Supporting Information

Kidney-targeted cytosolic delivery of siRNA using a small-sized mirror DNA tetrahedron for enhanced potency

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Experimental section

No unexpected or unusually high safety hazards were encountered.

Statistical analysis

Data were represented as mean ± standard deviation. Statistical analysis was performed with one-way ANOVA using the Origin software; the data with $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ were considered statistically significant.

Materials

Chlorpromazine (CPZ), ethylisopropylamiloride (EIPA), methyl-β-cyclodextrin (MβCD), poly-inosinate (poly-I), albumin (from mouse fraction V), IgG (from mouse serum), LDL (human low density lipoprotein, purified), HDL (human high density lipoprotein, purified), PE (L-alpha-phosphatidylethanolamine from egg yolk) and PC (L-alpha-phosphatidylcholine from egg yolk) were acquired from Sigma Aldrich (MO, USA). Exonuclease I and III and DNase I were obtained from New England Biolabs (MA, USA). Streptavidin coated magnetic beads (Dynabeads™ MyOne Streptavidin™ T1) and SYBR green Master Mix were purchased from ThermoFisher Scientific (CA, USA). Proteinase K, RNase A and primers for quantitative RT-PCR were obtained and synthesized by Bioneer (Daejeon, Korea). Other buffers, organic solvents and chemical reagents were purchased from Biosesang (Seongnam, Korea), Samchun chemicals (Seoul, Korea) and Sigma Aldrich (MO, USA), respectively.

Oligonucleotide synthesis

All oligonucleotide for assembly of sTds were synthesized on a 1 μmol scale by Mermaid-4 DNA/RNA synthesizer (Bioautomation, USA) using standard phosphoramidite chemistry. The L-DNA phosphoramidite was purchased from ChemGenes (MA, USA). Those of D-DNA, 2′-

OMe-RNA and 2′-F-RNA were from Sigma-Aldrich (MO, USA). 3′-fluorescein-modifier, 3′-amine-modifier CPG solid supports and 5'biotin-dT phosphoramidite were obtained from Glen Research (VA, USA). Solvents and reagents used for the oligonucleotide synthesis were acquired from sigma Aldrich (MO, USA) and prepared according to the manufacturer's indications. The oligonucleotides were cleaved from CPG and de-protected in NH_3 solution (33%) at 55˚C for 16 h. The crudes were purified by denaturing polyacrylamide gel electrophoresis (PAGE) followed by ethanol precipitation. All purified oligonucleotides were quantified by measuring UV absorbance at 260 nm (Genesys 10S UV/Vis Spectrophotometer, ThermoFisher Scientific, CA, USA) and characterized by ESI-MS analysis (molecular weight confirmation) performed by Novatia Inc. (PA, USA). Cy5.5-labeled oligonucleotides were prepared by conjugation of 3'-amine-modified oligonucleotides with Cy5.5 succinimidyl ester (NHS) (Lumiprobe, USA).

Preparation of self-assembled sTds

The solution containing four oligonucleotides (S1-S4, 300 nM, Table S1) in TM buffer (5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3) was heated to 95˚C for 10 min and slowly cooled down to 4˚C for overnight. The self-assembled structure was verified on SYBR-gold containing agarose gel (2%). Gels were run in 0.5X TAE buffer for 40 min at 4℃, 100V. After electrophoresis, gels were visualized by iBright FL1000 imaging system (ThermoFisher Scientific, CA, USA).

Melting temperature (T_m)

Fluorescence intensity of the mixture containing D-sTd (300 nM) and SYBR green (1×) in TM buffer was measured at varying temperature from 4 ˚C to 95 ˚C using a melting curve measurement program on a real time PCR machine (StepOne, Applied Biosystems, CA, USA).

T_m was determined as the temperature when the –dF/dT value was maximum (F, fluorescence; T, temperature).

Dynamic light scattering

The hydrodynamic sizes of sTds (1 μM) were measured by Zetasizer (Malvern Instruments, Worcestershire, UK).

Atomic force microscopy

The sTds assembled at 50-100 nM were diluted to 20 nM in TAE-Mg buffer (50 mM Tris-acetate, 2 mM EDTA, 12.5 mM $MgCl_2$) and mixed with an equivalent volume of TAE-Mg including 10 mM $NiCl_2$. These solutions were placed onto mica pre-treated with TAE-Mg including 5 mM $NiCl_2$, and incubate for 1 min at room temperature. The samples were imaged in the non-contact mode on an AFM instrument (SCANASYST Multimode, Bruker, USA) using SCANASYST-FLUID + tips (Bruker, USA) in fluid.

Serum nuclease resistance test

To test stability against serum nuclease, sTds (2 μM) were incubated in mouse serum (50%) at 37℃ for 1, 2, 3, 4, 5, 6 and 24 h. At each time points, reaction was quenched by adding EDTA (50 μM). Then, in order to degrade serum proteins, solutions were treated with proteinase K (80 μg) and SDS (0.1%) and incubated for 1 h at 37℃. Finally, the samples were analyzed on 1% agarose gel run in 0.5X TAE for 1 h at 120 V. Gels were stained by SYBR gold and visualized by iBright FL1000 imaging system.

Cellular uptake mechanisms

TCMK-1 (mouse tubular epithelial cell line) cells were obtained from Korean Cell Line Bank (Seoul, Korea). Cells were maintained in RPMI media (Welgene, Gyeongsan, Korea)

supplemented with 10% fetal bovine serum (heat-inactivated FBS, Welgene, Gyeongsan, Korea) and 1% penicillin-streptomycin under 37℃ in a 5% CO_2 incubator.

To investigate of endocytosis mechanism, cells (1×10^5) were pre-incubated with endocytosis inhibitor such as chlorpromazine (CPZ, 10 μM), methyl-β-cyclodextrin (MβCD, 1 mM) or ethylisopropylamiloride (EIPA, 50 μM) before addition of L-sTd. After 30 min, L-sTd (FAM-labeled, 200 nM) was added to each sample in serum-free RPMI media and incubated at 37℃ in a CO_2 incubator for 6 h. Cells were harvested, washed twice with PBS (1 mL) and resuspended in ice-cold PBS (500 μL). The internalized L-sTds were analyzed by a flow cytometer (Guava, Millipore, MA, USA).

To examine the scavenger receptor-mediated pathway, TCMK-1 cells were seeded($5X10^4$) on 24 well-plate (SPL, Pocheon, Korea) and pre-incubated with Poly-I (poly-inosinate, 50 μg/mL) in serum-free RPMI for 30 min before L-sTd (FAM-labeled, 100 nM) treatment. After 6 h, cells were trypsinized, washed twice with PBS and analyzed by a flow cytometer.

To investigate of megalin-mediated uptake, cells were pre-treated with siMeg (100 nM, sense; 5′-GCUAUUGUAUUAGAUCCUUdTdT-3′, antisense;5′-GGAUCUAAUACAA UAGCdTdT-3′) using lipofectamin RNAiMax (ThermoFisher Scientific, CA, USA) and incubated for 48 h. Before treatment of L-sTd, mRNA expression level of megalin was quantified by RT-PCR (Primer sequence for mouse megalin as follows; forward: 5'-GGAGGAACCAATCTGTTGTAATGT-3′, reverse: 5′-GATGGTTGCCTGGAGGG-3′). Then, L-sTd (FAM-labeled, 100 nM) was treated to both normal TCMK-1 and siMeg pre-treated TCMK-1 cells. Fluorescence intensity of the cells was measured using a flow cytometer 6 h post-transfection of L-sTd.

For analyzing endosomal escape of naked or L-sTd fused siP53, TCMK-1 cells (1×10^5) pre-treated with chloroquine (CQ) (100 μM) were incubated with fluorescein-labeled siP53 or siP53@L-sTd (200 nM) in serum-free RPMI for 6 h. The mean fluorescence intensity level was measured by flow cytometry and compared with CQ untreated group.

For flow cytometric analysis of all samples, 10,000 events were recorded in triplicates and internalization level was displayed with mean ± standard deviation (SD).

Pull-down assay of serum proteins bound to sTds

Streptavidin-coated magnetic beads (30 μL) were washed three times in Buffer A (60 μL, 0.5 mM EDTA, 1 M NaCl, 5 mM Tris-HCl, pH 7.5). After the final wash, the washed beads were re-suspended with 50% mouse serum (30 μL, Sigma-Aldrich, MO, USA) and the binding buffer (30 μL, 1 mM EDTA, 2 M NaCl, 10 mM Tris-HCl, pH 7.5) at 37 ˚C for 1 h. The proteins non-specifically bound to the magnetic beads were removed. The protein supernatant (60 μL) was added to biotinylated sTds (2 μM) immobilized streptavidin-coated magnetic beads and the mixture was incubated for 1 h at 37˚C. Magnetic beads were separated from unbound serum proteins and washed with Buffer A three times. Magnetic beads were resuspended in the loading buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 6% (v/v) glycerol, 2 mM DTT, 0.01% (w/v) Bromophenol Blue) and incubated at 95˚C for 10 min. Proteins bound on sTds-immobilized magnetic beads were analyzed on 5 - 12% SDS-PAGE and silver-stained by EzStain Silver reagent (Atto, Japan) according to manufacturer's instruction. Gel images were obtained by iBright FL1000 imaging system.

For determination of dissociation constant of sTds binding to albumin, IgG, LDL or HDL, the similar manner was used. Briefly, streptavidin-coated magnetic beads (10 μL) were incubated with sTds (20 pmol) in the binding buffer (50 μL,) for 30 min at room temperature and then

washed 3 times with Buffer A. The sTds-immobilized-beads were incubated at various concentration (80, 160, 400, 800 pmol) of proteins in the binding buffer (50 μL) at 37℃ for 1 h. After the unbound proteins were removed, the beads were washed with Buffer A. For elution of proteins bound to sTds, the beads were resuspended in 20 μL of the loading buffer and denatured at 95℃ for 10 min. Eluted samples were run in 5 - 10% SDS-PAGE and stained by Coomasie blue. For quantitative analysis of the protein band intensity, reference amounts of proteins (1, 2, 5, 10 μg for albumin, IgG and HDL, and 5, 10, 20, 50 μg for LDL) were also loaded to SDS-PAGE. The amount of bound proteins was estimated by the band intensity quantified by image J software (National Institute of Health, MD, USA). Dissociation constant (K_d) was calculated by curve-fitting with the ligand binding formula in SigmaPlot.

In-gel protein digestion

Protein bands of interest were excised from the gel. The bands were ground into 1mm^3 pieces and transferred into 1.5 mL low binding tubes. Excised gels were washed with LC/MS grade water (100 μL) for 15 min, suspended in acetonitrile (100 μL) and incubated for 30 min at 37˚C. After supernatant was removed, the gel pieces were resuspended in freshly prepared 10 mM dithiothreitol (DTT) in 50 mM ammonium bicarbonate (100 μL) and incubated at 37 °C for 45 min. After supernatant was removed, the gel pieces were incubated in freshly prepared 50 mM iodoacetic acid in 50 mM ammonium bicarbonate (100 μL) at room temperature in the dark for 30 min. The pieces were washed with 100% acetonitrile (100 μL), and dried in SpeedVac (SP Scientific, UK). The dried gel pieces were incubated in 20 ng/μL trypsin (100 μL, Promega, USA) at 37˚C overnight. Then, the suspension was added to 2.5% formic acid (50 μL), rigorously mixed for 15 min and transferred into a fresh low binding tube. The peptides were extracted by incubation with extraction buffer (100 μL, 1:2 (v/v) 5% formic acid/acetonitrile) for 15 min at 37˚C. Supernatants were pooled and dried in SpeedVac. The

residue was resuspended in 0.1% formic acid (100 µL) and desalted using C18 ZipTips (Millipore, MA, USA) prior to LC-MS/MS analysis. The C18 ZipTips were conditioned with acetonitrile and then equilibrated with 0.1% formic acid. The peptides were bound to the ZipTip by aspirating and dispensing the sample for 10 cycles, washed with 0.1% formic acid, and eluted by elution buffer (100 µL, 70% acetonitrile /0.1% formic acid).

LC-MS/MS analysis of serum proteins

Digested peptides were separated on a nanoAcquity (Waters, USA) LC system equipped with a 25-cm column (100 µm fused silica packed with C18 resin) and a 2-cm trap column, and Q-Executive Orbitrap mass spectrometer (ThermoFisher Scientific, USA). Gradient elution was performed from 5% to 50% acetonitrile in 0.1% formic acid with a flow rate of 250 nL/min and spray voltage of 2.0 kV. Neither sheath gas nor makeup liquid was used. The resulting MS/MS data was searched using Uniprot (Taxonomy: Mouse, 2019.08.02), and then analyzed using Proteome Discoverer software (v.2.4). Carbamidomethylation (C) was set as static modification, and oxidation (M) and acetyl (N-terminus) were set as variable modifications. The precursor and fragment mass tolerances were set to 10 ppm and 0.02 Da, respectively. The maximum peptide and site false discovery rates were specified as 0.01 using Percolator. Total number of proteins identified were 380 for M-sTd and 430 for F-sTd. These proteins were further sub-categorized for relevant tissues based on data mining of several databases using an online service DAVID 6.8.[1,2] The databases used for the analysis are as follows: GO, Biological Process; GO, Cellular Component; GO, Molecular Function; InterPro; KEGG Pathway; UniProt Keywords; UniProt Sequence Annotation; UniProt Tissue.

Animal imaging

The animal study was approved by the animal care and use committee of Korea Institute of Science and Technology and all mice were handled in accordance with institutional regulations (2018-082). BALB/c nude mice (22-24g, male, 6 weeks old) were purchased from Orient Bio Inc. (Sungnam, Korea), separated randomly into 4 group (n = 3 per group) and intravenously administrated Cy5.5-sTds (2 μM, 200 μL) through tail-vein. After injection of sTds, time-dependent *in vivo* fluorescence was monitored by an animal imaging system (emission at 710 nm, excitation at 660 nm, IVIS, Caliper Science, USA). After 7 or 24 h, mice were scarified for *ex vivo* imaging of major organs. The obtained images were analyzed by IVIS Living Imaging 3.0 software.

ESI-MS analysis of oligonucleotides of sTds

Biotin-labeled sTds (2 μM, 200 μL) were intravenously injected *via* tail vein (BALB/c mice, 20-22 g, male, 5 weeks old). After 7 h, blood was collected from heart, and kidney and liver were harvested. Serum solutions were prepared by clotting (RT, 30 min) and centrifugation (4℃, 2000g, 15 min) of the collected blood. Kidney and liver were homogenized under liquid nitrogen and lysed by RIPA buffer. The lysed tissues were centrifuged at 12,000 g for 10 min at 4 °C, and the supernatant was used for the next step. Streptavidin-coated magnetic beads (30 μL) pre-washed three times with Buffer A (0.5 mM EDTA, 1 M NaCl, 5 mM Tris-HCl, pH 7.5) were incubated with the prepared serum and tissue lysate solutions at 37℃ for 2 h. The beads were washed with PBS and 50 mM NaOH. Biotinylated S2 strands were eluted by incubation with D-biotin (1 mM in distilled water) at 95℃ for 20 min. Excess D-biotin was removed by G25 filtration column. Finally, the samples of the captured S2 strands were spiked with the internal standard strand (50 pmol). The samples were lyophilized, dissolved in distilled water and analyzed by ESI-MS. ESI-MS analysis was performed by Novatia Inc. (PA, USA).

Kidney section imaging

Kidney was excised from mice intravenously administrated with Cy5.5-labeled L-sTd at 7 h post-injection. A freshly dissected kidney tissue was embedded in optimum cutting temperature (OCT) compound (Leica Biosystems, Germany) and frozen completely at -80˚C. The frozen tissue block was sectioned with 15 μm thickness by Lab Core Incorporation (Seoul, Korea). The frozen-sections were washed with PBS, mounted with DAPI containing-mounting solution (Abcam, UK) and imaged using a fluorescence microscopy (Axio Observer 3, Carl Zeiss, Germany).

Treatment of acute kidney injury (AKI)

The AKI animal model was established by single intraperitoneal injection of the folic acid solution (350 μL, 250 mg/mL in 100 mM $NaHCO_3$, pH 8.8) into BALB/c mice by following the reported procedure.[3] AKI mice were divided randomly in 5 groups (n = 5 per group). At 2 h and 24 h after treatment of folic acid, each group of AKI mice were intravenously administered with PBS (200 μL), naked siP53 (2 μM, 200 μL), L-sTd (2 μM, 200 μL), siSC@L-sTd (2 μM, 200 μL) or siP53@L-sTd (2 μM, 200 μL). The siRNA dose in each group was approximately 0.25 mg/kg per injection. After 24 h, all mice were sacrificed and kidneys were harvested for further study.

Quantitative reverse-transcriptase PCR (qRT-PCR)

TCMK-1 cells seeded onto 12-well plate (2×10^5) were treated with PBS, siP53, siSC, L-sTd, siSC@L-sTd and siP53@L-sTd (200 nM) in serum-free RPMI at 37˚C in a 5% CO_2 incubator. Naked siP53 treated by a transfection reagent (lipofectamin RNAiMax) was used as a positive control. After 48 h, total RNA was isolated from the cells using RNeasy Mini kit (Qiagen, CA, USA). Concentration and purity of RNA were measured by Nanodrop (ThermoFisher Scientific, MA, USA). 2 μg total RNA was used for cDNA synthesis with Top script™ Reverse

Transcriptase and random hexamers primers in cDNA synthesis kit (Enzynomics, Daejeon, Korea). cDNA was mixed with Power SYBR®-Green PCR master mix (ThermoFisher Scientific, MA, USA) and primer pair, then amplified by a StepOne Real-Time PCR system (Applied Biosystems, USA). The 2-ΔΔCt method was used to analyze the relative changes in gene expression based on real-time quantitative PCR. The amount of amplified p53 mRNA level was normalized by those of amplified GAPDH. For estimation of p53 mRNA level *in vivo*, total RNA was extracted from homogenized kidney tissues and amplified by using the same manner described above. Primer sequences for qRT-PCR were as follows (Table. S4).

Western blot

TCMK-1 cells or homogenized kidney tissues were lysed in RIPA containing protease inhibitor cocktail (Roche Applied Science, Penzberg, Germany) at 4 ˚C for overnight. The mixture was centrifuged at 12,000 rpm for 20 min at 4 ˚C. Proteins (20 μg) in the supernatant were separated by 5 - 12% SDS-PAGE and transferred to PVDF membrane (4˚C, 100 min, 350 mA). After blocking with 5% BSA (w/v) in TBST buffer for 1 h at room temperature, the membrane was incubated with 5% BSA in TBST containing monoclonal anti-p53 antibody (1:1000), anti-caspase 3 (1:1000), anti-GADPH (1:1000), and anti-β-actin (1:1000) (Cell Signaling Technology, USA) at 4 ˚C for overnight. Then, they were washed 3 times by TBST and incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (1:3000) in 5% skim milk in TBST at room temperature for 1 h. After washing, the protein bands were visualized using Super Signal™ West Pico Chemiluminescent (ThermoFisher Scientific, USA) and imaged by iBright FL1000 imaging system.

Histological analysis of kidney tissue sections

Kidney tissues harvested from mice were fixed in 4% formaldehyde, paraffin embedded, cut in 4 μm sections, stained with hematoxylin and eosin (H&E), and analyzed by an optical microscopy (Eclipse Ti-S, Nikon, Japan). For fluorescent histological inspection, sections were de-paraffinized in xylene, re-hydrated with serial treatment of ethanol (100, 95, 75, and 50%), and washed with washing buffer (1 × TBST, 0.05% Tween 20, 0.03% Triton X-100). Antigen retrieval was conducted by boiling the sections with citrate buffer (10 mM sodium citrate, 0.05% Tween, pH 6.0) for 20 min and incubated with 0.3% (v/v) hydrogen peroxide in methanol for 15 min. Then, the sections were treated with M.O.M blocking buffer (Vector Laboratories, USA) for 1 h and 5% BSA/PBST for 5 min. Monoclonal anti-p53 antibody (1:200) solutions was added to the sections and incubated at 4 ˚C overnight. For visualization, slides were washed and treated with secondary antibody conjugated with AlexaFluor 488 (1:2000, ThermoFisher Scientific, USA) for 2 h. Stained-tissue were rinsed, dried, mounted with DAPI-containing mounting solution (Abcam, UK) and imaged by a fluorescence microscopy (Axio Observer 3, Carl Zeiss, Germany). To analyze apoptotic damage in the sections, the de-paraffinized sections were treated with Cy5-labeled annexin V (Abcam, UK) and imaged using a fluorescence microscopy. Results were obtained from at least 3 individual part of the section and all images were quantitatively analyzed by image J software.

Creatine and BUN analysis

Blood samples were collected from mice (n = 4 per group) at 48 h after AKI induction, clotted at room temperature for 30 min, and centrifuged at 2,000 g for 15 min at 4˚C. The amounts of creatine and BUN in the supernatant were analyzed by SCL Healthcare (Yongin, Korea).

Nuclease stability of siP53

SiP53 and siP53@L-sTd prepared with a 3′-fluorescein-labeled antisense strand were incubated with exonuclease I and III (20 units for exonuclease I, 100 units for exonuclease III), DNase I (4 units) and RNase A (80 μg) for 1, 4, 7, 24 h at 37℃. At each time point, the reaction was quenched by adding the loading buffer and heating at 95℃ for 10 min. Then, the samples were analyzed by denaturing PAGE (15%, 7 M UREA) run in 0.5×TBE for 1 h at 180 V. After electrophoresis, gels were visualized by iBright FL1000 imaging system.

For the stability test in plasma and kidney, siP53 and siP53@L-sTd prepared with a 3′-fluorescein-labeled antisense strand was intravenously administrated into BALB/c mice (2 μM, 200 μL). Mice were sacrificed at 1, 4, 7, and 24 h after administration. At each time point, blood was collected from, and heart and kidney were harvested. Serum solutions were prepared by clotting (30 min, room temperature) and centrifugation (2000 g, 4℃, 15 min) of the collected blood. Kidney was homogenized by LN_2 and lysed by RIPA buffer. Finally, 300 μL of serum plasma and 500 μL kidney lysate solution was collected. Among them, 1/20 volume of totally collected sample volume (serum plasma 15 μL, kidney lysate solution 25 μL) was used for PAGE analysis. The fluorescence-labeled antisense strand (5 pmol) was loaded as the control. Serum and kidney lysate samples were mixed with the loading buffer (0.5M EDTA, 98% formamide solution), heated at 95℃ for 10 min and analyzed by 20% denaturing PAGE (7M UREA). Gels were run in 0.5×TBE, 180 V for 1 h, then visualized by fluorescein channel of iBright FL1000 imaging system.

Liposome disruption test

For preparation of model liposomes, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (dissolved in $CHCl_3$) were mixed 5:2 molar ratios, mimicking the lipid composition of endosome.[4] After removal of $CHCl_3$ by overnight evaporation, liposomes were resuspended in PBS solution (pH 5 or pH 7.4) and sonicated for 10 min. The final concentration of liposomes

used in the disruption test was 5 μg/μL. Percent disruption of liposomes was quantified by measuring the decrease in optical density (OD) (595 nm) of liposomes at pH 5 or pH 7.4 upon treatment with various concentration (100, 250, 500 nM) of L-sTd, siP53@L-sTd, or siP53 at room temperature for 1 h. 2% Triton X-100 (TX-100) was used as the positive control.

Table S1. Sequences of the oligonucleotides used to construct sTds.* U instead of T was used for M-sTd and F-sTd. The 2′-OMe-RNA linker used for siP53@L-sTd was indicated with red.

Strand	**Sequence**
S1	GGGATCCCGATTCGAGACAGCATTTCTCCCACAC
S2	CGTGGTAGGTTTTGCTGTCTCGTTAGCGCCGGCC
S3	TCGGGATCCCTTCACGGGCAACTTGGCCGGCGCT
S4	ACCTACCACGTTGTTGCCCGTGTTGTGTGGGAGA
S4-linker	GGUGUAUGAAACCTACCACGTTGTTGCCCGTGTTGTGTGGGAGA

*3′-FAM-labeled S1 and 3′-Cy5.5-labeled S3 were used for serum stability analysis and *in vivo* experiments, respectively.

Table S2. ESI-MS characterization of oligonucleotides used for assembly of sTds.

Sequence		**Calculated [M-H]$^-$**	**Observed [M-H]$^-$**
D-sTd	D-S1 (3′-FAM)	10946.3	10949.3
	D-S2 (3′-NH_2)	10642.8	10643.5
	D-S3 (3′-NH_2)	10590.7	10590.0
	D-S4 (3′-NH_2)	10659.8	10660.0
L-sTd	L-S1 (3′-FAM)	10946.3	10945.9
	L-S2 (3′-NH_2)	10642.8	10643.4
	L-S3 (3′-NH_2)	10590.7	10590.7
	L-S4 (3′-NH_2)	10659.8	10660.0
M-sTd	M-S1 (3′-FAM)	11869.0	11872.0

	M-S2 (3′-NH_2)	11509.3	11510.0
	M-S3 (3′-NH_2)	11513.4	11513.8
	M-S4 (3′-NH_2)	11540.4	11541.0
F-sTd	F-S1 (3′-FAM)	11461.0	11460.5
	F-S2 (3′-NH_2)	11101.3	11099.5
	F-S3 (3′-NH_2)	11105.4	11104.7
	F-S4 (3′-NH_2)	11132.4	11130.4

Table S3. Sense (SS) and antisense (AS) sequences of siRNA used in this study. RNA is indicated with blue, and 2′-OMe-RNA is indicated with red. The DNA sequences complementary to the linker are underlined.

Strand	Sequence
siP53-SS	TTCATACACCGAGAAUAUUUCACCCUUCA
siP53-AS	UGAAGGGUGAAAUAUUCUC
siSC-SS	TTCATACACCACAUGAAGCAGCACGACUU
siSC-AS	AAGUCGUGCUGCUUCAUGU

Table S4. Primer sequences used for qRT-PCR.

Primer	Sequence
Mouse p53-F	ACAGCGTGGTGGTACCTTAT
Mouse p53-R	TATACTCAGAGCCGGCCT
Mouse GADPH-F	TGCACCACCAACTGCTTAG
Mouse GADPH-R	GGATGCAGGGATGATGTTC

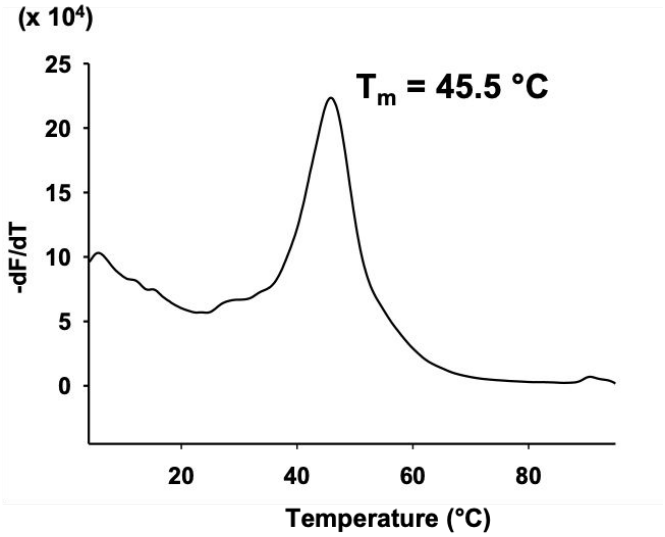

Figure S1. Melting temperature of D-sTd (300 nM) in TM buffer.

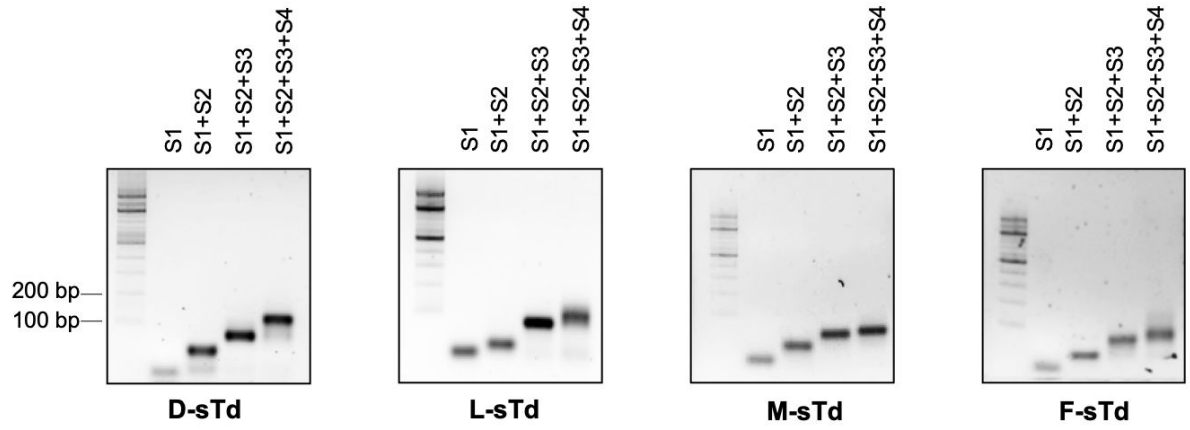

Figure S2. Agarose gel (2%) electrophoresis of sTds (300 nM)

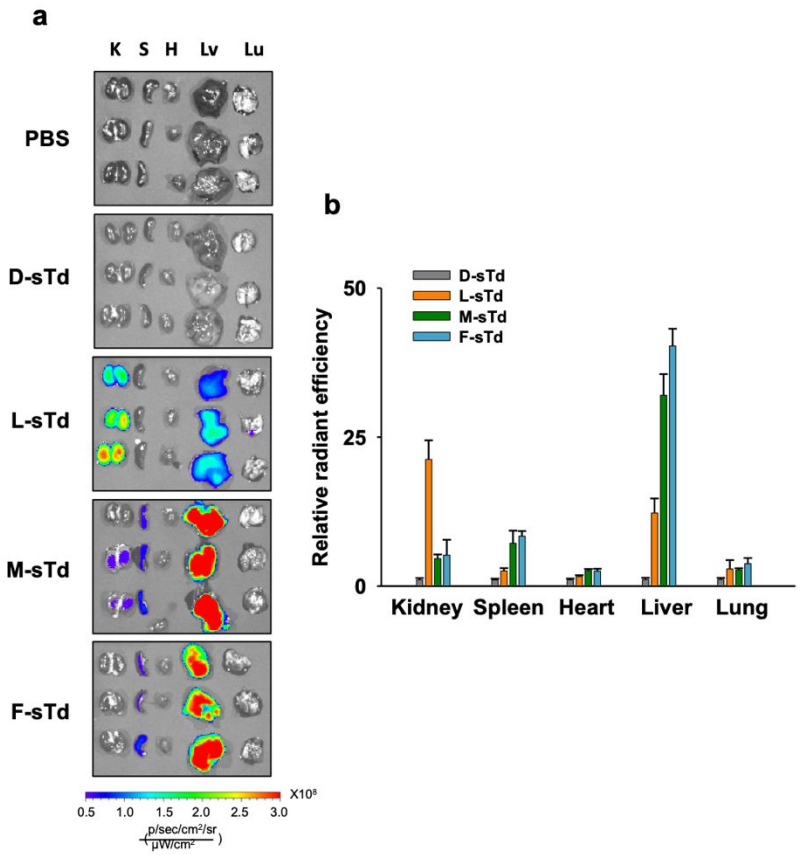

Figure S3. (a) *Ex vivo* imaging of major organs from healthy mice treated with sTds at 24 h post - injection. (K, kidney; S, spleen; H, heart; Lv, liver; Lu, lung). (b) Relative distribution levels of sTds in each organ (n = 3). Signals were normalized by the levels measured in each organ from PBS-treated mice.

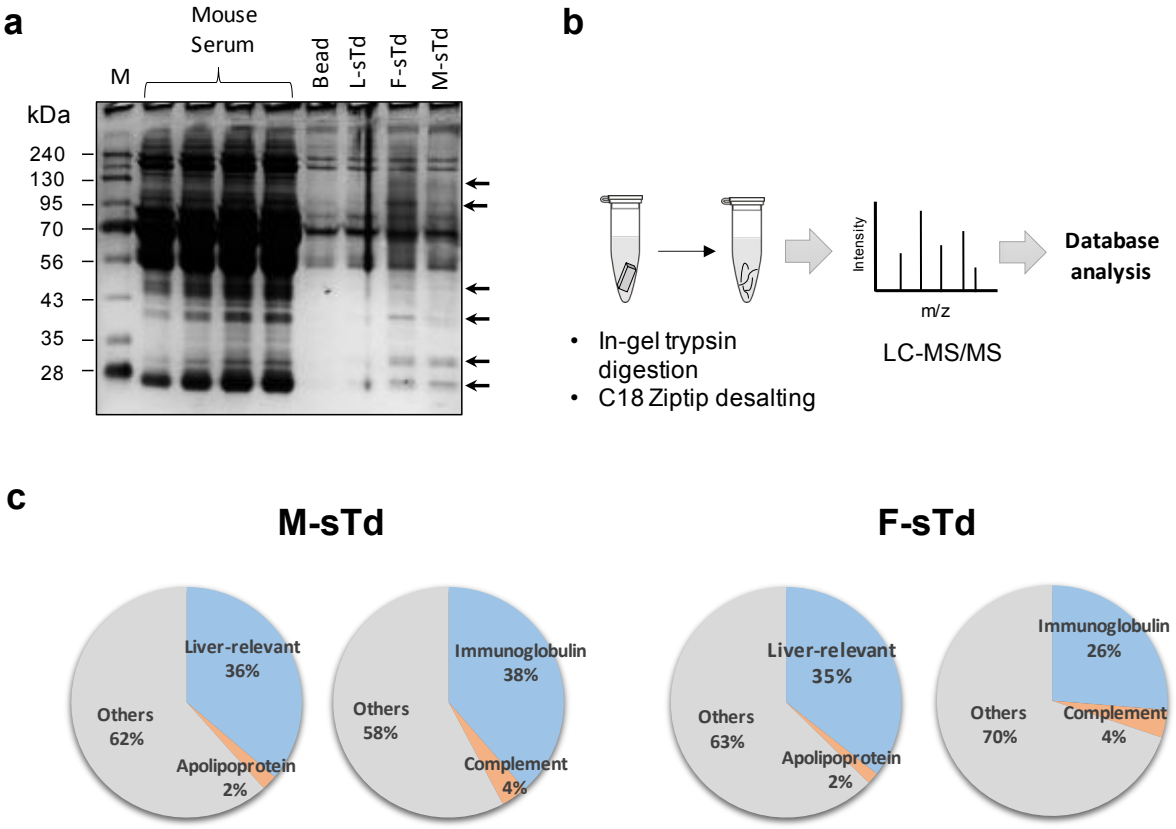

Figure S4. (a) SDS-PAGE analysis of proteins in mouse serum pulled down with sTds immobilized on magnetic beads (top-left). (b) The work-flow of proteome analysis was schematically displayed (top-right). (c) Liver-relevant or opsonizing proteins were identified from analysis of the proteins pulled down with M-sTd and F-sTd (bottom).

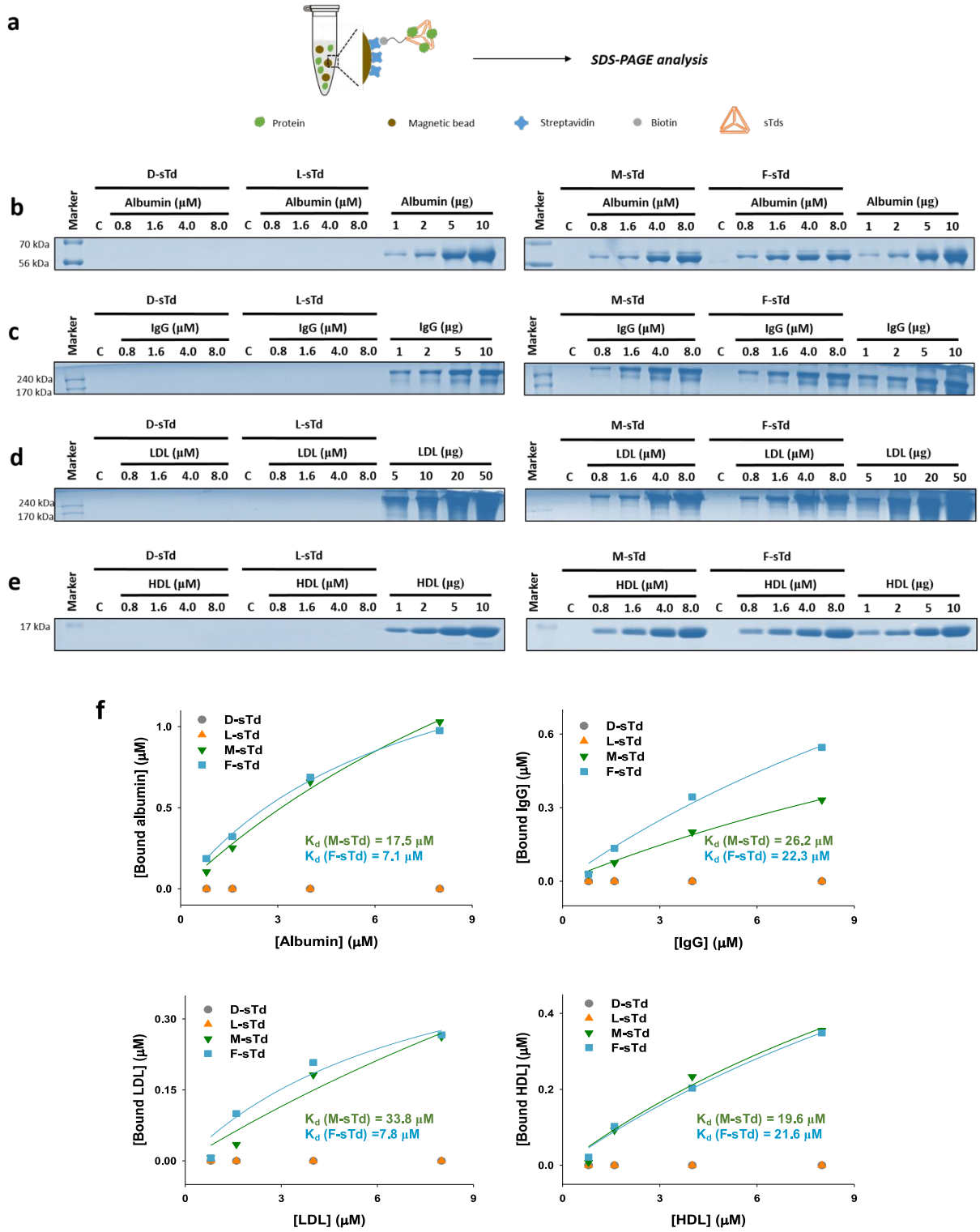

Figure S5. (a) The schematic presentation showing SDS-PAGE analysis of sTds binding to (b) albumin, (c) IgG, (d) LDL, and (e) HDL. (f) Dissociation constants (K_d) were obtained by curve-fitting with the ligand binding formula in SigmaPlot.

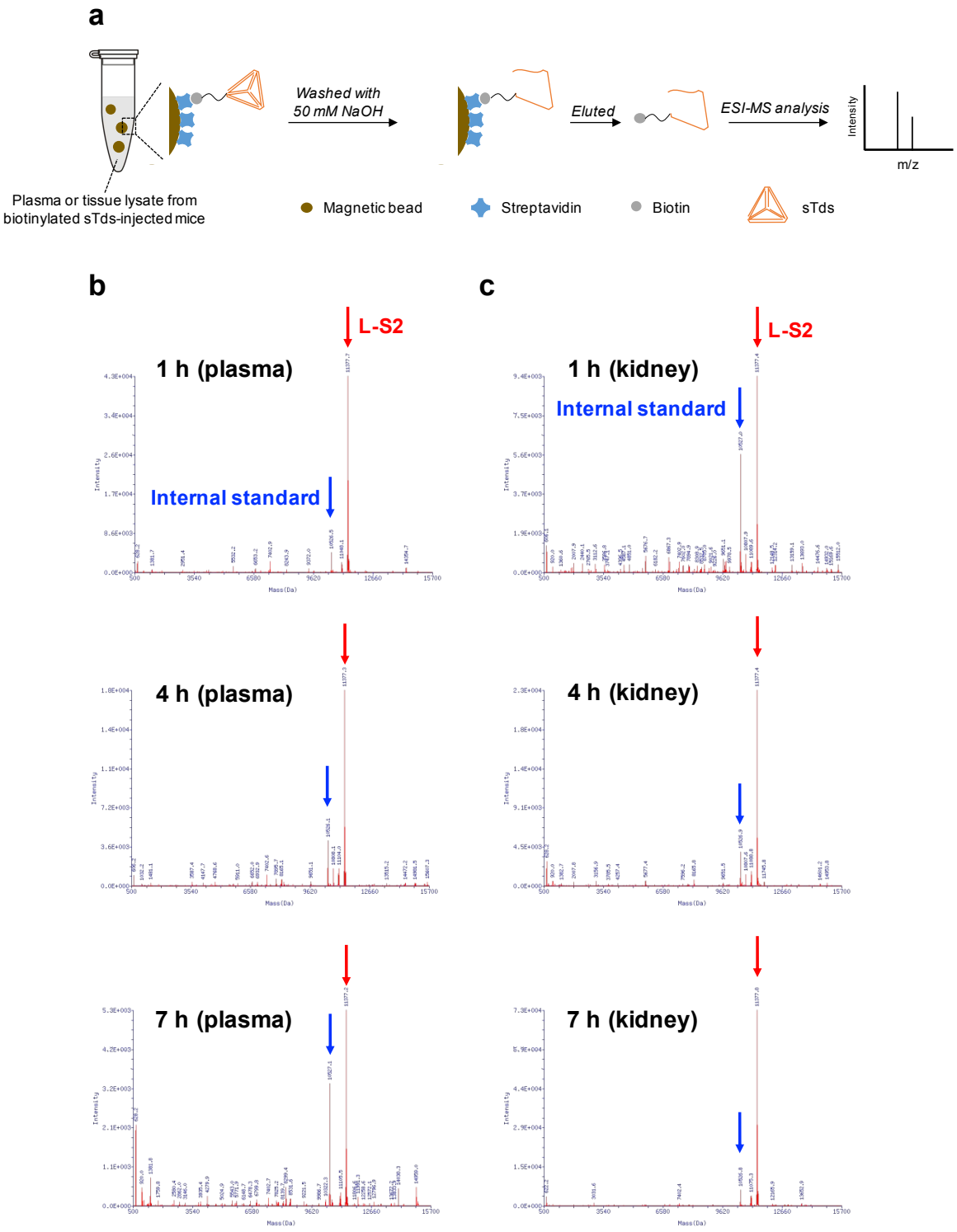

Figure S6. (a) Schematic illustration of ESI-MS analysis. ESI-MS spectra of biotinylated L-S2 captured in (b) plasma and (c) kidney lysate at 1, 4, and 7 h after intravenous injection of L-sTd.

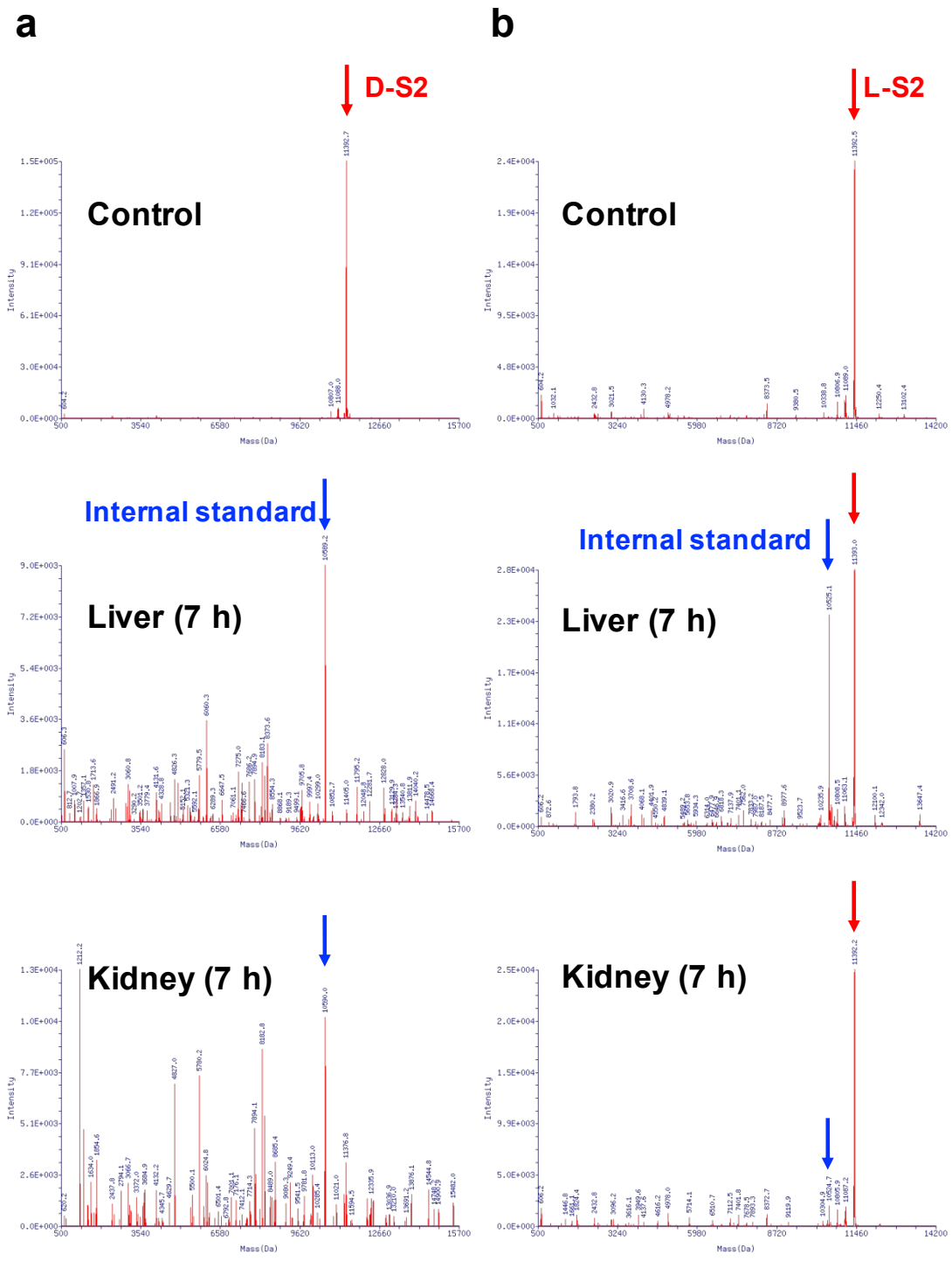

Figure S7. ESI-MS spectra of biotinylated (a) D-S2 and (b) L-S2 captured in kidney and liver lysate at 7 h after intravenous injection of D-sTd and L-sTd, respectively.

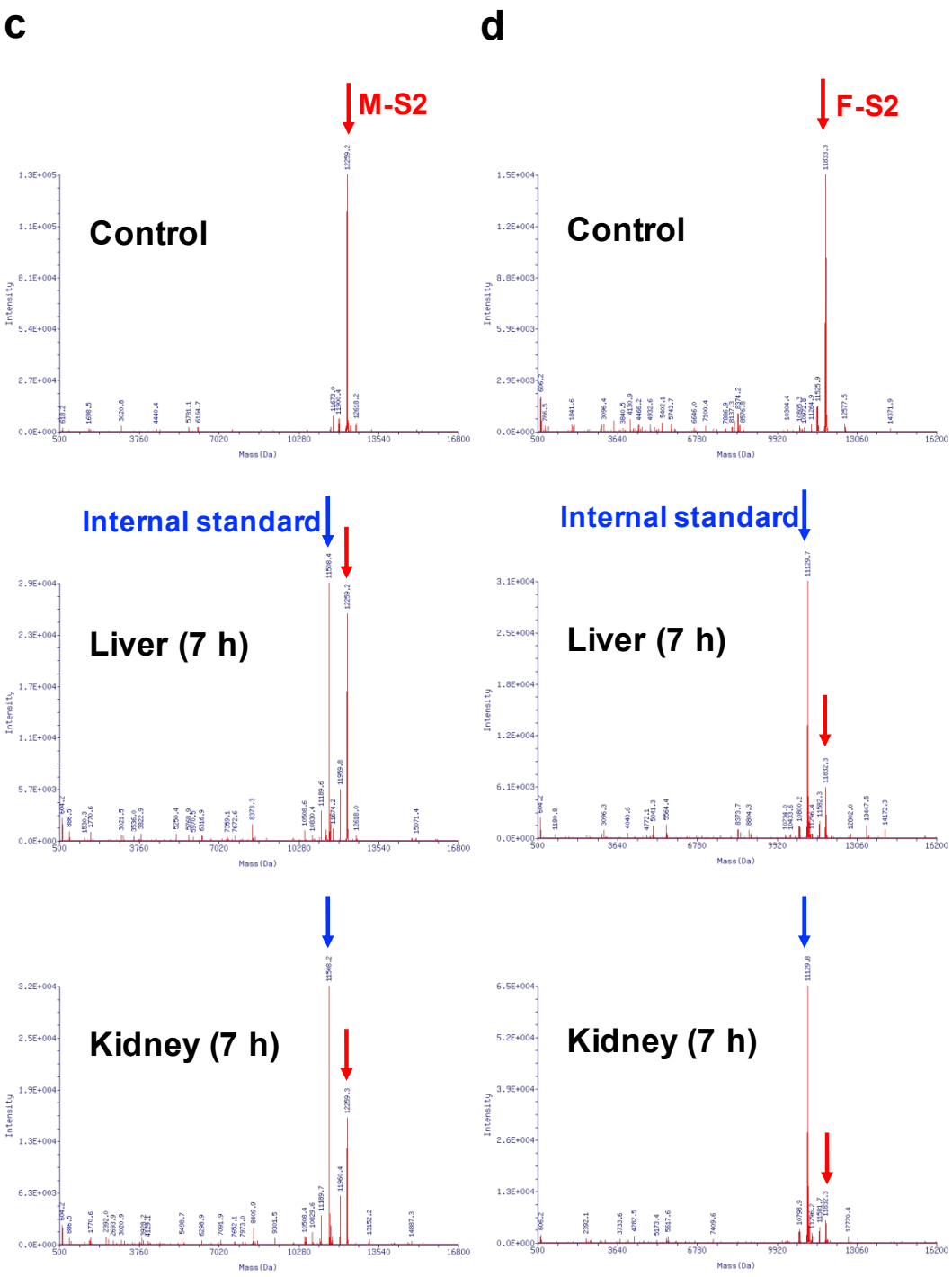

Figure S7 (continued). ESI-MS spectra of biotinylated (c) M-S2 and (d) F-S2 captured in kidney and liver lysate at 7 h after intravenous injection of M-sTd and F-sTd, respectively.

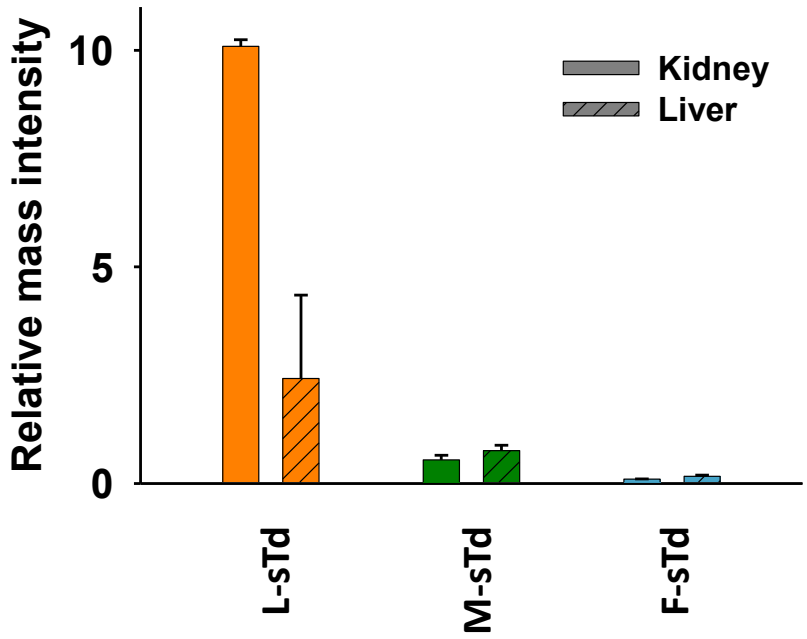

Figure S8. Relative mass intensity of L-sTd, M-sTd, and F-sTd estimated by normalization of intensity of S2 to intensity of the internal standard in ESI-MS spectra. Data are represented as mean ± standard deviation (SD) (n = 3).

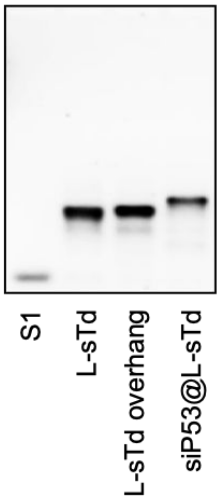

Figure S9. L-sTd loaded with siP53 (siP53@L-sTd) was characterized by agarose gel electrophoresis. Mobility of siP53@L-sTd was slightly reduced compared with that of L-sTd and L-sTd with the linker overhang.

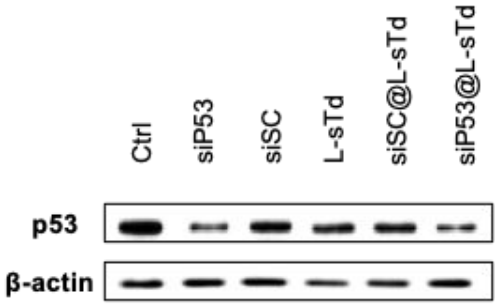

Figure S10. Representative image of western blotting of p53 in TCMK-1 cells. The β-actin level was used as the internal control to determine the relative p53 level.

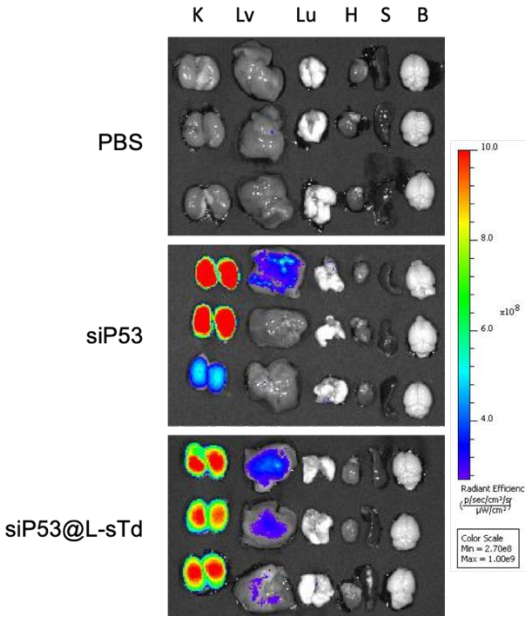

Figure S11. *Ex vivo* imaging of major organs from AKI mice treated with PBS, siP53, and siP53@L-sTd at 24 h post-injection (K, kidney; Lv, liver; Lu, lung; H, heart; S, spleen; B, brain).

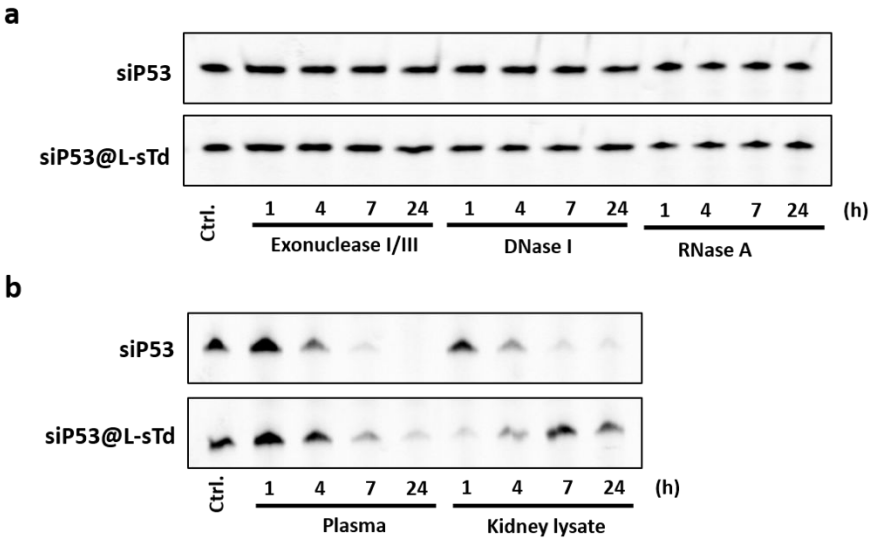

Figure S12. (a) 15% denaturing PAGE analysis of siP53 and siP53@ L-sTd after incubation with exonucleases I/III, DNase I, and RNase A. (b) 20% denaturing PAGE analysis of plasma and kidney lysate at 1, 4, and 7 h after intravenous injection of siP53 or siP53@L-sTd. The antisense strand was labeled with fluorescein for visualization.

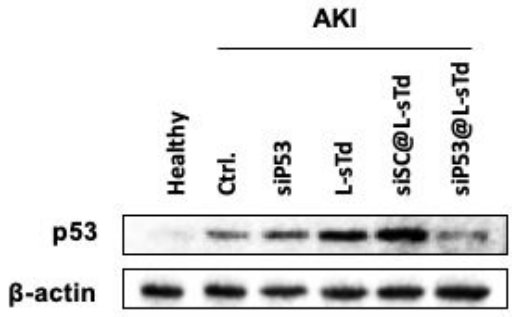

Figure S13. Representative image of western blotting of p53 in kidney lysate. The β-actin level was used as the internal control to determine the relative p53 level.

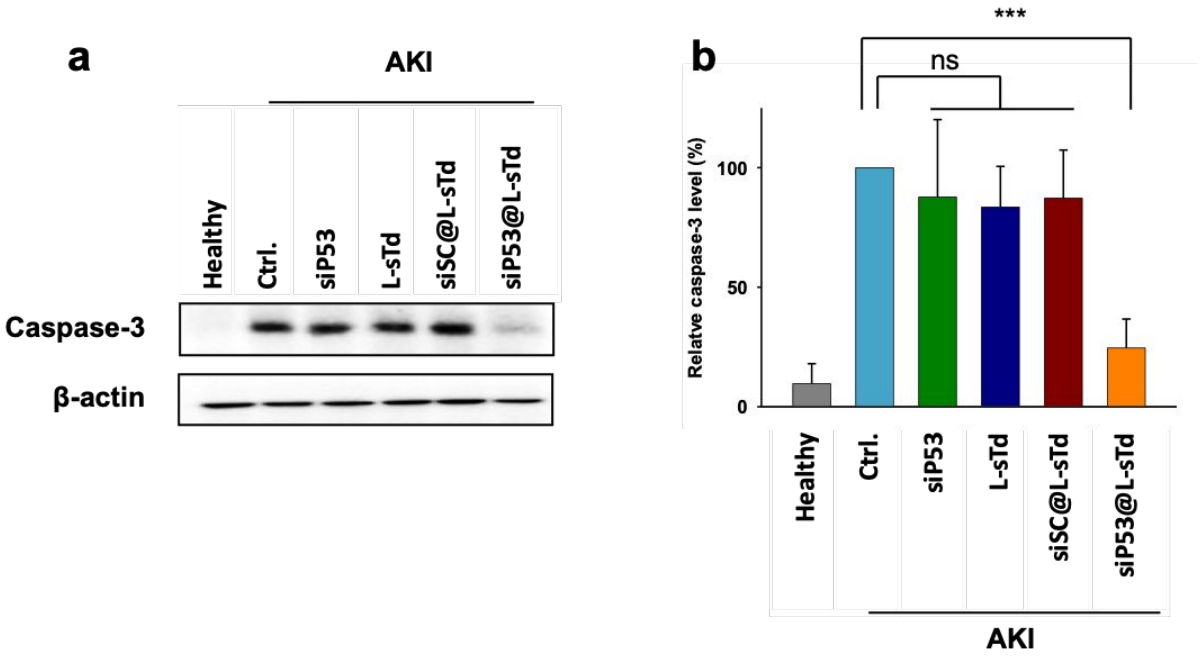

Figure S14. (a) Representative image of western blotting of caspase-3 in kidney lysate. (b) Relative caspase-3 levels in kidney lysate estimated by western blotting (mean ± SD; n = 5; $^{***}P < 0.001$; ns, non-significant). The β-actin level was used as the internal control to determine the relative caspase-3 level.

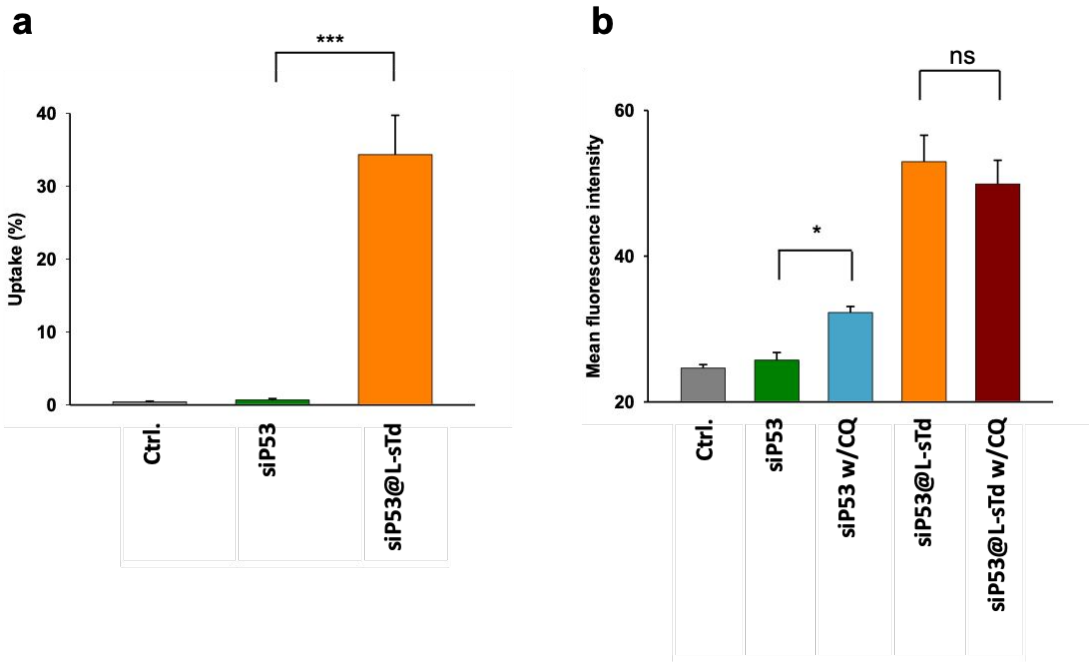

Figure S15. (a) Cellular uptake efficiency of siP53 and siP53@L-sTd in TCMK-1 cells. (b) Mean fluorescence intensity of TCMK-1 cells treated with siP53 and siP53@L-sTd in the presence or in the absence of chloroquine (CQ). (mean ± SD; n = 3; $^{*}P < 0.05$; $^{***}P < 0.001$; ns, non-significant).

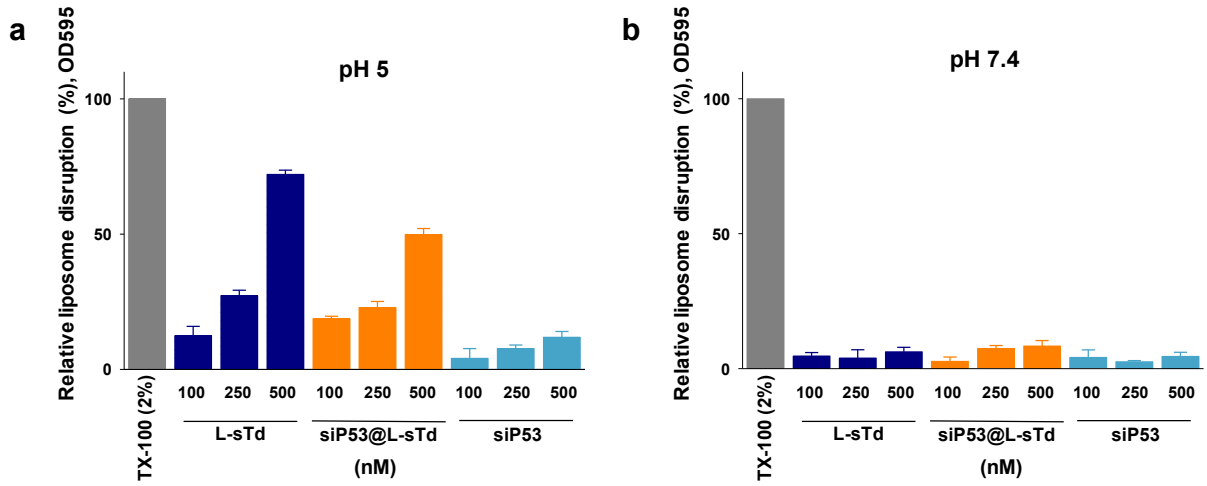

Figure S16. Relative liposome disruption (%) was estimated by the decrease of optical density at 595 nm (OD595) in the solution of liposomes upon treatment of L-sTd, siP53@L-sTd, and siP53 at (a) pH 5 and (b) pH 7.4. 2% Triton-X100 (TX-100) was used as the positive control (100%).

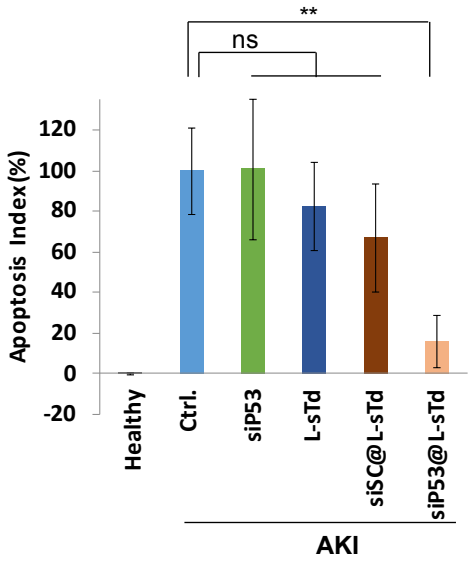

Figure S17. The apoptotic damage visualized by annexin V staining in kidney sections was quantified by using ImageJ (mean ± SD; n = 3; $^{**}P < 0.01$; ns, non-significant).

References

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(2) Huang, D. W.; Sherman, B. T.; Lempicki, R. A. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Research* **2008**, *37*(1), 1–13.

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