Supplementary Materials

Table S1. Physiological parameters of WT and *Tlr2*$^{-/-}$ diabetic mice

DM duration		Body weight (g)	Glucose (mg/dl)	Water intake (ml)	Urine output (ml)
1 m	WT	19.0±0.9 (15)	588±15 (15)	19.2±2.5 (12)	8.6±1.3 (12)
	Tlr2$^{-/-}$	22.2±0.6**(13)	578±29 (13)	14.4.±1.7 (12)	6.1±1.1 (12)
2 m	WT	24.7±1.9 (9)	550±71 (5)	14.7±2.0 (9)	7.6±1.2 (9)
	Tlr2$^{-/-}$	24.0±0.8 (11)	656±71 (5)	13.5±2.0 (11)	5.0±1.0 (11)
3 m	WT	22.7±1.6 (3)	472±55 (3)	27.6±9.9 (3)	22±11.9 (3)
	Tlr2$^{-/-}$	22.6±1.6 (5)	358±30 (5)	18.75±3.0 (5)	10±5.6 (5)

***P* <0.01 relative to WT mice. Numbers in parentheses indicate the number of mice for each group.

Table S2. Physiological parameters of WT and *Tlr4*$^{-/-}$ diabetic mice

DM duration		Body weight (g)	Glucose (mg/dl)	Water intake (ml)	Urine output (ml)
1 m	WT	23.1±1.0 (10)	649±11 (10)	18.0±1.6 (10)	9.4±1.2 (10)
	Tlr4$^{-/-}$	22.2±1.2 (8)	609±34 (8)	15.3±2.0 (8)	7.0±1.5 (8)
2 m	WT	20.9±0.7 (10)	611±39 (10)	25.9±1.6 (10)	15.1±1.4 (10)
	Tlr4$^{-/-}$	23.4±0.7* (11)	571±33 (11)	14.2±2.4*** (11)	7.2±1.5** (11)
3 m	WT	21.4± 2.5 (4)	740±33 (4)	27.0±1.1 (4)	17.3±2.1 (4)
	Tlr4$^{-/-}$	25±1.3* (5)	644±75 (5)	17.3±7.1*(5)	8.3±4.4**(5)

*P <0.05, **P <0.01, and ***P <0.001 relative to WT mice. Numbers in parentheses indicate the number of mice for each group.

Table S3.

Clinical data and immunohistochemical staining score of DN and non-diabetic control patients

Group	Age(y)/Sex	GLU-A.C (mg/dL)	HbA1C (%)	SCr (mg/dL)	UPCR	eGFR	Duration of diagnosed diabetes (y)	Immunohistochemical staining score (0~5)			
								TLR4	TLR2	HSP70	Cytoplasmic HMGB1
Control group (n=10)	73/F	111	N.A.	0.82	N.A.	68	N.A.	1	0	1	0
	63/M	112	N.A.	1.02	N.A.	74	N.A.	3	2	3	0
	34/M	111	N.A.	1.16	N.A.	73	N.A.	1	0	0	0
	56/F	95	N.A.	0.68	N.A.	≧90	N.A.	1	2	1	0
	64/M	110	N.A.	1.11	N.A.	67	N.A.	1	2	0	1
	45/M	79	N.A.	1.18	N.A.	67	N.A.	0	1	1	2
	54/F	111	N.A.	0.75	N.A.	81	N.A.	0	0	3	0
	59/M	102	N.A.	1.16	N.A.	65	N.A.	0	0	3	2
	57/M	106	N.A.	1.02	N.A.	75	N.A.	0	2	2	1
	58/F	95	N.A.	0.66	N.A.	≧90	N.A.	2	2	4	1
Mean±SD	56.3±10.7 F:M=4:6	103.2±10.7		0.96±0.2		71.3±5.4		0.9±1.0	1.1±1.0	1.8±1.4	0.7±0.8

Table S3. Continued

Group	Age(y)/Sex	GLU-A.C (mg/dL)	HbA1C (%)	SCr (mg/dL)	UPCR	eGFR	Duration of diagnosed diabetes (y)	Immunohistochemical staining score (0~5)			
								TLR4	TLR2	HSP70	Cytoplasmic HMGB1
DN group (n=11)	53/F	568	12.8	8.30	1220	5	10	5	4	5	4
	32/F	156	7.1	4.51	22693	10	3	2	3	5	1
	38/F	162	7.4	7.3	32877	6	10	5	1	5	0
	69/M	105	6.4	3.57	15296	17	14	5	5	3	4
	63/M	135	7.2	1.63	17817	43	5	4	5	5	1
	46/F	142	6.2	0.84	2174	74	4	5	5	4	0
	75/F	195	7.5	2.36	10349	20	10	5	5	5	0
	64/F	158	7.5	1.08	3504	51	7	5	4	4	5
	46/M	119	10.0	3.25	11995	21	11	5	4	5	3
	56/M	201	7.9	10.61	19247	5	4	1	3	5	5
	33/M	220	4.8	9.04	6171	7	2	4	3	5	2
Mean±SD	52.2±15.4 F:M=6:5	196.5±128.1	7.7±2.1	4.8±3.5	13031±9784	23.6±22.8	7.3±3.9	4.2±1.4	3.8±1.3	4.6±0.7	2.3±2.0

HbA1c, hemoglobin A1c; SCr, serum creatinine; UPCR, urinary protein/creatinine ratio; eGFR, estimated glomerular filtration rate (ml/min per 1.73 m^2);

F, female; M, male; N.A., not applicable/not available. Results are shown as mean ± standard deviation.

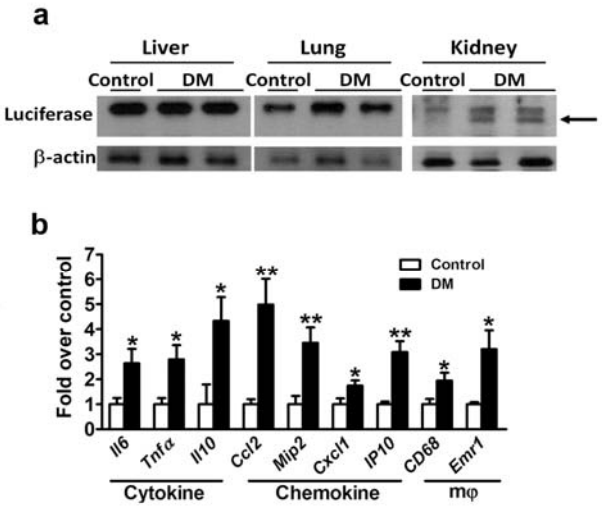

Fig. S1. Expression of inflammatory mediators in diabetic mice. (**a**) Immunoblot analysis on luciferase in the liver, lung, and kidney of 3-month diabetic and control NF-kB reporter mice. (**b**) Expression of inflammatory genes, including cytokines, chemokines, and macrophage markers, in the kidney of 3-month diabetic (n=6) relative to the control (n=5) C57BL/6 mice. *P<0.05 and **P<0.01.

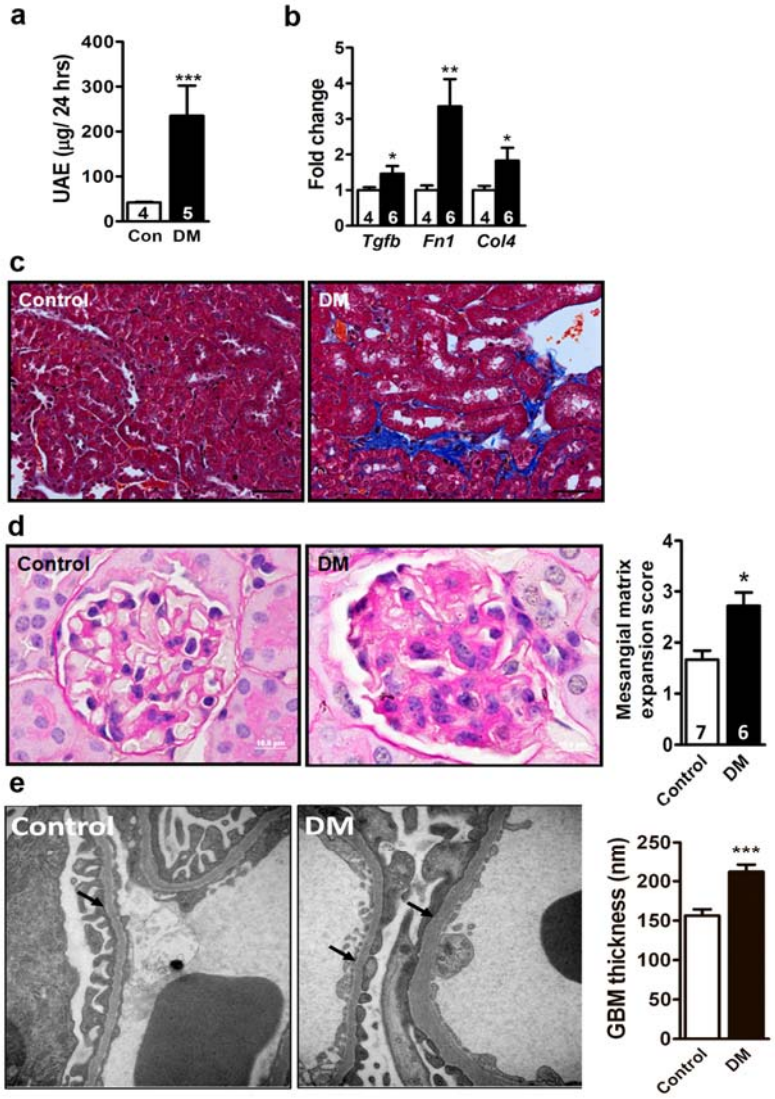

Fig. S2. Renal function and morphology in diabetic mice. (**a**) Daily urinary albumin excretion (UAE), (**b**) expression of fibrotic genes, and (**c**) masson trichrome stain in the kidney of 3-month diabetic and control mice. Scale bar, 50 µm. (**d**) Representative PAS-stained glomerular morphology and quantification of mesangial matrix expansion of 3-month diabetic and control mice. Scale bar, 30 µm. The scoring of mesangial matrix expansion was based on >30 glomeruli examined per mouse. (**e**) Representative image and thickness measurement of glomerular basement membrane (GBM, *arrows*) in the kidney of 3-month diabetic and control mice. Magnification, x30,000. Numbers inside bars indicate the mouse number for each group. **P*<0.05, ***P*<0.01, and ****P*<0.001.

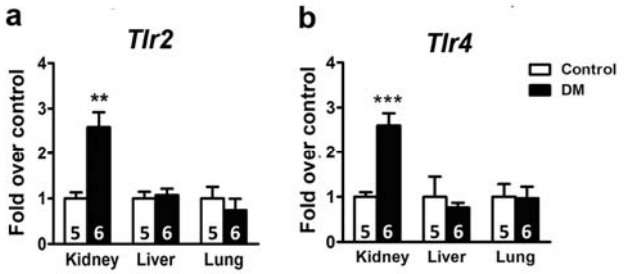

Fig. S3. Expression of TLRs in diabetic mice. Expression of (**a**) TLR2 and (**b**) TLR4 in the kidney, liver and lung of 1-month diabetic relative to control mice. Numbers inside bars indicate the mouse number for each group. $^{**}P<0.01$ and $^{***}P<0.001$.

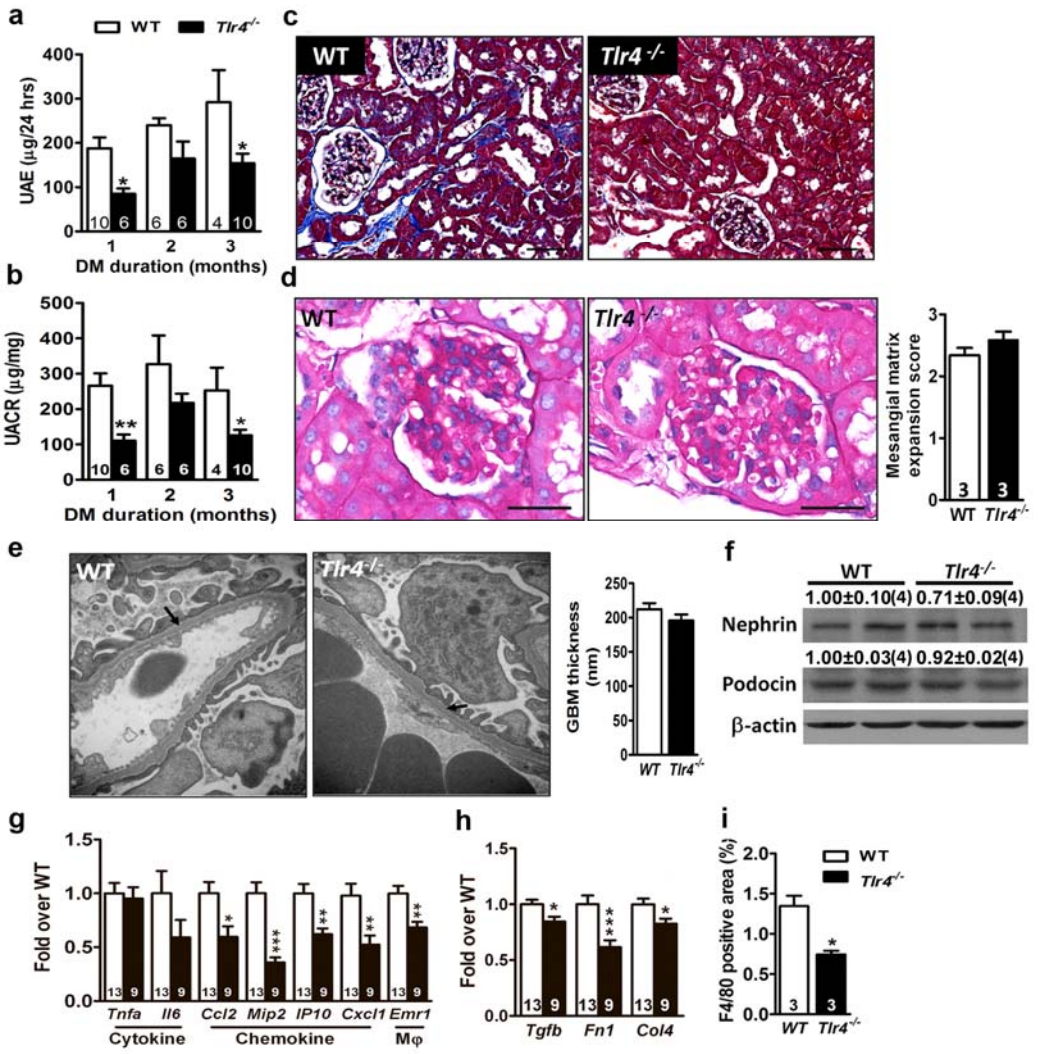

Fig. S4. Renal function, morphology, and gene expression of *Tlr4*$^{-/-}$ diabetic mice. (**a**) UAE and (**b**) urinary albumin-creatinine ratio (UACR) of *Tlr4*$^{-/-}$ and WT mice 1 to 3 months after induction of diabetes. (**c**) Masson trichrome stain in the kidney of *Tlr4*$^{-/-}$ and WT 3-month diabetic mice. Scale bar, 50 μm. (**d**) Representative PAS-stained glomerular morphology and quantification of mesangial matrix expansion of *Tlr4*$^{-/-}$ and WT 3-month diabetic mice. Scale bar, 30 μm. (**e**) Representative image and thickness measurement of GBM (*arrows*) of *Tlr4*$^{-/-}$ and WT 3-month diabetic mice. Magnification, x30,000. (**f**) Immunoblot analyses on nephrin and podocin from the kidney of *Tlr4*$^{-/-}$ and WT 1-month diabetic mice. The relative intensities of the bands by densitometric quantification to WT with the number of mice in parentheses are indicated. Expression of (**g**) inflammatory and (**h**) fibrotic genes in the kidney of *Tlr4*$^{-/-}$ and WT 1-month diabetic mice. (**i**) The percentage of F4/80 positive area quantified by the immunofluorescence analysis in the kidney of *Tlr4*$^{-/-}$ and WT 1-month diabetic mice. Numbers inside bars indicate the mouse number for each group. **P*<0.05, ***P*<0.01, and ****P*<0.001.

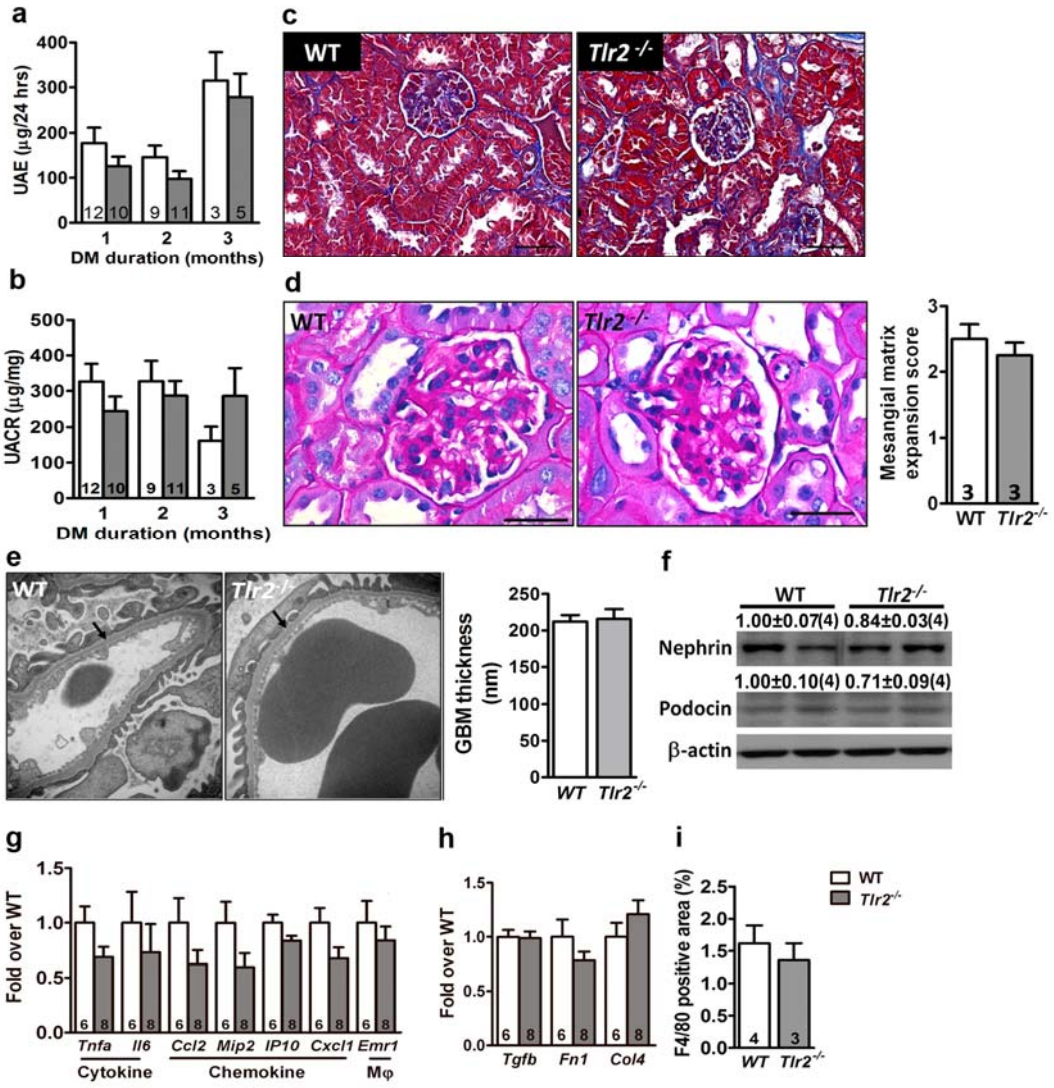

Fig. S5. Renal function, morphology, and gene expression of *Tlr2*$^{-/-}$ diabetic mice. (**a**) Daily urinary albumin excretion (UAE) and (**b**) urinary albumin-creatinine ratio (UACR) of *Tlr2*$^{-/-}$ and WT mice 1 to 3 months after induction of diabetes. (**c**) Masson trichrome stain in the kidney of 3-month *Tlr2*$^{-/-}$ and WT diabetic mice. Scale bar, 50 μm. (**d**) Representative PAS-stained glomerular morphology and quantification of mesangial matrix expansion of *Tlr2*$^{-/-}$ and WT 3-month diabetic mice. Scale bar, 30 μm. (**e**) Representative image and thickness measurement of GBM (*arrows*) of *Tlr2*$^{-/-}$ and WT 3-month diabetic mice. Magnification, x30,000. (**f**) Immunoblot analyses on nephrin and podocin from the kidney of *Tlr2*$^{-/-}$ and WT 1-month diabetic mice. The relative intensities of the bands by densitometric quantification to WT with the number of mice in parentheses are indicated. Expression of (**g**) inflammatory and (**h**) fibrotic genes in the kidney of *Tlr2*$^{-/-}$ and WT 1-month diabetic mice. (**i**) The percentage of F4/80 positive area quantified by the immunofluorescence analysis in the kidney of *Tlr2*$^{-/-}$ and WT 1-month diabetic mice. Numbers inside bars indicate the mouse number for each group.

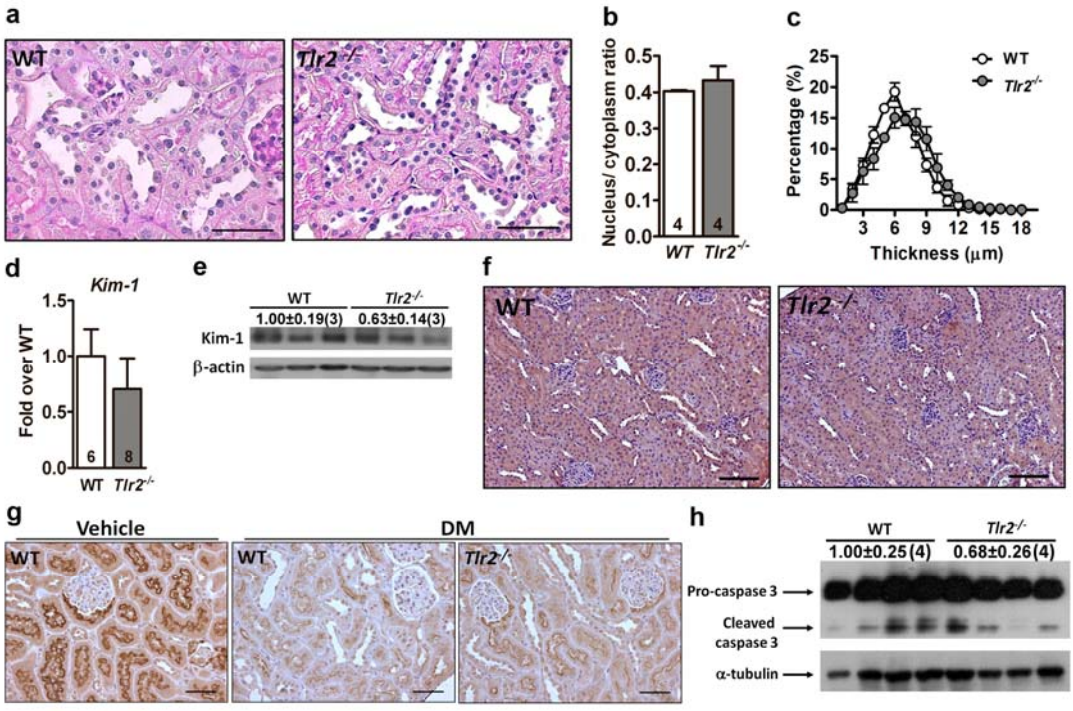

Fig. S6. Tubular injury and apoptosis in *Tlr2*$^{-/-}$ diabetic mice. (**a**) Representative tubular morphology, (**b**) quantification of the tubular nucleus-to-cytoplasm ratio, and (**c**) distribution of tubular epithelial thickness in *Tlr2*$^{-/-}$ and WT 1-month diabetic mice. Scale bar, 50 μm. (**d**) Expression of *Kim-1* in *Tlr2*$^{-/-}$ and WT 1-month diabetic mice. Numbers inside bars indicate the mouse number for each group. (**e**) Immunoblot analyses and (**f**) immunohistochemical staining of Kim-1 in the kidney of *Tlr2*$^{-/-}$ and WT 1-month diabetic mice. Scale bar, 100 μm. (**g**) Immunohistochemical staining of cubilin in the kidney of WT control, and WT and *Tlr2*$^{-/-}$ 3-month diabetic mice. Scale bar, 50 μm. (**h**) Immunoblot analyses of caspase 3 in the kidney of *Tlr2*$^{-/-}$ and WT 1-month diabetic mice. The relative intensities of the bands by densitometric quantification to WT with the number of mice in parentheses are indicated.