Arginine methylation-dependent LSD1 stability promotes invasion and metastasis of breast cancer

Jiwei Liu[1], Jingxin Feng[2], Lili Li[3], Luyao Lin[1], Jiafei Ji[2], Cong Lin[1], Lingxia Liu[2], Na Zhang[1], Dandan Duan[2], Zhongwei Li[1], Baiqu Huang[2], Yu Zhang[*,1] and Jun Lu[*,2]

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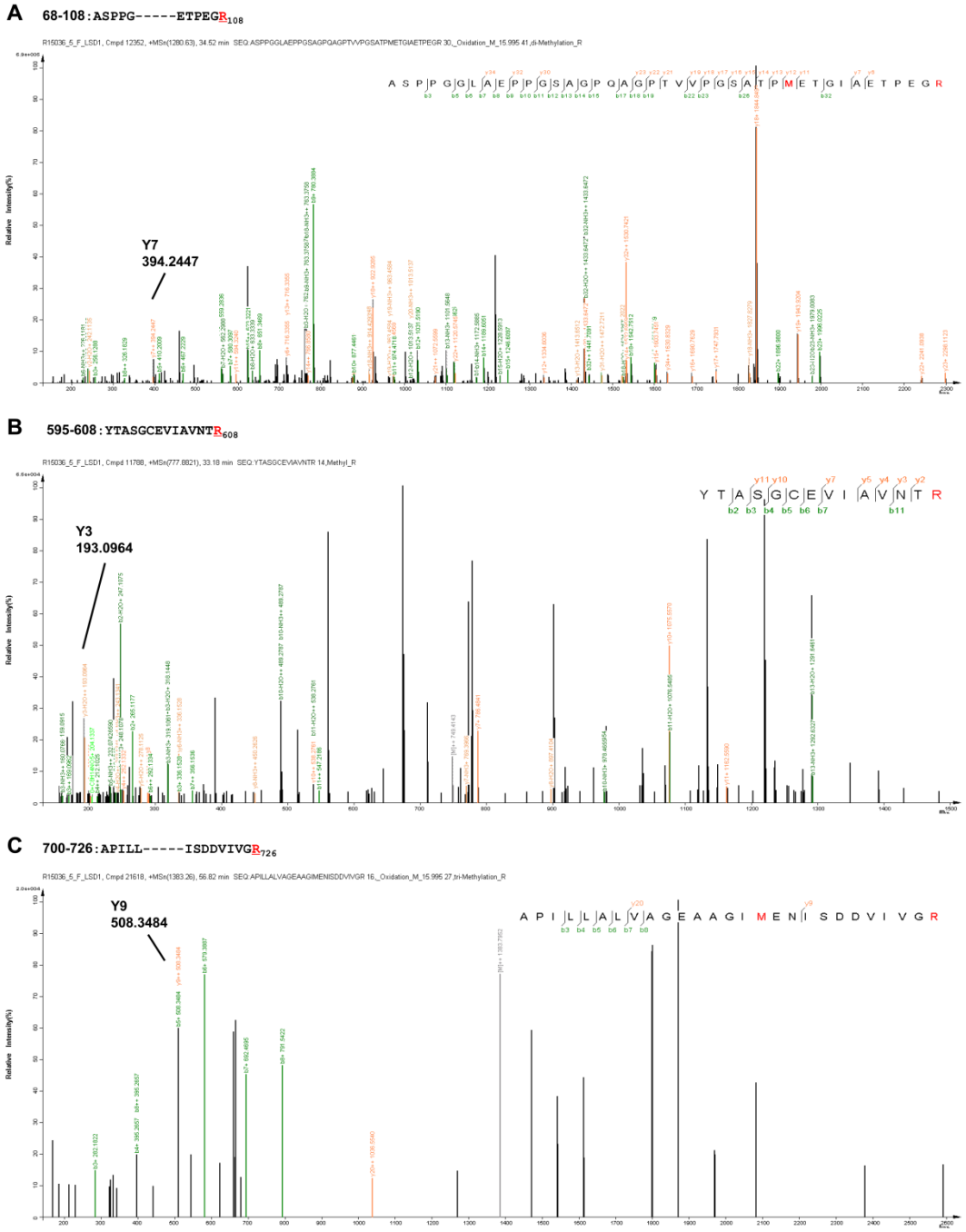
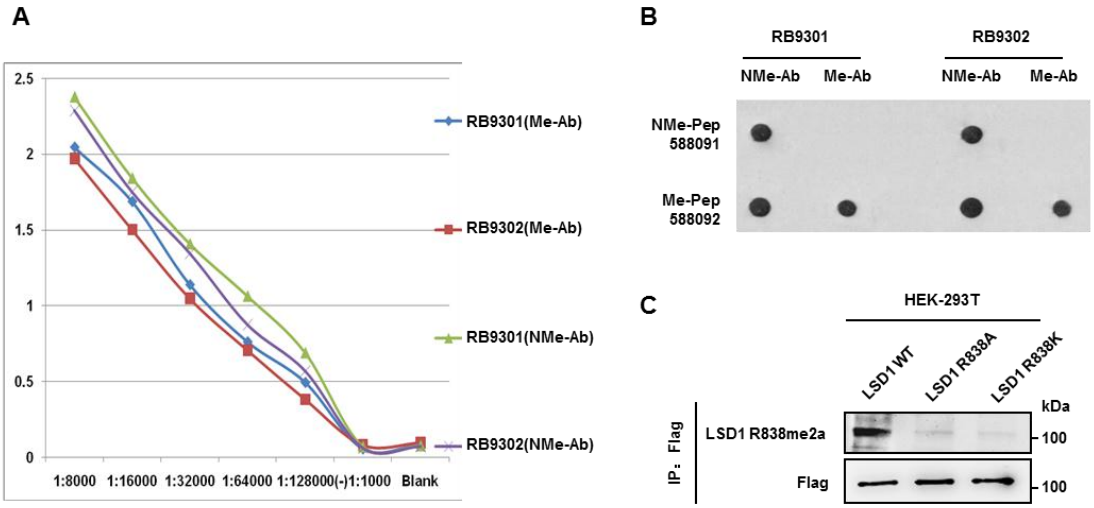
Appendix Figure S1



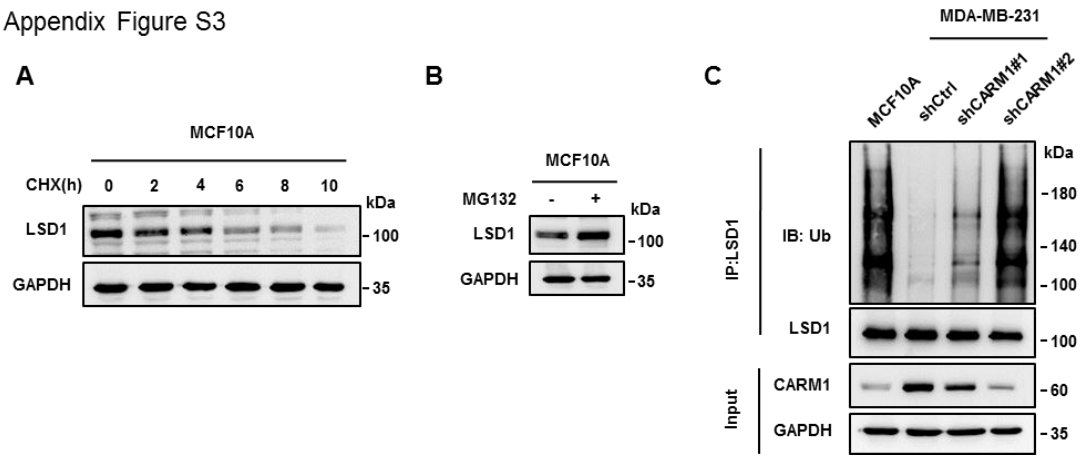
Appendix Figure S1. Mass spectrometric analysis of LSD1 methylation. Immunoprecipitation (IP) was performed using anti-Flag antibody in HEK-293T cells overexpressing Flag-tagged LSD1, followed by liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) analysis. Methylated residue of Arg108 **(A)**, Arg608 **(B)** and Arg726 **(C)** were identified.

Appendix Figure S2



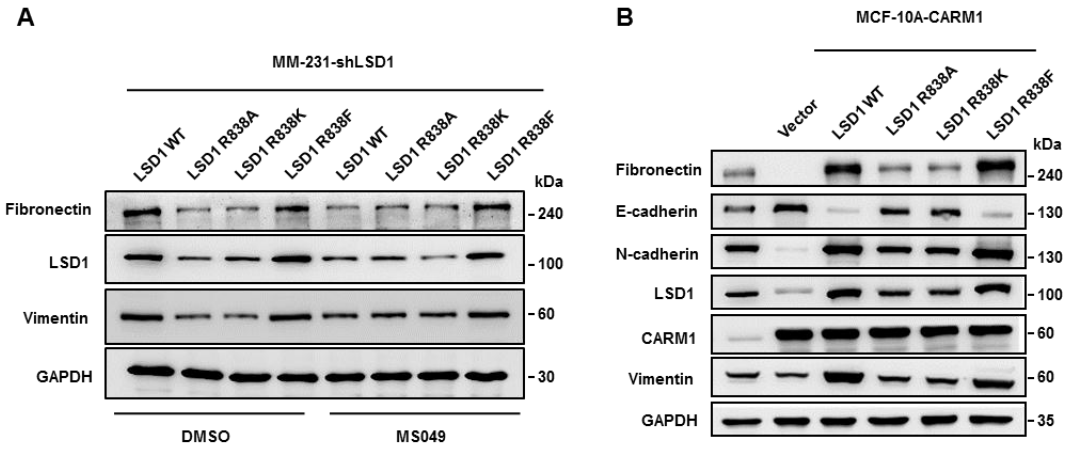
Appendix Figure S2. Generation and verification of antibody against LSD1 R838me2a. (A, B) The specificity of anti-LSD1R838me2a2 antibody was validated by ELISA **(A)** and dot blot analysis **(B)**. Data were provided by GL Biochem Co., Ltd (Shanghai, China) (Rabbit ID: RB9301, RB9301; **A**, Detailed ELISA data shown in Appendix Table S1. **B**, NMe-Pep: Non-asymmetric dimethylation peptide; Me-Pep: Asymmetric dimethylation peptide; NMe-Ab: Non-asymmetric dimethylation-Antibody; Me-Ab: Asymmetric dimethylation Antibody; Antibody Dilution: 1:500; Peptide: 5 ng). **(C)** HEK-293T cells transfected with Flag-LSD1 WT or Flag-LSD1 R838A/R838K, IP assay was performed using anti-Flag antibody followed by immunoblotting with anti-LSD1 R838me2a antibody.

Appendix Figure S3



Appendix Figure S3. Ubiquitination and stabilization of LSD1 in MCF10A cells. (A) MCF10A cells were treated with CHX for the indicated time, and LSD1 protein level was examined by western blotting. **(B)** MCF10A cells were treated with MG132 for 10 h, and the cell lysates were analyzed by western blotting. **(C)** The LSD1 ubiquitination status of MCF10A and CARM1-knockdown MDA-MB-231 cells were assessed by immunoblotting after IP using anti-LSD1 antibody..

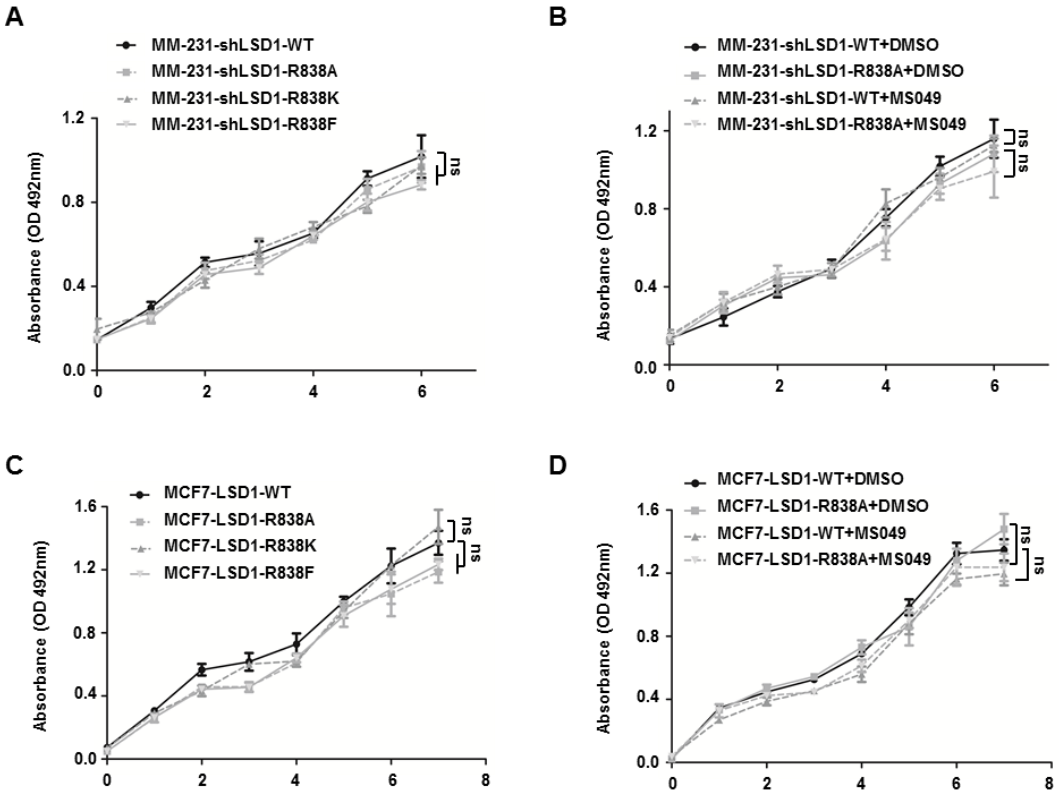
Appendix Figure S4



Appendix Figure S4. The effect of LSD1 Methylation on epithelial and mesenchymal markers. (A) MM-231-shLSD1 WT/R838A/R838K/R838F cells were treated with or without MS049. And the mesenchymal markers were analyzed by immunoblotting with indicated antibodies. **(B)** The epithelial and mesenchymal markers were analyzed by immunoblotting in MCF10A cells

overexpressing indicated protein(s). GAPDH was used as a protein lysate loading control.

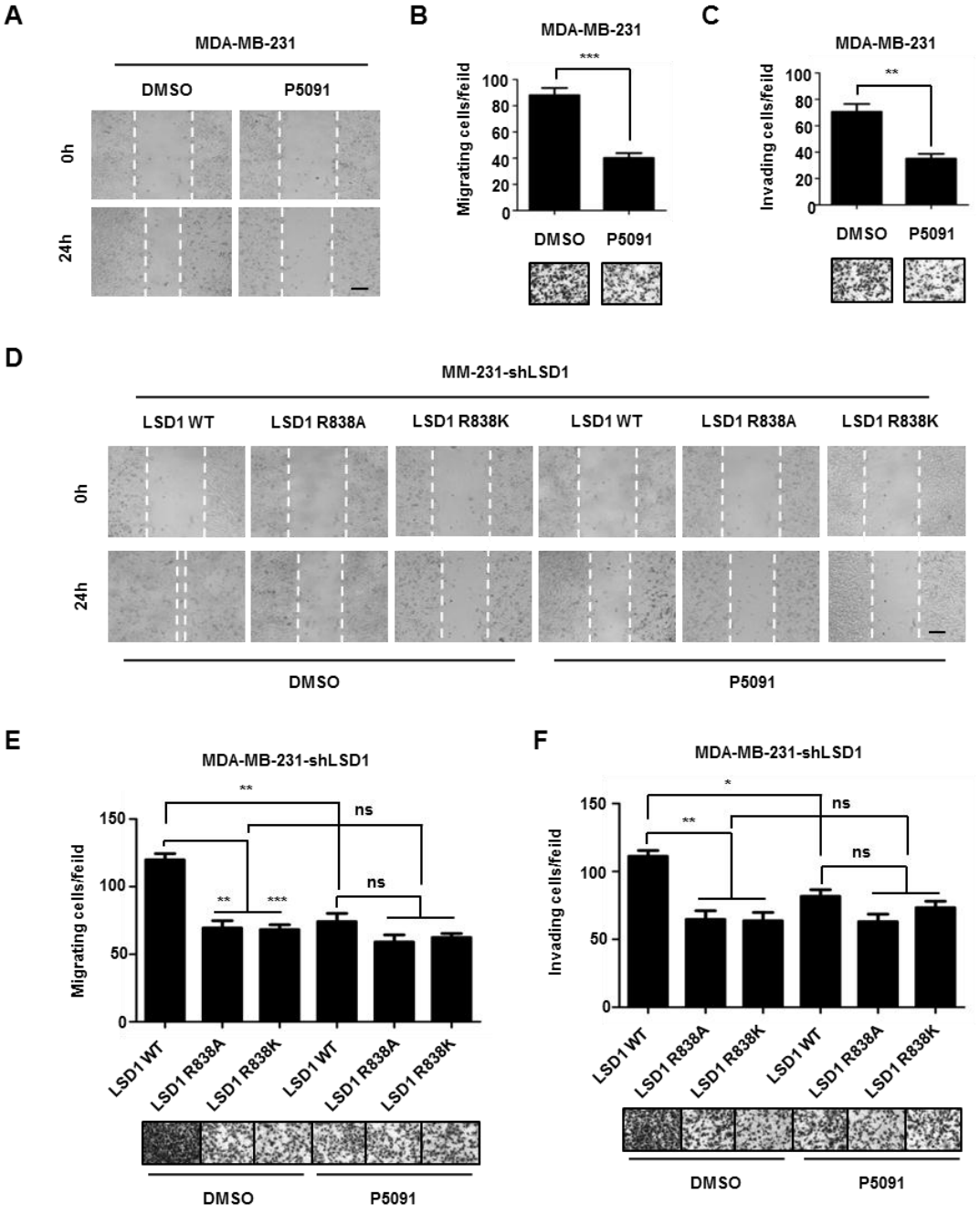
Appendix Figure S5



Appendix Figure S5. LSD1 R838 methylation has no impact on cell proliferation. (A, C) The growth rate of MM-231-shLSD1-WT/R838A/R838K/R838F cells **(A)** and MCF7-LSD1-WT/R838A/R838K/R838F cells **(C)** were measured by MTT assay. **(B, D)** The growth velocity of MDA-MB-231-shLSD1-WT/R838A cells **(B)** and MCF7-LSD1-WT/R838A cells under MS049 treatment **(D)** was assessed by MTT assays.

Data information: Data are the mean ± SD of five replicates per experiment, ns = not significant, t-test.

Appendix Figure S6



Appendix Figure S6. USP7 inhibitor attenuates LSD1-induced cell migration and invasion depending on LSD1 R838me2a. (A) Wound-healing assay of MDA-MB-231 cells treated with DMSO or P5091. The wounds were created by scraping the cells with sterile pipette tip. Scale bars, 100 μm. **(B, C)** Migration **(B)** and invasion **(C)** assays of MDA-MB-231 cells treated with DMSO or P5091. Representative images of migrated and invaded cells are

shown. Data represent the number of cells derived from mean cell counts of five fields. **(D)** Wound-healing assay of MM-231-shLSD1-WT/R838A/R838K cells with or without P5091 treatment. The wounds were created by scraping the cells with sterile pipette tip. Scale bars, 100 μm. **(E, F)** Migration **(E)** and invasion **(F)** assays of MM-231-shLSD1-WT/R838A/R838K cells treated with DMSO or P5091. Representative images of migrated and invaded cells are shown. Data represent the number of cells derived from mean cell counts of five fields.

Data information: Error bars represent mean ± SD, n = 3 experimental replicates, *P < 0.05, **P < 0.01, ***P < 0.001, ns = not significant, Student's t-test.

Appendix Table S1. Detailed ELISA data of Appendix Fig S1A

Animal ID	Blank	(-) 1:1000	SA 1:8000	SA 1:16000	SA 1:32000	SA 1:64000	SA 1:128000
RB9301(P-Ab)	0.084	0.07	2.755	2.291	1.865	1.539	1.248
RB9302(P-Ab)	0.072	0.052	2.673	2.216	1.812	1.481	1.169
RB9301(NP-Ab)	0.078	0.065	2.601	2.362	1.882	1.358	0.952
RB9302(NP-Ab)	0.063	0.058	2.683	2.377	2.069	1.597	1.162

ELISA:

Coating Antigens: free peptide

Coating Concentration: 2 ug/ml, 100ul / well

Coating Buffer: Phosphate Buffered Saline, pH7.4

Secondary Antibody: Goat Anti-Rabbit IgG (H+L), HRP Conjugated

(-):negative serum 1:1000 OD<0.2

SA:Antigen affinity purified Pab

OD 1:32000>1.0 ELISA titer>1:32000

Appendix Table S2. Correlation between LSD1R838me2a/LSD1/CARM1 levels and histopathological data in 70 breast cancer specimens

TNM Stage	Case（n）	High-level LSD1R838me2a (n)	High LSD1R838me2a (%)	*P*-Value
Stage I/II	43	15	34.9	**0.0095**
Stage III	27	18	66.7	
TNM Stage	Case（n）	High-level LSD1 (n)	High LSD1 (%)	*P*-Value
Stage I/II	43	14	32.6	**0.002**
Stage III	27	19	70.4	
TNM Stage	Case（n）	High-level CARM1 (n)	High CARM1 (%)	*P*-Value
Stage I/II	42	16	38.1	**0.0027**
Stage III	28	21	75	

N=70. *P* values are the result of x^2 tests.

Polyclonal antibody production

Step1： Antigen Preparation

Protein: provide 3mg antigen for immunization at least and 5mg for both ELISA test&Antigen Affinity Purification at least.

Peptide: design & Synthesis.conjugation with KLH.

Step2: Immunization (10 weeks for high titer,5-6weeks for Fast-titer)

Preparation of 2 Rabbits

Process flow chart:

	Process
Day0	1-2ml pre-immune sera and 1st-immunization with CFA
Day14	2nd-immunization with CFA
Day28	3rd-immunizaiton with IFA
Day35	4th-immunizaiton with IFA
Day42	5th-immunizaiton with NaCl(0.9%)
Day49	6th-immunizaiton with NaCl(0.9%)
Day56	7th-immunizaiton with NaCl(0.9%) & serum collected for ELISA test
Day63	8th-immunizaiton with NaCl(0.9%)
Day70	Bleed
We will provide ELISA data to you by e-mail	

If we get high serum titer (at least 1:8,000 dilution), we will do the followings:

Step3: Antigen Affinity Purification (1-2 weeks)

At least 3-5mg Antibody by Antigen Affinity Purification.

Step4: Identification

Elisa test titer >1:32000

13-15 weeks are needed for all the process in total.