Supplementary Information

# Living sensor display implanted on skin for long-term biomarker monitoring

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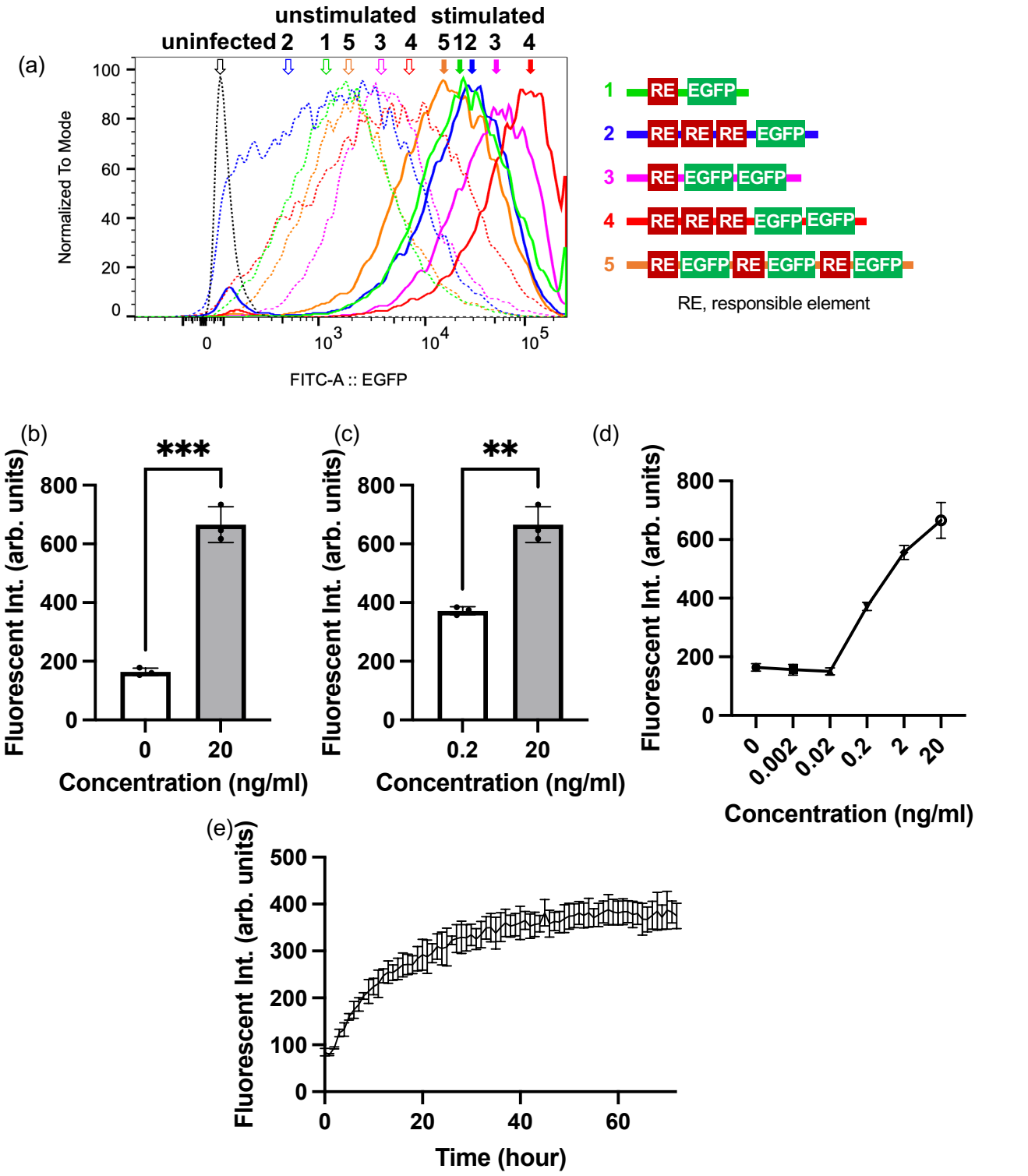


Fig. S1. In vitro characterization of NHEKs genetically modified with NF-κB-EGFP.
(a) Fluorescence intensity 24 hours after adding TNF-α at concentrations of 0 ng/mL and 20 ng/mL to the culture medium (n=3, biologically independent samples) P values = 0.0002. The statistical analysis was performed using t-tests (two-tailed). ***p < 0.001. (b) Fluorescence intensity 24 hours after adding TNF-α at concentrations of 0.2 ng/mL and 20 ng/mL to the culture medium (n=3, biologically independent samples) P values = 0.0012. The statistical analysis was performed using t-tests (two-tailed). **p < 0.01.
). (c) Dose-response curve of fluorescence intensity 24 hours after adding TNF-α at concentrations ranging from 0 ng/mL to 20 ng/mL (n = 3 , biologically independent samples). (d) Time course of fluorescence intensity over 72 hours after adding TNF-α at 20 ng/mL (n = 3 , biologically independent samples).

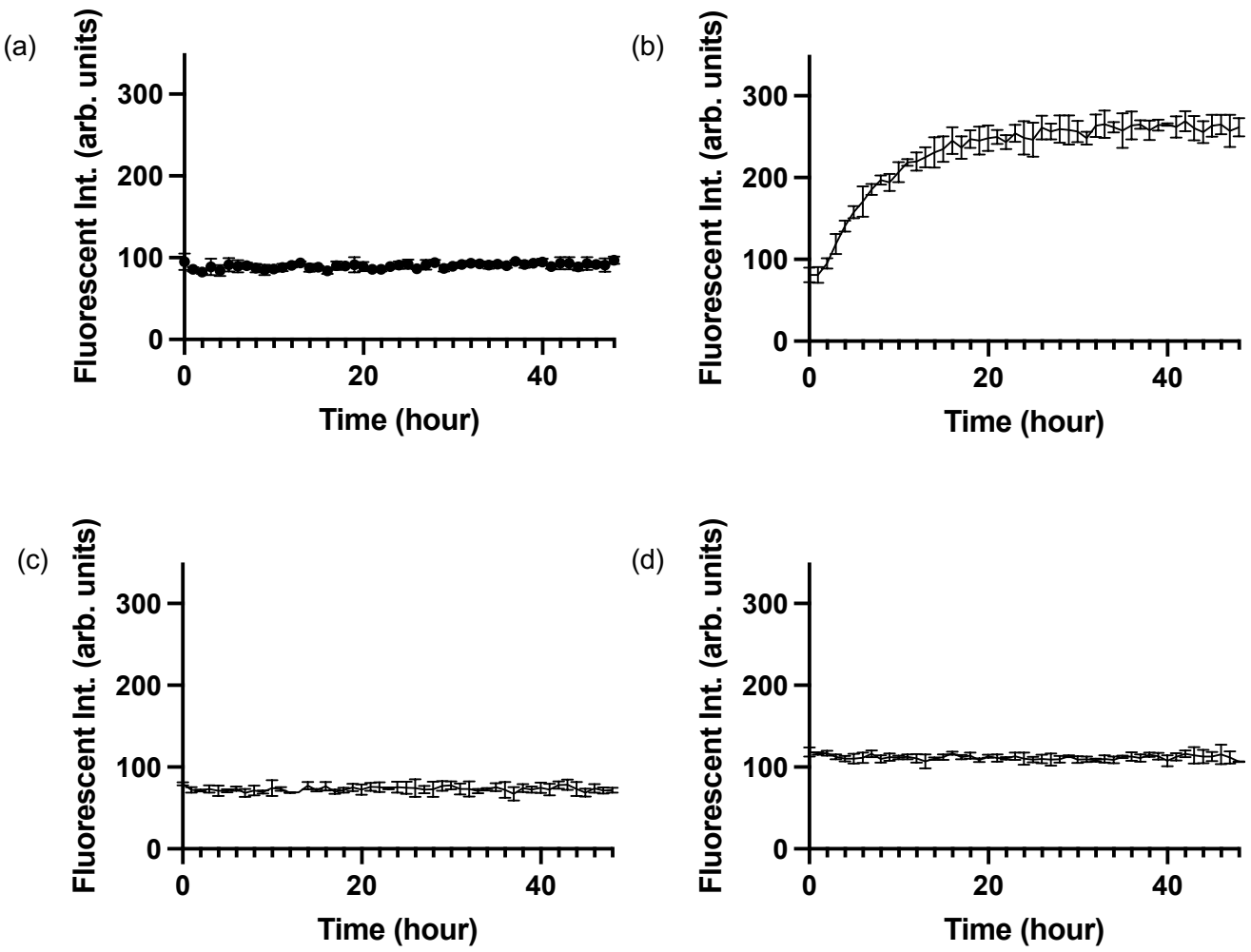


Fig. S2. Evaluation of cross selectivity in NHEKs genetically modified with NF-κB-EGFP (n = 3 , biologically independent samples).
(a) Culture medium only, (b) Addition of 20 ng/mL IL-1β, (c) Addition of 20 ng/mL IL-2,
(d) Addition of 20 ng/mL LPS.

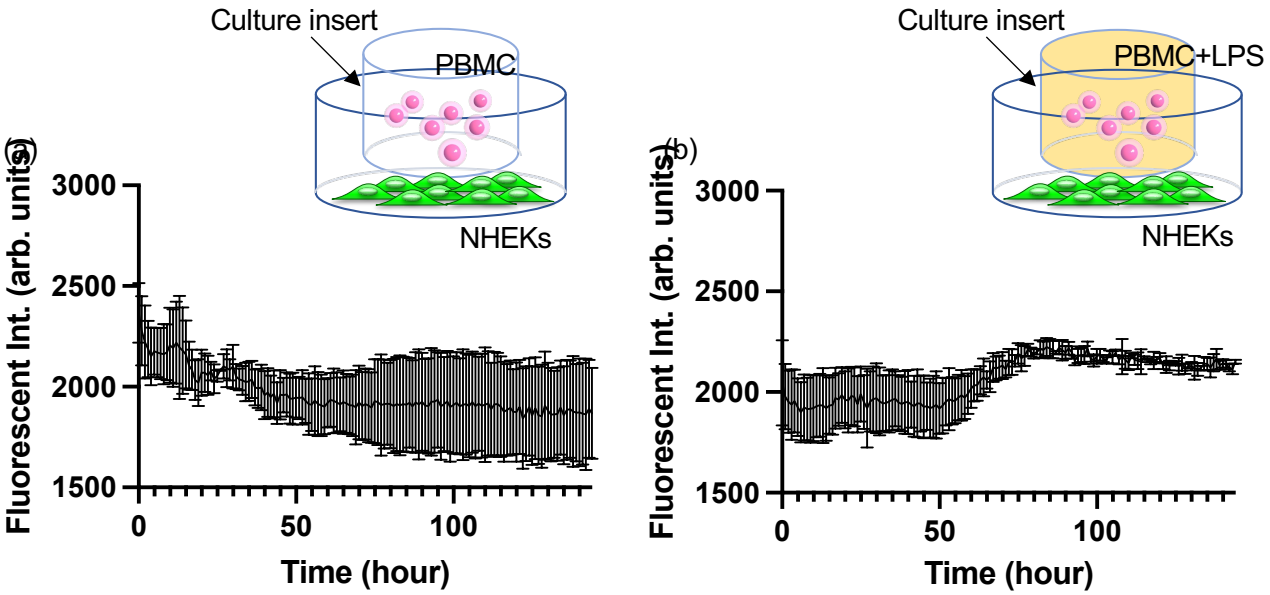


Fig. S3. Reconstitution of a human immune environment in a co-culture system using NHEKs genetically modified with NF-κB-EGFP and human PBMCs (n = 3 , biologically independent samples).
(a) Addition of 1 × $10^6$ PBMCs within the culture insert, (b) Addition of 1 × $10^6$ PBMCs along with LPS to achieve a final concentration of 20 ng/mL within the culture insert.

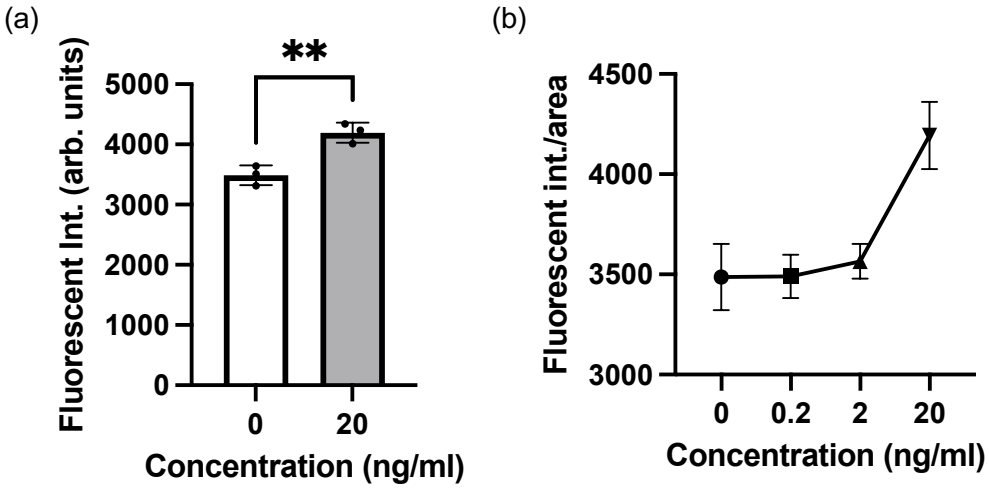


Fig. S4. In vitro characterization of tissue-engineered skin.
(a) Fluorescence intensity 17 hours after adding no TNF-α versus 20 ng/mL TNF-α to the medium (n=3, biologically independent samples) P values = 0.0065. The statistical analysis was performed using t-tests (two-tailed). **p < 0.01). (b) Dose-response curve of fluorescence intensity 24 hours after adding TNF-α at concentrations ranging from 0 ng/mL to 20 ng/mL (n = 3 , biologically independent samples).

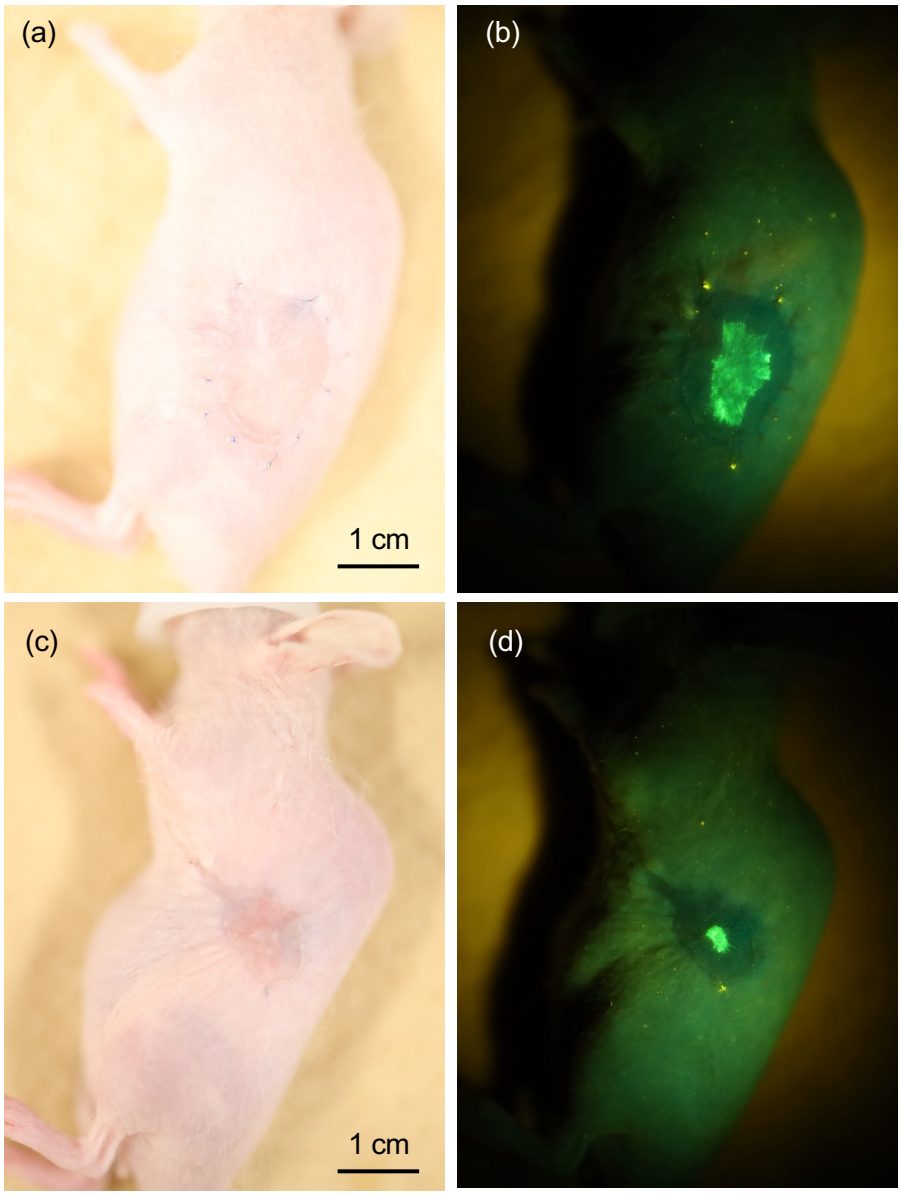


Fig. S5. (a,c) Brightfield and (b,d) fluorescence images of mice 4 weeks after transplantation.
At this time point, EGFP produced due to transplant-induced inflammation remains visible,
and fluorescence observation is feasible regardless of the size of the engrafted tissue-engineered skin.
Representative of at least three biologically independent repetitions with similar results.
Please note the mouse in picture (c) had the smaller engrafted tissue that that in (a).

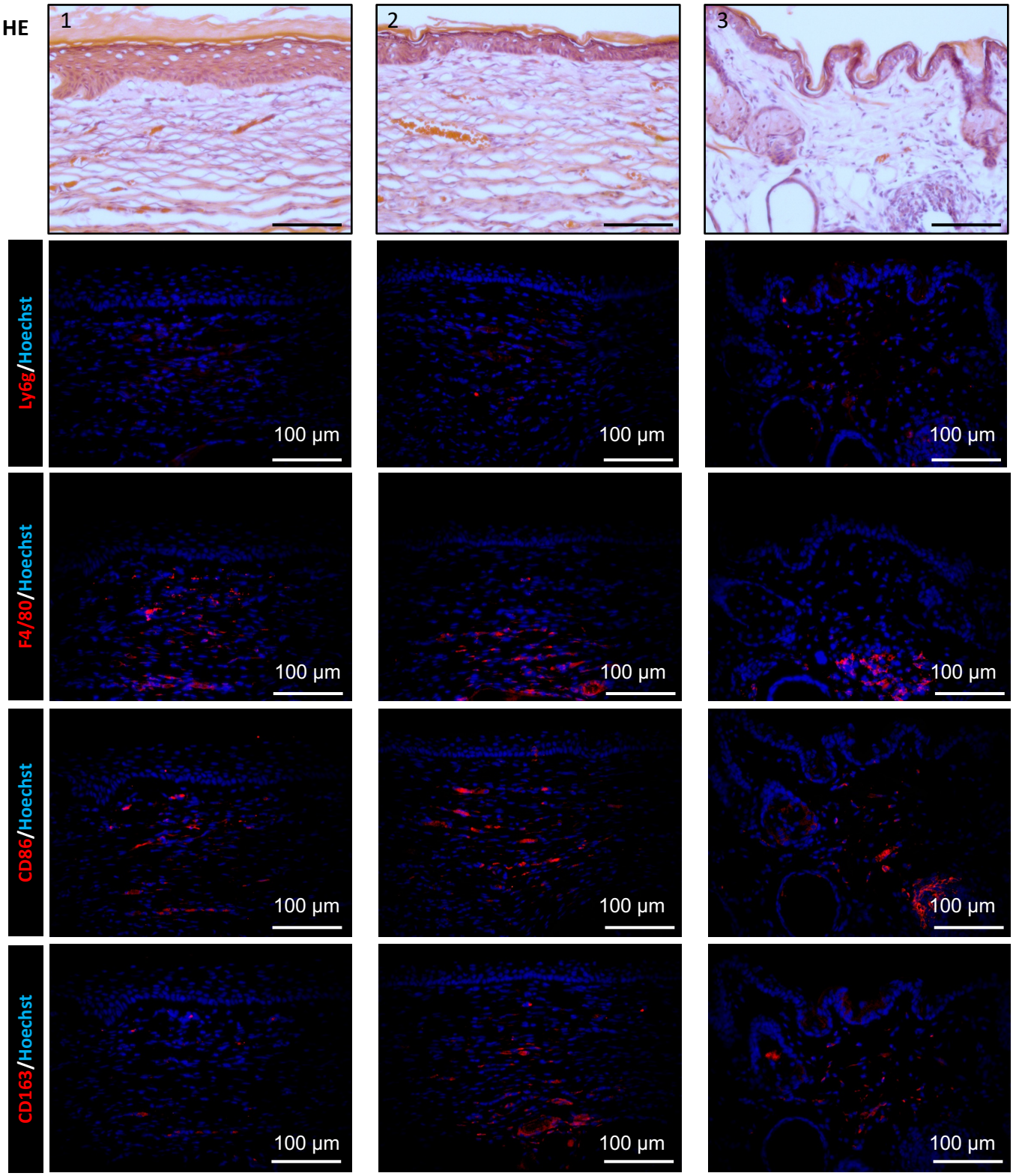


Fig. S6. Fluorescence images of immunostaining for inflammatory cells (Ly6g, F4/80, CD86, and CD163) in skin sections taken from around the transplantation site 3 weeks post-transplantation.
Representative of at least three independent repetitions with similar results.
At 3 weeks post-transplantation, macrophages and neutrophils
are observed, indicating that inflammation is still present around the transplant site.

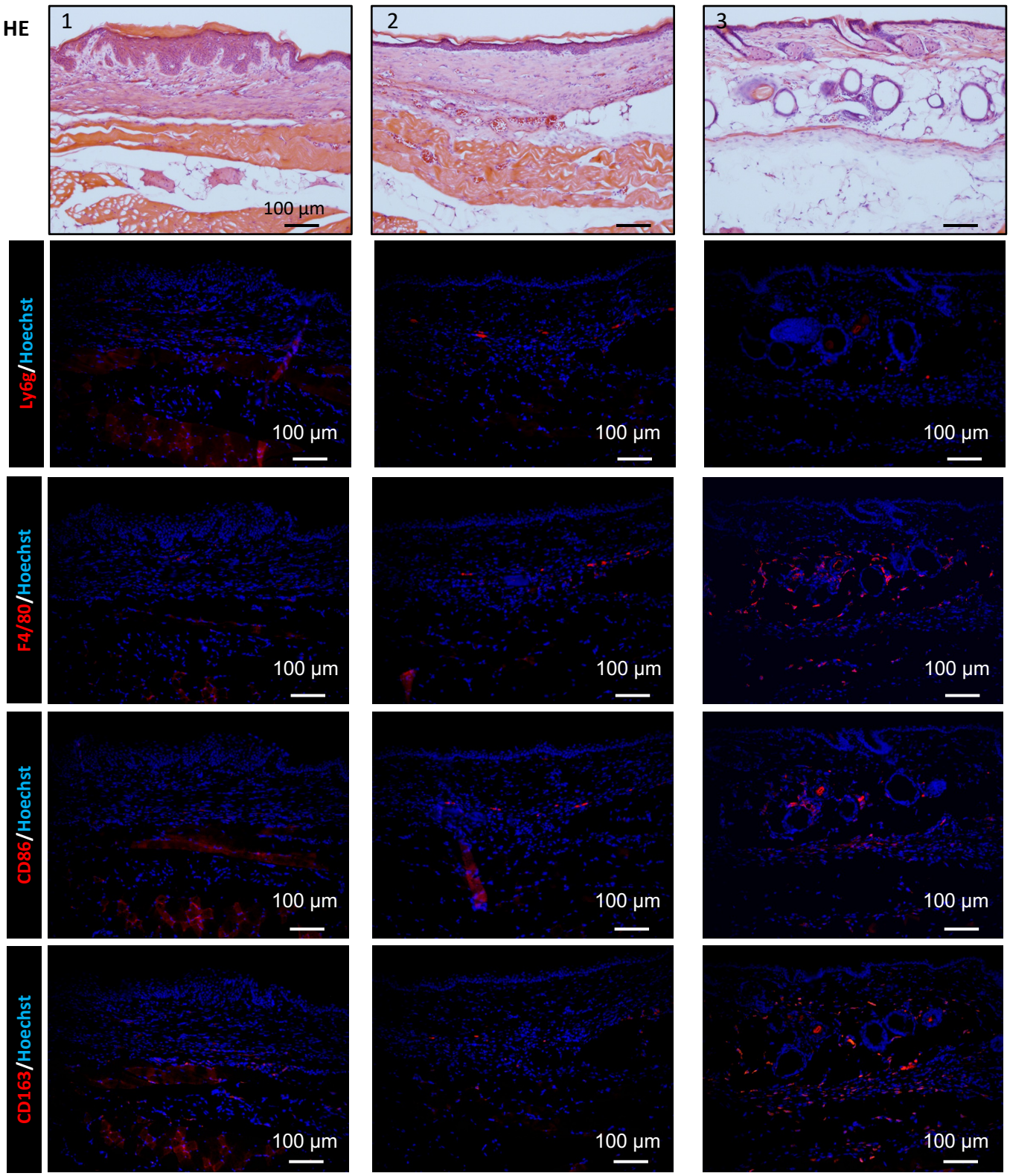


Fig. S7. Fluorescence images of immunostaining for inflammatory cells (Ly6g, F4/80, CD86, and CD163) in skin sections taken from around the transplantation site 4 weeks post-transplantation.
Representative of at least three independent repetitions with similar results.
At 4 weeks post-transplantation, macrophages and neutrophils are barely observed.
In fact, when inflammatory cells were counted, the number of macrophages decreased by at least 90% and neutrophils by at least 75% compared to 3 weeks post-transplantation (as shown in Fig. S6).

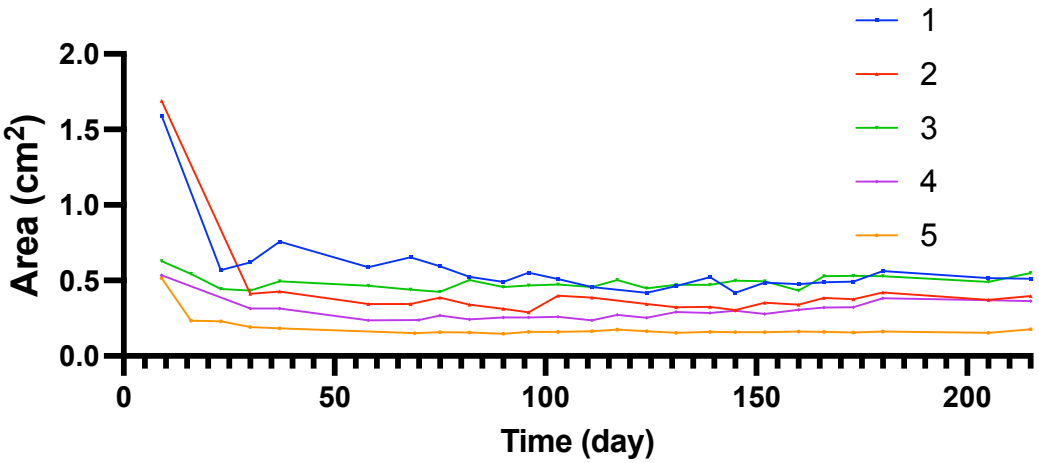


Fig. S8. Time course of changes in the area of the transplanted tissue-engineered skin (n = 5, biologically independent mice).
Approximately 30 days post-transplantation, the size of the transplant site becomes stable.

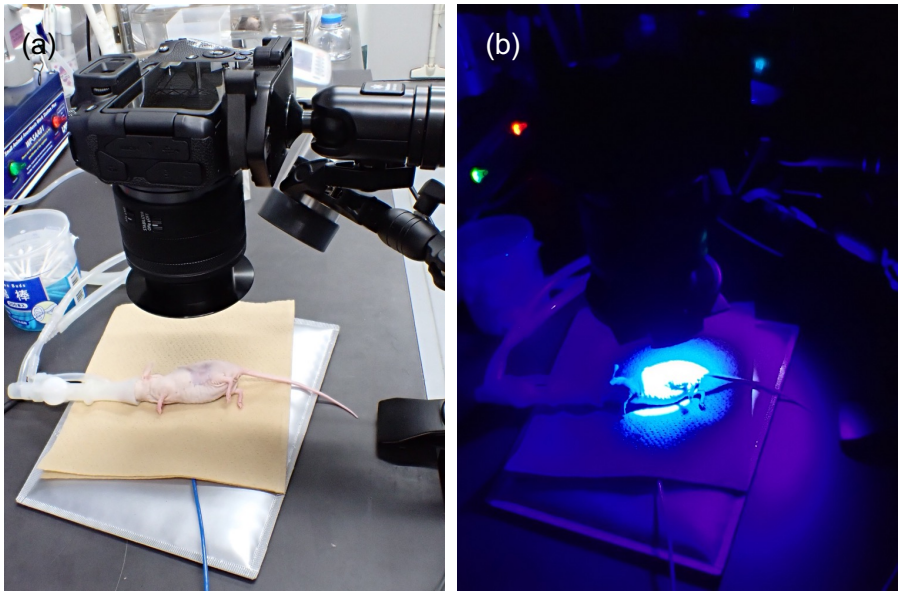


Fig.S9 *In vivo* fluorescence measurement
(a) View of the sample under bright-field observation.
(b) View during imaging while collimated light is applied as the excitation source.

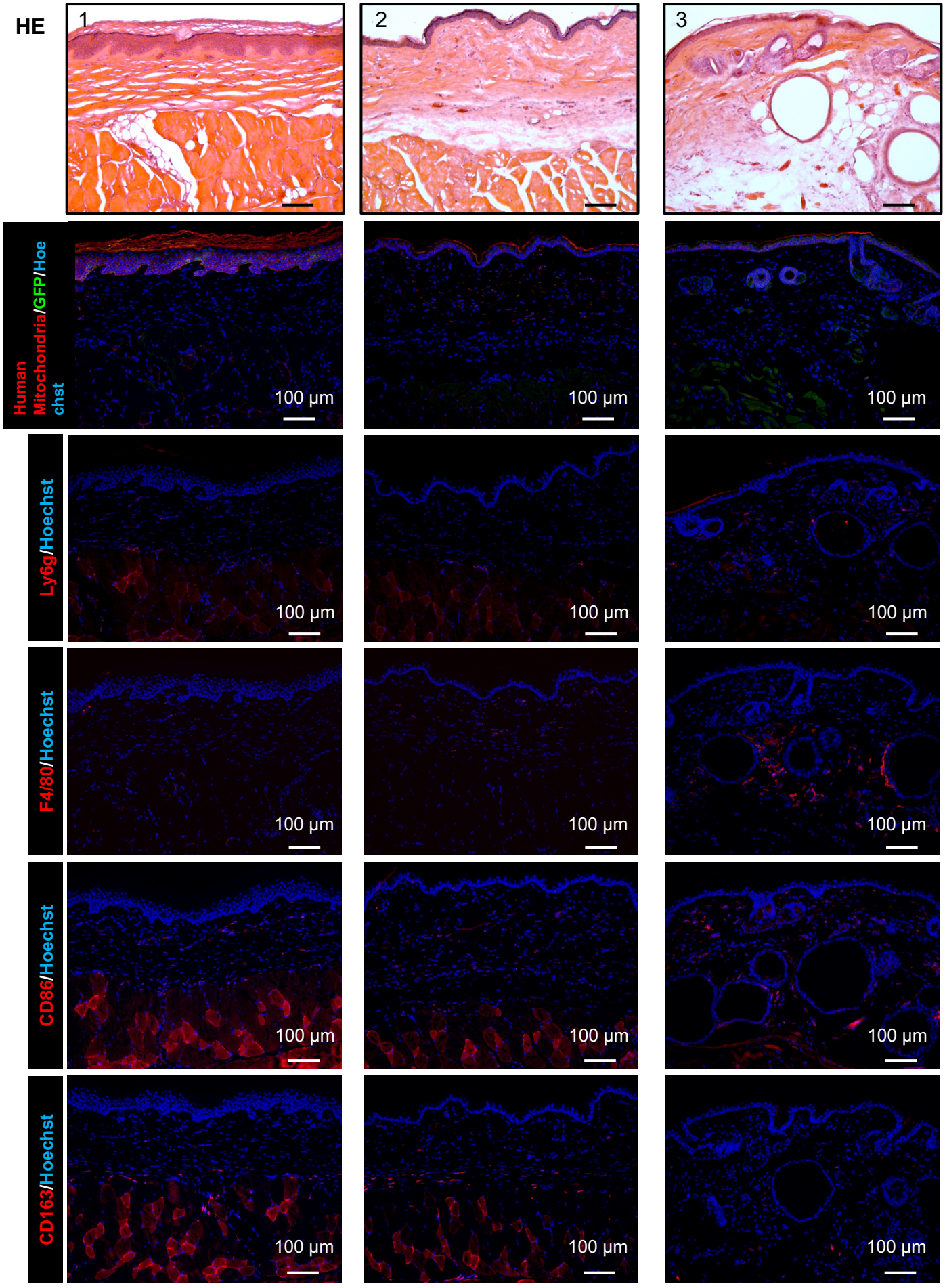


Fig. S10. Merged immunostaining images for human mitochondria and GFP from a skin section taken from around the transplantation site before TNF-α stimulation, and fluorescence images of immunostaining for inflammatory cells (Ly6g, F4/80, CD86, and CD163). Representative of at least three independent repetitions with similar results. Prior to TNF-α stimulation, inflammatory cells such as macrophages and neutrophils are barely observed.

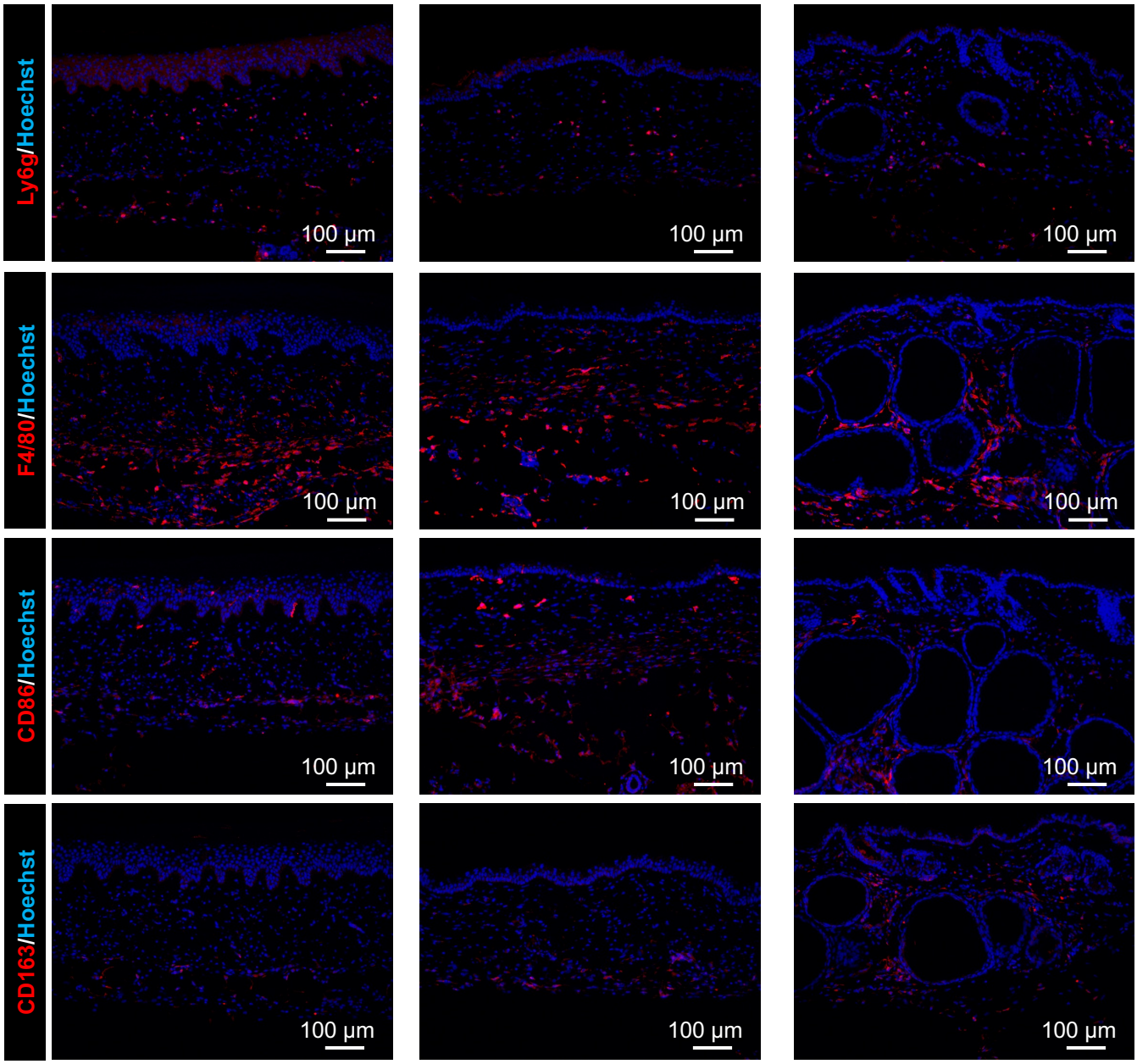


Fig. S11. Fluorescence images of immunostaining for inflammatory cells (Ly6g, F4/80, CD86, and CD163) in skin sections taken from around the transplantation site after TNF-α stimulation. Representative of at least three independent repetitions with similar results. TNF-α stimulation results in the accumulation of macrophages and neutrophils.

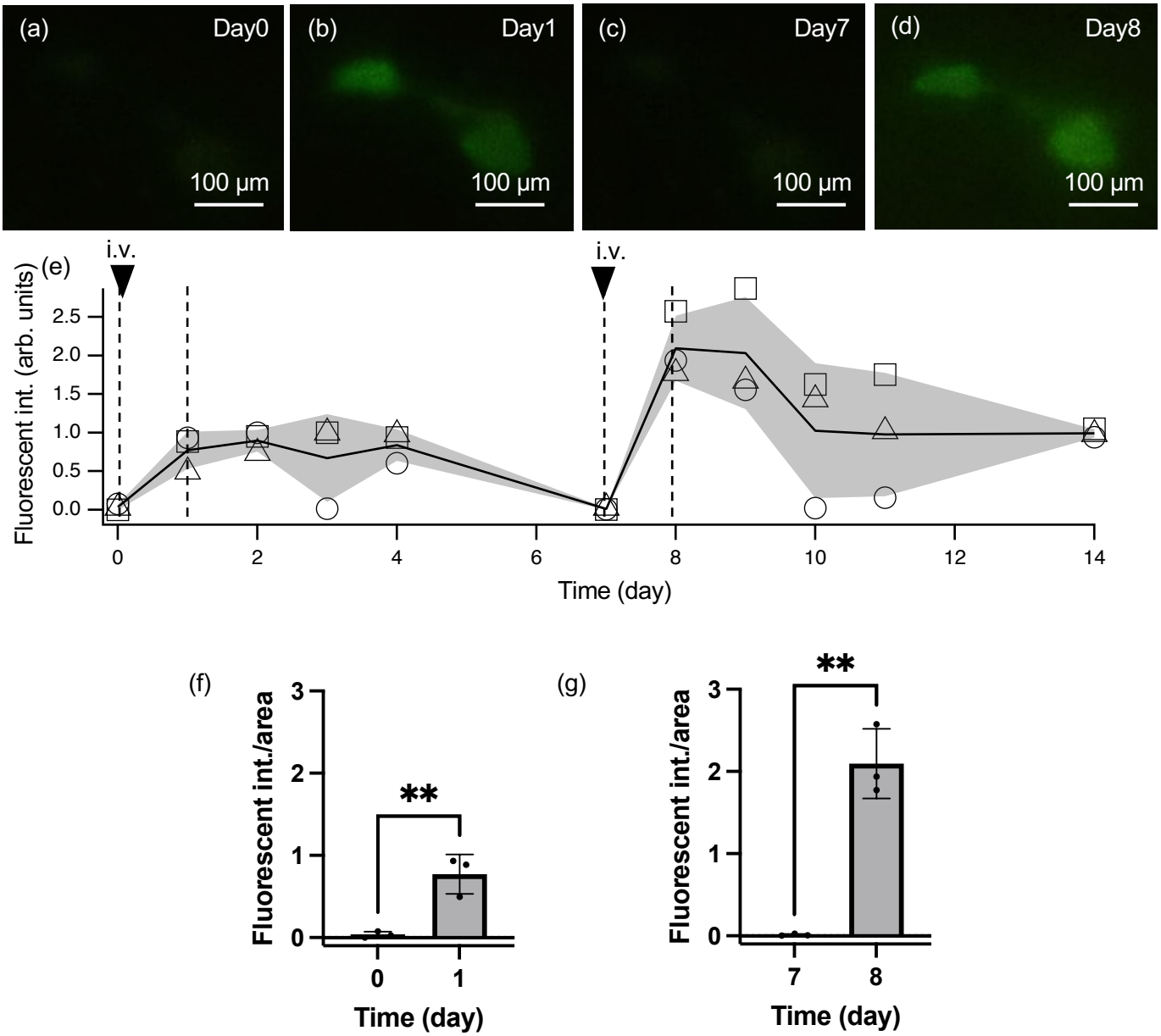


Fig. S12. Intravenous LPS stimulation in mice. Fluorescence images of the engrafted tissue-engineered skin (a) before LPS administration, (b) 1 day after intravenous administration of LPS, and (c) 7 days after administration. (d) Fluorescence image of the engrafted tissue-engineered skin 1 day after re-administration of LPS via the tail vein on day 8 following the initial administration. (e) Time course of fluorescence intensity calculated from image analysis of the tissue-engineered skin. (n=3, biologically independent mice) The vertical dotted lines indicate the time points at which fluorescence images were taken, and the downward-pointing triangles denote the timing of LPS administration. Due to inter-individual variability in fluorescence intensity following intravenous LPS administration, the fluorescence values were normalized to the maximum intensity observed after the first LPS injection.(f) Comparison of fluorescence intensities at 0 days and 1 days after administration (n=3, biologically independent mice) P values = 0.0063. The statistical analysis was performed using t-tests (two-tailed). **p < 0.01. (g) Comparison of fluorescence intensities at 7 days and 8 days after administration (administration (n=3, biologically independent mice) P values = 0.0010. The statistical analysis was performed using t-tests (two-tailed). **p < 0.01.

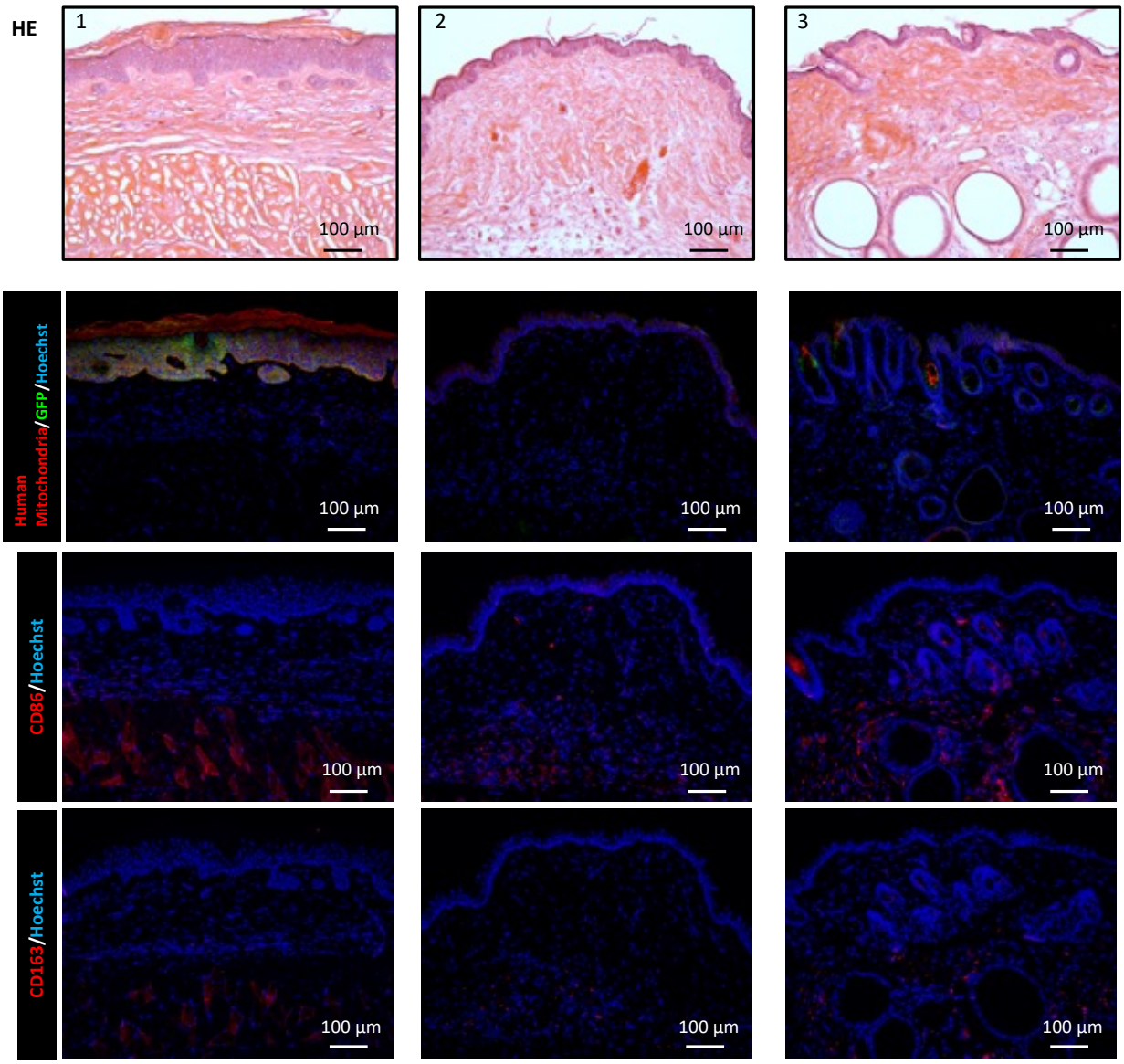


Fig. S13. Merged immunostaining images for human mitochondria and GFP from a skin section taken from around the transplantation site 9 days after intraperitoneal administration of LPS and 2 days after subcutaneous administration of LPS, along with fluorescence images of immunostaining for CD86 and CD163. Even after LPS administration, M1 and M2 macrophages are scarcely observed. Representative of at least three independent repetitions with similar results.

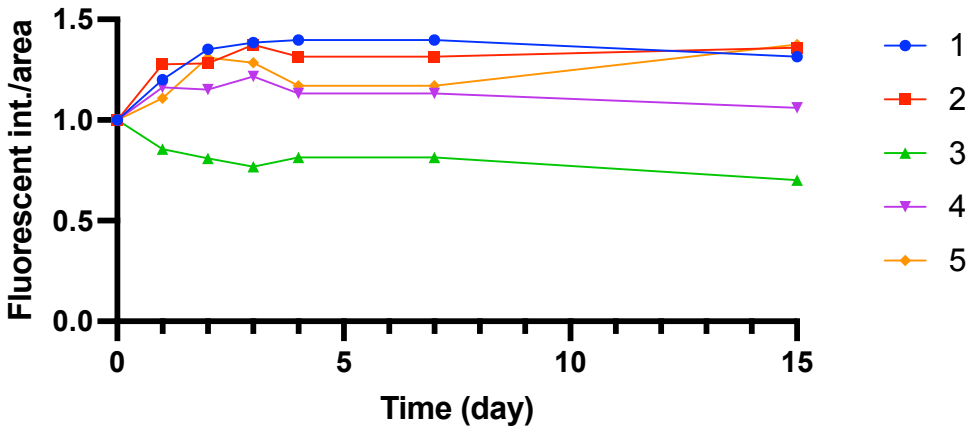


Fig.S14 Time course of fluorescence intensity calculated from image analysis of tissue-engineered skin after a single subcutaneous administration of LPS. (n=5, biologically independent mice)

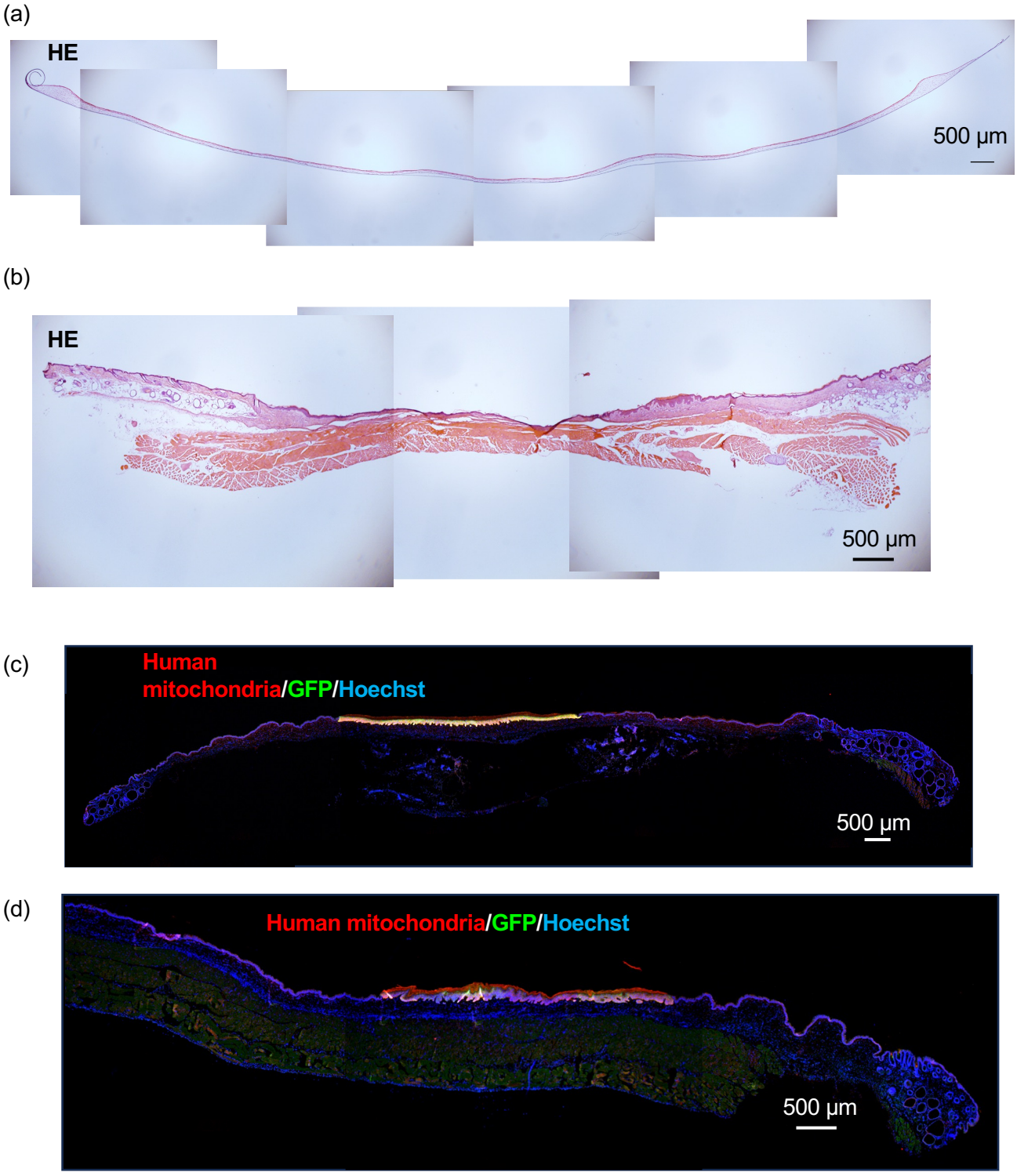


Fig. S15. Full uncropped scans corresponding to the cropped gel/blot images shown in the main figures. (a) Uncropped image corresponding to Fig. 2b.(b) Uncropped image corresponding to Fig. 3b. (c) Uncropped image corresponding to Fig. 4i.(d) Uncropped image corresponding to Fig. 5i. All images were obtained from biologically independent experiments repeated at least three times with similar results.