

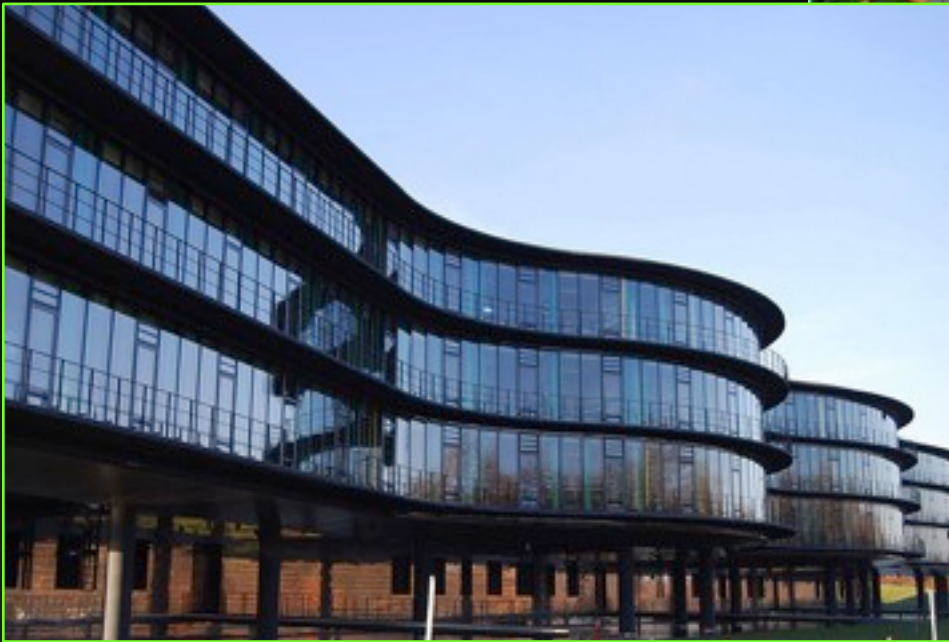
The Pros and Cons of siRNA Use in HCS

Eugenio Fava (fava@mpi-cbg.de)

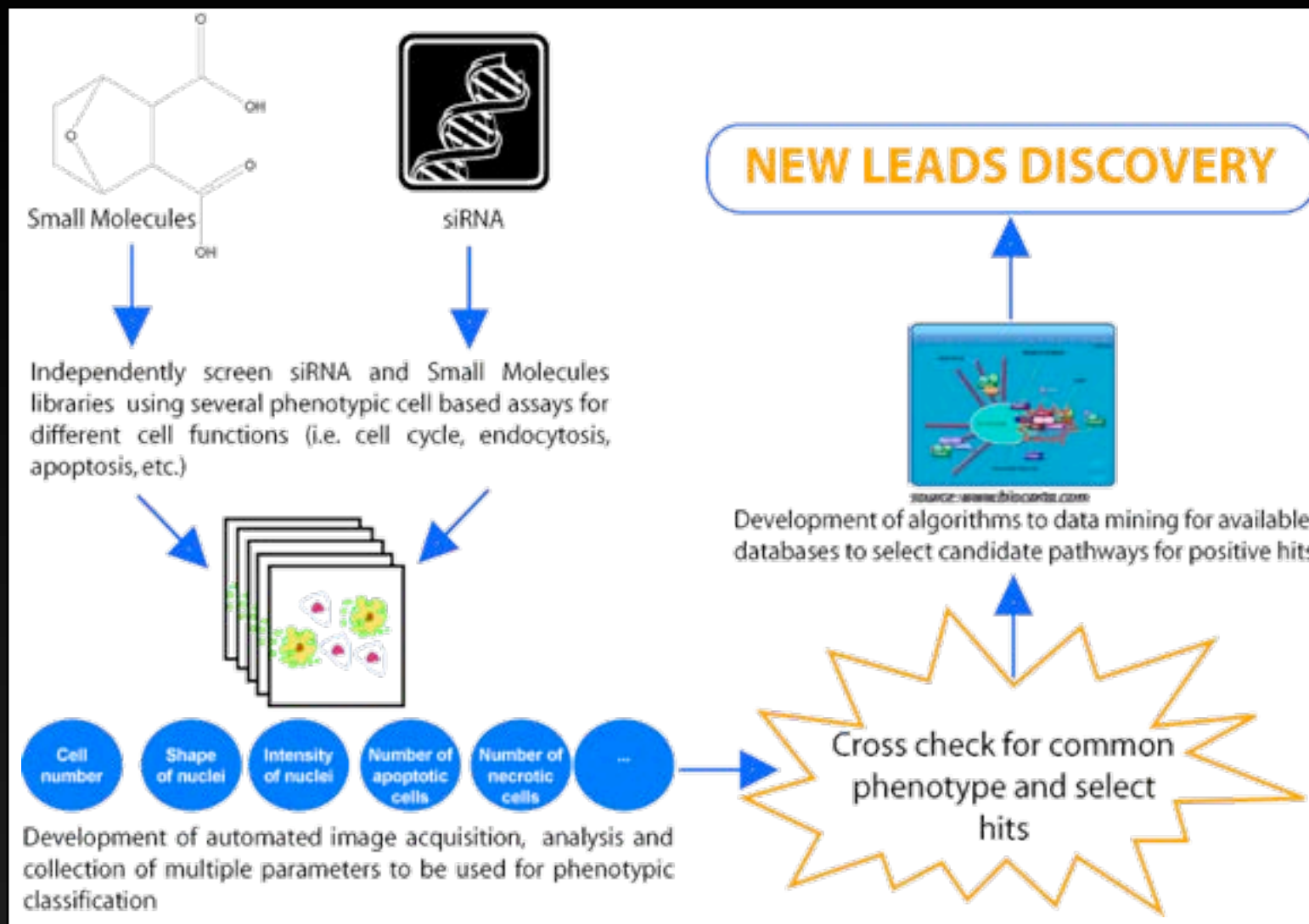
**DZNE - German Centre for
Neurodegenerative Diseases -
Bonn, Germany**



Max Plank Institute of Cell Biology
and Genetics
Technology Development Studio
Dresden, Germany



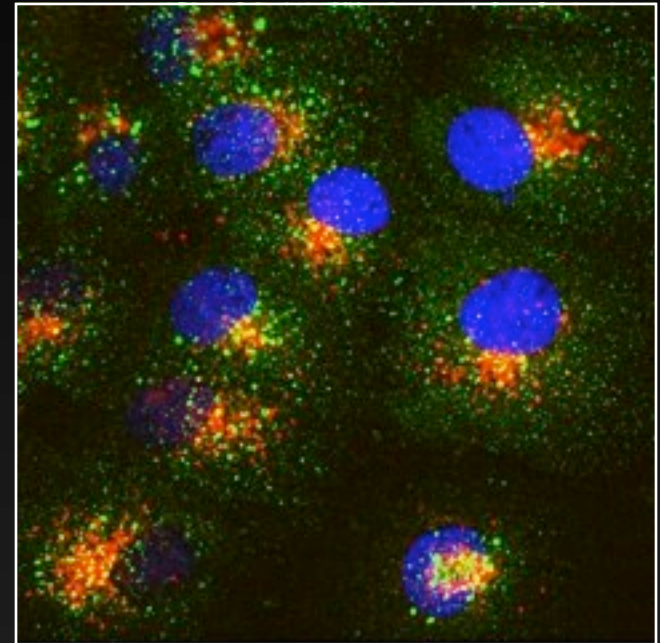
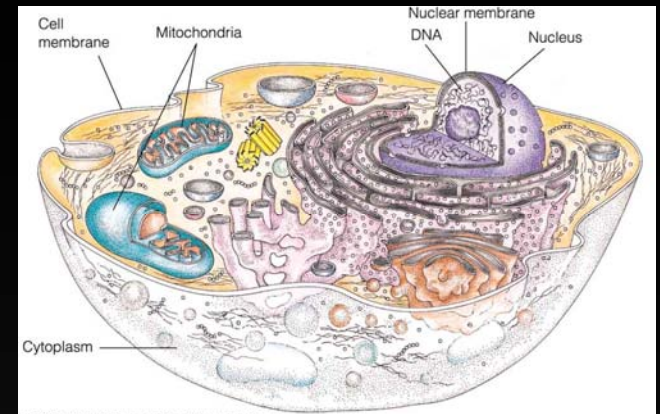
Why RNAi in HCS



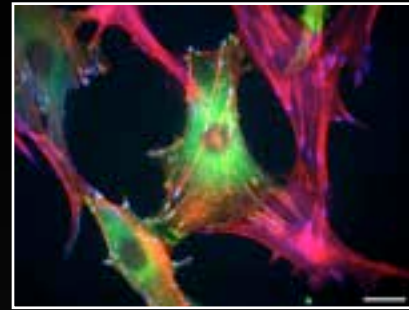
High Content: the cell as a test tube

Advantages in using cells as “test tube”:

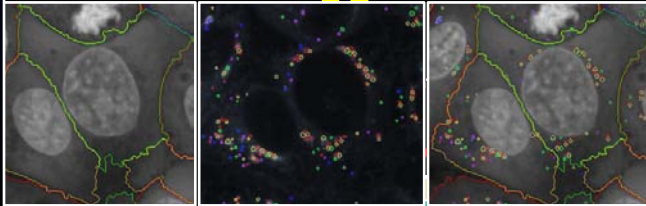
- Relevant physiological read-out
- **Spatial Information: Proteins sub-cellular localization**
- **In situ “molecular biology” (Protein-Protein interactions)**
- Toxicity data embedded in the system
- Bio-availability of compounds (membrane permeability, metabolic activity etc.)
- **Multiparametric**
- **Multiplexing**
 - Several markers simultaneous read-out
 - Several cell type
- Cell population study
 - Quantitative multi-parametric phenotypic changes measurements
- **Single-cell and/or sub-cellular level study**



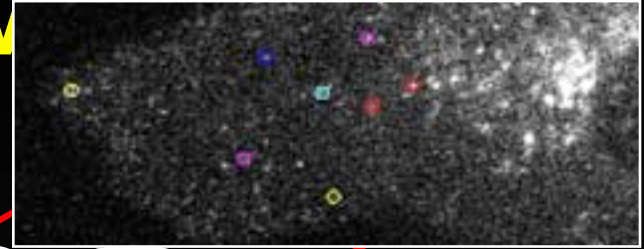
The cellular pathways approach to Cell-based phenotypic assays for drug discovery



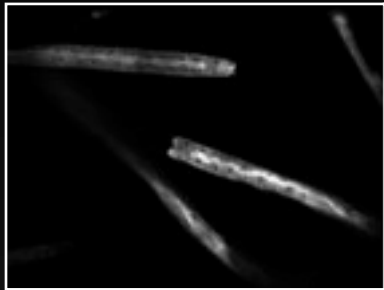
Cytoskeletal



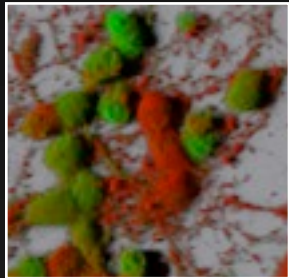
Lipid Metabolism



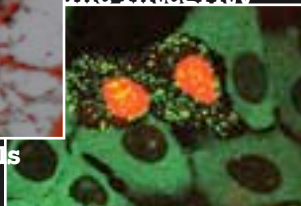
Endosome Distribution and motility



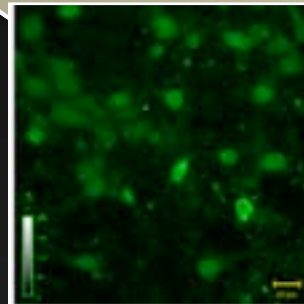
C. Elegans



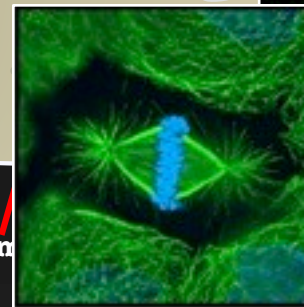
Primary Cells



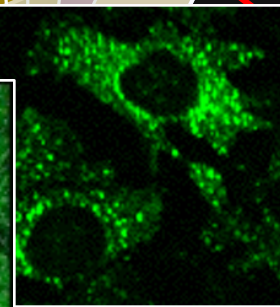
Translocation



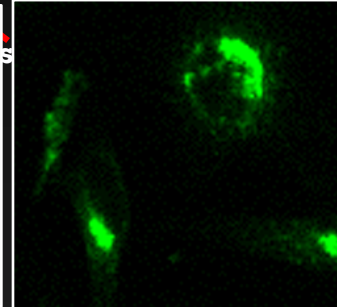
Calcium Homeostasis



Cell Cycle



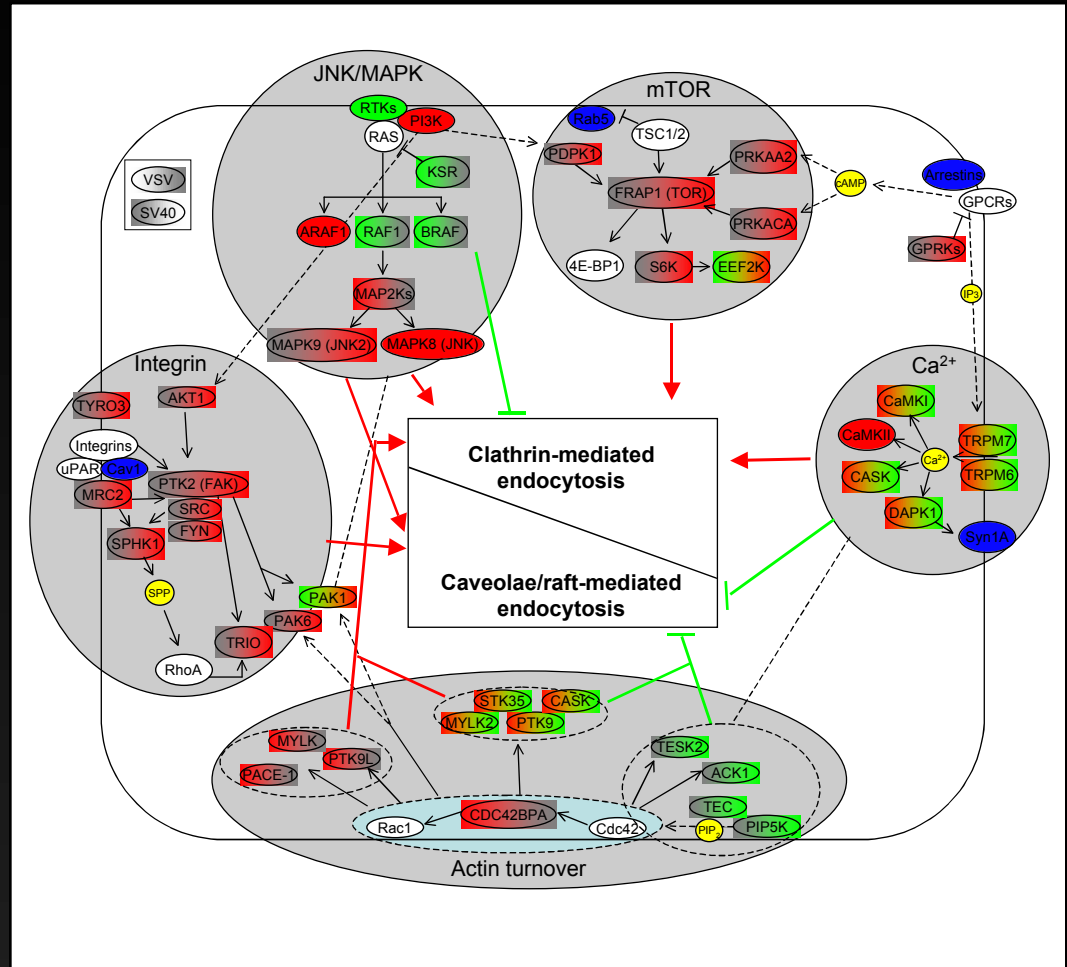
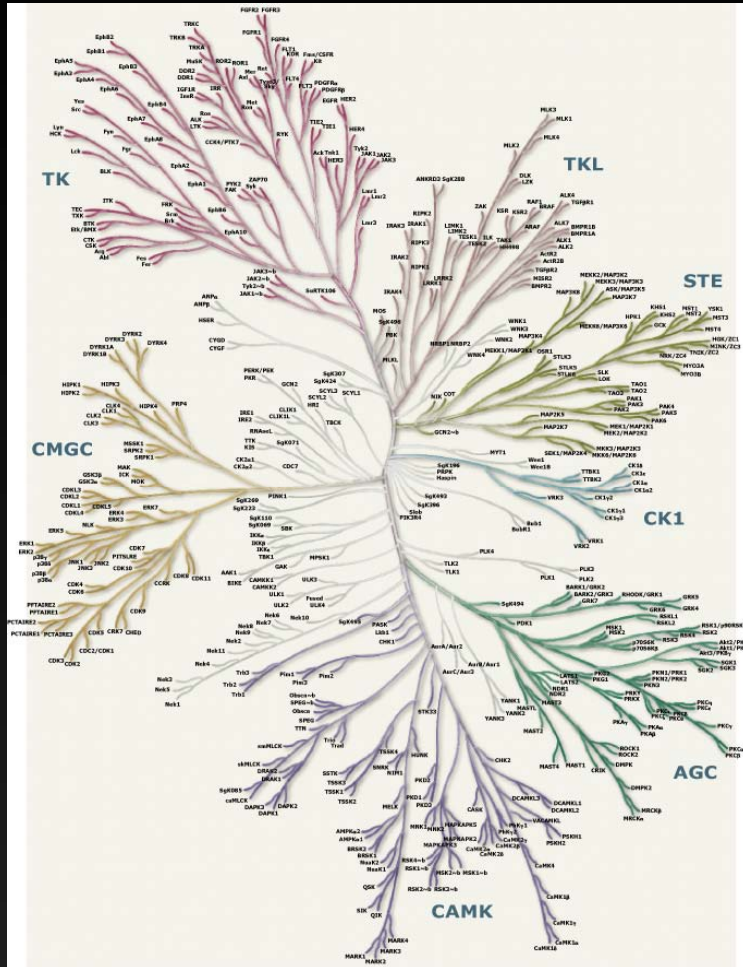
Golgi Morphology and Integrity



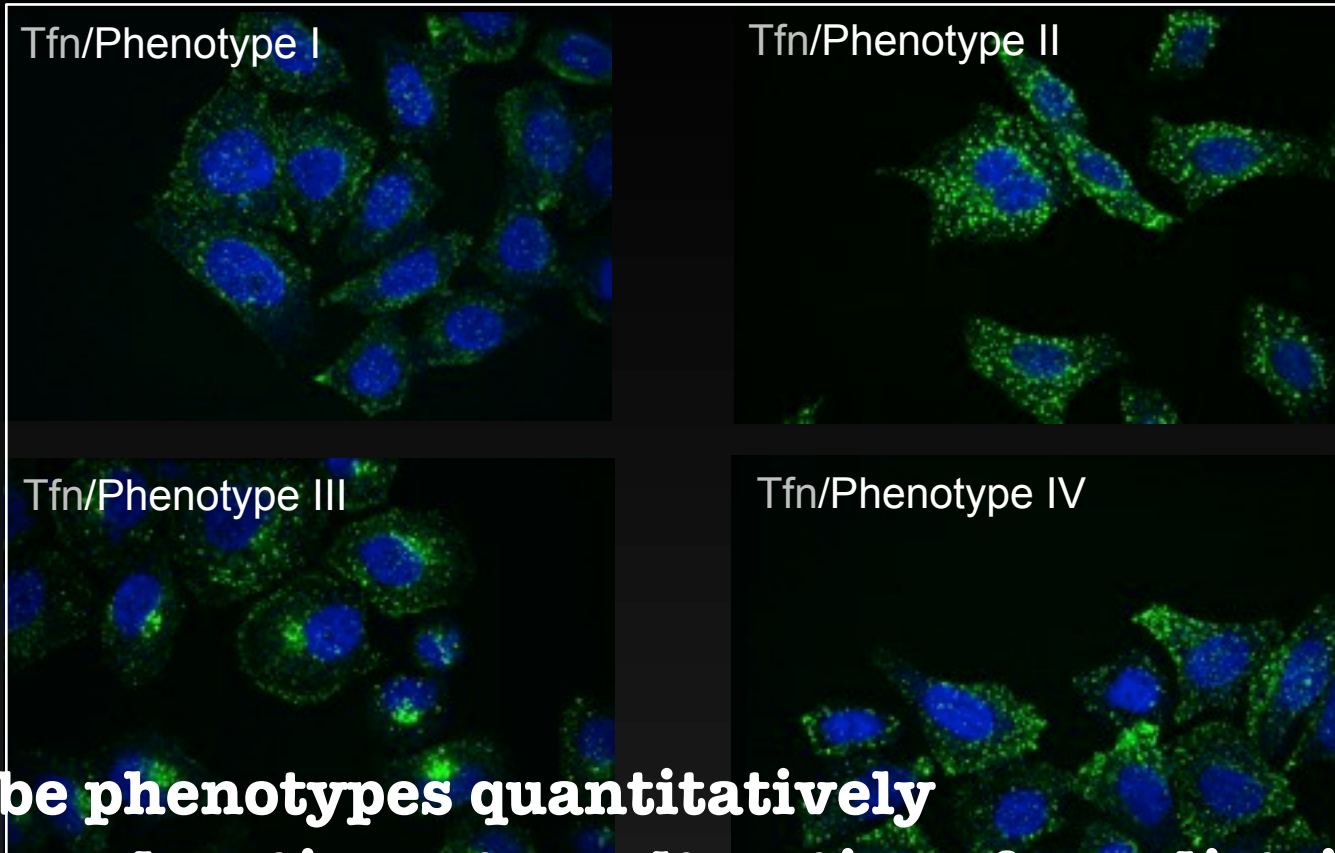
Apoptosis



RNAi in HCS proof of Principle



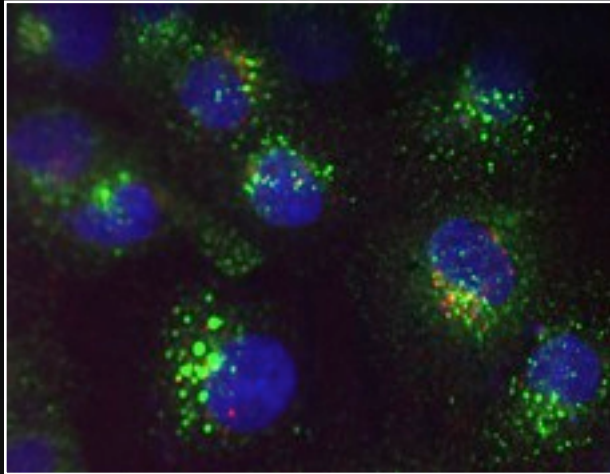
Lesson I: We need non supervised Automated Image Analysis



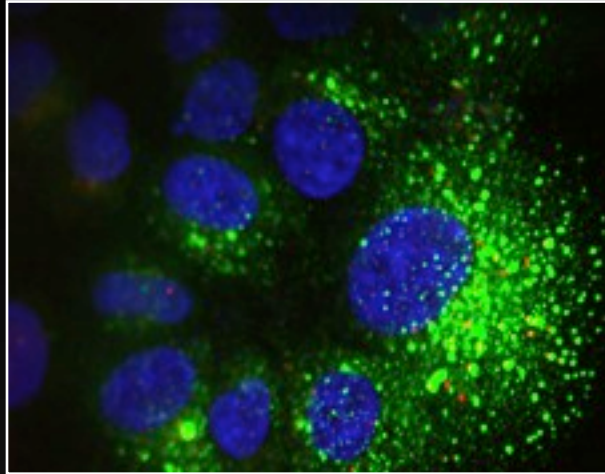
- **Describe phenotypes quantitatively**
- **Deduce endocytic system alterations from distribution**
- **Analyze dynamic process with end point assay**

Lesson II: how to handle RNAi off target

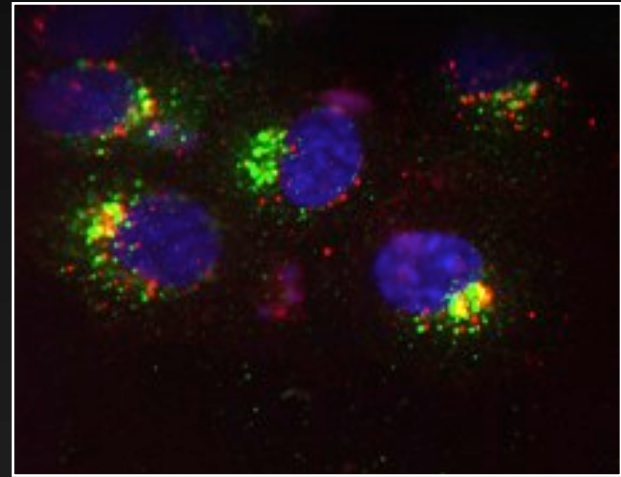
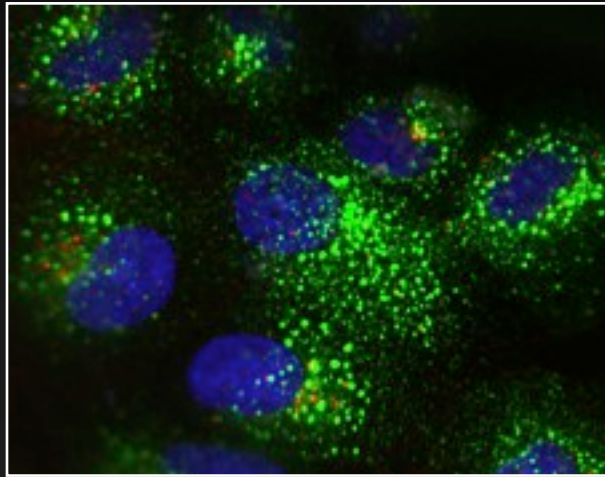
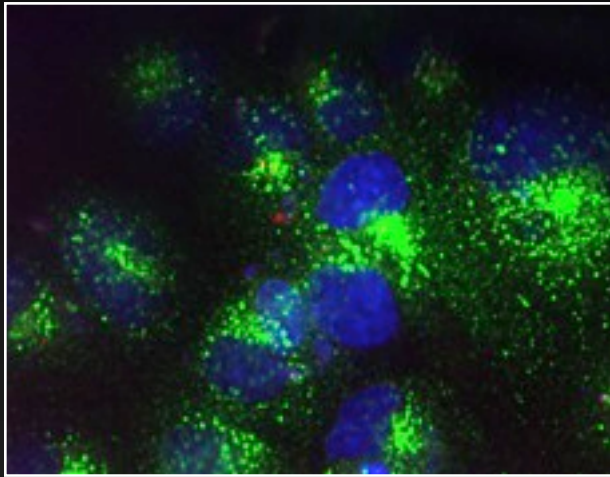
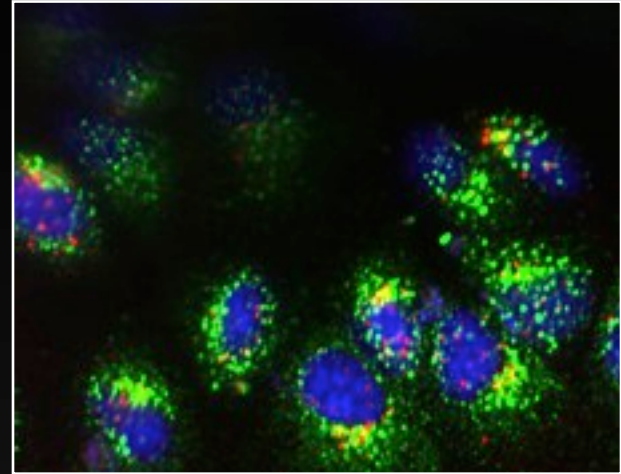
siRNA 1



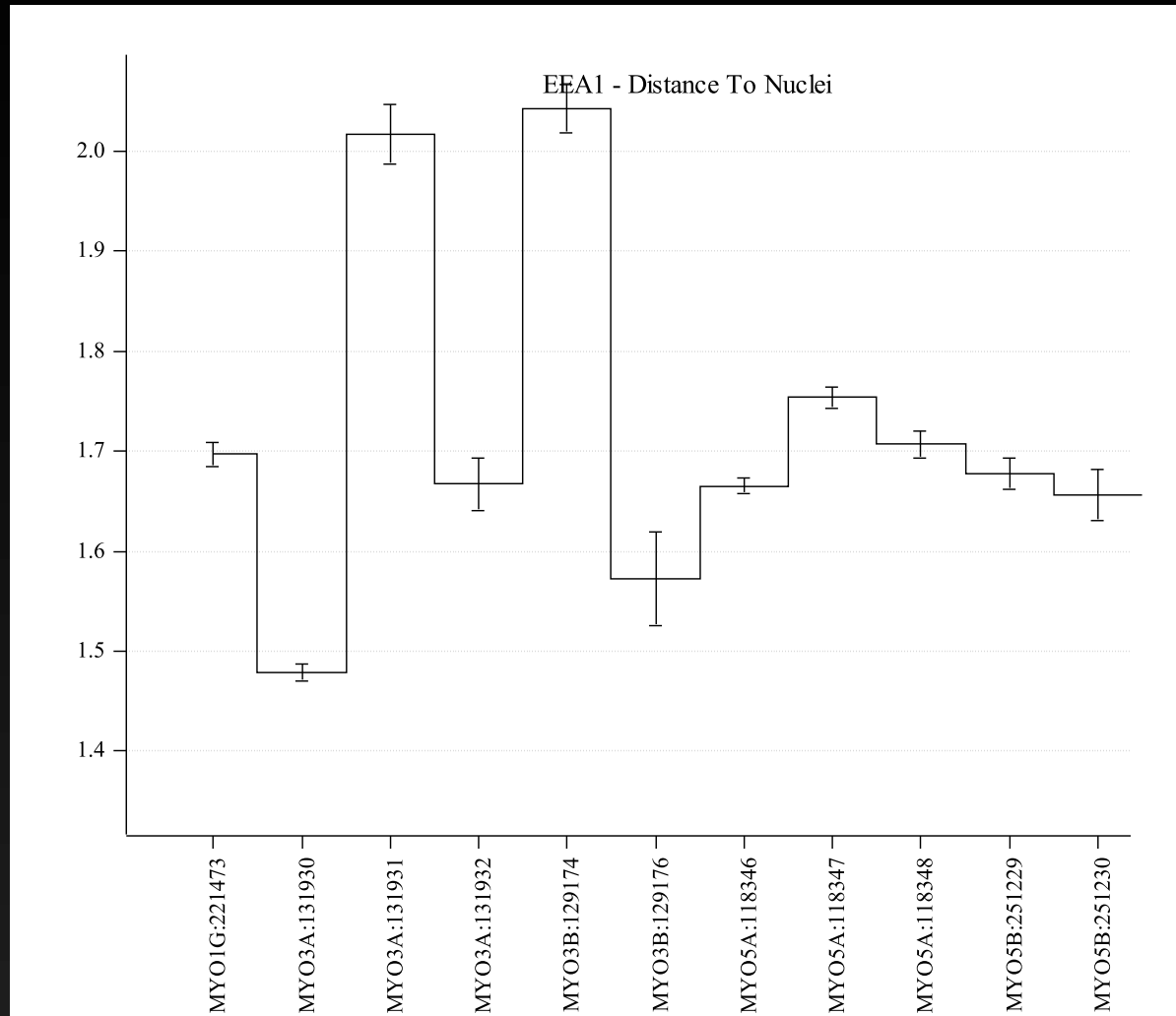
siRNA 2



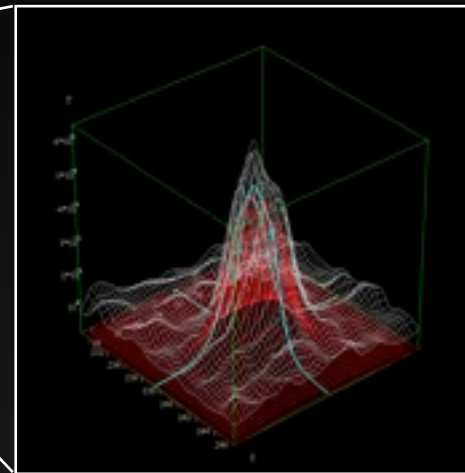
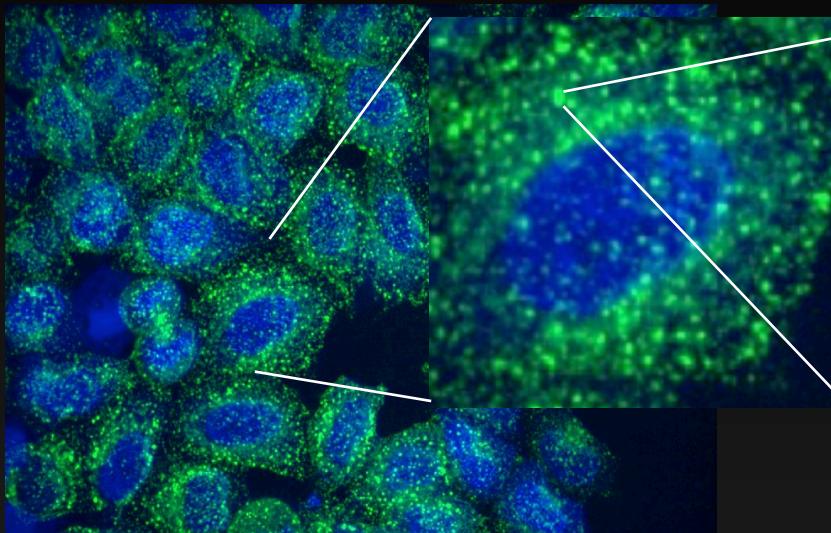
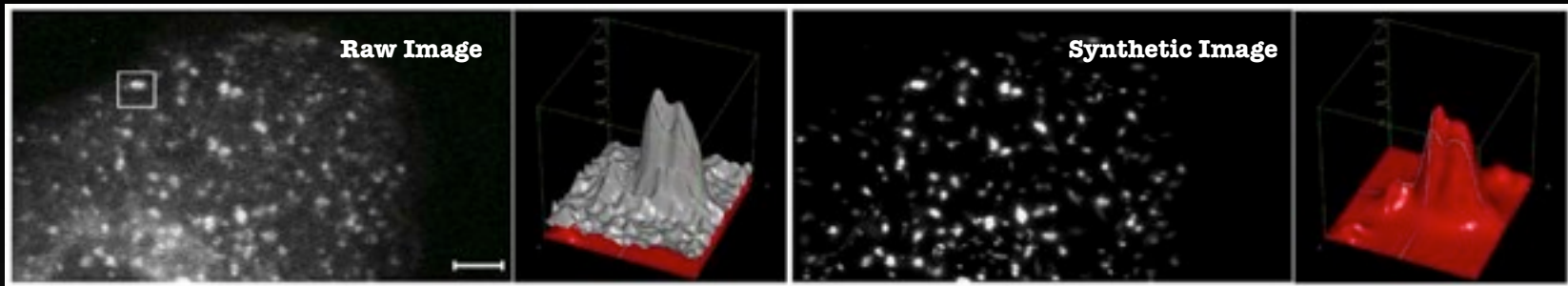
siRNA 3



Lesson III: single parameter analysis will not be sufficient to get out from the troubles

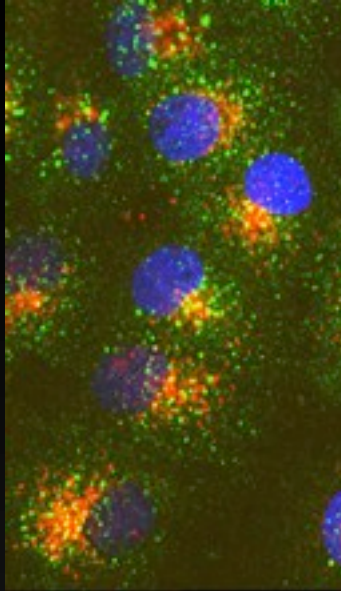


Automated image Analysis:



Motiontracking II by Yannis Kalaidzidis

Super Computing and Multi-parameter Analysis



File Name	Size	Intensity	Mean	Std	Min	Max	Area	Perim	CentX	CentY
vesicle_001.tif	100	100	100	100	100	100	100	100	100	100
vesicle_002.tif	100	100	100	100	100	100	100	100	100	100
vesicle_003.tif	100	100	100	100	100	100	100	100	100	100
vesicle_004.tif	100	100	100	100	100	100	100	100	100	100
vesicle_005.tif	100	100	100	100	100	100	100	100	100	100
vesicle_006.tif	100	100	100	100	100	100	100	100	100	100
vesicle_007.tif	100	100	100	100	100	100	100	100	100	100
vesicle_008.tif	100	100	100	100	100	100	100	100	100	100
vesicle_009.tif	100	100	100	100	100	100	100	100	100	100
vesicle_010.tif	100	100	100	100	100	100	100	100	100	100
vesicle_011.tif	100	100	100	100	100	100	100	100	100	100
vesicle_012.tif	100	100	100	100	100	100	100	100	100	100
vesicle_013.tif	100	100	100	100	100	100	100	100	100	100
vesicle_014.tif	100	100	100	100	100	100	100	100	100	100
vesicle_015.tif	100	100	100	100	100	100	100	100	100	100
vesicle_016.tif	100	100	100	100	100	100	100	100	100	100
vesicle_017.tif	100	100	100	100	100	100	100	100	100	100
vesicle_018.tif	100	100	100	100	100	100	100	100	100	100
vesicle_019.tif	100	100	100	100	100	100	100	100	100	100
vesicle_020.tif	100	100	100	100	100	100	100	100	100	100
vesicle_021.tif	100	100	100	100	100	100	100	100	100	100
vesicle_022.tif	100	100	100	100	100	100	100	100	100	100
vesicle_023.tif	100	100	100	100	100	100	100	100	100	100
vesicle_024.tif	100	100	100	100	100	100	100	100	100	100
vesicle_025.tif	100	100	100	100	100	100	100	100	100	100
vesicle_026.tif	100	100	100	100	100	100	100	100	100	100
vesicle_027.tif	100	100	100	100	100	100	100	100	100	100
vesicle_028.tif	100	100	100	100	100	100	100	100	100	100
vesicle_029.tif	100	100	100	100	100	100	100	100	100	100
vesicle_030.tif	100	100	100	100	100	100	100	100	100	100
vesicle_031.tif	100	100	100	100	100	100	100	100	100	100
vesicle_032.tif	100	100	100	100	100	100	100	100	100	100
vesicle_033.tif	100	100	100	100	100	100	100	100	100	100
vesicle_034.tif	100	100	100	100	100	100	100	100	100	100
vesicle_035.tif	100	100	100	100	100	100	100	100	100	100
vesicle_036.tif	100	100	100	100	100	100	100	100	100	100
vesicle_037.tif	100	100	100	100	100	100	100	100	100	100
vesicle_038.tif	100	100	100	100	100	100	100	100	100	100
vesicle_039.tif	100	100	100	100	100	100	100	100	100	100
vesicle_040.tif	100	100	100	100	100	100	100	100	100	100
vesicle_041.tif	100	100	100	100	100	100	100	100	100	100
vesicle_042.tif	100	100	100	100	100	100	100	100	100	100
vesicle_043.tif	100	100	100	100	100	100	100	100	100	100
vesicle_044.tif	100	100	100	100	100	100	100	100	100	100
vesicle_045.tif	100	100	100	100	100	100	100	100	100	100
vesicle_046.tif	100	100	100	100	100	100	100	100	100	100
vesicle_047.tif	100	100	100	100	100	100	100	100	100	100
vesicle_048.tif	100	100	100	100	100	100	100	100	100	100
vesicle_049.tif	100	100	100	100	100	100	100	100	100	100
vesicle_050.tif	100	100	100	100	100	100	100	100	100	100



2500 cpu

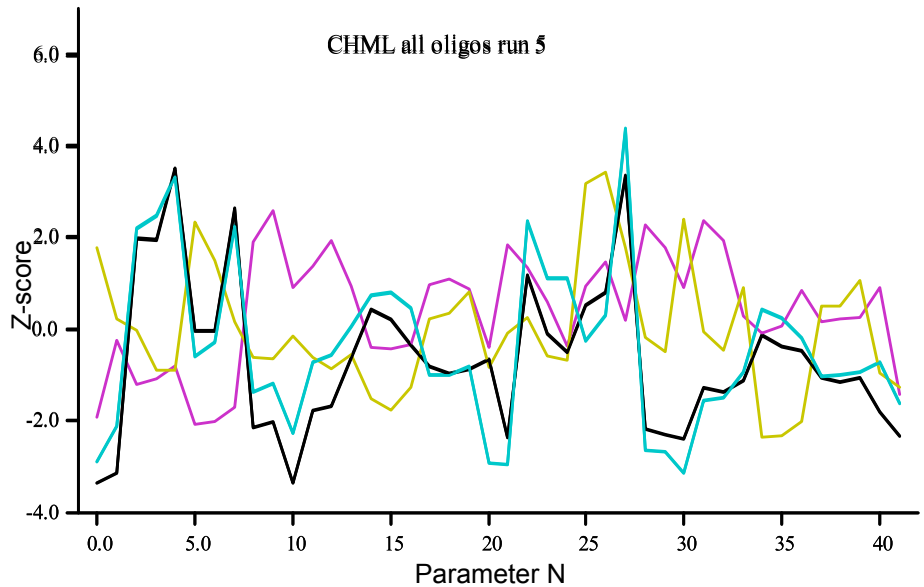
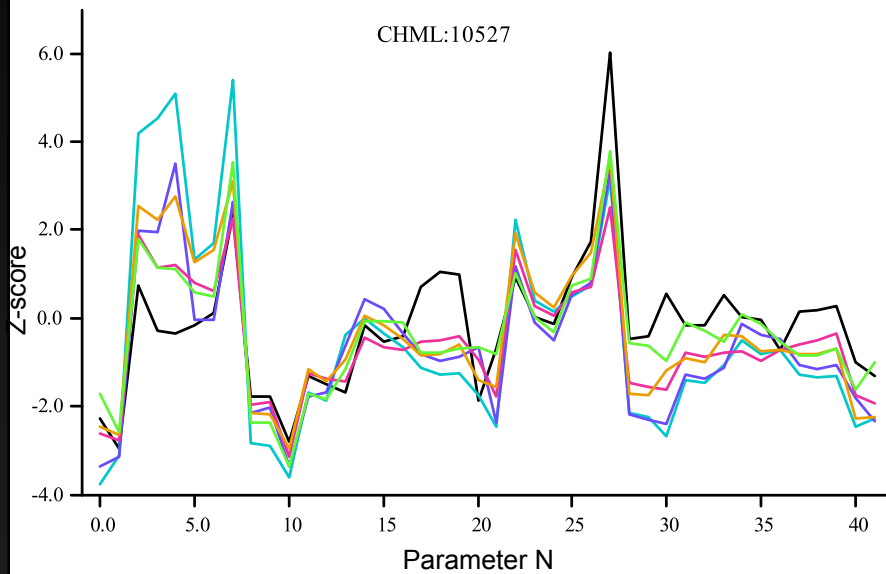
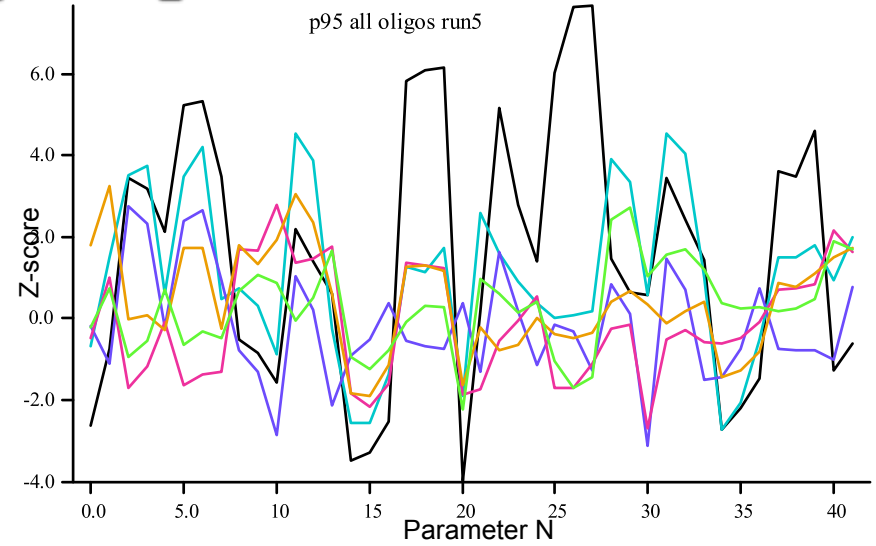
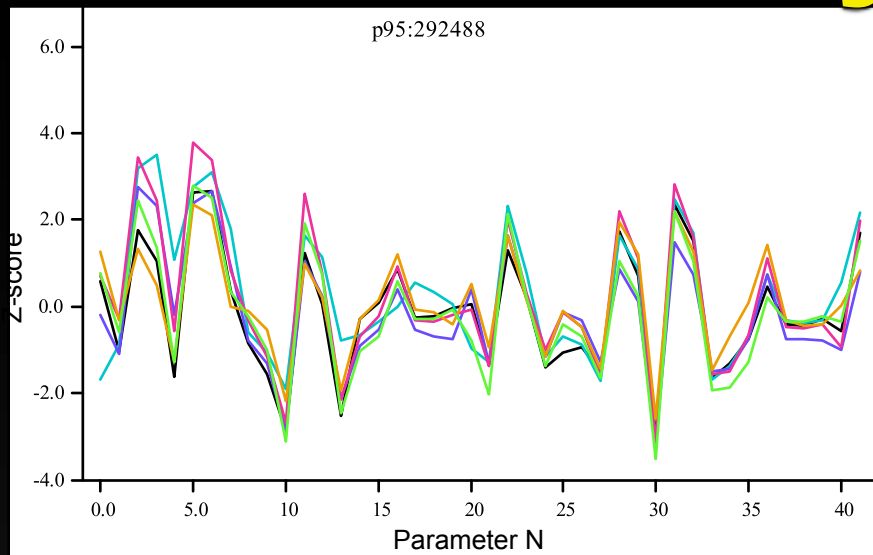
~ 60 PARAMETERS

- Total fluorescence intensity → Total signal in the image
- Number of vesicles
- Weighed mean size of vesicles
- Weighed mean intensity → Mean vesicle brightness
- Total integral vesicles intensity → Total signal associated with vesicles per image
- Mean integral intensity → Average total signal associated with vesicle per vesicle
- Peer to peer distance → Endosome clustering, size of cluster
- Distance to the nucleus → Average vesicle distance



Prof. Nagel, University of Technology Dresden

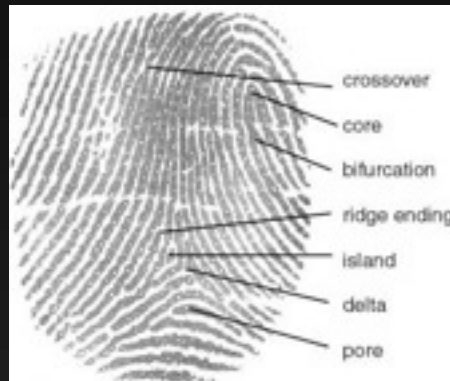
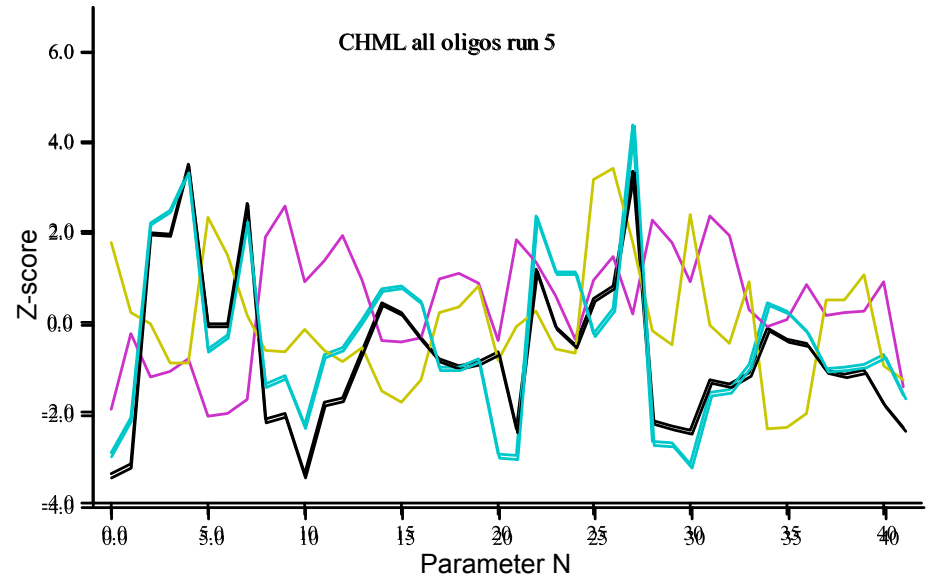
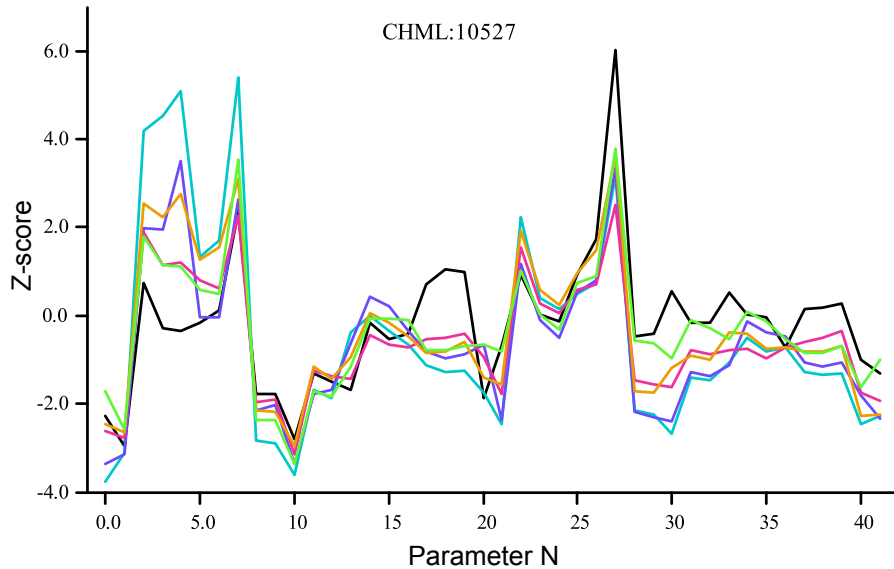
Phenotype profile



Phenotype profile

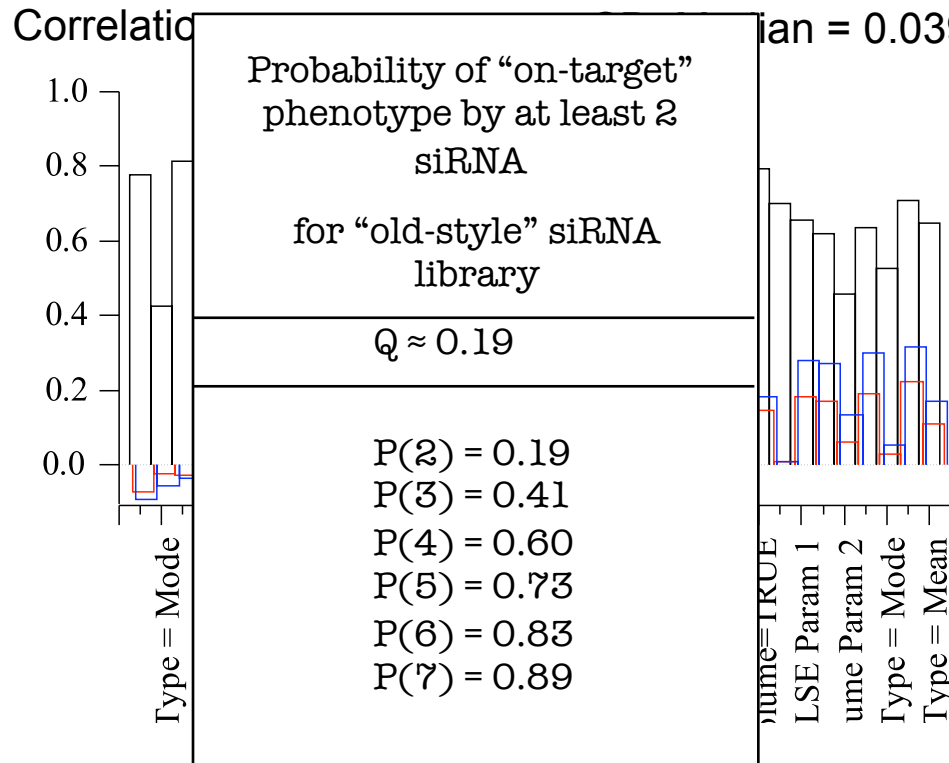
Same siRNA repeated 5 times

5 siRNA in one experiment



Pearson Correlation Coefficients for siRNA Kinome library (1206 gens, 3343 non-lethal siRNA)

Mean = 0.19
SD = 0.18
SD_Mean = 0.029
Median = 0.169
IQR = 0.039

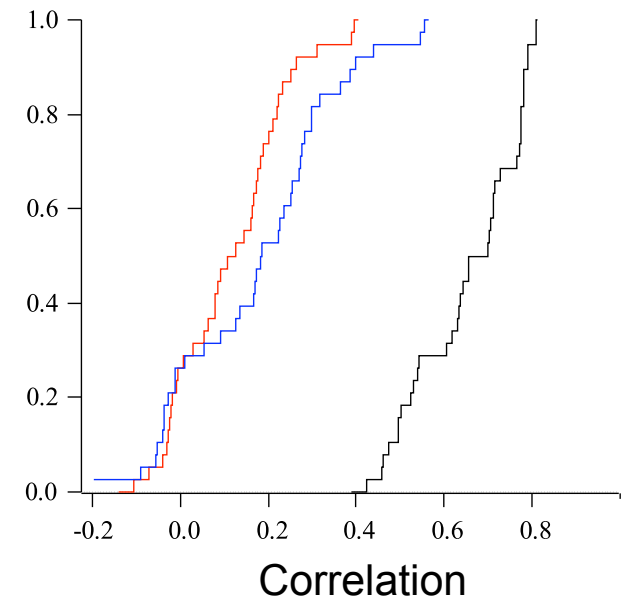


Correlation of Replicates

Correlation of siRNAs

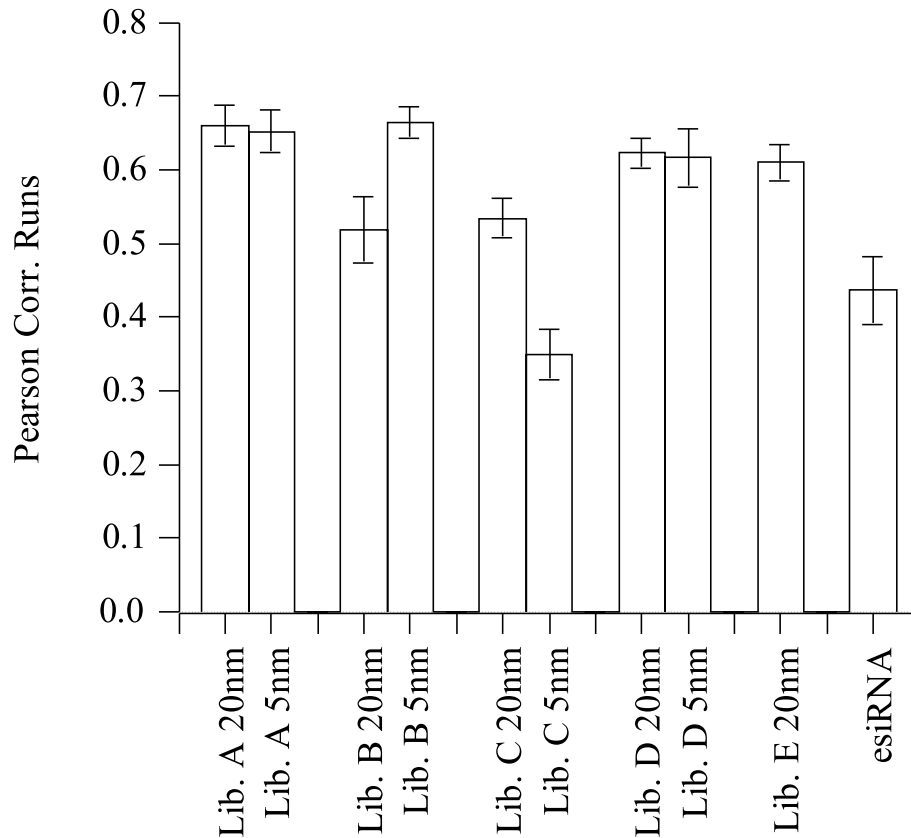
Ratio of Correlations

CPF

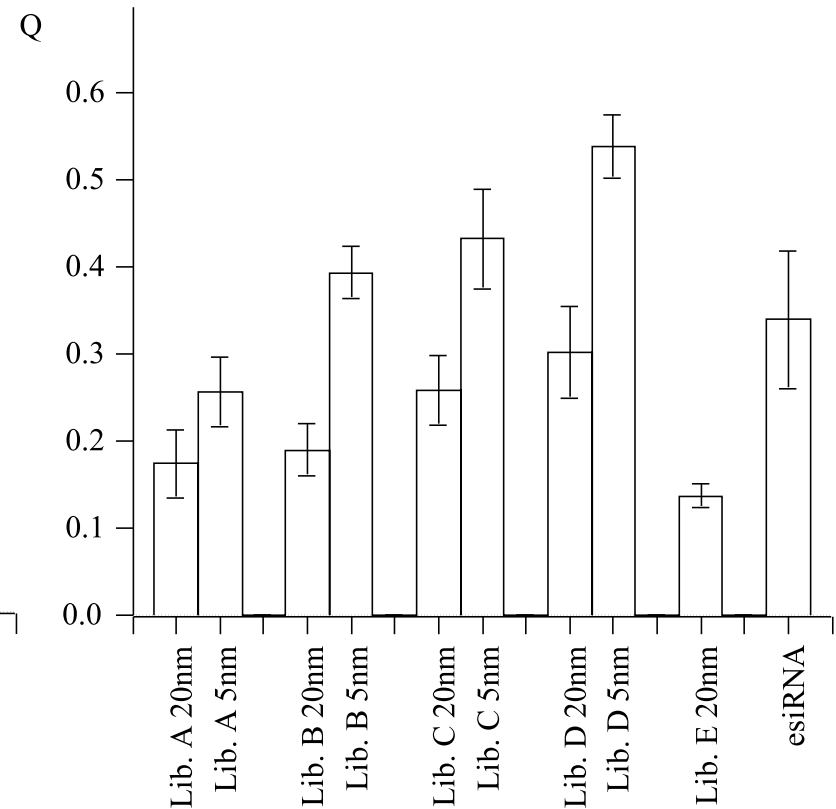


Library quality comparison

Phenotype stability (C_{p_r})

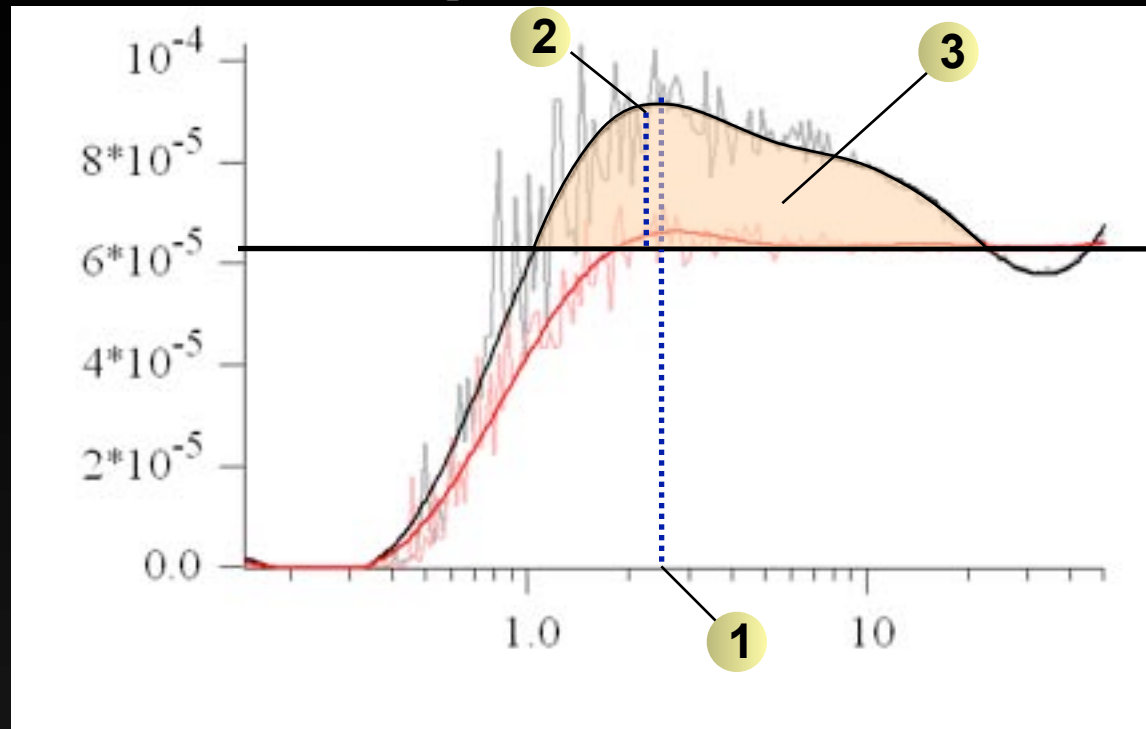


Phenotype specificity (C_{p_o}/C_{p_r})



Endosome Distribution Analysis

Peer- to peer distance Distribution



1.: Peak position
= Mean radius of clusters

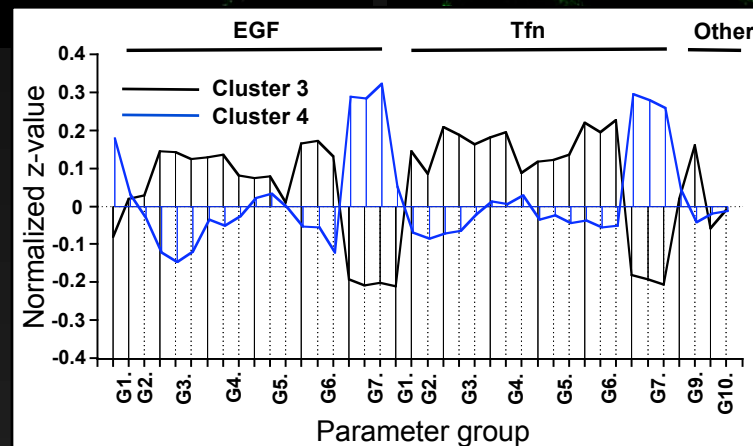
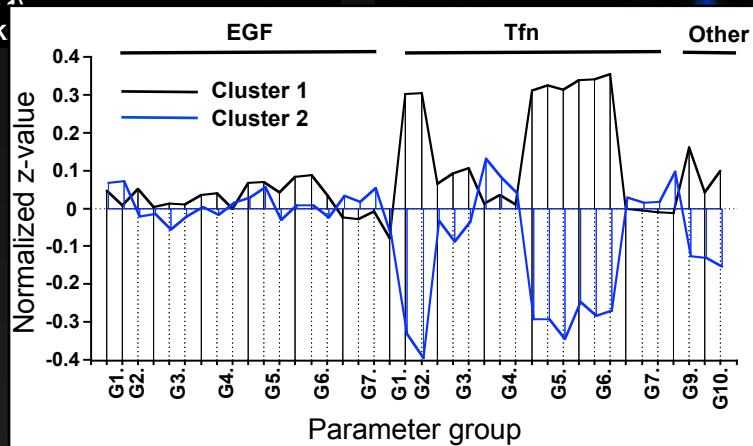
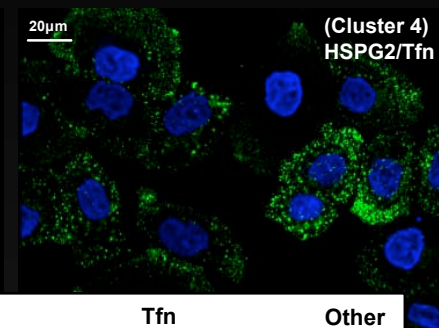
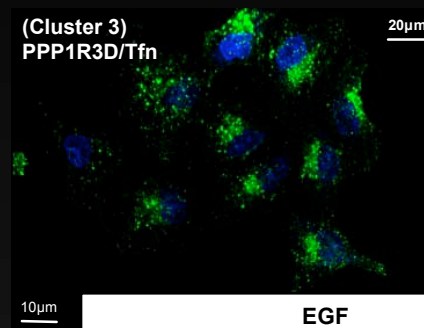
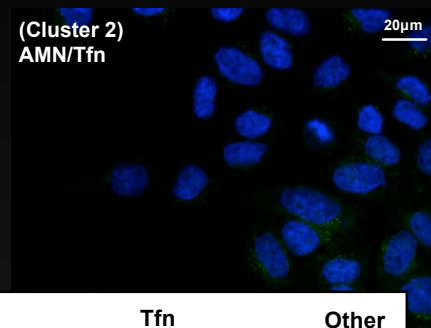
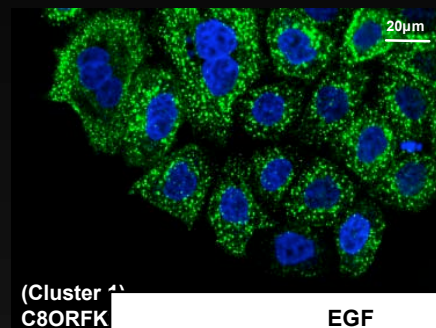
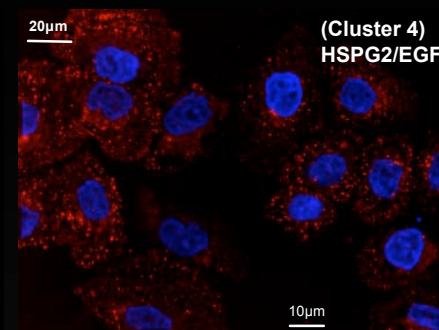
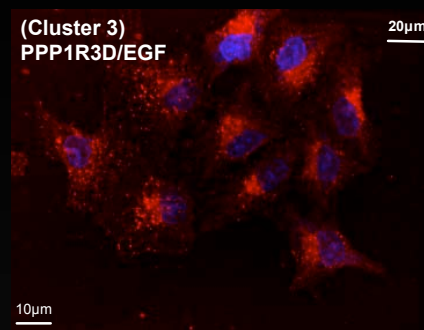
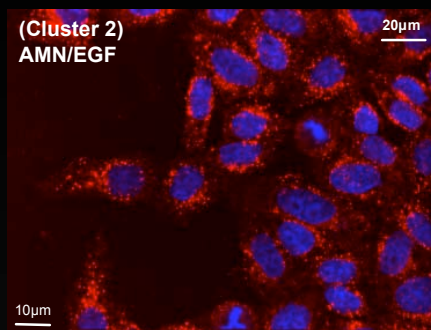
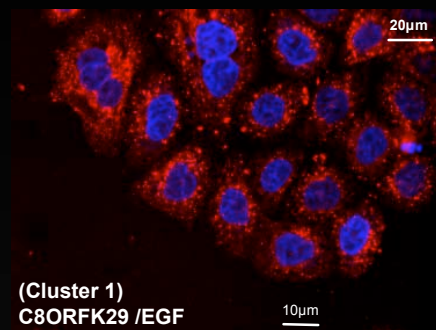
2.: Amplitude
= Peak Density in cluster (n of endosomes)

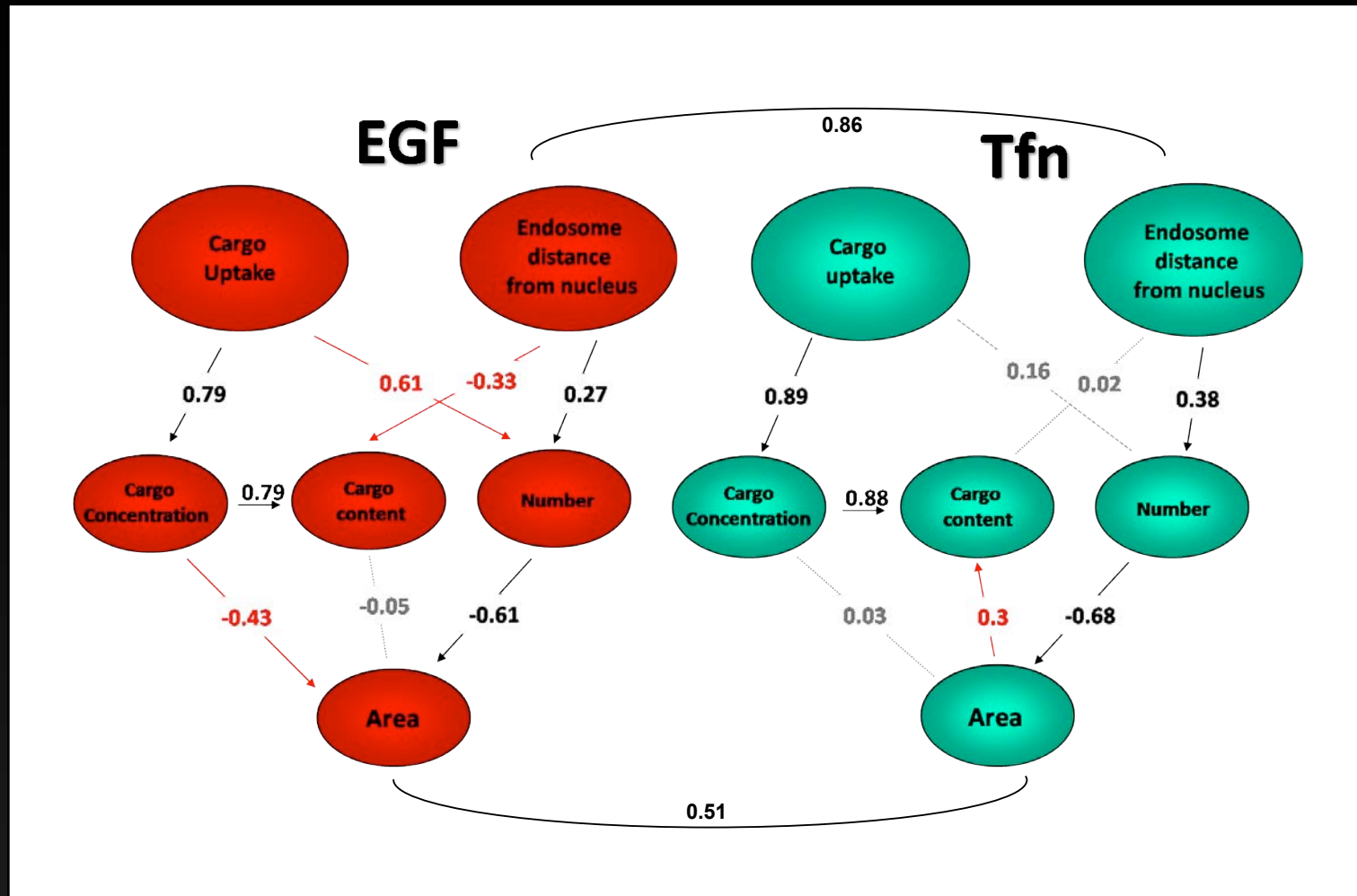
3.: Integral
= Proportional to clustered fraction

4.: Mean Distance to Nucleus
= subcellular position of Clusters

System Survey of Endocytosis by siRNA

- We performed a GWS on EGF and Tfr endocytosis
- We use 161.500 oligos
- We acquired and store 2.4 Milions images
- We generate 18 TB of Data
- The calculation used 3 milions of cpu hours





CG	Description	Hits
1	Selective up-regulation of Tfn endocytosis	932
2	Selective down-regulation of Tfn endocytosis	88
3	Specific effect on subcellular localization: endosomes appear clustered in the cell centre	801
4	Specific effect on subcellular localization: endosomes appear dispersed in the cell periphery	260
5	Opposite effects on EGF and Tfn endocytosis: EGF endocytosis is increased and Tfn endocytosis is decreased	224
6	Opposite effects on EGF and Tfn endocytosis: EGF endocytosis is decreased and Tfn endocytosis is increased	143
7	Effects on endocytosis of both markers: increased EGF and Tfn endocytosis	137
8	Effects on endocytosis of both markers: decreased EGF and Tfn endocytosis	799
9	Selective up-regulation of EGF endocytosis	178
10	Selective down-regulation of EGF endocytosis	324
11	Selective up-regulation of EGF endocytosis with accumulation of endosomes in cell centre	271
12	Reduced Tfn endocytosis with endosomes accumulated in the cell centre	204
13	Selective increase in EGF endosomes number and elongation	38
14	Increase in elongation of Tfn endosomes with mild increase of Tfn endocytosis	37