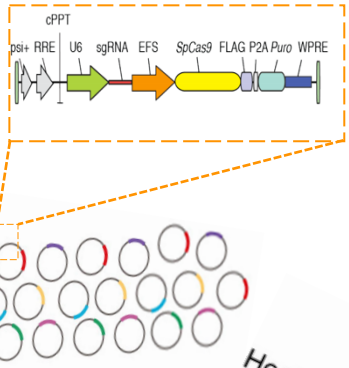
Application of CRISPR genome-editing tools in the evaluation of chemical hazards



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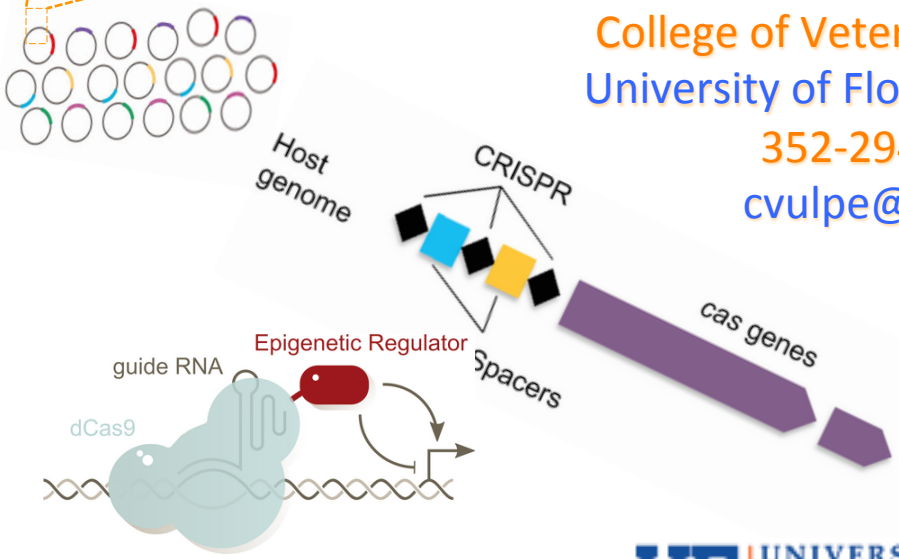
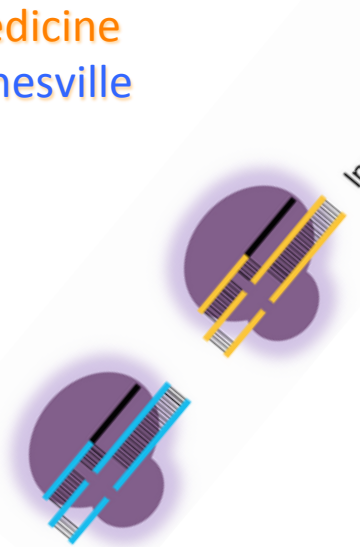
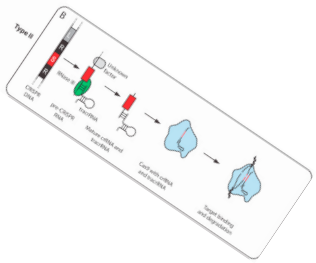




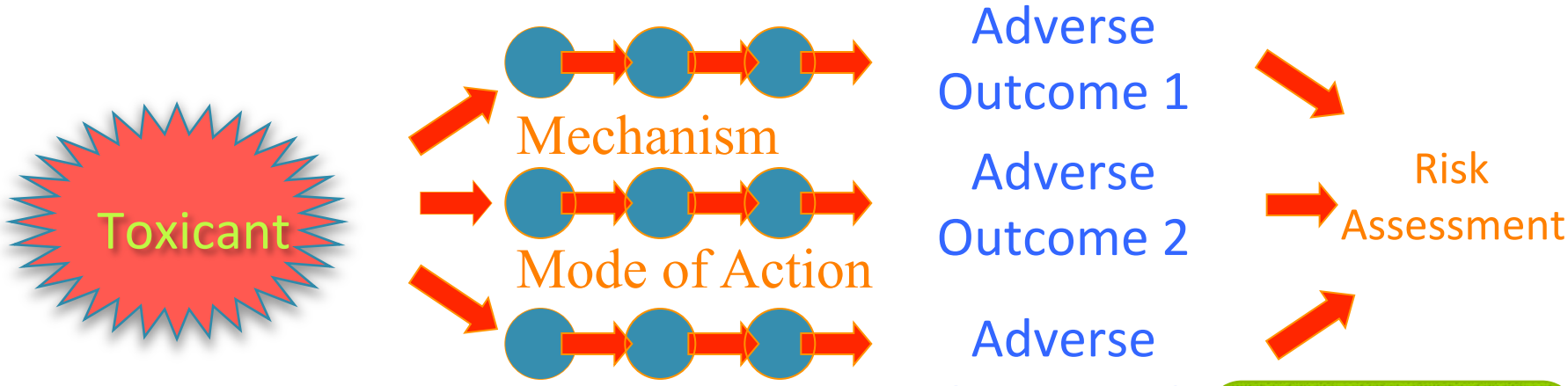


UF UNIVERSITY of FLORIDA

Superfund Research Program

Toxicity

Old School



Adverse outcome pathways

Reformed School

Functional Approaches in Toxicology

Mutate gene

Assess function in cell or organism

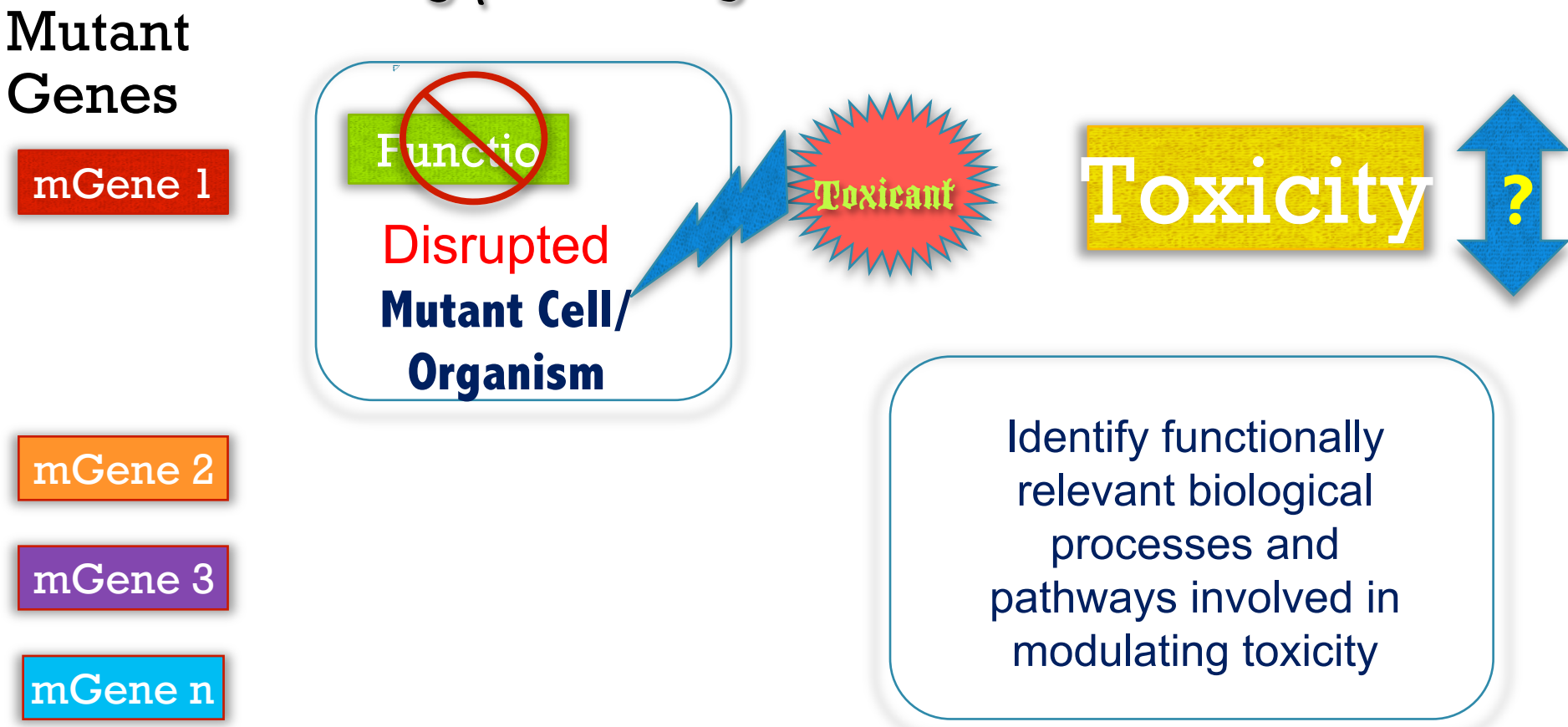
As related to role, if any, in toxicity

the study of the requirement for the biological activities of a gene and corresponding protein in the response to, and effect on, an organism by a toxicant

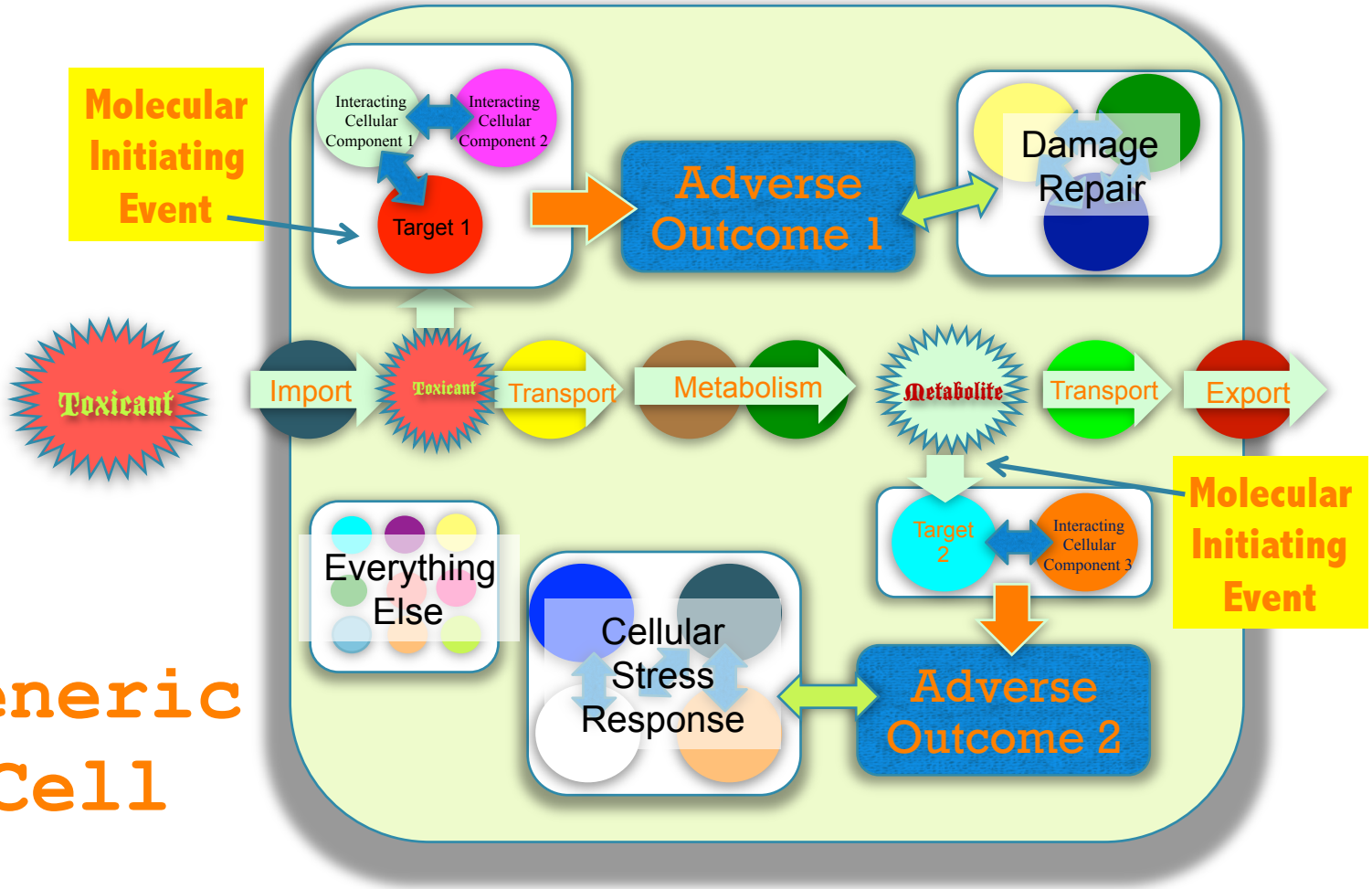
OR if you muck it up (the gene) & bad (or good) things happen, then it's probably important

Functional Profiling in Toxicology

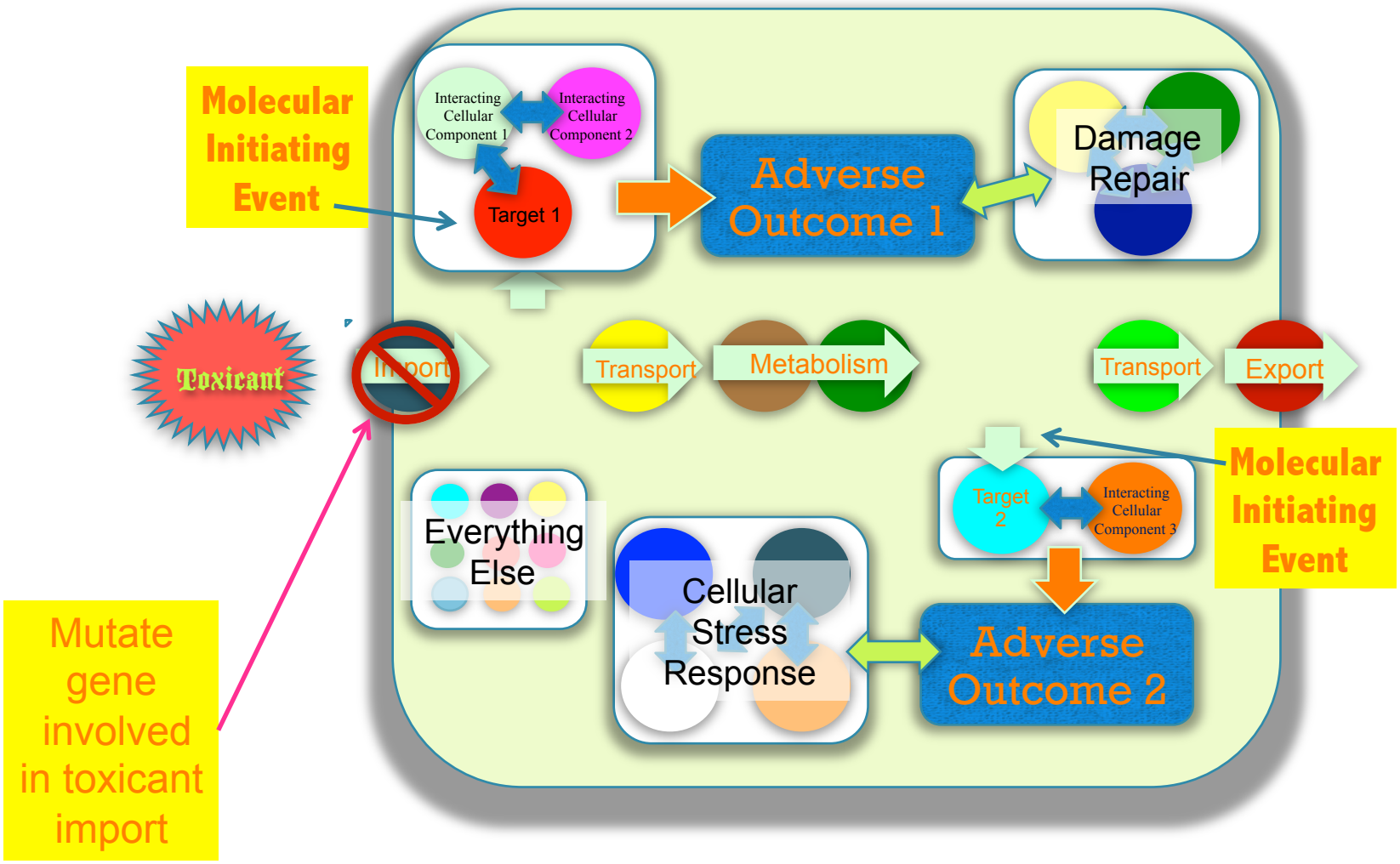
Systematically testing multiple (all) genes for their functional role, if any, in toxicity, by perturbing their function



Functional Toxicology to reveal Cellular Adverse Outcome Pathways

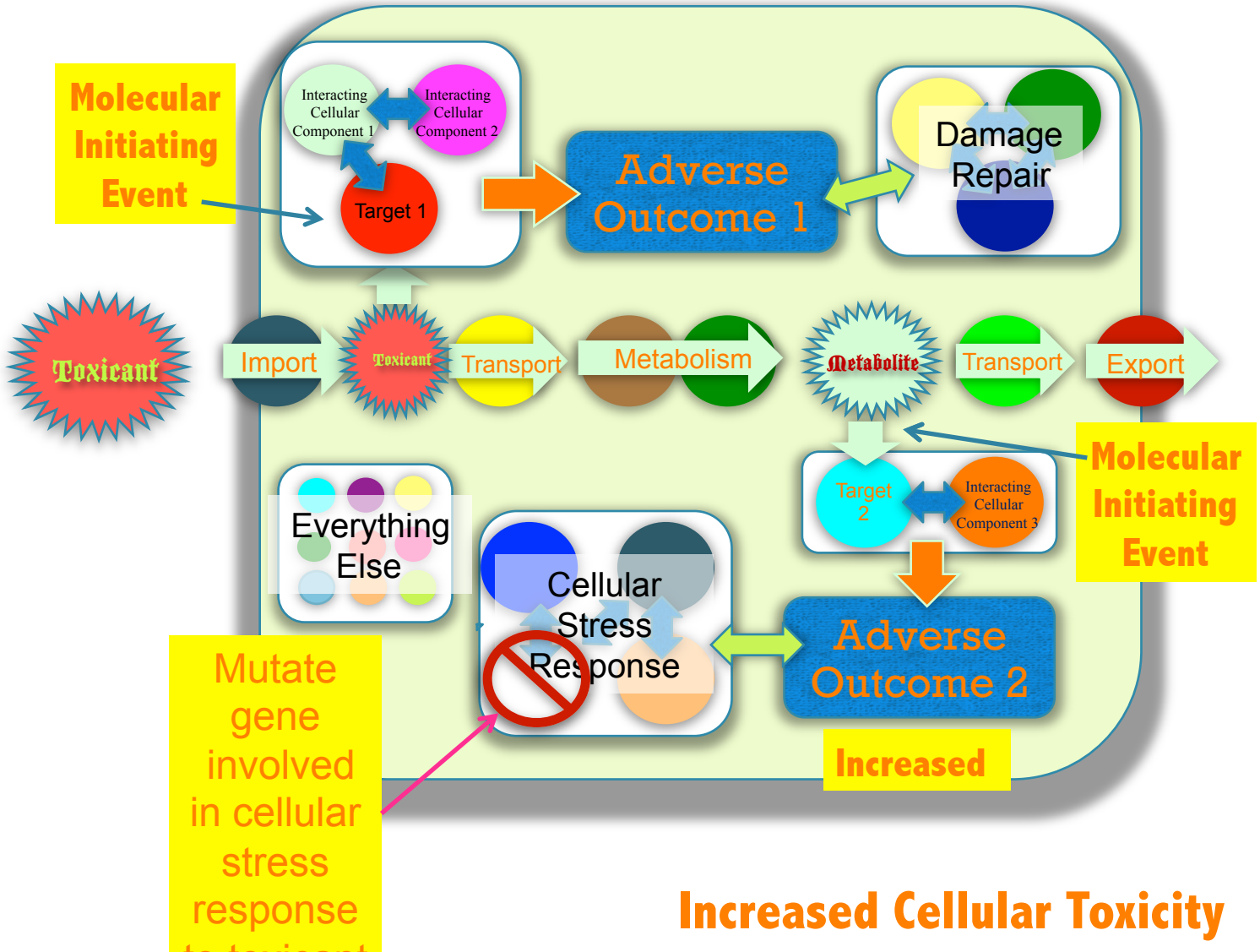


Functional Toxicology to reveal Cellular Adverse Outcome Pathways



Decreased Cellular Toxicity

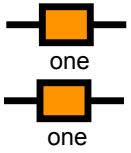
Functional Toxicology to reveal Cellular Adverse Outcome Pathways



CRISPR approaches to assess function of genes

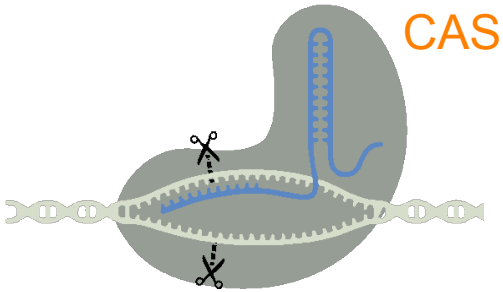
Targeted CRISPR vs Genome Wide CRISPR

Gene of Interest



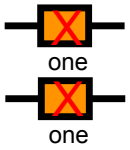
Targeting sgRNA to gene of interest

CAS9



Introduce into cell/organism

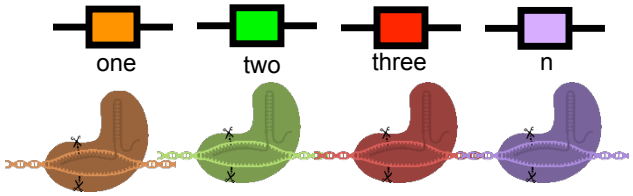
Screen for KO



Isolate KO cell/organism

Assess Function in cell/organism

Multiple genes of interest

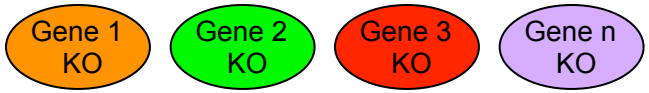


Targeting sgRNA to genes of interest

(CRISPR KO library)

Introduce into cell

One per cell



Generate pool of mutants

Screen for sensitivity to toxicant to identify and RANK the important genes

How do you do Genome Wide CRISPR Screening actually?

Use webtools to design sgRNA and make your own library e.g. http:// crispr.mit.edu/

Or purchase and amplify premade library from Addgene https://www.addgene.org/crispr/ https://www.addgene.org/pooled-library/

Wide Variety of Libraries available Human Mouse Drosophila

Design sgRNA

Pooled sgRNA oligonucleotide synthesis

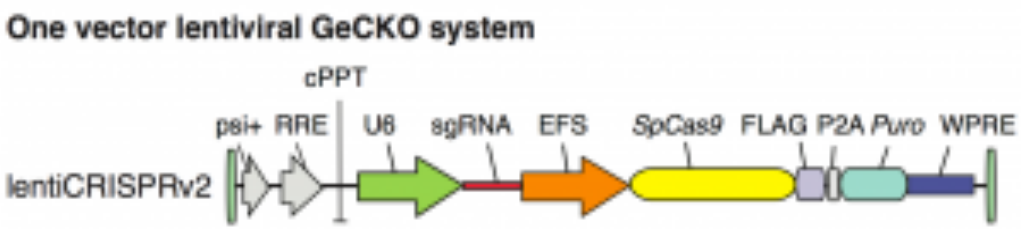
Gibson Cloning

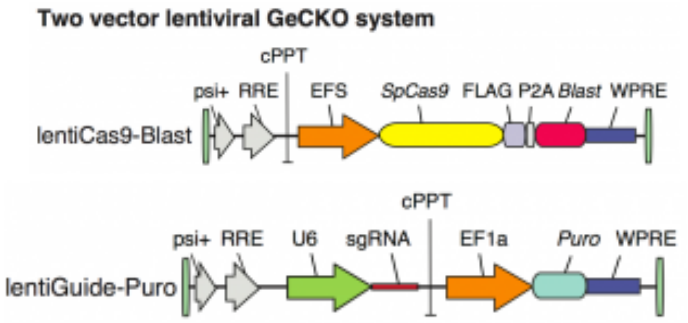
Clone into lentivirus vector

Pooled plasmid library

Lentivirus packaging

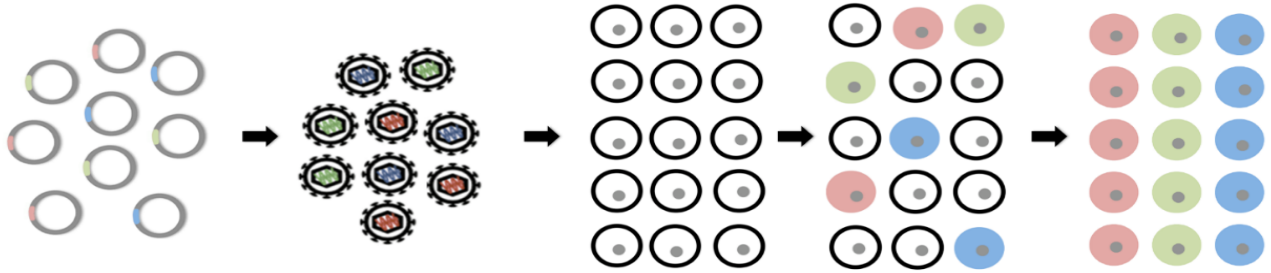
Infect Cells





http://genome-engineering.org/gecko/?page_id=34

Now what? – Make a mutant KO library



Pooled LentiCRISPR sgRNA Library

Package to make Lentiviral Pool

Low MOI Transduction

0.3-0.5 MOI

<1 virus per cell

Antibiotic Selection For integrated virus

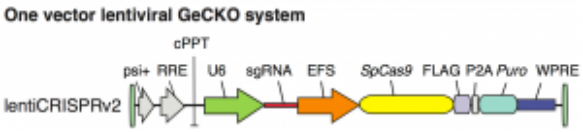
Each cell contains **1** sgRNA + Cas9

Library of Mutants

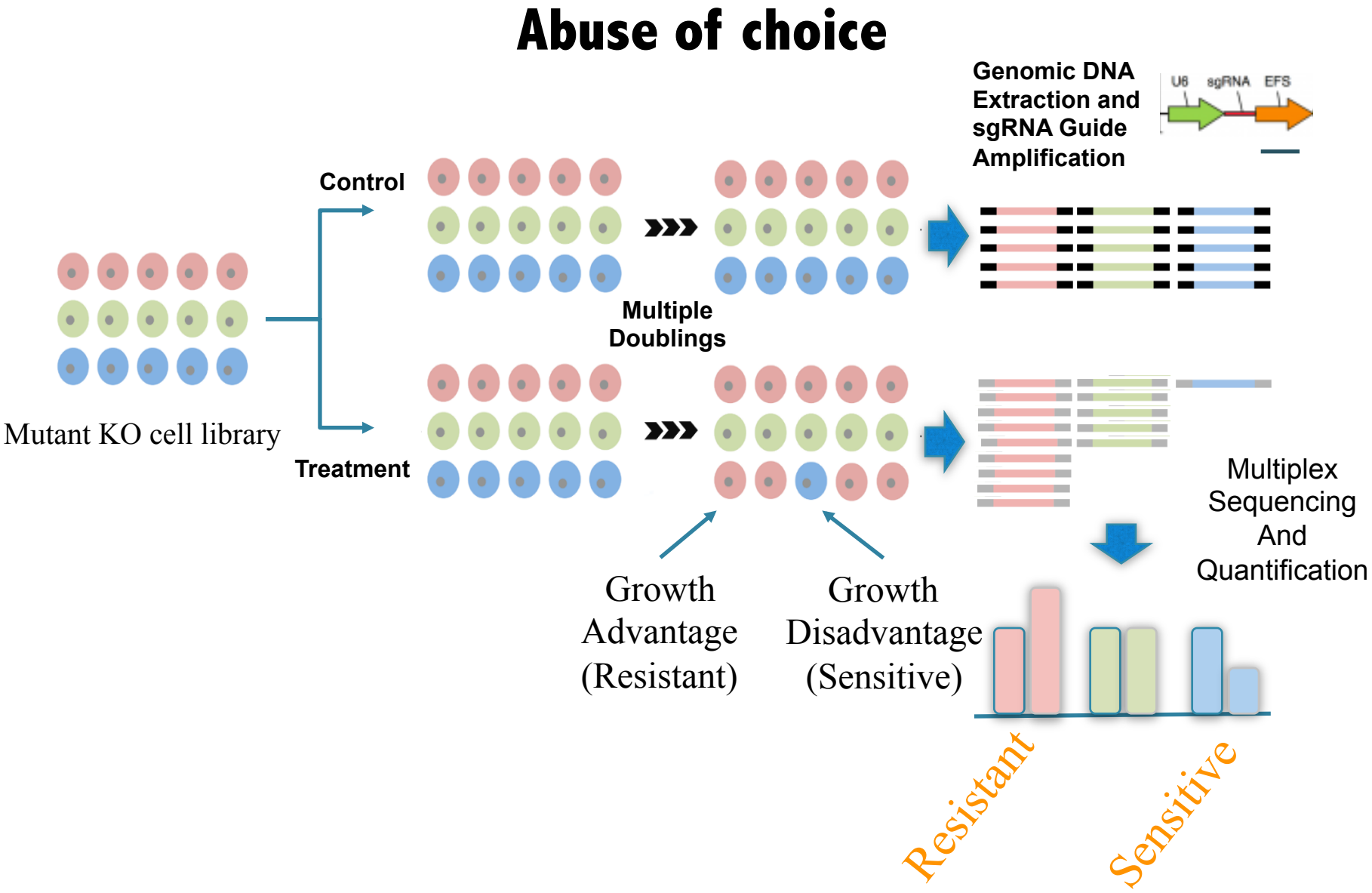
sgRNA targeted KO in each cell of one gene

Integrated sgRNA acts as Barcode for KO – uniquely identifies cell containing corresponding KO

sgRNA Barcode



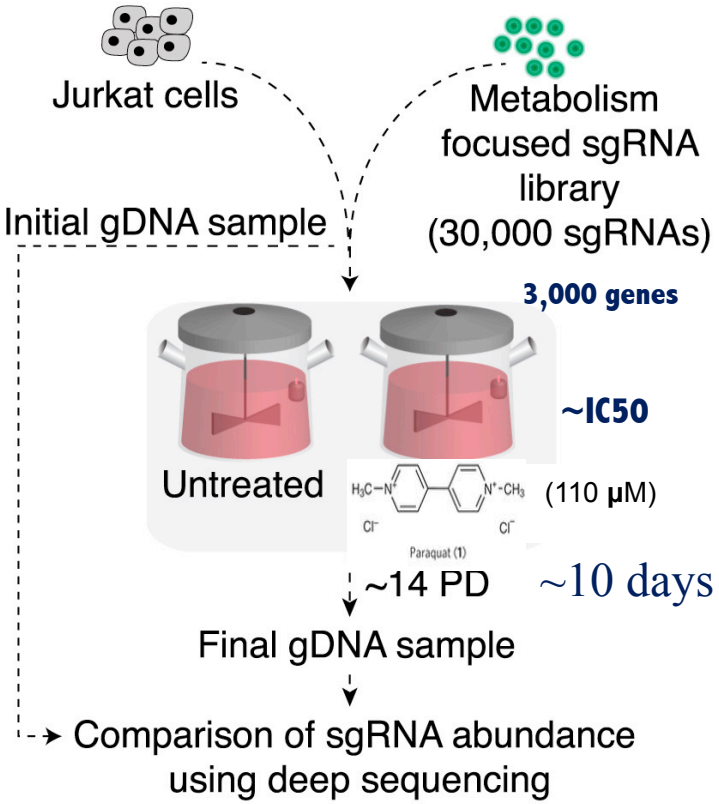
Proliferation/Survival Screen with CRISPR KO library



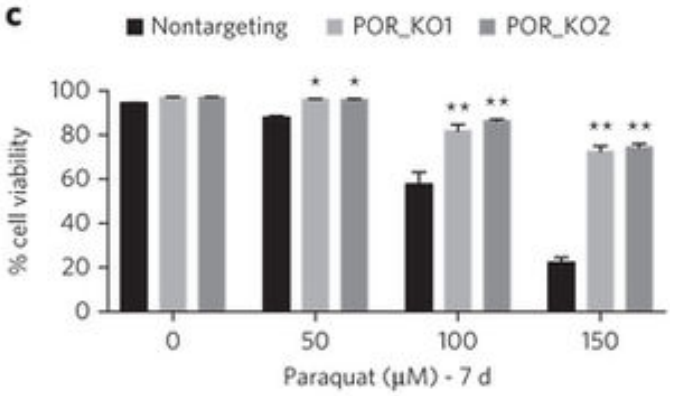
A CRISPR screen identifies a pathway required for paraquat-induced cell death

Colleen R Reczek, Kivancü Birsoy, Hyewon Kong, Inmaculada Mart'nez-Reyes, Tim Wang, Peng Gao, David M Sabatini & Navdeep S Chandel*

Nature Chemical Biology **13**, 1274–1279 (2017)



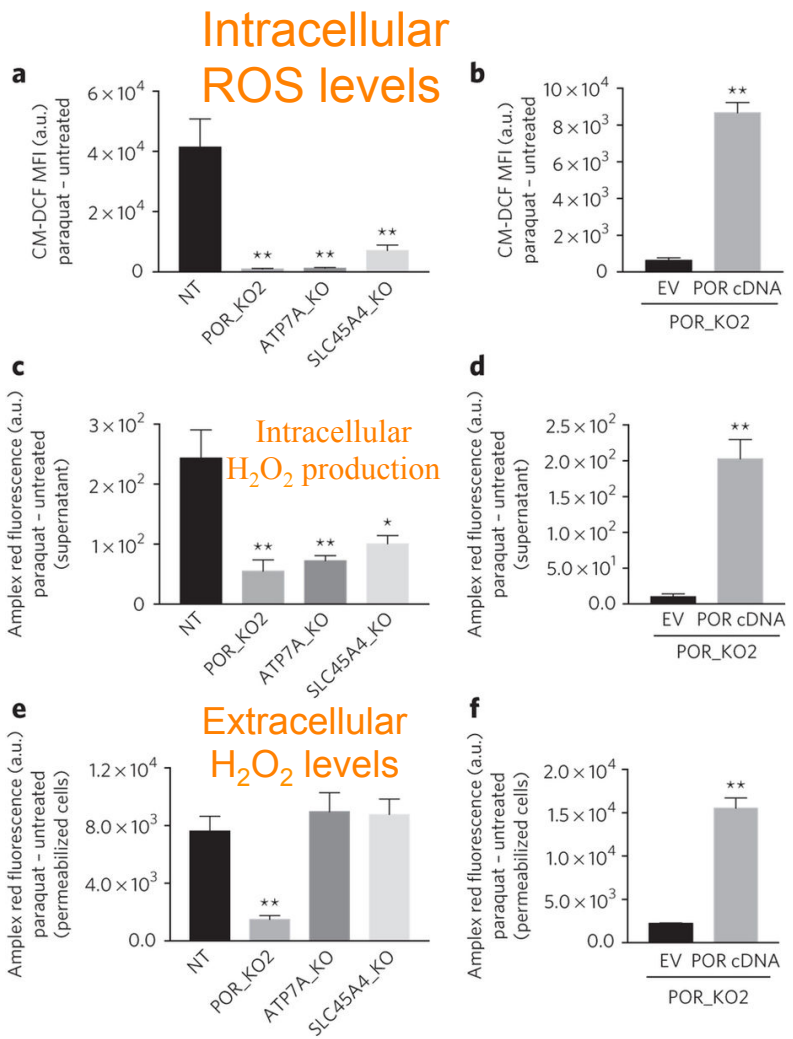
Top 25 Resistant Genes



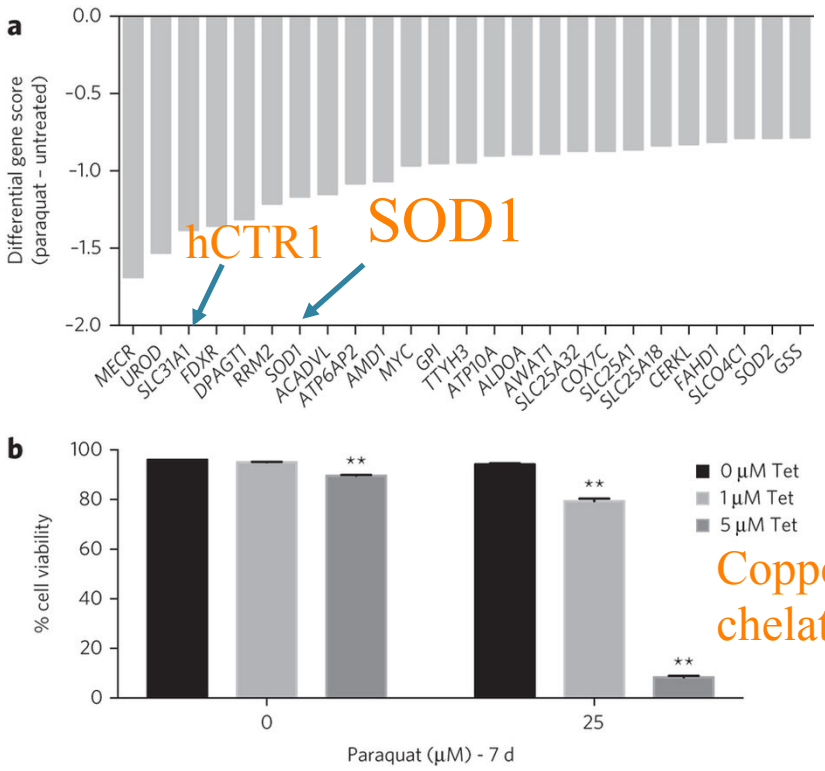
Individual KO sensitivity

Insight into mechanism of action of paraquat

ROS production by paraquat requires POR



Separate CRISPR Screen
Paraquat 25 **μ**M -10 days
identify sensitive clones



Copper chelator

Copper deficiency contributes to toxicity

Arsenic Trioxide whole genome CRISPR screen

Top 10 - Whole Genome Screen Candidates

Gene ID	Gene name	logFC	P Value	FDR
KEAP1	kelch-like ECH-associated protein 1	2.05	3.13E-59	6.87E-55
SEPHS2	selenophosphate synthetase 2	1.77	1.88E-23	2.06E-19
EEFSEC	eukaryotic elongation factor, selenocysteine-tRNA-specific	1.25	1.09E-17	7.97E-14
PSTK	phosphoseryl-tRNA kinase	1.49	3.23E-17	1.77E-13
KRT73	keratin 73	-2.5	2.88E-15	1.26E-11
ARID1B	AT rich interactive domain 1B (SWI1-like)	1.42	5.44E-13	1.99E-09
TXNDC17	thioredoxin domain containing 17	0.9	3.20E-10	1.00E-06
SLC6A12	solute carrier family 6 (neurotransmitter transporter), member 12	0.92	8.66E-10	2.37E-06
DCLRE1A	DNA cross-link repair 1A	-1.1	5.52E-09	1.34E-05
DLGAP5	discs, large (Drosophila) homolog-associated protein 5	-1.1	2.91E-08	6.38E-05

Log FC - relative abundance in treated vs control

FDR – False Discovery Rate

Secondary Screen

Customized CRISPR/Cas9 library enriched for candidate genes

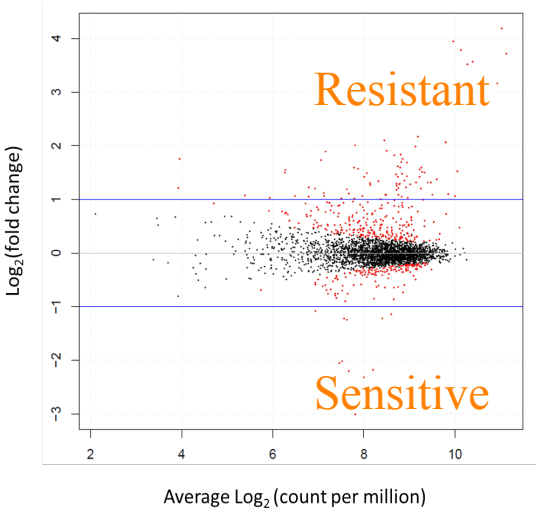
8 sgRNAs per gene

Multiple significant (FDR <0.001) sgRNAs per gene required for candidates selection

2° Screen (confirmatory)

20% sensitivity candidates

55% of resistance candidates



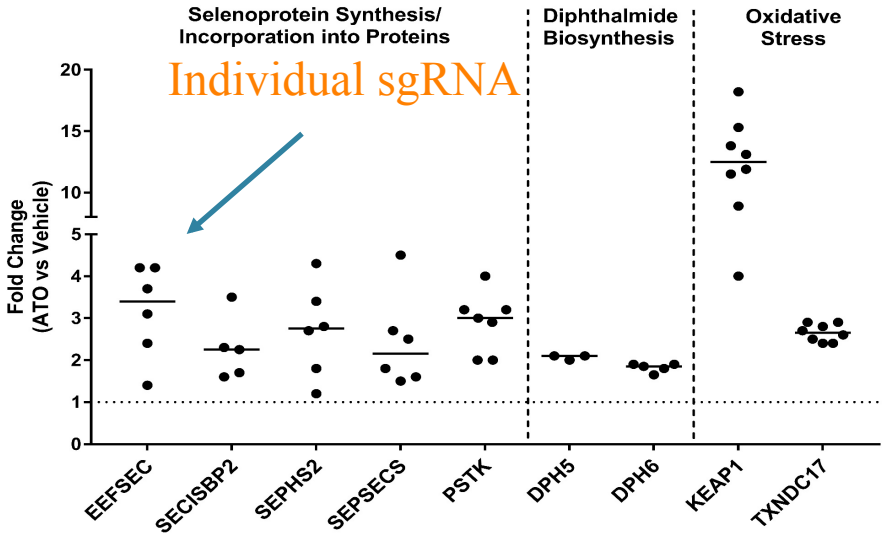
Arsenic Trioxide confirmatory CRISPR screen

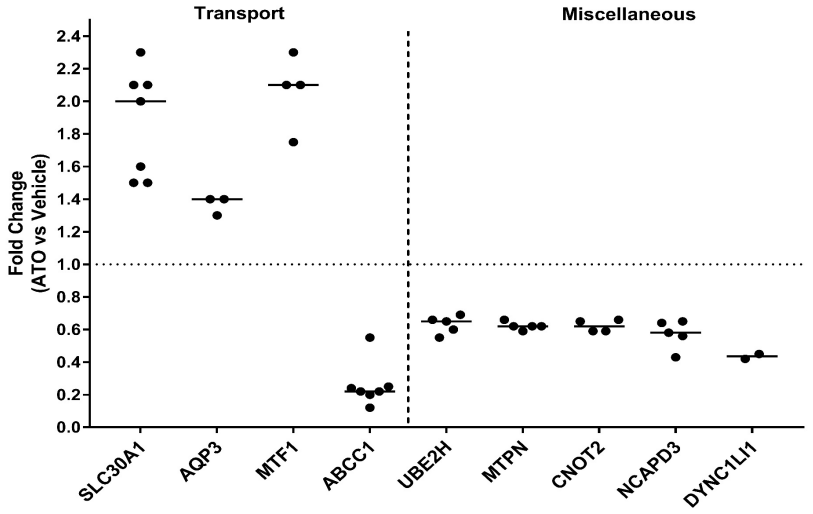
Resistant

Gene	sgRNA	FDR	Log FC
KEAP1	8/8	0.000354	3.6
TXNDC17	8/8	0.000354	1.4
PSTK	7/7	0.000354	1.6
GFI1B	7/7	0.000354	1.1
SLC30A1	7/7	0.000354	1
FLCN	7/7	0.000354	1.3
EED	7/7	0.000354	0.7
RRAGC	8/8	0.000354	1
EEFSEC	6/7	0.000354	1.6
C15orf41	7/7	0.000354	0.6
SET	7/8	0.000354	0.8
SEPHS2	6/7	0.000354	1.4
SEPSECS	7/8	0.000354	0.7
DPH6	6/7	0.000354	0.8
NAA38	8/8	0.000928	0.7

Sensitive

Gene	sgRNA	FDR	Log FC
ABCC1	8/8	0.000619	-2.1
MTPN	7/7	0.000619	-0.7
NCAPD3	6/7	0.000619	-0.7
DEPDC5	7/7	0.000619	-0.4
UBE2H	7/8	0.000619	-0.6
NPRL2	6/6	0.000619	-0.3
CNOT2	7/7	0.000619	-0.6
NDE1	7/8	0.000619	-0.7





sgRNA – the number of sgRNA for each gene

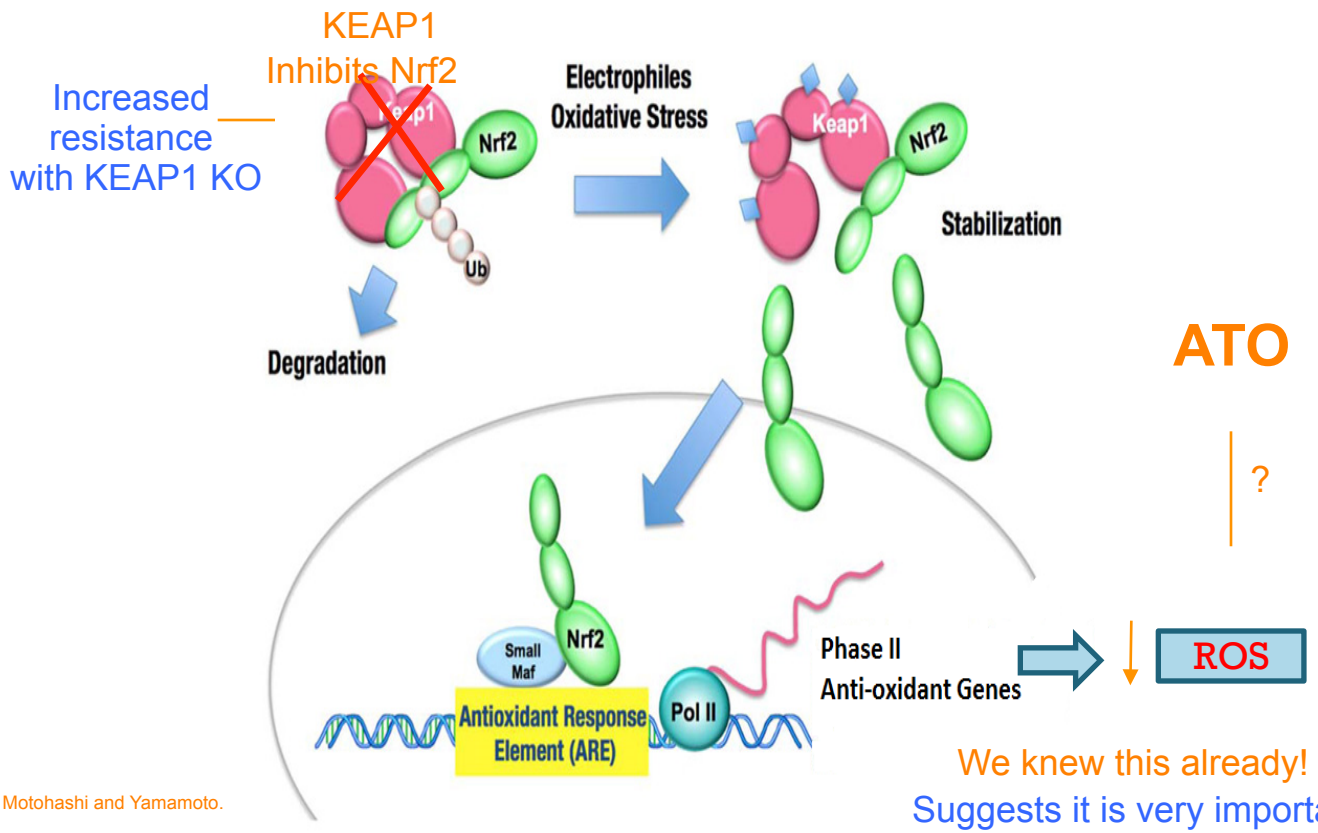
8/8 means 8 sgRNA out of 8 tested showed effect

Log FC – average relative abundance in treated vs control

FDR – False Discovery Rate

ATO Toxicity: Reactive Oxygen Species

Nrf2 primary anti-oxidant transcription factor – KEAP1 is REPRESSOR of NRF1

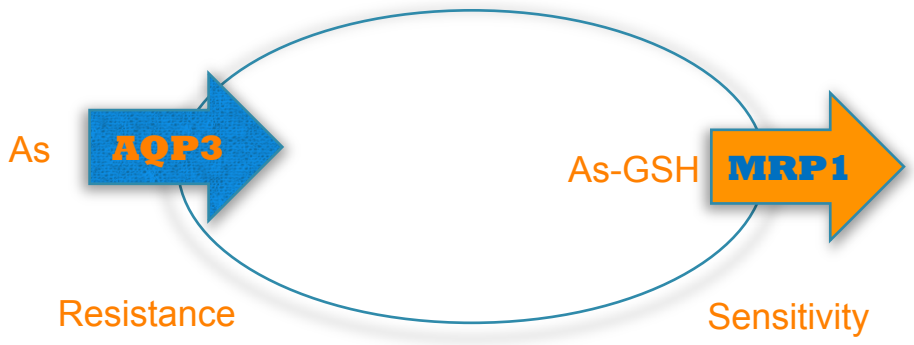


We knew this already!

Suggests it is very important in ACUTE short term toxicity

Mitsuishi *et al., Front. Oncol.*, 2012

Arsenic Transport



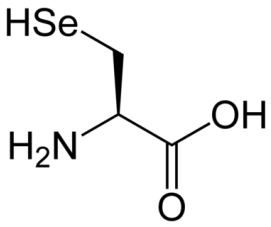
AQP3 SNPs associated with Arsenic Toxicity

Córdova EJ, Martínez-Hernández A, Uribe-Figueroa L, Centeno F, Morales-Marín M, Koneru H, et al. (2014) The NRF2-KEAP1 Pathway Is an Early Responsive Gene Network in Arsenic Exposed Lymphoblastoid Cells. PLoS ONE 9(2): e88069.

Te-Chang Lee, I-Ching Ho, Wen-Jen Lu, and Jin-ding Huang. Enhanced Expression of Multidrug resistance-associated Protein 2 and Reduced Expression of Aquaglyceroporin 3 in an Arsenic-resistant Human Cell Line *J. Biol. Chem. 2006 281: 18401-*

Michael W. Carew, Elaine M. Leslie; Selenium-dependent and -independent transport of arsenic by the human multidrug resistance protein 2 (MRP2/ABCC2): implications for the mutual detoxification of arsenic and selenium, *Carcinogenesis*, Volume 31, Issue 8, Pages 1450–1455,

Selenocysteine Incorporation into Proteins Increases Susceptibility to Arsenic Trioxide



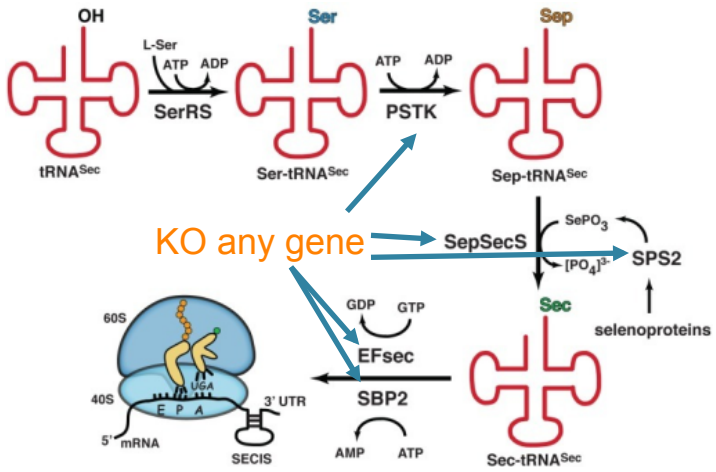
Selenocysteine
The 21st amino acid

Selenium
Previously known As binds Se

What did we find?

Loss of any gene needed for SeCys Incorporation in proteins leads to RESISTANCE

THIS IS NEW AND KINDA UNEXPECTED



Croat Med J. 2012 Dec; 53(6): 535–550.

Selenocysteine Synthesis/ Incorporation into Proteins

↓

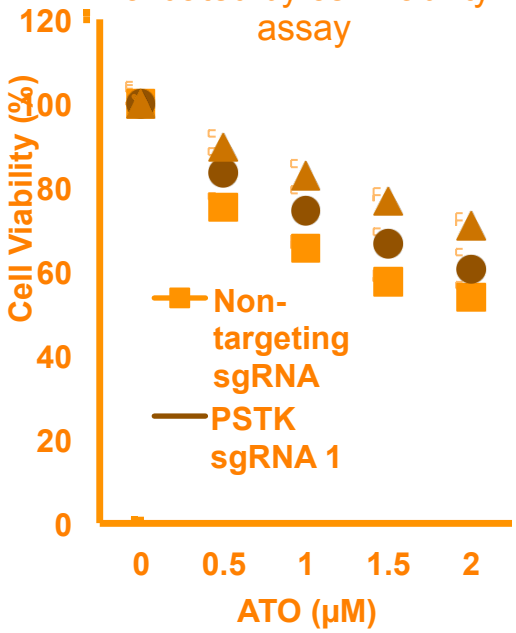
Proteins Lacking Selenocysteine

↓

Resistance to ATO

Many of these proteins involved in response to oxidative stress

Resistance of PSTK$^{-/-}$ K562 cells to ATO validated by cell viability assay



Se-As complex hypothesis

1938 – Moxon noted that Arsenic exposure can prevents Selenium poisoning (L.A. Moxon, Science, 88 (1938), p. 81)

Mutual antagonism (Se prevents As poisoning too) in multiple species including people (reviewed in Environment International 69 (2014) 148–158)

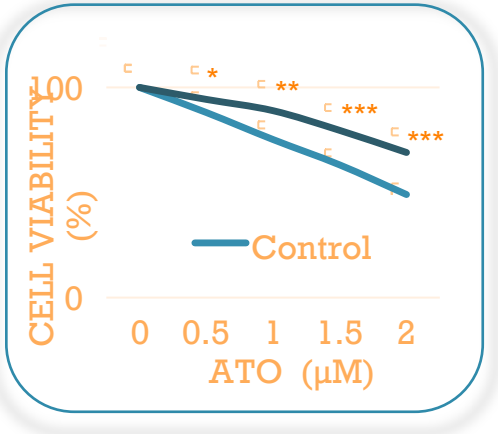
As-Se complexes formation may play a role

Inside the cell – glutathione complexes form

As + GSH → **(GS)2As–OH**

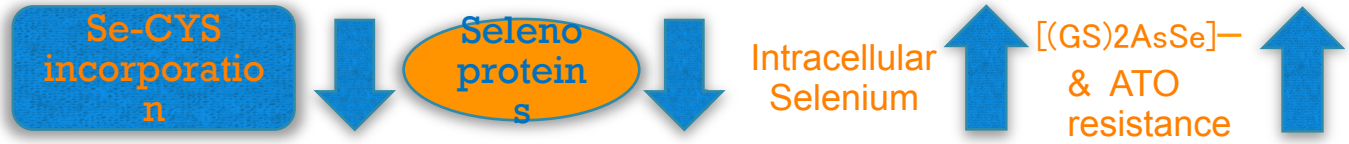
(GS)2As–OH + Se → [(GS)2AsSe]–

[(GS)2AsSe]– → Less toxic and possibly exported

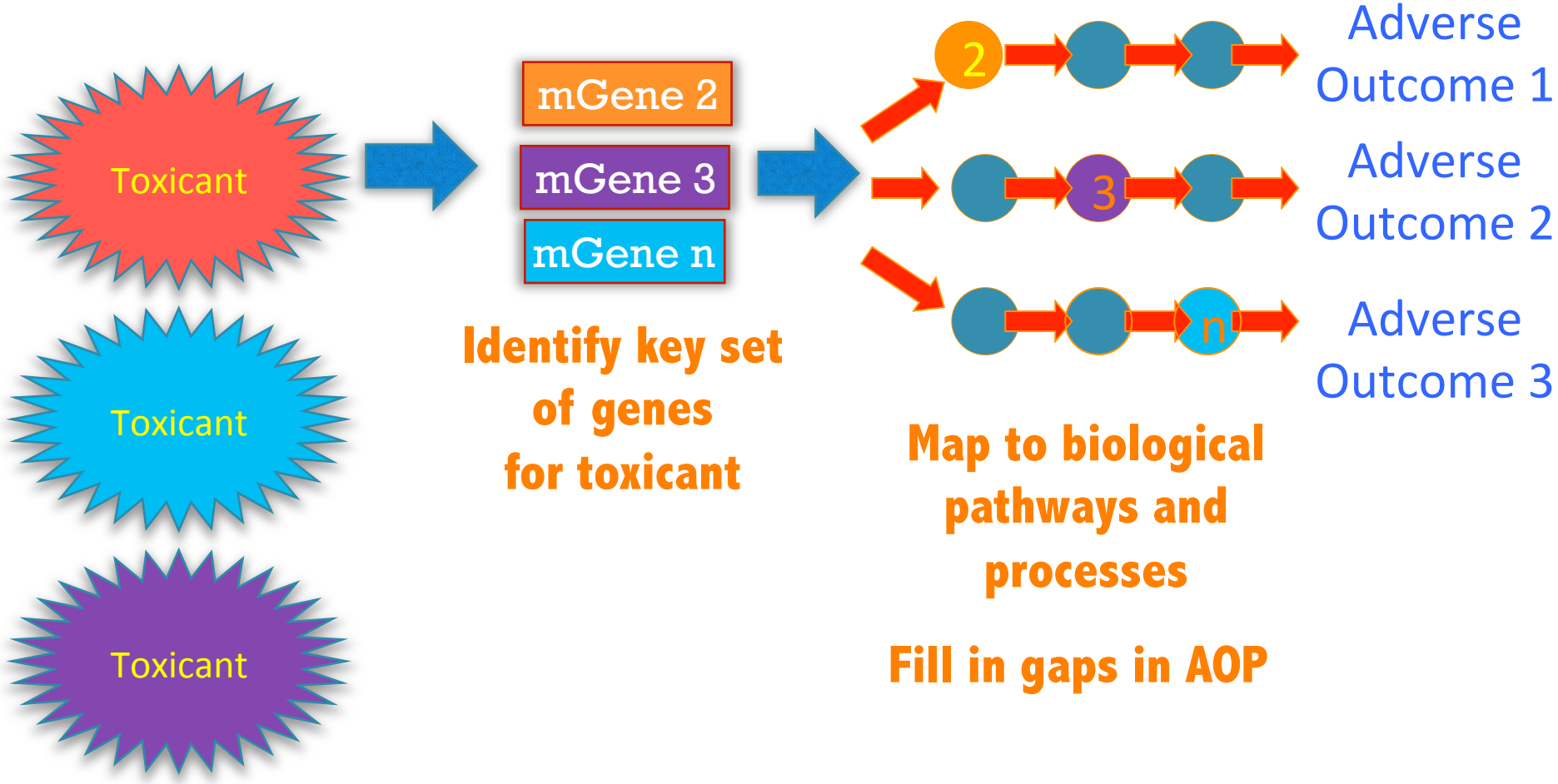


Protective effect of selenium (Na_2SeO_3) pre-treatment on ATO cytotoxicity in K562 cells.

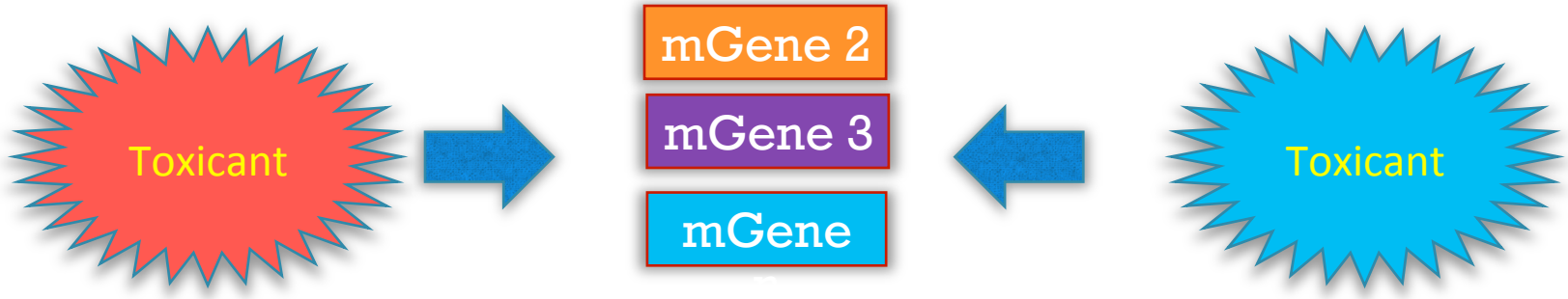
The Seleno Bis(S-glutathionyl) Arsinium Ion Is Assembled in Erythrocyte Lysate
Chemical Research in Toxicology **2006** *19* (4), 601-607



Functional screening to identify toxicity mechanisms and adverse outcome pathways

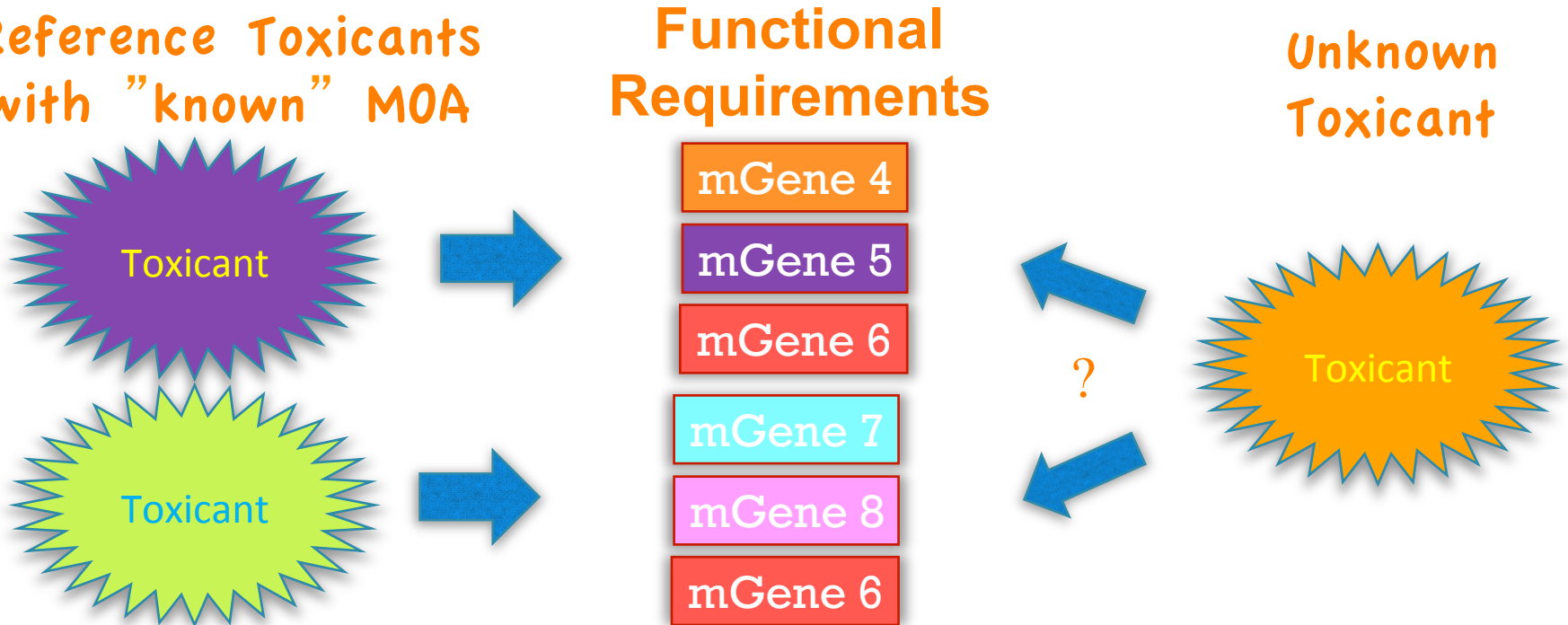


Infer mode of action by functional profiling



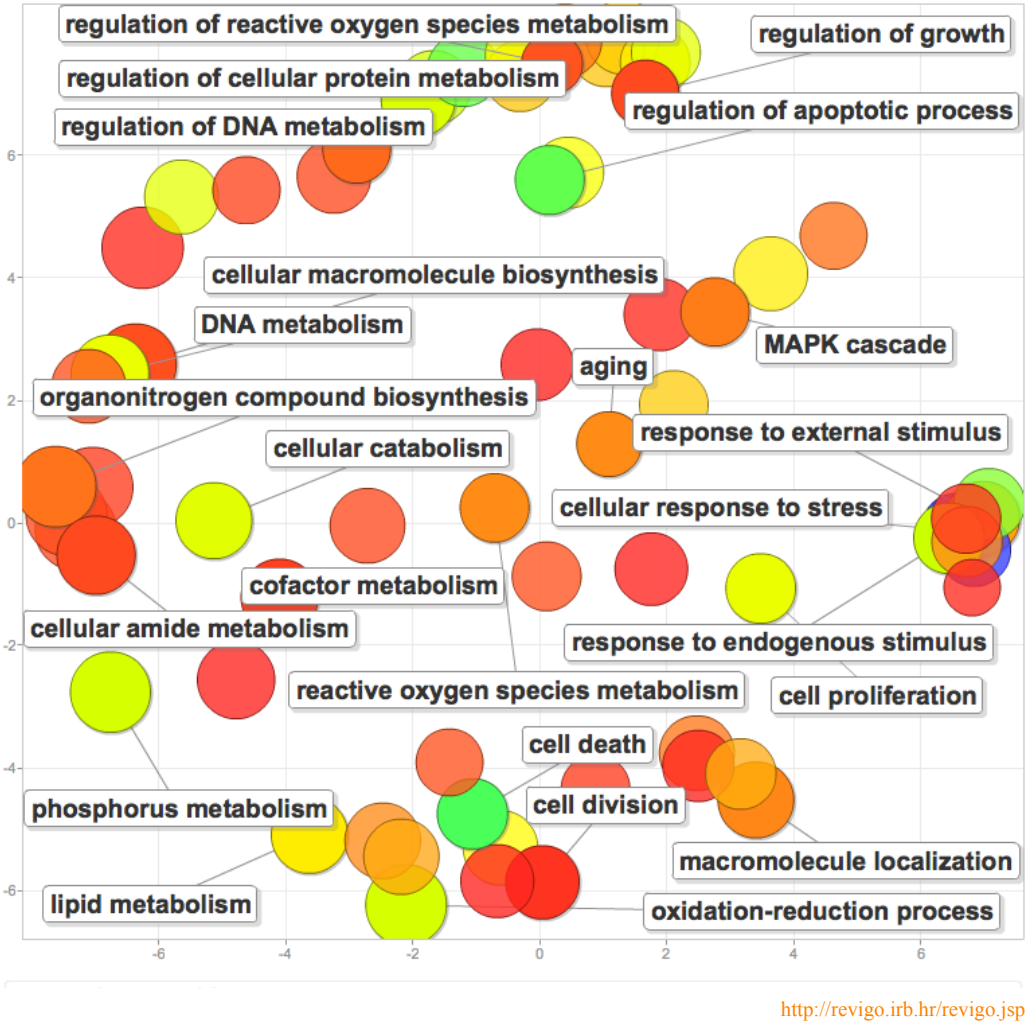
Shared key genes

Supports hypothesis of similar mode of action



ToxCRISPR – a focused CRISPR library for toxicology

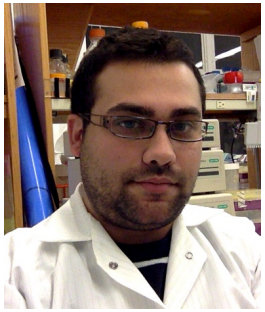
- Quan Lu, Luoping Zhang, Keith Houck
- 3675 Toxicology-related genes
  - S1500+ gene set prioritized by NIEHS/NTP/Tox21 program
  - 647 Environmental Genome Project (EGP) genes
  - Selected toxicant response-focused genes
- Subset CRISPR library for probing Mode of Action
  - Enable more rapid screening
  - Well annotated – understand function of most genes
  - Enable adverse outcome pathway determination



http://revigo.irb.hr/revigo.jsp

Revigo scatter plot of enriched GO terms

Acknowledgments



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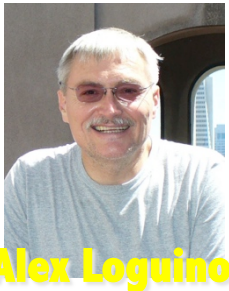

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NIEHS

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UF Center for Environmental & Human Toxicology
College of Veterinary Medicine

UF UNIVERSITY of FLORIDA

UC BERKELEY SUPERFUND RESEARCH PROGRAM
SCIENCE FOR A SAFER WORLD

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

References

Start Here: https://www.addgene.org/crispr/

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Abbreviations

- CRISPR -Clustered Regularly Interspaced Short Palindromic Repeats
- ToxCRISPR – toxicology subset library
- AOP – Adverse outcome pathway
- **sgRNA- single guide RNA**
- **MOI – multiplicity of infection**
- **KO - knockout**