

Supplementary Materials for

The Adaptor Protein p66Shc Inhibits mTOR-Dependent Anabolic Metabolism

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Other Supplementary Material for this manuscript includes the following:

(available at www.sciencesignaling.org/cgi/content/full/7/313/ra17/DC1)

Table S1. LC-MS/MS transitions for the metabolites measured in this study (Excel file).

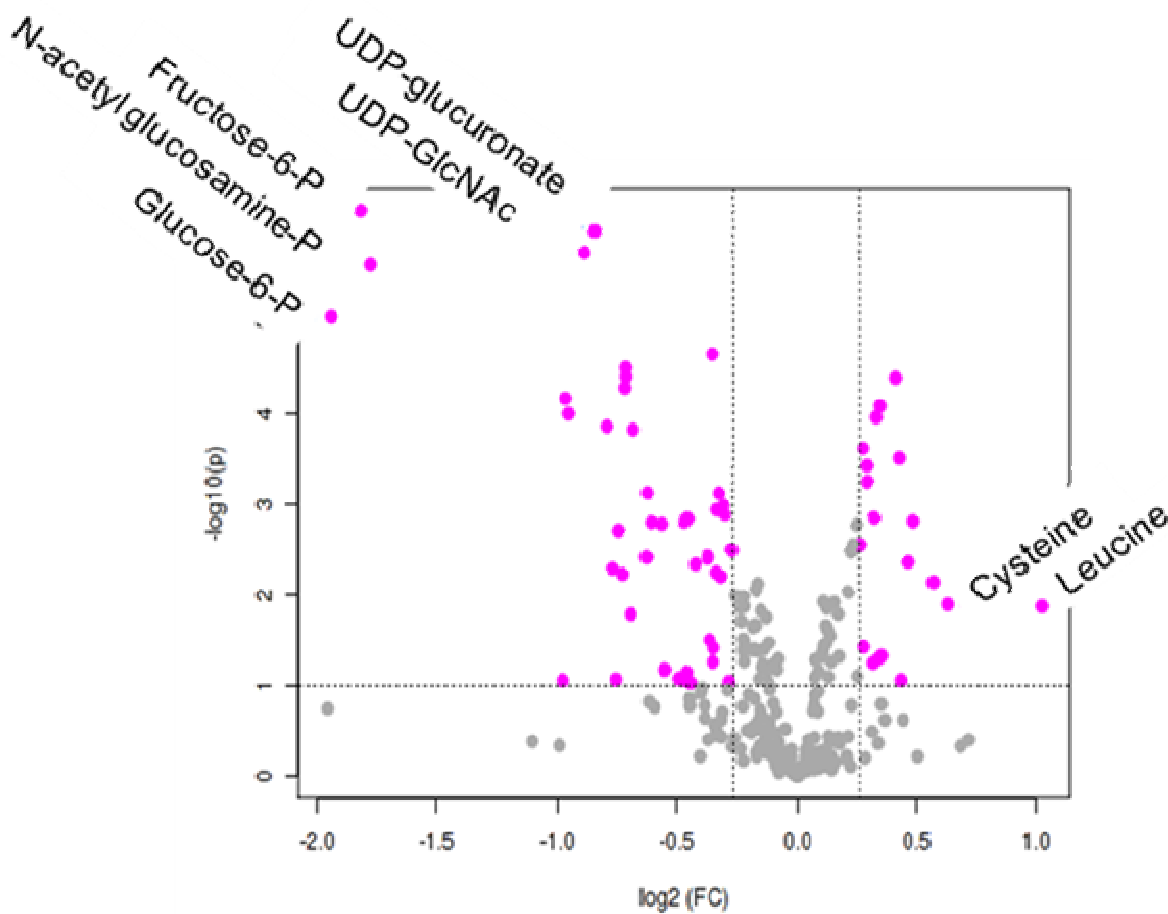


Fig. S1. A volcano plot for the metabolomics analysis of HeLa cells stably expressing shRNA targeting GFP or p66Shc. Significance is represented on the y-axis ($-\log_{10}(p)$) and fold change of p66Shc-competent over p66Shc-deficient HeLa cells is represented on the x-axis (\log_2). Representative metabolites with significant changes are labelled. N = 3 independent biological replicates.

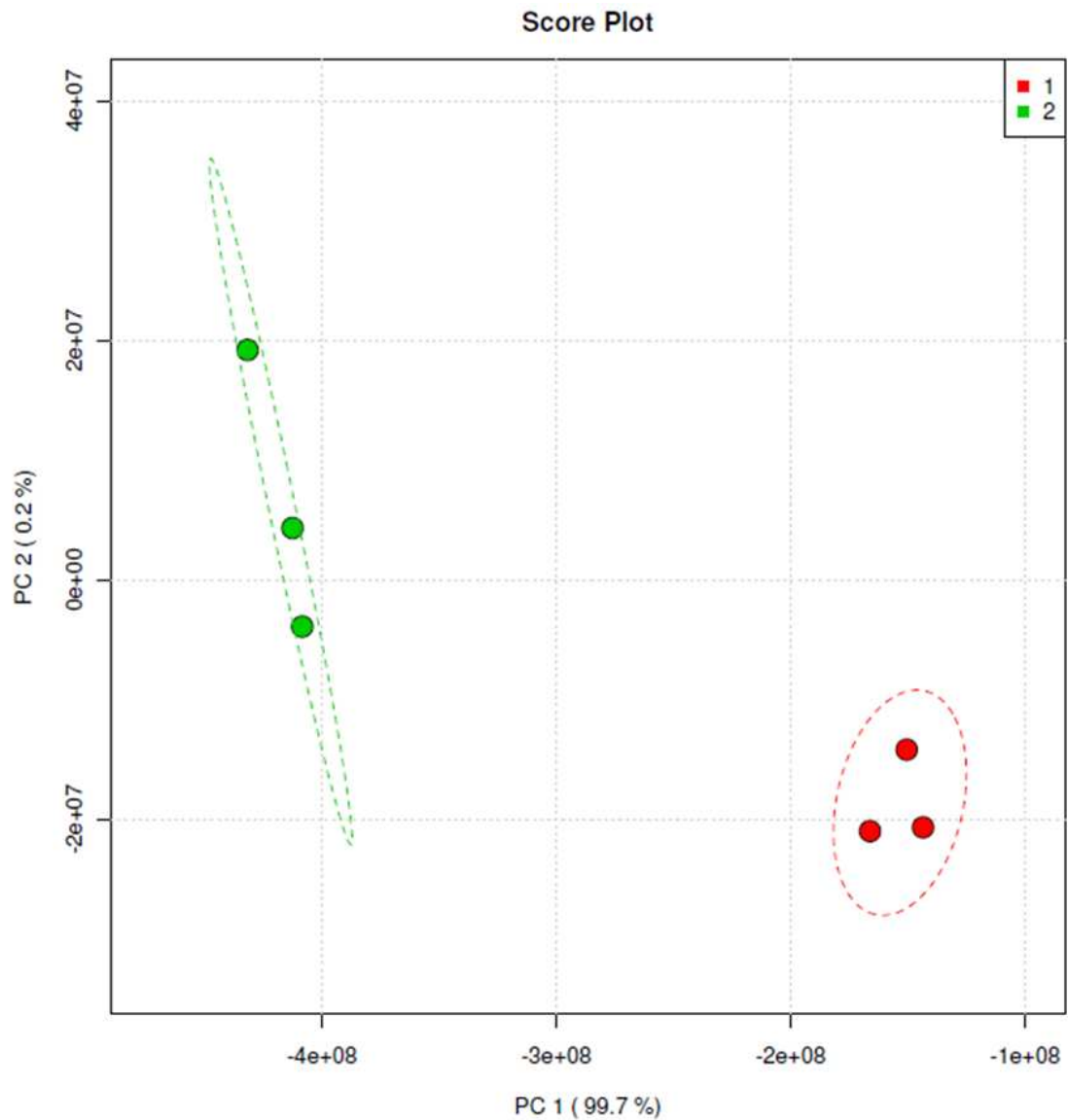


Fig. S2. Unsupervised principal component analysis for targeted metabolomic analysis of p66Shc KO and p66⁺ MEFs. Each dot represents a biological replicate. Red dots indicate replicates for p66Shc KO MEFs, and green dots indicate replicates for p66⁺ MEFs. The analysis demonstrates clear separation of overall metabolomic profiles of the two genotypes with statistical significance 99.7% (PC1) and 0.2% (PC2) (n=3, p < 0.05). The metabolites with the most significant changes in p66⁺ MEFs compared to KO MEFs are summarized in Table 1.

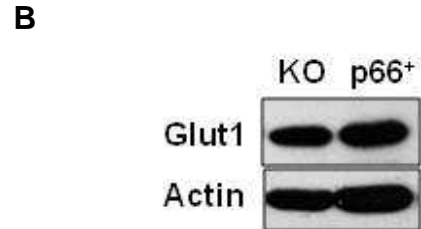


Fig. S3. The abundance of GLUT1 in p66Shc-competent and p66Shc-deficient HeLa cells and MEFs is similar. (A) HeLa cells stably transfected with indicated shRNAs were lysed and, and cell lysates were analyzed for the indicated proteins by immunoblotting. N = 2 independent biological replicates. (B) Lysates of p66Shc KO and p66⁺ MEFs were examined as in (A). N = 2 independent biological replicates.

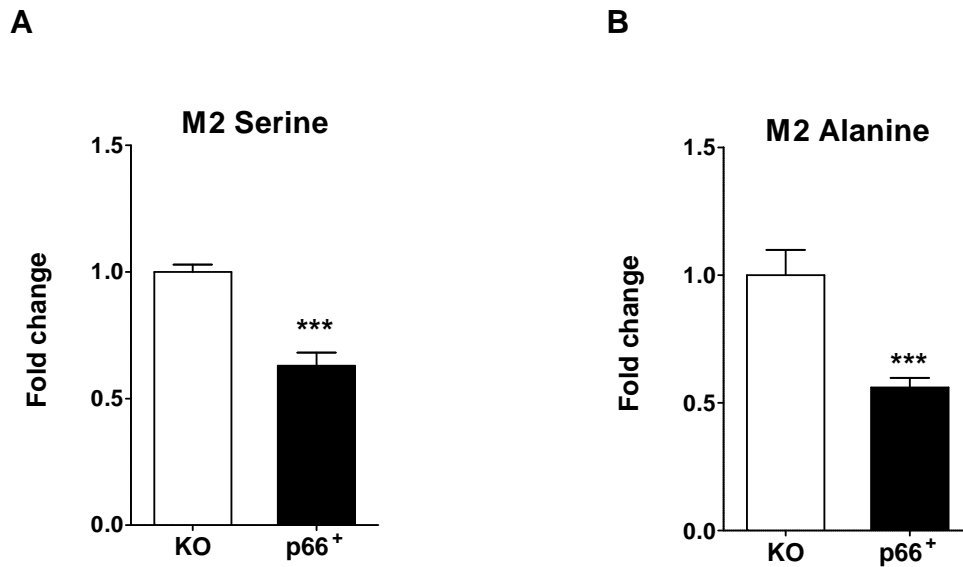


Fig. S4. p66Shc inhibits de novo synthesis of nonessential amino acids. Fold change of ¹³C-labeled serine (**A**) and alanine (**B**) in p66Shc KO (white) and p66⁺ (black) MEFs. Error bars represent SD of at least three independently prepared samples (***) p < 0.001).

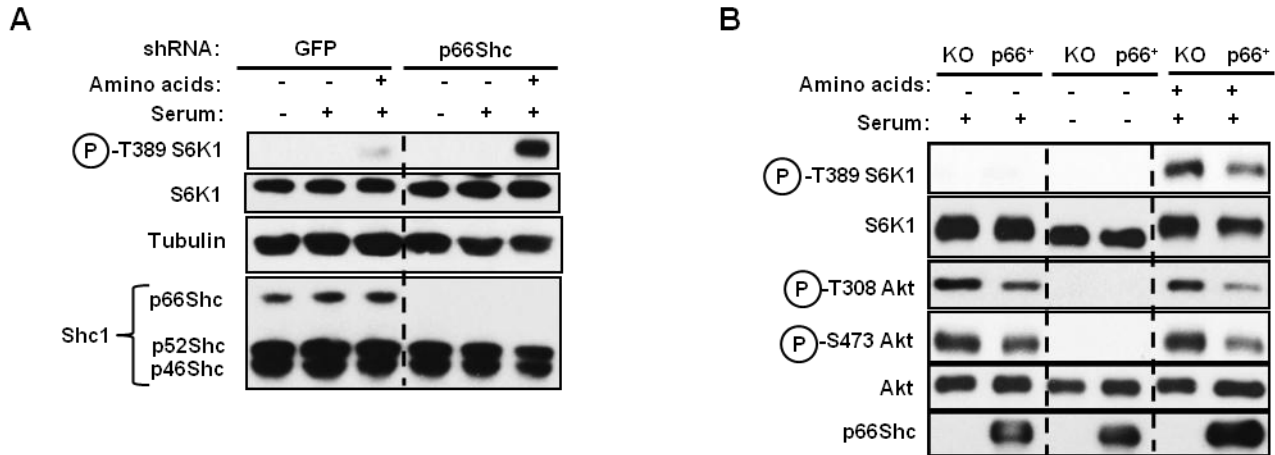


Fig. S5. Effect of amino acid deprivation on serum-mediated activation of mTORC1 in p66Shc-deficient cells. (A) HeLa cells stably transfected with shRNAs targeting the indicated genes were starved of amino acids for 50 min and stimulated either with dialyzed serum or normal serum for 10 min. Phosphorylation of S6K1 was analyzed by Western blotting. N = 3 independent biological replicates. (B) p66Shc KO and p66⁺ MEFs were treated and analyzed as in (A). N = 3 independent biological replicates.

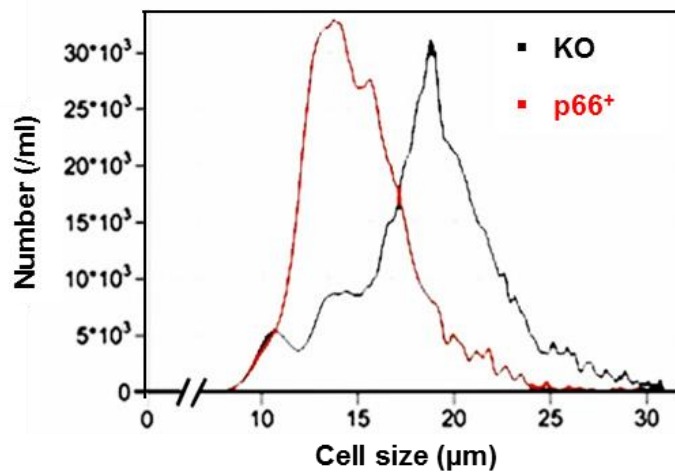
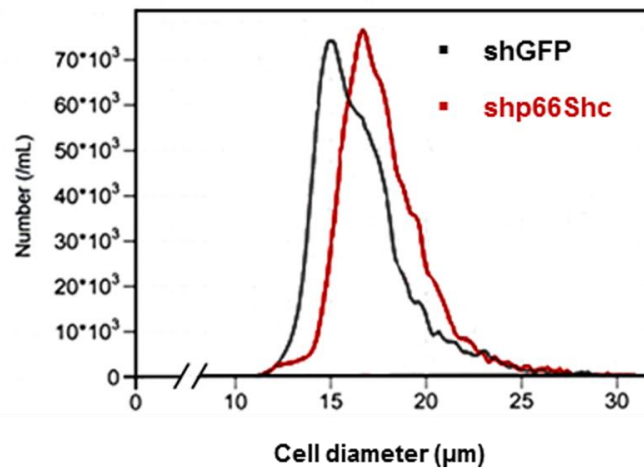
A**B**

Fig. S6. Effect of p66Shc expression on cell size. (A) Cell size measurement of KO and $p66^+$ cells. Cell size was measured using coulter counter. N = 3 biological replicates. (B) Cell size measurement of p66Shc-deficient and p66Shc-competent HeLa cells. Experiment was done as in (A). N = 3 biological replicates.

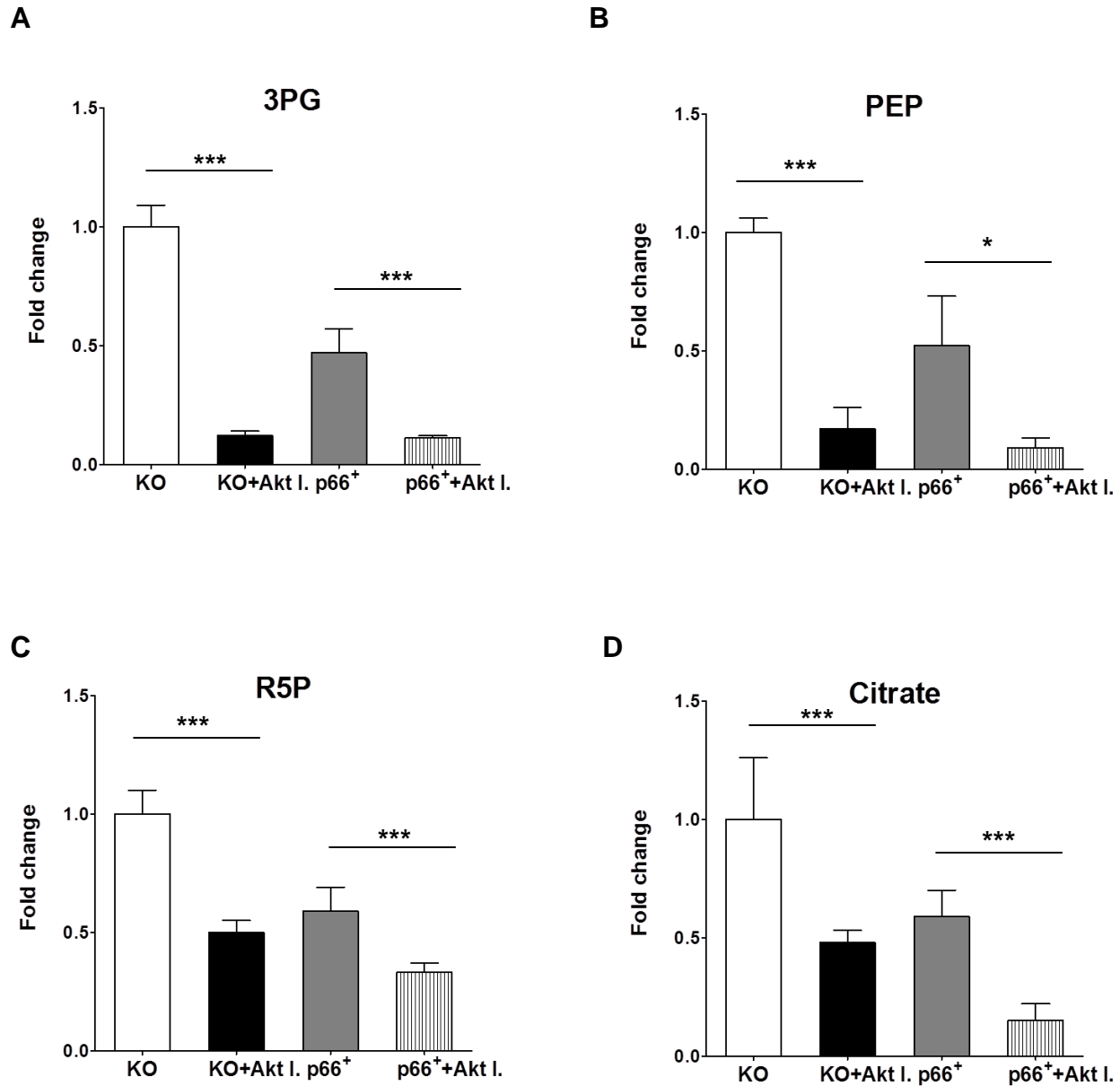


Fig. S7. Effect of Akt inhibition on the amounts of glycolytic metabolites in p66Shc-competent and p66Shc-deficient MEFs. (A-D) Fold change of 3PG (A), PEP (B), R5P (C), and citrate (D) in p66Shc KO MEFs (white), p66Shc KO MEFs treated with Akt inhibitor (black), p66⁺ (grey) and p66⁺ treated with Akt inhibitor (stripped) MEFs. Error bars represent SD of at least three independently prepared samples ($p < 0.05$, *** $p < 0.001$).

Table S1. LC-MS/MS transitions for the metabolites measured in this study (Excel file).
DP: de-clustering potential, CE: Collision energy, HMDB: Human Metabolome Database.

Table S2. LC-MS/MS Transitions for [1,2-¹³C₂]glucose intermediates. DP: de-clustering potential, EP: entrance potential, CE: Collision energy, CXP: collision cell exit potential

Q1	Q3	RT	ID	DP	CE	CXP
87	43	7.41	Pyruvate	-35	-14	-3
89	45		[1,2] ¹³ C-Pyruvate [M2]	-35	-14	-3
89	43	7.41	Lactate	-55	-16	-1
90	44		[1] ¹³ C-lactate [M1]	-55	-16	-1
91	45		[1,2] ¹³ C-lactate [M2]	-55	-16	-1
191	111	8.6	Citrate	-30	-18	-7
193	112		[1,2] ¹³ C-Citrate [M2]	-30	-18	-7
259	97	7.33	G6P 97	-40	-28	-5
261	79	7.33	[1,2] ¹³ C-G6P [M2]	-40	-28	-5
261	97		[1,2] ¹³ C-G6P [M2]	-40	-28	-5
259	79	8.26	D-Fructose-6P	-55	-72	-11
261	79	8.26	[1,2] ¹³ C-Fructose-6P [M2]	-55	-72	-11
300	79	7.7	GlcNAcP	-45	-76	-15
302	79		[1,2] ¹³ C-GlcNAcP [M2]	-60	-76	-15
606	159	8.5	UDP-GlcNAc	-110	-66	-1
608	159		UDP-[1,2] ¹³ C-GlcNAc [M2]	-110	-66	-1
808	159	8.5	Acetyl Co-enzyme A (ACoA)	-150	-88	-9
809	159		[2] ¹³ C-Acetyl Co-enzyme A (ACoA) [M1]	-150	-88	-9
810	159		[1,2] ¹³ C-Acetyl Co-enzyme A (ACoA) [M2]	-150	-88	-9

Table S3: List of genes showing differential regulation upon p66Shc expression.

Gene	Gene full Name	p66Shc KO - p66⁺ (average log₁₀)	p-value	SD
Lrp2	low density lipoprotein receptor-related protein 2	-1.915792811	0.00053	0.265341548
Lingo4	leucine rich repeat and Ig domain containing 4	-1.811543741	0.00053	0.08564836
Fam19a2	family with sequence similarity 19, member A2	-1.635935145	0.00053	0.094915819
Fras1	Fraser syndrome 1 homolog (human)	-1.632833069	0.00053	0.088349326
Epha7	Eph receptor A7	-1.617034208	0.00053	0.265811188
2810405K02Rik	RIKEN cDNA 2810405K02 gene	-1.545497146	0.00053	0.104072241
Pcdhgb8	protocadherin gamma subfamily B, 8	-1.505347374	0.00053	0.134934987
Nol4	nucleolar protein 4	-1.491293872	0.00053	0.147634015
Elavl2	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)	-1.475961437	0.00053	0.172756757
Slc38a4	solute carrier family 38, member 4	-1.450549921	0.00053	0.082508643
Slc16a7	solute carrier family 16 (monocarboxylic acid transporters), member 7	-1.422033949	0.00053	0.180917562
Fam132b	family with sequence similarity 132, member B	-1.408791619	0.00053	0.067851145
Fndc3c1	fibronectin type III domain containing 3C1	-1.317312614	0.00053	0.082532254
Cpa6	carboxypeptidase A6	-1.311506175	0.00053	0.39594187

Lrrtm1	leucine rich repeat transmembrane neuronal 1	-1.30232534	0.00053	0.261829654
Tmem178	transmembrane protein 178	-1.229814213	0.00053	0.252731011
Pcdh9	protocadherin 9	-1.209521591	0.00053	0.06012591
Rnf128	ring finger protein 128	-1.200148584	0.00053	0.295306529
Mfap3l	microfibrillar-associated protein 3-like	-1.184275636	0.00053	0.471271669
Nr3c2	nuclear receptor subfamily 3, group C, member 2	-1.179205418	0.00053	0.245215328
Epha3	Eph receptor A3	-1.141945788	0.00053	0.18094104
Pde3b	phosphodiesterase 3B, cGMP-inhibited	-1.126724114	0.00053	0.058435767
Armcx4	armadillo repeat containing, X-linked 4	-1.118182406	0.00053	0.009426341
Pcsk5	proprotein convertase subtilisin/kexin type 5	-1.110683672	0.00053	0.122133709
Mycn	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)	-1.109619722	0.00053	0.085221891
Fibin	fin bud initiation factor homolog (zebrafish)	-1.075052764	0.00053	0.053954081
Mei4	meiosis-specific, MEI4 homolog (<i>S. cerevisiae</i>)	-1.027144733	0.00054	0.404083607
Slc26a7	solute carrier family 26, member 7	-1.018616084	0.00053	0.265390359
Pmaip1	phorbol-12-myristate-13-acetate-induced protein 1	-0.991734978	0.00053	0.180923943
Sfrp2	secreted frizzled-related protein 2	-0.974846673	0.00053	0.172783959
Tll1	tolloid-like	-0.957443259	0.00053	0.175013394

Tmem151b	transmembrane protein 151B	-0.952357544	0.00053	0.148567314
Tnfrsf21	tumor necrosis factor receptor superfamily, member 21	-0.92950432	0.00053	0.067073319
Lef1	lymphoid enhancer binding factor 1	-0.929191993	0.00054	0.311455724
Hunk	hormonally upregulated Neu-associated kinase	-0.925378588	0.00054	0.488512276
Tdrkh	tudor and KH domain containing protein	-0.924298141	0.00053	0.016466993
Plcl1	phospholipase C-like 1	-0.922570232	0.00053	0.050416995
Pkia	protein kinase inhibitor, alpha	-0.917178399	0.00053	0.145255127
Tm6sf2	transmembrane 6 superfamily member 2	-0.909526811	0.00054	0.265433652
Mtap7d3	MAP7 domain containing 3	-0.905721395	0.00054	0.265381819
Zfp583	zinc finger protein 583	-0.89964319	0.00054	0.231461609
Fam38b	family with sequence similarity 38, member B	-0.886354776	0.00054	0.393893458
Sort1	sortilin 1	-0.883128964	0.00053	0.036959691
Hist1h2bg	histone cluster 1, H2bg	-0.870775698	0.00054	0.08807413
Snca	synuclein, alpha	-0.850845519	0.00054	0.101000827
Dok5	docking protein 5	-0.829479412	0.00054	0.098944847
Rell2	RELT-like 2	-0.824736054	0.00054	0.261803243
Gabre	gamma-aminobutyric acid (GABA) A receptor, subunit epsilon	-0.824104256	0.00054	0.134844879
Hist1h4k	histone cluster 1, H4k	-0.821945916	0.00054	0.296458294
Gm10406	predicted gene 10406	-0.808209064	0.00054	0.180904117
Cacna1b	calcium channel, voltage-dependent, N type, alpha 1B	-0.805835164	0.00054	0.143616773

	subunit			
Hoxb9	homeobox B9	-0.797828438	0.00054	0.198343907
Asxl3	additional sex combs like 3 (Drosophila)	-0.791955705	0.00054	0.054836257
Pcdhb2	protocadherin beta 2	-0.787980234	0.00054	0.160613469
Snx10	sorting nexin 10	-0.784827431	0.00054	0.100231686
Pcdhb3	protocadherin beta 3	-0.779873929	0.00054	0.114090528
4921528I01Rik	RIKEN cDNA 4921528I01 gene	-0.777509771	0.00054	0.192939047
Wnt2b	wingless related MMTV integration site 2b	-0.755864987	0.00054	0.0903634
Peg10	paternally expressed 10	-0.744421658	0.00054	0.10331376
Fndc5	fibronectin type III domain containing 5	-0.744227916	0.00054	0.074817703
Ntng2	netrin G2	-0.741595235	0.00054	0.121176342
Klf12	Kruppel-like factor 12	-0.738830144	0.00054	0.041258524
Npy1r	neuropeptide Y receptor Y1	-0.736048621	0.00054	0.125603467
Gpr137c	G protein-coupled receptor 137C	-0.73215916	0.00059	0.534608914
8430408G22Rik	RIKEN cDNA 8430408G22 gene	-0.724087278	0.00055	0.222465861
Bhlhe22	basic helix-loop-helix family, member e22	-0.718249387	0.00054	0.150545533
Nlr1	NLR family member X1	-0.713254896	0.00054	0.030703712
Elovl4	elongation of very long chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 4	-0.711290071	0.00055	0.223211263
Chdh	choline dehydrogenase	-0.705962742	0.00056	0.361115226
Sema6a	sema domain, transmembrane domain (TM), and cytoplasmic	-0.703651997	0.00054	0.059363453

	domain, (semaphorin) 6A			
Cthrc1	collagen triple helix repeat containing 1	-0.701838551	0.00055	0.173849535
Enox1	ecto-NOX disulfide-thiol exchanger 1	-0.699117306	0.00054	0.114088181
Fbxl7	F-box and leucine-rich repeat protein 7	-0.698448854	0.00054	0.044721972
Snrpn	small nuclear ribonucleoprotein N	-0.696287165	0.00055	0.123683742
1700010I14Rik	RIKEN cDNA 1700010I14 gene	-0.687747012	0.00056	0.2527405
Tmem169	transmembrane protein 169	-0.686456758	0.00057	0.395917183
Palm3	paralemmin 3	-0.684145211	0.00059	0.50659648
Tnfaip8l1	tumor necrosis factor, alpha-induced protein 8-like 1	-0.683349349	0.00055	0.029609797
Cd200	CD200 antigen	-0.681445884	0.00055	0.046093045
Pcdh8	protocadherin 8	-0.680995051	0.00056	0.27531672
Dclk2	doublecortin-like kinase 2	-0.676081914	0.00055	0.134788259
Igf2	insulin-like growth factor 2	-0.674359709	0.00055	0.036664029
Fut4	fucosyltransferase 4	-0.672636529	0.00057	0.404091035
F2rl1	coagulation factor II (thrombin) receptor-like 1	-0.664956186	0.00055	0.016152716
Tmem200a	transmembrane protein 200A	-0.663179322	0.00055	0.036468114
Tbx2	T-box 2	0.663640332	0.00055	0.021998753
Gm1661	predicted gene 1661	0.665248537	0.00055	0.108950542
Diras2	DIRAS family, GTP-binding RAS-like 2	0.667441057	0.00056	0.246125103
Rab3b	RAB3B, member RAS oncogene family	0.667673045	0.00055	0.087214478

Col28a1	collagen, type XXVIII, alpha 1	0.668464877	0.00055	0.103303578
Agt	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	0.668787912	0.00055	0.059258563
Slc22a23	solute carrier family 22, member 23	0.670209459	0.00055	0.036892269
Il13ra2	interleukin 13 receptor, alpha 2	0.670898039	0.00055	0.077902592
Syt5	synaptotagmin V	0.673105204	0.00055	0.134876587
Cfh	complement component factor h	0.673403902	0.00055	0.014587192
Cdh26	cadherin-like 26	0.674748099	0.00055	0.171407963
1700034H15Rik	RIKEN cDNA 1700034H15 gene	0.676046005	0.00056	0.339792831
Lgals9	lectin, galactose binding, soluble 9	0.678298366	0.00055	0.051080452
Gm14393	predicted gene 14393	0.680908099	0.00058	0.403850786
Adh7	alcohol dehydrogenase 7 (class IV), mu or sigma polypeptide	0.68327306	0.00055	0.152894719
Cygb	cytoglobin	0.683903613	0.00055	0.157736829
4-Sep	septin 4	0.684369152	0.00061	0.376028174
Nsg1	neuron specific gene family member 1	0.68443605	0.00055	0.027139644
Tmem117	transmembrane protein 117	0.6868859	0.00055	0.123397066
Siglecg	sialic acid binding Ig-like lectin G	0.687365882	0.00055	0.072380418
Cbln3	cerebellin 3 precursor protein	0.688270313	0.00055	0.066008871
Islr	immunoglobulin superfamily containing leucine-rich repeat	0.692047166	0.00054	0.019871733
Adamts1	ADAMTS-like 1	0.695033204	0.00054	0.104145348
Lama3	laminin, alpha 3	0.69513384	0.00054	0.108472544
Acy3	aspartoacylase (aminoacylase) 3	0.696801941	0.00054	0.086061403

Atf3	activating transcription factor 3	0.69773903	0.00054	0.024900306
Rsph1	radial spoke head 1 homolog (Chlamydomonas)	0.701830671	0.00055	0.207766755
Phactr1	phosphatase and actin regulator 1	0.703918435	0.00054	0.055588964
9930023K05Rik	RIKEN cDNA 9930023K05 gene	0.704413698	0.00058	0.462960081
Atp8b1	ATPase, class I, type 8B, member 1	0.704855288	0.00054	0.066155661
Timp3	tissue inhibitor of metalloproteinase 3	0.705980282	0.00054	0.009519119
Ticam2	toll-like receptor adaptor molecule 2	0.706980197	0.00055	0.216094637
Nhs12	NHS-like 2	0.708152301	0.00055	0.187820581
Tcf7	transcription factor 7, T cell specific	0.710676527	0.00054	0.079743011
Asgr1	asialoglycoprotein receptor 1	0.710908571	0.00054	0.102195858
Gem	GTP binding protein (gene overexpressed in skeletal muscle)	0.711129665	0.00054	0.074775012
Acss1	acyl-CoA synthetase short-chain family member 1	0.713136814	0.00056	0.332812454
Adrbk2	adrenergic receptor kinase, beta 2	0.71389249	0.00054	0.017370486
Mapkapk3	mitogen-activated protein kinase- activated protein kinase 3	0.717198672	0.00054	0.11169346
Map3k5	mitogen-activated protein kinase kinase kinase 5	0.722403796	0.00054	0.060276991
Islr2	immunoglobulin superfamily containing leucine-rich repeat 2	0.723526789	0.00054	0.137727559
Ggt7	gamma-glutamyltransferase 7	0.724810409	0.00054	0.121828837
Zfp296	zinc finger protein 296	0.726254956	0.00059	0.440252288

Sh2d1b1	SH2 domain protein 1B1	0.726373223	0.00054	0.086700286
B3gnt8	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8	0.729573708	0.00057	0.303349205
Tnni3	troponin I, cardiac 3	0.731254977	0.00058	0.514112037
9130019O22Rik	RIKEN cDNA 9130019O22 gene	0.732389495	0.00054	0.098879186
Ccbe1	collagen and calcium binding EGF domains 1	0.733942913	0.00054	0.137946031
Gstt1	glutathione S-transferase, theta 1	0.734574564	0.00054	0.095911655
Rtp4	receptor transporter protein 4	0.737249757	0.00054	0.050153752
Abi3	ABI gene family, member 3	0.739334553	0.00054	0.101934592
Aldh1a3	aldehyde dehydrogenase family 1, subfamily A3	0.742734742	0.00054	0.085524474
Rasl11a	RAS-like, family 11, member A	0.743417171	0.00054	0.069964518
Epb4.114a	erythrocyte protein band 4.1-like 4a	0.743720766	0.00054	0.067778459
Ica1	islet cell autoantigen 1	0.746449159	0.00058	0.532783978
Hyal3	hyaluronoglucosaminidase 3	0.746670131	0.00058	0.532544673
Ifi2712a	interferon, alpha-inducible protein 27 like 2A	0.748805838	0.00054	0.128921448
Matn4	matrilin 4	0.751296432	0.00055	0.309099514
Afap112	actin filament associated protein 1-like 2	0.754643517	0.00054	0.040200803
Abcc3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	0.755485412	0.00055	0.246203752
Deptor	DEP domain containing MTOR-interacting protein	0.758137134	0.00054	0.116223933
Smoc1	SPARC related modular calcium binding 1	0.759456057	0.00056	0.432661364

Dcn	decorin	0.759916709	0.00054	0.063105273
Cldn1	claudin 1	0.761833728	0.00054	0.04408684
Traf1	TNF receptor-associated factor 1	0.764048952	0.00054	0.065079109
Cfb	complement factor B	0.76455062	0.00054	0.088308557
Vtcn1	V-set domain containing T cell activation inhibitor 1	0.765821423	0.00055	0.267925708
Acta1	actin, alpha 1, skeletal muscle	0.765964795	0.00054	0.043835532
Gm12216	predicted gene 12216	0.76644448	0.00054	0.135900469
1700003F12Rik	RIKEN cDNA 1700003F12 gene	0.768055495	0.00054	0.089105998
Saa3	serum amyloid A 3	0.773580491	0.00054	0.173053452
Cdhr1	cadherin-related family member 1	0.774388432	0.00054	0.045177575
Ptgfr	prostaglandin F receptor	0.775513945	0.00054	0.090611927
Susd2	sushi domain containing 2	0.775594375	0.00054	0.097255044
Cdkn1c	cyclin-dependent kinase inhibitor 1C (P57)	0.776172936	0.00054	0.06379327
Mndal	myeloid nuclear differentiation antigen like	0.779296909	0.00055	0.379651909
Clec11a	C-type lectin domain family 11, member a	0.780058561	0.00054	0.055581965
Apol10b	apolipoprotein L 10B	0.782834626	0.00054	0.137041661
Cyp26b1	cytochrome P450, family 26, subfamily b, polypeptide 1	0.782836703	0.00054	0.117528419
Kctd14	potassium channel tetramerisation domain containing 14	0.784766049	0.00056	0.360430947
AI428936	expressed sequence AI428936	0.787294696	0.00054	0.099279262
Adam33	a disintegrin and metallopeptidase domain 33	0.788839092	0.00054	0.085605242

Aox1	aldehyde oxidase 1	0.790952518	0.00054	0.028503273
Blnk	B cell linker	0.790963356	0.00054	0.301575058
Ecsr	endothelial cell surface expressed chemotaxis and apoptosis regulator	0.793488682	0.00054	0.035874271
Rarres2	retinoic acid receptor responder (tazarotene induced) 2	0.793997302	0.00054	0.137749917
Epas1	endothelial PAS domain protein 1	0.794441813	0.00054	0.093825829
Fam179a	family with sequence similarity 179, member A	0.797152443	0.00054	0.085500996
Sncg	synuclein, gamma	0.797621076	0.00054	0.089113939
Angptl7	angiopoietin-like 7	0.79815192	0.00054	0.044478057
Eln	elastin	0.798869667	0.00054	0.018790951
Gprin3	GPRIN family member 3	0.800688631	0.00054	0.128589472
Rhox5	reproductive homeobox 5	0.801127842	0.00055	0.265045427
Pcdhga2	protocadherin gamma subfamily A, 2	0.802342483	0.00055	0.4339809
Ppl	periplakin	0.806596571	0.00054	0.017650785
Pdzn4	PDZ domain containing RING finger 4	0.816216272	0.00054	0.03802449
Bdh1	3-hydroxybutyrate dehydrogenase, type 1	0.818013017	0.00054	0.13315328
Gbp6	guanylate binding protein 6	0.820104623	0.00054	0.059587685
Auts2	autism susceptibility candidate 2	0.823419711	0.00054	0.017035484
Peg3	paternally expressed 3	0.824601124	0.00054	0.095374777
Slfn2	schlafen 2	0.828174735	0.00054	0.180890243
Espnl	espin-like	0.829729488	0.00054	0.187549219

Raet1d	retinoic acid early transcript delta	0.831300419	0.00054	0.084477615
Col10a1	collagen, type X, alpha 1	0.834622555	0.00054	0.043812899
Usp18	ubiquitin specific peptidase 18	0.841243699	0.00054	0.33200192
Rab40b	Rab40b, member RAS oncogene family	0.845386805	0.00054	0.216744132
Ddit4l	DNA-damage-inducible transcript 4-like	0.846972376	0.00054	0.038527775
Panx1	pannexin 1	0.847590486	0.00054	0.159315477
Isg15	ISG15 ubiquitin-like modifier	0.856119102	0.00054	0.114701267
Lxn	latexin	0.85697897	0.00054	0.065921894
Cercam	cerebral endothelial cell adhesion molecule	0.860725077	0.00054	0.094089887
Ankrd6	ankyrin repeat domain 6	0.861965839	0.00054	0.148669382
Naalad2	N-acetylated alpha-linked acidic dipeptidase 2	0.863873157	0.00054	0.043166595
Ceacam1	carcinoembryonic antigen-related cell adhesion molecule 1	0.868657678	0.00054	0.09876767
Nptx1	neuronal pentraxin 1	0.868733414	0.00055	0.432899662
Tnfrsf9	tumor necrosis factor receptor superfamily, member 9	0.869749964	0.00054	0.0822082
Apol6	apolipoprotein L 6	0.870822486	0.00054	0.096801623
Thsd4	thrombospondin, type I, domain containing 4	0.874631254	0.00054	0.375134475
Pcdhb13	protocadherin beta 13	0.877924773	0.00054	0.085463636
Acsbg1	acyl-CoA synthetase bubblegum family member 1	0.878723314	0.00054	0.30885885
Serpina3n	serine (or cysteine) peptidase inhibitor, clade A, member 3N	0.879110484	0.00053	0.041690783

Aoc3	amine oxidase, copper containing 3	0.881136113	0.00053	0.018828905
5430407P10Rik	RIKEN cDNA 5430407P10 gene	0.881624059	0.00053	0.072724759
Serpina3m	serine (or cysteine) peptidase inhibitor, clade A, member 3M	0.883754442	0.00053	0.060574541
Bst1	bone marrow stromal cell antigen 1	0.886785141	0.00056	0.540574601
Stac2	SH3 and cysteine rich domain 2	0.892804709	0.00054	0.147549381
Gjb3	gap junction protein, beta 3	0.893450437	0.00055	0.431657445
Nap1l3	nucleosome assembly protein 1-like 3	0.893947626	0.00054	0.139544026
Kng2	kininogen 2	0.895607628	0.00053	0.074813929
Il33	interleukin 33	0.896838083	0.00054	0.144757383
Ngfr	nerve growth factor receptor (TNFR superfamily, member 16)	0.898674402	0.00053	0.085524089
Sorl1	sortilin-related receptor, LDLR class A repeats-containing	0.899089977	0.00054	0.152335302
H2-T10	histocompatibility 2, T region locus 10	0.902292843	0.00054	0.380676837
Insc	inscuteable homolog (Drosophila)	0.90417236	0.00053	0.112156662
Spink2	serine peptidase inhibitor, Kazal type 2	0.905591442	0.00054	0.14676946
Clstn3	calsyntenin 3	0.908201748	0.00053	0.089411025
Gpr4	G protein-coupled receptor 4	0.909466117	0.00054	0.216203667
Cxcl17	chemokine (C-X-C motif) ligand 17	0.909828619	0.00055	0.537563025
Abcg1	ATP-binding cassette, sub-family G (WHITE), member 1	0.91071001	0.00054	0.274435175
Plcd4	phospholipase C, delta 4	0.913182402	0.00054	0.171352782

Ifit1	interferon-induced protein with tetratricopeptide repeats 1	0.918782586	0.00053	0.175317001
Cd74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	0.926534365	0.00053	0.095906798
Rhpn2	rhophilin, Rho GTPase binding protein 2	0.927138782	0.00053	0.08551548
Dgkg	diacylglycerol kinase, gamma	0.927520067	0.00053	0.100662246
Adh1	alcohol dehydrogenase 1 (class I)	0.928183835	0.00054	0.359441834
Edar	ectodysplasin-A receptor	0.928254958	0.00053	0.072900312
Il2rb	interleukin 2 receptor, beta chain	0.928805896	0.00054	0.267954993
Aim1	absent in melanoma 1	0.929351782	0.00053	0.057430612
Prex2	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2	0.93719502	0.00053	0.08997535
Ptk2b	PTK2 protein tyrosine kinase 2 beta	0.938135831	0.00053	0.027228824
Ldhd	lactate dehydrogenase D	0.93871423	0.00054	0.439647844
1700024P16Rik	RIKEN cDNA 1700024P16 gene	0.938819604	0.00054	0.360716271
Gstt3	glutathione S-transferase, theta 3	0.948569948	0.00053	0.024634112
Sim2	single-minded homolog 2 (Drosophila)	0.953991468	0.00053	0.143385491
Efhd1	EF hand domain containing 1	0.956252223	0.00053	0.103256292
Oasl2	2'-5' oligoadenylate synthetase-like 2	0.959954864	0.00053	0.023324485
Neurl3	neuralized homolog 3 homolog (Drosophila)	0.964136118	0.00053	0.134849684

Kng1	kininogen 1	0.977131516	0.00053	0.049991385
Sp7	Sp7 transcription factor 7	0.978273933	0.00054	0.309076084
Parm1	prostate androgen-regulated mucin-like protein 1	0.980508708	0.00053	0.058662293
Foxf2	forkhead box F2	0.981038713	0.00053	0.059632285
C1qtnf5	C1q and tumor necrosis factor related protein 5	0.982903387	0.00054	0.290011907
Spib	Spi-B transcription factor (Spi-1/PU.1 related)	0.983279881	0.00053	0.157542051
Fgd4	FYVE, RhoGEF and PH domain containing 4	0.984260836	0.00053	0.139318882
D14Ertd668e	DNA segment, Chr 14, ERATO Doi 668, expressed	0.988864035	0.00053	0.127634024
Dll1	delta-like 1 (Drosophila)	0.992361465	0.00054	0.432629062
Rnase1	ribonuclease, RNase A family, 1 (pancreatic)	0.993085648	0.00054	0.285009166
Acox2	acyl-Coenzyme A oxidase 2, branched chain	0.993184757	0.00053	0.094325878
Cybrd1	cytochrome b reductase 1	0.994028526	0.00053	0.225943878
Sult1a1	sulfotransferase family 1A, phenol-preferring, member 1	0.995373222	0.00053	0.12120022
Tfap2a	transcription factor AP-2, alpha	0.997539572	0.00053	0.135302675
Pdgfb	platelet derived growth factor, B polypeptide	0.998420792	0.00053	0.163272181
Hck	hemopoietic cell kinase	0.999070587	0.00053	0.250495191
C2cd4a	C2 calcium-dependent domain containing 4A	0.999639773	0.00053	0.208161313
Pcdhgb1	protocadherin gamma subfamily B,	1.005347499	0.00053	0.080214417

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9030224M15Rik	RIKEN cDNA 9030224M15 gene	1.013271323	0.00054	0.359742465
Mustn1	musculoskeletal, embryonic nuclear protein 1	1.015777183	0.00053	0.127381615
Tbxa2r	thromboxane A2 receptor	1.015799552	0.00053	0.044372957
Rapsn	receptor-associated protein of the synapse	1.017472248	0.00053	0.106670772
Mgst1	microsomal glutathione S-transferase 1	1.018475319	0.00053	0.0203985
Gas6	growth arrest specific 6	1.018597236	0.00053	0.012972127
Ptprq	protein tyrosine phosphatase, receptor type, Q	1.023762028	0.00053	0.09260246
Mrv1	MRV integration site 1	1.024551664	0.00053	0.047280673
Selenbp1	selenium binding protein 1	1.027124543	0.00053	0.136774659
H28	histocompatibility 28	1.027624929	0.00053	0.114131969
Pcdhga6	protocadherin gamma subfamily A, 6	1.027721531	0.00053	0.273393221
Serpina3f	serine (or cysteine) peptidase inhibitor, clade A, member 3F	1.031536232	0.00053	0.178581046
Ccl27a	chemokine (C-C motif) ligand 27A	1.03329066	0.00054	0.484304318
Iigp1	interferon inducible GTPase 1	1.036102758	0.00053	0.065017448
Apol9b	apolipoprotein L 9b	1.039598534	0.00053	0.153952589
Mmp13	matrix metalloproteinase 13	1.040888988	0.00053	0.083124011
Fmo4	flavin containing monooxygenase 4	1.046495119	0.00053	0.216815067
Steap4	STEAP family member 4	1.052428065	0.00053	0.002584317
Cln3	ceroid lipofuscinosis, neuronal 3, juvenile (Batten, Spielmeier-Vogt	1.058161466	0.00053	0.309026893

	disease)			
C1qtnf3	C1q and tumor necrosis factor related protein 3	1.062870795	0.00053	0.085885934
Dio2	deiodinase, iodothyronine, type II	1.07779171	0.00053	0.209791746
Tmprss6	transmembrane serine protease 6	1.079375769	0.00053	0.033600766
Xdh	xanthine dehydrogenase	1.085912708	0.00053	0.079133761
Pcdhgb6	protocadherin gamma subfamily B, 6	1.088637837	0.00053	0.059579985
Alox5ap	arachidonate 5-lipoxygenase activating protein	1.09044588	0.00053	0.166524406
Notum	notum pectinacetyl esterase homolog (Drosophila)	1.10405039	0.00053	0.094076015
Lbp	lipopolysaccharide binding protein	1.113722934	0.00053	0.03739157
Clec3b	C-type lectin domain family 3, member b	1.116350535	0.00053	0.076252528
Enpp2	ectonucleotide pyrophosphatase/phosphodiesterase 2	1.117753237	0.00053	0.347134136
Acpp	acid phosphatase, prostate	1.120588722	0.00053	0.137820192
Rragb	Ras-related GTP binding B	1.125262389	0.00053	0.309078515
Gdnf	glial cell line derived neurotrophic factor	1.132545993	0.00053	0.109641032
Hp	haptoglobin	1.13791102	0.00053	0.43130165
Lcn2	lipocalin 2	1.139064261	0.00053	0.08005039
Hspa12b	heat shock protein 12B	1.14116058	0.00053	0.085648791
Syt13	synaptotagmin XIII	1.143486055	0.00053	0.027679077
Slc16a2	solute carrier family 16 (monocarboxylic acid transporters),	1.159110478	0.00053	0.398843774

	member 2			
Sod3	superoxide dismutase 3, extracellular	1.167418756	0.00053	0.056023357
Ugt1a6b	UDP glucuronosyltransferase 1 family, polypeptide A6B	1.170555987	0.00053	0.128208789
Slc22a18	solute carrier family 22 (organic cation transporter), member 18	1.174411334	0.00053	0.145295089
Entpd3	ectonucleoside triphosphate diphosphohydrolase 3	1.180120689	0.00053	0.1037217
Cxcl10	chemokine (C-X-C motif) ligand 10	1.193671012	0.00053	0.140252908
Zfp941	zinc finger protein 941	1.194086034	0.00053	0.085421735
Elfn1	leucine rich repeat and fibronectin type III, extracellular 1	1.194848088	0.00053	0.020379081
Apol9a	apolipoprotein L 9a	1.197174371	0.00053	0.246164347
Gpbar1	G protein-coupled bile acid receptor 1	1.198285099	0.00053	0.135408239
Cda	cytidine deaminase	1.205054123	0.00053	0.237088505
Fam107a	family with sequence similarity 107, member A	1.205425335	0.00053	0.039519945
Nr1h3	nuclear receptor subfamily 1, group H, member 3	1.206406134	0.00053	0.091558614
Plcg2	phospholipase C, gamma 2	1.206711745	0.00053	0.398864245
Trim30a	tripartite motif-containing 30A	1.208785913	0.00053	0.144143522
Csmd1	CUB and Sushi multiple domains 1	1.210273167	0.00053	0.057302114
Pzca	prostate stem cell antigen	1.212806007	0.00053	0.091731027
Psmb9	proteasome (prosome, macropain) subunit, beta type 9 (large	1.213436685	0.00053	0.1345473

	multifunctional peptidase 2)			
Pcsk6	proprotein convertase subtilisin/kexin type 6	1.216391075	0.00053	0.032624856
Prep	proline arginine-rich end leucine-rich repeat	1.216786271	0.00053	0.051151785
A4galt	alpha 1,4-galactosyltransferase	1.22310685	0.00053	0.061975306
Vnn1	vanin 1	1.22704685	0.00053	0.016872923
Fxyd1	FXYP domain-containing ion transport regulator 1	1.231112772	0.00053	0.523075014
Trpv2	transient receptor potential cation channel, subfamily V, member 2	1.248179355	0.00053	0.216250134
Renbp	renin binding protein	1.248781807	0.00053	0.017383204
Grhl1	grainyhead-like 1 (Drosophila)	1.253278296	0.00053	0.270603819
Msl3l2	male-specific lethal 3-like 2 (Drosophila)	1.25986456	0.00053	0.137146309
Arhgap6	Rho GTPase activating protein 6	1.266639625	0.00053	0.180941556
Ccl5	chemokine (C-C motif) ligand 5	1.300214635	0.00053	0.131616389
H60b	histocompatibility 60b	1.301586423	0.00053	0.311046603
H2-Q4	histocompatibility 2, Q region locus 4	1.303535724	0.00053	0.084559364
C3	complement component 3	1.322856021	0.00053	0.063090714
AI607873	expressed sequence AI607873	1.346856052	0.00053	0.137647255
Cyp2j9	cytochrome P450, family 2, subfamily j, polypeptide 9	1.359477206	0.00053	0.086144262
Tnfsf10	tumor necrosis factor (ligand) superfamily, member 10	1.375656394	0.00053	0.185884401
C130074G19Rik	RIKEN cDNA C130074G19 gene	1.377624185	0.00053	0.13747556

Serping1	serine (or cysteine) peptidase inhibitor, clade G, member 1	1.386331531	0.00053	0.095017679
Nfe2l3	nuclear factor, erythroid derived 2, like 3	1.389071601	0.00053	0.032589724
Gda	guanine deaminase	1.400864825	0.00053	0.026537201
Crabp1	cellular retinoic acid binding protein I	1.404406104	0.00053	0.242432756
Htatip2	HIV-1 tat interactive protein 2, homolog (human)	1.407270603	0.00053	0.057078139
Plb1	phospholipase B1	1.44324694	0.00053	0.269786455
Oas2	2'-5' oligoadenylate synthetase 2	1.443352045	0.00053	0.137723246
Inmt	indolethylamine N-methyltransferase	1.460243408	0.00053	0.310783802
Pik3r5	phosphoinositide-3-kinase, regulatory subunit 5, p101	1.467004844	0.00053	0.08535067
Pdzk1ip1	PDZK1 interacting protein 1	1.474467624	0.00053	0.042997429
Mmp3	matrix metalloproteinase 3	1.479116108	0.00053	0.058432453
Tril	TLR4 interactor with leucine-rich repeats	1.50993966	0.00053	0.137914316
Tubg2	tubulin, gamma 2	1.527010062	0.00053	0.309441049
Pdpcn	podoplanin	1.555027038	0.00053	0.4399842
Sp140	Sp140 nuclear body protein	1.570560246	0.00053	0.250312212
Apod	apolipoprotein D	1.61198472	0.00053	0.221408291
Chi3l1	chitinase 3-like 1	1.636684153	0.00053	0.583086161
Unc93b1	unc-93 homolog B1 (C. elegans)	1.66968256	0.00053	0.360200982
H2-DMb1	histocompatibility 2, class II, locus Mb1	1.67565171	0.00053	0.359347716

Slc15a2	solute carrier family 15 (H ⁺ /peptide transporter), member 2	1.700204294	0.00053	0.385075496
Wnt4	wingless-related MMTV integration site 4	1.713137287	0.00053	0.360869216
Fmo2	flavin containing monooxygenase 2	1.815536475	0.00053	0.308692687
Sparcl1	SPARC-like 1	1.816996676	0.00053	0.063385442
Cyp2f2	cytochrome P450, family 2, subfamily f, polypeptide 2	1.83370284	0.00053	0.078208358
Klhl29	kelch-like 29 (Drosophila)	1.88351542	0.00053	0.138028824
Eml2	echinoderm microtubule associated protein like 2	1.970482521	0.00053	0.027241317
Slpi	secretory leukocyte peptidase inhibitor	2.08642332	0.00053	0.441915633
Lgals6	lectin, galactose binding, soluble 6	5.671892314	0.00053	0.198517766

Table S4: List of identified p66Shc-interacting proteins.

Identified protein	No. of peptides p66⁺ MEF – serum starvation	No. of peptides p66⁺ MEF – serum stimulation / 10 min
Shc1	900	920
Sgk269	22	23
FAM59A	4	27
Beta2-adaptin	5	23
Alpha1-adaptin	4	22
Grb2	0	12
Lrrk1	0	9
Anks1	1	7
PTPN12	1	5
Gab1	0	2
PP2C-beta	8	14
PP1A	2	2