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Supplementary Materials for

The CREB coactivator CRTC2 promotes oncogenesis in LKB1-mutant non–small cell lung cancer

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Published 24 July 2019, *Sci. Adv.* **5**, eaaw6455 (2019)
DOI: 10.1126/sciadv.aaw6455

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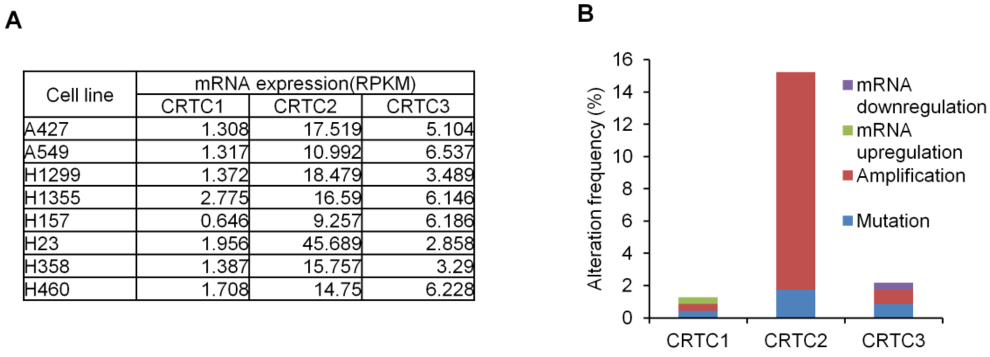

A

Cell line	mRNA expression(RPKM)		
	CRTC1	CRTC2	CRTC3
A427	1.308	17.519	5.104
A549	1.317	10.992	6.537
H1299	1.372	18.479	3.489
H1355	2.775	16.59	6.146
H157	0.646	9.257	6.186
H23	1.956	45.689	2.858
H358	1.387	15.757	3.29
H460	1.708	14.75	6.228

Fig. S1. mRNA expression and incidence of CRTC alterations in NSCLC. (A) Expression of CRTC1, CRTC2, and CRTC3 mRNA in a panel of NSCLC cell lines (n= 8). **(B)** Incidence of CRTC alterations in a panel of lung adenocarcinoma patient samples (n=230) from the TCGA.

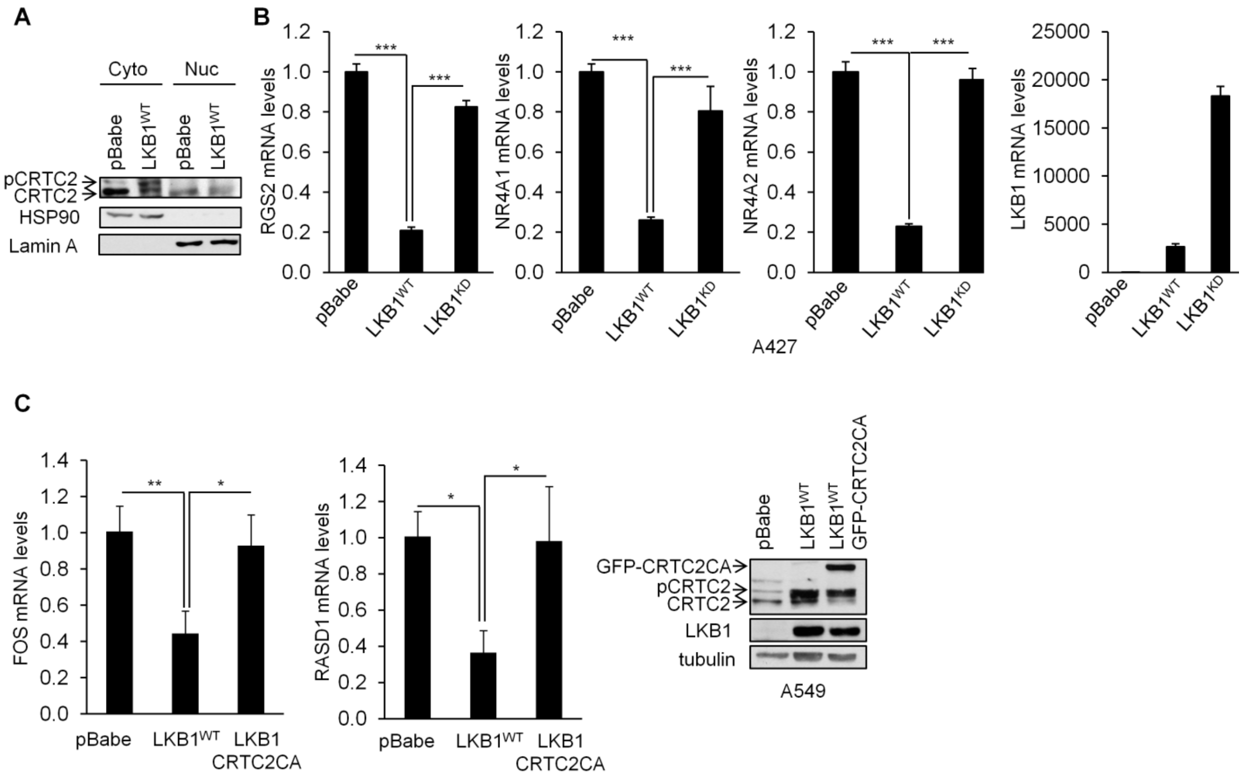

Fig. S2. LKB1 down-regulates the expression of CREB target genes. (A) Western blot analysis in nuclear and cytoplasmic fractions of A549 cells expressing the pBabe vector or full-length LKB1 (LKB1$^{\mathrm{WT}}$). **(B)** RGS2, NR4A1, NR4A2, and LKB1 mRNA levels in A427 cells expressing the pBabe vector, full-length LKB1 (LKB1$^{\mathrm{WT}}$) or kinase-dead LKB1 (LKB1$^{\mathrm{KD}}$). All values are expressed as means ± s.d. * $P<0.05$ ** $P<0.01$ *** $P<0.001$ determined by ANOVA with Tukeys method. **(C)** Immunoblot and qRT-PCR analysis in A549 cells expressing the pBabe vector, full-length LKB1 (LKB1$^{\mathrm{WT}}$) and/or phosphorylation-defective CRTC2 (GFP-CRTC2CA). All values are expressed as means ± s.d. * $P<0.05$ ** $P<0.01$ *** $P<0.001$ determined by ANOVA with Tukeys method.

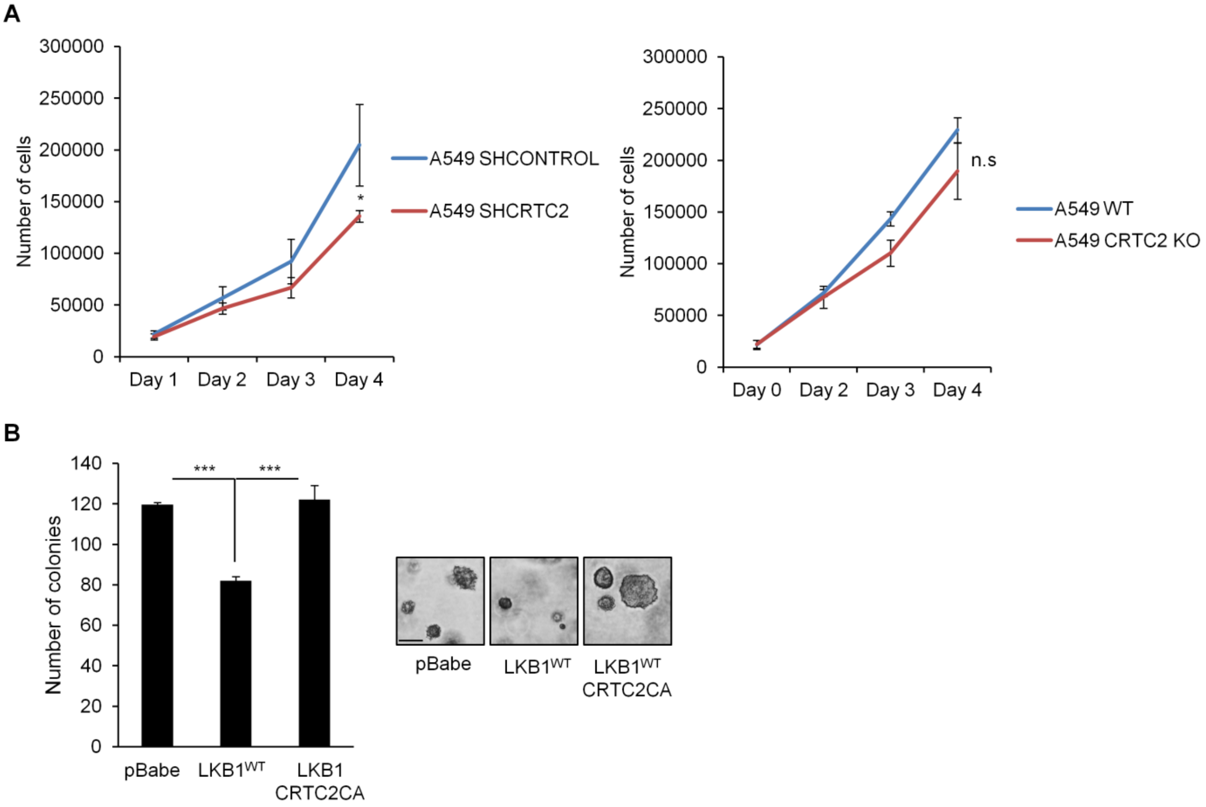

Fig. S3. CRTC2 promotes anchorage-independent growth in NSCLC cells. (A) Cellular proliferation in A549 shcontrol, A549 shCRTC2, A549 wild-type (WT) and A549 CRTC2-KO cells. All values are expressed as means ± s.d. * $P<0.05$ ** $P<0.01$ *** $P<0.001$ determined by two-sided student t-test. **(B)** Anchorage-independent growth assessed by soft agar assay in A549 cells expressing the pBabe vector, full-length LKB1 ($LKB1^{WT}$) and/or phosphorylation-defective CRTC2 (GFP-CRTC2CA). All values are expressed as means ± s.d. * $P<0.05$ ** $P<0.01$ *** $P<0.001$ determined by ANOVA with Tukeys method. Representative images are shown. Scale bar=100 μm.

gene_id	log ratio A549 LKB1/control (RNAseq)	log ratio CRTC2 binding A549 LKB1/control (ChipSeq)	log ratio CREB binding A549 LKB1/control (ChipSeq)	CRE site under the peak (ChipSeq)	log ratio LKB1 mutant/control lung adenocarcinoma TCGA (RNAseq)	q-VALUE
CYP1B1	-4.80	-1.22, -1.16	-1.03, -0.75	NO	-0.65	0.0115
RSPO3	-4.71	-2.12	-1.05	NO	0.76	0.245
EVA1C	-4.26	-2.20, -2.06	-1.49, -1.91	YES	0.63	6.40E-03
NR4A1	**-3.88**	**-1.59, -1.55**	**-1.1, -0.85**	**YES**	**1.36**	**4.53E-06**
PON3	-3.54	-1.17	-0.6	YES	-0.09	0.87
DUSP4	**-3.25**	**-1.48**	**-0.8**	**YES**	**2.09**	**1.29E-08**
MTUS1	-3.19	-1.30	-1.09	YES	0.53	7.36E-03
LIMCH1	-3.13	-1.64	-1.11	NO	0.73	4.70E-04
CPLX2	-3.06	-1.32	-0.71	YES	N/A	N/A
PDE4D	**-2.99**	**-1.96**	**-0.77**	**YES**	**1.88**	**5.00E-12**
GABRB3	-2.78	-2.28	-1.1	NO	0.59	5.29E-01
AVPI1	**-2.64**	**-1.39**	**-0.76**	**YES**	**1.17**	**3.84E-05**
GABRA5	-2.50	-1.52	-1.08	NO	N/A	N/A
NTRK3	-2.33	-3.21	-2.4	NO	N/A	N/A
THBD	-2.32	-1.43	-0.54	NO	-0.08	7.97E-01
DBNDD2	-2.27	-1.23	-0.3	NO	-0.2	2.74E-01
SFRP1	-2.23	-1.53	-1.07	NO	0.17	7.42E-01
MGAT5B	-2.04	-1.54	-0.93	NO	N/A	N/A
CD24	-1.99	-1.43	-0.72	NO	-0.46	2.53E-01
FMNL1	-1.96	-1.42	-0.91	NO	0.29	2.10E-01
ID1	**-1.87**	**-1.27**	**-0.57**	**YES**	**1.1**	**5.27E-04**
FBN2	-1.76	-2.90, -1.99	-1.79, -1.56	NO	N/A	N/A
STC2	-1.68	-1.09	-1.18	NO	0.3	3.65E-01
MMD	-1.61	-1.38	-0.83	NO	-0.61	5.25E-02
MFSD3	-1.60	-1.01	-0.67	YES	0.63	0.0113
PDE8A	-1.51	-1.51,-1.41	-0.82, -0.81	YES	0.34	0.0193
CPEB2	-1.46	-1.05	-0.43	YES	0.19	0.353
SLC40A1	-1.45	-1.04	-0.6	NO	-0.74	5.50E-03
ST6GAL2	-1.39	-2.42	-1.7	NO	N/A	N/A
PMP22	-1.36	-1.27	-0.85	NO	-0.33	0.0756
F2RL1	-1.36	-1.32	-0.74	NO	-0.67	8.41E-03
SLC16A3	-1.34	-1.28	-0.6	YES	0.1	0.77
TRAPPC5	-1.32	-1.01	-0.32	NO	-0.1	0.672
SIK1	**-1.32**	**-1.71**	**-0.59**	**YES**	**1.56**	**4.95E-10**
CYB561	-1.30	-1.46	-0.86	NO	0.33	0.0886
DENND5B	-1.29	-1.19	-0.65	NO	-0.1	0.8
RHOBTB2	-1.27	-1.38	-0.5	NO	1.05	1.56E-04
NPR3	-1.26	-1.56	-1.06	YES	-0.12	0.745
NRCAM	-1.21	-1.46	-0.96	NO	-0.61	0.0805
GRAMD1A	-1.16	-1.15	-0.86	YES	0.49	7.05E-03
FLYWCH2	-1.14	-1.07	-0.69	NO	0.08	0.625
NPEPL1	-1.11	-1.03	-0.6	NO	0.33	0.0653
CBX4	-1.11	-1.17	-0.92	YES	0.18	0.118
ST3GAL5	-1.08	-1.09	-0.54	YES	0.04	0.918
TGM2	-1.08	-1.67	-0.68	NO	-0.03	0.937
C9orf3	-1.07	-1.30	-0.92	YES	0.05	0.795
NDOR1	-1.05	-1.23	-0.88	YES	0.22	0.133
PRTFDC1	-1.03	-1.56	-0.57	YES	0.1	0.735
KHK	-1.03	-1.09	-0.28	NO	0.23	0.327

Fig. S4. Genes regulated by LKB1 at the mRNA level that show LKB1-regulated binding of CRTC2 to their promoters in NSCLC. RNAseq and ChIPseq experiments were performed in A549-LKB1^{WT} and A549-pBabe cells and datasets were cross-referenced. 49 protein-coding genes were identified with mRNA expression and CRTC2 binding log2 fold change below -1. For these 49 candidate genes, presence of canonical CRE sites and mRNA expression in a panel of LKB1-mutant and LKB1-wild type lung adenocarcinoma tumors was also analyzed.

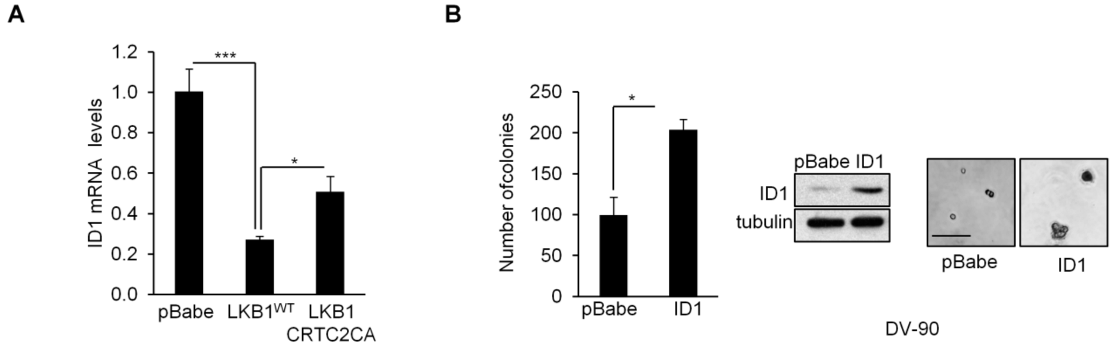

Fig. S5. ID1 expression is regulated by CRTC2 and promotes anchorage-independent growth in NSCLC cells. (A) ID1 mRNA levels in A549 cells expressing the pBabe vector, full-length LKB1 (LKB1^{WT}) and/or phosphorylation-defective CRTC2 (GFP-CRTC2CA). All values are expressed as means ± s.d. * $P<0.05$ ** $P<0.01$ *** $P<0.001$ determined by ANOVA with Tukeys method. **(B)** Anchorage-independent growth assessed by soft agar assay in LKB1 wild-type NSCLC DV-90 cells expressing the pBabe vector or full-length ID1. Representative images are shown. Lysates from DV-90 pBabe and DV-90-ID1 cells were immunoblotted with the indicated antibodies. Scale bar= 100 µm. All values are expressed as means ± s.d.* $P<0.05$ ** $P<0.01$ *** $P<0.001$ determined by two-sided student t-test**.**

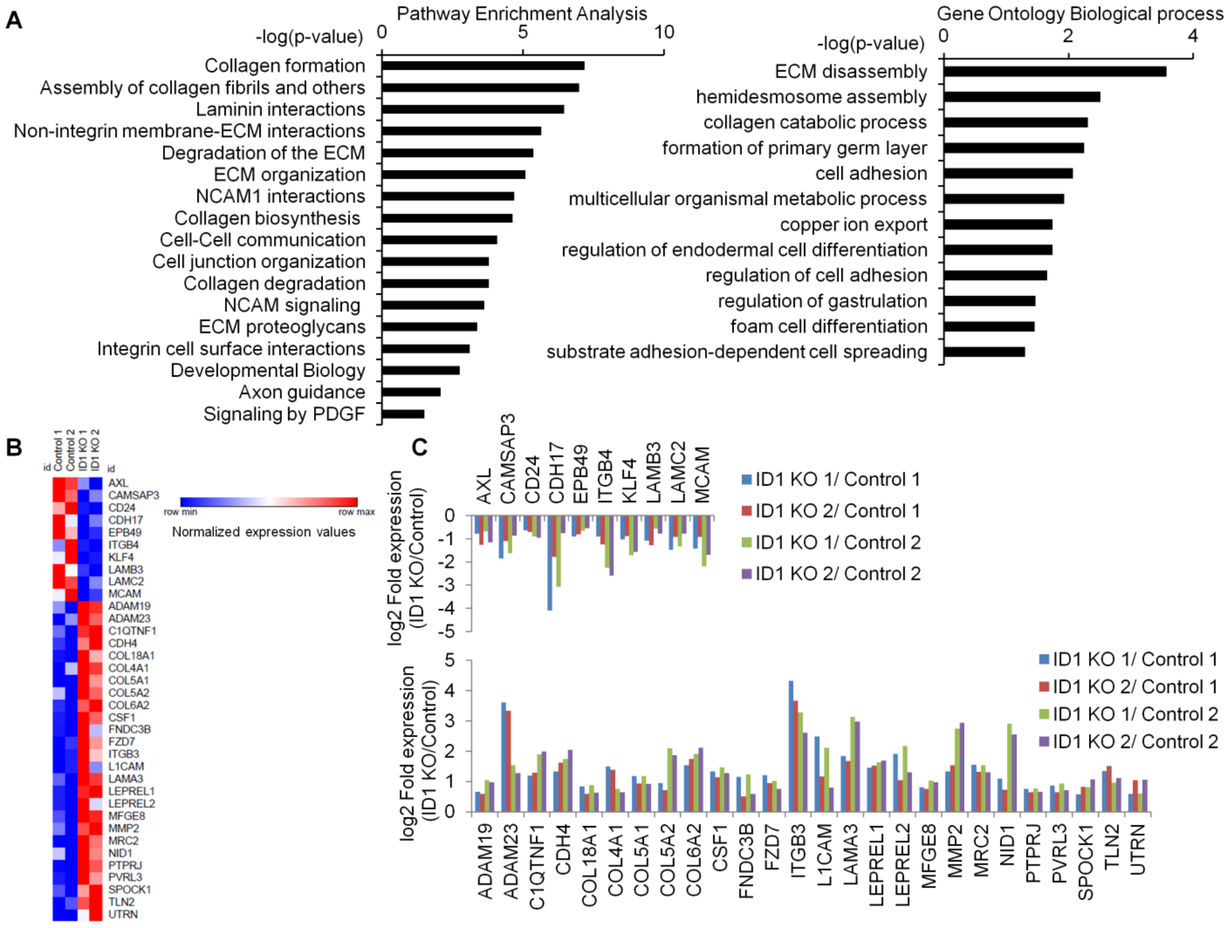

Fig. S6. Genes and molecular functions regulated by ID1 in NSCLC cells. (A) Pathway enrichment and Gene Ontology analysis of differentially expressed genes (log2 Fold change > 0.5 or < -0.5) in A549 ID1-KO (ID1 KO 1, ID1 KO 2) compared to A549 wild type clones (Control 1 and Control 2). **(B)** Heatmap showing differentially expressed genes involved in the regulation of the extracellular matrix, cellular cytoskeleton, cell-cell binding and cellular binding to the extracellular matrix in A549 ID1-KO (ID1 KO 1, ID1 KO 2) compared to A549 wild type (Control 1 and Control 2) clones. **(C)** Log2 Fold expression from the RNA-seq data for each gene in (B).