SUPPLEMENTARY APPENDIX

High VLA-4 expression is associated with adverse outcome and distinct gene expression changes in childhood B-cell precursor acute lymphoblastic leukemia at first relapse

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Online Supplementary Appendix

Design and Methods

Quantification of VLA-4 mRNA by quantitative real-time polymerase chain reeaction

Total RNA was isolated using RNeasy spin columns (Qiagen, Hilden, Germany) and reverse-transcribed using oligo (dT) primers and superscript II reverse transcriptase (Invitrogen, Karlsruhe, Germany). Quantitative real-time polymerase chain reaction (QRT-PCR) was performed using SYBR Green master-mix on an iCycler PCR system (both Bio-Rad, Hercules, CA, USA). The following primers and probes were used: VLA4-F: 5'-CTC gCC AAC gCT TCA gTg ATC-3’, VLA4-R: 5'-TCG TAA ATC AGG GGG CAC TCC-3’, ABL1-F: 5-Tgg AGA TAA CAC TCT AAG CAT AAC TAA AGG T-3’, ABL1-R: 5-GAT GTA GTT GCT TGG GAC CCA-3’, ABL-TM : 5`-6FAM-CCA TTT TTG GTT TGG GCT TCA CAC CAT XT-PH-3’. *VLA-4* expression was measured in triplicate and normalized to the expression of *ABL1*. Relative *VLA-4* mRNA expression was calculated as the fold difference by the comparative CT method (ΔCT).

Quantification of VLA-4 protein by flow activated cell sorting and immunocytochemistry

Cells were stained with monoclonal antibodies against VLA-4 (Acris Antibodies GmbH, Herford, Germany) and CD19 (BD Biosciences, PaloAlto, CA) and analyzed on a FACSCalibur flow cytometer with standard CellQuest software (BD Bioscience). For immunocytochemistry, cytospins of leukemia cells were fixed, incubated with anti-VLA-4 antibodies, washed with phosphate-buffered saline, stained with secondary antibodies (anti-mouse-Alexa488, Molecular Probes, Invitrogen) and counterstained with DAPI. As negative controls, samples were incubated with isotype-matched control antibodies. Slides were covered with Vectashield Mounting Medium (Vector Laboratories, Burlingame, CA USA) and examined using an Axioplan 200 microscope with AxioVision Release 4.5 software (Zeiss, Jena, Germany).

Gene expression analysis

From 43 of the study patients with ALL, RNA hybridization results on the Affymetrix HG-U133A microarray (Affymetrix, Santa Clara, CA, USA) were available, as previously described.[1] All microarray data were analyzed using Partek® Genomics Suits software, version 6.5 beta © 2009 (Partek Inc., St. Louis, MO, USA). Gene expression profiles were corrected for background measurements and normalized using robust multiarray analysis (RMA). Two-way ANOVA was used for comparison between three *VLA-4* expression groups. Pathway analysis of the differentially expressed genes was performed with Ingenuity Pathways Analysis (Ingenuity® Systems, www.ingenuity.com).

Cell lines and cell culture

The BCP-ALL cell lines REH and MHH-CALL2 were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Leukemia cell lines were cultured in RPMI 1640 at 37°C in 5% CO_2 in a humidified incubator, and media were supplemented with 10% fetal calf serum and 1% penicillin/streptomycin (Biochrom, Berlin, Germany). The stromal cell line L87/4 was kindly provided by K. Thalmeier.[2] Co-culture experiments were done as described previously.[3] Leukemia cells in monoculture or co-culture were treated with cytarabine (ARA-C: 1 μM; Sigma-Aldrich, St. Louis, MO, USA) and/or anti-VLA-4 blocking antibodies (Acris, Clone 44H6).

Cell proliferation and adhesion assay

The proliferation rate of leukemic cells was examined by MTS tetrazolium assay (Cell Titer96 Aqueous, Promega, Mannheim, Germany) as described previously.[3] REH cells were fluorescence-labeled with PKH27 (Sigma, St. Louis, MO, USA) and seeded into 24-well plates coated with poly-lysine or stromal cells. After collecting the supernatant, adherent cells were washed three times with medium and were shaken for 1 min to remove non-adherent and weakly adherent cells. The amount of adherent cells was measured using a fluorescence plate reader.

Western blot analysis

At the end of the monoculture and co-culture assays, cells were incubated with CD19 micromagnetic beads (Miltenyi Biotec, Bergisch-Gladbach, Germany) to separate leukemia cells. Western-blot analysis was done as described previously.[3] The separated leukemia cells were lysed using Chaps Cell Extract buffer (Cell Signaling, Beverly, MA, USA). Equal protein amounts were separated by 12%-15% SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Amersham Bioscience, Buckinghamshire, UK). The membranes were probed with rabbit antibody against human BCL-2 (Cell Signaling), followed by horseradish peroxidase (HRP)-

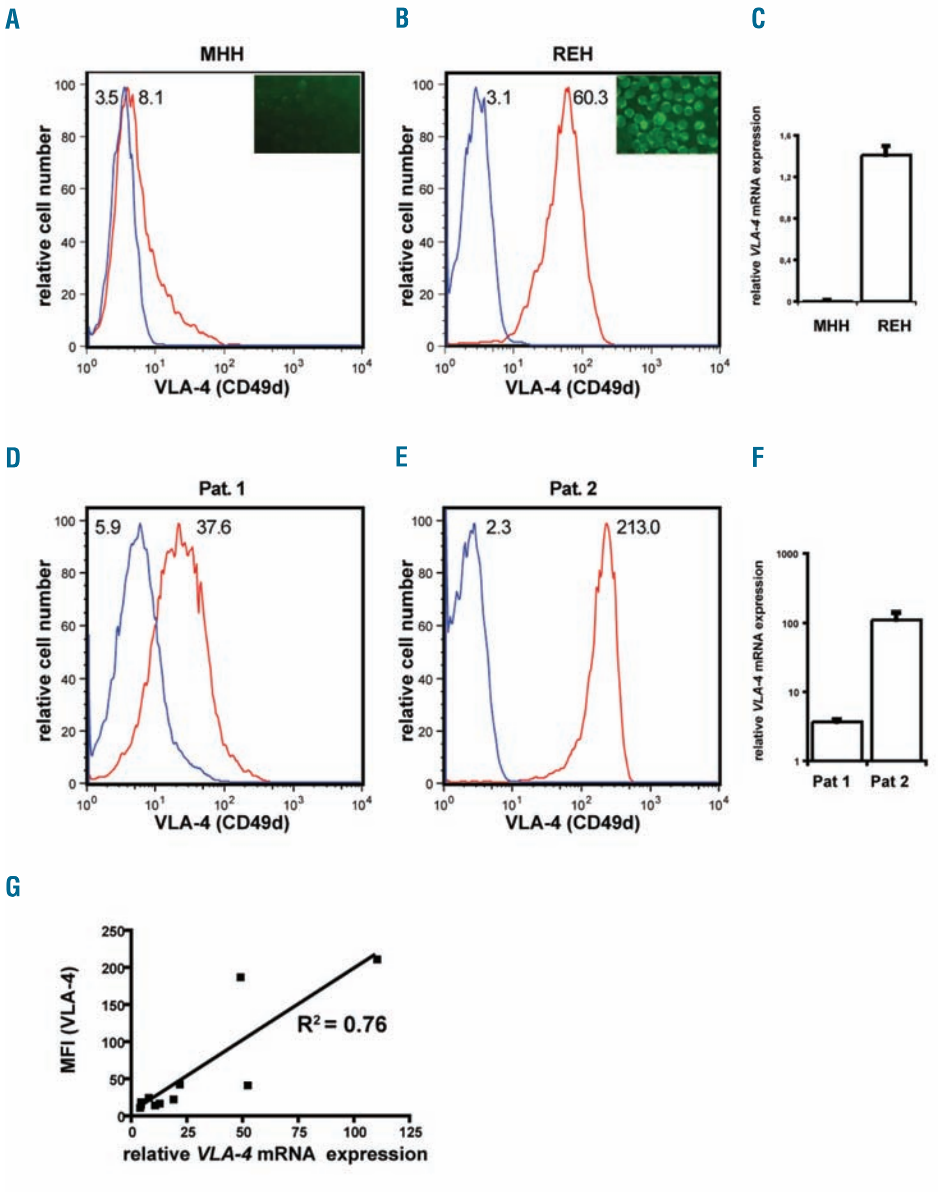
linked secondary antibodies. Blots were subsequently treated with anti-β actin antibodies as a control for equal loading. The bands were visualized by ECL (Amersham). The density of blots was analyzed using Quantity one®1-D software (Bio-Rad).

Statistical analysis

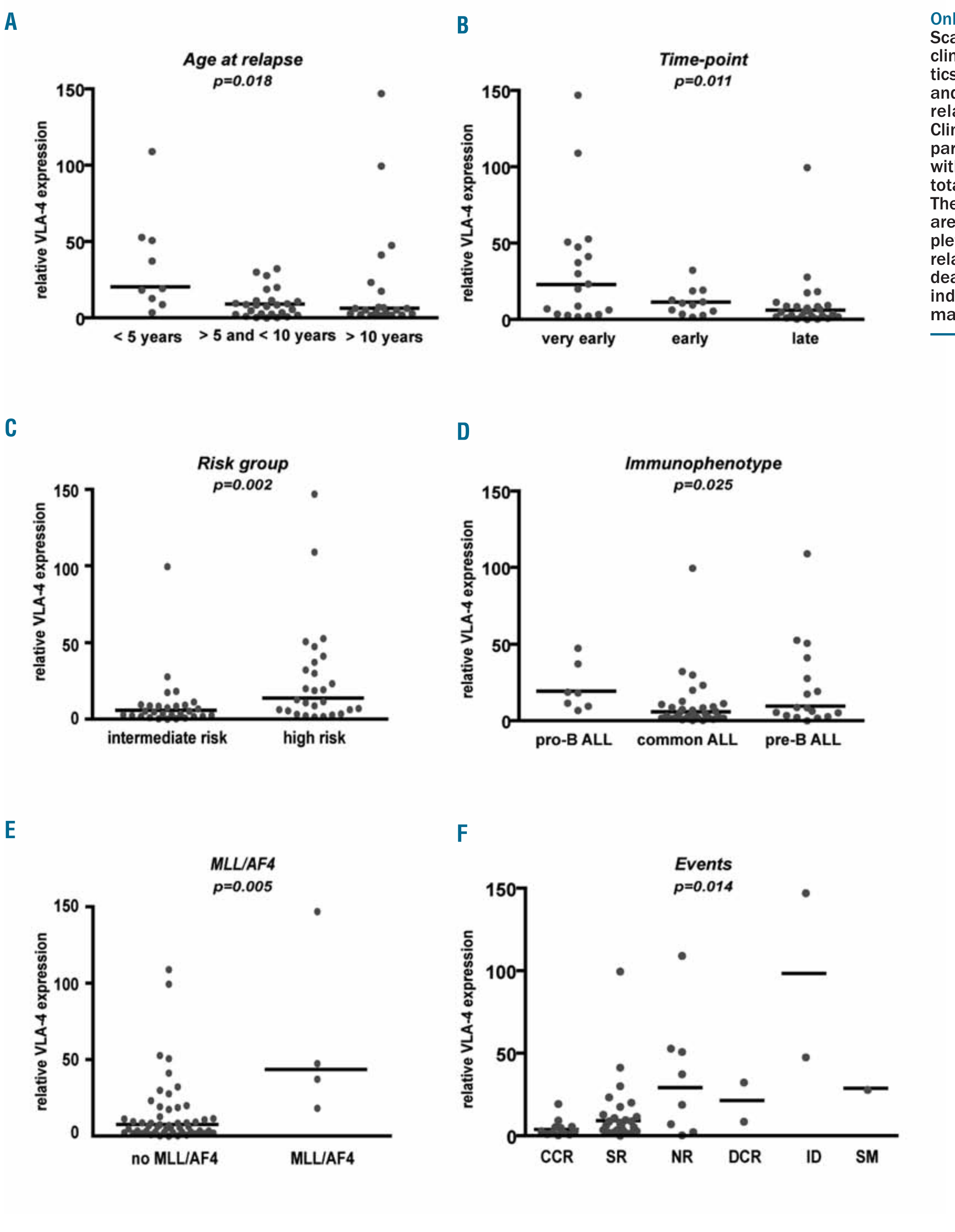
Mann-Whitney U and Kruskal-Wallis tests were performed to test the independence of two or more samples of a continuous parameter. Fisher's exact test and Pearson χ^2 test were used to test independence of categorical variables. Survival analyses were performed using Kaplan-Meier statistics and the log-rank test. Event-free survival was defined as the time interval between the diagnosis of relapse and a subsequent event including death in remission, second relapse, and second malignancy. The event-free survival time of patients who died in induction or had no response was set at 0. Overall survival was defined as the time interval between diagnosis of relapse and death. Multivariate Cox regression analysis was performed to test the independent significance of prognostic parameters for event-free survival. The univariate and multivariate analyses are provided with hazard ratios (HR) and 95% confidence intervals. *P* values less than 0.05 were considered statistically significant. Patients with relevant deviations from the ALL-REZ-BFM protocol treatment (n=5) were excluded from Kaplan-Meier analyses. As shown in Table 1, there was no difference in *VLA-4* expression of leukemia cells when different frontline protocols were used. Computations were performed using SPSS for Windows software, version 13.0 (SPSS Inc., Chicago, USA).

References

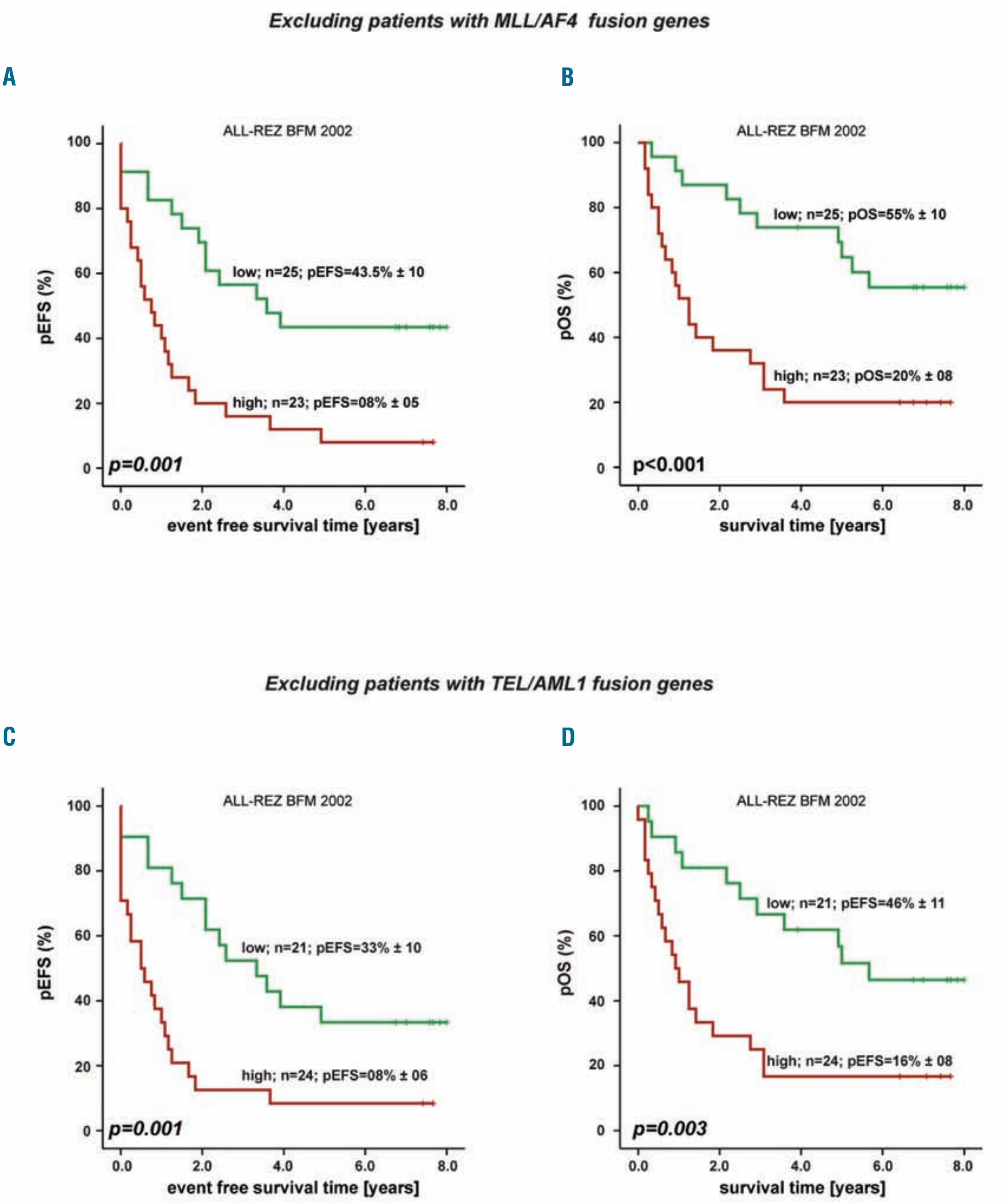
1. Kirschner-Schwabe R, Lottaz C, Todling J, Rhein P, Karawajew L, Eckert C, et al. Expression of late cell cycle genes and an increased proliferative capacity characterize very early relapse of childhood acute lymphoblastic leukemia. Clin Cancer Res. 2006;12 (15):4553-61.
2. Thalmeier K, Meissner P, Reisbach G, Hultner L, Mortensen BT, Brechtel A, et al. Constitutive and modulated cytokine expression in two permanent human bone marrow stromal cell lines. Exp Hematol. 1996;24(1):1-10.
3. Shalapour S, Zelmer A, Pfau M, Moderegger E, Costa-Blechschmidt C, van Landeghem FK, et al. The thalidomide analogue, CC-4047, induces apoptosis signaling and growth arrest in childhood acute lymphoblastic leukemia cells in vitro and in vivo. Clin Cancer Res. 2006;12(18): 5526-32.



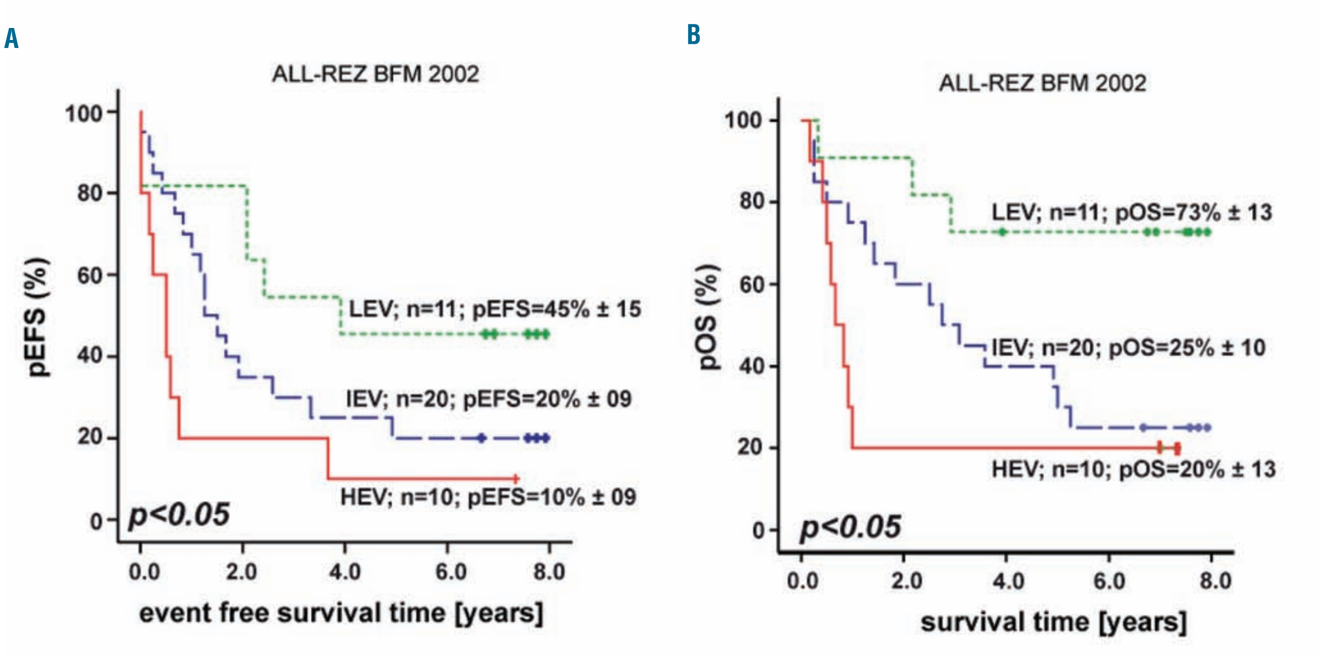
Online Supplementary Figure S1. ***VLA-4*** **mRNA correlates with VLA-4 protein level. (A-B) VLA-4 expression of BCP-ALL cell lines MHH and REH was analyzed using flow cytometry (histograms) and immunocytochemistry. Relative fluorescence intensities of VLA-4 (red) and isotype controls antibodies (blue) are shown. Mean of fluorescence intensities (MFI) of the corresponding results of VLA-4 and isotype control are shown in histograms. The immunocytochemistry results obtained with VLA-4 antibodies (green) are shown for MHH and REH in the upper right quadrants of the panels. (C) Relative *VLA-4* mRNA amounts of MHH and REH were quantified in relation to *ABL1* mRNA by QRT-PCR. (D-E) VLA-4 expression in leukemia cells of BCP-ALL patients (n=11) was analyzed using flow cytometry. Relative fluorescence intensities and MFI for VLA-4 (red) and isotype control antibodies (blue) of two representative patients are shown. (F) Relative *VLA-4* mRNA amounts quantified in relation to *ABL1* mRNA by QRT-PCR of the corresponding two patients are shown. (G) Correlation analysis (linear regression analysis) between relative *VLA-4* mRNA expression level and protein level (MFI) of leukemic cells from primary ALL samples (n=11) are shown. Abbreviation: Pat, patient.**



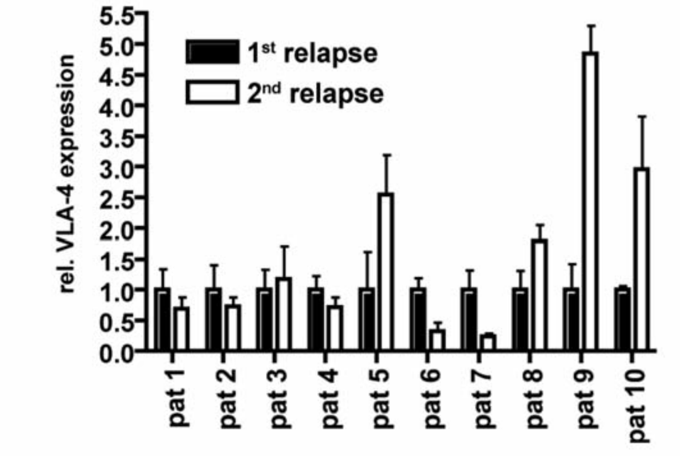
Online Supplementary Figure S2. Scatter dot plot presentation of clinical and biological characteristics of the studied BCP-ALL patients and *VLA-4* mRNA expression in correlation to these parameters. Clinical, diagnostic, and therapeutic parameters significantly associated with *VLA-4* mRNA expression in the total VLA-4 study cohort (n=56). The significant results from Table 1 are shown. CCR: continuous complete remission; SR: subsequent relapse; NR: non-responder; DCR: death in complete remission; ID: induction death; SM: secondary malignancy.



Online Supplementary Figure S3. *VLA-4* expression is associated with outcome of BCP-ALL at first relapse also after excluding patients with *MLL/AF4* and *TEL/AML1* fusion genes. (A) Kaplan-Meier analysis of event free-survival (EFS) is shown for BCP-ALL patients without *MLL/AF4* fusion genes (n=48) with *VLA-4* expression levels in leukemia cells lower and higher than the median (7.5). (B) Kaplan-Meier analysis of overall survival (OS) is shown for BCP-ALL patients without *MLL/AF4* fusion genes (n=48) with *VLA-4* expression levels in leukemia cells lower and higher than the median (7.5). (C) Kaplan-Meier analysis of EFS is shown for BCP-ALL patients without *TEL/AML1* fusion genes (n= 44) with *VLA-4* expression levels in leukemia cells lower and higher than the median (9.5). (D) Kaplan-Meier analysis of OS is shown for BCP-ALL patients without *TEL/AML1* fusion genes (n=44) with *VLA-4* expression levels in leukemia cells lower and higher than the median (9.5).

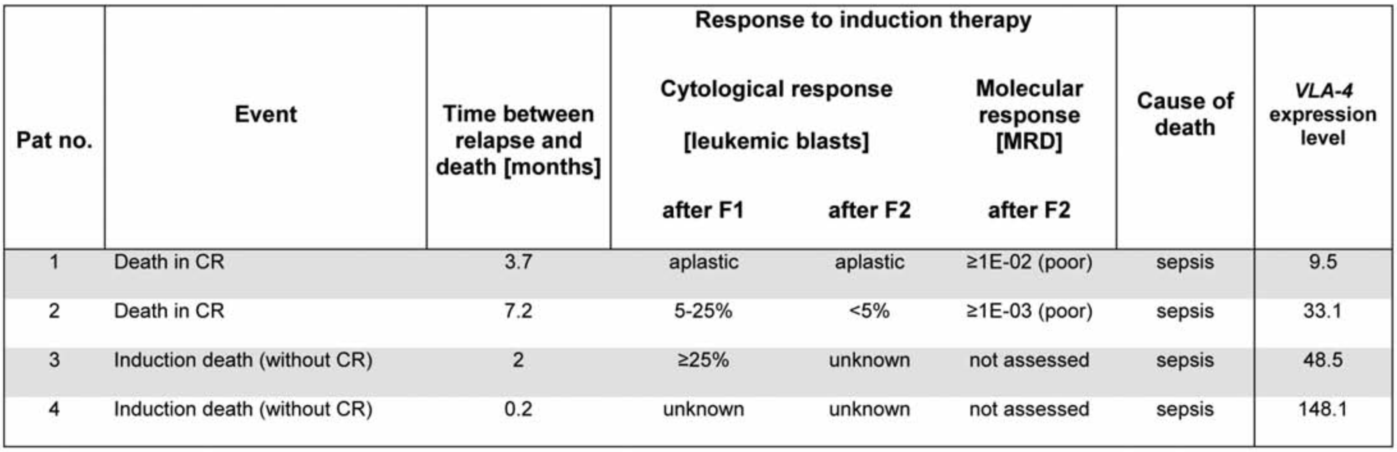


Online Supplementary Figure S4. Kaplan-Meier analysis of probability of event-free survival (pEFS) (A) and overall survival (pOS) (B) for the three groups (LEV, IEV, HEV) of BCP-ALL patients (n=41), who were included in gene expression profiling.



Online Supplementary Figure S5. Comparison analysis of *VLA-4* expression in leukemia cells from first and second relapse. Relative *VLA-4* mRNA amounts of bone marrow mononuclear cells were quantified in relation to *ABL1* mRNA by QRT-PCR, showing the relative *VLA-4* expression normalized to first relapse for each individual patient.

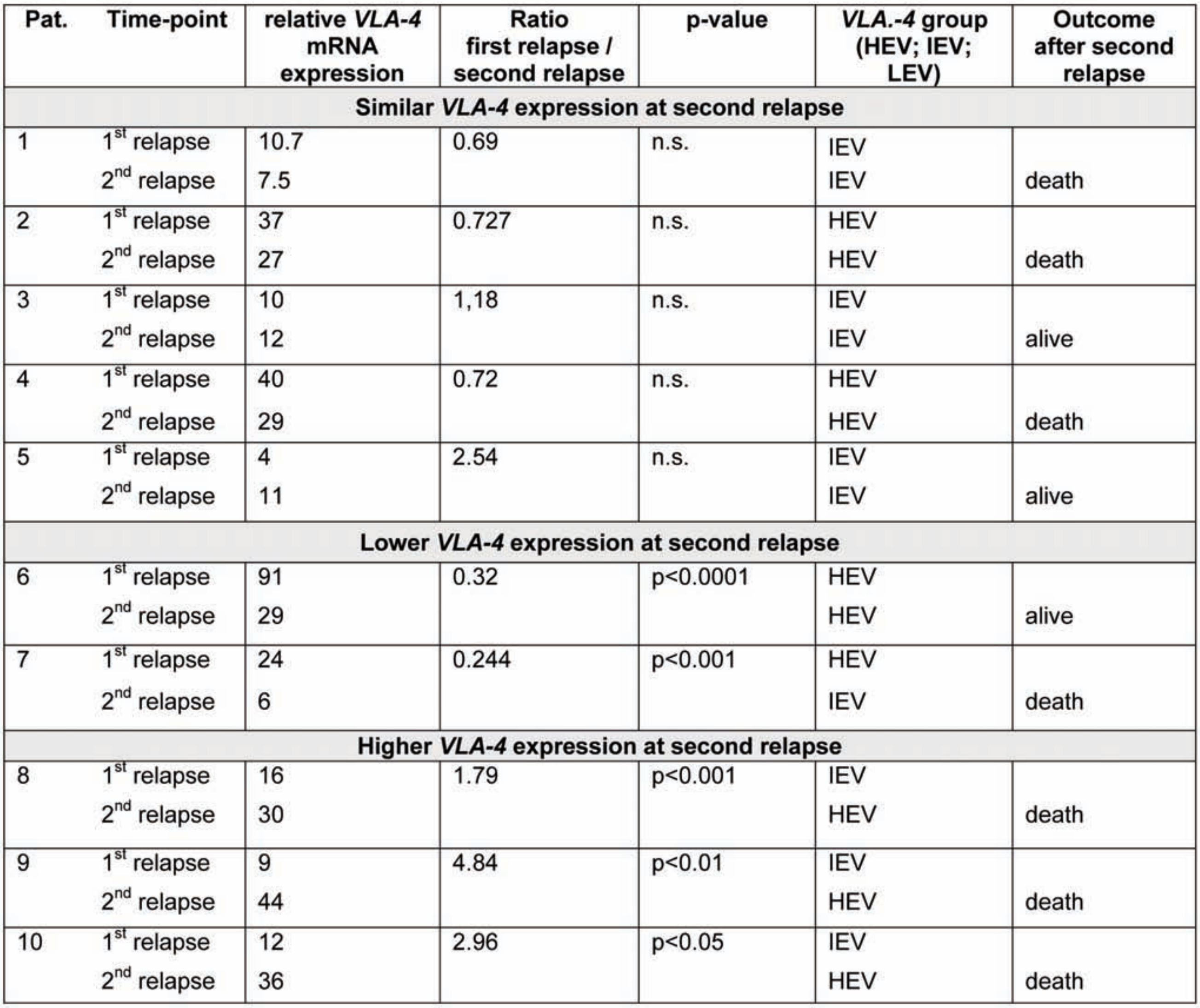
Online Supplementary Table S1. Cytological and molecular response to therapy of patients who suffered from death in induction or complete remission (CR). MRD high level (molecular poor response), equal to or more than 10^{-3} leukemia cells; MRD low level (molecular good response), less than 10^{-3} leukemia cells in BM aspiration after the second induction course (F2, week 5). Probabilities of event-free survival can be estimated at above 60% for MRD low-risk and at below 30% for MRD high intermediate-risk patients.



Pat no.	Event	Time between relapse and death [months]	Response to induction therapy			Cause of death	*VLA-4* expression level
			Cytological response [leukemic blasts]		Molecular response [MRD]		
			after F1	after F2	after F2		
1	Death in CR	3.7	aplastic	aplastic	≥1E-02 (poor)	sepsis	9.5
2	Death in CR	7.2	5-25%	<5%	≥1E-03 (poor)	sepsis	33.1
3	Induction death (without CR)	2	≥25%	unknown	not assessed	sepsis	48.5
4	Induction death (without CR)	0.2	unknown	unknown	not assessed	sepsis	148.1

CR: complete remission; MRD: minimal residual disease.

Online Supplementary Table S2. Comparison analysis of *VLA-4* expression in leukemia cells of matched samples from first and second relapse. Relative *VLA-4* mRNA amounts of bone marrow mononuclear cells were quantified in relation to *ABL1* mRNA by QRT-PCR (*see Online Supplementary Figure S5*).



Pat.	Time-point	relative *VLA-4* mRNA expression	Ratio first relapse / second relapse	p-value	*VLA.-4* group (HEV; IEV; LEV)	Outcome after second relapse
Similar *VLA-4* expression at second relapse						
1	1st relapse	10.7	0.69	n.s.	IEV	
	2nd relapse	7.5			IEV	death
2	1st relapse	37	0.727	n.s.	HEV	
	2nd relapse	27			HEV	death
3	1st relapse	10	1,18	n.s.	IEV	
	2nd relapse	12			IEV	alive
4	1st relapse	40	0.72	n.s.	HEV	
	2nd relapse	29			HEV	death
5	1st relapse	4	2.54	n.s.	IEV	
	2nd relapse	11			IEV	alive
Lower *VLA-4* expression at second relapse						
6	1st relapse	91	0.32	p<0.0001	HEV	
	2nd relapse	29			HEV	alive
7	1st relapse	24	0.244	p<0.001	HEV	
	2nd relapse	6			IEV	death
Higher *VLA-4* expression at second relapse						
8	1st relapse	16	1.79	p<0.001	IEV	
	2nd relapse	30			HEV	death
9	1st relapse	9	4.84	p<0.01	IEV	
	2nd relapse	44			HEV	death
10	1st relapse	12	2.96	p<0.05	IEV	
	2nd relapse	36			HEV	death

LEV: low expression of VLA-4 *(lower quartile, < 25%); IEM: intermediate expression of* VLA-4 *(intermediate quartiles, >25%-<75%) and HEV: high expression of* VLA-4 *(upper quartile, >75%).*

Online Supplementary Table S3. Differential expression, fold change and statistical analysis of the 142 down-regulated genes.

#	Probeset ID	Gene Symbol	Gene Title	p-value (VLA-4 grouped by quartile)	P-value (HEV vs. LEV)	Fold-Change (HEV vs. LEV)	RefSeq Transcript ID
1	203910_at	ARHGAP29	Rho GTPase activating protein 29	0.000	0.000	- 6.0	NM_004815
2	202932_at	YES1	v-yes-1 Yamaguchi sarcoma viral oncogene homolog 1	0.002	0.001	- 3.8	NM_005433
3	210517_s_at	AKAP12	A kinase (PRKA) anchor protein 12	0.013	0.005	- 3.8	NM_005100 /// NM_144497
4	204914_s_at	SOX11	SRY (sex determining region Y)-box 11	0.019	0.005	- 3.5	NM_003108
5	219737_s_at	PCDH9	protocadherin 9	0.030	0.008	- 3.5	NM_020403 /// NM_203487
6	207826_s_at	ID3	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	0.008	0.003	- 3.2	NM_002167
7	202723_s_at	FOXO1	forkhead box O1	0.000	0.000	- 3.1	NM_002015
8	219396_s_at	NEIL1	nei endonuclease VIII-like 1 (E. coli)	0.001	0.000	- 2.8	NM_024608
9	204790_at	SMAD7	SMAD family member 7	0.009	0.003	- 2.5	NM_005904
10	212762_s_at	TCF7L2	transcription factor 7-like 2 (T-cell specific, HMG-box)	0.006	0.001	- 2.5	NM_001146274 /// NM_001146283 /// NM_001146284 /// NM_001146285 /// NM_001146286
11	201912_s_at	GSPT1	G1 to S phase transition 1	0.024	0.007	- 2.3	NM_001130006 /// NM_001130007 /// NM_002094
12	201845_s_at	RYBP	RING1 and YY1 binding protein	0.010	0.003	- 2.2	NM_012234
13	204562_at	IRF4	interferon regulatory factor 4	0.007	0.005	- 2.2	NM_002460
14	211965_at	ZFP36L1	zinc finger protein 36, C3H type-like 1	0.019	0.007	- 2.2	NM_004926
15	221011_s_at	LBH	limb bud and heart development homolog (mouse)	0.008	0.002	- 2.1	NM_030915
16	202669_s_at	EFNB2	ephrin-B2	0.010	0.003	- 2.1	NM_004093
17	209184_s_at	IRS2	insulin receptor substrate 2	0.004	0.003	- 2.1	NM_003749
18	212450_at	SECISBP2L	SECIS binding protein 2-like	0.006	0.002	- 2.1	NM_014701
19	201461_s_at	MAPKAPK2	mitogen-activated protein kinase-activated protein kinase 2	0.001	0.000	- 2.1	NM_004759 /// NM_032960
20	210425_x_at	GOLGA8B	golgi autoantigen., golgin subfamily a, 8B	0.001	0.000	- 2.0	NM_001023567 /// NR_027410
21	218829_s_at	CHD7	chromodomain helicase DNA binding protein 7	0.004	0.001	- 2.0	NM_017780
22	209674_at	CRY1	cryptochrome 1 (photolyase-like)	0.013	0.004	- 2.0	NM_004075
23	214446_at	ELL2	elongation factor, RNA polymerase II, 2	0.023	0.007	- 2.0	NM_012081
24	203096_s_at	RAPGEF2	Rap guanine nucleotide exchange factor (GEF) 2	0.001	0.000	- 2.0	NM_014247
25	201367_s_at	ZFP36L2	zinc finger protein 36, C3H type-like 2	0.000	0.002	- 2.0	NM_006887
26	203593_at	CD2AP	CD2-associated protein	0.015	0.004	- 2.0	NM_012120
27	222024_s_at	AKAP13	A kinase (PRKA) anchor protein 13	0.016	0.007	- 2.0	NM_006738 /// NM_007200 /// NM_144767
28	211789_s_at	MLXIP	MLX interacting protein	0.014	0.005	- 2.0	NM_014938
29	205006_s_at	NMT2	N-myristoyltransferase 2	0.006	0.004	- 2.0	NM_004808
30	219855_at	NUDT11	nudix (nucleoside diphosphate linked moiety X)-type motif 11	0.002	0.001	- 2.0	NM_018159
31	212096_s_at	MTUS1	mitochondrial tumor suppressor 1	0.003	0.007	- 1.9	NM_001001924 /// NM_001001925 /// NM_001001931 /// NM_020749
32	214766_s_at	AHCTF1	AT hook containing transcription factor 1	0.011	0.003	- 1.9	NM_015446
33	203120_at	TP53BP2	tumor protein p53 binding protein, 2	0.008	0.002	- 1.9	NM_001031685 /// NM_005426
34	221230_s_at	ARID4B	AT rich interactive domain 4B (RBP1-like)	0.012	0.005	- 1.9	NM_016374 /// NM_031371
35	201369_s_at	ZFP36L2	zinc finger protein 36, C3H type-like 2	0.001	0.004	- 1.9	NM_006887
36	201677_at	C3orf37	Chromosome 3 open reading frame 37	0.002	0.001	- 1.9	NM_001006109 /// NM_020187
37	212838_at	DNMBP	dynamin binding protein	0.024	0.007	- 1.9	NM_015221
38	218268_at	TBC1D15	TBC1 domain family, member 15	0.012	0.003	- 1.9	NM_001146213 /// NM_001146214 /// NM_022771 /// NR_027449
39	204369_at	PIK3CA	phosphoinositide-3-kinase, catalytic, alpha polypeptide	0.019	0.006	- 1.9	NM_006218
40	201711_x_at	RANBP2	RAN binding protein 2	0.031	0.009	- 1.9	NM_006267
41	217100_s_at	UBXN7	UBX domain protein 7	0.001	0.000	- 1.9	NM_015562
42	201846_s_at	RYBP	RING1 and YY1 binding protein	0.007	0.002	- 1.8	NM_012234
43	202033_s_at	RB1CC1	RB1-inducible coiled-coil 1	0.018	0.005	- 1.8	NM_001083617 /// NM_014781
44	209037_s_at	EHD1	EH-domain containing 1	0.019	0.007	- 1.8	NM_006795
45	213457_at	MFHAS1	malignant fibrous histiocytoma amplified sequence 1	0.003	0.001	- 1.8	NM_004225
46	201471_s_at	SQSTM1	sequestosome 1	0.002	0.000	- 1.8	NM_001142298 /// NM_001142299 /// NM_003900
47	212689_s_at	KDM3A	lysine (K)-specific demethylase 3A	0.012	0.004	- 1.8	NM_001146688 /// NM_018433
48	201878_at	ARIH1	ariadne homolog, ubiquitin-conjugating enzyme E2 binding protein, 1 (Drosophila)	0.031	0.010	- 1.8	NM_005744
49	203706_s_at	FZD7	frizzled homolog 7 (Drosophila)	0.010	0.008	- 1.8	NM_003507
50	214792_x_at	VAMP2	vesicle-associated membrane protein 2 (synaptobrevin 2)	0.002	0.004	- 1.8	NM_014232
51	215498_s_at	MAP2K3	mitogen-activated protein kinase kinase 3	0.007	0.005	- 1.7	NM_002756 /// NM_145109
52	212231_at	FBXO21	F-box protein 21	0.002	0.001	- 1.7	NM_015002 /// NM_033624
53	201165_s_at	PUM1	pumilio homolog 1 (Drosophila)	0.001	0.000	- 1.7	NM_001020658 /// NM_014676
54	221643_s_at	RERE	arginine-glutamic acid dipeptide (RE) repeats	0.012	0.003	- 1.7	NM_001042681 /// NM_001042682 /// NM_012102
55	212840_at	UBXN7	UBX domain protein 7	0.001	0.001	- 1.7	NM_015562
56	210346_s_at	CLK4	CDC-like kinase 4	0.009	0.003	- 1.7	NM_020666
57	213579_s_at	EP300	E1A binding protein p300	0.019	0.005	- 1.7	NM_001429
58	218107_at	WDR26	WD repeat domain 26	0.000	0.000	- 1.7	NM_001115113 /// NM_025160
59	209102_s_at	HBP1	HMG-box transcription factor 1	0.029	0.010	- 1.7	NM_012257
60	212224_at	ALDH1A1	aldehyde dehydrogenase 1 family, member A1	0.027	0.009	- 1.7	NM_000689
61	202006_at	PTPN12	protein tyrosine phosphatase, non-receptor type 12	0.030	0.009	- 1.6	NM_001131008 /// NM_001131009 /// NM_002835

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62	218012_at	TSPYL2	TSPY-like 2	0.007	0.002	- 1.6	NM_022117
63	209339_at	SIAH2	seven in absentia homolog 2 (Drosophila)	0.009	0.002	- 1.6	NM_005067
64	218474_s_at	KCTD5	potassium channel tetramerisation domain containing 5	0.005	0.002	- 1.6	NM_018992
65	219492_at	CHIC2	cysteine-rich hydrophobic domain 2	0.029	0.008	- 1.6	NM_012110
66	207856_s_at	LOC150776 /// SMPD4	sphingomyelin phosphodiesterase 4, neutral membrane pseudogene /// sphingomyelin	0.002	0.001	- 1.6	NM_017751 /// NM_017951 /// NR_026922 /// XR_015221 /// XR_015765 /// XR_041691
67	210649_s_at	ARID1A	AT rich interactive domain 1A (SWI-like)	0.002	0.002	- 1.6	NM_006015 /// NM_139135
68	211297_s_at	CDK7	cyclin-dependent kinase 7	0.011	0.006	- 1.6	NM_001799
69	207361_at	HBP1	HMG-box transcription factor 1	0.013	0.004	- 1.6	NM_012257
70	204140_at	TPST1	tyrosylprotein sulfotransferase 1	0.000	0.000	- 1.6	NM_003596
71	208686_s_at	BRD2	bromodomain containing 2	0.001	0.001	- 1.6	NM_001113182 /// NM_005104
72	202102_s_at	BRD4	bromodomain containing 4	0.018	0.006	- 1.6	NM_014299 /// NM_058243
73	221860_at	HNRNPL	heterogeneous nuclear ribonucleoprotein L	0.026	0.008	- 1.6	NM_001005335 /// NM_001533
74	207753_at	ZNF304	zinc finger protein 304	0.003	0.003	- 1.6	NM_020657
75	202423_at	MYST3	MYST histone acetyltransferase (monocytic leukemia) 3	0.031	0.010	- 1.6	NM_001099412 /// NM_001099413 /// NM_006766
76	207108_s_at	NIPBL	Nipped-B homolog (Drosophila)	0.007	0.002	- 1.5	NM_015384 /// NM_133433
77	218344_s_at	RCOR3	REST corepressor 3	0.024	0.007	- 1.5	NM_001136223 /// NM_001136224 /// NM_001136225 /// NM_018254
78	212238_at	ASXL1	additional sex combs like 1 (Drosophila)	0.017	0.005	- 1.5	NM_015338
79	207164_s_at	ZNF238	zinc finger protein 238	0.010	0.004	- 1.5	NM_006352 /// NM_205768
80	212146_at	PLEKHM2	pleckstrin homology domain containing, family M (with RUN domain) member 2	0.015	0.005	- 1.5	NM_015164
81	213908_at	WHAMML1 /// WHAMML2	WAS protein homolog associated with actin, golgi membranes and microtubules-like	0.017	0.006	- 1.5	NR_003521 /// NR_026589 /// XM_926785 /// XR_042166
82	204937_s_at	ZNF274	zinc finger protein 274	0.022	0.007	- 1.5	NM_016324 /// NM_016325 /// NM_133502
83	212648_at	DHX29	DEAH (Asp-Glu-Ala-His) box polypeptide 29	0.011	0.009	- 1.5	NM_019030
84	218029_at	FAM65A	family with sequence similarity 65, member A	0.028	0.008	- 1.5	NM_024519
85	212069_s_at	BAT2L	HLA-B associated transcript 2-like	0.000	0.000	- 1.5	NM_013318
86	209675_s_at	HNRNPUL1	heterogeneous nuclear ribonucleoprotein U-like 1	0.015	0.006	- 1.5	NM_007040 /// NM_144732
87	215329_s_at	CDC2L1 /// CDC2L2	cell division cycle 2-like 1 (PITSLRE proteins) /// cell division cycle 2-like 2	0.003	0.001	- 1.5	NM_024011 /// NM_033486 /// NM_033487 /// NM_033488 /// NM_033489 /// NM_033492
88	202162_s_at	CNOT8	CCR4-NOT transcription complex, subunit 8	0.002	0.001	- 1.5	NM_004779
89	200898_s_at	MGEA5	meningioma expressed antigen 5 (hyaluronidase)	0.024	0.009	- 1.5	NM_001142434 /// NM_012215
90	205805_s_at	ROR1	receptor tyrosine kinase-like orphan receptor 1	0.009	0.008	- 1.5	NM_001083592 /// NM_005012
91	209456_s_at	FBXW11	F-box and WD repeat domain containing 11	0.007	0.002	- 1.5	NM_012300 /// NM_033644 /// NM_033645
92	207821_s_at	PTK2	PTK2 protein tyrosine kinase 2	0.019	0.006	- 1.5	NM_005607 /// NM_153831
93	221518_s_at	USP47	ubiquitin specific peptidase 47	0.023	0.007	- 1.5	NM_017944
94	202466_at	POLS	polymerase (DNA directed) sigma	0.002	0.006	- 1.5	NM_006999
95	222157_s_at	WDR48	WD repeat domain 48	0.007	0.002	- 1.5	NM_020839
96	209333_at	ULK1	unc-51-like kinase 1 (C. elegans)	0.015	0.004	- 1.5	NM_003565
97	214185_at	KHDRBS1	KH domain containing, RNA binding, signal transduction associated 1	0.008	0.002	- 1.5	NM_006559
98	219129_s_at	SAP30L	SAP30-like	0.011	0.003	- 1.5	NM_001131062 /// NM_001131063 /// NM_024632 /// NR_024084
99	216139_s_at	MAPK8IP3	mitogen-activated protein kinase 8 interacting protein 3	0.015	0.004	- 1.5	NM_001040439 /// NM_015133
100	212455_at	YTHDC1	YTH domain containing 1	0.025	0.007	- 1.5	NM_001031732 /// NM_133370
101	202683_s_at	RNMT	RNA (guanine-7-) methyltransferase	0.018	0.006	- 1.5	NM_003799
102	203334_at	DHX8	DEAH (Asp-Glu-Ala-His) box polypeptide 8	0.013	0.008	- 1.5	NM_004941
103	201364_s_at	OAZ2	ornithine decarboxylase antizyme 2	0.002	0.001	- 1.4	NM_002537
104	218706_s_at	GRAMD3	GRAM domain containing 3	0.000	0.000	- 1.4	NM_001146319 /// NM_001146320 /// NM_001146321 /// NM_001146322 /// NM_023927
105	211578_s_at	RPS6KB1	ribosomal protein S6 kinase, 70kDa, polypeptide 1	0.023	0.009	- 1.4	NM_003161
106	221809_at	RANBP10	RAN binding protein 10	0.003	0.001	- 1.4	NM_020850
107	210424_s_at	GOLGA8A /// GOLGA8B	golgi autoantigen, golgin subfamily a, 8A /// golgi autoantigen, golgin subfamil	0.023	0.010	- 1.4	NM_001023567 /// NM_181077 /// NR_027409 /// NR_027410 /// XM_001714558
108	202775_s_at	SFRS8	splicing factor, arginine/serine-rich 8 (suppressor-of-white-apricot homolog, Dr	0.028	0.009	- 1.4	NM_004592
109	201365_at	OAZ2	ornithine decarboxylase antizyme 2	0.006	0.002	- 1.4	NM_002537
110	218420_s_at	C13orf23	chromosome 13 open reading frame 23	0.021	0.007	- 1.4	NM_025138 /// NM_170719
111	209571_at	CIR	CBF1 interacting corepressor	0.034	0.010	- 1.4	NM_004882
112	201794_s_at	SMG7	Smg-7 homolog, nonsense mediated mRNA decay factor (C. elegans)	0.003	0.003	- 1.4	NM_173156 /// NM_201568 /// NM_201569
113	201354_s_at	BAZ2A	bromodomain adjacent to zinc finger domain, 2A	0.011	0.003	- 1.4	NM_013449
114	201715_s_at	ACIN1	apoptotic chromatin condensation inducer 1	0.006	0.006	- 1.4	NM_014977
115	215954_s_at	C19orf29	chromosome 19 open reading frame 29	0.007	0.002	- 1.4	NM_001080543 /// NM_021231
116	208200_at	IL1A	interleukin 1, alpha	0.009	0.005	- 1.4	NM_000575
117	220099_s_at	LUC7L2	LUC7-like 2 (S. cerevisiae)	0.019	0.005	- 1.4	NM_016019
118	206620_at	GRAP	GRB2-related adaptor protein	0.008	0.003	- 1.4	NM_006613
119	212553_at	RPRD2	regulation of nuclear pre-mRNA domain containing 2	0.000	0.001	- 1.4	NM_015203
120	203707_at	ZNF263	zinc finger protein 263	0.007	0.006	- 1.4	NM_005741
121	200083_at	USP22	ubiquitin specific peptidase 22	0.007	0.004	- 1.4	NM_015276
122	209361_s_at	PCBP4	poly(rC) binding protein 4	0.018	0.006	- 1.4	NM_020418 /// NM_033008 /// NM_033009 /// NM_033010
123	213121_at	SNRNP70	small nuclear ribonucleoprotein 70kDa (U1)	0.005	0.003	- 1.4	NM_003089
124	213954_at	FAM169A	family with sequence similarity 169, member A	0.018	0.006	- 1.3	NM_015566
125	213407_at	PHLPPL	PH domain and leucine rich repeat protein phosphatase-like	0.015	0.006	- 1.3	NM_015020

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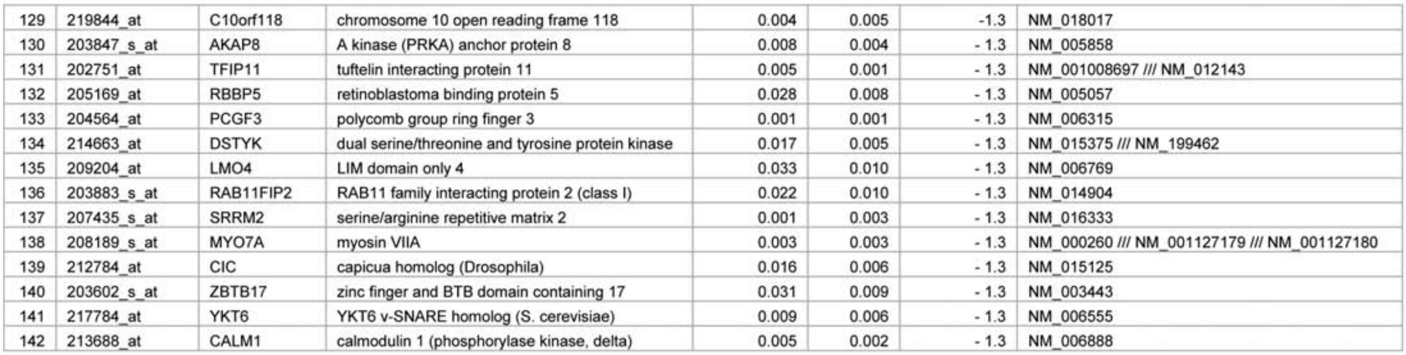

129	219844_at	C10orf118	chromosome 10 open reading frame 118	0.004	0.005	-1.3	NM_018017
130	203847_s_at	AKAP8	A kinase (PRKA) anchor protein 8	0.008	0.004	- 1.3	NM_005858
131	202751_at	TFIP11	tuftelin interacting protein 11	0.005	0.001	- 1.3	NM_001008697 /// NM_012143
132	205169_at	RBBP5	retinoblastoma binding protein 5	0.028	0.008	- 1.3	NM_005057
133	204564_at	PCGF3	polycomb group ring finger 3	0.001	0.001	- 1.3	NM_006315
134	214663_at	DSTYK	dual serine/threonine and tyrosine protein kinase	0.017	0.005	- 1.3	NM_015375 /// NM_199462
135	209204_at	LMO4	LIM domain only 4	0.033	0.010	- 1.3	NM_006769
136	203883_s_at	RAB11FIP2	RAB11 family interacting protein 2 (class I)	0.022	0.010	- 1.3	NM_014904
137	207435_s_at	SRRM2	serine/arginine repetitive matrix 2	0.001	0.003	- 1.3	NM_016333
138	208189_s_at	MYO7A	myosin VIIA	0.003	0.003	- 1.3	NM_000260 /// NM_001127179 /// NM_001127180
139	212784_at	CIC	capicua homolog (Drosophila)	0.016	0.006	- 1.3	NM_015125
140	203602_s_at	ZBTB17	zinc finger and BTB domain containing 17	0.031	0.009	- 1.3	NM_003443
141	217784_at	YKT6	YKT6 v-SNARE homolog (S. cerevisiae)	0.009	0.006	- 1.3	NM_006555
142	213688_at	CALM1	calmodulin 1 (phosphorylase kinase, delta)	0.005	0.002	- 1.3	NM_006888

142 differentially down-regulated genes between the HEV, IEV and LEV groups are shown. P *value, Two-way ANOVA statistical analysis; fold change, between HEV and LEV, probeset ID, Affymetrix GeneChip® U133A; HEV: high expression* VLA-4 *group; IEV: intermediate expression* VLA-4 *group; LEV: low expression* VLA-4 *group.*

Online Supplementary Table S4. Differential expression, fold change and statistical analysis of the 272 up-regulated genes.

#	Probeset ID	Gene Symbol	Gene Title	p-value (VLA-4 grouped by quartile)	p-value (HEV vs.LEV)	FoldChange (HEV vs.LEV)	RefSeq Transcript ID
1	203132_at	RB1	retinoblastoma 1	0.016	0.008	3.0	NM_000321
2	211675_s_at	MDFIC	MyoD family inhibitor domain containing	0.000	0.000	3.0	NM_199072
3	213416_at	ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	0.000	0.000	2.9	NM_000885
4	218469_at	GREM1	gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)	0.004	0.005	2.8	NM_013372
5	213620_s_at	ICAM2	intercellular adhesion molecule 2	0.001	0.001	2.7	NM_000873 /// NM_001099786 /// NM_001099787 /// NM_001099788 /// NM_001099789
6	218102_at	DERA	2-deoxyribose-5-phosphate aldolase homolog (C. elegans)	0.002	0.002	2.6	NM_015954
7	203305_at	F13A1	coagulation factor XIII, A1 polypeptide	0.006	0.002	2.6	NM_000129
8	201013_s_at	PAICS	phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinoca	0.014	0.005	2.6	NM_001079524 /// NM_001079525 /// NM_006452
9	205419_at	GPR183	G protein-coupled receptor 183	0.018	0.009	2.5	NM_004951
10	201302_at	ANXA4	annexin A4	0.030	0.009	2.5	NM_001153
11	205668_at	LY75	lymphocyte antigen 75	0.002	0.000	2.4	NM_002349
12	212174_at	AK2	adenylate kinase 2	0.006	0.002	2.4	NM_001625 /// NM_013411
13	213888_s_at	TRAF3IP3	TRAF3 interacting protein 3	0.000	0.000	2.3	NM_025228 /// XM_001718033 /// XM_001718553 /// XM_001720826
14	200791_s_at	IQGAP1	IQ motif containing GTPase activating protein 1	0.002	0.000	2.3	NM_003870
15	219463_at	C20orf103	chromosome 20 open reading frame 103	0.004	0.009	2.3	NM_012261
16	221581_s_at	LAT2	linker for activation of T cells family, member 2	0.022	0.007	2.3	NM_014146 /// NM_032463 /// NM_032464
17	200046_at	DAD1	defender against cell death 1	0.019	0.010	2.3	NM_001344
18	220952_s_at	PLEKHA5	pleckstrin homology domain containing, family A member 5	0.017	0.005	2.2	NM_001143821 /// NM_019012 /// NR_026568
19	213572_s_at	SERPINB1	serpin peptidase inhibitor, clade B (ovalbumin), member 1	0.006	0.002	2.2	NM_030666
20	209539_at	ARHGEF6	Rac/Cdc42 guanine nucleotide exchange factor (GEF) 6	0.004	0.001	2.2	NM_004840
21	209397_at	ME2	malic enzyme 2, NAD(+)-dependent, mitochondrial	0.008	0.002	2.2	NM_002396
22	201054_at	HNRNPA0	heterogeneous nuclear ribonucleoprotein A0	0.010	0.004	2.2	NM_006805
23	201528_at	RPA1	replication protein A1, 70kDa	0.025	0.008	2.2	NM_002945 /// XM_001717905
24	218163_at	MCTS1	malignant T cell amplified sequence 1	0.023	0.007	2.2	NM_001137554 /// NM_014060 /// XM_001715103
25	201036_s_at	HADH	hydroxyacyl-Coenzyme A dehydrogenase	0.003	0.004	2.2	NM_005327
26	208877_at	PAK2	p21 protein (Cdc42/Rac)-activated kinase 2	0.008	0.002	2.1	NM_002577
27	218477_at	TMEM14A	transmembrane protein 14A	0.002	0.001	2.1	NM_014051
28	208818_s_at	COMT	catechol-O-methyltransferase	0.021	0.006	2.1	NM_000754 /// NM_001135161 /// NM_001135162 /// NM_007310
29	213503_x_at	ANXA2	annexin A2	0.012	0.004	2.1	NM_001002857 /// NM_001002858 /// NM_001136015 /// NM_004039
30	202811_at	STAMBP	STAM binding protein	0.003	0.002	2.1	NM_006463 /// NM_201647 /// NM_213622
31	205260_s_at	ACYP1	acylphosphatase 1, erythrocyte (common) type	0.019	0.005	2.1	NM_001107 /// NM_203488
32	209773_s_at	RRM2	ribonucleotide reductase M2 polypeptide	0.007	0.005	2.1	NM_001034
33	209788_s_at	ERAP1	endoplasmic reticulum aminopeptidase 1	0.008	0.003	2.1	NM_001040458 /// NM_016442
34	209448_at	HTATIP2	HIV-1 Tat interactive protein 2, 30kDa	0.004	0.001	2.0	NM_001098520 /// NM_001098521 /// NM_001098522 /// NM_001098523 /// NM_006410
35	211963_s_at	ARPC5	actin related protein 2/3 complex, subunit 5, 16kDa	0.003	0.001	2.0	NM_005717
36	205412_at	ACAT1	acetyl-Coenzyme A acetyltransferase 1	0.010	0.004	2.0	NM_000019
37	218109_s_at	MFSD1	major facilitator superfamily domain containing 1	0.001	0.000	2.0	NM_022736
38	204835_at	POLA1	polymerase (DNA directed), alpha 1, catalytic subunit	0.029	0.008	2.0	NM_016937
39	221570_s_at	METTL5	methyltransferase like 5	0.019	0.007	2.0	NM_014168
40	36553_at	ASMTL	acetylserotonin O-methyltransferase-like	0.014	0.004	2.0	NM_004192 /// XM_001713702
41	201931_at	ETFA	electron-transfer-flavoprotein, alpha polypeptide	0.011	0.006	1.9	NM_000126 /// NM_001127716
42	203075_at	SMAD2	SMAD family member 2	0.030	0.009	1.9	NM_001003652 /// NM_001135937 /// NM_005901
43	220990_s_at	MIR21 /// TMEM49	microRNA 21 /// transmembrane protein 49	0.017	0.006	1.9	NM_030938
44	208002_s_at	ACOT7	acyl-CoA thioesterase 7	0.003	0.001	1.9	NM_007274 /// NM_181864 /// NM_181865 /// NM_181866
45	202546_at	VAMP8	vesicle-associated membrane protein 8 (endobrevin)	0.029	0.009	1.9	NM_003761
46	203126_at	IMPA2	inositol(myo)-1(or 4)-monophosphatase 2	0.003	0.007	1.9	NM_014214
47	204069_at	MEIS1	Meis homeobox 1	0.004	0.006	1.9	NM_002398
48	202854_at	HPRT1	hypoxanthine phosphoribosyltransferase 1	0.005	0.001	1.9	NM_000194
49	213599_at	OIP5	Opa interacting protein 5	0.014	0.006	1.9	NM_007280
50	220761_s_at	TAOK3	TAO kinase 3	0.004	0.002	1.9	NM_016281
51	201459_at	RUVBL2	RuvB-like 2 (E. coli)	0.007	0.002	1.9	NM_006666
52	202144_s_at	ADSL	adenylosuccinate lyase	0.003	0.001	1.8	NM_000026 /// NM_001123378
53	218946_at	NFU1	NFU1 iron-sulfur cluster scaffold homolog (S. cerevisiae)	0.001	0.000	1.8	NM_001002755 /// NM_001002756 /// NM_001002757 /// NM_015700
54	211761_s_at	CACYBP	calcyclin binding protein	0.030	0.010	1.8	NM_001007214 /// NM_014412
55	211932_at	HNRNPA3	heterogeneous nuclear ribonucleoprotein A3	0.008	0.003	1.8	NM_194247
56	208955_at	DUT	deoxyuridine triphosphatase	0.005	0.003	1.8	NM_001025248 /// NM_001025249 /// NM_001948

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57	203282_at	GBE1	glucan (1,4-alpha-), branching enzyme 1	0.013	0.008	1.8	NM_000158
58	202370_s_at	CBFB	core-binding factor, beta subunit	0.002	0.002	1.8	NM_001755 /// NM_022845
59	209384_at	PROSC	proline synthetase co-transcribed homolog (bacterial)	0.018	0.005	1.8	NM_007198
60	205077_s_at	PIGF	phosphatidylinositol glycan anchor biosynthesis, class F	0.002	0.001	1.8	NM_002643 /// NM_173074
61	214259_s_at	AKR7A2	aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)	0.003	0.001	1.8	NM_003689
62	219293_s_at	OLA1	Obg-like ATPase 1	0.002	0.002	1.8	NM_001011708 /// NM_013341
63	209123_at	QDPR	quinoid dihydropteridine reductase	0.010	0.003	1.8	NM_000320
64	204497_at	ADCY9	adenylate cyclase 9	0.000	0.002	1.8	NM_001116
65	211933_s_at	HNRNPA3 /// HNRNPA3P1	heterogeneous nuclear ribonucleoprotein A3 /// heterogeneous nuclear ribonucleop	0.003	0.001	1.8	NM_194247 /// NR_002726
66	201832_s_at	USO1	USO1 homolog, vesicle docking protein (yeast)	0.029	0.009	1.8	NM_003715
67	201569_s_at	SAMM50	sorting and assembly machinery component 50 homolog (S. cerevisiae)	0.010	0.003	1.8	NM_015380
68	213603_s_at	RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protei	0.008	0.007	1.8	NM_002872
69	217869_at	HSD17B12	hydroxysteroid (17-beta) dehydrogenase 12	0.013	0.004	1.8	NM_016142
70	202589_at	TYMS	thymidylate synthetase	0.020	0.009	1.8	NM_001071
71	203359_s_at	MYCBP	c-myc binding protein	0.008	0.002	1.8	NM_012333
72	218592_s_at	CECR5	cat eye syndrome chromosome region, candidate 5	0.028	0.009	1.8	NM_017829 /// NM_033070
73	204119_s_at	ADK	adenosine kinase	0.019	0.006	1.8	NM_001123 /// NM_006721
74	200812_at	CCT7	chaperonin containing TCP1, subunit 7 (eta)	0.011	0.007	1.8	NM_001009570 /// NM_006429
75	209702_at	FTO	fat mass and obesity associated	0.019	0.010	1.8	NM_001080432
76	221622_s_at	TMEM126B	transmembrane protein 126B	0.005	0.001	1.8	NM_018480
77	201209_at	HDAC1	histone deacetylase 1	0.022	0.006	1.7	NM_004964
78	219100_at	OBFC1	oligonucleotide/oligosaccharide-binding fold containing 1	0.001	0.000	1.7	NM_024928
79	209177_at	NDUFAF3	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 3	0.029	0.008	1.7	NM_199069 /// NM_199070 /// NM_199073 /// NM_199074 /// NM_199417
80	218919_at	ZFAND1	zinc finger, AN1-type domain 1	0.016	0.005	1.7	NM_024699
81	209444_at	RAP1GDS1	RAP1, GTP-GDP dissociation stimulator 1	0.012	0.004	1.7	NM_001100426 /// NM_001100427 /// NM_001100428 /// NM_001100429 /// NM_001100430
82	209272_at	NAB1	NGFI-A binding protein 1 (EGR1 binding protein 1)	0.002	0.005	1.7	NM_005966
83	219505_at	CECR1	cat eye syndrome chromosome region, candidate 1	0.025	0.007	1.7	NM_017424 /// NM_177405
84	204479_at	OSTF1	osteoclast stimulating factor 1	0.002	0.000	1.7	NM_012383
85	203781_at	MRPL33	mitochondrial ribosomal protein L33	0.002	0.001	1.7	NM_004891 /// NM_145330
86	200728_at	ACTR2	ARP2 actin-related protein 2 homolog (yeast)	0.018	0.005	1.7	NM_001005386 /// NM_005722
87	204386_s_at	MRP63	mitochondrial ribosomal protein 63	0.005	0.003	1.7	NM_024026
88	203095_at	MTIF2	mitochondrial translational initiation factor 2	0.004	0.004	1.7	NM_001005369 /// NM_002453
89	213616_at	C18orf10	chromosome 18 open reading frame 10	0.003	0.002	1.7	NM_015476
90	219373_at	DPM3	dolichyl-phosphate mannosyltransferase polypeptide 3	0.006	0.003	1.7	NM_018973 /// NM_153741
91	203621_at	NDUFB5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5, 16kDa	0.010	0.007	1.7	NM_002492
92	202942_at	ETFB	electron-transfer-flavoprotein, beta polypeptide	0.002	0.001	1.7	NM_001014763 /// NM_001985
93	209509_s_at	DPAGT1	dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminephosphotransfera	0.013	0.005	1.7	NM_001382 /// NM_203316
94	218870_at	ARHGAP15	Rho GTPase activating protein 15	0.018	0.008	1.7	NM_018460
95	201774_s_at	NCAPD2	non-SMC condensin I complex, subunit D2	0.015	0.004	1.7	NM_014865
96	210276_s_at	TRIOBP	TRIO and F-actin binding protein	0.003	0.001	1.7	NM_001039141 /// NM_007032 /// NM_138632
97	213733_at	MYO1F	myosin IF	0.009	0.007	1.7	NM_012335
98	217286_s_at	NDRG3	NDRG family member 3	0.022	0.006	1.7	NM_022477 /// NM_032013
99	205692_s_at	CD38	CD38 molecule	0.019	0.005	1.7	NM_001775
100	215411_s_at	TRAF3IP2	TRAF3 interacting protein 2	0.004	0.001	1.7	NM_147200 /// NM_147686
101	217118_s_at	C22orf9	chromosome 22 open reading frame 9	0.003	0.001	1.7	NM_001009880 /// NM_015264
102	209206_at	SEC22B	SEC22 vesicle trafficking protein homolog B (S. cerevisiae)	0.005	0.002	1.7	NM_004892
103	218654_s_at	MRPS33	mitochondrial ribosomal protein S33	0.000	0.000	1.7	NM_016071 /// NM_053035
104	218698_at	APIP	APAF1 interacting protein	0.016	0.006	1.7	NM_015957
105	201007_at	HADHB	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzy	0.000	0.000	1.7	NM_000183
106	209472_at	CCBL2	cysteine conjugate-beta lyase 2	0.030	0.009	1.7	NM_001008661 /// NM_001008662 /// NM_019610
107	212552_at	HPCAL1	hippocalcin-like 1	0.004	0.001	1.7	NM_002149 /// NM_134421
108	204646_at	DPYD	dihydropyrimidine dehydrogenase	0.015	0.008	1.7	NM_000110
109	217990_at	GMPR2	guanosine monophosphate reductase 2	0.013	0.004	1.7	NM_001002000 /// NM_001002001 /// NM_001002002 /// NM_016576
110	217989_at	HSD17B11	hydroxysteroid (17-beta) dehydrogenase 11	0.011	0.003	1.7	NM_016245
111	213379_at	COQ2	coenzyme Q2 homolog, prenyltransferase (yeast)	0.001	0.001	1.7	NM_015697
112	203494_s_at	CEP57	centrosomal protein 57kDa	0.003	0.001	1.7	NM_014679
113	208745_at	ATP5L	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G	0.000	0.001	1.7	NM_006476
114	208816_x_at	ANXA2P2	annexin A2 pseudogene 2	0.022	0.007	1.7	NR_003573
115	221488_s_at	CUTA	cutA divalent cation tolerance homolog (E. coli)	0.016	0.004	1.7	NM_001014433 /// NM_001014837 /// NM_001014838 /// NM_001014840 /// NM_015921
116	201268_at	NME1 /// NME1-NME2 /// NME2	non-metastatic cells 1, protein (NM23A) expressed in /// NME1-NME2 readthrough t	0.029	0.009	1.7	NM_000269 /// NM_001018136 /// NM_001018137 /// NM_001018138 /// NM_001018139 //
117	205726_at	DIAPH2	diaphanous homolog 2 (Drosophila)	0.003	0.001	1.7	NM_006729 /// NM_007309
118	218493_at	SNRNP25	small nuclear ribonucleoprotein 25kDa (U11/U12)	0.005	0.002	1.6	NM_024571

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119	212481_s_at	TPM4	tropomyosin 4	0.019	0.005	1.6	NM_001145160 /// NM_003290
120	202020_s_at	LANCL1	LanC lantibiotic synthetase component C-like 1 (bacterial)	0.000	0.004	1.6	NM_001136574 /// NM_001136575 /// NM_006055
121	208885_at	LCP1	lymphocyte cytosolic protein 1 (L-plastin)	0.003	0.002	1.6	NM_002298
122	207467_x_at	CAST	calpastatin	0.008	0.002	1.6	NM_001042440 /// NM_001042441 /// NM_001042442 /// NM_001042443 /// NM_001042444
123	201275_at	FDPS	farnesyl diphosphate synthase (farnesyl pyrophosphate synthetase, dimethylallylt	0.014	0.004	1.6	NM_001135821 /// NM_001135822 /// NM_002004
124	212687_at	LIMS1	LIM and senescent cell antigen-like domains 1	0.001	0.006	1.6	NM_004987
125	217854_s_at	POLR2E	polymerase (RNA) II (DNA directed) polypeptide E, 25kDa	0.013	0.004	1.6	NM_002695
126	201231_s_at	ENO1	enolase 1, (alpha)	0.030	0.008	1.6	NM_001428
127	215380_s_at	GGCT	gamma-glutamyl cyclotransferase	0.004	0.001	1.6	NM_024051
128	209659_s_at	CDC16	cell division cycle 16 homolog (S. cerevisiae)	0.004	0.001	1.6	NM_001078645 /// NM_003903
129	203837_at	MAP3K5	mitogen-activated protein kinase kinase kinase 5	0.015	0.005	1.6	NM_005923
130	204185_x_at	PPID	peptidylprolyl isomerase D	0.018	0.007	1.6	NM_005038
131	214096_s_at	SHMT2	serine hydroxymethyltransferase 2 (mitochondrial)	0.004	0.001	1.6	NM_005412
132	202529_at	PRPSAP1	phosphoribosyl pyrophosphate synthetase-associated protein 1	0.023	0.006	1.6	NM_002766
133	219043_s_at	LOC285359 /// PDCL3	phosducin-like 3 pseudogene /// phosducin-like 3	0.007	0.008	1.6	NM_024065 /// NR_002941
134	203537_at	PRPSAP2	phosphoribosyl pyrophosphate synthetase-associated protein 2	0.009	0.002	1.6	NM_002767
135	200955_at	IMMT	inner membrane protein, mitochondrial (mitofilin)	0.001	0.001	1.6	NM_001100169 /// NM_001100170 /// NM_006839
136	200846_s_at	PPP1CA	protein phosphatase 1, catalytic subunit, alpha isoform	0.013	0.004	1.6	NM_001008709 /// NM_002708 /// NM_206873
137	217870_s_at	CMPK1	cytidine monophosphate (UMP-CMP) kinase 1, cytosolic	0.016	0.004	1.6	NM_001136140 /// NM_016308
138	203696_s_at	RFC2	replication factor C (activator 1) 2, 40kDa	0.002	0.001	1.6	NM_002914 /// NM_181471
139	209083_at	CORO1A	coronin, actin binding protein, 1A	0.000	0.007	1.6	NM_007074
140	218288_s_at	CCDC90B	coiled-coil domain containing 90B	0.010	0.008	1.6	NM_021825
141	218375_at	NUDT9	nudix (nucleoside diphosphate linked moiety X)-type motif 9	0.019	0.007	1.6	NM_024047 /// NM_198038
142	218351_at	COMMD8	COMM domain containing 8	0.024	0.010	1.6	NM_017845
143	213045_at	MAST3	microtubule associated serine/threonine kinase 3	0.001	0.000	1.6	NM_015016
144	217726_at	COPZ1	coatomer protein complex, subunit zeta 1	0.002	0.001	1.6	NM_016057
145	205053_at	PRIM1	primase, DNA, polypeptide 1 (49kDa)	0.005	0.010	1.6	NM_000946
146	219079_at	CYB5R4	cytochrome b5 reductase 4	0.031	0.009	1.6	NM_016230
147	218153_at	CARS2	cysteinyl-tRNA synthetase 2, mitochondrial (putative)	0.005	0.001	1.6	NM_024537
148	221564_at	PRMT2	protein arginine methyltransferase 2	0.002	0.001	1.6	NM_001535 /// NM_206962
149	201274_at	PSMA5	proteasome (prosome, macropain) subunit, alpha type, 5	0.007	0.009	1.6	NM_002790
150	215096_s_at	ESD	esterase D/formylglutathione hydrolase	0.010	0.009	1.5	NM_001984
151	206752_s_at	DFFB	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase)	0.013	0.003	1.5	NM_004402
152	221726_at	RPL22	ribosomal protein L22	0.005	0.003	1.5	NM_000983
153	211703_s_at	TM2D1	TM2 domain containing 1	0.005	0.001	1.5	NM_032027
154	201818_at	LPCAT1	lysophosphatidylcholine acyltransferase 1	0.014	0.006	1.5	NM_024830 /// XM_001717124 /// XM_001720496
155	204821_at	BTN3A3	butyrophilin, subfamily 3, member A3	0.028	0.008	1.5	NM_006994 /// NM_197974
156	219055_at	SRBD1	S1 RNA binding domain 1	0.003	0.005	1.5	NM_018079
157	212936_at	FAM172A	family with sequence similarity 172, member A	0.017	0.004	1.5	NM_032042
158	205126_at	VRK2	vaccinia related kinase 2	0.019	0.005	1.5	NM_001130480 /// NM_001130481 /// NM_001130482 /// NM_001130483 /// NM_001136027
159	213365_at	ERI2	exoribonuclease 2	0.008	0.003	1.5	NM_001142725 /// NM_080663
160	222056_s_at	FAHD2A	fumarylacetoacetate hydrolase domain containing 2A	0.005	0.002	1.5	NM_016044
161	209833_at	CRADD	CASP2 and RIPK1 domain containing adaptor with death domain	0.000	0.005	1.5	NM_003805
162	200877_at	CCT4	chaperonin containing TCP1, subunit 4 (delta)	0.004	0.004	1.5	NM_006430
163	213698_at	ZMYM6	zinc finger, MYM-type 6	0.016	0.004	1.5	NM_007167
164	218158_s_at	APPL1	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper conta	0.011	0.009	1.5	NM_012096
165	204033_at	TRIP13	thyroid hormone receptor interactor 13	0.015	0.005	1.5	NM_004237
166	218450_at	HEBP1	heme binding protein 1	0.028	0.008	1.5	NM_015987
167	200006_at	PARK7	Parkinson disease (autosomal recessive, early onset) 7	0.023	0.010	1.5	NM_001123377 /// NM_007262
168	208658_at	PDIA4	protein disulfide isomerase family A, member 4	0.019	0.005	1.5	NM_004911
169	208667_s_at	ST13	suppression of tumorigenicity 13 (colon carcinoma) (Hsp70 interacting protein)	0.004	0.008	1.5	NM_003932
170	213838_at	NOL7	nucleolar protein 7, 27kDa	0.006	0.002	1.5	NM_016167
171	218549_s_at	FAM82B	family with sequence similarity 82, member B	0.011	0.003	1.5	NM_016033
172	213322_at	C6orf130	chromosome 6 open reading frame 130	0.016	0.004	1.5	NM_145063
173	201331_s_at	STAT6	signal transducer and activator of transcription 6, interleukin-4 induced	0.021	0.006	1.5	NM_003153
174	209485_s_at	OSBPL1A	oxysterol binding protein-like 1A	0.013	0.004	1.5	NM_018030 /// NM_080597
175	218253_s_at	LGTN	ligatin	0.006	0.002	1.5	NM_006893
176	203409_at	DDB2	damage-specific DNA binding protein 2, 48kDa	0.006	0.004	1.5	NM_000107
177	201764_at	TMEM106C	transmembrane protein 106C	0.012	0.003	1.5	NM_001143841 /// NM_001143842 /// NM_001143843 /// NM_024056
178	213333_at	MDH2	malate dehydrogenase 2, NAD (mitochondrial)	0.009	0.003	1.5	NM_005918
179	211623_s_at	FBL	fibrillarin	0.025	0.007	1.5	NM_001436
180	202306_at	POLR2G	polymerase (RNA) II (DNA directed) polypeptide G	0.024	0.009	1.5	NM_002696

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181	209248_at	GHITM	growth hormone inducible transmembrane protein	0.021	0.009	1.4	NM_014394
182	204168_at	MGST2	microsomal glutathione S-transferase 2	0.017	0.006	1.4	NM_002413
183	218923_at	CTBS	chitobiase, di-N-acetyl-	0.005	0.001	1.4	NM_004388
184	218567_x_at	DPP3	dipeptidyl-peptidase 3	0.030	0.009	1.4	NM_005700 /// NM_130443
185	217751_at	GSTK1	glutathione S-transferase kappa 1	0.027	0.009	1.4	NM_001143679 /// NM_001143680 /// NM_001143681 /// NM_015917
186	207002_s_at	PLAGL1	pleiomorphic adenoma gene-like 1	0.025	0.008	1.4	NM_001080951 /// NM_001080952 /// NM_001080953 /// NM_001080954 /// NM_001080955
187	202487_s_at	H2AFV	H2A histone family, member V	0.002	0.002	1.4	NM_012412 /// NM_138635 /// NM_201436 /// NM_201516 /// NM_201517
188	217874_at	SUCLG1	succinate-CoA ligase, alpha subunit	0.009	0.004	1.4	NM_003849
189	201553_s_at	LAMP1	lysosomal-associated membrane protein 1	0.008	0.002	1.4	NM_005561
190	217818_s_at	ARPC4	actin related protein 2/3 complex, subunit 4. 20kDa	0.008	0.002	1.4	NM_001024959 /// NM_001024960 /// NM_005718
191	45828_at	ATP5SL	ATP5S-like	0.005	0.002	1.4	NM_018035
192	202209_at	LSM3	LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae)	0.026	0.009	1.4	NM_014463
193	202239_at	PARP4	poly (ADP-ribose) polymerase family, member 4	0.025	0.008	1.4	NM_006437
194	216241_s_at	TCEA1	transcription elongation factor A (SII), 1	0.017	0.006	1.4	NM_006756 /// NM_201437
195	202338_at	TK1	thymidine kinase 1, soluble	0.019	0.005	1.4	NM_003258
196	219307_at	PDSS2	prenyl (decaprenyl) diphosphate synthase, subunit 2	0.022	0.009	1.4	NM_020381
197	222230_s_at	ACTR10	actin-related protein 10 homolog (S. cerevisiae)	0.023	0.008	1.4	NM_018477
198	203402_at	KCNAB2	potassium voltage-gated channel, shaker-related subfamily, beta member 2	0.016	0.004	1.4	NM_003636 /// NM_172130
199	202564_x_at	ARL2	ADP-ribosylation factor-like 2	0.023	0.010	1.4	NM_001667
200	219204_s_at	SRR	serine racemase	0.023	0.007	1.4	NM_021947
201	222129_at	FAM134A	family with sequence similarity 134, member A	0.026	0.008	1.4	NM_024293
202	212331_at	RBL2	retinoblastoma-like 2 (p130)	0.016	0.004	1.4	NM_005611
203	201290_at	SEC11A	SEC11 homolog A (S. cerevisiae)	0.004	0.001	1.4	NM_014300
204	202223_at	STT3A	STT3, subunit of the oligosaccharyltransferase complex, homolog A (S. cerevisiae	0.032	0.009	1.4	NM_152713
205	213454_at	APITD1	apoptosis-inducing, TAF9-like domain 1	0.012	0.007	1.4	NM_198544 /// NM_199294 /// NM_199295
206	201319_at	MYL12A	myosin, light chain 12A, regulatory, non-sarcomeric	0.005	0.001	1.4	NM_006471
207	201114_x_at	PSMA7	proteasome (prosome, macropain) subunit, alpha type, 7	0.009	0.006	1.4	NM_002792
208	209549_s_at	DGUOK	deoxyguanosine kinase	0.006	0.002	1.4	NM_080916 /// NM_080918
209	218471_s_at	BBS1	Bardet-Biedl syndrome 1	0.002	0.001	1.4	NM_024649
210	201433_s_at	PTDSS1	phosphatidylserine synthase 1	0.011	0.003	1.4	NM_014754
211	201318_s_at	MYL12A /// MYL12B	myosin, light chain 12A, regulatory, non-sarcomeric /// myosin, light chain 12B,	0.010	0.003	1.4	NM_001144944 /// NM_001144945 /// NM_001144946 /// NM_006471 /// NM_033546
212	217383_at	PGK1	Phosphoglycerate kinase 1	0.020	0.008	1.4	NM_000291
213	204126_s_at	CDC45L	CDC45 cell division cycle 45-like (S. cerevisiae)	0.025	0.008	1.4	NM_003504
214	202847_at	PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	0.024	0.007	1.4	NM_001018073 /// NM_004563
215	208107_s_at	LOC81691	exonuclease NEF-sp	0.002	0.001	1.4	NM_001144924 /// NM_030941
216	217794_at	PRR13	proline rich 13	0.002	0.001	1.4	NM_001005354 /// NM_018457
217	221972_s_at	SDF4	stromal cell derived factor 4	0.026	0.009	1.4	NM_016176 /// NM_016547
218	218488_at	EIF2B3	eukaryotic translation initiation factor 2B, subunit 3 gamma, 58kDa	0.020	0.008	1.4	NM_020365
219	200001_at	CAPNS1	calpain, small subunit 1	0.003	0.003	1.4	NM_001003962 /// NM_001749
220	210589_s_at	GBAP	glucosidase, beta; acid, pseudogene	0.002	0.003	1.4	NR_002188 /// XR_042183
221	218021_at	DHRS4 /// DHRS4L2	dehydrogenase/reductase (SDR family) member 4 /// dehydrogenase/reductase (SDR f	0.033	0.010	1.4	NM_021004 /// NM_198083
222	201576_s_at	GLB1	galactosidase, beta 1	0.020	0.008	1.4	NM_000404 /// NM_001079811 /// NM_001135602
223	210849_s_at	VPS41	vacuolar protein sorting 41 homolog (S. cerevisiae)	0.013	0.009	1.4	NM_014396 /// NM_080631
224	218568_at	AGK	acylglycerol kinase	0.005	0.002	1.4	NM_018238
225	220985_s_at	RNF170	ring finger protein 170	0.010	0.003	1.4	NM_030954
226	44120_at	ADCK2	aarF domain containing kinase 2	0.005	0.002	1.4	NM_052853
227	203299_s_at	AP1S2	adaptor-related protein complex 1, sigma 2 subunit	0.001	0.000	1.4	NM_003916
228	207623_at	ABCF2	ATP-binding cassette, sub-family F (GCN20), member 2	0.001	0.000	1.4	NM_005692 /// NM_007189
229	209734_at	NCKAP1L	NCK-associated protein 1-like	0.004	0.006	1.4	NM_005337
230	218741_at	CENPM	centromere protein M	0.016	0.007	1.4	NM_001002876 /// NM_001110215 /// NM_024053
231	213626_at	CBR4	carbonyl reductase 4	0.012	0.005	1.4	NM_032783
232	209608_s_at	ACAT2	acetyl-Coenzyme A acetyltransferase 2	0.012	0.003	1.4	NM_005891
233	203656_at	FIG4	FIG4 homolog (S. cerevisiae)	0.012	0.004	1.4	NM_014845
234	207143_at	CDK6	cyclin-dependent kinase 6	0.005	0.001	1.4	NM_001145306 /// NM_001259
235	213475_s_at	ITGAL	Integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen	0.011	0.008	1.4	NM_001114380 /// NM_002209
236	202159_at	FARSA	phenylalanyl-tRNA synthetase, alpha subunit	0.019	0.006	1.4	NM_004461
237	214765_s_at	NAAA	N-acylethanolamine acid amidase	0.025	0.008	1.4	NM_001042402 /// NM_014435
238	203270_at	DTYMK	deoxythymidylate kinase (thymidylate kinase)	0.005	0.003	1.4	NM_012145
239	210817_s_at	CALCOCO2	calcium binding and coiled-coil domain 2	0.032	0.009	1.4	NM_005831
240	214037_s_at	CCDC22	coiled-coil domain containing 22	0.012	0.004	1.4	NM_014008
241	208631_s_at	HADHA	hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzy	0.019	0.005	1.3	NM_000182
242	200793_s_at	ACO2	aconitase 2, mitochondrial	0.003	0.002	1.3	NM_001098
243	221104_s_at	NIPSNAP3B	nipsnap homolog 3B (C. elegans)	0.000	0.009	1.3	NM_018376
244	213822_s_at	UBE3B	ubiquitin protein ligase E3B	0.005	0.007	1.3	NM_130466 /// NM_183415

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245	204040_at	RNF144A	ring finger protein 144A	0.014	0.007	1.3	NM_014746
246	218809_at	PANK2	pantothenate kinase 2	0.005	0.001	1.3	NM_024960 /// NM_153638 /// NM_153640
247	219109_at	SPAG16	sperm associated antigen 16	0.007	0.009	1.3	NM_001025436 /// NM_024532
248	214482_at	ZBTB25	zinc finger and BTB domain containing 25	0.020	0.006	1.3	NM_006977
249	201494_at	PRCP	prolylcarboxypeptidase (angiotensinase C)	0.002	0.002	1.3	NM_005040 /// NM_199418
250	203257_s_at	C11orf49	chromosome 11 open reading frame 49	0.000	0.001	1.3	NM_001003676 /// NM_001003677 /// NM_001003678 /// NM_024113
251	221495_s_at	TCF25	transcription factor 25 (basic helix-loop-helix)	0.014	0.006	1.3	NM_014972
252	218838_s_at	TTC31	tetratricopeptide repeat domain 31	0.016	0.004	1.3	NM_022492
253	209665_at	CYB561D2	cytochrome b-561 domain containing 2	0.000	0.000	1.3	NM_007022
254	204808_s_at	TMEM5	transmembrane protein 5	0.005	0.009	1.3	NM_014254
255	222105_s_at	NKIRAS2	NFKB inhibitor interacting Ras-like 2	0.005	0.002	1.3	NM_001001349 /// NM_001144927 /// NM_001144928 /// NM_001144929 /// NM_017595
256	219036_at	CEP70	centrosomal protein 70kDa	0.011	0.004	1.3	NM_024491
257	201657_at	ARL1	ADP-ribosylation factor-like 1	0.000	0.000	1.3	NM_001177
258	219680_at	NLRX1	NLR family member X1	0.000	0.000	1.3	NM_024618 /// NM_170722
259	218124_at	RETSAT	retinol saturase (all-trans-retinol 13,14-reductase)	0.009	0.007	1.3	NM_017750
260	203014_x_at	SGSM3	small G protein signaling modulator 3	0.008	0.002	1.3	NM_015705
261	202795_x_at	TRIOBP	TRIO and F-actin binding protein	0.012	0.004	1.3	NM_001039141 /// NM_007032 /// NM_138632
262	209528_s_at	TELO2	TEL2, telomere maintenance 2, homolog (S. cerevisiae)	0.019	0.006	1.3	NM_016111
263	203740_at	MPHOSPH6	M-phase phosphoprotein 6	0.011	0.006	1.3	NM_005792
264	219060_at	WDYHV1	WDYHV motif containing 1	0.012	0.004	1.3	NM_018024
265	211536_x_at	MAP3K7	mitogen-activated protein kinase kinase kinase 7	0.015	0.004	1.3	NM_003188 /// NM_145331 /// NM_145332 /// NM_145333
266	218267_at	CINP	cyclin-dependent kinase 2-interacting protein	0.003	0.001	1.3	NM_032630
267	203500_at	GCDH	glutaryl-Coenzyme A dehydrogenase	0.014	0.004	1.3	NM_000159 /// NM_013976
268	211685_s_at	NCALD	neurocalcin delta	0.032	0.009	1.3	NM_001040624 /// NM_001040625 /// NM_001040626 /// NM_001040627 /// NM_001040628
269	205652_s_at	TTLL1	tubulin tyrosine ligase-like family, member 1	0.009	0.003	1.3	NM_001008572 /// NM_012263
270	218125_s_at	CCDC25	coiled-coil domain containing 25	0.003	0.002	1.3	NM_018246
271	221770_at	RPE	ribulose-5-phosphate-3-epimerase	0.009	0.003	1.3	NM_006916 /// NM_199229
272	201234_at	ILK	integrin-linked kinase	0.003	0.003	1.3	NM_001014794 /// NM_001014795 /// NM_004517

272 differentially up-regulated genes between the HEV, IEV and LEV groups are shown. P *value, Two-way ANOVA statistical analysis; fold change, between HEV and LEV, probeset ID, Affymetrix GeneChip® U133A; HEV, high expression* VLA-4 *group; IEV, intermediate expression* VLA-4 *group; LEV, low expression* VLA-4 *group.*