High Resolution Ultrasound Imaging for Repeated Measure of Wound Tissue Morphometry, Biomechanics and Hemodynamics under Fetal, Adult and Diabetic Conditions

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Running Title: *Non-invasive characterization of fetal and adult wounds*

Supplementary Figure Legends

Supplementary Fig S1a. Enlarged Doppler color flow images (from **Fig 1a**) of fetus at days (i) E6.5, (ii) E13.5 and (iii) E15.5 are shown. Scale bar = 5 mm

Supplementary Fig S1b. Flow chart of image processing and strain analysis.

Supplementary Fig S1c. Matlab code for enhancement of tissue density.

Supplementary Fig S2. Representative three-dimensional images of fetal wounds. (a) Enlarged view of Fig 2a. (b-d) 3D reconstructed images of the fetus at 3, 24, and 48 hours post-wounding. Fetal wounds sites are shown in cyan.

Supplementary Fig S5a. Morphometry of adult diabetic (db/db) cutaneous wound healing compared to the non-diabetic (db/+) mice d14 post-wounding in six different mice (i-vi) in each group. Grey: ultrasound B-mode image of full thickness stented wound bed (red arrow, white dotted region), wound edge (yellow arrows) of db/db and control db/+ mice. Color: enhanced anatomical images using Matlab code to show the cellular density. Density index low (blue) to high (red)

Supplementary Fig S5b. Histology of db/+ and db/db wounds at d14 post-wounding. Hematoxylin and Eosin staining. Scale bar = 500 μm for the mosaic containing the entire section. Scale bar = 100 μm for zoomed inset.

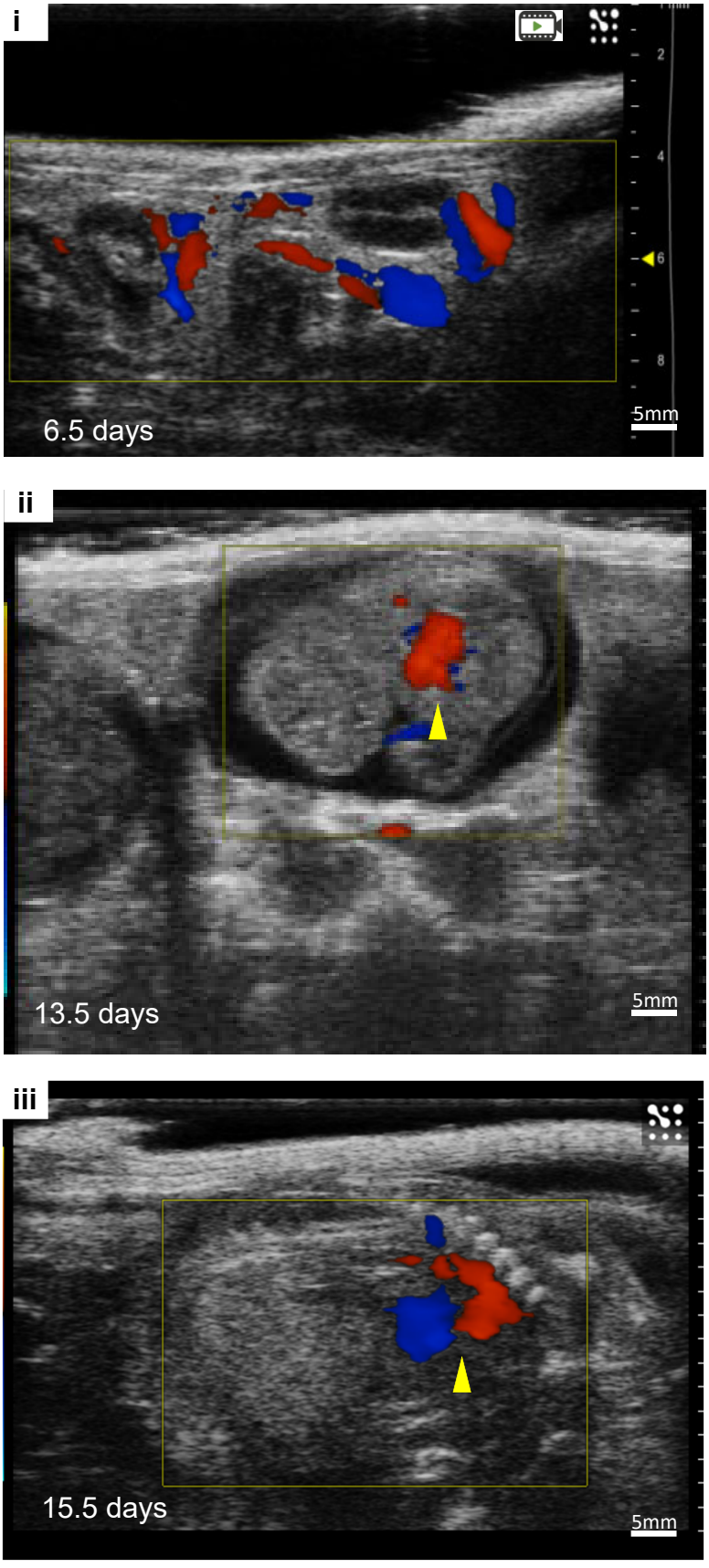
Supplementary Fig S6a. Adult wound tissue site for biomechanical strain measurement. A green line was traced around the wound site to help select wound edge and bed for strain analysis using 'VevoStrain®' software. Selected blue dot (i) for adult wound edge and (ii) wound bed to analyze biomechanical strain.

836 **Supplementary Fig S6b.** A green line was traced around the normal skin/wound site to help
837 select skin tissue strain analysis using 'VevoStrain®'. Adult tissue strain curves for
838 biomechanical elastic strain patterns and synchronicity. Blue curve for db/+ and red curve for
839 db/db (i). Wound strain synchronicity curves over time of wound edge (ii) wound bed (iii) to
840 analyze biomechanical strain.

841 **Supplementary Fig S7.** Color Doppler flow images of uterine artery imaged at E15.5. The
842 uterine artery as indicated by white arrow shows blood flowing across the placenta to the fetal
843 heart.

844 **Supplementary Videos. SV1** Ultrasound images of embryos at days (a) E6.5, (b) E13.5, and (c)
845 E15.5

846 **Supplementary Videos. SV2** (a) Representative three-dimensional reconstruction image of fetal
847 wounds. (bd) 3D reconstructed images of the fetal wound at (b) 3, (c) 24, and (d) 48 hours post-
848 wounding. Fetal wounds sites are shown by arrow.



Supplementary Fig. S1a

Flow chart: design of image processing and strain analysis

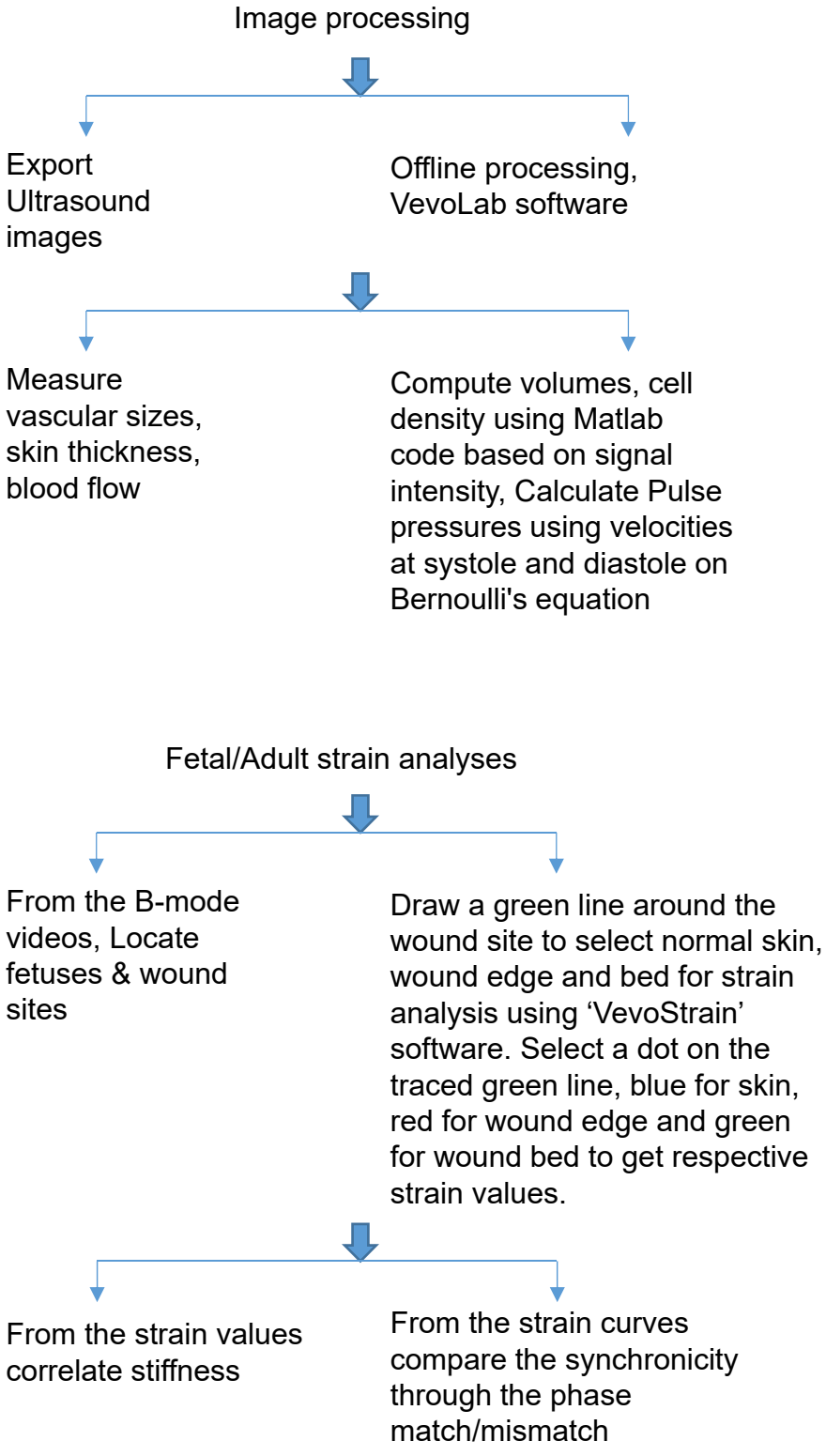
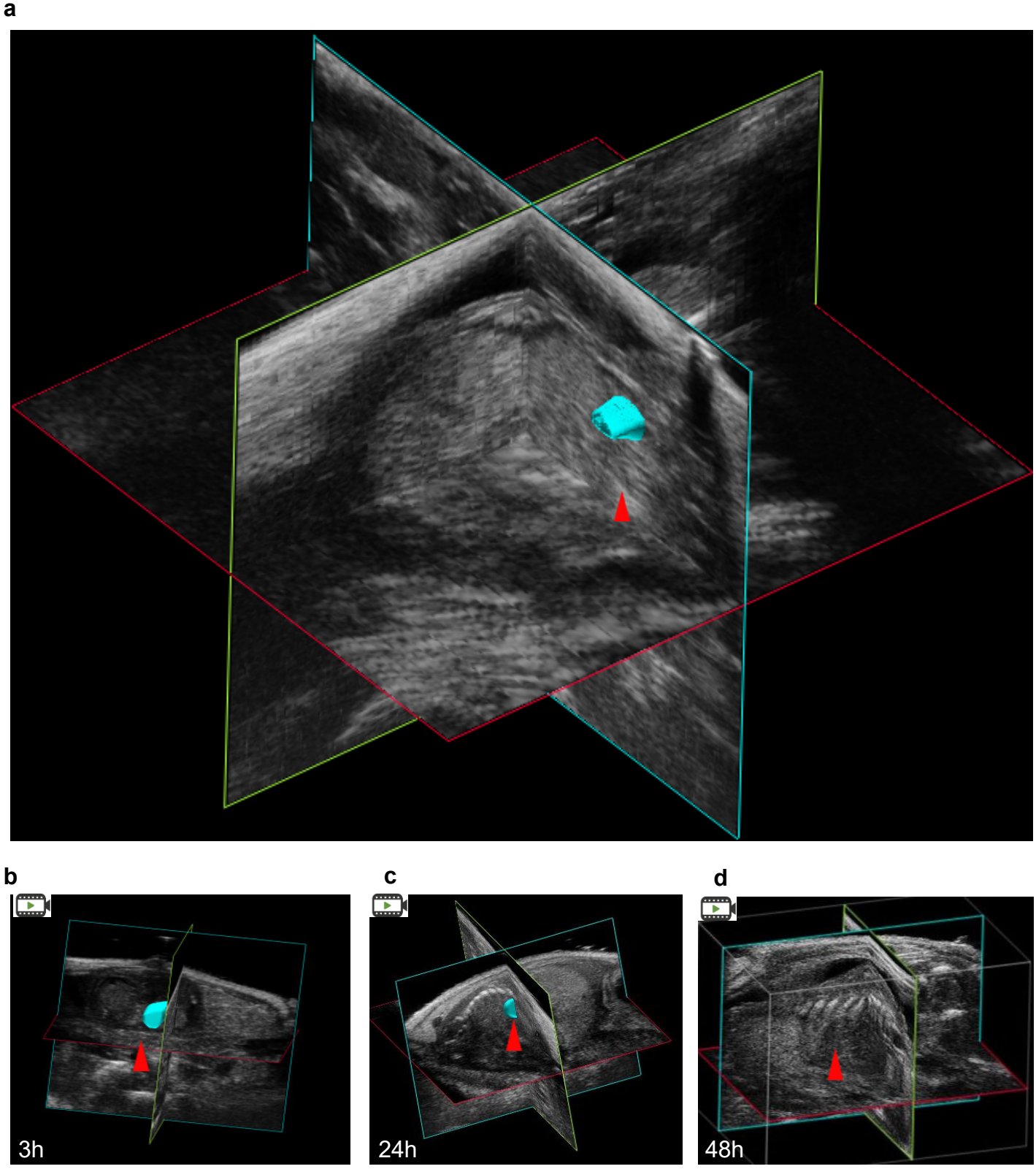


Figure S1b

```
Clc;
clear all;
close all;
im = imread('fetus1.jpg');
I = rgb2gray(im);
figure;
imshow(im);
rgbImage = ind2rgb(I, jet(256));
imshow(rgbImage);
maskedImage  = double(repmat(rgbImage, 1, 1, 3));
imshow(maskedImage);
% Enlarge figure to full screen.
set(gcf, 'Units', 'Normalized', 'OuterPosition', [0 0 1 1]);
%-----------------------------------------
%code below changes the pixels to green
Image1=im2double(rgbImage);

[row column page] = size(Image1)

mask = Image1(:,:,2) > Image1(:,:,1) & Image1(:,:,2) > Image1(:,:,3);

Image2 = Image1 .* mask(:,:,[1 1 1]);

figure,imshow(Image2);

title('light green');

mask = Image2(:,:,2) + 50 > Image2(:,:,1) & Image2(:,:,2) + 50 >
Image2(:,:,3);

Image3 = Image2 .* mask(:,:,[1 1 1]);

figure,imshow(Image3);

title('dark green');
```

Supplementary Fig. S1c



Supplementary Fig. S2

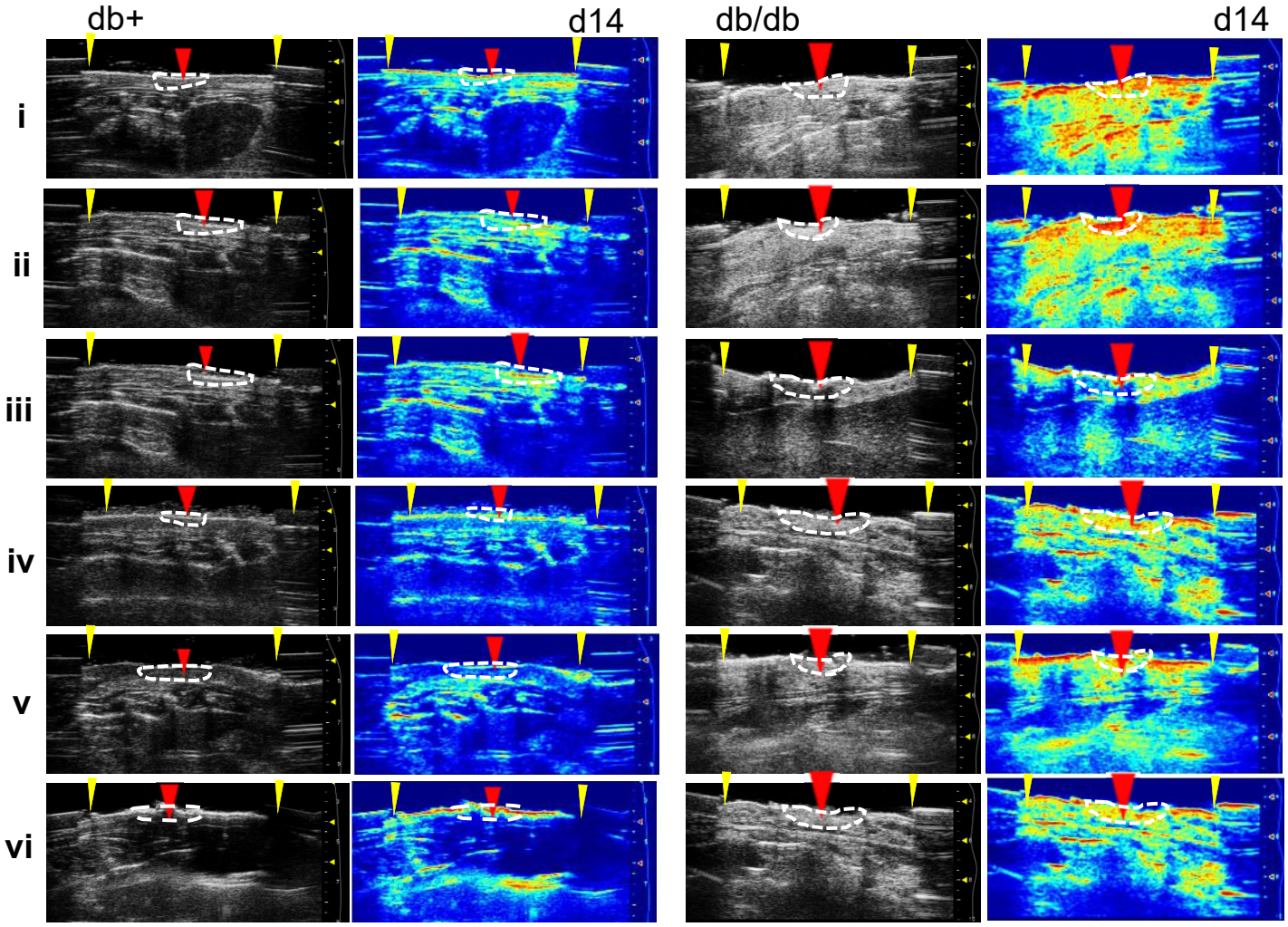


Figure S5a

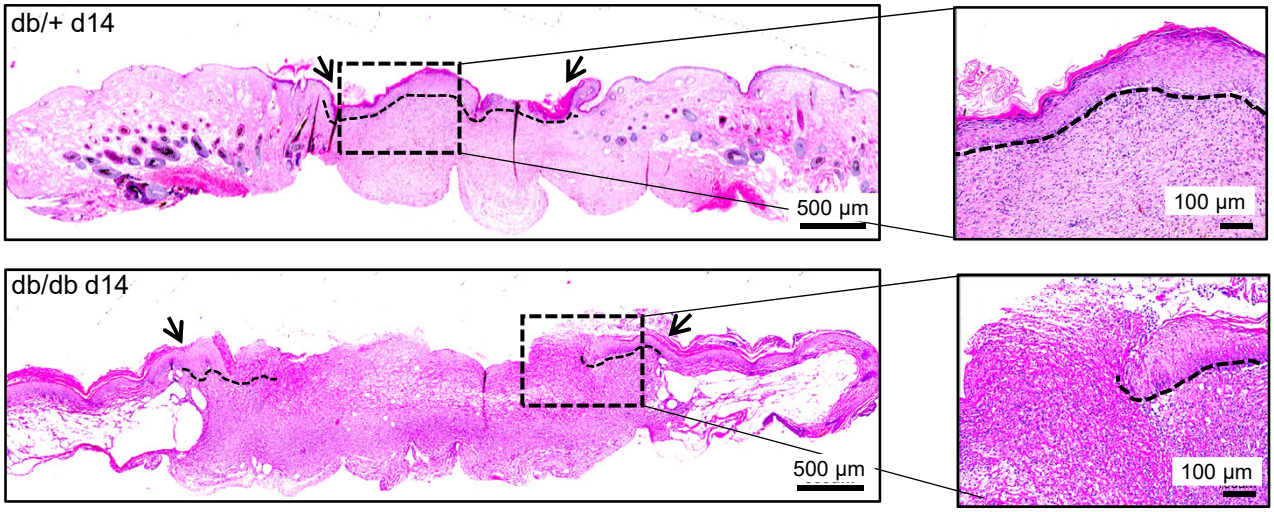


Figure S5b

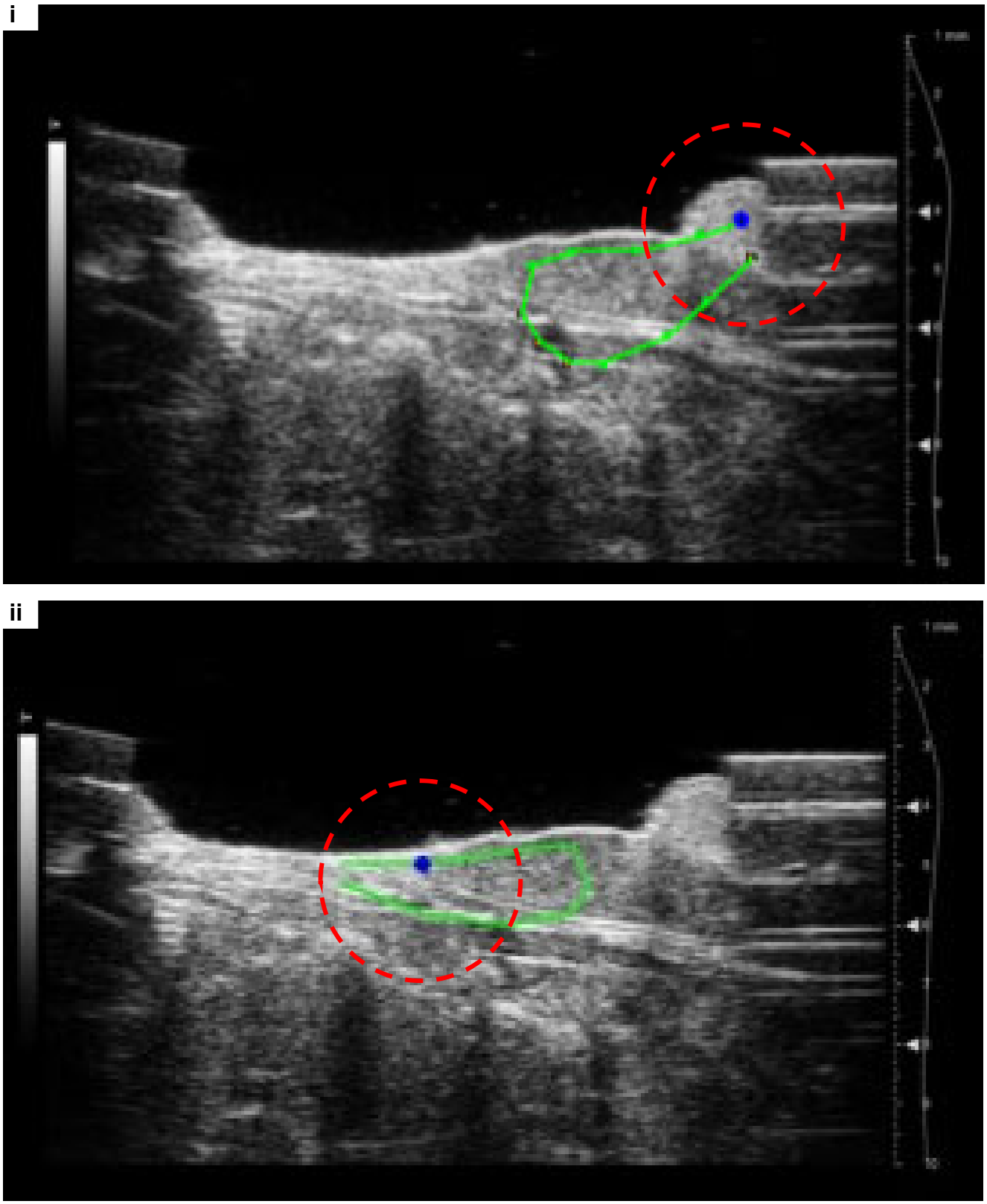


Figure S6a

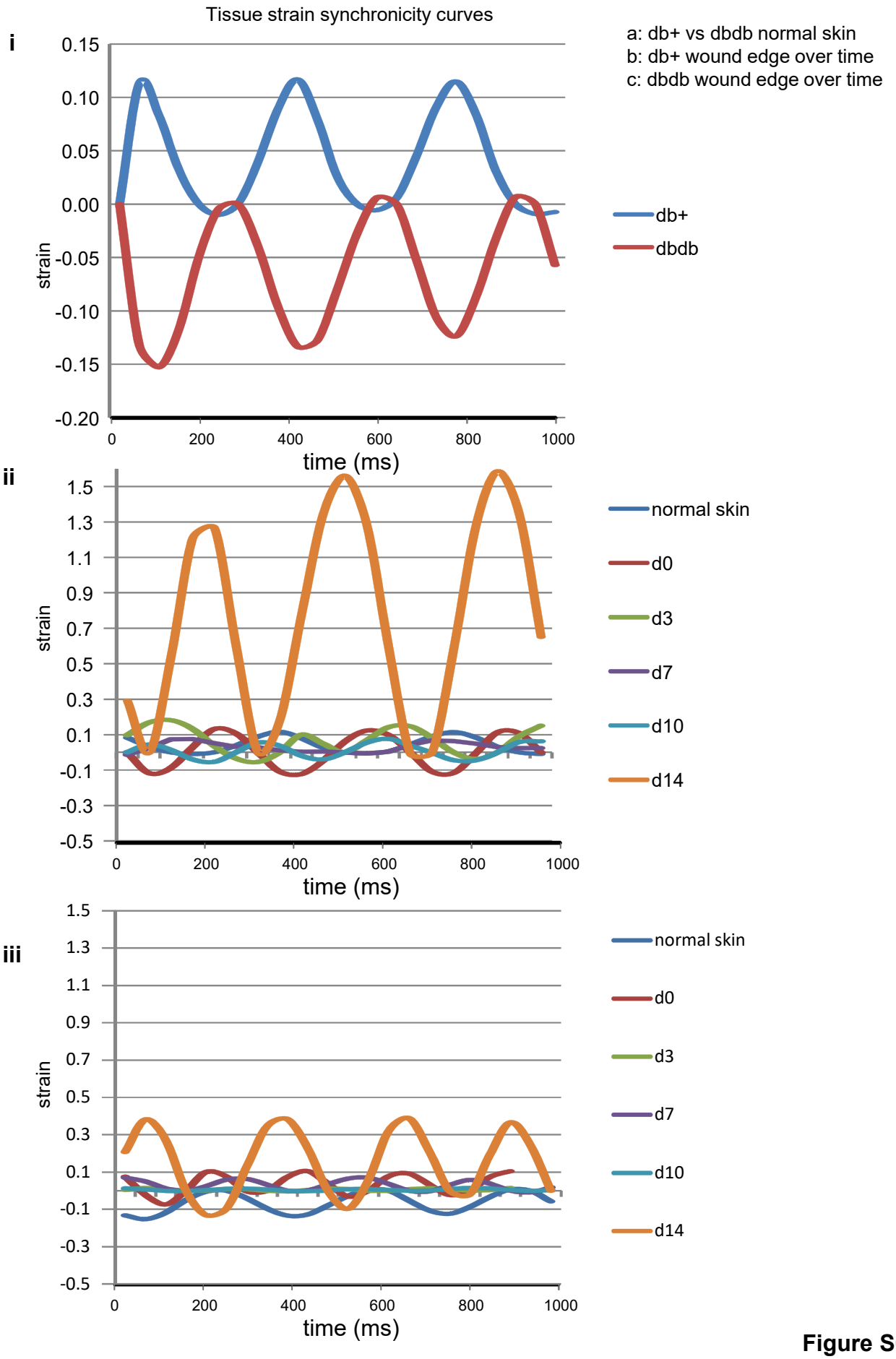

Tissue strain synchronicity curves
a: db+ vs dbdb normal skin
b: db+ wound edge over time
c: dbdb wound edge over time
i
strain
time (ms)
db+
dbdb
ii
normal skin
d0
d3
d7
d10
d14
iii
normal skin
d0
d3
d7
d10
d14

Figure S6b

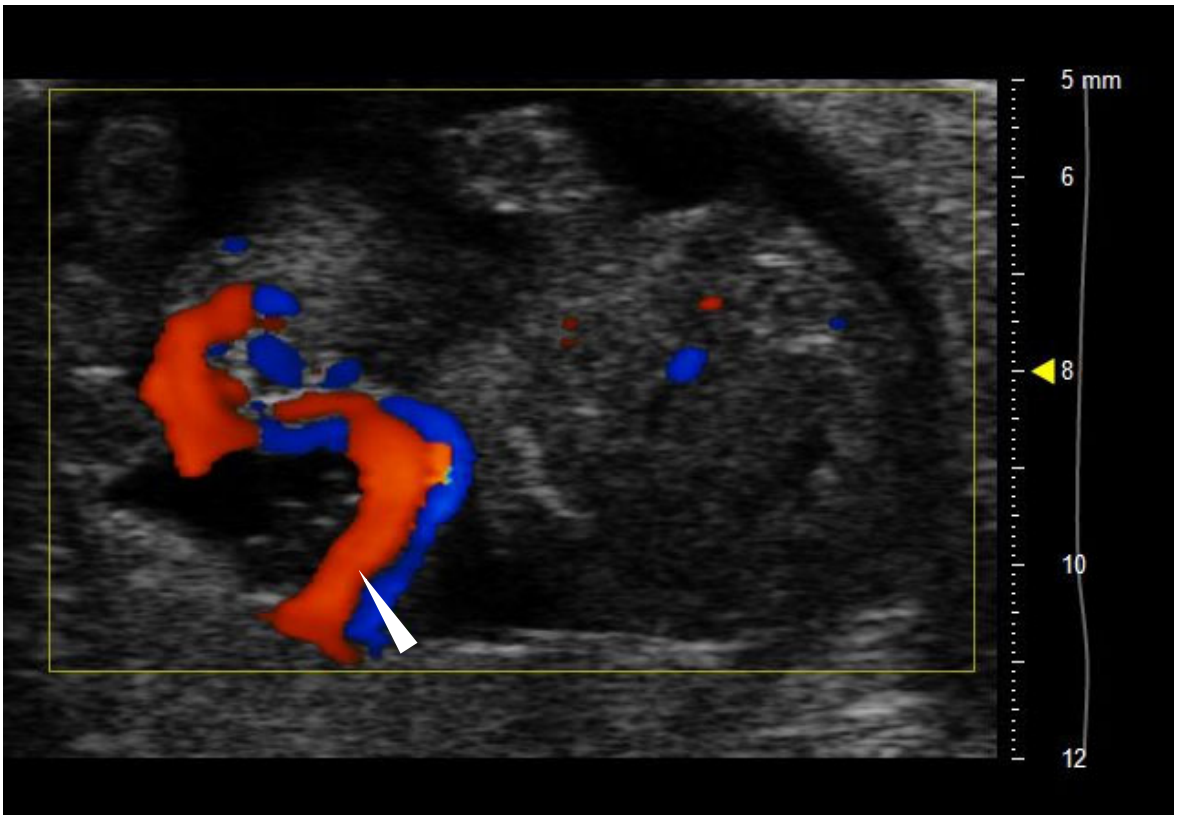


Figure S7