Quantitative proteomics reveal the anti-tumour mechanism of the carbohydrate recognition domain of Galectin-3 in Hepatocellular carcinoma

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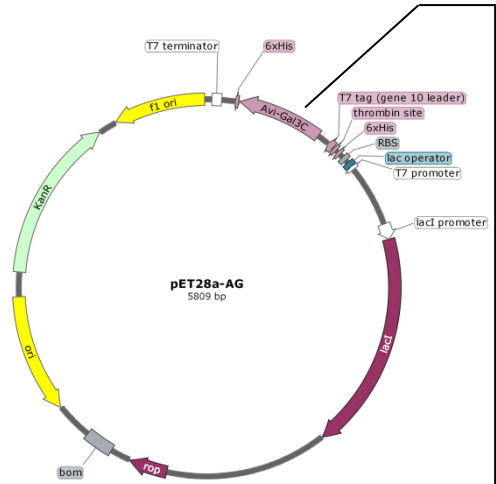
Running title: Anti-tumor mechanism revealed by quantitative proteomics

Key words: Hepatocellular carcinoma**,** Quantitative proteomics, Gal3C

A



>Avi-Gal3C

ATGGCTGGGCCACTGATTGTGCCTTATAACCTGCCTTTG
CCTGGGGGAGTGGTGCCTCGCATGCTGATAACAATTCTG
GGCACGGTGAAGCCCAATGCAAACAGAATTGCTTTAGA
TTTCCAAAGAGGGAATGATGTTGCCTTCCACTTTAACCC
ACGCTTCAATGAGAACAACAGGAGAGTCATTGTTTGCAA
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AAAAACTCAATGAAATCAGCAAACTGGGAATTTCTGGT
GACATAGACCTCACCAGTGCTTCATATACCATGATAGGA
CTGAATGACATCTTCGAAGCACAGAAAATCGAGTGGCA
TGAG

B

>Sequencing result of Avi-Gal3C

ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACT
GGTGGACAGCAAATGGGTCGCGGATCCGAATTCATGGCTGGGCCACTGATTGTGCCTTATAACCTGCCTTTGCCTG
GGGGAGTGGTGCCTCGCATGCTGATAACAATTCTGGGCACGGTGAAGCCCAATGCAAACAGAATTGCTTTAGATT
TCCAAAGAGGGAATGATGTTGCCTTCCACTTTAACCCACGCTTCAATGAGAACAACAGGAGAGTCATTGTTTGCAA
TACAAAGCTGGATAATAACTGGGGAAGGGAAGAAAGACAGTCGGTTTTCCCATTTGAAAGTGGGAAACCATTCAA
AATACAAGTACTGGTTGAACCTGACCACTTCAAGGTTGCAGTGAATGATGCTCACTTGTTGCAGTACAATCATCGG
GTTAAAAAACTCAATGAAATCAGCAAACTGGGAATTTCTGGTGACATAGACCTCACCAGTGCTTCATATACCATGA
TAGGACTGAATGACATCTTCGAAGCACAGAAAATCGAGTGGCATGAGCTCGAGCACCACCACCACCACCACTGA

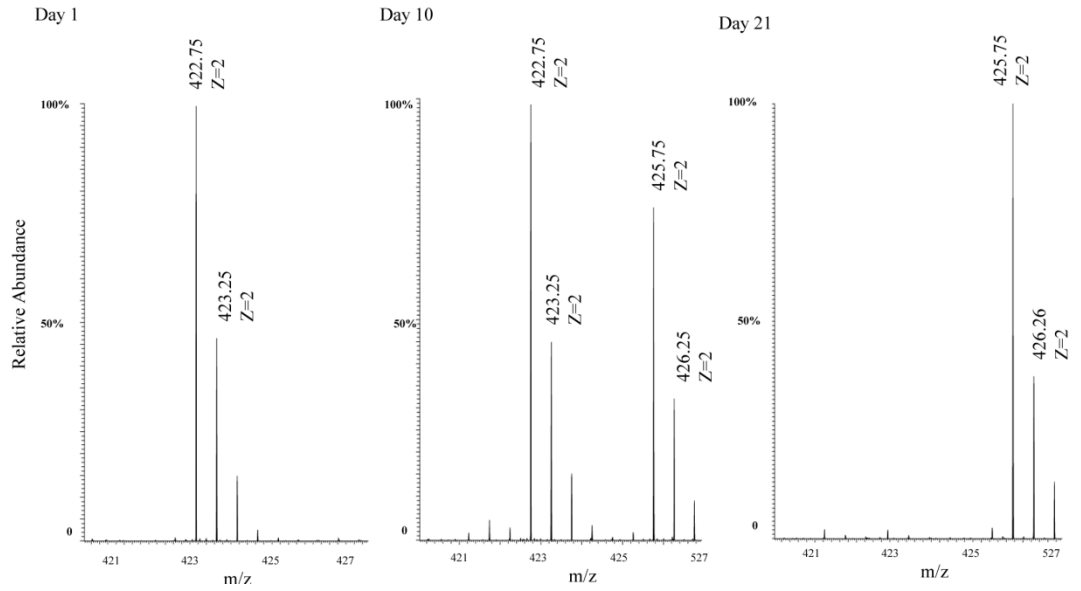
C

>rGal3C

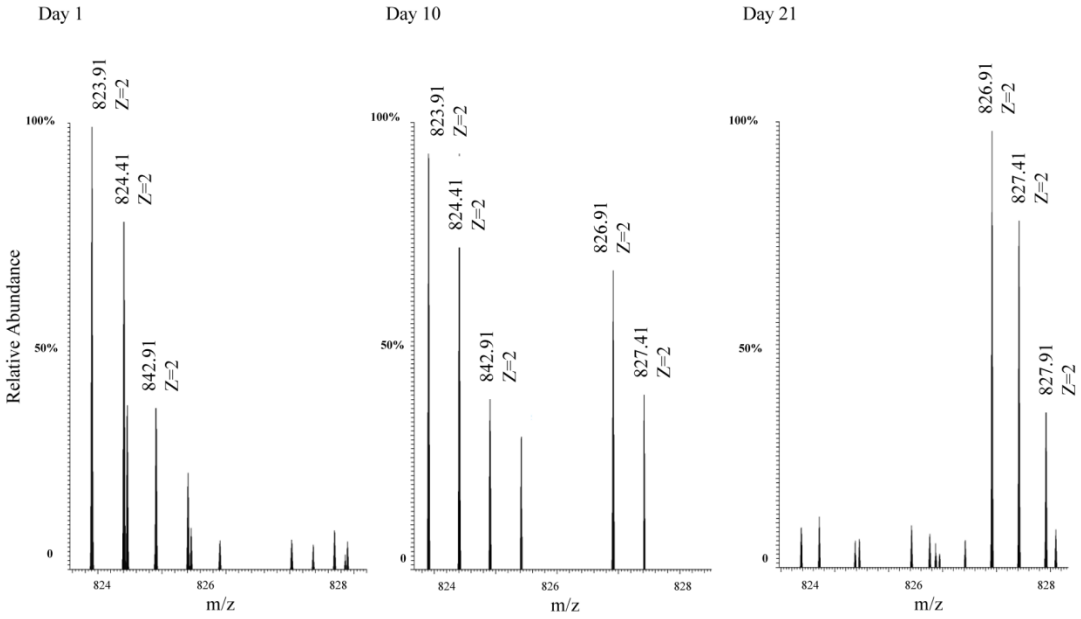
MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFMAGPLIVPYNLPLPGGVVPRMLITILGTVKPNANRIALDFQR
GNDVAFHFNPRFNENNRRVIVCNTKLDNNWGREERQSVFPFESGKPFKIQVLVEPDHFKVAVNDAHLLQYNHRVKKL
NEISKLGISGDIDLTSASYTMIGLNDIFEAQKIEWHELEHHHHHH

Supplementary Figure S1. Informtion of pET28a-AG and rGal3C.(A) Plasmid pET28a-AG was synthesized by inserting the 468 bp Avi-Gal3C DNA fragment into plasmid pET28a. (B) Nucleotide sequencing results of the inserted DNA fragment(Avi-Gal3C), the DNA fragment encoding carbohydrate recognize domain of Galectin-3 was underlined. (C) Amino acid sequence of rGal3C. The first 36 amine acids were the N-terminal histione tag (HisHisHisHisHisHis) and T7 tag(MetAlaSerMetThrGlyGlyGlnGlnMetGly), and underlined amine acids from 37-177 were the core sequence of carbohydrate recognition domain of galectin-3, and amine acids from 178-200 were the avi-tag(GlyLeuAsnAspIlePheGluAlaGlnLysIleClu) and C-terminal histione tag (HisHisHisHisHisHis).

IPAMTIA**K**



LLSELDQQSTEMP**R**



Supplementary Figure S2. Incorporation of $^{13}C_6$-L-lysine and $^{13}C_6$-L-arginine into HepG2 at various time points. The peptide IPAMTIAK (Mr=843.49) has a charge state of 2 and contains one lysine, the peptide LLSELDQQSTEMPR (Mr=1645.84) has a charge state of 2 and contains one arginine, and their heavy isotope is 6.032 Da higher than the light isotope. The ratio of intensity of heavy to light peak steadily increased. On day 21, this peptide was fully incorporated with $^{13}C_6$-L-lysine or $^{13}C_6$-L-arginine.

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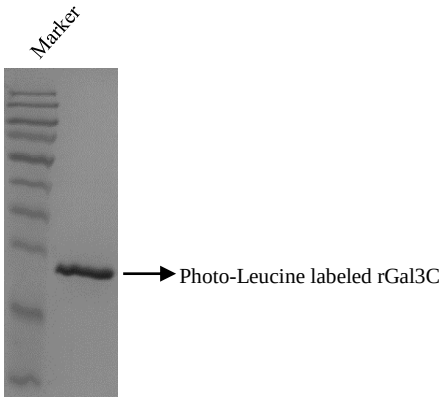
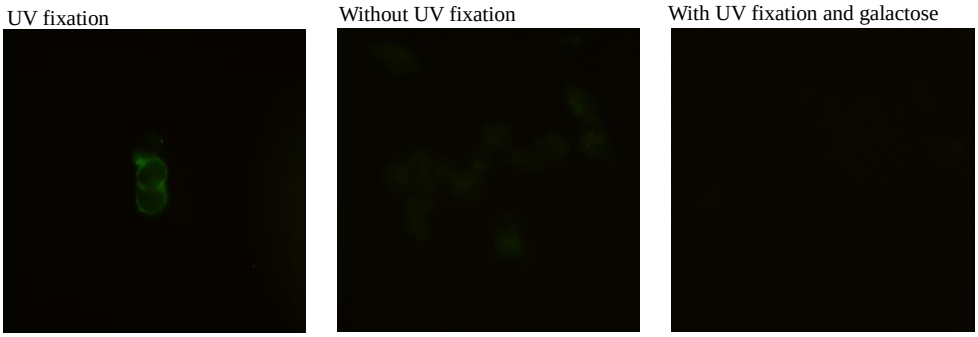


B



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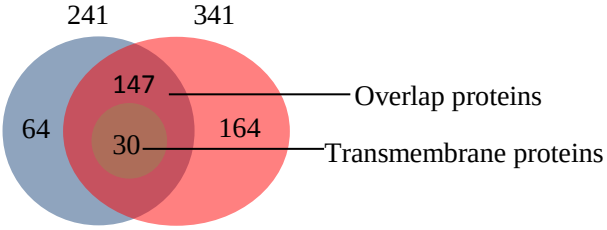
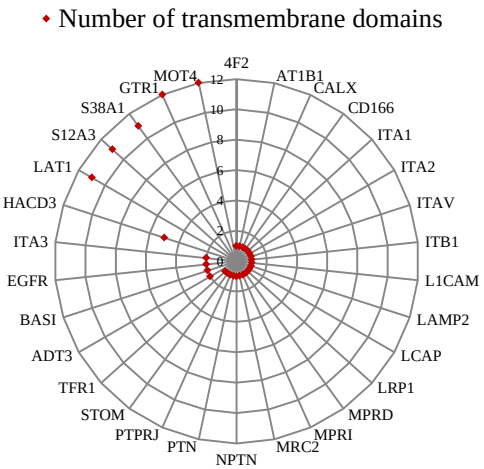


D



Supplementary Figure S3. (A) The Photo-Leucine labeled rGal3C was detected by 12% SDS-PAGE. (B) The binding of Photo-Leucine labeled rGal3C to HepG2 cell surface with UV fixation, without UV fixation or with UV fixation in the presence of lactose. (C) Overall 241 and 341 proteins were identified in two independent repeated experiments respectively, and 177 were repeated identified. In the 177 overlap proteins, 30 transmembrane proteins analyzed by TMHMM

Server v2.0 were considered as potential receptors of rGal3C. (D) A radar map displayed the number of transmembrane domians of the 30 potential receptors of rGal3C.

Supplementary table S1 List of up-regulated proteins

Protein IDs	Gene names	unique peptide number		SILAC ratio	
		H/L	L/H	H/L	L/H
P09455	RBP1	1	2	5.46	3.23
Q9UJX2	CDC23	3	1	1.31	3.05
P31949	S100A11	2	2	5.16	2.43
Q9NX74	DUS2	2	1	1.08	2.30
O95470	SGPL1	9	10	1.13	2.05
Q6PUV4	CPLX2	4	2	2.93	2.05
Q14764	MVP	8	5	2.54	2.03
P37802	TAGLN2	3	2	1.78	1.97
O75746	SLC25A12	3	2	1.03	1.87
Q9BQL6	FERMT1	2	3	1.72	1.86
P09382	LGALS1	4	4	2.87	1.85
P15559	NQO1	8	9	1.29	1.84
Q56P03	EAPP	1	2	1.01	1.81
P01011	SERPINA3	6	6	3.21	1.81
P52943	CRIP2	3	2	1.47	1.79
O43301	HSPA12A	2	2	1.78	1.77
Q92887	ABCC2	10	9	1.01	1.76
Q9Y3A2	UTP11L	3	2	1.01	1.76
P38571	LIPA	3	6	1.11	1.74
P31947	SFN	8	7	1.45	1.72
P53634	CTSC	7	6	1.12	1.68
Q14914	PTGR1	4	4	1.07	1.68
O14908	GIPC1	2	2	1.07	1.66
Q8N1I0	DOCK4	3	2	1.04	1.65
P56747	CLDN6	1	3	2.08	1.63
Q9UGH3	SLC23A2	1	1	1.23	1.63
P10644	PRKAR1A	9	8	1.13	1.61
O60443	DFNA5	6	5	1.10	1.59
Q99519	NEU1	4	3	1.91	1.54
O75312	ZPR1	5	3	1.06	1.54
Q8IYU8	MICU2	5	3	1.19	1.53
Q00653	NFKB2	3	3	1.19	1.53
P06280	GLA	2	3	1.03	1.53
P36955	SERPINF1	5	7	1.19	1.52
P48506	GCLC	10	12	1.14	1.51
O60678	PRMT3	2	3	1.21	1.51
Q13509	TUBB3	4	4	2.01	1.46
P13284	IFI30	2	2	1.78	1.44

P00966	ASS1	10	11	1.71	1.39
P60981	DSTN	7	9	1.50	1.38
P01009	SERPINA1	17	17	1.54	1.35
O60218	AKR1B10	16	16	2.31	1.33
Q16658	FSCN1	12	12	2.02	1.31
P23368	ME2	3	2	1.56	1.29
P04792	HSPB1	5	2	2.56	1.23
Q16643	DBN1	6	6	1.72	1.20
Q13501	SQSTM1	6	2	2.59	1.18
P21333	FLNA	66	67	1.54	1.16
Q01995	TAGLN	9	6	5.26	1.15
Q9NR12	PDLIM7	6	4	1.73	1.15
Q99536	VAT1	10	12	1.57	1.15
Q9NRZ9	HELLS	5	8	1.53	1.15
Q14807	KIF22	4	5	1.56	1.14
Q8IYB7	DIS3L2	3	4	1.57	1.14
P01034	CST3	3	2	1.55	1.13
Q16881	TXNRD1	12	14	1.79	1.13
P67936	TPM4	5	4	1.50	1.12
Q9HC07	TMEM165	2	2	1.68	1.11
Q7Z406	MYH14	3	2	1.83	1.10
P51948	MNAT1	1	1	1.67	1.10
P52292	KPNA2	6	6	1.85	1.10
Q5TFE4	NT5DC1	8	8	1.69	1.10
P78504	JAG1	3	2	1.68	1.09
P46821	MAP1B	3	3	1.93	1.09
Q9UK76	HN1	2	1	1.77	1.08
P42575	CASP2	2	1	1.53	1.07
P35579	MYH9	44	33	1.91	1.06
P02794	FTH1	3	2	1.91	1.05
Q9H0A8	COMMD4	6	2	1.52	1.05
Q01664	TFAP4	1	2	1.68	1.04
Q9UPY5	SLC7A11	1	2	2.47	1.04
Q9ULT8	HECTD1	11	3	1.63	1.03
P04035	HMGCR	6	2	2.50	1.03
O14530	TXNDC9	4	4	1.50	1.02
Q9Y371	SH3GLB1	6	2	1.70	1.01
P31153	MAT2A	5	2	1.60	1.01
P06400	RB1	5	3	1.58	1.01
P46013	MKI67	17	15	1.63	1.00
Q99439	CNN2	7	6	1.74	1.00

Supplementary Table S2 List of down-regulated proteins

Protein IDs	Gene names	unique peptide number		SILAC ratio	
		H/L	L/H	H/L	L/H
Q08AH3	ACSM2A	2	3	0.55	0.22
Q92597	NDRG1	9	9	0.37	0.38
P10909	CLU	11	6	0.29	0.53
P48029	SLC6A8	3	3	0.71	0.25
P37059	HSD17B2	5	6	0.86	0.21
P08833	IGFBP1	3	4	0.58	0.32
P05121	SERPINE1	9	12	0.59	0.34
P0C7P0	CISD3	1	2	0.61	0.33
P23141	CES1	26	27	0.95	0.27
P08319	ADH4	11	9	0.62	0.43
P02671	FGA	9	8	0.42	0.64
Q06033	ITIH3	6	2	0.32	0.85
P35575	G6PC	1	1	0.30	0.94
P21549	AGXT	9	9	0.84	0.35
O00584	RNASET2	7	9	0.46	0.64
Q16877	PFKFB4	5	4	0.57	0.54
Q13740	ALCAM	6	7	0.74	0.41
Q02338	BDH1	8	7	0.50	0.63
P02787	TF	30	28	0.61	0.53
Q6UWM9	UGT2A3	8	6	0.67	0.48
Q4G0N4	NADK2	6	5	0.60	0.54
Q8N6C5	IGSF1	14	8	0.46	0.72
P55268	LAMB2	6	4	0.70	0.49
P13674	P4HA1	15	17	0.52	0.66
P07148	FABP1	9	8	0.61	0.58
Q9Y646	CPQ	4	3	0.63	0.57
P52569	SLC7A2;SLC7A1	2	1	0.67	0.55
P56181	NDUFV3	1	1	0.43	0.88
P10646	TFPI	6	6	0.58	0.65
Q9C0E8	LNP	7	4	0.60	0.65
Q8TEQ8	PIGO	2	1	0.50	0.77
P51687	SUOX	2	1	0.74	0.54
P09972	ALDOC	9	10	0.64	0.62
Q9BQE5	APOL2	4	5	0.82	0.49
Q93088	BHMT	6	5	0.48	0.84
Q9UDR5	AASS	15	8	0.70	0.58
Q2QL34	MPV17L	2	2	0.73	0.56
Q02252	ALDH6A1	16	16	0.62	0.66
Q969G6	RFK	2	1	0.70	0.59
Q9UN36	NDRG2	7	5	0.63	0.66
Q96I45	TMEM141	2	1	0.64	0.66

Q9UBM7	DHCR7	3	2	0.47	0.90
P16591	FER	5	2	0.81	0.53
Q96EY8	MMAB	4	3	0.61	0.71
Q96A26	FAM162A	6	4	0.66	0.65
Q15120	PDK3	1	1	0.60	0.73
Q8NBP7	PCSK9	3	4	0.74	0.59
Q96HE7	ERO1L	12	13	0.58	0.76
P08754	GNAI3	2	3	0.46	0.97
P00450	CP	8	5	0.75	0.60
P28332	ADH6	9	10	0.65	0.69
Q16134	ETFDH	8	6	0.76	0.59
Q9BY49	PECR	11	12	0.55	0.83
P00734	F2	9	4	0.49	0.93
P16662	UGT2B7	4	5	0.95	0.48
P78549	NTHL1	1	1	0.52	0.88
Q86WA6	BPHL	8	6	0.54	0.86
Q16850	CYP51A1	15	12	0.84	0.55
Q00577	PURA	5	4	0.71	0.66
Q99424	ACOX2	6	5	0.67	0.71
Q9BPW8	NIPSNAP1	7	9	0.65	0.73
O00468	AGRN	16	11	0.63	0.75
P39060	COL18A1	6	6	0.74	0.65
O95479	H6PD	7	4	0.63	0.77
Q16762	TST	4	5	0.63	0.77
Q15118	PDK1	2	1	0.96	0.51
P05204	HMGN2;HMGN3	2	2	0.86	0.57
Q6JQN1	ACAD10	2	2	0.55	0.88
O14638	ENPP3	3	5	0.59	0.84
Q04446	GBE1	9	11	0.65	0.77
Q8N4Z0	RAB42	2	1	0.78	0.64
O00560	SDCBP	1	5	0.53	0.94
P00387	CYB5R3	8	7	0.62	0.81
Q9UKR5	C14orf1	2	3	0.62	0.81
O00469	PLOD2	16	18	0.60	0.85
Q96QD8	SLC38A2	6	5	0.50	1.00
P02679	FGG	13	14	0.63	0.80
Q969Z3	MARC2	3	4	0.80	0.63
O95573	ACSL3	17	18	0.89	0.57
P46019	PHKA2	6	7	0.66	0.77
O75600	GCAT	5	3	0.80	0.64
P31327	CPS1	21	10	0.61	0.84
Q7Z3D6	C14orf159	2	1	0.59	0.87
Q13011	ECH1	10	8	0.65	0.79
Q9NR19	ACSS2	13	11	0.81	0.65

P05026	ATP1B1	9	8	0.66	0.81
Q9Y5U8	MPC1	1	1	0.61	0.87
Q5T2W1	PDZK1;PDZK1P1	9	8	0.57	0.94
P04080	CSTB	6	5	0.89	0.60
P02771	AFP	17	22	0.67	0.80
Q13825	AUH	3	5	0.63	0.85
Q9H0W9	C11orf54	6	9	0.58	0.93
P50453	SERPINB9	11	7	0.92	0.58
Q9NPJ3	ACOT13	3	4	0.64	0.85
Q15345	LRRC41	2	4	0.66	0.83
Q53GQ0	HSD17B12	12	14	0.59	0.93
P07305	H1F0	7	6	0.63	0.89
Q9H6V9	C2orf43	3	5	0.65	0.87
P61225	RAP2B	2	1	0.65	0.87
P35914	HMGCL	6	9	0.62	0.92
Q8N4T8	CBR4	4	3	0.59	0.97
P53365	ARFIP2	2	2	0.92	0.62
Q96BR5	COA7	4	2	0.66	0.89
Q8TE04	PANK1	1	3	0.93	0.63
Q008S8	ECT2L	1	1	0.94	0.64
O00264	PGRMC1	4	3	0.66	0.94
Q96AB3	ISOC2	3	3	0.66	0.96
Q9P0M6	H2AFY2	13	12	0.63	1.00
Q9H7C9	AAMDC	3	5	0.67	0.97

Supplementary Table S3 List of potential receptors of rGal3C

Protein ID	Gene Name	Unique peptides_1	Unique peptides_2	iBAQ_1	iBAQ_2
P08195	4F2	13	15	51388000	102170000
P26006	ITA3	10	12	8734800	28183000
P56199	ITA1	5	12	2471600	8907500
Q01650	LAT1	4	4	27689000	31059000
P17301	ITA2	3	4	868750	3969000
P05026	AT1B1	2	3	1045300	28864000
P35613	BASI	2	3	9130800	20833000
P27824	CALX	2	3	979220	2728000
Q13740	CD166	2	5	816130	4586500
P00533	EGFR	2	6	1298000	5759300
P06756	ITAV	2	5	145470	2540000
P32004	L1CAM	2	6	423470	5865000
P13473	LAMP2	2	2	1830100	8407500
Q9UIQ6	LCAP	2	1	398200	322830
P02786	TFR1	2	1	352510	316730
P12236	ADT3	1	1	1446800	13671000
P11166	GTR1	1	2	2255600	3853300
Q9P035	HACD3	1	2	1331300	7679700
P05556	ITB1	1	5	0	11314000
Q07954	LRP1	1	1	53056	93367
O15427	MOT4	1	1	15661000	11441000
P20645	MPRD	1	1	1747600	2610500
P11717	MPRI	1	8	333210	1091100
Q9UBG0	MRC2	1	1	167160	888230
Q9Y639	NPTN	1	2	430810	2348500
P21246	PTN	1	1	1882300	25552000
Q12913	PTPRJ	1	5	427780	2018200
P55017	S12A3	1	1	301770	446250
Q9H2H9	S38A1	1	2	3849200	9622400
P27105	STOM	1	3	859220	6564700