Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Meybohm P, Bein B, Brosteanu O, et al. A multicenter trial of remote ischemic preconditioning for heart surgery. N Engl J Med. DOI: 10.1056/NEJMoa1413579

Supplementary Appendix

Remote Ischemic Preconditioning for Heart Surgery (RIPHeart-Study)

Corresponding author: Prof. Dr. Patrick Meybohm, MD, MHBA**;** Department of Anaesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt**,** Theodor-Stern-Kai 7**,** 60590 Frankfurt am Main Germany, Email: patrick.meybohm@kgu.de

Table of contents

*RIPHeart-Study Collaborators

Aachen (Department of Anaesthesiology, University Hospital Aachen, Aachen, Germany): Ana Stevanovic, Rolf Rossaint, Marc Felzen, (Department of Thoracic and Cardiovascular Surgery): Andreas Goetzenich; 195 patients;
Berlin (Department of Anaesthesiology and Intensive Care Medicine, Charité-Universitätsmedizin Berlin, Campus Charité Mitte, Berlin, Germany): Tobias Moormann, Katharina Chalk; 37 patients;
Bonn (Department of Anaesthesiology and Intensive Care Medicine, University Hospital Bonn, Bonn, Germany): Pascal Knuefermann, Olaf Boehm, Andreas Hoeft; 73 patients;
Duesseldorf (Department of Anaesthesiology and Intensive Care Medicine, University Hospital Duesseldorf, Germany): Michael Winterhalter; 65 patients;
Frankfurt am Main (Department of Anaesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt, Frankfurt am Main, Germany): Sonja Iken, Christian Weber, Carolin Wiedenbeck, Gerhard Schwarzmann, Karin Pense, (Department of Thoracic and Cardiovascular Surgery): Andreas Zierer, (Internal Medicine III: Cardiology, Angiology, Nephrology): Stephan Fichtlscherer; 117 patients;
Giessen (Department of Cardiovascular Surgery, University of Giessen, Germany): Gerold Goerlach, Matthias Wollbrueck, Ursula Boening; (Department of Anesthesiology): Markus Weigand; 148 patients;
Goettingen (Department of Anaesthesiology and Intensive Care Medicine, University Hospital Goettingen, Germany): Julia Strauchmann, Konrad August; 91 patients;
Jena (Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany): Kai U. Morsbach, Markus Paxian, Konrad Reinhard; 76 patients;
Kiel (Department of Anaesthesiology and Intensive Care Medicine, University Hospital Schleswig-Holstein, Campus Kiel, Germany): Jens Scholz, Jochen Renner, Ole Broch, Helga Francksen, Martin Albrecht, Bernd Kuhr; 237 patients;

Luebeck (Department of Anaesthesiology, University Hospital Luebeck, Luebeck, Germany): Hermann Heinze, Hauke Paarmann; (Department of Cardiac and Thoracic Vascular Surgery): Hans-Hinrich Sievers, Stefan Klotz; 56 patients;
Magdeburg (Department of Anaesthesiology, University Hospital Magdeburg, Germany); Thomas Hachenberg; 14 patients;
Mainz (Department of Anesthesiology, Medical Center of Johannes Gutenberg-University, Mainz, Germany): Christian Werner, Susanne Mauff; 116 patients;
Rostock (Clinic of Anaesthesiology and Intensive Care Medicine, University Hospital Rostock, Rostock, Germany): Angela Alms, Stefan Bergt; 146 patients;
Wuerzburg (Department of Anaesthesiology, University Hospital Wuerzburg, Wuerzburg, Germany): Norbert Roewer; 32 patients.

Role of Participating Investigators

Design of the Study
Patrick Meybohm, Berthold Bein, Jochen Cremer, Dirk Hasenclever, Oana Brosteanu, Kai Zacharowski
Writing Committee
Patrick Meybohm (principal investigator and chair), Dirk Hasenclever, Oana Brosteanu, Kai Zacharowski. No medical writer was involved. The writing committee decided to publish the paper. All Co-authors have approved submission.
Gathering of the Data
Patrick Meybohm, Berthold Bein, Jochen Cremer, Matthias Gruenewald, Christian Stoppe, Mark Coburn, Gereon Schaelte, Andreas Boening, Bernd Niemann, Jan Roesner, Frank Kletzin, Ulrich Strouhal, Christian Reyher, Rita Laufenberg-Feldmann, Marion Ferner, Ivo F. Brandes, Martin Bauer, Sebastian N. Stehr, Andreas Kortgen, Maria Wittmann, Georg Baumgarten, Tanja Meyer-Treschan, Peter Kienbaum, Matthias Heringlake, Julika Schoen, Michael Sander, Sascha Treskatsch, Thorsten Smul, Ewa Wolwender, Thomas Schilling, Kai Zacharowski, and the RIPHeart-Study Collaborators*
Study Statistician (Together with Clinical Trial Centre Leipzig Responsible for Data Analysis)
Dirk Hasenclever (Institute for Medical Informatics, Statistics and Epidemiology, University Leipzig, Germany).
Steering Committee
Patrick Meybohm, Dirk Hasenclever, Kai Zacharowski.
Clinical Endpoint Committee
Holger Thiele, Georg Fuernau (Department of Cardiology, University of Leipzig – Heart Center, Leipzig, Germany).
Clinical Monitoring, Project and Data Management
Holger Bogatsch, Oana Brosteanu, Matthias Collier, Madlen Doerschmann Manuela Engelmann, Silke Hauer, Marlen Heinke, Daniela Hesse, Tina Hoelscher, Thomas Junge, Daniela Krueger, Kathrin Scheibe, Vera Schleicher, Bianca Scholze (Clinical Trial Centre, University Leipzig, Germany).
Independent Data Monitoring and Safety Committee
Andreas Zierer (Department of Thoracic and Cardiovascular Surgery, University Hospital Frankfurt), Stephan Fichtlscherer (Internal Medicine III: Cardiology, Angiology, Nephrology, University Hospital Frankfurt)
Sponsor
University Hospital Schleswig-Holstein, Campus Kiel, Germany. There was no agreement concerning confidentiality of the data between the sponsor and the authors or the institutions.

Additional Methods for Eligibility Criteria

Inclusion Criteria
Patients (age ≥ 18 years, American Society of Anesthesiologists status 2-3) after written informed consent scheduled for all types of elective cardio-vascular surgery in which cardiopulmonary bypass was used.

Exclusion Criteria
1) Surgery related criteria (off-pump heart surgery, concomitant carotid surgery, minimal-invasive surgery, selective antegrade cerebral perfusion, previous heart surgery, aorta descendent surgery, emergency/urgent surgery),
2) Cardiac conditions (myocardial infarction up to 7 days, ejection fraction less than 30%, previous atrial fibrillation up to 6 months, drug therapy with antiarrhythmic agents, implanted pacemaker or defibrillator, instable angina pectoris (e.g. defined as elevated troponin T ≥ 0.05 µg/l up to 24 h)),
3) Others (stroke up to 2 months, severe renal failure, severe liver failure, severe alcohol abuse, severe chronic obstructive pulmonary disease, drug therapy with sulfonylureas and nicorandil (preconditioning-blocking and preconditioning-mimetic medication, respectively), acute infection with antibiotic therapy, severe peripheral artery occlusive disease (Fontaine stages 3 and 4), previous serious

neurological illness (e.g. Parkinson's disease, multiple sclerosis, epilepsy, Alzheimer's disease, preoperative delirium, use of psychiatric drugs), arteriovenous fistula or lymphedema at upper limbs, heparin-induced thrombocytopenia Type II, inclusion in other studies, and/or language problems barriers).

Additional Methods for Anesthesia and Management of Cardiopulmonary Bypass

Total intravenous anesthesia was performed in all patients. Volatile anesthetic agents were not allowed because they have been shown to induce preconditioning-like effects thereby reducing myocardial ischemia.[1,2] As only 7 out of 14 recruiting centers were able to run a vaporizer attached to the cardiopulmonary bypass circuit to administer volatile anesthetics, we used a total intravenous anesthesia technique. According to a recent review,[3] we standardized management of cardiopulmonary bypass as follows: use of non-pulsatile cardiopulmonary bypass, mean arterial blood pressure of 60–70 mmHg, hematocrit values 25-30%, α-stat acid-base management to regulate carbon dioxide tension, use of arterial line filters, and blood glucose levels < 200 mg/dl.

Additional Methods for Randomization

Patients were randomized to group RIPC or sham-RIPC. Randomization was performed centrally by the Clinical Trial Centre Leipzig. Randomization was stratified for i) centers and ii) individual risk for perioperative mortality using the European System for Cardiac Operative Risk Evaluation (EuroSCORE).[4] We used a cut-off value of higher than 5% for a 'Predicted mortality by logistic EuroSCORE' to stratify randomization of so-called 'high-risk patients'.[5] An online checklist for individual risk factors was used to calculate the logistic EuroSCORE at the time of registration. Randomization was performed by the minimization method described in Pocock et al.[6] including a random component. The trial statistician prepared the allocation algorithm, which was implemented at the Clinical Trial Centre. After written informed consent the Data Management (Clinical Trial Centre Leipzig) was contacted via an internet-based randomization tool.

Additional Methods for Outcome Measures

The primary endpoint was a composite (including all-cause mortality, non-fatal myocardial infarction, any new stroke, and/or acute renal failure) until hospital discharge (within a maximum of 14 days when length of hospital stay was longer).

I. All-cause mortality.
II. According to the ESC/ACC/AHA/WHF Task Force for the Redefinition of Myocardial Infarction,[7] non-fatal myocardial infarction was considered according to a type V myocardial infarction as follows: biomarker values more than five times the 99th percentile of the normal reference range combined with
    a. new pathological Q-waves within the first 72 h,
    b. new left bundle branch block within the first 72 h,
    c. standard clinical criteria for myocardial infarction from 72 h on,
    d. new ischemic finding by echocardiography/angiography, or
    e. myocardial infarction diagnosed at autopsy.

    A blinded clinical endpoint committee assessed all available electrocardiograms for reference reading.
III. Stroke was defined by
    a. any new, temporary or permanent, focal or global neurological deficit, or
    b. evidence of stroke on autopsy,

    and was evaluated according to the NIH stroke scale[8] (≥ 4 points) documented preoperatively and at hospital discharge (within a maximum of 14 days when length of hospital stay was longer).
IV. Acute renal failure was defined as
    a. any serum creatinine greater than or equal to two-fold increase from baseline to any time until hospital discharge or day 14,
    b. urine output ≤0.5 mL/kg/h for 12 h any time until hospital discharge (within a maximum of 14 days when length of hospital stay was longer) (RIFLE injury),[9]
    c. use of renal replacement therapy, or
    d. evidence of renal failure on autopsy.

Secondary Endpoints

- The occurrence of any individual component of the composite at 30 and 90 days after surgery (phone interview). As the last patient has been randomized in May 2014, follow up 12 months after surgery is not shown in this manuscript.
- Duration of mechanical ventilation, length of stay on the intensive care unit and in hospital.
- Troponin T/I (dependent on local standard) (preoperative, 6, 12, 24, and 48 h after surgery)
- Creatinine (preoperative, 24h and 48 postoperative as well as maximum creatinine). Modified RIFLE criteria were used to specify acute kidney injury.[10]

- Vasopressor and inotropic support.
- New onset of atrial fibrillation (within 4 days after surgery).
- Incidence of postoperative delirium was assessed with the CAM-ICU score[11] (preoperative, 24, 48, 72, and 96 h after surgery).

Additional Methods for Monitoring Quality

All study procedures, including development of the protocol, case report form and investigator site file, content of patient information and consent, application for ethics approval, data processing, central and on-site monitoring, and evaluation followed the Standard Operating Procedures (SOP) of the Clinical Trial Centre Leipzig (ZKS Leipzig).

The ZKS Leipzig was responsible for trial monitoring. Pre-study, initiation and regular interim visits were performed in all centers. A risk-based monitoring strategy was implemented, using the risk-based approach proposed by the ADAMON project group.[12] During trial conduct, central and statistical monitoring procedures were combined with on-site monitoring visits in order to achieve high protocol a and data quality, as well as to ensure patients' safety and rights. A first monitoring visit at a center was scheduled about 4 weeks after the inclusion of the site's first 3 – 4 patients, checking protocol adherence and preventing further systematic errors due to misunderstandings. All trial sites were then visited regularly. The frequency of further monitoring visits depended on the trial site's recruitment rate and on whether problems had been detected with the site, either in prior on-site visits or by central monitoring. Prior to every scheduled on-site visit, the trial statistician provided the monitor with patient synopses summarising the data already available in the database, and indicating possible protocol deviations or inconsistencies. During the visits, the monitor

- Checked informed consent forms of all patients enrolled.
- Performed source data verification on the documentation of the primary endpoint in all patients.
- Performed targeted source data verification for patients where the synopsis indicated possible derivations.
- Performed source data verification on further key data (eligibility criteria, surgery data, adverse events) in an additional random sample of 20 - 50% of the site´s patients.
- Discussed open queries raised by data management.
- Checked and updated the investigator site file.

Rationale for Initial Sample Size Calculation

Assumed event rates in the control group were based on the German data by the *"Bundesgeschäftsstelle für Qualitätssicherung GmbH"* (www.bqs-outcome.de/2007/ergebnisse/leistungsbereiche ; assessed 3rd May 2009) for all-cause mortality (ranging from 3.2 to 6.1%), any new stroke (1.3 to 2.3%), and acute renal failure (3.8 to 6.9%) depending on the type of surgery. Assuming a case-mix in the registry (72% isolated coronary artery graft surgery, 17% isolated aortic valve replacement, and 12% combined operations), we calculated a weighted mean of 3.7% for all-cause mortality, 1.5% for any new stroke, and 4.2% for acute renal failure. Non-fatal myocardial infarction event rate was not reported in the above data registry. A compilation of relevant studies that reported myocardial infarction event rates from 2.4 to 5.1,[2] 2.8 to 5.4,[13] 3.9 to 4.2,[14] and 6.2 to 7.9[15] resulted in a median rate of 4.7%. The sum of the four components for the composite endpoint was 14.1%. Considering the fact that a small proportion of patients dying after stroke or myocardial infarction would counted more than once in these figures, and that study populations would tended to be at lower risk than unselected patient populations, we conservatively assumed a baseline rate of 12% for the primary endpoint in the sham-RIPC group. Pharmacological studies in the field of cardiac surgery reported risk reductions ranging from 25 to 70%.[13,16-18] As a cheap, simple and safe intervention was investigated in our study, we targeted at 33% risk reduction to an event rate of 8% in the RIPC group.

Group sample sizes of 931 and 931 would achieve 80% power to detect a difference of 4% using a two-sided Chi-square test with continuity correction and with a significance level of 0.05.

We used a commercial available statistic software (NCSS 2007 and Power Analysis and Sample Size 2008; Kaysville, Utah, USA) for sample size and power calculation. To account for 10% follow up loss, 104 patients per group have been additionally calculated, resulting in a total of 1.035 patients per group.

Additional Results - ECG Reference Reading

A blinded clinical endpoint committee assessed all available electrocardiograms (ECG) for reference reading. Assessment was based on criteria published by Thygesen et al.[7]. The protocol required ECGs at baseline, 24h and 72h. All available ECGs of a patient were reviewed as a sequence by one reviewer. If additional ECGs were available these were also reviewed. The reference reader only documented presence of a Q-wave or a left bundle branch block for each individual ECG. The whole ECG sequence of the patient was resubmitted for a second review if in any ECG at any time point a first review assessment differed from the assessment of the local

investigator or there was a positive reference finding and the local assessment was missing. If the first reference reader did not detect an abnormality and the local assessment was missing, no second reading was performed. The second reader was aware of reviewing discrepant cases but was not informed about what the discrepancy was. The endpoint was determined based on the last reference assessment.
The algorithm to compute presence of a new abnormality had to deal with occasional transient abnormalities, with rare missing or not evaluable ECGs and with abnormalities already present at baseline. An abnormality counted for the composite primary endpoint if the abnormality was confirmed on the last available post surgery ECG (i.e. transient abnormalities seen at 24h, but not at 72h were ignored, but if the 72h ECG was missing or not evaluable, the 24h ECG was used). The abnormality was considered new unless the abnormality was positively confirmed to have been present at baseline. In cases of missing or not evaluable baseline ECGs and no further information it was assumed that no abnormality was present at baseline.
Abnormalities at baseline were reported in 16% of the patients.
In 97/1,385 (7%) patients no ECG at all or only a baseline ECG, but no postoperative ECG, was available for review despite multiple queries and training by monitors. In these patients, the local assessment concerning myocardial infarction yes/no was used.
Referees did not confirm the diagnosis of myocardial infarction in 3 patients, but found either new pathological Q-waves or new left bundle branch block in 76 patients. A sensitivity analysis re-running analysis of the primary endpoint disregarding the ECG review results leads to a lower overall PE rate but confirms the lack of treatment benefit (RIPC 9.7% versus sham-RIPC 8.7%; p-value=0.52).

Adherence to the Trial Intervention

Adherence to the trial intervention (RIPC / sham-RIPC) was assessed by the following variables:

- Intervention done (yes/no) as recorded on the case report form (CRF)
- Intervention as randomized (yes/no) as recorded on the CRF
- Blinding successful (yes/no) as recorded on the CRF
- Applied pressure according to protocol (yes/no). The patient's blood pressure before start of intervention as well as the applied pressure for each intervention cycle was recorded on the CRF. The indicator variable was defined as "yes" if the applied pressure was protocol conform for all intervention cycles.
- Number of intervention cycles. Each intervention cycle was documented with start and end time. The number of cycles for which a start time was recorded was calculated.
- Total intervention time ok (yes/no). As per protocol, every inflation cycles and every pause should have had a duration of 5 minutes each. We tolerated a deviation of ± 2 minutes per cycle or pause. The indicator variable "Total intervention time ok" was defined as "no", if in at least one inflation cycle or pause the duration was outside the tolerated interval.

Table S1. Performance of Intervention.

Variable	RIPC	Sham-RIPC
Intervention according to protocol — no./total no. (%)		
Yes	623/692 (90.0)	620/693 (89.5)
No	69/692 (10.0)	73/693 (10.5)
Time from first RIPC/ sham-RIPC cycle to cardiopulmonary bypass [minutes]	92 ± 37 / 672	94 ± 44 / 673
Subitems		
Intervention — no./total no. (%)		
Done	679/692 (98.1)	680/693 (98.1)
Not done (confirmed on CRF)	13/692 (1.9)	13/693 (1.9)
Intervention as randomized — no./total no. (%)		
Yes	678/679 (99.9)	679/680 (99.9)
No	1/679 (0.1)	1/680 (0.1)
Blinding successful — no./total no. (%)		
Yes	673/678 (99.3)	674/679 (99.3)
No	5/678 (0.7)	5/679 (0.7)
Target cuff inflation pressure OK — no./total no. (%)		
Yes	641/675 (95.0)	634/674 (94.1)
Too high	5/675 (0.7)	8/674 (1.2)
Too low	29/675 (4.3)	32/674 (4.7)
Number of inflation cycles — no./total no. (%)		
Four	675/676 (99.9)	675/675 (100.0)
Three	1/676 (0.1)	0/675 (0.0)
Total intervention time OK — no./total no. (%)		
Yes	652/676 (96.4)	651/675 (96.4)
No	24/676 (3.6)	24/675 (3.6)

Plus–minus values are means ± SD.

Intervention was accurately performed according to protocol in 1,243 patients.

Main reasons for inadequate performance were too low cuff pressure (N=61), inadequate duration of intervention (N=48), and missing intervention (N=26), respectively. This table refers to the patients included in the primary analysis (N=1,385). In addition, in 14 of the 18 patients excluded from the primary analysis, the intervention has not been done (see also Figure 1 in the main paper).

CRF denotes case report form, RIPC remote ischemic preconditioning.

Table S2. Intraoperative Data.

Variable	RIPC	Sham-RIPC
Type of surgery performed — no./total no. (%)		
Coronary artery bypass graft (alone)	313/692 (45.2)	317/693 (45.7)
Aortic valve replacement/ reconstruction (alone)	137/692 (19.8)	137/693 (19.8)
Mitral valve replacement/ reconstruction (alone)	19/692 (2.7)	24/693 (3.5)
Aorta ascendens replacement (alone)	18/692 (2.6)	22/693 (3.2)
Combined procedures	195/692 (28.2)	182/693 (26.3)
Other type of surgery*	10/692 (1.4)	11/693 (1.6)
Time of procedures — minutes / total no.		
Duration of cardiopulmonary bypass	115.1 ± 50.4 / 687	114.9 ± 50.1 / 690
Duration of aortic cross clamping	77.7 ± 34.8 / 683	76.7 ± 33.7 / 690
Duration of circulatory arrest	16.0 ± 10.4 / 34	16.2 ± 5.8 / 29
CPB management — no. (%) // total no.		
Priming (crystalloid/ mannitol/ colloid†/ albumin)	676 (97.7)/ 496 (71.7)/ 268 (38.7)/ 57 (8.2) // 692	685 (98.8)/ 506 (73.0)/ 275 (39.7)/ 67 (9.7) //693
Cardioplegic solution - type (Blood Buckberg/ Bretschneider / Calafiore)	272 (39.8)/ 215 (31.4)/ 197 (28.8) // 684	286 (41.5)/ 209 (30.3)/ 194 (28.2) // 689
Cardioplegic solution - temperature (Warm (32-37°C)/ Cold (4-10°C))	201 (29.4)/ 482 (70.6) // 683	193 (28.1)/ 495 (71.9) // 688
Anesthesia		
Type of anesthesia induction — no. (%)// total no.		
Propofol/ midazolam/ etomidate/ (S)-ketamine	537 (77.7)/ 184 (26.6)/ 87 (12.6)/ 0 (0.0) // 691	537 (77.5)/ 186 (26.8)/ 77 (11.1)/ 1 (0.1) // 693
Sufentanil/ remifentanil	686 (99.3)/ 1 (0.1) // 691	691 (99.7)/ 1 (0.1) // 693
Type of anesthesia maintenance — no. (%)// total no.		
Propofol/ midazolam/ volatile agents	676 (97.8)/ 84 (12.2)/ 22 (3.2) // 691	682 (98.6)/ 95 (13.7)/ 16 (2.3) // 692
Sufentanil/ remifentanil	618 (89.4)/ 153 (22.1) // 691	630 (91.0)/ 159 (23.0) // 692
Lowest hematocrit value — % / total no.	26.4 ± 4.4 / 641	26.2 ± 4.4 / 618
Hemodynamics		
Catecholamines — no./total no. (%)		
Any vasopressor‡	672/692 (97.1)	669/693 (96.5)
Any inotropic support§	225/692 (32.5)	226/693 (32.6)
Any inodilator¶	64/692 (9.2)	75/693 (10.8)
Use of any cardiac assist device‖ — no./total no. (%)	4/691 (0.6)	3/693 (0.4)
Intraoperative cumulative fluid intake		
Crystalloid fluid dose — ml / no.	1,500 (1,000; 2,000) / 688	1,500 (1,000; 2,000) / 690
Colloid fluid dose — ml / no.#	500 (500; 1,000) / 292	500 (500; 1,000) / 282
Number of patients transfused with RBC — no. (%)	283/692 (40.9)	300/693 (43.3)
Number of patients transfused with fresh frozen plasma— no. (%)	35/692 (5.1)	29/693 (4.2)
Number of patients transfused with thrombocytes — no. (%)	146/692 (21.1)	145/693 (20.9)

Plus–minus values are means ± SD. Fluid intake is shown as median (25%; 75% percentile).
*Other type of surgery includes isolated repair of atrium septum defect, pulmonary valve replacement, off-pump coronary artery bypass surgery, cardiac myxoma resection.
†Priming colloid (hydroxyethyl starch 6%, gelatin)
‡Vasopressor (norepinephrine, vasopressin, cafedrine/theodrenaline)
§Inotropic support (epinephrine, dobutamine)
¶Inodilatator (milrinone, enoximone, levosimendan)
‖ Cardiac assist device (intraaortic balloon pump)
#Colloids (hydroxyethyl starch 6% and 10%, gelatin, human albumin)
CPB denotes cardiopulmonary bypass, RBC packed red blood cell units, RIPC remote ischemic preconditioning.

Table S3. Composite Endpoint.

Variable	RIPC (N = 692)	Sham-RIPC (N = 693)
Primary outcome		
Composite endpoint — no. (%)*†	99 (14.3)	101 (14.6)
All-cause mortality	9 (1.3)	4 (0.6)
Myocardial infarction†	47 (6.8)	63 (9.1)
Biomarker values more than five times the 99th percentile plus		
New pathological Q-wave	31 (4.5)	35 (5.1)
New left bundle branch block	17 (2.5)	28 (4.0)
New ischemic finding by echocardiography/ angiography	5 (0.7)	6 (0.9)
Autopsy	0 (0.0)	0 (0.0)
Stroke	14 (2.0)	15 (2.2)
New, temporary or permanent, focal or global neurological deficit indicated by NIH stroke scale ≥ 4 points	14 (2.0)	15 (2.2)
Autopsy	0 (0.0)	0 (0.0)
Acute renal failure†	42 (6.1)	35 (5.1)
Serum creatinine ≥2-fold increase	40 (5.8)	30 (4.3)
Urine output <0.5 ml/kg/hour for >12 hours	13 (1.9)	13 (1.9)
Renal replacement therapy	10 (1.4)	14 (2.0)
Autopsy	0 (0.0)	0 (0.0)
Secondary outcomes		
Composite endpoint (30 days after surgery) — (%)‡	14.8 (12.1-17.4)	15.0 (12.3-17.6)
All-cause mortality‡	1.3 (0.5-2.1)	1.2 (0.4-2.0)
Myocardial infarction‡	6.8 (4.9-8.7)	9.1 (6.9-11.3)
Stroke‡	2.0 (1.0-3.1)	2.3 (1.2-3.5)
Acute renal failure‡	6.5 (4.6-8.4)	5.2 (3.5-6.9)
Composite endpoint (90 days after surgery) — (%)‡	15.5 (12.8-18.2)	15.6 (12.9-18.3)
All-cause mortality‡	2.0 (1.0-3.1)	1.8 (0.8-2.7)
Myocardial infarction‡	6.8 (4.9-8.7)	9.2 (7.0-11.4)
Stroke‡	2.0 (1.0-3.1)	2.6 (1.4-3.8)
Acute renal failure‡	6.7 (4.8-8.6)	5.4 (3.6-7.1)

*Primary endpoint was assessed until hospital discharge (within a maximum of 14 days when length of hospital stay was longer).

†Note that patients could have had multiple events, e.g. patients suffering from both stroke and acute renal failure; new pathological Q-wave and new left bundle branch block; or serum creatinine ≥2-fold increase and renal replacement therapy, respectively.

‡Dates of follow-up interviews varied about scheduled days. 30 days and 90 days rates were not calculated as proportions. Rate estimates with 95% confidence interval were derived from respective cumulative incidence curves; patients with shorter follow-up were censored at the end of their observation period. Death was treated as a competing risk factor.

RIPC denotes remote ischemic preconditioning

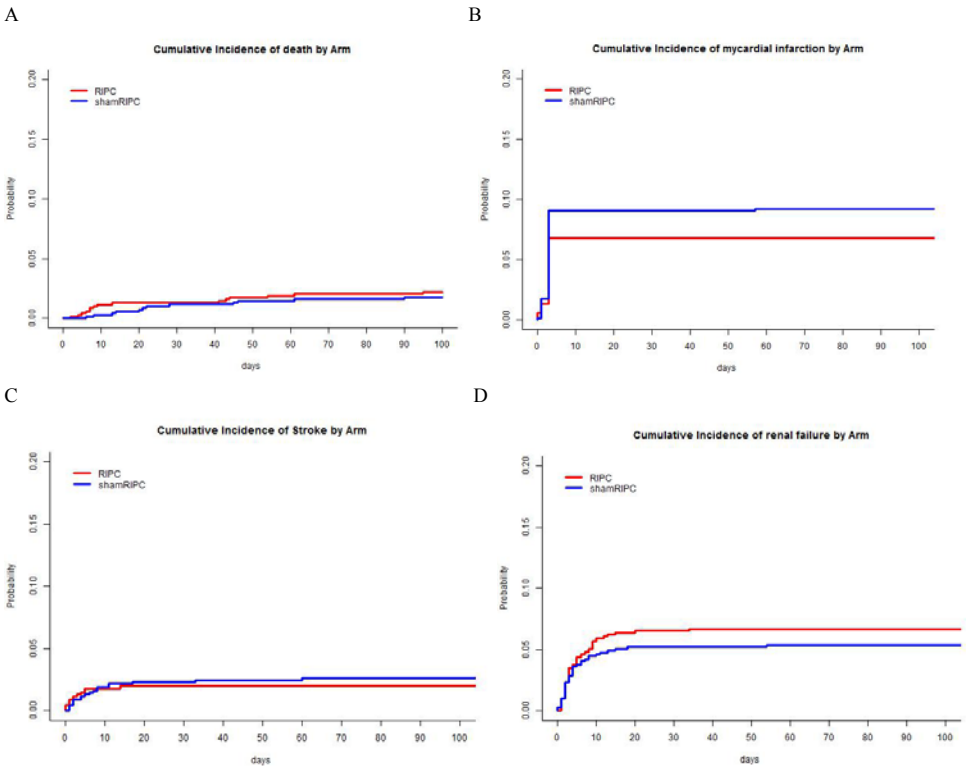

Figure S1. Cumulative Incidence of Composite Components.

Cumulative incidences curves are shown for the RIPC group (red line) and the Sham-RIPC group (blue line) for death of any cause (Panel A), myocardial infarction (Panel B), stroke (Panel C), and acute renal failure (Panel D). In Panel B-D death was treated as competing risk. The two treatment groups had similar rates of events. Model based p values were calculated with Cox regression (Panel A, p=0.76) or competing risks regression (Panel B-D, p=0.11, p=0.27, p=0.29 respectively) analysis.
RIPC denotes remote ischemic preconditioning.

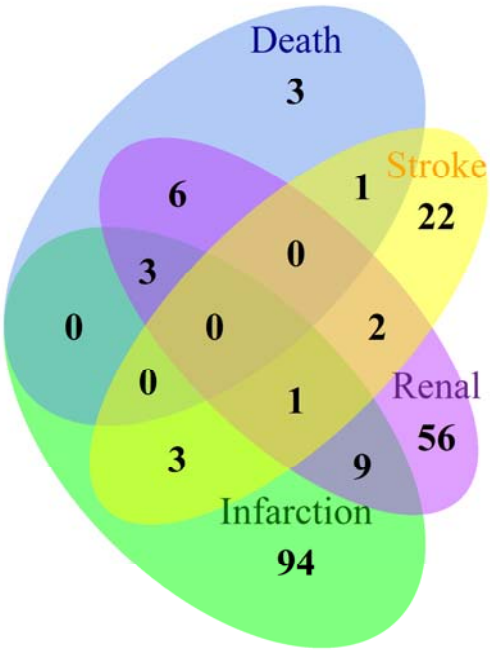

Figure S2. Venn Diagram of Multiplicity

This figure describes the multiplicity patterns for the individual components of the primary endpoint (including death, myocardial infarction (=Infarction), stroke, and acute kidney failure (=Renal)) until hospital discharge (within a maximum of 14 days when length of hospital stay was longer).

Table S4. Subgroup Analysis of the Primary Endpoint.

Subgroup	RIPC	Sham-RIPC	P Value test of interaction
All patients — no./total no. (%)	99/692 (14.3)	101/693 (14.6)	
Coronary artery bypass graft (alone)			0.13*
Yes	41/313 (13.1)	35/317 (11.0)	
No	58/379 (15.3)	66/376 (17.6)	
Use of cholesterol/ lipid lowering drugs			0.84*
Yes	64/434 (14.7)	70/473 (14.8)	
No	35/258 (13.6)	31/220 (14.1)	
Diabetes			0.86*
No	75/526 (14.3)	74/515 (14.4)	
Yes	24/166 (14.5)	27/178 (15.2)	
EuroSCORE			0.68*
0-2 points	20/213 (9.4)	21/192 (10.9)	
3-5 points	36/263 (13.7)	34/284 (12.0)	
≥6 points	43/216 (19.9)	46/217 (21.2)	

CI denotes confidence interval, RIPC remote ischemic preconditioning.

*Test of subgroups by treatment interaction within a logistic regression adjusting for EuroSCORE, diabetes mellitus status, concomitant treatment with cholesterol lowering drugs and centers as random effect. Odds ratios between treatment groups did not differ between subgroups.

Table S5. Postoperative Data.

Variable	RIPC	Sham-RIPC
Troponin T release — µg/l / no.*		
Baseline	0.01 (0.01; 0.02) / 449	0.01 (0.01; 0.02) / 436
6 hours after surgery	0.69 (0.43; 1.16) / 446	0.68 (0.40; 1.17) / 418
12 hours after surgery	0.53 (0.32; 0.86) / 428	0.50 (0.30; 0.87) / 408
24 hours after surgery	0.37 (0.22; 0.64) / 449	0.34 (0.20; 0.62) / 431
48 hours after surgery	0.27 (0.16; 0.47) / 424	0.25 (0.16; 0.41) / 412
Troponin I release — µg/l / no.*		
Baseline	0.01 (0.01; 0.02) / 203	0.02 (0.01; 0.02) / 221
6 hours after surgery	4.71 (3.17; 8.47) / 187	5.73 (3.51; 9.75) / 193
12 hours after surgery	3.13 (2.04; 5.48) / 178	3.79 (2.17; 6.58) / 191
24 hours after surgery	1.93 (1.19; 3.70) / 191	2.32 (1.31; 5.58) / 191
48 hours after surgery	0.94 (0.52; 1.88) / 145	1.09 (0.58; 2.23) / 155
Acute kidney injury (modified RIFLE criteria) — no./total no. (%)†		
No impairment	593/692 (85.7)	587/693 (84.7)
'Risk'	51/692 (7.4)	63/693 (9.1)
'Injury'	28/692 (4.0)	20/693 (2.9)
'Failure'	14/692 (2.0)	15/693 (2.2)
Missing data	6/692 (0.9)	8/693 (1.2)
Other outcomes — no./total no. (%)		
Sternal wound infection	6/690 (0.9)	18/693 (2.6)
Severe sepsis/ septic shock	6/690 (0.9)	8/693 (1.2)
Cardiopulmonary resuscitation	8/690 (1.2)	6/693 (0.9)
Re-thoracotomy	31/692 (4.5)	32/693 (4.6)
Use of any cardiac assist device‡	10/692 (1.4)	6/693 (0.9)
Use of any drug to treat symptoms of delirium§	205/689 (29.8)	212/690 (30.7)
Lowest hematocrit value within 48 hours — %/ total no.	28.4 ± 3.4 / 672	28.5 ± 3.2 / 677
Catecholamines < 24 hours — no./total no. (%)		
Any vasopressor¶	621/692 (89.7)	625/693 (90.2)
Any inotropic support‖	198/692 (28.6)	191/693 (27.6)
Any inodilator#	30/692 (4.3)	34/693 (4.9)
Cumulative fluid intake (within 24 hours after surgery)		
Crystalloid — ml / no.	4,340 (2,920; 6,500) / 690	4,540 (3,000; 6,900) / 692
Colloid — ml / no.**	1,000 (500; 1,500) / 279	1,000 (500; 1,500) / 257
Number of patients transfused with RBC — no./total no. (%)	174/692 (25.1)	175/693 (25.3)
Number of patients transfused with fresh frozen plasma— no./total no. (%)	50/692 (7.2)	46/693 (6.6)
Number of patients transfused with thrombocytes — no./total no. (%)	51/692 (7.4)	54/693 (7.8)
Cumulative fluid intake (until discharge from ICU; a maximum of 7 days after surgery)		
Crystalloid — ml / no.	6,100 (3,440; 9,700) / 673	6,318 (3,620; 10,048) / 679
Colloid — ml / no.**	1,000 (500; 2,000) / 296	1,000 (500; 1,500) / 279
Number of patients transfused with RBC — no./total no. (%)	242/692 (35.0)	244/693 (35.2)
Number of patients transfused with fresh frozen plasma— no./total no. (%)	54/692 (7.8)	54/693 (7.8)
Number of patients transfused with thrombocytes — no./total no. (%)	51/692 (7.4)	56/693 (8.1)

Plus–minus values are means ± SD. Troponin T/I values and fluid intake are shown as median (25%; 75% percentile).

*Troponin T/I was analyzed dependent on local standard.

†Acute kidney injury was defined according to the RIFLE criteria.[10] As data for urine output were not recorded hourly, modified RIFLE criteria based on the creatinine ratio (maximum creatinine post surgery to baseline)

were used: 'No impairment' = creatinine ratio ≤ 1.5; 'Risk' = 1.5 ≤ creatinine ratio < 2; 'Injury' = 2 ≤ creatinine ratio < 3 or urine output ≤0.5 mL/kg/h for 12 h; 'Failure' = 3 ≤ creatinine ratio or renal replacement therapy.
‡Cardiac assist device (intraaortic balloon pump, extracorporeal life support)
§Any drug to treat delirium (haloperidol, promethazine, risperidone, lorazepam, clonidine, dexmedetomidine, midazolam, other medications)
¶Any vasopressor (norepinephrine, vasopressin)
‖Any inotropic support (epinephrine, dobutamine)
#Any inodilator (enoximone, milrinone, levosimendan)
**Colloids (hydroxyethylstarch 6% and 10%, gelatin, human albumin 5% and 20%)
CI denotes confidence interval, ICU intensive care unit, RBC packed red blood cell units, RIPC remote ischemic preconditioning.

A B

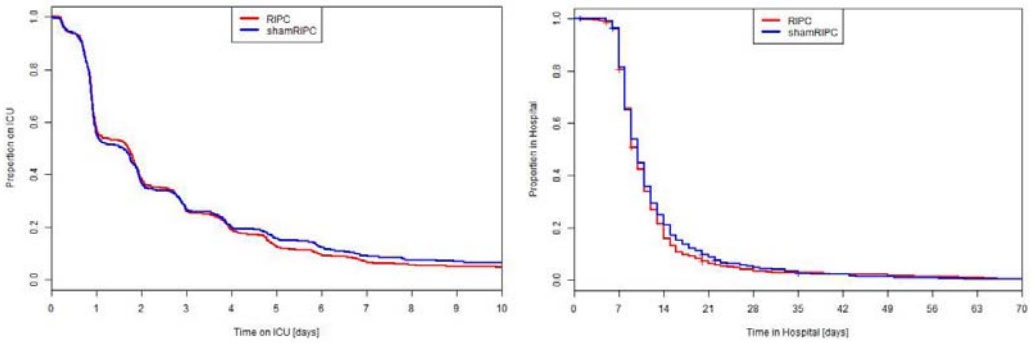

Figure S3. Length of Stay in the Intensive Care Unit (Panel A) and in the Hospital (Panel B).
There was no treatment difference (model based p= 0.82 and p=0.22 respectively).
ICU denotes intensive care unit, RIPC remote ischemic preconditioning.

Table S6. Length of stay in the Intensive Care Unit and in the Hospital.

Variable	RIPC (N = 692)	Sham-RIPC (N = 693)
Proportion of patients with stay on ICU — (%)		
Day 1 (24 hours after surgery)	58.1 (54.6-61.9)	55.6 (52.0-59.4)
Day 2 (48 hours after surgery)	38.5 (35.0-42.3)	37.1 (33.7-40.9)
Day 3 (72 hours after surgery)	26.0 (22.9-29.5)	26.9 (23.8-30.4)
Day 4 (96 hours after surgery)	18.9 (16.2-22.1)	20.0 (17.2-23.3)
Day 5 (120 hours after surgery)	12.8 (10.5-15.5)	15.6 (13.1-18.6)
Day 6 (144 hours after surgery)	9.3 (7.4-11.7)	12.3 (10.1-15.0)
Day 7 (148 hours after surgery)	6.7 (5.1-8.8)	8.9 (7.0-11.3)
Proportion of patients with stay in hospital — (%)		
Day 7 after surgery	80.6 (77.7-83.6)	81.5 (78.6-84.4)
Day 14 after surgery	16.0 (13.5-19.0)	21.1 (18.3-24.4)
Day 21 after surgery	6.2 (4.7-8.3)	8.8 (6.9-11.2)
Day 30 after surgery	3.4 (2.3-5.1)	4.8 (3.4-6.7)

*Proportion of patients (%) based on Kaplan-Meier point estimates with 95% confidence interval.
ICU denotes intensive care unit, RIPC remote ischemic preconditioning.

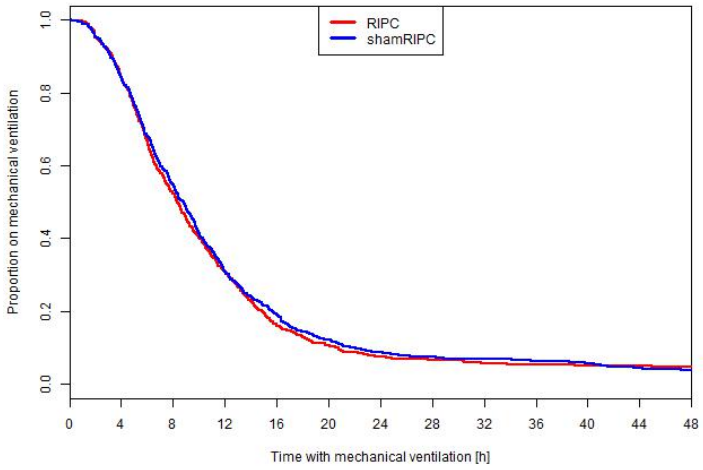

Figure S4. Time with Mechanical Ventilation

There was no treatment difference (model based p= 0.47).
RIPC denotes remote ischemic preconditioning.

Table S7. Time with Mechanical Ventilation.

Variable	RIPC (N = 692)	Sham-RIPC (N = 693)
Proportion of patients with ventilation — (%)		
4 hours after surgery	84.1 (81.4-86.9)	84.2 (81.5-86.9)
8 hours after surgery	52.3 (48.7-56.2)	54.7 (51.1-58.6)
12 hours after surgery	30.8 (27.6-34.5)	30.8 (27.5-34.5)
24 hours after surgery	7.5 (5.8-9.8)	8.7 (6.8-11.0)
36 hours after surgery	5.4 (4.0-7.4)	6.3 (4.7-8.4)

*Proportion of patients (%) based on Kaplan-Meier point estimates with 95% confidence interval.
RIPC remote ischemic preconditioning.

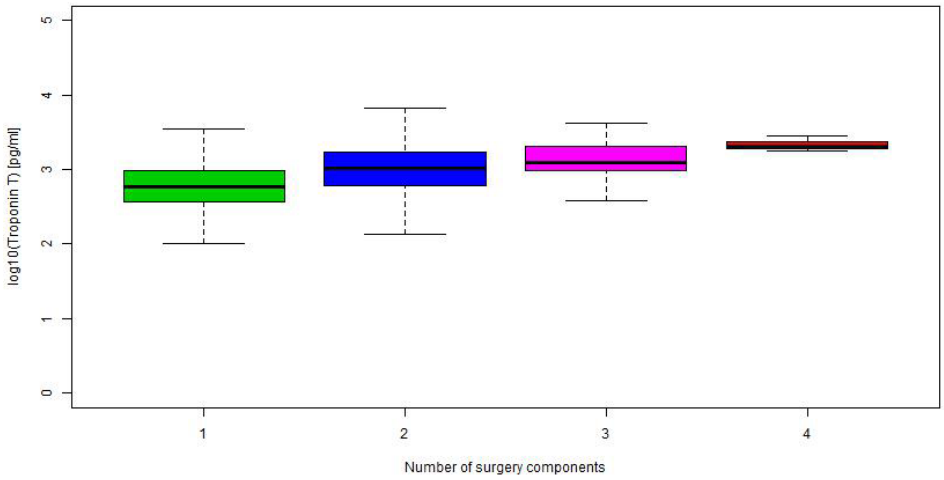

Figure S5. Maximum Postoperative Troponin T Release and Number of Surgical Procedures.

Using logistic regression analysis troponin T release was significantly affected by the number of surgical procedures ($p<0.001$) irrespective of the intervention arm.
Number of surgical procedures, total number of patients:
1 procedure (e.g. isolated valve surgery), N=1,009;
2 procedures (e.g. coronary artery bypass graft plus valve surgery), N=334;
3 procedures (e.g. triple valve surgery), N=39;
4 procedures (e.g. coronary artery bypass graft plus triple valve surgery), N=3.
Maximum troponin I release is also significantly associated with the number of surgery procedures ($p<0.001$; data not shown).

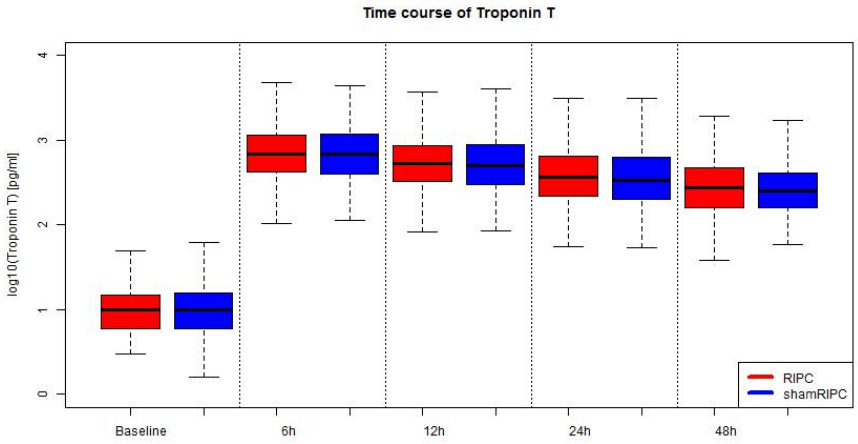

Figure S6. Postoperative Troponin T Release within 48 Hours after Surgery.

There was no treatment difference at 6, 12, 24, and 48 hours after surgery, respectively. Using Welch Two Sample t-test p values were p=0.44 (6h), p=0.39 (12h), p=0.38 (24h), and p=0.36 (48h), respectively.
Troponin I release was also not significantly different between intervention arms (data not shown).
RIPC denotes remote ischemic preconditioning.

Table S8. Outcomes (Per-Protocol Set).

Variable	RIPC (N = 583)	Sham-RIPC (N = 584)	Adjusted Odds ratio* (95% CI)	P Value adjusted*
Primary outcome				
Composite endpoint — no. (%)*	81 (13.9)	80 (13.7)	0.98 (0.70-1.37)	0.88
Death	6 (1.0)	3 (0.5)	0.53 (0.13-2.18)	0.38
Myocardial infarction	41 (7.0)	49 (8.4)	1.20 (0.78-1.85)	0.41
Stroke	11 (1.9)	12 (2.1)	1.12 (0.49-2.56)	0.80
Acute renal failure	33 (5.7)	27 (4.6)	0.83 (0.48-1.43)	0.50
Secondary outcomes				
New-onset atrial fibrillation — no. (%)	125 (21.5)	132 (22.6)	1.09 (0.82-1.45)	0.57
Delirium_CAM-ICU — no. (%)‡	82 (14.4)	77 (13.5)	0.88 (0.62-1.25)	0.47
Composite endpoint (30 days after surgery) — (%)§	14.4 (11.5-17.2)	14.1 (11.2-16.8)		
All-cause mortality§	1.1 (0.2-1.8)	1.0 (0.2-1.9)		
Myocardial infarction§	7.0 (4.9-9.2)	8.4 (6.1-10.7)		
Stroke§	1.9 (0.8-3.0)	2.2 (1.0-3.5)		
Acute renal failure§	6.2 (4.2-8.2)	4.6 (2.9-6.4)		
Composite endpoint (90 days after surgery) — (%)§	15.1 (12.2-18.0)	14.8 (11.8-17.6)		
All-cause mortality§	1.7 (0.7-2.8)	1.4 (0.4-2.3)		
Myocardial infarction§	7.0 (4.9-9.2)	8.6 (6.3-10.9)		
Stroke§	1.9 (0.8-3.0)	2.6 (1.3-3.9)		
Acute renal failure§	6.4 (4.3-8.4)	4.8 (3.0-6.6)		

*Odds ratios are shown for outcome variables. Logistic-regression analyses were adjusted for EuroSCORE, diabetes mellitus status, concomitant treatment with cholesterol lowering drugs and centers as random effect.

†The primary outcome was a composite (including death, myocardial infarction, acute kidney failure and stroke) until hospital discharge (within a maximum of 14 days when length of hospital stay was longer). Note that patients could have had multiple events, e.g. patients suffering from both stroke and acute renal failure.

‡Delirium was assessed by the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU Score).

§ Dates of follow-up interviews varied about scheduled days. 30 days and 90 days rates were not calculated as proportions. Rate estimates with 95% confidence interval were derived from respective cumulative incidence curves; patients with shorter follow-up were censored at the end of their observation period. Death was treated as a competing risk factor.

CI denotes confidence interval, RIPC remote ischemic preconditioning.

A B

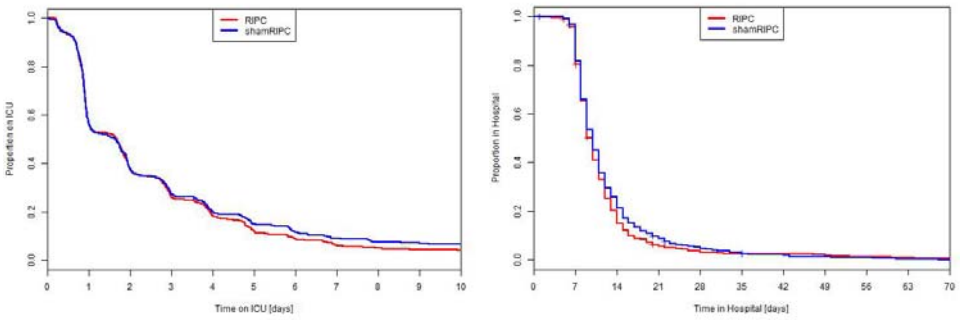

Figure S7. Length of Stay in the Intensive Care Unit (Panel A) and in the Hospital (Panel B; Per-Protocol Set).

There was not between-group difference (model based p= 0.83 and p=0.12 respectively).
ICU denotes intensive care unit, RIPC remote ischemic preconditioning.

Table S9. Length of Stay in the Intensive Care Unit and in the Hospital (Per-Protocol Set).

Variable	RIPC (N = 583)	Sham-RIPC (N = 584)
Proportion of patients with stay on ICU — (%)*		
Day 1 (24 hours after surgery)	57.4 (53.5-61.6)	56.5 (52.6-60.7)
Day 2 (48 hours after surgery)	37.9 (34.2-42.1)	37.9 (34.1-42.1)
Day 3 (72 hours after surgery)	25.9 (22.5-29.7)	27.3 (23.9-31.2)
Day 4 (96 hours after surgery)	18.3 (15.4-21.7)	20.0 (17.0-23.6)
Day 5 (120 hours after surgery)	12.2 (9.8-15.2)	15.1 (12.5-18.4)
Day 6 (144 hours after surgery)	8.8 (6.8-11.4)	11.7 (9.3-14.6)
Day 7 (168 hours after surgery)	6.0 (4.4-8.3)	9.0 (7.0-11.7)
Proportion of patients with stay in hospital — (%)*		
Day 7 after surgery	80.2 (77.0-83.5)	81.8 (78.7-85.0)
Day 14 after surgery	15.1 (12.4-18.3)	21.3 (18.2-24.9)
Day 21 after surgery	5.5 (3.9-7.7)	8.7 (6.7-11.4)
Day 30 after surgery	3.2 (2.0-5.0)	4.7 (3.2-6.7)

*Proportion of patients (%) are Kaplan-Meier point estimates with 95% confidence interval.
ICU denotes intensive care unit, RIPC remote ischemic preconditioning.

References

1. De Hert SG, Van der Linden PJ, Cromheecke S, et al. Choice of primary anesthetic regimen can influence intensive care unit length of stay after coronary surgery with cardiopulmonary bypass. Anesthesiology 2004;101:9-20.
2. Landoni G, Biondi-Zoccai GG, Zangrillo A, et al. Desflurane and sevoflurane in cardiac surgery: a meta-analysis of randomized clinical trials. J Cardiothorac Vasc Anesth 2007;21:502-11.
3. Murphy GS, Hessel EA, 2nd, Groom RC. Optimal perfusion during cardiopulmonary bypass: an evidence-based approach. Anesth Analg 2009;108:1394-417.
4. Nashef SA, Roques F, Michel P, Gauducheau E, Lemeshow S, Salamon R. European system for cardiac operative risk evaluation (EuroSCORE). Eur J Cardiothorac Surg 1999;16:9-13.
5. Gogbashian A, Sedrakyan A, Treasure T. EuroSCORE: a systematic review of international performance. Eur J Cardiothorac Surg 2004;25:695-700.
6. Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 1975;31:103-15.
7. Thygesen K, Alpert JS, White HD, et al. Universal definition of myocardial infarction. Circulation 2007;116:2634-53.
8. Wityk RJ, Pessin MS, Kaplan RF, Caplan LR. Serial assessment of acute stroke using the NIH Stroke Scale. Stroke 1994;25:362-5.
9. Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004;8:R204-12.
10. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: A systematic review. Kidney Int 2008;73:538-46.
11. Ely EW, Inouye SK, Bernard GR, et al. Delirium in mechanically ventilated patients: validity and reliability of the confusion assessment method for the intensive care unit (CAM-ICU). Jama 2001;286:2703-10.
12. Brosteanu O, Houben P, Ihrig K, et al. Risk analysis and risk adapted on-site monitoring in noncommercial clinical trials. Clin Trials 2009;6:585-96.
13. Mangano DT. Aspirin and mortality from coronary bypass surgery. N Engl J Med 2002;347:1309-17.
14. Liakopoulos OJ, Choi YH, Haldenwang PL, et al. Impact of preoperative statin therapy on adverse postoperative outcomes in patients undergoing cardiac surgery: a meta-analysis of over 30,000 patients. Eur Heart J 2008;29:1548-59.
15. Collard CD, Body SC, Shernan SK, Wang S, Mangano DT. Preoperative statin therapy is associated with reduced cardiac mortality after coronary artery bypass graft surgery. J Thorac Cardiovasc Surg 2006;132:392-400.
16. Magee MJ, Alexander JH, Hafley G, et al. Coronary artery bypass graft failure after on-pump and off-pump coronary artery bypass: findings from PREVENT IV. Ann Thorac Surg 2008;85:494-9.
17. Pan W, Pintar T, Anton J, Lee VV, Vaughn WK, Collard CD. Statins are associated with a reduced incidence of perioperative mortality after coronary artery bypass graft surgery. Circulation 2004;110:II45-9.
18. Karkouti K, Wijeysundera DN, Beattie WS. Risk associated with preoperative anemia in cardiac surgery: a multicenter cohort study. Circulation 2008;117:478-84.