Supplementary Information

Materials and Methods

Retrovirus-mediated small interfering RNA constructs

pSUPER-internal ribosome entry site-puro vector was digested with BglII and HindIII and annealed oligos (Slug: 5′-gatccGCTAGATTGAGAGAATAAAAGttcaagagaCTTTTATTCTCTCAATCTAGCttttttggaaa-3′; control: 5′-gatccTCGTATGTTGTGTGGAATTttcaagagaAATTCCACACAACATACGAtttttttggaaa-3′) were ligated into this vector. For the production of retroviruses, Plat-E cells were transfected with pSUPER and vesicular stomatitis virus G vectors using Effectene transfection reagent (QIAGEN). Culture supernatants of Plat-E cells were collected 60 hours following transfection with retroviral vectors. Target cell lines were infected in the presence of 10 μg/mL Polybrene.

Antibodies

The following antibodies were used in this study.

Rb (9309, Cell Signaling); β-Catenin (610154, BD Transduction Laboratories); Gli1 (NBP1-78259, NOVUS); Notch1 (2495, Cell Signaling); Phospho-Smad3 (Ser423/425) (9520, Cell Signaling); Smad3 (9523, Cell Signaling); Phospho-Smad2 (Ser465/467) (3108, Cell Signaling); Smad2 (5339, Cell Signaling); Phospho-Smad1/5(Ser463/465) (9516, Cell Signaling); Smad1 (6944, Cell Signaling); Smad5 (9517, Cell Signaling); Slug (sc-10436, SANTA CRUZ BIOTECHNOLOGY); E-cadherin (610181, BD Transduction Laboratories); Vimentin (5741, Cell Signaling); Pin1 (MAB2294, R&D Systems); Phospho-Pin1(Ser16) (3721, Cell Signaling); Phosphor-(Ser) CDKs Substrate Antibody (2324, Cell Signaling).

Supplementary Figure-S1 (Nishi et al.)

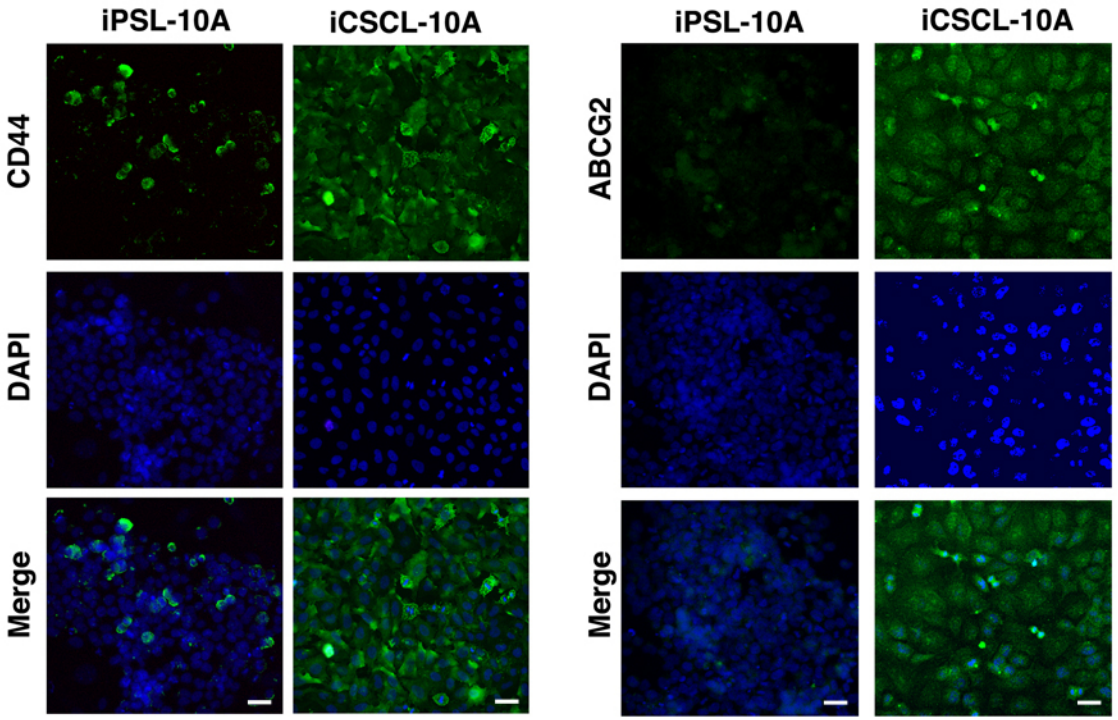

Figure S1. iPSL-10A partially express the CSC markers. Immunofluorescent analysis of CSC marker, CD44 and ABCG2 in cultured iPSL-10A and iCSCL-10A cells. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI). Scale bar, 200 μm.

Supplementary Figure-S2 (Nishi et al.)

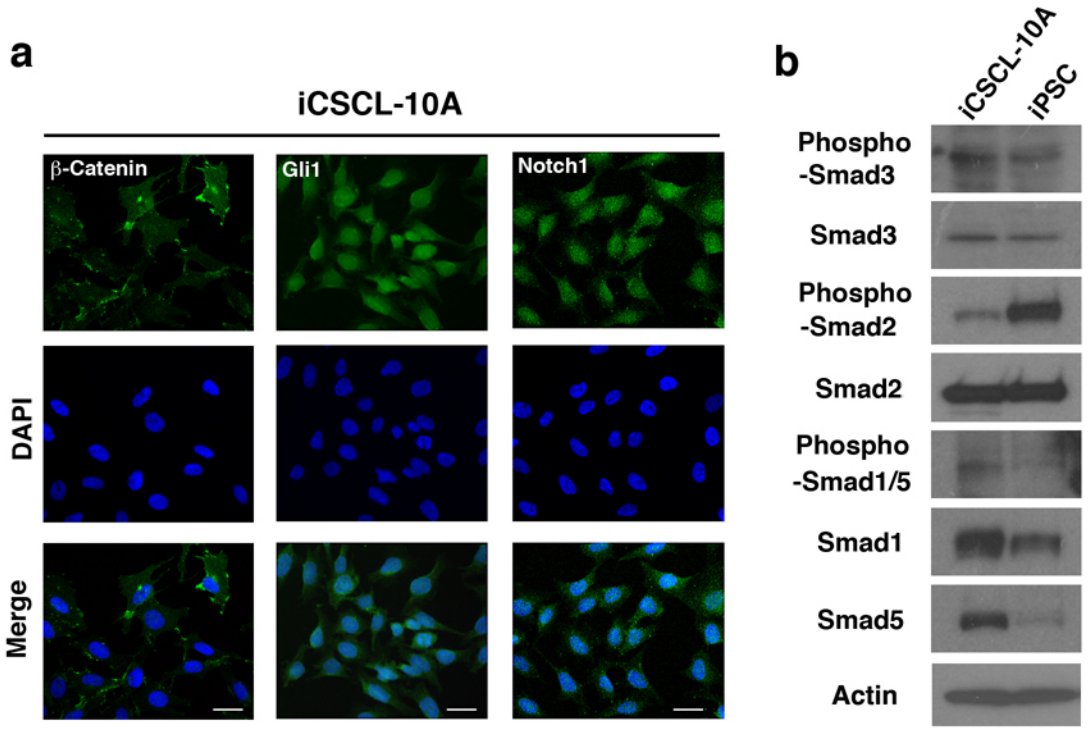

Figure S2. Activation of stem cell-related factors in iCSCL-10A. (a) Immunofluorescent analysis of cultured iCSCL-10A cells with indicated antibodies. Nuclei are stained with by 4',6-diamidino-2-phenylindole (DAPI). Scale bar, 200μm. (b) Immunoblotting analysis of Smad and Phospho-Smad specific antibodies in iCSCL-10A cells. Actin was used as a loading control.

Supplementary Figure-S3 (Nishi et al.)

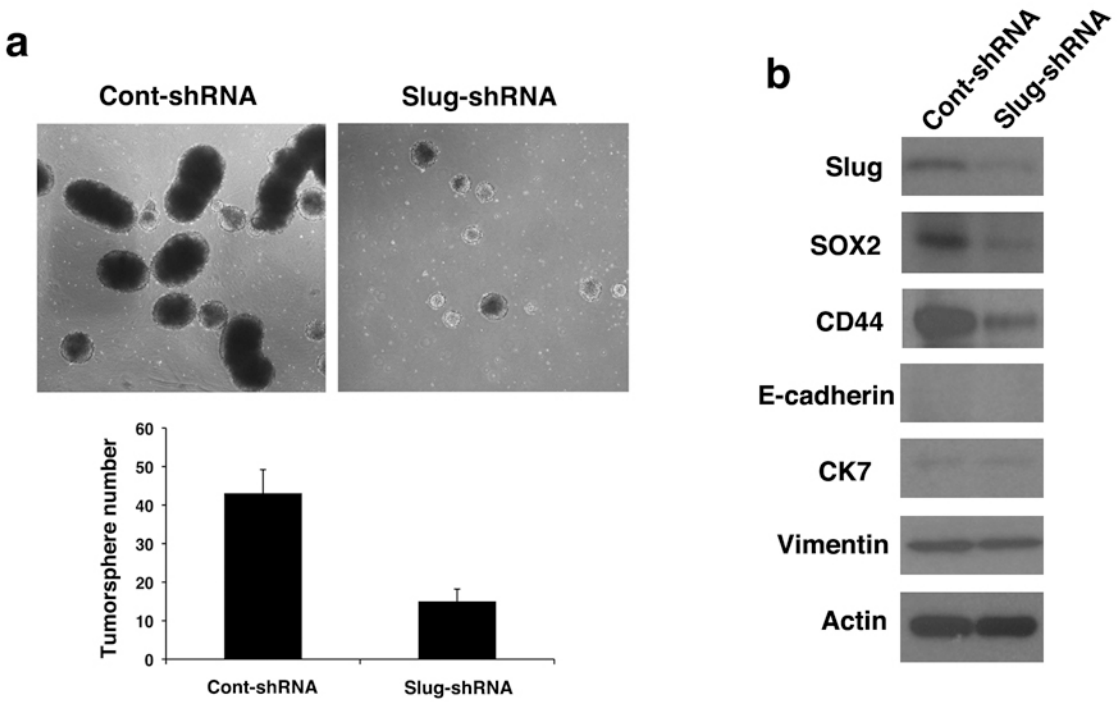

Figure S3. Slug-inhibition attenuates stem cell properties of iCSCL-10A cells. (a) Phase-contrast images of tumor spheres transduced with control- or slug-shRNA and quantification of tumor sphere-formation. Values represent the means ± s.e.m. (n = 3). (b) Immunoblotting of the stem cell and EMT marker proteins in iCSCL-10A cell transduced with control- or slug-shRNA.

Supplementary Figure-S4 (Nishi et al.)

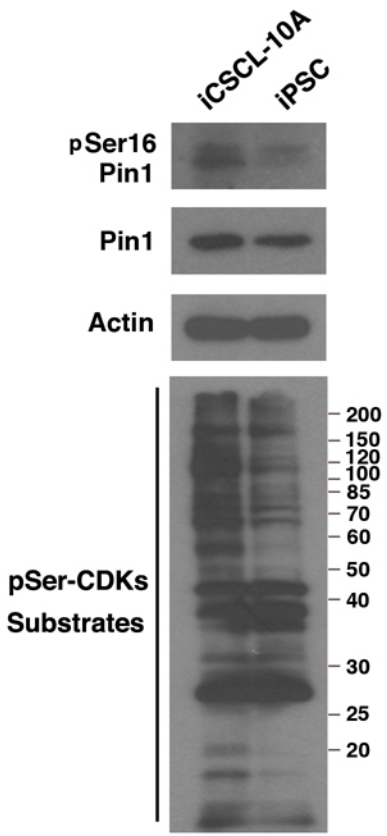

Figure S4. Phosphorylation of Pin1 and Pin1-substrate proteins in iCSCL-10A. iCSCL-10A and normal iPS cell lysates were subjected to immunoblotting analysis with anti-phospho-Pin1 (pSer16), anti-Pin1 or phospho-serine cyclin-dependent kinases (pSer-CDKs) substrate antibody as indicated. Actin was used as a loading control.

Supplementary Figure-S5 (Nishi et al.)

a

Tumor seeding (iCSCL-10A)		
Cell injected	EV	p16
$1X10^5$	12/12	0/12

b

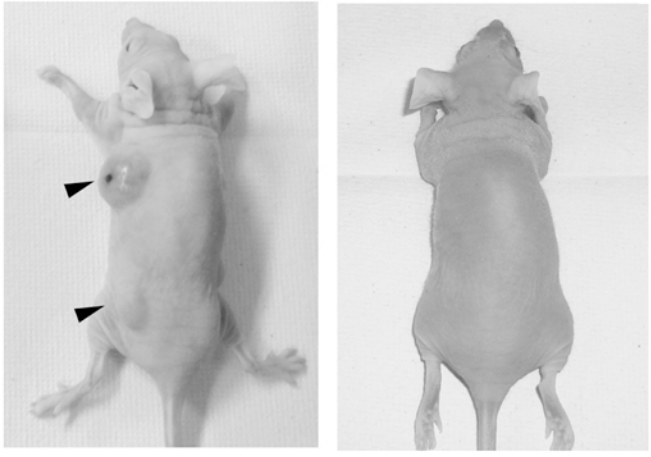

Figure S5. Re-expression of p16 abrogates the tumor-seeding ability of iCS-CL-10A cells. (a) The indicated numbers of iCSCL-10A cells transduced with either empty vector (EV) or p16 were injected into irradiated nude mice. The tumor initiation ability per injection was then monitored. (b) Representative pictures of nude mice with subcutaneous tumors. Arrows indicate the site of implantation and tumor growth from there.