Convergence in Biomedical Sciences in the 21st Century

Stephen T. C. Wong, Ph.D., P.E.
Houston Methodist and Weill Cornell Medicine

Weill Cornell Medicine

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Houston Methodist

- Established in 1919. 8 hospitals across Houston. Academically affiliated with Cornell and Texas A&M.
- HMH is the flagship hospital located in Texas Medical Center, the largest medical center in the world with 54 hospitals and biomedical institutions.
- Ranks #1 hospital in Texas and one of the top hospitals in the nation by US News and World Reports.

Downtown Houston

Convergency Research in Disease Problems

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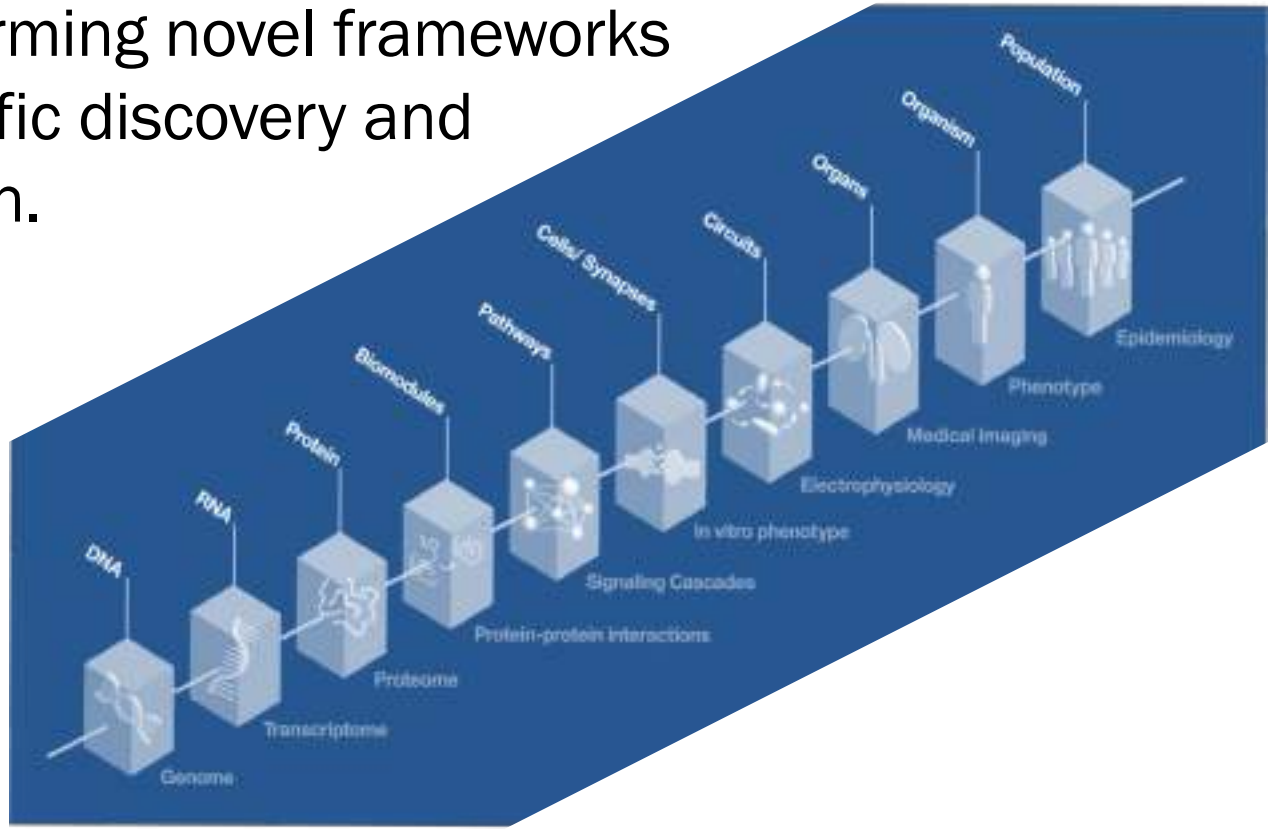
- Disease involves multiple scale of biology.
- It entails integrating knowledge, methods, and expertise from different disciplines and forming novel frameworks to catalyze scientific discovery and medical innovation.



Convergency Research Program in Wong Lab

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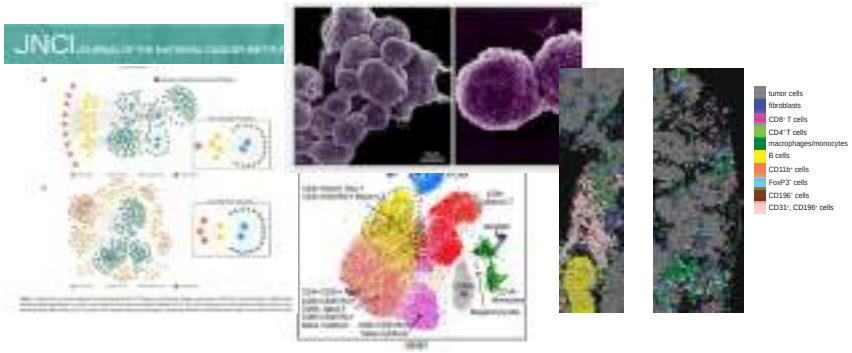
Drug Repositioning and Biomarker Discovery

Yeung TL, et. al, *J NCI*, March 2019; Huang L, et. al, *Bioinformatics*, Feb 2019; Huang L, et. al, *Sci Trans Med.*, Oct 2018; Ren D, et. al, *Cancer Research*, April 2018; Choi DS, et. al, *Stem Cells*, Sept 2014, Jin G, et. al, *Drug Discov Today*, May 2014; Zhao H, et. al, *Cancer Research*, Oct 2013; Jin G, et. al, *Cancer Research* Jan 2012.

Digital Medicine

Stubbins R, et. al, *JCO Clin. Cancer Informatics*, Dec 2018; Perez JA, et. al, *Academic Medicine*, March 2018; Alvarez PA, et. al, *Cardiovasc Ther.* Jun 2017; Islam AK, et. al, *Clinical Transplant*. Aug 2017; Weng S, et. al, *J Biomed Opt*. Oct 2017; Puppala M, et. al, *IEEE Trans Biomed Eng.*, Dec 2015; Andreu-Perez J, et. al. *IEEE J Biomed Health Informat*. Jul 2015.

Immuno-Tumor Microenvironment, Brain Microenvironment, Crosstalk, Metastases

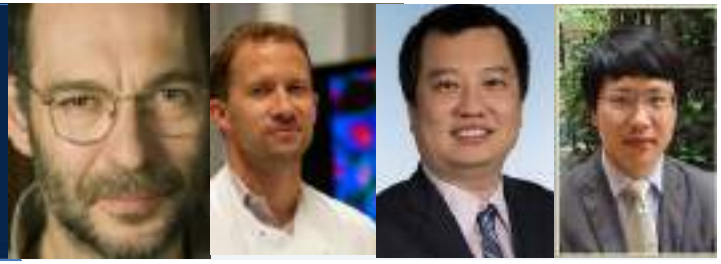


Bu W, et. al, *Cancer Research*, Jan 2019; Leung CS, et. al, *J Clin Invest*., Feb 2018; Markowitz GJ, et. al, *JCI Insight*, July 2018; Hu Q, et. al, *Clin. Cancer Research*, Sept 2017; Zhao Z, et. al, *Cancer Research*, April 2016; Fischer KR, et. al, *Nature*, Nov 2015; Wang H, et. al, *Cancer Cell*, Feb 2015; Choi H, et. al, *Cell Reports*, Feb 2015.

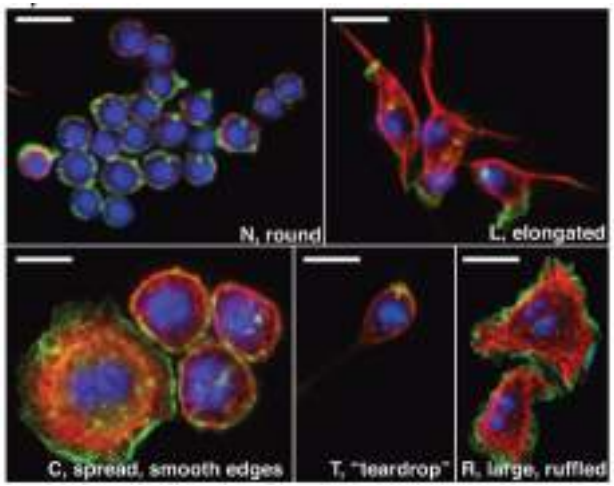
AI in Disease Management

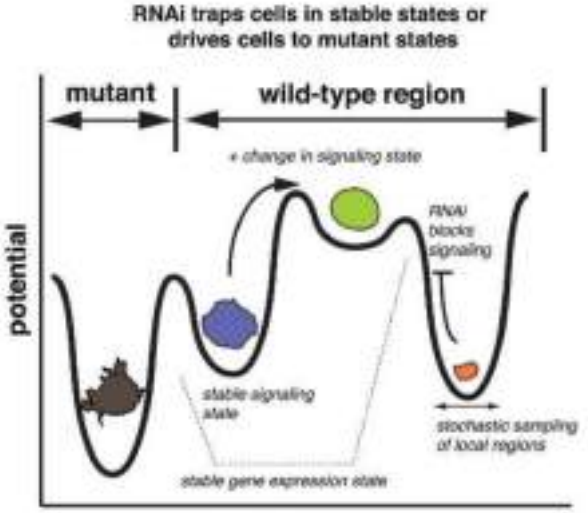
He T, et. al, *JCO Clin. Cancer Informatics*, May 2019; Bradley D, et. al, *Diabetes Care*, Mar 2019; Wong KK, et. al, *Cancers*, Jan 2019; Patel TA et al, *Cancer*, Jan 2017; Sheng J et. al, *IEEE J Biomed Health Informat*. Jul 2015.

Shape As Biomarker: Melanoma



- High content RNAi screening of 3 millions Drosophila Kc167 cells; dsRNAs targeting ~300 kinase and phosphatases; 3 channels/image, 16 images/well, 384 wells/plate, triplicates for each plate.
- Kc167 cell populations are dominated by five discrete phenotypes;
- Pheno-clusters defined amongst kinases and phosphatases show functional significance;
- Certain RNAi treatments increase morphological complexity by inducing intermediate phenotypes;
- PTEN deficiency leads to switch-like transitions between two morphologies;





Yin et al., *BMC Bioinformatics,* **9**:264;
Yin et al., *Pattern Recognition,* **42**(4): 509-522;
Yin et al., *Nature Cell Biology*, **15**: 860-871;

Switch-like Regulation of Morphology is Necessary during the Metastasis of Melanoma

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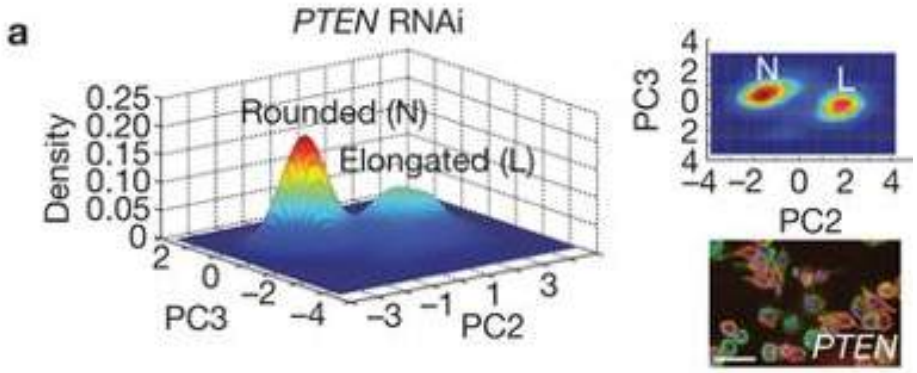
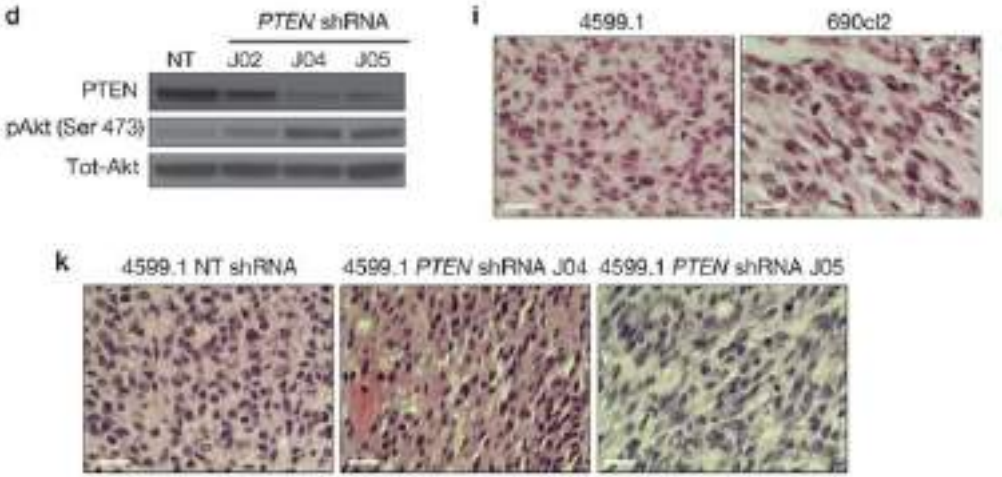
- A subset of genes centering tumor suppressor PTEN serve as highly conserved regulators of cell shape in fly cells as well as mouse and human melanoma cells

- Genes control cell shape behave more like light switches than teakettles coming to slow boil



Effect of PTEN deficiency in cultured fly cells (up) and xenograft human melanoma cells in mouse (down)



A Model for Morphological Landscape

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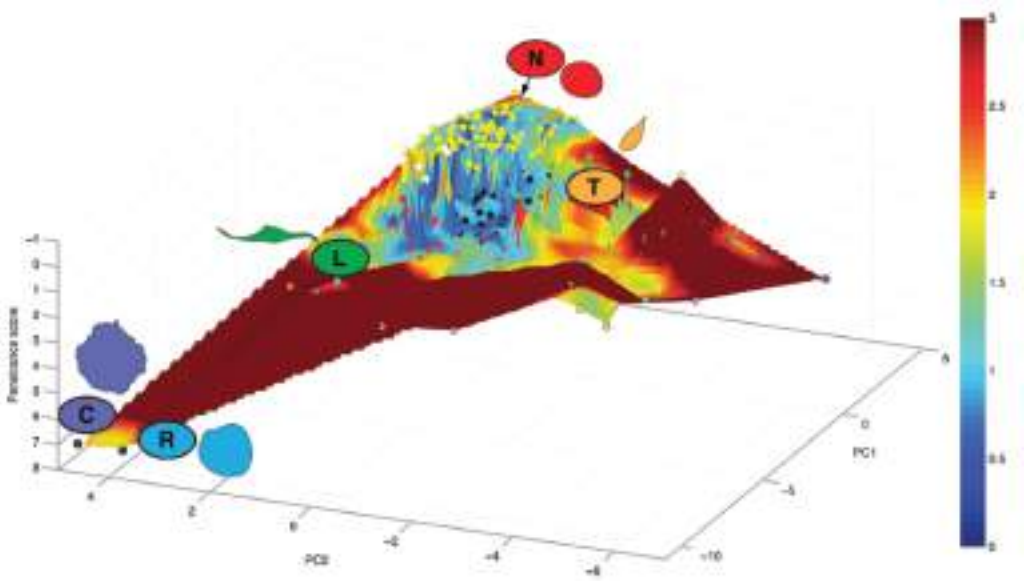
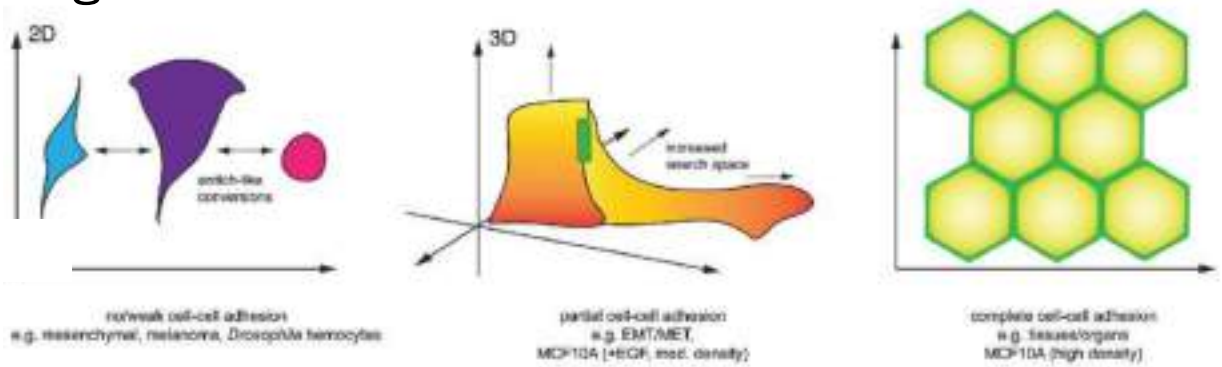
- Typical morphological phenotypes are conserved across cell types and species.
- Our morphological landscape models the shape space explored by cells under various RNAi treatments.
- Features like cell-cell adhesions can alter cell population's ability of exploring shape space.





Yin et al., *Bioessays*, **36**: 1195-1203

Great, But...

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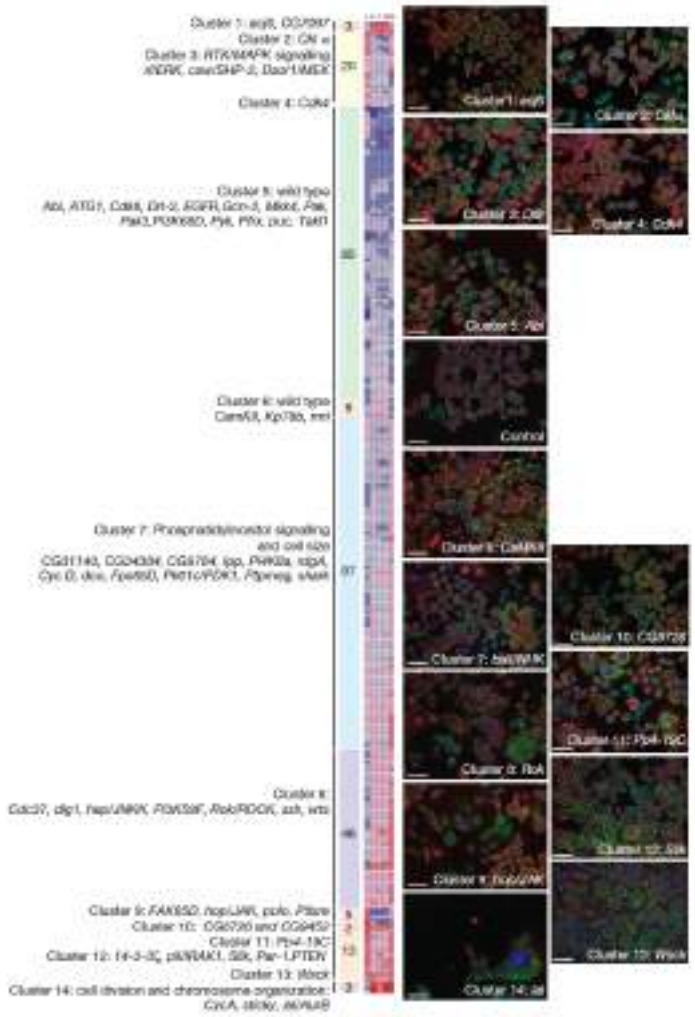
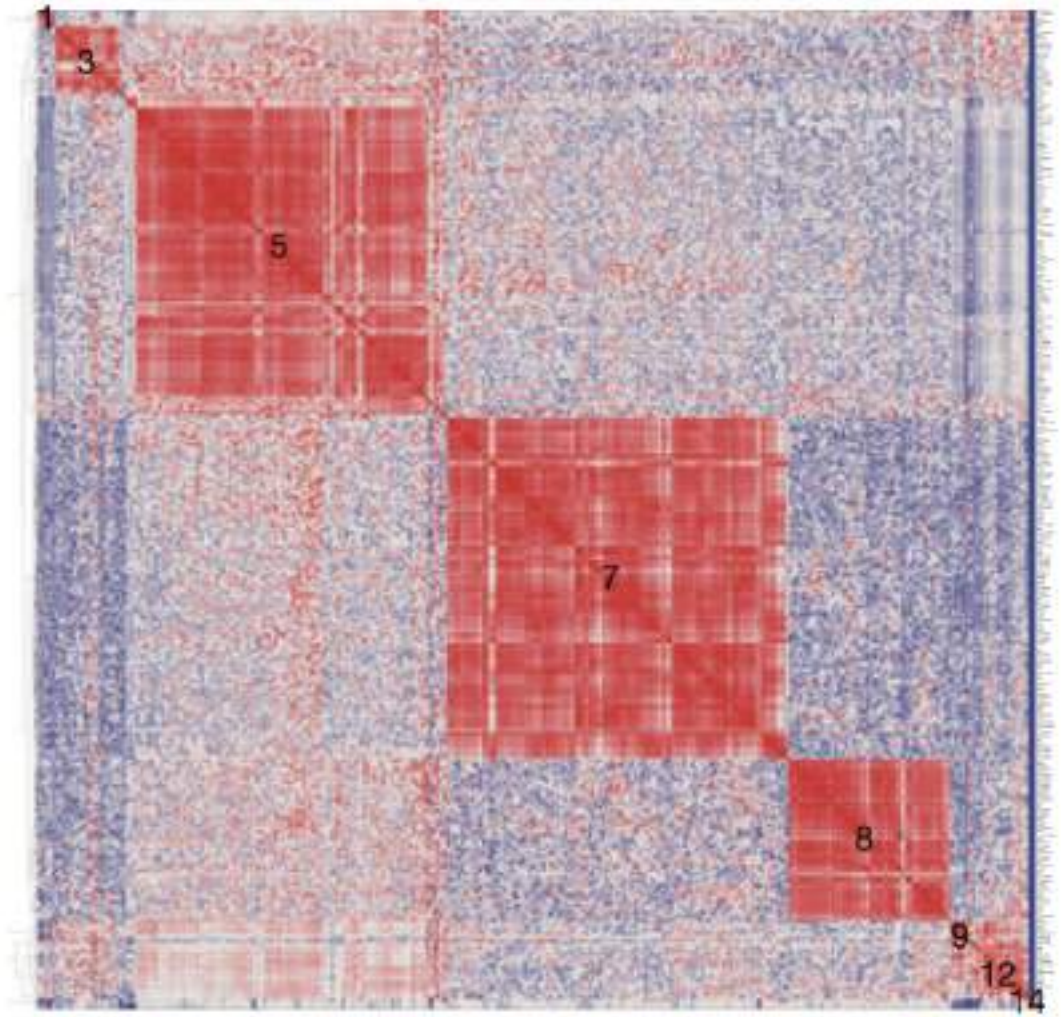
If only we had matching imaging and multiple-omics profiles for each RNAi





Yin et al., *Nature Cell Biology*, **15**: 860-871

Use Gene Function Data To Stratify Phenoclusters

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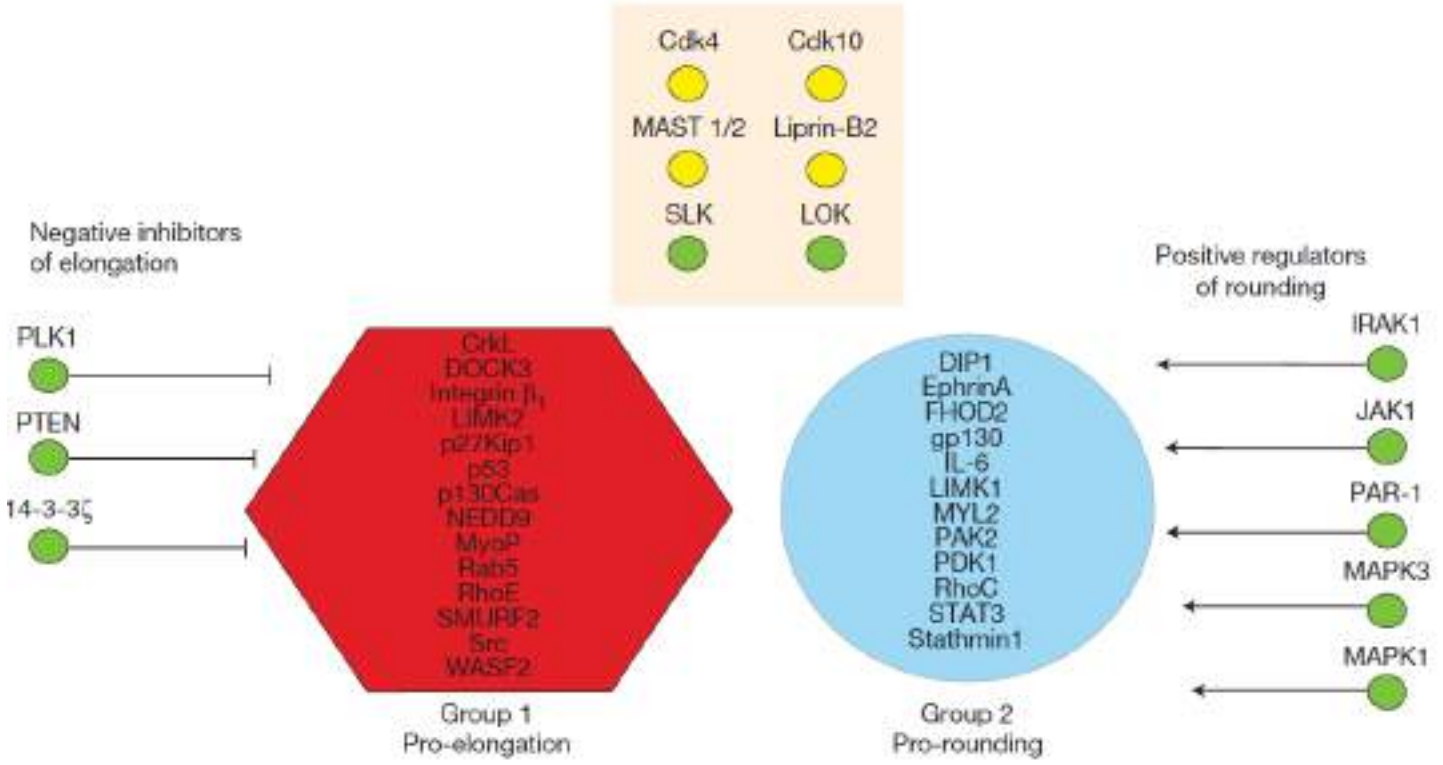
- **A subset of genes** centering tumor suppressor PTEN…
- They may induce similar phenotypes, but are they working the same mechanism?



Yin et al., *Nature Cell Biology*, **15**: 860-871

Converging Image, Proteomics and Genetics Data across 11 Mouse Melanoma Cell Lines

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- 3 cell lines with BRAF Kinase Activated
- 6 with NRAS GTPase Activated
- 2 with NRAS GTPase Activated and BRAF Kinase Dead

- Abundance of 4,800 proteins
- Intensity of 16,848 phosphorylation events
- 166 imaging features

Rich dataset to build a multi-scale causality network!
Every causality pair counts

Morphological Phenotype as Drug Targets

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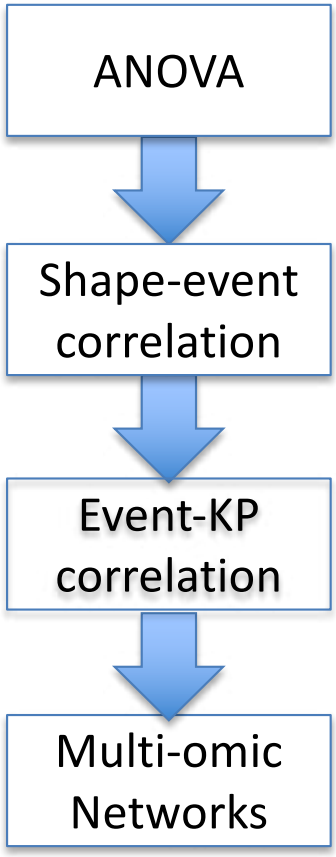
Shape features can serve as prognostic markers and drug targets



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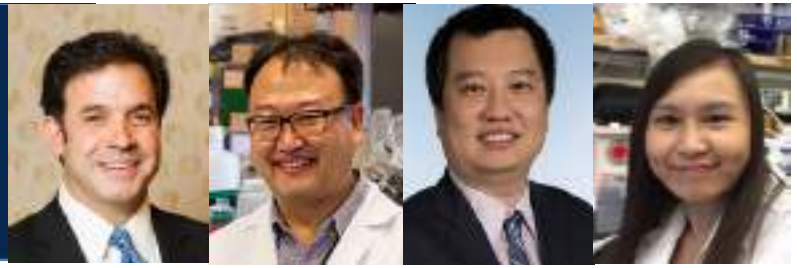
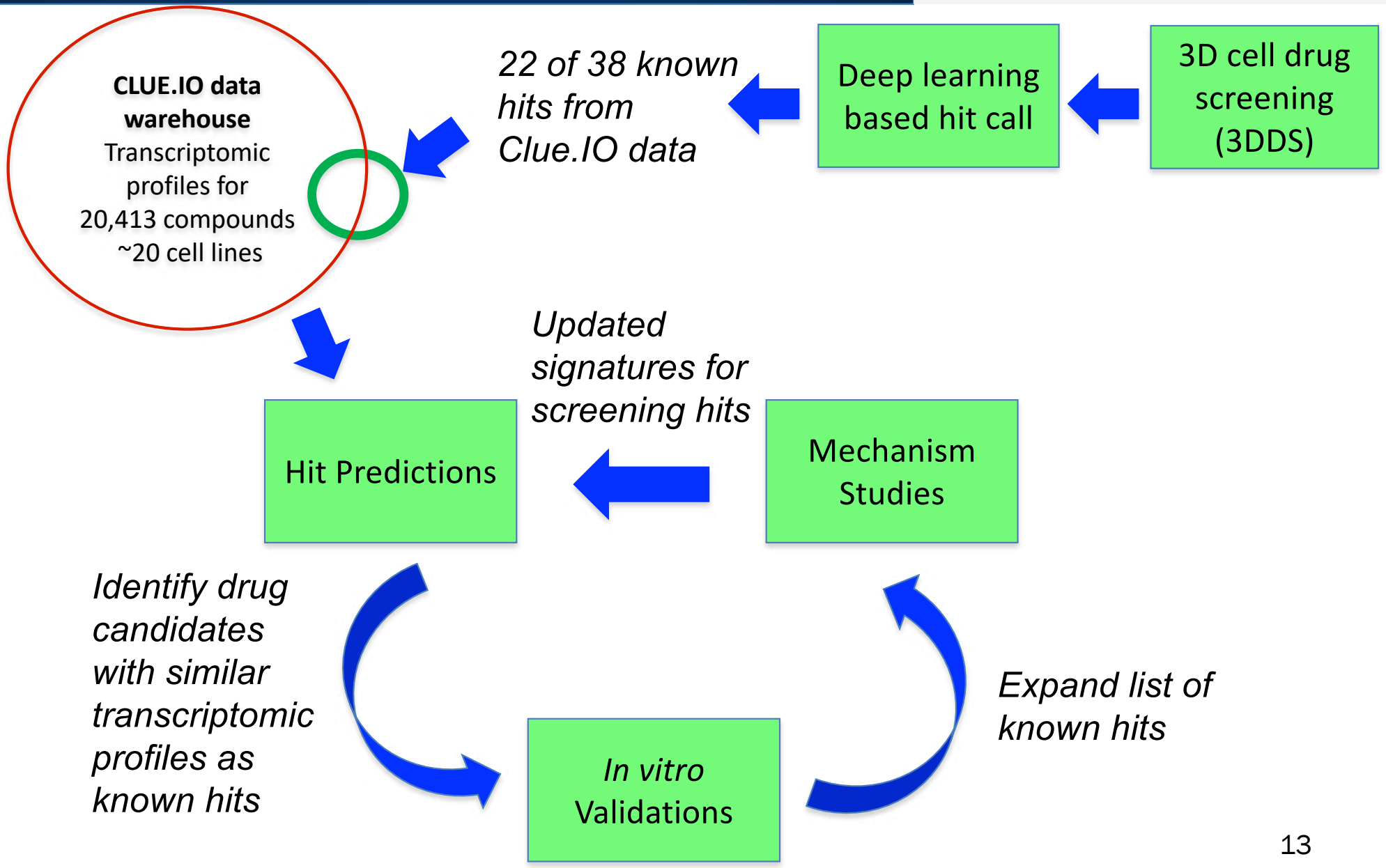
Converge Pharmacogenomics With HCS for Alzheimer's Drug Repositioning



- We hypothesize that modeling of large pharmacogenomic data with large drug screening data may identify new drug hits and delineate novel disease mechanism of Alzheimer's.
- So far we have accomplished the following:
  - Obtained 38 primary hits from high content screening 2,640 selected known drugs and bioactive compounds that strongly inhibit β-amyloid-driven p-tau accumulation and validate those 38 hits in separate assays.
  - Used 38 hits as a base to predict and validate novel drug candidates for Alzheimer's Disease using NIH LINCS database.
  - Gain insights of disease mechanisms from identified drug candidates to improve the success rate of predicted drug hits.

Systematic Drug Repositioning



3D Cell Drug Screening

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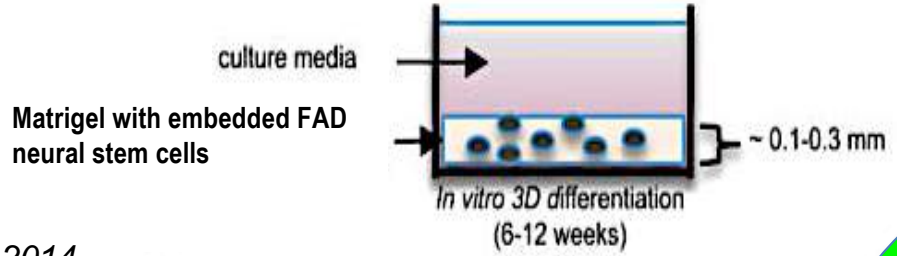
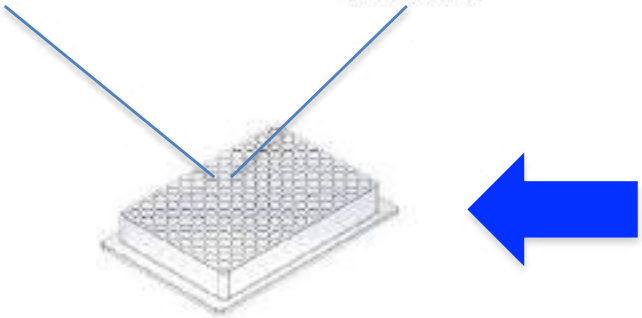
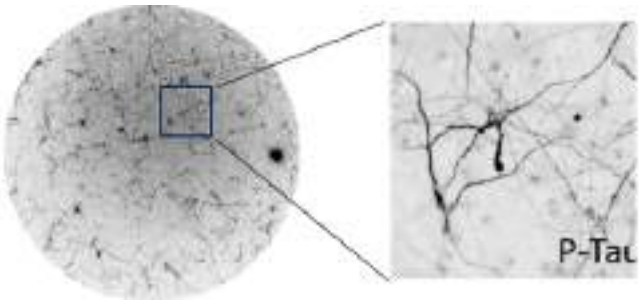


Choi SE et al., Nature, 2014



Library of 2640 compounds

Sigma-Aldrich LOPAC
Tocriscreen library
240 kinase inhibitors



Fluorescent microscopy imaging for pTau

Deep Learning Hit Call

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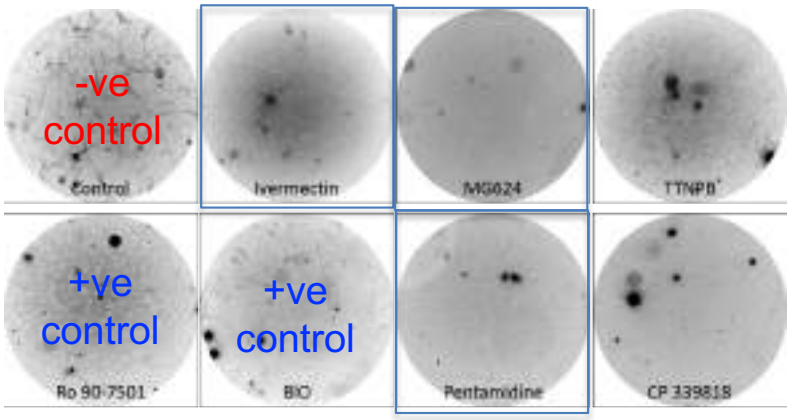
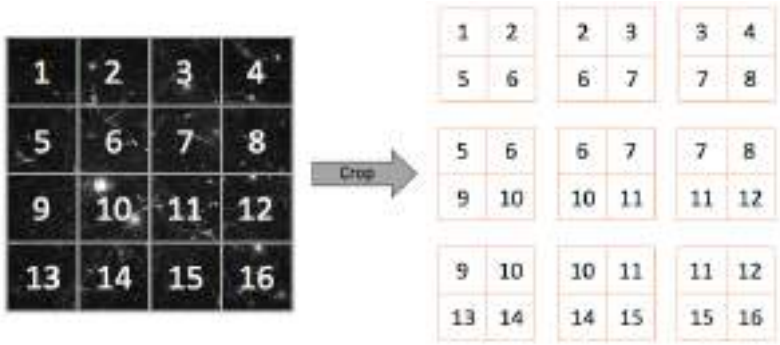
1. Positive/negative controls and observed top hits used as training set



Inhibitor of Aβ42 fibril formation

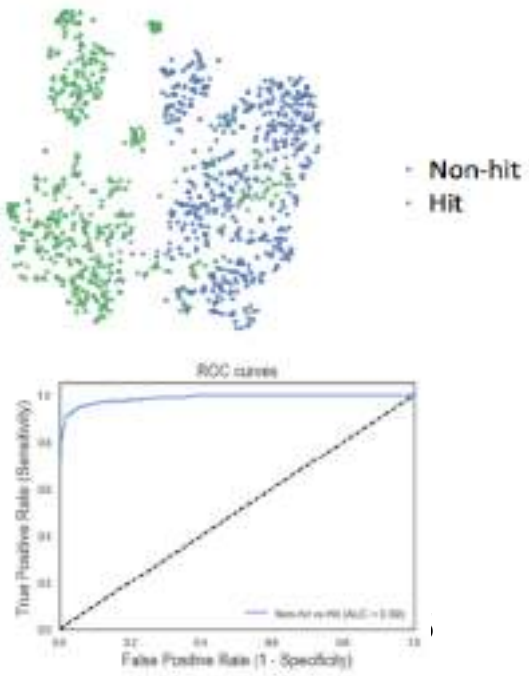
Potent, selective GSK-3 inhibitor

2. Generate balanced training sets



3. Deep Learning training for hit call

4. Results



38 Primary Hits

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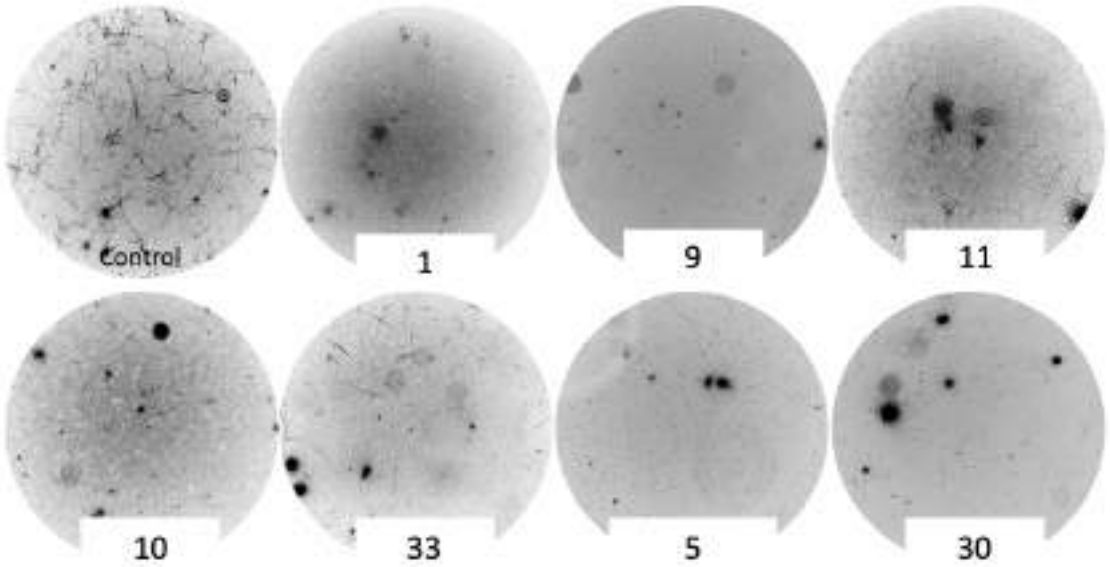

CLUE

BROAD INSTITUTE

Transcriptomic signatures regarding the cellular perturbation caused by 22 of the 38 hits are included in the Clue.IO data warehouse

Independent Confirmation of 33 Primary Hits

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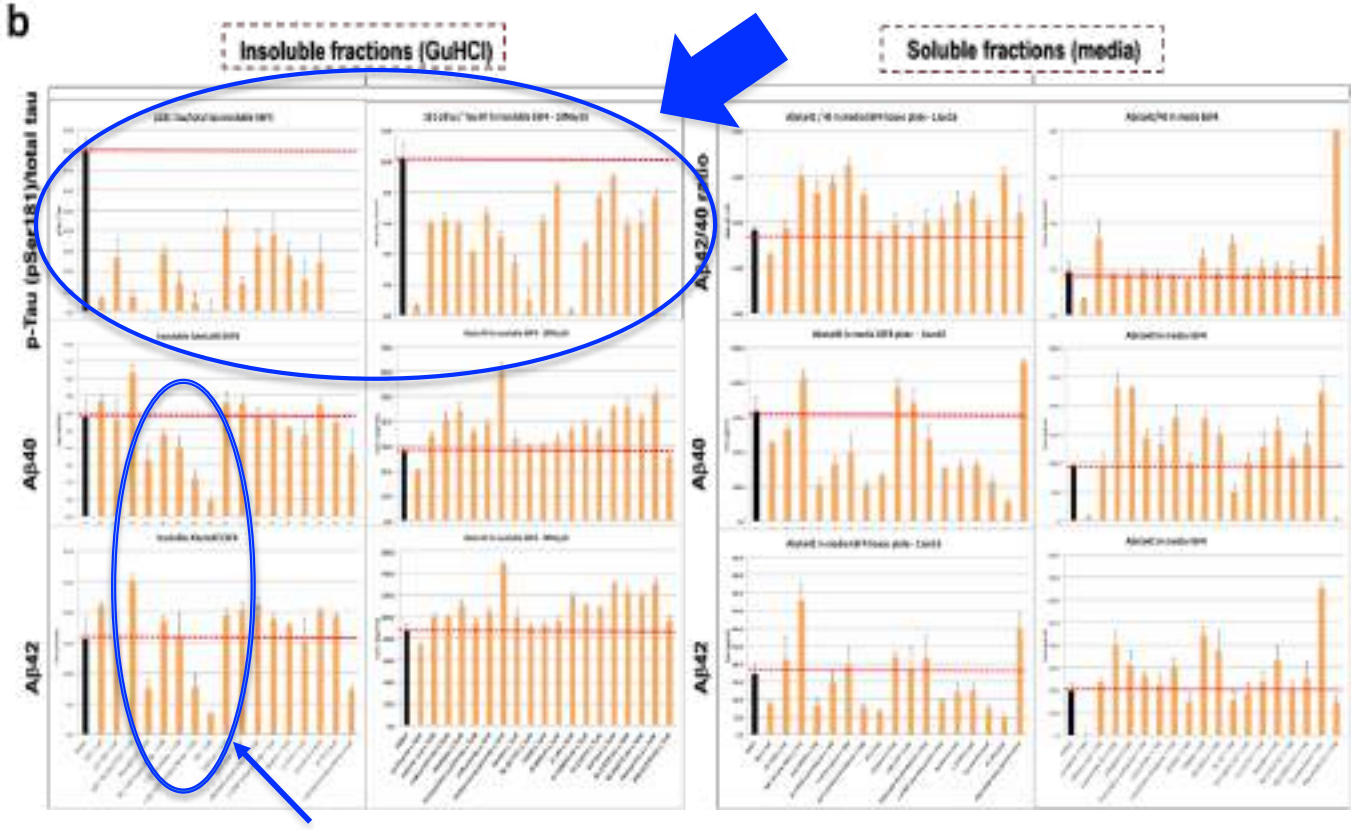
Validation of 33 hits using 3DDS assay

33 primary hits reduce p-tau /total-tau ratio



A few hits reduce Aβ level at the same time

- Selected 33 drug compounds (5 others are too toxic or unavailable) on Aβ and p-tau levels were analyzed by HT electrochemiluminescence /multi-array technology.
- Analysis of 33 primary hits for their effects on soluble (media) and insoluble (5M GuHCl) Aβ and phospho-tau (pSer181) levels.
- DMSO controls, black bar.

Unpublished Data from Tanzi and Kim labs, MGH

From Hits to More Hits

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22 hits have eligible data in CLUE.io LINCS

Compound digest APP

Set threshold as score_best4 >90

646 LINCS Compounds

Excluding 176 screened cmpds (already in 2,640 cmpd libraries)

470 unscreened Compounds

Categorization:

1. Approved drugs (70)
2. Clinical trials drugs (57)
3. Investigational compounds (163)
4. BRD-Series(180)

Pharmacology Analysis Selects 56 Candidates for Validation

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470 unscreened compounds

Select *Approved Drugs* (70) and clinical trials drugs (57) as repositioning candidates

127 Repositioning candidates:

70 approved drugs + 57 drugs in clinical trials

Pharmacological Filtering Criteria:

1. **Toxicity**: Exclude drugs requiring HSC review (GHS Cat. 1)
2. **Systemic effect**: Exclude non-systemic use drugs
3. **Commercial availability**: Exclude commercially unavailable drugs

56 Potential drug candidates for screening:

27 approved drugs

29 drugs in clinical trials

27 Approved Drugs Predicted From Primary 38

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- Original primary hits (**blue**).
- 5 validated predictions (**green**) with comparable effects to original top 3 hits (almost complete inhibition)
- 5 partial hits (**yellow**), 2 statin family drugs increased p-tau level (**red**)
- **>160-fold improvement** on hit rate (5/27 vs. 3/2,640, primary screening)

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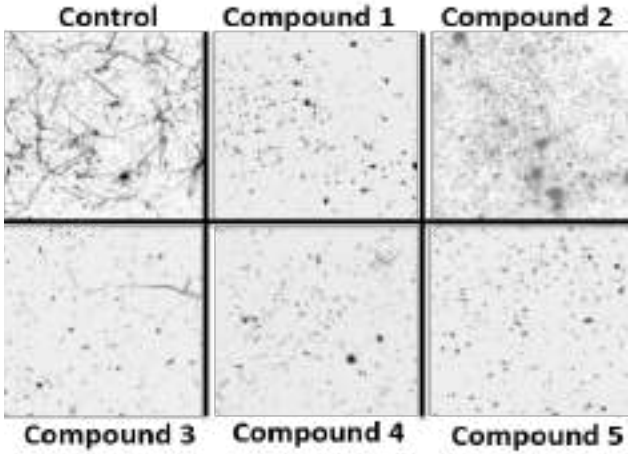
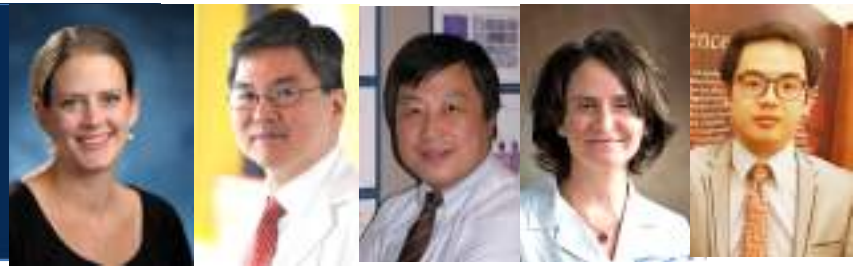
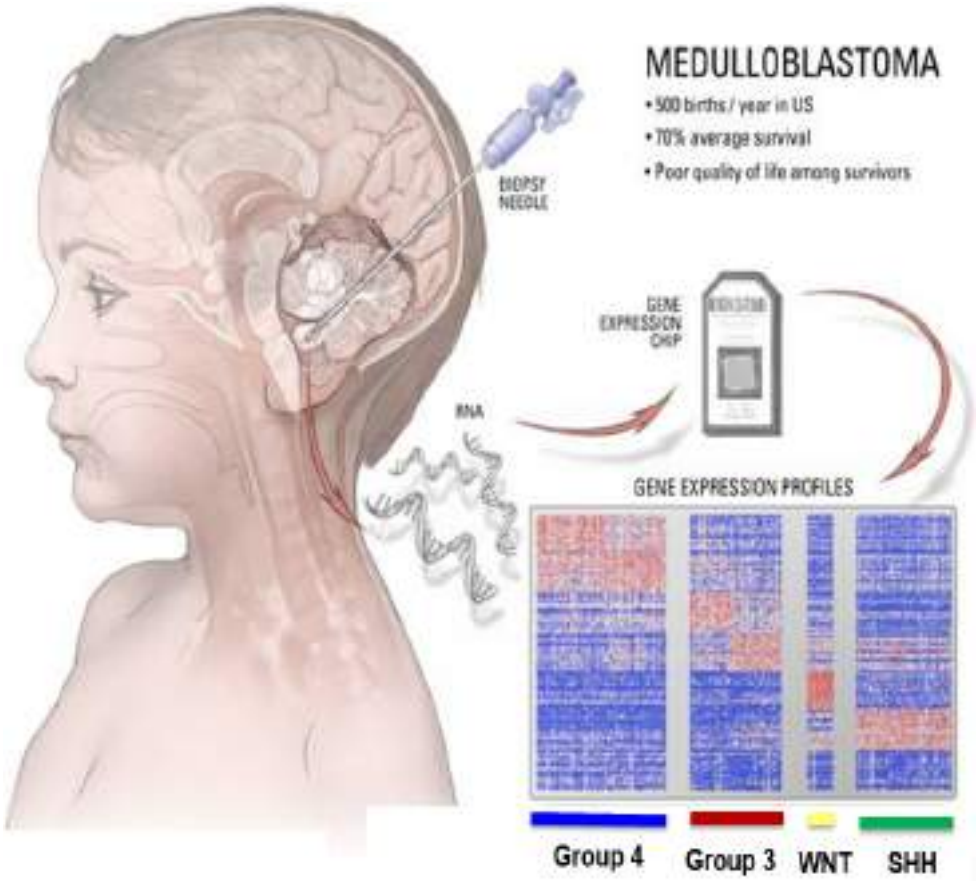


Network as a Biomarker



Medulloblastoma is the most common malignant brain tumor of childhood.



Northcott P et al, JCO, 2010; Cho YJ et al , JCO, 2010; Taylor M et al, Acta Neuropathologica, 2012

A Systems Biology-driven Drug Discovery Framework

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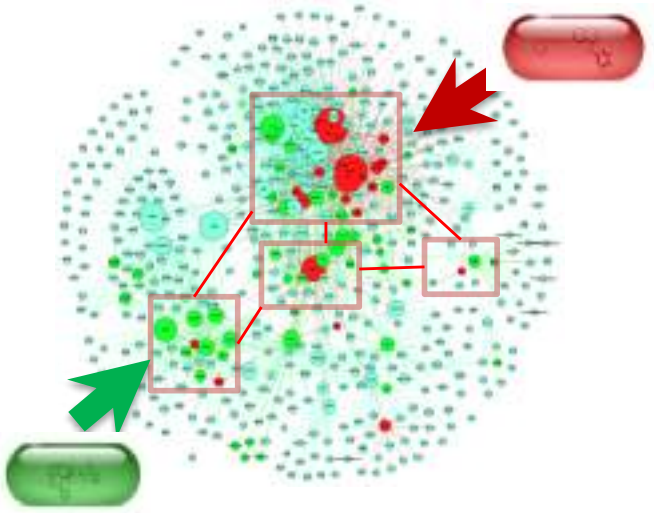
Based on Driver Signaling Network Identification (DSNI) and Drug Functional Network (DFN) Modeling

DSNI-DFN

① **Driver signaling networks identification (DSNI)**

② Construction of drug Functional network with potential mechanisms of action

③ Mapping targets of drug functional network to driver disease networks



L Huang et al Science Tran Med, Oct 2018

Driver Signaling Network Identification (DSNI)

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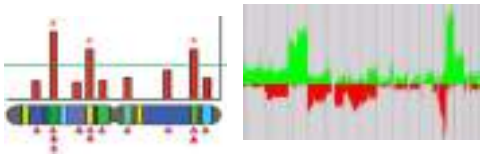
DNA-seq: mutated genes

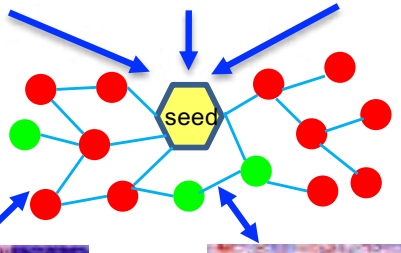
CNV: amplified genes



Pathway DBs: KEGG and NCI

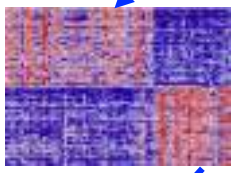
We analyzed multi-omics data (mRNA expression, DNA-copy number, DNA-methylation and DNA-seq profiles) of **>1,800 patients with Group 3/4 MBs**

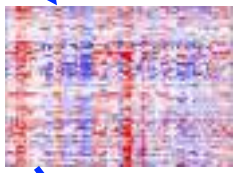
Network exploring



$$NetS(G) = \sum_{i \in S} \frac{1}{P_i \frac{1}{\sum_{i \in S} P_i}} \hat{\xi}_i - \frac{\lambda}{n} \sum_{(i,k) \in E} \left(\frac{\hat{\xi}_i}{\sqrt{d_i}} - \frac{\hat{\xi}_k}{\sqrt{d_k}} \right)^2 - \frac{\gamma}{m} \sum_{i \in S} (Z_i - \hat{\xi}_i)^2 / 2$$

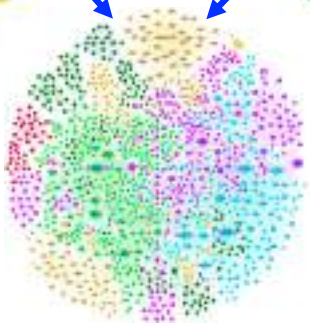
RNA-seq profiling





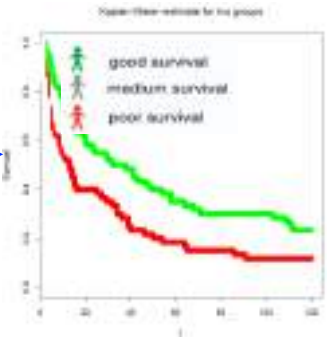
Methylation profiling

Subtype-specific disease driver Networks



Predict patients outcome

Kaplan-Meier-esimate for two survival populations



L Huang et al Science Tran Med, Oct 2018

Construct Drug Functional Network With Potential Mechanisms Of Action

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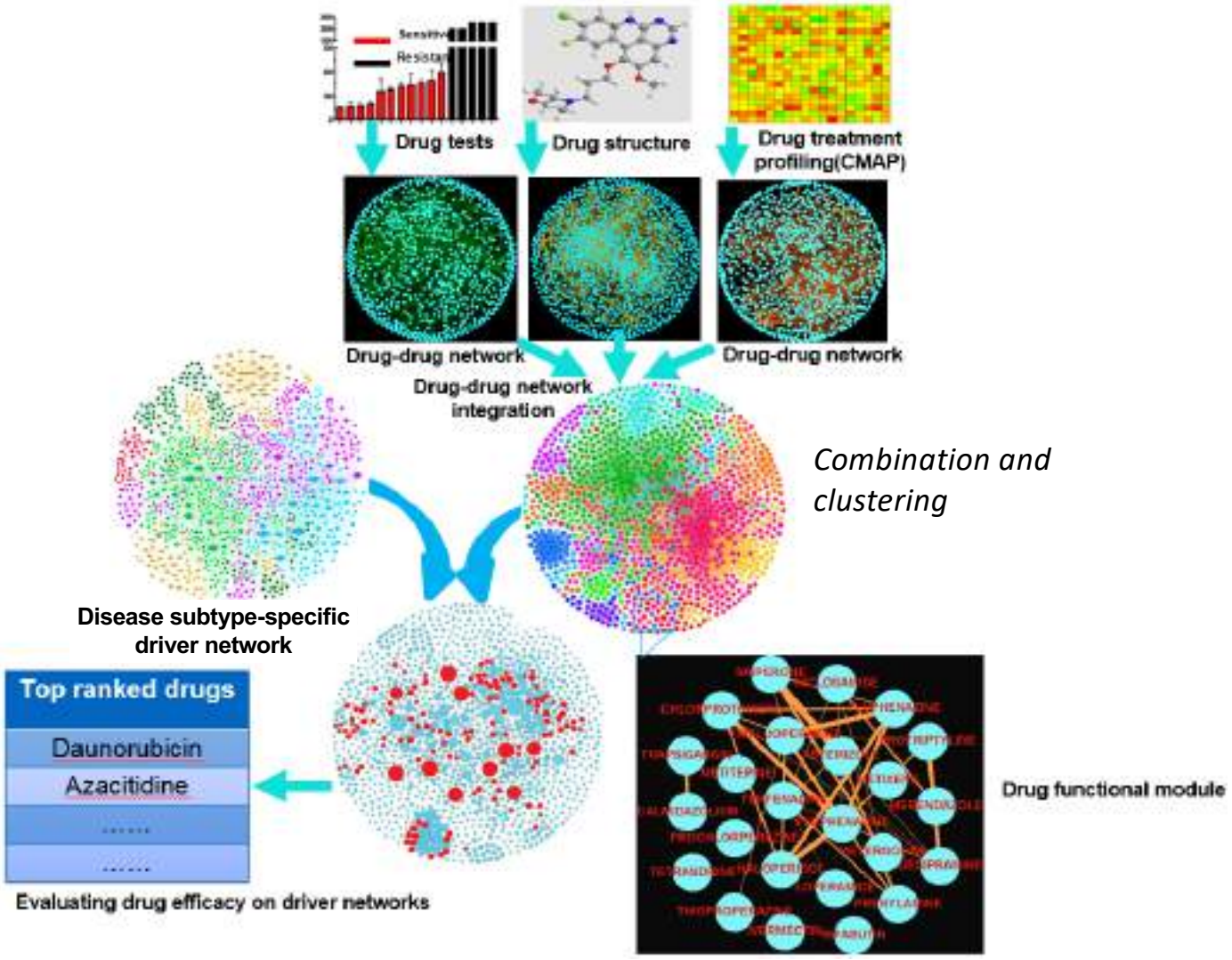

L Huang et al Bioinformatics, Feb 2019

Network As A Biomarker

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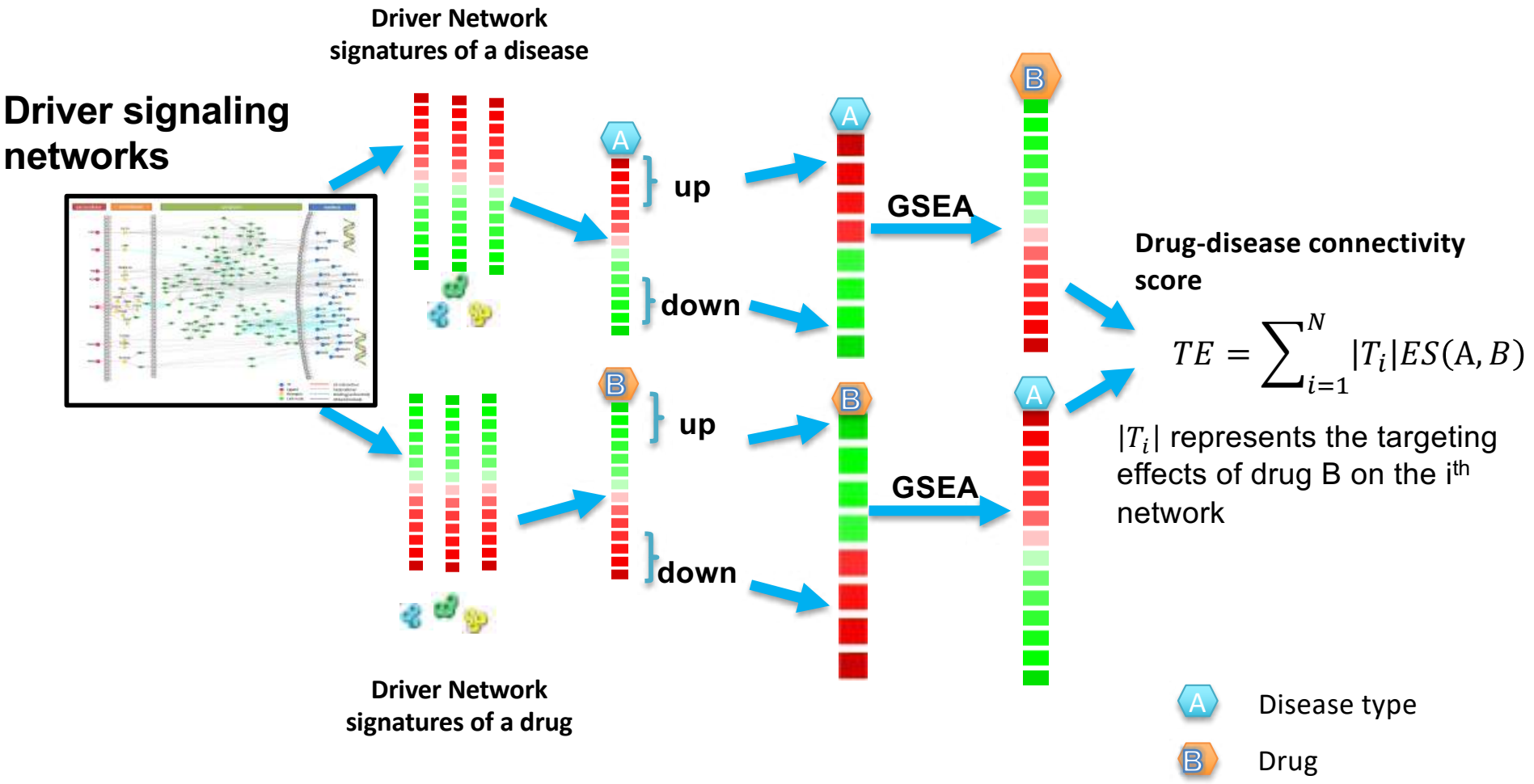

Candidate Drugs Identified for Groups 3 and 4 MB

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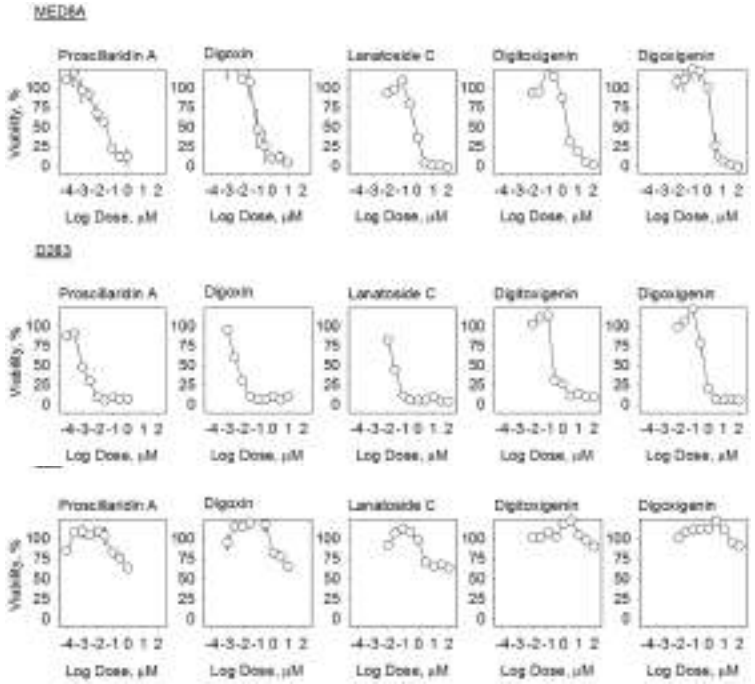
Apply DISNEEvaluate **1,300 known drugs** to find top ranking drug candidates for Group 3/4 MBs.

Multi-omics analysis

Data type	Group 3 MB	Group 4 MB	Control	Data source
mRNA expression	144	326	193	GSE85217
DNA copy number	316	261	Not applicable	GSE37384
DNA-seq	56	64	Not applicable	dbGaP(phs000504.v1.p1, phs000409); EGAS00001000215
DNA methylation	144	326	193	GSE85212

Cardiac glycosides (Ranking order)	Group 3	Group 4	SHH	WNT
Digoxin	9	48	80	1233
Digitoxigenin	14	49	1244	1239
Digoxigenin	27	40	1220	1098
Lanatoside C	28	15	1241	1252
Proscillaridin A	34	27	1240	1238

Drug Screening Validation



Huang L, et al. Science Tran Medicine, Oct, 2018

Successful Treatment in Orthotopic PDOX Model of Group 3/4 MBs

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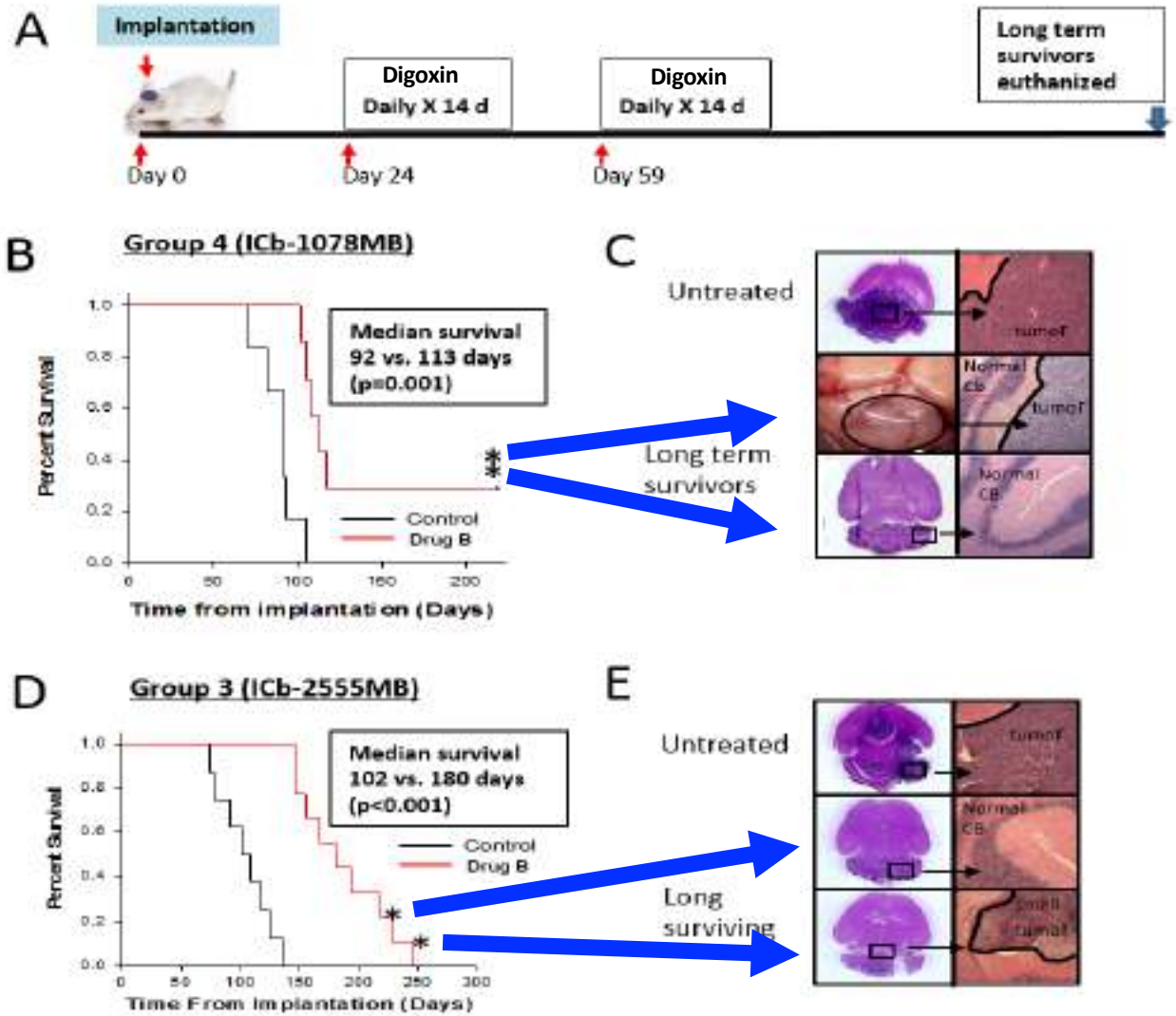

MB group 3 & 4 Mouse Models took Digoxin survived longer significantly

Comparison of The Effects of Digoxin and Ionizing Radiation

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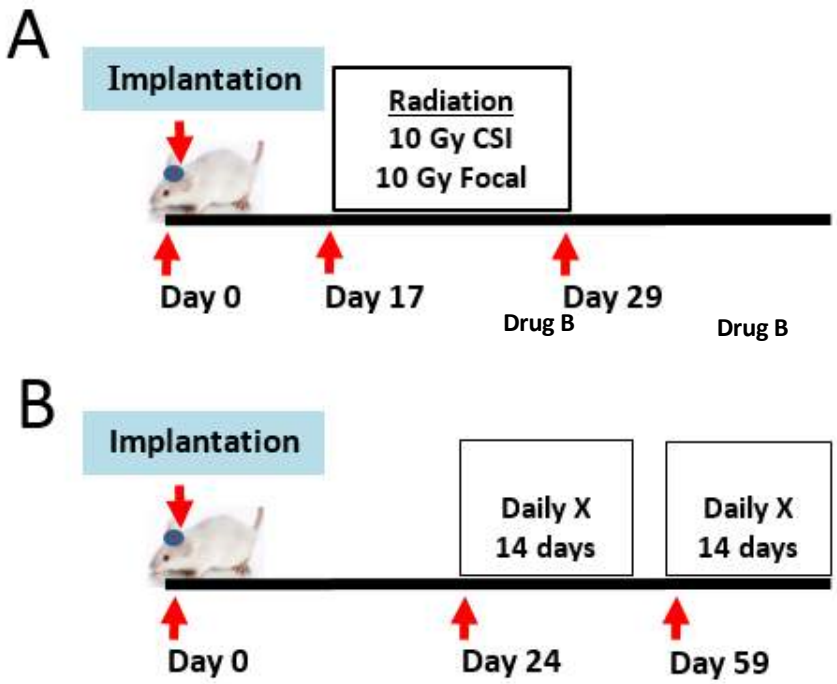
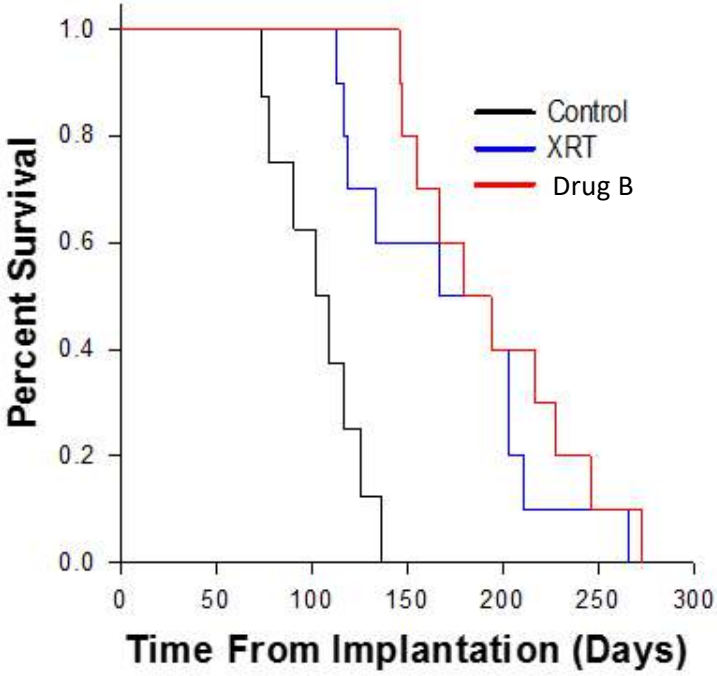
Group 3 model of MB



XRT vs control, p=0.0108
Drug B vs control, p<0.001



Huang L, et al. Science Tran Medicine, Oct, 2018

Comparison of The Effects of Digoxin and Ionizing Radiation

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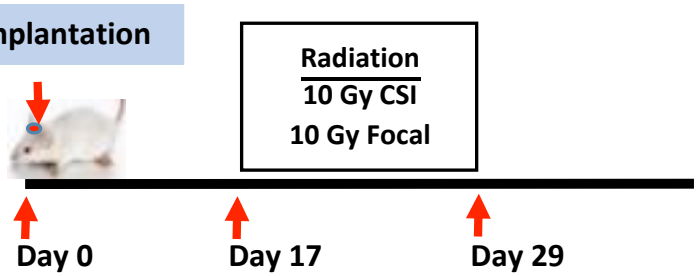
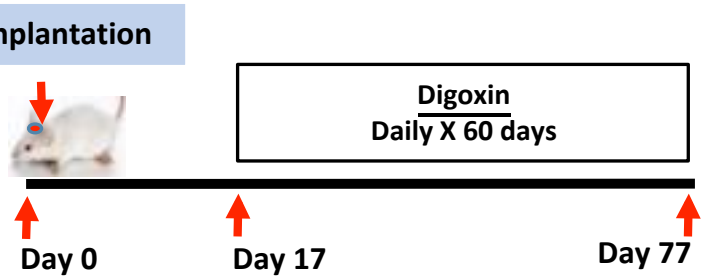
Group 3 model of MB

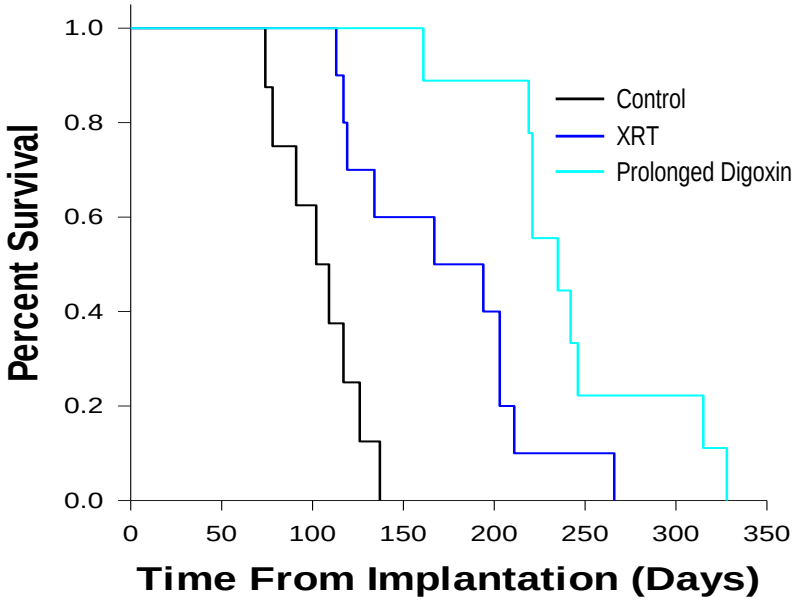
A



B



C

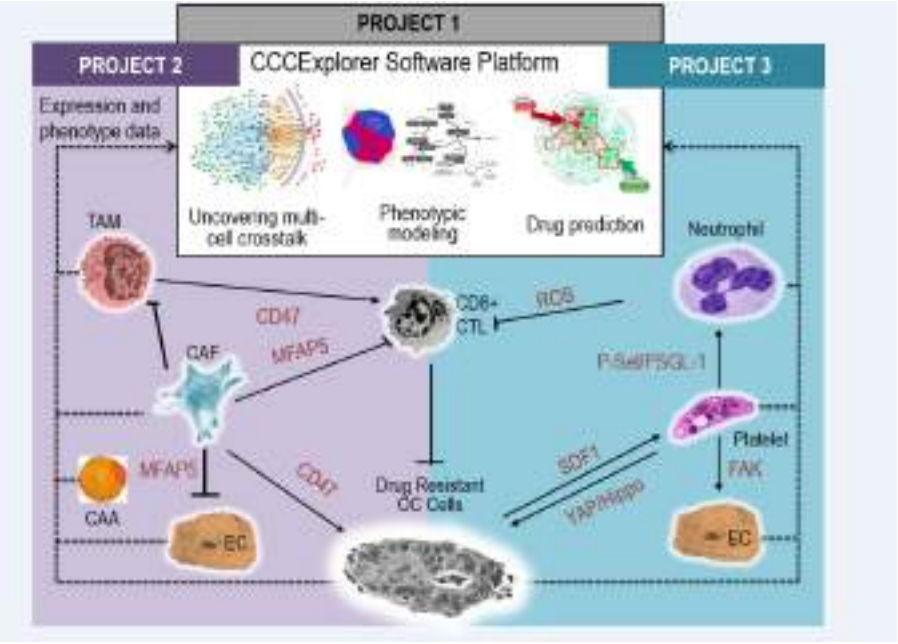


Prolonged digoxin showed a statistically significant prolongation of survival (235 vs 167 days, log rank $p<0.01$)

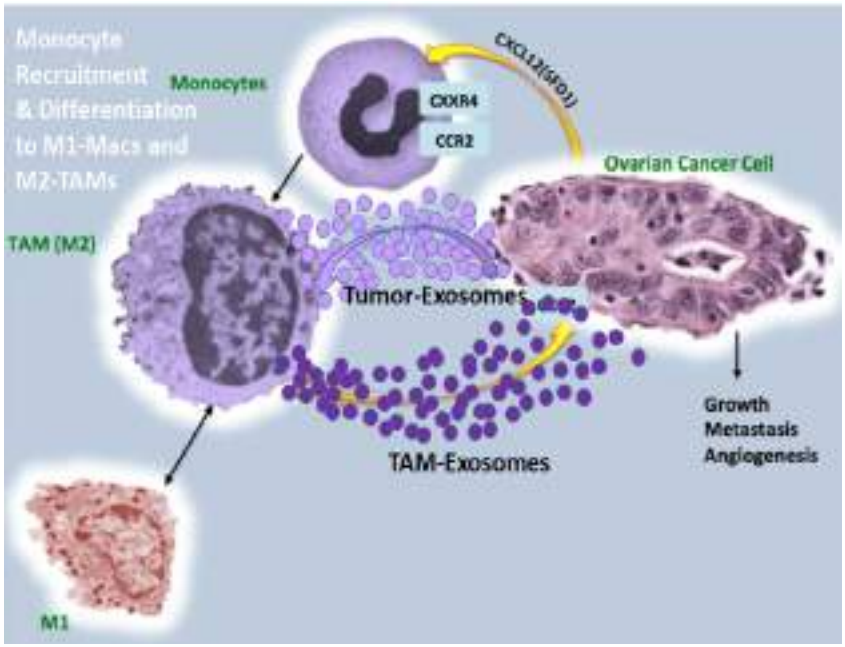
Crosstalk as Biomarker

Two major categories of cell-cell communications in tumor microenvironment

Receptor-mediated crosstalk



Exosome-mediated crosstalk



Identify Altered Pathways in the Stromal-Epithelial Environment that Drive Lung Carcinogenesis & Mediate Chemoresistance

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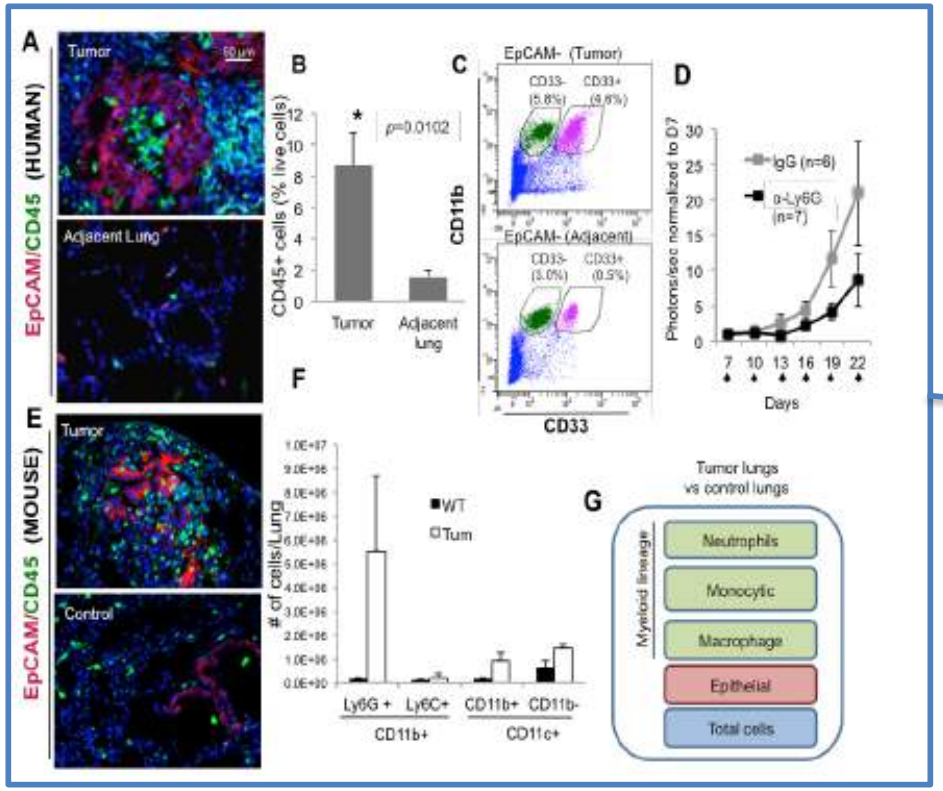
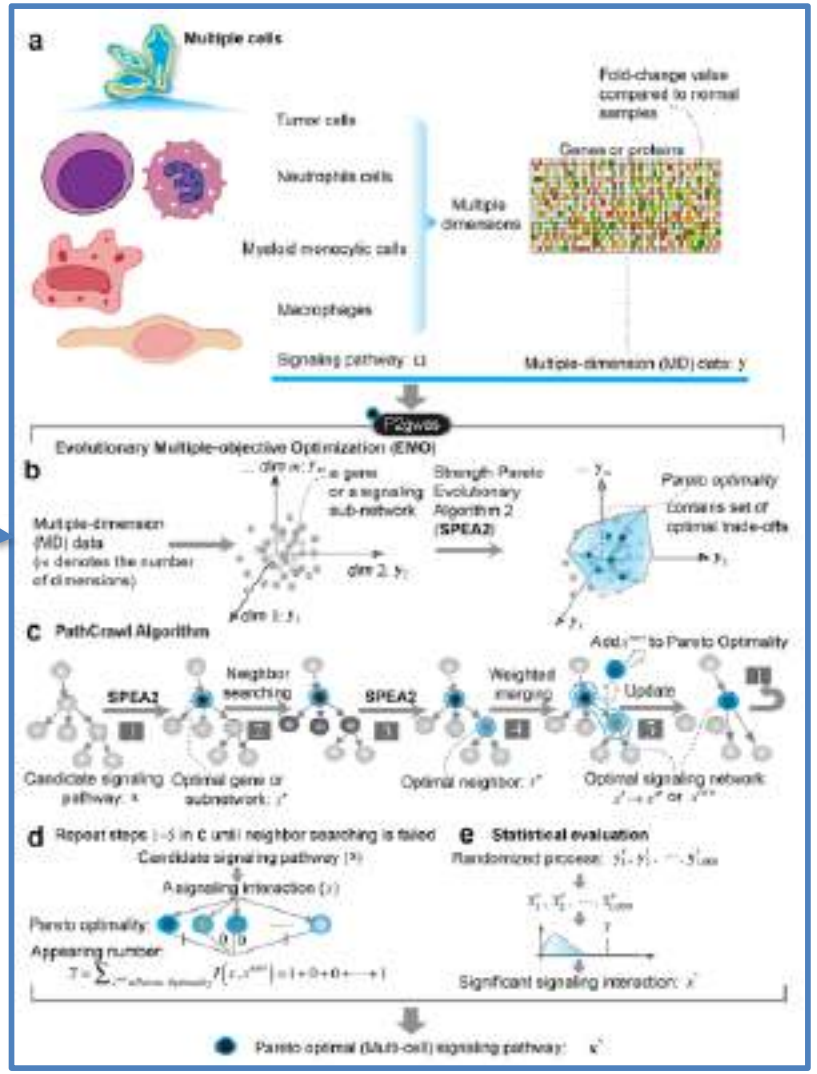
Myeloid cells infiltrate human and mouse NSCLC





Identify stroma-tumor crosstalk using multi-cellular data

Receptor-mediated Stromal-tumor Interaction

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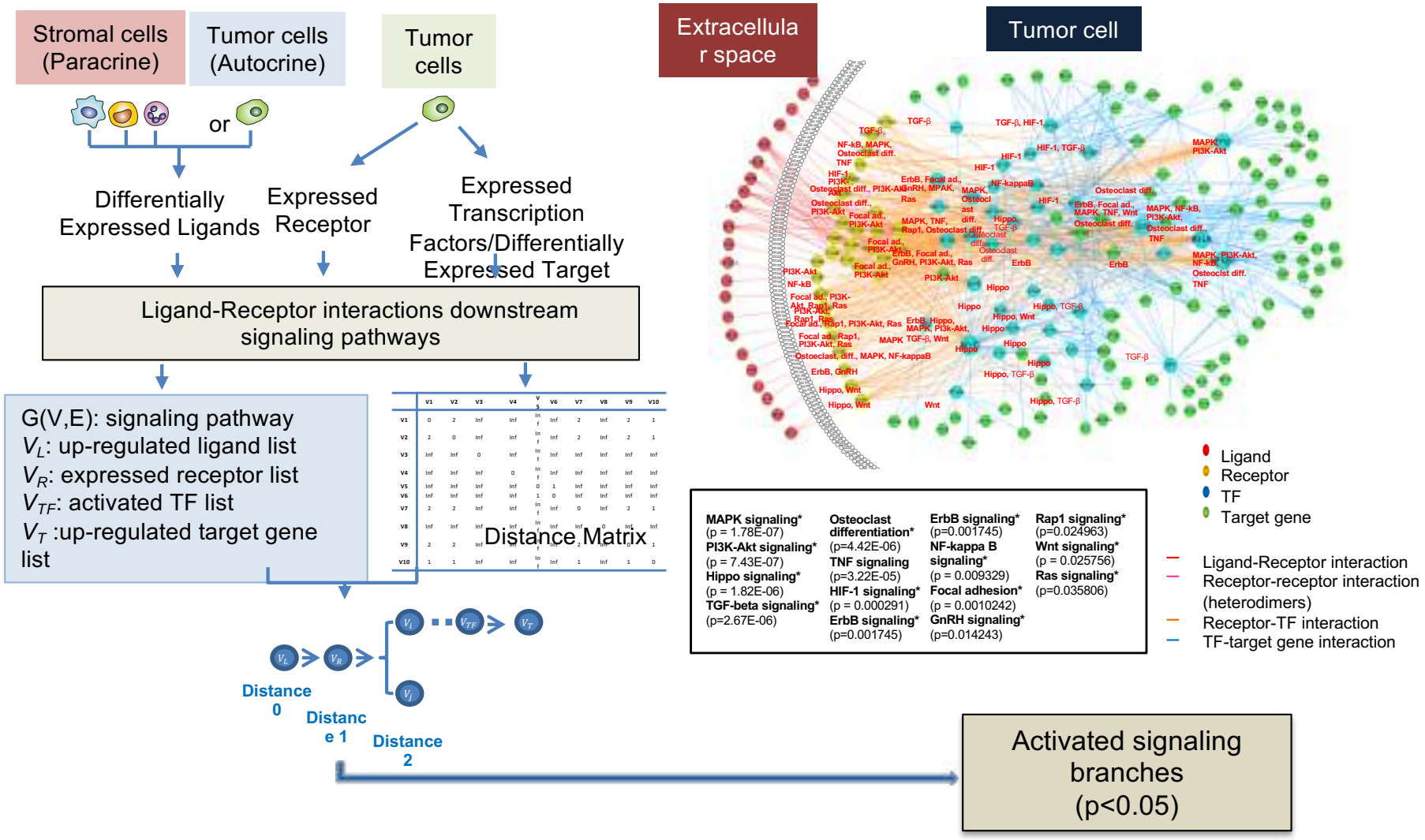
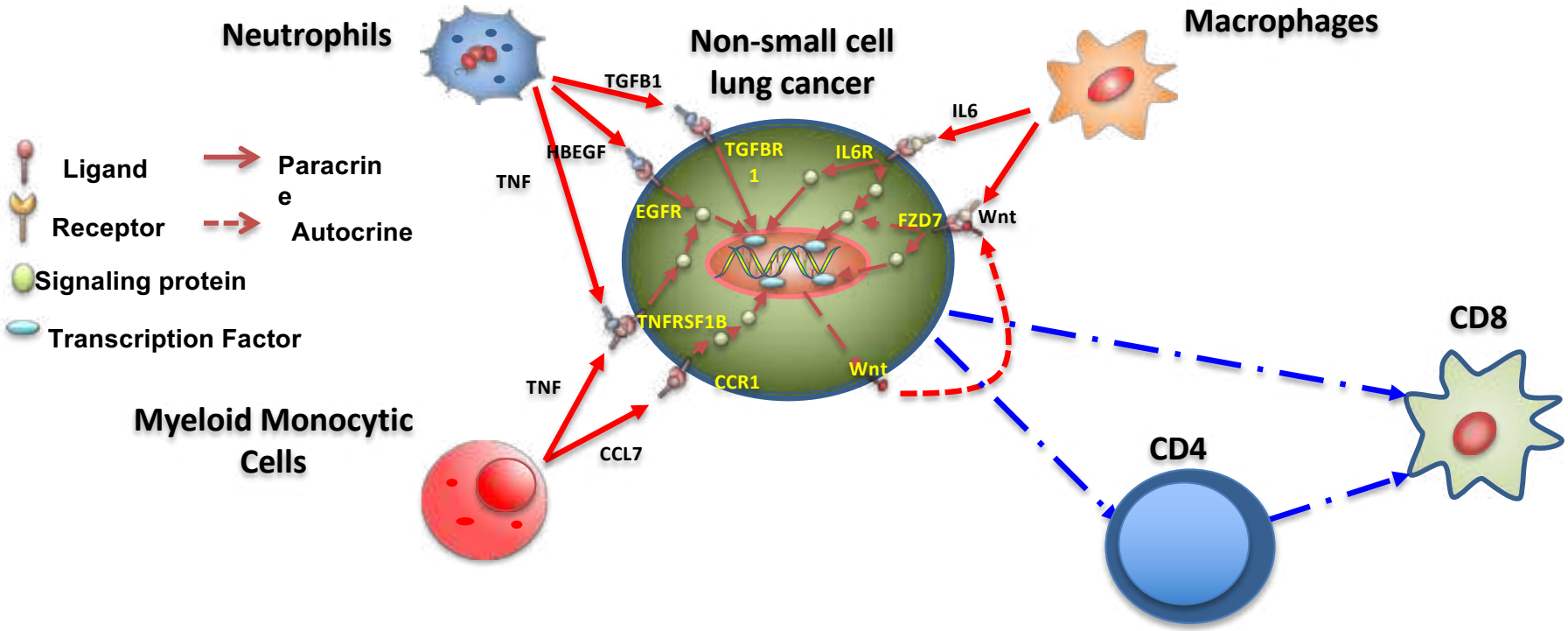


Receptor-mediated Stromal-tumor Interaction



- Common pathways: MAPK, PI3K-Akt, NF-kappa B, ErbB, Ras, TGF-, and TNF
- Unique pathways: HIF-1(MMC&Macrophage), Wnt(Macrophase&tumor), Hippo(Neu&Macrophage&tumor), FoxO(Neu&tumor)
- Novel paracrine crosstalk: IL6-IL6R, WNT11-FZD7
- Validation: IL6-IL6R-Stat3

Choi, et al, Cell Report, 2015

CCCExplorer

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Systematic Identification Of Druggable Epithelial–stromal Crosstalk Signaling Networks In Ovarian Cancer

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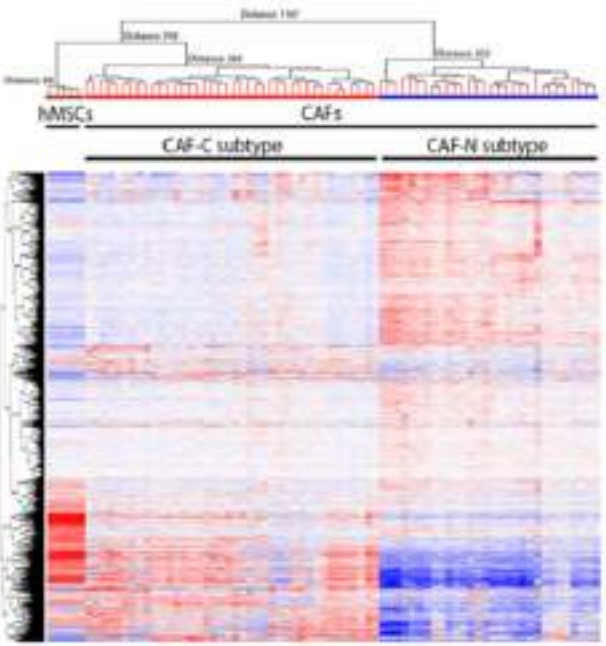
Unsupervised clustering of Cancer Associated Fibroblast(CAFs) and bone marrow Mesenchymal Stem Cells (MSCs) identified two major CAF subtypes

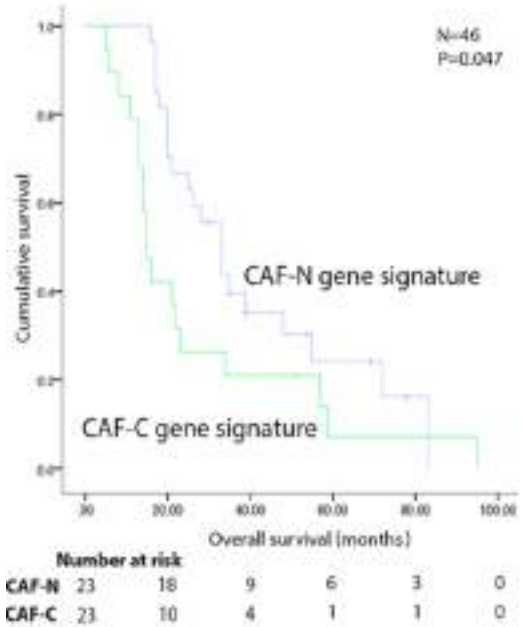
A)



B)

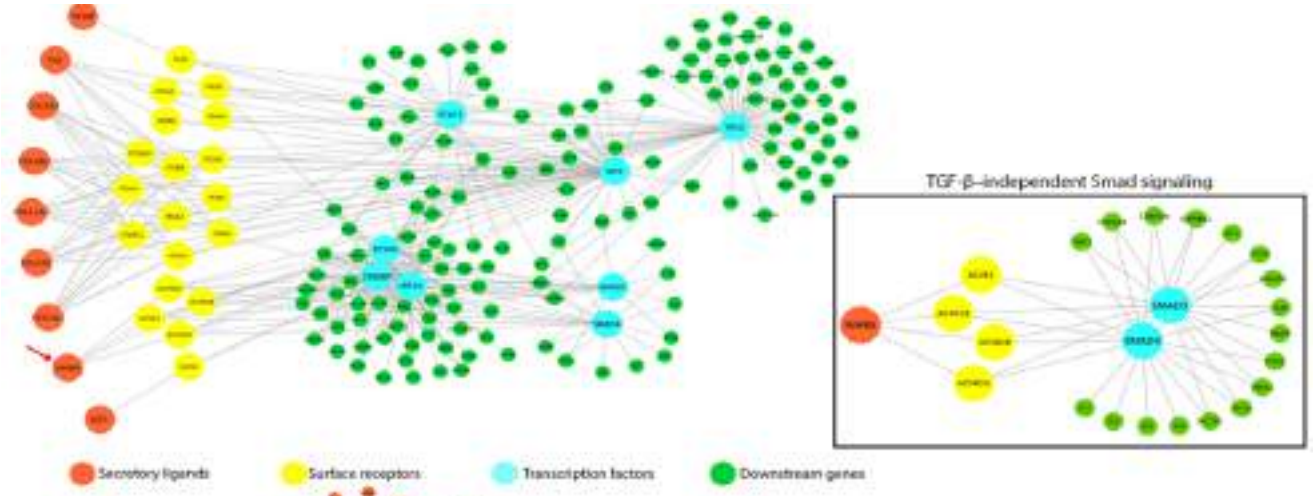
Number of clusters	Average silhouette score k means	Average silhouette score hierarchical
3	0.520779	0.506461
4	0.44033	0.459211
5	0.212553	0.327744
6	0.288765	0.303304
7	0.253932	0.248601
8	0.271511	0.231292
9	0.276265	0.213131
10	0.257753	0.21664

C)

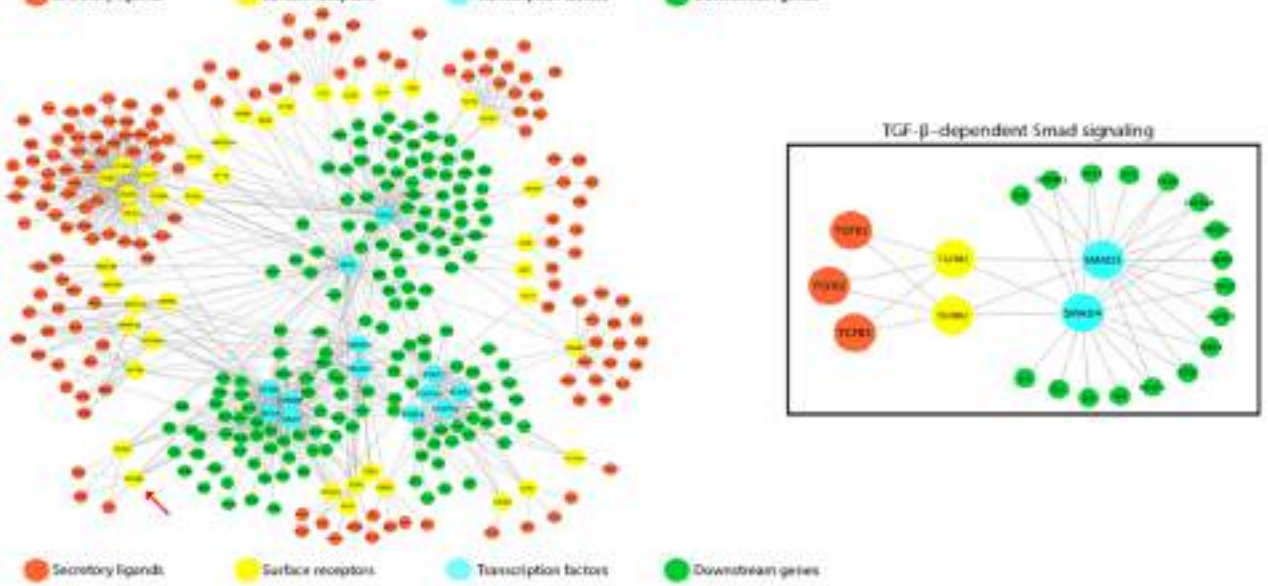


Yueng TL, Sheng J, et al. JNCI, Oct

Tumor-CAF Crosstalk



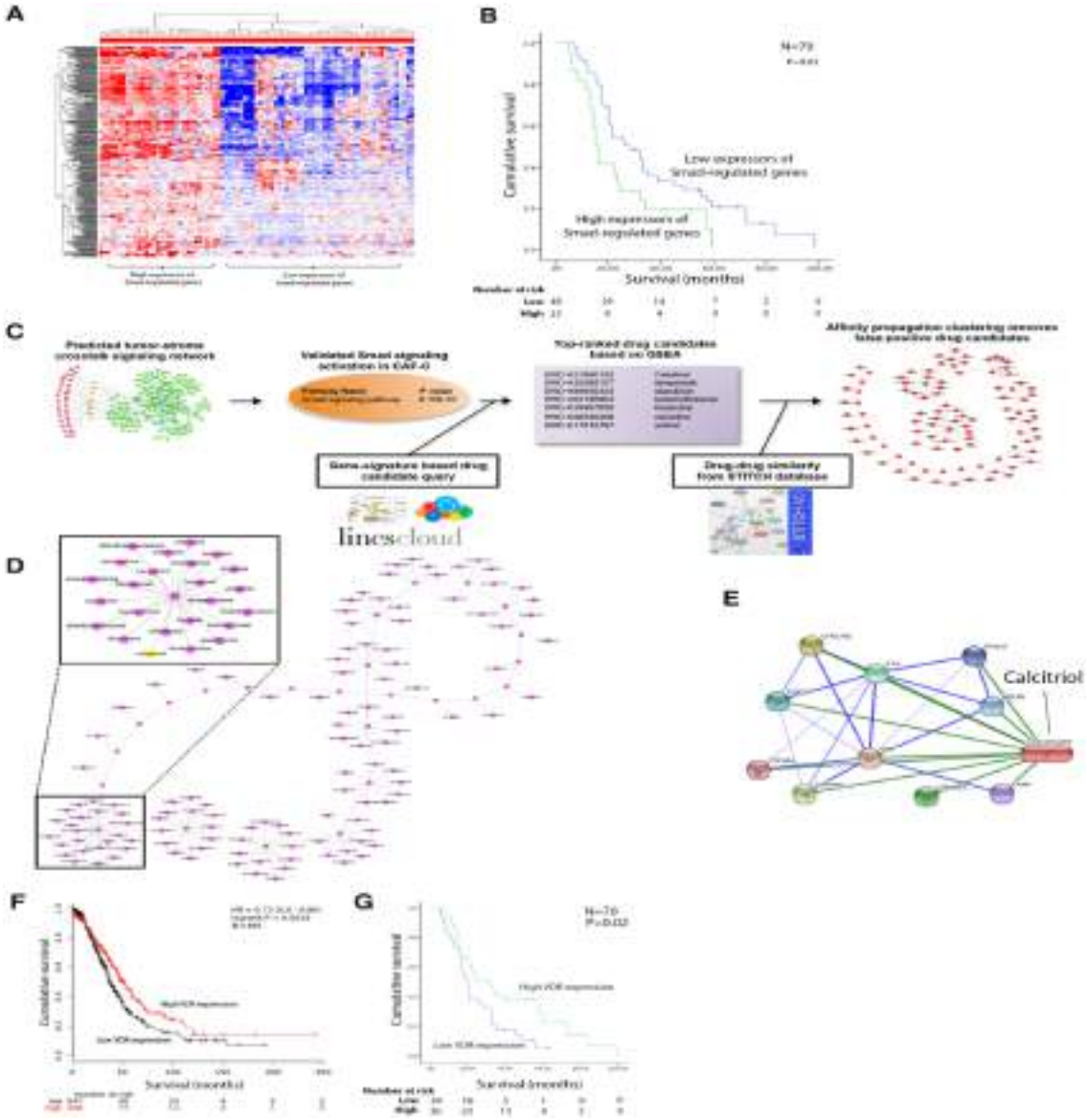
Activated signaling pathways in CAF-C were predicted through overexpressed secretory **ligands** in cancer cells and activated transcription factors in CAFs in the CAF-C patient cohort.



Activated signaling pathways in CAF-C were predicted through the identification of overexpressed **receptors** and activated transcription factors in CAFs in the CAF-C patient cohort

Yeung, et al, JNCI, 2018

Targeting Crosstalk



Identification of a prescription drug, calcitriol, that target activated Smad signaling in cancer-associated fibroblasts (CAFs).

Yeung, et al, JNCI, 2018

Exosomes Represent a Rich Source of Biomarkers

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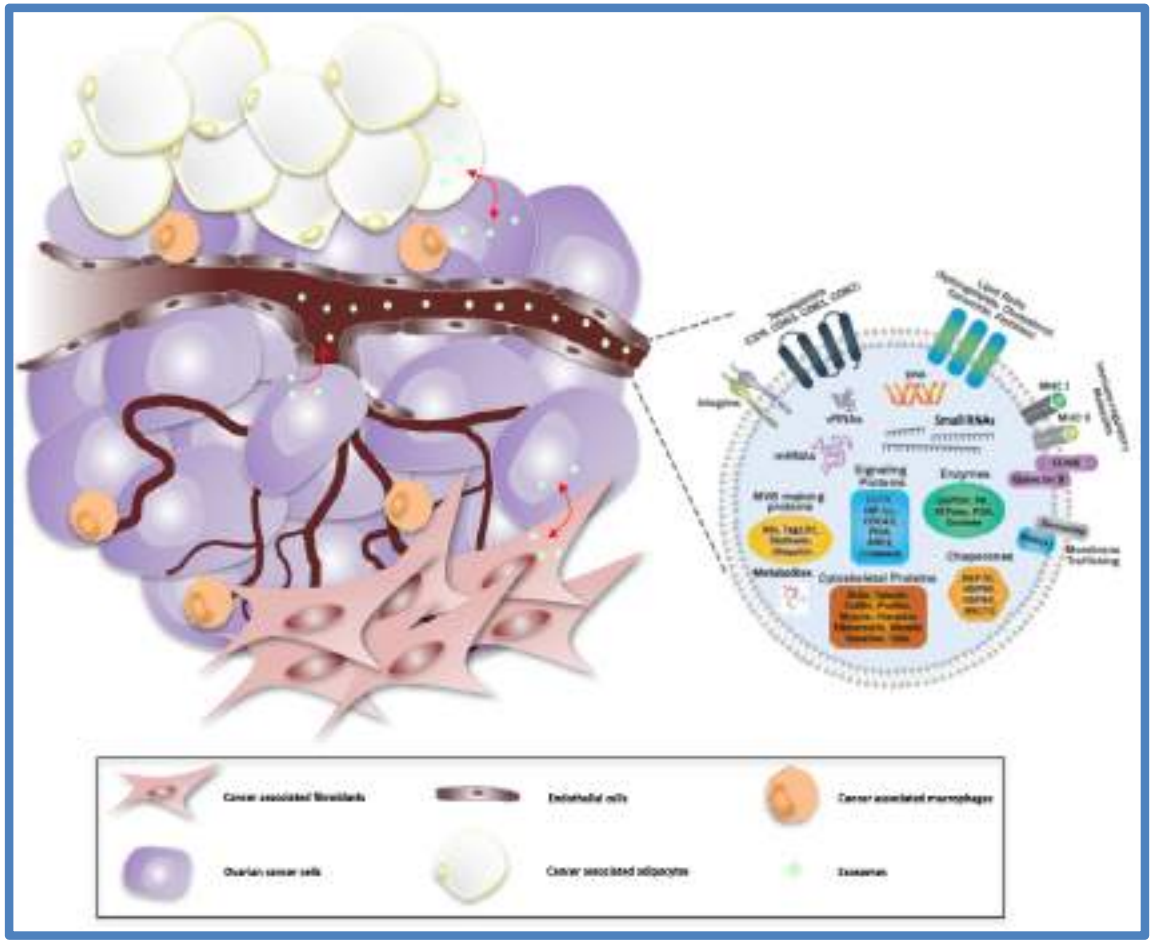

Advantages of Using Exosomal Small RNA-Based Biomarkers for Cancer Detection

- Stable at -20°C for 5 years, largely unaffected after 2 weeks at 4°C and resistant to freeze-thaw cycles.
- Investigate exosomal miRNAs as well as other exosomal small RNAs such as non-coding RNAs (tRNAs, rRNAs, lincRNAs, piRNAs, snoRNAs) as potential new biomarkers.

Exosome-mediated Stromal-tumor Interaction

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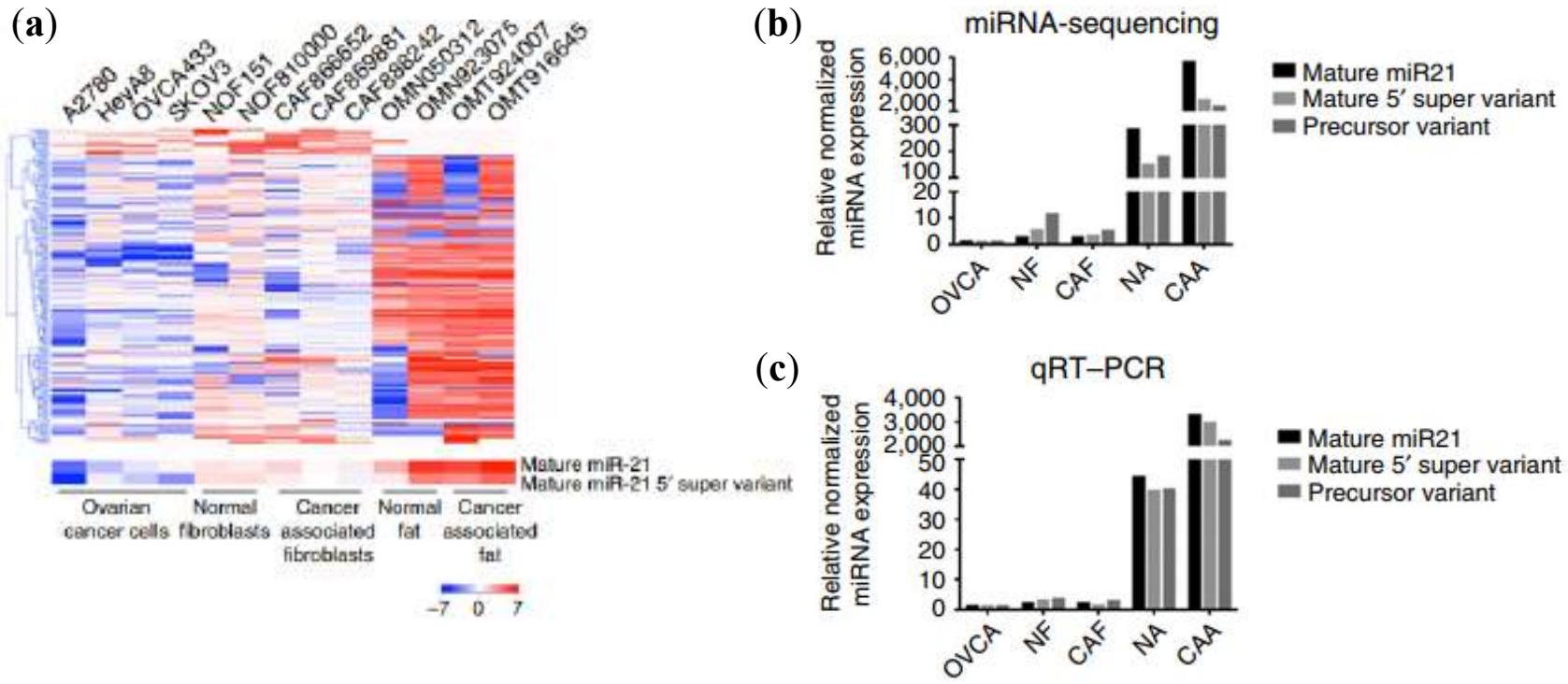
Exosomal transfer of miR21 derived from cancer associated fibroblasts (CAF) and cancer associated adipocytes (CAA) confers paclitaxel resistance in ovarian cancer cells through targeting APAF1.

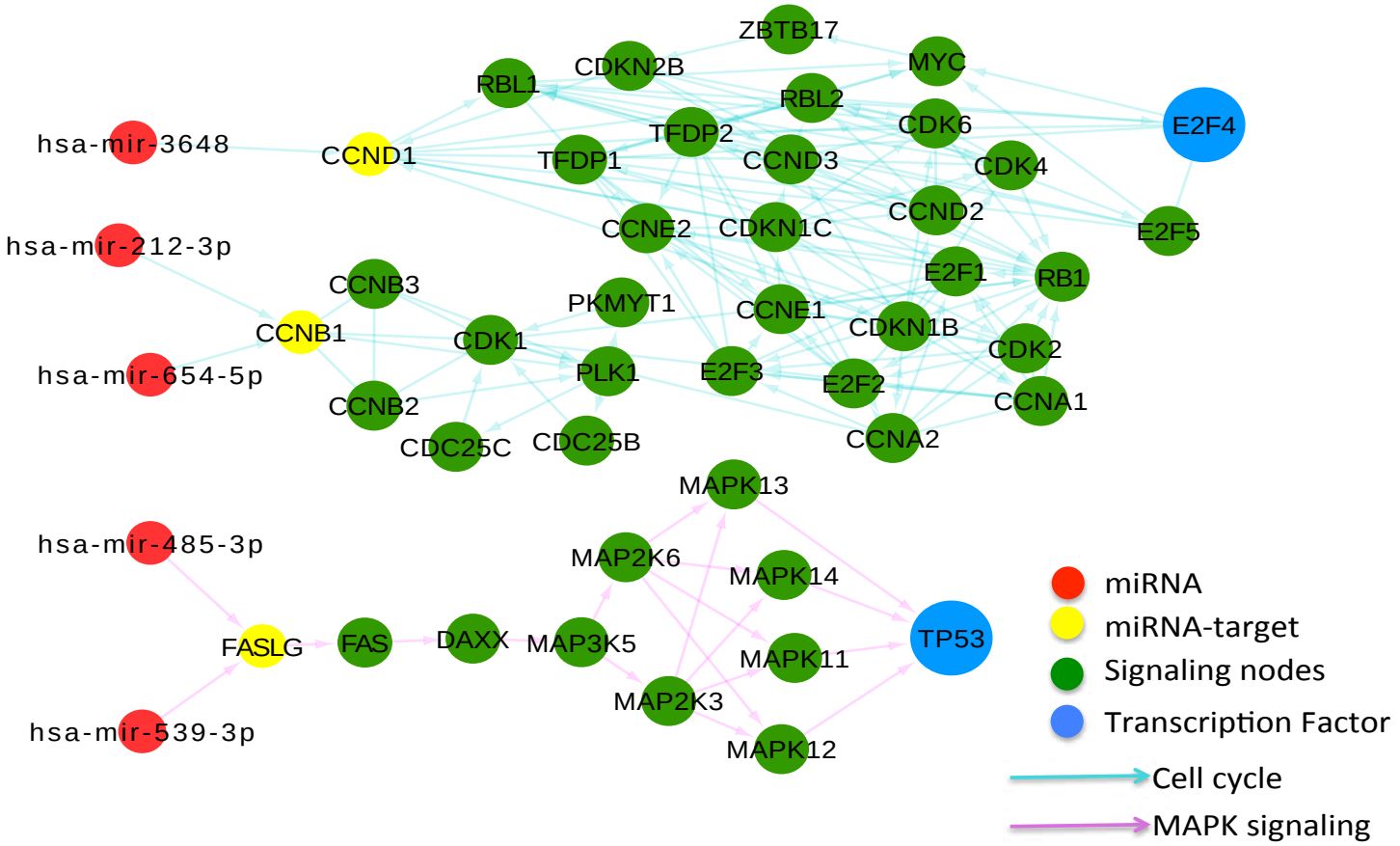


Chi Lam Au Yeung, et, al. Nat Commun. 2016

Exosomal Crosstalk

Crosstalk analysis identified two ovarian cancer pathways that are altered by 5 up-regulated exosomal miRNAs from CAF

Software: CCCExplorer



Identify Differentially Expressed Exosomal Small RNAs

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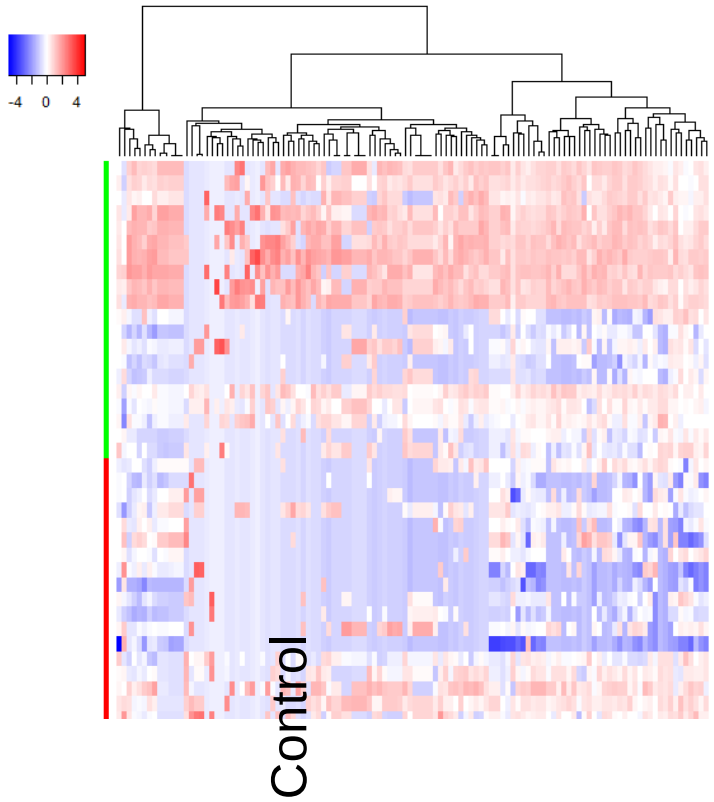
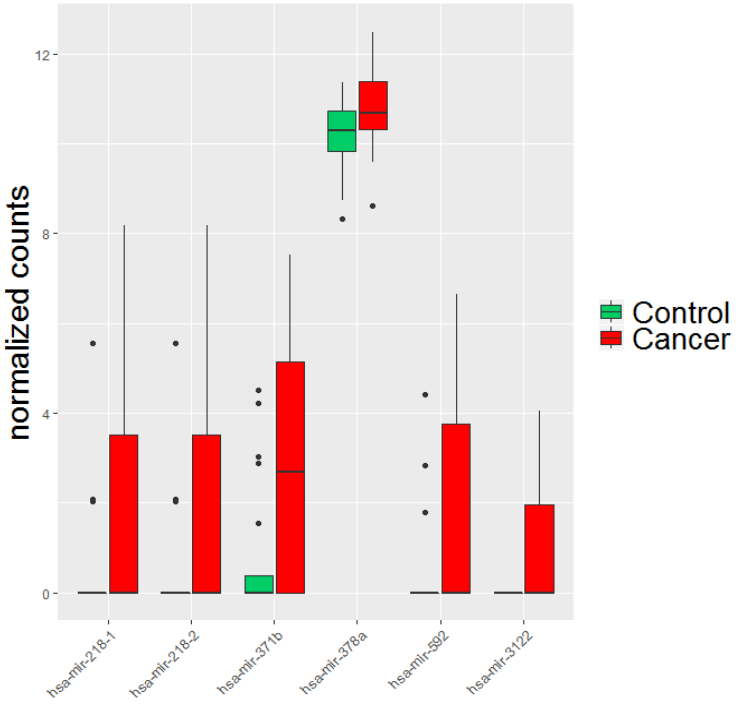
Differentially expressed exosomal miRNAs



Top 6 up-regulated exsomal miRNAs
Cancer *vs.* Control



Inter-cellular Communication at Primary and Metastatic Sites

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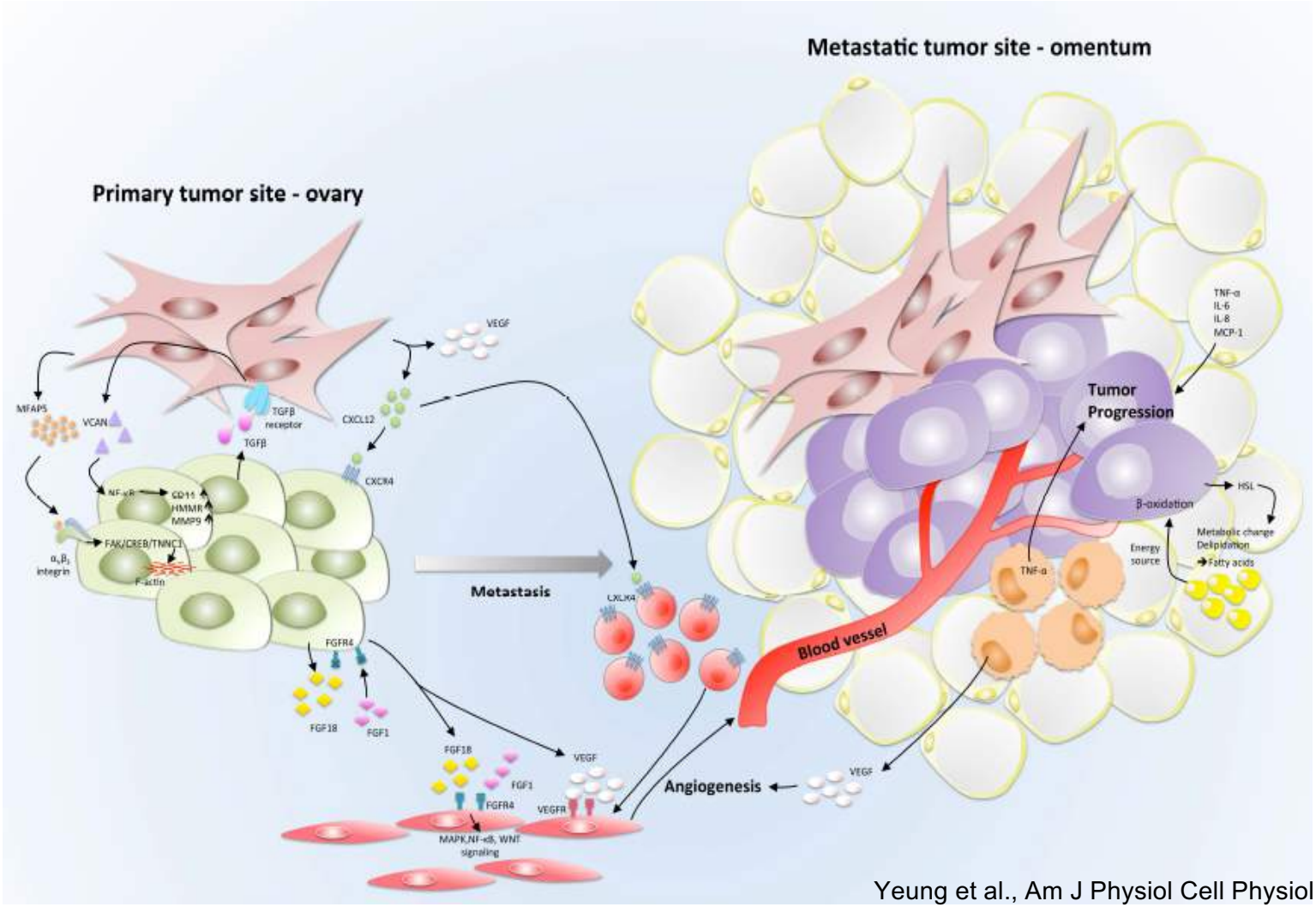

Yeung et al., Am J Physiol Cell Physiol, 2015

Discovery of Cancer Drug Targets Crosstalk

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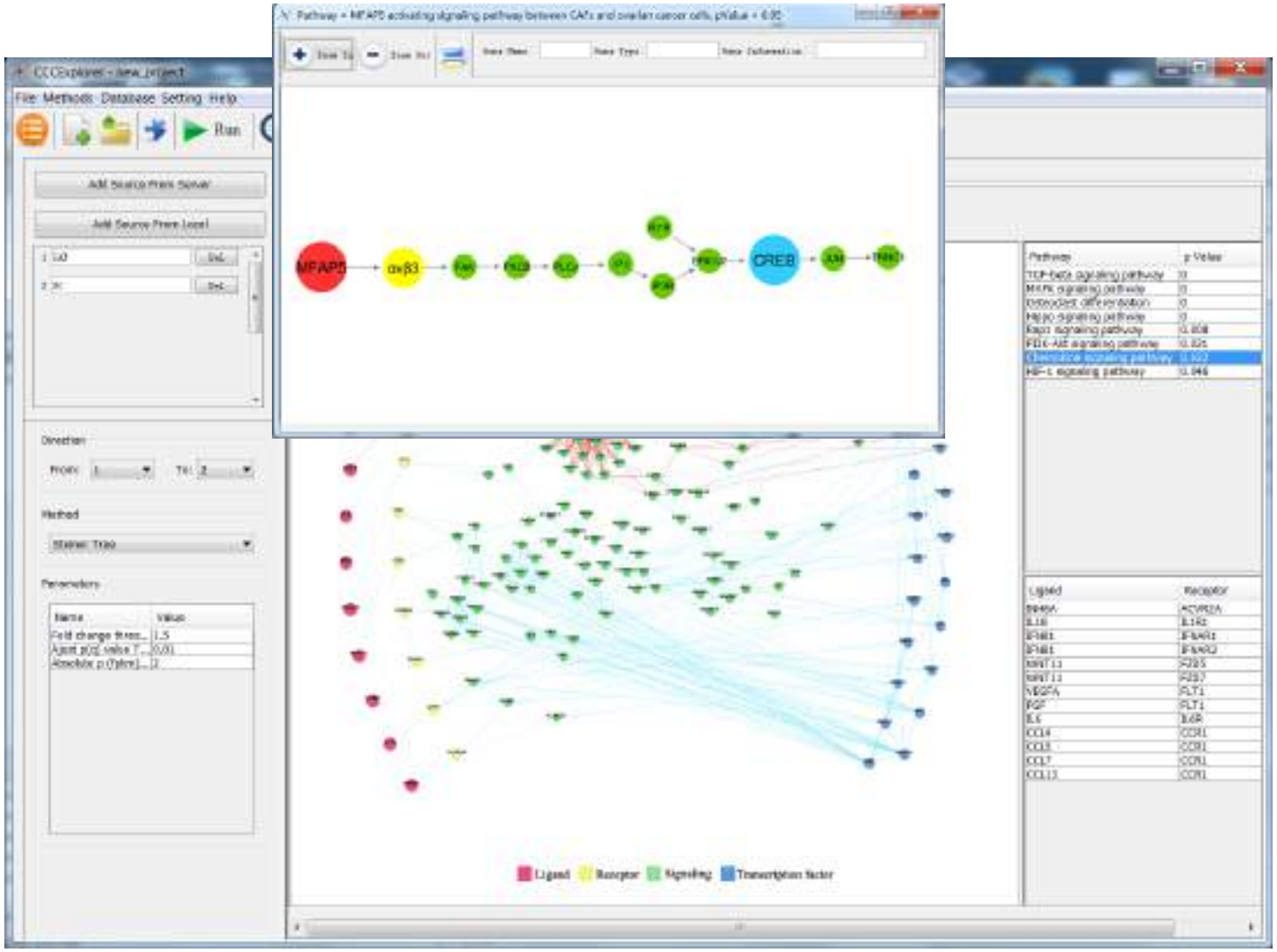

MFAP5 is a Novel Target for Cancer Treatment

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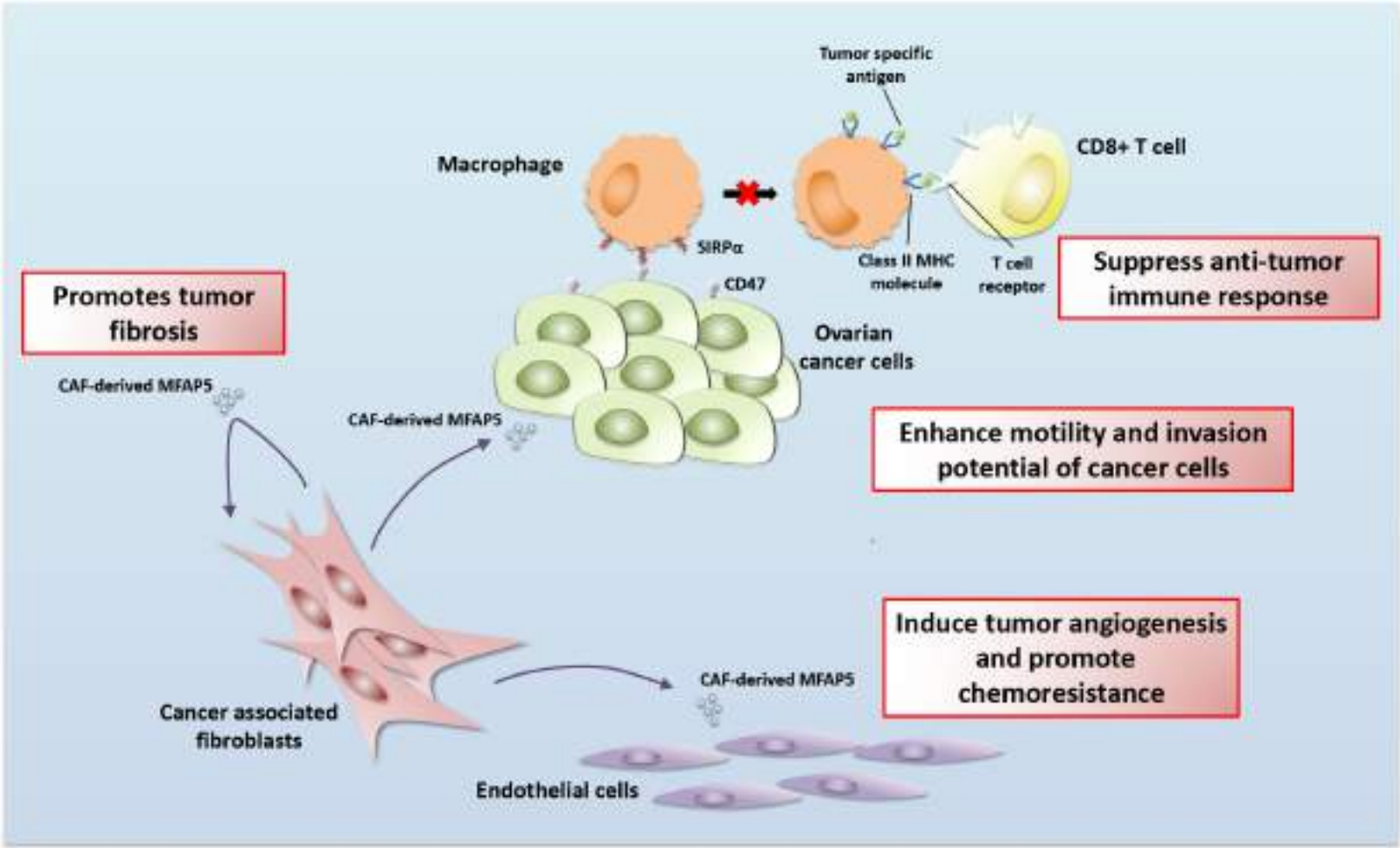

Leung et al., J Clin Invest., 2018, Leung et al., Clin Cancer Research, 2019

MFAP5-targeting Monoclonal Antibody In Cancer Treatment

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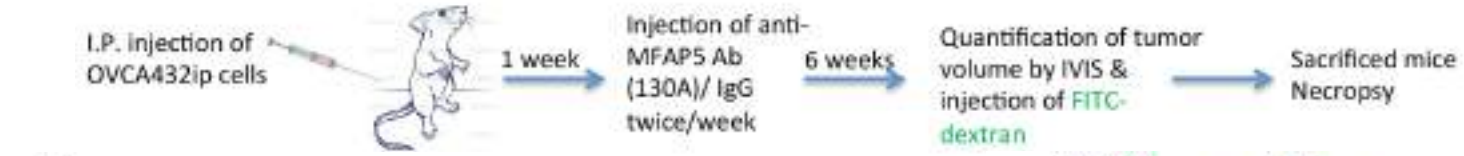
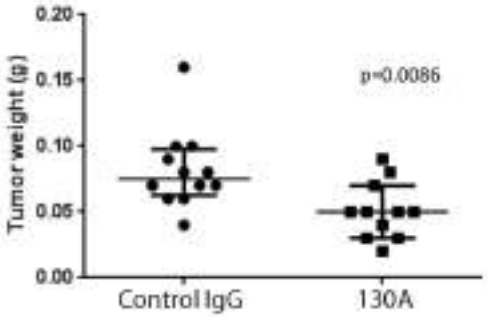
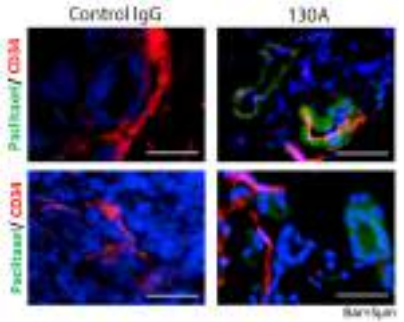
Ovarian cancer model



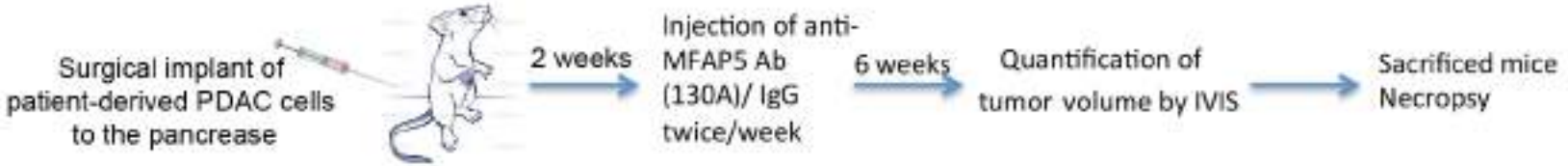


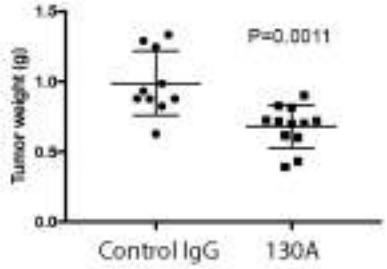
Reduce Tumor Size



Enhance Drug Uptake in Tumor Tissue

Pancreatic cancer model





Reduce Tumor Size

Humanized Monoclone Antibody Targeting MFAP5

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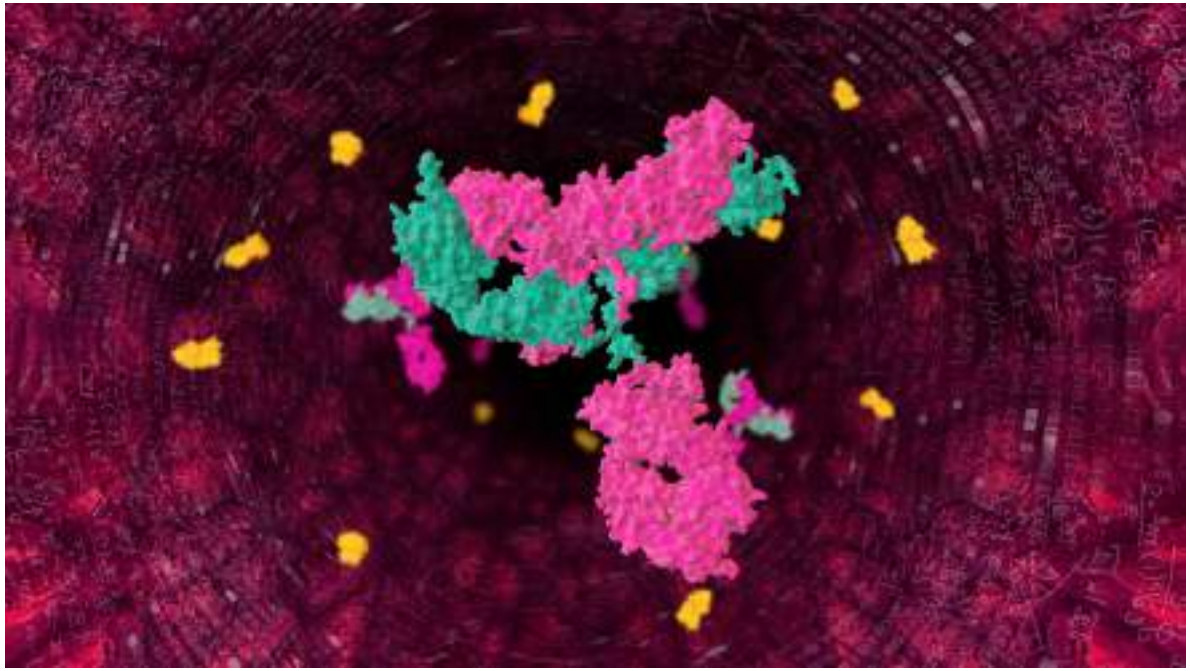
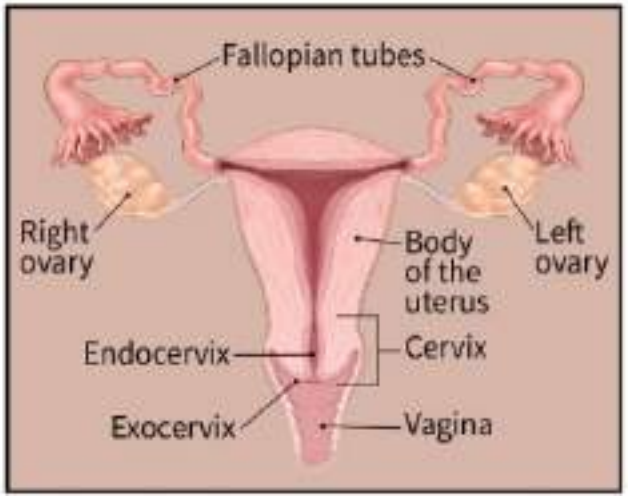
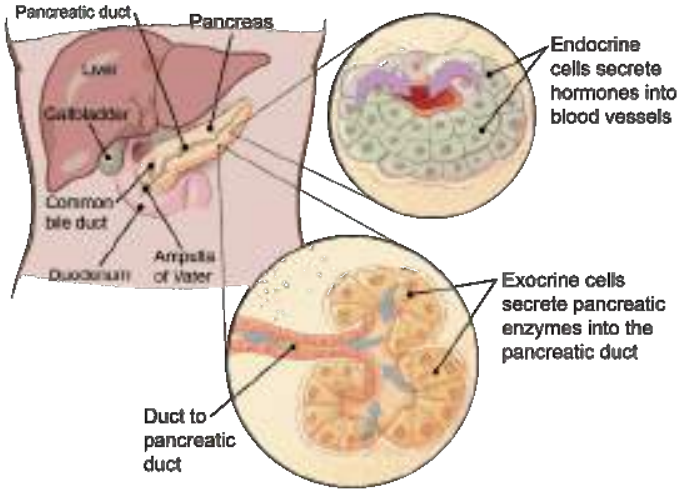


Ovarian Cancer



Pancreatic Cancer



Patent pending

ACKNOWLEDGEMENTS

SMAB Colleagues:

Zheng Yin, PhD, Jianting Sheng, PhD; Xuping Li, PhD; Lin Wang, PhD; Xin Wang, MSc; Yunjie He, MSc; Hong Zhao, PhD; Xiang Nan, MSc; Mamta Puppala, MSc; Tian Cheng He, PhD; Chika Ezeana, MD; Rebecca Danforth, PhD; Xiaohui Xu, MS; Shenyi Chen, PhD, Dongbing Gao, MSc.

SMAB Alumni:

Fuhai Li, PhD (Univ Washington St Louis); Lei Huang, PhD (Cincinnati Children's); Guangxu Jin, PhD (Wake Forest), PhD; Kelly Somelar, MSc (Univ. Tartu, Estonia); James Mancuso, PhD (MD Anderson Cancer Center); Xiaobo Zhou, PhD (UT Health); Jared Gilliam, PhD (MD Anderson Cancer Center); Ming Zhan, PhD (NIMH); Zheng Xia, PhD (Oregon Health & Sci U); Joshua Chakranarayan, MD (Baylor College of Medicine); Jing Fan, PhD (Humana); Bo Geng, PhD (Aalborg University, Denmark); Zhuhong You, PhD (Chinese Academy of Sciences); Xiaofeng Xia, PhD (ATGC, Inc, Philadelphia); Xiaoyun Xu, PhD (Lonza Group, AG); Kong Ren, PhD (Jiangsu Polytech, China); Yaping Yu, MD, PhD (Xiangya School of Medicine, China); Min Deng, MD, PhD (Sun Yat-sen University, China); Xuemei Yuan, MSc (Sorbonne U, France)

Collaborators:

- Norbert Perrimon, PhD, Harvard Medical School
- Rudi Tanzi, PhD; Doo Yoon Kim, PhD, Mass General Hospital, Harvard Medical School
- Chris Bakal, PhD, Heba Sailem, PhD, Institute of Cancer Research, London, U.K.
- Ching Lau, MD, PhD, Jackson Lab and Connecticut Children's Medical Center
- Xian-nan Li, MD, PhD, Northwestern University
- Sarah Injah, MD, PhD, Patricia Baxter, MD, Texas Children Hospital, Baylor College of Medicine
- Vivek Mittel, PhD, Weil Cornell Medicine, NY
- Sam Mok, PhD, Sammy Ferri-Borgogno, PhD, Univ of Texas MD Anderson Cancer Center
- Swaminathan Iyer, MD, Jing Yang, PhD, Jin He, PhD, Huan Liu, PhD, U Texas MD Anderson Cancer Center

Funding support:

R01AG057635, R01AG028928, R01AG057635, R01CA121225, R01LM08696, R01ES024165, U54CA149196, U01CA188388, CPRIT RP101334, Tsing Tsung and Wei Fong Chao Foundation, John S Dunn Research Foundation, Cure Alzheimer's Fund, Gillson Longenbaugh Foundation, Huffington Foundation, Johnsson Estate Endowment

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