SUPPLEMENTAL MATERIAL

Doni et al., http://www.jem.org/cgi/content/full/jem.20141268/DC1

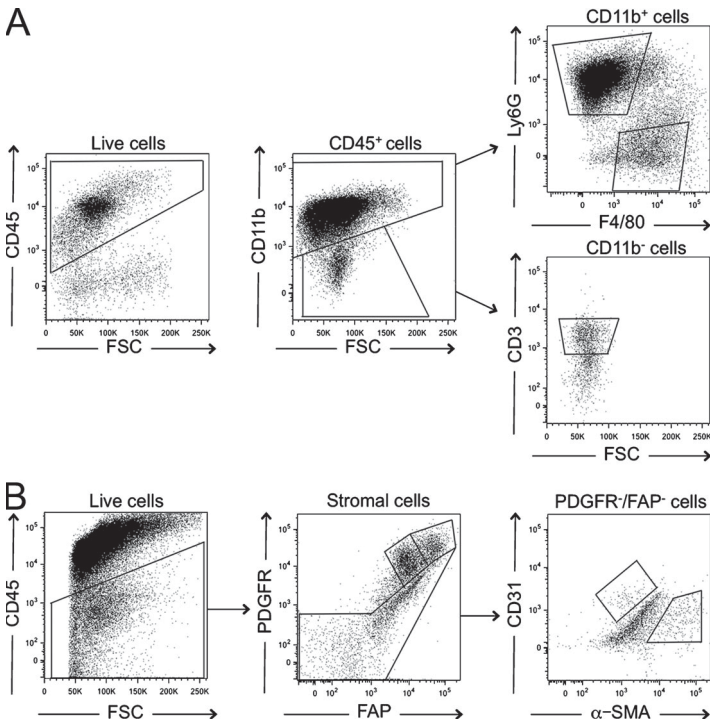

Figure S1. Gating strategy used in flow cytometry analysis for immunophenotyping and sorting leukocytes and stromal cells in the course of skin repair

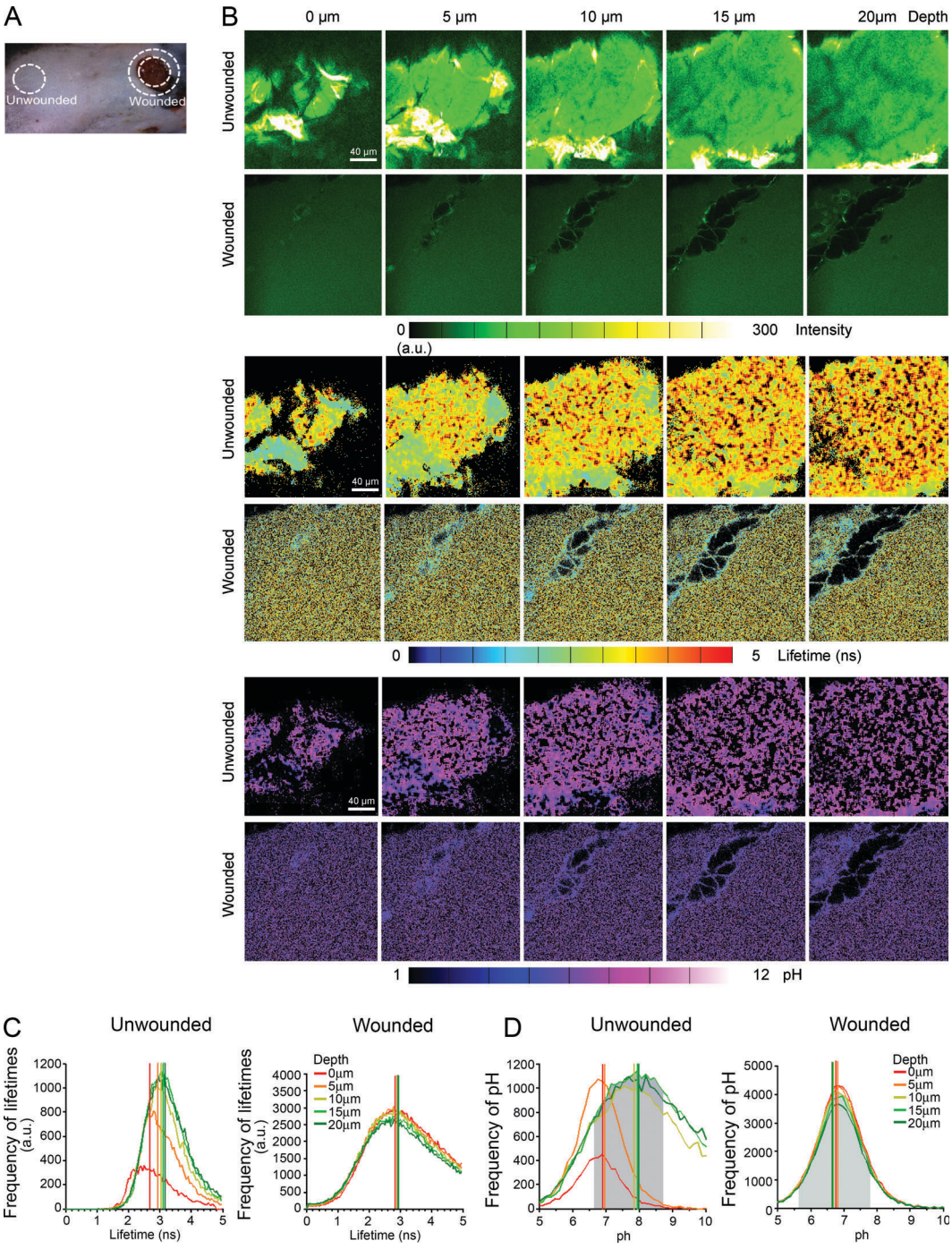

Figure S2. Method used to measure the pH in wounded skin by two-photon microscopy. (A) Regions of analysis of unwounded or wounded skin included in the dotted line. (B, top) BCECF (free acid; 80 µM) fluorescence intensity images of unwounded and wounded skin areas at different depth (from 0 to 20 µm). The decrease in mean fluorescence intensity measured in the wounded skin area suggests a more protonated configuration of BCECF (HBCECF), and hence a more acidic environment compared to the unwounded skin. Care was taken in checking that autofluorescence and Second Harmonic Generation (SHG) signal were lower (8–10 fold) than BCECF signal. (middle) Lifetime fits measured in unwounded and wounded regions as a function of depth. An off-peak time gate was set to reduce the contribution from SHG. (bottom) pH maps, calculated as a fraction of contributions from lifetime of HBCECF and unprotonated BCECF (BCECF2), which show the more acidic regions measured in wounded area of the skin. (C) Lifetime distribution as taken from B (middle). Lines refer to mean lifetimes calculated with Gaussian fits of the distributions. (D) pH distribution as taken from B (bottom). Lines refer to average pH calculated with Gaussian fits. Gray area refers to range of pH considering the mean $\pm$ σ in the derma (10-20 µm). Data show results of one representative mouse out of 5 analyzed.