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RUNNING TITLE: Sema3A and diabetic nephropathy

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Supplementary Table 1. Patient's characteristics and relationship of albumin creatinine categories based on ACR with variables of interest.

	Albumin creatinine				
	Control	Normo	Micro	Macro	
Dependent Variables	**n=42**	**n=40**	**n=38**	**n=9**	**p-value**
Sex, n (% Female) #	20 (48)	10 (25)	13 (34)	2 (22)	0.15
Age, y *	53.3±13.1 b	59.0±12.4 a,b	64.0±11.9 a	62.3±15.6 a,b	0.0028
Weight, kg *	84.3±18.3	91.3±14.8	93.9±18.0	92.4±10.3	0.065
Height, m *	1.76±0.08	1.75±0.07	1.72±0.09	1.73±0.09	0.092
BMI, kg/m^2 *	27.2±6.3 b	30.0±5.3 a,b	31.9±6.4 a	31.5±5.2 a,b	0.0055
SBP, mmHg *	131.9±16.7 b	138.7±15.4 b	141.8±17.5 b	155.5±15.7 a	0.0007
DBP, mmHg *	74.1±9.1	77.7±10.2	76.7±9.5	80.2±12.4	0.24
Hypertension, n (% Yes) #	14 (33)	33 (85)	35 (97)	8 (100)	<0.0001
Hypertension drug use, n (% Yes) #	6 (14)	27 (71)	33 (94)	8 (100)	<0.0001
Smoking, n (% Yes) #	9 (21)	4 (10)	11 (29)	3 (33)	0.16
Cardiovascular history, n (% Yes) #	0 (0)	9 (23)	16 (42)	6 (67)	<0.0001
Statin use, n (% Yes) #	8 (19)	17 (45)	21 (60)	6 (75)	0.0006
HbA1c, % Mean±SD *	5.37±0.33 b	7.67±1.06 a	7.69±1.33 a	7.74±0.76 a	<0.0001
Serum creatinine, µmol/L **	72 (54, 97) b	74 (54, 155) b	89 (55, 168) b	107 (72, 312) a	<0.0001
eGFR *	86.3±14.3 a	85.3±21.3 a	72.7±21.8 a	51.8±27.3 b	<0.0001
Urinary creatinine, mmol/L**	11.4 (3.7, 24) a	8.5 (1.5, 30) a,b	6.6 (2.6, 18) b	5.7 (2.3, 14) b	<0.0001
Urinary albumin, mg/L **	9.4 (2.2, 82) c	5.5 (2.1, 21) c	57 (16, 564) b	515 (171, 1738) a	<0.0001
Albumin creatinine, mg/mmol **	0.56 (0.29, 7.22) c	0.70 (0.17, 3.33) c	8.38 (3.40, 31.6) b	115.4 (41.8, 262.1) a	<0.0001

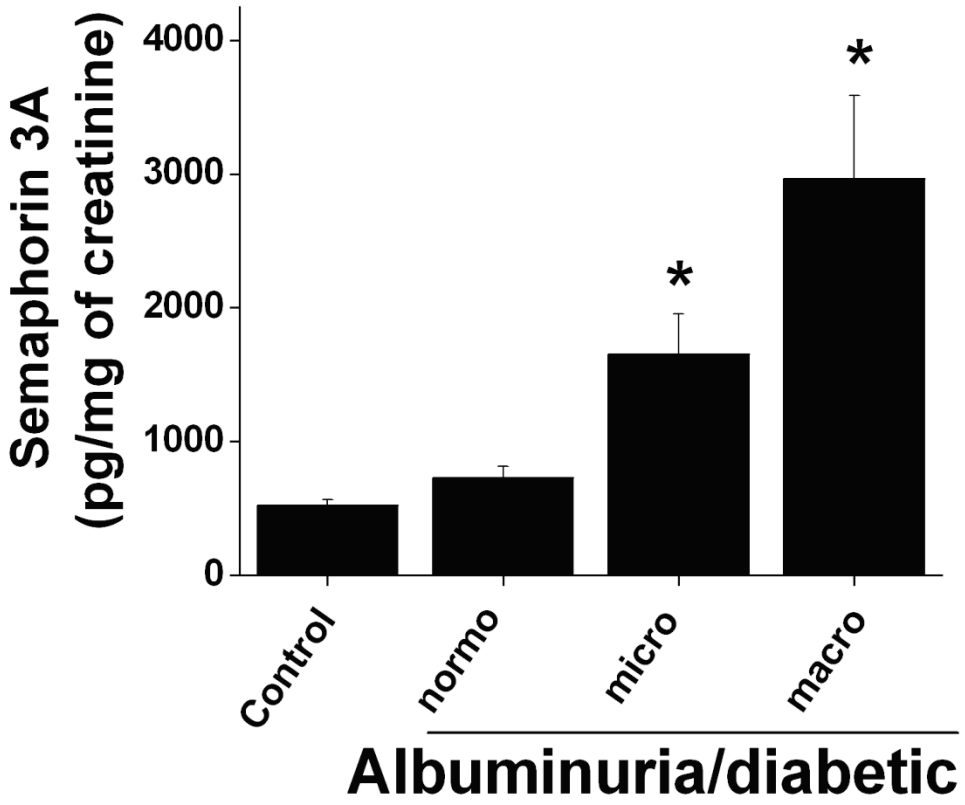
column %, p-value reflects result from chi-square test of Albumin creatinine group proportion equality
* Mean±SD, p-value reflects result from one-way ANOVA test for Albumin creatinine group mean equality
a,b,c Pair of means with different letters are significantly different, $p<0.05$, Tukey HSD
** Log value used in analysis, median (min, max) represented

Statistical analysis of supplementary table 1.

Data were assessed for normality and appropriate transformations were used when necessary. Median (min, max) is reported for continuous non-normal data, mean±SD is reported otherwise. Chi-square tests were used to assess the relationship between control and albuminuria groups with categorical variables. One-way ANOVA was used to assess the relationship of control and albuminuria groups with continuous variables. Tukey's multiple comparison tests were used to determine group differences for significant ANOVAs. To investigate the association between albumin creatinine ratio (albuminuria), eGFR, or hypertension status and log netrin-1 we performed a linear regression using log-netrin-1 as the dependent variable and the albuminuria eGFR or hypertension as dependent variables. Various

models were built to adjust for possible confounding. SAS© 9.3 (SAS Institute, Inc., Cary, NC) was used for all analyses.

Supplementary Figure S1.



Supplementary Figure 1. Sema3A excretion in non-diabetic controls and patients with different stages of diabetic nephropathy. Sema3A was quantified by ELISA as described in Methods. A significant increase in sema3A excretion was seen in patients with both microalbuminuria and macroalbuminuria. *, $p<0.001$ vs. non-diabetic control.

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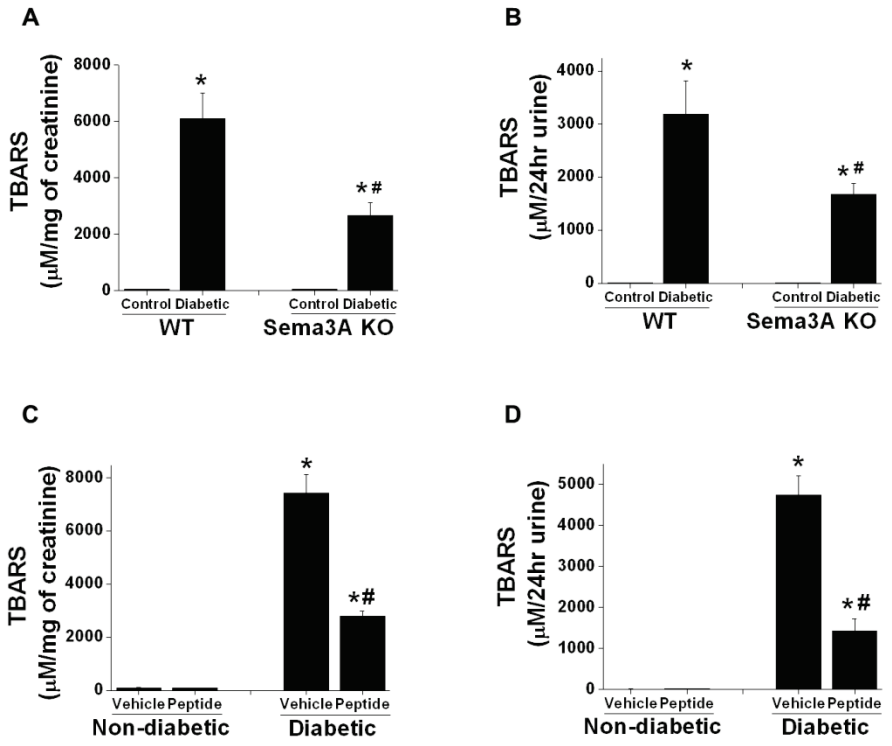
Supplementary Figure S2.



Supplementary Figure 2. Quantification of oxidative stress as measured by the excretion of thiobarbituric acid reactive substances (TBARS) in urine. TBARS excretion was normalized to 24hr urine (B&D) and to micromoles per mg of creatinine (A&C). WT diabetic mice showed increased excretion of TBARS in urine as compared to control (non-diabetic) mice, which was significantly suppressed by sema3A gene ablation (A & B). *, $P<0.001$ vs. control. #, $p<0.05$ vs. WT diabetic. n=4-6. Likewise, the increased excretion of TBARS in the urine of diabetic mice was significantly suppressed by treatment with the sema3A inhibitory peptide (C&D). *, $P<0.001$ vs. control. #, $p<0.001$ vs. WT diabetic. n=4-6.