SUPPLEMENTARY INFORMATION

4-Hydroxytamoxifen probes for light-dependent spatiotemporal control of Cre-ER mediated reporter gene expression

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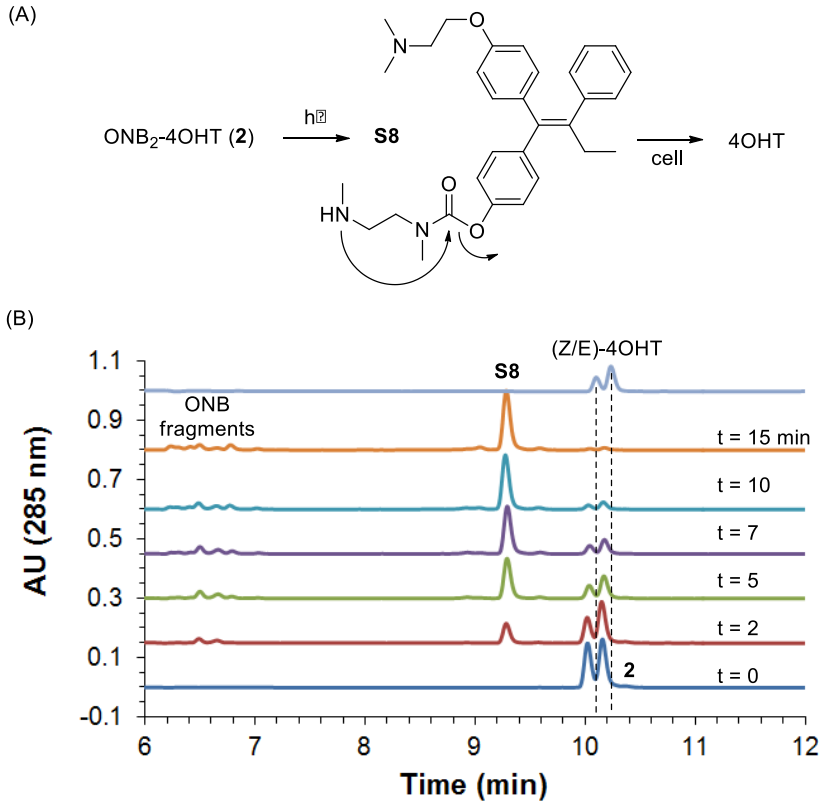
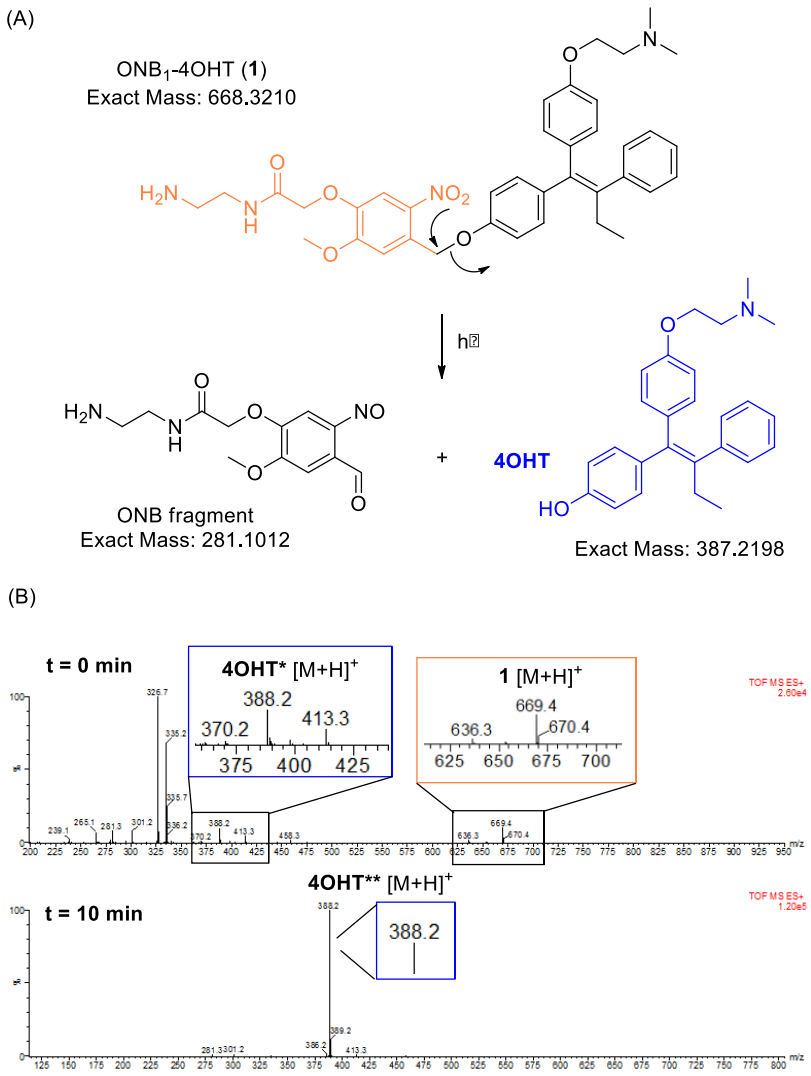
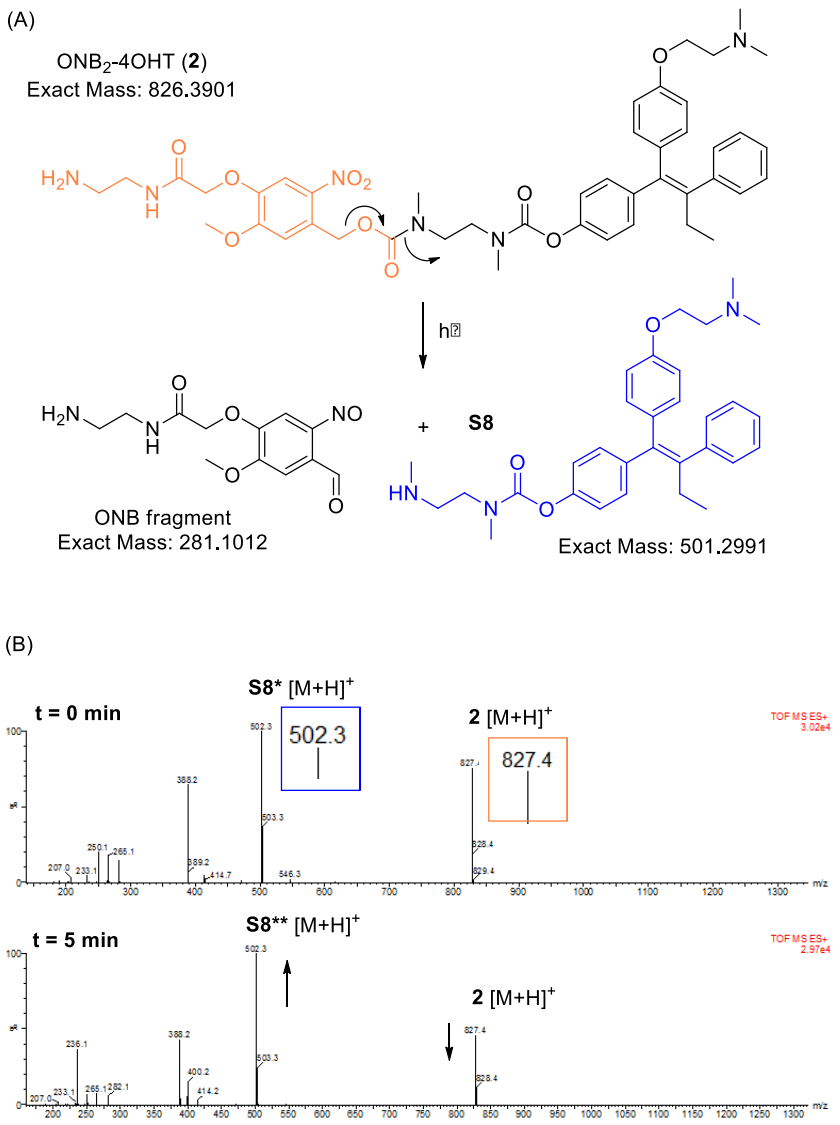


Figure S1. (A) Photochemical cleavage reaction of ONB_2-4OHT (**2**) to release **S8** (Z and E), a 4OHT derivative that can undergo intramolecular metabolic transformation[1] of its methyl(2-(methylamino)ethyl)carbamate to release free 4OHT; (B) HPLC traces (detection at 285 nm) for ONB_2-4OHT (**2**) after UVA exposure as a function of time.



4OHT*: mass fragmentation; 4OHT** attributable to release

Figure S2. (A) Photochemical cleavage reaction of ONB$_1$-4OHT (**1**; M_r = 668.32) to release free 4OHT (M_r = 387.22) and ONB fragments; (B) Two mass (ESI) spectra, each measured before (t = 0 min) or after UVA exposure (10 min), suggest full release of 4OHT, which is in agreement with HPLC analysis (cf, **Figure 1**).



S8*: mass fragmentation; S8** attributable to release and fragmentation

Figure S3. (A) Photochemical cleavage reaction of ONB_2-4OHT (**2**; M_r = 826.39) to release **S8** (M_r = 501.30) and ONB fragments; (B) Comparison of two mass (ESI) spectra, each measured before (t = 0 min) or after UVA exposure (5 min), suggests growth of **S8** relative to **2**, which is in agreement with HPLC analysis (cf, **Figure S1**).

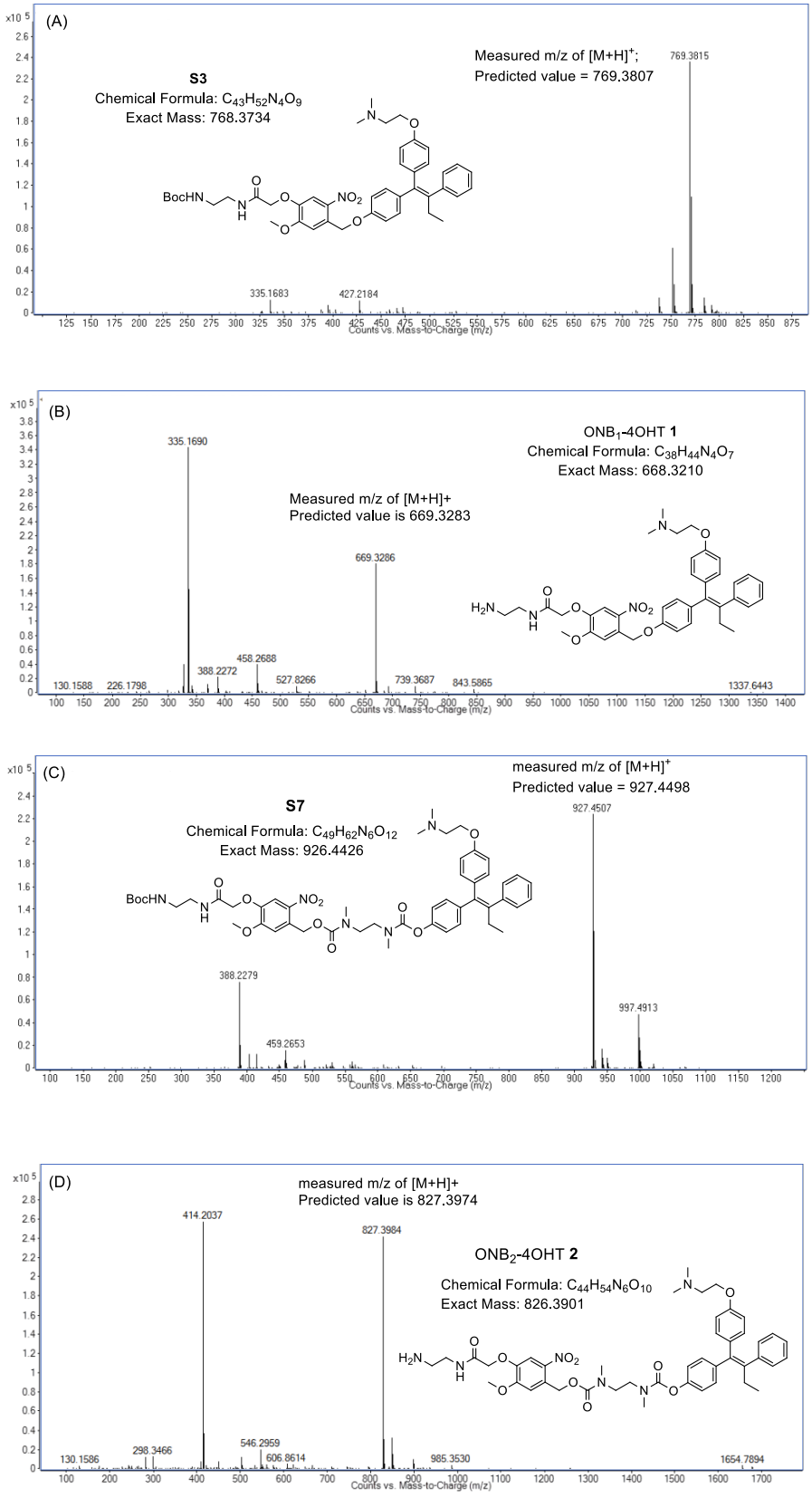


Figure S4a. High resolution mass spectra (HRMS) of **S3** (A), **1** (B), **S7** (C) and **2** (D)

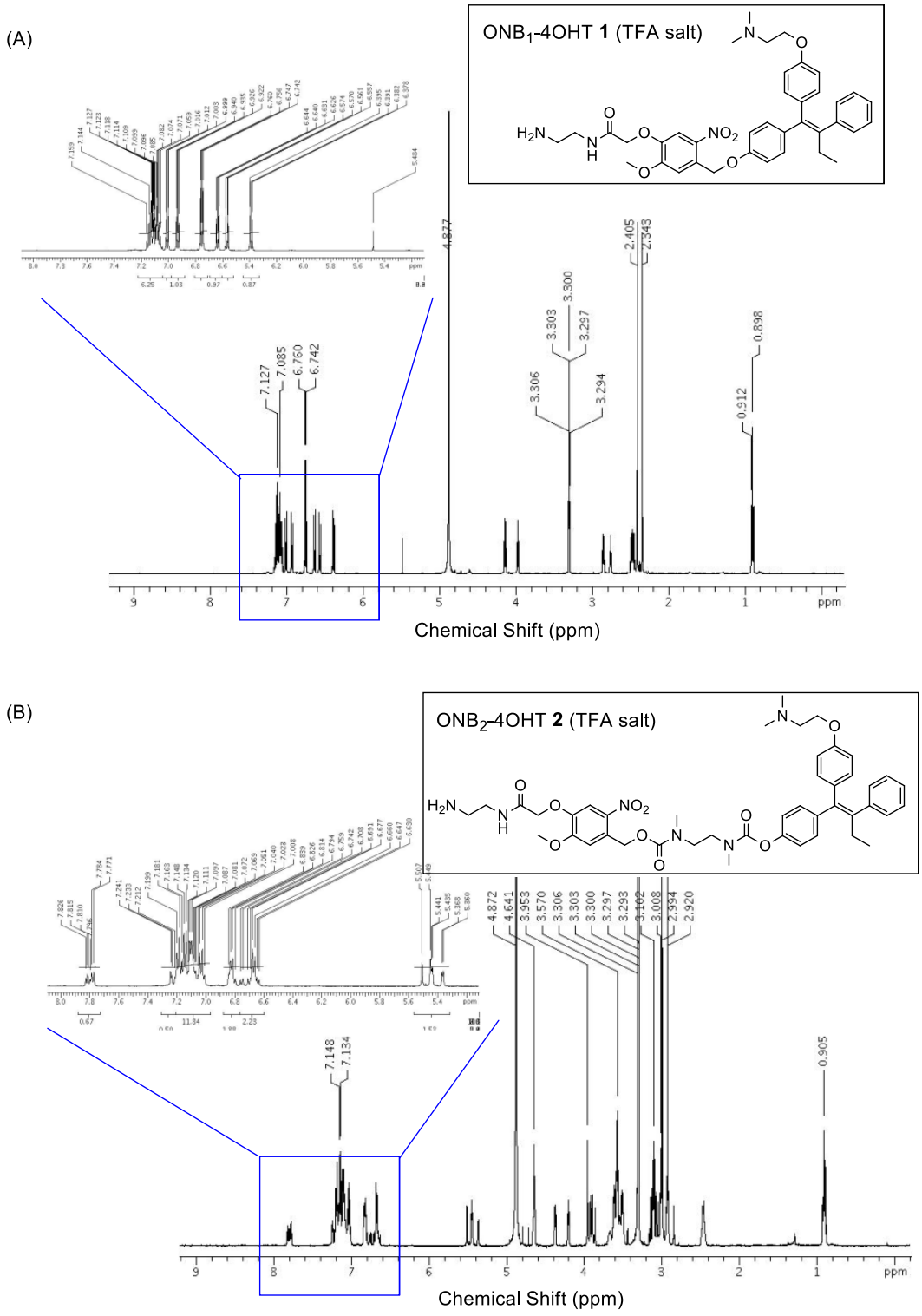


Figure S4b. ^{1}H-NMR spectra of ONB$_1$-4OHT **1** (CD_3OD) and ONB$_2$-4OHT **2** (CD_3OD)

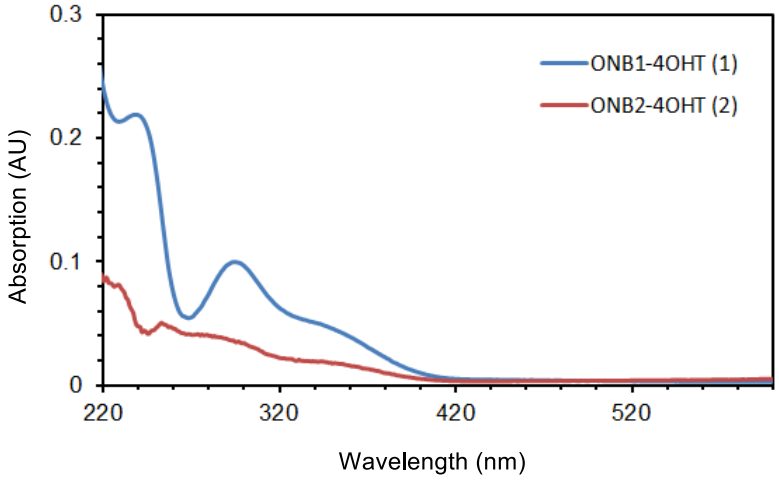


Figure S4c. UV–vis spectra of ONB_1-4OHT **1** and ONB_2-4OHT **2** (CD_3OD), each measured in 10% aq MeOH (0.1 mg/mL)

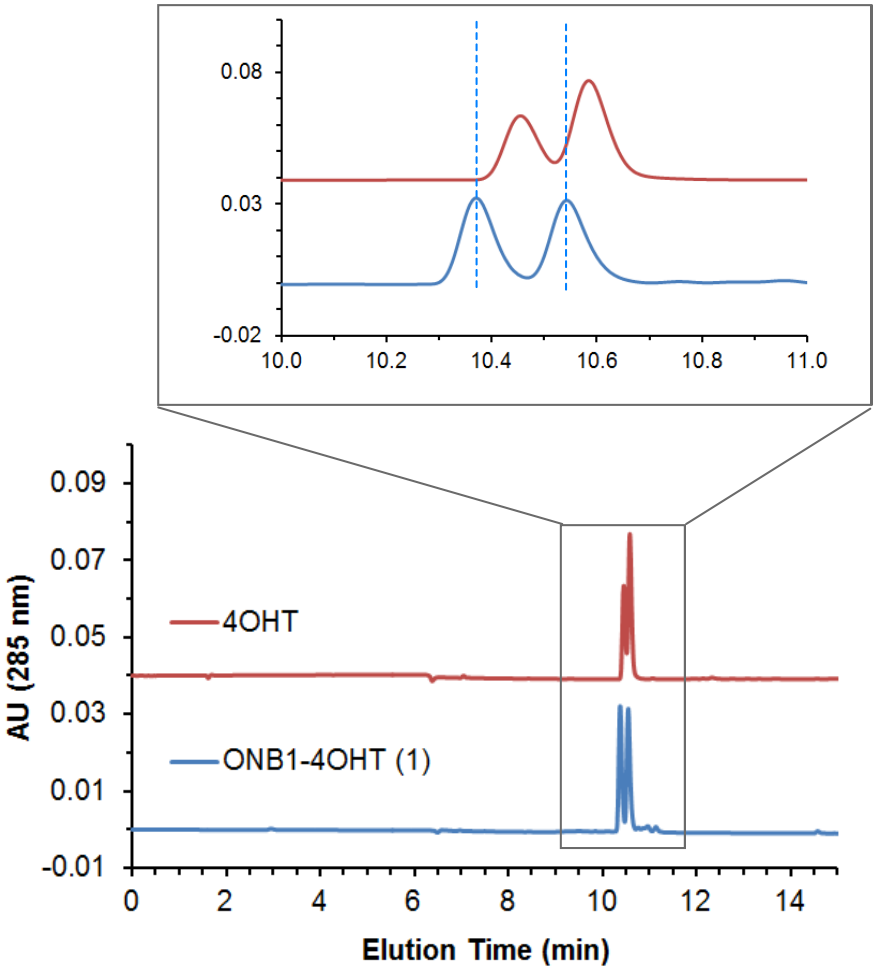


Figure S4d. HPLC traces of ONB_1-4OHT **1** and 4OHT.

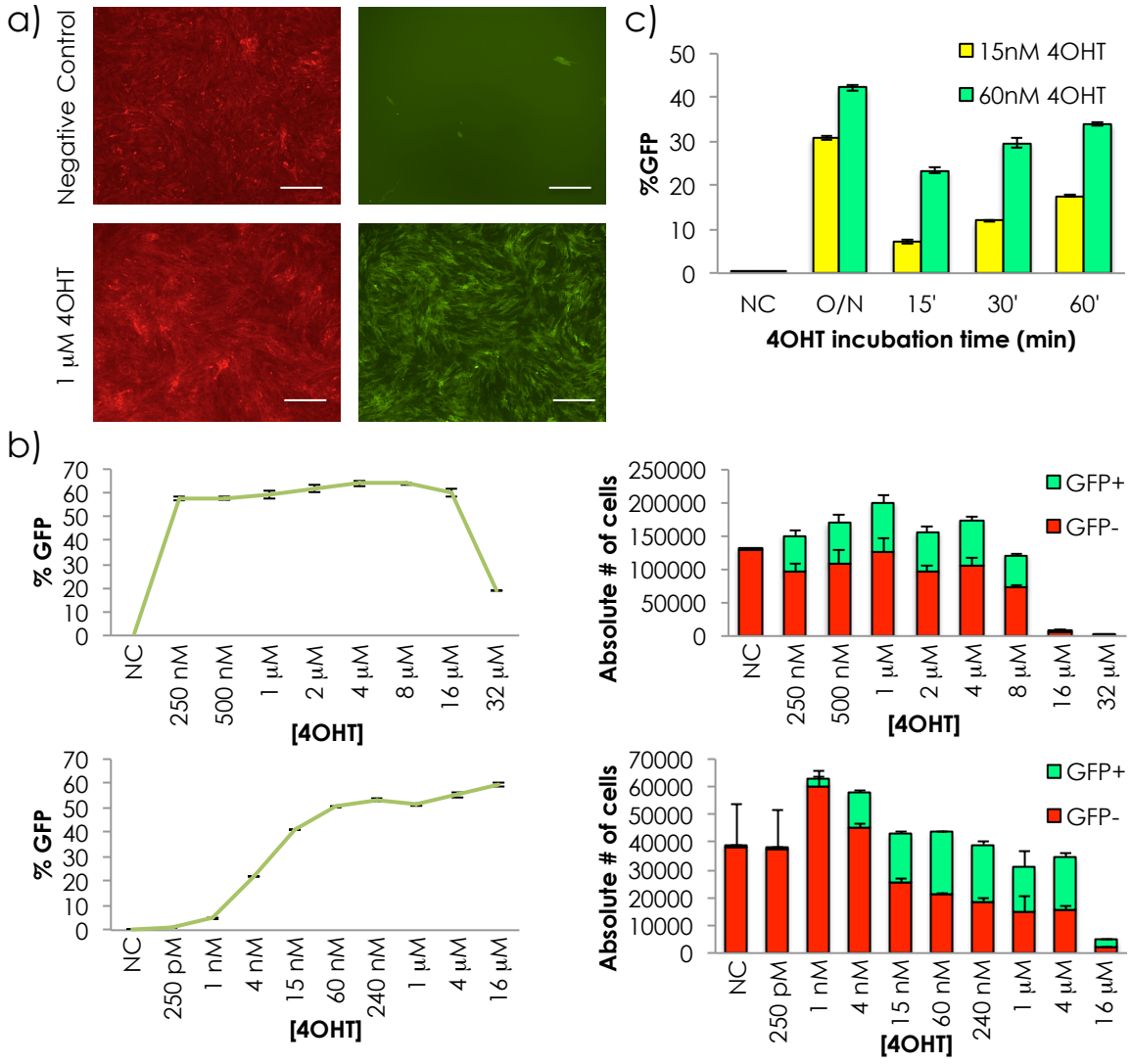


Figure S5. Optimization of 4OHT mediated recombination and GFP reporter activation in UT MEFs. (**a**) UT MEFs were incubated overnight in media only (negative control, top row) or media with 1 µM 4OHT (bottom row), then imaged at 24 h for the presence of Tomato (left plots) and the activation of GFP expression (right plots). At 24 h, all UT MEFs should retain Tomato expression, but only those cells in which 4OHT activated Cre-ER recombinase will express GFP. (**b**) Overnight incubation of UT MEFs in various concentrations of 4OHT, either 2-fold (top graphs) or 4-fold (bottom graphs) serial dilutions, then analyzed by FACS for percentage of GFP-expressing cells (left graphs) and absolute number (right graphs) of GFP^+ (green) and GFP^- (red) cells. Robust GFP activation was observed between 15 nM and 16 µM 4OHT. UT MEFs incubated with 4OHT concentrations of 16 µM and above exhibited a decrease in viability. Error bars are SD (N = 3). (**c**) Incubation of UT MEFs with 4OHT for short timepoints. UT MEFs were incubated in 15 nM (yellow) and 60 nM (green) concentrations of 4OHT for 15, 30, and 60 min, or overnight (O/N), then washed in PBS and analyzed 24 hours post-treatment. Robust activation of GFP expression was observed at all treatment times, indicating that 4OHT entered the cells rapidly upon incubation. Error bars are SD (N = 3).

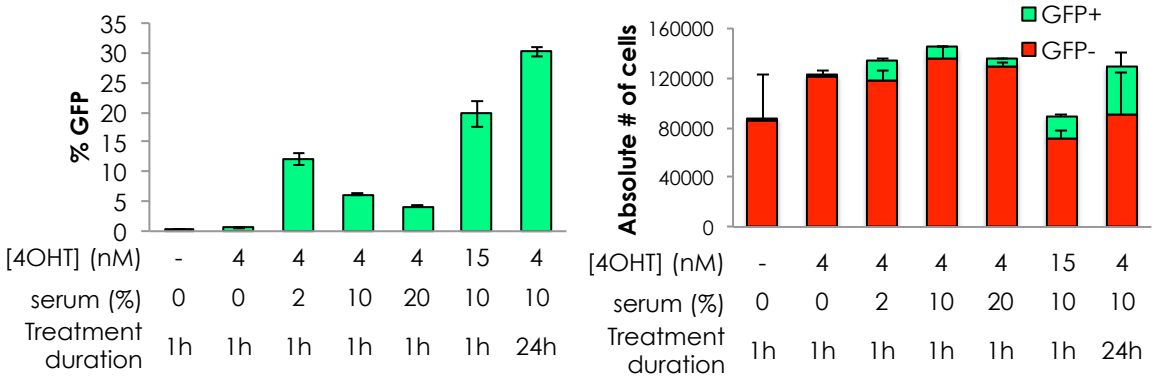


Figure S6. Effects of serum concentration on 4OHT mediated GFP recombination. We previously observed that increasing serum concentrations could inhibit recombination efficiency by TAM treatment.[2] To determine the effects of serum on 4OHT-mediated recombination efficiency, UT MEFs were treated for 1 h with 4 nM 4OHT in media containing 0%, 2%, 10%, or 20% serum, then washed and replaced with media containing 10% serum and cultured overnight. Percentage (left graph) and absolute number (right graph) of GFP^+ and GFP^- cells is indicated. Controls include an untreated control, a 15 nM 4OHT 1hour treatment in 10% serum, and a 24 h 4OHT treatment (4 nM, 10% serum). Error bars are SD (N = 3). Increasing the percentage of serum in the media appeared to slightly decrease recombination efficiency, but the effect was mild and was less significant at higher 4OHT concentrations.

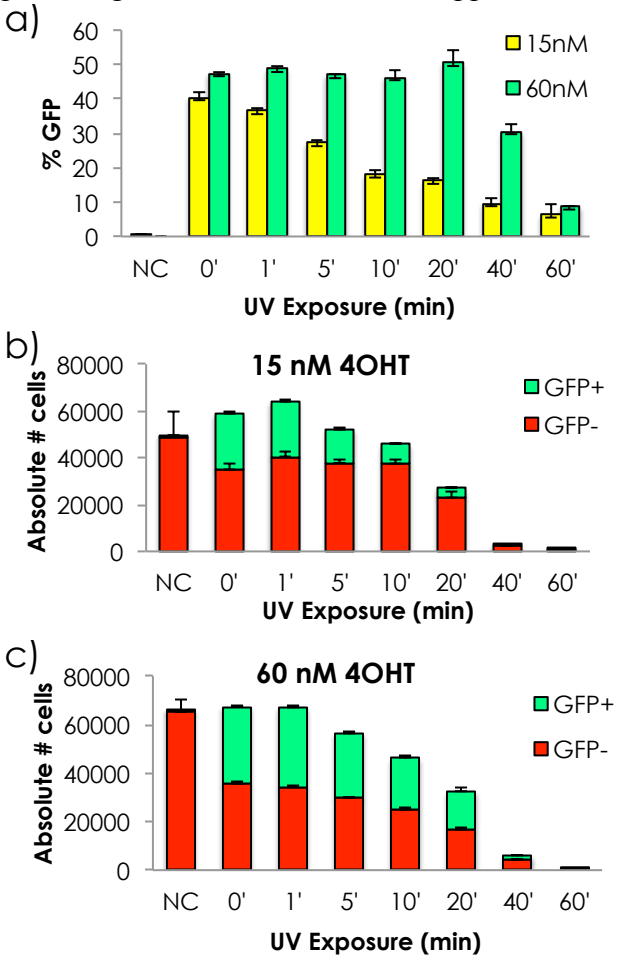


Figure S7. Effect of UV exposure on 4OHT mediated recombination efficiency and MEF viability. It has been reported that UV can rapidly degrade 4OHT.[3] We examined the effects of UV exposure on 4OHT recombination efficiency and MEF viability. (**a**) 15 nM (yellow) or 60 nM (green) 4OHT was exposed (in media) to UV for different lengths of time, then added to UT MEFs and incubated for 24 h. Percentage of GFP^+ cells is indicated. Error bars are SD (N = 3). The absolute cell number of GFP^+ (green) and GFP^- (red) is shown for 15 nM (**b**) and 60 nM (**C**) 4OHT concentrations. Error bars are SD (N = 3). We observed a modest decrease in recombination efficiency with increasing UV exposure lengths for 15 nM 4OHT, but this decrease was less evident in 60 nM 4OHT. At UV exposure times of 40 min or greater, the viability of MEFs decreased dramatically. We conclude that at shorter UV exposure times of 10 min or less, 4OHT activity and MEF viability is not significantly decreased.

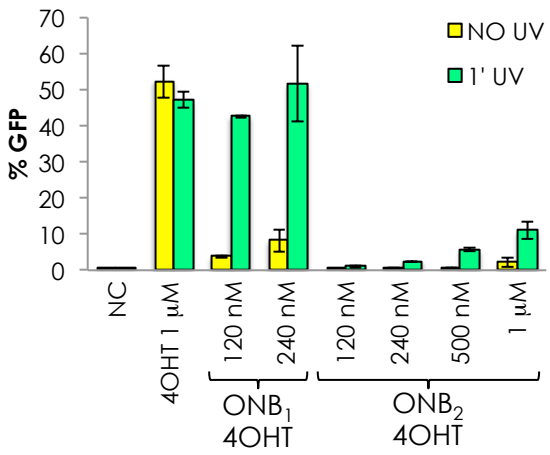
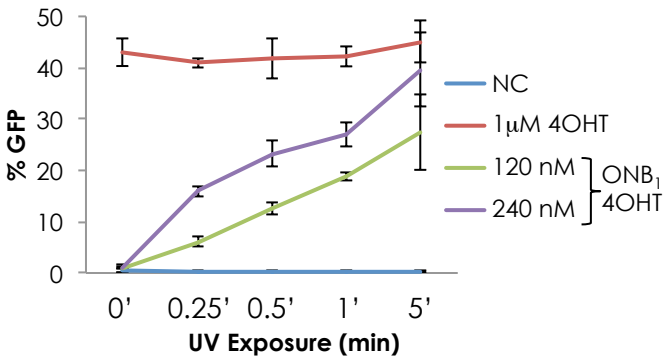


Figure S8. Comparison of ONB_1-4OHT and ONB_2-4OHT recombination efficiency upon exposure to UV light. UT MEFs were treated with 4OHT (1 µM), ONB_1-4OHT (120 nM and 240 nM), and ONB_2-4OHT (120 nM, 240 nM, 500 nM, and 1 µM) for 1 hour, then washed and exposed to 1 min UV light. Percentage of GFP^+ cells either unexposed ("No UV", yellow) or exposed ("1' UV", green) to UV light is shown. Untreated negative control (NC) is shown. Error bars are SD (N =3). ONB_2-4OHT had much worse recombination efficiency than ONB_1-4OHT at the range of concentrations and UV exposures tested. We conclude that ONB_1-4OHT has much greater potential for spatial activation than ONB_2-4OHT.

Figure S9. Recombination efficiency of intracellular ONB_1-4OHT at shorter UV exposure times. UT MEFs were treated with 120 nM or 240 nM ONB_1-4OHT for 1 h, then washed and exposed to UV at short timepoints (0.25, 0.5, 1, and 5 min). Percentage of GFP^+ cells is shown. Error bars are SD (N = 3). We find that ONB_1-4OHT can be uncaged in as little as 15 s, but longer exposure times lead to greater uncaging and activation of GFP expression.



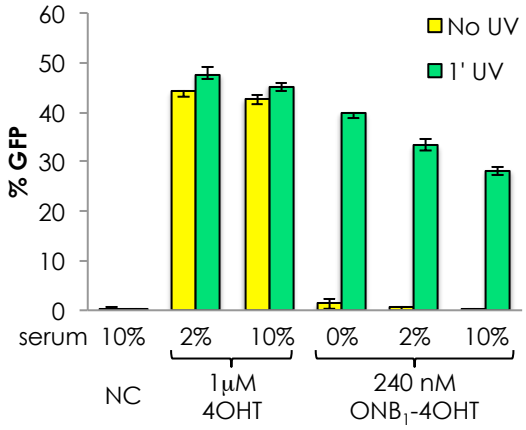


Figure S10. Effect of serum concentration on ONB_1-4OHT recombination efficiency. UT MEFs were treated for 1 h in 1 µM 4OHT or 240 nM ONB_1-4OHT in media containing 0%, 2%, and 10% serum, then washed and exposed to UV for 1 min. Negative control (NC) was untreated. Percentage of GFP^+ cells either unexposed ("No UV", yellow) or exposed ("1' UV", green) to UV light are shown. Error bars are SD (N = 3). Our data indicate that decreasing the serum concentration during ONB^1-4OHT treatment from the standard 10% in MEF media leads to only a modest improvement in recombination efficiency.

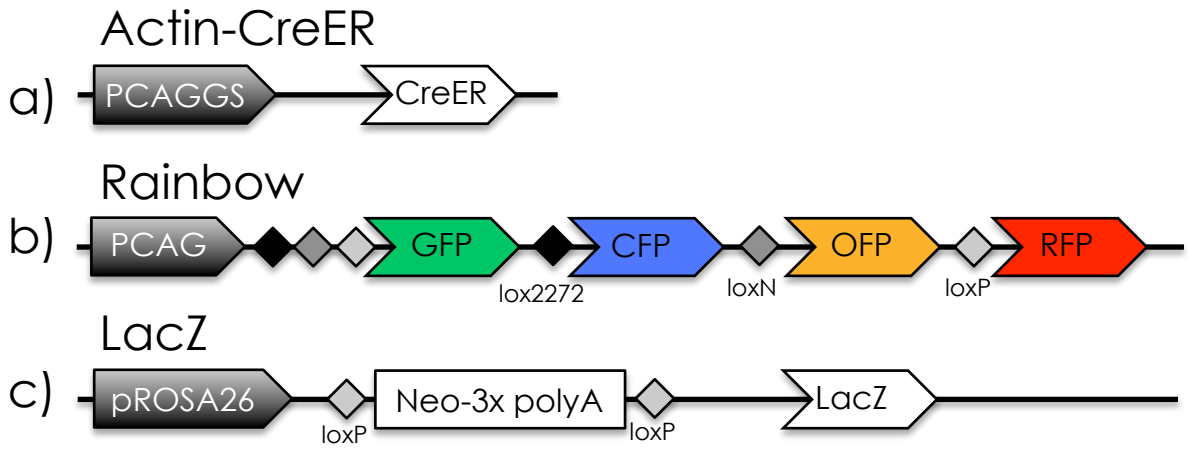


Figure S11. ACRL MEF constructs. ACRL MEFs are comprised of three constructs that induce expression of multiple reporter genes upon incubation with 4OHT. **a)** The Actin-CreER driver constitutively expresses Cre-ER via a chicken β-actin promoter (pCAGGS).[4] **b)** The Rainbow reporter system initially expresses GFP until acted upon by Cre recombinase to delete GFP and randomly initiate expression of either CFP, OFP, or RFP.[5] **c)** In the LacZ reporter system**,** loxP-flanked DNA stop sequence prevents the expression of the lacZ gene unless acted upon by Cre recombinase.[6] ACRL MEFs possess one copy of each construct. ACRL MEFs initially are GFP^{+}, but after addition of 4OHT, they will switch off GFP, turn on LacZ, and randomly turn on either CFP, OFP, or RFP.

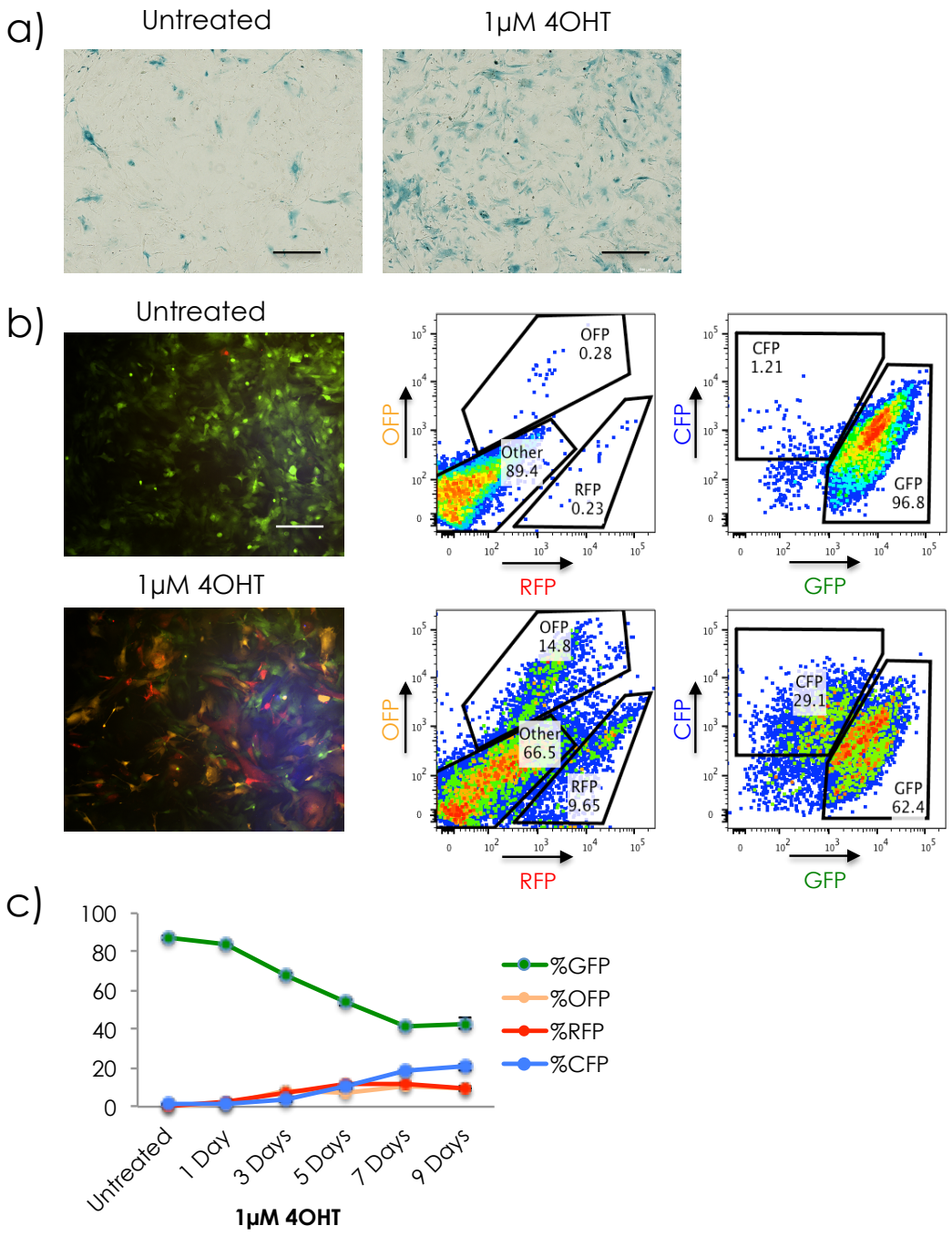


Figure S12. Characterization of 4OHT mediated genetic recombination in ACRL MEFs. ACRL MEFs were cultured on 3cm dishes and treated with 1 μM 4OHT for 1 hour then cultured for 24 hours or more. **a)** 24 hours following 4OHT treatment, ACRL MEFs were fixed in 0.2% glutaraldehyde, treated with X-gal, and imaged for LacZ. Robust LacZ expression can be observed in cultures treated with 4OHT. **b)** 7 days following 4OHT treatment ACRL MEFs were imaged and analyzed for expression of fluorescent genes. Images and FACS plots are shown for activation of OFP, RFP, and CFP. **c)** Graphs for OFP, RFP, and CFP expression following ACRL MEF pretreatment with 4OHT and cultured between 1 to 9 days. Error bars are SD (N = 3).

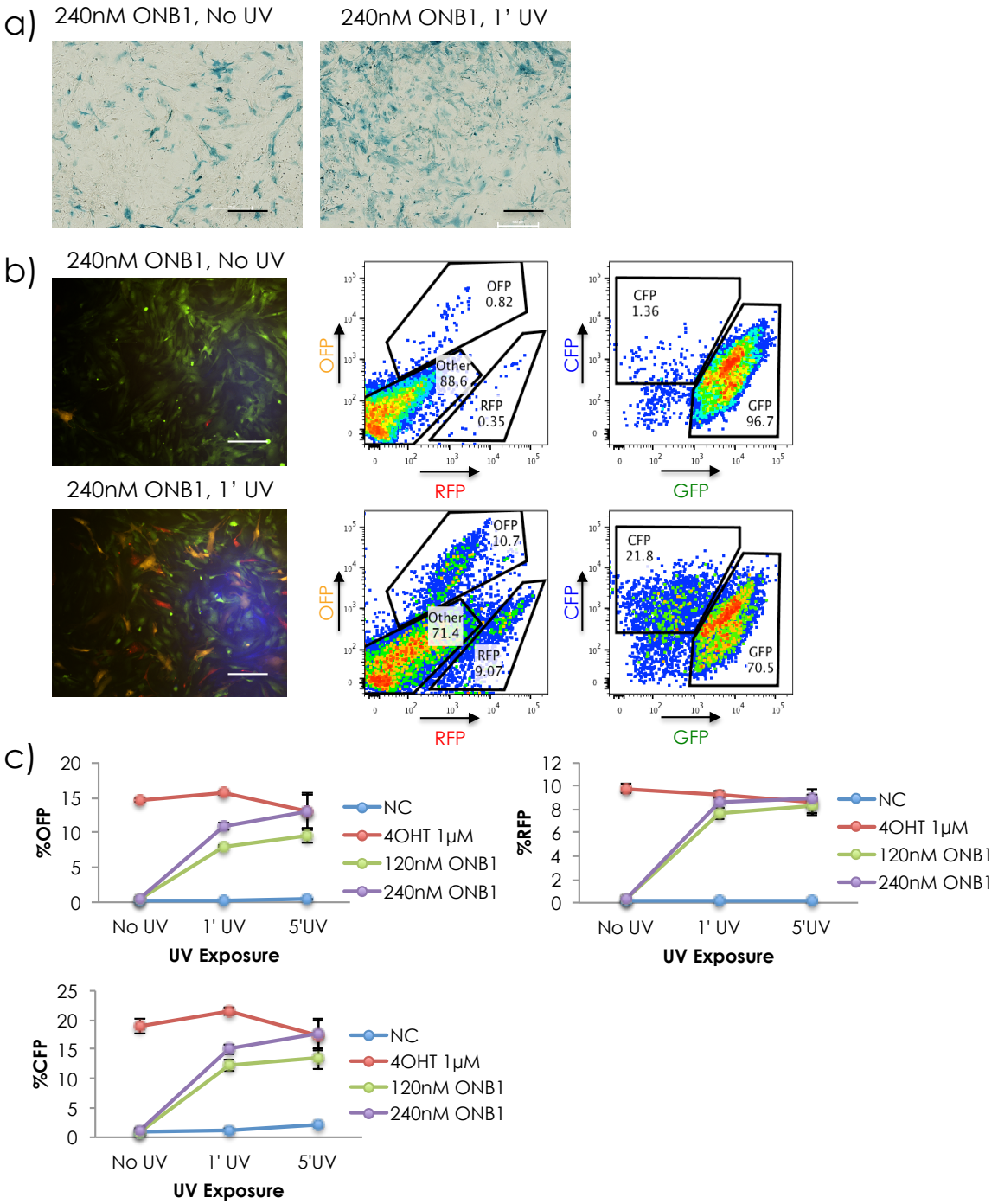


Figure S13. Characterization of ONB1-mediated genetic recombination in ACRL MEFs. ACRL MEFs were plated on 3cm tissue culture dishes and treated with caged ONB1-4OHT for 1 hour, then exposed or unexposed to 1' UV and cultured for 24 hours or 7 days. **a)** 24 hours following ONB1 treatment cells were imaged for LacZ expression. **b)** Representative fluorescent images and FACS analysis are shown for the activation of OFP, RFP, and CFP in ACRL MEFs in culture for 7 days following ONB1 treatment. **c)** Graphs for OFP, RFP, and CFP expressing cells at different UV exposure times and following 1 hr incubation with ONB1-4OHT and analyzed after 7 days in culture. Error bars are SD (N = 3).

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