Supplementary method and materials

Procurement and preparation of the heart

The hearts were procured and prepared, as described in detail before (1, 2). Briefly, an intramuscular dose of ketamine (20 mg/kg) and atropine (0.05 mg/kg) was injected as premedication. Orotracheal intubation was established, and general anesthesia was maintained with isoflurane (1-2%). The heart was exposed via a standard median sternotomy. After intravenous delivery of heparin (1000 U/kg bolus) and Ringer's lactate solution (1000 mL), 850 ml of whole blood was retrieved through a two-stage venous cannula placed into the right atrium and was used to prime the ESHP circuit. The pigs were then euthanized by exsanguination. After a cross-clamp was placed across the ascending aorta, the hearts were arrested with 500 ml of reverse 4:1 blood cardioplegia (modified Del Nido) and were excised, weighed, immediately mounted on the custom ESHP apparatus and perfused for 12 hours.

Assessment of myocardial metabolism

Myocardial oxygen consumption ($MVO_2$) was measured as described previously (1).

Venoarterial lactate difference was calculated using the arterial and venous perfusate lactate concentrations measured with blood gas analysis (ABL 800 blood gas analyzer, Radiometer, ON, CA).

Myocardial injury

Myocardial edema

The weight gained by the heart over the 12-hour ESHP (edema formation) was measured and reported as a percentage of initial heart weight as described previously (1, 4).
**Troponin-I**

The perfusate concentration of cardiac troponin-I (adjusted with heart weight) was determined at T1, T5, and T11 using a porcine ELISA Kit (Life Diagnostics, US).

**Histological changes**

Histologic sections (6 µm thick) of the OCT-embedded myocardial tissue samples collected from the anterolateral wall of the LV in ex-situ perfused, and in-vivo hearts were stained with hematoxylin and eosin (H & E). All the histological assessments were performed by a single investigator (CS) who was blinded to the groups. Each slide was covered, and no labeling was associated with the slide at the time of the evaluation. The cardiomyocyte characteristics (nuclear number, size, area) was assessed using an operator-interactive, semi-automated method for quantification of the fiber data as previously reported (5-9). This method is at least equivalent to Cavalieri stereology (10, 11) with minimal differences but harbors more accuracy than the Cavalieri stereology. The parameters measured in the H & E-stained sections of LV were the variation of area, perimeter, and width of the nuclei as well as the variation of their angle, circularity, the Feret diameter (as the longest distance between any two points along the selection boundary), skewness, and kurtosis.

**Statistical analysis**

The normally-distributed data (assessed with the Shapiro-Wilk test and evaluation of the histogram plot of the data distributions) were reported as the mean ± the standard error of the mean. The non-normally-distributed continuous data were transformed to normality using the “two-step transformation to normality” as described before (12) and were reported as median [Interquartile Range (IQR)]. The data were compared between the groups using the independent samples t-test, or the analysis of variance (one way-ANOVA), followed by the Fisher’s Least Significant Difference (LSD) or Games-Howell post hoc tests where appropriate. The Welch correction was applied when the assumption of equal variances was not met. The overtime trend
of changes within each group was assessed using a linear regression model. A 2-sided p-value < 0.05 was considered statistically significant. The analysis was performed with the IBM SPSS statistics software (Version 21.0).

**Supplementary Table 1.** Basic characteristics of perfusion and animal weight

Data are presented as mean±SD. NWM-S, non-working mode perfusion in supported position; NWM-H, non-working mode perfusion in hanging position; WM-S, working mode perfusion in supported position; WM-H, working mode perfusion in hanging position.
References: