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Upregulation of Gingival Tissue miR-200b in Obese Periodontitis Subjects

Abstract

Increased local immune and inflammatory responses in obese individuals with periodontitis may explain the aggressive clinical presentation and altered treatment response when compared to that of normal weight subjects. Our goal was to identify any differences in microRNA (miRNA) expression profiles of gingival tissue in periodontitis when obesity is present, which may suggest novel molecular pathways that this miRNA network may affect. Total RNA was extracted from gingival tissue biopsies collected from normal weight and obese individuals with periodontitis; miRNA expression profiling was performed with Affymetrix GeneChip miRNA 3.0 arrays; and results were validated with quantitative reverse transcription polymerase chain reaction (qRT-PCR). *In silico* identification of previously confirmed miRNA gene targets was conducted through miRTarBase and miRWalk databases, and pathway enrichment analysis identified enriched miRNA gene sets. Expression of selected genes in the same biopsy samples was tested with qRT-PCR. The gingival tissue miRNA profile of obese patients, compared to that of normal weight patients, showed 13 upregulated and 22 downregulated miRNAs, among which miR-200b was validated by qRT-PCR to be significantly increased in obesity. Functional analysis of 51 experimentally validated miR-200b gene targets identified enrichment of genes involved in cell motility, differentiation, DNA binding, response to stimulus, and vasculature development -pathways not previously identified in the obesity-specific disease profile. Furthermore, the expression of the miR-200b gene targets *ZEB1/2, GATA2,* and *KDR* was confirmed by qRT-PCR as being lower in obese patients with periodontitis versus normal weight patients, suggesting a role of miR-200b in regulation of a set of gene targets and biological pathways relevant to wound healing and angiogenesis. Functional studies to explore the role of miR-200b in the above processes may offer new insights on putative therapeutic targets for this group of patients.

Keywords

obesity, periodontal disease, microRNA-200b, epithelial, wound healing, angiogenesis

Periodontitis is a chronic polymicrobial oral disease presenting with dysregulated immune and inflammatory responses at the level of connective tissue and bone support surrounding the teeth, leading to tooth loss if left untreated (Kornman and Van Dyke 2008; Nahid et al. 2011). Obesity is associated with a higher prevalence of periodontitis where adipocytes surpass their lipid storage role and exert a number of endocrine functions, resulting in a state of low-grade inflammation and insulin resistance (Suvan et al. 2011). This may explain the more severe clinical presentation of periodontitis before treatment and poor response to periodontal therapy with less pocket resolution and higher bleeding levels (Suvan et al. 2014). In obesity, factors such a hyperglycemic status, the presence of advanced glycation end products, modifications of saliva's pH, and neutrophil dysfunction amplify the immune stress and affect the biological phenotype of periodontitis (Boesing et al. 2009).

MicroRNAs (miRNAs) are small (20- to 24-nucleotide) noncoding RNAs that mediate translational inhibition or degradation, by binding most commonly at the 3′ UTR of their messenger RNA (mRNA) targets (Bartel 2009). The latest miRBase release (v. 21, June 2014) contains 2,588 human mature miRNAs, estimated to regulate 20% to 30% of protein-coding genes. Microarray and polymerase chain reaction (PCR) array analyses have identified a number of differentially expressed miRNAs in individuals with periodontitis compared to healthy controls (Xie et al. 2011; Perri et al. 2012). Studies using experimental animal models of periodontitis have confirmed the overexpression of miR-146a in maxillary biopsies, coupled with increased expression of inflammatory cytokines (Nahid et al. 2011). A comprehensive screening of periodontitis-affected gingiva (Stoecklin-Wasmer et al. 2012) identified a specific miRNA profile and examined the mRNA expression of predicted targets in a transcriptomic dataset. Obese individuals were included only in one of these profiling studies, which used a selective miRNA array and a limited number of obese individuals (n = 5) with periodontitis (Perri et al. 2012).

MiRNAs regulate gene expression by providing negative feedback in a broad array of cellular processes that occur in periodontitis and that may be affected by obesity. Therefore, we hypothesized that obese periodontitis patients present with a different miRNA profile than that of normal weight patients with periodontitis and that some of these miRNAs would be associated with novel gene targets involved in the disease pathology. Using an Affymetrix miRNA microarray, quantitative reverse transcription PCR (qRT-PCR), and bioinformatics techniques we identified novel miRNA-regulated molecular processes that may contribute to an aggravated pathogenesis of periodontitis in obesity.

Materials and Methods

For full version of the methods, see Appendix.

Study Participant Selection and Sample Collection. A flowchart of the study design is presented in Appendix Figure 1. Thirty-six eligible individuals were identified among participants of a larger ongoing trial within the Unit of Periodontology, UCL Eastman Dental Institute and Hospital. All participants gave informed consent to participate in the study, which was approved by the Surrey Borders Research Ethics Committee, London (09/H0806/43). The investigators were blinded to group allocation until all laboratory work was concluded. Participants were classified as normal weight (body mass index, 20–24.9 kg/m^2) or obese (≥30 kg/m^2), according to the World Health Organization (2012) classification for defining body composition. All recruited subjects were required to have severe periodontitis (probing pocket depths >5 mm and marginal alveolar bone loss >30% with >50% of the teeth affected; Suvan et al. 2014) and >15 natural teeth, to be older than 35 y of age, and to be in good general health, free of diabetes or any systemic infection or disease. They were excluded if they were on chronic antibiotic (≥2 wk), anti-inflammatory, or anticoagulant therapy during the month preceding the baseline exam. Details of the additional inclusion and exclusion criteria are provided in Appendix Table 1. A gingival excess tissue sample was collected from each individual during the nonsurgical therapy phase and maintained in RNAlater solution (Ambion, TX, USA) at –20°C until further analysis.

RNA Isolation. Total RNA was isolated with the *mir*Vana miRNA Isolation Kit (Ambion). Assessment of sample quality was performed with the Agilent 2100 Bioanalyzer (Agilent Technologies, USA), and only 10 samples of the best RNA integrity (estimated as RNA integrity number, 8.65 ± 0.21) were selected for subsequent analyses (due to limited resources available for the GeneChip set of assays). The smaller group of patients shared similar characteristics with the larger group (that contained it), which was included in subsequent analyses.

GeneChip miRNA Microarray Analysis. The FlashTag Biotin RNA Labeling Kit for Affymetrix GeneChip miRNA Arrays (Genisphere, PA, USA) was used to label 500 ng of total RNA by the addition of polyA-polymerase. Samples were hybridized on Affymetrix GeneChip miRNA 3.0 arrays (Affymetrix, CA, USA) composed of 179,217 probes representing 19,913 mature miRNAs, of which 1,733 are human. The Affymetrix Fluidics protocol (FS450_0003) was followed for hybridization, washing, and scanning of the slides with the GeneChip Scanner 3000. The Affymetrix miRNA QC tools were used for data normalization and background correction. Data analysis was done with Partek 6.6 software and the fold change level set to 1.2, which was in agreement with previous miRNA microarray studies based on similar size platforms and number of samples (Dalman et al. 2012; Pritchard et al. 2012).

qRT-PCR Analysis of miRNA Expression. We used qRT-PCR to validate the expression levels of 6 selected miRNAs from the GeneChip miRNA microarray data that had the highest fold change or the lowest *P* value between the two groups, according to single TaqMan MicroRNA expression assays (Life Technologies, CA, USA) testing all available samples (normal weight, $n = 17$; obese, $n = 19$). TaqMan Assay IDs with primer information from manufacturer are presented in Appendix Table 2.

Bioinformatics Analysis for miR-200b-5p Gene Targets. Bioinformatics analysis was conducted exclusively for miR-200b-5p, the only confirmed target by qRT-PCR. Experimentally validated gene targets for miR-200b were identified in two databases: miRWalk (Dweep et al. 2011) and miRTarBase (Hsu et al. 2011). We combined these gene lists and confirmed the curated interactions (direct miRNA-mRNA binding confirmed by strong experimental evidence) by accessing the published manuscripts.

Functional Enrichment Analysis of miR-200b Targets. The g:profiler functional enrichment tool g:GOSt (http://biit.cs.ut.ee/gprofiler/index.cgi; Reimand et al. 2011) was used to look for overrepresentation of Gene Ontology terms in the miR-200b gene targets list relative to the whole human proteome.

qRT-PCR Analysis of miR-200b Gene Targets Expression. A selected group of genes representative for relevant processes and pathways was measured with TaqMan gene expression assays (Life Technologies, CA, USA) in all RNA samples that miR-200b-5p was previously measured. The "shortlisted" miR-200b gene targets were *ZEB1, ZEB2, CDH1, GATA2, PLCG1, KDR,* and *UBC* (normalization control), as well as *SMAD3*, which has been reported to regulate miR-200b (Ahn et al. 2012).

Statistical Analysis. Data from microarray were analyzed with Partek 6.6 and one-way analysis of variance, and qRT-PCR expression data were analyzed in the R-statistical environment. Differences between obese and normal weight groups were analyzed through a nonparametric test (Mann-Whitney *U* test) and results were presented as median ± interquartile range. Log transformation normalized the distributions and improved visualization and interpretation of results. The correlation between miR-200b-5p and the combined group of target genes was analyzed by calculating partial correlation coefficients, after removing the effects of obesity. For all analyses, $P < 0.05$ was considered significant.

Results

Study Demographics

The mean body mass index of the obese group was 36 kg/m^2, as compared to 22.36 kg/m^2 for the normal weight group. There were no statistically significant differences in age, sex, or ethnicity between the two groups, as presented in Appendix Table 3.

miRNA Expression Profiles of Gingival Tissue by Microarray Expression Analysis

Principal component analysis (PCA) did not show any set of miRNAs clustering in a distinct manner in any group (mapping percentage of 29.9%; Appendix Fig. 2). Moreover, a hierarchical clustering analysis resulted in segregation of samples into two distinct groups (obese and normal weight; Fig. 1). After normalization and using low stringency parameters (fold change ≥1.2 or ≤–1.2; $P \leq 0.05$), we detected 13 upregulated and 22 downregulated mature human miRNAs in obese individuals with periodontitis compared to normal weight (Table 1), with only three downregulated miRNAs exceeding a 1.5-fold expression difference (miR-4721, miR-557, miR-4327; fold changes: 1.55, 1.53, 1.50, respectively) (Table 1). The volcano plot depicts these miRNAs in Appendix Figure 3. The control miRNA RNU6B was not differentially expressed in the study tissues, which confirmed that it is a suitable normalization control for qRT-PCR experiments.

Figure 1.

Unsupervised hierarchical cluster analysis diagram of the differentially expressed microRNAs (miRNAs) in normal weight versus obese individuals with periodontitis. The heatmap highlights miRNA expression (colored grid) linked by a dendrogram (a tree diagram) to hierarchically cluster miRNAs. Each row represents individual human miRNAs, and each column represents a different sample. Each cell is colored according to the level of expression of that gene in that sample. Downregulated miRNAs are illustrated in blue and upregulated in red. A distinct miRNA expression pattern is presented for the normal weight group (left side) and the obese (right side).

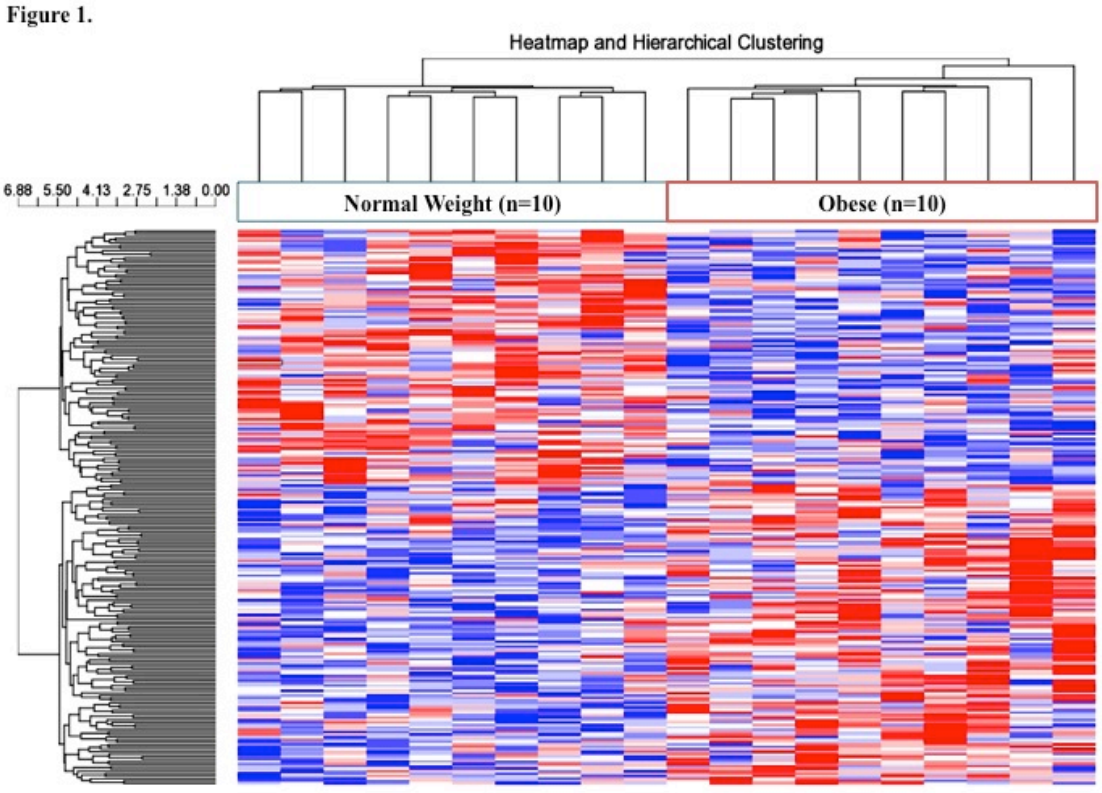

Table 1.

List of All Human (hsa-) Mature miRNAs That Are Down- or Upregulated with a Fold Change Threshold of 1.2 and an Associated *P* Value < 0.05

miRNA	Fold Change	*P* Value	Accession No
	Downregulated		
miR-4721 [a]	–1.55	0.003	MIMAT0019835
miR-557 [a]	–1.53	0.003	MIMAT0003221
miR-4327	–1.50	0.045	MIMAT0016889
miR-409-3p	–1.45	0.038	MIMAT0001639
miR-1183	–1.42	0.020	MIMAT0005828
miR-642b	–1.41	0.038	MIMAT0018444
miR-132	–1.39	0.030	MIMAT0000426
miR-3622a-5p	–1.37	0.012	MIMAT0018003
miR-299-5p	–1.34	0.019	MIMAT0002890
miR-196a [b]	–1.32	0.000	MIMAT0000226
miR-212	–1.32	0.038	MIMAT0000269
miR-764	–1.31	0.030	MIMAT0010367
miR-654-3p	–1.29	0.046	MIMAT0004814
miR-1238	–1.29	0.031	MIMAT0005593
miR-4269	–1.28	0.045	MIMAT0016897
miR-7-2-3p	–1.28	0.010	MIMAT0004554
miR-3198	–1.26	0.039	MIMAT0015083
miR-563	–1.26	0.012	MIMAT0003227
miR-1284	–1.26	0.007	MIMAT0005941
miR-433	–1.24	0.010	MIMAT0001627
miR-2276	–1.21	0.004	MIMAT0011775
miR-4683	–1.20	0.013	MIMAT0019768
miR-4721 [a]	–1.55	0.003	MIMAT0019835
	Upregulated		
miR-4725-5p	1.22	0.043	MIMAT0019843
miR-1253	1.22	0.012	MIMAT0005904
miR-765	1.23	0.047	MIMAT0003945
miR-323-3p [b]	1.23	0.003	MIMAT0000755
miR-200b-5p [b]	1.27	0.006	MIMAT0004571
miR-4642	1.31	0.024	MIMAT0019702
miR-4704-5p	1.31	0.045	MIMAT0019803
miR-200c-5p	1.31	0.047	MIMAT0004657
miR-1911-3p	1.31	0.036	MIMAT0007886
miR-3128	1.32	0.043	MIMAT0014991
miR-720 [c]	1.36	0.031	MI0006654
miR-4454	1.37	0.047	MIMAT0018976
miR-188-5p [a]	1.41	0.048	MIMAT0000457

Earlier naming convention used the miR/miR* nomenclature to identify the mature miRNA that was predominantly expressed from a precursor stem loop. This nomenclature has been replaced with -5p/-3p in miRBase. The miRandola database (http://atlas.dmi.unict.it/mirandola/tools.php; Russo et al. 2012) identified the most current name for each miRNA included in our Affymetrix array that did not have an updated nomenclature.

miRNA, microRNA.

Bold font indicates miRNAs selected to be validated with qRT-PCR because they had:

[a]the highest fold changes between groups or

[b]the lowest *P* values in the comparison of the observed differences.

[c]Furthermore, MiRBase (http://www.mirbase.org) noted that the sequence annotated as miR-720 is likely to be a fragment of a tRNA and so was removed from the database.

Validation of Microarray miRNA Expression Profiles by qRT-PCR

A total of six miRNAs—three upregulated (miR-323a-3p, miR-200b-5p, miR-188-5p) and three downregulated (miR-4721, miR-557, miR-196a)—were selected for validation experiments with qRT-PCR. As highlighted in Table 1, these were based on criteria such as the greatest fold change (miR-4721 and miR-557 from the downregulated group; miR-188-5p from upregulated group) and lowest *P* value (miR-323-3p and miR-200b-5p from upregulated group; miR-196a from downregulated group). Using TaqMan MicroRNA expression assays, we measured the expression of these miRNAs using U6snoRNA and RNU6B as endogenous controls (Fig. 2). As distribution was not normal, data was log transformed to improve interpretation. In all qRT-PCR experiments independent of exclusion of outliers, miR-200b-5p was expressed in significantly higher levels in gingival tissue biopsies from obese patients (64.2% increase in obese patients; $P = 0.007$). However, qRT-PCR expression of miR-557 and miR-4721 (previously downregulated in obesity in microarray) appeared significantly increased ($P = 0.002$ and 0.05, respectively). The expression of miR-196a (downregulated in obesity in microarray) and miR-188-5p and miR-323-3p (upregulated in obesity in microarray) were not statistically different between the two groups ($P = 0.75$, 0.3, 0.28, respectively), although they followed the trend of changes observed in the microarray.

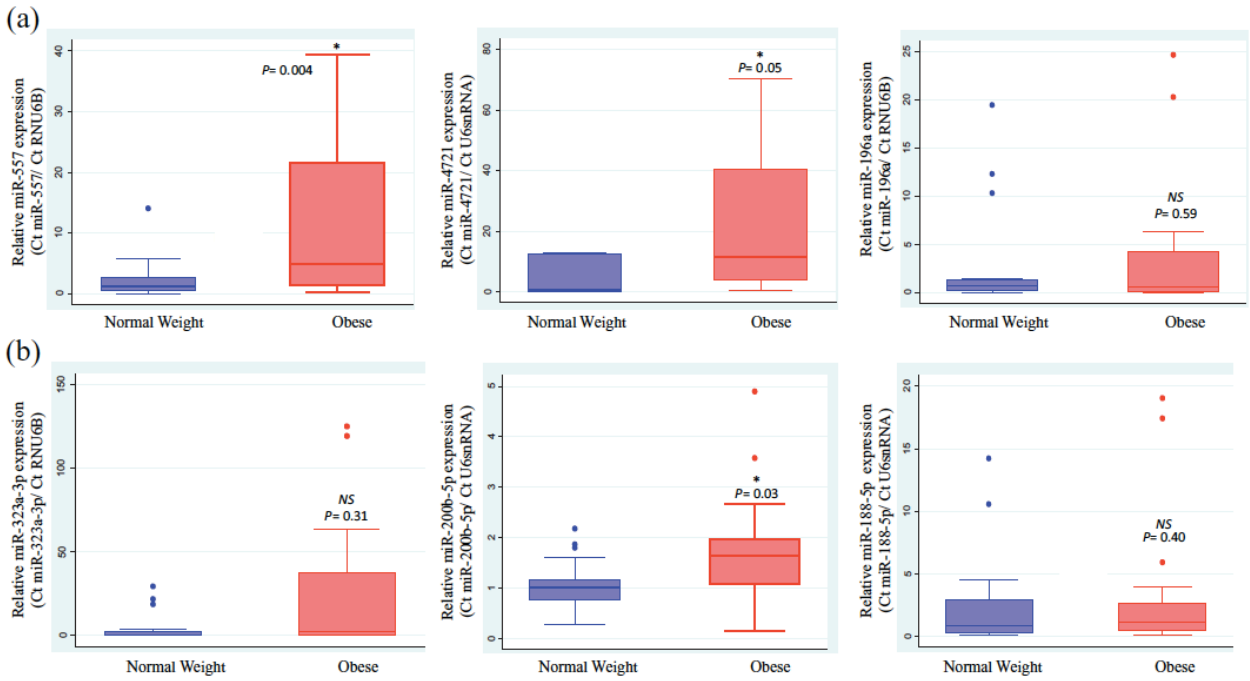

Figure 2.

Box and whisker plots of expression levels of selected microRNAs (miRNAs) by quantitative reverse transcription polymerase chain reaction: (**a**) the decreased miRNAs (miR-4721, miR-557, miR-196a) and (**b**) the increased miRNAs (miR-323a-3p, miR-200b-5p and miR-188-5p) in miRNA microarray experiments. The thick horizontal line indicates the mean in each group; the box in diagram represents the interquartile range; and the two whiskers extend from the lower quartile to the minimum value and from the upper quartile to the maximum value (±1.5 × interquartile range). Points outside of whiskers represent the mild/severe outliers per group. Significance was determined through a nonparametric test (Mann-Whitney *U* test), as the distribution was not normal. $^{*}P \leq 0.05$.

In Silico miR-200b Gene Target Identification

We used two databases to identify likely gene targets of the validated miRNAs. The target analysis was performed exclusively for the primary transcript of miR-200b-5p (as explained in detail in the Methods section [in the Appendix] and in Appendix Fig. 4). The miRWalk database included 203 entries for experimentally validated miR-200b targets. Filtering this list to remove indirect or falsely curated targets identified 62 entries of direct and true interactions, which included 23 unique gene targets (Appendix Table 4). To confirm these targets, we used the miRTarBase algorithm, which identified 39 manually curated and experimentally validated unique gene targets (Appendix Table 4), of which 11 genes were also curated by miRWalk; these combined approaches provided 51 experimentally validated unique miR-200b gene targets.

Functional Annotation Analysis of miR-200b Gene Targets Using Gene Ontology

We performed a functional enrichment analysis on the 51 validated miR-200b gene targets, using the Gene Ontology dataset (Ashburner et al. 2000), to understand the biological processes and molecular functions that these genes were associated with. The complete enriched Gene ontology terms for the above miR-200b targets, along with their statistical significance, are presented in Appendix Table 5 (summarized version in Table 2). All the genes included in the analysis had at least one annotation within the three Gene Ontology domains of "biological process," "molecular function," and "cellular component."

The 51 miR-200b gene targets were enriched for many Gene Ontology processes and suggested that many of these gene targets have roles in "cell differentiation" (24 genes), specifically "epithelial cell differentiation" (9 genes) and "stem cell differentiation" (7 genes), "angiogenesis" (11 genes), "epithelial cell migration" (6 genes), "immune system process" (18 genes), "transcription" (19 genes), "cellular response to stimulus" (33 genes), and "signaling" (32 genes; Table 2 and Appendix Table 5). Interestingly, our Gene Ontology analysis predicted involvement of pathways relevant to cell motility, cell differentiation, cell response to stimulus, and tissue development not identified before as possible pathways in an obesity-specific disease profile.

Table 2.

Gene Ontology Functional Analysis Summary.

Parent GO Term [a]: GO Term Name	miR-200b Gene Targets (Q = 51)					
	P Value	T	Q&T	Q&T/Q (R)	Q&T/T (P)	Common Genes
Biological process						
Tissue development: epithelium development	4.09E-04	827	13	0.26	0.02	*BCL2, CREB1, EGFR, EPHA2, ERRFI1, FLT1, GATA4, HOXB5,* ***KDR****, SMAD2, VEGFA,* ***ZEB1****,* ***ZEB2***
Vasculature development: angiogenesis	1.09E-05	387	11	0.22	0.03	*EPHA2, ETS1, FLT1, FN1,* ***GATA2****, GATA4,* ***KDR****,* ***PLCG1****, PTEN, SHC1, VEGFA*
Immune system process: immune system process	1.59E-02	2,182	18	0.35	0.01	*BCL2, BMI1, CREB1, EGFR, ELMO2, EP300, EPHA2, ETS1, FLT1, FN1,* ***GATA2****,* ***KDR****,* ***PLCG1****, PTEN, SHC1, VEGFA, XIAP,* ***ZEB1***
Cell differentiation						
Cell differentiation	6.25E-04	3,113	24	0.47	0.01	*BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1,* ***GATA2****, GATA4, HOXB5,* ***KDR****,* ***PLCG1****, PTEN, PTPRD, SMAD2, SUZ12, TCF7L1, VEGFA, WASF3,* ***ZEB1****,* ***ZEB2****, ZFPM2*
Epithelial cell differentiation	2.17E-02	527	9	0.18	0.02	*BCL2, CREB1, EPHA2, ERRFI1, GATA4, HOXB5,* ***KDR****, VEGFA,* ***ZEB1***
Stem cell differentiation	3.55E-02	304	7	0.14	0.02	*BCL2, EP300,* ***GATA2****, GATA4, SMAD2, TCF7L1,* ***ZEB2***
Cell motility						
Cell migration	2.72E-03	977	13	0.26	0.01	*BCL2, EGFR, ELMO2, EPHA2, ETS1, FLT1, FN1,* ***KDR****,* ***PLCG1****, PTEN, SHC1, VEGFA,* ***ZEB2***
Endothelial cell migration	1.97E-03	122	6	0.12	0.05	*EPHA2, ETS1,* ***KDR****,* ***PLCG1****, PTEN, VEGFA*

Epithelial cell migration	1.14E-02	165	6	0.12	0.04	*EPHA2, ETS1,* ***KDR***, ***PLCG1***, *PTEN, VEGFA*
Epithelium migration	1.14E-02	165	6	0.12	0.04	*EPHA2, ETS1,* ***KDR***, ***PLCG1***, *PTEN, VEGFA*
Cell adhesion: cell adhesion	2.73E-02	1,022	12	0.24	0.01	*ATP2A2, BCL2, EGFR, EPHA2, ERBB2IP, FN1,* ***KDR***, *PTEN, PTPRD, RND3, SHC1, VEGFA*
Transcription from RNA polymerase II promoter: transcription from RNA polymerase II promoter	3.00E-05	1,617	19	0.37	0.01	*BMI1, CREB1, E2F3, EGFR, EP300, ETS1,* ***GATA2***, *GATA4, KLF11, LITAF, NFAT5, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, XIAP,* ***ZEB1***, *ZFPM2*
Signaling						
Signaling	3.06E-04	5,247	32	0.63	0.01	*ATP2A2, BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, FLT1,* ***GATA2***, *GATA4,* ***KDR***, *LITAF, NFAT5,* ***PLCG1***, *PTEN, PTPRD, RASSF2RIN2, RND3, SHC1, SLC9A3R2, SMAD2, TCF7L1, VAC14, VEGFA, WASF3, XIAP,* ***ZEB1***, ***ZEB2***
ERBB signaling pathway	6.59E-03	235	7	0.14	0.03	*CREB1, EGFR, ERBB2IP, ERRFI1,* ***PLCG1***, *PTEN, SHC1*
Epidermal growth factor receptor signaling pathway	5.71E-03	230	7	0.14	0.03	*CREB1, EGFR, ERBB2IP, ERRFI1,* ***PLCG1***, *PTEN, SHC1*
Response to stimulus						
Cellular response to stimulus	6.49E-04	5,730	33	0.65	0.01	*ATP2A2, BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1,* ***GATA2***, *GATA4,* ***KDR***, *KLF11, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, SHC1, SLC9A3R2, SMAD2, , TCF7L1, VAC14, VEGFA, XIAP,* ***ZEB1***, ***ZEB2***
Cellular response to chemical stimulus	1.03E-02	2,117	18	0.35	0.01	*BCL2, CREB1, EGFR, ELMO2, EP300, EPHA2, ETS1, FLT1, GATA4,* ***KDR***, *KLF11, LITAF,* ***PLCG1***, *PTEN, SHC1, SMAD2, VEGFA,* ***ZEB1***
Cellular response to growth factor stimulus	1.42E-02	640	10	0.20	0.02	*CREB1, EGFR, FLT1,* ***KDR***, ***PLCG1***, *PTEN, SHC1, SMAD2, VEGFA,* ***ZEB1***
Molecular function						
DNA binding						
Nucleic acid binding transcription factor activity	5.15E-04	1,167	15	0.29	0.01	*CREB1, E2F3, EP300, ETS1,* ***GATA2***, *GATA4, HOXB5, KLF11, NFAT5, RERE, SMAD2, TCF7L1,* ***ZEB1***, ***ZEB2***, *ZFPM2*
Sequence-specific DNA binding transcription factor activity	3.27E-03	1,166	14	0.28	0.01	*CREB1, E2F3, EP300, ETS1,* ***GATA2***, *GATA4, HOXB5, KLF11, NFAT5, RERE, SMAD2, TCF7L1,* ***ZEB1***, ***ZEB2***
Transcription factor binding	9.39E-03	475	9	0.18	0.02	*BCL2, CREB1, EP300, ETS1,* ***GATA2***, *GATA4, SMAD2,* ***ZEB1***, *ZFPM2*
Cellular component						
Protein complex						
Transcription factor complex	2.39E-02	286	7	0.14	0.02	*CREB1, E2F3, EP300, ETS1, SMAD2, TCF7L1,* ***ZEB1***
PcG protein complex	2.70E-03	30	4	0.08	0.13	*BAP1, BMI1, RNF2, SUZ12*
Shc-EGFR complex	1.60E-02	2	2	0.04	1.00	*EGFR, SHC1*

A selection of enriched Gene Ontology (GO) terms identified by functional analysis through g:profiler for the 51 validated gene targets extracted from the miRWalk and miRTarBase databases. Based on the g:SCS statistical method, a $P < 0.05$ was considered significant. Bold font highlights the genes that were selected to measure their mRNA expression in our gingival tissue samples. An extended version of this GO analysis summary table is presented in the Appendix Table 5.

T, the number of human genes associated with the GO term; Q, the number of genes analyzed (query dataset); Q&T, the number of genes in the query dataset that are also associated with the GO term (common genes); Q&T/Q, an index of recall; Q&T/T, an index of precision.

[a]Included to group-enriched terms.

mRNA Expression of miR-200b Gene Targets in Gingival Tissue Biopsies

After outlier exclusion, statistical analysis showed a significant negative correlation between the expression of miR-200b-5p and transcription factors *ZEB2* and *GATA2* in human gingival tissue biopsies from obese patients with periodontitis (Fig. 3b: $r = –0.46$, $P = 0.008$; Fig. 3d: $r = –0.42$, $P = 0.02$, respectively). The same inverse correlation trend toward significance was observed for miR-200b-5p expression and genes *ZEB1* and *KDR* in the same samples from obese patients (Fig. 3a: $r = –0.34$, $P = 0.06$; Fig. 3d: $r = –0.33$, $P = 0.06$, respectively). The correlation for the combined groups is presented in Appendix Table 6. Expression of *SMAD3* was not significantly correlated with

changes in miR-200b-5p expression (P = 0.14), nor was expression levels of E-cadherin (P = 0.89), a known downstream target of *ZEB1/2. PLCG1* expression in our gingival tissue samples in both groups was too low to allow comparisons. These findings suggest that the correlation between increased expression of miR-200b-5p and periodontitis in obesity is closely related to the miR-200b-5p regulation of *ZEB1/2, GATA2*, and *KDR* genes in these periodontitis patient specimens.

Figure 3.

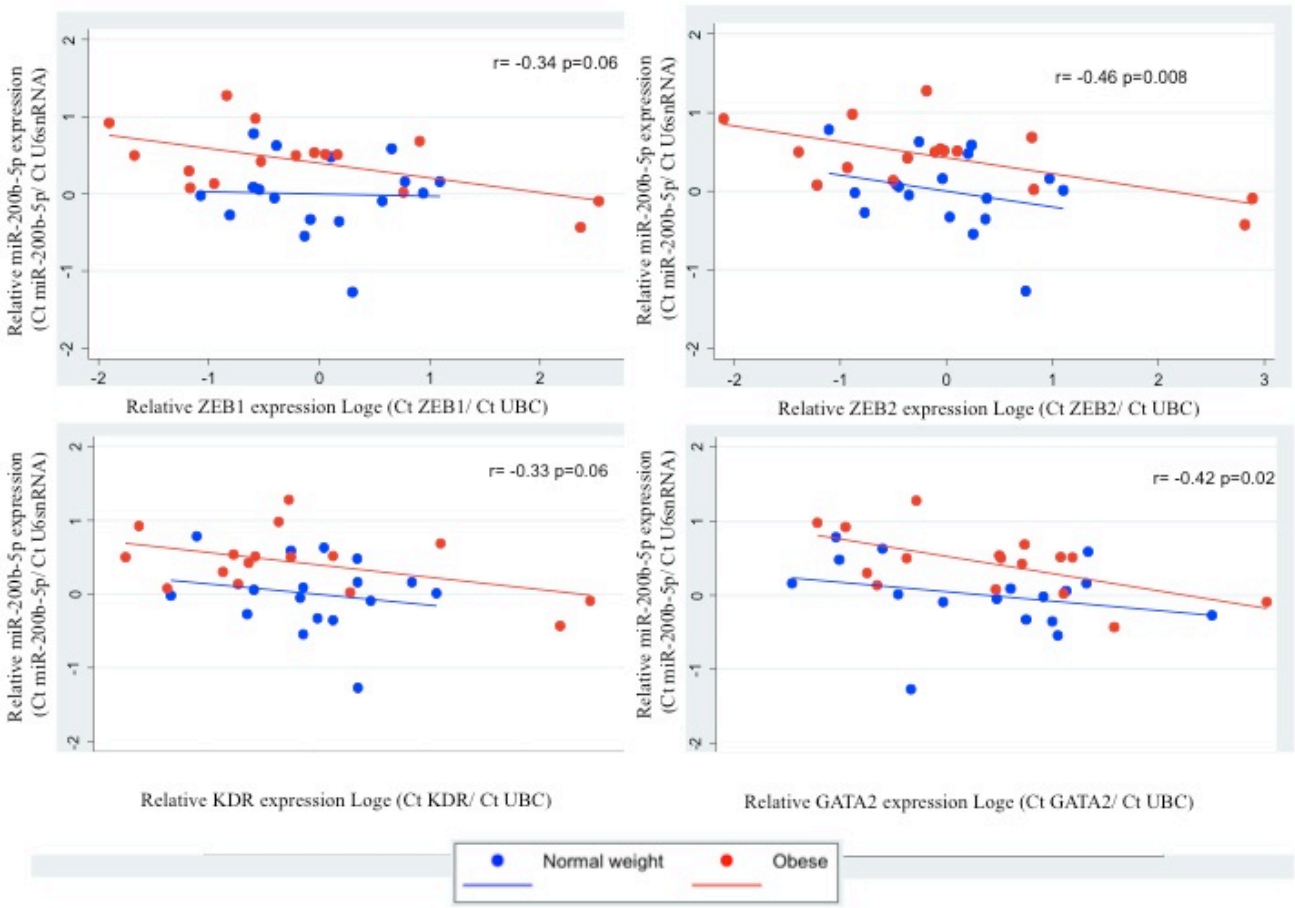

Figure 3.

Inverse correlation between the miR-200b-5p expression and its target genes *ZEB1, ZEB2, KDR,* and *GATA2* in gingival tissue biopsies. (**b**, **d**) There was a statistically significant correlation between the expression of miRNA-200b and *ZEB2* (r = –0.46, P = 0.008) and between miR-200b-5p and *GATA2* (r = –0.42, P = .02). (**a**, **c**) The correlations between miR-200b-5p expression and *ZEB1* and *KDR* followed the same trend (r = –0.34, P = 0.06; r = –0.33, P = 0.06, respectively) but did not reach statistical significance.

Discussion

In the present study, microarray miRNA profiling of gingival tissue samples revealed a differential miRNA expression in obese individuals with periodontitis when compared to normal weight. Among the 13 upregulated miRNAs and 22 downregulated miRNAs in the presence of obesity, miR-200b-5p expression was found to be 1.6 times higher in obese patients, as replicated with qRT-PCR. We further showed an inverse correlation between miR-200b-5p expression and mRNA expression of its target genes *ZEB1, ZEB2, GATA2,* and *KDR* in gingival tissue biopsies from periodontal disease patients, with *ZEB2* and *GATA2* being statistically significant and *ZEB1* and *KDR* approaching a substantial trend toward significance. Previous studies have demonstrated that miRNA-200b

interacts directly with the 3′ UTR of these genes and regulates their expression at the translational level (Ahn et al. 2012). The above genes play central roles in pathways involved in reepithelialization of gingival wounds (Tomikawa et al. 2012), which is an important process in periodontal regeneration and reestablishment of tissue integrity, affecting treatment outcome.

MiR-200b is expressed in a variety of cells and modulates key cellular functions, such as cell proliferation, motility, apoptosis, and stem cell properties, and it controls signals in angiogenesis and epithelial-mesenchymal transition, a process in which epithelial cells acquire mesenchymal characteristics (Brabletz and Brabletz 2010). Regulation of miR-200b/c modifies TLR4 signaling in macrophages, with effects on host innate defenses against periodontal pathogens (Wendlandt et al. 2012), while miR-200c-5p was found to be increased in inflammatory bowel disease patients (Paraskevi et al. 2012). There have been no studies reporting miR-200b expression in gingival tissue in obesity. However, in experimental models of obesity, diet-induced liver injury was correlated with increased miR-200b levels in mouse plasma (Tryndyak et al. 2012) and in mouse and rat liver tissue (Alisi et al. 2011; Tryndyak et al. 2012). Decreased miR-200b expression was reported in obese mouse adipose tissue (Oger et al. 2014), suggesting a cell-specific regulation of its expression.

One of the known factors regulating hepatic miR-200b expression (Hu et al. 2012), as well as the regenerative capacity of periodontium (Nokhbehsaim et al. 2014), is the metabolic hormone leptin, which was not measured in our subjects. In obese patients, gingival miR-200b upregulation could affect reprogramming epithelial-mesenchymal transition (Korpal et al. 2008), and stem cell pluripotency (Miyazaki et al. 2012) is highly relevant to regenerative biological pathways that occur in periodontitis. Since *ZEB1* 3′ UTR contains 5 highly conserved miR-200b binding sites and the *ZEB2* 3′ UTR contains 6 (Gregory et al. 2008; Brabletz and Brabletz 2010), the suppression of gingival *ZEB1/2* expression in the obese biopsies is expected. As ZEB factors are strong epithelial-mesenchymal transition inducers, in obese patients miR-200b overexpression could hinder mesenchymal cell growth over the epithelium and reduce periodontal healing. Furthermore, the association of *ZEB2* with the TGFβ/BMP/Wnt pathway (Gregory et al. 2008; Shin et al. 2012; Cong et al. 2013) makes it a particularly important factor for the reestablishment of periodontal attachment, as downregulation of Wnt signaling leads to a pathologic widening of the periodontal ligament space (Lim, Liu, Cheng, et al. 2014) and to root resorption (Lim, Liu, Hunter, et al. 2014). Both *ZEB1* and *ZEB2* bind to E-boxes in the E-cadherin promoter and control its expression (Ahn et al. 2012). Contrary to our expectations, the expression of E-cadherin in the obese gingival biopsies was similar to normal weight. However, a miR-200b effect on other ZEB1- and ZEB2-mediated junctional proteins cannot be excluded (Howe et al. 2011).

Angiogenesis plays a pivotal role in periodontitis, as it facilitates transporting oxygen and nutrients to the injury site and removal of the cellular debris from the inflamed tissue (Artese et al. 2010). Downregulation of endothelial miR-200b enables upregulation of *GATA2* transcription factor activity, which regulates the promoters of many endothelial genes and thus, successful angiogenic outcome (Chan et al. 2012). In obese subjects, upregulation of miR-200b correlated with a decreased expression of *GATA2* as well as *KDR*, both of which play central roles in the angiogenic response and wound closure.

Previous studies in normal weight individuals with periodontitis reported increased expression of miRNAs linked to inflammatory/immune response pathways (Xie et al. 2011; Stoecklin-Wasmer et al. 2012). In our study, none of these periodontitis-specific miRNAs were found differentially expressed among our samples, as all the study participants had periodontitis. This adds to the novelty of our findings, which describes the upregulation of miR-200b in the gingival tissue of obese periodontitis subjects.

Although the miRNA microarray allowed the analysis of a large number of miRNAs in parallel, the low sensitivity of the assay required more sensitive methods (qRT-PCR) to quantify the

differences (Draghici et al. 2006). Even though miR-200b expression reached statistical significance when measured by qRT-PCR, confirming the differences in other miRNAs (miR-557, miR-4721, and miR-196a) was challenging due to the small differences between the groups and the low expression levels, as previously noted (Fichtlscherer et al. 2010). Further limitations of this study were the small sample size and tissue availability. We selected a limited number of miRNAs for qRT-PCR validation, using fold change and *P* value criteria. Our cutoff for microarray data comparisons between the two groups was set to 1.2-fold, which is in agreement with previous studies on miRNA expression suggesting that, unlike gene expression microarrays that use higher cutoff values, a smaller 1.2-fold change in miRNA expression can be biologically relevant (Dalman et al. 2012; Pritchard et al. 2012). Furthermore, a more focused investigation in a larger sample set on the association of miR-557 and miR-4721 with obesity may be interesting, as they appeared decreased in the microarray in obese samples but significantly increased when measured by qRT-PCR.

To identify relevant miR-200b mRNA targets, we assumed that the gene targets of the primary transcript miR-200b are the same as the gene targets of miR-200b-5p. A search in TargetScan identified 1,057 transcripts with conserved sites as putative targets of human miR-200bc/429/548a. However, as the reliability of target predictions is still highly debatable (Pio et al. 2014), we limited our functional analysis to "experimentally confirmed" miR-200b targets. This filtering might have excluded novel genes that could be regulated by miR-200b. Despite the smaller number of genes included, a substantial proportion of miR-200b genes were linked to biological pathways associated with miR-200b alone, such as cell migration and epithelium development. Comprehensive annotation of all the confirmed miR-200b target genes would aid the interpretation of this dataset.

The pathophysiologic mechanisms underlying periodontitis in the presence of obesity are complex and currently not fully understood. Here we report that in obese patients with periodontitis gingival tissue, miR-200b is increased, suggesting a suppressing effect on a group of genes and transcription factors that may affect cell plasticity, tissue homeostasis, and angiogenesis. Addressing periodontitis with more effective treatments will reduce the risk of secondary events in remote tissues and organs, such as the cardiovascular system. Further extensive functional experiments *in vitro* and in animal models of periodontal disease are required to validate the suitability of the miR-200b regulatory network as a therapeutic target.

Data Deposition

The Affymetrix miRNA microarray data have been submitted to Gene Expression Ominbus (GSE59398).

Author Contributions

A.Z. Kalea, R.C. Lovering, contributed to design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; R. Hoteit, contributed to data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; J. Suvan, V.K. Khodiyar, contributed to data acquisition, analysis, and interpretation, critically revised the manuscript; J. Palmen, contributed to data acquisition and analysis, critically revised the manuscript; J.A. Cooper, contributed to design, data acquisition, analysis, and interpretation, critically revised the manuscript; Z. Harrington, contributed to data acquisition, critically revised the manuscript; S.E. Humphries, contributed to conception, design, and data interpretation, critically revised the manuscript; F. D'Aiuto, contributed to conception, design, data acquisition, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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Clinical Investigations

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JDR Clinical Research Supplement Appendix

Appendix *JDR Clinical Research Supplement*

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Upregulation of Gingival Tissue miR-200b in Obese Periodontitis Subjects

APPENDIX

METHODS

RNA Isolation

Tissues were weighed, and total RNA including microribonucleic acids (miRNAs) was isolated using the *mir*Vana miRNA Isolation Kit (Ambion, TX, USA) following the manufacturer's instructions, and RNA was eluted in DEPC-treated water. The RNA concentration and quality were assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, DE, USA), accepting a ratio of 2.0 (± 5%) for sample absorbance at 260/280. Samples were further treated with DNase I (Invitrogen, CA, USA) to exclude genomic DNA contamination. Further assessment of sample quality was performed using the Agilent 2100 Bioanalyzer (Agilent Technologies Inc, USA) in order to select ten samples of the best RNA integrity (estimated as RNA integrity number), in order to proceed with the miRNA microarray platform, at the UCL Genomics Centre. All samples from both groups were later used for the validation of selected miRNA and target gene expression analyses using quantitative reverse transcription polymerase chain reaction (qRT-PCR).

GeneChip miRNA Microarray Analysis

Agilent 2100 Bioanalyzer measured RNA integrity number, RNA concentration, and the ribosomal ratio (28S/18S rRNA), using a fluorescent assay combined with electrophoresis. The FlashTag Biotin RNA Labeling Kit for Affymetrix GeneChip miRNA arrays (Genisphere, PA, USA) was used to label 500ng of total RNA by the addition of polyA-polymerase, following the manufacturer's instructions. Samples were hybridized on Affymetrix GeneChip miRNA 3.0 arrays (Affymetrix, CA, USA) comprised of 179 217 probes that represent 19 913 mature miRNAs of which 1 733 are human. The Affymetrix Fluidics protocol (FS450_0003) was followed for hybridization, washing, and scanning of the slides using the GeneChip Scanner 3000. The Affymetrix miRNA QC tools were used for data normalization and background correction. This was done using RMA (robust multiarray average) and DABG (detection above background) methods to down-weigh the poorly performing probes and to detect the *P* value for each probe set, respectively. Data analysis was done

using Partek 6.6 software and the fold change level was set to 1.2, which was in agreement with previous miRNA microarray studies using similar size platforms and number of samples (Pritchard et al. 2012).

Functional Enrichment Analysis of Validated miR-200b Targets

The g:profiler functional enrichment tool g:GOSt (http://biit.cs.ut.ee/gprofiler/index.cgi) (19) was used to look for over-representation of Gene Ontology (GO) terms in the miR-200b gene targets list relative to the whole human proteome. The annotation datasets used for the analysis were based on the Ensembl 75 and Ensembl Genomes 22 (May 26, 2014) releases. Effective domain size for GO:18656. The query gene lists (as HGNC symbols) were pasted into the "Query Set" field (unrecognized or ambiguous HGNC symbols were replaced with a UniProtKB ID). The hypergeometric test, with the 'g:SCS statistical threshold' was used for enrichment analysis as GO consists of hierarchically related general and specific terms. The g:SCS method computes multiple testing correction for *P* values gained from GO enrichment analysis, while g:SCS analytically approximates a threshold *t* corresponding to the 5% upper quintile of randomly generated queries of that size. All actual *P* values resulting from the query are transformed to corrected *P* values by multiplying these to the ratio of the approximate threshold *t* and the initial experiment-wide threshold a = 0.05. The default options selected were 'significant only', 'hierarchical sorting', 'undefined maximum size of functional category', and 'only annotated genes' as the 'statistical domain size'.

qRT-PCR Analysis of miRNA Expression

We used qRT-PCR to validate the expression levels of six selected miRNAs from the GeneChip miRNA microarray. Data that had the highest fold-change or the lowest *P* value between the two groups were validated by single TaqMan MicroRNA expression assays. TaqMan Assay IDs with Primer information from manufacturer are presented in Appendix Table 2. For this analysis all available samples were used (n = 17 for normal weight and n = 19 for obese group). TaqMan MicroRNA reverse transcription kit (Applied Biosystems, USA) was used to specifically transcribe miRNAs to cDNA. Each reaction included 10ng of diluted RNA, 1.5 µl of 10X RT buffer, 0.15µl of 100mM dNTPs, 3µl of 5X RT primers specific for the miRNAs of interest, as well as for the endogenous control miRNAs, 0.19µl of 20U/µl RNase inhibitor, 1µl of 50 U/µl MultiScribe Reverse Transcriptase and nuclease-free water to a total volume of 15µl. The reverse transcription reaction was performed at 16°C for 30 min followed by 42°C for 30 min, 85°C for 5 min and at 4°C on hold in a Bio-Rad C1000 Thermocycler. The cDNA from the samples was stored at -20°C before performing the qRT-PCR. For normalization we used two small non-coding RNAs (U6snoRNA and RNU6B) with stable and moderate expression across a large number of human tissues that were used by other studies (Perri et al. 2012). The qRT-PCR was done following the standard 384-well protocol on the Applied Biosystems 7900HT RT-PCR System. For each miRNA, the reaction included 1µl of the corresponding 20X TaqMan Gene Expression (primer/probe) mix, 10µl of 2X TaqMan Universal master mix II, 1.2µl of cDNA and nuclease-free water to a total volume of 20µl per reaction. Triplicate reactions were run and each reaction consisted of 40 cycles of 95°C for 15 sec and 60°C for 1 min. Amplification data was analyzed using RQ Manager (Applied Biosystems, Foster City, CA, USA). The expression of all miRNAs was calculated using the -ΔΔCt method (relative quantification, $RQ = 2^{-\Delta\Delta Ct}$).

Bioinformatic Analysis for Identification of miR-200b-5p Targets

Bioinformatics analysis was conducted exclusively for miR-200b-5p, which was the only confirmed target by qRT-PCR. To gain further insight into the genes and biological pathways regulated by miR-200b-5p, we followed the curated information on experimentally validated targets of miR-200b using two different databases, miRWalk and miRTarBase. MiRWalk (http://www.umm.uni-

heidelberg.de/apps/zmf/mirwalk/index.html updated on March 2013) (Dweep et al. 2011) is a comprehensive database with eight established target prediction programs, as well as information on experimentally validated miRNA interactions associated with genes and pathways including the manuscripts (PubMed IDs) curated to extract the information. MiRTarBase (http://mirtarbase.mbc.nctu.edu.tw) curates experimentally validated miRNA-target interactions by manually reviewing relevant literature including functional assays on miRNA binding (Hsu et al. 2011). We combined the outputs of miRWalk and miRTarBase, extracted the list of experimentally validated targets for miR-200b and confirmed the curated interactions (for direct miRNA to mRNA binding at the 3'UTR with functional assays to confirm this) by accessing the published manuscripts.

Functional Enrichment Analysis of Validated miR-200b Targets

The g:profiler functional enrichment tool g:GOSt (http://biit.cs.ut.ee/gprofiler/index.cgi) (Reimand et al. 2011) was used to look for over-representation of GO terms in the miR-200b gene targets list relative to the whole human proteome.

qRT-PCR Analysis of miR-200b Gene Targets Expression

After identification of possible miR-200b gene targets, a selected list of genes representative for interesting processes and pathways was measured in all RNA samples that miR-200b-5p was previously measured. A High-Capacity RNA-to-cDNA reverse transcription kit (Applied Biosystems, Life Technologies, CA, USA) was used to specifically transcribe DNAse I treated RNA to cDNA, following the manufacturer's instructions. Each 20µl reaction included 1µg of RNA, 10µl of 10X RT buffer and 1µl of 20X Enzyme Mix. The reverse transcription reaction was performed at 37°C for 60 min, followed by 95°C for 5 min and at 4°C on hold in a Bio-Rad C1000^{TM} Thermocycler. We then used the cDNA to measure expression of the "shortlisted" miR-200b gene targets using TaqMan® gene expression assays (Life Technologies, CA, USA) for *ZEB1, ZEB2, GATA2, KDR, PLCG1, CHD1, SMAD3* (which has been reported to regulate miR-200b (Ahn et al. 2012)) and *UBC* (as a normalization control). As before, the qRT-PCR was done following the standard 384-well protocol on the Applied Biosystems 7900HT RT-PCR System. Each reaction included 1µL of 20X TaqMan® Gene Expression Assay and 10µL of 2X TaqMan® Gene Expression Universal Master Mix, 1.8µl of cDNA and nuclease-free water to a total volume of 20µl per reaction. Triplicate reactions were run and each reaction consisted of 40 cycles of 95°C for 15 sec and 60°C for 1 min. Amplification data was analyzed using RQ Manager (Life Technologies, CA, USA). The expression of all miR-200b target genes was calculated using the -ΔΔCt method (relative quantification, $RQ = 2^{-\Delta\Delta Ct}$).

TaqMan Assay IDs with Primer information and more details on the protocols are presented in Appendix Table 2.

Statistical Analysis

Data from microarray were analyzed using Partek 6.6 and 1-way analysis of variance. A 1.2-fold change was chosen as cut-off for comparisons in agreement with previous studies on miRNA expression that suggest that, unlike gene expression microarrays that use a cut-off value of 1,5 to 2 (Dalman et al. 2012) a small 1.2-fold change in miRNA expression can be biologically relevant (Choe et al. 2005).**[*AQ7*]** Identification of experimentally validated miRNA targets was performed using miRWalk and miRTarBase databases. In this study, we only focused on mature human miRNAs since the target validation for hairpin miRNAs is not possible with the available algorithms. qRT-PCR expression data were analyzed in the R statistical environment. Differences between obese and non-obese groups were analyzed using a nonparametric test (Mann-Whitney *U* test) and results presented as median (± interquartile range), as the data were not normally distributed and the sample size was small. Log-transformation normalized the distributions and was used to improve

visualization and interpretation of results, to allow regression lines to be fitted and interactions with obesity to be tested. Few outliers were excluded from the samples after transformation. The correlation between miR-200b-5p and the combined group of target genes was analyzed by calculating partial correlation coefficients after removing the effects of obesity. For all analyses, a *P* value < 0.05 was considered significant.

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Appendix Table 1.

Inclusion and Exclusion Criteria for Study Participants.

Inclusion criteria	>35 y of age
	In good general health
	Required to have >15 natural teeth
	Been diagnosed with severe chronic periodontitis (defined as probing pocket depths of >5 mm and marginal alveolar bone loss of >30% with >50% of the teeth affected)
Exclusion criteria	Body mass index between 25 and 29.9 (World Health Organization overweight)
	Smoking (within the previous 5 y)
	History of alcohol or drug abuse
	Diagnosis of diabetes
	Treatment for serious systemic medical conditions (hepatic disease, renal disease, transmittable diseases, cancer, or HIV)

Appendix Table 2.

TaqMan Expression Assay IDs (Life Technologies, CA, USA).

Genes	Assay ID
hsa-miR-106b	000442
hsa-miR-188-5p	002320
hsa-miR-196a	241070_mat
hsa-miR-200b-5p	002274
hsa-miR-323	002695
hsa-miR-557	001525
hsa-miR-4721	463604_mat
RNU6B	001093
snoU6	001973
ZEB1	Hs00232783_m1
ZEB2	Hs00207691_m1
PLCG1	Hs01008225_m1
KDR	Hs00911700_m1
GATA2	Hs00231119_m1
SMAD3	Hs00969210_m1
CDH1	Hs01023894_m1
UBC	Hs01871556_s1

Appendix Table 3.

Demographics of the Study Participants.

Variables	Obese	Normal Weight	*P* Value
Mean age	50.5 ± 4.93	50.3 ± 9.41	0.95
Mean body index	36 ± 4.08	22.36 ± 1.11	<0.001
Sex			0.66
Male	6 (60)	7 (70)	
Female	4 (40)	3 (30)	
Ethnicity			0.83
Caucasian	7 (70)	7 (70)	
African	1 (10)	1 (10)	
Asian	1 (10)	1 (10)	
Afro-Caribbean	1 (10)	1 (10)	

Values in mean ± SD or *n* (%).

Appendix Table 4.

Candidate Genes as Experimentally Validated miRNA Gene Targets Associated in miRWalk and miRTarBase Databases with the Differentially Expressed miRNAs in the Obesity Group.

Gene Symbol	Entrez ID	Official Name	MiRWalk	MiRTarBase
ATP2A2	488	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2	×	
BAP1	8314	BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase)		×
BCL2	596	B-cell CLL/lymphoma 2		×
BMI1	648	BMI1 proto-oncogene, polycomb ring finger		×
CCNE2	9134	Cyclin E2		×
CREB1	1385	cAMP responsive element binding protein 1		×
E2F3	1871	E2F transcription factor 3	×	×
EGFR	1956	Epidermal growth factor receptor	×	
ELMO2	63916	Engulfment and cell motility 2		×
EP300	2033	E1A binding protein p300		×
EPHA2	1969	Ephrin (EPH) receptor A2	×	
ERBB2IP	55914	erbb2 interacting protein		×
ERRFI1	54206	ERBB receptor feedback inhibitor 1		×
ETS1	2113	v-ets avian erythroblastosis virus E26 oncogene homolog1	×	×
FLT1	2321	fms-related tyrosine kinase 1		×
FN1	2335	Fibronectin 1		×
GATA2	**2624**	**GATA binding protein 2**	×	
GATA4	2626	GATA binding protein 4		×
HOXB5	3215	Homeobox B5		×
KDR	**3791**	**Kinase insert domain receptor (a type III receptor tyrosine kinase)**	×	×
KLF11	8462	Kruppel-like factor 11		×
KLHL20	27252	Kelch-like family member 20		×
LITAF	9516	Lipopolysaccharide-induced TNF factor	×	
MATR3	9782	Matrin 3	×	×
NFAT5	10725	Nuclear factor of activated T-cells 5, tonicity-responsive	×	

PLCG1	**5335**	**Phospholipase C, gamma 1**	×	
PTEN	5728	Phosphatase and tensin homolog	×	
PTPN12	5782	Protein tyrosine phosphatase, non-receptor type 12		×
PTPRD	5789	Protein tyrosine phosphatase, receptor type, D		×
RASSF2	9770	Ras association (RalGDS/AF-6) domain family member 2		×
RERE	473	Arginine-glutamic acid dipeptide (RE) repeats	×	×
RIN2	54453	Ras and Rab interactor 2		×
RND3	390	Rho family GTPase 3	×	×
RNF2	6045	Ring finger protein 2		×
SEPT7	989	Septin 7		×
SFRS2IP	9169	SR-related CTD-associated factor 11	×	
SHC1	6464	SHC (Src homology 2 domain containing) transforming protein 1		×
SIP1	8487	Gem (nuclear organelle) associated protein 2	×	
SLC9A3R2	9351	Solute carrier family 9, subfamily A (NHE3, cation proton antiporter 3), member 3 regulator 2	×	
SMAD2	4087	SMAD family member 2		×
SUPV3L1	6832	Suppressor of var1, 3-like 1		×
SUZ12	23512	SUZ12 polycomb repressive complex 2 subunit	×	×
TCF7L1	83439	Transcription factor 7–like 1 (T-cell specific, HMG-box)		×
VAC14	55697	Vac14 homolog (S. cerevisiae)		×
VEGFA	7422	Vascular endothelial growth factor A	×	×
WASF3	10810	WAS protein family, member 3	×	×
WDR37	22884	WD repeat domain 37		×
XIAP	331	X-linked inhibitor of apoptosis		×
ZEB1	**6935**	**Zinc finger E-box binding homeobox 1**	×	×
ZEB2	**9839**	**Zinc finger E-box binding homeobox 2**	×	×
ZFPM2	23414	Zinc finger protein, FOG family member 2		×

Bold font highlights the genes that were later selected for quantitative reverse transcription polymerase chain reaction expression experiments.

miRNA, microRNA.

Appendix Table 5.

Complete Gene Ontology Functional Analysis Using G:Profiler.

Parent GO Term Name [a]	GO Term ID	GO Term Name	*P* Value	T	Q	Q&T	Q&T/Q	Q&T/T	Term Type	Depth in Group	Common Genes
GO:0007389 pattern specification process	GO:0007389	Pattern specification process	6.49E-05	460	51	11	0.216	0.024	BP	3	*BMI1, EP300, FLT1, GATA4, HOXB5, RNF2, SMAD2, TCF7L1, VEGFA, ZEB1, ZEB2*
GO:0007389 pattern specification process	GO:0003002	Regionalization	5.75E-03	331	51	8	0.157	0.024	BP	4	*BMI1, EP300, GATA4, HOXB5, RNF2, SMAD2, TCF7L1, ZEB2*
GO:0007389 pattern specification process	GO:0009952	Anterior/posterior pattern specification	2.41E-03	202	51	7	0.137	0.035	BP	5	*EP300, GATA4, HOXB5, RNF2, SMAD2, TCF7L1, ZEB2*
GO:0008283 cell proliferation	GO:0042127	Regulation of cell proliferation	7.89E-06	1317	51	18	0.353	0.014	BP	4	*BAP1, BCL2, BMI1, E2F3, EGFR, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, PTEN, SHC1, SMAD2, SUZ12, VEGFA, XIAP, ZEB1*
GO:0009790 embryo development	GO:0009790	Embryo development	9.38E-07	1002	51	17	0.333	0.017	BP	4	*EGFR, EP300, EPHA2, FLT1, GATA2, GATA4, HOXB5, KDR, PLCG1, RNF2, SEPT7, SMAD2, TCF7L1, VEGFA, ZEB1, ZEB2, ZFPM2*
GO:0009790 embryo development	GO:0009792	Embryo development ending in birth or egg hatching	1.05E-04	603	51	12	0.235	0.02	BP	5	*EGFR, EP300, EPHA2, GATA2, GATA4, HOXB5, PLCG1, SMAD2, VEGFA, ZEB1, ZEB2, ZFPM2*
GO:0009790 embryo development	GO:0043009	Chordate embryonic development	9.38E-05	597	51	12	0.235	0.02	BP	6	*EGFR, EP300, EPHA2, GATA2, GATA4, HOXB5, PLCG1, SMAD2, VEGFA, ZEB1, ZEB2, ZFPM2*
GO:0009790 embryo development	GO:0048598	Embryonic morphogenesis	2.74E-03	532	51	10	0.196	0.019	BP	5	*EP300, EPHA2, FLT1, GATA2, GATA4, HOXB5, RNF2, SMAD2, ZEB1, ZEB2*

GO:0016043 cellular component organization	GO:0007045	Cell-substrate adherens junction assembly	2.36E-02	51	51	4	0.078	0.078	BP	2	*BCL2, KDR, PTEN, VEGFA*
GO:0016043 cellular component organization	GO:0048041	Focal adhesion assembly	2.36E-02	51	51	4	0.078	0.078	BP	3	*BCL2, KDR, PTEN, VEGFA*
GO:0030154 cell differentiation	GO:0030154	Cell differentiation	6.25E-04	3113	51	24	0.471	0.008	BP	4	*BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, PLCG1, PTEN, PTPRD, SMAD2, SUZ12, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0030154 cell differentiation	GO:0045595	Regulation of cell differentiation	1.60E-04	1234	51	16	0.314	0.013	BP	4	*BCL2, CREB1, EP300, ERRFI1, ETS1, GATA2, GATA4, KDR, PTEN, PTPRD, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0030154 cell differentiation	GO:0000904	Cell morphogenesis involved in differentiation	1.05E-02	768	51	11	0.216	0.014	BP	5	*BCL2, CREB1, EGFR, EP300, EPHA2, FN1, PLCG1, PTEN, PTPRD, SMAD2, VEGFA*
GO:0030154 cell differentiation	GO:0045597	Positive regulation of cell differentiation	7.34E-04	585	51	11	0.216	0.019	BP	3	*BCL2, CREB1, EP300, ETS1, GATA2, GATA4, KDR, PTPRD, SMAD2, VEGFA, ZEB1*
GO:0030154 cell differentiation	GO:0030855	Epithelial cell differentiation	2.17E-02	527	51	9	0.176	0.017	BP	5	*BCL2, CREB1, EPHA2, ERRFI1, GATA4, HOXB5, KDR, VEGFA, ZEB1*
GO:0030154 cell differentiation	GO:0048863	Stem cell differentiation	3.55E-02	304	51	7	0.137	0.023	BP	5	*BCL2, EP300, GATA2, GATA4, SMAD2, TCF7L1, ZEB2*
GO:0032989 cellular component morphogenesis	GO:0022604	Regulation of cell morphogenesis	9.08E-04	358	51	9	0.176	0.025	BP	4	*EP300, FN1, KDR, PTEN, PTPRD, SEPT7, SMAD2, VEGFA, WASF3*
GO:0032989 cellular component morphogenesis	GO:0008360	Regulation of cell shape	2.77E-02	112	51	5	0.098	0.045	BP	5	*FN1, KDR, SEPT7, VEGFA, WASF3*
GO:0048513 organ development	GO:0048513	Organ development	6.13E-03	2742	51	21	0.412	0.008	BP	5	*ATP2A2, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, GATA2, GATA4, HOXB5, KDR, PTEN, SHC1, SMAD2, TCF7L1, VEGFA, ZEB1, ZEB2, ZFPM2*
GO:0048513 organ	GO:0009887	Organ	3.60E-03	839	51	12	0.235	0.014	BP	6	*BCL2, EP300, EPHA2, ERRFI1,*

development		morphogenesis									*GATA2, GATA4, HOXB5, PTEN, SMAD2, VEGFA, ZEB1, ZFPM2*
GO:0048513 organ development	GO:0048568	Embryonic organ development	2.19E-04	404	51	10	0.196	0.025	BP	5	*EGFR, EPHA2, GATA2, GATA4, HOXB5, KDR, SMAD2, VEGFA, ZEB1, ZFPM2*
GO:0048513 organ development	GO:0060541	Respiratory system development	4.65E-06	194	51	9	0.176	0.046	BP	5	*CREB1, EGFR, EP300, ERRFI1, GATA4, KDR, SMAD2, VEGFA, ZFPM2*
GO:0048513 organ development	GO:0030324	Lung development	1.52E-06	171	51	9	0.176	0.053	BP	6	*CREB1, EGFR, EP300, ERRFI1, GATA4, KDR, SMAD2, VEGFA, ZFPM2*
GO:0048513 organ development	GO:0048732	Gland development	2.50E-03	296	51	8	0.157	0.027	BP	6	*BCL2, CREB1, EGFR, EPHA2, ETS1, GATA2, PTEN, VEGFA*
GO:0048513 organ development	GO:0048286	Lung alveolus development	7.96E-03	39	51	4	0.078	0.103	BP	7	*CREB1, ERRFI1, KDR, VEGFA*
GO:0048870 cell motility	GO:0048870	Cell motility	6.46E-03	1056	51	13	0.255	0.012	BP	4	*BCL2, EGFR, ELMO2, EPHA2, ETS1, FLT1, FN1, KDR, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0048870 cell motility	GO:0016477	Cell migration	2.72E-03	977	51	13	0.255	0.013	BP	5	*BCL2, EGFR, ELMO2, EPHA2, ETS1, FLT1, FN1, KDR, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0048870 cell motility	GO:2000145	Regulation of cell motility	1.64E-02	509	51	9	0.176	0.018	BP	2	*BCL2, EGFR, EPHA2, ETS1, FLT1, KDR, PLCG1, PTEN, VEGFA*
GO:0048870 cell motility	GO:0030334	Regulation of cell migration	1.08E-02	483	51	9	0.176	0.019	BP	3	*BCL2, EGFR, EPHA2, ETS1, FLT1, KDR, PLCG1, PTEN, VEGFA*
GO:0048870 cell motility	GO:0001667	Ameboidal cell migration	8.68E-03	245	51	7	0.137	0.029	BP	6	*EPHA2, ETS1, KDR, PLCG1, PTEN, VEGFA, ZEB2*
GO:0048870 cell motility	GO:2000147	Positive regulation of cell motility	2.13E-02	281	51	7	0.137	0.025	BP	3	*BCL2, EGFR, ETS1, FLT1, KDR, PLCG1, VEGFA*
GO:0048870 cell motility	GO:0030335	Positive regulation of cell migration	1.85E-02	275	51	7	0.137	0.025	BP	4	*BCL2, EGFR, ETS1, FLT1, KDR, PLCG1, VEGFA*
GO:0048870 cell motility	GO:0010631	Epithelial cell migration	1.14E-02	165	51	6	0.118	0.036	BP	5	*EPHA2, ETS1, KDR, PLCG1, PTEN, VEGFA*
GO:0048870 cell	GO:0043542	Endothelial cell	1.97E-03	122	51	6	0.118	0.049	BP	6	*EPHA2, ETS1, KDR, PLCG1, PTEN,*

motility		migration									*VEGFA*
GO:0048870 cell motility	GO:0010632	Regulation of epithelial cell migration	3.72E-02	119	51	5	0.098	0.042	BP	4	*EPHA2, ETS1, KDR, PLCG1, VEGFA*
GO:0048870 cell motility	GO:0010594	Regulation of endothelial cell migration	7.57E-03	86	51	5	0.098	0.058	BP	5	*EPHA2, ETS1, KDR, PLCG1, VEGFA*
GO:0048870 cell motility	GO:0010595	Positive regulation of endothelial cell migration	2.76E-02	53	51	4	0.078	0.075	BP	6	*ETS1, KDR, PLCG1, VEGFA*
GO:0051179 localization	GO:0051674	Localization of cell	6.46E-03	1056	51	13	0.255	0.012	BP	1	*BCL2, EGFR, ELMO2, EPHA2, ETS1, FLT1, FN1, KDR, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0055123 digestive system development	GO:0048565	Digestive tract development	3.57E-02	118	51	5	0.098	0.042	BP	3	*BCL2, EGFR, EP300, GATA4, SMAD2*
GO:0055123 digestive system development	GO:0048546	Digestive tract morphogenesis	2.76E-02	53	51	4	0.078	0.075	BP	3	*BCL2, EGFR, GATA4, SMAD2*
GO:0001944 vasculature development	GO:0001944	Vasculature development	4.08E-06	560	51	13	0.255	0.023	BP	5	*EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, SHC1, VEGFA, ZFPM2*
GO:0001944 vasculature development	GO:0001568	Blood vessel development	2.74E-05	534	51	12	0.235	0.022	BP	6	*EPHA2, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, SHC1, VEGFA, ZFPM2*
GO:0001944 vasculature development	GO:0048514	Blood vessel morphogenesis	6.12E-06	467	51	12	0.235	0.026	BP	7	*EPHA2, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, SHC1, VEGFA, ZFPM2*
GO:0001944 vasculature development	GO:0001525	Angiogenesis	1.09E-05	387	51	11	0.216	0.028	BP	8	*EPHA2, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, SHC1, VEGFA*
GO:0001944 vasculature development	GO:1901342	Regulation of vasculature development	9.17E-05	192	51	8	0.157	0.042	BP	5	*EPHA2, ETS1, FLT1, GATA2, GATA4, KDR, PLCG1, VEGFA*
GO:0001944 vasculature	GO:0045765	Regulation of angiogenesis	4.26E-05	174	51	8	0.157	0.046	BP	6	*EPHA2, ETS1, FLT1, GATA2, GATA4, KDR, PLCG1, VEGFA*

development											
GO:0001944 vasculature development	GO:0045766	Positive regulation of angiogenesis	1.80E-05	99	51	7	0.137	0.071	BP	3	*ETS1, FLT1, GATA2, GATA4, KDR, PLCG1, VEGFA*
GO:0002376 immune system process	GO:0002376	Immune system process	1.59E-02	2182	51	18	0.353	0.008	BP	1	*BCL2, BMI1, CREB1, EGFR, ELMO2, EP300, EPHA2, ETS1, FLT1, FN1, GATA2, KDR, PLCG1, PTEN, SHC1, VEGFA, XIAP, ZEB1*
GO:0006366 transcription from RNA polymerase II promoter	GO:0006366	Transcription from RNA polymerase II promoter	3.00E-05	1617	51	19	0.373	0.012	BP	1	*BMI1, CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZFPM2*
GO:0006366 transcription from RNA polymerase II promoter	GO:0006357	Regulation of transcription from RNA polymerase II promoter	2.39E-05	1413	51	18	0.353	0.013	BP	2	*BMI1, CREB1, EGFR, EP300, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZFPM2*
GO:0006366 transcription from RNA polymerase II promoter	GO:0045893	Positive regulation of transcription, DNA-templated	9.43E-03	1093	51	13	0.255	0.012	BP	3	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, NFAT5, SMAD2, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0006366 transcription from RNA polymerase II promoter	GO:0045892	Negative regulation of transcription, DNA-templated	8.17E-03	908	51	12	0.235	0.013	BP	3	*BMI1, CREB1, EP300, GATA2, KLF11, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0006366 transcription from RNA polymerase II promoter	GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	1.56E-03	775	51	12	0.235	0.015	BP	2	*CREB1, EGFR, EP300, ETS1, GATA2, GATA4, NFAT5, SMAD2, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0006366 transcription from RNA polymerase II promoter	GO:0000122	Negative regulation of transcription from RNA polymerase II promoter	5.50E-03	575	51	10	0.196	0.017	BP	2	*BMI1, EP300, GATA2, KLF11, RNF2, SMAD2, SUZ12, VEGFA, ZEB1, ZFPM2*
GO:0007154 cell communication	GO:0007154	Cell communication	4.29E-04	5317	51	32	0.627	0.006	BP	3	*ATP2A2, BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, FLT1, GATA2, GATA4, KDR, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, SHC1, SLC9A3R2, SMAD2, TCF7L1, VAC14,*

											VEGFA, WASF3, XIAP, ZEB1, ZEB2
GO:0007154 cell communication	GO:0010646	Regulation of cell communication	4.46E-05	2490	51	23	0.451	0.009	BP	4	*BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, GATA2, GATA4, KDR, LITAF, NFAT5, PLCG1, PTEN, RIN2, SHC1, SLC9A3R2, SMAD2, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2*
GO:0007154 cell communication	GO:0010647	Positive regulation of cell communication	4.48E-02	1074	51	12	0.235	0.011	BP	3	*BCL2, EGFR, FLT1, GATA4, KDR, LITAF, PTEN, SHC1, SMAD2, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0009987	Cellular process	7.91E-03	13683	51	49	0.961	0.004	BP	1	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPN12, PTPRD, RASSF2, RERE, RIN2, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0044699	Single-organism process	4.20E-02	12323	51	46	0.902	0.004	BP	1	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RERE, RIN2, RND3, RNF2, SEPT7, SHC1, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0044763	Single-organism cellular process	1.87E-02	11036	51	44	0.863	0.004	BP	2	*ATP2A2, BAP1, BCL2, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, RNF2, SEPT7, SHC1, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0065007	Biological regulation	6.59E-05	9480	51	44	0.863	0.005	BP	1	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1,*

											FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, RNF2, SEPT7, SHC1, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2
GO:0008150 biological process	GO:0050789	Regulation of biological process	7.83E-06	8970	51	44	0.863	0.005	BP	2	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, RNF2, SEPT7, SHC1, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0050794	Regulation of cellular process	8.85E-07	8483	51	44	0.863	0.005	BP	3	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, RNF2, SEPT7, SHC1, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0044238	Primary metabolic process	7.29E-03	9374	51	41	0.804	0.004	BP	1	*BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPN12, PTPRD, RERE, RIN2, RND3, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0044237	Cellular metabolic process	9.63E-03	9455	51	41	0.804	0.004	BP	2	*BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPN12, PTPRD, RERE, RIN2, RND3, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*

GO:0008150 biological process	GO:0071704	Organic substance metabolic process	2.15E-02	9694	51	41	0.804	0.004	BP	1	*BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPN12, PTPRD, RERE, RIN2, RND3, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0043170	Macromolecule metabolic process	2.11E-03	7803	51	38	0.745	0.005	BP	2	*BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPN12, PTPRD, RERE, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0044260	Cellular macromolecule metabolic process	2.00E-04	7222	51	38	0.745	0.005	BP	3	*BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN, PTPN12, PTPRD, RERE, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0044767	Single-organism developmental process	8.68E-05	4995	51	32	0.627	0.006	BP	2	*ATP2A2, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, LITAF, PLCG1, PTEN, PTPRD, RERE, RNF2, SEPT7, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:1901360	Organic cyclic compound metabolic process	1.52E-02	6149	51	32	0.627	0.005	BP	2	*BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, PTEN, RERE, RIN2, RND3, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0006725	Cellular aromatic compound metabolic process	6.61E-03	5939	51	32	0.627	0.005	BP	3	*BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, GATA2, GATA4, KLF11, LITAF,*

											NFAT5, PTEN, RERE, RIN2, RND3, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2
GO:0008150 biological process	GO:0019222	Regulation of metabolic process	5.00E-03	5543	51	31	0.608	0.006	BP	3	*BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, LITAF, NFAT5, PLCG1, PTEN, RIN2, RNF2, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0080090	Regulation of primary metabolic process	2.36E-04	4884	51	31	0.608	0.006	BP	4	*BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, LITAF, NFAT5, PLCG1, PTEN, RIN2, RNF2, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0031323	Regulation of cellular metabolic process	4.04E-04	4992	51	31	0.608	0.006	BP	4	*BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, LITAF, NFAT5, PLCG1, PTEN, RIN2, RNF2, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0032501	Multicellular organismal process	3.24E-02	6011	51	31	0.608	0.005	BP	1	*ATP2A2, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RERE, RNF2, SEPT7, SHC1, SMAD2, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0044707	Single-multicellular organism process	1.32E-02	5780	51	31	0.608	0.005	BP	2	*ATP2A2, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RERE, RNF2, SEPT7, SHC1, SMAD2, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0048518	Positive regulation of biological process	2.17E-06	4061	51	31	0.608	0.008	BP	1	*ATP2A2, BCL2, BMI1, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, LITAF, NFAT5, PLCG1, PTEN, PTPRD, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, WASF3,*

										XIAP, ZEB1, ZEB2, ZFPM2
GO:0008150 biological process	GO:0046483	Heterocycle metabolic process	2.37E-02	5929	51	31	0.608	0.005	BP	3	*BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, PTEN, RERE, RIN2, RND3, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0006139	Nucleobase-containing compound metabolic process	1.19E-02	5753	51	31	0.608	0.005	BP	4	*BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, PTEN, RERE, RIN2, RND3, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0071840	Cellular component organization or biogenesis	1.71E-03	4985	51	30	0.588	0.006	BP	1	*BAP1, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERBB2IP, ETS1, FN1, GATA2, KDR, KLHL20, PLCG1, PTEN, PTPRD, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, WASF3*
GO:0008150 biological process	GO:0016043	Cellular component organization	1.07E-03	4885	51	30	0.588	0.006	BP	2	*BAP1, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERBB2IP, ETS1, FN1, GATA2, KDR, KLHL20, PLCG1, PTEN, PTPRD, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, WASF3*
GO:0008150 biological process	GO:0060255	Regulation of macromolecule metabolic process	1.40E-03	4639	51	29	0.569	0.006	BP	4	*BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, ERRFI1, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, LITAF, NFAT5, PLCG1, PTEN, RNF2, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0048522	Positive regulation of cellular process	2.27E-05	3616	51	28	0.549	0.008	BP	2	*BCL2, BMI1, CREB1, E2F3, EGFR, EP300, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, LITAF, NFAT5, PLCG1, PTEN, PTPRD, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0048519	Negative regulation of biological process	8.72E-05	3567	51	27	0.529	0.008	BP	1	*ATP2A2, BAP1, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1,*

											GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, PTEN, RNF2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZFPM2
GO:0008150 biological process	GO:0090304	Nucleic acid metabolic process	3.86E-02	4776	51	27	0.529	0.006	BP	5	*BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, PTEN, RERE, RNF2, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0048869	Cellular developmental process	8.70E-05	3307	51	26	0.51	0.008	BP	3	*BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, PLCG1, PTEN, PTPRD, SEPT7, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0043412	Macromolecule modification	2.37E-05	2869	51	25	0.49	0.009	BP	3	*BAP1, BCL2, BMI1, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, FN1, GATA2, KDR, KLHL20, PLCG1, PTEN, PTPN12, PTPRD, RNF2, SHC1, SMAD2, SUZ12, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0019538	Protein metabolic process	3.54E-02	4160	51	25	0.49	0.006	BP	3	*BAP1, BCL2, BMI1, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, FN1, GATA2, KDR, KLHL20, PLCG1, PTEN, PTPN12, PTPRD, RNF2, SHC1, SMAD2, SUZ12, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0036211	Protein modification process	1.08E-05	2763	51	25	0.49	0.009	BP	4	*BAP1, BCL2, BMI1, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, FN1, GATA2, KDR, KLHL20, PLCG1, PTEN, PTPN12, PTPRD, RNF2, SHC1, SMAD2, SUZ12, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0044267	Cellular protein metabolic process	2.64E-03	3626	51	25	0.49	0.007	BP	4	*BAP1, BCL2, BMI1, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, FN1, GATA2, KDR, KLHL20, PLCG1, PTEN, PTPN12, PTPRD, RNF2, SHC1, SMAD2, SUZ12, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0006464	Cellular protein modification process	1.08E-05	2763	51	25	0.49	0.009	BP	5	*BAP1, BCL2, BMI1, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1,*

											FLT1, FN1, GATA2, KDR, KLHL20, PLCG1, PTEN, PTPN12, PTPRD, RNF2, SHC1, SMAD2, SUZ12, VEGFA, XIAP, ZEB2
GO:0008150 biological process	GO:0051171	Regulation of nitrogen compound metabolic process	1.20E-02	3925	51	25	0.49	0.006	BP	4	*BMI1, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, PTEN, RIN2, RNF2, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0019219	Regulation of nucleobase-containing compound metabolic process	7.68E-03	3833	51	25	0.49	0.007	BP	5	*BMI1, CREB1, E2F3, EGFR, EP300, EPHA2, ERRFI1, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, PTEN, RIN2, RNF2, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0048523	Negative regulation of cellular process	3.00E-04	3248	51	25	0.49	0.008	BP	2	*BAP1, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, GATA2, GATA4, KDR, KLF11, KLHL20, PTEN, RNF2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0016070	RNA metabolic process	3.90E-02	4182	51	25	0.49	0.006	BP	6	*BMI1, CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, PTEN, RERE, RNF2, SCAF11, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0051239	Regulation of multicellular organismal process	8.72E-07	2037	51	23	0.451	0.011	BP	3	*ATP2A2, BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, GATA2, GATA4, KDR, LITAF, PLCG1, PTEN, PTPRD, SEPT7, SMAD2, TCF7L1, VEGFA, WASF3, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0009893	Positive regulation of metabolic process	1.02E-05	2307	51	23	0.451	0.01	BP	2	*BCL2, BMI1, CREB1, E2F3, EGFR, EP300, ETS1, FLT1, GATA2, GATA4, KDR, NFAT5, PLCG1, PTEN, SHC1, SMAD2, SUPV3L1, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0010604	Positive regulation of macromolecule metabolic process	1.46E-06	2090	51	23	0.451	0.011	BP	3	*BCL2, BMI1, CREB1, E2F3, EGFR, EP300, ETS1, FLT1, GATA2, GATA4, KDR, NFAT5, PLCG1, PTEN, SHC1, SMAD2, SUPV3L1, TCF7L1, VEGFA,*

											XIAP, ZEB1, ZEB2, ZFPM2
GO:0008150 biological process	GO:0031325	Positive regulation of cellular metabolic process	3.51E-06	2185	51	23	0.451	0.011	BP	3	*BCL2, BMI1, CREB1, E2F3, EGFR, EP300, ETS1, FLT1, GATA2, GATA4, KDR, NFAT5, PLCG1, PTEN, SHC1, SMAD2, SUPV3L1, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0008150 biological process	GO:0010556	Regulation of macromolecule biosynthetic process	2.18E-02	3494	51	23	0.451	0.007	BP	1	*BCL2, BMI1, CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, KDR, KLF11, LITAF, NFAT5, PTEN, RNF2, SHC1, SMAD2, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0050793	Regulation of developmental process	2.16E-07	1713	51	22	0.431	0.013	BP	3	*BCL2, CREB1, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, PTPRD, SEPT7, SMAD2, SUZ12, TCF7L1, VEGFA, WASF3, ZEB1, ZFPM2*
GO:0008150 biological process	GO:2000112	Regulation of cellular macromolecule biosynthetic process	4.76E-02	3383	51	22	0.431	0.007	BP	1	*BCL2, BMI1, CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, KLF11, LITAF, NFAT5, PTEN, RNF2, SHC1, SMAD2, SUZ12, TCF7L1, VEGFA, XIAP, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0006793	Phosphorus metabolic process	2.93E-02	3021	51	21	0.412	0.007	BP	3	*BCL2, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, KDR, PLCG1, PTEN, PTPN12, PTPRD, RIN2, RND3, SHC1, SMAD2, SUPV3L1, VAC14, VEGFA, ZEB2*
GO:0008150 biological process	GO:0006796	Phosphate-containing compound metabolic process	2.32E-02	2977	51	21	0.412	0.007	BP	4	*BCL2, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, KDR, PLCG1, PTEN, PTPN12, PTPRD, RIN2, RND3, SHC1, SMAD2, SUPV3L1, VAC14, VEGFA, ZEB2*
GO:0008150 biological process	GO:0006996	Organelle organization	1.70E-02	2670	51	20	0.392	0.007	BP	3	*BAP1, BCL2, BMI1, CREB1, EP300, ERBB2IP, ETS1, GATA2, KLHL20, PTEN, RERE, RND3, RNF2, SEPT7, SHC1, SUPV3L1, SUZ12, TCF7L1, VEGFA, WASF3*
GO:0008150 biological process	GO:0008283	Cell proliferation	1.46E-05	1735	51	20	0.392	0.012	BP	2	*BAP1, BCL2, BMI1, E2F3, EGFR, EPHA2, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, PTEN, SHC1, SMAD2, SUZ12, VEGFA, XIAP, ZEB1, ZEB2*

GO:0008150 biological process	GO:0009892	Negative regulation of metabolic process	2.63E-03	1717	51	17	0.333	0.01	BP	2	*BCL2, BMI1, CREB1, EGFR, EP300, ERRFI1, GATA2, GATA4, KLF11, PTEN, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0031324	Negative regulation of cellular metabolic process	4.26E-03	1573	51	16	0.314	0.01	BP	3	*BCL2, BMI1, CREB1, EP300, ERRFI1, GATA2, GATA4, KLF11, PTEN, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0016310	Phosphorylation	2.53E-02	1593	51	15	0.294	0.009	BP	5	*BCL2, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, KDR, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZEB2*
GO:0008150 biological process	GO:0006468	Protein phosphorylation	1.06E-03	1234	51	15	0.294	0.012	BP	6	*BCL2, CCNE2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, KDR, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZEB2*
GO:0008150 biological process	GO:0006928	Cellular component movement	2.24E-02	1577	51	15	0.294	0.01	BP	3	*BCL2, CREB1, EGFR, ELMO2, EPHA2, ETS1, FLT1, FN1, GATA4, KDR, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0008150 biological process	GO:0032989	Cellular component morphogenesis	3.47E-04	1132	51	15	0.294	0.013	BP	4	*BCL2, CREB1, EGFR, EP300, EPHA2, FN1, KDR, PLCG1, PTEN, PTPRD, SEPT7, SMAD2, SUPV3L1, VEGFA, WASF3*
GO:0008150 biological process	GO:0051128	Regulation of cellular component organization	1.81E-02	1549	51	15	0.294	0.01	BP	3	*BAP1, BCL2, EP300, EPHA2, ETS1, FN1, GATA2, KDR, PTEN, PTPRD, SEPT7, SMAD2, SUPV3L1, VEGFA, WASF3*
GO:0008150 biological process	GO:0051173	Positive regulation of nitrogen compound metabolic process	2.79E-03	1332	51	15	0.294	0.011	BP	3	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, NFAT5, SHC1, SMAD2, SUPV3L1, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0045935	Positive regulation of nucleobase-containing compound metabolic process	2.15E-03	1305	51	15	0.294	0.011	BP	4	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, NFAT5, SHC1, SMAD2, SUPV3L1, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0010605	Negative regulation of macromolecule metabolic process	2.18E-02	1573	51	15	0.294	0.01	BP	3	*BMI1, CREB1, EGFR, EP300, ERRFI1, GATA2, KLF11, PTEN, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*

GO:0008150 biological process	GO:0009891	Positive regulation of biosynthetic process	5.99E-03	1416	51	15	0.294	0.011	BP	1	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, KDR, NFAT5, SHC1, SMAD2, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0010557	Positive regulation of macromolecule biosynthetic process	1.97E-03	1296	51	15	0.294	0.012	BP	1	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, KDR, NFAT5, SHC1, SMAD2, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0031399	Regulation of protein modification process	6.01E-04	1181	51	15	0.294	0.013	BP	2	*BCL2, BMI1, CCNE2, EGFR, EP300, ERRFI1, FLT1, GATA2, KDR, PLCG1, PTEN, SHC1, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0032268	Regulation of cellular protein metabolic process	1.06E-02	1483	51	15	0.294	0.01	BP	1	*BCL2, BMI1, CCNE2, EGFR, EP300, ERRFI1, FLT1, GATA2, KDR, PLCG1, PTEN, SHC1, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0031328	Positive regulation of cellular biosynthetic process	2.62E-02	1394	51	14	0.275	0.01	BP	2	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, NFAT5, SHC1, SMAD2, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0051254	Positive regulation of RNA metabolic process	3.04E-03	1159	51	14	0.275	0.012	BP	1	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, NFAT5, SMAD2, SUPV3L1, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0010628	Positive regulation of gene expression	2.42E-02	1192	51	13	0.255	0.011	BP	1	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, NFAT5, SMAD2, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0042325	Regulation of phosphorylation	1.05E-02	1104	51	13	0.255	0.012	BP	1	*BCL2, CCNE2, EGFR, EP300, EPHA2, ERRFI1, FLT1, KDR, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0008150 biological process	GO:1902680	Positive regulation of RNA biosynthetic process	1.45E-02	1137	51	13	0.255	0.011	BP	2	*CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, NFAT5, SMAD2, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0044093	Positive regulation of molecular function	3.54E-02	1235	51	13	0.255	0.011	BP	1	*BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0008150 biological process	GO:0008284	Positive regulation of cell proliferation	1.06E-03	747	51	12	0.235	0.016	BP	3	*BCL2, BMI1, E2F3, EGFR, ETS1, FLT1, GATA4, KDR, PTEN, SHC1, SUZ12, VEGFA*

GO:0008150 biological process	GO:0051253	Negative regulation of RNA metabolic process	1.43E-02	959	51	12	0.235	0.013	BP	5	*BMI1, CREB1, EP300, GATA2, KLF11, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:1902679	Negative regulation of RNA biosynthetic process	1.00E-02	926	51	12	0.235	0.013	BP	3	*BMI1, CREB1, EP300, GATA2, KLF11, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0001932	Regulation of protein phosphorylation	9.15E-03	918	51	12	0.235	0.013	BP	3	*BCL2, CCNE2, EGFR, EP300, ERRFI1, FLT1, KDR, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0008150 biological process	GO:0043085	Positive regulation of catalytic activity	2.50E-02	1013	51	12	0.235	0.012	BP	2	*BCL2, BMI1, CREB1, EGFR, EPHA2, ERRFI1, FLT1, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0008150 biological process	GO:0051172	Negative regulation of nitrogen compound metabolic process	4.44E-02	1073	51	12	0.235	0.011	BP	3	*BMI1, CREB1, EP300, GATA2, KLF11, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0045934	Negative regulation of nucleobase-containing compound metabolic process	3.82E-02	1057	51	12	0.235	0.011	BP	4	*BMI1, CREB1, EP300, GATA2, KLF11, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0010558	Negative regulation of macromolecule biosynthetic process	4.40E-02	1072	51	12	0.235	0.011	BP	2	*BMI1, CREB1, EP300, GATA2, KLF11, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:2000113	Negative regulation of cellular macromolecule biosynthetic process	2.75E-02	1023	51	12	0.235	0.012	BP	2	*BMI1, CREB1, EP300, GATA2, KLF11, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0051247	Positive regulation of protein metabolic process	2.75E-02	1023	51	12	0.235	0.012	BP	1	*BCL2, BMI1, EGFR, EP300, FLT1, KDR, PLCG1, PTEN, SHC1, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0032270	Positive regulation of cellular protein metabolic process	1.13E-02	937	51	12	0.235	0.013	BP	2	*BCL2, BMI1, EGFR, EP300, FLT1, KDR, PLCG1, PTEN, SHC1, VEGFA, XIAP, ZEB2*
GO:0008150 biological process	GO:0031401	Positive regulation of protein modification process	3.30E-03	832	51	12	0.235	0.014	BP	3	*BCL2, BMI1, EGFR, EP300, FLT1, KDR, PLCG1, PTEN, SHC1, VEGFA, XIAP, ZEB2*
GO:0008150	GO:0010629	Negative regulation	3.01E-02	1032	51	12	0.235	0.012	BP	4	*BMI1, CREB1, EP300, GATA2,*

biological process		of gene expression									*KLF11, RNF2, SMAD2, SUZ12, TCF7L1, VEGFA, ZEB1, ZFPM2*
GO:0008150 biological process	GO:0040007	Growth	2.99E-02	858	51	11	0.216	0.013	BP	1	*BAP1, BCL2, CREB1, EP300, ERBB2IP, GATA4, PTEN, SHC1, SMAD2, SUPV3L1, VEGFA*
GO:0008150 biological process	GO:0051270	Regulation of cellular component movement	5.58E-03	576	51	10	0.196	0.017	BP	1	*BCL2, EGFR, EPHA2, ETS1, FLT1, GATA4, KDR, PLCG1, PTEN, VEGFA*
GO:0008150 biological process	GO:0006325	Chromatin organization	1.27E-02	632	51	10	0.196	0.016	BP	1	*BAP1, BMI1, EP300, GATA2, RERE, RNF2, SUPV3L1, SUZ12, TCF7L1, VEGFA*
GO:0008150 biological process	GO:0040008	Regulation of growth	3.78E-02	565	51	9	0.176	0.016	BP	3	*BAP1, BCL2, CREB1, EP300, GATA4, PTEN, SHC1, SUPV3L1, VEGFA*
GO:0008150 biological process	GO:0018212	Peptidyl-tyrosine modification	1.31E-02	261	51	7	0.137	0.027	BP	1	*EGFR, EPHA2, ERRFI1, FLT1, KDR, SHC1, VEGFA*
GO:0008150 biological process	GO:0018108	Peptidyl-tyrosine phosphorylation	1.25E-02	259	51	7	0.137	0.027	BP	2	*EGFR, EPHA2, ERRFI1, FLT1, KDR, SHC1, VEGFA*
GO:0008150 biological process	GO:0040017	Positive regulation of locomotion	2.98E-02	296	51	7	0.137	0.024	BP	2	*BCL2, EGFR, ETS1, FLT1, KDR, PLCG1, VEGFA*
GO:0008150 biological process	GO:0051272	Positive regulation of cellular component movement	2.44E-02	287	51	7	0.137	0.024	BP	2	*BCL2, EGFR, ETS1, FLT1, KDR, PLCG1, VEGFA*
GO:0008150 biological process	GO:0048511	Rhythmic process	4.65E-02	211	51	6	0.118	0.028	BP	1	*BCL2, EGFR, ETS1, KDR, PTEN, VEGFA*
GO:0008150 biological process	GO:0042698	Ovulation cycle	1.37E-02	97	51	5	0.098	0.052	BP	1	*BCL2, EGFR, ETS1, KDR, VEGFA*
GO:0009888 tissue development	GO:0009888	Tissue development	5.29E-05	1487	51	18	0.353	0.012	BP	3	*ATP2A2, BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, GATA4, HOXB5, KDR, PTEN, SMAD2, TCF7L1, VEGFA, ZEB1, ZEB2, ZFPM2*
GO:0009888 tissue development	GO:0060429	Epithelium development	4.09E-04	827	51	13	0.255	0.016	BP	4	*BCL2, CREB1, EGFR, EPHA2, ERRFI1, FLT1, GATA4, HOXB5, KDR, SMAD2, VEGFA, ZEB1, ZEB2*
GO:0009888 tissue	GO:0048729	Tissue	2.58E-04	527	51	11	0.216	0.021	BP	3	*BCL2, EGFR, EPHA2, FLT1, GATA4, KDR, SMAD2, TCF7L1, VEGFA,*

development		morphogenesis									*ZEB2, ZFPM2*
GO:0009888 tissue development	GO:0002009	Morphogenesis of an epithelium	3.35E-02	421	51	8	0.157	0.019	BP	4	*BCL2, EGFR, EPHA2, FLT1, GATA4, KDR, VEGFA, ZEB2*
GO:0022610 biological adhesion	GO:0022610	Biological adhesion	2.87E-02	1027	51	12	0.235	0.012	BP	1	*ATP2A2, BCL2, EGFR, EPHA2, ERBB2IP, FN1, KDR, PTEN, PTPRD, RND3, SHC1, VEGFA*
GO:0022610 biological adhesion	GO:0007155	Cell adhesion	2.73E-02	1022	51	12	0.235	0.012	BP	3	*ATP2A2, BCL2, EGFR, EPHA2, ERBB2IP, FN1, KDR, PTEN, PTPRD, RND3, SHC1, VEGFA*
GO:0023052 signaling	GO:0023052	Signaling	3.06E-04	5247	51	32	0.627	0.006	BP	1	*ATP2A2, BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, FLT1, GATA2, GATA4, KDR, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, SHC1, SLC9A3R2, SMAD2, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2*
GO:0023052 signaling	GO:0044700	Single organism signaling	3.06E-04	5247	51	32	0.627	0.006	BP	2	*ATP2A2, BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, FLT1, GATA2, GATA4, KDR, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, SHC1, SLC9A3R2, SMAD2, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2*
GO:0023052 signaling	GO:0007165	Signal transduction	1.01E-04	4719	51	31	0.608	0.007	BP	4	*ATP2A2, BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, FLT1, GATA2, GATA4, KDR, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, SHC1, SLC9A3R2, SMAD2, TCF7L1, VAC14, VEGFA, XIAP, ZEB1, ZEB2*
GO:0023052 signaling	GO:0007166	Cell surface receptor signaling pathway	1.83E-04	2682	51	23	0.451	0.009	BP	5	*BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, FLT1, GATA2, GATA4, KDR, PLCG1, PTEN, PTPRD, SHC1, SMAD2, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2*
GO:0023052 signaling	GO:0023051	Regulation of signaling	4.19E-05	2482	51	23	0.451	0.009	BP	3	*BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, GATA2, GATA4, KDR, LITAF, NFAT5, PLCG1, PTEN, RIN2, SHC1, SLC9A3R2, SMAD2, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2*

GO:0023052 signaling	GO:0009966	Regulation of signal transduction	3.22E-05	2229	51	22	0.431	0.01	BP	4	*BCL2, EGFR, EP300, EPHA2, ERRFI1, FLT1, GATA2, GATA4, KDR, LITAF, NFAT5, PLCG1, PTEN, RIN2, SHC1, SLC9A3R2, SMAD2, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2*
GO:0023052 signaling	GO:0035556	Intracellular signal transduction	1.43E-03	2282	51	20	0.392	0.009	BP	5	*BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, KDR, LITAF, NFAT5, PLCG1, PTEN, RIN2, RND3, SHC1, SLC9A3R2, SMAD2, VEGFA, XIAP, ZEB2*
GO:0023052 signaling	GO:0007167	Enzyme linked receptor protein signaling pathway	4.79E-06	963	51	16	0.314	0.017	BP	6	*CREB1, EGFR, EPHA2, ERBB2IP, ERRFI1, FLT1, GATA4, KDR, PLCG1, PTEN, PTPRD, SHC1, SMAD2, VEGFA, XIAP, ZEB1*
GO:0023052 signaling	GO:1902531	Regulation of intracellular signal transduction	5.66E-04	1353	51	16	0.314	0.012	BP	5	*BCL2, EGFR, EPHA2, ERRFI1, FLT1, KDR, LITAF, NFAT5, PLCG1, PTEN, RIN2, SHC1, SLC9A3R2, VEGFA, XIAP, ZEB2*
GO:0023052 signaling	GO:0023056	Positive regulation of signaling	4.32E-02	1070	51	12	0.235	0.011	BP	2	*BCL2, EGFR, FLT1, GATA4, KDR, LITAF, PTEN, SHC1, SMAD2, VEGFA, XIAP, ZEB2*
GO:0023052 signaling	GO:0009967	Positive regulation of signal transduction	2.73E-02	1022	51	12	0.235	0.012	BP	3	*BCL2, EGFR, FLT1, GATA4, KDR, LITAF, PTEN, SHC1, SMAD2, VEGFA, XIAP, ZEB2*
GO:0023052 signaling	GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	3.62E-03	688	51	11	0.216	0.016	BP	7	*CREB1, EGFR, EPHA2, ERBB2IP, ERRFI1, FLT1, KDR, PLCG1, PTEN, SHC1, VEGFA*
GO:0023052 signaling	GO:0000165	MAPK cascade	4.47E-02	577	51	9	0.176	0.016	BP	6	*CREB1, EGFR, EPHA2, FLT1, KDR, PLCG1, SHC1, VEGFA, ZEB2*
GO:0023052 signaling	GO:0038127	ERBB signaling pathway	6.59E-03	235	51	7	0.137	0.03	BP	8	*CREB1, EGFR, ERBB2IP, ERRFI1, PLCG1, PTEN, SHC1*
GO:0023052 signaling	GO:0007173	Epidermal growth factor receptor signaling pathway	5.71E-03	230	51	7	0.137	0.03	BP	9	*CREB1, EGFR, ERBB2IP, ERRFI1, PLCG1, PTEN, SHC1*
GO:0023052 signaling	GO:0043491	Protein kinase B signaling	4.71E-02	125	51	5	0.098	0.04	BP	6	*EGFR, EP300, EPHA2, PTEN, VEGFA*

GO:0032502 developmental process	GO:0032502	Developmental process	1.12E-04	5045	51	32	0.627	0.006	BP	1	*ATP2A2, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, LITAF, PLCG1, PTEN, PTPRD, RERE, RNF2, SEPT7, SHC1, SMAD2, SUPV3L1, SUZ12, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0032502 developmental process	GO:0048856	Anatomical structure development	5.66E-04	4458	51	29	0.569	0.007	BP	2	*ATP2A2, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, PLCG1, PTEN, PTPRD, RNF2, SEPT7, SHC1, SMAD2, SUPV3L1, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0032502 developmental process	GO:0007275	Multicellular organismal development	4.24E-04	4402	51	29	0.569	0.007	BP	3	*ATP2A2, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, PLCG1, PTEN, PTPRD, RERE, RNF2, SEPT7, SHC1, SMAD2, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0032502 developmental process	GO:0009653	Anatomical structure morphogenesis	1.97E-09	2247	51	27	0.529	0.012	BP	2	*BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, PLCG1, PTEN, PTPRD, RNF2, SEPT7, SHC1, SMAD2, SUPV3L1, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0032502 developmental process	GO:2000026	Regulation of multicellular organismal development	7.41E-07	1295	51	19	0.373	0.015	BP	4	*BCL2, CREB1, EP300, EPHA2, ERRFI1, ETS1, FLT1, GATA2, GATA4, KDR, PLCG1, PTEN, PTPRD, SEPT7, SMAD2, VEGFA, WASF3, ZEB1, ZFPM2*
GO:0032502 developmental process	GO:0022603	Regulation of anatomical structure morphogenesis	4.16E-08	697	51	16	0.314	0.023	BP	4	*BCL2, EP300, EPHA2, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, PTPRD, SEPT7, SMAD2, VEGFA, WASF3*
GO:0032502 developmental process	GO:0048646	Anatomical structure formation involved in morphogenesis	1.97E-04	925	51	14	0.275	0.015	BP	2	*EP300, EPHA2, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZEB2*
GO:0032502 developmental process	GO:0051094	Positive regulation of developmental process	3.48E-05	805	51	14	0.275	0.017	BP	2	*BCL2, CREB1, EP300, ETS1, FLT1, GATA2, GATA4, KDR, PLCG1, PTPRD, SMAD2, VEGFA, ZEB1, ZFPM2*

GO:0035295 tube development	GO:0035295	Tube development	4.84E-08	485	51	14	0.275	0.029	BP	4	*BCL2, CREB1, EGFR, EP300, EPHA2, ERRFI1, FLT1, GATA4, KDR, SMAD2, VEGFA, ZEB1, ZEB2, ZFPM2*
GO:0035295 tube development	GO:0030323	Respiratory tube development	1.78E-06	174	51	9	0.176	0.052	BP	5	*CREB1, EGFR, EP300, ERRFI1, GATA4, KDR, SMAD2, VEGFA, ZFPM2*
GO:0035295 tube development	GO:0035239	Tube morphogenesis	6.87E-03	339	51	8	0.157	0.024	BP	5	*BCL2, EPHA2, FLT1, GATA4, KDR, VEGFA, ZEB1, ZEB2*
GO:0040011 locomotion	GO:0040011	Locomotion	2.72E-02	1399	51	14	0.275	0.01	BP	1	*BCL2, CREB1, EGFR, ELMO2, EPHA2, ETS1, FLT1, FN1, KDR, PLCG1, PTEN, SHC1, VEGFA, ZEB2*
GO:0040011 locomotion	GO:0040012	Regulation of locomotion	3.38E-02	557	51	9	0.176	0.016	BP	3	*BCL2, EGFR, EPHA2, ETS1, FLT1, KDR, PLCG1, PTEN, VEGFA*
GO:0048468 cell development	GO:0048468	Cell development	3.79E-04	1691	51	18	0.353	0.011	BP	4	*BCL2, CREB1, EGFR, EP300, EPHA2, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, PTPRD, SMAD2, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2*
GO:0048468 cell development	GO:0000902	Cell morphogenesis	1.07E-03	1062	51	14	0.275	0.013	BP	5	*BCL2, CREB1, EGFR, EP300, EPHA2, FN1, KDR, PLCG1, PTEN, PTPRD, SEPT7, SMAD2, VEGFA, WASF3*
GO:0048731 system development	GO:0048731	System development	1.70E-03	3816	51	26	0.51	0.007	BP	4	*ATP2A2, BCL2, BMI1, CREB1, EGFR, EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, PLCG1, PTEN, PTPRD, SHC1, SMAD2, TCF7L1, VEGFA, WASF3, ZEB1, ZEB2, ZFPM2*
GO:0048731 system development	GO:0072359	Circulatory system development	6.98E-06	844	51	15	0.294	0.018	BP	5	*EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZFPM2*
GO:0048731 system development	GO:0072358	Cardiovascular system development	6.98E-06	844	51	15	0.294	0.018	BP	5	*EP300, EPHA2, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZFPM2*
GO:0050896 response to stimulus	GO:0050896	Response to stimulus	5.17E-03	7281	51	36	0.706	0.005	BP	1	*ATP2A2, BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLF11, KLHL20, LITAF, NFAT5, PLCG1, PTEN,*

											PTPRD, RASSF2, RIN2, RND3, SHC1, SLC9A3R2, SMAD2, TCF7L1, VAC14, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2
GO:0050896 response to stimulus	GO:0051716	Cellular response to stimulus	6.49E-04	5730	51	33	0.647	0.006	BP	2	*ATP2A2, BCL2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, GATA2, GATA4, KDR, KLF11, LITAF, NFAT5, PLCG1, PTEN, PTPRD, RASSF2, RIN2, RND3, SHC1, SLC9A3R2, SMAD2, TCF7L1, VAC14, VEGFA, XIAP, ZEB1, ZEB2*
GO:0050896 response to stimulus	GO:0048583	Regulation of response to stimulus	1.82E-04	2921	51	24	0.471	0.008	BP	3	*BCL2, CREB1, EGFR, ELMO2, EP300, EPHA2, ERRFI1, FLT1, GATA2, GATA4, KDR, LITAF, NFAT5, PLCG1, PTEN, RIN2, SHC1, SLC9A3R2, SMAD2, TCF7L1, VEGFA, XIAP, ZEB1, ZEB2*
GO:0050896 response to stimulus	GO:0070887	Cellular response to chemical stimulus	1.03E-02	2117	51	18	0.353	0.009	BP	1	*BCL2, CREB1, EGFR, ELMO2, EP300, EPHA2, ETS1, FLT1, GATA4, KDR, KLF11, LITAF, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZEB1*
GO:0050896 response to stimulus	GO:0010033	Response to organic substance	2.80E-02	2272	51	18	0.353	0.008	BP	1	*BCL2, CREB1, EGFR, EP300, EPHA2, ETS1, FLT1, GATA4, KDR, KLF11, KLHL20, LITAF, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZEB1*
GO:0050896 response to stimulus	GO:0048584	Positive regulation of response to stimulus	9.13E-03	1465	51	15	0.294	0.01	BP	2	*BCL2, CREB1, EGFR, ELMO2, FLT1, GATA4, KDR, LITAF, PLCG1, PTEN, SHC1, SMAD2, VEGFA, XIAP, ZEB2*
GO:0050896 response to stimulus	GO:0071310	Cellular response to organic substance	4.89E-02	1683	51	15	0.294	0.009	BP	2	*BCL2, CREB1, EGFR, EP300, FLT1, GATA4, KDR, KLF11, LITAF, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZEB1*
GO:0050896 response to stimulus	GO:0070848	Response to growth factor	2.31E-03	657	51	11	0.216	0.017	BP	2	*CREB1, EGFR, EPHA2, FLT1, KDR, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZEB1*
GO:0050896 response to stimulus	GO:0071363	Cellular response to growth factor stimulus	1.42E-02	640	51	10	0.196	0.016	BP	3	*CREB1, EGFR, FLT1, KDR, PLCG1, PTEN, SHC1, SMAD2, VEGFA, ZEB1*
GO:0090130 tissue migration	GO:0090130	Tissue migration	1.36E-02	170	51	6	0.118	0.035	BP	3	*EPHA2, ETS1, KDR, PLCG1, PTEN, VEGFA*
GO:0090130 tissue	GO:0090132	Epithelium migration	1.14E-02	165	51	6	0.118	0.036	BP	4	*EPHA2, ETS1, KDR, PLCG1, PTEN,*

migration											*VEGFA*
GO:0005575 cellular component	GO:0005622	Intracellular	1.06E-02	13081	51	48	0.941	0.004	CC	1	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, PTPN12, RASSF2, RERE, RIN2, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0005575 cellular component	GO:0044424	Intracellular part	6.23E-03	12922	51	48	0.941	0.004	CC	2	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, PTPN12, RASSF2, RERE, RIN2, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0005575 cellular component	GO:0043226	Organelle	1.07E-03	11800	51	47	0.922	0.004	CC	1	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, PTPN12, RASSF2, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0005575 cellular component	GO:0043229	Intracellular organelle	1.07E-04	11178	51	47	0.922	0.004	CC	3	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, PTPN12, RASSF2, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*

GO:0005575 cellular component	GO:0043227	Membrane-bounded organelle	1.29E-02	10924	51	44	0.863	0.004	CC	2	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, RASSF2, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0005575 cellular component	GO:0043231	Intracellular membrane-bounded organelle	5.92E-04	10046	51	44	0.863	0.004	CC	4	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, RASSF2, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0005575 cellular component	GO:0044422	Organelle part	4.52E-02	6791	51	33	0.647	0.005	CC	1	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, ERBB2IP, ETS1, FN1, GATA2, GATA4, KLHL20, LITAF, MATR3, PLCG1, PTEN, PTPN12, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, VAC14, VEGFA, ZFPM2*
GO:0005575 cellular component	GO:0044446	Intracellular organelle part	2.31E-02	6604	51	33	0.647	0.005	CC	4	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, ERBB2IP, ETS1, FN1, GATA2, GATA4, KLHL20, LITAF, MATR3, PLCG1, PTEN, PTPN12, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, VAC14, VEGFA, ZFPM2*
GO:0005575 cellular component	GO:0031974	Membrane-enclosed lumen	9.90E-04	2937	51	23	0.451	0.008	CC	1	*BMI1, CCNE2, CREB1, E2F3, EP300, ETS1, FN1, GATA2, GATA4, KLHL20, MATR3, PTEN, RERE, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, VEGFA, ZFPM2*
GO:0005575 cellular component	GO:0043233	Organelle lumen	6.73E-04	2876	51	23	0.451	0.008	CC	2	*BMI1, CCNE2, CREB1, E2F3, EP300, ETS1, FN1, GATA2, GATA4, KLHL20, MATR3, PTEN, RERE, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SMAD2,*

										SUPV3L1, SUZ12, VEGFA, ZFPM2
GO:0005575 cellular component	GO:0070013	Intracellular organelle lumen	9.41E-03	2815	51	21	0.412	0.007	CC	5	*BMI1, CCNE2, CREB1, E2F3, EP300, ETS1, GATA2, GATA4, KLHL20, MATR3, PTEN, RERE, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, ZFPM2*
GO:0005634 nucleus	GO:0005634	Nucleus	1.07E-05	6221	51	37	0.725	0.006	CC	5	*BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, ERBB2IP, ERRFI1, ETS1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, RASSF2, RERE, RNF2, SEPT7, SCAF11, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0005634 nucleus	GO:0044428	Nuclear part	1.41E-05	2568	51	24	0.471	0.009	CC	6	*BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, EP300, ERBB2IP, ETS1, GATA2, GATA4, KLHL20, MATR3, PLCG1, PTEN, RERE, RNF2, SEPT7, SCAF11, GEMIN2, SMAD2, SUZ12, ZFPM2*
GO:0005634 nucleus	GO:0031981	Nuclear lumen	3.71E-03	2193	51	19	0.373	0.009	CC	7	*BMI1, CCNE2, CREB1, E2F3, EP300, ETS1, GATA2, GATA4, KLHL20, MATR3, PTEN, RERE, RNF2, SEPT7, SCAF11, GEMIN2, SMAD2, SUZ12, ZFPM2*
GO:0005634 nucleus	GO:0005654	Nucleoplasm	2.35E-03	1504	51	16	0.314	0.011	CC	7	*BMI1, CCNE2, CREB1, E2F3, EP300, ETS1, GATA2, GATA4, KLHL20, PTEN, RERE, RNF2, GEMIN2, SMAD2, SUZ12, ZFPM2*
GO:0043234 protein complex	GO:0043234	Protein complex	2.00E-02	3751	51	24	0.471	0.006	CC	1	*BAP1, BCL2, BMI1, CREB1, E2F3, EGFR, EP300, ETS1, FLT1, FN1, GATA2, KLHL20, PLCG1, PTPN12, RERE, RNF2, SEPT7, SHC1, GEMIN2, SMAD2, SUPV3L1, SUZ12, TCF7L1, ZEB1*
GO:0043234 protein complex	GO:0005667	Transcription factor complex	2.39E-02	286	51	7	0.137	0.024	CC	3	*CREB1, E2F3, EP300, ETS1, SMAD2, TCF7L1, ZEB1*
GO:0043234 protein complex	GO:0031519	PcG protein complex	2.70E-03	30	51	4	0.078	0.133	CC	7	*BAP1, BMI1, RNF2, SUZ12*

GO:0043234 protein complex	GO:0070435	Shc-EGFR complex	1.60E-02	2	51	2	0.039	1	CC	2	*EGFR, SHC1*
GO:0003677 DNA binding	GO:0003677	DNA binding	1.75E-02	2432	51	19	0.373	0.008	MF	1	*BCL2, CREB1, E2F3, EGFR, EP300, ETS1, GATA2, GATA4, HOXB5, KLF11, NFAT5, RERE, SMAD2, SUPV3L1, SUZ12, TCF7L1, ZEB1, ZEB2, ZFPM2*
GO:0003677 DNA binding	GO:0001071	Nucleic acid binding transcription factor activity	5.15E-04	1167	51	15	0.294	0.013	MF	1	*CREB1, E2F3, EP300, ETS1, GATA2, GATA4, HOXB5, KLF11, NFAT5, RERE, SMAD2, TCF7L1, ZEB1, ZEB2, ZFPM2*
GO:0003677 DNA binding	GO:0003700	Sequence-specific DNA binding transcription factor activity	3.27E-03	1166	51	14	0.275	0.012	MF	2	*CREB1, E2F3, EP300, ETS1, GATA2, GATA4, HOXB5, KLF11, NFAT5, RERE, SMAD2, TCF7L1, ZEB1, ZEB2*
GO:0003677 DNA binding	GO:0043565	Sequence-specific DNA binding	7.57E-04	724	51	12	0.235	0.017	MF	2	*BCL2, CREB1, EP300, ETS1, GATA2, GATA4, HOXB5, RERE, SUZ12, TCF7L1, ZEB1, ZEB2*
GO:0003677 DNA binding	GO:0001067	Regulatory region nucleic acid binding	1.50E-03	380	51	9	0.176	0.024	MF	1	*CREB1, E2F3, EP300, GATA2, GATA4, HOXB5, KLF11, TCF7L1, ZEB1*
GO:0003677 DNA binding	GO:0000975	Regulatory region DNA binding	1.50E-03	380	51	9	0.176	0.024	MF	2	*CREB1, E2F3, EP300, GATA2, GATA4, HOXB5, KLF11, TCF7L1, ZEB1*
GO:0003677 DNA binding	GO:0044212	Transcription regulatory region DNA binding	1.25E-03	372	51	9	0.176	0.024	MF	3	*CREB1, E2F3, EP300, GATA2, GATA4, HOXB5, KLF11, TCF7L1, ZEB1*
GO:0003677 DNA binding	GO:0000981	Sequence-specific DNA binding RNA polymerase II transcription factor activity	4.02E-02	310	51	7	0.137	0.023	MF	3	*CREB1, EP300, ETS1, GATA2, GATA4, HOXB5, KLF11*
GO:0003677 DNA binding	GO:0001012	RNA polymerase II regulatory region DNA binding	2.89E-02	113	51	5	0.098	0.044	MF	4	*CREB1, EP300, GATA2, GATA4, HOXB5*
GO:0003677 DNA binding	GO:0000977	RNA polymerase II regulatory region sequence-specific	2.42E-02	109	51	5	0.098	0.046	MF	5	*CREB1, EP300, GATA2, GATA4, HOXB5*

		DNA binding									
GO:0003677 DNA binding	GO:0035326	Enhancer binding	5.74E-03	36	51	4	0.078	0.111	MF	4	*CREB1, GATA2, GATA4, HOXB5*
GO:0003677 DNA binding	GO:0001158	Enhancer sequence-specific DNA binding	3.53E-03	32	51	4	0.078	0.125	MF	1	*CREB1, GATA2, GATA4, HOXB5*
GO:0003677 DNA binding	GO:0000980	RNA polymerase II distal enhancer sequence-specific DNA binding	3.27E-02	18	51	3	0.059	0.167	MF	2	*CREB1, GATA2, HOXB5*
GO:0003682 chromatin binding	GO:0003682	Chromatin binding	8.09E-06	376	51	11	0.216	0.029	MF	2	*BAP1, EGFR, EP300, GATA2, GATA4, RERE, RNF2, SMAD2, SUZ12, TCF7L1, ZEB1*
GO:0005488 binding	GO:0005488	Binding	3.26E-04	12782	51	49	0.961	0.004	MF	1	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, HOXB5, KDR, KLF11, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, PTPN12, PTPRD, RASSF2, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0005515 protein binding	GO:0005515	Protein binding	3.69E-10	8396	51	47	0.922	0.006	MF	2	*ATP2A2, BAP1, BCL2, BMI1, CCNE2, CREB1, E2F3, EGFR, ELMO2, EP300, EPHA2, ERBB2IP, ERRFI1, ETS1, FLT1, FN1, GATA2, GATA4, KDR, KLHL20, LITAF, MATR3, NFAT5, PLCG1, PTEN, PTPN12, PTPRD, RASSF2, RERE, RND3, RNF2, SEPT7, SCAF11, SHC1, GEMIN2, SLC9A3R2, SMAD2, SUPV3L1, SUZ12, TCF7L1, VAC14, VEGFA, WASF3, XIAP, ZEB1, ZEB2, ZFPM2*
GO:0005515 protein binding	GO:0008134	Transcription factor binding	9.39E-03	475	51	9	0.176	0.019	MF	3	*BCL2, CREB1, EP300, ETS1, GATA2, GATA4, SMAD2, ZEB1, ZFPM2*
GO:0005515 protein binding	GO:0033613	Activating transcription factor binding	2.76E-02	53	51	4	0.078	0.075	MF	4	*CREB1, EP300, GATA4, SMAD2*

GO:0043234 protein complex	GO:0031519	PcG protein complex	2.70E-03	30	51	4	0.078	0.133	CC	7	*BAP1, BMI1, RNF2, SUZ12*

This appendix table groups the enriched Gene Ontology (GO) terms (e.g., "positive regulation of endothelial cell migration," "transcription factor binding," "nucleoplasm") into "broader" GO term categories within the biological process (BP), molecular function (MF), and cellular component (CC) ontologies (e.g., "cell motility," "protein binding," "nucleus," respectively). We summarize this GO analysis in Table 2, focusing on the more specific terms—the processes and functions relevant to our pathology and terms high in their "precision index" (Q*T/T),**[*AQ8*]** which is a ratio of the number of genes in our list (Q = 51 genes) that are associated with the GO term divided by the total number of genes curated to be associated with the GO term (T, differs for each term).

T, term genes; Q, query genes; Q&T, common genes; Q&T/Q, recall; Q&T/T, precision.

[a]Included to group-enriched terms.

Appendix Table 6.

Association of Variables with miR-200b-5p for Normal Weight and Obese Patients (
(*SE*), Adjusted for Group.

	r (*P*)	*B* (*SE*)
ZEB1	–0.34 (0.06) [b]	–0.155 (0.079)
ZEB2	–0.46 (0.008) [a]	–0.203 (0.072)
KDR	–0.33 (0.06) [b]	–0.154 (0.079)
GATA2	–0.42 (0.02) [a]	–0.220 (0.087)
SMAD3	–0.27 (0.14)	–0.273 (0.181)
CDH1	–0.005 (0.98)	–0.009 (0.374)

B (*SE*) was calculated with log-transformed data; *r* values are partial correlations adj
group. The data are graphically presented in Figure 4.

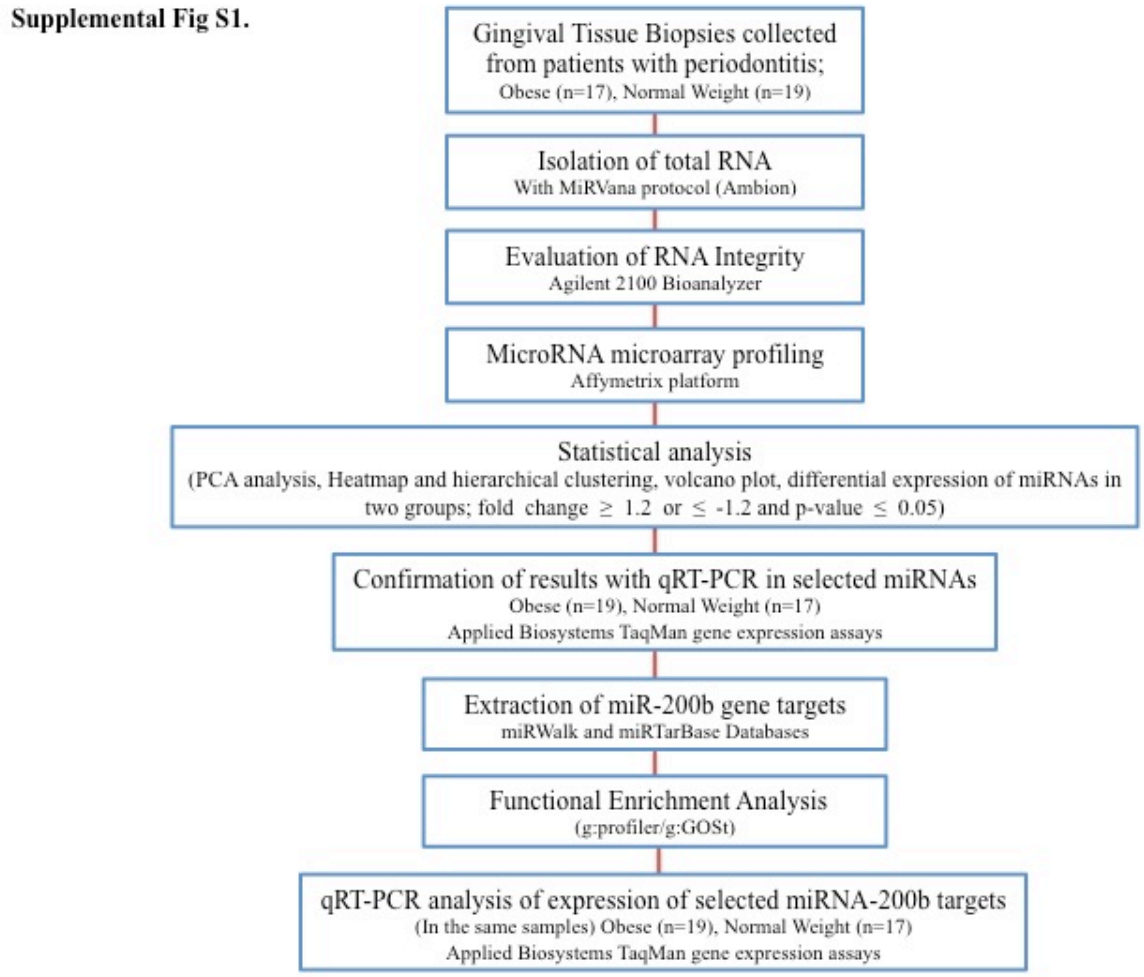
[a] $P < 0.05$

[b] $P < 0.1$.

Appendix Figures

Appendix Figure 1.

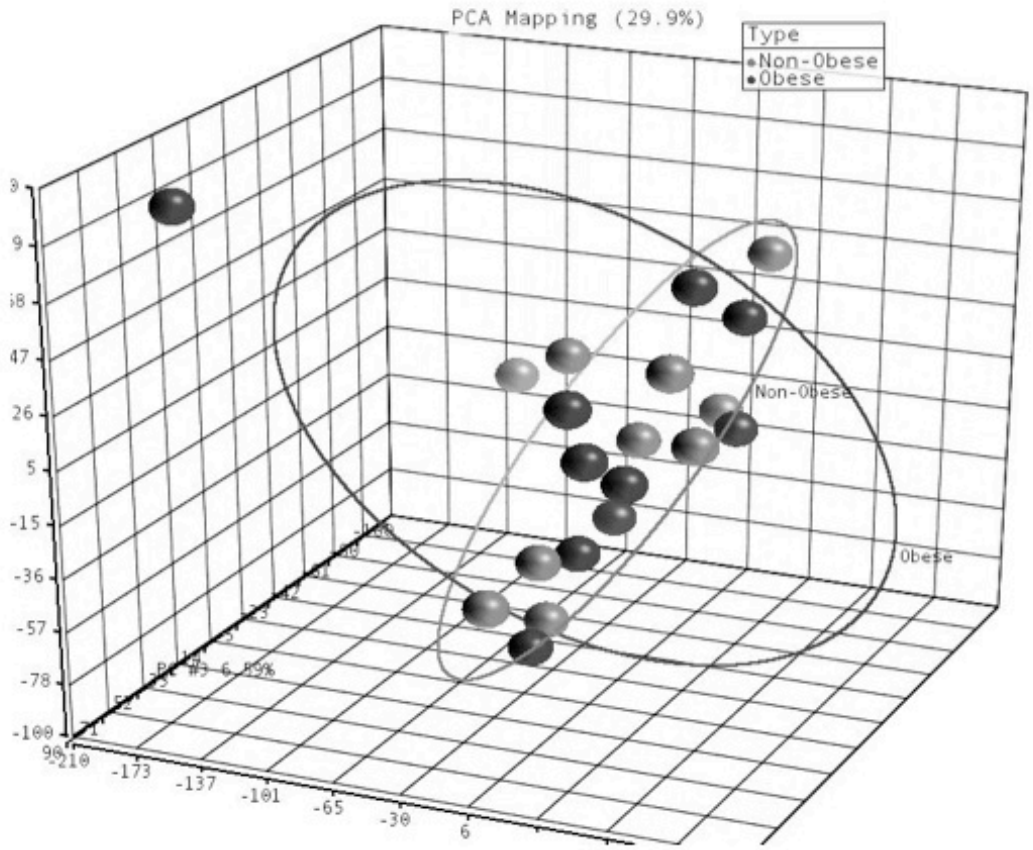
Diagram of the approach used for analysis of genes regulated by microRNAs (miRNAs) in the periodontium in the presence of obesity. PCA, principal component analysis; qRT-PCR, quantitative reverse transcription polymerase chain reaction.



Appendix Figure 2.

Principal component analysis (PCA) showed that no group of microRNAs (miRNAs) clustered in a distinct manner in obese patients (black dots when compared to normal weight controls [light gray dots]; mapping percentage of 29.9%).

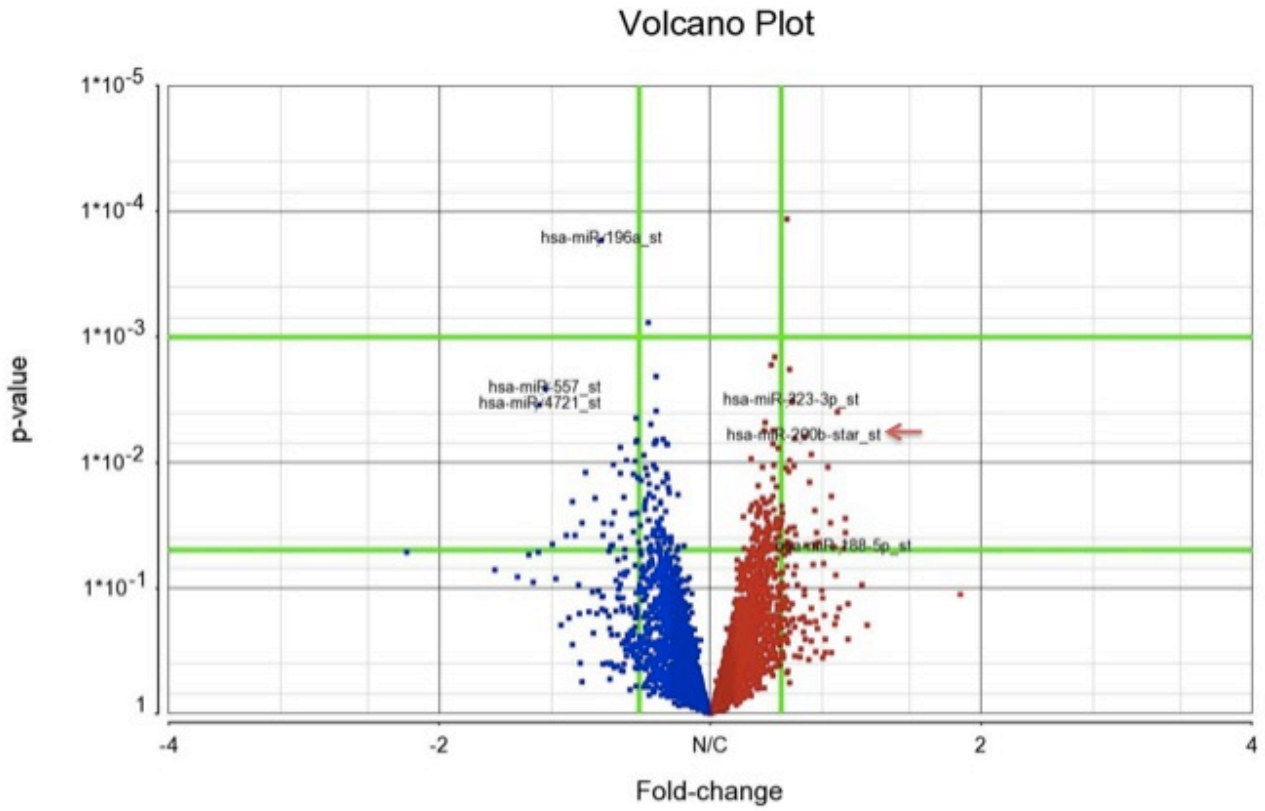
Supplemental Figure S2. Principal Component Analysis (PCA) showed that no group of miRNAs clustered in a distinct manner in obese patients (black dots when compared to normal weight controls (light grey dots) (mapping percentage of 29.9%).



Appendix Figure 3.

Volcano plot of all human microRNAs (miRNAs) with fold change differences against *P* values of differential expression in 20 samples. The *x*-axis represents the fold change of miRNA expression levels between the two groups; the *y*-axis is the unadjusted *P* value based on –log10. The green horizontal lines represent the unadjusted $P = 0.05$ and $P = 0.01$ values; the two green vertical lines represent the 1.2- and –1.2-fold change lines, respectively. Blue and red dots within the left and right vertical green lines represent the down- and upregulated miRNAs, respectively, with unadjusted *P* value ≤ 0.05. N/C, no change.

Supplemental Figure S3.



Appendix Figure 4.

Sequence alignment by Clustal Omega of mature sequences of miR-200b-5p and miR-200b-3p having > 5nt overlap out of 22nt (EBI tools; http://www.ebi.ac.uk/Tools/msa/clustalo/). Published literature comprised information mainly on the primary transcript (miR-200b) and not on the mature sequences that derive from its 5′ and 3′ strands (miR-200b-5p and miR-200b-3p, respectively). As shown here, the base sequences of miR-200b-5p and miR-200b-3p have 5nt overlap out of 22nt, which cannot exclude their binding to similar messenger RNA despite the differences in their sequence. As the effect of strand-specific mature microribonucleic acids in regard to target binding has not been fully understood, we proceeded with the analysis focusing on the primary transcript miR-200b and its experimentally validated gene targets.

Supplemental Figure S4.