

Figure S1. TargetScan results for miR-203 binding sites on (A) Snail2 and (B) Phf12 3'UTRs. We want to highlight that Snail2 3'UTR has 2 binding sites, one conserved and other poorly conserved, while Phf12 3'UTR have only one conserved site.

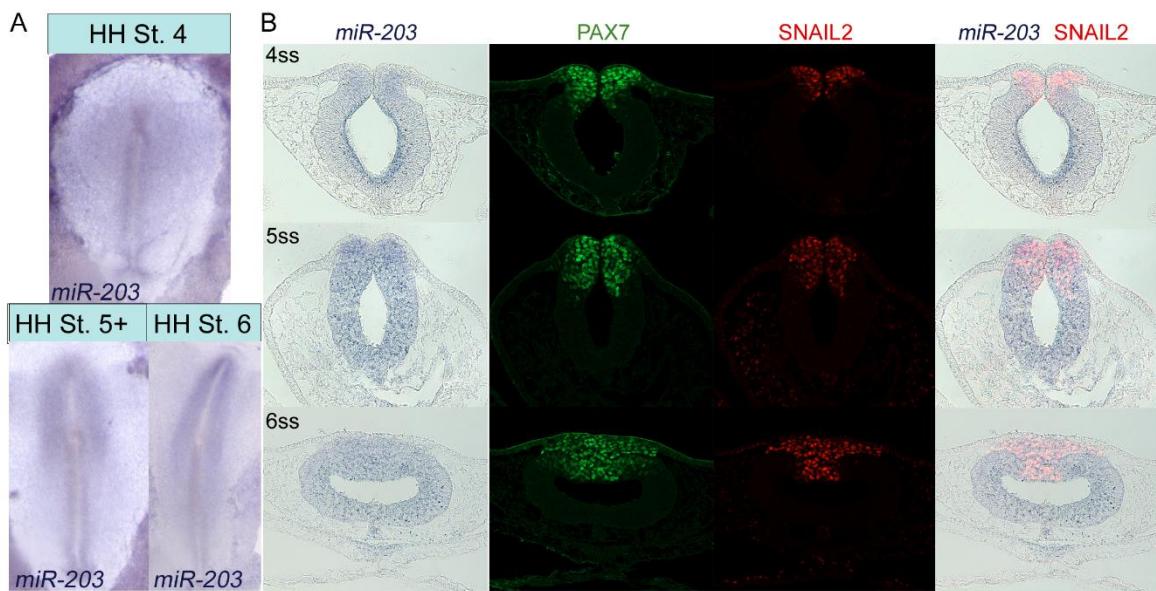


Figure S2. (A) Whole-mount *in situ* hybridization using DIG-labeled LNA probes (Exiqon) against miR-203 at early chick developmental stages. (B) miR-203 is present in the pre-migratory neural crest from 4ss (somites stage) until 6ss. miR-203 expression overlaps with well-known neural crest markers: Pax7 (green) and Snail2 (red).

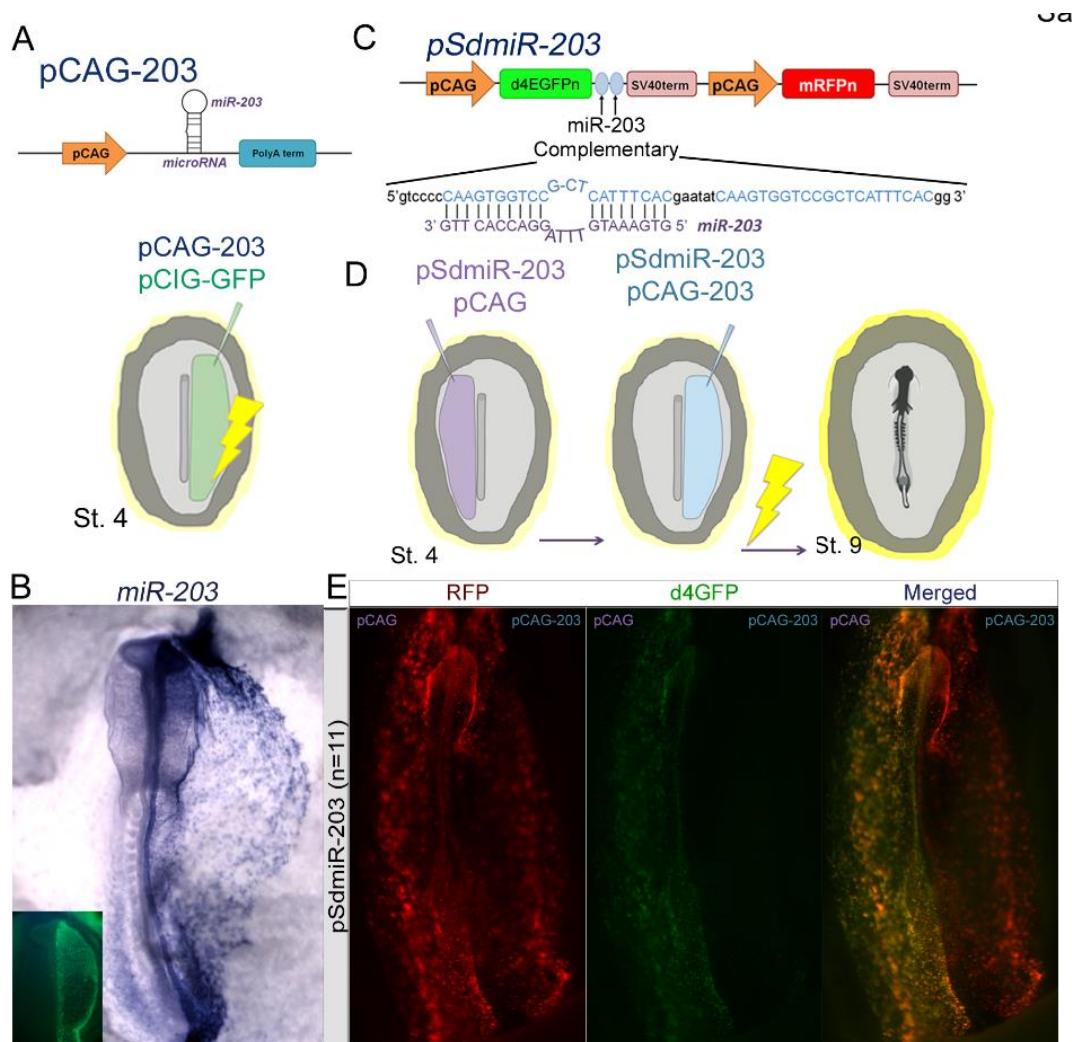


Figure S3. (A) Scheme of pCAG-203 vector to overexpress miR-203. Diagram of electroporation assay for gain-of-function experiments. pCAG-miR-203 does not have a fluorescent marker, for this reason, was co-electroporated with a fluorescent vector that express GFP downstream the CAG promoter. We injected the vectors in the right side of the embryos at stage 4. Following injection, embryos were electroporated and cultured until stage 9. (B) Electroporation of pCAG-203 vector (right side) successfully overexpress a mature miR-203 evidenced by *in situ* hybridization using LNA probes. (C) Schematic drawing of the miRNA dual-sensor vector (pSdmir-203) containing two copies of complementary sequences to the mature miR-203. (D) Illustration of bilateral electroporation assay to evaluate if pCAG-203 express a functional miR-203. (E) Co-electroporation pSdmir-203 and the empty pCAG vector (left side) caused that most of the cells are yellow because of the expression of both green and red reporters. Whereas, co-electroporation pSdmir-203 and pCAG-203 vector (right side) caused that most of the cells are only red, because of the strong repression of the green reporter. pCAG, Chick β-actin promoter; d4EGFP_N nuclear-localized destabilized EGFP with a half-life of 4 h; mRFP_N, nuclear-localized monomeric red fluorescent protein.

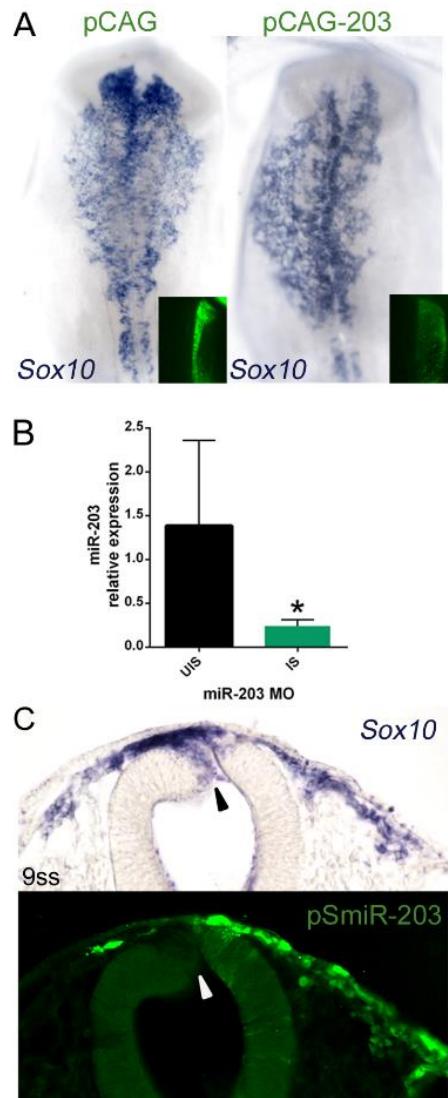


Figure S4. (A) miR-203 overexpression causes inhibition of neural crest migration. *In situ* hybridization for *Sox10* confirms that inhibition of NCC migration is caused by pCAG-203. (B) Real time qPCR confirm morpholino-mediated loss of miR-203 (miR-203 MO) on the IS compared with the UIS of the same group of embryos (n=6). Asterisk (*) indicate significant differences ($P<0.05$) by Student's t-test. (C) Neural crest cells from the sponge IS complete their delamination in advance compare with the UIS where many *Sox10* expressing cells are still on the neural tube (see black arrowhead).

A

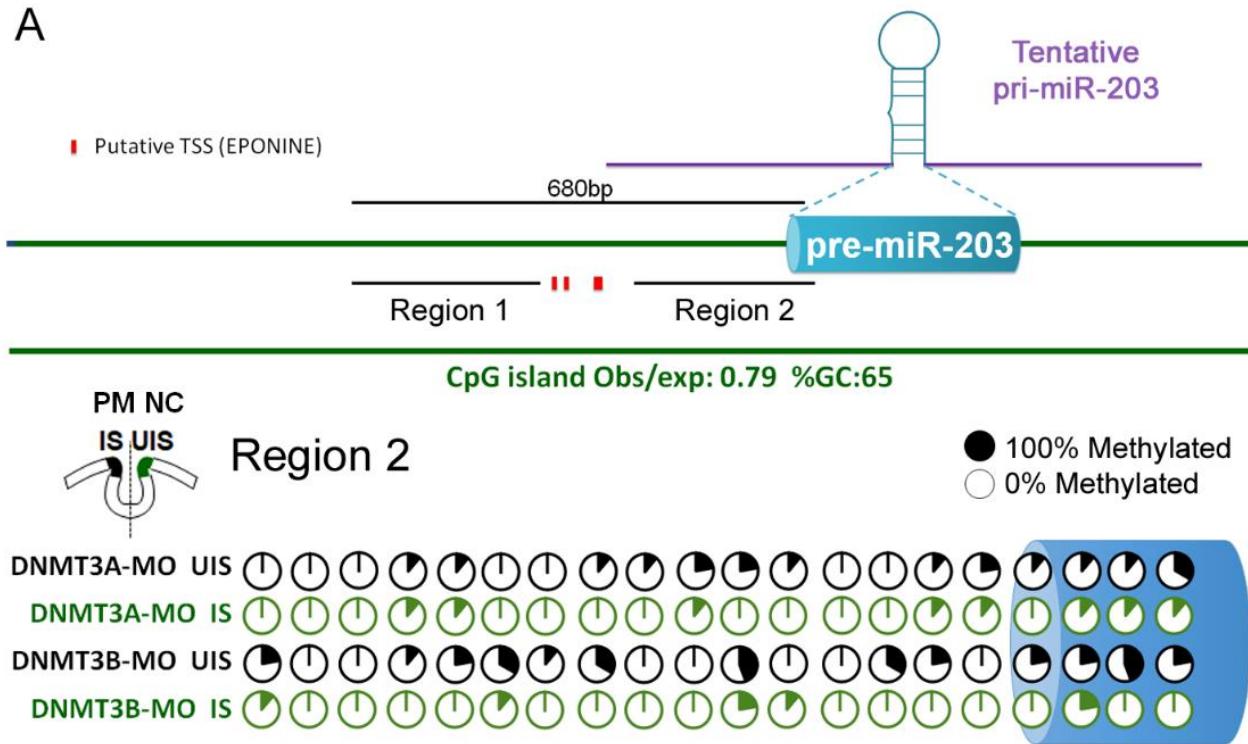


Figure S5. Bisulfite sequencing profiles of CpGs methylation on the region 2 on the injected side (IS) compared with the uninjected side (UIS) of the same group of embryos ($n=6$) electroporated with morpholinos against DNMT3A (DNMT3A-MO) and DNMT3B (DNMT3B-MO). Independent clones ($n=10$) were sequenced for each sample. Percentage of each methylated CpG sites are shown with filled (100% methylated) and open (0% methylated) circles. Total percentage of methylation in each condition is normalized with the UIS to make graphs in figure 5 F.

Table S1. *In silico* analyzes of conserved and poorly conserved microRNA-binding sites on Snail2 and Phf12 3'UTRs (TargetScan), their known functions, demonstrated targets, and chick expression (GEISHA).

GEISHA microRNAs expression in chick development

http://geisha.arizona.edu/geisha/quick_search.jsp?table=mir

miR-1 (Heidersbach et al. 2013; Wystub et al. 2013; Wei et al. 2014)

miR-19 (Olive et al. 2009; Mavrakis et al. 2010; Liu et al. 2011)

miR32-5p/92-3p/367 (Zhu et al. 2013; Zhu et al. 2015; Sharifi and Salehi 2016)

miR-33-5p (Mi et al. 2016; Wang et al. 2016)

miR130-3p/301-3p/454-3p (Leone et al. 2015; Xia et al. 2015; Egawa et al. 2016; Lv et al. 2016)

miR-142 (Lu et al. 2013; Sonda et al. 2013; Borges et al. 2016; Dickman et al. 2017)

miR-155 (Gracias et al. 2013; Robertson et al. 2014; Forzati et al. 2017)

miR-181-5p (Korhan et al. 2014; Li et al. 2015; Ma et al. 2015)

miR-200b-3p/429-3p (Ye et al. 2014; Wu et al. 2016; Gui et al. 2017)

miR-203 (Yi et al. 2008; Wei et al. 2010; Zhang et al. 2011; Benaich et al. 2014; Shi et al. 2015)

miR-221-3p/222-3p (Takigawa et al. 2016; Yan et al. 2016; Wu et al. 2017)

miR-365-3p (Wang et al. 2013; Gastaldi et al. 2014)

miR-455-5p (Chai et al. 2015; Li et al. 2016; Liu et al. 2016)

	Conserved sites			Poorly conserved sites				Known function	Known Target Gene	Reference	Expression (GEISHA) Stages 3-25		
	8mer	7mer-m8	7mer-1A	8mer	7mer-m8	7mer-1A	6mers						
Phf12	miR-19-3p	1	0	0	0	0	0	1	0,85	Oncogene	Pten, Bim, TNF- α	Olive et al. 2009; Mavrakis et al. 2010; Liu et al. 2011	Broadly expressed but at much reduced levels in the heart
	miR-130-3p /301-3p /454-3p	1	0	0	0	0	0	0	0,77	Oncogene	Coiled-coil domain-containing protein 6 (CCDC6), Smad4, Pten	Leone et al. 2015; Xia et al. 2015; Egawa et al. 2016; Lv et al. 2016	Hindbrain, spinal cord, widespread expression after stage 21 except in heart
	miR-365-3p	0	1	0	0	0	0	1	0,41	Tumor suppressor	KRAS, MAX, PAX6	Wang et al. 2013; Gastaldi et al. 2014	No expression detected until stage 14, extraembryonic, notochord, surface ectoderm, widespread expression after stage 19
	miR-142-3p	0	0	1	0	0	0	0	0,4	Tumor suppressor, hematopoietic development	Interferon regulatory factor 7 (Irf7), interleukin 6 signal transducer (IL6st), transforming growth factor beta receptor 1 (TGFBR1)	Lu et al. 2013; Sonda et al. 2013; Borges et al. 2016; Dickman et al. 2017	No expression detected until stage 20, atria, sinus venosus
	miR-455-5p	0	0	1	0	0	0	0	0,38	Tumor suppressor	RAB18, ZEB1, RA F proto-oncogene serine/threonine-protein kinase (RAF1)	Chai et al. 2015; Li et al. 2016; Liu et al. 2016	Unknown
	miR-221-3p /222-3p	0	0	1	0	0	0	0	0,36	Osteoblasts/osteocytes develop	RUNX2, Smad5, tartrate-resistant acid phosphatase (TRAP), ADP-ribosylation factor 4 (ARF4)	Takigawa et al. 2016; Yan et al. 2016; Wu et al. 2017	No expression detected until stage 14, low level ubiquitous from stage 16-25
	miR-33-5p	1	0	0	0	0	0	0	0,32				
	miR-203	0	1	0	0	0	0	0	0,31				
Snai1/2	miR-7467-3p	0	0	1	0	0	0	0	0,24	Unknown	Unknown	Unknown	Unknown
	miR-1-3p/206	0	1	0	0	1	0	0	0,72	Cardiovascular development	ER β 1, myocardin, telokin	Heidersbach et al. 2013; Wystub et al. 2013; Wei et al. 2014	Atria, heart, myocardium, myotome, somites, ventricles
	miR-181-5p	0	0	1	0	1	0	1	0,47	Tumor suppressor	KRAS, matrix metalloproteinase MMP-14, c-Met	Korhan et al. 2014; Li et al. 2015; Ma et al. 2015	Ubiquitous
	miR-200b-3p /429-3p	0	1	0	0	0	0	1	0,43	Tumor suppressor	Zeb1, multiple members of RAB family, extracellular-regulated protein kinase 5 (ERK5)	Ye et al. 2014; Wu et al. 2016; Gui et al. 2017	Surface ectoderm from stage 13-18, diverse expression before stage 20
	miR-203	1	0	0	0	0	0	1	0,38	Skin development, tumor suppressor	p63, Snail2, LIM and SH3 domain protein 1 (LASP1)	Yi et al. 2008; Wei et al. 2010; Zhang et al. 2011; Benach et al. 2014; Shi et al. 2015	Ubiquitous after stage 15, no expression detected at stage 5
	miR-33-5p	0	0	1	0	1	0	1	0,31	Osteoblasts/osteocytes develop	Special AT-rich sequence-binding protein 2 (SATB2), high-mobility group AT-hook 2 (HMGA2)	Mi et al. 2016; Wang et al. 2016	No expression detected until stage 21, amnion, atria, extraembryonic
	miR-155	0	0	0	0	1	0	0	0,23	Oncogene	ELK3 (ETS Transcription Factor), several mRNAs	Gracias et al. 2013; Robertson et al. 2014; Forzati et al. 2017	No expression detected stage 3-24
miR-32-5p /92-3p/367	miR-32-5p /92-3p/367	0	0	0	0	0	1	0	0,22	Oncogene	Pten, Smad7, estrogen-related receptor β (ER β 1)	Zhu et al. 2013; Zhu et al. 2015; Sharifi and Salehi 2016	Neural Plate/Tube, spinal cord, widespread expression

Table S2. Results obtained with the Jaspar 2018 (<http://jaspar.genereg.net/>) for SNAI2 binding site in the tentative promoter of miR-203. High binding sites (>9) are mapped in figure 2A. We also show the sequence analyzed in Jaspar 2018.

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA0745.1	SNAI2	12,6931	0.999707221414	miR_203_tentative_promoter	-1162	-1153	+	AACAGGTGC
MA0745.1	SNAI2	9,71729	0.94034684341	miR_203_tentative_promoter	-1552	-1543	+	GGCAGGTAC
MA0745.1	SNAI2	8,48966	0.915858769456	miR_203_tentative_promoter	-1089	-1080	-	CACAGGTTG
MA0745.1	SNAI2	7,98092	0.905710714071	miR_203_tentative_promoter	-1273	-1264	-	ATCAGGTTG
MA0745.1	SNAI2	4,63965	0.839061031445	miR_203_tentative_promoter	-1599	-1590	-	TGCATGTTT
MA0745.1	SNAI2	3,94346	0.825173896668	miR_203_tentative_promoter	-1527	-1518	+	TCAAGGTGT
MA0745.1	SNAI2	3,5435	0.817195747557	miR_203_tentative_promoter	-1199	-1190	-	TGGAGGTTG
MA0745.1	SNAI2	3,51455	0.816618237138	miR_203_tentative_promoter	-1293	-1284	+	AGAAAGTGA
MA0745.1	SNAI2	3,24005	0.811142584031	miR_203_tentative_promoter	-1082	-1071	+	TGCCAGTGC

>miR_203_tentative_promoter Chr 5:50767590-50768212
AGGACTGGCTTGAGTTGCCTATATAATTATAAGAGCCAAAGATCATAGGATCTGGAGTGCAGAACATTACACAGCACATATAACAGCTCTTAAACATGCAAAACACTCTATTAACATGA
GTGAAGCCTCATAAGATGA**GGCAGGTAC**GCATTATCTCCTCCTCAAGGTGTGGCGAAGTGACTTGGTAAGGCCTGAGGCTGAGTCGCTGGCAGAGCAGGGCCGACTATCCACA
TCCTACACACACAGGCTGCCCCGCTCCAGCATGGCAAAGCGTGAAGGCCTCCCGTCCACCCCAGCAGCAGGGCTCGAAAGCCCAGTGGTTGTTTCATTCTCTCTATAGACA
AGGGTGAATAATTAAATGGAAGAAAGTCAAATTAGAGAAAAGTGACTCCGGCAAGCAACCTGATTCTGAAAGTTCATGAAATCATACAATTGTTGAGTTGGAAGGGACCCCTAAAG
CCATCCAGTCCAACCTCCATGCATAAGTAGGGACTCCACGGCTCC**AACAGGTGC**TCAAGAGCCCCGTCAGCCTGACCTGGCTGTCTAAGGACAGGGACCCACCATCTCTGGCA
ACCTGTGCCAGTGCCTC

Table S3. Complete list of utilized primers, LNA probe, and morpholinos

Gene/Direction	Sequence
LNA Probe	
dre-miR-203a(gga-miR-203)	CAAGTGGTCCTAACATTCAC
RT-qPCR Primer Sequences	
stem-loop-gga-miR-203	GTCTCCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGGGAGACCAAGTG
stem-loop-gga-miR-16	GTCTCCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGGGAGACCAAGTG
qPCR-gga-miR-203 Fwd	CCGGCGTGAATGTTAGG
qPCR-gga-miR-16 Fwd	CGCCGCTAGCAGCACGTAAA
qPCR-gga-miR-universal Rev	GAGGTATTTCGACCAGAGGA
qPCR-Snail2 Fwd	GCCAAACTACAGCGAATGG
qPCR-Snail2 Rev	CGGAGAGAGGTATTGGTA
qPCR-Phf12-Fwd	CTGAGGAACCCTTGCAGAAG
qPCR-Phf12-Rev	AGAGTCCCAAAGCGAAGTC
qPCR-HPRT1-Fwd	TGGTGAAGTGGCCAGTTG
qPCR-HPRT1-Rev	TCATTGTAGTCGAGGGCGTATC
Bisulfite Primer Sequences	
Proximal region	
P-miR-203 Fwd	AGGTAGTTGGAAAAATTGGTT
P-miR_203 Rev	CTCCTTAAAAACATTACAACCC
PN-miR-203 Fwd	AAGTTTGTGTTGTTATT
PN-miR-203 Rev	TAAAATTTAAAAACCACTACACCA
Distal region	
D-miR-203 Fwd	TTTATTTGTTGAGGGGAAGG

D-miR-203 Rev	TTTCCAAACTACCTTCTCCCTA
DN-miR-203 Fwd	TTGTGTGAGGTTGGTAGTTAGG
DN-miR-203 Rev	ATCATCATCATCTAAAACAACCC
pCAG-miR-203 cloning	
Xhol-gga-miR-203 Fwd	AAACTCGAGCTCCGAGCTGAGAAGAATGG
EcoRV-gga-miR-203 Rev	AAAGATATCCGCGCACTACAAGCCTATT
Sponge cloning	
miR-203 sponge Fwd	gtcccCAAGTGGTCCGCTCATTTCACGaatatCAAGTGGTCCGCTCATTTCACgg
miR-203 sponge Rev	gacccGTGAAATGAGCGGACCACTTGatattcGTGAAATGAGCGGACCACTTGgg
Scramble sponge Fwd	gtcccATCTAGCTGATCTAACGAAcatatATCTAGCTGATCTAACGAAACgg
Scramble sponge Rev	gacccGTTCGATTAGATCAGCTAGATatattGTTCGATTAGATCAGCTAGATgg
pUTRs cloning	
Snail2-3'UTR Fwd	ACCGGTGTCATGCAGTCATGTTAC
Snail2-3'UTR Rev	GCTAGCTTCACCTCACGCTTCTTC
mutSnail2-3'UTR SITIO A Fwd	ATGCATGAGACCCGCAGTAGATCTAAACG
mutSnail2-3'UTR SITIO A Rev	GCAGGGTCTCATGCATGGCATCTTCCCC
mutSnail2-3'UTR SITIO B Fwd	CAAGCGACCCGCACCAAAGAAACAGTATTTAATGG
mutSnail2-3'UTR SITIO B Rev	GGTGCAGGGTCGCTGGCAGGAATGTATTAGTAAC
Phf12-3'UTR SanDI Fwd	AAAGGGTCCCGAATTGGAGGAAGGGAGCT
Phf12-3'UTR Rev	GCTAGCTACAGTGGAGCTAGCTGGC
mutPhf12-3'UTR Rev	AAAACCGTGCTGCTCGCTGCAGTTCTTTAAAAGCGGGCTATAG
Morpholinos Sequences	
DNMT3A MO	TGGGTGTGTCACTGCTTCCACCAT
DNMT3B MO	CGAGGCTCGTTACCATGCTCATCGC
SNAIL2 MO	TCTTGACCAG GAAGGAGC
miR-203 MO	GGTCAAGTGGTCCTAACATTTCAC

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