Visualization of plasmid delivery to keratinocytes in mouse and human epidermis

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FIGURE S1. Real time imaging of single cells expressing hMGFP reporter under the control of the CMV promoter in mouse footpad skin. A. Schematic representation of pCMV-hMGFP/CBL plasmid. **B,C.** *In vivo* imaging of hMGFP-positive keratinocytes following intradermal injection of pCMV-hMGFP/CBL into paw skin using the Lucid confocal fluorescence system (see Materials and Methods). Fluorescence mode (488 nm laser, left panel) images at 20.8 (**B**) and 32 (**C**) μm depths are shown together with their corresponding reflectance mode images (right panels). Scale bar is 100 μm. **D.** Fluorescence microscopy of the skin sections obtained from previously imaged paws (see above panels **B** and **C**) injected with pCMV-hMGFP/CBL. Scale bar is 20 μm**.** DAPI stain (blue) was used to identify cellular nuclei. Abbreviations: Ampr, ampicillin resistance; hMGFP, humanized Montastrea green fluorescence protein; CBL, click beetle luciferase; CMV, cytomegalovirus.

FIGURE S2: Immunofluorescence of the human skin xenograft/mouse skin border. **A.** Microscopy of a 10 μm frozen skin section showing the junction of human and mouse skin stained with human keratin 1 antibody (red). **B.** Microscopy of a 10 μm section showing the junction of human and mouse skin stained with mouse keratin 1 antibody (red). Top panels, brightfield-fluorescence overlay. Blue fluorescence from DAPI stains

shows nuclei. Scale bar is 20 μm. **C.** Hematoxilin-eosin (H&E) staining of the human skin xenograft-mouse skin border. Microscopy of a 10 μm section showing the junction (white dots) of human (right) and mouse (left) skin stained with hematoxilin-eosin. Scale bar is 20 μm.

FIGURE S3: Long-term reporter expression of following administration of pUbc-luc2/eGFP by microneedle arrays. Representative bioluminescence image (see Materials and Methods) of pUbc-luc2/eGFP expression 72 days after application of metal microneedles (top) coated with DNA (0.6 μg/microneedle) or PAD (bottom) with less DNA (0.02 μg/microneedle). In vivo imaging of bioluminescence is a sensitive assay and this signal likely results from small numbers of transfected cells since GFP positive cells could not be found in the skin after 7 days.

FIGURE S4: Temporal analysis of reporter expression following administration of pCMV-CBL/hMGFP by microneedle arrays. Bioluminescence data of pCMV-CBL/hMGFP expression after application of metal microneedles coated with reporter plasmid DNA (0.6 μg/microneedle) or PAD with less reporter plasmid DNA (0.02 μg/microneedle). Controls are salmon sperm DNA (metal) and β-gal plasmid DNA (PAD).

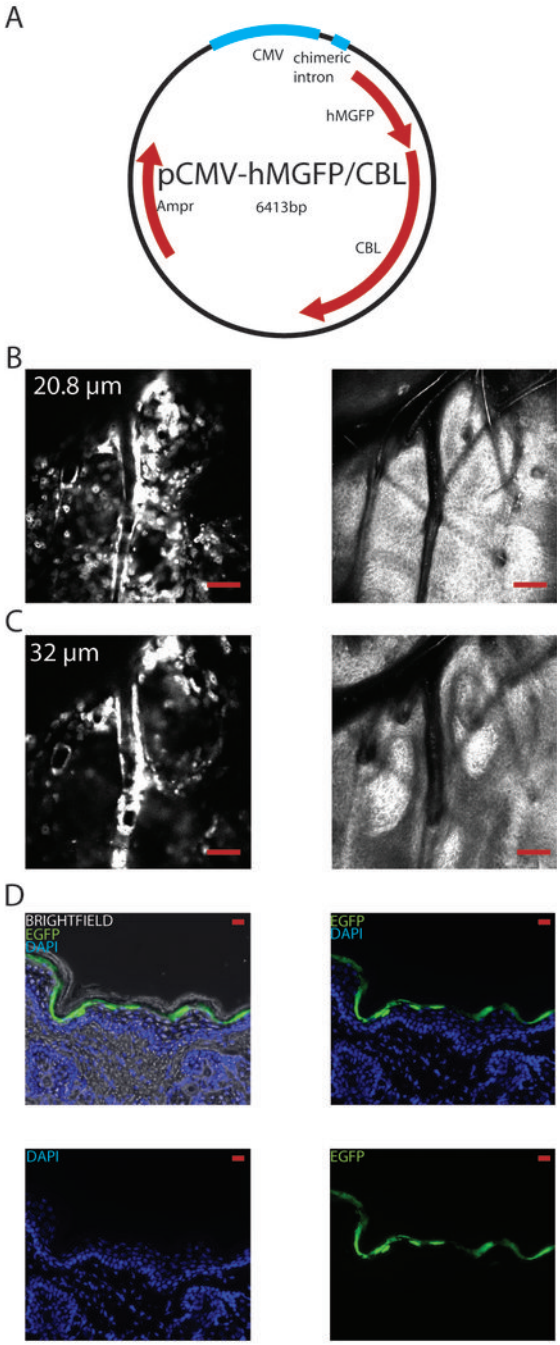
SUPPLEMENTAL VIDEO FILE 1: Video of eGFP expression 24 h after intradermal (ID) injection of pUbc-luc2/eGFP plasmid in mouse footpad using the

VivaScope 2500 (Lucid) confocal fluorescence microscope. A 2D image stack video of the corresponding 3-D image in Fig. 2 was created with ImageJ software. 2D en face images are translated in depth from the surface to 64 μm depth.

SUPPLEMENTAL VIDEO FILE 2: Video of hMGFP expression 24 h after ID injection of pCMV-hMGFP/CBL plasmid in mouse footpad using the VivaScope 2500 (Lucid) confocal fluorescence microscope. A 2D image stack video of the corresponding en face image in Fig. S1 was created with ImageJ software. 2D en face images are translated in depth from the surface to 64 μm deep.

SUPPLEMENTAL VIDEO FILE 3: Video of eGFP expression 24 h after injection of pUbc-luc2/eGFP plasmid in human skin xenografts using the VivaScope 2500 (Lucid) confocal fluorescence microscope. A 2D image stack video of the corresponding en face image in Fig. 3 was created with Image J software. 2D en face images are translated in depth from the surface to 64 μm depth.

FIGURE S1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hMGFP
pCMV-hMGFP/CBL
Ampr
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CBL
B
20.8 µm
C
32 µm
D
BRIGHTFIELD
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EGFP
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DAPI
EGFP

FIGURE S2

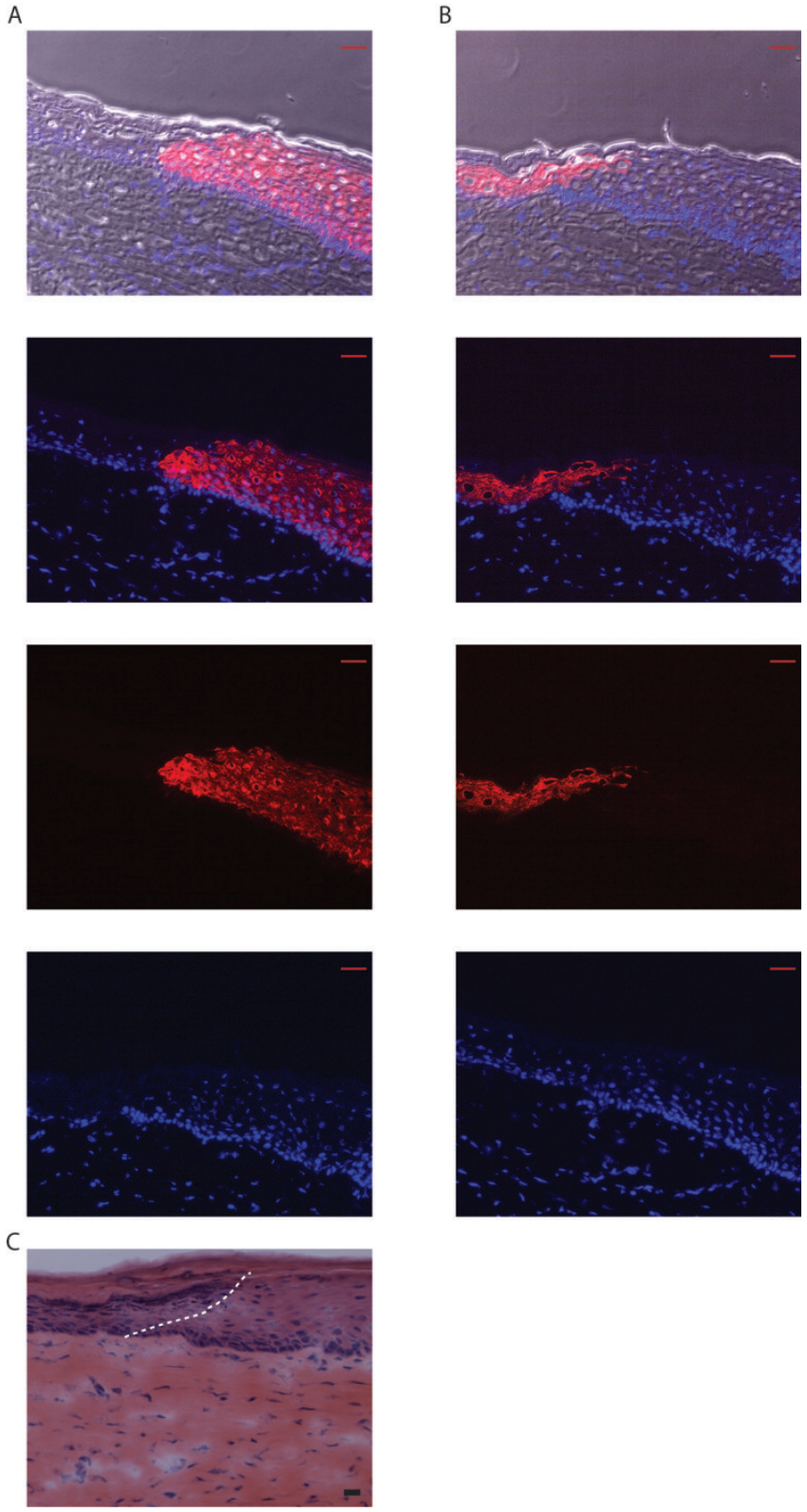


FIGURE S3

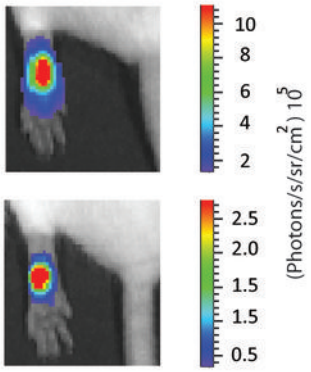
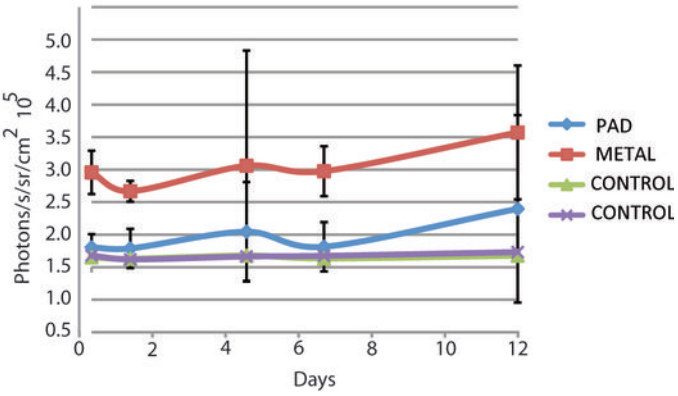


FIGURE S4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CONTROL