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

| RT-qPCR primer | Sequence          |
|----------------|-------------------|
| KDM6B-F        | ACCCAGTGGCTGTTGTTGAC |
| KDM6B-R        | AATCATCAACCGGTCTCCTC |
| LDHA-F         | GCCCTGTCGTCCATCAGTA |
| LDHA-R         | CAAGGCAAGGCTGAAA   |
| VEGFA-F        | GCTGCTGCAATGACGAG  |
| VEGFA-R        | GACTTATAACCGGATTTCTT |
| GAPDH-F        | CTAAGGCAAACGGTGA   |
| GAPDH-R        | AGAAGGAGGCTGAAAA  |

Table S2. Sequences of ChIP-qPCR Primers

| ChIP-qPCR primer | Sequence          |
|------------------|-------------------|
| LDHA-P1-F        | TCCAGAAGCAGACAGGAGAG |
| LDHA-P1-R        | CTTGTCCGATCCACCC  |
| LDHA-P2-F        | TAAACAAATAGGAGACGAAAG |
| LDHA-P2-R        | TGCGAGGAGGATGGGATGA |
| VEGFA-P1-F       | CGCAGCGGTTAGGTGGAC |
| VEGFA-P1-R       | GGGGAATGGCAAGCAAAA |
| FGF1-P1-F        | AGGCTGAAGTGGGATTA  |
| FGF1-P1-R        | CTGAAACGGAGATGTGAT |
### Table S3. Luciferase intensity of orthotopic tumor and lung metastasis

|                  | lung (cps)               | in situ (cps)         |
|------------------|--------------------------|-----------------------|
|                  | Control                  | Control               |
| Day 7            | 0                        | 2315                  |
| Day 14           | 15762 3382 11897 12654  | 80330 125690 60254 72115 141451 |
| Day 21           | 102067 64056 98741 75236 113022 |
| Day 23           | 660769 424373 682231 439587 502475 |
| Day 7            | 0                        | 51                    |
| Day 14           | 0                        | 49                    |
| Day 21           | 0                        | 123                   |
| Day 23           | 0                        | 62                    |

**shJMJD3-1 (KDM6B-KD1)**

|                  | lung (cps)               | in situ (cps)         |
|------------------|--------------------------|-----------------------|
|                  | Control                  | Control               |
| Day 7            | 0                        | 2315                  |
| Day 14           | 0                        | 80330 125690 60254 72115 141451 |
| Day 21           | 0                        | 248488 478999 523547 178952 102479 |
| Day 23           | 0                        | 1502495 2813695 1724781 1925415 2712852 |
| Day 7            | 0                        | 51                    |
| Day 14           | 0                        | 28590 32574 113252 64124 14126 |
| Day 21           | 0                        | 104212 184805 175412 95417 162415 |
| Day 23           | 0                        | 1851850 869488 1724784 881147 1625414 |

**shJMJD3-2 (KDM6B-KD2)**

|                  | lung (cps)               | in situ (cps)         |
|------------------|--------------------------|-----------------------|
|                  | Control                  | Control               |
| Day 7            | 0                        | 2315                  |
| Day 14           | 0                        | 80330 125690 60254 72115 141451 |
| Day 21           | 0                        | 248488 478999 523547 178952 102479 |
| Day 23           | 0                        | 1502495 2813695 1724781 1925415 2712852 |
| Day 7            | 0                        | 22                    |
| Day 14           | 0                        | 40738 74444 112365 35744 65317 |
| Day 21           | 0                        | 257395 148773 265412 162114 152119 |
| Day 23           | 0                        | 1880931 588281 1571478 1721475 624173 |