Supplementary Information

Overexpression of miR-194 Reverses HMGA2-driven Signatures in Colorectal Cancer

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Table S1. List of miRNA targeting genes co-occurred with HMGA2 in CRC patient (NES > 1.85)

miRNA	size	ES	NES	NOM p-val	FDR q-val	Onco miR	TS miR	targeting HMGA2
miR-101	227	0.5300	2.0500	0.0000	0.0760		✓	
miR-194	93	0.5700	2.0300	0.0020	0.0500	✓	✓	
miR-499	62	0.5800	2.0300	0.0020	0.0360		✓	
miR-202	89	0.5600	1.9700	0.0020	0.0440		✓	
miR-196a / miR-196b	123	0.5000	1.9700	0.0000	0.0370	✓		✓
miR-495	210	0.4800	1.9600	0.0020	0.0340	✓	✓	
miR-518a-2	178	0.4900	1.9600	0.0030	0.0290		✓	
miR-217	96	0.5600	1.9400	0.0000	0.0300			
miR-141 / miR-200a	268	0.4600	1.9300	0.0000	0.0300	✓	✓	
miR-139	110	0.5200	1.9200	0.0030	0.0300		✓	
miR-29a / miR-29b / miR-29c	441	0.4500	1.9100	0.0000	0.0280		✓	
miR-182	32	0.6300	1.9100	0.0040	0.0260	✓		
miR-524	382	0.4500	1.8800	0.0020	0.0310		✓	
miR-490	52	0.5500	1.8800	0.0020	0.0300		✓	✓
miR-203	242	0.4500	1.8800	0.0020	0.0290	✓	✓	✓
miR-9	203	0.4800	1.8700	0.0000	0.0280	✓	✓	✓
miR-369-3p	172	0.5200	1.8700	0.0020	0.0270		✓	
miR-188	69	0.5200	1.8600	0.0020	0.0280		✓	
miR-186	230	0.4600	1.8600	0.0030	0.0270		✓	✓
miR-200a	42	0.5600	1.8600	0.0070	0.0270		✓	

Table S2. Primers used for detecting gene expression levels in this study

Name	Direction	Sequence (5'-3')
hsa-miR-194-5p	Forward	UGUAACAGCAACUCCAUGUGGA
	Reverse	mRQ 3' Primer*
HMGA2	Forward	CCCAAAGGCAGCAAAAACAA
	Reverse	GCCTCTTGGCCGTTTTTCTC
VAPA	Forward	AGGCTCAGAAAGGTAGCACA
	Reverse	GTGAAGGAAGAGGACTGGTGA
GAPDH	Forward	TGCACCACCAACTGCTTAGC
	Reverse	GGCATGGACTGTGGTCATGAG
RNU44	Forward	CCTGGATGATGATAAGCAAAT G
	Reverse	GAGCTAATTAAGACCTTCATGTTCA
miR-194-1 promoter	Forward	TGGCTGATGTCATAGTTGCTCA
	Reverse	GTGGGTGAAACCACTTACCAGT
miR-194-2 promoter	Forward	TCTTGCAAAGCGTGTTCTCG
	Reverse	TATGCACCACTGGCAAAGGG
LIN28B	Forward	TGATAAACCGAGAGGGAAGC
	Reverse	TGTGAATTCCACTGGTTCTCC
ERGIC2	Forward	CTGAGGAGCACATGCCATTC
	Reverse	CCAAGTCTGAAACGACAGCAA
ZBTB39	Forward	AAGAATGTTGCCAGTGACGC
	Reverse	AATGGACGTGAAGGGAGCAG
MIB1	Forward	ATGAGTAACTCCCGGAATAACCG
	Reverse	GCCGTTGTCCCACACTACC
EPC2	Forward	CAGCGAGCAATTTCAGCACA
	Reverse	TGCCTCAGGAACAGGAATGAC
FBXW7	Forward	CGACGCCGAATTACATCTGTC
	Reverse	CGTTGAAACTGGGGTTCTATCA
SUMO2	Forward	GACGGGCAACCAATCAATGAA
	Reverse	GTTCTGGAGTAAAGAAGCAGGT
EHBP1	Forward	ACCTCTACTTTACCACCACCAC
	Reverse	CTCCAATACCAATCACTCACAAGG
SEPHS1	Forward	ACTTGTGTCATTCCTTTGAGGC
	Reverse	CCCATCATGTAAGGGTCGTCTA
CHD6	Forward	GCTTCACAAGCACACGGCTA
	Reverse	GCAGGCTCCAGAAAGAACCT
STX16	Forward	TTGGCTCTTCCATTGCCACT
	Reverse	CACGCTCACACAAACGAGG
DEPDC1B	Forward	AGATTTGCTGCCTTCTCCTACC
	Reverse	GTCCACTTCATCCTTGGAACAC
ARHGAP5	Forward	TACATTCTGGCAGTCGGTGT
	Reverse	TCATCCACGGGTTTACTTGG
RSBN1	Forward	CACACTCAGGTCAACAGGACA
	Reverse	ACGAGACTTATCTGCCGCAT
QKI	Forward	GTCGAAATAGGTGTTCACAGGTC
	Reverse	TGCAGTTGGCTTGAGAGGAT
CHD4	Forward	GTAAGAGACCTGCGAGGCAA
	Reverse	GTAAGGACATGCTGGCGAGA
NCL	Forward	AAGCGTTGGAACTCACTGGT
	Reverse	AAGTGTTCTCGCATCTCGCT
CEP350	Forward	TTGGGTCTCGGGAAATGCTC
	Reverse	CTACAGCTCAACATCACAAGCC
KLF12	Forward	GCCACTTGTCATGGTTTAGCC
	Reverse	TCACACTCTGTTGAGGAGGGA
PAN3	Forward	CTTTGGTGCCCTCAACATCTC
	Reverse	ATCCCATCGGAACTAGCCTCT
YTHDF1	Forward	TCAACCGCAGTATCAGAGCC
	Reverse	AGGAGAGTTGCTATCGCTGC
AP1G1	Forward	GCGCCTACAAGCAAACCATC
	Reverse	AGCCCATCCAACAAGAAGGG

*mRQ 3' Primer were supplied with Mir-X™ miRNA First-Strand Synthesis and SYBR qRT-PCR kit (Takara Bio Inc., Japan)

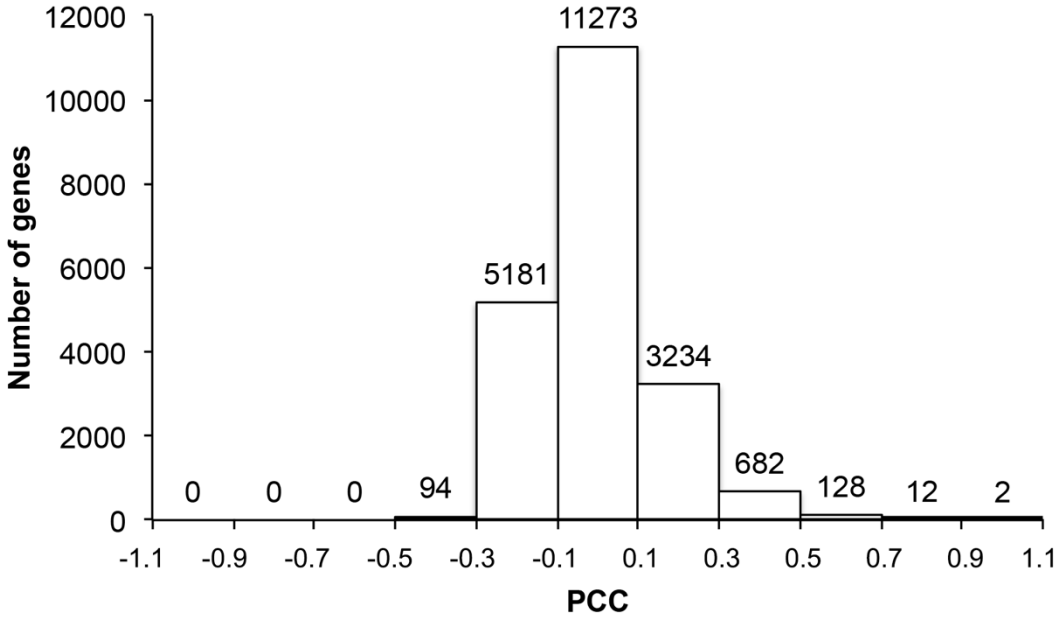

Figure S1. Distribution of global gene expression correlations to HMGA2 in CRC patients. To perform the correlation analysis between 20,606 genes and HMGA2 expressed in CRC patients, Pearson's correlation coefficient (PCC) was calculated and the frequency is shown.

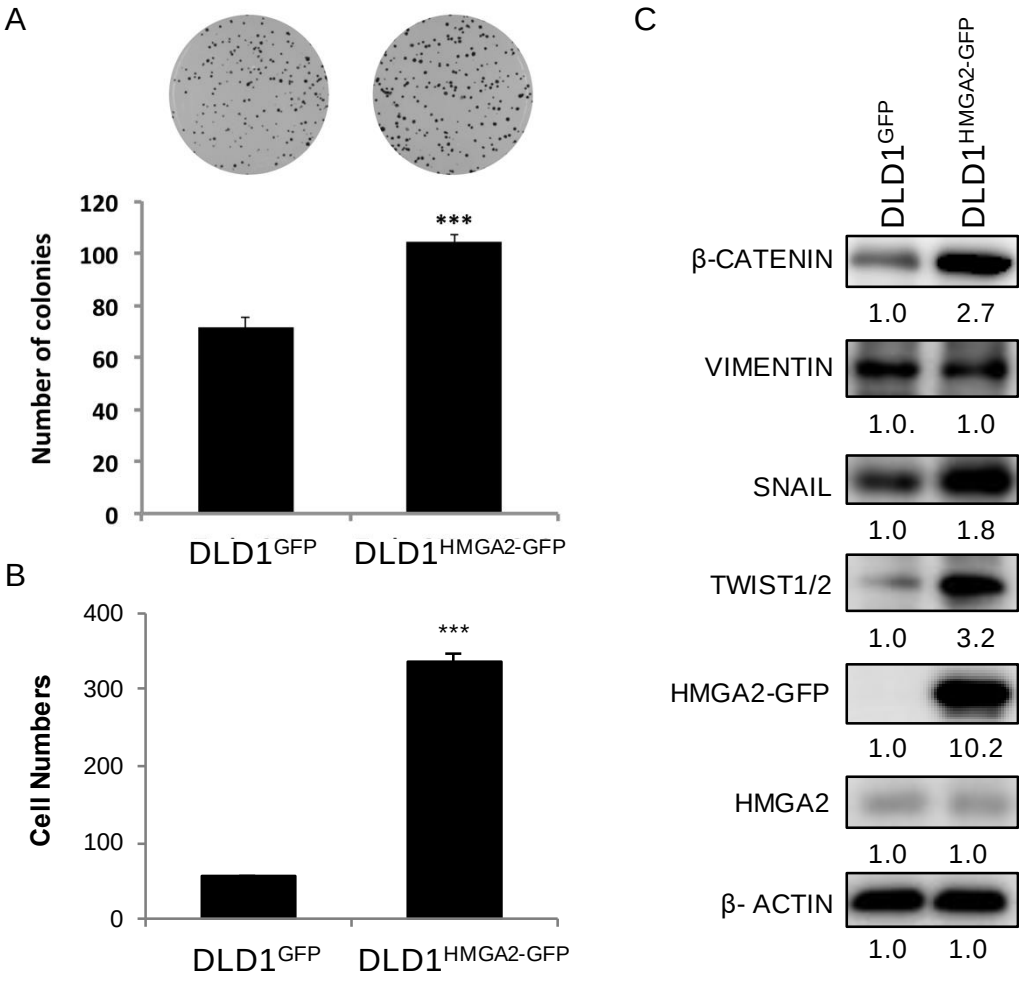

Figure S2. HMGA2 overexpression enhanced the tumorigenesis ability of DLD1 cells. To examine the effects of HMGA2 on cell grow, we chose DLD1, a cell line express low levels of HMGA2, to perform the colony formation assay. (A) DLD1 cells overexpressing TurboGFP-HMGA2 or TurboGFP were seeded onto 6-well plates for 10 days until the colonies formed, and the number of colonies was counted. *** $P < 0.001$. (B) Cell migration was measured using transwell migration assay. Migrated cells were counted. Data was obtained from three independent experiments. *** $P < 0.001$. (C) Protein level of EMT markers. Quantification results were normalized to the level of β-ACTIN and compared to $DLD1^{GPF}$ control cells.

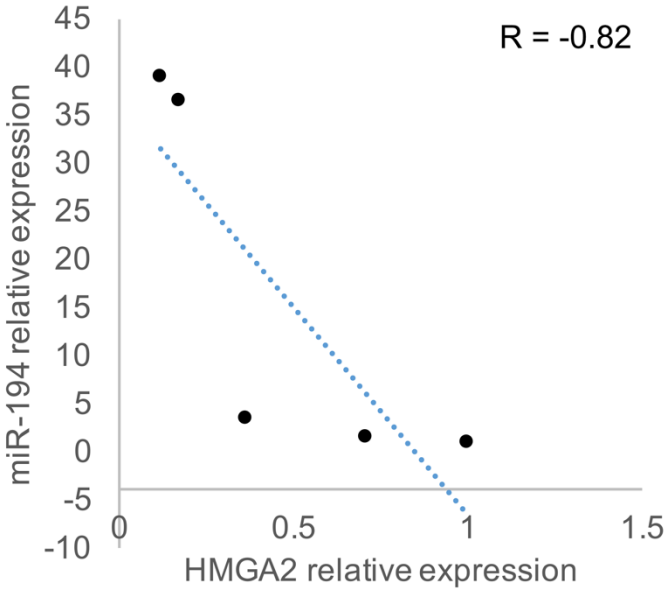

Figure S3. Relative expression of miR-194 and HMGA2 in CRC cells. RNA was extracted by Trizol and reverse-transcribed into cDNA. The relative level of HMGA2 mRNA and miR-194 were determined by real-time PCR and normalized to GAPDH and RUN44, respectively. Data is presented by the difference between the internal controls (-ΔCt).

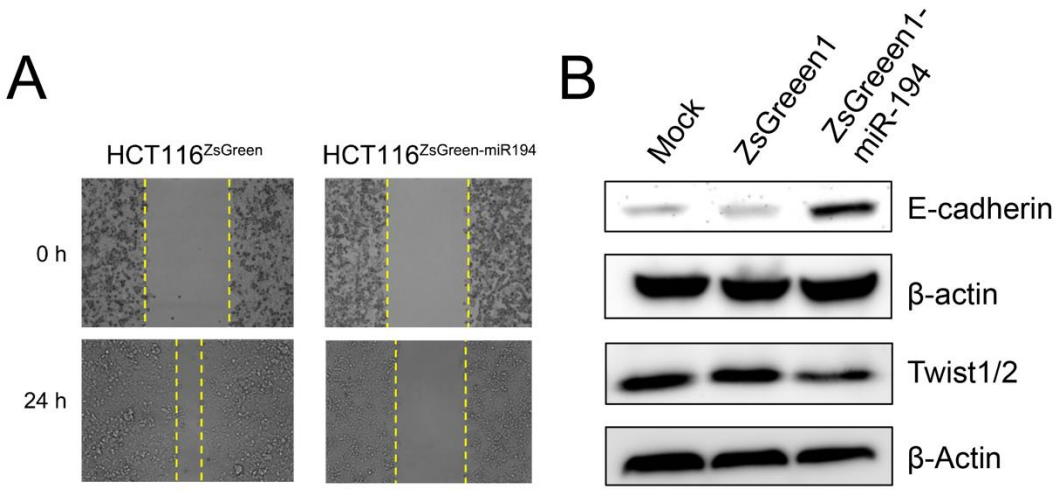

Figure S4. Overexpression of miR-194 reduced cell migration and EMT process. (A) Wound-healing assay was performed to evaluate the cell migratory activity. were compared. Wound gap migration of stable clones harboring ZsGreen1 or ZsGreen1-miR-194 was imaged at time 0-hour (upper panel) and after 24 hours (lower panel). The yellow dashed lines mark the migratory front edges. (B) Protein levels of two EMT markers, E-cadherin and Twist1/2 were measured by western blot and β-Actin was used as loading control.

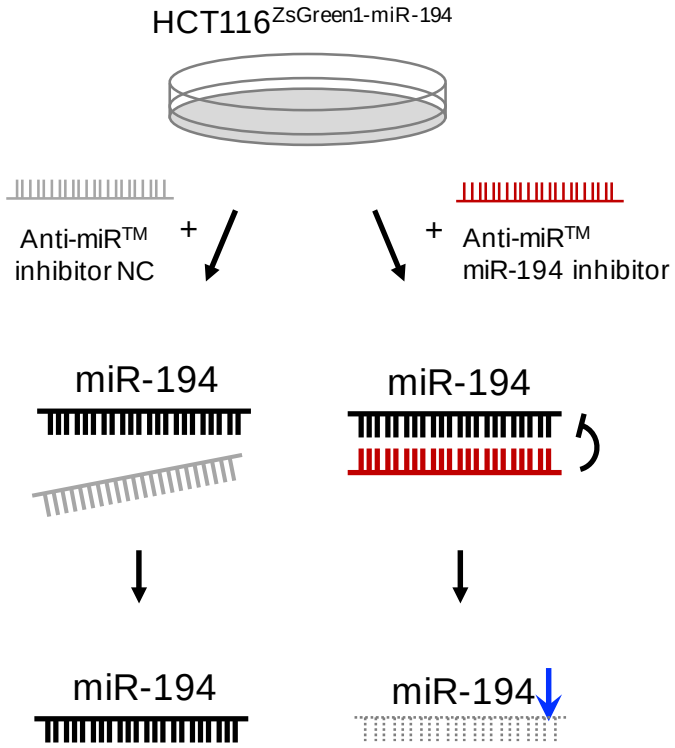

Figure S5. Knockdown of miR-194. To deplete miR-194 expression, we applied miRNA inhibitor, Anti-miR™ miR-194 inhibitor and the Anti-miR™ inhibitor negative control (NC). miR-194 inhibitor complementary binds to endogenous miR-194 and inhibits miR molecules.

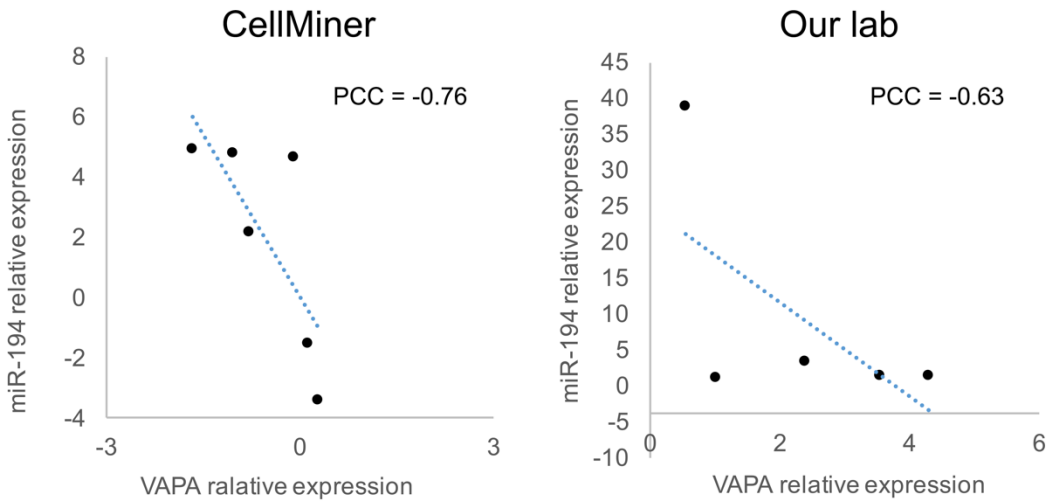

Figure S6. Correlation of miR-194 and VAPA. Correlation between expression of HMGA2 and miR-194 in CRC cell lines in (A) CellMiner database and (B) our lab (data was adopted from Figure 5A and 5B).

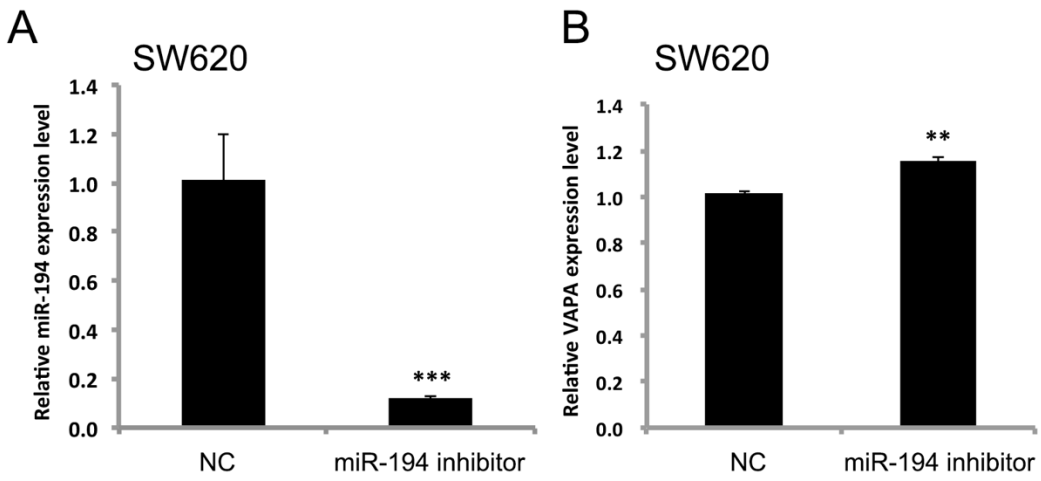

Figure S7. VAPA was regulated by miR-194 in SW620. Expression levels of (A) miR-194 and (B) VAPA in miR-194 inhibitor transfected SW620 cells were measured and compared with those of scramble negative control (NC) transfected ones. RUN44 and GAPDH were used as internal control for normalizing miR-194 and VAPA, respectively.

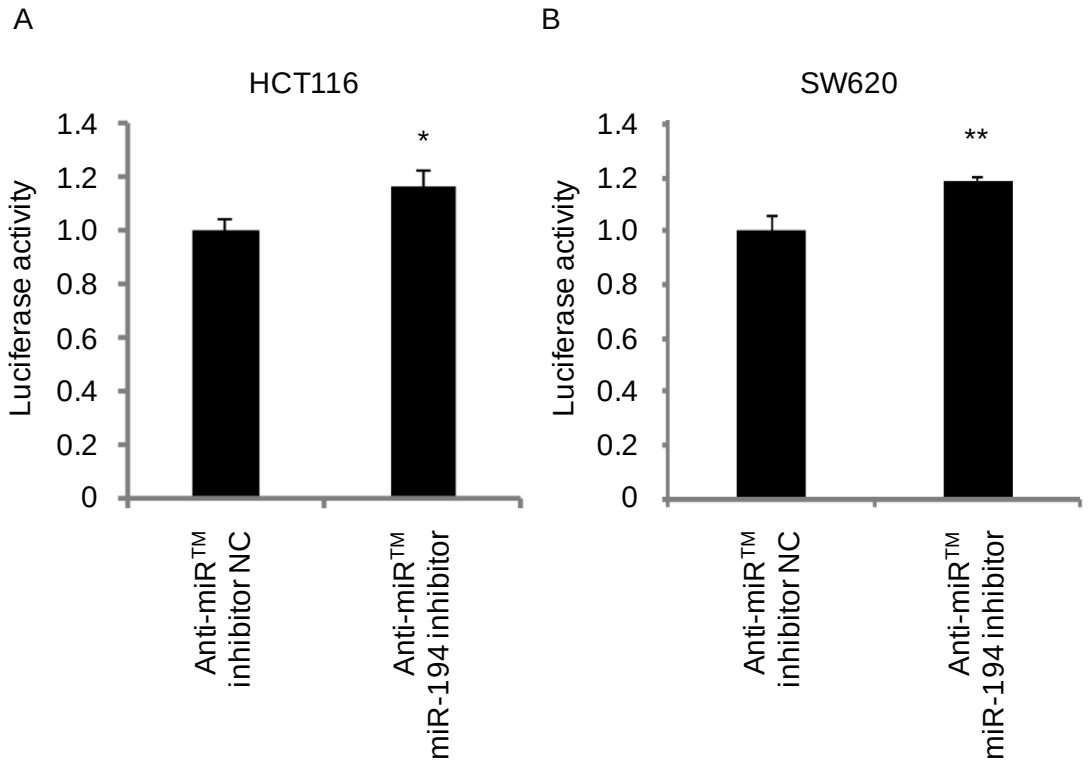

Figure S8. 3'UTR luciferase assay. pMIR-Luc-VAPA 3'UTR and internal control β-galactosidase reporter were transfected with anti-miR miR-194 inhibitor or scramble negative control (NC) into (A) HCT116 or (B) SW620 cells. Dual-light reporter assay was performed and compared with cells transfected with anti-miR inhibitor negative control (NC). ** $P < 0.005$.

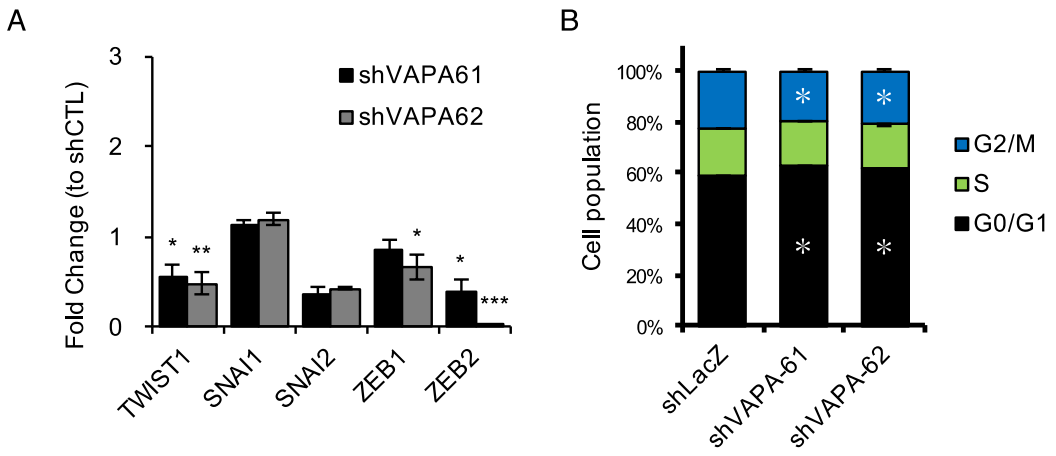

Figure S9. Knockdown of VAPA reduced EMT markets and increased cell population in G_0/G_1 phase. Knockdown of VAPA in HCT116 cells was performed to inspect the function of VAPA. (A) mRNA level of selected EMT markers were measured by qPCR, normalized to GAPDH, and compared with that in cells transfected with shCTL. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (B) DNA content was analyzed with flow cytometry. Cells were fixed with 70% ethanol, RNA was digested, DNA was stained with propidium iodide (PI). * $P < 0.05$.