SUPPLEMENTAL MATERIAL

Data S1.

SUPPLEMENTAL METHODS

BOEC culture

The present study was enabled by our prior developmental, standardization, and validation studies performed in 2000-2003 and described elsewhere (text references 2-5). These involved BOEC cultures from >150 unique individuals, with gene expression surveyed on >80. At our period of peak activity, we successfully established BOEC cultures 2-3 times per week, with a success rate of ~90%. As developed by the Hebbel Lab (Univ of Minnesota) our method includes taking each of the following extraordinary precautions to avoid culture variation effects and to maximize reproducibility.

[a] Necessary supplies, reagents, microarray chips, culture medium ingredients are acquired in sufficient quantity prior to study onset so there is no risk that lot number of anything could change in mid-study.

[b] At venipuncture, the first few ml of peripheral venous blood is discarded.

[c] Blood is maintained at room temperature between venipuncture and culture by using special shipping boxes [8] that provide this protection.

[d] Although in the past we have successfully established BOEC cultures up to 8 hours after venipuncture, we make every effort to minimize the venipuncture-to-culture delay. In the present study the interval between blood attainment and starting culture set-up was <4 hours.

[e] Any single step of the overall process (i.e., culture set-up/maintenance, quality control assessments, RNA preparation, cell biology experiments) is always performed by the same trained and highly-experienced technician known to consistently achieve highly reproducible results. For example, for the present study the single BOEC culture technician was available around-the-clock daily for ~2 years.

[f] All cultures are set up in the same culture room, using the same culture hood, using the same temperature/humidity/gas-controlled incubator. For our studies, a culture room is dedicated to BOEC specifically, and only the designated BOEC culture technician has access.

[g] All cultures are passed to the same extent, a nominal million-fold expansion providing ~$3x10^7$ BOEC. They are always harvested 4 hours after the last change of culture medium and when at 85-90% confluence. This degree of expansion is solidly within what we previously found to be a “safe window” of expansion: deliberately acquired gene expression changes (from IL-1/TNF) have completely washed out; and the cells are several logs of expansion shy of developing instability of phenotype or gene expression.

[h] Quality Control: All cultures are subjected to rigorous quality control measures, with success indicated by: cobblestone morphology; positive for VE-cadherin and vWF and P1H12(CD146); negative for CD45 and CD14; single population of cells at level of light microscopy. In addition, all cultures are submitted for cytogenetics analysis (to enable later exclusion of data from any exhibiting culture-acquired cytogenetic abnormalities).

Our prior standardization and validation studies revealed that BOEC cultures passing this multi-parameter screen additionally: are negative for CD133; 100%

endothelial at level of FACS; positive for multiple endothelial antigens (flk1, PECAM-1, VCAM-1, ICAM-1, CD34, CD51, thrombomodulin); exhibit typical endothelial behaviors such as “in vitro angiogenesis”, acLDL uptake, VCAM-1 upregulation in response to TNF/IL-1. Also, gene expression profiling confirms endothelial lineage identity, and EM reveals Weibel Palade bodies.

Antibodies Used

Antibody	Source	catalog #	Working dilution or concentration
anti-vWF	Sigma	F3520	1 μg/ml
anti-VE-cadherin	Santa Cruz	sc-6458	1 μg/ml
anti-CD146	Hebbel lab	P1H12	5 μg/ml
anti-CD45	Santa-Cruz	sc-25590	1 μg/ml
anti-CD14	Santa Cruz	sc-9150	1 μg/ml
anti-HMGB1	ABCAM	ab190377	1:500 dilution
anti-LAMC1	Sigma	sab 4051727	1 μg/ml

Code used for samir:

```
require(samr)
x = as.matrix(expressionDat)
y = c(rep(2,13),rep(1,6))
data=list(x=x,y=y, geneid=probe.id, genenames= gene.id, logged2=F)
samr.obj<-samr(data, resp.type="Two class unpaired", nperms=500)
delta.table <- samr.compute.delta.table(samr.obj)
delta=0.719
samr.plot(samr.obj,delta)
siggenes.table<-samr.compute.siggenes.table(samr.obj,delta, data, delta.table)
```

Table S1. **Coronary reactivity assessment summary.**

Definition(s) of abnormal coronary endothelial function:
less than 50% increase in coronary blood flow (CBF) in response to highest dose acetylcholine
and/or
more than 20% reduction in coronary artery diameter (CAD) in response to highest-dose acetylcholine

subject	% change CBF in response to highest-dose acetylcholine	% CAD change in response to highest-dose acetylcholine	subject group assignment
A	52	-15	NL
B	99	-9	NL
C	133	0	NL
D	129	0	NL
E	555	49	NL
F	57	-20	NL
G	63	-30	ABNL
H	20	-42	ABNL
I	-24	-28	ABNL
J	56	-30	ABNL
K	48	7	ABNL
L	7	-4	ABNL
M	31	-21	ABNL
N	46	-10	ABNL
O	-10	-26	ABNL
P	-24	-36	ABNL
Q	22	-33	ABNL
R	-57	-35	ABNL
S	-100	-100	ABNL

Table S2. Differentially expressed transcripts at threshold of Welch P<0.001,listed in order of P value.

PROBE SET	GENE	FDR (%)	P	FOLD (ABNL/NL)	EXPANDED NAME
209041_s_at	UBE2G2	≤0.1	$2.9x10^{-6}$	1.28	ubiquitin conjugating enzyme E2G2
209181_s_at	RABGGTB	≤0.1	$8.5x10^{-6}$	1.27	Rab geranylgeranyltransferase subunit beta
203622_s_at	PNO1	≤0.1	$1.3x10^{-5}$	1.42	partner of NOB1 homolog
202855_s_at	SLC16A3	≤0.1	$2.0x10^{-5}$	1.84	solute carrier family 16 member 3
218447_at	CMC2	6.75	$3.2x10^{-5}$	1.35	C-X9-C containing motif containing 2
220890_s_at	DDX47	6.75	$4.1x10^{-5}$	1.22	DEAD box helicase 47
208996_s_at	POLR2C	≤0.1	$4.3x10^{-5}$	1.34	RNA polymerase II, subunit C
214938_x_at	HMGB1	≤0.1	$5.7x10^{-5}$	1.40	high mobility group box 1
216149_at	LRRC37BP1	6.75	$5.7x10^{-5}$	1.13	leucine rich repeat containing 37B pseudogene 1
212714_at	LARP4	≤0.1	$1.1x10^{-4}$	1.20	La ribonucleoprotein domain family member 4
202564_x_at	SNX15	8.64	$1.1x10^{-4}$	0.86	sorting nexin 15
213825_at	OLIG2	≤0.1	$1.3x10^{-4}$	1.11	oligodendrocyte transcription factor 2
219082_at	AMDHD2	≤0.1	$1.3x10^{-4}$	0.82	N-acetylglucosamine-6-phosphate deacetylase
200770_s_at	LAMC1	8.64	$1.5x10^{-4}$	0.71	laminin subunit gamma 1
212601_at	ZZEF1	>10	$1.5x10^{-4}$	0.88	zinc finger, ZZ type with EF hand domain
213826_s_at	H3F3B	>10	$1.8x10^{-4}$	0.84	H3 histone, family 3B
44120_at	ADCK2	>10	$2.1x10^{-4}$	0.91	aarF domain containing kinase 2
220016_at	AHNAK	6.75	$2.3x10^{-4}$	1.15	AHNAK nucleoprotein
203202_at	KRR1	>10	$2.8x10^{-4}$	1.29	KRR1, small subunit processome component homolog
208765_s_at	HNRNPR	>10	$3.5x10^{-4}$	1.18	heterogeneous nuclear ribonucleoprotein R
207734_at	LAX1	>10	$3.7x10^{-4}$	1.08	lymphocyte transmembrane adaptor 1
211999_at	H3F3B	6.75	$3.7x10^{-4}$	1.24	H3 histone family member 3B
205822_s_at	HMGCS1	>10	$4.0x10^{-4}$	1.48	3-hydroxy-3methylglutary-Coenzyme A synthase 1
217370_x_at	FUS	>10	$4.2x10^{-4}$	1.29	FUS RNA binding protein
214150_x_at	ATP6V0E	8.64	$4.2x10^{-4}$	0.80	ATPase H+ transporting V0 subunit e1

221255_s_at	EMC6	>10	$4.4x10^{-4}$	1.21	ER membrane protein complex subunit 6
217370_x_at	FUS	>10	$4.2x10^{-4}$	1.29	RNA binding protein FUS
222382_x_at	NUP205	>10	$5.1x10^{-4}$	1.16	nuclear pore complex protein Nup205
201965_s_at	SETX	>10	$5.7x10^{-4}$	0.85	senataxin
208672_s_at	SFRS3	6.75	$5.7x10^{-4}$	1.30	serine and arginine rich splicing factor 3
212394_at	EMC1	6.75	$6.1x10^{-4}$	1.13	ER membrane protein complex subunit 1
219836_at	ZBED2	>10	$6.2x10^{-4}$	1.14	zinc finger, BED-type containing 2
202722_at	HMGCL	>10	$6.7x10^{-4}$	0.86	hydroxymethylglutaryl-CoA lyase mitochondrial
202856_s_at	SLC16A3	6.75	$7.0x10^{-4}$	1.72	solute carrier family 16 member 3
208990_s_at	HNRPH3	>10	$7.1x10^{-4}$	1.31	heterogeneous nuclear ribonucleoprotein H3 (2H9)
211933_s_at	HNRNPA3	>10	$7.2x10^{-4}$	1.21	heterogeneous nuclear ribonucleoprotein A3
214409_at	RFPL3S	>10	$7.3x10^{-4}$	1.19	RFPL3 antisense [ncRNA]
215558_at	C6orf133	>10	$8.1x10^{-4}$	1.11	chromosome 6 open reading frame 13315
204647_at	HOMER3	>10	$8.2x10^{-4}$	0.74	homer scaffold protein 3
201574_at	ETF1	6.75	$8.2x10^{-4}$	1.22	eukaryotic translation termination factor 1
214882_s_at	SFRS2	>10	$8.7x10^{-4}$	1.27	splicing factor, arginine-serine rich 2
200700_s_at	KDELR2	6.75	$8.7x10^{-4}$	1.17	KDEL endoplasmic reticulum protein retention R2
201862_s_at	LRRFIP1	6.75	$9.7x10^{-4}$	1.49	LRR binding FLII interacting protein 1
210269_s_at		>10	$9.9x10^{-4}$	0.85	DNA segment on X & Y 155 expr. sequence

Table S3. ABNLs vs NLs did not significantly differ in expression of 40 inflammation-response genes (for each gene, all transcripts are listed).

Gene `	Probest	Fold Difference ABNL/NL)	P
AGER	210081_at	1.00	0.987
	217046_s_at	0.87	0.076
CAT	215573_at	0.98	0.616
C3	217767_at	0.90	0.077
	211922_s_at	0.81	0.110
	201432_at	0.83	0.044
C4	214428_x_at	1.02	0.554
	208451_s_at.	1.00	0.985
C5	205500_at	1.04	0.600
CCL2	216598_s_at	0.90	0.827
CCL3	205114_s_at	1.00	0.974
CRP	205753_at	0.99	0.885
	37020_a	0.94	0.215
F3	204363_at	1.00	0.971
HMOX1	203665_at	1.01	0.978
ICAM1	215485_s_at	0.87	0.305
	202638_s_at	0.73	0.294
	202637_s_at	0.84	0.371
IFNB1	208173_at	0.97	0.631
IL1β	205067_at	1.00	0.936
	39402_at	0.98	0.515
IL2	207849_at	0.98	0.645
IL6	205207_at	1.20	0.213
IL8	202859_x_at	0.90	0.678
	211506_s_at	0.68	0.412
JUN	213281_at	1.00	0.981
	201466_s_at	0.94	0.607
	201465_s_at	0.92	0.281
	201464_x_at	0.91	0.313
MYC	202431_s_at	1.09	0.272
NFKB1	209239_at	1.02	0.785
NFKB2	209636_at	0.88	0.089
	207535_s_at	0.84	0.309
	211524_at	1.04	0.384
NFKBIA	201502_s_at	0.86	0.267
NOS1	207309_at	1.06	0.202
	207310_s_at	0.98	0.576
NOS3	205581_s_at	1.08	0.781
PPARG	208510_s_at	0.74	0.154
PTK2	207821_s_at	0.95	0.514
	208820_at	0.92	0.241

REL	206036_s_at	0.99	0.862
RELA	201783_s_at	1.01	0.717
	209878_s_at	1.01	0.901
RELB	205205_at	0.88.	0.236
SELE	206211_at	0.65	0.357
SEL	206049_at	0.91	0.617
SP1	214732_at	1.04	0.434
TGFβ	203084_at	0.94	0.230
	203085_s_at	0.96	0.750
TLR2	204924_at	0.79	0.216
TLR4	221060_s_at	0.81	0.410
TP53	211300_s_at	1.06	0.660
	201746_at	0.98	0.857
TNF	207113_s_at	1.01	0.770
SOD1	200642_at	1.02	0.757
SRC	221281_at	0.95	0.370
	213324_at	0.97	0.668
	221284_s_at	0.92	0.225
VCAM1	203868_s_at	1.14	0.799
VEGF	212171_x_at	0.95	0.368
	210513_s_at	0.91	0.402
	211527_x_at	0.87	0.244
	210512_s_at	0.84	0.407
	211527_x_at	0.87	0.244
	212171_x_at	0.95	0.368

Figure S1. Hierarchical clustering analyses. A. Clustering using the universe of all 43 transcripts significant at P<0.001 suggests *HMGB1* is important but not the sole discriminator between NLs vs ABNLs. The low-*HMGB1* subjects in the ABNL group are outlined in the inset. **B**. Clustering using only the 9 transcripts significant at FDR<0.1% reveals substructure discriminating all lowest *HMGB1* expressers (those encircled in the inset) from the highest *HMGB1* expressers, regardless of subject group.

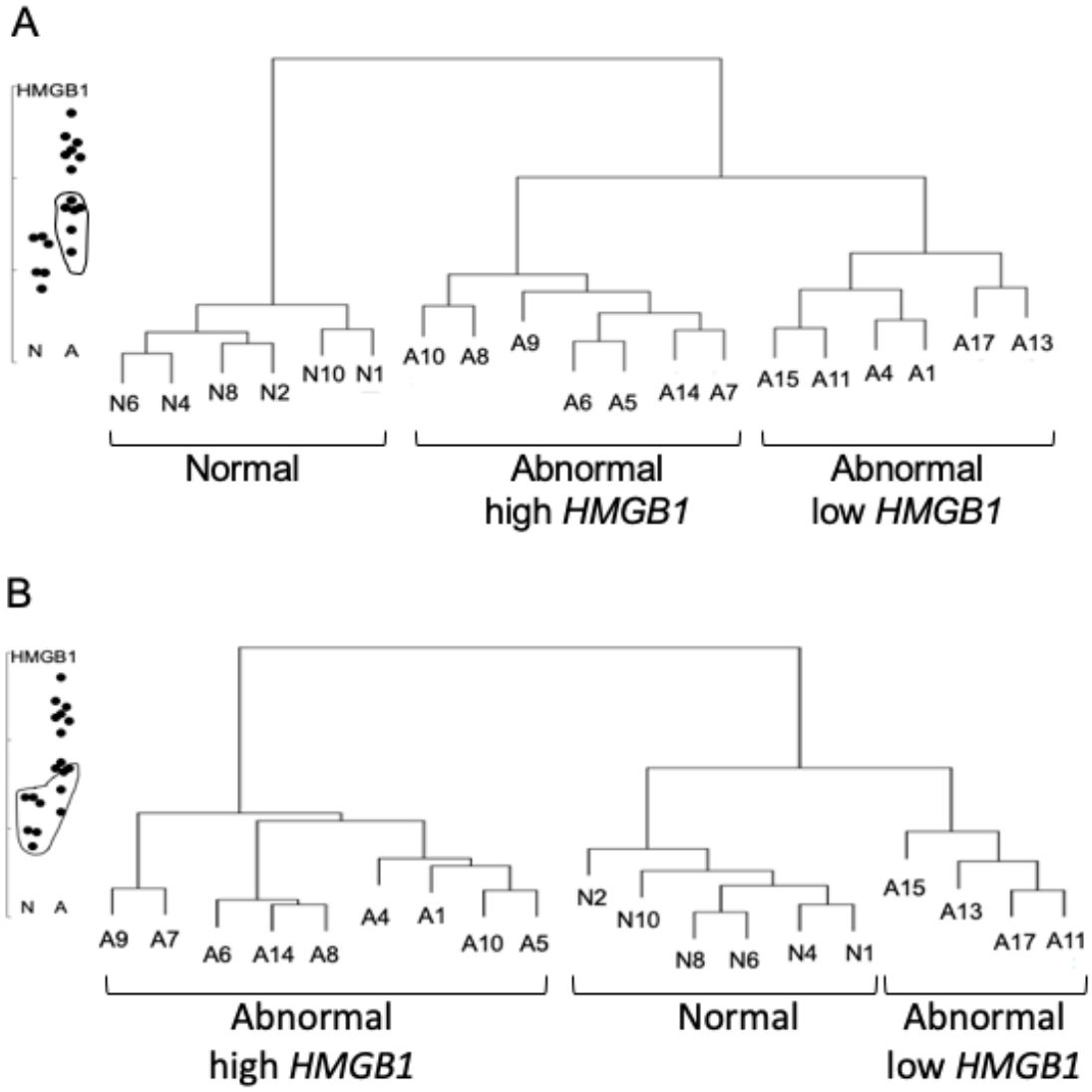
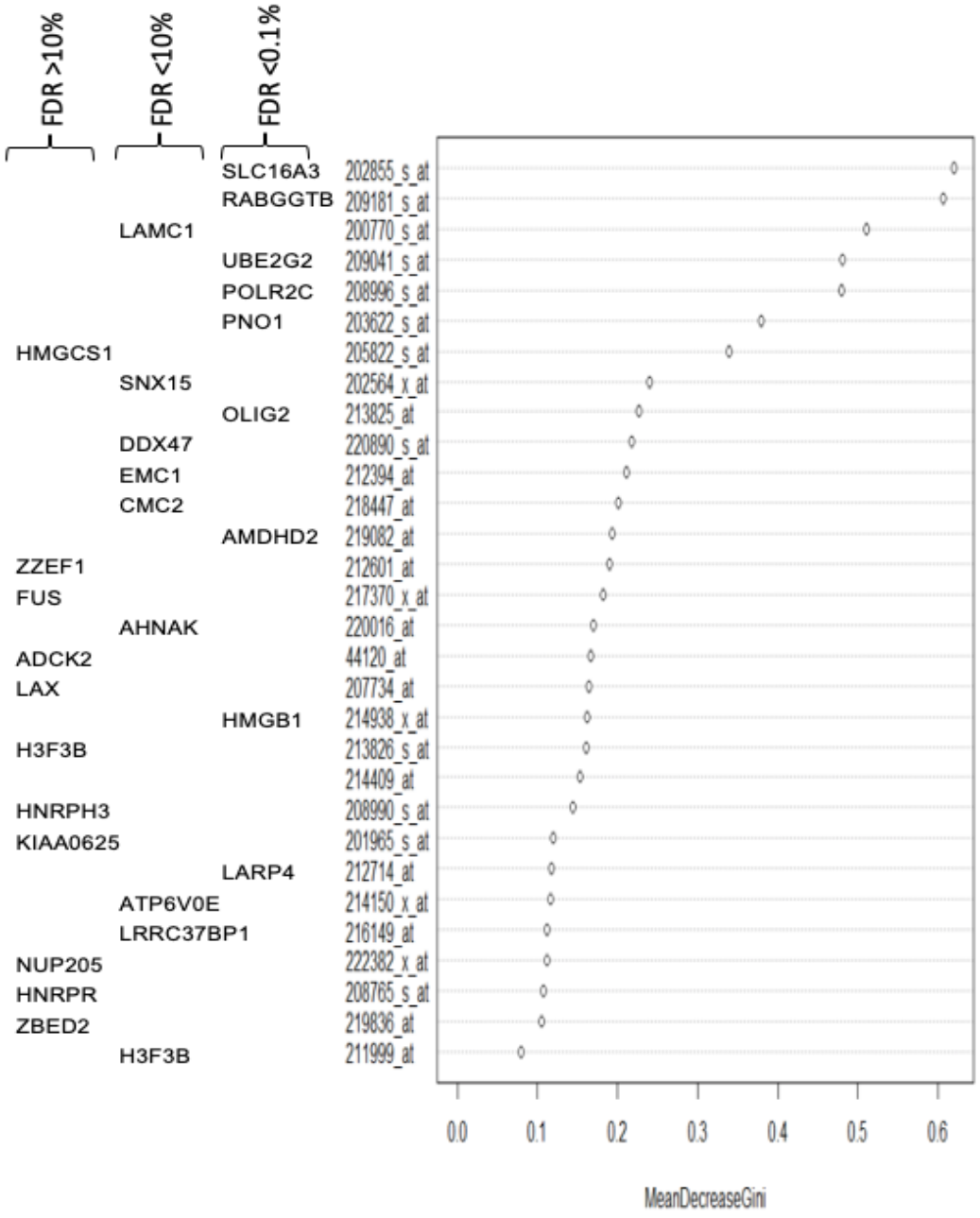


Figure S2. Random forest analysis.



We applied a random forest approach (using the universe of 43 transcripts significant at P<0.001 that yielded the clustering pattern shown in Supplemental Figure 1A) to estimate relative degree of contribution made by individual transcripts/genes in correctly separating ABNLs from NLs. Stronger contribution is rightwards on the horizontal axis. At the left, transcripts are listed by gene names in one of three columns indicating their significance level: FDR>10% (far left), FDR<10% but >0.1% (middle), FDR <0.1% (right).