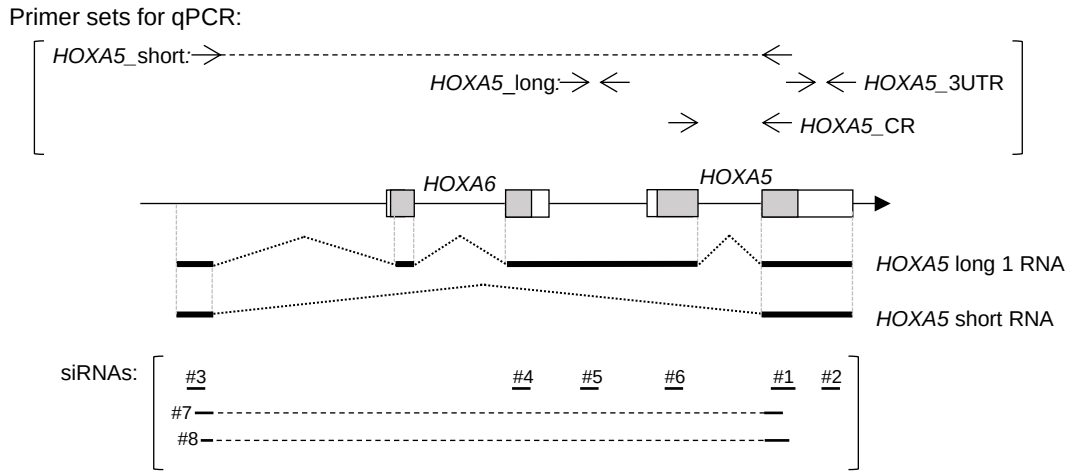
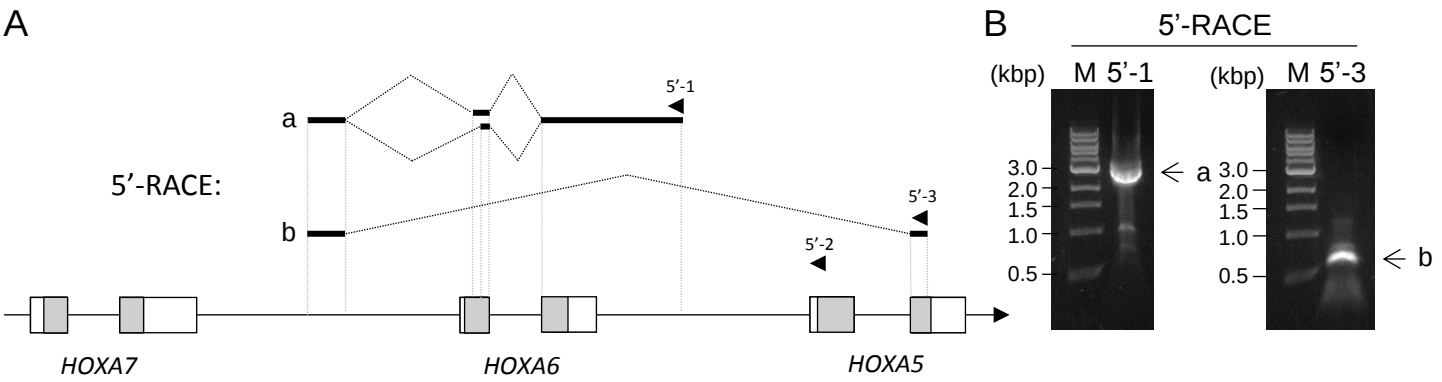


Figure S1. Schematic representation of the primer sets used for the qPCR assay and siRNAs targeting of the *HOXA6-HOXA5* locus. Primers for qPCR amplification of the indicated transcripts or genomic regions are indicated by the left/right arrows. Transcripts, *HOXA5* short and *HOXA5* long 1; regions, *HOXA5* coding region (CR) and 3' UTR. Targeted sequences of siRNAs #1 to #8 are indicated by the solid lines and the dashed lines for siRNAs #7 and #8 indicate the skipped sequences of the siRNAs.

Figure S2



C

>5'-1 (2,182 nt): corresponding to the band "a" (partial sequence of long 1)

CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGTGGGACCGGCGCCAGCTGCAGCCCGCCTCTTGCAGCCTGGCGCGACTGCTGTACTGGCCCCTC
AGAGATTTCCCAGCAAGCAGGGTAGAGGGGGACGCAAGGGGACCTGGGGGGCGATCACCCAGAGCCTATATGGCCGGGAAATCTCCCCGCCTCGGCTACCCCTGACC
GGAGCTGGACGCCGTTATAGACTCGGGTCCTCCCCCACCCCCACTCCAGCACCATGGAGACTGGTTGCACAGGCCGCCTGGCACGTCATGGCTGCTTCTGTCCAAAC
TGCGCGTCCCAAGGCGTTCTGGGGGCGCACGCTGGTTCAGGGAACACTCTGGCTTTGGGATTCTGCAATCGGAGAGACTACCTGTAGTCCTGAGGCCTGTAGGCCTG
GCTGCTCTGAGGAGGATTGGCTATGGCAAAGAAGGACGGCTTTGGCTCCAGGAGAGGGCTGTGAAGGGCAGAGGCCGCACCTGTGTGCTTTGGGCTCTGGTCTGGGC
TAGCGAGCTGGCTGGACCGAGGTGGCCTTCAAGGTCCCACACCTACATACTCCCAGACCACCCTCCAGGCTGGCAGAGGTGGAGCTGTTCAATGGGCAGCCCAGGGC
AAGGGCAGACAGTGGGGCCCAGCTCGCCGTCCTGCCCCCCTTGGAGAGAAACTGAAAGTCCAACTCGGTCCTGGCCTGCAACCGGGCGTCCTACGAGTACGGGGCCT
CGTGTTTCTATTCTGATAAGGACCTCAGTGGCGCCTCGCCCTCGGGCAGTGGCAAGCAGAGGGGCCCCGGGGACTACCTGCACTTTTCTCCCGAGCAGCAGTACAAA
CCCGACAGCAGCAGCGGGCAGGGCAAAGCACTCCATGACGAAGGCGCCGACCGGAAGTACACGAGCCCGGTTTACCCTTGGATGCAGCGGATGAACTCCTGCGCGGGG
TGCTGTGTATGGGAGCCATGGGCGCCGAGGCCGCCAGACCTACACGCGCTACCAGACACTGGAGCTGGAGAAGGAGTTCCACTTCAACCGCTACCTGACACGGCGCC
GCCGCATCGAGATCGCCAACGCGCTCTGCCTCACCGAGCGCCAGATCAAGATCTGGTTCCAGAACCGCCGCATGAAGTGGAAAAAGGAAAACAAGCTCATCAATTCC
ACGCAGCCCAGCGGGGAGGACTCAGAGGCAAAGGCGGGCGAGTAGATGCCTGGGCAGGGACCAGGCCAGCGCTGCAACCTCCTTCGGCTTTGCCCCCTTGCCCTCGC
CTGTTCCCCAACTTTTCTCCCCGCCTGCTCCCATCTGGGGGCTTCCGCAGCTTCAGGGGAGCCCGGAGCTTTGCAAGCGTCTGTGCATTTATTTCTTACAAAACAAA
ACAAAACAAAACAAAACAAAACAAAACAAAACAAAACCTCACACACAGCCAATACCAGCGGTGGAGCGGGGCGCACTGCACACACCCAGTCCCGCTCCACAA
GGATGCCCAAACCGGCTCCGGAGTTCTGGGGTGCGCCCTGAATGCGTCTGGGGCTCCTGTGCTAGTGTCTCTGAGCTCCCCGACCTCGGGACGCGGGGCTGCTGGGG
AGGAAAGAGCCCTGGGCCGAGCCCCGTTTCGTGTCTGGGCGTCTCTGCCTAAGTCCCTCGCCAGTAAGTCCCGAAGGGCATCATCAAATCATCTCTGGCCAGTGGAA
AGAGGGGGTCTAAGAGCTGGAGCCCGGCCTTGTCGCGGCCTCCCAAGCCGCCTCCCAGAACAGGAAGGAAGCTCGAGGAACAAAGGGGGCCCCAACAGAGCCCAGTC
TCTCGGTCCCGCGTGTGCAACCGTCAGTGGAAGAGAAGCAGCCTCAGCCGAGGCGAGTTAACCTGGGCGCGGGTGGAACATTACAGCCCGGGGTAGGCTCCCGGCTC
CGGATTCCCGCTCCCAGCCTCCGATAGCGCCCGCGTCGCCGCCACAGCAGCGTTCAGGACCCAACGAGGGGCCCAGGGCCATGGGAGCCGCCAGAGTCCTGGCTTCC
AGACGTGCCAGGGGCGCCTGCAACGCCGGGCCTCCAGCGGGGAGACTCCACTTGCCTCTCAGCTATATTTGTGATTTACTTGAGTCTTTCGGAGCCGTGGAAAATAA
CTACAGAGATACTAGGCACTGCCAAGGAATGCTTCCATTATCGCCCTCATTTTACTCCTGGAGGTCTAAGCAAGACCCCAGTAACCC

>5'-1 (2,035 nt): corresponding to the band "a" (partial sequence of long 2)

CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGTGGGACCGGCGCCAGCTGCAGCCCGCCTCTTGCAGCCTGGCGCGACTGCTGTACTGGCCCCTC
AGAGATTTCCCAGCAAGCAGGGTAGAGGGGGACGCAAGGGGACCTGGGGGGCGATCACCCAGAGCCTATATGGCCGGGAAATCTCCCCGCCTCGGCTACCCCTGACC
GGAGCTGGACGCCGTTATAGACTCGGGTCCTCCCCCACCCCCACTCCAGCACCATGGAGACTGGTTGCACAGGCCGCCTGGCACGTCATGGCTGCTTCTGTCCAAAC
TGCGCGTCCCAAGGCGTTCTGGGGGCGCACGCTGGTTCAGGGAACACTCTGGCTTTGGGATTCTGCAATCGGAGAGACTACCTGTAGTCCTGAGGCCTGTAGGCCTG
GCTGCTCTGAGGAGGATTGGCTATGGCAAAGAAGGACGGCTTTGGCTCCAGGAGAGGGCTGTGAAGGGCAGAGGCCGCACCTGTGTGCTTTGGGCTCTGGTCTGGGC
TAGCGAGCTGGCTGGACCGAGGTGGCCTTCAAGGTCCCACACCTACATACTCCCAGACCACCCTCCAGGCTGGCAGAGGTGGAGCTGTTCAATGGGCAGCCCAGGGC
AAGGGCAGACAGTGGGGCCCAGCTCGCCGTCCTGCCCCCCTTGGAGAGAAACTGAAAGCCAGTACAAACCCGACAGCAGCAGCGGGCAGGGCAAAGCACTCCATGACG
AAGGCGCCGACCGGAAGTACACGAGCCCGGTTTACCCTTGGATGCAGCGGATGAACTCCTGCGCGGGTGCTGTGTATGGGAGCCATGGGCGCCGAGGCCGCCAGACC
TACACGCGCTACCAGACACTGGAGCTGGAGAAGGAGTTCCACTTCAACCGCTACCTGACACGGCGCCGCCGCATCGAGATCGCCAACGCGCTCTGCCTCACCGAGCG
CCAGATCAAGATCTGGTTCCAGAACCGCCGCATGAAGTGGAAAAAGGAAAACAAGCTCATCAATTCCACGCAGCCCAGCGGGGAGGACTCAGAGGCAAAGGCGGGCG
AGTAGATGCCTGGGCAGGGACCAGGCCAGCGCTGCAACCTCCTTCGGCTTTGCCCCCTTGCCCTCGCCTGTTCCCCAACTTTTCTCCCCGCCTGCTCCCATCTGGGG
GCTTCCGCAGCTTCAGGGGAGCCCGGAGCTTTGCAAGCGTCTGTGCATTTATTTCTTACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAA
ACCTCACACACAGCCAATACCAGCGGTGGAGCGGGGCGCACTGCACACACCCAGTCCCGCTCCACAAGGATGCCCAAACCGGCTCCGGAGTTCTGGGGTGCGCCCTG
AATGCGTCTGGGGCTCCTGTGCTAGTGTCTCTGAGCTCCCCGACCTCGGGACGCGGGGCTGCTGGGGAGGAAAGAGCCCTGGGCCGAGCCCCGTTTCGTGTCTGGGC
GTCTCTGCCTAAGTCCCTCGCCAGTAAGTCCCGAAGGGCATCATCAAATCATCTCTGGCCAGTGGAAAGAGGGGGTCTAAGAGCTGGAGCCCGGCCTTGTCGCGGCC
TCCCAAGCCGCCTCCCAGAACAGGAAGGAAGCTCGAGGAACAAAGGGGGCCCCAACAGAGCCCAGTCTCTCGGTCCCGCGTGTGCAACCGTCAGTGGAAGAGAAGCA
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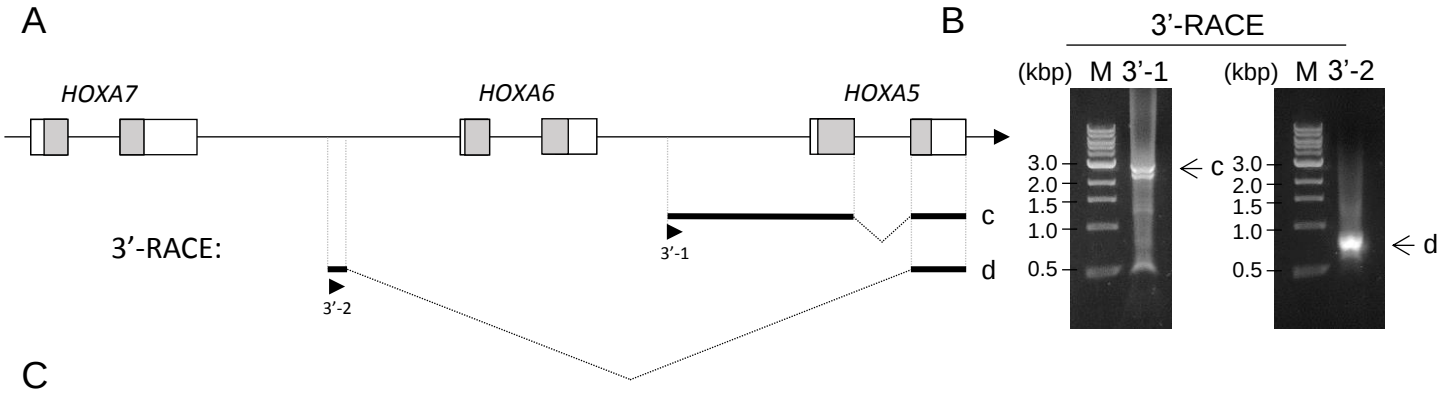
GCCACAGCAGCGTTCAGGACCCAACGAGGGGCCCAGGGCCATGGGAGCCGCCAGAGTCCTGGCTTCCAGACGTGCCAGGGGCGCCTGCAACGCCGGGCCTCCAGCGG
GGAGACTCCACTTGCCTCTCAGCTATATTTGTGATTTACTTGAGTCTTTCGGAGCCGTGGAAAATAACTACAGAGATACTAGGCACTGCCAAGGAATGCTTCCATTA
TCGCCCTCATTTTACTCCTGGAGGTCTAAGCAAGACCCCAGTAACCC

>5'-3 (757 nt) : corresponding to the band "b" (partial sequence of short)

CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGTGGGACCGGCGCCAGCTGCAGCCCGCCTCTTGCAGCCTGGCGCGACTGCTGTACTGGCCCCTC
AGAGATTTCCCAGCAAGCAGGGTAGAGGGGGACGCAAGGGGACCTGGGGGGCGATCACCCAGAGCCTATATGGCCGGGAAATCTCCCCGCCTCGGCTACCCCTGACC
GGAGCTGGACGCCGTTATAGACTCGGGTCCTCCCCCACCCCCACTCCAGCACCATGGAGACTGGTTGCACAGGCCGCCTGGCACGTCATGGCTGCTTCTGTCCAAAC
TGCGCGTCCCAAGGCGTTCTGGGGGCGCACGCTGGTTCAGGGAACACTCTGGCTTTGGGATTCTGCAATCGGAGAGACTACCTGTAGTCCTGAGGCCTGTAGGCCTG
GCTGCTCTGAGGAGGATTGGCTATGGCAAAGAAGGACGGCTTTGGCTCCAGGAGAGGGCTGTGAAGGGCAGAGGCCGCACCTGTGTGCTTTGGGCTCTGGTCTGGGC
TAGCGAGCTGGCTGGACCGAGGTGGCCTTCAAGGTCCCACACCTACATACTCCCAGACCACCCTCCAGGCTGGCAGAGGTGGAGCTGTTCAATGGGCAGCCCAGGGC
AAGGGCAGACAGTGGGGCCCAGCTCGCCGTCCTGCCCCCCTTGGAGAGAAACTGAAAGACAACATAGGCGGCCCGGAAGGCAAAAGGGCCCGGACGGCCTACACGCG
CTACCAGACCCTGGAGCTGGAGAAGGAGTTCCACTTCAACCGTTACCTGACCC

Figure S2. 5'-rapid amplification of cDNA ends (RACE) experiments of the *HOXA6-HOXA5* locus. A. Scheme diagram of the gene-specific primers used for 5'-RACE experiment. **B.** Electrophoretic analysis of PCR amplification products. **C.** Nucleotide sequences of the PCR products. Primers used are underlined. Grey boxes indicate the junctions between different exons. M, DNA ladder marker.



>3′-1 (2,792 nt): corresponding to the band "c" (partial sequence of long 1 and 2)

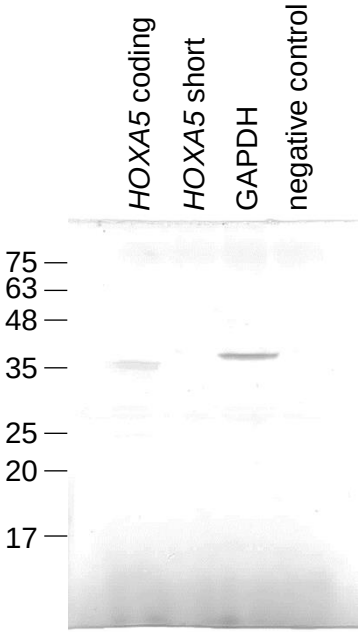
GACTCCACTTGCCTCTCAGCTATATTTGTGATTTACTTGAGTCTTTCGGAGCCGTGGAAAATAACTACAGAGATACTAGGCACTGCCAAGGAATGCTTCCATTATCG
CCCTCATTTTACTCCTGGAGGTCTAAGCAAGACCCCAGTAACCCGCCCCCACGGATCCCCAGACCCCAGGCAGCCTCTGTGGCTGCAGGGAGCCTGGGCCCACTCGCT
GGGTTCAAGGAGAACCCTCCGACTTGGGGCCCGGCTGGCTCGAGGAACCGGACCCTGTGATGAAGATTTTCCAGGCTGGATACGTAAGCAAATCAAATCGCAAACTA
ATGACACGAAAACCATATTCACACGAAAGAAAAATCGACTACGGTTATAAAAGTGTATGGAATTTGACCTCGCCTTGGAGAAACACTACACAAAAGCTATCTTTAAT
AGACGCCTGTCATTTAAGAGCGGCAGGGACACTGTCCCTCATGCGCTCGCAAAAACAGAGCCGTAATTGTAGCTGCTGCTGCTGCGGGCAGGATTTATTTCTCCAAT
TGGCTAAATGGCTTTCCCCCTTCCCCGAAGGTGATATCTGTATTTTCAAAATTCAGAGCTGCTGGCAGGACGGTCAGTACCGACCCCAACCTCGACACAAAAATAAG
AGGGGCTGCAAAACGGGGGAAATAAAGTTGTTGTAAATAAAATGCAAGTCACCACCTCCCCCCAATCCTCTGCATCCTCGCCGGGCGCGCGATCGGCAGCTGACGGC
CTAACAATTGGTACATCCTAATGGAACTGCGAGGGAAATGCAATAATTTTGCCATAATGGGCTGTAACCTCAATTCGACCCCGGCCCTTGCAGCCCCCGGTCGGAAG
CTGGGCGATGAGCCCTGCCTCCAGCGGGTGGCGCTCGAGTCCGGCTGAACGGCGGCAACTGGCGGCGGGCACGCGCCCGGGGCGCGCGCGCCACCCCCCTCGCCTCC
ACCCAACTCCCCTATTAGTGCACGAGTTTACCTCTAGAGGTCATCAGGCAGGATTTACGACTGGACAACAAAAGCACGTGATTCGAAGTCGTACCCCATATTTGGGT
GCCTACGTAGGAGGGAACCAAGTACATGTCCCAGTCATTTCCATAATTCATCATAAATTGTGCAAGGGTGCTATAGACGCACAAACGACCGCGAGCCACAAATCAAG
CACACATATCAAAAAACAAATGAGCTCTTATTTTGTAAACTCATTTTGCGGTCGCTATCCAAATGGCCCGGACTACCAGTTGCATAATTATGGAGATCATAGTTCCG
TGAGCGAGCAATTCAGGGACTCGGCGAGCATGCACTCCGGCAGGTACGGCTACGGCTACAATGGCATGGATCTCAGCGTCGGCCGCTCGGGCTCCGGCCACTTTGGC
TCCGGAGAGCGCGCCCGCAGCTACGCTGCCAGCGCCAGCGCGGCGCCCGCCGAGCCCAGGTACAGCCAGCCGGCCACGTCCACGCACTCTCCTCAGCCCGATCCGCT
GCCCTGCTCCGCCGTGGCCCCCTCGCCCGGCAGCGACAGCCACCACGGCGGGAAAAACTCCCTAAGCAACTCCAGCGGCGCCTCGGCCGACGCCGGCAGCACCCACA
TCAGCAGCAGAGAGGGGGTTGGCACGGCGTCCGGAGCCGAGGAGGACGCCCCTGCCAGCAGCGAGCAGGCGAGTGCGCAGAGCGAGCCGAGCCCGGCGCCGCCCGCC
CAACCCCAGATCTACCCCTGGATGCGCAAGCTGCACATAAGTCATGACAACATAGGCGGCCCGGAAGGCAAAAGGGCCCGGACGGCCTACACGCGCTACCAGACCCT
GGAGCTGGAGAAGGAGTTCCACTTCAACCGTTACCTGACCCGCAGAAGGAGGATTGAAATAGCACATGCTCTTTGCCTCTCCGAGAGACAAATTAAAATCTGGTTCC
AAAACCGGAGAATGAAGTGGAAAAAAGATAATAAGCTGAAAAGCATGAGCATGGCCGCGGCAGGAGGGGCCTTCCGTCCCTGAGTATCTGAGCGTTTAAAGTACTGA
GCAGTATTAGCGGATCCCGCGTAGTGTCAGTACTAAGGTGACTTTCTGAAACTCCCTTGTGTTCCTTCTGTGAAGAAGCCCTGTTCTCGTTGCCCTAATTCATCTTT
TAATCATGAGCCTGTTTATTGCCATTATAGCGCCTGTATAAGTAGATCTGCTTTCTGTTCATCTCTTTGTCCTGAATGGCTTTGTCTTGAAAAAAAATAGATGTTTT
AACTTATTTATATGAAGCAAGCTGTGTTACTTGAAGTAACTATAACAAAAAAAGAAAAGAGAAAAAAAAACACACAAAAAGTCCCCCTTCAATCTCGTTTAGTGCCA
ATGTTGTGTGTTGCACTCAAGTTGTTTAACTGTGCATGTGCGTGGAAGTGTTCCTGTCTCAATAGCTCCAAGCTGTTAAAGATATTTTTATTCAAACTACCTATATT
CCTTGTGTAATTAATGCTGTTGTAGAGGTGACTTGATGAGACACAACTTGTTCGACGTGTAGTGACTAGTGACTCTGTGATGAAAACTGTGACTCCAAGCGGTGTGT
CCCTGCGTGCCTTTATAGGACCCTTTGCACGAACTCTGGAAGTGGCTCTTATAAGCGCAGCTTCAGTGATGTATGTTTTTGTGAACAAAGTTACAAATATTGTCCAA
GTCTGGCTGTTTTAAGCAAACTGTGATCAGCTTTTTTTTTTTTTTTTTTTTTTTTGTATTTGTTTTTAAGGAAAAAATACTGACTGGAACAAAAAATAAACTTTCTA
TTGTAAGTTCCAAAAAAAAAAAAAAAAAAAGTACTCTGCGTTGATACCACTGCTTGCCCTATAGTGAGTCGTATTAG

>3′-2 (1,124 nt): corresponding to the band "d" (partial sequence of short)

GAGGTGGAGCTGTTCAATGGGCAGCCCAGGGCAAGGGCAGACAGTGGGGCCCAGCTCGCCGTCCTGCCCCCCTTGGAGAGAAACTGAAAGACAACATAGGCGGCCCG
GAAGGCAAAAGGGCCCGGACGGCCTACACGCGCTACCAGACCCTGGAGCTGGAGAAGGAGTTCCACTTCAACCGTTACCTGACCCGCAGAAGGAGGATTGAAATAGC
ACATGCTCTTTGCCTCTCCGAGAGACAAATTAAAATCTGGTTCCAAAACCGGAGAATGAAGTGGAAAAAAGATAATAAGCTGAAAAGCATGAGCATGGCCGCGGCAG
GAGGGGCCTTCCGTCCCTGAGTATCTGAGCGTTTAAAGTACTGAGCAGTATTAGCGGATCCCGCGTAGTGTCAGTACTAAGGTGACTTTCTGAAACTCCCTTGTGTT
CCTTCTGTGAAGAAGCCCTGTTCTCGTTGCCCTAATTCATCTTTTAATCATGAGCCTGTTTATTGCCATTATAGCGCCTGTATAAGTAGATCTGCTTTCTGTTCATC
TCTTTGTCCTGAATGGCTTTGTCTTGAAAAAAAATAGATGTTTTAACTTATTTATATGAAGCAAGCTGTGTTACTTGAAGTAACTATAACAAAAAAAGAAAAGAGAA
AAAAAAACACACAAAAAGTCCCCCTTCAATCTCGTTTAGTGCCAATGTTGTGTGTTGCACTCAAGTTGTTTAACTGTGCATGTGCGTGGAAGTGTTCCTGTCTCAAT
AGCTCCAAGCTGTTAAAGATATTTTTATTCAAACTACCTATATTCCTTGTGTAATTAATGCTGTTGTAGAGGTGACTTGATGAGACACAACTTGTTCGACGTGTAGT
GACTAGTGACTCTGTGATGAAAACTGTGACTCCAAGCGGTGTGTCCCTGCGTGCCTTTATAGGACCCTTTGCACGAACTCTGGAAGTGGCTCTTATAAGCGCAGCTT
CAGTGATGTATGTTTTTGTGAACAAAGTTACAAATATTGTCCAAGTCTGGCTGTTTTAAGCAAACTGTGATCAGCTTTTTTTTTTTTTTTTTTTTTTGTATTTGT
TTTTAAGGAAAAAATACTGACTGGAACAAAAAATAAACTTTCTATTGTAAGTTCCAAAAAAAAAAAAAAAAAAAAAGTACTCTGCGTTGATACCACTGCTTGCCC
TATAGTGAGTCGTATTAG

Figure S3. 3'-rapid amplification of cDNA ends (RACE) experiments of the *HOXA6-HOXA5* locus. A. Scheme diagram of the gene-specific primers used for 3'-RACE experiment. **B.** Electrophoretic analysis of PCR amplification products. **C.** Nucleotide sequences of the PCR products. Primers used are underlined. Grey boxes indicate the junctions between different exons. M, DNA ladder marker.

A



B

RNA Name	Coding Probability	Coding Label
HOXA5 short	0.0448	no
HOXA5 coding	0.997	yes
MALAT1	0.0142	no
GAPDH	0.999	yes

Figure S4. Analysis of translation potency of the *HOXA5* short RNA. A. A T7 promoter-containing DNA fragments encoding full-length HOXA5 RNA, *HOXA5* short RNA, or GAPDH were generated by PCR amplification and the resultant PCR products were subjected to *in vitro* transcription and translation assays, which included the incorporation of fluorescent lysine. The synthesized proteins were analyzed by 15% SDS-PAGE and detected using a fluoro-imaging instrument. **B.** The translation potency of *HOXA5* short RNA was calculated using Coding-Potential Assessment Tool (CPAT) software. Sequences of the coding regions of *HOXA5* and *GAPDH* were used as translatable sequences and that of *MALAT1,* known as a functional long non-coding RNA, was used as an untranslatable sequence.

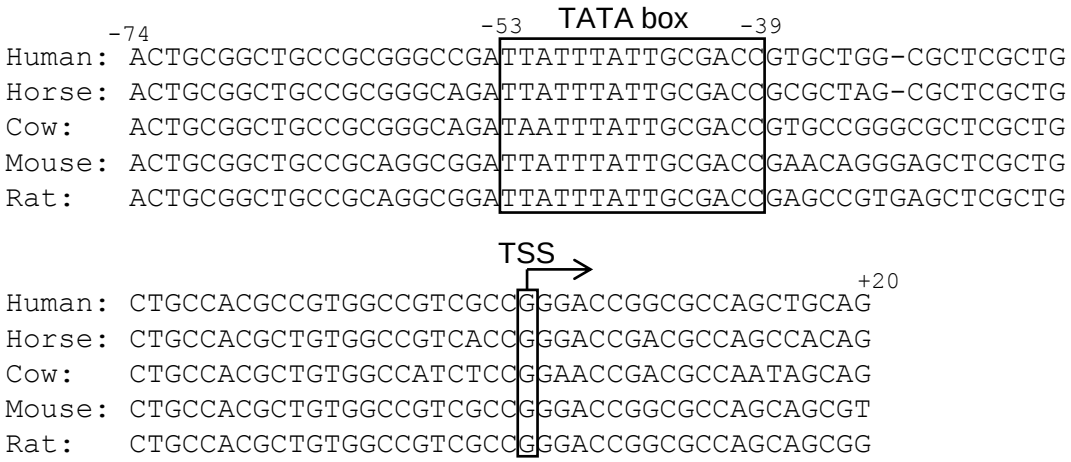


Figure S5. Evolutionary conserved sequences of a transcriptional start site of the *HOXA5 short RNA.* Sequence alignment of the upstream sequences of a transcriptional start site (TSS) in *HOXA5* short RNA indicates the presence of a consensus TATA box and a TSS in most species.

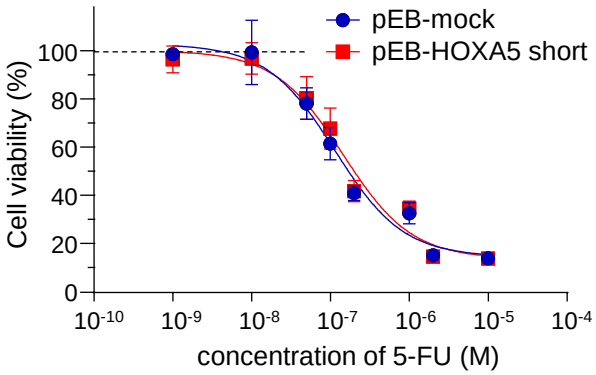


Figure S6. Intrinsic chemoresistance to 5-FU in HOXA5 short RNA expressing HCT116 cells. The cell viability of pEB-HOXA5 short or pEB-mock HCT116 cells was determined by Cell Count Reagent *SF* after treatment with increasing doses of 5-FU for 48 h.

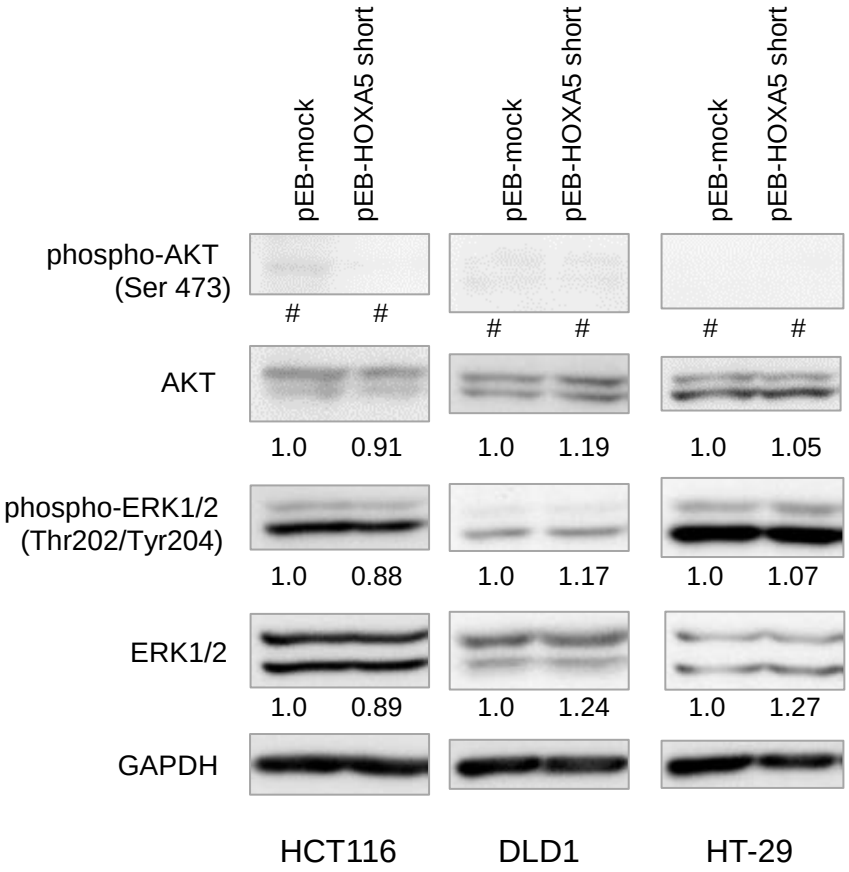


Figure S7. Effects of *HOXA5* short RNA on AKT and ERK activation. Protein levels of phosphorylated AKT (Ser473; #9271, Cell Signaling Tech.), total AKT (#9272, Cell Signaling Tech.), phosphorylated ERK1/2 (#9101, Cell Signaling Tech.) and total ERK1/2 (#9102, Cell Signaling Tech.) were measured by western blot analysis. GAPDH levels were used as an endogenous quantitative control. The level of phospho-AKT, phosphor-ERK1/2, AKT or ERK1/2 band relative to that of GAPDH was quantitatively analyzed by densitometry. #: The band corresponding to phospho-AKT was not sufficiently detected for densitometry analyses.