

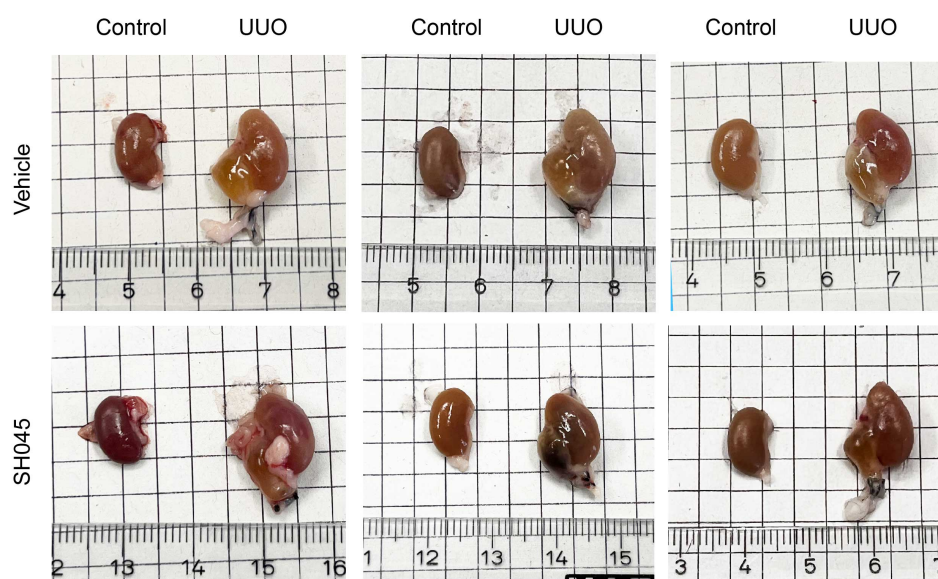
# ***In vivo* inhibition of TRPC6 by SH045 attenuates renal fibrosis in the New Zealand obese (NZO) mouse model of metabolic syndrome**

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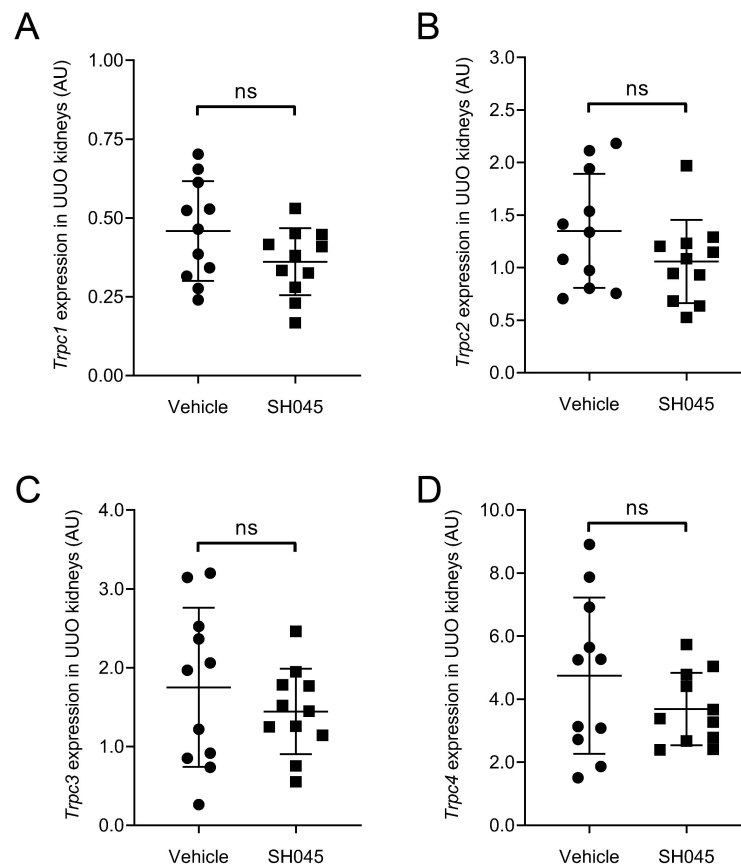
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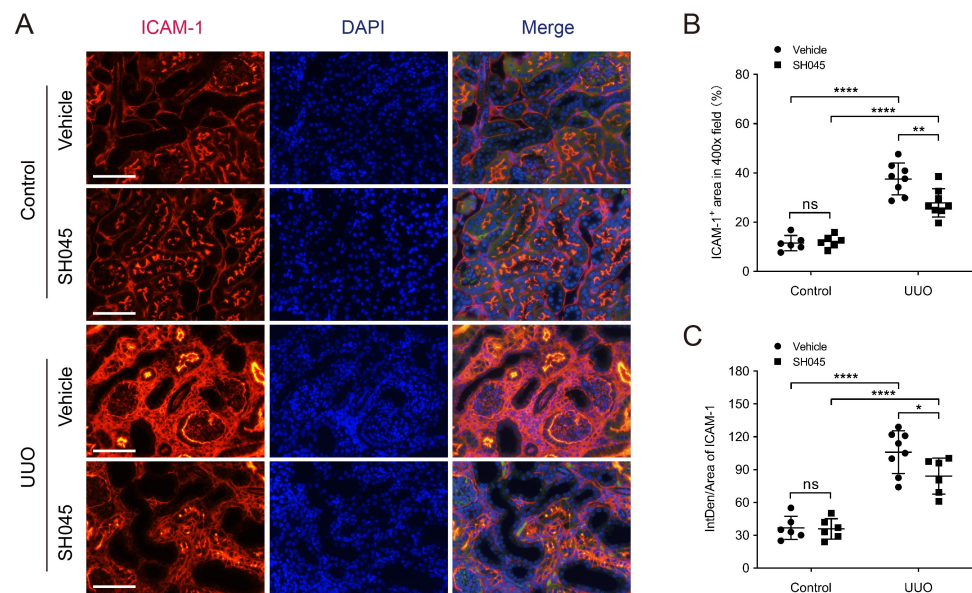
**Figure S1**



**Figure S1.** Macroscopic comparison of the kidneys. Images of control kidneys (left) and UUO kidneys (right). The UUO kidney increased in size (hydronephrosis) compared to control kidney. Scale bar: 1cm.

**Figure S2**

**Figure S2.** The impact of SH045 on renal *Trpc* gene expression in UUO kidneys. (A) Renal mRNA levels of *Trpc1*, (B) *Trpc2*, (C) *Trpc3*, and (D) *Trpc4* (n=11 each). Data expressed as means  $\pm$  SD. Two-way ANOVA followed by Sidak's multiple comparisons post hoc test. ns, not statistically significant. AU, arbitrary units.

**Figure S3**

**Figure S3.** SH045 impact on renal ICAM-1 expression after UUO. (A) Renal immunofluorescence of ICAM-1. (B) Quantification of renal ICAM-1 expression in area proportion (Control n=6 each, UUO n=8 each, respectively). (C) Quantification of renal ICAM-1 expression in mean density (Control n=6 each, UUO n=8 each, respectively). Data expressed as means  $\pm$  SD. Two-way ANOVA followed by Sidak's multiple comparisons post hoc test. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\*\* $p < 0.0001$  defined as significant. ns, not statistically significant. IntDen, integrated density.

## Table S1

**Table S1.** SH045 impact on blood parameters in NZO-UUO mice. Blood parameters after 7-days UUO (n=9, 8 for UUO vehicle and UUO SH045, respectively). Data expressed as means  $\pm$  SD. Two-tailed unpaired t-test. n.a. = not applicable.

		UUO+Vehicle		UUO+SH045		p-value
Serum parameter	Unit	Mean	SD	Mean	SD	t-test
Sodium	mmol/L	147.30	1.25	146.88	2.17	0.608
Potassium	mmol/L	4.57	0.30	4.49	0.78	0.760
Chloride	mmol/L	109.40	1.17	110.75	1.49	0.047
Ionized Calcium	mmol/L	1.38	0.20	1.33	0.05	0.563
Total Carbon Dioxide	mmol/L	27.20	1.40	26.44	1.81	0.320
Glucose	mg/dL	240.10	43.83	229.56	62.19	0.672
Hematocrit	% PCV	40.10	4.43	41.63	3.62	0.444
Hemoglobin	g/dL	13.63	1.51	14.16	1.22	0.432
Anion Gap	mmol/L	16.40	2.27	15.25	1.28	0.221

## Table S2

**Table S2.** Primers used in qRT-PCR experiments.

Primers	Forward	Reverse
<i>Havcr1</i> (KIM-1)	5'-CTGGAGTAATCACACTGAAGCAATG-3'	5'-GATGCCAACATAGAAGCCCTTAGT-3'
<i>Lcn2</i> (NGAL)	5'-ATGTACACCTCCATCCTGGTCAG-3'	5'-GCCACTTGACATTGTAGCTCTG-3'
<i>Trpc1</i>	5'-CATCTAGCGATGAGCCTCTTGAC-3'	5'-CCAAACCGTGTTCAGGAAGTGC-3'
<i>Trpc2</i>	5'-AAGACTGCGGTTGGCTGTCAAC-3'	5'-GGCAACAAGAGCTTCCAGATGG-3'
<i>Trpc3</i>	5'-ATTCCTCGCCATCGGCTATTGG-3'	5'-GAGGCGTTGAATACAAGCAGACC-3'
<i>Trpc4</i>	5'-CTTGAACAGGCAAGGTCCACCA-3'	5'-TGTAATCCTGGAGTCCGCCATC-3'
<i>Trpc6</i>	5'-GACCGTTCATGAAGTTGTAGCAC-3'	5'-AGTAATCTTTGGGCGCTTGAAGTCC-3'
<i>Col1a1</i>	5'-CATGTTGAGCTTTGTGGACCT-3'	5'-GCAGCTGACTTCAGGGATGT-3'
<i>Col3a1</i>	5'-CTCACCTTCTTCATCCCACTCTTA-3'	5'-ACATGGTTCTGGCTTCCAGACAT-3'
<i>Col4a1</i>	5'-TTAAAGGACTCCAGGACCAC-3'	5'-CCCCTGAGCCTGTACAC-3'
<i>Acta2</i> ( $\alpha$ -SMA)	5'-ACTGGGACGACATGGAAG-3'	5'-CATCTCCAGAGTCCAGCACA-3'
<i>Ccn2</i> (CTGF)	5'-TGCGAAGCTGACCTGGAGGAAA-3'	5'-CCGCAGAACTTAGCCCTGTATG-3'
<i>Fln1</i> (Fibronectin)	5'-CCCTATCTCTGATACCGTTGTCC-3'	5'-TGCCGCAACTACTGTGATTCGG-3'
<i>Ccl2</i>	5'-GCTACAAGAGGATCACCAGCAG-3'	5'-GTCTGGACCCATTCTTCTTGG-3'
<i>Ccl5</i>	5'-CCTGCTGCTTTGCCTACCTCTC-3'	5'-ACACACTTGGCGGTTCTTCGA-3'
<i>Ccr2</i>	5'-GCTGTGTTTGCCTCTTACCAG-3'	5'-CAAGTAGAGGCAGGATCAGGCT-3'
<i>Cxcl1</i>	5'-TCCAGAGCTTGAAGGTGTTGCC-3'	5'-AACCAAGGGAGCTTCAGGGTCA-3'
<i>Cxcl2</i>	5'-CATCCAGAGCTTGAGTGTGACG-3'	5'-GGCTTCAGGGTCAAGGCAAACT-3'
<i>Icam1</i>	5'-CTGGGCTGGAGACTCAGT-3'	5'-CCACACTCTCCGAAACGAA-3'
<i>18S</i>	5'-ACATCCAAGGAAGGCAGCAG-3'	5'-TTTTCGTCACTACCTCCCG-3'