Table S1 Urinary microvesicles of patients with diabetic nephropathy and proteinuric controls

	DN (n=49)		Proteinuric controls (MN/MCD, n=29)		P
	DN alone (n=42)	DN+MN (n=7)	DM+MN/MCD (n=10)	MN/MCD (n=19)	
Podocyte MVs					
Nephrin[+]	142.9 (47.2–834.7)	73.4 (9.7–325.7)	15.6 (9.1,72.9)*	33.6 (19.6–52.5)*	<0.001
Podocin[+]	226.9 (92.7–852.6)	97.8 (18.9–815.7)	54.8 (16.2–248.3)*	75.3 (24.9–140.5)*	0.001
Nephrin[+] podocin[+]	44.5 (8.9–383.0)	14.6 (2.7–68.0)	7.1 (3.4–40.0)*	12.0 (1.9–19.5)*	0.001
Proximal tubular MVs					
AQP1[+]	230.3 (123.0–913.4)	395.8 (19.4–1,588.3)	72.3 (36.8–238.4)	105.9 (45.2–256.7)	0.053
Megalin[+]	219.6 (78.5–650.8)	188.4 (53.1–671.7)	56.97 (35.7–107.9)*	95.1 (16.3–227.0)	0.031
AQP1^{+}megalin^{+}	39.4 (5.0–148.3)	148.3 (8.2–366.1)	1.7 (0.8–9.3)*[†]	10.9 (2.1–46.0)	0.017
Endothelial MVs					
CD31^{+}	52.4 (17.5–353.1)	45.24 (20.1–237.2)	18.1 (13.3–87.4)	71.0 (13.2–108.2)	0.549
CD144^{+}	376.0 (134.8–2,782.4)	366.6 (49.1–1,309.1)	78.3 (8.6–332.8)*	298.8 (71.4–927.1)	0.028
CD31^{+}CD144^{+}	33.4 (6.9–123.4)	10.3 (3.7–58.4)	11.6 (1.2–65.1)	16.0 (4.1–59.0)	0.281

DN, diabetic nephropathy; DM, diabetic mellitus; MN, membranous nephropathy; MCD, minimal change disease; AQP1, Aquaporin 1; MVs, microvesicles. MVs levels are presented as median (interquartile range) 10^3/μmol creatinine and analyzed using ANOVA followed by Student's t test with Bonferroni correction. P values listed in the table are ANOVA comparing four groups. *P<0.05 compared to pure DN. [†]P<0.05 compared to DN+MN.

Table S2 Combination of urinary nephrin+ MVs and diabetic retinopathy in relation to diabetic nephropathy

Combination of urinary nephrin[+] MV and DR	Diabetic nephropathy		Total
	Positive	Negative	
Positive	40	3	43
Negative	5	26	31
Total	45	29	

MVs, microvesicles; DR, diabetic retinopathy.

Table S3 Comparisons of clinical-pathological characteristics among diabetic nephropathy patients of different classification

	DN-I (n=4)	DN-II (n=12)	DN-III (n=29)	DN-IV (n=4)	P value
Gender male, n (%)	2 (50.0)	10 (83.3)	23 (79.3)	3 (75.0)	0.564
Age, years	54.3±12.7	51.7±9.9	44.6±11.0	44.0±10.4	0.138
Urine protein, g/24 h	6.2±2.8	4.3±3.9	6.7±4.1	7.4±3.2	0.299
Serum creatinine, μmol/L	81.4 (70.4–90.5)	109.1 (87.1–151.7)	128.2 (95.9–283.3)*	477.2 (265.5–689.0)*†‡	0.001
Pathological features					
Glomerular lesions					
Glomerular sclerosis	0 (0–0)	8.0 (0–15.6)	6.7 (0–21.5)	55.6 (38.1–60.5)*†‡	0.001
Ischemic sclerosis	1.6 (0–5.0)	17.7 (10.1–30.4)*	4.8 (0–14.2)†	15.5 (7.7–30.6)	<0.001
Mesangial expansion	1 (0.25–1)	2 (2–2.75)*	3 (3–3)*†	3 (2.25–3)*	<0.001
Microaneuysms or mesangiolysis, n (%)	1 (25.0)	3 (25.0)	23 (29.0)*†	3 (75.0)*†	0.004
Kimmelstiel–Wilson lesion, n (%)	0	0	29 (100.0)*†	3 (75.0)*†	<0.001
Tubulointerstitial lesions					
Tubular injury score	2 (2–3)	2 (2–2)	2 (2–3)	2.5 (2–4)	0.657
Chronic tubulointersitial score	2 (2–2)	2.5 (2–4)	4 (2–5)*	6 (6–8)*†‡	0.024
Interstitial inflammation score	1 (1–2)	1 (1–2)	2 (1–2)	3 (3–4)*†‡	0.010
Vasicular score	1 (1–2)	2 (2–3)*	3 (2–3)*	3 (3–3)*	0.023

Normal range: serum creatinine, 44–133 mmol/L; urine protein, 0–0.15 g/24 h. Data are presented as n (%), mean ± SD or median (interquartile range) and analyzed using Student's *t* test, or Mann-Whitney rank sum test or a chi-square test wherever applicable without correction. P values listed in the table are ANOVA comparing the four groups. *P<0.05 compared to DN I; †P<0.05 compared to DN II; ‡P<0.05 compared to DN III. DN, diabetic nephropathy.

Table S4 Correlations between urinary microvesicles and pathological features in diabetic nephropathy

	Glomerular sclerosis		Ischemic sclerosis		Mesangial expansion		Tubular injury score		Chronic tubulointerstitial score		Interstitial inflammation score		Vascular score	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Podocyte MVs														
nephrin+	0.151	0.300	0.109	0.462	0.278	0.053	0.098	0.501	–0.059	0.687	–0.109	0.456	0.217	0.135
podocin+	0.036	0.808	0.118	0.430	0.209	0.153	0.203	0.165	–0.141	0.340	–0.202	0.169	0.225	0.124
nephrin+ podocin+	0.122	0.404	0.088	0.554	0.270	0.061	0.067	0.646	–0.075	0.609	–0.132	0.365	0.166	0.254
Proximal tubular MVs														
AQP1+	–0.058	0.694	0.161	0.276	0.277	0.054	0.458	<0.001	–0.070	0.635	–0.140	0.337	0.251	0.082
megalin+	0.088	0.549	0.065	0.659	0.404	0.004	0.352	0.013	–0.031	0.834	–0.096	0.512	0.442	<0.001
AQP1+ megalin+	–0.134	0.360	–0.020	0.893	0.354	0.013	0.349	0.014	–0.008	0.959	0.033	0.821	0.325	0.023
Endothelial MVs														
CD31+	0.065	0.669	–0.063	0.679	0.306	0.039	0.503	<0.001	0.100	0.507	0.022	0.884	0.349	0.017
CD144+	0.072	0.635	0.020	0.899	0.385	0.008	0.446	0.002	0.110	0.466	–0.005	0.975	0.473	<0.001
CD31+ CD144+	0.121	0.421	0.046	0.763	0.388	0.008	0.481	<0.001	0.138	0.361	0.039	0.797	0.391	0.007

DN, diabetic nephropathy; AQP1, Aquaporin 1; MVs, microvesicles.

Table S5 Ordinal regression models of microvesicles for pathological features in diabetic nephropathy

	Univariant			Multivariant Stepwise		
	OR	95% CI	P	OR	95% CI	P
Mesangial expansion						
Proximal tubular MVs						
lg(megalin$^+$)	3.5	1.3–9.4	0.015	2.0	0.7–6.3	0.219
lg(AQP1$^+$megalin$^+$)	2.5	1.2–5.5	0.021			
Endothelial MVs						
lg(CD31$^+$)	2.4	1.0–5.9	0.064			
lg(CD144$^+$)	2.6	1.1–6.2	0.025			
lg(CD31$^+$CD144$^+$)	2.8	1.2–7.0	0.024	2.2	0.8–6.0	0.140
Tubular injury score						
Proximal tubular MVs						
lg(AQP1$^+$)	5.2	1.8–15.6	0.003	2.9	0.9–9.7	0.078
lg(megalin$^+$)	3.0	1.1–8.0	0.027			
lg(AQP1$^+$megalin$^+$)	2.6	1.2–5.8	0.018			
Endothelial MVs						
lg(CD31$^+$)	5.4	2.0–15.0	0.001	3.9	1.3–11.3	0.013
lg(CD144$^+$)	3.5	1.5–8.0	0.003			
lg(CD31$^+$CD144$^+$)	3.5	1.5–8.3	0.005			
Vascular score						
Endothelial MVs						
lg(CD31$^+$)	2.5	1.1–5.7	0.035			
lg(CD144$^+$)	3.4	1.5–7.8	0.003	3.1	1.2–8.4	0.025
lg(CD31$^+$CD144$^+$)	2.6	1.2–5.6	0.020			
Proximal tubular MVs						
lg(megalin$^+$)	3.5	1.4–8.8	0.007	1.3	0.4–4.4	0.712
lg(AQP1$^+$megalin$^+$)	2.2	1.1–4.4	0.024			

AQP1, aquaporin 1; MVs, microvesicles; lg, log$_{10}$; CI, confidence interval; OR, odds ratio.

Table S6 Correlations between urinary microvesicles and three potential insults contributing to tubular injury

	Proteinuric controls						DN					
	Urine protein		Interstitial inflammation score		vascular score		Urine protein		Interstitial inflammation score		Vascular score	
	r	P	r	P	r	P	r	P	r	P	r	P
Proximal tubular MVs												
AQP1+	0.505	0.005	0.337	0.074	0.191	0.320	0.050	0.731	−0.067	0.649	0.251	0.082
megalin+	0.383	0.040	0.398	0.033	0.515	0.004	0.167	0.252	−0.119	0.415	0.442	0.001
AQP1+ megalin+	0.497	0.006	0.523	0.004	0.389	0.037	0.084	0.567	0.042	0.776	0.325	0.023
Podocyte MVs												
Nephrin+	0.201	0.296	0.424	0.022	0.246	0.198	0.067	0.650	−0.068	0.643	0.217	0.135
Podocin+	0.197	0.307	0.155	0.421	0.035	0.857	0.029	0.844	−0.146	0.323	0.225	0.124
Nephrin+ podocin+	−0.018	0.927	0.212	0.269	0.187	0.331	0.011	0.940	−0.074	0.613	0.166	0.254
Endothelial cell MVs												
CD31+	0.278	0.235	0.496	0.026	0.444	0.050	0.191	0.204	0.076	0.616	0.349	0.017
CD144+	0.338	0.145	0.407	0.075	0.350	0.131	0.156	0.301	0.049	0.747	0.473	0.001
CD31+CD144+	0.227	0.336	0.478	0.033	0.494	0.027	0.095	0.531	0.102	0.502	0.391	0.007

DN, diabetic nephropathy; AQP1, Aquaporin 1; MVs, microvesicles.

Table S7 Vascular score between diabetic nephropathy and proteinuric controls with low proteinuria and interstitial inflammation

	Vascular score
Urine protein ≤4 g	
Proteinuric controls (n=12)	1 (0.25–2)
DN (n=20)	2 (1–3)
P	0.024
Interstitial inflammation score =1	
Proteinuric controls (n=18)	1 (1–1.3)
DN (n=23)	2 (1–3)
P	0.002

DN, diabetic nephropathy.