Table 1

Demographic data of patients comparing the different CADI groups.

Table 2

Significantly enriched biological processes in upregulated DEGs are provided for the six histological parameters. The number in parenthesis depicts the number of DEGs that could be assigned to at least one category in the respective group. The number of genes for each category and histological parameter is provided where significantly enriched categories are indicated with an asterisk.

Table 3

Linear regression model of histological and clinical parameters to predict creatinine values of one year after engraftment.

Table 4

Linear regression using molecular biomarkers to predict creatinine values of one year after engraftment.

Figure 1

Distribution of histological parameters. ati … acute tubular injury; ta … tubular atrophy; ii … interstitial inflammation; if … interstitial fibrosis; as … arteriolosclerosis; gs … glomerulosclerosis

Supplementary table 1 Linear regression model of histological parameters represented as the CADI score to predict creatinine values of one year after engraftment.

Supplementary table 2 Model selection process with the seven molecular markers as independent variables to predict creatinine values one year after engraftment.

Supplementary table 3 Linear regression using molecular biomarkers in combination with clinical parameters to predict creatinine values of one year after engraftment.

Supplementary figure 1 Number of differentially expressed genes (DEGs) between damaged and healthy tissue. The Venn diagram shows the overlap of identified DEGs when comparing damaged versus healthy tissue based on the six histological parameters. Two genes, namely IGJ and CD52 were upregulated in damaged tissue samples no matter which histological parameter was investigated.

Supplementary figure 2 Interaction network of DEGs and interacting proteins. DEGs are represented as grey filled nodes whereas interacting proteins connecting at least two DEGs are represented as smaller white nodes. Node border colors of DEGs depict the cluster assignment based on gene expression

patterns. Node size of DEGs corresponds to the number of significant findings associated with the six histological parameters.

Supplementary figure 3 Scatterplot of predicted creatinine values to measured creatinine values after leave-one-out cross validation. The Pearson correlation coefficient between predicted and measured creatinine values was 0.45.

Table 1 Demographic data of patients comparing the different CADI groups.

	No of drugs	CADI <2	CADI 2 - <4	CADI ≥ 4	p-value
No. of samples		52	21	9	
Donor (Living / deceased)		2/50	0/21	0/9	0.554
Donor creatinine (mg/dl)		0.81 (0.34)	0.80 (0.50)	0.97 (0.38)	0.290
Donor age (years)		41 (23)	59 (19)	63 (6)	**<0.001**
Recipient age (years)		52.1 (19.5)	61.8 (11.9)	65.4 (10.2)	**0.006**
Recipient weight (kg)		77 (18)	77.8 (11.5)	77.3 (21)	0.626
sex (male / female)		37 / 15	15 / 6	5 / 4	0.628
BCAR (%)		27	14	56	0.065
BCAR (days post TX)		13 (135)	8 (3)	14 (6)	0.205
PRA latest		2 (4)	0 (2)	0 (0)	0.101
CIT (hours)		12 (10)	12 (11)	9 (17)	0.821
DM		11	2	4	0.104*
Delayed graft function (no / yes)		50 /2	18 /3	8 / 1	0.160*
CMV (negative / positive)		26 / 10	10 / 6	3 / 4	0.316*
Pyelonephritis (negative / positive)		44 / 8	20 / 1	9 / 0	0.421*
Hyperlipidemia (number of drugs 0 / 1)		33 / 19	12 / 9	5 / 4	0.829
Cholesterol	0	199 (68)	220 (103)	208 (14)	0.785
	1	183 (76)	229 (121)	181 (45)	0.241
Hypertension (number of drugs 0 / 1 / 2 / 3 / 4 / 5)		4 / 11 / 16 / 9 / 10 /2	3 / 1 / 2 / 5 / 6 / 4	0 / 1 / 2 / 2 / 4 / 0	0.134*
Mean arterial pressure (1 year post TX)	0	97 (17)	--	--	
	1	90 (18)	--	--	

	No of drugs	CADI <2	CADI 2 - <4	CADI ≥ 4	p-value
	2	93 (8)	90 (0)	98 (10)	0.263
	3	100 (13)	106 (12)	96 (0)	0.239
	4	94 (11)	92 (15)	95 (3)	0.487
	5	101 (12)	103 (10)	--	0.564
Immunosuppression: (Standard IS / steroid-free / else)		43/5 / 1	21/ 0 / 0	6/3 / 0	0.050*

Data represent median and interquartile range.

* Fisher test

Kruskal-Wallis test and Chi-square test were used for computation of p-values

nd no data; na not applicable, standard IS … steroids+MMF+CNI

Table 2

Significantly enriched biological processes in upregulated DEGs are provided for the six histological parameters. The number in parenthesis depicts the number of DEGs that could be assigned to at least one category in the respective group. The number of genes for each category and histological parameter is provided where significantly enriched categories are indicated with an asterisk.

Biological Process	**ati (17)**	**ta (30)**	**ii (26)**	**if (16)**	**as (5)**	**gs (12)**
Immunity and defense	4*	6*	9*	4*	3*	6*
Cell communication	3*	7*	5*	5*	1	3*
Natural killer cell mediated immunity	2*	0	0	1*	1*	1*
Ligand-mediated signaling	2*	1	2	2*	1	2*
Cell adhesion-mediated signaling	0	3*	2	2*	0	2*
Cytokine and chemokine mediated signaling pathway	1	1	2*	2*	1*	1
Cell motility	2*	3*	1	1	1	1
Signal transduction	5	13*	10*	5	1	4
Cytokine/chemokine mediated immunity	1	0	1	2*	1*	0
Apoptosis	0	2	2	1	2*	3*
B-cell- and antibody-mediated immunity	2*	1	1	1	0	0
Cell cycle control	3*	0	0	0	0	0
Cell cycle	3*	1	0	0	0	0
Oncogenesis	2*	1	0	0	0	0
Cell structure and motility	2	6*	1	2	1	1
Intracellular protein traffic	0	4*	2	1	0	1
Cell structure	0	3*	0	1	0	0
Cell adhesion	0	3*	2	2	0	1
Endocytosis	0	3*	1	0	0	1
Proteolysis	0	2	1	2	2*	2
Cell surface receptor mediated signal transduction	2	4	4	2	1	3*
Sensory perception	0	1	0	0	0	2*

Table 3 Linear regression model of histological and clinical parameters to predict creatinine values of one year after engraftment.

	parameter estimate	p-value	adj R^2
intercept	0.245	**0.0051**	
CADI 2 vs CADI 1	0.179	0.0816	
CADI 3 vs CADI 1	0.369	**0.0115**	
PRA (%)	0.005	**0.0387**	
BCAR	0.052	0.6257	
per HLA-MM	0.033	0.2381	
total		**0.0231**	**0.1434**

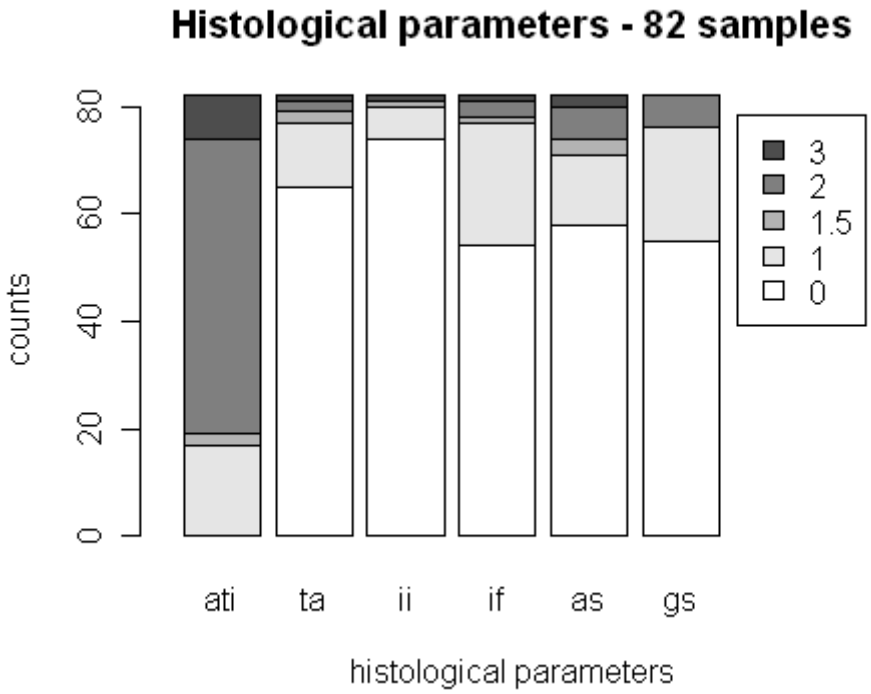
The adjusted R^2 provides an estimate of the variability of one year creatinine. It is penalized for additional covariables fitted into the model. The use of CADI scores only without the additional parameters resulted in an adjusted R^2 of 0.08.

Table 4 Linear regression using molecular biomarkers to predict creatinine values of one year after engraftment.

	parameter estimate	p-value	adj R^2
intercept	0.353	0.0075	
NLRP2 (AU)	0.076	0.0769	
RGS5 (AU)	0.155	**0.0009**	
IGJ (AU)	0.039	0.0780	
total		**< 0.0001**	**0.281**

Biomarkers units represent relative expression values as compared to the Stratagene Universal human reference RNA. AU … arbitrary units

Figure 1


Histological parameters - 82 samples
counts
0
20
40
60
80
ati
ta
ii
if
as
gs
histological parameters
3
2
1.5
1
0

Supplementary Table 1 Linear regression model of histological parameters represented as the CADI score to predict creatinine values of one year after engraftment.

	parameter estimate	**p-value**	**adj R^2**
intercept	0.388	**< 0.0001**	
CADI 2 vs CADI 1	0.176	0.0880	
CADI 3 vs CADI 1	0.319	**0.0256**	
total		**0.0398**	**0.077**

Supplementary table 2 Model selection process with the seven molecular markers as independent variables to predict creatinine values one year after engraftment.

No. of genes	adj R^2	R^2	Variables in Model
4	0.2840	0.3333	SELL NLRP2 RGS5 IGJ
3	0.2815	0.3187	NLRP2 RGS5 IGJ
5	0.2712	0.3340	SELL NLRP2 RGS5 IGJ IGL@
5	0.2706	0.3335	RGS1 SELL NLRP2 RGS5 IGJ
5	0.2705	0.3334	SELL NLRP2 RGS5 IGJ IL7R
4	0.2702	0.3205	RGS1 NLRP2 RGS5 IGJ
4	0.2688	0.3192	NLRP2 RGS5 IGJ IGL@
4	0.2684	0.3189	NLRP2 RGS5 IGJ IL7R
3	0.2672	0.3051	NLRP2 RGS5 IGL@
4	0.2593	0.3104	SELL NLRP2 RGS5 IGL@
6	0.2574	0.3343	RGS1 SELL NLRP2 RGS5 IGJ IGL@
5	0.2573	0.3213	RGS1 NLRP2 RGS5 IGJ IGL@
6	0.2572	0.3340	SELL NLRP2 RGS5 IGJ IL7R IGL@
5	0.2570	0.3210	RGS1 NLRP2 RGS5 IGJ IL7R
6	0.2566	0.3335	RGS1 SELL NLRP2 RGS5 IGJ IL7R
5	0.2554	0.3196	NLRP2 RGS5 IGJ IL7R IGL@
4	0.2540	0.3055	RGS1 NLRP2 RGS5 IGL@
4	0.2536	0.3051	NLRP2 RGS5 IL7R IGL@
2	0.2529	0.2787	NLRP2 RGS5
2	0.2526	0.2784	RGS5 IGJ
4	0.2502	0.3019	SELL RGS5 IGJ IL7R
3	0.2500	0.2888	SELL RGS5 IGJ
3	0.2480	0.2869	RGS5 IGJ IL7R
5	0.2454	0.3105	SELL NLRP2 RGS5 IL7R IGL@
5	0.2453	0.3104	RGS1 SELL NLRP2 RGS5 IGL@
6	0.2438	0.3220	RGS1 NLRP2 RGS5 IGJ IL7R IGL@
7	0.2429	0.3343	RGS1 SELL NLRP2 RGS5 IGJ IL7R IGL@
3	0.2424	0.2816	NLRP2 RGS5 IL7R
3	0.2408	0.2800	RGS1 NLRP2 RGS5
3	0.2402	0.2795	RGS5 IGJ IGL@
5	0.2400	0.3055	RGS1 NLRP2 RGS5 IL7R IGL@
3	0.2394	0.2787	SELL NLRP2 RGS5
3	0.2391	0.2784	RGS1 RGS5 IGJ
5	0.2378	0.3035	RGS1 SELL RGS5 IGJ IL7R
4	0.2375	0.2901	SELL RGS5 IGJ IGL@
4	0.2372	0.2898	RGS1 SELL RGS5 IGJ
5	0.2364	0.3022	SELL RGS5 IGJ IL7R IGL@

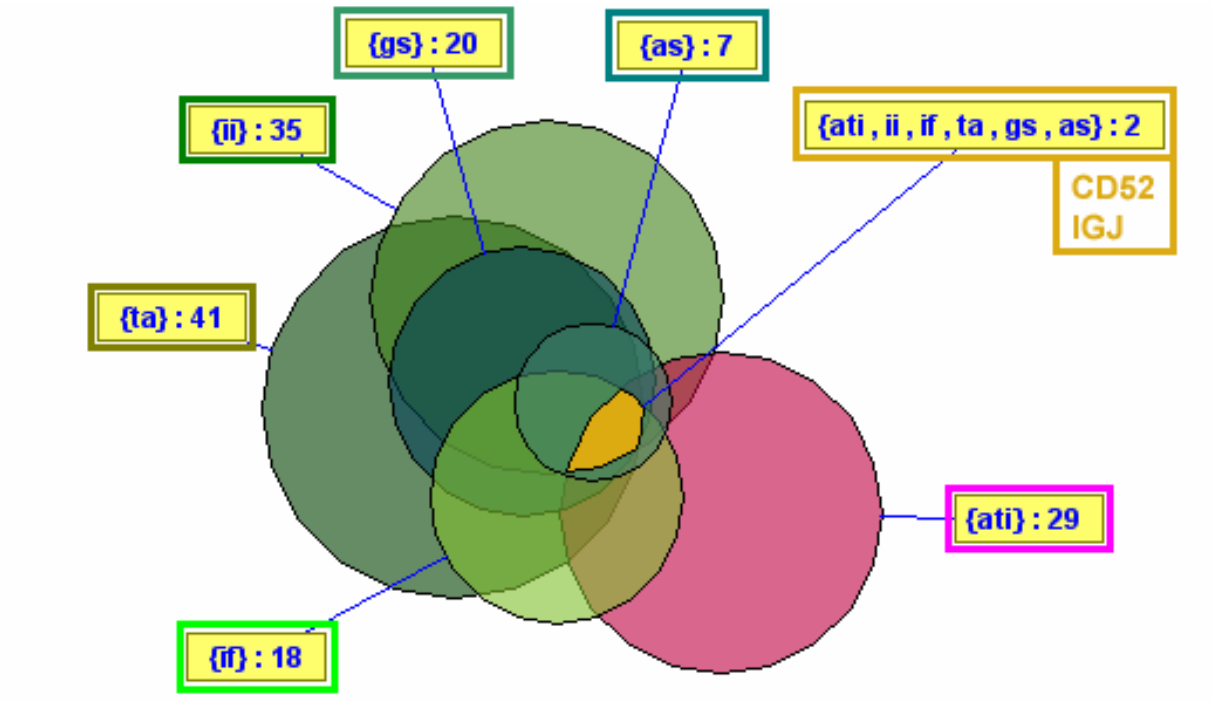
2	0.2356	0.2619	RGS5 IGL@
4	0.2344	0.2872	RGS5 IGJ IL7R IGL@
4	0.2341	0.2869	RGS1 RGS5 IGJ IL7R
3	0.2336	0.2732	RGS5 IL7R IGL@
6	0.2310	0.3105	RGS1 SELL NLRP2 RGS5 IL7R IGL@
4	0.2299	0.2830	RGS1 NLRP2 RGS5 IL7R
4	0.2290	0.2821	SELL NLRP2 RGS5 IL7R
4	0.2272	0.2805	RGS1 SELL NLRP2 RGS5
2	0.2268	0.2535	RGS5 IL7R
4	0.2261	0.2795	RGS1 RGS5 IGJ IGL@
4	0.2256	0.2790	SELL RGS5 IL7R IGL@
3	0.2242	0.2644	SELL RGS5 IGL@
5	0.2240	0.2909	RGS1 SELL RGS5 IGJ IGL@
6	0.2234	0.3037	RGS1 SELL RGS5 IGJ IL7R IGL@
3	0.2226	0.2628	RGS1 RGS5 IGL@
4	0.2203	0.2741	RGS1 RGS5 IL7R IGL@
5	0.2200	0.2872	RGS1 RGS5 IGJ IL7R IGL@
3	0.2187	0.2591	RGS1 RGS5 IL7R
5	0.2174	0.2848	RGS1 SELL NLRP2 RGS5 IL7R
5	0.2147	0.2824	RGS1 SELL RGS5 IL7R IGL@
3	0.2136	0.2543	SELL RGS5 IL7R
4	0.2124	0.2667	RGS1 SELL RGS5 IGL@
1	0.2110	0.2246	RGS5
4	0.2087	0.2633	RGS1 SELL RGS5 IL7R
2	0.2065	0.2339	RGS1 RGS5
2	0.1979	0.2255	SELL RGS5
3	0.1922	0.2340	RGS1 SELL RGS5
2	0.1545	0.1837	IGJ IL7R
2	0.1521	0.1814	IL7R IGL@
3	0.1503	0.1942	RGS1 IGJ IL7R
3	0.1492	0.1932	RGS1 IL7R IGL@
3	0.1490	0.1930	IGJ IL7R IGL@
3	0.1433	0.1876	NLRP2 IGJ IL7R
3	0.1427	0.1870	NLRP2 IL7R IGL@
4	0.1416	0.2008	RGS1 IGJ IL7R IGL@
3	0.1412	0.1856	NLRP2 IGJ IGL@
2	0.1408	0.1705	NLRP2 IGL@
4	0.1393	0.1987	NLRP2 IGJ IL7R IGL@
3	0.1392	0.1837	SELL IGJ IL7R
3	0.1372	0.1818	SELL IL7R IGL@
4	0.1371	0.1966	RGS1 NLRP2 IGJ IL7R
4	0.1370	0.1965	RGS1 NLRP2 IL7R IGL@
4	0.1366	0.1962	RGS1 SELL IGJ IL7R
2	0.1362	0.1660	NLRP2 IGJ
2	0.1349	0.1648	IGJ IGL@

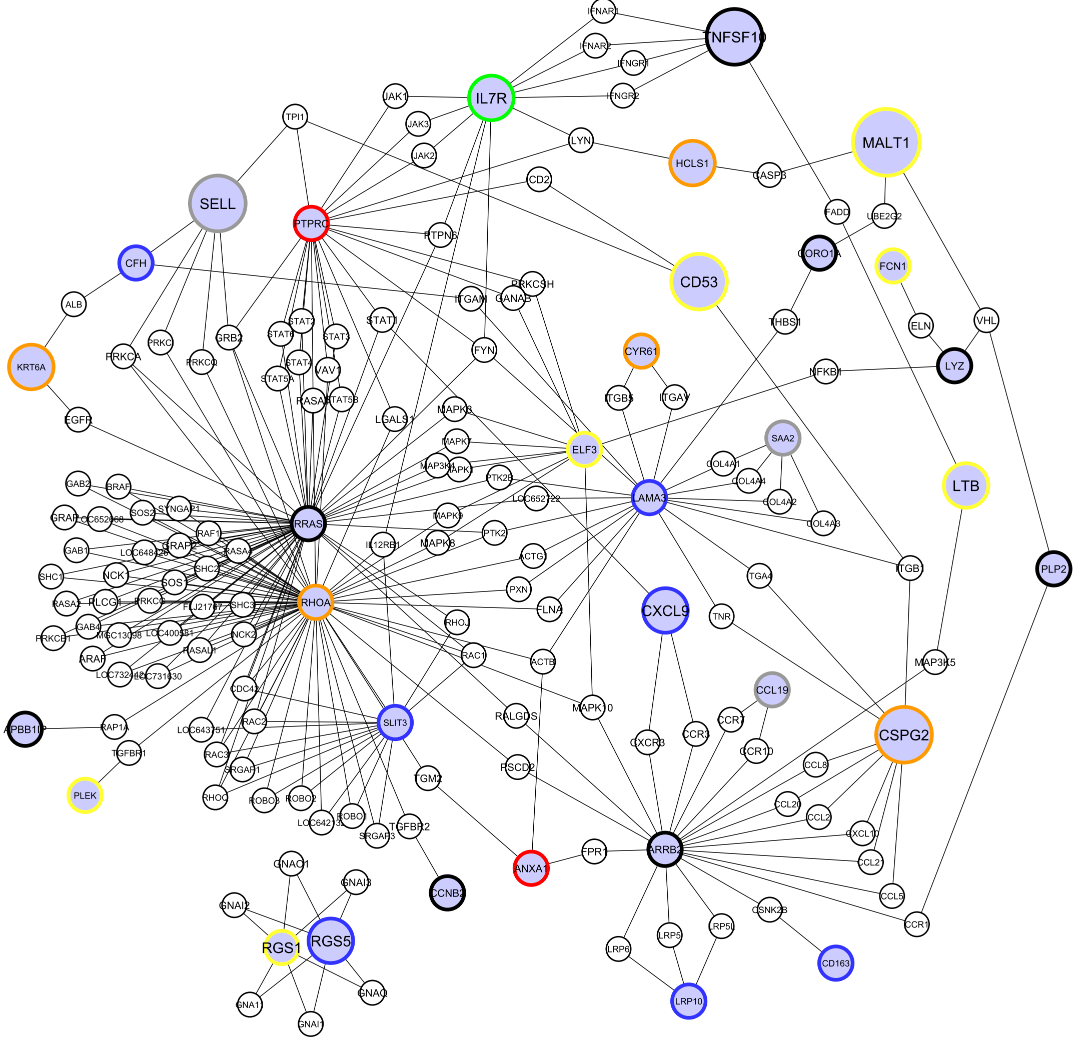
3	0.1347	0.1794	RGS1 NLRP2 IGL@
4	0.1340	0.1937	RGS1 SELL IL7R IGL@
4	0.1334	0.1932	SELL IGJ IL7R IGL@
1	0.1321	0.1471	IGL@
2	0.1316	0.1616	RGS1 IGL@
4	0.1306	0.1906	RGS1 NLRP2 IGJ IGL@
1	0.1302	0.1452	IGJ
2	0.1302	0.1602	RGS1 IL7R
3	0.1301	0.1751	SELL NLRP2 IGL@
3	0.1300	0.1750	RGS1 NLRP2 IGJ
5	0.1294	0.2045	RGS1 NLRP2 IGJ IL7R IGL@
2	0.1290	0.1590	RGS1 IGJ
3	0.1285	0.1736	RGS1 IGJ IGL@
4	0.1278	0.1879	SELL NLRP2 IL7R IGL@
5	0.1277	0.2029	RGS1 SELL IGJ IL7R IGL@
4	0.1274	0.1876	SELL NLRP2 IGJ IL7R
4	0.1262	0.1865	SELL NLRP2 IGJ IGL@
5	0.1231	0.1987	SELL NLRP2 IGJ IL7R IGL@
3	0.1231	0.1684	SELL NLRP2 IGJ
2	0.1225	0.1527	SELL IGL@
5	0.1221	0.1978	RGS1 SELL NLRP2 IGJ IL7R
5	0.1209	0.1967	RGS1 SELL NLRP2 IL7R IGL@
3	0.1203	0.1658	SELL IGJ IGL@
4	0.1199	0.1806	RGS1 SELL NLRP2 IGL@
2	0.1176	0.1480	SELL IGJ
3	0.1168	0.1625	RGS1 SELL IGL@
3	0.1149	0.1607	RGS1 SELL IL7R
3	0.1147	0.1604	RGS1 NLRP2 IL7R
5	0.1143	0.1906	RGS1 SELL NLRP2 IGJ IGL@
4	0.1142	0.1753	RGS1 SELL NLRP2 IGJ
6	0.1141	0.2057	RGS1 SELL NLRP2 IGJ IL7R IGL@
3	0.1133	0.1591	RGS1 SELL IGJ
4	0.1124	0.1736	RGS1 SELL IGJ IGL@
1	0.1047	0.1201	IL7R
2	0.1026	0.1336	SELL IL7R
4	0.0990	0.1611	RGS1 SELL NLRP2 IL7R
2	0.0897	0.1211	NLRP2 IL7R
3	0.0891	0.1362	SELL NLRP2 IL7R
3	0.0701	0.1182	RGS1 SELL NLRP2
2	0.0684	0.1005	RGS1 SELL
2	0.0681	0.1002	RGS1 NLRP2
1	0.0675	0.0836	RGS1
2	0.0648	0.0971	SELL NLRP2
1	0.0547	0.0710	SELL
1	0.0162	0.0332	NLRP2

Supplementary table 3 Linear regression using molecular biomarkers in combination with clinical parameters to predict creatinine values one year after engraftment.

	parameter estimate	p-value	adj R^2
intercept	0.321	0.0336	
NLRP2	0.058	0.2226	
RGS5	0.133	**0.0041**	
IGJ	0.054	0.0224	
PRA (%)	0.006	**0.0221**	
BCAR	0.030	0.7585	
per HLA-MM	0.028	0.2674	
total		**0.0003**	**0.3127**

Webfigure 1

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MALT1
CD2
SELL
PTPRC
PTPN6
FADD
UBE2G2
CORO1A
FCN1
CFH
PRKCSH
CD53
ITGAM
GANAB
ALB
STAT1
STAT2
STAT6
STAT3
STAT4
STAT5A
STAT5B
VAV1
RASA1
GRB2
PRKC
PRKCA
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FYN
THBS1
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KRT6A
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MAPK3
MAPK7
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PTK2B
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COL4A4
GAB2
BRAF
SOS2
SYNGAP1
RRAS
LAMA3
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COL4A3
LTB
GRAP
RAF1
MAPK9
PTK2
IL12RB1
MAPK8
GAB1
GRAP2
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SHC2
ACTG1
SHC1
NCK1
SOS1
PXN
ITGB1
PLP2
RASA3
PLCG1
PRKCE
SHC3
RHOA
FLNA
CXCL9
TNR
PRKCB1
GAB4
NCK2
RASAL1
RHOJ
ARAF
RAC1
ACTB
MAP3K5
CCL19
MAPK10
RALGDS
CDC42
APBB1IP
RAP1A
RAC2
SLIT3
CCR7
CSPG2
TGFBR1
RAC3
CXCR3
CCR3
CCR10
SRGAP1
TGM2
PSCD2
CCL8
PLEK
RHOC
ROBO3
ROBO2
ROBO1
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CCNB2
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GNAI2
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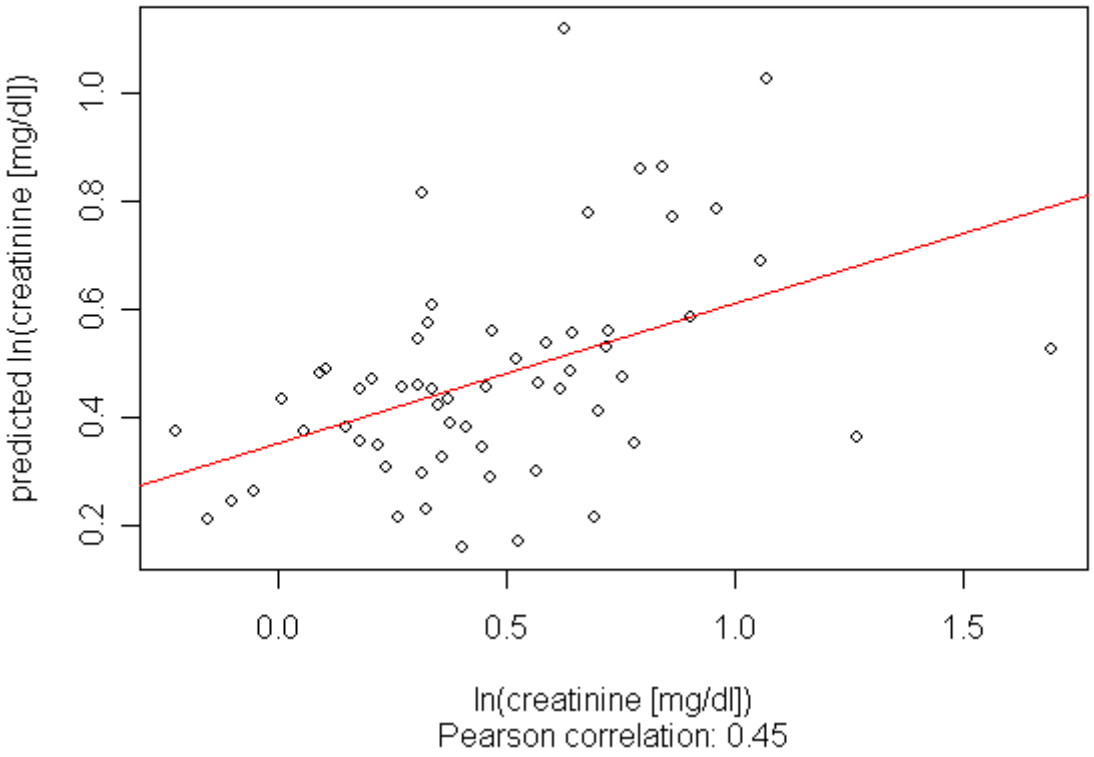
Webfigure 3


predicted ln(creatinine [mg/dl])
0.2
0.4
0.6
0.8
1.0
0.0
0.5
1.0
1.5
ln(creatinine [mg/dl])
Pearson correlation: 0.45