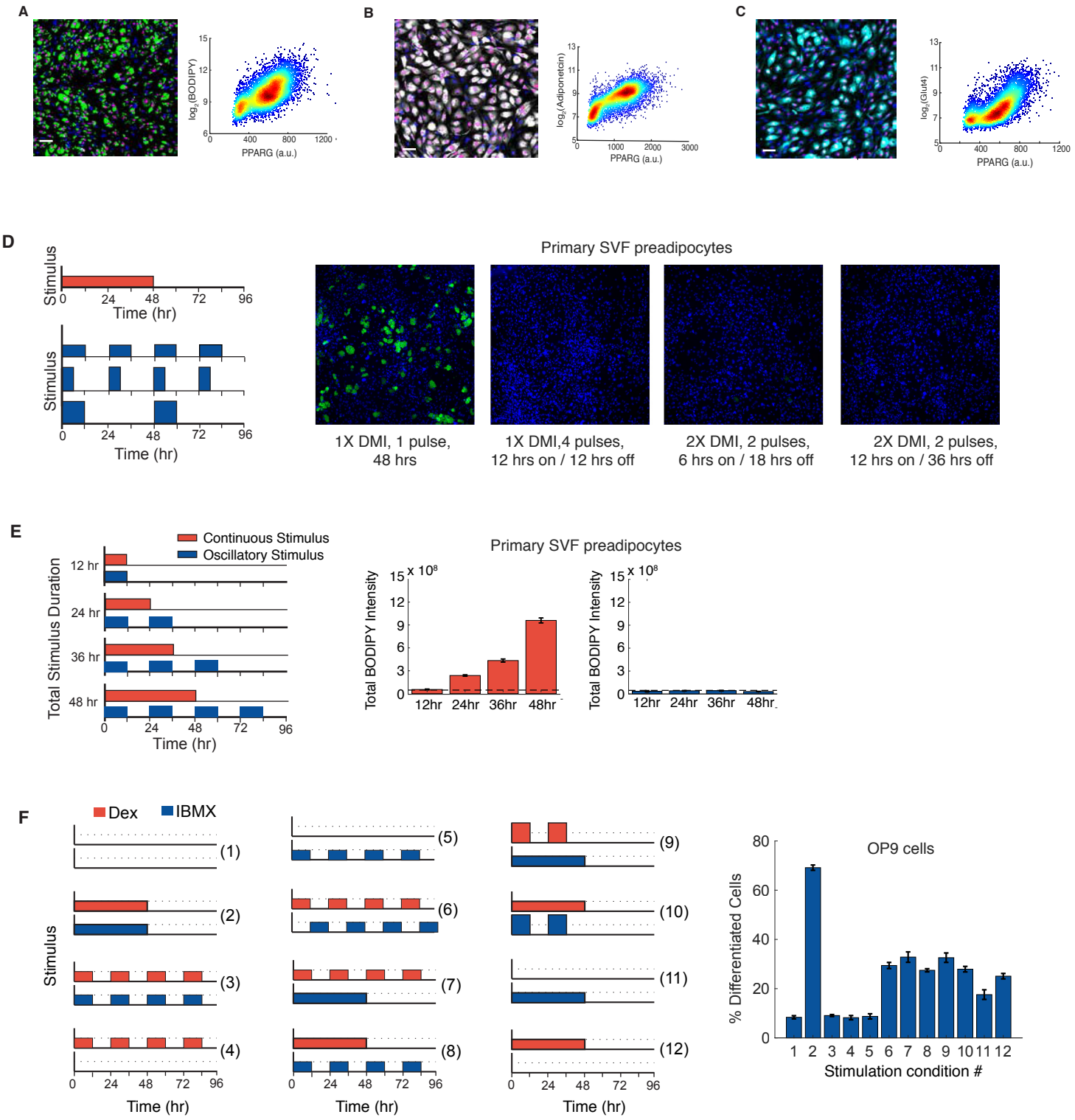

Figure S1. Adipogenesis creates two populations of cells with either low PPARG (undifferentiated cells) or high PPARG (differentiated cells) and the rejection of circadian hormonal pulses occurs also in primary SVF preadipocytes.

(A-C) A 48hr DMI stimulus was applied at t=0 hrs to mouse OP9 cells. At t=96 hours, the cells were fixed and stained with Hoescht (blue) to mark nuclei and anti-PPARG (red), plus either (A) BODIPY (green), (B) anti-Adiponectin (white), or (C) anti-GLUT4 (turquoise). Images and scatter plots show that the high PPARG correlates closely with lipid accumulation (BODIPY) and markers of mature adipocytes (Adiponectin and GLUT4).

(D-E) Primary SVF preadipocytes treated with the same pulse protocols as in Figure 1D and 1E show the same rejection of circadian hormonal pulses and the same gradual increase in adipogenesis for increasing continuous stimuli with durations greater than 12 hours.

(A,B,C,E) Scale bar represents 20 uM.

(F) Preadipocytes filter both pulses of glucocorticoids (dexamethasone) and cAMP (cellular increase mediated by IBMX). Dexamethasone (1 uM) and/or IBMX (0.25 mM) were added and removed for the indicated durations of time to cell media consisting of MEMalpha with Pen-Strep, L-glutamine, and 1.75nM insulin.

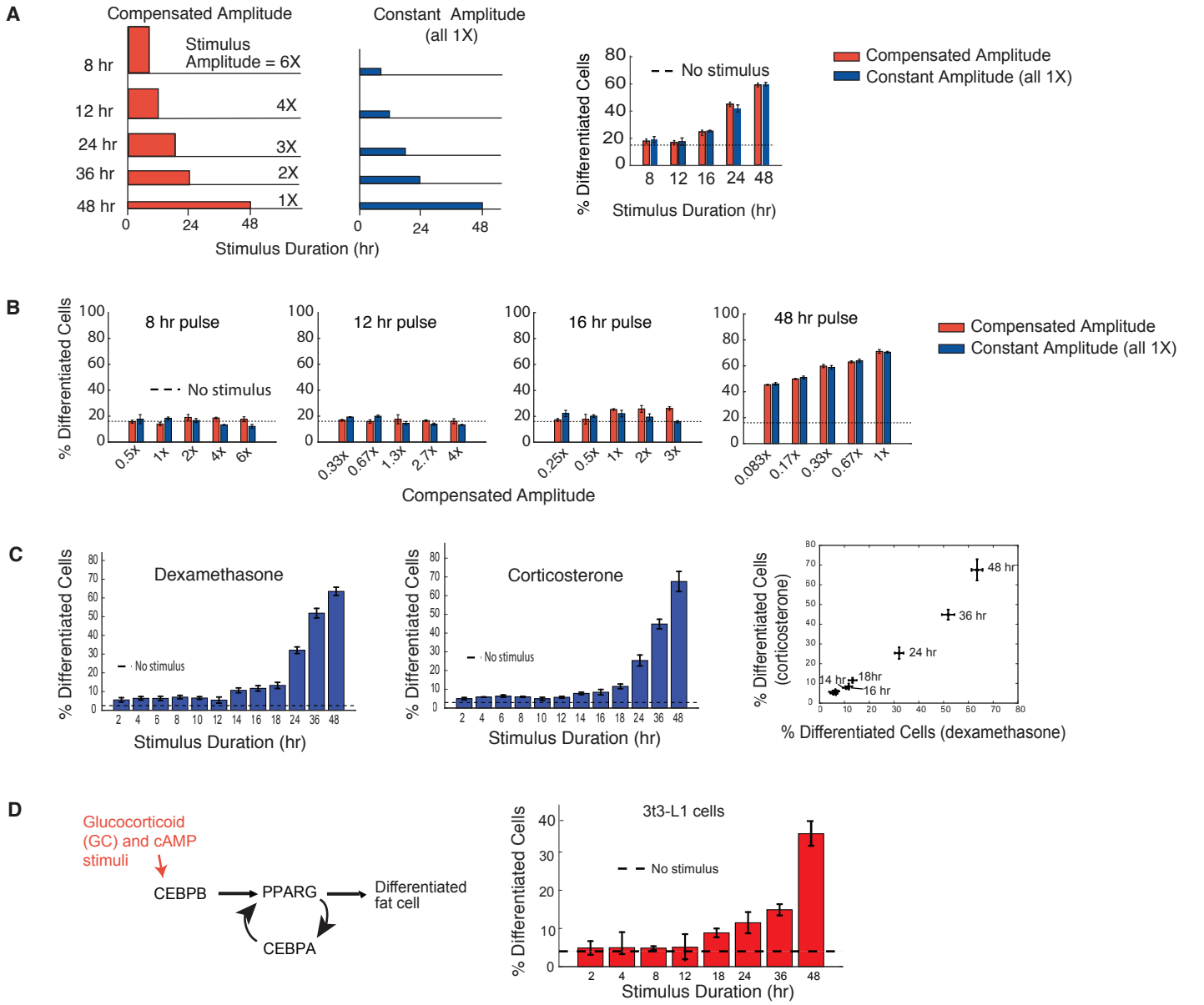

Figure S2. Verifying that the concentration of the applied DMI pulses is not saturating and that using corticosterone, a physiological glucocorticoids, instead of dexamethasone has the same filtering effect. Also, like OP9 cells, 3T3-L1 cells filter out short and circadian glucocorticoid input signals.

(A)Test of whether the rate of preadipocyte differentiation is controlled by the integrated strength of the stimulus or simply by the stimulus duration within a wide-range of stimulus amplitudes. (Left) Schematic showing tested protocols in which DMI stimuli was applied to OP9 preadipocyte cells for different durations at a single concentration (blue) versus at increasing concentrations that kept the total stimulus exposure constant (red). As an example of the latter, if the pulse durations were decreased by two-fold, the pulse amplitudes were increased two-fold to compensate. The cells were fixed 96 hours after application of the stimulus. (Right) Bar plots showing percent of differentiated cells for each protocol. The results show that the same rejection of differentiation stimuli less than 12 hours in duration and graded increase in differentiation rates for stimuli longer than 12 hours is seen whether or not the stimulus amplitude is increased to keep the integrated strength of the stimulus constant.

(B) Verification that the DMI stimulus concentration used is not saturating for pulsatile stimuli. The constant amplitude (1X) of the DMI stimulus was varied across a 50-fold range. Strikingly, the filtering of short duration stimuli was observed for all dilutions tested for both compensated (red bars) and constant 1X (blue bars) amplitude conditions. In contrast, increased differentiation was observed with increasing amplitudes for long 48-hour duration stimuli. (A-B)In all experiments, the 1X concentration of DMI used was 1 uM dexamethasone, 250 uM IBMX, and 1.76 nM insulin. Percent of differentiated cells, measured at 96 hours as in Figure 1B, represents mean +/- s.e.m from 3 technical replicates with approximately 7000 cells per replicate. All data shown are representative of 3 independent experiments.

(C) Using a physiological glucocorticoid, corticosterone, shows the same differentiation ability and filtering behavior as when the synthetic glucocorticoid, dexamethasone, is used. Stimuli pulses of different durations were applied to OP9 cells to indirectly activate PPARG via activation of the glucocorticoid receptor and CEBPB (DMI). For the corticosterone experiments, 1 uM corticosterone was used instead of 1uM dexamethesone in the normal DMI stimulus. The results plotted as barplots (left and middle) or in a single scatter plot (right) show that pulsing with corticosterone has the same filtering behavior as pulsing with dexamethasone. Percent of differentiated cells, quantified as in Figure 1B at 96 hours after stimuli was applied to OP9 cells to initiate adipogenesis, shows mean +/- s.e.m. from 3 technical replicates, representing 3 independent experiments.

(D) Bar plots showing percent of differentiated cells 96 hours after different durations of stimuli were applied to 3T3-L1 cells to initiate adipogenesis by indirectly activating PPARG via activation of the glucocorticoid receptor and CEBPB upstream of PPARG expression using DMI. Percent of differentiated cells, quantified as in Figure 1B at 96 hours after stimuli was applied to the cells to initiate adipogenesis, shows mean +/- s.e.m. from 3 technical replicates, representing 3 independent experiments.

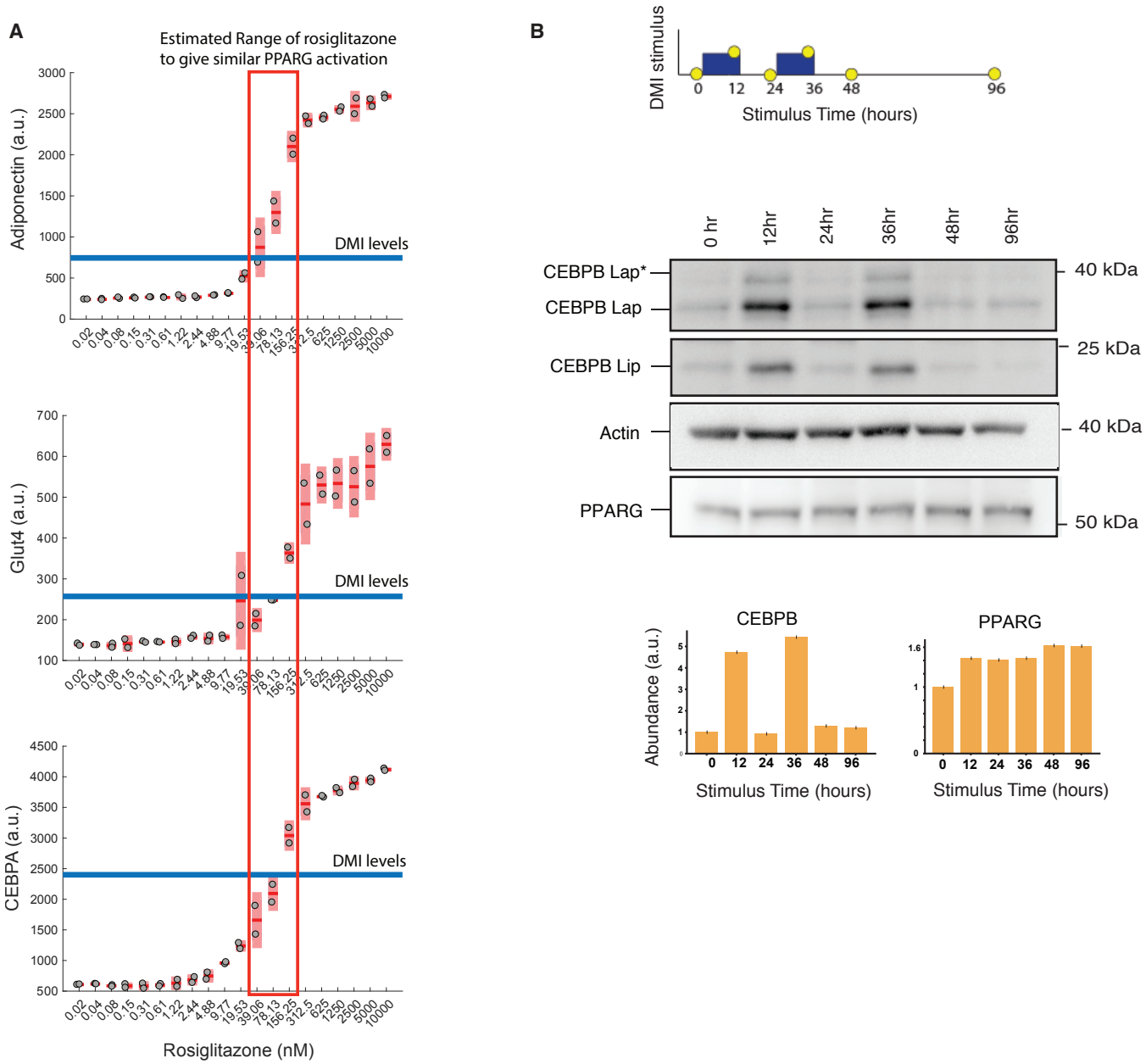

Figure S3. Determining the concentration of Rosiglitazone that gives comparable PPARG activation as a DMI stimulus and measuring CEBPB and PPARG dynamics by western blots.

(A) To estimate a level of Rosiglitazone that gives similar PPARG activation as DMI, different amounts of Rosiglitazone were added to the media of undifferentiated mouse OP9 cells plated in 96-well wells in order to induce the cells to differentiate. Forty-eight hours when the cells were maximally differentiated by Rosiglitzone, the cells were fixed and stained for Adiponectin, Glut4, and CEBPA protein levels The titration curves showed that ~40 to 100 nM of Rosiglitazone for 48 hours matches the expression of the respective genes induced by the standard DMI differentiation protocol (marked by the blue vertical lines). Thus, a dose of 100nM Rosiglitazone was used in Figure 2A in order to compare differentiation effects to those resulting from a DMI stimulus.

(B) The average PPARG and CEBPB concentrations in OP9 cells pulsed with DMI were measured by western blot. The barplots show a quantification of the western blots measured using ImageJ analysis software.

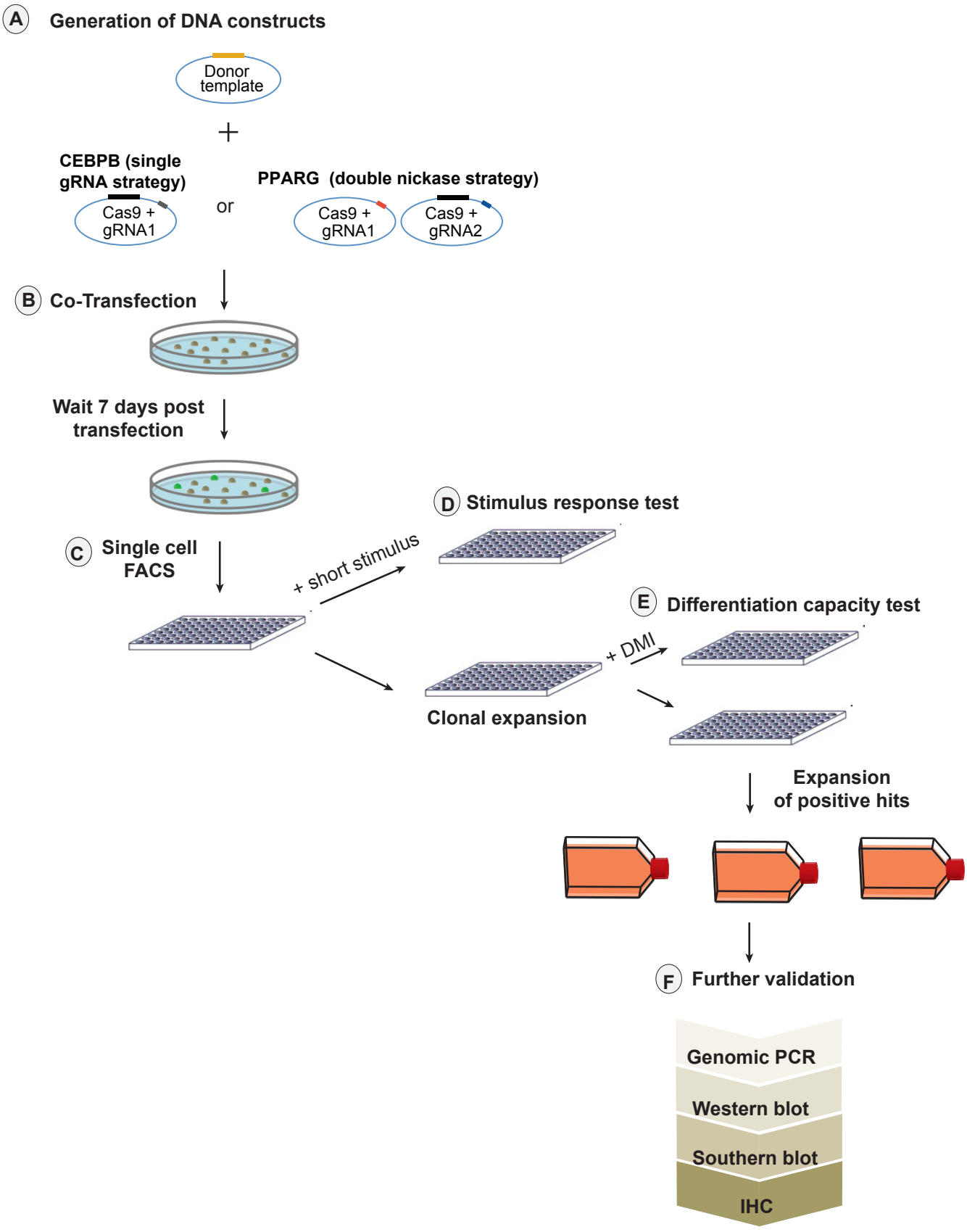

Figure S4. Workflow for generating and validating single clones with endogenously tagged CEBPB and PPARG using CRISPR-mediated genome editing. The different steps in the workflow are described in detail in the Methods section.

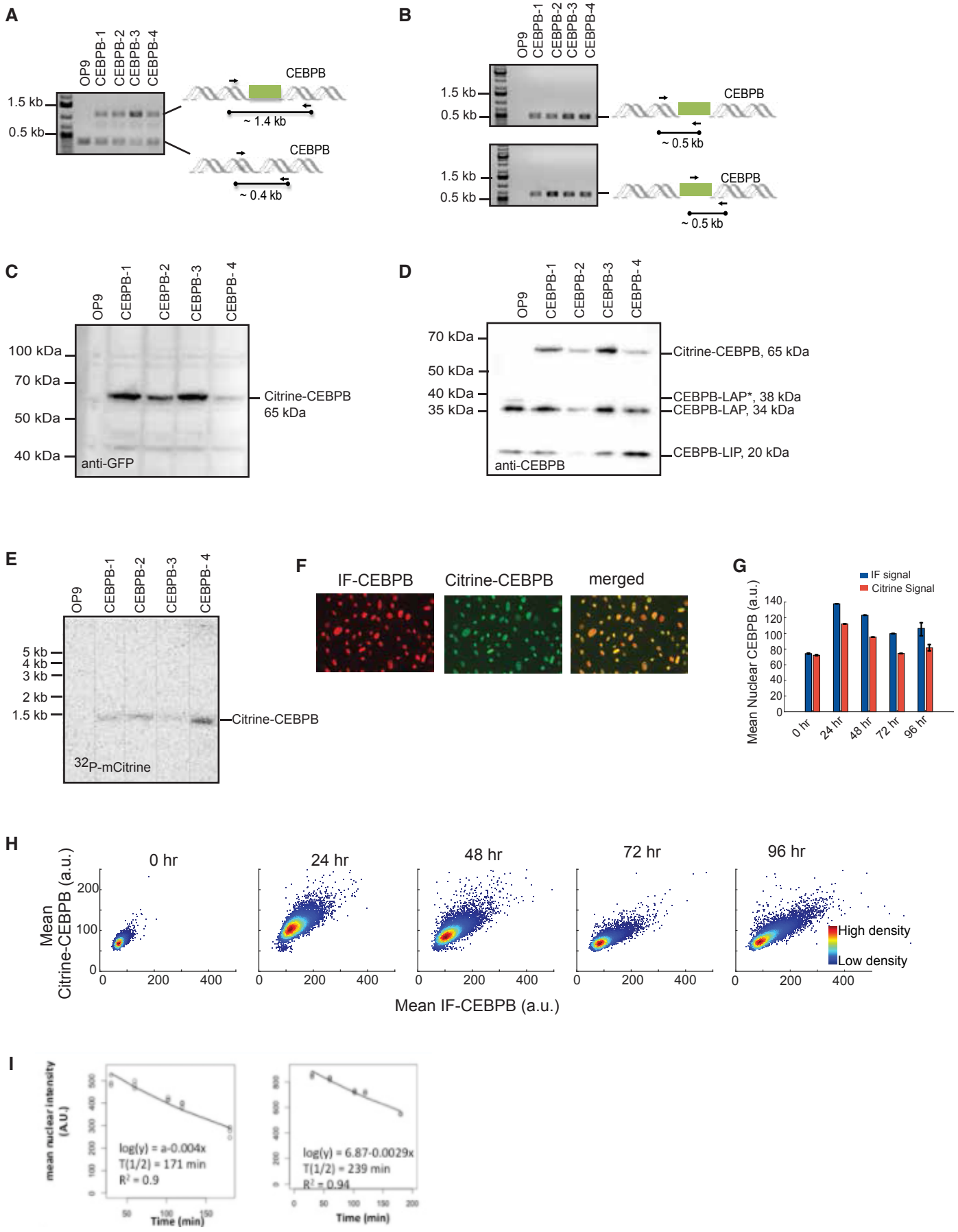

Figure S5. Validation of CEBPB clones. The different steps of the validation are described in detail in the Methods section.

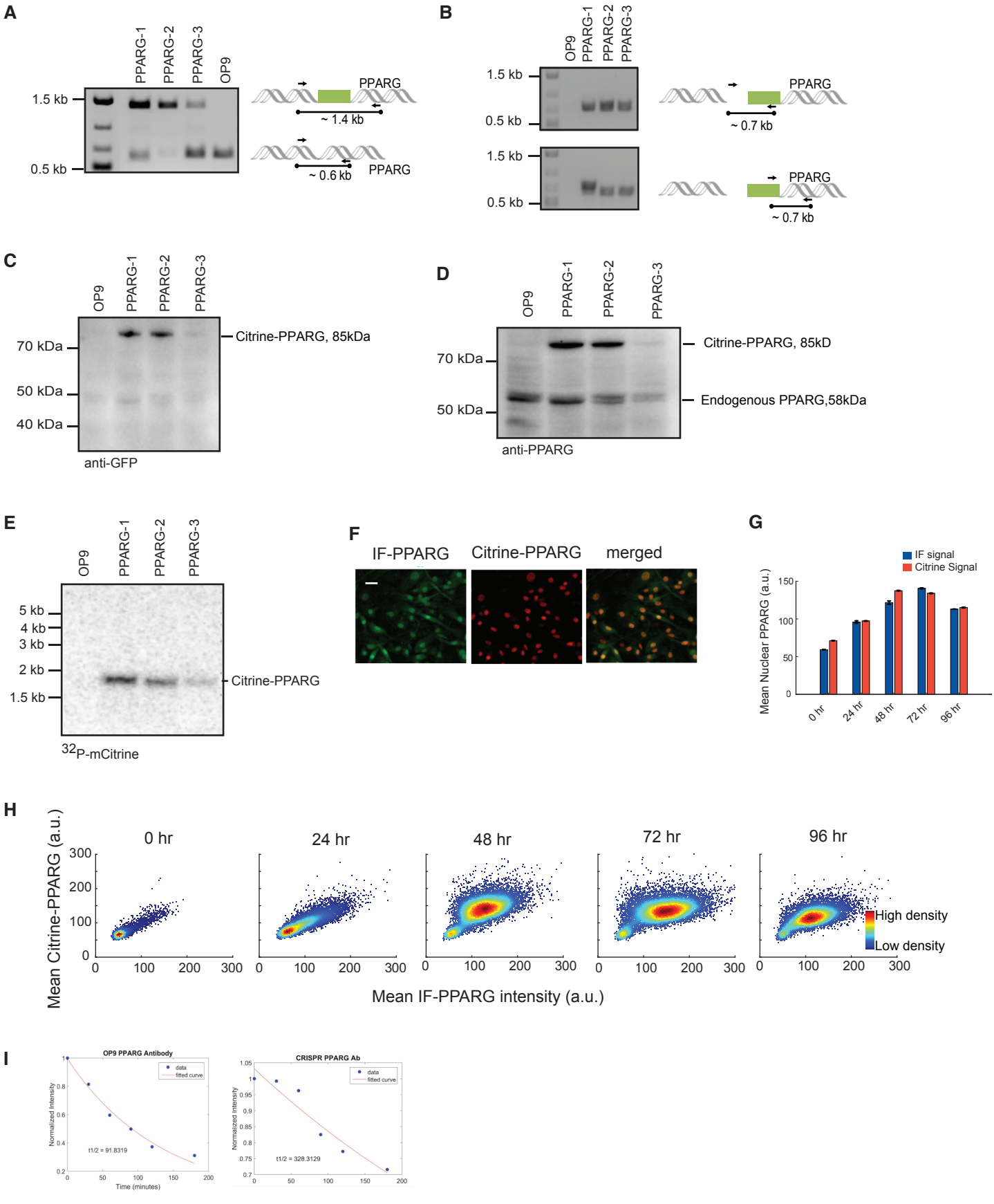

Figure S6. Validation of PPARG clones. The different steps of the validation are described in detail in the Materials and Methods section.

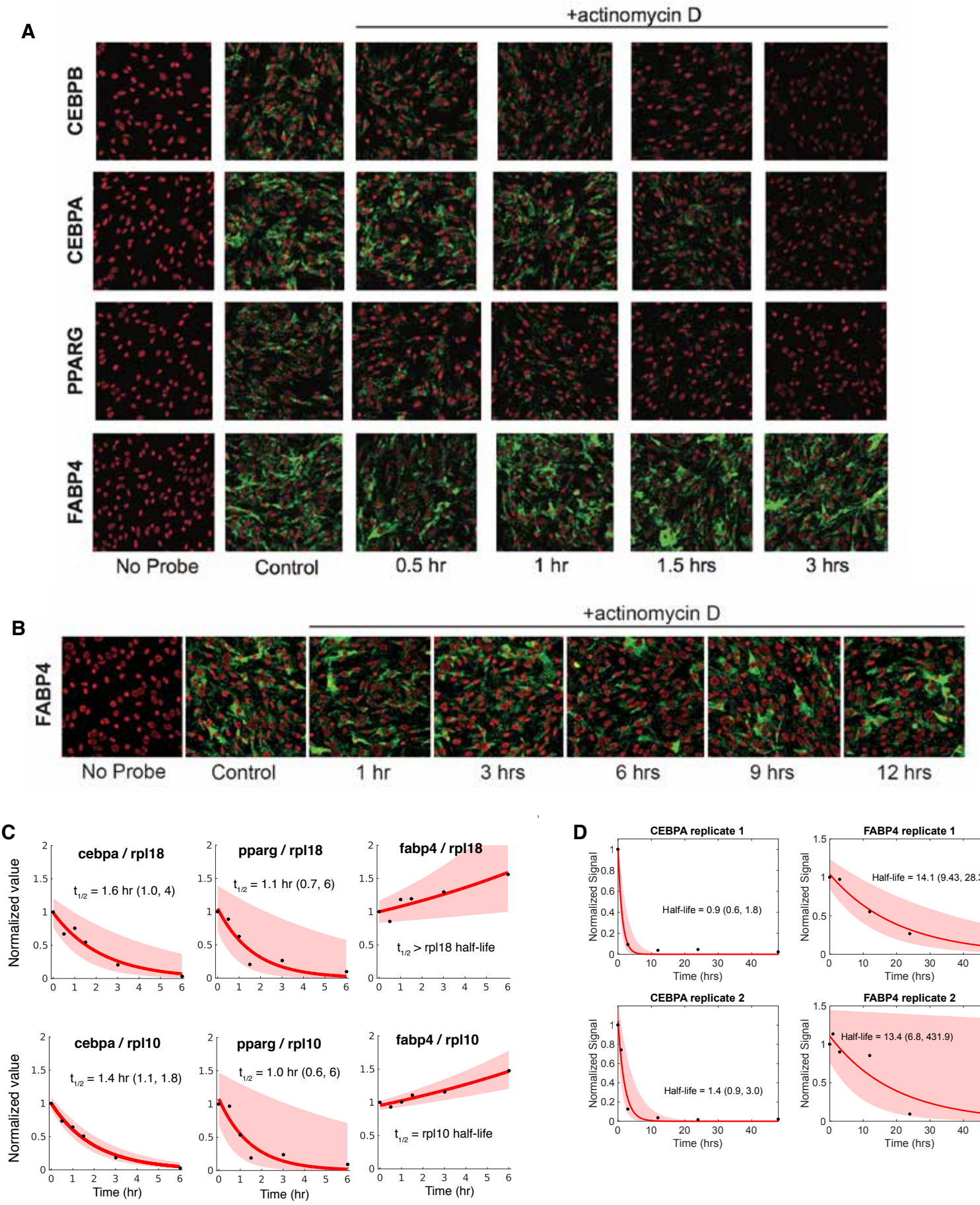

Figure S7. Measurements of mRNA decay after addition of actinomycin D.

(A-B) RNA FISH images of actinomycin D experiments. The long half-life of FABP4 mRNA is apparent by eye, even before carrying out quantitative analysis.

(C) RT-PCR measurements confirm the half-life measurements obtained by RNA FISH.

(D) 5EU measurements confirm the half-life measurements obtained by RNA FISH.