Supplementary Data:

Supplemental Methods

Development of the methylation risk score

A methylation risk score was developed to predict chronic injury (CADI-score > 2) at 1 year after transplantation. For this, we first selected all 66 CpG islands that were hypermethylated due to transplantation-induced ischemia in two cohorts (i.e., the paired biopsy cohort and the pre-implantation biopsy cohort). These 66 CpG islands contained 1,634 CpGs. From these, we selected all 1,238 CpGs that are also measured using 450K arrays (to allow our 850K array-based methylation data to be replicated in the post-implantation biopsy cohort, which was profiled using 450K Illumina arrays only). Then, we correlated methylation (beta) values from each of the 1,238 CpGs located in these 66 CpG islands with chronic injury (CADI>2) in the pre-implantation cohort. For this, a logistic regression model containing each of the 1,238 CpGs was fit using ridge regression to penalize the coefficient estimates. Ridge regression was chosen because it is better suited for logistic models with many input variables and also because it can handle input variables that are dependent from each other (which is necessary here because CpGs that belong to a CpG island are often co-regulated at the methylation level). This resulted in a logistic model, in which a coefficient was assigned to each individual CpG. Next, the methylation risk score was defined as the sum of methylation (beta) values at each CpG in 66 ischemia-hypermethylated CpG islands, weighted by marker-specific effect sizes (i.e., multiplied by the coefficient obtained for this CpG in the logistic regression model).

The formula is therefore: methylation risk score or MRS= intercept + $c_1ß_1$+ $c_2ß_2$ + $c_3ß_3$ + … + $c_{1238}ß_{1238}$

The methylation risk score, consisting of the same coefficients that were determined in the pre-implantation discovery cohort (c_1, c_2, c_3, c_4, …, c_{1238}) was subsequently validated in the post-reperfusion cohort.

Supplemental Results

Potential confounding by diabetes mellitus

Although diabetes mellitus can also influence the renal epigenome (Reddy, Natarajan, Epigenetics in Diabetic Kidney Disease. Journal of the American Society of Nephrology, 22: 2182-2185, 2011), diabetes of the recipient will not have confounded our results, since biopsies were obtained at the time of implantation. Moreover, none of the donors in the longitudinal cohort and only 2 out of 82 in the implantation cohort had diabetes mellitus. Since we selected only those CpGs that were significantly hypermethylated upon ischemia in both cohorts, we can conclude that these CpGs were not hypermethylated because of diabetes.

Correlation of the Methylation Risk Score (MRS) with other clinical endpoints

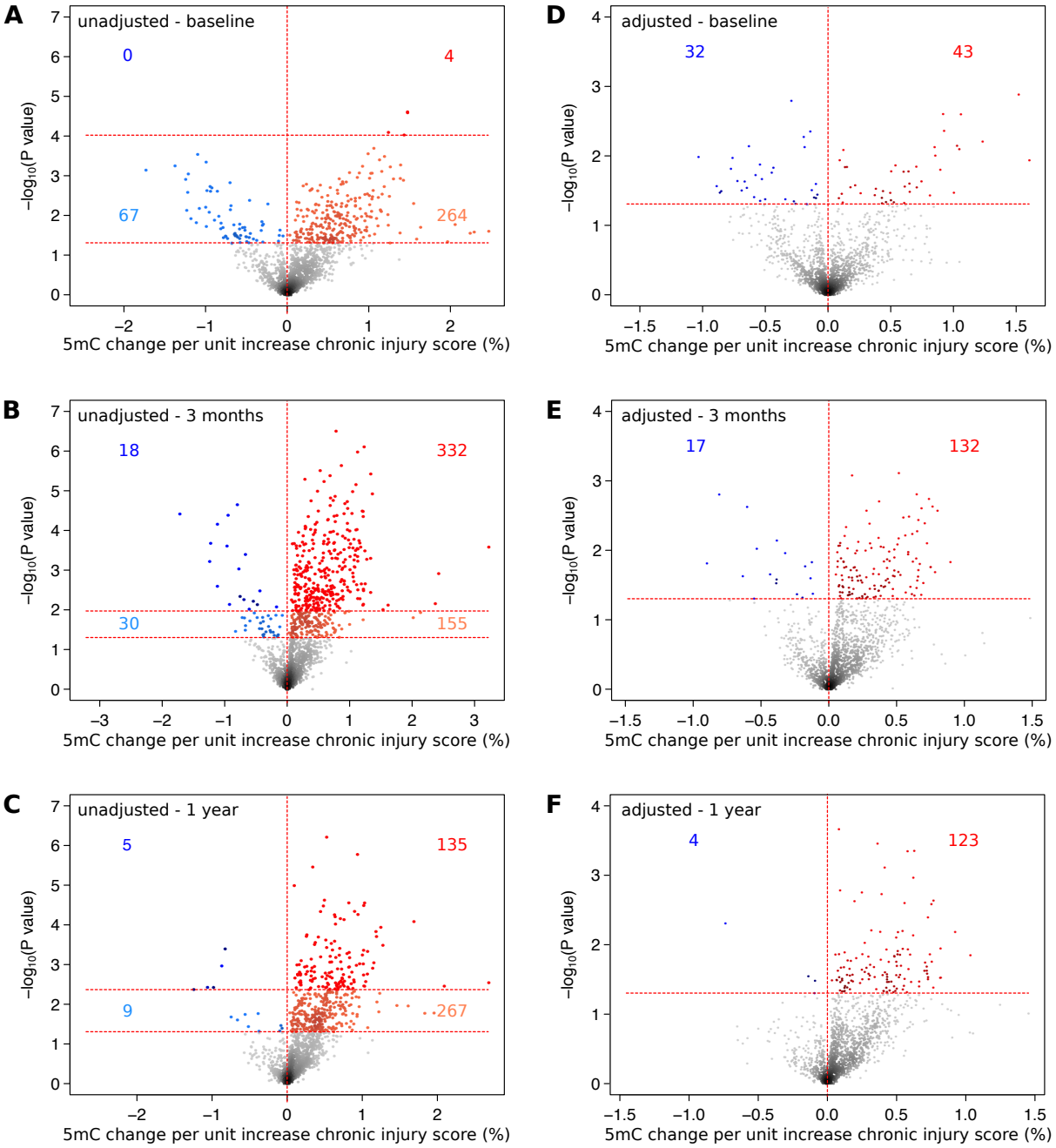
1. Delayed graft function and MRS: The methylation risk score did not differ between transplants with or without delayed graft function (*P*=0.46 in the pre-implantation cohort, *P*=0.21 in the post-reperfusion cohort).
2. Acute rejection and MRS: The methylation risk score did also not differ between recipients who experienced acute rejection *versus* those who did not (*P*=0.59 in the implantation cohort, *P*=0.51 in the post-reperfusion cohort).
3. eGFR levels at 3 months after transplantation and MRS: There was only a non-significant trend for reduced eGFR levels in recipients with the highest methylation score (by ANOVA: *P*=0.09 in the implantation cohort and *P*=0.09 in the post-reperfusion cohort).
4. eGFR levels at 12 months after transplantation and MRS: eGFR levels at 12 months after transplantation significantly differed according to methylation risk score tertiles in both cohorts as assessed by ANOVA (*P*=0.05 in the pre-implantation cohort and *P*=0.01 in the post-reperfusion cohort). There was also a significant correlation between the methylation risk score and eGFR levels at 12 months in both cohorts as assessed by Pearson correlation (*P*=0.032 in the pre-implantation cohort and *P*=0.009 in the post-reperfusion cohort). More specifically, in the pre-implantation cohort, eGFR levels in recipients with the lowest methylation risk score were 54.32 ± 11.47 ml/min/1.73m^2 *versus* 45.58 ± 14.90 ml/min/1.73m^2 in recipients with the highest tertile score. In the post-

reperfusion cohort, eGFR levels in recipients with the lowest methylation risk score were 61.76 ± 23.10 ml/min/1.73m^2 *versus* 44.25 ± 15.16 ml/min/1.73m^2 in recipients with the highest scores.

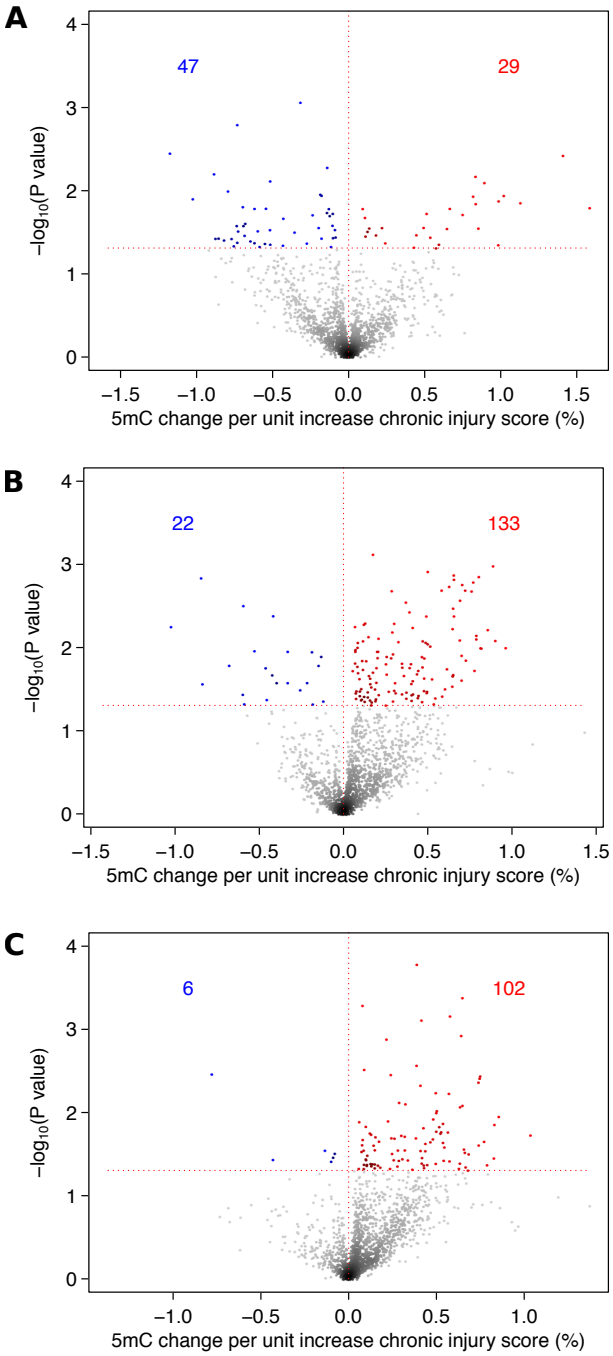
5. Decrease in allograft function and MRS: We subsequently calculated the change in eGFR levels between 3 and 12 months (eGFR at 12 months minus eGFR at 3 months). In the pre-implantation cohort, the methylation risk score was higher when eGFR decreased more between 3 and 12 months, but this negative correlation did not reach significance (Pearson correlation r = -0.19, *P*=0.15). In the second post-reperfusion cohort, this association did exceed the significance level (r=-0.35, *P*=0.02). However, when both cohorts were combined, effects became more significant (r=-0.29, *P*=0.004). The fact that combining the two cohorts decreases the P value points towards an increased power to detect this effect by increasing the number of patients, and suggests the effect is not solely apparent in one cohort only (i.e., the post-reperfusion cohort).
6. Chronic allograft injury at 3 months after transplantation and MRS: Although high methylation risk scores immediately after implantation correlate with higher CADI scores at 3 months after transplant (Spearman correlation, *P*=0.000001 in the implantation cohort and *P*=0.01 in the post-reperfusion cohort), the methylation risk score failed to predict a CADI>2 at 3 months post-transplantation. Indeed, the methylation risk score predicted a CADI>2 at 3 months post-transplant in the pre-implantation cohort (*P*=0.001), but not in the post-reperfusion cohort (*P*=0.11). Overall, this suggests that the methylation risk score at implantation is able to predict chronic injury after transplantation, i.e., a CADI>2 at 12 months, but not at 3 months.
7. Progression of chronic allograft injury and MRS: To assess how the methylation risk score correlated with changes in CADI, we also evaluated CADI scores at the time of transplantation. 'Progression of CADI' was defined as an increase in CADI score of at least 2 units compared to baseline (i.e., at the time of implantation; as previously reported by O'Connell *et al. in The Lancet*). These analyses were done on both cohorts combined as information on CADI progression were available for less patients (n=51 in the pre-

implantation cohort and n=40 in the post-reperfusion cohort), adjusted for the type of cohort (as these biopsies were performed at different time-points and were analyzed using a different methylation array). We observed that the methylation risk score was significantly higher in transplants exhibiting an increase in CADI score compared to those who had a stable CADI (*P*=0.02). Interestingly, transplants in the highest tertile of the methylation risk score also had a significantly increased risk of CADI progression compared to the lowest methylation risk scores (*P*=0.03, OR 3.48, 95% CI 1.16-25.27).

Supplemental Figures

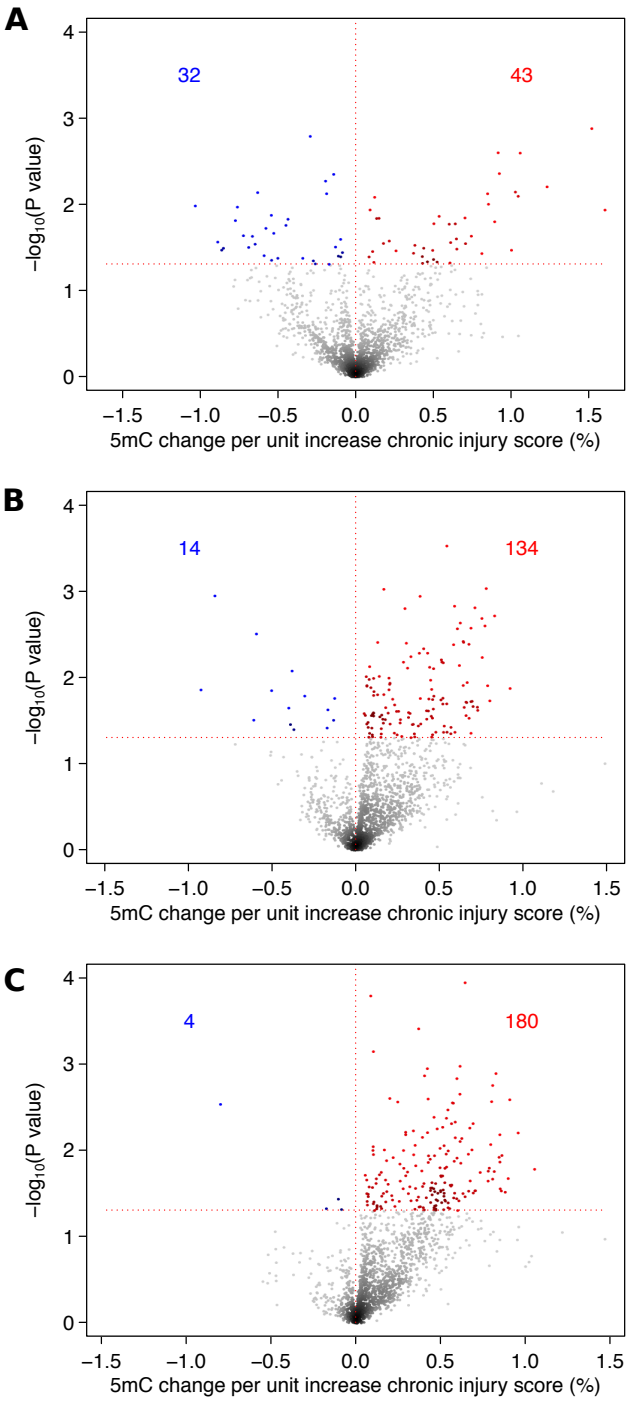


Supplemental Figure 1: Correlation of ischemia-induced hypermethylation of kidney transplants with chronic allograft injury. Logarithmic *P* values obtained for individual CpGs that were correlated with the CADI score at the time of implantation (A and D), and 3 months (B and E) and 1 year (C and F) after transplantation, both unadjusted (A-C) as well as adjusted for donor age and gender (D-F). Positive (red) and negative (blue) correlations are highlighted at FDR<0.05 and *P*<0.05 (light colors).



Supplemental Figure 2: Correlation of ischemia-induced hypermethylation of kidney transplants with chronic allograft injury, adjusted for cold and warm ischemia time, donor age and donor gender. Logarithmic *P* values obtained for individual CpGs that were correlated with the CADI score at the time of implantation (A), and 3 months (B) and 1 year (C) after transplantation, adjusted for cold and warm ischemia time, donor age and donor gender. Positive (red) and negative (blue) correlations are highlighted

at $P<0.05$. Consistent with the hypermethylation upon ischemia, future injury was also mainly associated with hypermethylation: hypermethylation in 133 of 155 (85.8%) and 102 of 108 (94.4%) significant CpGs associated with chronic injury at 3 months and 1 year respectively (binomial test $P<0.00001$ for both comparisons). Again, DNA methylation correlated more with future injury than with injury already evident at the time of transplantation (evaluated in the same biopsies on which DNA methylation was measured): in 76 of CpGs, methylation correlated with chronic injury at baseline ($P<0.0001$, χ^2 test, for both time points), in 29 (38.2%) with a positive correlation. Thus, whether or not ischemia time itself was included in the model did not the skewing towards positive correlation between methylation and chronic injury, nor the skewing towards more correlation with future injury than with injury already evident at the time of transplantation. This eliminates the possibility that methylation merely translated a correlation between ischemia time and chronic injury in the abovementioned analyses that were not adjusted for ischemia time.



Supplemental Figure 3: Correlation of ischemia-induced hypermethylation of kidney transplants with chronic allograft injury, adjusted for donor age and donor gender, and for acute rejection (concerning the correlations at 3 months and 1 year, the correlation with chronic injury already apparent at the time of implantation was not adjusted for acute rejection as this evidently occurs post-implantation). Logarithmic *P* values obtained for individual CpGs that were correlated with the CADI score at the time of

implantation (A), and 3 months (B) and 1 year (C) after transplantation. Positive (red) and negative (blue) correlations are highlighted at $P<0.05$. Consistent with the hypermethylation upon ischemia, future injury was also mainly associated with hypermethylation: hypermethylation in 134 of 148 (90.5%) and 180 of 184 (97.8%) significant CpGs associated with chronic injury at 3 months and 1 year respectively (binomial test $P<0.00001$ for both comparisons). Again, DNA methylation correlated more with future injury than with injury already evident at the time of transplantation (evaluated in the same biopsies on which DNA methylation was measured): in 75 of CpGs, methylation correlated with chronic injury at baseline ($P<0.0001$, χ^2 test, for both time points), in 43 (57.3%) with a positive correlation. Thus, whether or not acute rejection was included in the model did not the skewing towards positive correlation between methylation and chronic injury, nor the skewing towards more correlation with future injury than with injury already evident at the time of transplantation. This eliminates the possibility that acute rejection confounded the correlation between ischemia time and chronic injury in the abovementioned analyses that were not adjusted for acute rejection.