

Supplementary material for

Pancreatic ductal deletion of S100A9 alleviates acute pancreatitis by targeting VNN1-mediated ROS release to inhibit NLRP3 activation

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Supplementary Table S1 Primer sequences used for qPCR assay

Gene	species	Primers (5'-3')
β -actin	Human	Forward: CCTGGGCATGGAGTCCTGTG Reverse: TCTTCATTGTGCTGGGTGCC
IL-1 β	Human	Forward: ATGATGGCTTATTACAGTGGCAA Reverse: GTCGGAGATTCGTAGCTGGA
IL-6	Human	Forward: AAGCCAGAGCTGTGCAGATGAGTA Reverse: TGTCCTGCAGCCACTGGTTC
IL-8	Human	Forward: CACTGTGTGTAAACATGACTTCCAA Reverse: TGTGGTCCACTCTCAATCACTCTC
IL-18	Human	Forward: TCTTCATTGACCAAGGAAATCGG Reverse: TCCGGGGTGCATTATCTCTAC
S100A8	Human	Forward: AAATTGCTAGAGACCGAGTGTCTCT Reverse: CACGCCCATCTTTATCACCA
S100A9	Human	Forward: TCCACCAATACTCTGTGAAGCTG Reverse: CCTCCATGATGTGTTCTATGACC
HSPA1B	Human	Forward: GGTCAGGCCCTACCATTGAG Reverse: TCCTTGAGTCCCAACAGTCCA
HSPA8	Human	Forward: AAGCTATGTCGCCTTTACGGACAC Reverse: CATCATCAAATCTGCGTCCAATC
HNRNP2	Human	Forward: ACCGTCTCGCTATAGCCGTTTG Reverse: CATCACTTCATCGGCTGAGCA
PABPC1	Human	Forward: GGTTCCAAGGGCTATGGATTTGTA Reverse: GCCCTAGCTCCAAGTTCAGCTTC
TUBB4A	Human	Forward: CCATGGACTCTGTCCGTTCT Reverse: CCCTTTGCCCAGTTGTTG
TUBA1C	Human	Forward: CTGAGCAACTCATCACAGGCAAG Reverse: CCATGAGCAGCGAGGTGAAC
TBC1D2	Human	Forward: CAAGACTCCCAGCCGGGTTA Reverse: GGAATTCCCAGCGCTTCATC
VNN1	Human	Forward: TGGCACTTTCGGAACCCAGTA Reverse: TCAGACTAAACAAGCGTCCGTCAG
β -actin	Mouse	Forward: CATCCGTAAAGACCTCTATGCCAAC Reverse: ATGGAGCCACCGATCCACA
IL-1 β	Mouse	Forward: AAATCTCGCAGCAGCACATCAA Reverse: CCACGGGAAAGACACAGGTAGC
IL-6	Mouse	Forward: CCTTCTTGGGACTGATGATGCTG Reverse: TTGGGAGTGGTATCCTCTGTGA
IL-18	Mouse	Forward: CAGGCCTGACATCTTCTGCAA Reverse: TCTGACATGGCAGCCATTGT
CXCL1	Mouse	Forward: ATGGCTGGGATTCACCTCAAGAAC Reverse: AGTGTGGCTATGACTTCGGTTTGG
CXCL2	Mouse	Forward: CACTGGTCCTGCTGCTGCTG Reverse: GCGTCACACTCAAGCTCTGGATG
CXCL5	Mouse	Forward: ATCCCCAGCGGTTCCATCTCG Reverse: CGTTGCGGCTATGACTGAGGAAG
CXCR2	Mouse	Forward: CAGCTGCCTTAACCCCATC Reverse: CTTGAGAAGTCCATGGCGAAA

Supplementary Table S2 Statistical data of protein identification

Samples	Total spectrogram number	Number of identification spectrogram	Identification of peptide number	Identification of protein number	Unique-2
IgG	4713	493	154	56	32
IP	15232	420	80	20	13

Supplementary Table S3 Unique proteins of pull-down in S100A9 IP group

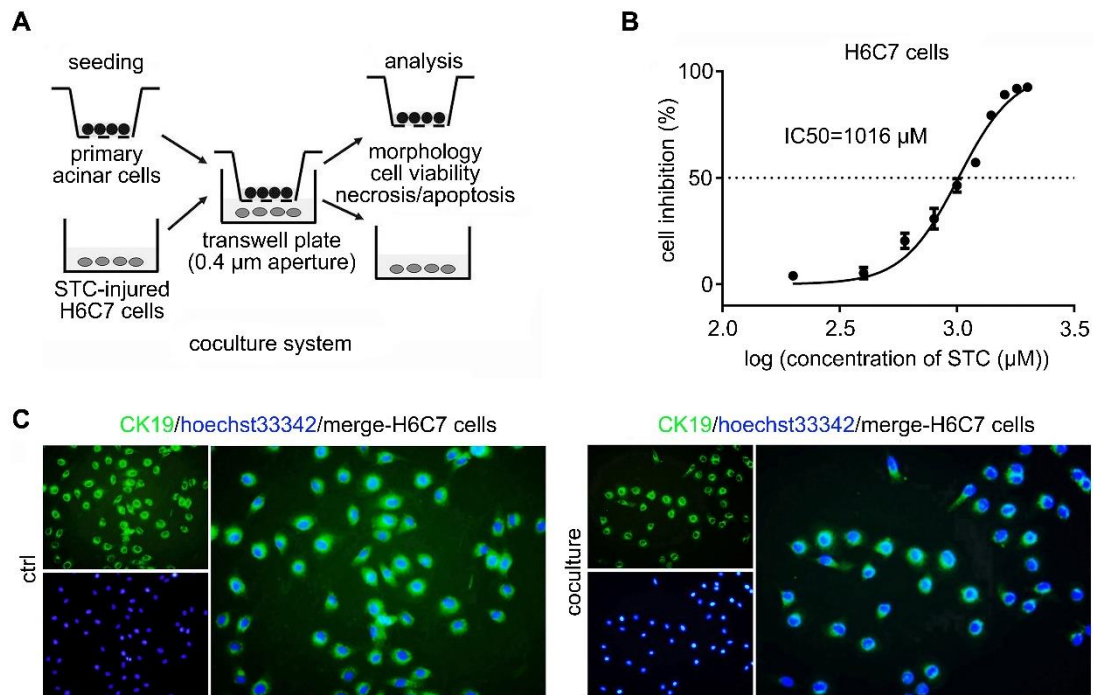
Accession	Names	Length	Mass	Unused	Coverage(%)
sp P0DMV9 HS71B_HUMAN	Heat shock 70 kDa protein 1B	641	70051.60	3.33	9.20
sp P11142 HSP7C_HUMAN	Heat shock cognate 71 kDa protein	646	70897.60	14.69	13.78
sp P55795 HNRH2_HUMAN	Heterogeneous nuclear ribonucleoprotein H2	449	49229.30	2.00	3.79
sp P11940 PABP1_HUMAN	Polyadenylate-binding protein 1	636	70670.40	10.43	9.43
sp P04350 TBB4A_HUMAN	Tubulin beta-4A chain	444	49585.50	2.00	2.70
sp Q9BQE3 TBA1C_HUMAN	Tubulin alpha-1C chain	449	49894.90	2.08	4.45
sp Q9BYX2 TBD2A_HUMAN	TBC1 domain family member 2A	928	105412.80	6.14	4.31
sp O95497 VNN1_HUMAN	Pantetheinase	513	57011.20	2.28	4.09
sp P04432 KVD39_HUMAN	Immunoglobulin kappa variable 1D-39	117	12737.30	2.00	13.68
sp P0DOX7 IGK_HUMAN	Immunoglobulin kappa light chain	214	23378.90	2.00	8.41
sp P01860 IGHG3_HUMAN	Immunoglobulin heavy constant gamma 3	377	41286.60	7.24	15.38

Supplementary Table S4 Contact list between S100A9 and VNN1

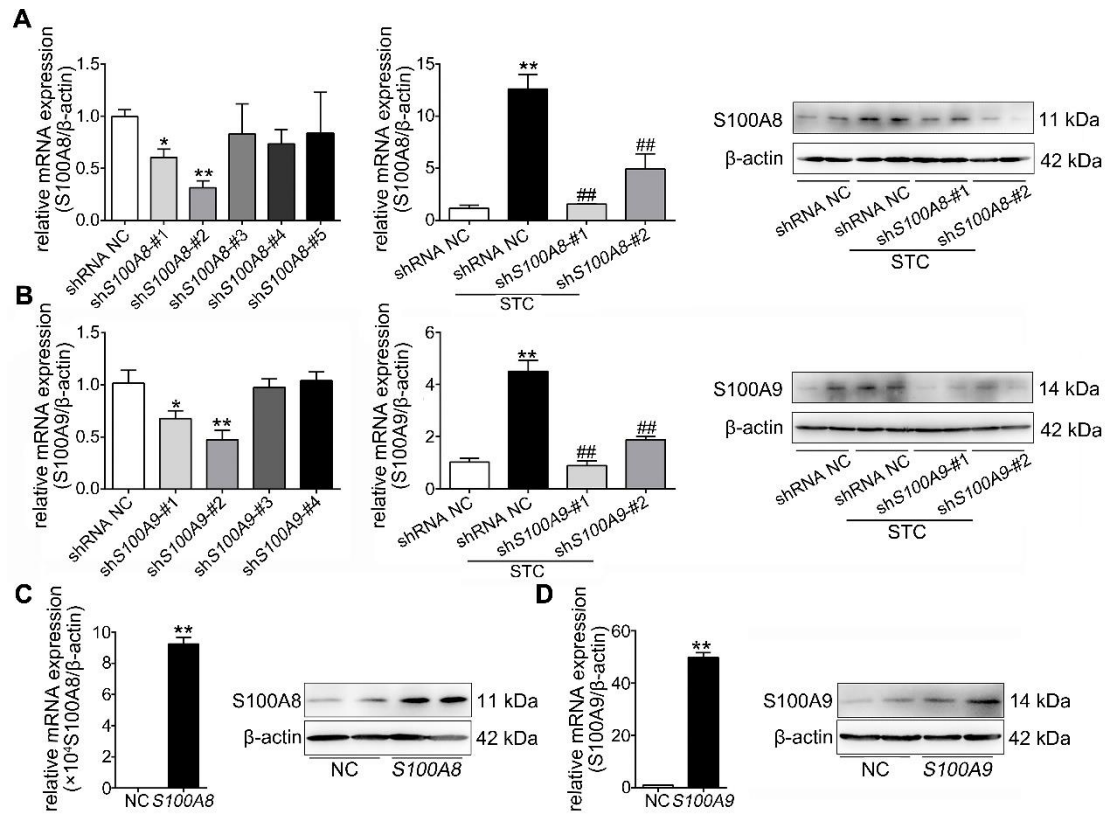
The residues in S100A9	The residues in VNN1	Interaction type
Oxygen atom of carboxyl group of Glu92	Nitrogen atoms of guanidine group of Arg259	Salt bridge
Nitrogen atom of amino group of Lys57	Oxygen atoms of carboxyl group of Glu324	Salt bridge
Sulfur atom of thioether group of Met94	Nitrogen atom of imidazolyl group of His228	Hydrogen bond
Oxygen atom of backbone of Gly100	Oxygen atom of hydroxyl group of Ser309	Hydrogen bond
Nitrogen atom of backbone of Gly102	Oxygen atom of hydroxyl group of Ser309	Hydrogen bond
Oxygen atom of backbone of Glu92	Nitrogen atom of imidazolyl group of His310	Hydrogen bond
Nitrogen atom of imidazolyl group of His105	Oxygen atom of backbone of His310	Hydrogen bond
Nitrogen atom of imidazolyl group of His103	Oxygen atom of hydroxyl group of Ser311	Hydrogen bond
Oxygen atom of carboxyl group of Glu92	Carbon atom of backbone of Val313	Hydrogen bond
Oxygen atom of carboxyl group of Glu92	Nitrogen atom of backbone of Val314	Hydrogen bond
Oxygen atom of carboxyl group of Glu52	Nitrogen atom of amido group of Asn315	Hydrogen bond
Carbon atom of sidechain of Lys57	Oxygen atom of backbone of Ser321	Hydrogen bond
Nitrogen atom of amino group of Lys57	Oxygen atom of backbone of Ile323	Hydrogen bond
Nitrogen atom of guanidine group of Arg85	Oxygen atom of backbone of Phe431	Hydrogen bond
Nitrogen atom of guanidine group of Arg85	Oxygen atom of amido group of Gln434	Hydrogen bond

Supplementary Table S5 Contact list between three compounds and S100A9

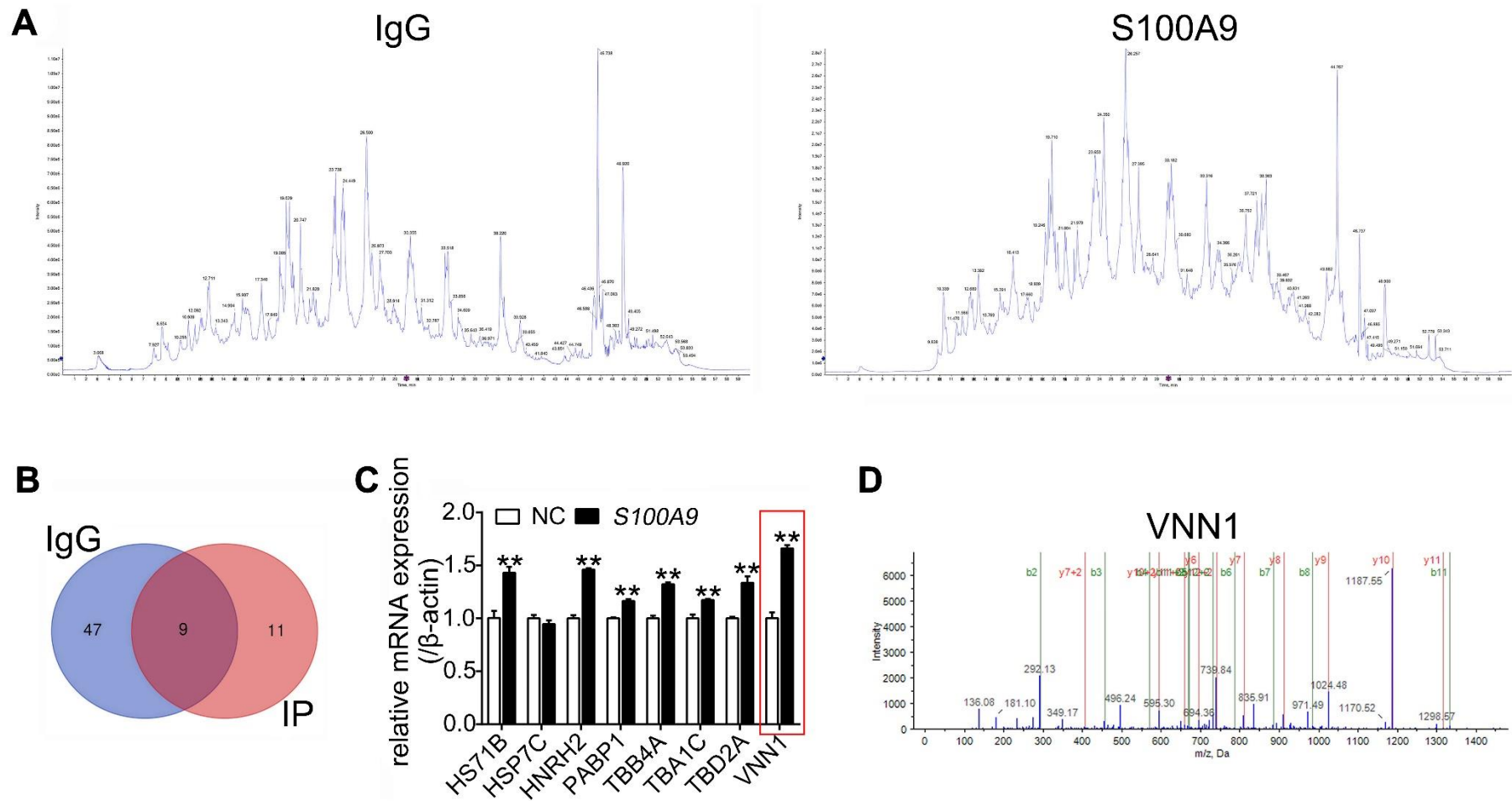
Compounds	Chemical groups in compound	The residues in S100A9	Interaction type
C ₄₂ H ₆₀ N ₄ O ₆	Oxygen atom in one hydroxyl group (hydrogen bond acceptor)	Side chain nitrogen atom of Arg85	Hydrogen bond
	Oxygen atom in one hydroxyl group (hydrogen bond donor)	Side chain oxygen atom of Glu92	Hydrogen bond
	Carbon atom (hydrogen bond donor)	Backbone oxygen atom of His103	Hydrogen bond
	Others	Gly97, Gly100, Ala89, Trp88 and Val58	Hydrogen bond
C ₂₈ H ₂₉ F ₃ N ₄ O ₅ S	Oxygen atom (hydrogen bond acceptor)	Side chain nitrogen atom of Lys51	Hydrogen bond
	Nitrogen atom (hydrogen bond donor)	Backbone oxygen atom of Trp88	Hydrogen bond
	Nitrogen atom	Side chain oxygen atom of Glu92	Salt bridge
	Others	Val58, Ile62, Leu49, Phe48 and Leu109	Hydrogen bond
C ₃₀ H ₃₂ N ₄ O ₆ S ₄	Nitrogen atom (hydrogen bond donor)	Side chain oxygen atom of Glu92	Hydrogen bond
	Sulfur atom (hydrogen bond acceptor)	Backbone nitrogen atom of Gly102	Hydrogen bond
	Oxygen atom (hydrogen bond acceptor)	Backbone nitrogen atom of His105	Hydrogen bond
	Others	Leu109, Ala89, Trp88, Gly97 and Gly100	Hydrogen bond



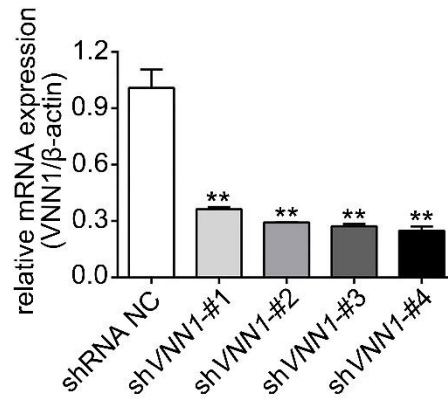
Supplementary Figure S1 (A) Pattern diagram of the coculture system for STC-injured ductal cells and primary acinar cells. (B) MTT results showed that the IC₅₀ value of STC in H6C7 cells was 1016 μM ($n = 6$). (C) IF staining proved that the expression of the ductal cell marker CK19 clearly decreased in STC-treated H6C7 cells. Data are presented as the mean \pm SEM.



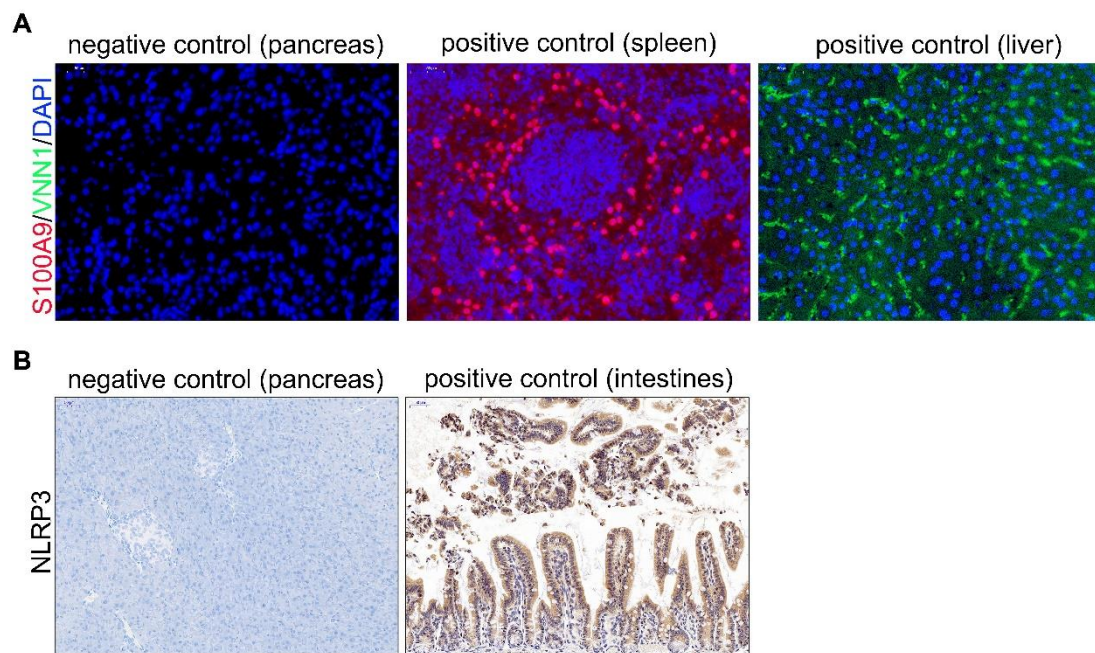
Supplementary Figure S2 (A) shS100A8-#1 and -#2 significantly downregulated S100A8 mRNA (n = 3) and protein expressions. (B) shS100A9-#1 and -#2 significantly downregulated S100A9 mRNA (n = 3) and protein expressions. (C) S100A8 mRNA and protein expression levels were upregulated in S100A8-overexpressing H6C7 cells (n = 3). (D) S100A9 mRNA and protein expression levels were upregulated in S100A9-overexpressing H6C7 cells (n = 3). Data are presented as the mean ± SEM; *P < 0.05 and **P < 0.01 vs. shRNA NC or NC group; ##P < 0.01 vs. shRNA NC+STC.



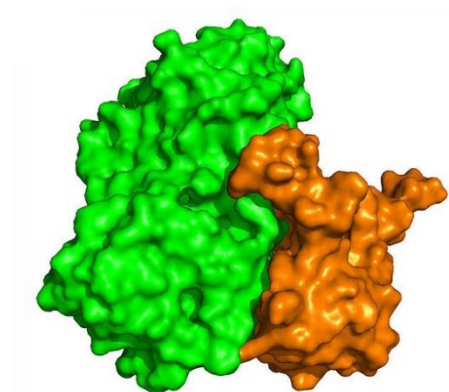
Supplementary Figure S3 (A) LC-MS/MS was performed to identify and analyze the binding proteins of S100A9. (B) 56 and 20 kinds of proteins were identified in the IgG and S100A9 pull-down samples, respectively. (C) Gene expression levels of 8 proteins (HS71B, HSP7C, HNRH2, PABP1, TBB4A, TBA1C, TBD2A and VNN1) in S100A9-overexpressing H6C7 cells were detected by qPCR (n = 3). (D) Mass spectrogram of the VNN1 protein. Data are presented as the mean \pm SEM; **P < 0.01 vs. NC group.



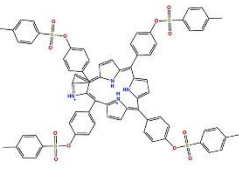
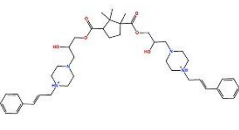
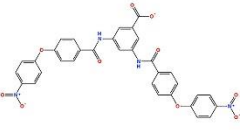
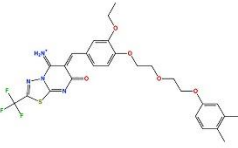
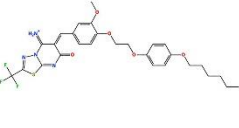
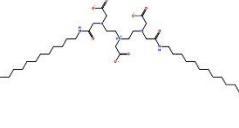
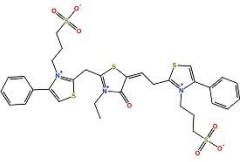
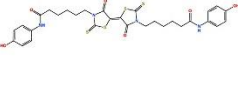
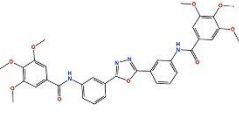
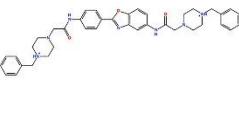
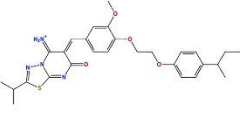
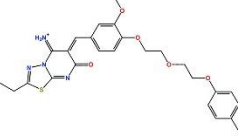
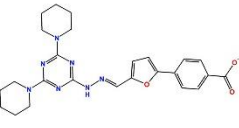
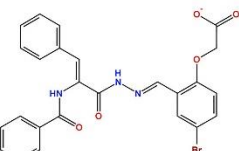
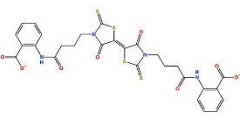
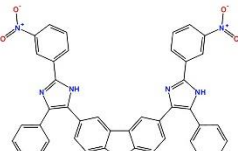
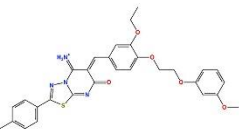
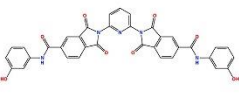
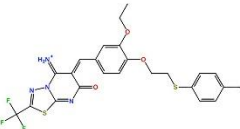
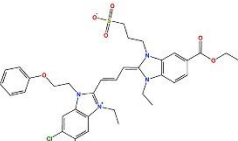
Supplementary Figure S4 shVNN1s significantly downregulated S100A9 mRNA level compared to shRNA NC group (n = 3). Data are presented as the mean \pm SEM; **P < 0.01 vs. shRNA NC group.

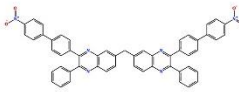
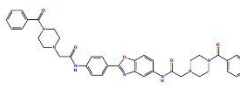
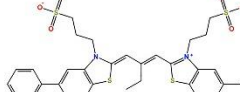
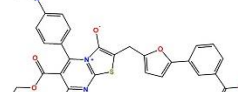
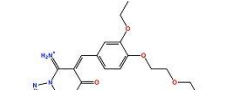
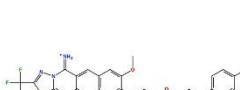
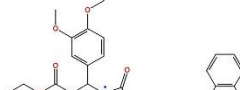
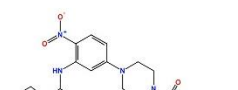
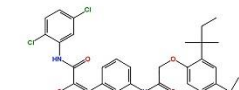
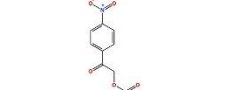
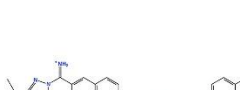
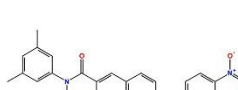
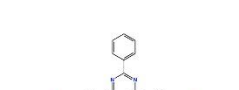
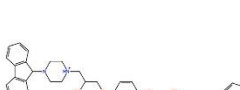

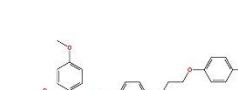
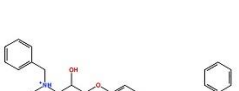
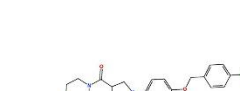

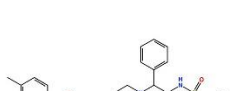


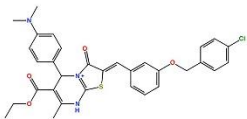
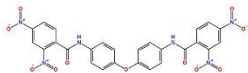
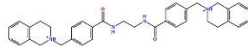
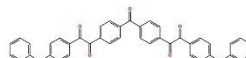
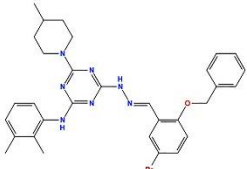

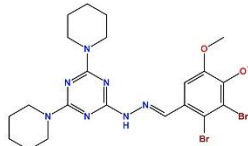
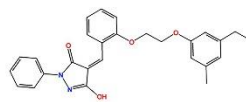
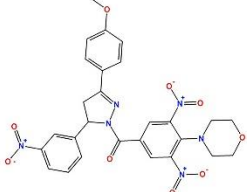
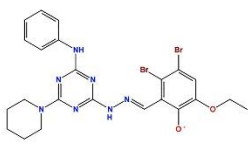
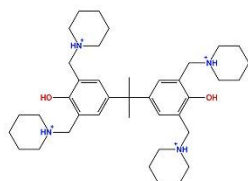
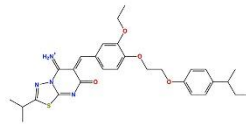
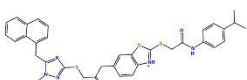
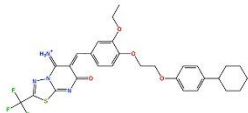
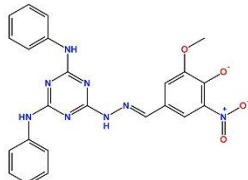
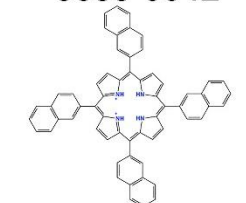
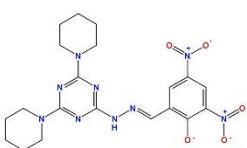
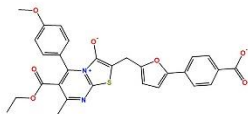
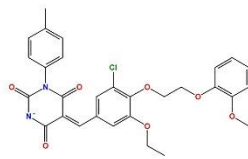
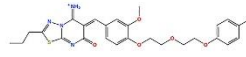
Supplementary Figure S5 (A-B) Negative and positive controls of S100A9, VNN1 and NLRP3 expressions.

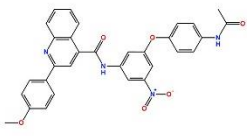
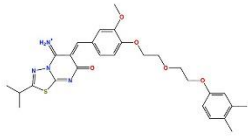
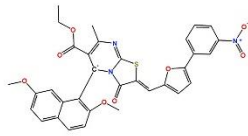
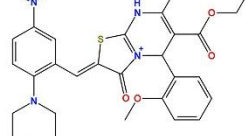
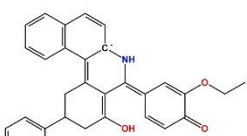
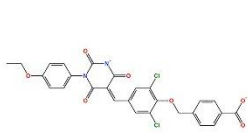
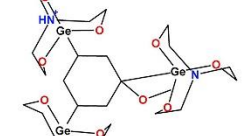
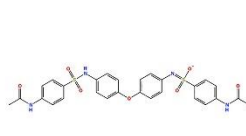
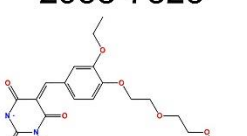
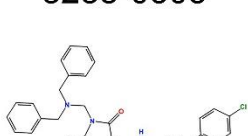
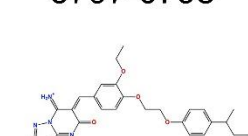
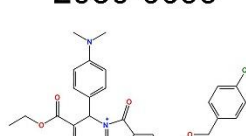
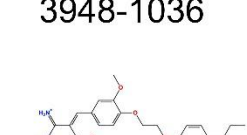
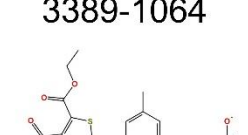
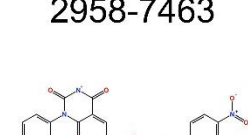
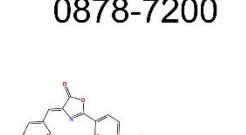
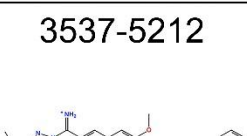
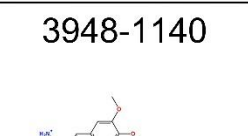
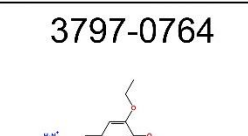
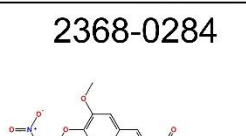


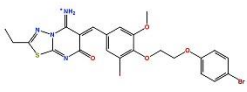
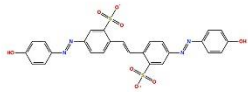
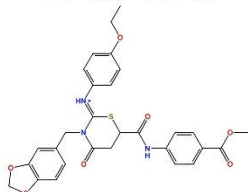
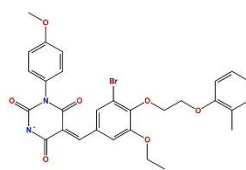
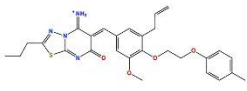
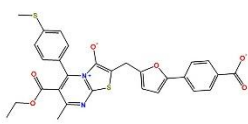
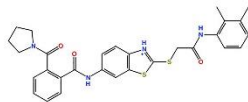
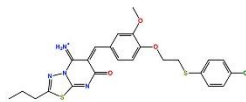
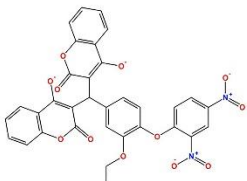
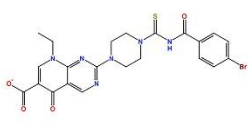
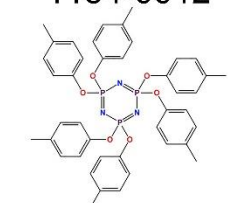
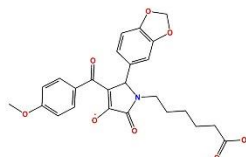
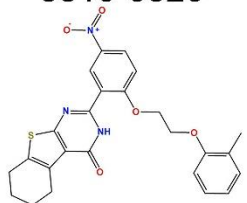
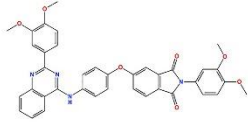
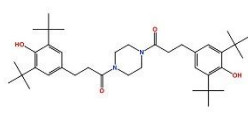
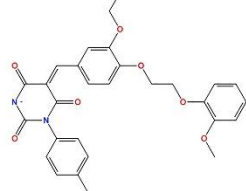
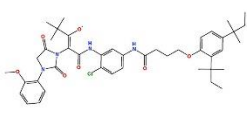
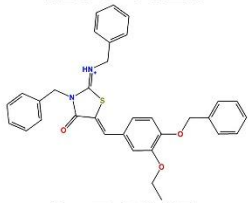
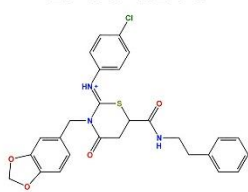
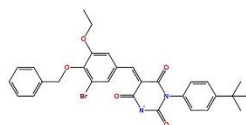
Supplementary Figure S6 Binding model between the S100A9 protein and the VNN1 protein; the surfaces of S100A9 and VNN1 are colored orange and green, respectively.

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<p>3948-1191</p>  <p>S:-8.1575</p>	<p>0249-0003</p>  <p>S:-8.0931</p>	<p>2324-0140</p>  <p>S:-8.0680</p>	<p>3232-0780</p>  <p>S:-8.0591</p>
<p>3289-7235</p>  <p>S:-8.0092</p>	<p>3861-0047</p>  <p>S:-7.9737</p>	<p>3797-0694</p>  <p>S:-7.9408</p>	<p>3537-5285</p>  <p>S:-7.9036</p>
<p>0927-0035</p>  <p>S:-7.8888</p>	<p>2040-0304</p>  <p>S:-7.8013</p>	<p>3232-0776</p>  <p>S:-7.7541</p>	<p>0669-0140</p>  <p>S:-7.7270</p>
<p>4011-0808</p>  <p>S:-7.7152</p>	<p>2036-0830</p>  <p>S:-7.6875</p>	<p>3948-0977</p>  <p>S:-7.6810</p>	<p>2324-0157</p>  <p>S:-7.6803</p>

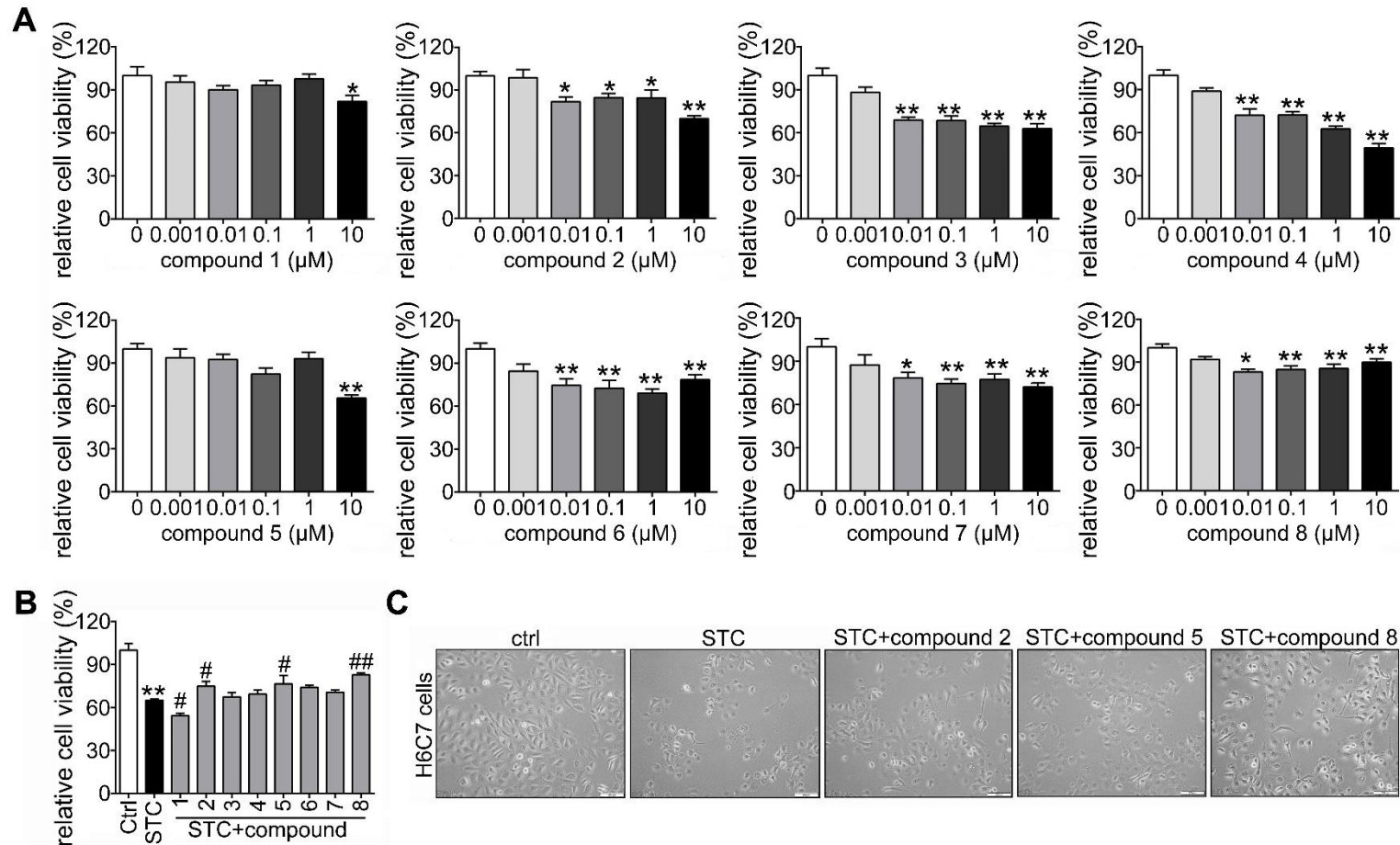
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<p>3948-1099</p>  <p>S:-7.5964</p>	<p>3948-1042</p>  <p>S:-7.5867</p>	<p>2033-0102</p>  <p>S:-7.5851</p>	<p>3819-1354</p>  <p>S:-7.5820</p>
<p>0105-0143</p>  <p>S:-7.5818</p>	<p>3257-1297</p>  <p>S:-7.5771</p>	<p>3773-3548</p>  <p>S:-7.5752</p>	<p>2368-0020</p>  <p>S:-7.5750</p>
<p>2036-0525</p>  <p>S:-7.5608</p>	<p>0940-0013</p>  <p>S:-7.5265</p>	<p>2036-0823</p>  <p>S:-7.5255</p>	<p>3202-0346</p>  <p>S:-7.5175</p>
<p>4013-2645</p>  <p>S:-7.5161</p>	<p>3448-6069</p>  <p>S:-7.5141</p>	<p>2324-0213</p>  <p>S:-7.5076</p>	<p>1477-0001</p>  <p>S:-7.5034</p>

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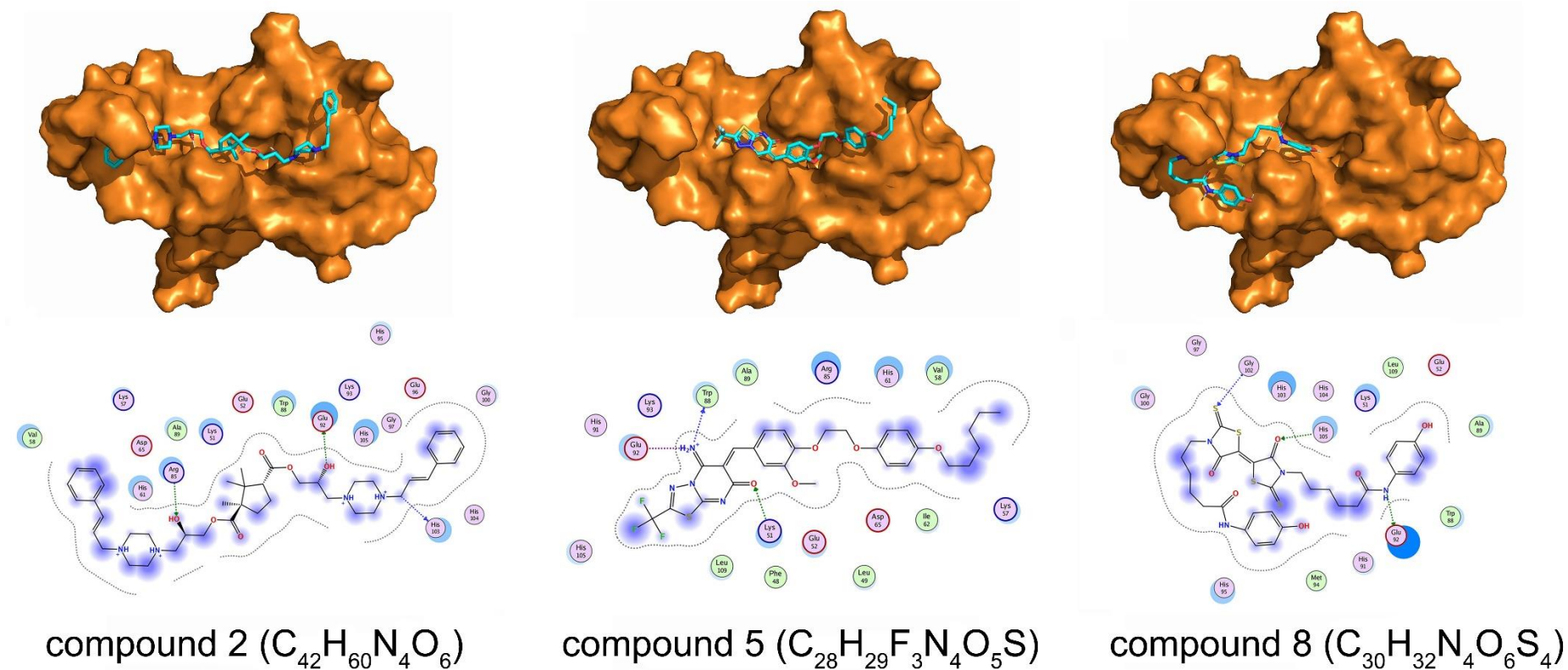
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<p>2641-1586</p>  <p>S:-7.3775</p>	<p>2897-0360</p>  <p>S:-7.3720</p>	<p>0102-0089</p>  <p>S:-7.3577</p>	<p>2425-3824</p>  <p>S:-7.3517</p>
<p>2958-7523</p>  <p>S:-7.3485</p>	<p>3235-0305</p>  <p>S:-7.3385</p>	<p>3797-0798</p>  <p>S:-7.3286</p>	<p>2030-0099</p>  <p>S:-7.3274</p>
<p>3948-1036</p>  <p>S:-7.3232</p>	<p>3389-1064</p>  <p>S:-7.3223</p>	<p>2958-7463</p>  <p>S:-7.3214</p>	<p>0878-7200</p>  <p>S:-7.3059</p>
<p>3537-5212</p>  <p>S:-7.2984</p>	<p>3948-1140</p>  <p>S:-7.2984</p>	<p>3797-0764</p>  <p>S:-7.2961</p>	<p>2368-0284</p>  <p>S:-7.2832</p>

<p>3537-5171</p>  <p>S:-7.2732</p>	<p>0083-0100</p>  <p>S:-7.2663</p>	<p>2738-1113</p>  <p>S:-7.2640</p>	<p>3202-0313</p>  <p>S:-7.2625</p>
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<p>1804-0074</p>  <p>S:-7.2373</p>	<p>3368-0273</p>  <p>S:-7.2353</p>	<p>1154-0012</p>  <p>S:-7.2337</p>	<p>3448-2506</p>  <p>S:-7.2311</p>
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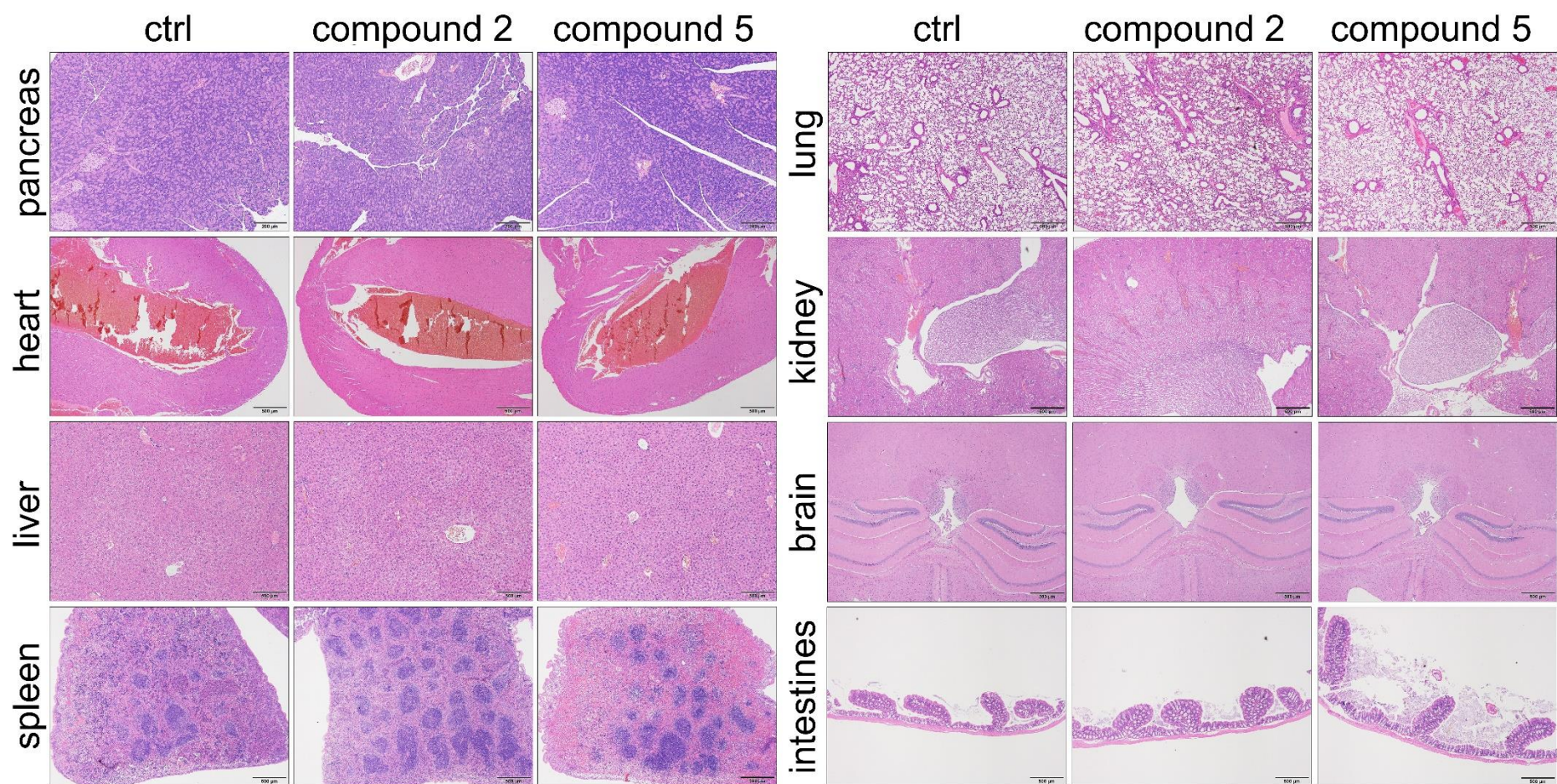
Supplementary Figure S7 Structures and docking scores of the top 100 inhibitors of the S100A9-VNN1 interaction.



Supplementary Figure S8 (A) Toxicities of the top 8 compounds *in vitro* (n = 6). (B) Pharmacodynamics of the top 8 compounds *in vitro* (n = 6). (C) Compounds 2, 5 and 8 decreased STC-induced H6C7 cells injury. Data are presented as the mean \pm SEM; *P < 0.05 and **P < 0.01 vs. ctrl group; #P < 0.05 and ##P < 0.01 vs. STC group.



Supplementary Figure S9 Binding models of $C_{42}H_{60}N_4O_6$, $C_{28}H_{29}F_3N_4O_5S$ and $C_{30}H_{32}N_4O_6S_4$ with S100A9 protein.



Supplementary Figure S10 HE staining results of pancreas, heart, liver, spleen, lung, kidney, brain and intestines also proved that compounds 2 and 5 have no obvious toxicities at the doses of 10 mg/kg/day for 2 days.