SUPPLEMENTARY MATERIALS

Fig. S1

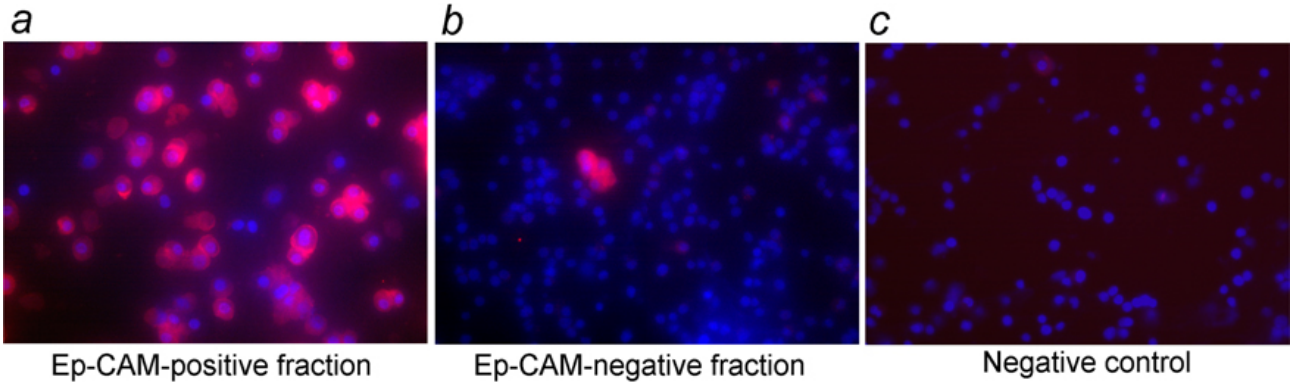

Fig. S1. Ep-CAM staining in sorted cells. (a) Ep-CAM-positive fraction showed Ep-CAM staining (red color) in 84±6% cells. (b) Ep-CAM-negative fraction showed few Ep-CAM stained cells, 9±7%, $p<0.001$. (c) Negative control without primary antibody showed no staining. Nuclei counterstained with DAPI. Orig. Mag. x 200. Cytospun cells were fixed in 4% paraformaldehyde in PBS for 10 min and blocked with 3% goat serum in PBS for 1 h before anti-EpCAM (MCA850H, Serotec Ltd. Raleigh, NC) for 1 h at room temperature. After three washes with PBS for 10 min each, rhodamine-conjugated mouse-specific goat IgG (1:300; #715-295-150, Jackson Immuno Research, West Grove, PA) was added for 1 h.

Fig. S2

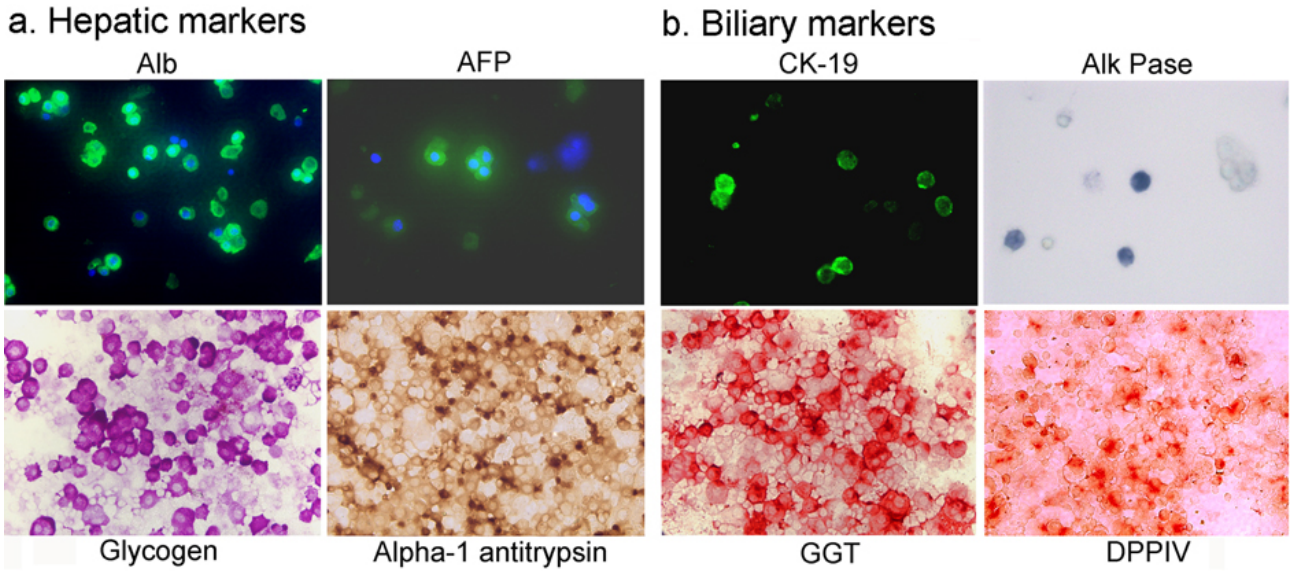

Fig. S2. Immunohistochemical staining for specific liver markers in Ep-CAM-positive cells. Shown are freshly isolated Ep-CAM-positive fetal liver cells with stainings for hepatic and biliary markers as indicated. Most cells expressed hepatic and biliary markers. Quantitative analysis of gene expression is given below in Table 4. Orig. Mag. x 200. Histochemical stainings for the hepatobiliary markers, glycogen, dipeptidyl peptidase IV (DPPIV), γ-glutamyltranspeptidase (GGT), and glucose-6-phosphatase (G-6-P), utilized published protocols. [S8] To colocalize glycogen and cytokeratin (CK)-19, cells were first immunostained for CK-19 and then stained for glycogen content.

Fig. S3

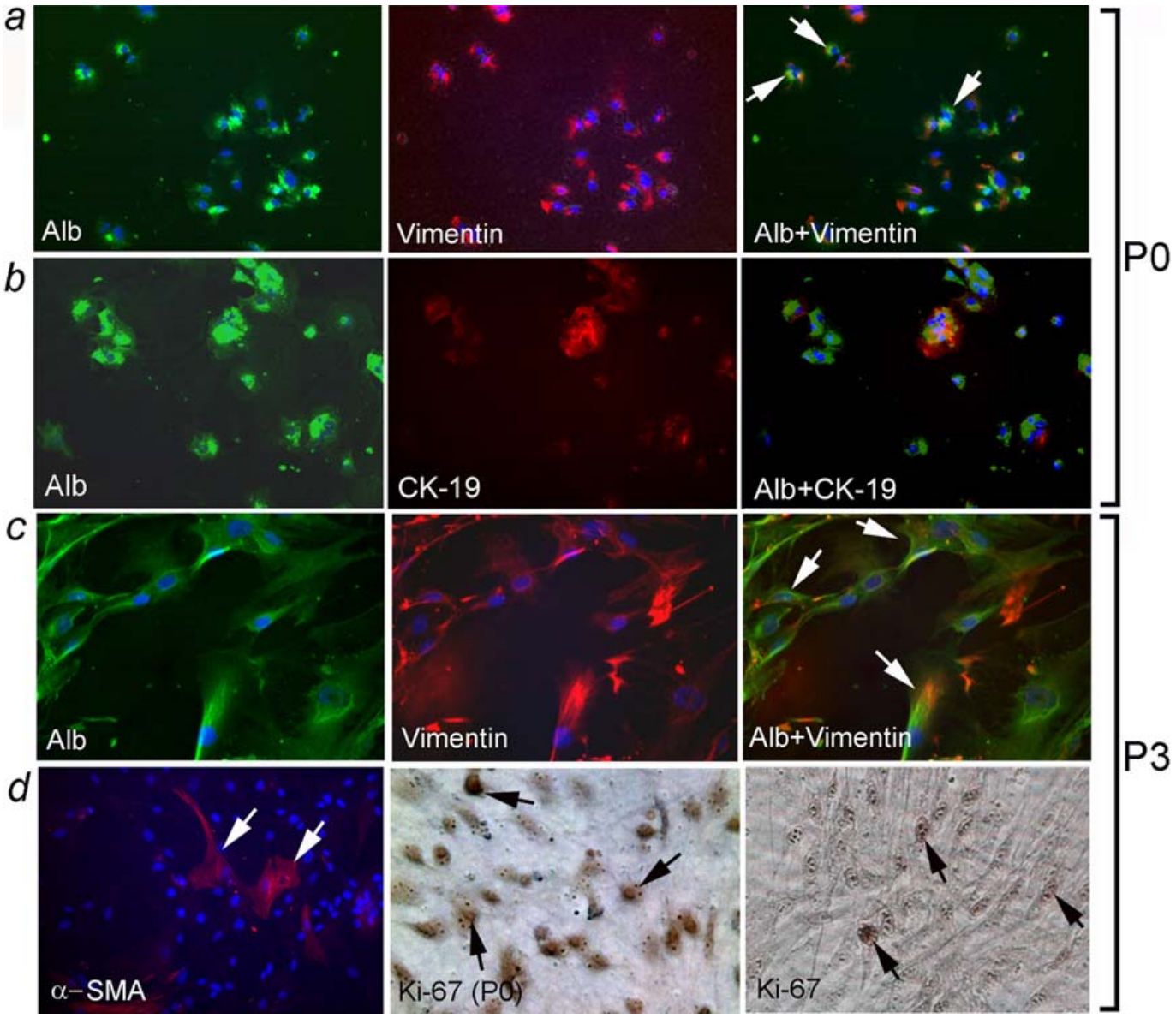

Fig. S3. Phenotypic perturbation in cultured fetal cells. Panels a, b and middle panel in d show primary cultures (P0) and panels c and d show cells passaged thrice (P3). Panels a-c, left, show albumin staining with green FITC signal. Middle panels in a-c show vimentin or CK-19 staining (red color, rhodamine), whereas panels on right show merged images. Panel d on left shows expression of α-smooth muscle actin (red color). Middle and right panels in d show cell proliferation with Ki-67 immunostaining using DAB for visualization. DAPI nuclear counterstain, blue color, all other panels; Orig. Mag. x200.

Fig. S4

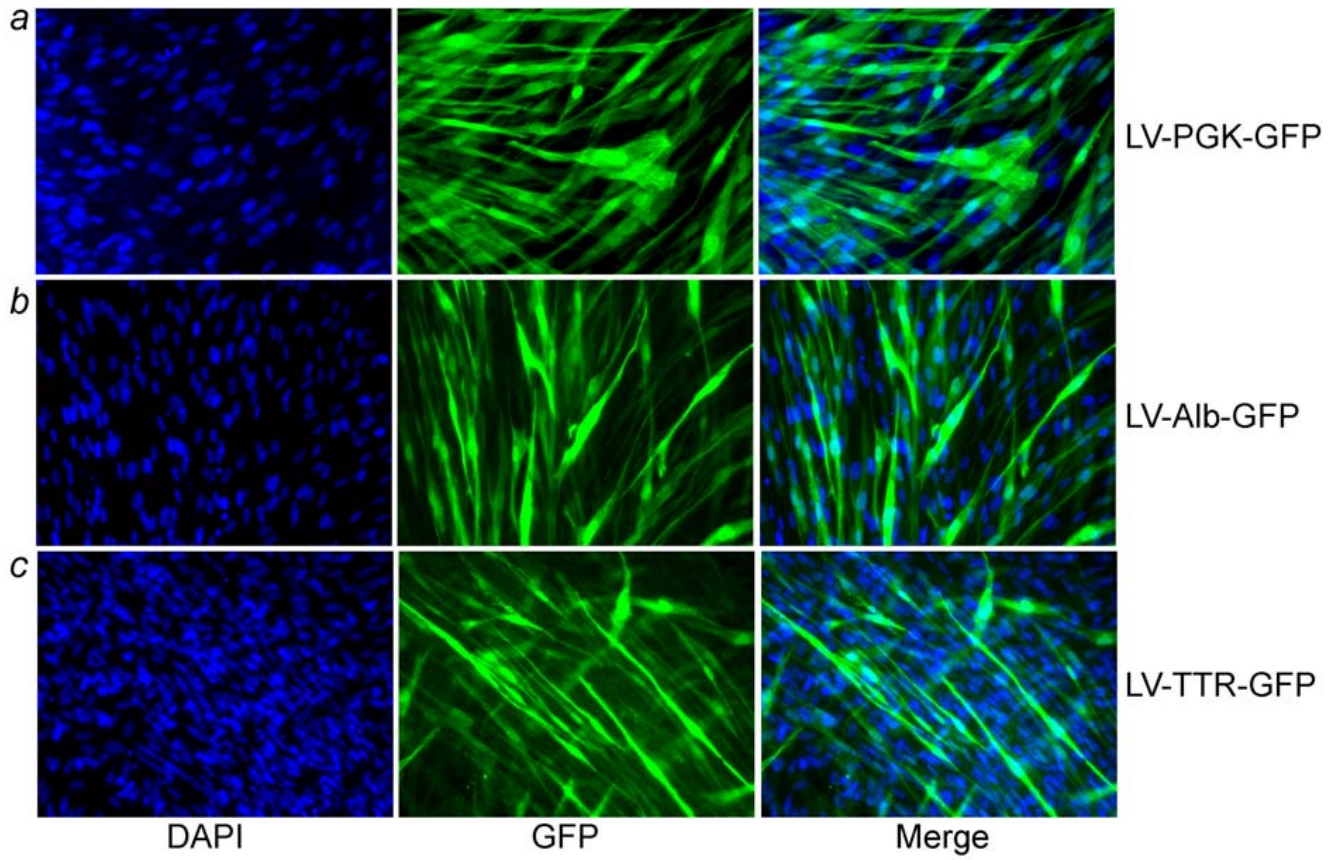

Fig. S4. Efficiency of LV-transduction in cultured fetal cells. Panels a, b and c show cells passaged once (P1) with trznsduction using LVs expressing GFP under PGK, Alb and TTR promotes, respectively. The panels show DAPI staining on left to visualize cell nuclei, GFP staining in the middle to demonstrate expression of the transgene and merged images on the right to show the extent of cell transduction. The majority of cells were transduced under the conditions used. Orig. Mag. x200.

Fig. S5

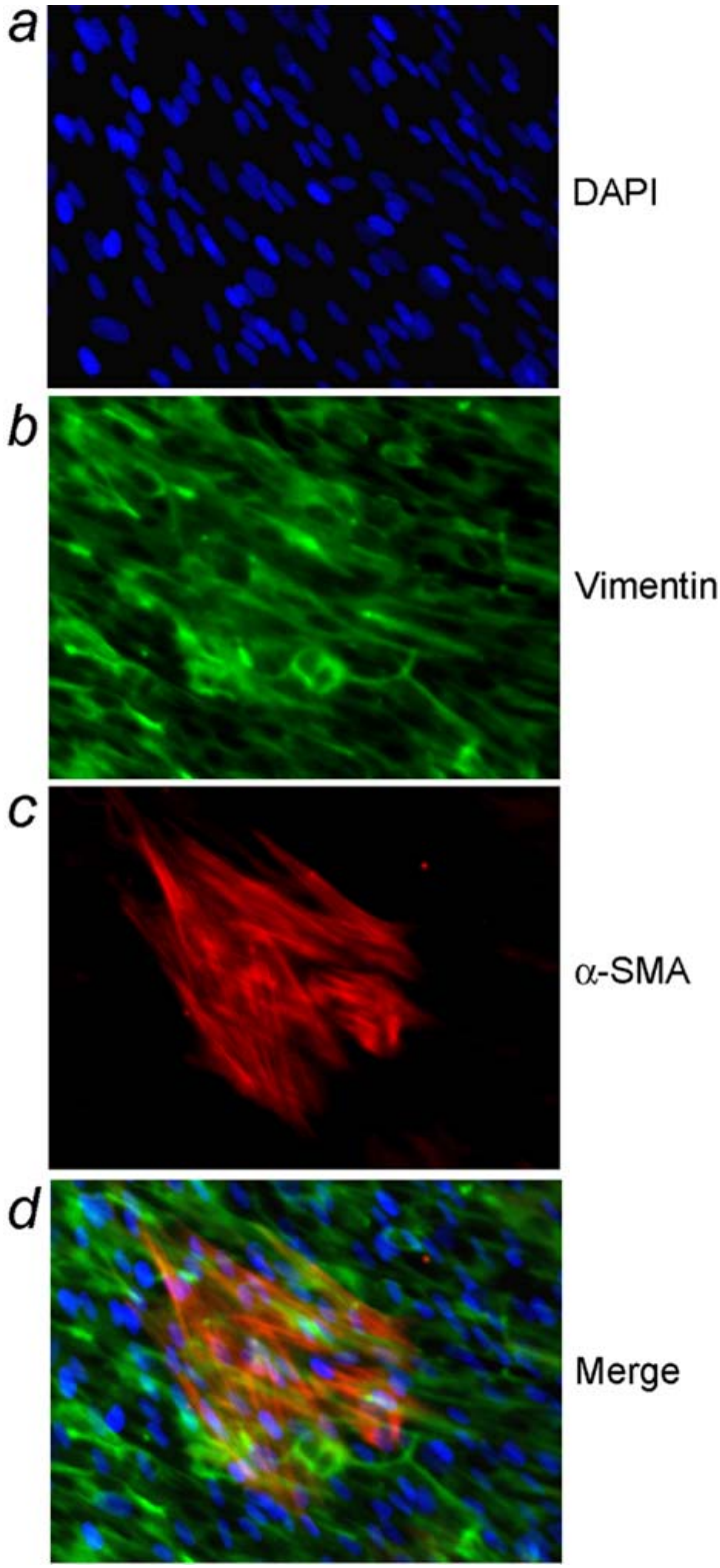

Fig. S5. Differential regulation of vimentin and α-SMA expression in cultured fetal liver cells. The panels show DAPI staining of nuclei (a) and immunostaining for vimentin (b) or α-SMA (c) with merged images (d) to demonstrate that while vimentin was expressed in all cells, α-SMA was expressed in only some cells. Orig. Mag. x200.

Fig. S6

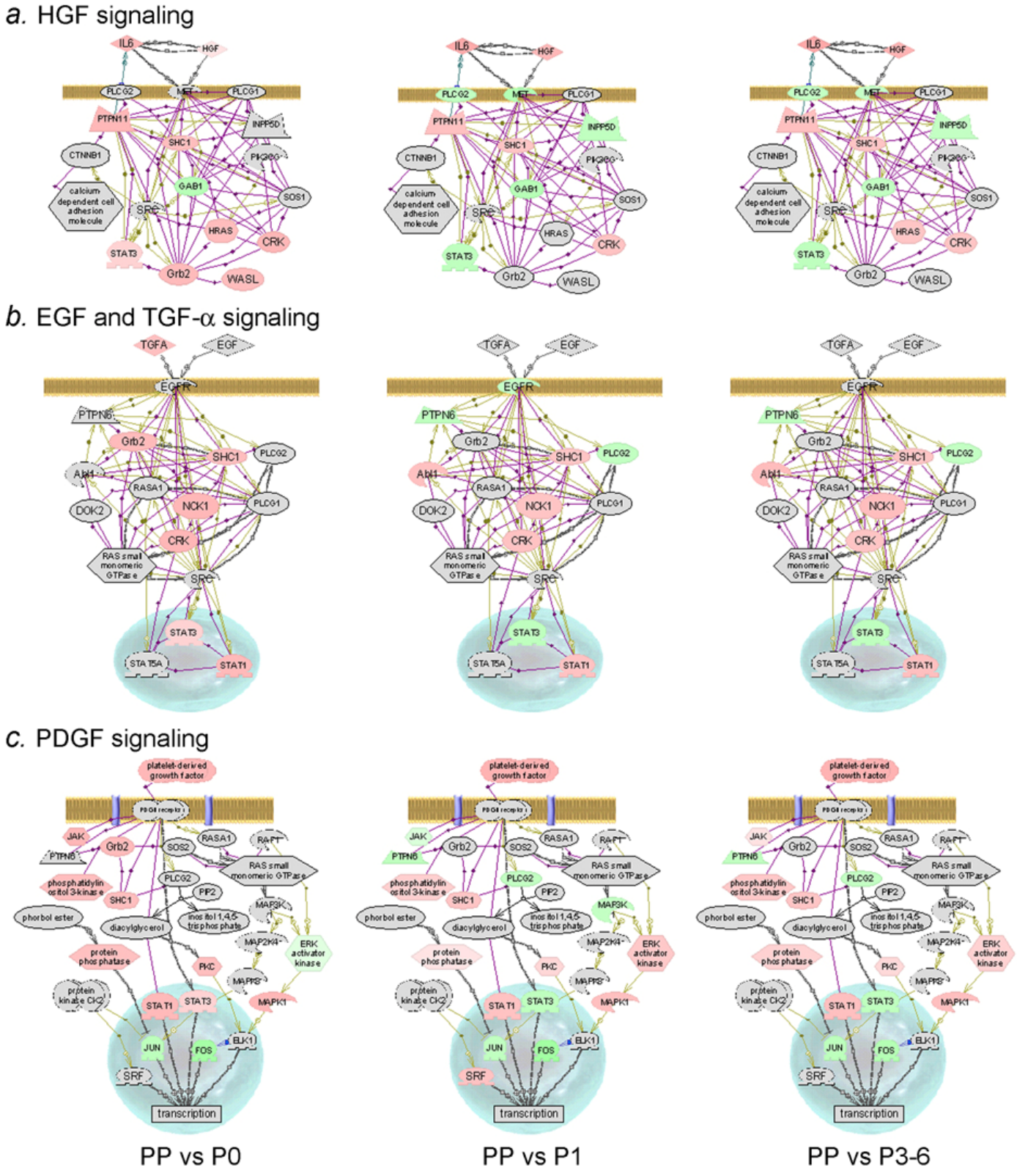
a. HGF signaling
IL6
HGF
PLCG2
MET
PLCG1
PTPN11
INPP5D
SHC1
CTNNB1
PIK3CG
GAB1
calcium-dependent cell adhesion molecule
SOS1
SRC
HRAS
CRK
STAT3
Grb2
WASL
b. EGF and TGF-α signaling
TGFA
EGF
EGFR
PTPN6
Grb2
SHC1
PLCG2
Abl1
RASA1
DOK2
NCK1
PLCG1
CRK
RAS small monomeric GTPase
SRC
STAT3
STAT5A
STAT1
c. PDGF signaling
platelet-derived growth factor
PDGF receptor
JAK
PTPN6
Grb2
SOS2
RASA1
RAFT
RAS small monomeric GTPase
phosphatidylinositol 3-kinase
PLCG2
SHC1
PP2
MAP3K
phorbol ester
diacylglycerol
inositol 1,4,5-trisphosphate
MAP2K4
ERK activator kinase
protein phosphatase
PKC
MAPK8
protein kinase CK2
STAT1
STAT3
MAPK1
JUN
FOS
ELK1
SRF
transcription
PP vs P0
PP vs P1
PP vs P3-6

Fig. S6. Microarray analysis showing changes in HGF, EGF/TGF-α and PDGF signaling. The data were obtained by PathwayStudio analysis and represent genes expressed better in PP cells (pink) or less well in PP cells (green). Boxes in grey indicate genes that were not in the query lists of differentially expressed genes. Panel a shows regulation of several critical members transducing HGF signaling in cultured cells compared with PP cells. These included c-Met and phospholipase C gamma 2 (PLCG2) receptors, inositol phosphate-5-phosphatase D (INPP5D), the adapter molecule GRB2 (growth factor receptor bound 2), the docking protein GAB1 (GRB2-associated binding protein1), which recruits phosphatidylinositol-3 kinase, STAT3 (signal tranducer and activator of transcription-3), which directs the acute phase response, and WASL (Wiskott-Aldrich syndrome-like) gene, which can regulate actin polymerization. Similarly, as shown in Panel b, TGF-α and EGF/TGF-α receptor were regulated during cell culture with perturbations in STAT3 expression. By contrast, as shown in Panel c, PDGF signaling seemed to be mostly unperturbed in fetal cells.

Fig. S7

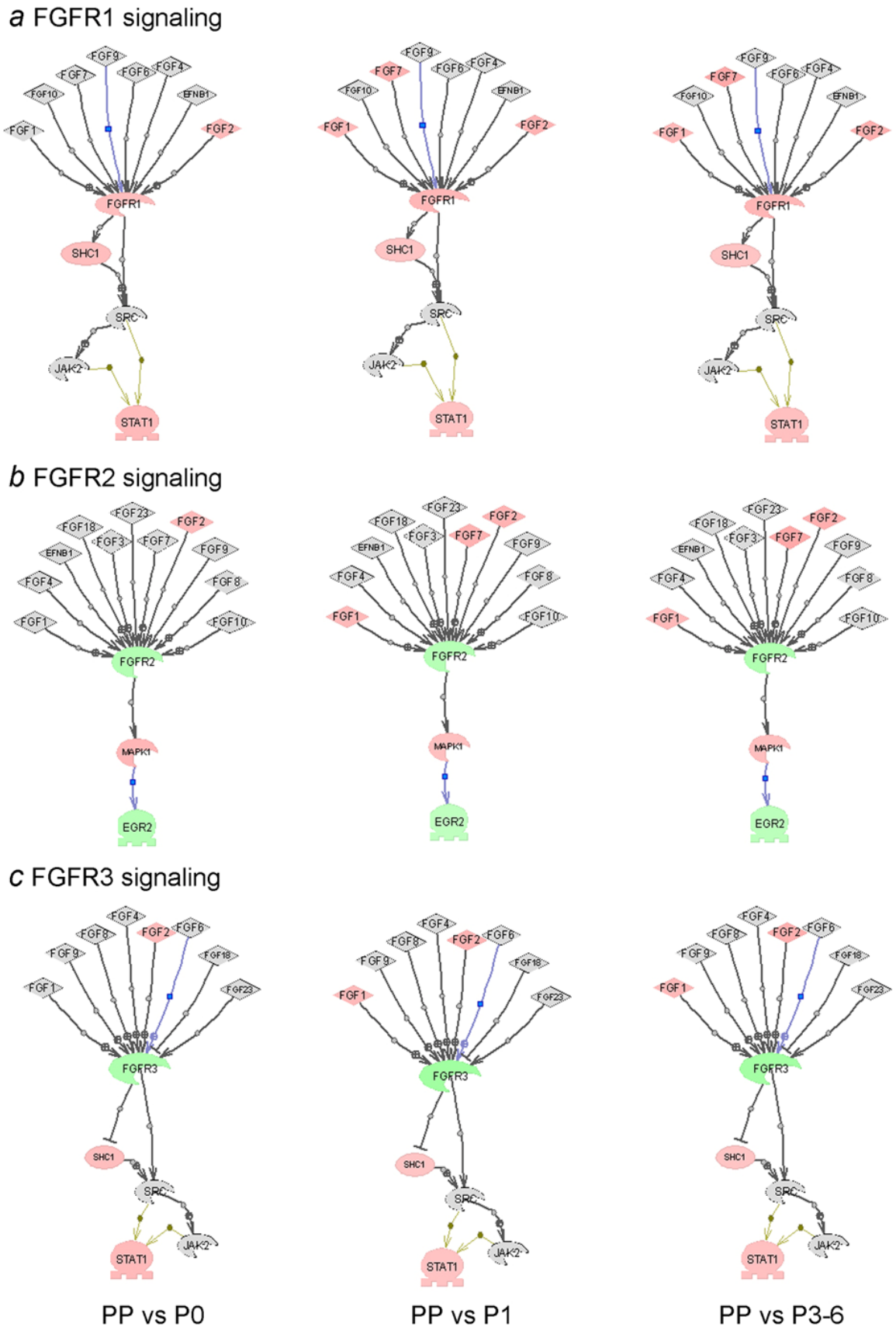
a FGFR1 signaling
FGF9
FGF7
FGF6
FGF4
FGF10
EFNB1
FGF1
FGF2
FGFR1
SHC1
SRC
JAK2
STAT1
b FGFR2 signaling
FGF23
FGF18
FGF2
FGF3
FGF7
EFNB1
FGF9
FGF4
FGF8
FGF1
FGF10
FGFR2
MAPK1
EGR2
c FGFR3 signaling
FGF4
FGF8
FGF2
FGF6
FGF9
FGF18
FGF1
FGF23
FGFR3
SHC1
SRC
STAT1
JAK2
PP vs P0
PP vs P1
PP vs P3-6

Fig. S7. Regulation of FGF receptor pathways in fetal liver cells. These data demonstrate the overall regulation of FGF receptor pathways in PP cells vs cultured cells. Genes expressed better in PP cells are in pink, expressed less well in PP are in green, and those not represented in the lists are in grey. Panel a shows that FGFR1 was expressed better in PP cells, although in contrast with P0 cells, FGF-1 and FGF-7 were expressed at lower levels in P1 and P3-6 cells. By contrast, FGFR2 and FGFR3 were less well expressed in PP cells. FGFR2 signaling appeared to be associated with superior expression of the downstream EGR2 (early growth response-2) gene in cultured cells versus PP cells.

Fig. S8

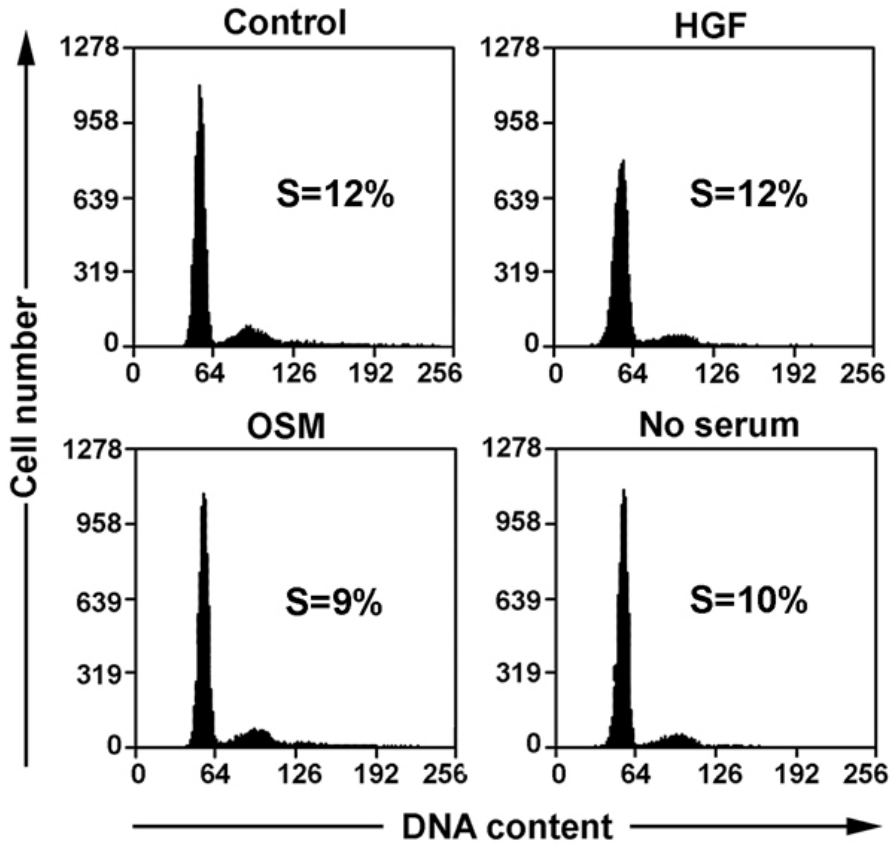

Fig. S8. Cell cycle profiles during various manipulations. The data were from P3 fetal cells shown in main Fig. 8. The flow cytometric profiles of cells with FBS (control) or after treatment with HGF, OSM or no serum were similar. These findings excluded the possibility that altered cell proliferation confounded changes in gene expression under these conditions.

Table S1

Gene expression profiles in sorted fetal liver cells

Marker	Ep-CAM-positive fraction	Ep-CAM-negative fraction
Hepatic markers		
Albumin	64±9%*	5±2%
AFP	38±5%*	3±2%
Glycogen	57±8%*	8±4%
Biliary markers		
CK-19	72±9%*	2±1%
GGT	51±6%*	6±3%
DPPIV	52±5%*	0.2±0.3%
Alkaline phosphatase	8±4%	ND

P<0.001 vs. Ep-CAM-negative fraction, ANOVA; ND = not done

Table S2. Immunostaining protocols

Antigen	Fixation	Blocking	Primary Antibody	Dilution	Secondary Antibody	Dilution	Color development	Counter-stain
AFP	4% PAF, RT	3% Goat serum	MFCD00162111 (#A8452, Sigma)	1:500	Peroxidase-conjugated anti-mouse IgG (#A3682, Sigma)	1:200	DAB	-
Alb	4% PAF, RT	3% Goat serum	HSA-11 clone (#A6684, Sigma)	1:1000	FITC-conjugated IgG (#F9137, Sigma)	1:300	-	DAPI
Alb	4% PAF, RT	3% Goat serum	IgG fraction antiserum (#A0433, Sigma)	1:1000	FITC-conjugated IgG (#F0382, Sigma)	1:300	-	-
CK-19	4% PAF, RT	3% Goat serum	RPN 1165 (Amersham Pharmacia Biotech Inc., Piscataway, NJ)	1:100	Peroxidase-conjugated IgG (#A3682, Sigma)	1:200	DAB	-
CK-19	4% PAF, RT	5% Donkey serum	RPN 1165 (Amersham Pharmacia Biotech)	1:100	Rhodamine-conjugated IgG (#715-295-150, Jackson ImmunoRes., West Grove, PA)	1:500	-	DAPI
Ep-CAM	4% PAF, RT	5% Donkey serum	MCA850H (Serotec Ltd., Raleigh, NC)	None	Rhodamine-conjugated anti mouse IgG (#715-295-150, Jackson Immuno Research)	1:500	-	DAPI
GFP	4% PAF, RT	5% goat serum 1% BSA, 0.1% Triton X-100	Rabbit anti-GFP (#A-6455, Molecular Probes, Eugene, OR)	1:300	FITC-conjugated anti-rabbit IgG (#F0382, Sigma)	1:40	-	DAPI
Ki-67	Ethanol, 4°C	3% Goat serum	Mouse monoclonal (#550609, Pharmingen Inc., San Diego)	1:25	Peroxidase-conjugated anti-mouse IgG (#A3682, Sigma)	1:200	DAB	-
α-SMA	4% PAF, RT		Anti-α smooth muscle-Cy3™ (#C6198, Sigma)	1:800	-	-	-	DAPI
Vimentin	4% PAF, RT	5% Donkey serum	V212210 (United States Biological, Swampscott, MA)	1:100	Rhodamine-conjugated anti mouse IgG (#715-295-150, Jackson Immuno Research)	1:500	-	DAPI
Common	10 min	1 h, RT	2 h incubation, RT	-	1 h incubation, RT	-	-	-

RT= room temperature; PFA= paraformaldehyde; DAPI = 4',6-Diamidino-2-phenylindole; DAB = diaminobenzidine

Table S3. PCR Primers

Gene	Primer pairs (5'-3'; Forward and Reverse)	Tm (°C)	Product (bp)	Reference
ABCG-2	GGGTTCTCTTCTTCCTGACGACC TGGTTGTGAGATTGACCAACAGACC	65	398	CW Scharenberg et al. Blood 2002;99: 507
Alb	TGCTTGAATGTGCTGATGACAGGG AAGGCAAGTCAGCAGGCATCTCATC	60	160	R Schwartz et al. J Clin Invest 2002; 109: 1291
AFP	TGCAGCCAAAGTGAAGAGGGAAGA CATAGCGAGCAGCCCAAAGAAGAA	60	160	R Schwartz et al. J Clin Invest 2002; 109: 1291
β-Actin	AGAGCTATGAGCTGCCTGAC CTGATCCACATCTGCTGGAA	65	361	Y. Heremans et al. J Cell Biol 2002;159: 303
β2-microglobulin	AGCAAGGACTGGTCTTT CTCGATCCCACTTAACTATCT	54	124	Current authors (MI)
CK-19	ATGGCCGAGCAGAACCGGAA CCATGAGCCGCTGGTACTCC	60	308	R Schwartz et al. J Clin Invest 2002; 109: 1291
E-Cadherin	TTGGTCTACGCCTGGG AGTTGGGAAATGTGAGCA	60	139	Current authors (MI)
EGF receptor	ATTCCGAGACGAAGCC CACGAGCCGTGATCTG	60	162	Current authors (MI)
Frizzled-1	GAACTTTCCTCCAACTTCATGGC CATTCCATTTTACAGACCGG	60	398	YW Qiang et al. Oncogene 2003;22:1536
Frizzled -2	GGTGAGCCAGCACTGCAAGAG CCTAAAAGTGAAATGGTTTCGATCG	60	310	YW Qiang et al. Oncogene 2003;22:1536
Frizzled -3	GCTGTACTCACAGTTAACATG GCTAAAATACCCTTGCTGATTT	45	450	YW Qiang et al. Oncogene 2003;22:1536
Frizzled -4	TGCCTTTTCAGGGCAAAGTG ACAGGAAGAGATTTATGGAATG	55	378	YW Qiang et al. Oncogene 2003;22:1536
Frizzled -5	TACCCAGCCTGTCGCTAAAC AAAACCGTCCAAAGATAAACTGC	55	247	YW Qiang et al. Oncogene 2003;22:1536
Frizzled -6	ACATCTCTGCTTGTTCAC GATCTGTGAAATTCCTAA	45	732	YW Qiang et al. Oncogene 2003;22:1536
Frizzled -7	GTTTGGATGAAAAGATTTCAGGC GACCACTGCTTGACAAGCACAC	60	294	YW Qiang et al. Oncogene 2003;22:1536
Frizzled -8	ACAGTGTTGATTGCTATTAGCATG GTGAAATCTGTGTATCTGACTGC	55	268	YW Qiang et al. Oncogene 2003;22:1536
Frizzled -9	CCCTAGAGACAGCTGACTAGCAG CGGGGGTTTATTCCAGTCACAGC	60	270	YW Qiang et al. Oncogene 2003;22:1536

Frizzled -10	ACACGTCCAACGCCAGCATG ACGAGTCATGTTGTAGCCGATG	60	160	YW Qiang et al. Oncogene 2003;22:1536
HNF-1α	GTGTCTACAACTGGTTTGCC TGTAGACACTGTCACTAAGG	52	251	I Lemm I. et al. Mol Carcinogenesis 1999; 24:305
HNF-1β	GAAACAATGAGATCACTTCCTCC CTTTGTGCAATTGCCATGACTCC	56	374	I Lemm et al. Mol Carcinogenesis 1999; 24:305
HNF-3α	CAACATGTTCGAGAACGGCT CCACTGTGGTCCAGAGTCTG	56	263	RK Giri (unpublished)
HNF-3β	CACCCTACGCCTTAACCAC GGTAGTAGGAGGTATCTGCGG	52	235	Current authors (MI)
HNF-4	CTGCTCGGAGCCACAAAGAGATCCATG ATCATCTGCCACGTGATGCTCTGCA	58	370	L Suaud et al. Biochem Biophys Res Commun 1997;235: 820
HGF	ACCACACGAACACAGC AGACTTCGTAGCGTACC	60	134	Current authors (MI)
c-Met	ACAACCCGAATACTGCC AGGATACGGAGCGACA	54	190	Current authors (MI)
Nanog	AGAACTCTCCAACATCCTG GGGTAGGTAGGTGCTG	54	147	Current authors (MI)
Notch-1	GCGGCCGCCTTTGTGGTTCTGTTC GCCGGCGCGTCCTCCTCTTCC	65	500	J. Walsh et al. APMIS, 2003; 111: 197-210
Notch-2	TCGTGCAAGAGCCAGTTACCC AATGTCATGGCCGCTTCAGAG	65	530	J. Walsh et al. APMIS, 2003; 111: 197-210
Oct-4	AGTGAGAGGCAACCTG CGTTGTGCAAGTCGC	53	165	Current authors (MI)
α-SMA	AGTACCCGATAGAACATGG TTTTCTCCCGGTTGGC	60	153	Current authors (MI)
TGF-β1	TGATGTCACCGGAGTTG GAACCCGTTGATGTCCA	58	124	Current authors (MI)
TGF-β2	CAACAGACCAACCGGC GTACCCTTTGGGTTCGT	58	144	Current authors (MI)
TGF-β1 Receptor	CGTGCTGACATCTATGCAAT AGCTGCTCCATTGGCATAC	54	251	U Wulbrand et al. Eur J Clin Invest 1998; 28:1038
TGF–β2 Receptor	TGCGTCTGGACCCTAC ACTGCATTACAGCGAGAT	58	187	RK Giri (unpublished)
Vimentin	CACCTACAGCCTCTACG AGCGGTCATTCAGCTC	60	170	Current authors (MI)