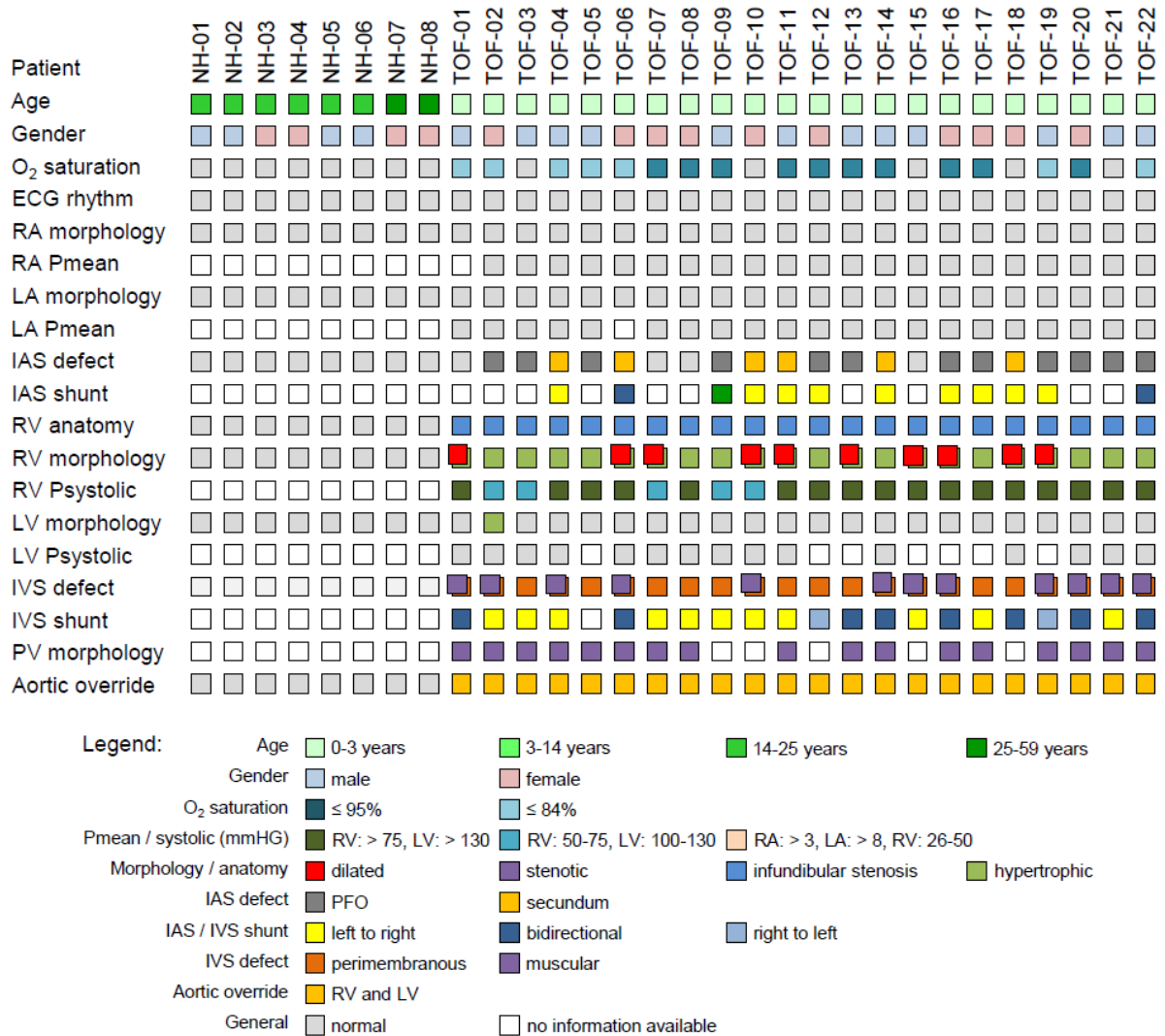


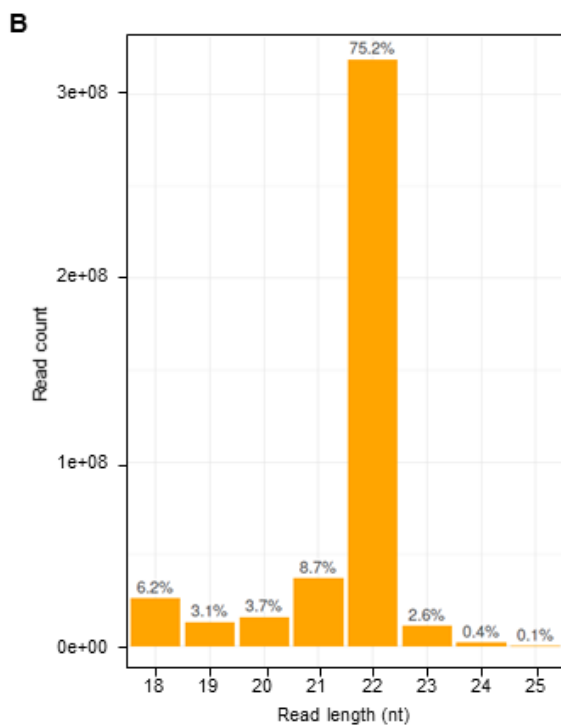
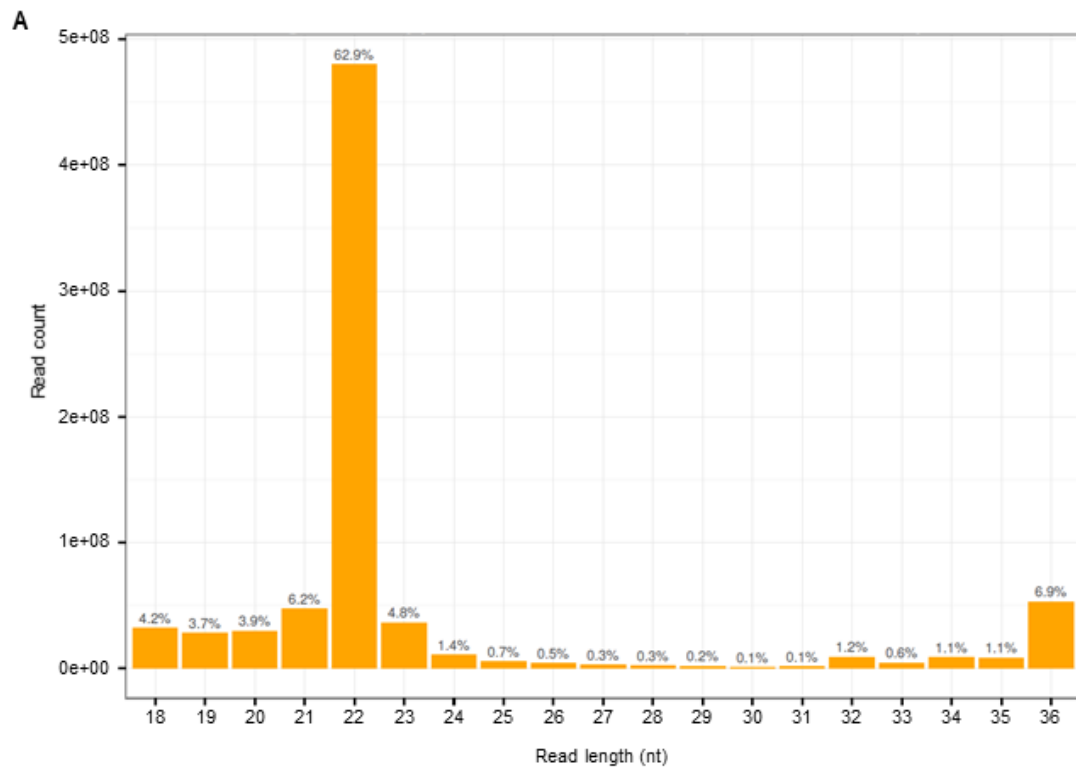
Altered microRNA and target gene expression related to Tetralogy of Fallot

Marcel Grunert, Sandra Appelt, Ilona Dunkel, Felix Berger & Silke R. Sperling

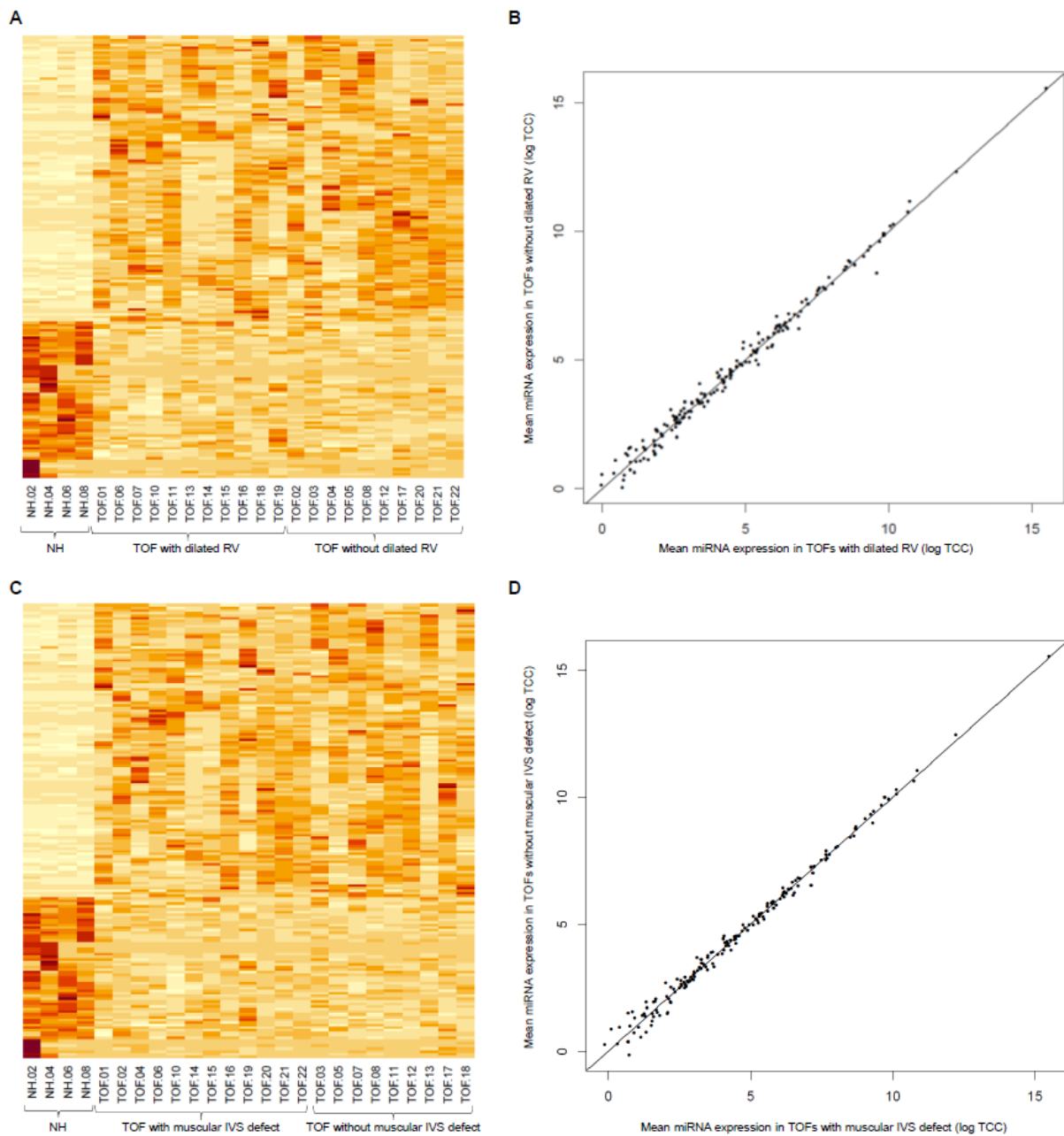
Supplementary Information



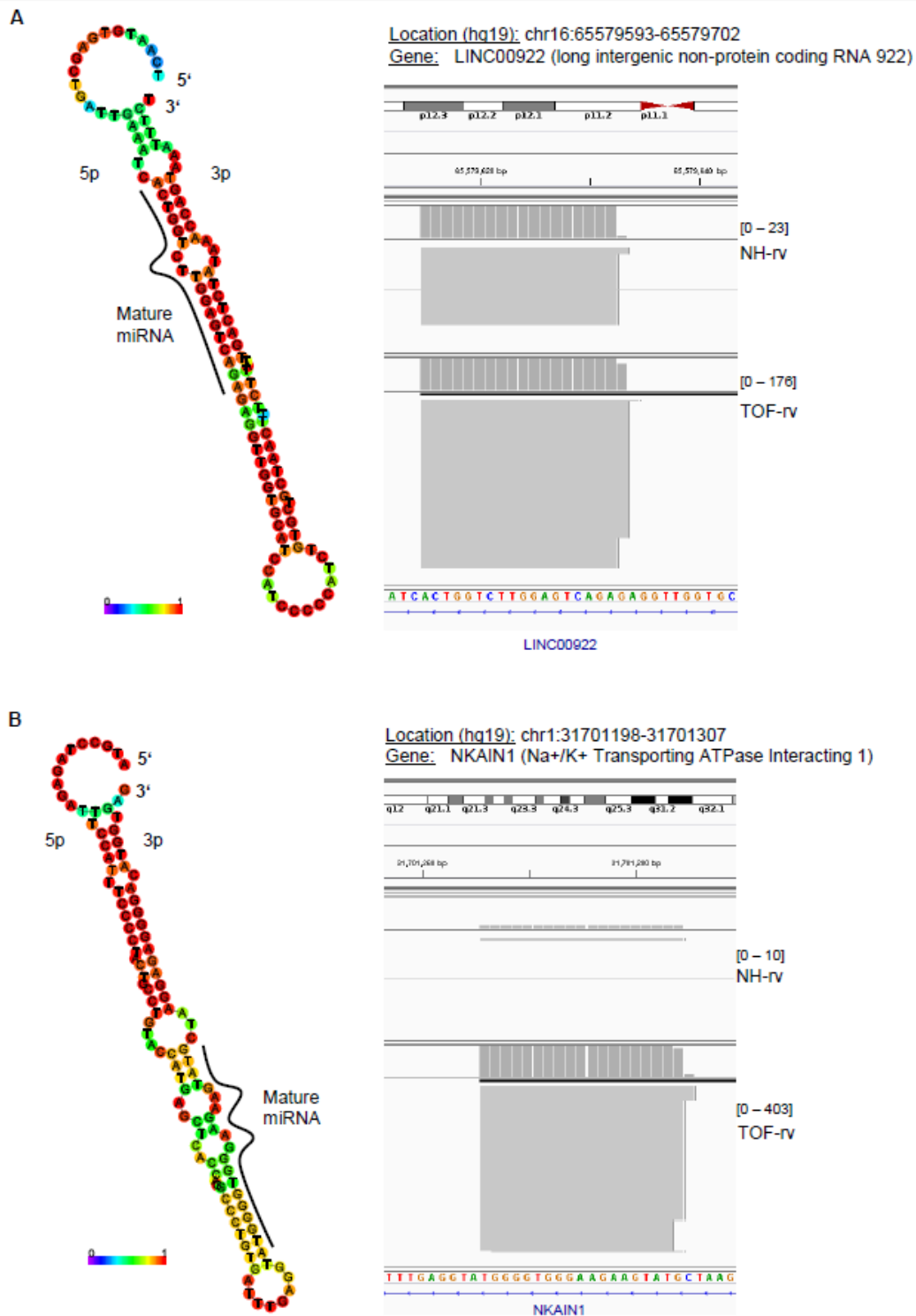
Supplementary Figure S1. Phenotype matrix for TOF patients and normal heart controls. Each individual is represented by one column. In addition to general information, all pathological features are indicated. Double boxes are used for more than one piece of information per row. See legend for color-coding. The samples NH-01, NH-03, NH-05 and NH-07 were taken from left ventricle whereas all other normal heart and TOF samples were taken from right ventricle. ECG indicates electrocardiogram; IAS, intra-atrial septum; IVS, intra-ventricular septum; LA, left atrium; LV, left ventricle; NH, normal heart; PFO, patent foramen ovale; Pmean, mean pressure; Psystolic, systolic pressure; PV, pulmonary valve; RA, right atrium; RV, right ventricle; TOF, Tetralogy of Fallot.



Supplementary Figure S2. Read length distribution over all TOF-rv, NH-lv and NH-rv samples of (A) all mapped sequences and (B) mapped mature miRNA sequences. nt: nucleotides.



Supplementary Fig. S3: Expression of significantly differentially expressed miRNAs (n=172; TOF-rv vs. NH-rv) in TOFs with and without dilated RV or muscular IVS defect. (A+C): Heatmap based on hierarchical clustering of identified miRNAs (TCC normalized expression values) among the different groups of samples/individuals. (B+D): MiRNA correlation of expression values in the two groups of patients. IVS: intra-ventricular septum; NH: normal heart (samples from right ventricle); RV: right ventricle; TOF: Tetralogy of Fallot (samples from right ventricle).

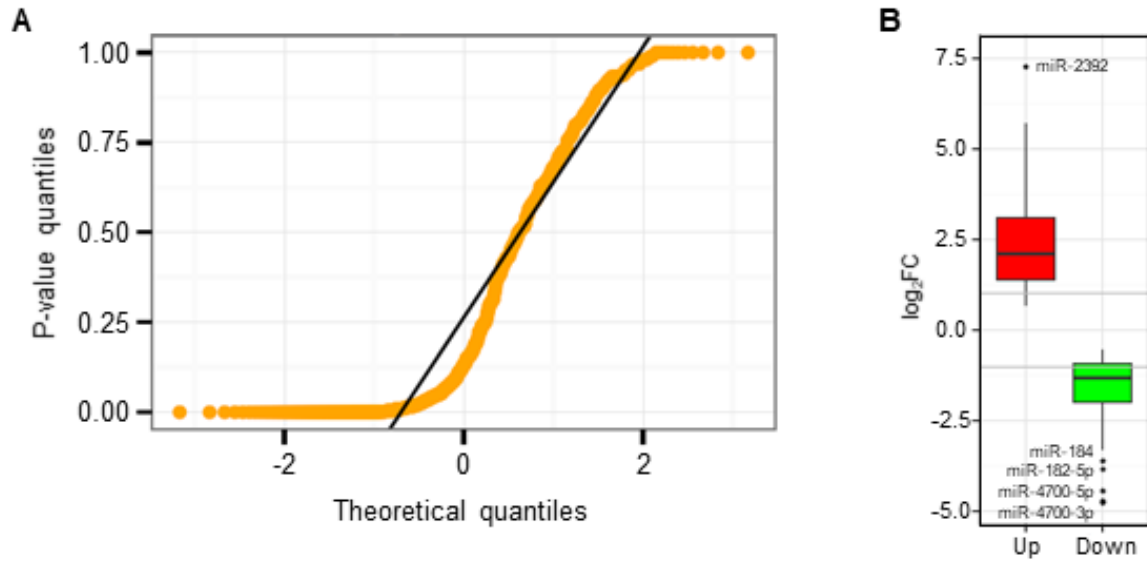


Supplementary Figure S4. Predicted novel miRNA candidates. (A) Novel miRNA located at chromosome 16 and overlapping the long non-coding RNA LINC00922. (B) Novel miRNA located at chromosome 1 and in an intron of NKAIN1. (Left) Centroid secondary structure drawing encoding base-pair probabilities based on RNAfold WebServer. (Right) Graphical representation of the novel miRNA location with read count over all NH-rv and TOF-rv samples.

#miRDeep information for novel miRNA located at chr1:31228351-31228460

```
score_nucleus 3
score_star -1.3
score_mfe 1.4
score_freq 0
score 3.4
flank_first_end 38
flank_first_seq ATGCCTAGAGATTTCATTCCCTACTGCCTGTACCA
flank_first_struct .....(((((.....)))....))
flank_second_beg 88
flank_second_seq CTAAGGAGAGGGGACATGGTGAG
flank_second_struct ...)))))))).)))).)
freq 388
loop_beg 59
loop_end 68
loop_seq ATTTGAGGTA
loop_struct .....))
mature_arm second
mature_beg 69
mature_end 87
mature_query read_65782186_x255
mature_seq TGGGGTGGGAAGAAGTATG
mature_strand +
mature_struct )))))).))....))
pre_seq TGAGCTCACCAGGCCCTGTGATTGAGGTATGGGGTGGGAAGAAGTATG
pre_struct ((.....(((.....)))....))
pri_beg 1
pri_end 110
pri_id chr1_542
pri_mfe -36.20
pri_seq
ATGCCTAGAGATTTCATTCCCTACTGCCTGTACCATGAGCTCACCAGGCCCTGTGATTGAGGTATGGGGTGGGAAGAA
GTATGCTAAGGAGAGGGGACATGGTGAG
pri_struct .....(((((.....)))....))
star_arm first
star_beg 39
star_end 58
star_seq TGAGCTCACCAGGCCCTGTG
star_struct ((.....(((.....)))....))
```

Supplementary Figure S7. Predicted novel miRNA candidate located at chromosome 1 and in an intron of NKAIN1. Given are the various miRDeep information.



Supplementary Figure S8. Distribution of p-values and fold changes for significantly differentially expressed miRNAs in TOF-rv versus NH-rv. (A) QQ-plot for p-values derived from exact test for negative binomially distributed read counts after TCC normalization. (B) Fold change (FC) of up- (red) and down-regulated (green) miRNAs.

miRNA	q-value	m-value	FC	miRNA	q-value	m-value	FC	miRNA	q-value	m-value	FC
hsa-let-7a-3p	3.04E-02	-0.82	-1.77	hsa-let-7i-5p	8.18E-10	2.23	4.68	hsa-miR-378g	1.62E-04	1.46	2.75
hsa-miR-1	7.16E-03	-0.68	-1.61	hsa-miR-101-5p	4.00E-02	1.46	2.75	hsa-miR-378i	2.85E-05	4.25	19.02
hsa-miR-10a-5p	2.99E-13	-2.89	-7.40	hsa-miR-105-5p	3.16E-02	1.67	3.19	hsa-miR-378j	2.54E-03	2.50	5.65
hsa-miR-124-3p	4.54E-05	-2.96	-7.79	hsa-miR-122-3p	1.56E-03	1.89	3.71	hsa-miR-381-3p	8.23E-05	2.10	4.28
hsa-miR-126-3p	9.06E-03	-0.77	-1.70	hsa-miR-1261	3.27E-08	4.19	18.29	hsa-miR-382-5p	9.56E-04	1.36	2.57
hsa-miR-133b	6.88E-04	-1.51	-2.84	hsa-miR-1262	3.17E-03	1.43	2.70	hsa-miR-3917	4.00E-02	2.72	6.59
hsa-miR-135a-5p	3.98E-02	-1.26	-2.39	hsa-miR-126-5p	1.01E-03	1.39	2.63	hsa-miR-422a	3.78E-04	2.10	4.27
hsa-miR-139-3p	7.72E-04	-1.47	-2.77	hsa-miR-1271-3p	8.42E-06	3.53	11.53	hsa-miR-423-3p	3.86E-03	0.99	1.99
hsa-miR-139-5p	1.64E-09	-1.78	-3.44	hsa-miR-127-3p	3.33E-02	0.85	1.81	hsa-miR-424-3p	1.71E-02	0.82	1.76
hsa-miR-140-3p	3.33E-02	-0.54	-1.45	hsa-miR-1285-3p	3.41E-02	1.18	2.27	hsa-miR-424-5p	2.88E-05	2.29	4.87
hsa-miR-140-5p	3.86E-10	-2.01	-4.02	hsa-miR-130a-3p	1.97E-10	1.69	3.23	hsa-miR-4306	4.81E-02	3.32	9.95
hsa-miR-143-5p	8.61E-03	-0.64	-1.56	hsa-miR-130b-3p	4.23E-03	1.14	2.20	hsa-miR-433-3p	2.31E-02	0.93	1.90
hsa-miR-146b-5p	6.97E-04	-1.32	-2.50	hsa-miR-136-5p	5.77E-05	4.52	22.92	hsa-miR-4429	1.90E-07	3.39	10.46
hsa-miR-148a-3p	1.39E-10	-1.57	-2.97	hsa-miR-144-3p	1.26E-03	2.54	5.83	hsa-miR-4454	1.87E-02	1.74	3.33
hsa-miR-148b-3p	1.65E-11	-1.65	-3.14	hsa-miR-146a-5p	4.00E-02	1.29	2.44	hsa-miR-4510	6.58E-07	3.20	9.20
hsa-miR-150-5p	2.02E-03	-1.12	-2.17	hsa-miR-15b-5p	1.81E-02	1.26	2.39	hsa-miR-451a	1.25E-03	2.33	5.04
hsa-miR-151a-5p	3.36E-02	-0.58	-1.50	hsa-miR-181a-5p	2.83E-03	0.82	1.76	hsa-miR-454-3p	1.37E-03	2.49	5.61
hsa-miR-151b	4.54E-05	-1.20	-2.30	hsa-miR-181b-5p	2.23E-08	1.76	3.39	hsa-miR-455-5p	1.28E-04	1.36	2.57
hsa-miR-182-5p	3.92E-09	-4.45	-21.79	hsa-miR-181c-3p	1.20E-02	0.90	1.87	hsa-miR-487b-5p	3.46E-03	4.07	16.78
hsa-miR-183-5p	8.42E-06	-3.62	-12.27	hsa-miR-181c-5p	2.32E-02	0.94	1.91	hsa-miR-488-5p	9.93E-03	1.77	3.41
hsa-miR-184	3.72E-07	-3.85	-14.38	hsa-miR-181d-5p	9.91E-11	2.26	4.80	hsa-miR-493-5p	4.44E-02	1.32	2.49
hsa-miR-187-5p	4.18E-03	-2.39	-5.24	hsa-miR-1827	9.58E-04	3.41	10.61	hsa-miR-4999-5p	6.54E-03	3.32	9.99
hsa-miR-193b-3p	5.24E-03	-0.89	-1.86	hsa-miR-186-5p	1.53E-02	0.89	1.85	hsa-miR-499a-3p	3.39E-02	0.86	1.81
hsa-miR-215-5p	1.71E-02	-1.34	-2.53	hsa-miR-187-3p	2.10E-05	3.74	13.34	hsa-miR-499b-5p	3.39E-02	0.86	1.81
hsa-miR-21-5p	6.82E-03	-0.76	-1.69	hsa-miR-204-3p	6.56E-03	2.47	5.55	hsa-miR-508-3p	3.33E-02	3.15	8.88
hsa-miR-223-3p	4.61E-05	-1.14	-2.20	hsa-miR-204-5p	3.19E-05	3.05	8.31	hsa-miR-508-5p	1.62E-03	3.60	12.10
hsa-miR-22-3p	1.06E-04	-0.89	-1.86	hsa-miR-206	1.15E-05	2.85	7.23	hsa-miR-509-3-5p	1.09E-04	4.12	17.43
hsa-miR-23c	1.33E-08	-2.72	-6.58	hsa-miR-20b-3p	7.22E-04	2.63	6.17	hsa-miR-509-3p	3.28E-02	3.01	8.05
hsa-miR-29a-3p	7.46E-04	-1.00	-2.01	hsa-miR-20b-5p	1.11E-04	2.62	6.14	hsa-miR-509-5p	4.03E-02	3.18	9.03
hsa-miR-29b-3p	3.16E-16	-2.32	-4.99	hsa-miR-210-3p	3.46E-03	0.92	1.89	hsa-miR-542-3p	2.49E-04	1.84	3.58
hsa-miR-29c-3p	5.22E-05	-1.35	-2.55	hsa-miR-221-3p	8.42E-06	2.18	4.53	hsa-miR-551b-3p	8.61E-04	1.93	3.80
hsa-miR-29c-5p	1.87E-03	-1.10	-2.14	hsa-miR-221-5p	1.38E-05	1.91	3.75	hsa-miR-584-5p	6.58E-07	2.83	7.13
hsa-miR-3065-5p	2.26E-04	-1.10	-2.14	hsa-miR-222-3p	2.05E-06	1.50	2.83	hsa-miR-6128	1.76E-02	1.17	2.26
hsa-miR-30a-5p	2.54E-02	-0.71	-1.64	hsa-miR-2355-3p	2.39E-03	4.00	15.98	hsa-miR-6129	2.59E-08	5.63	49.46
hsa-miR-30c-2-3p	8.26E-04	-0.82	-1.77	hsa-miR-2392	1.33E-06	7.27	153.97	hsa-miR-6130	6.96E-06	3.09	8.50
hsa-miR-30d-5p	1.67E-02	-0.83	-1.78	hsa-miR-23b-5p	2.49E-02	0.88	1.84	hsa-miR-6131	1.62E-04	3.77	13.61
hsa-miR-320d	3.45E-02	-1.03	-2.05	hsa-miR-28-3p	7.20E-07	1.71	3.26	hsa-miR-6133	3.10E-04	5.40	42.33
hsa-miR-338-3p	2.26E-04	-1.10	-2.14	hsa-miR-29a-5p	4.00E-02	1.59	3.02	hsa-miR-6134	4.76E-08	5.68	51.26
hsa-miR-345-3p	1.25E-05	-3.27	-9.65	hsa-miR-30a-3p	7.16E-03	0.90	1.87	hsa-miR-618	4.46E-02	1.97	3.91
hsa-miR-34a-3p	1.20E-02	-1.63	-3.09	hsa-miR-3117-3p	3.20E-07	2.56	5.90	hsa-miR-6515-5p	1.00E-02	2.98	7.90
hsa-miR-3690	8.84E-03	-1.63	-3.10	hsa-miR-3124-5p	3.18E-02	1.12	2.18	hsa-miR-663a	1.36E-02	4.69	25.72
hsa-miR-4485	2.83E-03	-1.54	-2.90	hsa-miR-3127-5p	7.29E-04	2.04	4.11	hsa-miR-6809-3p	3.16E-02	1.28	2.43
hsa-miR-4521	9.77E-04	-1.49	-2.81	hsa-miR-3168	4.51E-07	4.46	22.07	hsa-miR-769-5p	2.92E-02	1.74	3.35
hsa-miR-4532	2.85E-08	-2.34	-5.07	hsa-miR-3184-5p	3.86E-03	0.99	1.99	hsa-miR-8052	1.02E-02	4.07	16.74
hsa-miR-4634	3.08E-02	-1.22	-2.33	hsa-miR-32-5p	4.25E-07	2.20	4.59	hsa-miR-92a-3p	3.86E-03	1.04	2.06
hsa-miR-4700-3p	2.32E-10	-4.78	-27.39	hsa-miR-335-3p	4.72E-02	2.59	6.01	hsa-miR-95-3p	8.42E-06	1.86	3.63
hsa-miR-4700-5p	1.65E-11	-4.72	-26.44	hsa-miR-33a-5p	1.54E-05	1.65	3.14	hsa-miR-95-5p	1.01E-03	3.59	12.05
hsa-miR-598-3p	7.32E-03	-0.82	-1.76	hsa-miR-33b-5p	1.18E-08	3.21	9.26	hsa-miR-9-5p	1.40E-03	1.45	2.74
hsa-miR-628-5p	1.76E-02	-1.12	-2.17	hsa-miR-34c-5p	4.60E-03	1.23	2.35	hsa-miR-99a-3p	3.48E-02	0.70	1.62
hsa-miR-642a-3p	4.51E-02	-0.94	-1.91	hsa-miR-3591-5p	1.55E-03	1.89	3.71	hsa-miR-99b-3p	6.56E-03	0.98	1.98
hsa-miR-642b-5p	4.56E-02	-0.94	-1.91	hsa-miR-3613-5p	2.37E-02	3.01	8.07				
hsa-miR-6503-5p	3.66E-03	-1.45	-2.74	hsa-miR-3615	1.13E-02	1.39	2.61				
hsa-miR-6723-5p	7.72E-04	-1.47	-2.77	hsa-miR-363-3p	2.10E-05	1.45	2.74				
hsa-miR-676-5p	3.83E-02	-1.07	-2.10	hsa-miR-371a-5p	8.42E-06	2.37	5.18				
hsa-miR-7704	3.39E-02	-0.76	-1.69	hsa-miR-371b-3p	8.42E-06	2.37	5.18				
hsa-miR-7975	2.45E-03	-2.29	-4.88	hsa-miR-372-3p	2.90E-08	3.30	9.82				
hsa-miR-8062	1.90E-04	-2.33	-5.01	hsa-miR-378a-3p	1.98E-07	2.03	4.09				
hsa-miR-9-3p	5.63E-03	-1.31	-2.48	hsa-miR-378b	9.56E-11	4.80	27.87				
hsa-miR-940	2.02E-03	-1.44	-2.72	hsa-miR-378c	3.86E-10	2.12	4.33				
hsa-miR-98-3p	1.22E-03	-1.58	-3.00	hsa-miR-378e	1.64E-05	2.40	5.27				
hsa-miR-98-5p	8.78E-03	-2.16	-4.47	hsa-miR-378f	5.97E-04	2.50	5.64				

Supplementary Table S1. Significantly differentially expressed miRNAs in TOF-rv versus NH-rv. (Left) Down-regulated miRNAs and (center & right) up-regulated miRNAs in TOF compared to controls. MiRNAs are ordered by names. FC indicates the fold change between TOF-rv versus NH-rv.

miRNAs at chr6	miRNA at chr16	miRNA at chr1
ELK4	CAMK2B	CDC42BPG
EYS	CBFB	CHRM1
GRIN2A	HEBP1	DNAJC8
H2AFV	IL22	EEF1A1
IGF1	NBR1	EIF5A
MAF	PLEC	EXTL3
MAPK1	PTBP1	FAIM3
PLXNA4	SRA1	GTDC1
POFUT1	TLL1	HDGF
PRLR	ZFYVE26	KCND1
RBMS3		LRP4
SAMD12		MLEC
SGCD		NR4A1
VPS53		NRIP2
ZXDC		NXF1
		PALLD
		PCDHGA7
		PHB
		PLXNB2
		RNF121
		RTN4R
		SEMA4G
		SRCIN1
		SSH2
		TMEM61
		TPM3
		VAMP5
		VAT1
		YBX2
		YTHDF2
		ZCCHC17

Supplementary Table S2. Predicted target genes of the novel miRNA candidates at chromosome 6 (left, n=15), at chromosome 16 (center; n=10) and at chromosome 1 (right; n=31).