

## Supplemental figures and tables

### Figure S1. Depletion of Shoc2 affects phospho-ERK signaling by EGF.

(A) T47D-NT, -LV1, and -SR cells were serum-starved for 18h and stimulated with EGF for the indicated time. Cell lysates were probed for Shoc2, phosphorylated ERK1/2, and total ERK1/2. Percent change of phosphorylated ERK1/2 was normalized to total ERK in arbitrary units (pERK1/2/total ERK).

(B) MCF7-NT, -LV1, and -SR cells were serum-starved for 18h and stimulated with EGF for the indicated time. Cell lysates were probed for Shoc2, phosphorylated ERK1/2, and total ERK1/2. Percent change of phosphorylated ERK1/2 was normalized to total ERK in arbitrary units (pERK1/2/total ERK).

(C) Equal numbers of Cos-NT, Cos-LV1, Cos-SR and Cos1 cells were plated onto 24-wells plate, and the numbers were counted 24, 48, and 72 h after seeding. The graph depicts the mean number from triplicate experiments  $\pm$ SD.

(D) Equal numbers of T47D-NT, T47D-LV1, T47D-SR and T47D cells were plated onto 24-wells plate, and the numbers were counted 48 and 72 h after seeding. The graph depicts the mean number from triplicate experiments  $\pm$ SD.

### Figure S2. The effect of Shoc2 depletion on cell attachment to extracellular matrixes.

(A)  $5 \times 10^4$  of Cos-NT, Cos-LV1, and Cos1 cells depleted of KSR1 (KSR1#2) were seeded on a collagen-coated 96-well plate (10 ng/mL) and incubated for the indicated time. Cells were fixed and stained with crystal violet. Images were obtained using Nikon Eclipse E600 microscope.

(B, C)  $5 \times 10^4$  of Cos-NT and Cos-LV1 were seeded on a fibronectin (5 ug/mL) (B) or laminin (1 ug/mL) (C) coated 96-well plate and incubated for the indicated time. Cells were fixed and stained with crystal violet. Images were obtained using Nikon Eclipse E600 microscope.

(D)  $5 \times 10^4$  of T47D-NT and T47D-LV1 were seeded on a fibronectin-coated 96-well plate (5 ug/mL) and incubated for the indicated time. Cells were fixed and stained with crystal violet. Images were obtained using Nikon Eclipse E600 microscope.

### Figure S3. RNA-seq analysis.

(A) Flow chart of a workflow for RNA-seq transcriptional profiling.

(B) Cos1, Cos-NT, -LV1, and -SR cells were serum-starved for 18h and stimulated with EGF for the indicated time. Cell lysates were probed with indicated antibodies.

(C) Raw sequencing reads were trimmed and aligned to the reference genome. The number of reads successfully aligned for each of the three samples is shown.

(D) Heat map illustrating gene expression changes as compared with Shoc2-depleted (LV1) to control (NT) reads.

(E) Pie charts present percentage of reads distributed to exons, introns, and intergenic regions.

**Figure S4. Gene expressions is not affected in cells expressing Shoc2-tRFP or depleted of KSR1.**

(A) Total RNA was extracted from cells depleted of endogenous Shoc2 and expressing Shoc2-tRFP (SR). Levels of expression of genes were quantified by RT-PCR. Data are presented as the fold change of mRNA levels normalized to control (NT) (mean  $\pm$  SD,  $n = 4$ ).

(B) Total RNA was extracted from cells depleted of KSR1 (KSR1#2). Levels of expression of genes were quantified by RT-PCR. Data are presented as the fold change of mRNA levels normalized to control (NT) (mean  $\pm$  SD,  $n = 4$ ;  $a$  vs.  $b$ ,  $p < 0.05$ ).

**Figure S5. Conditional media of Cos-NT rescues cell attachment.**

(A) Cell attachment assays were performed in the presence of conditional media of either Cos-NT (Cos-LV1+NT-CM) or Cos-LV1 (Cos-LV1+ LV-CM) cells. Images of cells fixed and stained with crystal violet were obtained using Nikon Eclipse E600 microscope.

(B) Cells from the experiments in (A) were solubilized with 2% SDS and subjected to colorimetric densitometry measurement (OD550). Data from three independent experiments was analyzed. Bars represent mean values ( $\pm$  SD,  $n = 3$ ;  $a$  vs.  $b$ ,  $p < 0.05$ ).

(C) Serum-starved Cos-NT, Cos-LV1, Cos-SR and Cos1 cells were treated with U0126 (10  $\mu$ M) or dimethyl sulfoxide (DMSO) for 2 h at 37°C. The cells were then incubated with 0.2 ng/mL of EGF at 37°C and lysed. The indicated proteins were analyzed using specific antibodies.

(D) Cos-NT cells treated with either DMSO or 10  $\mu$ M U0126 for 2 h. The cells were stimulated with 0.2 ng/mL of EGF at 37 °C for 90min. Total RNA was then extracted. Levels of expression of the indicated genes were quantified by RT-PCR. Data are presented as the fold change of mRNA levels normalized to untreated cells (mean  $\pm$  SD,  $n = 4$ ).

(E) Serum-starved Cos-NT, Cos-LV1, Cos-SR and Cos1 cells were treated with PD98059 (50 uM) or dimethyl sulfoxide (DMSO) (vehicle) for 2 h at 37°C. The cells were then incubated with 0.2 ng/mL of EGF at 37°C and lysed. The indicated proteins were analyzed using specific antibodies.

(F) Cos-NT cells treated with either DMSO or 10 uM PD98059 for 2 h. The cells were stimulated with 0.2 ng/mL of EGF at 37 °C for 90min. Total RNA was then extracted. Levels of expression of genes were quantified by RT-PCR. Data are presented as the fold change of mRNA levels normalized to untreated cells (mean  $\pm$  SD,  $n = 4$ ).

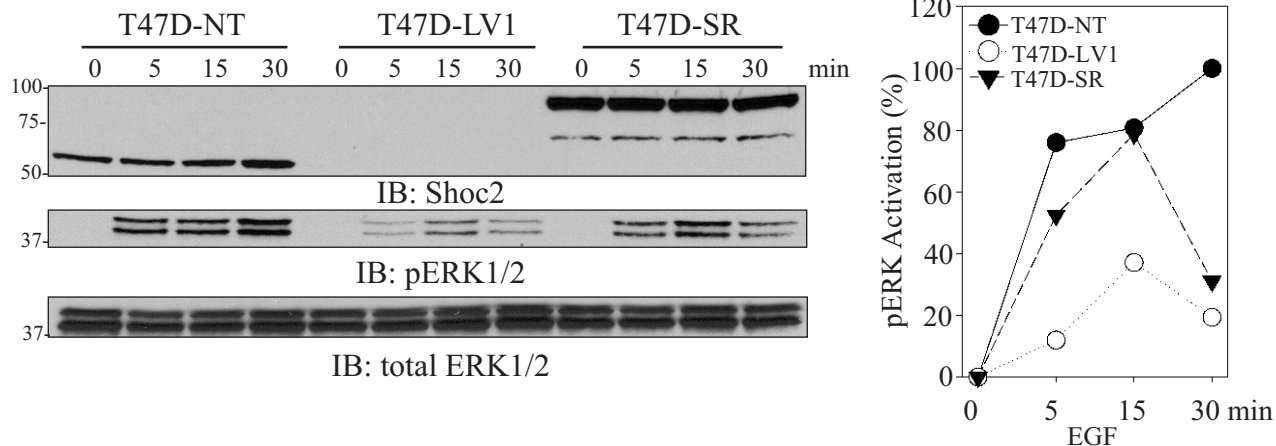
**Table S1. Top 12 differentially expressed transcription factors (fold change > 2, FDR < 0.005).**

**Table S2. Differentially expressed cell adhesion related genes (fold change > 1.5, FDR < 0.0001).**

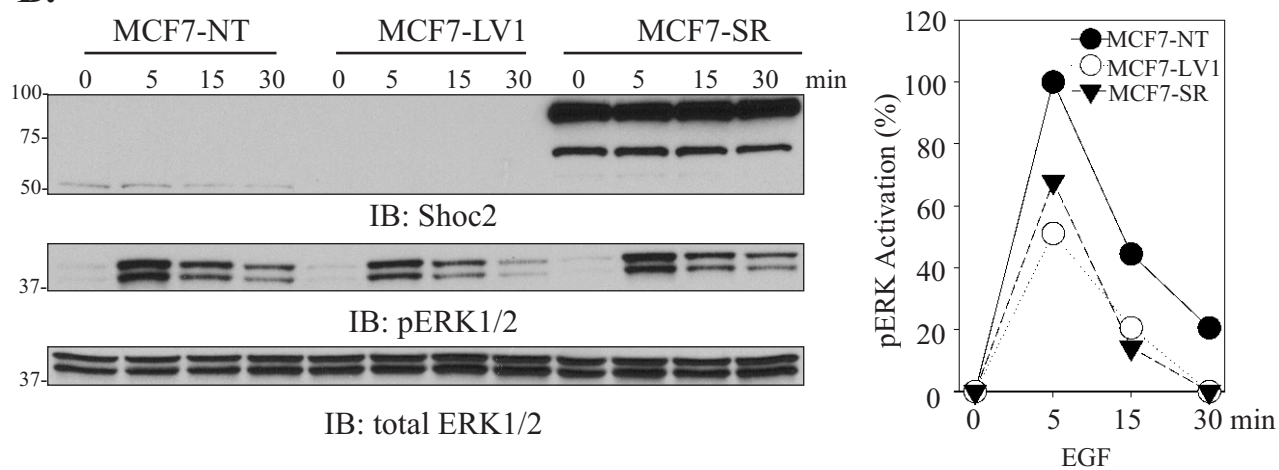
**Table S3. Primers used for RT-PCR.**

# Supplemental Figure 1.

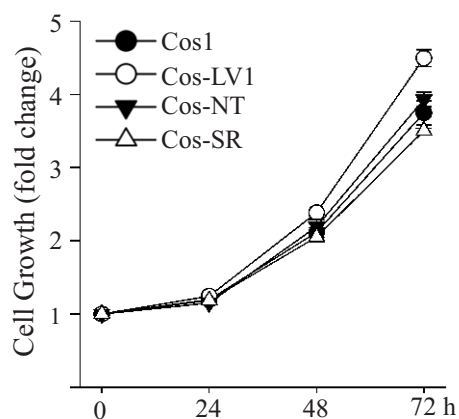
**A.**



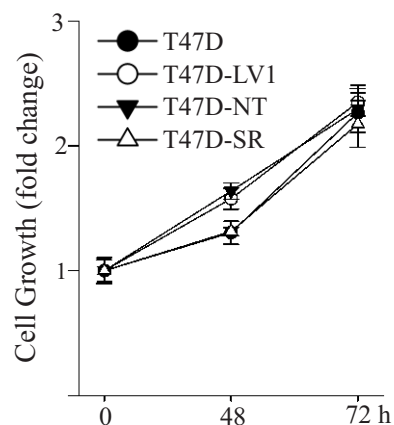
**B.**



**C.**

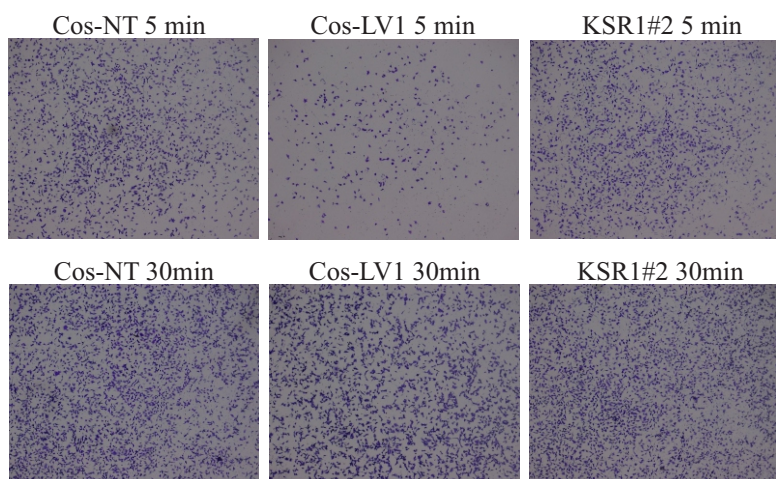


**D.**

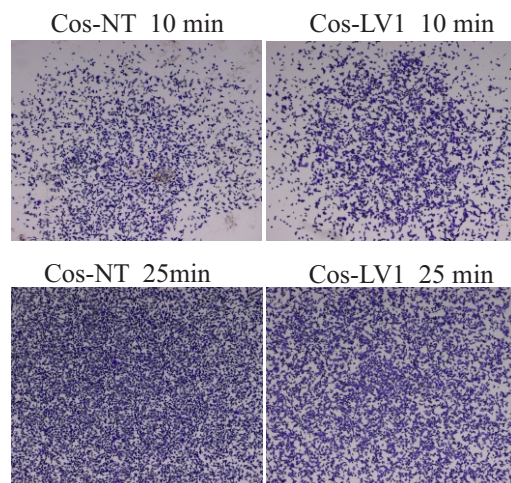


## Supplemental Figure 2

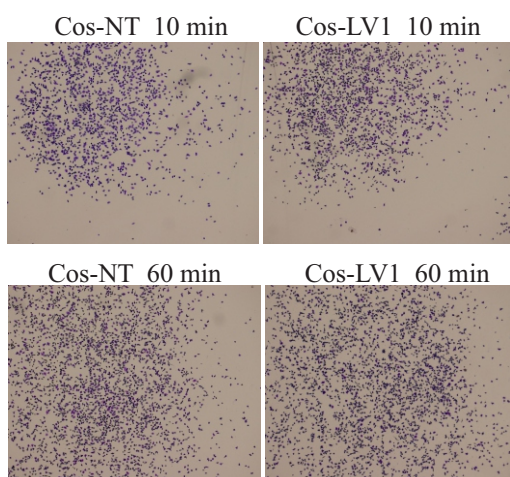
**A.**



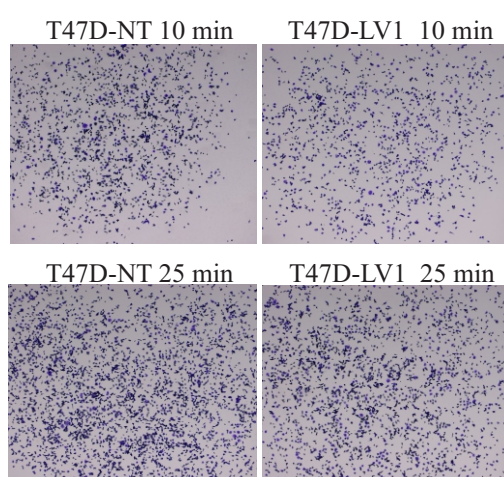
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**C.**

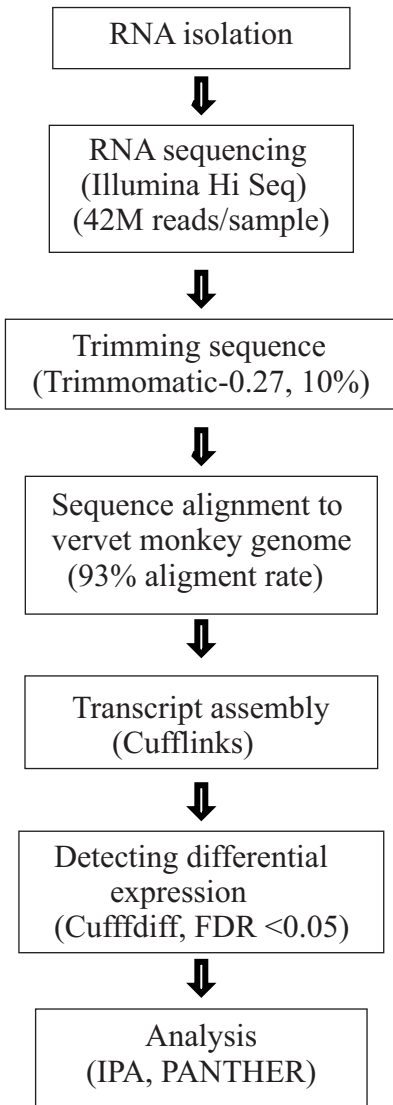


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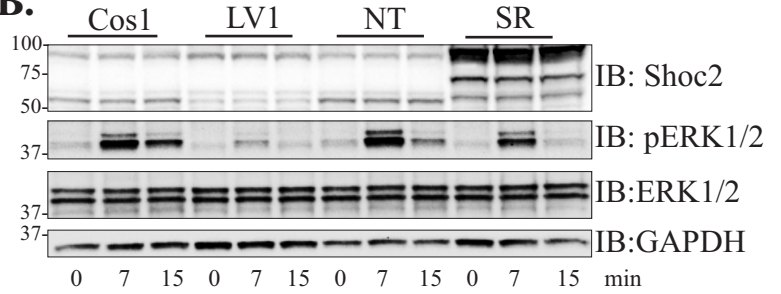


# Supplemental Figure 3.

**A.**



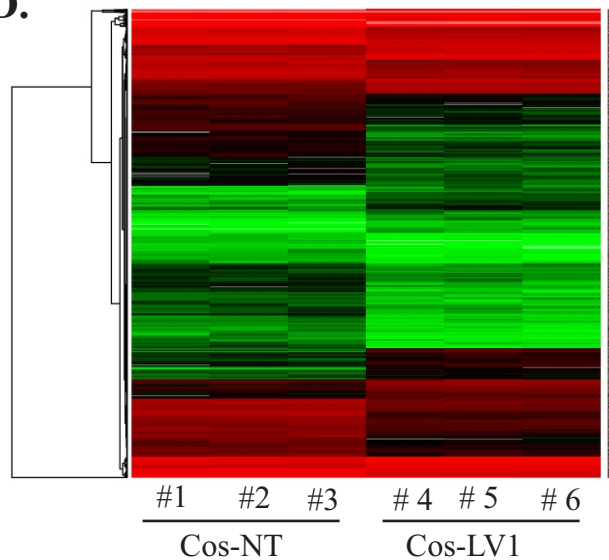
**B.**



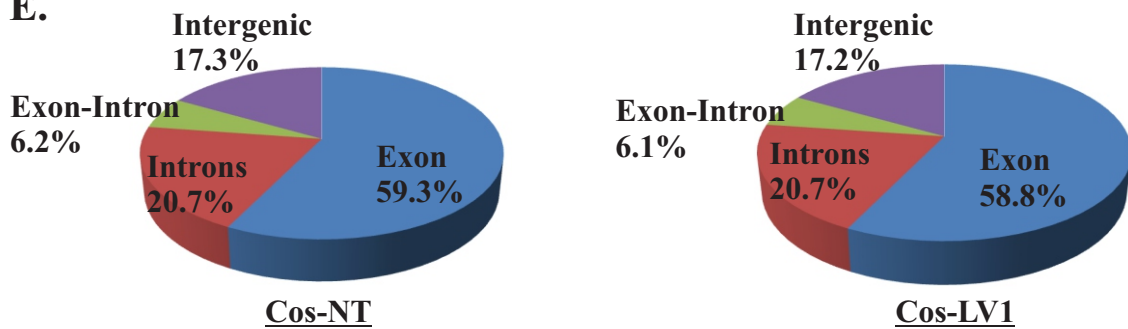
**C.**

	Replicate	Total reads	Reads After Treammung	Aligned Reads
Cos-NT	1	42,952,132	38,607,172	36,207,885
	2	38,735,131	34,782,083	32,331,306
	3	44,044,083	39,381,284	36,772,731
	Average	41,910,449	37,590,180	35,103,974
Cos-LV1	1	43,474,834	39,011,336	36,519,959
	2	48,201,304	43,104,467	39,878,182
	3	40,265,842	35,969,848	33,394,305
	Average	43,980,660	39,361,884	36,597,482

**D.**

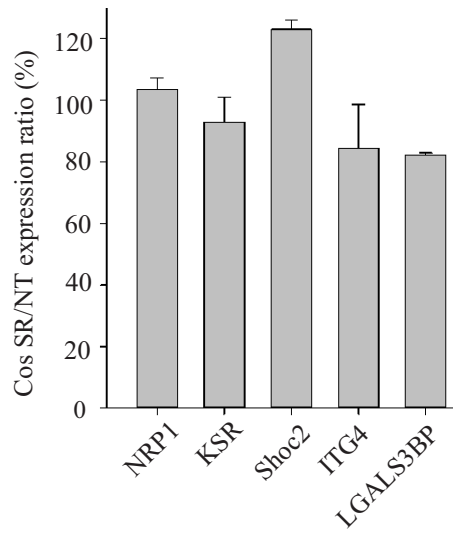


**E.**

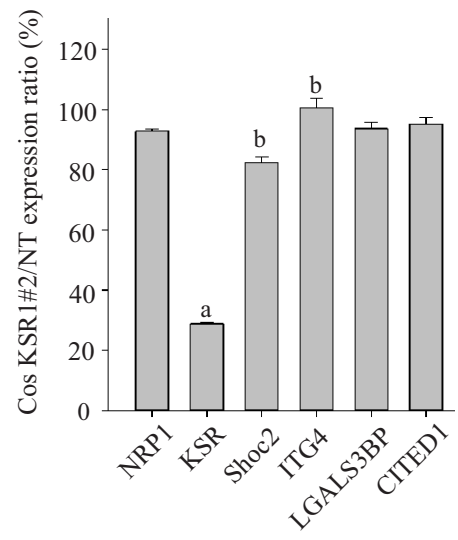


## Supplemental Figure 4

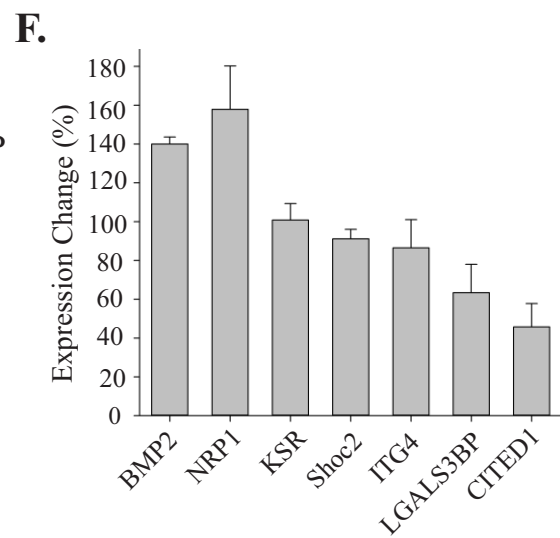
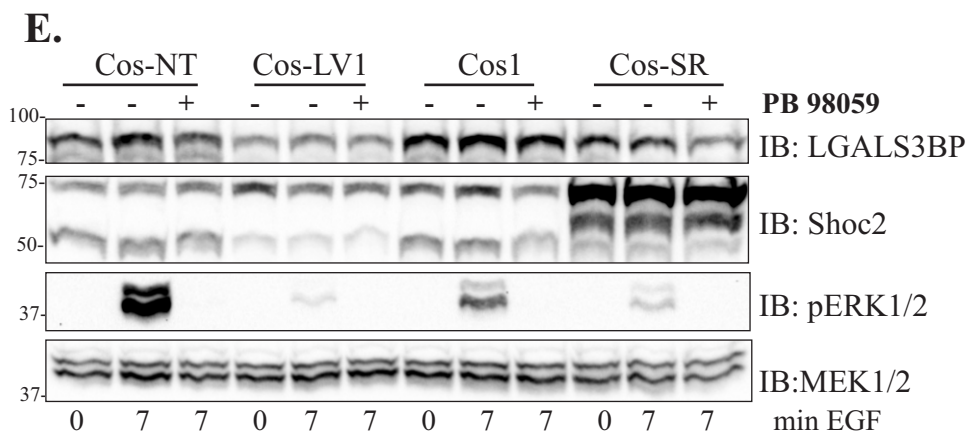
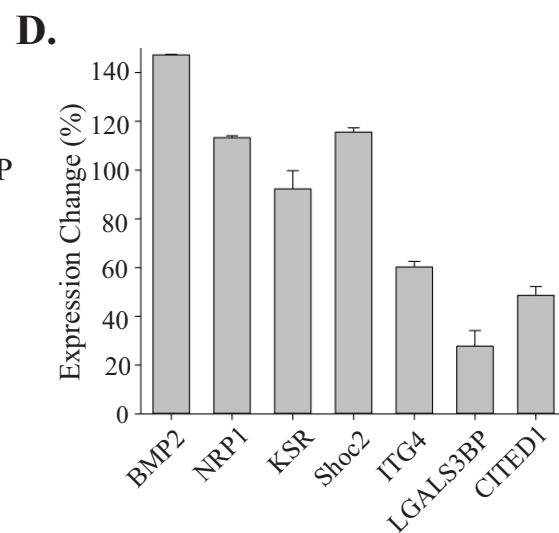
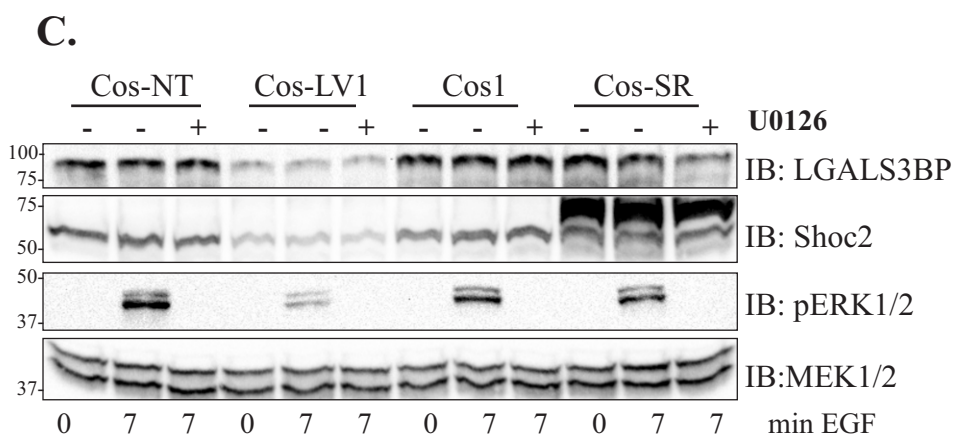
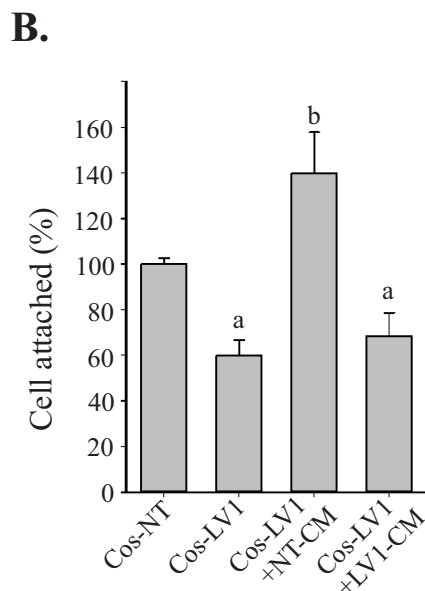
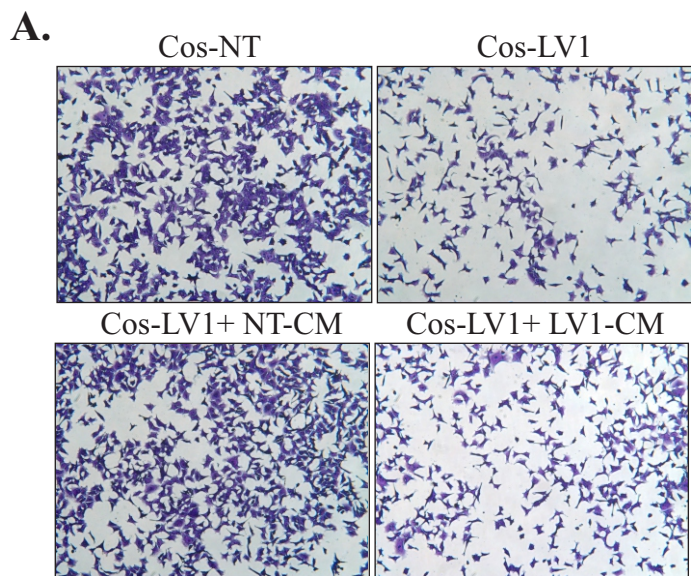
**A.**



**B.**



# Supplemental Figure 5





## Supplemental Table 1

EntrezGene.ID	Gene name	Log <sub>2</sub> foldchange	p-value	q-value	
388585	HES5	-1.517	5.00E-05	0.000642	Among its related pathways are Signaling by GPCR and Disease
57167	SALL4	-1.304	5.00E-05	0.000642	zinc finger transcription factor. Defects in this gene are a cause of Duane-radial ray syndrome (DRRS).
390010	NKX1-2	-1.277	5.00E-05	0.000642	NK1 Homeobox 2
4609	MYC	-1.183	5.00E-05	0.000642	plays a role in cell cycle progression, apoptosis and cellular transformation a variety of hematopoietic tumors, leukemias and lymphomas, including Burkitt lymphoma.
9734	HDAC9	-1.12376	5.00E-05	0.000642	transcriptional regulation, cell cycle progression, and developmental events.
389692	MAFA	-1.078	5.00E-05	0.000642	binds RIPE3b, a conserved enhancer element that regulates pancreatic beta cell-specific expression of the insulin gene
84667	HES7	-1.021	5.00E-05	0.000642	transcriptional repressor, and is implicated in correct patterning of the axial skeleton
367	AR	-1.01	5.00E-05	0.000642	stimulates transcription of androgen responsive genes
602	BCL3	-1.00031	0.00085	0.007354	transcriptional co-activator that activates through its association with NF-kappa B homodimers
59272	ACE2	1.508	5.00E-05	0.000642	regulation of cardiovascular and renal function, as well as fertility
3428	IFI16	1.908	5.00E-05	0.000642	modulates p53 function, and inhibits cell growth in the Ras/Raf signaling pathway
2313	FLI1	2.275	5.00E-05	0.000642	undergo a t(11;22)(q24;q12) translocation with the Ewing sarcoma gene on chromosome 22, which results in a fusion gene that is present in the majority of Ewing sarcoma cases

## Supplemental Table 2

FPKM	Log Ratio	Symbol	log2FC	logCPM	PValue	FDR	Entrez Gene Name
4089	-1.234	MYC	-1.234	7.994	7.06E-152	1.09E-148	v-myc myelocytomatosis viral oncogene homolog (avian)
2254	1.096	SLIT2	1.096	8.261	1.89E-170	5.83E-167	slit homolog 2 (Drosophila)
1514	-1.437	CXCR4	-1.437	6.573	4.21E-110	5.20E-107	chemokine (C-X-C motif) receptor 4
1338	0.714	FN1	0.714	7.213	6.63E-38	1.49E-35	fibronectin 1
603	0.755	NRP1	0.755	6.153	2.50E-37	5.05E-35	neuropilin 1
492	-1.083	PROCR	-1.083	4.926	6.13E-35	1.10E-32	protein C receptor, endothelial
447	-0.590	CD97	-0.590	4.983	5.87E-15	1.91E-13	CD97 molecule
334	-0.683	RGS16	-0.683	4.602	1.44E-16	5.63E-15	regulator of G-protein signaling 16
258	-1.586	ITGA4	-1.586	3.975	3.60E-53	1.59E-50	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)
160	0.788	FLT1	0.788	4.212	1.00E-15	3.56E-14	fms-related tyrosine kinase 1
156	0.605	FBLN5	0.605	4.055	4.51E-10	7.01E-09	fibulin 5
147	-0.637	ARAP3	-0.637	3.537	5.02E-07	4.32E-06	ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 3
131	-2.179	PLTP	-2.179	2.904	3.08E-42	8.10E-40	phospholipid transfer protein
125	-0.649	MITF	-0.649	3.311	7.08E-08	7.36E-07	microphthalmia-associated transcription factor
101	-1.324	APOE	-1.324	2.891	1.98E-13	5.07E-12	apolipoprotein E
74	0.621	NEDD9	0.621	3.326	2.76E-06	2.01E-05	neural precursor cell expressed, developmentally down-regulated 9
70	1.453	BMPER	1.453	3.562	5.56E-23	4.09E-21	BMP binding endothelial regulator
31	1.786	ETS1	1.786	2.689	1.96E-27	2.08E-25	v-ets erythroblastosis virus E26 oncogene homolog 1 (avian)

**Supplemental Table 3.**

Gene Name	Primer Sequences	RefSeq ID
ADAM12	F-CCCCTGGGATCTGCTTTGAG R-CTTCCTGCAGGGGGATGTTT	XM_001087980.2
BMPER	F-ATTACCTGCTGCGTCTTGCT R-ATGCAAGGGTTGTTCGGTGAT	XM_001105988.2
BMP2	F-ACTCGAAATTCCTCCGTGACC R-GGGTCACTTCCACCACGAAT	XM_005568382.1
BMP3	F-TCTCCCCCAAGTCCTTCGAT R-ACCCCCACAGCTCTCACTAT	XM_001089525.2
CITED1	F-AGTGGCCATTCTGCACTACC R-GCAGGTTGAAGGATGGGGAT	NM_001266177.1
CCND1	F-GGCAGAATAGCGCGAGGGA R-TTGAGGAGGTTGGCATCGGG	NM_001278446.1
ITGA4	F-AGCTGCTTACCACCTTGGTC R-AGCCATGCTATTGCCAGTGT	XM_005573626.1
p27(Kip1)	F-GGCAAGTACGAGTGGCAAGA R-AGCCGGAGCCCCAATTAAG	NM_001261005.1
LGALS3BP	F-CTGCGACCTGTCCATCAGTG R-CGATGTCGATCCTTCGGGAG	XM_001109047.2
MYC	F-TCTCACCGTCCTCGGATTCT R-CCGACTCTGACCTTTTGCCA	NM_001142873.1
NRP1	F-CCTCATCGGGCATTCTCTCC R-TCAGGATAGTTCAGGCGGGA	NM_001265816.1
SMAD7	F-TCCTCGGAAGTCAAGAGGCT R-TGGACAGTCTGCAGTTGGTTT	XM_005586745.1
SHOC2	F-TGCAGTCCCTCCCAGCAGAGG RGCCGTAATCAAGCATCCGCAGC	NM_001257588.1
TGF $\beta$ 1	F-CGCGTGCTAATGGTGGAAAC R-TCTGGTACAGCTCCACATGC	XM_001100842.2
HPRT1	F-CCTGGCGTCGTGATTAGTGA R-CGAGCAAGACGTTTCAGTCCT	NM_001283594.1