Supporting Information

MicroRNA-122-functionalized DNA tetrahedron stimulate hepatic differentiation of human mesenchymal stem cells for acute liver failure therapy

Hongyan Wei[a,b,1], Fenfang Li [a,b,1], Tiantian Xue[a], Haixia Wang[a], Enguo Ju[a], Mingqiang Li[a,b,*], Yu Tao[a,b,*]

[a] Laboratory of Biomaterials and Translational Medicine, Center for Nanomedicine, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou 510630, China

[b] Guangdong Provincial Key Laboratory of Liver Disease Research, Guangzhou 510630, China

* Corresponding authors

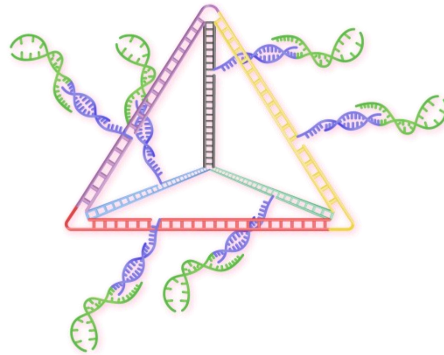
E-mail addresses: limq567@mail.sysu.edu.cn (M. Li), taoy28@mail.sysu.edu.cn (Y. Tao)

[1] These authors contributed equally to this work.

1. Materials

All HPLC-purified single-stranded DNAs (see Table S1-S2) with specific sequences were synthesized from Sangon Biological Engineering (Shanghai, China). The TDN was designed and evaluated by using the computer program SEQUIN [1] and UNPACK [2]. AFM measurements were performed by using a Nanoscope V multimode atomic force microscope (Dimension Icon (Bruker AXS, US)). The hydrodynamic diameter was measured by dynamic light scattering (DLS) (Malvern, UK). The zeta potentials were analyzed by Malvern Zetasizer (Nano ZS, Malvern, UK). Human adipose tissue-derived mesenchymal stem cells (hMSCs) were purchased from Saliai Stem Cell Science and Technology Co, LTD (Guangzhou, China). Human normal liver cells (L-O2) were taken from Sun Yat-sen University's First Affiliated Hospital (Guangzhou, China). The HiScript II One Step qRT-PCR SYBR® Green Kit was purchased from Vazyme (Nanjing, China). The RNAiso Plus was obtained from TAKARA (Japan). Cell Counting Kit 8 (CCK-8) and dimethyl sulfoxide (DMSO) were purchased from Solarbio (Beijing Solarbio Science Technology Co., Ltd, China). Hoechst 33342 and LysoTracker were purchased from Thermo Fisher Scientific (Gibco, USA). Oil Red O was obtained from Meilunbio (Guangzhou, China). The cytokines were determined by enzyme-linked immunosorbent assay (ELISA) using an OptEIA set (Pharmingen, San Diego, CA, USA). The PAS Kit was purchased from Sigma-Aldrich (USA). The Albumin antibody was purchased from Invitrogen (USA). Human HNF4A antibody, ECAD antibody and β-Tubulin antibody were purchased from Cell Signaling (USA). Paraformaldehyde solution (4% w/v) and methanol were obtained from Aladdin (Shanghai, China). All chemicals were reagent grade or higher and used without further purification. Milli-Q water (18.2 $M\Omega \cdot cm^{-1}$) was used in all experiments.

Table S1. Structure and nucleic acid sequences of TDN-miR122. The dark green capital letters represented ribonucleotides (RNA), the other colors capital letters represent deoxyribonucleic acid (DNA). The underlined capital letters represented a 2'-O-methyl base. All oligodeoxyribonucleotides have a phosphodiester backbone.



Name	Sequences (5'-3')
S_1	GTCTGAGGCAGTTGAGAGATCTCGAACATTCC-overhang$_1$
S_2	TAAGTCTGAAGATCCATTTATCACCAGCTGCTGCACGCCA TAGTAGACGTATCACCTGTCC-overhang$_1$
S_3	AGCTACTTGCTACACGAGGATCTTCAGACTTAGGAATGT TCGAGATCACATGCGAGGACTCGGTCCAATACCGTACTAAC GATTACAGATCAA-overhang$_1$
S_4	CAGCTGGTGATAAAACGTGTAGCAAGTAGCTTTGATCTG TAATCGACTCTACGGGAAGAGC-overhang$_1$
S_5	ATGCCCATCCGGCTCACTACTATGGCGTGCAG-overhang$_1$
S_6	CGAGTCCTCGCATGACTCAACTGCCTCAGACGGACAGG TGATACGAGAGCCGGATGGGCATGCTCTTCCCGTAGAGATA

	GTACGGTATTGGAC-overhang$_1$
miR-122 sense	CAAACGCCAUUAUCACACUAAAUA
miR-122 antisense	UGGAGUGUGACAAUGGUGUUUG-overhang$_{1-1}$
FAM-S$_1$	GTCTGAGGCAGTTGAGAGATCTCGAACATTCC-overhang$_1$
Cy7-S$_1$	GTCTGAGGCAGTTGAGAGATCTCGAACATTCC-overhang$_1$
Overhang$_1$	TTTTTTTTTTTTTTTTTTTTT
Overhang$_{1-1}$	AAA AAA AAA AAA AAA AAA AAA

Table S2. Primers sequences of housekeeping gene β-actin and hepatocyte-associated functional genes for qRT-PCR.

mRNA	Primer (5’-3’)	Sequence
β-actin	Forward	CTGGAACGGTGAAGGTGACA
	Reverse	AAGGGACTTCCTGTAACAATGCA
ALB	Forward	GAGACCAGAGGTTGATGTGATG
	Reverse	AGGCAGGCAGCTTTATCAGCA
HNF4A	Forward	CAGTGGAGAGTTCTTACGACAC
	Reverse	GGCTAAATCTGCAGGAGTACAT
FOXA2	Forward	CTCCATGAACATGTCGTCGTA
	Reverse	ATGGAGTTCATGTTGGCGTAG
AFP	Forward	CATGAGCACTGTTGCAGAGGAGA
	Reverse	CGTGGTCAGTTTGCAGCATTCTG

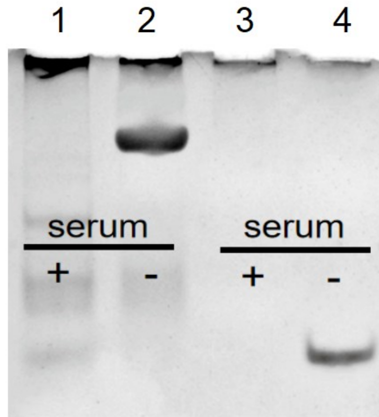


Fig. S1. The stability of TDN-miR122. Native PAGE analysis of the disassembly of TDN-miR122 incubated with serum at 37 °C (lane 1: TDN-miR122 in the serum for 2 hours, lane 2: TDN-miR122 in the water for 2 hours, lane 3: miR122 in the serum for 2 hours, lane 4: miR122 in the water for 2 hours).

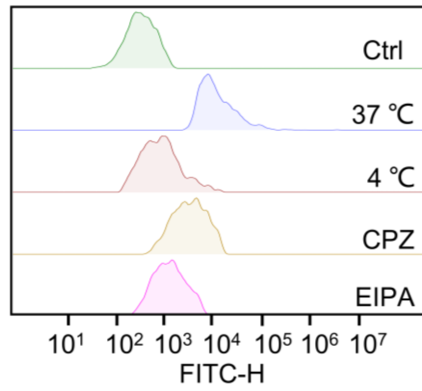


Fig. S2. Cellular uptake efficiency of TDN-miR122-FAM in hMSCs in the presence of endocytosis inhibitors: chlorpromazine (CPZ, 10 μM, clathrin-mediated endocytosis), 5-(N-ethyl-N-isopropyl) amiloride (EIPA, 50 μM, macropinocytosis).

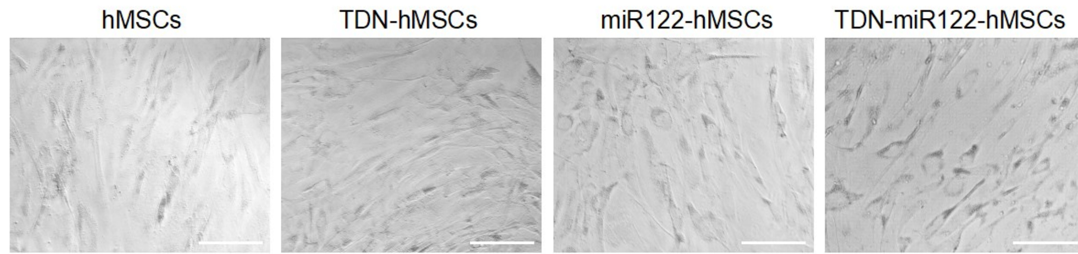


Fig. S3. Morphological changes (day 21) of hMSCs differentiation into functional hepatocyte-like cells. Undifferentiated hMSCs (hMSCs and TDN-hMSCs) assume a typical fibroblast-like cell shape. hMSCs-derived hepatocyte-like cells (miR122- hMSCs and TDN-miR122-hMSCs) were round to polygonal morphology and densely packed (scale bar: 25 μm).

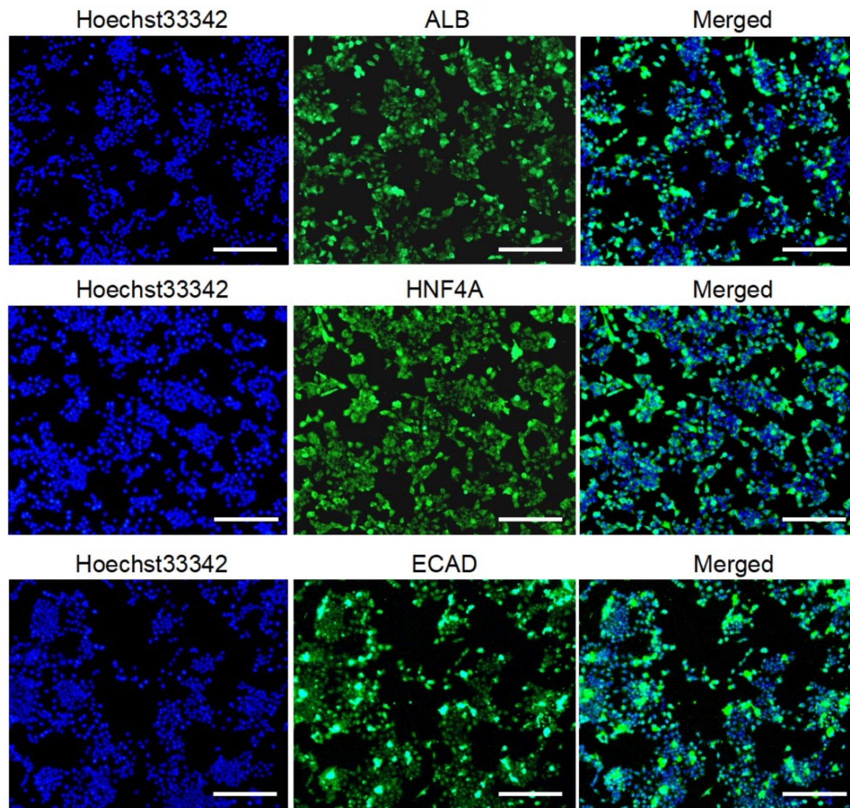


Fig. S4. Immunofluorescent staining of hepatocyte marker ALB, HNF4A, and ECAD expressions in human normal liver cells (LO2) (scale bar: 100 μm).

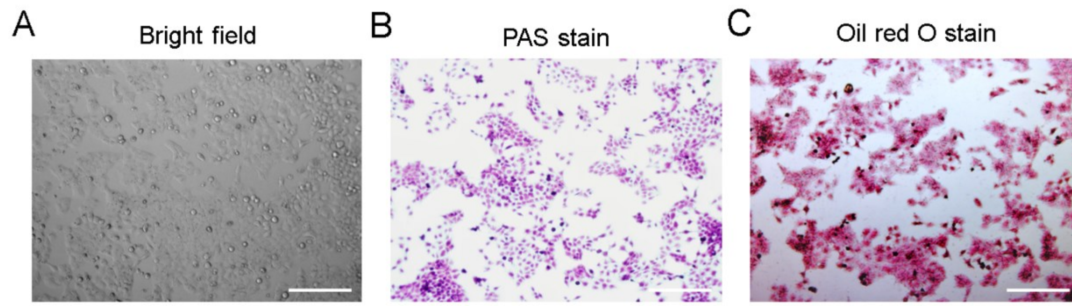


Fig. S5. (A) Morphological observation on human normal liver cell (LO2). (B) Abilities for glycogen synthesis in LO2 cells. (C) Oil red O stain in LO2 cells (scale bar: 100 μm).

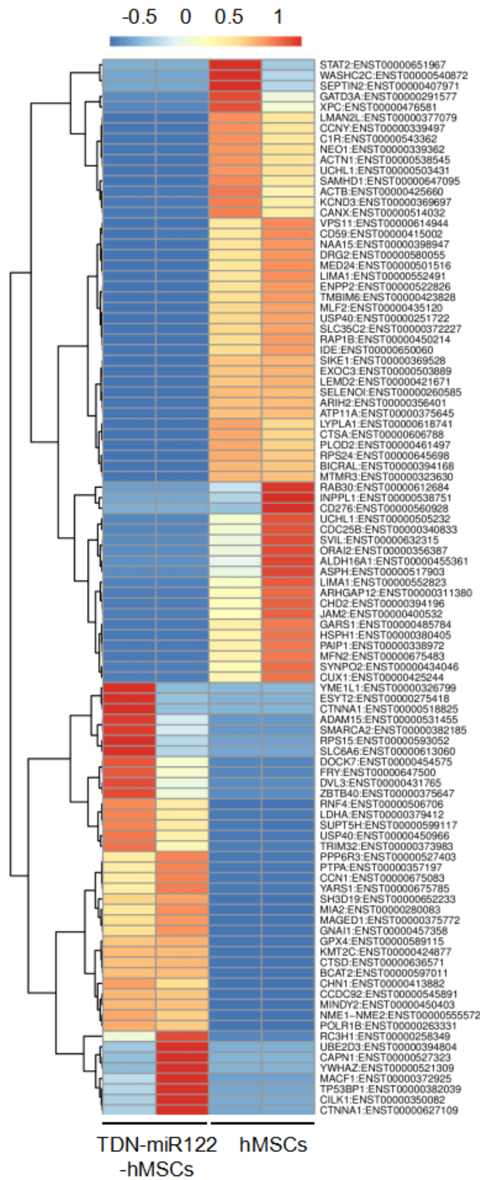


Fig. S6. Heat map representation and cluster analysis of these differentially expressed genes in TDN-miR122-hMSCs and hMSCs.

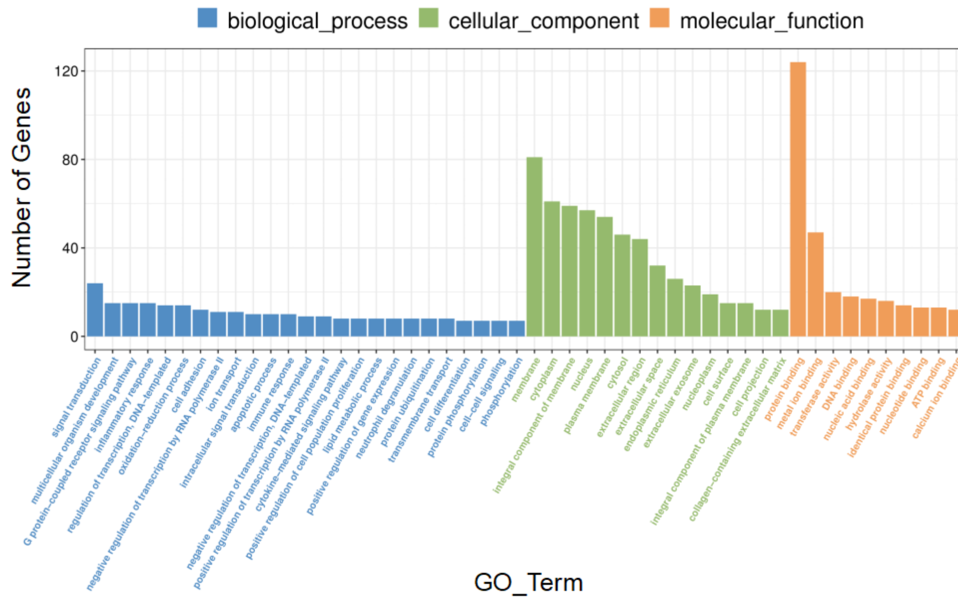


Fig. S7. Gene ontology (GO) analysis of the identified differentially expressed genes. The differentially expressed genes were grouped into three main categories: molecular function (MF), biological process (BP), and cellular component (CC). The number of genes in a specified category is plotted on the y-Axis.

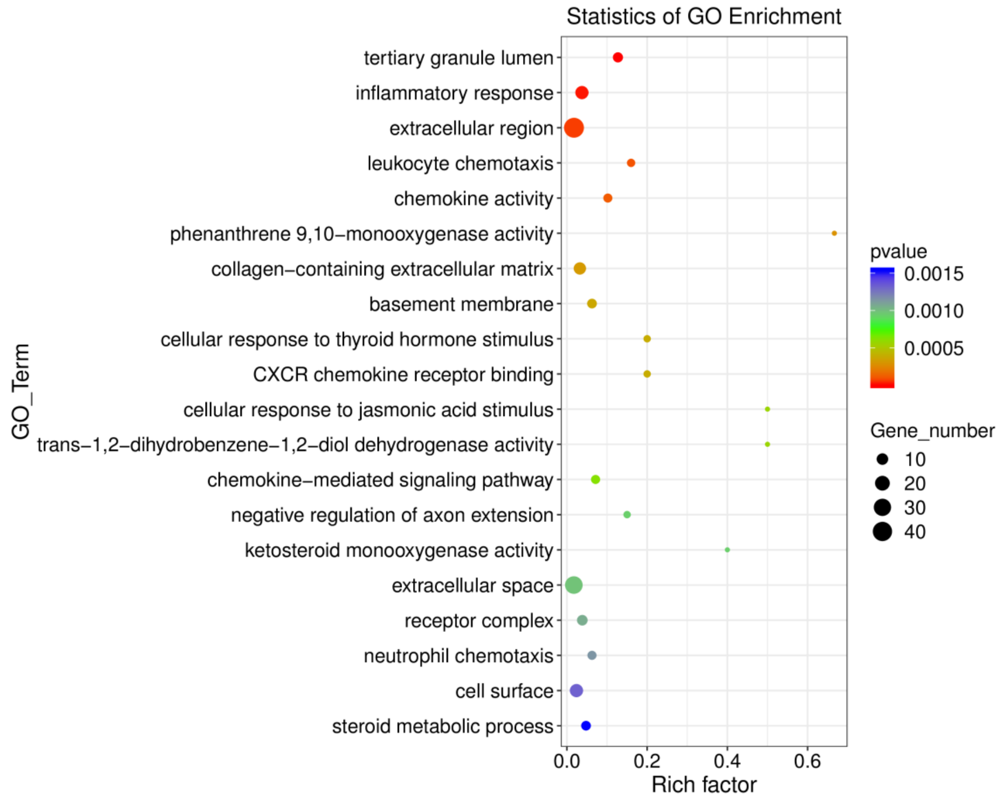


Fig. S8. Using the OmicShare tools, KEGG pathway analysis of the differentially expressed genes.

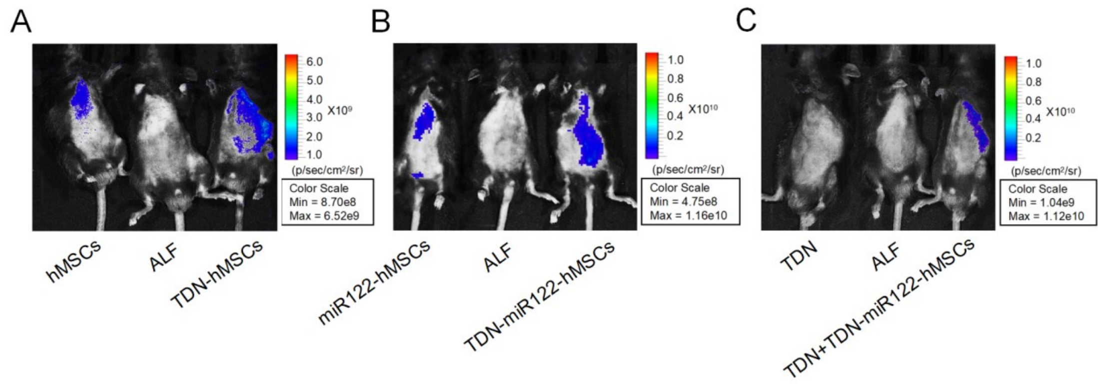


Fig. S9. *In vivo* fluorescence distribution imaging of the saline (ALF), DiD-hMSC$_S$ treatment group, DiD-TDN-hMSC$_S$ treatment group (A), DiD-miR122-hMSC$_S$ treatment group, DiD-TDN-miR122-hMSC$_S$ treatment group (B) and TDN+DiD-TDN-miR122-hMSC$_S$ treatment group (C) on day 3 in mice with ALF, using the IVIS spectrum imaging system at ex/em = 644/665 nm. The color changes indicate the fluorescence signal intensities from high (red) to low (blue).

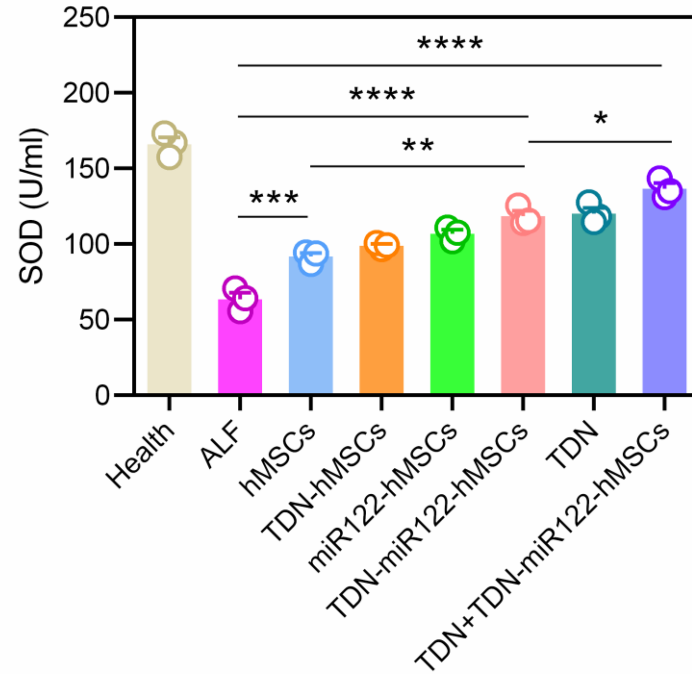


Fig. S10. Analysis of the SOD from ALF mice treated with different groups on day 3.

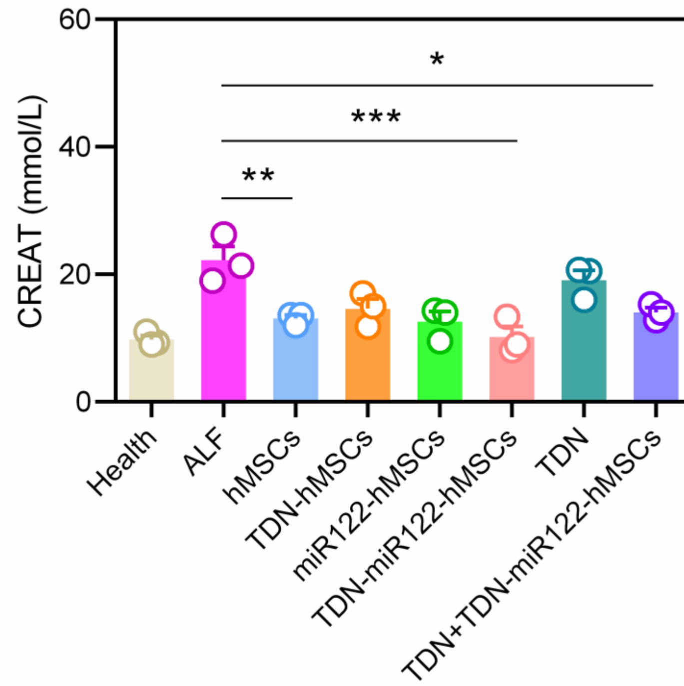


Fig. S11. Analysis of hematological markers of kidney damage (CREAT) in the ALF mice after different treatments.

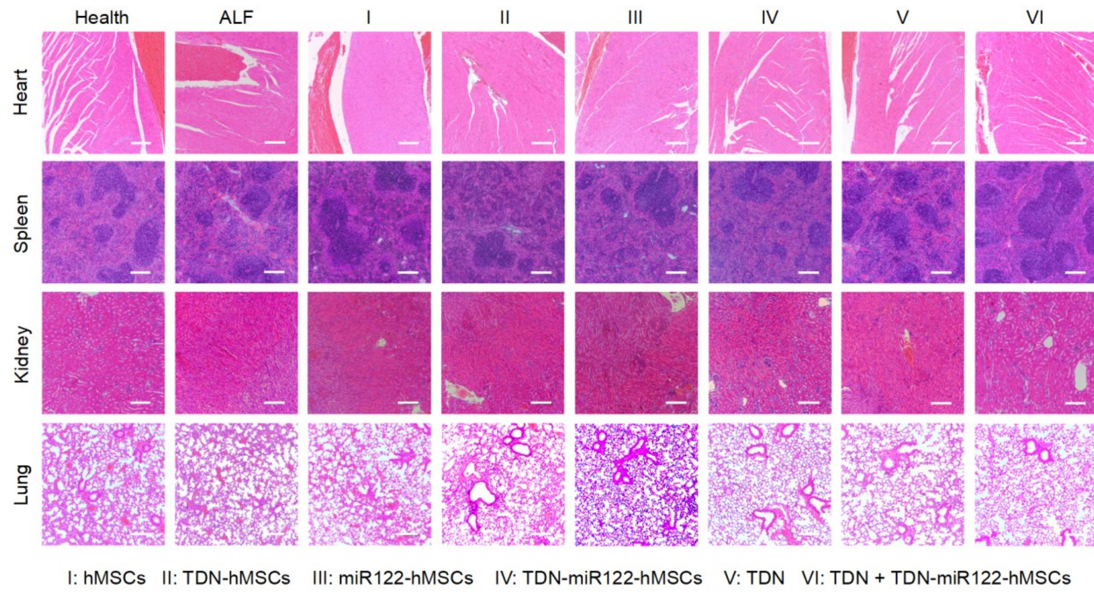


Fig. S12. H&E staining of major organs from ALF mice treated with different groups (scale bar: 100 μm).

References

[1] Q. Liu, D. Wang, M. Yuan, B.F. He, J. Li, C. Mao, G.S. Wang, H. Qian, Capturing intracellular oncogenic microRNAs with self-assembled DNA nanostructures for microRNA-based cancer therapy, Chem. Sci. 9(38) (2018) 7562-7568.

[2] H. Wei, Z. Zhao, Y. Wang, J. Zou, Q. Lin, Y. Duan, One-step self-assembly of multifunctional DNA nanohydrogels: an enhanced and harmless strategy for guiding combined antitumor therapy, ACS Appl. Mater. Interfaces 11(50) (2019) 46479-46489.