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# HCA Redistribution assays: Adaptation of assays designed for compound screening to RNAi screening procedures

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# RNAi screening

- RNAi screening is a wholly complementary approach to classical small-molecule library screening and can significantly augment such a program in moving a drug candidate quickly through the drug discovery pipeline.

- Combining automated primary screens and cell imaging analysis methods can help to examine multiple intracellular events induced by RNAi-based gene silencing in a high-throughput manner.

- Pre-defined siRNA Libraries: Thermo Scientific Dharmacon® siGENOME siRNA libraries are available in collections arranged by gene family and function, from small focused subsets up to whole genome collections.

# Small molecule screening vs. RNAi screening

| | **Small Molecules** | **RNAi Reagents** |
|---|---|---|
| **Target** | Protein or complex | mRNA |
| **Delivery method** | None | Variable |
| **Controls** | Reference compound | RNAi control reagent |
| **Time course** | Minutes to hours | Several days |
| **Statistical analysis*** | Sample-based normalization | Control-based normalization |

*Birmingham A, et al. (2009) Statistical methods for analysis of high-throughput RNA interference screens. Nat Methods. Aug;6(8):569-75

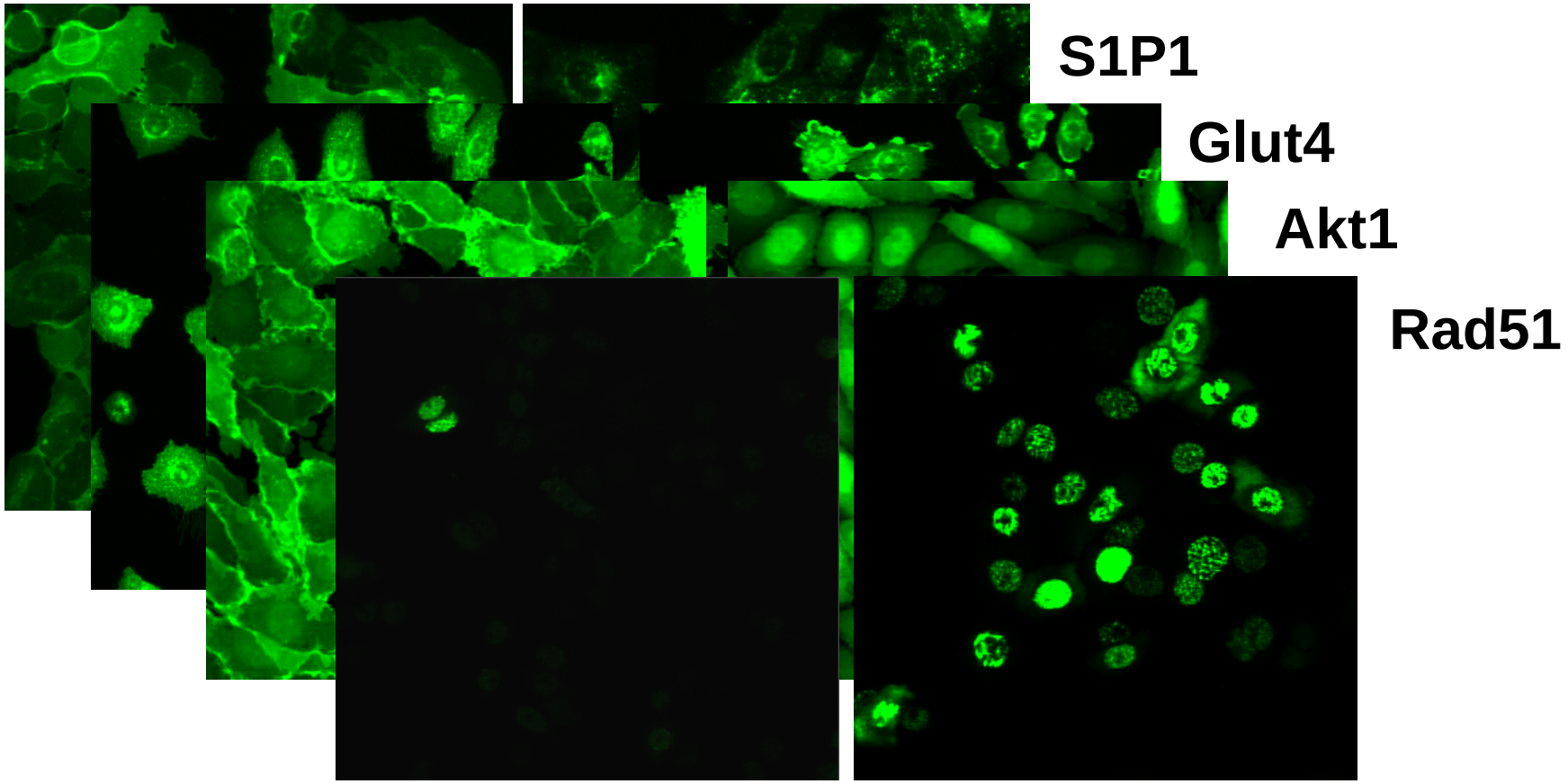
# Redistribution® assay technology

- Thermo Scientific Redistribution® technology is a cell based assay technology that uses protein translocation as a readout for the activity of cellular signaling pathways.

- Redistribution assays monitors the cellular translocation of EGFP-tagged proteins in response to drug compounds or other stimuli.

- Fluorescent proteins enable a genetically encoded experimental system that is
  - *relatively simple*
  - *less time consuming*
  - *very low in operational cost*
  - *indefinitely scalable.*

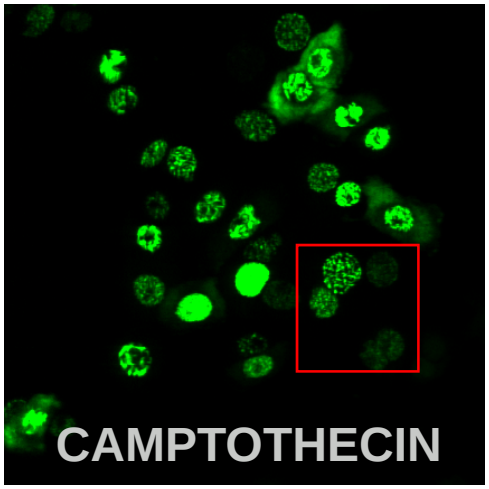
# Redistribution assays monitors the cellular translocation of EGFP-tagged proteins in response to stimuli.

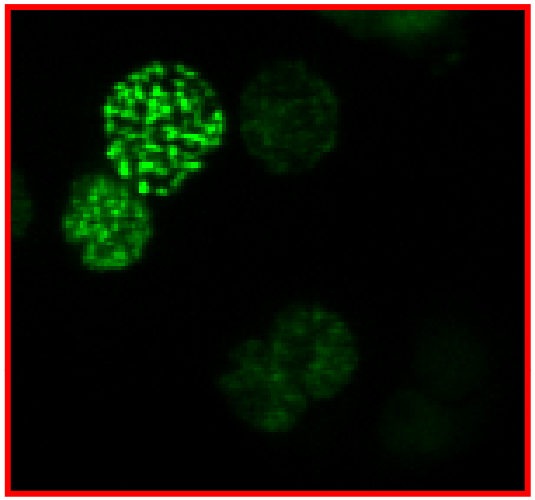


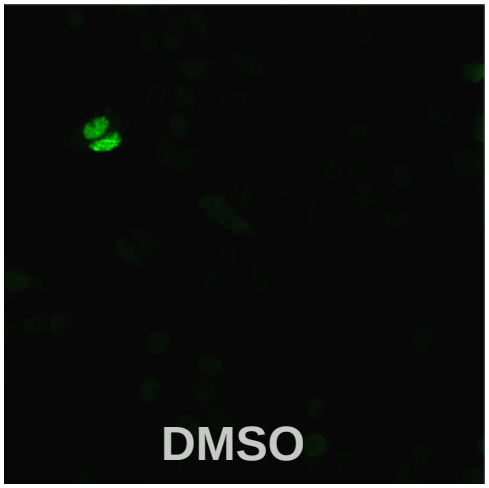
# Small molecule screening vs. RNAi screening

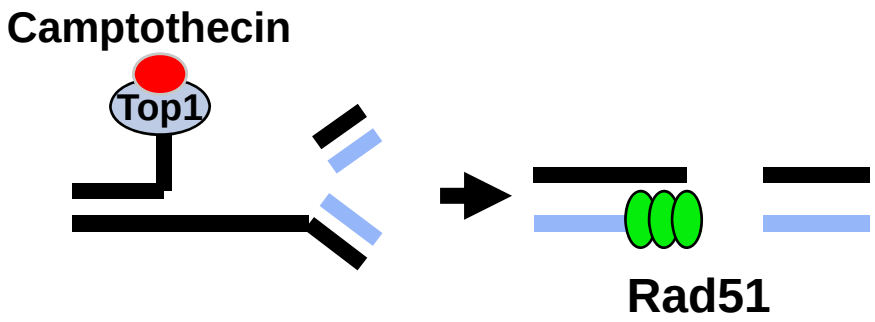
- **Redistribution assays were initially developed for screening and profiling of small molecule drug candidates however these assays are also amenable to use with RNAi reagents for functional genomic screening in mammalian cells.**

# Selection of an assay: Rad51_SW480 Redistribution assay

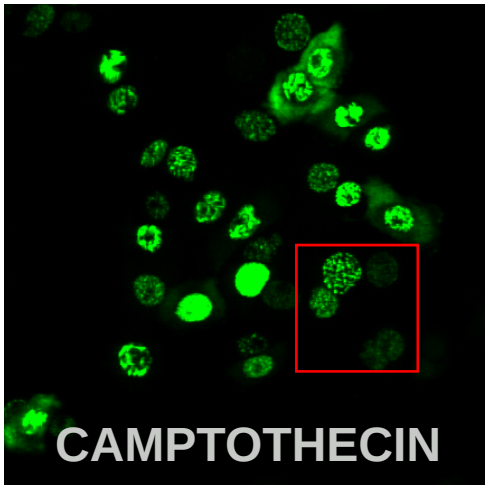


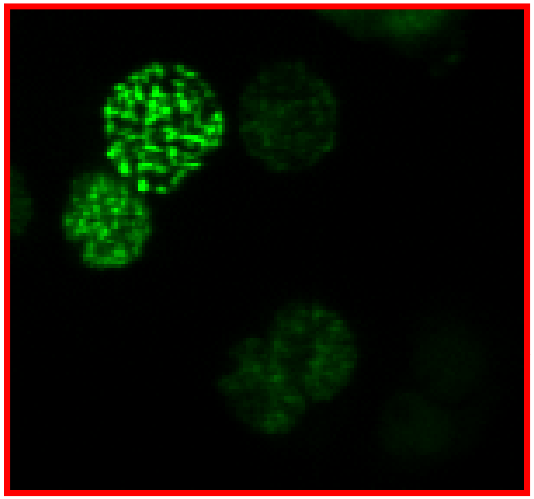



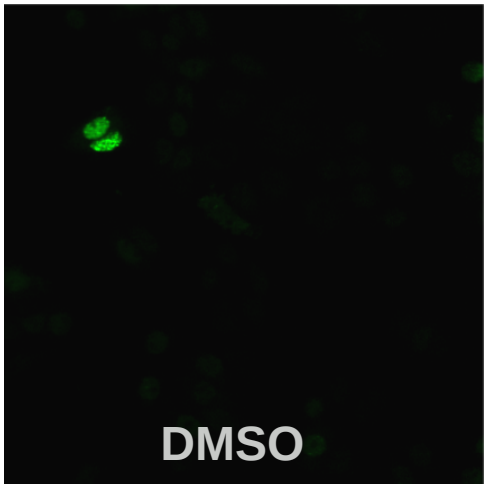


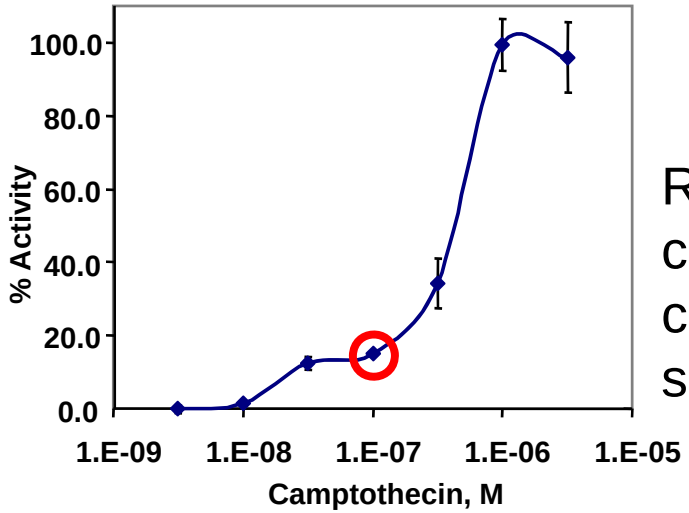



# Selection of an assay: Rad51_SW480 Redistribution assay



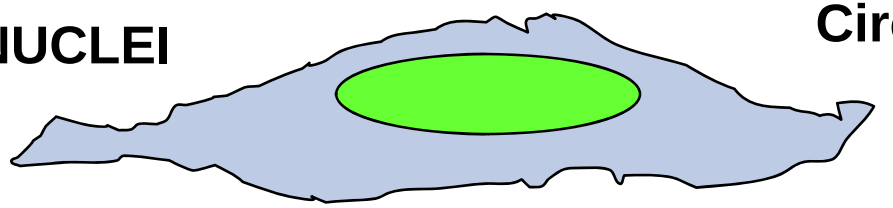



Rad51_SW480 assay can be used for chemotherapy sensitization screening

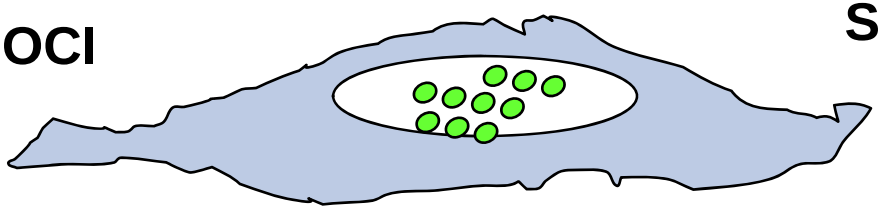
# HCA readouts from Rad51 assay

**Rad51**
**accumulation- NUCLEI**



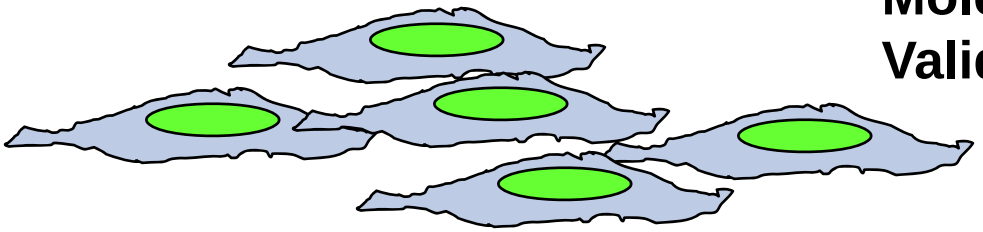
**Molecular Translocation**
**CircAvgInten**

**Rad51**
**accumulation- FOCI**



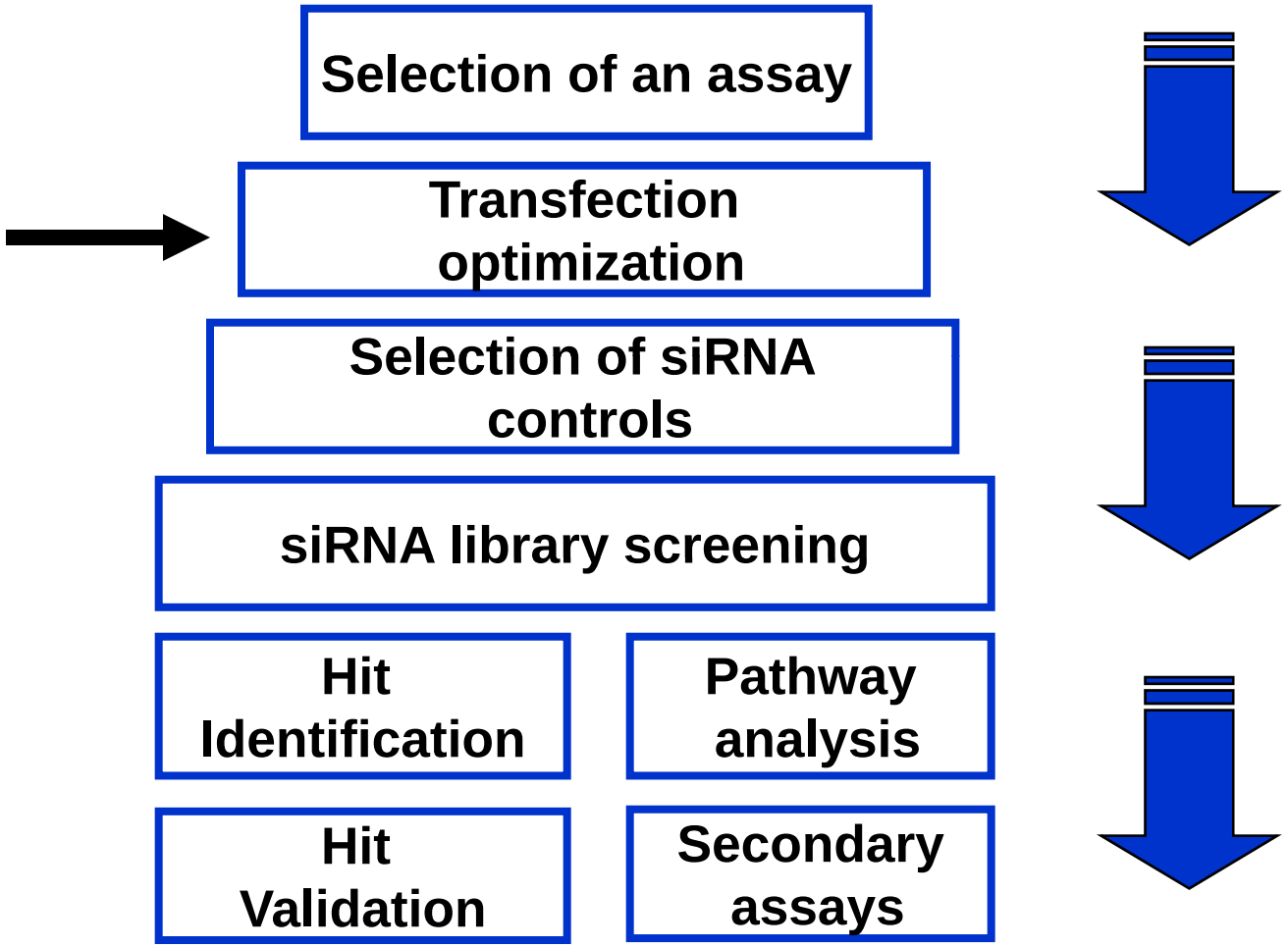
**Spot Detector**
**SpotTotalInten**

**Cell Count**

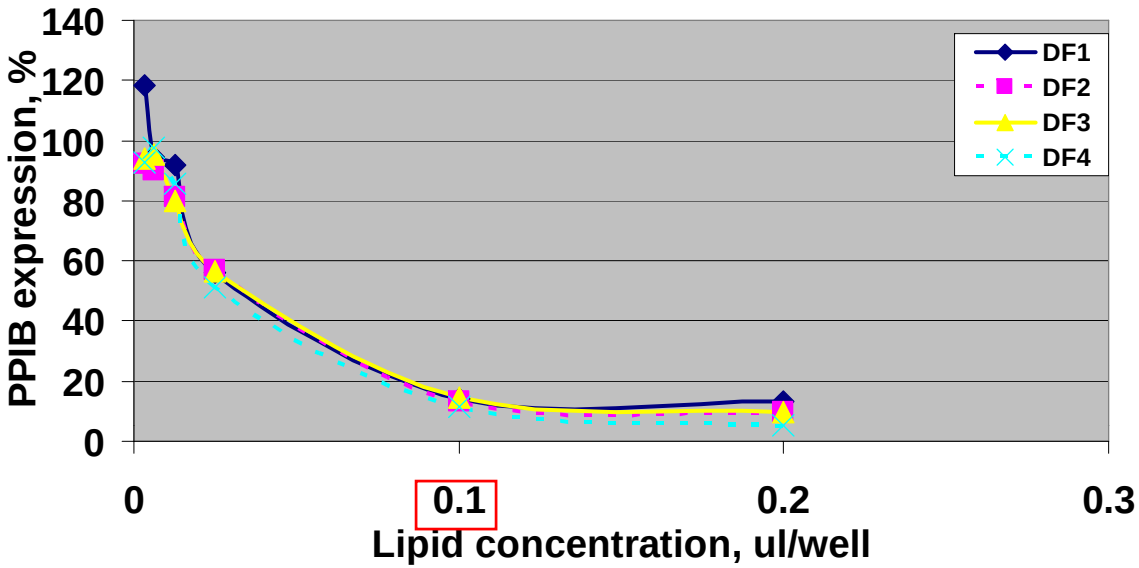


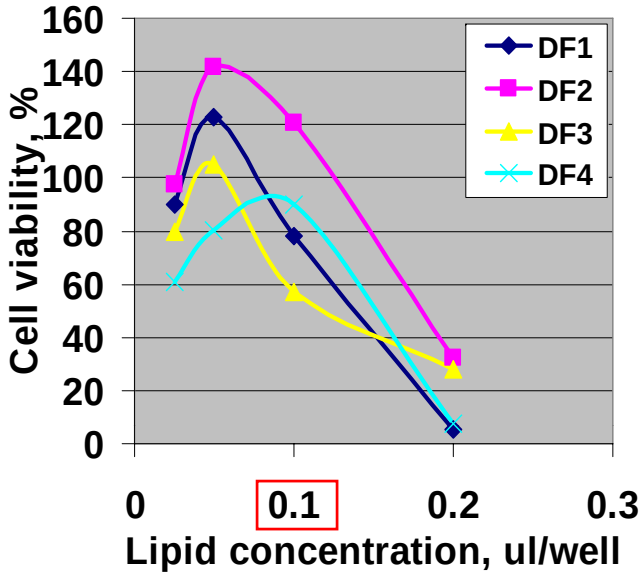
**Molecular Translocation**
**ValidCellCount**

# RNAi high throughput screening workflow



# Transfection optimization



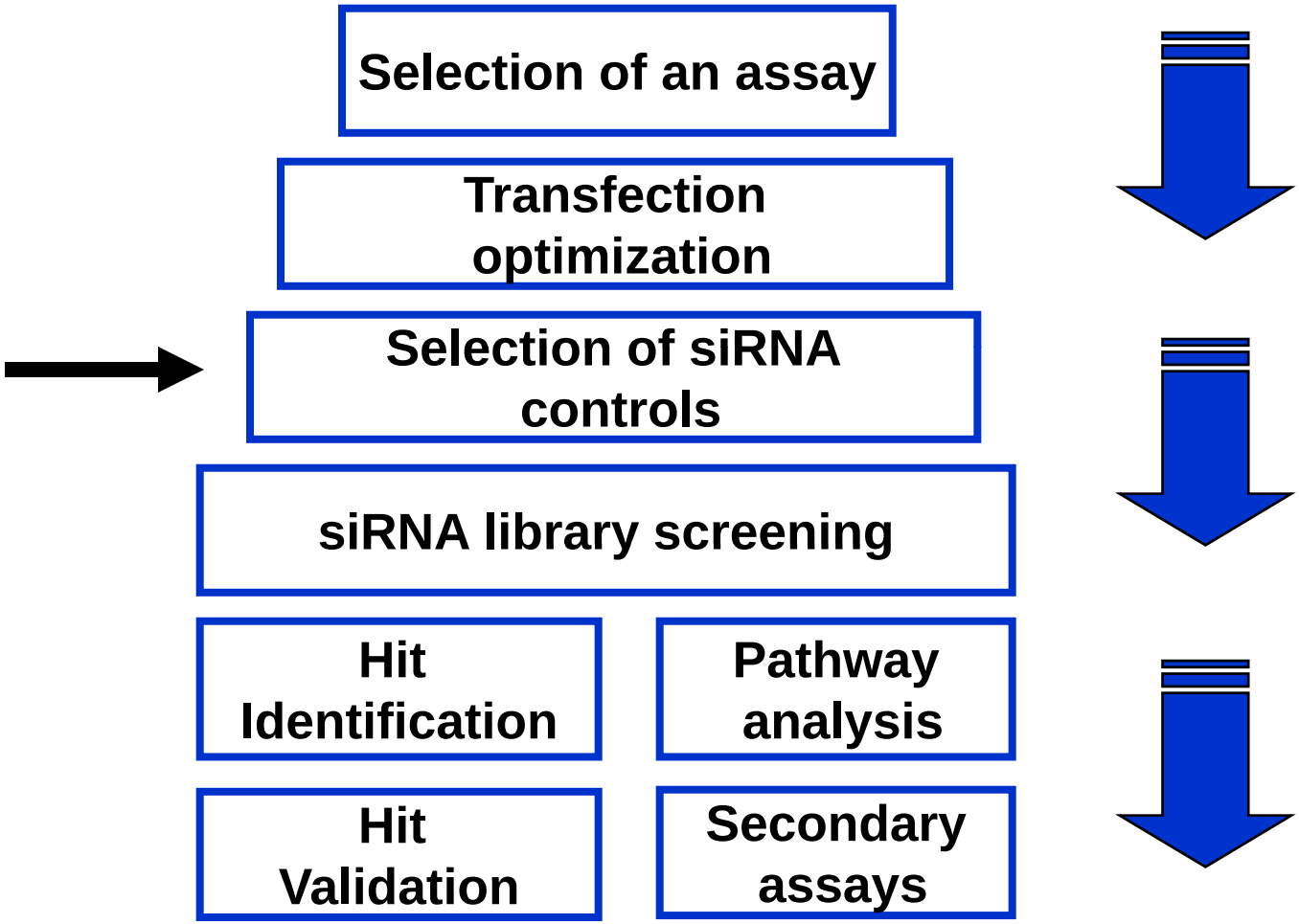




**Rad51 accumulation- NUCLEI**

| | DF1 | DF2 | DF3 | DF4 |
|---|---|---|---|---|
| **Z' factor** | 0.53 | 0.82 | 0.6 | 0.17 |
| **CV%** | 3 | 10 | 20 | 17 |

Dharmafect ® 2 was selected as the best performing lipid transfection reagent.

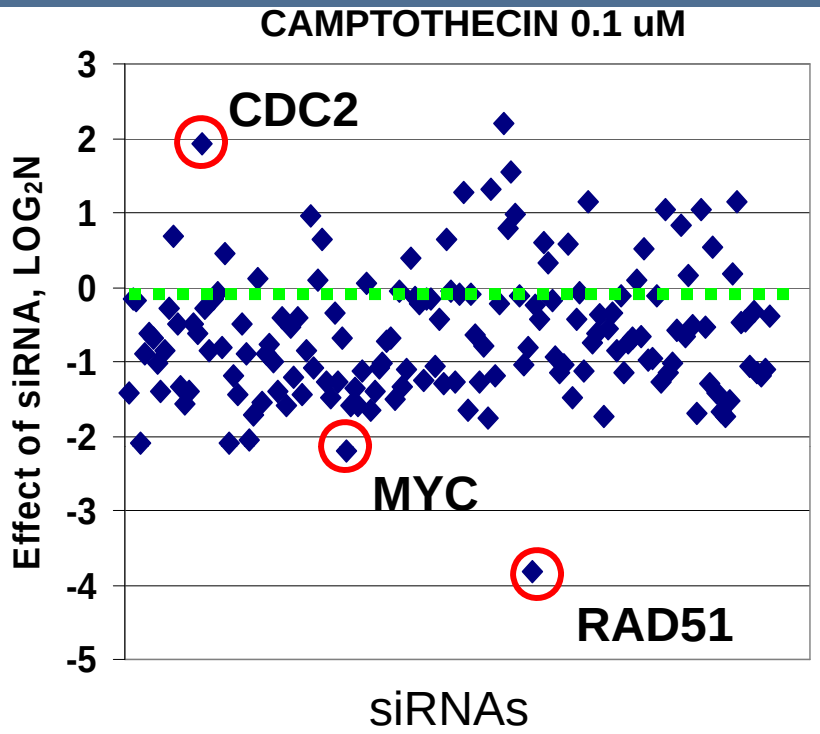
# RNAi high throughput screening workflow

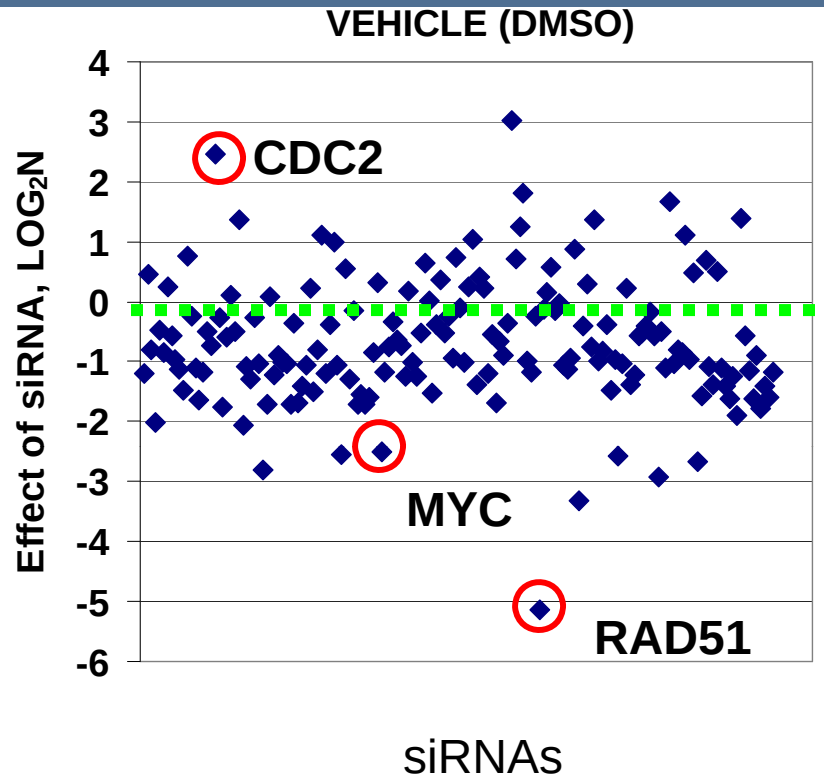


# Selection of proper siRNA controls – CircAveInt readout

- For the selection of positive siRNA controls we utilized RNAi based screening of Dharmacon siGENOME® human Cell Cycle siRNA Library :
  - Dharmacon siGENOME® human Cell Cycle siRNA Library allows for comprehensive, systematic study of cell cycle proteins via siRNA-induced gene silencing using a convenient multi-well format.
  - The library is comprised of 111 siRNA SMARTpools targeting individual genes.

# Selection of proper siRNA controls

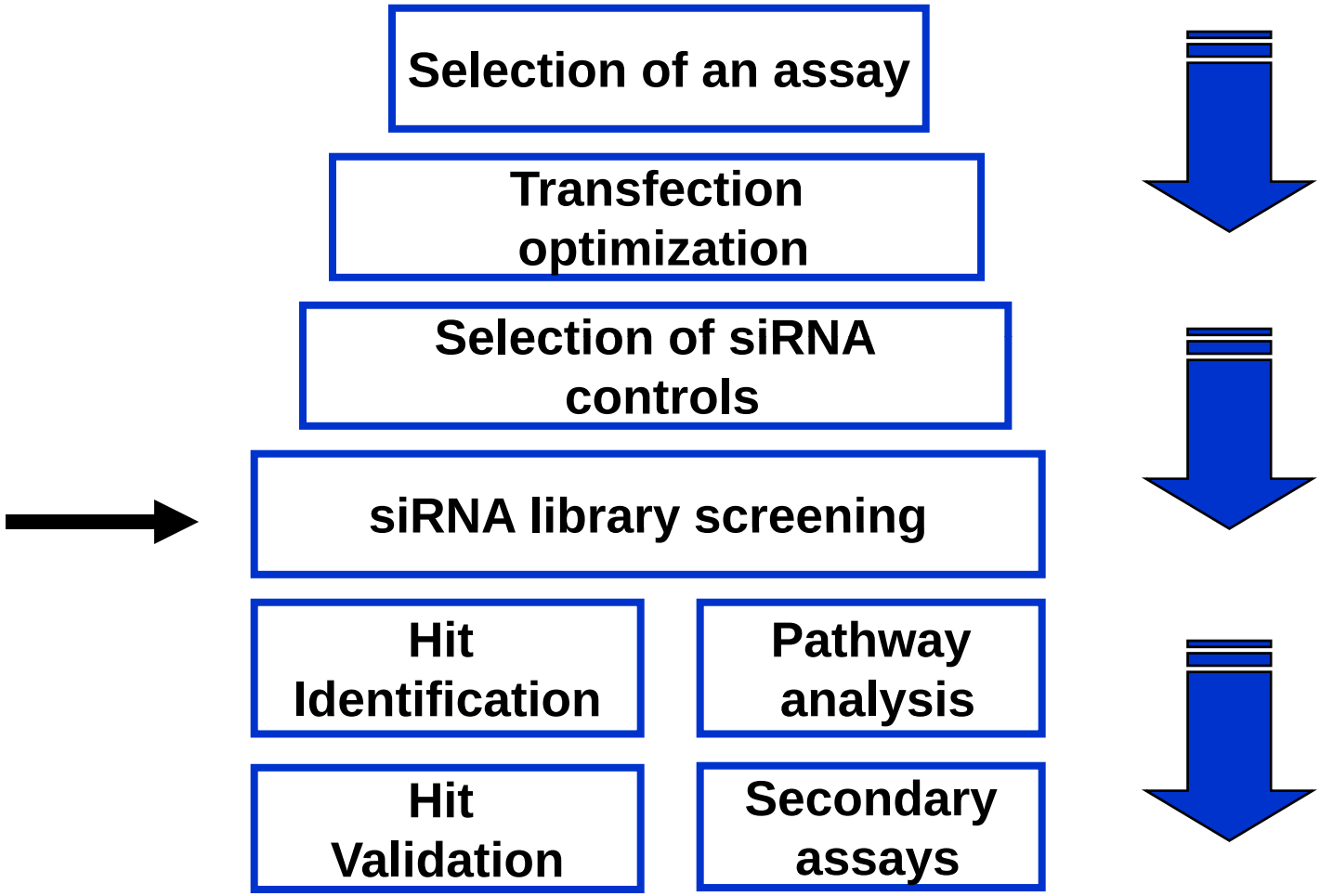



**Rad51 accumulation- NUCLEI**

| | CDC2 | MYC | RAD51 |
|---|---|---|---|
| **Z' factor (C 0.1)** | 0.66 | 0.71 | 0.74 |
| **Z' factor (DMSO)** | 0.71 | 0.55 | 0.53 |

# RNAi high throughput screening workflow



# RNAi screening with Redistribution® assays

**Thermo Scientific Dharmacon siGENOME siRNA SMARTpool siRNA Libraries**

**Individually arrayed SMARTpool siRNAs, 96-well plate format**

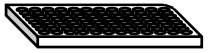


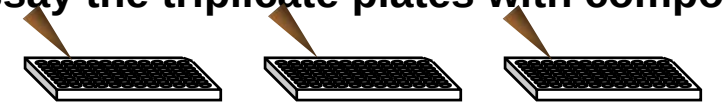
**Plate cells and incubate 24 hours**

**Transfect plates in triplicate**

**Controls in columns 1 & 12, corners excluded**

**Incubate 72 hours**

**Assay the triplicate plates with compound**

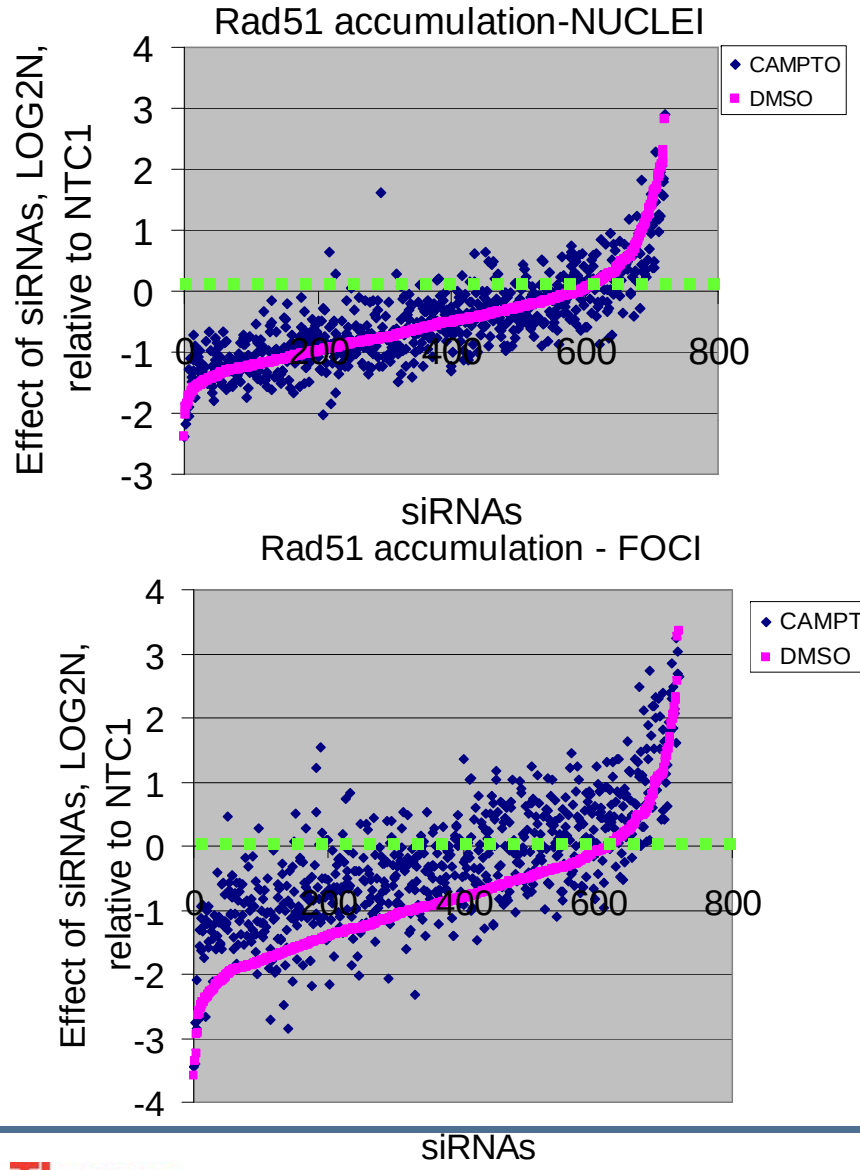


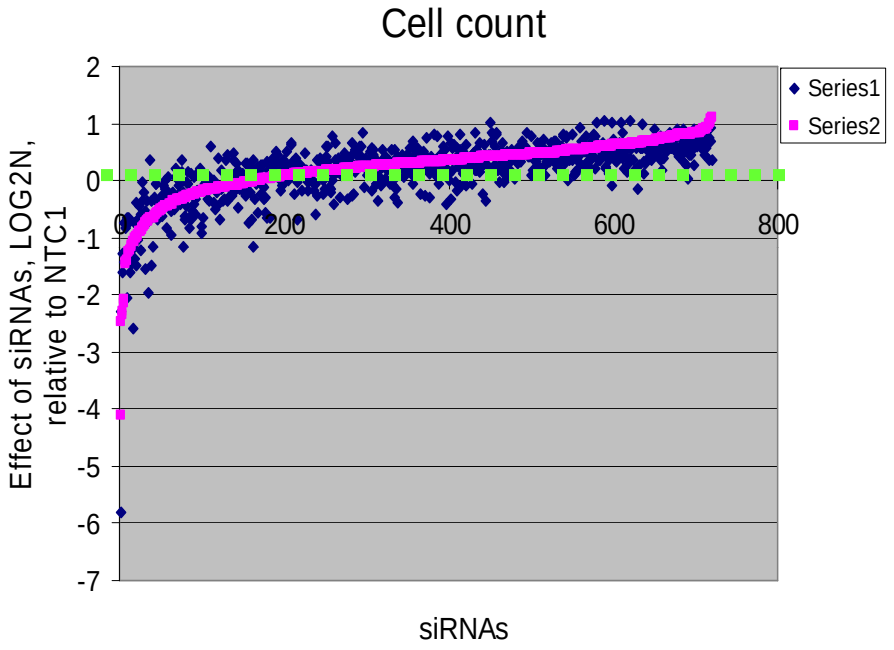
**Incubate 24 hours**

**Endpoint analysis**

**HCA readout**

# Kinome-wide camptothecin sensitization screen with Rad51 Redistribution assay

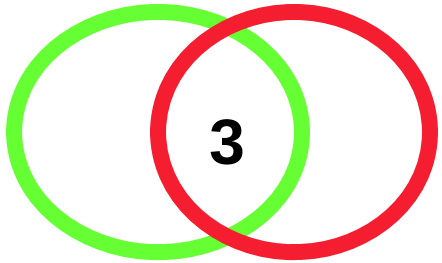



Library data was normalized to cells transfected with **N**on **T**argeting **C**ontrol siRNA pool #1, NTC1 on a per plate basis.

# siRNA hit selection – EGFP intensity readouts

**Rad51 - NUCLEI** **9**



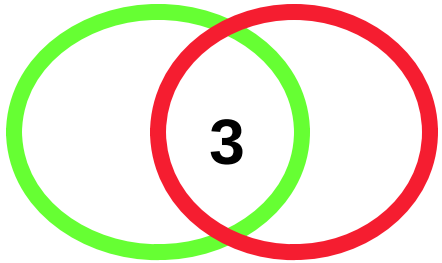

**7** **Rad51 - FOCI**

- **EGFP intensity readouts:** hit identification was performed based on following criteria:
  - 1. CAMPTOTHECIN: Student's t-test p-values ($p<0.05$)
  - 2. CAMPTOTHECIN: fold change, (>1.5, or 33% increase).
  - 3. DMSO, change, fold (<1.2 or 17% increase)

- **Rad51 NUCLEI and FOCI hits were combined into one group, 16 hits.**

# siRNA hit selection – cell count

**Rad51 NUCLEI +FOCI**



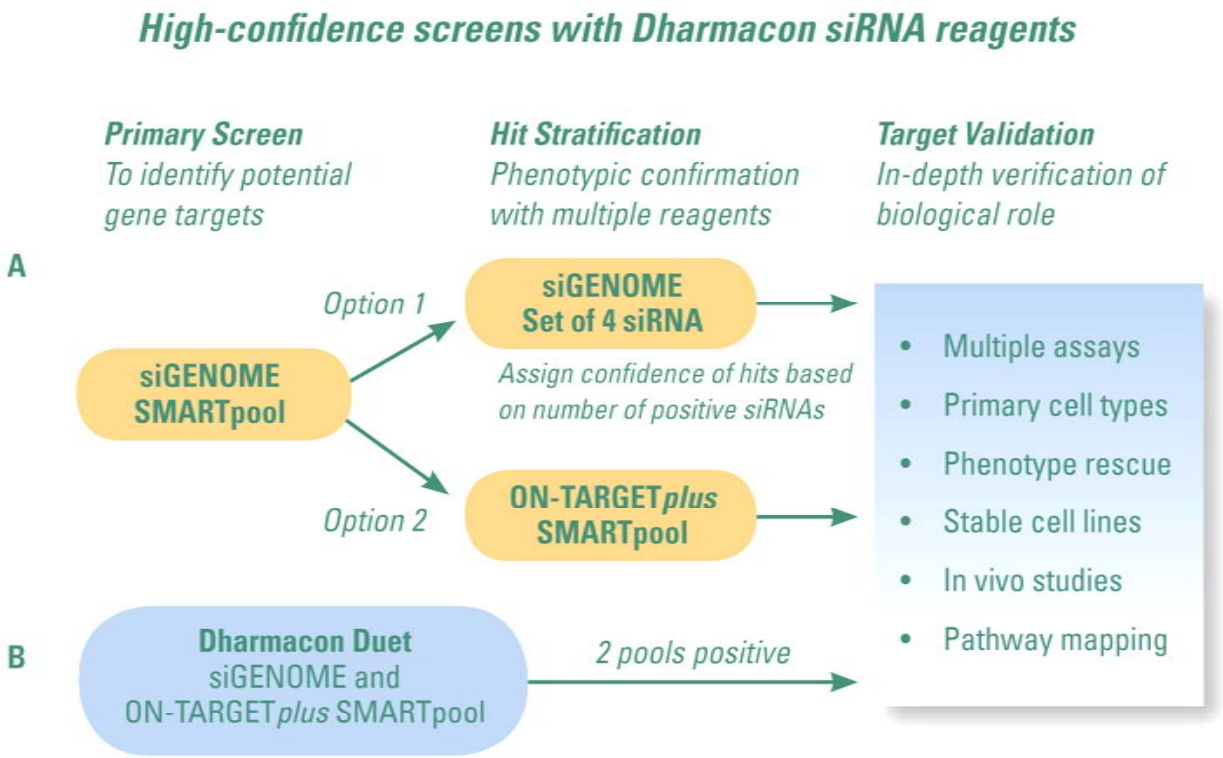

**Cell Count**

- **Cell Count readout:** hit identification was performed based on following criteria:
  - 1. CAMPTOTHECIN, Student's t-test p-values ($p<0.05$)
  - 2. CAMPTOTHECIN, fold change, (<-1.5, or 33% decrease).
  - 3 . DMSO, fold change, (> -1.1 or 9% decrease)

- **Cell Count readout: Four out of eight selected hits were previously identified as potential targets for anti-cancer therapy.**

# Hit validation

- Overall, 18 candidate hits were selected for hit validation and follow-up studies.
- All SMARTpool siRNA reagent-derived candidate hits that were selected in primary screening will be subjected to independent procedures for hit prioritization and subsequent validation.



# Conclusions

- Resdistribution assays can be easily adapted to RNAi-based screens
- Combination of RNAi screening and HCA allows simultaneous collection and  analysis of multiple parameters
- The redistribution analysis of Rad51-EGFP fusion protein in cells where targets of Kinome  library were individually knockdown allowed us to examine their role in the DNA damage/repair pathways