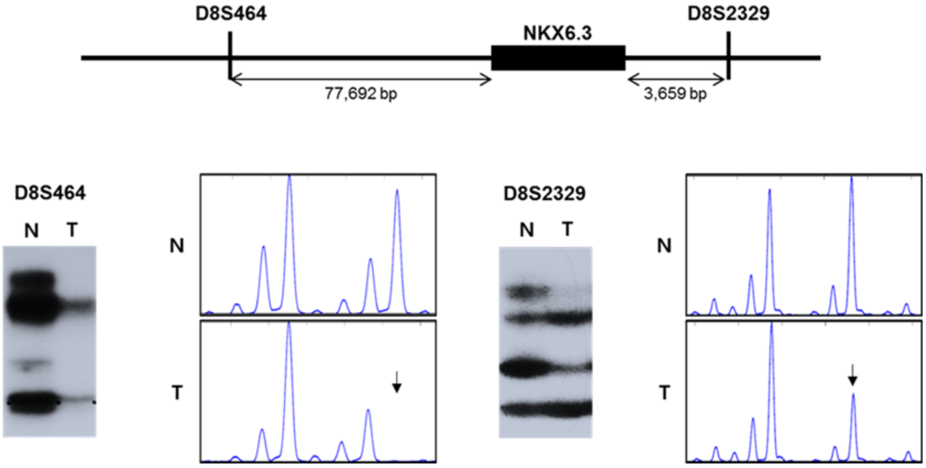
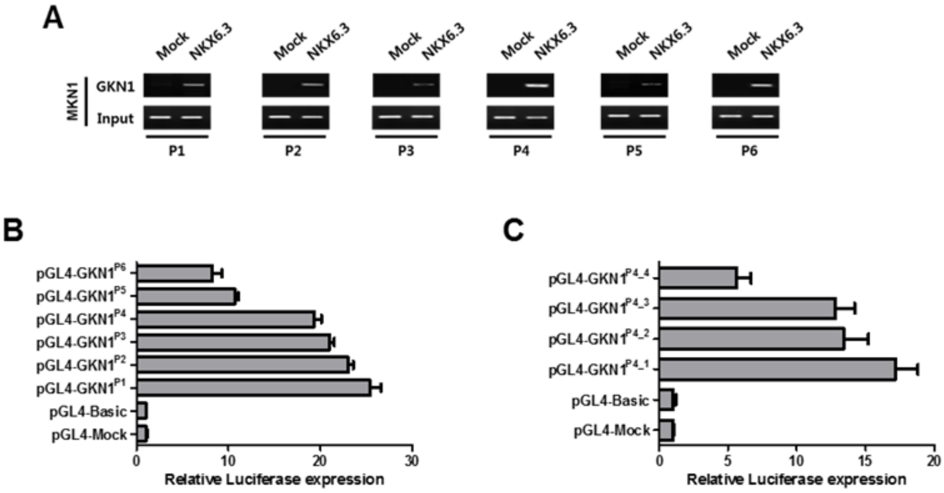
NKX6.3 controls gastric differentiation and tumorigenesis

Supplementary Material



Supplementary Figure 1. Allelic loss of NKX6.3 gene in gastric cancer.

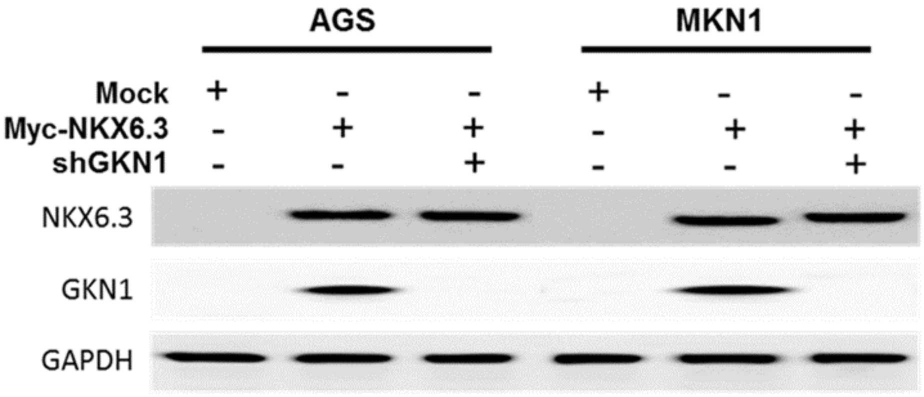
Allelic loss of NKX6.3 gene in gastric cancer with microsatellite markers D8S464 and D8S2329, which are located -77.692 kb and +3.659 kb from the *NKX6.3* locus, respectively. Representative autoradiograph and fluorescent microsatellite analysis showed loss of heterozygosity (LOH) at the *NKX6.3* locus.



Supplementary Figure 2. Binding activity of NKX6.3 to the GKN1 promoter. **A.** Putative promoter activity was characterized between -5 kb and +1 kb relative to transcription start site (TSS) of GKN1 by ChIP and QPCR.

B. Reporter gene luciferase assays showed that NKX6.3 occupancy was 18-fold enriched at the P4 region compared to control. Normalized luciferase activity values for each construct (N=3) are represented as mean ± SD.

C. Activity analysis of 5’-deletion constructs at the P4 promoter region demonstrated a significant decrease in promoter activity. Further deletion of the 5’ binding motifs resulted in a progressive loss of activity, indicating that NKX6.3 binding motifs at P4 construct are required for the GKN1 transcription in MKN1 cells.



Supplementary Figure 3. GKN1 silencing by shRNA in NKX6.3 stable cells.

NKX6.3 induced the GKN1 expression, whereas GKN1 silencing with *shGKN1* completely ablated NKX6.3-induced GKN1 expression.

Supplementary Table 1. The primer sequences used for studies of the *NKX6.3* binding activity on *GKN1* promoter region

Primer name	Sequences
ChIP-PCR	
P1	F: 5’- GTACCTGAGCATTGTTTGTTTTACATCTT -3’
	R: 5’- CGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P2	F: 5’- TACCTCATACACATGATGCAATTACTCA -3’
	R: 5’- CGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P3	F: 5’- TACCATCTGATTGACATCTTTAAATGAG -3’
	R: 5’- CGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P4	F: 5’- TACCGGAAAACGATCAGGTTCTGTAGTC -3’
	R: 5’- CGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P5	F: 5’- TACCAGGAAAGTTCTATCTATCAGATGG -3’
	R: 5’- CGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P6	F: 5’- TACCTGACCCCACCTGATAACACCTAGT -3’
	R: 5’- CGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
Luciferase assay	
P1	F: 5’- CTTCGGGGTACCTGAGCATTGTTTGTTTTACATCTTTAAAT -3’
	R: 5’- GTTGCCCTCGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P2	F: 5’- CTTCGGGGTACCTCATACACATGATGCAATTACTCAGGA -3’
	R: 5’- GTTGCCCTCGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P3	F: 5’- CTTCGGGGTACCATCTGATTGACATCTTTAAATGAGCAG -3’
	R: 5’- GTTGCCCTCGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P4	F: 5’- CTTCGGGGTACCGGAAAACGATCAGGTTCTGTAGTCA -3’
	R: 5’- GTTGCCCTCGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P5	F: 5’- CTTCGGGGTACCAGGAAAGTTCTATCTATCAGATGGGG -3’

	R: 5’- GTTGCCCTCGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P6	F: 5’- CTTCGGGGTACCTGACCCCACCTGATAACACCTAGTT -3’
	R: 5’- GTTGCCCTCGAGGCCGTAACGTTTTCCAGTGTGTAG -3’
P4_1	F: 5’- CTTCGGGGTACCTGACTACTGGTGGCTGAGTTTAAAC -3’
	R: 5’- GTTGCCCTCGAGCCCCATCTGATAGATAGAACTTTCCTA -3’
P4_2	F: 5’- CTTCGGGGTACCCCAGTTTTGAAGAGATGTTGATAGCGAAT -3’
	R: 5’- GTTGCCCTCGAGCCCCATCTGATAGATAGAACTTTCCTA -3’
P4_3	F: 5’- CTTCGGGGTACCCGAAATACAGTTTTCCTCCTTGGCAAA -3’
	R: 5’- GTTGCCCTCGAGCCCCATCTGATAGATAGAACTTTCCTA -3’
P4_4	F: 5’- CTTCGGGGTACCCCTACTCTCTGCTTTTGATGAAAATATTTTT -3’
	R: 5’- GTTGCCCTCGAGCCCCATCTGATAGATAGAACTTTCCTA -3’

Supplementary Table 2. The primer sequences used for studies of the *NKX6.3* gene

Primer name	Sequences
Mutation analysis	
E1-1	F: 5’- CGGTGTGCCAGTACTCTGTG -3’
	R: 5’- CCTGGGGGCTGTAGTAGACC -3’
E1-2	F: 5’- CTCGCAGGGGGTCTACTACA -3’
	R: 5’- GGACCCGTACTCACTGTTGC -3’
E2	F: 5’- AACCCCACACTTCCTTCCTC -3’
	R: 5’- TCTCCCGCACAGACTTACCT -3’
E3-1	F: 5’- TGTCCTTCCCCAACAGAAAC -3’
	R: 5’- CTCTTCTTCCGCCACTTGGT -3’
E3-2	F: 5’- TCTTTCTCATCGCAGGTGTG -3’
	R: 5’- GATCTTCTCGTCGTCCGAGT -3’
E3-3	F: 5’- CGAGGACGACGAGTACAACA -3’
	R: 5’- GAAGGGGAGGGGAAGGTA -3’
Methylation	
NKX6.3 M	F: 5’- ATATTTATTGGGTATGATCGAGTCG -3’
	R: 5’- GTTATATAACCACCTCTCTCCAACG -3’
NKX6.3 U	F: 5’- TATTTATTGGGTATGATTGAGTTGT -3’
	R: 5’- ATTATATAACCACCTCTCTCCACCAC -3’
DNA copy number	
NKX6.3	F: 5’- AACCCCACACTTCCTTCCTC -3’
	R: 5’- TCTCCCGCACAGACTTACCT -3’
GAPDH	F: 5’-ACCCAGAAGACTGTGGATGG-3’
	R: 5’-TTCTAGACGGCAGGTCAGGT-3’
Real time-RT-PCR	
NKX6.3	F: 5’- TCTTTCTGCTTCTGGGGTGT -3’
	R: 5’- GTCCAGCGGCTTGTTGTACT -3’

GAPDH	F: 5’-AAATCAAGTGGGGCGATGCTG-3’ R: 5’-GCAGAGATGATGACCCTTTTG-3’

Supplementary Table 3. The primer sequences used for studies of differentiation related gene expresion

Primer name	Sequences
Muc5ac	F: 5'- GCTCAGCTGTTCTCTGGATGAG -3' R: 5'- TTACTGGAAAGGCCCAAGCA -3'
Muc2	F: 5'- CTTCGACGGACTCTACTACAGC -3' R: 5'- CTTTGGTGTTGTTGCCAAAC -3'
CDX2	F: 5'- GCGGAACCTGTGCGAGTG -3' R: 5'- GACTGTAGTGAAACTCCTTCTCCAGC -3'

Supplementary Table 4. The primer sequences used for predicted NKX6.3 target gene expression

Primer name	Sequences
BAK1	F: 5’- AGAGCTGTCTGAACTCACGT -3’
	R: 5’- TTACACTGTGCCAGAGCCAT -3’
BBC3	F: 5’- GACCTCAACGCACAGTA -3’
	R: 5’- CTAATTGGGCTCCATCT -3’
BCL2	F: 5’- GATTGTGGCCTTCTTTGAG -3’
	R: 5’- CAAACTGAGCAGAGTCTTC -3’
BCL3	F: 5’- GAAAACAACAGCCTTAGCATGGT -3’
	R: 5’- CTGCGGAGTACATTTGCG -3’
BNIP3	F: 5’- TGGACGGAGTAGCTCCAAGAGC -3’
	R: 5’- AGAAGCCCTGTTGGTATCTTGTG -3’
GKN1	F: 5’- CAAAGTCGATGACCTGAGCA -3’
	R: 5’- CTTGCCTCTTGCATCTCCTC -3’
MCL1	F: 5’- AAAGCCTGTCTGCCAAAT -3’
	R: 5’- CCTATAAACCCACCACTC -3’
MDM2	F: 5’- CAGCAGGAATCATCGGACTCA -3’
	R: 5’- CCTTATTACACACAGAGCCAGGC -3’
PDCD4	F: 5’- ATGGATATAGAAAATGAGCAGAC -3’
	R: 5’- CCAGATCTGGACCGCCTATC -3’
SIVA1	F: 5’- TTCAGAACCACACGGCTAC -3’
	R: 5’- TTCCTCTCTTTTTCCTCCC -3’
TP53INP1	F: 5’- CTTCCTCCAACCAAGAACCA -3’
	R: 5’- GATGCCGGTAAACAGGAAAA -3’
CCNB1	F: 5’- GGCCAAAATGCCTATGAAGA -3’
	R: 5’- AGATGTTTCCATTGGGCTTG -3’

CCND3	F: 5’- CTGGCCATGAACTACCTGGA -3’
	R: 5’- CCAGGAAATCATGTGCAATC -3’
CCNE1	F: 5’- CCACACCTGACAAAGAAGATGATGAC -3’
	R: 5’- GAGCCTCTGGATGGTGCAATAAT -3’
CDKN1A	F: 5’- GGCAGACCAGCATGACAGATT -3’
	R: 5’- GCGGATTAGGGCTTCCTCT -3’
CDKN2B	F: 5’- CACCGTTGGCCGTAAACTTAAC -3’
	R: 5’- TAATGAAGCTGAGCCCAGGTCT -3’
CDKN2D	F: 5’- TCACACTGCTGTGGTCAGCTT -3’
	R: 5’- CGTCCCTGCGATGGAGAT -3’
CCKBR	F: 5’- TCACACTGCTGTGGTCAGCTT -3’
	R: 5’- CGTCCCTGCGATGGAGAT -3’
CDH1	F: 5’- GAACAGCACGTACACAGCCCT -3’
	R: 5’- GCAGAACTGTCCCTGTCCCAG -3’
GAST	F: 5’- TCACACTGCTGTGGTCAGCTT -3’
	R: 5’- CGTCCCTGCGATGGAGAT -3’
LIPF	F: 5’- CTCTAGCTCCTGTTGCCACT -3’
	R: 5’- TACACATCCAAGCGACTCGT -3’
SOX2	F: 5’- TTGCTGCCTCTTTAAGACTAGGA -3’
	R: 5’- CTGGGGCTCAAACTTCTCTC -3’
TWSG1	F: 5’- TGAGCAAATGCCTCATTCAG -3’
	R: 5’- GGTTGCACATACCGACACAG -3’