Table S1. The microarray expression data sets used in this study are publicly available (continued).

Entity	GEOAccession codes		Title of Samples
	Series	**Sample**	
OS cell line (n=29)	GSE70414	GSM1727193 GSM1727194 GSM1727195 GSM1727196 GSM1727197	1. MG63 [mRNA] 2. Saos [mRNA] 3. HOS [mRNA] 4. NY [mRNA] 5. Hu09 [mRNA]
	GSE30807	GSM764200	6. osteosarcoma U2OS cells
	GSE37552	GSM921514 GSM921515 GSM921516 GSM921517	7. Human_Osteosarcoma_Non-Metastatic_SaOS-2_Parental Line 8. Human_Osteosarcoma_ Metastatic_LM7_Subline of SaOS-2 9. Human_Osteosarcoma_Non-Metastatic_HOS_Parental Line 10. Human_Osteosarcoma_ Metastatic_143B_Subline of HOS
	GSE18947	GSM469260 GSM469261 GSM469264	11. low metastatic potential cell subline of Sosp-9607, biological rep1 12. low metastatic potential cell subline of Sosp-9607, biological rep2 13. low metastatic potential cell subline of Saos-2
	GSE16089	GSM402655 GSM402656 GSM402657	14. Saos-2 parental cells replicate 1 15. Saos-2 parental cells replicate 2 16. Saos-2 parental cells replicate 3
	GSE7454	GSM180459	17. U-2OS (no-treatment) expression profile (AffyChip U133 Plus 2.0 platform)
	GSE41828	GSM1025090 GSM1025091 GSM1025092 GSM1025093 GSM1025094	18. U2OS, Untreated 24 hours rep1 19. U2OS, Untreated 24 hours rep2 20. U2OS, Untreated 24 hours rep3 21. U2OS, Untreated 24 hours rep4 22. U2OS, Untreated 24 hours rep5
	GSE46493	GSM1131226 GSM1131227 GSM1131228	23. U2OS No Treatment, Replicate 1 24. U2OS No Treatment, Replicate 2 25. U2OS No Treatment, Replicate 3

Table S1. *Cont.*

Entity	GEOAccession codes		Title of Samples
	Series	**Sample**	
OS cell line (n=29)	GSE41445	GSM1017514	26. U2OS_21a
		GSM1017515	27. U2OS_21b
		GSM1017516	28. U2OS_21c
	GSE55957	GSM1349294	29. ZOS osteosaracoma cell line
Primay OS cells (n=3)	GSE85537	GSM2276632	30. Bone1, biological rep1
		GSM2276633	31. Bone2, biological rep2
		GSM2276634	32. Bone3, biological rep3
Healthy whole blood (n=43)	GSE93272	GSM2449608	33. Whole blood from healthy control(HC003_1)
		GSM2449609	34. Whole blood from healthy control(HC004_1)
		GSM2449610	35. Whole blood from healthy control(HC005_1)
		GSM2449611	36. Whole blood from healthy control(HC006_1)
		GSM2449612	37. Whole blood from healthy control(HC007_1)
		GSM2449613	38. Whole blood from healthy control(HC008_1)
		GSM2449614	39. Whole blood from healthy control(HC009_1)
		GSM2449615	40. Whole blood from healthy control(HC010_1)
		GSM2449616	41. Whole blood from healthy control(HC011_1)
		GSM2449617	42. Whole blood from healthy control(HC012_1)
		GSM2449618	43. Whole blood from healthy control(HC013_1)
		GSM2449619	44. Whole blood from healthy control(HC015_1)
		GSM2449620	45. Whole blood from healthy control(HC016_1)
		GSM2449621	46. Whole blood from healthy control(HC017_1)
		GSM2449622	47. Whole blood from healthy control(HC018_1)
		GSM2449623	48. Whole blood from healthy control(HC019_1)
		GSM2449624	49. Whole blood from healthy control(HC020_1)
		GSM2449625	50. Whole blood from healthy control(HC021_1)
		GSM2449626	51. Whole blood from healthy control(HC022_1)
		GSM2449627	52. Whole blood from healthy control(HC023_1)
		GSM2449628	53. Whole blood from healthy control(HC024_1

Table S1. *Cont.*

Entity	GEOAccession codes		Title of Samples
	Series	**Sample**	
Healthy whole blood (n=43)	GSE93272	GM2449629	54. Whole blood from healthy control(HC025_1)
		GSM2449630	55. Whole blood from healthy control(HC026_1)
		GSM2449631	56. Whole blood from healthy control(HC027_1)
		GSM2449632	57. Whole blood from healthy control(HC028_1)
		GSM2449633	58. Whole blood from healthy control(HC030_1)
		GSM2449634	59. Whole blood from healthy control(HC031_1)
		GSM2449635	60. Whole blood from healthy control(HC034_1)
		GSM2449636	61. Whole blood from healthy control(HC035_1)
		GSM2449637	62. Whole blood from healthy control(HC036_1)
		GSM2449668	63. Whole blood from healthy control(AU_2)
		GSM2449669	64. Whole blood from healthy control(AW_2)
		GSM2449670	65. Whole blood from healthy control(AY_2)
		GSM2449671	66. Whole blood from healthy control(BA_2)
		GSM2449672	67. Whole blood from healthy control(BB_2)
		GSM2449673	68. Whole blood from healthy control(HC003_2)
		GSM2449674	69. Whole blood from healthy control(HC005_2)
		GSM2449675	70. Whole blood from healthy control(HC008_2)
		GSM2449676	71. Whole blood from healthy control(HC023_2)
		GSM2449677	72. Whole blood from healthy control(HC025_2)
		GSM2449678	73. Whole blood from healthy control(HC026_2)
		GSM2449679	74. Whole blood from healthy control(HC034_2)
		GSM2449680	75. Whole blood from healthy control(HC036_2)

Table S2. Clinical characteristics of OS patients and healthy donors (primary cells and PBMCs, respectively).

Parameters		**OS patients (n=24)**	**Healthy donors (n=12)**
Median age [range]		19 [5–61]	22 [19–29]
Gender	Male	12 (50.0%)	5 (41.7%)
	Female	12 (50.0%)	7 (58.3%)
Enneking stage	IIB	19 (79.2%)	-
	III	5 (20.8%)	-
Tumor location	Femur	13 (51.2%)	-
	Tibia	6 (25.0%)	-
	Other	5 (20.8%)	-
Metastasis	Bone	1 (4.2%)	-
	Lung	3 (12.5%)	-
	Bone and lung	1 (4.2%)	-
	None	19 (79.2%)	

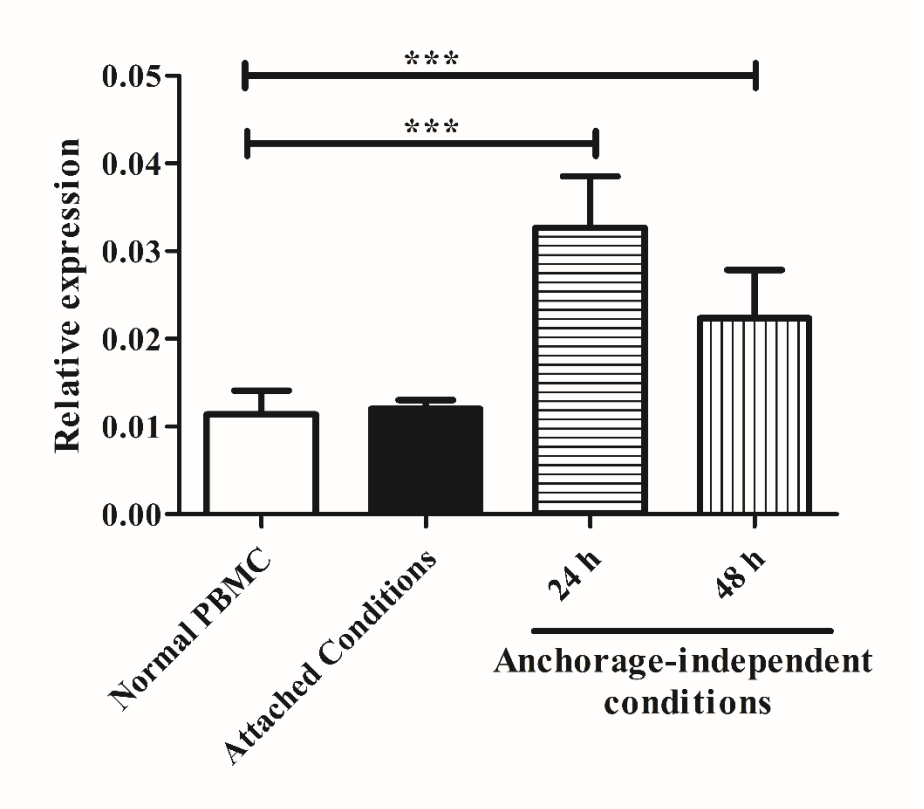

Figure S1. Comparison of the expression of ezrin among normal PBMCs, SaOS-2 (Human OS cell lines) in attached conditions and anchorage-independent conditions. The relative expression of *ezrin* was quantified by qRT-PCR in 12 samples of normal PBMCs and SaOS-2. Expression data were normalized on β-actin RNA level by the $2^{-\Delta Ct}$ method. Each sample was analyzed in triplicate. Data are displayed as vertical scatter plots with bars representing mean ± SD. The One-Way ANOVA test was used to determine *p*-values: ***, $p < 0.0001$.

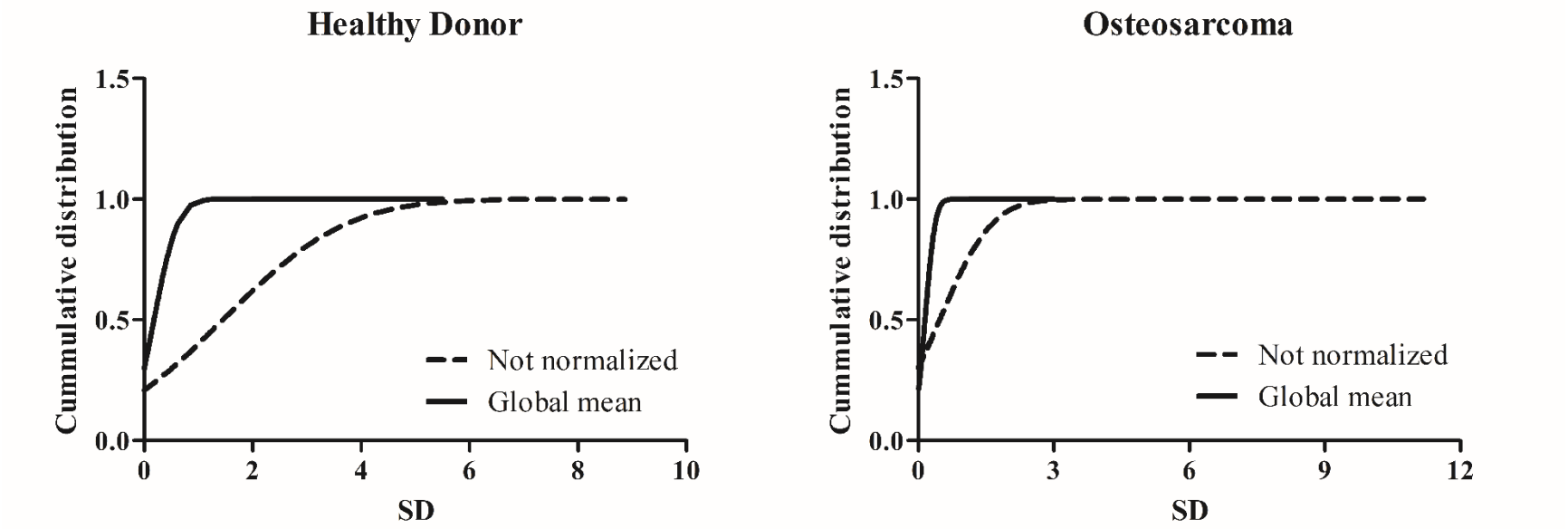

Figure S2. Cumulative distributions (CV) of the standard deviation (SD). Standard deviations (SDs) for each individual gene in the heathy and OS buffy coat samples presented not normalized (- -) and global mean normalized expression data (—).

Materials and Methods

Cell culture as anchorage-independent conditions

To mimic circulating tumor cell condition, Saos-2 osteosarcoma cell line was cultured on 6-well culture plate which coated with 450 μl of 6 mg/ml poly(2-hydroxyethyl methacrylate) or polyHEMA (Sigma-Aldrich, St. Louis, MO, USA) each well for 24 and 48 hours. The resistant osteosarcoma cells were observed under light microscope.