Expanded View Figures

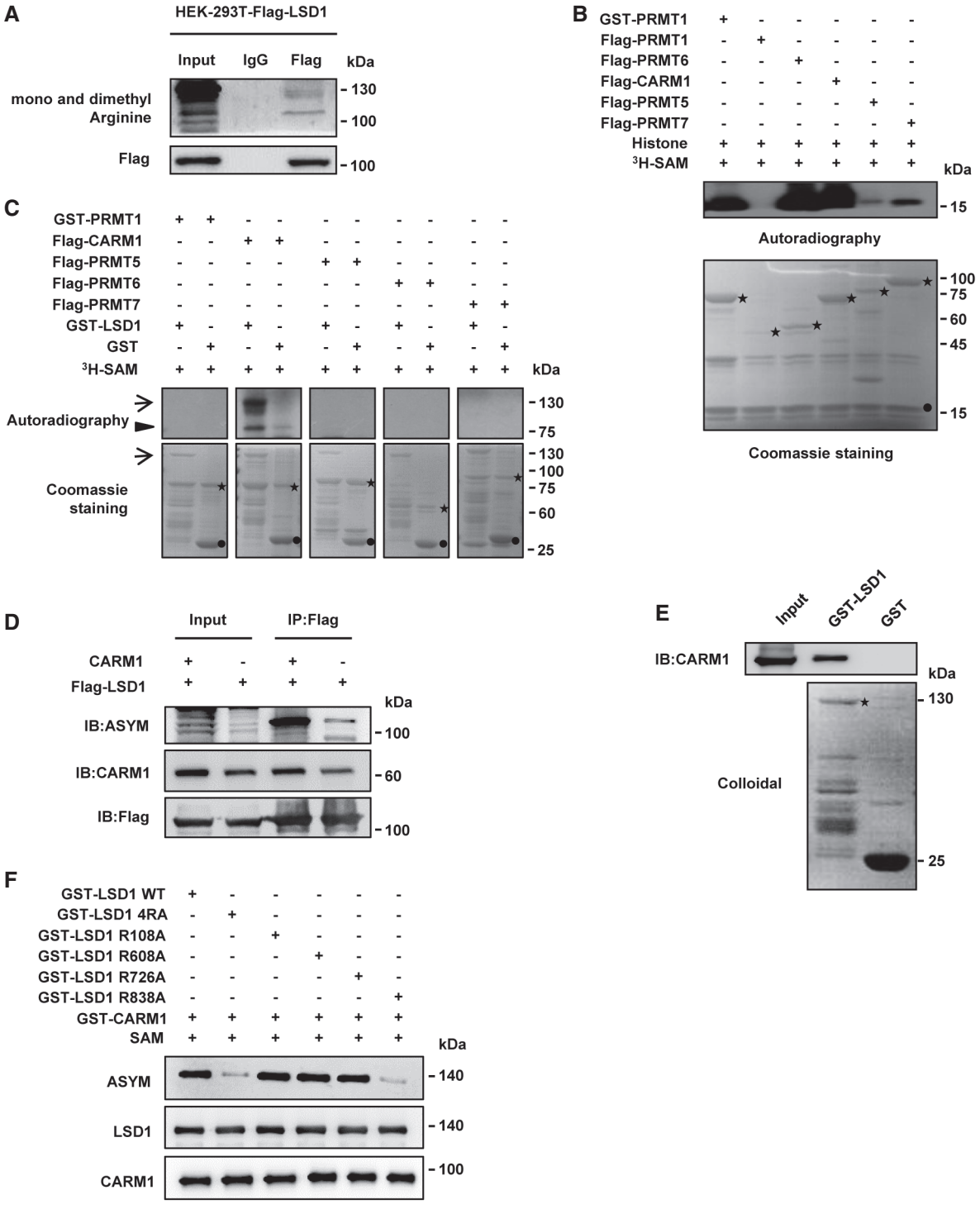

Figure EV1.

Figure EV1. LSD1 is methylated by and interacts with CARM1.

A HEK-293T cells were transfected with Flag-tagged LSD1, and cell lysates were immunoprecipitated by anti-Flag antibody and then subjected to immunoblotting.

B, C *In vitro* methylation assays. Purified fusion proteins of GST-tagged or Flag-tagged PRMTs were incubated with core histones (B) and GST or GST-LSD1 (C) in the presence of ^{3}H-SAM. Methylated proteins were detected by autoradiography, and the total amounts of proteins were visualized by Coomassie Blue staining (B, asterisks: PRMTs, filled circles: core histones; C, asterisks: PRMTs, arrows: LSD1, arrowheads: automethylation of CARM1, filled circles: GST).

D LSD1 asymmetric dimethylation status was examined by IP assay with anti-Flag in HEK-293T cells that transfected with the indicated plasmids.

E Purified GST-tagged LSD1 was pulled down with the Flag-CARM1 purified from HEK-293T cells. The amounts of GST and GST-tagged LSD1 were visualized by Coomassie Blue staining (asterisks: LSD1).

F *In vitro* methylation assays. Purified fusion protein of GST-tagged LSD1 WT or its mutants was incubated with GST-CARM1 in the presence of SAM. Methylation of LSD1 protein was analyzed by Western blot.

Source data are available online for this figure.

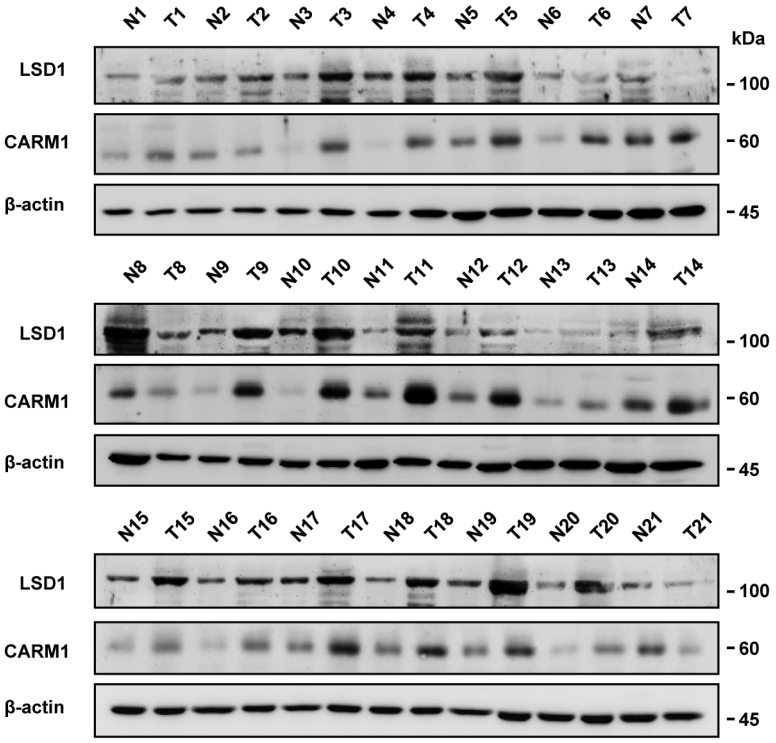

Figure EV2. CARM1 and LSD1 are highly expressed in human breast cancerous tissues and positively correlated with each other.

Immunoblotting analysis of LSD1 and CARM1 expression in human breast cancerous tissues and adjacent tissues (n = 21). N, normal; T, tumor.

Source data are available online for this figure.

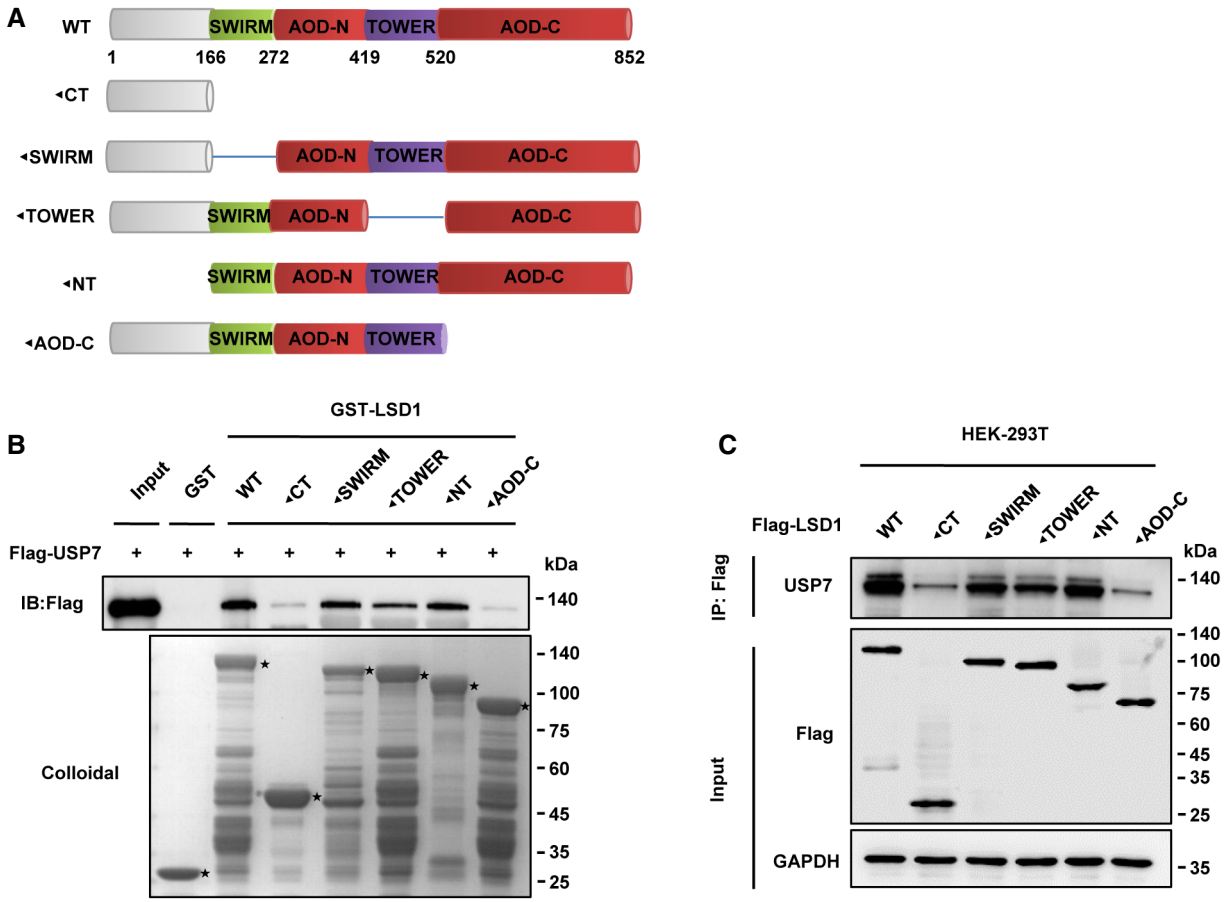

Figure EV3. Carboxyl AOD domain (AOD-C) of LSD1 is important for its interaction with USP7.

A Schematic diagram of LSD1. LSD1 encodes a polypeptide of 852 aas with a SWIRM domain, a TOWER domain in between the amino amine oxidase (AOD) domain (AOD-N) and carboxyl AOD domain (AOD-C). NT amino terminus; CT carboxyl terminus.

B The GST-tagged LSD1 deletion mutants were applied for GST pull-down assays with Flag-USP7 purified from HEK-293T cells. The amounts of GST or GST-tagged mutants were visualized by Coomassie Blue staining (asterisk: GST or GST-LSD1 mutants).

C Flag-tagged LSD1 deletion mutants were overexpressed in HEK-293T cells, followed by Co-IP assays using anti-Flag antibody, and their interaction with USP7 was assessed by Western blot.

Source data are available online for this figure.

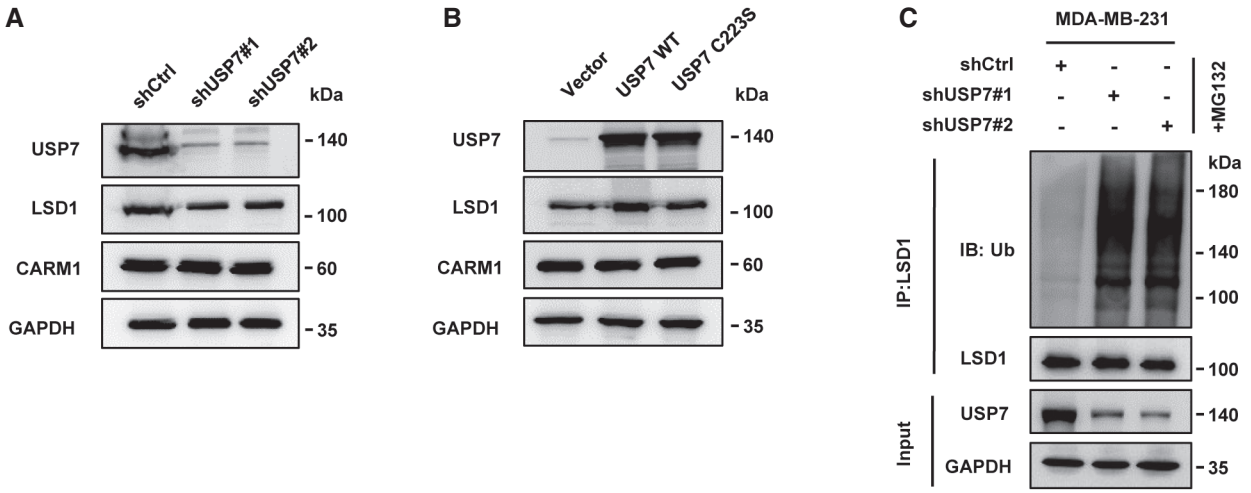

Figure EV4. Ubiquitin-specific protease 7 increases LSD1 protein level.

A MDA-MB-231 cells were transfected with USP7 shRNAs or control vector, and LSD1 protein level was assessed by Immunoblotting.

B HEK-293T cells were transfected with USP7 WT or USP7 C223S, and LSD1 protein level was assessed by immunoblotting.

C MDA-MB-231 cells were transfected with USP7 shRNAs or control vector, and cell lysates were immunoprecipitated by anti-LSD1 antibody and then subjected to immunoblotting.

Source data are available online for this figure.

Figure EV5. Methylation of LSD1 promotes migration and invasion of MDA-MB-231, MCF7, and MCF10A cells.

A, B Wound-healing assay of MM-231-shLSD1-WT/R838A/R838K/R838F cells (A) and MCF7-LSD1-WT/R838A/R838K/R838F cells (B) with or without MS049 treatment. The wounds were created by scraping the cells with sterile pipette tip. Scale bars, 100 μm.

C, D Migration (C) and invasion assays (D) were conducted in MCF10A cells that co-expressed CARM1 with LSD1 WT, or one of LSD1 mutants (R838A/R838K/R838F). Representative images of migrated and invaded cells are shown. Data represent the number of cells derived from mean cell counts of five fields (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).

E Wound-healing assays of MCF10A cells that co-expressed CARM1 with LSD1 WT, or one of LSD1 mutants (R838A/R838K/R838F). The wounds were created by scraping the cells with sterile pipette tip. Scale bars, 100 μm.

Source data are available online for this figure.

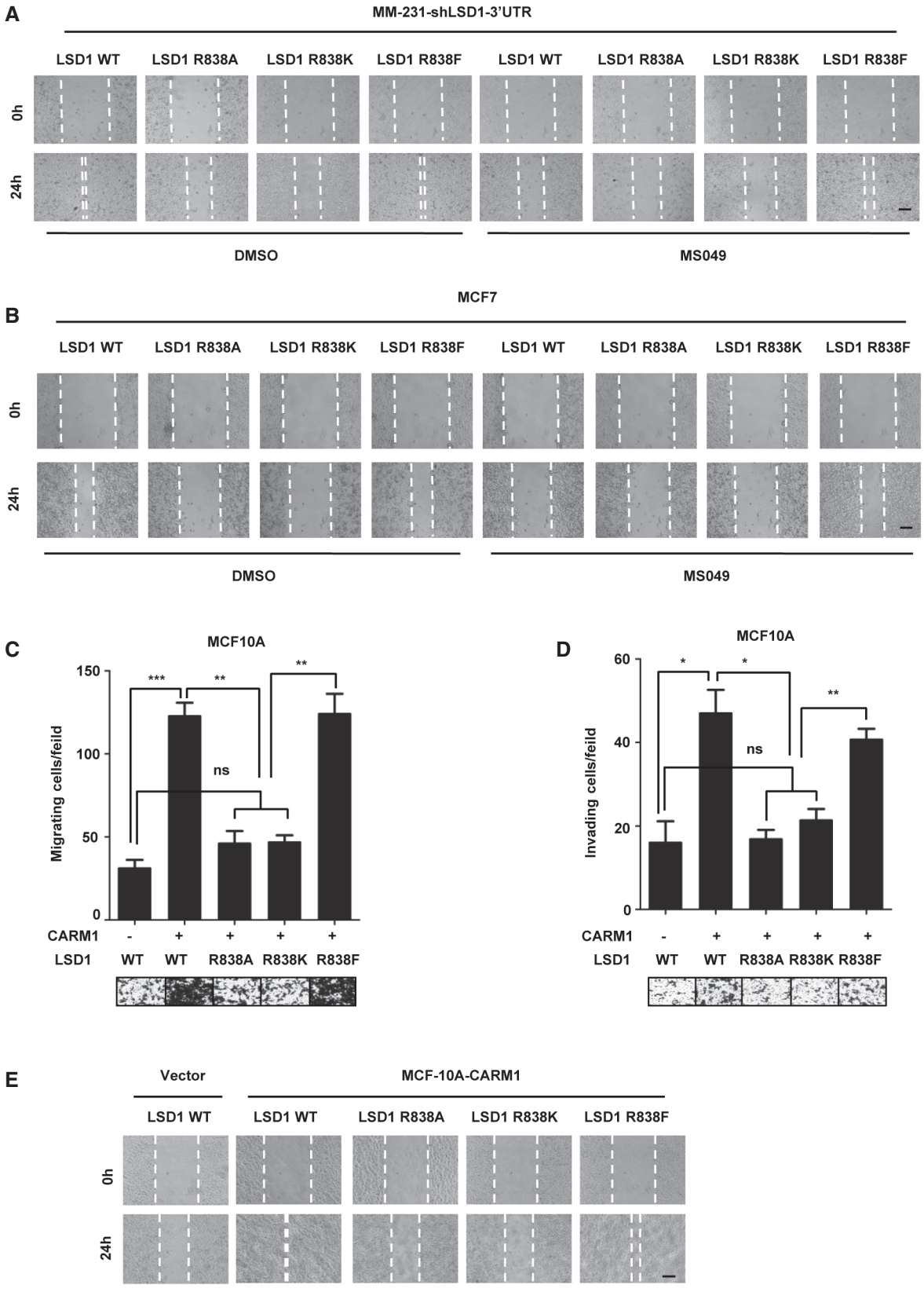

Figure EV5.