



Supporting Information

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OTUD7B Deubiquitinates LSD1 to Govern Its Binding Partner Specificity, Homeostasis and Breast Cancer Metastasis

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Supporting Information

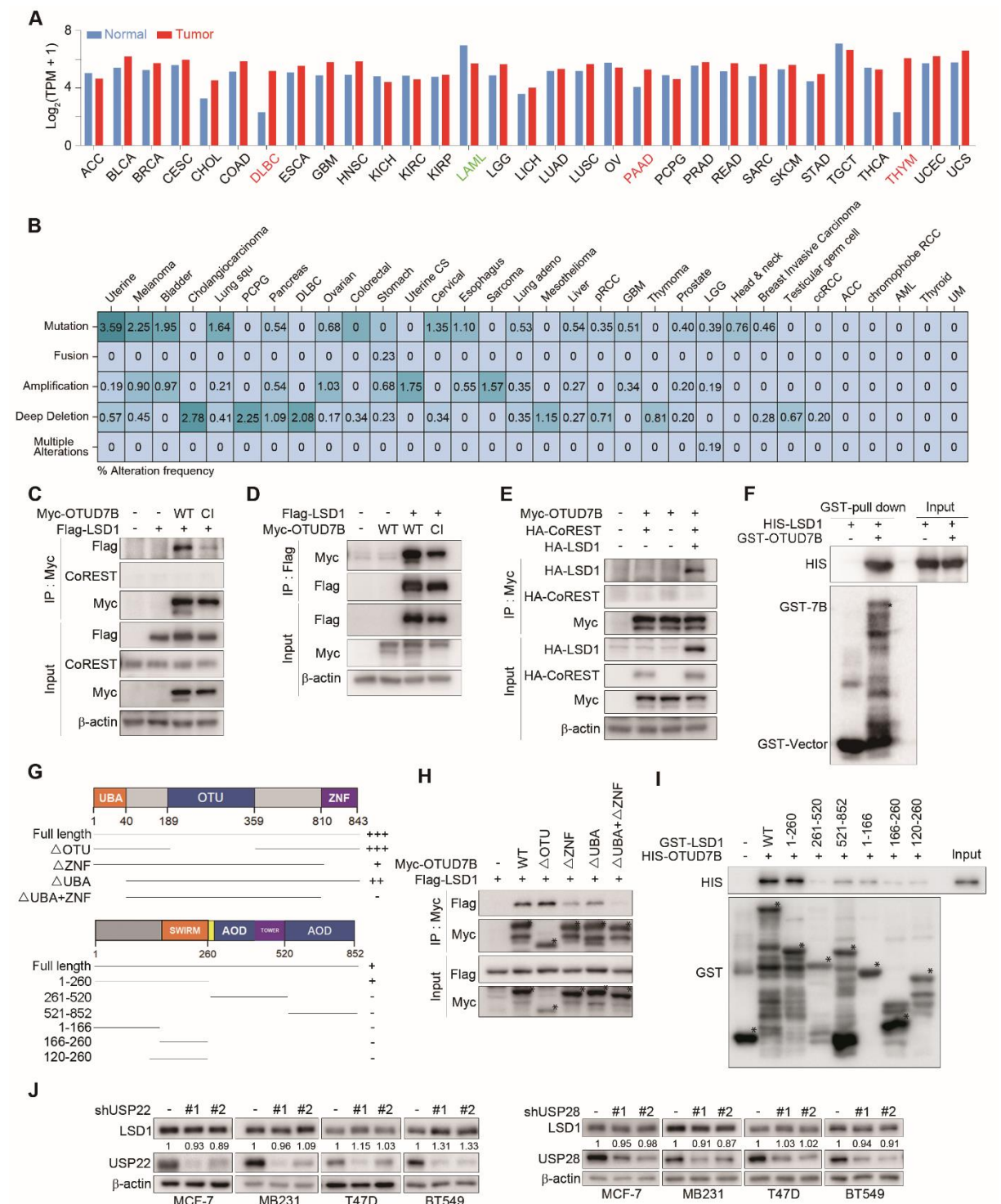


Figure S1. OTUD7B binds LSD1 and regulates its stability. A) Histogram shows the median mRNA expression profile of *LSD1* across the indicated tumor samples (red) and paired tissues (blue) using TCGA database. The cancer types with *LSD1* expression levels greater than 2-fold are highlighted in red. B) TCGA DNA sequencing results showing *LSD1* genomic alteration frequencies in the indicated tumor samples ($n = 10953$). (C-E) HEK293T cells were

transfected with the indicated plasmids, followed by IP and IB analysis as indicated. F) Purified His-LSD1 was incubated with GST or GST-OTUD7B coupled to GSH-Sepharose. Proteins retained on sepharose were analyzed by western blotting with the indicated antibodies. G) Schematic diagrams of deletion mutants of OTUD7B (top panel) and LSD1 (bottom panel). H) HEK293T cells were co-transfected with Flag-LSD1 and the indicated OTUD7B constructs, followed by IP and IB analysis as indicated. I) Purified His-OTUD7B was incubated with the indicated GST proteins coupled to GSH-sepharose. Proteins retained on sepharose were analyzed by western blotting. J) IB analysis of breast cancer cell lines infected with the indicated lentiviral shRNAs constructs.

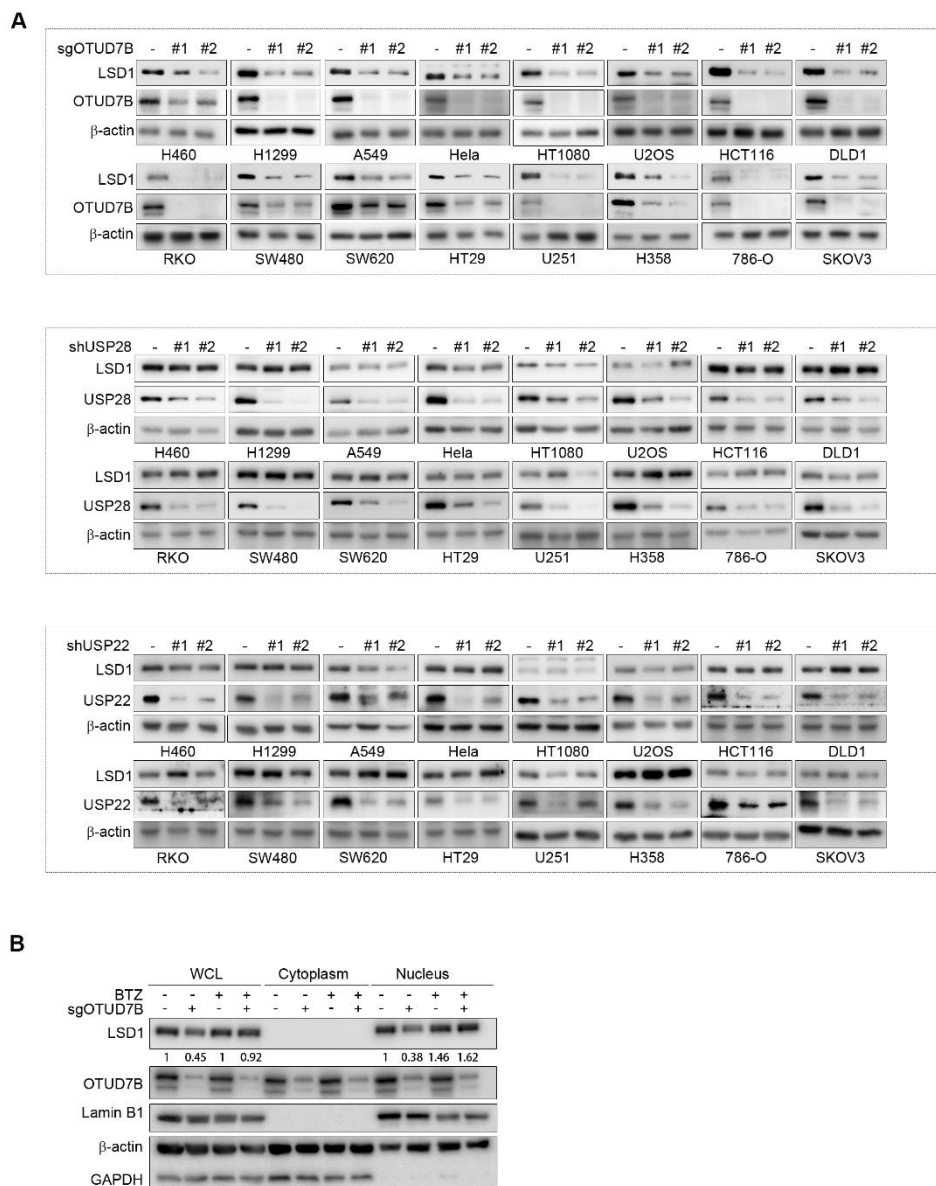


Figure S2. OTUD7B binds LSD1 and regulates its stability. A) IB analysis of WCL derived from 16 different cancer cell cells expressing the indicated lentiviral constructs. B) MDA-MB-231 cells expressing OTUD7B sgRNA were treated with BTZ for 5 hr, then lysed and subjected to IB analysis of LSD1 and OTUD7B in nuclear and cytoplasmic fractions using Lamin B1 (nuclear), GAPDH (cytoplasm) and β -actin (WCL) as controls.

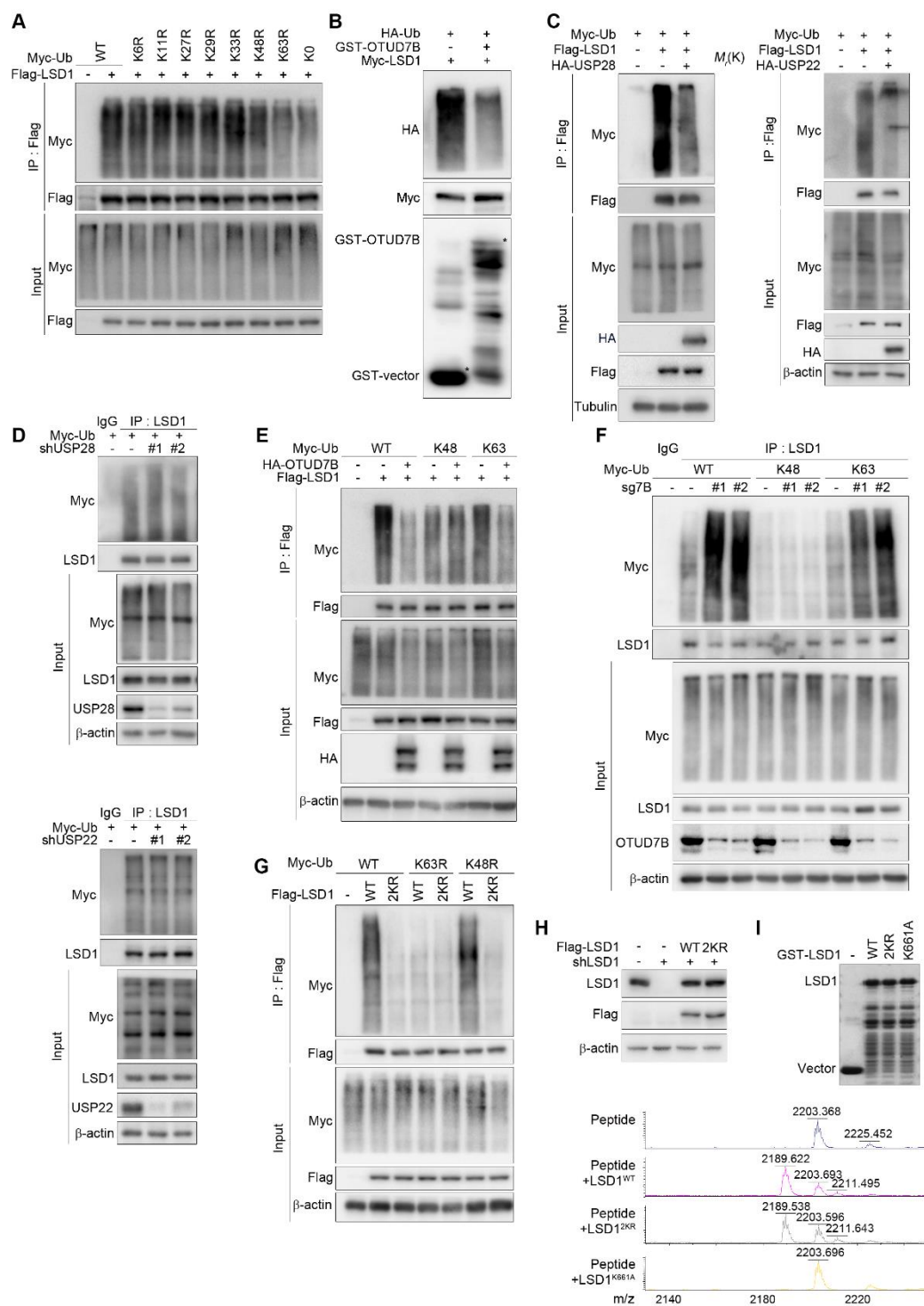


Figure S3. OTUD7B is a bona fide LSD1 deubiquitinase. A) HEK293T cells were co-transfected with Flag-LSD1 and the indicated Ubiquitin constructs, collected for Flag-IP, followed by IB analysis with the indicated antibodies. B) Ubiquitinated LSD1 obtained from HEK293T cells transfected with Myc-LSD1 and HA-ubiquitin followed by Myc-immunoprecipitation was incubated with GST or GST-OTUD7B. Proteins retained were analyzed by western blot with indicated antibodies. C) HEK-293T cells were co-transfected

with the indicated plasmids, collected for Flag-IP, followed by IB analysis with the indicated antibodies. D) HEK-293T cells infected with lentiviruses encoding the indicated shRNAs were transfected with Myc-Ubiquitin, treated with MG132 (25 μM) for 8 hr. Cell lysates were collected for IP with antibody against LSD1 and IB analysis as indicated. E) HEK293T cells were co-transfected with the indicated constructs, collected for Flag-IP, followed by IB analysis with the indicated antibodies. F) HEK293T cells stably expressing control or OTUD7B sgRNA were transfected with indicated Ub constructs and collected for LSD1-IP, followed by western blotting analysis with indicated antibodies. G) HEK293T cells were co-transfected with the indicated constructs, collected for Flag-IP, followed by IB analysis with the indicated antibodies. H) Western blotting analysis of LSD1 protein levels in LSD1^{WT} and LSD1^{2KR} reconstituting MDA-MB-231 cells. I) Coomassie Brilliant Blue (CBB) staining showing expression levels of the indicated GST-LSD1 recombinant proteins (top panel). In vitro demethylation assay was performed by incubating H3K4me1 peptides with the indicated GST-LSD1 constructs and analyzed by mass spectrometry (bottom panel).

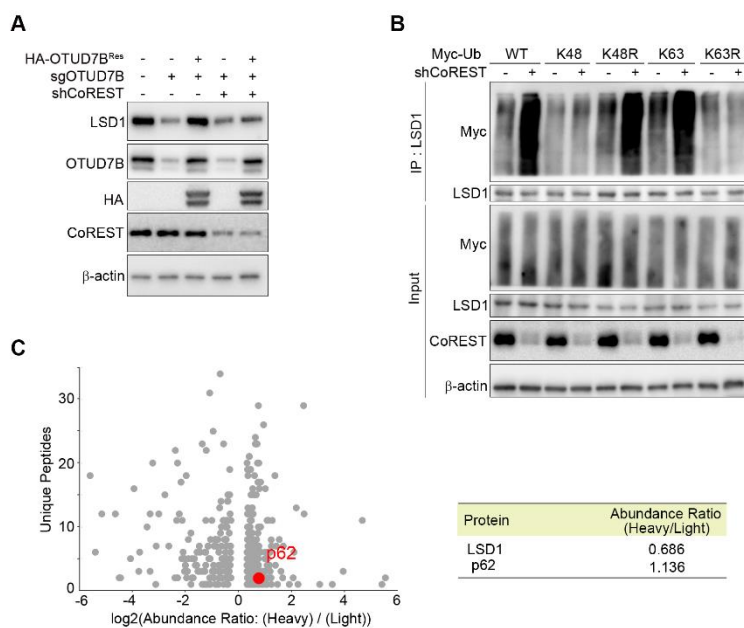


Figure S4. OTUD7B-mediated removal of K63-linked poly-Ub chains on LSD1 determines LSD1/ CoREST/HDACs integrity. A) MDA-MB-231 infected with lentiviruses encoding the indicated constructs, followed by IB analysis with the indicated antibodies. B) HEK293T cells expressing control or CoREST shRNA were transfected with the indicated Myc-tagged ubiquitin constructs, treated with BTZ for 5 hr, collected for IP with LSD1 antibody and IB analysis with the indicated antibodies. C) MDA-MB-231 cells labelled by SILAC were infected with lentiviruses encoding control or CoREST shRNA, treated with BTZ for 5 hr. Cell lysates were collected for IP with LSD1 specific antibody, followed by mass spectrometry analysis. Scatter plot represents the indicated LSD1-interacting proteins (p62 highlighted by red) (left panel); abundance ratio of p62 upon CoREST-depleted versus control condition (right panel).

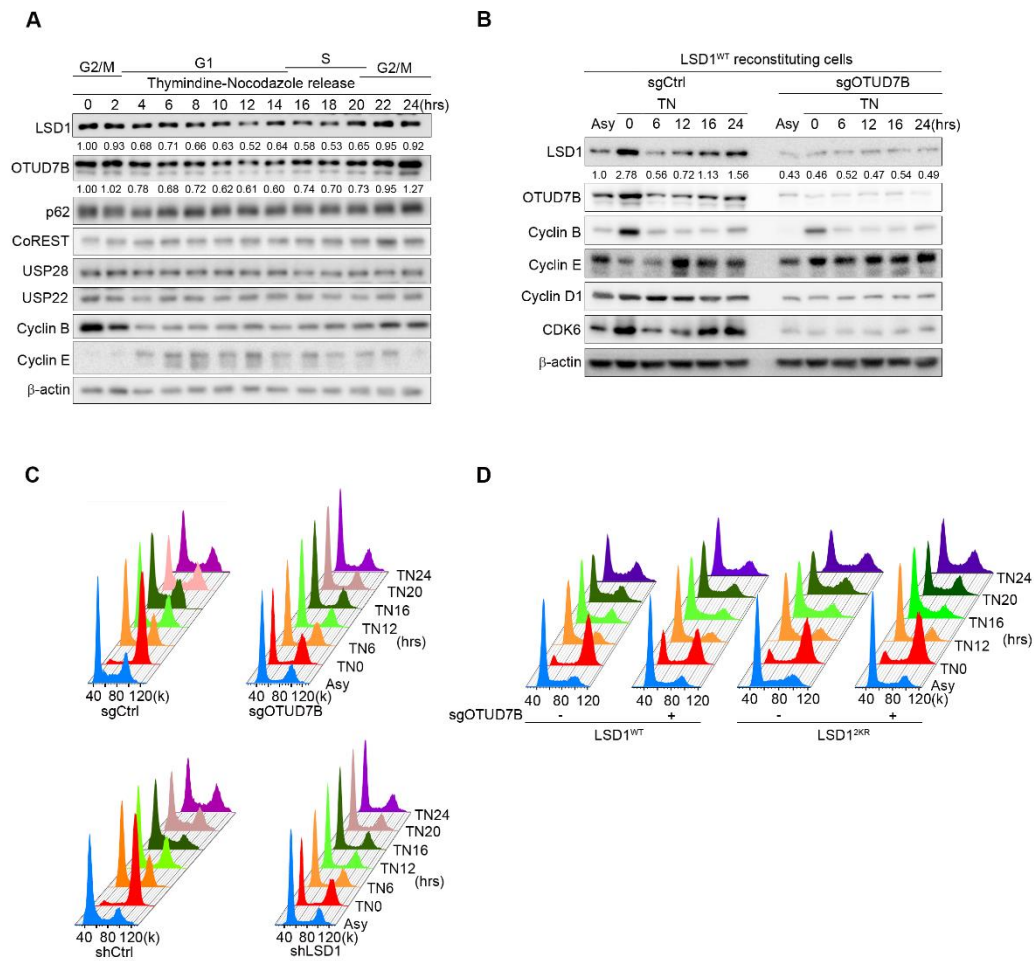


Figure S5. OTUD7B-dependent regulation of LSD1 fluctuation is crucial for cell cycle progression. A) MDA-MB-231 cells were synchronized by thymidine/nocodazole treatment, released and harvested at the indicated times, followed by western blotting analysis as indicated. B) LSD1^{WT} reconstituting MDA-MB-231 cells stably expressing control or OTUD7B sgRNA were synchronized by thymidine/nocodazole treatment, released and harvested at the indicated times, followed by western blotting analysis as indicated. (C-D) Parental (C) or LSD1 reconstituting D) MDA-MB-231 cells stably expressing the indicated shRNAs or sgRNAs were synchronized by thymidine/nocodazole treatment, released and harvested at the indicated times, followed by FACS analysis.

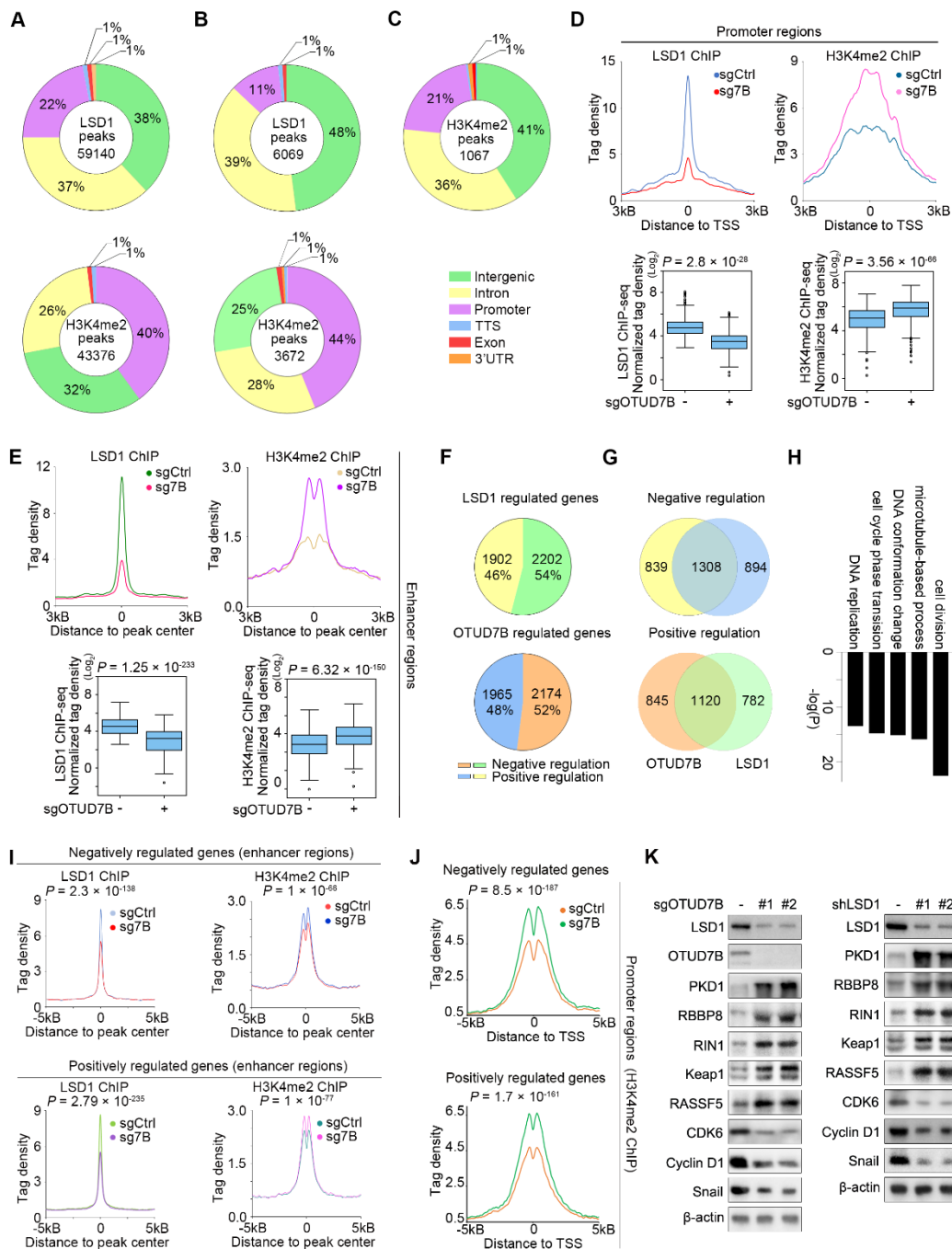


Figure S6. OTUD7B regulates gene transcription in a LSD1-dependent manner. A) Pie charts showing genomic distribution of LSD1-bound peaks (59,140) (top panel) and H3K4me2 peaks (43,376) (bottom panel). B) Pie charts showing genome-wide distribution of reduced LSD1-bound peaks ($n = 6069$, $FC > 2$) (top panel) and elevated H3K4me2 peaks ($n = 3672$, $FC > 2$) (bottom panel) upon OTUD7B loss. C) Pie chart showing genomic distribution of H3K4me2 peaks gained upon OTUD7B depletion, centered on reduced LSD1 peaks (± 3 kB) ($n = 1067$, $FC > 1.5$). (D-E) Fold enrichment of normalized LSD1 and H3K4me2 ChIP-seq reads at promoter regions ($n = 223$) (D) and enhancer regions ($n = 844$) (E) in MDA-MB-231

cells depleted of OTUD7B. Top panels show tag density distribution, and bottom panels show box plot. *P* values were calculated by student's *t* test. F) Pie charts showing differentially expressed genes detected by RNA-seq in LSD1-silenced or OTUD7B-depleted MDA-MB-231 cells, compared to control cells. G) Venn diagram showing the overlap in transcripts from (F) categorized by positively-regulated and negatively-regulated gene sets. H) Gene ontology analysis of transcripts negatively co-regulated by OTUD7B and LSD1 ($FC > 1.5$). I) Fold enrichment of normalized LSD1 and H3K4me2 ChIP-seq reads at enhancer regions of OLco-regulated transcripts. Negatively-regulated genes ($n = 1308$); positively regulated genes ($n = 1120$). *P* values were calculated by student's *t* test. J) Fold enrichment of normalized H3K4me2 ChIP-seq reads at promoter regions of OLco-regulated transcripts. *P* values were determined by student's *t* test. K) IB analysis of MDA-MB-231 cells infected with the indicated lentiviral constructs with the indicated antibodies.

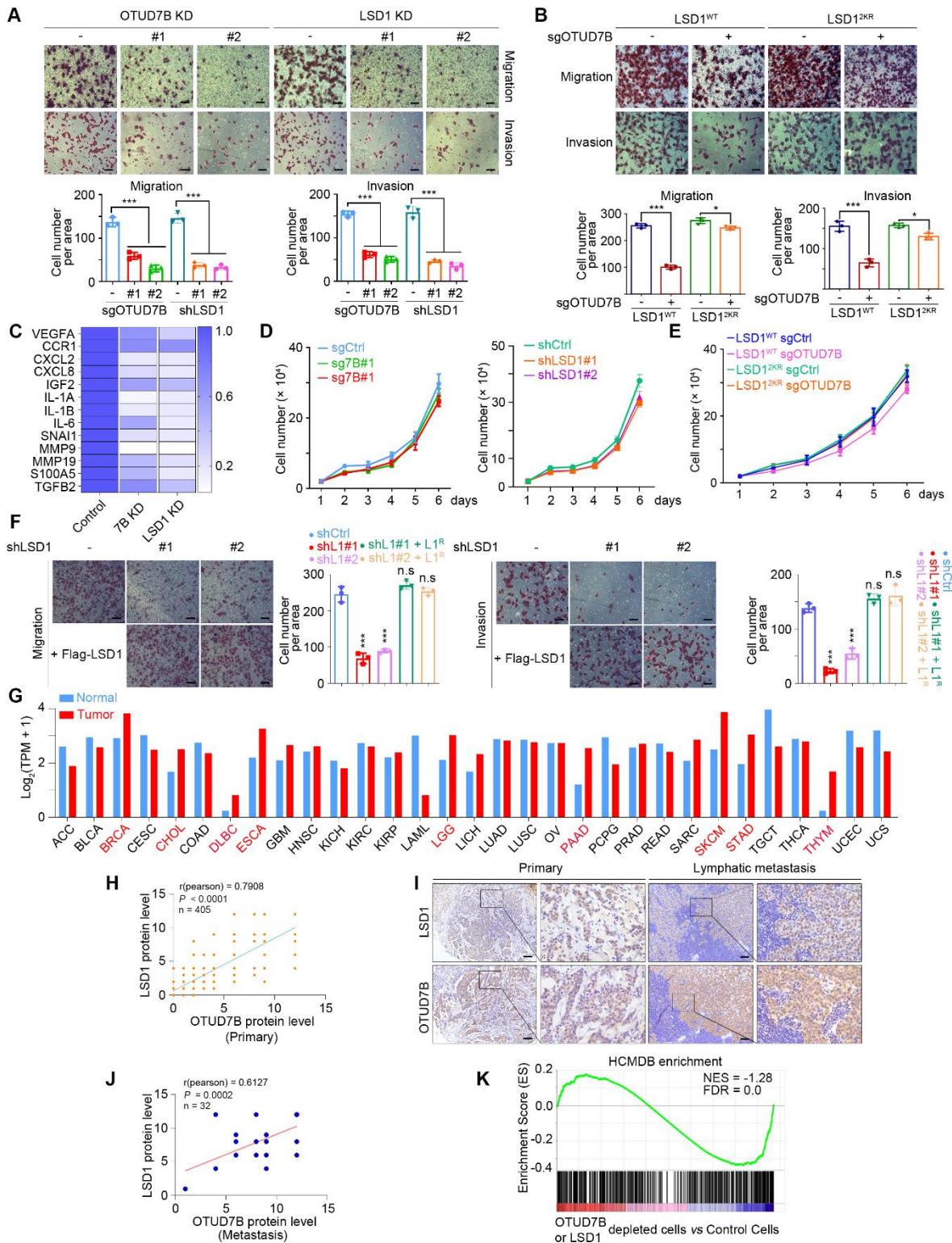


Figure S7. OTUD7B promotes metastasis via deubiquitinating LSD1. A) MDA-MB-231 cells expressing the indicated sgRNAs or shRNAs were subjected to Transwell migration or invasion assays. Scale bars, 50 μm . P values were determined by two-tailed unpaired t test. B) LSD1 reconstituting LM2 cells expressing the indicated sgRNAs were subjected to Transwell migration or invasion assays as indicated. Scale bars, 50 μm . *** $P < 0.001$. P values were

determined by two-tailed unpaired t test. C) Heatmap revealed fold change of indicated pro-metastasis genes upon OTUD7B or LSD1 depletion by RNA-seq analysis. (D-E) Parental (D) or LSD1 reconstituting (E) LM2 cells infected with lentiviruses encoding the indicated shRNAs or sgRNAs were subjected to cell proliferation assay. F) LM2 infected with lentivirus encoding the indicated constructs were subjected to Transwell migration or invasion assays as indicated. Scale bars, 50 μm . *** $P < 0.001$. P values were determined by two-tailed unpaired t test. G) Histogram shows the median mRNA expression profile of OTUD7B across the indicated tumor samples (red) and paired tissues (blue) using TCGA database. The cancer types with OTUD7B expression levels greater than 2-fold are highlighted in red. Data were presented as mean \pm SEM of three independent experiments. H) Pearson's correlation coefficient is shown to indicate the correlation between LSD1 and OTUD7B protein levels based on (Figure 7D). P values were calculated by Fisher's exact test. I) Immunohistochemical staining of LSD1 and OTUD7B in paired primary and lymphatic metastatic ($n = 27$) breast cancer samples. Scale bar, 100 μm . J) Pearson's correlation coefficient is shown to indicate the correlation between LSD1 and OTUD7B protein levels based on (I). K) GSEA of pro-metastatic genes associated with breast cancer in HCMDB database compared with OLco-regulated gene sets.

Table S1. Summary of DUB screening results.

| USP | Interaction | USP | Interaction | OTU | Interaction | ZUP | Interaction | UCH | Interaction | JAMM | Interaction | MJD | Interaction |
|--------------|-------------|--------------|-------------|---------------|-------------|------|-------------|-------|-------------|---------|-------------|--------|-------------|
| USP1 | - | USP41 | - | ALG13 | - | ZUP1 | - | UCHL1 | - | COPS5 | - | ATXN3 | - |
| USP2A | - | USP43 | - | YOD1 | - | | | UCHL3 | - | COPS6 | - | ATXN3L | - |
| USP2B | - | USP44 | - | OTUD1 | - | | | UCHL5 | - | PSMD14 | - | JOSD1 | - |
| USP3 | - | USP45 | - | OTUD3 | - | | | BAP1 | - | BRCC3 | - | JOSD2 | - |
| USP4 | - | USP46 | - | OTUD5 | - | | | | | MYSM1 | - | JOSD3 | - |
| USP5S | - | USP48 | - | OTUD4 | - | | | | | PSMD7 | - | | |
| USP5L | - | USP49 | - | OTUD6A | +/- | | | | | EIF3F | - | | |
| USP6 | - | USP50 | - | OTUD6B | - | | | | | EIF3H | - | | |
| USP7 | +/- | USP51 | - | OTUD7A | - | | | | | STAMBP | - | | |
| USP8 | - | USP53 | - | OTUD7B | ++ | | | | | STAMBPL | - | | |
| USP9X | - | USP54 | - | A20 | - | | | | | MPND | - | | |
| USP9Y | - | CYLD | - | OTULIN | - | | | | | PRPF8 | - | | |
| USP10 | - | DUB3 | - | OTULINL | - | | | | | | | | |
| USP11 | - | USPL1 | - | OTUB1 | - | | | | | | | | |
| USP12 | - | USP27X | - | OTUB2 | - | | | | | | | | |
| USP13 | - | USP28 | ++ | VCPIP1 | - | | | | | | | | |
| USP14 | - | USP29 | - | ZRANB1 | - | | | | | | | | |
| USP15 | - | USP30 | - | PARP11 | - | | | | | | | | |
| USP16 | - | USP31 | - | | | | | | | | | | |
| USP18 | - | USP32 | - | | | | | | | | | | |
| USP19 | - | USP33 | - | | | | | | | | | | |
| USP20 | - | USP34 | - | | | | | | | | | | |
| USP21 | - | USP35 | - | | | | | | | | | | |
| USP22 | ++ | USP36 | - | | | | | | | | | | |
| USP24 | - | USP37 | - | | | | | | | | | | |
| USP25 | - | USP38 | - | | | | | | | | | | |
| USP26 | - | USP39 | - | | | | | | | | | | |
| USP47 | - | USP40 | - | | | | | | | | | | |

Table S2. Case number across different cancer types for analyzing *LSD1* and *OTUD7B* mRNA expression profile

| <i>TCGA ID</i> | <i>Cancer types</i> | <i>Tumor</i> | <i>Normal</i> |
|----------------|--|--------------|---------------|
| ACC | Adrenocortical carcinoma | 77 | 128 |
| BLCA | Bladder Urothelial Carcinoma | 404 | 28 |
| BRCA | Breast invasive carcinoma | 1085 | 291 |
| CESC | Cervical squamous cell carcinoma and endocervical adenocarcinoma | 306 | 13 |
| CHOL | Cholangiocarcinoma | 36 | 9 |
| COAD | Colon adenocarcinoma | 275 | 349 |
| DLBC | Lymphoid Neoplasm Diffuse Large B-cell Lymphoma | 47 | 337 |
| ESCA | Esophageal carcinoma | 182 | 286 |
| GBM | Glioblastoma multiforme | 163 | 207 |
| HNSC | Head and Neck squamous cell carcinoma | 519 | 44 |
| KICH | Kidney Chromophobe | 66 | 53 |
| KIRC | Kidney renal clear cell carcinoma | 523 | 100 |
| KIRP | Kidney renal papillary cell carcinoma | 286 | 60 |
| LAML | Acute Myeloid Leukemia | 173 | 70 |
| LGG | Brain Lower Grade Glioma | 517 | 207 |
| LIHC | Liver hepatocellular carcinoma | 369 | 160 |
| LUAD | Lung adenocarcinoma | 483 | 347 |
| LUSC | Lung squamous cell carcinoma | 486 | 338 |
| OV | Ovarian serous cystadenocarcinoma | 426 | 88 |
| PAAD | Pancreatic adenocarcinoma | 179 | 171 |
| PCPG | Pheochromocytoma and Paraganglioma | 182 | 3 |
| PRAD | Prostate adenocarcinoma | 492 | 152 |
| READ | Rectum adenocarcinoma | 92 | 318 |
| SARC | Sarcoma | 262 | 2 |
| SKCM | Skin Cutaneous Melanoma | 461 | 558 |
| STAD | Stomach adenocarcinoma | 408 | 211 |
| TGCT | Testicular Germ Cell Tumors | 137 | 165 |
| THCA | Thyroid carcinoma | 512 | 337 |
| THYM | Thymoma | 118 | 339 |
| UCEC | Uterine Corpus Endometrial Carcinoma | 174 | 91 |
| UCS | Uterine Carcinosarcoma | 57 | 78 |
| Total cases | | 9497 | 5360 |

TCGA PanCan 2018

Table S3. Case numbers of different cancer types utilized for analyzing genetic alteration of *LSD1*

| <i>Cancer types (TCGA PanCan 2018)</i> | <i>Case number</i> |
|--|---------------------|
| Uterine | 529 |
| Melanoma | 444 |
| Bladder | 411 |
| Cholangiocarcinoma | 36 |
| Lung squ | 487 |
| PCPG | 178 |
| Pancreas | 184 |
| DLBC | 48 |
| Ovarian | 584 |
| Colorectal | 594 |
| Stomach | 440 |
| Uterine CS | 57 |
| Cervical | 297 |
| Esophagus | 182 |
| Sarcoma | 255 |
| Lung adeno | 566 |
| Mesothelioma | 87 |
| Liver | 372 |
| pRCC | 283 |
| GBM | 592 |
| Thymoma | 123 |
| Prostate | 494 |
| LGG | 514 |
| Head & neck | 523 |
| Breast Invasive Carcinoma Breast | 1084 |
| Testicular germ cell | 149 |
| ccRCC | 511 |
| ACC | 92 |
| chromophobe RCC | 65 |
| AML | 200 |
| Thyroid | 500 |
| Uveal melanoma | 80 |
| <i>Total cases</i> | <i>10961</i> |

Table S4. shRNA or sgRNA sequences in detail.

| Target | Sequence (5'—3') |
|-------------------|-----------------------|
| h. Ctrl sgRNA | GACCAGGATGGGCACCACCC |
| h. OTUD7B sgRNA#1 | GCTGAGTCTGTTGGTAACGG |
| h. OTUD7B sgRNA#2 | ATAGAAGCTGCAGTTCGGTT |
| h. Ctrl shRNA | GCAAAGAAGGCCACTACTATA |
| h. LSD1 shRNA#1 | GCCACATTCGCAAAGGAAAC |
| h. LSD1 shRNA#2 | GCAGTTGTGGTTGGATAATCC |
| h. p62 shRNA | GCGGCTTCCAGGCGCACTACC |
| h. CoREST shRNA | GCATCATAACCTTTATCATGC |
| h. USP22 shRNA#1 | GGAACACATGCTTCATGAACT |
| h. USP22 shRNA#2 | AGTCTCAACAATGACAACAA |
| h. USP28 shRNA#1 | GCTGCCAACAAGGAAGTATTA |
| h. USP28 shRNA#2 | GCTGGAATTCCTCAGATTAT |

Table S5. Antibodies list

| | | |
|---|-------------|-----------------|
| mouse monoclonal anti-LSD1 (Immunoblotting, immunofluorescence, IHC and immunoprecipitation) | CST | Cat# 4218 |
| rabbit polyclonal anti-LSD1 (Immunoblotting, ChIP-seq and ChIP-qPCR) | BETHYL | Cat# A300-215A |
| rabbit polyclonal anti- OTUD7B (Immunoblotting, immunofluorescence, IHC and immunoprecipitation) | Proteintech | Cat# 16605-1-AP |
| mouse monoclonal anti- OTUD7B (immunoblotting) | Proteintech | Cat# 66276-1-Ig |
| rabbit polyclonal anti-CoREST (Immunoblotting) | CST | Cat# 14567 |
| mouse monoclonal anti- H3K9me2 (ChIP-seq and ChIP-qPCR) | Abcam | Cat#1220 |
| rabbit polyclonal anti- H3K4me2 | Millipore | Cat# 07-030 |
| rabbit polyclonal anti-HDAC1 (immunoblotting) | Proteintech | Cat# 10197-1-AP |
| rabbit polyclonal anti-HDAC2 (immunoblotting) | Proteintech | Cat# 12922-3-AP |
| rabbit polyclonal anti-USP22 (immunoblotting) | Proteintech | Cat# 55110-1-AP |
| rabbit polyclonal anti-USP28 (immunoblotting) | Proteintech | Cat# 17707-1-AP |
| rabbit polyclonal anti-Flag (immunoblotting) | Proteintech | Cat# 20543-1-AP |
| rat monoclonal anti-HA (immunoblotting) | Roche | Cat# 3F10 |
| mouse monoclonal anti-Actin (immunoblotting) | Sigma | Cat# A1978 |
| mouse monoclonal anti-Myc (immunoblotting) | CST | Cat# 2276 |
| rabbit polyclonal anti-Myc (immunoblotting) | Proteintech | Cat# 16286-1-AP |
| mouse monoclonal anti-GFP (immunoblotting) | Proteintech | Cat# 66002-1-Ig |
| rabbit polyclonal anti-p62 (immunoblotting) | Proteintech | Cat# 18420-1-AP |
| mouse monoclonal anti-Cyclin B (immunoblotting) | CST | Cat# 4135 |
| mouse monoclonal anti-Cyclin E (immunoblotting) | CST | Cat# 4129 |

| | | |
|--|-------------|-----------------|
| mouse monoclonal anti-Cyclin D1 (immunoblotting) | Proteintech | Cat# 60186-1-Ig |
| rabbit polyclonal anti-CDK6 (immunoblotting) | CST | Cat# 14052-1-AP |
| mouse monoclonal anti-Tubulin (immunoblotting) | Proteintech | Cat# 66240-1-Ig |
| rabbit polyclonal anti-PKD1 (immunoblotting) | ABclonal | Cat# A14200 |
| rabbit polyclonal anti-RIN1 (immunoblotting) | Proteintech | Cat# 16388-1-AP |
| rabbit polyclonal anti-RBBP8 (immunoblotting) | Proteintech | Cat# 12624-1-AP |
| rabbit polyclonal anti-Keap1 (immunoblotting) | Proteintech | Cat# 10503-2-AP |
| rabbit polyclonal anti-RASSF5 (immunoblotting) | SAB | Cat# 27727 |
| rabbit monoclonal anti-Snail (immunoblotting) | CST | Cat# 3879 |
| mouse monoclonal anti-His (immunoblotting) | Proteintech | Cat# 66005-1-Ig |
| rabbit polyclonal anti-GST (immunoblotting) | Proteintech | Cat# 10000-0-AP |
| mouse monoclonal anti-Lamin B1 (immunoblotting) | Proteintech | Cat# 66095-1-Ig |
| mouse monoclonal anti-GAPDH (immunoblotting) | Santa cruz | Cat# sc32233 |

Table S6. Primers for quantitative real-time PCR

| Target | Forward primer (5'—3') | Reverse primer (5'—3') |
|------------------|------------------------|-------------------------|
| <i>Keap1</i> | ATCGATGGCCACATCTATG | GATCCTTCGTGTCAGCATTG |
| <i>Sox15</i> | GGACAGGGAAGAGGCAACCT | GTTTGCAGTGGGAAGAGCCATA |
| <i>RIN1</i> | TCTTCCTGAAGCGAAGGAGC | CCTGGGCTGGCTTTTCTCTC |
| <i>RASSF5</i> | GGAGCATCTTCGAGCAGCC | GTGAATTTACAGTTAGTGCAGCG |
| <i>ANAPC2</i> | GCCACGTGCAAGGTTCTT | TCTGGGAAGTCTCGGACGAT |
| <i>BMP2</i> | TCCATGTGGACGCTCTTTCA | ACCATGGTCGACCTTTAGGAG |
| <i>PKD1</i> | CCTTCCTCACGTTCTCAGGC | GCCAGCACACCAGACTCTTA |
| <i>RBBP8</i> | GGAGCACTCTGTGTGTGCAA | ATGTGCTTTGGCCATTGGAG |
| <i>Cyclin D1</i> | GACCCCGCACGATTTTATTG | CATGGAGGGCGGATTGGAAA |
| <i>CDK6</i> | GTCTGATTACCTGCTCCGCG | TTACATAGCCTCTGCCCAAGC |
| | A | |
| <i>Snail</i> | CGAGTGGTTCTTCTGCGCTA | GGGCTGCTGGAAGGTAAACT |
| <i>Gapdh</i> | TCGGAGTCAACGGATTTGGT | TGGAATTTGCCATGGGTGGA |

Table S7. Primers for LSD1 ChIP-qPCR.

| Target | Forward primer (5'—3') | Reverse primer (5'—3') |
|------------------|------------------------|------------------------|
| <i>Keap1</i> | CCTCCACCTCAGCCTC | AATTAGCCGGGTGTGGTG |
| <i>Sox15</i> | CCCTGGGGCAAGAAAGGC | CACGGCCCAAGCACCTGC |
| <i>RINI</i> | GTAAAGCGGCCTCAGTC | TGAGGCCAGGGGCAGATG |
| <i>RASSF5</i> | CTACTAACAAGGGGAAAG | CTGTCTGGTCATCCAGGC |
| <i>ANAPC2</i> | GGAGGCCCGCCCTCCGC | CCTCTATGCCTGGGGGC |
| <i>BMP2</i> | ATTATCCCAAACGTTTGAGC | GGTCTCCCATTTGGCTGGCG |
| <i>PKD1</i> | TTTTGAGACAGGGTCTTG | TGAGCTGTGATCACACCAC |
| <i>RBBP8</i> | GACCTGGCTGGAAAGCC | GGGACATTTCAAGGGGTCC |
| <i>Cyclin D1</i> | GCCGCCAGGTATGCGGCTGC | GGGTGGTGCAGGGACCAGTC |
| <i>CDK6</i> | GATTATCACACGGCATCCTC | TGGGTGCGTTTTAGGGACAG |
| <i>Snail</i> | AGCCGGGCGGAGGAAATTTC | CGGGACACCTGACCTTCCG |

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Table S8. Primers for H3K4me2 ChIP-qPCR.

| Target | Forward primer (5'—3') | Reverse primer (5'—3') |
|------------------|------------------------|------------------------|
| <i>Keap1</i> | CCCTTCTCACTGTCCCTTC | AGATGGTGGCGCGCAGC |
| <i>Sox15</i> | CACCACCCCAACCCCTTTC | CTCGGCCTTTCTTGCCCC |
| <i>RINI</i> | GGGAGGAGAAGGATGTC | CGGGCCCTGCTCCTTG |
| <i>RASSF5</i> | CCTGAGCGGCCCCGAGC | GCGCCGGGACACAGAG |
| <i>ANAPC2</i> | TGGCATTCCAGAACTCAG | CCTGGAGGAGTGGTTCG |
| <i>BMP2</i> | GGAGCTAGCGCGGAGCGCCC | CCCGAGGGACGCGTGGCCCC |
| <i>PKD1</i> | TCCCAAAGTCTGGGATTC | ATTCAACAACAGACAGG |
| <i>RBBP8</i> | ATCCGCGTCCATACCCC | TGCAGGGAAGCATGTGTAG |
| <i>Cyclin D1</i> | GCCAGCCGCCCTGGTGG | GGTGCAGGGACCAGTCC |
| <i>CDK6</i> | GATTATCACACGGCATCC | GCCAACCTGAGACATGC |

Table S9. Primers for H3K9me2 ChIP-qPCR.

| Target | Forward primer (5'—3') | Reverse primer (5'—3') |
|------------------|------------------------|------------------------|
| <i>Keap1</i> | CCCTTCTCACTGTCCCTTC | AGATGGTGGCGCGCAGC |
| <i>Sox15</i> | CACCACCCCAACCCCTTTC | CTCGGCCTTTCTTGCCCC |
| <i>RIN1</i> | GGGAGGAGAAGGATGTC | CGGGCCCTGCTCCTTG |
| <i>RASSF5</i> | CCTGAGCGGCCCCGAGC | GCGCCGGGACACAGAG |
| <i>ANAPC2</i> | GGAGGCCCGCCCTCCGC | CCTCTATGCCTGGGGGC |
| <i>BMP2</i> | GGAGCTAGCGCGGAGCGCCC | CCCGAGGGACGCGTGGCCCC |
| <i>PKD1</i> | TTTTGAGACAGGGTCTTG | TGAGCTGTGATCACACCAC |
| <i>RBBP8</i> | ATCCGCGTCCATACCCC | TGCAGGGAAGCATGTGTAG |
| <i>Cyclin D1</i> | CCGACCATCCGCCAGG | CTGAAATCACCTTCACG |
| <i>CDK6</i> | CTGTCTCTGCTCTCTGTC | CCTTGTGGTGCTTGGCAG |
| <i>Snail</i> | ATTGCGGGCTCGGGAGAC | CTGCCCTCTACACGGCAC |

Table S10. Gene sets utilized in GSEA analysis.

| Gene Set Utilized | |
|---|--|
| HALLMARK_EPITHELIAL MESENCHYMAL TRANSITION | CD44, VCAN, DAB2, FOXC2, GJA1, CXCL1, IGFBP2, IL6, CXCL8, ITGAV, ITGB3, JUN, LOX, LOXL2, MMP1, MMP2, MMP3, MMP14, TNFRSF11B, SERPINE1, PLAUR, HTRA1, PTHLH, CXCL12, SNAI2, SPP1, TAGLN, TGFB1, TGM2, TIMP3, VCAM1, VEGFA, VEGFC, VIM, WNT5A, ADAM12, SLIT2, CADM1 |
| HALLMARK_TNFA SIGNALING VIA NFKB | BIRC2, ATF3, BMP2, BTG1, CD44, CEBPB, KLF6, HBEGF, EGR1, F3, CXCL1, CXCL2, CXCL3, ICAM1, IL6, JUN, SMAD3, MYC, NFKB1, SERPINE1, PER1, PLAU, PLAUR, PTGS2, CCL5, STAT5A, TAP1, TNF, VEGFA, FOSL1, IRS2, SPHK1, KLF4, TRIP10, ACKR3 |
| HALLMARK_IL6 JAK STAT3 SIGNALING | BAK1, CBL, CD44, CXCL1, CXCL3, HMOX1, IL2RG, IL6, ITGB3, JUN, LEPR, STAT1, STAT3, TGFB1, TNF, TYK2 |
| HALLMARK_TGF BETA SIGNALING | XIAP, RHOA, BMP2, CDH1, CTNNB1, ID1, SMAD3, SERPINE1, SKIL, TGFB1, ARID4B |
| GO_Positive Regulation of Cell Motility | AAMP, ACTN4, AKT1, AKT2, ANXA1, ANXA3, ARF6, RHOA, RHOC, BMP2, BMP4, BMP7, C1QBP, CAV1, CD74, CD151, CCR7, DAB2, DOCK1, HBEGF, EGFR, F2R, F3, PTK2B, FER, FGFR1, VEGFD, FOXC2, FLT4, GATA3, GCNT2, GRN, HGF, HIF1A, HMGB1, HMOX1, HSPA5, HSPB1, ICAM1, IGF1R, IGFBP5, IL6, CXCL8, ITGA6, ITGAV, ITGB3, KDR, LGALS3, LYN, SMAD3, MDK, MMP9, MMP14, NEDD9, DDR2, SERPINE1, PAK1, PDGFB, PFN1, PIK3CG, PLAU, MAPK1, MAPK3, PTGS2, PTK2, RAC1, RAC2, RET, RPS6KB1, S100A7, CCL5, SDCBP, CXCL12, SELE, SNAI2, SNAI1, SOX9, SP1, SRC, STAT3, STAT5A, TAC1, TACR1, TERT, TGFB1, TGFB2, TNF, TWIST1, VEGFA, VEGFC, WNT5A, CXCR4, IRS2, FADD, NRP2, CCN4, SPHK1, CLDN1, ARHGEF2, CXCL14, BCAR1, IQSEC1, AKT3, GPNMB, SEMA4D, ACKR3, RAB25, PREX1, FERMT3, STK11, SLIT2, ANGPT2, ATP6V1C1, AXL, BMP5, CBL, CBLB, CDC42, EFN1, ERBB2, ERBB4, EFEMP1, FGFR4, FOXC1, FOXO1, GSK3B, NRG1, IGFBP2, LEP, LOX, MMP2, MST1, MST1R, NTRK1, ENPP1, PIK3CA, PLAUR, PRL, PRLR, PTEN, PTPN3, PXN, ROBO1, SDC2, SHC1, SYK, TSC2, VAV2, YES1, WASL, ADIPOQ, ELMO1, VAV3, AGR2, TXNIP, GIT1, CCDC88A, EPHA10, XIAP, BMP6, RUNX2, CCNA2, CD44, CREB1, CTNNB1, E2F1, EGR1, GDF2, IBSP, ID1, IL10, JUN, SMAD4, MYC, PPARG, HTRA1, SKIL, CLDN5, TP53, VCAM1, KLF4, PPARGC1A, F11R, DLL4, HTRA3 |

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| GO_Chemotaxis | AGTR1, ALCAM, ANGPT2, ANXA1, RHOA, BMP4, BMP7, BSG, C1QBP, CD74, CCR5, CCR7, CREB1, HBEGF, EFN1, ERBB2, F3, PTK2B, FER, FGFR1, VEGFD, GATA3, CXCL1, CXCL2, CXCL3, HGF, NRG1, HMGB1, HOXB9, HSPB1, IL6, CXCL8, IL10, ITGAV, ITGB3, KDR, L1CAM, LGALS3, LOX, LYN, SMAD3, SMAD4, MDK, MIF, MST1, NOTCH3, NTRK1, SERPINE1, PDGFB, PIK3CA, PIK3CG, PLAU, PLAUR, MAPK1, MAPK3, PTK2, RAC1, RAC2, RET, ROBO1, S100A7, S100A8, S100A9, SCN1B, CCL5, CCL14, CCL18, CXCL12, SHC1, SRC, SYK, TGFB1, TSC2, VCAM1, VEGFA, VEGFC, EZR, VLDLR, WNT5A, CXCR4, FOSL1, TNFSF11, IRS2, TNFRSF11A, NRP2, SLIT2, CXCL14, BCAR1, VAV3, GPNMB, SEMA4D, EVL, ENAH, ACKR3, PREX1, WNT3A, EPHA10, AKT1, BMP5, CAV1, CD44, CDC42, CEACAM5, HMOX1, ICAM1, ITGA6, LEP, EPCAM, MMP1, MMP9, MMP14, CD200, SDC2, SELE, TNF, YES1, FADD, WASL, F11R |
| GO_Regulation of Cell Adhesion | ACTN4, AKT1, ANGPT2, ANXA1, RHOA, ARHGDI, ARHGDIB, BMP2, BMP4, BMP6, BMP7, C1QBP, CAV1, CBLB, CD44, CD74, CDC42, CDH1, CDKN2A, CEBPB, CCR7, DOCK1, EFN1, ERBB2, PTK2B, FOXC2, GATA3, GCNT2, GSK3B, HMGB1, IBSP, ICAM1, IGFBP2, IL6, CXCL8, IL10, ILK, IDO1, IRAK1, ITGA6, ITGAV, JAK1, KDR, LEP, LGALS3, LYN, EPCAM, SMAD3, MDK, MMP14, MUC1, MYB, NEDD9, SERPINE1, PAK1, PDGFB, PIK3CA, PIK3CG, PLAU, PLAUR, PTEN, PTK2, RAC1, RAC2, RAC3, RET, CCL5, CXCL12, SELE, SLC9A1, SNAI2, SOX9, SRC, SYK, TGFB1, TGFB2, TGM2, TNF, UTRN, VCAM1, VEGFA, WNT5A, YES1, CXCR4, TNFSF11, FADD, DNAJA3, KLF4, ADIPOQ, DLC1, VAV3, GPNMB, SEMA4D, AGR2, F11R, FOXP3, PREX1, PEAK1, CD276, FERMT3, WNT3A, S100A8, S100A9, EZR, AHR, AXL, BAK1, BST2, CD151, KLF6, EGR1, EZH2, LEPR, MIF, NTRK1, PRKDC, RAG2, STAT3, STK11, TAC1, TLR4, TP53, TNFRSF4, IRS2, CADM1, TLR9, DLL4, FER, GRN, HMOX1, IL13RA2, MMP8, CD200, SPHK1, IL1RL1, ADGRF5, CRP, ACE, MAPK1, MAPK3 |
| GO_Vasculature Development | AAMP, ADM, AGTR1, AHR, AKT1, ANGPT2, ANXA1, ANXA2, ANXA3, RHOA, BAK1, BMP4, BMP7, BRCA1, BSG, BTG1, CAV1, CDC42, CTNNA1, EGR1, ERBB2, F3, PTK2B, VEGFD, FOXC1, FOXC2, FLT4, GDF2, GRN, HGF, HIF1A, HK2, HMGB1, HMOX1, HSPB1, ID1, IL6, CXCL8, IL10, ITGAV, ITGB3, JAK1, JUN, KDR, LEP, LEPR, LOX, LOXL2, MDK, MMP2, MMP14, NFATC1, |

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| | SERPINE1, PDGFB, PIK3CA, PIK3CG, PPARG, MAPK1, PRL, PTEN, PTGS2, PTK2, PTK7, ROBO1, S100A7, SHC1, SIX1, SP1, STAT1, STK11, SYK, TERT, TGFB1, TGFBR2, CLDN5, TNF, TWIST1, VAV2, VEGFA, VEGFC, WNT5A, CXCR4, ADAM12, FOSL1, HMGA2, RECK, NRP2, SPHK1, KLF4, SLIT2, AKT3, PAK4, YAP1, VAV3, GPNMB, HTATIP2, DLL4, FOXJ2, ACKR3, SOX17, ADGRF5, BMP2, BMP5, BMP6, BRCA2, CEBPB, EGFR, ESR1, FGFR1, GATA3, GJA1, GLI1, SFN, IGFBP5, SMAD3, MST1, MYC, NME1, PGR, SERPINB5, PRKDC, HTRA1, RB1, CXCL12, SNAI2, SOX9, STAT5A, TLR4, TNFSF11, CLDN1, ODAM, WNT3A, RICTOR, ARF6, ARHGDIB, C1QBP, DOCK1, HBEGF, EFNB1, ERBB4, FER, ITGB4, MMP9, DDR2, PAK1, PFN1, PXN, RAC1, RET, SRC, TAC1, PDLIM1, MTA2, BCAR1, IQSEC1, SEMA4D, PTP4A3, EVL, RAB25 |
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