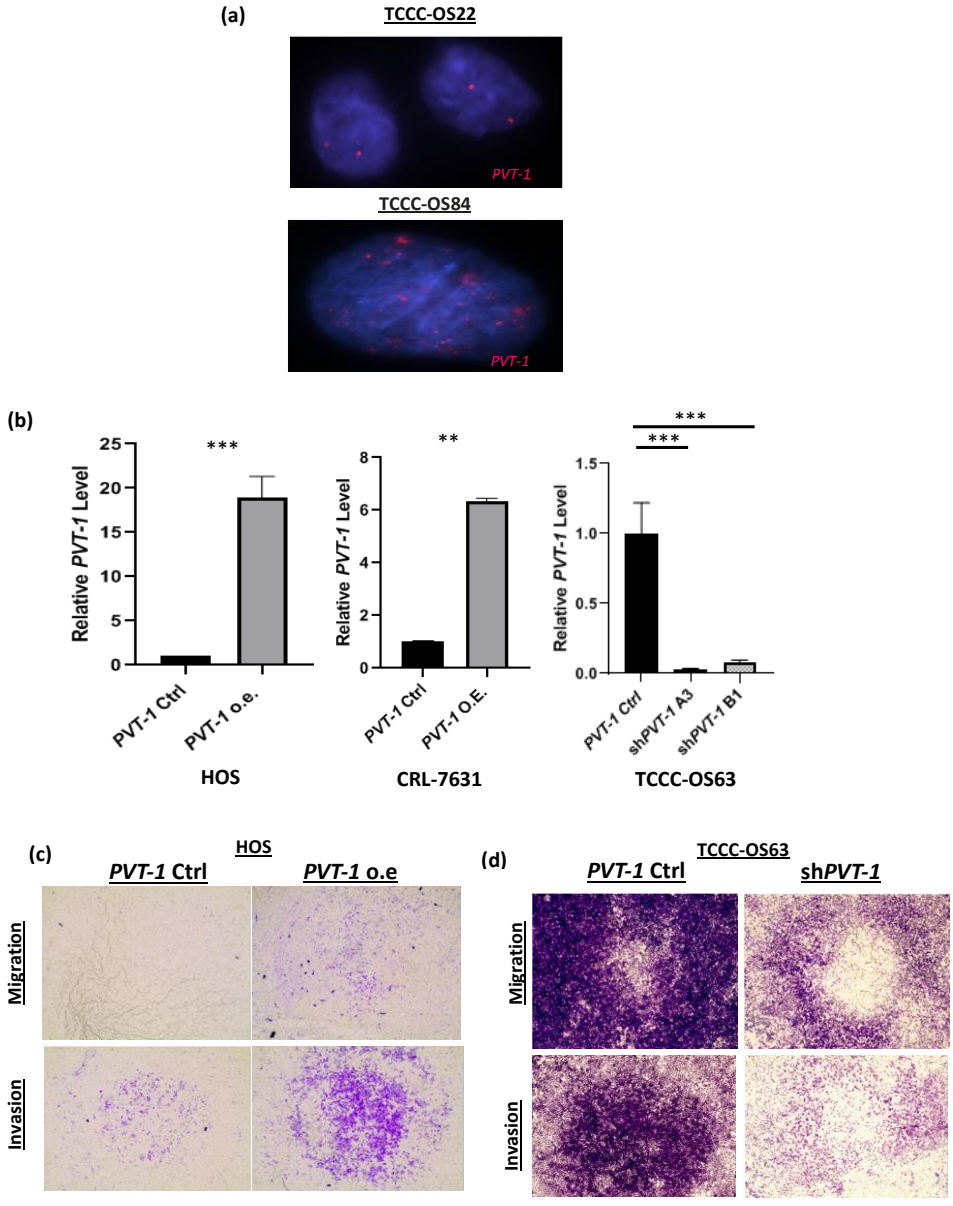
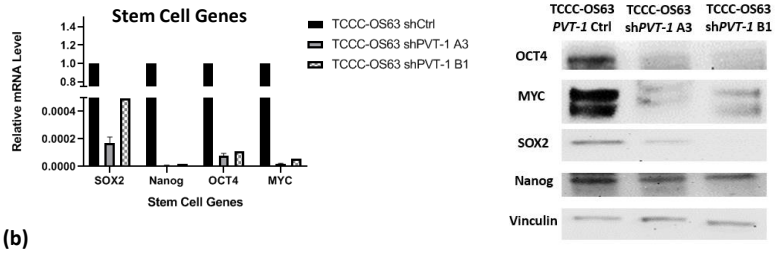
	***PVT-1* Copy Number**		***PVT-1* Copy Number**
Cell Line		**Patient Derived Xenograft**	
HOS	2 Copies	TCCCC-OS22	2 Copies
CRL-7631	2 Copies	TCCCC-OS57	2 Copies
CRL-7642	2 Copies	TCCCC-OS13	>6 Copies
U2OS	5 Copies	TCCCC-OS23	>6 Copies
Saos-2	5 Copies	TCCCC-OS46	>6 Copies
TCHOS-1	>6 Copies	TCCCC-OS51	>6 Copies
MG63	>6 Copies	TCCCC-OS52	>6 Copies
G-292	>6 Copies	TCCCC-OS 54,63,74 and 84	>6 Copies

Supplemental Table 1: ***PVT-1* copy number in osteosarcoma cell lines and patient-derived xenografts.**

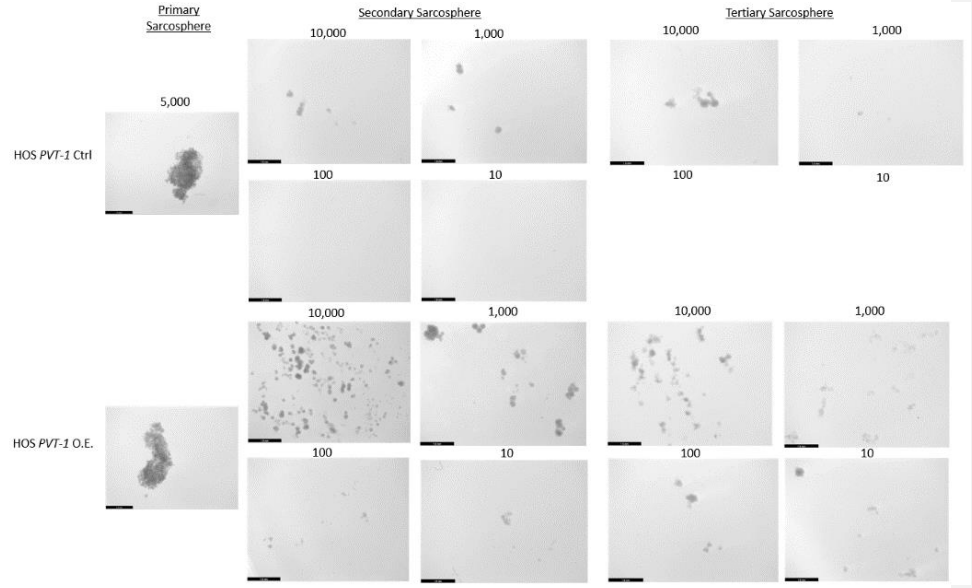


Supplemental Figure 1: ***PVT-1* promotes tumorigenic behaviors in osteosarcoma.** (a) Fluorescence *In Situ* Hybridization identifies *PVT-1* copy number in patient-derived xenografts (Red Dots). (b) Stable *PVT-1* o.e. produced in HOS and CRL-7631 cell lines and sh*PVT-1* cell lines in TCCC-OS63 were established and confirmed using RT-qPCR. (c) Representative images of HOS *PVT-1* o.e. cell lines regulation of migration and invasion behaviors. (d) Migration and invasion characterization of TCCC-OS63 cells through the use of transwell assay. Error bars represent S.D.; a Student's t-test or one-way ANOVA was used to calculate statistical significance values: *p<.05; p<.001; *** p<.0001. Data represents three independent experiments.

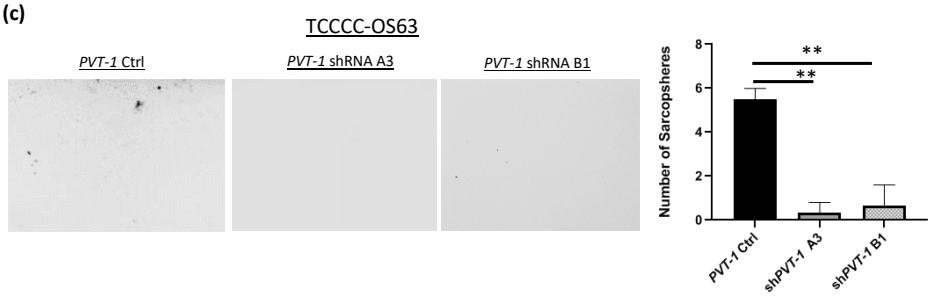
(a)


Stem Cell Genes
Relative mRNA Level
1.4
1.2
1.0
0.8
0.0004
0.0002
0.0000
SOX2
Nanog
OCT4
MYC
Stem Cell Genes
TCCC-OS63 shCtrl
TCCC-OS63 shPVT-1 A3
TCCC-OS63 shPVT-1 B1
TCCC-OS63 PVT-1 Ctrl
TCCC-OS63 shPVT-1 A3
TCCC-OS63 shPVT-1 B1
OCT4
MYC
SOX2
Nanog
Vinculin

(b)

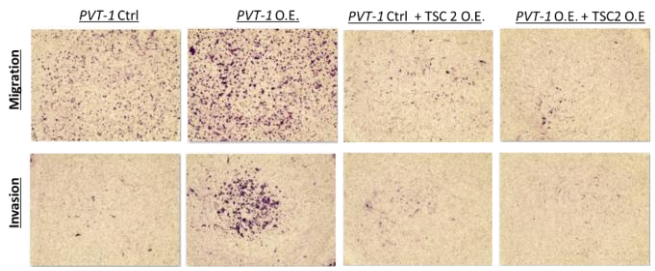

Primary Sarcosphere
Secondary Sarcosphere
Tertiary Sarcosphere
5,000
10,000
1,000
100
10
HOS PVT-1 Ctrl
HOS PVT-1 O.E.

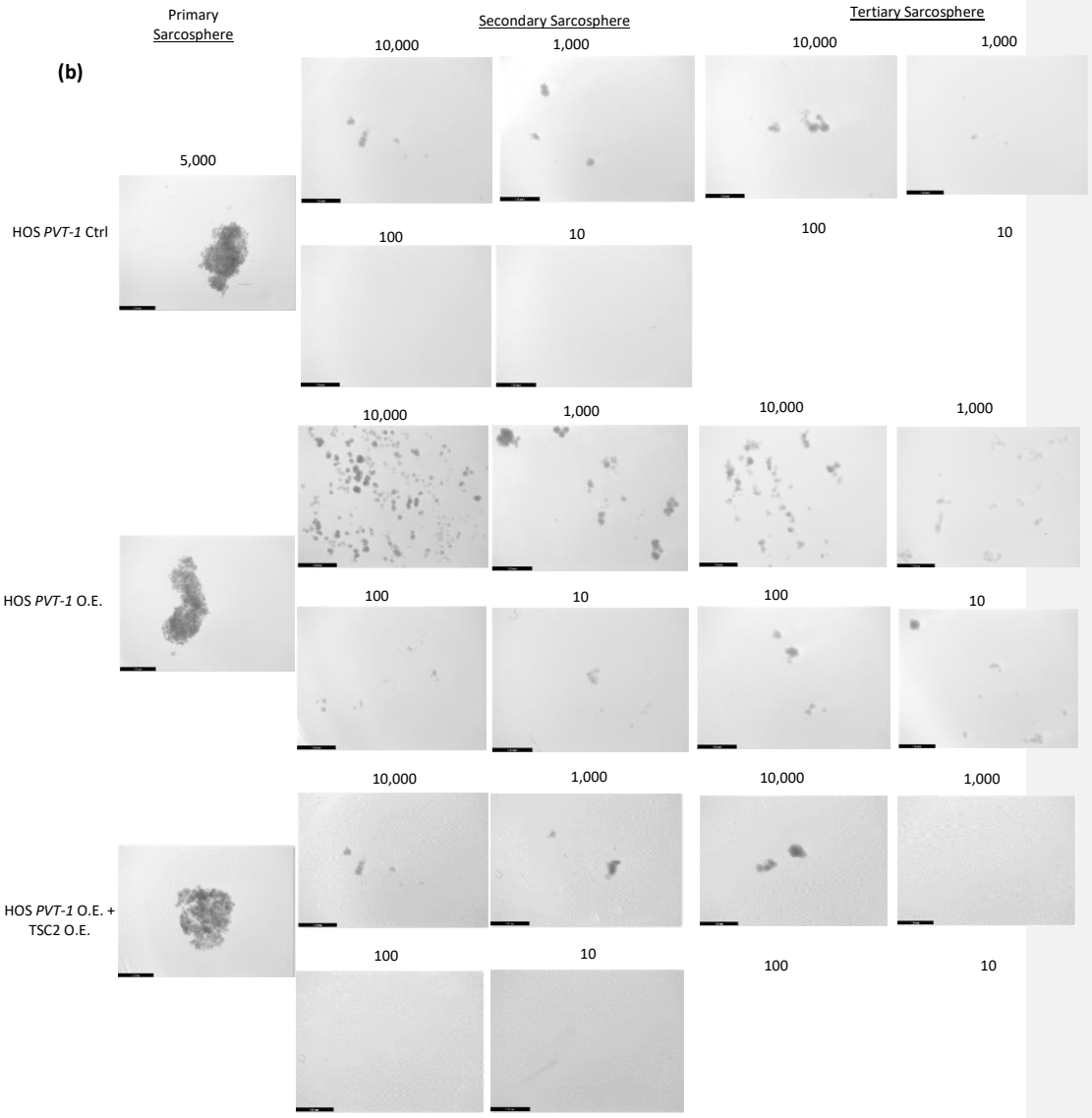
(c)

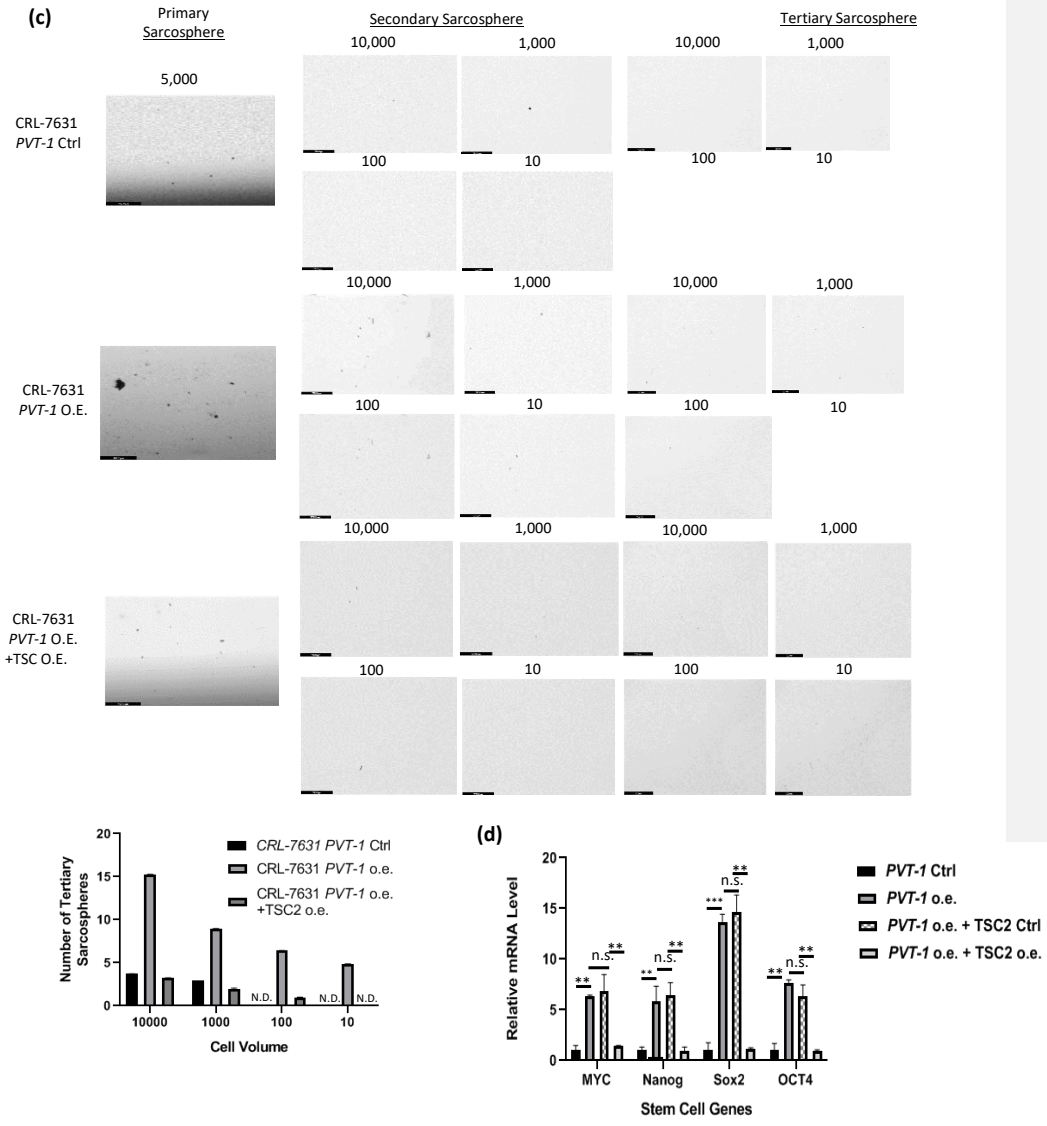

TCCCC-OS63
PVT-1 Ctrl
PVT-1 shRNA A3
PVT-1 shRNA B1
Number of Sarcopsheres
8
6
4
2
0
**
**
PVT-1 Ctrl
shPVT-1 A3
shPVT-1 B1

Supplemental Figure 2: ***PVT-1* enhances cancer stem-like behaviors.** (a) RNA and protein expression of stem cell markers was assessed in TCCC-OS63 shCtrl and sh*PVT-1* cells using qPCR and western blot. (c) Representative images of secondary and tertiary serial dilution sarcospheres formed from HOS *PVT-1* Ctrl and *PVT-1* o.e cells. (d) Representative sphere formation images of TCCC-OS63 cell lines. (e) Error bars represent S.D.; one-way ANOVA was used to calculate statistical significance values: *p<.05; p<.001; *** p<.0001. Data represents three independent experiments.

(a)


PVT-1 Ctrl
PVT-1 O.E.
PVT-1 Ctrl + TSC 2 O.E.
PVT-1 O.E. + TSC2 O.E
Migration
Invasion


(b)
Primary Sarcosphere
Secondary Sarcosphere
Tertiary Sarcosphere
10,000
1,000
10,000
1,000
5,000
HOS PVT-1 Ctrl
100
10
100
10
10,000
1,000
10,000
1,000
HOS PVT-1 O.E.
100
10
100
10
10,000
1,000
10,000
1,000
HOS PVT-1 O.E. + TSC2 O.E.
100
10
100
10



Supplemental Figure 3: **TSC2 is downstream of *PVT-1*.** (a) Brightfield images of transwell assay to assess TSC2 role in regulating migration and invasion in HOS cells. (c) Representative images of serial dilution sarcospheres formed from HOS cells dependent upon *PVT-1* and TSC2 expression. (d) Representative images from CRL-7631 serial dilution sarcosphere assay based upon *PVT-1* and TSC2 expression. Quantification of tertiary sarcosphere. None Detected (N.D.) (e) With the use of qPCR, mRNA level of stem cell genes due to rescuing TSC2 expression in HOS *PVT-1* o.e cells. Error bars represent S.D.; Student's t-test and one-way ANOVA was used to calculate statistical significance values: *p<.05; p<.001; *** p<.0001. Data represents three independent experiments.

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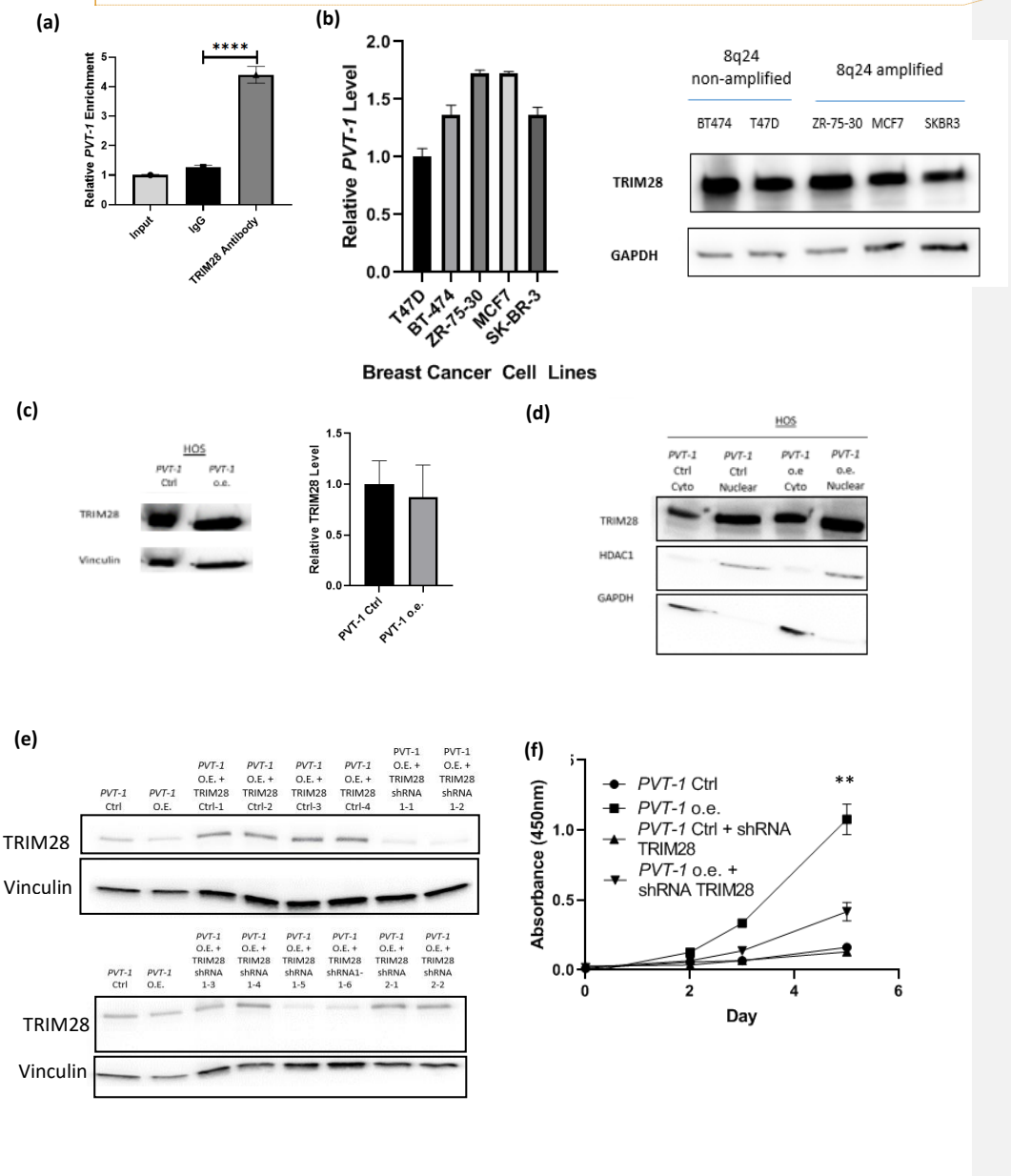
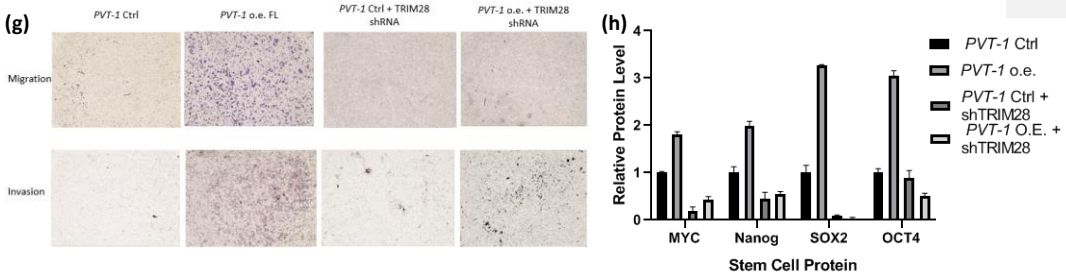
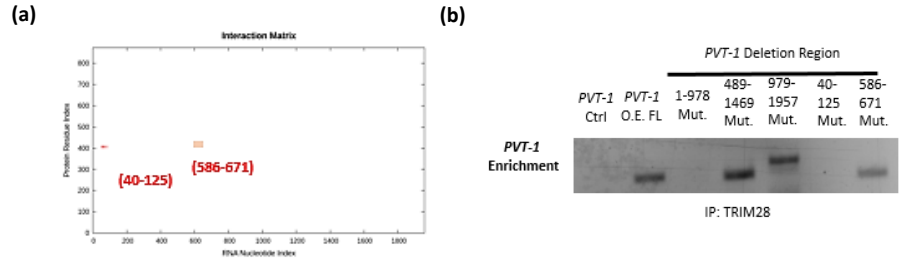
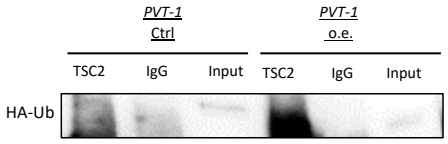




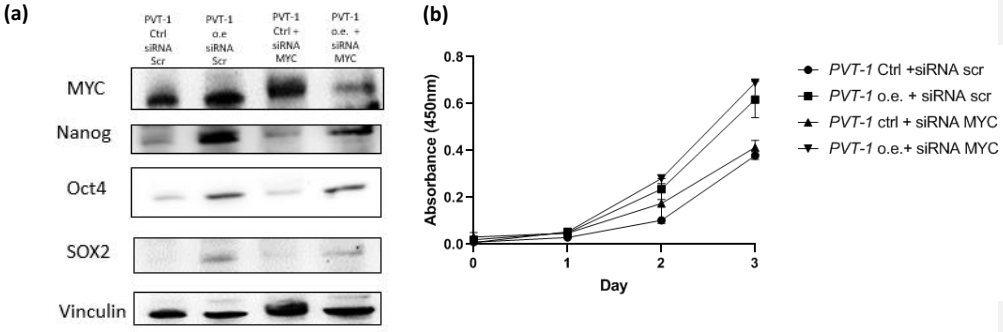
Supplmental Figure 4: **TRIM28 is a protein binding partners of *PVT-1*.** (a) TRIM28 RIP-qPCR performed using TCCOS-63 parental cell line. (b) *PVT-1* expression level in 8q24 non-amplified (BT474 and T47D) and amplified (ZR-75-30, MAF7, and SKBR3) breast cancer cell lines. TRIM28 level in the breast cancer cell lines. (c) Western Blot to assess TRIM28 level in HOS *PVT-1* Ctrl vs. *PVT-1* o.e.. (d) Cellular localization of TRIM28 based on *PVT-1* perturbation in HOS *PVT-1* o.e. cells. (e) Development of *PVT-1* o.e. clones +/-shTRIM28 (f) Cell viability assay to assess proliferation of *PVT-1* in the absence of TRIM28. (g) Representative images of migration and invasion assay to assess TRIM28 importance to *PVT-1*-induced behaviors. (h) Quantification of western blot assessing stem cell gene set comparing HOS *PVT-1* o.e. vs. *PVT-1* o.e. + shTRIM28. Error bars represent S.D.; a Student's t-test or one-way ANOVA was used to calculate statistical significance values: *p<.05; p<.001; *** p<.0001. Data represents three independent experiments.



Supplemental Figure 5: **Mutation of *PVT-1*/TRIM28 interaction region negates *PVT-1*-induced behaviors.** (a) catRAPID algorithm was used to predict the *PVT-1*/TRIM28 binding region. (b) Agarose run of the qPCR product to verify the presence of *PVT-1* strand.



Supplemental Figure 6: ***PVT-1*/TRIM28 axis promotes TSC2 ubiquitination.** Western blot from TSC2 pulldown in HOS cells. Blot is to probe for HA-Ub protein level in either the TRIM28 pulldown, IgG, and Input samples from both *PVT-1* Ctrl and *PVT-1* o.e. cell lines.



Supplemental Figure 7: **The role of MYC in *PVT-1* induced behaviors.** (a) Western blot to assess efficiency to siMYC and assess the expression of stem cell proteins in HOS cell line. (b) Cell viability of HOS cells with overexpression of *PVT-1* +/- MYC. Error bars represent S.D.; a one-way ANOVA was used to calculate statistical significance values: *p<.05; p<.001; *** p<.0001. Data represents three independent experiments.

Supplemental Table 2. qPCR primer sequences		
Primer	**5' Sequence**	**3' Sequence**
PVT-1-1	CATCCGGCGCTCAGCT	TCATGATGGCTGTATGTGCC
PVT-1-2	CAGCACTCTGGACGGAC	CAACAGGAGAAGCAAACA
MYC	GCATTTCTGACAGCCGGAGA	AGAATCGGACACATCCTCGC
OCT-4	CTTGAATCCCGAATGGAAAGG	GTGTATATCCCAGGGTGATCCTC
SOX-2	GCCGAGTGGAAACTTTTGTCG	GGCAGCGTGTACTTATCCTTCT
NANOG	TGATTTGTGGGCCTGAAGAAAA	GAGGCATCTCAGCAGAAGACA
TRIM28-1	TTTCATGCGTGATAGTGGCAG	GCCTCTACACAGGTCTCACAC

TRIM28-2	TGAGACCTGTGTAGAGGCG	CGTTCACCATCCCGAGACTT
18s	GGCCCTGTAATTGGAATGAGTC	CCAAGATCCAACTACGAGCTT
TSC2	CCGTCTTCCACATCGCCACCCTG	ACGATCACGTGGACAAAGTTGAACTG
1-978 Mutant	TCCAGTGGATTTCCTTGCGG	GCGTTATTCCCCAGACCACT
457-1457 Mutant	TCCCCTTTTACTGCCAGGAC	CTCAAGTCCGTCCAGAGTGC
979-1957 Mutant	TCCTTTCAGCACTCTGGACG	ACACAGAGCACCAAGACTGG
40-125 Mutant	CGACGAGCTGCGAGCAAA	GACCGCCAACATCCTTTCC
586-671 Mutant	CAGCTGGGCTTGAGCTGAC	TGTATGTGCCAAGGTCACCC

Table 3: Antibodies for western blot and Co-Immunoprecipitation

Antibody	**Company/Catalog Number**
MYC	Abcam/ab32072
Vinculin	Cell Signaling/ 13901
SOX2	Cell Signaling/9093T
OCT4	Cell Signaling/9093T
Nanog	Cell Signaling/9093T
TRIM28	Bethyl Laboratories/ A300-275A
TSC-1	Bethyl Laboratories/ A300-316A
VPS34	Echelon Biosciences/ Z-R015
TSC2	Abcam/ ab32554
Sumo	Thermo Fisher/33-2400

Table 4: Therapeutic Agent

Drug	**Company**	**Catalog Number**
TAK-981	Takeda	TAK-981-001-Z

Table 5: ChIRP Biotin Labeled Probe Sequences

Sequence Name	Sequence
lacZ neg ctrl_1	gtgtgaaattgttatccgct
lacZ neg ctrl_2	tgtgctgcaaggcgattaag
lacZ neg ctrl_3	aaaccaggcaaagcgccatt

lacZ neg ctrl_4	tgaccgtaatgggataggtt
lacZ neg ctrl_5	aaaataattcgcgtctggcc
PVT1-1	ATC CTT TCC GCA AGG AAA TC
PVT1-2	TTG AAC GAA GCT CCA TGC AG
PVT1-3	TAG TAT CCT GAA ATG TGC CG
PVT1-4	AAC AGA GAT CTC AAC CCT CT
PVT1-5	GGG AGT ATG GTC AGC TCA AG
PVT1-6	GGC AGT AAA AGG GGA ACA CC
PVT1-7	AAT ACT CCA GCT TTA GGT CA
PVT1-8	TAG CAG CAA CAG GAG AAG CA
PVT1-9	CGA GAG GAC ATT TCC TGC TG
PVT1-10	TAA GCT GCA AGG TCA GTA GT

Supplemental Methods

Cell Lines and Cell Culture: Established osteosarcoma cell lines were acquired from the ATCC cell line repository. Cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin and streptomycin (P/S). Osteosarcoma patient-derived xenograft cell line, TCCC-OS63, has been previously described (Nomura et al. 2019). SK-BR-3 and BT-474 were generously provided by Dr. Suzanne Fuqua (Baylor College of Medicine) and T47D, MCF7, and ZR-75-30 were provided by Dr. Xiang Zhang (Baylor College of Medicine). The SK-BR-3 cells were grown in McCoy's 5A medium (containing 1.5 mM L-glutamine and 2.2 g/L Sodium Bicarbonate) and supplemented with 10% FBS and 100 U/ml P/S while BT-474 and T47D were cultured in DMEM with 10% FBS and 100 U/ml P/S. MCF-7 cells were grown in DMEM supplemented with 10% FBS, 10 ug/mL of human insulin, and 1uM of 4-hydroxytamoxifen. ZR-75-30 was maintained in RPMI-1640 containing 10% FBS and 100 U/ml P/S. All cells were routinely tested for mycoplasma contamination (Lonza MycoAlert: Sugar Land, TX). Cell lines were maintained at 37° C and 5% CO_2 conditions. All cells

were only passaged up to eight times. Osteosarcoma cell lines were authenticated using short tandem repeat DNA fingerprinting methods through the MD Anderson Cancer Center facility.

Cell Viability Assay: Cell proliferation was assessed by seeding 1000-2000 cells/well into a 96-well dish. Cell viability was monitored over a 72-144 hour time frame. Before each time point, colorimetric reagent, CCK-8 was added to the corresponding wells (Dojindo: Rockville, MD). Absorbance was read 3 hours after addition of reagent using Thermo Fisher Plate Reader.

Kaplan-Meier Analysis: 8q24 event-free survival analysis consisted of 18 participants in the 8q24 amplified group vs. 12 participants in the 8q24 non-amplified group. For the *PVT-1* event-free survival and overall survival the data was obtained from TARGET database.

Real-Time Polymerase Chain Reaction: RNA was isolated from cells using RNeasy Kit (Qiagen: Hilden. Germany). Reverse-transcription was conducted using qScript cDNA synthesis kit (Quanta). RT-qPCR reaction consisted of SYBR Green Supermix and was conducted on a StepOne Plus real-time PCR System (Bio-Rad, Applied Bioscience: Waltham, MA). Results were normalized to 18s expression. Primer sequences are shown in the supplemental table 2.

Fluorescence *In Situ* Hybridization: FISH was performed using Texas Red labeled *PVT-1* gene on interphase nuclei and metaphase spreads according to the manufacturer's protocol (Cytocell: Tarrytown, NY). The slides were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and the images were captured using 10X/25 aperture at magnification of 100X/1.40 oil R on Nikon E800 microscope equipped with a

cooled-charge coupled devices camera. The cells were analyzed using Smart Capture Imaging System. A total of 200 interphase nuclei and 5 metaphase spreads were analyzed for copy number changes.

Plasmids and transfection: Human *PVT-1* plasmids for overexpression or *PVT-1* shRNA plasmids were obtained through Applied Biological Materials: LV278976 and Transcriptomic, respectively. The shRNA plasmids consist of GFP reporter and puromycin selection marker and the overexpression plasmid consisted of puromycin selection marker. The cells were transfected by using Lipofectamine 2000 for 48 hours. The cells with shRNA plasmid were GFP sorted with the goal of collecting cells with the highest 10% GFP intensity and stable selection was observed by growing the cell in puromycin selection media. The cell transfected with overexpression plasmid was treated with puromycin.

The TSC2 expression plasmid, pFlag-TSC2WT, was purchased from Addgene. Cells were selected using G418. shTSC2 plasmids were acquired from Horizon Discovery and selection occur with hygromycin. shTRIM28 cells were developed with the use of GIPZ Lentiviral shRNA plasmids. The two plasmid clones used for this study were V3LHS_358502 and V3LHS_640068. The shRNA TRIM28 plasmids consist of a GFP marker and hygromycin selection marker. After transfections, cells were sorted for GFP and stable selection was ensured with hygromycin treatment.

Reverse phase protein array (RPPA): Protein was isolated from HOS-*PVT-1* ctrl and HOS-*PVT-1* cells. Reverse phase protein array assays were carried out as previously described (Satterfield et al. 2017).

Western Blots: Cells were lysed using lysis buffer (1% RIPA buffer supplemented with protease and phosphatase inhibitors (RIPA buffer: Millipore, Protease inhibitor: Roche, Phosphatase Inhibitor: Sigma). Protein concentration was quantified using the BCA method and lysate was denatured in Lammeli Buffer and Beta-Mercaptoethanol (BioRad). Protein lysate was run on a SDS gel and then transferred onto a PVDF membrane (Gel: Bio-Rad, Transmembrane: Invitrogen). The membrane was blocked with either BSA or dry milk. Primary antibodies were then added and allowed to incubate for 24 hours. Antibodies are listed in the supplemental table 3.

RNA Immunoprecipitation (RIP): RIP was performed based upon RIP kit instructions (Millipore).

Drug Sensitivity Study: *PVT-1* Ctrl and *PVT-1* o.e. cells were seeded 1500 cells/well in a 96-well plate. Cell were then treated with serial dilution of TAK-981 (Millennium Pharmaceuticals: Cambridge, MA). Cell viability was assessed at 72 hours after addition of treatment with the use of CCK-8 reagent. Data was normalized to cells treated with only the corresponding drug solvent.