

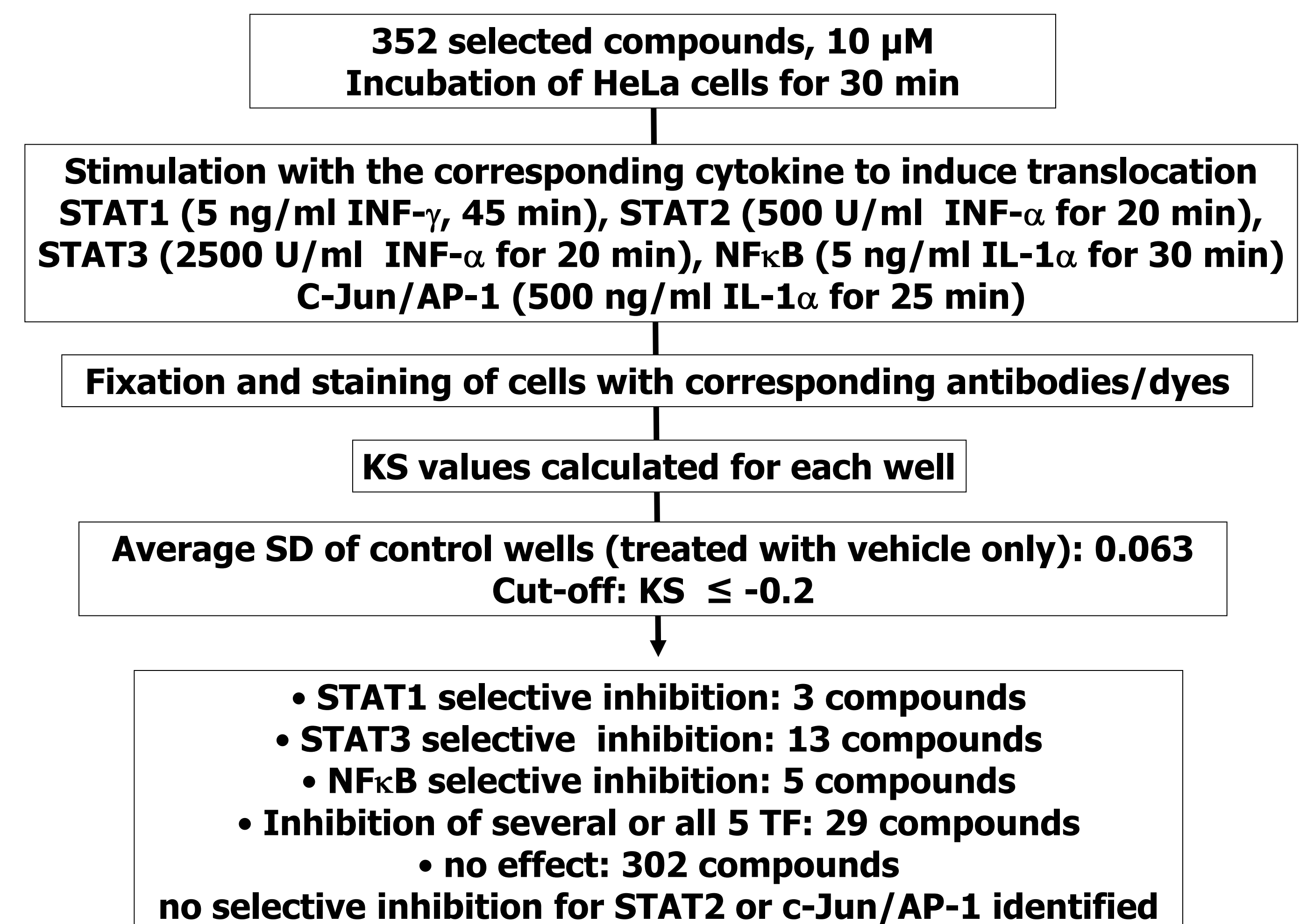
Introduction

Transcription factors are regulatory proteins which bind to target specific DNA sites when activated by receptor-specific mediators. Many transcription factors form homo- or hetero-dimers upon activation and translocate from the cytoplasm into the nucleus. Quantification of nuclear translocation of activated transcription factors with automated fluorescence microscopy represents a High Content (HC) screening method in medium- to high-throughput format.

BioFocus has established a panel of cell-based assays for quantification of nuclear translocation of several STAT proteins and further transcription factors, offering a platform for compound profiling. Translocation assays for STAT1, STAT2, STAT3, STAT6, NF κ B and c-Jun/AP-1 are available. Most assays are based on immunofluorescence staining. STAT6 translocation is quantified with a proprietary cell line stably expressing a STAT6-ZsGreen fusion protein.

Here we present the profiling of 352 compounds with a panel of transcription factor assays in HC format. Compounds were selected from the BioFocus library by a similarity search based on structures of known kinase inhibitors. Influence of the selected compounds on activation pathways of the following five transcription factors was tested: STAT1, STAT2, STAT3, NF κ B and on c-Jun/AP-1. Assays were run in 384-well format, measured with an ArrayScanII and acquired data were analyzed using in-house BioFocus data miner software. Several compounds were identified which inhibited only one specific activation pathway. Other compounds showed a rather unspecific profile and inhibited translocation of several transcription factors. The high content transcription factor assays at BioFocus provide a useful platform for compound profiling in a variety of signal transduction pathways.

Compound testing, results



STAT1 translocation assay

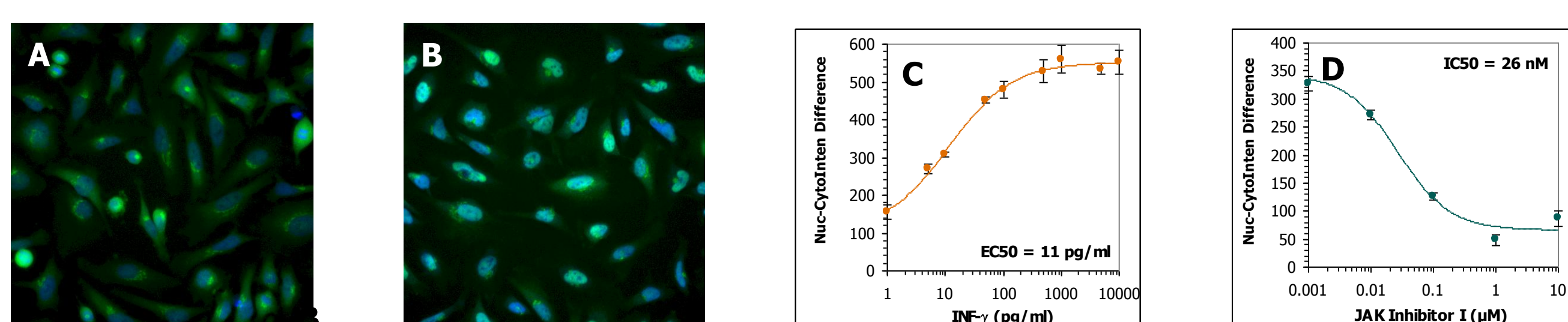


Figure 1: Composite images of HeLa cells treated with vehicle (A) or 10 ng/ml INF- γ (B) for 45 min. Dose response of INF- γ , 45 min incubation (C) and dose-dependent inhibition of STAT1 translocation by JAK Inhibitor I (D). Cells were pre-incubated with inhibitor for 20 min before stimulation with 2 ng/ml INF- γ for 45 min.

STAT2 translocation assay

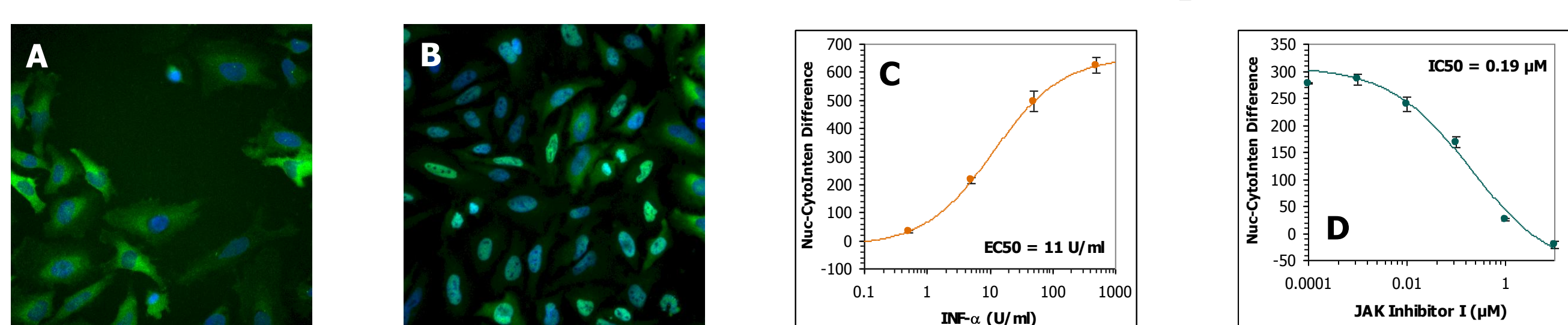


Figure 2: Composite images of HeLa cells treated with vehicle (A) or 5 U/ml INF- α (B) for 45 min. Dose response of INF- α , 45 min incubation (C) and dose-dependent inhibition of STAT2 translocation by JAK Inhibitor I (D). Cells were pre-incubated with inhibitor for 20 min before stimulation with 300 U/ml INF- α for 25 min.

STAT3 translocation assay

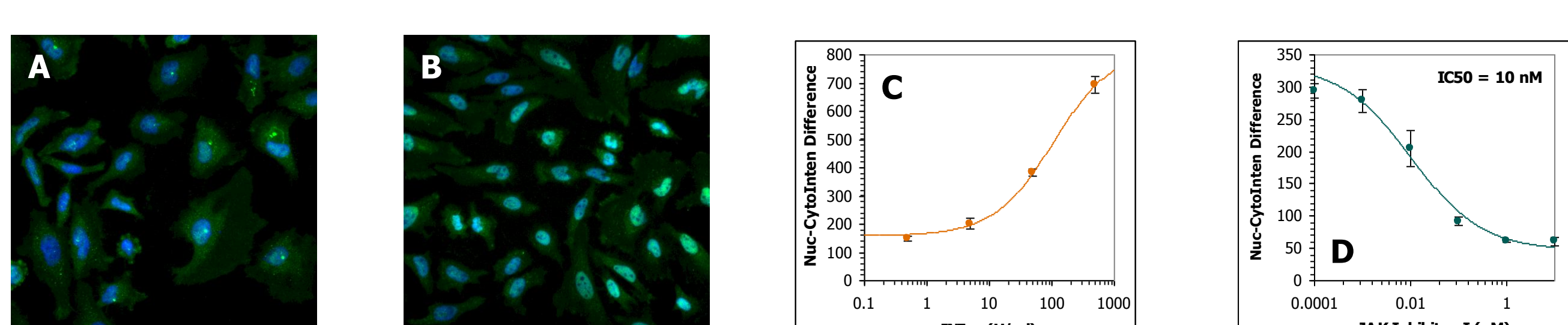


Figure 3: Composite images of HeLa cells treated with vehicle (A) or 500 U/ml INF- α (B) for 45 min. Dose response of INF- α , 45 min incubation (C) and dose-dependent inhibition of STAT3 translocation by JAK Inhibitor I (D). Cells were pre-incubated with inhibitor for 20 min before stimulation with 500 U/ml INF- α for 25 min.

Materials and methods

Reagents were purchased from Sigma or GIBCO, compounds were from the BioFocus compound collection, and HeLa cells were obtained from ECACC (93021013). Hit kits including antibodies and dyes were purchased from Cellomics, Inc. HeLa-STAT6 cells stably expressing STAT6-ZsGreen were generated in-house. Cells were seeded in PDL-coated 384-well microplates and treated the next day with compounds or with DMSO for 30 min. After compound incubation, the corresponding activator was added and cells were incubated at 37 $^{\circ}$ C accordingly. Cells were fixed with 3.7% formaldehyde, permeabilized, and stained with Hoechst 33342 and the corresponding transcription factor specific antibodies. HeLa-STAT6-ZsGreen cells were stained with Hoechst 33342 only. Microplates were measured and analyzed with the ArrayScanII (Cellomics). Approximately 500-1000 cells per well were analyzed and KS values (Kolmogorov-Smirnov analysis) of CircRingAvgIntenDiff were calculated for each sample well. Significant changes were defined by calculating KS values for replicate control samples and by using these data to set a threshold, below which, a cellular response would be considered significant. A KS lower or equal to -0.2 was regarded as significant, which corresponds to the three fold standard deviation of all KS values of the reference wells (3×0.063).

STAT6 translocation assay

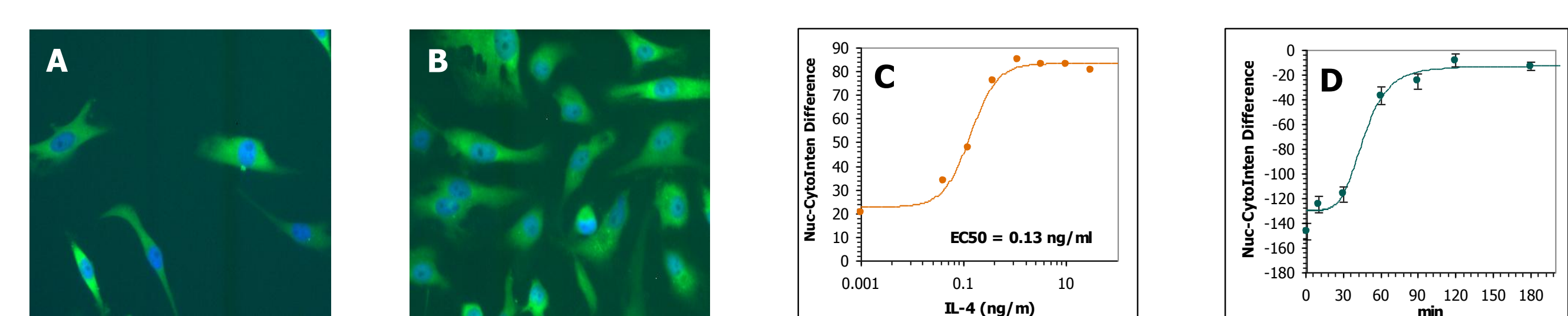


Figure 4: Composite images of HeLa cells expressing STAT6-ZsGreen treated with vehicle (A) or 3 ng/ml IL-4 (B) for 90 min. Dose response of IL-4, 90 min incubation (C) and time course with 1 ng/ml IL-4 (D).

NF κ B translocation assay

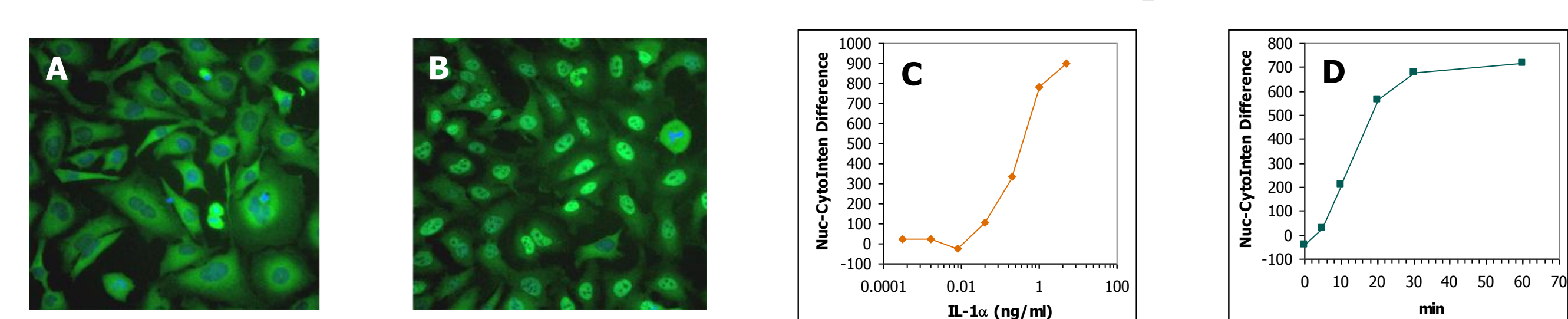


Figure 5: Composite images of HeLa cells treated with vehicle (A) or 2 ng/ml IL-1 α (B) for 30 min. Dose response of IL-1 α , 20 min incubation (C) and time course with 2 ng/ml IL-1 α (D).

c-Jun/AP-1 activation assay

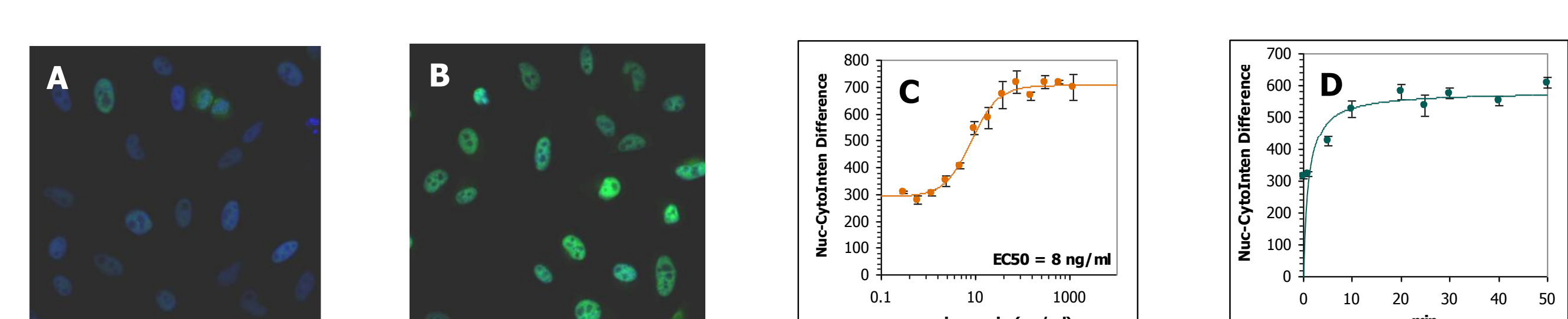


Figure 6: Composite images of HeLa cells treated with vehicle (A) or 100 ng/ml anisomycin (B) for 25 min. Dose response of anisomycin, 25 min incubation (C) and time course with 100 ng/ml anisomycin (D).

Table 1. Transcription factor assays, activators and detection methods used for quantification of nuclear translocation in human cell lines using the ArrayScanII

TF	Activator	Pathway	Detection method
NF κ B	IL-1 α	IKK1, IKK2, IKK3, MEKK1	NF κ B p65 specific antibody
c-Jun/AP-1	Anisomycin	MEKKK1, MEK4, MEK7, JNK	Phospho-c-Jun specific antibody
STAT1	IFN- γ	JAK1, JAK2	STAT1 specific antibody
STAT2	IFN- α	JAK1, Tyk2	STAT2 specific antibody
STAT3	IFN- α	JAK1, Tyk2	STAT3 specific antibody
STAT6	IL-4	JAK1, JAK3	HeLa cells stably expressing STAT6-ZsGreen

Conclusions

- A panel of transcription factor assays is available to profile compounds for their influence on a variety of signal transduction pathways
- These assays are performed in high content format
- Screening can be performed for inducers or for inhibitors of nuclear translocation of the corresponding transcription factor.
- Pilot screens on 5 different transcription factors identified pathway-specific hits.

For further information please contact BioFocus at info@glpg.com