

## **Supplemental Figures and Tables**

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Line and subline name	Method used to generate subline	Passage analyzed	% nuclei with H3K27me3 foci
HSF-6	Start	57	40%
HSF-6 (1)	Colony pick	61	70%
HSF-6 (2)	Colony pick	61	30%
HSF-6 (3)	Colony pick	61	30%
HSF-6 (4)	Colony pick	61	70%
HSF-6 (5)	Colony pick	61	50%
HSF-6 (6)	Colony pick	61	50%
HSF-6 (7)	Colony pick	61	95%
HSF-6 (8)	Colony pick	61	95%
HSF-6 (9)	Colony pick	61	50%
HSF-6 (10)	Colony pick	61	95%
HSF-6 (S6)	Single cell	64	0%
HSF-6 (S7)	Single cell	64	0%
HSF-6 (S8)	Single cell	64	0%
HSF-6 (S9)	Single cell	64	0%
H9	Start	37	36%
H9 (1)	Colony pick	41	0%
H9 (2)	Colony pick	41	0%
H9 (3)	Colony pick	41	0%
H9 (4)	Colony pick	41	0%
H9 (5)	Colony pick	41	50%
H9 (6)	Colony pick	41	50%
H9 (7)	Colony pick	41	0%
H9 (8)	Colony pick	41	0%
H9 (9)	Colony pick	41	0%
H9 (10)	Colony pick	41	0%
HSF-6	Regular passaging	61	0%
H9	Regular passaging	41	0%

Supplemental Table 1: Percentage of nuclei in HSF-6 and H9 lines and sublines containing a single focal dot of H3K27me3.

### **Supplemental Figure Legends:**

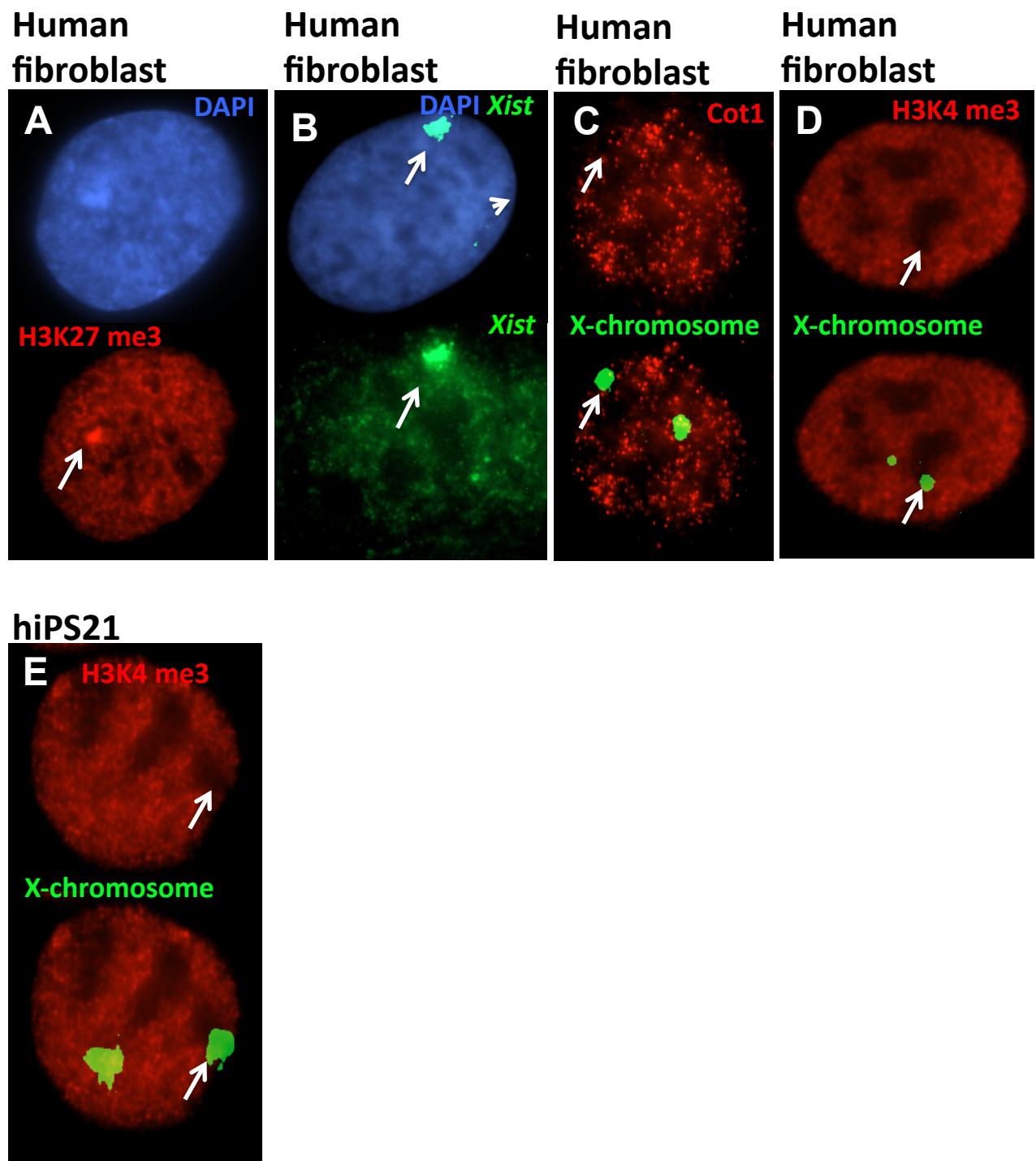
**Supplemental Figure 1:** Comparison of Cot 1/X chromosome FISH and H3K27me3/X chromosome FISH techniques for identifying XCI in female fibroblasts and hIPS21. **(A)** Female fibroblast nuclei stained with H3K27me3 and DAPI. Arrow indicates focal dot identified in n=328/328 nuclei **(B)** Female fibroblast demonstrating a single *XIST* focal dot in a DAPI stained nucleus indicating *XIST*-dependent XCI. **(C)** Cot1/X chromosome FISH of female fibroblasts, where one X chromosome is localized to a nuclear exclusion zone in n=18/18 nuclei (arrow). **(D)** H3K4me3/X chromosome FISH technique of female fibroblasts, one X chromosome localized to a nuclear exclusion zone in n=30/30 nuclei (arrow). **(E)** H3K4me3/X chromosome FISH of hIPS21 which was previously reported as exhibiting *XIST*-independent XCI in the self-renewing state shows one X chromosome to a nuclear exclusion zone (n=27 nuclei analyzed).

**Supplemental Figure 2:** Teratoma analysis in class III lines. **(A)** Teratomas were generated by transplantation into the testicles of immunocompromized mice. n= number of testicles that were transplanted. **(B)**. Representative image of endoderm, mesoderm and ectodermal lineages (arrow) identified in each of the tumors by histology.

**Supplemental Figure 3:** Karyotype and Flow cytometry of new UCLA hESC lines. **(A)** Karyotype was performed by Cell Line Genetics at the passage (p) indicated for all six hESC lines. **(B)** Flow cytometry was performed to evaluate TRA-1-81 and SSEA4 positive cells in all six hESC lines at the passage (p) indicated.

**Supplemental Figure 4:** Teratomas were generated from the six new UCLA lines as indicated. All lines generated teratomas with evidence of ectoderm, mesoderm and endoderm as shown in the representative picture. In UCLA5 n=4 replicates generated tumors, however n=2/4 developed cystic tumors with no solid regions for diagnosis of teratoma and n=2/4 developed solid tumors where representative lineages from ectoderm, endoderm and mesoderm could be identified. n=number of testicles transplanted.

**Supplemental Figure 5:** Images of the human blastocysts used to generate UCLA1-6 hESC lines. Images were taken within 24 hours of plating on MEFs



Supplemental figure 1

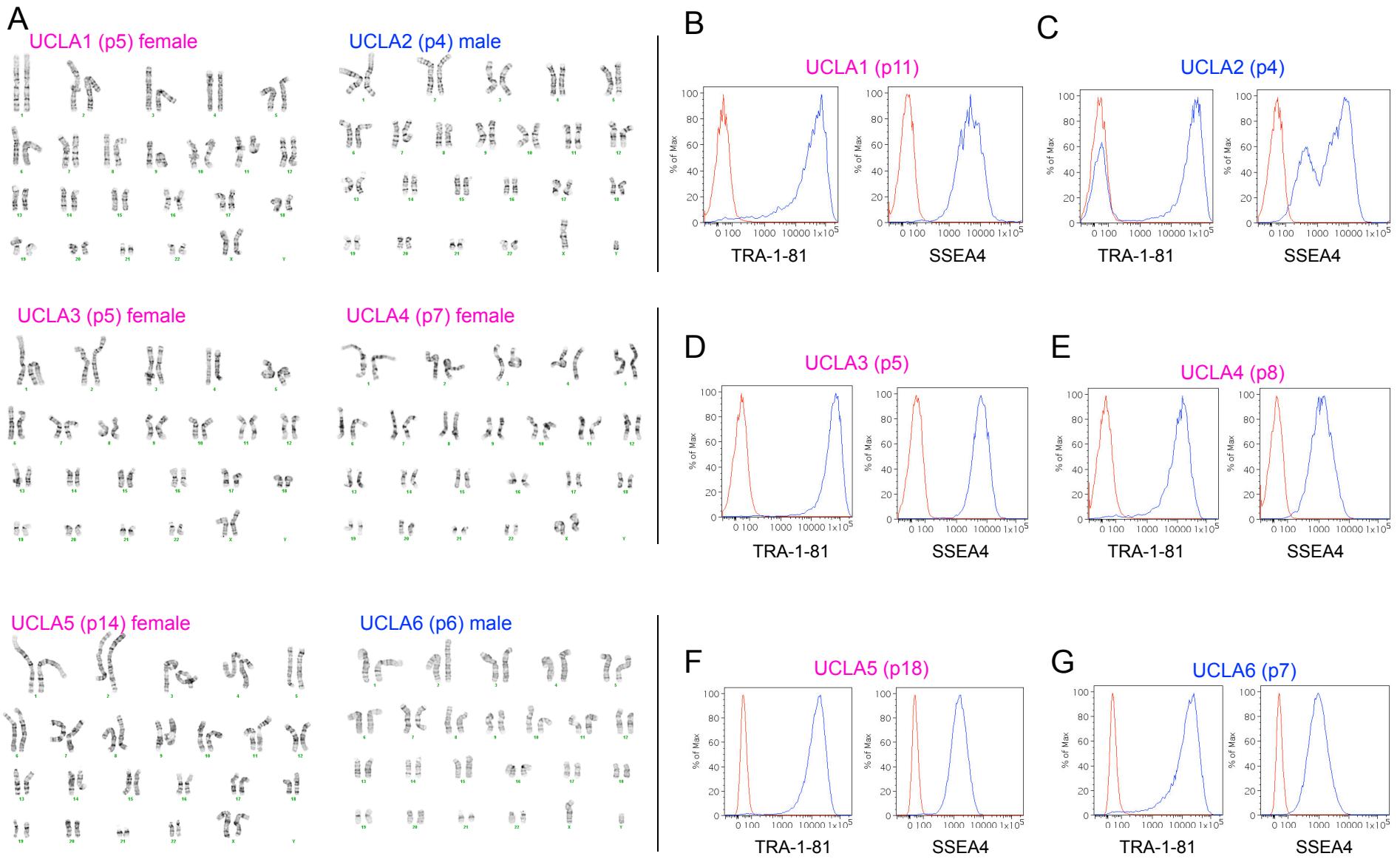
**A**

Line and Subline name	Differentiation <i>in vivo</i>	Result
HSF-6 (S7)	SCID-Beige testicles	4/4 teratomas
HSF-6 (S8)	SCID-Beige testicles	4/4 teratomas
HSF-6 (S9)	SCID-Beige testicles	4/4 teratomas
HSF-6	SCID-Beige testicles	4/4 teratomas
H9 (7)	SCID-Beige testicles	4/4 teratomas
H9 (8)	SCID-Beige testicles	4/4 teratomas
H9 (9)	SCID-Beige testicles	4/4 teratomas
H9	SCID-Beige testicles	4/4 teratomas

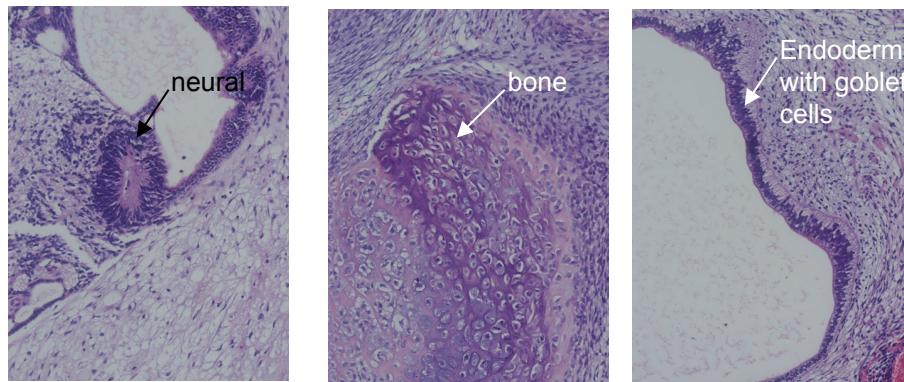
Table: Teratomas generated from class III hESC lines and sublines

**B**

Supplemental Figure 2



Supplemental Figure 3



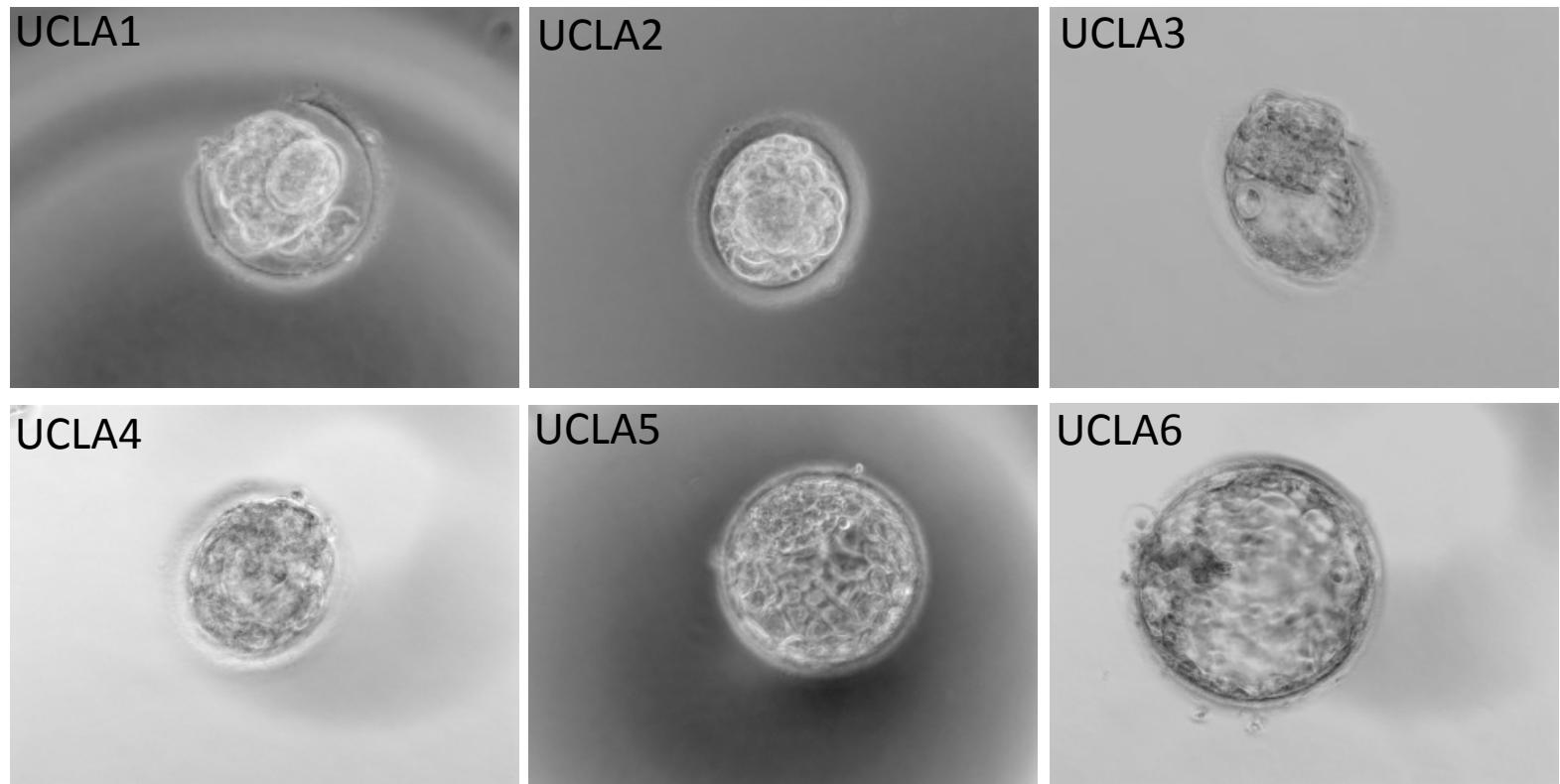
Ectoderm

Mesoderm

Endoderm

<b>Cell Line</b>	<b>Positive</b>	<b>Positive</b>	<b>Positive</b>
UCLA1	n=4	n=4	n=4
UCLA2	n=2	n=2	n=2
UCLA3	n=2	n=2	n=2
UCLA4	n=2	n=2	n=2
UCLA5*	n=2/4	n=2/4	n=2/4
UCLA6	n=4	n=4	n=4

Supplemental Figure 4



Supplemental Figure 5



218208_at	PQLC1: PQ loop repeat containing 1	NM_025078	80148 g13376629	1.8	5.394	0.005714
224593_at	ZNF646: zinc finger protein 646	BE965646	144268 Hs.61976.0	1.8	5.557	0.009259
205347_s_at	TMSL8: thymosin-like 8	NM_021992	11013 g11496272	1.77	6.235	0.003572
210512_s_at	VEGFA: vascular endothelial growth factor A	AF022375	7422 g3719220	1.77	4.947	0.007883
201727_s_at	ELAVL1: ELAV (embryonic lethal, abnormal vision, Drosophila)-like 1 (Hu antigen R)	NM_001419	1994 g4503550	1.76	5.822	0.004335
226868_at	GLT8D3: glycosyltransferase 8 domain containing 3	BF977231	283464 Hs.173044.0	1.75	7.52	0.002678
201200_at	CREG1: cellular repressor of E1A-stimulated genes 1	NM_003851	8804 g4503036	1.74	6.086	0.004667
201650_at	KRT19: keratin 19	NM_002276	3880 g4504916	1.74	6.95	0.003423
202815_s_at	HEXIM1: hexamethylene bis-acetamide inducible 1	NM_006460	10614 g5453681	1.74	8.447	0.001632
207551_s_at	MSL3L1: male-specific lethal 3-like 1 (Drosophila)	NM_006800	10943 g5803103	1.74	5.316	0.006178
212195_at	IL6ST: interleukin 6 signal transducer (gp130, oncostatin M receptor)	AL049265	3572 Hs.71968.0	1.73	6.903	0.002358
226786_at	RFX1: regulatory factor X, 1 (influences HLA class II expression)	BF507952	5989 Hs.73677.0	1.73	9.595	0.001692
213135_at	TIAM1: T-cell lymphoma invasion and metastasis 1	U90902	7074 Hs.82141.0	1.72	6.625	0.00273
202083_s_at	SEC14L1: SEC14-like 1 (S. cerevisiae)	A1017770	6397 Hs.75232.0	1.71	5.312	0.007319
202743_at	PIK3R3: phosphoinositide-3-kinase, regulatory subunit 3 (p55, gamma)	BE622627	8503 Hs.88051.0	1.71	6.49	0.003045
41387_r_at	JMD3: jumonji domain containing 3	AB002344	23135 g4861272_RC	1.7	5.309	0.006103
204944_at	PTPRG: protein tyrosine phosphatase, receptor type, G	NM_002841	5793 g11386168	1.69	4.802	0.009016
213146_at	JMD3: jumonji domain containing 3	AA521267	23135 Hs.103915.0	1.69	6.114	0.004094
202931_x_at	BIN1: bridging integrator 1	NM_004305	274 g4757747	1.68	5.437	0.005553
225543_at	GTF3C4: general transcription factor IIIc, polypeptide 4, 90kDa	BF434224	9329 Hs.21734.0	1.68	5.626	0.006163
225864_at	FAM84B: family with sequence similarity 84, member B	AL039862	157638 Hs.49136.0	1.68	12.45	0.000254
235520_at	SUHW3: suppressor of hairy wing homolog 3 (Drosophila)	AA805654	55609 Hs.121668.0	1.68	11.296	0.000933
212980_at	USP34: ubiquitin specific peptidase 34	AL050376	9736 Hs.322645.0	1.66	4.865	0.009106
226713_at	CCDC50: coiled-coil domain containing 50	AI247881	152137 Hs.10198.0	1.66	5.692	0.005566
211107_s_at	AURKC: aurora kinase C	AB017332	6795 g4521232	1.64	5.219	0.00644
202447_at	DECRL1: 2,4-dienoyl CoA reductase 1, mitochondrial	NM_001359	1666 g450300	1.63	6.51	0.005614
226715_at	FOXP1: forkhead box K1	AW007319	221937 Hs.11556.0	1.63	7.096	0.005404
227559_at	Hs.5232.1	AL521129	Hs.5232.1	1.63	5.121	0.009701
223043_at	TMEM85: transmembrane protein 85	AF151018	51234 g7106757	1.62	9.041	0.000883
200720_s_at	ACTR1A: ARP1 actin-related protein 1 homolog A, centracin alpha (yeast)	AL532341	10121 Hs.153961.0	1.61	6.856	0.002893
218913_s_at	GMIP: GEM interacting protein	NM_016573	51291 g7706106	1.61	5.549	0.007898
224374_s_at	EMILIN2: elastin microfibril interfacer 2	AF270513	84034 g13661555	1.61	6.103	0.00419
225617_at	ODF2: outer dense fiber of sperm tails 2	AL138382	4957 Hs.129055.2	1.61	6.197	0.003449
41386_i_at	JMD3: jumonji domain containing 3	AB002344	23135 g4861272_RC	1.6	7.018	0.002316
208112_x_at	EHD1: EH-domain containing 1	NM_006795	10938 g5803008	1.59	6.513	0.002872
211980_at	COL4A1: collagen, type IV, alpha 1	AI922605	1282 Hs.119129.0	1.59	5.587	0.005114
223547_at	C1orf100: chromosome 14 open reading frame 100	AL038769	51528 Hs.170219.0	1.58	8.087	0.001305
212484_at	FAM89B: family with sequence similarity 89, member B	BF974389	23625 Hs.18686.0	1.57	6.076	0.004273
221954_at	C2orf111: Chromosome 20 open reading frame 111	A1A60474	51526 Hs.75798.1	1.57	8.226	0.005339
1568629_s_at	PIK3R2: phosphoinositide-3-kinase, regulatory subunit 2 (p85 beta)	BC033311	5296 Hs.2390523.1	1.57	6.565	0.003144
205823_at	RGS12: regulator of G-protein signaling 12	AI824113	6002 Hs.78281.0	1.56	6.326	0.005412
238041_at	Transcribed locus	AA151712	Hs.82572.0	1.56	6.035	0.007326
203329_at	PTPRM: protein tyrosine phosphatase, receptor type, M	NM_002845	5797 g4506318	1.55	6.983	0.002561
203943_at	KIF3B: kinesin family member 3B	NM_004798	9371 g4758645	1.54	5.075	0.007109
209039_x_at	EHD1: EH-domain containing 1	AF001434	10938 g2529706	1.54	6.304	0.003255
218853_s_at	MOSPD1: motile sperm domain containing 1	NM_019556	56180 g9506542	1.54	5.186	0.00701
224996_at	CDNA FLJ39064 f1, clone NT2RP7014583	N30209	Hs.332422.0	1.54	5.407	0.0059
205406_s_at	SPA17: sperm autoantigenic protein 17	NM_017425	53340 g8394342	1.53	5.112	0.006931
222221_x_at	EHD1: EH-domain containing 1	AY007161	10938 Hs.155119.2	1.53	7.118	0.002215
205002_at	AHDC1: AT hook, DNA binding motif, containing 1	NM_015699	27245 g7661559	1.52	5.419	0.007801
218699_at	RAB7L1: RAB7, member RAS oncogene family-like 1	BG338251	8934 Hs.115325.0	1.52	5.718	0.005165
226077_at	RNF145: ring finger protein 145	AL553942	153830 Hs.104893.0	1.52	12.745	0.000303
212905_at	CSTF2T: cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa, tau variant	AI872408	23283 Hs.21929.1	1.5	5.179	0.008063
227373_at	ATXN1L: ataxin 1-like	AW299653	342371 Hs.20787.0	1.5	5.923	0.004382
227656_at	C6orf70: chromosome 6 open reading frame 70	AW968493	55780 Hs.47546.0	1.5	5.761	0.004509
202005_at	ST14: suppression of tumorigenicity 14 (colon carcinoma)	NM_021978	6768 g11415039	-1.58	-5.331	0.007794
220615_s_at	MLSTD1: male sterility domain containing 1	NM_018099	55711 g8922433	-1.63	-6.496	0.003841
209615_s_at	PAK1: p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)	U51120	5058 g1256421	-1.67	-14.176	0.000237
213010_at	PRKCD8P: protein kinase C, delta binding protein	AI088622	112464 Hs.85181.1	-1.7	-5.468	0.007638
219073_s_at	OSBP10: oxysterol binding protein-like 10	NM_017784	114884 g8923336	-1.71	-5.375	0.006349
205229_s_at	COCH: coagulation factor C homolog, cochlin (Limulus polyphemus)	AA669336	1690 Hs.21016.0	-1.77	-8.122	0.001592
201058_s_at	MYL9: myosin, light chain 9, regulatory	NM_006097	10398 g5174602	-1.83	-6.453	0.00347
210999_s_at	GRB10: growth factor receptor-bound protein 10	U66065	2887 g1518100	-1.83	-10.351	0.00074
226370_at	KLHL15: kelch-like 15 (Drosophila)	BG149487	80311 Hs.61603.0	-1.89	-6.302	0.004331
226913_s_at	SOX8: SRY (sex determining region Y)-box 8	BF527050	30812 Hs.243678.0	-1.98	-5.549	0.005264
216905_s_at	ST14: suppression of tumorigenicity 14 (colon carcinoma)	U20428	6768 Hs.56937.1	-2	-7.114	0.005012
1554242_a_at	COCH: coagulation factor C homolog, cochlin (Limulus polyphemus)	BC007230	1690 Hs.21016.2	-2.02	-7.047	0.002937
232553_at	PCYT1B: phosphate cytidylyltransferase 1, choline, beta	AI857508	9468 Hs.214282.0	-2.08	-4.915	0.008033
219032_x_at	OPN3: opsin 3 (encephalopsin, panopsin)	NM_014322	23596 g7657070	-2.1	-7.58	0.00307
235649_at	ADAMTS8: ADAM metallopeptidase with thrombospondin type 1 motif, 8	AW207389	11095 Hs.126758.0	-2.25	-7.094	0.002162
227846_at	GPR176: G protein-coupled receptor 176	AA526584	11245 Hs.55144.0	-2.38	-8.879	0.003939
226863_at	FAM110C: family with sequence similarity 110, member C	AI674565	642273 Hs.8379.0	-2.42	-9.89	0.00063
206673_at	GPR176: G protein-coupled receptor 176	NM_007223	11245 g6005771	-2.43	-6.91	0.002324
1554887_at	Hs.2375106.1	BC032716	Hs.2375106.1	-2.66	-8.714	0.001211
209496_at	RARRES2: retinoic acid receptor responder (tazarotene induced) 2	BC000069	5919 g12652642	-2.85	-9.965	0.001791
202771_at	FAM38A: family with sequence similarity 38, member A	NM_014745	9780 g7662013	-2.91	-9.669	0.001187
222033_s_at	FLT1: Fms-related tyrosine kinase 1 (vascular endothelial growth factor/vascular perfrillin AA058828	2321 Hs.138671.2	-2.94	-11.662	0.000765	
216915_s_at	PTPN12: protein tyrosine phosphatase, non-receptor type 12	S69182	5782 Hs.62.1	-5.43	-6.742	0.005342

\$ 170 genes satisfied the comparison filtering criteria