Morphology profiling driven by deep learning characterizes functional changes in CRISPR knockout cell lines

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Deepcell

Key points

- The Deepcell platform characterizes & sorts cells based on high-dimensional morphology analysis without labels, eliminating the need for specific biomarkers.
- We demonstrate high-dimensional morphology profiling of CRISPR knockout (KO) cell lines can determine phenotypic differences arising from single gene perturbations.
- The REM-I platform can detect rare morphological phenotypes (~1% of the population).

Introduction

- CRISPR/Cas9-based screens have revolutionized functional genomics by enabling the knockout (KO) or expression modulation of hundreds or thousands of genes in parallel.
- The REM-I platform enables high-dimensional morphology analysis and enrichment of unlabeled single cells using artificial intelligence (AI), advanced imaging, and microfluidics, for high resolution profiling of population heterogeneity.
- A library of 11 single gene KOs were generated in three cell lines (HEK293, Jurkat, and K562) and compared to a Cas9 negative control.
- Results showed distinct responses among cell lines, indicating the same gene perturbation translates to context-dependent phenotypic effects
- KOs targeting similar intracellular pathways resulted in consistent phenotypic patterns within cell lines, suggesting that high-dimensional morphological signatures are robust and orthogonal.

Methods

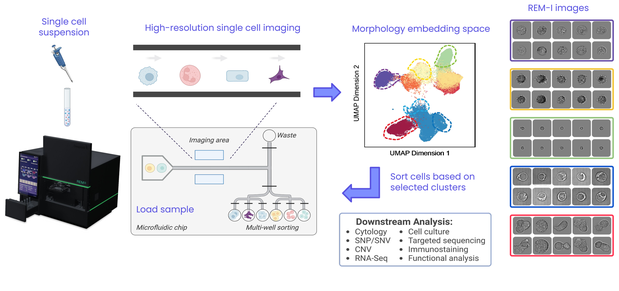

Figure 1. The REM-I workflow. *A single cell suspension is loaded onto a microfluidic chip where bright-field images of single cells are captured and analyzed in real-time by the Deepcell Human Foundation Model (HFM). High-dimensional morphological features are visualized by UMAP, and user-defined cell groups can be sorted for downstream functional or molecular analysis. Morphology embeddings are used to profile the morphological heterogeneity of cells, identify which morphology features differentiate each condition, and train a random forest classifier to predict each class of cells.*

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Results

Morphology profiling reveals large shifts associated with single gene KOs

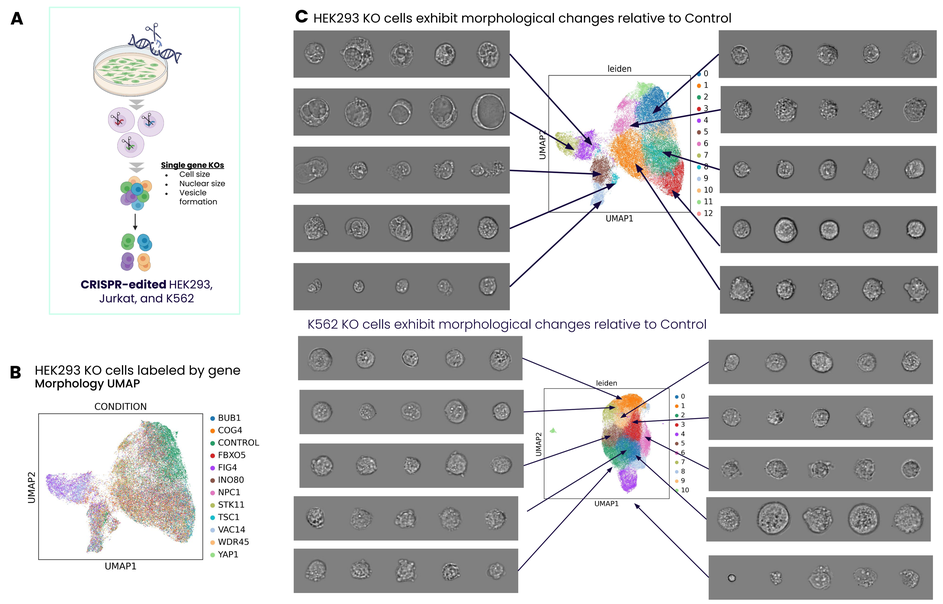
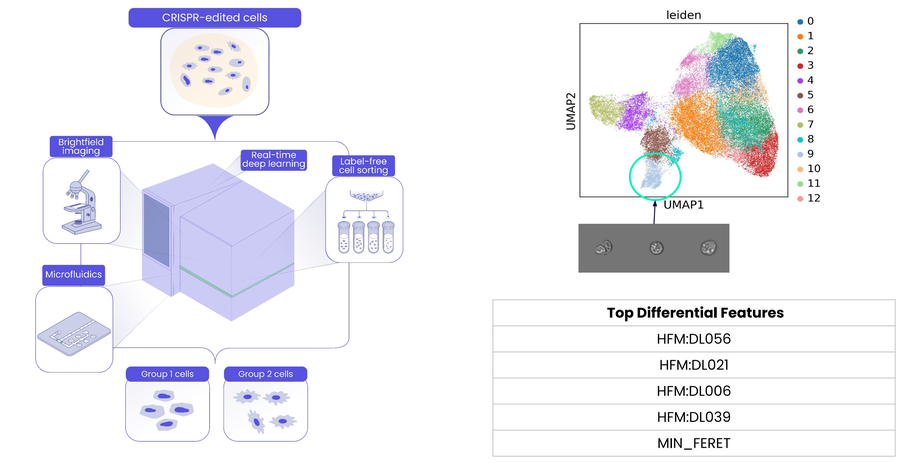


Figure 2. Single gene CRISPR/Cas9 knockout result in distinct phenotypic response (A) *A library of 11 single gene KOs were generated in HEK293 (Human Embryonic Kidney cells), Jurkat (T Lymphocyte), and K562 (Myelogenous Leukemia). Individual genes were selected based on previously reported effects on cell morphology, such as vesicle formation, cell size, and nuclear size.* ***(B)*** *We trained a self-supervised model using 115 deep learning and morphometric features extracted from images of single cells. Morphology UMAP dimensionality reduction of HEK293 cell morphological profiles with indicated single gene KOs.* ***(C)*** *Leiden clustering reveals morphological diversity driven by distinct responses among cell lines.*

A combination of deep learning and morphometric features enables cell group differentiation for label-free sorting



Top Differential Features
HFM:DL056
HFM:DL021
HFM:DL006
HFM:DL039
MIN_FERET

Figure 3. Imaging with REM-I enables high dimensional, morphology based, label-free cell sorting (A) *Label-free cells are imaged with the Deepcell REM-I platform for real-time AI classification.* ***(B)*** *User-defined cell groups, guided by leiden clustering, are examined using machine learning top differential features to characterize deep morphological characteristics.*

Results cont.

HFM reveals distinct morphotypes associated with known molecular pathways

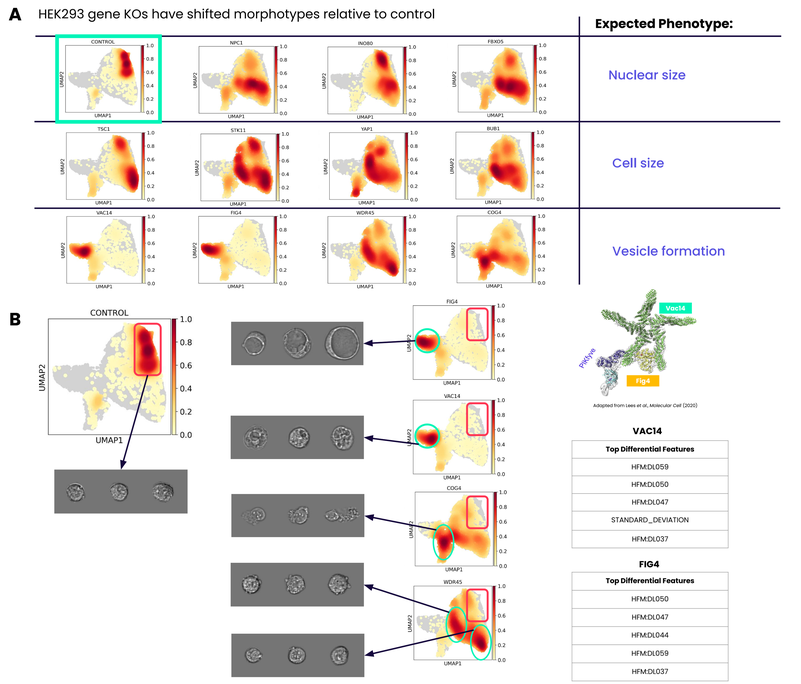


Figure 4. Morphology profiling of CRISPR-edited HEK293 cell lines. ***(A)*** *Density plots of UMAP projections demonstrate morphotype shifts caused by each gene KO. Morphotype groups localize according to known expected phenotypes.* ***(B)*** *A closer look at vesicle formation gene KOs unveils consistent morphological groups linked to biochemical and molecular functions. Machine learning top differential features further show morphological characteristics driving morphotype distinctions.*

High-dimensional analysis elucidates rare populations in use cases with subtle morphological diversity

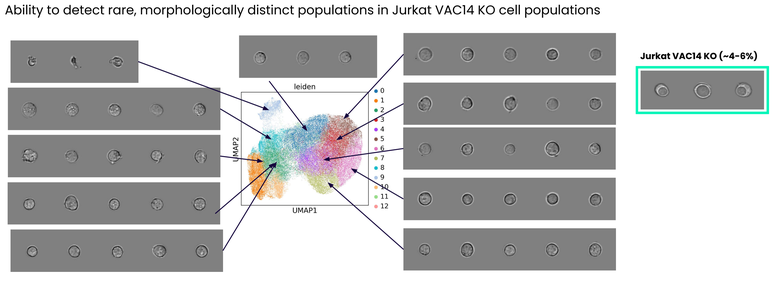


Figure 5. Jurkat single gene CRISPR/Cas9 knockouts show subtle changes in morphology. ***(A)*** *Across the library of 11 single gene KOs in Jurkat, morphological changes were less pronounced in comparison to HEK293 and K562. Deepcell Axon suite analysis showed a rare morphotype that resemble phenotypic shifts associated with VAC14 KO HEK293 and K562 cells. This demonstrated exploratory power of REM-I to assess both pooled screening and individual sample analysis.*

Conclusions

- Morphology analysis of CRISPR KO cells reveals dramatic shifts relative to Control.
- CRISPR KO cells induced expected morphological changes and similar KOs exhibited similar morphology patterns.
- Morphology analysis enables detection of rare, morphologically distinct subpopulations in CRISPR KO cells.