

Supplementary Material

Targeted single-cell gene induction by optimizing the dually regulated CRE/*loxP* system by a newly defined heat-shock promoter and the steroid hormone in *Arabidopsis thaliana*

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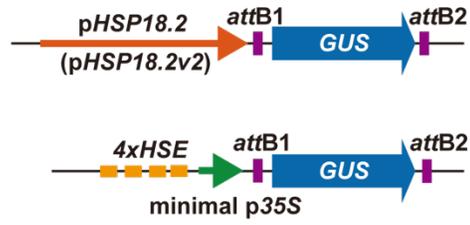
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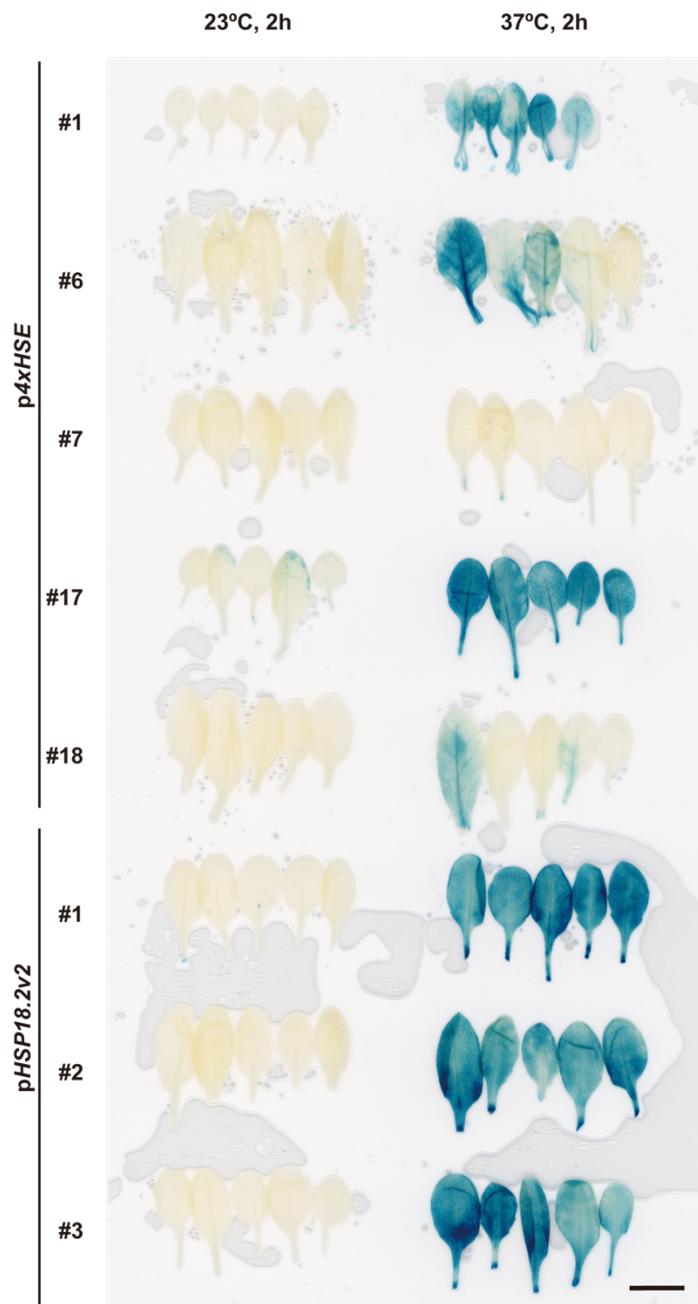
ykamei@nibb.ac.jp

Figure S1

(A)

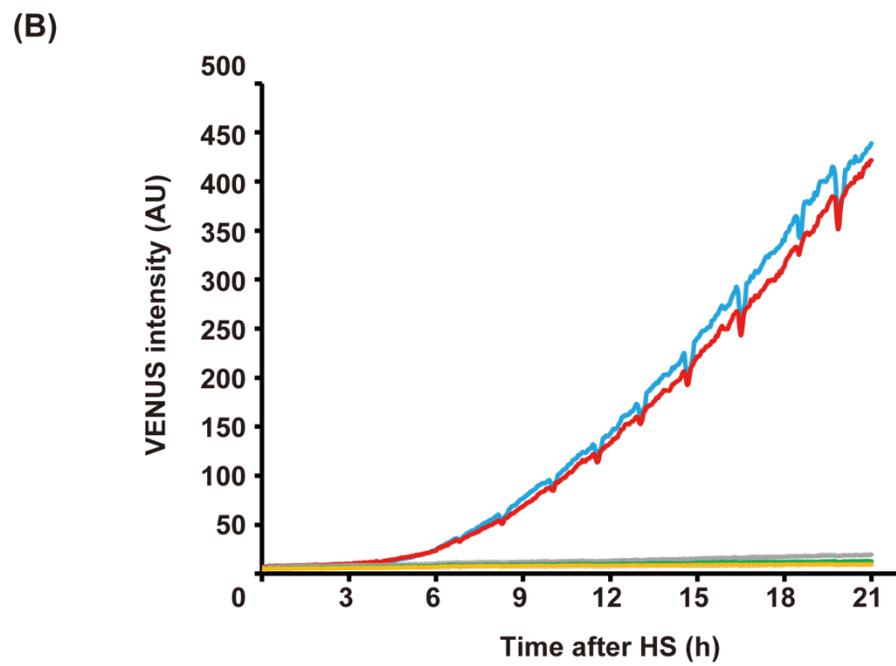
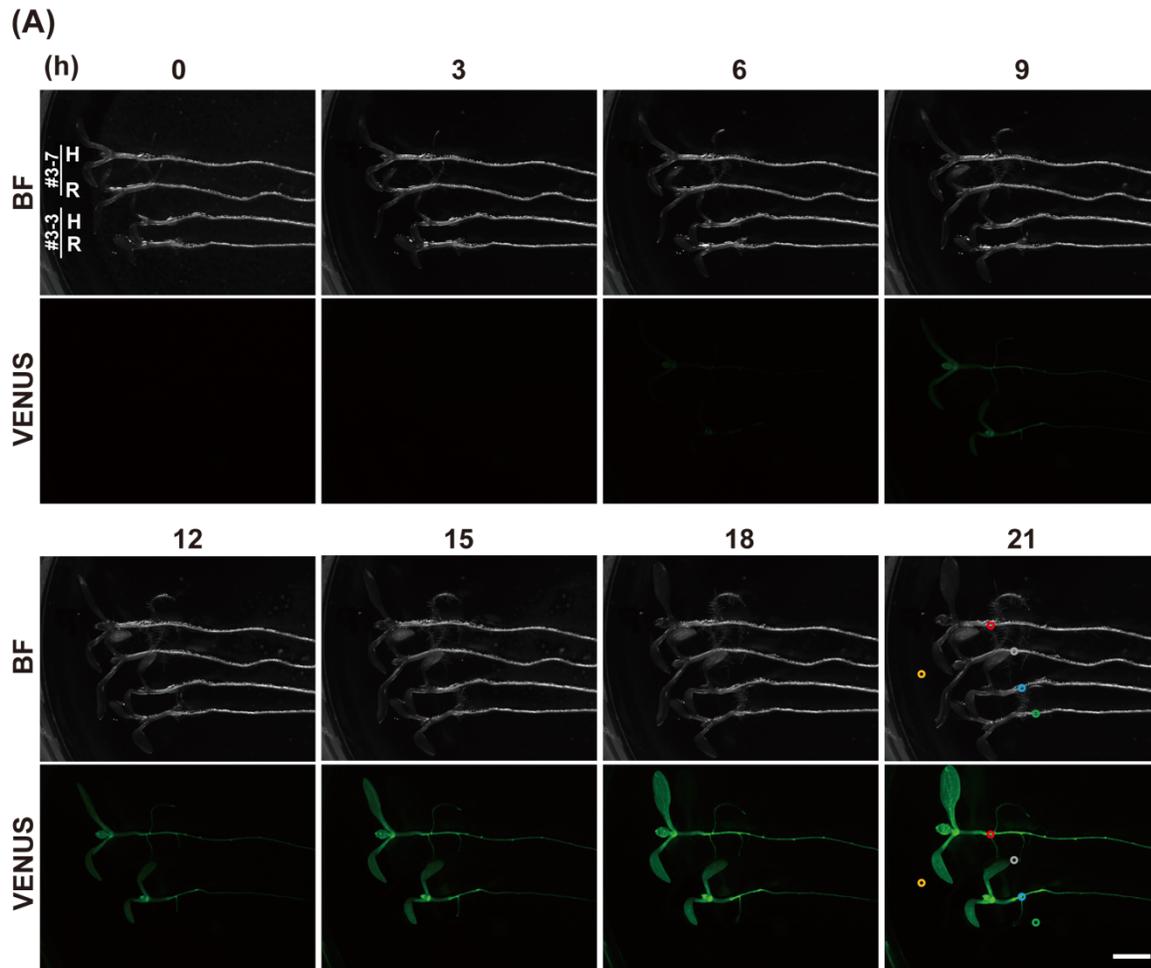


(B)



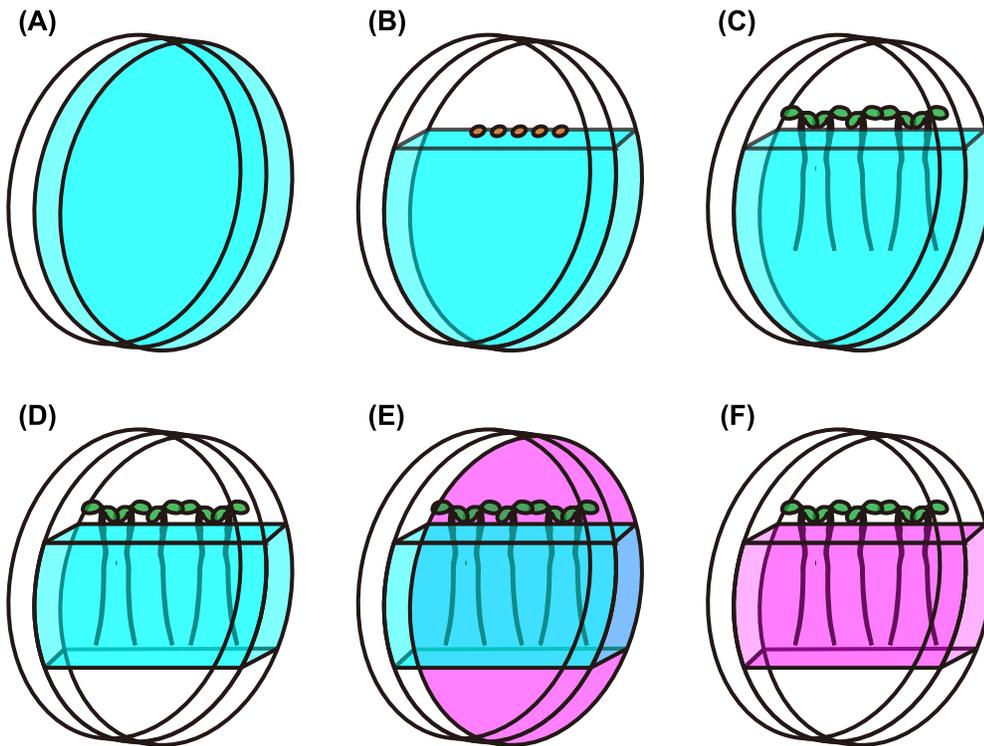
Supplementary Figure 1. Comparison of GUS reporter activity between *pHSP18.2v2:GUS* and *p4xHSE:GUS* reporter plants. **(A)** Schematic drawing of the reporter constructs used in this study. Either *pHSP18.2v2* or *p4xHSE* containing four tandem repeats of HSE fused to the minimal p35S promoter sequences were cloned into the pAM-PAT-GW vector, and the *GUS* reporter gene was introduced downstream of the promoters. **(B)** Images of GUS-stained *p4xHSE::GUS* and *pHSP18.2v2:GUS* reporter leaves. Five leaves derived from five different plants of a reporter line (five and three homozygous lines for *p4xHSE:GUS* and *pHSP18.2v2:GUS*, respectively) were treated at either 23 °C or 37 °C for 2 h. GUS staining of leaves was performed at 37 °C overnight. Scale bar represents 1 cm.

Figure S2



Supplementary Figure 2 Temporal kinetics of VENUS induction upon HS treatment. **(A)** Images of the transgenic seedlings containing both the driver and reporter constructs upon various treatments. Two seedlings of two lines (Lines #3-3 and #3-7) grown on 1/2MS media containing DEX were treated either at 23 °C (R) or at 37 °C (H) for 60 min, respectively. Bright field (BF) and VENUS images at the indicated time points (h) after the treatments were presented. **(B)** VENUS intensity profiles of the five regions-of-interests (ROIs). Five ROIs are shown as colored circles in the BF and VENUS images at 21 h in **(A)**, and the same colors are used in the profiles. ROI 5 shown as an orange circle was used as a background. Scale bar represents 2.5 mm

Figure S3



Supplementary Figure 3. Schematic images of the procedure used to make root samples for IR-laser irradiation. **(A)** The medium in glass bottom dishes. **(B)** Part of the medium was removed, and seeds were placed onto the line between the glass and cut surface. **(C)** Three-day-old seedlings after 3-d stratification. **(D)** The medium below the root tips was removed. **(E)** PI solution was added to the upper and lower parts of the sample. **(F)** Excess PI solution was removed and washed with distilled water.

Figure S4

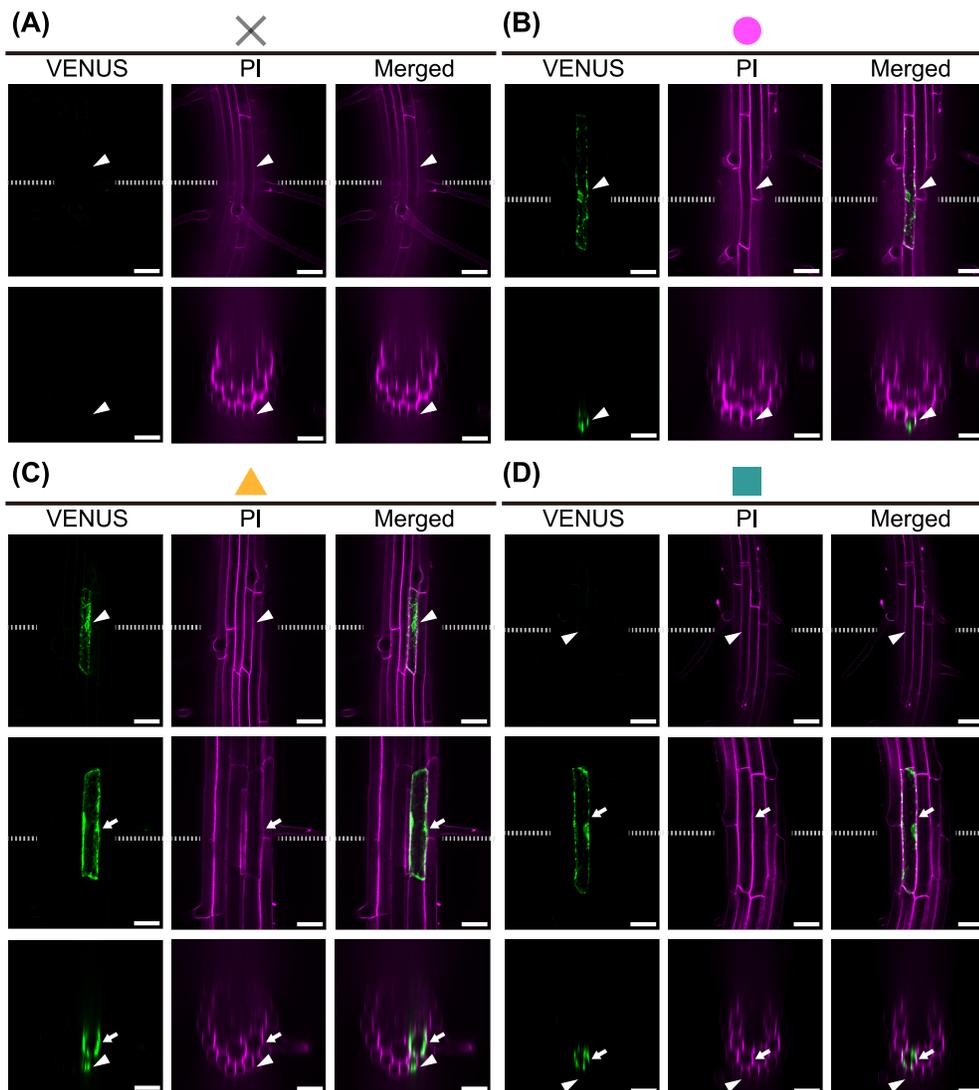
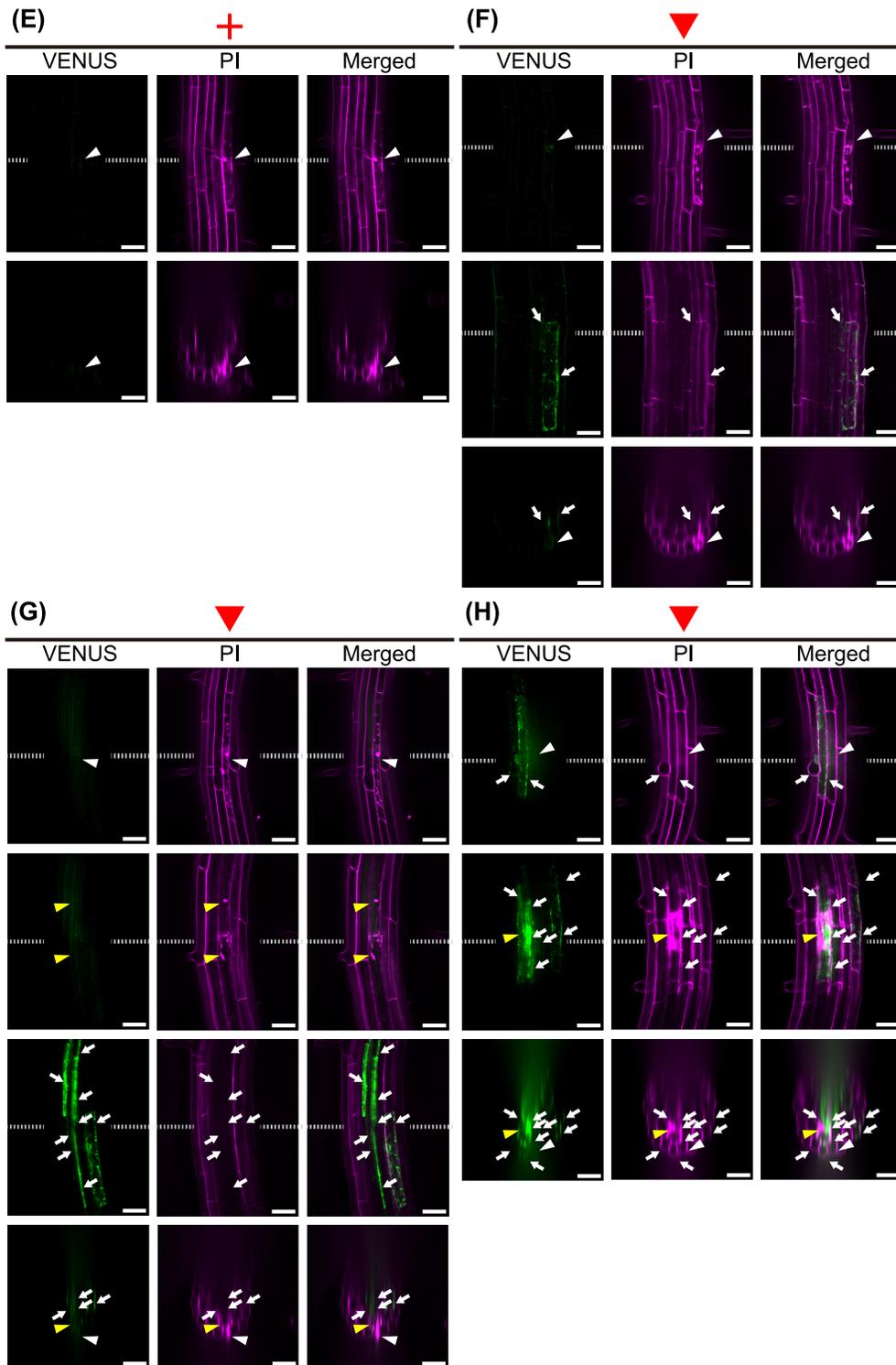
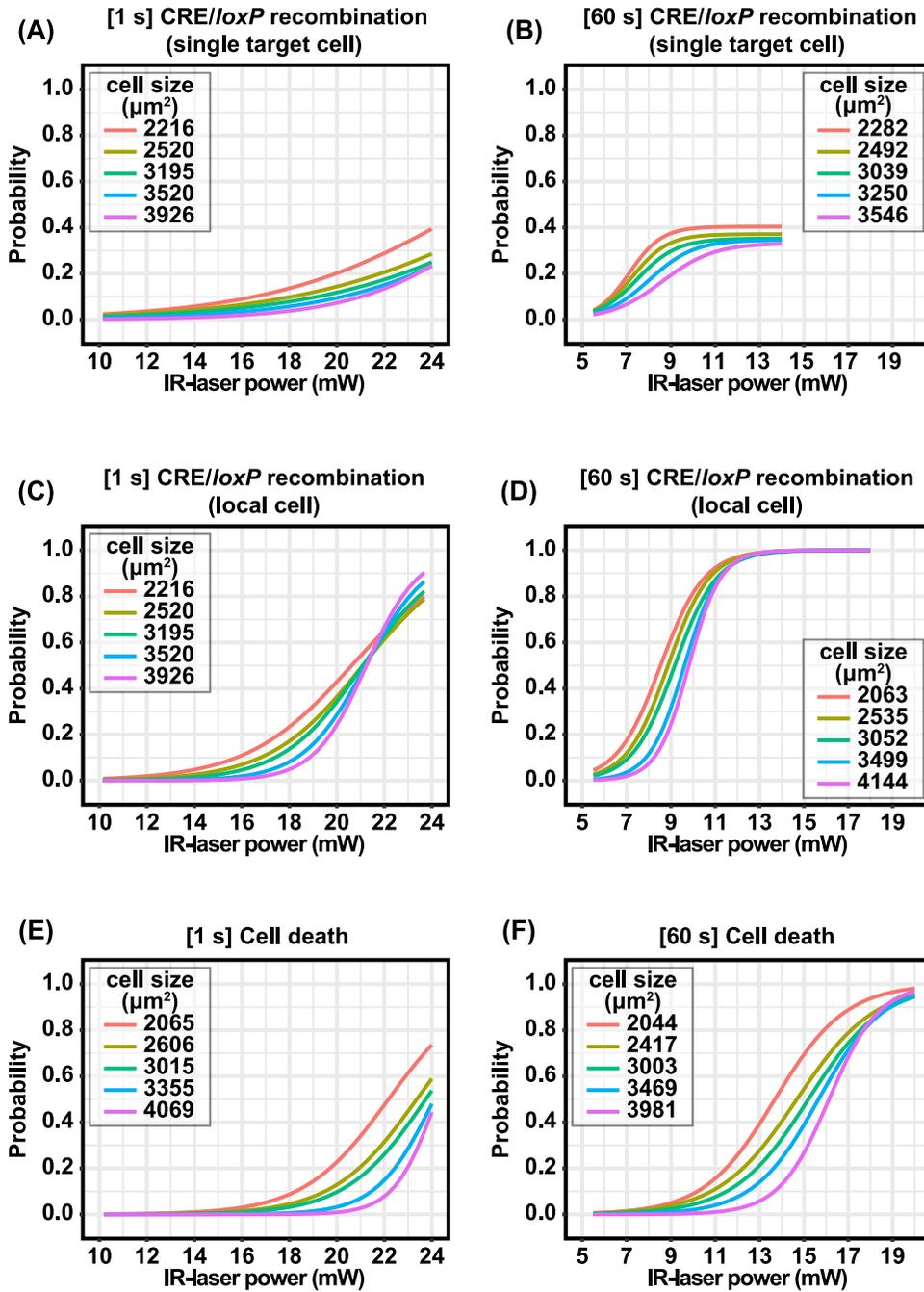


Figure S4



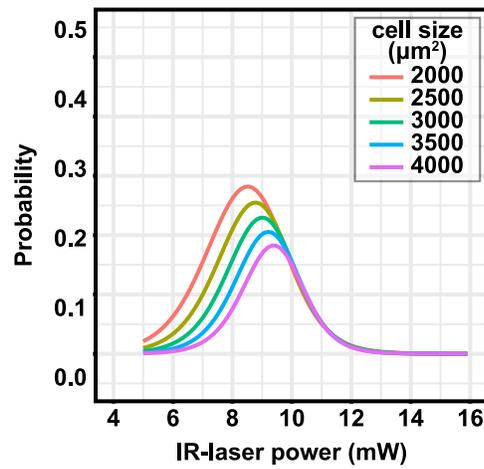
Supplementary Figure 4. Expression patterns of VENUS after IR-laser irradiation. **(A)** No fluorescence of VENUS. **(B)** VENUS fluorescence only in the irradiated cell. **(C)** VENUS fluorescence in the irradiated cell and other cells. **(D)** VENUS fluorescence only in non-irradiated cell(s). **(E)** PI fluorescence (cell death) in the irradiated cells without VENUS fluorescence. **(F)** PI fluorescence (cell death) in the irradiated cells with VENUS fluorescence in the other cells. **(G)** PI fluorescence (cell death) in the irradiated cells and VENUS fluorescence in the other cells. **(H)** PI fluorescence (cell death) and VENUS fluorescence only in non-irradiated cell(s). Dead cells were determined using the PI signal in the cells. The dotted lines correspond to the positions of the optical cross section shown below. White arrowheads, yellow arrowheads, and white arrows indicate the irradiated cells, non-irradiated cells with PI fluorescence (cell death), and non-irradiated cells with VENUS fluorescence, respectively. The meaning of cross, circle, triangle, square, plus, and inverted triangle above each panel is the same as in Figure 3. Scale bars represent 20 μm .

Figure S5



Supplementary Figure 5. Effect of cell size on the CRE/*loxP* recombination and cell death upon IR-laser irradiation. A series of probability curves from subset data with different cell-size filterings are shown. The irradiation duration of the IR-laser was 1 s (**A, C, E**) or 60 s (**B, D, F**). (**A, B**) CRE/*loxP* recombination at the single target cell. (**C, D**) CRE/*loxP* recombination at the single target and/or the neighboring cells. (**E, F**) Cell death at the single target and/or the neighboring cells. The cell sizes indicate the mean cell sizes in μm^2 of the corresponding subset data.

Figure S6



Supplementary Figure 6. The estimated optimal laser powers at different cell sizes. The Y-axis indicates the probabilities of recombination occurrence at the targeted cell without recombination at neighboring cells. Each of the recombination probabilities at the targeted (P_{target}) and neighboring cells ($P_{neighboring}$) was estimated using the logistic regression model including cell size and the interaction between cell size and IR-laser power as the explanatory variables; then, $P_{target} \times (1 - P_{neighboring})$ was calculated, giving rise to the curves shown.