130kDa				IB:DNMT3a			IB:DNMT3a
124kDa				IB:HDAC7			IB:HDAC7
62kDa				IB:HDAC1			IB:HDAC1
60kDa				IB:HDAC2			IB:HDAC2
49kDa				IB:HDAC3			IB:HDAC3
106kDa				IB:HDAC4			IB:HDAC4
122kDa				IB:HDAC5			IB:HDAC5
160kDa				IB:HDAC6			IB:HDAC6
43kDa				IB:β-actin			IB:β-actin

− #1 #2 LV-shDNMT3a   − + LV-DNMT3a

A549   SK-LU-1

1   **Fig. S1 The relationship between DNMT3a and HDACs.** Representative results

2   of western blot of HDACs expression in DNMT3a knockdown and overexpression

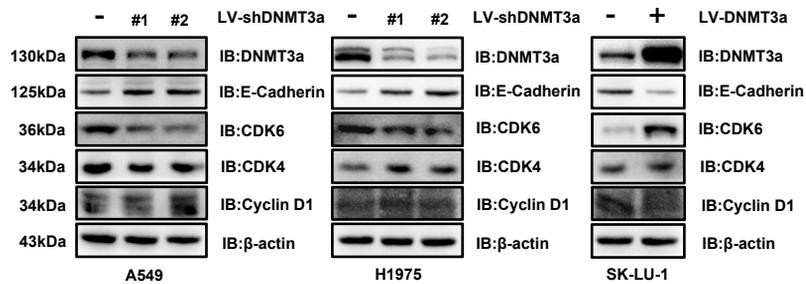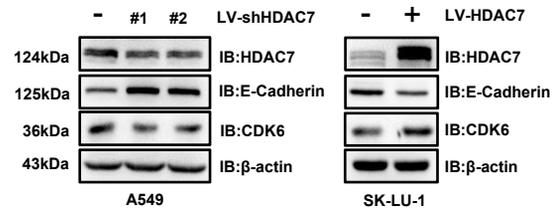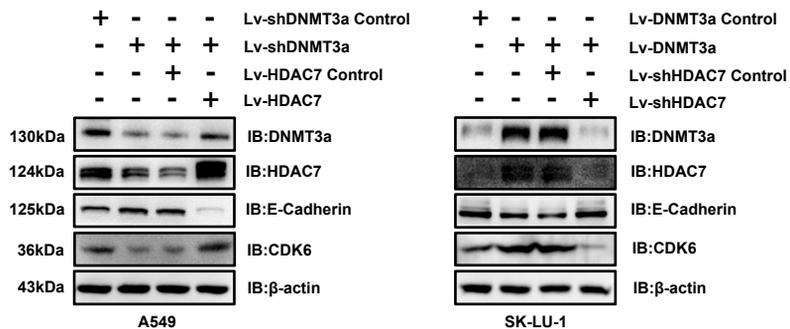3   LUAD cells.

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	-	#1	#2	LV-shDNMT3a		-	#1	#2	LV-shDNMT3a		-	+	LV-DNMT3a

130kDa — IB:DNMT3a — IB:DNMT3a — IB:DNMT3a

125kDa — IB:E-Cadherin — IB:E-Cadherin — IB:E-Cadherin

36kDa — IB:CDK6 — IB:CDK6 — IB:CDK6

34kDa — IB:CDK4 — IB:CDK4 — IB:CDK4

34kDa — IB:Cyclin D1 — IB:Cyclin D1 — IB:Cyclin D1

43kDa — IB:β-actin — IB:β-actin — IB:β-actin

A549   H1975   SK-LU-1

b

-	#1	#2	LV-shHDAC7		-	+	LV-HDAC7

124kDa — IB:HDAC7 — IB:HDAC7

125kDa — IB:E-Cadherin — IB:E-Cadherin

36kDa — IB:CDK6 — IB:CDK6

43kDa — IB:β-actin — IB:β-actin

A549   SK-LU-1

c

+	-	-	-	Lv-shDNMT3a Control
-	+	+	+	Lv-shDNMT3a
-	-	+	-	Lv-HDAC7 Control
-	-	-	+	Lv-HDAC7

130kDa — IB:DNMT3a

124kDa — IB:HDAC7

125kDa — IB:E-Cadherin

36kDa — IB:CDK6

43kDa — IB:β-actin

A549

+	-	-	-	Lv-DNMT3a Control
-	+	+	+	Lv-DNMT3a
-	-	+	-	Lv-shHDAC7 Control
-	-	-	+	Lv-shHDAC7

IB:DNMT3a

IB:HDAC7

IB:E-Cadherin

IB:CDK6

IB:β-actin

SK-LU-1

21   **Fig. S2 DNMT3a or HDAC7 knockdown upregulates the expression of E-**

22   **cadherin and CDK6 in LUAD cells. a** Representative results of western blot of E-

23   cadherin, CDK6, CDK4 and cyclin D1 expression in DNMT3a knockdown and

24   overexpression LUAD cells. **b** Representative results of western blot of E-cadherin and

25   CDK6 expression in HDAC7 knockdown and overexpression LUAD cells. **c**

26   Representative western blot analysis of DNMT3a, HDAC7, E-cadherin and CDK6

27   expression in the indicated groups. β-actin was used as an internal control.

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LV-shDNMT3a+LV-HDAC7 Control

LV-shDNMT3a+LV-HDAC7

DNMT3a   HDAC7

LV-shDNMT3a+LV-HDAC7 Control

LV-shDNMT3a+LV-HDAC7

ZEB1   c-Myc

DNMT3a   HDAC7   ZEB1   c-Myc

IHC score

LV-shDNMT3a+LV-HDAC7 Control
LV-shDNMT3a+LV-HDAC7

b

+ + + + +   + + + + +   LV-shDNMT3a+LV-HDAC7 Control
- - - - -   + + + + +   LV-shDNMT3a+LV-HDAC7

130kDa   IB:HDAC7
124kDa   IB:DNMT3a
200kDa   IB:ZEB1
60kDa   IB:c-Myc
43kDa   IB:β-actin

A549 xenograft tumour

DNMT3a   HDAC7   ZEB1   c-Myc

Relative protein level(/β-actin)

LV-shDNMT3a+LV-HDAC7 Control
LV-shDNMT3a+LV-HDAC7

41   **Fig. S3 HDAC7 overexpression partially reversed the effects of DNMT3a**

42   **knockdown.**   **a** Representative images and statistical analysis of DNMT3a, HDAC7,

43   ZEB1 and c-Myc IHC staining in xenograft tumours from nude mice in the indicated

44   group. Scale bars, 50 μm and 30 μm (inset). **b** Representative results of western blot

45   analysis of DNMT3a, HDAC7, ZEB1 and c-Myc expression in xenograft tumour cells

46   from nude mice in the indicated group. β-actin was used as an internal control. $^{*}p <$

47    $0.05,\ ^{**}p < 0.01.$

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a

130kDa — IB:DNMT3a
124kDa — IB:HDAC7
200kDa — IB:ZEB1
60kDa — IB:c-Myc
43kDa — IB:β-actin

SK-LU-1

b

SK-LU-1

c

SK-LU-1
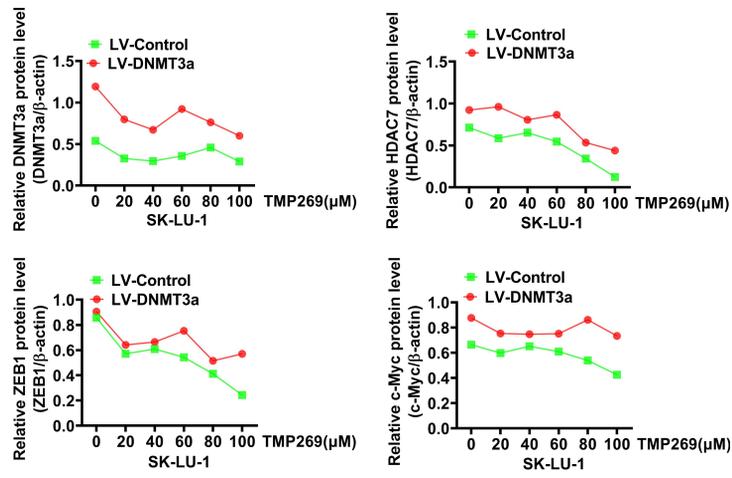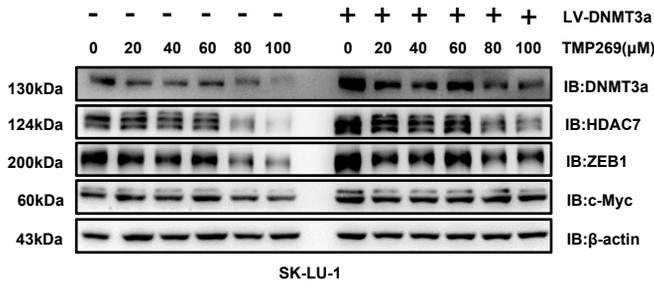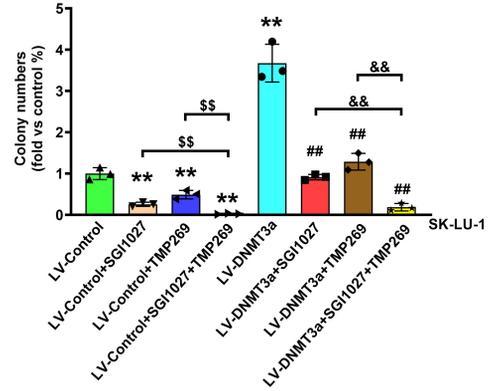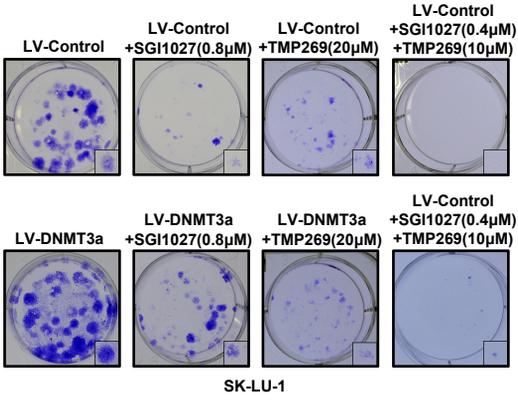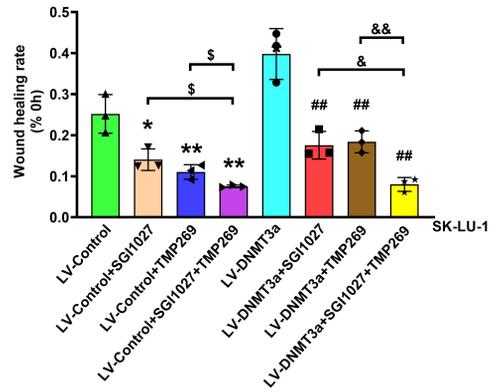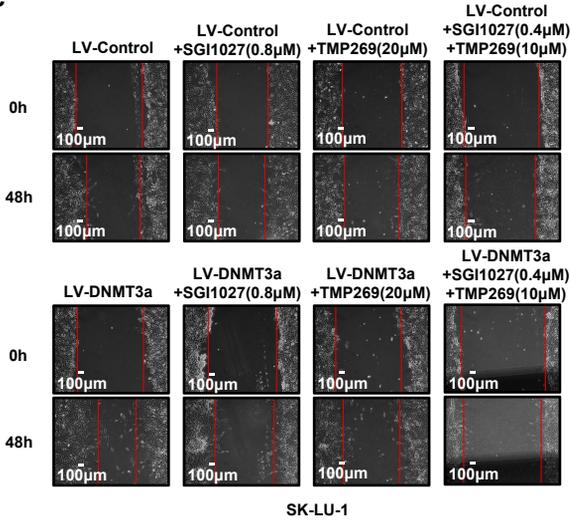
62   **Fig. S4 TMP269 reversed the DNMT3a-induced changes in the expression of**

63   **proteins in LUAD cells. a** Representative western blot and statistical analysis of

64   DNMT3a, HDAC7, ZEB1 and c-Myc expression in DNMT3a overexpression LUAD

65   cells and control cells after treatment with TMP269 for 48 h. β-actin was used as an

66   internal control. **b** Representative images and statistical analysis of the colony

67   formation assay in the indicated groups after treatment with SGI1027 and/or TMP269

68   for 48 h. Colonies were visualized by crystal violet staining. **c** Representative wound

69   healing assay images and statistical analysis in the indicated groups after treatment with

70   SGI1027 and/or TMP269 for 48 h. The migration ability was quantified as the mean

71   scratch area at each time point. The initial scratch area (0 h) was set as 100%. Scale

72   bars, 100 μm (inset). $^{*}p < 0.05$ vs. the LV-Control group, $^{**}p < 0.01$ vs. the LV-Control

73   group, $^{##}p < 0.01$ vs. the LV-DNMT3a group, $^{$$}p < 0.01$ vs. the LV-

74   Control+SGI1027+TMP269 group, $^{\&\&}p < 0.01$ vs. the LV-DNMT3a+

75   SGI1027+TMP269 group.

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a

A549 xenograft tumour

A549 xenograft tumour

b

DNMT3a   HDAC7   ZEB1   c-Myc

82   **Fig. S5 DNMT3a and HDAC7 inhibitors decreased LUAD cell progression in**

83   **vivo. a** Gross photograph of subcutaneous xenograft tumours and data showing the

84   changes in subcutaneous tumour weight, tumor volume and nude mouse body weight

85   in each group. **b** Representative images and statistical analysis of DNMT3a, HDAC7,

86   ZEB1 and c-Myc IHC staining in xenograft tumours from nude mice in the indicated

87   group. Scale bars, 50 μm and 30 μm (inset). $^{*}p < 0.05$, $^{**}p < 0.01$.
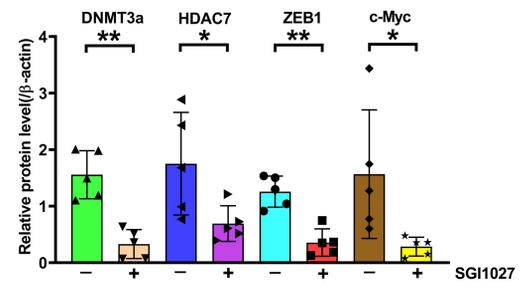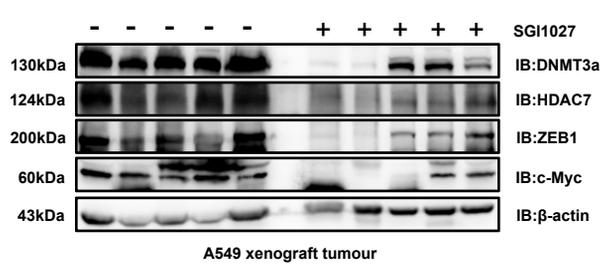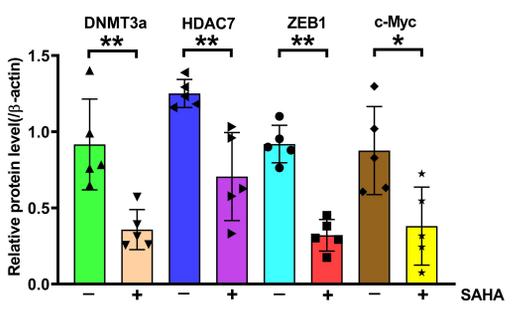
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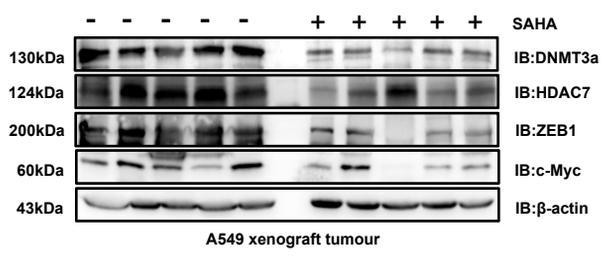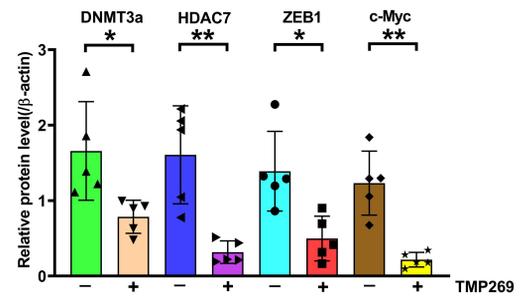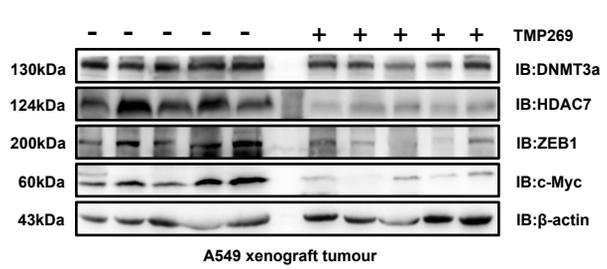
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102   **Fig. S6 SGI1027, SAHA and TMP269 reversed the DNMT3a-induced**

103   **changes in the expression of proteins in vivo. a** Representative western blot and

104   statistical analysis of DNMT3a, HDAC7, ZEB1 and c-Myc expression in xenograft

105   tumours from nude mice after treatment with SGI1027. **b** Representative western blot

106   and statistical analysis of DNMT3a, HDAC7, ZEB1 and c-Myc expression in xenograft

107   tumours from nude mice after treatment with SAHA. **c** Representative western blot and

108   statistical analysis of DNMT3a, HDAC7, ZEB1 and c-Myc expression in in xenograft

109   tumours from nude mice after treatment with TMP269. β-actin was used as an internal

110   control. $^{*}p < 0.05$, $^{**}p < 0.01$.

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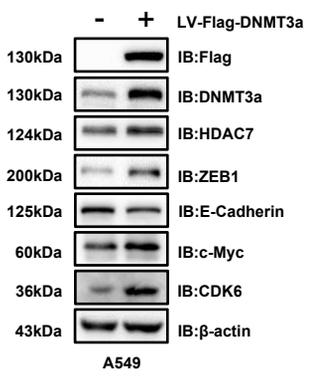|	−	+	LV-Flag-DNMT3a
130kDa			IB:Flag
130kDa			IB:DNMT3a
124kDa			IB:HDAC7
200kDa			IB:ZEB1
125kDa			IB:E-Cadherin
60kDa			IB:c-Myc
36kDa			IB:CDK6
43kDa			IB:β-actin

A549

122   **Fig. S7 Overexpression of DNMT3a upregulates the expression of HDAC7,**

123   **ZEB1 and c-Myc in LUAD.** Representative results of western blot of HDAC7,

124   DNMT3a, ZEB1, and c-Myc expression in DNMT3a overexpression LUAD cells. β-

125   actin was used as an internal control.
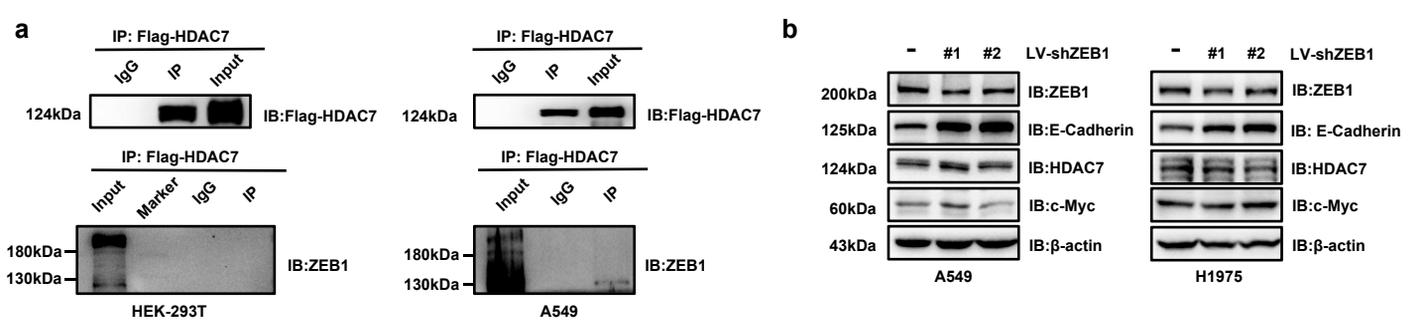
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IP: Flag-HDAC7

| IgG | IP | Input |

124kDa   IB:Flag-HDAC7

IP: Flag-HDAC7

| Input | Marker | IgG | IP |

180kDa
130kDa   IB:ZEB1

HEK-293T

IP: Flag-HDAC7

| IgG | IP | Input |

124kDa   IB:Flag-HDAC7

IP: Flag-HDAC7

| Input | IgG | IP |

180kDa
130kDa   IB:ZEB1

A549

b

	-	#1	#2	LV-shZEB1
200kDa				IB:ZEB1
125kDa				IB:E-Cadherin
124kDa				IB:HDAC7
60kDa				IB:c-Myc
43kDa				IB:β-actin

A549

	-	#1	#2	LV-shZEB1
				IB:ZEB1
				IB: E-Cadherin
				IB:HDAC7
				IB:c-Myc
				IB:β-actin

H1975

142   **Fig. S8 HDAC7 and ZEB1 do not interact directly, and ZEB1 does not**

143   **regulate the expression of HDAC7 in LUAD. a** Co-IP analysis and western blotting

144   were performed to confirm that there was no interaction between HDAC7 and ZEB1 in

145   either HEK-293T or A549 cells. **b** Representative western blot analysis of ZEB1, E-

146   cadherin, HDAC7, and c-Myc expression in ZEB1 knockdown LUAD cells. β-actin

147   was used as an internal control.

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163 **Supplementary Information**

164 **Screening criteria for clinical patients**

165 The patients with LUAD who underwent surgical treatment at Department of

166 Thoracic Surgery, Tangdu Hospital, Air Force Military Medical University from May

167 2009 to January 2014 were enrolled in this retrospective study.Patients were screened

168 according to the following criteria: (1) Pathologically confirmed LUAD without special

169 pathological components such as adenosquamous carcinoma; (2) None of the patients

170 had received chemotherapy, radiotherapy, targeted therapy, immunotherapy and other

171 tumor-related therapies before surgery. (3) The patient had no tumor in other organs.

172 All patients had signed the informed consent, and the study had been approved by the

173 Ethics Committee of the Second Affiliated Hospital of Air Force Medical University.

174 **Follow-up content and requirements of clinical patients**

175 The follow-up contents and requirements were as follows: (1) Basic information

176 of patients, including age, gender, smoking history and other basic information; (2)

177 Surgical and pathological information: including operation time, postoperative

178 pathological results, pathological grade, TNM clinical stage of lung cancer according

179 to the 8th AJCC edition (including T stage, N stage, M stage), etc. (3) Follow-up content:

180 including overall survival, medication and physical examination; (4) Follow-up

181 methods: outpatient and telephone follow-up; (5) Follow-up cycle: once every 3 months

182 within 2 years after surgery, once every 6 months after 2 years; (6) Follow-up time: up

183   to January 2019 or death of the patient, whichever came first. The study was conducted

184   in accordance with the Declaration of Helsinki and clinical guidelines.