Supplementary Materials

Solutions and buffers

Phosphate buffered saline pH 7.3 containing 0.6% (w/v) bovine serum albumin (PBS/BSA)
100mM sodium phosphate buffer pH 7.4 (PB)
10mM sodium citrate buffer pH 5 (CB)
50mM Tris-HCl buffer, pH 7.6 (TB 7.6)
100mM Tris-HCl buffer, pH 8.4 (TB 8.4)
0.05% (w/v) 3,3'-diaminobenzidine + 1.5µl/ml 30% H_2O_2 in PB (DAB)
0.05% (w/v) 3,3'-diaminobenzidine + 1.5µl/ml 30% H_2O_2 + 5mM $NiCl_2$ in TB (DAB-Ni)

Primary antibodies

: cardiac troponin-T (anti-cTnT)	rabbit polyclonal, working dilution 1:500, Abcam ab45932
: von Willebrand Factor (anti-vWF)	mouse monoclonal (clone 2Q2134), working dilution 1:200, Abcam ab68545
: vimentin (anti-Vim)	mouse monoclonal (clone V9), working dilution 1:500, Dako M0725
: α-smooth muscle actin (anti-αSMA)	mouse monoclonal (clone 1A4), working dilution 1:500, Abcam ab7817

All antibody dilutions were in PBS/BSA.

Secondary antibodies

: goat anti-rabbit IgG - horseradish peroxidase conjugate (GARIgHRP) - Santa Cruz sc-2005
: goat anti mouse IgG - horseradish peroxidase conjugate (GAMIgHRP) - Santa Cruz sc-2004
: goat anti-mouse IgG - alkaline phosphatase conjugate (GAMIgAP) - Santa Cruz sc-2008
All secondary antibodies were used at a dilution of 1:150 (v/v) in PBS/BSA.

Other reagents

Gallyas's physical developer pH 5 [1]
Vector Red™ (Vector Laboratories, SK-5100)
0.1% (v/v) Nuclear Fast Red™ in 5% (w/v) $KAl(SO_4)_2$
0.1% (v/v) light green + 0.05% (w/v) methyl blue in saturated aqueous picric acid (picro-LGMB)

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LAD coronary artery occlusion and MI induction

Health adult female domestic pigs (*S. scrofa domesticus*; 40-60kg) were pre-treated with intravenous (IV) amiodarone (300 mg), atenolol (10 mg) and potassium (2g) in 500 ml saline per hr (total 1L) to maintain a heart rate of 60-70 beats / min and were then anaesthetised (ketamine (2g) / propofol (10mg/ml) total 600-800 mg), intubated and ventilated. The right carotid artery was surgically exposed with arterial access obtained with an 8F sheath. Coronary angiography was performed with a 5F Judkins right 4.0 guide catheter (Launcher, Medtronic). The left anterior descending (LAD) coronary artery was occluded between the second and third diagonal branches for 150 min using a 2.7 Fr 3.5 X 12 mm angioplasty balloon (Quantum Maverick, Boston Scientific) expanded to 6 - 8 atm, over a 0.014" (0.36 mm) BMW guide wire (Abbott). Unfractionated heparin was administered immediately following inflation of the balloon. ECG monitoring was performed throughout the balloon occlusion to confirm a transmural infarct. After 150 min of coronary occlusion, the balloon was deflated and catheters removed. Following extubation, the pigs were observed and monitored for 3 hours until ambulation was recovered. Sham-operated pigs, which were subjected to identical pre-surgical and surgical procedures, except for the step involving LAD coronary occlusion, were used as controls. The pigs were housed in the animal facility within separate pens with daily feeds for 6 weeks before follow-up EP study.

EP mapping and programmed electrical stimulation (PES)
Six weeks following MI, pigs were intubated, and anesthetised as above in the absence of any anti-arrhythmic drugs. After the administration of systemic intravenous heparin, a 3.5-mm-tip mapping catheter with 2-mm inter-electrode spacing (Thermocool, Biosense-Webster, Inc) was advanced into the LV retrograde via the left carotid artery. PES was performed from the LV apex and the LV outflow tract, using the Thermocool Biosense Webster catheter. All pacing was performed at twice the pacing threshold. Extra stimuli were delivered after eight ventricular drive beats (pacing cycle length 250, 300, and 350 ms). The first extra stimulus (S2) was initially set 200-220 ms after the last pacing stimulus of the drive train (S1). S2 was then delivered at progressively shorter coupling intervals, scanning in 10 ms steps until the effective refractory period (ERP) was reached. If no arrhythmias were observed, S2 was reset to a point 30 ms outside the ERP. A second extra stimulus (S3) was then added 170-200 ms after S2 and scanning in 10 ms decrements was repeated until S2 and S3 were both refractory or equal to 140 ms. Again, if no arrhythmias were induced, a third extra stimulus (S4) was similarly introduced. After reaching a coupling interval of 140 ms or refractoriness with all extra stimuli, burst pacing was performed by delivering 20 beats at a cycle length of 280 ms. If no arrhythmias were observed, the burst cycle length was progressively decreased in 10 ms steps until arrhythmia induction, 2:1 conduction, or a minimum cycle length of 200 ms was reached (the minimum for the stimulator).

Tissue processing and immunohistochemistry
Heart tissue was sliced into 5mm transverse sections from the apex upwards to include all 3 epicardial location markers (Figure 1). Tissue blocks were processed into paraffin wax and orientated for sectioning from the cranial surface. 4μm-thick sections were cut and collected onto Vectabond™-treated slides (Vector Laboratories, Peterborough, UK). For preliminary examination of tissue integrity, sections were stained for 5 min each in Nuclear Fast red and picro-LGMB.

Sections were subjected to antigen retrieval with CB for 30 mins at 95°C, washed thoroughly in water and equilibrated in PBS/BSA for 10 min. Sections were incubated with primary antibodies diluted in PBS/BSA (see Methods), for 1 h at room temperature (RT) followed by washing (3 x 1 min) in PBS/BSA and then a subsequent incubation step with the appropriate HRP-conjugated secondary antibody for 1 hr at RT.

Anti-cTnT immunostained sections were washed in PBS/BSA for 1 min; PB for 2 mins and incubated in DAB for 3min followed by rinsing in water.

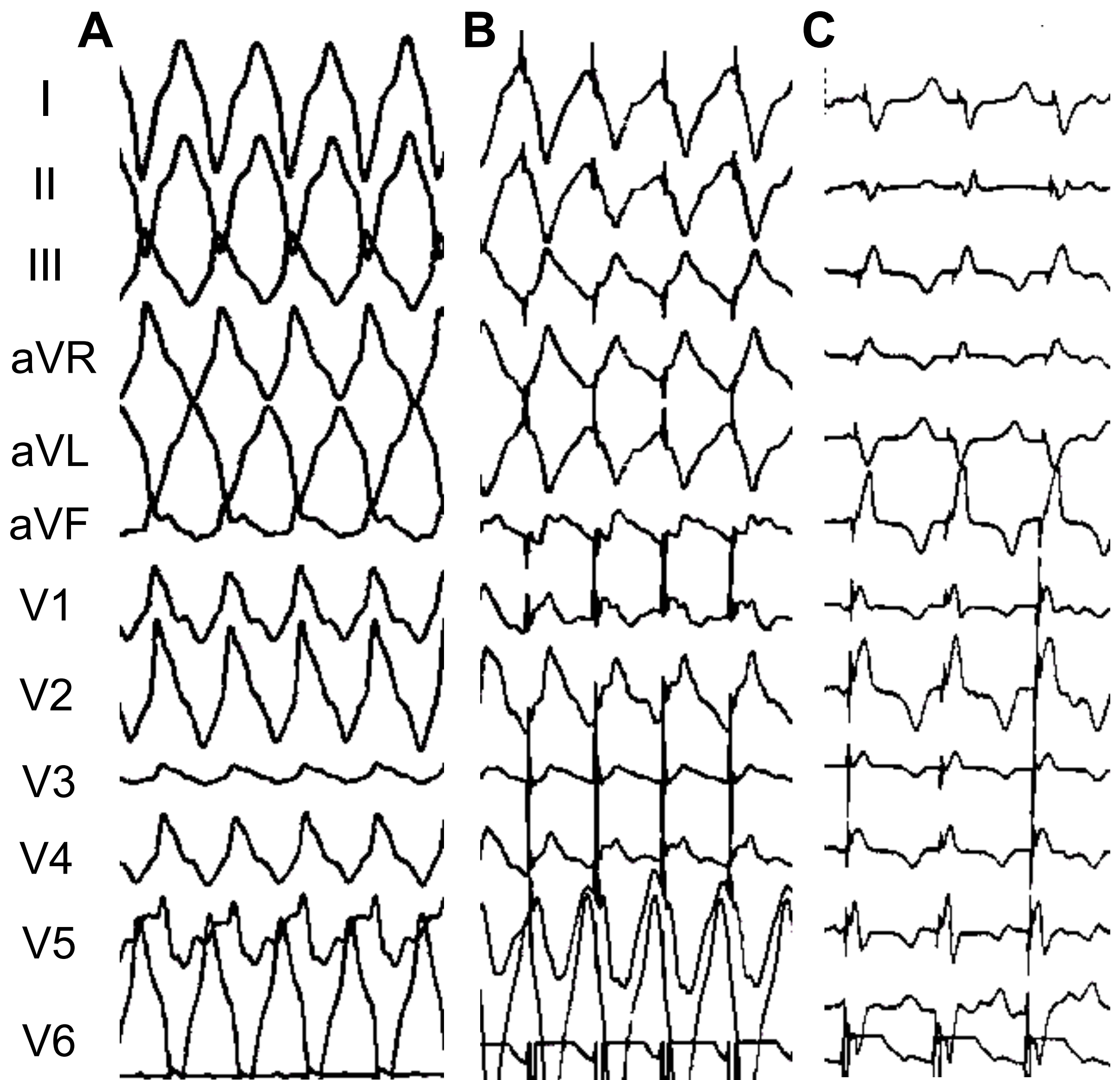
For double IHC staining, vWF- and Vim-immunostained sections were washed in PBS/BSA for 1 min; TB 7.6 for 2 mins and incubated in DAB-Ni for 3 mins. Following rinsing in water, Vim-stained sections were equilibrated in PBS/BSA for 10 mins whereas vWF-sections were intensified in Gallyas' developer for 3 mins prior to thorough rinsing in water and equilibrating for 10 min in PBS/BSA. Sections were subsequently incubated with anti-αSMA diluted as above in PBS/BSA, for 1 hr at RT prior to washing (3 x 1 min) in PBS/BSA, and then incubated in GAMIgAP for 1 h. Sections were washed in PBS/BSA for 1 min; TB 8.4 for 2 mins, developed with Vector Red™ for 10 min, and thoroughly rinsed with water. All sections were lightly counterstained with picro-LGMB for 10 s, dehydrated, cleared and mounted in Gurr's neutral mountant. Operators were blinded to the location of tissue sampling during IHC processing and analysis. Imaged was performed using an Olympus BX51 light microscope using a 20X objective and a Zeiss Axiocam digital camera.

References
1. Gallyas F. Light insensitive physical developers. Stain Technology 1979;54:173-75.

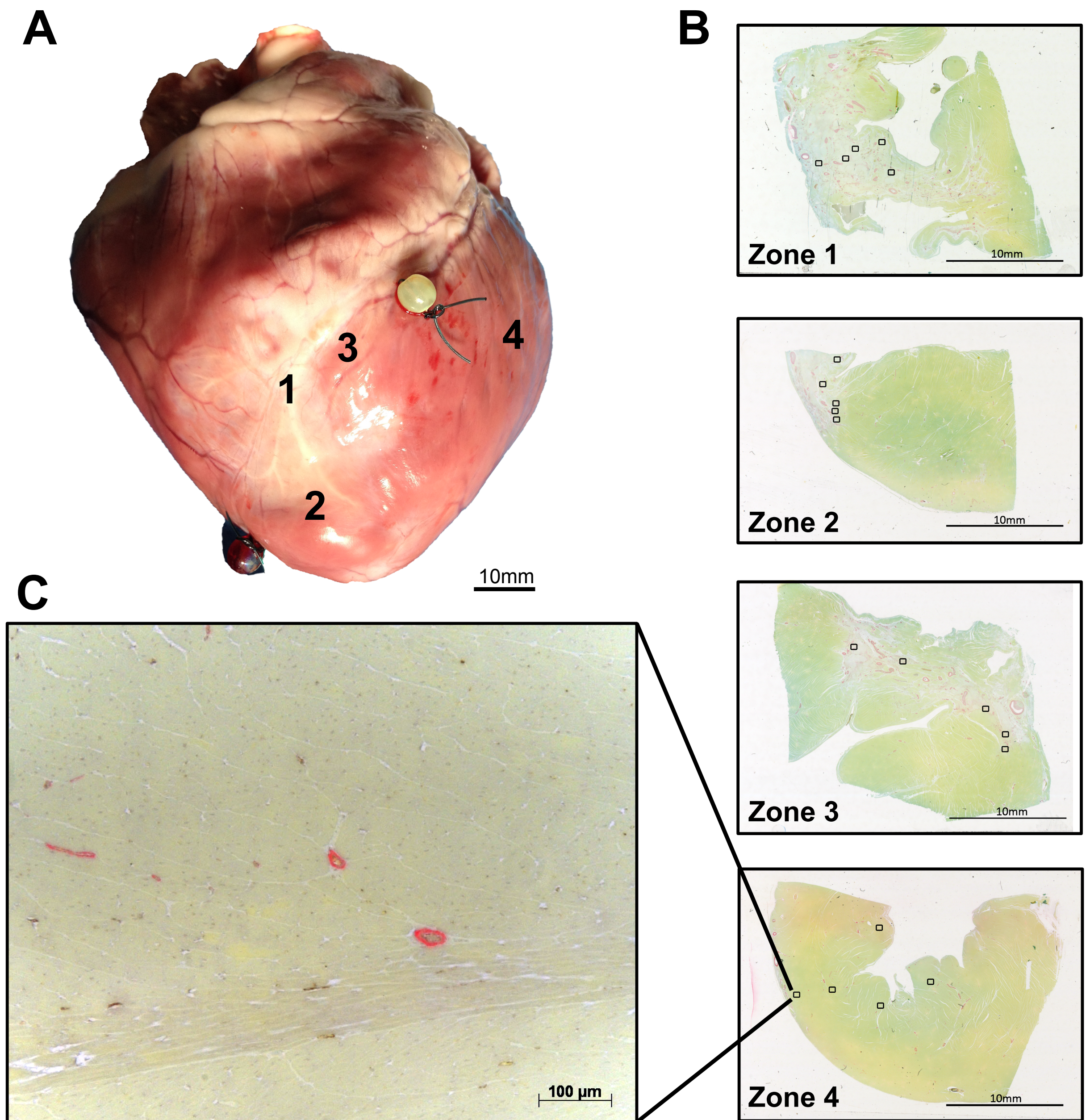
Online Table. Characterisation of post-MI myocardium

Pig no.	MI survival	CARTO points	VT induction	VT CL (ms)	VT exit point border location	VT exit point voltage (mV) (unipolar)	LV mass (g)	Voltage area bipolar < 2 mV (cm^2)	Voltage area bipolar < 1 mV (cm^2)	Border zone area (cm^2)	Border zone % of scar	Unipolar Low Voltage Area < 8.3 mV (cm^2)	Unipolar: Bipolar LVA Penumbra (cm^2)
1	No	-	POVF 22 min										
2	No	-	POVF 28 min										
3	No	-	POVF 33 min										
4	Yes	389	Yes	320	Infero-septal	0.79 mV (7.85 mV)	145	29.4	10.3	19.1	65 %	23.6	- 5.8
5	Yes	506	No	-	-	-	144	19.8	11.4	8.4	42 %	14.6	4.8
6	Yes	335	No	-	-	-	141	21.9	12.4	9.5	43 %	29.4	7.5
7	Yes	390	Yes *	480	Infero-septal	0.37 mV (6.04 mV)	162	19.4	6.8	12.6	65 %	32.6	13.2
8	Yes	397	Yes	288	Antero-apical	1.06 mV (10.72 mV)	155	26.7	10.4	16.3	61 %	Errors	-
9	Yes	330	Yes	224	Apical-septal	1.21 mV (3.04 mV)	138	26.2	9.2	17.0	66 %	28.9	2.7
10	Yes, POVF 30 min	298	No	-	-	-	148	16.5	10.5	6.0	36 %	2.2	-4.3
11	Yes	370	Yes	496	Antero-apical	0.70 mV (1.07 mV)	148	25.1	10.0	15.1	61 %	42.4	17.3
12	No	-	POVF 35 min		-	-	-	-	-	-	-		
13	Yes	572	No	-	-	-	137	24.6	15.1	9.5	39%	nil	-
14	Yes	544	No	-	-	-	151	31.0	17.7	13.3	43%	nil	-
15	Yes	467	No	-	-	-	155	49.9	29.5	20.4	41%	nil	-
16	Yes	481	Yes	290	Apical-septal	1.15 mV (12.74 mV)	121	31.1	10.5	20.6	66%	nil	-
17	Sham	-	-	-	-	-	-	-	-	-	-	-	-
18	Sham	-	-	-	-	-	-	-	-	-	-	-	-

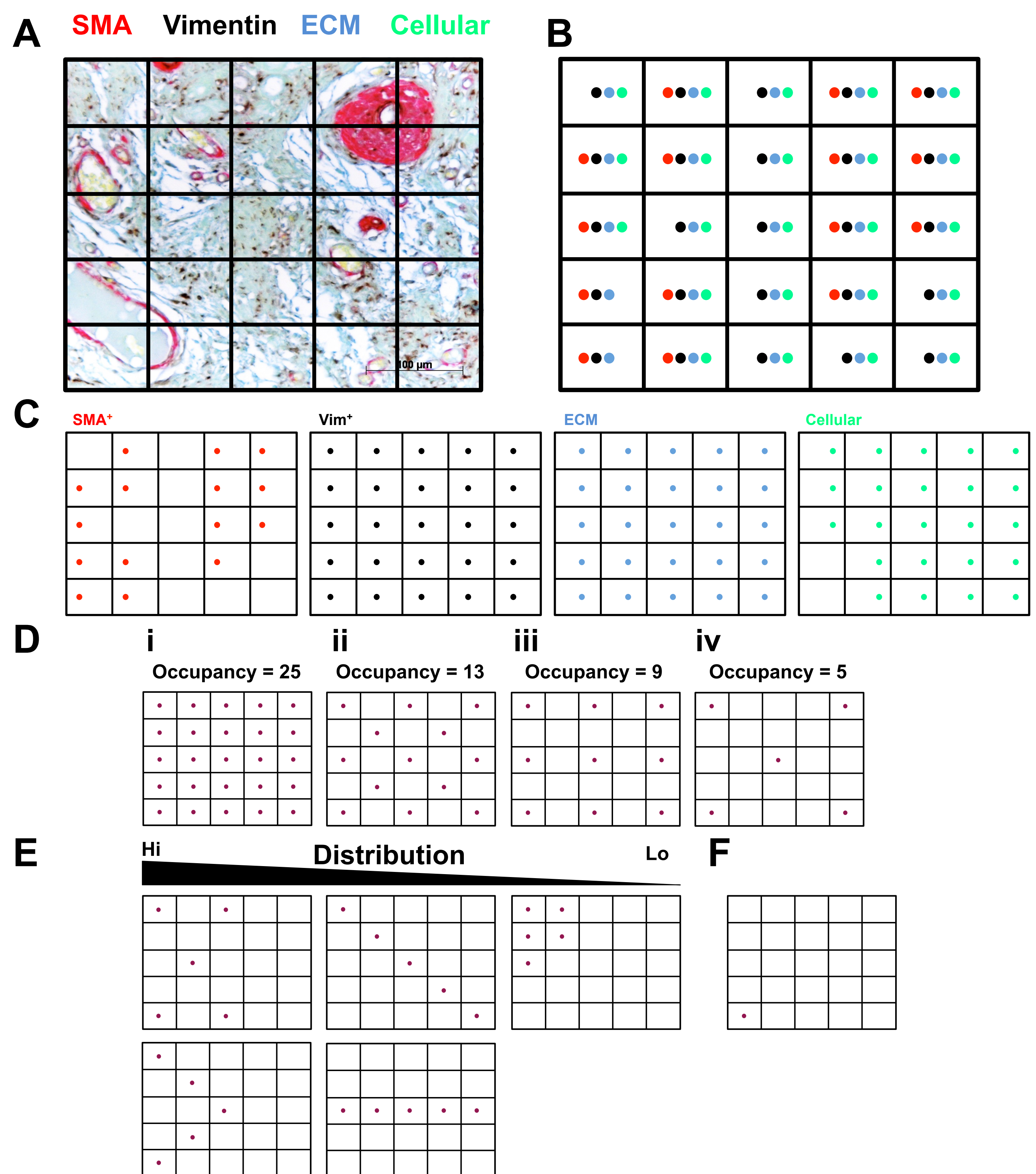
POVF= post-occlusion ventricular fibrillation, * mechanically-induced



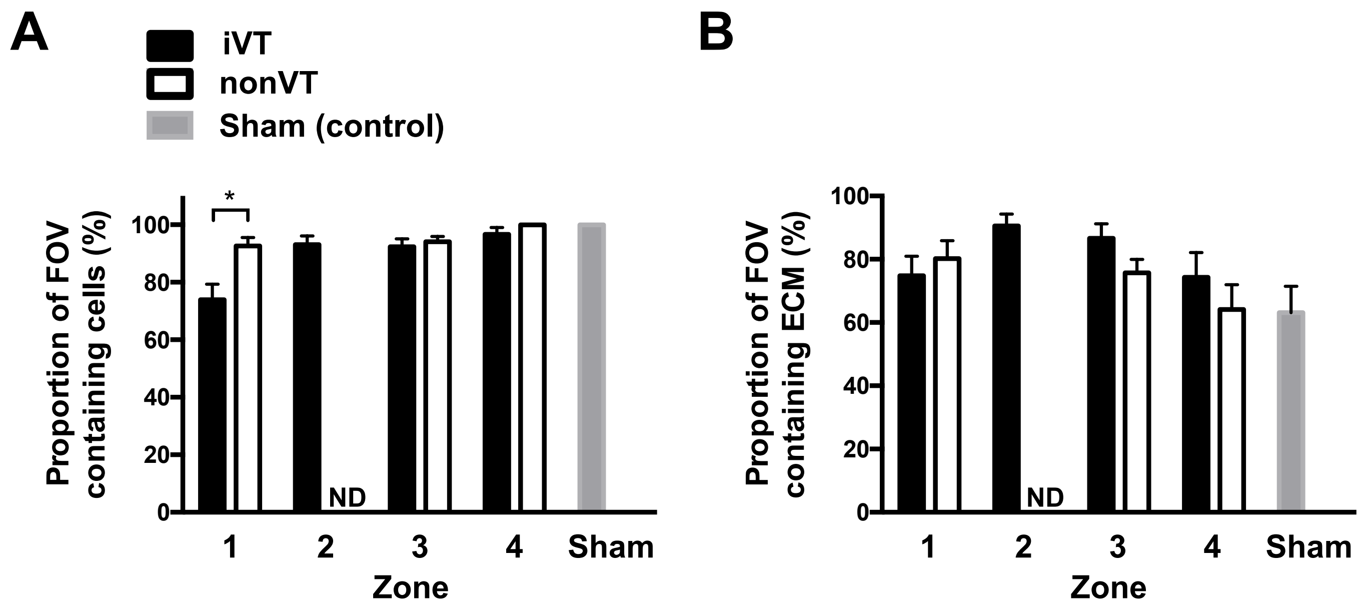
Online Figure 1. VT pace-mapping.
A representative example of induced VT (CL = 288 ms) **(A)** with a good pace map (matching 11/12 patterns) **(B)** compared with a poor pace map **(C)**.



Online Figure 2. Zonal sectioning and regional selection for IHC analysis. Zones 1-4 from pig hearts **(A)** that had been identified to electrophysiological catheter-based mapping (Supplementary Table) were sectioned into 4 µm-thick sections as described in Supplementary Methods **(B)**. Following IHC (see Supplementary Materials and Methods), five regions in each section were randomly selected (black boxes (0.6 mm^2), **(B)**) for detailed examination and imaging **(C)**. The representative sections in **(B)** and **(C)** were stained for cTNT and counterstained with LGMB as described in Supplementary Materials and Methods.

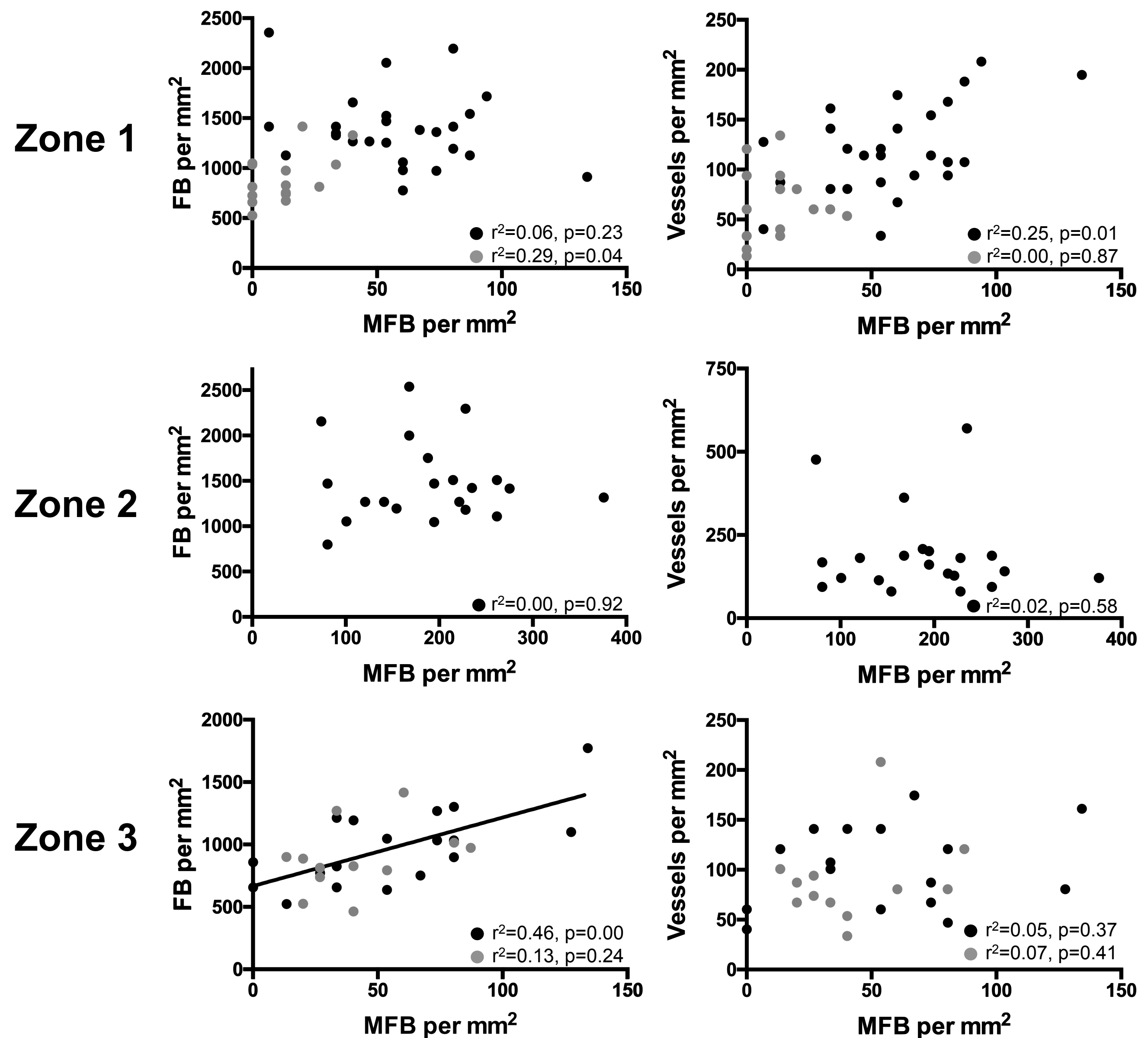


Online Figure 3. A system for calculating the spatial distribution of cells in zones 1 to 4. In this example, an IHC section stained for αSMA/Vim and LGMB is overlaid with a 5x5 grid **(A)**. In each box of the grid, signal intensities corresponding to αSMA (red), vimentin (black), ECM (blue) and cells (green) that exceed a threshold (i.e. typically 1.5 x background signal levels) are scored **(B).** Deconvolution of this information into component signals yields separate matrices **(C)**. The distribution of the signals within each matrix is calculated using a method based on the grid occupancy and is assigned a value between 1 (maximum) or 0 (minimum). In **(D)**, the maximal distribution of signal possible for different occupancies is described. If each of the 25 boxes contains a signal then the distribution is (by default) maximum and is assigned 1 **(i)**. For lower occupancy (e.g. matrix constructed from the occupancy of 13, 9 or 5 boxes (**ii-iv**, respectively)), maximum distributions can still be achieved if this occupancy is achieved via the configurations in panels **(ii)**, **(iii)** and **(iv)**. In **(E)**, an example of a signal occupancy of '5' is used to illustrate configurations that exhibit progressively reduced distributions (horizontal) and the scenarios in which different arrangements can give similar distributions (vertical). If all of the signals map to a single box of the grid, distribution is minimal and is assigned zero **(F)**.



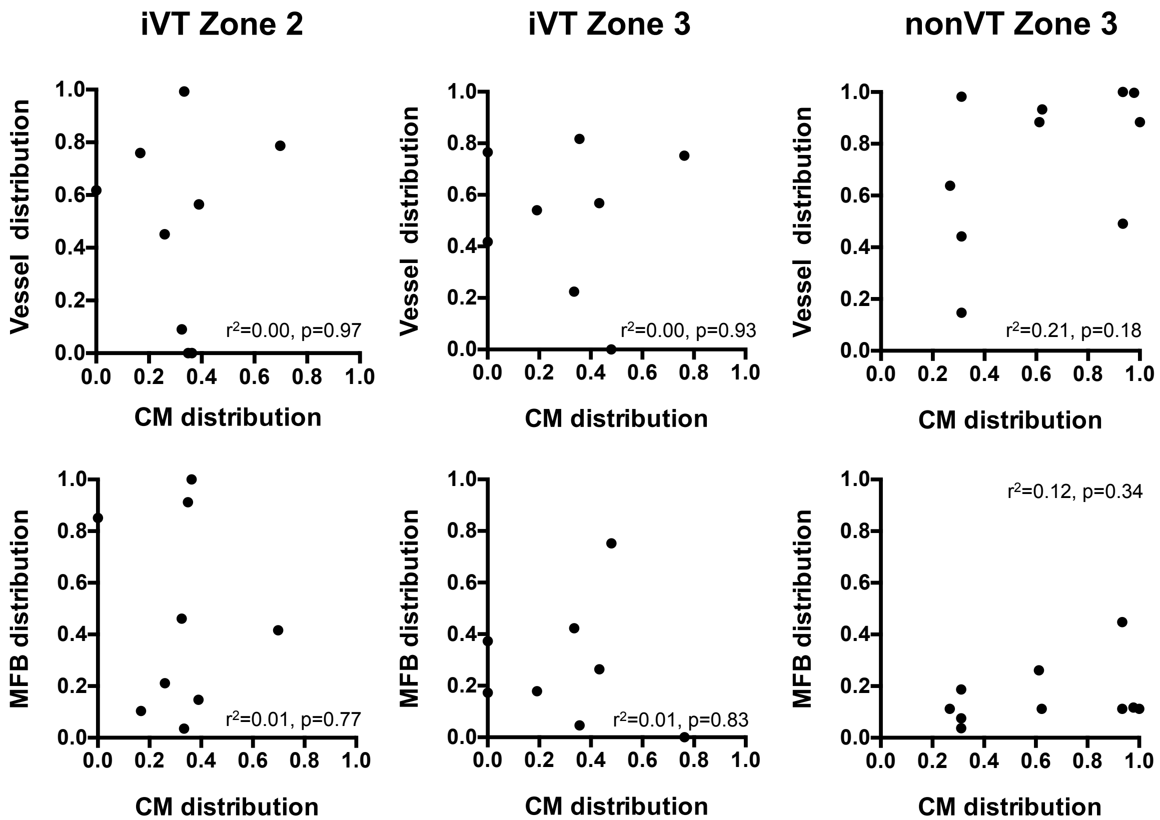
Online Figure 4. Assessing the cellularity and abundance of ECM in zones 1 to 4 in iVT and nonVT hearts.

The proportion of fields of view (FOV) imaged from zones 1 to 4 from iVT and nonVT hearts that were categorized as containing cells (i.e. light-green positive, methyl blue negative) **(A)** or with evidence of ECM and acellularity (light-green negative, methyl blue positive) **(B)** was calculated using a grid-based system as described in Supplementary Methods. For example, if an imaged FOV from a particular section had 25/25 regions of the grid evidencing cells and 20/25 regions with evidence of ECM, values of cellularity and ECM in that FOV would be 100% and 80% respectively. Data are given as mean ± SEM and are from the analysis of the following number of sections (iVT / nonVT): cellularity; zone 1 (39/36), zone 2 (54/ND), zone 3 (45/73), zone 4 (9/9), sham (9)) and ECM; zone 1 (50/42), zone 2 (61/ND), zone 3 (52/104), zone 4 (23/36), sham (23)). * indicates $p<0.05$.



Online Figure 5. Regression analysis of cellular composition of zones 1 to 4 in iVT and nonVT hearts.

Regression analysis was performed on the densities of MFB, FB and vessels in zones 1 to 3 in iVT (black circles) and nonVT hearts (grey circles) shown in Figure 3B. Zone 4 (normal myocardium) contained so few MFB that regression analysis involving MFB was not possible. Each point represents the analysis of a separate IHC section. Linear regression lines were only plotted where the Pearson r^2 coefficient > 0.3 and the p-value of the slope of the regression line < 0.05 i.e. our arbitrary threshold of correlation. No data from nonVT hearts conform to these criteria.



Online Figure 6. The spatial distribution of CMs within BZ of iVT and nonVT hearts does not correlate with the distribution of blood vessels or MFB.
Regression analysis was performed using individual data points that are plotted in histogram form in Figure 4C. As described in Supplementary Methods and Supplementary Figure 5, the spatial distribution of cells and vessels within BZ (zones 2 and 3) of iVT and nonVT hearts scales between zero (i.e. maximum clustering of cells/vessels in the minimum area) and 1 (i.e. maximum spatial distribution of cells/vessels throughout the entire area). A Pearson r^2 value > 0.3 and a p-value of the slope of the regression line < 0.05 was used as arbitrary threshold of correlation.