Supplementary Figures

HaCaT Keratinocytes: A Differentiation-Competent Platform for Episomal Replication of HPV Type 11

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Academic Editors: Veronica Sanchez and Elizabeth White

Received: 18 December 2025
Revised: 29 January 2026
Accepted: 10 February 2026
Published: 12 February 2026

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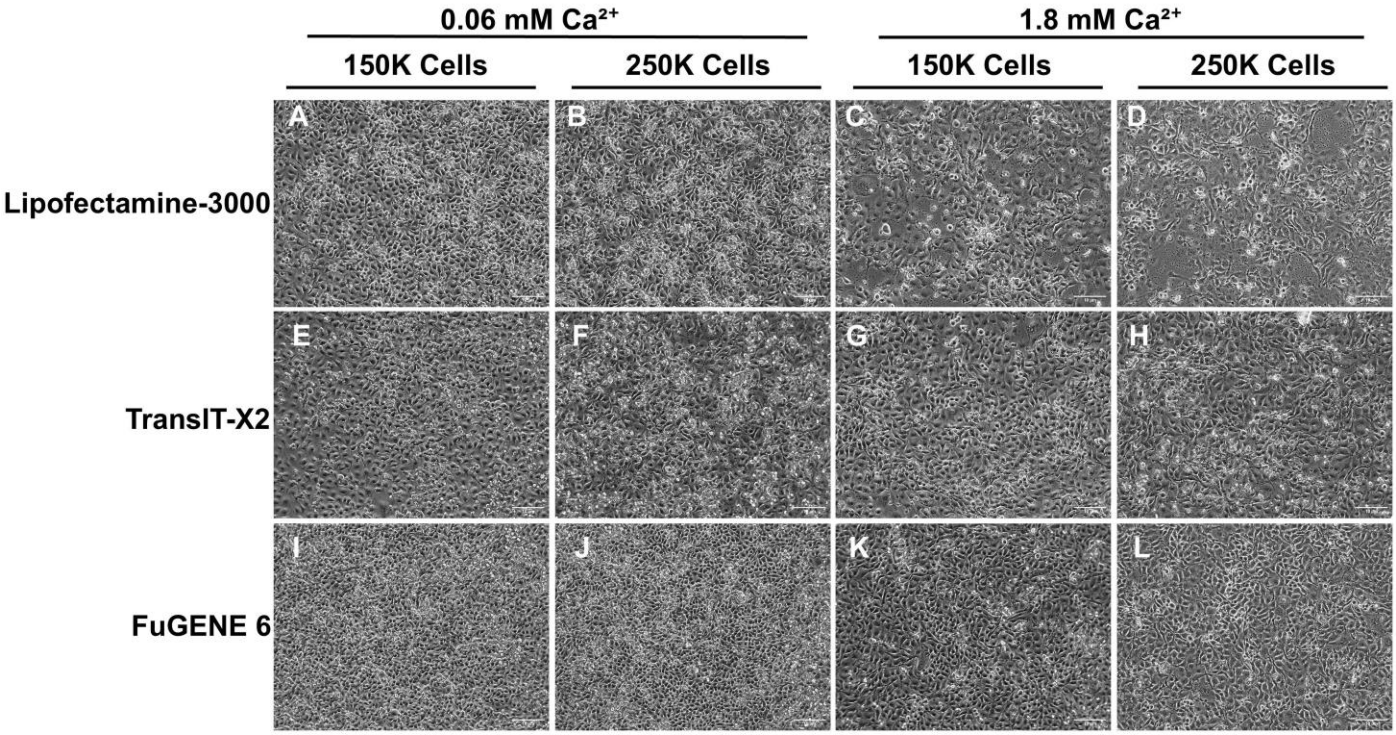

Figure S1. Effect of calcium concentration, cell density, and transfection reagent on DNA delivery efficiency in HaCaT cells.

HaCaT cells were maintained for at least three passages in Dulbecco's Modified Eagle Medium (DMEM) containing either low calcium [0.06 mM Ca^{2+}] or high calcium [1.8 mM Ca^{2+}]. Cells were seeded in 12-well plates at approximately 1.5×10^5 cells per well (Panels A, C, E, G, I, K) or 2.5×10^5 cells per well (Panels B, D, F, H, J, L), corresponding to ~30–50 % confluence, to assess the effect of plating density on transfection efficiency. Three commercially available transfection reagents were evaluated: (A–D) Lipofectamine 3000 (LF-3000) (E–H), TransIT-X2, and (I–L) FuGENE 6. Cells were grown and refreshed in media containing either low Ca^{2+} [0.06 mM] (Panels A, B, E, F, I, J) or high Ca^{2+} [1.8 mM] (Panels C, D, G, H, K, L) both before and after transfection. Each well was transfected with 1 μg of a CMV-GFP reporter plasmid using reagent-to-DNA ratios recommended by the respective manufacturers. Following transfection, cultures were maintained for 72 h and evaluated for GFP expression (see Figure 3). This figure provides a morphological comparison of each condition. Phase-contrast images were captured using a Motic AE31 Elite inverted microscope equipped with a GT5.0 camera and a 10× objective. White Scale Bar = 10 μm at lower left corners. This figure illustrates the influence of extracellular calcium concentration and cell density on HaCaT cell morphology across the three reagents tested.

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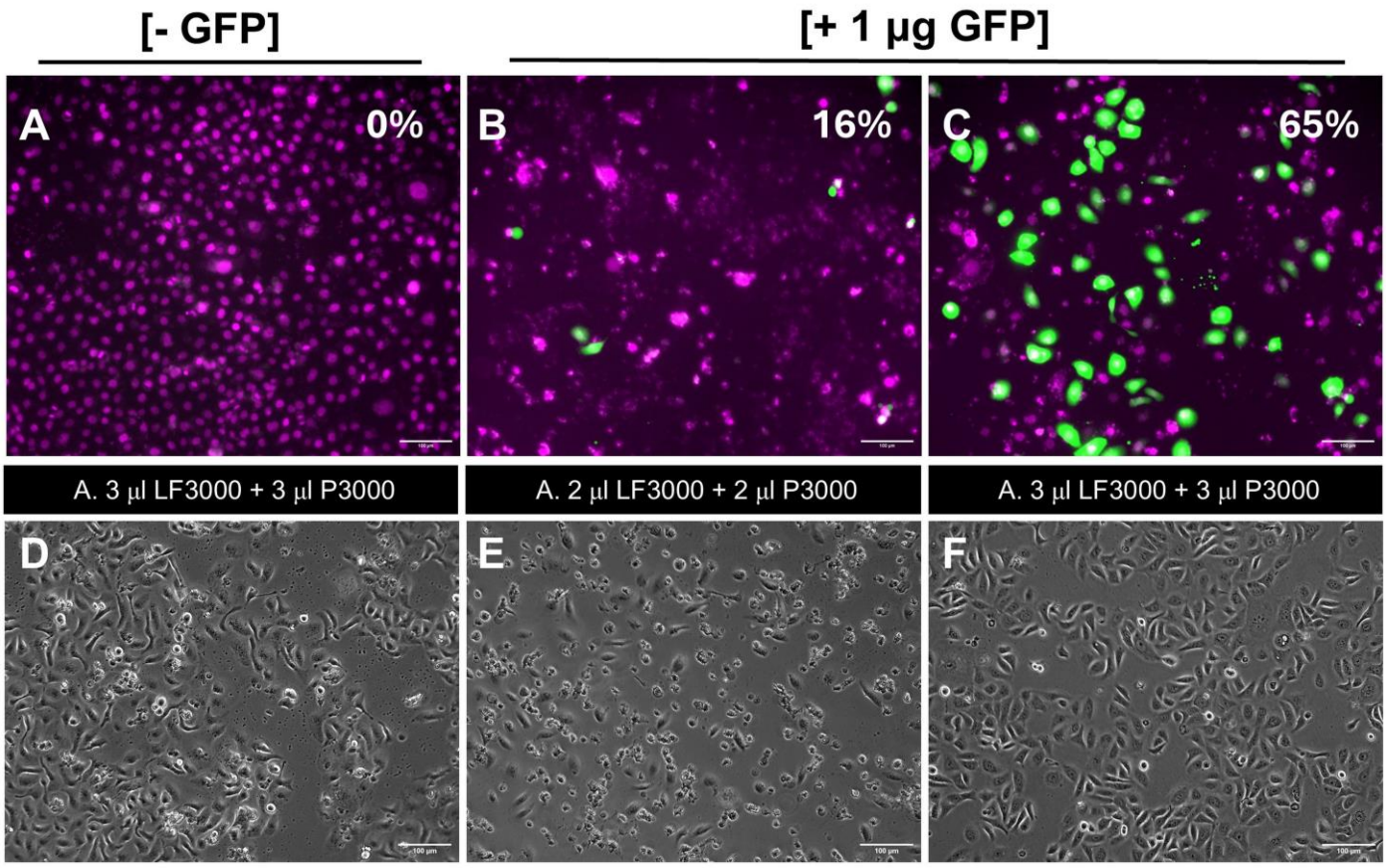

Figure S2. Optimization of Lipofectamine 3000 (LF3000)–mediated transfection in HaCaT cells under low-calcium conditions.

HaCaT cells were seeded at 1.5 × 10^5 cells per well in 12-well plates and cultured overnight at 37 °C in 5 % CO_2 using Dulbecco's Modified Eagle Medium (DMEM) containing low calcium [0.06 mM Ca^{2+}]. Cells were transfected with plasmid DNA encoding GFP under the following conditions:(A) 0 µg GFP + 3 µL P3000 + 3 µL LF3000 (negative control); (B) 1 µg GFP + 2 µL P3000 + 2 µL LF3000; (C) 1 µg GFP + 3 µL P3000 + 3 µL LF3000. Lipid–DNA complexes were prepared in 100 µL Opti-MEM and added dropwise to the cultures with gentle agitation. Cells were maintained in 2 mL of low-calcium [0.06 mM Ca^{2+}] growth medium during transfection. After 4-6 h, the medium was replaced with fresh low-calcium medium, and cells were incubated for 72 h post-transfection. Images were obtained by fluorescence microscopy using DAPI (magenta) and FITC (green) channels, and transfection efficiency was quantified using ImageJ. Panels A–C show overlaid DAPI–FITC fluorescence images. Corresponding phase-contrast micrographs (D–F) were separately obtained from the same wells. White Scale Bar = 100 µm in lower left corner . Notably, cells in panels B and E appear sparse with numerous floating cells, indicating suboptimal viability at intermediate transfection reagent ratios.

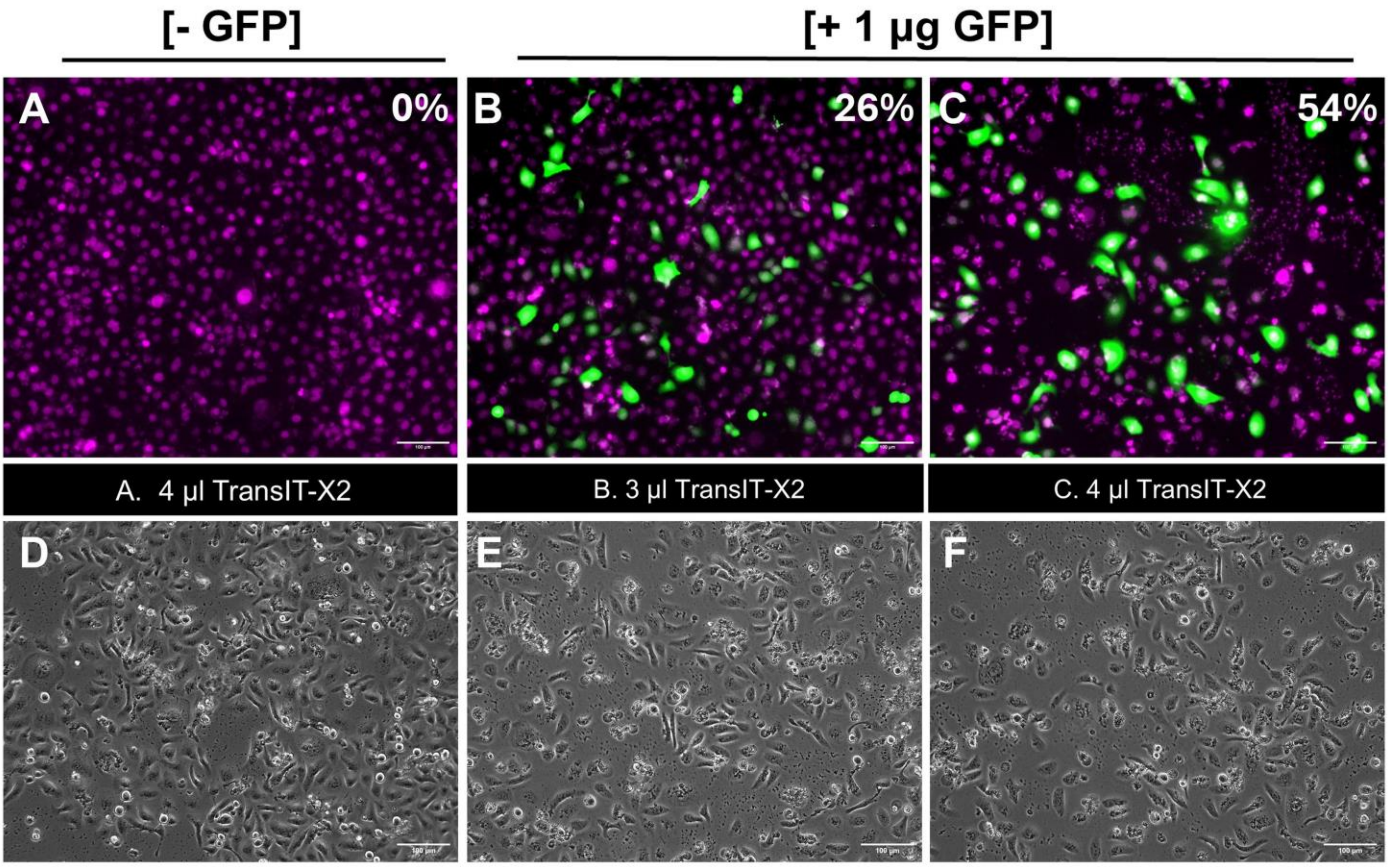

Figure S3. Optimization of TransIT-X2–mediated transfection in HaCaT cells under low-calcium conditions.

HaCaT cells were seeded at 1.5 × 10^5 cells per well in 12-well plates and incubated overnight at 37 °C in 5 % CO_2 using Dulbecco's Modified Eagle Medium (DMEM) containing low calcium [0.06 mM Ca^{2+}]. Cells were transfected with plasmid DNA encoding GFP under the following conditions: (A) 0 µg GFP + 4 µL TransIT-X2 (negative control); (B) 1 µg GFP + 3 µL TransIT-X2; (C) 1 µg GFP + 4 µL TransIT-X2. DNA–lipid complexes were prepared in 100 µL Opti-MEM and incubated for 20 min at room temperature before being added dropwise to the cells with gentle mixing. Cells were maintained in 2 mL of low-calcium [0.06 mM Ca^{2+}] medium during transfection. After 6 h, the medium was replaced with fresh low-calcium medium, and incubation was continued for 72 h post-transfection. Fluorescence microscopy was performed using DAPI (magenta) and FITC (green) channels, and transfection efficiency was quantified using ImageJ. Panels A–C show overlaid DAPI–FITC fluorescence images, while panels D–F display the corresponding phase-contrast micrographs of the same wells obtained separately. White Scale Bar = 100 µm in lower left corner.

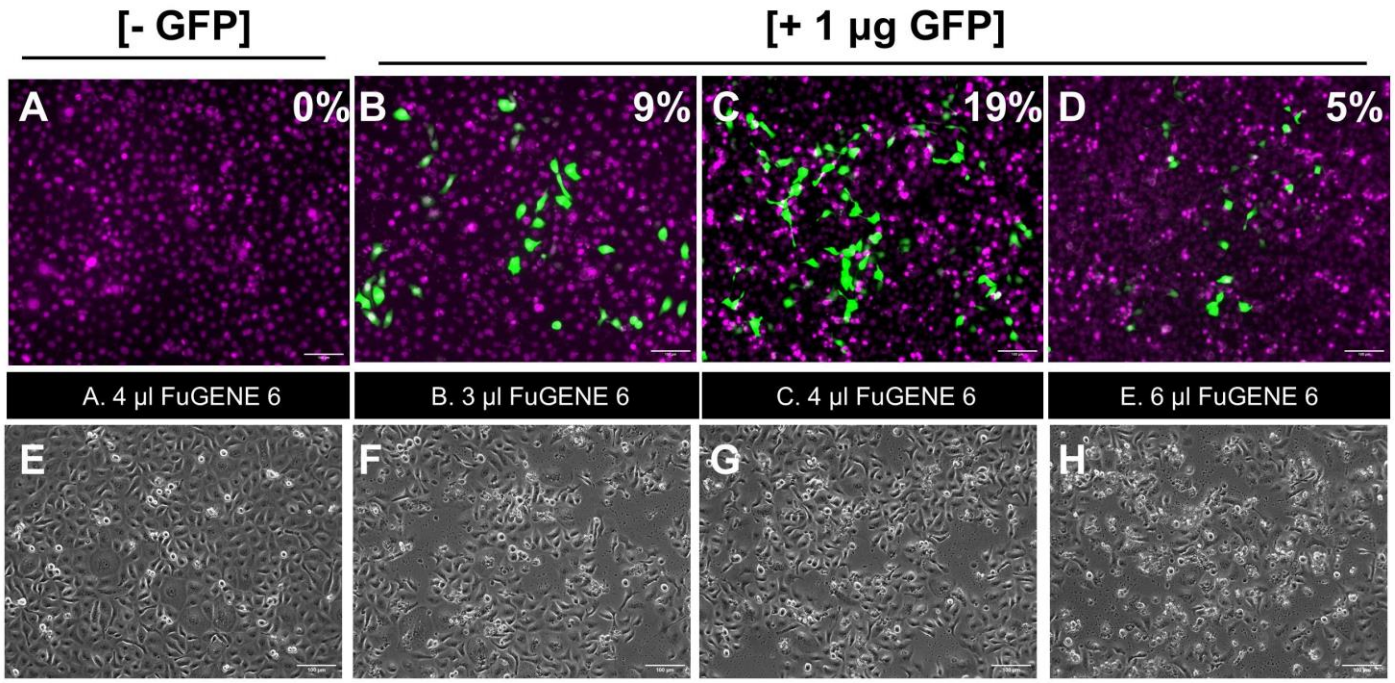

Figure S4. Optimization of FuGENE 6–mediated transfection in HaCaT cells under low-calcium conditions.

HaCaT cells were seeded at 1.5×10^5 cells per well in 12-well plates and cultured overnight (O/N) at 37 °C in 5 % CO_2 using Dulbecco's Modified Eagle Medium (DMEM) containing low calcium [0.06 mM Ca^{2+}]. Cells were transfected with GFP-expressing plasmid DNA under the following conditions: (A) 0 µg GFP + 4 µL FuGENE 6 (negative control); (B) 1 µg GFP + 3 µL FuGENE 6; (C) 1 µg GFP + 4 µL FuGENE 6; (D) 1 µg GFP + 6 µL FuGENE 6. DNA–lipid complexes were prepared in 100 µL Opti-MEM and added dropwise to the cells maintained in 1 mL of low-calcium [0.06 mM Ca^{2+}] growth medium. The medium was replaced 6 h after transfection, and cells were maintained for 72 h post-transfection under low-calcium conditions. Fluorescence microscopy was performed using DAPI (blue) and FITC (green) filters, and transfection efficiency was quantified using ImageJ. Panels A–D show overlaid DAPI–FITC fluorescence images, while panels E–H display the corresponding phase-contrast micrographs acquired separately from the same wells. Scale Bar = 100 µm.