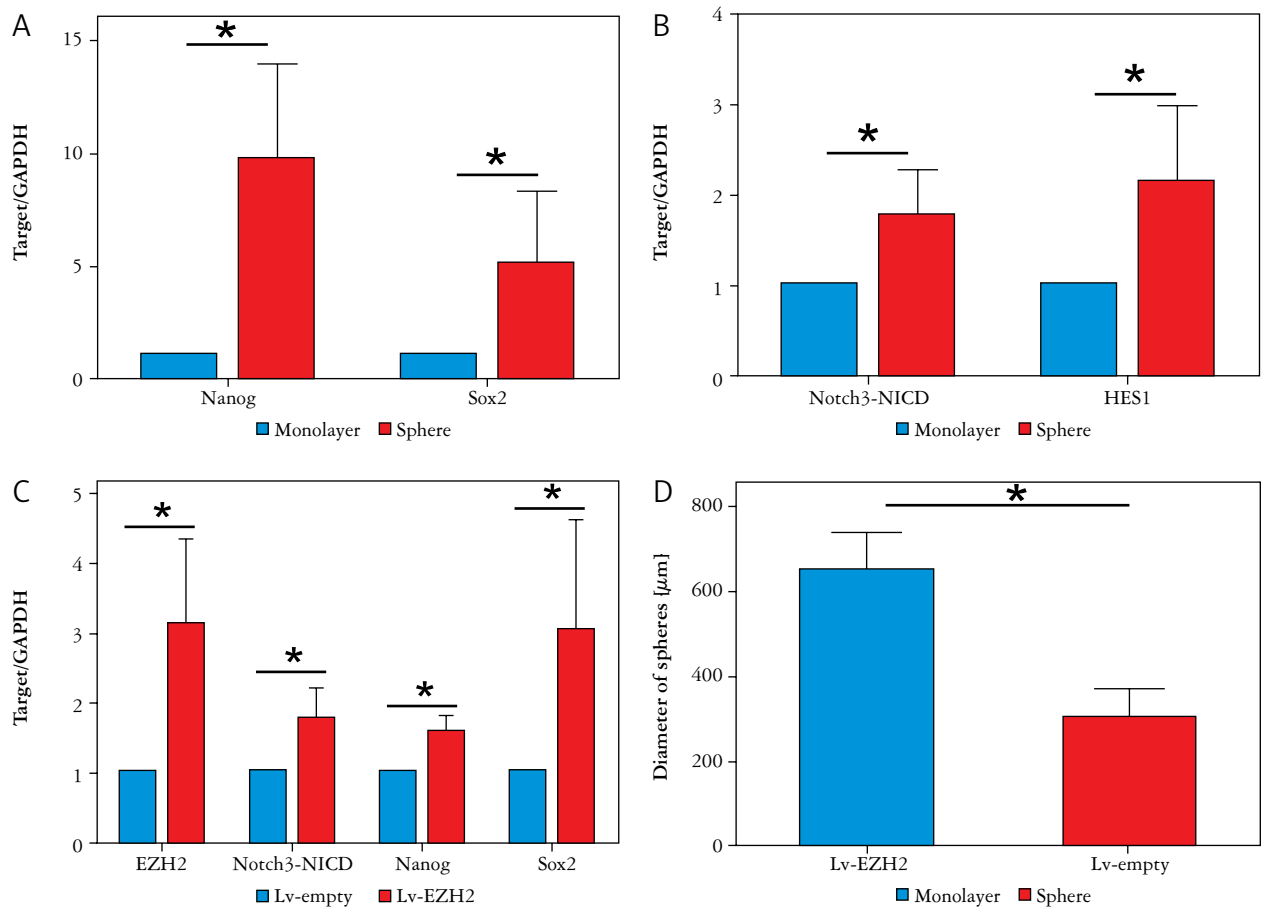


Suppl. Table 1. Sequences of primer pairs used in this study

GENE	PRIMER SEQUENCE (5'→3')
GAPDH	F: GGAGCGAGATCCCCTCCAAAAT
	R: GGCTGTTGTCATACTTCTCATGG
Nanog	F: TTTGTGGGCCTGAAGAAAAC
	R: AGGGCTGTCCTGAATAAGCAG
Sox2	F: GCCGAGTGGAACTTTTGTGC
	R: GGCAGCGTGTACTTATCCTTCT
Oct4	F:GTGTTTCAGCCAAAAGACCATCT
	R: GGCCTGCATGAGGGTTTCT
Notch1	F:GAGGCGTGGCAGACTATGC
	R: CTTGTACTCCGTCAGCGTGA
Notch2	F:CCTTCCACTGTGAGTGTCTGA
	R: AGGTAGCATCATTCTGGCAGG
Notch3	F:CGTGGCTTCTTTCTACTGTGC
	R: CGTTCACCGGATTTGTGTCAC
Notch4	F:GATGGGCTGGACACCTACAC
	R: CACACGCAGTGAAAGCTACCA
Hes1	F:CCTGTCATCCCCGTCTACAC
	R: CACATGGAGTCCGCCGTA
Hey1	F:GTTCCGGCTCTAGGTTCCATGT
	R: CGTCGGCGCTTCTCAATTATTC

Suppl. Table 2. Characteristics of osteosarcoma patients

MALE/FEMALE	37/21						
Age (years)	Range	Mean	Median	≤ 24	> 24		
	7–74	24.31	18	38 (65.5%)	20 (34.5%)		
Tumour diameter [cm]	Range	Mean	Median	≤ 8	> 8		
	1.4–18	8.4	8	32 (55.2%)	26 (44.8%)		
Metastatic location	Lung	Pelvis	Scapula	Skull			
	29 (90.6%)	1 (3.1%)	1 (3.1%)	1 (3.1%)			
Enneking staging	IA	IB	IIA	IIB	III		
	1 (1.7%)	1 (1.7%)	12 (20.7%)	39 (67.2%)	5 (8.6%)		
Locations	Femur	Tibia	Fibula	Humerus	Pelvis	Clavicle	Outside bone
	35 (60.3%)	8 (13.8%)	5 (8.6%)	4 (6.9%)	4 (6.9%)	1 (1.7%)	1 (1.7%)
Surgery method	Excision	Amputation					
	49 (84.5%)	9 (15.6%)					



Suppl. Fig. 1. Densitometry analysis of protein expressions in osteosarcoma stem cell (OSC) spheres. A) Densitometry analysis of protein expressions demonstrated that Nanog and Sox2 were upregulated in OSC spheres compared to monolayer cells ($*p < 0.05$); B) Densitometry analysis of protein expressions demonstrated that Notch3 intracellular domain (Notch3-NICD) and HES1 were upregulated in OSC spheres compared to monolayer cells ($*p < 0.05$); C) Densitometry analysis of protein expressions showed that after overexpression of enhancer of zeste homologue 2, Notch3-NICD, Nanog, and Sox2 were upregulated in osteosarcoma cells ($*p < 0.05$)