

## Supporting Information for

### ORIGINAL ARTICLE

#### **A pentapeptide enabled AL3810 liposome-based glioma-targeted therapy with immune opsonic effect attenuated**

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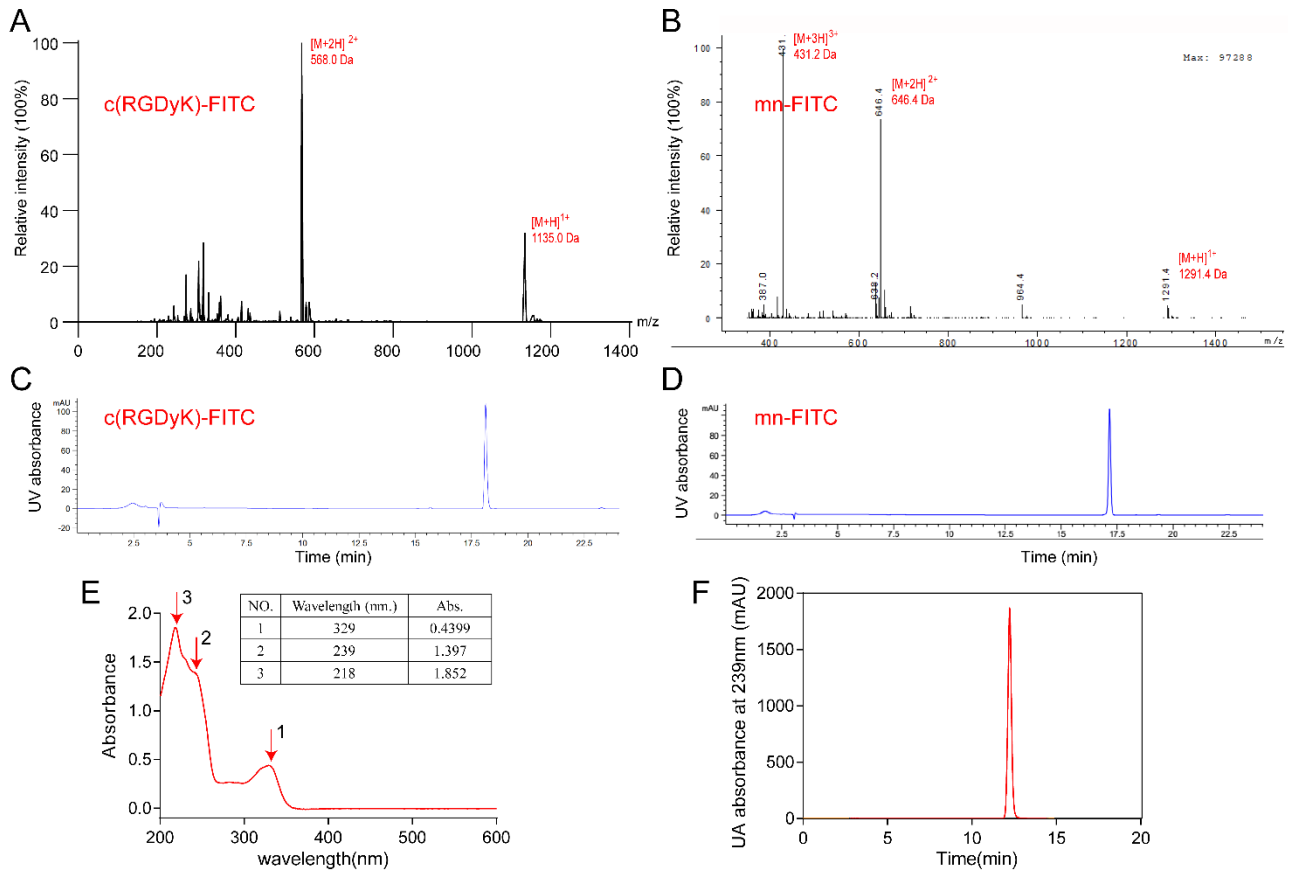
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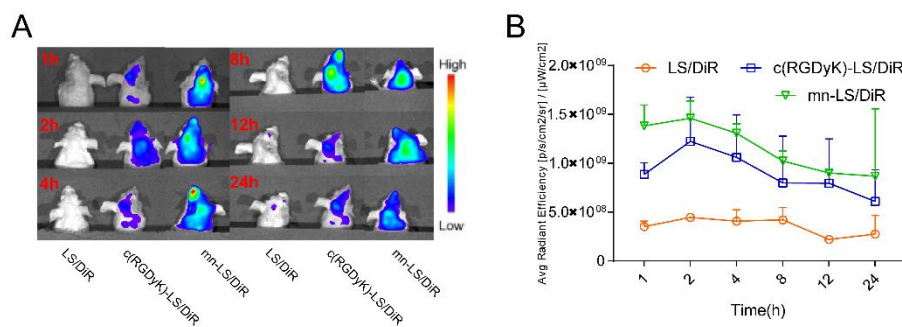
\*Corresponding author. Tel: +86 21 5198 0090.

E-mail address: wylu@shmu.edu.cn (Weiyue Lu).

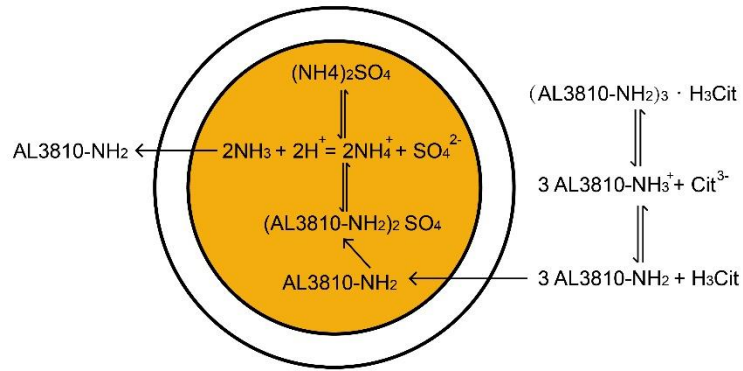
Running title: Immunocompatible mn-modified liposome for glioma-targeted therapy



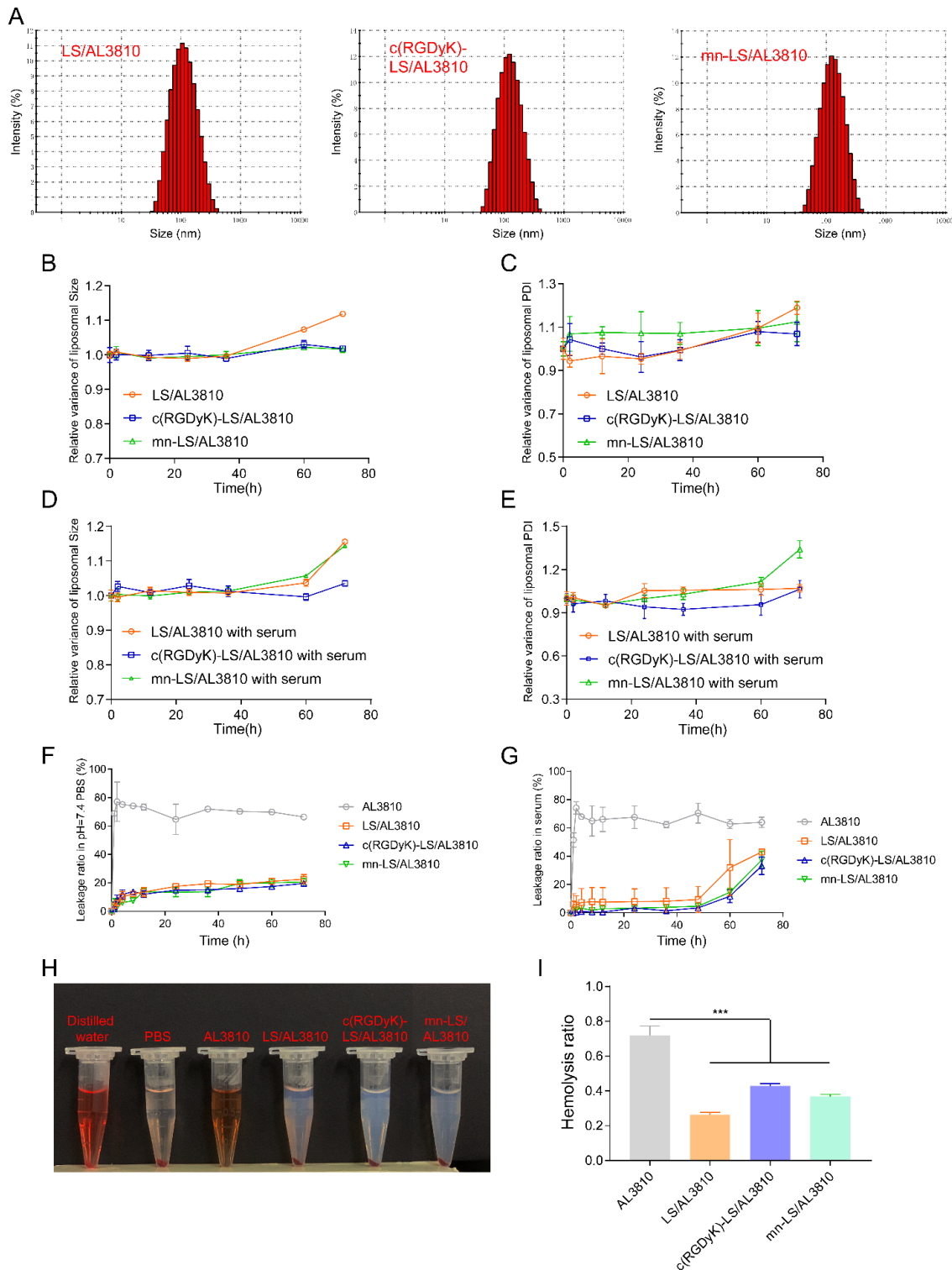
**Figure S1** Physicochemical characterization of peptide-fluorescein and AL3810. (A)–(D) Analysis of conjugates c(RGDyK)-FITC and mn-FITC by ESI-MS (A), (B) and RP-HPLC (C), (D). (E) UV scanning of molecule AL3810 in 200 –600 nm wavelength. (F) Analysis of molecule AL3810 by RP-HPLC.



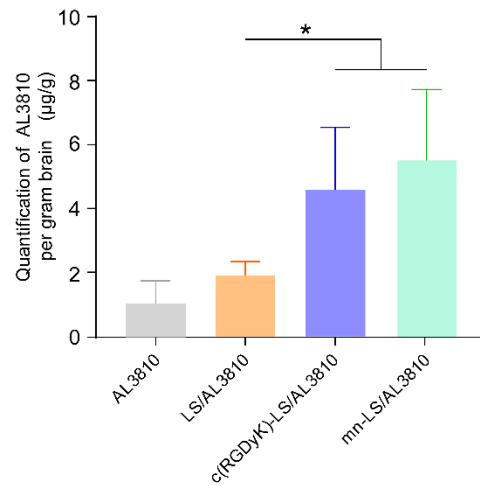
**Figure S2** *In vivo* glioma targeting ability. (A) and (B) *In vivo* fluorescence imaging of glioma orthotopic xenograft at predesignated time point after intravenous injection with DiR-labeled liposomes (A). Semi-quantitative ROI analysis of *in vivo* fluorescent images of the brain at different time points (B). Data are mean ± SD,  $n = 3$ .



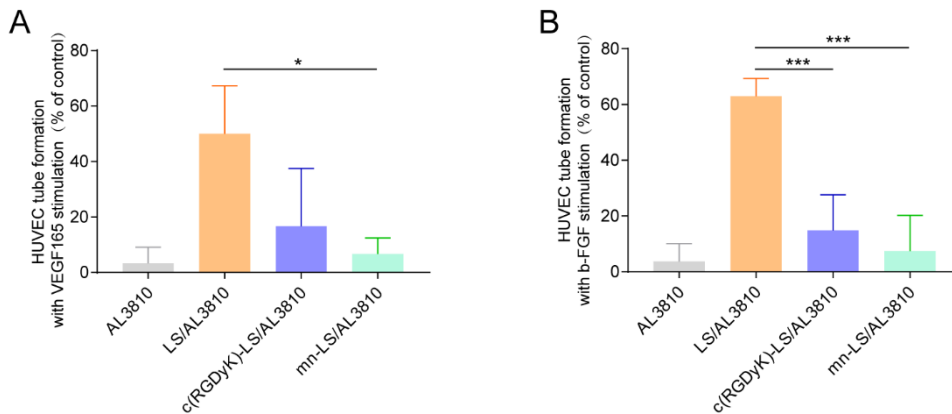
**Figure S3** The illustration of liposomal AL3810 loading principle by ammonium sulfate gradient method.



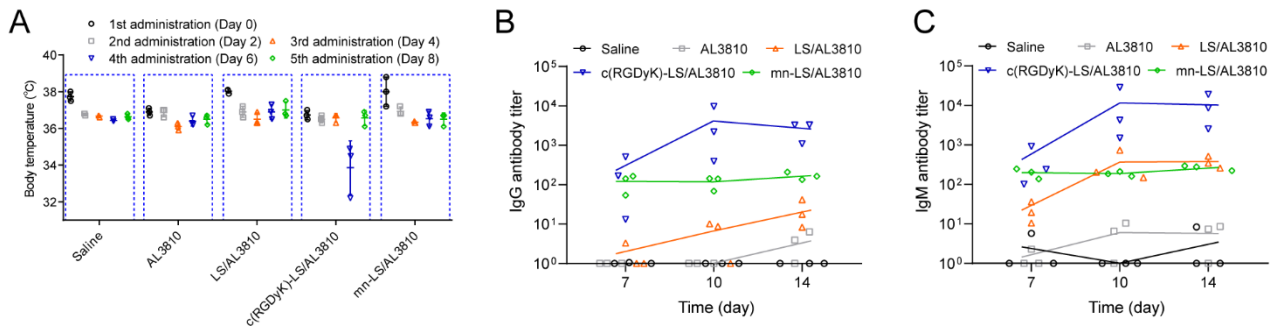
**Figure S4** Characterization of AL3810-loaded liposome. (A) Size distribution of AL3810-loaded liposomes detected by DLS. Stability of AL3810-loaded liposomes in size (B) and PDI (C) after incubation in 37 °C. Stability of AL3810-loaded liposomes in size (D) and PDI (E) after incubation with 50% serum in 37 °C. *In vitro* leakage kinetics of AL3810 from AL3810-loaded liposomes at 37 °C in PBS (F) or in 30% serum (G). Hemolytic test of AL3810 formulations (H). Hemolysis ratio was defined as the percentages of distilled water group, which was set at 100% (I). Data are mean  $\pm$  SD,  $n = 3$ ; \*\*\*  $P < 0.001$ .



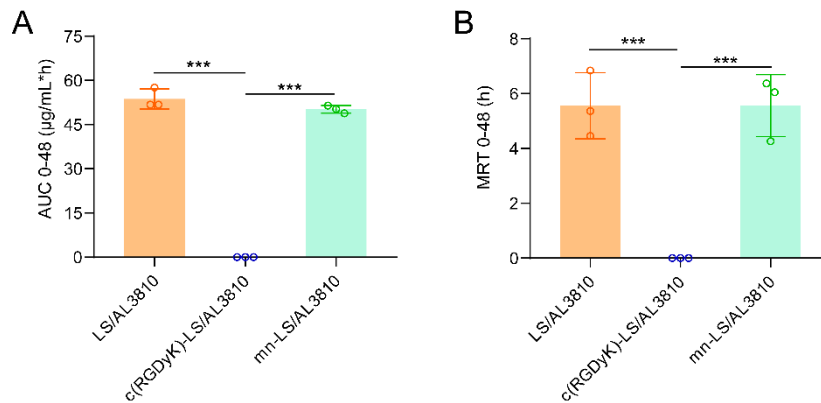
**Figure S5** Brain penetration of ligand-tethered AL3810-loaded liposomes. AL3810 concentration of different AL3810 formulations in brain of U87 MG orthotopic xenograft bearing nude mice at 4 h post injection. Data are mean  $\pm$ SD,  $n = 3$ ; \*  $P < 0.05$ .



**Figure S6** Semiquantitative analysis of *in vitro* angiogenesis inhibition of AL3810 formulations. (A)–(B) destruction of different AL3810 loaded liposomes on neovascularization formed by HUVECs with VEGF165 (A) and b-FGF (B) stimuli. Cells incubated with drug-free DMEM served as the control. Data are presented as the percentages of the control group, which was set at 100%. Data are mean  $\pm$ SD,  $n = 3$ ; \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .



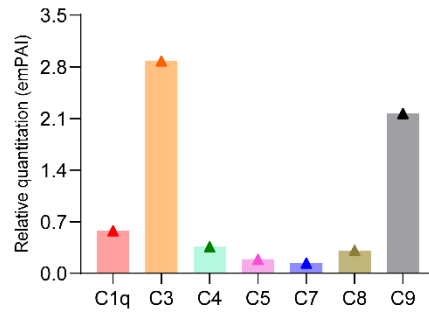
**Figure S7** Biosafety assessment of the glioma-targeted AL3810-loaded liposomes on ICR mice. (A) The body temperature record of ICR mice 0.5 h post various remedies at AL3810 each dose of 4 mg/kg. Data was collected from abdominal thermometer. Time course of anti-liposome IgG (B) and IgM (C) antibody production of ICR mice on day 7, 10 and 14 from onset of administration. Blood was sampled from the retro-orbital sinus and analyzed *via* ELISA assay. Corresponding liposome served as antigen. Data are mean  $\pm$  SD,  $n = 3$ .



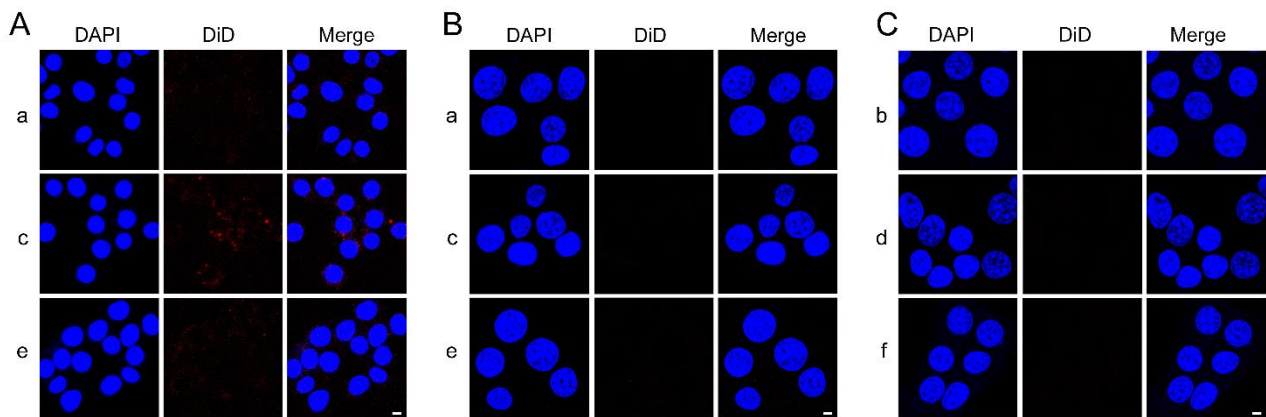
**Figure S8** AUC<sub>0-48</sub> (A) and MRT<sub>0-48</sub> (B) of various AL3810-loaded liposomal formulations calculated by DAS 2.0. Data are mean  $\pm$  SD,  $n = 3$ ; \*\*\*  $P < 0.001$ .



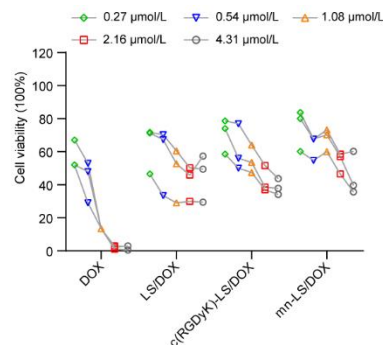
**Figure S9** Specificity between immune opsonin and peptide-tethered liposome. The photograph of 50  $\mu$ L mn-LS/AL3810 incubated with 50  $\mu$ L plasma sampled from c(RGDyK)-LS/AL3810 injected SD rat at 0 h.



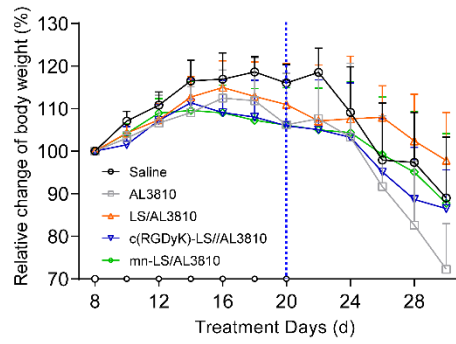
**Figure S10** The exponentially modified Protein Abundance Index (emPAI) of complements in group d using nano-LC/MS/MS.



**Figure S11** Reciprocity of immune opsonized liposomes with macrophage and hepatocyte. (A) The cellular uptake of pristine DiD-loaded liposomes by RAW264.7 cells at 37 °C for 4 h. (B) The cellular uptake of pristine DiD-loaded liposomes by AML12 cells at 37 °C for 4 h. (C) The cellular uptake of immune opsonized DiD-loaded liposomes by AML12 cells at 37 °C for 4 h. Intracellular fluorescence was captured by a confocal laser scanning microscope. Scale bar = 5  $\mu$ m. Group a: pristine LS/AL3810; Group b: LS/AL3810 with 10% volume of plasma sampled from LS/AL3810 injected group at 0 h; Group c: pristine c(RGDyK)-LS/AL3810; Group d: c(RGDyK)-LS/AL3810 with 10% volume of plasma sampled from c(RGDyK)-LS/AL3810 injected group at 0 h; Group e: pristine mn-LS/AL3810; Group f: mn-LS/AL3810 with 10% volume of plasma sampled from mn-LS/AL3810 injected group at 0 h.

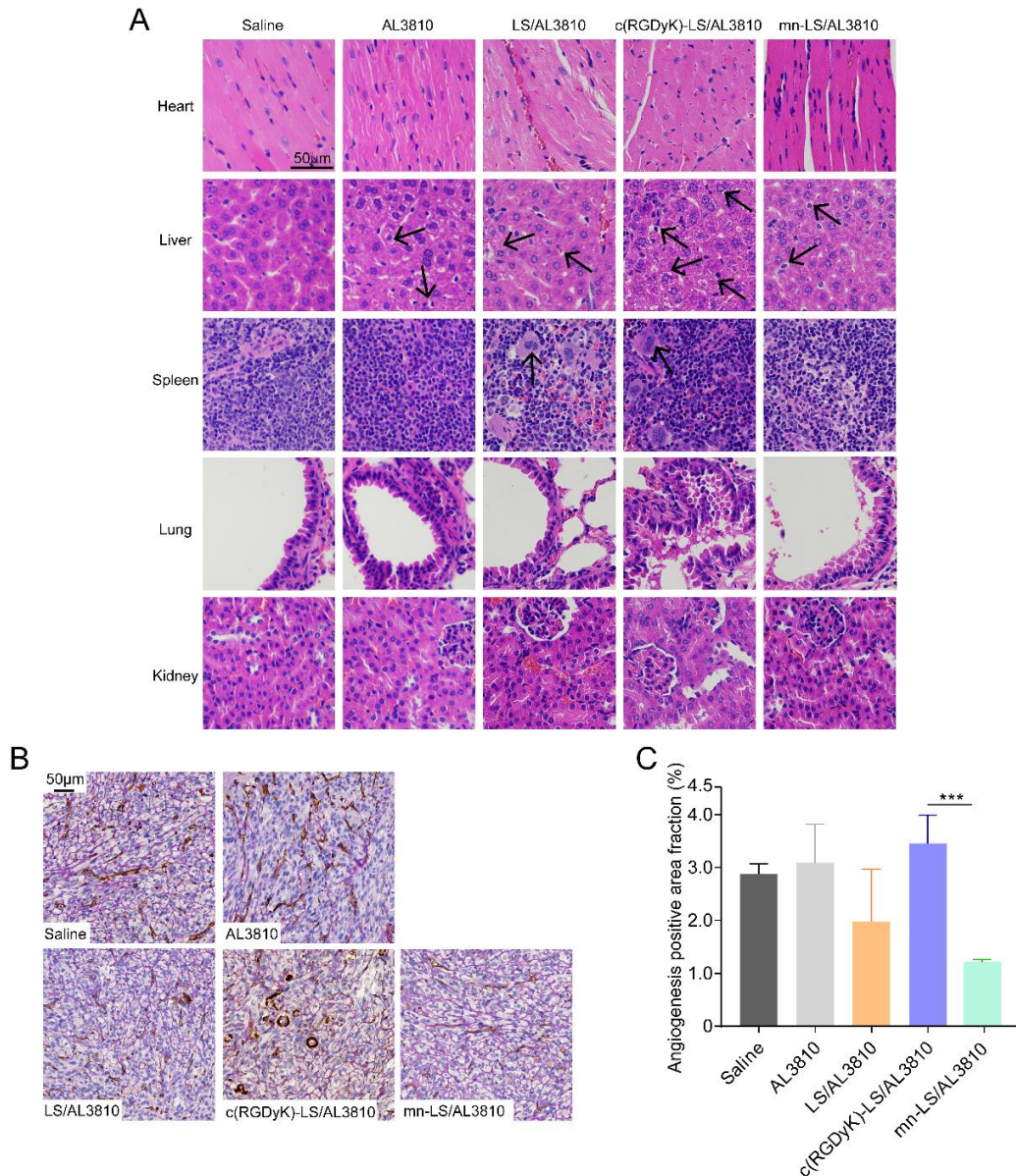


**Figure S12** Anti-proliferative effect of DOX liposomal formulations on RAW264.7 cells after 48 h exposure. Cell viability was measured by MTT assay.

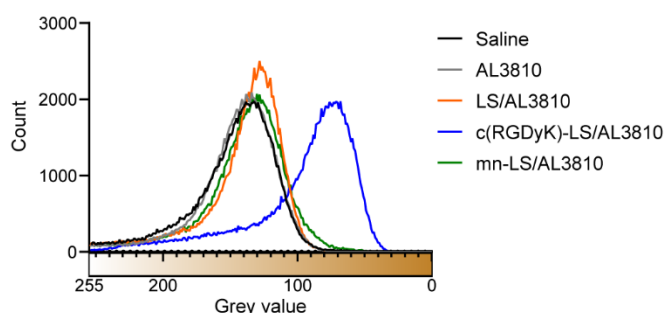


**Figure S13** Body weight change of nude mice bearing U87 MG orthotopic xenograft receiving different AL3810 formulations. The relative body weight was calculated as the ratio of body weight to the initial body weight. Data are mean  $\pm$ SD,  $n = 10$ .





**Figure S14** Safety and efficacy evaluation of AL3810 liposome treatment course. (A) Hematoxylin-eosin staining of organ sections of the mice bearing U87 MG orthotopic xenograft after various treatments. The black arrows pointed at hepatocytes cytoplasmic loosening in liver sections. The black arrows pointed at multinucleated giant cells in spleen sections. CD31 immunohistochemical staining of the glioma tissues of the mice bearing U87 MG orthotopic xenograft after various treatments (B). The amount of glioma angiogenesis was counted within three randomly selected vision fields (C). Data are mean  $\pm$  SD,  $n = 3$ ; \*\*\*  $P < 0.001$ .



**Figure S15** Histogram of the positive area of C3 deposition in liver after treating various AL3810 formulations.

**Table S1** Size distribution, encapsulation efficiency and loading capacity of AL3810-loaded liposomes.

Formulations	Size (nm)	PDI	Encapsulation efficiency ( % )	Loading capacity ( % )
LS/AL3810	100.12±1.03	0.195±0.010	96.32±2.19	6.43±0.14
mn-LS/AL3810	117.43±0.81	0.164±0.006	94.68±3.05	5.94±0.18
c(RGDyK)-LS/AL3810	111.87±2.43	0.169±0.002	95.12±1.96	5.99±0.12

The size distribution of AL3810-loaded liposomes measured by dynamic light scattering. The encapsulation efficiency (EE) and loading capacity (LC) of AL3810-loaded liposomes. Data are represented with mean ± SD ( $n = 3$ ).

**Table S2** The list of identified proteins in Band-1.

#	prot_acc	prot_desc	prot_score	prot_mass	prot_matches
1	A0A0G2JVP4	Immunoglobulin heavy constant mu OS=Rattus norvegicus OX=10116 GN=Ighm PE=1 SV=1	4938	52360	260
2	A0A0G2JSH5	Serum albumin OS=Rattus norvegicus OX=10116 GN=Alb PE=1 SV=1	1817	70710	83
3	M0RBF1	Complement C3 OS=Rattus norvegicus OX=10116 GN=C3 PE=1 SV=1	1235	187746	68
4	F1LZ11	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	937	14286	38
5	P12346	Serotransferrin OS=Rattus norvegicus OX=10116 GN=Tf PE=1 SV=3	703	78512	39
6	P04639	Apolipoprotein A-I OS=Rattus norvegicus OX=10116 GN=Apoa1 PE=1 SV=2	404	30100	32
7	A0A0G2JV52	Complement C4A (Fragment) OS=Rattus norvegicus OX=10116 GN=C4a PE=4 SV=4	342	151663	16
8	A0A0G2K151	Apolipoprotein E OS=Rattus norvegicus OX=10116 GN=ApoE PE=1 SV=1	337	41573	12
9	A0A0G2K290	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	308	14124	10
10	F7F389	Complement component C9 OS=Rattus norvegicus OX=10116 GN=C9 PE=1 SV=1	305	66555	16
11	A0A0G2JVX7	Apolipoprotein A-IV OS=Rattus norvegicus OX=10116 GN=Apoa4 PE=1 SV=1	285	44335	13
12	A0A0G2K4I8	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	270	12790	16
13	M0R9U2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	263	10952	9
14	F1LWD1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=3	262	14376	9
15	P20762	Ig gamma-2C chain C region OS=Rattus norvegicus OX=10116 PE=2 SV=1	251	37062	14
16	F1M3H2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	250	11295	16

17	A0A096P6L8	Fibronectin OS=Rattus norvegicus OX=10116 GN=Fn1 PE=1 SV=2	242	275925	19
18	P20059	Hemopexin OS=Rattus norvegicus OX=10116 GN=Hpx PE=1 SV=3	236	52060	17
19	D4ACR1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	226	13876	6
20	A0A096P6L9	Complement C5 OS=Rattus norvegicus OX=10116 GN=C5 PE=1 SV=2	226	190786	13
21	Q7TQ70	Ac1873 OS=Rattus norvegicus OX=10116 GN=Fga PE=1 SV=1	225	87344	12
22	P14046	Alpha-1-inhibitor 3 OS=Rattus norvegicus OX=10116 GN=A1i3 PE=1 SV=1	225	165038	17
23	A0A0G2JUY4	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	214	12526	6
24	F1LWDO	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	213	12027	9
25	M0R7Q2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	212	13315	8
26	P02680	Fibrinogen gamma chain OS=Rattus norvegicus OX=10116 GN=Fgg PE=1 SV=3	205	51228	17
27	A0A0G2JYK0	Serine protease inhibitor A3N OS=Rattus norvegicus OX=10116 GN=LOC299282 PE=1 SV=1	200	68226	10
28	D3ZC54	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	200	13293	6
29	A0A0G2JZW1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	183	24607	6
30	A0A0G2K3G0	Histidine-rich glycoprotein OS=Rattus norvegicus OX=10116 GN=Hrg PE=1 SV=1	181	61358	8
31	M0RDF2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	177	11290	8
32	D4A5Y5	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	175	13184	9
33	F1LXY6	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	163	11354	9
34	F1M3E9	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=3	160	13894	6
35	F1M3X3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	153	13177	6
36	P24090	Alpha-2-HS-glycoprotein OS=Rattus norvegicus OX=10116 GN=AhsG PE=1 SV=2	151	38757	6
37	P05544	Serine protease inhibitor A3L OS=Rattus norvegicus OX=10116 GN=Serpina3l PE=1 SV=3	147	46419	10
38	D3ZJW6	RCG21066 OS=Rattus norvegicus OX=10116 GN=rCG_21066 PE=1 SV=1	130	13226	5
39	A0A0G2K548	A-kinase-anchoring protein 9 OS=Rattus norvegicus OX=10116 GN=Akap9 PE=1 SV=1	130	444371	8
40	A0A0G2JXB7	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	128	13110	9
41	D4A3D1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	124	11253	8
42	Q6P0K8	Junction plakoglobin OS=Rattus norvegicus OX=10116 GN=Jup PE=1 SV=1	123	82490	10
43	Q63041	Alpha-1-macroglobulin OS=Rattus norvegicus OX=10116 GN=A1m PE=1 SV=1	122	168388	17
44	F1M1R0	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 GN=LOC690813 PE=1 SV=2	116	15055	4
45	P14480	Fibrinogen beta chain OS=Rattus norvegicus OX=10116 GN=Fgb PE=1 SV=4	113	54828	11
46	A0A0G2K1T8	Serine protease inhibitor A3N OS=Rattus norvegicus OX=10116 GN=LOC299282 PE=1 SV=1	112	46447	4
47	A0A0G2K3K2	Actin, cytoplasmic 1 OS=Rattus norvegicus OX=10116 GN=Actb PE=1 SV=1	109	42401	4
48	A0A0G2K980	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	109	11344	6
49	A0A0G2JY31	Alpha-1-antiproteinase OS=Rattus norvegicus OX=10116 GN=Serpina1 PE=1 SV=1	108	46264	11
50	Q3KR94	Vitronectin OS=Rattus norvegicus OX=10116 GN=Vtn PE=1 SV=1	107	55489	4
51	A0A0G2JSK1	RCG20603 OS=Rattus norvegicus OX=10116 GN=Serpina3c PE=3 SV=1	104	46676	9
52	Q4KM75	CD5 antigen-like OS=Rattus norvegicus OX=10116 GN=Cd5l PE=1 SV=1	95	39375	8
53	D3ZBB2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=3	95	13251	4
54	D3ZFC6	Inter-alpha-trypsin inhibitor heavy chain 4 OS=Rattus norvegicus OX=10116 GN=Itih4 PE=1 SV=3	93	104027	9
55	A0A0G2JZL1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	92	15112	5
56	A0A0G2K828	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	91	13442	3
57	F1LMV6	Desmoplakin OS=Rattus norvegicus OX=10116 GN=Dsp PE=1 SV=1	89	334582	8
58	M0RA79	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	83	11066	3
59	A0A0G2K7X7	Complement C7 OS=Rattus norvegicus OX=10116 GN=C7 PE=1 SV=1	82	96854	4
60	D3ZFF8	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	81	13148	6

61	A0A0G2K5D2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	78	13658	3
62	A0A0G2K7S9	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	77	13164	4
63	A0A0G2K896	Similar to RIKEN cDNA 1300017J02 OS=Rattus norvegicus OX=10116 GN=RGD1310507 PE=1 SV=1	77	78724	4
64	P10959	Carboxylesterase 1C OS=Rattus norvegicus OX=10116 GN=Ces1c PE=1 SV=3	76	60479	3
65	M0RDR6	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	69	12884	5
66	G3V9R9	Afamin OS=Rattus norvegicus OX=10116 GN=Afm PE=1 SV=1	69	71135	5
67	M0RBK4	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	69	11674	1
68	A0A0G2K7P6	IgV domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	68	10337	2
69	D3Z9Z0	Ankyrin 1 OS=Rattus norvegicus OX=10116 GN=Ank1 PE=1 SV=1	65	188206	1
70	M0R693	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	62	12811	3
71	A0A0G2K5X3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	60	13425	2
72	D3ZM39	Desmoglein 1 OS=Rattus norvegicus OX=10116 GN=Dsg1 PE=4 SV=1	59	114980	3
73	Q5M7T5	Serine (Or cysteine) peptidase inhibitor, OS=Rattus norvegicus OX=10116 GN=Serpinc1 PE=1 SV=1	58	52714	4
74	A0A0G2JUY3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	58	12903	2
75	A0A0H2UHA1	Mannan-binding lectin serine protease 1 OS=Rattus norvegicus OX=10116 GN=Masp1 PE=1 SV=1	56	81715	3
76	A0A0G2K9R5	Apolipoprotein C-I OS=Rattus norvegicus OX=10116 GN=ApoC1 PE=4 SV=1	53	7557	4
77	M0R3Q0	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	53	11877	2
78	D3ZWD6	Complement C8 alpha chain OS=Rattus norvegicus OX=10116 GN=C8a PE=1 SV=1	52	67884	6
79	A0A0G2JSW3	Globin a4 OS=Rattus norvegicus OX=10116 GN=Hbb PE=1 SV=1	50	16069	2

The yellow highlighted immunoglobulin, the orange highlighted Ig-like domain-containing protein, the blue highlighted complement C3, the green highlighted serum albumin and the red highlighted other complement proteins.

**Table S3** The list of identified proteins in Band-2.

#	prot_acc	prot_desc	prot_score	prot_mass	prot_matches
1	P20762	Ig gamma-2C chain C region OS=Rattus norvegicus OX=10116 PE=2 SV=1	2253	37062	98
2	A0A0G2JVP4	Immunoglobulin heavy constant mu OS=Rattus norvegicus OX=10116 GN=Ighm PE=1 SV=1	867	52360	47
3	A0A0G2JSH5	Serum albumin OS=Rattus norvegicus OX=10116 GN=Alb PE=1 SV=1	696	70710	31
4	F1LZ11	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	656	14286	31
5	Q4KM75	CD5 antigen-like OS=Rattus norvegicus OX=10116 GN=Cd51 PE=1 SV=1	533	39375	30
6	P02680	Fibrinogen gamma chain OS=Rattus norvegicus OX=10116 GN=Fgg PE=1 SV=3	513	51228	26
7	M0RBF1	Complement C3 OS=Rattus norvegicus OX=10116 GN=C3 PE=1 SV=1	508	187746	31
8	Q6P0K8	Junction plakoglobin OS=Rattus norvegicus OX=10116 GN=Jup PE=1 SV=1	288	82490	21
9	F1LWD1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=3	231	14376	8
10	A0A0G2JY31	Alpha-1-antiproteinase OS=Rattus norvegicus OX=10116 GN=Serpina1 PE=1 SV=1	222	46264	20
11	F1M3X3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	220	13177	9
12	M0R9U2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	216	10952	7
13	D4ACR1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	204	13876	7
14	F7F389	Complement component C9 OS=Rattus norvegicus OX=10116 GN=C9 PE=1 SV=1	202	66555	14
15	F1M3H2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	200	11295	13

16	Q68FY4	Group specific component OS=Rattus norvegicus OX=10116 GN=Gc PE=1 SV=1	194	55080	12
17	A0A0G2JYK0	Serine protease inhibitor A3N OS=Rattus norvegicus OX=10116 GN=LOC299282 PE=1 SV=1	193	68226	12
18	A0A0G2K9B1	Serine protease inhibitor A3N OS=Rattus norvegicus OX=10116 GN=LOC299282 PE=1 SV=1	190	48570	11
19	FILMV6	Desmoplakin OS=Rattus norvegicus OX=10116 GN=Dsp PE=1 SV=1	183	334582	23
20	A0A0G2K980	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	166	11344	6
21	A0A0G2K290	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	151	14124	6
22	P14480	Fibrinogen beta chain OS=Rattus norvegicus OX=10116 GN=Fgb PE=1 SV=4	150	54828	14
23	F1LXY6	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	147	11354	6
24	F1LP05	ATP synthase subunit alpha OS=Rattus norvegicus OX=10116 GN=Atp5f1a PE=1 SV=1	136	59890	10
25	F1M1R0	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 GN=LOC690813 PE=1 SV=2	133	15055	4
26	M0R7Q2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	132	13315	7
27	P20761	Ig gamma-2B chain C region OS=Rattus norvegicus OX=10116 GN=Igh-1a PE=1 SV=1	123	37101	5
28	A0A0G2JUG1	IF rod domain-containing protein OS=Rattus norvegicus OX=10116 PE=3 SV=1	115	57880	7
29	D3ZC54	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	106	13293	4
30	A0A0G2JUP5	Murinoglobulin-1 OS=Rattus norvegicus OX=10116 GN=Mug1 PE=4 SV=1	99	162631	3
31	A0A0G2K151	Apolipoprotein E OS=Rattus norvegicus OX=10116 GN=ApoE PE=1 SV=1	99	41573	7
32	F1M3E9	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=3	99	13894	4
33	A0A0G2JZW1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	96	24607	7
34	A0A0G2JXK5	Serine protease inhibitor A3N OS=Rattus norvegicus OX=10116 GN=LOC299282 PE=1 SV=1	87	46361	10
35	G3V6D3	ATP synthase subunit beta OS=Rattus norvegicus OX=10116 GN=Atp5f1b PE=1 SV=1	83	56309	9
36	A0A0G2K477	Immunoglobulin heavy constant mu OS=Rattus norvegicus OX=10116 GN=Ighm PE=4 SV=1	80	51903	10
37	A0A0G2K4I8	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	80	12790	9
38	F1LWD0	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	78	12027	3
39	A0A0G2QC06	Serotransferrin OS=Rattus norvegicus OX=10116 GN=Tf PE=1 SV=1	76	109507	6
40	M0R757	Elongation factor 1-alpha OS=Rattus norvegicus OX=10116 GN=LOC100360413 PE=3 SV=1	75	50418	5
41	A0A0G2JXB7	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	74	13110	5
42	M0RDF2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	71	11290	5
43	A0A0G2K304	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	71	12752	4
44	P04639	Apolipoprotein A-1 OS=Rattus norvegicus OX=10116 GN=Apoa1 PE=1 SV=2	70	30100	4
45	D3ZM39	Desmoglein 1 OS=Rattus norvegicus OX=10116 GN=Dsg1 PE=4 SV=1	69	114980	2
46	D3ZFF8	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	69	13148	3
47	Q7TQ70	Ac1873 OS=Rattus norvegicus OX=10116 GN=Fga PE=1 SV=1	66	87344	13
48	A0A0G2K9Y0	Immunoglobulin heavy constant mu OS=Rattus norvegicus OX=10116 GN=Ighm PE=4 SV=1	63	52301	8
49	A0A0G2JWK0	Integrin beta OS=Rattus norvegicus OX=10116 GN=Itgb3 PE=1 SV=1	62	64287	1
50	A0A0G2K3K2	Actin, cytoplasmic 1 OS=Rattus norvegicus OX=10116 GN=Actb PE=1 SV=1	62	42401	4
51	D3ZY51	Plakophilin 1 OS=Rattus norvegicus OX=10116 GN=Pkp1 PE=4 SV=2	60	68486	4
52	A0A0G2JUY3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	56	12903	1
53	D3ZJW6	RCG21066 OS=Rattus norvegicus OX=10116 GN=rCG_21066 PE=1 SV=1	53	13226	2
54	A0A0G2JT70	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	50	10463	2
55	P01048	T-kininogen 1 OS=Rattus norvegicus OX=10116 GN=Map1 PE=1 SV=2	50	48828	4
56	A0A0G2K5D2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	50	13658	1

The yellow highlighted immunoglobulin, the orange highlighted Ig-like domain-containing protein, the blue highlighted complement C3, the green highlighted serum albumin and the red highlighted other complement proteins.

**Table S4** The list of identified proteins in Band-3.

#	prot_acc	prot_desc	prot_score	prot_mass	prot_matches
1	F1LTN6	Uncharacterized protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	2856	25184	100
2	P01836	Ig kappa chain C region, A allele OS=Rattus norvegicus OX=10116 PE=1 SV=1	1399	11896	46
3	A0A0G2JVP4	Immunoglobulin heavy constant mu OS=Rattus norvegicus OX=10116 GN=Ighm PE=1 SV=1	972	52360	49
4	M0RBF1	Complement C3 OS=Rattus norvegicus OX=10116 GN=C3 PE=1 SV=1	876	187746	43
5	P20762	Ig gamma-2C chain C region OS=Rattus norvegicus OX=10116 PE=2 SV=1	863	37062	39
6	A0A0G2K5D2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	788	13658	24
7	P02770	Serum albumin OS=Rattus norvegicus OX=10116 GN=Alb PE=1 SV=2	745	70682	29
8	A0A0G2K0N6	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	664	10860	19
9	F1M1R0	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 GN=LOC690813 PE=1 SV=2	663	15055	28
10	M0RC23	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	523	11302	15
11	D3ZBB2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=3	513	13251	17
12	A0A0G2K3S3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	503	14930	14
13	F1M7B3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	453	11304	13
14	M0RD98	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	436	13229	9
15	M0RAV0	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	403	10237	12
16	A0A0G2K7P6	IgV domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	385	10337	20
17	A0A0G2JTG4	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	341	10359	13
18	A0A0G2JY98	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	299	15486	10
19	F1LYF1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=3	283	15381	10
20	A0A0G2JUY3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	260	12903	9
21	A0A0G2K9Z5	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	236	13196	6
22	Q63041	Alpha-1-macroglobulin OS=Rattus norvegicus OX=10116 GN=A1m PE=1 SV=1	232	168388	8
23	P02680	Fibrinogen gamma chain OS=Rattus norvegicus OX=10116 GN=Fgg PE=1 SV=3	226	51228	12
24	D3ZZ08	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	219	13297	7
25	G3V7N9	Adiponectin a OS=Rattus norvegicus OX=10116 GN=C1qb PE=1 SV=1	212	26859	8
26	A0A0G2K9B1	Serine protease inhibitor A3N OS=Rattus norvegicus OX=10116 GN=LOC299282 PE=1 SV=1	196	48570	6

6						
2	Q4KM75	CD5 antigen-like OS=Rattus norvegicus OX=10116 GN=Cd5l PE=1 SV=1	184	39375	17	
7						
2	A0A0G2K4K2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	181	10515	7	
8						
2	A0A0G2JWX0	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	181	15610	9	
9						
3	A0A0G2K6T8	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	173	10452	8	
0						
3	F1LZ11	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	171	14286	5	
1						
3	P14480	Fibrinogen beta chain OS=Rattus norvegicus OX=10116 GN=Fgb PE=1 SV=4	168	54828	13	
2						
3	M0R8G6	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	166	11167	7	
3						
3	A0A0G2JYK0	Serine protease inhibitor A3N OS=Rattus norvegicus OX=10116 GN=LOC299282 PE=1 SV=1	164	68226	8	
4						
3	D3ZWC1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	152	12466	6	
5						
3	G3V836	Clusterin OS=Rattus norvegicus OX=10116 GN=Clu PE=1 SV=1	151	52015	9	
6						
3	A0A0G2QC06	Serotransferrin OS=Rattus norvegicus OX=10116 GN=Tf PE=1 SV=1	130	109507	8	
7						
3	A0A0G2K3K2	Actin, cytoplasmic 1 OS=Rattus norvegicus OX=10116 GN=Actb PE=1 SV=1	129	42401	11	
8						
3	Q7TQ70	Ac1873 OS=Rattus norvegicus OX=10116 GN=Fga PE=1 SV=1	121	87344	8	
9						
4	M0R9U2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	117	10952	4	
0						
4	A0A0G2JZV7	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	114	12261	2	
1						
4	A0A0G2JXK5	Serine protease inhibitor A3N OS=Rattus norvegicus OX=10116 GN=LOC299282 PE=1 SV=1	111	46361	7	
2						
4	P31720	Complement C1q subcomponent subunit A OS=Rattus norvegicus OX=10116 GN=C1qa PE=1 SV=2	108	26186	5	
3						
4	F1M5L5	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	108	13019	11	
4						
4	P20767	Ig lambda-2 chain C region OS=Rattus norvegicus OX=10116 PE=4 SV=1	104	11482	5	
5						
4	D4A3L8	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	95	14798	6	
6						
4	F1LXY6	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	92	11354	3	
7						
4	P04639	Apolipoprotein A-I OS=Rattus norvegicus OX=10116 GN=Apoa1 PE=1 SV=2	90	30100	5	

8						
4	A0A0G2JY31	Alpha-1-antiproteinase OS=Rattus norvegicus OX=10116 GN=Serpina1 PE=1 SV=1	89	46264	13	
9						
5	A0A0G2JZS9	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	87	10918	3	
0						
5	M0RAB8	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	85	11205	1	
1						
5	D3ZPL2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=3	84	11433	2	
2						
5	M0R7Q2	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	80	13315	2	
3						
5	G3V6G1	Immunoglobulin joining chain OS=Rattus norvegicus OX=10116 GN=Jchain PE=1 SV=1	80	18229	3	
4						
5	D3ZQR5	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	80	12873	2	
5						
5	D3ZC54	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	76	13293	2	
6						
5	F7F389	Complement component C9 OS=Rattus norvegicus OX=10116 GN=C9 PE=1 SV=1	75	66555	6	
7						
5	A0A0G2K290	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	75	14124	4	
8						
5	P55159	Serum paraoxonase/arylesterase 1 OS=Rattus norvegicus OX=10116 GN=Pon1 PE=1 SV=3	74	39561	3	
9						
6	Q6P0K8	Junction plakoglobin OS=Rattus norvegicus OX=10116 GN=Jup PE=1 SV=1	71	82490	8	
0						
6	M0R4Z4	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	71	13448	5	
1						
6	A0A0G2K151	Apolipoprotein E OS=Rattus norvegicus OX=10116 GN=ApoE PE=1 SV=1	71	41573	4	
2						
6	A0A0G2K4I8	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	70	12790	3	
3						
6	F1M3X3	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=2	69	13177	2	
4						
6	A0A0G2K7I1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	68	10253	2	
5						
6	A0A0G2K9Y0	Immunoglobulin heavy constant mu OS=Rattus norvegicus OX=10116 GN=IGHM PE=4 SV=1	66	52301	3	
6						
6	M0R5W6	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	65	12874	4	
7						
6	M0R816	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	65	10746	2	
8						
6	M0RBK4	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	64	11674	5	
9						
7	M0RBP7	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	64	12890	3	



0					
7	A0A0G2K099	Uncharacterized protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	64	54490	3
1					
7	A0A0G2K458	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=4 SV=1	63	12809	2
2					
7	A0A0G2JZN1	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=1	61	10470	4
3					
7	A0A0G2K3E0	Aquaporin-1 OS=Rattus norvegicus OX=10116 GN=Aqp1 PE=1 SV=1	59	26524	2
4					
7	A0A0G2JSK1	RCG20603 OS=Rattus norvegicus OX=10116 GN=Serpina3c PE=3 SV=1	59	46676	4
5					
7	FILMV6	Desmoplakin OS=Rattus norvegicus OX=10116 GN=Dsp PE=1 SV=1	58	334582	6
6					
7	FILWD0	Ig-like domain-containing protein OS=Rattus norvegicus OX=10116 PE=1 SV=2	56	12027	3
7					
7	D3ZFC6	Inter-alpha-trypsin inhibitor heavy chain 4 OS=Rattus norvegicus OX=10116 GN=Itih4 PE=1 SV=3	55	104027	2
8					
7	P19939	Apolipoprotein C-I OS=Rattus norvegicus OX=10116 GN=Apoc1 PE=3 SV=1	55	9854	4
9					
8	G3V7I0	Peroxiredoxin 3 OS=Rattus norvegicus OX=10116 GN=Prdx3 PE=1 SV=1	55	28567	2
0					
8	D3ZY51	Plakophilin 1 OS=Rattus norvegicus OX=10116 GN=Pkp1 PE=4 SV=2	53	68486	1
1					
8	A0A0G2JSW3	Globin a4 OS=Rattus norvegicus OX=10116 GN=Hbb PE=1 SV=1	52	16069	2
2					
8	A0A0G2JUP5	Murinoglobulin-1 OS=Rattus norvegicus OX=10116 GN=Mug1 PE=4 SV=1	50	162631	2
3					

The yellow highlighted immunoglobulin, the orange highlighted Ig-like domain-containing protein, the blue highlighted complement C3, the green highlighted serum albumin and the red highlighted other complement proteins.