Supplementary Information

ONECUT2* upregulation is associated with CpG hypomethylation at promoter-proximal DNA in gastric cancer and triggers *ACSL5

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Supplementary Materials and Methods

Statistical analysis

A paired *t*-test was used to examine differences in mRNA level and methylation between paired gastric tumor and adjacent non-tumor tissues. Values are expressed as the mean ± standard deviation (SD). All data are representative of at least three separate experiments, and the results are expressed as the group mean ± SD. Correlations between *OC2* expression and CpG methylation or between the levels of two different genes were determined using Pearson's correlation coefficient. All statistical analyses were performed using the R package.

Supplementary Tables

Table S1. Origin and characteristics of gastric cancer cell lines

	SNU1	SNU5	SNU16	SNU216	SNU484	SNU520	SNU601	SNU620	SNU638	SNU668	SNU719	MKN1	MKN45	MKN74	AGS	KATOIII
Origin	stomach	stomach; ascites	stomach; ascites	stomach; lymph node	stomach	stomach	stomach; ascites	stomach; ascites	stomach; ascites	stomach; ascites	stomach	stomach; lymph node metastasis	stomach; liver metastasis	stomach; liver metastasis	stomach	stomach; pleural effusion
Species (age)	human	female (33) Mongoloid	female (33) Mongoloid	female (46) Mongoloid	male (53) Mongoloid	female (60) Mongoloid	male (34) Mongoloid	female (59) Mongoloid	male (48) Mongoloid	male (63) Mongoloid	male (53) Mongoloid	male (72) Mongoloid	female (62) Mongoloid	male (37) Mongoloid	female (54) Caucasian	male (55) Mongoloid
Growth pattern	suspension	suspension	suspension	monolayer	monolayer	suspension	monolayer	suspension	monolayer	monolayer	monolayer	monolayer		monolayer	monolayer	adherent and suspension
Histo-pathology	adenocarcinoma	adenocarcinoma	adenocarcinoma	adenocarcinoma	adenocarcinoma, primary	adenocarcinoma, primary	signet ring cell carcinoma	adenocarcinoma, metastatic	adenocarcinoma, metastatic	signet ring cell carcinoma	adenocarcinoma, primary	carcinoma, adenosquamous	adenocarcinoma	adenocarcinoma, tubular	adenocarcinoma	signet ring cell carcinoma
Differentiation	poorly differentiated	poorly differentiated	poorly differentiated	moderately differentiated	poorly differentiated	poorly differentiated	poorly differentiated	poorly differentiated	poorly differentiated	poorly differentiated	moderately differentiated		poorly differentiated	well-differentiated	well-differentiated to poorly differentiated	poorly differentiated
Lauren's classification				intestinal		diffuse	diffuse	diffuse		diffuse	intestinal	-	diffuse	intestinal	mixed	diffuse
Reference*	1, 2	1, 2	1, 2	1, 3	1, 3	1, 3	1, 3,	1, 3	1, 3	1, 3	1, 3	1, 4	1, 4	1,4	1, 5	1, 4

*1. Korean Cell Line Bank; 2. Park et al. (1990); 3. Park et al. (1997); 4. Yokozaki (2000); 5. Barranco et al. (1983)

Table S2. Primer sequences used in this study

Primer	Sequence(s)	T_m (℃)	Cycles	Product (bp)
(a) For RT-PCR and qRT-PCR				
OC2	F: 5′-AAATCTGGCAGGGAGACCTT-3′	64	39	151
	R: 5′-CTGCTGGGAAATGGTGATCT-3′			
β-actin	F: 5′-CAAGAGATGGCCACGGCTGCT-3′	68	25	283
	R: 5′-TCCTTCTGCATCCTGTCGGCA-3′			
(b) For bisulfite sequencing of OC2				
Region 1	F: 5′-GTAGTTTGGGGTGTATATTTGTAT-3′	62	35	159
	R: 5′-CAACCTTCATTCAATCCATCAAAA-3′			
Region 2	F: 5′-GGGTATTTTTTTGTAGGGATTGTAGAG-3′	62	35	477
	R: 5′-AAACTACTAACCAACCCCAACCC-3′			
(c) For pyrosequencing				
OC2-pyro-F	5′-GAGGGTGGTGGAATTTGTTAGA-3′	59	40	199
OC2-pyro-R	5′-ACCATAATCACCAACATAACCTC-3′			
OC2-pyro-S	5′-TTGTTTATGTTTATGTTAGG-3′			
(d) For cloning of OC2 gene				
XbaI-OC2-F	5′-GTATCTAGAGCCACCATGAAGGCTGCCTACACCGC-3′			
NotI-OC2-R	5′-GTCGCGGCCGCTCATGCTTTGGTACACGTGCTG-3′			
(e) For ChIP-PCR				
Region 1	5′-CCTCCACAGCCACTGGTAG-3′	60	35	184
	5′-GACGTCTTCTGTTTGGGTGAG-3′			
Region 2	5′-CAGCATGGCCTCGATCCT-3′	60	35	187
	5′-AGGGTGGTGGAACTTGTCAG-3′			
Region 3	5′-GCCAGCTGGAAGAAATCAAC-3′	60	35	177
	5′-TCTCCCTGCCAGATTTGAGT-3′			

Table S3. *OC2* peaks significantly increased over 4-fold at the promoter region in *OC2*-transfected AGS cells compared with the control (MS Excel file)

Table S4. *OC2* peaks significantly increased over 4-fold at the promoter region of *OC2*-transfected MKN74 cells compared with the control (MS Excel file)

Table S5. Genes significantly upregulated in *OC2*-transfected AGS cells compared with the control (MS Excel file)

Table S6. Genes significantly upregulated in *OC2*-transfected MKN74 cells compared with the control (MS Excel file)

Supplementary Figures

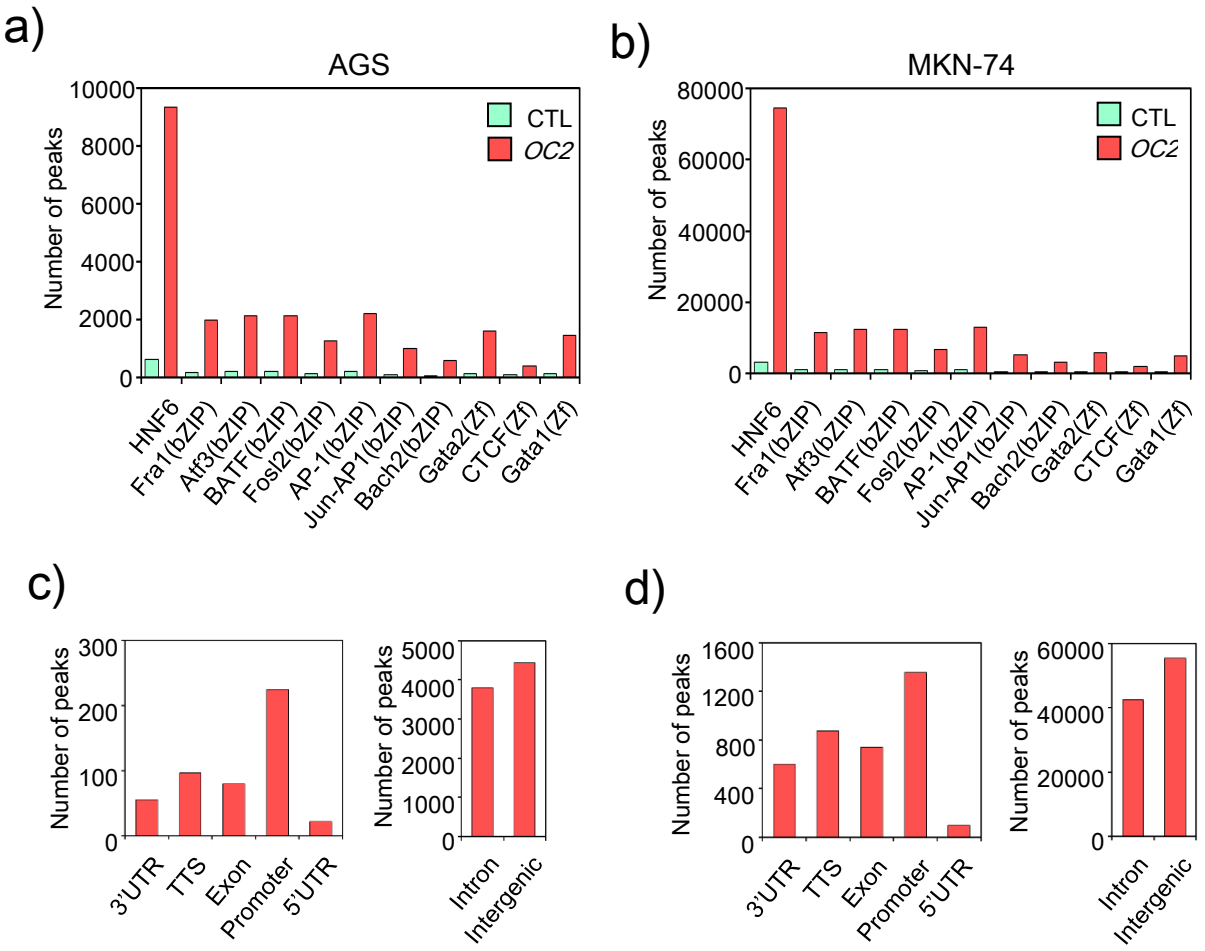

Figure S1. OC2-binding motif as determined with ChIP-seq analysis. (*a*) Top OC2-binding motifs in *OC2*-AGS cells. The consensus motif with the highest enrichment of an individual *OC2*-binding site was *HNF6* with 9,336 peaks of total OC2 peaks in *OC2*-AGS cells (p = 1e–7601) and 652 peaks in control cells (p = 1e–50). (*b*) Top OC2-binding motifs in *OC2*-MKN-74 cells. The consensus motif with the highest enrichment of an individual OC2-binding site was also *HNF6* with 74,543 peaks of total OC2 peaks in *OC2*-MKN-74 cells (p = 1e–54622) and 3,023 peaks in control cells (p = 1e–1678). (*c*, *d*) Distribution of OC2-binding motifs based on genomic features in *OC2*-AGS (*c*) and *OC2*-MKN74 (*d*) cells significantly increased following transfection of cells with *OC2*: 224 peaks in *OC2*-AGC cells (Supplementary Table S3) and 1353 peaks in *OC2*-MKN-74 cells (Supplementary Table S4) were significantly increased by ≥4-fold compared with the control-transfected cells, respectively (p < 0.0001).

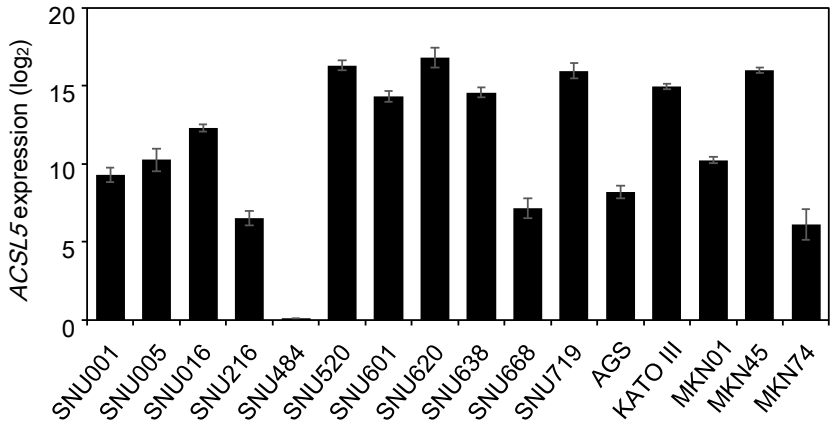

Figure S2. qRT-PCR analysis for *ACSL5* expression in GC cell lines. This analysis was performed to select *ACSL5*-expressed GC cell for KD experiment by siRNA. Actually, MKN01 was selected and treated with siRNAs in this study.

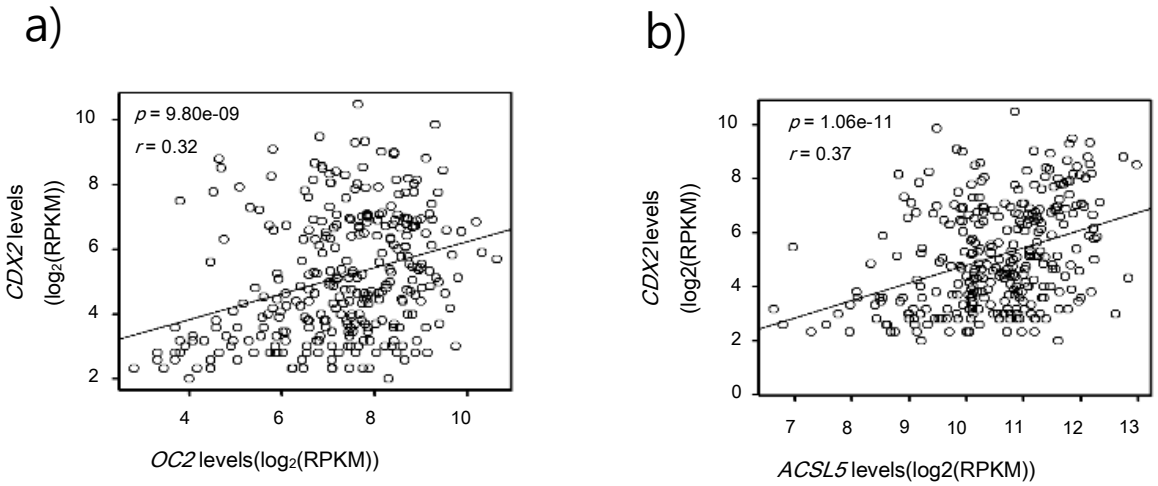

Figure S3. Positive correlation between *CDX2* and *OC2* (*a*) or *ACSL5* (*b*) based on data from 311 GC tissues in the GENT database. This data suggests that three IM markers, such as CDX2, OC2, and ACSL5, may be associated with intestinal differentiation or development of IGC.

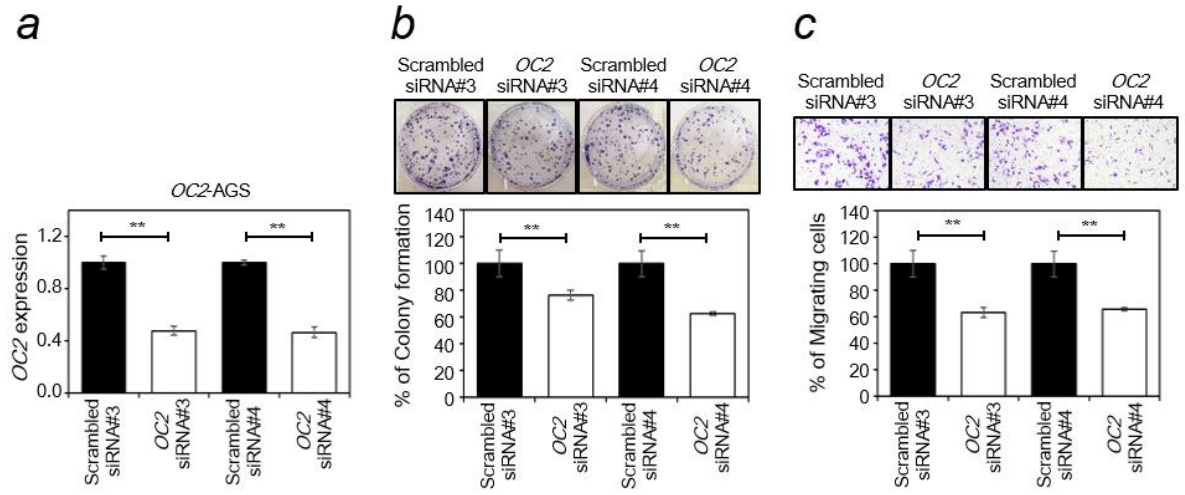

Figure S4. The loss of function of *OC2* in ectopic *OC2* expression cells (*OC2*-AGS). (*a*) Knockdown of *OC2* by siRNA. *OC2*-AGS cells were transfected with two different *OC2*-specific or scrambled siRNAs, and *OC2* mRNA level was examined by qRT-PCR. (*b*, *c*) Colony formation and migration assays for *OC2*-KD OC2-AGS cells. All assays were performed as described in Fig. 6*h* and 6*i*. Potential of colony forming and migration in siRNA KD cells were compared with cells transfected with scrambled siRNAs.

Supplementary References

1. Korean Cell Line Bank (http://cellbank.snu.ac.kr/main/index.html)
2. Park JG, Frucht H, LaRocca RV, Bliss DP Jr, Kurita Y, Chen TR, Henslee JG, Trepel JB, Jensen RT, Johnson BE, Bang YJ, Kim JP, Gazdar AF. Characteristics of cell lines established from human gastric carcinoma. Cancer Res. 1990;50:2773-80.
3. Park JG, Yang HK, Kim WH, Chung JK, Kang MS, Lee JH, Oh JH, Park HS, Yeo KS, Kang SH, Song SY, Kang YK, Bang YJ, Kim YH, Kim JP. Establishment and characterization of human gastric carcinoma cell lines. Int J Cancer. 1997;70(4):443-9.
4. Yokozaki H. Molecular characteristics of eight gastric cancer cell lines established in Japan. Pathol Int. 2000;50:767-77.
5. Barranco SC, Townsend CM Jr, Casartelli C, Macik BG, Burger NL, Boerwinkle WR, Gourley WK. Establishment and characterization of an in vitro model system for human adenocarcinoma of the stomach. Cancer Res. 1983;43:1703-9.