Supplemental Information

A Transcriptional Signature and Common Gene Networks Link Cancer with Lipid Metabolism and Diverse Human Diseases

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Inventory:

Hirsch et al, Figure S1

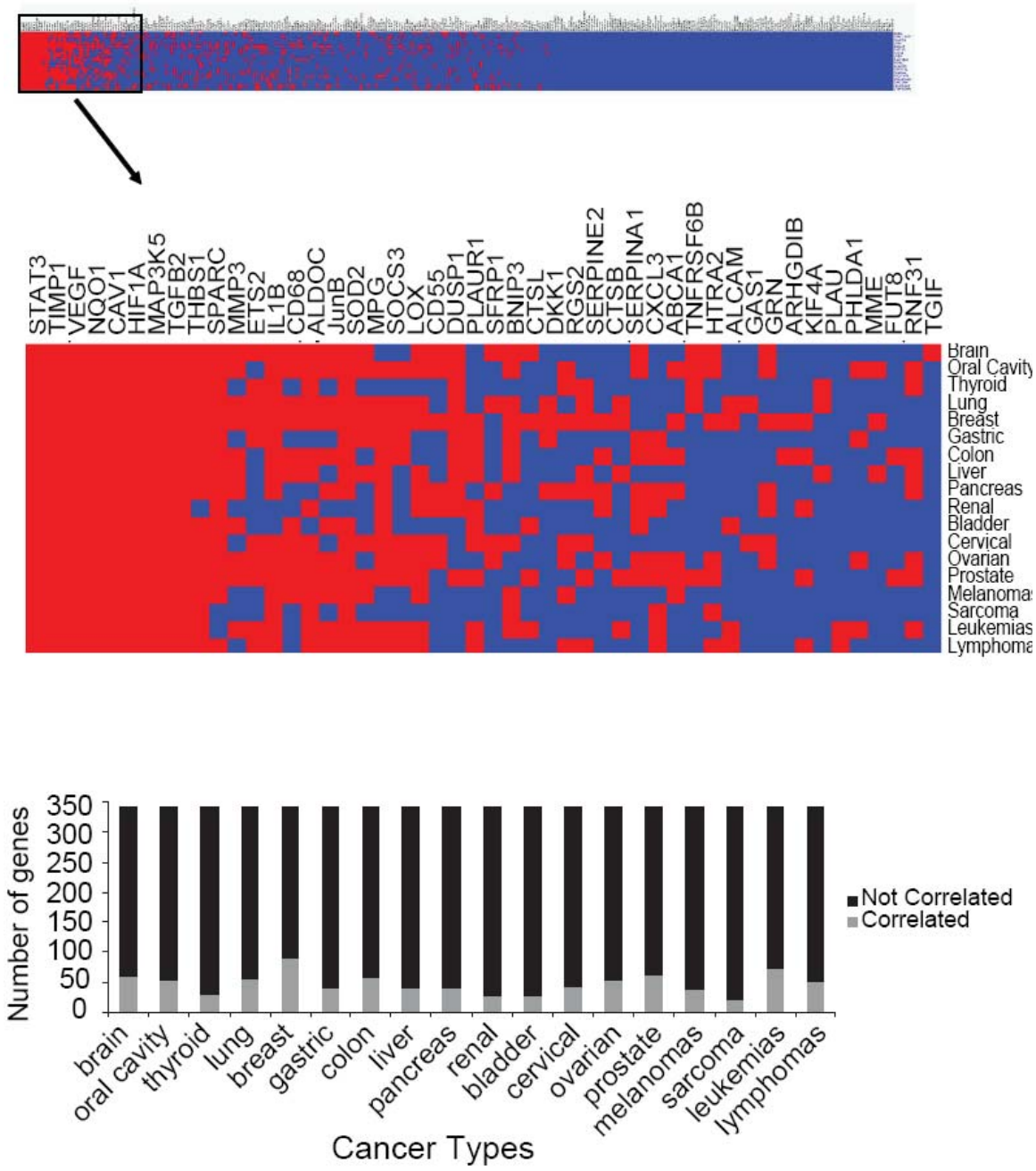

Figure S1, related to Figure 1: MCF10A and BJ Fibroblast cell transformation models correlate with clinical cancer gene data sets**. a,** Heat-map representation of common genes (red color) between 343 cancer gene signature and 18 most common types of cancer. K-means clustering revealed 50 genes being involved in most cancer types. **b,** Number of genes correlated (grey) with each type of cancer.

Hirsch et al, Figure S2

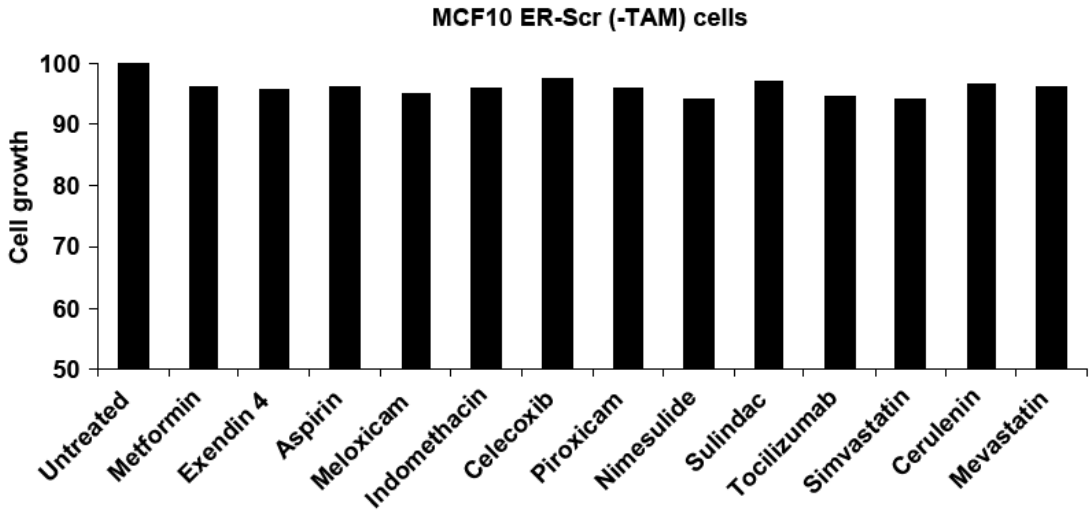

Figure S2, related to Figure 3: Cellular growth of drug-treated ER-Src (-TAM) cells. ER-Src cells were treated with different drugs and cellular growth was evaluated 72h post treatment. The results suggest that the drugs concentrations that were used above do not affect ER-Src cell growth and do not have cytotoxic effects.

Hirsch et al, Figure S3

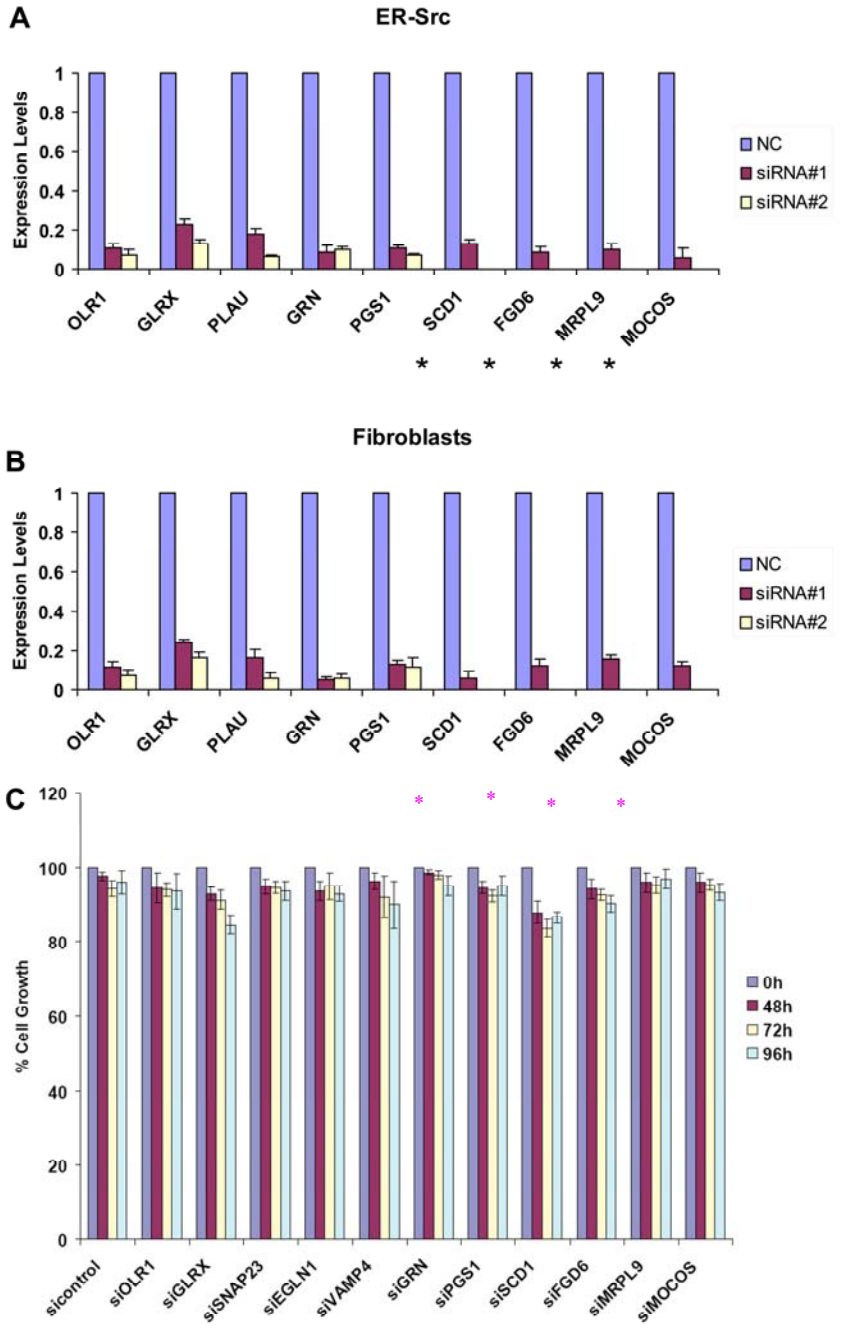

Figure S3, **related to Figure 4:** Efficiency of siRNA transfections in ER-Src cells and ELR fibroblasts. ER-Src (A) cells and ELR fibroblasts (B) were treated with two different siRNAs against OLR1, GLRX, PLAU, GRN, PGS1 and one siRNA (asterisk) against SCD1, FGD5, MRPL9, MOCOS. The mRNA expression levels for all these genes was evaluated 48h post siRNA transfections by real-time PCR assay. The data represent the mean ± SD of three independent experiments. (C) Depletetion of metabolism related genes does not adversely affect MCF10A cell growth. MCF10A cells were transfected with either control siRNAs or siRNAs specific to the 11 metabolism related genes of interest. Cells were counted at time 0, 24 hours, 48 hours, 72 hours, and 96 hours and expressed as a percentage as compared to time non-treated cells.

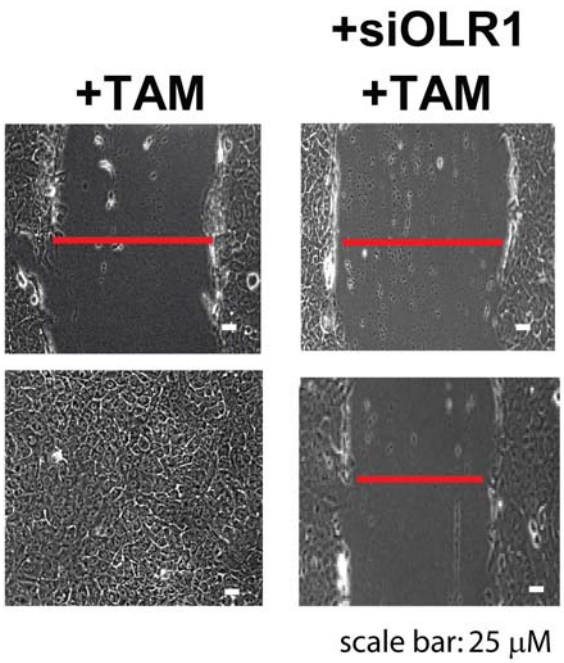

Figure S4, related to Figure 5: Depletion of OLR1 reduces inflammatory and invasion response in a wound healing assay. MCF10A ER-Src monolayer cells were scratched with a p10 pipet tip and then treated with tamoxifen or EtOH in the presence and absence of siOLR1. White scale bars measure 25 μm. Red lines on photos show width of scratch before and after treatments.

Hirsch et al, Figure S5

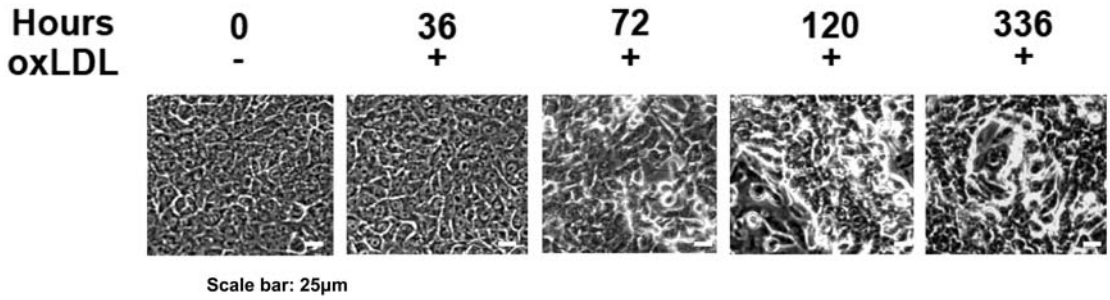

Figure S5, related to Figure 7: Treatment of normal MCF10A Cells with oxLDL results in transformation. MCF10A cells were treated with oxLDL (2ug/ml) and morphology monitored my microscopy Representative phase-contrast images of MCF10A cells treated with 2ug/ml oxidized LDL (oxLDL) for 36, 72, 120 and 336 hours.

Table S1, related to Figure 1: 1201 Genes Differentially Expressed at an FDR of 1% in any time point during MCF10A ER-Src Transformation: For each of the 1201 genes (identified by symbol and Entrez number) the mean fold enrichment (log2) and Q value at each time point is listed.

See Excel File

Table S2, related to Figure 1: Differential Gene Expression between Isogenic Fibroblast Lines at 1% FDR For each differentially expressed gene (identified by symbol and Entrez number), the mean fold enrichment (log2) and Q value is listed. Tab#1 EH vs. EL; Tab#2 EL vs. ELR; Tab# EH vs. ELR

See Excel File

Table S3, related to Figure 1: 343 Common Gene Signature from MCF10A ER-Src and Fibroblast Cell lines: The list of differentially regulated genes in the MCF10A cell line were compared to the differentially regulated genes in the fibroblast system. In order to be considered a common gene, the gene must be regulated in the same direction in both systems.

Upregulated				Downregulated	
ABCA1	EREG	LY96	ROBO3	ADI1	NAV2
ACSL3	ETHE1	MAP3K5	RPL36	AIM1	NFIC
ALAS1	ETS2	MAP3K7IP2	RRAGC	ALCAM	NPM1
ALDOC	EVI2A	MAPK14	RY1	ANKRD25	NQO1
ANGEL2	EVI2B	MID1IP1	SALF;STON1	ARHGDIB	NR2F2
ANKRD28	EVL	MME	SAT	ARHGEF17	PARN
ANKRD46	FBXL5	MMP3	SEMA4C	ARPC2	PCMT1
ANXA3	FBXO28	MOCOS	SERPINA1	ARS2	PDLIM4
ANXA7	FGD6	MOSPD2	SERPINB1	ASF1A	PLS3
ARHGEF18	FHL2	MPG	SERPINE2	ATP10D	PRKACB
ARHGEF2	FLJ20245	MRPL9	SIAH2	ATP1B3	PTDSS1
ARNTL2	FOSL1	MRPS18A	SIGIRR	BAG1	PTMS
ARPC5L	FST	MTMR11	SLC12A7	BCLAF1	PTPLB
BCL2A1	FUT8	MTUS1	SLC1A4	BRP44L	PTPRK
BCL2L1	FYN	MYD88	SLC2A3	C10ORF116	PTRF
BCL3	GALE	NARF	SLC33A1	C11ORF51	RACGAP1
BNIP3	GALNT2	NEDD4L	SLC39A8	C14ORF108	RAI17
C17ORF62	GAS1	NIT1	SLCO4A1	C22ORF9	RAMP1
C1R	GATAD1	NMI	SNAP23	CALD1	RAP2A
C1S	GCH1	NP	SNAPC1	CAV1	RGS20
C2ORF33	GLRX	NPC1	SOCS3	CCND3	RNASEN
C9ORF82	GPR126	NR1H2	SOD2	CENPF	RUSC1
CA9	GRN	NT5E	STAM	CHD9	S100A4
CANT1	GSDMDC1	OLR1	STAMBP	COL4A6	SCHIP1
CARHSP1	GTPBP2	OSMR	STAT3	COMMD8	SFRP1
CASP4	HAX1	OXR1	STC1	COTL1	SFRS2
CD55	HERC5	P2RX4	TAP1	CRIM1	SFRS6
CD68	HEXA	P4HA1	TEAD4	CXORF6	SLCO2A1
CD97	HEXB	PAPPA	TGIF	DDX46	SPARC
CDCP1	HIF1A	PARP8	TIMP1	DENR	SPRY2
CFHR3;CFH	HRB	PCGF1	TJP2	DKK1	STARD7
CLEC2B	HTRA2	PDE4B	TM4SF1	DLG5	SYNCRIP
CPSF3L	IBRDC3	PELI1	TMEFF1	DMD	TCEAL1
CREM	IER2	PGS1	TMEM22	DNAPTP6	TCF4
CSDA	IFI35	PHLDA1	TMEM59	DPM3	TGFB2
CSF3	IFITM1	PIP5K3	TNFAIP1	DPYSL2	THBS1
CSNK2B	IFNGR1	PLAU	TNFAIP8	DST	TNS3
CTBS	IFRD1	PLAUR	TNFRSF21	DSTN	TOB1
CTSB	IL15RA	PLOD1	TNFRSF6B	EPS8	TPM2
CTSL	IL1A	PLSCR1	TNIP2	FBLN1	TSPAN14
CXCL3	IL1B	PMAIP1	TRAF4	FLJ38984	TTC3
CYB5R2	IL1R1	PNRC1	UAP1	GALNT7	UBP1
DCTN6	IL1RAP	PPM1D	UBE1L2	GAS2L1	UPF3B
DENND1A	IL6	PPM2C	UBXD2	GBP1	WDR73
DFNA5	IL7	PROCR	UCKL1	GCAT	
DGUOK	IRF2	PROS1	UGCG	GPR125	ZWINT
DHRS7	IRF7	PSCD1	UPP1	HARS	
DIP13B	ISG20	PTPN2	VAMP4	HCAP-G	
DOCK4	ITPKA	PTX3	VEGF	HEXIM1	
DTWD1	IVNS1ABP	PYGL	VRK2	HMGN3	
DUSP1	JUNB	RAB7L1	WARS	HTRA1	
EDG1	KCNG1	RABGGTA	WIPI1	IFIT5	
EEF1A2	KIAA0963	RBM13	YTHDC2	IQWD1	
EGLN1	KLF2	RBM7	ZFAND1	ISOC2	
EHD4	LAMP3	RGS17	ZMYM1	KIAA0100	
EIF1	LARP6	RGS2	ZMYM6	KIF4A	
EIF1B	LGALS8	RIPK2	ZNF140	LASP1	
EPAS1	LHFPL2	RND3	ZNF200	MLLT11	
EPHA2	LOC57149	RNF31;ISGF3G	ZNF45	MRPL20	
EPN2	LOX	RNF41		MYH10	

Table S4, related to Figure 1: Motif Analysis (Lever) for 2KB and 10 KB Up and Downstream of Start Site for Selected Biofunction Gene Groups:
Tab#1 2KB analysis Tab#2 10Kb analysis. The numbers in the header columns represent AUC values as described in Badis et al 2009.

See excel file

Table S5, related to Figure 1: Genes from Common Gene Signature Known to be Involved in Cancer: The 343 genes from the common gene signature were researched using Ingenuity Pathway Analysis databases and extensive literature searches resulting in the categorization of 208 genes previously known to play a role in cancer. Genes highlighted in red are up regulated in our set. Genes highlighted in green are down regulated in our data set.

ABCA1	CSDA	GAS2L1	LOX	PROCR	STAM
ACSL3	CSF3	GBP1	LY96	PROS1	STARD7
ADI1	CSNK2B	GLRX	MAP3K5	PTPLB	STAT3
AIM1	CTSB	GRN	MAP3K7IP2	PTPN2	STC1
ALAS1	CTSL	GSDMDC1	MAPK14	PTPRK	SYNCRIP
ALCAM	CXCL3	GTPBP2	MME	PTX3	TAP1
ALDOC	DFNA5	HARS	MMP3	RACGAP1	TCEAL1
ANKRD28	DKK1	HAX1	MPG	RAMP1	TCF4
ANXA3	DLG5	HEXB	MTMR11	RAP2A	TEAD4
ANXA7	DMD	HEXIM1	MTUS1	RGS2	TGFB2
ARHGDIB	DOCK4	HIF1A	MYD88	RGS20	TGIF
ARHGEF2	DPM3	HMGN3	MYH10	RIPK2	THBS1
BAG1	DPYSL2	HRB	NEDD4L	RNASEN	TIMP1
BCL2A1	DST	HTRA1	NFIC	RND3	TJP2
BCL2L1	DUSP1	HTRA2	NIT1	RNF31;ISGF3G	TM4SF1
BCL3	EDG1	IER2	NMI	ROBO3	TMEFF1
BCLAF1	EEF1A2	IFITM1	NPC1	RRAGC	TMEM22
BNIP3	EGLN1	IFNGR1	NPM1	S100A4	TMEM59
C1R	EIF1	IFRD1	NQO1	SAT	TNFAIP8
C1S	EPAS1	IL15RA	NR2F2	SERPINA1	TNFRSF21
CA9	EPHA2	IL1A	NT5E	SERPINB1	TNFRSF6B
CALD1	EPS8	IL1B	OLR1	SERPINE2	TNIP2
CANT1	EREG	IL1R1	OSMR	SFRP1	TOB1
CASP4	ETHE1	IL1RAP	OXR1	SIAH2	TPM2
CAV1	ETS2	IL7	PDE4B	SIGIRR	TRAF4
CCND3	EVI2A	IRF2	PDLIM4	SLC12A7	TTC3
CD55	EVI2B	IRF7	PHLDA1	SLC1A4	UBXD2
CD68	EVL	ISG20	PLAU	SLC2A3	UGCG
CD97	FBLN1	ITPKA	PLAUR	SLCO4A1	UPP1
CDCP1	FHL2	JUNB	PLOD1	SNAP23	VAMP4
CENPF	FOSL1	KIF4A	PLS3	SNAPC1	VEGF
CLEC2B	FST	KLF2	PLSCR1	SOCS3	VRK2
COL4A6	FUT8	LAMP3	PMAIP1	SOD2	ZWINT
COTL1	FYN	LASP1	PNRC1	SPARC	
CREM	GAS1	LGALS8	PPM1D	SPRY2	

Table S6, related to Figure 1: Overlap Between Common Gene Set and Cancer Gene Sets or Disease Gene Sets: The common gene signature was compared to the data set from Lerebours F *et al,* Ellmark P *et al,* Logsdon CD *et al,* Delys L *et al,* Lee *et al,* Skogsberg *et al,* Sluimer *et al,* and Schadt EE *et al.* In order to be considered an overlap, a gene must be differentially regulated in the same direction in each set.

	Breast Cancer	Inflammatory Gastric Cancer	Pancreatic Cancer	Thyroid Cancer	Obesity	Metabolic Syndrome	Atherosclerosis	Atherosclerosis 2
Upregulated Genes	BCL2A1 CTSB IL1A IL1B IL6 IRF7 PLAU VEGF	CSF3 IFNG IL1A IL1B IL1R1 IL1RAP IL6 TNF	IRF7 PLAUR SERPINB1 SLC2A3 TAP1	ARNTL2 BCL2A1 CD97 CTSB DUSP1 EPHA2 EREG ETHE1 FYN GALE GAS1 IER2 IL1RAP JUNB MAP3K5 MTUS1 NP NPC1 NT5E OLR1 P4HA1 PLAU PMAIP1 PROS1 RGS17 SERPINA1 STAM TIMP1 TM4SF1 TNFAIP8 TNFRSF21 UPP1 WARS	ACSL3 BCL2A1 BNIP3 C1R C1S CREM CXCL3 FYN GALNT2 GRN IER2 IFI35 IFITM1 IL1B IL1R1 OSMR PHLDA1 PIP5K3 PLAU PLAUR PROS1 PTPN2 RABGGTA RGS2 SERPINE2 SLC39A8 SOD2 TIMP1 UPP1 VEGF	ANXA3 BCL2A1 CD68 CD97 CSF3 CTSB DUSP1 EHD4 EVI2A EVL FGD6 FHL2 GCH1 GLRX GRN HEXA HEXB HTRA2 IFNGR1 LOX MMP3 MOCOS MTMR11 OLR1 P2RX4 PARP8 PGS1 PLAU PLAUR PLSCR1 PMAIP1 PTPN2 RAB7L1 RGS2 SERPINA1 SERPINB1 TNFAIP8 TNIP2 VAMP4	ABCA1 CD68 CSF IFNGR1 IL1A IL1B IL1R1 MMP3 SERPINB1 SERPINE2 SLC2A3 TIMP1	CARHSP1 CLEC2B DENND1A DTWD1 EDG1 EEF1A2 GALNT2 GAS1 IL6 IRF2 PDE4B PELI1 PHLDA1 SERPINE2 STC1 ZFAND1
Down-regulated Genes	CCND3 GBP1	TGFB	NQO1 TPM2	AIM1 ATP1B3 CCND3 DMD EPS8 FBLN1 GCAT MYH10 PDLIM4 PRKACB RAMP1 S100A4 SFRP1 STARD7 THBS1 TOE1	ARHGDIB BAG1 DPYSL2 NQO1 PDLIM4 RNASE S100A4 TGFB2 TNS3 TPM2	ARHGDIB ARPC2 COTL1 EPS8 GAS2L1 HTRA1 LASP1 NQO1 PDLIM4 PTRF RNASEN SLCO2A1 SPARC TCEAL1 TGFB2	THBS1	ADI1 FBLN1 PTPRK SFRP1 SFRS2 SPRY2 TCF4

Table S7, related to Figure 2: Biofunctions and Diseases for Common Gene Set: The common gene set was entered into Ingenuity Pathways Analysis suite and examined for significant biofunctions with P-values better than 1E-05 by Fisher's exact test.

See excel file

Table S8, related to Figure 2: Literature Curated Gene Sets for 32 Human Diseases and Central Node Overlap Between Common Gene Set Networks and 32 Human Disease Networks:

Tab#1 Literature Curated gene sets for each disease. Genes marked in red overlap with transformation signature.

Tab#2 Overlapping nodes between disease and transformation signature networks. Gene sets for 32 human diseases were generated using the extensive disease database in Ingenuity Pathways. Genes identified as important for each disease were analyzed using Ingenuity Pathway Analysis. The genes were organized into networks and central nodes identified. The table includes the p-value score (stated as -10log), central nodes, # of molecules of interest from the data set, predicted biofunctions, and total molecules in each network. The lists of central nodes for the common gene set was compared against the list of central nodes for each disease.Nodes in red overlap with transformation signature

See Excel File

Table S9, related to Figure 2: Validation Of Predicted Central Nodes As Important Regulators During Transformation: 23 out of 42 predicted nodes were rigorously tested for role in transformation by transformation assays, colony assays, foci assays, mammosphere assays, migration assays, invasion assays, wound healing assays, and mouse xenograft experiments. Nodes were perturbed by chemical inhibitors, siRNA, antibodies, drugs, or addition of exogenous cytokine/signaling molecules. A summary of the extent of testing is shown in the table. Notably all 23 nodes tested were determined to be important for transformation/tumor formation.

Node	Number of Disease Associations	Tested for Inhibition of Transformation	Chemical Inhibitor	siRNA	antibody	drug	exogenous addition of factor
NFKB	25	yes	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing Mouse Model	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing Mouse Model		Transformation Colony Assay Mammosphere Migration Invasion	
TNF	18	yes				Transformation	
AKT	15	yes	Transformation Colony Assay Mammosphere Migration Invasion	Transformation Colony Assay			
P38 MAPK	13	yes	Transformation				
AP1	12	no					
JNK	11	yes	Transformation Colony Assay Mammosphere Migration Invasion				
MAPK	11	no					
IL6	9	yes	Transformation Colony Assay Mammosphere Migration Invasion	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing Mouse Model	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing Mouse Model	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing Mouse Model	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing Mouse Model
PI3K	9	yes	Transformation				
PDGF	8	yes	Transformation				
TGFB1	8	yes	Transformation				Transformation
TP53	8	yes	Transformation				
CREB	7	no					
ESR1	7	no					
IFNG	7	no					
LDL	7	yes				Transformation Colony Assay Foci Assay Mammosphere Migration Invasion Wound Healing Mouse Model	Transformation Colony Assay Migration Invasion Wound Healing Mouse Model
Beta Estradic	6	no					
Insulin	6	yes	Transformation			Transformation Colony Assay Foci Assay Mammosphere Migration Wound Healing Mouse Model	
NR3C1	6	no					
RAS	6	yes	Transformation Colony Assay Mammosphere Migration Invasion	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing			
VEGF	6	yes	Transformation	Transformation Colony Assay		Transformation	Transformation
AGT	5	no					
MYC	5	yes	Transformation Colony Assay	Transformation Colony Assay Migration Invasion			

STAT3	5	yes	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing Mouse Model	Transformation Colony Assay Mammosphere Migration Invasion Wound Healing			
ERBB2	4	yes	Transformation Colony Assay				
IGF1	4	yes	Transformation				
IL13	4	no					
NFAT	4	yes	Transformation				
IL1B	3	yes	Transformation Colony Assay		Transformation	Transformation Foci Assay	Transformation
Ret Acid	3	no					
EGFR	2	no					
HIF1A	2	no					
JUNB	2	no					
SP1	2	no					
ARRB2	1	no					
CCND3	1	no					
CTNNB1	1	no					
CXCL3	1	no					
PLAU	1	yes		Transformation Colony Assay Mammosphere Migration Invasion Wound Healing			
PTEN	1	yes	Transformation				
SOCS3	1	yes		Transformation Colony Assay Mammosphere Migration Invasion			