

Myosin heavy chain-derived peptide of *Gadus morhua* promotes the proliferation and differentiation in osteoblasts and bone formation and maintains bone homeostasis in ovariectomized mice

Meilian Yang^a, Shuzhen Cheng^a, Wuchao Ma^a, Di Wu^a, Hesham R. El-Seedi^b,

Zhenyu Wang^a, Ming Du^{a, *}

^a School of Food Science and Technology, Collaborative Innovation Center of Seafood Deep Processing, National Engineering Research Center of Seafood, Dalian Polytechnic University, Dalian 116034, China.

^b Pharmacognosy Group, Department of Pharmaceutical Biosciences, Uppsala University, Biomedical Centre, SE 751 24 Uppsala, Sweden and International Research Center for Food Nutrition and Safety.

* Corresponding Authors: Prof. Ming Du (E-mail: duming@dlpu.edu.cn;

Fax: +86-411-86323262; Tel: +86-411-86332275)

Table S1 Peptides from GMP-c identified by UPLC-MS/MS.

No.	Start	AA sequence	End	Length	m/z	z	Mr.calc.	Protein	pI ^a	GRAVY ^b	-CDOCKER	
											interaction energy	
											3VI4	1L5G
		RGDN ^c									83.65	-
		c(RGDF-MVA) ^d									-	84.69
1	E	MNKKREAEFQ	K	10	1279.6458	1	1280.4661	Myosin heavy chain, fast skeletal muscle	8.34	-1.980	161.30	212.27
2	K	SRKVQHELEEAEE	R	13	792.3865	2	1582.7587	Myosin heavy chain, fast skeletal muscle-like	4.64	-1.815	163.04	166.94
3	I	DQDKSDFVEDELK	L	14	848.8760	2	1695.7474	A Chain A, Parvalbumin beta	3.90	-1.843	131.14	179.50
4	L	GETNPADSKPGSIR	G	14	476.9074	3	1427.7004	Nucleoside diphosphate kinase B-like	6.07	-1.350	126.59	176.85
5	M	EEPVTIPEKPHSEEE	L	15	875.4088	2	1748.8104	Calsequestrin-2-like	4.20	-1.713	156.76	142.61
6	A	GDSDGDGKIGVDE	F	13	632.2709	2	1262.5262	Parvalbumin beta-2	3.71	-1.162	138.66	158.14
7	F	KPSAPKIPDGEK	V	12	422.9062	3	1265.6979	Fast skeletal muscle troponin T	8.50	-1.533	134.29	156.12
8	I	IDQDKSDFVEE	D	11	1324.5891	1	1323.5830	Parvalbumin beta	3.77	-1.291	122.31	166.52
9	E	KSYELPDGQVITIG	N	14	760.4033	2	1518.7930	Fast skeletal muscle alpha-actin	4.37	-0.186	129.30	151.31
10	K	LEQQVDDLEGSLE	Q	13	737.8459	2	1473.6835	Myosin heavy chain, partial	3.39	-0.777	120.43	159.80
11	D	KSDFVEEDELK	L	11	669.8236	2	1337.6350	Parvalbumin beta	4.18	-1.391	126.05	148.65
12	E	NEKLMEEYE	K	9	1200.5061	1	1199.5016	Alpha-actinin-3	4.09	-1.889	140.50	128.92
13	L	EDLKDEDIGF	A	10	1180.5378	1	1179.5295	Calsequestrin-1-like isoform X1	3.77	-1.070	145.79	123.57
14	A	LEEAEGTLEHEES	K	13	736.8214	2	1471.6313	Myosin heavy chain, fast skeletal muscle-like isoform X1	3.89	-1.285	135.25	133.39
15	M	TDAEQDQLINDH	F	12	699.8110	2	1397.6058	Creatine kinase M-type	3.84	-1.525	117.30	149.05
16	D	FVEEDELKLF	L	10	1268.6421	1	1267.6335	A Chain A, Parvalbumin beta	4.00	-0.050	120.23	142.22
17	K	LEQQVDDLEGS	L	11	616.7822	2	1231.5568	Myosin-1B-like	3.43	-0.945	123.17	136.48
18	F	KDLLDPIISD	R	10	1128.6157	1	1127.6074	Ceatine kinase, partial	3.93	-0.020	116.07	142.38

19	F	KVEDEFPDL	S	9	1091.5293	1	1090.5182	Creatine kinase muscle type A, partial	3.92	-0.967	114.58	143.56
20	K	LLPVLYPPVVEE	V	12	1367.7822	1	1366.7748	Myosin heavy chain, fast skeletal muscle-like isoform X3	3.79	0.908	135.18	121.96
21	Q	QVDDLEGSLE	Q	10	1087.4761	1	1086.4717	Myosin heavy chain, fast skeletal muscle-like isoform X3	3.43	-0.690	106.84	149.15
22	E	SHQQTLLDDL	Q	9	528.7491	2	1055.4883	Myosin heavy chain, fast skeletal muscle-like isoform X1	4.20	-1.233	103.61	150.84
23	E	EIVDPNVDEL	S	10	1142.5603	1	1141.5503	LOW QUALITY PROTEIN: filamin-C-like	3.43	-0.240	108.85	143.81
24	Q	EEGWDRDLLDPA	W	13	764.8678	2	1527.7205	Alpha-actinin-3	3.77	-0.900	113.40	138.83
25	S	DFVEEDELKLF	L	11	1383.6693	1	1382.6605	Parvalbumin beta	3.83	-0.364	100.63	147.68
26	F	SGDEEFPDLS	L	10	1095.4429	1	1094.4403	Creatine kinase M-type-like	3.43	-1.100	97.93	149.27
27	W	PVIYLLFWGFL	L	11	1367.7822	1	1366.7689	Large neutral amino acids transporter small subunit 2-like	5.95	1.955	106.90	136.81
28	R	EQFEEQEAK	A	10	633.7778	2	1265.5411	Myosin-7	3.98	-2.380	103.69	139.63
29	M	TDAEQDQLIN	D	10	1146.5283	1	1145.5200	Creatine kinase muscle type A, partial	3.49	-1.160	99.76	141.78
30	K	FSGDEEFPDLSLH	N	13	746.8330	2	1491.6517	Creatine kinase M-type-like	3.91	-0.585	115.27	125.93
31	A	LEEAEGTLE	H	9	990.4592	1	989.4553	Myosin heavy chain, fast skeletal muscle-like	3.58	-0.633	88.99	145.51
32	L	REQFEEQE	A	9	612.2603	2	1222.5101	Myosin heavy chain, fast skeletal muscle-like isoform X1	3.98	-2.911	110.59	123.81
33	K	FSGDEEFPDLSL	H	12	1355.6008	1	1354.5928	Creatine kinase M-type-like	3.43	-0.367	93.43	140.25
34	R	EFDDLPISEQ	A	10	1192.5334	1	1191.5295	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	3.43	-0.880	95.04	136.44
35	F	SGDEEFPDL	S	9	1008.4145	1	1007.4083	Creatine kinase M-type-like	3.43	-1.133	108.66	122.75
36	Q	ITGTEREAEQE	A	12	696.3177	2	1390.6212	PDZ and LIM domain protein 5 isoform X1	3.98	-1.750	118.59	112.06
37	K	SDFVEEDELK	L	10	1210.5497	1	1209.5400	A Chain A, Parvalbumin beta	3.83	-1.140	95.45	135.02
38	V	LDPDLGLEE	E	9	1000.4854	1	999.4760	MYCBP-associated protein isoform X1	3.43	-0.511	94.26	134.52

39	E	EIVDPNVDE	H	9	1029.4713	1	1028.4662	Filamin-C-like isoform X1	3.43	-0.689	108.71	119.05
40	N	EVDFYEPF	M	8	1045.4507	1	1044.4440	Calsequestrin-1-like	3.57	-0.450	87.90	139.22
41	A	SEGDNLNDAEER	C	12	674.7847	2	1347.5538	Myosin heavy chain, fast skeletal muscle-like isoform X1	3.83	-2.050	100.84	126.22
42	F	SGDEEFPDLSL	H	11	1208.5310	1	1207.5244	Creatine kinase M-type-like	3.43	-0.655	89.83	135.03
43	R	EFDDLPISE	Q	9	1064.4766	1	1063.4709	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	3.43	-0.589	93.29	131.29
44	K	DLLDPIISD	R	9	500.7639	2	999.5124	Creatine kinase M-type	3.42	0.411	109.93	114.39
45	E	LDEIIEE	V	7	860.4259	1	859.4174	Troponin C, skeletal muscle-like	3.50	-0.171	101.97	121.77
46	S	DFVEEDELKL	F	10	1236.5959	1	1235.5920	A Chain A, Parvalbumin beta	3.83	-0.680	97.35	126.30
47	A	PEAGPEPEPE	P	10	1051.4565	1	1050.4506	Troponin T, fast skeletal muscle isoforms-like isoform X7	3.58	-1.900	107.00	114.29
48	E	KTIDDLEDE	L	9	1077.4896	1	1076.4873	Tropomyosin alpha-1 chain isoform X1	3.77	-1.533	100.73	118.04
49	L	QEAAEQIE	A	8	479.7020	2	957.3927	Myosin heavy chain, fast skeletal muscle-like isoform X1	3.58	-1.837	105.38	113.20
50	V	EDEFPDLS	K	8	951.3950	1	950.3868	Creatine kinase muscle type A, partial	3.43	-1.225	90.37	126.73
51	F	DYDPAEDNIQ	S	10	1179.4769	1	1178.4728	Nebulin isoform X1	3.37	-1.760	100.42	116.09
52	D	FVEEDELK	L	8	1008.4822	1	1007.4811	A Chain A, Parvalbumin beta	4.00	-0.887	84.38	129.90
53	E	QQVDDLEGSLE	Q	11	1215.5352	1	1214.5303	Myosin heavy chain, partial	3.43	-0.945	96.30	117.94
54	Y	DVIEDPVE	I	8	915.4259	1	914.4233	Calsequestrin-1-like isoform X1	3.43	-0.338	81.09	132.84
55	K	VEDEFPDLS	K	9	1050.4604	1	1049.4553	Creatine kinase M-type	3.43	-0.622	90.27	117.55
56	Q	GEVDDSVQE	A	9	977.4075	1	976.3985	Myosin-8-like	3.43	-1.144	87.99	112.08
57	Q	GEVDDSIQE	A	9	991.4146	1	990.4142	Myosin heavy chain, fast skeletal muscle-like	3.43	-1.111	94.34	104.81
58	V	DDLEGSLE	Q	8	877.3809	1	876.3712	Myosin heavy chain, fast skeletal muscle-like	3.43	-0.950	91.99	107.04
59	I	TEPEAIPDQ	V	9	1000.4440	1	999.4396	Calphotin-like isoform X1	3.57	-1.289	83.19	114.31
60	S	GDEEFPDL	S	8	921.3809	1	920.3763	Creatine kinase M-type-like	3.43	-1.175	72.04	123.67

61	C	DDEETAL	V	8	935.3776	1	934.3767	Actin, alpha cardiac-like	3.43	-1.225	78.06	117.39
62	E	QQVDDLE	G	7	829.3526	1	828.3501	Myosin-6-like	3.49	-1.357	102.14	86.76
63	K	KDIDDLE	L	7	847.4007	1	846.3970	Myosin-6-like	3.84	-1.371	83.62	102.98
64	T	ERE	K	3	433.2028	1	432.1968	Ataxin-2-like protein isoform X2	-	-	90.05	96.06
65	R	EFDDLPI	E	8	935.4338	1	934.4283	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	3.49	-0.225	74.79	107.26
66	E	EVFE	G	4	523.2391	1	522.2325	Inactive rhomboid protein 1-like isoform X1	-	-	66.77	108.14
67	P	SSLDP	F	5	518.2436	1	517.2384	Transcription factor HIVP2	3.80	-0.580	79.16	94.97
68	A	LDENE	M	6	766.3256	1	765.3181	Actin, alpha cardiac-like	3.57	-1.233	72.78	97.46
69	L	REQFEEQEA	K	10	647.7809	2	1293.5472	Myosin-6-like	3.98	-2.440	83.64	84.69
70	L	TLIT	G	5	560.3636	1	559.3581	Myosin-7-like	5.19	2.280	74.18	92.31
71	Q	TITPEYE	V	7	852.3963	1	851.3912	Nebulin isoform X1	3.79	-0.971	81.51	84.39
72	K	DIDDLE	L	6	701.2943	1	700.2915	Myosin heavy chain, fast skeletal muscle-like isoform X1	3.37	-0.950	70.80	87.45
73	K	VFL	K	3	378.2387	1	377.2314	Collagen alpha-2(VI) chain-like isoform X1	-	-	67.63	82.76
74	T	HPST	F	4	441.2098	1	440.2019	Nebulin isoform X1	-	-	67.34	69.17

Theoretical properties determined by ProtParam tool (<https://web.expasy.org/protparam/>).

^a pI: isoelectric point; ^b GRAVY: Grand average of hydropathicity; ^c RGDN is a short peptide acting as the ligand and is bound by the integrin $\alpha 5 \beta 1$ (PDB: 3VI4) receptor pro-active site. ^d c(RGDf-MVA) is a cyclic peptide acting as the ligand and is bound by the integrin $\alpha v \beta 3$ (PDB: 1L5G) receptor pro-active site.

Table S2 The interaction site between integrin (PDB: 3VI4) and P-GM-1 (MNKKREAEFQ).

Interaction residue	Distance (Å)	Category	Types
MNKKREAEFQ:H53 - C:ASP228:OD2	1.98316	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
MNKKREAEFQ:H73 - D:GLU229:OE2	1.86837	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
MNKKREAEFQ:H96 - C:ASP227:OD2	2.00172	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
D:LYS182:NZ - MNKKREAEFQ:O139	3.92794	Electrostatic	Attractive Charge
MNKKREAEFQ:N1 - D:GLU320:OE2	2.8988	Electrostatic	Attractive Charge
D:SER134:HN - MNKKREAEFQ:O114	2.5815	Hydrogen Bond	Conventional Hydrogen Bond
D:SER134:HG - MNKKREAEFQ:O114	2.58277	Hydrogen Bond	Conventional Hydrogen Bond
D:LYS182:HZ3 - MNKKREAEFQ:O173	2.09118	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H51 - C:ASP227:O	1.81578	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H52 - C:ASP228:O	2.92792	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H75 - D:SER227:O	3.00913	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H99 - C:ASP227:OD2	1.98186	Hydrogen Bond	Conventional Hydrogen Bond
C:ILE225:HA - MNKKREAEFQ:O28	2.66334	Hydrogen Bond	Carbon Hydrogen Bond
D:SER177:HB1 - MNKKREAEFQ:O138	2.87872	Hydrogen Bond	Carbon Hydrogen Bond
D:SER177:HB2 - MNKKREAEFQ:O138	2.67452	Hydrogen Bond	Carbon Hydrogen Bond
MNKKREAEFQ:H70 - D:SER134:OG	2.51207	Hydrogen Bond	Carbon Hydrogen Bond
MNKKREAEFQ:H71 - D:SER227:O	2.40846	Hydrogen Bond	Carbon Hydrogen Bond
MNKKREAEFQ:N94 - C:PHE187	4.27687	Electrostatic	Pi-Cation
MNKKREAEFQ:H163 - C:TRP157	2.9863	Hydrogen Bond	Pi-Donor Hydrogen Bond

Table S3 The interaction site between integrin (PDB: 3VI4) and RGD.

Interaction residue	Distance (Å)	Category	Types
RGD:H4 - D:GLU229:OE2	1.82364	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
D:LYS182:NZ - RGD:O43	3.4447	Electrostatic	Attractive Charge
RGD:N19 - C:ASP227:OD2	5.43225	Electrostatic	Attractive Charge
D:SER134:HN - RGD:O26	2.73664	Hydrogen Bond	Conventional Hydrogen Bond
D:SER134:HG - RGD:O26	2.10986	Hydrogen Bond	Conventional Hydrogen Bond
D:LYS182:HZ2 - RGD:O42	1.89479	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H2 - D:SER227:O	1.89724	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H24 - C:ASP227:OD2	3.01201	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H28 - D:ASN224:O	2.84598	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H35 - D:ASN224:O	2.44018	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H31 - D:ASN224:O	2.29132	Hydrogen Bond	Carbon Hydrogen Bond
RGD:H21 - C:PHE187	2.51331	Hydrogen Bond; Electrostatic	Pi-Cation; Pi-Donor Hydrogen Bond

Table S4 The interaction site between integrin (PDB: 1L5G) and P-GM-1 (MNKKREAEFQ).

Interaction residue	Distance (Å)	Category	Types
MNKKREAEFQ:H2 - B:ASP127:OD2	2.77435	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
MNKKREAEFQ:H3 - B:ASP251:OD2	2.07426	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
MNKKREAEFQ:H74 - B:GLU220:OE2	1.93446	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
A:LYS119:NZ - MNKKREAEFQ:O179	2.72285	Electrostatic	Attractive Charge
B:ARG214:NH1 - MNKKREAEFQ:O114	4.68449	Electrostatic	Attractive Charge
B:ARG216:NH1 - MNKKREAEFQ:O139	5.10515	Electrostatic	Attractive Charge
MNKKREAEFQ:N1 - B:ASP126:OD2	3.06328	Electrostatic	Attractive Charge
MNKKREAEFQ:N94 - A:ASP150:OD2	4.23321	Electrostatic	Attractive Charge
MNKKREAEFQ:N94 - A:ASP218:OD2	5.30428	Electrostatic	Attractive Charge
A:LYS119:HZ3 - MNKKREAEFQ:O178	1.91168	Hydrogen Bond	Conventional Hydrogen Bond
B:TYR166:HH - MNKKREAEFQ:O138	2.25999	Hydrogen Bond	Conventional Hydrogen Bond
B:ARG214:HH21 - MNKKREAEFQ:O114	2.2212	Hydrogen Bond	Conventional Hydrogen Bond
B:ARG214:HH21 - MNKKREAEFQ:O126	2.14244	Hydrogen Bond	Conventional Hydrogen Bond
B:ARG216:HH21 - MNKKREAEFQ:O139	1.95465	Hydrogen Bond	Conventional Hydrogen Bond
B:ASN313:HD21 - MNKKREAEFQ:S13	2.74028	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H3 - B:SER123:O	1.893	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H4 - B:ASP127:OD1	2.09672	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H21 - B:SER123:O	2.18061	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H21 - B:ASP251:OD1	2.27035	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H35 - B:ASP251:OD1	2.40762	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H73 - B:GLU220:OE1	2.08292	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H74 - B:ASP217:O	1.83656	Hydrogen Bond	Conventional Hydrogen Bond

MNKKREAEFQ:H74 - B:ALA218:O	2.8467	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H75 - B:ALA218:O	2.52906	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H92 - A:ASP218:OD1	2.0331	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H143 - B:ASP179:OD2	2.80773	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H143 - B:ASP179:O	2.33354	Hydrogen Bond	Conventional Hydrogen Bond
MNKKREAEFQ:H176 - B:ASP179:OD1	2.60855	Hydrogen Bond	Conventional Hydrogen Bond
A:LYS119:HE2 - MNKKREAEFQ:O178	2.98249	Hydrogen Bond	Carbon Hydrogen Bond
MNKKREAEFQ:H6 - B:ASP251:OD1	2.54079	Hydrogen Bond	Carbon Hydrogen Bond
MNKKREAEFQ:H70 - B:ASP217:O	2.60383	Hydrogen Bond	Carbon Hydrogen Bond
MNKKREAEFQ:H71 - B:GLU220:OE1	3.08051	Hydrogen Bond	Carbon Hydrogen Bond

Table S5 The interaction site between integrin (PDB: 1L5G) and RGD.

Interaction residue	Distance (Å)	Category	Types
A:ARG248:HH22 - RGD:O42	3.09416	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
B:LYS253:HZ3 - RGD:O42	1.96134	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
RGD:H18 - B:GLU220:OE1	1.85456	Hydrogen Bond; Electrostatic	Salt Bridge; Attractive Charge
A:TYR178:HH - RGD:O72	2.59884	Hydrogen Bond	Conventional Hydrogen Bond
B:LYS253:HZ1 - RGD:O41	2.18312	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H14 - B:ASN215:O	2.4394	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H17 - B:ASP217:O	2.11876	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H20 - B:TYR122:O	2.25997	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H21 - B:SER121:OG	2.53508	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H21 - B:GLU220:OE1	2.27468	Hydrogen Bond	Conventional Hydrogen Bond
RGD:H12 - B:TYR122:O	2.33875	Hydrogen Bond	Carbon Hydrogen Bond
RGD - A:ALA215	3.78369	Hydrophobic	Pi-Alkyl

Figure S1 The ligand–protein interactions between P-GM-1 (MNKKREAEFQ) and integrin (PDB: 3VI4), i.e., the H-bond interactions, interpolated charge interactions, and hydrophobic interactions, respectively.

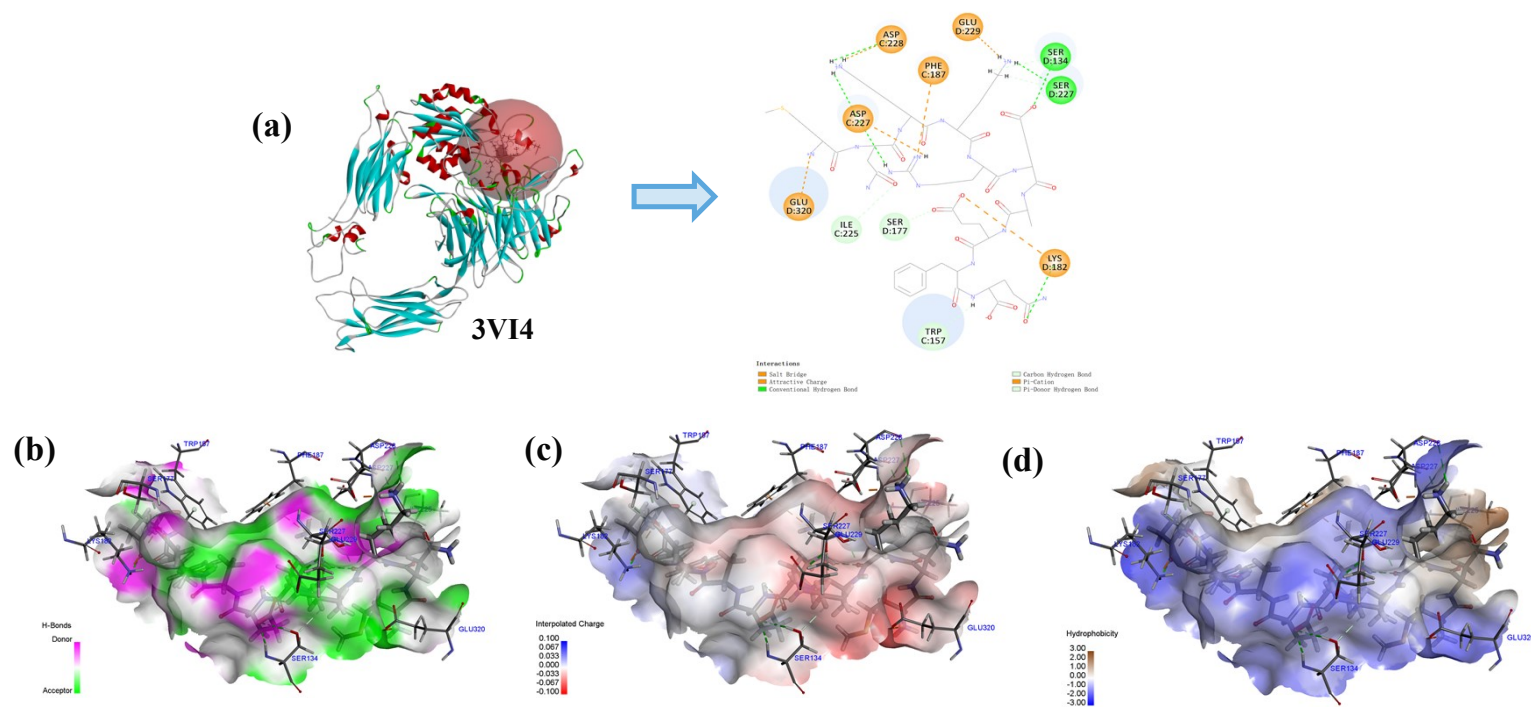
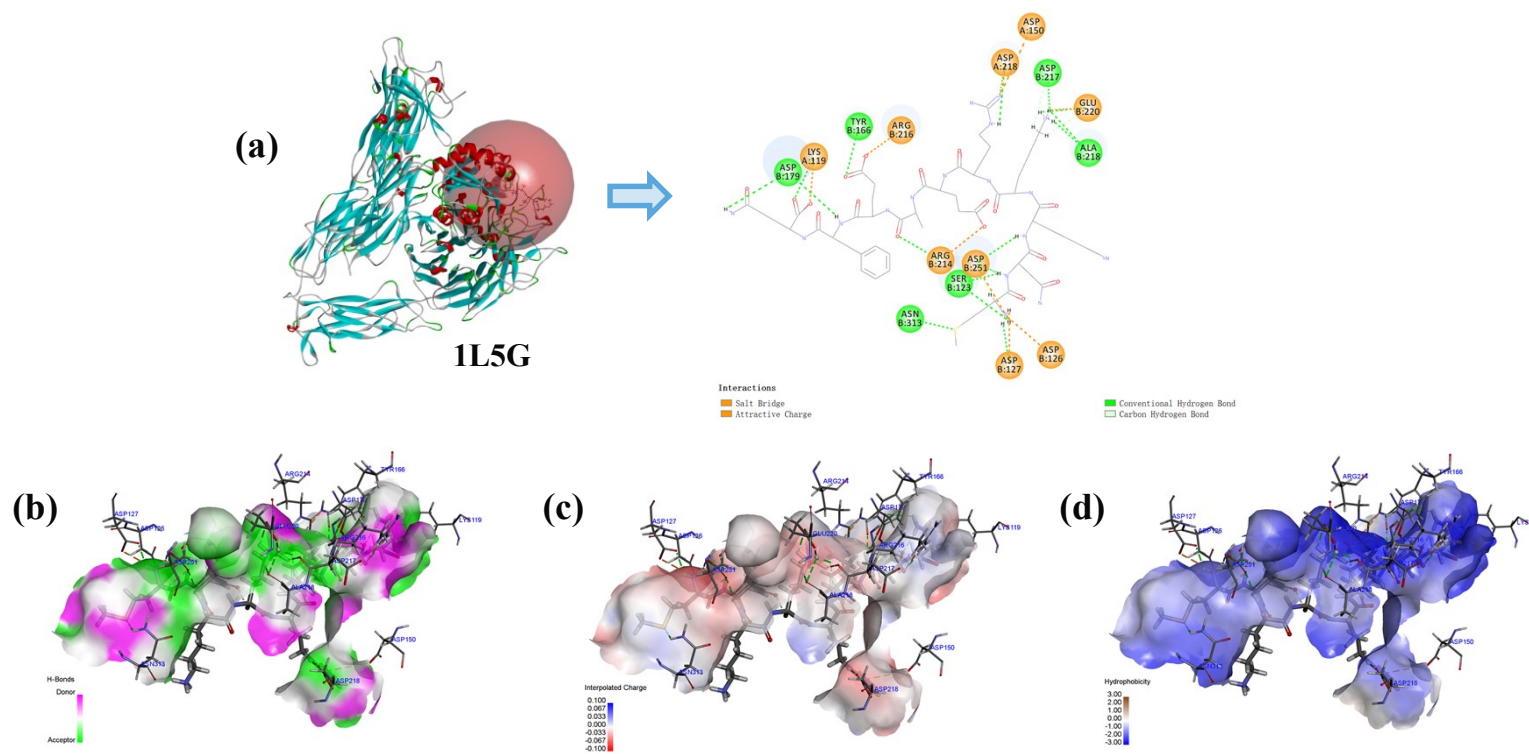


Figure S2 The ligand–protein interactions between P-GM-1 (MNKKREAEFQ) and integrin (PDB: 1L5G), i.e., the H-bond interactions, interpolated charge interactions, and hydrophobic interactions, respectively.



Raw images of Western blot data
Figure S3

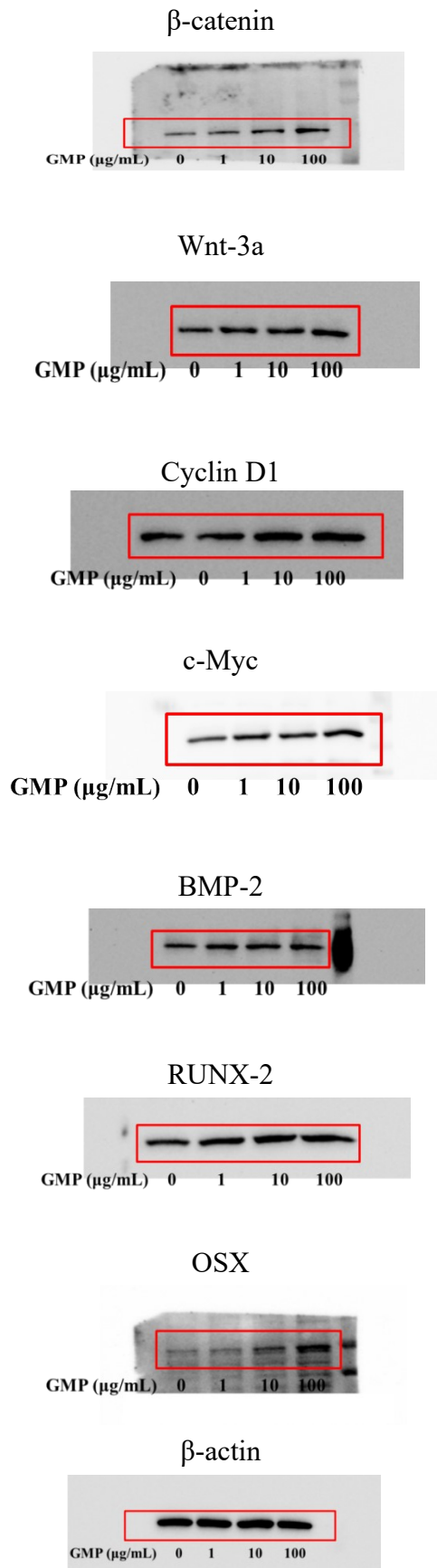


Figure S4

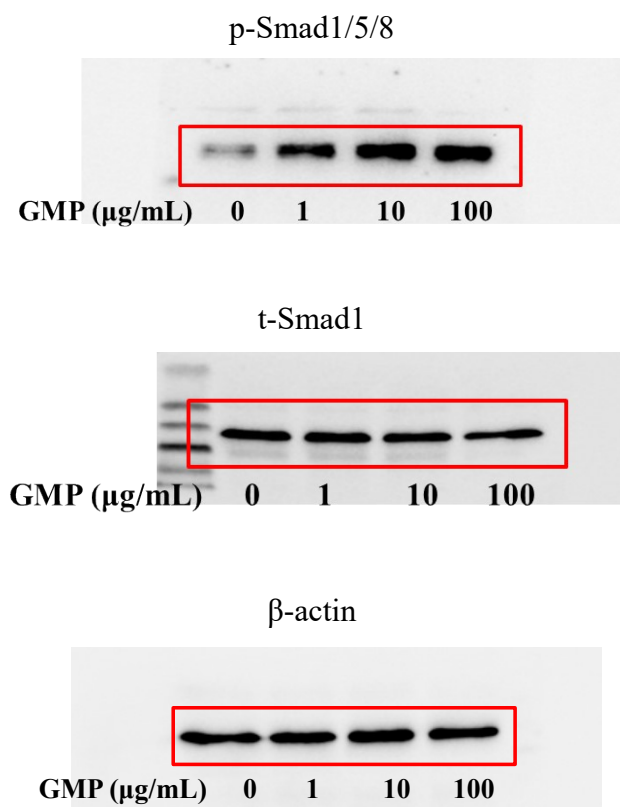


Figure S5

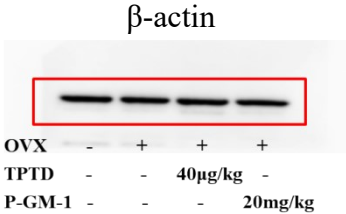
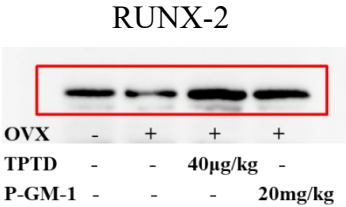
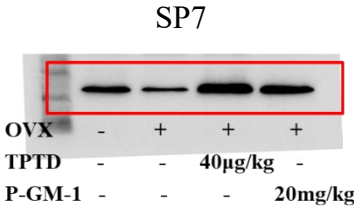
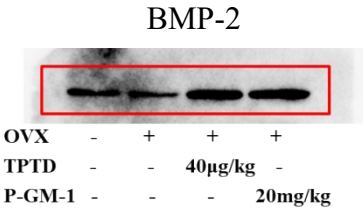


Figure S6

