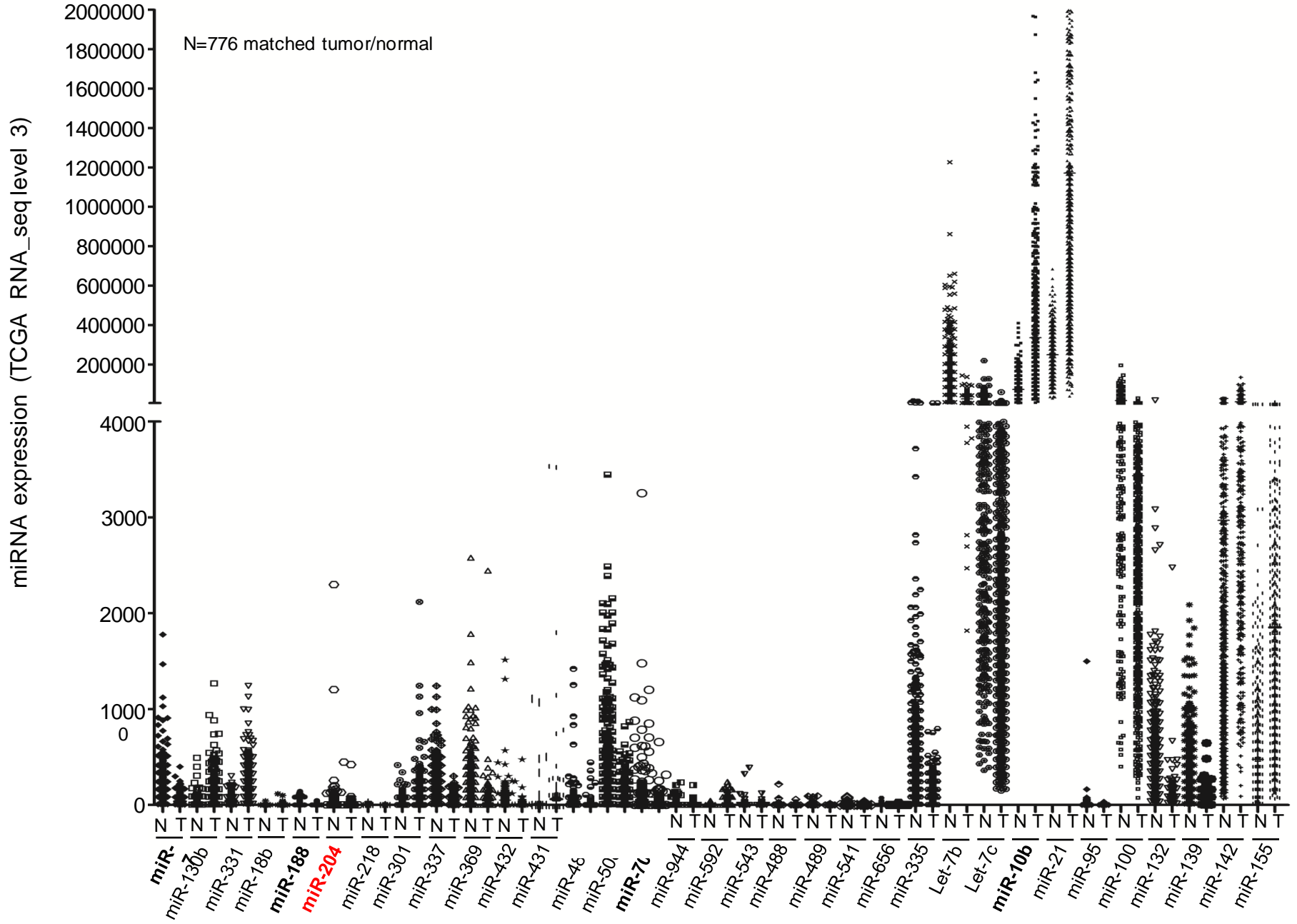


## **Supplementary information**

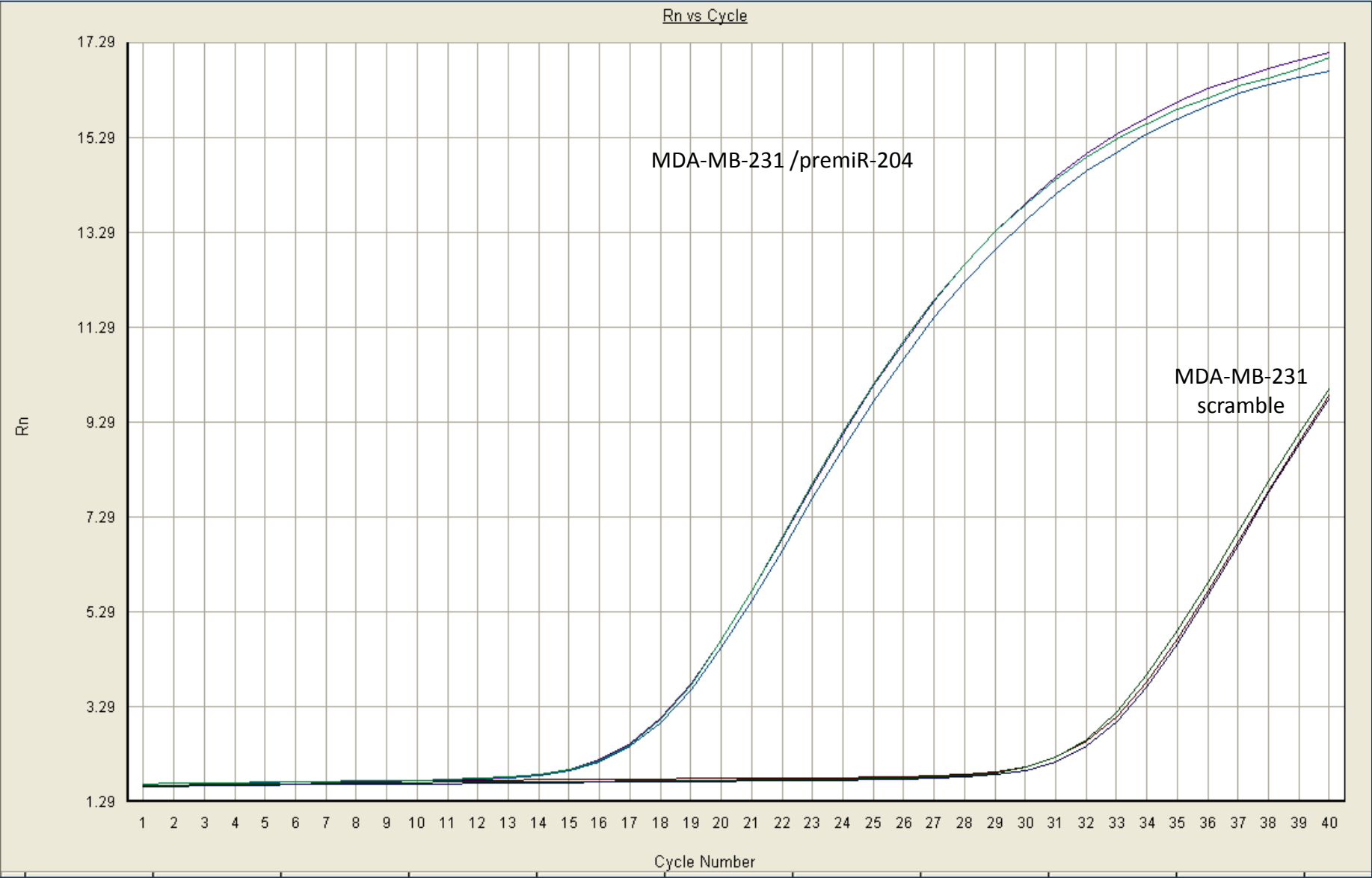
### **Dual targeting of ANGPT1 and TGFBR2 genes by miR-204 controls angiogenesis in breast cancer**

Ali Flores-Pérez, Laurence A. Marchat, Sergio Rodríguez-Cuevas, Verónica Bautista-Piña, Alfredo Hidalgo-Miranda, Elena Aréchaga Ocampo, Mónica Sierra Martínez, Carlos Palma-Flores, Miguel A. Fonseca-Sánchez, Horacio Astudillo-de la Vega, Erika Ruiz-García, Juan Antonio González-Barrios, Carlos Pérez-Plasencia, María L. Streber, César López-Camarillo

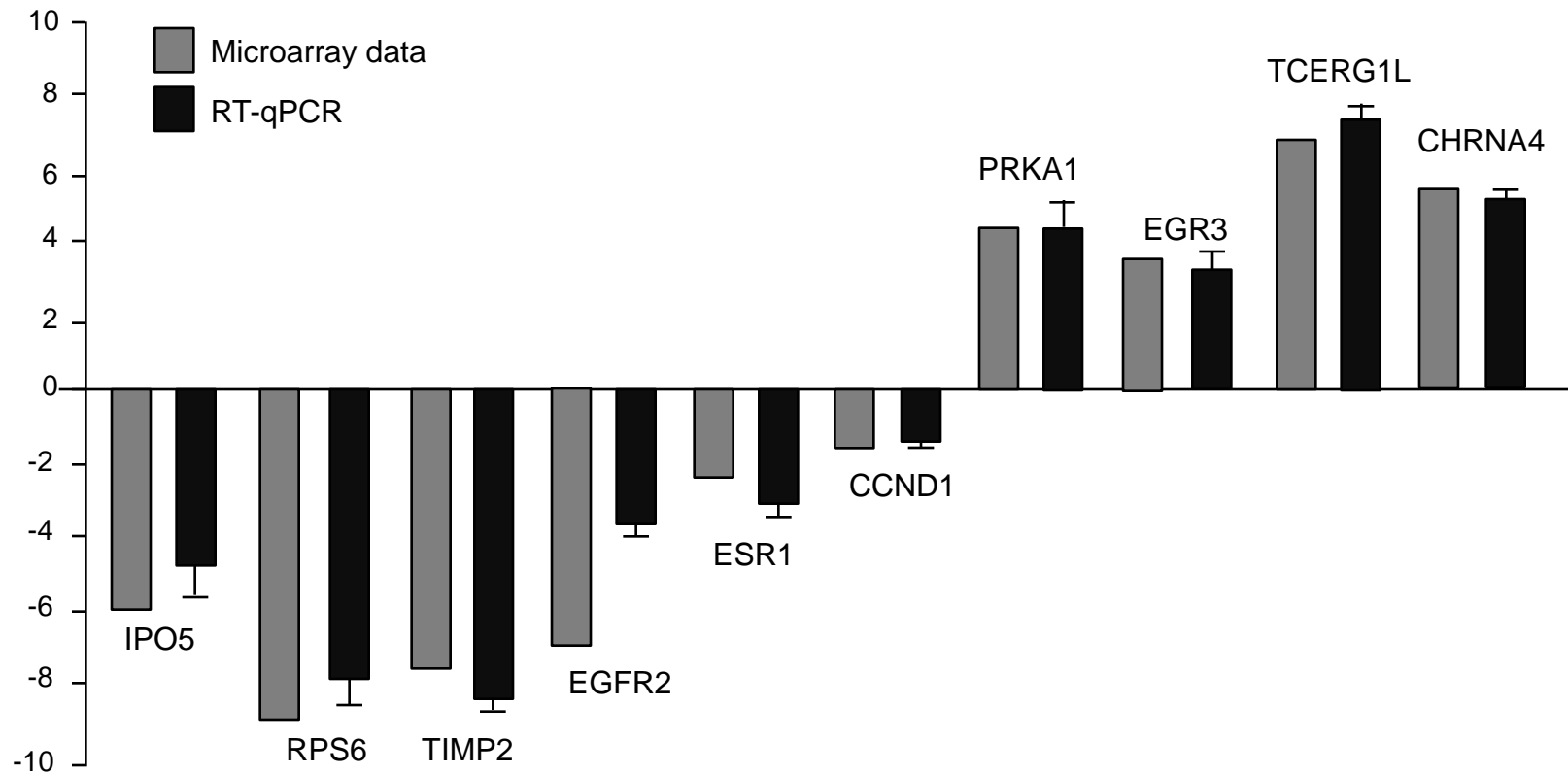
**Supplementary figure 1.** Validation of expression of 33 out of 54 miRNAs detected by TLDA (discovery cohort) using TCGA data sets (validation cohort) in 776 tumor and normal adjacent matched tissues. In bold are denoted 4 miRNAs with discordant expression between the discovery and validation cohort.



Supplementary figure 2. Restoration of miR-204 expression in MDA-MB-231 breast cancer cell line



Supplementary figure 3. Validation of DNA microarrays analysis using RT-qPCR for ten selected genes.



**Supplementary table 1.** MicroRNAs regulated in the complete set of breast tumors (9/9). Fold change (log2RQ) values are indicated for individual microRNAs in breast cancer patients (T).

Up-regulated	Number of samples found	Breast cancer patient (T)								
		T 15	T 30	T 75	T 76	T 78	T 85	T 88	T 105	T 191
hsa-let-7g*	9/9	1.23	11.38	5.39	5.08	5.65	3.79	10.40	4.15	5.70
hsa-miR-148b*	9/9	1.14	1.53	0.40	2.67	2.98	1.02	2.33	1.18	3.59
hsa-miR-454*	9/9	1.49	2.85	2.26	1.00	1.40	1.02	1.00	1.79	1.94
hsa-miR-592	9/9	2.92	5.18	2.71	5.26	5.36	2.54	5.00	3.28	3.79
Downregulated	Number of samples found	Breast cancer patient (T)								
		T 15	T 30	T 75	T 76	T 78	T 85	T 88	T 105	T 191
hsa-let-7c	9/9	-3.65	-2.21	-3.30	-2.29	-2.38	-6.17	-1.20	-1.89	-0.84
hsa-miR-100	9/9	-3.41	-2.72	-2.16	-1.01	-1.36	-4.29	-1.07	-1.21	-1.26
hsa-miR-10b*	9/9	-1.75	-1.01	-1.65	-0.64	-0.55	-3.61	-0.81	-3.03	-1.86
hsa-miR-10b	9/9	-2.83	-1.65	-2.53	-1.24	-1.05	-4.84	-1.35	-0.80	-1.58
hsa-miR-132	9/9	-2.80	-1.21	-1.48	-4.10	-5.32	-3.96	-2.13	-0.22	-1.86
hsa-miR-139-3p	9/9	-6.48	-2.43	-3.25	-8.90	-8.83	-6.63	-9.38	-3.33	-11.29
hsa-miR-1	9/9	-2.44	-0.79	-1.79	-4.90	-3.20	-5.76	-3.23	-1.35	-1.66
hsa-miR-195	9/9	-3.19	-1.00	-3.00	-1.46	-1.47	-4.37	-0.97	-1.48	-0.88
hsa-miR-204	9/9	-6.73	-4.67	-2.87	-6.84	-7.51	-9.04	-5.85	-6.47	-6.42
hsa-miR-218	9/9	-3.08	-2.08	-2.19	-1.63	-1.64	-4.86	-1.21	-0.77	-2.34
hsa-miR-26b*	9/9	-2.13	-0.40	-1.48	-2.34	-2.16	-2.92	-0.14	-2.29	-0.07
hsa-miR-376a	9/9	-2.05	-2.18	-2.36	-1.13	-1.27	-5.16	-1.35	-1.15	-3.04
hsa-miR-376c	9/9	-1.60	-2.33	-2.36	-1.88	-2.08	-5.64	-2.40	-1.20	-3.62
hsa-miR-424*	9/9	-5.16	-3.19	-0.74	-0.12	-0.18	-2.88	-1.91	-4.21	-2.68
hsa-miR-432	9/9	-1.34	-0.76	-1.15	-1.34	-0.40	-3.98	0.35	-2.60	-0.91
hsa-miR-433	9/9	-2.49	-2.25	-0.98	-3.32	-2.03	-5.41	-1.34	-1.08	-3.47
hsa-miR-489	9/9	-2.42	-2.04	-3.78	-4.13	-4.26	-5.67	-2.91	-3.34	-4.22
hsa-miR-656	9/9	-1.51	-0.46	-2.26	-2.01	-2.96	-2.28	0.32	-2.59	-0.24
hsa-miR-944	9/9	-0.99	-1.80	-2.55	-0.94	-1.25	-3.69	-1.30	-1.66	-2.52

Cellular pathway KEGG	Total genes	-ln (p-value)
MAPK signaling pathway	114	22.63
Focal adhesion	90	19.83
Wnt signaling pathway	75	22.37
Axon guidance	71	28.93
Ubiquitin mediated proteolysis	63	15.39
Colorectal cancer	54	27.37
Prostate cancer	52	21.86
Adherens junction	48	27.51
TGF-beta signaling pathway	48	15.61
ErbB signaling pathway	47	15.38

**Supplementary table 2.** Cellular pathways potentially affected by microRNAs deregulated in breast tumors

Gene	Sequence (5' - 3')	Product length (bp)	Nucleotide position
GAPDH	FW- CCCACCCACACTGAATCTCC RV- GTACATGACAAGGTGCGGCT	90	1266-1355
IPO5	FW- AGGAGAAATGCACGAGGCAA RV- TTAAGGCCCTTCACGCAGAG	173	3134-3306
RPS6	FW- TGTTACTCCACGTGTCCTGC RV- AAGTCTGCGTCTCTTCGCAA	166	579-744
TIMP2	FW- AGCTTTGCTTTATCCGGGCT RV- ATGCTTAGCTGGCGTCACAT	175	1868-2042
EGFR2	FW- GGTGGCATTAGGGGTGACT RV- CAAGGGAACAGGAAATATGTCG	173	1365-1537
ESR1	FW- ACTCAACAGCGTGTCTCCG RV- CACATTTTCCCTGGTTCTGTAG	173	529-701
CCND1	FW- GCTGTAGTGGGGTTCTAGGC RV- GGCACGCTACGCTACTGTAA	160	1626-1785
PRKAA1	FW- AGTGTGCGCTTTTTGAATAGTTTG RV- AGCAGTCTAGGCCAACAAGC	243	661-903
EGR3	FW- GGTGACCATGAGCAGTTTGC RV- TAGGTCACGGTCTTGTGGCC	225	388-612
ESR1	FW- CGCTGCGTCGCCTCTAA RV- TCCAGCTCGTTCCCTTGAT	182	124-305
TCERG1L	FW- TACAGGTTTCCAAGGGTGGC RV- TGCAAACCTCCGGGCATACAA	162	2405-2528
CHRNA4	FW- AATGTCACCTCCATCCGCATC RV- GTCAGCATTGTTGTAGAGGG	72	550-598
ANGPT1	FW- CGTGAATCTGGAGCCGTTTG RV- AGTAGTTTGAAGCACAGCAAGC	141	3'UTR 200-341
TGFBR2	FW- TGTCAGAGGATACTGTGGCTTG RV- TGAGTACAGCTGAAGTGTTCAT	162	3' UTR 2250-2412
CREB5	TCTTCAAAGACTGGCTTTTCATTTT AAAGCACCAAAGCGTTTCCC	125	3' UTR 150-275

**Supplementary table 4.** Primers used to validate DNA microarrays data.

**Supplementary table 5.** Cell proliferation and migration genes modulated by miR-204

Expression	Biological Process	Number of genes	Gene Symbol
Downregulated	Proliferation	38	E2F1, EXO1, TXNIP, CLSPN, DBF4B, PDS5A, PDPN, TIPIN, SKP2, CDK4, TACC1, TACC2, CDT1, PSMA2, TUBB, EREG, NPM1, <b>TGFBR2*</b> , <b>SMAD4*</b> CHTF18, <b>MAPRE2*</b> , FANCA, DSCC1, CDK4, ID2, NPM1, FOXC1, ASNS, ID3, GPR3, <b>CREB5*</b> ,EXTL3, AKT1S1, <b>ARHGAP5*</b> , SPTBN1, NEFL, CDH4, <b>RAB22A*</b>
	Migration/Invasion	24	CAP2, MARK1, LLGL1, HOOK3, TACC2, EPB41L2, CORO2B, TUBB, ANK3, NPM1, CAP1, NEFL, LCP1, TUBA8, KIF21A, TACC2,FMOD, EID2, ID1, <b>TGFBR2*</b> , <b>SMAD4*</b> , <b>FOXC1*</b> , <b>FRAS1*</b> , <b>LRP8*</b>
Upregulated	Proliferation	32	btg1, GJA1, BDKRB2, TRIB1, GPNMB, ptch1, Wisp2, Dbp, BTG4, Hyal1, Nppc, mmp7, il6r, irs2, Spn, KCTD11, ESR2, eglN3, TNFRSF14, APC, nupr1, ADRA1A, RARRES1, Hyal1, LAMA5, Ang, Kiss1r, Ifitm1, Hyal1, Scgb3a1 ,LAMA5 ,GPNMB
	Migration/Invasion	29	TMEM8B, RET, fn1, GPNMB, ITGA1, RHOB, ITGA7, CNTN2, Cntn4, Wisp2, ROR2, Itgax, PCDHB9, PCDHB10, PCDHB11, CD72, PCDHB5, Wisp2, Nedd9, sspN, CDH1, fn1, APC, RHOB, Selp1g, LAMA5, GPNMB, LAMA5, CCR1

\*Genes with miR-204 binding sites



Primer name	Sequence 5'-3'	Nucleotide position in target gene
ANGPT1.1-S	GATCCGGATTTTCAGAAGCCTAATTCCTTCAAGAGAGGAATTAGGCT TCTGAAATCCTTTTTT GGAAA	2533-2554
ANGPT1.1-AS	AGCTTTTCCAAAAAAGGATTTTCAGAAGCCTAATTCCTCTCTTGAAG GAATTAGGCTTCTGAAATCCG	2533-2554
ANGPT1.2-S	GATCCGCCATAATGAACTGTAGTACATTCAAGAGA TGACTACAGTTCATTATGGCTTTTTTGGAAA	2617-2638
ANGPT1.2-AS	AGCTTTTCCAAAAAAGCCATAATGAACTGTAGTACATCTCTTGAATG TACTACAGTTCATTATGGCG	2617-2638
TGFβ2 1.1-S	GATCC GGAGGAGAAGATTCCTGAAGA TTCAAGAGA TCTTCAGGAATCTTCTCCTCC TTTT TT GGAAA	2041-2062
TGFβ2 1.1-AS	AGCTTTTCCAAAAAAGGAGGAGAAGATTCCTGAAGATCTCTTGAAT CTTCAGGAATCTTCTCCTCCG	2041-2062
TGFβ2 1.2-S	GATCCGGTGTTAAGATTTGAAGTTGGTTCAAGAGACCAACTTCAA TCTTAACACCTTTTTTGGAAA	3566-3587
TGFβ2 1.2-AS	AGCTTTTCCAAAAAAGGTGTTAAGATTTGAAGTTGGTCTCTTGAAC CAACTTCAAATCTTAACACCG	3566-3587

**Supplementary table 6.** Primers used to silencing ANGPT1 and TGFβ2 gene expression