Supplementary Table S1

		Cell Numbers		RELA Translocation	
Compound	Concentration (µM except DMSO)	Average Normalized Cell Numbers	Standard Deviation	Average Normalized RELA Intensity Ratios (Nucleus/Cytoplasm)	Standard Deviation
Puromycin (Positive control)	125	0.53	0.09	1.58	0.08
Glycine (Negative control)	500	0.99	0.01	1.00	0.01
DMSO (Vehicle control)	0.5 %	1.00	0.01	1.00	0.00
ICA-1	500.0	0.94	0.01	1.03	0.00
	250.0	0.95	0.01	1.03	0.00
	125.0	0.95	0.00	1.03	0.00
	62.5	0.96	0.00	1.03	0.00
	31.0	0.96	0.01	1.03	0.00
	15.0	0.96	0.02	1.03	0.00
	2.0	0.97	0.01	1.03	0.00

Effects of ICA-1 on HepaRG Cells

Supplementary Table S2

Renal PTC Model	Phenotypic Features	Description

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5 Features	glcm_corr_mean:Actin:cell_region	Mean correlation of the whole-cell F-actin GLCM
	ccoeff_normed:DNA-Actin:cell_region	Correlation coefficient of DNA and F-actin intensities at the whole-cell region
	glcm_contrast_std:Actin:cell_region	Standard deviation in contrast of the whole-cell F-actin GLCM
	ccoeff_normed:DNA_gH2AX:cell_region	Correlation coefficient of DNA and γH2AX intensities at the whole-cell region
	glcm_contrast_mean:Actin:cell_region	Mean contrast of the whole-cell F-actin GLCM

Hepatocyte Model	Phenotypic Features	Description
5 DNA Features	total_intensity_ratio:DNA-DNA:nondna_region-cell_region	Ratio between total DNA intensity at the cytoplasmic region over the whole-cell region
	total_intensity:DNA:nondna_region	Total DNA intensity at the cytoplasmic region
	glcm_diff_var_mean:DNA:dna_region	Mean difference variance of the nuclear DNA GLCM
	cv_intensity:DNA:dna_chromosome	Coefficient of variation of DNA intensity at the chromosome region
	cv_intensity:DNA:nondna_region	Coefficient of variation of DNA at the cytoplasmic region

Hepatocyte Model	Phenotypic Features	Description
8 RELA Features	total_intensity_ratio:RELA-RELA:nondna_peridna-cell_region	Ratio between total REL-A intensity at the perinuclear region over the whole-cell region
	glcm_sum_ent_mean:RELA:cell_region	Mean sum entropy of the whole-cell REL-A GLCM
	total_intensity_ratio:RELA-RELA:nondna_region-cell_region	Ratio between total REL-A intensity at the cytoplasmic region over the whole-cell region
	total_intensity_ratio:RELA-RELA:nondna_inner-cell_region	Ratio between total REL-A intensity at the inner cytoplasmic region over the whole-cell region
	total_intensity_ratio:RELA-RELA:nondna_outer-cell_region	Ratio between total REL-A intensity at the outer cytoplasmic region over the whole-cell region
	total_intensity_ratio:RELA-RELA:dna_region-cell_region	Ratio between total REL-A intensity at the nuclear region over the whole-cell region
	fraction_obj_intensity:RELA:dna_chromosome-RELA_object	Fraction of total REL-A object intensity at the chromosomal region over the whole-cell region
	fraction_obj_intensity:RELA:nondna_peridna-RELA_object	Fraction of total REL-A object intensity at the perinuclear region over the whole-cell region
14 F-Actin Features	total_intensity:F-actin:cell_region	Total F-actin intensity at the whole-cell region
	glcm_diff_var_mean:F-actin:nondna_region	Mean difference variance of the cytoplasmic F-actin GLCM

Hepatocyte Model	Phenotypic Features	Description
	mean_intensity:F-actin:dna_region	Mean F-actin intensity at the nuclear region
	total_intensity_ratio:F-actin-F-actin:nondna_inner-cell_region	Ratio between total F-actin intensity at the inner cytoplasmic region over the whole-cell region
	mean_intensity:F-actin:nondna_outer	Mean F-actin intensity at the cytoplasmic region
	glcm_diff_var_mean:F-actin:dna_region	Mean difference variance of the nuclear F-actin GLCM
	mean_intensity:F-actin:cell_region	Mean F-actin intensity at the whole-cell region
	glcm_contrast_mean:F-actin:cell_region	Mean contrast of the whole-cell F-actin GLCM
	glcm_contrast_mean:F-actin:dna_region	Mean contrast of the nuclear F-actin GLCM
	fraction_obj_intensity:F-actin:nondna_region-F-actin_object	Fraction of total F-actin object intensity at the cytoplasmic region over the whole-cell region
	total_intensity:F-actin:nondna_inner	Total F-actin intensity at the inner cytoplasmic region
	total_intensity:F-actin:nondna_peridna	Total F-actin intensity at the perinuclear region
	total_intensity:F-actin:dna_region	Total F-actin intensity at the nuclear region

Hepatocyte Model	Phenotypic Features	Description
	fraction_obj_intensity:F-actin:nondna_peridna-F-actin_object	Fraction of total F-actin object intensity at the perinuclear region over the whole-cell region
2 Combined Features, 1 Nuclear Morphology Feature	ccoeff_normed:RELA-F-actin:dna_chromosome	Correlation coefficient of REL-A and F-actin intensities at the chromosomal region
	ccoeff_normed:RELA-F-actin:dna_region	Correlation coefficient of REL-A and F-actin intensities at the nuclear region
	perimeter:mask:cell_region	Cell perimeter length

Supplementary Table S1: Effects of ICA-1S on HepaRG cell numbers and cytoplasmic-to-nuclear translocation of RELA. The results show the average (n = 3) relative cell numbers and ratios of RELA intensity (nucleus / cytoplasm). The vehicle control was 0.5% DMSO (same DMSO concentration as contained in samples treated with 500 M ICA-1S, which was dissolved in 100% DMSO). All results were normalized to the results obtained with the vehicle control, which were set to 1 (average, n = 3). The results obtained with all tested concentrations of ICA-1S are shown, as well as the results obtained with the positive (puromycin) and negative (glycine) controls. Only 1 concentration was tested in case of the controls. A decline in cell numbers indicates cell death. An increase in the nuclear / cytoplasmic intensity ratio of RELA indicates cytoplasmic-to-nuclear translocation of RELA.

Supplementary Table S2: Predictive phenotypic features. The table lists the constitutive cellular phenotypic features of the HCI-based models that were evaluated for predicting renal PTC toxicity (5 features, top) and hepatocyte toxicity (30 features) in humans. The 5 features used for predicting renal PTC toxicity included 3 features related to the F-actin cytoskeleton and 2 combined features (related to DNA and F-actin or DNA and H2AX; features 2 and 4). The GLCM of a marker summarizes the distribution of spatial transitions between different intensity levels of the marker, and feature 1 (PTC-based model) describes textural patterns of F-actin in the cell. Features 2 and 4 describe the spatial correlations between marker intensities.

F-actin features were also important for predicting hepatocyte toxicity, and 14 of the 30 selected features were F-actin features. 8 features were RELA features, 5 features were related to DNA, 2 features were combined features, and 1 feature described nuclear morphology. The coefficient of variation (CV), which is equal to standard deviation by the mean, is a standardized measure of the dispersion of a set of values as, for instance, DNA staining intensity levels.