Nanoscale

ROYAL SOCIETY OF CHEMISTRY

ADI PEG ADI ff [11,12] PEG ADI. H ADI PEG. A , PEG ADI 50% [10] F , ff PEG ADI, PEG [13] PEG- PEG ADI, ff . N ADI *via* ffi ADI. F ADI ADI ADI. [14] ffi ADI. H

CL-4B
PB .

ADI, I - (ADI),
I - (ADI) (DL ,
BI-90P , B I L , A),
(EM, JEM-2100F, JEOL L , J),
2%
F -IR ADI,
I - (ADI), I - (ADI) KB
F IR (N I 10,
A). N ADI, I - (ADI), I - (ADI)
(0.2 L^{-1})
(9, P I , C). C
(CD)
(M J-810, J , , J). N ADI, I - (ADI),
I - (ADI) 0.2 L^{-1}.
M
250–190 .

2.3 Specific activity test of WIT-n(ADI)

ADI, I - (ADI), I -
(ADI) . B , 0.1
L^{-1} ADI, I - (ADI), I - (ADI)
50 M 37 C 10 . A
. O
1.0 μ
I - (ADI), ADI,
I - (ADI), I - (ADI) . (
1 : 1) 4 C 37 C ,
ADI, I - (ADI),
I - (ADI)
60 .

2.4 The hydrophilicity determination of WIT-n(ADI)

ADI, I - (ADI),
I - (ADI) L
L A 60 . (1 L^{-1})
N_2
. F ,

2.5 The cellular uptake of WIT-n(ADI)

H (H EC),
-22 (H22) , RA 264.7
( )
1×10^4
37 C , 24 .
ADI, I - (ADI),
I - (ADI) B (200 L^{-1}).
A 24 , PB
(4%) 10 . F
PB ,
FI C- DAPI O
I 81 .

H EC , H22 , RA 264.7
1×10^5
B- ADI, I -
(ADI), I - (ADI) (200 L^{-1}) 24 , ,
, (FAC)
B- . F ,
10 000 B . E

2.6 Cell viability assay

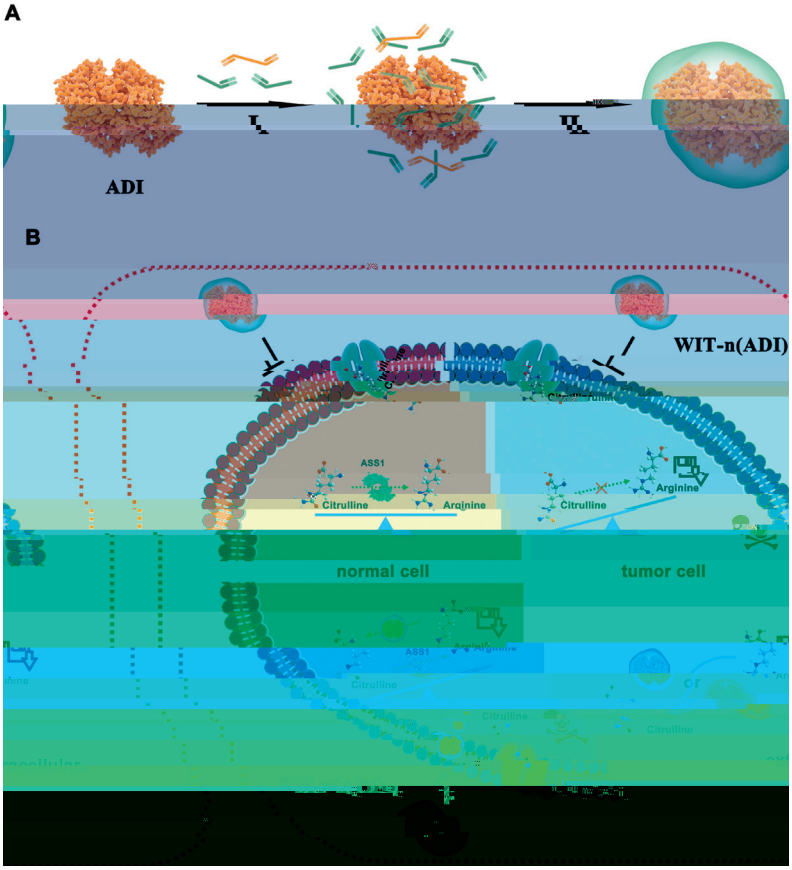
C ff ADI, I - (ADI), I -
(ADI) CCK-8 . F , H EC ,
H22 , RA 264.7 96-
4000
37 C 24 . ,
ff
ADI, I - (ADI), I - (ADI). A
48 , CCK ,
(450)

2.7 Test of the intracellular ratio of citrulline to arginine

H EC , H22 , RA 264.7
ff ADI,
I - (ADI), I - (ADI). A 24 , ,
PB . D
14 000
30 ,

2.8 Blood circulation of WIT-n(ADI)

I - (ADI),
K (
ADI, I - (ADI), I - (ADI)) 6 .
B- ADI, I - (ADI),
I - (ADI) *via*
ADI ~1 . A
A
E Affi H Q
C A
E . A ,



Scheme 1 Preparation and therapeutic mechanism of WIT-n(ADI). (A) Preparation of ADI nanocapsules. Step I: monomers and crosslinkers were enriched around individual ADI *via* electrostatic and hydrogen bond interactions; step II: free radical polymerization was initiated to form a thin polymeric shell around ADI. AAM and MPC were respectively used as monomers to prepare SIT-n(ADI) and WIT-n(ADI). (B) Therapeutic mechanism of WIT-n(ADI). WIT-n(ADI) could catalyze extracellular arginine into citrulline and indirectly reduce the intracellular arginine concentration of ASS1-deficient tumor cells, efficiently suppressing their growth. Furthermore, WIT-n(ADI) could not be taken up by normal cells, leading to their negligible influence on the intracellular arginine concentration and the undetectable side effects. In contrast, SIT-n(ADI) could be efficiently taken up by normal cells, resulting in the imbalance of intracellular arginine and citrulline and the consequent toxicity.

. H , PC I - (ADI) ~80%, I - (ADI) ADI (F . 1†) EM[25] I - (ADI) I - (ADI) (F . 1A B) ADI . C I - (ADI), I - (ADI) . C EM (DL) (F . 1C) I - (ADI) 27 3 (PDI ~0.112), I - (ADI) 28 10 (PDI ~0.357). I - (ADI) I - (ADI), I - (ADI) ff PMPC.[26] A , ADI −10.46 . I - (ADI) I - (ADI) 0 ff AAM MPC- (F . 1D). I - (ADI) F ,[27] I - (ADI) I - (ADI). F -IR I - (ADI) 1710, 1240, 1090, 970 $^{-1}$ (F . 1E), MPC-[28] I - (ADI) 1617, 1560, 1350, 1119 $^{-1}$ AAM- ADI.[29] ff ADI. D ff ADI, I - (ADI) I - (ADI)

280 (F . 1F).
ADI I - (ADI) I -
(ADI) , (CD) (F . 2†). I -
ADI .

3.2 Activity maintenance of WIT-n(ADI)

ADI
. I - (ADI) I - (ADI)
ffi ff ADI
(F . 2A),
ADI. C
PEG , , ADI.
ADI ,
. N ADI, I - (ADI),
I - (ADI)
(1 : 1) 4 C 37 C. A
F . 2B, ADI 20

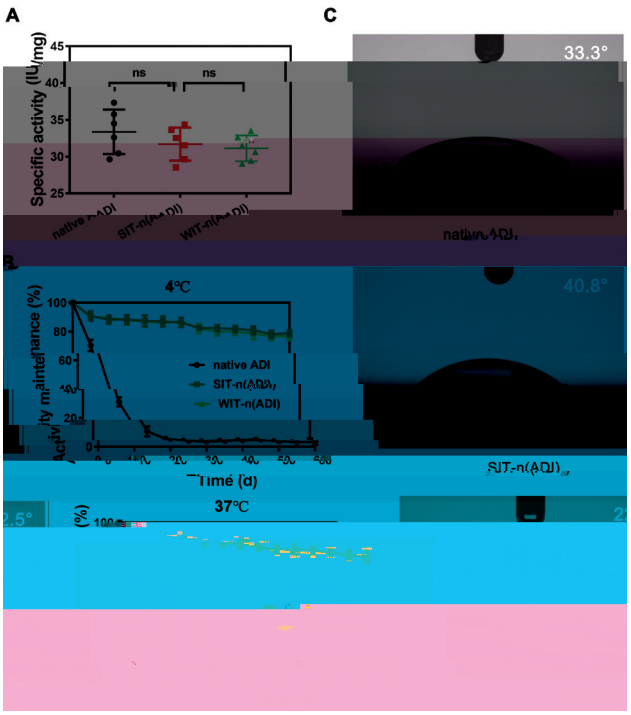


Fig. 2 Activity maintenance of WIT-n(ADI). (A) The specific activity measurement of native ADI, SIT-n(ADI), and WIT-n(ADI). The significance level is shown as $^{ns}p > 0.05$. (B) After incubation with mouse serum (volume ratio was 1 : 1), native ADI, SIT-n(ADI), and WIT-n(ADI) wtakADItakADparti 6, andction

ffiς I - (ADI). ff MPC AAM, [23] F ff FI C FI C- B- (F . 3A,†). ffiς (F . 3B†). ffiς ADI, I - (ADI), I - (ADI). H EC 2.20%, 13.93%, 4.32%, ADI, I - (ADI), I - (ADI). H22 15.52%, 66.54%, 13.19%. RA 264.7 I - (ADI). ffiς I - (ADI). RA 264.7 6.74%, ADI I - (ADI) 34.72% 77.85%. I - (ADI) ffiς PC- . H PC ffiς . O

PC ffiς [30,31] O , PC- ffiς [24,32] I PC ff . A PC PC- PC [33] PC [34] A PC PC 58.3%, ffiς PC 80%, ffiς I - (ADI) I - (ADI). M ff - PC- ff . F , PC- ff ff ffiς [35] I - (ADI) I - (ADI) . A F . 3A, ( ) I - (ADI) I - (ADI) PB . H PB I - (ADI) I - (ADI) ff I - (ADI) . O I - (ADI) ff I - (ADI) PB ffiς I - (ADI) . H EC , H22 , RA 264.7 18.26%, 28.13%, 34.65% (F . 3B). I ffiς I - (ADI) . PB - H EC , H22 , RA 264.7 0.77%, 0.94%, 1.29%, I - (ADI) . I [36] I - (ADI) I - (ADI). I I - (ADI) . H EC ff I - (ADI) . I (EPR ff). I

I - (ADI) c cc , c
c c c c c c
c , c I - (ADI) ff c
c ff c .

3.4 Cell viability and the intracellular ratio of citrulline to arginine

c H ﹣ EC c , H22 c , RA 264.7
c c . F . 4A c
H ﹣ EC c ff c c .
A c c (<30 L^{-1}), ADI, I - (ADI),
I - (ADI) . c c H ﹣ EC.
c c c ,
, , c H ﹣ EC c . I -
(ADI) c . A 100 L^{-1}, c
H ﹣ EC c ~65%. c c
c c , . c
c H ﹣ EC c c I - (ADI) c -
c . O c , ADI I - (ADI) c
H ﹣ EC c c -
c c , c -
c . D ff H ﹣ EC c , H22 c
A 1 c c c c .
H c , c
c ADI, I - (ADI),

RA 264.7 I - (ADI) ffi A 1. I ff A 1-

ADI ff [37] ADI I - (ADI) ff H EC ADI.[37] A ADI, . I , A 1- H22 ADI ffi I ADI

I - (ADI) H EC ADI I - (ADI) ADI A 1-

3.5 *In vivo* activity and immune response of WIT-n(ADI)

in vivo ADI, I - (ADI), I - (ADI) I - (ADI) . A F . 5A, ADI I - (ADI) ~2 . ~8 . , I - (ADI) ~90 . , 240 . , ~25% I - (ADI) . F . 5B 0.55 0.17, ADI, I - (ADI), I - (ADI). I ADI I - (ADI) . A 96 . , ADI I - (ADI) 144 . . I - (ADI)

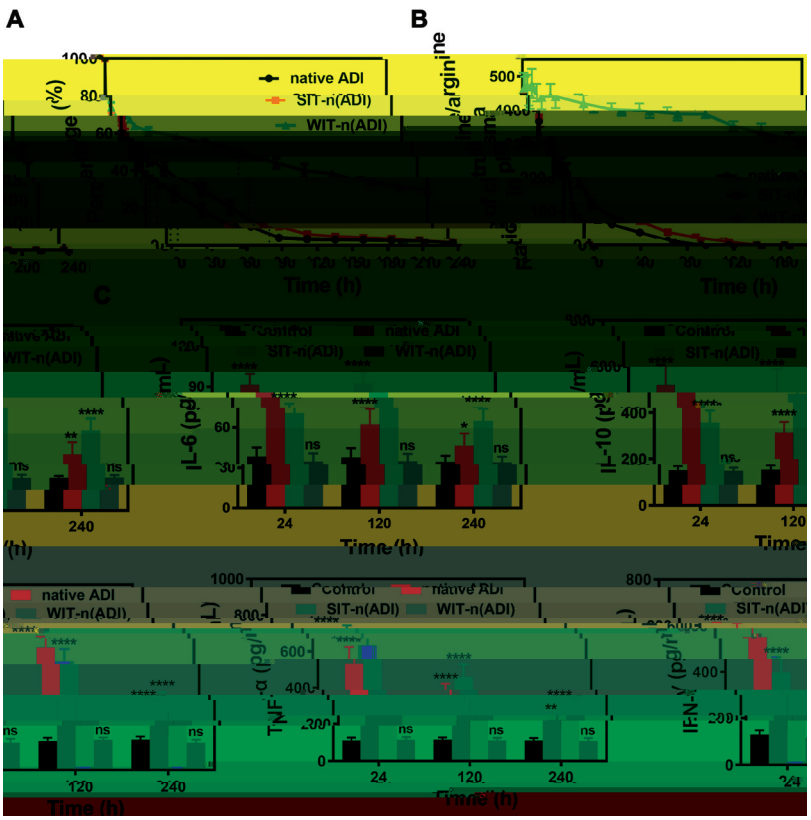


Fig. 5 *In vivo* activity measurement. (A) Circulation time of native ADI, SIT-n(ADI), and WIT-n(ADI). ADI was labeled with rhodamine B. (B) Ratio of citrulline to arginine in the plasma of mice, which were injected with native ADI, SIT-n(ADI), and WIT-n(ADI). The ADI equivalent was ~1 mg. (C) Cytokine levels in mouse blood after different times of sample administration. Significantly different from the control group, $^{ns}p > 0.05$, $*p < 0.05$, $**p < 0.01$, and $****p < 0.001$.

. A 240 . ,
I - (ADI) 280.
F
. A F . 4,† I - (ADI)
I - (ADI)
I - (ADI) -
I - (ADI) -
A 1- ,
in vivo I - (ADI) .

3.7 Tumor suppression by a single injection of WIT-n(ADI)

ff c I - (ADI) H22- . A 4 , - c c ADI, I - (ADI), I - (ADI) c . c ff (F . 7†), c ff c . c ADI I - (ADI), c c

ff c . c , c 3 c c (2)/2 . c . PB c 13 (F . 7A). N ADI c , c ADI c 22 . I - (ADI) c c I - (ADI), c ADI 13 . H ,

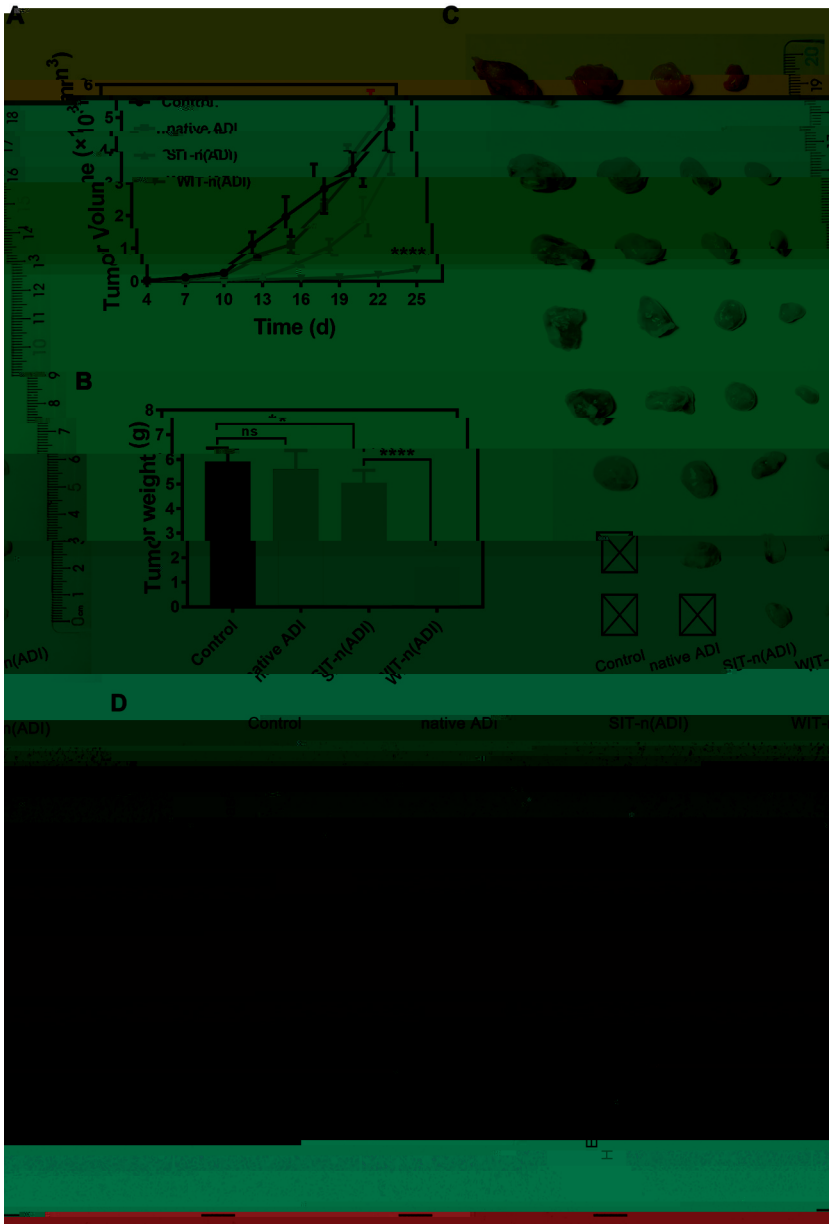


Fig. 7 Tumor suppression. (A) Growth evaluation of H22 subcutaneous tumor in Kunming mice after sample administration. Tumor volume was examined every 3 days for 21 consecutive days. (B) Average mass of collected tumor tissues. (C) H22 tumor tissues obtained from euthanized mice after 21 days of sample administration and a rectangle represented the dead mice. (D) Immunohistochemistry analyses of the expression of caspase-3 and Bcl-2 in each group. Nuclei were stained blue, and the proteins were stained brown. The bar was 200 μm. (E) H&E staining and significance levels are shown as $^{ns}p > 0.05$, $^{*}p < 0.05$, and $^{****}p < 0.001$.

13 I - (ADI). I , I - (ADI) - I - (ADI) 25 PB , ADI, I - (ADI), I - (ADI) 25 ~5.92 , ~5.61 , ~5.04, ~1.62 , (F . 7B). F . 7C - 25, I - (ADI) ffi I - (ADI). I - .[38] H , -3 B -2 (F . 7D). I - (ADI) H22 B -2 -3 . H&E (F . 7E). I - (ADI) A 1-

3.8 Biological safety of WIT-n(ADI)

ff (, , , , ,)

H&E (F . 8A). I ADI I - (ADI) . I ADI I - (ADI) , (F . 8B). (P), (AL), (A), (ALP) ADI I - (ADI) I - (ADI) , PEG ADI ADI, . A , ADI-PEG20 H , PEG ADI . F I/II ADI-PEG20 , 12 (43%) . 3 , 3 (8.5%) . 4 .[39] ADI-PEG20. , . PEG ADI, I - (ADI) I

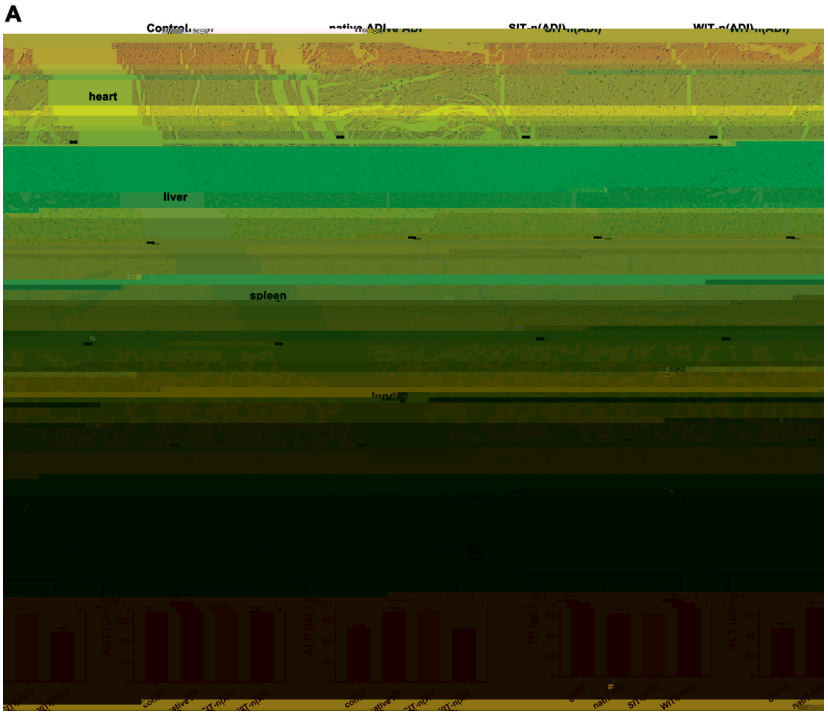


Fig. 8 Biological safety. (A) Histological sections of organs stained with H&E, and the bar was 20 μm. (B) Serum levels of total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in mouse blood of different groups. The significance level is shown as ****$p < 0.001$.

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