicines with any reliability and regularity, far more work needs to be done. Defining the role of pathways in complex diseases will undoubtedly take many years…

Classical Approaches

Defining the target (could be enzymes or isolated protein(s)

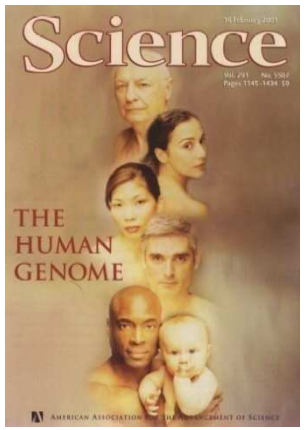
Structural information on the target – finding the pocket



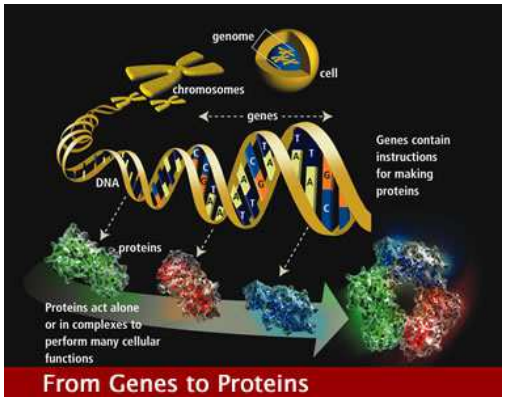
Structural guided chem /med chem

Lipinski's Rule of 5

From genes to pathways:



tough journey



- Complex, multiple protein-protein interactions
- Dynamic and temporal processes!
- Regulation (normal) and de-regulation (disease)

REVIEW

Assembly of Cell Regulatory Systems Through Protein Interaction Domains

Tony Pawson[1,2*] and Piers Nash[1]

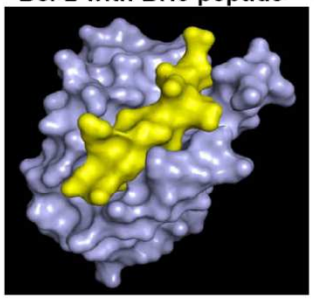
The sequencing of complete genomes provides a list that includes the proteins responsible for cellular regulation....

However, this does not immediately reveal what these proteins do, nor how they are assembled into the molecular machines and functional networks that control cellular behavior.

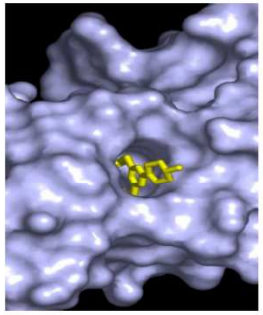
Science **2003**, *300*, 445-452

Extended Binding Motifs: a need for a new drug modality

Bcl-2 with BH3 peptide



Abl Kinase with Inhibitor Bound



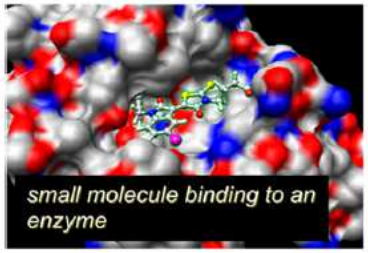
Extended Binding Motifs:
- Large, flat interface surface area
- Diversity of weak interactions
- Evolved to selectively recognize specific substrates/proteins

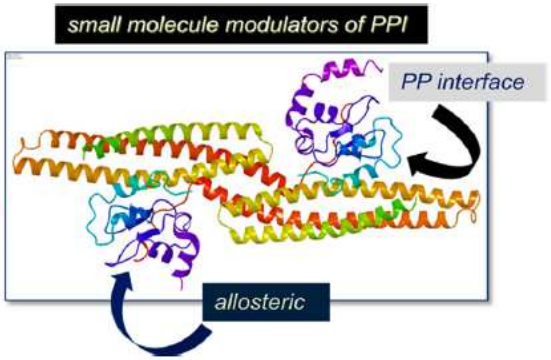
Compact Binding Motifs:
- Discrete, concave binding site
- Evolved to recognize small substrate or co-factor
- Known 'rule-of-5' small molecule starting points

- Map large surface area
- Shallow surface
- Combination of several weak interactions
- Extended hydrophobic interactions
- Possible *hot spots*

CHEMICAL REVIEWS

2014



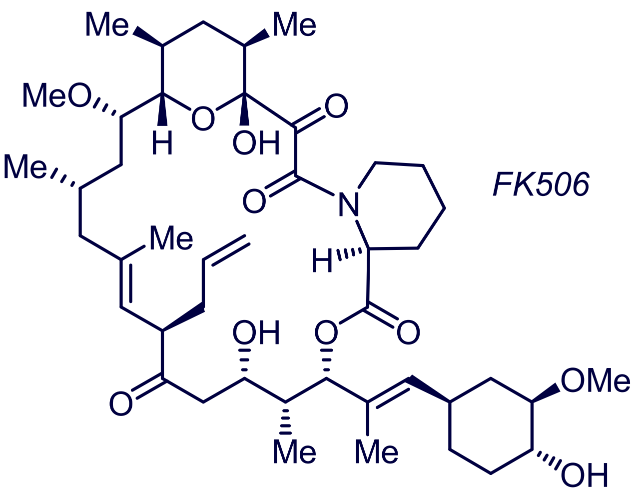


Small Molecule Modulators of Protein–Protein Interactions: Selected Case Studies

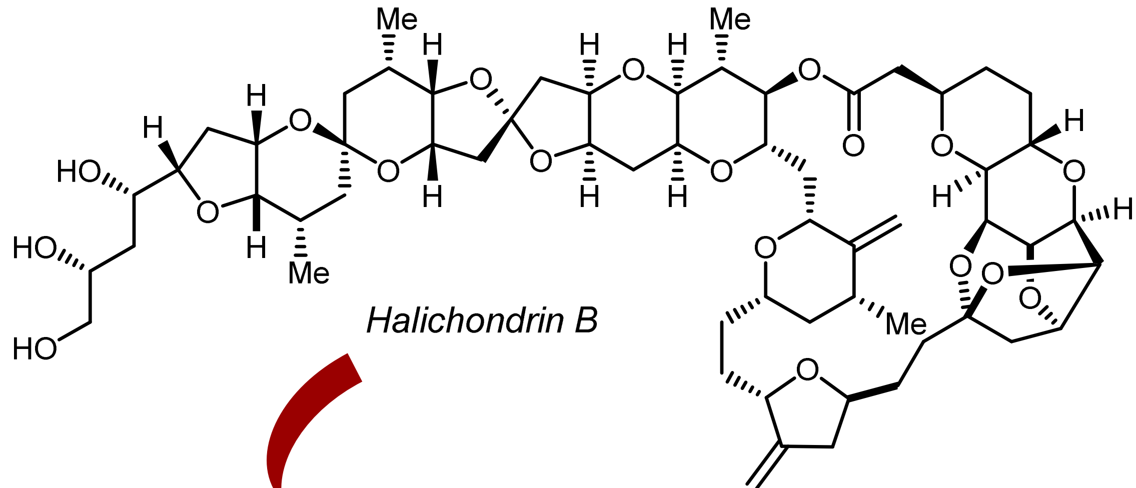
Madhu Aeluri,[†] Srinivas Chamakuri,[†] Bhanudas Dasari,[†] Shiva Krishna Reddy Guduru,[†] Ravikumar Jimmidi,[†] Srinivas Jogula,[†] and Prabhat Arya*

Dr. Reddy's Institute of Life Sciences (DRILS), University of Hyderabad Campus Gachibowli, Hyderabad 500046, India

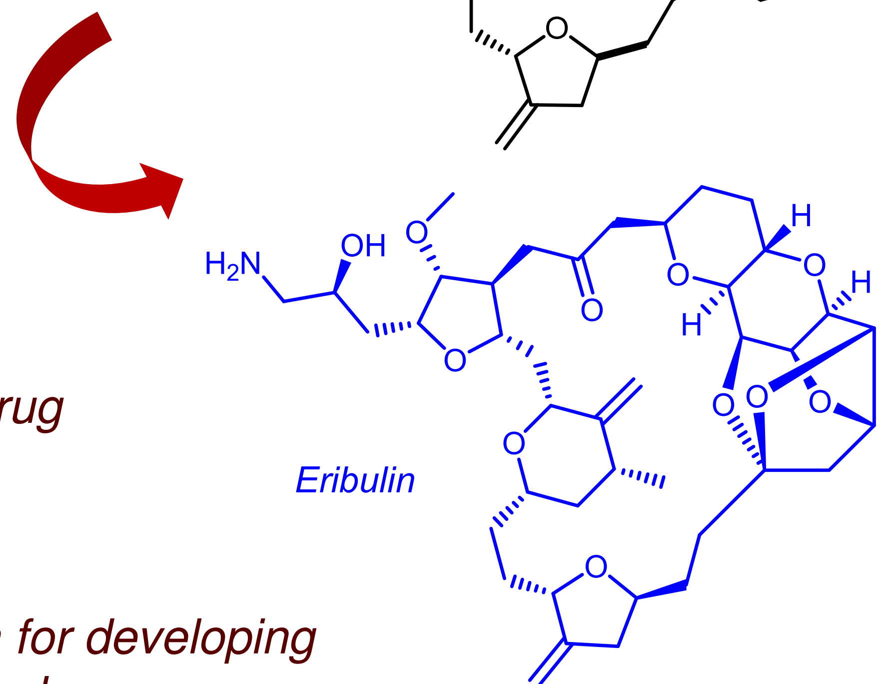
Finding Molecules as Effective Modulators of Pathways!



FK506



Halichondrin B



Eribulin

- present complex 3D architectures
- dense display of stereo-defined groups
- *challenging task in placing them on the drug discovery path!*
- excellent track record as small molecule modulators of protein-protein interactions
- *can serve as a good source of inspiration for developing novel scaffolds in the drug discovery arena!*

Building A Chemical Toolbox for Challenging "Undruggable" Targets

Bioactive Natural Products → Natural Product Inspired Sub-structures / Natural Product Fragments as Sub-structures

Key features in our design:

- 3D architectures
- sufficient complexity
- stereochemical and skeletal diversity
- synthesis in a reasonable time-scale
- easy to follow-up medicinal chemistry studies

Our working model

Exploring Macrocyclic Chemical Space!

- Cyclic compounds
- Large surface area
- Pre-organization
- Less freely rotating bonds
- Enhanced cell permeation

From Arya Research Team:

*Chem. & Biol.*163 (2005)
Curr. Opin. Chem. Biol. 247 (2005)
Chem. Rev. 1999 (2009)

Diversity-Based Organic Synthesis in the Era of Genomics and Proteomics**

Prabhat Arya,* Doug T. H. Chou, and Myung-Gi Baek

Angew Chem 2001

RESCUING COMBICHEM

Diversity-oriented synthesis aims to pick up where traditional combinatorial chemistry left off

STU BORMAN, C&EN WASHINGTON

The natural-product-like compounds produced in DOS have a much better shot at interacting with desired molecular targets and exhibiting interesting biological activity.

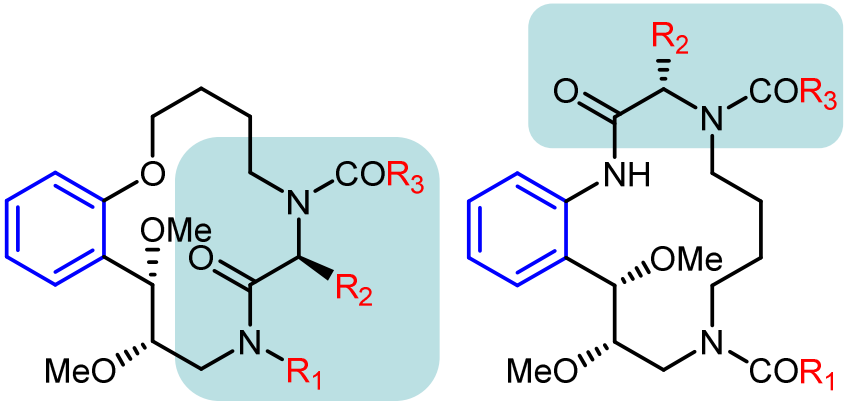
Chem & Eng News 2004

Examples of Our Early Work

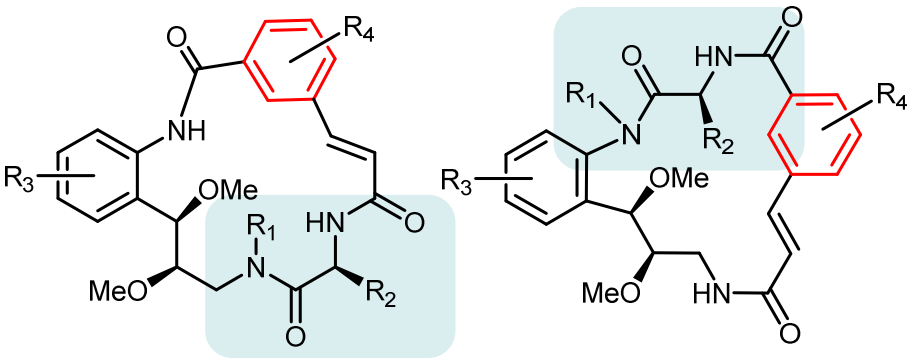
1 Natural Product-Inspired, Functionalized 14- and 17-Membered Rings Macrocyclic Toolbox

2 Indoline / Tetrahydroquinoline (Alkaloids) and Benzofuran (Flavonoids)-Inspired Macrocyclic Toolbox

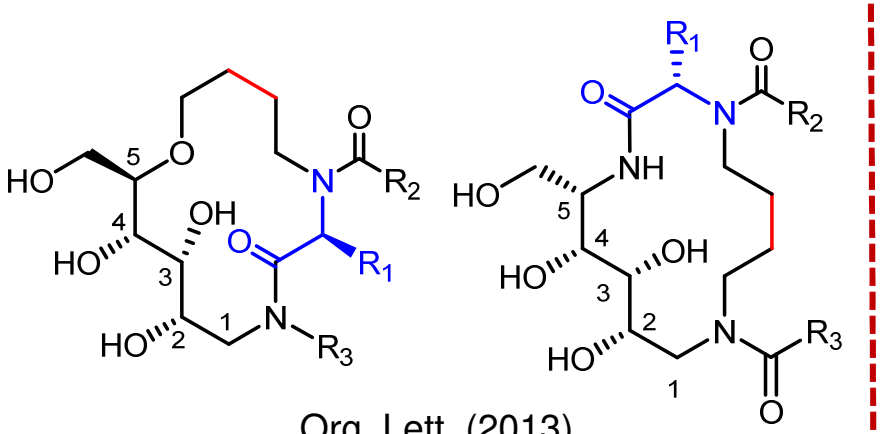
3 Glyco-based Macrocyclic Toolbox



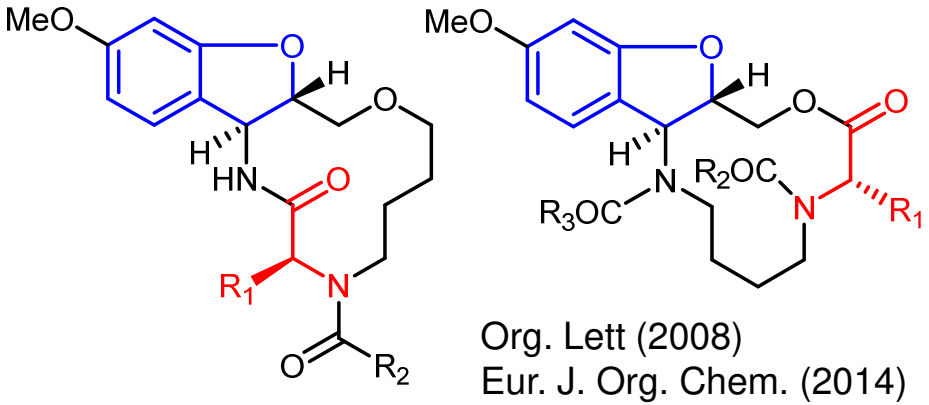
Org. Lett. (2013)



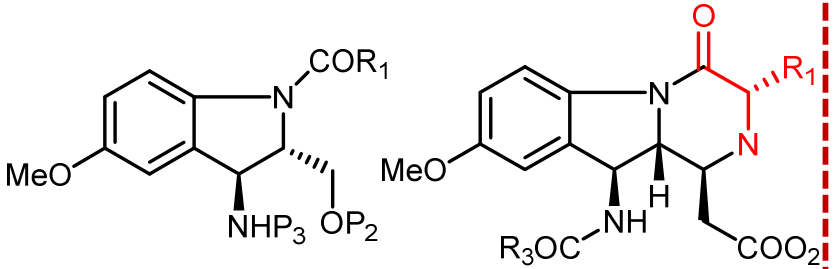
Eur. J. Org. Chem. (2013)



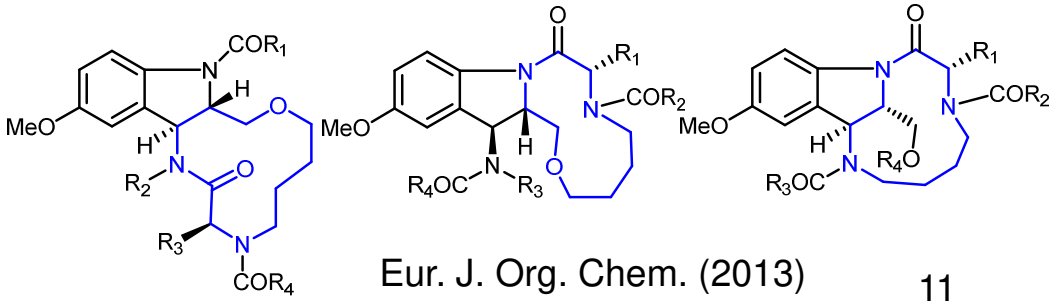
Org. Lett. (2013)



Org. Lett (2008)
Eur. J. Org. Chem. (2014)



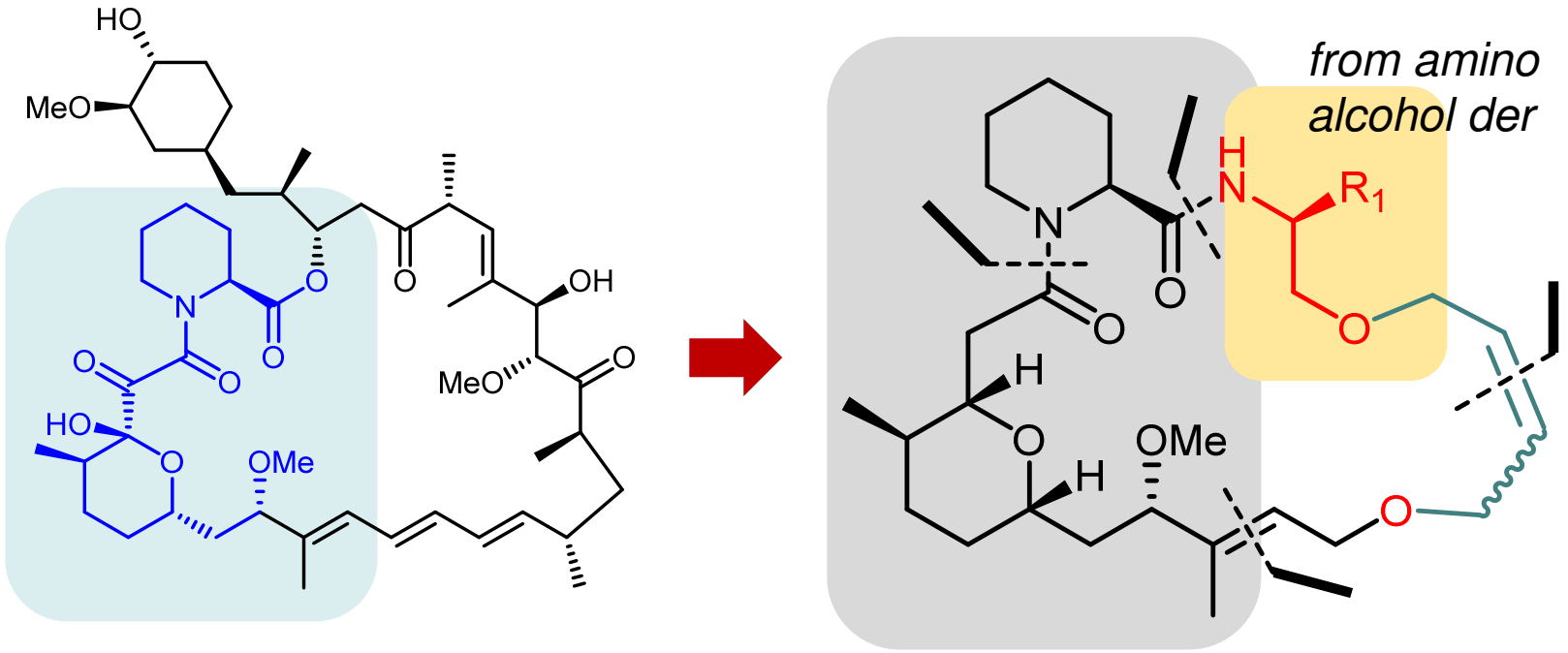
Angew Chem (2005)



Eur. J. Org. Chem. (2013)

Recent Examples

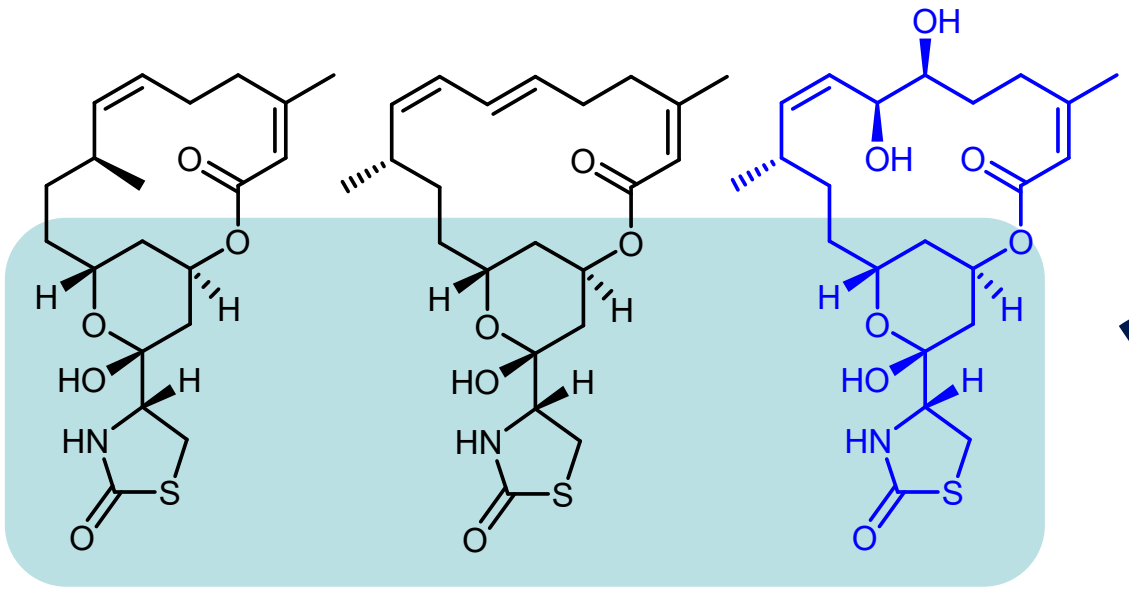
Example 1:
Rapamycin fragment-based Macrocyclic Toolbox



Rapamycin

Org. Lett. 480-483 (2015)

Example 2: Latrunculin-derived Macrocyclic Toolbox

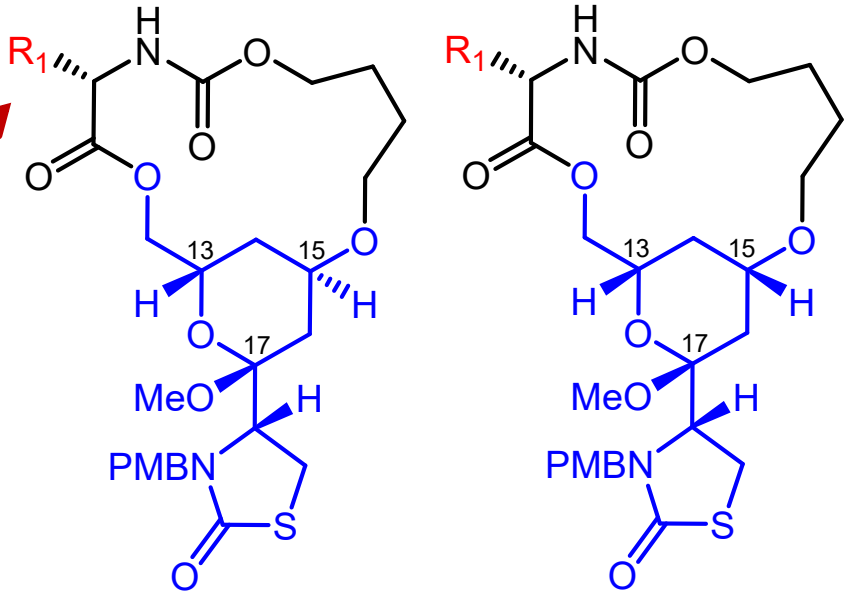


Latrunculin B *Latrunculin A* *Latrunculol A*

- Isolated from Red Sea sponge *Negombata magnifica*
- Potent G-actin Inhibitors
- Latrunculol A is more potent compared to other family members

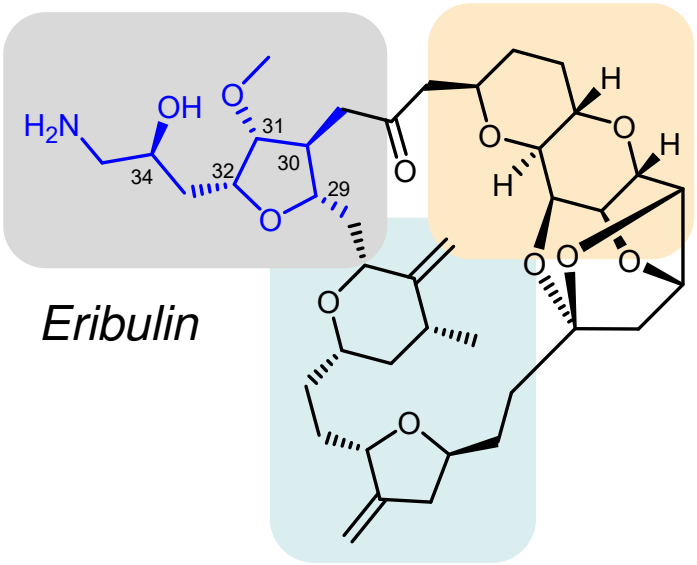
15-Membered ring

Diversity site



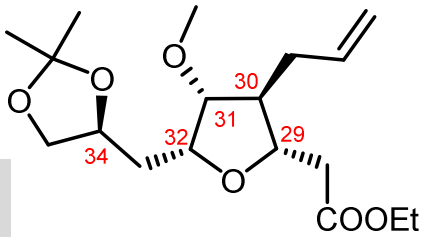
Org. Lett. 472-475 (2015)

Example 3: Macrocyclic Toolbox based on Eribulin Sub-structures



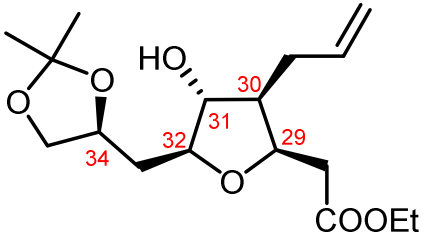
Practical and scalable synthesis

B

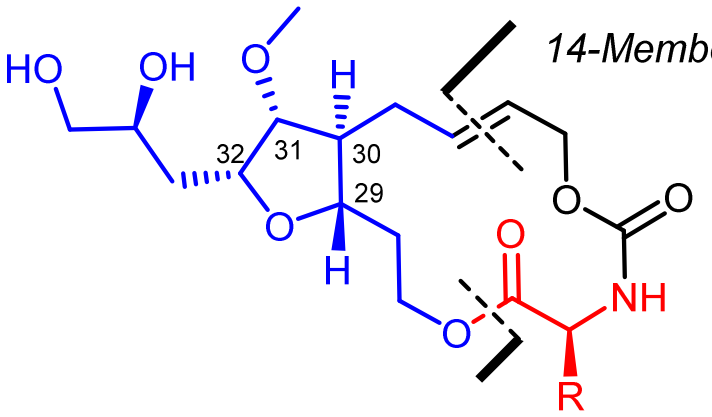


A

Precise fragment-based

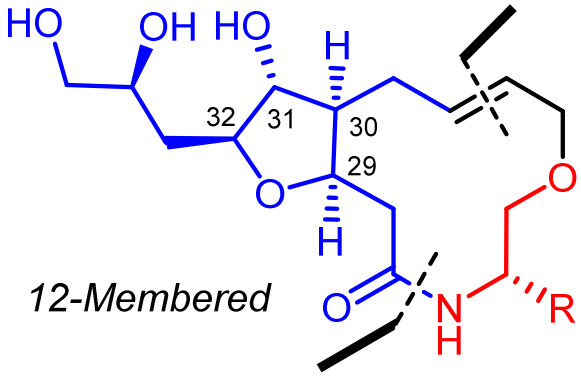


Diastereomeric fragment-based



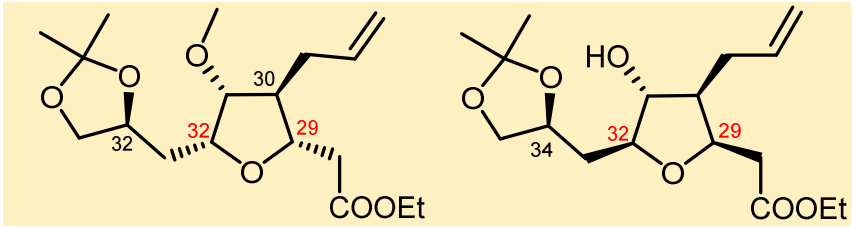
A

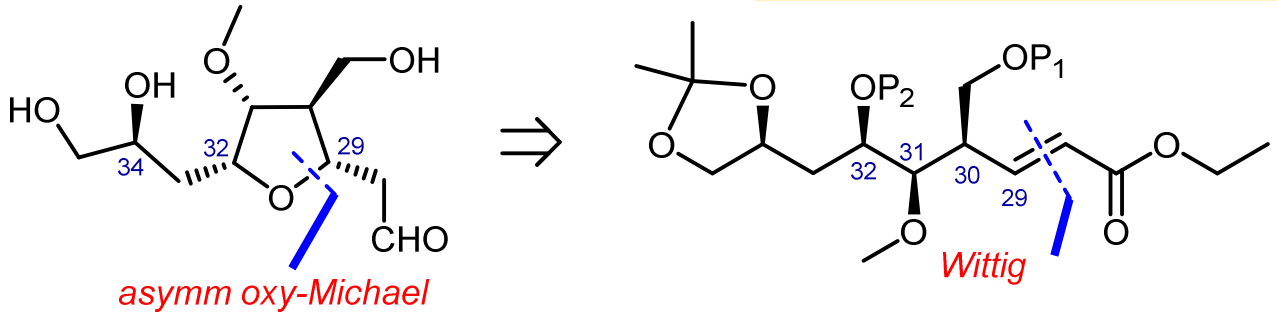
B

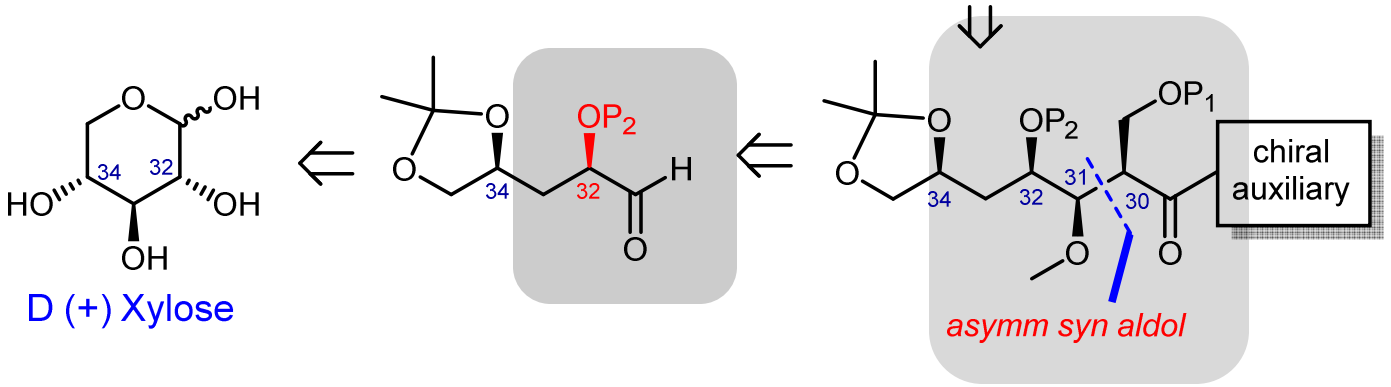


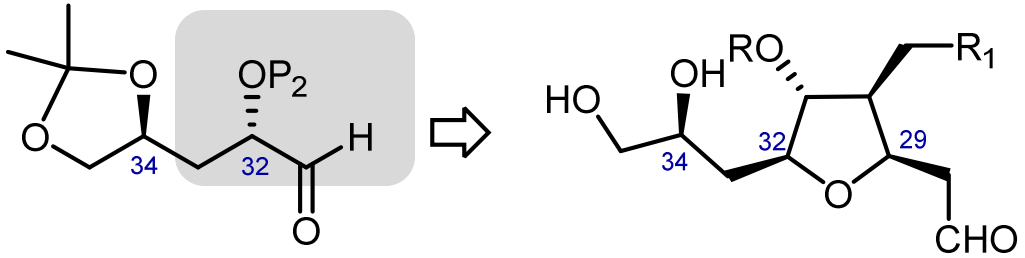
Org. Lett. 468-471 (2015)

Our Approach



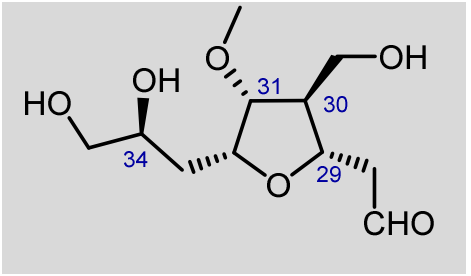




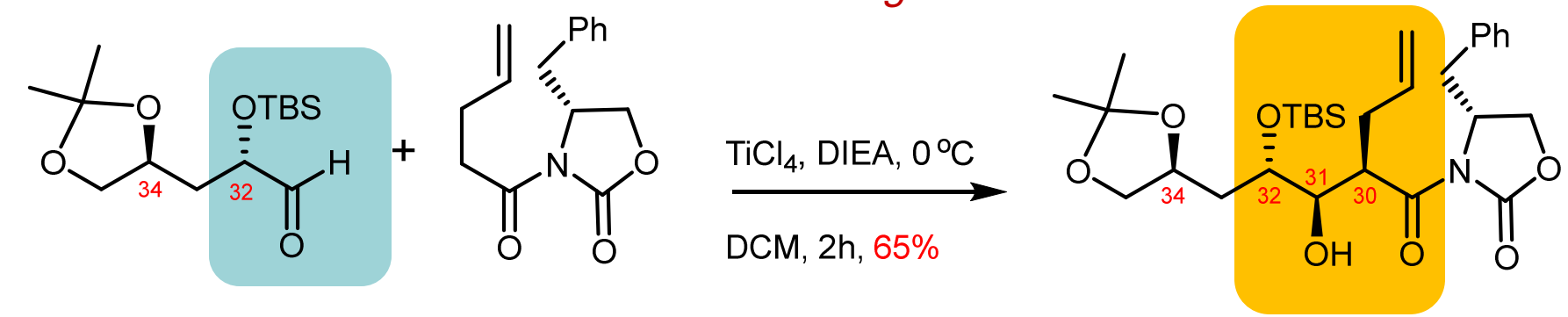


Jimmidi, Krishna Reddy, Arya *Org. Lett.* **2015**, *17*, 468-471

Synthesis of Diastereomeric Fragment

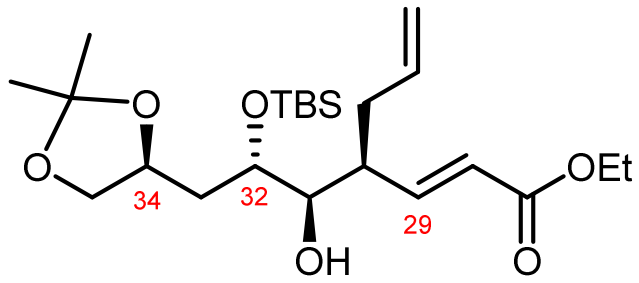


single diastereomer

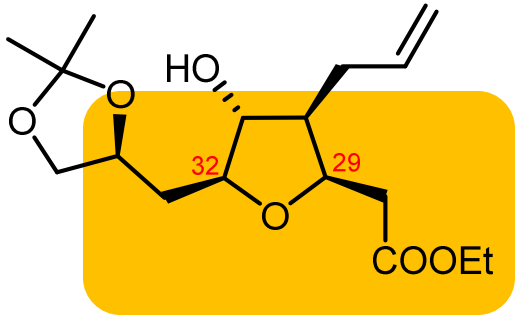


i. $NaBH_4$, THF:H_2O (4:1), 0 °C-RT, 3h, 75%

ii. TEMPO, $PhIO_4$, DCM, 4 h, $PPh_3CHCOOEt$, 80%

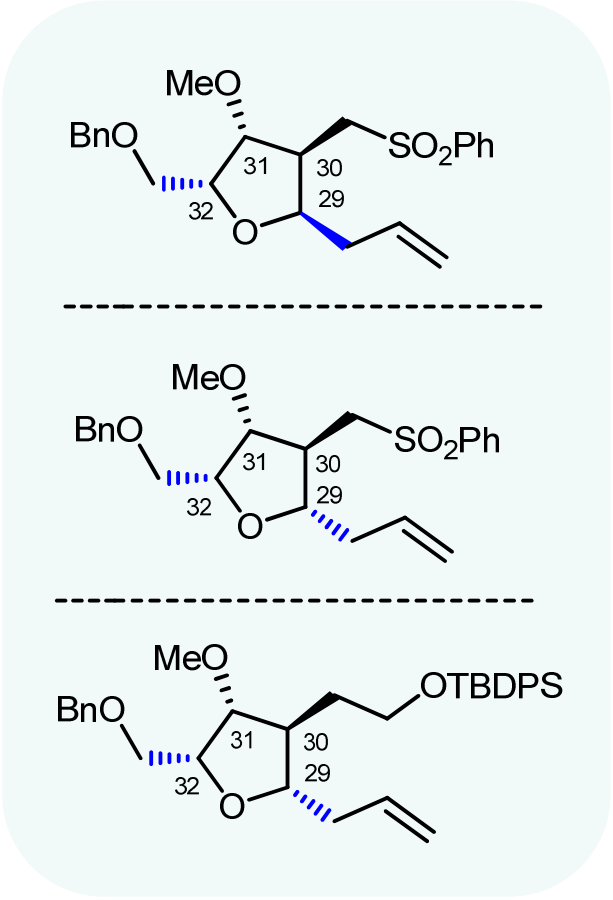


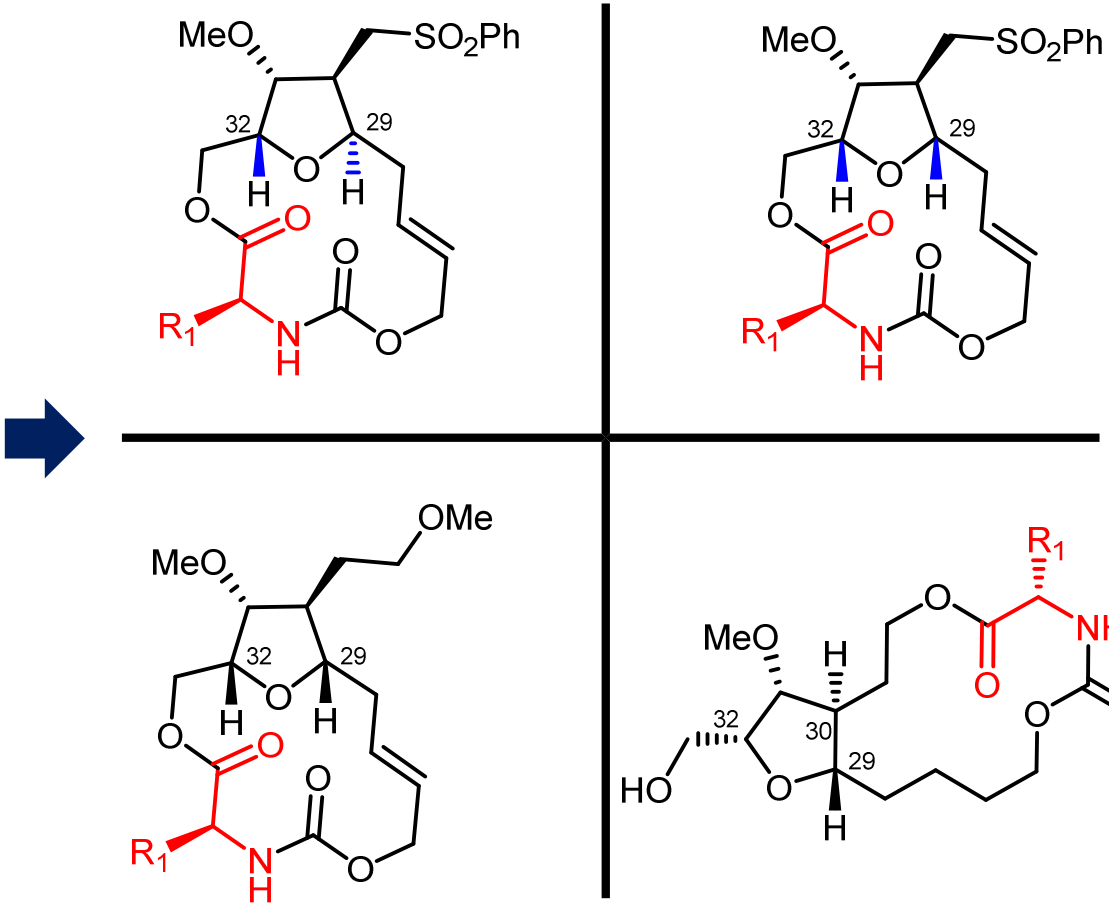
TBAF, THF, 0 °C-RT, 70%



single diastereomer

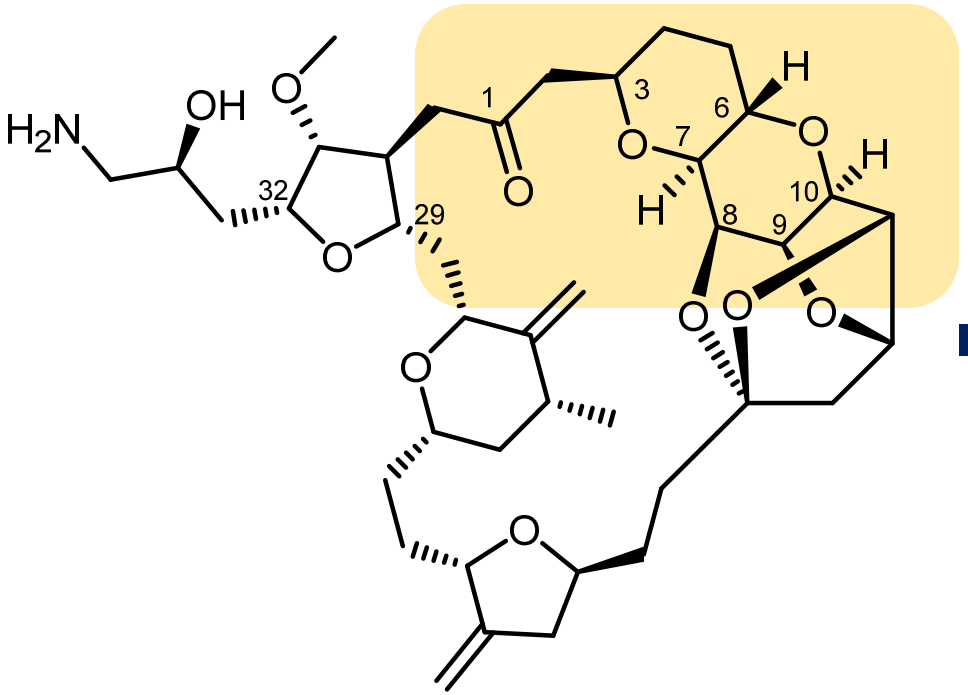
Different Macrocyclic Architectures



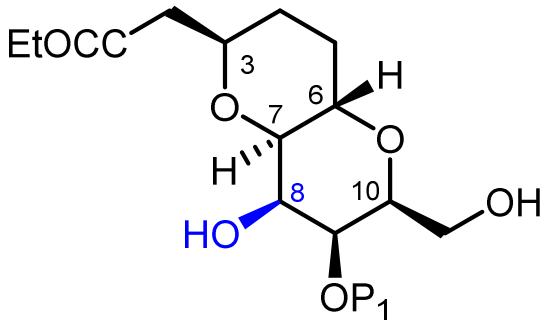


Synthesis Special Issue (2017)

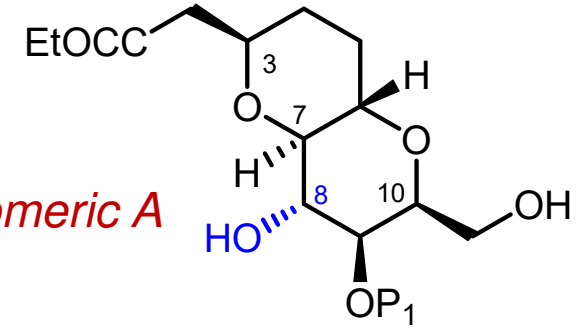
Leading to A New Family of Macrocyclic Architectures



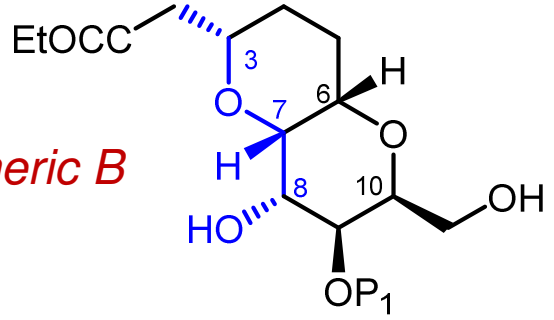
Precise



Isomeric A



Isomeric B



Submitted for publication

Growing Faith in Emerging Chemical Biology Model

clinically-relevant questions

Modern Organic Synthesis

novel chemical toolbox to explore the scope of biologically relevant chemical space
// natural product-inspired compounds

Cell Signaling Biology (biological questions/ novel assays)

cellular/zebrafish studies
in vivo studies

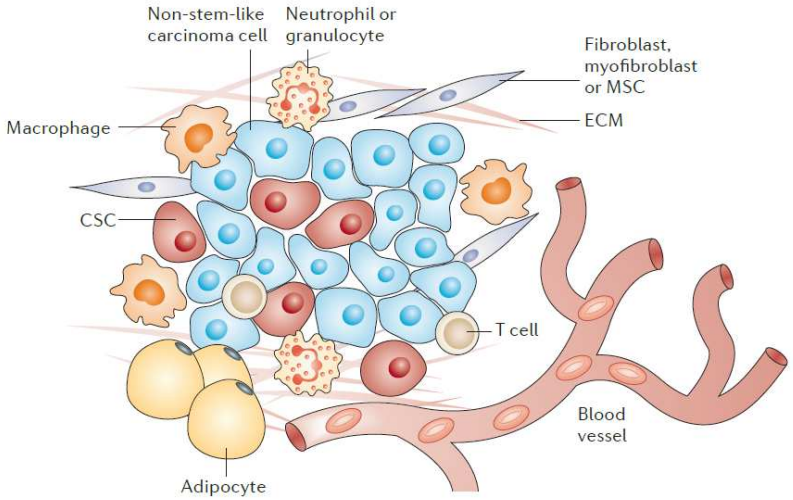
Phenotypic Unbiased Screens

Discovery of functional small molecules

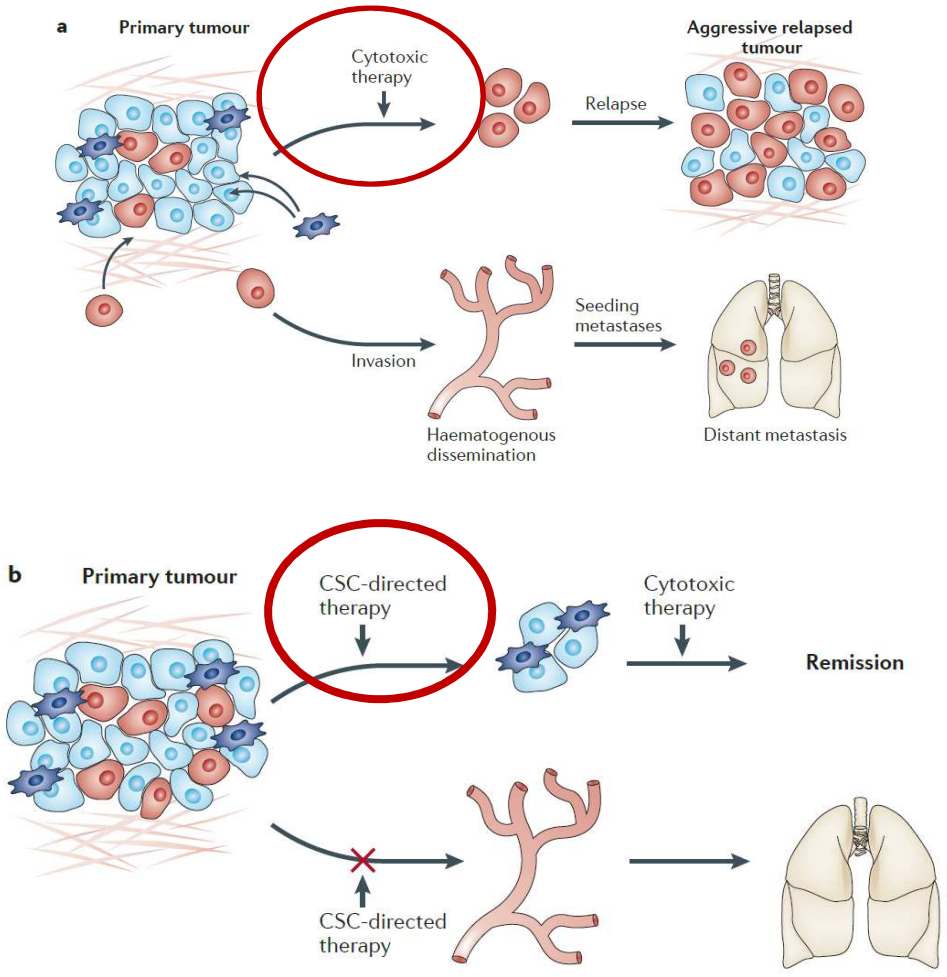
placing them onto the drug discovery path!

understanding the mode of action – biophysics, genomic, CRISPR-Cas9 tools

Challenges in Cancer

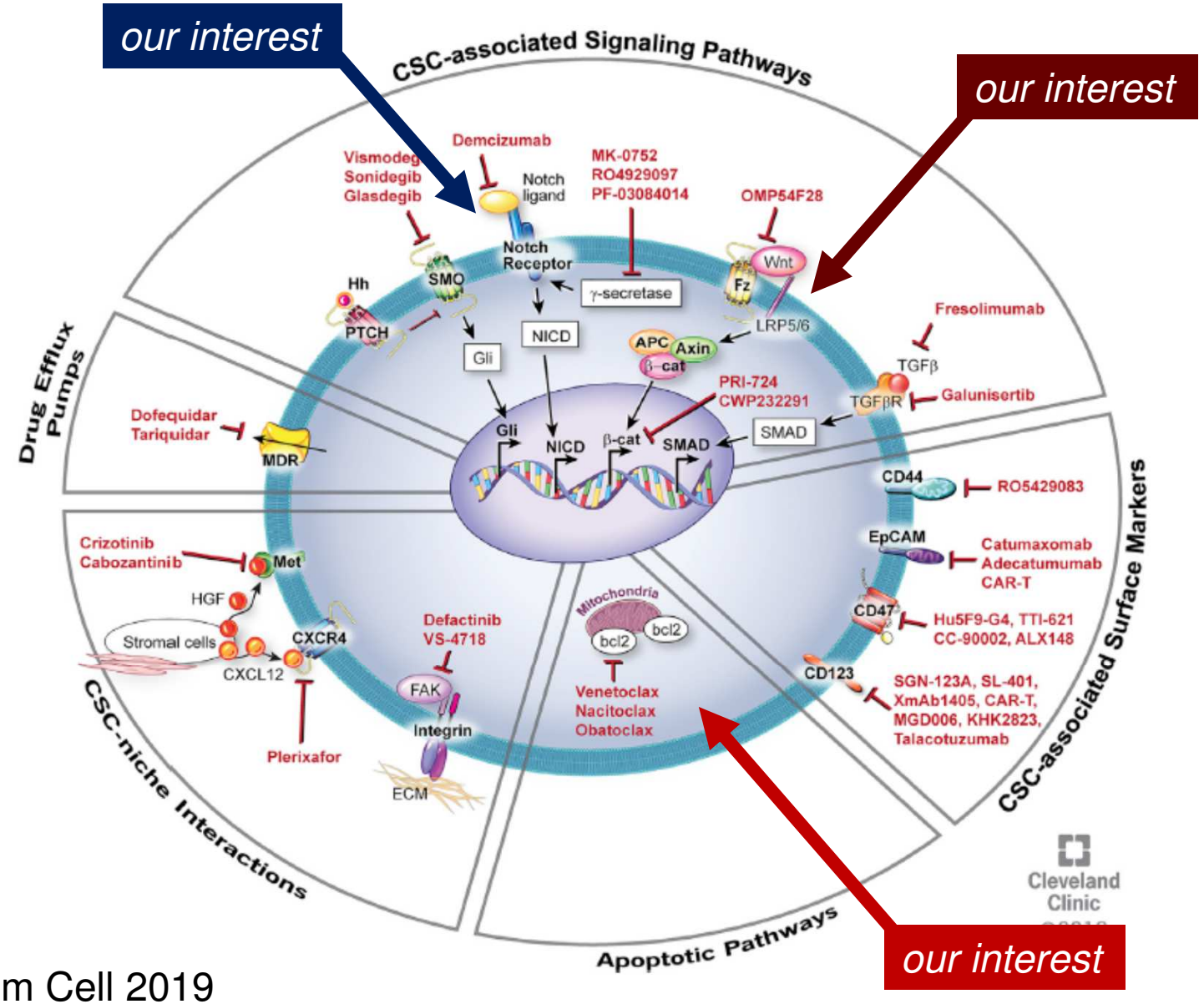


- Tumor heterogeneity
- Every patient is different!
- *Cancer cells vs cancer stem cells/tumor initiating cells*
- Selective killing – understanding of signaling pathways involved
- Tumor metastasis – Understanding of signaling pathways involved

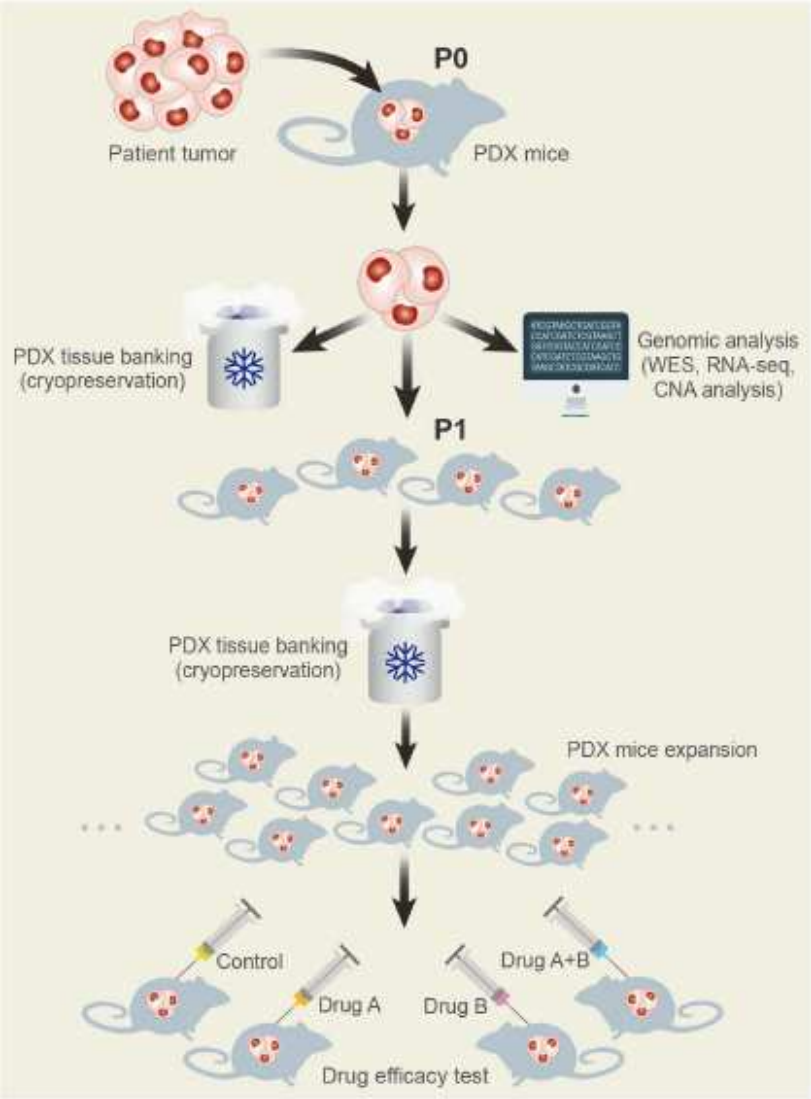


Nat Rev Drug Discov 2014

Cancer Stem Cell-derived Signaling Pathways



Cancer Patient-derived Banking Platform



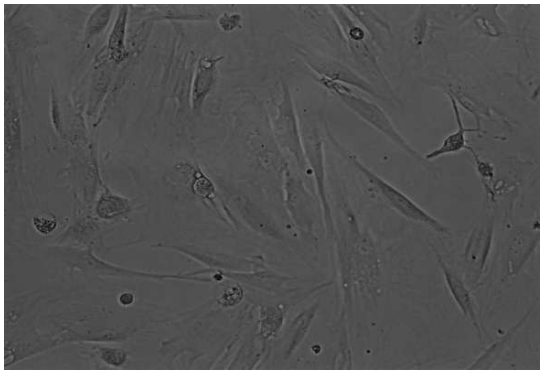
TRANSCELL Oncologics

- >900 Indian patient samples banking with primary cancer cells, tumorspheres (enriched with cancer stem cells).
- Patient-derived tumor xenograft (1st generation, 10 samples as proof of concept).
- Novel procedure for obtaining tumorspheres that are rich in tumor initiating cells/cancer stem cells

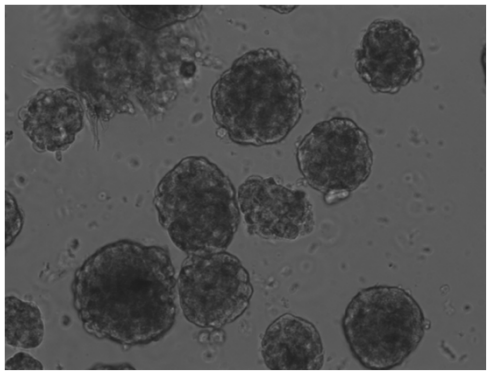
Indian Patient-derived Transcriptomic Study

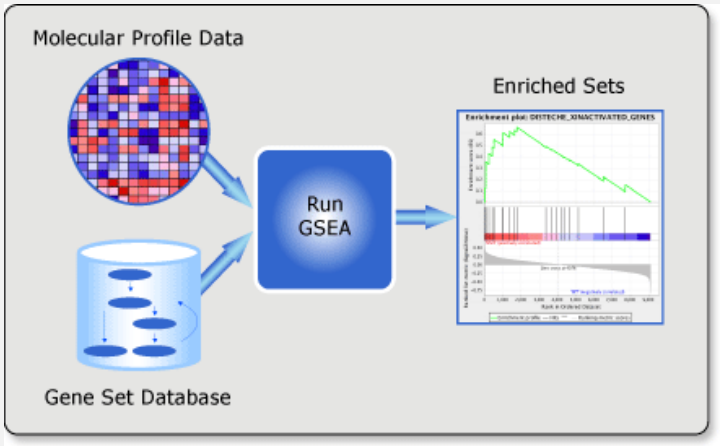
Tumor Biopsy

Primary Cancer Cells



Tumorspheres





- Oncogenic Signatures (Broad Inst)
- Cancer Hall marks (Broad Inst)
- Apoptosis

→ K-RAS, c-Myc, Wnt

Unpublished

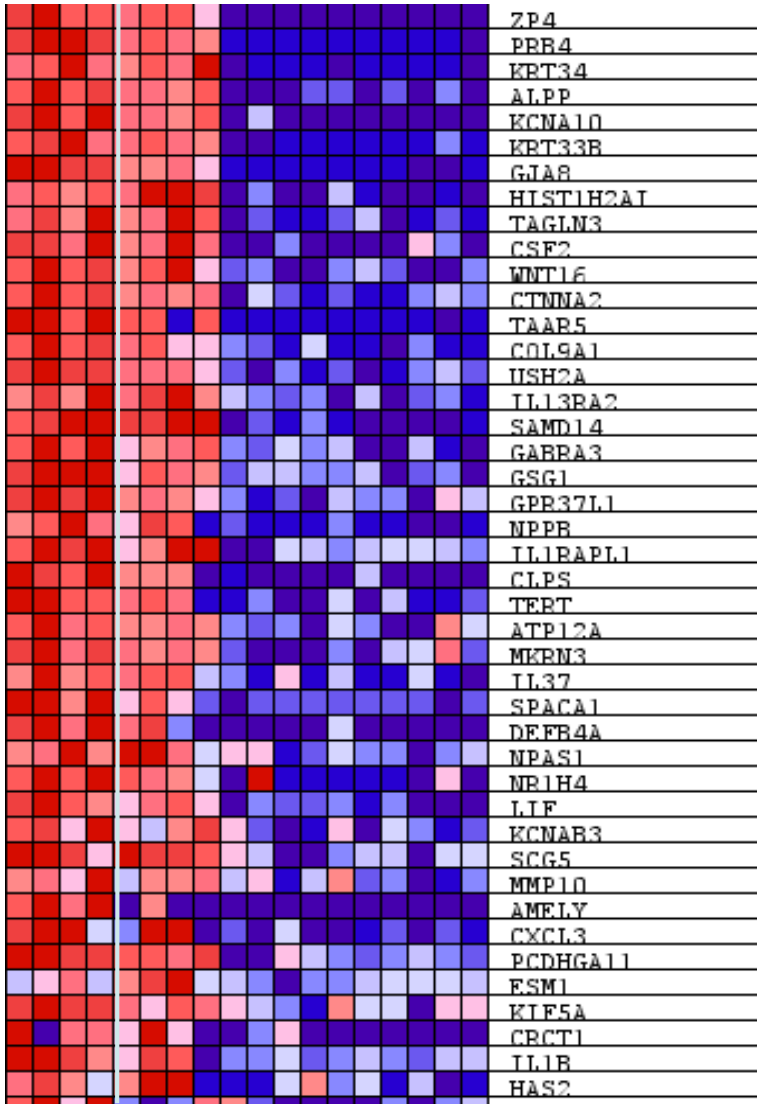
Transcriptomic Data – Breast Cancer

Down
Up

TS: Tumorspheres
PCC: Primary Cancer Cells

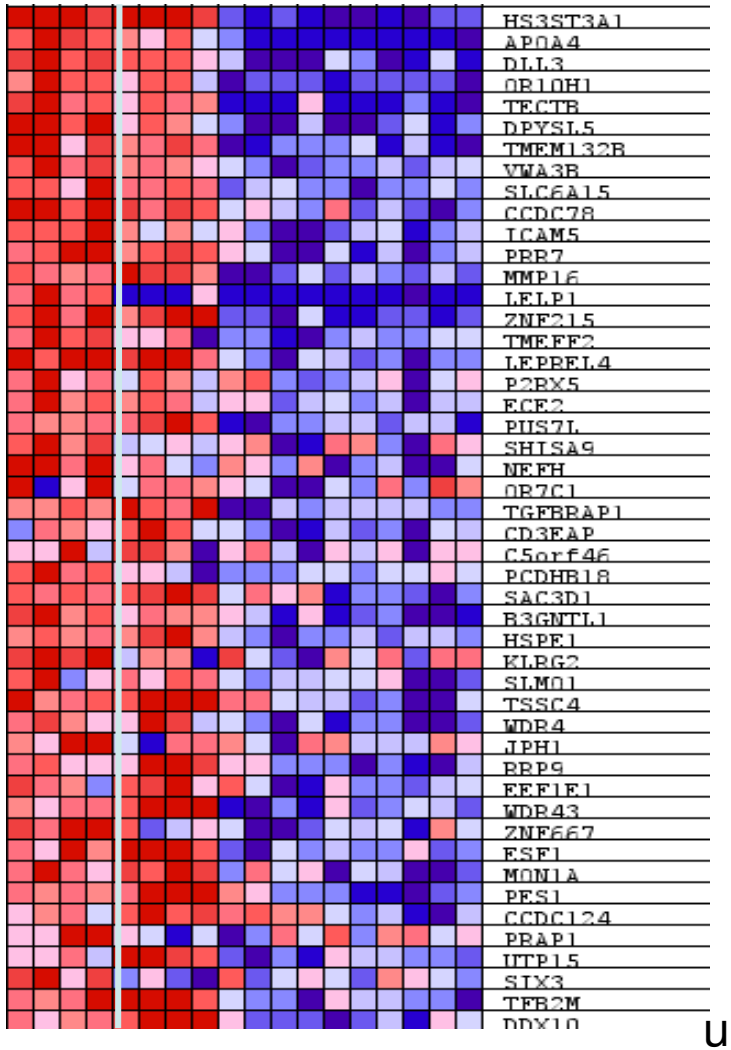
TS PCC Control

KRAS target genes



TS PCC Control

Myc target genes



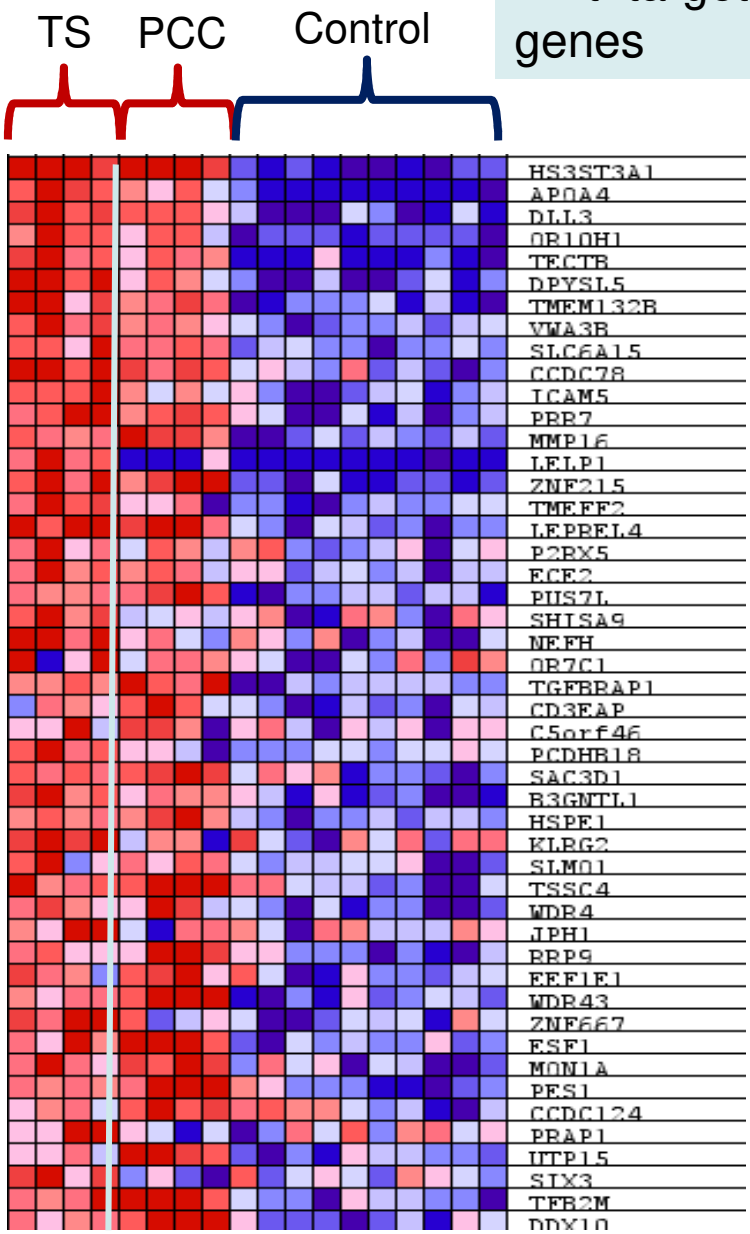
unpublished

Breast Cancer

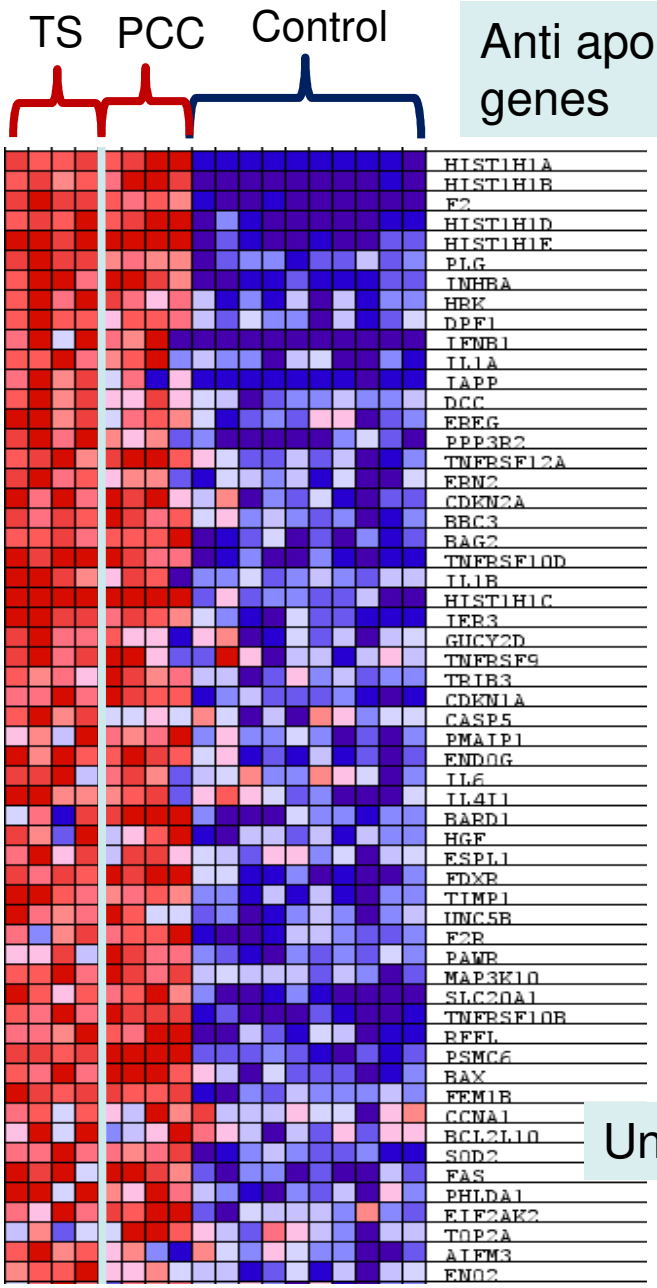
Down
Up

TS: Tumorspheres
PCC: Primary Cancer Cells

Wnt target genes

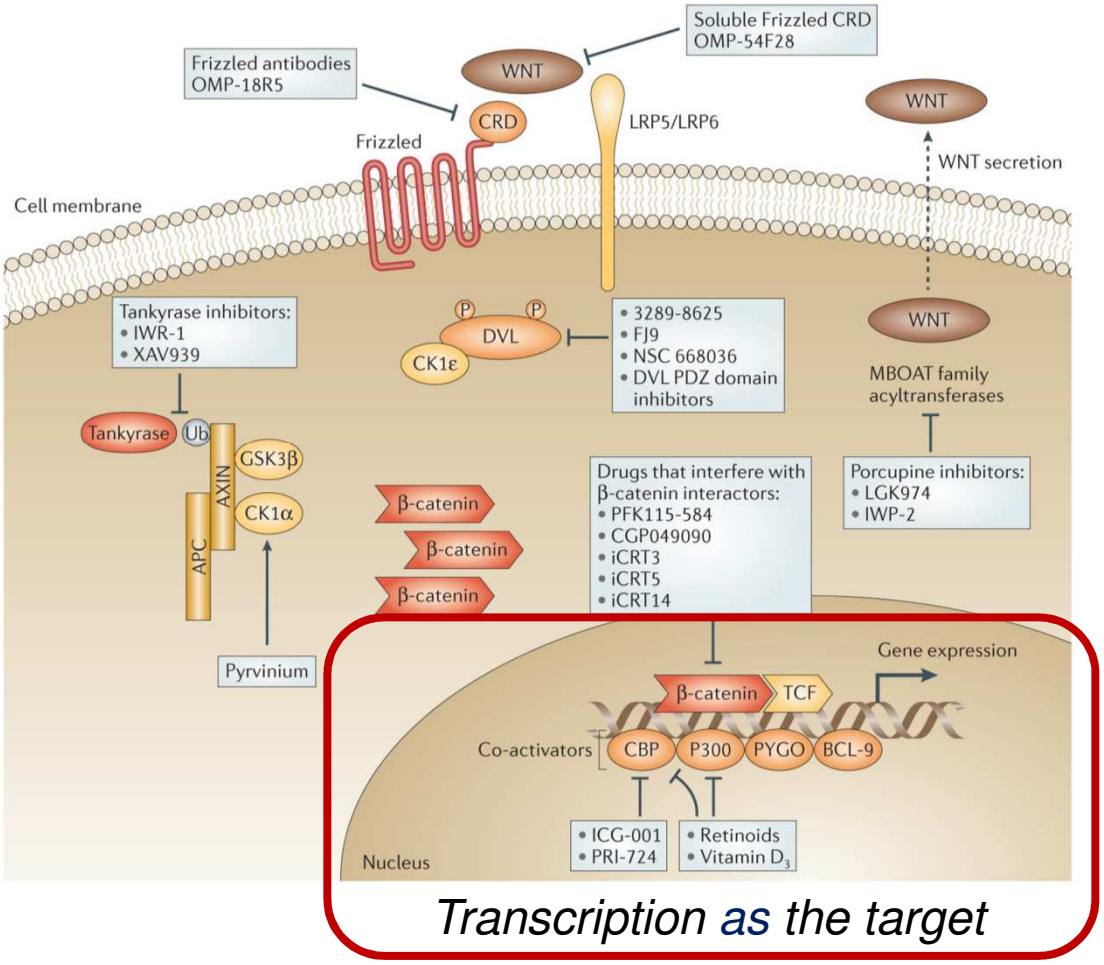


Anti apoptotic genes



Unpublished

Wnt Signaling and Stemness



Transcription as the target

- "Undruggable" domain
- Multiple cell surface interactions
- Multiple protein-protein interactions (Cytoplasm)
- Multiple DNA-protein-protein interactions (Transcription/Nucleus)
- Play a key role to maintain stemness / epithelial to mesenchymal transition / critical for cancer stem cells / metastasis

Nat Rev Drug Disc 2014

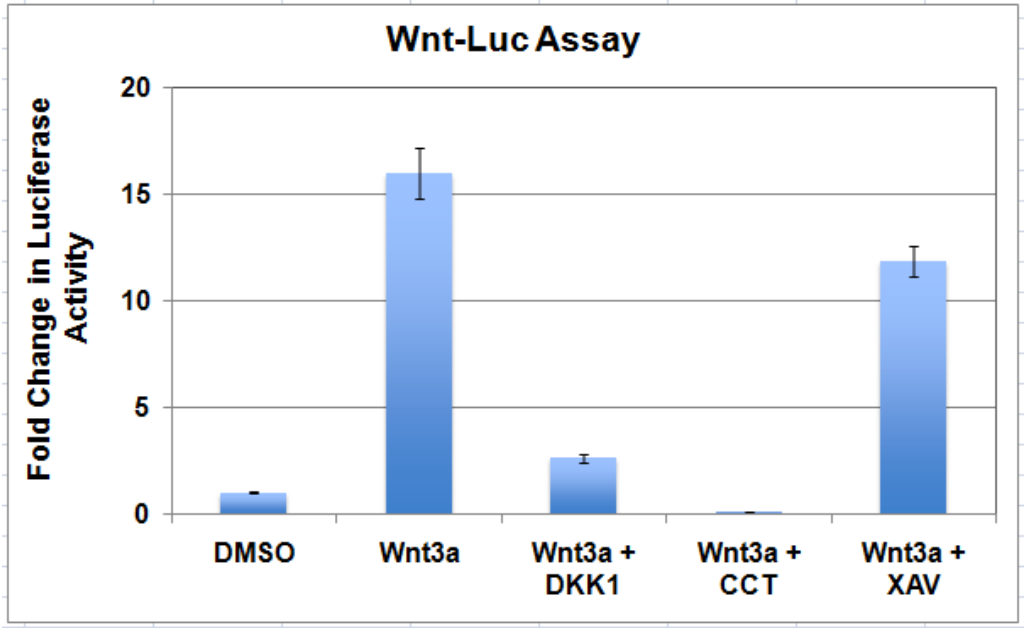
Primary Screening – WnT Inhibitors

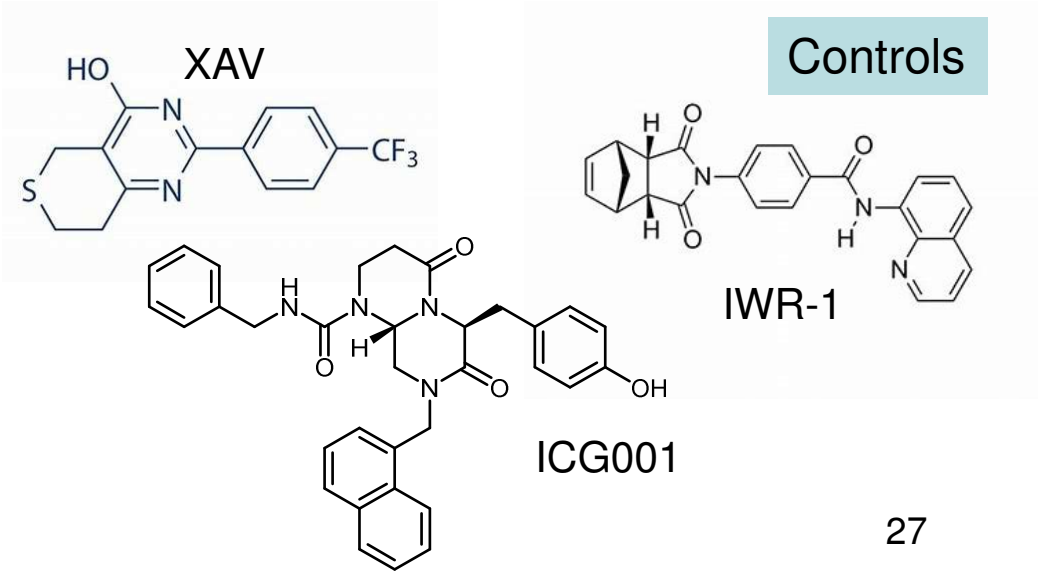
30,000 cells per well //
Incubate the plates
overnight in CO2 incubator

Add agonist (Wnt 3a), antagonist (DKK1), control (DMSO)

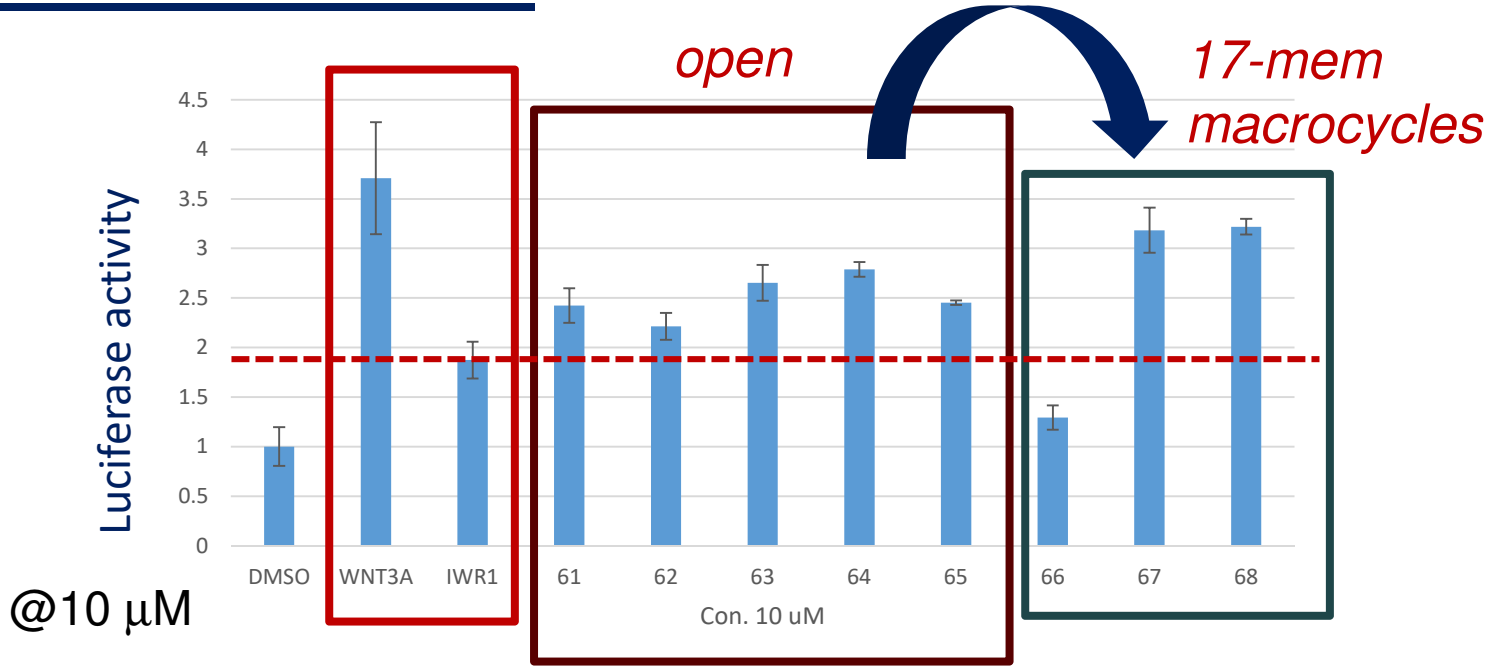
Add 100 µL luciferase substrate mixture

Incubate for 10 min and then read the chemi-luminescence signal



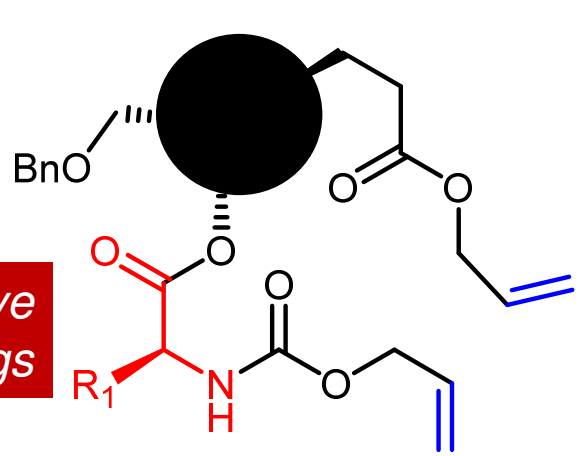


Wnt Luciferase Screening Data

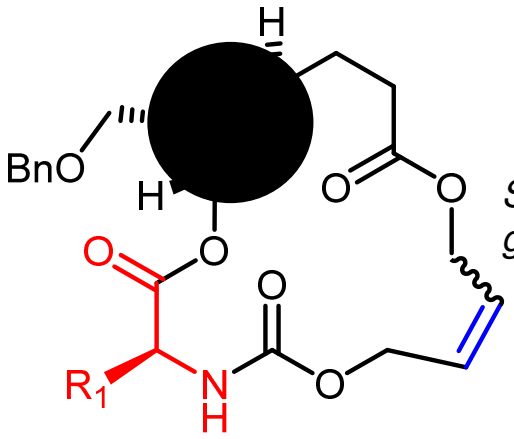


@10 μM

Five Analogs



SM-2015-61/62/63/64/65



Single olefin geometry

SM-2015-66/67/68/69/70

Family 1 Hits

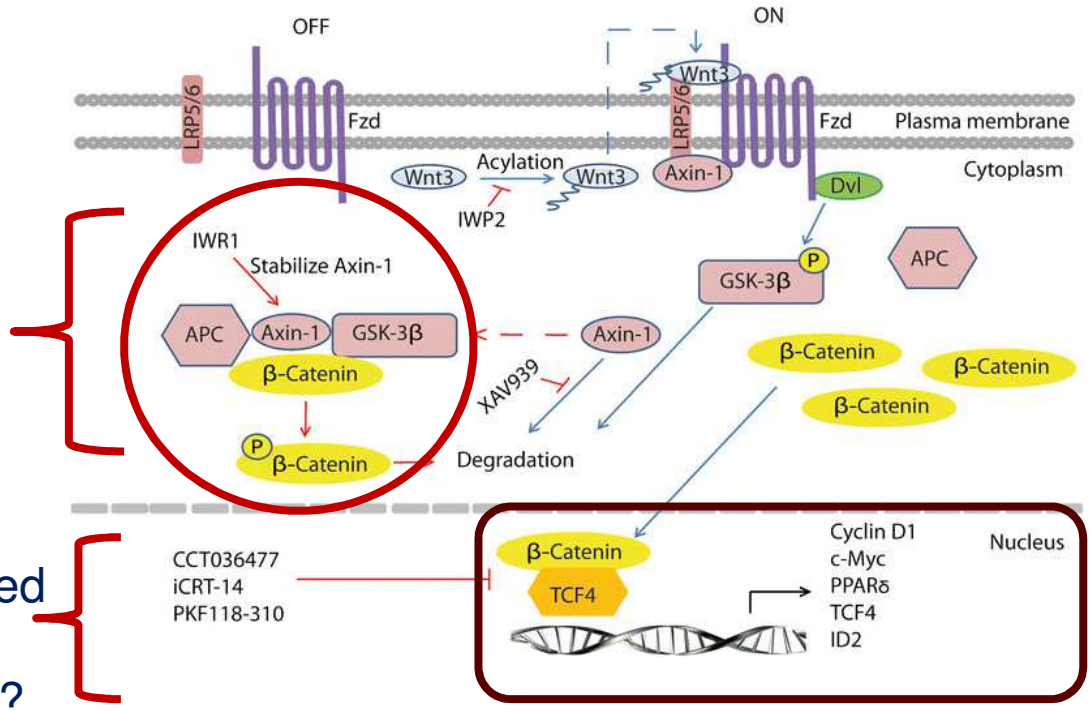
Our Chemical Biology Journey!

The effect of small molecule on cytoplasmic protein complexes?

The effect of small molecule on transcriptional machinery – related to multiple protein-protein // DNA-protein-protein interactions?

Tools and Methods:

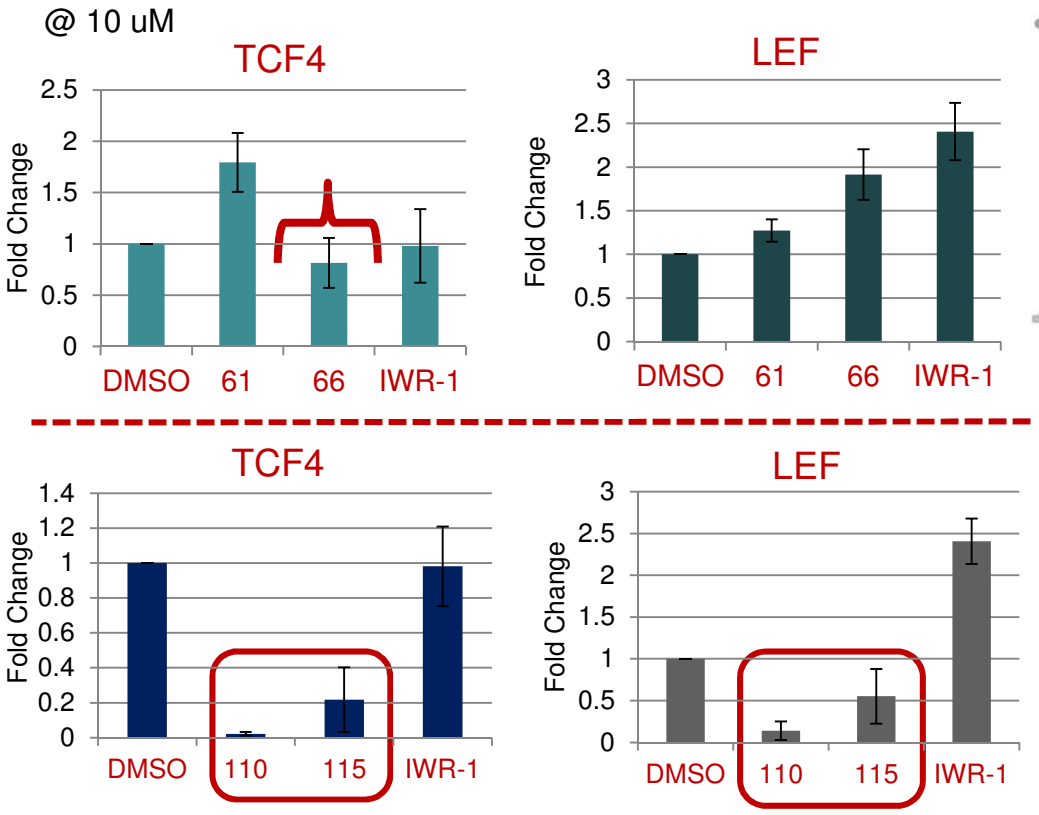
RT-PCR
Western blots
RNA sequencing
Quantitative proteomics
Finally, the target pull-down plan

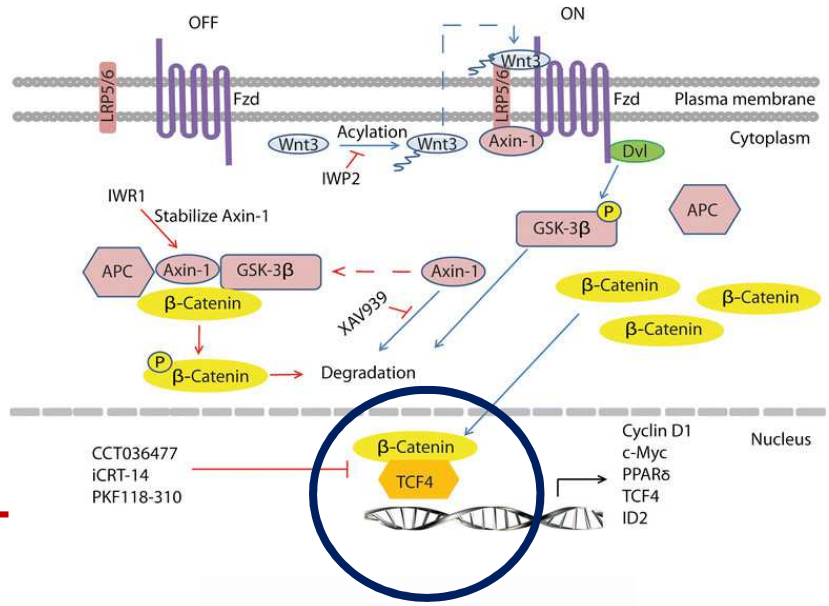


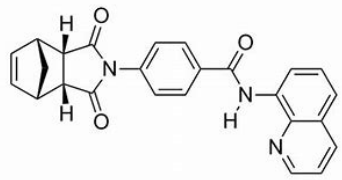
On the Functional Biology Front:

- The effect on tumorspheres from established cell lines?
- The effect on tumorspheres from patients-derived cells?
- The effect on organoids synthesized from patient organs/tissues?
- The effect on stemness / EMT / cancer stem cells?

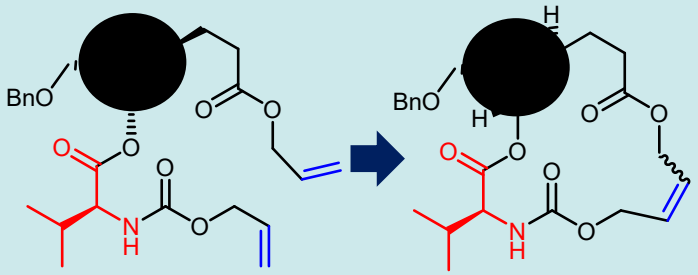
RT-PCR Data – Transcription Genes





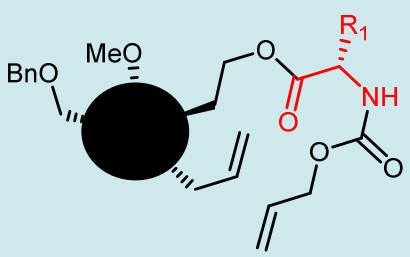


IWR-1 (control)

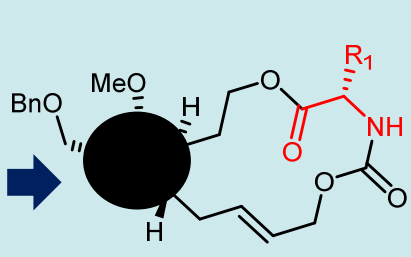


SM-61

SM-66



SM-110

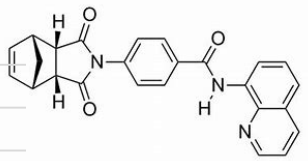


SM-115

RT-PCR Data (contd.)

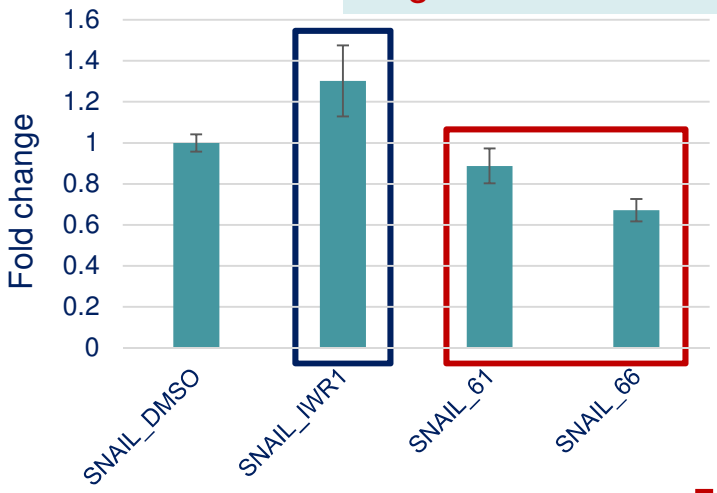
Selected genes related to…

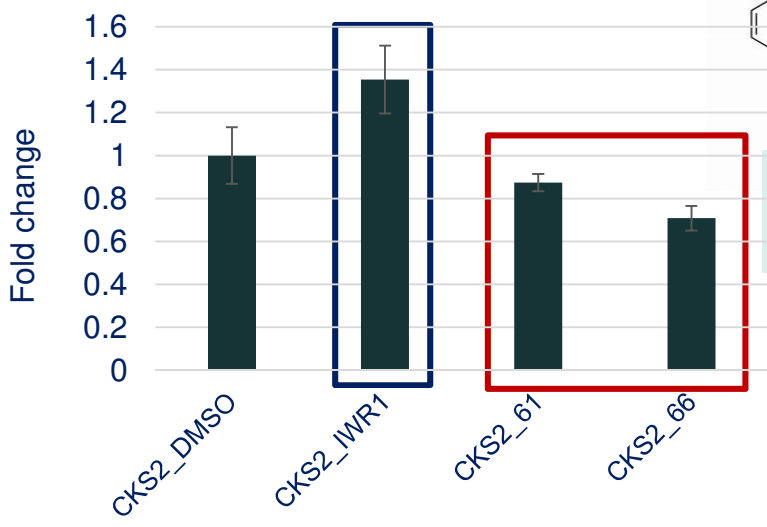
IWR-1

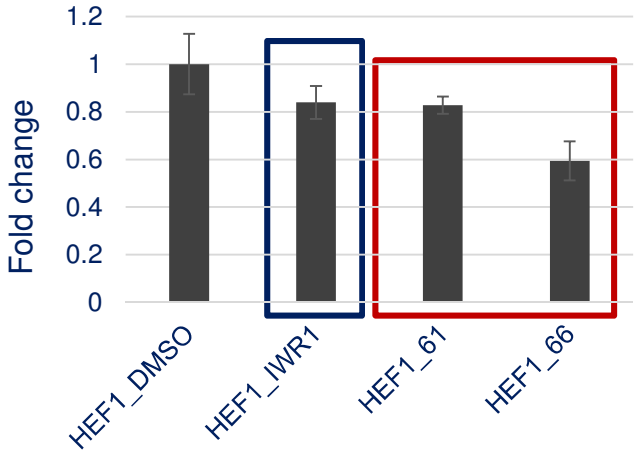


66: macrocycle
61: precursor

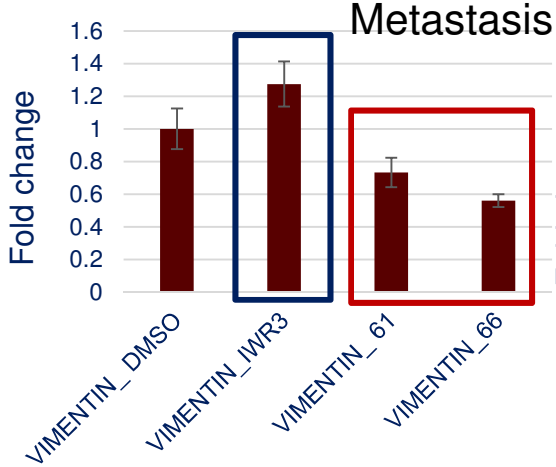
Migration / Invasion



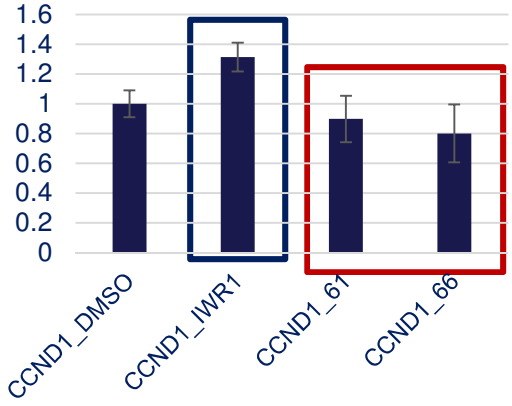




Metastasis



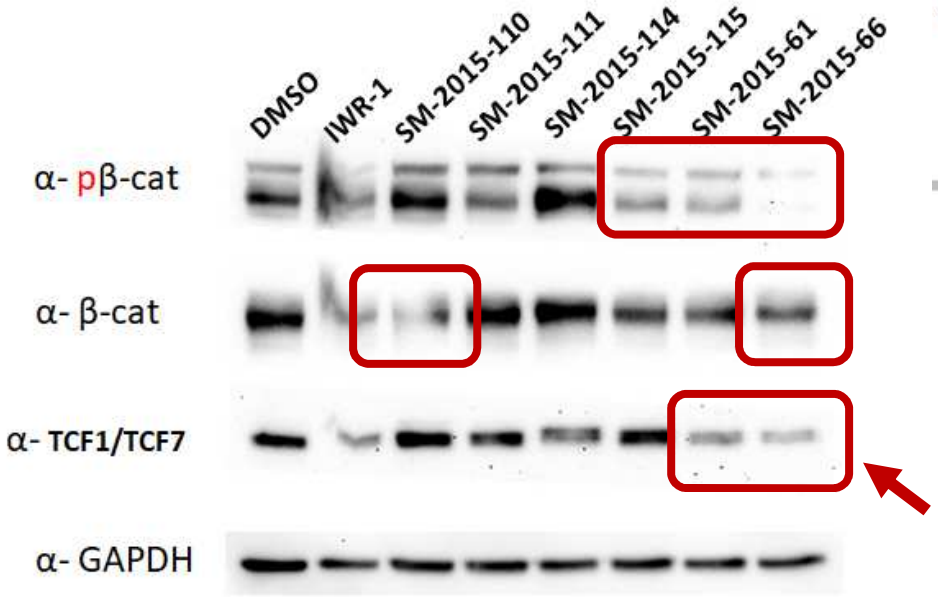
Proliferation

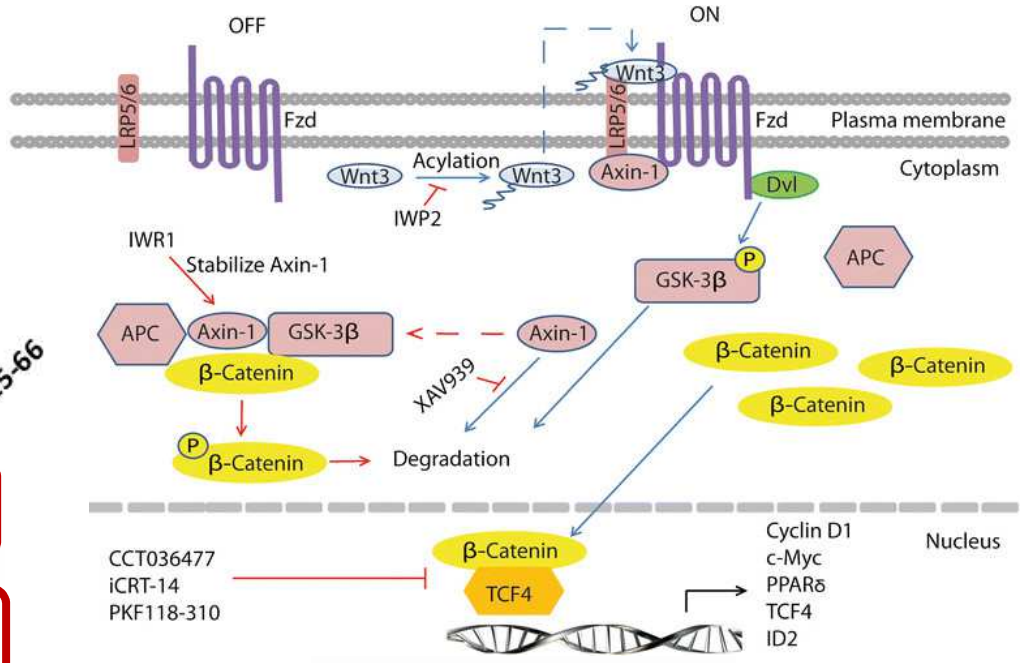


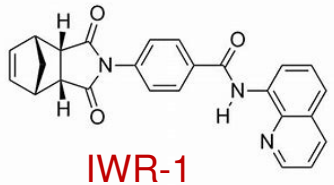
With Family 1 hits

Moving on to proteins

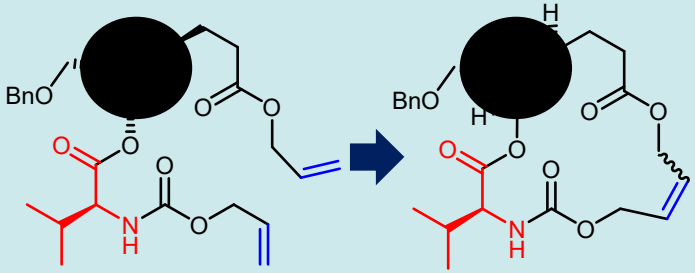
Western Blots





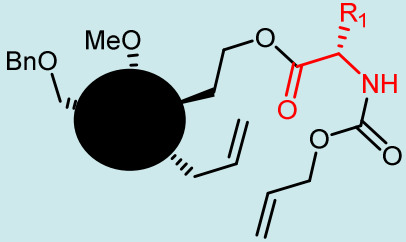


IWR-1

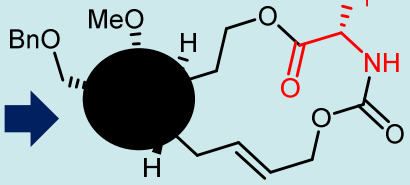


SM-61

SM-66

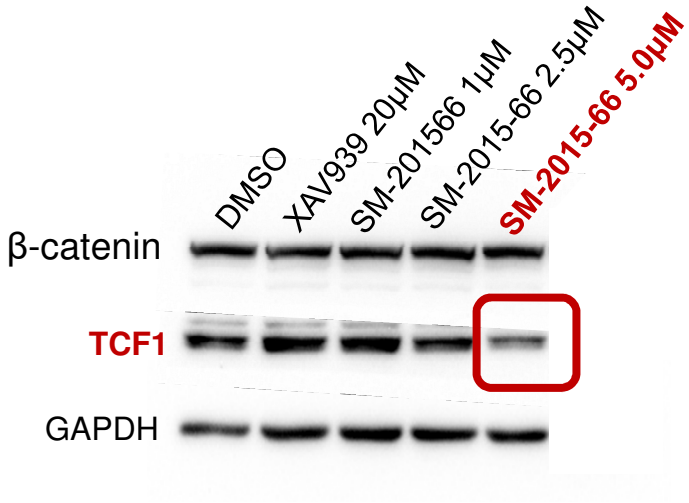


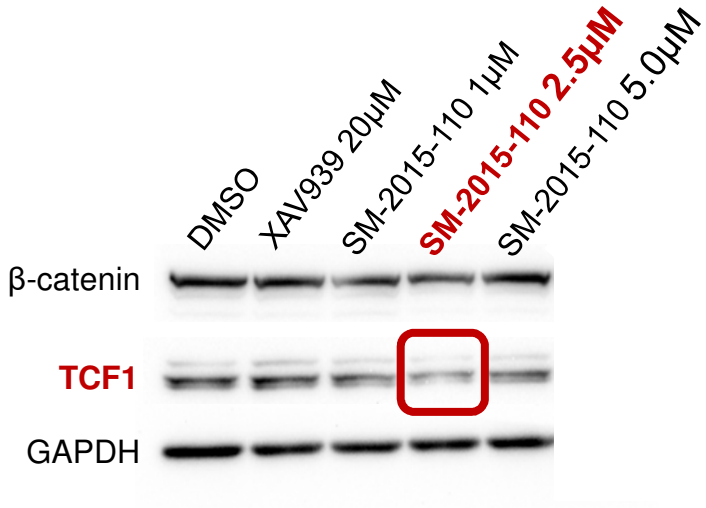
SM-110

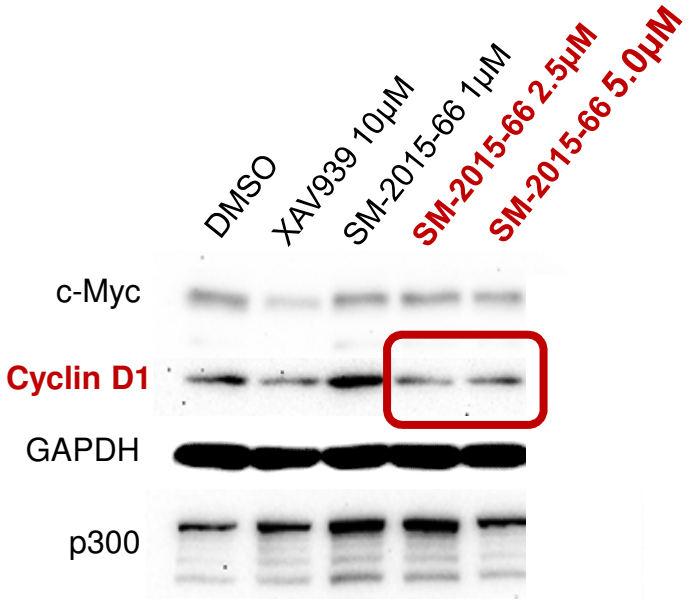


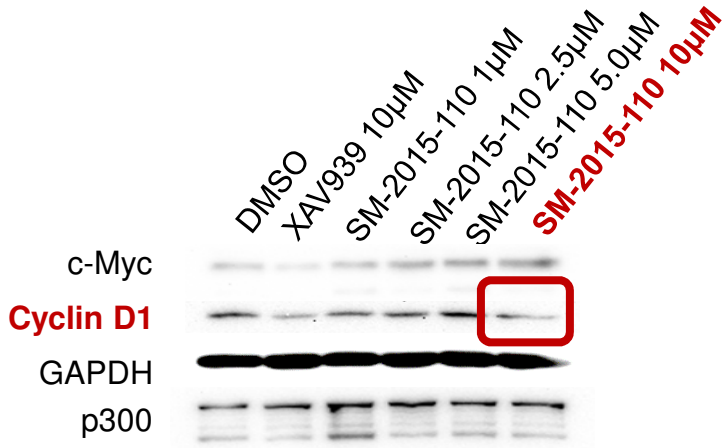
SM-115

Western Blots (contd)

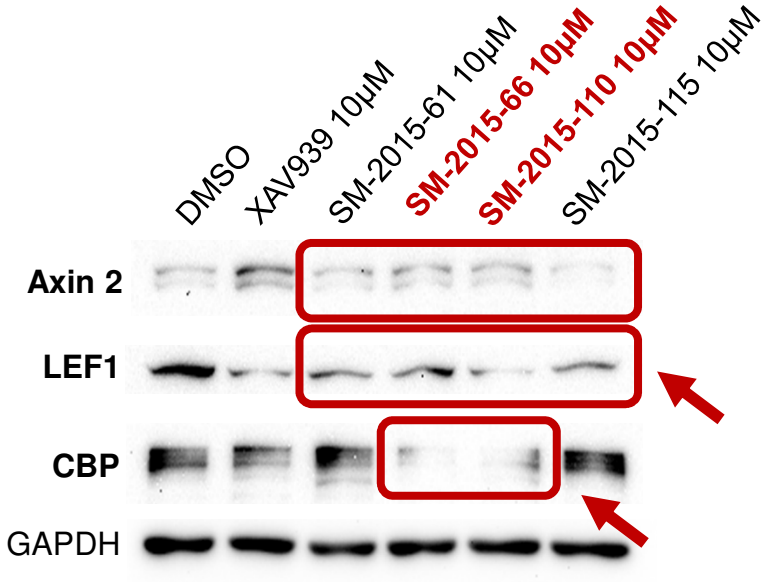








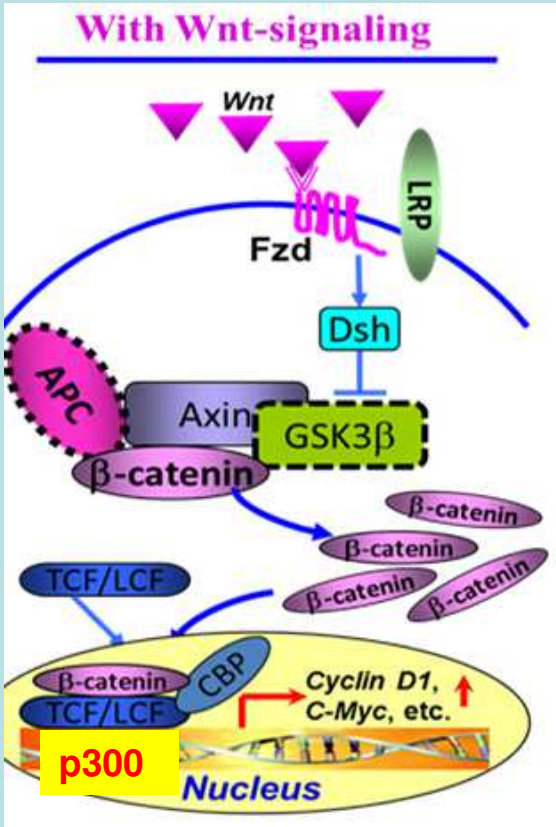
Western Blots (contd)



Axin 2: 66 Showed Axin 2 stabilization compared to 61. 110 Showed Axin 2 stabilization compared to 115.

LEF 1: 61, 66, 110 and 115, all showed decreased expression levels of LEF1 but 110 was more potent.

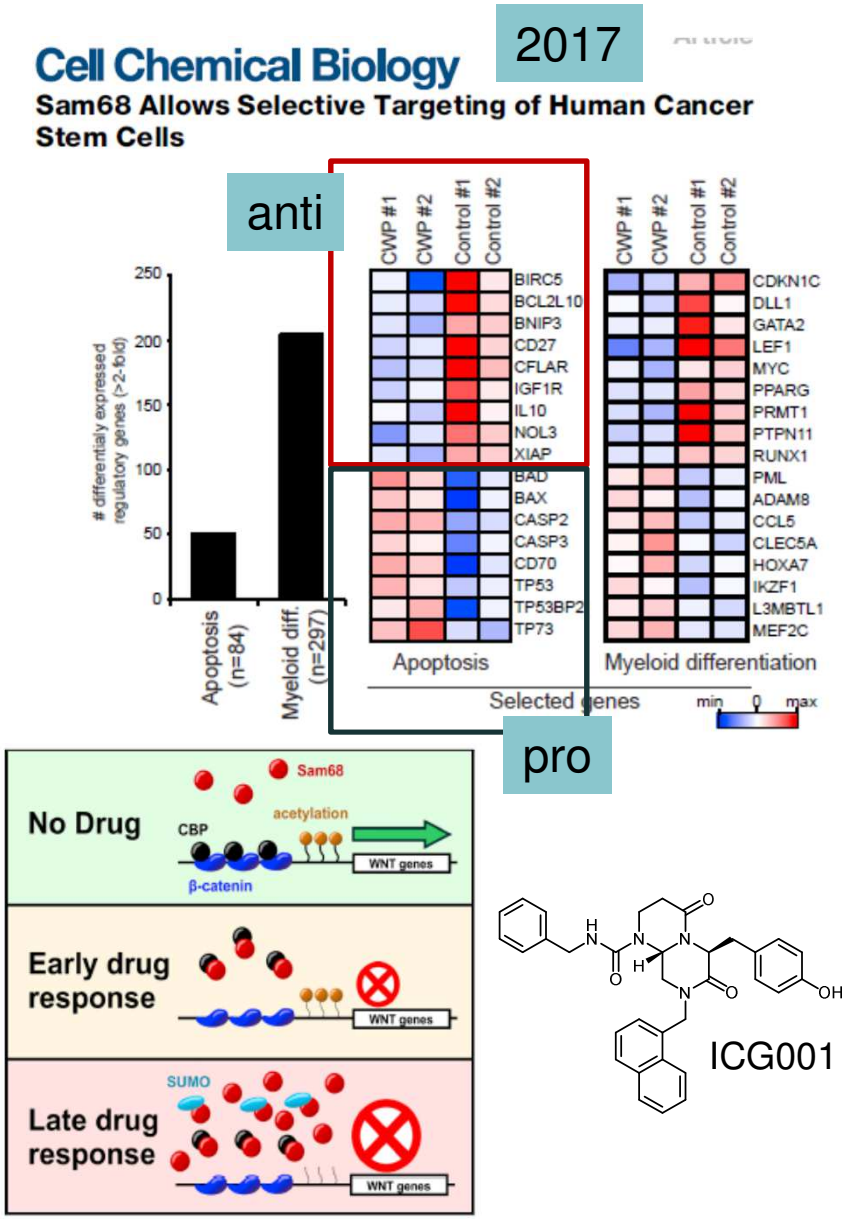
CBP: 66 and 110 decreased the expression of CBP



Source: http://www.jnsci.org/content/36

CBP: (CREB, cAMP response element-binding protein)-binding protein

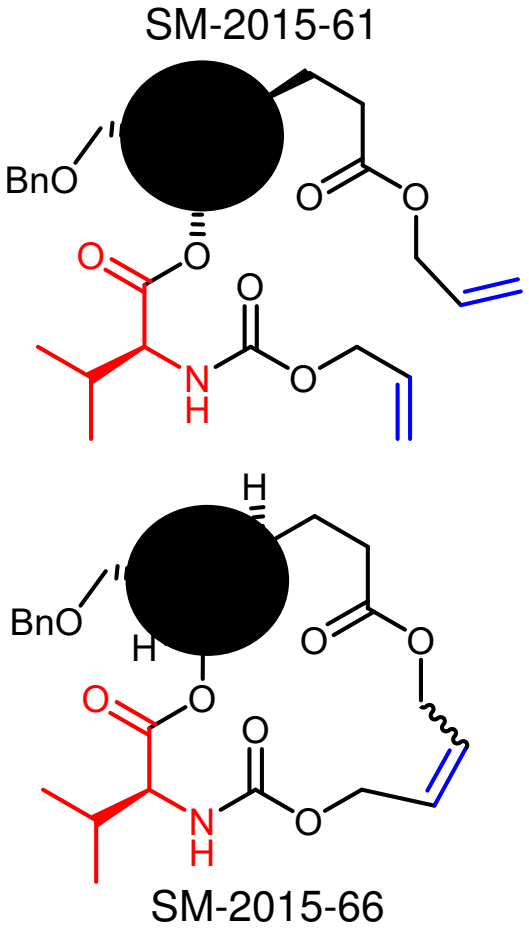
RNA Seq Study



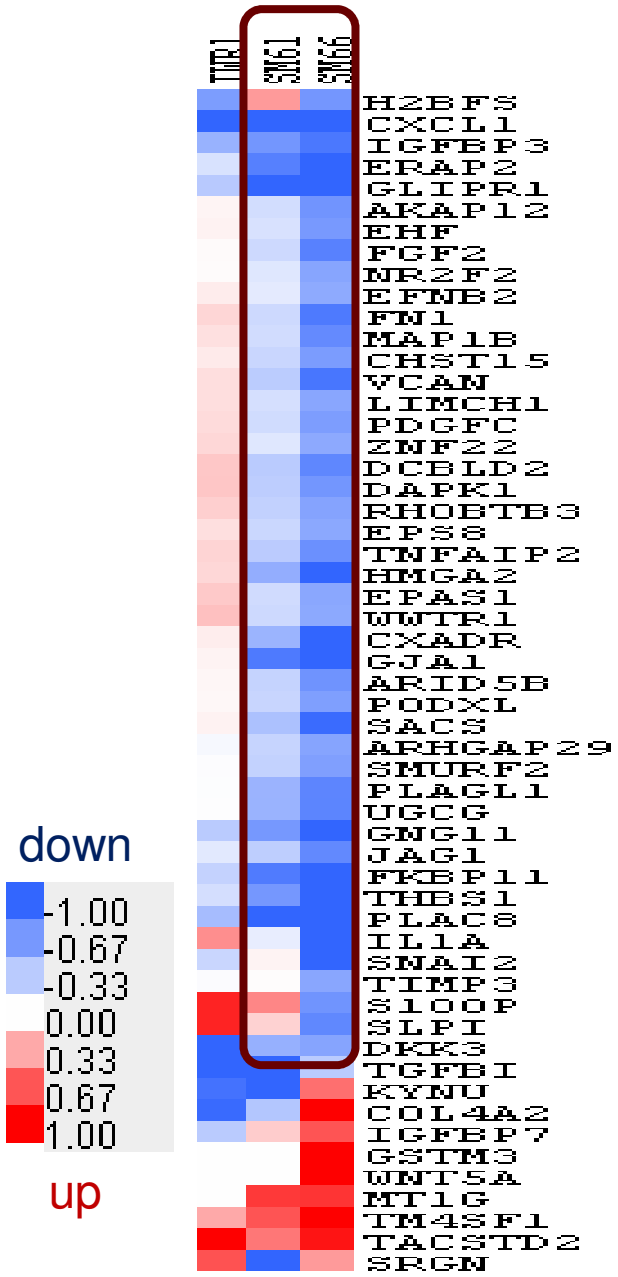
Bhatia's lab, McMaster

What's next

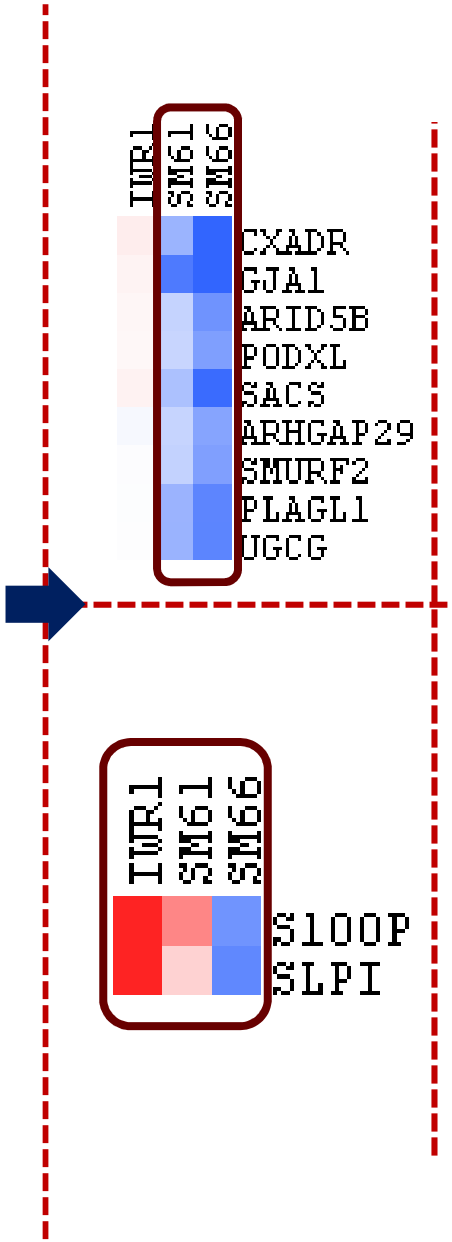
SM-2015-61 vs SM-2015-66 small molecule treated transcriptome study

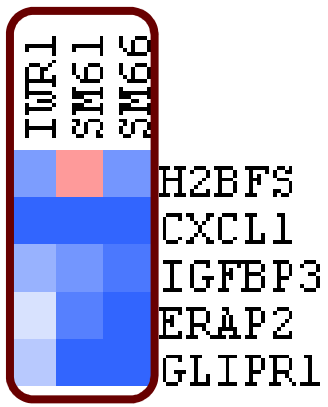


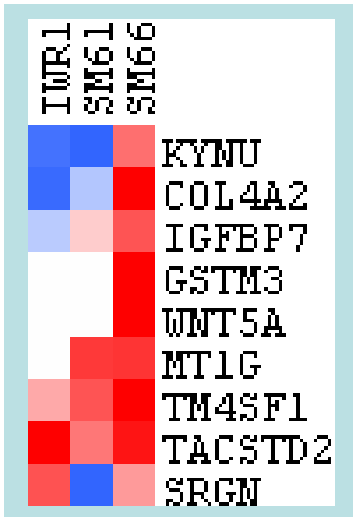
RNA Seq Study



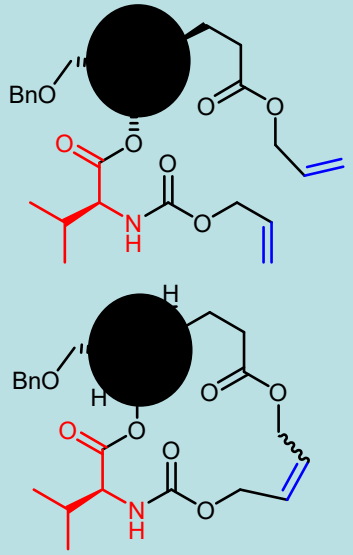
Cell Migration Gene Set





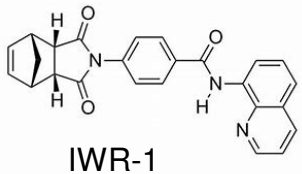


SM-2015-61



SM-2015-66

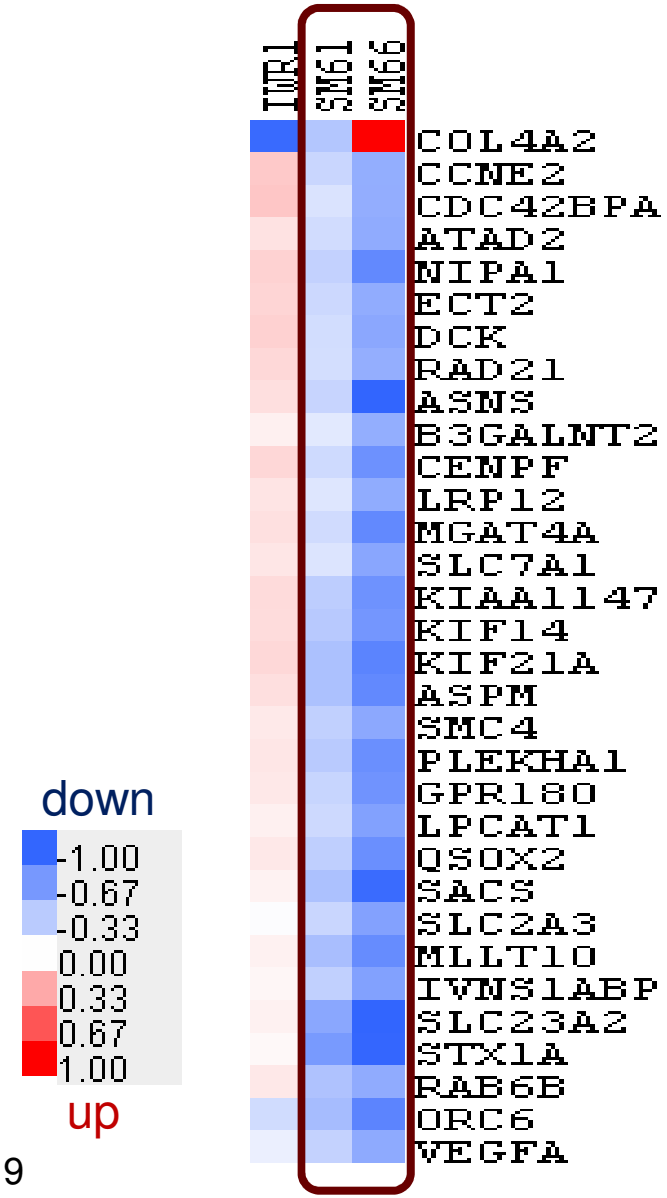
Family 1 Hits



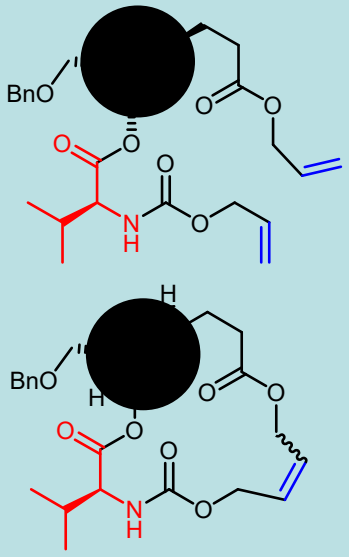
IWR-1
(control)

RNA Seq Study (contd)

Metastasis Breast Cancer Gene Set

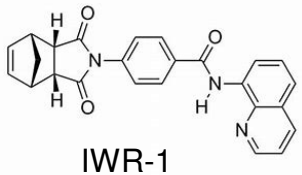


SM-2015-61



SM-2015-66

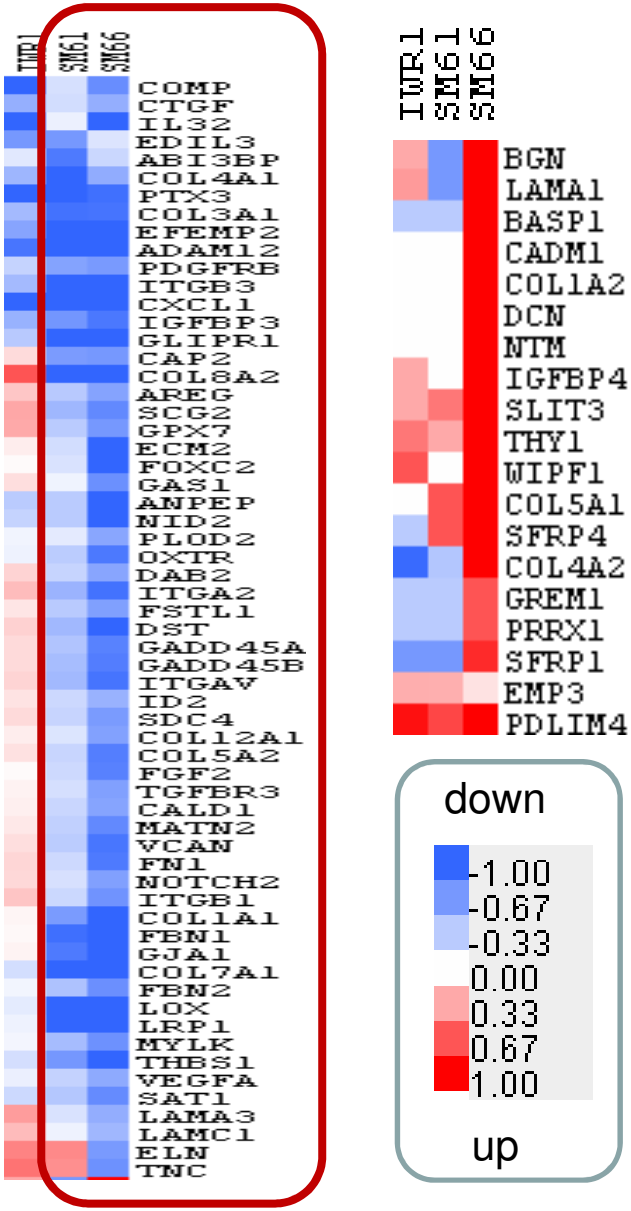
Family 1 Hits



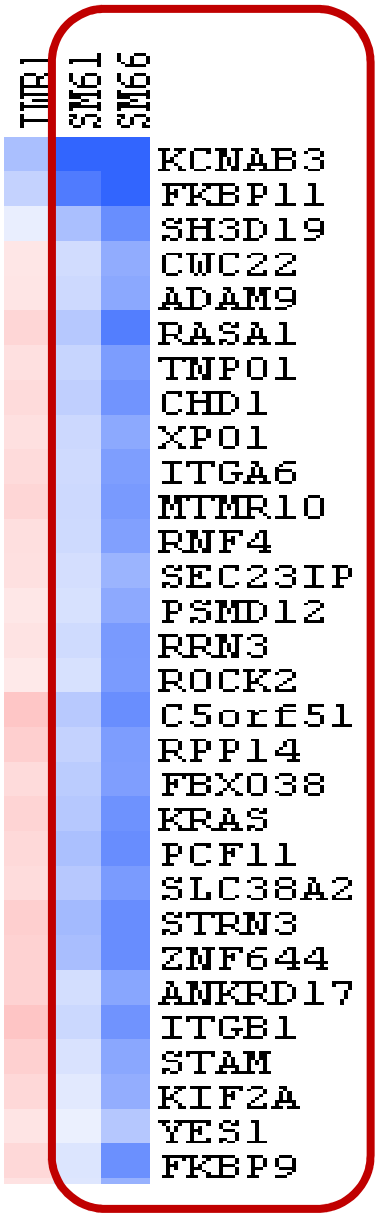
IWR-1
(control)

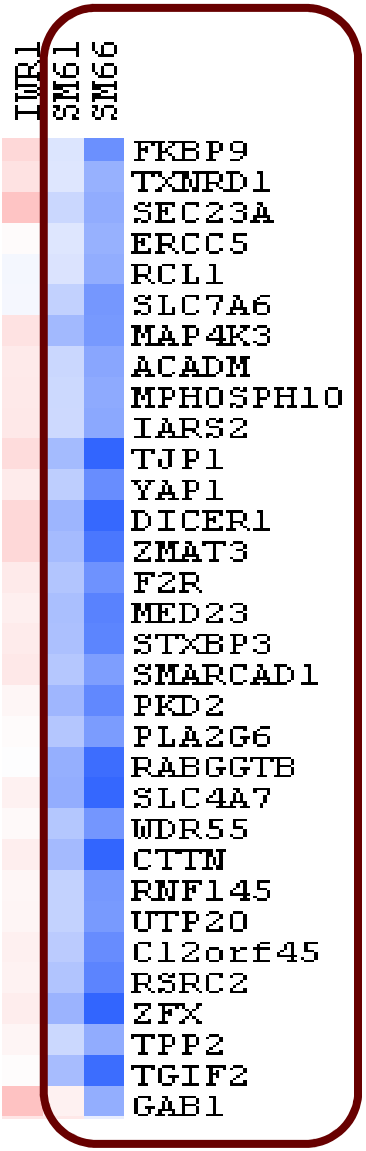
RNA Seq Study (contd)

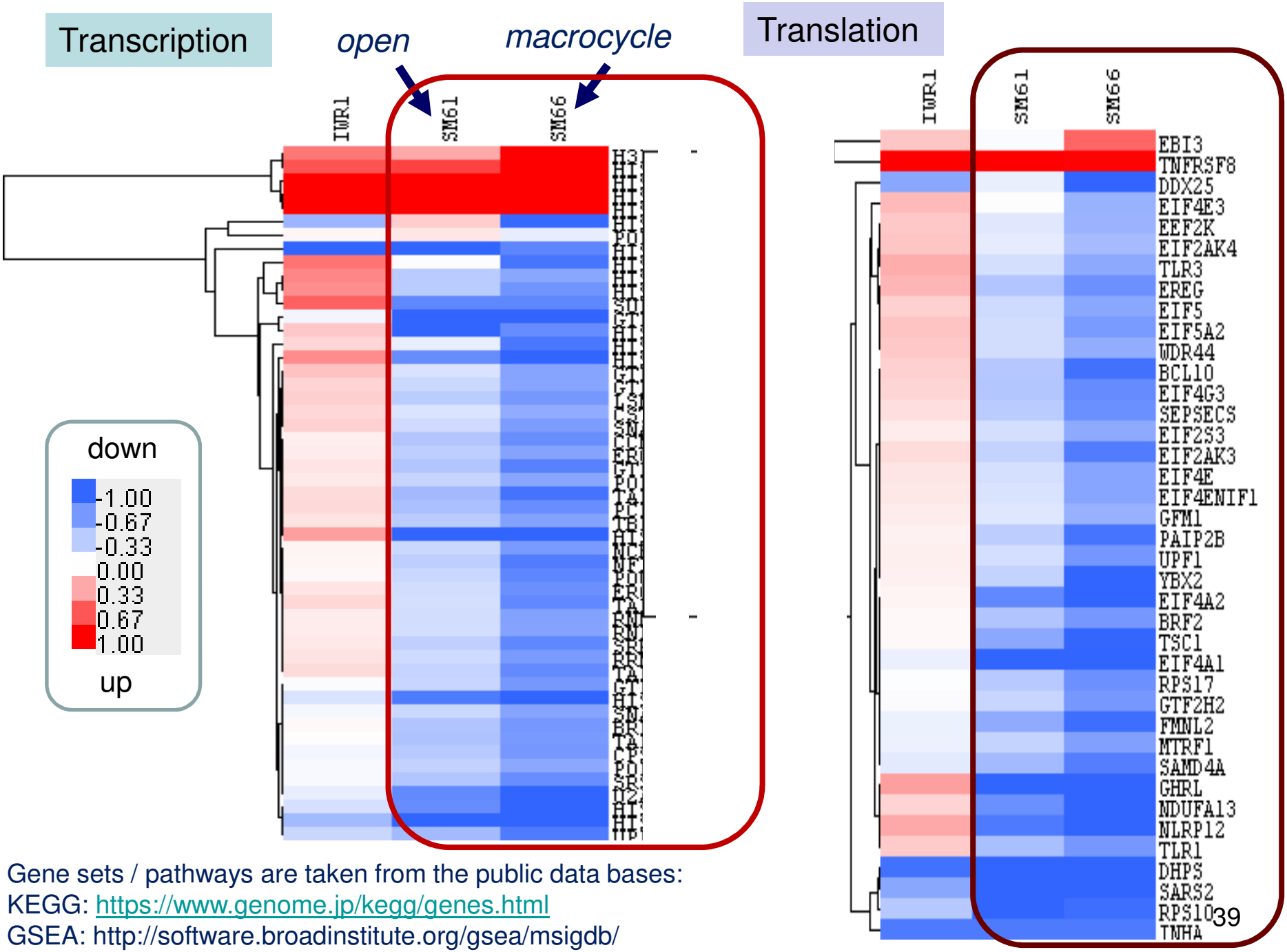
Epithelial to Mesenchymal Transition



Stemness

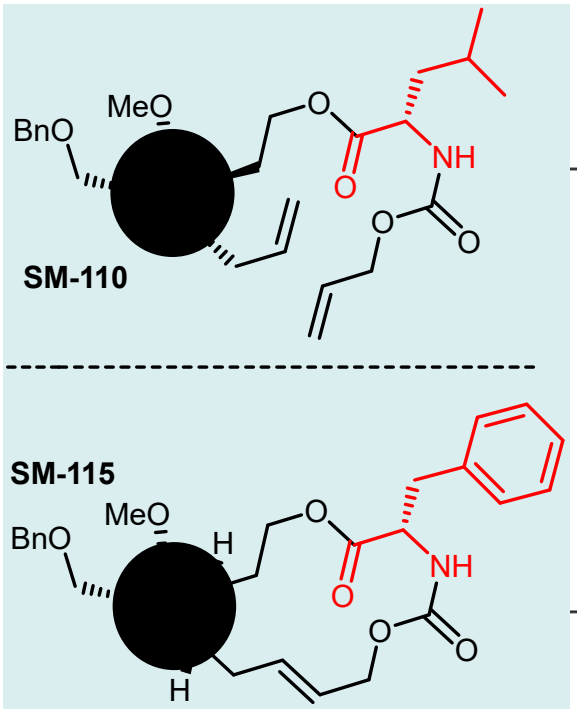




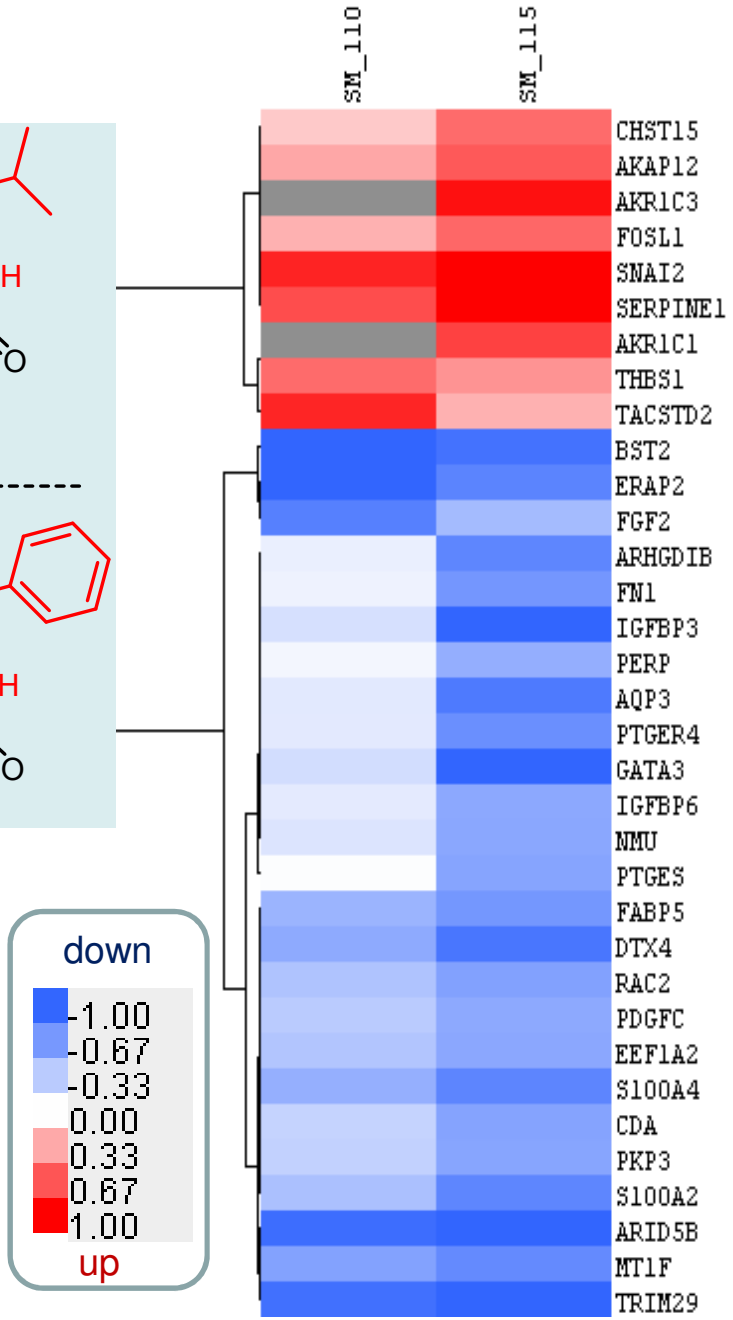


Gene sets / pathways are taken from the public data bases:
KEGG: https://www.genome.jp/kegg/genes.html
GSEA: http://software.broadinstitute.org/gsea/msigdb/
REACTOME: https://reactome.org/

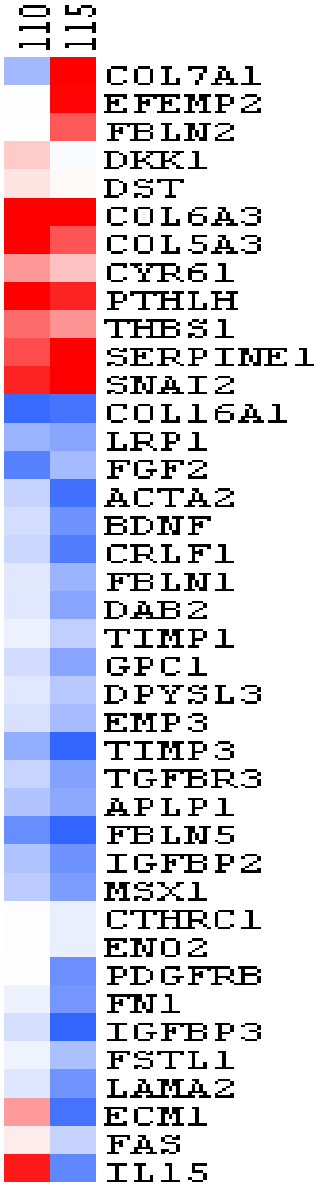
Family 2 Hits



Cell Migration Gene Set

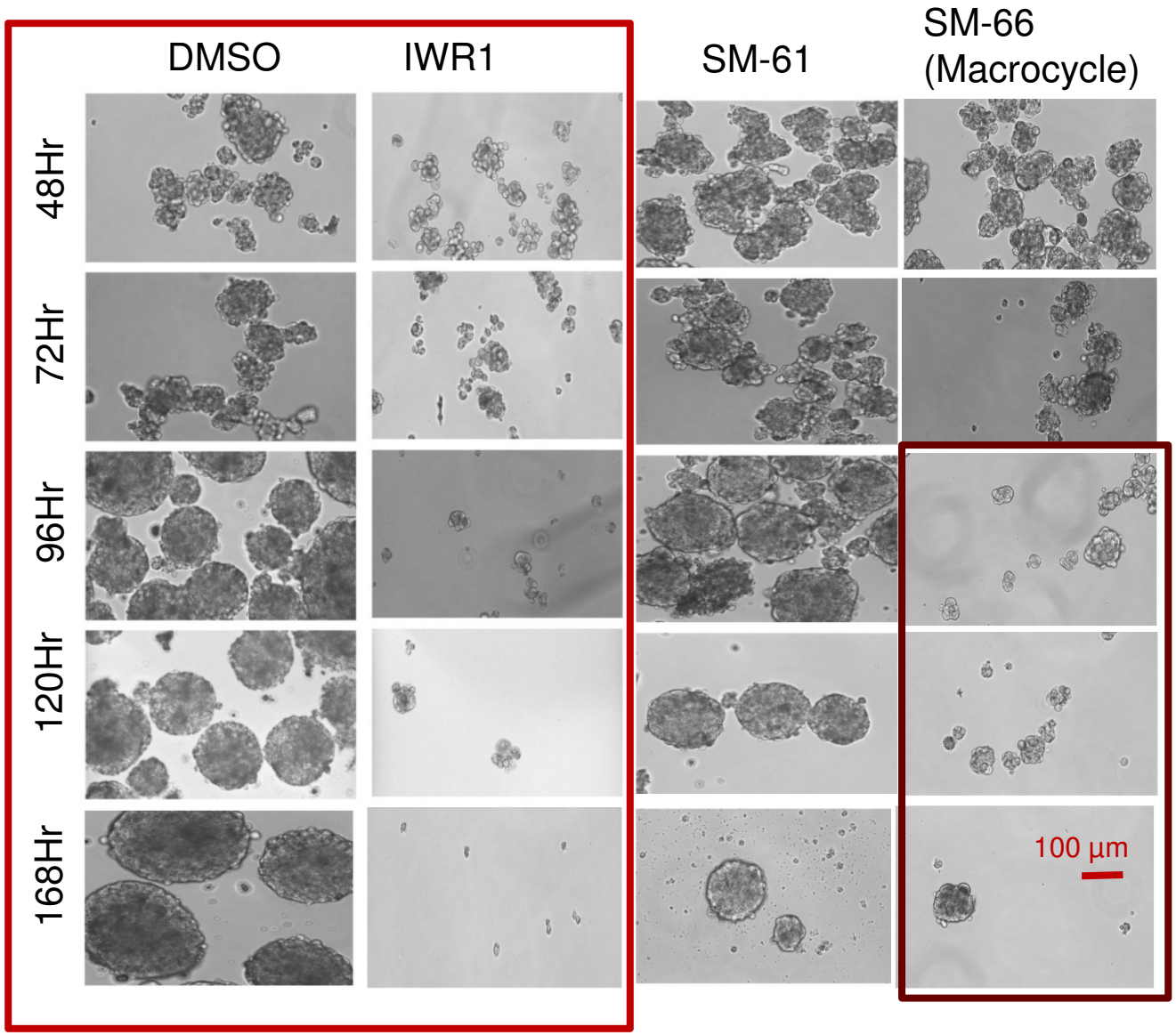


Epithelial to Mesenchymal Transition Gene Set

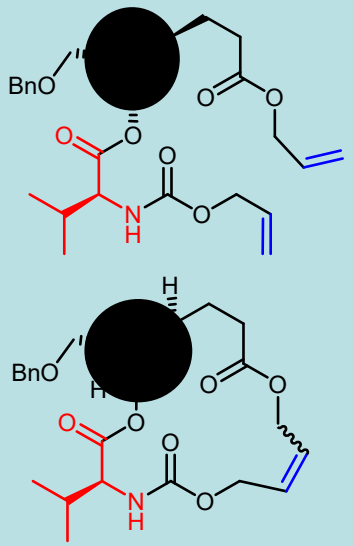


Effect on Tumorspheres (G2*) from HCT 116

G2* = 2nd generation



SM-2015-61

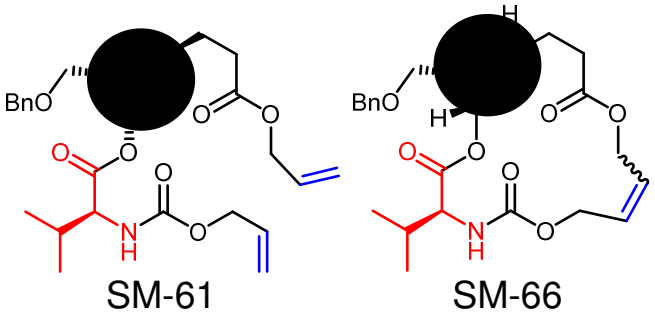


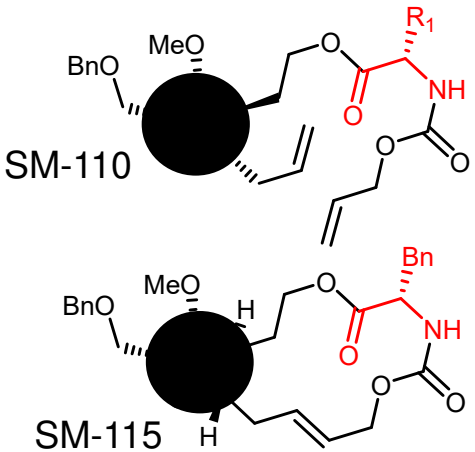
SM-2015-66

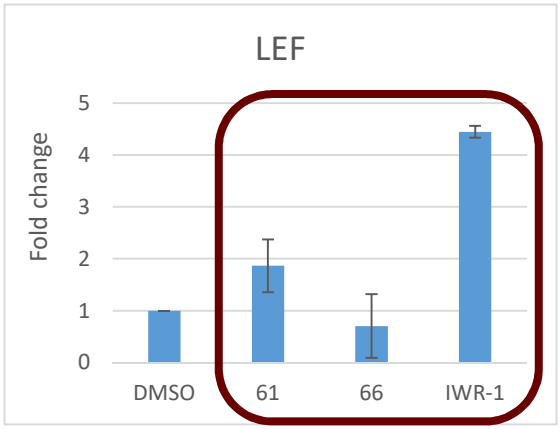
Family 1 Hits

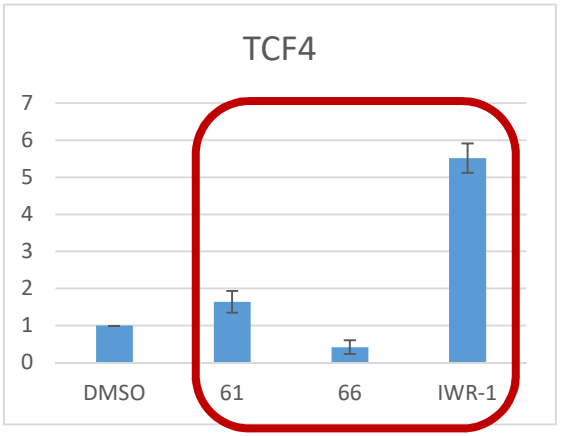
RT-PCR Analysis: Small Molecule Treated Tumorsphere (G2) from HCT 116 (contd)

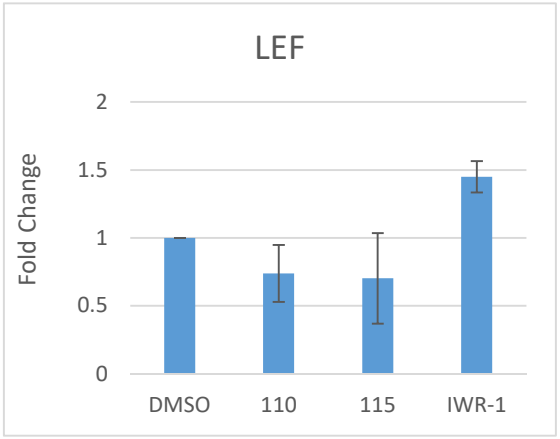
Wnt Transcription-related Genes

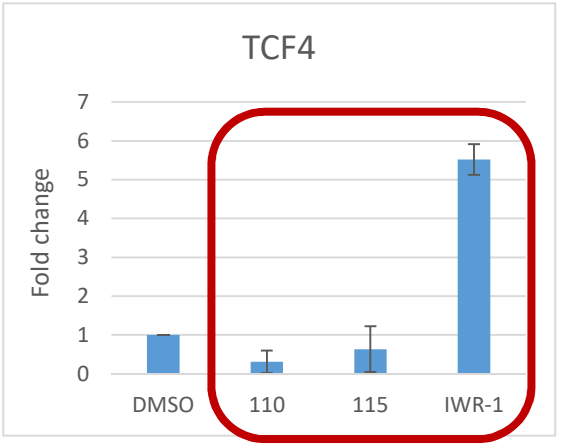








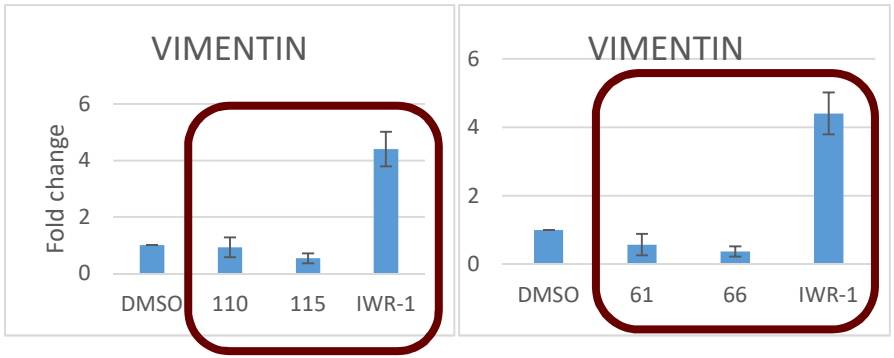


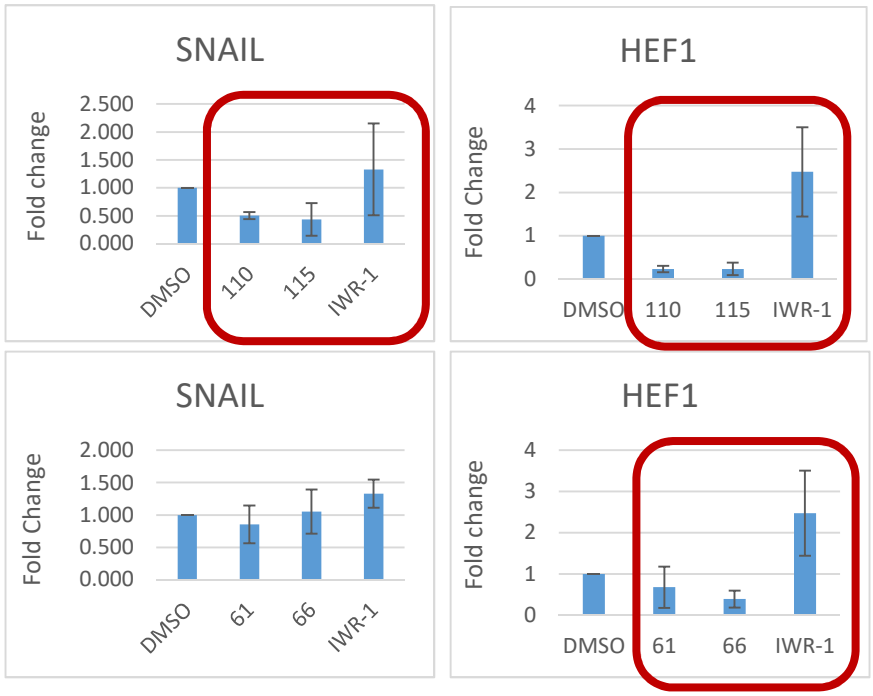


RT-PCR Analysis: Small Molecule Treated Tumorsphere (G2) from HCT 116

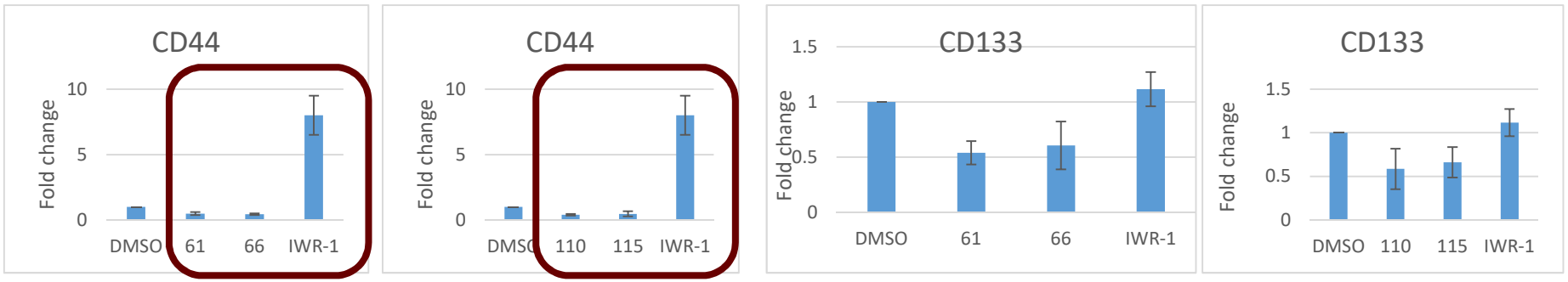
Migration and Invasion

Metastasis



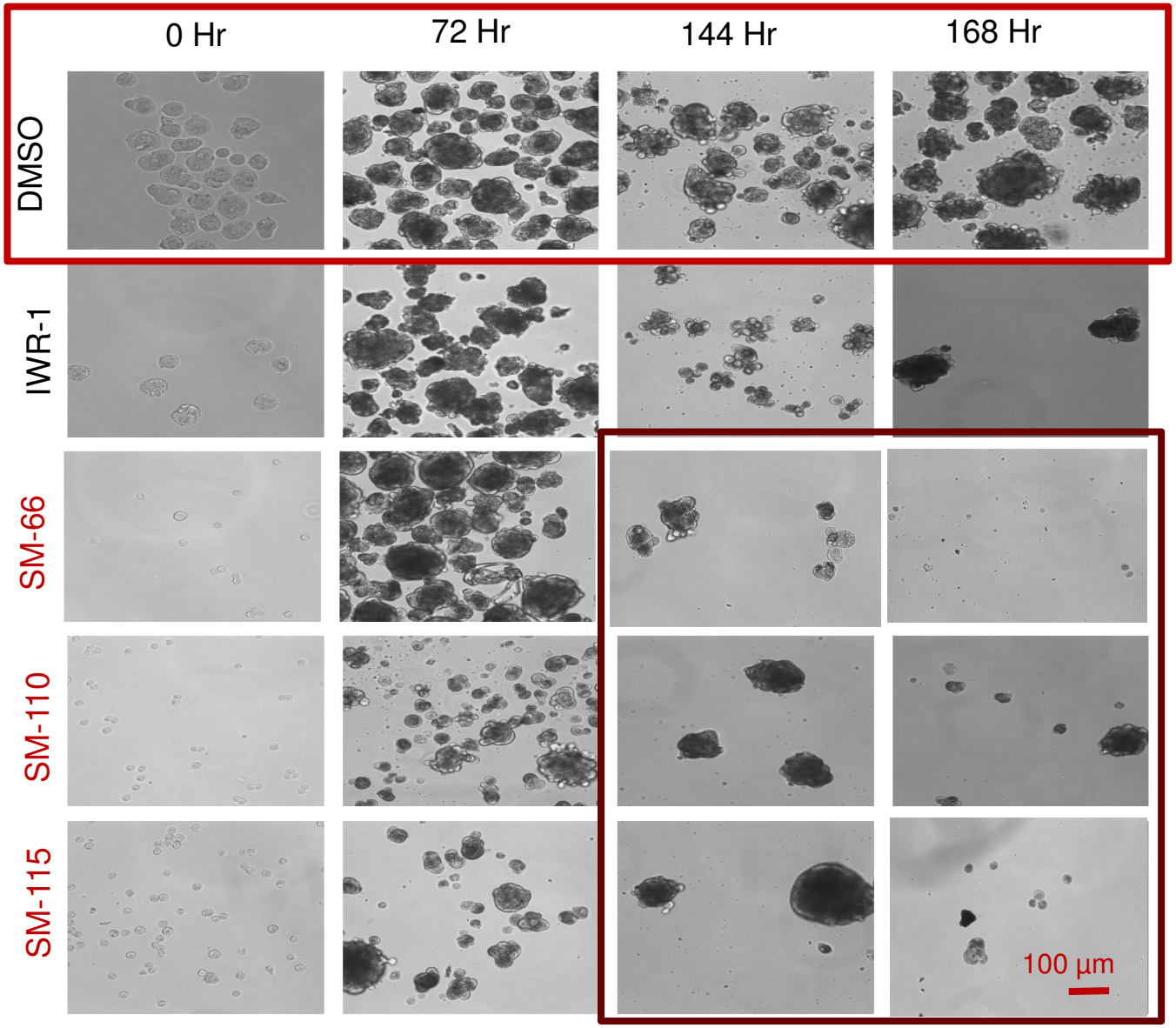


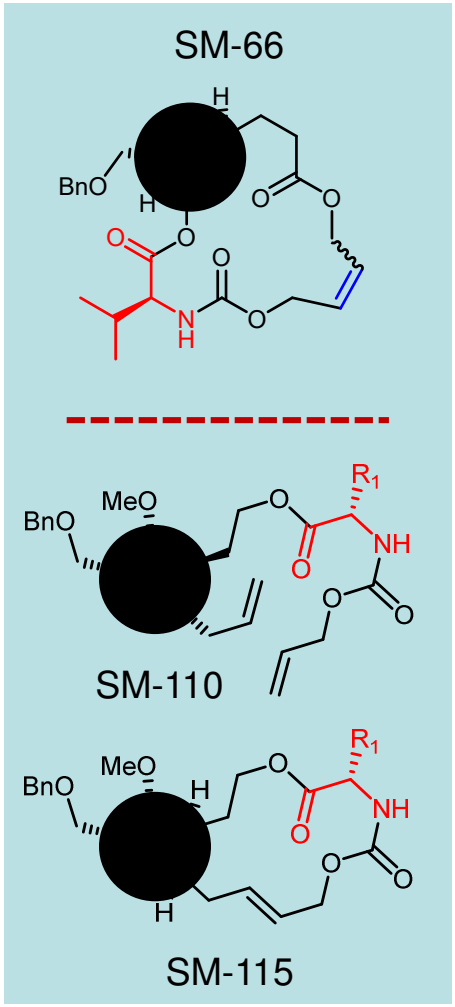
Stemness



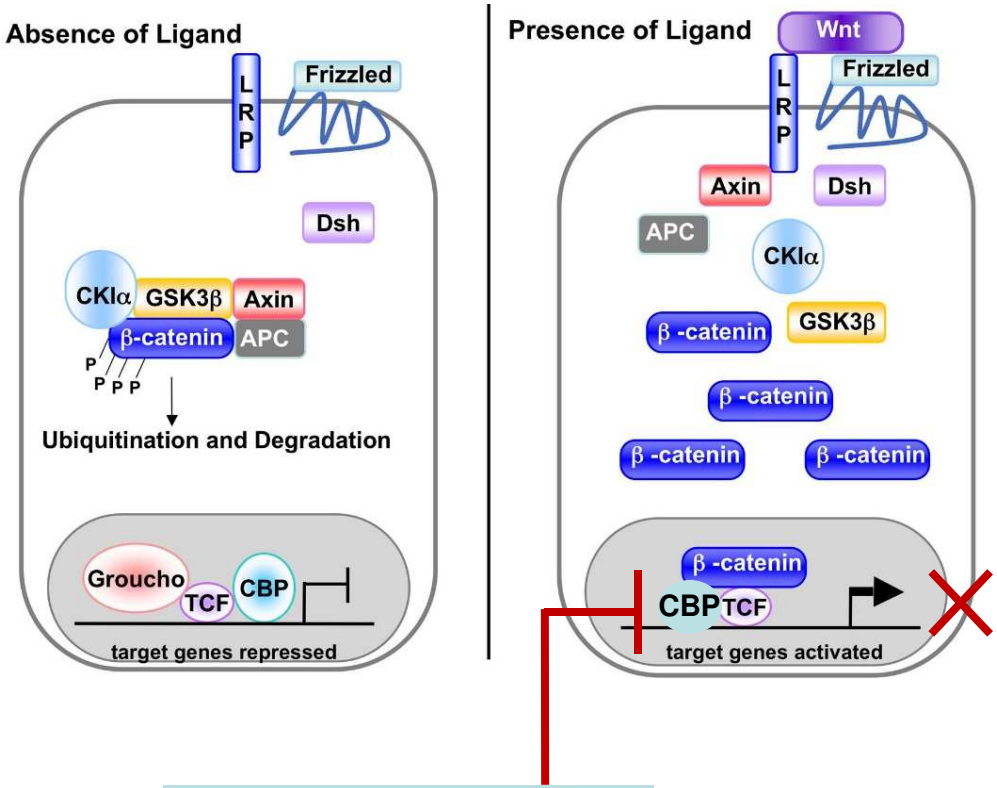
Effect on Tumorspheres (G2*) from Patient Buccal Mucosa (Indian Patient 001)

G2 = 2nd generation





Our Working Hypothesis



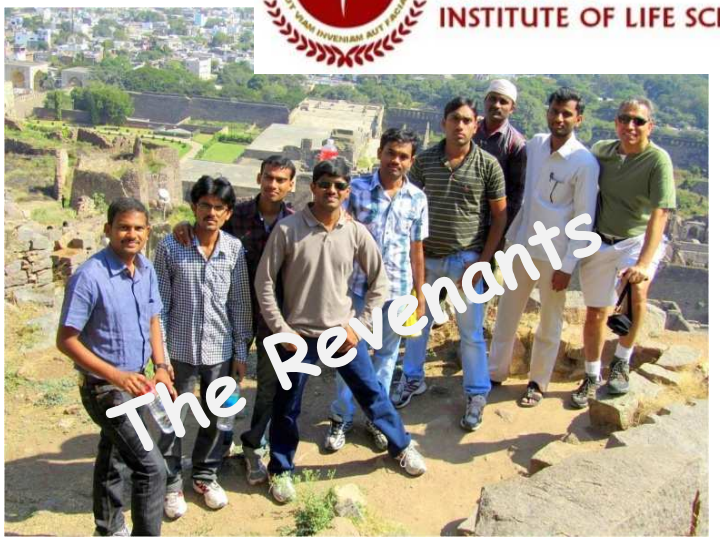
Our small molecules as transcription inhibitors

Ongoing Studies

- RNA seq with 110/115
- Quant proteomics with 66, 110 and 115
- Evaluation on patient-derived organoids
- Target pull-down studies
- Medicinal chemistry // Protein degradation direction

Filing two US provisional patents related to macrocyclic inhibitors of Wnt pathway / b-catenin-CBP-TCF interactions

Academic (India) - Graduate Students (2009-2018):
Madhu Aeluri
Srinivas Chamakuri
Ravikumar Jimmidi
Shiva Krishna Reddy
Bhanudas Dasari
Srinivas Jogula
Saidulu Konda
Mahender Khatravath
Naveen Kumar
Jagan Gaddam



DR. REDDY'S INSTITUTE OF LIFE SCIENCES

MEDGENOME

Institute of Bioinformatics

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NGS:
Madhu Mohan
Chem toolbox/med chem:
Jagan Gaddam
Naveen Kumar
Mahender Khatravath
Anand
Neha Kardam
Prabhat Arya

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