Figure 4 and 5 Real-time polymerase chain reaction with SYBR Green master mix were used to quantify the expression levels of 84 genes ontologically related and regulated by TGFβ/BMP Signalling pathway, or the 84 genes of Signal Transduction Pathway Finder specific arrays (SA Biosciences, Frederick, MD). The heat-map shows absolute mRNA copy numbers which were calculated from PCR cycle thresholds (Cts, Fig. 4). For example, on the color coded log2 scale, a value of 10 represents 2^{10} or 1024 transcripts. Two endogenous controls, GAPDH and ACTB, were used for normalization. Functional gene clustering (with major groups according to the array manual) indicated at right. In the Signal Transduction Pathway Finder Array (Fig. 5**),** the fold expression differences were analyzed through the SA Biosciences webpage, then transferred into MATLAB and visualized with the Bioinformatics Toolbox Clustergram function. All experiments were run in triplicates.

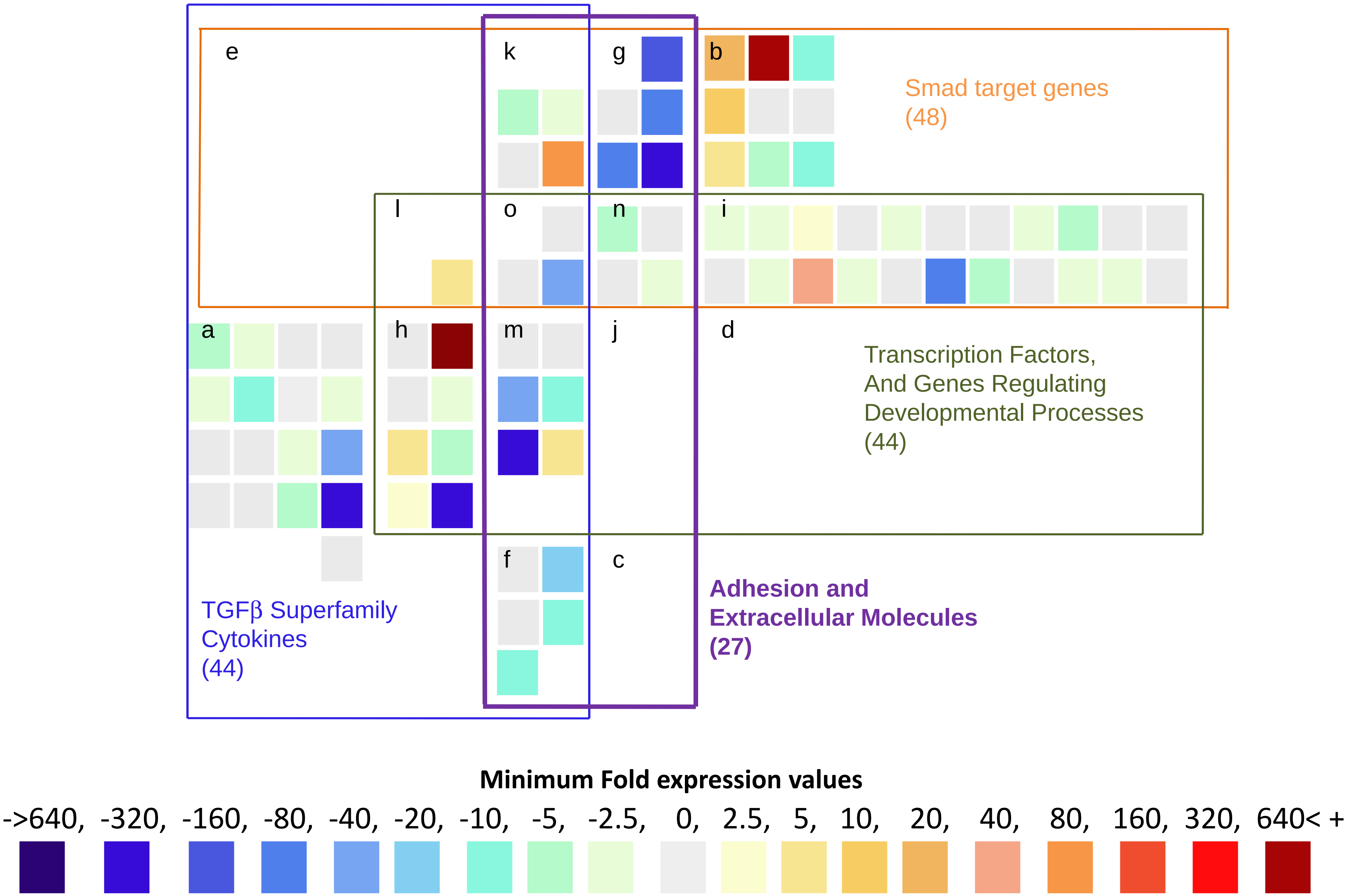
Figure 6 Parallel triplicate experiments were plated and synchronized as described in the Material and Methods section. A-083-01 (5μM) pre-treatment were used (30 minutes) on selected samples followed by LAM and TGFβ1 exposure alone and in combination with the inhibitor. Samples were harvested after 4-hour incubation to enhance the stable expressional profile changes. Selected genes were assayed on cDNA library generated (Materials and Methods) by ABI TaqMan probes (Supplemental Table 2) on ABI 7900 HT Fast Real Time QPCR instrument. The results were transferred to Excel (Microsoft) and graphed with error bars generated by standard deviation of Ct values from the three independent experiments.

Supplemental Material: The plate is normalized to the average of 5 house-keeping genes B2M, HPRT1, RPL13A, GAPGH, and ACTB. The p=0.001 and below was considered highly significant. From the original description and functional grouping of product specification sheet four groups were created by combining the Transcription Factors and Regulators (group 4, 21 genes) with the Genes Involving in Cellular and Developmental Processes (group 5, 28 genes).

The Venn analysis was performed through (http://www.pangloss.com/seidel/Protocols/venn4.cgi) site. The generated group lists (Supplemental Table 1) were used to create the figure (Supplemental Fig. 1) in which genes are represented by squares, grouped according to their function and coloured according to their expression levels in MCF-7 as compared to MCF-10A.

The SuperArray (SA) results were validated with individual TaqMan assays designed against selective targets from the same cDNA pools that were used to generate the array data sets (Supplemental Table 2). Expressional fold changes are shown. From the total of 33 validated genes, 11 presented. The TaqMan data considered more accurate, because of specifically targeted assays vs. the less sensitive SYBR Green detection system/chemistry employed in Super Arrays. The difference between the two systems is greater when expressional changes are higher. Some of the genes are not analyzed in all experiments (N/A) due to depletion of representative cDNA archives.

Gene Expressional Differences in MCF-7 vs. MCF-10A cells, Venn Groups



Supplemental Figure 1

Supplemental Table 1.

Change of Basal Gene Expressional values in MCF-7 as compared to MCF-10A cell line.

Gene # /Position	GeneBank	Symbol	Fold Up/Down Regulation	T-test p-Value	Description

Venn Group a

01 /A01	NM_001105	ACVR1	**-4.96**	**0.0000**	Activin A receptor, type I
02 /A02	NM_001616	ACVR2A	**-2.62**	**0.0007**	Activin A receptor, type IIA
03 /A03	NM_000020	ACVRL1	-1.25	0.7013	Activin A receptor type II-like 1
05 /A05	NM_020547	AMHR2	1.08	0.8043	Anti-Mullerian hormone receptor, type II
16 /B04	NM_004329	BMPR1A	-2.40	0.0011	Bone morphogenetic protein receptor, type IA
17 /B05	NM_001203	BMPR1B	**-7.37**	**0.0000**	Bone morphogenetic protein receptor, type IB
36 /C12	NM_000557	GDF5	-1.38	0.4911	Growth differentiation factor 5 (cartilage-derived morphogenetic protein-1)
37 /D01	NM_001001557	GDF6	1.12	0.9002	Growth differentiation factor 6
38 /D02	NM_182828	GDF7	-1.52	0.4995	Growth differentiation factor 7
53 /E05	NM_020997	LEFTY1	-1.76	0.0529	Left-right determination factor 1
59 /E11	NM_018055	NODAL	**-3.62**	**0.1290**	Nodal homolog (mouse)
77 /G05	NM_003238	TGFB2	**-4.52**	**0.0566**	Transforming growth factor, beta 2
78 /G06	NM_003239	TGFB3	-1.12	0.2902	Transforming growth factor, beta 3
80 /G08	NM_004612	TGFBR1	**-2.53**	**0.0000**	Transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53kDa)
81 /G09	NM_003242	TGFBR2	**-36.72**	**0.0001**	Transforming growth factor, beta receptor II (70/80kDa)
82 /G10	NM_003243	TGFBR3	**-268.88**	**0.0000**	Transforming growth factor, beta receptor III (betaglycan, 300kDa)
83 /G11	NM_004257	TGFBRAP1	-1.34	0.1339	Transforming growth factor, beta receptor associated protein 1

Venn Group b

06 /A06	NM_012342	BAMBI	**25.26**	**0.0000**	BMP and activin membrane-bound inhibitor homolog (Xenopus laevis)
15 /B03	NM_133468	BMPER	**11.24**	**0.0000**	BMP binding endothelial regulator
19 /B07	NM_001789	CDC25A	**5.45**	**0.0018**	Cell division cycle 25A
21 /B09	NM_004936	CDKN2B	**651.26**	**0.0000**	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
22 /B10	NM_005454	CER1	-1.68	**0.4798**	Cerberus 1, cysteine knot superfamily, homolog (Xenopus laevis)
27 /C03	NM_000099	CST3	**-4.81**	**0.0010**	Cystatin C (amyloid angiopathy and cerebral hemorrhage)
31 /C07	NM_004116	FKBP1B	**-14.45**	**0.0001**	FK506 binding protein 1B, 12.6 kDa
42 /D06	NM_002166	ID2	1.08	**0.5613**	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
58 /E10	NM_005380	NBL1	**-13.16**	**0.0000**	Neuroblastoma, suppression of tumorigenicity 1

Venn Group f

34 /C10	NM_016204	GDF2	-1.27	0.6842	Growth differentiation factor 2
35 /C11	NM_020634	GDF3	1.10	0.8533	Growth differentiation factor 3
54 /E06	NM_000627	LTBP1	**-9.20**	**0.0000**	Latent transforming growth factor beta binding protein 1
55 /E07	NM_000428	LTBP2	**-16.70**	**0.0003**	Latent transforming growth factor beta binding protein 2
56 /E08	NM_003573	LTBP4	**-7.69**	**0.0000**	Latent transforming growth factor beta binding protein 4

Venn Group g

26 /C02	NM_000090	COL3A1	**19.06**	**0.0116**	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)
29 /C05	NM_000118	ENG	**-76.91**	**0.0000**	Endoglin (Osler-Rendu-Weber syndrome 1)
63 /F03	NM_002658	PLAU	**-163.80**	**0.0000**	Plasminogen activator, urokinase
65 /F05	NM_000602	SERPINE1	**-48.87**	**0.0004**	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
79 /G07	NM_000358	TGFBI	**-246.19**	**0.0000**	Transforming growth factor, beta-induced, 68kDa

Venn Group h

10 /A10	NM_001201	BMP3	1.12	0.7571	Bone morphogenetic protein 3 (osteogenic)
11 /A11	NM_130851	BMP4	1.19	0.2907	Bone morphogenetic protein 4
12 /A12	NM_021073	BMP5	**4.80**	0.5417	Bone morphogenetic protein 5
13 /B01	NM_001718	BMP6	**2.82**	**0.0201**	Bone morphogenetic protein 6
14 /B02	NM_001719	BMP7	**2234.86**	**0.0000**	Bone morphogenetic protein 7 (osteogenic protein 1)
18 /B06	NM_001204	BMPR2	**-3.38**	**0.0003**	Bone morphogenetic protein receptor, type II (serine/threonine kinase)
61 /F01	NM_000475	NR0B1	**-5.73**	**0.0319**	Nuclear receptor subfamily 0, group B, member 1
74 /G02	NM_000660	TGFB1	-1.38	**0.0509**	Transforming growth factor, beta 1 (Camurati-Engelmann disease)

Venn Group i

20 /B08	NM_000389	CDKN1A	-2.26	**0.0000**	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
23 /B11	NM_003741	CHRD	1.84	**0.0371**	Chordin
28 /C04	NM_004405	DLX2	-2.03	**0.0308**	Distal-less homeobox 2
30 /C06	NM_005241	EVI1	-2.15	**0.1681**	Ecotropic viral integration site 1
32 /C08	NM_005252	FOS	2.08	**0.0136**	V-fos FBJ murine osteosarcoma viral oncogene homolog
39 /D03	NM_173849	GSC	**43.78**	**0.0005**	Goosecoid
40 /D04	NM_022740	HIPK2	-1.59	**0.0486**	Homeodomain interacting protein kinase 2
41 /D05	NM_002165	ID1	**-2.84**	**0.0004**	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein
51 /E03	NM_002228	JUN	**-3.01**	**0.0015**	V-jun sarcoma virus 17 oncogene homolog (avian)
52 /E04	NM_002229	JUNB	-1.70	**0.0223**	Jun B proto-oncogene
57 /E09	NM_002467	MYC	-1.72	**0.0030**	V-myc myelocytomatosis viral oncogene homolog (avian)
60 /E12	NM_005450	NOG	**-90.39**	**0.0000**	Noggin
64 /F04	NM_001754	RUNX1	1.05	**0.7004**	Runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)
66 /F06	NM_005900	SMAD1	**-3.95**	**0.0001**	SMAD, mothers against DPP homolog 1 (Drosophila)
67 /F07	NM_005901	SMAD2	**-3.01**	**0.0001**	SMAD, mothers against DPP homolog 2 (Drosophila)
68 /F08	NM_005902	SMAD3	-1.61	**0.0352**	SMAD, mothers against DPP homolog 3 (Drosophila)
69 /F09	NM_005359	SMAD4	**-4.54**	**0.0006**	SMAD, mothers against DPP homolog 4 (Drosophila)
70 /F10	NM_005903	SMAD5	**-3.29**	**0.0001**	SMAD, mothers against DPP homolog 5 (Drosophila)
71 /F11	NM_020429	SMURF1	-1.12	**0.3781**	SMAD specific E3 ubiquitin protein ligase 1
72 /F12	NM_003107	SOX4	**-3.16**	**0.0000**	SRY (sex determining region Y)-box 4
84 /G12	NM_003244	TGIF	1.37	**0.7693**	TGFB-induced factor (TALE family homeobox)
76 /G04	NM_006022	TSC22D1	-1.30	**0.0283**	TSC22 domain family, member 1

Venn Group k

45 /D09	NM_000600	IL6	**-4.98**	**0.0118**	Interleukin 6 (interferon, beta 2)
49 /E01	NM_002213	ITGB5	1.05	**0.6171**	Integrin, beta 5
50 /E02	NM_000889	ITGB7	**-2.80**	**0.0009**	Integrin, beta 7
62 /F02	NM_002608	PDGFB	**115.14**	**0.0000**	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)

Venn Group l

73 /G01	NM_007315	STAT1	**3.78**	**0.0003**	Signal transducer and activator of transcription 1, 91kDa

Venn Group m

04 /A04	NM_000479	AMH	-1.08	0.3811	Anti-Mullerian hormone
08 /A08	NM_006129	BMP1	**-44.45**	**0.0000**	Bone morphogenetic protein 1
09 /A09	NM_001200	BMP2	**-290.42**	**0.0000**	Bone morphogenetic protein 2
46 /D10	NM_002191	INHA	1.87	0.0114	Inhibin, alpha
47 /D11	NM_002192	INHBA	**-8.30**	**0.0002**	Inhibin, beta A (activin A, activin AB alpha polypeptide)
48 /D12	NM_002193	INHBB	**4.73**	**0.0001**	Inhibin, beta B (activin AB beta polypeptide)

Venn Group n

07 /A07	NM_199173	BGLAP	**-3.77**	**0.0001**	Bone gamma-carboxyglutamate (gla) protein (osteocalcin)
24 /B12	NM_000088	COL1A1	-1.79	**0.1686**	Collagen, type I, alpha 1

25 /C01	NM_000089	COL1A2	-1.47	**0.3629**	Collagen, type I, alpha 2
33 /C09	NM_006350	FST	**-2.69**	**0.0014**	Follistatin

Venn Group o

43 /D07	NM_000618	IGF1	-1.34	0.6914	Insulin-like growth factor 1 (somatomedin C)
44 /D08	NM_000598	IGFBP3	1.11	0.3014	Insulin-like growth factor binding protein 3
75 /G03	NM_015927	TGFB1I1	**-37.76**	**0.0001**	Transforming growth factor beta 1 induced transcript 1

House-keeping gene controls

H01	NM_004048	B2M	**-4.76**	**0.0000**	Beta-2-microglobulin
H02	NM_000194	HPRT1	6.57	**0.0003**	Hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome)
H03	NM_012423	RPL13A	**-2.08**	**0.0000**	Ribosomal protein L13a
H04	NM_002046	GAPDH	-1.10	0.3978	Glyceraldehyde-3-phosphate dehydrogenase
H05	NM_001101	ACTB	1.65	**0.0002**	Actin, beta

The sub-groups that contained no genes (**c, e, j, d in Figure 3**) are not listed.
The significant negative values are blue and the positive change is pink. The relevant p-values are highlighted in red.

Table 2

SuperArray Validation with TaqMan Assay
(Fold Change)

Genes	MCF-7/MCF-10A Un-treated controls		Treatment											
			CII				LAM				TGFβ			
			MCF-10A		MCF-7		MCF-10A		MCF-7		MCF-10A		MCF-7	
	SA	TaqMan	SA	TaqMan	SA	TaqMan	SA	TaqMan	SA	TaqMan	SA	TaqMan	SA	TaqMan
BMP2	-290.42	-15.43	-1.08	1.13	1.08	-1.05	-1.43	2.01	3.59	1.68	2.17	1.92	1.35	1.25
BMP7	2234.86	32360.94	1.10	1.59	1.02	1.22	4.33	10.59	2.43	3.28	-2.35	-2.01	1.16	1.84
CDKN2B	651.26	10890.94	1.37	1.03	-1.05	1.08	1.20	-1.02	-1.35	1.68	1.12	1.05	1.51	2.24
FST	-2.69	-2.16	1.05	1.28	1.06	1.36	-2.14	-1.85	-1.25	1.32	16.37	14.93	2.47	3.80
SERPINE1	-48.87	-14.56	1.63	1.71	1.27	1.07	13.95	16.38	5.02	4.61	120.85	118.03	7.56	10.69
PDGFB	115.14	79.71	1.32	N/A	1.02	-1.01	-2.03	N/A	-2.00	N/A	-1.60	N/A	1.57	1.16
PLAU	-163.8	-12.34	2.25	N/A	1.03	-1.39	1.61	N/A	-1.34	N/A	-1.06	N/A	2.67	2.29
TGFBR1	-2.53	-1.44	-1.21	N/A	-1.02	1.09	2.24	N/A	1.20	N/A	1.46	N/A	1.09	-1.09
TGFBR2	-36.72	-7.00	1.31	N/A	-1.01	N/A	-2.06	N/A	-1.09	N/A	-1.81	N/A	1.21	N/A
TGFBR3	-268.88	-67.21	1.09	N/A	-1.00	N/A	-1.45	N/A	1.74	N/A	-3.54	N/A	-1.06	N/A
TGFBI	-246.19	-39.46	1.10	N/A	1.06	N/A	-1.44	N/A	-1.50	N/A	1.36	N/A	1.12	N/A

TaqMan Probes Used:

BMP2	**Hs00154192_m1**	**CCND1**	**Hs00277039_m1**
BMP7	**Hs00233476_m1**	**FASN**	**Hs00188012_m1**
CDKN2B	**Hs00793225_m1**	**FN1**	**Hs00415006_m1**
FST	**Hs00246260_m1**	**IGFBP3**	**Hs00426287_m1**
SERPINE1	**Hs00167155_m1**	**MMP10**	**Hs00233987_m1**
PDGFB	**Hs00234042_m1**	**MYC**	**Hs00905030_m1**
PLAU	**Hs00170182_m1**	**NOG**	**Hs00271352_m1**
TGFBR1	**Hs00610319_m1**		
TGFBR2	**Hs00559661_m1**		
TGFBR3	**Hs00234259_m1**		
TGFBI	**Hs00165908_m1**		