**SUPPLEMENTARY MATERIAL**

# Next-generation sequencing reveals a novel role of lysine-specific demethylase 1 in adhesion of rhabdomyosarcoma cells

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## I. Supplementary Methods

### Cell cycle analysis

DNA content was determined by flow cytometric measurement of fixed and PI-stained nuclei and analyzed with FlowJo software (Tree Star Inc., Ashland, OR, USA) according to manufacturer's instructions.

### Invasion Assay

For invasion assays, cells were reversely transfected with siRNA against LSD1 or control siRNA for 72 hours in a 10 cm dish. Knockdown cells were trypsinized, counted and reseeded at 50.000 cells/$cm^2$ on matrigel-coated (dilution 1:3) transwell inserts (Millicell cell culture plate inserts, 12 mm diameter, pore size 8 µm, Millicell, Burlington, MA; USA) in serum-free (0.1% FCS) media to achieve a serum gradient in the setting with 10% FCS for attraction. Cells were allowed to invade and transmigrate for 24 hours and stained with crystal violet solution (0.5% crystal violet, 30% ethanol, and 3% formaldehyde) after removing the matrigel on the top site of the transwell insert. Stained cells were washed with tap water, dried and resolved in 1% SDS for comparative measurement at 550 nm with a spectrophotometer.

### Wound Healing Assay

For invasion assays, cells were reversely transfected with siRNA against LSD1 or control siRNA for 72 hours in a 6-well plate. After 72 hours of knockdown, confluent cell layers were scratched with a yellow (20-200 µl) pipette tip, rinsed and the media was changed to starvation medium with 0.5% FCS to avoid overgrowth of the cell layers. Pictures were taken with an Olympus IX71 microscope with a CC-12 camera system on start and every 24 hours until complete closure in the first condition. Before the picture was taken, the well was once washed with PBS and fresh 0.5% FCS-

medium was applied. Open area was measured with Image J software and calculated in relation to the initial open area.

## II. Supplementary Tables

Supplementary Table T1: Cells and cell culture

| Cell line | Obtained from | Culture Medium |
|---|---|---|
| RD | ATCC | DMEM |
| RH30 | DSMZ | RPMI 1640 |
| RMS13 | ATCC | RPMI 1640 |
| T174 | Unknown source | DMEM |
| RH41 | DSMZ | RPMI 1640 |
| TE381.T | ATCC | DMEM |
| TE671 | ATCC | DMEM |
| Kym-1 | JCRB | RPMI 1640 |
| TE441.T | ATCC | DMEM (with 20% FCS) |
| RH18 | DSMZ | DMEM |
| RH36 | ATCC | DMEM |

Supplementary Table T2: Primer Sequences targeting mRNA

| Target | Forward Primer (5'-3') | Reverse Primer (5'-3') |
|---|---|---|
| 28S | TTGAAAATCCGGGGGAGAG | ACATTGTTCCAACATGCCAG |
| C3AR1 | GAAGCCTTCAGCTACTGTCTCAG | AGAATTACTGGGGGCTCATTC |
| COL2A1 | GTGAACCTGGTGTCTCTGGTC | TTTCCAGGTTTTCCAGCTTC |
| COL9A1 | GAGCTTGGCCGTGTAGGAC | AAAGCCAATTGTTCCTCTGG |
| FLNC | GCCTCCCTCTCGGATGAC | GGCTGGTTCACCTTGAGC |
| GAPDH | CAAGGTCATCCATGACAACTTTG | GGGTCCAAGTTGTCCAGAATGC |
| ITGA10 | GTTCTTGCCCCTGGTGTTC | GGGTGATGTTCATCCAGGTT |
| ITGA5 | CCCATTGAATTTGACAGCAA | TGCAAGGACTTGTACTCCACA |
| ITGB8 | GCATTATGTCGACCAAACTTCA | GCAACCCAATCAAGAATGTAACT |
| KDM1A | CCATGGTGGTAACAGGTCTTG | TGATCTTGGCCAGTTCCATA |
| MET | TGAAATTCATCCAACCAAATCTT | AATAGAAAACTGACAATGTTGAGAGG |
| PDGFRB | CATCTGCAAAACCACCATTG | GAGACGTTGATGGATGACACC |
| PLAU | TTGCTCACCACAACGACATT | GGCAGGCAGATGGTCTGTAT |
| PLAUR | CTGCAAGGGGAACAGCAC | GCTTTGGTTTTTCGGTTCG |
| SERPINE1 | CCCAGGACTAGGCAGGTG | AAGGCACCTCTGAGAACTTCA |
| SPP1 | GAGGGCTTGGTTGTCAGC | CAATTCTCATGGTAGTGAGTTTTCC |
| MEF2C | CAGGTGCTGACGGGTACAA | TCACAGTTTGCAACTCTTCTTTG |
| Myogenin | GCTCAGCTCCCTCAACCA | GCTGTGAGAGCTGCATTCG |
| MyoD | CACTACAGCGGCGACTCC | TAGGCGCCTTCGTAGCAG |

Supplementary Table T3: Primer Sequences targeting genomic DNA

| Target | Forward Primer (5'-3') | Reverse Primer (5'-3') |
|---|---|---|
| C3AR1 | ATACCCCTGGCTAGAGTGTT | GTTGGGGAGGAATGACCTGAA |
| ITGA10 | CGCTCAGTGTTTTCCTTGGTC | ATCTTCCATCCCCACCAGAAA |
| SERPINE1 | CTCACCCCCTTGCCCTAAAAG | TGAACGGGACCAGGGGTTAC |
| SPP1 | ACTGCCTGCCCCTCTTAAAAA | CTGACTGAGAGCAGGATGAC |
| negCtrl | TGCTGTTACTTTTTACAGGGAGTT | TTTGAGCAAAATGTTGAAAACAA |

Supplementary Table T4: Table of buffer concentrations used for chromatin-immunoprecipitation experiments

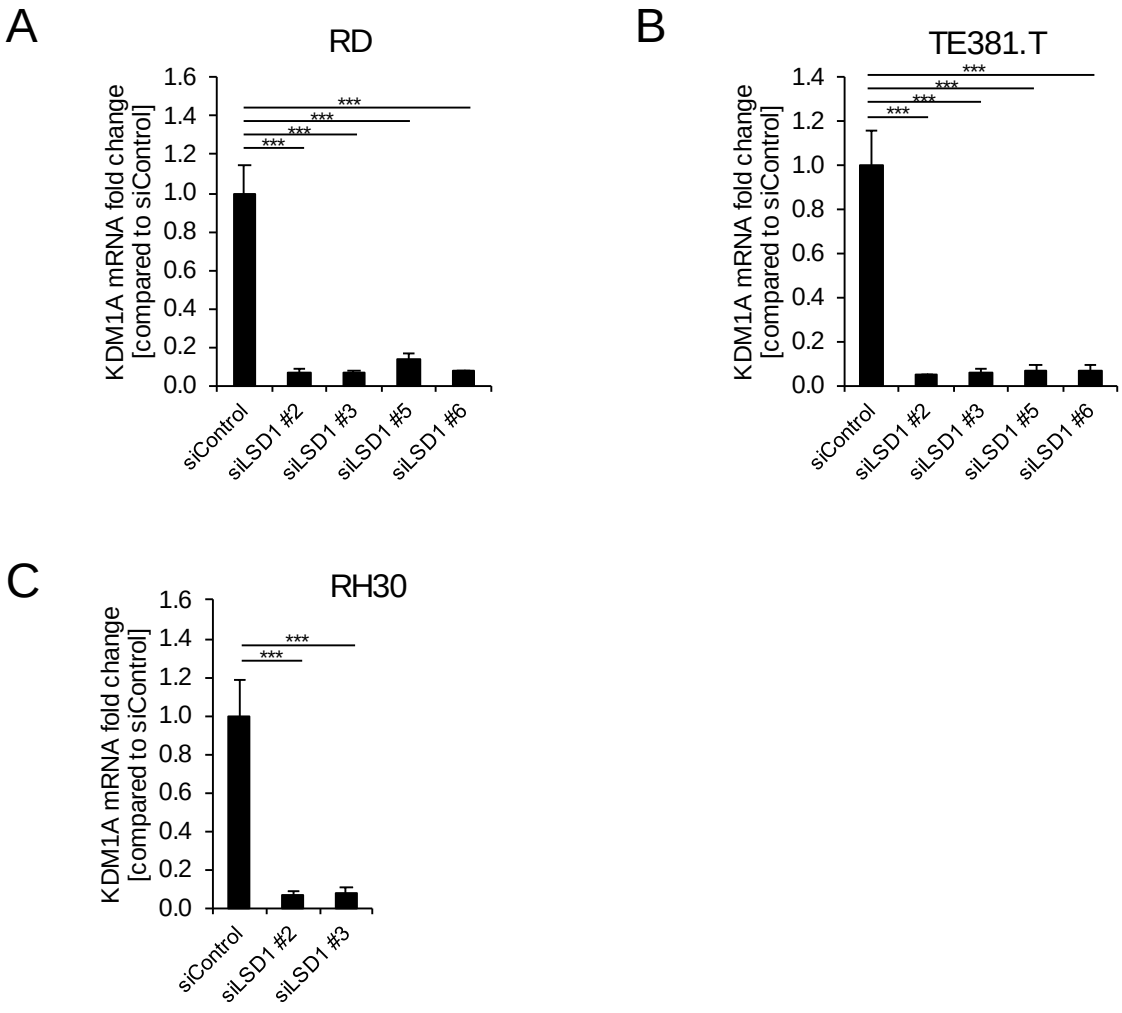
| **ChIP Buffer** | **Final Concentrations** |
|---|---|
| Incubation Buffer | 15 mM TrisHCl pH 7.4<br>15 mM NaCl<br>60 mM KCl<br>0.1 mM EDTA pH 8.0<br>0.5 mM EGTA pH 8.0<br>0.5 mM spermidine<br>0.15 mM spermine |
| Douncing Buffer | Incubation buffer + 0.6 % NP40 |
| TSE I Buffer | 20 mM TrisHCl pH 8.0<br>2 mM EDTA<br>150 mM NaCl<br>0.1 % SDS<br>1 % TritonX-100 |
| Dilution Buffer | 20 mM TrisHCl pH 8.0<br>2 mM EDTA<br>150 mM NaCl<br>1 % TritonX-100 |
| TSE II Buffer | 20 mM TrisHCl pH 8.0<br>2 mM EDTA<br>500 mM NaCl<br>0.1 % SDS<br>1 % TritonX100 |
| Buffer III Buffer | 10 mM TrisHCl pH 8.0<br>1 mM EDTA<br>1 % NP40<br>1 % Deoxycholate<br>250 mM LiCl |
| TE Buffer | 10 mM TrisHCl pH 8.0<br>1 mM EDTA |
| Elution Buffer | 1 % SDS<br>100 mM $NaHCO_3$ |

Supplementary Table T5: Differential expression of RNA-Sequencing siControl (WT) vs. siLSD1 #2 (KD2)

Supplementary Table T 6: Differential expression of RNA-Sequencing siControl (WT) vs. siLSD1 #3 (KD3)

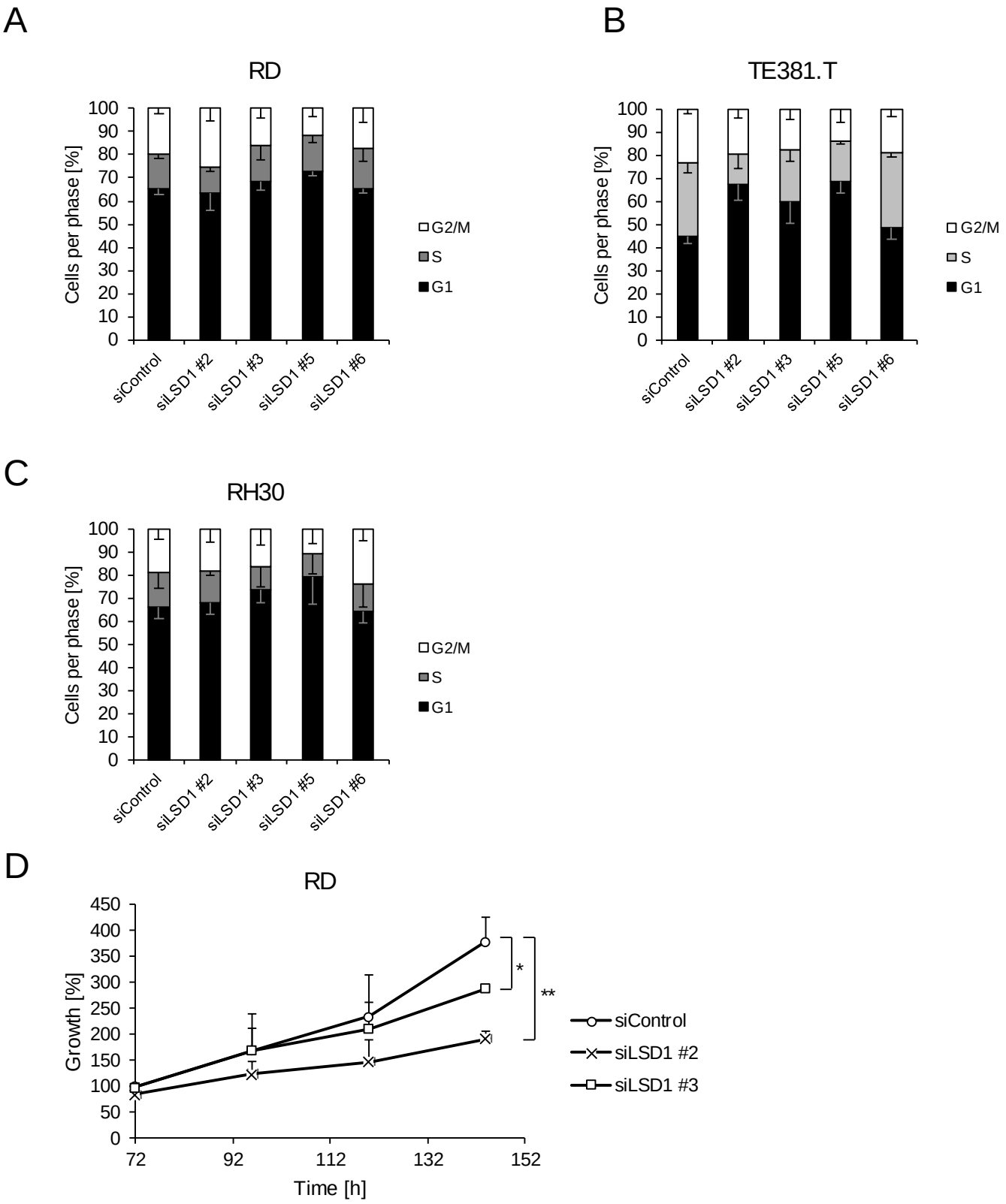
## III. Supplementary Figures

Supplementary Figure 1



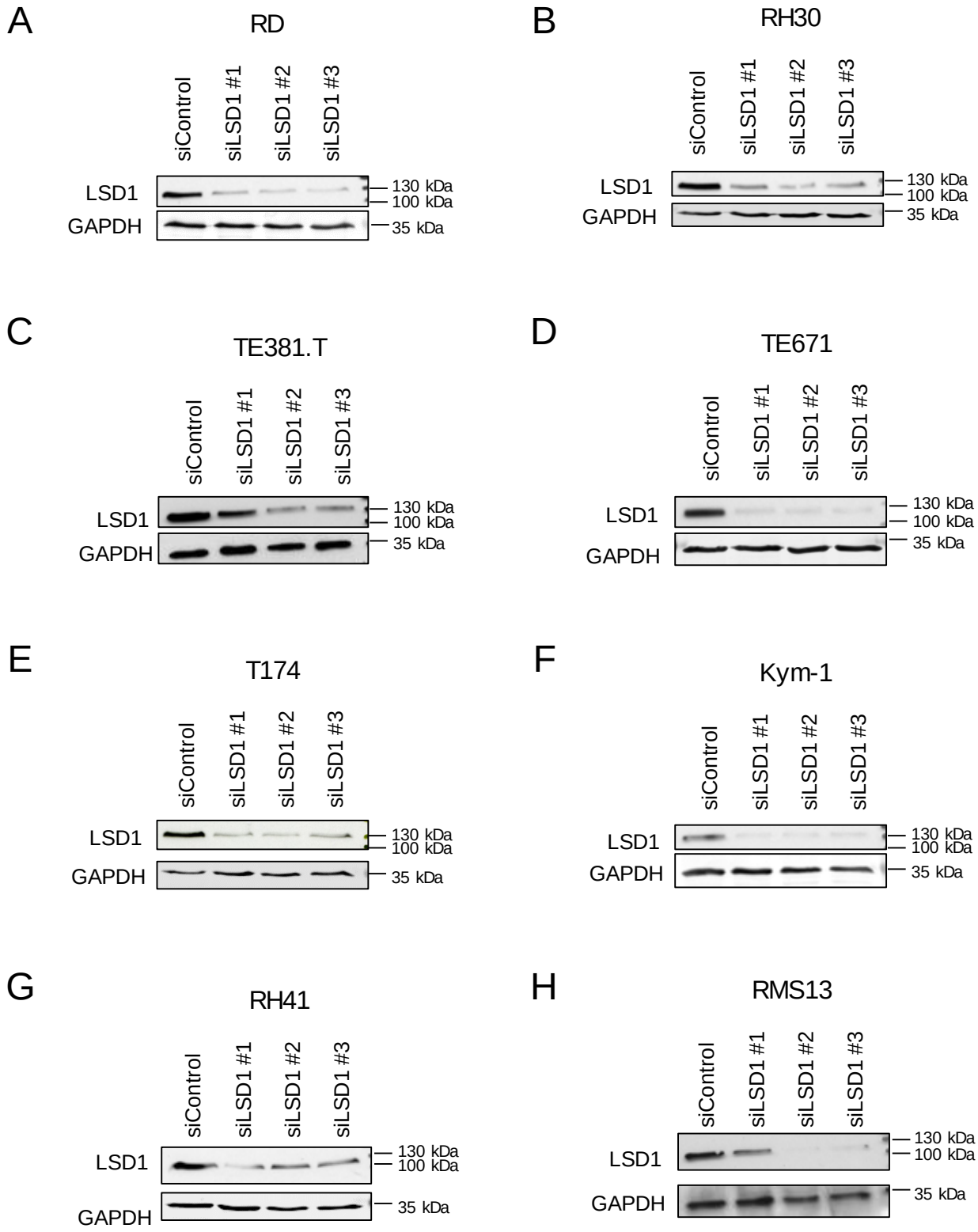

**Supplementary Figure 1: LSD1 mRNA is reduced upon siRNA-mediated knockdown**
RD (**A**), TE381.T (**B**) and RH30 (**C**) cells were reversely transfected with siRNA against LSD1 or siControl for 96 hours. LSD1 mRNA levels were measured by using qRT-PCR with GAPDH and β-Actin as reference and for normalization. Mean and SD of three independent experiments are shown; ***P<0.001.

Supplementary Figure 2




**Supplementary Figure 2: LSD1 knockdown does not affect the cell cycle of RMS cell lines**
**A-C** RD (A), TE381.T (B) and RH30 (C) cells were reversely transfected with siRNA against LSD1 or siControl for 96 hours. Nuclei were stained with PI and fluorescence was measured with flow cytometry using PerCP-Channel. FlowJo software was used for conducting cell cycle analysis. Mean and SD of three independent experiments are shown. **D** RD cells were reversely transfected with siRNA against LSD1 or siControl for 72 hours. After 72 hours of knockdown, the media was changed to 0.5% FCS and cell growth was measured every 24 hours up to 144 hours of knockdown in total with crystal violet staining. Mean and SD of three independent experiments are shown; *P<0.5, **P<0.1.

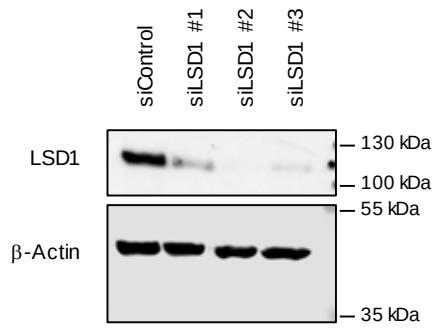
Supplementary Figure 3




**Supplementary Figure 3: siRNA-mediated knockdown of LSD1 strongly reduces LSD1 protein levels**
RD (**A, J**), RH30 (**B, K**), TE381.T (**C**), TE671 (**D**), T174 (**E**), Kym-1 (**F**), RH41 (**G**) and RMS13 (**H**) cells were reversely transfected with siRNA against LSD1or siControl for 48 (**A-H**) or 144 (**J,K**) hours. Knockdown of LSD1 was confirmed using Western blotting, with GAPDH as loading control. SiLSD1 construct #1 was excluded from functional analysis, since its efficacy varied between the cell lines, raising doubts on the target specificity for LSD1. **L** A panel of aRMS (RH30, RMS13, RH41), eRMS (RD, TE381.T, T174, RH36, RH18, TE441.T) and the not finally specified Kym-1 cells were assessed for their basal LSD1 protein expression using Western blotting, with β-Actin as loading control.

Supplementary Figure 3 (continued)

J

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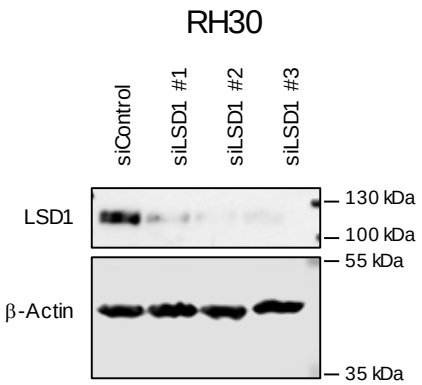


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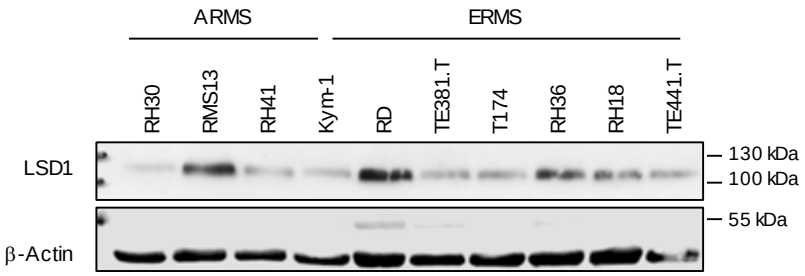
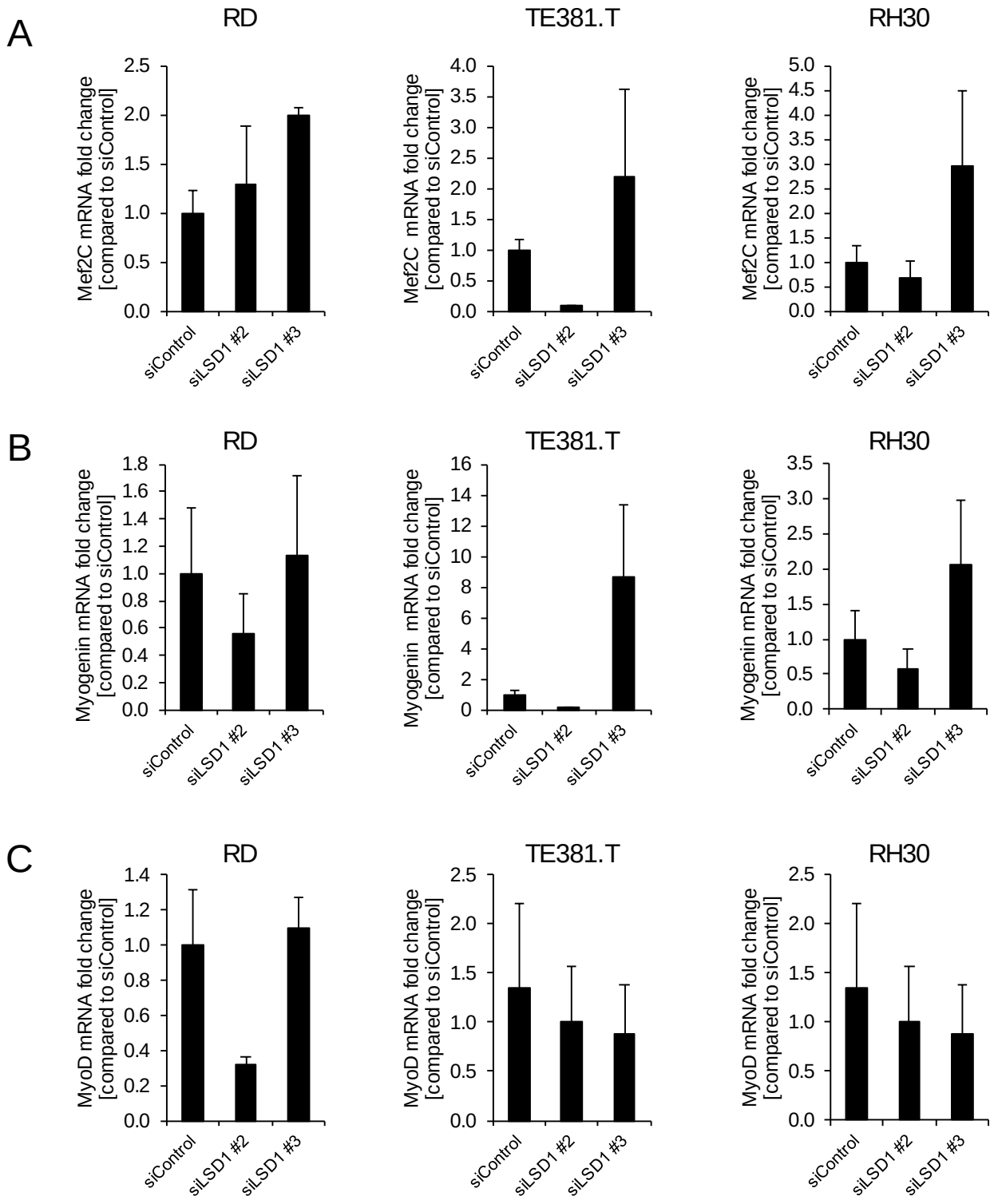


Supplementary Figure 4

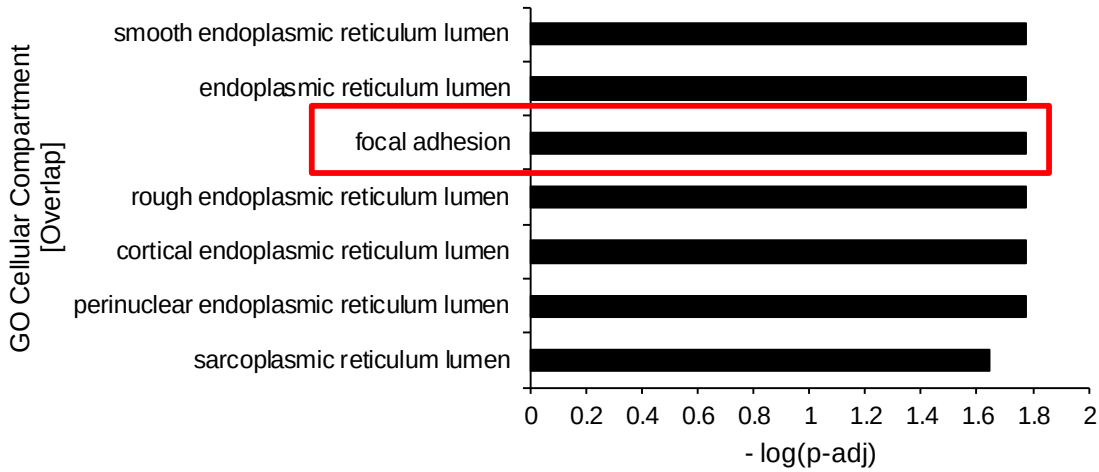



**Supplementary Figure 4: Knockdown of LSD1 does not change the expression of myogenic differentiation markers in RMS cell lines**

RD, TE381.T and RH30 cells were reversely transfected with siRNA against LSD1 or siControl for 96 hours. The myogenic differentiation markers Mef2C (**A**), Myogenin (**B**) and MyoD (**C**) were analyzed with RT-qPCR. GAPDH and 28S-rRNA were used as reference and for normalization. Values are displayed relative to siControl. Mean and SD of three independent experiments in triplicate are shown.

Supplementary Figure 5

A



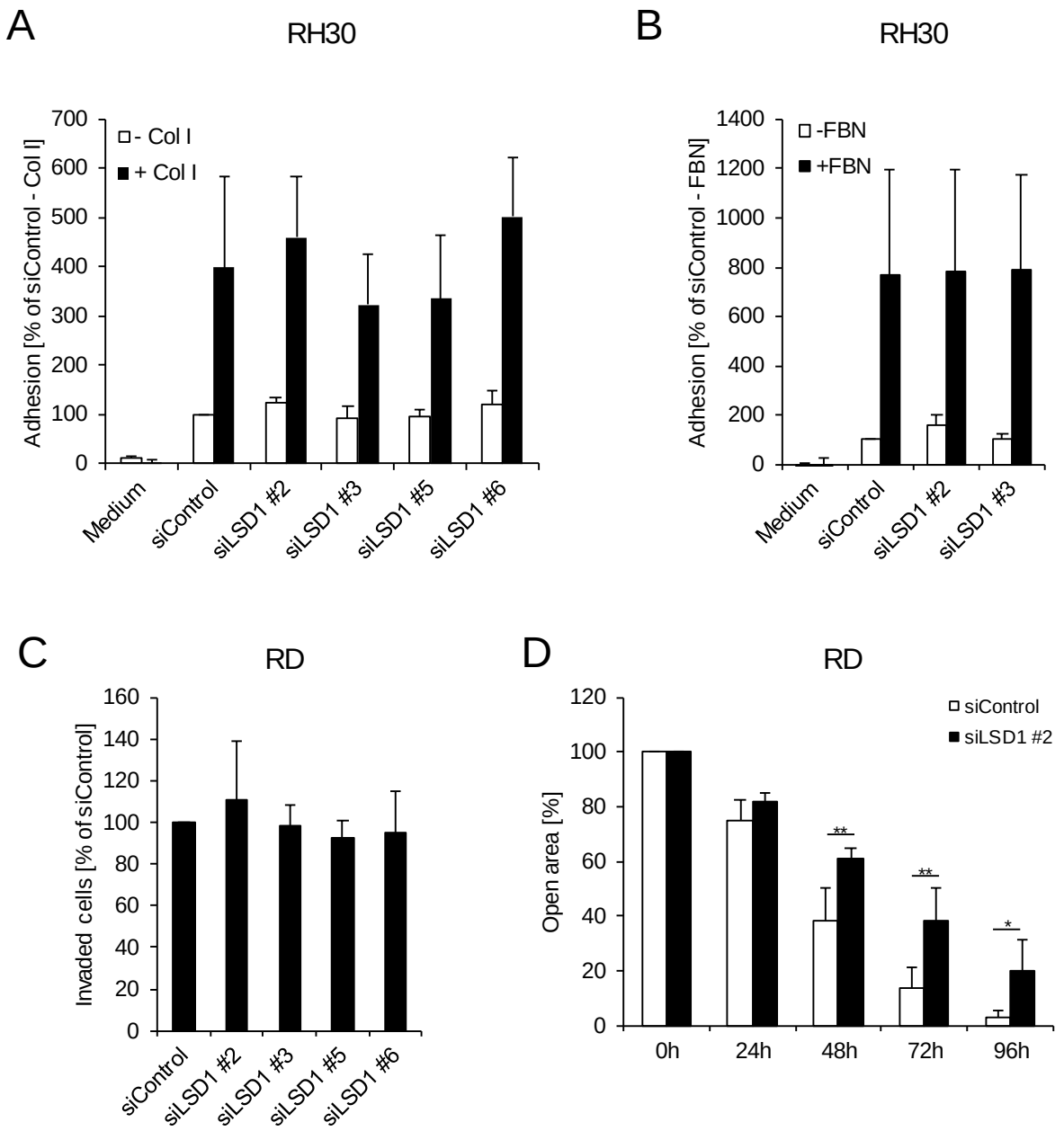

B

| Gene | Protein | siLSD1 #2 | | siLSD1 #3 | |
|---|---|---|---|---|---|
| | | log2 Fold change | p-Value | log2 Fold change | p-Value |
| BMP4 | bone morphogenetic protein 4 | 0.79 | 5.29E-07 | -0.71 | 3.12E-04 |
| COL26A1 | collagen type XXVI alpha 1 chain | -1.20 | 6.96E-20 | -1.24 | 3.32E-12 |
| COL2A1 | collagen type II alpha 1 chain | -1.05 | 2.65E-10 | -1.61 | 8.72E-17 |
| ASPH | aspartate beta-hydroxylase | 0.67 | 5.60E-12 | 0.55 | 3.55E-04 |
| COL5A3 | collagen type V alpha 3 chain | -1.02 | 4.01E-11 | -0.88 | 4.52E-07 |
| EBI3 | Epstein-Barr virus induced 3 | 4.31 | 1.66E-51 | 2.70 | 1.18E-13 |
| COL9A1 | collagen type IX alpha 1 chain | -0.96 | 8.86E-05 | -1.30 | 2.17E-06 |
| SPP1 | secreted phosphoprotein 1 | 1.44 | 8.86E-26 | 0.55 | 1.40E-05 |
| PLAUR | plasminogen activator, urokinase receptor | 2.25 | 2.24E-57 | 0.94 | 2.76E-13 |
| GAS6 | growth arrest specific 6 | 1.32 | 3.19E-10 | -1.53 | 3.35E-13 |
| SCG2 | secretogranin II | 2.27 | 7.50E-11 | 1.29 | 1.65E-08 |
| CTSC | cathepsin C | 0.89 | 4.22E-10 | 0.86 | 5.55E-08 |
| TMEM97 | transmembrane protein 97 | -0.87 | 9.44E-13 | -0.68 | 3.90E-08 |

**Supplementary Figure 5: Analysis of GO Cellular Compartment associates LSD1-regulated genes with focal adhesion and ER**

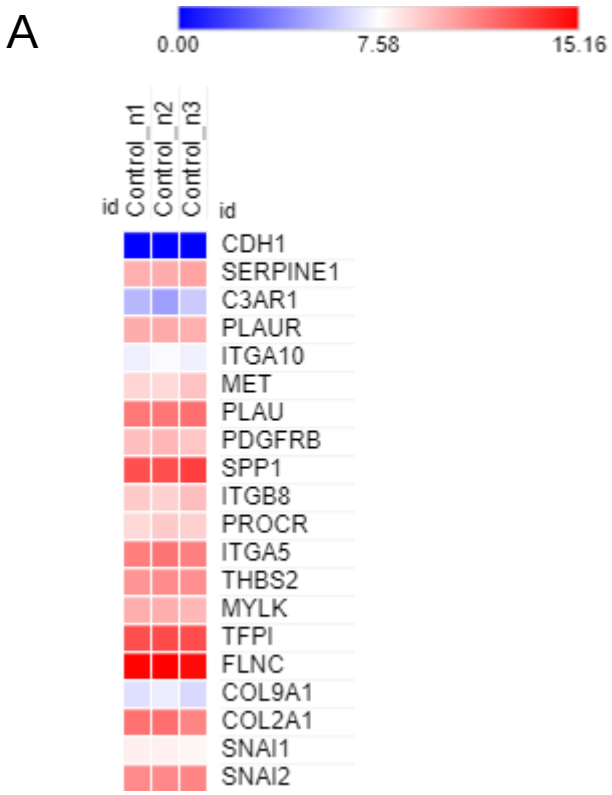
**A** GO analysis Cellular Compartment for the overlap done with EnrichR and sorted for adjusted p-value. Enriched pathways with padj<=0.5 are shown. **B** Table of genes contributing to the enrichment of GO terms from (A) with the exception of the term "focal adhesion (GO:0005925)".

Supplementary Figure 6



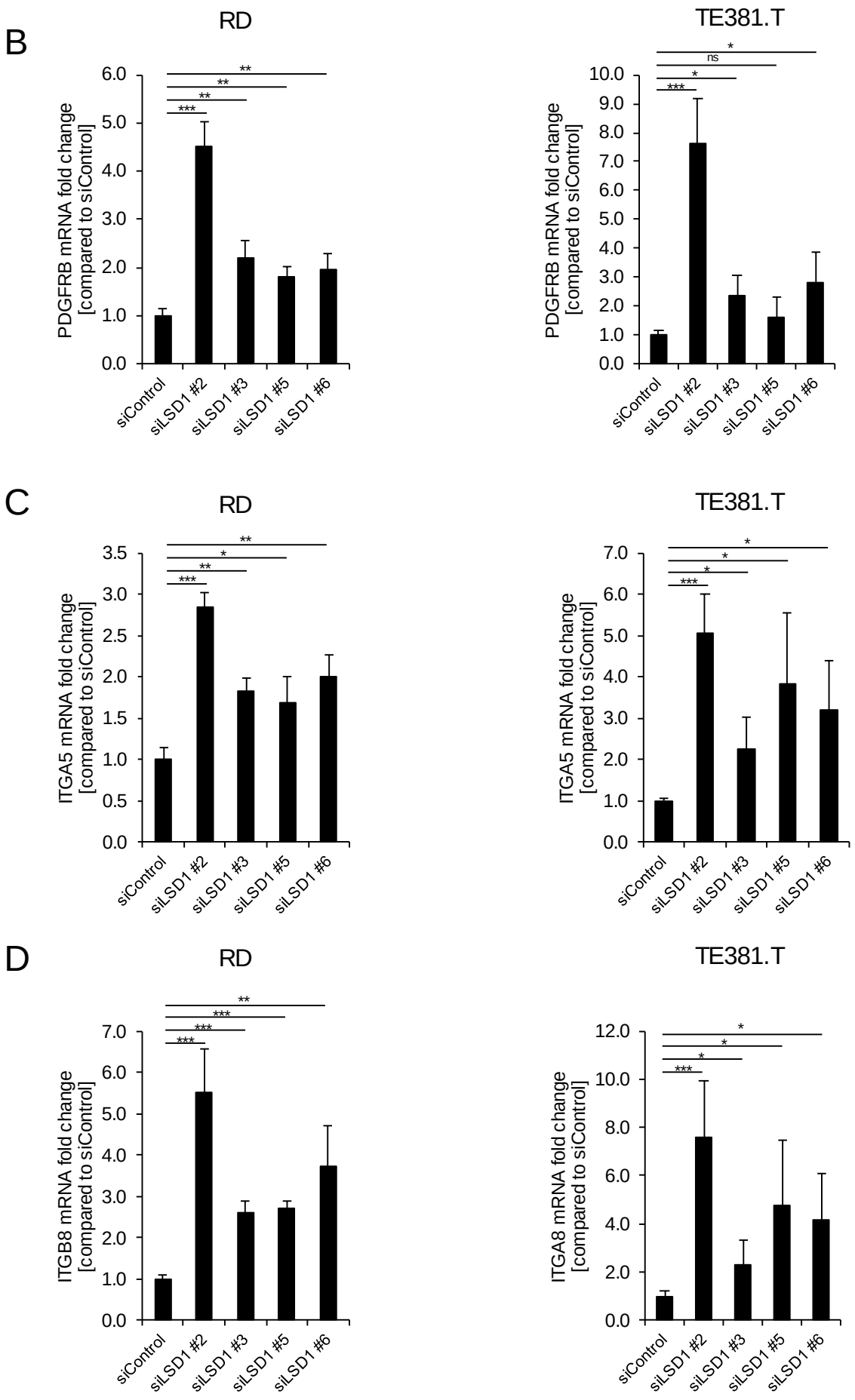

**Supplementary Figure 6: Adhesion to collagen I and fibronectin is not changed by LSD1 knockdown in RH30 cells**
**A** Plates were coated with 5 µg/cm$^2$ collagen I. RH30 cells were reversely transfected with siRNA against LSD1 or siControl for 96 hours. Cells were detached, counted and seeded for adhesion for 60 minutes. Adherent cells were stained with crystal violet after extensive washing. Mean and SD of three independent experiments in triplicate are shown. **B** Plates were coated with 1 µg/cm$^2$ fibronectin. RH30 cells were reversely transfected with siRNA against LSD1 or siControl for 96 hours. Cells were detached, counted and seeded for adhesion for 60 minutes. Adherent cells were stained with crystal violet after extensive washing. Mean and SD of three independent experiments in triplicate are shown. **C** RD cells were reversely transfected with siRNA against LSD1 or siControl for 72 hours. Cells were reseeded on matrigel-coated transwell assays for 24 hours and transmigration was analyzed with crystal violet staining. Mean of three independent experiments are shown. **D** RD cells were reversely transfected with siRNA against LSD1 or siControl for 72 hours. The confluent cell layer was scratched and wound closure was measured every 24 hours in starvation medium (0.5% FCS) up to complete closure in one condition. Mean and SD of three independent experiments with six replicates for each condition are shown; *P<0.5, **P<0.1.

Supplementary Figure 7




**Supplementary Figure 7: LSD1-dependent gene expression changes in RNA sequencing are verified by qRT-PCR**
**A** Analysis of expression of the 17 target genes as well as CHD1, SNAI1 and SNAI2 with heat mapping using log2-transformed counts from siControl RNA sequencing. The heat map was generated using the Morpheus tool (Broad Institute). Counts below 1 are displayed as zero values. **B-K** Validation of differential regulation by siLSD1 knockdown in the RNA sequencing with qRT-PCR. For this, RD and/or TE381.T cells were reversely transfected with siRNA against LSD1 or siControl for 96 hours. GAPDH and 28S-rRNA were used as reference and for normalization. Values are displayed relative to siControl. Mean and SD of three independent experiments in triplicate are shown. *P<0.05, **P<0.01, ***P<0.001.

Supplementary Figure 7 (continued)



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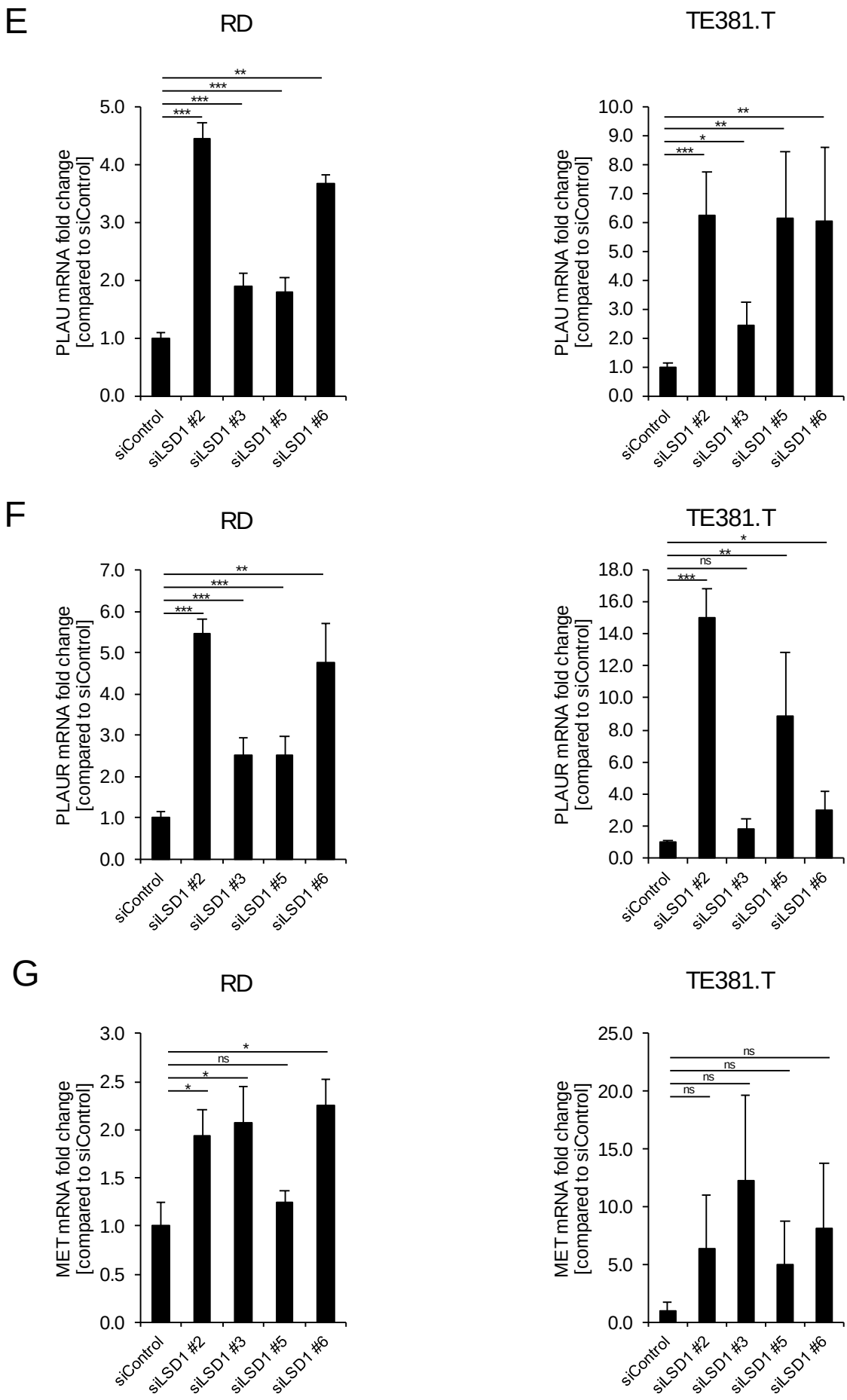
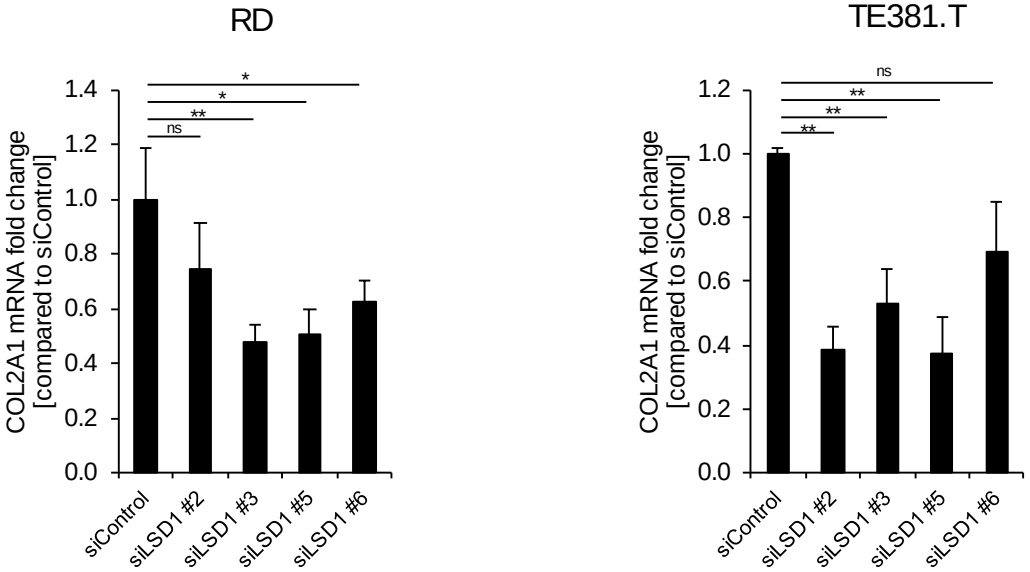


Supplementary Figure 7 (continued)

H

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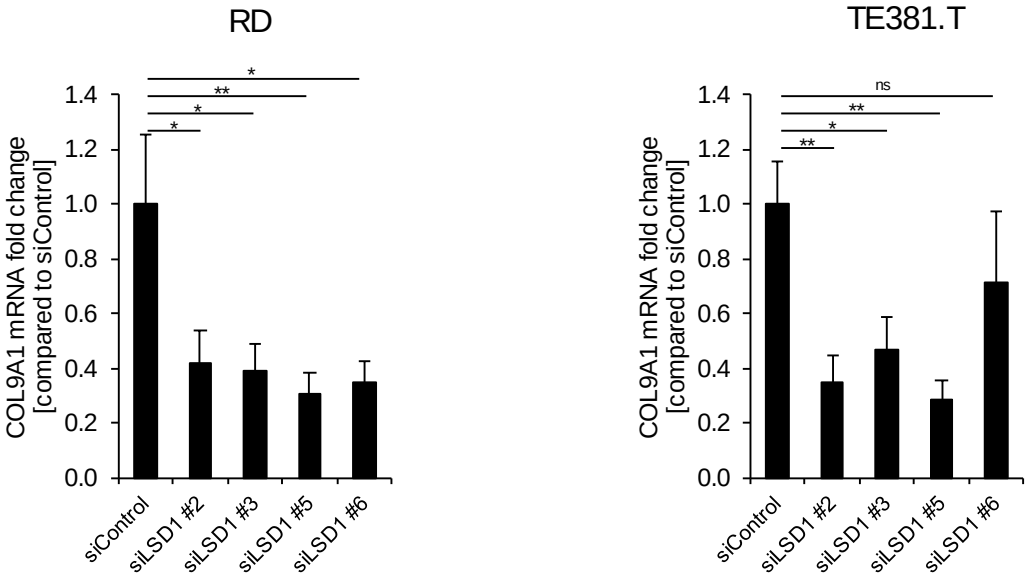


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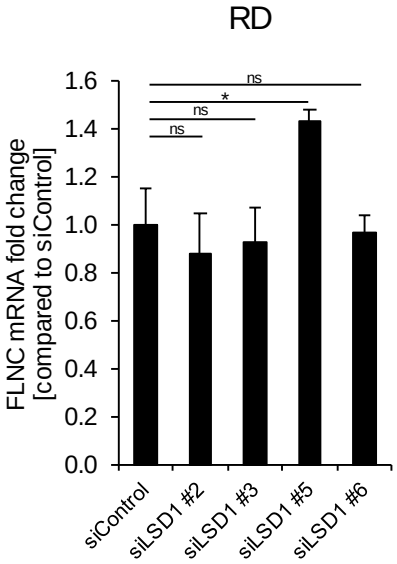
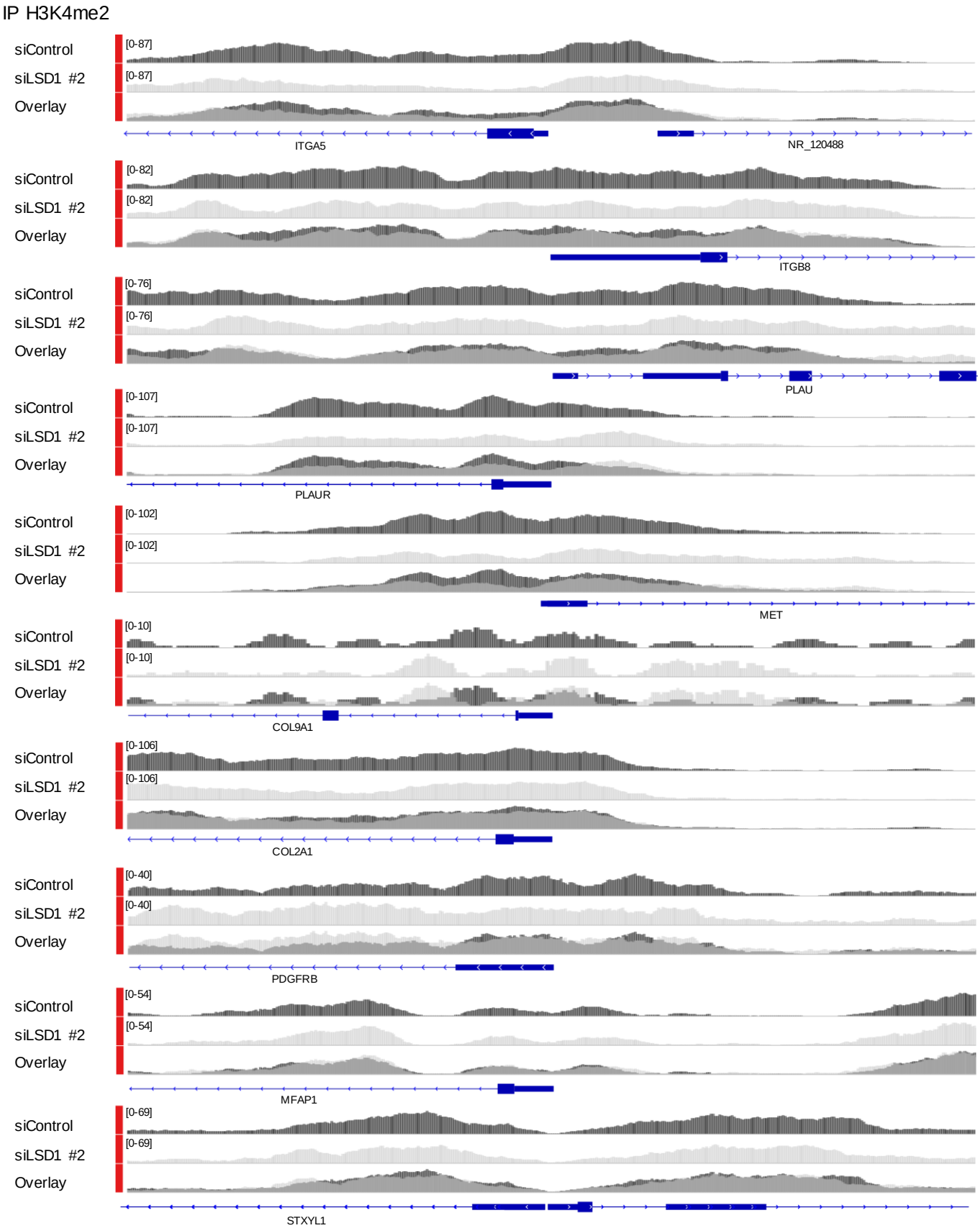


# Supplementary Figure 8




**Supplementary Figure 8: Changes in intensity and distribution of H3K4me2 peaks at the genomic regions of LSD1-regulated genes**

Visualization of H3K4me2 peaks in siControl and siLSD1 at the promoter regions of the genes from the list depicted in Fig. 5A. The tracks are shown separately and as overlay (siControl in black, siLSD1 in light grey). Further, the localization of the counted peaks is shown below together with the RefSeq annotation for the gene (blue bars). Additionally, two non-siLSD1-regulated genes are shown (MFAP1, STYXL1).