UPAP attenuates renal fibrosis by modulating mitochondrial unfolded protein response via HSP60-ClpP complex

Short title: UPAP in renal fibrosis

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Supplemental methods

UPAP antibody production. The polyclonal antibody against the UPAP microprotein was produced according to standard procedures by HuaBio. The polyclonal antibodies were obtained from inoculated rabbits and purified using affinity chromatography. The specificity of our customed antibody was validated by immunoblotting and immunofluorescence using UPAP knockout cells.

CRISPR/Cas9-based genome editing. Designed guide RNA (gRNA) was cloned into a lentiCRISPR v2 vector. Donor oligo was designed and commercially synthesized (GenScript Biotech). The targeting vector was co-transfected with donor oligos into HEK293T cells, followed by selection with puromycin (2 μg/mL). A DNA extraction kit (Beyotime) was used for genomic DNA extraction, and PCR was performed to amplify the targeting region. The obtained PCR products were subsequently sequenced to confirm successful genome editing. The gRNA and donor oligos sequences used in the study are listed in Supplemental Table 5.

LC-MS/MS analysis. Proteins were fractionated by SDS-PAGE and stained with Coomassie Brilliant Blue. The protein bands were cut, destained, and dehydrated. Samples were dissolved in 0.1% TFA and 2% acetonitrile, and the resulting peptides were analyzed with a QExactive mass spectrometer (Thermo Fisher Scientific) coupled with an Easy-nLC instrument (Thermo Fisher Scientific). The linear gradient consisted of (A) 0.1% formic acid, 99.9% water and (B) 0.1% formic acid, 99.9% acetonitrile at a flow rate of 300 nL/min. The QExactive was operated in data-dependent mode with survey scans acquired at a resolution of 70,000 at m/z 350-1600. The parent ion with TOP20 ion strength over the whole scan was selected for fragmentation by the HCD method and identified by secondary mass spectrometry. The library database was searched for calculation and analysis using the Proteome Discoverer software.

RNA isolation and real-time quantitative PCR (RT-PCR). Total RNA was isolated from cells using SteadyPure Quick RNA Extraction Kit (Accurate Biology) according to the

manufacturer's protocol. cDNA was synthesized using Evo M-MLV RT Mix Kit with gDNA Clean for qPCR (Accurate Biology). Real-time quantitative PCR was performed using SYBR Green Premix Pro Taq HS qPCR Kit (Accurate Biology). The expression levels of target genes were normalized to GAPDH. Primer sequences are listed in Supplemental Table 6.

Immunoblotting analysis. Cell or tissue lysates were added to SDS-PAGE and transferred to PVDF membranes (MilliporeSigma). Membranes were incubated overnight with primary antibodies. HRP-linked goat anti-rabbit/mouse IgG was used as the secondary antibody. GAPDH and α-tubulin were used as loading controls. The blots were visualized using ECL reagent. Blot images were captured using Image Lab software (Bio-Rad). Full-length immunoblotting images for all figures included in this manuscript are provided in the Unedited gel blots file.

Immunofluorescence. Cells were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton X-100, blocked with 5% BSA, and incubated overnight at 4°C with primary antibodies, and subsequently incubated with anti-rabbit (or mouse) IgG. Nuclei were counterstained with DAPI. Fluorescence images were captured using an Evident SpinSR confocal microscope (Olympus). Images for each channel were acquired using the same settings.

Immunohistochemistry (IHC). Kidney sections were deparaffinized and subsequently blocked with 3% H_2O_2 for 20 minutes. The sections were then incubated with EDTA/EGTA buffer (pH 9.0) at 100°C for 20 minutes for antigen retrieval. Following subsequent blocking with 5% bovine serum albumin (BSA) for 1 hour at room temperature, the sections were incubated with primary antibody overnight at 4°C. Staining was performed using the GTVision™ III Detection System (Gene Tech). Images were obtained using a Pannoramic SCAN II (3DHISTECH), and the quantification of IHC staining density was measured using ImageJ software (NIH).

Immunoprecipitation assay. Cells were collected and lysed on ice in Pierce™ IP lysis

buffer (Thermo Scientific) containing 1 mM PMSF for 30 minutes. The lysates were incubated with the indicated primary antibodies or IgG in combination with protein A/G beads (Beyotime) at 4°C overnight under rotary agitation. After three washes with 1× TBST, the bound proteins were eluted using 1×SDS-loading buffer, followed by SDS-PAGE and immunoblotting analysis.

GST pull-down assay. GST-tagged protein was incubated with His-tagged protein in 1 mL of incubation buffer (50 mM Tris-HCl, 10% glycerol, 100 mM KCl, 5 mM MgCl2, 10 mM β-mercaptoethanol, and 0.1% NP-40, pH 8.0) supplemented with corresponding Glutathione MagBeads (Sangon Biotech) at room temperature for 2 hours with gentle rotation. Then the MagBeads was washed with washing buffer 3 times. The MagBeads was boiled with 1× SDS loading buffer, and the protein enriched in the MagBeads was detected by immunoblotting analysis.

Mitochondrial isolation and proteinase K gradient assay. Mitochondria were isolated using a cell mitochondria isolation kit (Beyotime). Briefly, 2×10^7 cells were washed twice with pre-cold 1×PBS and lysed in mitochondrial isolation buffer, incubated on ice for 10 minutes, and homogenized with a 2 mL Dounce grinder. Homogenates were centrifuged at 600×g, and the supernatants were collected and centrifuged again at 11,000×g. The pellet was resuspended in mitochondrial storage buffer. For the proteinase K gradient assay, purified mitochondria were incubated with a range of PK concentrations from 1 ng/mL to 1 mg/mL at 37°C for 20 minutes, followed by adding PMSF (Beyotime) and proteinase inhibitors (Beyotime) to terminate PK digestion. Pellets were collected by centrifugation at 12,000×g, 4°C for 5 min, homogenized in lysis buffer containing protease inhibitors and PMSF, and then subjected to immunoblotting analysis. Mitochondria were solubilized using Triton X-100 at a final concentration of 1%.

Mitochondrial respiration rate measurement. The oxygen consumption rate was determined by XF Cell Mito Stress Test using XF-96 Extracellular Flux Analyzer (Seahorse Bioscience). A total of 1×10^4 cells were seeded in XF96-well cell culture

microplates and incubated overnight. Different inhibitors (Agilent Technologies, 103015-100) were injected at the indicated time points: 1.5 μM oligomycin was added to assess respiration related to ATP turnover; 1.5 μM carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) was used to induce maximal respiration; rotenone/antimycin A (Rot/Anti-A) was added at a final concentration of 0.5 μM to inhibit electron transport and assess the nonmitochondrial basal respiration level.

Measurement of cellular ATP. The ATP detection assay kit (Beyotime) was used for the measurement of cellular ATP levels according to manufacturer's instructions. Briefly, cells were collected and lysed in lysis buffer. 100 μL ATP detection reagent was added to each plate well and incubated for 5 minutes at room temperature. Add 20 μL sample into each well and mixed, and the luminescence was measured on a luminometer. The values were normalized to the control group in each individual experiment.

Measurement of mitochondrial ROS. The mitochondrial ROS measurement was performed using MitoSox Red probe (Beyotime). Cells were collected and centrifuged at 600 ×g for 5 minutes. Cells were then incubated in 1mL MitoSox Red in PBS for 30 minutes at 37℃. Cells were centrifuged at 1500 rpm for 5 minutes, and the pellets were re-suspended in 1× PBS and 10,000 cells were analyzed using flow cytometry (Beckman). Data were analyzed by FlowJo software and presented as the percentage of positive cells.

Transmission electron microscopy (TEM). Cells and kidney tissues were fixed in glutaraldehyde and osmic acid, dehydrated in ethanol and acetone, postfixed, and dyed with uranyl acetate. After these procedures, the tissues were embedded and polymerized overnight at 37°C. The samples were then sectioned to generate ultrathin sections. The sections were sequentially stained with uranyl acetate and lead citrate. The stained sections were imaged using a transmission electron microscope (HITACHI).

Cellular thermal shift assay (CETSA). To assess the binding of DCEM1 with HSP60 within cells, 2 million HK-2 cells were treated with either DMSO or DCEM1 (20 μM)

for 1 hour at room temperature. Protease inhibitors were directly added to the media, and cells were harvested by scrapping and washed once with PBS, then resuspended in 500 µL PBS supplemented with 1× protease inhibitors and DCEM1 (20 µM). 100 µL cell suspension was distributed into three PCR tubes and incubated at 55°C, 57°C and 58°C for 3 minutes in a PCR machine. Immediately after heating, cell suspensions were further incubated at room temperature for 3 minutes and then snap frozen in liquid nitrogen. Cells were lysed by three freeze-thaw cycles in liquid nitrogen was performed. Cell lysates were collected and centrifuged at 20,000 g for 20 minutes at 4°C to remove cell debris and aggregated proteins. Supernatant was collected and 10 µL was heated with SDS sample buffer at 100°C for 5 minutes and subjected to immunoblotting analysis for detection of HSP60 and GAPDH.

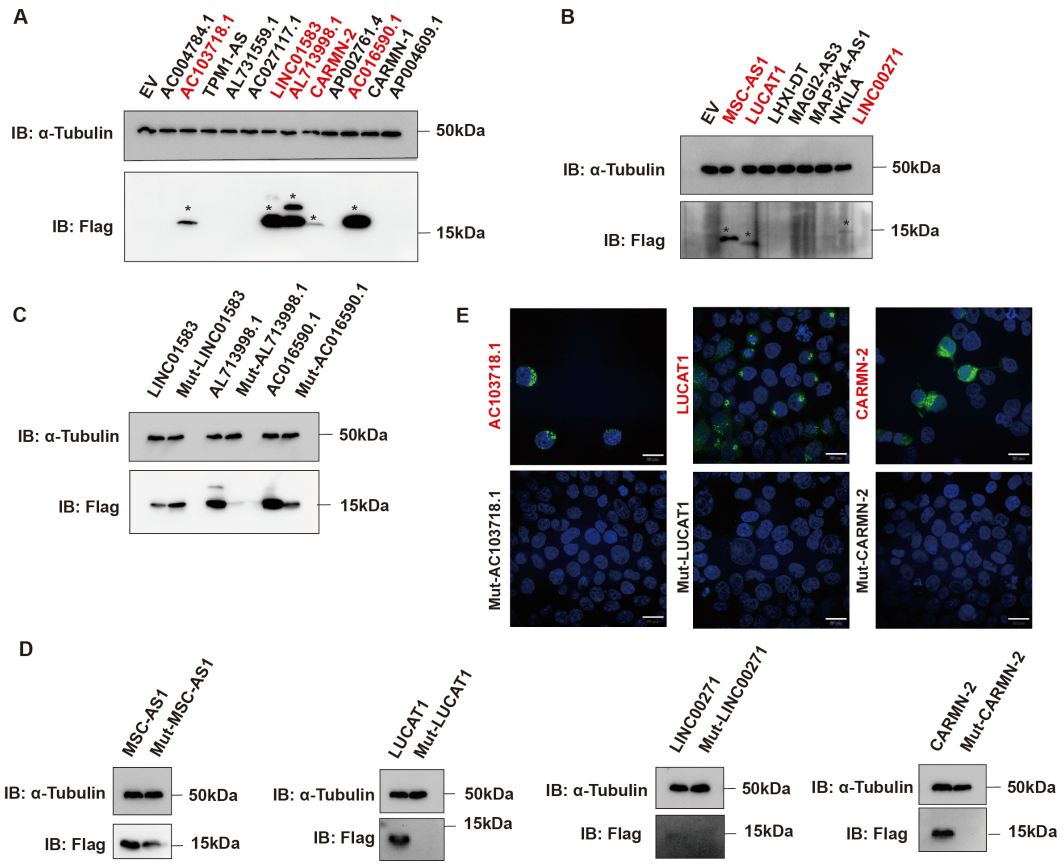
Proximity ligation assays (PLA). PLA was used to analyze the protein-protein interaction along with the subcellular localization of the observed interaction in HK-2 cells and kidney tissues using Duolink® *In Situ* Starter Kit (Sigma) according to manufacturer's instructions. Briefly, Cells were fixed with 4% paraformaldehyde (Beoytime) in 1 × PBS for 30 minutes at room temperature and then washed twice with 1× PBS. Blocking reagents was added for 1h at 37°C, and primary antibodies specific for UPAP and HSP60 were diluted (1:100) with antibody diluents and added directly on the coverslip overnight at 4°C. Washed twice with Duolink *in situ* wash buffer A (8.8 g NaCl, 1.2 g Tris base, 0.5 mL Tween 20, pH 7.4) with gentle agitation. Then, 40 µL of probe mix was added on to the coverslip and subsequently transferred into a humidified chamber for 1 hour at 37°C. The samples were washed with Duolink *in situ* wash buffer A twice for 5 min under gentle agitation and the probes were ligated with two circleforming DNA oligonucleotides by ligation-ligase solution for 30 minutes at 37 °C (4 µL 5× Ligation buffer, 0.5 µL ligase and 15.5 µL ddH2O). The samples were washed in 1× wash buffer A twice at 5 minutes interval under gentle agitation and added fluorescently labelled oligonucleotides buffer (4 µL of amplification red buffer, 0.25 µL polymerase and 15.75 µL ddH2O) to complete the enzymatic ligation via rolling

circle amplification under dark conditions for 100 minutes at 37 °C in the humidified chamber. The samples were washed in 1× wash buffer B (5.84 g NaCl, 4.24 g Tris base, 26.0 g Tris-HCL, pH 7.4) twice for 10 minutes followed by 0.01× wash buffer B for 1 minute by diluting 1× buffer B 1:100 in high purity water under dark conditions and mounted onto a slide in a minimal volume of ProLong Gold antifade reagent with DAPI. Slides were stored at 4°C in the dark. For human kidney tissues slides, the same methods were followed as mentioned in the IHC protocol up to antigen retrieval and further followed the exact protocol of the *in situ* PLA protocol mentioned above in this section. Subcellular localization of protein-protein interactions was detected using the Evident SpinSR confocal microscope (Olympus).

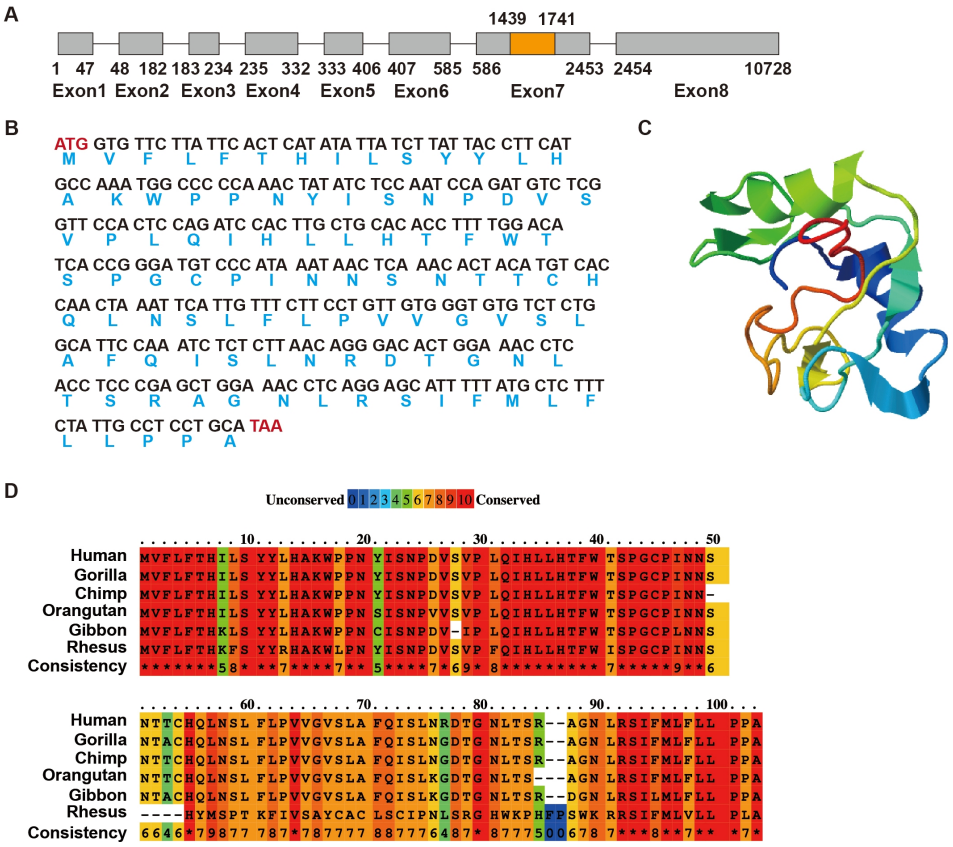
Mitochondrial chaperonin activity assay (MiCAA). To assess the endogenous mitochondrial chaperonin (protein folding) activity, HK-2 cells were co-transfected with pmCherry-N1 and EYFP-Mito plasmids, which were obtained from Vigen Biotechnology. After 24 hours, cells were collected and analyzed by flow cytometry using PE Texas Red and FITC channels. The average fluorescent intensity for both channels was determined using FACS Diva software. YFP signal intensity was normalized to RFP signal intensity and plotted as a fold change compared to control.

Histopathology. Kidney tissues were fixed in 4% paraformaldehyde and embedded in paraffin. The kidney sections were stained with hematoxylin and eosin (H&E) and Masson's trichrome. Tubular injury was defined as tubular sloughing, cast formation, dilation, degeneration, or atrophy, and was assessed according to the following scoring system: 0 (no injury), 1 (<25% affected area), 2 (25-50% affected area), 3 (51-75% affected area), and 4 (>75% affected area). Images of the kidney slides were obtained on a Pannoramic SCAN II (3DHISTECH), and the stained areas were quantified using ImageJ software (NIH).

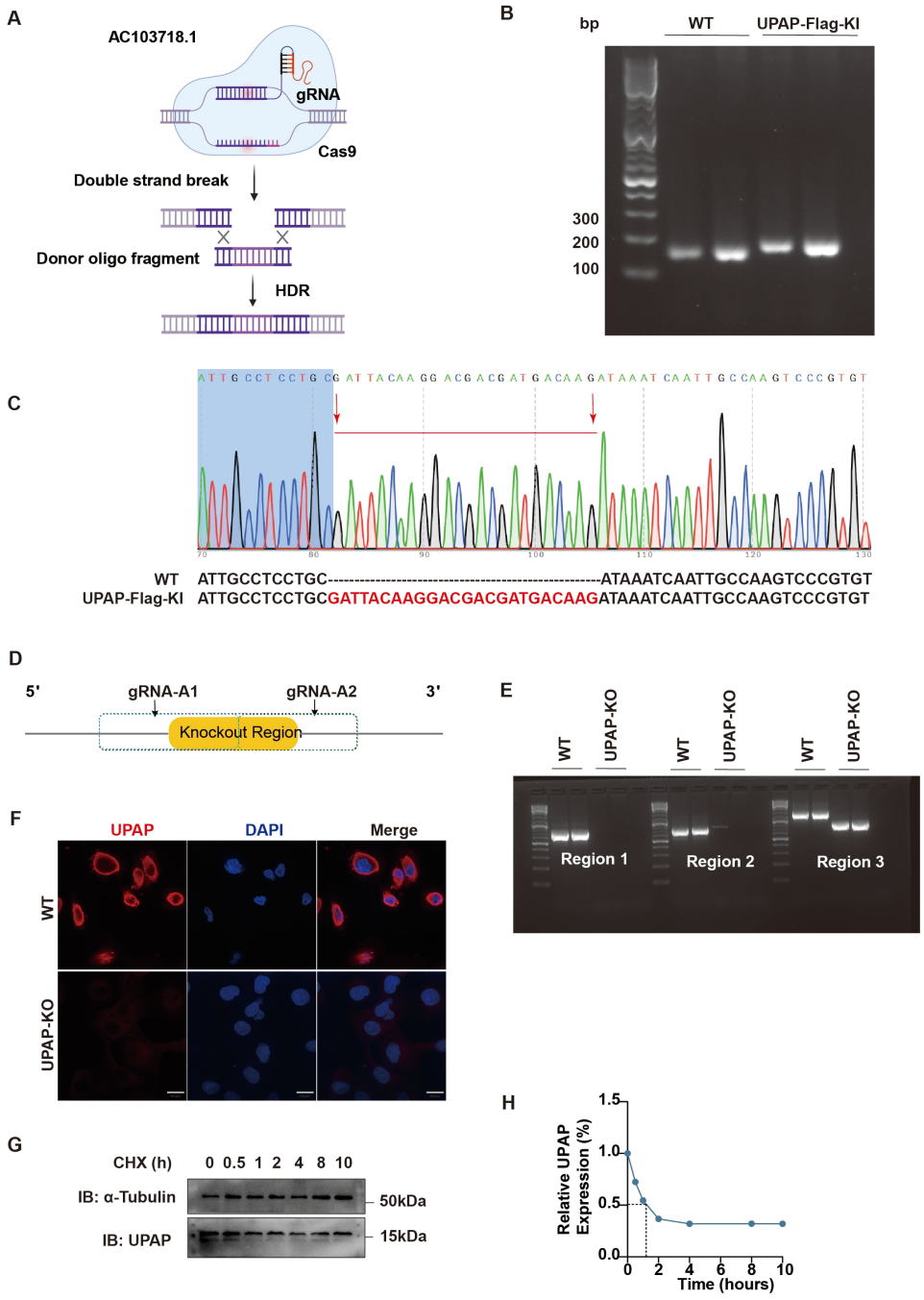
Supplemental Figures and Tables



Supplemental Figure 1. Characterization of the coding potential of lncRNA AC103718.1. (A-B) Nineteen ORF-Flag fusion constructs were transfected into HEK293T cells, and the Flag signal was detected by immunoblotting analysis. The red front indicates candidate coding potential lncRNAs. **(C-D)** Indicated constructs were expressed in HEK293T cells, and the Flag signal was detected by immunoblotting analysis. **(E)** Indicated constructs were expressed in HEK293T cells, and Flag signal (green) was detected by immunofluorescence staining analysis. Nuclei were stained with DAPI (blue). Scale bar: 20 μm.



Supplemental Figure 2. Sequence, 3D structures, conservative analysis of UPAP peptides. (A) Schematic of the ORF location at the lncRNA AC103718.1 locus. The eight exons of lncRNA AC103718.1 are shown. **(B)** The amino acid sequence of the UPAP encoded by the lncRNA AC103718.1 of *H.sapiens*. The ATG start codon and TAA stop codon are highlighted in red. **(C)** The 3D structures of UPAP were modeled by C-I-TASSER algorithm. **(D)** Multi-species alignment of the UPAP was performed using the PRALINE algorithm after *in silico* translation of the conserved homologous ORFs in the indicated species.



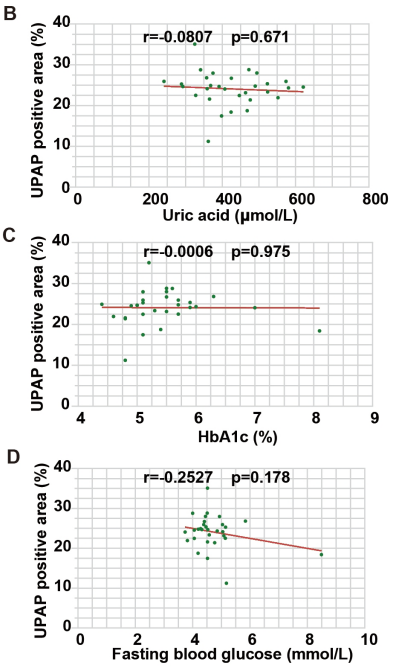
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209 **Supplemental Figure 3. UPAP is a microprotein encoded by lncRNA AC103718.1**
210 **and is endogenously expressed. (A)** The targeting strategy using CRISPR/Cas9-
211 mediated homologous recombination to knock a Flag epitope tag into the UPAP locus
212 in HEK293T cells. **(B)** PCR results for the wild type (WT) and UPAP-Flag knockin (KI)
213 alleles. **(C)** Sanger sequencing results of WT and UPAP-Flag KI locus from genomic
214 DNA of the HEK293T cells. **(D)** Schematic of the CRISPR/Cas9-mediated KO strategy.

The blue front indicates primers for Region 1; The green front indicates primers for Region 2; The black front indicates primers for Region 3. **(E)** PCR results for the WT and UPAP knock-out (KO) alleles. **(F)** The expression of UPAP (red) in HK-2 cells with UPAP-KO was detected by immunofluorescence using anti-UPAP antibody. Nuclei were stained with DAPI (blue). Scale bar, 20 μm. **(G-H)** The degradation half-life of UPAP was measured in HK-2 cells.

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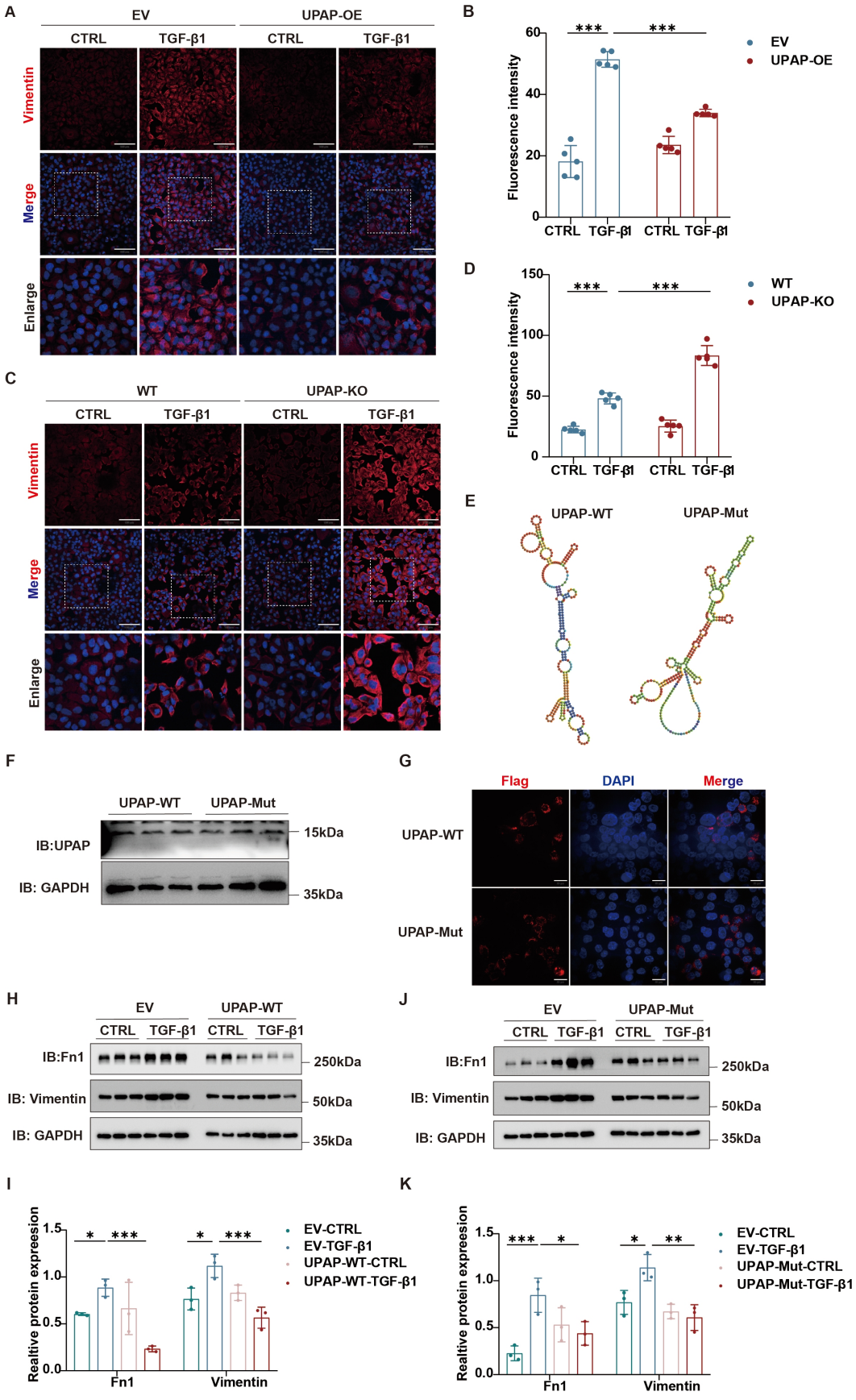
Characteristics	MF (n=16)	SF (n=14)
Age (years)	36.06±12.60	41.79±8.22
Gender (male,n, %)	7 (43.8%)	5 (35.7)
HB (g/L)	132.50±19.18	122.93±16.09
Fasting blood glucose (mmol/L)	4.53±0.49	4.90±1.08
HbA1c (%)	5.53±0.58	5.36±0.85
Serum albumin (g/L)	38.15±3.40	35.25±2.38
BUN (mmol/L)	5.21±1.80	8.07±1.69
Serum creatinine (μmol/L)	75.78±17.85	130.00±27.32
eGFR (ml/(min*1.73m2))	99.00±27.93	51.14±13.12
Uric acid (μmol/L)	387.93±79.63	466.29±89.31



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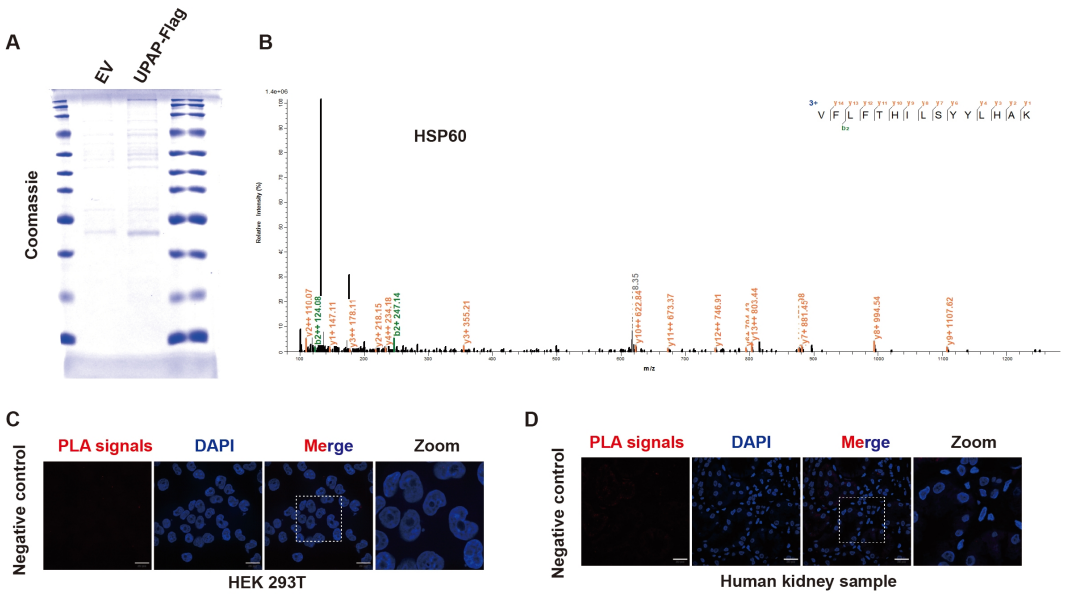
222 **Supplemental Figure 4. Clinical significance of UPAP in CKD patients. (A)** Clinical
223 characteristics of CKD patients with mild renal fibrosis (MF) or severe renal fibrosis
224 (SF). **(B-D)** Correlation analysis showing the relationship between UPAP staining
225 intensity and uric acid **(B)**, HbA1c **(C)**, and fasting blood glucose **(D)**. Data are
226 presented as mean ± SD or number (percent).

227


A
EV
UPAP-OE
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CTRL
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Enlarge
B
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Fluorescence intensity
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40
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CTRL
TGF-β1
CTRL
TGF-β1
EV
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WT
UPAP-KO
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TGF-β1
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Fluorescence intensity
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TGF-β1
CTRL
TGF-β1
WT
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UPAP-Mut
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IB: GAPDH
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UPAP-Mut-CTRL
UPAP-Mut-TGF-β1

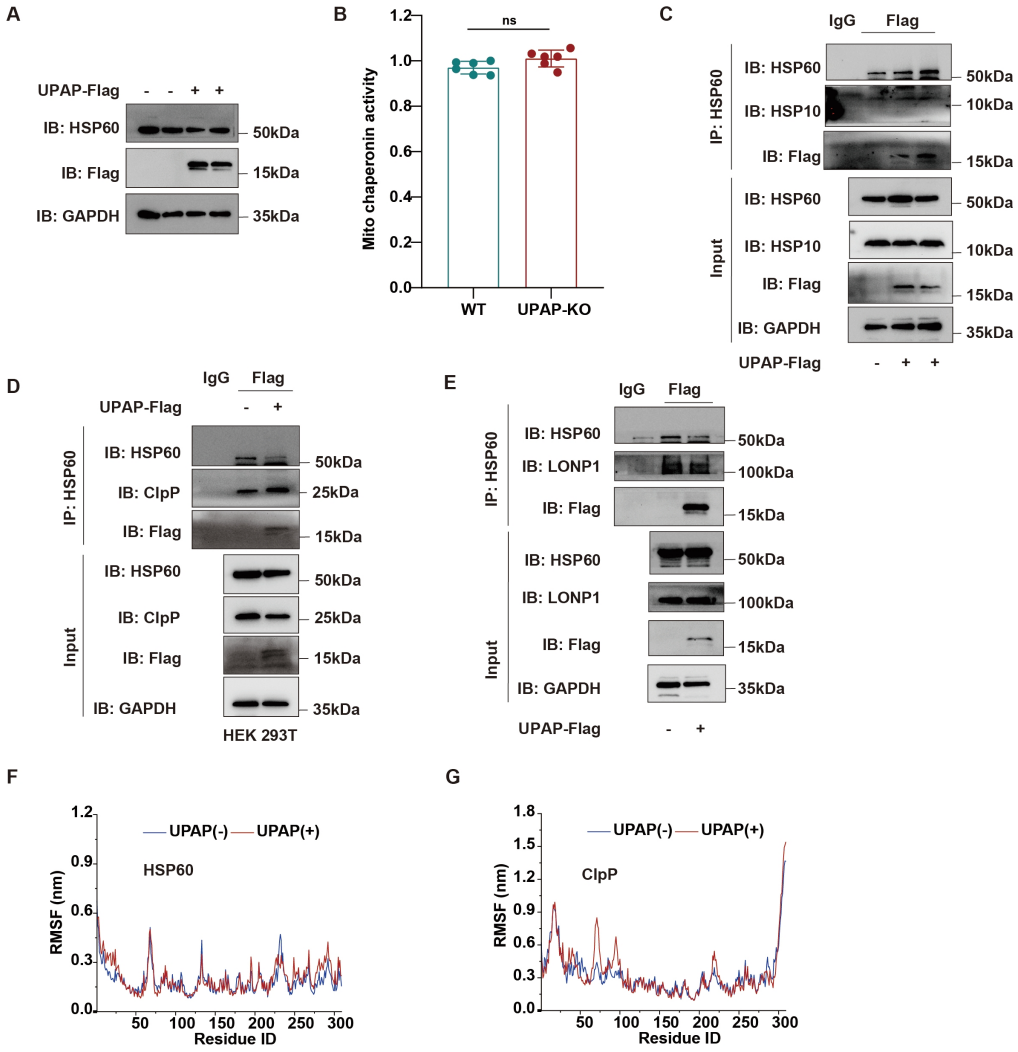
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Supplemental Figure 5. UPAP alleviates the TGF-β1-induced partial EMT. (A-B) Representative images and quantification of fluorescence intensity of vimentin (red) in UPAP-OE HK-2 cells treated with TGF-β1 (n = 5). Nuclei were stained with DAPI (blue). Scale bar: 100 μm. **(C-D)** Representative images and quantification of fluorescence intensity of vimentin (red) in UPAP-KO HK-2 cells treated with TGF-β1 (n = 5). Nuclei were stained with DAPI (blue). Scale bar: 100 μm. **(E)** Predicted minimal free energy of RNA folding for wild-type (WT) and mutant (Mut) UPAP RNA. **(F-G)** The same protein products of UPAP-WT and UPAP-Mut were detected by immunoblotting and immunofluorescence analysis with the UPAP antibody. **(H-K)** Immunoblotting analysis and quantification of Fn1 and vimentin in HK-2 cells after overexpression of UPAP-WT or UPAP-Mut (n = 3). Data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

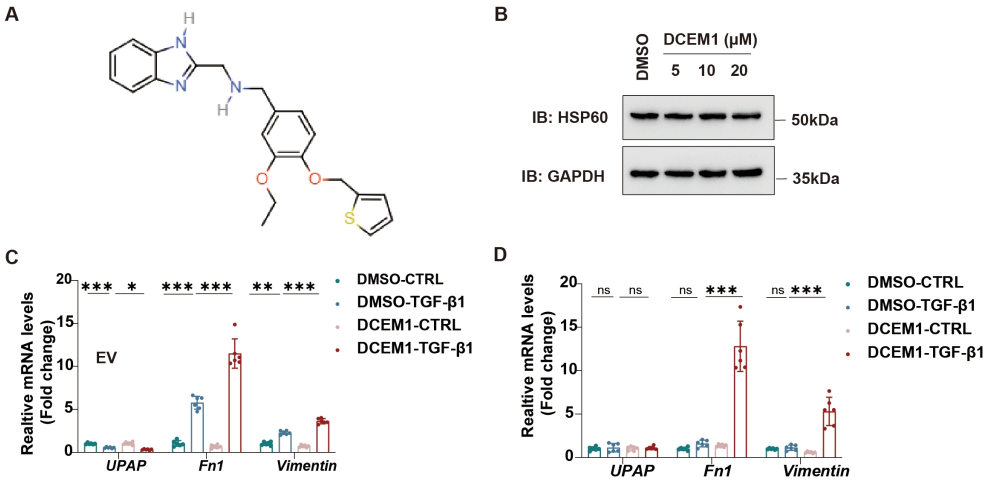


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242 **Supplemental Figure 6. UPAP interacts with HSP60. (A)** Coomassie brilliant blue
243 staining of FLAG immunoprecipitations was performed in HEK293T cells transfected
244 with empty vehicle or UPAP-Flag plasmid. **(B)** LC/MS-MS spectra of HSP60 from
245 Flag-IP eluates. **(C-D)** Isotype controls using normal mouse IgG and normal rabbit IgG
246 antibodies served as negative controls for PLA between UPAP and HSP60 in HEK293T
247 cells **(C)** and kidney tissues **(D)**.



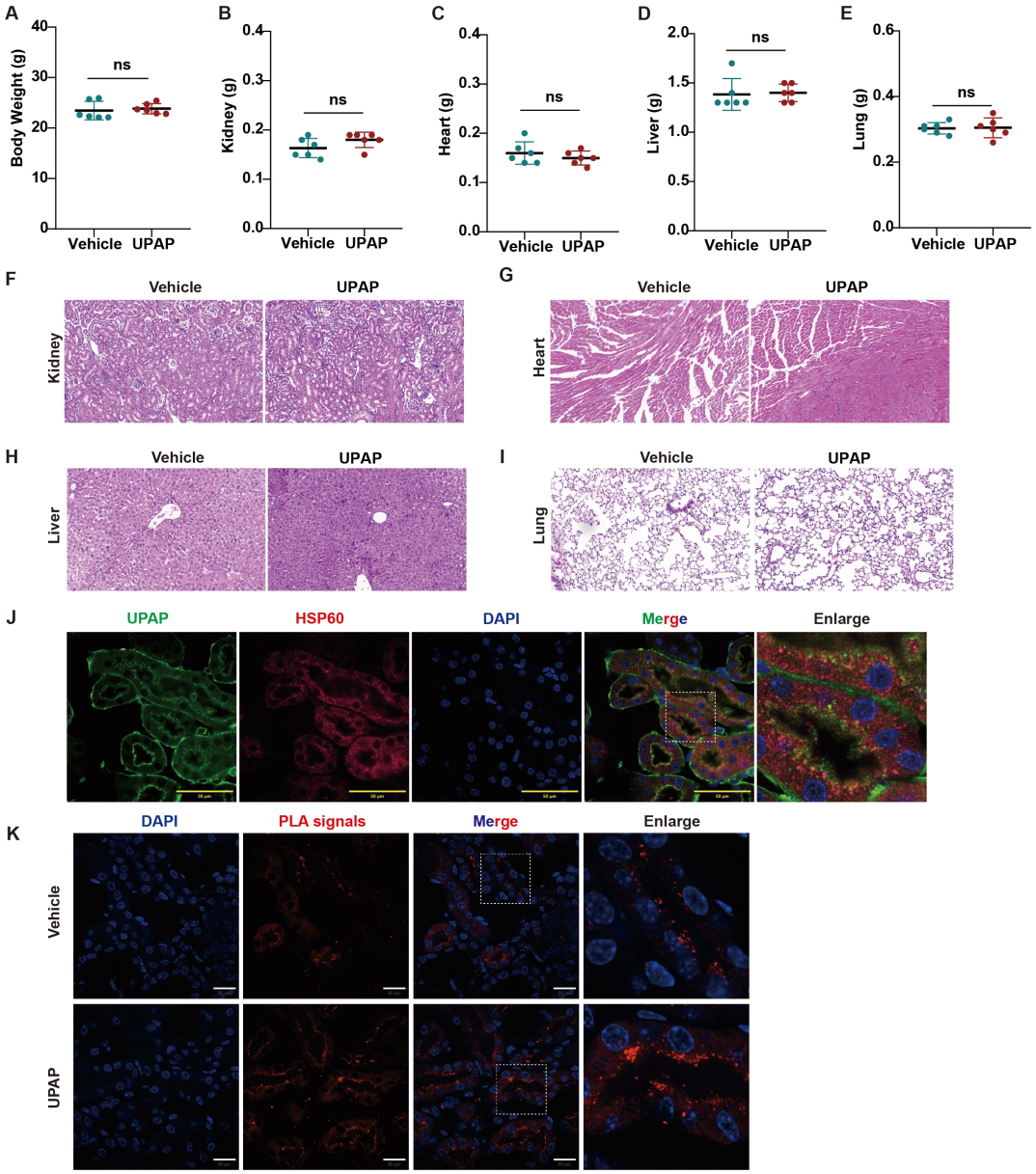
Supplemental Figure 7. UPAP enhances the interaction between HSP60 and ClpP. **(A)** Immunoblotting analysis of UPAP in UPAP overexpression HEK293T cells. **(B)** Mitochondrial chaperonin activity was analyzed in HK-2 cells with UPAP-KO ($n = 6$). **(C)** The interaction between HSP60 and HSP10 was detected by Co-IP assays in HEK293T cells transfected with UPAP-Flag construct. **(D)** The interaction between HSP60 and ClpP was detected by Co-IP assays in HEK293T cells transfected with UPAP-Flag construct. **(E)** The interaction between HSP60 and LONP1 was detected using Co-IP assays in HEK293T cells transfected with the UPAP-Flag plasmid. **(F)** Root mean square fluctuations (RMSF) values of HSP60, with or without UPAP. **(G)** Root mean square fluctuations (RMSF) values of ClpP, with or without UPAP.



Supplemental Figure 8. UPAP regulates partial EMT through enhancing the HSP60-ClpP interaction. (A) Structure of DCEM1. **(B)** Immunoblotting analysis of HSP60 expression in HK-2 cells treated with DCEM1. **(C)** Quantification of mRNA levels of UPAP, Fn1 and vimentin in HK-2 cells treated with DCEM1 and TGF-β1 (*n* = 6). **(D)** Quantification of mRNA levels of UPAP, Fn1 and vimentin in UPAP-OE HK-2 cells treated with DCEM1 and TGF-β1 (*n* = 6). Data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.



Supplemental Figure 9. Human and mouse HSP60 share highly homology. Alignment of full-length HSP60 amino acid sequences from human (Black) and mouse (gray). Non-conserved amino acid sequences are marked in red.



Supplemental Figure 10. UPAP treatment did not induce organ or systemic toxicities. (A) Body weight of mice treated with either vehicle or UPAP was measured at the time of termination. **(B-I)** At the time of termination, major organs were harvested, weighed **(B-E)** and sections were cut and stained with H&E for histopathological evaluation **(F-I)**. Scale bars: 20 μm. **(J)** Colocalization analysis of exogenous UPAP (green) with HSP60 (red) in uIRI renal tissues. Nuclei were stained with DAPI (blue). Scale bars: 50 μm. (K) PLA between HSP60 and ClpP was performed in uIRI renal tissues collected from both vehicle-treated and exogenous UPAP-treated mice subjected

to uIRI injury. Nuclei were stained with DAPI (blue). Scale bars: 20 μm. Data are presented as mean ± SD. ns-not significant.

Supplemental Table 1: LncRNAs with coding potential beginning with ATG start codon.

Gene_id	Gene_type	Chrom	GENOME START	GENOME END	Length (aa)
ENSG00000066405	protein_coding	3	138010240	138031138	217
ENSG00000140416	protein_coding	15	63042830	63060940	188
ENSG00000140416	protein_coding	15	63042857	63060940	179
ENSG00000149591	protein_coding	11	117203014	117204356	201
ENSG00000149591	protein_coding	11	117203382	117203939	87
ENSG00000149591	protein_coding	11	117203457	117203939	62
ENSG00000149591	protein_coding	11	117203814	117203939	42
ENSG00000165898	protein_coding	14	74493775	74494997	154
ENSG00000178882	protein_coding	12	124295430	124314522	216
ENSG00000227706	lncRNA	6	67887051	67887344	98
ENSG00000234456	lncRNA	7	79460319	79460366	16
ENSG00000235531	lncRNA	8	72055483	72055566	28
ENSG00000245869	lncRNA	11	118886853	118886912	20
ENSG00000248323	lncRNA	5	91286464	91286553	30
ENSG00000249669	lncRNA	5	149424841	149424903	21
ENSG00000249669	lncRNA	5	149421100	149421276	59
ENSG00000253671	lncRNA	8	17821596	17821676	27
ENSG00000259498	lncRNA	15	63046217	63046273	19
ENSG00000259518	lncRNA	15	82096398	82097537	43
ENSG00000260401	lncRNA	11	73240912	73240965	18
ENSG00000267605	lncRNA	19	37267452	37267685	78
ENSG00000272841	lncRNA	6	160990827	160990883	19
ENSG00000277268	lncRNA	17	36933362	36933439	26
ENSG00000278709	lncRNA	20	57711173	57711313	47

ENSG00000282943	lncRNA	19	43170218	43170271	18
ENSG00000285108	lncRNA	8	129696156	129696458	101
ENSG00000285994	lncRNA	10	12565708	12565767	20
ENSG00000231028	lncRNA	6	135498552	135498815	88

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Supplemental Table 2: List of mitochondria-associated proteins enriched in the eluate from UPAP-overexpressing cells.

Description	Coverage [%]	# Peptides	# PSMs	# Unique Peptides
HSPD1	34	14	17	14
ATP5B	33	11	11	11
HSPE1	32	3	3	3
MDH2	25	7	7	7
GLS	21	5	6	3
PRDX6	21	4	4	4
MGST3	18	2	2	2
VDAC2	15	3	3	3
PHB2	15	3	3	3
MRPL16	12	1	1	1
SHMT2	11	6	6	6
ACAT1	11	3	3	3
SOD2	11	2	2	2
PRDX4	11	2	2	1
CYCS	11	1	1	1
DECR1	9	1	1	1
ATP5F1D	8	1	2	1
TIMM50	8	1	1	1
SOD1	8	1	1	1
CS	7	3	3	3
MTCH2	5	1	1	1
AIFM1	5	2	2	2
ATP5PB	5	1	1	1
BDH1	5	1	1	1
UQCRC2	4	1	1	1

CHCHD3	4	1	1	1
PRDX3	4	1	1	1
PDHB	3	1	1	1
APEX1	3	1	1	1
PAICS	3	1	1	1
FASN	2	4	4	4
LRPPRC	2	2	2	2
LETM1	2	1	1	1
LAP3	2	1	1	1
MRPS5	2	1	1	1
COASY	2	1	1	1
SPHK2	2	1	1	1
GARS1	1	1	1	1

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Supplemental Table 3: The binding free energy of HSP60 and ClpP with or without UPAP.

Energy	Without UPAP（kcal/mol）	With UPAP（kcal/mol）
$\triangle E_{vdw}$	-165.28	-238.84
$\triangle E_{ele}$	-98.84	-128.72
$\triangle E_{GB}$	65.26	52.63
$\triangle E_{surf}$	-37.55	-34.81
$\triangle E_{Gas}$	-264.12	-367.56
$\triangle E_{solv}$	27.71	17.82
$\triangle E_{Bind}$	-236.41	-349.74

Supplemental Table 4: List of antibodies used in the study.

Antibodies	Provider	Catalog
GAPDH	Proteintech	60004-1-Ig
α-Tubulin	Proteintech	66031-1-Ig
TOM20	Proteintech	11802-1-AP
Ubiquitin	Proteintech	10201-2-AP
ATP5A1	Beyotime	AG1187
GM130	ABclonal	A11408
Calreticulin	Proteintech	27298-1-AP
MT-CO2	Proteintech	55070-1-AP
Fn1	Abcam	ab2413
Vimentin	Huabio	ET1610-39
α-SMA	Huabio	ET1607-53
HSP60	Abcam	ab46798
HSP60	Sangon	D190788
HSP10	Sangon	D261766
ClpP	Huabio	HA721503
LONP1	Sangon	D261864
FLAG	Abmart	M20008
MYC	Abmart	M20002
HA	Abmart	M20003
GST	Sangon	D110271
His	Abmart	M20001
Anti-rabbit IgG, Alexa Fluor 647	Abcam	ab150079
Anti-mouse IgG, Alexa Fluor 488	Abcam	ab150113
Anti-rabbit IgG, HRP	Beyotime	A0208
Anti-mouse IgG, HRP	Beyotime	A0216

Supplemental Table 5: The sequence of gRNA, donor oligos and siRNAs used in the study.

Flag knocking in in cell line:

gRNA	CAATTGCCAAGTCCCGTGTG
Donor oligos	AATCTCTCTTAACAGGGACACTGGAAACCTCACCTCCCG AGCTGGAAACCTCAGGAGCATTTTTATGCTCTTTCTATTG CCTCCTGCAGATTACAAGGACGACGATGACAAGTAAATC AATTGCCAAGTCCCGTGTGTGATGTTGGTGTTTTAGGTGT GACTCTTGATCCATTGCCATATGTCTATCCTCATTGTCTTT T

UPAP knocking out in cell line:

gRNA1	CAATTGCCAAGTCCCGTGTGTGG
gRNA2	TTTATGAACCTACTTACCAGTGG

298 **Supplemental Table 6**: List of primers used for RT-PCR.

Gene name		Sequence (5'-3')
	Reverse	TCAGGTGTGGTGATGTATGAAGA
UPAP	Forward	ATGTCTCGGTTCCACTCCAG
	Reverse	GCCAGAGACACACCCACAAC
LUCAT1	Forward	TGTGATGAACATTCCCAAAGC
	Reverse	AGCAAGTAGGAGAACAGATCACA
CARMN	Forward	TTGTTAAGGGGCCTGGGTGT
	Reverse	TGTGAGAAACTCCAAGCTCCAA
FN1	Forward	AAGAAGGGCTCGTGTGACAG
	Reverse	GAGAGCTTCTTGTCCTGTCTT
VIMENTIN	Forward	GGACCAGCTAACCAACGACA
	Reverse	AAGGTCAAGACGTGCCAGAG
GAPDH	Forward	CCTCAACGACCACTTTGTCA
	Reverse	TTACTCCTTGGAGGCCATGT

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