Supporting Information

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Optimization of Retroviral Transduction of Human Cancer Cell Lines

First, we introduced *Slc7a1* (mouse receptor gene) into human cancer cell lines with lentivirus to improve the transduction efficiency (6, 7). Second, we introduced several combinations of retroviruses into human cancer cell lines with *Slc7a1* (summarized in Table S1). According to the protocol summarized in Fig. 1*A*, eight days after transduction, cells were harvested by trypsinization and plated onto a Matrigel-coated plate (BD Biosciences, Bedford, MA) or mitomycin C-treated and provided feeder cells with mouse embryonic fibroblasts (MEF) (6, 7). The next day, the medium (DMEM with 10% FBS) was replaced with the medium suitable for the culture of ES cells, mTeSR1 medium (StemCell Technologies, Vancouver, BC), or MEF-conditioned maintenance medium. We first introduced green fluorescent protein (GFP) into cancer cell lines with amphotropic retrovirus produced in PLAT-A packaging cells. To assess the transfection efficiency, we introduced GFP or DsRed (Clontech, Palo Alto, CA) into cancer cell lines with ecotropic retrovirus produced in PLAT-E packaging cells. We manufactured a specific tool, GFP–*NANOG* promoter clone11, which contained a specific sequence in the promoter region for pluripotent-associated gene *NANOG*, and could detect the pluripotent state in a living cell on based on previous studies. GFP expressions of transfectants were visualized with all-in-one-type fluorescence microscopy (BZ-8000; Keyence, Osaka). To determine the differentiation ability of iPC cells, we transferred the embryoid body (EB)-like structures to EB culture condition as gelatin-coated plates maintained in DMEM/F12 containing 20% knockout-certified serum replacement (KSR; Invitrogen, Carlsbad, CA), 2 mM L-glutamine, 100 μM nonessential amino acids, 100 μM 2-mercaptoethanol (Invitrogen), and 0.5% penicillin and streptomycin at 37 °C under a 5% humidified CO_2 atmosphere.

Real-Time Reverse Transcription PCR (RT-PCR)

For quantitative assessments of PCR amplification products with various sizes, quantitative real-time RT-PCR using a LightCycler TaqMan Master Kit (Roche Diagnostics, Tokyo) for specific genes and *GAPDH* (Tables S2 and S3). The amplification protocol consisted of 35 cycles of denaturation at 95 °C for 10 s, and annealing and elongation at 60 °C for 30 s. If Universal Probe Library probe (Roche Diagnostics) was not selected with the specific primers of the target gene for PCR analysis of retroviral transgenes, quantitative real-time RT-PCR using a LightCycler FastStart DNA Master SYBR Green I kit was carried out (Roche Diagnostics). The amplification protocol consisted of denaturation at 95 °C for 10 s, annealing at 60 °C for 10 s, and elongation at 72 °C for 10 s. The products were then subjected to a temperature gradient of 55–95 °C with continuous fluorescence monitoring to produce a melting curve of the products. The expressions of mRNA copies were normalized against *GAPDH* mRNA expression.

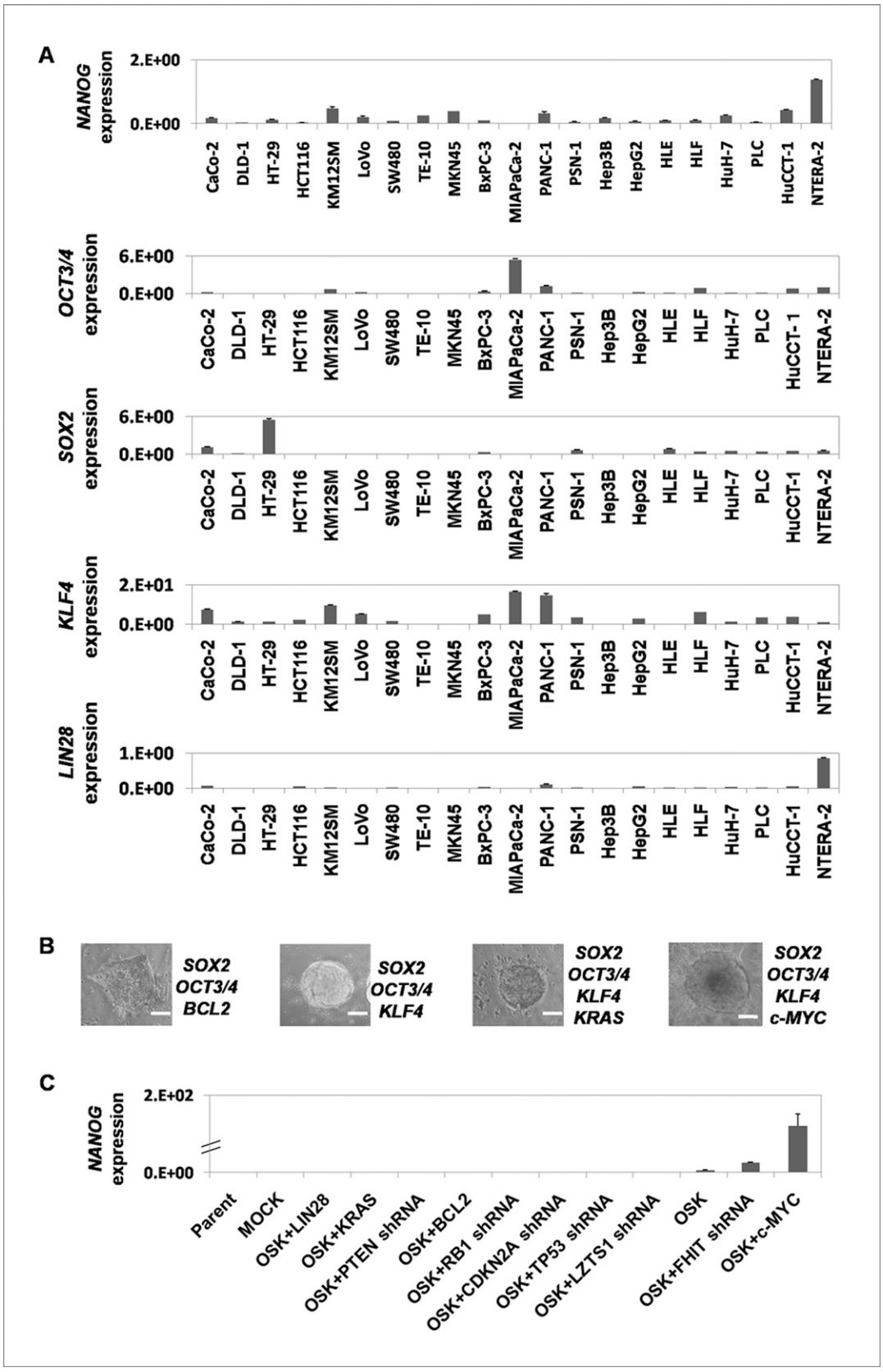

Fig. S1. Immature status of gastrointestinal cancer cell lines. (*A*) Real-time RT-PCR analysis showed *NANOG*, *OCT3/4*, *SOX2*, *KLF4,* and *LIN28* in 20 gastrointestinal cancer cell lines. The expression of *NANOG* mRNA is low in four cancer cell lines: DLD-1 and HCT116 (colorectal cancer), MIAPaCa-2 (pancreatic cancer), and PLC (hepatocellular carcinoma). (*B*) Immature status induced by combinations of several defined factors was shown by the morphology of DLD-1. (*C*) Real-time RT-PCR analysis of *NANOG* expression in cell line DLD-1 that was transfected by combinations of several defined factors. The expressions of mRNA copies were normalized against *GAPDH* mRNA expression.

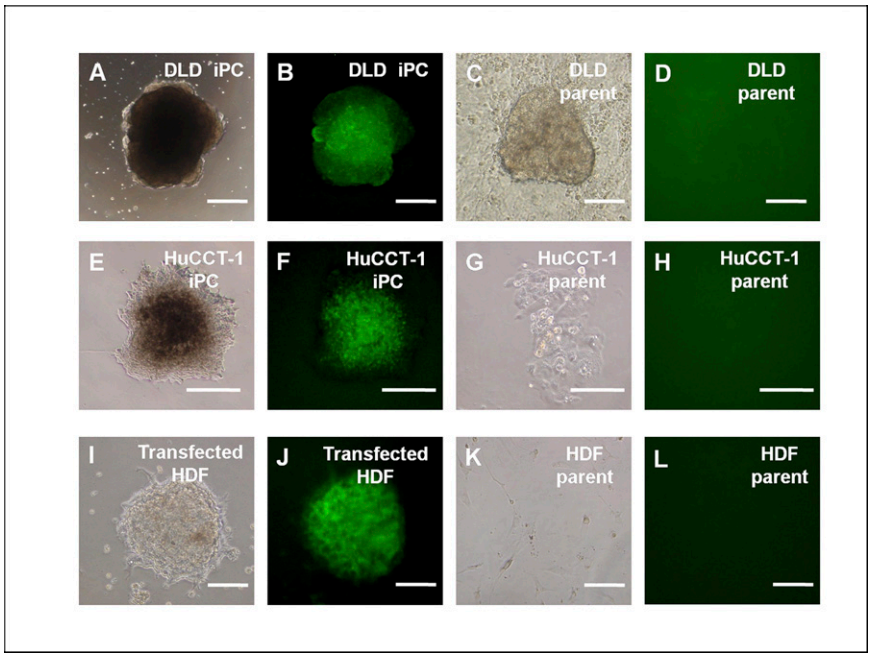

Fig. S2. Evaluation of *NANOG* promoter activity *NANOG* promoter activity was evaluated by *GFP-NANOG* promoter clone11 in DLD-1, which was or was not induced. (*A* and *B*) Morphology and fluorography of *GFP* of DLD1-induced cells. (*C* and *D*) Morphology and fluorography of parental cells. (*E–H*) Morphology and fluorography of GFP of iPC cells (*E* and *F*) and those of parental cells (G and H) in HuCCT-1. (*I–L*) Morphology and fluorography of GFP of transfected HDF (*I* and *J*) and those of parental cells (*K* and *L*) in HDF. GFP expression of transfectants was visualized with all-in-one-type fluorescence microscopy (BZ-8000; Keyence, Osaka). Scale bars: 200 μm.) (Original magnification, ×200)

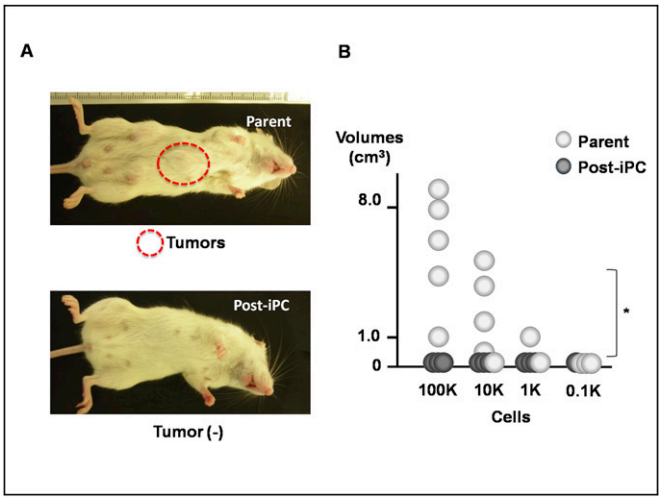

Fig. S3. In vivo analyses of proliferation assay. To determine proliferative properties in vivo, we s.c. transplanted differentiated iPC cells at several concentrations into the dorsal flanks of NOD/SCID mice. (*A*) Four weeks after injection, mice were killed and observed for tumor formation. (*B*) Significant differences of tumorigenesis between PostiPC and parental cancer cells ($n = 5$, *, $P < 0.01$, Wilcoxon rank test).

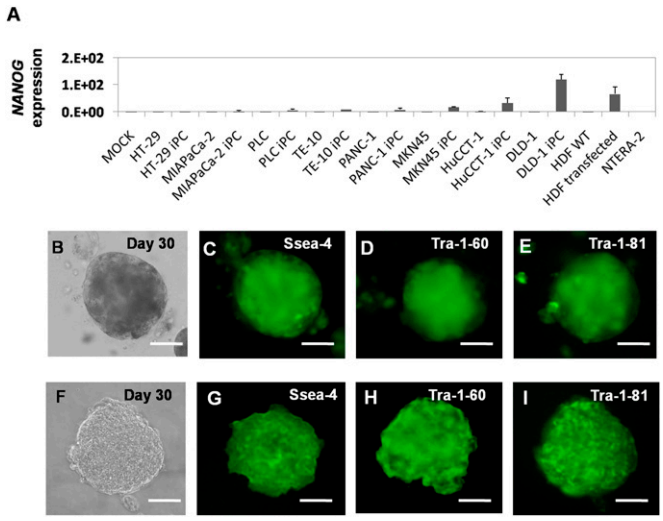

Fig. S4. iPC cells from several gastrointestinal cancers. (*A*) Real-time RT-PCR analysis of *NANOG* mRNA expressions in eight iPC cells lines (colorectal cancer, DLD-1 and HT-29; esophageal cancer, TE-10; gastric cancer, MKN45; pancreatic cancers, MIAPaCa-2 and PANC-1; hepatocellular carcinoma, PLC; cholangiocellular carcinoma, HuCCT-1. Transfected HDF and NTERA-2 were used as references for *NANOG* expression. The expressions of *NANOG* mRNA copies were normalized against *GAPDH* mRNA expression. (*B–I*) iPC cells from PLC and HuCCT-1 were analyzed for surface antigens, phase contrast (*B* and *F*), Ssea-4 (*C* and *G*), Tra-1-60 (*D* and *H*), and Tra-1-81 (*E* and *I*).

Table S1. Plasmid used for transfection

Name	Type	Company	Product No.
pMIG Bcl-2	Retrovirus	Addgene	8793
pMXs-hOCT3/4	Retrovirus	Addgene	17217
pMXs-hSOX2	Retrovirus	Addgene	17218
pMXs-hKLF4	Retrovirus	Addgene	17219
pMXs-hc-MYC	Retrovirus	Addgene	17220
pBabe K-Ras 12V	Retrovirus	Addgene	12544
pSin-EF2-Nanog-Pur	Lentivirus	Addgene	16578
pSin-EF2-LIN28-Pur	Lentivirus	Addgene	16580
pLenti6/UbC/mSlc7a1	Lentivirus	Addgene	17224
pRetroQ-DsRed Monomer-C1	Retrovirus	Clontech	632508
pMXs Retroviral Vector (negative control)	Retrovirus	Cell Biolabs	RTV-010
NM_000546 shRNAmir	Lentivirus	Open Biosystems	RHS4430-99365288
NM_000077 shRNAmir	Lentivirus	Open Biosystems	RHS4696-99703685
NM_000314 shRNAmir	Lentivirus	Open Biosystems	RHS4740-NM_000314
NM_002012 shRNAmir	Lentivirus	Open Biosystems	RHS4740-NM_002012
NM_000321 shRNAmir	Lentivirus	Open Biosystems	RHS4531-NM_000321
NM_021020 shRNAmir	Lentivirus	Open Biosystems	RHS4740-NM_021020

Table S2. Primer sequences corresponding to universal probe libraries (UPL)

Primer	Sequence 5′-3′	UPL No.	Applications
hNANOG-S	ATG CCT CAC ACG GAG ACT GT	66	*NANOG* RT-PCR
hNANOG-AS	AGG GCT GTC CTG AAT AAG CA		
hREX1-S	TCT GAG TAC ATG ACA GGC AAG AA	65	*REX1* RT-PCR
hREX1-AS	TCT GAT AGG TCA ATG CCA GGT		
hOCT3/4-S	AGC AAA ACC CGG AGG AGT	35	*OCT3/4* RT-PCR
hOCT3/4-AS	CCA CAT CGG CCT GTG TAT ATC		
hSOX2-S	CTC CGG GAC ATG ATC AGC	70	*SOX2* RT-PCR
hSOX2-AS	CTG GGA CAT GTG AAG TCT GC		
hKLF4-S	ATT GGA CCC GGT GTA CATTC	66	*KLF4* RT-PCR
hKLF4-AS	AGC ACG AAC TTG CCC ATC		
hc-MYC-S	CAC CAG CAG CGA CTC TGA	34	*c-MYC* RT-PCR
hc-MYC-AS	GAT CCA GAC TCT GAC CTT TTG C		
hGAPDH-S	AGC CAC ATC GCT CAG ACA C	60	*GAPDH* RT-PCR
hGAPDH-AS	GCC CAA TAC GAC CAA ATC C		
hLIN28-S	CTG TCC AAA TGC AAG TGA GG	79	*LIN28* RT-PCR
hLIN28-AS	GCA GGT TGT AGG GTG ATT CC		
hKRT19-S	GTC ATG GCC GAG CAG AAC	89	*KRT19* RT-PCR
hKRT19-AS	CCG GTT CAA TTC TTC AGT CC		
hCDH1-S	CCC GGG ACA ACG TTT ATT A	35	*CDH1* RT-PCR
hCDH1-AS	GCT GGC TCA AGT CAA AGT CC		
hVIM-S	AAA GTG TGG CTG CCA AGA AC	16	*VIM* RT-PCR
hVIM-AS	AGC CTC AGA GAG GTC AGC AA		
hFABP4-S	GGA TGA TAA ACT GGT GGT GGA	85	*FABP4* RT-PCR
hFABP4-AS	CAC AGA ATG TTG TAG AGT TCA ATG C		
hMAP2-S	GCT CAA CAT AAA GAC CAG ACT GC	13	*MAP2* RT-PCR
hMAP2-AS	TGG AGA AGG AGG CAG ATT AGC		
hPAX6-S	TCA CCA TGG CAA ATA ACC TG	20	*PAX6* RT-PCR
hPAX6-AS	CAG CAT GCA GGA GTA TGA GG		
hTP16-S	GTG GAC CTG GCT GAG GAG	34	*CDKN2A* RT-PCR
hTP16-AS	CTT TCA ATC GGG GAT GTC TG		
hTP53-S	CCC CAG CCA AAG AAG AAA C	58	*TP53* RT-PCR
hTP53-AS	AAC ATC TCG AAG CGC TCA C		
hDNMT3A-S	TGG TGC ACT GAA ATG GAA AG	48	*DNMT3A* RT-PCR
hDNMT3A-AS	GCT CAT GTT GGA GAC GTC AG		
hDNMT3B-S	AGA GGG ACA TCT CAC GGT TC	84	*DNMT3B* RT-PCR
hDNMT3B-AS	GGT TGC CCC AGA AGT ATC G		

Table S3. Primer sequences for specific applications

Primer	Sequence 5′-3′	Applications
hOCT3/4-ChIP-S2	TTG CCA GCC ATT ATC ATT CA	*OCT3/4* ChIP
hOCT3/4-ChIP-AS2	TAT AGA GCT GCT GCG GGA TT	
hNANOG-ChIP-S2	GAT TTG TGG GCC TGA AGA AA	*NANOG* ChIP
hNANOG-ChIP-AS2	GGA AAA AGG GGT TTC CAG AG	
hSOX2-ChIP-S1	GAG AAG GGC GTG AGA GAG TG	*SOX2* ChIP
hSOX2-ChIP-AS1	AAA CAG CCA GTG CAG GAG TT	
hPAX6-ChIP-S1	TTG TGT GAG AGC GAG CGG TGC ATT TG	*PAX6* ChIP
hPAX6-ChIP-AS1	CAC CGC TCC TCA CTG GCC CAT TAG C	
hMSX2-ChIP-S1	TTC TGG CGG TAG AGG GAG AGT GGG ATG G	*MSX2* ChIP
hMSX2-ChIP-AS1	ATC ACG CCG AAA CTG AAA AGC CCG AGA C	
mehNANOG-F1-S1	TGG TTA GGT TGG TTT TAA ATT TTT G	Bisulfite sequencing
mehNANOG-F1-AS1	AAC CCA CCC TTA TAA ATT CTC AAT TA	
hOCT3/4-pl	CCA CAT CGG CCT GTG TAT ATC	*OCT3/4* Tg genomic and RT-PCR
hSOX2-pl	TTC TCC CCC CTC CAG TTC G	*SOX2* Tg genomic and RT-PCR
hKLF4-pl	GAC GCC TTC AGC ACG AAC	*KLF4* Tg genomic and RT-PCR
hc-MYC-pl	GAT CCA GAC TCT GAC CTT TTG C	*c-MYC* Tg genomic and RT-PCR
hGAPDH-S1	TTG GTA TCG TGG AAG GAC TCA	*GAPDH* PCR
hGAPDH-AS1	TGT CAT CAT ATT GGC AGG TT	