Supplementary Data

Growth hormone induces TNF-α in podocytes and contributes to monocyte-to-macrophage differentiation and glomerulosclerosis

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Fig.S1:

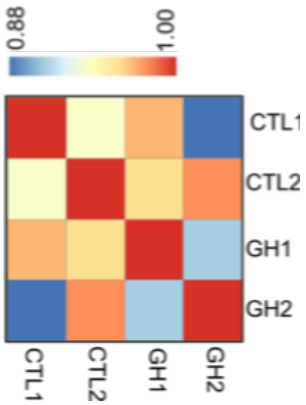

Fig.S1: Pearson correlation between all experimental conditions:

The Pearson correlation coefficient and their significance represented as a scale bar (0.88-1). CTL=Control and GH=Growth hormone.

Fig.S2:

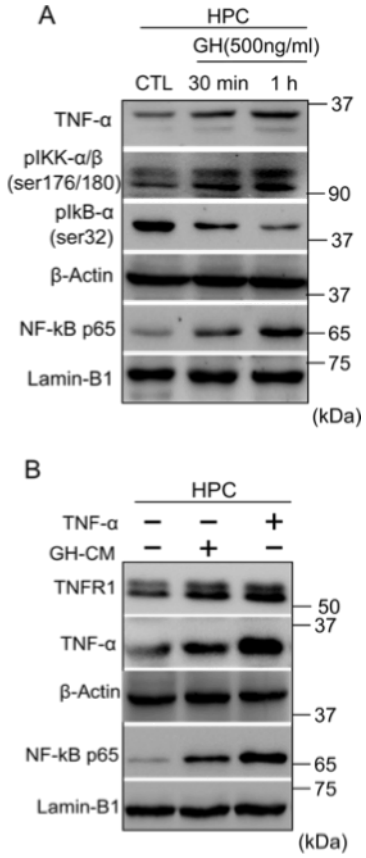

Fig.S2. GH induces TNF-α signaling in human podocytes (HPC):

A. Representative immunoblot from HPC showing expression of TNF-α, pIKK-α/β (ser176/180), pIkB-α (ser32) and NF-kB-p65 in CTL, 30 min and 1 h GH (500ng/ml) treatment (n=3). **B.** Representative immunoblot from HPC showing expression of TNFR1, TNF-α and NF-kB-p65 in with or without GH-CM and with or without recombinant TNF-α (2ng/ml) treatment for 1 h (n=3). **A & B.** β-Actin and Lamin-B1 served as loading control.

Fig.S3:

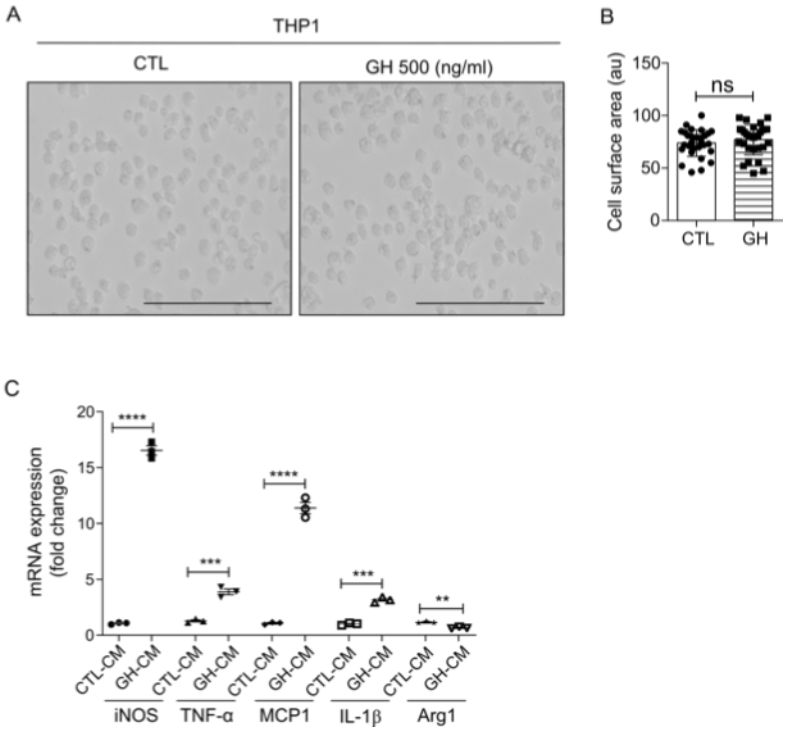

Fig.S3. GH exposed THP1 cells unable to differentiate, but GH-CM induces differentiation:

A. Representative image of TPH1 cells from CTL and GH (500ng/ml) treatment for 72 h. Scale bar= 50 μm. Magnification=x200. **B.** Quantification of cell surface area of THP1 cells (n=30) from with or without GH treatment and presented as a dot plot. (n=3). **C.** qRT-PCR analysis showing the expression of iNOS, TNF-α, MCP1, IL-1β and Arg1 in THP1 cells exposed to CTL-CM and GH-CM. β-Actin was used as an internal control. ****p<0.0001, ***p<0.001, and **p<0.01. Data presented as mean ±S.D. (n=3) and statistical significance was analyzed by one-way ANOVA post hoc Dunnett test. ns= not significant. au=arbitrary unit.

Fig.S4:

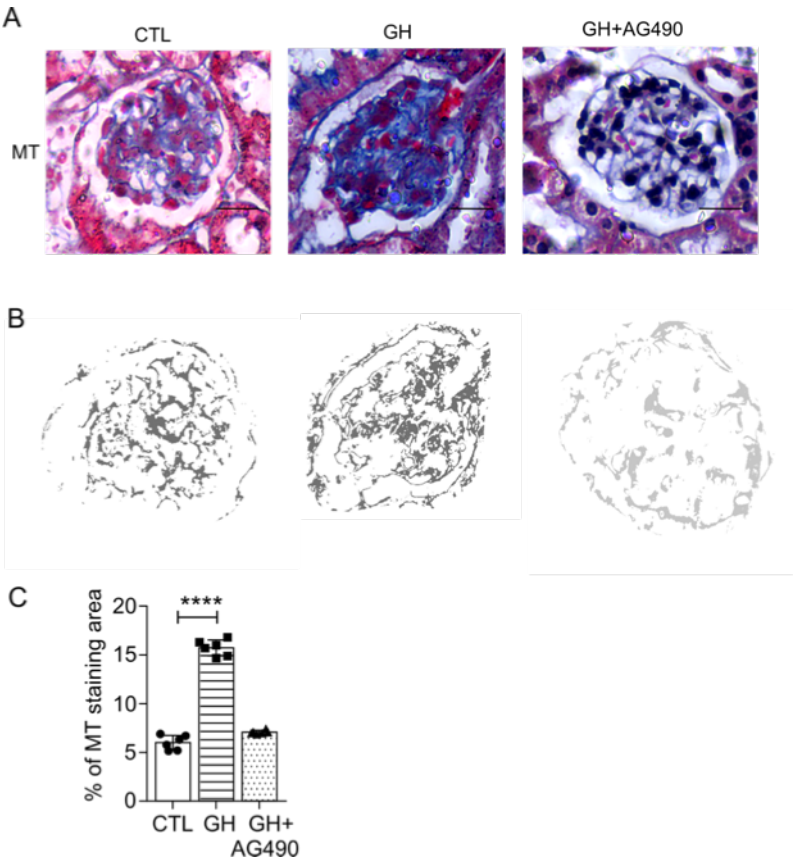

Fig.S4. AG490 administered GH treated mice protected from glomerular fibrosis:

A. Masson's trichrome (MT) staining to glomerular sections from with or without GH and GH+AG490 treated mice group (n=6, each group). Scale bar= 20µm. Magnification=x630. **B.** Representative images of quantified area for MT **s**taining in with or without GH and GH+AG490 treated mice group using GIMP 2.10.22 and ImageJ (NIH). **C.** The percentage of staining area from with or without GH and GH+AG490 treated mice group were presented as dot plot. Each dot represents the average value of five glomerular sections form each mouse. ***p<0.0001. Data presented as mean ±S.D. (n=3) and statistical significance was analyzed by one-way ANOVA post hoc Dunnett test.

Fig.S5:

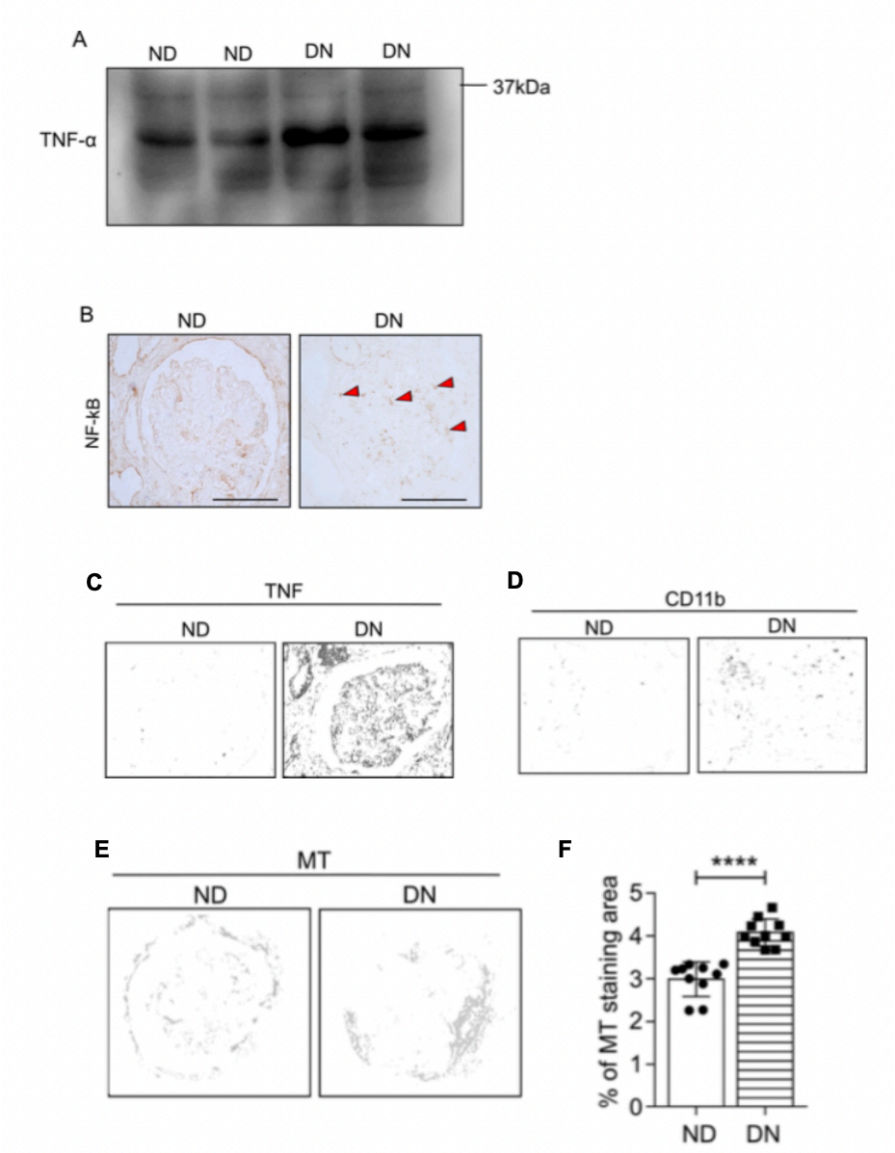

Fig.S5. Elevated serum TNF-α correlates with glomerular TNF-α signaling and macrophage activation in DN patients.

A. Representative immunoblot from non-diabetic (ND) and diabetic nephropathy (DN) serum sample for TNF-α. (n=3). **B.** Representative images of immunohistochemical staining for TNF-α (indicated by red arrow head) in ND and DN biopsy samples. Scale bar=50μm. Magnification= x400. **C&D.** Representative images of quantified area for TNF-α, CD11b and MT staining in ND and DN biopsy samples using GIMP 2.10.22 and ImageJ (NIH). **E&F.** Representative images of quantified area for MT staining and presented as a dot plot from ND and DN biopsy samples using GIMP 2.10.22 and ImageJ (NIH). Each dot represents the average value of five glomeruli from each biopsy samples (n=10). ****p<0.0001.

Table S1: List of the primers and siRNA used in the study.

TNF-α	Human CACAGTGAAGTGCTGGCAAC
	Human AGGAAGGCCTAAGGTCCACT
	Mice CCCTCACACTCACAAACCAC
	Mice ACAAGGTACAACCCATCGGC
NF-kB	Human CCAACAGATGGCCCATACCT
	Human AACCTTTGCTGGTCCCACAT
	Mice GTCAAAATTTGCAACTATGTGGGG
	Mice GTTTGCAAAGCCAACCACCA
IL-1B	Human CAGAAGTACCTGAGCTCGCC
	Human AGATTCGTAGCTGGATGCCG
	Mice TGCCACCTTTTGACAGTGATG
	Mice TGATGTGCTGCTGCGAGATT
IL-10	Human TGCTCTTGCAAAACCAAACC
	Human CAGCTGTTCTCAGACTGGGTG
	Mice CCAAGGTGTCTACAAGGCCA
	Mice GCTCTGTCTAGGTCCTGGAGT
MCP1	Human GATCTCAGTGCAGAGGCTCG
	Human TTTGCTTGTCCAGGTGGTCC
	Mice TACATGGGAGACTCTGGGGG
	Mice AGAGAGCTCTGGCTTGGAGA
CXCL1	Human CTGGCTTAGAACAAAGGGGCT
	Human TAAAGGTAGCCCTTGTTTCCCC
CXCL2	Human TTCACAGTGTGTGGTCAACAT
	Human TCTCTGCTCTAACACAGAGGGA
NPHS1	Human AGAGCCCCATTCAAAGGCTC
	Human AGAAGGAGCTCACGGTTTCG
NPHS2	Human CATCTGGTTCTGCGTAAAGGTTG

	Human ACATGTCTTTGGTCACGATCTCA
IkB-α	Human AAGTGATCCGCCAGGTGAAG
	Human CTGCTCACAGGCAAGGTGTA
	Mice CCTGACCTGGTTTCGCTCTT
	Mice CTGTATCCGGGTACTTGGGC
iNOS	Human TTCAAGACCAAATTCCACCAC
	Human ATTCTGCTGCTTGCTGAGGT
	Mice TGCATGGACCAGTATAAGGCAAGC
	Mice GCTTCTGGTCGATGTCATGAGCAA
Arginase1	Human TGATGTTGACGGACTGGACC
	Human ATCTAATCCTGAGAGTAGCCCTGT
	Mice CAGAAGAATGGAAGAGTCAG
	Mice CAGATATGCAGGGAGTCACC
siRNA-TNF-α	sense, 5′-GCCGAUGGGUUGUACCUUG-3′