Organ-specific adaptive signaling pathway activation in metastatic breast cancer cells

Supplementary Material

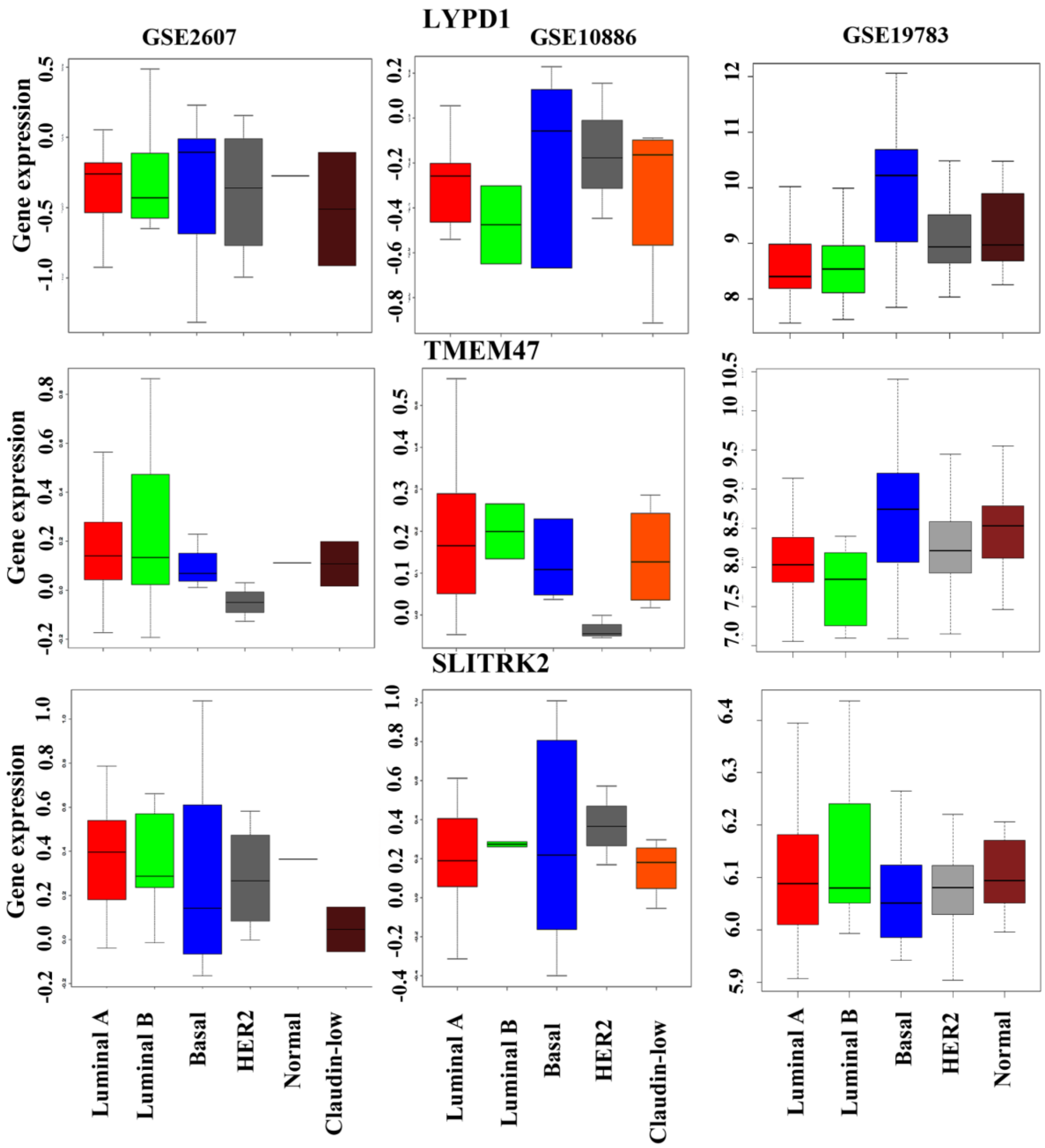
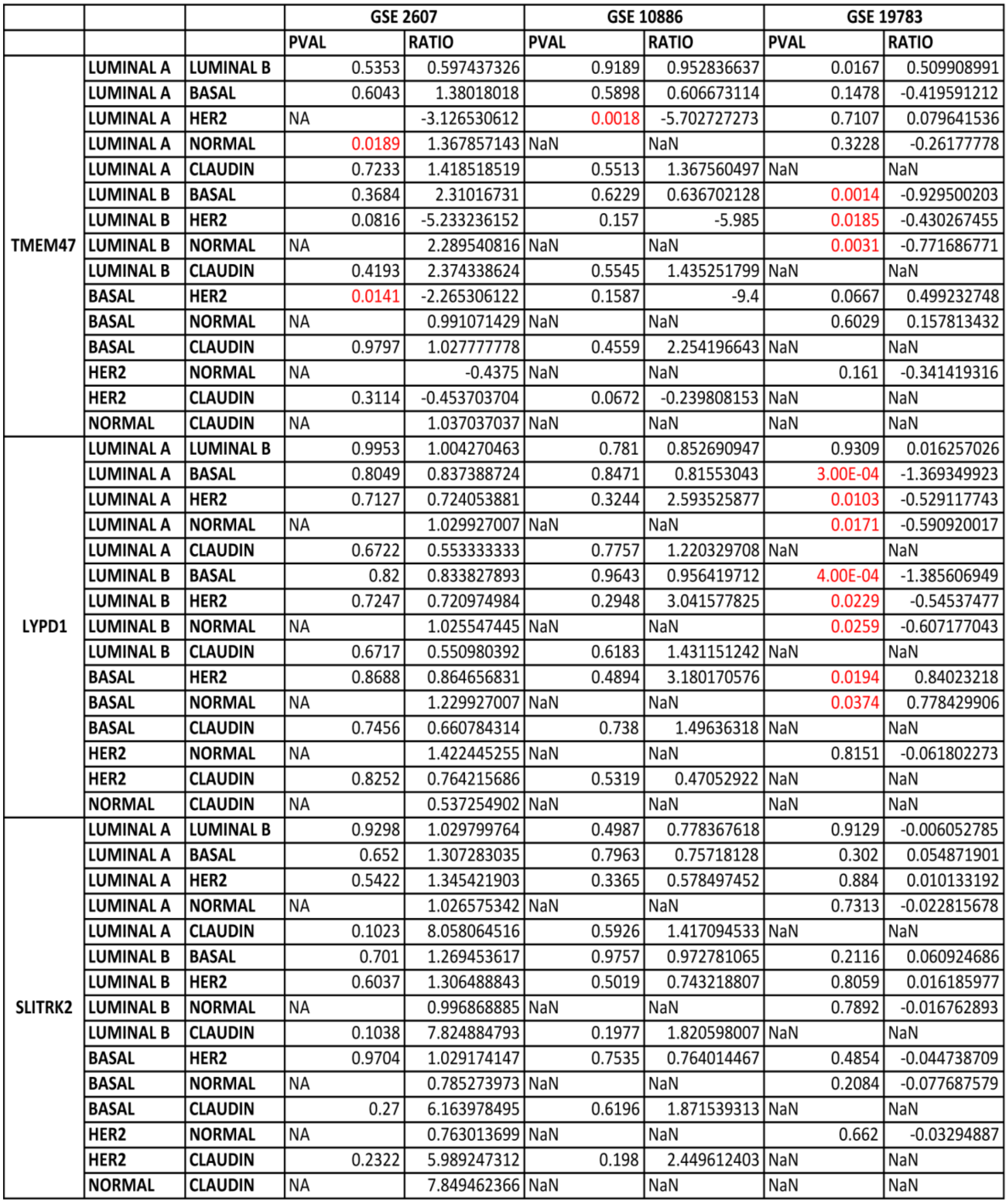


Figure S1: Expression levels of LYPD1, TMEM47, and SLITRK2 in various subtypes of breast cancer. Datasets used for the analyses are indicated. Normalized data were used for these analyses.



			GSE 2607		GSE 10886		GSE 19783	
			PVAL	RATIO	PVAL	RATIO	PVAL	RATIO
TMEM47	**LUMINAL A**	**LUMINAL B**	0.5353	0.597437326	0.9189	0.952836637	0.0167	0.509908991
	LUMINAL A	**BASAL**	0.6043	1.38018018	0.5898	0.606673114	0.1478	-0.419591212
	LUMINAL A	**HER2**	NA	-3.126530612	0.0018	-5.702727273	0.7107	0.079641536
	LUMINAL A	**NORMAL**	0.0189	1.367857143	NaN	NaN	0.3228	-0.26177778
	LUMINAL A	**CLAUDIN**	0.7233	1.418518519	0.5513	1.367560497	NaN	NaN
	LUMINAL B	**BASAL**	0.3684	2.31016731	0.6229	0.636702128	0.0014	-0.929500203
	LUMINAL B	**HER2**	0.0816	-5.233236152	0.157	-5.985	0.0185	-0.430267455
	LUMINAL B	**NORMAL**	NA	2.289540816	NaN	NaN	0.0031	-0.771686771
	LUMINAL B	**CLAUDIN**	0.4193	2.374338624	0.5545	1.435251799	NaN	NaN
	BASAL	**HER2**	0.0141	-2.265306122	0.1587	-9.4	0.0667	0.499232748
	BASAL	**NORMAL**	NA	0.991071429	NaN	NaN	0.6029	0.157813432
	BASAL	**CLAUDIN**	0.9797	1.027777778	0.4559	2.254196643	NaN	NaN
	HER2	**NORMAL**	NA	-0.4375	NaN	NaN	0.161	-0.341419316
	HER2	**CLAUDIN**	0.3114	-0.453703704	0.0672	-0.239808153	NaN	NaN
	NORMAL	**CLAUDIN**	NA	1.037037037	NaN	NaN	NaN	NaN
LYPD1	**LUMINAL A**	**LUMINAL B**	0.9953	1.004270463	0.781	0.852690947	0.9309	0.016257026
	LUMINAL A	**BASAL**	0.8049	0.837388724	0.8471	0.81553043	3.00E-04	-1.369349923
	LUMINAL A	**HER2**	0.7127	0.724053881	0.3244	2.593525877	0.0103	-0.529117743
	LUMINAL A	**NORMAL**	NA	1.029927007	NaN	NaN	0.0171	-0.590920017
	LUMINAL A	**CLAUDIN**	0.6722	0.553333333	0.7757	1.220329708	NaN	NaN
	LUMINAL B	**BASAL**	0.82	0.833827893	0.9643	0.956419712	4.00E-04	-1.385606949
	LUMINAL B	**HER2**	0.7247	0.720974984	0.2948	3.041577825	0.0229	-0.54537477
	LUMINAL B	**NORMAL**	NA	1.025547445	NaN	NaN	0.0259	-0.607177043
	LUMINAL B	**CLAUDIN**	0.6717	0.550980392	0.6183	1.431151242	NaN	NaN
	BASAL	**HER2**	0.8688	0.864656831	0.4894	3.180170576	0.0194	0.84023218
	BASAL	**NORMAL**	NA	1.229927007	NaN	NaN	0.0374	0.778429906
	BASAL	**CLAUDIN**	0.7456	0.660784314	0.738	1.49636318	NaN	NaN
	HER2	**NORMAL**	NA	1.422445255	NaN	NaN	0.8151	-0.061802273
	HER2	**CLAUDIN**	0.8252	0.764215686	0.5319	0.47052922	NaN	NaN
	NORMAL	**CLAUDIN**	NA	0.537254902	NaN	NaN	NaN	NaN
SLITRK2	**LUMINAL A**	**LUMINAL B**	0.9298	1.029799764	0.4987	0.778367618	0.9129	-0.006052785
	LUMINAL A	**BASAL**	0.652	1.307283035	0.7963	0.75718128	0.302	0.054871901
	LUMINAL A	**HER2**	0.5422	1.345421903	0.3365	0.578497452	0.884	0.010133192
	LUMINAL A	**NORMAL**	NA	1.026575342	NaN	NaN	0.7313	-0.022815678
	LUMINAL A	**CLAUDIN**	0.1023	8.058064516	0.5926	1.417094533	NaN	NaN
	LUMINAL B	**BASAL**	0.701	1.269453617	0.9757	0.972781065	0.2116	0.060924686
	LUMINAL B	**HER2**	0.6037	1.306488843	0.5019	0.743218807	0.8059	0.016185977
	LUMINAL B	**NORMAL**	NA	0.996868885	NaN	NaN	0.7892	-0.016762893
	LUMINAL B	**CLAUDIN**	0.1038	7.824884793	0.1977	1.820598007	NaN	NaN
	BASAL	**HER2**	0.9704	1.029174147	0.7535	0.764014467	0.4854	-0.044738709
	BASAL	**NORMAL**	NA	0.785273973	NaN	NaN	0.2084	-0.077687579
	BASAL	**CLAUDIN**	0.27	6.163978495	0.6196	1.871539313	NaN	NaN
	HER2	**NORMAL**	NA	0.763013699	NaN	NaN	0.662	-0.03294887
	HER2	**CLAUDIN**	0.2322	5.989247312	0.198	2.449612403	NaN	NaN
	NORMAL	**CLAUDIN**	NA	7.849462366	NaN	NaN	NaN	NaN

Figure S2: Statistical comparisons between different subtypes for the expression of LYPD1, TMEM47, and SLITRK2.

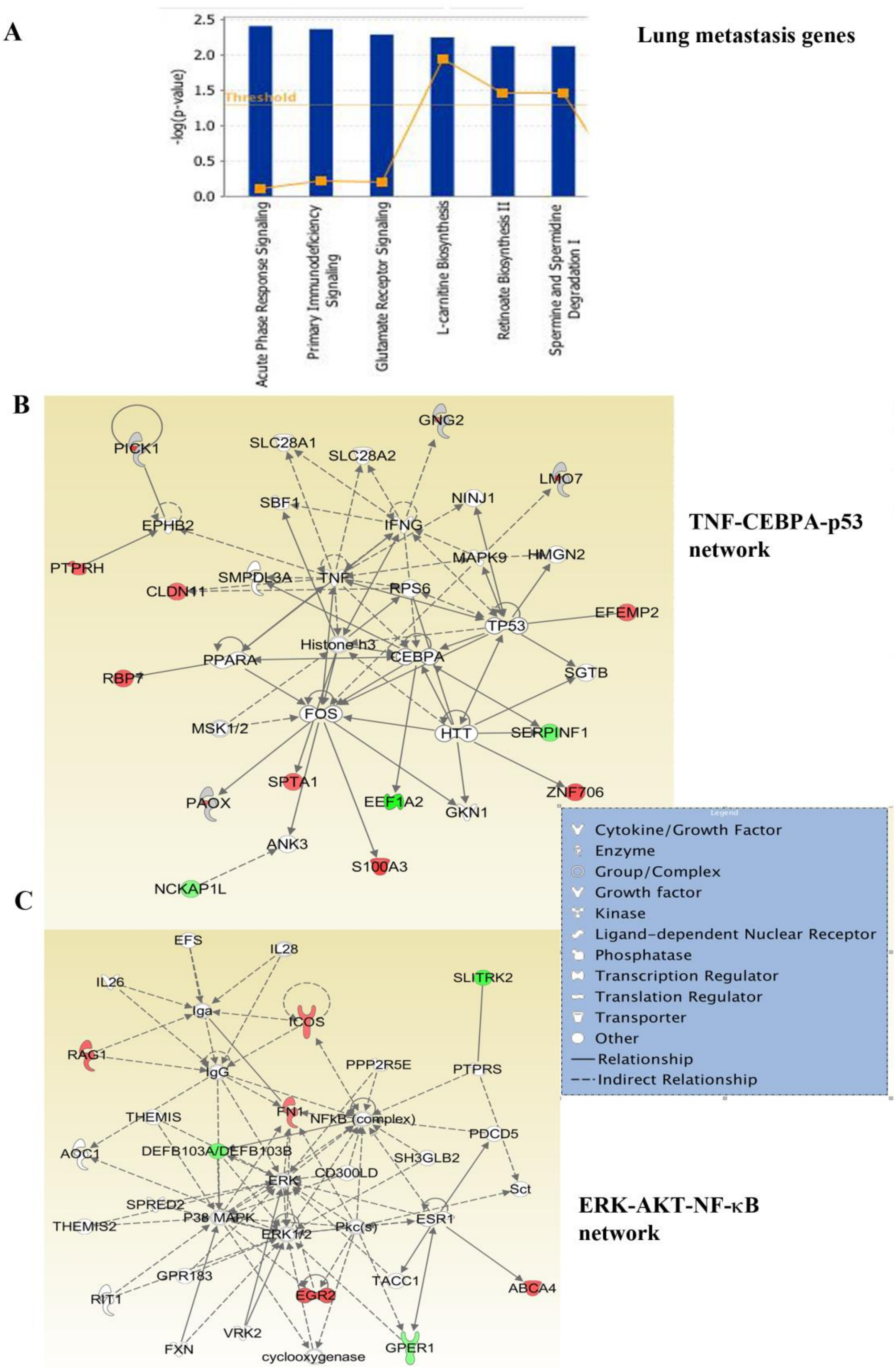
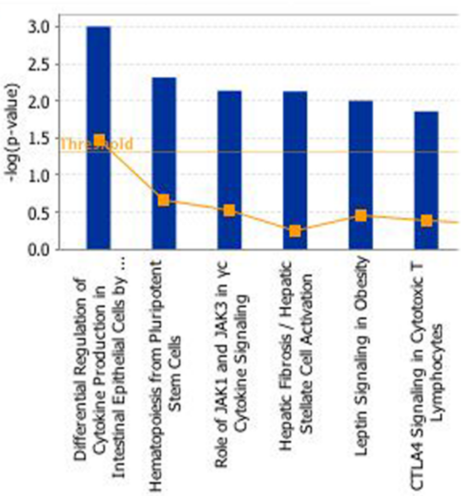

A
Lung metastasis genes
-log(p-value)
2.5
2.0
1.5
1.0
0.5
0.0
Threshold
Acute Phase Response Signaling
Primary Immunodeficiency Signaling
Glutamate Receptor Signaling
L-carnitine Biosynthesis
Retinoate Biosynthesis II
Spermine and Spermidine Degradation I
B
TNF-CEBPA-p53 network
PICK1
SLC28A1
SLC28A2
GNG2
LMO7
SBF1
NINJ1
EPHB2
IFNG
MAPK9
HMGN2
PTPRH
SMPDL3A
TNF
RPS6
CLDN11
EFEMP2
TP53
Histone h3
PPARA
CEBPA
RBP7
SGTB
MSK1/2
FOS
HTT
SERPINF1
SPTA1
ZNF706
PAOX
EEF1A2
GKN1
ANK3
S100A3
NCKAP1L
C
ERK-AKT-NF-κB network
EFS
IL28
IL26
SLITRK2
Iga
ICOS
RAG1
IgG
PPP2R5E
PTPRS
THEMIS
FN1
NFkB (complex)
PDCD5
AOC1
DEFB103A/DEFB103B
SH3GLB2
ERK
CD300LD
Sct
SPRED2
THEMIS2
P38 MAPK
ERK1/2
Pkc(s)
ESR1
GPR183
TACC1
RIT1
EGR2
ABCA4
FXN
VRK2
cyclooxygenase
GPER1
Legend
Cytokine/Growth Factor
Enzyme
Group/Complex
Growth factor
Kinase
Ligand-dependent Nuclear Receptor
Phosphatase
Transcription Regulator
Translation Regulator
Transporter
Other
Relationship
Indirect Relationship

Figure S3: Ingenuity pathway analysis of genes differentially expressed in LMD-231 cells. A) Major signaling pathways in LMD-231 cells. B) LMD-231 cells show activation of TNF-CEBPA-p53 network. C) ERK-AKT-NF-κB signaling network is active in LMD-231 cells.

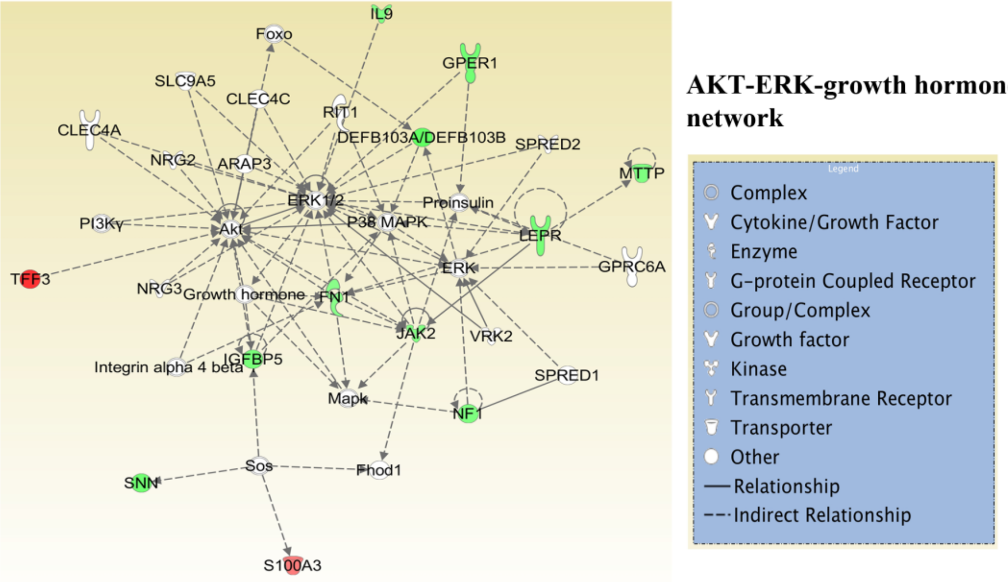
A

Bone metastasis genes



B

AKT-ERK-growth hormone network



C

TNF-p53 network

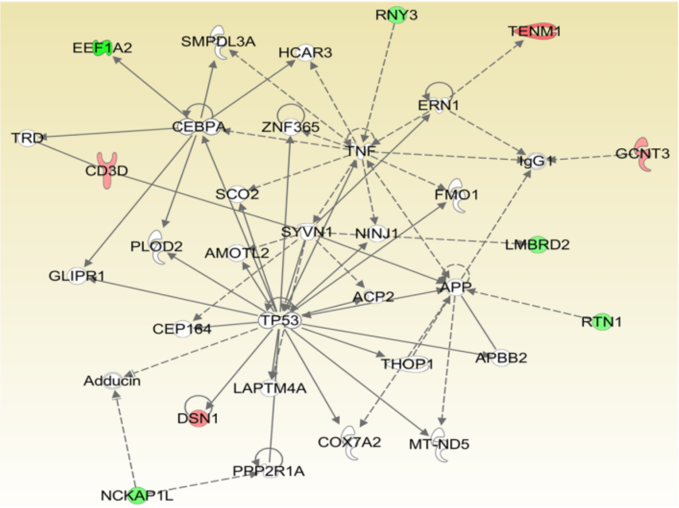


Figure S4: Ingenuity pathway analysis of genes differentially expressed in BMD-231 cells. A) Major signaling pathways in BMD-231 cells. B) BMD-231 cells show activation of AKT-ERK-Growth hormone network. C) TNF-p53 signaling network is active in BMD-231 cells.

Table S1: PAM classifier analysis of microarray results to identify organ-specific metastasis signature.

Table S2: Various comparisons of genes differentially expressed in different cell types. ANOVA analysis was performed to measure genes differentially expressed in different cell types.

Note: Tables S1 and S2 are in supplementary Information in oncotarget site.

Table S3: Sequences of primers used for qRT-PCR

Gene	Forward	Reverse
β-Actin	TGGATCAGCAAGCAG	GCATTTGCGGTGGAC
CXCL1	CCAGACCCGCCTGCTG	CCTCCTCCCTTCTGGTCAGTT
ESM1	TAATTATGCGGTGGACTGCCCTCA	TTCATGCCATCCATGCCTGAGACT
LYPD1	TTGCGGATTGTTCTTGCTTCCAGG	TGATGATGCACAGGACTTGCGGTA
SGK1	AGGCATGTAGCAGGGAGGCTTATT	TTTCATTCTTCGGGTTGCCCAAGG
SLITRK2	TCGGTTACTAATGACTTGGCGGCT	TTAGGCACCTTCGGTGTCAACTCA
TMEM47	TTACCGCCTGCTTATCCTACCCAA	TCCCATTAGATGCTGAAGGGCAGT