Doc S1 | Supplementary Materials and Methods

Establishment of LLC-GFP-derived cancer stem-like cells

To establish cancer stem-like cells, LLC-GFP cells were striped and cultured under floating condition using 6 well ultra-low attachment plate (Corning). Spheroids were cultured using DMEM/F12 (Sigma-Aldrich) / B27/RA(-) (Life technologies) / 10 μg/ml EGF (Sigma-Aldrich) / 10 μg/ml FGF2 (Peprotech) / 50 U/mL penicillin and 0.5% streptomycin (Life technologies) and maintained at 37 ˚C in a 5 % CO_2 atmosphere to establish LLC-GFP-derived cancer stem-like cells (LLC-GFPstem).

Infection of lentivirus

pLKO.1/TRC1-based MISSION shRNA lentivirus particles were purchased from Sigma-Aldrich. The clones chosen were described in Table S1. Scramble shRNA was used as control. shRNA-encoding lentivirus were added to culture media and LLC-GFP cells were cultured for 24 hr. After maintaining for 24 hr, LLC-GFP or LLC-GFPstem cells were selected with 50 μg/ml puromycin (Wako) for 3 days. Cells stably expressing shRNA were maintained with10 μg/ml puromycin.

Growth curve assay

1,000 LLC-GFP cells / 200 μl were passaged on collagen-coated 96-well plate (Iwaki) and cultured at 37 ˚C in 5 % CO_2 atmosphere. Cells were striped and counted every 2, 4 and 6 days.

WST-8 assay

1,000 LLC-GFP cells / 200 μl were seeded onto collagen-coated 96-well plate (Iwaki) and cultured at 37 ˚C in 5 % CO_2 atmosphere. Next day, culture media were replaced with new media containing 100 ng/ml recombinant SEMA6B (rSEMA6B) (R&D systems) or 100 ng/ml recombinant SEMA3A (rSEMA3A) (R&D systems) and then cultured for 4 days. WST-8 reagent (Dojindo) was added, and cells were incubated for 30 min to compare the wavelength at 450nm using infinite F200 Pro (TECAN). Each value was normalized to 1xPBS-treated LLC/scramble.

RT-qPCR

TRIZOL (Life technologies) was used to extract total RNA samples. Reverse transcription of 1 μg total RNA to cDNA samples was performed using PrimeScript Reverse Transcriptase (Takara). RT-qPCR was performed using GoTaq®qPCR Master Mix (Promega) and MiniOpticon real-time PCR system (BioRad). PCR cycle used in this study was as follows: denaturation 95 ˚C for 30 sec, annealing at 58 ˚C for 30 sec, and elongation 72 ˚C for 30 sec. All data were normalized to *Actb* values. Table S2 describes the sequences of each primer.

Sphere forming assay

LLC-GFPstem cells were dissociated into single cells with Accutase and the suspended in were suspended in DMEM/F12 (Sigma-Aldrich) / B27/RA(-) (Life technologies) / 10 μg/ml EGF (Sigma-Aldrich) / 10 μg/ml FGF2 (Peprotech) / 50 U/mL penicillin and 0.5% streptomycin (Life technologies) / 1% methylcellulose (Wako) (10^3 cells / 200 μl). 200 μl cell suspensions were seeded to 96 well Ultra-low attachment plates (Corning) and cultured for 1 week. Spheroids with the diameter of greater than 100 μm were counted.

Intrafemoral inoculation

Before intra-femoral inoculation, cells stripped with 0.05 % Trypsin/EDTA were counted and re-suspended in 5% FBS/ 1x PBS at a concentration of 2 x 10^3 cells/μl. Mice were anesthetized with isoflurane and 26-gauge needles were used to penetrate their femoral cartilage to make

small holes. 5 µl cell suspension was intrafemorally inoculated and the effects of Sema6b knockdown were tested.

Weight bearing test

Before performing pain assessment, mice were placed in the test environment for at least 30 min. The hind-limb weight bearing was measured Incapacitance tester (IITC, U.S.A.) was used to measure the weight bearing of ipsilateral or contralateral hind-limb on 1 week, 2 weeks and 3 weeks after intrafemoral inoculation of LLC-GFP cells.

GFP detection

Three weeks after transplantation, mice were deeply anesthetized with 150 mg/kg body-weight pentobarbital and perfused with 4% paraformaldehyde (PFA). Dissected femur samples were soaked in 4% PFA overnight and decalcified with OSTEOSOFT solution (Merck). Femurs were dehydrated with 30% sucrose (Wako) and embedded into OCT compound (Tissue Teck). Samples were sectioned into 20 µm slices and nuclei were counterstained with Hoechst 33342 (Sigma-Aldrich). All photos were taken with LSM700 (Carl Zeiss).

Table S1 | shRNA clones used in this study.

	Names of each pLKO.1/TRC1-based MISSION shRNA clone	
Gene	**clone#1**	**clone#2**
Sema3a	**TRCN0000067328**	**TRCN0000067329**
Plxa1	TRCN0000079188	TRCN0000079189
Sema6b	TRCN0000112313	TRCN0000112311

Table S2 | Primers used in this study.

	RT-qPCR primers	
Gene	**Forward primer sequence**	**Reverse primer sequence**
Sema6b	GCCCTGTCGTTTTTCCTGCT	ACGGGATAGTGGCTCAAGTAG
Ccnd1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
Pcna	TTTGAGGCACGCCTGATCC	GGAGACGTGAGACGAGTCCAT
Sox2	GCGGAGTGGAAACTTTTGTCC	CGGGAAGCGTGTACTTATCCTT
Cd44	TCGATTTGAATGTAACCTGCCG	CAGTCCGGGAGATACTGTAGC
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT