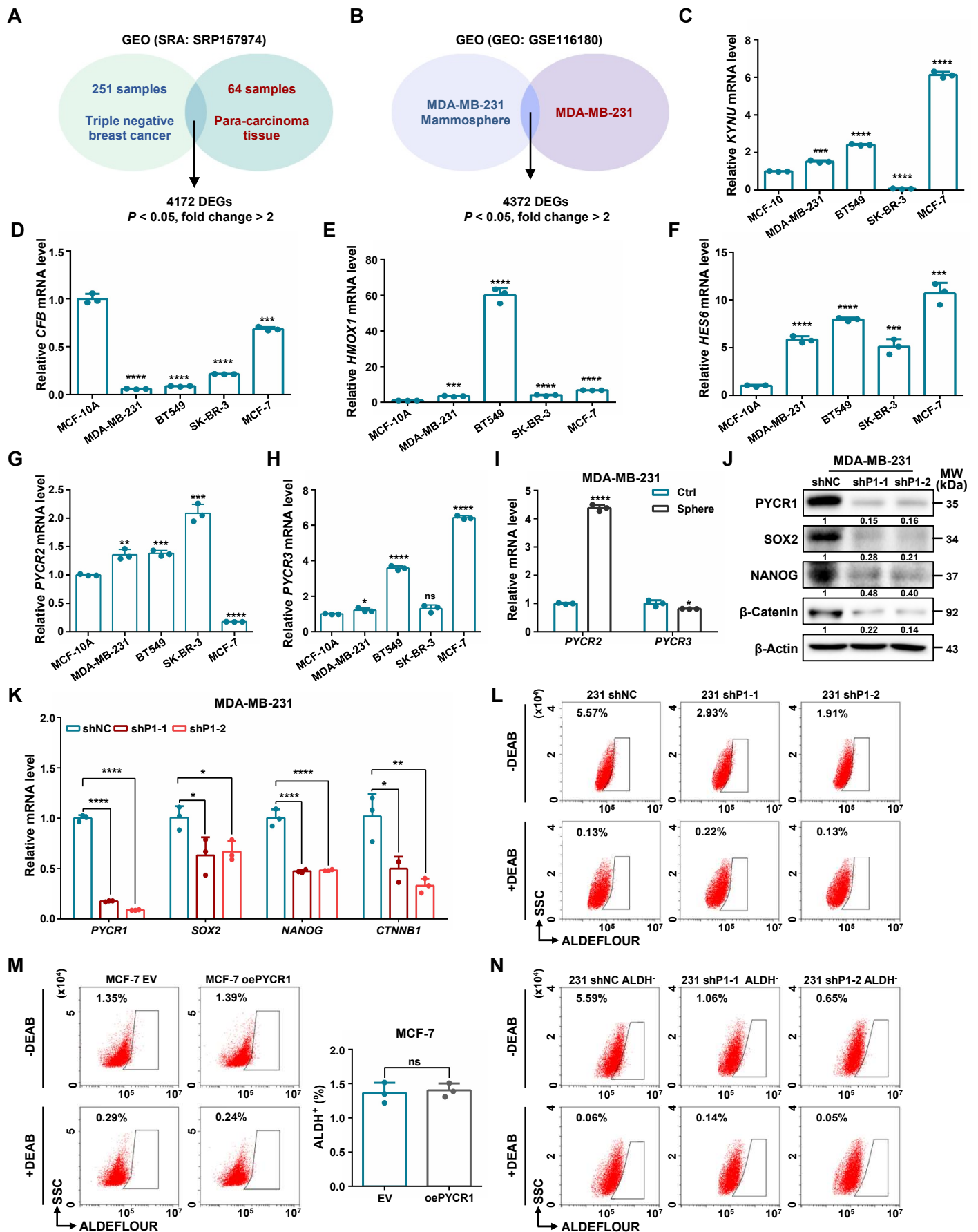
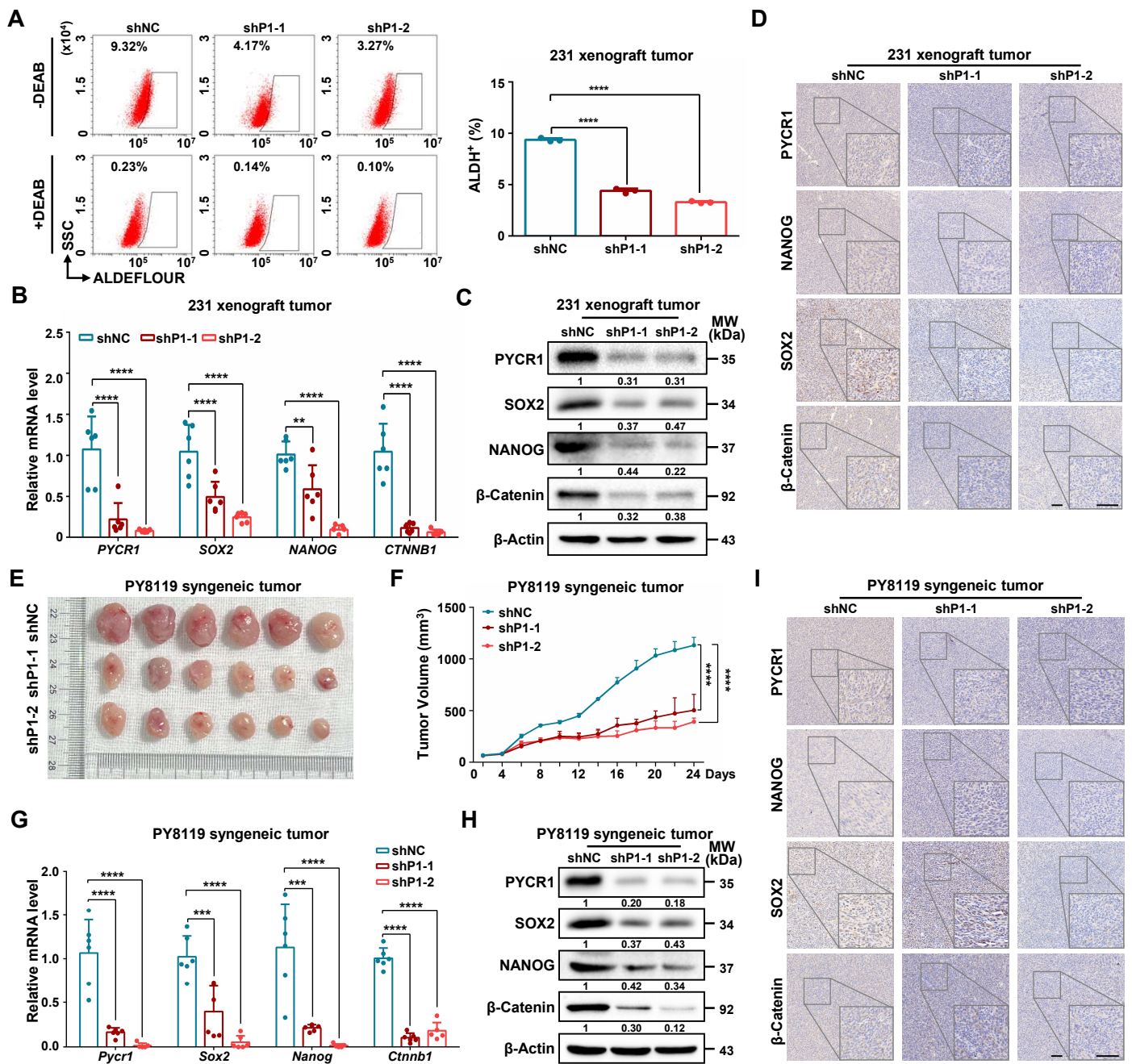




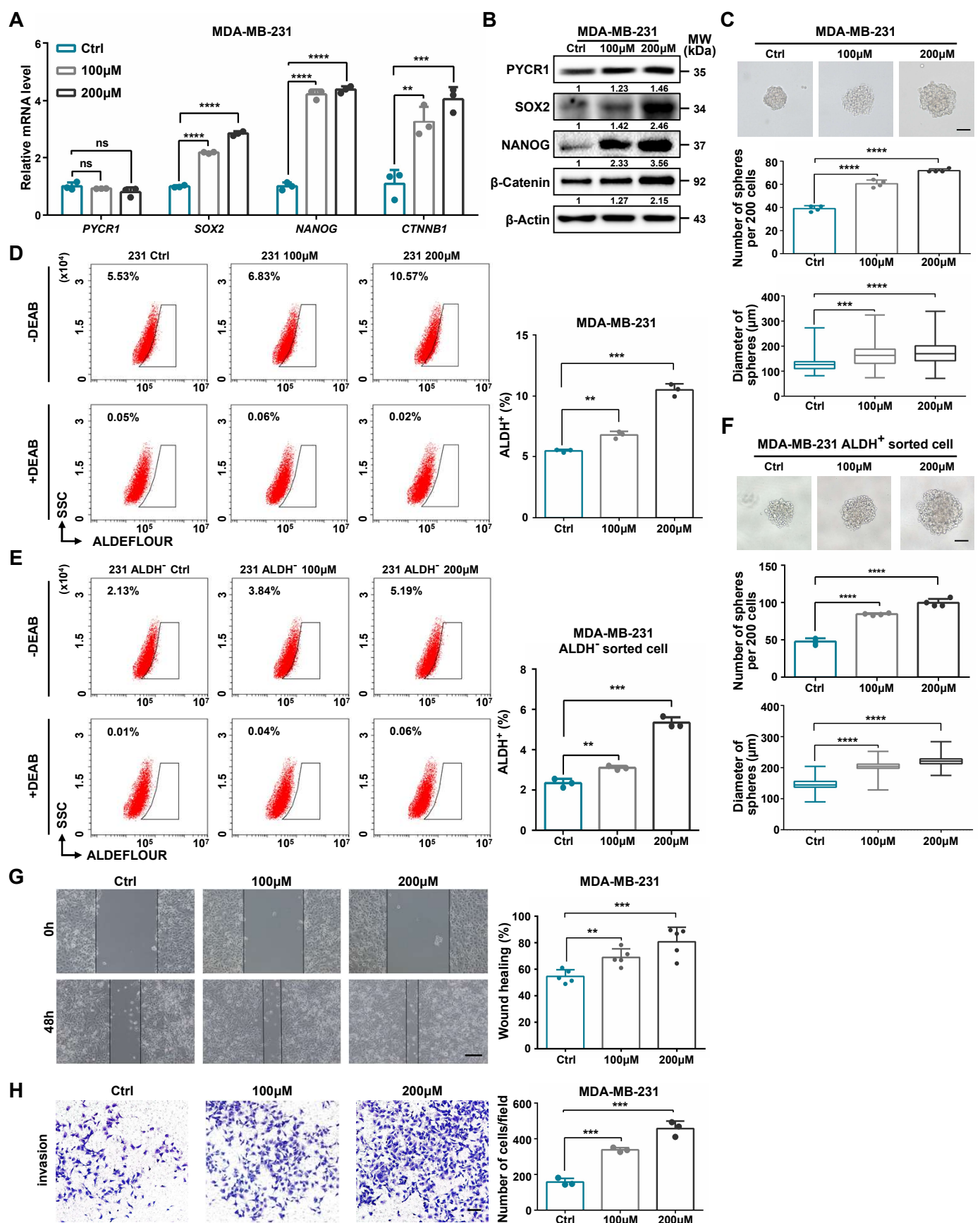
Supplementary Fig. 1

(A) Overlapping expressed genes from TNBC and normal breast tissues to obtain DEGs (SRP157974) ($FC > 2$, $P < 0.05$). (B) Overlapping expressed genes from spheroids and adherent MDA-MB-231 cells to obtain DEGs (GSE116180) ($FC > 2$, $P < 0.05$). (C-H) Relative mRNA levels of typical xenobiotic metabolism gene *KYNU* (C), *CFB* (D), *HMOX1* (E), *HES6* (F), *PYCR2* (G) and *PYCR3* (H) in breast cancer cell lines ($n = 3$). (I) Relative mRNA levels of *PYCR2* and *PYCR3* in sphere MDA-MB-231 cells ($n = 3$). (J) Relative protein levels of *PYCR1*, *SOX2*, *NANOG* and β -Catenin were determined in *PYCR1* knockdown MDA-MB-231 cells. (K) Relative mRNA levels of *PYCR1*, *SOX2*, *NANOG* and *CTNNB1* were determined in *PYCR1* knockdown MDA-MB-231 cells ($n = 3$). (L) Flow cytometry analysis for ALDH-positive cells in MDA-MB-231 cells following *PYCR1* depletion. (M) Flow cytometry analysis for ALDH-positive cells in MCF-7 cells (Left). Differences of ALDH-positive cells were analyzed (Right, $n = 3$). (N) The ALDH-positive cells in sorted ALDH⁻ MDA-MB-231 shNC, shP1-1, shP1-2 cells were detected by flow cytometry after 6 days monolayer culture. Graph data were presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. P values were calculated with two-tailed, unpaired Student's t -test.



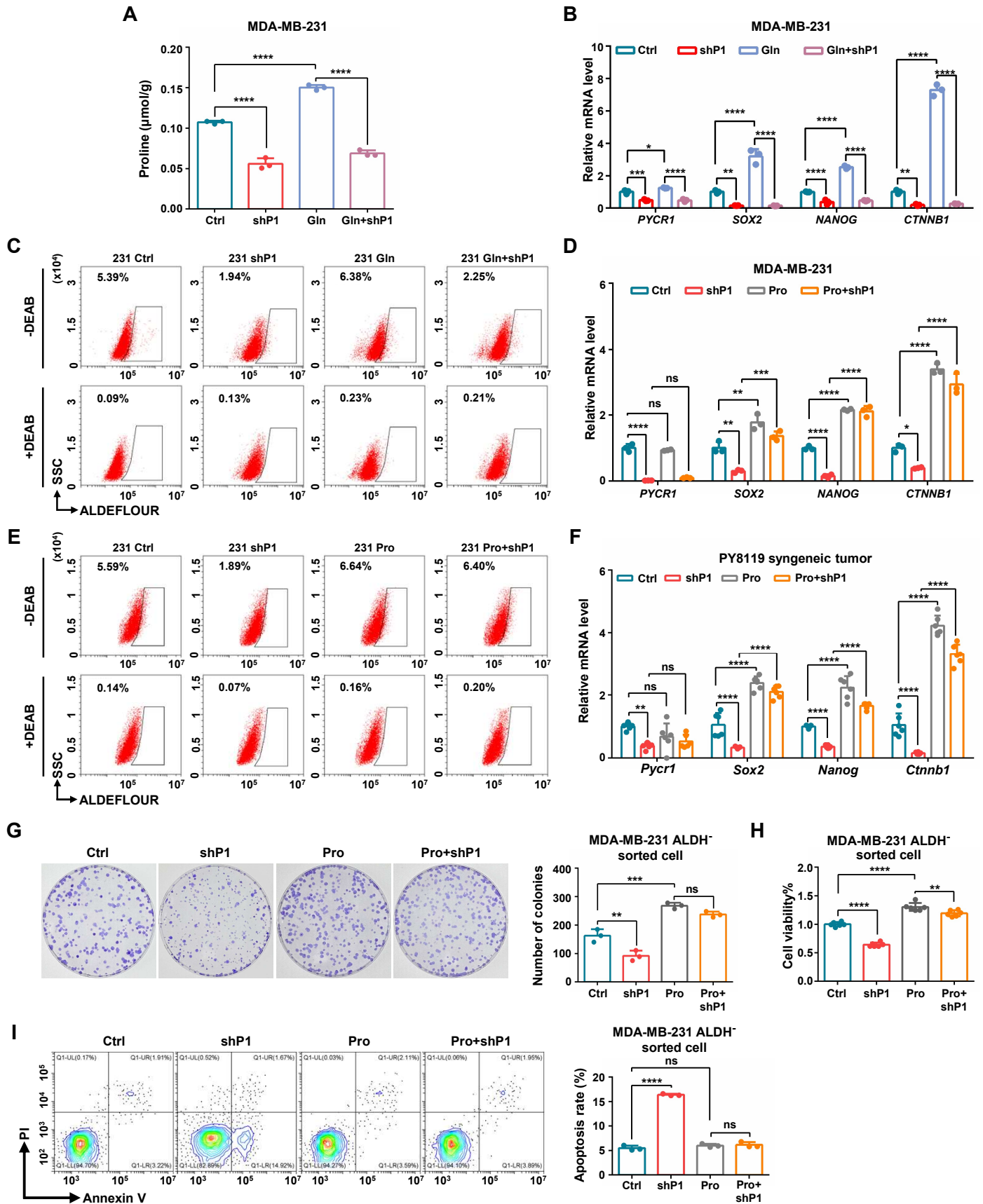
Supplementary Fig. 2

(A) ALDH-positive populations were analyzed in xenograft tumor (from Fig. 1L) (Left). Differences of ALDH-positive cells among groups were analyzed ($n = 3$, Right). (B) Relative mRNA levels of *PYCR1*, *SOX2*, *NANOG* and *CTNNB1* were determined between MDA-MB-231 shNC and shP1 (shP1-1, shP1-2) tumor ($n = 6$). (C) Relative protein levels of PYCR1, SOX2, NANOG and β -Catenin were determined between MDA-MB-231 shNC and shP1 (shP1-1, shP1-2) tumor. (D) Representative images of PYCR1, NANOG, SOX2 and β -Catenin IHC staining in MDA-MB-231 shNC and shPYCR1 (shP1-1, shP1-2) tumor. Scale bars, 50 μ m. (E-F) Immunocompetent mice ($n = 6$) were subcutaneously inoculated with shPycr-1 (shP1-1) and shPycr-2 (shP1-2) PY8119 cells (E) and tumor volumes were analyzed (F). (G) Relative mRNA levels of *Pycr1*, *Sox2*, *Nanog* and *Ctnnb1* were determined between PY8119 shNC and shPycr1 (shP1-1, shP1-2) tumor ($n = 6$). (H) Relative protein levels of PYCR1, SOX2, NANOG and β -Catenin were determined between PY8119 shNC and shP1 (shP1-1, shP1-2) tumor. (I) Representative images of PYCR1, NANOG, SOX2 and β -Catenin IHC staining in tumor. Scale bars, 50 μ m. Graph data were presented as mean $\pm$ SD. $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. P values were calculated with two-tailed, unpaired Student's t -test.



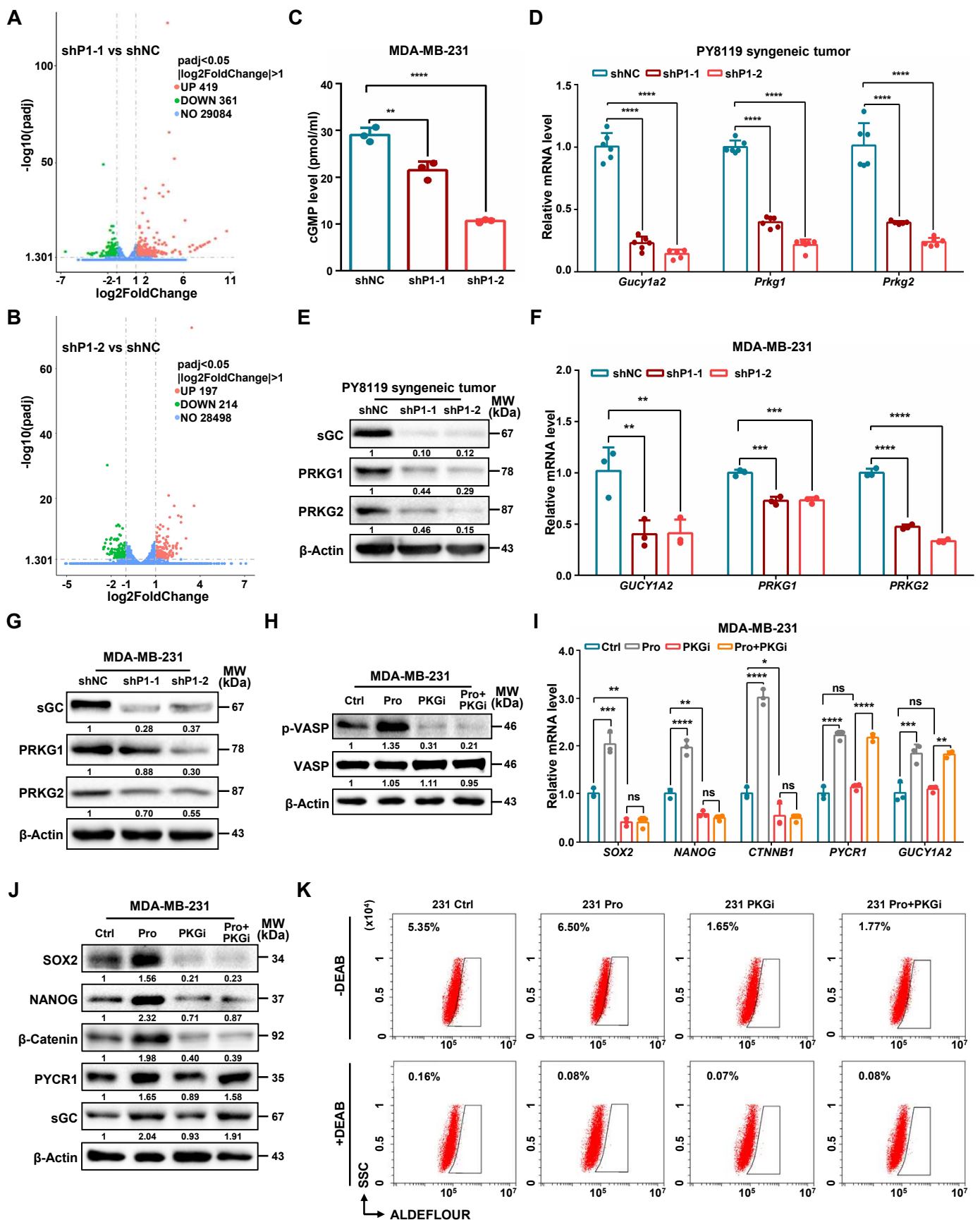
Supplementary Fig. 3

(A) Relative mRNA levels of *PYCR1*, *SOX2*, *NANOG* and *CTNNB1* were determined in MDA-MB-231 cells treated with proline (100 μM or 200 μM) (n = 3). (B) Relative protein levels of *PYCR1*, *SOX2*, *NANOG* and β-Catenin were determined in MDA-MB-231 cells treated with proline (100 μM or 200 μM). (C) Sphere formation was performed in MDA-MB-231 cells treated with proline (100 μM or 200 μM). The representative images were presented (Upper, scale bar = 100 μm) and the number (Middle) and diameter (Bottom) of spheroids were measured and counted (n = 4). (D) ALDH-positive populations were analyzed following supplemental addition of proline to the MDA-MB-231 cells (Left). Differences of ALDH-positive cells among groups were analyzed (n = 3) (Right). (E) Flow cytometry analysis for ALDH-positive cells in sorted ALDH⁺ MDA-MB-231 cells treated proline (100 μM or 200 μM) after 3 days monolayer culture. Differences of ALDH-positive cells among groups were analyzed (n = 3). (F) Sphere formation assay was performed in sorted ALDH⁺ MDA-MB-231 cells treated proline (100 μM or 200 μM). The representative images were presented (Upper, scale bar = 100 μm) and the number (Middle) and diameter (Bottom) of spheroids were measured and counted (n = 4). (G) Representative images of MDA-MB-231 cells treated proline (100 μM or 200 μM) in wound healing assay (Left). Statistical significance was analyzed (n = 5) (Right, scale bar = 100 μm). (H) Representative images of MDA-MB-231 cells treated proline (100 μM or 200 μM) in transwell invasion assays (Left). Statistical significance was analyzed (n = 3) (Right, scale bar = 100 μm). Graph data were presented as mean ± SD. ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. *P* values were calculated with two-tailed, unpaired Student's t-test.



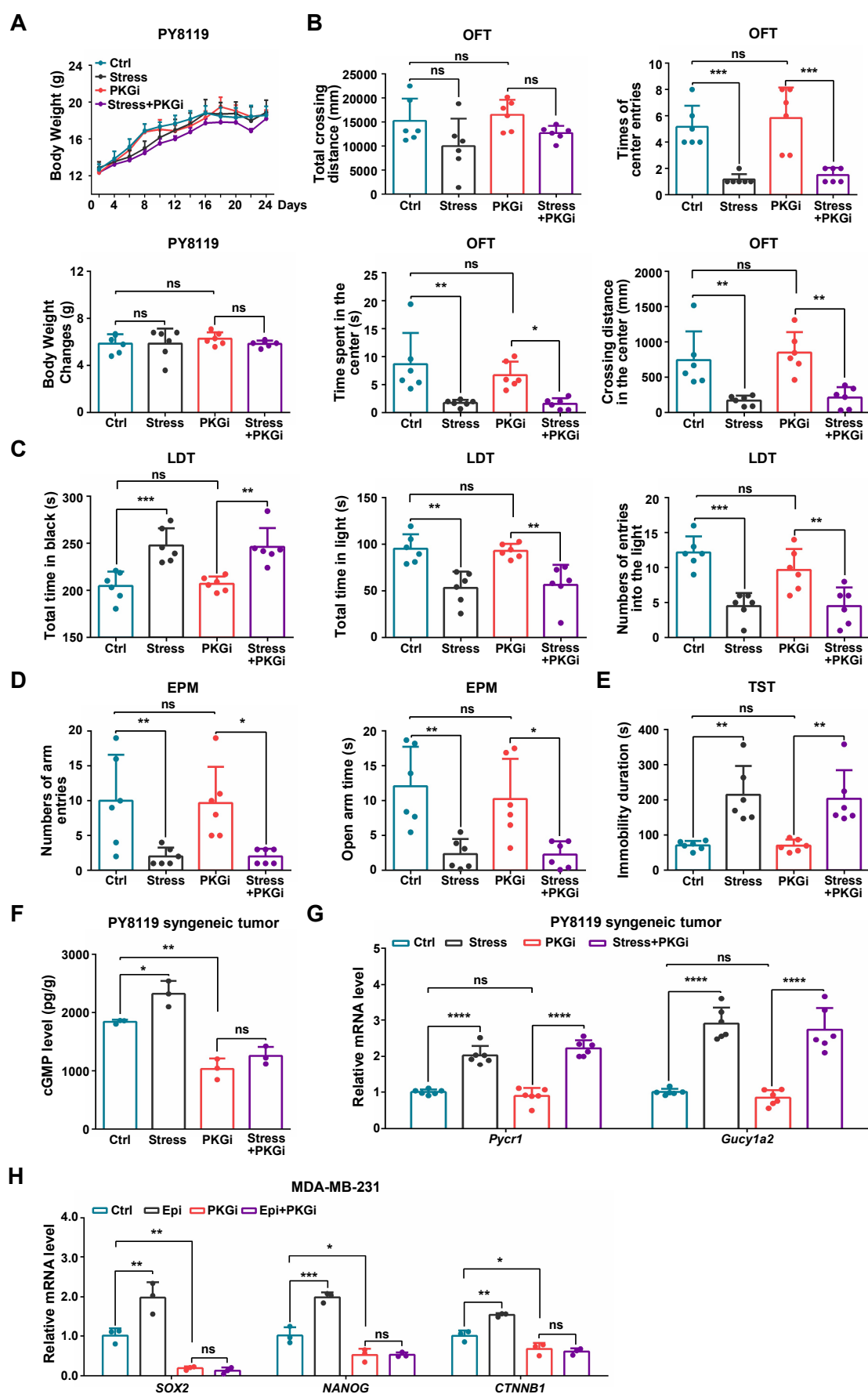
Supplementary Fig. 4

(A) The proline levels were measured in PYCR1-deficient MDA-MB-231 cells treated glutamine (n = 3). (B) Relative mRNA levels of *PYCR1*, *SOX2*, *NANOG* and *CTNNB1* were determined in PYCR1-deficient MDA-MB-231 cells treated with glutamine (n = 3). (C) Flow cytometry analysis for ALDH-positive cells in PYCR1-deficient MDA-MB-231 cells treated glutamine. (D) Relative mRNA levels of *PYCR1*, *SOX2*, *NANOG* and *CTNNB1* were determined in MDA-MB-231 cells treated with proline (n = 3). (E) Flow cytometry analysis for ALDH-positive cells in PYCR1-deficient MDA-MB-231 cells treated proline. (F) Relative mRNA levels of *Pycri1*, *Sox2*, *Nanog* and *Ctnnb1* were determined in PY8119 xenograft tumor (n = 6). (G) Representative images of colonies formed by sorted ALDH⁺ MDA-MB-231 cells (Ctrl and shP1) following treated proline (Left). The numbers of colonies were analyzed (Right) (n = 3). (H) CCK-8 assay for sorted ALDH⁺ MDA-MB-231 cells (Ctrl and shP1) following treated proline for 12 h. The cell viability was analyzed (n = 6). (I) Flow cytometry with Annexin V/PI double staining was used to detect the percentage of apoptosis in sorted ALDH⁺ MDA-MB-231 cells (Ctrl and shP1) following treated proline (Left). Differences of apoptosis rate among groups were analyzed (n = 3) (Right). Graph data were presented as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. *P* values were calculated with one-way ANOVA.



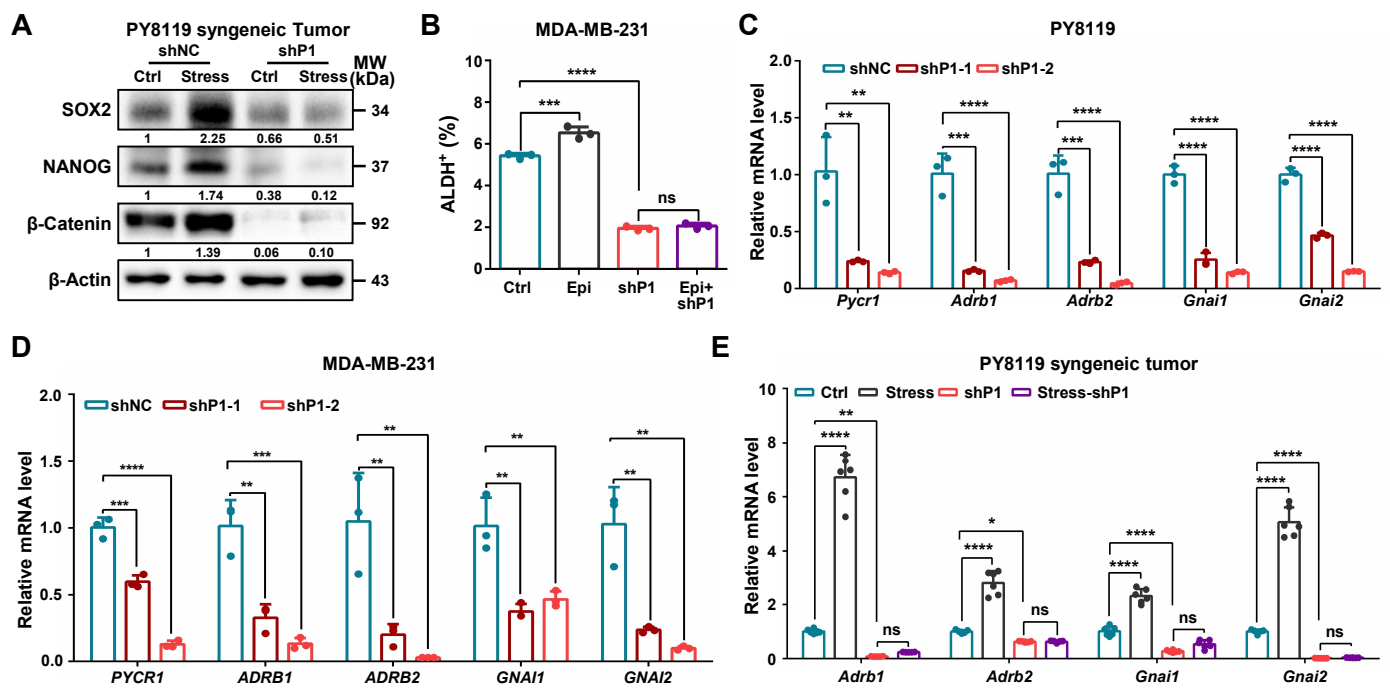
Supplementary Fig. 5

(A-B) Volcano plots displaying downregulated genes in MDA-MB-231 shP1-1 cells (A) and shP1-2 cells (B). The number of significantly variant genes ($FC > 2$, $padj < 0.05$) was shown. Vertical dashed lines indicate cut-off of FC (2), whereas the horizontal dashed lines indicate cut-off of P value (0.05). (C) The cGMP levels were measured following PYCR1 knockdown in MDA-MB-231 cells ($n = 3$). (D) Relative mRNA levels of *Gucy1a2*, *Prkg1* and *Prkg2* were determined following PYCR1 knockdown in PY8119 tumor ($n = 6$). (E) The protein levels of sGC, PRKG1 and PRKG2 were determined following PYCR1 knockdown in PY8119 tumor. (F) Relative mRNA levels of *GUCY1A2*, *PRKG1* and *PRKG2* were determined following PYCR1 knockdown in MDA-MB-231 cells ($n = 3$). (G) The protein levels of sGC, PRKG1 and PRKG2 were determined following PYCR1 knockdown in MDA-MB-231 cells. (H) The phosphorylation level of VASP (Ser239) was determined following treatment proline and PKGi in MDA-MB-231 cells. (I) Relative mRNA levels of *SOX2*, *NANOG*, *CTNNB1*, *PYCR1* and *GUCY1A2* were determined following treatment proline and PKGi in MDA-MB-231 cells ($n = 3$). (J) Relative protein levels of *SOX2*, *NANOG*, β-Catenin, *PYCR1* and sGC were determined following treatment proline and PKGi in MDA-MB-231 cells. (K) Flow cytometry analysis for ALDH-positive cells in MDA-MB-231 cells treated proline and PKG inhibitor. PKGi, PKG inhibitor. Graph data were presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. P values were calculated with two-tailed, unpaired Student's t-test (C, D, F) and one-way ANOVA (I).



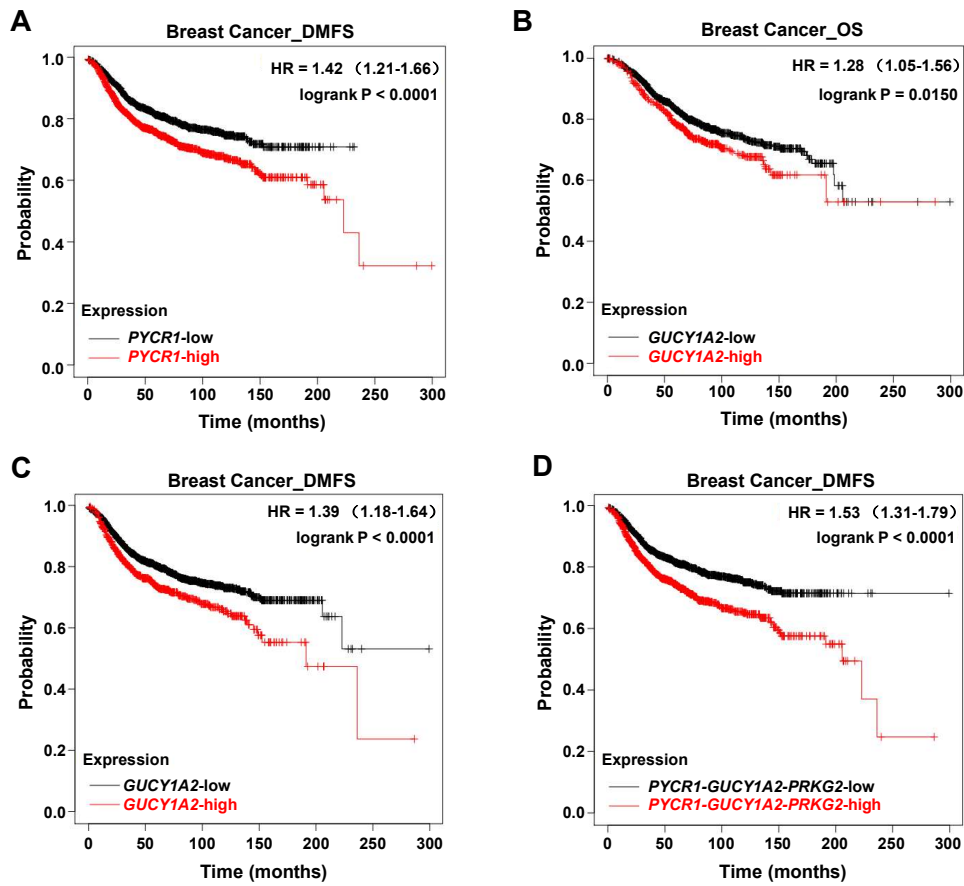
Supplementary Fig. 6

(A) Body weight changes of Ctrl, Stress, PKGi and Stress+PKGi PY8119 tumor in mice ($n = 6$). (B) Total crossing distance, times of center entries, time spent in the center and crossing distance in the center were analyzed in OFT ($n = 6$). (C) Total times in black, total time in light and numbers of entries into the light were analyzed in LDT ($n = 6$). (D) Numbers of arm entries and open arm times were analyzed in EPM ($n = 6$). (E) Immobility duration was analyzed in TST ($n = 6$). (F) The cGMP levels of PY8119 tumor ($n = 3$). (G) Relative mRNA levels of *Pycr1* and *Gucy1a2* were determined in PY8119 tumor ($n = 3$). (H) Relative mRNA levels of *SOX2*, *NANOG* and *CTNNB1* were determined following Epi and PKGi treatment in MDA-MB-231 cells ($n = 3$). OFT, open-field tests. LDT, light–dark box test. EPM, elevated plus maze test. TST, tail suspension test. Graph data were presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. P values were calculated with one-way ANOVA.



Supplementary Fig. 7

(A) Relative protein levels of stemness factors in PY8119 tumor. (B) Differences of ALDH-positive cells following PYCR1 knockdown and Epi treatment in MDA-MB-231 cells were analyzed ($n = 3$). (C-D) Relative mRNA levels of *PYCR1*, β -receptors (*ADRB1* and *ADRB2*) and G protein (*GNAI1* and *GNAI2*) related genes were determined following PYCR1 knockdown in PY8119 cells (C) and MDA-MB-231 cells (D) ($n = 3$). (E) Relative mRNA levels of β -receptors (*Adrb1* and *Adrb2*) and G protein (*Gnai1* and *Gnai2*) related genes were determined in PY8119 tumor ($n = 6$). Graph data were presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. P values were calculated with two-tailed, unpaired Student's t -test (C, D) or one-way ANOVA (B, E).



Supplementary Fig. 8

(A) Kaplan-Meier distant metastasis-free survival (DMFS) plots of breast cancer patients created using Kaplan-Meier Plotter network. Patients were classified into *PYCR1* high and *PYCR1* low and analyzed as indicated. (B-C) Kaplan-Meier overall survival (OS) (B) and distant metastasis-free survival (C) plots of breast cancer patients created using Kaplan-Meier Plotter network. Patients were classified into *GUCY1A2* high and *GUCY1A2* low subgroups, and analyzed as indicated. (D) Kaplan-Meier distant metastasis-free survival plots of breast cancer patients created using Kaplan-Meier Plotter network. Patients were classified into *PYCR1-GUCY1A2-PRKG2* high and *PYCR1-GUCY1A2-PRKG2* low subgroups and analyzed as indicated.

Primers of shRNAs	Sense (5'-3')	Antisense (5'-3')
shNC	TTCTCCGAACGTGTCACGT	ACGTGACACGTTCCGAGAA
Human-shPYCR1-1	CACAGTTTCTGCTCTCAGGAA	CACAGTTTCTGCTCTCAGGAA
Human-shPYCR1-2	GCCCACAAGATAATGGCTA	GCCCACAAGATAATGGCTA
Mus-shPycr1-1	GGCTTTCGCTCCTTGCTTATC	GGCTTTCGCTCCTTGCTTATC
Mus-shPycr1-2	GCCAAGATGCTACTAGACTCA	GCCAAGATGCTACTAGACTCA
Primers of plasmid construction	Sense (5'-3')	Antisense (5'-3')
pLVX-PYCR1	CGGAATTCGCCACCATGAGCGTGGGCT TCATCGGCGC	CGGGATCCCGATCCTTGCCCGCTGGGGCCA

Supplementary Table 1: Sequences of primers for plasmid construction

Primers of RT-qPCR	Sense (5'-3')	Antisense (5'-3')
Human-PYCR1	TGGCTGCCCACAAGATAATGG	TCCTCAATGTCGGCGCCTATT
Human-PYCR2	AAGCCAGACACATCGTGGTC	CGTACACTGTAGCGCCTTC
Human-PYCR3	CTCATCAGAGCAGGAAAAGTG	TTGGGCAAGACCCGC
Human-SOX2	TGGACAGTTACGCGCACAT	CGAGTAGGACATGCTGTAGGT
Human-NANOG	ACCTATGCCTGTGATTGTGG	AGTGGGTTGTTTGCCTTTGG
Human-CTNNB1	ATGGAGCCGGACAGAAAAGC	TGGGAGGTGTCAACATCTTCTT
Human-ACTB	TTGCCGACAGGATGCAGAAGGA	AGGTGGACAGCGAGGCCAGGAT
Human-PRKG1	GGACAGGACTCATCAAGCATAC	CTTACGAGTGACATTTACCGTT
Human-PRKG2	ATCCCAACTCCACCATTTCCT	GTACAATTCTGCATACAGATACCAA
Human-GUCY1A2	TGCTGGGGATGATTAAGGCTG	GTTGATGCTAATTCTGAGGTCCG
Human-ADRB1	ATCGAGACCCTGTGTGTCATT	GTAGAAGGAGACTACGGACGAG
Human-ADRB2	TTGCTGGCACCCAATAGAAGC	CAGACGCTCGAACTTGGA
Human-GNAI1	TTAGGGCTATGGGGAGGTTGA	GGTACTCTCGGGATCTGTTGAAA
Human-GNAI2	TACCGGGCGGTTGTCTACA	GGGTGGCAAAGTCGATCTG
Mus-Pycr1	ATGAGCGTAGGCTTCATCGG	GTGTCAGGTTACCCCTATCT
Mus-Sox2	CGGCACAGATGCAACCGAT	CCGTTTCATGTAGGTCTGCG
Mus-Nanog	CACAGTTTGCCTAGTTCTGAGG	GCAAGAATAGTTCTCGGGATGAA
Mus-Ctnnb1	ATGGAGCCGGACAGAAAAGC	TGGGAGGTGTCAACATCTTCTT
Mus-Actb	GAGGTATCCTGACCCTGAAGTA	CACACGCAGCTCATTGTAGA
Mus-Prkg1	ATCCGAGAGGTCTGAAGGATCT	ATTCCACGGGGTACATACAGT
Mus-Prkg2	TGAAGAACGGGATCAACGAC	CGCTGTGTCACTGATTCTCA
Mus-Gucy1a2	GCTCTAGAGAGGCCGCTAA	GAATGGTCTGCATAGGAGCA
Mus-Adrb1	CTCATCGTGGTGGGTAACGTG	ACACACAGCACATCTACCGAA
Mus-Adrb2	ATGTCGGTTATCGTCCTGGC	GGTTTGTAGTCGCTCGAACTTG
Mus-Gnai1	GGTTTACAGACACGTCCATCAT	GCCTGCATATTCTGGGTAGCAT
Mus-Gnai2	CAGATCGACTTTGCTGATCCC	TAAGCGGCTGAGTCATTGAGC

Supplementary Table 2: Primers for RT-qPCR

Antibodies	Company	Catalog
β -Catenin (Rabbit monoclonal)	CST	Cat#8480 RRID:AB_11127855
NANOG (Rabbit polyclonal)	Abcam	Cat#ab80892 RRID:AB_2150114
SOX-2 (E-4) (Mouse monoclonal)	Santa Cruz Biotechnology	Cat#sc-365823 RRID:AB_10842165
PYCR1(Rabbit polyclonal)	Proteintech	Cat#13108-1-AP RRID:AB_2174878
PRKG1 (Rabbit polyclonal)	Proteintech	Cat#21646-1-AP RRID:AB_2878897
GUCY1B3 (Rabbit polyclonal)	Proteintech	Cat#19011-1-AP RRID:AB_2115817
VASP (Rabbit polyclonal)	Proteintech	Cat#13472-1-AP RRID:AB_2213418
Anti-cGKII antibody (Rabbit polyclonal)	Huabio	Cat#ER63000 RRID:AB_2925218
Phospho-VASP (Ser239) (Rabbit polyclonal)	Affinity	Cat#AF3338 RRID:AB_2834753
β -Actin (Rabbit monoclonal)	abclonal	Cat#AC038 RRID:AB_2863784
Chemicals, peptides, and recombinant proteins	Company	Catalog
Mammary Epithelial Cell Growth Medium BulletKit	Lonza/Clonetics Corporation	Cat#CC-3150
Minimum Essential Medium	Gibco	Cat#A1048901
fetal bovine serum (FBS)	Gibco	Cat#10270
Leibovitz's L-15 Medium	Gibco	Cat#11415064
McCoy's 5a Medium	Gibco	Cat#16600082
RPMI-1640	Gibco	Cat#C11875500BT
F12K	HyClone	Cat#SH30526.01
Anti-mycoplasma reagent Savelt	Hanbio	Cat#HB-SV-1000
Penicillinstreptomycin	Thermo	Cat#15140122
DMEM	Gibco	Cat# C11995500BT
Insulin	Sigma,	Cat#91077C
Matrigel	Corning	Cat#354234
Lipo2000	Invitrogen	Cat#11668500
Puromycin	Sigma	Cat#P8833
L-proline	MCE	Cat#HY-Y0252
KT5823	MCE	Cat#HY-N6791
Epinephrine bitartrate	Selleck	Cat#S2521
L-Glutamine	Solarbio	Cat#G0200
TRIzol	Life Technologies	Cat#15596-026
EasyScript One-Step gDNA Removal cDNA Synthesis SuperMix	Transgen	Cat#AE311-03
Evo M-MLV RT Kit with gDNA Clean for qPCR	ACCURATE BIOTECHNOLOGY	Cat#AG11705
2X Universal SYBR Green Fast qPCR Mix	ABclonal	Cat#RK21203
ChamQTM Universal SYBR qPCR Master Mix	Vazyme	Cat#Q711-02
protease inhibitor cocktail	MCE	Cat#HY-K0010
Phosphatase Inhibitor Cocktail	Bimake	Cat#B15001
PageRuler Prestained Protein Ladder	Thermo Fisher Scientific	Cat#26617
ECL kits	Thermo Fisher Scientific	Cat#34580
SPLink Detection Kits	ZSGB-BIO	Cat#SP9000
ALDEFLUOR Kit	STEMCELL Technologies	Cat#01700
DMEM/F12 medium	Gibco	Cat#C11330500BT
methylcellulose	R&D Systems	Cat#HSC001
basic fibroblast growth factor	Peprtech	Cat#100-18B
B-27	Gibco	Cat#17504044
epidermal growth factor	Sigma	Cat#E9644
proline assay kit	Solarbio	Cat#BC0295
cGMP ELISA kit	Cloud-Clone Corp	Cat#CEA577Ge
Bovine serum albumin (BSA)	Sigma	Cat#V900933
Annexin V-EGFP/PI kit	Abbkine	Cat#KTA0005
Cell Counting Kit-8	Meilunbio	Cat#MA0218
Dnase I	Merck	Cat#10104159001
Collagenase I	Gibco	Cat#17018029

Supplementary Table 3: Reagents