Toxicant Analysis via High-content Screening of Cellular Circuits
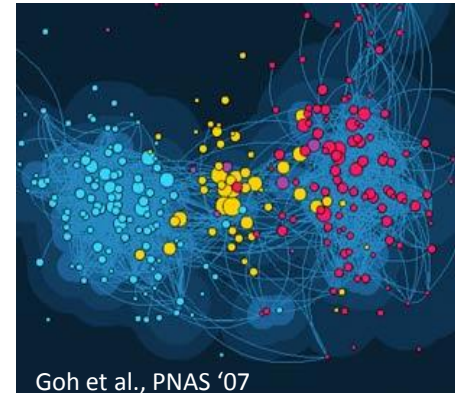
11/18/2010

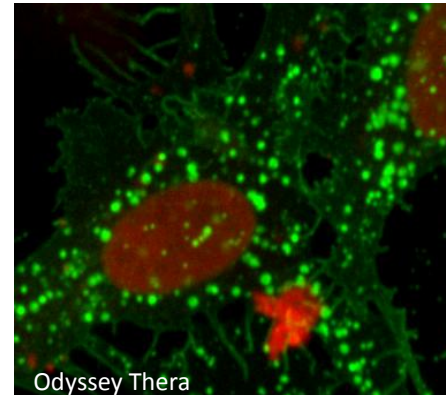Computational Toxicology Communities of Practice
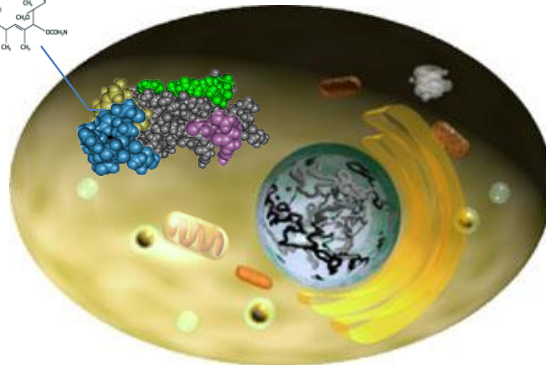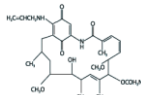
John K. Westwick, Ph.D.

Challenges

Complex Adaptive Systems

Visible Human Explorer (NLM)

Complex molecular and cellular networks drive cell signaling and toxicity
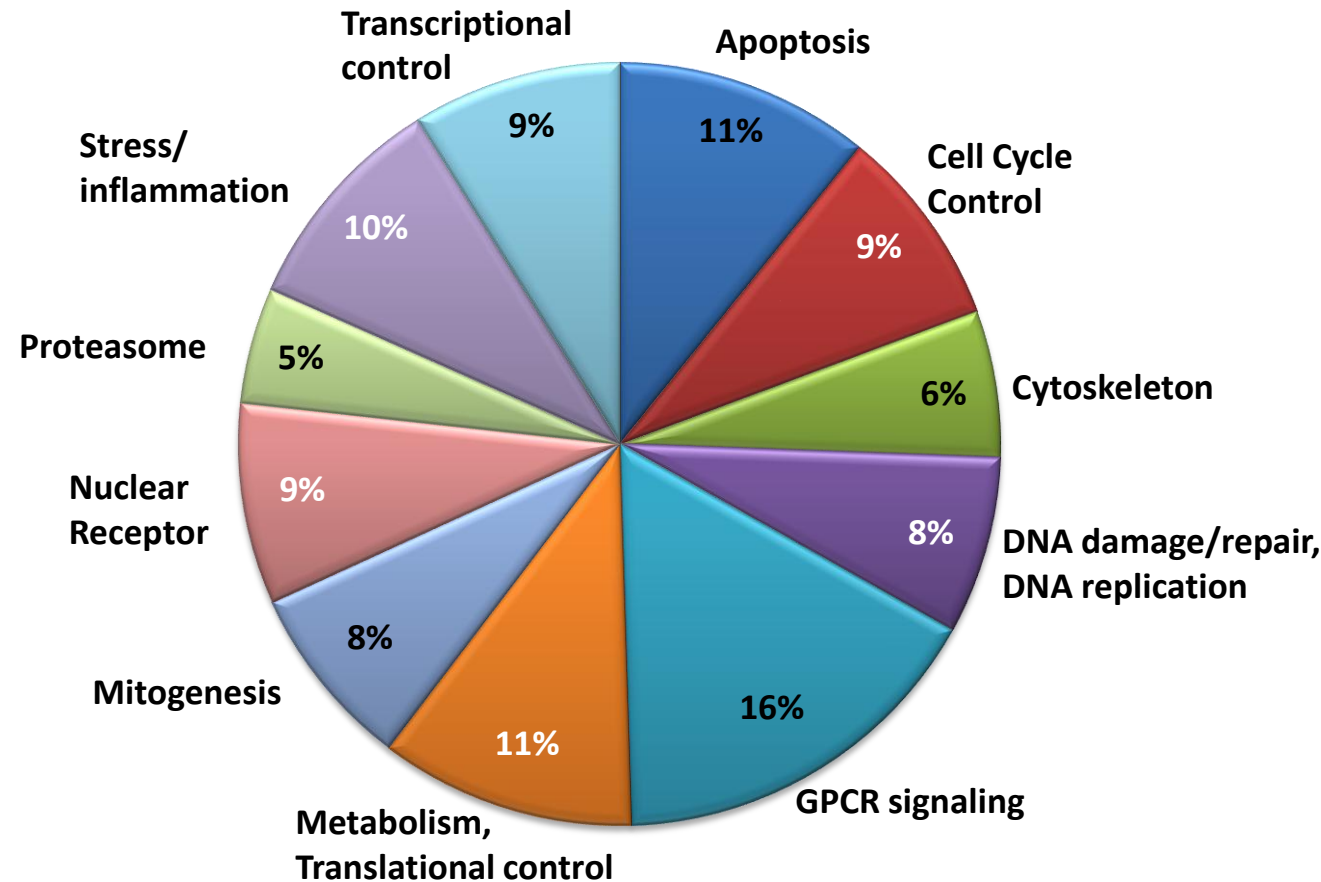

Odyssey Thera

Goh et al., PNAS '07

Large and growing number of agents requiring analysis

Need for **assay diversity, biological context** *and* **throughput** in analytical strategies

Odyssey Thera
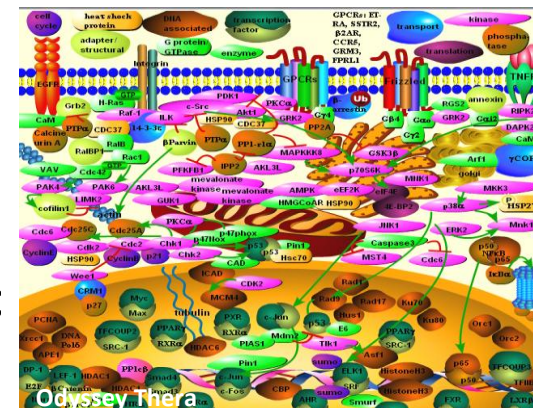
Assay Diversity (performed in living human cells)

Pie chart segments:
- Apoptosis 11%
- Cell Cycle Control 9%
- Cytoskeleton 6%
- DNA damage/repair, DNA replication 8%
- GPCR signaling 16%
- Metabolism, Translational control 11%
- Mitogenesis 8%
- Nuclear Receptor 9%
- Proteasome 5%
- Stress/inflammation 10%
- Transcriptional control 9%

- Odyssey has created and validated the world's largest high-content assay collection

- Capability covers all target classes, including 'un-drugable' targets

- Panel size and breadth enables systems-based profiling

- Major ongoing development program around key target classes and cell types

ODYSSEY THERA

# Biological context addressed with PCA, HCS and other technologies

- Protein-fragment Complementation Assay (PCA) technology
  - A reporter protein is rationally dissected into two fragments which are fused to two proteins that are known to interact within a signaling complex
  - Fusion proteins are expressed in living human cells
  - Assembly of the reporter protein from its fragments can only happen if the test proteins exist in a complex
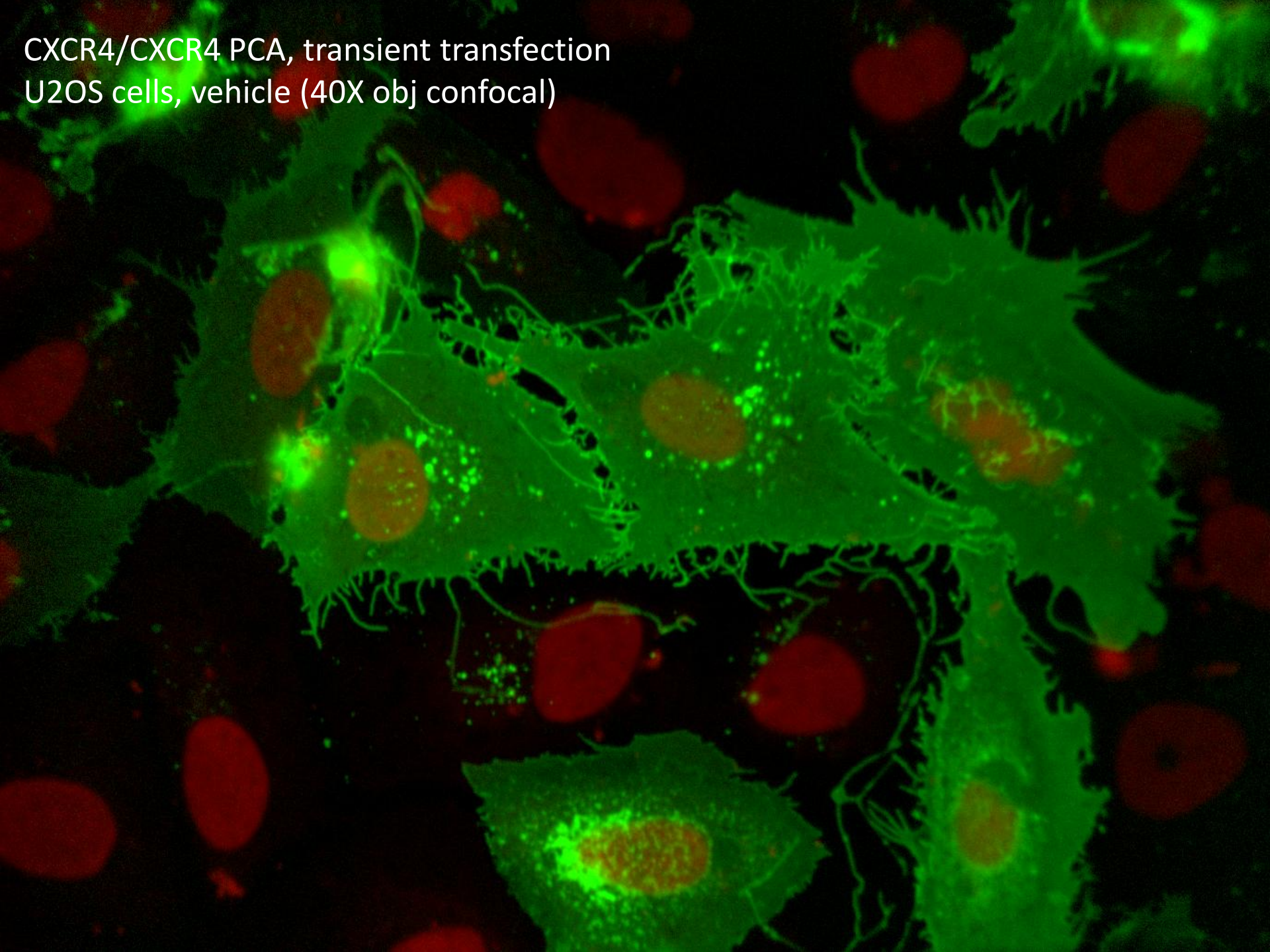 - Test agent activity is measured via changes in signal intensity and location (e.g. fluorescence)
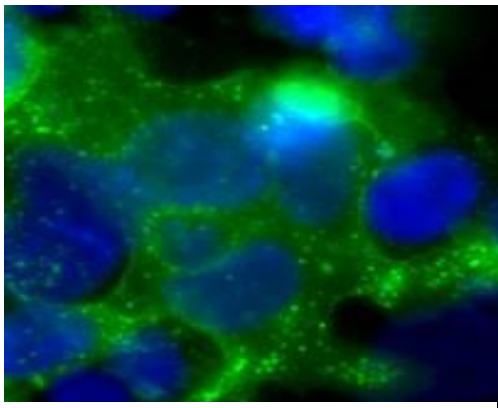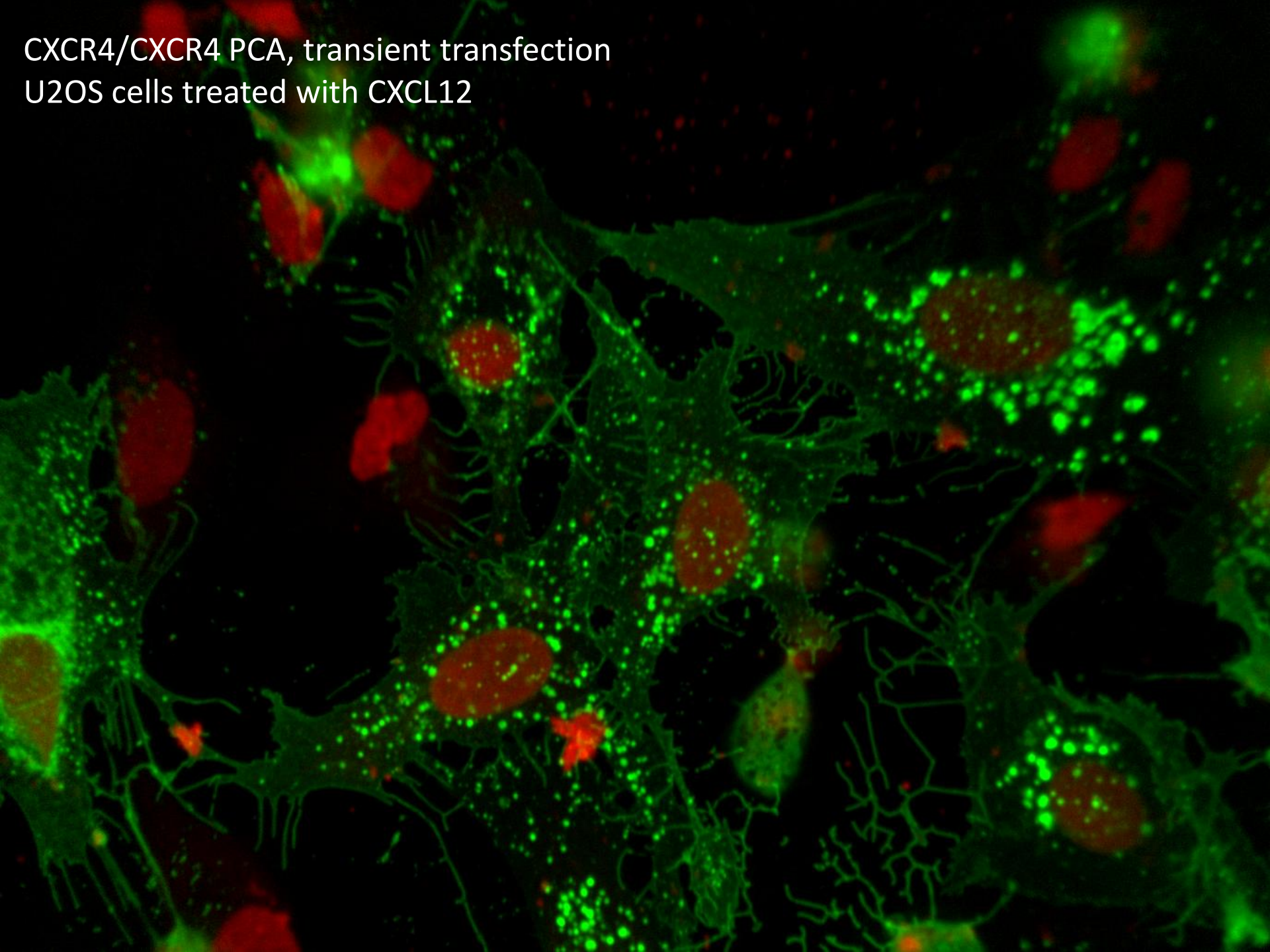

Frizzled4/GRK
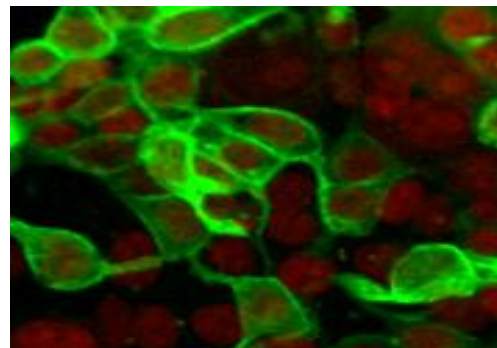
A_{2A}/D_{2L}GPCR heterodimer

ODYSSEY THERA
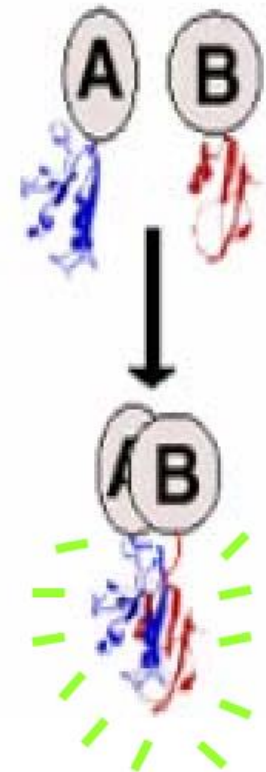
CXCR4/CXCR4 PCA, transient transfection
U2OS cells, vehicle (40X obj confocal)

CXCR4/CXCR4 PCA, transient transfection
U2OS cells treated with CXCL12

Cellular assays proposed for ToxCast (examples)

- GPCR, developmental paths
  - Frizzled PCA (Wnt pathway), mGLuR3 PCA, Smo (smoothened) PCA

- Kinases, phosphatases, GTPases, GEFs, metabolic, nucleases/proteases, structural, proteasome
 - TGFβR1 PCA, p38α/Mnk1, Vav/Cdc42

- Apoptosis, Damage, transport, hypoxia, immunomodulation, 2nd messenger signaling
  - p53/Pin1, Rad51 PCA, ARF/γCOP, BAD/BclxL, Hif1α PCA

- Nuclear receptors and other transcription and translation regulators
  - HDAC PCA, CAR PCA, ERRγ PCA, HCA of active ER units, myc/max

- Protein level and post-translational modification
  - GRP78 IF

- PCAs + metabolic activation
  - Androgen receptor PCA (+ compound metabolism)

- Functional Analyses of Stress and Toxicity
  - Human ESC-derived motorneurons (Wst-8); ESC-derived hu hepatocytes (multiTox)

NB: >90% of the assays involve high content screening and automated image analysis

ODYSSEY THERA

# Development Program: LXRα Homodimer PCA (transient probe transfection)
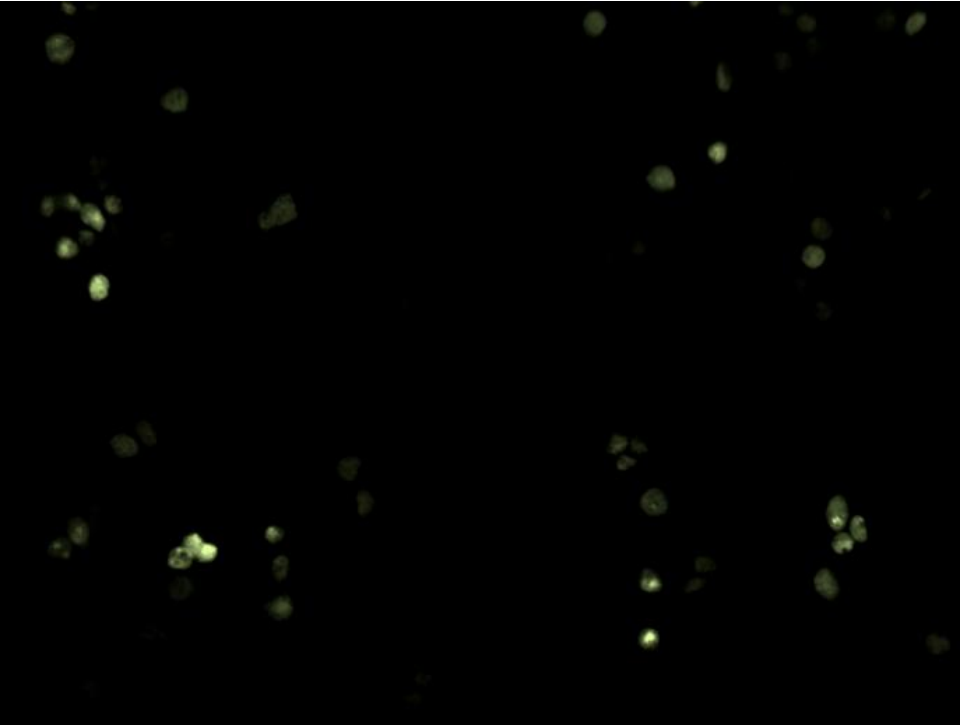
Vehicle-treated cells show weak nuclear staining
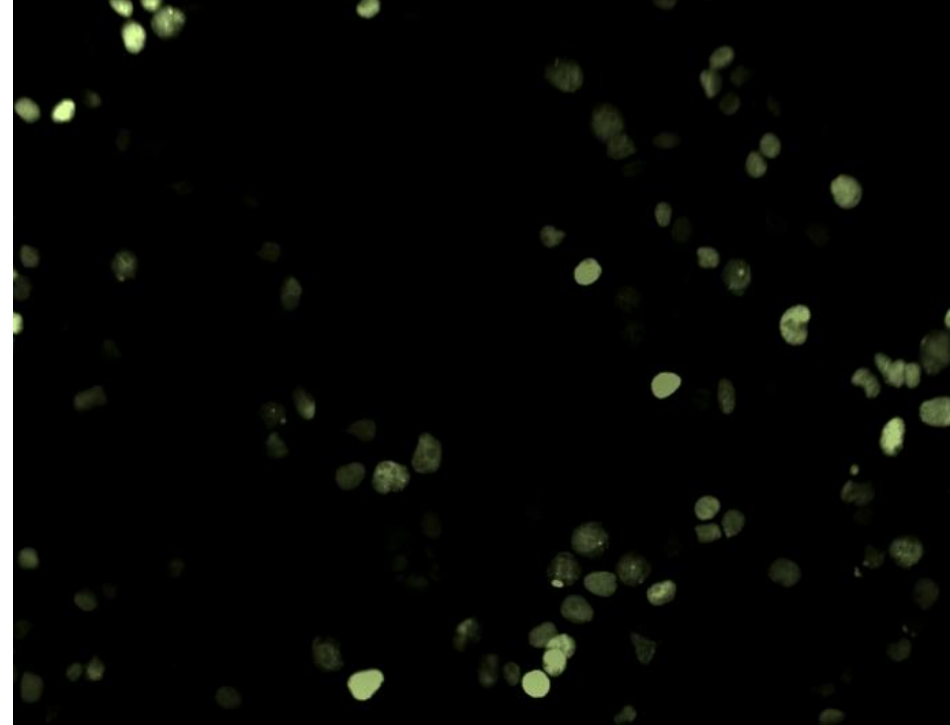
Agonist-stimulated cells show strong nuclear staining (T0901317)

- Dose-responsive induction seen with known agonist (TO compound)
- Status: results with transient led to decision to develop stable line (in progress)

ODYSSEY THERA

Development Program: Androgen Receptor/SRC-1 PCA (transient probe transfection)
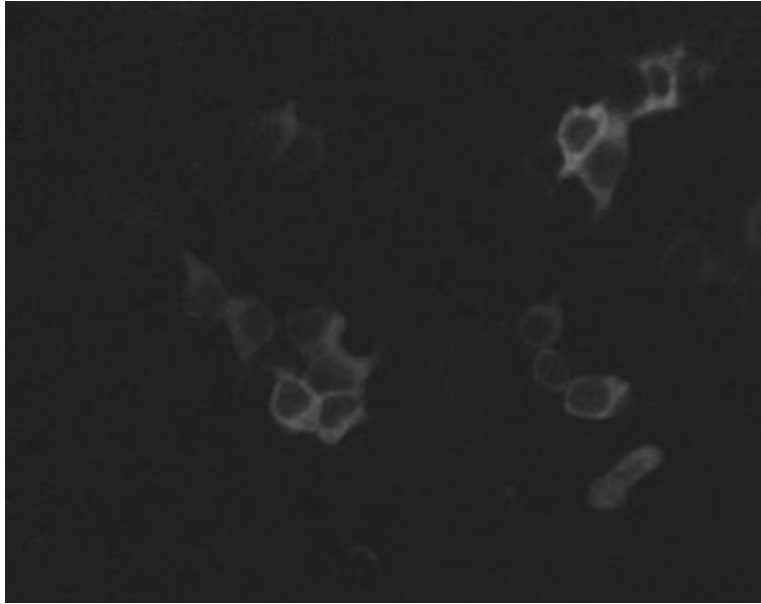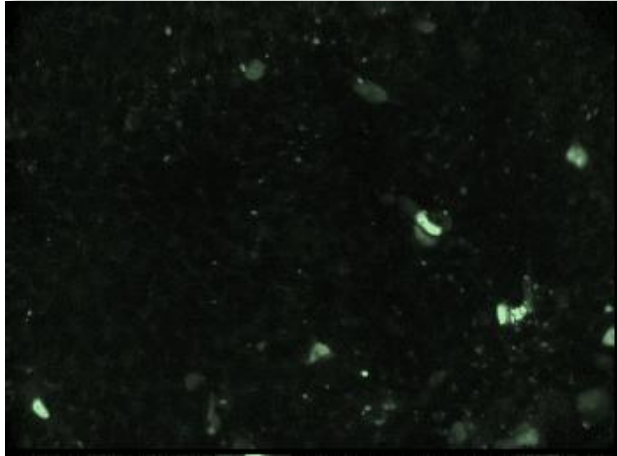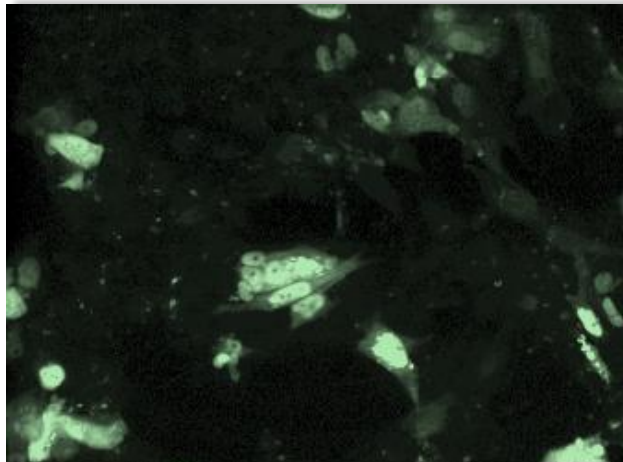

Vehicle-treated cells show diffuse cytoplasmic staining
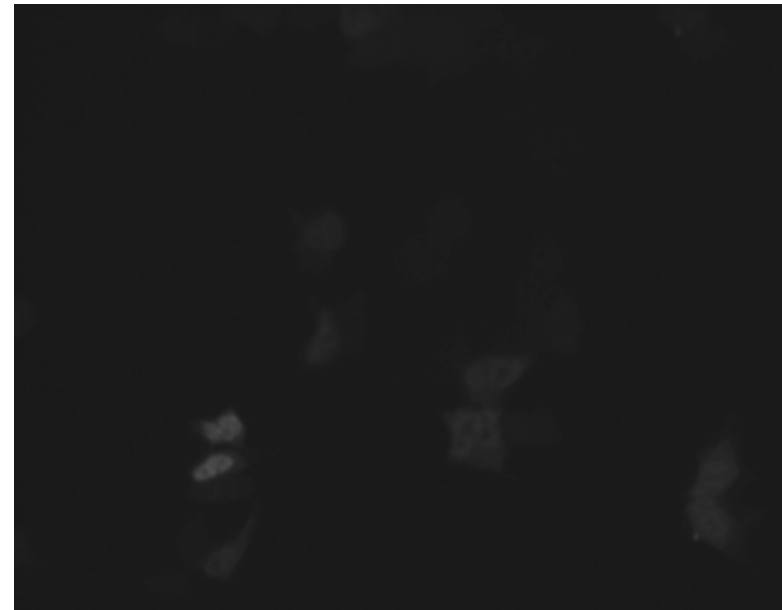
Ligand-stimulated cells (4-HT) demonstrate nuclear
staining and cytoplasmic speckling

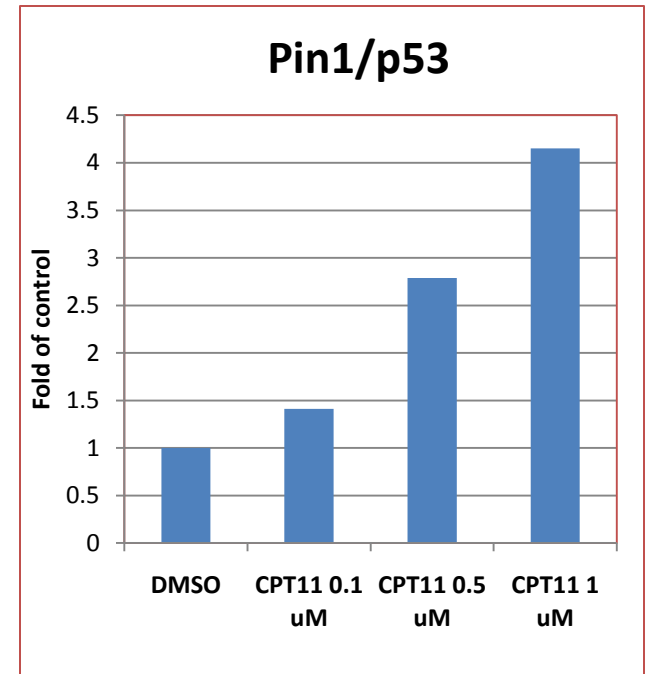status: selecting stable cell lines

ODYSSEY
THERA

Development Program: Dose responsive PCA in human stem cells

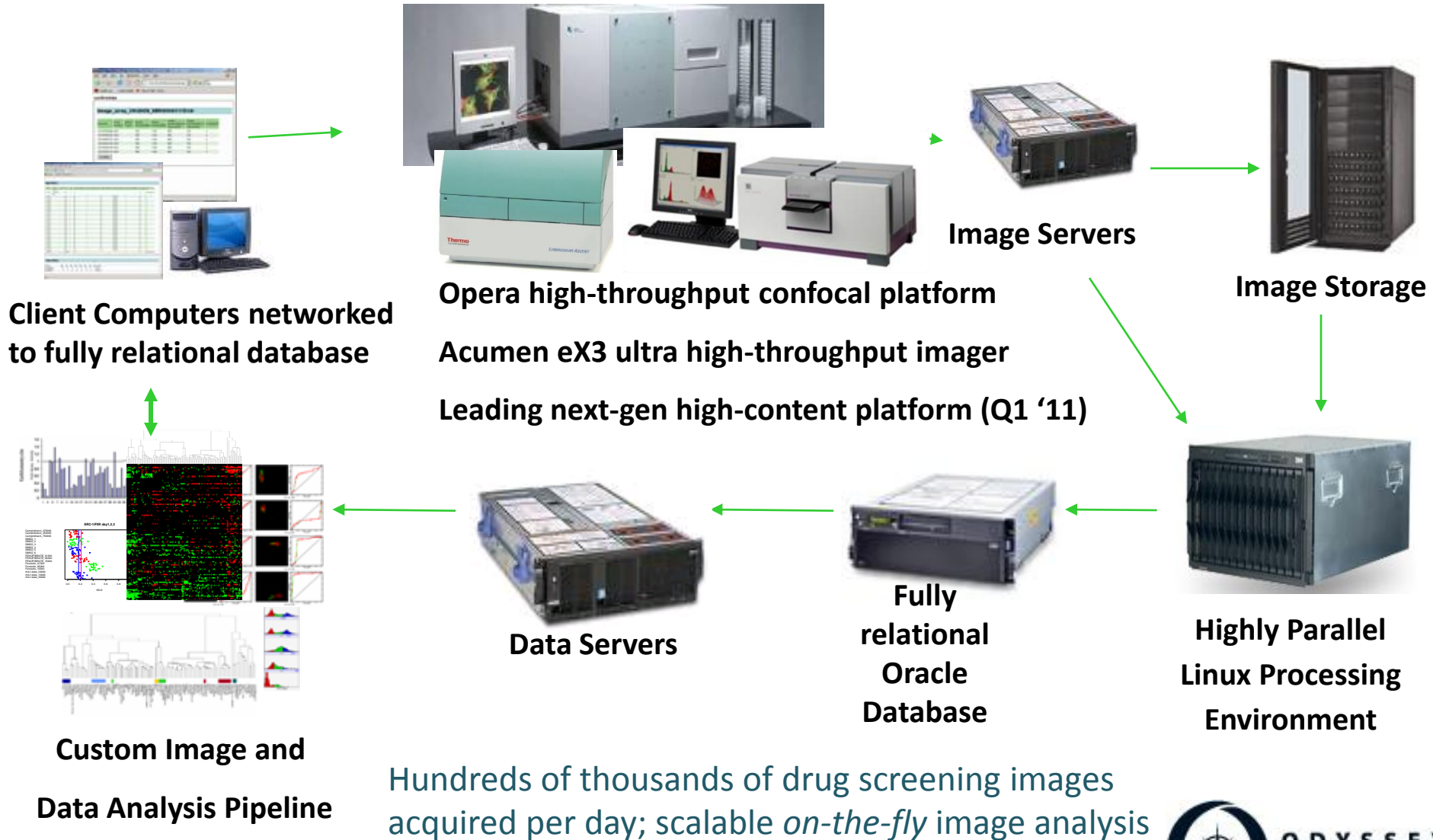Pin1/p53 transient transfection (vehicle)

Pin1/p53
topoisomerase inhibitor

Dose-response from high-throughput, high-content screening

Throughput Requirements Addressed by Integrated Technology Platform and LIMS

Image Servers

Image Storage

Client Computers networked to fully relational database

Opera high-throughput confocal platform

Acumen eX3 ultra high-throughput imager

Leading next-gen high-content platform (Q1 '11)

Data Servers

Fully relational Oracle Database

Highly Parallel Linux Processing Environment

Custom Image and Data Analysis Pipeline

Hundreds of thousands of drug screening images acquired per day; scalable *on-the-fly* image analysis

ODYSSEY THERA

Automated image analysis quantifies pixels from defined sub-cellular compartments @ 2 or 3 wavelengths
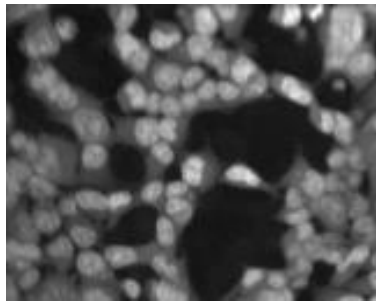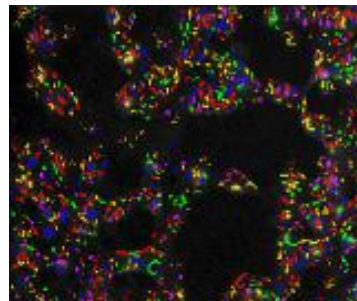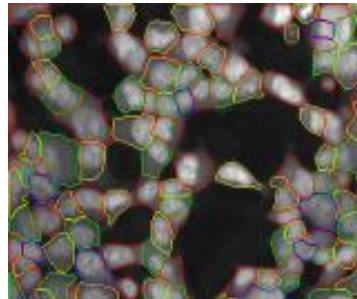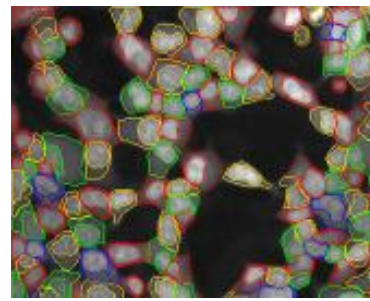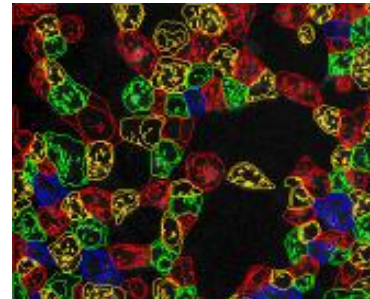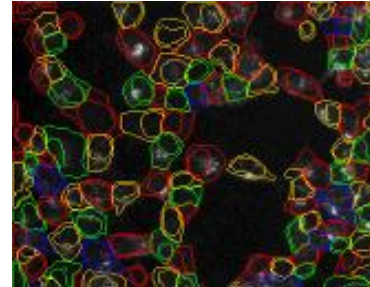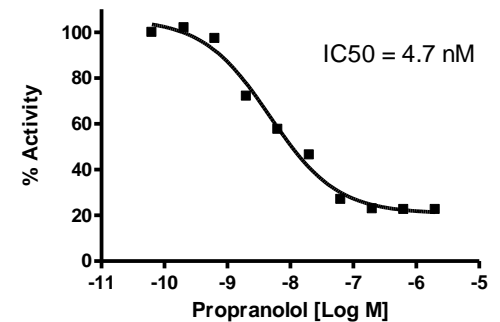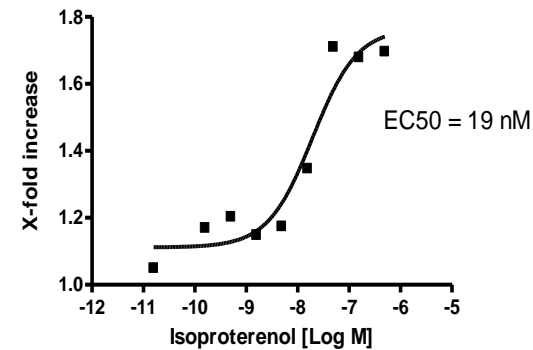
Quantitation of complex biology
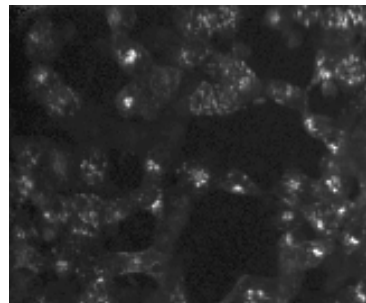
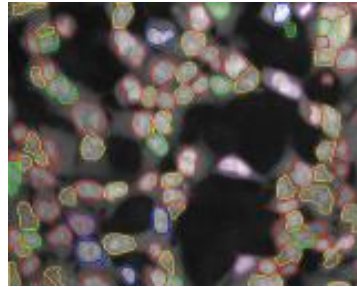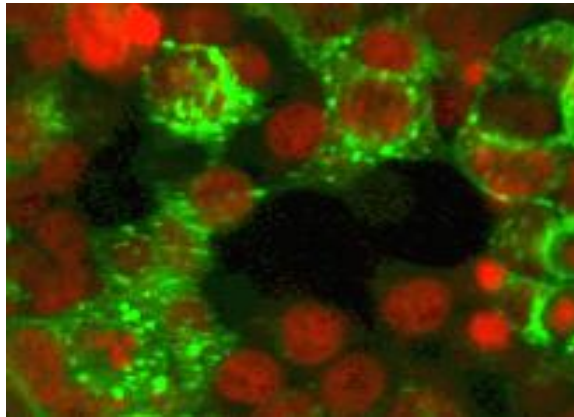

vehicle
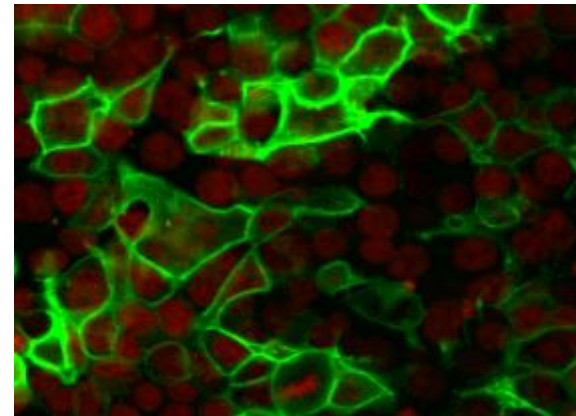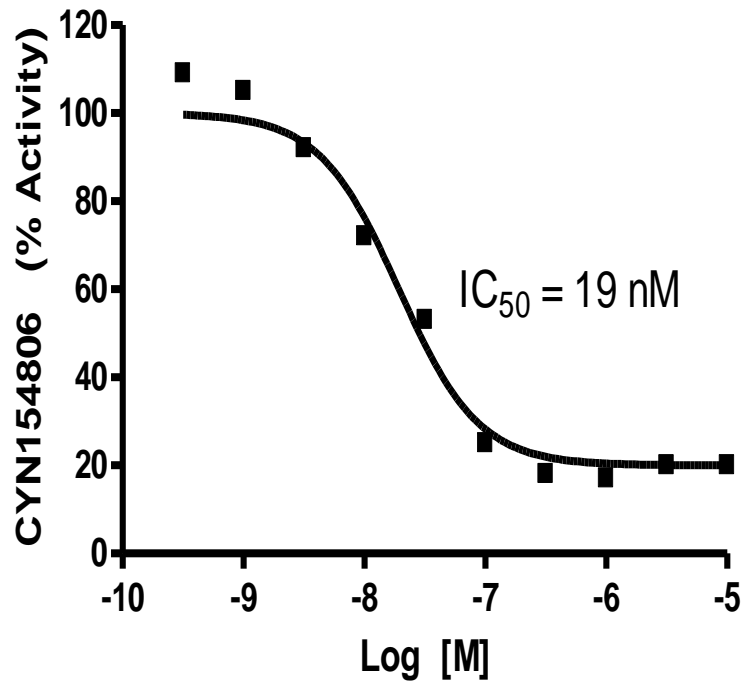
CYN 154806 (10μM)

IC_{50} = 19 nM

Image-based QC

- nucSize filter
  - Cell type / assay dependent
  - Both upper and lower size limits are defined per cell type

- Nuclear stain intensity filter
  - Stain, cell type and assay dependent
  - Upper and lower intensity limits are defined
  - Cells that don't meet criteria are filtered out

- Cell count filter
  - Max and min values defined for each cell type
  - Images that don't meet criteria are filtered out

O D Y S S E Y
T H E R A

Plate-Based Assay QC for High Content Data

Confocal images
(for each assay plate)

β2AR/βARR2 +isoproterenol (90' drug treatment)

Vehicle control image   Reference compound image

Mean fluorescence intensity and standard deviation calculated for reference compound and vehicle control(s)
(Based on 8 wells for reference compound & vehicle controls)

Z' for reference compound meets assay specific criteria

Z' for unstimulated controls for stimulated assays meets assay specific criteria

Well-to Well CV in mean intensity for vehicle controls and min mean intensity (if applicable) meet criteria established for assay

Min cell # and Well-to-well CV in cell number meet criteria established for assay

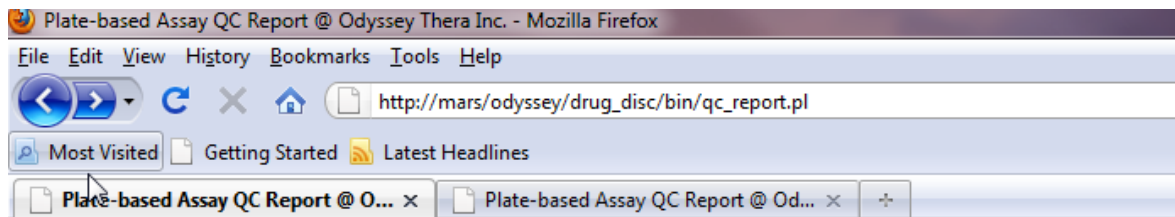Core QC criteria

If an assay plate meets criteria, the plate PASSES

If an assay plate fails one or more criteria, the plate is FAILED and repeated

ODYSSEY THERA

QC Team reviews quantitative results via custom web interfaces

Flow Chart of SOP's Governing Performance of OT Cell-based Assays

Compounds (Samples)

Compound Receipt and Registration	*SOP-XL-001, -003*
Compound (CMP) and Daughter Plate Creation	*SOP-XL-007*

Instrument QC

MultiDrop Cassette Calibration and QC	SOP-IQC-003	Opera Calibration& QC	SOP-IQC-005
Biomek F/X Multichannel Dispenser Calibration	SOP-IQC-004	Acumen Operation	*SOP-IQC-007*

Cell-based Assays

Stable PCA Assays (stimulated with agonist)	SOP-CCM-005	SOP-CP-012	SOP-DRG-004	SOP-STM-002	SOP-FIX-002, or -004	SOP-IQC-005 or -007
Stable PCA Assays (no agonist)	SOP-CCM-005	SOP-CP-012	SOP-DRG-004	SOP-FIX-002 or -004	SOP-IQC-005 or -007	
Transient PCA and artifact detection assays (no agonist)	SOP-CCM-005	SOP-CP-012	SOP-DNA QC-002	SOP-TF-009	SOP-DRG-004	SOP-FIX-002 or -003	SOP-IQC-005 or -007
Immuno-fluorescence assays	SOP-CCM-005	SOP-CP-012	SOP-DRG-004	SOP-IF-028, -029, or -031	SOP-IQC-005 or -007	
LDH assays	SOP-CCM-005	SOP-CP-008 (HEK) or -011 (hepatocyte)	SOP-DRG-004	SOP-CV-009 (HEK) or -010 (hepatocyte)		

Assay/Data QC

Data Analysis	Data flow for compound profiling
Data QC	QC of High Content Assay data

ODYSSEY THERA

Every compound (and target) generates a unique signature

Mdm2/p53

Vehicle

Bortezomib
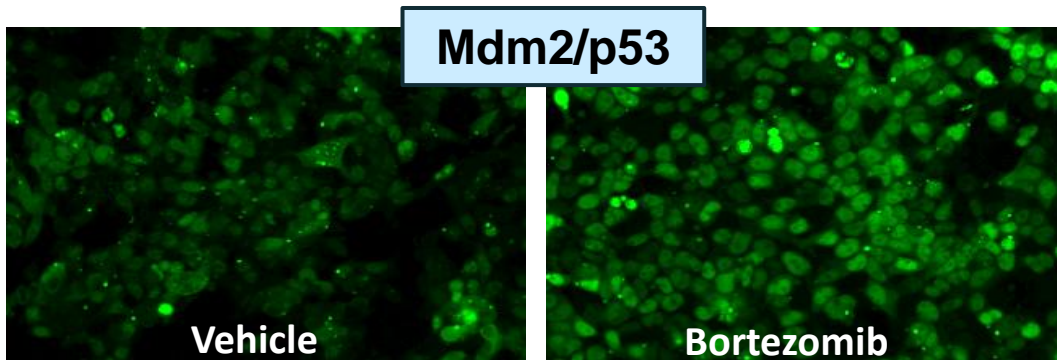
Quantitative image analysis
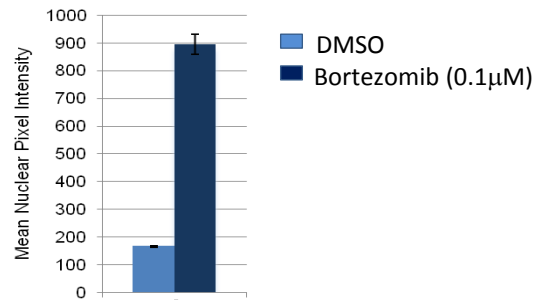
Compound profile: activity across assay panel

Mean Nuclear Pixel Intensity

- DMSO
- Bortezomib (0.1μM)

Bortezomib (0.1μM)

fold change relative to vehicle control
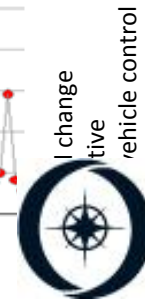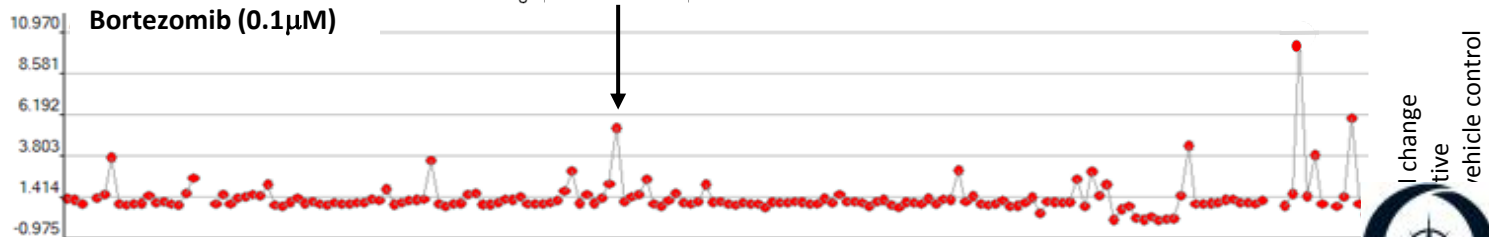
O D Y S S E Y
T H E R A

Odyssey internal efforts: Database of drugs, toxicants and targets

- Thousands of drugs, toxicants, targets (si/miRNA) represented in this evolving resource
- New test agents are compared to database -> mechanistic and safety information
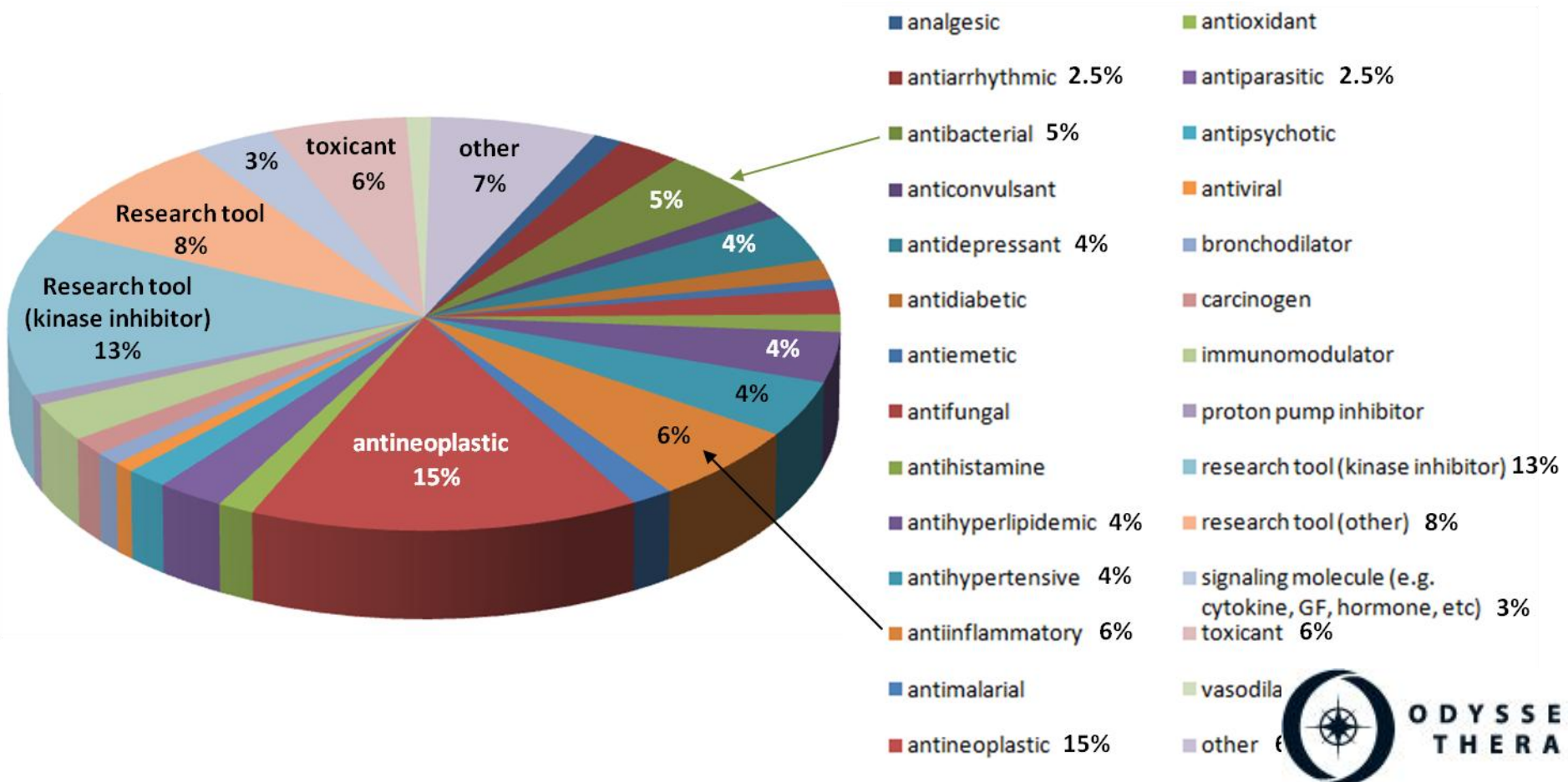
The final frontier (or, full circle?)
Visualizing/confirming toxicant activity *in vivo* with PCA
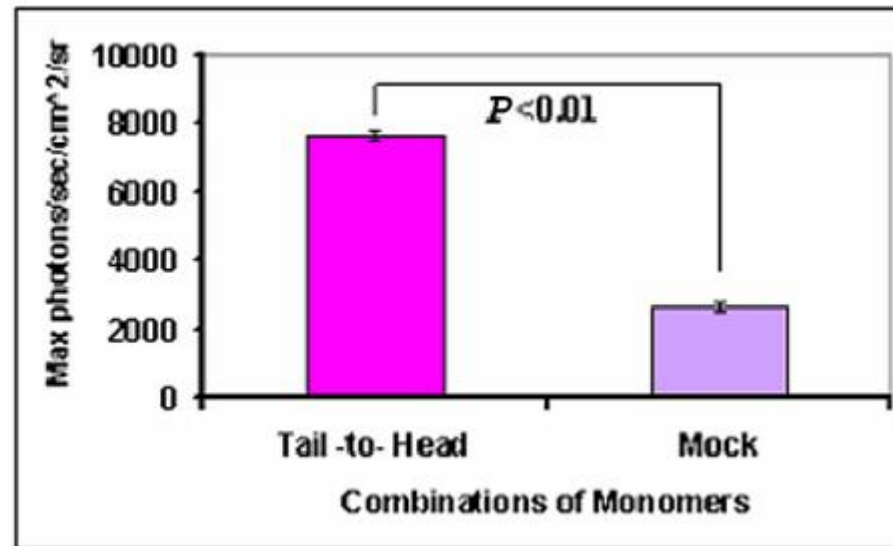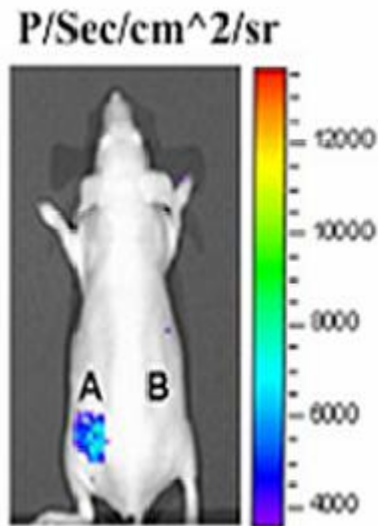
Massoud *et al*. 2004

Toxicant Analysis via High-content Screening of Molecular Circuits
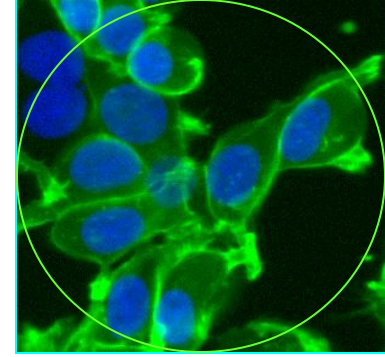
- We believe that the combination of highly contextual assays + HT/HC analysis + diverse parallel screens will contribute to toxicant identifiers and classifiers

- We've engaged internally in a process similar to that underway with ToxCast computational biologists, namely looking for patterns or "signatures" of activity that represent a particular biological phenotype

- (NB: the majority of our time has been devoted to engineering the capability and not mining the data)

- We look forward to continued partnership with EPA scientists to better define the functional relevance of particular patterns of activity

ODYSSEY
THERA

Thanks

ODYSSEY THERA

Biology and Screening:
Jane Lamerdin
Jennifer Dias
Jon Melnick
Graham Bilter
Eddie Wehri
Shayla Hart
Veronica Alves
Helen Chan
Tomoe Minami

Bioinformatics:
Zhengping Huang
Cedric Wiesner
Jie Hu

EPA
David Dix
Keith Houk
Jennifer Hill
David Reif

John Westwick   jwestwick@odysseythera.com   (925) 242-5011
Jane Lamerdin   jlamerdin@odysseythera.com   (925) 242-5007

References

- Yu, H., West, M., Keon, B.H., Bilter, G.K., Owens, S., Lamerdin, J., Westwick, J.K. Measuring drug action in the cellular context using protein-fragment complementation assays, Assay and Drug Discovery Technologies, 6: 811-822, (2004).
- Westwick, J.K. and Lamerdin, J.L. Improving Drug Discovery with Contextual Assays and Cellular Systems Analysis. In: Signal Transduction (Methods in Molecular Biology, Louis Luttrell and S. Ferguson, Ed.), in press (2010).
- Macdonald, M.L., Lamerdin, J., Owens, S., Keon, B.H., Bilter, G.K., Shang, Z., Huang, Z., Yu, H., Dias, J., Minami, T., Michnick, S.W., and Westwick, J.K. Identifying Off-Target Effects and Hidden Phenotypes of Drugs in Human Cells. Nature Chemical Biology, 2(6): 329-337, (2006).
- Murray, B.W., Guo, C., Piraino, J., Westwick, J.K., Lamerdin J., Dagostino E., Knighton D., Zhang, C., Loi, C-M., Zager, M., Kraynov, E., Bouzida, D., Martinez, R., Karlicek, S., Bergqvist, S., Kephardt, S., Marakovits, J., Zhang, J., Smeal, T. Small-molecule p21-activated kinase-4 inhibitor PF-3758309 is a potent inhibitor of oncogenic signaling and tumor growth. Proceedings of the National Academy of Science; 107(20): 9446-51, (2010).
- Michnick SW, Ear PH, Manderson EN, Remy I, Stefan E. Universal strategies in research and drug discovery based on protein-fragment complementation assays. Nat Rev Drug Discov. (7):569-82 (2007).
- Remy I, Montmarquette A, Michnick SW. PKB/Akt modulates TGF-beta signaling through a direct interaction with Smad3. Nat Cell Biol. 6(4):358-65 (2004).
- MacDonald, M.L. and Westwick, J.K. Exploiting Network Biology to Improve Drug Discovery. In: High Content Screening (Methods in Molecular Biology, volume 356: 221-232; Lansing Taylor, Ed.), (2007).
- Westwick, J.K. and Michnick, S.W., Use of Protein-fragment Complementation Assays (PCA) in small GTPase research and drug discovery. Methods in Enzymology, (C. Der, Ed.) 407:388-401, (2005).
- Ding, Z., Liang, J., Li, J., Lu, Y., Ariyarantna, V., Davies, M.A., Westwick, J.K., and Mills, G.B. Physical Association of PDK1 and AKT1 is sufficient for pathway activation independent of membrane localization and PI3-kinase. PLoS, in press (2010).
- Michnick, S.W., Macdonald, M.L., and Westwick, J.K. Chemical genetic strategies to delineate MAP kinase signaling pathways using protein-fragment complementation assays (PCA). Methods, 40(3):287-293, (2006).
- Auld, D.S., Johnson, R.L., Zhang, Y., Veith, H., Jadhav, A., Yasgar, A., Simenov, A., Zheng, W., Martinez, L., Westwick, J.K., Austin, C.P., and Inglese, J. GFP-based cellular assays analyzed by laser scanning microplate cytometry in 1536-well plate format. Methods in Enzymology (J. Inglese, Ed.), 414: 566-589, (2006).

ODYSSEY
THERA