

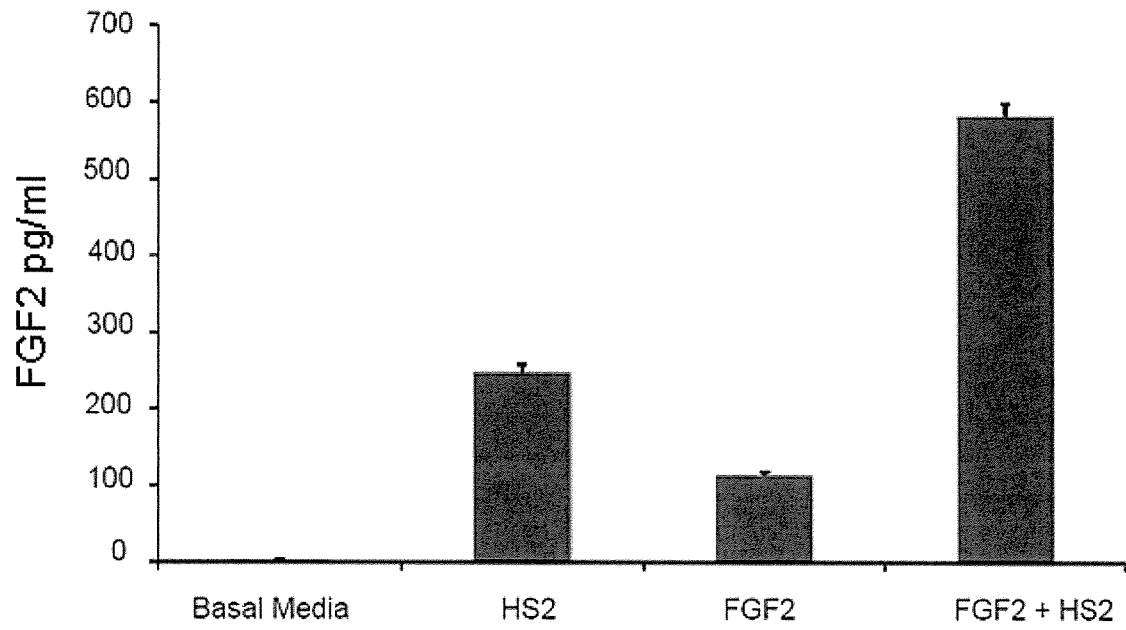


Figure 1

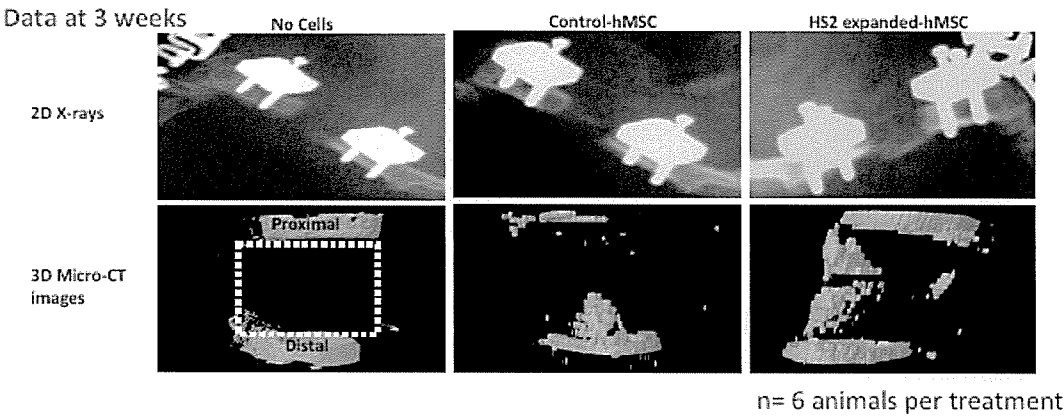


Figure 2A

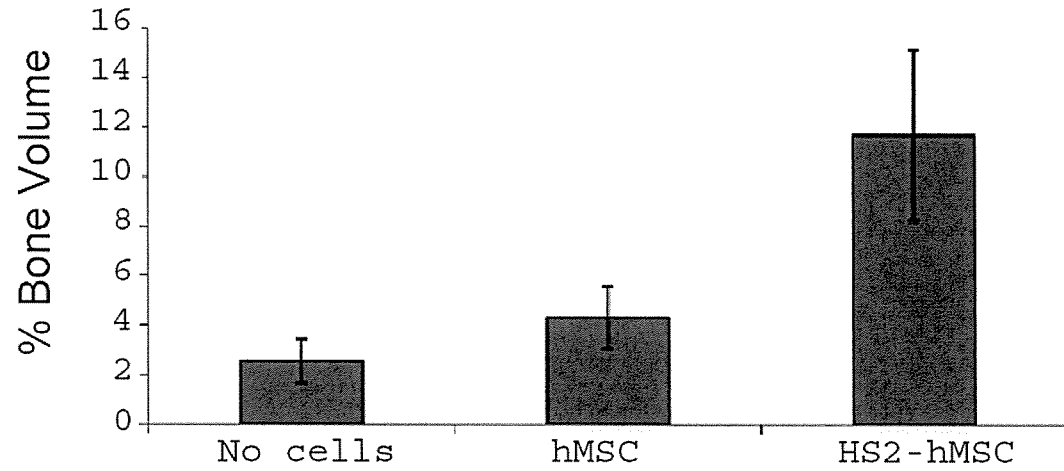


Figure 2B

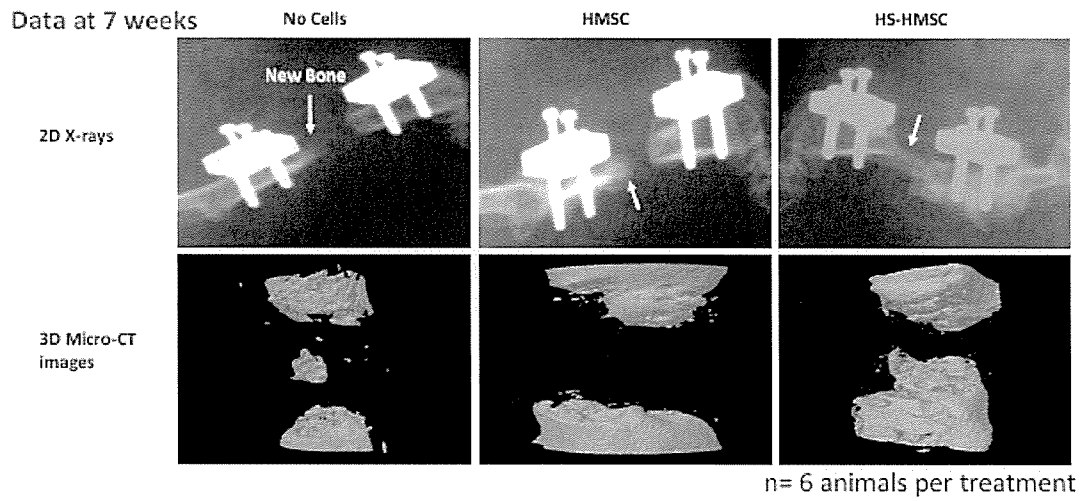


Figure 3A

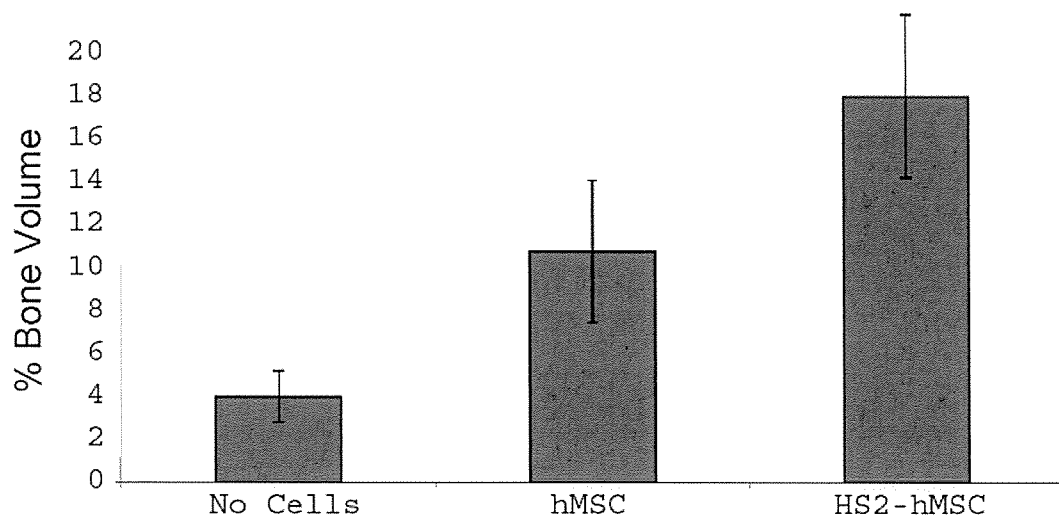


Figure 3B

4/28

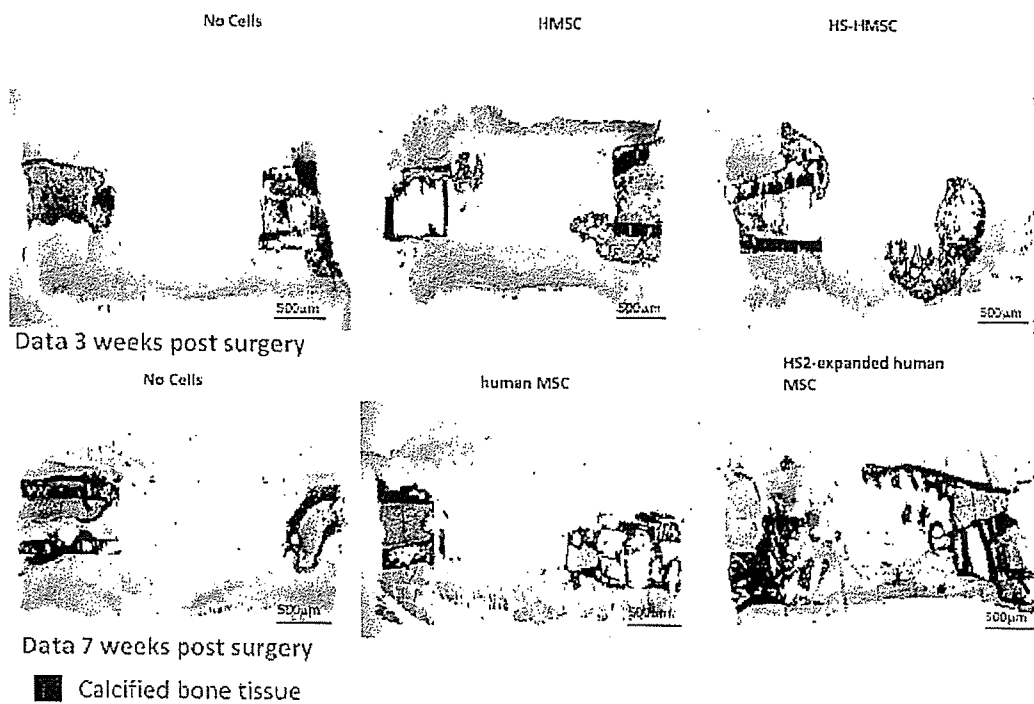


Figure 4

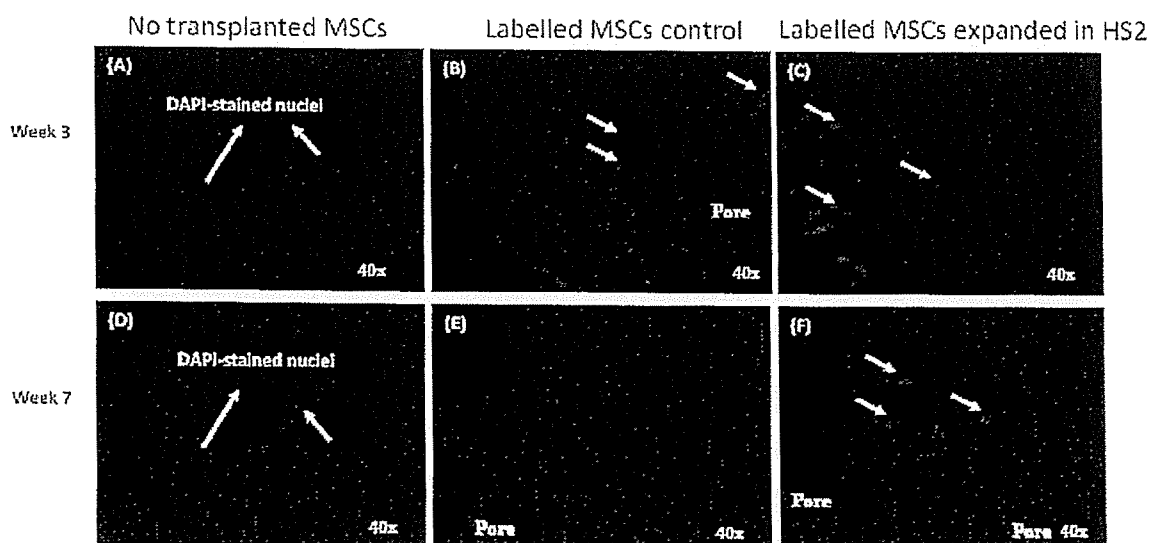


Figure 5

5/28

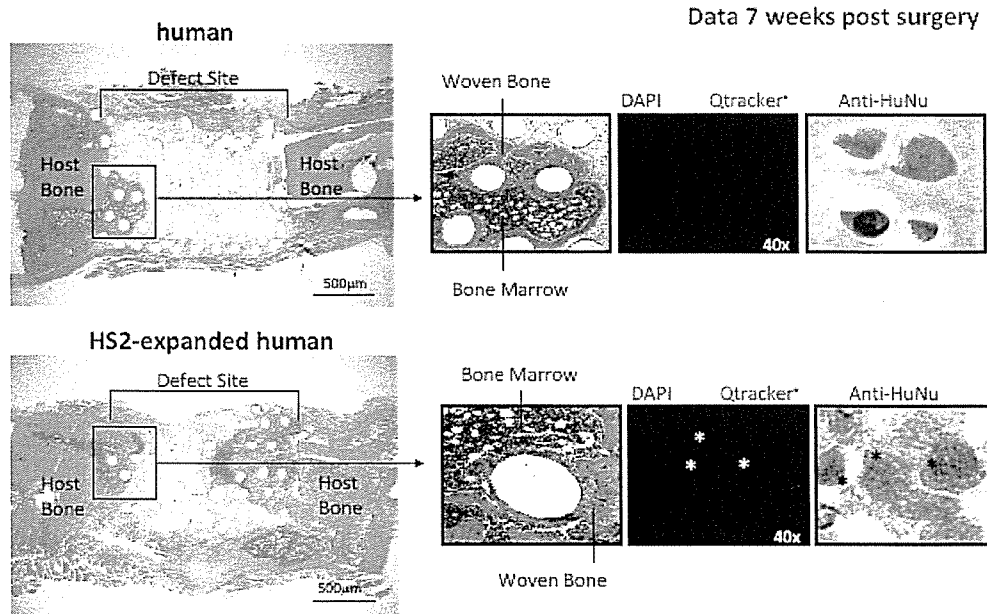


Figure 6

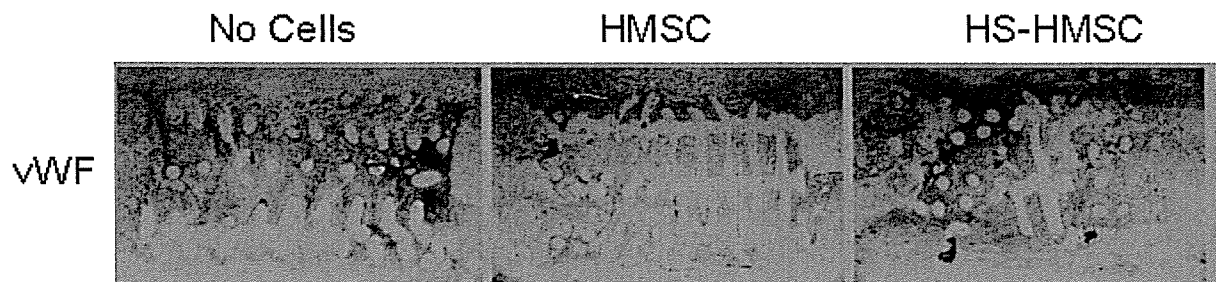


Figure 7

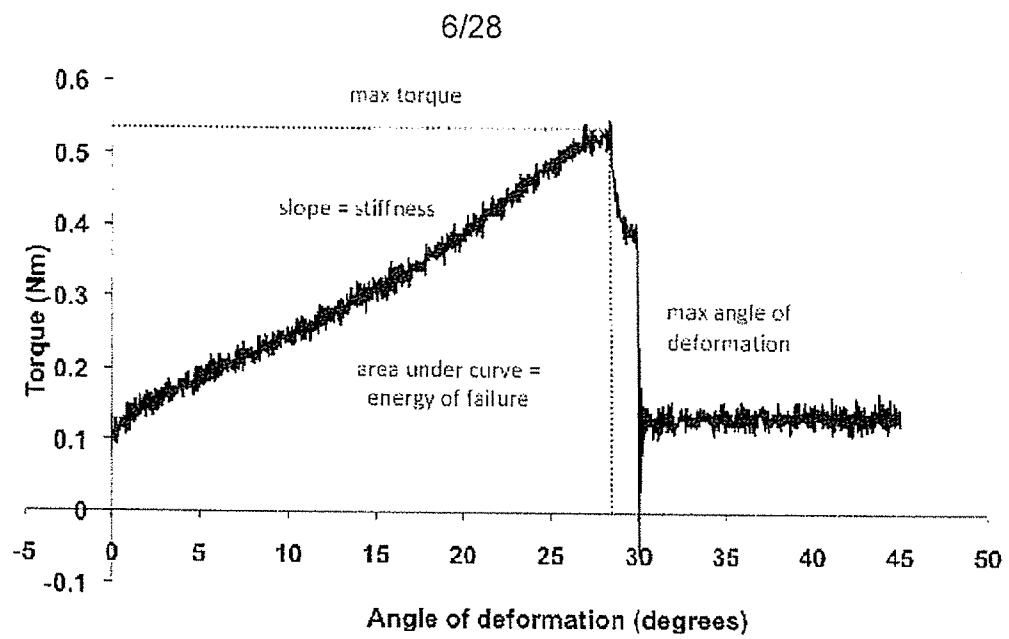


Figure 8A

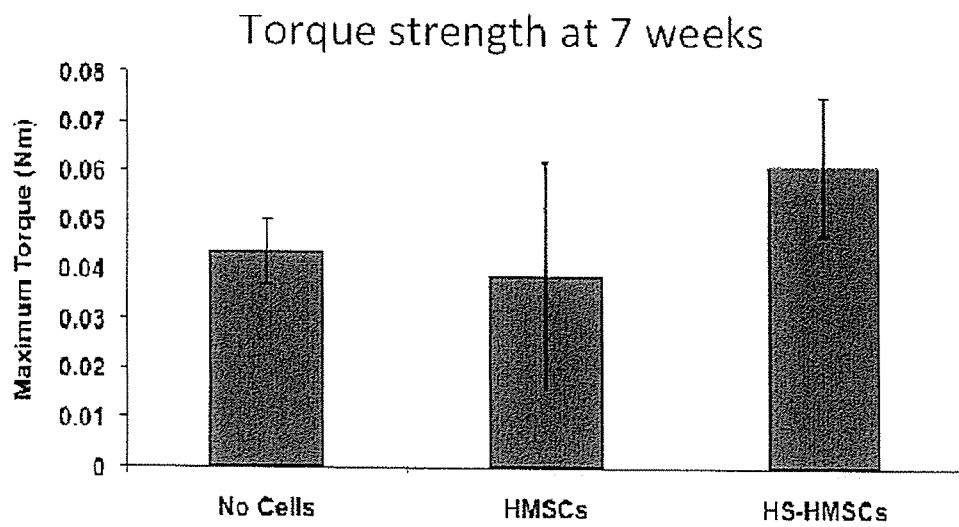


Figure 8B

CONDITION	E 10
pronase	40,000
mild alkali treatment	25,000
heparinase	7,000
number of heparinase resistant domains	2

Figure 9

	% E10
TREATMENT	
Heparitinase	61.5
Heparinase	15.3
HNO₂	49.0

Figure 10

	%E10 GAG
DISACCHARIDE	
IdoA/GlcA-AMann _R	12.9
IdoA(2S)-AMann _R	53.4
GlcA-AMann _R (6S)	10.25
IdoA-AMann _R (6S)	3.4
IdoA(2S)-AMann _R (6S)	18.7
GlcA(2S)-AMann _R	1.0
GlcA-AMann _R (3S)	0.30
GlcA-AMann _R (3,6S)	0.15
UNKNOWN	0.0

Figure 11

	% IN E10
sulfation number : peak number	
Non-sulfated : 1	37.2
Mono-sulfated : 1	1.5
Mono-sulfated : 2	1.0
Mono-sulfated : 3	0.3
Mono-sulfated : 4	0.3
Mono-sulfated : 5	7.7
Mono-sulfated : 6	23.9
Mono-sulfated : 7	18.4
Mono-sulfated : 8	0.7
Mono-sulfated : 9	0.4
Di-sulfated : 1	1.8
Di-sulfated : 2	0.9
Di-sulfated : 3	2.7
Di-sulfated : 4	1.5
Tri-sulfated : 1	1.0
Tri-sulfated : 2	0.0
Tri-sulfated : 3	0.3
Tri-sulfated : 4	0.4
TOTAL	100

Figure 12

PEAK NUMBER	DISACCHARIDE	% in E10 GAG
1	Δ HexUA- GlcNAc	44.8
3	Δ HexUA- GlcNSO ₃	21.5
2	Δ HexUA- GlcNAc(6S)	8.0
7	Δ HexUA(2S)- GlcNAc	2.4
4	Δ HexUA- GlcNSO ₃ (6S)	4.0
5	Δ HexUA(2S)- GlcNSO ₃	12.4
8	Δ HexUA(2S)- GlcNAc(6S)	0.2
6	Δ HexUA(2S)- GlcNSO ₃ (6S)	4.1
9	unknown	2.4

Figure 13

SULFATION	% in E10 GAG
Total sulfation/100 disaccharides	77.4
6-O-Sulfate	16.3
2-O-Sulfate	19.1
N-Sulfate	42.0
O-Sulfate	35.4
ratios of sulfations	
2-O-Sulfate/6-O-Sulfate	1.17
N-Sulfate/O-Sulfate	1.19
N-Sulfate/2-O-Sulfate	2.2
N-Sulfate/6-Sulfate	2.58

Figure 14

11/28

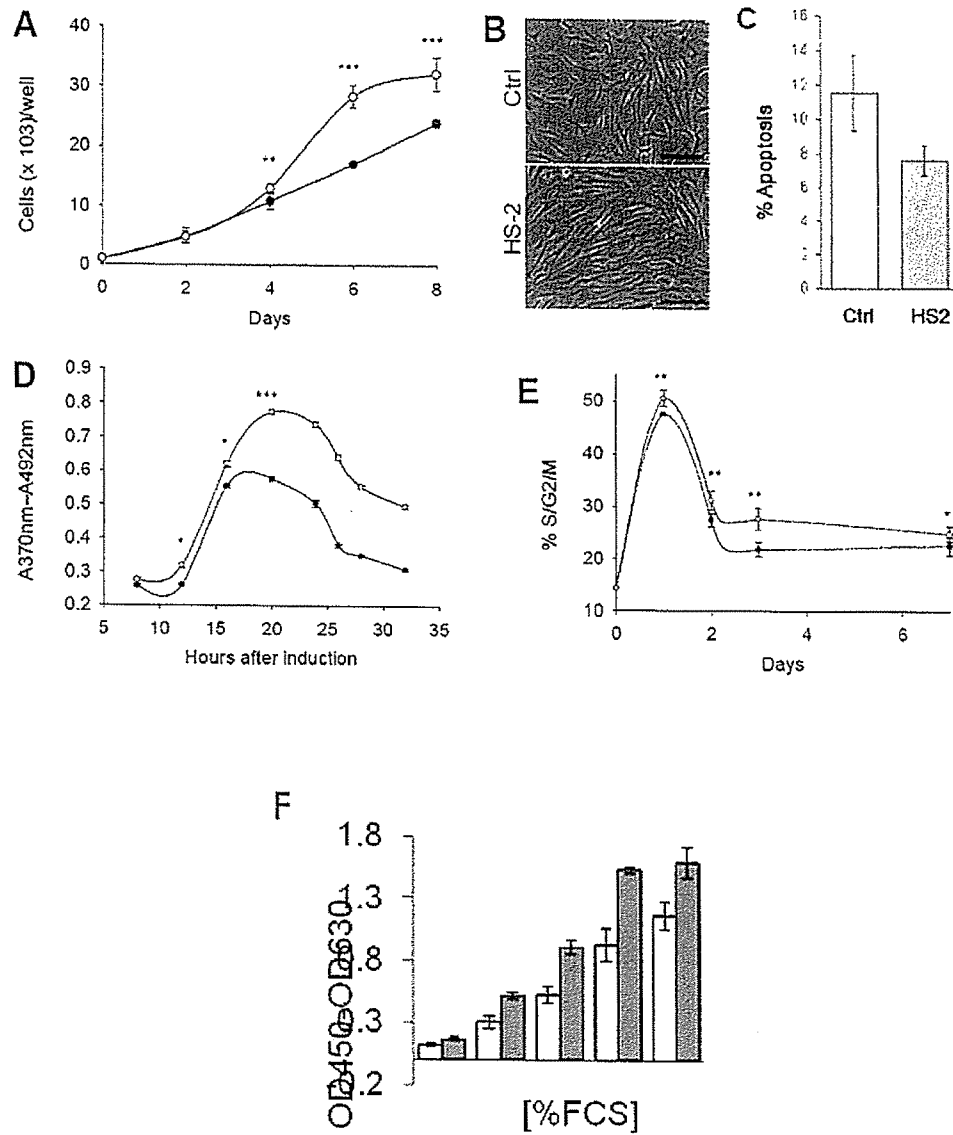


Figure 15

12/28

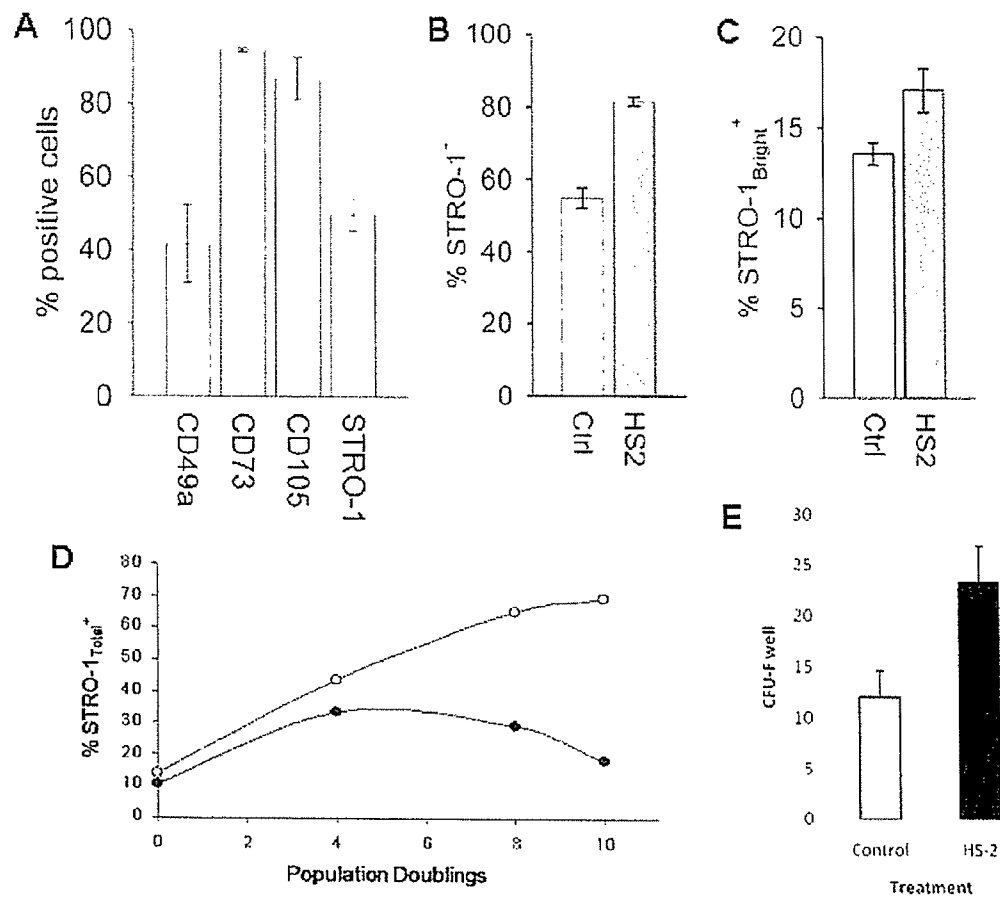
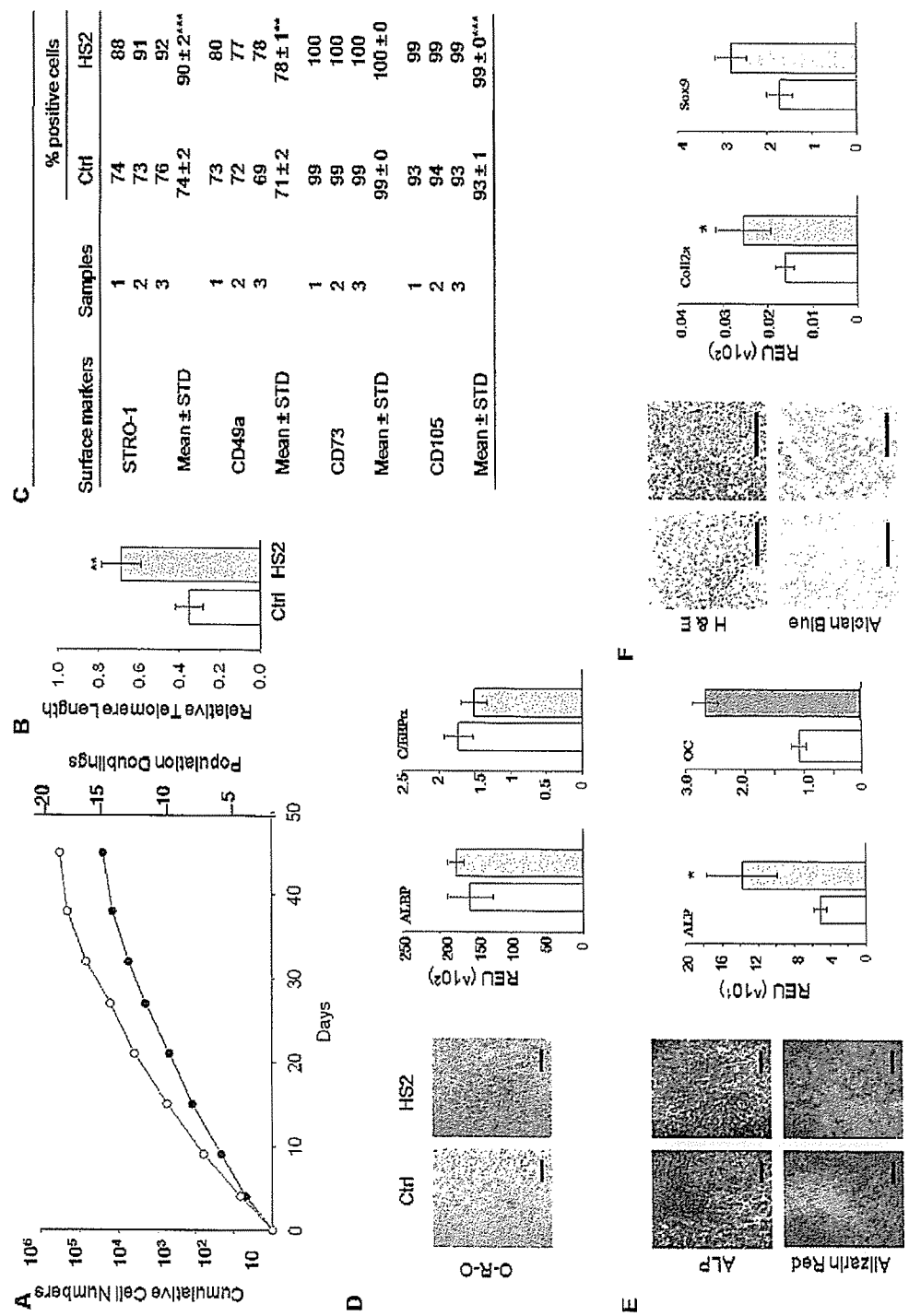


Figure 16



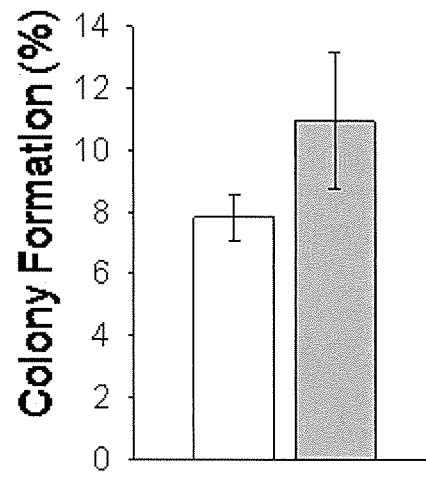
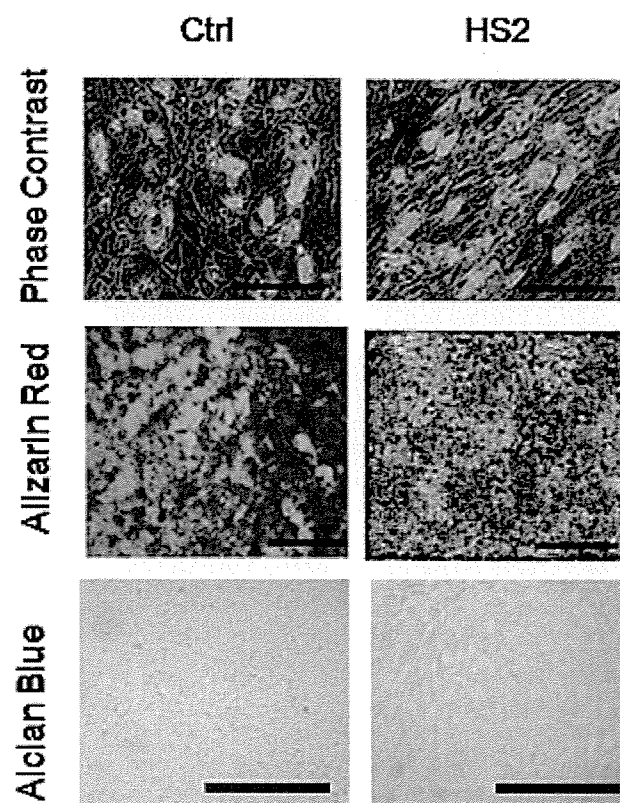
A**B**

Figure 18

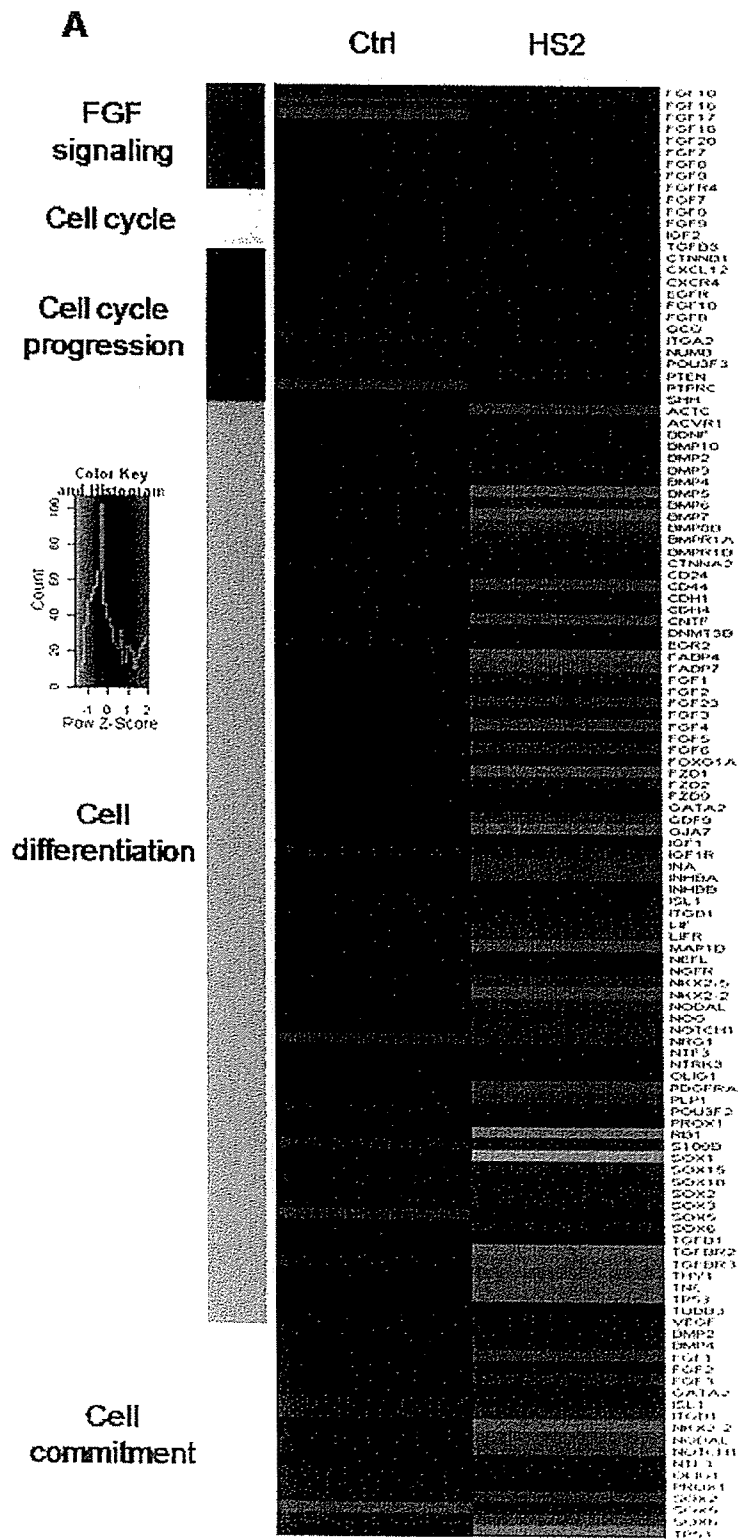
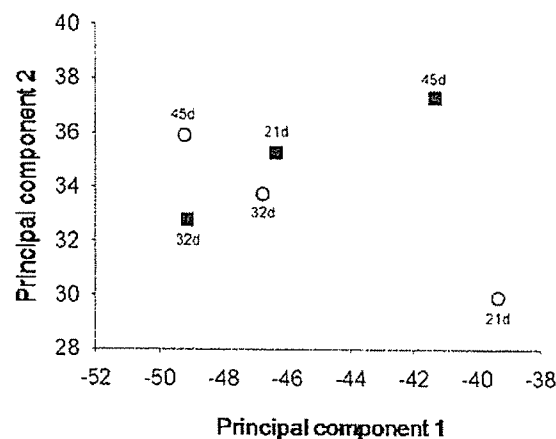


Figure 19

B**C**

Symbol	Description	Fold Change
Up regulated		
VCAM1	Vascular cell adhesion molecule 1	5.858986499
GCM2	Glial cells missing homolog 2 (Drosophila)	5.695428926
IGF2R	Insulin-like growth factor 2 receptor	4.222693908
ITGA4	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	4.138188185
CTNNAL1	Catenin (cadherin-associated protein), alpha-like 1	4.042307815
MYL4	Myosin, light polypeptide 4, alkali; atrial, embryonic	3.707959606
LASS1	LAG1 longevity assurance homolog 1 (S. cerevisiae)	3.385028243
ITGA2	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	3.37002629
ICAM5	Intercellular adhesion molecule 5, telencephalin	3.231415232
NCAM2	Neural cell adhesion molecule 2	3.214941542
NEUROG1	Neurogenin 1	3.200451234
FGF17	Fibroblast growth factor 17	3.153615349
FGF19	Fibroblast growth factor 19	3.096687725
SLC1A2	Solute carrier family 1 (glial high affinity glutamate transporter), member 2	3.085489342
FGF12	Fibroblast growth factor 12	3.031896211
Down regulated		
FZD1	Frizzled homolog 1 (Drosophila)	105.5488977
FZD3	Frizzled homolog 3 (Drosophila)	29.59892173
GDF9	Growth differentiation factor 9	28.11525787
IL6R	Interleukin 6 receptor	24.17353641
IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	22.10213584
BMP5	Bone morphogenetic protein 5	17.62639701
TGFB2	Transforming growth factor, beta receptor II (70/80kDa)	14.61376967
FABP4	Fatty acid binding protein 4, adipocyte	12.70374367
GJA7	Gap junction protein, alpha 7, 45kDa (connexin 45)	11.84183258
GFAP	Glial fibrillary acidic protein	11.73796254
FZD4	Frizzled homolog 4 (Drosophila)	10.5989069
CD9	CD9 antigen (p24)	8.572876617
FABP7	Fatty acid binding protein 7, brain	8.416440381
FABP7	Fatty acid binding protein 7, brain	8.416440381
ACTA2	Actin, alpha 2, smooth muscle, aorta	8.135049403
GDF8	Growth differentiation factor 8	7.861982352
NKX2-5	NK2 transcription factor related, locus 5 (Drosophila)	7.29208558
FABP6	Fatty acid binding protein 6, ileal (gastrotropin)	7.247190687
FZD7	Frizzled homolog 7 (Drosophila)	7.184131073

Figure 19 cont...

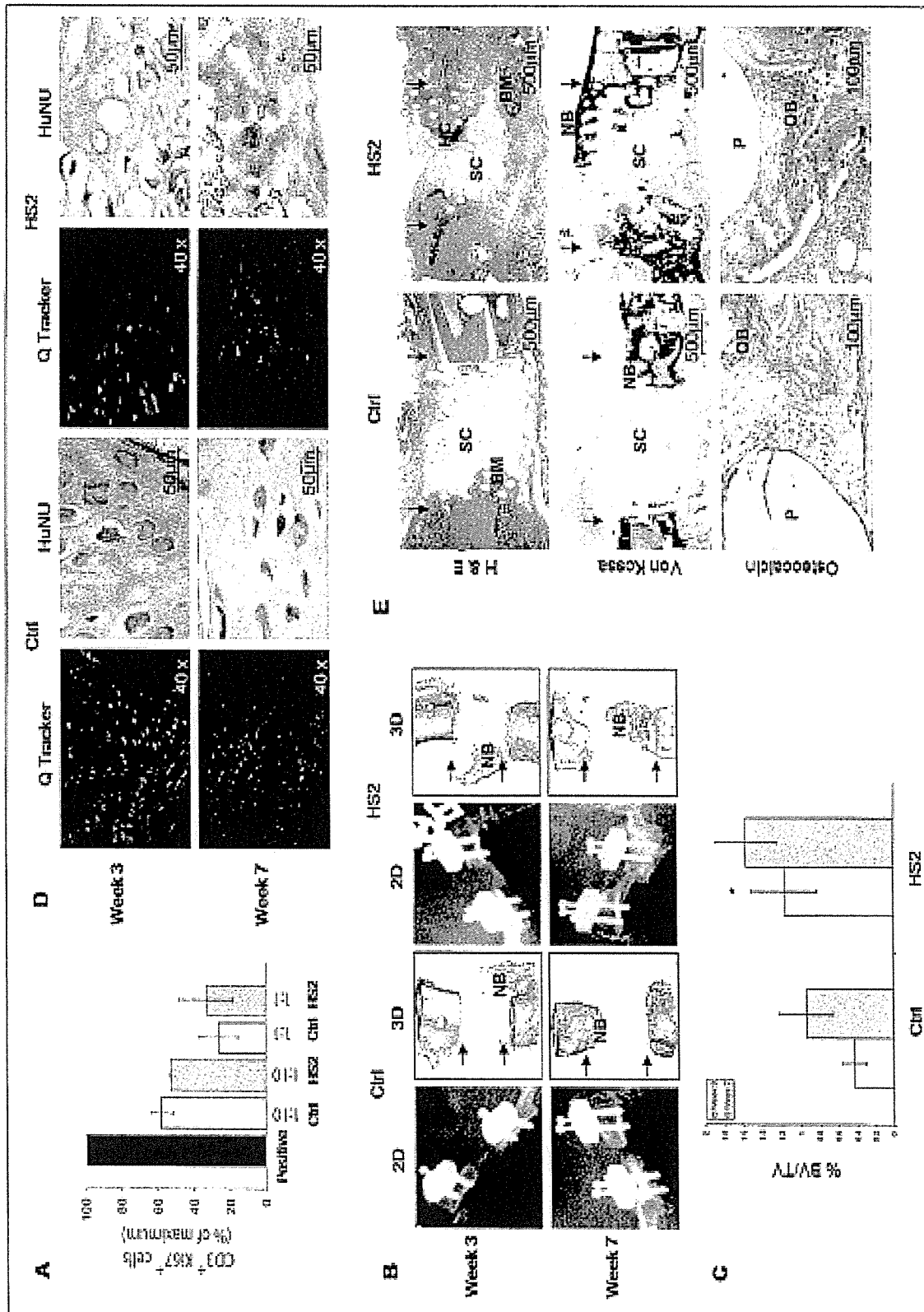


Figure 20

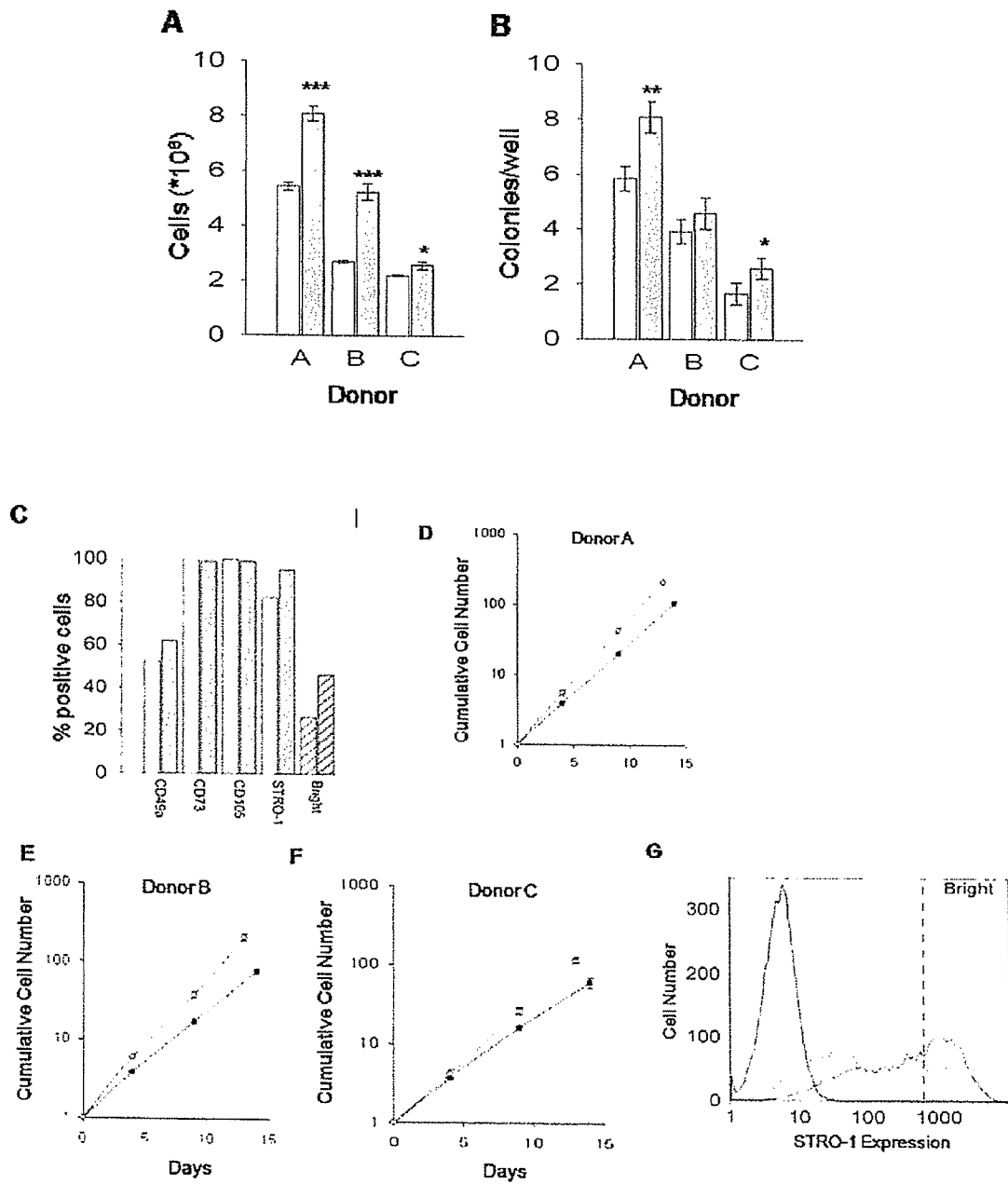


Figure 21

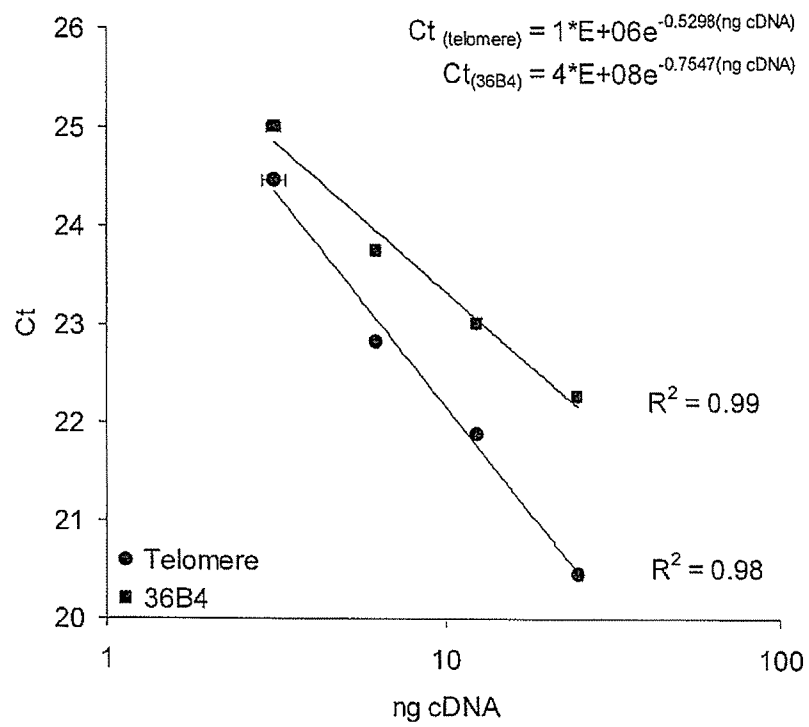
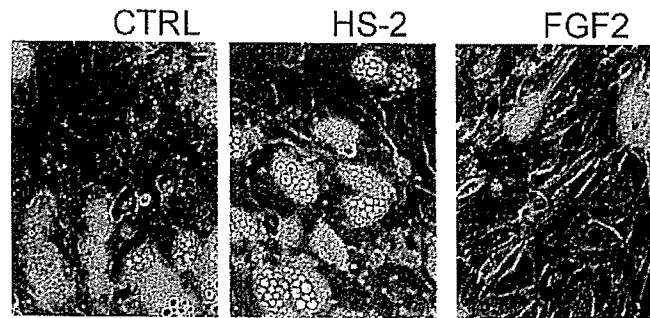


Figure 22

A)



B)

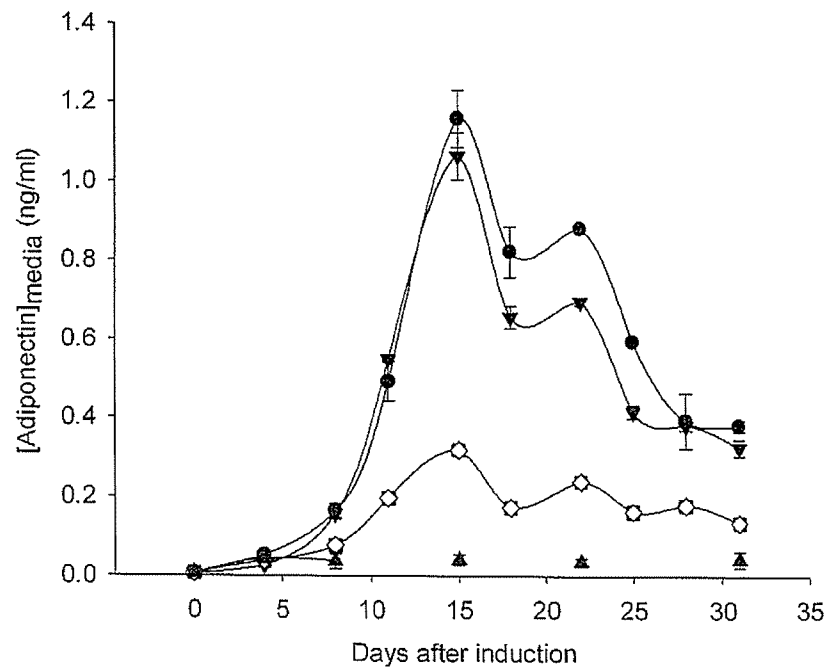
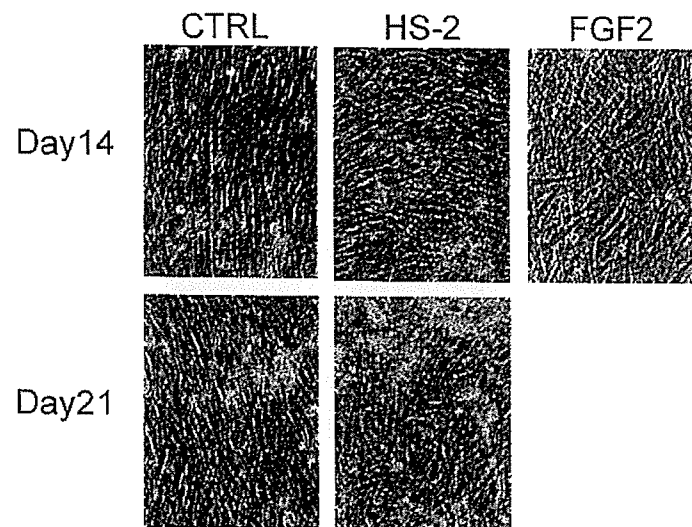


Figure 23

A)



B)

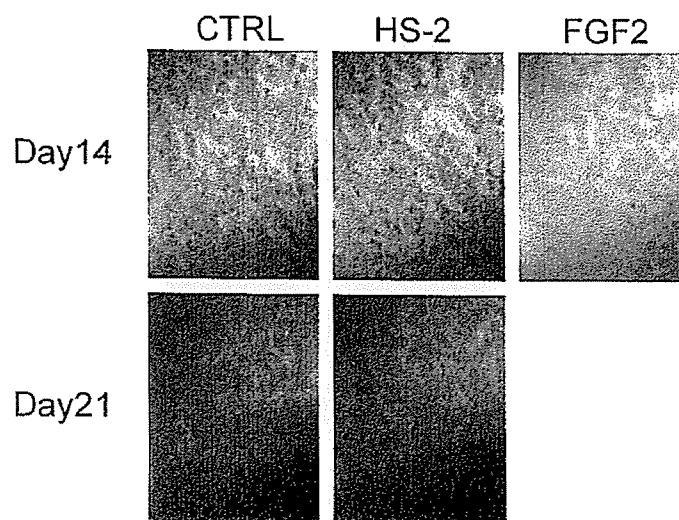


Figure 24

Gene	Primers	Probe	ACC number
18S	F: TTCGAGGCCCTGTAATTGGA R: GCAGCAACTTTAATATACGCTATTGG	AGTCCACTTTAAATCCTT	AY248756
AlkPhos	F: ATGCCCTGGAGCTTCAGAAG R: TGGTGGAGCTGACCCTTGAG	acgtTGgCtAaGaAtGtCaTc	NM_000478
BSPII	F: AGAGGAAGCAATCACCAAAATGA R: TTGAGAAAGCACAGGCCATTC	ctgCtTTaaTtTtgCtCagc	NM_004967
ALBP	F: GGAAAGTCAAGAGCACCATAACCT R: TTCCACCACCAGTTTATCATCCT	aaatCaAcCaCcaTaAaGaGa	BC003672.1
C/EBP α	F: GAGGGACCGGAGTTATGACAAG R: GGCACAGAGGCCAGATACAAG	aataTtTtGcTtTatCaGcCgat	NM_004364.2
Coll2a	F: GTACTTTCCAATCTCAGTCACTCTAGGA R: TTTTATTTTGCAGTCTGCCAGTT	ccCcTcTtTtCTaAgaga	NM_033150
SOX9	F: AAAGGCAACTCGTACCCAAATTT R: AGTGGGTAATGCGCTTGGAT	caAgaCaCaAaCAtgAcc	Z46629

Figure 25

M vs A Plot

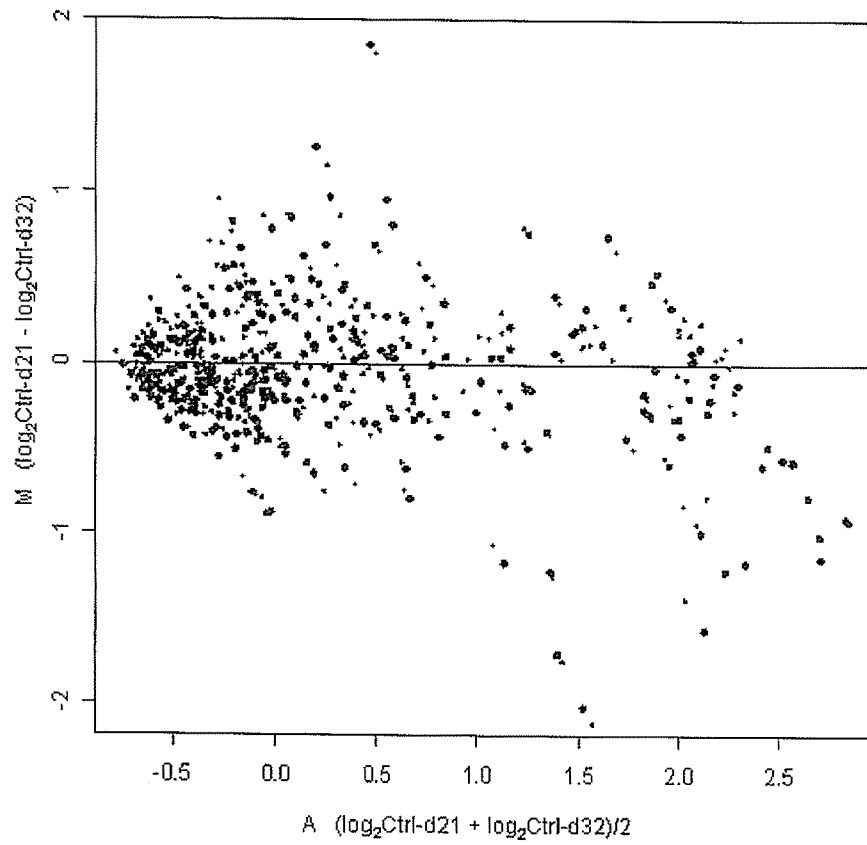


Figure 26

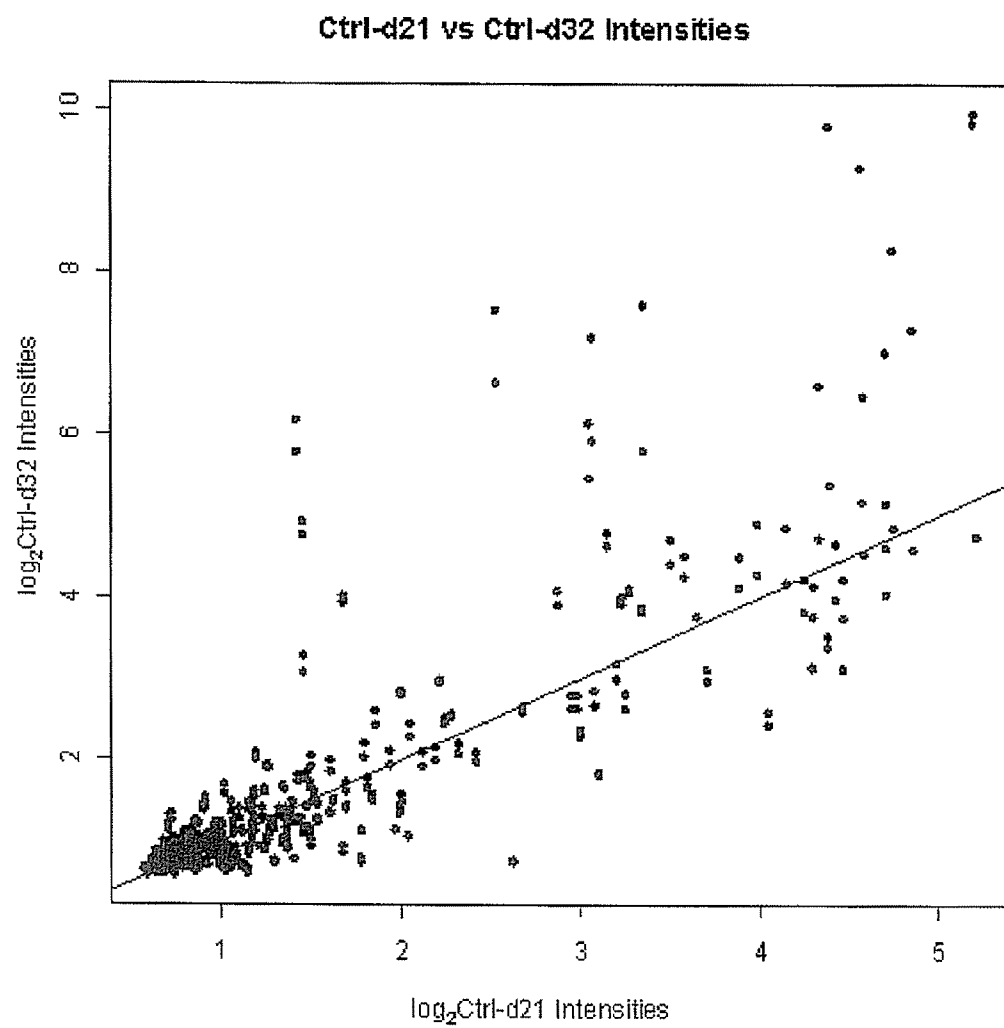


Figure 27

Table S2	No.	Unigene	RefSeq #	Symbol	Description
Cluster 1	1	Hs.522891	NM_000609	CXCL12	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
	2	Hs.471119	NM_001204	BMPR2	Bone morphogenetic protein receptor, type II (serine/threonine kinase)
	3	Hs.453951	NM_013957	NRG1	Neuregulin 1
	4	Hs.429052	NM_002211	ITGB1	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
	5	Hs.420269	NM_001849	COL6A2	Collagen, type VI, alpha 2
	6	Hs.369849	NM_002392	MDM2	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)
Table S2	No.	Unigene	RefSeq #	Symbol	Description
Cluster 1	7	Hs.470316	NM_001105	ACVR1	Activin A receptor, type I
	8	Hs.513609	NM_005611	RBL2	Retinoblastoma-like 2 (p130)
	9	Hs.74615	NM_006206	PDGFRA	Platelet-derived growth factor receptor, alpha polypeptide
	10	Hs.502328	NM_000610	CD44	CD44 antigen (homing function and Indian blood group system)
	11	Hs.28792	NM_002192	INHBA	Inhibin, beta A (activin A, activin AB alpha polypeptide)
	12	Hs.500483	NM_001613	ACTA2	Actin, alpha 2, smooth muscle, aorta
	13	Hs.467824	NM_015317	PUM2	Pumilio homolog 2 (Drosophila)
	14	Hs.436873	NM_002210	ITGAV	Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
	15	Hs.532082	NM_002184	IL6ST	Interleukin 6 signal transducer (gp130, oncostatin M receptor)
Cluster 2	16	Hs.516105	NM_001615	ACTG2	Actin, gamma 2, smooth muscle, enteric
	17	Hs.95577	NM_000075	CDK4	Cyclin-dependent kinase 4
	18	Hs.201671	NM_005686	SOX13	SRY (sex determining region Y)-box 13
	19	Hs.521461	NM_006158	NEFL	Neurofilament, light polypeptide 68kDa
	20	Hs.238990	NM_004064	CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)
	21	Hs.73793	NM_003376	VEGF	Vascular endothelial growth factor
	22	Hs.244723	NM_001238	CCNE1	Cyclin E1
	23	Hs.512234	NM_000600	IL6	Interleukin 6 (interferon, beta 2)
	24	Hs.527971	NM_006617	NES	Nestin
	25	Hs.376032	NM_002607	PDGFA	Platelet-derived growth factor alpha polypeptide

Figure 28

	26	Hs.125331	XM_291161	Dppa5	Similar to developmental pluripotency associated 5; embryonal stem cell specific gene 1
	27	Hs.482390	NM_003243	TGFB3	Transforming growth factor, beta receptor III (betaglycan, 300kDa)
	28	Hs.37092	NM_005247	FGF3	Fibroblast growth factor 3 (murine mammary tumor virus integration site (v-int-2) oncogene homolog)
Cluster 3	29	Hs.370414	NM_018055	NODAL	Nodal homolog (mouse)
	30	Hs.408528	NM_000321	RB1	Retinoblastoma 1 (including osteosarcoma)
	31	Hs.46366	NM_000614	CNTF	Ciliary neurotrophic factor
	32	Hs.133421	NM_002310	LIFR	Leukemia inhibitory factor receptor
	33	Hs.95582	NM_006942	SOX15	SRY (sex determining region Y)-box 15

Figure 28 cont...

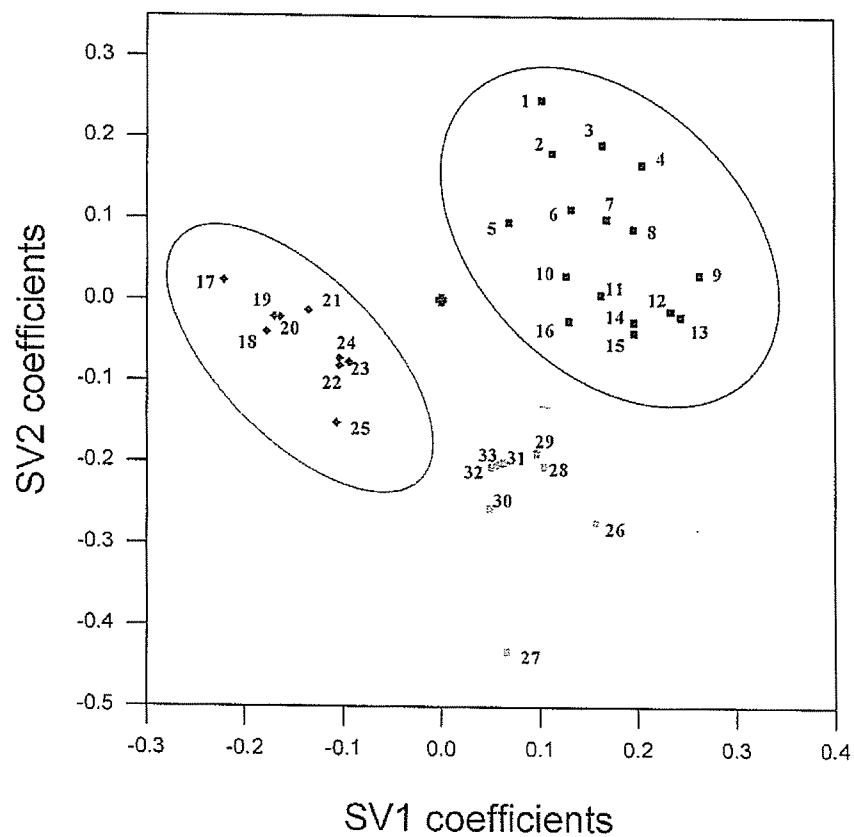


Figure 29

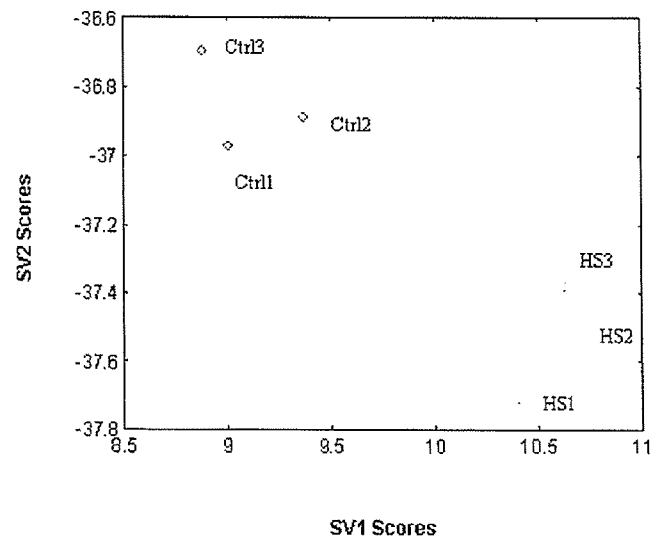


Figure 30

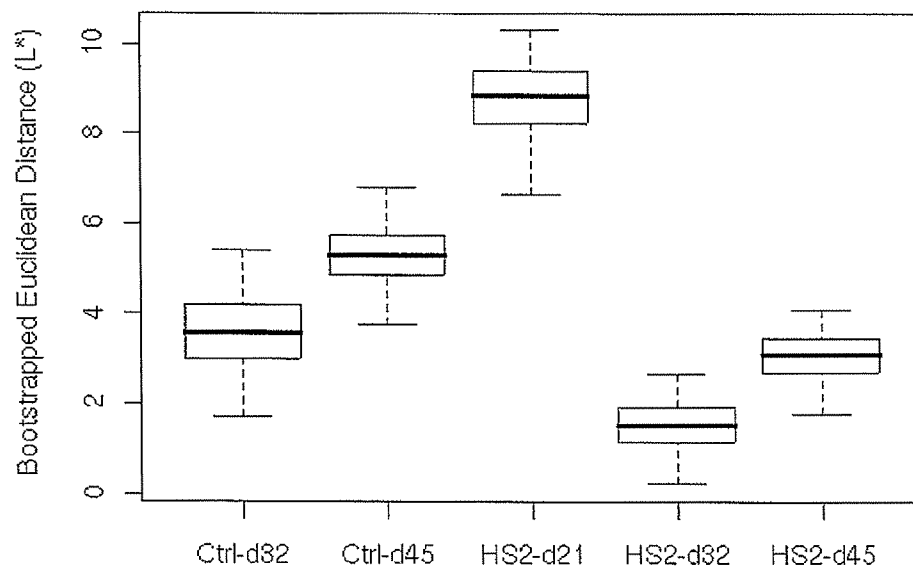


Figure 31

Table S3	Estimate () ^a	Lower CL ^b	Upper CL ^c
Ctrl-d45 vs Ctrl-d21	3.704	3.018	4.416
Ctrl-d45 vs Ctrl-d21	5.373	4.976	5.744
HS-2-d21 vs Ctrl-d21	8.761	7.921	9.653
HS-2-d32 vs Ctrl-d21	1.57	1.28	1.844
HS-2-d45 vs Ctrl-d21	3.008	2.758	3.328

Figure 32