Supplementary Figure S1

A

![Graph showing cell growth (x10^4 cells)]

|          | 1 Day | 3 Days | 5 Days |
|----------|-------|--------|--------|
| NANOGW   |       |        |        |
| HT1080   | □     | □      | □      |
| SK-LMS-1 | □     | □      | □      |
| DDLS8817 | □     | □      | □      |

B

![Western blots showing NANOG WT, Nanog, CD133, Sox2, Oct4, c-Myc, β-actin expression]}

C

|          | HT1080 Spheroids | SK-LMS-1 Spheroids | DDLS8817 Spheroids |
|----------|------------------|--------------------|--------------------|
| NANOGW   | -                | +                  | -                  |
| DAPI     |                  |                    |                    |
| CD133    |                  |                    |                    |
| MERGE    |                  |                    |                    |
| Scale: 100 μm |

D

![Graph showing cell growth (x10^4 cells)]

|          | 1 Day | 3 Days | 5 Days |
|----------|-------|--------|--------|
| sh.NANOG |       | □      | □      |
| HT1080   | □     | □      | □      |
| SK-LMS-1 | □     | □      | □      |
| DDLS8817 | □     | □      | □      |

E

|          | HT1080 Spheroids | SK-LMS-1 Spheroids | DDLS8817 Spheroids |
|----------|------------------|--------------------|--------------------|
| sh.NANOG | -                | +                  | -                  |
| Nanog    |                  |                    |                    |
| CD133    |                  |                    |                    |
| β-actin  |                  |                    |                    |

F

|          | HT1080 Spheroids | SK-LMS-1 Spheroids | DDLS8817 Spheroids |
|----------|------------------|--------------------|--------------------|
| sh.NANOG | -                | +                  | -                  |
| DAPI     |                  |                    |                    |
| Nanog    |                  |                    |                    |
| CD133    |                  |                    |                    |
| Scale: 100 μm |

G

![Images showing HT1080, sh. Scr, 6 μm; 20 μm; 42 μm; 77 μm; 1 Day; 4 Days; 7 Days; 10 Days]}

![Images showing SK-LMS-1, sh. Scr, 6 μm; 25 μm; 40 μm; 81 μm]}

![Images showing DDLS8817, sh. Scr, 6 μm; 23 μm; 38 μm; 79 μm]}

![Images showing sh. NANOG, 6 μm; 20 μm; 23 μm]
Supplementary Figure S1

**CD133**
- Red: Mutation/amplification of CD133
- Blue: No mutation/amplification of CD133
- N=427
- p=0.0389

**NANOG**
- Red: Mutation/amplification of NANOG
- Blue: No mutation/amplification of NANOG
- N=428
- p=0.008591

Progression-free Survival (%) vs. Months
Supplementary Figure S2

| sh.NANOG | HT1080 Spheroids | SK-LMS-1 Spheroids | DDLS8817 Spheroids |
|----------|------------------|--------------------|--------------------|
| -        | -                | -                  | -                  |
| +        | +                | +                  | +                  |

- Nanog
- β-actin
Supplementary Figure S3

A

HT1080

Monolayer

Spheroid

B

HT1080 SK-LMS-1 DDLS8817

Monolayers Spheroids Monolayers Spheroids Monolayers Spheroids

p-AKT\textsuperscript{Thr308}
p-AKT\textsuperscript{Ser473}
AKT1/2
\beta-actin

C

HT1080 SK-LMS-1 DDLS8817

Spheroids Spheroids Spheroids

CD133 (-) CD133 (+) CD133 (+)

p-AKT\textsuperscript{Thr308}
p-AKT\textsuperscript{Ser473}
AKT1/2
\beta-actin

D

HT1080 SK-LMS-1 DDLS8817

Spheroids Spheroids Spheroids

LY294002 - + - + - +
p-AKT\textsuperscript{Thr308}
p-AKT\textsuperscript{Ser473}
AKT1/2 CD133 Nanog Sox2 Oct4 c-Myc \beta-actin

E

HT1080 SK-LMS-1 DDLS8817

CD133 (+) CD133 (+) CD133 (+)

cells cells cells

LY294002 - + - + - +
p-AKT\textsuperscript{Thr308}
p-AKT\textsuperscript{Ser473}
AKT1/2 CD133 Nanog \beta-actin

F

HT1080 CD133 (+) cells

LT1080 CD133 (+) cells

Days 1 4 7 10

DMSO 6 \mu m 16 \mu m 38 \mu m 68 \mu m

LY294002 16 \mu m

sh.Scr 6 \mu m 15 \mu m 33 \mu m 62 \mu m

sh.AKT1/2

SK-LMS-1 CD133 (+) cells

DMSO 6 \mu m 19 \mu m 40 \mu m 67 \mu m

LY294002 18 \mu m

sh.Scr 6 \mu m 19 \mu m 38 \mu m 65 \mu m

sh.AKT1/2

DDLS8817 7 CD133 (+) cells

DMSO 6 \mu m 18 \mu m 38 \mu m 64 \mu m

LY294002 18 \mu m

sh.Scr 6 \mu m 18 \mu m 37 \mu m 62 \mu m

sh.AKT1/2
Supplementary Figure S4

**A**

Bar graph showing proliferation (%) of HT1080, SK-LMS-1, and DDLS8817 spheroids treated with DMSO, Dox, LY294002, and LY294002 + Dox. Asterisks indicate significant differences.

**B**

Western blot analysis of HT1080 spheroids treated with DMSO or LY294002.

**C**

Western blot analysis of HT1080 spheroids treated with sh.Scr or sh.AKT1/2 and Dox or 6 Gy.

**D**

Western blot analysis of HT1080 spheroids treated with sh.Scr or sh.AKT1/2 and Dox or 6 Gy.
Supplementary Figure S5

A

Mice bearing HT1080 xenografts

- sh.Scr + DMSO
- sh.Scr + Dox.
- sh.AKT1/2 + DMSO
- sh.AKT1/2 + Dox.