

Plac1 Is a Key Regulator of the Inflammatory Response and Immune Tolerance In Mammary Tumorigenesis

Hongyan Yuan, Xiaoyi Wang, Chunmei Shi, Lu Jin, Jianxia Hu, Alston Zhang, James Li, Nairuthya Vijayendra, Venkata Doodala, Spencer Weiss, Yong Tang, Louis M. Weiner and Robert I. Glazer.

Supplementary Data

Supplementary Figure 1. EO771/sh490/sh187 Plac1 tumor growth

Supplementary Figure 2. SB225002 cytotoxicity assay

Supplementary Figure 3. MC/Plac1 cells

Supplementary Figure 4. Immune signaling pathways

Supplementary Table 1. Antibodies for IHC and FACS

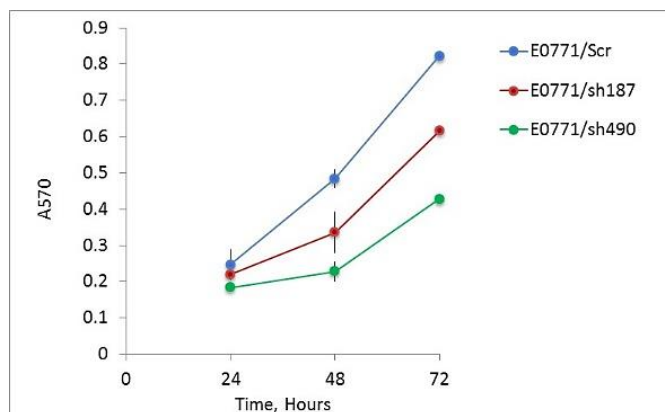
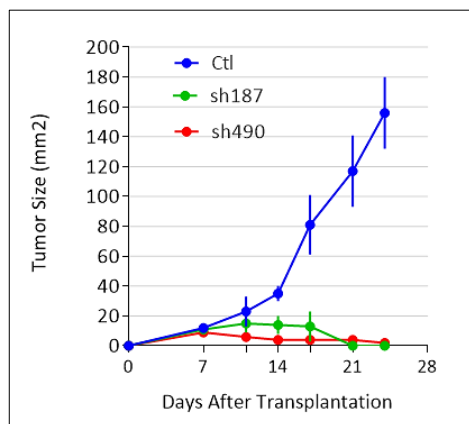
Supplementary Table 2. Gene expression in EO771/shPlac1 cells

Supplementary Table 3. Gene expression in EO771/shCxcl1 cells

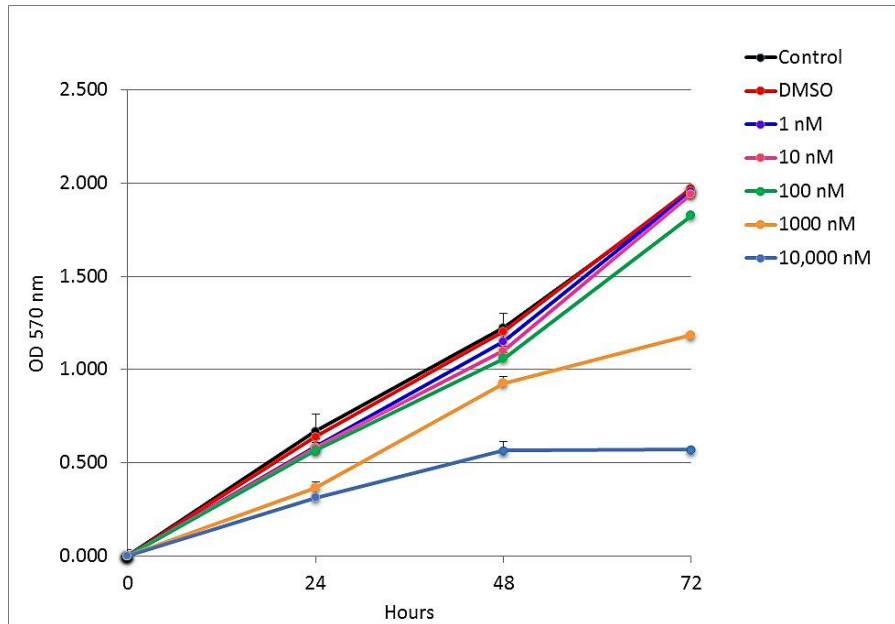
Supplementary Table 4. Gene expression in EO771 tumors+SB25002 treatment

Supplementary Table 5. Gene expression for MC/Plac1 cells

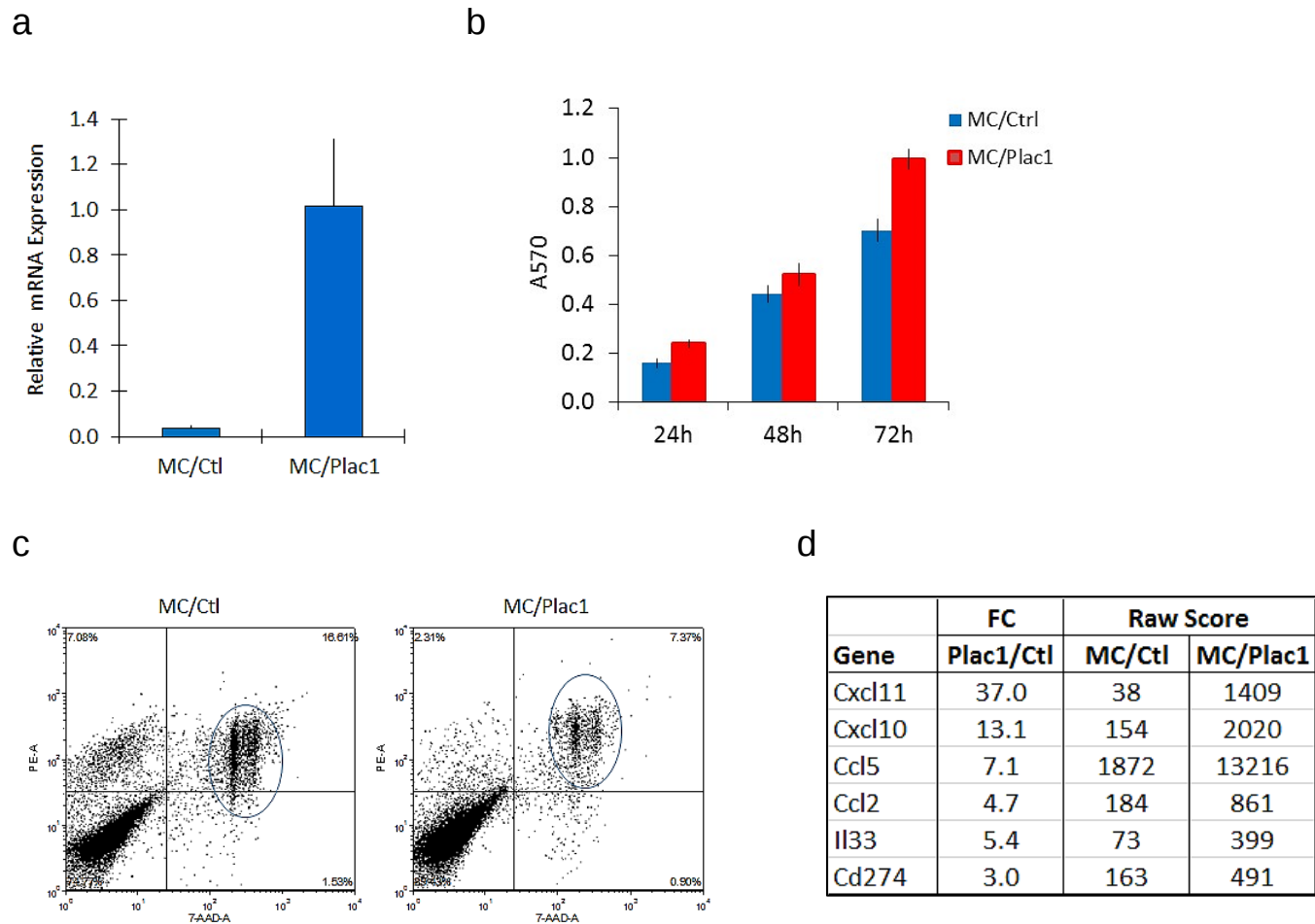
Supplementary Table 6. List of primers for qRT-PCR analysis.

a**b**

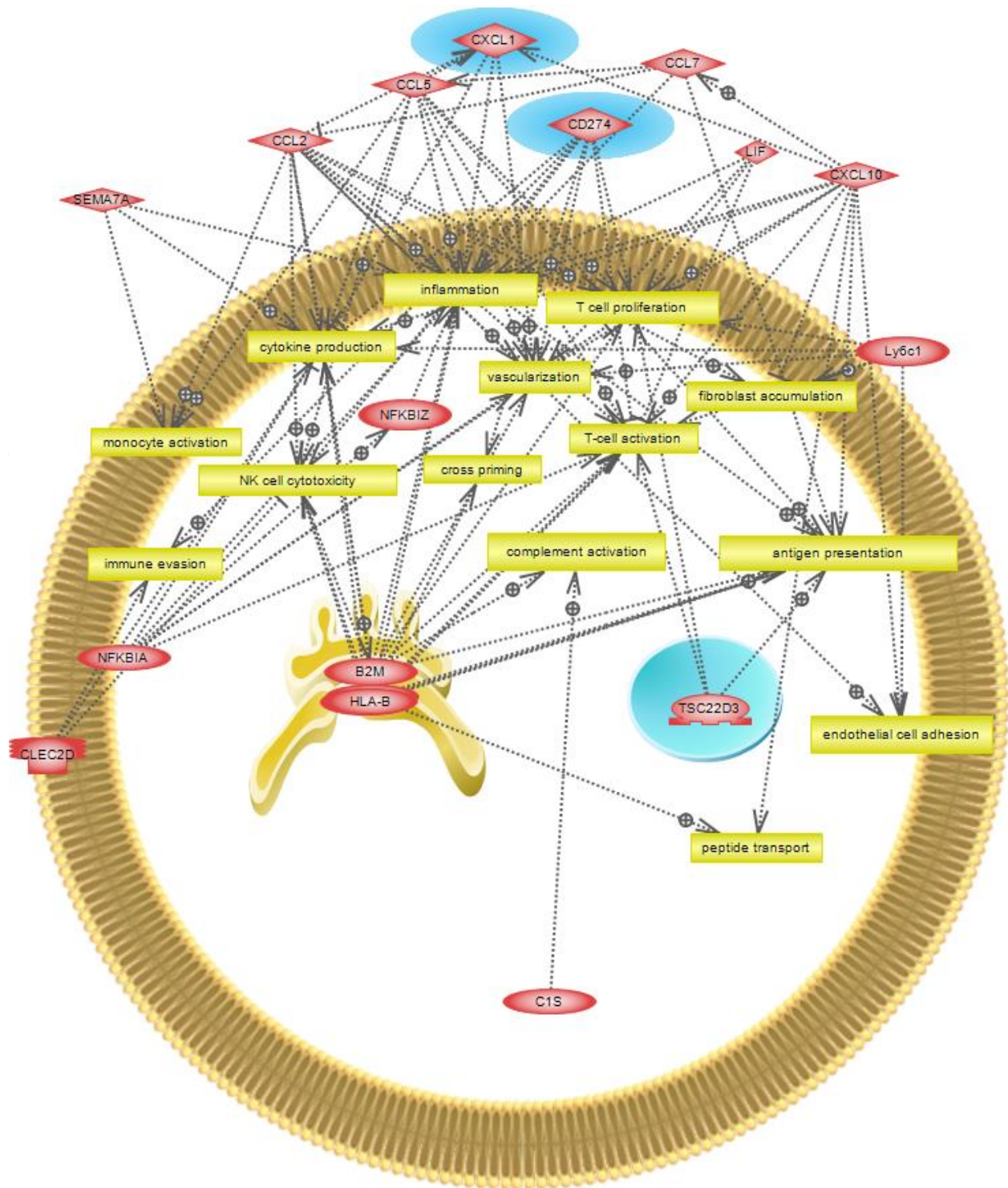
Supplementary Figure 1. Growth of EO771/Scr, EO771/sh187 and EO771/sh490 cells, and the effect of Cxcl1 co-expression in EO771/sh490 cells. (a) EO771/Scr, EO771/sh187 and EO771/sh490 cells were grown as monolayers and cell density determined by sulforhodamine B staining and measuring the absorbance at 570 nm. Shown is the mean±S.D. of triplicate determinations. The growth of EO771/sh490 cells differed significantly ($P<0.001$) from EO771/Scr cells at 24-72 hr using the two-sided Student's t test. (b) C57BL/6 mice at five weeks of age, were inoculated in the mammary gland with 1×10^6 cells, and tumor size determined by caliper measurement in two dimensions. The growth of EO771/sh187 or EO771/sh490 cells differed significantly from EO771/Scr ($P<0.01$) by the unpaired Student's t test. Shown is the mean±SD, N=6 per group.



Supplementary Figure 2. In vitro cytotoxicity of SB225002 to EO771 cells. EO771 cells were grown in 96-well plates at an initial density of 5,000 cells per well, and treated with 0.001% DMSO or SB225002. Cell density was determined after 24, 48 and 72 hr by sulforhodamine B staining and measuring absorbance at 570 nm. Indicated is the final concentration (nM) of SB225002. Shown is the mean $\pm$ SD of triplicate determinations.



Supplementary Figure 3. Overexpression of Plac1 in MC mammary tumor cells. (a) qRT-PCR analysis of MC/Plac1 cells transfected with a vector expressing murine Plac1 and selected in G-418. MC/Plac1 cells expressed significantly greater levels of Plac1 than control MC/Ctl cells by the two-sided Student's t test ($P < 0.001$, $N = 3$). (b) MC/Plac1 cells were grown in a 96-well plate at an initial density of 5,000 cells per well for 24-72 hr, and cell density determined by sulforhodamine B staining and measuring absorbance at 570 nm. Shown is the mean $\pm$ SD of triplicate determinations. (c) Analysis of apoptosis in MC/Plac1 cells by fluorescence of 7-aminoactinomycin D as a measure of cell viability. The scatter determined by FACS is *circled* and is a measure of late apoptosis, which decreased from 16.6% in MC/Ctl cells to 7.3% in MC/Plac1 cells. (d) Table of chemokine and immune-related gene expression in MC/Ctl and MC/Plac1 cells as determined by Affymetrix gene expression profiling (see Supplementary Table 5 for full list).



Supplementary Figure 4. Schematic of immune signaling by Plac1 in EO771 cells. Based on data in Table 1, EO771 cells overexpress several chemokines and immune factors, which regulate inflammation, cytokine production, fibroblast activation, antigen presentation, and monocyte and T cell activation. Plac1 maintained high CXCL1 expression, a chemokine that activates CXCR2 on MDSC, whereas, CD274/ PD-L1 expressed by MDSC and tumor cells activates Treg cells; both pathways contribute to immune tolerance. Inhibition of CXCR2 with an antagonist and treatment with a PD-L1 mAb counteracts in part activation of a tolerogenic tumor microenvironment.

Supplementary Table 1. Antibodies for IHC and FACS.

	Catalog #	Source	Dilution	
			IHC	FACS
anti-Plac1	sc-365919	Santa Cruz	400	
anti-PD-L1	17952-I-AP	Proteintech	200	
anti-Cxcl1	sc-1374	Santa Cruz	120	
anti-CD8a	14-0808-82	eBioscience	40	
anti-SMA	sc130617	Santa Cruz	100	
anti-Ki67	CRM325	Biocare	50	
anti-CD31	ab56299	abcam	100	
anti-Foxp3	14-5773-82	eBioscience	50	
CD16/32	101301	Biolegend		100
FITC anti-mouse CD4	100509	Biolegend		200
PE/Cy7 anti-mouse CD8a	100721	Biolegend		40
APC anti-mouse CD3 epsilon	100311	Biolegend		100
APC anti-mouse CD25	102011	Biolegend		400
PE anti-mouse CD80	104707	Biolegend		400
PE anti-mouse CD86	105007	Biolegend		125
APC anti-mouse F4/80	123115	Biolegend		80
APC/Cy7 anti-mouse CD45	103115	Biolegend		80
PE anti-mouse NK1.1	108707	Biolegend		80
APC anti-mouse CD11c	117309	Biolegend		80
APC anti-mouse/human CD11b	101211	Biolegend		80
PE anti-mouse Foxp3	12-4771-80	eBioscience		80
PE/Cy7 anti-mouse Ly6g/Ly6c (Gr-1)	108415	Biolegend		200

Supplementary Table 2. Gene expression in EO771/shPlac1 cells. Shown are genes with ≥ 3 -fold change in expression and a raw score ≥ 300 in EO771/shPlac1 or EO771/Scr cells.

Function	log2		Raw Score		FC	Gene Name
	Scr	shPlac1	Scr	shPlac1	shPlac1/Scr	
Adhesion/ECM						
Pkp2	6.63	8.27	99	308	3.1	plakophilin 2
P4ha2	11.33	9.66	2567	811	-3.1	prolyl 4-hydroxylase, alpha polypeptide II
Sdc1	11.58	9.88	3064	939	-3.2	syndecan 1
Col18a1	10.35	8.42	1301	343	-3.7	collagen, type XVIII, alpha 1
Apoptosis						
Birc5	8.74	10.53	427	1478	3.5	baculoviral IAP repeat containing 5; Survivin
Ebag9	6.75	8.43	107	345	3.2	estrogen receptor binding site associated, antigen, 9
CD82	10.83	8.96	1823	498	-3.7	Kangai 1 (suppression of tumorigenicity 6, prostate;
Trp53inp1	8.30	6.34	314	81	-3.8	tumor protein p53 inducible nuclear protein 1
Differentiation						
Pter	6.83	8.62	114	394	3.5	phosphotriesterase related
Krt8	10.83	12.42	1817	5489	3.0	keratin 8
Gdf15	9.71	8.12	840	279	-3.3	growth differentiation factor 15
DNA Repair						
Fign1	7.05	9.56	132	757	5.7	fidgetin-like 1
Rad51	6.51	8.75	91	431	4.7	RAD51 recombinase
Fen1	6.89	9.08	119	540	4.6	flap structure-specific endonuclease 1
Rad51ap1	6.37	8.51	83	365	4.5	RAD51 associated protein 1
Ercc6l	6.71	8.83	105	456	4.4	excision repair cross-complementation group 6-like
Rad54l	6.24	8.25	75	305	4.1	RAD54-like (S. cerevisiae)
Ddit3	10.19	8.16	1166	286	-4.0	DNA-damage-inducible transcript 3
Gadd45b	10.55	7.31	1499	159	-12.5	growth arrest and DNA-damage-inducible, beta
DNA Synthesis						
Mki67	7.10	9.99	137	1020	7.4	marker of proliferation Ki-67
Tyms	5.99	8.56	64	378	5.9	thymidylate synthetase
Mcm5	6.42	8.87	86	467	5.4	minichromosome maintenance complex component 5
Cdc6	6.00	8.36	64	328	5.1	cell division cycle 6
Rrm2	9.22	11.28	596	2487	4.4	ribonucleotide reductase M2
Rrm1	8.32	10.34	320	1296	4.1	ribonucleotide reductase M1
Pola1	7.15	9.10	142	549	3.9	polymerase (DNA directed), alpha 1, catalytic subunit
Mcm3	8.10	9.88	274	942	3.5	minichromosome maintenance complex component 3
Mcm4	8.11	9.90	276	955	3.5	minichromosome maintenance complex component 4
Prim1	7.73	9.47	212	709	3.4	primase, DNA, polypeptide 1 (49kDa)
Nasp	8.10	9.73	275	850	3.1	nuclear autoantigenic sperm protein (histone-binding)
Mcm7	8.85	10.45	462	1399	3.0	minichromosome maintenance complex component 7
Smarcb1	7.83	9.43	228	690	3.0	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
Immune/Inflammation						
Tapbp	11.71	10.11	3356	1102	-3.0	TAP binding protein (tapasin)
CD274	8.24	6.50	302	91	-3.3	programmed cell death 1 ligand 1
Cxcl10	8.84	7.02	459	130	-3.6	chemokine (C-X-C motif) ligand 10
Sema7a	8.74	6.93	426	122	-3.6	semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group)
Ifi44	9.35	7.50	652	181	-3.6	interferon-induced protein 44
Ly6c1	10.23	8.36	1198	329	-3.7	lymphocyte antigen 6 complex, locus C
Clec2d	8.51	6.62	366	99	-3.7	C-type lectin domain family 2, member D
Nfkbi2	9.22	7.17	596	144	-4.4	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
Ly6a	12.27	10.18	4937	1160	-4.3	lymphocyte antigen 6 complex, locus A, stem cell antigen-1
H2-K1	11.02	8.90	2069	478	-4.5	major histocompatibility complex, class I, K
Tsc22d3	11.15	8.90	2265	478	-4.8	TSC22 domain family, member 3
Ccl5	11.90	9.61	3812	784	-4.8	chemokine (C-C motif) ligand 5
H2-D1	11.20	8.88	2353	471	-5.0	major histocompatibility complex, class I, D
B2m	8.58	6.16	383	72	-5.3	beta-2-microglobulin
Nfkbia	12.08	9.02	4330	517	-7.1	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
C1s	9.84	7.00	918	128	-7.1	complement component 1, s subcomponent
Lif	9.37	6.32	664	80	-8.3	leukemia inhibitory factor
Mndal	10.63	7.53	1585	185	-8.3	myeloid cell nuclear differentiation antigen
Ccl2	11.83	8.25	3640	305	-11.9	chemokine (C-C motif) ligand 2
CD68	9.86	5.78	929	55	-16.9	scavenger receptor class D, member 1
Ccl7	11.48	7.25	2854	153	-18.5	chemokine (C-C motif) ligand 7
Cxcl1	12.78	6.73	7054	106	-66.7	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
Invasion/Motility						
Diap3	5.59	8.59	48	386	9.1	diaphanous-related formin 3
Hmmr	7.51	9.33	182	644	3.5	hyaluronan-mediated motility receptor (RHAMM)
Racgap1	8.47	10.21	355	1184	3.3	Rac GTPase activating protein 1
Serpine1	10.98	9.35	2021	651	-3.1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
Ntn1	9.52	7.84	736	229	-3.2	netrin 1
Serpinf1	11.60	9.75	3097	860	-3.4	serpin peptidase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1
Ctsh	10.92	8.47	1931	354	-5.6	cathepsin H
Plau	9.34	6.31	646	80	-7.1	plasminogen activator, urokinase
Metabolism						
Pdss1	6.40	8.33	84	322	3.8	prenyl (decaprenyl) diphosphate synthase, subunit 1
Mgat2	7.70	9.33	208	643	3.1	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
Sephs2	7.93	9.52	244	733	3.0	selenophosphate synthetase 2
Npc2	13.22	11.54	9553	2987	-3.2	Niemann-Pick disease, type C2
Idua	8.35	6.65	327	100	-3.2	iduronidase, alpha-L-
ND5	11.77	9.98	3486	1008	-3.4	mitochondrially encoded NADH dehydrogenase 5
Lipg	8.32	6.54	320	93	-3.6	lipase, endothelial
Ip6k1	9.86	7.72	932	211	-4.3	inositol hexakisphosphate kinase 1
St3gal1	8.53	6.33	370	80	-4.5	ST3 beta-galactoside alpha-2,3-sialyltransferase 1
Dnpep	8.79	6.46	444	88	-5.0	aspartyl aminopeptidase
Fabp4	10.76	7.33	1734	161	-11.4	fatty acid binding protein 4, adipocyte

Mitosis						
Melk	6.62	8.96	98	498	5.1	maternal embryonic leucine zipper kinase
Nuf2	6.74	9.05	107	529	5.0	NUF2, NDC80 kinetochore complex component
Cenpf	7.87	10.14	233	1126	4.8	centromere protein F, 350/400kDa
H1f0	8.62	10.75	392	1726	4.6	H1 histone family, member 0
Kif22	7.69	9.83	207	910	4.4	kinesin family member 22
Ccnb1	7.44	9.59	174	771	4.2	cyclin B1
Ndc80	6.37	8.43	83	345	4.2	NDC80 kinetochore complex component
Kif2c	7.23	9.26	150	613	4.1	kinesin family member 2C
Aspm	7.42	9.45	171	699	4.1	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)
Ccna2	9.00	10.92	512	1941	3.8	cyclin A2
Ect2	8.08	10.01	270	1030	3.8	epithelial cell transforming 2
Ska1	6.72	8.63	106	396	3.7	spindle and kinetochore associated complex subunit 1
Spag5	6.65	8.42	100	342	3.5	sperm associated antigen 5
Cdca5	7.77	9.50	219	724	3.4	cell division cycle associated 5
Cenpt	6.81	8.55	112	376	3.4	centromere protein T
Nek2	7.03	8.76	130	434	3.3	NIMA-related kinase 2
Sgo1	7.74	9.47	214	710	3.3	shugoshin-like 1 (S. pombe)
Spc25	7.44	9.18	174	582	3.3	SPC25, NDC80 kinetochore complex component
Tacc3	7.88	9.59	236	772	3.3	transforming, acidic coiled-coil containing protein 3
Ncapd2	8.00	9.71	256	836	3.3	non-SMC condensin I complex, subunit D2
Ncapg	7.14	8.87	141	466	3.3	non-SMC condensin I complex, subunit G
Ncaph	7.99	9.58	255	766	3.2	non-SMC condensin I complex, subunit H
Aurka	8.74	10.37	428	1322	3.1	aurora kinase A
Tubb2a	7.48	9.10	178	550	3.1	tubulin, beta 2A class IIa
Cdc20	10.38	11.99	1333	4063	3.1	cell division cycle 20
Ndrp4	10.82	9.14	1808	564	-3.2	NDRG family member 4
Plk2	9.70	7.88	830	236	-3.6	polo-like kinase 2
Pafah1b1	8.89	7.01	474	129	-3.7	platelet-activating factor acetylhydrolase 1b, regulatory subunit 1 (45kDa)
Zwint	9.11	6.72	553	106	-5.3	ZW10 interacting kinetochore protein
Protein Trafficking/Degradation						
Diap2	5.59	8.59	48	386	8.0	diaphanous-related formin 2
Tubb4b	10.34	12.40	1299	5409	4.2	tubulin, beta 4B class IVb
Dnajc9	6.76	8.78	109	440	4.1	DnaJ (Hsp40) homolog, subfamily C, member 9
Uhrf1	7.38	9.22	167	598	3.6	ubiquitin-like with PHD and ring finger domains 1
Hspa4	8.20	9.98	294	1013	3.4	heat shock 70kDa protein 4
Ide	7.85	9.54	231	744	3.2	insulin-degrading enzyme
Fnbp4	8.37	6.78	330	110	-3.0	formin binding protein 4
Mapk8ip3	8.32	6.73	319	106	-3.0	mitogen-activated protein kinase 8 interacting protein 3
Trim30a	8.45	6.81	349	112	-3.1	tripartite motif containing 30
Gramd1a	10.03	8.39	1048	335	-3.1	GRAM domain containing 1A
Hspa5	13.38	11.63	10689	3170	-3.3	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)
Calr	11.78	10.01	3517	1031	-3.4	calreticulin
Rcn3	11.45	9.61	2801	780	-3.6	reticulocalbin 3, EF-hand calcium binding domain
Vcp	8.26	6.36	308	82	-3.7	valosin containing protein
Srprb	9.14	7.18	564	145	-3.8	signal recognition particle receptor, B subunit
Rab14	8.39	6.38	336	83	-4.0	RAB14, member RAS oncogene family
Tmed3	11.31	9.24	2534	604	-4.2	transmembrane emp24 protein transport domain containing 3
Arih2	9.46	7.40	707	169	-4.2	ariadne RBR E3 ubiquitin protein ligase 2
Hspa1b	8.91	6.73	481	106	-4.3	heat shock 70kDa protein 1B
Fkbp7	9.45	7.34	700	162	-4.3	FK506 binding protein 7
Zfand2a	10.49	8.33	1438	321	-4.5	zinc finger, AN1-type domain 2A
Arl4d	10.49	8.33	1438	321	-5.0	ADP-ribosylation factor-like 4D
Dnajb6	9.54	7.19	746	146	-5.3	DnaJ (Hsp40) homolog, subfamily B, member 6
Cryab	10.39	7.84	1340	229	-5.3	crystallin, alpha B
Hspa8	10.16	7.42	1142	171	-6.7	heat shock 70kDa protein 8
Signaling						
Shcbp1	7.11	9.69	138	829	6.0	SHC SH2-domain binding protein 1
Adcy7	6.96	9.25	124	609	5.0	adenylate cyclase 7
Procr	10.78	9.14	1761	565	-3.1	protein C receptor, endothelial
Igf1bp4	10.46	8.81	1409	449	-3.2	insulin-like growth factor binding protein 4
Grb14	9.52	7.82	734	225	-3.2	growth factor receptor-bound protein 14
Snx12	10.85	9.01	1849	517	-3.6	sorting nexin 12
Ramp3	11.55	9.30	3007	629	-2.8	receptor (G protein-coupled) activity modifying protein 3
Appl2	11.20	8.67	2352	407	-5.9	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 2
Rgs16	10.86	7.93	1859	244	-7.7	regulator of G-protein signaling 16
Transcription/Translation						
Krcc1	7.45	9.56	175	757	4.4	lysine-rich coiled-coil 1
Srsf1	8.41	10.40	341	1350	4.0	serine/arginine-rich splicing factor 1
Gatad2a	6.29	8.27	78	309	3.9	GATA zinc finger domain containing 2A
Srsf2	9.85	11.53	920	2962	3.2	serine/arginine-rich splicing factor 2
Hnrnpd	9.74	11.38	858	2674	3.1	heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa)
Neat1	8.59	10.21	387	1186	3.1	nuclear paraspeckle assembly transcript 1 (non-protein coding)
Lmnb1	7.32	8.91	160	480	3.0	laminin B1
Malat1	10.59	8.91	1543	483	-3.2	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)
Taf1d	9.14	7.36	566	164	-3.4	TATA box binding protein (TBP)-associated factor, RNA polymerase I, D, 41kDa
Hnrpdl	12.03	10.23	4197	1203	-3.4	heterogeneous nuclear ribonucleoprotein D-like
Rpl41	8.37	6.56	330	94	-3.6	ribosomal protein L41
Luc7l	10.50	8.64	1448	398	-3.6	LUC7-like (S. cerevisiae)
Ccnl1	11.77	9.85	3500	922	-3.8	cyclin L1
Clk1	11.64	9.69	3199	825	-3.8	CDC-like kinase 1
Rps10	8.29	6.29	314	79	-4.0	ribosomal protein S10
Pabpc1	9.92	7.88	971	236	-4.2	poly(A) binding protein, cytoplasmic 1
Luc7l2	11.64	9.30	3185	632	-5.0	LUC7-like 2 (S. cerevisiae)
Txnip	10.11	7.41	1105	171	-7.1	thioredoxin interacting protein
Transport						
Chac2	6.77	8.65	109	403	3.7	ChaC, cation transport regulator homolog 2 (E. coli)
Slc22a4	9.90	8.25	955	304	-3.1	solute carrier family 22 (organic cation/zwitterion transporter), member 4
Slc35a2	8.95	7.28	493	156	-3.1	solute carrier family 35 (UDP-galactose transporter), member A2
Nptx1	9.53	7.84	737	229	-3.2	neuronal pentraxin I
Slc25a37	10.84	9.14	1830	565	-3.2	solute carrier family 25 (mitochondrial iron transporter), member 37

Supplementary Table 3. Gene expression in EO771/shCxc1. Shown are ≥ 3.0 -fold changes in gene expression with a raw score ≥ 300 in EO771/shCxc1 or EO771/Scr cells.

Function	log 2		Raw Score		FC	Gene Name
	Scr	shCxc1	Scr	shCxc1	sh/Scr	
Adhesion/ECM						
Plet1	9.07	7.15	538	142	-3.8	Placenta Expressed Transcript 1, ECM, wound repair
Apoptosis						
CD82	11.42	9.78	2747	878	-3.1	Kangai 1 (Suppression Of Tumorigenicity 6, Prostate
Immune/Inflammation						
Ly6a	12.93	11.29	7787	2512	-3.1	Lymphocyte antigen 6 complex, locus A, Stem cell antigen-1, Sca-1
Il23a	8.84	7.06	459	133	-3.5	Innate and adaptive immunity, autoimmunity, inflammatory cytokines
C3	8.48	6.55	358	94	-3.8	Complement Component 3, innate and adaptive immunity, inflammation
Cxcl1	11.06	9.07	2137	536	-4.0	Chemokine (C-X-C Motif) Ligand 1 (Melanoma Growth Stimulating Activity, Alpha)
CD68	8.62	6.54	394	93	-4.2	Macrophage phagocytosis
Invasion/Motility						
Plau	9.89	7.05	949	133	-7.3	Plasminogen activator urokinase
Mitosis						
Clip4	8.61	6.91	391	120	-3.6	CAP-GLY Domain Containing Linker Protein Family, Member 4, centrosome
Piwi2	9.46	7.64	707	199	-3.6	Piwi-Like RNA-Mediated Gene Silencing 2, meiosis, oncogene when overexpressed
Signaling						
Rgs16	10.79	9.15	1771	568	-3.2	Regulator of G-Protein Signaling 16
Transport						
Slc7a3	7.76	9.39	217	673	3.1	Solute Carrier Family 7 (Cationic Amino Acid Transporter, Y+ System), Member 3

Supplementary Table 4. Gene expression in EO771 isografts from SB25002-treated mice. Shown are genes with ≥ 3 -fold change in expression and a raw score ≥ 300 in mammary tumors from mice treated with vehicle or 20 mg/kg SB25002.

Function	log2		Raw Score		FC	Gene Name
	Vehicle	SB25002	Vehicle	SB25002		
Adhesion/ECM						
Cgref1	11.6121	13.3081	3130	10142	3.2	cell growth regulator with EF hand domain 1
Nrcam	8.0089	9.6418	258	799	3.1	neuronal cell adhesion molecule
Apoptosis						
Cidea	8.7618	7.1531	434	142	-3.0	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A
Differentiation						
Krt18	13.0294	14.6218	8360	25211	3.0	keratin 18
Lor	9.5320	6.3053	740	79	-9.4	loricrin
Immune/Inflammation						
Ly6c1	14.9712	13.4000	32121	10809	-3.0	lymphocyte antigen 6 complex, locus C1
Ly6d	8.8947	7.3091	476	159	-3.0	lymphocyte antigen 6 complex, locus D
Tmem176a	10.6826	9.0431	1644	528	-3.1	transmembrane protein 176A
C1qtnf1	10.4737	8.6785	1422	410	-3.5	C1q and tumor necrosis factor related protein 1
Tmem176b	15.2836	13.2279	39886	9594	-4.2	transmembrane protein 176B
Invasion/Motility						
Tspan2	9.2743	10.8752	619	1878	3.0	tetraspanin 2
Tnnt2	9.8600	11.4300	929	2759	3.0	troponin T2, cardiac
Serpinb1a	12.8849	10.8336	7564	1825	-4.1	serine (or cysteine) peptidase inhibitor, clade B, member 1a
Metabolism						
Tex101	8.5414	10.3233	373	1281	3.4	testis expressed gene 101
Cpt1c	6.8928	8.5777	119	382	3.2	carnitine palmitoyltransferase 1c
Pla2g5	12.3834	10.8200	5343	1808	3.0	phospholipase A2, group V
Gsta2	8.9964	7.1455	511	142	-3.6	glutathione S-transferase, alpha 2 (Yc2)
Plin4	10.3196	7.7437	1278	214	-6.0	perilipin 4
Gpd1	9.8930	7.1120	951	138	-6.9	glycerol-3-phosphate dehydrogenase 1 (soluble)
Hp	14.7010	11.5504	26634	2999	-8.9	haptoglobin
Proliferation						
Figf	10.8829	9.3714	1888	640	3.0	c-fos induced growth factor
Greb1	12.9176	14.7121	7737	26840	3.5	gene regulated by estrogen in breast cancer protein
Signaling						
F3	9.0419	10.6820	527	1643	3.1	coagulation factor III
P2rx3	9.2673	7.6281	616	198	-3.1	purinergic receptor P2X, ligand-gated ion channel, 3
Snca	11.6458	9.5825	3204	767	-4.2	synuclein, gamma
Transcription/Translation						
Anp32a	12.4230	14.0442	5491	16894	3.1	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A
Pcbd1	11.4736	9.7331	2844	851	-3.3	pterin 4 alpha carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1) 1
Glod5	9.8789	7.7521	942	216	-4.4	glyoxalase domain containing 5
Transport						
Scara5	11.5306	9.7621	2958	868	-3.4	scavenger receptor class A, member 5 (putative)
Mup-ps16	9.3871	7.5667	670	190	-3.5	major urinary protein, pseudogene 16
Mup4	10.2305	8.1747	1201	289	-4.2	major urinary protein 4
Retn	9.6222	7.5379	788	186	-4.2	resistin
Mup19	11.7530	8.9928	3451	509	-6.8	major urinary protein 19

Supplemental Table 5. Gene expression in MC/Plac1 cells. Shown are genes with ≥ 3 -fold change in expression with a raw score ≥ 300 in MC/Ctl or MC/Plac1 cells.

	log2		Raw Score		FC	
Function	MC/Ctl	MC/Plac1	MC/Ctl	MC/Plac1	Plac1/Ctl	Gene Name
Adhesion/ECM						
Cldn1	8.07	10.78	269	1758	6.5	Claudin 1
Hs6st2	6.51	8.59	91	385	4.2	Heparan Sulfate 6-O-Sulfotransferase 2
Vcan	6.72	8.63	105	396	3.8	Versican
Lgals9	7.32	9.22	160	596	3.7	Lectin, Galactoside-Binding, Soluble, 9
Fn1	8.56	6.93	377	122	-3.1	Fibronectin 1
Plec	10.97	9.34	2006	648	-3.1	Plectin
Matn2	8.44	6.66	347	101	-3.4	Matrilin 2
Emp2	11.52	9.73	2937	849	-3.4	Epithelial membrane protein 2
Lrrc8a	8.5	6.54	362	93	-3.9	Leucine Rich Repeat Containing 8 Family, Member A
Differentiation						
Krt16	6.12	10.4	70	1351	19.4	Keratin 16
Fam198b	7.26	9.21	153	592	3.9	Family With Sequence Similarity 198, Member B
Sema3c	7.96	9.85	249	923	3.7	Sema Domain, Immunoglobulin Domain (Ig), Short Basic Domain, Secreted
Pard6g	8.22	9.89	298	949	3.2	Par-6 Family Cell Polarity Regulator Gamma
Scara5	9.28	7.64	622	199	-3.1	Scavenger Receptor Class A, Member 5
Cwh43	8.98	7.12	505	139	-3.6	Cell Wall Biogenesis 43 C-Terminal Homolog
Immune/Inflammation						
Cxcl11	5.25	10.46	38	1409	37.0	Chemokine (C-X-C Motif) Ligand 11
Iigp1	7.05	11.43	133	2759	21.0	Interferon inducible GTPase 1
Tgtp1	8.95	13.22	495	9541	19.3	T-cell specific GTPase 1
Cxcl10	7.27	10.98	154	2020	13.1	Chemokine (C-X-C Motif) Ligand 10
Ifi2712a	7.4	11.03	169	2091	12.4	Interferon, Alpha-Inducible Protein 27-Like 2
Irg1	6.19	9.74	73	855	11.7	Immunoresponsive 1 Homolog (Mouse)
Trim30a	8.62	11.99	393	4068	10.5	Tripartite Motif Containing 5
Gbp6	7.9	11.24	239	2419	10.1	Guanylate Binding Protein Family, Member 6
Ifit3	9.91	13.22	962	9541	9.9	Interferon-Induced Protein With Tetratricopeptide Repeats 3
Zbp1	7.68	10.8	205	1783	8.8	Z-DNA Binding Protein 1
Rsad2	9.62	12.7	787	6654	8.6	Radical S-Adenosyl Methionine Domain Containing 2
Ifit1	10.63	13.62	1585	12590	7.9	Interferon-Induced Protein With Tetratricopeptide Repeats 1
Gbp3	10.39	13.22	1342	9541	7.1	Guanylate Binding Protein 3
Ccl5	10.87	13.69	1872	13216	7.1	Chemokine (C-C Motif) Ligand 5
Ddx60	8.53	11.35	370	2610	7.1	DEAD (Asp-Glu-Ala-Asp) Box Polypeptide 60
Irf7	8.59	11.4	385	2702	7.0	Interferon Regulatory Factor 7
Il33	6.21	8.64	74	399	5.4	Interleukin-33
Oasl2	9.92	12.29	969	5008	5.2	2'-5'-Oligoadenylate Synthetase-Like
H2-Q7	6.63	8.94	99	491	5.0	Histocompatibility 2, Q region locus 7
Ifit2	10.32	12.6	1278	6208	4.9	Interferon-Induced Protein With Tetratricopeptide Repeats 2
Ifi44	10.03	12.28	1046	4973	4.8	Interferon-Induced Protein 44
Ccl2	7.52	9.75	184	861	4.7	Chemokine (C-C Motif) Ligand 2
Usp18	10.98	13.2	2020	9410	4.7	Ubiquitin Specific Peptidase 18
Isg15	11.38	13.56	2665	12077	4.5	ISG15 Ubiquitin-Like Modifier
Dhx58	7.63	9.73	198	849	4.3	DEXH (Asp-Glu-X-His) Box Polypeptide 58
Igtp	10.94	12.95	1965	7913	4.0	Interferon gamma induced GTPase
Gbp2	11.13	12.98	2241	8079	3.6	Guanylate Binding Protein 2, Interferon-Inducible
Ifih1	10.17	11.94	1152	3929	3.4	Interferon Induced With Helicase C Domain 1
Ifi47	10.92	12.69	1938	6608	3.4	Interferon gamma inducible protein 47
Irgm1	11.47	13.2	2837	9410	3.3	Immunity-related GTPase
Lrr1	7.12	8.83	139	455	3.3	Leucine Rich Repeat Protein 1
Cd274	7.35	8.94	163	491	3.0	Programmed Cell Death 1 Ligand 1
Oasl1	8.03	9.69	261	826	3.2	2'-5'-Oligoadenylate Synthetase-Like1
Gbp7	9.52	11.17	734	2304	3.1	Guanylate Binding Protein 7
Aebp1	9.79	11.43	885	2759	3.1	Adipocyte Enhancer Binding Protein 1
Oas1a	11.21	12.81	2369	7181	3.0	2'-5'-Oligoadenylate Synthetase 1, 44/46kDa
Cxcl2	9.38	7.69	666	207	-3.2	Chemokine (C-X-C Motif) Ligand 2
Cd68	10.49	8.48	1438	357	-4.0	Scavenger Receptor Class D, Member 1
Il1a	8.44	5.93	347	61	-5.7	Interleukin 1, Alpha
Invasion/Motility						
Fhod3	5.32	9.92	40	969	24.3	Formin Homology 2 Domain Containing 3
Serpinb2	5.20	9.30	37	630	17.1	Serpin Peptidase Inhibitor, Clade B (Ovalbumin), Member 2
Plat	7.68	9.68	205	820	4.0	Plasminogen Activator, Tissue
Pfn2	7.82	9.77	226	873	3.9	Profilin 2
Myo10	9.93	11.63	976	3169	3.3	myosin X
Psmb9	9.68	11.42	820	2740	3.3	Proteasome (Prosome, Macropain) Subunit, Beta Type, 9
Hmmr	7.89	9.54	237	744	3.2	Hyaluronan-Mediated Motility Receptor
Malat1	12.05	10.16	4240	1144	-4.0	Metastasis Associated Lung Adenocarcinoma Transcript 1
Tnfrsf2	10.8	8.69	1783	413	-4.3	Tumor Necrosis Factor, Alpha-Induced Protein 2
Mmp3	9.06	6.9	534	119	-4.5	Matrix Metallopeptidase 3 (Stromelysin 1)
Ctsc	8.72	6.48	422	89	-4.7	Cathepsin C
Mmp10	10.39	7.88	1342	236	-5.7	Matrix Metallopeptidase 10 (Stromelysin 2)
Metabolism						
Cmpk2	7.56	10.03	189	1046	5.9	CMP (UMP-CMP) Kinase 2, Mitochondrial
Cyp11b1	7.84	9.98	229	1010	4.6	Cytochrome P450, Family 1, Subfamily B, Polypeptide 11
Ugt1a1	9.91	11.59	962	3083	3.2	UDP Glucuronosyltransferase 1 Family, Polypeptide A1
Ckb	10.01	8.41	1031	340	-3.0	Creatine kinase, brain
Pck2	10.36	8.67	1314	407	-3.2	Phosphoenolpyruvate Carboxykinase 2
Shmt2	10.48	8.85	1428	461	-3.1	Serine Hydroxymethyltransferase 2 (Mitochondrial)
Mthfd2	11.21	9.48	2369	714	-3.2	Methylenetetrahydrofolate Dehydrogenase (NADP+ Dependent) 2

Psph	12.25	10.51	4871	1458	-3.4	Phosphoserine Phosphatase1
Aldoc	10.29	8.63	1252	396	-3.6	Aldolase C, Fructose-Bisphosphate
Stbd1	11.22	9.37	2385	662	-3.6	Starch Binding Domain 1
Ero1l	11.6	9.65	3104	803	-3.7	Endoplasmic Oxidoreductin-1-Like Protein
Aspa	10.03	8.07	1046	269	-3.9	Aspartoacylase
Acot2	8.52	6.52	367	92	-4.0	Acyl-CoA Thioesterase 2
Gpt2	10.54	10.22	1489	1193	-4.0	Glutamic Pyruvate Transaminase
Pycr1	9.5	7.49	724	180	-4.0	Pyrraline-5-Carboxylate Reductase 1
Hmox1	12.11	10.07	4421	1075	-4.1	Heme Oxygenase (Decycling) 1
Acsbg1	8.56	6.44	377	87	-4.3	Acyl-CoA Synthetase Bubblegum Family Member 1
Cth	9.87	7.55	936	187	-5.0	Cystathionine Gamma-Lyase
Gsta1/Gsta2	11.5	7.27	2896	154	-16.7	Glutathione S-Transferase Alpha 1/Alpha 2
Inmt	11.59	6.63	3083	99	-31.3	Indolethylamine N-Methyltransferase
Mitosis						
Kif20a	8.35	10.35	326	1305	4.0	Kinesin Family Member 20A1
Nusap1	8.07	10.06	269	1067	4.0	Nucleolar And Spindle Associated Protein 1
Plk1	7.52	9.35	184	653	3.6	Polo-like Kinase 1
Ttk	7.18	9.01	145	516	3.6	TTK Protein Kinase
Kif23	6.93	8.7	122	416	3.4	Kinesin Family Member 23
Prc1	8.62	10.36	393	1314	3.4	Protein Regulator Of Cytokinesis 1
Aurkb	7.83	9.55	228	750	3.3	Aurora B Kinase
Kif2c	8.09	9.8	272	891	3.3	Kinesin Family Member 2c
Aspm	7.57	9.25	190	609	3.2	Asp (Abnormal Spindle) Homolog, Microcephaly Associated
Zwilch	8.05	9.73	265	849	3.2	Zwilch Kinetochore Protein
Ncapg	7.26	8.92	153	484	3.2	Non-SMC Condensin I Complex, Subunit G
Birc5	9.69	11.34	826	2592	3.1	Baculoviral IAP Repeat Containing 5
Mastl	6.62	8.26	98	307	3.1	Microtubule Associated Serine/Threonine Kinase-Like
Kif22	7.1	8.72	137	422	3.1	Kinesin Family Member 22
Nuf2	7.54	9.17	186	576	3.1	NUF2, NDC80 Kinetochore Complex Component
Cenpe	8.28	9.87	311	936	3.0	Centromere protein E
Fam110c	9.45	7.83	699	228	-3.1	Family With Sequence Similarity 110, Member C
Proliferation						
Plac1	7.51	11.37	182	2647	14.5	Placental-specific 1
Shcbp1	8.21	10.16	296	1144	3.9	SHC SH2-Domain Binding Protein 11
Wnt4	8.42	10.2	343	1176	3.4	Wingless-Type MMTV Integration Site Family, Member 4
Rab32	8.22	10.09	298	1090	3.7	RAB32, Member RAS Oncogene Family
Afp	6.4	8.23	84	300	3.6	Alpha-fetoprotein
Mertk	6.55	8.29	94	313	3.3	C-Mer Proto-Oncogene Tyrosine Kinase
Ccna2	8.84	10.53	458	1479	3.2	Cyclin A2
Smpd13b	8.33	9.97	322	1003	3.1	Sphingomyelin Phosphodiesterase, Acid-Like 3B1
Ghr	9.88	11.52	942	2937	3.1	Growth hormone receptor
Bnip3	11.61	9.96	3126	996	-3.1	BCL2/Adenovirus E1B 19kDa Interacting Protein 3
Trib3	11.13	9.25	2241	609	-3.6	Tribbles Pseudokinase 3
Gas5	9.82	7.91	904	241	-3.8	Growth Arrest-Specific 5
Phlda2	9.71	7.75	838	215	-3.9	Pleckstrin Homology-Like Domain, Family A, Member 2
Vegfa	11.76	9.67	3468	815	-4.2	Vascular endothelial growth factor A
Areg	11.03	8.94	2091	491	-4.3	Amphiregulin
Reep6	10.85	8.69	1846	413	-4.5	Receptor Accessory Protein 6
Csf1r	9.98	7.78	1010	220	-4.6	Colony Stimulating Factor 1 Receptor
Prkg2	11.34	9.11	2592	553	-4.7	Protein Kinase, CGMP-Dependent, Type II
Artn	9.45	7.15	699	142	-4.9	Artemin
Ndrp1	11.85	9.48	3692	714	-5.0	N-Myc Downstream Regulated 1
Chac1	11.53	9.02	2957	519	-5.7	ChaC, Cation Transport Regulator Homolog 1
Fgf21	8.46	5.95	352	62	-5.7	Fibroblast growth factor 21
Fam129a	9.37	6.76	662	108	-6.1	Family With Sequence Similarity 129, Member A
Secretion						
Inhbb	8.76	11.76	434	3468	8.0	Activin Beta-B Chain
Fst	7.18	9.81	145	898	6.2	Follistatin
Tac1	7.03	8.91	131	481	3.7	Tachykinin, Precursor 1
Transport						
Rtp4	7.03	8.84	131	458	3.5	Receptor (Chemosensory) Transporter Protein
Abca1	8.81	10.5	449	1448	3.2	ATP-Binding Cassette, Sub-Family A (ABC1), Member 1
Slc1a4	9.29	7.59	626	193	-3.2	Solute Carrier Family 1 (Glutamate/Neutral Amino Acid Transporter), Member 4
Fxyd2	10.25	8.26	1218	307	-4.0	FXD Domain Containing Ion Transport Regulator 2
Slc7a11	8.84	5.84	458	57	-8.0	Solute carrier family 7 (anionic amino acid transporter light chain, xc-system), member 11
Transcription/Translation						
Hist1h2b	8.77	12.55	437	5997	13.9	Histone cluster 1, H2b
Cspsr	5.81	8.28	56	311	5.9	Component of Sp100-rs
Gadd45g	9.95	12.36	989	5257	5.3	Growth Arrest And DNA-Damage-Inducible, Gamma
Hist1h3b	6.22	8.58	75	383	5.1	Histone cluster 1, H3b
Hist1h4a	6.16	8.3	72	315	4.6	Histone cluster 1, H4a
Hopx	7.44	9.53	174	739	4.3	HOP Homeobox
Depdc1a	7.35	9.26	163	613	3.8	DEP Domain Containing 1
Foxm1	6.6	8.45	97	350	3.6	Forkhead Box M1
Hist2h2aa	8.44	10.23	347	1201	3.5	Histone cluster 2, H2aa
Nr4a2	7.12	8.92	139	484	3.5	Nuclear Receptor Subfamily 4, Group A, Member 2
Stat1	9.86	11.59	929	3083	3.3	Signal transducer and activator of transcription 1, 91kDa
Atxn7l1	7.07	8.8	134	446	3.3	Ataxin 7-Like 1
Id3	9.42	11.09	685	2180	3.2	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
Sp100	7.39	9.02	168	519	3.1	SP100 Nuclear Antigen
Txnip	10.35	8.65	1305	402	-3.2	Thioredoxin Interacting Protein
Rpl30	8.36	6.51	329	91	-3.6	Ribosomal Protein L30

Supplementary Table 6. List of primers for qRT-PCR analysis

Gene Accession #	Primer Name	Sequence	Amplicon size (bp)	Primer Location
Cxcl1 NM_008176.3	mCxcl1 rRNA-F mCxcl1 rRNA-R	GTGTCTAGTTGGTAGGGCATAAT CAGTCCTTTGAACGTCTCTGT	94	823-846 896-917
Ccl2 NM_011333.3	mCcl2 rRNA-F mCcl2 rRNA-R	CTCGGACTGTGATGCCTTAAT TGGATCCACACCTTGCAATTA	106	538-559 623-644
Ccl5 NM_013653.3	mCcl5 rRNA-F mCcl5 rRNA-R	CCAGAGAAGAAGTGGGTTCAAG AGCAATGACAGGGAAGCTATAC	100	283-305 361-383
Ccl7 NM_013654.3	mCcl7 rRNA-F mCcl7 rRNA-R	GGATAGGAGCTGTCTGTAGGA CATGAGGTCTCCAGAGCTTTAC	128	472-493 578-600
CD274 NM_021893.3	mCD274 rRNA-F mCD274 rRNA-R	GAGTGCAGATTCCTGTAGAAC CTCCTCTCCTGCCACAAAC	99	198-220 227-297
Lif NM_008501.2	mLif rRNA-F mLif rRNA-R	CGCCAATGCTCTCTTCATTTT ATGGGAAGTCTGTCATGTTAGG	98	177-198 253-275
Plac1 NM_019538.4	mPlac1 rRNA-F mPlac1 rRNA-R	GAGGTCCTTCTTGTGTAGTCATC ATCTGGGCACTATATGGGTTTC	96	790-813 864-886
18S rRNA NR_003278	m18S rRNA-F m18S rRNA-R	TCGGAACTGAGGCCATGATT CCTCCGACTTTTCGTTCTTGATT	146	900-919 1024-1045
CD68 NM_001291058.1	mCD68 rRNA-F mCD68 rRNA-R	CCCACCTGTCTCTCTCATTTT GTATTCCACCGCCATGTAGT	107	545-566 632-652
Ly6a NM_001271416.1	mLy6a rRNA-F mLy6a rRNA-R	CTCAGGAGGCAGCAGTTATT GTACCCAGGATCTCCATACTTTC	106	164-184 247-270
IL23a NM_031252.2	mIL23a rRNA-F mIL23a rRNA-R	CCAGCGGGACATATGAATCTAC TGTGGGTCAACAACCATCTTC	95	148-170 223-243
Plau NM_008873.3	mPlau rRNA-F mPlau rRNA-R	GATTCTGGAGGACCGCTTATC GTAGACACCGGGCTTGTTT	105	1129-1150 1215-1234