Supplemental Digital Content

Figure S1. Raw data of miR expression profiling in renal biopsies (A) and urine sediments (B). Inter-plate corrected Cq values are shown, median miR Cq values (used for normalization) are displayed with red lines. AR: acute rejection. C: controls (no rejection).

Figure S2. Marker levels are not dependent on proteinuria and eGFR. (A) Subgroups of rejection and no-rejection were compared for patients having similar urinary protein level (left panel) or having similar eGFR (right panel). Significant differences for marker levels were still found between rejection and no-rejection. (B) Correlation plots (spearman rank tests) of eGFR with marker levels within the total no-rejection group (upper panel) or within the rejection group (lower panel). No significant correlations were found, except CXCL-9 versus eGFR, which showed a weak rho correlation coefficient. (C) In the whole group, urine CXCL-9 protein concentrations correlated highly with CXCL-9 protein levels that had been corrected for urinary creatinine excretion, showing minimal effect of urinary output on biomarker levels.

Figure S3. MiR- and CXCL-9 levels in according to rejection severity. Medians with 95% confidence interval are shown. Sample sizes (TCMR / AMR / mixed) are 81 / 10

/ 7 for miRs and 88 / 13 / 7 for CXCL-9. TCMR: T-cell-mediated rejection. AMR: antibody-mediated rejection.

Figure S4. Expression of miRs in urine sediments of recipients with rejection, BKVN infection or CMV infection. Scatter plots of relative miR expression levels are shown on a logarithmic scale. Medians are presented by horizontal lines. Within the BKV group, samples collected at the Leiden University Medical Center are presented as filled dots and samples collected at the Radboud University Medical Center are presented as open circles. Differences were tested by Mann-Whitney U tests. * indicates P < 0.001.

Table S1. MiR expression profiling in renal biopsies [1]

MicroRNA	Rej vs. No-rej	MWU	FDR
hsa-miR-155-5p	6.20	0.001	0.254
hsa-miR-142-3p	4.79	0.001	0.127
hsa-miR-21-5p	2.94	0.002	0.169
hsa-miR-615-3p	0.46	0.002	0.127
hsa-miR-150-5p	5.27	0.003	0.152
hsa-miR-192-5p	0.55	0.003	0.127
hsa-miR-378a-3p	0.49	0.004	0.145
hsa-miR-29b-2-5p	0.40	0.004	0.127
hsa-miR-124-3p	0.22	0.004	0.113
hsa-miR-146b-5p	2.47	0.004	0.102
hsa-miR-29c-5p	0.28	0.004	0.092
hsa-miR-142-5p	3.47	0.004	0.085
hsa-miR-193b-3p	0.47	0.005	0.098
hsa-miR-223-3p	3.98	0.005	0.091
hsa-miR-188-5p	0.44	0.005	0.085
hsa-miR-30d-5p	0.63	0.008	0.127
hsa-miR-874	0.26	0.008	0.120
hsa-miR-22-3p	0.32	0.008	0.113
hsa-miR-148a-3p	0.56	0.008	0.107
hsa-miR-215	0.73	0.009	0.114
hsa-miR-30c-5p	0.54	0.011	0.133
hsa-miR-130a-3p	0.29	0.011	0.127
hsa-miR-491-5p	0.68	0.011	0.121
hsa-miR-18b-5p	1.33	0.011	0.116
hsa-let-7c	0.59	0.011	0.112
hsa-miR-365a-3p	0.62	0.011	0.107
hsa-miR-30c-2-3p	0.34	0.011	0.103
hsa-miR-378a-5p	0.39	0.011	0.100
hsa-miR-221-3p	0.59	0.015	0.131
hsa-miR-500a-5p	0.69	0.015	0.127
hsa-miR-126-3p	0.68	0.015	0.123
hsa-miR-940	0.52	0.015	0.119
hsa-miR-187-3p	0.38	0.015	0.115
hsa-miR-107	0.51	0.015	0.112
hsa-miR-29c-3p	0.58	0.015	0.109
hsa-let-7d-5p	0.60	0.015	0.106
hsa-miR-145-5p	0.49	0.015	0.103
hsa-miR-502-5p	0.33	0.015	0.100
hsa-miR-27b-3p	0.74	0.015	0.098
hsa-miR-193b-5p	0.31	0.015	0.095
hsa-miR-190a	0.26	0.015	0.093
hsa-miR-342-5p	1.63	0.018	0.109

hsa-miR-887	0.65	0.018	0.106
hsa-miR-7-1-3p	3.71	0.020	0.115
hsa-miR-194-5p	0.68	0.021	0.119
hsa-miR-362-5p	0.62	0.021	0.116
hsa-miR-199a-5p	0.40	0.021	0.113
hsa-miR-26b-3p	0.76	0.021	0.111
hsa-miR-99a-3p	0.26	0.021	0.109
hsa-miR-101-5p	0.16	0.021	0.107
hsa-miR-338-3p	0.39	0.024	0.120
hsa-miR-98-5p	0.49	0.028	0.137
hsa-miR-204-5p	0.61	0.028	0.134
hsa-miR-324-5p	0.71	0.028	0.132
hsa-miR-214-3p	0.51	0.028	0.129
hsa-miR-324-3p	0.62	0.028	0.127
hsa-miR-744-5p	0.56	0.028	0.125
hsa-let-7a-5p	0.47	0.028	0.123
hsa-miR-532-3p	0.64	0.028	0.121
hsa-let-7e-5p	0.57	0.028	0.119
hsa-miR-106b-3p	0.47	0.028	0.117
hsa-miR-143-3p	0.61	0.037	0.152
hsa-miR-141-3p	0.80	0.037	0.149
hsa-miR-342-3p	1.93	0.037	0.147
hsa-miR-196a-5p	0.64	0.037	0.145
hsa-miR-29b-3p	0.47	0.037	0.142
hsa-miR-30b-5p	0.59	0.037	0.140
hsa-miR-135a-5p	0.62	0.037	0.138
hsa-miR-424-3p	0.57	0.037	0.136
hsa-miR-149-5p	0.58	0.037	0.134
hsa-let-7e-3p	0.59	0.037	0.132
hsa-miR-1247-5p	0.53	0.037	0.131
hsa-miR-551b-3p	0.21	0.037	0.129
hsa-miR-431-5p	0.43	0.037	0.127
hsa-miR-29a-3p	0.65	0.043	0.146
hsa-miR-99b-3p	0.60	0.043	0.144
hsa-miR-497-5p	0.35	0.049	0.162
hsa-miR-133a	0.32	0.049	0.160
hsa-miR-128	0.74	0.049	0.158
hsa-miR-151a-5p	0.68	0.049	0.156
hsa-miR-139-5p	0.59	0.049	0.154
hsa-miR-502-3p	0.60	0.049	0.152
hsa-miR-1972	1.82	0.049	0.150
hsa-miR-133b	0.53	0.049	0.148
hsa-miR-188-3p	0.35	0.049	0.146

[1] The second column shows fold change differences between rejection- and no-rejection group. MWU column shows P values of the Mann-Whitney U test. FDR column shows P values after correction for multiple comparisons by false discovery rate approach.

Table S2. MiR expression profiling in urine sediments [1]

MicroRNA	Rej vs. No-rej	MWU	FDR
hsa-miR-92b-5p	2.97	0.001	0.568
hsa-miR-296-3p	6.18	0.006	1.704
hsa-miR-20b-5p	3.02	0.012	2.272
hsa-miR-95	0.23	0.012	1.704
hsa-mir-1908	15.48	0.012	1.363
hsa-miR-221-5p	0.25	0.016	1.515
hsa-miR-34b-5p	0.27	0.016	1.298
hsa-miR-200c-5p	0.30	0.016	1.136
hsa-mir-203a	0.13	0.021	1.325
hsa-miR-33a-3p	0.33	0.021	1.193
hsa-miR-153	0.04	0.021	1.084
hsa-miR-224-5p	0.28	0.024	1.136
hsa-miR-25-3p	3.09	0.027	1.180
hsa-miR-141-3p	0.29	0.027	1.095
hsa-miR-126-5p	9.85	0.027	1.022
hsa-mir-147a	0.23	0.027	0.959
hsa-mir-184	0.15	0.027	0.902
hsa-miR-125b-2-3p	0.23	0.027	0.852
hsa-miR-452-5p	0.21	0.036	1.076
has-miR-615-3p	0.60	0.036	1.022
hsa-mir-375	0.22	0.036	0.974
hsa-miR-708-5p	0.41	0.036	0.929
hsa-miR-126-3p	6.97	0.036	0.889
hsa-miR-410	3.06	0.036	0.852
hsa-miR-149-5p	0.44	0.036	0.818
hsa-miR-486-3p	8.82	0.036	0.786
hsa-mir-451a	9.82	0.046	0.968
has-miR-195-3p	1.96	0.046	0.933
hsa-miR-1237-3p	0.21	0.046	0.901
hsa-miR-891a	3.13	0.046	0.871
hsa-miR-573	0.35	0.046	0.843

[1] The second column shows fold change differences between rejection- and no-rejection group. MWU column shows P values of the Mann-Whitney U test. FDR column shows P values after correction for multiple comparisons by false discovery rate approach.

Table S3. The rationale for the selection of miRs studied in the independent cohort of urines.

MicroRNA	Rationale
miR-92b-5p	Increased during rejection in urines.
miR-296-3p	Increased during rejection in urines.
miR-25-3p	Increased during rejection in urines.
miR-203a	Decreased during rejection in urines.
miR-224-5p	Decreased during rejection in urines.
miR-149-5p	Decreased during rejection in both biopsies and urines.
miR-141-3p	Decreased during rejection in both biopsies and urines.
miR-615-3p	Decreased during rejection in both biopsies and urines.
miR-126-3p	Decreased during rejection in biopsies, but increased in urines.
miR-210-3p	Shown to be decreased in urine in previous study [9].
miR-155-5p	Increased in rejection biopsies. Shown in previous biopsy studies [5, 22].
miR-142-3p	Increased in rejection biopsies. Shown in previous biopsy studies [5, 22].
miR-21-5p	Increased in rejection biopsies. Shown in previous biopsy studies [5, 22].
miR-142-5p	Increased in rejection biopsies. Shown in previous biopsy studies [5, 22].
miR-223-3p	Increased in rejection biopsies. Shown in previous biopsy studies [5, 22].