Correspondence: MicroRNA-212/132 family is required for epithelial stromal interactions necessary for mouse mammary gland development

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Supplementary Note

Materials and Methods

Animal studies.
All animal studies were performed in accordance with the relevant guidelines and regulations and with the approval of the local and national authorities.

Whole mount analyses of mammary glands.
Whole mount carmine-alum staining of mammary glands was performed as described previously[1].

Quantitative RT-PCR analyses.
Total RNA samples from dissected mammary gland samples were isolated with Tri Reagent (Sigma). cDNAs were synthesized using SuperScript II (Life Technologies) and random hexamers according to the manufacturer's protocol. Real time PCR was performed in triplicates using the Power-SYBR Green master mix (Life Technologies) and the Applied Biosystems 7300 light cycler. The results were analyzed by the ddCt method using HPRT1 expression levels for normalizations. Student's *t*-test is used for the calculation of the significances in expression level differences. The used primer sequences are as following:
HPRT1-Frw: GATTAGCGATGATGAACCAGGTT
HPRT1-Rev: CCTCCCATCTCCTTCATGACA
Hic1 variant 1 specific Frw: CATGCCCCCCAGGAGAGTGTGCTG
Hic1 variant 2 specific Frw: GGACATTTTACTTAAATCGGGAGAGTGTGCTG
Hic1 Rev (common for both variants): CGGTGTAGATGAAGTCCAGCACCAG
EpCAM-Frw: TTGCTCCAAACTGGCGTCTA
EpCAM-Rev: TCCCAGACTTGCTGTGAGTCA

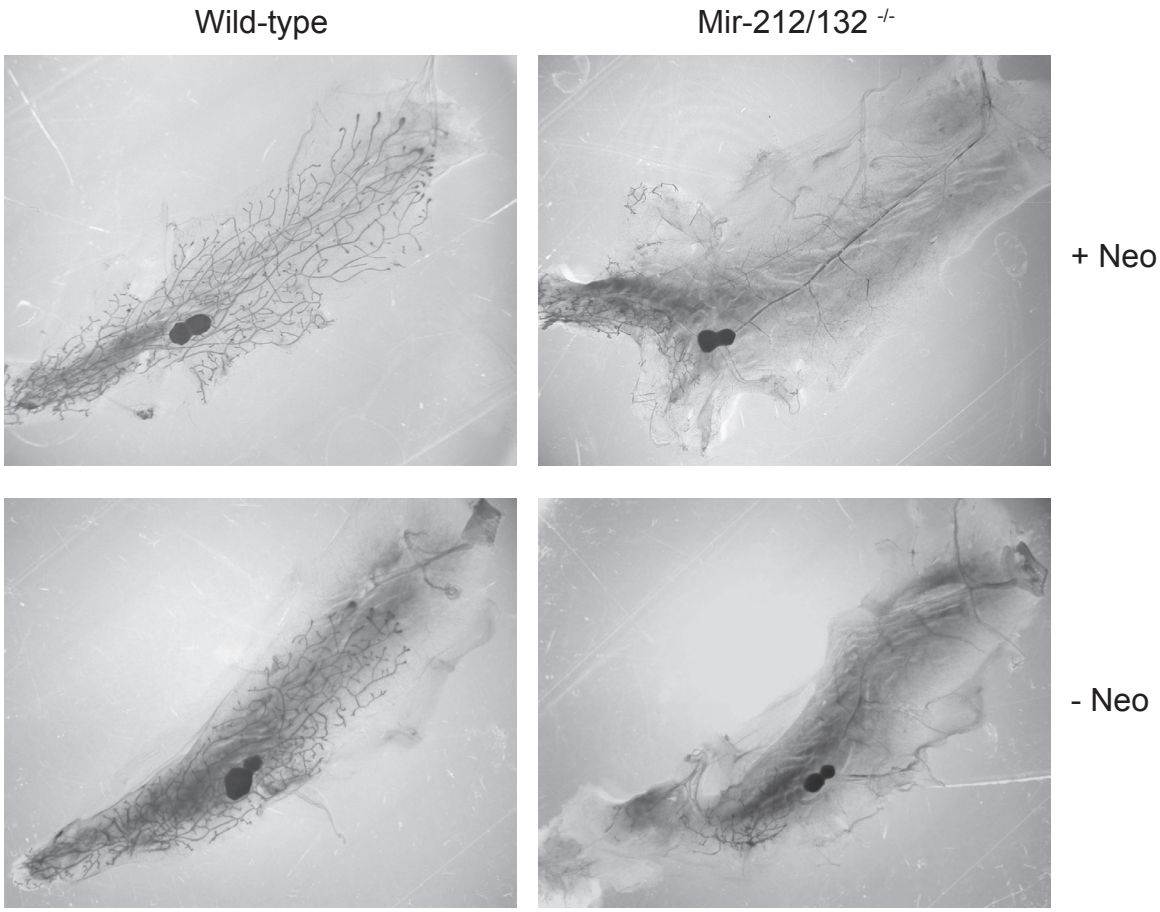
Bioinformatical analyses.
The evolutionary conservation ratios of corresponding genomic regions was analyzed by and exported from ECR browser (http://ecrbrowser.dcode.org).

The putative binding sites for transcription factors within the 290 bp long genomic sequence, which was differentially deleted by our targeting strategy, has been analyzed using the following databases and search tools:
TFSEARCH (http://www.cbrc.jp/research/db/TFSEARCH.html),
MotifMap (http://motifmap.ics.uci.edu),
JASPAR (http://jaspar.genereg.net), and
PROMO(http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3)

The CpG islands within the same 290 bp long genomic sequence was performed by using the CpG Island Searcher tool (http://cpgislands.usc.edu)

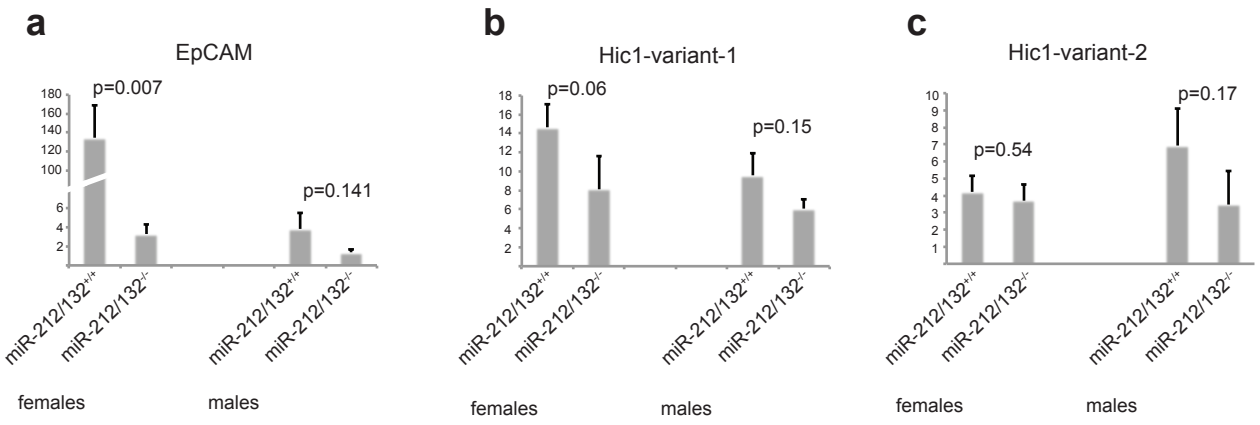
The sequence of this 290bp long sequence is shown below:

GAGAAGGAAGAAGTCACTTTTGCCAGCAGAAAGCTGAAGGGGGAGGAGAAGGAAGT
TTTATCACACCTTTCTTCCAGAAAACCCTAGGTGAGCAGGTAGAGGGGAAAGGGCAA
AGAATCCCTTTCCTGATAGAGCCATAACTCTGTCCCCCAACTTTCTCCTGGGCCATCA
TTAAAAGGGTCCTTAACACAGCAAGAAGCTGGCAGGCTAGTGAAATTCACCTGGCCC
CTAATTCATTGTCTGAAGGTATCAGCCAGCAGTCTTCCCTCAACTGGTCTGTAAGTA
ATGCA



Supplementary Figure 1. Presence of neomycin cassette do not influence the impaired ductal outgrowth phenotype.

Whole mount Carmine-Alum staining of mammary glands of 10 week old female mice in 129SV background. Upper panels are from mice containing neomycin cassette in the miR-212/132 loci and lower panels are from mouse lines for which the neomycin cassette had been removed from the genomic loci.



Supplementary Figure 2. Expression of EpCAM and two major spliced variants of Hic1 in adult male and female mammary glands.
Transcript levels of EpCAM (a), and variant 1 (b) and variant 2 (c) of Hic1 was determined by quantitative RT-PCR and demonstrated in relative levels on y-axis. Mice used in these experiments were in C57BL6/N background that did not contain Neo cassette in their genome.
p values determined by t-test is shown above the corresponding samples. n=3