

Supplementary Materials

Establishment and characterization of a highly metastatic human osteosarcoma cell line from osteosarcoma lung metastases

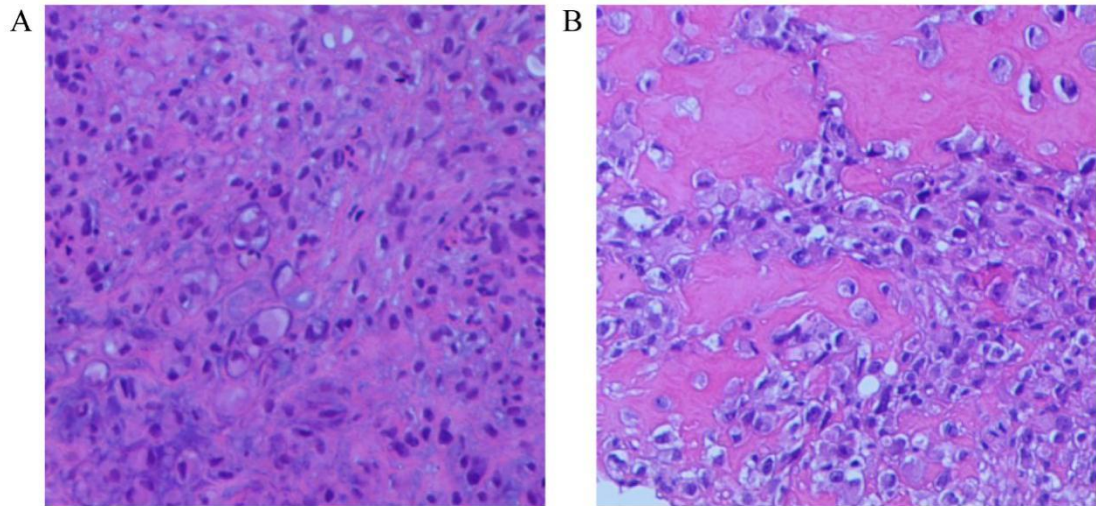
Supplementary Table S1: List of primers used in the RT-PCR analysis

Gene	Forward (5'to3')	Reverse (5'to3')
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
ALP	ACTGGGGCCTGAGATACCC	TCGTGTTGCACTGGTTAAAGC
ACAN	GTGCCTATCAGGACAAGGTCT	GATGCCTTTCACCACGACTTC
COL II	TGGACGCCATGAAGGTTTTCT	TGGGAGCCAGATTGTCATCTC
COL X	GGGGCTAAGGGTCAAAGGG	GGTCCTCCAACCTCCAGGATCA
OC	GGCGCTACCTGTATCAATGG	GTGGTCAGCCAACCTCGTCA
OP	CTCCATTGACTCGAACGACTC	CAGGTCTGCGAAACTTCTTAGAT
FAS	GCCAATTCTGCCATAAGCCCTGTCC	GCTGGTGAGTGTGCATTTCCTTGAT
CD44	CTGCCGCTTTCAGGTGTA	CATTGTGGGCAAGGTGCTATT
CDH11	AGAGGTCCAATGTGGGAACG	GGTTGTCCTTCGAGGATACTGT
SCARB1	AATAAGCCCATGACCCTGAAGC	GCCCCACATGATCTCACCC
EZR	ATGCCCCACGTCTGAGAATC	TCCTGCGGCGCATATAACAAC
NF2	CCAACGGGACCATCGAATCTC	CCAGCCAATCGTACTTGCAGT
GNAS	TGCCTCGGGAACAGTAAGAC	GCCGCCCTCTCCATTAAAC
RUNX2	TGGTACTGTCATGGCGGGTA	TCTCAGATCGTTGAACCTTGCTA
CXCR4	ACGCCACCAACAGTCAGAG	AGTCGGGAATAGTCAGCAGGA

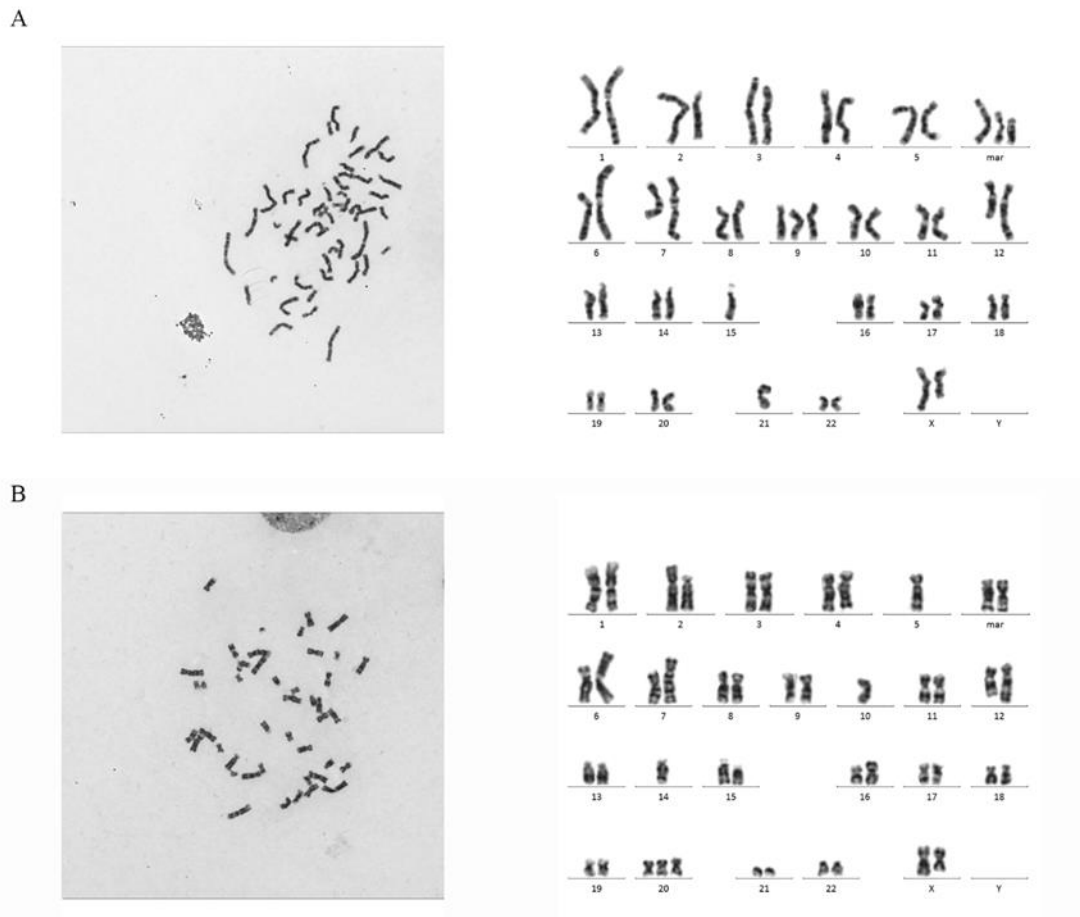
RT-PCR, real-time polymerase chain reaction; osteocalcin (OC), osteopontin (OP), alkaline phosphatase (ALP), aggrecan (ACAN), type II collagen (COL II) and type X collagen (COL X), GNAS complex locus (GNAS), scavenger receptor class B member 1 (SCARB1), Fas cell surface death receptor (FAS), CD44 molecule (CD44), cadherin 11 (CDH11), neurofibromin 2 (NF2), ezrin (EZR), RUNX2 (RUNX family transcription factor 2), **C-X-C motif chemokine receptor 4 (CXCR4)**.

Supplementary Table S2: Results of whole-exon sequencing analysis

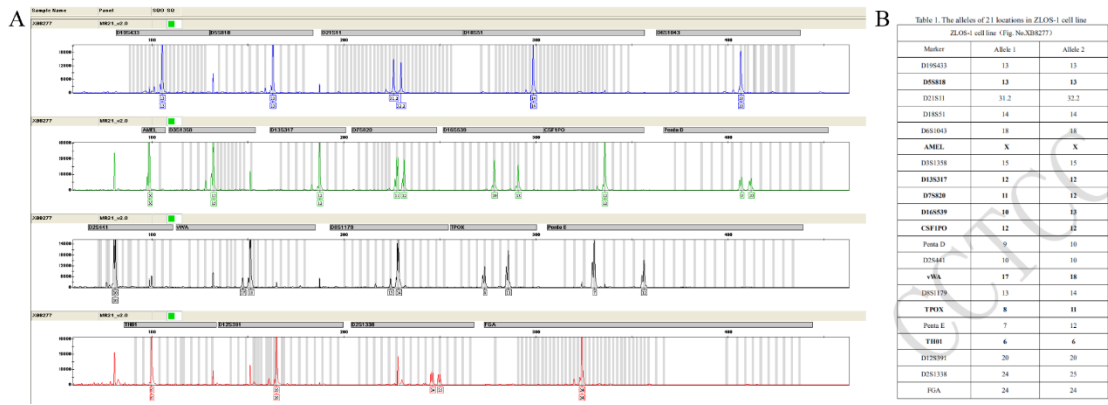
Summary statistics for identified SNPs					
Total SNPs	Fraction of SNPs in dbSNP (%)		Fraction of SNPs in 1000genomes (%)		Novel
80273	98.26		92.66		1397
Functional categories for coding SNPs					
Synonymous	Missense	Stop gain	Stop loss	Start loss	Splicing
9240	9166	113	40	26	138



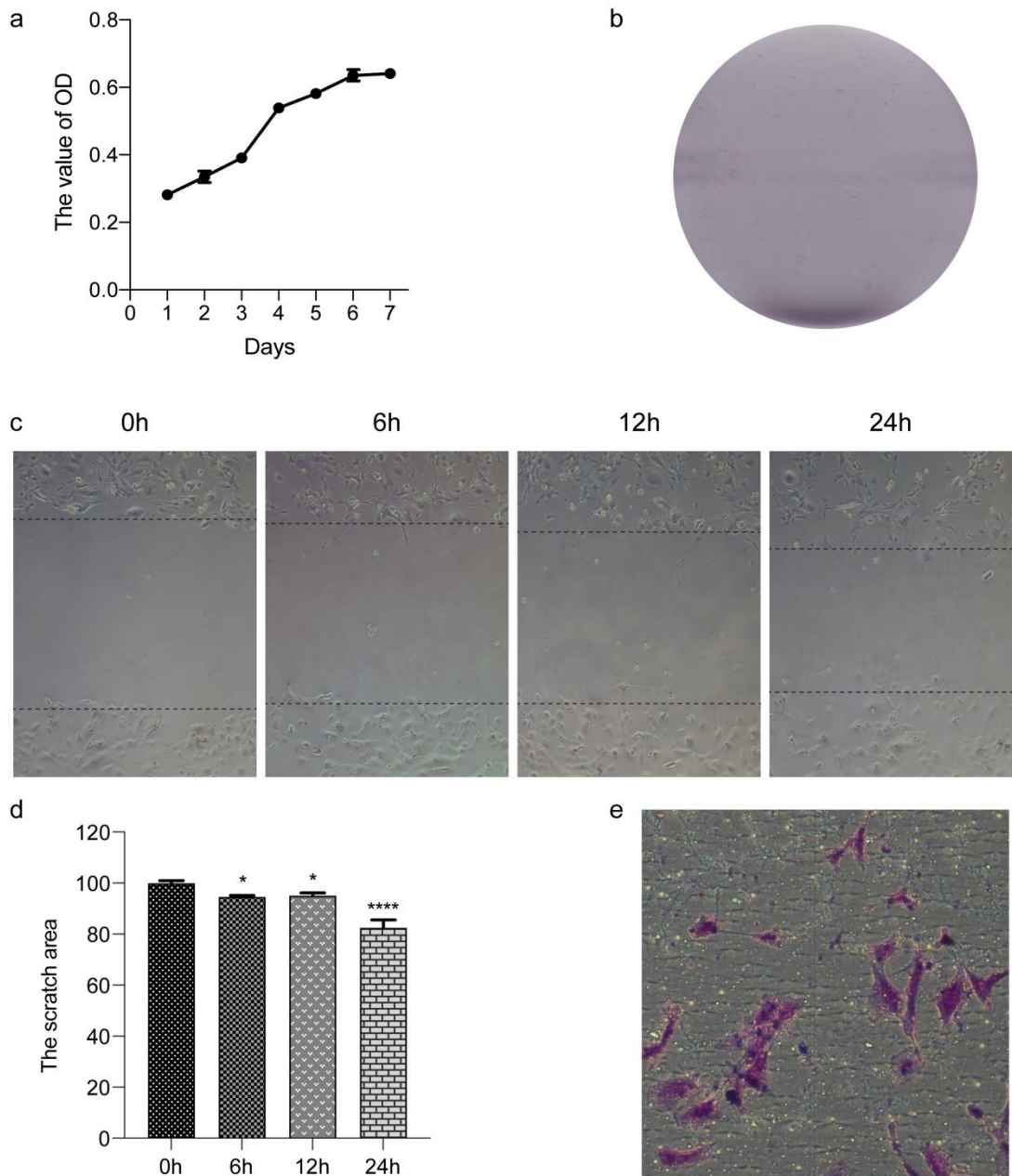
Supplementary Figure S1: HE staining of preoperative lung.



Supplementary Figure S2: Karyotype analysis of the 14th generation ZOSL-1 cell (different sight). (A)Sight No. 0004; (B) Sight No. 0251.



Supplementary Figure S3: The alleles of 21 locations in ZOSL-1 cell line.



Supplementary Figure S4: Proliferation, migration and invasion capability of hFOB 1.19. a: The result of CCK-8. b: The result of cloning. c: Scratch detection within 24 hours. d: Quantification of scratch results. ***: $p < 0.0001$; **: $p < 0.001$; *: $p < 0.01$, *: $p < 0.05$. e: The cell migration through the matrigelcoated filter in the transwell chamber.