

ZNF350 promoter methylation accelerates colon cancer cell migration

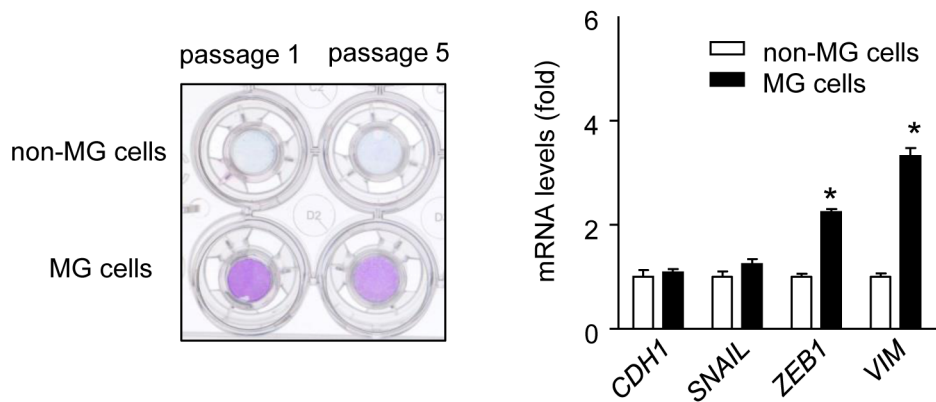
SUPPLEMENTARY MATERIALS

Supplementary Table 1: Primer sets used for qPCR and cloning ZNF350 sequence, and oligonucleotide sequences for siRNA

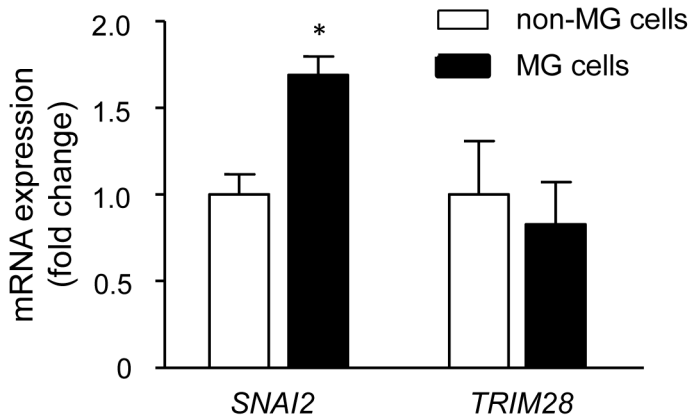
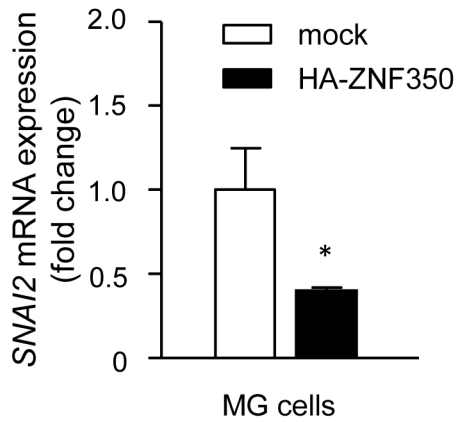
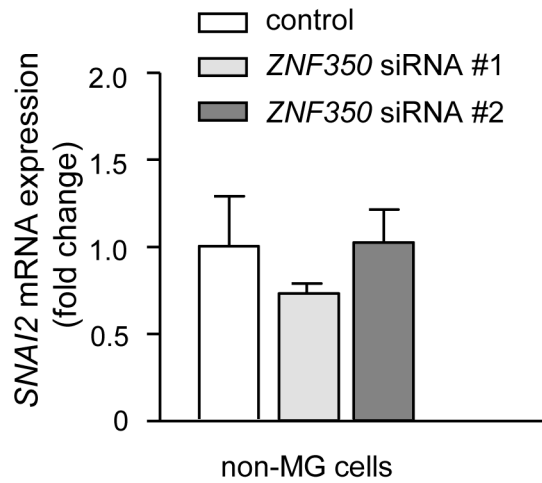
Primers for qPCR		
Targets		primer sequences (5'-3')
<i>CDHI</i>	forward	TGGAGGAATTCTTGCTTTGC
	reverse	CGCTCTCCTCCGAAGAAAC
<i>SNAIL1</i>	forward	GCTGCAGGACTCTAATCCAGA
	reverse	ATCTCCGGAGGTGGGATG
<i>ZEB1</i>	forward	GGAGGATGACACAGGAAAGG
	reverse	TCTGCATCTGACTCGCATTC
<i>VIM</i>	forward	TGTCCAAATCGATGTGGATGTTTC
	reverse	TTGTACCATTCTTCTGCCTCCTG
<i>ZNF350</i>	forward	TCTTGTGTATCTGGAGAAAATAGAGGT
	reverse	AAGAAATGGTGAACCCAAA
<i>GAPDH</i>	forward	AGCCACATCGCTCAGACAC
	reverse	GCCCAATACGACCAAATCC
Primers for <i>ZNF350</i> promoter cloning		
<i>ZNF350</i> (-297)	forward	AAAAACTCGAGTGATAAAGCCTGAGTCTCTGAAAATCTGC
<i>ZNF350</i> (-161)	forward	AAAAACTCGAGTTTCAAACATGGCTGCCGTCAGGAGC
<i>ZNF350</i> (-56)	forward	AAAAACTCGAGTTCTCCTCGCCGCCGTAGGTGGACCATAAAC
<i>ZNF350</i> (-29)	forward	AAAAACTCGAGTAAACCCGTGCGAGGACTCCAGAAG
<i>ZNF350</i> (-13)	forward	AAAAACTCGAGCTCCAGAAGTAGGAGCAGTTTACGGAAG
<i>ZNF350</i> (+49)	reverse	AAAAAAAGCTTTCTCCAGATACACAAGAAGGGCCTC
Primers for <i>ZNF350</i> coding sequence cloning		
<i>ZNF350</i> (ENST00000243644)	forward	AAAAAGGATCCATGATCCAGGCCCAGGAATC
	reverse	AAAAAGCGGCCGCCTATGGGTTTTCTGTAACATA
Sequence of siRNAs		
Name		Sequence (5'-3')
<i>ZNF350</i> siRNA #1		GAAAUCAGGUCUCAUAAA
<i>ZNF350</i> siRNA #2		ACAGGAACGUAGUCCUUGU

Supplementary Table 2: Primers used for pyrosequencing experiment

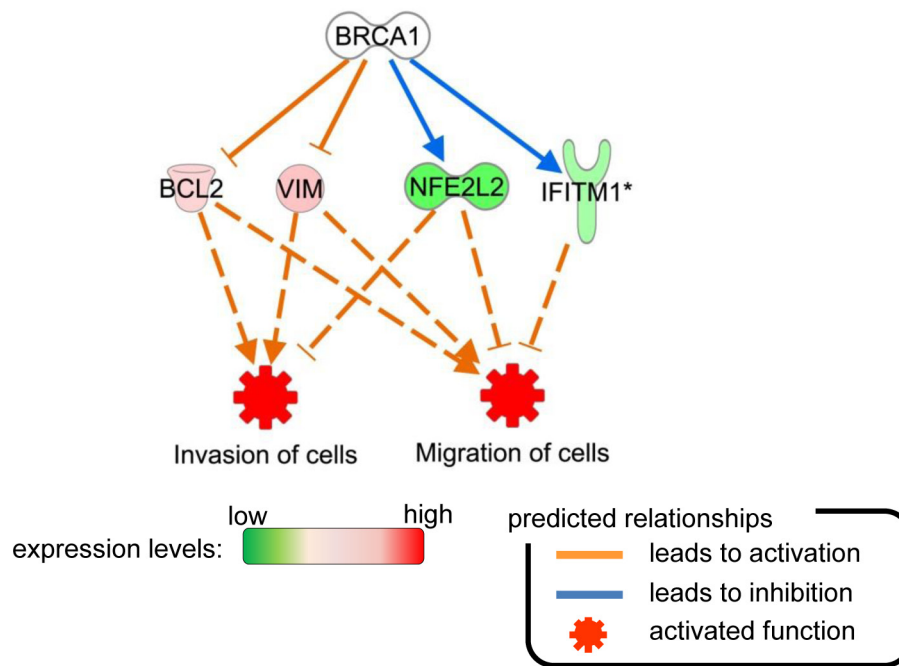
Region	Forward primer (5'-3')	Reverse primer (5'-3')	Sequencing primer (5'-3')
1	GGAGTTAGGGAAG AAGAGAAGTT	Biotin-AACAATTTAACTT ACCCCATATTTACC	GGAAGAAGAGAAGTTATTG
2	Biotin-ATTTAAAATGTTTA AAAGAGTAAGGATAAG	TAACCTCTCTTCT TCCCTAACTCC	CTATACCTCCAATTTTCAAACATAA
3	GGTTTTTGGTTTAA AAATTTGTTATTGT	Biotin-AAACCACACACTA ACCTCTATTT	TTGTTTTTTTAAATATTTTAGGTTT
4	AGGATTTTAGAAG TAGGAGTAGT	Biotin-ACCACACAC TAACCTCTATT	ATTTTAGAAGTAGGAGTAGTTT



Supplementary Figure 1: Visualization of HCT116 cell migration for isolate MG and non-MG cells after one- or five passages under standard cell culture conditions. The migrating cells were visualized by Diff-Quick staining (left panel). Expression of *CDH1*, *SNAIL1*, *ZEB1*, and *VIM* mRNA in the both types of cells were assayed by qPCR using *GAPDH* mRNA as an endogenous quantitative control. Data are expressed as the mean relative changes \pm SD (n = 4) compared to those in control non-MG cells. * $P < 0.05$, unpaired Student's *t*-test.

A**B****C**

Supplementary Figure 2: (A) *SNAI2* and *TRIM28* mRNA levels in the MG and non-MG cells were assayed by qPCR. mRNA expression in the MG cells was calculated with the comparative $\Delta\Delta C_t$ method using *GAPDH* mRNA as an endogenous quantitative control and are expressed as relative changes compared with their expression in the control non-MG cells. Data are presented as the means \pm SD (n = 4). * $P < 0.05$, unpaired Student's *t*-test. (B and C) *SNAI2* mRNA levels in the MG cells transfected with mock or *ZNF350* vector and in the non-MG cells transfected with siRNAs targeting *ZNF350* were assayed by qPCR. mRNA expression in the MG cells was calculated with the comparative $\Delta\Delta C_t$ method using mock or control siRNA as an endogenous quantitative control and are expressed as the relative changes compared with expression in the control MG cells and non-MC cells. Data are presented as the means \pm SD (n = 4). * $P < 0.05$, unpaired Student's *t*-test.



Supplementary Figure 3: Ingenuity pathway analysis (IPA) of differentially expressed *BRCA1*-related genes in the MG cells, focusing on the functions of migration and invasion of cells. Up-regulated and down-regulated genes in the MG cells are shown in red and green, respectively.