Supplementary Material

Supplementary Material A:

Hybridization Protocol

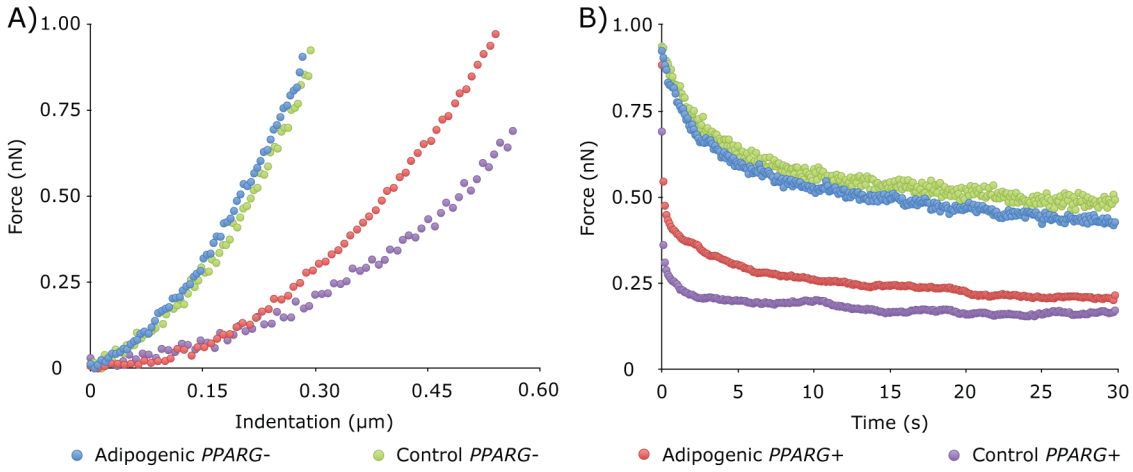
Molecular beacons were resuspended at 100 μM in Tris-EDTA buffer (pH 7.4) (ThermoFisher Scientific, Waltham, MA). A three-stage hybridization assay was used to verify beacon specificity for its designated target (Bratu et al., 2011; Tyagi and Kramer, 1996). Briefly, a SPECTRAmax GEMINI XS microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA) was used to record measurements of raw fluorescent units (RFUs) (excitation: 495nm; emission: 520nm) at 15-second intervals at 37˚C. First, three wells of a 96-well plate were loaded with 200 μL of molecular beacon hybridization buffer solution, composed of 20 mM Tris-HCL with 1 mM $MgCl_2$ (pH~8.5), and readings were taken for 2 minutes. Then, 2 μL of *PPARG* beacon were added, and measurements were collected for 5 minutes. Finally, 4 μL of target oligo DNA, designed to be complementary to the loop region of the *PPARG* beacon, was added to each of the wells, and readings were taken over 40 minutes to measure the hybridization response. To confirm that hybridization was only achieved in the presence of the beacon's complementary target sequence, this experiment was repeated with a non-complimentary, off-target sequence.

References

Bratu, D.P., Catrina, I.E., Marras, S.A., 2011. Tiny molecular beacons for in vivo mRNA detection. Methods in molecular biology 714, 141-157.

Tyagi, S., Kramer, F.R., 1996. Molecular beacons: probes that fluoresce upon hybridization. Nat Biotechnol 14, 303-308.

Supplementary Material B:



Supplementary Fig. 1. Representative elastic and viscoelastic AFM data. Morphologically spread ASCs were indented over the perinuclear region to obtain (A) elastic and (B) viscoelastic responses. A set of four, representative, single cells is shown. *PPARG*+ cells exhibited a more compliant and less viscous phenotype that *PPARG*- cells, regardless of medium environment. Average cell heights were approximately 4 μm, resulting in overall strains ranging between 5-15% (most tests <10%).

Supplementary Material C:

Noise Removal during Image Processing and Beacon Expression Quantification

To calculate the percentage of cells associated with positive molecular beacon signal, a custom MATLAB program was written to take advantage of the built in blob detection functionality included with the image processing toolbox. A consequence of using the lipofection reagent X-tremeGENE HP to deliver our beacons was the presence of brightly fluorescent, punctate debris throughout the sample, which complicated quantification of true, beacon-mRNA hydridization. To address this issue, an ImageJ macro was created to isolate the debris signal, subtract it from the original image, and save the adjusted images in a user defined output folder for analysis with the MATLAB algorithm. The macro first opens a dialog box to prompt the user for input and output folders and stores the image names in a vector. The script then iterates through each of the images, storing two copies of each image (one at a time) as separate variables. The script then adjusts the contrast of one of the image copies to remove weaker signals, to retain only the brightest signal (debris). After isolation of the debris signal, the image is converted to an 8-bit image and the edges of the remaining signals are detected using "Find Edges". Once edges have been detected, the image is converted to binary and the "Fill Holes" function is used to refill the detected regions of debris. The isolated debris signal is then subtracted from the original image using the "Transparent-zero create" function. The resulting image is saved as a "*.tiff" file in the user defined output folder. The ImageJ macro script is available upon request.

After debris removal, the images were ready to be passed through the MATLAB program, which takes several user-defined inputs including: intensity threshold (number of intensity values above the mean background), minimum area threshold, pixel ratio of the camera used to obtain the images, number of sections to break the image into for analysis (helps with variable background intensities), and number of pixels away from the nucleus to look for beacon signal. Firstly, the program converts the incoming image to a 0-255 intensity scale and subtracts the minimum pixel intensity value from each point. The image is then segmented into an n-by-n matrix of equally sized images, where n is the user defined section number (default = 8, yielding 64 sections). Each of the smaller, sectioned image matrices are then sorted by pixel intensity, and the average of the lowest 25, non-zero pixel values is calculated for each column. The average of each column's background intensity value is then computed and stored in another n-by-n matrix, where each value corresponds to the average background intensity for its respective image section. Using the background values, a binary image was created where any pixels with an intensity below the sum of the calculated background (which included the removed debris pixels) and the user defined intensity threshold are set to zero, while the remaining pixels are set equal to one. The imaging toolbox is then used to detect the areas of positive beacon signal and select only those regions larger than the user-defined area threshold. The imaging toolbox assigns each detected region an index and generates a matrix in which all of the pixels within a detected area

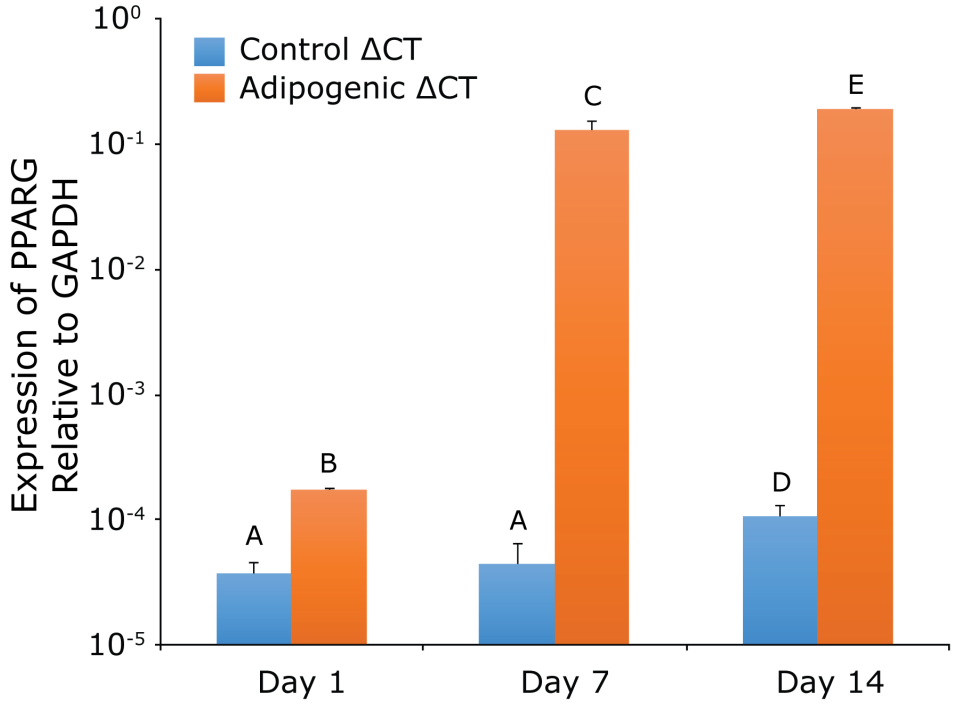
are set equal to the corresponding index. A similar process is implemented for images of Hoechst-stained nuclei to label each nucleus with an index for determining cell number and additional computations. Since the molecular beacon signal is often purely cytoplasmic (with little to no signal in the nucleus), nuclei are expanded by a user-defined number of pixels (2 pixels used for this study) using the same index value for the detected nucleus. This expansion is performed in two phases: (i) first, the program cycles through pixels from left to right, progressing from the top to the bottom of the image, and expanding all indexed regions in the reverse direction (up and to the right) and (ii) second, the program cycles through and expands pixels in the opposite direction (from right to left while moving from the bottom to the top of the image). The expansion is performed in the reverse direction of pixel interrogation to prevent infinite expansion as the next pixel to be examined would always be replaced with a positive value and expanded further. After expansion, each pixel is examined and a two-dimensional vector is used to store the indices of both the detected beacon signal and the expanded nuclei where they both store non-zero values. To prevent the assignment of one beacon region to multiple nuclei, each beacon signal was assigned to whichever nuclear index it overlapped with the most using the mode function. The number of unique nuclei indices were summed and compared to the total nuclei count for the calculation of percent of *PPARG*± cells. Several images are saved in subfolders of various steps in this process for reference and assistance in debugging and/or setting threshold values. The molecular beacon expression quantification m-file is available upon request.

Supplementary Material D:

Lipid Aggregate Quantification

Successful differentiation was confirmed through the formation of significantly larger lipid aggregates, quantified with a custom MATLAB program, in ASCs exposed to adipogenic induction medium compared to control medium. The quantification program prompts the user for several inputs including: file type (*.ext format), an intensity threshold (0-255), a lipid area threshold (μm^2), the pixel ratio of the camera used to acquire the images (pixels/μm), and binning parameters (number of bins and maximum x-axis value) for optimizing histogram outputs for viewing. After assigning the user inputs to variables, the program scans the folder for images matching the defined file type. The program then enters a loop to iterate through each image. First the image is converted to a 0-255 intensity scale and then inverted (255-image) so that the darker lipids appear bright. Although the image processing could be performed without this step, it is in place to help with visualization of positive signals. Next, a binary image is generated using the user-defined intensity threshold, setting all values below or equal to the threshold equal to zero and pixels with intensities greater than the threshold equal to one. The regions of signal are then detected and indexed using the functions of the image processing toolbox. Three matrices are then initialized for storing all lipid aggregate areas, as well as those above and below the size threshold. The matrices are populated by iterating through each detected lipid region, comparing the values to the user-defined threshold and then storing both the area and lipid number in two of the corresponding matrices. Additionally, the program outputs a figure that includes the inverted original image, a histogram of the intensity values (with a line designating the intensity threshold), the binary version of the detected regions, a binary version of only the lipid aggregates over the area threshold, and finally a histogram of the lipid aggregate areas (with a line designating the area threshold) to provide the user with an idea of the distribution. The lipid aggregate quantification m-file is available upon request.

Supplementary Material E:

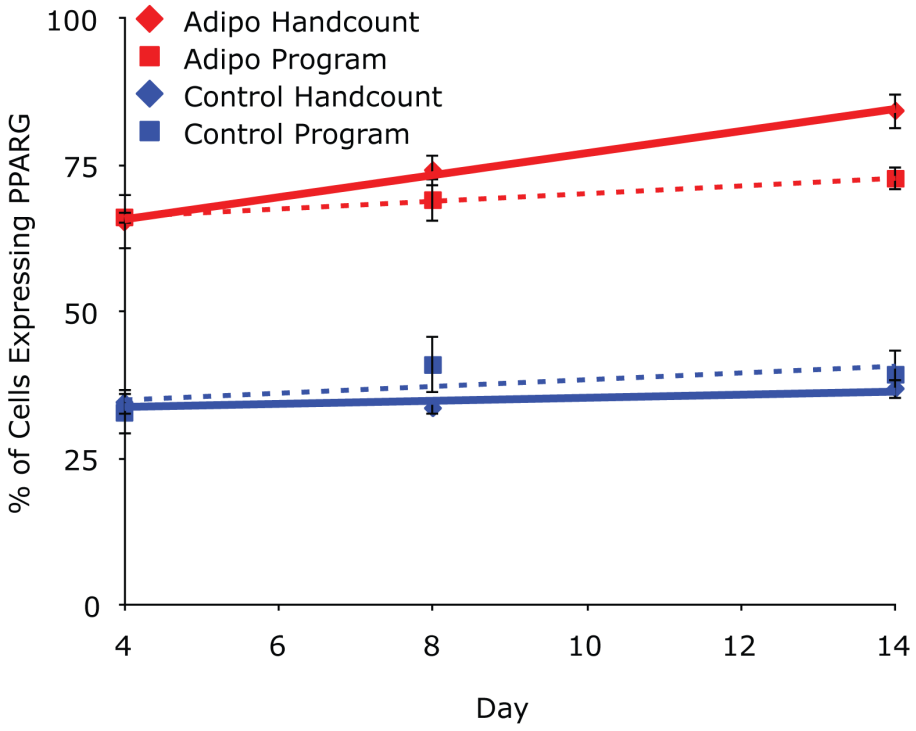


Supplementary Fig. 2. *PPARG* is upregulated in ASCs exposed to adipogenic induction medium. Adipogenic samples (orange bars) expressed *PPARG* 3.7-fold more than controls (blue bars) after just one day of induction. Within adipogenic samples, relative *PPARG* expression increased 1100-fold by day 14 of the induction period. Control samples showed a nominal, 2-fold increase over the same period. Statistical significance existed for groups with non-matching letters ($p < 0.015$).

Supplementary Material F:

Program error assessment

Error associated with the beacon detection program was assessed by comparing program-processed and hand-counted data for a single image set from each of the three iterations on three randomly chosen days (Day 4 [Iteration 1], Day 8 [Iteration 3], Day 14 [Iteration 2]). The values obtained from both approaches were within ±10% of each other on each of the days for both adipogenic and control samples (Supplementary Fig. S2). Further analysis indicated the program loses accuracy when assessing samples with >80% *PPARG*+ expression due to an overcorrection of regional background thresholding in densely populated areas of individual images. It should be noted that actual *PPARG*+ expression differences between adipogenic and control samples are likely greater than reported, since the program tends to slightly overestimate expression in control samples and underestimate in adipogenic samples, artificially bringing the reported values closer together.



Supplementary Fig. S3. Beacon detection error assessment. Percent expression values returned by the beacon detection program (square data points fit linearly with dotted lines) showed agreement within ±10% of values calculated from hand-counting the number of PPARG+ cells (diamond data points fit linearly with solid lines) for both adipogenic (red curves) and control (blue curves) samples. Each data point represents the average and standard deviation of percent expression from the six-image set of a single experimental iteration.