

## Supplementary materials for

### **KDM6B-mediated histone demethylation of LDHA promotes lung metastasis of osteosarcoma**

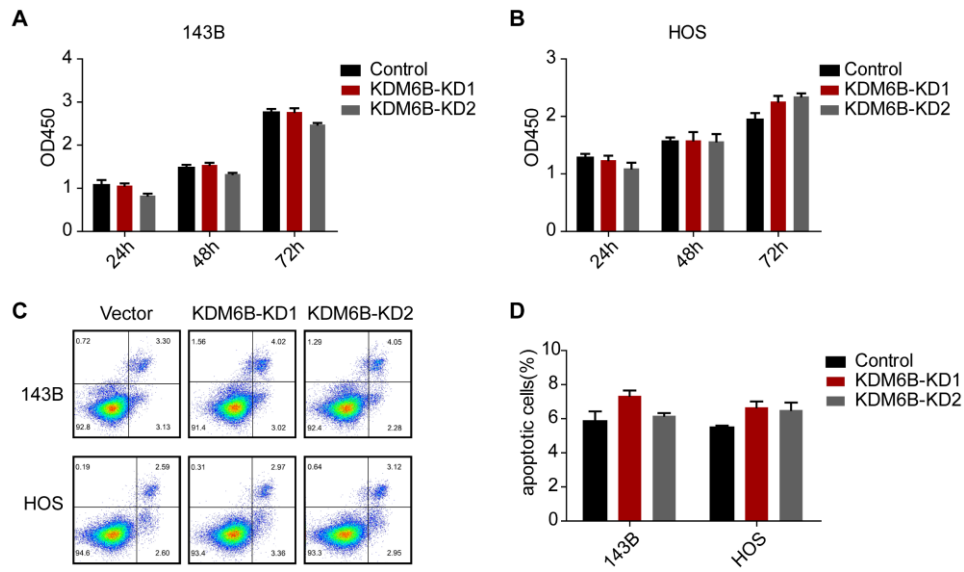
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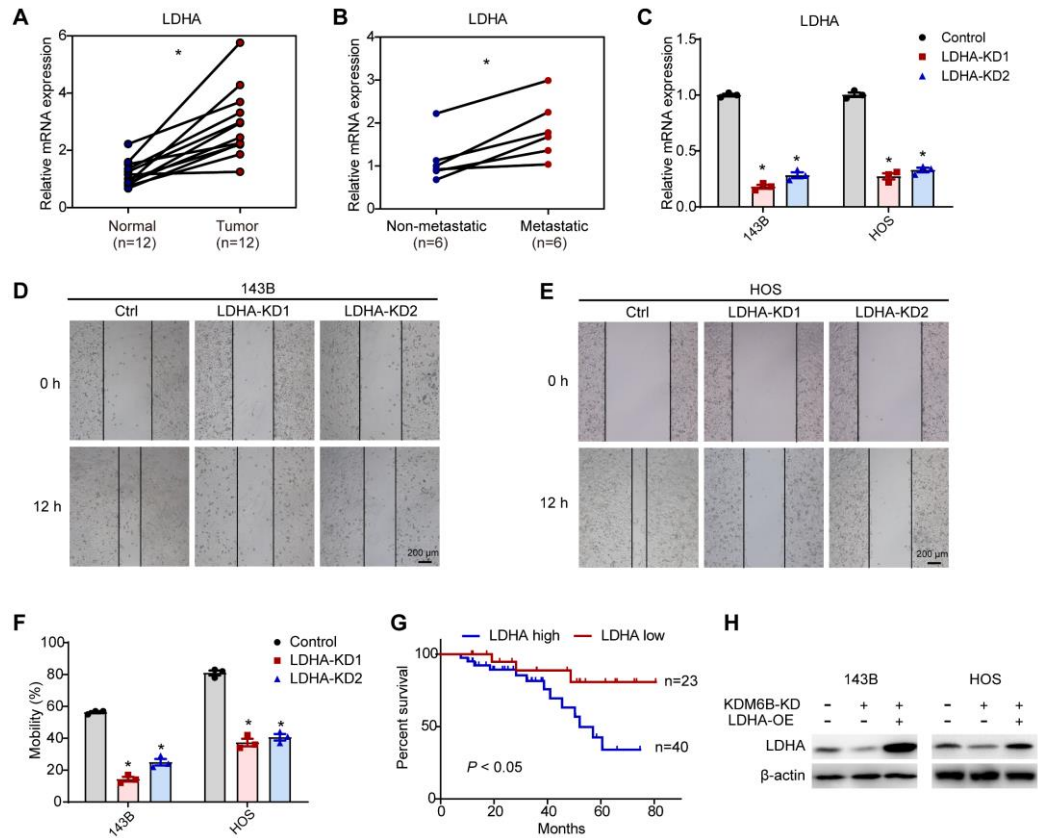


**Figure S1. Intact cell growth and survival in KDM6B-KD OS cells**

(A and B) CCK-8 assays in control and KDM6B-knockdown 143B and HOS cells.

(C) Flow cytometry profiling of Annexin-V/7-AAD-stained control and KDM6B-knockdown 143B and HOS cells.

(D) Quantitation results for (C).



**Figure S2. Elevated LDHA expression and function in OS**

- (A) Expression of LDHA in 12 pairs of primary OS patients determined by RT-qPCR.
- (B) LDHA mRNA expression in patients with metastatic and non-metastatic osteosarcoma.
- (C) LDHA mRNA expression checked by RT-qPCR in control/LDHA-KD 143B and HOS cells.
- (D and E) Wound-healing assay of control and LDHA-KD 143B and HOS cells.
- (F) Quantitative results for (D, E).
- (G) Kaplan-Meier plot of overall survival of 63 OS patients based on LDHA expression levels. A log-rank test was used for statistical analysis.

(H) Immunoblots for LDHA expression in control/KDM6B-KD and LDHA-OE OS cells. \* $P < 0.05$ .

**Table S1. Sequences of the RT-qPCR Primers**

<b>RT-qPCR primer</b>	
KDM6B-F	ACCCACTGTGGTCTGTTGTAC
KDM6B-R	AATCATCACCCGTGTCTCC
LDHA-F	GGCCTGTGCCATCAGTA
LDHA-R	CAAGCCACGTAGGTCAA
VEGFA-F	GCTGCTGCAATGACGAG
VEGFA-R	GACTTATACCGGGATTTCTT
GAPDH-F	CTAAGGCCAACCGTGAAA
GAPDH-R	AGAAGGAAGGCTGGAAAA

**Table S2. Sequences of ChIP-qPCR Primers**

<b>ChIP-qPCR primer</b>	
LDHA-P1-F	TCCAGAAGCACAGCCCAGAG
LDHA-P1-R	CTTGTTCCGCATCCACCC
LDHA-P2-F	TAACAAATAGGGAGACCGAAAG
LDHA-P2-R	TGGGAGGGCATGGGATGA
VEGFA-P1-F	CGCAGCGGTTAGGTGGAC
VEGFA-P1-F	GGGGAATGGCAAGCAAAA
FGF1-P1-F	AGGCTGAAGTTTAGGATTA
FGF1-P1-R	CTGAAACGAGGATGTGAT

**Table S3. Luciferase intensity of orthotopic tumor and lung metastasis**

lung (cps)	Control				
Day 7	0	5	0	0	0
Day 14	15762	3382	11897	12654	3579
Day 21	102067	64056	98741	75236	113022
Day 23	660769	424373	682231	439587	502475
	shJMJD3-1 (KDM6B-KD1)				
Day 7	0	0	0	0	0
Day 14	0	0	0	0	0
Day 21	0	0	0	0	0
Day 23	0	0	722	0	0
	shJMJD3-2 (KDM6B-KD2)				
Day 7	0	0	0	0	0
Day 14	0	0	0	0	0
Day 21	0	0	0	0	0
Day 23	0	0	0	0	0
in situ (cps)	Control				
Day 7	2315	1429	123	55	87
Day 14	80330	125690	60254	72115	141451
Day 21	248488	478999	523547	178952	102479
Day 23	1502495	2813695	1724781	1925415	2712852
	shJMJD3-1 (KDM6B-KD1)				
Day 7	51	49	1239	62	118
Day 14	28590	32574	113252	64124	14126
Day 21	104212	184805	175412	95417	162415
Day 23	1851850	869488	1724784	881147	1625414
	shJMJD3-2 (KDM6B-KD2)				
Day 7	22	139	1148	24	112
Day 14	40738	74444	112365	35744	65317
Day 21	257395	148773	265412	162114	152119
Day 23	1880931	588281	1571478	1721475	624173