

Figure A1. DROSHA mRNA stability and miRNA-mediated regulation. **a**, DROSHA mRNA remaining in non-sphere (red) and sphere (blue) cells over time. **b**, ChIP-seq tracks for DROSHA at the DROSHA gene locus. **c**, Relative m⁶A abundance in P1-3 and NC cells. **d**, Relative DR mRNA levels in NC, T1, T2, T3, and T4 cells. **e**, Western blots for DROSHA and GAPDH. **f**, Relative m⁶A abundance in NC, T1, T2, T3, and T4 cells. **g**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **h**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **i**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **j**, DROSHA mRNA remaining over time in MDA-MB-231 cells treated with DZNeP. **k**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **l**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **m**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **n**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **o**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **p**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **q**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **r**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **s**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **t**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **u**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **v**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **w**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **x**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **y**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP. **z**, DROSHA mRNA levels in MDA-MB-231 cells treated with DZNeP.

P1-3 P3-M
298P ME L14
DROSHA (v m F. 6). C
ME L14 NA (v m
ME L14 DROSHA NA A KA- fi
(F. 4). P1-3 DROSHA (F. 4)
D O HA (F. 4) A KA

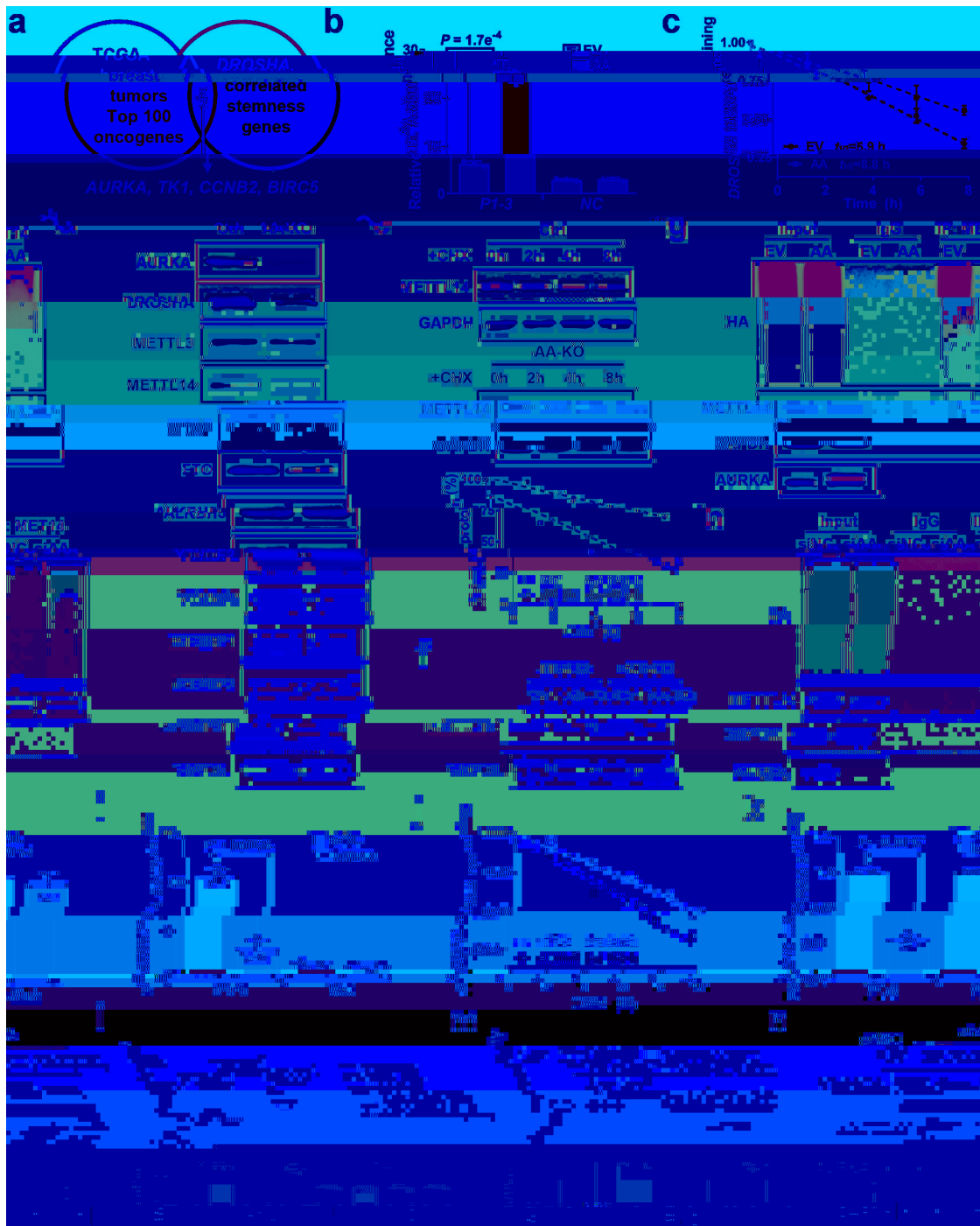


Fig. 4 AURKA-stabilized METTL4 maintains DROSHA transcript via m⁶A deposition. **a** Heatmap showing gene expression levels across various cell lines and conditions. The top row highlights TCGA tumors (red) and DROSHA-correlated stemness genes (green). Key genes include AURKA, TK1, CGNB2, and BIRC5. The heatmap shows expression levels for AURKA, DROSHA, METTL4, METTL14, GAPDH, and HA across different cell lines and treatments (+CIX, 0h, 2h, 4h, 8h). **b** Bar graph showing relative mRNA levels for P1-3 and NC conditions. The y-axis is labeled 'Relative mRNA level' and the x-axis shows 'P1-3' and 'NC'. A p-value of $P = 1.7e^{-4}$ is indicated. The graph shows a significant increase in relative mRNA levels for P1-3 compared to NC. **c** Line graph showing DROSHA mRNA levels over time (h) for EV and AA-KO conditions. The y-axis is labeled 'DROSHA mRNA level' and the x-axis is 'Time (h)'. The EV condition shows a higher level of DROSHA mRNA compared to the AA-KO condition. The half-life ($t_{1/2}$) is indicated as 6.9 h for EV and 8.8 h for AA-KO.

DROSHA⁶A
BC C

DISCUSSION

D O HA, ⁶H BC C
D O HA BC C
D O HA STC1
β-C BC C O A KA
DROSHA NA (1)
ME L14
(2) IGF2BP2 A-fm DROSHA
D O HA BC C
DROSHA A fi C
D O HA C1
M DROSHA A
D O HA ME L14
A KA
DROSHA
D O HA
BC C
NA D O HA
G EA
D O HA CD44⁺/
CD24⁻ BC C
NA D O HA
BC C P
D O HA
CBP80 NA P II.⁸ D O HA
β-C STC1
CED (390-875) D O HA
β-C STC1
D O HA C1
C1 D O HA BC C
C1 D O HA BC C
DROSHA NA -M E2F1
NA
NA DROSHA
A NA
NA
HDF2 A-fm

NA²⁸ C IGF2BP
NA
DROSHA¹⁶ NA
D O HA
DROSHA NA
DROSHA NA
NA
A D N P NA
D N P DROSHA A
BC C O D N P
fi E H2
P C2 H3K27³
BC C D N P
4K27³

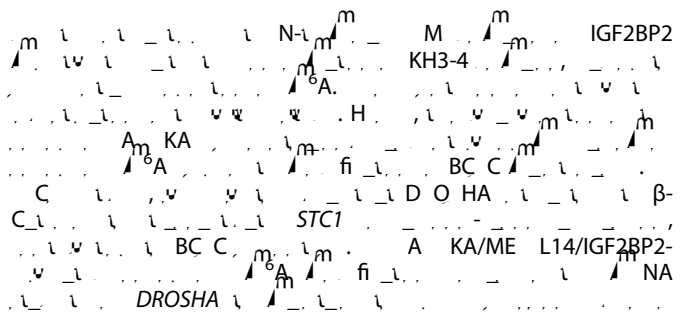
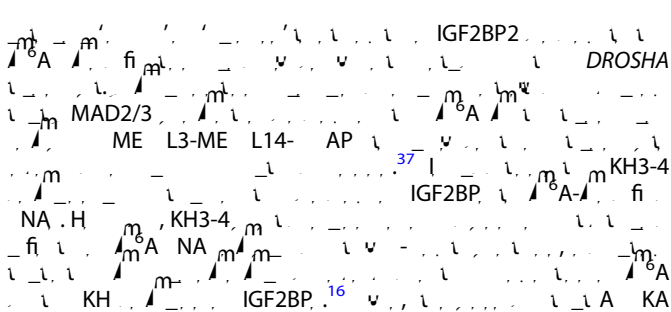
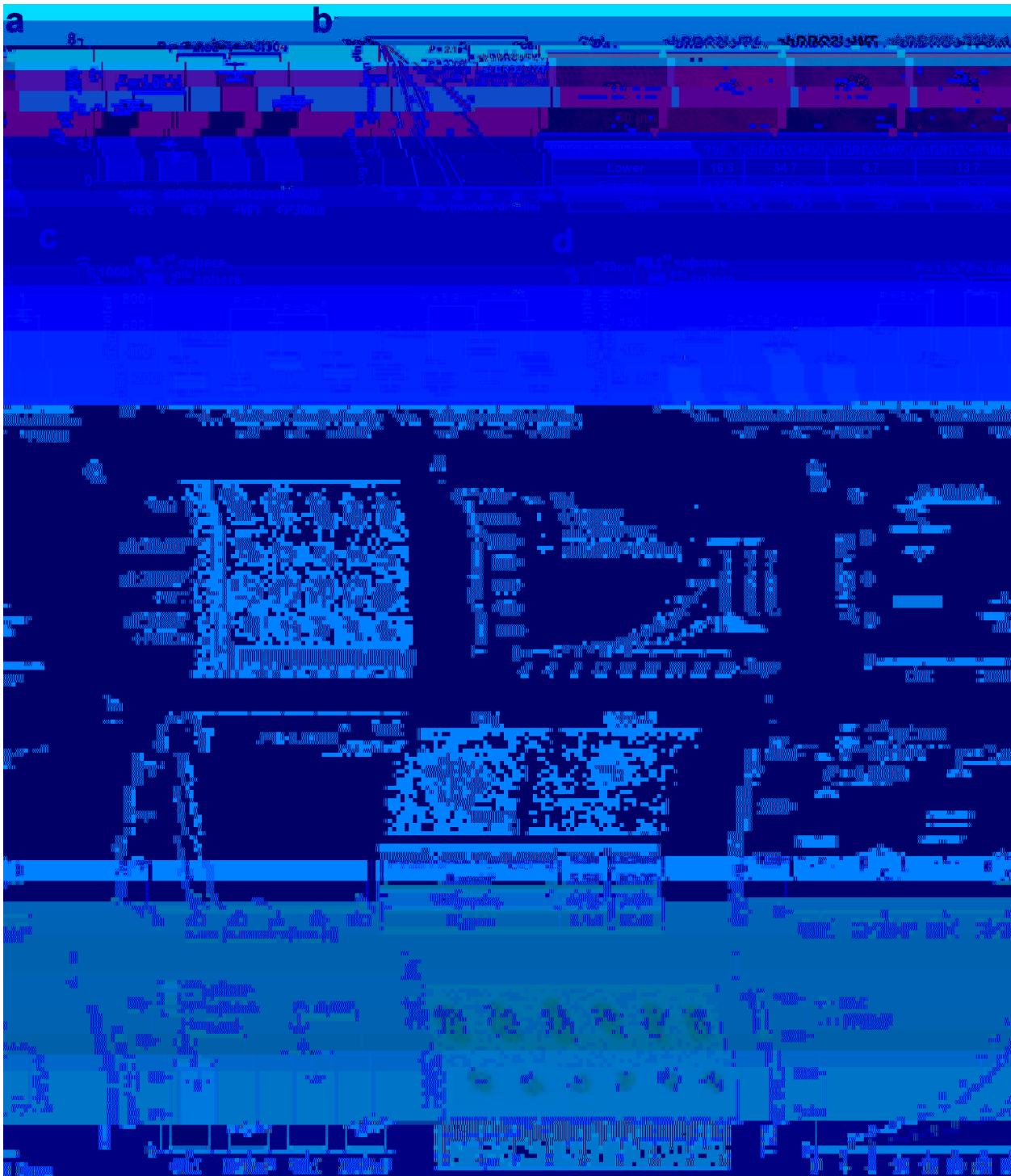


Fig. 6 Suppression of DROSHA m⁶A modification attenuates BCSC traits. **a** ALDH⁺ DROSHA WT P3M DROSHA MDA-MB-231 DRO3 RNA DROSHA 3'UTR. **b** ELDA MDA-MB-231 DROSHA WT P3M DROSHA. T 95% 529611.112889999961(000)439999980) 441(S999T614 500.938351T .95-2.21TDTD() F111T 2.302T WT-27) -444.10000663520

BC C. ... m⁶A m⁶ fi ... DROSHA ... (v ... F. 8).

MATERIALS AND METHODS

C ... MDA-MB-231 (H B-26), K-B ... MCF-7 (H B-22), ... (A CC) ... L-15 M ... (G ... 11415056), M C ... (G ... 16600082), PMI-1640 M ... (G ... 11876093) ... 0.0231 ... (... 91077C) ... E ... M ... E ... M ... (G ... 11095080) ... 0.01 ... (...) ... 10% ... (FB, G ... 10100147), 0.1% ... (... 15140122) ... HEK293 (C L-11268) ... A CC ... 10569044) ... 10% FB, 0.1% ... 37 C ... 5% CO₂ ... MDA-MB-231 ... 3 ... CELL A ... ING (N C & M ... B ... C40100). A ... PC ... B ... F ... C ... Et ... B ... A fi ... H ... D ... M ... (N ... 2019-028). ... 5.

NA (G ... P ... C ...) ... 3000 (L ... 3000, L3000015) ... HEK293 ... PA 2 (A ... #12260) ... MD2.G (A ... #12259). HEK293 ... 6m ... 80% ... (L ... C1, L -ME L14), ... PA 2 ... MD2.G ... HEK293 ... L ... 3000. ... 24 ... 48 ... 72

... NA (...) ... D O HA ... NA ... 1. ... 2μ ... (... P8833) ... 72 ... MDA-MB-231 (... A KA) ... A KA ... C I P -C_9 ... (... C I P ... 2, A ... #52961) ... MDA-MB-231 ... NA ... A KA ... 5'- GAG CACGAGAACACG -3'. ... DNA6 ... (1-875 ... 876-1374, D875) D O HA ... DNA6 ... H ... D O HA (P1-3) ... CHECK2 ... ME L14 ... C1 ... L -D ... M ... -N ... 500, 1000, 1500 ... (D500, D1000, D1500), ... GL3-B ... STC1 ... DROSHA ... L -O - GFP ... P ... CHECK2-D O HA (P1-M ... P2-M ... P3-M ...) ... L -ME L14 ... 298P ... DNA6-D O HA (E1045 ... III ...) ... DNA6-D O HA (P3M ...) ... GL3- C1 (M ... 1, M ... 2 ... M ... 1 ... M ... 2 ...) ... CF- ... PC ... A ... KA ... GE -6P-1 ... H ... A ... 1. ... DNA6 -F -A KA ... DNA6 -M C ... HA ... 39 ... (B ... L ... B ... C ...).

NA ... - PC ... NA ... AG NA P ... (AG21101, ACC A E BIO ECHNOLOG , H NAN) ... NA ... (... K ... 9109) ... DNA ... E ... O ... DNA ... DNA ... M (... G ... B ... AE311-02). E ... M-ML ... DNA C ... PC (AG11705, ACC A E BIO ECHNOLOG , H NAN). ... PC ... C ... B ... PC M ... M (... B ... M ... C ... L ... 711-02) ... PC ... (A ... M ... 3005P). ACTB ... GAPDH ... NA ... PC ... 1.

μ g A...
 NA... (K...)
 NA... D... NA...
 (A... 61006).
 NA... 10 μ L... NA...
 (50%... 2.5%... 0.5 MOP) 55 C...
 15... NA...
 A... H... -N⁺... (GEH... , PN303B)
 B... D... A... (B...). A...
 M... 0.02%... (MB) 0.3
 M... 1 PB
 5%... PB...
 A... (#202003) 4 C.
 A... G... (H+L)
 A... F... (31460),
 B... ECL (A... K-12045-D50)
 C... D... (B...).
 M... D... N... P...
 F... M...
 P... B... (0.137 M N₂C, 2.7 M KC, 1.1 M
 KH₂PO₄, 0.5 M M₂C₂, 8.1 M N₂HPO₄, 0.9 M C₂C₂ H 7.4),
 21013024) A... F... D... N... P...
 10 μ M D... N... P... (7120) A...
 D... N... P... (8...)

NA...
 B...
 D... N... P... 24...
 5 μ L... D (A... D, CA1201)
 NA...
 NA... R... (K...)... PC...

C... IPA... (50 M... H 7.5, 120 M
 N₂C, 1%... -100, 0.5%... D... 0.1%... D, 5
 M ED A) (M... C... E... H-
 K001) 30... A...
 6... Ev...
 D... PAGE...
 (M... HA F00010)
 B... ECL... B...
 H... : D O HA (3364), C-
 M C (9402), P... -A... A (288) (3079), H... m H3
 (9715), F O (14386)... G (2625)
 (C)... MA... A. OC 4 (G 101497)
 NANOG (G 100863) G... C...
 A. GAPDH (60004-1- J), B... A... (66009-1- I), G B10 (23591-
 1-AP), LCO4A1 (26399-1-AP), O B 2 (24643-1-AP), ME L3
 (15073-1-AP), ME L14 (26158-1-AP), AP (60188-1-IG), HDF2
 (24744-1-AP), HDF3 (25537-1-AP), IGF2BP1 (14642-1-AP),
 IGF2BP2 (11601-1-AP), IGF2BP3 (22803-1-AP) HA... (66006-
 2- I) P... G... C... β -C... (06-
 734), A... A (07-648) ALKBH5 (ABE547) M...
 MA... A... C1 (229477) A... C... M...
 K. PA P (-8007) G... B... CA... A...
 G... (H+L) A... (31460) G...
 (H+L) A... (31430) v...
 F... MA... A...
 IP...
 IP...
 40... C...
 F... C... F...
 (19... 10) A... M...

D... MAC 2, A...
 C... C...
 IP... G E60213, G E29714, G E90642... G E54365
 NA... IP- PC
 NA... (K...)
 NA... D...
 NA... (A... 61006). F... IP...
 41... NA... (5 μ)
 DN... (M0303, NEB)
 200-... 95 C... 25...
 NA F... (A... AM8740), A...
 (10 μ ... 5 μ NA; A...
 40 μ L P... A... (P9424) IPP
 (150 M N₂C, 0.1% NP-40, 10 M... HC, H 7.4) 2
 NA (5 μ)
 4 C. B... 0.5 M L... NA... (BE & A OCI-
 A E, P3732) m IPP m... NA...
 E... C... (H < 5.0,
 P1025) DNA... A... O...
 M... M (ABM, G490). A...
 PC... PC...

D... GL3- C1 (,
 D500, D1000, D1500, M... M... M... M...
 (F...) (v...) (L...
 K)
 HEK293... A... 24, F... v... m...
 D... A... (P... E1910)
 NA... CHECK2-D O HA (, P1-
 M... P2-M... P3-M...
 NA... D... N... P...
 D... A...
 24...
 F...
 F...
 C... M... G... (NE...
 801009). A... 3.6%...
 P1110) 10%... (K... 6762007) 15...
 55 C... 55 C... 2...
 DIG-
 P (, 11277073910) v...
 2... C (20... C, 3.0 M N₂C, 0.3 M... H 7.0).
 A... F... M 555... I G (1:200, m)
 F... (1:100, A...) IGF2BP2 (1:100, P...
 G...) A... F... M 488... I G
 (1:200, m... F...) N...
 DAPI... (L... C... P5II).
 DROSHA, m... D O HA PC...
 MEGA... 7... K...
 (F... AM1333) v... NA... R...
 (K...) P... D O HA PC...

IP... IP... M... IP Ki...
E... IP... M... IP Ki...
B fl... IP... 30...
... 4 C...
... A... G... 5...
... I G... (M...)... A KA... ()...
A1231), IGF2BP2... (P... , 11601-1-AP). B...
(30... 55... NA...
E... C... (H... 5.0...)
... PC...
... 1.

C...
C... (20... -HC, H 7.4, 10... M N_C, 3...
M M C₂)... 15... N... 0.9% NP-40 (fi...
... 10... 3000... 4 C...
... E... 3...
... (100... -HC, H 7.4, 2... M N_C, 1%...
... -100, 1... M EG A, 0.1% D, 1... M N_F, 20... M N_P₂O₇).
A... 10... 30... 4 C...
... 14,000 g... 30... 4 C...

P... -IP... LC/M /M...
C... 15... 70%-80%... -IP...
... (50... M... H 7.4, 150... M N_C, 1% NP-40, 0.5%...
... 0.1% D, 1... M...
... N... A... F... 5...
50... L N... A...
... Ev...
... A KA... (A1231), IGF2BP2...
(P... , 11601-1-AP), β-C... (M... , 06-734),
D O HA... (C , 3364), F... (P... ,
20543-1-AP), H... (C , 12698), ME L14...
(A... , 252562)... I G... (C , 7074). A...
... P... A/G P...
A... (C... B... C-2003)
... F...
... A KA, D O HA... I G...
... 10% D...
... 20...
... 42... L... O... E... ()...
D... M... 3000 LC... ()...
E... M... ()... 16,011...
... fi... A...
... 2... 4.

A KA... BL21 *E. coli*...
... GE 6P1-G -A KA-H... B... G -A KA...
G... 1... L... PB...
... 200... M KC. G...

... fi... (8611, P... C...)...
50... (H... 8.0) ... 10... M... C...
... IGF2BP2 (1-220)...
N... fi... (9 6M1).
... IGF2BP2 (1-220)...
(5μ) ... 30μL...
... -A KA (5μ). G (1.8μm)...
... 300μL... (20... M HEPE,
H 7.5, 200... M KC, 5... M M C₂, 0.5... M EG A, 1... M...
... 0.05%... -100) ... 3... 4 C...
... 3...
... /10... M... (H 8.0)...

NA...
B... D O HA... PC...
... 7... MEGA... 7... (A... ,
AM1333) ... -16- P (A... , AM8452) ...
NA... P... (K...).
... (1...)
... 5μ... fi...
... 25 C... C... A...
(... A10004).
... P... PC...
... 1.

NA...
... MDA-MB-231 (... A KA)
... 72...
N... (B... , C...). NA...
... N... (... , 74104). L...
... NEBN... M NA L... P... Ki...
... (NEB)...
... A... H...
... 125... NA...
... 19... H... 2.0.5
... DEG
DE 2.43 G... P... <0.05... FC> 1.5
... D...
... GEO... G E128428.

M...
... NA... MDA-MB-231. D O HA...
... NC... NA... P...
G... C... L... (... , C...)
... (-)394 u...

(P_m E^m).
 (v^m m⁰), (v^m m¹),
 (v^m m²), (v^m m³)
 D
 0-3
 N
 H
 (0 300)
 (%
 1 1) (%
 2 2) (%
 3 3).

G EA
 (G EA. 2.1.0)
 D O HA
 P < 0.05 FC > 1.25
 G⁴⁵

M (MD)
 β-C₁ (PDB ID: 3 7)
 C B P D B 3D
 D O HA I A E⁴⁶
 β-C₁ D O HA
 G 5.1.5 A 99 A MD
 B (MM/PB A)
 β-C₁ D O HA.¹⁸
 LIGPLO (.4.4.2).⁴⁷

A KA (PDB ID: 5G1)
 IGF2BP3 M C B P D B 3D
 M IGF2BP2 (PDB ID: 6F 1)
 A KA IGF2BP2
 G 5.1.5 A 99 A MD
 B (MM/PB A)
 A KA IGF2BP2.¹⁸
 LIGPLO (.4.4.2).⁴⁷

ALDH⁺
 ALDEFLO
 (EMCELL 01700)
 B fl ALDEFLO
 ALDH⁺ BODIP (BAAA)
 30 37 C ALDH⁺
 ALDH⁻
 ALDH⁺ D
 ALDH⁺ fl
 (B C v C FLE).

F
 (C m 3471)
 A 7
 (O m DP73).
 6-
 1 10³

1 10³ / A
 F E (ELDA)
 96 m (C 3474)
 1, 5, 10, 20, 50, 100 / m (24
 A 7
 ELDA⁴⁹

DMEM/F12 (G C11330500B), 20
 (P 100-18B), 20 μL
 B27 (F 17504044) 20 μL
 (P 100-05).

A
 F BALB/ (4-6 B
 A C L)
 MDA-MB-231 (10⁵ / 0.1 μL/
 = 0.5 m²
 M fi m m l

F
 C0130
 H1136)
 (10⁵, 10⁴, 10³, 10²)
 (4-6)
 A
 EL L D v A
 A⁴⁹
 C M I A C
 AAE18017) (IAC C) D M (N

C IP
 C IP A C IP-I E C m
 K (A M) l fi 1 10⁷
 10
 1 PB 5 PB
 fi G fi
 5
 PM F (fi 0.6 M)
 2500 10 4 C 1 L
 B 30

DNA 10 μL
 DNA -20 C
 2 μ D O HA (3364, C)
 I G (C 7074) A/G
 C IP B I C IP B II E
 50 μL B B AM2. A 50 μL
 C B
 2 μL P K 37 C 1 DNA
 PC STC1
 1.

JA PA
 (F) C1 " " +402
 -1810 ()⁵⁰
 100
 G E P fi A (GEPIA)
 D O HA
 (ME AB IC) m B P⁵²
 A " MB 2.0" ⁵³ H
 (H.)

K₁-M (KM), χ^2 (O), $P < 0.05$

(ANOVA), χ^2 (O), $P < 0.05$

DATA AVAILABILITY

NA: GEO E125705, CGA, IP: G E128428 (NA), G E125705, CGA, IP: G E60213, G E29714, G E90642, G E54365, IP: G E7513, G E15192

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AUTHOR CONTRIBUTIONS

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ADDITIONAL INFORMATION

Supplementary information <https://doi.org/10.1038/41422-020-00397-2>

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34. D., ... MO ... 6A- NA ... ME L3 ...
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