SPARCS, a platform for genome-scale CRISPR screening for subcellular spatial phenotypes

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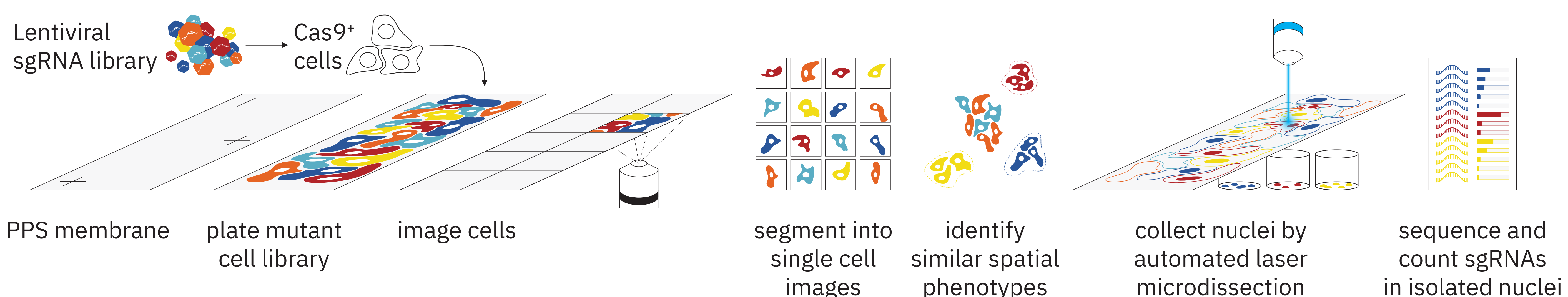
Highlights

Screening of any cell library size that can be imaged

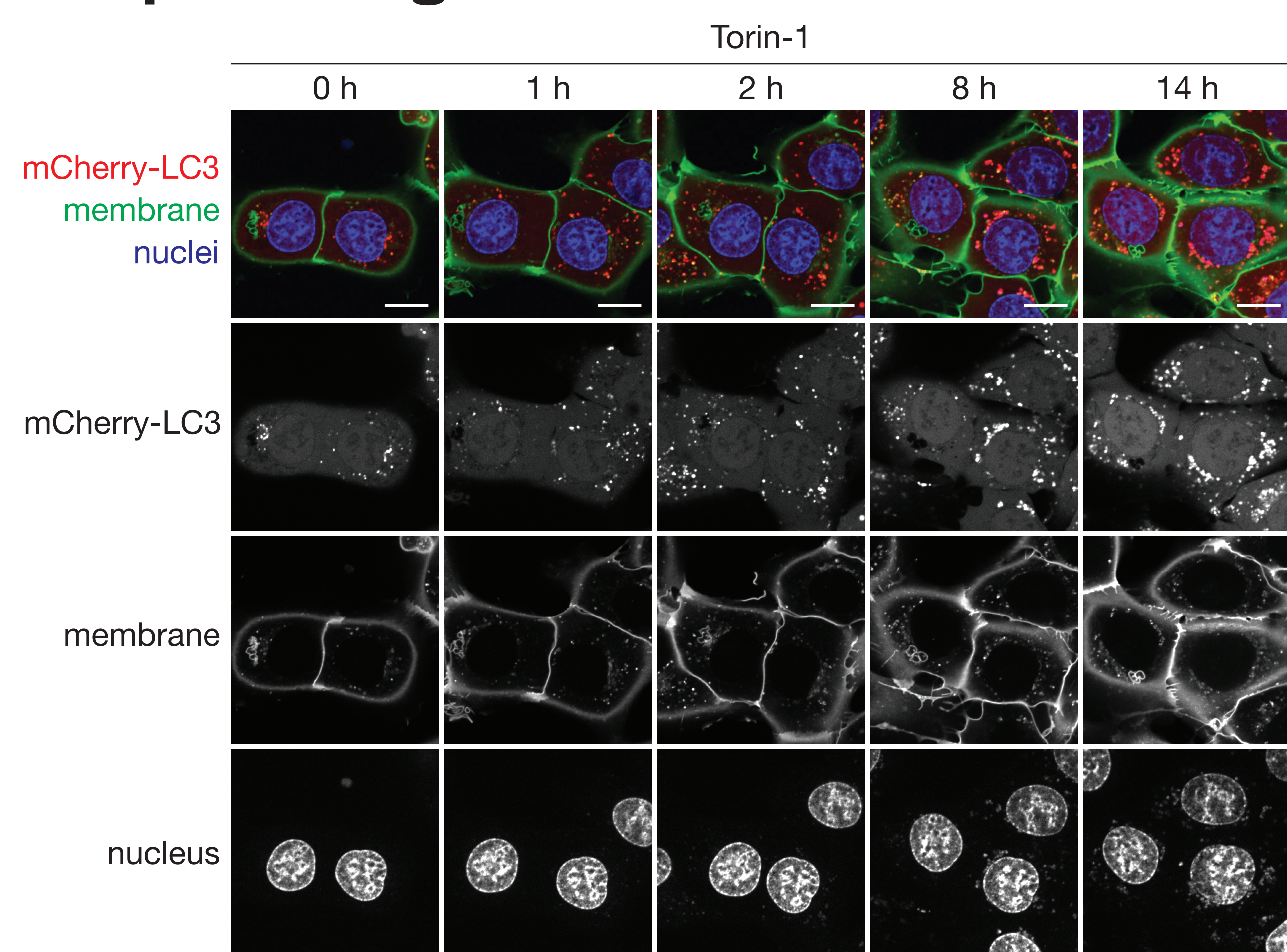Compatible with state-of-the-art microscopy technologies

Screens can be archived for reanalysis

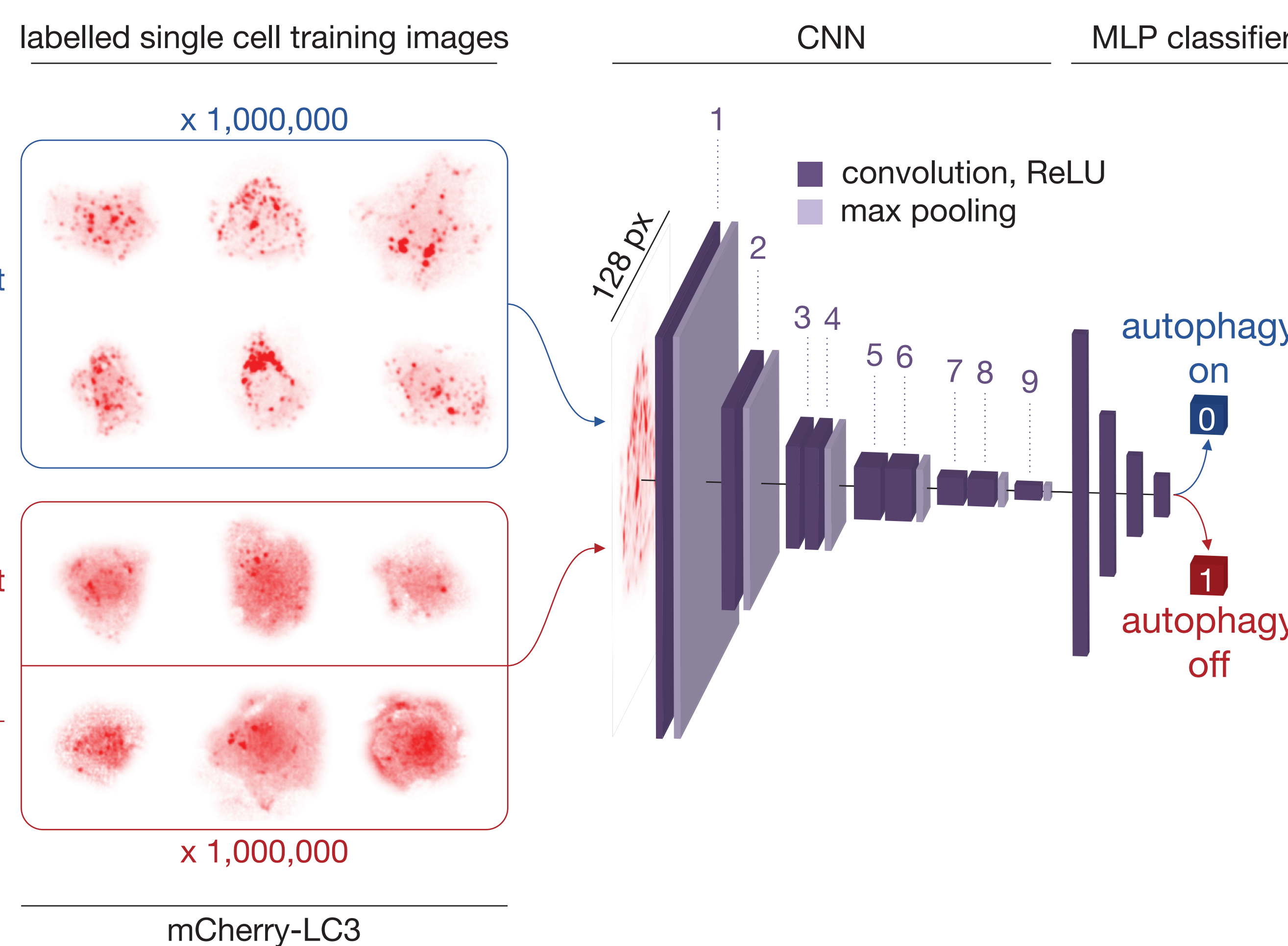Deep learning-based detection of before-unseen phenotypes
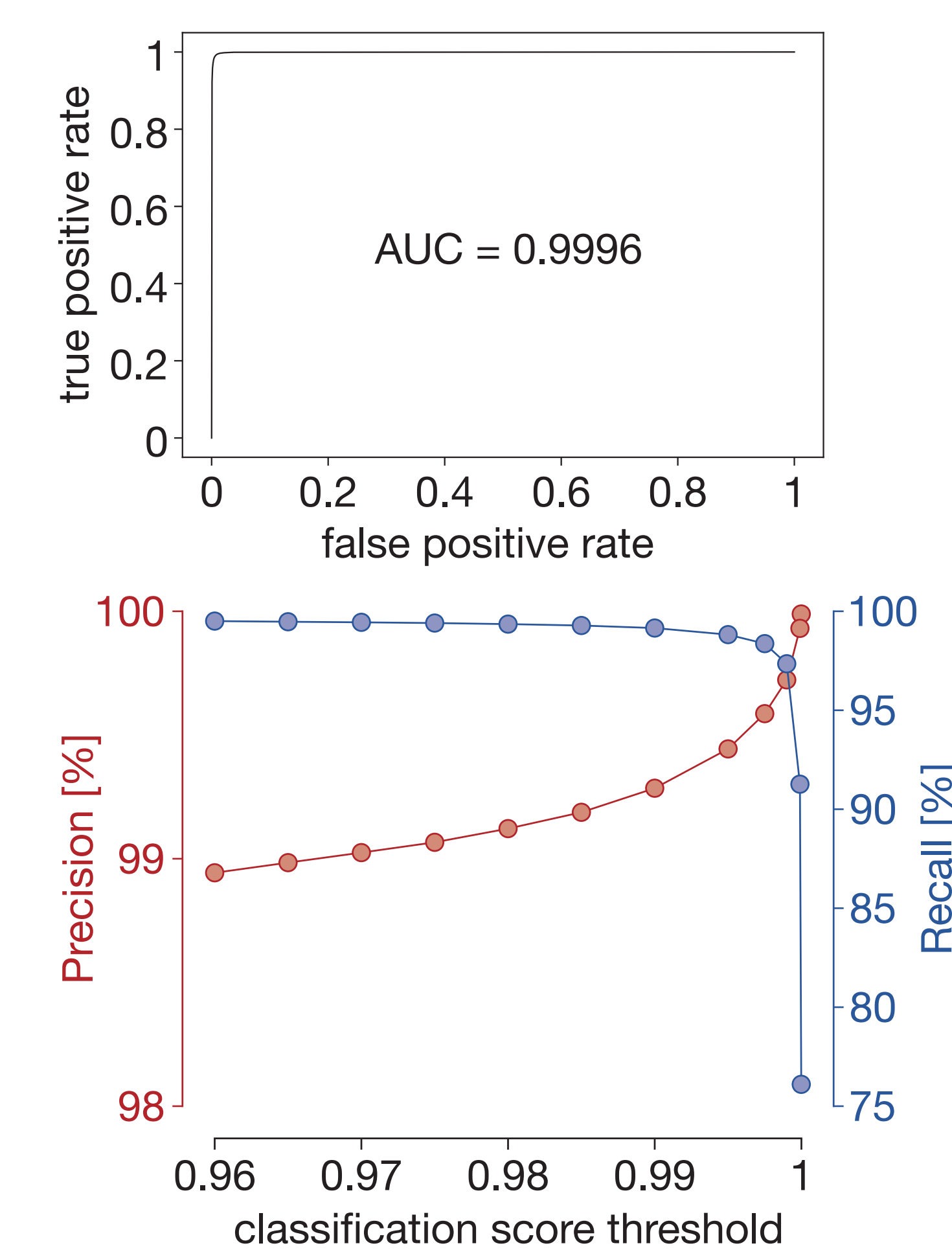
The SPARCS screening pipeline

Lentiviral sgRNA library → Cas9+ cells

PPS membrane | plate mutant cell library | image cells | segment into single cell images | identify similar spatial phenotypes | collect nuclei by automated laser microdissection | sequence and count sgRNAs in isolated nuclei

Deep learning-based classification of autophagosome formation

Torin-1
0 h | 1 h | 2 h | 8 h | 14 h

mCherry-LC3
membrane
nuclei

mCherry-LC3

membrane

nucleus

Microscopy images of autophagosome formation in human U2OS cells stably expressing mCherry-LC3 and mNeon fused to the lipidation signal of Lck (membrane). 100x, scalebars represent 15µm.

labelled single cell training images | CNN | MLP classifier

x 1,000,000

wt + Torin-1

128 px

convolution, ReLU
max pooling

autophagy on 0

wt
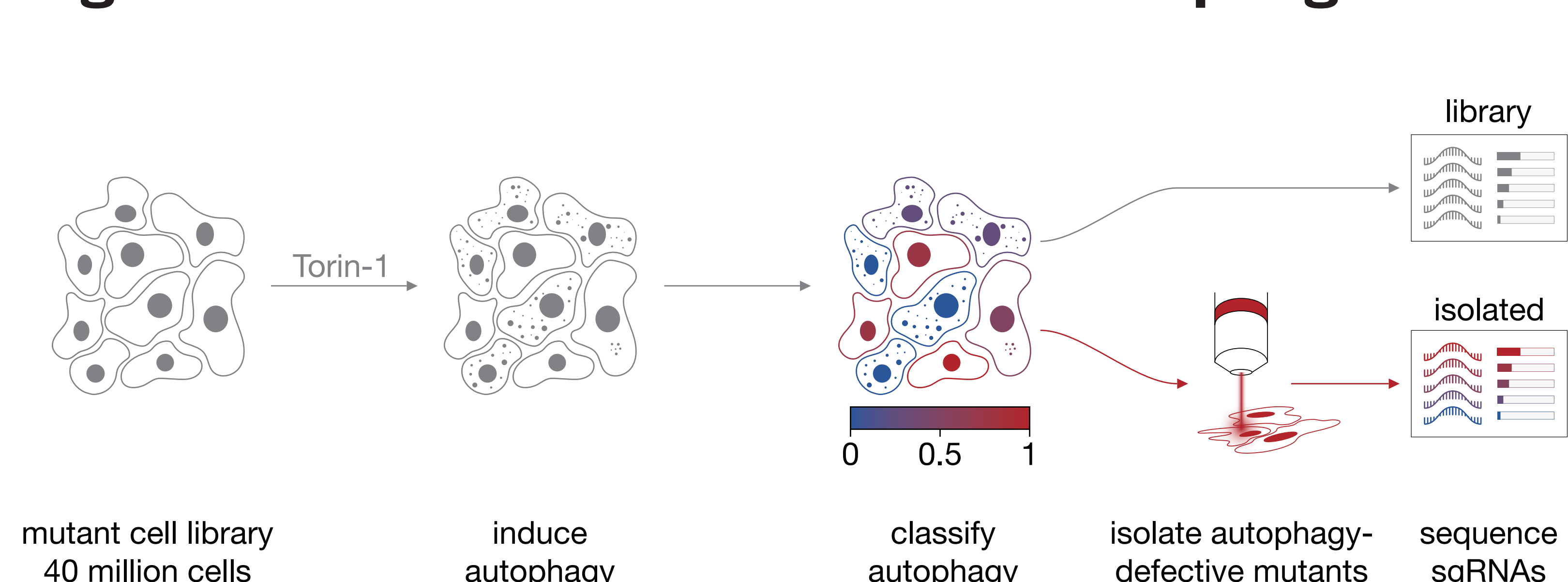ATG5−/− + Torin-1

autophagy off 1

x 1,000,000

mCherry-LC3

Overview of binary single cell image classifier and training strategy. Only the LC3 channel of segmented cells was used in training. CNN: convolutional neural network. MLP: multilayer perceptron.
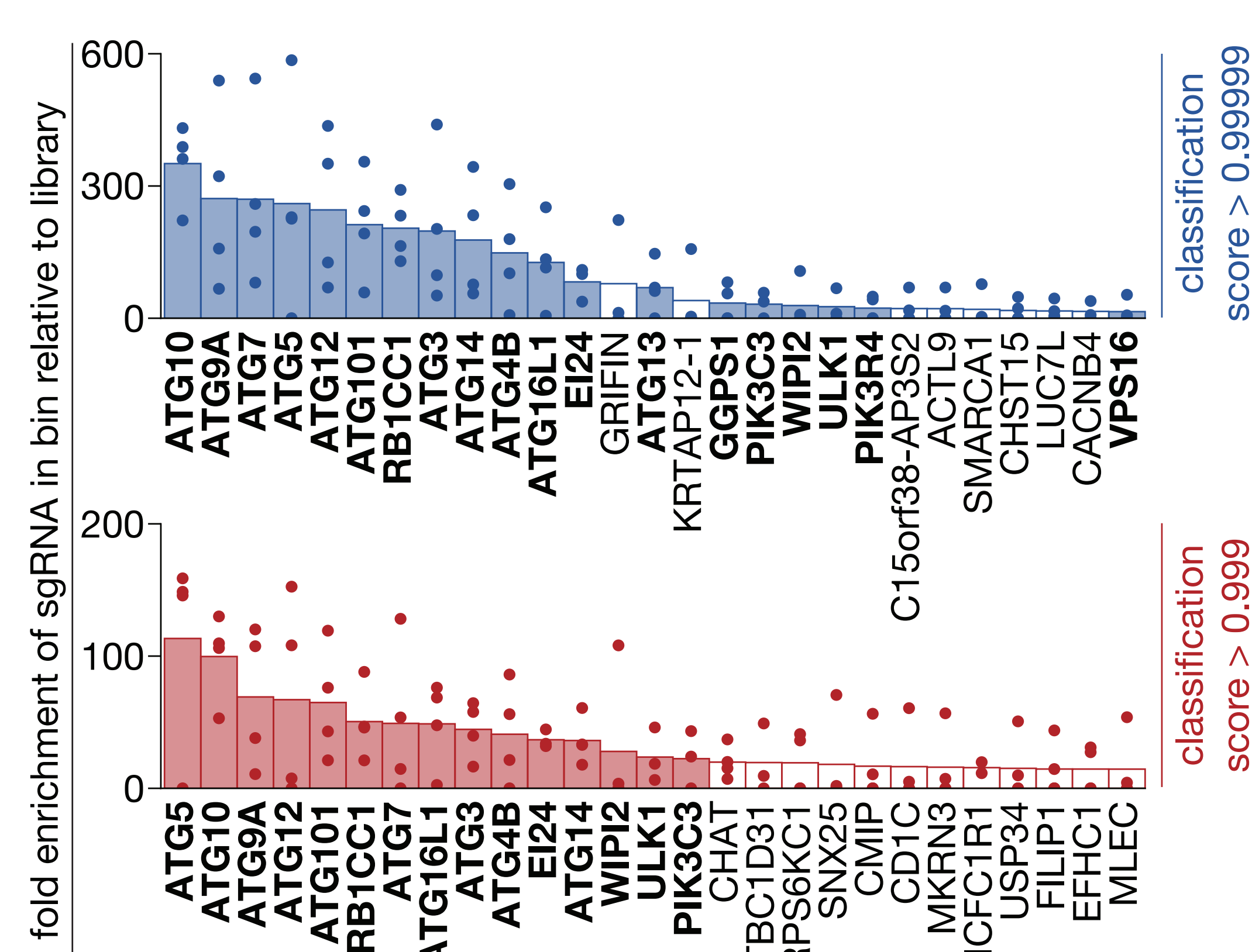
AUC = 0.9996

true positive rate / false positive rate

Precision [%] / Recall [%] / classification score threshold

Autophagy classifier performance metrics on biolgically independent test set.

A genome-wide CRISPR screen for autophagosome formation with SPARCS

Torin-1

mutant cell library 40 million cells | induce autophagy | classify autophagy | isolate autophagy-defective mutants | sequence sgRNAs

library

isolated

0 0.5 1

Genome-wide CRISPR knockout screening strategy for autophagosome formation in human U2OS reporter cells described above. Cells were stimulated with the mTOR inhibitor Torin-1 for 14 hrs to induce autophagosome formation. Mutant cells with defects in autophagosome formation were identified using the image classifier described above. 399,193 mutants were isolated with laser microdissection. The abundance of sgRNAs in these isolated mutants was determined by sequencing.

fold enrichment of sgRNA in bin relative to library

classification score > 0.99999
classification score > 0.999

ATG10 ATG9A ATG7 ATG5 ATG101 RB1CC1 ATG3 ATG4B ATG16L1 EI24 GRIFIN ATG13 GGPS1 PIK3C3 WIPI2 PIK3R4 KRTAP12-1 SNX25 C15orf38-AP3S2 ACTL9 SMARCA1 CHST15 LUC7L CACNB4 VPS16

ATG5 ATG10 ATG9A ATG7 ATG12 ATG101 RB1CC1 ATG16L1 ATG3 ATG4B EI24 ATG14 WIPI2 ULK1 PIK3C3 CHAT TBC1D31 RPS6KC1 CMIP CD1C MKRN3 HCFC1R1 USP34 FILIP1 EFHC1 MLEC

Sequencing results for highest scoring hits. Dots represent individual sgRNAs, bars represent gene-wise mean of sgRNAs. Bold: known regulators of autophagy.

Torin-1 ⊣ mTORC1

ULK1 ATG13 RB1CC1 ATG101

ER isolation membrane

PIK3R4 PIK3C3 ATG14 Beclin-1 AMBRA1 USO1

ATG9A

ATG4B ATG7 ATG10 ATG3 ATG12 ATG5 ATG16L1 WIPI2

LC3-I
LC3-II
LC3-II autophagosome

fold enrichment 512 32 2
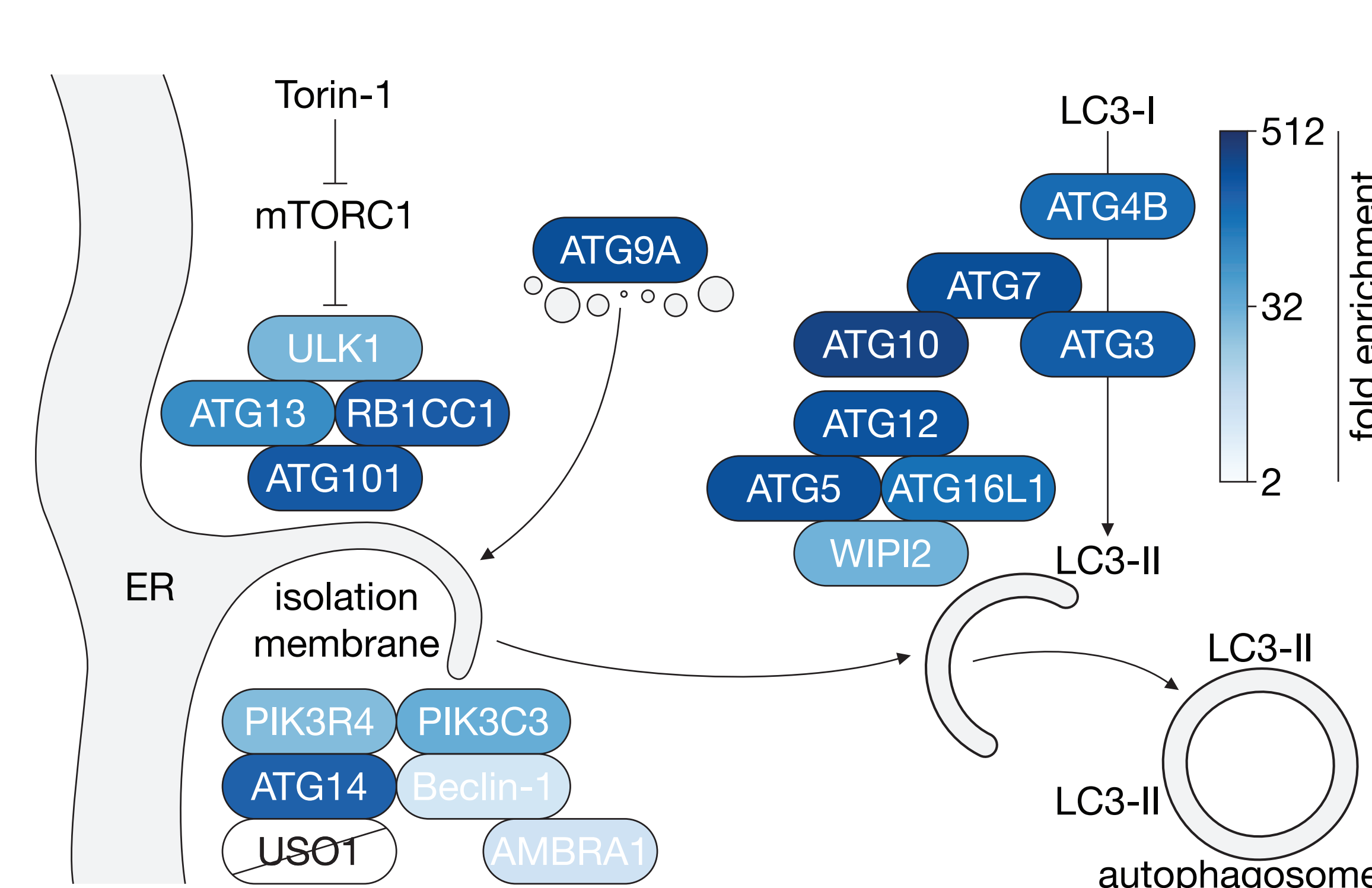
Overview of the canonical macroautophagy pathway. Autophagy regulators identified in our screen are highlighted in blue. Colours represent mean sgRNA enrichment per gene.