Low dose IL-17 therapy prevents and reverses diabetic nephropathy, metabolic syndrome and associated organ fibrosis

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Supplementary Figure S1.

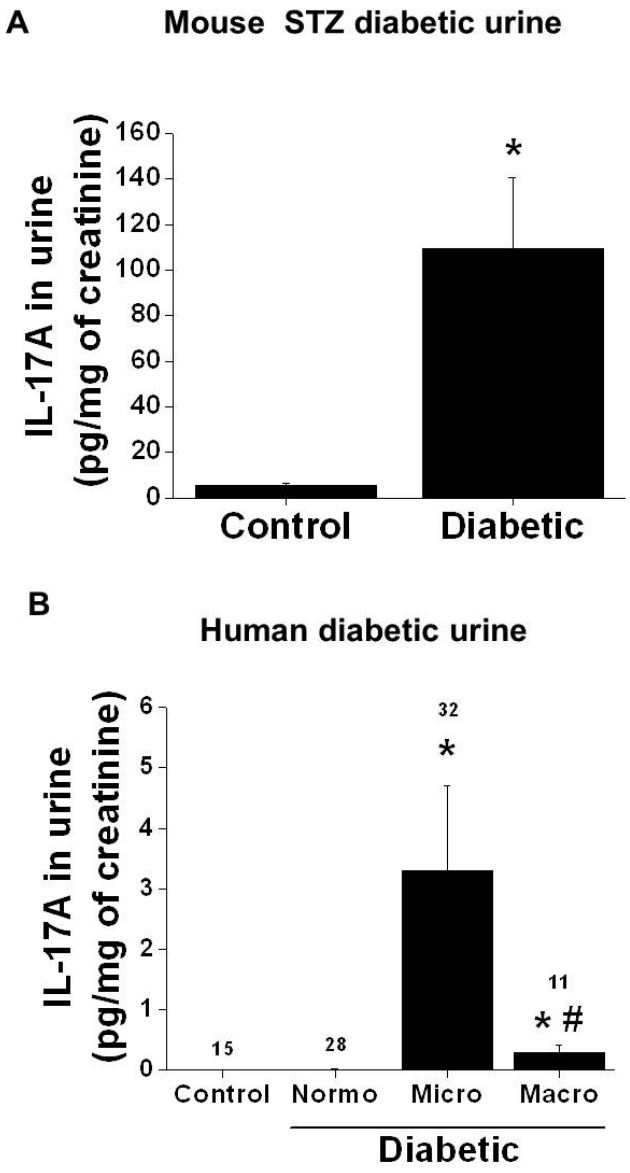

Figure S1. Quantification of IL-17A excretion in diabetic mouse (STZ induced) and human patients. IL-17A was quantified by ELISA. IL-17A excretion was significantly increased after 8 weeks of diabetes over non-diabetic control mouse. *, $p<0.001$ vs. control. n=8-10. Excretion of IL-17A in healthy control and diabetes without microalbuminuria (normo) showed low levels of IL-17A excretion, which was significantly increased in patients with microalbuminuria and macroalbuminuria. However, macroalbuminuria patients had significantly lower levels of IL-17A as compared to microalbuminuria patients. *, $p<0.05$ vs. other groups. #, $p<0.05$ vs. microalbuminuria group. Number patients analyzed in each group is indicated at top of each bar.

Supplementary Figure S2.

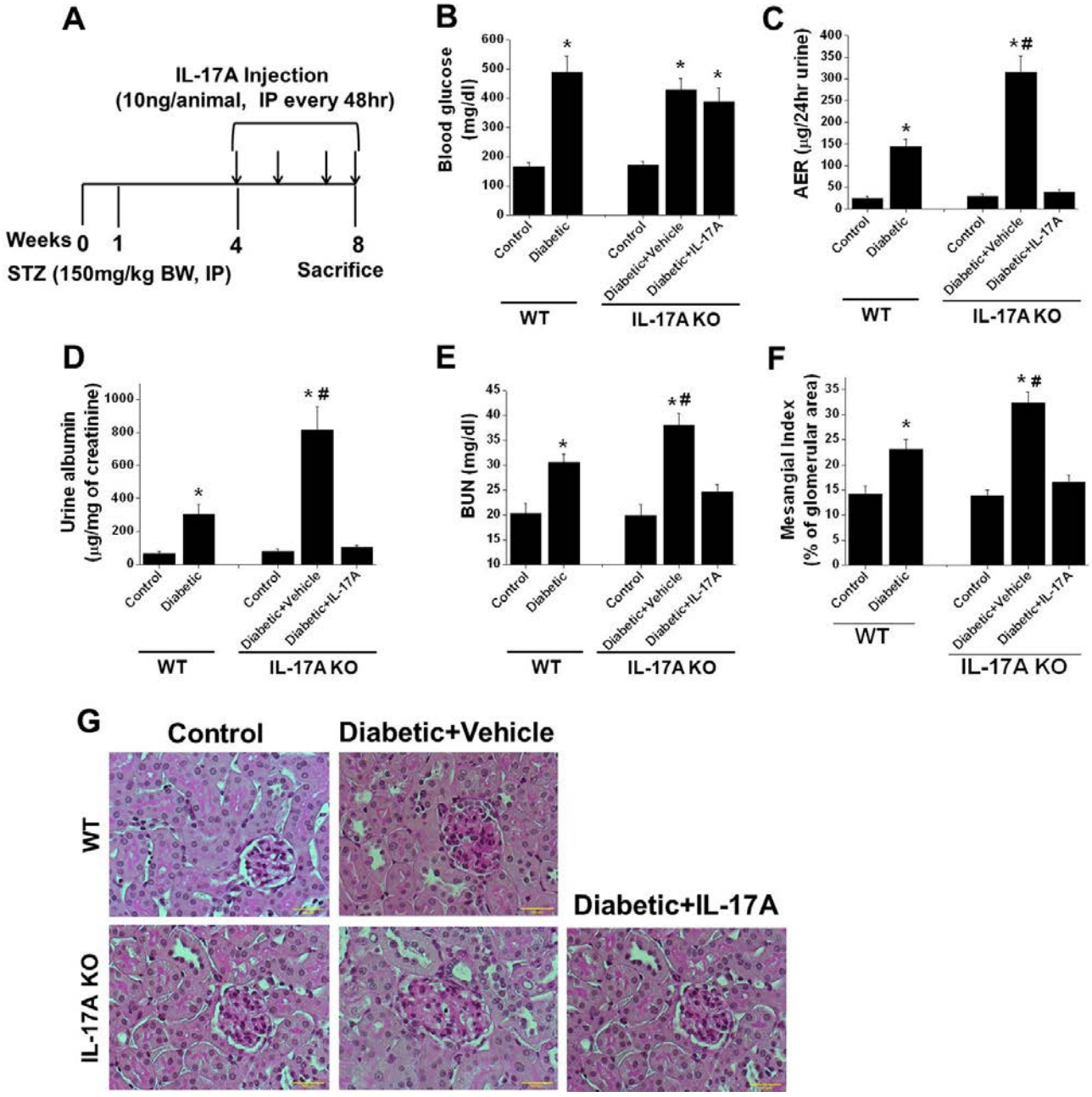

Figure S2. IL-17A administration to diabetic IL-17A knockout mice suppresses diabetic nephropathy. A. Diabetes was induced by administering a single dose of STZ, and 4 weeks after induction of diabetes, animals were received either vehicle or IL-17A for additional 4 weeks (10ng/animals/every 48hr). Animals were sacrificed at 8 weeks after induction of diabetes and tissues were processed for histopathology. B. Blood glucose in WT and IL-17A knockout (KO) mice. *, $p<0.001$ vs. control. C. Albumin excretion rate in WT and IL-17A KO diabetic mice. *, $p<0.001$ vs. control. #, $p<0.05$ vs. vehicle treated IL-17A KO diabetic. D. Albumin excretion normalized to mg of creatinine. *, $p<0.01$ vs. control. #, $p<0.05$ vs. vehicle treated IL-17A KO diabetic. E. Renal function was

determined by measuring blood urea nitrogen (BUN). *, $p<0.05$ vs. control. #, $p<0.05$ vs. vehicle treated IL-17A KO diabetic. F. Quantification of mesangial expansion. *, $p<0.05$ vs. control. #, $p<0.05$ vs. WT diabetic. #, $p<0.001$ vs. vehicle treated IL-17A KO diabetic. G. PAS stained section from WT and IL-17A KO control and diabetic mice treated with vehicle or IL-17A. Diabetes induced glomerular hypertrophy and mesangial expansion in WT mice and IL-17A KO mice which was suppressed in IL-17A treated IL-17A KO mice kidney. Scale bar: 100 µM. N=6.

Supplementary Figure S3.

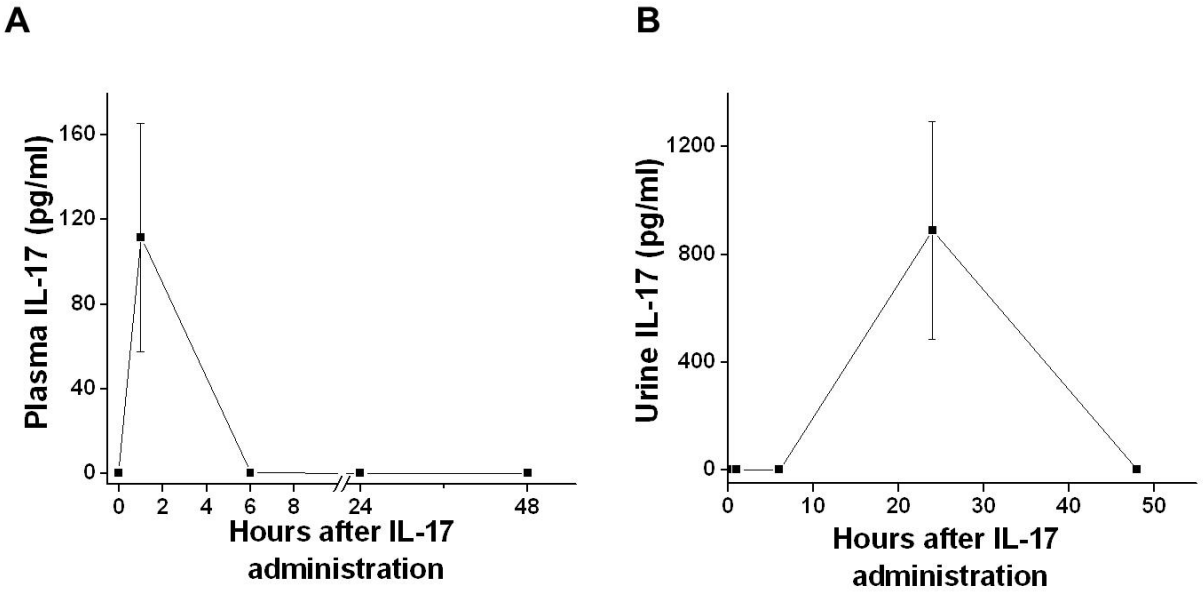

Figure S3. IL-17A plasma clearance kinetics in WT mice. 10ng of recombinant IL-17A was administered intraperitoneally, after which blood (A) and urine (B) samples were collected at various time points. Plasma and urine IL-17A were quantified by ELISA as described in Materials and Methods.

Supplementary Table S1. Effect of IL-17 isoforms at different doses on diabetes induced albuminuria, polyuria, kidney weight/body weight ratio and blood glucose

Animals (number of animal)	Treatment	Blood glucose (mg/dl)	Urine glucose (mg/dl)	Urine Volume (ml)	KW/BW¶ (mg/gm)	Urine albumin (µg/24hr)
Non-Diabetic (12)	Vehicle	198±11	98±12	0.8±0.2	9±1	24±5
Non-Diabetic (5)	IL-17A	205±9	61±10	0.9±0.2	10±0.1	19±3
Diabetic (15)	Vehicle	634±115*	10900±1648*	26±6*	16±2*	200±7*
Diabetic (12)	10ng IL-17A	622±43*	10144±667*	24±3*	13±1	110±12*#
Diabetic (6)	50ng IL-17A	512±132*	14225±2345*	31±5*	14±2	98±4*#
Diabetic (6)	100ng IL-17A	480±55*	12240±912*	18±6*	15±1*	109±36*#
Diabetic (6)	10ng IL-17E	630±66*	11650±2847*	20±5*	13±1	233±44*
Diabetic (6)	10ng IL-17F	576±101*	8860±1786*	18±6*	15±2*	128±29*#
Diabetic (6)	10ng IL-17C	658±17*	11067±1768*	18±3*	15±1*	283±74*

*, $p<0.05$ vs. Non diabetic vehicle or IL-17A treated.

#, $p<0.05$ vs. Diabetic vehicle treated.

¶, kidney weight/body weight ratio.

Supplementary Figure S4.

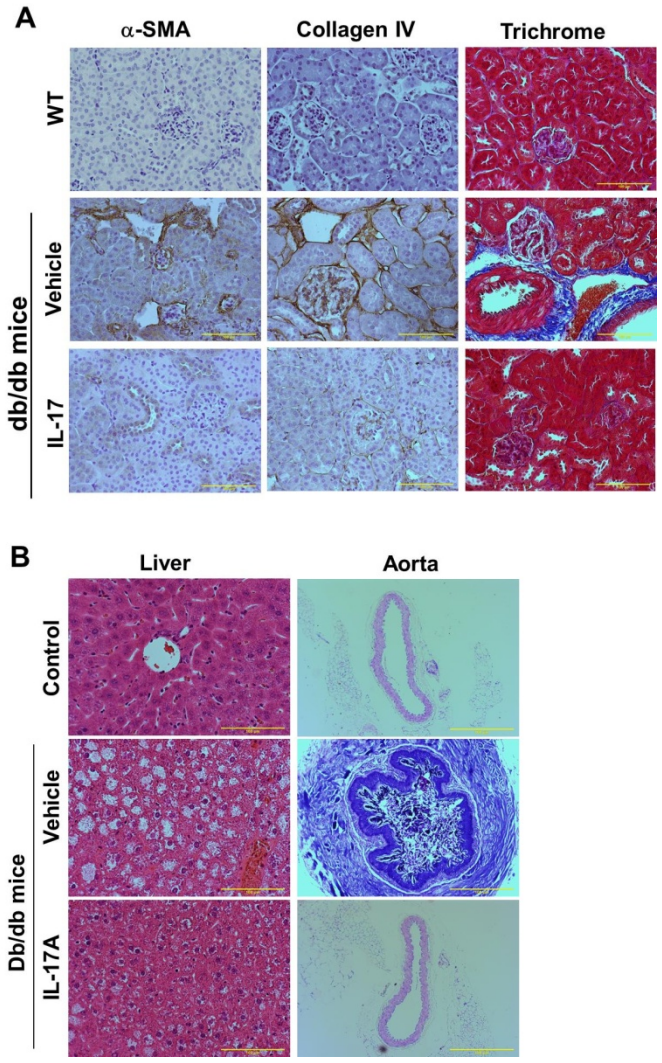

Figure S4. Fibrosis was determined by immunohistochemical staining for α-SMA, collagen IV and Masson's trichrome staining. Scale bar: 100 µM. Vehicle treated diabetic mouse kidney shows increased expression of α-SMA, collagen IV and trichrome staining, which was reduced in IL-17A treated mouse kidney. B. Lipid deposition and fibrosis in liver was determined by Masson's trichrome staining. Scale bar: 100 µM. Fat deposition in and around the aorta was determined by Toludine blue staining. Vehicle treated liver and aorta show a large amount of lipid deposition that was entirely suppressed in IL-17A treated db/db mice.

Supplementary Figure S5.

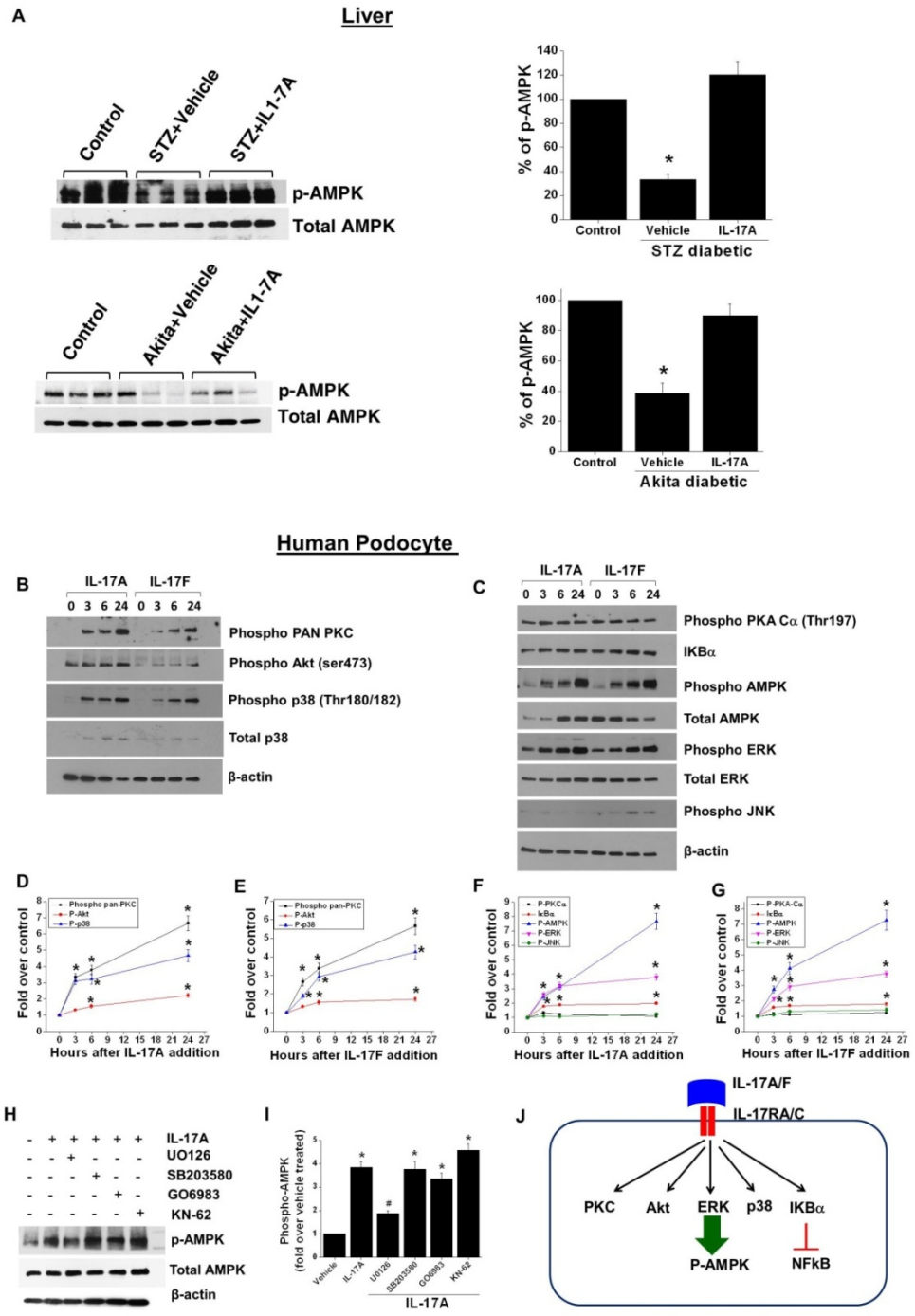

Figure S5. IL-17A administration suppresses diabetes induced downregulation of AMPK activation in liver (A) and increases AMPK activation through ERK MAPK (B-J) in human podocytes. A. Western blot analysis of phosphor and total AMPK in control and diabetic animals (left panel) and densitometric quantification of AMPK expression (right panel). *, p<0.001 vs. other groups. B & C. Western blot analysis of

phosphorylation of different kinases in podocytes in response to IL-17A or IL-17F treatment (50ng/ml) at different time points. D-G. Quantification of Western blots by densitometry. Phospho kinase levels were expressed as a fold increase over control, and protein loading was normalized to total kinase and actin level. *, $p<0.05$ vs. control (0hr). n=4. H-I. ERK pathway inhibitor (U0126) but not p38 (SB203580) or PKC (GO6983) or calmodulin kinase (KN-62) inhibitor suppressed IL17A induced AMPK phosphorylation, suggesting in part that ERK is an upstream activator of AMPK. *, $p<0.001$ vs. vehicle treated. #, $p<0.05$ vs. IL-17A treated. n=3. J. Schematic representation of IL17A/F mediated activation of AMPK in human podocytes.

Supplementary Figure S6.

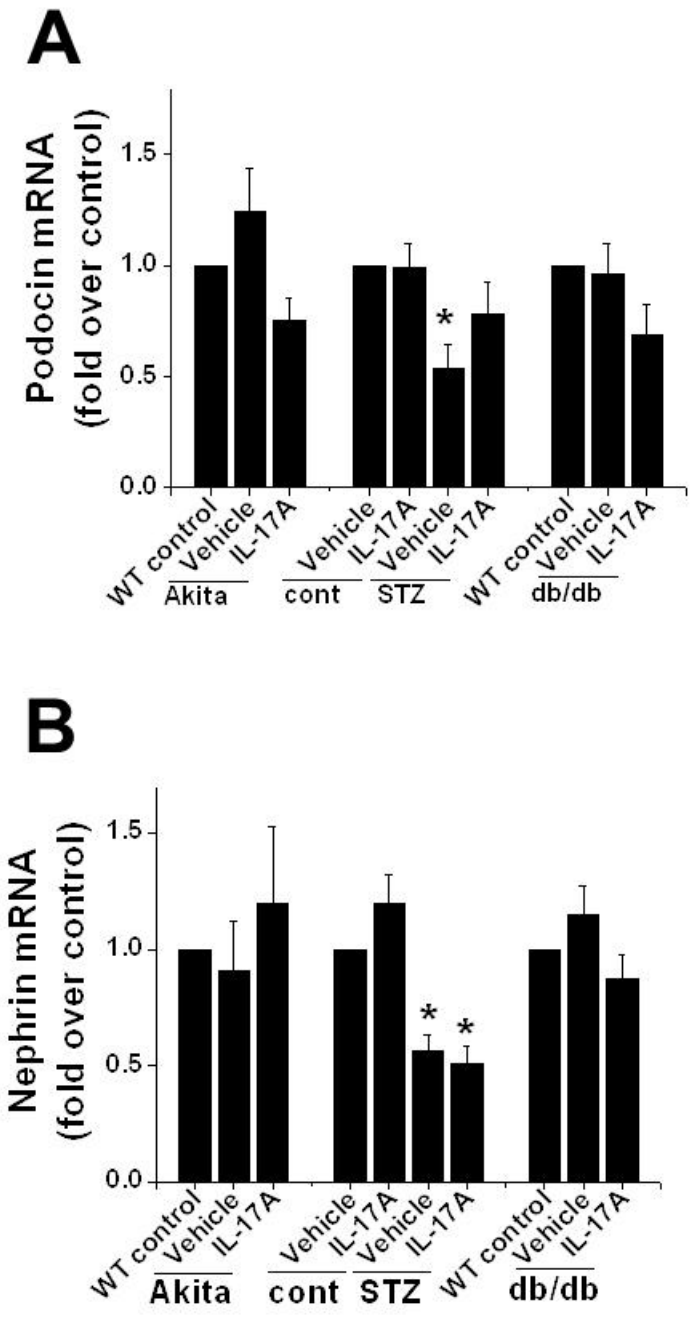

Figure S6. Quantification of podocin and nephrin mRNA in the diabetic mouse kidney by real time RT-PCR analysis. Kidney from WT, Ins2Akita, db/db and STZ diabetic mice that are treated with vehicle or IL-17A as described in Materials and Methods were used for mRNA quantification. *, $p<0.05$ vs. control. N=4-6.

Supplementary Figure S7.

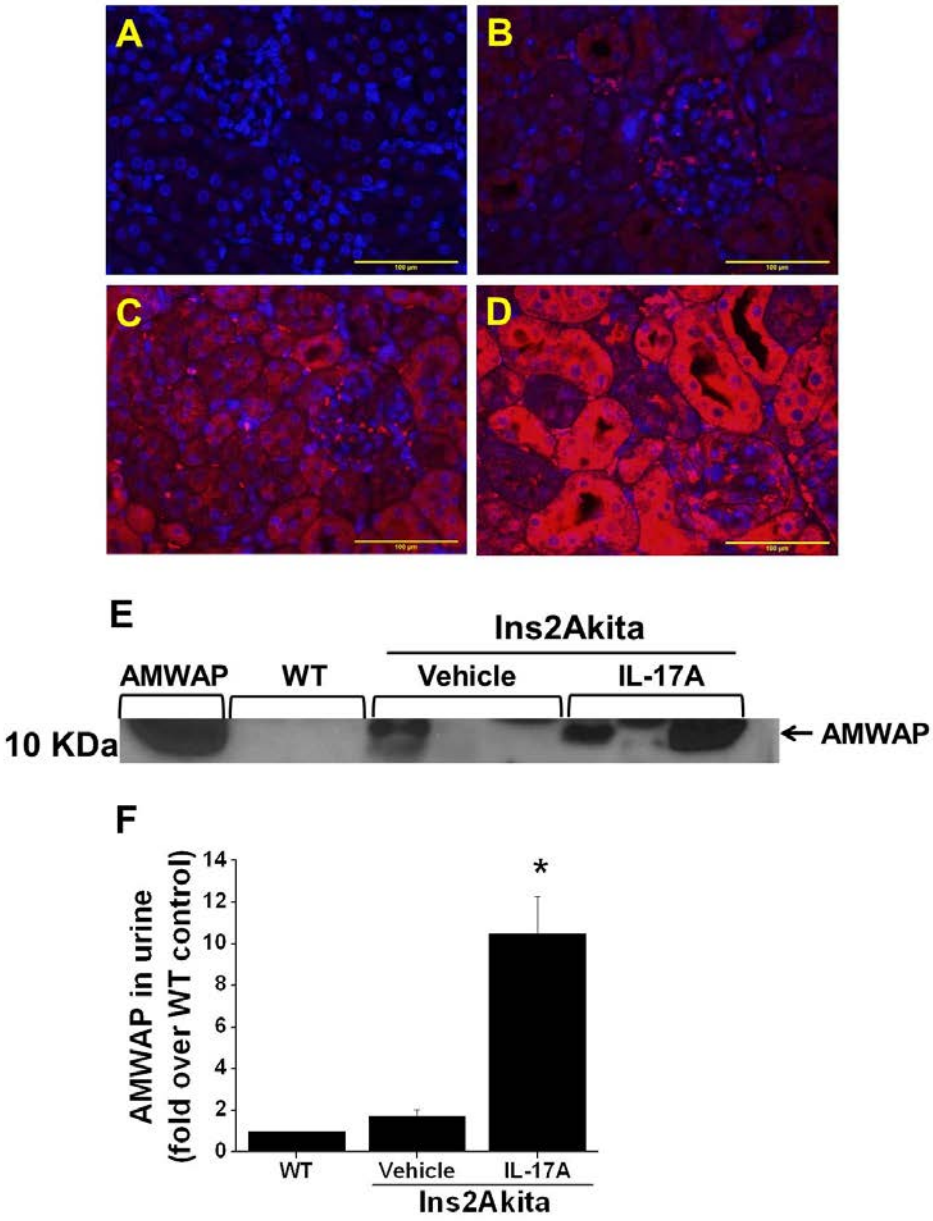

Figure S7. AMWAP immunolocalization in the kidney. A. 2nd antibody control. B. Wild type non-diabetic control. C. Vehicle treated Ins2Akita mouse kidney. D. IL-17A treated Ins2Akita mouse kidney showing intense staining in the tubular epithelium and podocytes. Scale bar: 100 µM. E. Western blot analysis of AMWAP excretion in urine from WT, vehicle and IL-17A treated Ins2Akita mice. F. Densitometric quantification of AMWAP Western blots. *, $p<0.001$ vs. other groups. n=4-6.

Supplementary Figure S8.

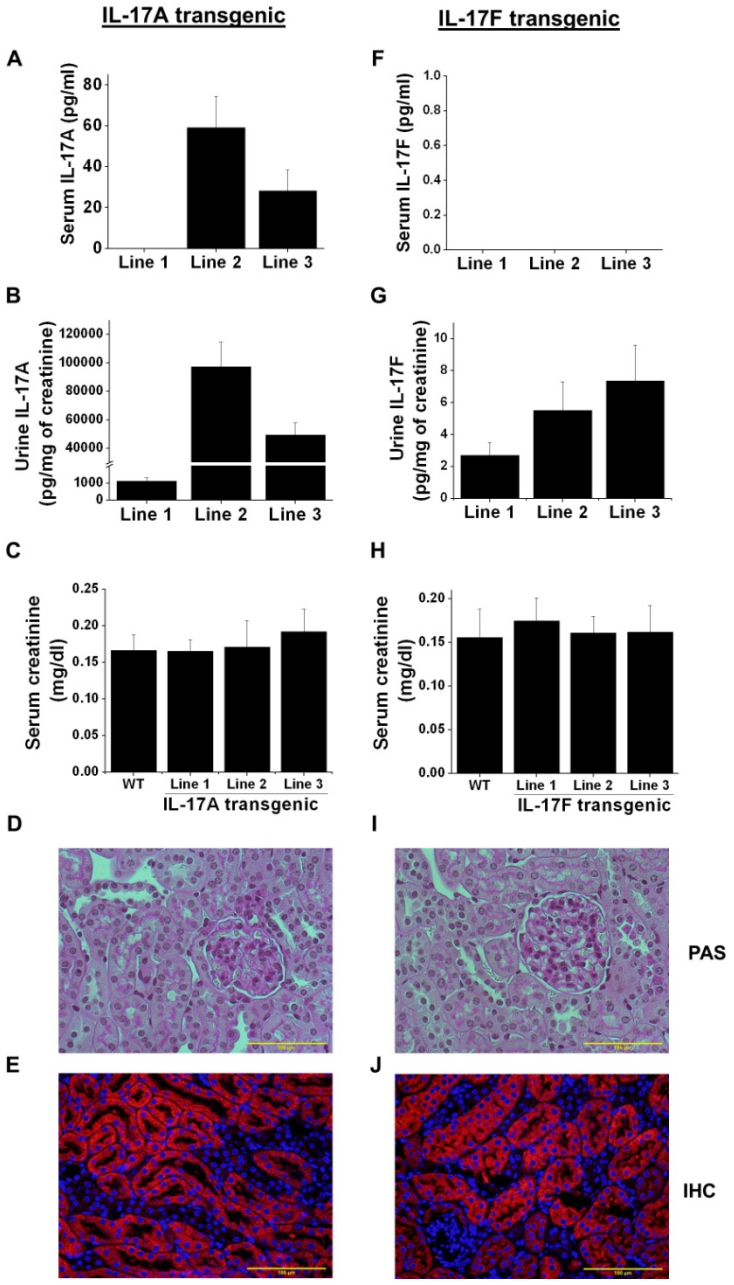

Figure S8. Characterization of 12 weeks old IL-17A (A-E) transgenic mice and IL-17F (F-J) transgenic mice for transgene expression and kidney function. A. Quantification of IL-17A levels in serum by ELISA. B. Quantification of IL-17A levels in urine by ELISA. C. Kidney function was determined by measuring serum creatinine. D. PAS-hematoxylin stained kidney section showing normal morphology. E. Immunohistochemical localization of IL-17A in line 2 kidney. Characterization of 12-week old IL-17A (F-J) transgenic mice. A. Quantification of IL-17F levels in serum by ELISA. B. Quantification of IL-17F levels in urine by ELISA. C. Kidney function was determined by measuring serum creatinine. D. PAS-hematoxylin stained kidney section showing normal morphology. E. Immunohistochemical localization of IL-17F in line 2 kidney. Scale bar: 100 µM. N=6-8.

Supplementary Figure S9.

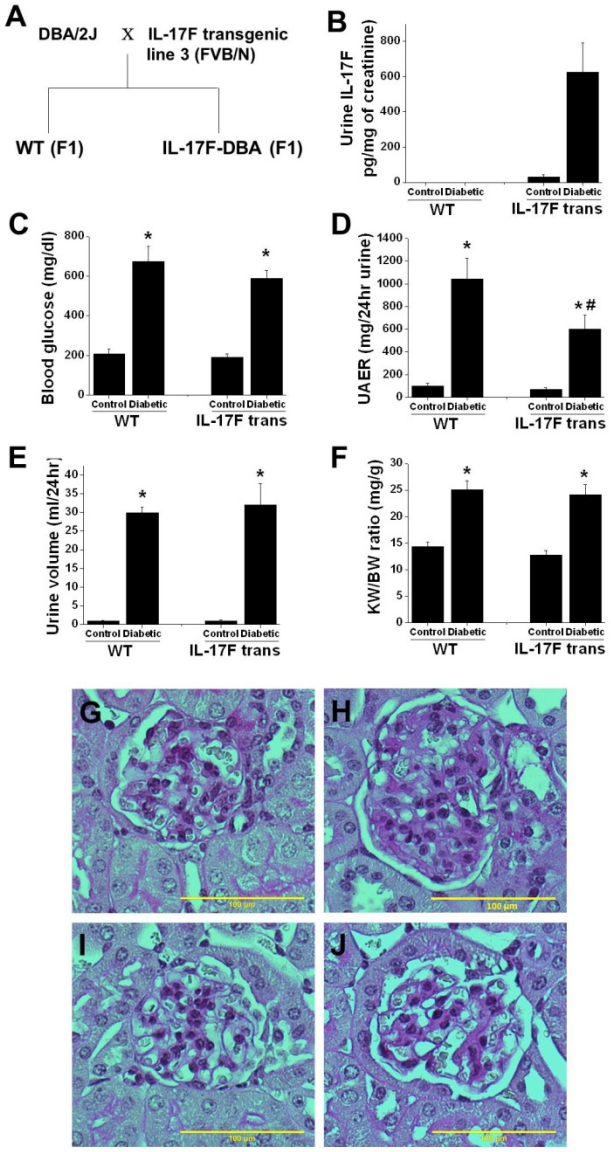

Figure S9. Epithelial specific overexpression of IL-17F is sufficient to suppress diabetic nephropathy. Data from Line 3. A. IL-17F transgenic mice were crossed with nephropathy prone strain DBA/2J. 6-week old WT and IL-17A positive F1 mice were given a single dose of STZ (150mg/kg BW). Mice were sacrificed 8 weeks after STZ administration, and albuminuria was quantified. B. Quantification of IL-17F excretion in urine. IL-17F is only detectable in IL-17F transgenic mouse urine but not in plasma (not shown). C. Blood glucose level at 8 weeks of diabetes. D. Urine albumin excretion rate (UAER) expressed as microgram per 24hr urine. E. Urine volume per 24hr. F. Kidney hypertrophy was calculated as ratio of kidney weight and body weight (KW/BW). G-J. PAS-hematoxylin stained kidney section. G. WT control. H. WT diabetes. I. IL-17F transgenic control. J. IL-17A transgenic diabetic mouse kidney. Scale bar: 100 µM. *p<0.001 vs. other groups. #, p<0.05 vs. WT diabetic. N=8-12.

Supplementary Table 2. Plasma lipid profile from WT and IL-17A transgenic mice in a mixed background (FVB/N vs. DBA/2J).

Animals	Treatment	Triglycerides (mg/dl)	HDL (mg/dl)	LDL/VLDL (mg/dl)	Total cholesterol (mg/dl)
WT	Non-diabetic	254±17	108±15	29±2	90±17
WT	Diabetic	390±26*****	67±17*****	33±5	83±17
IL-17A (line 1)	Non-diabetic	278±33	105±4	29±3	114±16
IL-17A (line 1)	Diabetic	163±31#	92±6	21±5	95±16
IL-17A (line 2)	Non-diabetic	303±24	109±8	33±5	104±19
IL-17A (line 2)	Diabetic	220±29#	88±9	20±5$	97±22
IL-17F (line 3)	Non-diabetic	317±21	99±13	30±2	107±9
IL-17F (line 3)	Diabetic	223±19#	81±8	26±3	87±5

6-week old transgenic and WT mice were made diabetic with a single dose of STZ. Animals were sacrificed at 8 weeks after induction of diabetes, and plasma lipid levels were quantified. *, $p<0.01$ vs. non-diabetic control. #, $p<0.001$ vs. WT diabetic and corresponding transgenic non-diabetic controls. $, $p<0.05$ vs. line 2 non-diabetic control and WT diabetic. N=6-8.

Supplementary Figure S10.

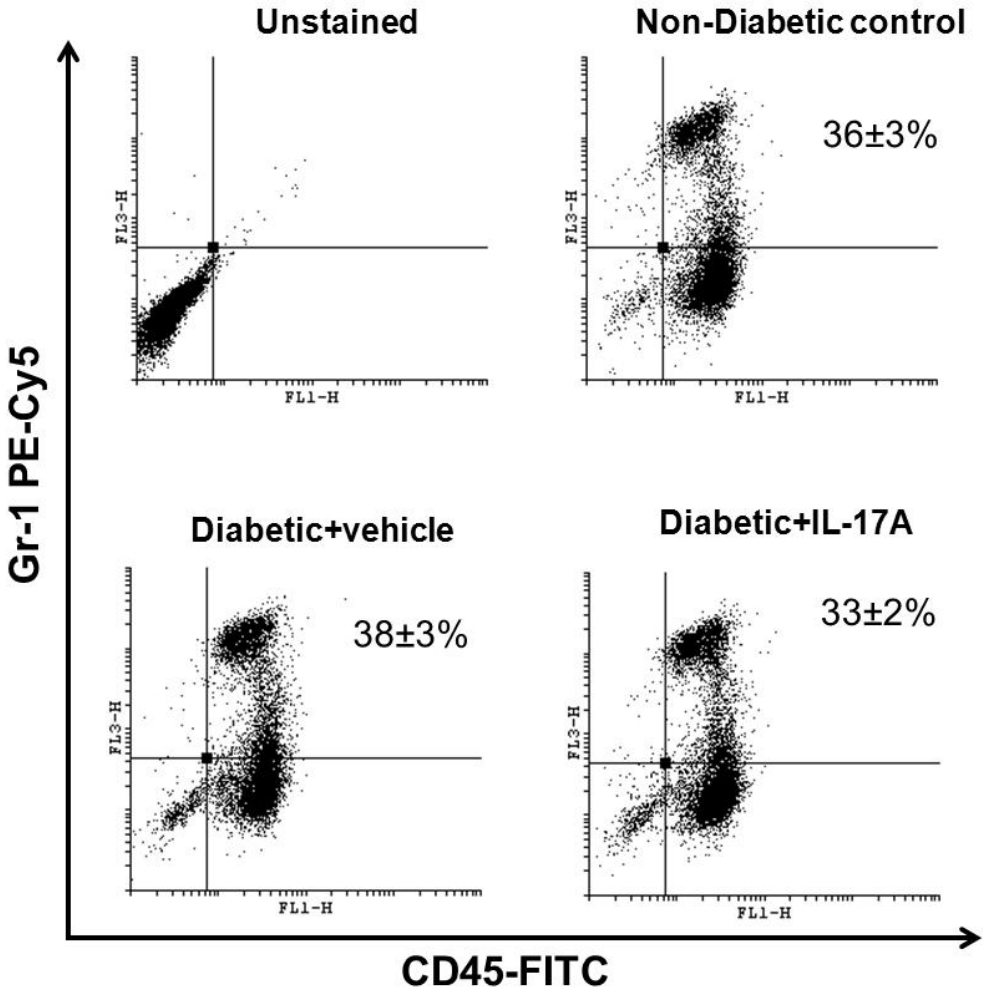

Figure S10. Flow cytometric analysis of neutrophils in blood from non-diabetic control mice, vehicle-treated diabetic mice and diabetic mice treated for 6 week with IL-17A (10ng/animal). N=4.

Supplementary Figure S11.

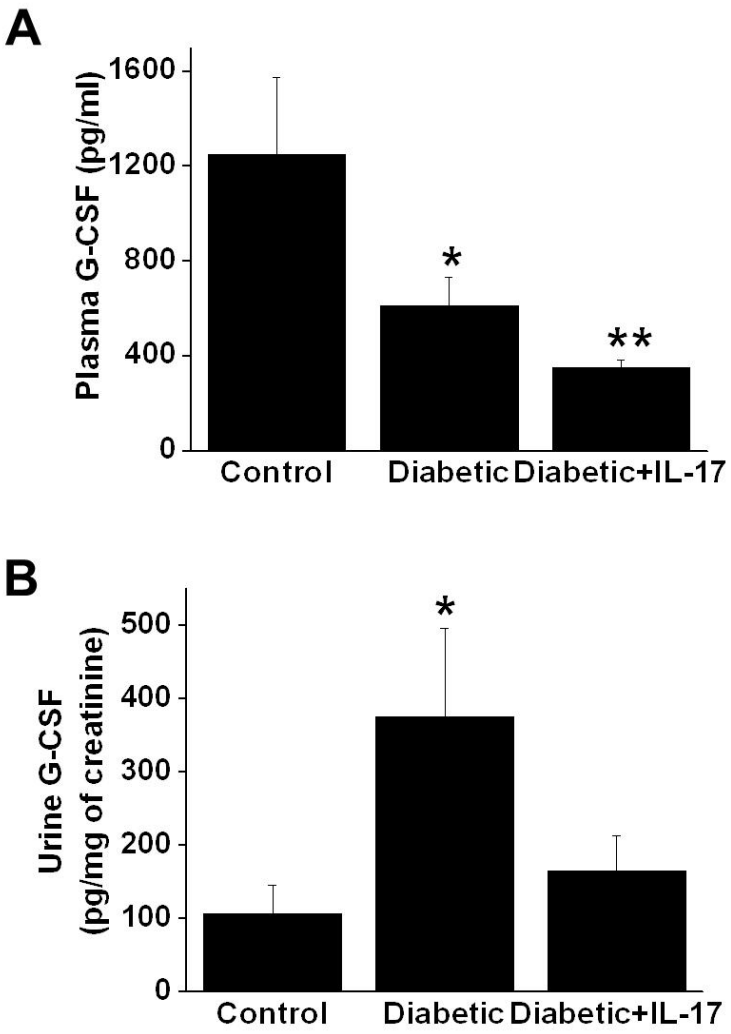

Figure S11. Quantification of plasma and urine granulocyte colony stimulating factor (G-CSF) by ELISA in control and diabetic animals treated with vehicle or IL-17A. Diabetes was induced by administering STZ, and G-CSF was quantified at 12 weeks after STZ administration. *, $p<0.001$ vs. other groups. **, $p<0.05$ vs. diabetic animals. N=4-6.

Supplementary Figure S12.

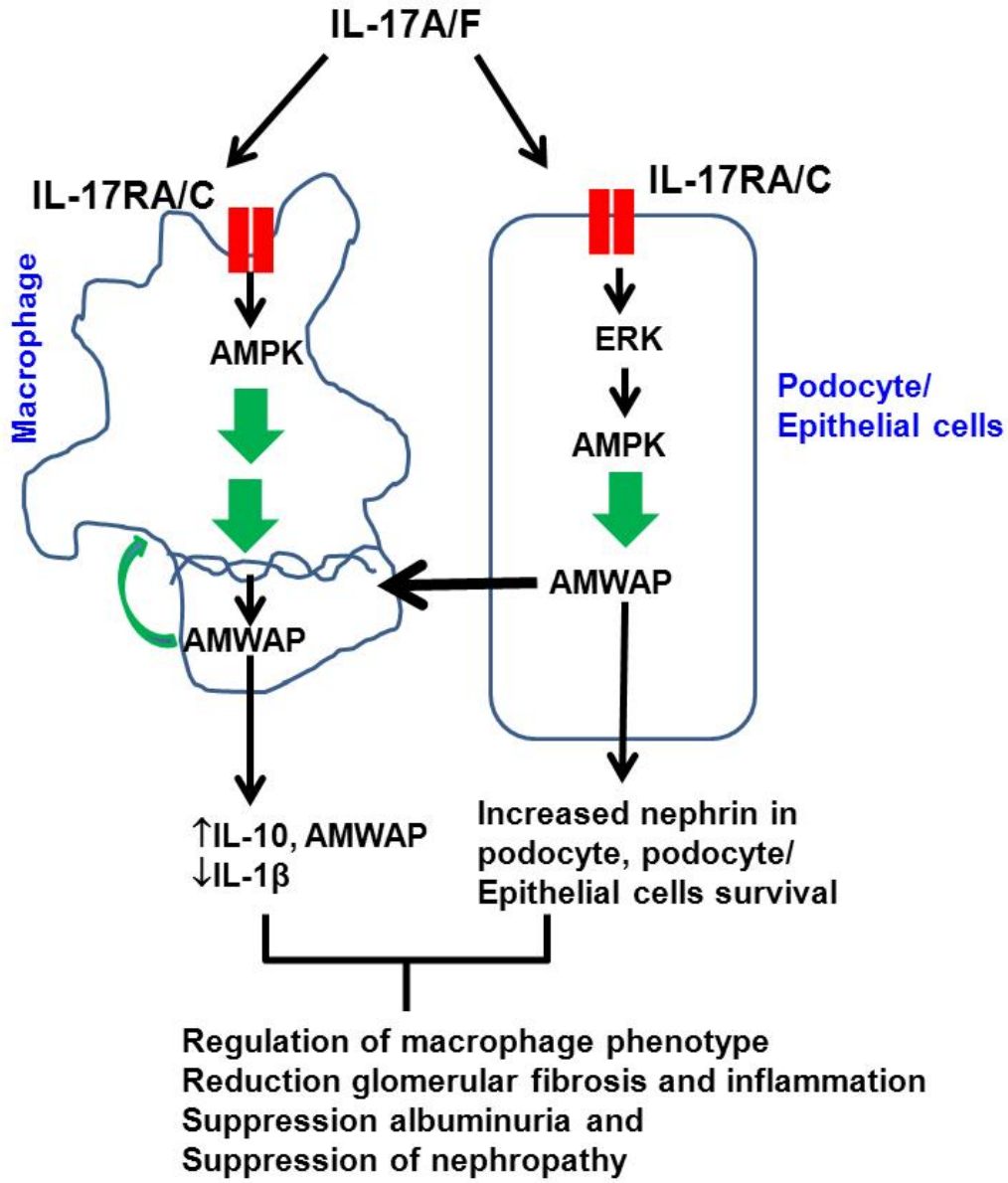

Figure S12. Model depicts the pathways through which IL-17A/F may protect kidney against diabetic nephropathy. IL-17A/F may act through IL-17RA and IL-17RC in macrophage, podocyte and epithelial cells. Binding of IL-17 to its receptors causes the activation of AMPK in an ERK MAPK dependent manner which then may increase the AMWAP expression. AMWAP, acting in autocrine or paracrine manner, will increase IL-10 production and suppression of macrophage inflammatory phenotype. AMPK may also increase podocyte survival independent of AMWAP. Together, IL-17A/F suppresses diabetic nephropathy.