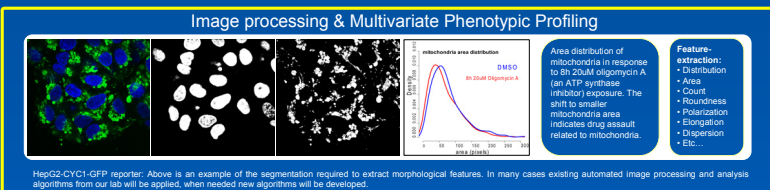
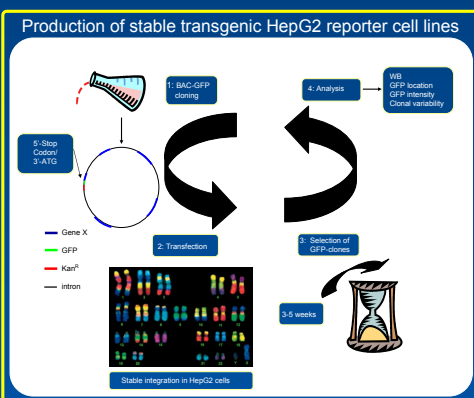
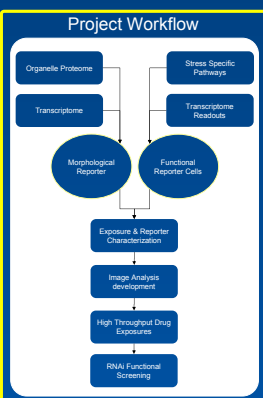


# Automated High Content Imaging of Cell Organelle Morphometry and Function in Cellular Stress Responses

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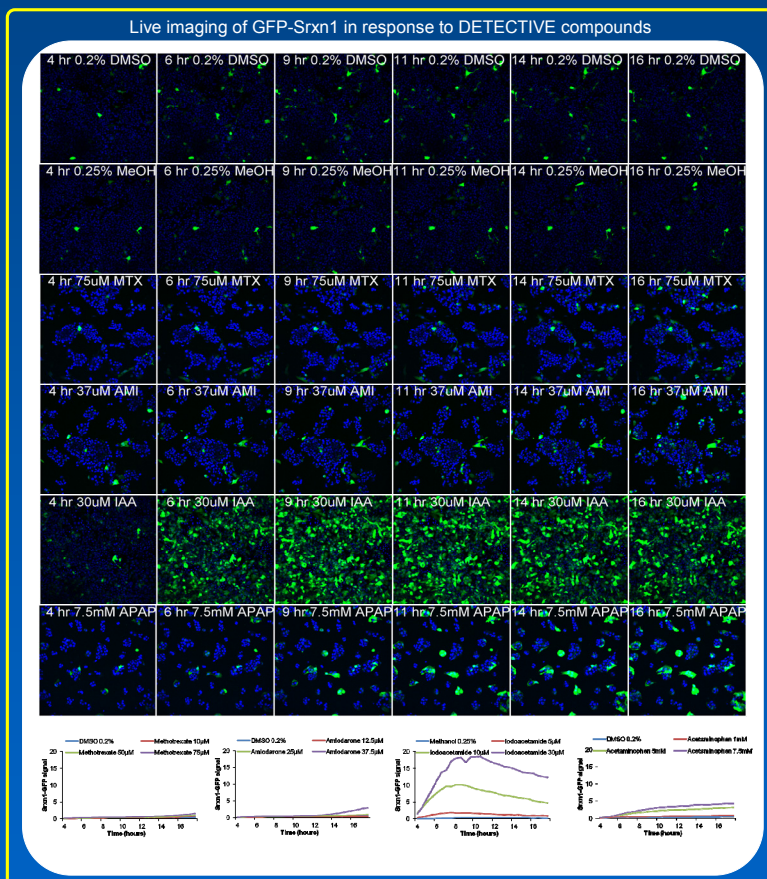
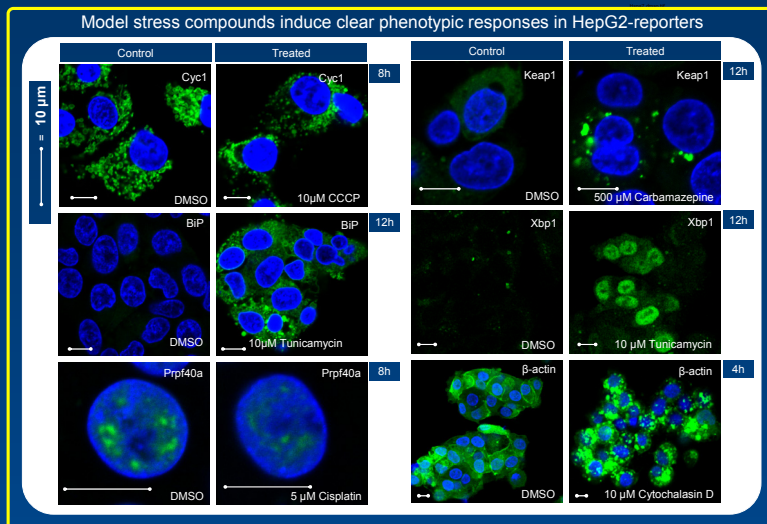
## Introduction

As part of the DETECTIVE consortium our goal is to establish reporter cell models for candidate biomarkers relevant to repeated dose toxicity. To test the feasibility that repeated dose toxicity can be monitored by early intracellular alterations, we are generating a large panel of HepG2 fluorescent reporter cell lines using BAC-GFP transgeneomics strategies to monitor cell organelle disturbances at the morphological and functional level. We anticipate that such a panel together may function as a cell-phenotyping array to classify the adverse activity of chemicals. We already successfully established reporter cell lines for mitochondrial, actin cytoskeleton, nuclear and endoplasmic reticulum (ER) morphology as well as functional reporters for oxidative stress and ER stress. We report the current status of the BAC-GFP reporter HepG2 library and demonstrate that individual reporter cell lines are sensitive to their corresponding model stress responses. We further demonstrate that these reporter cell lines can also be used in time lapse microscopy experiments to follow e.g. oxidative stress on a cell-to-cell basis in time. By testing known stress routes versus (repeated) incubations with the DETECTIVE model compounds, we anticipate to build a compound effect classification system, that incorporates high-precision robotic liquid handling, high throughput automated fluorescence microscopy and multivariate profiling analysis techniques. In addition, established target constructs can be incorporated in other cell models including human stem cells.



**Selected marker genes & selection of cell lines**

Gene target	Reporter for	GFP construct	Stable cell line
Srxn1	Oxidative stress	X	X
NFE2L2	Oxidative stress	X	-
NFKBIA	NFKB-signaling	X	-
RELA	NFKB-signaling	X	-
TP53	DNA damage	X	-
BRCA1	DNA damage	X	-
TP53BP1	DNA damage	X	-
PRPF40A	Nuclear morphology	X	X
ACTB	Actin	X	X
VIM	Cytoskeleton	X	-
CDH1	Cell-Cell Junctions	X	-
ACTN1	Focal Adhesion	X	-
VCL	Focal Adhesion	X	-
HSPA5	ER stress	X	X
PDIA6	ER stress	X	X
XPB1	ER stress	X	X
ATF4	ER stress	X	-
DDIT3	ER stress	X	-
LC3	Autophagy	X	X
KEAP1	Ox. Stress / Autophagy	X	X
TMM23	Mitochondria	X	X
CYC1	Mitochondria	X	X
CYC5	Mitochondria	X	-
MYH9	Vesicles	X	-



## Conclusions and future plans:

- We successfully established a panel of HepG2 BAC-GFP reporter cells.
- Established reporter cell lines allow identification of functional and phenotypic responses.
- Reporter cell lines allow quantitative live cell imaging of stress responses (e.g. Srxn1-GFP).
- We will expand the reporter panel with markers for e.g. Golgi and endosomes.
- We will select for each stress type 1 or 2 well-responding reporters
- We will build an image database for stress phenotype profiling
- We will test consortium compounds and classify their effect using the cell models
- Develop multi parameter phenotypic profiling pipe-line