

Table S1, Primers, siRNAs and LNA-probe used in this study

For cloning

GAS5-R1-5.1
TTCTAGAGCTAGCGAATTCTTTTCGAGGTAGGAGTCGACT
GAS5-Not1-3.1
TCGCAGATCCTTGCGGCCGCTGGATTGCAAAAATTTATTAA
miR-21-pCDH-R1-5.1
ATTCTAGAGCTAGCGAATTCTGATTGAACTTGTTTCATTTT
miR-21-pCDH-Not1-3.1
TCGCAGATCCTTGCGGCCGCAAGACTATCCCCATTTCTCCA
GAS5-miR21-BS-M-5.1
ACAAATGAATTACAGATCAGGTGAAGTTGAAATGGTGGAG
GAS5-miR21-BS-M-3.1
ACCTGATCTGTAATTCATTTGTGTGCCAATGGCTTGAG
miR-21-GAS5-mt-5.1
TCTTCCATCAGACCGCGATTGACTGTTGAATCTCATG
miR-21-GAS5-mt-3.1
TCAATCGCGGTCTGATGGAAGACCCGACAAGGTGGTACAG

For RNA precipitation

T7-GAS5-5.1
TAATACGACTCACTATAGGGTTTCGAGGTAGGAGTCGACT
T7-GAS5-Not1-3.1
GCGGCCGCTGGATTGCAAAAATTTATTAA

For RT-PCR

GAS5-RT-5.1
CTTCTGGGCTCAAGTGATCCT
GAS5-RT-3.1
TTGTGCCATGAGACTCCATCAG
GAS5-RT-3.1A
CTTGCTCCACACAGTGTAGT

U77-RT-5.1
CAGATACTATGATGGTTGCA
U77-RT-3.1
GACAGATAGTACATCTCTTC
U44-RT-5.1
CCTGGATGATGATAAGCAA
U44-RT-3.1
AGTCAGTTAGAGCTAATtaa
Pre-miR-21-RT-5.1
GCTTATCAGACTGATGTTGACTG
Pre-miR-21-RT-3.1
CAGCCCATCGACTGGTG

Pri-miR-21-RT-5.1
TGTTTTGCCTACCATCGTGA
Pri-miR-21-RT-3.1
AAGTGCCACCAGACAGAAGG

siRNAs

GAS5-siRNA-1
CUUGCCUGGACCAGCUUAAdTdT
GAS5-siRNA-2
AAGCUGGAAGUUGAAAUGGdTdT

LNA oligo

GAS5-LNA probe
Bio-T+T+A+CCCAAGC+A+AGTCATCCATGGA+T+A+A

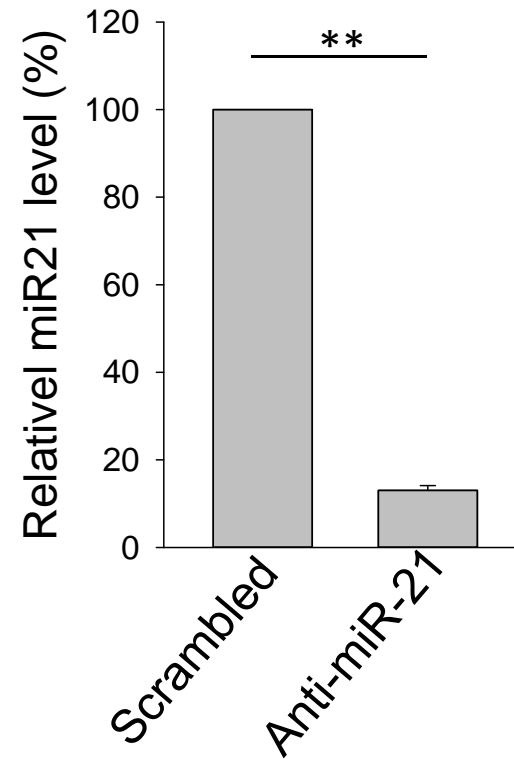


Fig. S1 Suppression of miR-21 by anti-miR-21. Total RNA was isolated from the cells 24 h after transfection, followed by qRT-PCR. Error bars represent SEM, n = 3. **, p < 0.01.

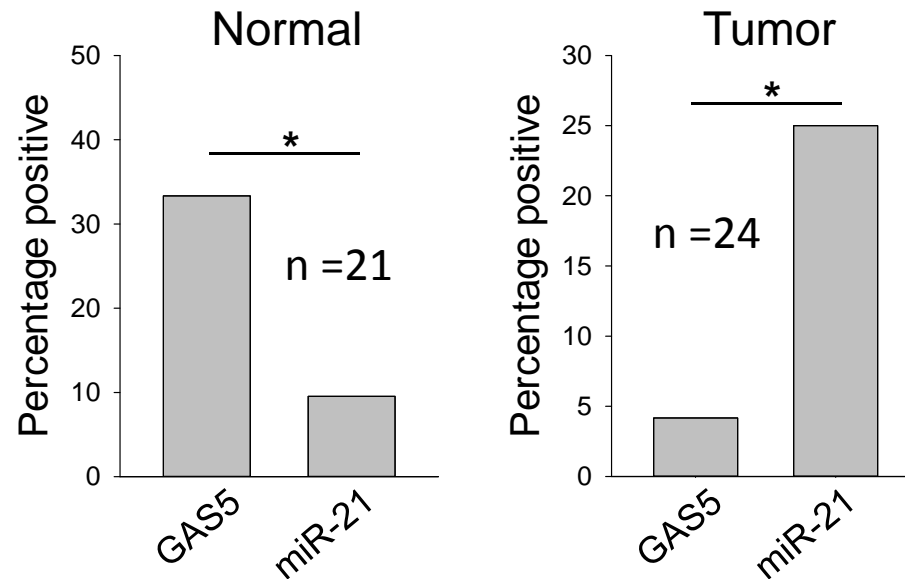
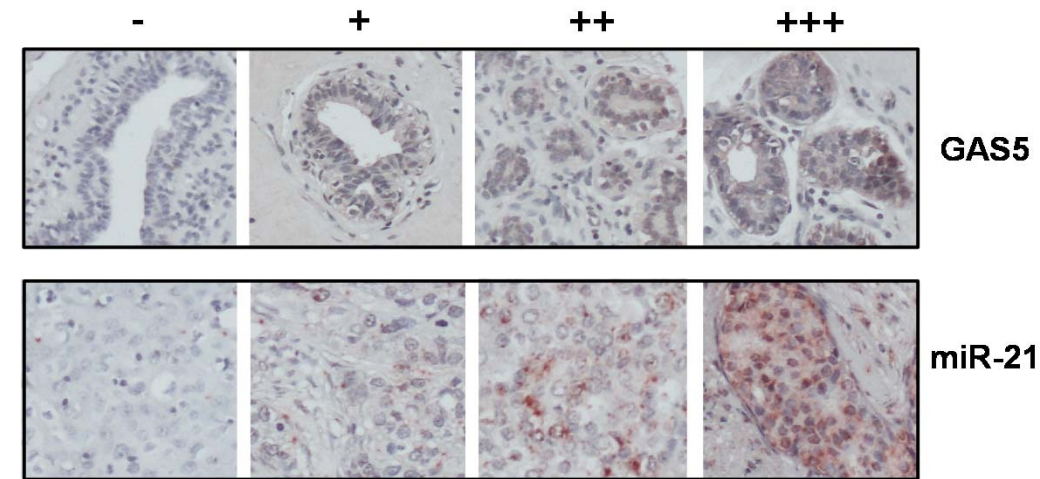


Fig. S2. A, Detection of GAS5 and miR-21 in breast cancer tissue microarrays by in situ hybridization. A, Signal intensity scale. B, Statistical analysis with McNemar's Test. The TMA consists of 24 normal and 24 tumor cores. No cells were seen in three cores and they were removed. Positive: ++ or +++; Negative: - or +. *, $p < 0.05$.

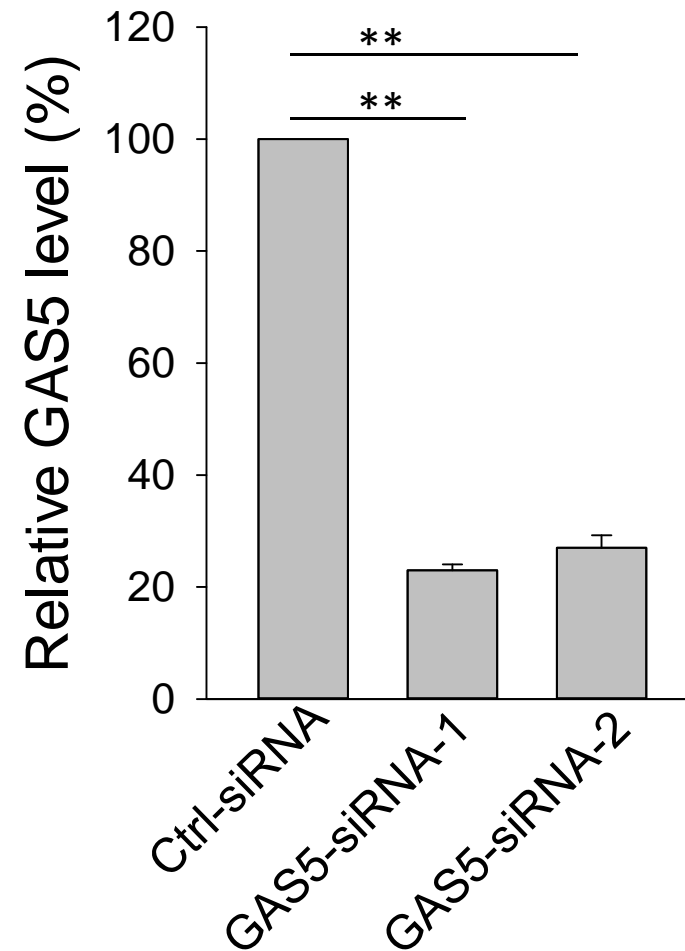


Fig. S3 Suppression of GAS5 by GAS5-siRNAs. Total RNA was isolated from the cells 24 h after transfection, followed by qRT-PCR. Error bars represent SEM, n = 3. **, p < 0.01.

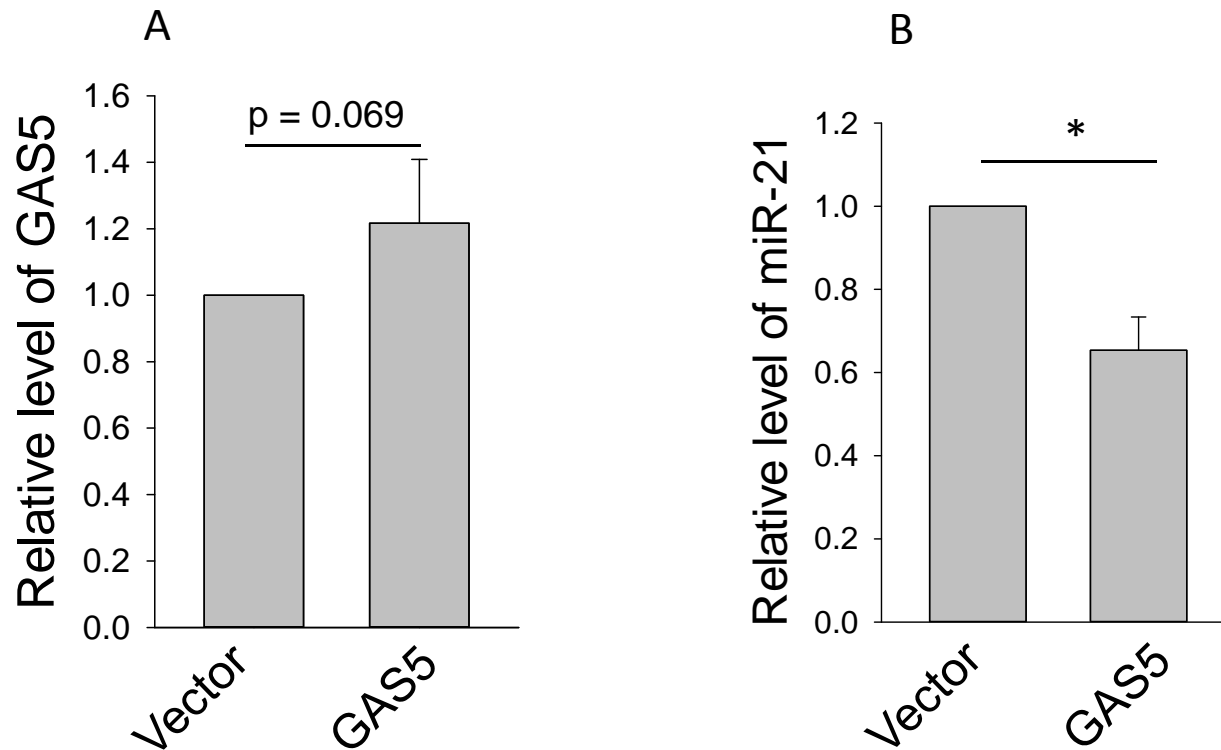


Fig. S4 Effect of GAS5 on miR-21 in the xenograft-derived tumors . Total RNA was isolated from freshly frozen tumors and subject to qRT-PCR. A, Detection of GAS5 in tumors derived from GAS5 infected cells. B, Detection of miR-21 in the same tumor samples. Error bars represent SEM, n = 3. *, p < 0.05.

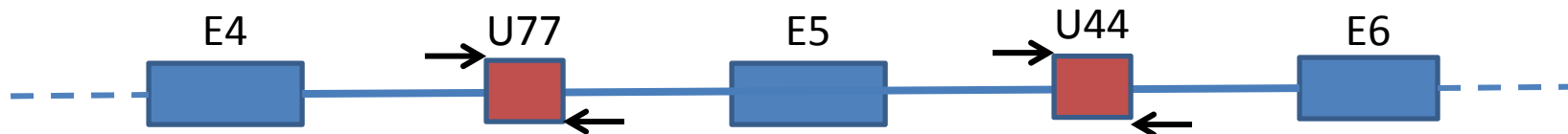


Fig. S5 Relative positions of snoRNA U77 and U44 to GAS5. U77 is between exon4 (E4) and exon 5 (E5); U44 is between exon5 and exon6 (E6). Arrows are primers used to amplify U77 and U44, respectively.