

1 **Oncometabolite L-2-Hydroxyglurate Directly Induces Vasculogenic Mimicry**
2 **Through PHLDB2 in Renal Cell Carcinoma**

3

4 Huan Wang, Liya Wang, Qiming Zheng, Zeyi Lu, Yuanlei Chen, Danyang Shen, Dingwei Xue,
5 Minxiao Jiang, Lifeng Ding, Jie Zhang, Haiyang Wu, Liqun Xia, Jun Qian, Gonghui Li and
6 Jieyang Lu

7

8

9 **Table of contents**

10

11 --Supplementary Materials & Methods

12 --Supplementary Tables 1-2

13 --Supplementary Figures 1-4

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45 **Supplementary Materials and Methods**

46

47 **RNA-seq read mapping**

48 The Illumina reads (150 bp paired end) were mapped to the annotated genome of GRCh37(hg19)
49 using HISAT2. Approximately 38~55 million 150bp paired-end total read per sample were
50 obtained. To ensure the quality of information analysis, total reads were trimmed to remove any
51 remaining adapter sequences or low-quality reads by TrimGalore
52 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Then the clean reads were
53 mapped to the annotated genome with parameters. Then FPKM was calculate with the reference
54 genome by Stringtie strategy for further analysis. Sequencing statistics per sample was showed in
55 supplementary Table 2.

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89 Supplementary Table 1: Patient characteristics from TCGA datasets

90

Variables		Case number (N=517) or mean (range)
Gender	Male	336
	Female	181
Age(years)		60.64 (34-90)
Stage	I	266
	II	54
	III	121
	IV	76

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

116

117

118

119

120

121

122

123

124

125 Supplementary Table 2: RNA-seq statistics

126

Sample	Total reads	Clean reads	Mapped reads	Mapped rate (%)
786-O	56316634	55915234	54233628	96.99
786-O+L-2HG	38540642	38277802	37048773	96.79
A-498	53859884	53471064	51840938	96.95
A-498+L-2HG	55105450	54698764	52972770	96.84

127

128

129

130

131

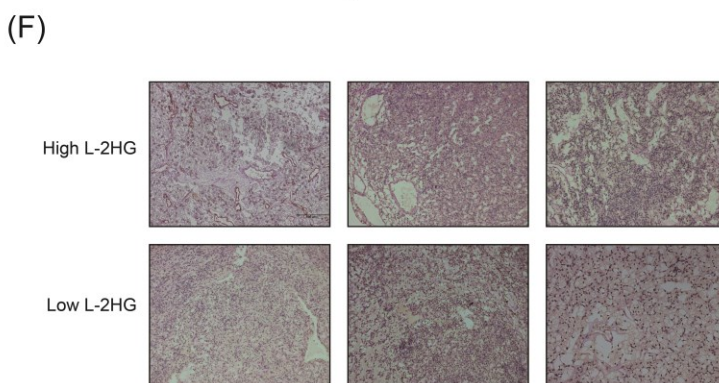
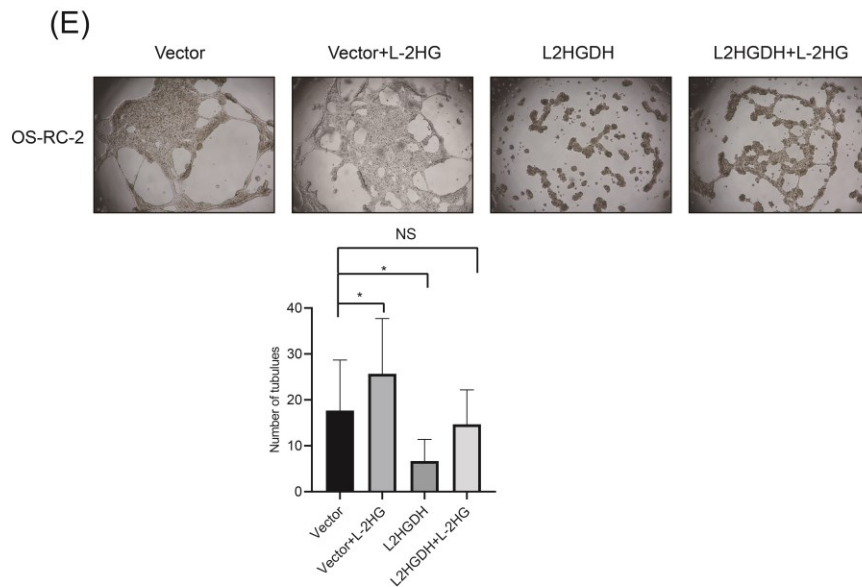
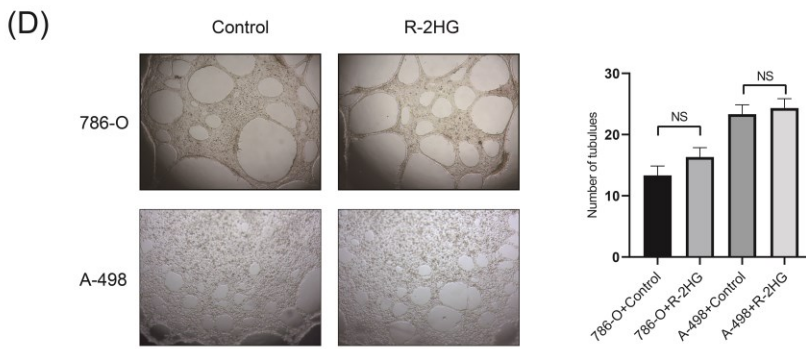
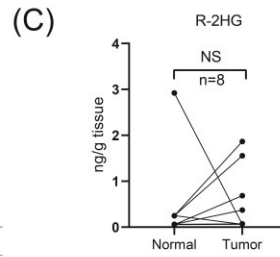
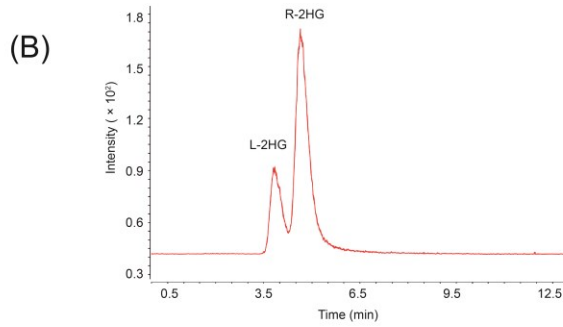
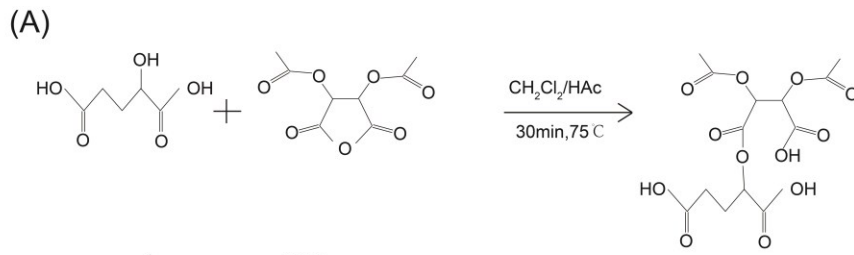
132

133

134

135

136



138

139 **Figure S1**

140 (A) Reaction scheme for the derivatization of 2HG. DATAN and 2HG was processed in the CH₂Cl₂ and
141 HAc in 75°C for 30min.

142 (B) Standard solutions of L-2HG and R-2HG were analyzed by LC-MS/MS. Two well resolved peaks
143 for the derivatized L-2HG and derivatized R-2HG were recorded.

144 (C) R-2HG level was measured by LC-MS/MS in the RCC tissues and adjacent normal tissues, n=8
145 NS= no significant.

146 (D) Images (40X) of VM in 786-O and A-498 cells after R-2HG treatment for 24h. VM tubes were
147 quantified in the right panel. NS=no significant.

148 (E) Images (40X) of VM in OS-RC-2 cells after L-2HG treatment or L2HGDH plasmid transfection.
149 Quantification of VM including three kinds of cell lines (786-O, A-498, OS-RC-2) for each
150 experimental group was presented in the bottom. *p<0.05, NS=no significant.

151 (F) The presence of VM in tumor tissues (High L-2HG vs Low L-2HG). PAS positive and CD34
152 negative was considered the VM channel. The quantification data was in the Fig1E.

153

154

155

156

157

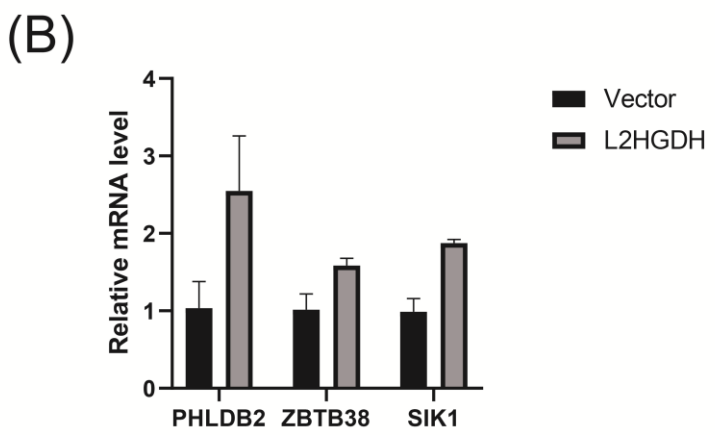
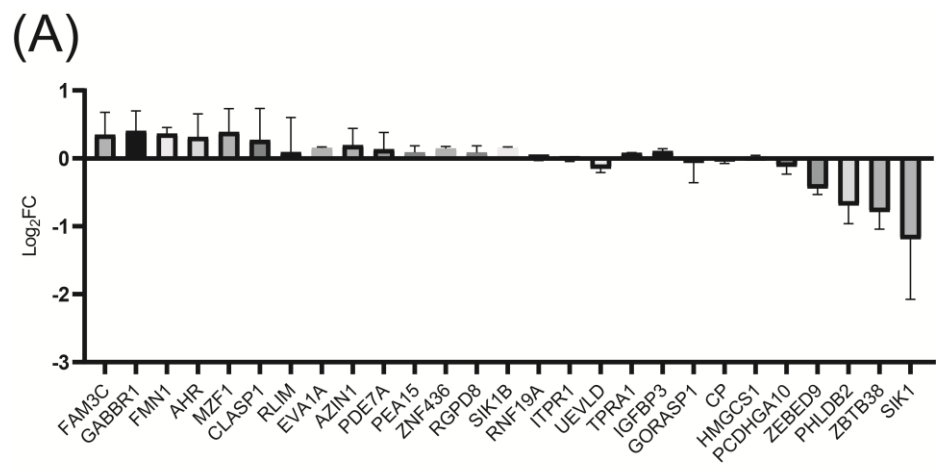
158

159

160

161

162

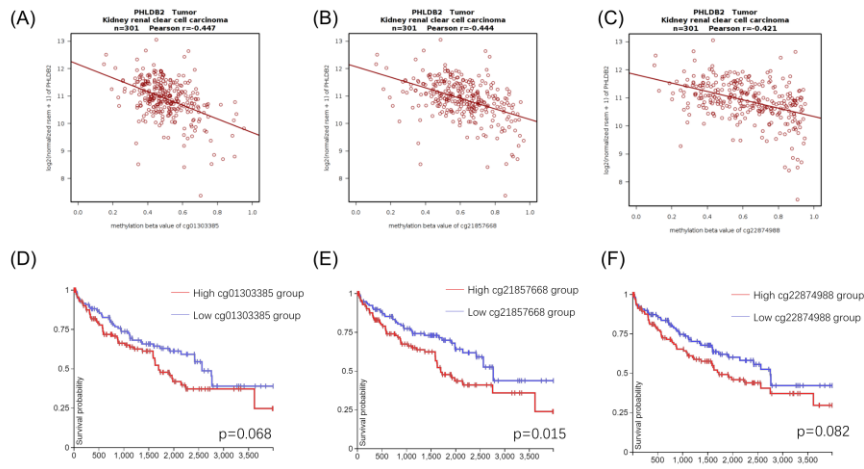


163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185

Fig S2

(A) In 786-O cells, the mRNA level of DEGs was confirmed via qPCR. The data was shown with the Log₂FC (fold change). SIK1, ZBTB38 and PHLDB2 were the most down-regulated genes.

(B) In 786-O cells, PHLDB2, ZBTB38, SIK1 relative mRNA level increased after L2HGDH plasmid transfection.

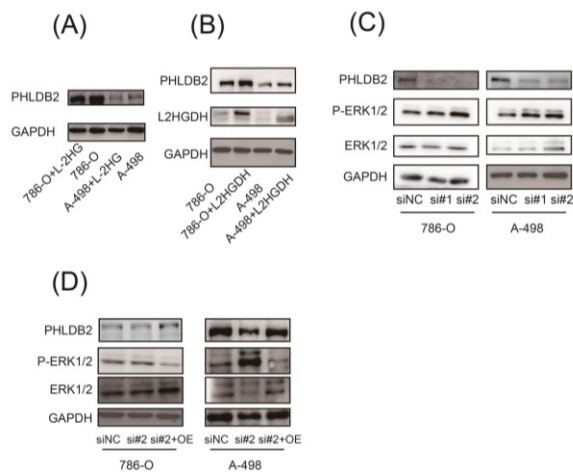


187
 188
 189
 190
 191
 192
 193
 194
 195
 196
 197
 198
 199
 200
 201
 202
 203
 204
 205
 206
 207
 208
 209
 210
 211
 212
 213
 214
 215
 216

Figure S3

Correlation of methylation with PHLDB2 expression and OS of RCC.

(A, B, C) Pearson correlation of cg01303385, cg21857668 and cg22874988 with PHLDB2 expression.
 (D) Kaplan-Meier analysis of overall survival (OS) in RCC patients with cg01303385 level (p=0.068).
 (E) Kaplan-Meier analysis of OS in RCC patients with cg21857668 level (p=0.015). (F) Kaplan-Meier analysis of OS in RCC patients with cg22874988 level (p=0.082)



218

219

Fig S4

221 (A) Western blot analysis to compare the PHLDB2 expression for 786-O and A-498 cells after
 222 L-2HG treatment.

223 (B) Western blot analysis to compare the PHLDB2 expression for 786-O, 786-O+L2HGDH
 224 transfection, A-498, A-498+L2HGDH transfection.

225 (C) Western blot analysis to compare the ERK1/2 phosphorylation for siNC, siPHDB2#1,
 226 siPHDB2#2 in 786-O and A-498 cells.

227 (D) Western blot analysis to compare the ERK1/2 phosphorylation for siNC, siPHDB2#2,
 228 siPHDB2#2+ OE PHLDB2 in 786-O and A-498 cells.

229

230

231

232

233

234

235