Supplementary Figure

Fig. S1

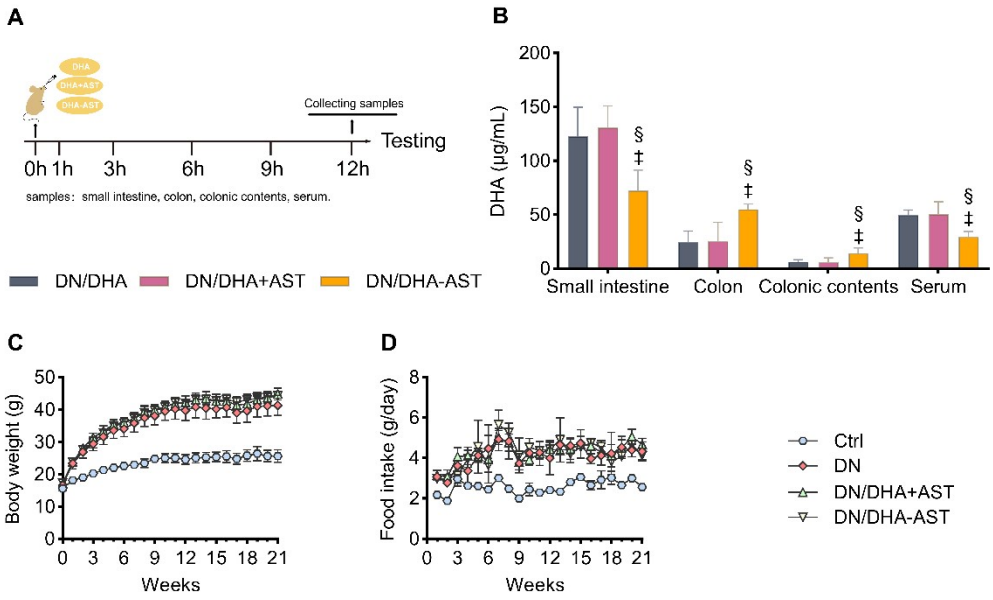


Fig. S1. DHA-AST enables colonic enrichment of DHA in DN mice and has no effects on body weight or food intake. (A) Schematic diagram for the verification of colon-targeting effect of DHA-AST in KKAy mice; **(B)** DHA levels of DN mice in the small intestine, colon, colonic contents, and serum; **(C)** Body weight; **(D)** Food intake; All data are expressed as means ± SD (n=3-10, **n represents the number of independent biological replicates per group**). $P < 0.05$ indicates significant differences among groups. $^{\dagger}P < 0.05$, DN/DHA+AST vs DN/DHA group; $^{\ddagger}P < 0.05$, DN/DHA-AST vs DN/DHA group; $^{\S}P < 0.05$, DN/DHA-AST vs DN/DHA+AST group. Abbreviations: Ctrl, control; DN, diabetic nephropathy; DHA, docosahexaenoic acid; AST, astaxanthin.

Fig. S2

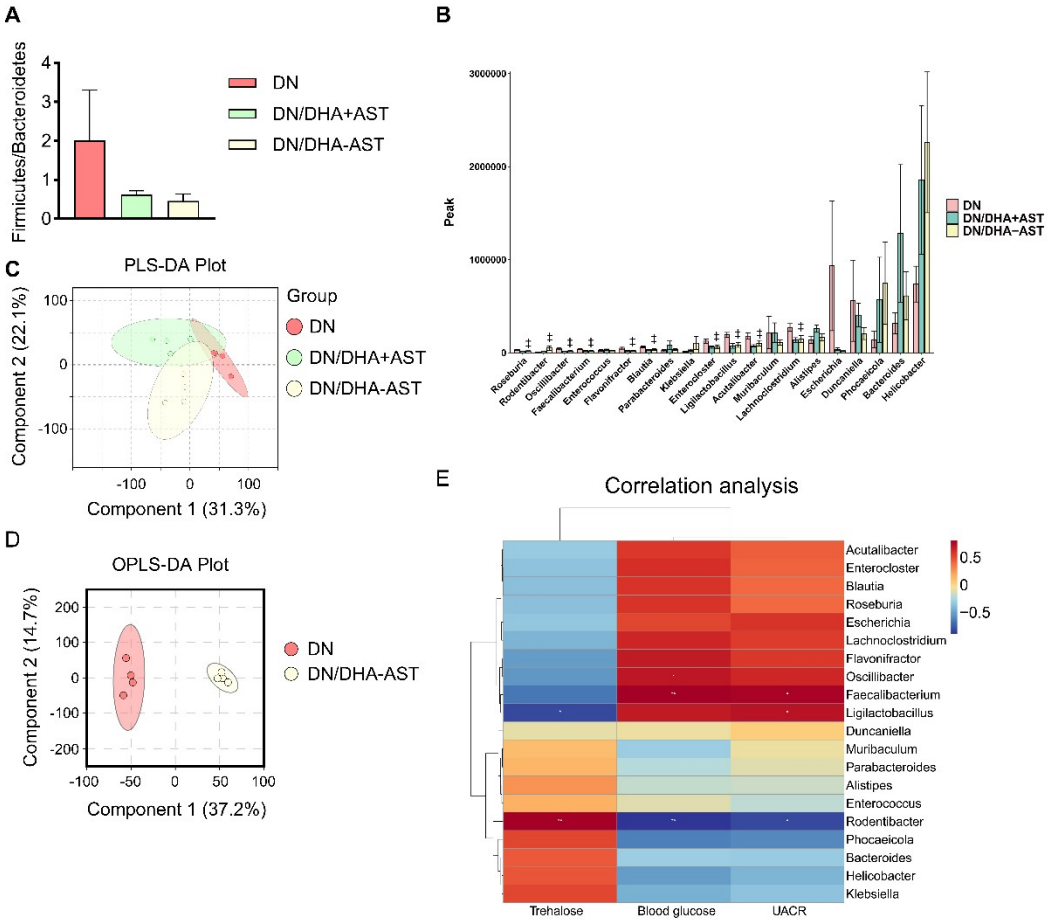


Fig. S2. Supplementary analysis of metagenomics and metabolomics. (A) The *Firmicutes*/*Bacteroidetes* ratio derived from metagenomic analysis; **(B)** Differential abundance analysis of the top 20 most abundant genera at the genus level; **(C)** PLS-DA plot derived from metabolomic analysis and **(D)** OPLS-DA plot (R^2X=0.519, R^2Y=0.994, Q^2=0.751, R^2Y-Q^2 < 0.3); (E) Spearman correlation analysis of differential genera with trehalose levels and kidney injury phenotypes (UACR and fasting blood glucose) (*, $P < 0.05$; **, $P < 0.01$). All data are expressed as means ± SD (n=3-4, n represents the number of independent biological replicates per group). $P < 0.05$ indicates significant differences among groups. $^{\dagger}P < 0.05$, DN/DHA+AST vs DN

group; ‡$P < 0.05$, DN/DHA-AST vs DN group; §$P < 0.05$, DN/DHA-AST vs DN/DHA+AST group. Abbreviations: PLS-DA, partial least squares discriminant analysis; OPLS-DA, orthogonal projections to latent structures discriminant analysis; Other abbreviations are the same as in **Fig. S1**.

Fig. S3

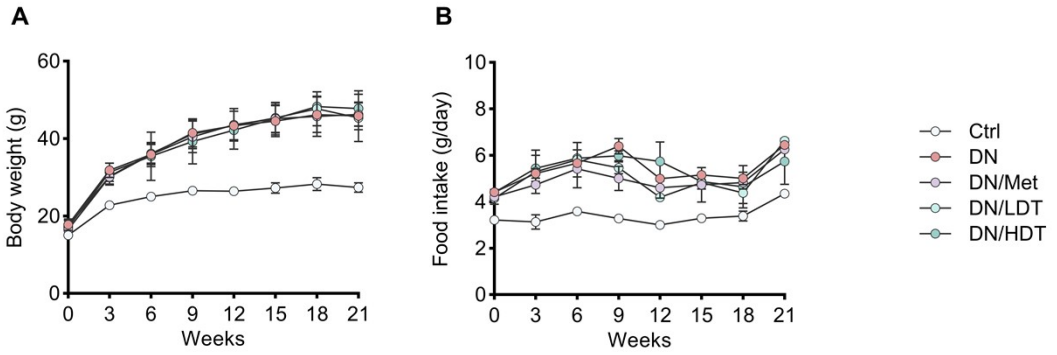


Fig. S3. The effects of trehalose on body weight and food intake in DN mice. (A) Body weight; **(B)** Food intake; All data are expressed as means ± SD (n=10, n represents the number of independent biological replicates per group). *$P < 0.05$ indicate significant differences among groups. Abbreviations are the same as in **Fig. S1**.

Fig. S4

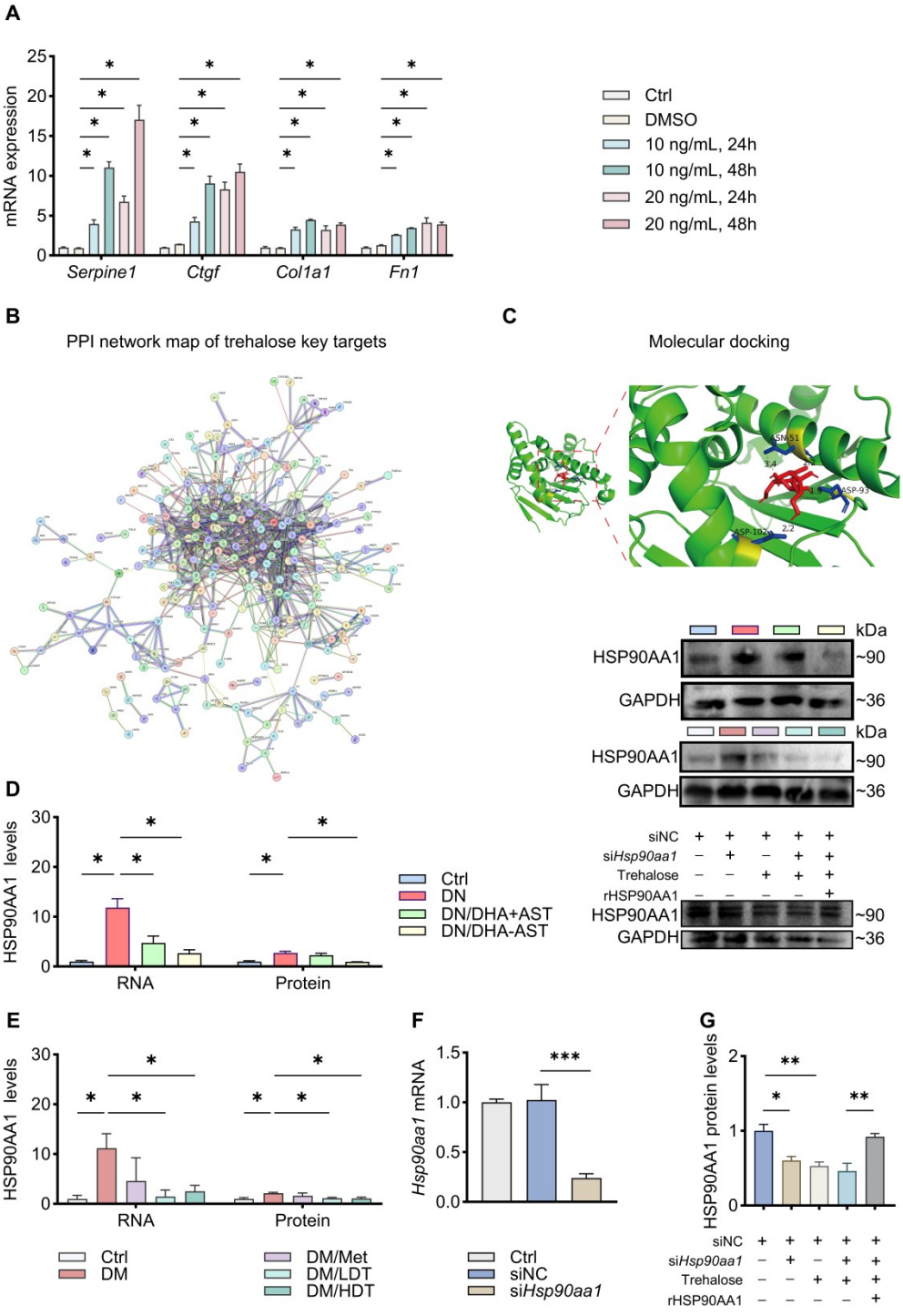


Fig. S4. HSP90AA1 may be a key target for trehalose in ameliorating renal fibrosis. **(A)** The mRNA expression of *Serpine1*, *Ctgf*, *Col1a1* and *Fn1* in fibrotic GMCs induced by different concentrations of TGF-β1; **(B)** PPI network diagram of 296

key target proteins for trehalose; (**C)** Three-dimensional binding model of trehalose interacting with HSP90AA1 (Green, overall backbone of HSP90AA1; red sticks, bound ligand trehalose; blue sticks, key ligand-interacting amino acid residues; yellow, α-helix functional domain; black numerals, atomic distances between ligand and residues.); The mRNA and protein levels of HSP90AA1 in DN mice with **(D)** DHA-AST and **(E)** trehalose; **(F)** The mRNA expression of *Hsp90aa1*; (G) The protein levels of HSP90AA1 after individual or combined treatment with si*Hsp90aa1* (50 mM), trehalose (10 mg/mL), and rHSP90AA1 (100 ng/mL). *Gapdh* was used as the endogenous control gene for qRT-PCR analysis. GAPDH was employed as endogenous control genes for western blot analysis. Data are presented as means ± SD (n=3-10, n represents the number of independent biological replicates per group). *$P < 0.05$ indicates significant differences among groups;**, $P < 0.01$; ***, $P < 0.001$. Abbreviations: PPI, protein-protein interaction; HSP90AA1, heat shock protein 90 alpha family class A member 1; si*Hsp90aa1*, small interfering RNA targeting Hsp90aa1; rHSP90AA1, recombinant HSP90AA; siNC, negative control small interfering RNA; Other abbreviations are the same as in **Fig. S1**.

Fig. S5

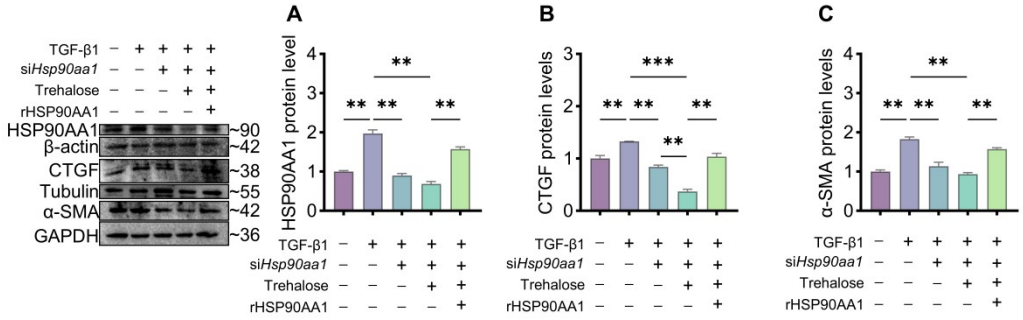


Fig. S5. HSP90AA1 knockdown attenuated TGF-β1-induced injury and potentiated trehalose protection, which was reversed by recombinant HSP90AA1 supplementation. The protein levels of (A) HSP90AA1, (B) CTGF, and (C) α-SMA were detected after individual or combined treatment with siHsp90aa1 (50 mM), TGF-β1 (10 ng/mL), trehalose (10 mg/mL), and rHSP90AA1 (100 ng/mL). GAPDH, Tubulin and β-actin was employed as endogenous control genes for western blot analysis. Data are presented as means ± SD (n=3, n represents the number of independent biological replicates per group). *$P < 0.05$ indicates significant differences among groups; **, $P < 0.01$; ***, $P < 0.001$. Abbreviations: PPI, protein-protein interaction; HSP90AA1, heat shock protein 90 alpha family class A member 1; si*Hsp90aa1*, small interfering RNA targeting Hsp90aa1; rHSP90AA1, recombinant HSP90AA; siNC, negative control small interfering RNA; Other abbreviations are the same as in **Fig. S1**

Supplementary Table

Table 1. Dietary ingredient composition of H100293G

Components	Weight Ratio (g%)	Weight (g)
Protein	20	-
Carbohydrates	64	-
Fat	7	-
Total	-	-
Casein	-	200
Cystine	-	3
Corn Starch	-	397
Maltodextrin	-	132
Sucrose	-	100
Cellulose	-	50
Soybean Oil	-	70
Mineral Premix M1003G	-	35
Vitamin Premix V1002	-	10
Choline Bitartrate	-	2.5
Antioxidant TBHQ (tert-Butylhydroquinone)	-	0.014
Total	-	1000

Table 2. Dietary ingredient composition of H10045

Components	Weight Ratio (g%)	Weight (g)
Protein	24	-
Carbohydrates	41	-
Fat	24	-
Total	-	-
Casein	-	233.06
Cystine	-	3.5
Corn Starch	-	84.83
Maltodextrin	-	116.53
Sucrose	-	201.36
Cellulose	-	58.26
Soybean Oil	-	29.13
Lard	-	206.84
Mineral Mixture M1002	-	11.65
Calcium Hydrogen Phosphate	-	15.15
Calcium Carbonate	-	6.41
Potassium Citrate	-	19.23
Vitamin Mixture V1001	-	11.56
Choline Bitartrate	-	2.33
Food Red Dye	-	0.058
Total	-	1000

Table 3. Gene primer sequence.

Gene Name	Primer Sequence5′-3′
Il6 (Mus musculus)	F: CTGCATTGGCATGAGGTTTG
	R: TCAGAGGGATCTGTGTCTTCT
Il1b (Mus musculus)	F: AAATGCCACCTTTTGACAGTGATG
	R: GCAGCCCTTCATCTTTTGGG
Tnf (Mus musculus)	F: TTGTCTTAATAACGCTGATTTGGT
	R: GGGAGCAGAGGTTCAGTGAT
Ccl2 (Mus musculus)	F: TTAAAAACCTGGATCGGAACCAA
	R: GCATTAGCTTCAGATTTACGGGT
Sele (Mus musculus)	F: GGTGCATCTGGGGAACTAGA
	R: CCAGAGACCCGAGGAGAGTT
Nos2 (Mus musculus)	F: TGCCTCTCACTCTTCCTTGG
	R: CCCAAAGTGCTTCAGTCAGG
Cybb (Mus musculus)	F: CTTCTTGGGTCAGCACTGGC
	R: GCAGCAAGATCAGCATGCAG
Nox4 (Mus musculus)	F: CTTGGTGAATGCCCTCAACT
	R: TTCTGGGATCCTCATTCTGG
Ptgs2 (Mus musculus)	F: GCGACATACTCAAGCAGGAGCA
	R: AGTGGTAACCGCTCAGGTGTTG
Serpine1 (Mus musculus)	F: AGGATCGAGGTAAACGAGAGC
	R: GCGGGCTGAGATGACAAA

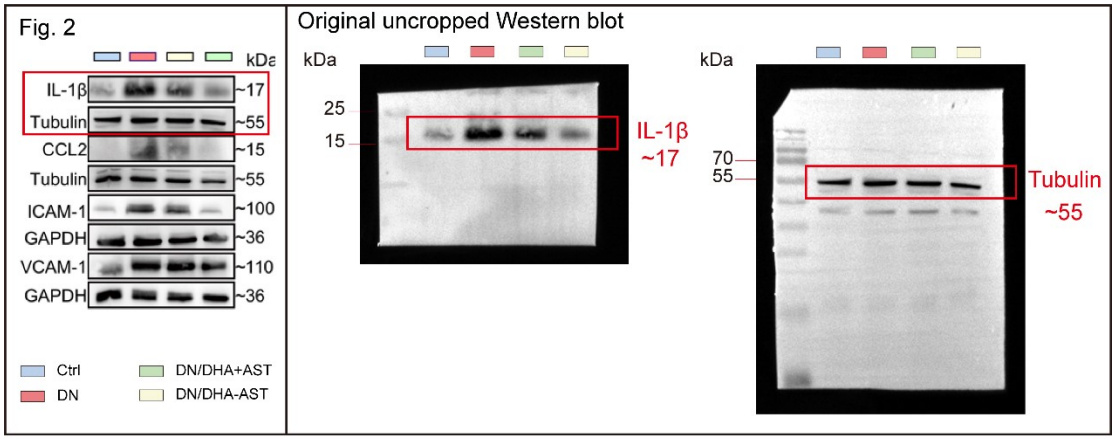
Ctgf (Mus musculus)	F: TGACCTGGAGGAAAACATTAAGA
	R: AGCCCTGTATGTCTTCACACTG
Tgfb1 (Mus musculus)	F: GACTCTCCACCTGCAAGACCAT
	R: GGGACTGGCGAGCCTTAGTT
Col1a1 (Mus musculus)	F: CGGATAGCAGATTGAGAACATCCG
	R: CGGCTGAGTAGGGAACACACA
Acta2 (Mus musculus)	F: TAACCCTTCAGCGTTCAGC
	R: ACATAGCTGGAGCAGCGTCT
Fn1 (Mus musculus)	F: CGGAGAGAGTGCCCCTACTA
	R: CGATATTGGTGAATCGCAGA
Hsp90aa1 (Mus musculus)	F: CAGAGGCGGACAAGAACGACAAG
	R: GATCCTGTTGGCGTGCGTCTG
HSP90AA1 (Homo sapiens)	F: CAGAGGCGGACAAGAACGACAAG
	R: GATCCTGTTGGCGTGCGTCTG
Gapdh (Mus musculus)	F: TGGTGAAGGTCGGTGTGAAC
	R: GCTCCTGGAAGATGGTGATGG
siHsp90aa1 (Mus musculu)	ACTGTCATCACGAAGCATAAC

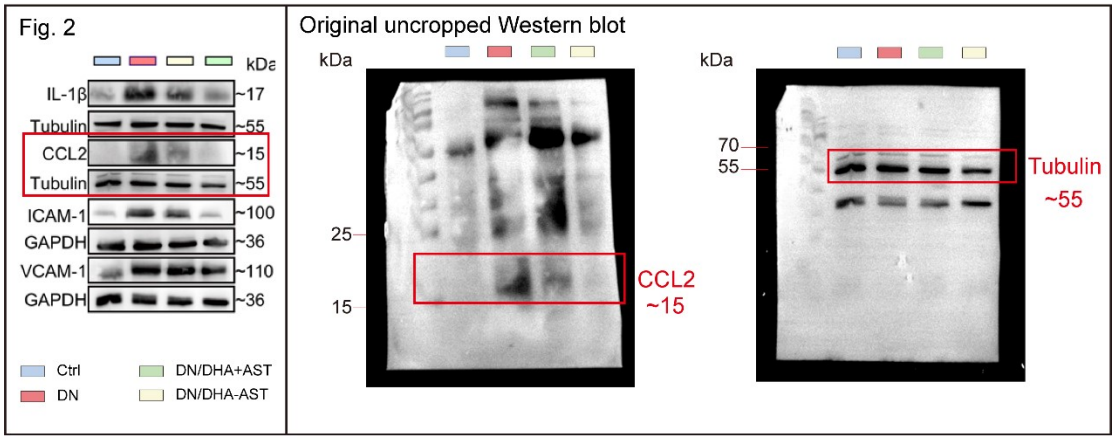
Table 4. Binding energies of trehalose with candidate protein targets.

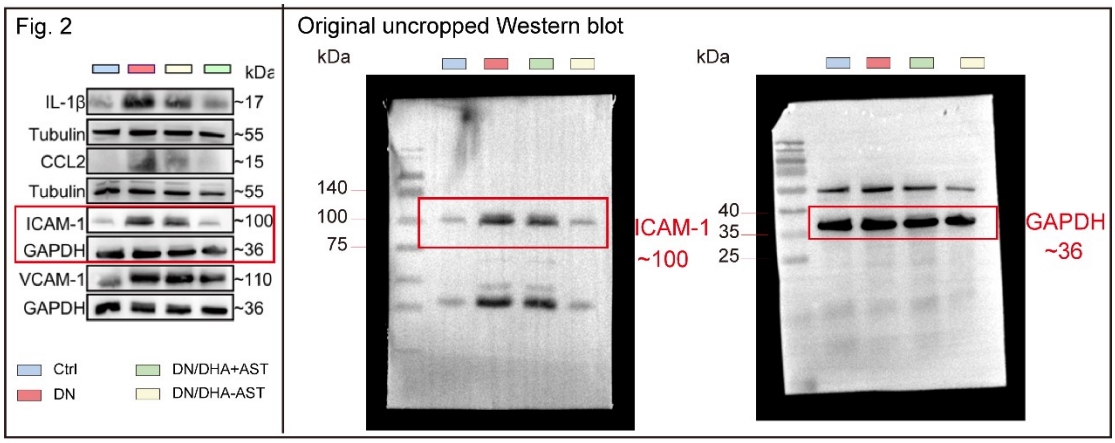
Compound	Protein Target	Binding Energy (kcal/mol)
Trehalose	HSP90AA1	-7.073
	STAT3	1.05
	ESR1	-0.28
	SRC	1.47
	AKT1	-0.9

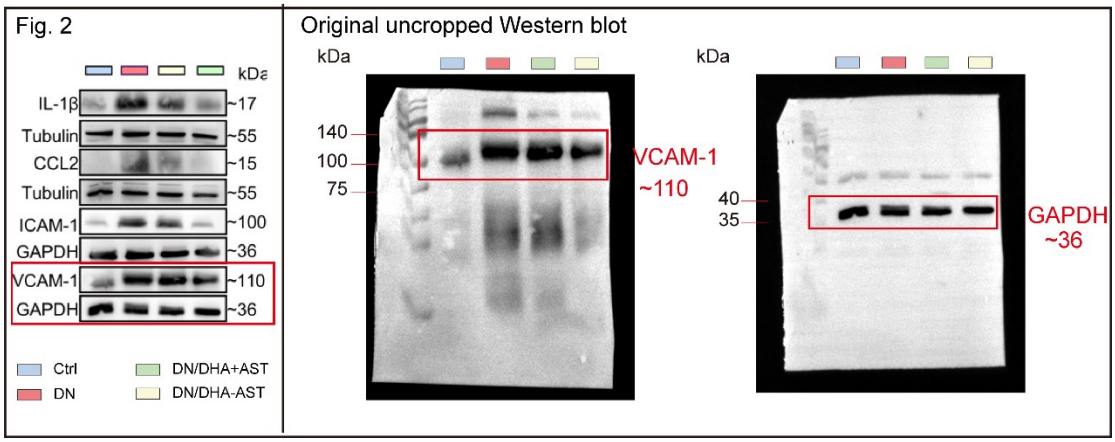
Abbreviation: HSP90AA1, heat shock protein 90 alpha family class A member 1; STAT3, signal transducer and activator of transcription 3; ESR1, Estrogen Receptor 1; SRC, SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase; AKT1, AKT Serine/Threonine Kinase 1.

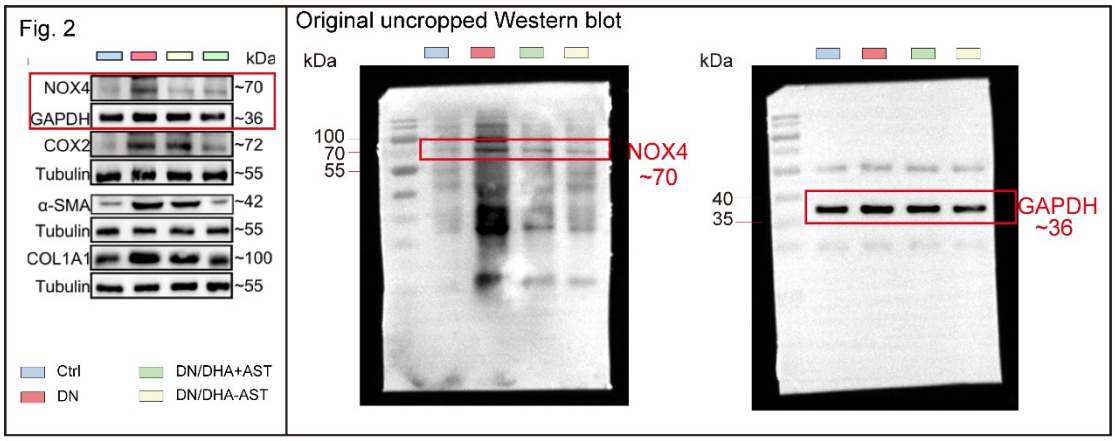
Uncropped original WB images







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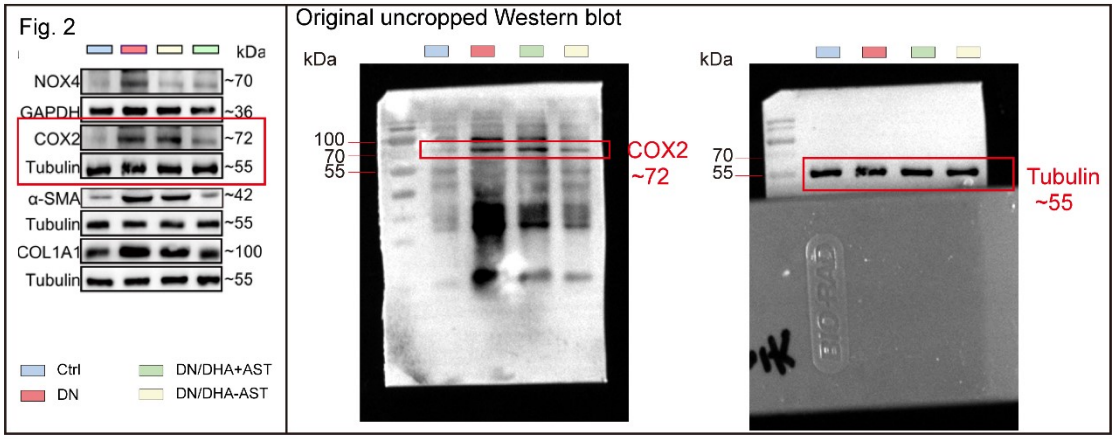
kDa
NOX4 ~70
GAPDH ~36
COX2 ~72
Tubulin ~55
α-SMA ~42
Tubulin ~55
COL1A1 ~100
Tubulin ~55
Ctrl
DN
DN/DHA+AST
DN/DHA-AST
Original uncropped Western blot
kDa
100
70
55
NOX4
~70
kDa
40
35
GAPDH
~36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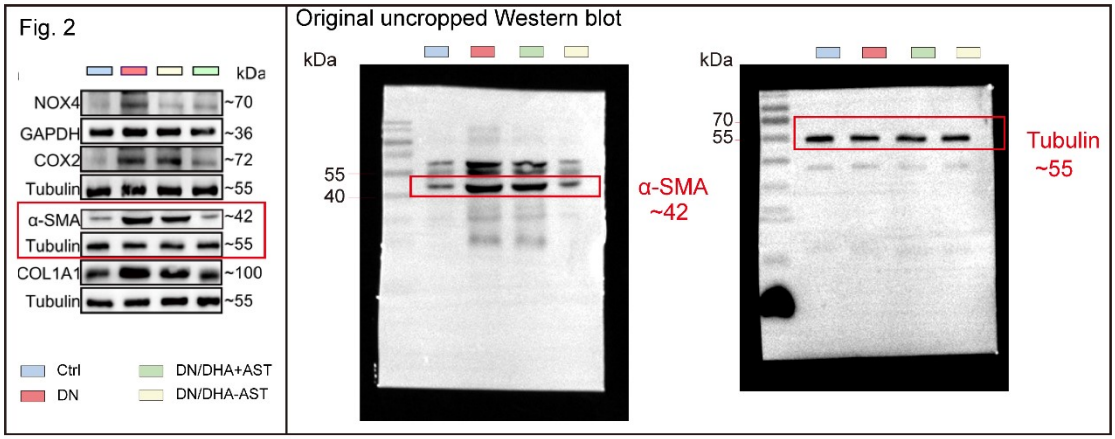
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Ctrl
DN
DN/DHA+AST
DN/DHA-AST
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kDa
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α-SMA
~42
kDa
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55
Tubulin
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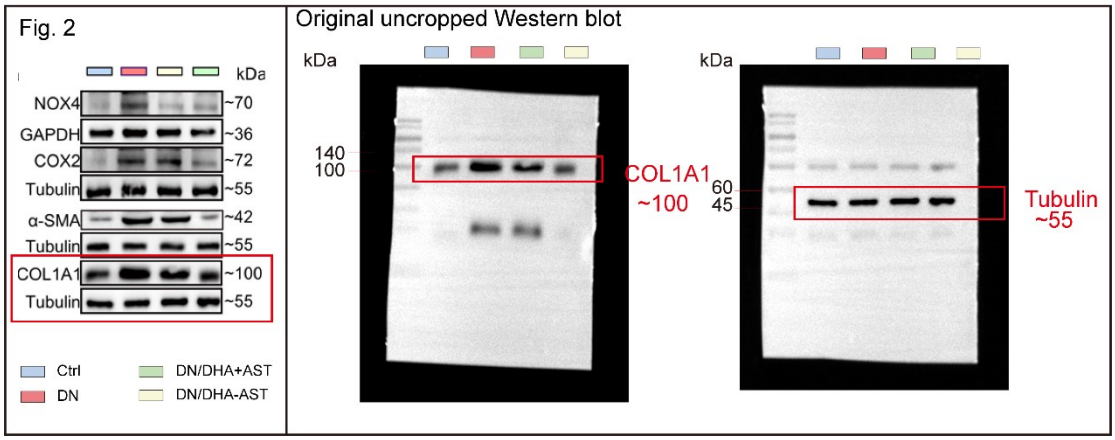
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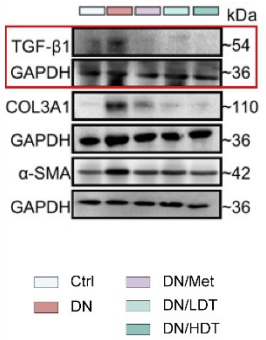
Tubulin ~55
α-SMA ~42
Tubulin ~55
COL1A1 ~100
Tubulin ~55
Ctrl
DN
DN/DHA+AST
DN/DHA-AST
Original uncropped Western blot
kDa
140
100
COL1A1
~100
kDa
60
45
Tubulin
~55

Fig. 5



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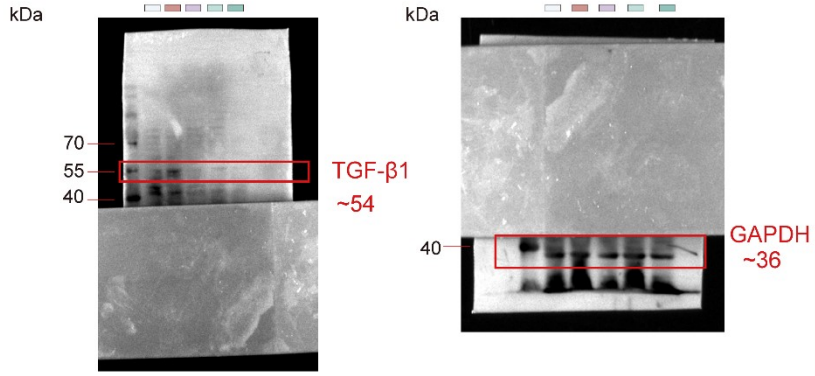

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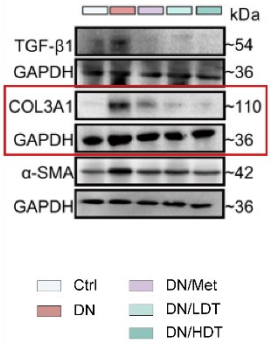

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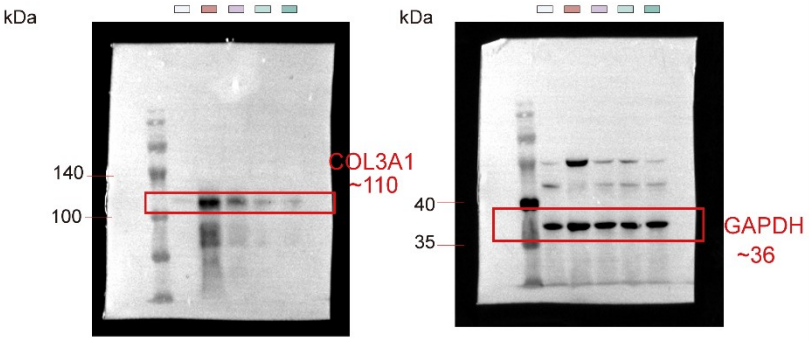

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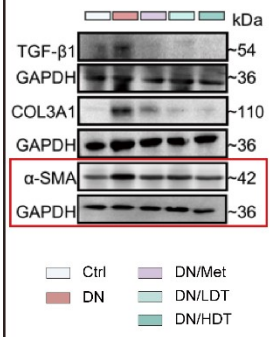

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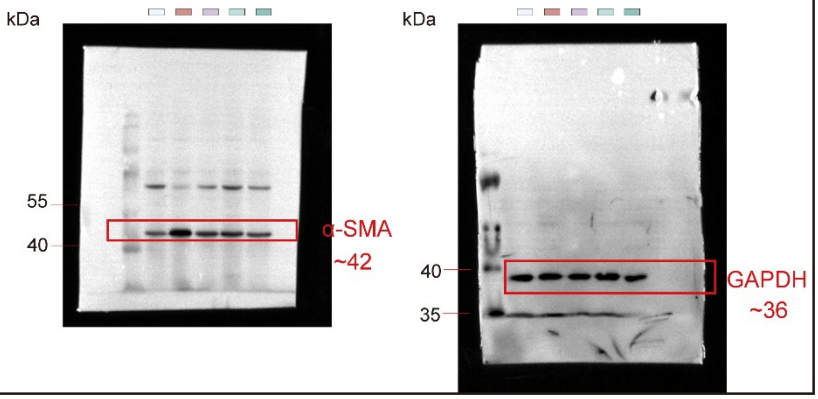
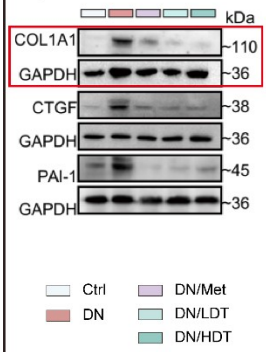


Fig. 5



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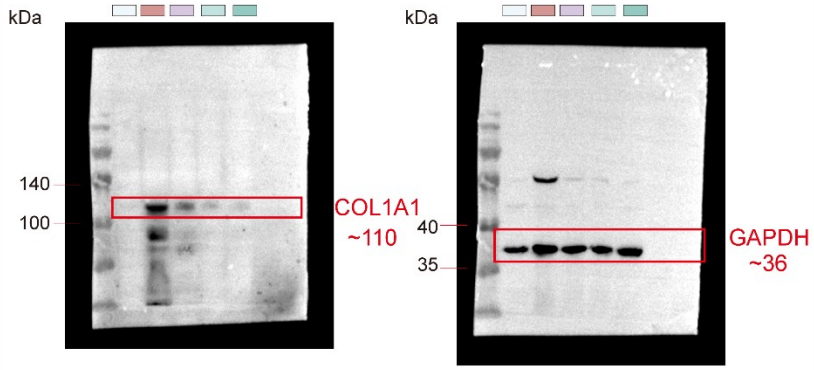

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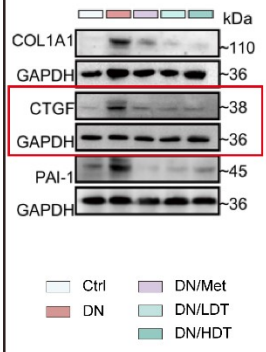

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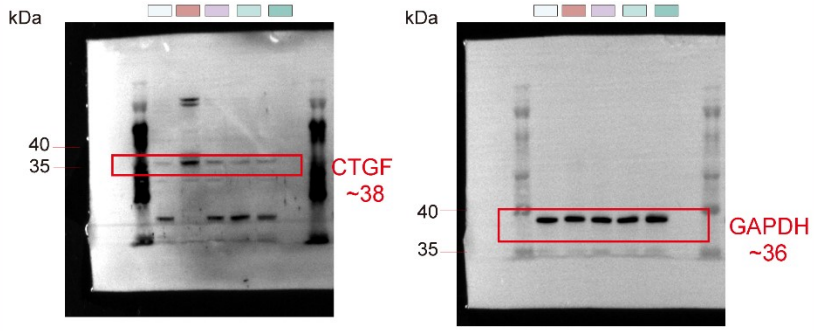

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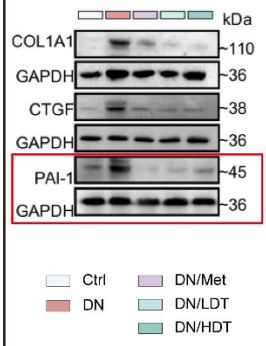

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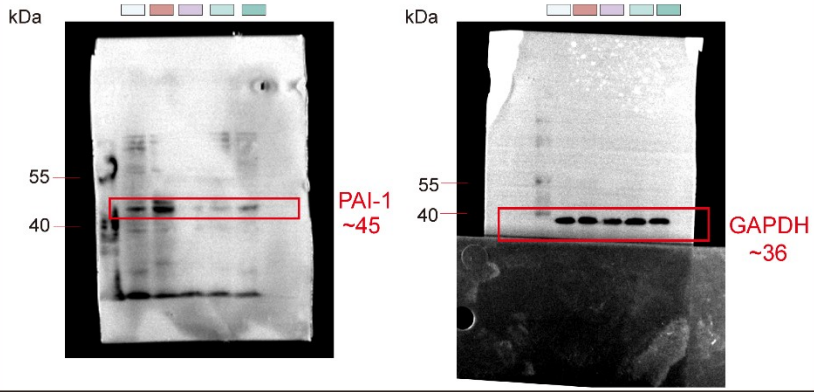

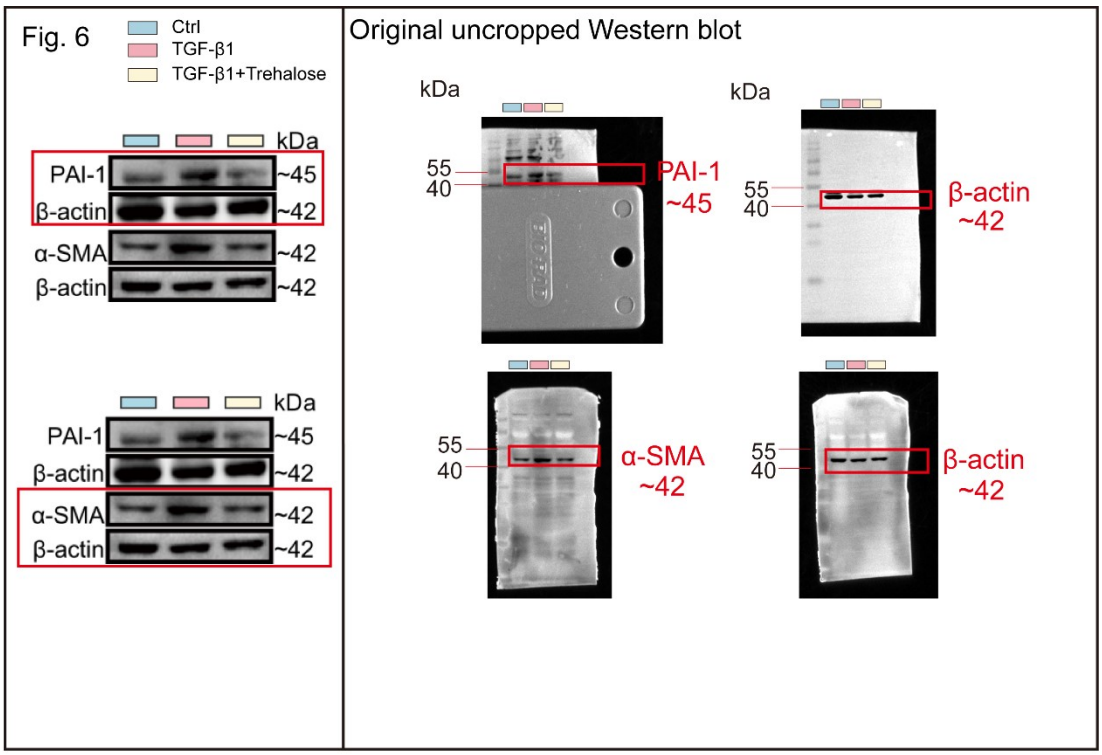
Fig. 6
Ctrl
TGF-β1
TGF-β1+Trehalose
kDa
PAI-1
β-actin
α-SMA
~45
~42
Original uncropped Western blot
55
40
PAI-1
~45
β-actin
~42
α-SMA
~42

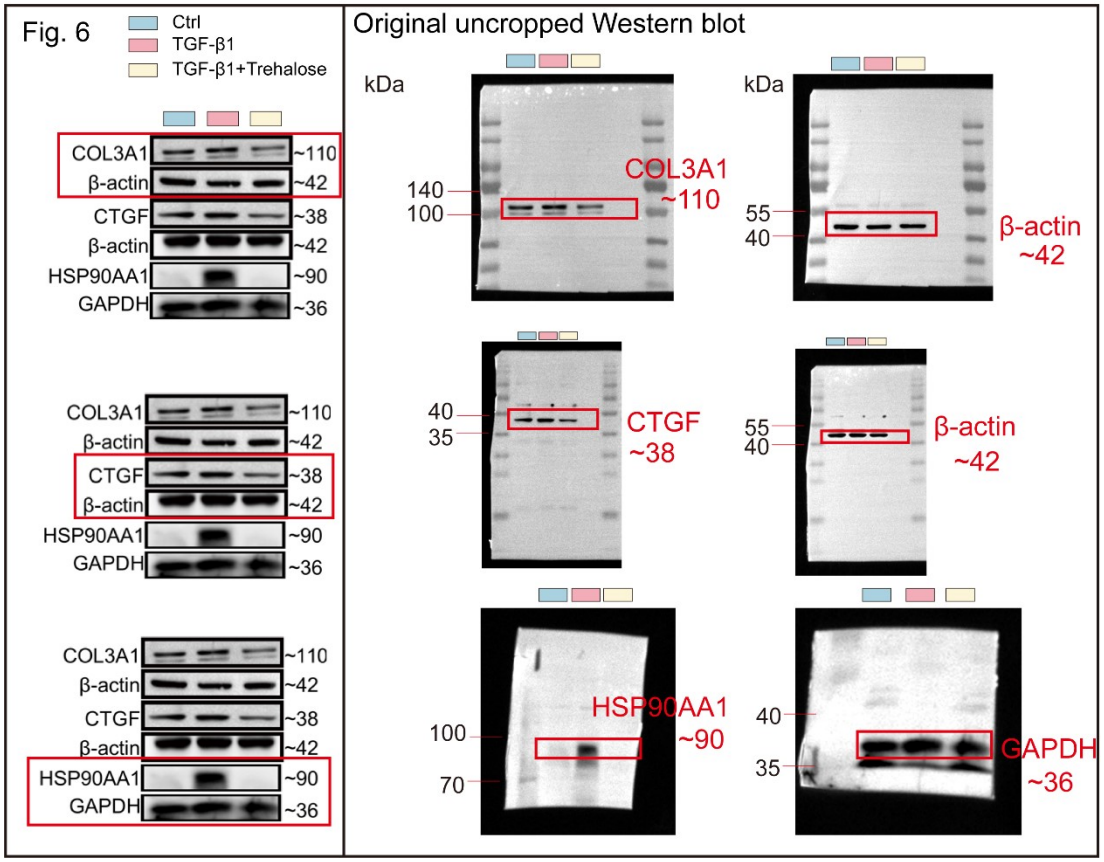
Fig. 6
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TGF-β1+Trehalose
COL3A1
β-actin
CTGF
HSP90AA1
GAPDH
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~90
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kDa
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100
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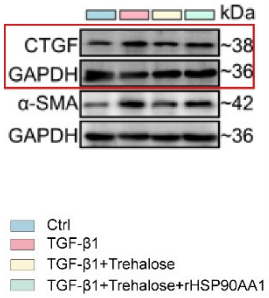
GAPDH
~36

Fig. 6



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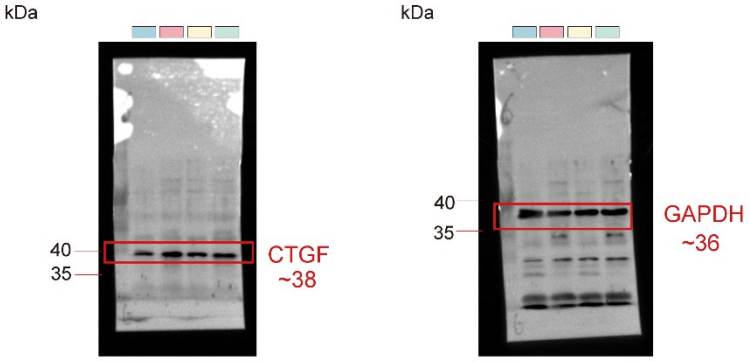

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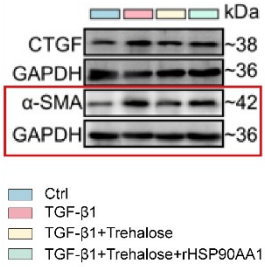

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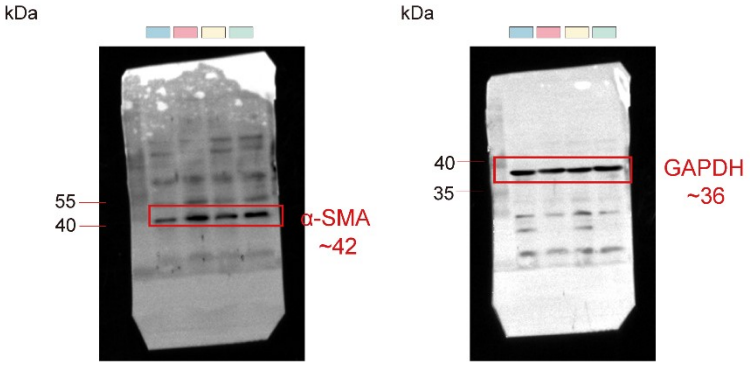
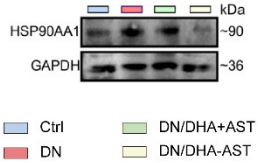


Fig. S4



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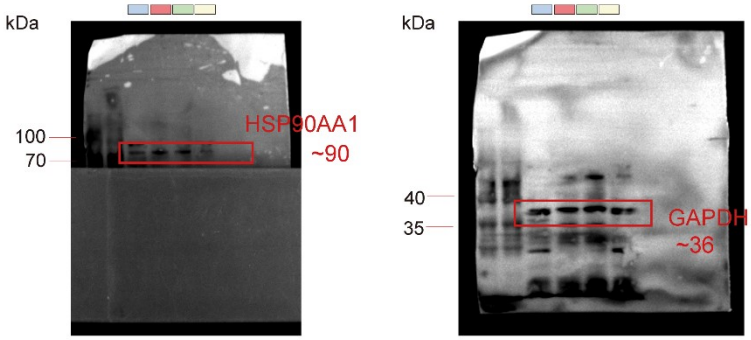
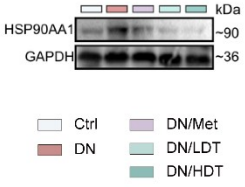


Fig. S4



Original uncropped Western blot

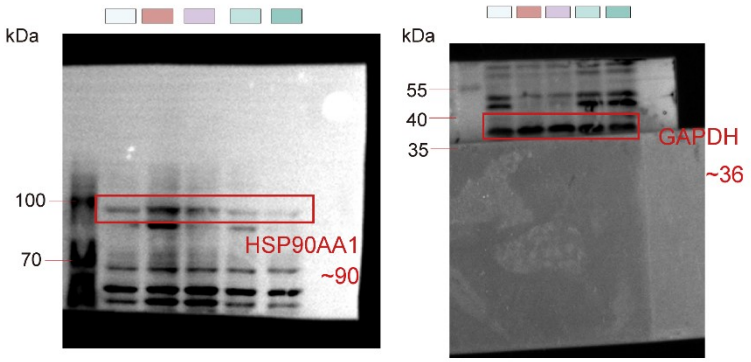
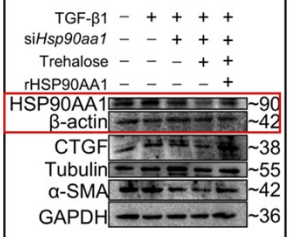


Fig. S5



Original uncropped Western blot

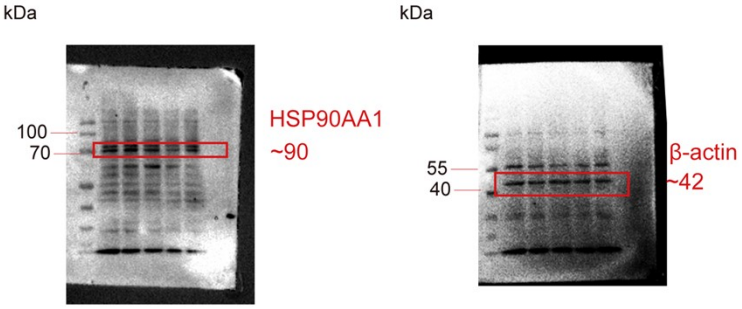
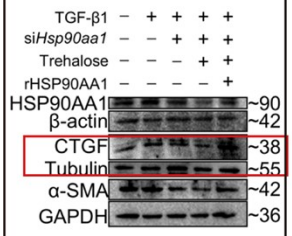


Fig. S5



Original uncropped Western blot

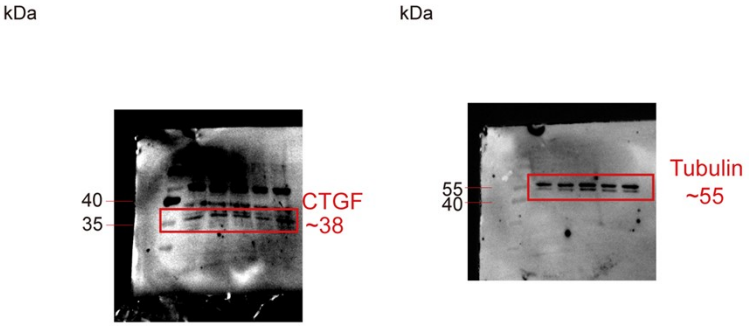
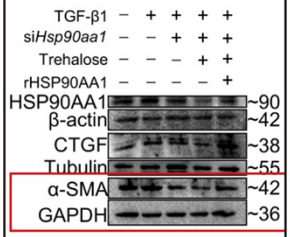


Fig. S5



Original uncropped Western blot

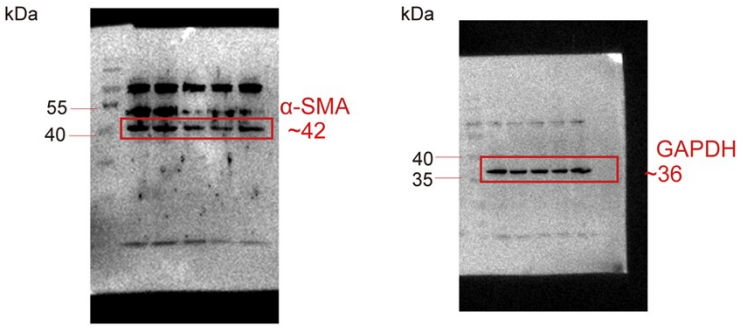
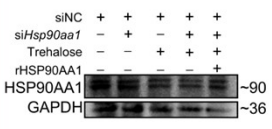


Fig. S4



Original uncropped Western blot