Supplemental Information

Negative MAPK-ERK regulation sustains CIC-DUX4 oncoprotein expression in undifferentiated sarcoma.

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The supplemental data contains eight figures and three datasets.

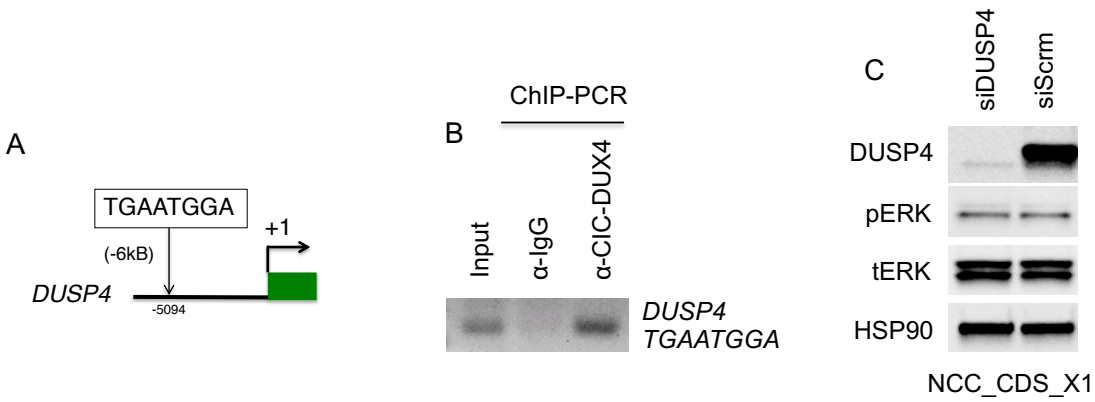

Fig. S1. CIC-DUX4 binds a *DUSP4* upstream regulatory element.

A) CIC-binding motif in the upstream regulatory element of *DUSP4*. B) ChIP PCR demonstrating CIC-DUX4 occupancy of the upstream regulatory element of *DUSP4*. C) Immunoblot of DUSP4 and pERK in NCC_CDS_X1 cells expressing *siDUSP4* or *siScrm* control.

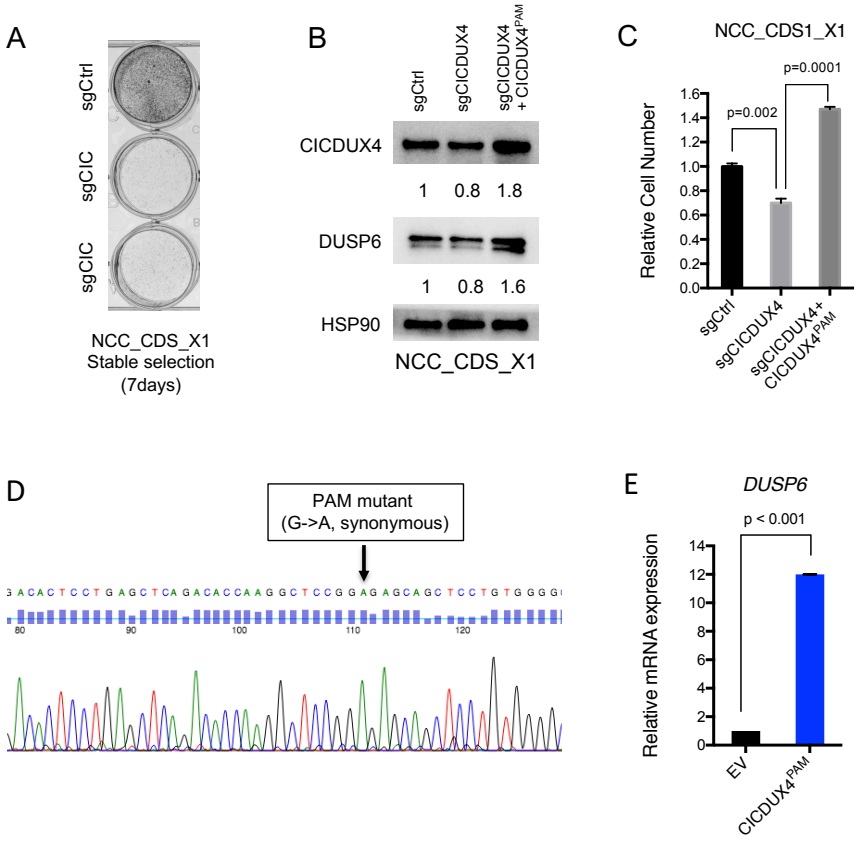

Fig. S2. CRISPR-resistant CIC-DUX4 cDNA (CIC-DUX4^{PAM}) rescues sgCIC-DUX4 mediated DUSP6 and tumor growth suppression.

A) Crystal violet viability assay of NCC_CDS_X1 cells stably expressing sgCIC. 7 days after selection. B) Immunoblot of CIC-DUX4 and DUSP6 in NCC_CDS_X1 cells transiently expressing sgCtrl, sgCIC-DUX4, or co-expressing sgCIC-DUX4 and a CRISPR-resistant CIC-DUX4 cDNA, CIC-DUX4^{PAM}. C) Relative number of NCC_CDS_X1 cells expressing either sgCtrl, sgCIC-DUX4, or co-expressing sgCIC-DUX4 and a CRISPR-resistant CIC-DUX4 cDNA, CIC-DUX4^{PAM}. p-value, one-way ANOVA. Error bars, SEM. D) Sequence tracing of CIC-DUX4^{PAM} demonstrating the silent mutation in the PAM-recognition sequence. E) Relative *DUSP6* mRNA expression in 293T cells expressing EV or CIC-DUX4^{PAM}. p-value, student's t-test. Error bars, SEM.

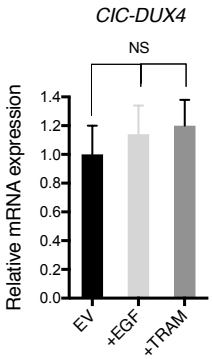

Fig. S3. Relative CIC-DUX4 mRNA expression in NCC_CDS_X1 cells treated with epidermal growth factor (EGF), trametinib (TRAM), or EV with no treatment for 6 hours. p-value, one-way ANOVA. Error bars, SEM.

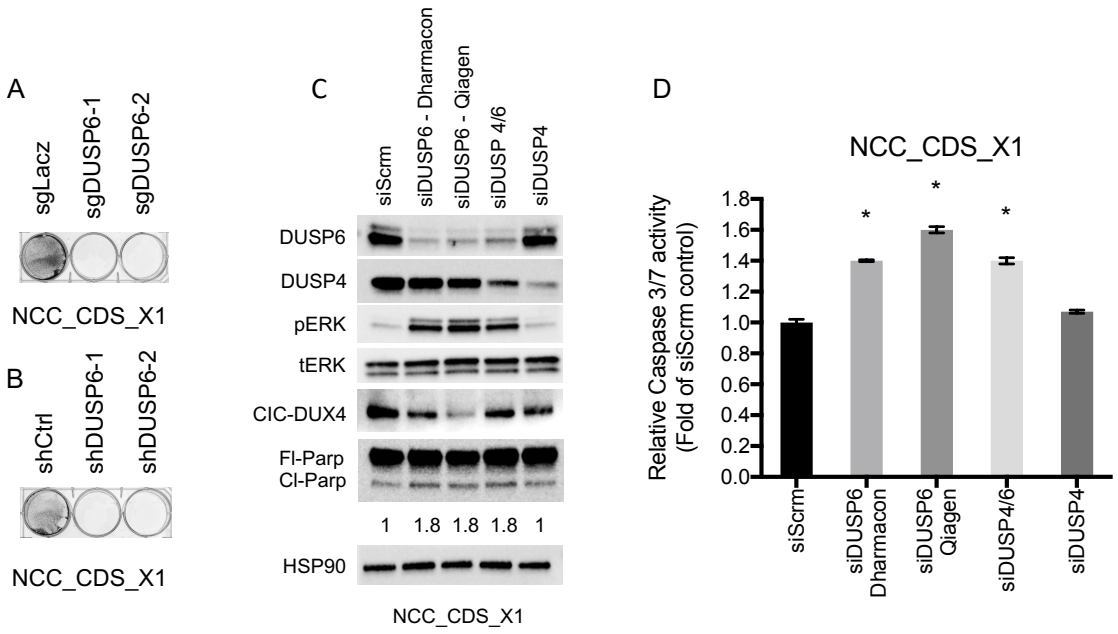

Fig. S4. Genetic silencing of *DUSP6* decreases viability and enhances apoptosis in NCC_CDS_X1 cells.

A-B) Crystal violet assay of NCC_CDS_X1 cells expressing validated *sgDUSP6* (A) or *shDUSP6* constructs (B). 7 days after selection. C) Immunoblot of pERK and Parp in NCC_CDS_X1 cells expressing either *siScrm* control, *siDUSP6-8* from Dharmacon, *siDUSP6* from Qiagen, combination *siDUSP6* and *siDUSP4*, or *siDUSP4*. D) Caspase 3/7 activity in NCC_CDS_X1 cells expressing either *siScrm* control, *siDUSP6-8* from Dharmacon, *siDUSP6* from Qiagen, combination *siDUSP6* and *siDUSP4*, or *siDUSP4*. *<0.05. p-value, one-way ANOVA. Error bars, SEM.

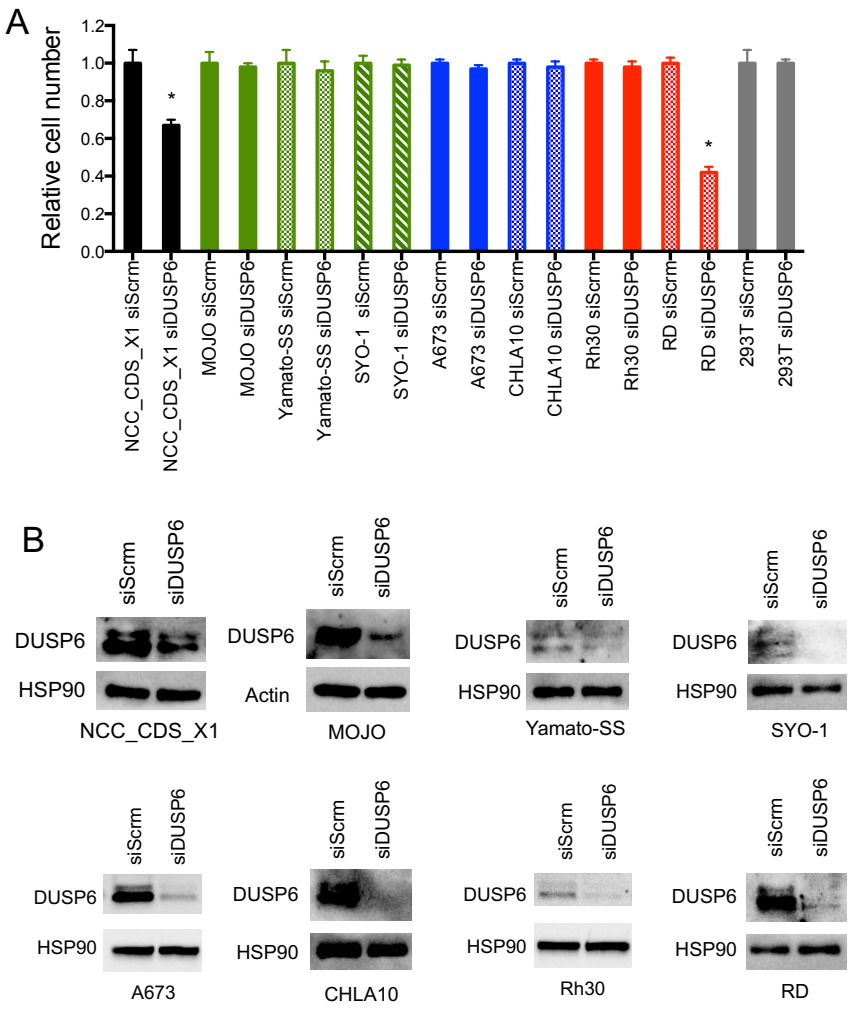

Fig. S5. Genetic inhibition of *DUSP6* decreases CIC-DUX4 sarcoma growth relative to other fusion-positive sarcomas.

A) Cell titer glo assay in a panel of fusion positive and fusion negative sarcoma cell lines expressing either *siScrm* or *siDUSP6*. NCC_CDS_X1 (CIC-DUX4 sarcoma), MOJO, Yamato-SS, SYO-1 (Synovial Sarcoma), A673 and CHLA10 (Ewing Sarcoma), Rh30 and RD (Rhabdomyosarcoma) cells. RD is a fusion-negative rhabdomyosarcoma that harbors an NRAS mutation. $P < 0.05$, one-way ANOVA. Error bars, SEM. B) Immunoblots demonstrating DUSP6 knockdown.

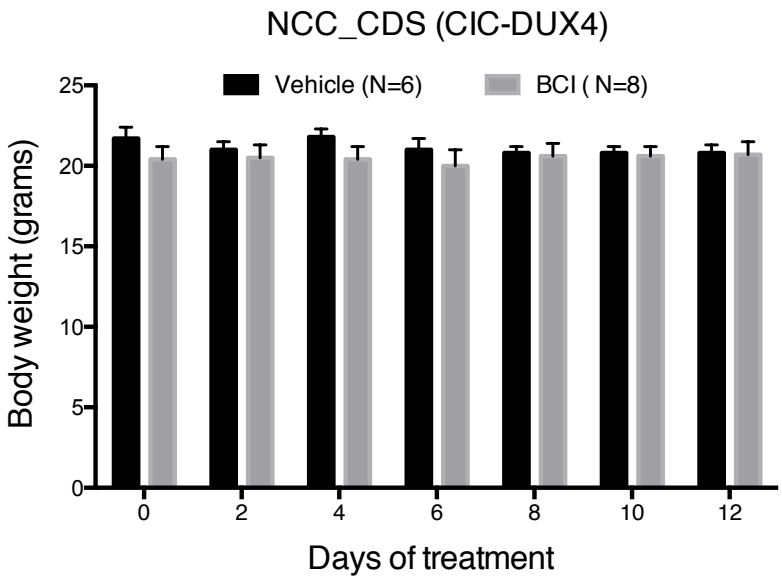

Fig. S6. Serial weights from NCC_CDS_X1 tumor bearing mice treated with BCI (N=8) or vehicle (N=6). Error bars, SEM.

Fig. S7. ERK1 or ERK2 inhibition does not independently suppress CIC-DUX4 tumor growth.

A) Crystal violet assay of NCC_CDS_X1 cells expressing either *siScrm* control, *siERK1*, *siERK2*, or combination *siERK1* and *siERK2*. B) Cell-titer glo assay comparing NCC_CDS_X1 cells expressing either *siScrm* control, *siERK1, siERK2*, or combination *siERK1* and *siERK2* (72 hours). p-value not signficant, one-way ANOVA. Error bars, SEM.

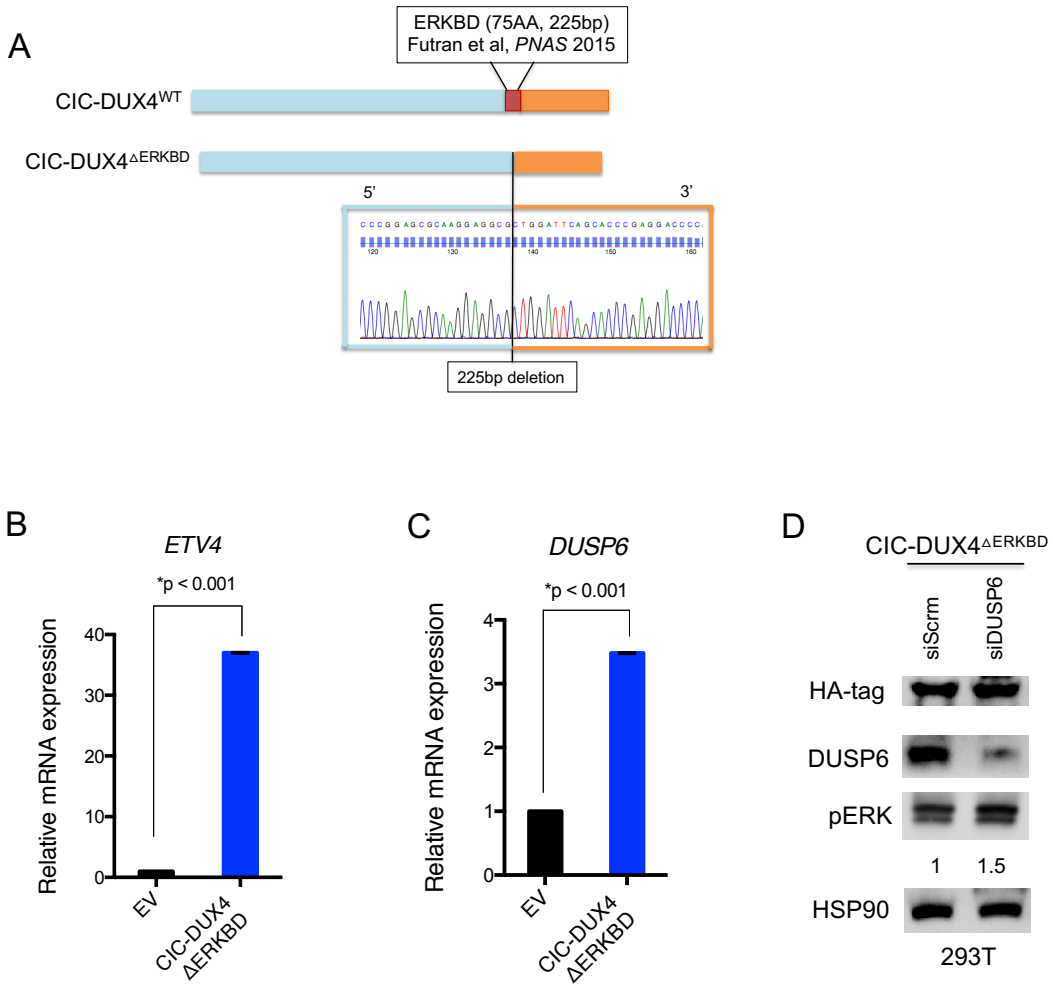

Fig. S8. The ERK-CIC binding interface is conserved and regulates ERK mediated degradation of the CIC-DUX4 fusion oncoprotein.

A) Structural schematic of the highly conserved 75 AA ERK-binding domain in CIC-DUX4. Sequence verification of the 225bp deletion in CIC-DUX4, which produced the ERK-resistant CIC-DUX4$^{\Delta ERKBD}$ mutant isoform. B-C) Relative *ETV4* and *DUSP6* mRNA expression in 293T cells expressing either empty vector (EV) or the CIC-DUX4$^{\Delta ERKBD}$ variant. p-value, student's t-test. Error bars, SEM. D) Immunoblot of 293T cells expressing CIC-DUX4$^{\Delta ERKBD}$ and either *siScrm* control or *siDUSP6*.