CTRL: a label-free method for dynamic measurement of single-cell volume:

Materials and Methods and Supplementary Data

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Fig. S1

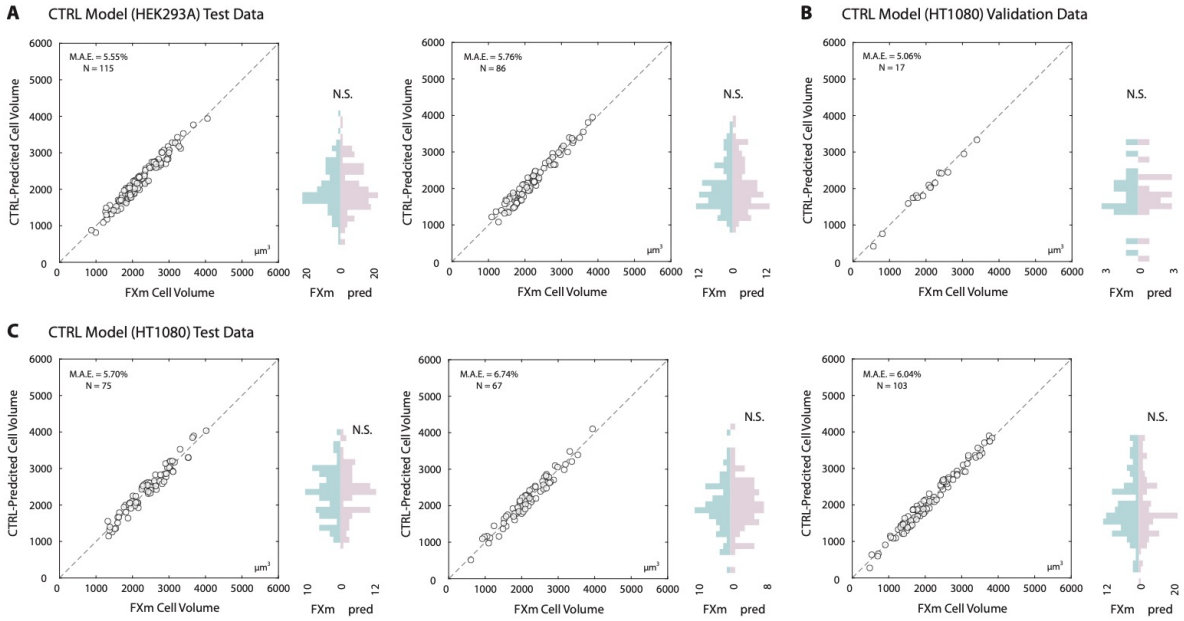

Fig. S1 Validation and test data on CTRL models. (A) CTRL model (HEK-293A) test (2 more biological repeats). The trained CTRL model of HEK-293A cells was applied to test data (cells in a different microchamber). CTRL-predicted volume is compared to the measured FXm cell volume for every single cell. Volume distributions are plotted as histograms. No statistical significance is found between FXm volume and predicted volume. Cell number and Mean Absolute Error (M.A.E.) are indicated in the top-left corner of the panel. **(B) CTRL model (HT1080) validation.** The trained CTRL model of HT1080 cells was applied to validation data (same microchamber, the other 20% of the cells). CTRL-predicted volume is compared to the measured FXm cell volume for every single cell. Volume distributions are plotted as histograms. No statistical significance is found between the measured FXm volume and the predicted volume. Cell number and Mean Absolute Error (M.A.E.) are shown in the top-left corner. **(C) CTRL model (HT1080) test (3 biological repeats).** The trained CTRL model of HT1080 cells was applied to test data (cells in a different microchamber). CTRL-predicted volume is compared to the measured FXm cell volume for every single cell. Volume distributions are plotted as histograms. No statistical significance is found between the measured FXm volume and the predicted volume. Cell number and Mean Absolute Error (M.A.E.) are indicated in the top- left corner of the panel.

Fig. S2

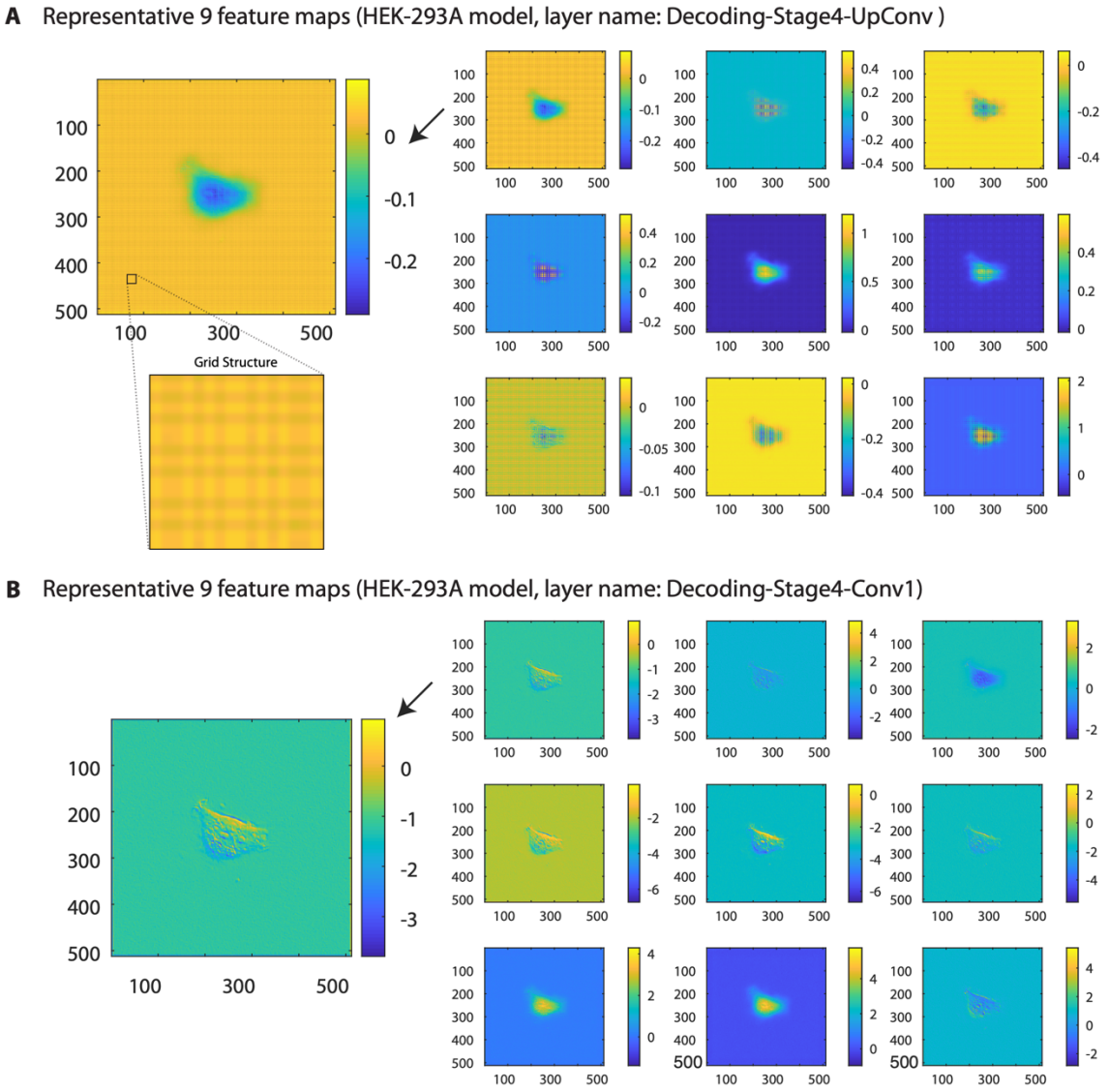

Fig. S2 Feature maps display a grating-like local structure. (A) Representative 9 feature maps of layer 'Decoding-Stage4-UpConv'. Representative 9 feature maps of the layer after the last up-convolutional operation in the upsampling top level (the 4th last layer) with grating-like local structure highlighted, potentially indicating U-NetR is learning the optic features of the microscope images. **(B) Representative 9 feature maps of layer 'Decoding-Stage4-Conv1'.** Representative 9 feature maps of the layer after the layer in (A) (the 3rd last layer). The grid-like local structure is too fine to be seen in these feature maps.

Fig. S3

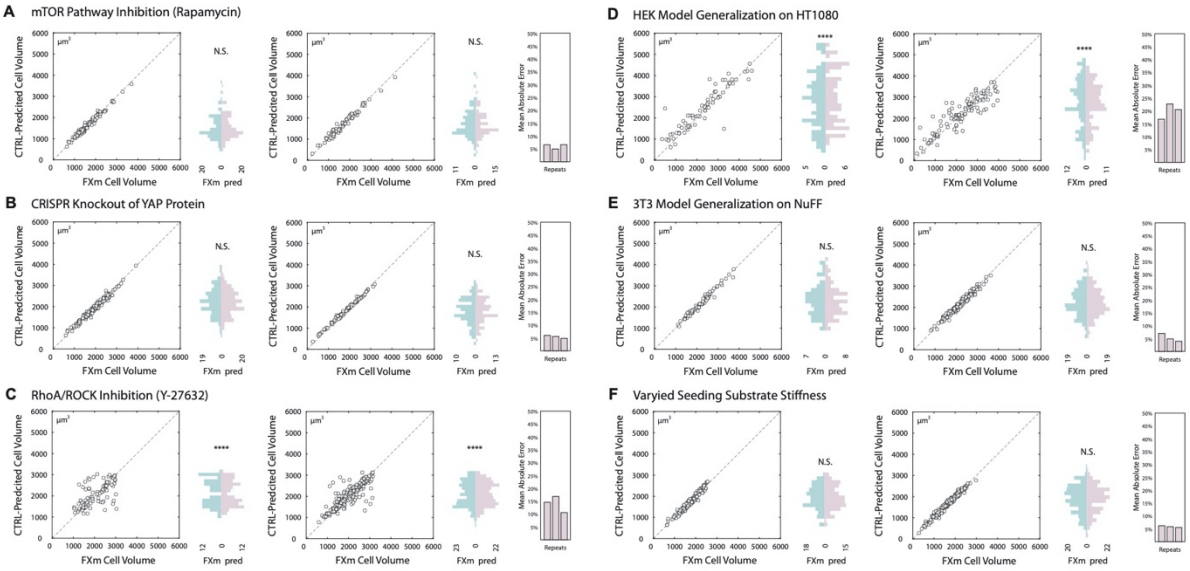

Fig. S3 Biological repeats of generalization investigation of CTRL method. (A) Two more biological repeats of mTOR pathway inhibition (rapamycin). Two more biological repeats are displayed corresponding to Fig. 2A. The Mean Absolute Error (M.A.E.) of all 3 biological repeats are displayed on the right. **(B) Two more biological repeats of CRISPR YAP protein knockout.** Two more biological repeats are displayed corresponding to Fig. 2B. The Mean Absolute Error (M.A.E.) of all 3 biological repeats are displayed on the right. **(C) Two more biological repeats of ROCK pathway inhibition (Y-27632).** Two more biological repeats are displayed corresponding to Fig. 2C. The Mean Absolute Error (M.A.E.) of all 3 biological repeats are displayed on the right. **(D) Two more biological repeats of HEK model tested on HT1080 cells.** Two more biological repeats are displayed corresponding to Fig. 2D. The Mean Absolute Error (M.A.E.) of all 3 biological repeats are displayed on the right. **(E) Two more biological repeats of NIH-3T3 model tested on NuFF cells.** Two more biological repeats are displayed corresponding to Fig. 2F. The Mean Absolute Error (M.A.E.) of all 3 biological repeats are displayed on the right. **(F) Two more biological repeats of test on varied seeding substrate stiffness (3kPa).** Two more biological repeats are displayed corresponding to Fig. 2D. The Mean Absolute Error (M.A.E.) of all 3 biological repeats are displayed on the right.

Fig. S4

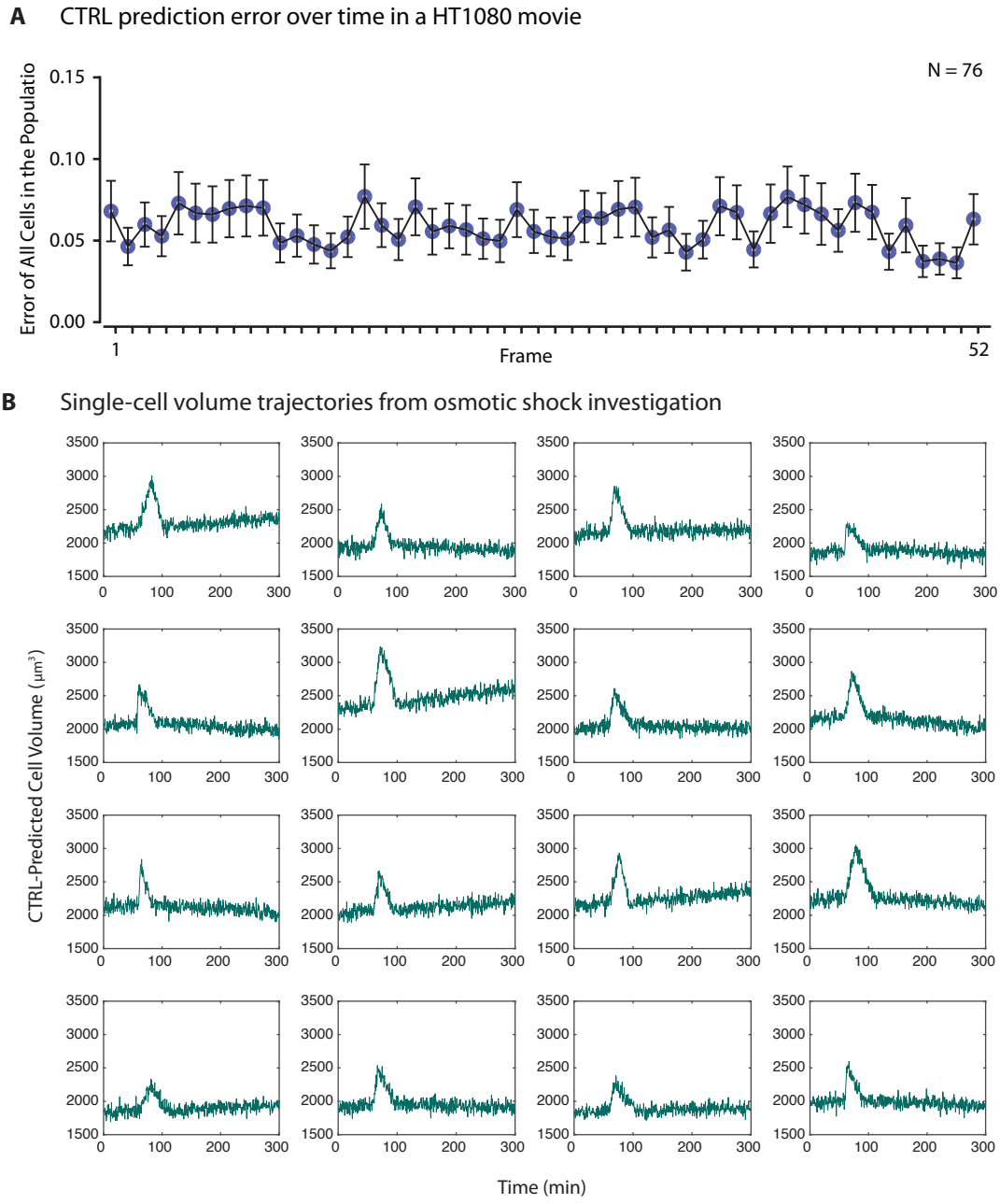

Fig. S4 CTRL volume prediction error overtime in an HT1080 movie and osmotic shock volume trajectories. (A) The error of CTRL volume prediction overtime in an HT1080 movie. Mean prediction error averaged over all HT1080 cells in the population of the movie in one experiment (N=76) is plotted overtime against the frame number in the movie. Error bars are standard deviation of the error. **(B) Single-cell osmotic shock volume trajectories.** 16 single-cell volume trajectories from 2 biological repeats are displayed. The time length of the movie is 300 min and the time resolution is 30s.