

## SUPPLEMENTARY TABLES

Table S1

Selected miR	Direction of aberrant expression per esophageal tissue type (compared to NSE)				Type of miR based on literature	Reference(s)
	BE-ND	LGD	HGD	EAC		
miR199a/b-3p	-	-	-	↑	Unknown	(1, 2)
miR-199a-5p	↑	-	↑	↑	BE-miR	(1-3)
miR-199b-5p	↑	-	↑	↑	BE-miR	(3)
miR-200b	=/↓	-	-	↓	Indefinite	(4)
miR-200c	=/↓	-	-	↓	Indefinite	(4)
miR-223	-	-	-	↑	Unknown	(5)
miR-375	↓	-	↓	↓	Neo-miR	(3, 6, 7)

Abbreviations: miR, microRNA; NSE, normal squamous epithelium; BE-ND, non-dysplastic Barrett's esophagus; LGD/HGD, low/high-grade dysplasia; EAC, esophageal adenocarcinoma

Table S1: Literature-based miR selection for qRT-PCR validation in a chemo-radiation naïve EAC cohort. Two types of miRs were chosen; miRs that may play a role in the development of BE-ND (BE-miRs) and miRs that may be involved in the neoplastic progression of BE (Neo-miRs).

Table S2

Quantitative real-time PCR primers		
Gene	Forward 5' to 3'	Reverse 5' to 3'
GAPDH	AGCCTCCCGCTTCGCTCTCT	GCGCCAATACGACCAAATCCGT
PARP1	CGAGGCGGCAGCGTGTTTCT	GCCCGCTCTTGGCGTACTCG
MT1E	AGAGCAGCCAGTTGCAGGGC	GAGCCACCAGTGGCGCAAGA
CYB5A	GGTGGAGCTGGCTGAGTCGC	GCCTCGTCCGACTGCTCTGC
SMARCD1	ACCCCGCCTAGCTCGACTC	TCCCGCTCGTGAGGGTCCTG
STMN1	CTCTCGGCCAATGCGGAGCC	CGAGCCGCCTGACCACACTC
SLC11A2	TGGGCTTTCACCGGACCAGG	GGAGCAGCAGCCCCACAAGG
SLC39A1	AGCCTTGACGTGACGCTCC	AGGTGACGGCCCTGACTGCT
WDR40A	ACCCGCCGTCCTGCTATCCT	GGCGCTTCCGCTTCCTGCT
GFPT1	CGGGAGGCGGAGAAGGCTGT	GGGGGTGCACACACGAGCTT
KIAA1279	ATGGCGAACGTTCCGTGGGC	GCCCGGGCGCTGTATTTGGA
LMO2	TTGGGTCCTGCAGGGCTTGCT	GGCGGAGCCTTACCCTTGC

Table S2: Quantitative real-time PCR primers used for the validation of the Illumina microarray results.

Table S3

Gene	Full name	Average fold change miR-223 versus Mock OE33 cells (Illumina microarray)	Binding of miR-223 to 3'UTR gene predicted by:		Match miR-223 and 3'UTR gene
			MicroCosm	TargetScan	
PARP1	Poly (ADP-ribose) polymerase 1	-2.42	Yes	Yes	8mer
MT1E	Metallothionein 1E	-2.27	Yes	Yes	7mer-1A
TNFRSF12A	Tumor necrosis factor receptor superfamily, member 12A	-2.22	No	No	
FAM104A	Family with sequence similarity 104, member A	-2.13	No	No	
CXCL1	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity)	-1.91	No	No	
CYB5A	Cytochrome b5 type A (microsomal)	-1.91	Yes	Yes	8mer
TUBAP2	Tubulin, alpha pseudogene 2	-1.88	Not listed	Not listed	
SLC11A2	Solute carrier family 11, member 2	-1.84	Yes	Yes	8mer
SLC39A1	solute carrier family 39 , member 1	-1.84	Yes	Yes	7mer-m8
HS.574590	-	-1.80	Not listed	Not listed	
SMARCD1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 1	-1.79	No	Yes	8mer
LOC643272	-	-1.78	Not listed	Not listed	
MT1IP	Metallothionein 1L, pseudogene	-1.78	Not listed	Not listed	
MANBAL	Mannosidase, beta A, lysosomal-like	-1.77	Yes	Yes	8mer
STMN1	Stathmin 1	-1.75	Yes	Yes	7mer-m8
PURB	Purine-rich element binding protein B	-1.74	No	Yes	8mer
AIDA	Axin interactor, dorsalization associated	-1.73	Not listed	Yes	7mer-1A
ARL2BP	ADP-ribosylation factor-like 2 binding protein	-1.70	No	No	
IGSF3	Immunoglobulin superfamily, member 3	-1.69	Yes	Yes	8mer
UBXN1	UBX domain protein 1	-1.69	Not listed	Yes	7mer-1A
ATP1B1	ATPase, Na <sup>+</sup> /K <sup>+</sup> transporting, beta 1 polypeptide	-1.67	Yes	Yes	7mer-m8
BLCAP	Bladder cancer associated protein	-1.67	No	No	
TMEM14D	Transmembrane protein 14 D (pseudogene)	-1.66	Not listed	Not listed	
LOC653631	-	-1.66	Not listed	Not listed	
FOSL1	FOS-like antigen 1	-1.66	No	No	

Table S3 (continued)

Gene	Full name	Average fold change miR-223 versus Mock OE33 cells (Illumina microarray)	Binding of miR-223 to 3'UTR gene predicted by:		Match miR-223 and 3'UTR gene
			MicroCosm	TargetScan	
GFPT1	Glutamine-fructose-6-phosphate transaminase 1	-1.65	Yes	Yes	8mer
TOMM5	Translocase of outer mitochondrial membrane 5 homolog (yeast)	-1.64	Not listed	Yes	7mer-m8
ARL6IP1	ADP-ribosylation factor-like 6 interacting protein 1	-1.64	No	No	
BOLA3	Bola homolog 3	-1.63	No	No	
SLMO2	Slowmo homolog 2	-1.62	No	Yes	7mer-m8
ATP6V1D	ATPase, H <sup>+</sup> transporting, lysosomal 34kDa, V1 subunit D	-1.62		No	
RAC1	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1)	-1.61	No	No	
TMED10	Transmembrane emp24-like trafficking protein 10	-1.60	No	Yes	7mer-1A
LMO2	LIM domain only 2 (rhombotin-like 1)	-1.60	No	Yes	8mer
DCAF12	DDB1 and CUL4 associated factor	-1.60	Not listed	Yes	7mer-m8
ARHGDI A	Rho GDP dissociation inhibitor (GDI) alpha	-1.59	No	No	
ABRACL	ABRA C-terminal like	-1.58	Not listed	Not listed	
MPP5	membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5)	-1.58	Not listed	Yes	8mer
CTSL2	Cathepsin L2	-1.58	Yes	Yes	7mer-m8
GCLM	Glutamate-cysteine ligase, modifier subunit	-1.57	Not listed	Yes	7mer-m8
TMEM14B	Transmembrane protein 14B	-1.57	Not listed	Not listed	
KIAA1279	KIAA1279	-1.56	Yes	Yes	7mer-m8
DESI2	Desumoylating isopepidase 2	-1.56	Not listed	Not listed	
THUMPD1	THUMP domain containing 1	-1.55	No	Yes	7mer-m8/7mer-1a
EPHX1	Epoxide hydrolase 1, microsomal (xenobiotic)	-1.55	No	No	
PSMD11	Proteasome (prosome, macropain) 26S subunit, non-ATPase	-1.55	No	No	
DBT	Dihydrolipoamide brached chain transacylase E2	-1.54	No	Yes	7mer-m8/7mer-1a

Table S3 (continued)

Gene	Full name	Average fold change miR-223 versus Mock OE33 cells (Illumina microarray)	Binding of miR-223 to 3'UTR gene predicted by:		Match miR-223 and 3'UTR gene
			MicroCosm	TargetScan	
SETP18	SET pseudogene 18	-1.53	Not listed	Not listed	
RCN2	Reticulocalbin 2, EF-hand calcium binding domain	-1.53	Yes	Yes	8mer
CMPK1	Cytidine monophosphate (UMP-CMP) kinase 1, cytosolic	-1.53	Not listed	Yes	7mer-m8
NEU1	Sialidase 1 (lysosomal sialidase)	-1.52	No	No	
CCT3	Chaperonin containing TCP1, subunit 3	-1.52	Yes	Yes	7mer-1A
SLC19A1	Solute carrier family 19 (folate transporter), member 1	-1.52	No	Yes	7mer-m8
PTRF	Polymerase I and transcript release factor	-1.51	No	No	
DLST	Dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)	-1.51	No	No	
NIPAL3	NIPA-like domain containing 3	-1.51	Not listed	Yes	8mer
MT1P3	Metallothionein 1 pseudogene 3	-1.51	Not listed	Not listed	
FKBP1C	FK506 binding protein 1C	-1.50	Not listed	Not listed	

Abbreviations:

8mer: an exact match to positions 2-8 of miR-223 (the seed + position 8) followed by an 'A';

7mer-m8: an exact match to positions 2-8 of miR-223 (the seed + position 8);

7mer-1A: an exact match to positions 2-7 of miR-223 (the seed) followed by an 'A'.

Table S3: Illumina microarray results of OE33 miR-223 cells compared to OE33 mock cells (two biological replicates). Genes more than 1.5 fold down-regulated are listed in this table. The prediction models MicroCosm (<http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/>) and TargetScan (8) were queried to find out whether binding of miR-223 to the 3'UTR of the potential target gene is likely. The type of match between miR-223 and the 3'UTR is also given.

## REFERENCES

1. Feber A, Xi L, Luketich JD, Pennathur A, Landreneau RJ, Wu M, et al. MicroRNA expression profiles of esophageal cancer. *The Journal of thoracic and cardiovascular surgery*. 2008;135(2):255-60; discussion 60. Epub 2008/02/05.
2. Yang H, Gu J, Wang KK, Zhang W, Xing J, Chen Z, et al. MicroRNA expression signatures in Barrett's esophagus and esophageal adenocarcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2009;15(18):5744-52. Epub 2009/09/10.
3. Leidner RS, Ravi L, Leahy P, Chen Y, Bednarchik B, Streppel M, et al. The microRNAs, MiR-31 and MiR-375, as candidate markers in Barrett's esophageal carcinogenesis. *Genes, chromosomes & cancer*. 2012;51(5):473-9. Epub 2012/02/04.
4. Smith CM, Watson DI, Leong MP, Mayne GC, Michael MZ, Wijnhoven BP, et al. miR-200 family expression is downregulated upon neoplastic progression of Barrett's esophagus. *World journal of gastroenterology : WJG*. 2011;17(8):1036-44. Epub 2011/03/31.
5. Mathe EA, Nguyen GH, Bowman ED, Zhao Y, Budhu A, Schetter AJ, et al. MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2009;15(19):6192-200. Epub 2009/10/01.
6. Bansal A, Lee IH, Hong X, Anand V, Mathur SC, Gaddam S, et al. Feasibility of microRNAs as biomarkers for Barrett's Esophagus progression: a pilot cross-sectional, phase 2 biomarker study. *The American journal of gastroenterology*. 2011;106(6):1055-63. Epub 2011/03/17.
7. Nguyen GH, Schetter AJ, Chou DB, Bowman ED, Zhao R, Hawkes JE, et al. Inflammatory and microRNA gene expression as prognostic classifier of Barrett's-associated esophageal adenocarcinoma. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2010;16(23):5824-34. Epub 2010/10/16.
8. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. 2005;120(1):15-20. Epub 2005/01/18.