

Supplementary Material

1 Supplementary Figures

1.1 Figure S1: Reference card of EN2 lateral flow test.

- Hold the card and align it to the cassette and test line (T) and read the result.
- Do not use the card beyond expiration date. Do not use if damaged.
- This card must be used only with BCT tests of the LOT number written on the card.
- Store the card in the kit box away from the direct sunlight.

LIODetect® BCT Rapid Test
Reference card



LIONEX Diagnostics
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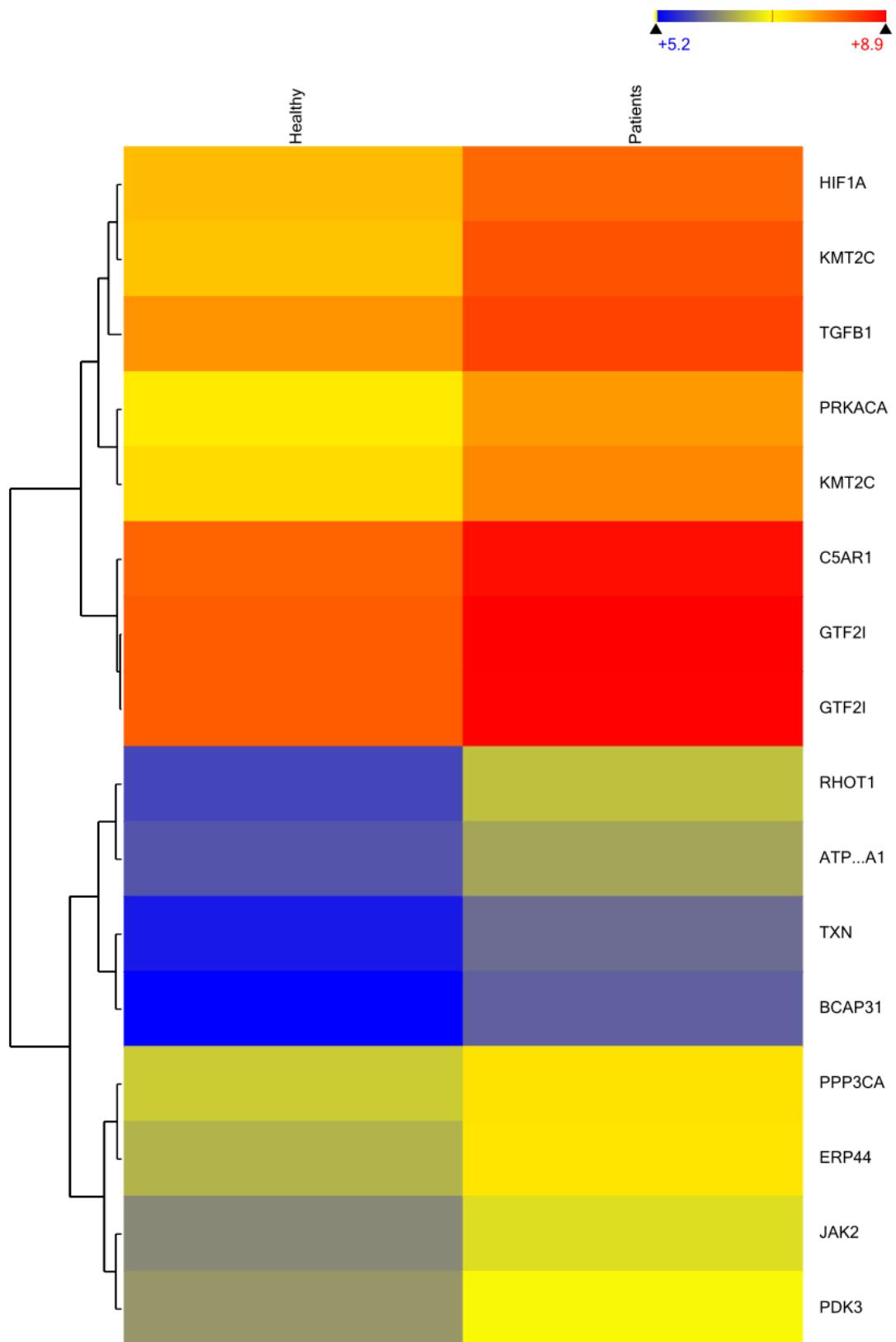
Salzdahlumer Str. 196

38124 Braunschweig, GERMANY

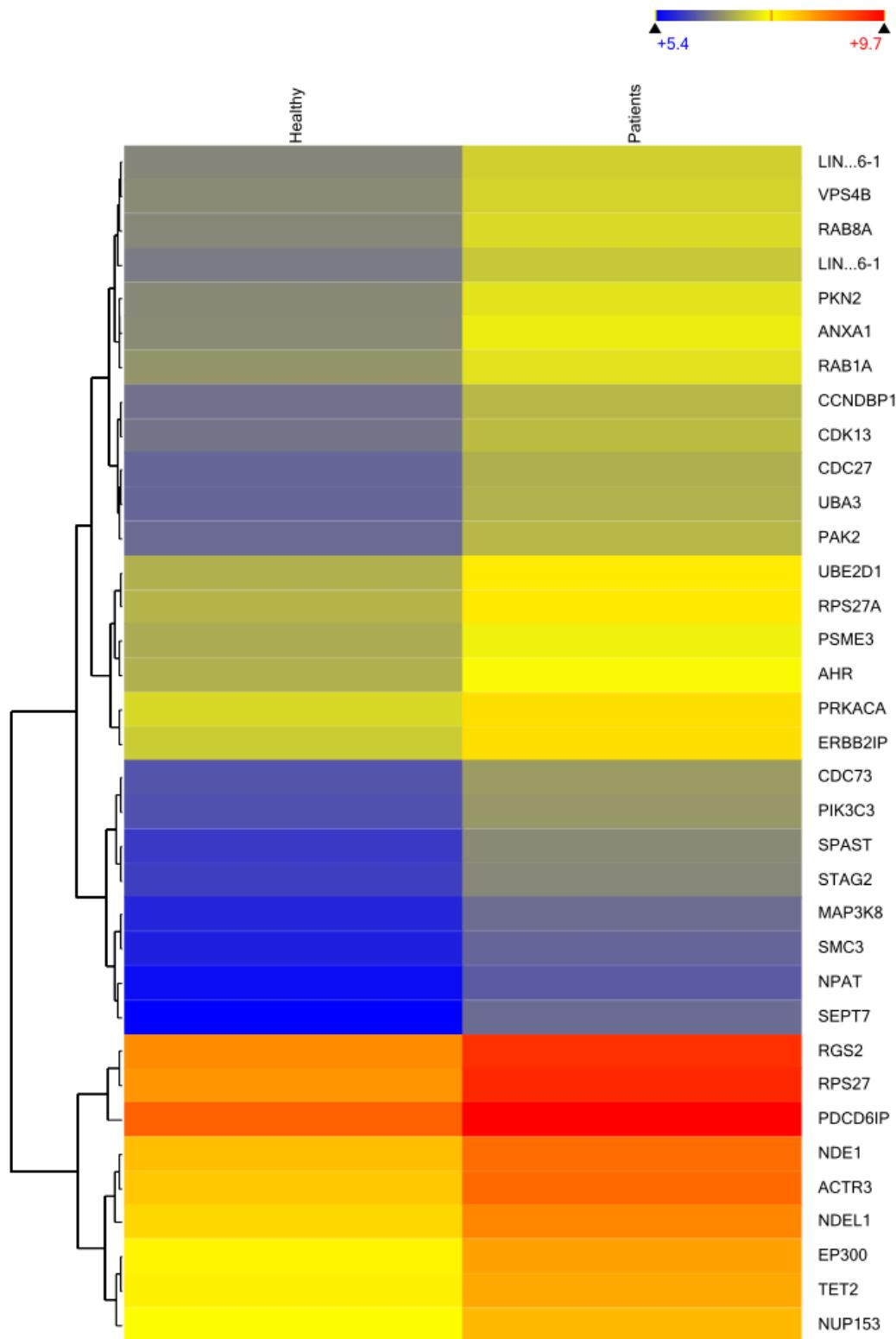
info@lionex.de /www.lionex.de

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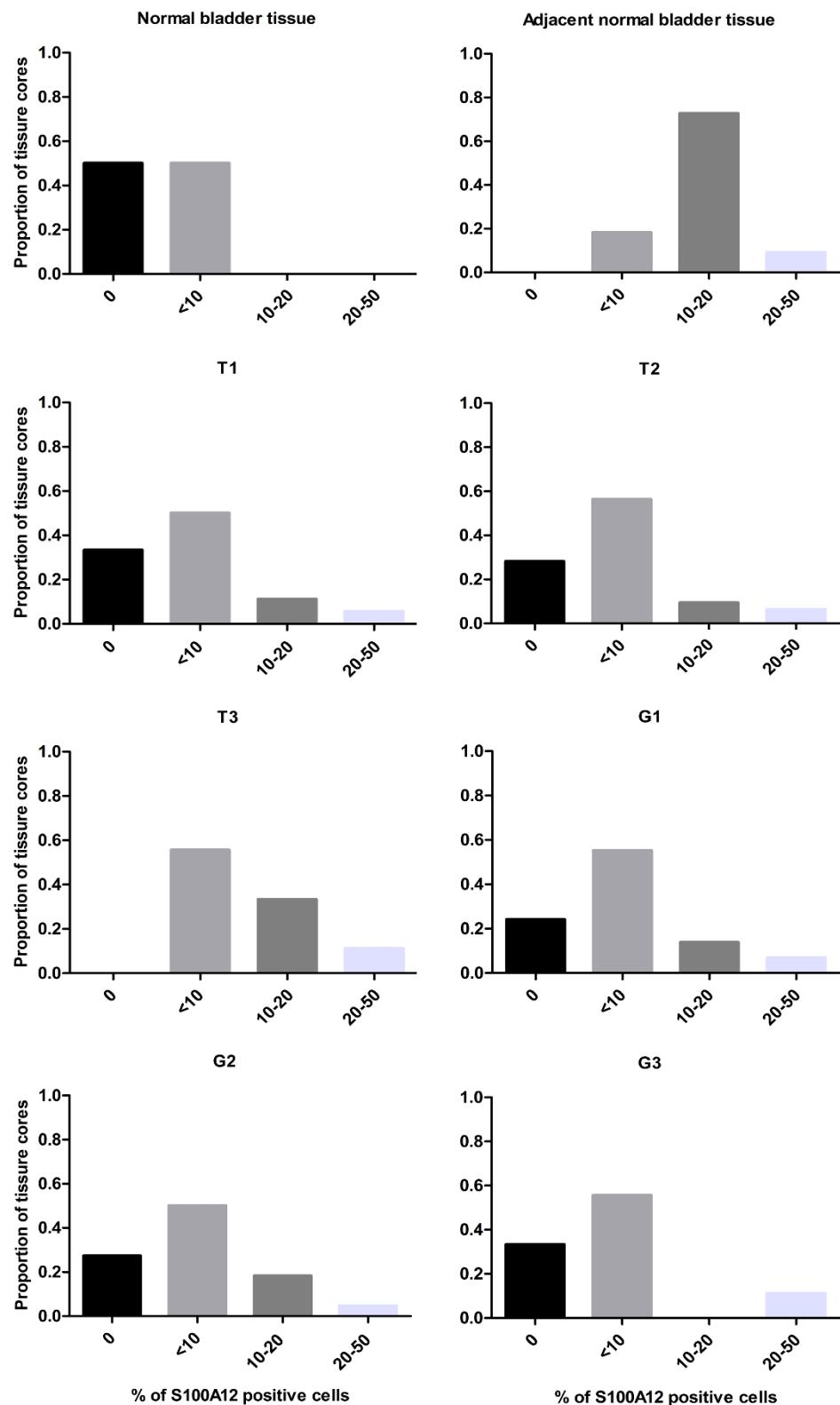
REF BCT_10_EN LOT IXXXXXX 2021-01-25

1.2 Figure S2: Hierarchical clustering of significantly gene expression across the list of the cellular homeostasis targets.

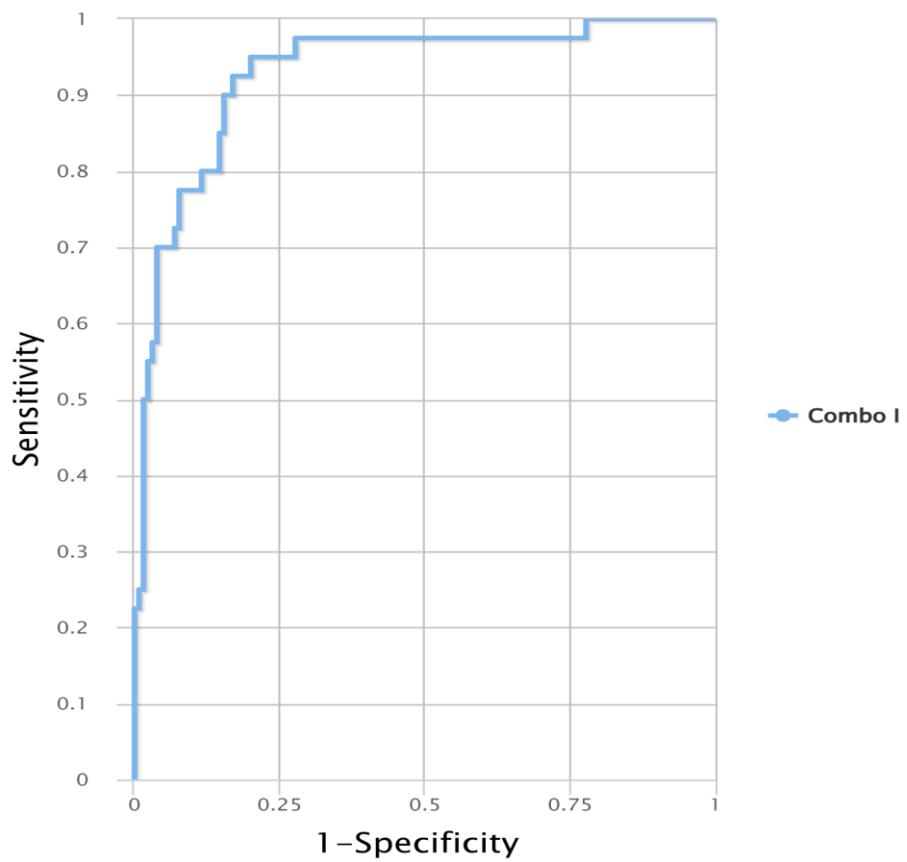
1.3 Figure S3: Hierarchical clustering of significantly gene expression of the list of the cell cycle genes ranked according to their p-values using the Gene Ontology (GO) Enrichment tool.



1.4 Figure S4: Immunohistochemical analysis of S100A12 expression. The percentages of positive cells for S100A12 for stage and grade groups in bladder cancer tissue array.



1.5 Figure S5: A ROC analysis of S100A12 and EN2 as combination using CombiROC method.



2 Supplementary Tables

2.1 **Table S1:** Primers used in the qPCR analysis.

Gene Symbol	Type	Sequence (5'-3')	Amplicon size [bp]
<i>S100A12</i>	Forward	GCAAACACCATCAAGAATATCAA	119
	Reverse	GCAATGGCTACCAAGGGATA	
<i>S100A8</i>	Forward	GCCAAGCCTAACCGCTATAA	82
	Reverse	CCCACCCAGGTCTTCTGAAAG	
<i>NAMPT</i>	Forward	TAT TGC CTT CGG TTC TGG TG	139
	Reverse	TTT GTT GGG ATC AGC AAC TG	
<i>JUP</i>	Forward	AAC CAG GAG AGC AAG CTG AT	128
	Reverse	CAC ACG GAT AGC ACC TTG AG	
<i>KLRF1</i>	Forward	ATG ACC AAC TTG AAA TGG CT	148
	Reverse	GGT CCC TTT ATG AAGAATATCTTG	
<i>PTGDR</i>	Forward	CTC TGC CCG TAA TTT ATC GC	110
	Reverse	CAC AGA TAG AAA TCG CAA GGC	
<i>GAPDH</i>	Forward	GGAAGGTGAAGGTCGGAGTC	101
	Reverse	GTCATTGATGGCAACAATATCCACT	

2.2 **Table S2:** Exclusive differentially regulated gene set between recurrent and new positive samples.

Gene Symbol	Gene description	Fold change	p-value
<i>AP001434.2</i>	lincRNA	2.22	<0.001
<i>MYBL1</i>	Myb-related protein A	1.67	0.001
<i>EEF1DP3</i>	pseudogene	1.64	<0.001
<i>RP11-841O20.2</i>	antisense RNA	1.59	<0.001
<i>FGRBP2</i>	fibroblast growth factor binding protein 2	1.59	<0.001
<i>SAMD3</i>	Sterile alpha motif domain-containing protein 3	1.53	<0.001
<i>MME-AS1</i>	antisense RNA	1.52	<0.001
<i>RP11-1143G9.4</i>	antisense RNA	1.51	<0.001

<i>MIR606</i>	miRNA	1.51	<0.001
<i>AFF3</i>	AF4/FMR2 family member 3	-1.50	0.001
<i>GTSF1L</i>	Gametocyte-specific factor 1-like	-1.50	<0.001
<i>ROR1</i>	Inactive tyrosine-protein kinase transmembrane receptor ROR1	-1.51	<0.001
<i>SLC9A7</i>	Sodium/hydrogen exchanger 7	-1.54	0.001
<i>RNU6-517P</i>	snRNA	-1.56	0.001
<i>MIR7641-2</i>	miRNA	-1.59	<0.001
<i>KLHL14</i>	Kelch-like protein 14	-1.63	<0.001
<i>CD200</i>	OX-2 membrane glycoprotein	-1.67	<0.001
<i>FCRL5</i>	Fc receptor-like protein 5	-1.68	<0.001

Data analysis was performed using the software Partek® Genomic Suite (The significant regulated genes were selected using ANOVA test with fold change >1.5 and fdr-corrected p-value <0.05).

2.3 **Table S3:** Differentially regulated gene set in risk group analysis.

Gene Symbol	Gene description	Fold change	p-value	Comparison
<i>JUP</i>	Junction plakoglobin	1,64	<0,001	High vs. Healthy
<i>RNU6-707P</i>	RNA, U6 small nuclear 707, pseudogene	1,66	0,001	Intermediate vs. Healthy
<i>S100A12</i>	Calcium binding Protein S100A12	1,61	0,001	Intermediate vs. Healthy
<i>CLEC12A</i>	C-type lectin domain family 12 member A	1,59	0,012	Intermediate vs. Healthy
<i>RNU6-237P</i>	RNA, U6 small nuclear 237, pseudogene	1,58	0,002	Intermediate vs. Healthy
<i>IGHV1-2</i>	immunoglobulin heavy variable 1-2	-1,51	0,004	Intermediate vs. Healthy
<i>TRAJ42</i>	T cell receptor alpha joining 42	-1,52	0,022	Intermediate vs. Healthy
<i>TRAJ56</i>	T cell receptor alpha joining 5	-1,53	0,027	Intermediate vs. Healthy
<i>IGKV1-17</i>	immunoglobulin kappa variable 1-17	-1,55	0,012	Intermediate vs. Healthy
<i>TRAJ19</i>	T cell receptor alpha joining 19	-1,56	0,043	Intermediate vs. Healthy

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<i>SNORA5A</i>	small nucleolar RNA, H/ACA box 5A	-1,58	0,003	Intermediate vs. Healthy
<i>HIST1H3H</i>	histone H3.1	-1,58	0,028	Intermediate vs. Healthy
<i>IGKV2-24</i>	immunoglobulin kappa variable 2-24	-1,59	0,020	Intermediate vs. Healthy
<i>TRDJ4</i>	T cell receptor delta joining 4	-1,63	0,0348	Intermediate vs. Healthy
<i>IGHG1</i>	Ig gamma-1 chain C region	-1,63	0,054	Intermediate vs. Healthy
<i>IGLV1-40</i>	immunoglobulin lambda variable 1-40	-1,64	0,011	Intermediate vs. Healthy
<i>TRDV1</i>	T cell receptor delta variable 1	-1,66	0,011	Intermediate vs. Healthy
<i>RNU4ATAc</i>	RNA, U4atac small nuclear (U12-dependent splicing	-1,67	0,057	Intermediate vs. Healthy
<i>KLRC3</i>	NKG2-E type II integral membrane protein	-1,71	0,004	Intermediate vs. Healthy
<i>TRAJ17</i>	T cell receptor alpha joining 1	-1,71	0,001	Intermediate vs. Healthy
<i>TRAJ29</i>	T cell receptor alpha joining 29	-1,80	0,001	Intermediate vs. Healthy