

Supplemental Methods

CD99 and CDKN1A siRNA sequences

siRNA CD99-1	5'GGCUGGCCAUUAUUAAGUC3'
siRNA CD99-2	5'ACCCAGUGCUGGGGAUGAC3'
siRNA CD99-3	5'AUGACGACCCACGACCACCA 3'
siRNA CD99-4	5'AUGCCAACGCAGAGCCAGC3'
siRNA CD99-5	5' CUUCGAGUCCCCGGGCUUCG 3'
siRNA CD99-6	5'CCCUAGUUCUCCGGUAGC3'

siRNA CDKN1A-1	5'CGACUGUGAUGCGCUAAUG3'
siRNA CDKN1A-2	5'CCUAAUCCGCCACAGGAA3'
siRNA CDKN1A-3	5'CGUCAGAACCCAUGCGGCA 3'
siRNA CDKN1A-4	5'AGACCAGCAUGACAGAUUU3'

CD99 siRNA sequences (all from Dharmacon Inc, USA, except siRNA CD99-1 from Ambion) and Scrambled sequences (ON-TARGETplus siCONTROL, Dharmacon) were administered at a final concentration of 80nM and cells were collected after 48h from transfection.

CDKN1A siRNA sequences (Smart pool from Dharmacon Inc, USA) and Scrambled sequences (ON-TARGETplus siCONTROL, Dharmacon) were administered at a final concentration of 100 nM and cells were harvested after 24 h from transfection.

PCR Primers

CD99: Taq Man Gene Expression Assay (Applied Biosystems) (Assay ID Hs00365982_m1, Ref. Seq. NM_002414.3).

GAPDH: Taq Man specific primers and probe were designed by using Primer Express software (Applied Biosystems) as follows (all 5'–3' direction): forward GAAGGTGAAGGTCGGAGTC, reverse GAAGATGGTGATGGGATTTC, probe: FAM-GAAGCTTCCCGTTCTCAGCC.

Western Blotting analysis

Western blotting experiments were performed as previously described (1). The following antibodies were used for western blotting analysis: anti-CD99 12E7 antibody (DAKO, Glostrup, Denmark) (diluted 1:10,000), anti-GAPDH (Santa Cruz Biotechnology Inc., Santa Cruz, CA) (diluted 1:2,000), anti-phospho ERK 1/2

(phospho-p44/p42 MAPK Thr202/Tyr204) (Covance, Emeryville, CA) (diluted 1:1,000), anti-ERK 1/2 (p44/p42 MAPK) (Cell Signalling Technology, Inc., Beverly, MA, USA) (diluted 1:2,000), anti-p21 (Oncogene Science, Cambridge, MA) (diluted 1:500), anti-p27 (Cell Signalling Technology, Inc., Beverly, MA, USA) (diluted 1:1,000), anti-Cyclin D1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA) (diluted 1:1,000), anti-Cyclin A (Santa Cruz Biotechnology Inc., Santa Cruz, CA) (diluted 1:2,000), anti Cyclin B1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA) (diluted 1:2,000), anti-Cdk2 (Santa Cruz Biotechnology Inc., Santa Cruz, CA) (diluted 1:1,000), anti-Cdk4 (Santa Cruz Biotechnology Inc., Santa Cruz, CA) (diluted 1:1,000), anti-Cdc2 (Calbiochem, San Diego, CA,) (diluted 1:1,000), anti-Bcl-2 (Chemicon International, Temecula, CA, USA) (diluted 1:3,000). Anti-mouse or anti-rabbit secondary antibodies conjugated to horseradish peroxidase were from GE Healthcare (Chicago, IL). Proteins were visualized with ECL (GE Healthcare) according to manufacturer's protocols.

Chromatin Immunoprecipitation assay (ChIP)

ChIP assay was performed as described previously (2) Briefly, TC71 and IOR/BRZ cells were washed twice with PBS and crosslinked with 1% formaldehyde at 37 C for 10 min. Next, the cells were washed twice with PBS at 4 C, collected and resuspended in lysis buffer (1% SDS, 10mM EDTA, 50mM Tris-HCl pH 8.1) and left on ice for 10 min. Then, the cells were sonicated four times for 10 s at 30% of maximal power and collected by centrifugation at 4 C for 10 min at 14 000 rpm. The supernatants were collected and diluted in IP buffer (0.01% SDS, 1.1% Triton X-100, 1.2mM EDTA, 16.7mM Tris-HCl pH 8.1, 16.7mM NaCl) and precleared with sonicated salmon sperm DNA/ protein A agarose (UBI) for 1 h at 4C. The precleared chromatin was immunoprecipitated for 12 h with rabbit polyclonal Fli1C-19 (Santa Cruz) antibody. Salmon sperm DNA/protein A agarose was added and precipitation was continued for 4 h at 4 C. After pelleting, precipitates were washed sequentially for 5 min with the following buffers: Wash A (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl pH 8.1, 150mM NaCl), Wash B (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl pH 8.1, 500mM NaCl), and Wash C (0.25M LiCl, 1% NP-40, 1% sodium deoxycholate, 1mM EDTA, 10mM Tris-HCl pH 8.1), and then twice with TE buffer (10mM Tris, 1mM EDTA). The immune complexes were eluted with elution buffer (1% SDS, 0.1M NaHCO₃). The eluates were reverse crosslinked by heating at 65 C for 12 h and digested with proteinase K (0.5 mg/ml) at 45 C for 1 h. DNA was obtained by phenol/chloroform/isoamylalcohol (25:24:1) extractions. Yeast tRNA was added to each sample and DNA was precipitated with EtOH for 12 h at 4 C and then resuspended in TE buffer. PCR was performed with primers flanking Ets-containing CD99 promoter fragment: forward 5'-TTGTTAAGTGTGGAAGGGC-3', and reverse 5'-CTGCTCACCTCAGGGAGTTC-3' (417bp). To validate the efficiency of ChIP assay, PCR for the presence of TGFβR2 promoter sequences, an established direct target of EWS-FLI1, was performed with following pairs of primers: forward 5'-GTGTGGGAGGGCGGTGAGGGGC-3' and reverse 5'-GAGGGAAGCTGCACAGGAGTCCGGC-3' (285bp) (3). The amplification products obtained in 35 cycles were analyzed in a 2% agarose gel and visualized by

ethidium bromide staining. The negative control promoter primers used for ChIP were previously described (S4, S5)

Determination of ERK 1/2 phosphorylation by cell-based enzyme-linked immunosorbent assay

To determine the phosphorylation status of ERK 1/2, cells were seeded into 96-well microplates at the density of 1×10^4 cells/well in 10% FBS IMDM. 48 h after seeding, cells were fixed in 4% formaldehyde at room temperature. a cell-based CASE enzyme-linked immunosorbent assay kit (SuperArray) according to manufacturer's instructions was used to measure ERK1/2 phosphorylation. Absorbance readings were normalized to relative cell number as determined by a cell staining solution. Experiments were done in triplicate.

Proliferation assay

Cell proliferation was assessed by an MTT [3 (4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay according to the manufacturer's recommendations (Roche Diagnostic. Mannheim, Germany). Experiments were done in triplicate.

Motility assay

Motility assay was done using Transwell chambers (Costar). Cells in IMDM or DMEM plus 10% FBS were seeded in the upper compartment, whereas IMDM or DMEM plus 10% FBS was placed in the lower compartment of the chamber. Migrated cells were fixed by methanol, counterstained by Giemsa and counted. Experiments were done in triplicate.

Soft agar assay

Anchorage-independent growth was determined in 0.33% agarose (SeaPlaque, FMC BioProducts) with a 0.5% agarose underlay. Cell suspensions were plated in semisolid medium (IMDM or DMEM 10% FBS plus agar 0.33%) and incubated at 37°C in a humidified 5% CO₂ atmosphere. Colonies were counted after 7 days. Experiments were done in triplicate.

ECM adhesion

The adhesive ability was analyzed by using the CytoMatrix cell adhesion strips coated with human collagen type IV or collagen type I (Chemicon International, Temecula, CA, USA) according to the manufacturer's instructions. Experiments were performed in triplicate.

Analysis of Apoptosis

Detection and quantification of apoptotic cells was obtained by flow cytometric analysis (FACSCalibur, Becton Dickinson, San Jose, CA, USA) of annexin-V-FITC-labelled cells according to the manufacturer's instructions (Medical & Biological Laboratories, Naka-ku Nagaya, Japan).

Cell Cycle Analysis

For the evaluation of BrdUrd labeling index, cell cultures were incubated with 10 μ M bromodeoxyuridine (BrdUrd) (Sigma-Aldrich) for 1 h in CO₂ atmosphere at 37°C. Harvested cells were fixed in 70% ethanol for 30 min. After DNA denaturation with 2 N HCl for 30 min at room temperature, cells were washed with 0.1 M Na₂B₄O₇, pH 8.5, processed for indirect immunofluorescence staining, using α -BrdUrd (Becton Dickinson, Milan, Italy) diluted 1:4 as a primary MAb, and analyzed by flow cytometry (FACSCalibur; Becton Dickinson). For the cell cycle analysis, 70% ethanol-fixed cells were pretreated with 100 μ g/ml RNase for 30 min at 37°C and stained with 20 μ g/ml propidium iodide before flow cytometric analysis.

Neural differentiation

EWS cells were seeded at low density (25,000/dish 60mm) in standard medium. After 24 hours, cells were exposed to low serum medium (1% IMDM) or to NGF (20 ng/ml). 72h later, Western blotting or immunofluorescence analysis was made for H-neurofilament and β III tubulin.

Flow cytometry

Cell lines were analysed on a FACSCalibur flow cytometer (Becton Dickinson). Following antibodies were used: anti-CD99 O13 MAb (dilution 1:80) (Signet, Dedham, MA); anti-vitronectin receptor (α V β ₃ clone LM609, Chemicon International, Temecula CA, USA) (dilution 1:10); CDw49b VLA2 (anti-alpha 2 chain, α 2 β 1) (dilution 1:10), CDw49d VLA4 (anti-alpha 4 chain, α 4 β 1) (dilution 1:10); CDw49e VLA5 (anti-alpha 5 chain, α 5 β 1) (dilution 1:10), CDw49f VLA6 (anti-alpha 6 chain, α 6 β 1) (dilution 1:10); anti-CD45, anti-CD34, anti-CD166, anti-CD105, anti-CD44, anti-CD90 (all from Immunotech S.A., Marseille, France) (dilution 1:2); P1B5 (anti-alpha3 chain, α 3 β 1) (dilution 1:20); anti-IGF-IR (clone α IR3) (dilution 1:10); anti- α PDGF-R β (clone MM95) (dilution 1:20); anti-EGF-R (dilution 1:10); anti-trkA (dilution 1:10); anti-FGF β (dilution 1:40) (all from Calbiochem-Novabiochem, San Diego CA, USA); anti-c-kit monoclonal antibody YB5.B8 (dilution 1:40) (Pharmingen, San Diego CA, USA); anti-HER2 (clone TAB 250) (dilution 1:400) (Zymed Laboratories Inc. San Francisco, CA); anti-TGF receptor β (clone C-16) (dilution 1:5) (Santa Cruz).

Immunostaining and laser scanning confocal microscopy

Immunofluorescence was performed on adherent cells grown on coverslips for 48 h and fixed in 4% para-formaldehyde or in methanol/acetone 3:7, and permeabilized with 0.15% Triton X-100 in phosphate buffered saline (PBS), and incubated with the following antibodies: anti-phospho ERK 1/2 (phospho-p44/p42 MAPK Thr202/Tyr204) (dilution 1:10) (Covance Inc.); anti-ERK 1/2 (p44/p42 MAPK) (Cell signaling) (dilution 1:10); anti-TUB β III (dilution 1:50) (Sigma), anti-CD99 O13 MAb (dilution 1:80) (Signet), anti-H neurofilament 200kD (clone NE14) (dilution 1:5) (Roche Diagnostic Mannheim). After fixation procedure, murine cell lines were incubated with anti-mouse normal horse serum 1:50 to avoid aspecific binding of secondary antibody. Nuclei were counterstained by propidium iodide (0.05 μ g/ml) or with bisbenzimidazole Hoechst 33258 (0.5 μ g/ml).

In human primary bone marrow MSCs, CD99 and H-neurofilament were detected by avidin-biotin immunostaining according to standard protocol.

Cell fluorescence was then evaluated with a QUIPS-XL image analysis system (Vysis Inc., Downers Grove, IL) and with laser scanning confocal microscopy using a Leica TCS SP2 spectral confocal microscope equipped with argon-helium neon (Ar-HeNe) lasers (Leica Microsystems, Mannheim, Germany). Signals from different fluorescent probes were taken in sequential double fluorescence mode, which allows the elimination of channels cross-talk, the colocalization was detected in an overlay model. Images acquisition and processing were conducted using the SCANware and Multicolor Analysis Leica programs. Acquisition parameters were as follow: 63.0/1.4 NA objective; image size: 1024x1024; pinhole size: 1 Airy; step size: 0.5 μ m.

Alizarin Red S staining

Alizarin Red S staining was used to visualize bone mineralization. The cells were fixed in 10% formalin (Sigma Aldrich) at room temperature and stained with 2% ARS (Sigma Aldrich).

Microarray Experiments

Agilent Microarray Experiments

Hybridizations were performed on Human 1A (V2) Oligo Microarray slides (Agilent Technologies) containing 18,716 oligo probes (60-mer length). Total RNA was used to obtain labeled cRNA, according to the manufacturer's instructions (Low RNA Input Fluorescent Linear Amplification Kit-Agilent Technologies). TCsiCD99/23, TCsiCD99/24, TCsiCTR, BRZsiCD99/36, BRZsiCD99/78 and BRZsiCTR cRNAs were labeled with Cyanine 5-CTP (Cy5) (Perkin Elmer Life Sciences Inc., Boston, MA), while TC-71 and IOR/BRZ parental cell lines were labeled with Cyanine 3-CTP (Cy3) and used as a common reference for all the comparisons. In brief, 5 μ g of total RNA were reverse-transcribed with a T7 promoter primer using Moloney murine leukaemia virus reverse transcriptase. Next cRNA was synthesized from the double-stranded cDNA using T7 RNA polymerase to incorporate Cy3-CTP or Cy5-CTP. Labeled cRNAs were purified with RNeasy Mini Protocol for RNA Cleanup (Qiagen, GmbH, Germany). After purification, Cy3 and Cy5 labeled cRNAs were fragmented by 25X fragmentation buffer at 60°C in water bath for 30' minutes and hybridized to microarray slides at 60°C for 17 hours according to the manufacturer's

instructions. The slides were washed with 6X SSPE + 0.005% N-Lauroylsarcosine for 1 minute, with 0.06x SSPE + 0.005% N-Lauroylsarcosine for 1 minute and with Drying solution for 30 seconds (Agilent Technologies). Hybridized slides were scanned using GenePix 4000B microarray scanner (Axon Instruments) that acquires data at two wavelengths simultaneously [532nm (green) and 635nm (red)]. Signal and background intensities for both channels were calculated with GenePix 3.0 (Axon Instruments). Spots showing evident blemishes were flagged and excluded from analysis.

Raw expression data was normalized with LOWESS regression function. In the lowess normalization, a non-linear lowess smoother function is fit to the graph of un-normalized log-ratio on the y-axis versus average log intensity (i.e., $[\log(\text{Red}) + \log(\text{Green})]/2$) on the x-axis. This is the so-called M-A plot. The lowess smoother is based on a concatenation of linear regressions for points in overlapping intervals on the x-axis of the M-A plot. Lowess smoother value is 0.4 and that is subtracted from the un-normalized log-ratios for the array in order to obtain the normalized log-ratios. All datasets were filtered according to log expression variation filter, the variance of the log-ratios for each gene is compared to the median of all the variances, setting the significance threshold at <0.05 . Genes not significantly more variable than the median gene are filtered out. Specifically, the quantity $(n-1) \text{ Vari} / \text{Varmed}$ is computed for each gene i . Vari is the variance of the log intensity for gene i across the entire set of n arrays and Varmed is the median of these gene-specific variances. This quantity is compared to a percentile of the chi-square distribution with $n-1$ degrees of freedom. (BRB-ArrayTools <http://linus.nci.nih.gov/BRB-ArrayTools.html>).

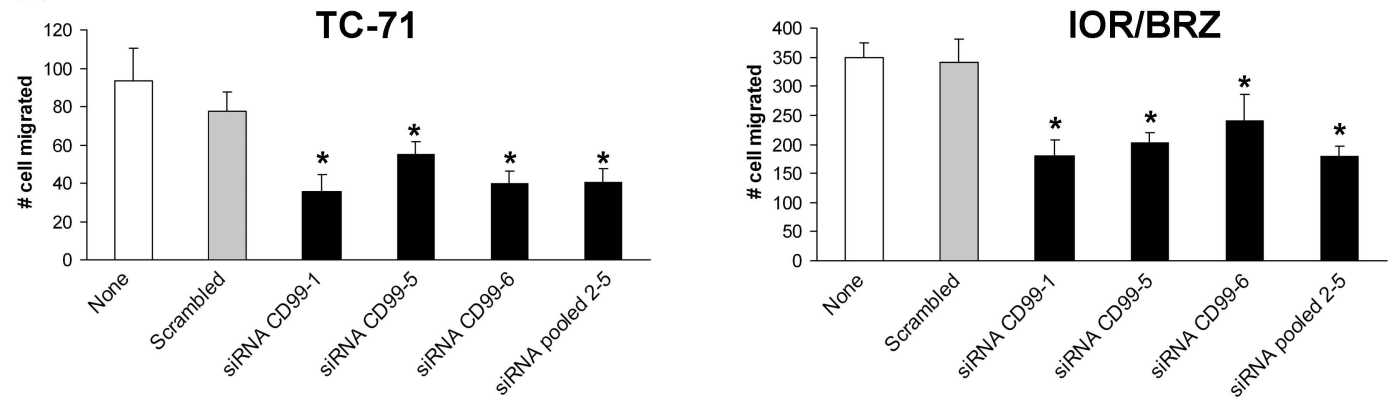
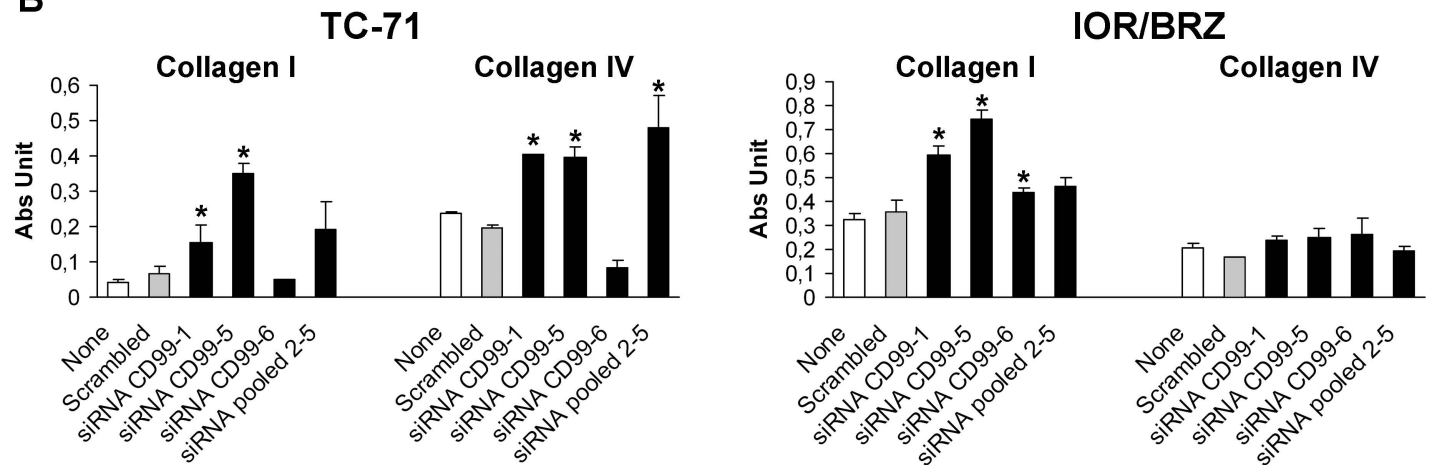
To identify genes whose expression changes in response to CD99 expression level, we rank-ordered the genes using a supervised approach. We first filtered the complete dataset as described above. Statistical comparison between siCD99 and siCTR was performed with BRB Array Tools software, applying a Random Variance Model (RVM) t-test setting p-val threshold for significance <0.05 .

Reference:

- S1. Scotlandi, K., Zuntini, M., Manara, M.C., Sciandra, M., Rocchi, A., Benini, S., Nicoletti, G., Bernard, G., Nanni, P., Lollini, P.L., et al. 2007. CD99 isoforms dictate opposite functions in tumour malignancy and metastases by activating or repressing c-Src kinase activity. *Oncogene* 26:6604-6618.
- S2. Garofalo, C., Sisci, D., and Surmacz, E. 2004. Leptin interferes with the effects of the antiestrogen ICI 182,780 in MCF-7 breast cancer cells. *Clin Cancer Res* 10:6466-6475.
- S3. Siligan, C., Ban, J., Bachmaier, R., Spahn, L., Kreppel, M., Schaefer, K.L., Poremba, C., Aryee, D.N., and Kovar, H. 2005. EWS-FLI1 target genes recovered from Ewing's sarcoma chromatin. *Oncogene* 24:2512-2524.
- S4. Zupanska, A., Adach, A., Dziembowska, M., and Kaminska, B. 2007. Alternative pathway of transcriptional induction of p21WAF1/Cip1 by

cyclosporine A in p53-deficient human glioblastoma cells. *Cell Signal* 19:1268-1278.

- S5. Herrero-Martin, D., Osuna, D., Ordonez, J.L., Sevillano, V., Martins, A.S., Mackintosh, C., Campos, M., Madoz-Gurpide, J., Otero-Motta, A.P., Caballero, G., et al. 2009. Stable interference of EWS-FLI1 in an Ewing sarcoma cell line impairs IGF-1/IGF-1R signalling and reveals TOPK as a new target. *Br J Cancer* 101:80-90.

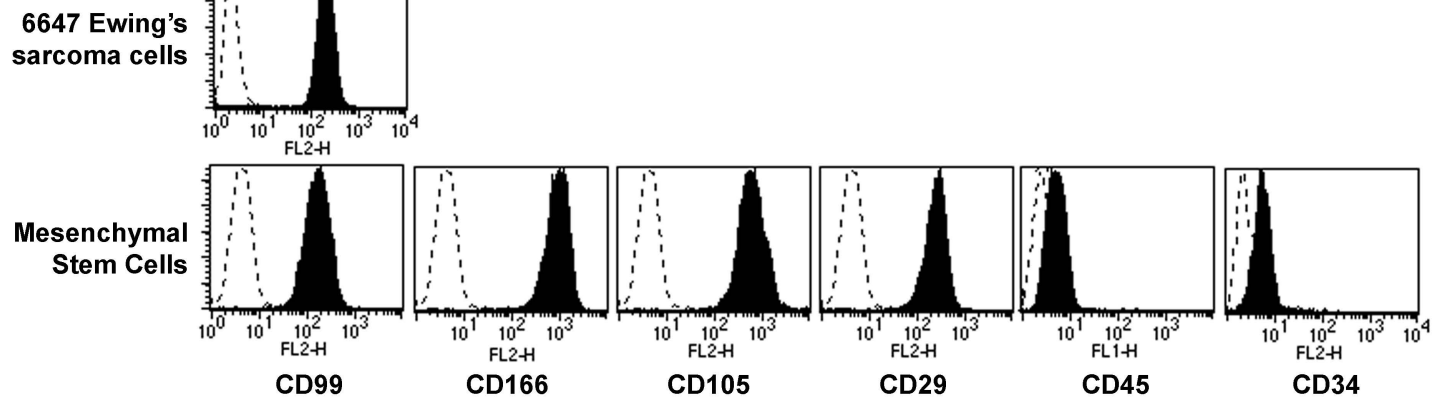
A**B**

Rocchi *et al.*, Supplemental Figure 1

(A) Migratory inhibition due to CD99 loss was confirmed in transient conditions by using three additional sequences plus seq.1 that was the basis for plasmid-RNAi construction.

(B) Increased adhesion to collagen I and IV due to CD99 loss was substantially confirmed in transient conditions by using three additional sequences plus seq.1 that was the basis for plasmid-RNAi construction.

A



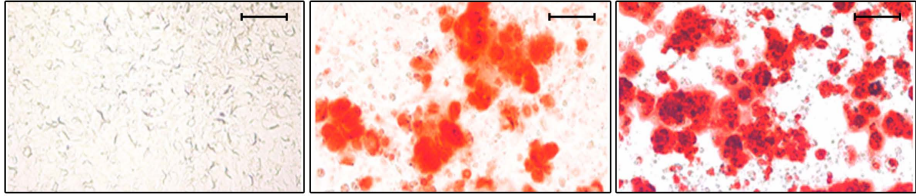
B

Osteoblastic differentiation

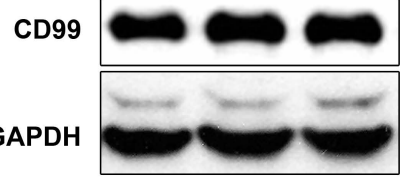
day 0

day 7

day 14



day 0 day 7 day 14



Rocchi *et al.*, Supplemental Figure 2

A. Expression of CD99 in normal mesenchymal stem cells (MSCs), which were positive for three cell surface markers (CD166, CD105, CD29) and negative for CD45 and CD34, two markers of hematopoietic stem cells. MSCs expressed CD99 at levels comparable to that found on patient-derived 6647 Ewing's sarcoma cells.

B. MSCs were cultured in osteogenic medium and terminal differentiation was monitored by Alizarin Red-S staining that visualized the appearance of mineralized bone nodules. Scale bar = 600µm. CD99 expression is maintained during osteoblastic differentiation, as indicated by Western blotting.

(A) We applied Principal component analysis (PCA) on EWS-FLI-1-silenced Ewing's sarcoma cells derived from SK-N-MC, EW24 (data set from Tirode et al, 2007) , EWS502 and TC-71 cell line (data set from Kinsey et al, 2006) and showed that the CD99 signature allowed for a clear separation of EWS/FLI knockdown cells from controls. Circles indicate EWS-FLI1 silenced cells, triangles indicated controls.

(B) Hierarchical clustering of EWS-FLI-1-silenced Ewing's sarcoma cells using the CD99-associated signature. Data set for SK-N-MC and EW24 derives from Tirode et al, 2007, while that for EWS502 and TC-71 cell lines derives from Kinsey et al, 2006.

Rocchi et al, Supplemental Table 2.

CD99 molecular signature based on TC-71 CD99-shRNA cells.

Agilent ID	Gene Symbol	p-value	Mean Log Ratio TC-CD99-shRNA clones
A_23_P208031	SYT4	0.0405273	2.941211373
A_23_P150609	IGF2	0.0318831	2.742986359
A_23_P38244	APOH	0.01938	2.369150506
A_23_P82651	NPTX2	0.0381212	2.260888688
A_23_P214950	PERP	0.0030853	2.258936841
A_23_P378450	MBD3L2	0.0119145	1.880065069
A_23_P377284	TGFA	0.0256738	1.758038413
A_23_P369298	PERP	0.0127363	1.72997142
A_23_P160117	EDG1	0.000174	1.715002417
A_23_P205757	DLK1	0.0002983	1.654226176
A_23_P326931	TTC18	0.0491484	1.62125732
A_23_P327380	TP73L	0.0276321	1.386691481
A_23_P40108	COL9A3	0.0465385	1.378460646
A_23_P164706	ZNF177	0.0262984	1.375578102
A_23_P207521	COL1A1	0.0319283	1.33636548
A_23_P361014	TSHZ3	0.0388648	1.318601746
A_23_P94800	S100A4	0.003106	1.230329823
A_23_P30776	HIST1H2BE	0.0282291	1.227724753
A_23_P134125	MAP3K5	0.0358448	1.215717837
A_23_P42178	HIST1H2BF	0.0212113	1.191524841
A_23_P360213	ENST00000361227	0.0143736	1.152079358
A_23_P8013	HIST1H2BL	0.0204959	1.136855911
A_23_P402081	HIST1H2BN	0.036295	1.127129491
A_23_P64029	AK128061	0.0032798	1.106967594
A_23_P93180	HIST1H2BC	0.0140897	1.075146217
A_23_P111042	HIST1H2BI	0.0179712	1.070788075
A_23_P19379	HIST1H2BD	0.0288574	1.058214039
A_23_P131846	SNAI1	0.0049027	1.049023587
A_23_P161595	MARK2	0.0445185	1.047482554
A_23_P133656	LAMA4	0.0204524	1.046562996
A_23_P204870	CAB39L	0.0025135	1.0209004
A_23_P145237	HIST1H2BK	0.0140436	1.008825459
A_23_P133851	HIST1H2BJ	0.0161961	0.960445415
A_23_P95899	IGSF21	0.0298913	0.938628793
A_23_P52697	CD248	0.0260357	0.93115347
A_23_P132936	SPCS3	0.0056178	0.923937887
A_23_P109539	APOBEC3B	0.0209941	0.888087038
A_23_P108075	SLC7A10	0.0128831	0.886838019
A_23_P111054	HIST1H2BB	0.0025503	0.883088248
A_23_P428184	HIST1H2AD	0.0280682	0.875172198
A_23_P413696	C21orf129	0.009915	0.855599605
A_23_P19408	HIST1H2BM	0.0405951	0.853875615
A_23_P332992	HIST3H2BB	0.0464222	0.84800107
A_23_P96340	CLCN5	0.0239009	0.810598738
A_23_P18223	ITIH1	0.0499999	0.803701422
A_23_P104833	KIAA0999	0.0417597	0.787340404
A_23_P143906	MLF1	0.005817	0.781416528
A_23_P44436	GKN1	0.0160068	0.780783478
A_23_P431851	ENST00000361453	0.0118651	0.773061804

A_23_P63736	MGC16291	0.0317136	0.769561361
A_23_P15272	ABCC6	0.0460371	0.748084281
A_23_P142986	ENST00000258092	0.0103909	0.740770273
A_23_P85371	RABGGTB	0.0288905	0.735202815
A_23_P31873	RAB11FIP1	0.0466888	0.731655583
A_23_P22134	BNC1	0.0099923	0.726386986
A_23_P81795	LRFN2	0.006393	0.70404401
A_23_P255587	ATF7IP	0.0006165	0.70384109
A_23_P126225	PAX7	0.008566	0.703336701
A_23_P75430	C11orf75	0.0014712	0.692198226
A_23_P109974	RAB6B	0.0203309	0.68604067
A_23_P134058	C6orf114	0.0177721	0.685173362
A_23_P102113	WNT10A	0.027559	0.669707302
A_23_P336040	GOPC	0.000476	0.669347592
A_23_P11874	MPZL1	0.0279357	0.668336973
A_23_P31460	STEAP1	0.0076353	0.661805214
A_23_P401718	CCDC74B	0.0004001	0.65894505
A_23_P428748	FAM84B	0.0364633	0.658719194
A_23_P59397	RSHL2	0.038756	0.658476144
A_23_P142830	PLA2R1	0.0349044	0.648851529
A_23_P62901	BTG2	0.0151346	0.644287556
A_23_P354704	ST8SIA1	0.0424912	0.631246649
A_23_P53217	EED	0.0362112	0.629588721
A_23_P420749	MLL	0.0021891	0.62819129
A_23_P67708	TCF3	0.0388994	0.626052447
A_23_P32938	DDX10	0.0238145	0.62436435
A_23_P162640	GABARAPL1	0.0365693	0.6223801
A_23_P63807	NRBF2	0.0175478	0.621433362
A_23_P253963	TMEM70	0.0454959	0.619689435
A_23_P206626	ENST00000300061	0.0187859	0.619109228
A_23_P73632	NR0B1	0.0285254	0.601819694
A_23_P258477	HIST1H2AL	0.0318099	0.595471967
A_23_P112159	EIF2C2	0.0142335	0.593117647
A_23_P54367	RAB27A	0.0286349	0.592501038
A_23_P127175	SAR1A	0.0433987	0.592001107
A_23_P203767	LGR4	0.0352305	0.585235514
A_23_P203027	RDX	0.0212833	0.580936843
A_23_P54992	DYNLL2	0.0413321	0.580279709
A_23_P112950	ATF7IP	0.0089446	0.57938312
A_23_P201628	LAMC1	0.0142526	0.578952294
A_23_P156976	HUS1	0.0163166	0.560082532
A_23_P88404	TGFB3	0.0442309	0.553091208
A_23_P13524	TMEM126A	0.0395512	0.522183524
A_23_P119554	PSG8	0.0435814	0.518566249
A_23_P71718	KCNT1	0.0459437	-0.529202402
A_23_P107701	ERCC1	0.0487344	-0.530297883
A_23_P214151	SENP6	0.0464815	-0.530310767
A_23_P88012	A_23_P88012	0.0369656	-0.536661374
A_23_P20392	PSD3	0.0264572	-0.549365973
A_23_P68068	WDR54	0.0273989	-0.549569549
A_23_P350551	C12orf57	0.032345	-0.549715512
A_23_P65448	C14orf94	0.0438966	-0.552167042
A_23_P65208	ZMYM5	0.0191832	-0.579881024
A_23_P253958	LRRC17	0.0087933	-0.590367781

A_23_P211631	FBLN1	0.04765	-0.59298075
A_23_P5541	PUM2	0.0447673	-0.599440896
A_23_P111037	HIST1H3A	0.0102079	-0.603640366
A_23_P138271	ARL8A	0.0103723	-0.605751967
A_23_P416581	GNAZ	0.0038287	-0.616890132
A_23_P503182	ABR	0.0085518	-0.623210154
A_23_P144150	DCUN1D1	0.0174666	-0.625319619
A_23_P167256	PHF17	0.0078064	-0.632884853
A_23_P112061	TMEM76	0.0331887	-0.636093277
A_23_P111701	GNG11	0.0451842	-0.636474254
A_23_P137909	HIST3H3	0.0223402	-0.645246764
A_23_P433071	BECN1	0.0023617	-0.647198895
A_23_P207811	PAIP1	0.0044542	-0.648687389
A_23_P23141	H3F3A	0.0094545	-0.650338602
A_23_P166360	PRAME	0.0159409	-0.658593792
A_23_P46767	NHLRC2	0.0494833	-0.664297547
A_23_P164797	ZNF580	0.0051911	-0.665267556
A_23_P250607	PLS3	0.0028573	-0.674951304
A_23_P99404	ZMYM2	0.0417751	-0.67833804
A_23_P327551	CPNE4	0.0310572	-0.68059307
A_23_P335329	GNG4	0.0392083	-0.694732264
A_23_P170927	ASMTL	0.0451061	-0.694991289
A_23_P150566	BSCL2	0.0017316	-0.696981713
A_23_P21017	RP6-213H19.1	0.0156372	-0.711270161
A_23_P138226	OLFM3	0.0429171	-0.713382646
A_23_P134925	BNIP3L	0.0335403	-0.713835981
A_23_P96652	ENST00000382832	0.0025731	-0.716658235
A_23_P431305	FAM69B	0.0257191	-0.717545887
A_23_P352266	BCL2	0.0027955	-0.720220865
A_23_P94159	FBXO25	0.0011961	-0.72328493
A_23_P18692	ADH5	0.0123283	-0.744395227
A_23_P217470	IDS	0.003063	-0.749112671
A_23_P118266	ATBF1	0.0395259	-0.768768108
A_23_P217952	HK1	0.0115614	-0.790338963
A_23_P66017	PRRT2	0.0340476	-0.816737567
A_23_P201711	S100A6	0.002356	-0.816759735
A_23_P148308	RBM3	0.0253811	-0.87483176
A_23_P431360	ZNF219	0.0447954	-0.908176344
A_23_P105251	GLI1	0.0151693	-0.943512334
A_23_P6321	CLDN5	0.0074693	-1.010853695
A_23_P169766	THC2370681	0.0092948	-1.015125461
A_23_P43675	ZNF618	0.0390967	-1.141353872
A_23_P69208	TIMP4	0.0131217	-1.331186548
A_23_P217510	CD99	0.011674	-1.95666207

Rocchi *et al*, Supplemental Table 3.

CD99 molecular signature based on TC-71 and IOR/BRZ CD99-shRNA cells.

Agilent ID	Gene Symbol	p-value	Mean Log Ratio CD99-shRNA clones
A_23_P205757	DLK1	0.0025802	2.138670963
A_23_P214950	PERP	0.0051832	1.434286937
A_23_P124084	LOXL1	0.0435225	1.22080599
A_23_P253446	GAP43	0.0012648	1.174257917
A_23_P103981	HIST2H2AA3	0.0119917	1.057585432
A_23_P145237	HIST1H2BK	0.0013596	1.056667589
A_23_P56746	FAP	0.026535	1.026336379
A_23_P326931	TTC18	0.0219652	0.97938699
A_23_P353035	IGFBP7	0.0397349	0.976251507
A_23_P160117	EDG1	0.0071108	0.960235663
A_23_P369298	PERP	0.0220699	0.9572848
A_23_P259442	CPE	0.0427876	0.935968458
A_23_P35066	SNX7	0.0002432	0.903396833
A_23_P377284	TGFA	0.0381581	0.898789976
A_23_P79069	FLJ21438	0.0265307	0.895271467
A_23_P19379	HIST1H2BD	0.0011393	0.879820317
A_23_P30776	HIST1H2BE	0.003332	0.859314814
A_23_P8013	HIST1H2BL	0.0014899	0.854682207
A_23_P402081	HIST1H2BN	0.0076317	0.852393095
A_23_P122445	HIST1H1C	0.0416025	0.848184359
A_23_P401718	CCDC74B	0.0000121	0.837083058
A_23_P133851	HIST1H2BJ	0.0005106	0.823949302
A_23_P42178	HIST1H2BF	0.0029356	0.822447218
A_23_P131935	C20orf42	0.0019001	0.813567759
A_23_P86059	KIAA1822L	0.0311689	0.812631471
A_23_P366216	HIST1H2BH	0.0058338	0.802678376
A_23_P54996	TEX14	0.0432853	0.795937872
A_23_P209625	CYP1B1	0.0111001	0.786585723
A_23_P40470	H2BFS	0.0135808	0.784497162
A_23_P170713	A_23_P170713	0.0227754	0.780104253
A_23_P93180	HIST1H2BC	0.0032179	0.773949673
A_23_P111042	HIST1H2BI	0.003238	0.762525602
A_23_P155197	PTPLB	0.0428478	0.752538562
A_23_P19408	HIST1H2BM	0.0050134	0.736187808
A_23_P94800	S100A4	0.0358012	0.730786167
A_23_P59069	HIST1H2BO	0.017257	0.728847684
A_23_P111054	HIST1H2BB	0.0001832	0.726864097
A_23_P139500	BHLHB3	0.0219376	0.720681389
A_23_P7791	OGFRL1	0.0239989	0.720366418
A_23_P202520	ABLIM1	0.0467583	0.718269275
A_23_P36795	SYT1	0.0241737	0.716673627
A_23_P86838	SLC36A4	0.0037152	0.68837339
A_23_P202232	MKI67	0.0448069	0.662191248
A_23_P156036	FSIP2	0.0362458	0.656895481
A_23_P82526	ABCB1	0.0230264	0.638820461
A_23_P10902	FRZB	0.0203446	0.638468117
A_23_P109974	RAB6B	0.0104225	0.637452386
A_23_P52697	CD248	0.0411987	0.619940313
A_23_P421401	PDGFRB	0.0395072	0.61911751

A_23_P64029	AK128061	0.0197762	0.610087415
A_23_P144668	LOC134147	0.0101668	0.59938874
A_23_P259270	CDKL2	0.01755	0.59705201
A_23_P204870	CAB39L	0.013634	0.596115342
A_23_P131846	SNAI1	0.0065915	0.585274189
A_23_P147431	LYN	0.0118176	0.583200846
A_23_P94533	CTSL	0.025502	0.567542573
A_23_P108143	GAMT	0.0339881	0.556719963
A_23_P428184	HIST1H2AD	0.0222939	0.539880982
A_23_P138811	CHORDC1	0.0293676	0.539801608
A_23_P132936	SPCS3	0.0495494	0.532257497
A_23_P63736	MGC16291	0.0239819	0.516176933
A_23_P139825	YAF2	0.0168744	0.516017469
A_23_P251002	A_23_P251002	0.0094296	0.500372306
A_23_P57709	PCOLCE2	0.0419818	0.498708833
A_23_P45025	MAPK10	0.0395662	0.498606871
A_23_P39525	FLJ22746	0.0111329	0.49093552
A_23_P37205	NDRG2	0.049115	0.48608632
A_23_P40445	CBR3	0.0321906	0.480516389
A_23_P122662	GFOD1	0.0488522	0.471556881
A_23_P363936	HSPA4L	0.0339658	0.460061939
A_23_P34744	CTSK	0.03895	0.452345707
A_23_P97826	TECTB	0.0406975	0.435994007
A_23_P54992	DYNLL2	0.0439261	0.429621903
A_23_P339098	SLC35F2	0.0456297	0.42452458
A_23_P328074	SALL1	0.0483558	0.417079319
A_23_P170927	ASMTL	0.0471427	-0.415666611
A_23_P502832	RBM12	0.0428965	-0.421403549
A_23_P431305	FAM69B	0.0357512	-0.428195062
A_23_P91019	PRKRA	0.049216	-0.465495368
A_23_P46767	NHLRC2	0.0250755	-0.470098215
A_23_P89801	ACAA2	0.0202473	-0.471044533
A_23_P215060	PODXL	0.0441064	-0.473469069
A_23_P379645	BMF	0.0378	-0.489488429
A_23_P4353	WSB1	0.0221401	-0.493825138
A_23_P17914	PNPLA3	0.011091	-0.493939505
A_23_P201789	JARID1B	0.0098654	-0.497396161
A_23_P33196	COL5A2	0.0399512	-0.500510186
A_23_P21017	RP6-213H19.1	0.006092	-0.515096936
A_23_P211631	FBLN1	0.0301087	-0.524627794
A_23_P20502	SLC39A4	0.0350531	-0.594505822
A_23_P125387	TMEFF2	0.002439	-0.611035898
A_23_P138635	BNIP3	0.035822	-0.63067394
A_23_P110122	CCNG2	0.0091373	-0.672591831
A_23_P66017	PRRT2	0.0075199	-0.69610803
A_23_P63343	UTS2	0.0496601	-0.733197661
A_23_P163440	RKHD3	0.0176879	-0.76649414
A_23_P335329	GNG4	0.0008995	-0.770754876
A_23_P19482	DDAH2	0.0171026	-0.812375048
A_23_P252928	MAGEA12	0.0179149	-0.87688188
A_23_P148255	MAGEA2B	0.0164974	-0.924126213
A_23_P53126	LMO2	0.0107592	-1.024355941
A_23_P217510	CD99	0.0002708	-1.596820507
A_23_P159637	CSAG2	0.022494	-1.961808983