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Supporting Information

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Multi-Arm PEG/Peptidomimetic Conjugate Inhibitors of DR6/APP Interaction Block Hematogenous Tumor Cell Extravasation

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Table S1. Amino acid sequences of twenty-four peptides and the corresponding K_D values. [a]

ID	Sequence	$K_D \pm SEM$[b] (nM)
AHP-01	WTLSINS	1153.7 ± 7.94
AHP-02	FQFRLQS	NB[c]
AHP-03	YLPAPLP	688.5 ± 11.20
AHP-04	ANLLYES	NB
AHP-05	YYSGNNL	1096 ± 10.04
AHP-06	KLGPVCP	NB
AHP-07	YSLHASP	NB
AHP-08	HSTAPQL	NB
AHP-09	KLVPEPS	NB
AHP-10	NGSTHER	527.8 ± 12.09
AHP-11	GFSHYFP	NB
AHP-12	TIDATTP	321.8 ± 11.24
AHP-13	SHTDTNQ	1153.7 ± 7.94
AHP-14	AFMYSLP	457.3 ± 11.56
AHP-15	TIPKYER	NB
AHP-16	KFLQVTN	NB
AHP-17	HSYPYTQ	NB
AHP-18	TTYMPKM	NB
AHP-19	MPPFRHD	NB
AHP-20	LPRSPLA	NB
AHP-21	AGHFSLW	NB
AHP-22	LMTTKSD	NB
AHP-23	GGLSMTR	4458.8 ± 5.85
AHP-24	NHQRLLP	1093.5 ± 262

[a] K_D values of peptides to DR6 were assessed as described in the Materials and Methods.

[b] K_D values shown are the average of three independent experiments in triplicate.

[c] NB = No binding.

Table S2. Amino acid sequences of D-peptides and DRI-peptides and the corresponding K_D values.

ID	Sequence	$K_D \pm$ SEM (nM)
AHP-D-03	D-(YLPAPLP)	4734.0 ± 8.80
AHP-D-10	D-(NGSTHER)	27110.0 ± 47383
AHP-D-12	D-(TIDATTP)	55130.0 ± 27585
AHP-D-13	D-(SHTDTNQ)	3174 ± 8.80
AHP-DRI-03	D-(PLPAPLY)	1219 ± 7.02
AHP-DRI-10	D-(REHTSGN)	17810 ± 37081
AHP-DRI-12	D-(PTTADIT)	225.19 ± 4.30
AHP-DRI-13	D-(QNTDTHS)	862.7 ± 6.59

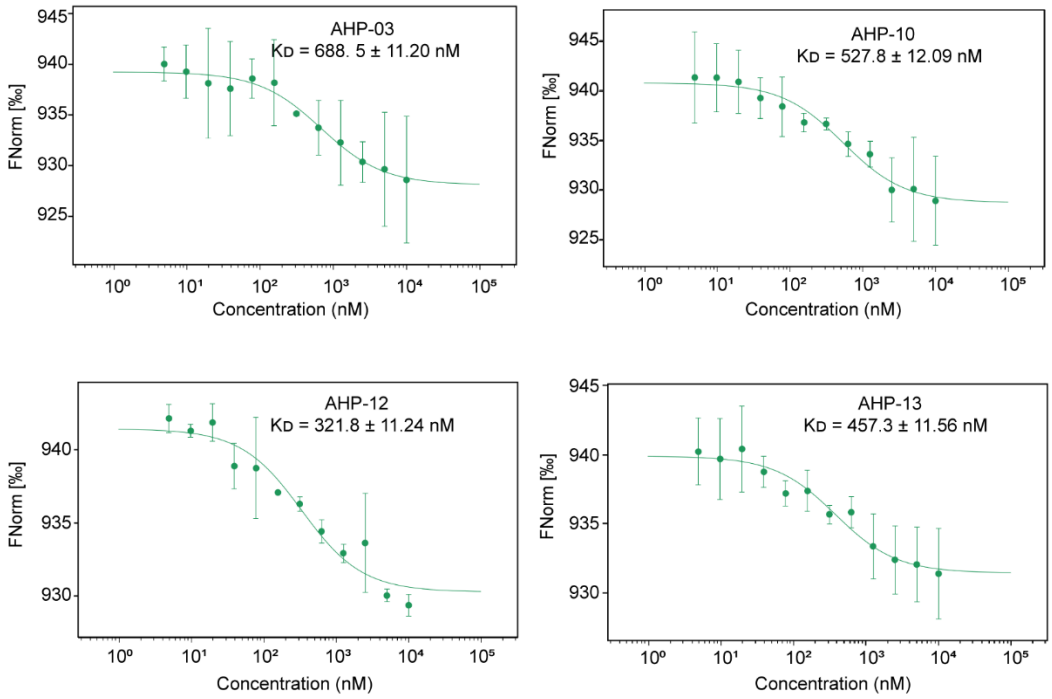
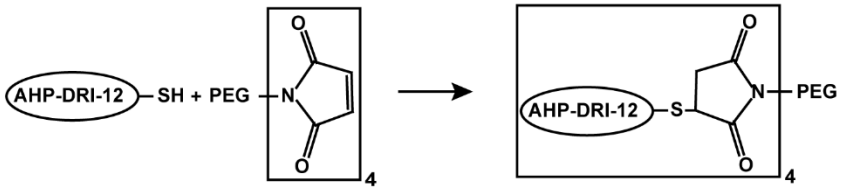
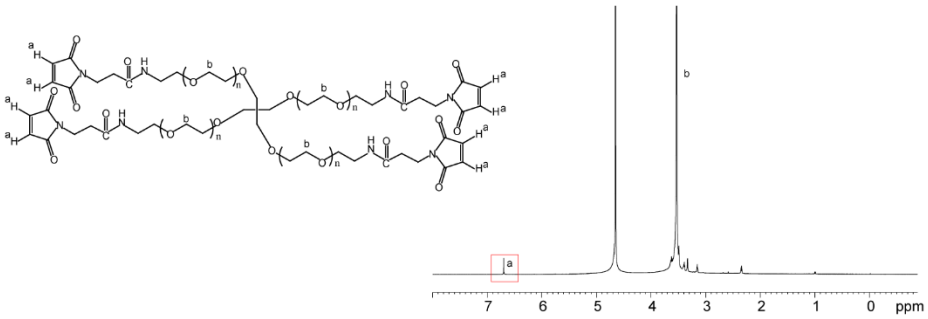


Figure S1. The binding curve of AHP-03, AHP-10, AHP-12 and AHP-13 to DR6 protein were measured by MST, respectively.

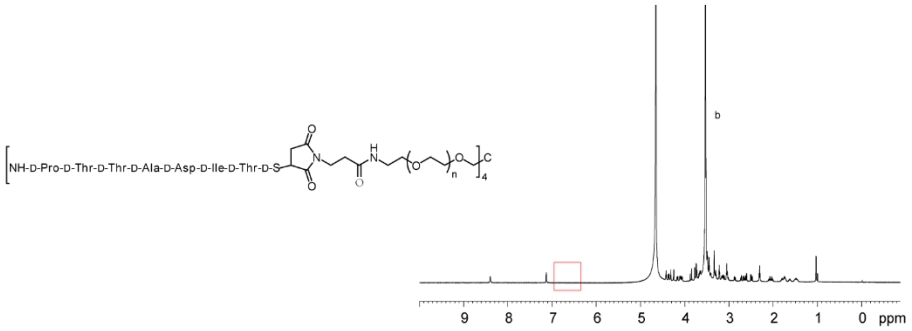
A


AHP-DRI-12
SH + PEG
4
AHP-DRI-12
S
N
PEG
4

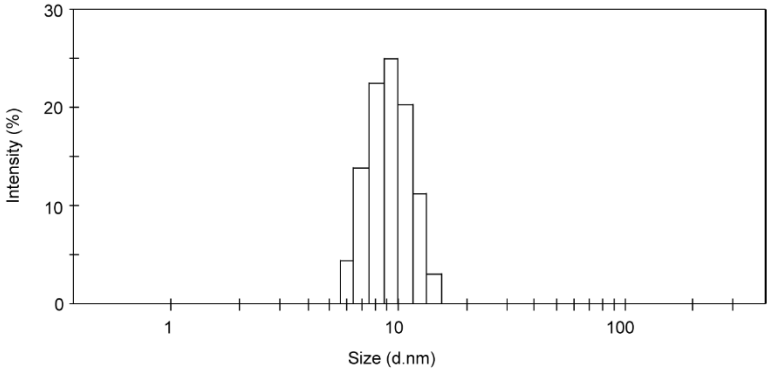
B


a
b
ppm

C


NH-D-Pro-D-Thr-D-Thr-D-Ala-D-Asp-D-Ile-D-Thr-D-S
b
ppm

D


Intensity (%)
Size (d.nm)

E

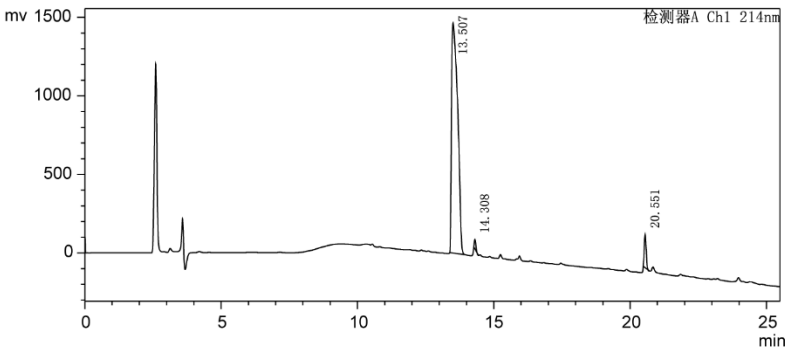
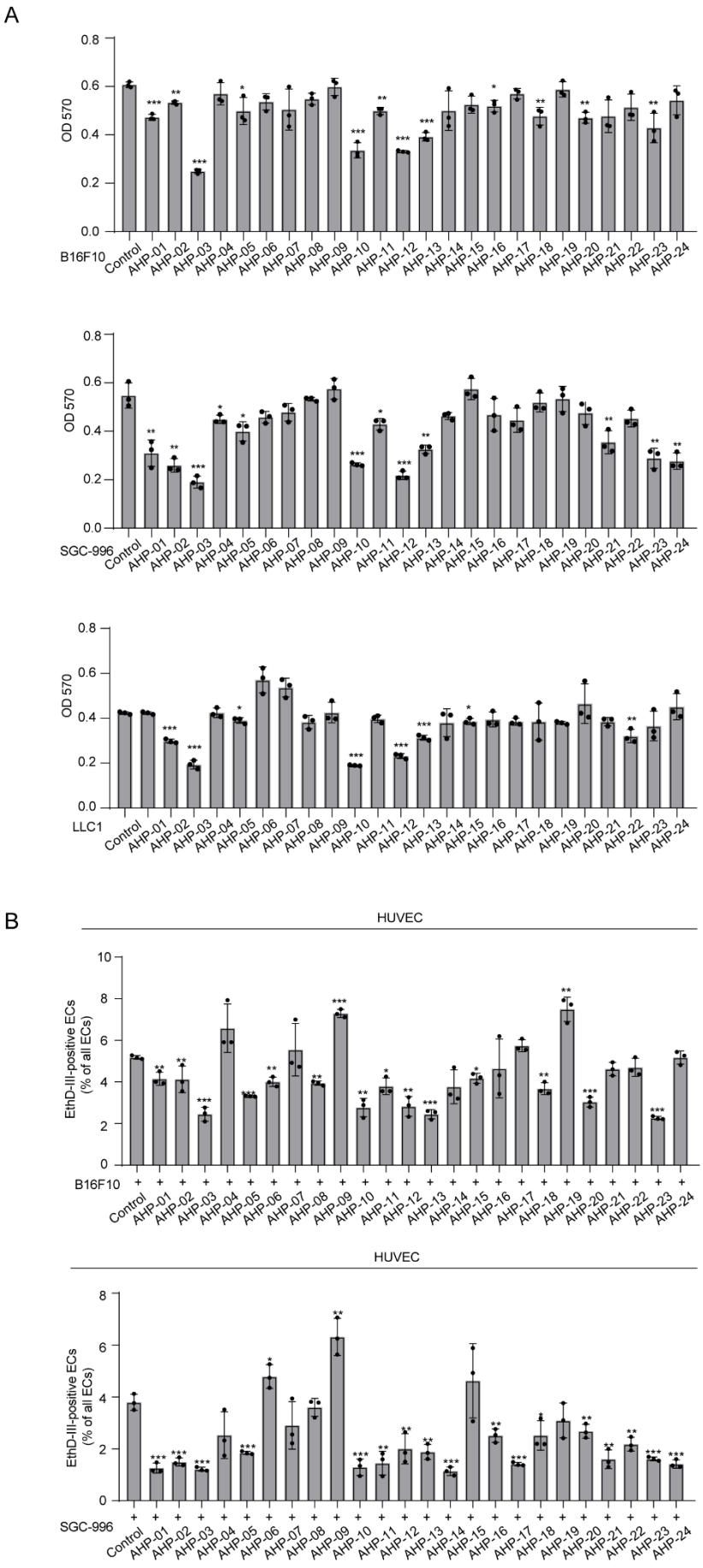

mv
检测器A Ch1 214nm
13.507
14.308
20.551
min

Figure S2. Synthesis of PEG-tAHP-DRI. A) Schematic presentation of PEG-tAHP-DRI. **B)** ^{1}H NMR spectra of 4-arm-PEG_{5k} in D_2O. **C)** ^{1}H NMR spectra of PEG-tAHP-DRI in D_2O. **D)** Size distribution of PEG-tAHP-DRI. **E)** HPLC curve of PEG-tAHP-DRI.


A
OD 570
B16F10
SGC-996
LLC1
Control
AHP-01
AHP-02
AHP-03
AHP-04
AHP-05
AHP-06
AHP-07
AHP-08
AHP-09
AHP-10
AHP-11
AHP-12
AHP-13
AHP-14
AHP-15
AHP-16
AHP-17
AHP-18
AHP-19
AHP-20
AHP-21
AHP-22
AHP-23
AHP-24
B
HUVEC
EthD-III-positive ECs (% of all ECs)
B16F10
SGC-996

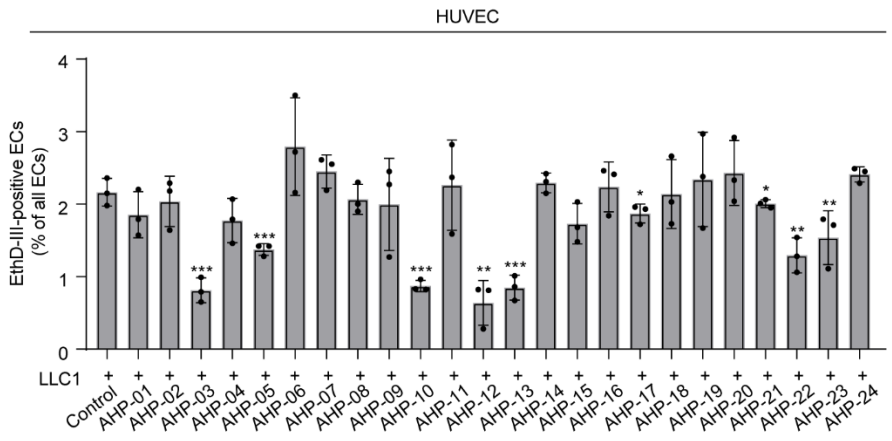
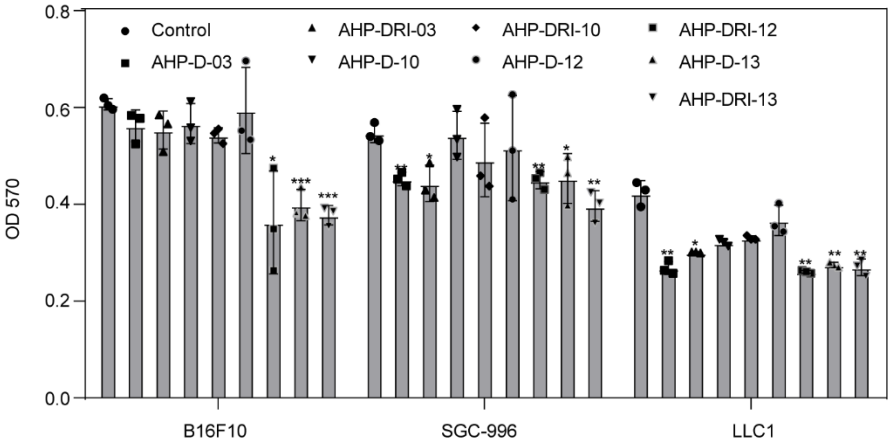
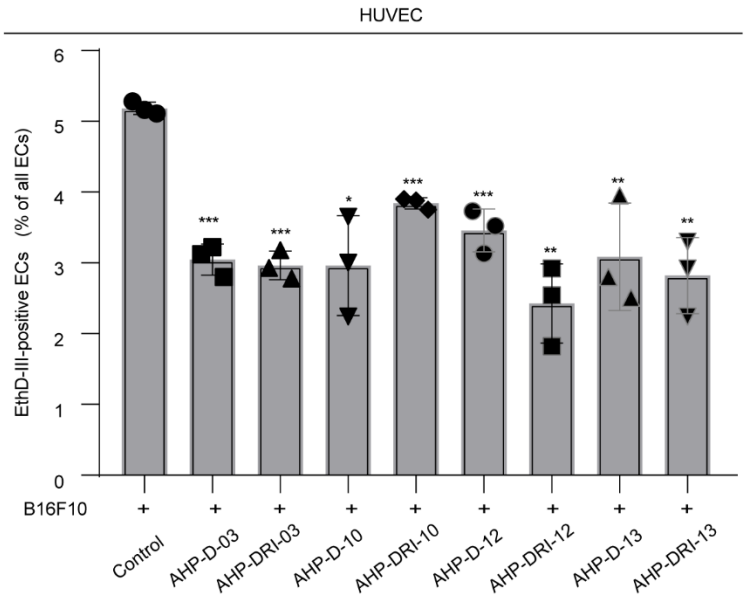


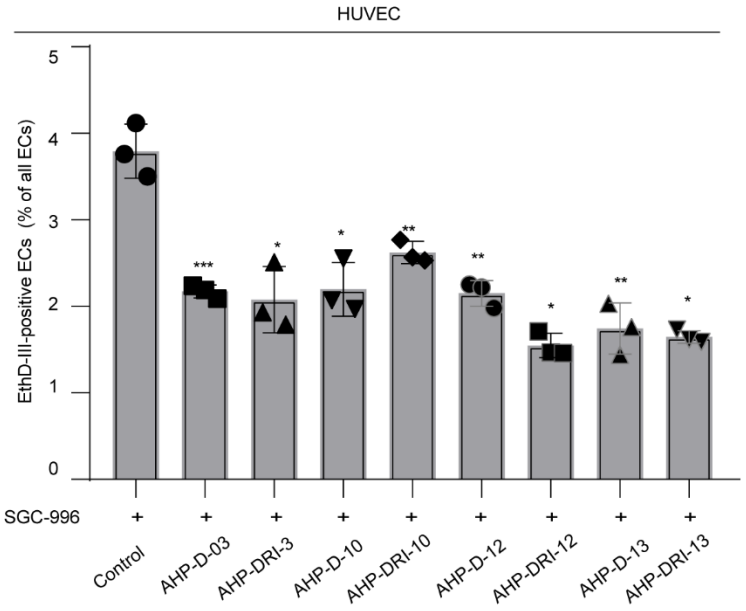
Figure S3. Screening of twenty-four DR6-targeting peptides. A) Cell attachment of different TCs to culture plate coated with DR6 protein after treated twenty-four peptides. **B)** HUVECs necroptosis when co-cultured with different TCs after treated with twenty-four peptides. Data are means ± SD (n = 3), and analyzed with GraphPad Prism 8.0. *P < 0.05, **P < 0.01, ***P < 0.005 calculated by unpaired two-tailed Student's t-test. NS indicates P > 0.05.

A


Control
AHP-D-03
AHP-DRI-03
AHP-D-10
AHP-DRI-10
AHP-D-12
AHP-DRI-12
AHP-D-13
AHP-DRI-13
OD 570
0.0
0.2
0.4
0.6
0.8
B16F10
SGC-996
LLC1

B


HUVEC
EthD-III-positive ECs (% of all ECs)
0
1
2
3
4
5
6
B16F10
Control
AHP-D-03
AHP-DRI-03
AHP-D-10
AHP-DRI-10
AHP-D-12
AHP-DRI-12
AHP-D-13
AHP-DRI-13


HUVEC
EthD-III-positive ECs (% of all ECs)
0
1
2
3
4
5
SGC-996
Control
AHP-D-03
AHP-DRI-3
AHP-D-10
AHP-DRI-10
AHP-D-12
AHP-DRI-12
AHP-D-13
AHP-DRI-13

C

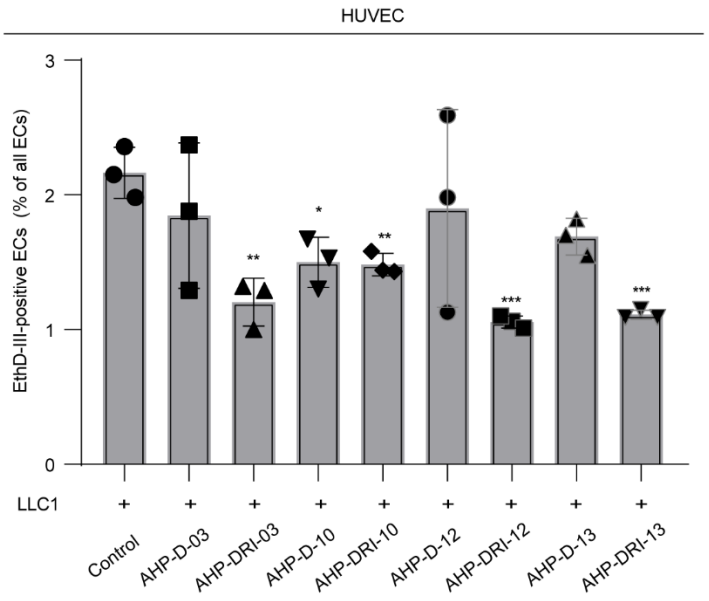


Figure S4. Screening of D-peptides and DRI-peptides. A) Cell attachment of different TCs to culture plate coated with DR6 protein after treated with eight peptides. **B)** HUVECs necroptosis when co-cultured with different TCs after treated with eight peptides. Data are means ± SD (n = 3), and analyzed with GraphPad Prism 8.0. *P < 0.05, **P < 0.01, ***P < 0.005 calculated by unpaired two-tailed Student's t-test. NS indicates P > 0.05.

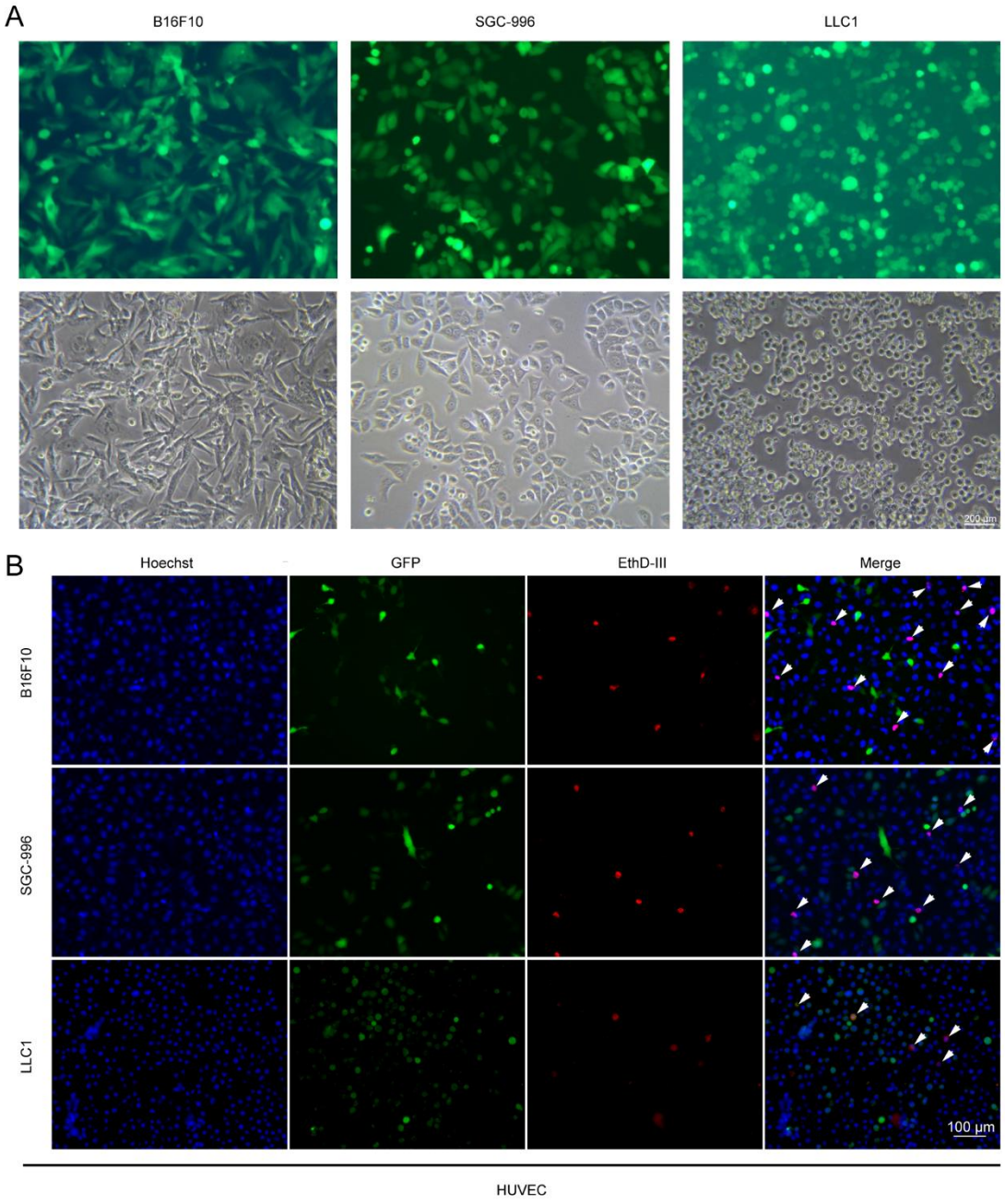


Figure S5. A) B16F10, SGC-996, LLC1 cells stably expressed GFP. **B) F**luorescent images of HUVECs cultured in the presence of GFP-TCs (green) and stained as indicated. Arrowheads, EthD-III-positive HUVECs (red).

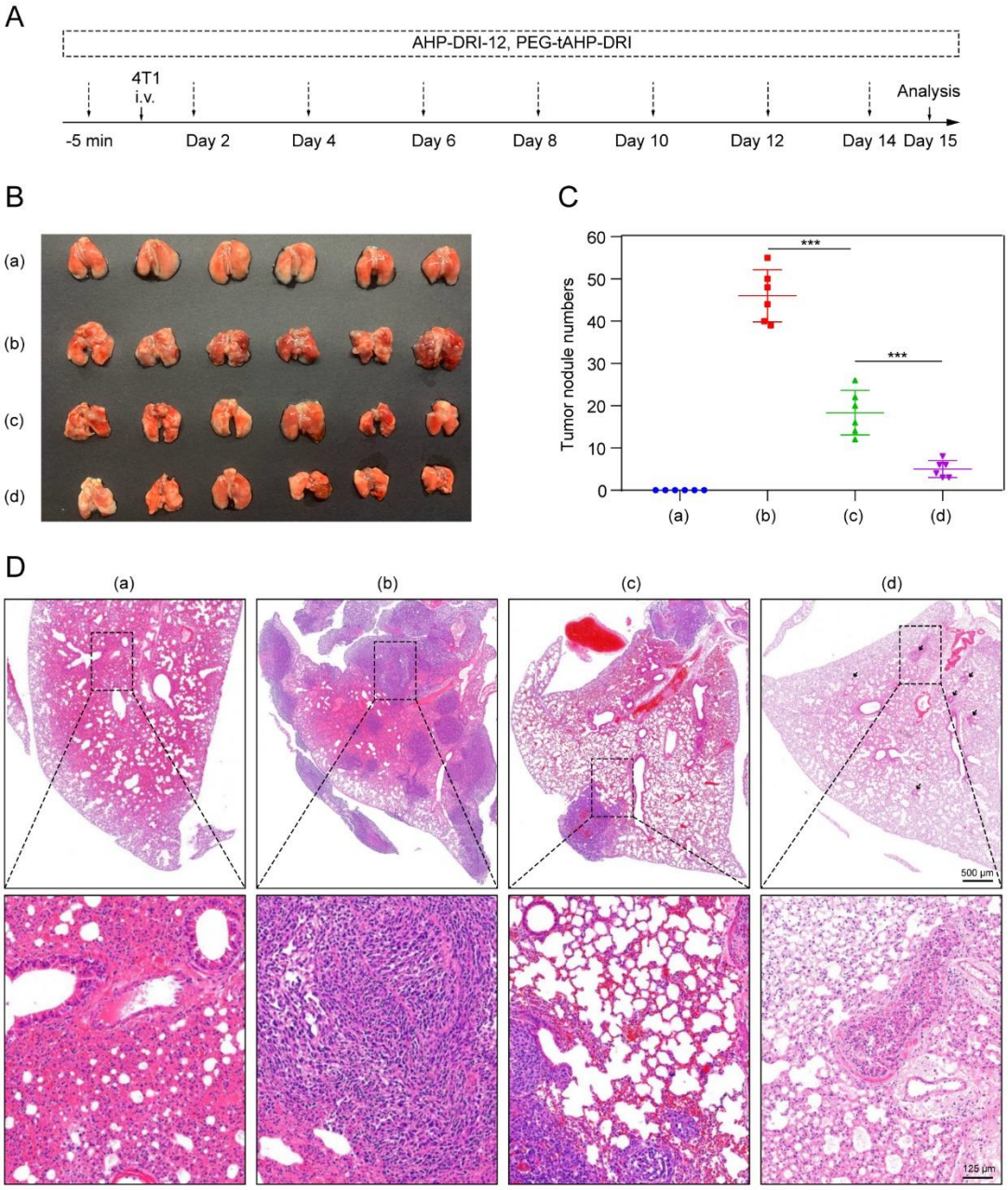


Figure S6. A) Experimental design. **B**) Photographs of the lungs 15 days after i.v. injection of 4T1. (a) saline, (b) 4T1 + saline (vehicle), (c) 4T1 + AHP-DRI-12, (d) 4T1 + PEG-tAHP-DRI. **C**) Metastasis nodules of lungs were quantified. **D**) H&E staining of lung sections. Data are means ± SD (n = 6), and analyzed with GraphPad Prism 8.0. *P < 0.05, **P < 0.01, ***P < 0.005 calculated by unpaired two-tailed Student's t-test. NS indicates P > 0.05.

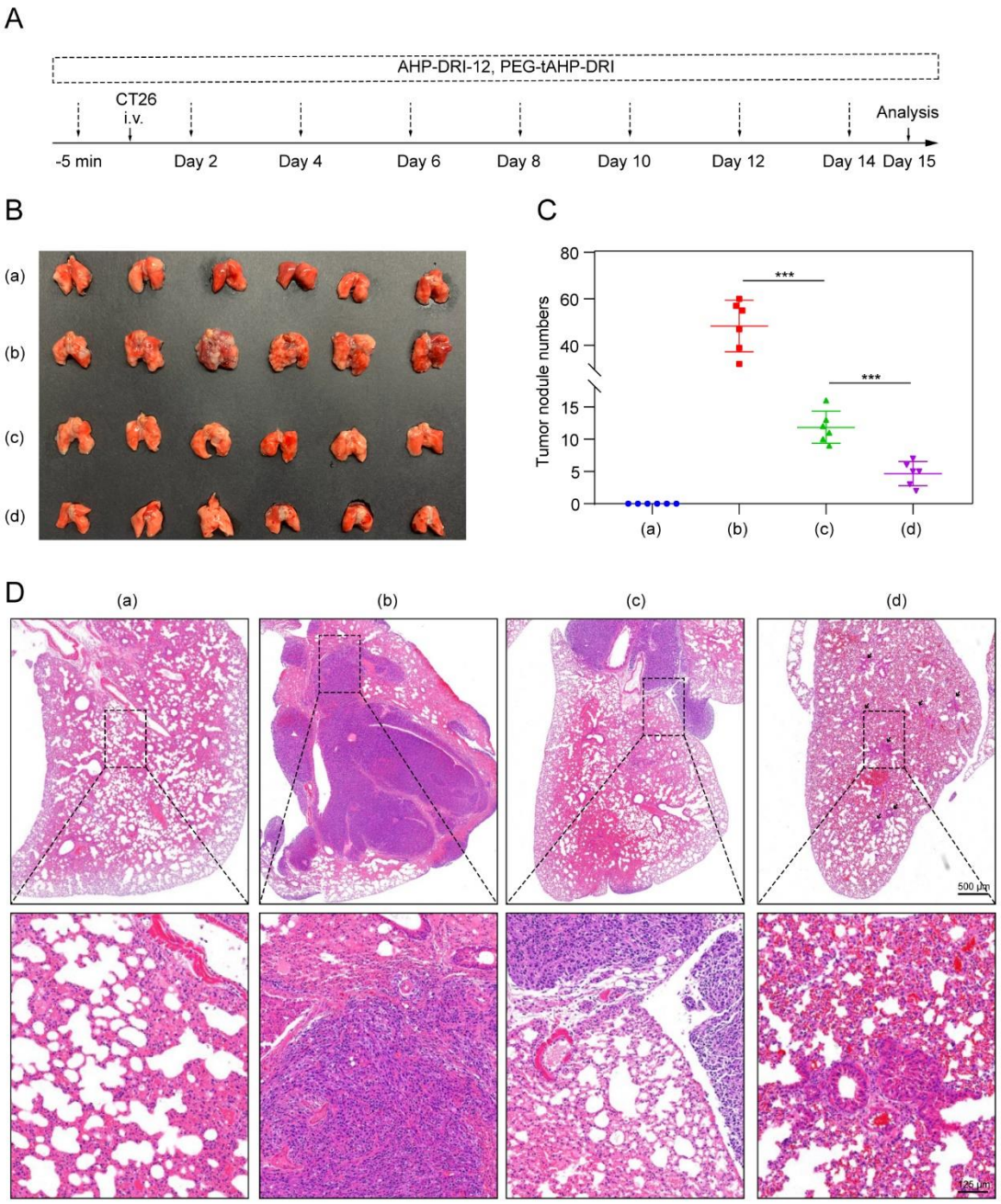


Figure S7. A) Experimental design. **B)** Photographs of the lungs 15 days after i.v. injection of CT26. (a) saline, (b) CT26 + saline (vehicle), (c) CT26 + AHP-DRI-12, (d) CT26 + PEG-tAHP-DRI. **C)** Metastasis nodules of lungs were quantified. **D)** H&E staining of lung sections. Data are means ± SD (n = 6), and analyzed with GraphPad Prism 8.0. *P < 0.05, **P < 0.01, ***P < 0.005 calculated by unpaired two-tailed Student's t-test. NS indicates P > 0.05.

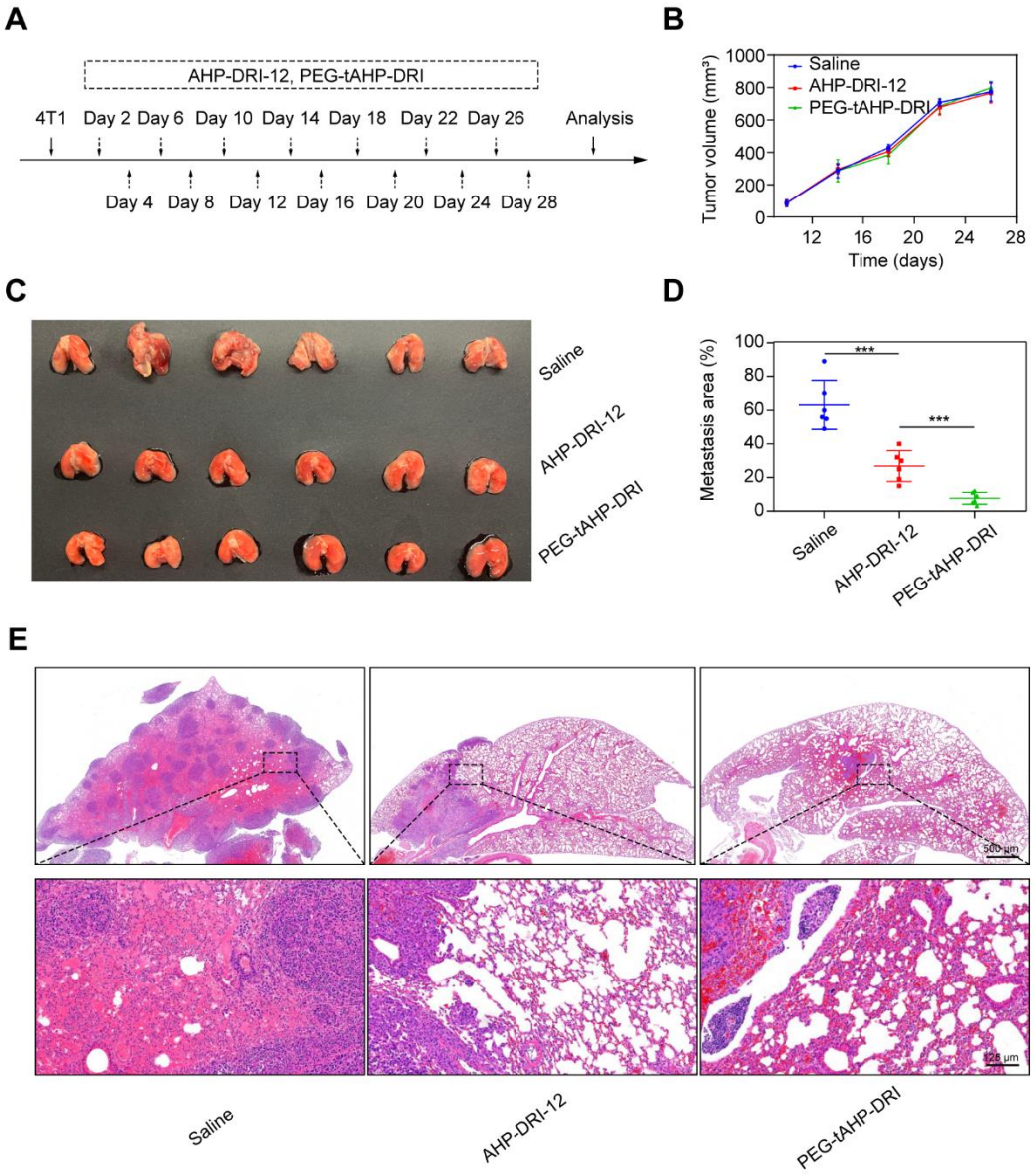
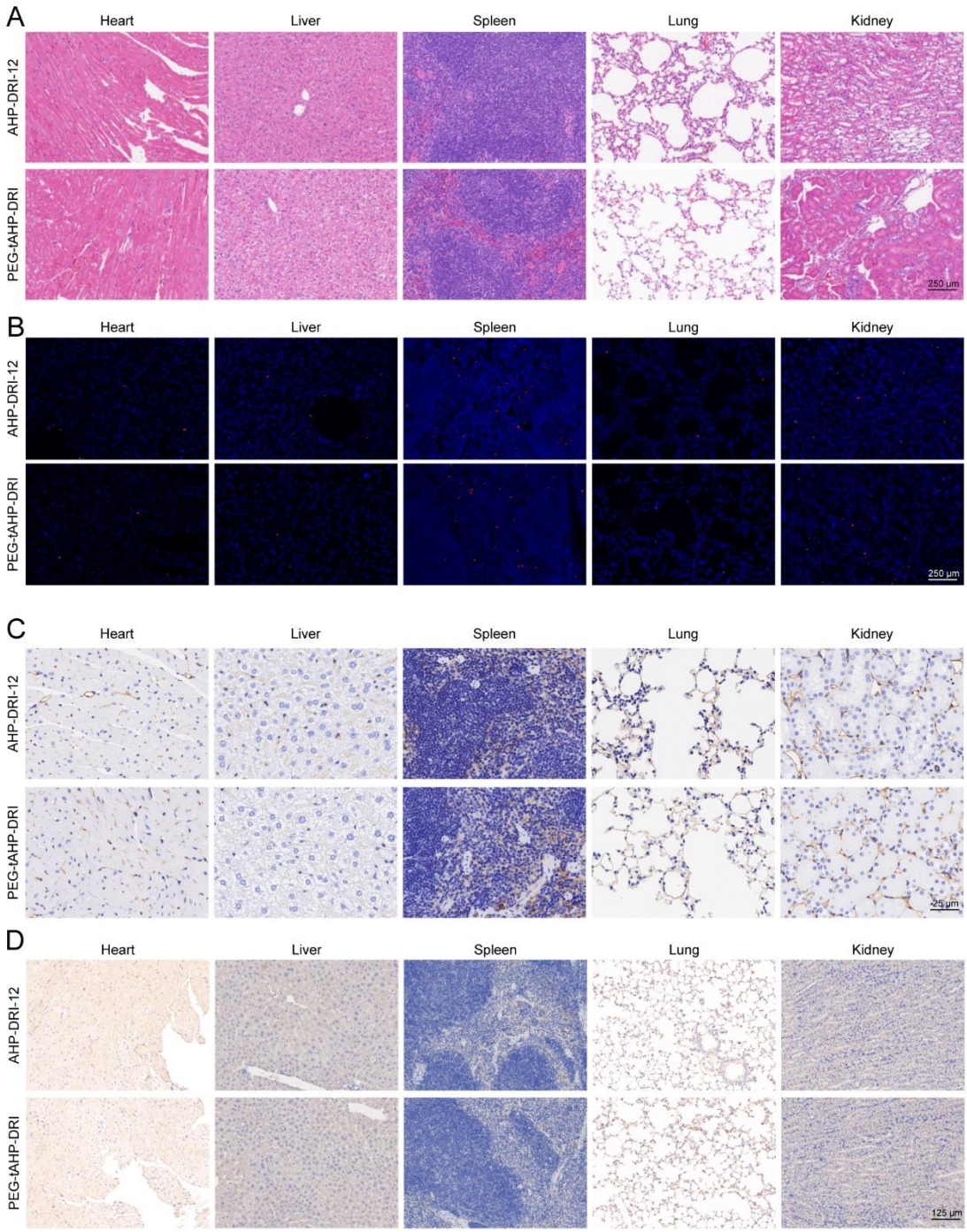


Figure S8. A) Experimental design. **B)** Orthotopic tumor growth of 4T1. **C)** Photographs of the lungs 30 days after 4T1 injection. **D)** Metastasis areas of lungs were quantified. **E)** H&E staining of lung sections. Data are means ± SD (n = 6), and analyzed with GraphPad Prism 8.0. *P < 0.05, **P < 0.01, ***P < 0.005 calculated by unpaired two-tailed Student's t-test. NS indicates P > 0.05.


A
Heart
Liver
Spleen
Lung
Kidney
AHP-DRI-12
PEG-tAHP-DRI
250 μm
B
Heart
Liver
Spleen
Lung
Kidney
AHP-DRI-12
PEG-tAHP-DRI
250 μm
C
Heart
Liver
Spleen
Lung
Kidney
AHP-DRI-12
PEG-tAHP-DRI
25 μm
D
Heart
Liver
Spleen
Lung
Kidney
AHP-DRI-12
PEG-tAHP-DRI
125 μm

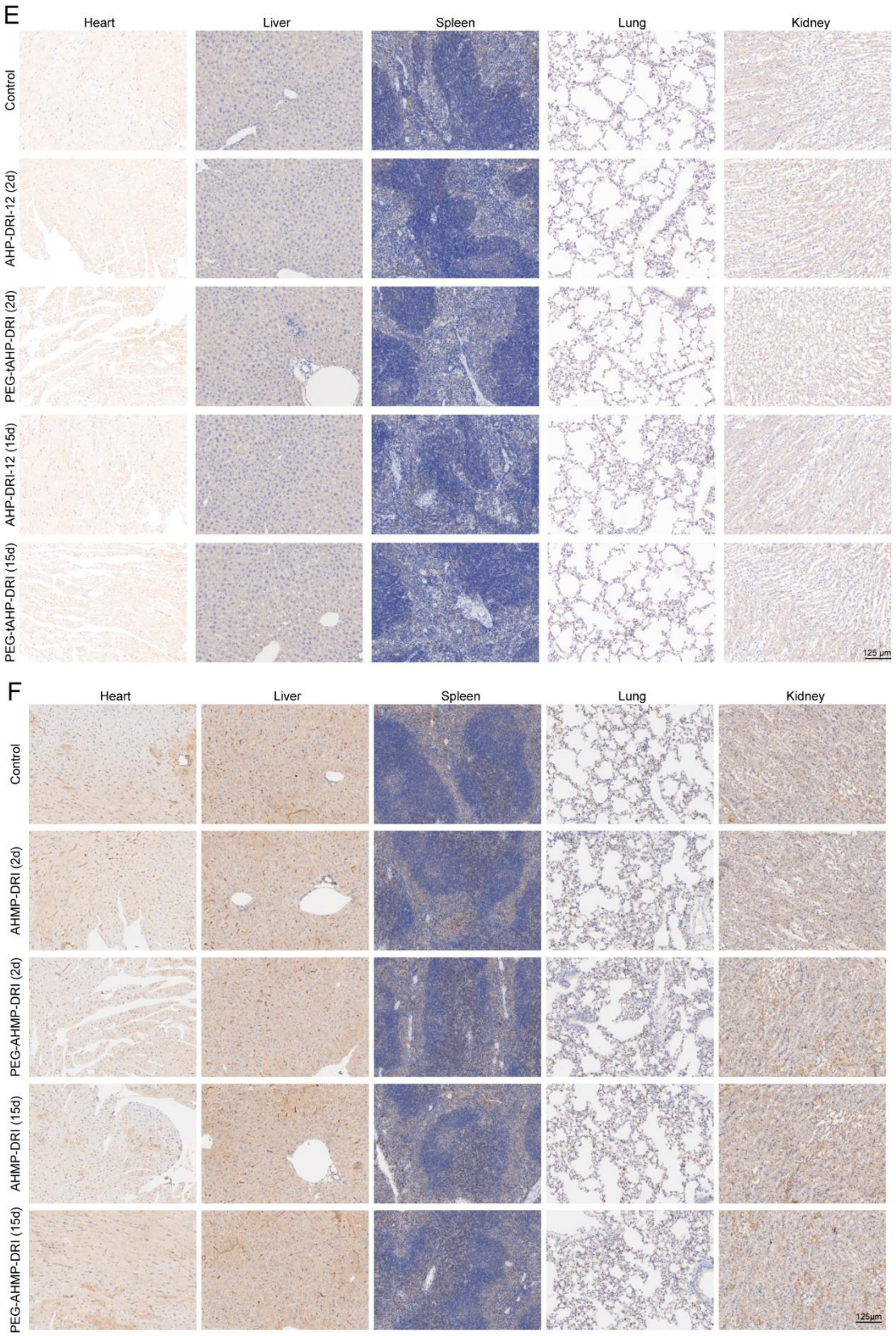


Figure S9. A, **B)** Tissue sections (heart, liver, spleen, lung and kidney) of healthy mice 2 days after injected with AHP-DRI-12 or PEG-tAHP-DRI were stained with H&E (**A**) and Tunel (**B**). **C, D)** Tissue

sections (heart, liver, spleen, lung and kidney) of healthy mice 2 days after injected with AHP-DRI-12 or PEG-tAHP-DRI were stained with CD31 **(C)** and p-RIPK1 **(D)**. **E**, **F)** Tissue sections (heart, liver, spleen, lung and kidney) of healthy mice 2 days and 15 days after injected with AHP-DRI-12 or PEG-tAHP-DRI were stained with p-IκB-α (**E**) and caspase 8 (**F**).

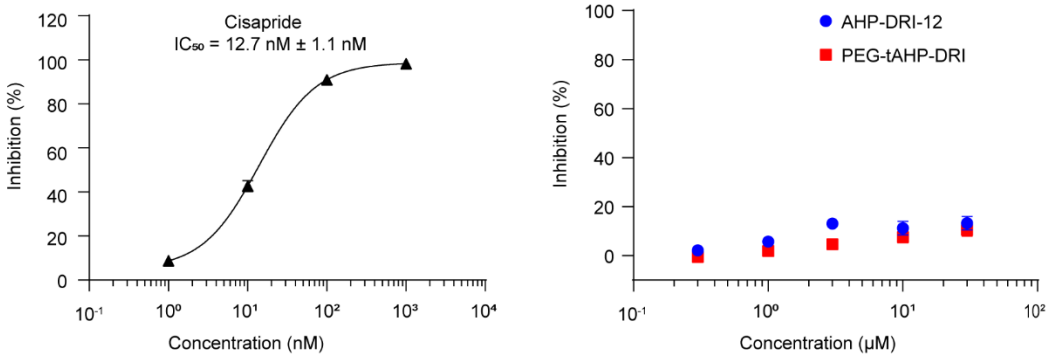


Figure S10. Current change of hERG induced by cisapride, AHP-DRI-12 or PEG-tAHP-DRI.

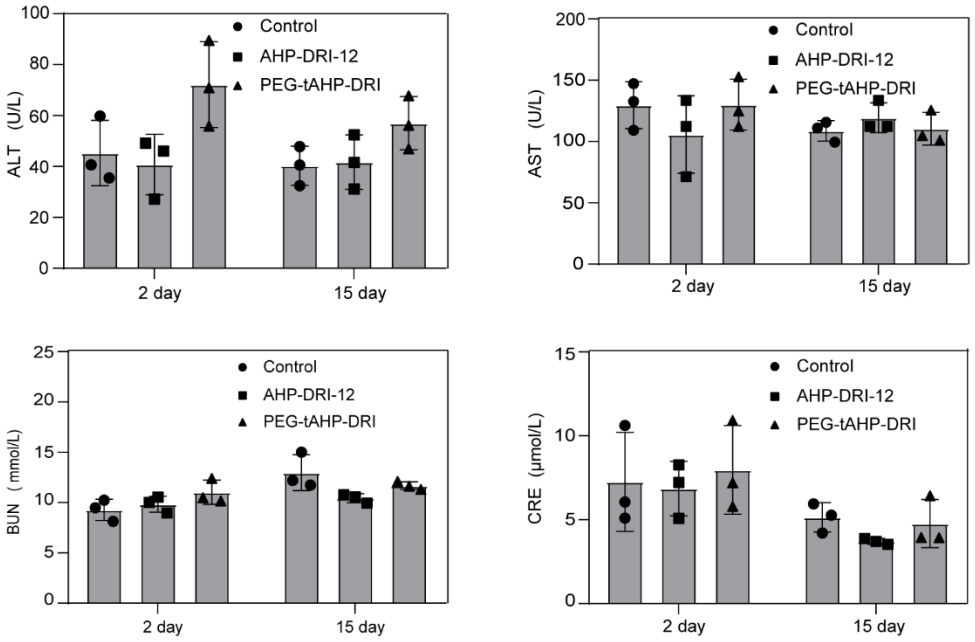
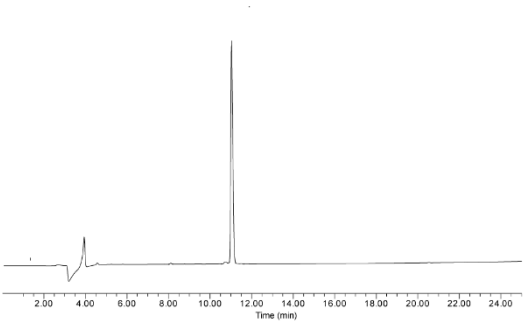
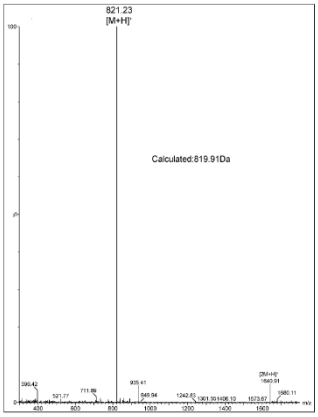


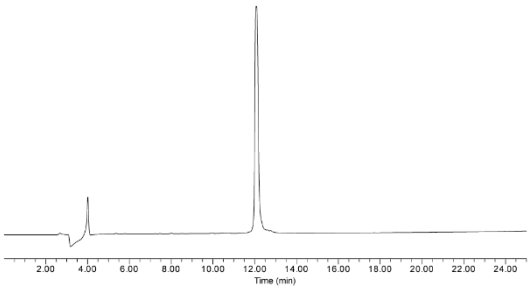
Figure S11. Serum ALT, AST, BUN and CRE levels after i.v. injection of AHP-DRI-12 and PEG-tAHP-DRI. Data are means ± SD (n = 3), and analyzed with GraphPad Prism 8.0. *P < 0.05, **P < 0.01, ***P < 0.005 calculated by unpaired two-tailed Student's t-test. NS indicates P > 0.05.

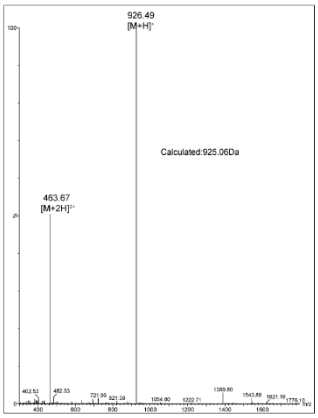
AHP-01: WTLSINS



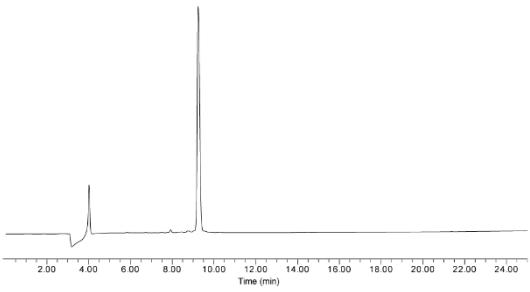


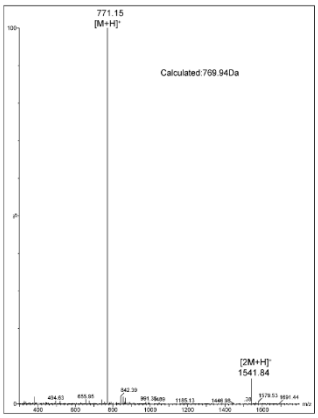
AHP-02: FQFRLQS



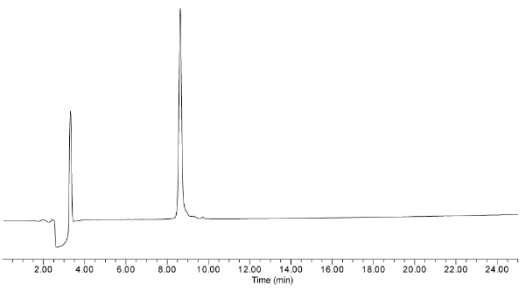


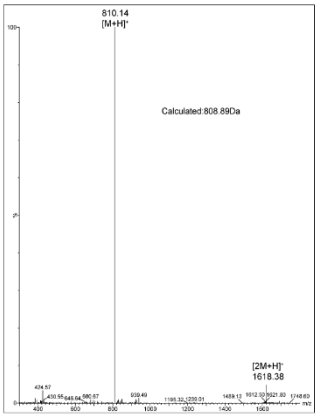
AHP-03: YLPAPLP





AHP-04: ANLLYES





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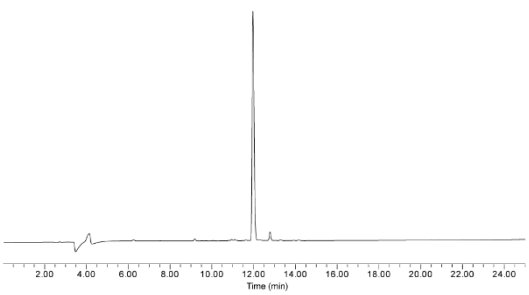
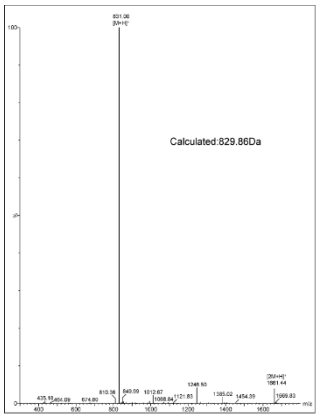
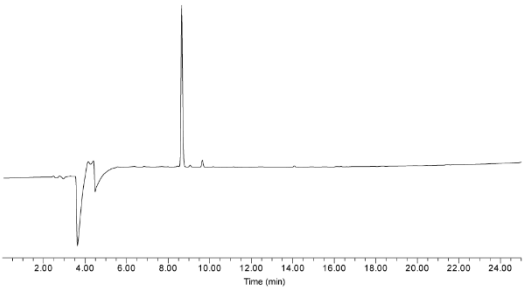
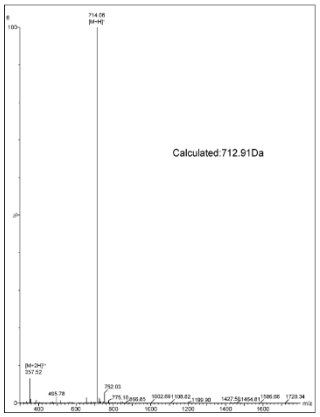


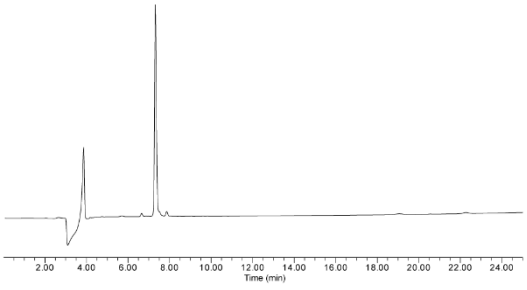


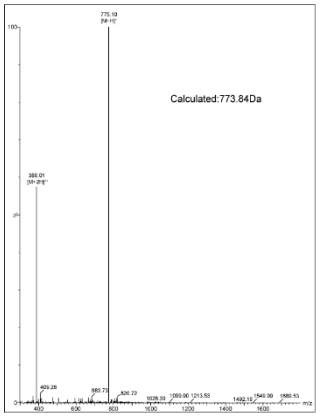
AHP-06: KLGPVCP



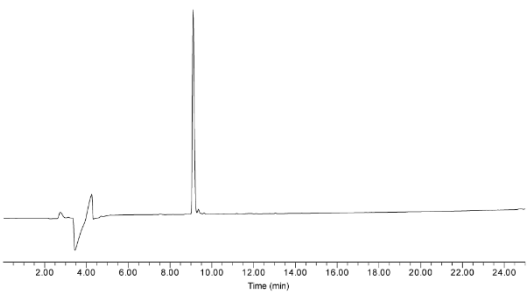


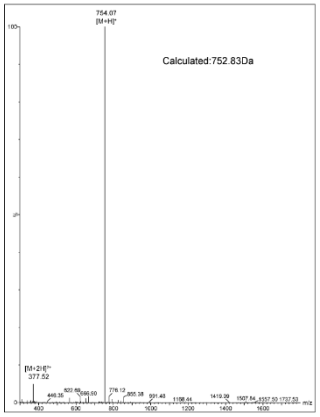
AHP-07: YSLHASP



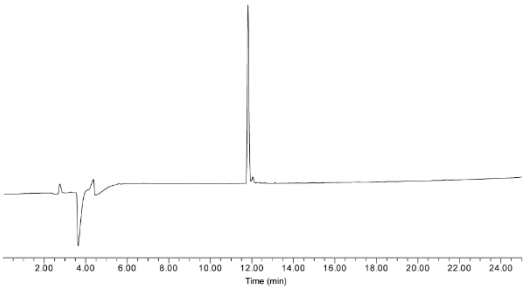


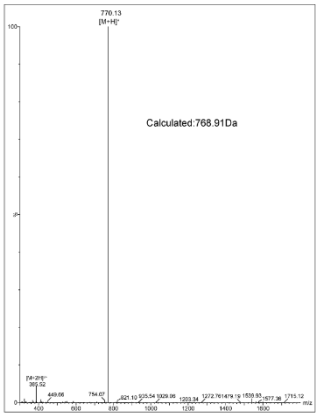
AHP-08: HSTAPQL



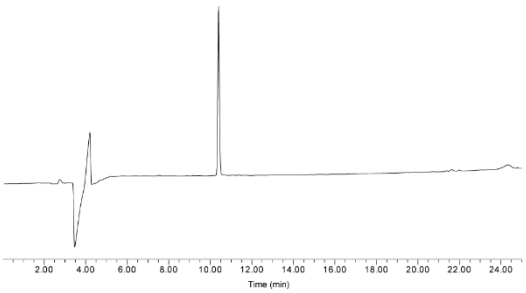


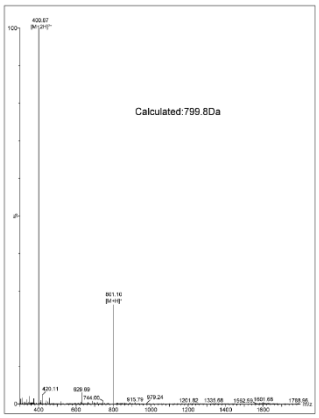
AHP-09: KLVPEPS





AHP-10: NGSTHER





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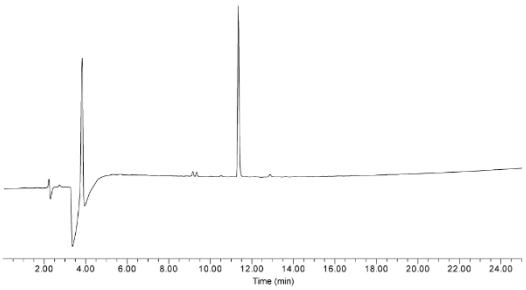

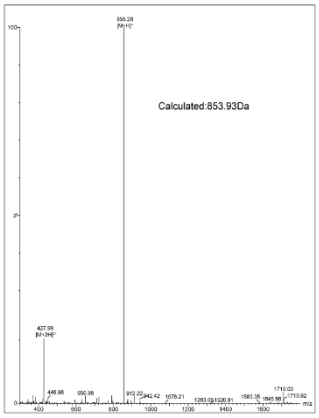

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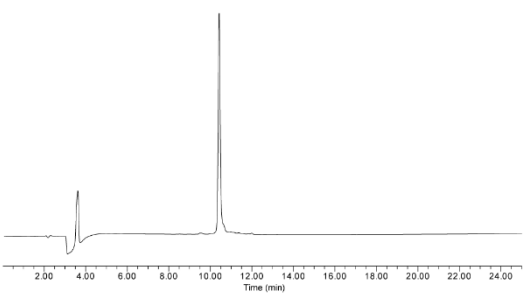
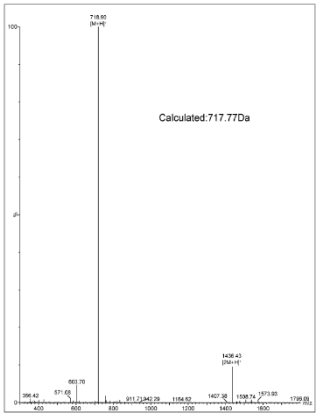
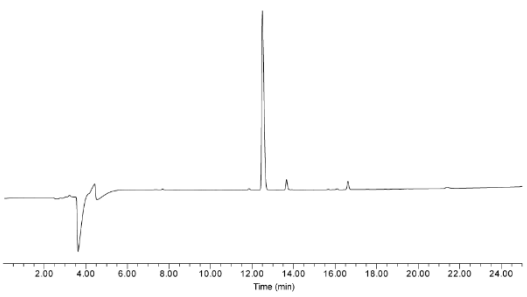
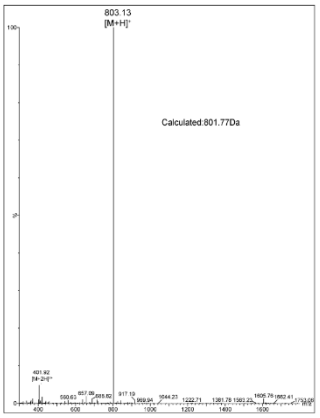




AHP-13: SHTDTNQ





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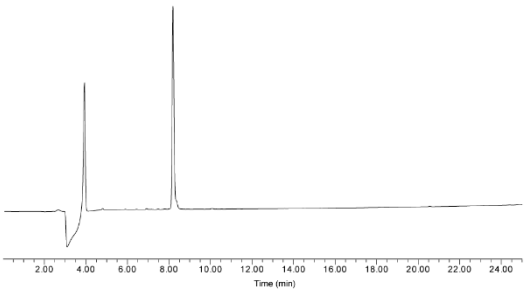
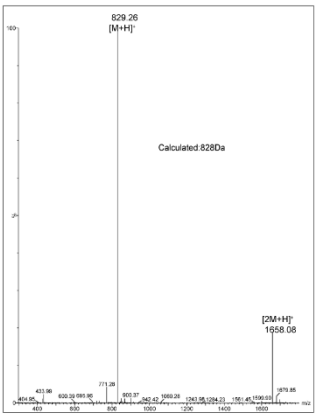
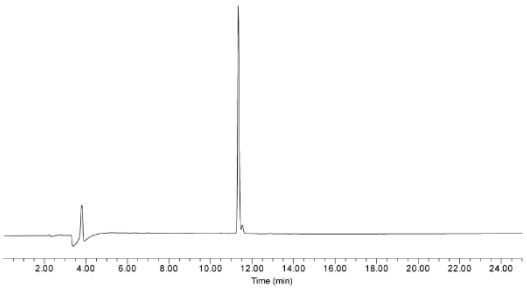
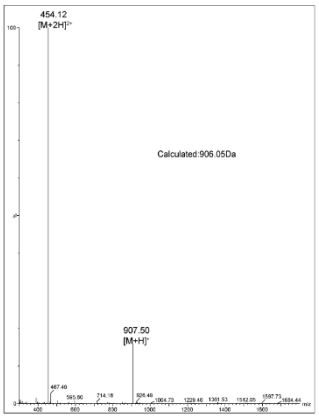




AHP-15: TIPKYER





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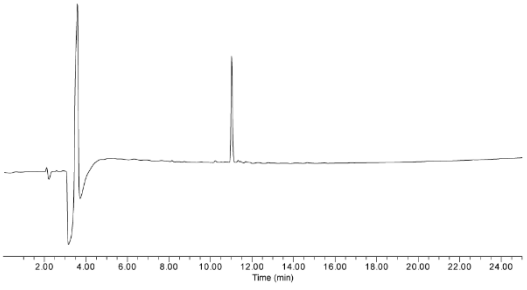

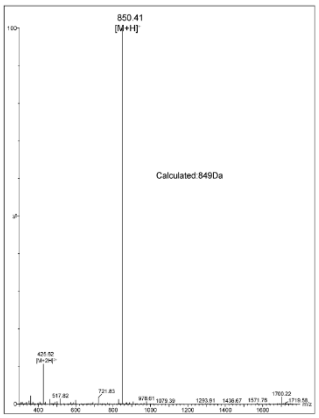

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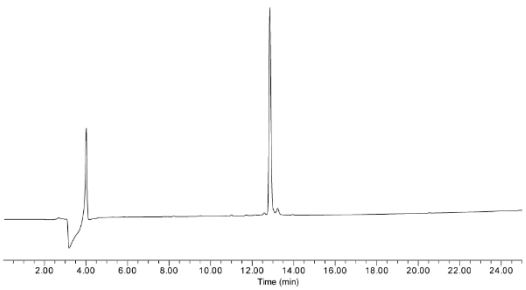
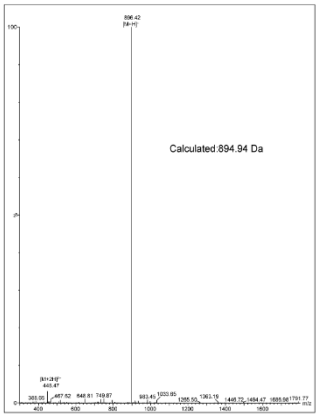
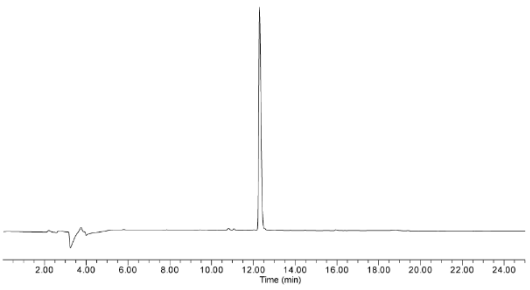
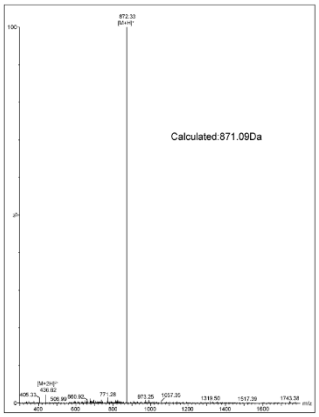


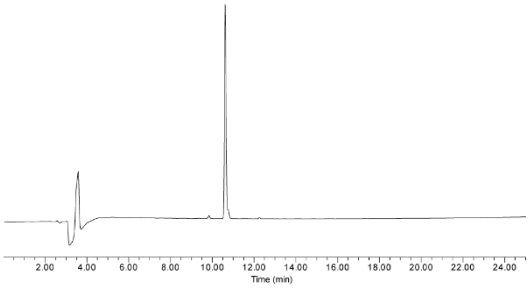


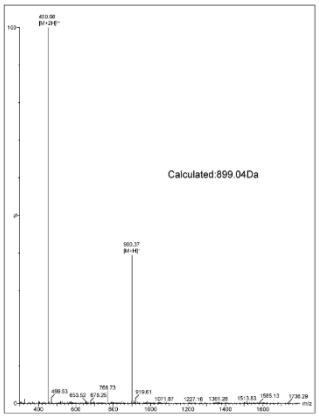
AHP-18: TTYMPKM



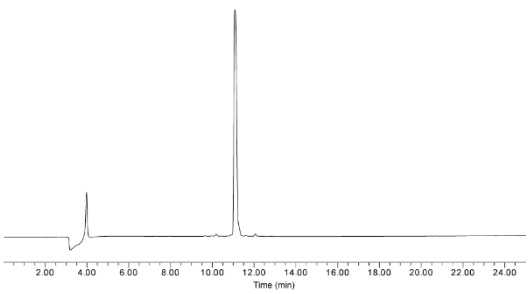


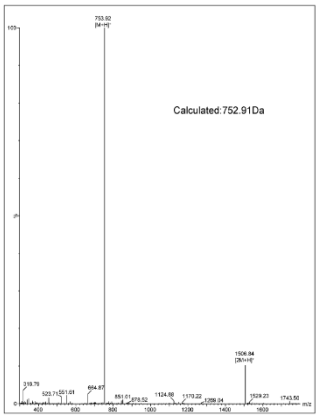
AHP-19: MPPFRHD



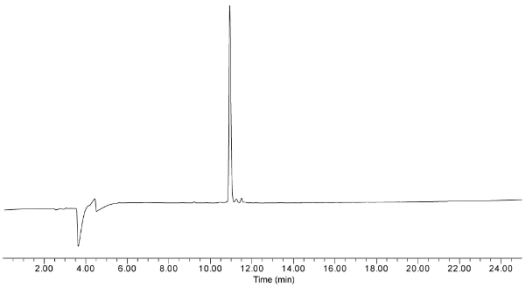


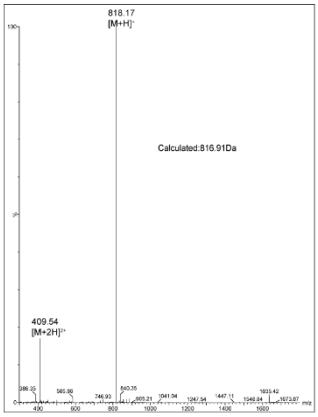
AHP20: LPRSPLA



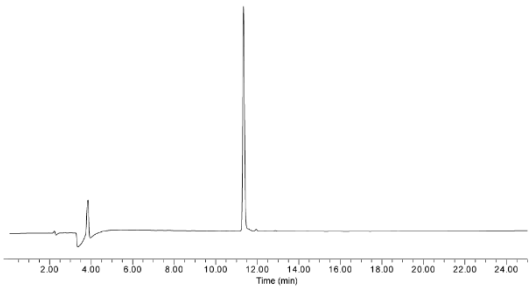


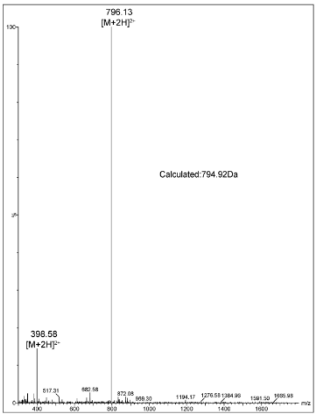
AHP-21: AGHFSLW



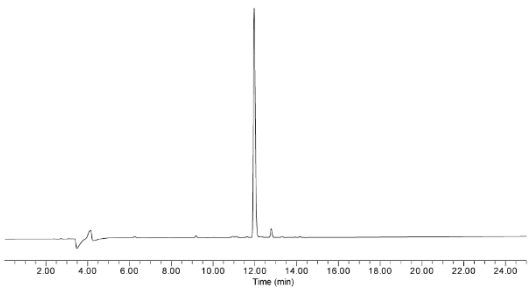


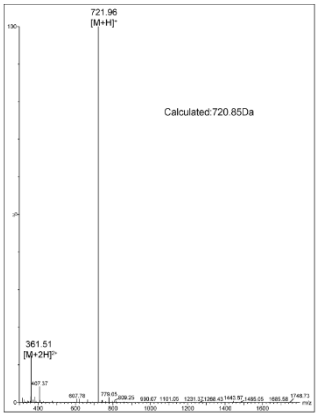
AHP-22: LMTTKSD



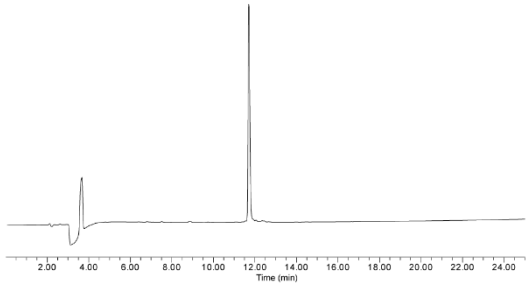


AHP-23: GGLSMTR





AHP-24: NHQRLLP



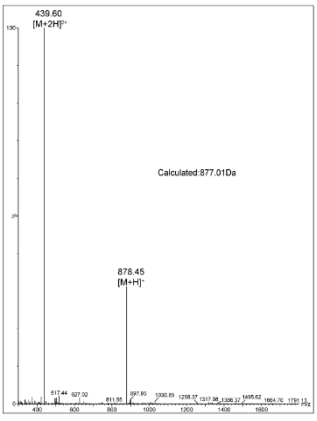
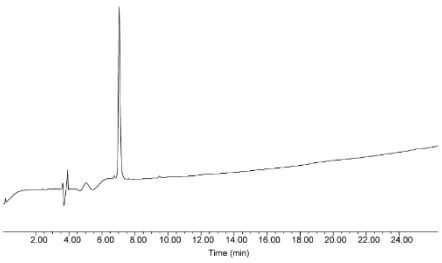
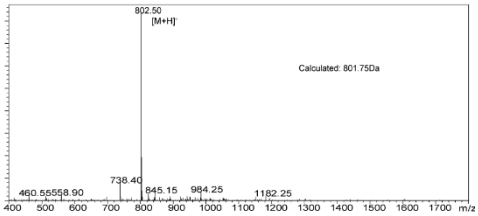


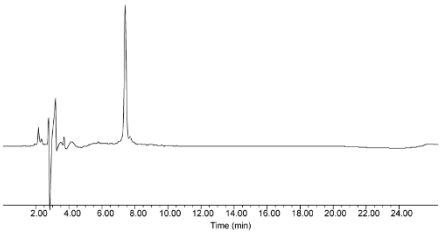
Figure S12. Characterization of twenty-four DR6-targeting peptides by HPLC (λ = 220 nm) and ESI-MS.

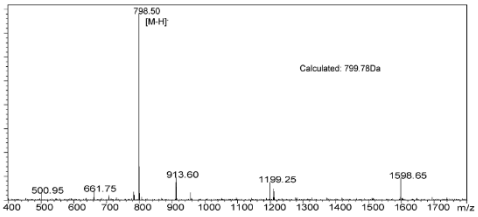
AHP-D-03: D-(YLPAPLP)



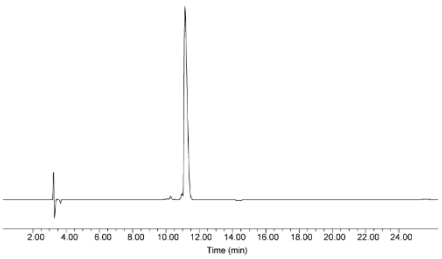


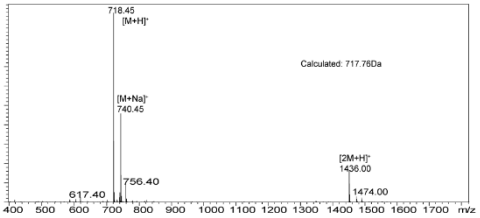
AHP-D-10: D-(NGSTHER)



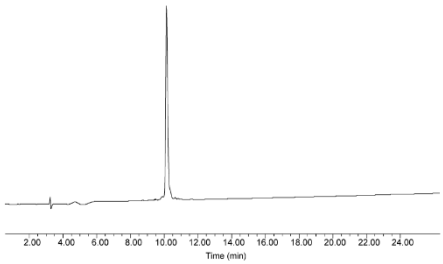


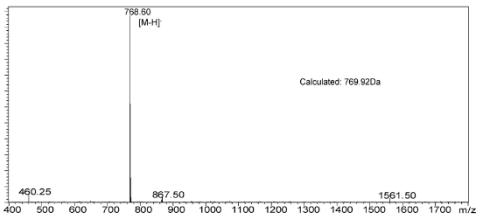
AHP-D-12: D-(TIDATTP)



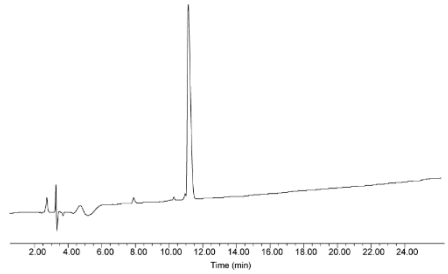


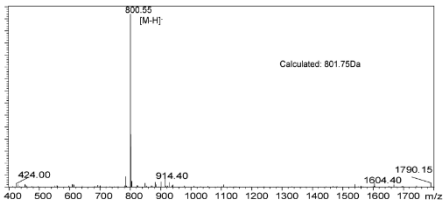
AHP-D-13: D-(SHTDTNQ)





AHP-DRI-03: D-(PLPAPLY)





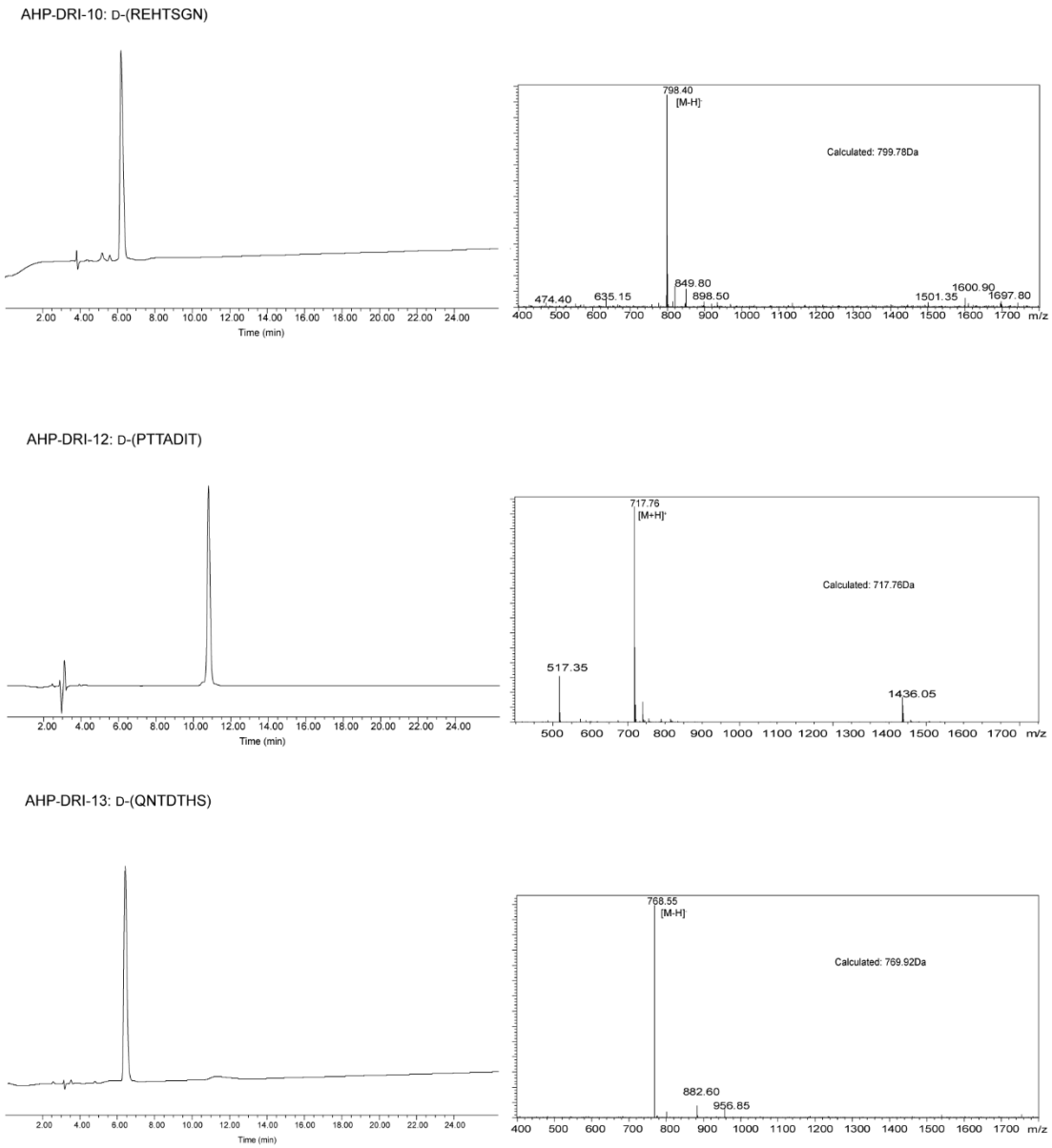


Figure S13. Characterization of D-peptides and DRI-peptides (totally composed of D amino acids) by HPLC (λ = 220 nm) and ESI-MS.