SUPPLEMENTARY INFORMATION

Synthesis of A Photocaged Tamoxifen for Light-dependent Activation of Cre-ER Recombinase-driven Gene Modification

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1. Synthesis of photocaged tamoxifen

General. (Z)-Tamoxifen was purchased as citrate salt (≥99%) from Sigma-Aldrich, and it was converted to a free base form by the acid-base extraction method. All solvents and reagents were purchased from commercial suppliers, and used without further purification. Compound characterization was typically carried out by standard analytical methods including ^{1}H NMR spectroscopy, ESI mass spectrometry, and UV-vis spectrometry. For the NMR measurement, each sample was dissolved in a deuterated methanol (CD_3OD), and the spectrum was acquired under a standard observation condition with a Varian nuclear magnetic resonance spectrometer at 400 MHz. UV-vis absorption spectra were recorded on a Perkin Elmer Lambda 20 spectrophotometer. Purity of compounds was determined by analytical HPLC on a Waters Acquity Peptide Mapping System equipped with a Waters photodiode array detector. Each sample was dissolved in an aqueous acetonitrile solution (50%) run on a C4 BEH column (150 x 2.1 mm, 300 Å) connected to Waters Vanguard column. Elution was performed in a linear gradient beginning with 98:2 (v/v) water/acetonitrile (with trifluoroacetic acid at 0.14 wt % in each of the eluents) at a flow rate of 1 mL/min.

Compound 2. ONB linker[1-3] **1** (0.1 g, 0.25 mmol) was dissolved in dry CH_2Cl_2 (10 mL), and the solution was cooled with an ice bath. To this solution was added triethylamine (0.073 mL, 0.53 mmol), and methanesulfonyl chloride (0.021 mL, 0.28 mmol). The mixture was stirred for 90 min at 0^{o}C. At the end of the reaction, it was diluted with DCM (20 mL) and poured into a separatory funnel that contained water (20 mL). The mixture was shaken and the organic layer was collected. It was dried with sodium sulfate, and evaporated to dryness *in vacuo*, yielding ONB-methanesulfonate **2** as pale beige solid (105 mg, 87.5%). This material was not further purified and used immediately for the next step. R_f = 0.23 in EtOAc/Hexane (1:1).

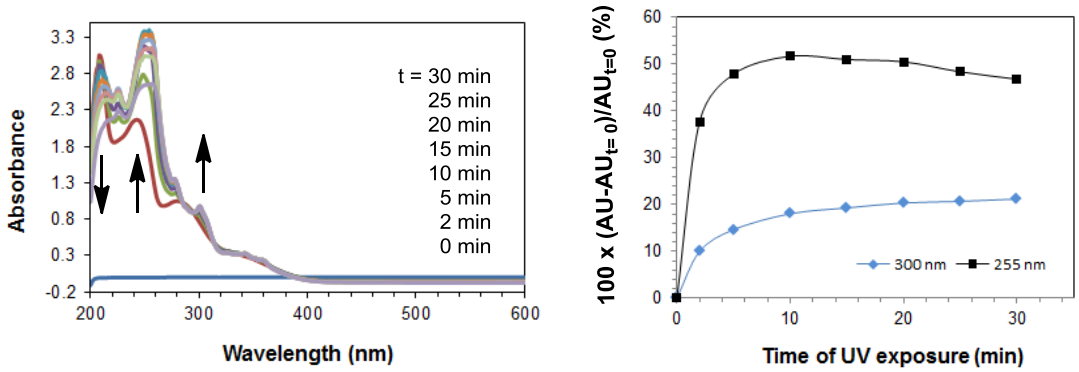
TAM-ONB 3. To a solution of (Z)-tamoxifen (10 mg, 0.027 mmol) dissolved in dry acetone (0.6 mL) was added ONB-methanesulfonate **2** (14 mg, 0.03 mmol) dissolved in 0.5 mL of acetone. The capped reaction vessel was wrapped with aluminum foil and left in a water bath set at 37^{o}C. During the reaction, the product was precipitated slowly as pale beige solid. After 3 days at the same bath, the mixture was cooled in a refrigerator for a day to promote and complete the precipitation of the product. The mixture was transferred to a plastic tube (2 mL) and the tube was spun down at 4500 rpm for 5 min. The supernatant was gently discarded and the pellet was resuspended in acetone (4 mL) followed by brief sonication (10 s). The suspension was spun down, and the pellet was collected. The acetone wash was repeated twice more, and the final pellet was dried under nitrogen atmosphere, yielding TAM-ONB **3** (R = Boc) as beige solid (6.1 mg; 27%). ^{1}H NMR (400 MHz, CD_3OD; **Fig. S1**): δ 7.88 (s, 1H), 7.37–7.35 (m), 7.33 (m), 7.26–7.13 (m), 6.86–6.83 (d, 2H), 6.70–6.68 (m, 3H), 5.12 (s, 2H), 4.70 (s, 2H),

4.45 (br m, 2H), 4.02 (s, 3H), 3.88-3.87 (q, 2H), 3.37 (m, 2H), 3.30 (m, 2H), 3.21 (s, 6H), 3.12 (s, 3H), 2.46–2.43 (m, 2H), 1.41 (s, 9H), 0.92-0.88 (t, 3H) ppm. HRMS (ESI) calcd for $C_{43}H_{53}N_4O_8$ 753.3858, found 753.3856 (**Fig. S2**). Anal. HPLC: t_r (min) = 7.60 (purity ≥99%; **Fig. S3**). UV-vis spectrometry (10% aq EtOH; 0.1 mg/mL): absorption peaks at 350, 270 nm (**Fig. S4**).

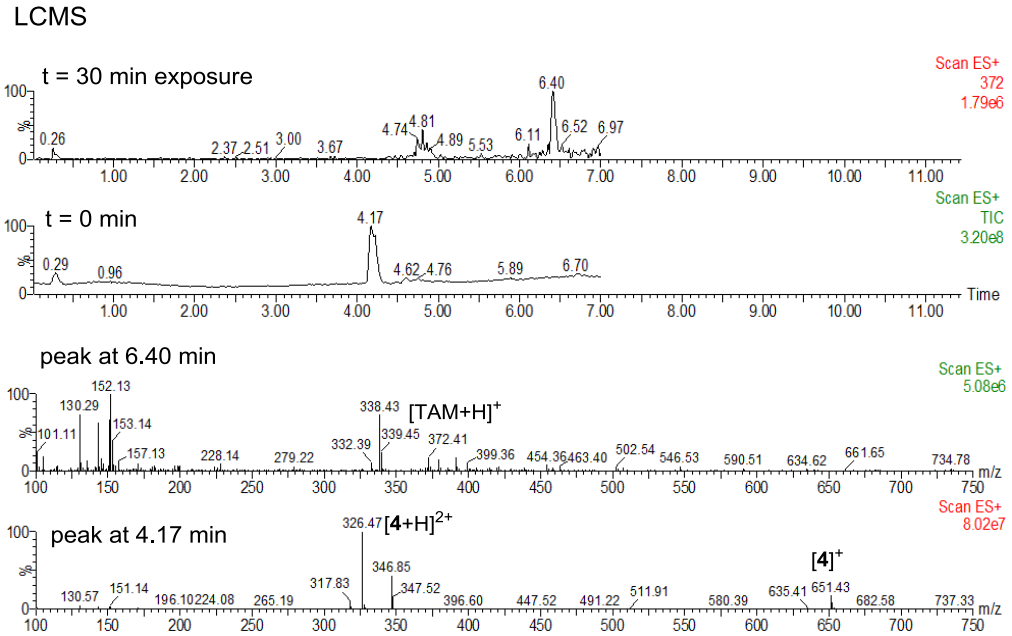
TAM-ONB 4. To the *N*-Boc-protected TAM-ONB **3** (30.9 mg, 0.041 mmol) dissolved in MeOH (1 mL) was added 6M HCl (1 mL). The solution was stirred for 6 h at RT, and concentrated to dryness *in vacuo*, yielding TAM-ONB **4** (R = H; HCl salt). This solid was dissolved in water (2 mL), and freeze-dried, yielding fluffy solid (27.4 mg; 96%). ^{1}H NMR (CD_3OD) spectrum of the product indicated lack of the *N*-Boc group. ^{1}H NMR (400 MHz, CD_3OD; **Fig. S1**): δ 7.90 (s, 1H), 7.40 (s, 1H), 7.37–7.33 (m, 3H), 7.29–7.25 (m), 7.22–7.18 (m), 7.16–7.11 (m), 6.85–6.83 (d, 2H), 6.71–6.68 (d, 2H), 5.14 (s, 2H), 4.76 (s, 2H), 4.45 (br m, 2H), 4.03 (s, 3H), 3.90-3.79 (q, 2H), 3.61-3.58 (t, 2H), 3.37 (m, 2H), 3.31 (m, 2H), 3.14 (s, 3H), 2.69 (s, 3H), 2.47–2.42 (quart, 2H), 0.92-0.88 (t, 3H) ppm. MS (ESI) calcd for $C_{38}H_{45}N_4O_6$ 653.3, found 653.3 $[M]^+$, 654.3 $[M+H]^+$ (**Fig. S2**). Anal. HPLC: t_r = 7.03 min (**Fig. S3)**. UV-vis spectrometry (phosphate buffered saline, pH 7.4; 0.33 mg/mL): absorption peaks at 340, 280 nm (**Fig. S4**). Solubility in water ≥20 mg/mL.

Photolysis experiments of TAM-ONB 4. Photolysis experiments were carried out using Spectroline® UV bench lamps (XX-15A; emission wavelength at 365 or 254 nm). **TAM-ONB 4** was dissolved in EtOH (67 μM), and was placed in a quartz cuvette without any cover under UV lamps at the distance of ~ 5 cm from lamps. The solution was irradiated over up to 30 min, and the progress of the photolysis was monitored by UV-vis spectrometry at specific time points.

UV-vis absorption spectra of TAM-ONB **4** as a function of UV exposure time



LCMS spectra of TAM-ONB **4** before and after 30 min of UV exposure time: **TAM**: calculated for $C_{26}H_{29}NO$ 371.22, found $[M+H]^+$ 372.41; **TAM-ONB 4**: calculated for $C_{38}H_{45}N_4O_6$ 653.33, found $[M]^+$ 652.43, 651.43, $[M+H]^{2+}$ 326.47



2. Copies of ^{1}H NMR spectra, mass spectra, HPLC traces, and UV-vis spectra

Fig. S1. ^{1}H NMR spectra of Tamoxifen (citrate salt), TAM-ONB **3**, and TAM-ONB **4** (HCl salt). Each spectrum was acquired in CD_3OD at 2 mg/mL concentration.

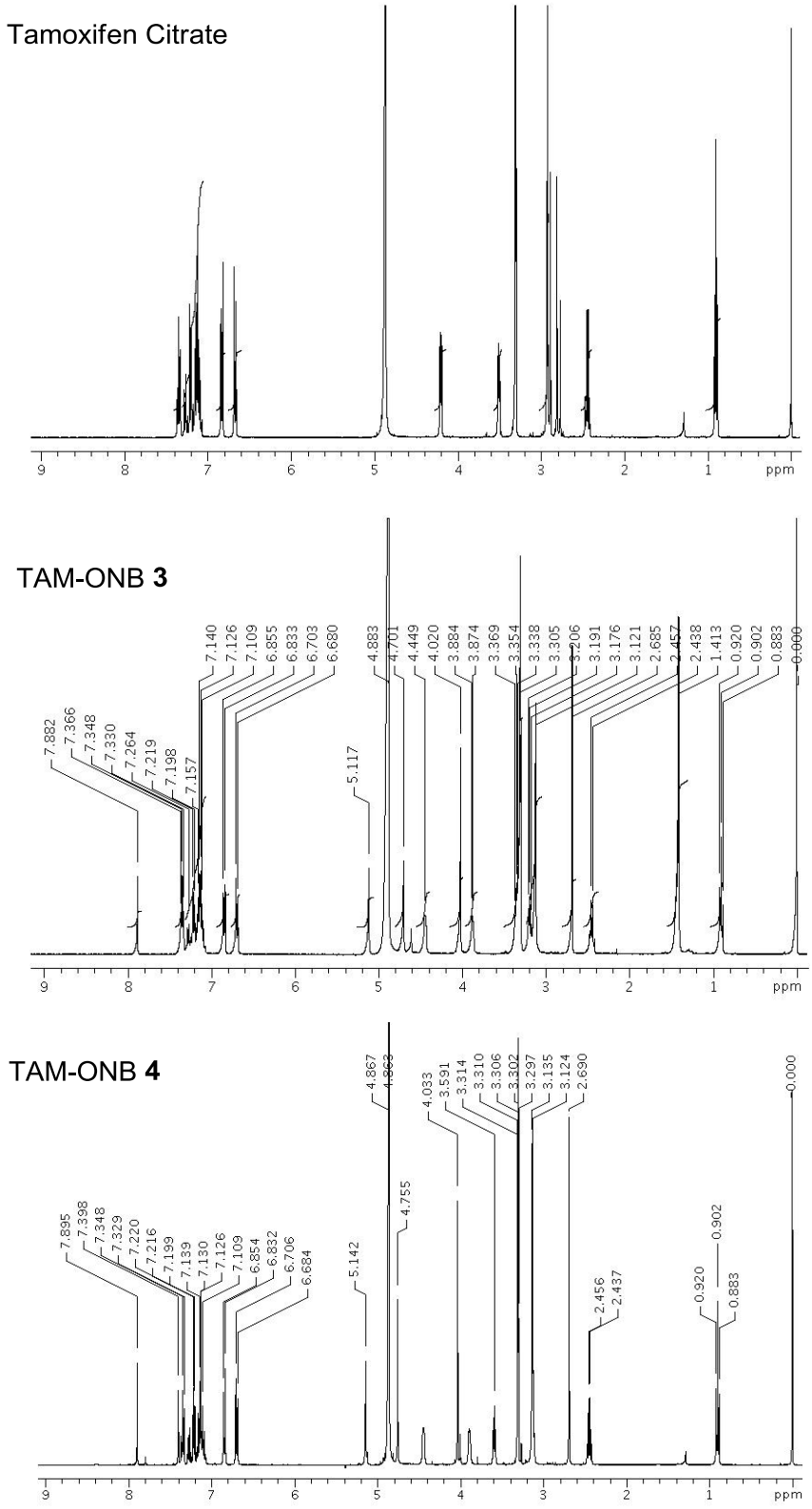


Fig. S2. High resolution mass spectrum of TAM-ONB **3**, and ESI mass spectrum of TAM-ONB **4**.

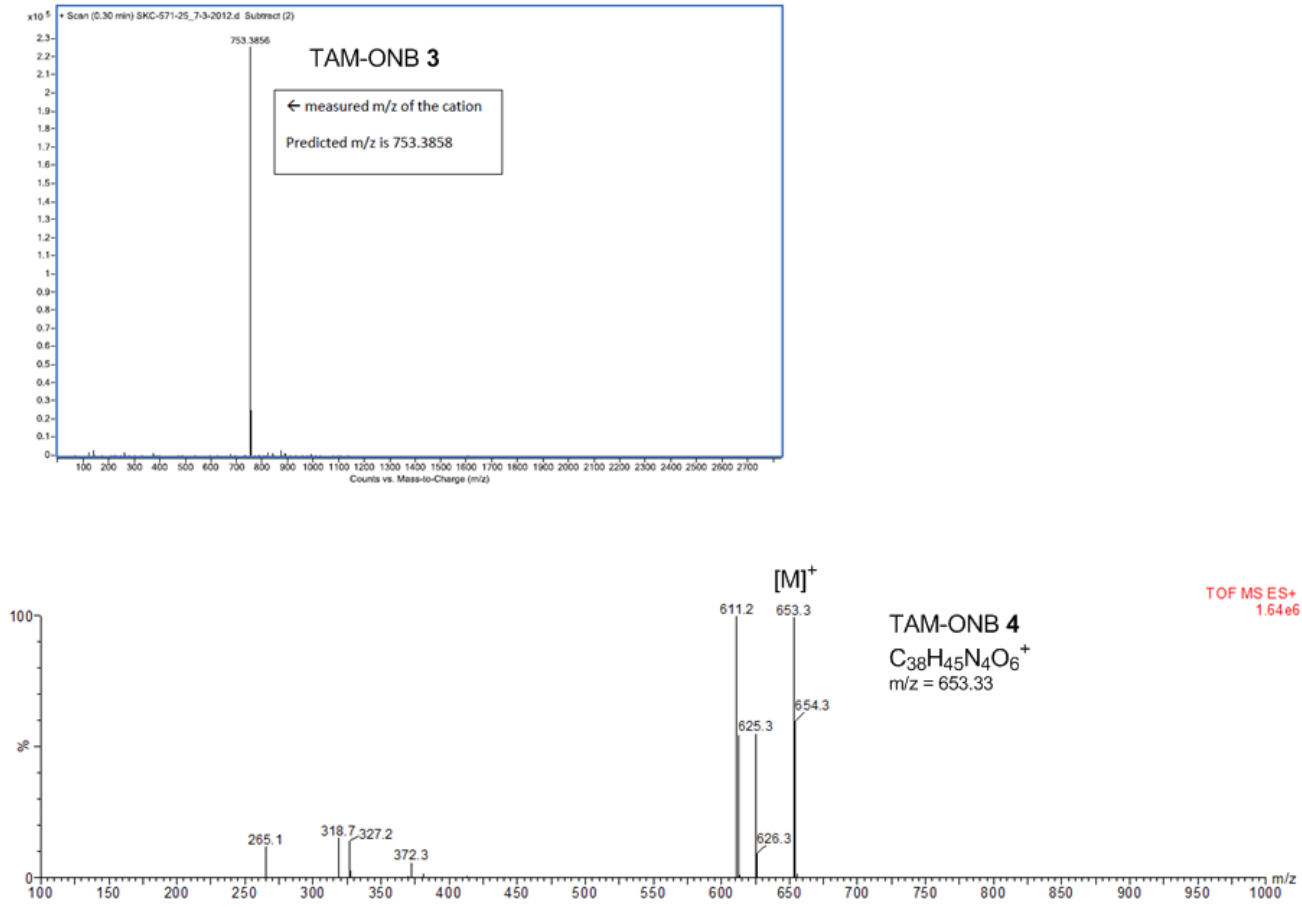


Fig. S3. Analytical HPLC traces of Tamoxifen citrate (TAM), TAM-ONB **3**, and TAM-ONB **4.**

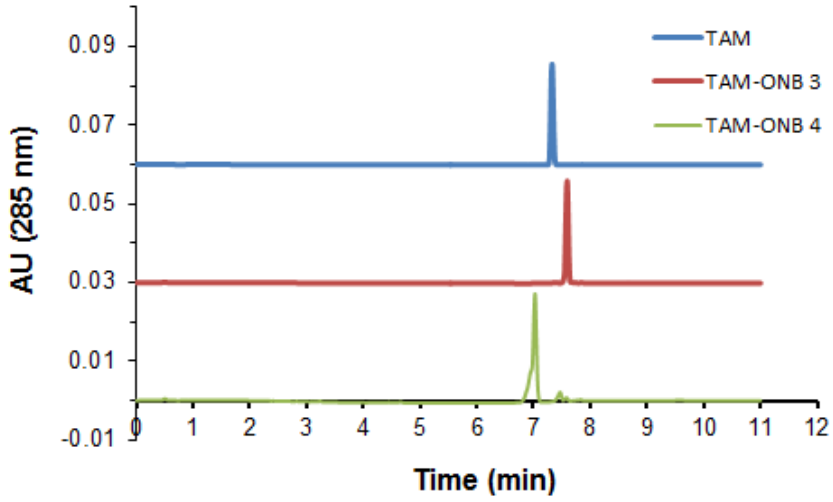
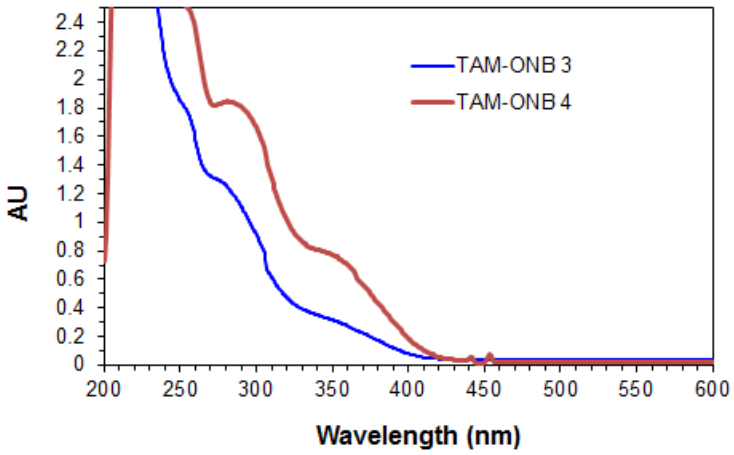


Fig. S4. UV-vis spectral characterization of TAM-ONB **3** and TAM-ONB **4**, each acquired in 10% aq. EtOH (conc = 0.1 mg/mL), and phosphate buffered saline (PBS, pH 7.4) (conc = 0.33 mg/mL), respectively.



3. Methods for Cell Studies

Generation of UT MEFs

Tamoxifen-detecting mouse embryonic fibroblasts (MEFs) were generated by crossing two transgenic lines, one with a constitutive promoter (human Ubiquitin C, UBC) driving expression of Cre-ER^{T2} as described in literature,[4] and another line with the mT/mG reporter inserted into the constitutively active Rosa26 locus as described in literature.[5] The mT/mG reporter initially expresses TdTomato, but when Cre-ER^{T2} recombinase is active, will recombine to remove TdTomato and activate GFP. e14.5 Embryos were harvested from UBC-Cre-ER^{T2} x mT/mG crosses, head and livers were removed and the remaining tissue was diced and treated in 0.25% Trypsin (Invitrogen 25200-114) to dissociate tissues, then plated onto gelatinized tissue-culture treated dishes (Fisher 353003) in MEF media (DMEM (high glucose, Invitrogen 10566-024), 10% FCS (Omega Scientific FB-11), 100 U/mL Penicillin 100 μg/mL Streptomycin (Invitrogen 15140-155), and 1x GlutaMAX (Invitrogen 35050)). UT MEFs were passaged 1:3 with Trypsin every 4–5 days, and discarded after 6-8 passages. UT MEFs were plated at least 1 day prior to treatment. Unless indicated, all treatments and incubations were done in MEF media.

All animal procedures were approved by the International Animal Care and Use Committee, and the Stanford Administrative Panel on Laboratory Animal Care.

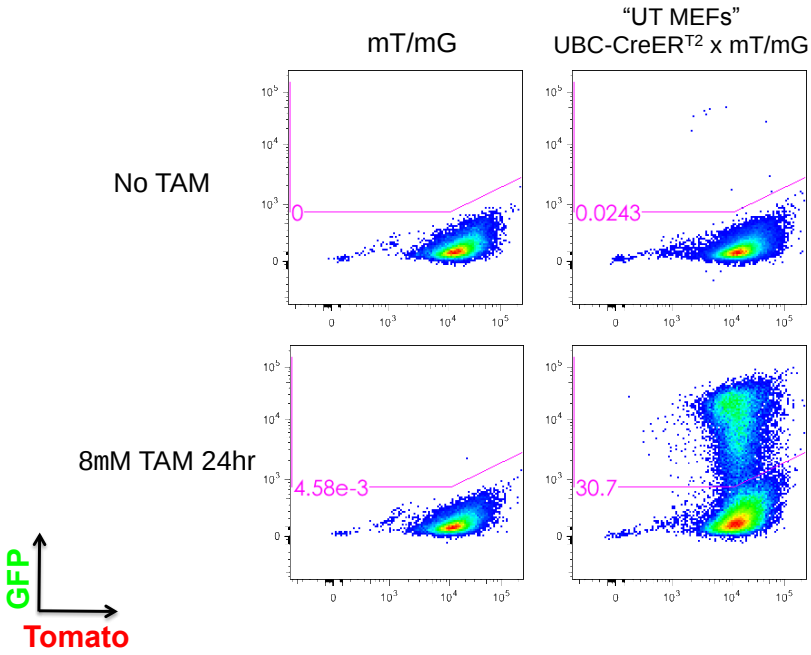
Treatments of UT MEFs with TAM and TAM-ONB 4

Tamoxifen (Sigma T5648) was diluted in DMSO at 8 mM concentration. TAM-ONB4 was resuspended in water at 8 mM. TAM-ONB **4** was stored in the dark at 4^{o}C for up to 1 week, and

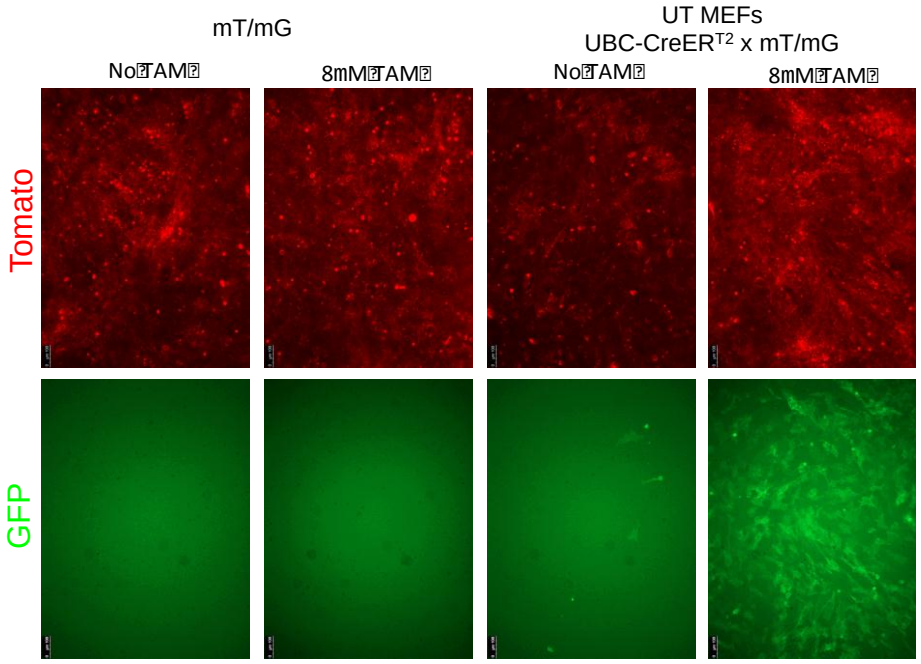
concentration reconfirmed by UV-vis spectroscopy with each use ($\varepsilon_{350\ nm}$ = 3541.3 $M^{-1}cm^{-1}$). UV light (λ = 365 nm) was provided by an 8-watt VWR Transluminator (VWR, LM-20E). For initial TAM-ONB **4** tests (Fig. 2, 3) TAM-ONB **4** was diluted in PBS to 800 μM concentration, exposed to UV light, then diluted in MEF media and added to UT MEFs. Also for initial experiments (Fig. 2, 3, S7), the UV light source was placed upside-down 6.5 cm above uncovered dishes. For later experiments (Fig. 4, S7, S8, S9), TAM-ONB **4** was added directly to UT MEFs at final concentrations, then uncaged by exposure of TAM-ONB **4**/UT MEFs to UV light. Also for later experiments (Fig. 4, S8, S9), dishes containing UT MEFs were placed directly onto the UV source (0.1 cm). For pre-treatments, TAM and TAM-ONB **4** were added at final concentrations onto UT MEFs in serum-free MEF media for 1 hr, then removed and the wells washed once in PBS. Pre-treated UT MEFs were exposed dry to UV light, then MEF media was added. After treatment and 24 hr incubation, UT MEFs were photographed with a Leica DMI 6000B microscope with LAS AF software. UT MEFs were then harvested by Trypsin and analyzed for Tomato/GFP expression on a BD FACS Fortessa analyzer (BD Biosciences) and analyzed with FACS Diva (BD Biosciences) and Flowjo software (Treestar). Absolute cell numbers were estimated by adding 10,000 accudrop beads (BD Biosciences 34529) to the sample tubes prior to analysis, then calculating the fraction of sample run based on the number of accudrop beads recovered.

Fig. S5. Validation of UBC-CreER^{T2} x mT/mG MEFs (UT MEFs).

A.

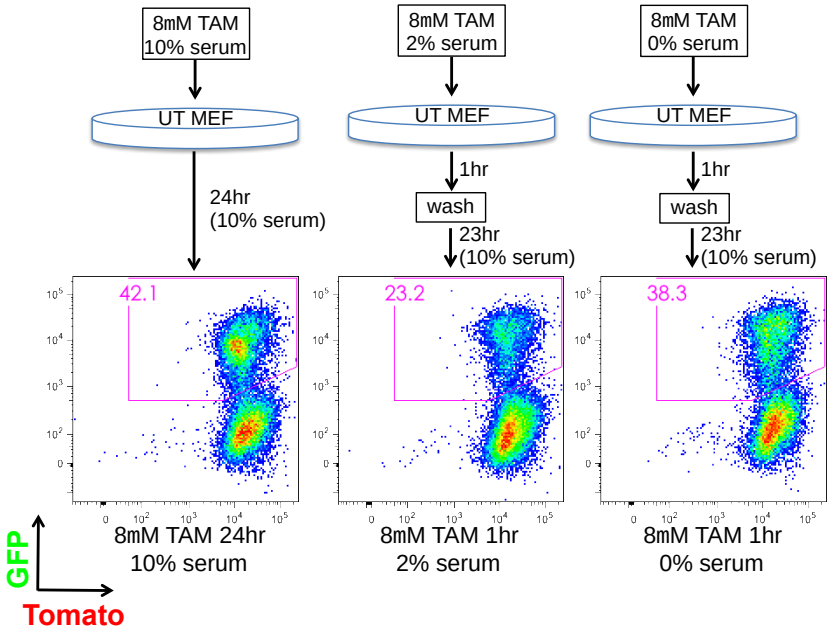


B.



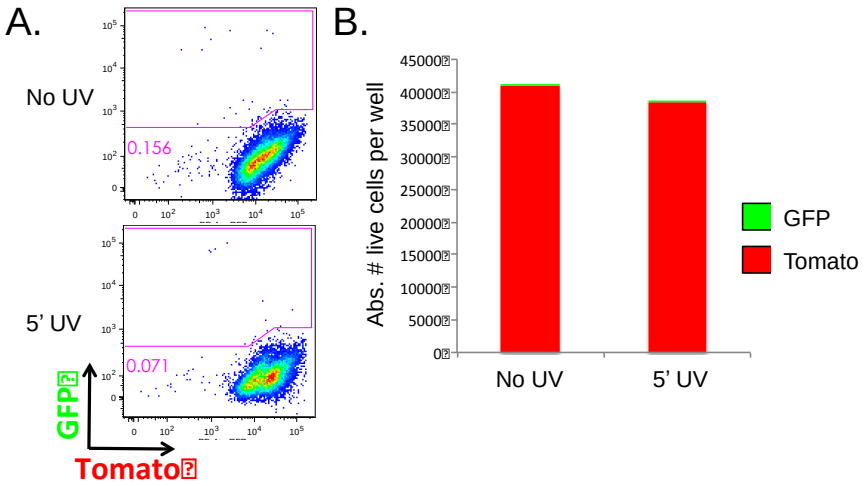
a) FACS analysis of mT/mG only MEFs and UBC-CreER^{T2} x mT/mG MEFs (UT MEFs) treated with or without 8 μM tamoxifen (TAM) for 24 hr. Activated Cre recombinase will induce permanent GFP expression. Although the TdTomato gene is excised, expression is still visible in recombined cells at 24 hrs. b) Tomato (top row) and GFP (bottom row) fluorescence in mT/mG only MEFs and UT MEFs 24 hr after treatment with or without 8 μM TAM.

Fig. S6. One hour treatment with TAM is sufficient for near maximum recombination, but is inhibited by serum.



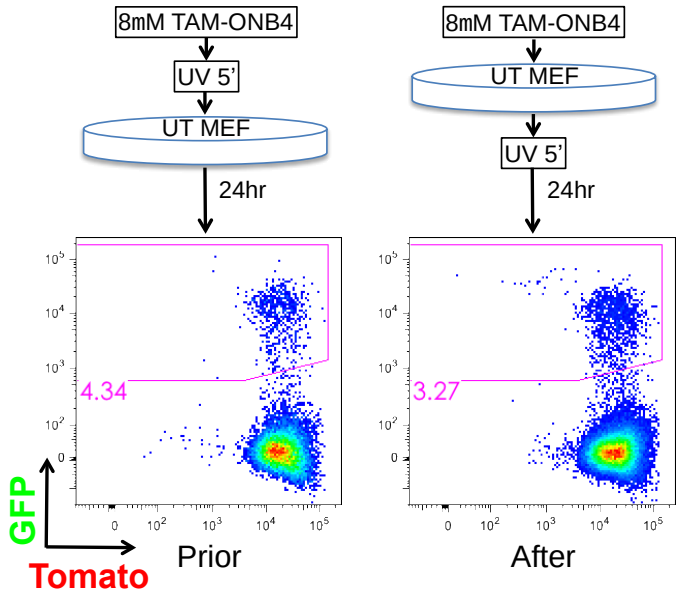
UT MEFs were treated with 8 μM TAM either overnight (left), or for 1 hr in media with 2% serum (middle), or in serum free media (right). After 1 hr treatment, UT MEFs were washed and replaced with media with 10% serum. While 1 hr TAM treatment was sufficient for robust recombination, only in the absence of serum did recombination efficiency approach that of UT MEFs with 24 hr TAM treatment. See Figure S9 for effects of serum on TAM-ONB **4**.

Fig. S7. Viability and background recombination of UT MEFs unaffected by exposure to UV light.



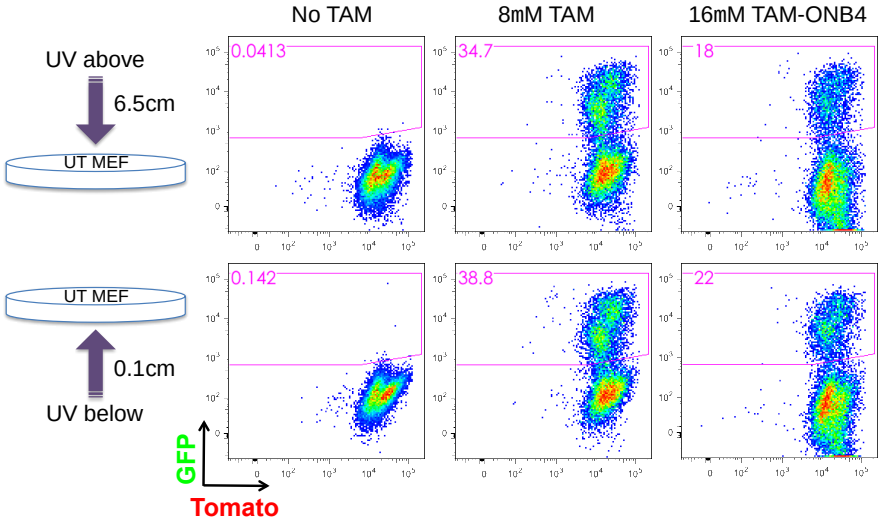
UT MEFs were unexposed (No UV) or exposed (5' UV) to UV light for 5 min. No change in recombination frequency (A) or number of live cells (B) was observed.

Fig. S8. TAM-ONB4 **4** equally effective if UV-uncaged immediately prior to or immediately after adding to UT MEFs.



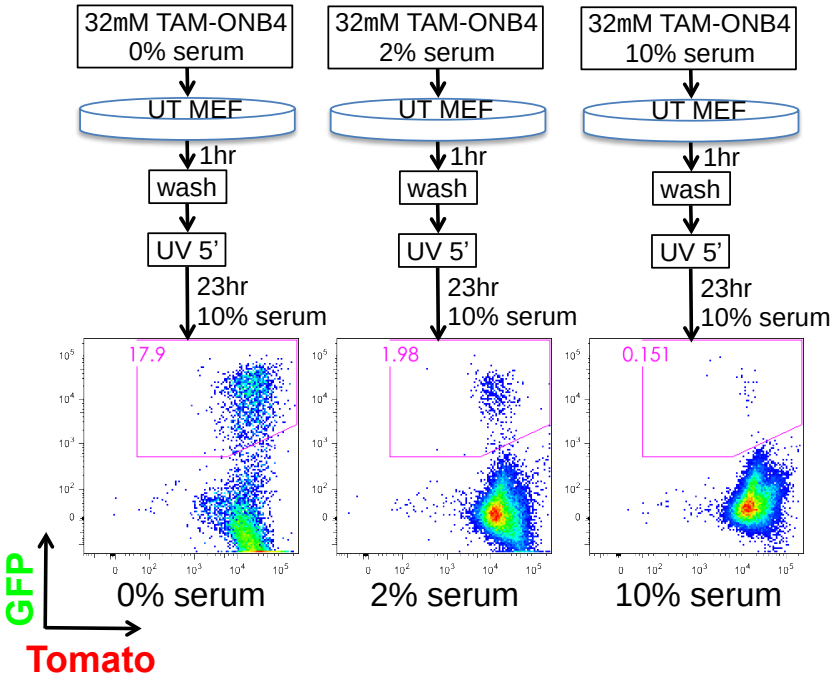
8 μM TAM-ONB **4** was exposed to UV for 5 min either prior to (left) or after (right) adding to MEFs. Recombination efficiency appeared to be equal, and MEF viability was equivalent whether they were exposed to UV light or not.

Fig. S9. Tissue culture plastic does not inhibit photolysis of TAM-ONB **4** by UV light.



UT MEFs were untreated (left column) or treated with 8 μM TAM (middle column), 16 μM TAM-ONB **4** (right column), then exposed to UV for 5 min with the UV source placed 6.5 cm above uncovered dishes (top row), or the dishes placed directly onto the UV source (bottom row). Recombination efficiency appeared slightly greater when the UV source was below the UT MEFs, likely due to the shorter distance from the UV source to the cells (0.1 cm). Our data indicate that the plastic from the dishes does not inhibit UV light from uncaging TAM-ONB **4**.

Fig. S10. Serum inhibits binding of caged TAM-ONB **4** to UT MEFs during pre-treatment.



UT MEFs were treated with 32 μM caged TAM-ONB **4** for 1 hr in media containing 0% (left), 2% (right), and 10% (serum), then washed and uncaged with 5 min UV exposure. The presence of serum during pretreatment dramatically reduced the efficiency of binding of caged TAM-ONB **4**.

References

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