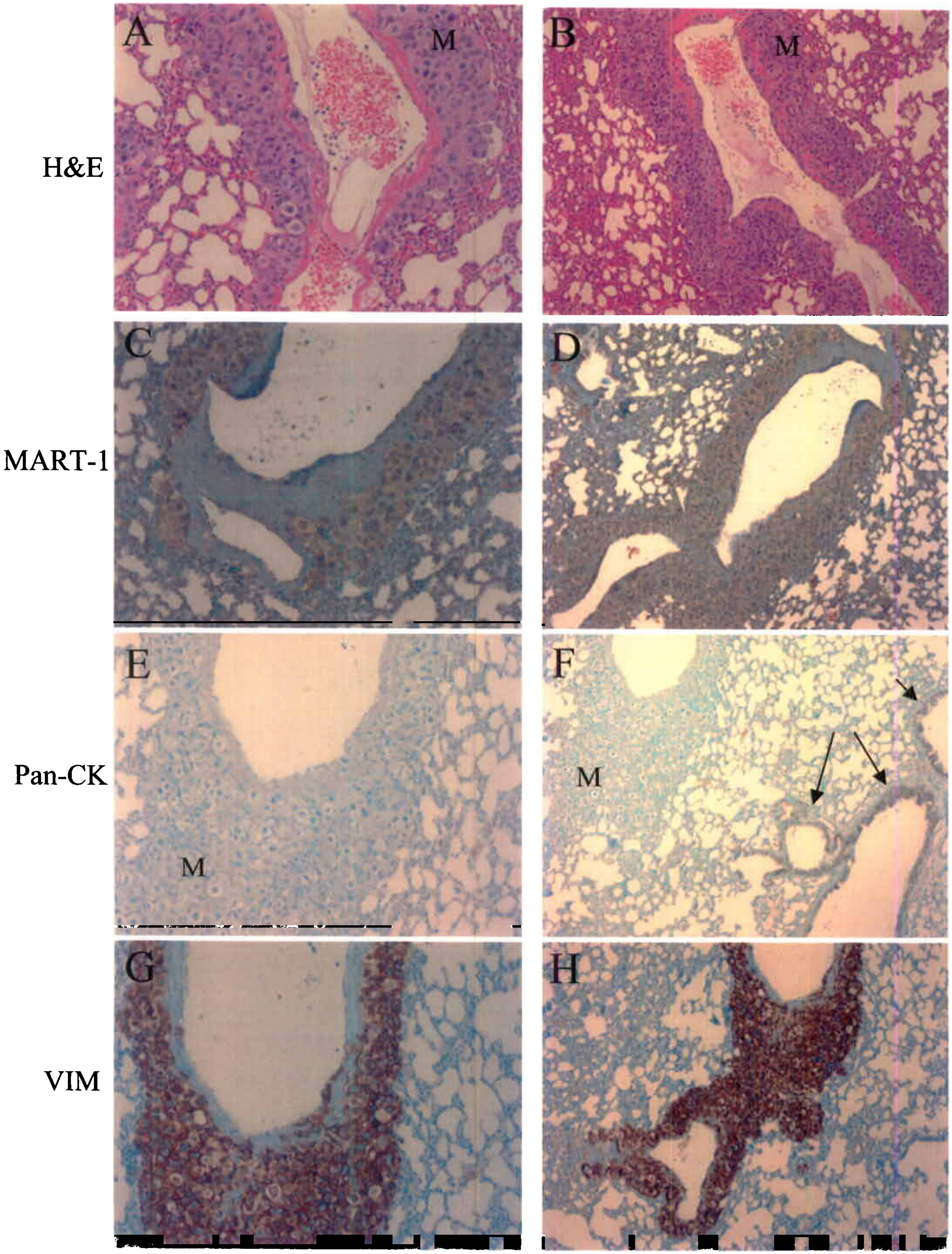

Figure S1

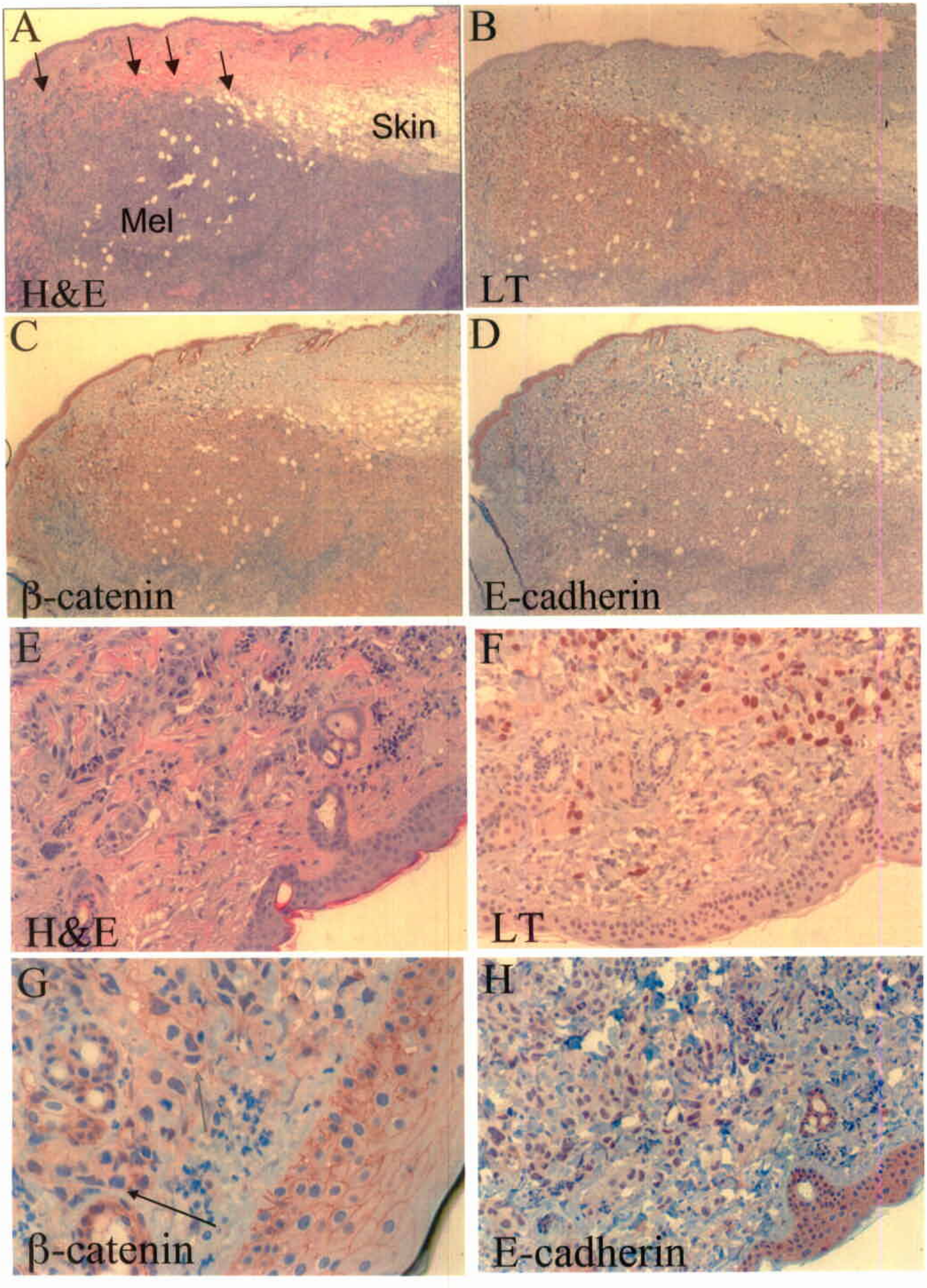
A
Skin
Mel
H&E
B
LT
C
β-catenin
D
E-cadherin
E
H&E
F
LT
G
β-catenin
H
E-cadherin

Figure S2

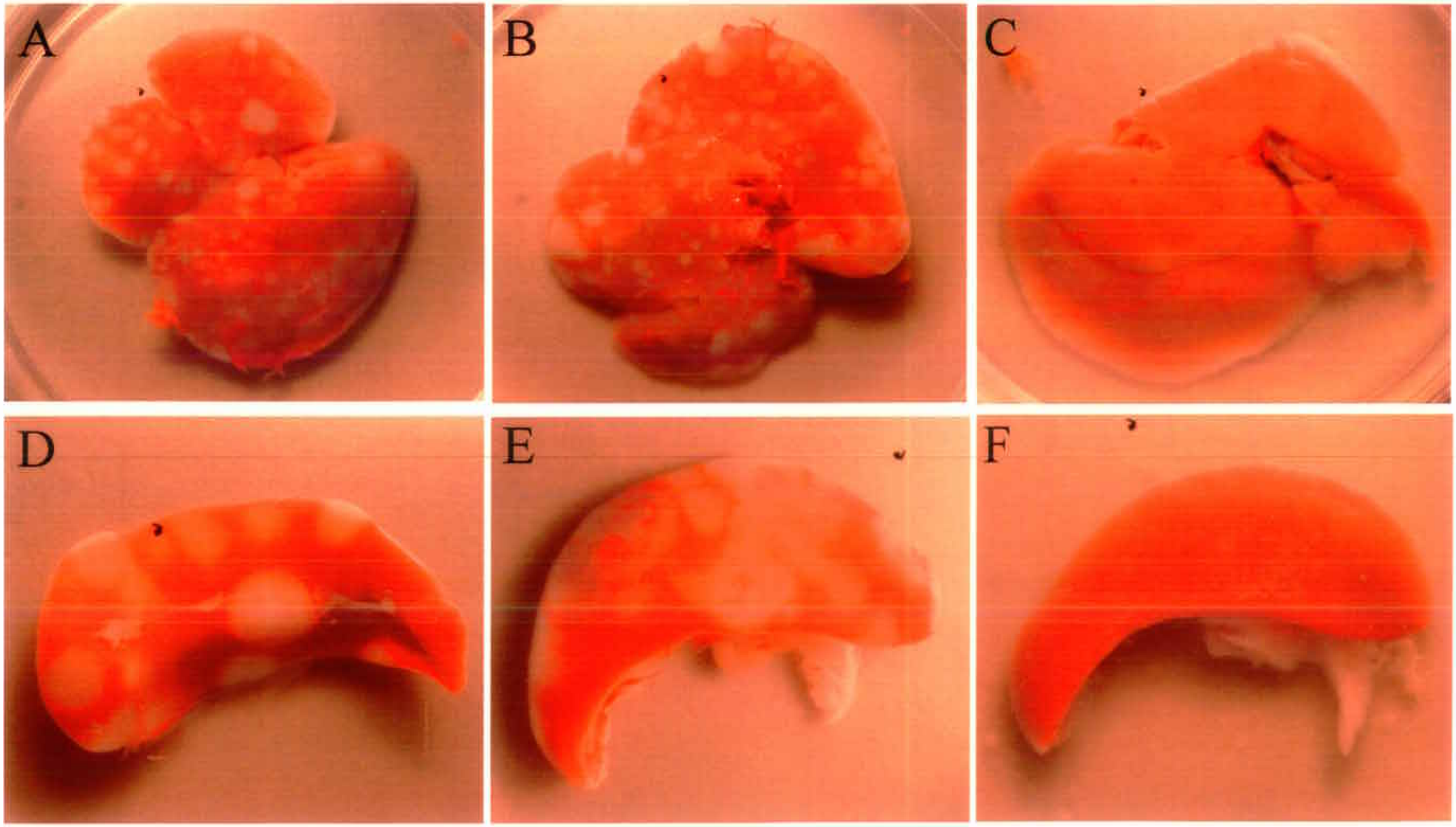

Figure S3

Supplementary Table 1 Genes significantly correlated in their expression patterns with Slug in human nevus samples (p<0.05).

Genes significantly correlated with SLUGH in a dataset of 9 nevus samples

Probe	Name	Correlation	p-value
213139_at	slug homolog, zinc finger protein (chicken)	1	0
200984_s_at	CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3A5, EJ16, EJ30, EL32 and G344)	0.986642	0
211971_s_at	leucine-rich PPR-motif containing	0.984621	0
222171_s_at	PBX/knotted 1 homeobox 2	0.979982	0
204671_s_at	ankyrin repeat domain 6	0.96746	0.002
213122_at	KIAA1750 protein	0.962889	0.002
221524_s_at	Rag D protein	0.950736	0.002
201735_s_at	chloride channel 3	0.949528	0.002
204489_s_at	CD44 antigen (homing function and Indian blood group system)	0.958454	0.004
210357_s_at	hypothetical protein	0.953514	0.004
207040_s_at	suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)	0.950795	0.004
201178_at	F-box only protein 7	0.946218	0.004
204271_s_at	endothelin receptor type B	0.945601	0.004
212018_s_at	DKFZP564M182 protein	0.943323	0.004
200811_at	cold inducible RNA binding protein	0.951932	0.005
202454_s_at	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	0.928209	0.005
208717_at	oxidase (cytochrome c) assembly 1-like	0.928969	0.006
31835_at	histidine-rich glycoprotein	0.926137	0.006
206701_x_at	endothelin receptor type B	0.925976	0.006
200005_at	eukaryotic translation initiation factor 3, subunit 7 (zeta, 66/67kD)	0.923322	0.006
208319_s_at	RNA binding motif protein 3	0.93197	0.007
213446_s_at	IQ motif containing GTPase activating protein 1	0.92619	0.007
217354_s_at	calcium channel, voltage-dependent, gamma subunit 2	0.92418	0.007
217530_at		0.921495	0.007
63305_at	PBX/knotted 1 homeobox 2	0.920606	0.007
212361_s_at	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	0.932176	0.008
213817_at		0.929063	0.008

219578_s_at	cytoplasmic polyadenylation element binding protein	0.92445	0.008
201635_s_at	fragile X mental retardation, autosomal homolog 1	0.930481	0.009
205535_s_at	BH-protocadherin (brain-heart)	0.926768	0.009
215945_s_at	tripartite motif-containing 2	0.91736	0.009
213854_at	synaptogyrin 1	0.935979	0.01
202475_at		0.935009	0.01
201734_at	chloride channel 3	0.929102	0.01
200972_at	tetraspan 3	0.918361	0.011
213725_x_at		0.917605	0.011
209185_s_at	insulin receptor substrate 2	0.916226	0.011
210470_x_at	non-POU-domain-containing, octamer-binding	0.912525	0.011
209118_s_at	tubulin, alpha 3	0.913627	0.012
218561_s_at	CGI-203 protein	0.908361	0.012
214046_at		0.914242	0.013
207236_at	zinc finger protein 345	0.913669	0.013
206913_at	bile acid Coenzyme A: amino acid N-acyltransferase (glycine N-choloyltransferase)	0.907673	0.013
202412_s_at	ubiquitin specific protease 1	0.932244	0.014
208931_s_at	interleukin enhancer binding factor 3, 90kD	0.911572	0.014
201982_s_at	pregnancy-associated plasma protein A	0.910563	0.014
203417_at	microfibrillar-associated protein 2	0.908614	0.014
58780_s_at	hypothetical protein FLJ10357	0.90837	0.014
203657_s_at	cathepsin F	0.909389	0.015
217886_at	epidermal growth factor receptor pathway substrate 15	0.932524	0.016
202993_at	ilvB (bacterial acetolactate synthase)-like	0.905077	0.016
201031_s_at	heterogeneous nuclear ribonucleoprotein H1 (H)	0.902419	0.016
205337_at		0.905207	0.017
212261_at	trinucleotide repeat containing 15	0.903697	0.017
206645_s_at	nuclear receptor subfamily 0, group B, member 1	0.903083	0.017
201416_at	SRY (sex determining region Y)-box 4	0.90129	0.018
209789_at	coronin, actin binding protein, 2B	0.900728	0.018
220474_at	solute carrier family 25 (mitochondrial oxodicarboxylate carrier), member 21	0.898865	0.018
207323_s_at	myelin basic protein	0.898588	0.018
204490_s_at	CD44 antigen (homing function and Indian blood group system)	0.897909	0.018
215266_at	hypothetical protein DKFZp434N074	0.894685	0.018

AFFX-BioB-5_at		0.899179	0.019
202975_s_at	KIAA0878 protein	0.895783	0.019
213564_x_at	lactate dehydrogenase B	0.893949	0.019
220193_at	hypothetical protein FLJ22938	0.893499	0.019
212746_s_at	KIAA0470 gene product	0.899723	0.02
204653_at	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)	0.896881	0.02
91920_at	chondroitin sulfate proteoglycan BEHAB/brevican	0.896098	0.02
203587_at	ADP-ribosylation factor 4-like	0.894923	0.02
211728_s_at	hyaluronoglucosaminidase 3	0.892751	0.02
212070_at	G protein-coupled receptor 56	0.891706	0.02
202976_s_at	KIAA0878 protein	0.891169	0.02
213047_x_at	SET translocation (myeloid leukemia-associated)	0.890923	0.02
201050_at	similar to vaccinia virus HindIII K4L ORF	0.892127	0.021
219107_at	chondroitin sulfate proteoglycan BEHAB/brevican	0.887016	0.026
214724_at	KIAA1735 protein	0.884387	0.027
205029_s_at	fatty acid binding protein 7, brain	0.883224	0.027
200985_s_at	CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3A5, EJ16, EJ30, EL32 and G344)	0.882335	0.027
209563_x_at	calmodulin 2 (phosphorylase kinase, delta)	0.887328	0.028
214730_s_at	Golgi apparatus protein 1	0.884493	0.028
214500_at	H2A histone family, member Y	0.882136	0.028
201417_at		0.887357	0.029
201046_s_at	RAD23 homolog A (S. cerevisiae)	0.884712	0.029
213900_at	Friedreich ataxia region gene X123	0.881244	0.029
220753_s_at	lambda-crystallin	0.88061	0.029
218211_s_at	melanophilin	0.880119	0.029
201101_s_at	KIAA0164 gene product	0.887742	0.031
201380_at	cartilage associated protein	0.885436	0.032
49111_at		0.88526	0.032
211937_at	eukaryotic translation initiation factor 4B	0.884939	0.032
213516_at	lymphoid blast crisis oncogene	0.878349	0.033
205372_at	pleiomorphic adenoma gene 1	0.878605	0.035
209184_s_at	insulin receptor substrate 2	0.877252	0.037
205188_s_at	MAD, mothers against decapentaplegic homolog 5 (Drosophila)	0.858837	0.037
200810_s_at	cold inducible RNA binding protein	0.858558	0.037
219046_s_at	PBX/knotted 1 homeobox 2	0.858392	0.037
203548_s_at	lipoprotein lipase	0.87599	0.038
212114_at		0.859012	0.038

217989_at	retinal short-chain dehydrogenase/reductase retSDR2	0.85127	0.038
202341_s_at	tripartite motif-containing 2	0.851217	0.038
219831_at	cyclin-dependent kinase-like 3	0.849561	0.038
220927_s_at	heparanase-like protein	0.849166	0.038
210493_s_at		0.848502	0.038
219376_at	hypothetical protein FLJ23393	0.874143	0.039
218316_at	translocase of inner mitochondrial membrane 9 homolog (yeast)	0.873713	0.039
209515_s_at	RAB27A, member RAS oncogene family	0.870522	0.039
211520_s_at	glutamate receptor, ionotropic, AMPA 1	0.870307	0.039
219114_at	g20 protein	0.864071	0.039
209199_s_at	MADS box transcription enhancer factor 2, polypeptide C (myocyte enhancer factor 2C)	0.864012	0.039
214739_at	hypothetical protein MGC4126	0.863715	0.039
204105_s_at	neuronal cell adhesion molecule	0.863197	0.039
213543_at		0.862624	0.039
215146_s_at	KIAA1043 protein	0.861997	0.039
220261_s_at	hypothetical protein FLJ10479	0.8618	0.039
205100_at	glutamine-fructose-6-phosphate transaminase 2	0.861232	0.039
211876_x_at	protocadherin gamma subfamily A, 11	0.859347	0.039
211576_s_at	solute carrier family 19 (folate transporter), member 1	0.859035	0.039
209672_s_at	hypothetical protein	0.854843	0.039
202990_at	phosphorylase, glycogen; liver (Hers disease, glycogen storage disease type VI)	0.852225	0.039
200701_at	Niemann-Pick disease, type C2 gene	0.851336	0.039
222145_at		0.850919	0.039
221823_at	hypothetical gene supported by AF038182; BC009203	0.850219	0.039
216512_s_at		0.849388	0.039
218841_at	hypothetical protein MGC5540	0.847755	0.039
201910_at	FERM, RhoGEF (ARHGEF) and pleckstrin domain protein 1 (chondrocyte-derived)	0.846595	0.039
214890_s_at	DKFZP564J102 protein	0.845768	0.039
216375_s_at	ets variant gene 5 (ets-related molecule)	0.845112	0.039
209878_s_at	v-rel reticuloendotheliosis viral oncogene homolog A, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, p65 (avian)	0.844985	0.039
207842_s_at	MLN51 protein	0.84498	0.039
218457_s_at	DNA (cytosine-5-)-methyltransferase 3 alpha	0.844665	0.039
210613_s_at	synaptogyrin 1	0.874532	0.04

200707_at	protein kinase C substrate 80K-H	0.864363	0.04
209859_at	tripartite motif-containing 9	0.86301	0.04
213452_at	zinc finger protein 184 (Kruppel-like)	0.855515	0.04
213591_at	aldehyde dehydrogenase 7 family, member A1	0.85515	0.04
207170_s_at	DKFZP586A011 protein	0.853579	0.04
213063_at		0.852748	0.04
212936_at		0.852449	0.04
203697_at	frizzled-related protein	0.846031	0.04
216112_at		0.845886	0.04
222297_x_at	ribosomal protein L18	0.844504	0.04
214464_at	Ser-Thr protein kinase related to the myotonic dystrophy protein kinase	0.844379	0.04
209162_s_at	PRP4/STK/WD splicing factor	0.840095	0.04
202356_s_at	general transcription factor IIF, polypeptide 1 (74kD subunit)	0.839953	0.04
208974_x_at	karyopherin (importin) beta 1	0.839859	0.04
214154_s_at	plakophilin 2	0.83907	0.04
205202_at	protein-L-isoaspartate (D-aspartate) O-methyltransferase	0.838986	0.04
203139_at	death-associated protein kinase 1	0.876579	0.041
203562_at	fasciculation and elongation protein zeta 1 (zygin I)	0.870968	0.041
210517_s_at	A kinase (PRKA) anchor protein (gravin) 12	0.870631	0.041
40640_at	arylsulfatase A	0.868854	0.041
203632_s_at	G protein-coupled receptor, family C, group 5, member B	0.865927	0.041
206479_at	melastatin 1	0.865683	0.041
217853_at	tumor endothelial marker 6	0.865437	0.041
213843_x_at	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	0.865317	0.041
212419_at		0.860383	0.041
218228_s_at	tankyrase, TRF1-interacting ankyrin-related ADP-ribose polymerase 2	0.860381	0.041
200030_s_at	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 3	0.855859	0.041
210916_s_at		0.855229	0.041
212776_s_at	KIAA0657 protein	0.853712	0.041
201647_s_at	CD36 antigen (collagen type I receptor, thrombospondin receptor)-like 2 (lysosomal integral membrane protein II)	0.851666	0.041
218831_s_at	Fc fragment of IgG, receptor, transporter, alpha	0.851389	0.041
207469_s_at	Pirin	0.847035	0.041

201089_at	ATPase, H+ transporting, lysosomal (vacuolar proton pump), beta polypeptide, 56/58kD, isoform 2	0.846828	0.041
201671_x_at	ubiquitin specific protease 14 (tRNA-guanine transglycosylase)	0.845122	0.041
221204_s_at	cartilage acidic protein 1	0.843208	0.041
219394_at	Phosphatidylglycerophosphate Synthase	0.843126	0.041
218213_s_at	chromosome 11 open reading frame 10	0.842591	0.041
217613_at		0.841753	0.041
217887_s_at	epidermal growth factor receptor pathway substrate 15	0.84015	0.041
220974_x_at	similar to rat tricarboxylate carrier-like protein	0.838575	0.041
208857_s_at	protein-L-isoaspartate (D-aspartate) O-methyltransferase	0.876099	0.042
213763_at		0.87455	0.042
203042_at	lysosomal-associated membrane protein 2	0.872363	0.042
216048_s_at	KIAA0878 protein	0.871	0.042
218450_at	heme binding protein 1	0.868135	0.042
201923_at	peroxiredoxin 4	0.867464	0.042
216295_s_at		0.867395	0.042
221234_s_at	BTB and CNC homology 1, basic leucine zipper transcription factor 2	0.867321	0.042
217777_s_at	butyrate-induced transcript 1	0.866611	0.042
219001_s_at	hypothetical protein MGC10765	0.866007	0.042
214751_at		0.864645	0.042
202219_at	solute carrier family 6 (neurotransmitter transporter, creatine), member 8	0.864441	0.042
212263_at	homolog of mouse quaking QKI (KH domain RNA binding protein)	0.859435	0.042
202179_at	bleomycin hydrolase	0.856247	0.042
208213_s_at	potassium voltage-gated channel, shaker-related subfamily, beta member 1	0.856238	0.042
47550_at	leucine zipper, putative tumor suppressor 1	0.856106	0.042
205353_s_at	prostatic binding protein	0.85401	0.042
209856_x_at	abl-interactor 12 (SH3-containing protein)	0.852863	0.042
209737_at	atrophin-1 interacting protein 1; activin receptor interacting protein 1	0.84324	0.042
202192_s_at	growth arrest-specific 7	0.841928	0.042
213818_x_at	collagen, type V, alpha 1	0.838269	0.042
203329_at	protein tyrosine phosphatase, receptor type, M	0.867882	0.043
212094_at	paternally expressed 10	0.859515	0.043
202967_at	glutathione S-transferase A4	0.853883	0.043
218594_at	hypothetical protein FLJ10359	0.840508	0.043

201306_s_at	acidic protein rich in leucines	0.840395	0.043
200840_at	lysyl-tRNA synthetase	0.840373	0.043
212398_at	radixin	0.83763	0.043
40148_at	amyloid beta (A4) precursor protein-binding, family B, member 2 (Fe65-like)	0.837249	0.043
214039_s_at	putative integral membrane transporter	0.874634	0.044
221644_s_at	AIM-1 protein	0.871668	0.044
215074_at	myosin IB	0.871112	0.044
206426_at	melan-A	0.840705	0.045
200645_at	GABA(A) receptor-associated protein	0.837255	0.045
220617_s_at	hypothetical protein FLJ10697	0.836003	0.045
216032_s_at	serologically defined breast cancer antigen 84	0.835737	0.045
214042_s_at		0.836483	0.046
218251_at	hypothetical protein STRAIT11499	0.835766	0.046
213981_at	catechol-O-methyltransferase	0.835388	0.046
202629_at	amyloid beta precursor protein (cytoplasmic tail) binding protein 2	0.835351	0.046
212352_s_at		0.833417	0.046
202554_s_at	glutathione S-transferase M3 (brain)	0.833964	0.047
218220_at	MYG1 protein	0.832766	0.047
202236_s_at	solute carrier family 16 (monocarboxylic acid transporters), member 1	0.832679	0.047
215152_at	v-myb myeloblastosis viral oncogene homolog (avian)	0.830679	0.047
33148_at	zinc finger RNA binding protein	0.834432	0.048
203698_s_at	frizzled-related protein	0.833765	0.048
220361_at	hypothetical protein FLJ12476	0.833687	0.048
208027_s_at		0.83368	0.048
215318_at	hypothetical gene CG012	0.832432	0.048
219450_at	hypothetical protein FLJ11017	0.830702	0.048
201919_at	hypothetical protein FLJ10618	0.829874	0.048
201002_s_at	ubiquitin-conjugating enzyme E2 variant 1	0.829588	0.048
209434_s_at	phosphoribosyl pyrophosphate amidotransferase	0.831385	0.049
221197_s_at	choline acetyltransferase	0.830122	0.049
203662_s_at	tropomodulin	0.829734	0.049
214879_x_at	upstream transcription factor 2, c-fos interacting	0.828896	0.049
203316_s_at	small nuclear ribonucleoprotein polypeptide E	0.828806	0.049
219905_at	erythroblast membrane-associated protein	0.828185	0.049
212892_at	zinc finger protein 282	0.827964	0.049
211623_s_at	casein kinase 2, beta polypeptide	0.827885	0.049
202246_s_at	cyclin-dependent kinase 4	0.827297	0.049

211774_s_at	DKFZP564I122 protein	0.82698	0.049
220201_at	olfactory receptor, family 1, subfamily K, member 1	0.81166	0.049

Estimation of the frequency of metastasis-enabling alterations

Under the assumption that the ability to metastasize is acquired by the Mel-STR melanoma cells after injection into the host animal, we estimate a conservative lower bound on the frequency with which such an alteration must occur in order to give rise to our observed data.

Let N_0 be the number of tumor cells that survive the initial subcutaneous injection. The results of the tumor resection experiments indicate that numerous metastatic cells have already been seeded in the lungs within the 18 days after the initial injection. Thus, the total number of elapsed cell divisions prior to seeding metastasis depends on N_0 and N_f, where N_f is the number of cancer cells in the tumor mass at the time of surgical resection. Given the size of the primary tumors at the time of resection, we can estimate that $N_f = 10^7$. Therefore, $\alpha = \log_2 (N_f/N_0)$, where α is the number of population doublings that occur *in vivo* prior to surgical resection of the primary tumor mass. Since 10^3 injected Mel-STR cells are sufficient to form a primary Mel-STR tumor (data not shown) we can infer that the frequency of injected cells that survive is $> 10^{-3}$. It follows that $N_0 > 10^3$, and therefore $\alpha < \log_2 (10^7/10^3) = \log_2 (10^4) < 14$. This, in turn, implies that the total number of individual cellular divisions (a quantity distinct from population doublings) that elapse prior to surgical resection of the primary tumor is less than

$$[(N_0) + (2)(N_0) + (2^2)(N_0) + (2^3)(N_0) + \ldots + (2^{\alpha-1})(N_0)] = (2^\alpha - 1)(N_0)$$

It follows that the total number of conversions *in vivo* from a non-metastatic clone to a metastatic clone is at most $(2^\alpha - 1)(N_0)(p)$, where p is the probability that a non-metastatic cell becomes metastatic after a single division. Assuming that such a conversion event will occur with equal probability in any of the $(2^{\alpha}-1)(N_0)$ cell divisions that occur *in vivo*, one can determine that the average number of cellular descendants derived from a newly formed metastatic cell is α. Thus, the total number of metastatic cells in the primary tumor mass at the moment of surgical resection is at most $(2^{\alpha}-1)(N_0)(p)(\alpha)$.

The expected number of metastatic nodules in the lungs at the endpoint of the experiment can be calculated as:

(*number of metastasis-competent cells released into the circulation before resection*) x (*the probability that a metastasis-competent cell released into the circulation will ultimately form a visible nodule in the lung*)

The second quantity in the product above can be estimated to be less than one in 10^5 cells, since the injection of 10^5 Mel-STR cells directly into the murine tail-vein failed to yield any visible lung metastases after a 2 month period (data not shown). The first quantity in the product above clearly depends on the number of tumor cells that are shed daily into the circulation. For a ≈1g tumor, this number has been previously estimated to be 10^6 cells per day [43]. Therefore,

$$(10^6 \text{ cancer cells shed per day}) \times (18 \text{ days}) \times$$
$$[(2^{\alpha}-1)(N_0)(p)(\alpha) \text{ metastatic cells in the tumor}] \div [N_f \text{ total cancer cells in the tumor}]$$

is a conservative upper bound on the total number of metastatic cells released into the circulation prior to surgical resection of the primary tumor mass. This number is $\approx(2 \times 10^7)(p)(\alpha)$. As discussed above, it follows that

$$(10^{-5})(2 \times 10^7)(p)(\alpha) < (10^{-5})(2 \times 10^7)(p)(15) = 3000p$$

is an upper bound on the expected number of lung nodules at the endpoint of the experiment. Since greater than 30 metastatic nodules were observed in the mouse lungs at the endpoint of the tumor resection experiments, we have that

$$3000p > 30 \text{ or } p > 1/100$$

This frequency is significantly higher than known rates of genomic mutation.