

clAP2-Mediated IGF2BP2 Ubiquitination and Degradation Promote Cardiomyocyte Apoptosis via Stabilizing m⁶A-modified BAX mRNA in Myocardial Infarction

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Table S1. Antibodies, compounds and reagents.

Table S2. Sequences of oligonucleotides.

Table S3. List of *Birc3* co-expressed genes.

Figure S1. Volcano plots of DEGs distribution in GSE775 (a) and GSE97494 (b). The nodes in purple represent down-regulated genes; nodes in red represent up-regulated genes; and nodes in grey represent genes without significant changes.

Figure S2. METTL3 regulates clAP2 protein level. **a** METTL3 protein level in AC16 cells under Norm or OGD condition. **b** Knockout of METTL3 decreased clAP2 protein level in AC16 cells.

Figure S3. clAP2 overexpression protected against MI. **a** clAP2 protein level in mouse hearts 4 weeks after administration of AAV-Vector or AAV-cTnT-clAP2 (1×10^{11} vg/mouse) by tail vein injection. **b** C57BL/6J mice were administered AAV-Vector or AAV-cTnT-clAP2 by tail vein injection 4 weeks before Sham or LAD ligation surgery. Western blot assay was used to detect IGF2BP2, Caspase 3, cleaved Caspase 3 (c-Caspase 3) levels in mouse hearts of each group.

Figure S4. The effect of clAP2 on IGF2BP2 mRNA. RT-qPCR was used to detect IGF2BP2 mRNA levels in AC16 cells 48 hours after transfected with *Flag-clAP2* or *sgclAP2* plasmids and control cells. ns $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure S5. Protein levels of GADD45 α , NF- κ B p65, HMGB2, p21, Lamin A/C in AC16 cells 48 hours after transfected with Myc-IGF2BP2 plasmid and control cells.

Figure S6. CIW1-2 inhibits cell viability of A549 cells. A549 cells were treated with DMSO or CIW1-2 for 24 hours and subjected to CCK8 assay. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure S7. IGF2BP2 upregulates and stabilizes cIAP2 mRNA. **a, b** RIP-qPCR was performed to detect IGF2BP2 bound with cIAP2 mRNA in AC16 **(a)** and HL-1 **(b)** cells. **c** Validation of the cIAP2 protein level in IGF2BP2 overexpression (OE) AC16 cells by immunoblotting assay. **d** RT-qPCR was used to detect IGF2BP2 and cIAP2 mRNA levels in IGF2BP2-OE AC16 cells. **e** cIAP2 mRNA levels in IGF2BP2-OE AC16 cells treated with Actinomycin D (25 µg/mL) at different time points. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure S8. Landscape of immune infiltration in the heart. **(a)** Proportion of different immune cells in mouse heart samples. **(b)** Comparisons of immune cells between Sham and MI mice. MI, myocardial infarction. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table S1. Antibodies, compounds and reagents.

Antibody	Source	Cat. No	Application
clAP2 Polyclonal Antibody	Invitrogen	PA5-8750	Immunohistochemistry, Western blot
c-IAP2 (58C7) Rabbit mAb	Cell Signaling Technology	3130S	Western blot
Polyclonal Anti-BIRC3 Antibody	Atlas Antibodies	HPA002317	Western blot
Caspase-3 (3CSP03) Antibody	Santa Cruz Biotechnology	sc-56046	Western blot
Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb	Cell Signaling Technology	9664S	Western blot, immunofluorescence
GAPDH Antibody (6C5)	Santa Cruz Biotechnology	sc-32233	Western blot
IGF2BP2 (D4R2F) Rabbit mAb	Cell Signaling Technology	14672s	Western blot, immunoprecipitation, RNA
ANTI-FLAG® antibody	M2 Sigma-Aldrich	F1804	Western blot, immunoprecipitation
9E10 Anti-Myc Monoclonal Antibody	OriGene	TA150121	Western blot, immunoprecipitation
β-Actin	Sigma-Aldrich	A5316	Western blot
Anti-HA tag-ChIP (ab9110)	Abcam	ab9110	Western blot
m ⁶ A antibody	Synaptic Systems	#202003	m ⁶ A RNA immunoprecipitation
anti-rabbit IgG HRP	Santa Cruz Biotechnology	sc-2004	Western blot
anti-mouse IgG HRP	Santa Cruz Biotechnology	sc-2005	Western blot
Compounds and reagents		Source	Cat. No
Actinomycin D		Selleck Chemicals	S8964
MG132		Selleck Chemicals	S2619
Cycloheximide		MCE	HY-12320
CWI1-2		Selleck Chemicals	E1327
DMSO		Sigma-Aldrich	D2650
Tris		Sigma-Aldrich	T1503
NaCl		Sigma-Aldrich	S1679
SDS		Sigma-Aldrich	L3771

APS

Sigma-Aldrich

A3678

Table S2. Sequences of oligonucleotides

Gene	Primer Sequence (5'-3')
<i>BIRC3</i> (human)	F: CTGTGATGGTGGACTCAGGTGTTG R: TGGCTTGAAGTTGACGGATGAACTC
<i>IGF2BP2</i> (human)	F: CATCATCGGAAAGGAGGGCTTGAC R: GCATGGATGGTGACAGGCTTCTC
<i>BAX</i> (human)	F: TCAGGATGCGTCCACCAAGAAG R: TGTGTCCACGGCGGCAATCATC
<i>GAPDH</i> (human)	F: GTCTCCTCTGACTTCAACAGCG R: ACCACCCTGTTGCTGTAGCCAA
<i>Birc3</i> (mice)	F: GGACATTAGGAGTCTTCCCACAG R: GAACACGATGGATACCTCTCGG
<i>Igf2bp2</i> (mice)	F: CGCCAGACGAGAATGAGGAAGTG R: GTATCTCTGCTCCTGCTGCTTCAC
<i>Bax</i> (mice)	F: AGGATGCGTCCACCAAGAAGCT R: TCCGTGTCCACGTCAGCAATCA
<i>Gapdh</i> (mice)	F: CATCACTGCCACCCAGAAGACTG R: ATGCCAGTGAGCTTCCCGTTCAG
sgRNA	Sequence (5'-3')
<i>sgControl</i>	F: CACCGACTAGCCTGTTGCGGAGTAG R: AAACCTACTCGCGAACAGGCTAGTC
<i>sgclAP2#1</i>	F: CACCGGTTAGTAGACTATCCAGGAT R: AAACATCCTGGATAGTCTACTAACC
<i>sgclAP2#2</i>	F: CACCGGACTTGTGTAATTCCAATCC R: AAACGGATTGGAATTACACAAGTCC

Abbreviation: F, forward; R, reverse.

Table S3. List of Birc3 co-expressed genes.

Target Gene	Co-expressed Gene	p value	r value
Birc3	Hspa1a	0.000273	0.970901
Birc3	Hspa1b	0.000323	0.968871
Birc3	Selp	0.000678	0.958012
Birc3	Cxcl1	0.000182	0.975308
Birc3	Sphk1	0.000365	0.967309
Birc3	Il6	0.000204	0.9741
Birc3	Cyr61	0.000387	0.966521
Birc3	Atf3	0.000111	0.979762
Birc3	Il4ra	0.000657	0.958544
Birc3	Hbegf	0.000433	0.964977
Birc3	Hmox1	0.000415	0.965567
Birc3	Litaf	0.000369	0.967161
Birc3	Sele	0.000726	0.956835
Birc3	Lox	0.000782	0.955522
Birc3	Junb	4.18E-05	0.986306
Birc3	Ankrd2	0.000878	0.953404
Birc3	Ier3	0.000801	0.955091
Birc3	Wsb1	0.000811	0.954873
Birc3	Il1r2	0.000614	0.959668
Birc3	Mt2	0.000543	0.96162
Birc3	Tgif1	0.000968	0.951517
Birc3	Socs3	0.000598	0.960103
Birc3	Igf2bp2	4.03E-05	0.986505
Birc3	Acsl4	0.000697	0.95755
Birc3	Fam107b	0.000248	0.972015
Birc3	Plk2	0.000834	0.954362
Birc3	Nfkbiz	0.000192	0.974723
Birc3	Atf4	0.000412	0.965647
Birc3	D8Wsu26e	0.000249	0.971953
Birc3	Ppp1r15a	0.000104	0.980238
Birc3	Errf1	7.67E-06	0.993063
Birc3	Epha2	0.000699	0.957508
Birc3	Pgm1	0.000332	0.968517
Birc3	Hamp	0.000896	0.953022
Birc3	Ereg	0.000828	0.954487
Birc3	Klf6	6.02E-05	0.98415
Birc3	Ptgs2	0.000566	0.960972
Birc3	Gm15453	0.00093	0.952301
Birc3	Smad1	0.00075	0.956268
Birc3	Akap12	0.000533	0.961899
Birc3	Ero1l	0.000196	0.974551
Birc3	Egr1	7.82E-05	0.982399
Birc3	Rel1	0.000423	0.965297
Birc3	Btg3	0.000649	0.958753
Birc3	Fos	1.27E-05	0.991512
Birc3	Xirp1	0.000535	0.961835
Birc3	Cebpd	1.70E-06	0.996203
Birc3	Fosl1	0.000695	0.957595
Birc3	Mafk	0.000877	0.953418
Birc3	Ptx3	2.80E-05	0.988346
Birc3	Odc1	0.000722	0.956934
Birc3	Mmp14	0.00022	0.973309
Birc3	Tiparp	0.000813	0.954817
Birc3	Sptlc2	0.000439	0.964772
Birc3	Arg2	0.000293	0.970077
Birc3	Eno2	0.000206	0.974024

Birc3	Adss	0.000323	0.968877
Birc3	Fhl1	0.00065	0.958724
Birc3	Sowahc	0.000799	0.955147
Birc3	Igfbp3	0.000992	0.951033
Birc3	Hif1a	5.82E-05	0.984366
Birc3	Map2k3	0.000604	0.959928
Birc3	Abca4	2.59E-05	0.988701
Birc3	Casp4	0.000205	0.97405
Birc3	Ier2	0.000546	0.961535
Birc3	Pdlim1	0.000796	0.955208
Birc3	Gch1	0.000658	0.958516
Birc3	Dnajb1	0.000572	0.960808
Birc3	Slc23a2	0.000193	0.974716
Birc3	Rnf19a	0.000633	0.959167
Birc3	Slc10a6	0.000138	0.977879
Birc3	Grid1	0.000324	0.968833
Birc3	Il1r1	0.000492	0.963112
Birc3	Nfkbia	5.63E-05	0.984576
Birc3	Snhg6	6.55E-05	0.98361
Birc3	Ppp2r5c	0.000939	0.952119
Birc3	Ccl5	0.000171	0.975895
Birc3	Slc25a25	0.000702	0.957421
Birc3	Serpina3n	0.00036	0.967487
Birc3	Eif5	0.00072	0.956991
Birc3	Btg2	0.000265	0.971245
Birc3	Csf1	0.000264	0.971308
Birc3	Efhd2	0.000572	0.960798
Birc3	Gclc	0.000761	0.956021
Birc3	Pim1	0.000217	0.973458
Birc3	Gadd45a	7.00E-05	0.98316
Birc3	Cdr2l	0.000643	0.95891
Birc3	Fkbp5	0.000889	0.953162
Birc3	Rbm18	0.000448	0.964488
Birc3	Casp3	9.47E-05	0.980989
Birc3	Zfp326	0.000736	0.956603
Birc3	Palld1	0.000886	0.95322
Birc3	Csnk1d	0.000843	0.954167
Birc3	Nxf1	0.000534	0.961888
Birc3	Smoc1	0.00044	0.964743
Birc3	Ell	0.000477	0.963559
Birc3	Gpr3	0.000585	0.960442
Birc3	Tnfrsf1b	0.000648	0.958789
Birc3	Cd9	0.000212	0.973738
Birc3	Clca1	0.000808	0.954931
Birc3	Hnrnpa1	0.000519	0.962314
Birc3	Nus1	7.09E-05	0.983078
Birc3	Cryab	0.000879	0.953388
Birc3	Zfp36	0.000507	0.962663
Birc3	Itgb7	0.000117	0.979294
Birc3	Tfpi	0.000462	0.964044
Birc3	Chd7	0.00035	0.967837
Birc3	Krt6a	0.000347	0.96795
Birc3	LOC102635048	0.000814	0.95481
Birc3	D9Ert306e	4.08E-05	0.986439
Birc3	Akap2	0.00104	0.950098
Birc3	Nupr1	0.000579	0.960616
Birc3	Tnip1	0.00081	0.954903
Birc3	Sh3bgrl	9.80E-05	0.980731

Birc3	Ewsr1	0.000834	0.954349
Birc3	Abrac1	0.000729	0.956778
Birc3	Rdh7	0.000496	0.962979
Birc3	Mdfic	0.000361	0.967445
Birc3	Tsc22d1	0.000476	0.963614
Birc3	Rad	0.001015	0.950593
Birc3	Cited2	0.000195	0.974604
Birc3	Kcnj2	0.000276	-0.9708
Birc3	Thy1	0.000215	-0.97359
Birc3	Gab1	0.000298	-0.96984
Birc3	Pstpip2	0.000259	-0.97152
Birc3	Myoc	0.000334	-0.96844
Birc3	Epb4.1	0.00097	-0.95148
Birc3	Coq9	0.000257	-0.97159
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Birc3	Dbp	0.000947	-0.95195
Birc3	Creg1	0.000469	-0.96383
Birc3	Pdrg1	0.00033	-0.96859
Birc3	Pptc7	0.000385	-0.96659
Birc3	Hccs	0.000244	-0.97218
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Birc3	Polrmt	0.000954	-0.9518
Birc3	Pik3r2	0.000825	-0.95457
Birc3	Mrpl23	0.000574	-0.96074
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Birc3	Txnrd2	0.000733	-0.95667
Birc3	Tspan13	0.000235	-0.97262
Birc3	Cdh2	6.84E-05	-0.98332
Birc3	Dpysl4	0.000423	-0.96531
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Birc3	Slc6a8	0.001012	-0.95064
Birc3	Dgcr6	0.000238	-0.97245
Birc3	Ube2a	0.000357	-0.96759
Birc3	St6galnac6	0.000789	-0.95537
Birc3	Agtrap	0.000973	-0.95143
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Birc3	Rhbdl3	8.41E-05	-0.98187
Birc3	Ndufaf4	0.000138	-0.97787
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Birc3	Naa38	0.000403	-0.96598
Birc3	Maf1	0.000422	-0.96534
Birc3	Dennd4c	0.000513	-0.96249
Birc3	ErbB3	0.000456	-0.96423
Birc3	Serac1	0.000682	-0.95792
Birc3	Nr1h3	8.79E-05	-0.98156
Birc3	Spr	0.000899	-0.95295
Birc3	Dynl1a	0.000154	-0.97687
Birc3	Amz2	0.000966	-0.95156
Birc3	Lym2	0.00071	-0.95723
Birc3	Mrpl21	0.000275	-0.97084
Birc3	Sorbs1	0.000977	-0.95135
Birc3	Bcas3	0.000156	-0.97674
Birc3	Slc37a4	0.000468	-0.96386

Birc3	Krt10	0.000722	-0.95694
Birc3	B3galt2	0.000569	-0.96089
Birc3	Ilvbl	0.000824	-0.95458
Birc3	Ndufb11	0.000386	-0.96656
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Birc3	0610012G03Rik	0.000825	-0.95456
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Birc3	Pde6d	0.000877	-0.95342
Birc3	Aifm1	0.000502	-0.96283
Birc3	Ubr7	0.000363	-0.96737
Birc3	Lamtor3	0.00028	-0.9706
Birc3	Cnot8	0.000206	-0.97404
Birc3	Dgcr2	0.000155	-0.97684
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Birc3	Nsmce4a	0.000569	-0.96088
Birc3	Cox8b	0.000731	-0.95673
Birc3	St3gal5	0.000809	-0.95492
Birc3	Pradc1	0.000983	-0.95121
Birc3	Tex261	2.50E-05	-0.98886
Birc3	Itsn1	0.000369	-0.96714
Birc3	Rnaseh2c	0.000712	-0.95717
Birc3	Limch1	0.000695	-0.95761
Birc3	Rnf34	0.000512	-0.96252
Birc3	Armc1	0.00049	-0.96317
Birc3	Slc12a7	0.000349	-0.96787
Birc3	Flcn	0.001009	-0.95071
Birc3	Stk11ip	0.00039	-0.96642
Birc3	Ipo8	0.000391	-0.96637
Birc3	Nkx2-5	0.000179	-0.97544
Birc3	Rbl2	0.000515	-0.96243
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Birc3	Sh3d19	0.000356	-0.96761
Birc3	Larp4b	0.000111	-0.97972
Birc3	Rpap1	0.000978	-0.95131
Birc3	Rufy1	0.000335	-0.96841
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Birc3	Mphosph9	0.000259	-0.97152
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Birc3	Osbp	0.000477	-0.96357
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Birc3	Mrps12	0.001041	-0.95008
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Birc3	Mocs2	0.000537	-0.9618
Birc3	Pde4dip	0.000927	-0.95237
Birc3	Gnpda1	0.000876	-0.95344
Birc3	Grpel1	0.000382	-0.96668
Birc3	Rnpepl1	0.000225	-0.97309
Birc3	Rab14	0.000467	-0.96388
Birc3	Pigq	3.88E-05	-0.98671
Birc3	Qars	0.000365	-0.96728
Birc3	Odf2	0.000983	-0.95122
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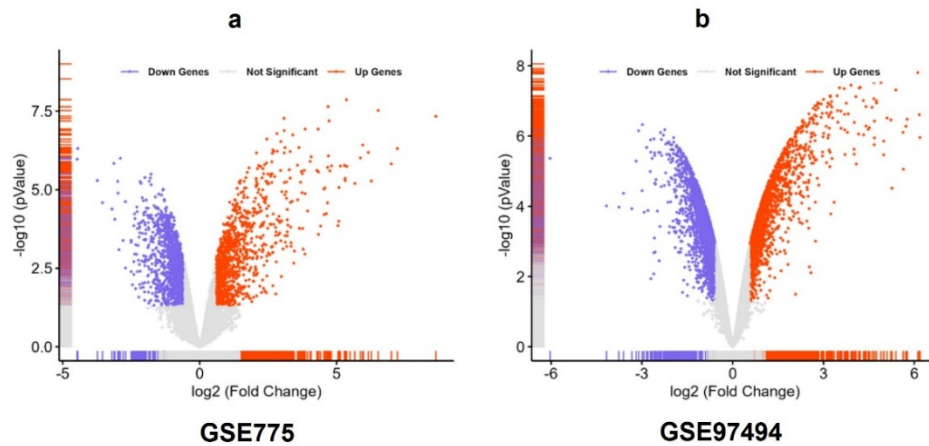


Figure S1. Volcano plots of DEGs distribution in GSE775 (a) and GSE97494 (b). The nodes in purple represent down-regulated genes; nodes in red represent up-regulated genes; and nodes in grey represent genes without significant changes.

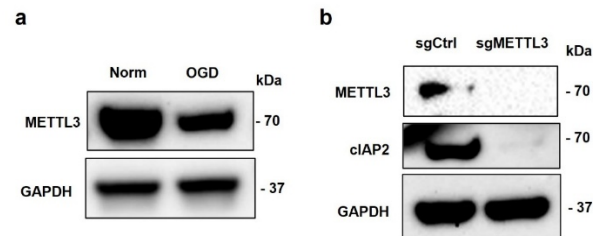


Figure S2. METTL3 regulates cIAP2 protein level. **a** METTL3 protein level in AC16 cells under Norm or OGD condition. **b** Knockout of METTL3 decreased cIAP2 protein level in AC16 cells.

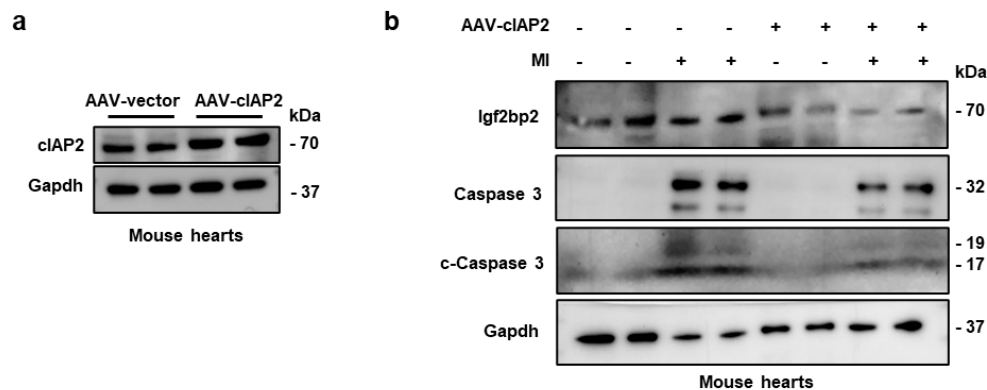


Figure S3. cIAP2 overexpression protected against MI. **a** cIAP2 protein level in mouse hearts 4 weeks after administration of AAV-Vector or AAV-cTnT-cIAP2 (5×10^{11} viral genomes/mouse) by tail vein injection. **b** C57BL/6J mice were administered AAV-Vector or AAV-cTnT-cIAP2 by tail vein injection 4 weeks before Sham or LAD ligation surgery. Western blot assay was used to detect IGF2BP2, Caspase 3, cleaved Caspase 3 (c-Caspase 3) levels in mouse hearts of each group.

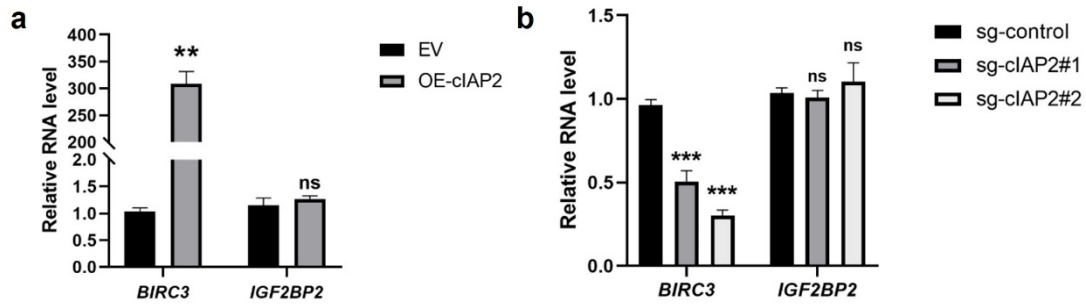


Figure S4. The effect of clAP2 on *IGF2BP2* mRNA. RT-qPCR was used to detect *IGF2BP2* mRNA levels in AC16 cells 48 hours after transfected with *Flag-clAP2* or *sgclAP2* plasmids and control cells. ns $p \geq 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

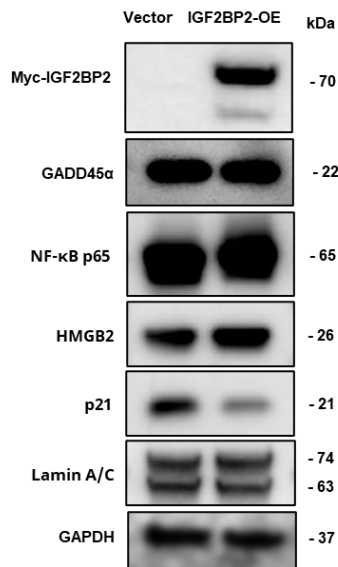


Figure S5. Protein levels of GADD45α, NF-κB p65, HMGB2, p21, Lamin A/C in AC16 cells 48 hours after transfected with Myc-IGF2BP2 plasmid and control cells.

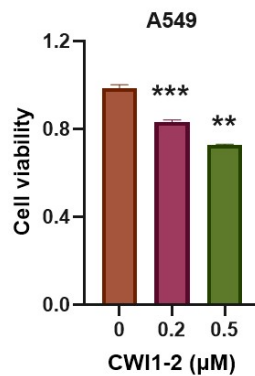


Figure S6. CWI1-2 inhibits cell viability of A549 cells. A549 cells were treated with DMSO or CWI1-2 for 24 hours and subjected to CCK8 assay. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

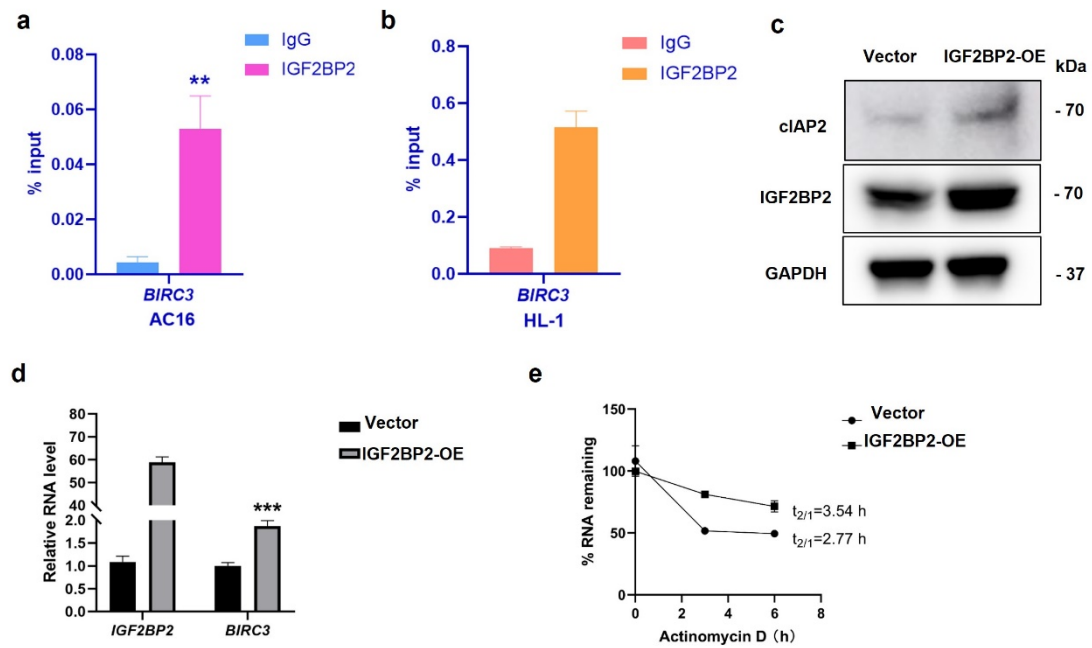


Figure S7. IGF2BP2 upregulates and stabilizes cIAP2 mRNA. **a, b** RIP-qPCR was performed to detect IGF2BP2 bound with cIAP2 mRNA in AC16 (**a**) and HL-1 (**b**) cells. **c** Validation of the cIAP2 protein level in IGF2BP2 overexpression (OE) AC16 cells by immunoblotting assay. **d** RT-qPCR was used to detect IGF2BP2 and cIAP2 mRNA levels in IGF2BP2-OE AC16 cells. **e** cIAP2 mRNA levels in IGF2BP2-OE AC16 cells treated with Actinomycin D (25 µg/mL) at different time points. *p<0.05, **p<0.01, ***p<0.001.

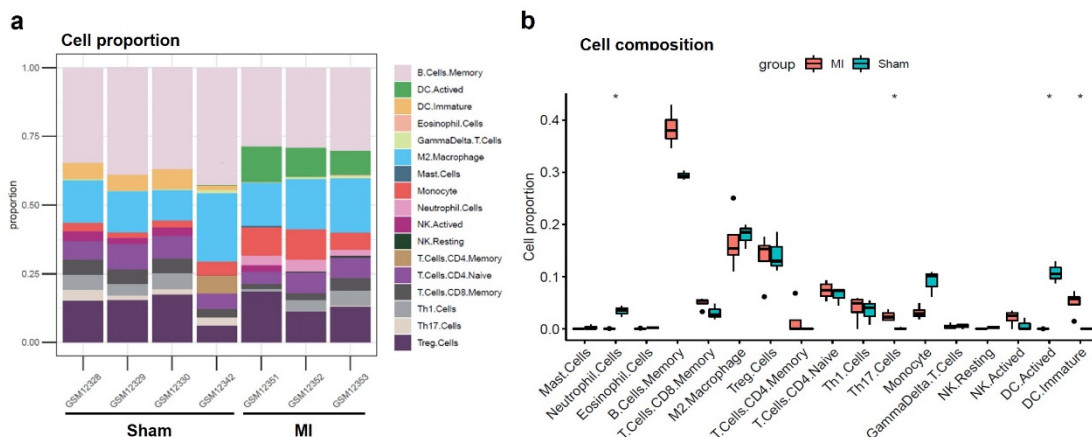


Figure S8. Landscape of immune infiltration in the heart. **(a)** Proportion of different immune cells in mouse heart samples. **(b)** Comparisons of immune cells between Sham and MI mice. MI, myocardial infarction. * p<0.05; ** p<0.01; *** p<0.001.