

## Supplementary Appendix

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

Supplement to: Serra AL, Poster D, Kistler AD, et al. Sirolimus and kidney growth in autosomal dominant polycystic kidney disease. *N Engl J Med* 2010;363:820-9. DOI: 10.1056/NEJMoa0907419.

## Supplementary Material

### Statistical Methods

The original sample size calculation was based on detecting a difference in mean percentage change in total kidney volume over the 18 month treatment period. However, an analysis of covariance gives a more precise estimate of the effect of treatment than an analysis of percentage change.<sup>1</sup> Percentage change neither appropriately adjusts for any chance imbalance at baseline between groups nor is its mean necessarily normally distributed.<sup>1</sup> When clinical interest focuses on percentage change, this suggests that the effect of treatment should be measured on a ratio scale and then a log transformation is appropriate.<sup>2</sup> The log transformation is particularly useful for positively skewed data, so that data become approximately normally distributed (Supplementary Appendix, Fig. 1) and then the residuals in an analysis of covariance are likely to follow a normal distribution as required for this method of analysis.<sup>3,4</sup> Hence when percentage change is of clinical interest, the recommended method of analysis is an analysis of covariance of the log outcome at the end of the trial with the log outcome at baseline as a covariate.<sup>5</sup>

The analysis proposed in the trial protocol was therefore amended in an analysis plan prepared by Biometrical Practice BIOP AG. The following analysis of covariance model was specified in the analysis plan for the primary outcome:

$$\log_{10}(TKV_{18}) = \beta_0 + \beta_1 \log_{10}(TKV_0) + \beta_2 T,$$

where  $TKV_{18}$  and  $TKV_0$  are the total kidney volume at 18 months and randomization respectively,  $T$  is a treatment group indicator, and  $\beta_0, \beta_1$ , and  $\beta_2$  are model parameters.

$\beta_2$  represents the effect of treatment and its estimate is the difference between group means on the log scale, after adjustment for any imbalance between the groups in log  $TKV$  at randomization. Back-transforming either a point or interval estimate for  $\beta_2$  to the original scale gives the ratio of the geometric mean in the sirolimus group to the geometric mean in

the control group.<sup>4</sup> In the intent to treat analysis of the primary outcome, the point estimate for  $\beta_2$  is  $7.11 \times 10^{-3}$  with 95% confidence interval -5.26 to  $19.49 \times 10^{-3}$ . Back-transforming these estimates gives a ratio of 1.02 with 95% confidence interval 0.99 to 1.05 (Supplementary Appendix, Table 1).

When data are skewed, the median is a more stable and more representative measure of the center of a distribution. The log of the ratio of the median under treatment relative to control –  $\log(\text{median } TKV_{(T=1)} / \text{median } TKV_{(T=0)})$  – is just the difference between the logs –  $\log(\text{median } TKV_{(T=1)})$  minus  $\log(\text{median } TKV_{(T=0)})$ . Because the log transformation is monotonic, data are ordered the same way on both log and original scales so that the log of the median is equal to the median of the logs. Hence this ratio of medians is equal to the median  $\log(TKV_{(T=1)})$  minus the median  $\log(TKV_{(T=0)})$ . If the log transformation makes the data approximately normal, then the mean and median are roughly the same on the log scale. So in this situation, the difference between group means on the log scale – mean  $\log(TKV_{(T=1)})$  minus mean  $\log(TKV_{(T=0)})$  – is an approximate estimate of the log of the ratio of medians on the original scale. Back-transforming this estimate gives an approximate estimate of the ratio of the median in the treatment group to the median in the control group.

The sample size calculation can also be re-expressed in a way that illustrates the power of the trial to detect differences on a ratio scale. In the sample size calculation, an annual growth rate of 6% was assumed in the control group, and the trial powered to detect a growth rate in the treatment group of half that in the control group. A 6% annual increase in the control group implies  $(\text{mean } TKV_{12(T=0)} - \text{mean } TKV_{0(T=0)}) / \text{mean } TKV_{0(T=0)} = 6/100$ , or mean  $TKV_{12(T=0)} = 1.06 \times \text{mean } TKV_{0(T=0)}$ . Over 18 months, compounding the annual increase implies  $TKV_{18(T=0)} = (1.06)^{1.5} \times \text{mean } TKV_{0(T=0)}$ . Likewise a 3% increase in the treatment group implies mean  $TKV_{18(T=1)} = (1.03)^{1.5} \times \text{mean } TKV_{0(T=1)}$ . So the ratio of means at the end of the trial is  $TKV_{18(T=1)} / TKV_{18(T=0)} = TKV_{0(T=1)} / TKV_{0(T=0)} \times (1.0453/1.0913)$ . Since randomization ensures that the ratio of means at baseline is approximately one, the trial was powered to

detect any ratio of means at the end of the trial less than 0.96 or, with a two-sided test, any ratio more than 1.04. This in turn suggests that if the ratio is estimated to be around 1.00, then the width of its confidence interval should be in the order of 8 percentage points. This level of power was achieved in the trial: confidence intervals for the effect of treatment have a width of around 6 percentage points, slightly better than expected (Supplementary Appendix, Table 1).

Of the two secondary outcomes, the urinary albumin to creatinine ratio was analyzed in exactly the same way as the primary outcome, while the estimated glomerular filtration rate was analyzed by a standard analysis of covariance without the log transformation.

#### References for Supplementary Appendix, Statistical Methods

1. Vickers AJ. The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. *BMC Med Res Methodol* 2001;1:6.
2. Keene ON. The log transformation is special. *Stat Med* 1995;14(8):811-819.
3. Bland JM, Altman DG. Transformations, means, and confidence intervals. *BMJ* 1996;312(7038):1079.
4. Bland JM, Altman DG. The use of transformation when comparing two means. *BMJ* 1996;312(7039):1153.
5. Senn S. *Statistical issues in drug development*. Chichester: Wiley; 1997, p 102.

**Supplementary Table 1.** Total kidney volume at 18 months: parameter estimates from analyses of covariance.\*

Analysis	Patients	Variable	Estimate (back-transformed)		
			Ratio	95% CI	P value
Intent-to-treat†	100	Treatment (0=control, 1=sirolimus)	1.02	0.99 to 1.05	0.26
Per-protocol‡	94	Treatment (0=control, 1=sirolimus)	1.01	0.98 to 1.04	0.35
Planned secondary analysis†	100	Treatment (0=control, 1=sirolimus)	1.01	0.99 to 1.05	0.24
		Age (per 10 years)	1.01	0.98 to 1.03	0.64
		Gender (0=male, 1=female)	0.98	0.95 to 1.02	0.38
		Log <sub>10</sub> urinary albumin excretion at randomization§	0.99	0.96 to 1.03	0.77
Unplanned secondary analysis†	100	Treatment (0=control, 1=sirolimus)	1.01	0.97 to 1.05	0.67
		ACEi/ARB use (0=no, 1=yes)	1.00	0.96 to 1.05	0.83
		Interaction between treatment and ACEi/ARB use	1.02	0.96 to 1.08	0.53

\* All analyses are of log<sub>10</sub> total kidney volume at 18 months and in addition to the variables listed above, all linear regression models include an intercept and log<sub>10</sub> total kidney volume at randomization as a covariate (see Supplementary Appendix, Statistical Methods).

† Conservative replacement of 4 missing values (2 in the control group, 2 in the sirolimus group) – see the Statistical Analysis subsection of the Methods section for details.

‡ Patients excluded if no measurement available after 18 months (4 patients) or if not known to have taken at least 50% of trial medication (2 patients).

§ In this ratio, albumin was measured in milligrams per liter and creatinine in millimoles per liter.

ACEi/ARB denotes angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker.

**Supplementary Table 2.** Sirolimus dosage, sirolimus steady-state blood levels and parameters of treatment adherence in patients assigned to the sirolimus group.\*

<b>Months</b>	<b>Sirolimus dosage</b>	<b>Sirolimus levels</b>	<b>Continuation†</b>	<b>Adherence‡</b>
	<b>(milligram per day)</b>	<b>(microgram per liter)</b>	<b>(%)</b>	<b>(%)</b>
<b>3 months</b>	1.5 (0.6)	4.7 (2.2)	100	96 (3)
<b>6 months</b>	1.4 (0.7)	4.1 (1.7)	100	95 (3)
<b>9 months</b>	1.5 (0.7)	4.4 (2.0)	98	94 (4)
<b>12 months</b>	1.6 (0.6)	4.9 (2.2)	96	94 (4)
<b>18 months</b>	1.5 (0.7)	4.5 (2.1)	96	90 (4)

\* Table shows the mean (standard deviation).

† Continuation was defined as the proportion of patients who remained on the sirolimus treatment.

‡ Adherence was defined for each specific time interval as the average daily proportion of patients who took their sirolimus dose as prescribed among those still on the sirolimus treatment.

**Supplementary Table 3.** Blood pressure and antihypertensive treatment from randomization to 18 months.\*

<b>Blood pressure –</b>		<b>Randomization</b>	<b>6 months</b>	<b>12 months</b>	<b>18 months</b>
mm Hg					
	<b>Sirolimus</b>	<b>N = 50</b>	<b>N = 49</b>	<b>N = 48</b>	<b>N = 48</b>
	<b>Control</b>	<b>N = 50</b>	<b>N = 50</b>	<b>N = 50</b>	<b>N = 49</b>
Systolic	Sirolimus	130 (14)	128 (14)	126 (12)	130 (14)
Diastolic	Sirolimus	84 (11)	82 (9)	80 (9)	82 (11)
Systolic	Control	130 (15)	130 (14)	126 (13)	126 (16)
Diastolic	Control	83 (10)	84 (10)	81 (9)	82 (12)
<b>Hypertension – no. (%)†</b>		<b>Randomization</b>	<b>6 months</b>	<b>12 months</b>	<b>18 months</b>
	<b>Sirolimus</b>	<b>N=36</b>	<b>N=33</b>	<b>N=33</b>	<b>N=34</b>
	<b>Control</b>	<b>N=32</b>	<b>N=32</b>	<b>N=32</b>	<b>N=34</b>
<b>Antihypertensive treatment – no.</b>	Sirolimus	27 (75)	31 (94)	31 (94)	32 (94)
	Control	23 (72)	27 (84)	29 (91)	30 (88)
	(%)‡				
ACEi/ARB	Sirolimus	22 (61)	28 (85)	28 (85)	29 (85)
	Control	21 (66)	25 (78)	26 (81)	28 (82)
Diuretics	Sirolimus	4 (11)	5 (15)	5 (15)	6 (18)
	Control	9 (28)	9 (28)	9 (28)	8 (24)
Others	Sirolimus	7 (19)	6 (18)	8 (24)	8 (24)
	Control	5 (16)	6 (19)	8 (25)	8 (24)

\* Table shows either the mean (standard deviation) or number of patients (percent).

† Hypertension was defined as systolic blood pressure above 140 mm Hg or diastolic blood pressure above 90 mm Hg or antihypertensive treatment.

ACEi/ARB denotes angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker.

**Supplementary Table 4.** Initiation of antihypertensive treatment and incidence of hypertension during the 18-month treatment period in the safety-analysis population, according to randomized group.\*

Category	Sirolimus (N=49)	Control (N=50)
	Number of patients (%)	
<b>Initiation of antihypertensive treatment</b>		
ACEi/ARB	9 (26)	7 (21)
Diuretics	2 (6)	0 (0)
Others	1 (3)	3 (9)
<b>Change of initial antihypertensive treatment</b>	6 (12)	8 (16)
<b>New onset of hypertension†</b>	0 (0)	2 (4)

\* The safety-analysis population consisted of all randomized patients who underwent at least one follow-up visit.

§ Macrohematuria was associated with urinary tract infection in 2 patients of the sirolimus group and in one patient of the control group.

† Hypertension was defined as systolic blood pressure above 140 mm Hg or diastolic blood pressure above 90 mm Hg or antihypertensive treatment.

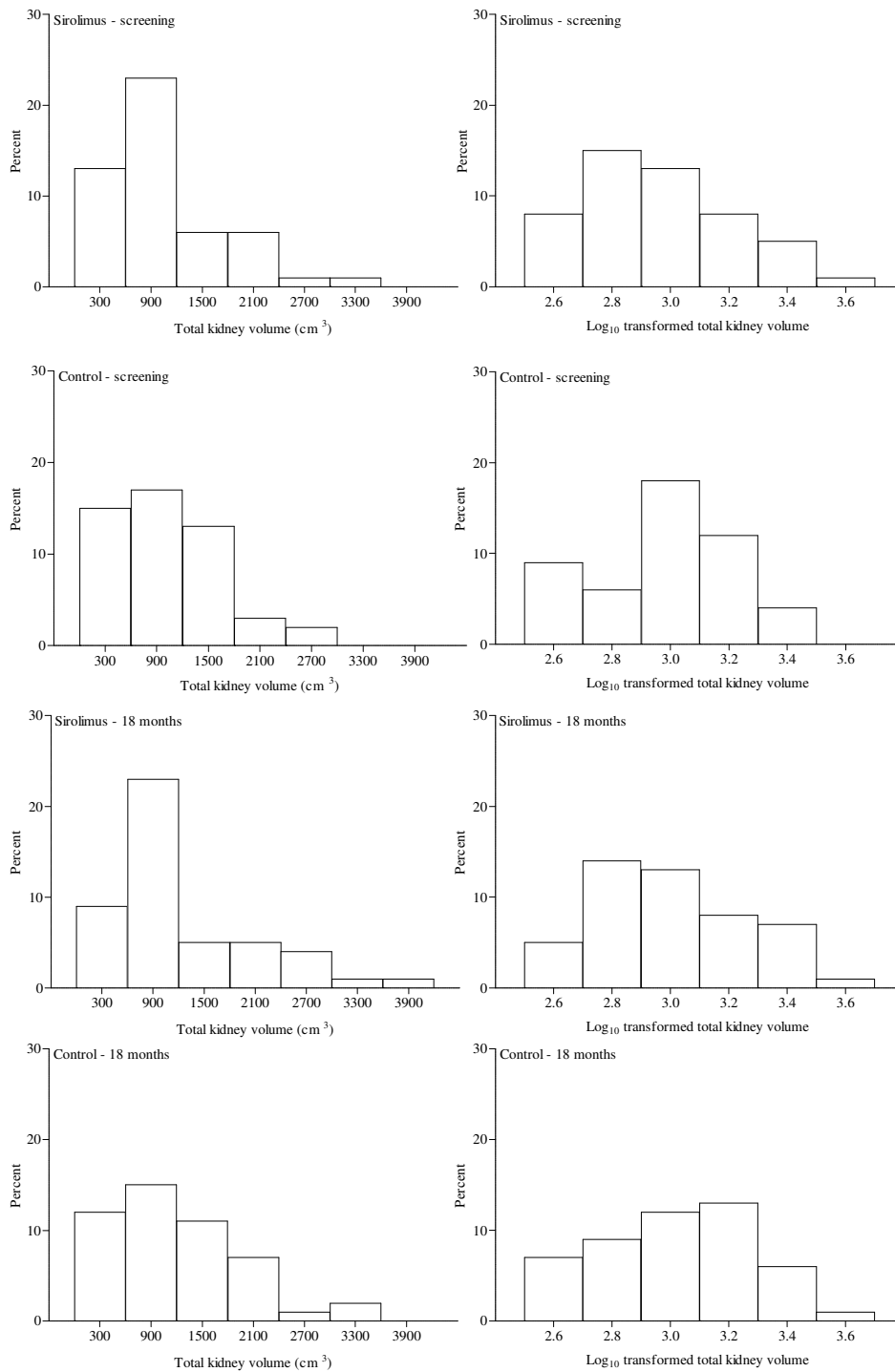
ACEi/ARB denotes angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker.

**Supplementary Table 5.** Laboratory parameters at 18 months. \*

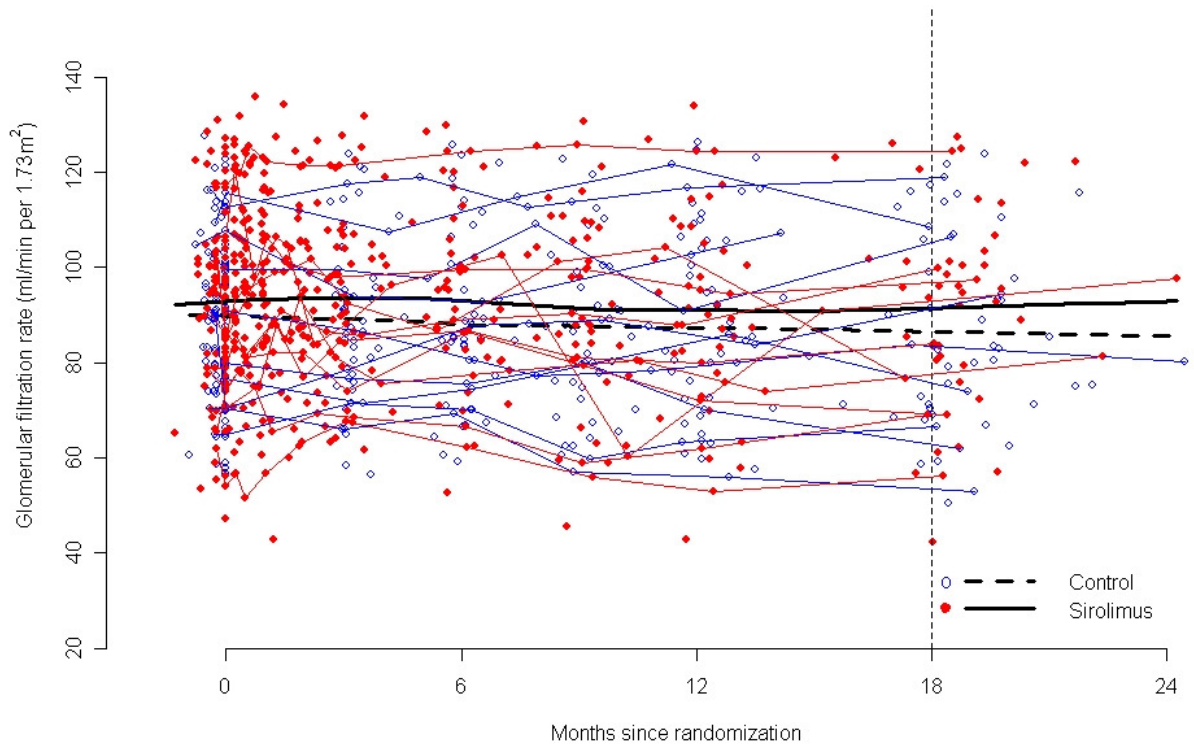
<b>Parameter</b>	<b>Sirolimus</b>	<b>Control</b>
<b>Triglycerides</b> (millimol per liter)		
Mean (SD)	1.5 (1.6)	1.0 (0.4)
Range	0.4 – 10.7	0.4 – 2.7
Number of patients $\geq 4.5$	1	0
<b>Total cholesterol</b> (millimol per liter)		
Mean (SD)	5.3 (1.1)	4.8 (0.9)
Range	3.2 – 9.3	2.6 – 6.3
Number of patients $\geq 6.2$	10	2
<b>LDL cholesterol</b> (millimol per liter)		
Mean (SD)	3.2 (1.1)	2.9 (0.7)
Range	1.4 – 7.0	1.4 – 4.4
Number of patients $\geq 4.1$	12	3
<b>HDL cholesterol</b> (millimol per liter)		
Mean (SD)	1.5 (0.4)	1.5 (0.6)
Range	0.7 – 3.1	0.7 – 4.4
Number of patients $\leq 1$	4	7
<b>Liver enzymes</b> (Units per liter) – mean (SD)		
AST	30 (12)	27 (11)
ALT	34 (23)	27 (20)
$\gamma$ GT	29 (19)	20 (10)
<b>Hemoglobin</b> (gram per deciliter)		
Mean (SD)	13.9 (0.9)	13.7 (1.2)
Range	11.9 – 15.5	11.4 – 17.1
<b>White-cell count</b> ( $\times 10^3$ per $\text{mm}^3$ )		
Mean (SD)	6.1 (1.6)	6.0 (1.5)
Range	3.1 – 10.1	3.6 – 10.6
<b>Platelet count</b> ( $\times 10^3$ per $\text{mm}^3$ )		
Mean (SD)	274 (65)	258 (62)
Range	159 – 412	112 – 419

\* To convert values for triglycerides to milligrams per deciliter, divide by 0.01129. To convert values for cholesterol, LDL and HDL to milligrams per deciliter, divide by 0.02586. LDL denotes low-density lipoprotein, HDL high-density lipoprotein, SD standard deviation, AST aspartate aminotransferase, ALT alanine aminotransferase and  $\gamma$ GT gamma glutamyl-transferase.

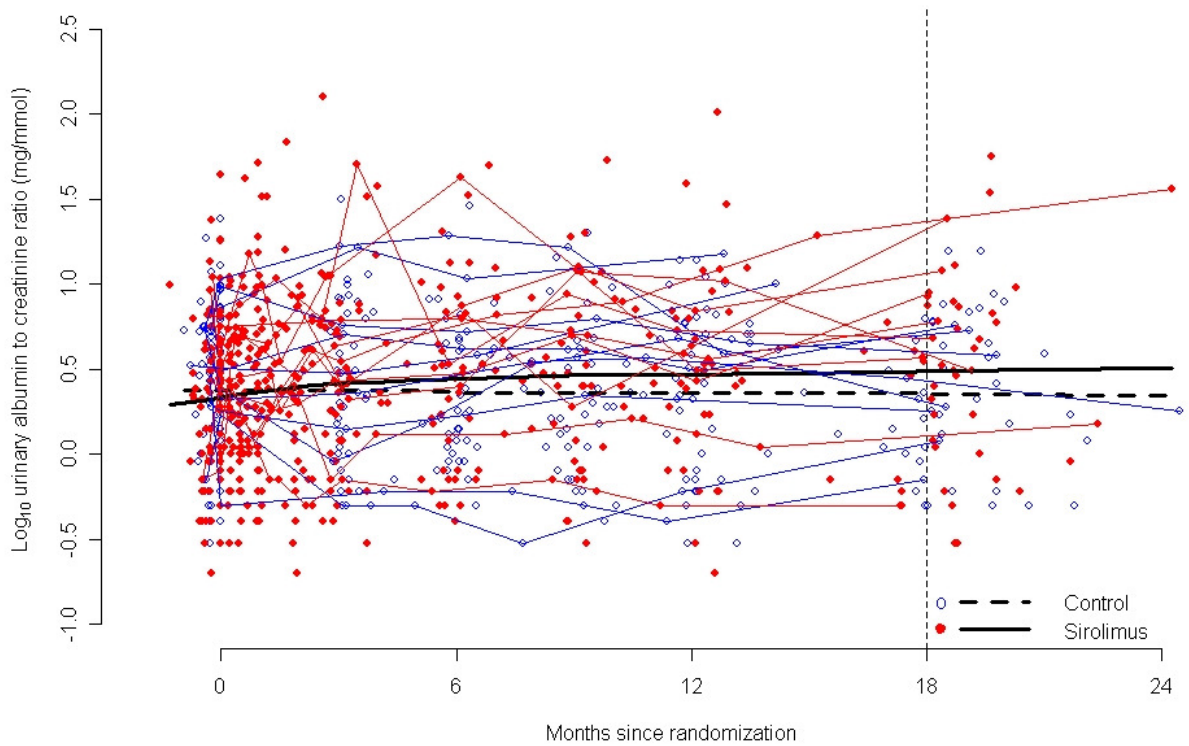
**Supplementary Figure 1.** Relative frequency of total kidney volume and  $\log_{10}$  transformed total kidney volume in the sirolimus and control groups at randomization and at 18 months.



**Supplementary Figure 2.** Estimated glomerular filtration rate (milliliter per minute per 1.73 m<sup>2</sup>) over time in the sirolimus and control groups. A non-parametric mean response curve is shown for each group, calculated using the default LOESS function in R version 2.10.1. All measurements are shown, with lines showing measurements over time for a random sample of 10 patients in each group. Note that patients in the sirolimus group had 4 additional measurements during the first 6 months.



**Supplementary Figure 3.**  $\text{Log}_{10}$  urinary albumin to creatinine ratio (milligram per millimol) over time in the sirolimus and control groups. A non-parametric mean response curve is shown for each group, calculated using the default LOESS function in R version 2.10.1. All measurements are shown, with lines showing measurements over time for a random sample of 10 patients in each group. Note that patients in the sirolimus group had 4 additional measurements during the first 6 months.



**Supplementary Figure 4.** Time course of adherence parameters (persistence, adherence) in patients assigned to the sirolimus group. Persistence curve shows a Kaplan Meier estimation of the proportion of patients on treatment. Adherence curve indicates the day-to-day proportion of patients who took sirolimus as prescribed. Data from 48 individual dosing histories were used for analysis (one patient refused to be monitored).

