## Supplementary Note 1: Preparation of cells

### *Transfection and preparation of cells*

The plasmids encoding for BRD4 and CECR2 fused with GFP were from reference[1]. Cells were reverse transfected 24 hours before a FRAP experiment. For each FRAP condition, 0.2 µg of DNA and 0.5 µl of Lipofectamine 2000 (Life Technologies, UK) were each diluted in 50 µl of OptiMEM (LifeTechnologies, UK) and then thoroughly mixed before incubating for 20 minutes at room temperature. During the incubation period, cells were trypsinized, resuspended in antibiotics-free culture medium and counted. For each FRAP condition, a lipofectamine-plasmid complex was mixed with 150,000 cells and seeded into a well of a 24-well optical grade flat-bottom plate (Thistle Scientific, UK). Only the left half of the plate was seeded starting with the WT DMSO control at the upper left corner (A1). The plate was incubated at 37 degrees and 5 % $CO_2$ for 6 hours, where the medium was later changed to fresh McCoy's 5A medium with HEPES supplemented with 10 % foetal calf serum (Life technologies, UK), and 1 % GlutaMAX (Life technologies, UK) and containing SAHA at 2.5 µM before incubating the plate overnight at 37 degrees and 5 % $CO_2$. 500 µl of this medium was also added to the corresponding compound wells on the right of the plate in advance to allow the temperature and pH to reach equilibrium.

The microscope incubator should be switched on and left overnight to reach 37 degrees Celsius prior to the FRAP experiment. For added temperature stability, several bottles of water were placed inside the incubator to increase the heat latency.

### *Compound addition*

On the day of the FRAP experiment, the plate was observed on an inverted widefield fluorescence microscope (Carl Zeiss, UK) to assess transfection efficiency and viability. Generally, transfection efficiencies of 20 % or more were achievable.

Each test compound was pipetted onto the empty side of the plate containing 500 µl HEPES buffered medium in each corresponding well. For example, compounds pipetted into well A4, A5, and A6 will be dosed by the fluid handler onto the cells in wells A1, A2, and A3 respectively. The DMSO concentration was maintained constant throughout the wells at 0.2 %. The first well (A4) was manually transferred to its corresponding well (A1) before the plate was incubated for 1 hour at 37 degrees and 5 % $CO_2$. The plate was then ready to be transferred to the microscope for a FRAP experiment.

## Supplementary Note 2: Microscopy system

The FRAP setup used in Frapid was adopted from Philpott et al. (2014)[1] and consisted of a Zeiss LSM710 (Carl Zeiss, UK) inverted confocal microscope fitted with a 488 nm argon-ion laser, incubator capable of reaching 37 degrees, 10x 0.45 N.A. and 20x 0.8 N.A. Plan Apochromat objectives, and a motorized stage and objective turret. The system was also equipped with laser autofocus. An appropriate multi-well plate insert was necessary to seat culture plates onto the stage. The system PC's ran Windows 7 Ultimate 64-bit and had an Intel Core i5-2500 @3.30GHz processor with 32 GB RAM and an integrated video card. The microscope was controlled with ZEN 2009 with Visual Basic 6 macro editing features and MATLAB 2012a (Mathworks, USA) with the fuzzy logic and image processing toolboxes. For editing of Visual Basic macros, a ZEN hardware system key was required. The drivers, Microsoft Excel, and the Visual Basic libraries of the USB to digital I/O interface were also installed following the manufacturer's manual.

## Supplementary Note 3: Technical details of fluid handler

A fluid handler was custom designed and built to allow compounds to be aspirated and dispensed onto cells at the desired time points. The microscope condenser must be removed in order to install this device. The fluid handler consists of purchased components:

- A stepper motor (cat. No. : SM-42BYG011-25-090327, Hobbytronics, UK) controlled by an EasyDriver motor driver (cat. No.: ROB-10267, Hobbytronics, UK) to extend and retract the collection tube
- A rack and pinion gear set (cat. No.: 917D/1, Maplin, UK)
- Silicon tubing (1.5 mm I.D. X 3 mm O.D.) (Advanced Fluid Solutions, UK)
- A stepper motor-driven peristaltic pump (William Manufacturing Company Limited, UK) controlled by a Pololu Stepper motor driver (cat. No.: DRV8825, Hobbytronics, UK) to facilitate aspiration and dispension of fluid
- A 24-channel digital I/O USB interface (cat. No. USB-1024, Measurement Computing, USA) to control the motor drivers from the host computer

All components were connected as per the circuit diagram in supplementary fig. 4. The schematics for the remaining parts are shown in supplementary fig. 5-9.

## Supplementary Note 4: Frapid : Operating Instructions

### *Installation and initial setup*

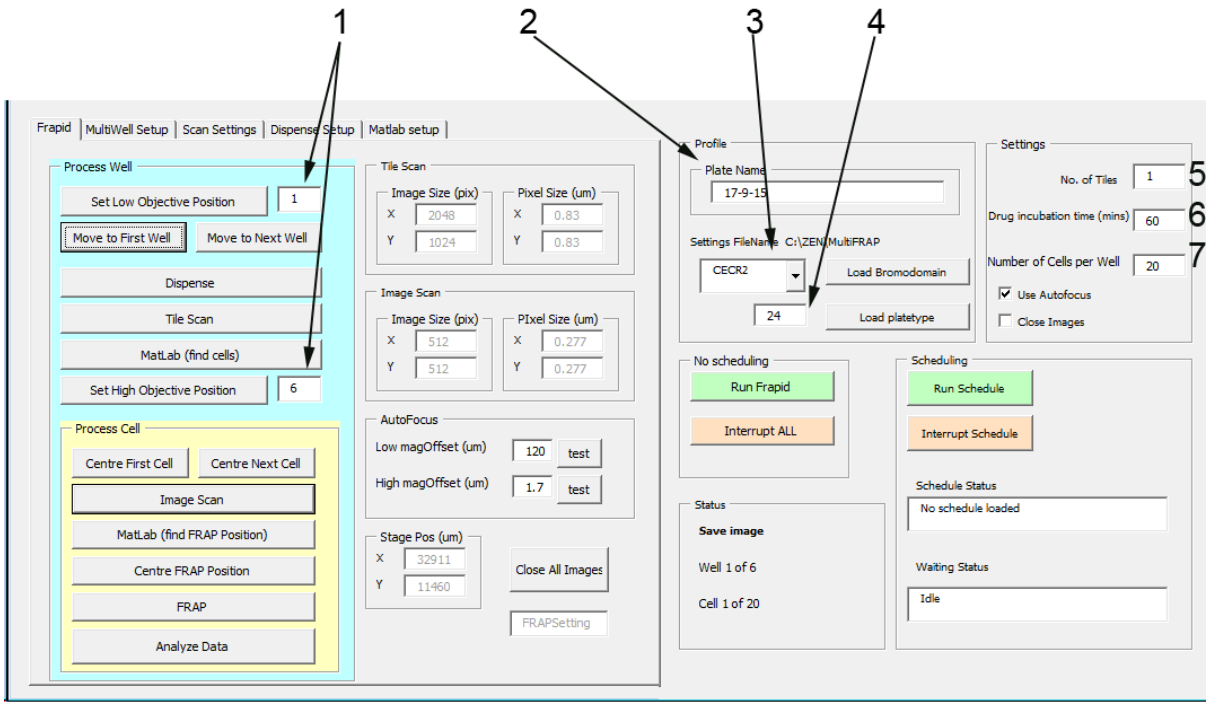
1. Install drivers of USB-1024 interface
2. Install MATLAB with fuzzy logic and image analysis toolbox
3. Copy all MATLAB scripts (.m) and fuzzy logic files (.fis) into a folder called C:\mfrap
4. Copy the frapid.ini configuration file and frapid.lvb macro file to C:\windows\zen
5. Setup tilescan, imagescan, and FRAP configuration profiles in ZEN as listed in *FRAP configuration profiles.xlsx* in supplementary methods

### *Setup instructions for each experiment*

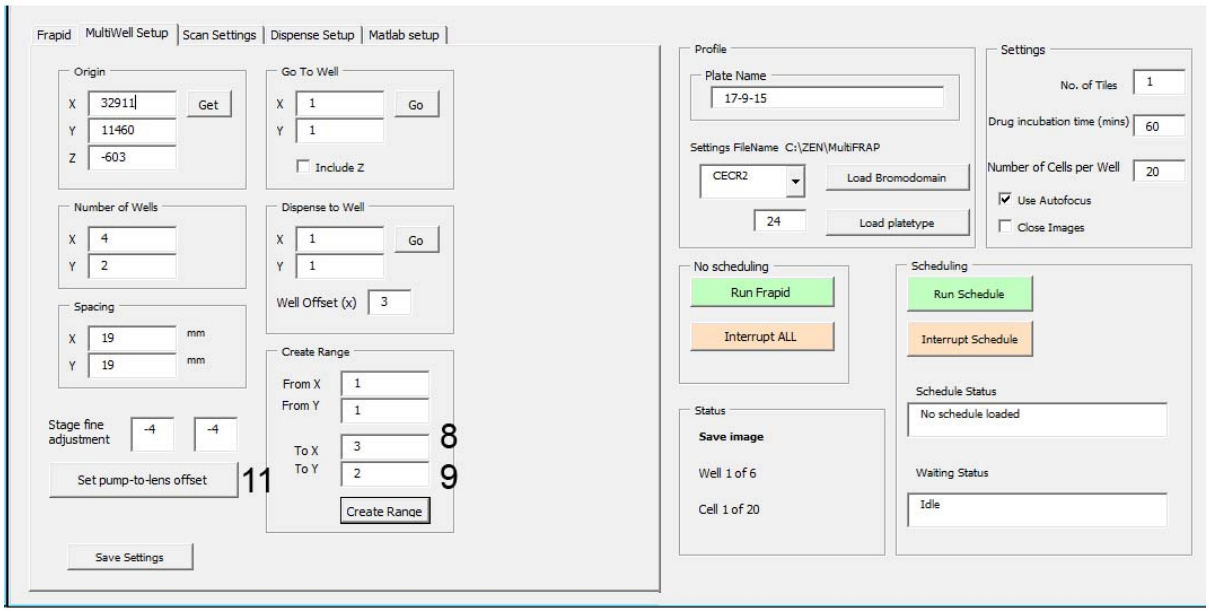
1. Follow the transfection and preparation of cells protocol
2. Turn on incubator and set temperature to 37 degrees
3. Plug in USB-1024 interface into USB port of host computer. Remove microscope condenser and replace with fluid handler
4. On day of experiment, add compound as per compound addition protocol
5. Switch on 488 nm argon-ion laser to high power and allow to stabilize (5 to 10 minutes)
6. Place 24-well plate or 8-well slide onto stage and move stage to the first well (well A1)
7. Load and run Frapid macro from c:\windows\zen in ZEN. Press ‘OK’ to confirm loading MATLAB and coordinates of first well

### *Operation instructions of software*

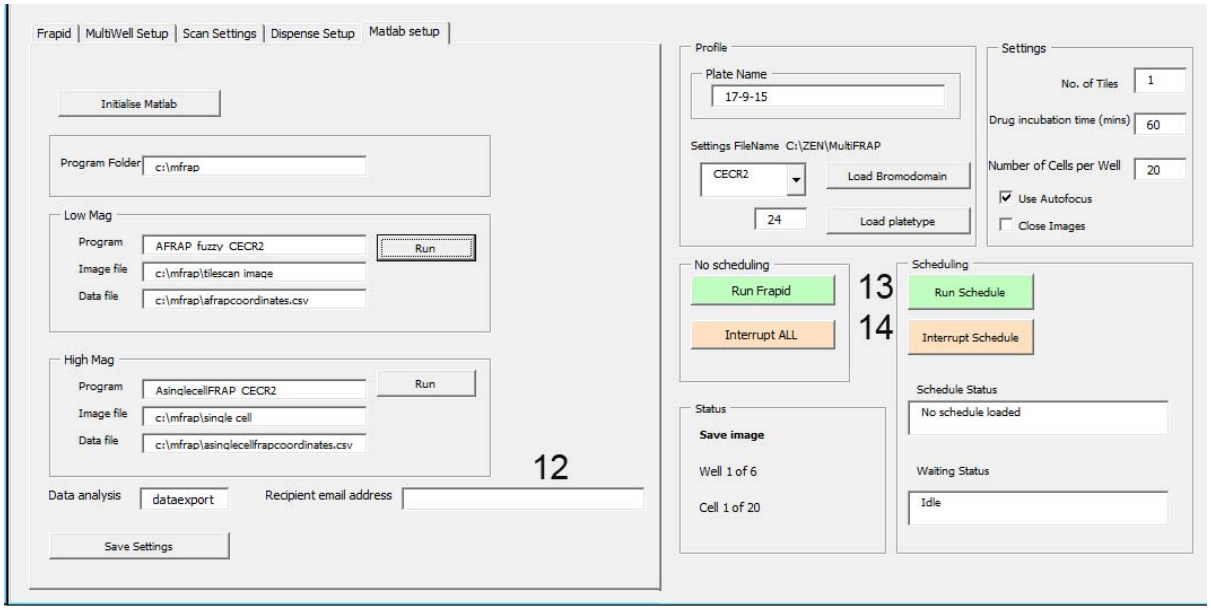
- Set experimental details in Frapid
    1. Enter the positions of the 10x and 20x objectives in panel A
    2. Enter the plate name
    3. Select the bromodomain to be tested in the drop down menu. Press ‘Load bromodomain’ button
    4. Enter the plate type (24 –well as default). Press ‘Load plate type’ button
    5. Enter the number of rows of tiles for low resolution scanning
    6. If testing a compound, enter the drug incubation time
    7. Enter the number of cells to be tested



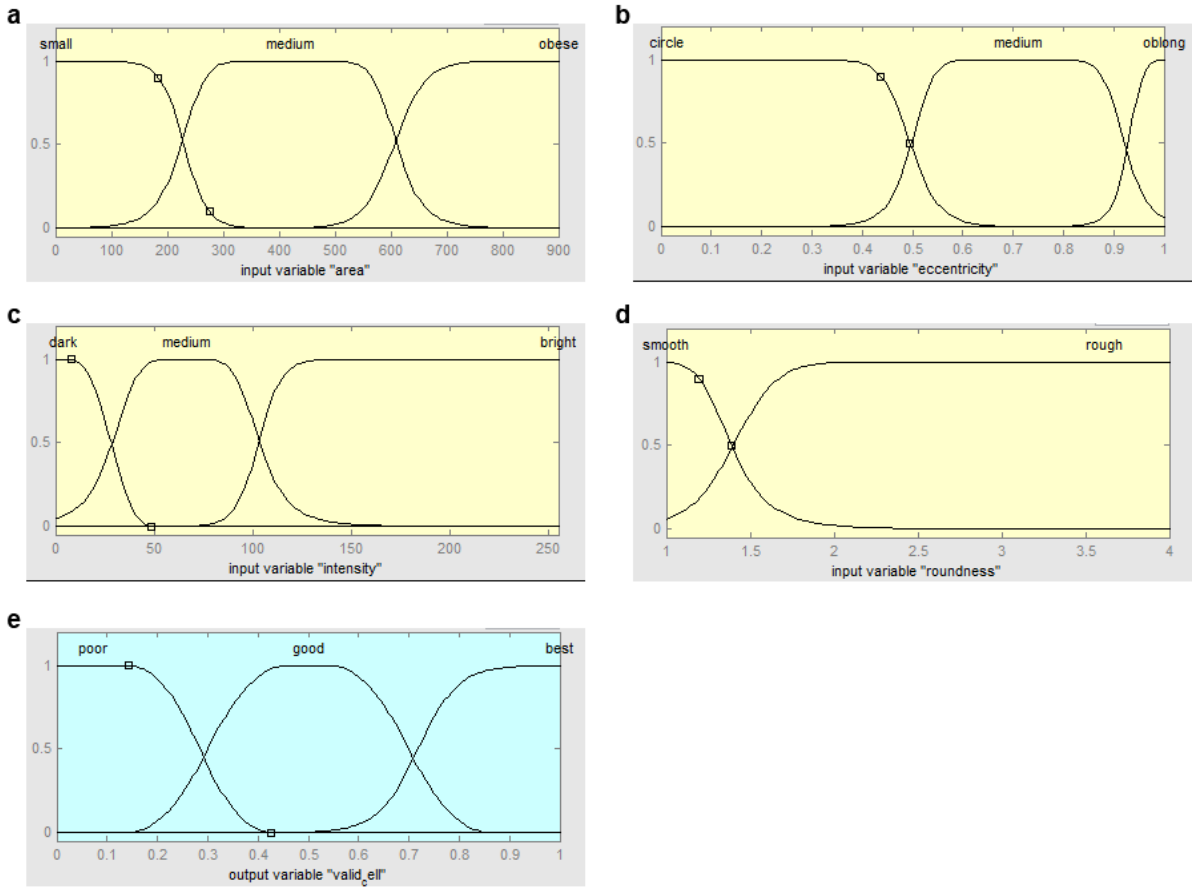

- Set plate parameters in MultiWell Setup
  8. Enter the number of columns of wells to be tested in ‘To X’
  9. Enter the number of rows of well to be tested in ‘To Y’
  10. Move the stage in the x-y directions so that the fluid handler lowers into well without missing
  11. Press ‘Set pump to lens offset’ button to confirm the adjustment



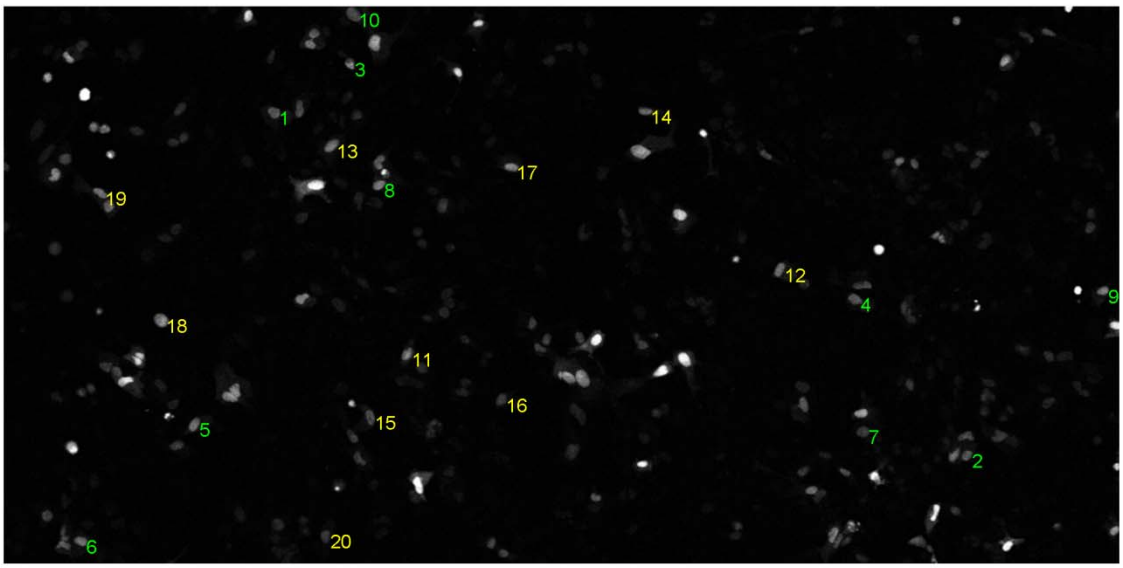

- Set email address
  12. Enter user’s email address
- Run experiment
  13. If testing a compound, press ‘Run Schedule’. If not, press ‘Run Frapid’
  14. Press Interrupt Schedule and Interrupt ALL to halt the experiment



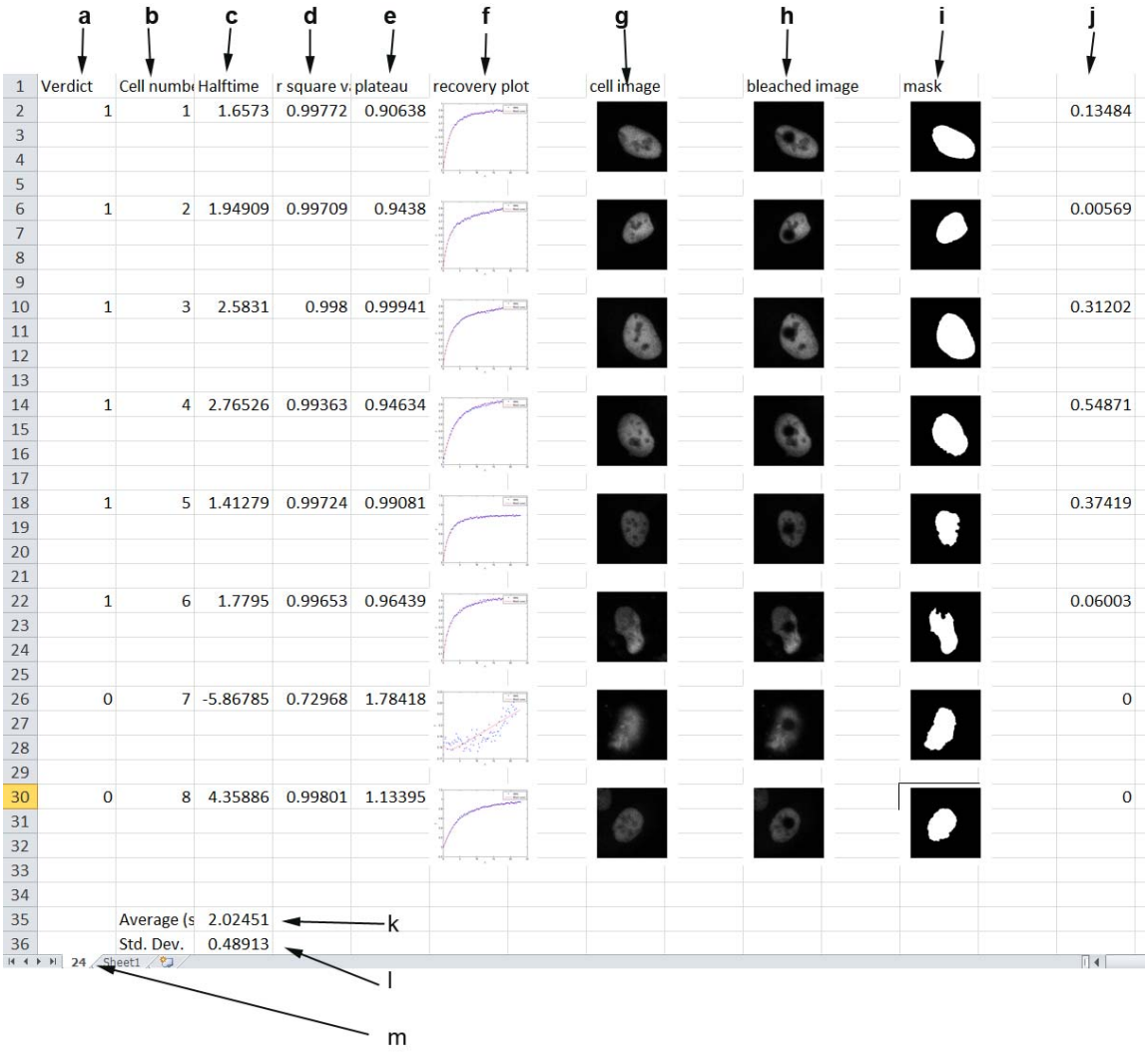

For a more detailed explanation of controls, see supplementary figures 10-14



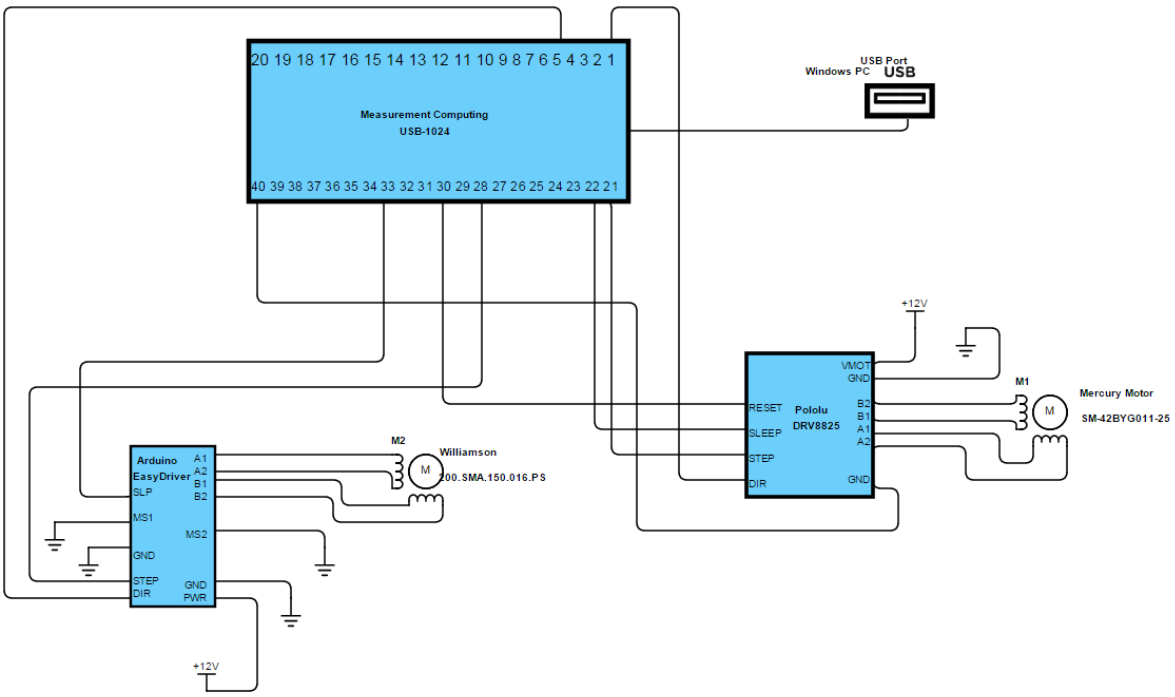

Supplementary fig. 1. Membership rules. Fuzzy logic membership rules for the identification of cells based on a) area, b) eccentricity, c) average fluorescence intensity, d) roundness. Since the membership rules are not rigid, cells are given more tolerance in contrast to traditional Boolean logic. e) Each cell is given a weighted score which is then ranked



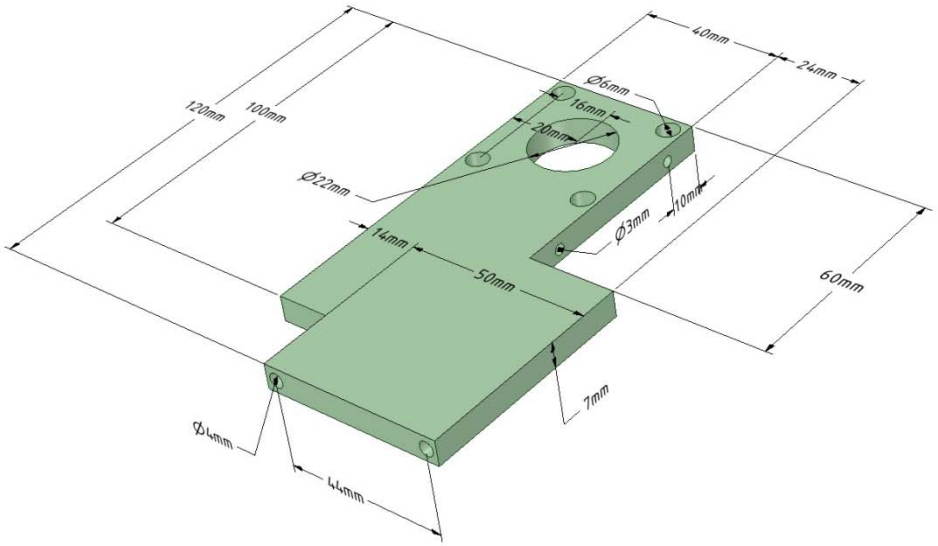

Supplementary fig. 2. Tilescan of identified and ranked cells for FRAP. Identification of 20 suitable cells in a tilescan for automated FRAP. Suitable cells are labeled with a number indicating their ranking (with 1 being the best) and order in which they will be processed. The first 10 cells are indicated in green, the next 10 cells are indicated in yellow



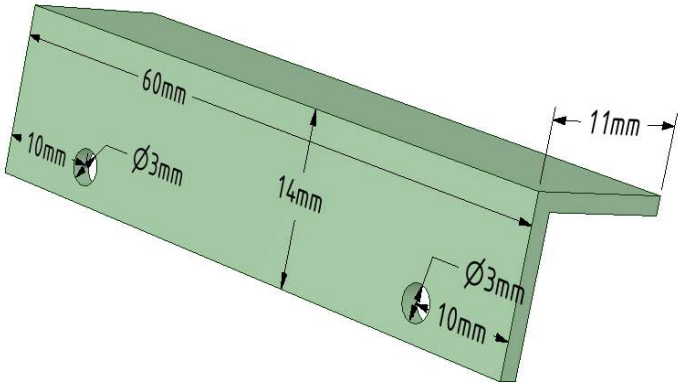

Supplementary fig. 3. Data presented in spreadsheet format with average and standard deviation half times for each well. Results displayed in an Excel spreadsheet detailing the a)- verdict, b) – cell number, c)- recovery halftime, d)- $R^2$ value for goodness of curve fit, e)- the plateau from curve fitting, f)- normalized recovery plot showing data points (blue) and curve fit (red), g) – image of cell during pre-bleach, h) – image of cell immediately after bleach, i) – segmentation mask of cell, j) – automated pre-calculation for determining the sample standard deviation. Specifically, the square of the difference between the individual cell's recovery half time and the population average recovery half time, k) – average recovery half time of cells with verdict '1', l) – standard deviation of individual half times of cells with verdict '1', m) – the position of the well represented by a two digit number that designates the column and row numbers respectively. Cells 7 and 8 were rejected due to their $R^2$ value and/or plateau of curve fit. Users can manually change the verdict of any cell, causing the average and standard deviation to update automatically.



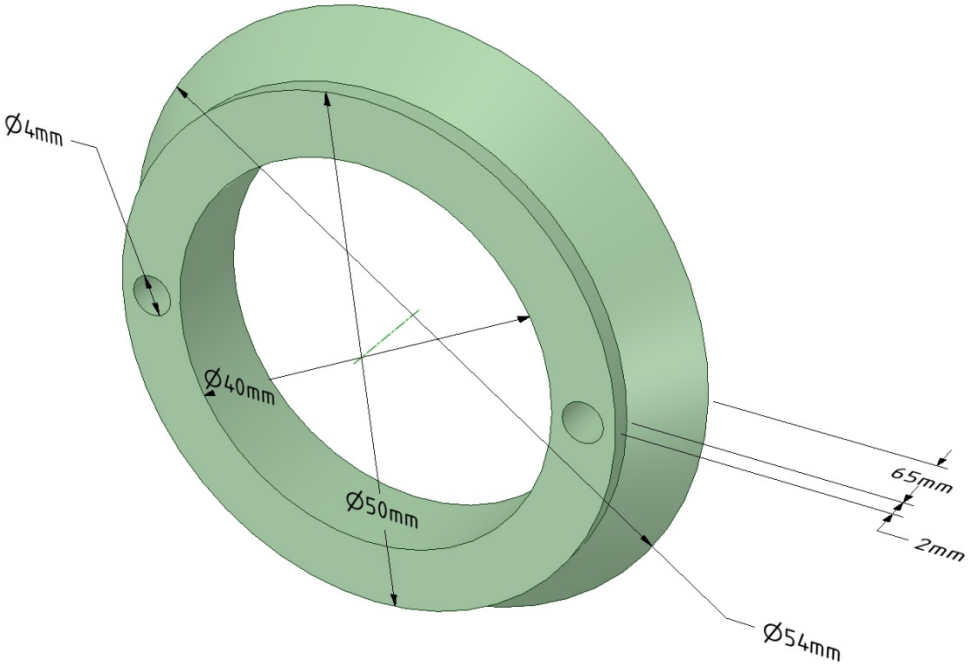

Supplementary fig. 4. Circuit diagram for connecting fluid handler. Circuit diagram for connecting the fluid handler and the host computer through the USB interface



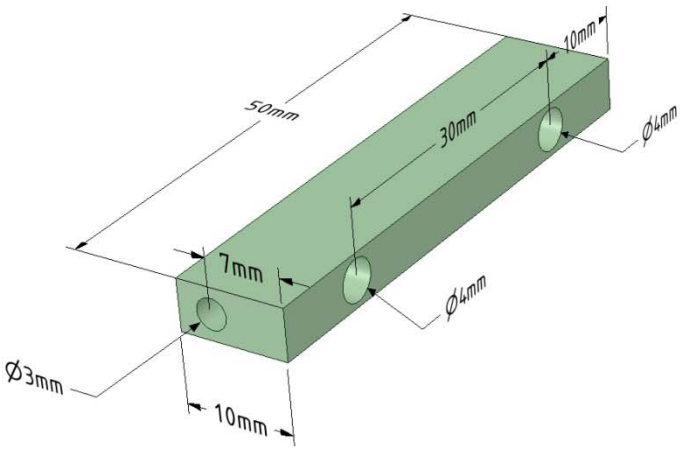

Supplementary fig. 5. Design drawing of main bracket. Isometric view of main bracket machined from acrylic



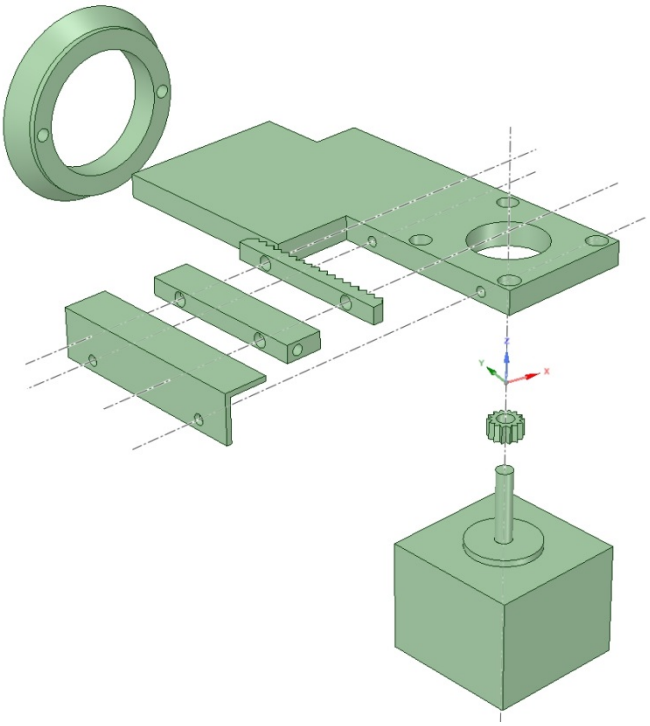

Supplementary fig. 6. Design drawing of bracket. Isometric view of bracket machined from 1 mm thick stainless steel



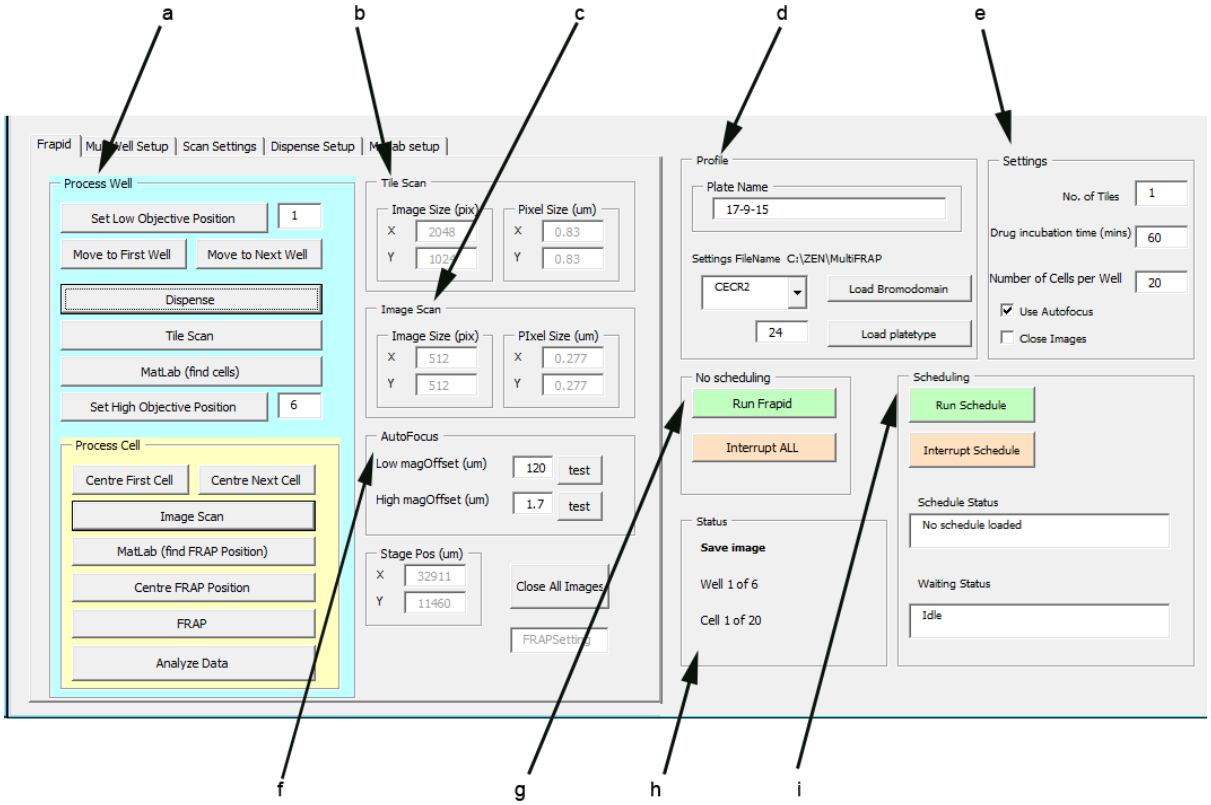

Supplementary fig. 7. Design drawing of ring mount. Ring mount lathed from stainless steel




Supplementary fig. 8. Design drawing of tube holder. Tube holder machined from acrylic

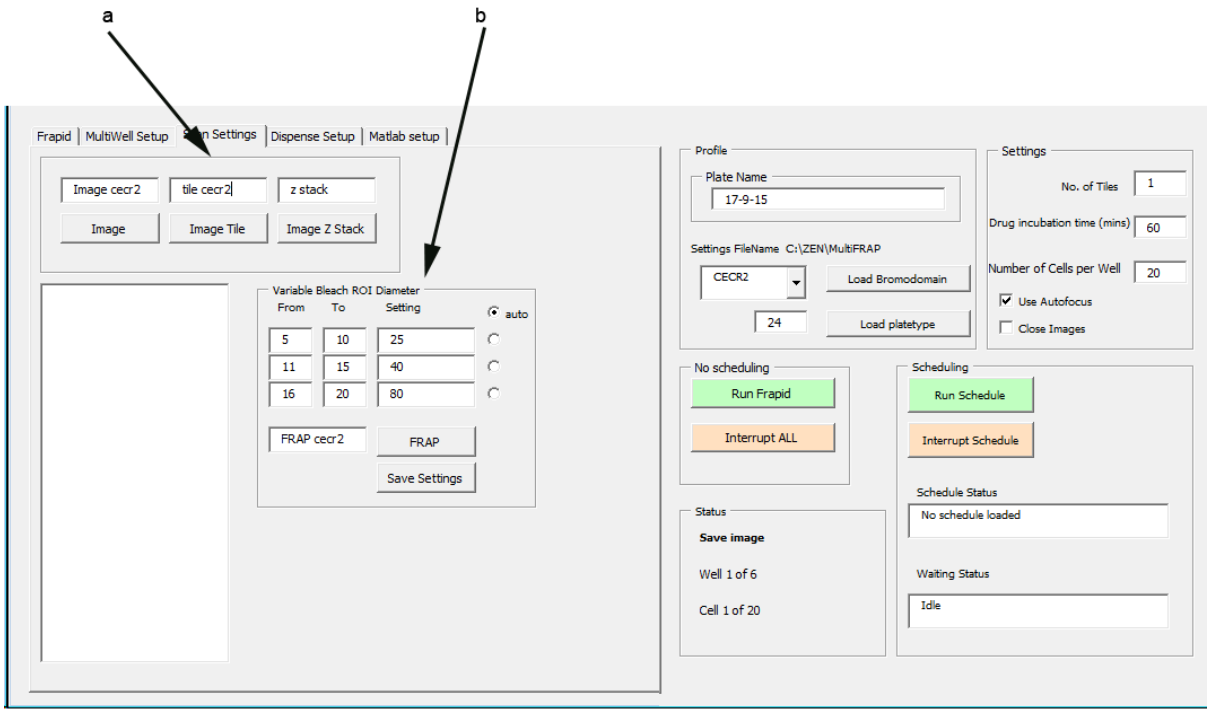


Supplementary fig. 9. Exploded view of fluid handler showing ring mount, main bracket, rack, tube holder, bracket, pinion gear and stepper motor.



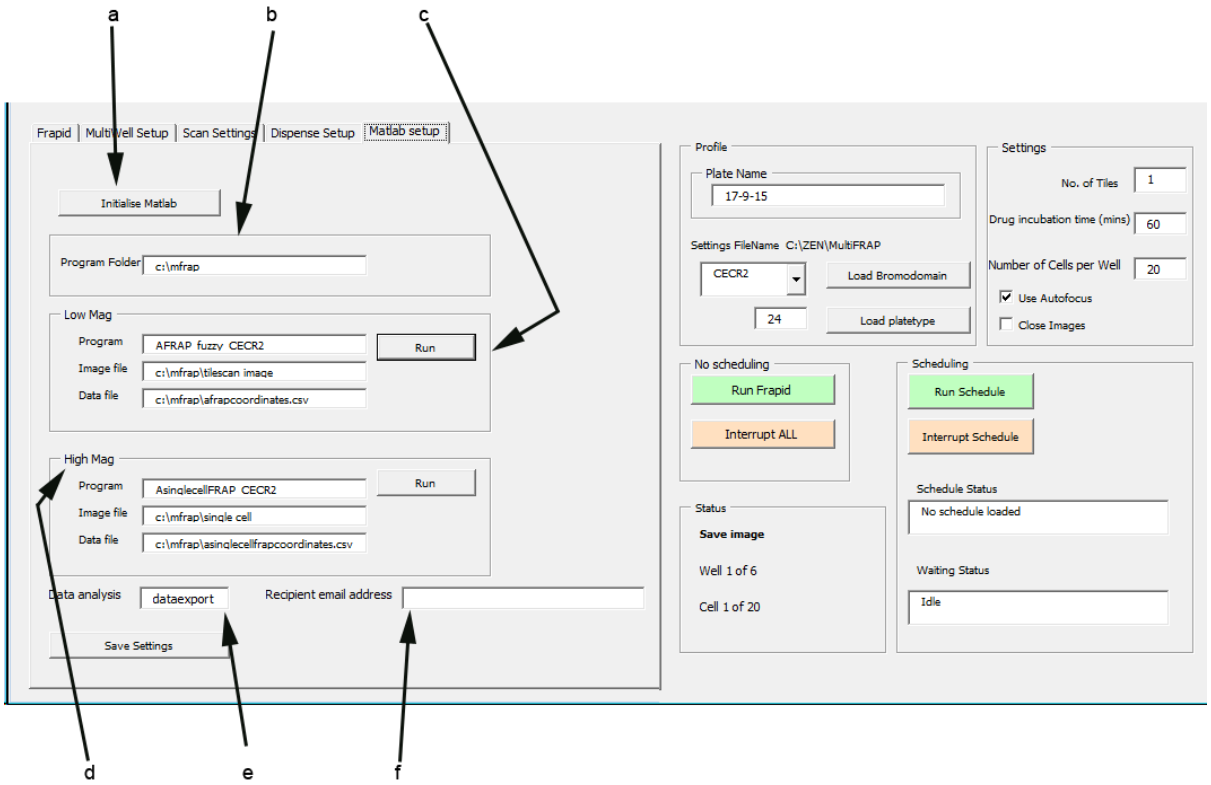

Supplementary fig. 10. User interface: Frapid. Frapid setup controls the main operations of running a FRAP experiment. a) – Series of buttons that control each step of FRAP. The buttons for locating cells and FRAP are organized within the frames of 'Process Well' and 'Process Cell' respectively. The numerical text boxes next to the objective position buttons allow user to enter the position of the 10x and 20x objectives respectively. b) – Image details of low magnification tilescan. c) – Image details of high magnification scan. d) – User enters 'Plate name' as the experiment folder name to store images. This is also used as the filename for the excel analysis file. Users can also select the bromodomain and enter the plate type (8-well chamber slide or 24-well plate) for the experiment. e) – 'No. of tiles' changes the number of rows acquired per tilescan. Increasing this number will allow more cells to be discovered but may produce out-of-focus artifacts if set too high. 'Drug incubation time' is the number of minutes between dispensing compound to running FRAP for each well. 'Number of cells per well' is the target number of cells that the user wants to achieve. Once this number of cells has been reached, the system moves to the next well. If this number is not achievable after the first tilescan, up to 4 more tilescans will be attempted before moving onto the next well. f) – Autofocus offsets must be empirically determined for each objective and plate manufacturer. g) – Controls to perform FRAP on an entire plate without scheduling drug dispensing. Useful for troubleshooting or preliminary experiments. 'Interrupt ALL' stops experiment. h) – Status window to display the current step, the current well, and the number of cells that have been successfully acquired. i) – Controls for running a full FRAP experiment with scheduled drug dosing. 'Schedule status' describes the current action such as 'Dispensing' or 'FRAP'. A countdown timer is displayed in the 'Waiting Status' during drug incubation.


Supplementary fig. 11. User interface: MultiWell Setup. MultiWell setup controls the aspects of the plate map. a) – denotes the current position of the stage and focus motor. b) – button to test the transfer of fluid from one well to another by enter the x and y coordinate of the target well. 'Well offset' denotes the number of spaces in the horizontal direction between the target well and drug-containing well. For a 24-well plate, between well A1 and A4, this will be 3. c) – The spacing between each well should be set beforehand in the frapid.ini configuration file, but can be altered manually. Obtain dimensions from multiwell plate manufacturer. d) – Control for specifying the offset between the midpoints of the pump and microscope objective axis in the x-y plane. To do this, start by using Frapid to visit the first well. Then, manually adjust and move the stage in the x-y direction so that the pump is centered in the well. Confirm this adjustment by clicking 'Set pump-to-lens offset'. e) – Specify the number of rows and columns of wells containing cells for FRAP and click 'Create Range' to confirm.




Supplementary fig. 12. User interface: Scan Settings. ZEN profile settings region-of-interest size are obtained automatically from the frapid.ini configuration file, but can be manually altered in the 'Scan Settings' page. a) – denotes the ZEN configuration profiles to operate an Image scan, and Tilescan, and a Z-stack. Buttons allow manual and immediate running of these profiles for testing purposes. b) – Sets the bleach region diameter in pixels for different sizes of cells.


Supplementary fig. 13. User interface: Dispense Setup. Dispense setup for manually testing motor functions of the fluid handler. All parameters are loaded from frapid.ini configuration file but can be altered here. a) – 'Set' – confirm current position as home position of actuator, 'Go home' – force pump to return to home position. b) – Increasing the numbers will decrease the speed of the motors. c) & d) – Set the limit of travel in the vertical direction of pump by specifying the number of steps the motor should take. Positive and negative numbers denote different directions. 'Suck Steps' and 'Pump Steps' denote the number of steps the pump motor will take to fully aspirate and dispense the required amount of medium. Dispension is higher to ensure no liquid is leftover. All settings are loaded from frapid.ini configuration file.




Supplementary fig. 14. User interface: Matlab setup. The corresponding MATLAB scripts for each bromodomain are controlled in the Matlab setup page. All parameters are automatically loaded from the frapid.ini configuration file but can be altered here. a) – button to reload MATLAB if it has been prematurely closed. b) – folder name containing all MATLAB scripts for running FRAP. c)- filenames of tilescan images and list of coordinates for cells. d) – filenames for high magnification images and coordinates of bleach region. e) – filename of MATLAB script for analyzing FRAP data. f) – email address for automatically sending analysis file to once entire experiment has been completed.

## Supplementary References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