Modular transcriptional repertoire and MicroRNA target analyses characterize genomic dysregulation in the thymus of down syndrome infants

Supplementary Material

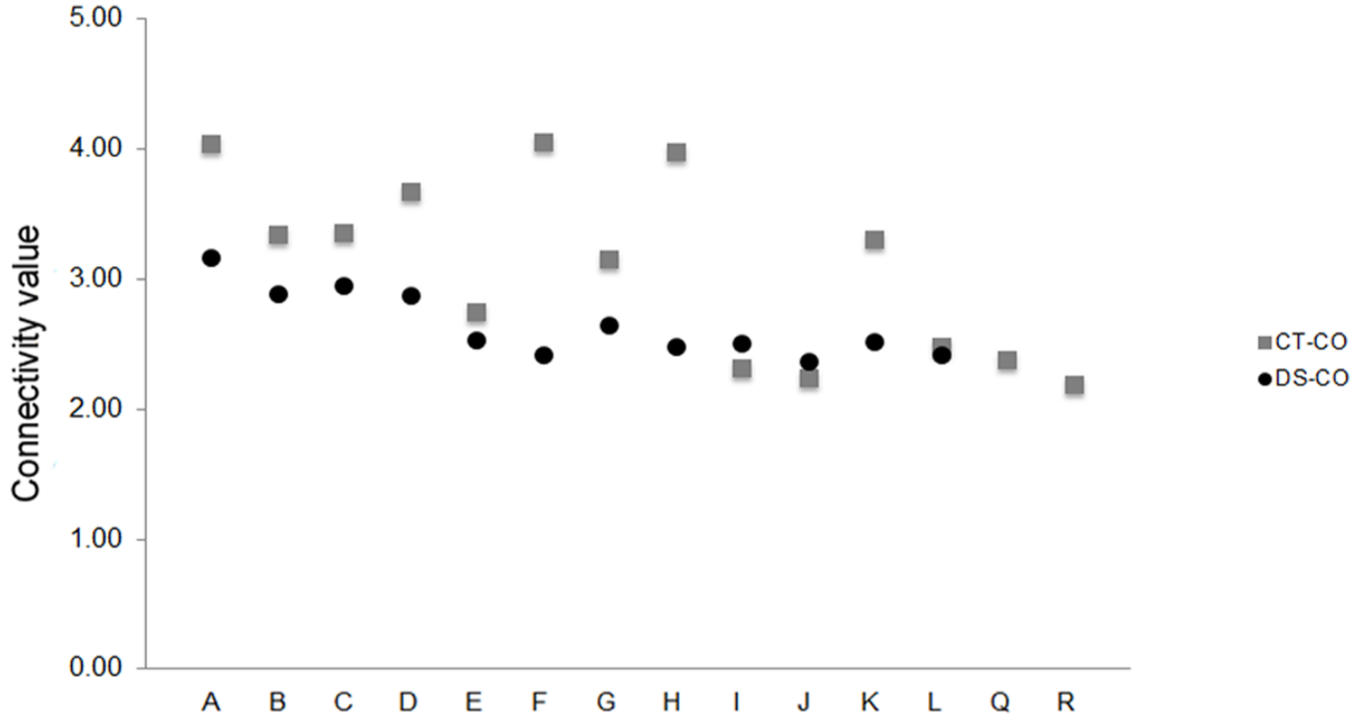


Figure S1. Community subnetwork connectivities in CO networks. Dispersion graph of connectivity values for communities' subnetworks in CT-CO (square dots) and DS-CO (circle dots) networks.

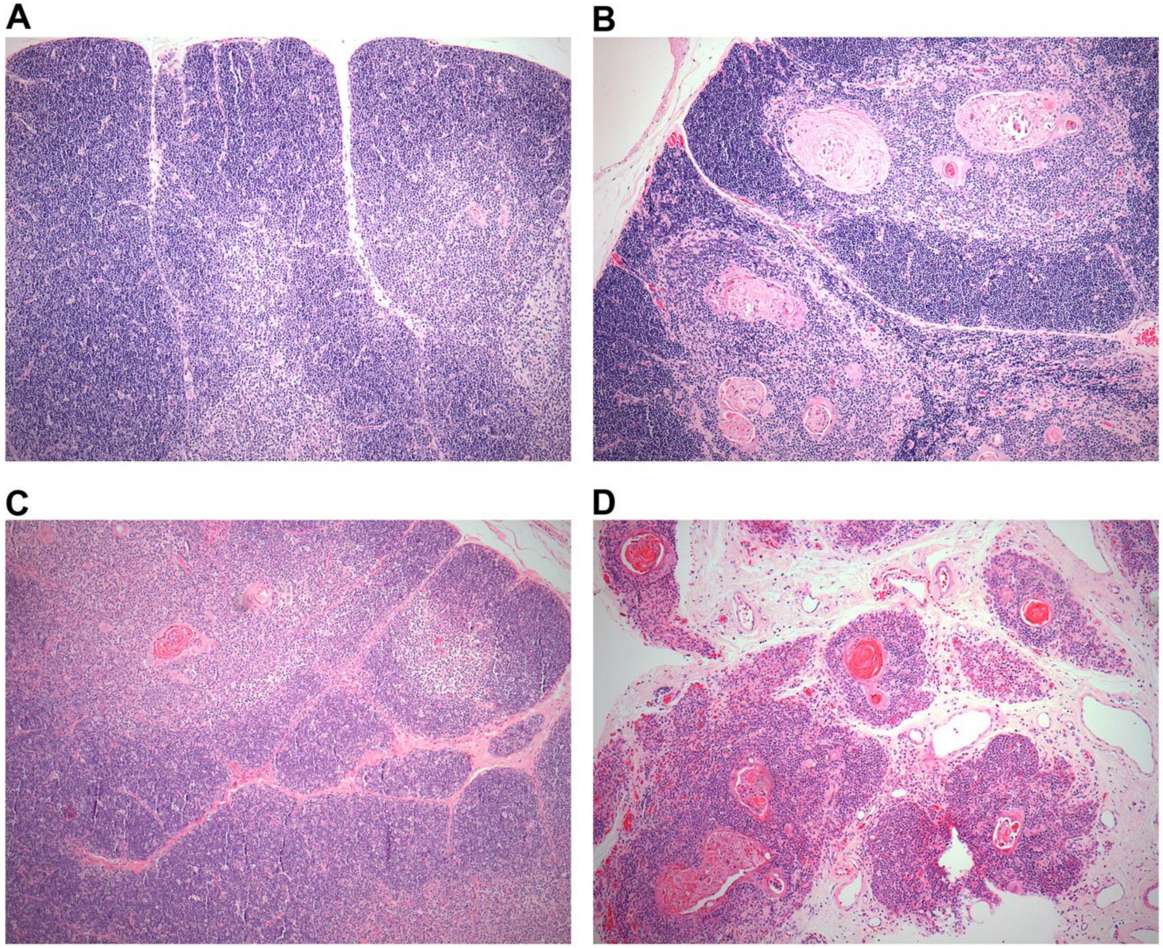
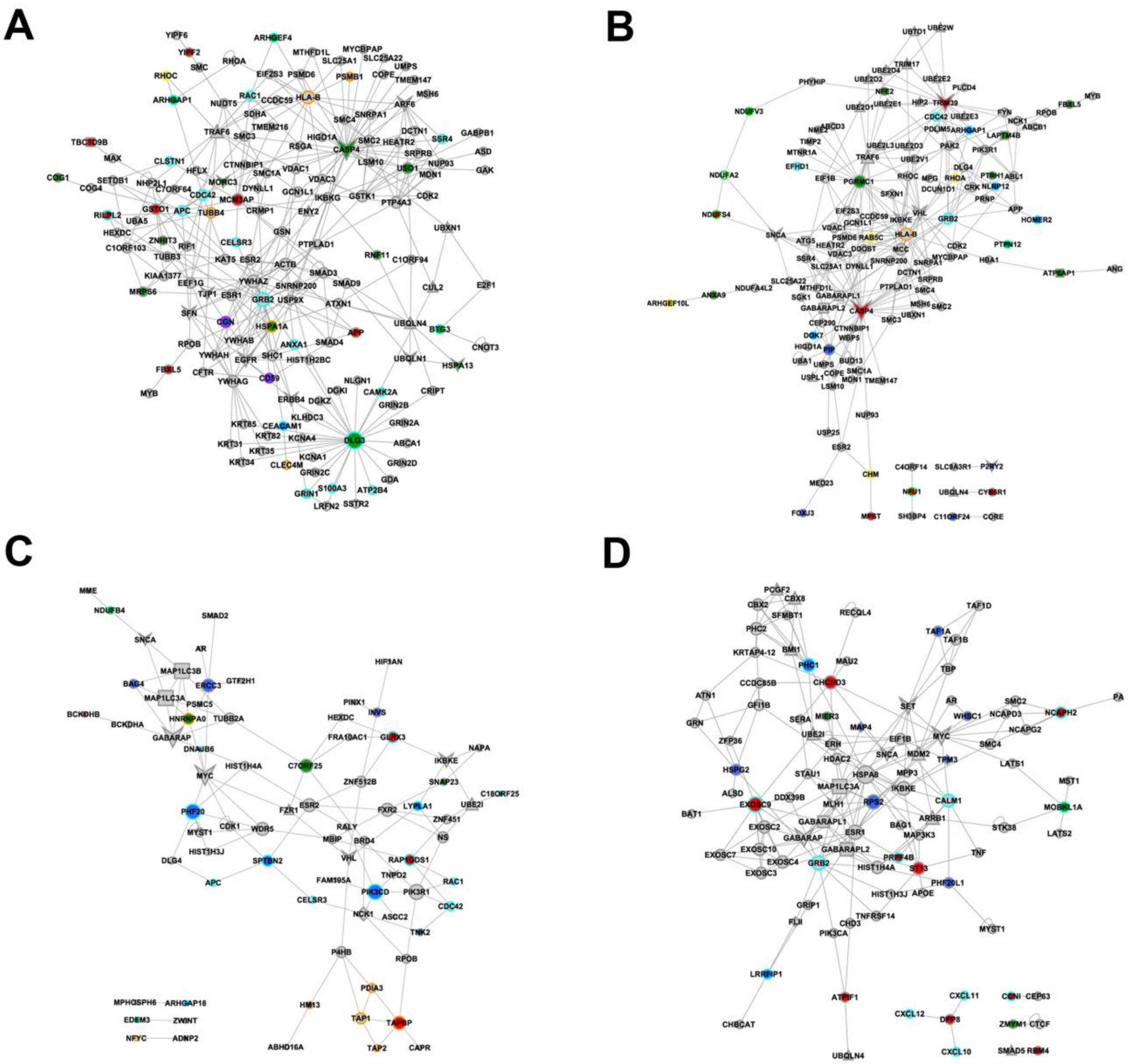


Figure S2. Histology of the Thymus. Histological aspect of thymuses obtained from karyotipically normal (**A** and **C**) and Down syndrome patients (**B** and **D**) included in this study. In **A** and **B** the subjects were 9 months old (patients CT9 and DS7). In **C** and **D** the subjects were 18 months old (patients CT4 and DS5). Cortical atrophy is present in Down syndrome thymuses. The 18 months old DS patient had more evident morphological changes, with marked cortical atrophy. All figures HE, 100X.


A
YIPF6
YIPF2
SMC
RHOA
RHOC
ARHGAP1
ARHGEF4
MTHFD1L
MYCBPAP
SLC25A1
SLC25A22
EIF2S3
PSMD6
PSMB1
COPE
UMPS
TMEM147
RAC1
CCDC59
HLA-B
NUDT5
MSH6
ARF6
SDHA
SMC4
SNRPA1
TRAF6
SMC3
TMEM216
HIGD1A
SMC2
HEATR2
DCTN1
SSR4
GABPB1
TBC1D9B
RSGA
CASP4
SRPRB
ASD
MAX
CLSTN1
HFLX
CTNNBIP1
VDAC1
LSM10
USO1
NUP93
MDN1
GAK
COG1
SETDB1
NHP2L1
MORC3
SMC1A
VDAC3
COG4
C7ORF64
CDC42
DYNLL1
MCM3AP
GCN1L1
IKBKG
GSTK1
CDK2
PTP4A3
RILPL2
GSTO1
APC
TUBB4
CRMP1
ENY2
UBA5
HEXDC
UBXN1
GSN
ZNHIT3
CELSR3
PTPLAD1
C1ORF103
RIF1
RNF11
C1ORF94
TUBB3
KAT5
ESR2
ACTB
KIAA1377
SMAD3
EEF1G
YWHAZ
SNRNP200
SMAD9
CUL2
E2F1
MRPS6
TJP1
ESR1
GRB2
USP9X
ATXN1
SFN
CGN
HSPA1A
APP
UBQLN4
BTG3
RPOB
YWHAB
ANXA1
SMAD4
CNOT3
YWHAH
EGFR
SHC1
HIST1H2BC
UBQLN1
HSPA13
FBXL5
CFTR
YWHAG
CD59
NLGN1
MYB
DGKI
CAMK2A
CRIPT
ERBB4
DGKZ
GRIN2B
KLHDC3
KRT85
CEACAM1
GRIN2A
KRT82
KCNA4
DLG3
ABCA1
KRT31
KRT35
KCNA1
KRT34
CLEC4M
GRIN2D
GRIN2C
GDA
GRIN1
S100A3
ATP2B4
LRFN2
SSTR2
B
UBTD1
UBE2W
TRIM17
UBE2D4
UBE2D2
UBE2E2
PHYHIP
NFE2
PLCD4
UBE2D1
UBE2E1
HIP2
TRIM39
NDUFV3
CDC42
UBE2E3
FYN
MYB
FBXL5
RPOB
NCK1
NME2
ABCD3
PDLIM5
ARHGAP1
ABCB1
LAPTM4B
TIMP2
UBE2L3
UBE2D3
PAK2
PIK3R1
MTNR1A
TRAF6
UBE2V1
EFHD1
DLG4
NDUFA2
EIF1B
PGRMC1
RHOC
MPG
RHOA
PTRH1
ABL1
CRK
NLRP12
DCUN1D1
SFXN1
PRNP
APP
NDUFS4
EIF2S3
CCDC59
VHL
IKBKE
GRB2
HOMER2
GCN1L1
VDAC1
SNCA
ATG5
PSMD6
RAB5C
HLA-B
CDK2
PTPN12
HEATR2
DDOST
MCC
VDAC3
MYCBPAP
SSR4
SNRNP200
SNRPA1
HBA1
SLC25A1
DYNLL1
ATP6AP1
ANG
DCTN1
SLC25A22
SRPRB
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SMC4
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ANXA9
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MSH6
SMC2
GABARAPL2
CASP4
UBXN1
SMC3
CEP290
CTNNBIP1
DOK7
WBP5
HIGD1A
PIP
UBA1
BUD13
UMPS
SMC1A
COPE
USPL1
MDN1
TMEM147
LSM10
NUP93
USP25
ESR2
C4ORF14
SLC9A3R1
P2RY2
CHM
MED23
NFU1
UBQLN4
CYB5R1
FOXJ3
MPST
SH3BP4
C11ORF24
CORE
C
MME
NDUFB4
SMAD2
SNCA
AR
MAP1LC3B
BAG4
ERCC3
GTF2H1
MAP1LC3A
PSMC5
HIF1AN
BCKDHB
HNRNPA0
TUBB2A
PINX1
INVS
BCKDHA
HEXDC
GABARAP
DNAJB6
FRA10AC1
GLRX3
NAPA
IKBKE
HIST1H4A
C7ORF25
MYC
ZNF512B
SNAP23
C18ORF25
PHF20
ESR2
FZR1
FXR2
LYPLA1
UBE2I
ZNF451
MYST1
CDK1
WDR5
RALY
HIST1H3J
MBIP
BRD4
NS
DLG4
SPTBN2
VHL
RAP1GDS1
APC
TNPO2
FAM195A
RAC1
PIK3CD
PIK3R1
CELSR3
CDC42
NCK1
ASCC2
TNK2
P4HB
RPOB
PDIA3
MPHOSPH6
ARHGAP18
HM13
EDEM3
ZWINT
TAP1
TAPBP
NFYC
ADNP2
ABHD16A
TAP2
CAPR
D
PCGF2
CBX8
CBX2
SFMBT1
RECQL4
TAF1D
PHC2
TAF1A
TAF1B
KRTAP4-12
BMI1
PHC1
MAU2
TBP
CCDC85B
CHCHD3
ATN1
GFI1B
SET
AR
SMC2
PA
WHSC1
NCAPD3
NCAPH2
GRN
SERA
MIER3
MAP4
MYC
NCAPG2
UBE2I
EIF1B
ZFP36
ERH
SMC4
HDAC2
SNCA
MDM2
TPM3
LATS1
HSPG2
STAU1
HSPA8
MPP3
MST1
MAP1LC3A
IKBKE
ALSD
DDX39B
CALM1
BAT1
EXOSC9
MLH1
RPS2
MOBKL1A
GABARAPL1
ARRB1
STK38
EXOSC2
ESR1
BAG1
LATS2
GABARAP
MAP3K3
EXOSC10
GABARAPL2
TNF
EXOSC7
PRPF4B
EXOSC4
GRB2
HIST1H4A
ST13
EXOSC3
PHF20L1
HIST1H3J
APOE
GRIP1
TNFRSF14
FLII
CHD3
MYST1
PIK3CA
LRRFIP1
CXCL11
CCNI
CEP63
ATPIF1
CXCL12
DPP8
ZMYM1
CTCF
CHBCAT
CXCL10
SMAD5
RBM4
UBQLN4

Figure S3. Interactome for DE and CO networks. Interactome for CT-DE (**A**), DS-DE (**B**), CT-CO (**C**) and DS-CO (**D**) selected hubs (depicted in blue), VIPs (depicted in red) and high-hubs (depicted in green) using MINT and IntAct databases. Node size is related to node degree (number of links). Node shapes and border colors represent biological processes, as follows: circle for cell process; circle with light blue border for calcium ion binding, thymic or T cell development and T cell differentiation, circle with orange, purple, yellow or light green border stand for, antigen presentation, Hassall´s corpuscles, signaling/GTPase activity or mitochondrial respiratory chain respectively; diamond for actin/cytoskeleton; square for autophagy; triangle for ubiquitination; vee for apoptosis.

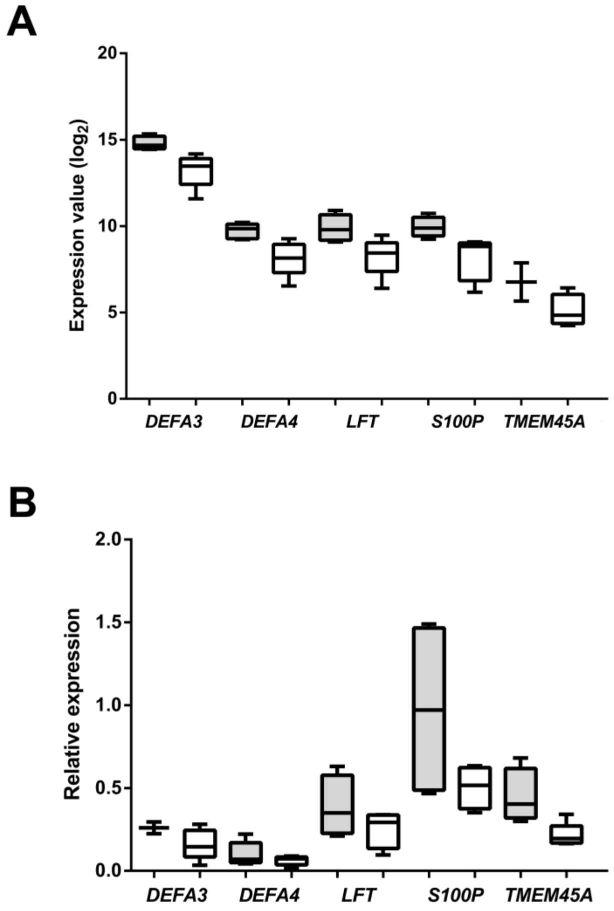

Figure S4. qPCR validation of DNA microarray data. In **A** the boxplots representing DNA microarray expression values of five selected genes in DS (gray) and CT (white) groups. In **B** qPCR expression fold changes boxplots of DS and CT groups for the same genes. Values for DS group indicate upregulation.

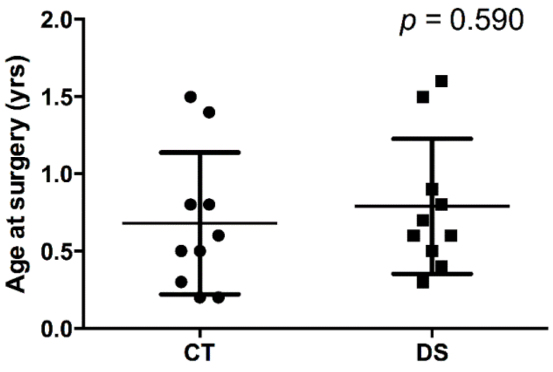

Figure S5. Patients' age at surgery. Scatter-plot of age at surgery (in years) of subjects with Down Syndrome and karyotypically normal control subjects and t-test p-value.

Table S1: Number of genes per community in DE networks

	Number of genes	
Communities	**CT-DE Network**	**DS-DE Network**
A	41	55
B	40	52
C	39	50
D	30	38
E	26	32
F	25	30
G	18	18
H	14	14
I	3	11

Table S2: Number of genes per community in CO networks

Communities	Number of genes	
	CT- CO Network	DS-CO Network
A	404[a]	587[a]
B	400[a]	533[a]
C	397[a]	436[a]
D	293[a]	436[a]
E	290[a]	393[a]
F	248[a]	316[a]
G	235[a]	312[a]
H	226[a]	289[a]
I	213[a]	252
J	188	199
K	180[a]	189[a]
L	165[a]	172[a]
M	152	145
N	147	119
O	132	105
P	124	100
Q	117[a]	95
R	106[a]	82
S	97	70[a]
T	95	69
U	92[a]	56
V	83	54
W	75	53

X	35	51
Y	32	48[a]
Z	30	46
A'	21	14
B'	-	8
C'	-	8
D'	-	7
E'	-	7
F'	-	6

[a] Communities harboring at least one high hierarchy gene and included in the coarse-grained community structure (CGCS) diagrams showed in Figure 4.

Table S3: Differentially expressed micro RNAs

microRNA	**Mean**	**Mean**	***p* value**	**fold (DS/CT)**
	DS group	**CT group**		
Up-regulated in DS group				
hsa-miR-548d-5p	5.771	3.265	0.007	1.768
hsa-miR-196a-5p	4.847	3.071	0.040	1.578
hsa-miR-449a	3.470	1.990	0.028	1.744
hsa-miR-563	5.820	4.401	0.050	1.323
hsa-miR-30b-3p	2.811	1.665	0.035	1.688
hsa-miR-609	3.499	2.358	0.029	1.484
Down-regulated in DS group				
hsa-miR-548am-5p	2.528	3.526	0.034	0.717
hsa-miR-301a-3p	1.199	2.316	0.049	0.518
hsa-miR-129-2-3p	1.909	3.464	0.037	0.551
hsa-miR-139-5p_v18.0	0.886	2.667	0.023	0.332
hsa-let-7f-1-3p	5.290	7.083	0.041	0.747
hsa-miR-602	7.426	9.674	0.044	0.768
hsa-miR-484	4.532	6.849	0.015	0.662
hsa-miR-200b-5p	2.395	5.195	0.018	0.461
hsa-let-7b-3p	3.866	6.775	0.016	0.571
hsa-miR-1267	2.588	5.593	0.006	0.463
hsa-miR-191-3p	10.223	13.346	0.032	0.766
hsa-miR-204-5p	4.519	7.983	0.035	0.566
hsa-miR-486-5p	2.616	6.393	0.005	0.409
hsa-miR-127-3p	3.282	7.107	0.025	0.462
hsa-miR-550a-5p	6.955	10.836	0.043	0.642

hsa-miR-296-5p	4.230	8.499	0.010	0.498
hsa-miR-361-3p	3.714	8.529	0.009	0.436
hsa-miR-766-3p	13.347	18.531	0.046	0.720
hsa-miR-625-5p	9.784	15.070	0.043	0.649
hsa-miR-149-5p	8.622	14.136	0.018	0.610
hsa-miR-497-5p	5.445	11.516	0.019	0.473
hsa-miR-1249	6.347	14.024	0.007	0.453
hsa-miR-197-3p	23.434	32.666	0.035	0.717
hsa-miR-99b-5p	7.604	16.944	0.024	0.449
hsa-miR-30d-5p	15.219	26.102	0.035	0.583
hsa-miR-140-3p	16.540	27.855	0.032	0.594
hsa-miR-193b-3p	10.971	22.687	0.028	0.484
hsa-miR-424-3p	8.870	20.677	0.033	0.429
hsa-miR-125a-5p	13.624	26.490	0.016	0.514
hsa-miR-145-5p	13.777	27.115	0.016	0.508
hsa-miR-1280_v18.0	20.575	34.153	0.024	0.602
hsa-miR-331-3p	16.576	30.223	0.019	0.548
hsa-miR-151a-5p	16.946	35.105	0.018	0.483
hsa-miR-23b-3p	32.651	54.707	0.045	0.597
hsa-miR-455-3p	12.723	35.789	0.003	0.356
hsa-miR-324-3p	39.682	69.553	0.046	0.571
hsa-miR-200c-3p	52.551	88.058	0.034	0.597
hsa-miR-30c-5p	61.840	100.202	0.044	0.617
hsa-miR-1274b_v16.0	30.730	76.706	0.026	0.401
hsa-miR-29a-3p	52.271	120.050	0.030	0.435
hsa-miR-100-5p	75.757	153.017	0.031	0.495
hsa-miR-205-5p[a]	230.549	469.303	0.011	0.491
hsa-miR-720_v18.0[a]	309.105	575.821	0.034	0.537

hsa-miR-15b-5p[a]	456.515	737.685	0.040	0.619
hsa-miR-181a-5p[a]	478.509	827.574	0.037	0.578
hsa-miR-125b-5p[a]	663.695	1123.982	0.017	0.590
hsa-miR-150-5p[a]	531.064	1256.764	0.025	0.423

[a] microRNAs abundantly expressed in DS and CT groups.