

**Supplemental Table 1. Patient and tumor characteristics.**

Characteristic	miRNA sequencing cohort, n=90		RT-qPCR cohort 1, n=29*		RT-qPCR cohort 1 extended, n=45 <sup>†</sup>		RT-qPCR cohort 2, n=20 <sup>‡</sup>	
	No	%	No	%	No	%	No	%
<b>Age (years)</b>								
Median	52.9		58.2		58.1		56	
Range	22-84		22-78		22-81		30-81	
<b>Tumour histology</b>								
Squamous	90	100	29	100	45	100	20	100
<b>Tumor stage (FIGO)</b>								
1b -2b	70	78	19	65	33	73	13	65
3b-4a	20	22	10	35	12	27	7	35
<b>Tumor volume (cm<sup>3</sup>) §</b>								
Median	26		41		31		60	
Range	3-267		3-218		3-218		3-321	
<b>Lymph node status<sup>  </sup></b>								
Positive	44	49	15	51	19	42	11	55
Negative	46	51	14	49	26	58	9	45
<b>HPV type<sup>¶</sup></b>								
HPV16	50	56	10	34	23	51	13	60
HPV18	14	16	11	38	14	31	2	10
HPV other	16	18	6	21	6	13	2	
HPV negative	8	9	2	7	2	4	0	
HPV16+HPV18	1	1					3	15
<b>Hypoxia status<sup>**</sup></b>								
More hypoxic	26	29	10	34	18	40	10	50
Less hypoxic	64	71	19	66	27	60	10	50
<b>Observation time (months)<sup>††</sup></b>								
Median	39		27.3		36		36	
Range	5-60		4.6-60		4.6-60		2-60	
<b>Recurrence<sup>‡‡</sup></b>	19	21	10	34	11	24	10	50
<b>Disease specific death<sup>‡‡</sup></b>	16	18	9	31	10	22	9	45

FIGO, Federation International de Gynecologie et d'Obstetrique.

\* Subgroup of patients in sequencing cohort.

<sup>†</sup> Subgroup of patients in sequencing cohort, includes patients in RT-qPCR cohort 1.

<sup>‡</sup> Patients not in sequencing cohort.

<sup>§</sup> Determined from MR-images before treatment. Calculated based on 3 orthogonal diameters (a,b,c) as  $(\pi/6)abc$ . Undetermined for 6 tumors in sequencing cohort, two in RT-qPCR cohort 1, four in RT-qPCR cohort 1 extended, and one in RT-qPCR cohort 2.

<sup>||</sup> Detected by MRI or CT at diagnosis, according to the response evaluation criteria in solid tumors version 1.1.

<sup>¶</sup> Collected from (Lando, M. *et al*, 2013).

<sup>\*\*</sup> Output from 6-gene hypoxia classifier collected from (Fjeldbo, C.S. *et al*, 2016b).

<sup>††</sup> Based on patients without recurrence.

<sup>‡‡</sup> Follow-up data up to 60 months.

**Supplemental Table 2. Exiqon microRNA LNA™ PCR primer sets**

Candidate reference RNA	Product no.
miR-151a-5p	204007
miR-152-3p	204294
miR-505-3p	204214
miR-532-5p	204221
miR-652-3p	204387
miR-671-3p	204024
miR-28-5p	204322
miR-423-5p	204488
RNU6	203907
<b>MiRNA of investigation</b>	
miR-210-3p	204333
miR-9-5p	204513

**Supplemental Table 4. Ranking of 20 candidate reference miRNAs in RefFinder**

MiRNA	Overall ranking		Comparative $\Delta Ct$		BestKeeper		NormFinder		GeNorm	
	Rank	Stability	Rank	Stability	Rank	Stability	Rank	Stability	Rank	Stability
miR-28-5p	1	1.78	1	0.79	2	1.27	1	0.39	5	0.48
miR-423-3p	2	2.45	3	0.80	1	1.24	3	0.44	4	0.42
miR-505-3p	3	3.46	2	0.79	7	1.31	2	0.40	6	0.52
miR-151a-5p	4	3.98	4	0.82	5	1.31	9	0.50	1	0.00
miR-151b	5	4.47	5	0.82	6	1.31	10	0.50		
miR-532-5p	6	4.74	6	0.82	3	1.28	4	0.48	7	0.56
miR-671-3p	7	5.21	7	0.82	8	1.31	7	0.49	3	0.34
miR-152-3p	8	6.16	8	0.84	4	1.30	5	0.48	9	0.61
miR-652-3p	9	8.49	9	0.84	9	1.32	8	0.50	8	0.58
miR-140-5p	10	8.80	10	0.84	10	1.34	6	0.49	10	0.63
miR-664a-3p	11	11.00	11	0.91	11	1.37	11	0.62	11	0.65
miR-96-5p	12	12.47	12	0.94	14	1.45	12	0.66	12	0.67
miR-425-3p	13	13.00	13	0.94	13	1.44	13	0.66	13	0.69
miR-181c-3p	14	13.47	14	0.97	12	1.39	14	0.69	14	0.72
miR-126-3p	15	15.00	15	0.99	15	1.46	15	0.72	15	0.75
miR-598-3p	16	16.00	16	1.03	16	1.54	16	0.79	16	0.77
miR-708-3p	17	17.73	17	1.06	19	1.66	17	0.83	18	0.83
miR-708-5p	18	18.24	18	1.08	18	1.65	18	0.86	19	0.85
miR-301b-3p	19	18.72	19	1.09	20	1.70	19	0.87	17	0.80
miR-214-5p	20	19.20	20	1.10	17	1.60	20	0.88	20	0.87

**Supplemental Table 5. MiRNA family affiliation of candidate reference miRNAs.**

MiRNA	Family ID	Family Accession	Chromosome
miR-28-5p			chr3
miR-151a-5p	mir-28	MIPF0000057	chr8
miR-151b			chr14
miR-152-3p	mir-148	MIPF0000056	chr17
miR-423-3p	mir-423	MIPF0000329	chr17
miR-505-3p	mir-505	MIPF0000217	chrX
miR-532-5p	mir-188	MIPF0000113	chrX
miR-652-3p	mir-652	MIPF0000333	chrX
miR-671-3p	mir-671	MIPF0000358	chr7

**Supplemental Table 6. PCR efficiency.**

Candidate reference RNA	PCR efficiency	Comment
miR-151a-5p	99%	
miR-152-3p	101%	
miR-505-3p	99%	
miR-532-5p	70 %	Low efficiency *
miR-652-3p	93%	
miR-671-3p	NA	High Cq values (>33 at 80x) *
miR-28-5p	111%	Suboptimal efficiency compared to miR-151a-5p *
miR-423-5p	102 %	
RNU6	108%	
MiRNA of investigation		
miR-210-3p	95%	
miR-9-5p	101%	

\* indicate exclusion from further analysis

**Supplemental Table 7. RT-qPCR Cq values for candidate reference RNAs**

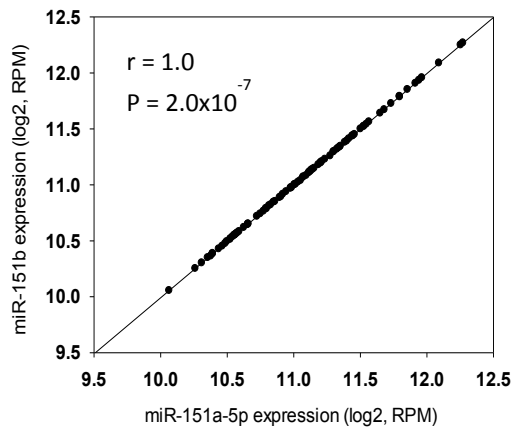
Reference RNA	Cq Range	Cq Max	Cq Min	Mean	Std Dev
miR-151a-5p	2.13	29.14	27.00	28.09	0.52
miR-152-3p	2.10	29.86	27.76	28.81	0.51
miR-505-3p	3.65	33.28	29.63	31.93	0.70
miR-652-3p	2.32	31.14	28.81	29.86	0.53
miR-423-3p	2.10	30.01	27.91	28.73	0.56
RNU6	6.32	27.50	21.19	23.50	1.60

# Supplemental figure 1

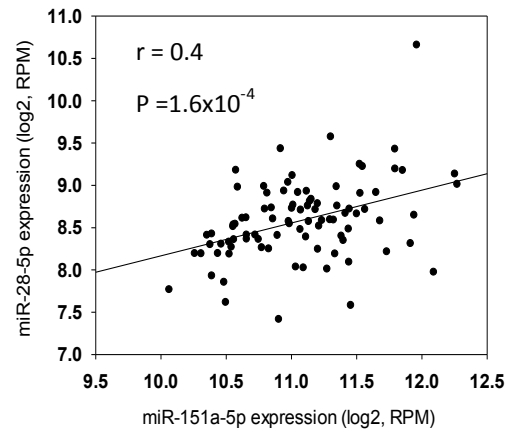
A)

miR-151a-5p	UCGAGGAGCUCACAGUCUAGU.....
miR-151b	UCGAGGAGCUCACAGUCU.....
miR-28-5p	..AAGGAGCUCACAGUCUAUUGAG..

B)

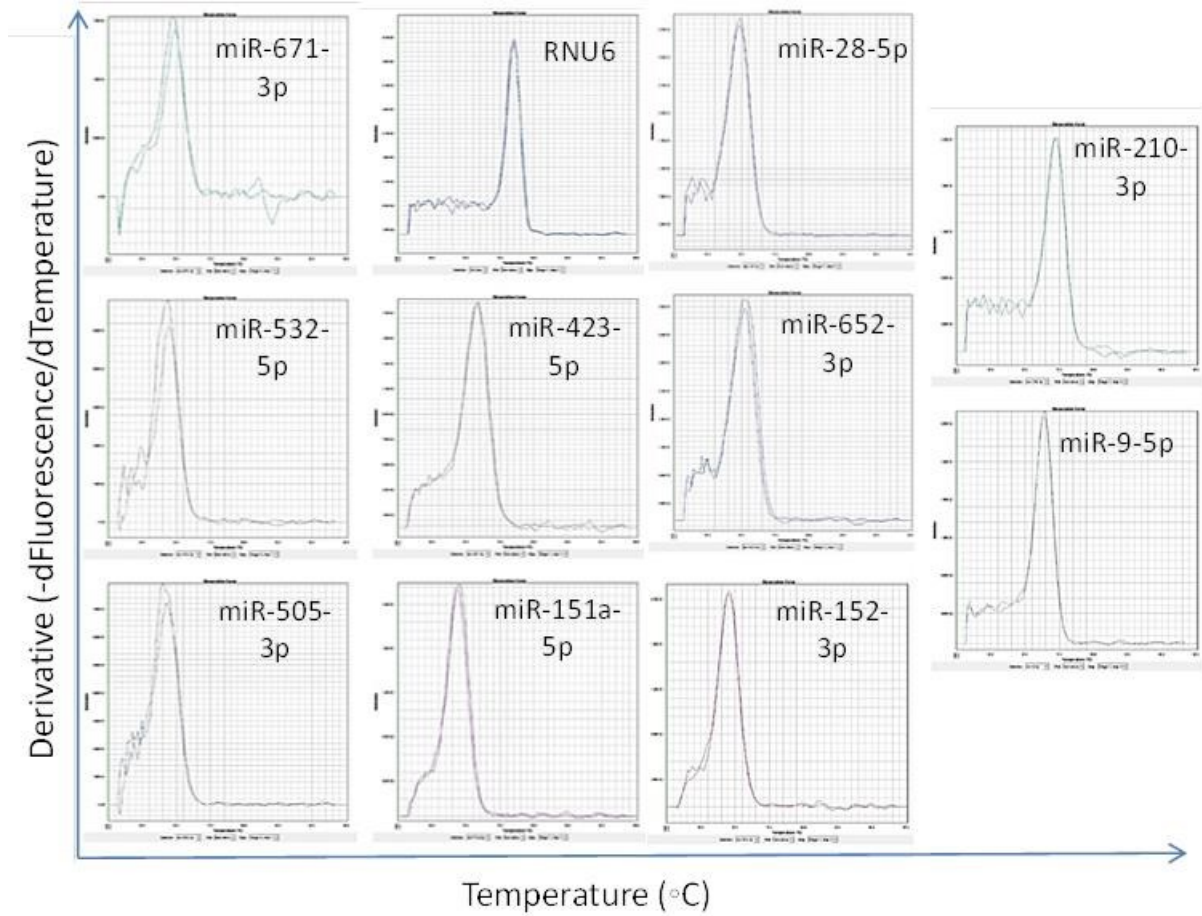


C)



Supplemental Fig. 1. **MiR-28 family members of candidate reference miRNAs.** A) Sequence alignment of miR-151a-5p, miR-151b and miR-28-5p. Regression plots of miR-151a-5p vs miR-151b (B) and miR-28-5p (C) expression levels (log2, RPM) and corresponding correlation coefficient (r) and p-value from Pearson's correlation analysis.

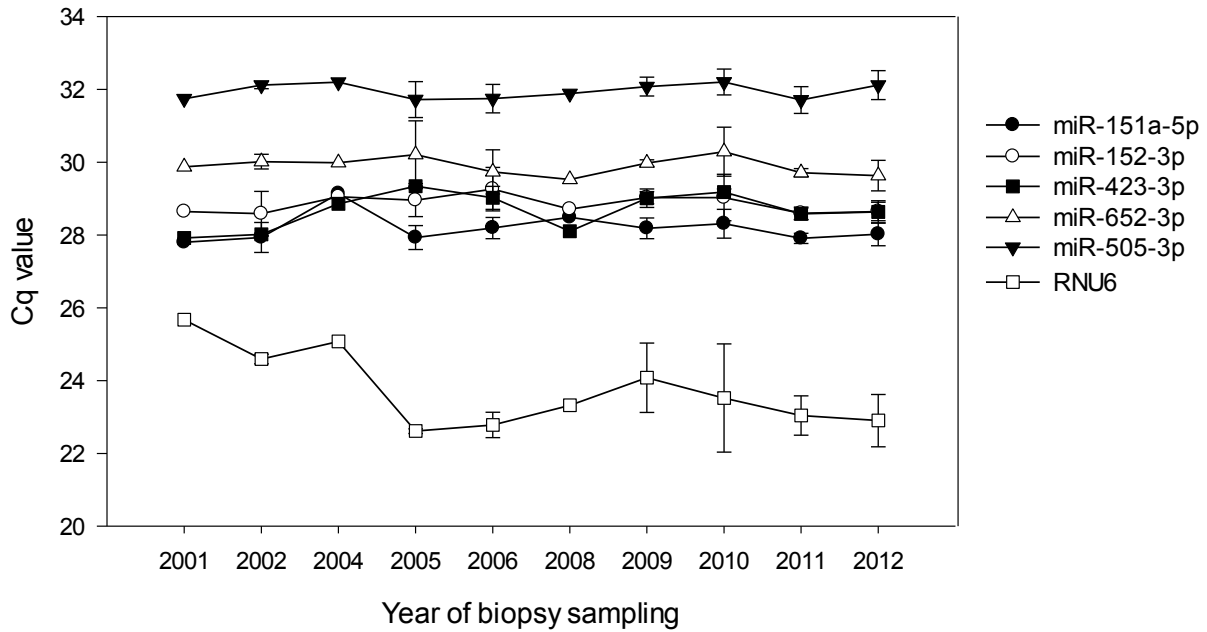
## Supplemental figure 2



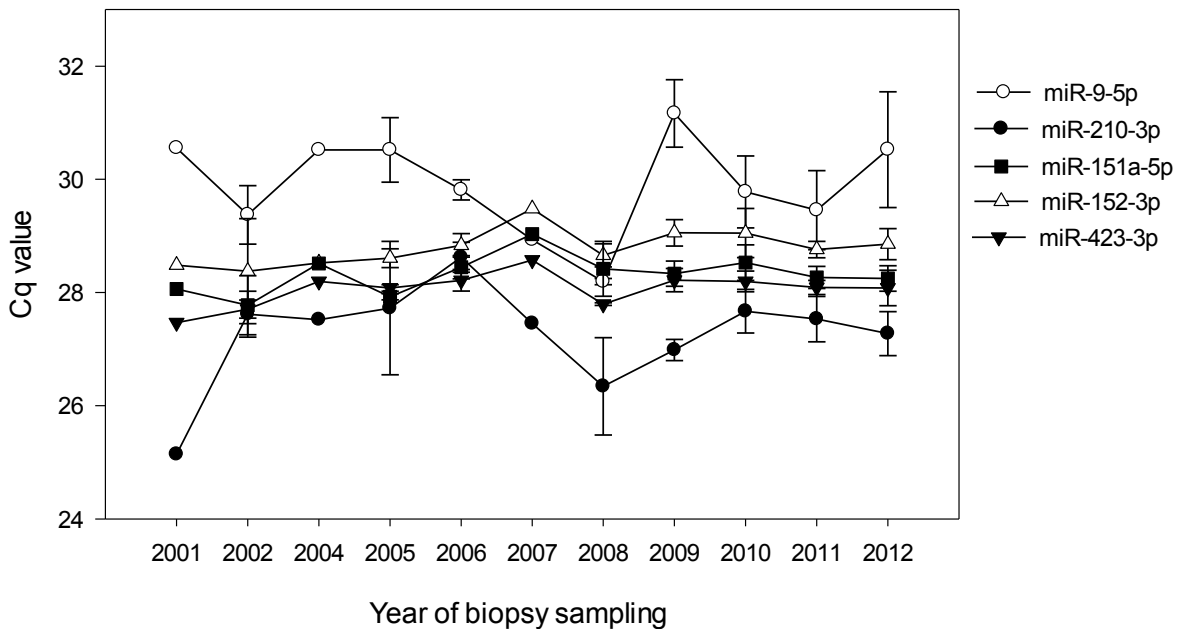
Supplemental Fig. 2. **Specificity of the PCR products.** Melting curve analysis generated for the amplified product of candidate reference RNAs and miRNAs of investigation. Change in fluorescence is plotted versus temperature.

### Supplemental figure 3

A)

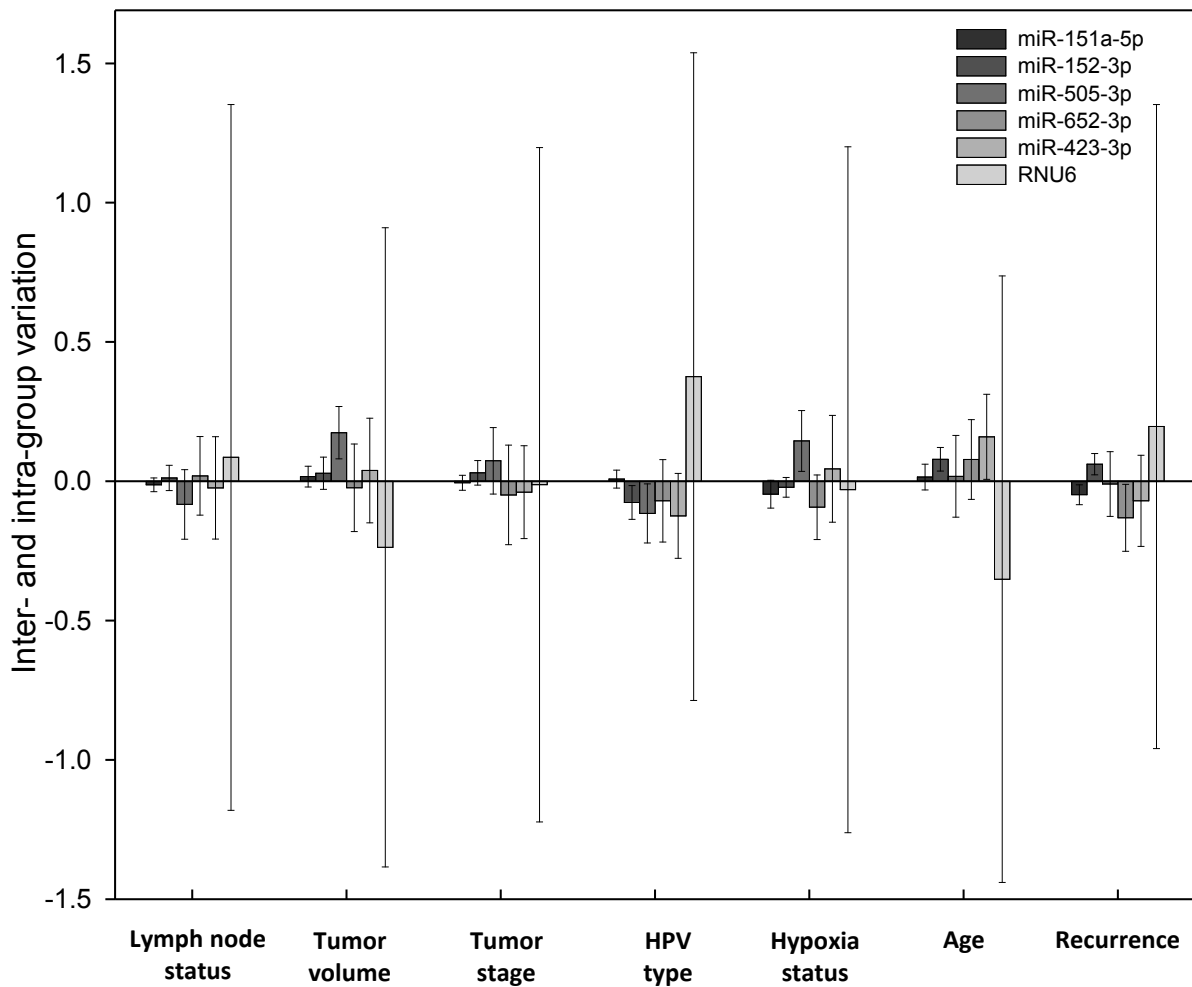


B)



Supplemental Fig. 3. **Cq values versus year of biopsy sampling.** Raw Cq values from RT-qPCR analyses of A) candidate reference miRNAs and RNU6 versus year of biopsy sampling in RT-qPCR cohort 1 (29 samples) and B) the selected reference miRNAs and miRNAs of investigation versus year of biopsy sampling in RT-qPCR cohort 1 extended. Points and error bars indicate mean Cq value and standard deviation. Spearman rank order correlation analysis showed no significance between the miRNAs or RNU6 and the year of biopsy sampling.

## Supplemental figure 4



Supplemental Fig. 4. **Inter- and Intra-group variation calculated by NormFinder.** The columns represent the inter-group variation and the error bars the average of the intra-group variances. The top-ranked gene has an inter-group variation as close to zero as possible and as small error bars as possible.