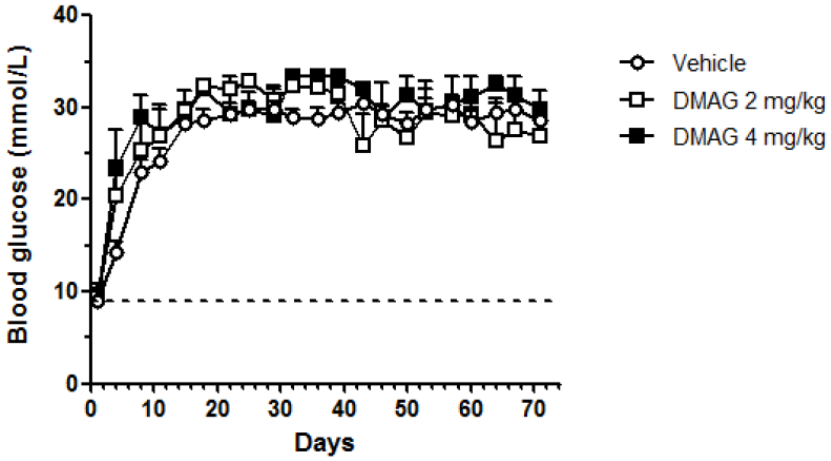
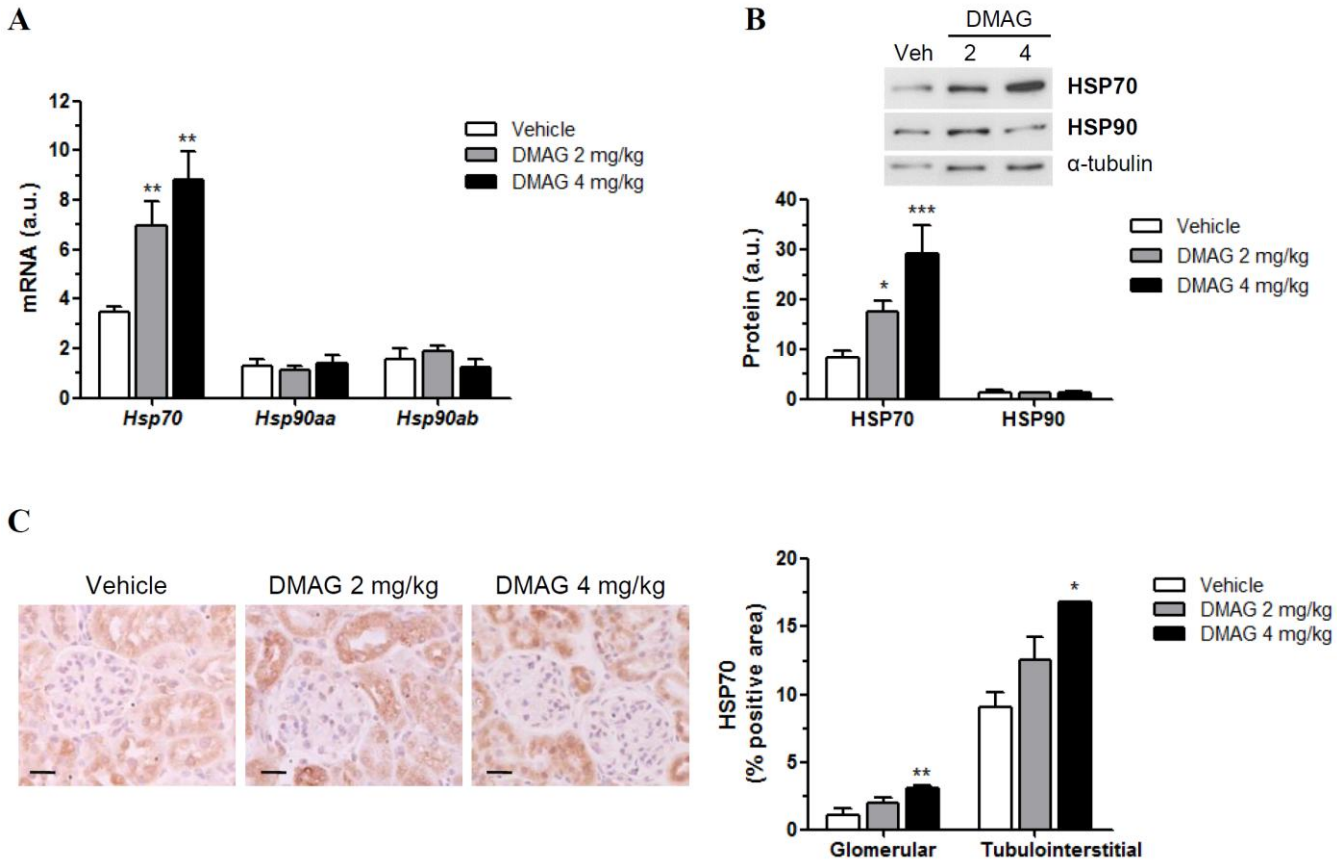
SUPPLEMENTARY DATA

Supplementary Figure 1. Evolution of blood glucose in diabetic apoE$^{-/-}$ mice treated with either vehicle or DMAG (2 and 4 mg/kg) along the 10-week follow-up. Horizontal dashed-line represents the average levels of blood glucose in non-diabetic mice. White circles indicate vehicle mice; white squares, DMAG 2 mg/kg; black squares, DMAG 4 mg/kg. Values represented are the mean±SEM of 6-9 animals per group.



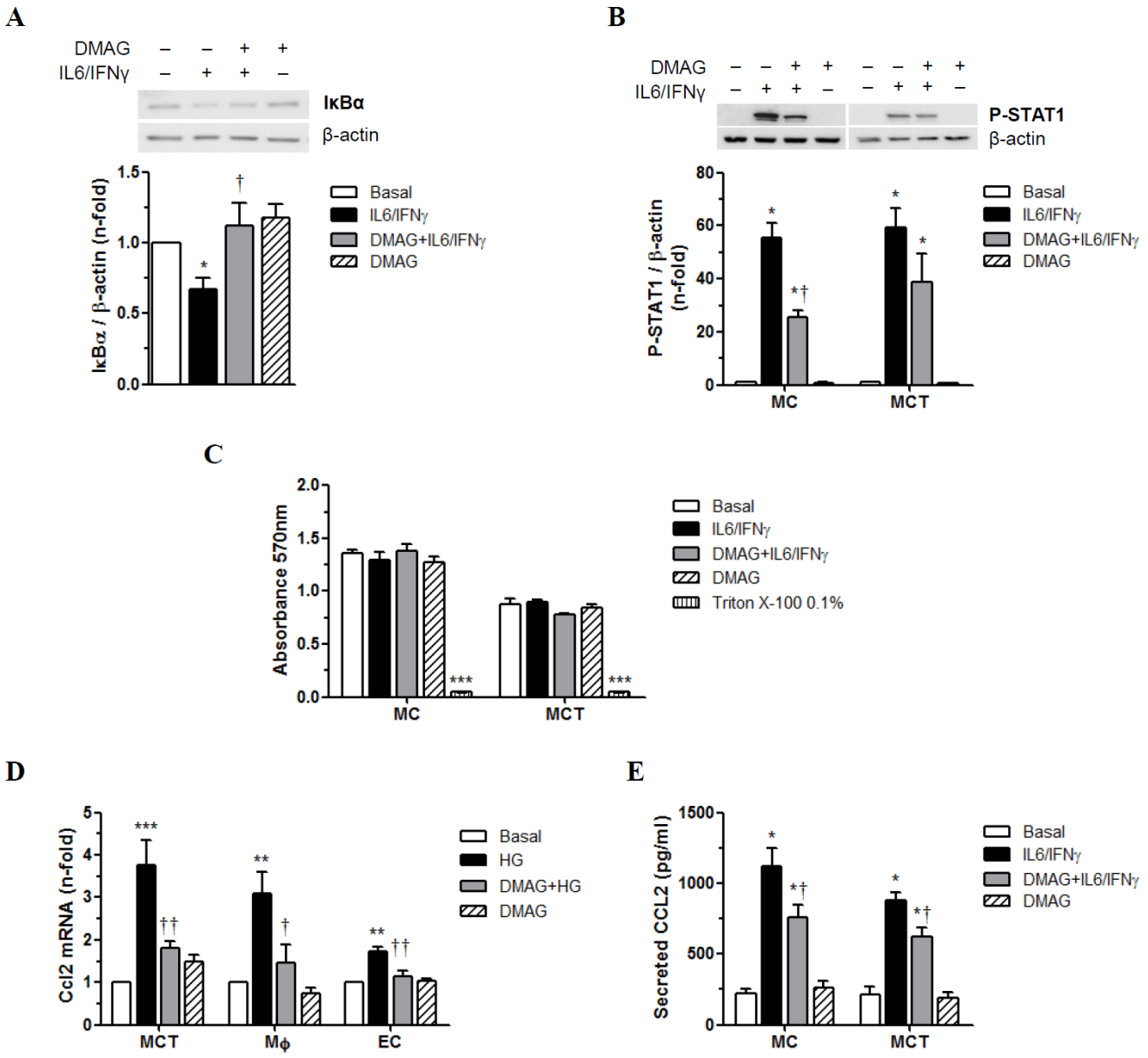
SUPPLEMENTARY DATA

Supplementary Figure 2. HSP90 inhibition modulates HSP70 expression in diabetic kidneys. *A:* Real-time PCR analysis of HSP70 and HSP90 (Hsp90aa, subunit α; Hsp90ab, subunit β) mRNA expression in renal cortex from control and DMAG-treated mice. Results were normalized to 18S and expressed in arbitrary units (a.u.). *B:* Immunoblot analysis of HSP70 and HSP90 proteins in renal cortical lysates from vehicle- and DMAG-treated groups. Representative images and summary of normalized densitometric quantification are shown. *C:* Immunolocalization of HSP70 in kidney sections from diabetic apoE$^{-/-}$ mice. Representative micrographs (scale bar, 20 μm) and quantification of positive staining in both glomerular and tubulointerstitial compartments are shown. White bars indicate diabetes-vehicle mice; gray bars, diabetes-DMAG 2 mg/kg; black bars, diabetes-DMAG 4 mg/kg. Values are expressed as mean±SEM (n=6-9 animals per group). **P*<0.05; ***P*<0.01; ****P*<0.001 vs. diabetes-vehicle group.



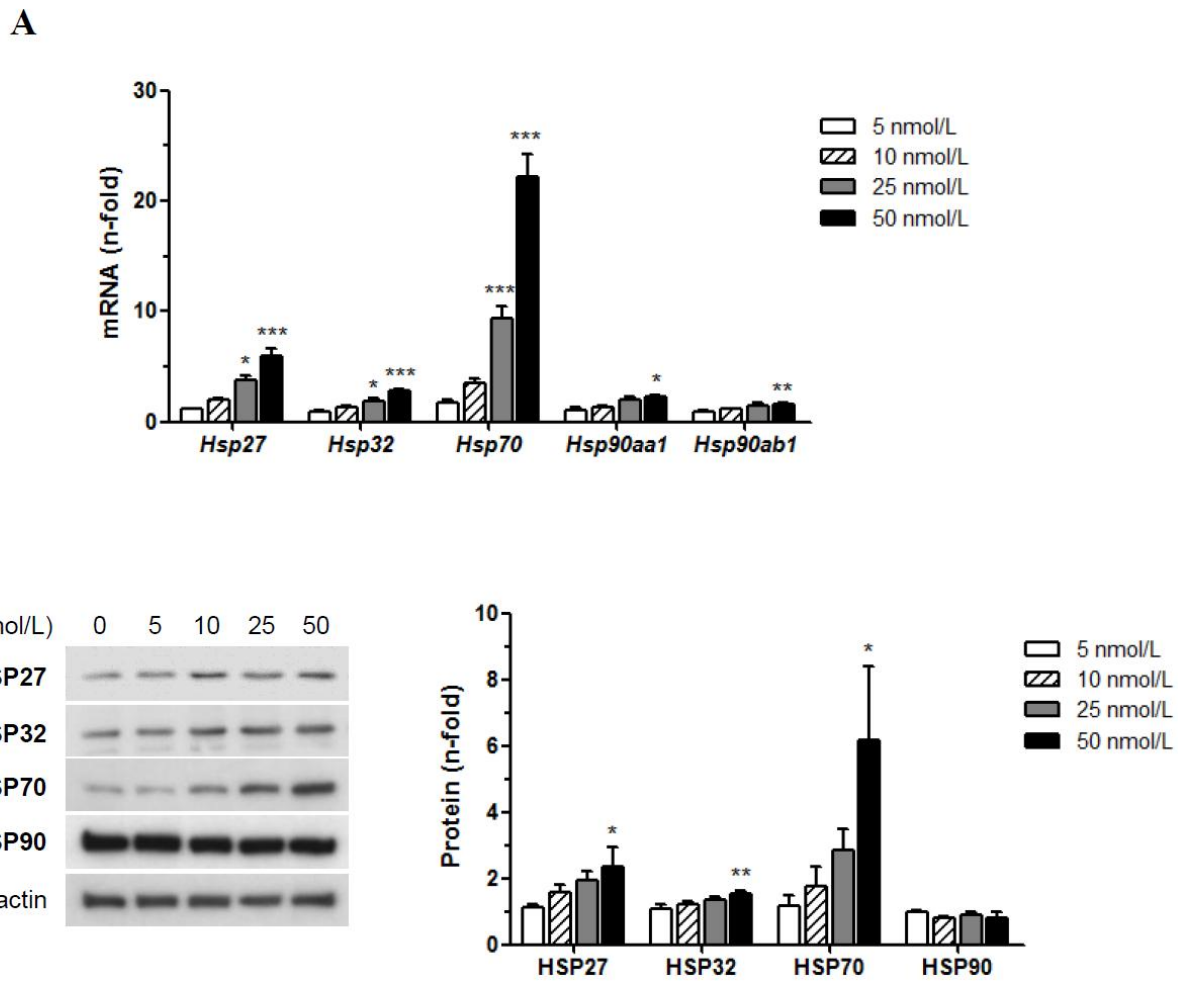
SUPPLEMENTARY DATA

Supplementary Figure 3. *In vitro* effects of DMAG treatment. Quiescent tubuloepithelial cells (MCT), mesangial cells (MC), macrophages (MΦ) and endothelial cells (EC) were pretreated with DMAG (50 nmol/L, 4 hours) before stimulation with either cytokines (IL6 10^2 U/mL and IFNγ 10^3 U/mL) or high glucose (HG, D-glucose 30 mmol/L). *A-B*: Western blot analysis of IκBα in MCT (*A*) and P-STAT1 in MC and MCT (*B)* at 1 hour of cytokine stimulation. Representative immunoblots and summary of normalized densitometric quantification are shown. *C:* MTT viability assay in MC and MCT at 24 hours. *D:* Real-time PCR analysis of *Ccl2* gene expression in MCT, MΦ and EC stimulated with HG for 24 hours. Results are expressed as the relative increase over basal conditions. *E:* CCL2 secretion by MC and MCT was measured in cell supernatants at 24 hours by ELISA. White bars indicate basal condition; black bars, stimulus; gray bars, DMAG+stimulus; hatched bars, DMAG alone; and, vertical-striped bars, Triton X-100 at 0.1%. Values represent the mean±SEM of 3-7 independent experiments. **P*<0.05, ***P*<0.01, ****P*<0.001 vs. basal; †*P*<0.05, ††*P*<0.01 vs. stimulus.



SUPPLEMENTARY DATA

Supplementary Figure 4. DMAG treatment modulates HSP90-associated intracellular transduction *in vitro*. *A:* Real-time PCR analysis of mRNA expression of HSP family members in primary mouse mesangial cells after 4 hours of treatment with different concentrations of DMAG (5-50 nmol/L). Values were normalized to 18S. *B:* Mesangial expression levels of HSP proteins at 24 hours. Representative immunoblots and summary of normalized densitometric quantification are shown. Results expressed as relative increase (n-fold) over non-treated cells are the mean±SEM of 3-4 independent experiments. **P*<0.05; ***P*<0.01; ****P*<0.001 vs. non-treated cells.



SUPPLEMENTARY DATA

Supplementary Figure 5. siRNA targeting HSP70 in renal cells. *A:* Real-time PCR analysis of HSP70 in MCT after 24 hours of transfection with negative control scramble (scr) or HSP70 specific (si70) siRNA. Values normalized to 18S are expressed as the relative increase (n-fold) over vehicle (-) condition. Mean±SEM of 4-6 independent experiments. *** $P<0.001$ vs. scrambled. *B:* Representative immunoblots (n = 4 experiments) of HSP70 and HSP90 protein expression in MCT.