Supplemental Information

Jeter et al., NANOG promotes cancer stem cell characteristics and prostate cancer resistance to castration

Supplemental Methods

Cells, reagents, and animals
Cancer cell lines including prostate (Du145, LNCaP and PC3 cells), breast (MCF-7) and colon (COLO320), teratocarcinoma cells (NTERA), and human embryonic kidney (HEK) 293T cells were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA) and cultured in the recommended media. Xenograft human prostate tumors LAPC-4 and LAPC-9 were kindly provided by Dr. C. Sawyers (Klein et al., 1997; Reiter and Sawyers, 2001). NOD/SCID Interleukin-2 Receptor (IL2) knockout mice (NOD/SCID-γ) and NOD/SCID mice were obtained from the Jackson Laboratories (Bar Harbor, ME, USA) and maintained in standard conditions in the American Association for Accreditation of Laboratory Animal Care (AAALAC) approved MDACC Animal Facility. All chemicals were obtained from Sigma unless specified otherwise. Primary antibodies used in this study were summarized in Supplemental Table S2.

Purification of tumor cells from xenografts
Basic procedures have been described in our previous publications (Patrawala et al., 2006, 2007; Jeter et al., 2009; Li et al., 2009). In brief, xenograft prostate tumors (Du145, PC3, LAPC-4, and LAPC-9) were
harvested and minced into pieces and digested with 1x Accumax (Innovative Cell Technologies, Inc, San Diego, CA, USA). Dissociated cells were filtered through a 40-µm cell strainer (BD Falcon, Bedford, MA) and further separated from RBC and debris by Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA) density gradient centrifugation. To deplete lineage-positive host cells, the crude tumor preparation was stained with biotinylated anti-H2Kd (BD Pharmingen, San Jose, CA, USA) mouse haplotype marker and streptavidin-conjugated Qdot 655 (Invitrogen, Carlsbad, CA, USA). Separation was subsequently performed via FACS.

**Tumor transplantation and castration**

The basic procedures for subcutaneous (s.c) tumor cell implantation have been previously described (Patrawala et al., 2005, 2006, 2007). Briefly, NOD/SCID mice (6-8 week old) were injected s.c with different numbers of tumor cells in 50-µl of medium containing 50% Matrigel. Dorsal prostate (DP) orthotopic injections (25 µl) were performed as previously described (Patrawala et al., 2007; Li et al., 2009). Tumor development was monitored starting from the second week. Tumorigenicity was measured by tumor weight and tumor incidence. All animals were terminated at 1-5 months after tumor cell injection depending on tumor burden and morbidity. Tumors harvested were fixed in formalin and paraffin sections were cut for HE staining or IHC analysis. Castrations to assay androgen-independent growth *in vivo* were performed by surgical bilateral removal of the testis, vas deferens and testicular fat pad. Bicalutamide (5 mg/kg) intra-dermal injections were performed 3 days prior to the castration to further reduce androgen signaling.

**Fluorescence-activated cell sorting (FACS) and ALDEFLUOR assays**

GFP+ and GFP (7-AAD- and Lin−) tumor cells were sorted using a FACSria flow cytometer (BD Biosciences, San Jose, CA, USA). ALDEFLUOR Assay (Stem Cell Technologies, Vancouver, Canada) was performed according to the manufacturers’ protocol. In brief, trypsinized cells were suspended in
ALDEFLUOR Assay buffer and ALDEFLUOR substrate with or without the ALDH1 inhibitor diethylaminobenzaldehyde (DEAB). Following a 30-min. incubation at 37°C, the stained cells were plunged into an ice bucket, centrifuged and re-suspended in ice-cold ALDEFLUOR Assay buffer, followed immediately by FACS analysis.

**Immunofluorescence (IF) and immunohistochemistry (IHC) Staining**

Basic procedures have been previously described (Jeter et al., 2009). IF detection of NANOG was performed via permeabilization and denaturation pretreatment (0.5% Triton X100, 0.25% sodium dodecyl sulfate). Fixed cells on coverslips were blocked with Background Sniper (Biocare Medical, Concord, CA, USA) for 15 min followed by the NANOG primary antibody (Santa Cruz Biotech, Santa Cruz, CA, USA) and incubated for 2 h at room temperature. Biotinylated anti-rabbit (Vector Laboratories, Burlingame, CA, USA) was used to amplify the signal followed by streptavidin-Alexafluor 594 (Invitrogen). CD133 staining was performed by incubating with a directly conjugated CD133-APC (Miltenyi Biotec). Coverslips were mounted in Prolong Gold Anti Fade (Invitrogen). Immunofluorescence images were captured either on an Olympus BX40 fluorescence microscope or a Zeiss confocal microscope.

For IHC, formalin fixed, paraffin-embedded tissue sections were deparaffinized and hydrated. Endogenous peroxidase activity was blocked (3% \( \text{H}_2\text{O}_2 \)) and antigen retrieval was performed (10 mM citrate buffer; pH 6.0). After blocking with Biocare Blocking Reagent (Biocare), 1° antibodies (Supplemental Table S2) were incubated at appropriate dilutions for 30 min to 2 h at room temperature. Slides were washed in PBS twice and then incubated in biotinylated goat-anti-rabbit or mouse IgG (Vector Laboratories) at a 1:500 dilution for 30 min at room temperature, followed by streptavidin-conjugated horseradish peroxidase (BioGenex Laboratories Inc., San Ramon, CA) and DAB (BioGenex Laboratories Inc.) development.
In vitro proliferation, drug resistance, clonal, and clonogenic assays

pLVX cells, doxycycline-dosed as indicated, either alone or in the presence of the indicated drugs, were trypsinized and counted using a hemocytometer at the appropriate time point. BrdU staining procedures and basic clonal and clonogenic growth procedures have been previously described (Jeter et al., 2009; Li et al., 2009; Patrawala et al., 2006). To assay androgen-independent growth in vitro, androgen-deprivation was achieved by culturing cells in charcoal-dextran stripped serum (CDSS) and/or in 20 μM bicalutamide. For clonogenic assays, cells were plated at low density (ranging from 1-5K cells/well) in Methocult (StemCell Technologies) diluted according to manufacturers’ specifications in DMEM/F12 plus B27 (Invitrogen) and N2 supplements (Invitrogen). Dissociated cells were added together with doxycycline (500 ng/ml) to the Methocult, mixed well and transferred to UltraLow Attachment 24-well dishes (Corning, Corning, NY, USA). Spheres (> 100 μm) that arose within 10-16 days were scored and imaged.

Migration assays

Migration properties of various pLVX LNCaP cells were observed via Biostation IM time-lapse video microscopy (Nikon, Melville, NY, USA). In brief, cells plated ~12 h prior and at 90% confluence were scored with a P20 pipette tip to generate a ‘scratch’. Images were captured over the indicated time course. The NIS-Elements microscope imaging software system was used to analyze the videos and measure migration rates. A line with at least 3 cells intersecting was drawn across the field parallel to the scratch on each side to measure the initial distance apart (D1). At the appropriate time point (30 h later) a second set of parallel lines was drawn to measure the final distance apart (D2) and the distance traveled (μm) was calculated as D1-D2.
Supplemental References

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Klein KA, Reiter RE, Redula J, Moradi H, Zhu XL, Brothman AR et al (1997). Progression of metastatic human prostate cancer to androgen independence in immunodeficient SCID mice. Nat Med 3: 402-8.

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Reiter RE, Sawyers CL (2001). Xenograft models and the molecular biology of human prostate cancer. In: Chung LWK, Isaacs WB and Simons JW (eds). Prostate cancer: Biology, Genetics, and the New Therapeutics. Humana Press Inc.: Totowa, NJ. pp 163-174.
Supplemental Figure Legends

Supplemental Figure S1: Fluorescence images and clonal analysis of PGK-GFP transduced Du145 cells.

a) LNCaP, Du145 and MCF-7 cells (50K/well in a 6-well dish) were transduced with the PGK-GFP or NP8-GFP reporters at 20-µl virus/well. Images (x100) were taken about one week after infection. Note that the majority of PGK-GFP infected cancer cells were positive for GFP whereas only a small percentage of cells infected with NP8-GFP were GFP+.

b) PGK-GFP+/− Du145 cells exhibit similar cloning efficiency (C.E.). Du145 cells were infected with the respective lentiviruses (7 d) and viable (7AAD−) GFP+/GFP− cells were purified by FACS and plated (200 cells/well in a 6-well dish). Colonies were counted 14 d later.

Supplemental Figure S2: Biological characterization of NP8-GFP+/− PCa cells in vitro and in vivo

a) NP8-GFP+ LNCaP cells exhibit higher clonal capacity in androgen-deprived conditions compared to the NP8-GFP− cells. LNCaP cells transduced (7-10 d prior) with the NP8-GFP lentivirus were purified by FACS and plated either in regular media (200 cells/well), or in media supplemented with charcoal-dextran stripped serum (CDSS) or 20 µM bicalutamide. Colonies were stained with Giemsa and imaged at d 14.

b) IHC characterization of NP8-GFP+ versus NP8-GFP− Du145 cell-derived tumors. Note that the NP8-GFP+ cell derived Du145 tumors contain more NANOG+ and Ki-67+ cells. IHC staining with mouse IgG and rabbit (Rb) IgG was used as staining control for Ki-67 and NANOG staining, respectively.

Supplemental Figure S3: MCF7 cells transduced with pLVX-NANOGP8 or pLVX-NANOG1 express NANOG protein in the nucleus.
IF staining using a Rb polyclonal anti-NANOG antibody (H-155; Santa Cruz) revealed nuclear NANOG (red) following Dox induction (1 \(\mu\)g/ml, 48 h) in MCF7-pLVX-NANOG1/P8 cells. Note that in the absence of Dox, only a small percentage of MCF7 cells expressed NANOG protein, mostly on nuclear membrane and/or perinuclear regions. Original magnifications, x200.

**Supplemental Figure S4: Diagram of promoter regions of NANOG target genes analyzed by ChIP**

Arrows indicate the nucleotide positions of PCR primers relative to the transcription start site (TSS) used to amplify the chromatin-bound DNA associated with immunoprecipitated NANOG and the lengths of PCR products in base pairs (bp). Chromosomal locations of the promoters assayed and the gene name of the putative target are also indicated.

**Supplemental Figure S5: Short-term NANOG overexpression in cancer cells is insufficient to promote proliferation or alter expression of proliferative or CSC markers.**

a) NANOG induction does not increase LNCaP or Du145 cell numbers in 72 h. 50K cells of each type were plated in a 12-well dish and doxycycline dosed (\(\mu\)g/ml) as indicated. Cells were trypsinized and counted 72 h later and cell number (x1,000) is indicated.

b) BrdU incorporation assays. Cells indicated were treated with doxycycline for 3 days followed by a 4-h BrdU pulse. Staining was performed with Bu20A anti-BrdU mouse mAb and goat anti-mouse IgG conjugated to Alexafluor 594.

c-d) Western blot analysis. Cells (as indicated) were either un-induced or induced with doxycycline (500 ng/ml) for 3 d. Whole cell lysates were run on SDS-PAGE gels and Western blotting performed with the indicated antibodies (see Supplemental Table S2). In both experiments, N-tera cells were used as controls.
Supplemental Figure S6: Cellular and molecular characterizations of NANOG-overexpressing prostate tumors and PCa cells

a) qRT-PCR analysis of Nanog mRNA levels in LNCaP tumors in intact male mice (left) or Du145 s.c or DP tumors (right) derived from the pLVX-control, pLVX-NANOG1 or pLVX-NANOGP8 cells. The Nanog mRNA levels in pLVX-control tumors were set at 1. Bars represent the mean ± S.D (n = 2).

b) NANOG IHC staining in LNCaP tumors harvested in intact male NOD/SCID mice. The IHC staining was performed using a goat polyclonal anti-NANOG antibody (R & D) and under non-denaturing and non-amplified conditions to detect the transgene-derived NANOG protein (detection of endogenous NANOG protein in cancer cells requires denaturation and amplifications; see Jeter et al., 2009).

c) Ki-67 staining in pLVX-control versus pLVX-NANOG1/P8 LNCaP cell-derived tumors in intact NOD/SCID-γ mice. Original magnifications, x200.

d) NANOG1/P8 overexpression in Du145 cells increases ALDEFLUOR-positive cells. The three types of Du145 cells as indicated were treated with doxycycline (500 ng/ml) for 10 d and then used in Aldefluor assays.
a

LNCaP

| Condition          | GFP- | GFP+ |
|--------------------|------|------|
| Regular medium     |      |      |
| 200 cells/well     |      |      |
| + bicalutamide     |      |      |
| 2000 cells/well    |      |      |
| CDSS               |      |      |
| 2000 cells/well    |      |      |


b

Du145 tumors

| Condition          | NP8-GFP- | NP8-GFP+ |
|--------------------|-----------|-----------|
| HE                 |           |           |
| Ki-67 Mouse IgG (mouse mAb) |           |           |
| Nanog (Rb pAb)     | Rb IgG    |           |
| Rb IgG             |           |           |
a

| pLVX | Nanog1 | NanogP8 |
|------|--------|---------|
| ![Graph](image1.png) | ![Graph](image2.png) |

b

- pLVX
- Nanog1
- NanogP8

![Images](image3.png)

LNCaP tumors in intact hosts

| pLVX (#2283) | NanogP8 (#2285) | Nanog1 (#2284) |
|---------------|----------------|---------------|
| ![Images](image4.png) |

c

- HE
- Ki-67

![Images](image5.png)

d

Du145 Aldefluor

| pLVX | Nanog1 | NanogP8 |
|------|--------|---------|
| ![Images](image6.png) | ![Images](image7.png) | ![Images](image8.png) |

Fluorescence
### Supplemental Table S1. qPCR (TaqMan or SYBR Green) primer/probe sequences

#### TaqMan

**Primer/probe sequences**

| mRNA  | F primer                           | R primer                                   | Probe                  |
|-------|------------------------------------|--------------------------------------------|------------------------|
| GAPDH | Cat# 4352339E                      |                                            |                        |
| Nanog1| CGCCCTGCTAGAAAGACATTT              | AGAAGCGGTCTTGGCTATAGATAA                   | CTGCTAAGGACAACTTTGAT*  |
| NanogP8| CGCCCTGCTAGAAAGACATTT             | ACGAGTTGGAATCTTTAGGTTTAAATGC             | CTTGGCTGCCGTCTCG*      |

For Nanog1-specific qPCR, the probe spans a portion of the 23-bp region in the 3'UTR unique to Nanog1mRNA (see Figure 1A).

For NanogP8-specific qPCR, the probe spans the upstream and downstream regions flanking the 23-bp region (junction site).

*FAM-MGB

#### SYBR Green

**Primer sequences**

| mRNA       | Accession | Forward Primers | Reverse Primers |
|------------|-----------|-----------------|-----------------|
| α2-integrin| NM 002203.3| CCGACAATAACAGCTGCTCAAG | TCCAGTGTTGTGACACTTTG |
| ABCG2      | NM 004827.2| ACAACACATTGCTTGGCTGTC | GCTGCAAGGCGTAATCCATAC |
| ALDH1A1    | NM 00681.3 | TGTGGGTGGTGGACAAATTCAG | ACATCGCATTGAGGGCCTTCCC |
| β1INTG     | NM 002113.3| TGGGTTGGTGGTGGACAAATTCAG | AGTCTTCAGAGACAGCCAGAG |
| BCL2       | NM 00633.2 | CTGGGATGGCTTGGTGGACAGTG | AGTCTTCAGAGACAGCCAGAG |
| CD44       | NM 00610.3 | AGAACAACAGAGATGCCTGATG | TCCAGITCTTCTCTACAGAGTTG |
| CDH1 (E-cad)| NM 00222.2 | TGGGTTGGTGGTGGACAAATTCAG | AGTCTTCAGAGACAGCCAGAG |
| C-MYC      | NM 002467 | TTCTTCTGCTTCTGGAGATCTG | TCCAGITCTTCTCTACAGAGTTG |
| CXCR4      | M 001085.4 | TCCCTGCTTGTCCCTCGGCTG | TGAAGGTGCTGATCCATTTCCC |
| CD133 (PROM1)| NM 004360.3 | AGAAGCAGATCGCCACATACATC | ACTGACATTTCTCCGACAGAG |
| IGFBP5     | NM 00599.3 | TACGGCGAAGCTGACAGCGAG | CTTGACTGCTGATCCGAGAC |
| MMP9       | NM 00359.2 | AGTGTAGGCTGAGCCAGCAT | TCAGAATGCAGCCCTACAGCC |
| NANO       | NM 00286.2 | TAGCAATGGTGAGCCAGCGAG | TCTGAGTCTCCACATGGAGG |
| OCT4       | NM 00270.1 | GAGCAACAATGGAATTTGGTCC | ATGAGTCTGAGTCTGAGAG |
| CD133 (PROM1)| NM 006017.2 | ACAATTTCCAGCAACAGAGTCC | GACGCTTCTGAGTAGAGTCTG |
| SNAIL1     | NM 00385.2 | CCAATGTGAAGGCCCTAATTAGT | GCTGCTGAGGAGTAAACTTGG |
| SOX2       | NM 003106.2 | CATCACCCAGACAGAAAGACAG | TGGGCTGAGTGGGATCTGAGG |
| TWIST1     | NM 000474.3 | ACAAGCTGAGCAAGATTTG | TTGGCAATTTCCGAGTGGCTG |
Supplemental Table S2: Primary antibodies used in the present study

| Antibody Specificity | Company                          | Catalogue # | Host/Type         | Application |
|----------------------|----------------------------------|-------------|-------------------|-------------|
| Nanog                | Kamiya Biomedical Company        | PC-102      | rabbit polyclonal | WB          |
| Nanog                | Santa Cruz Biotechnology         | sc-33759    | rabbit polyclonal | WB/IF/IHC   |
| C-Myc                | Santa Cruz Biotechnology         | sc-40       | mouse monoclonal  | WB          |
| Gli-1                | Santa Cruz Biotechnology         | sc-20687    | rabbit polyclonal | WB          |
| AR                   | Santa Cruz Biotechnology         | sc-7305     | mouse monoclonal  | WB/IHC      |
| IGFBP-5              | Santa Cruz Biotechnology         | sc-6006     | goat polyclonal   | WB          |
| E-Cadherin           | Santa Cruz Biotechnology         | sc-7870     | rabbit polyclonal | WB          |
| PSA                  | Santa Cruz Biotechnology         | sc-7316     | mouse monoclonal  | WB/IHC      |
| Snail                | Cell Signaling Technology        | #3895       | mouse monoclonal  | WB          |
| Slug                 | Cell Signaling Technology        | #9585       | rabbit monoclonal | WB          |
| Phospho-ERK1/2       | Cell Signaling Technology        | #9101       | rabbit polyclonal | WB          |
| Phospho-STAT3        | Cell Signaling Technology        | #9145       | rabbit polyclonal | WB          |
| ERK1                 | Santa Cruz Biotechnology         | sc-93       | rabbit polyclonal | WB          |
| ERK2                 | Santa Cruz Biotechnology         | sc-1647     | mouse monoclonal  | WB          |
| STAT3                | Cell Signaling Technology        | #9132       | rabbit polyclonal | WB          |
| OCT-4                | Chemicon International           | MAB4306     | mouse monoclonal  | WB          |
| SOX-2                | Chemicon International           | AB5603      | rabbit polyclonal | WB          |
| CDK6                 | Santa Cruz Biotechnology         | sc-177      | rabbit polyclonal | WB          |
| NKKX3.1              | Abcam                            | ab78008     | rabbit polyclonal | WB          |
| Cdc25A               | Santa Cruz Biotechnology         | sc-97       | rabbit polyclonal | WB          |
| CyclinD1             | Santa Cruz Biotechnology         | sc-717      | rabbit polyclonal | WB          |
| β-actin              | Sigma                            | A-5441      | mouse monoclonal  | WB          |
| CD44                 | Abcam                            | ab51037     | mouse monoclonal  | WB          |
| CD133 (APC)          | Miltenyi Biotec                  | 130-090-826 | mouse monoclonal  | IF          |
| Ki-67                | Dako                             | M-7249      | rat polyclonal    | IHC         |
Supplemental Table S3: qRT-PCR
Quantification of relative mRNA levels in response to NANOG overexpression

Shown are the mean mRNA values in pLVX-Nanog1 (N1) or pLVX-NanogP8 (NP8) relative to pLVX control
Red: upregulation >2X (200%); Blue: downregulation < 0.5X (50%)
Underlined indicates fold change > 2x (or less than 0.5x) with P < 0.05

| LNCaP spheres, AI conditions (CDSS) and + bicalutamide (bic) | Gene | LNCaP N1 Spheres | LNCaP NP8 Spheres | LNCaP N1 AI | LNCaP NP8 AI | LNCaP N1 Bic | LNCaP NP8 Bic |
|-------------------------------------------------------------|------|------------------|------------------|------------|------------|------------|------------|
| IGFbp5                                                      | 0.9100 | 0.8186 | 2.7712 | 3.4917 | 5.3649 | 6.9555 |
| CXC4R                                                      | 0.4820 | 0.4525 | 2.9874 | 2.2719 | 11.5014 | 9.6778 |
| CD133                                                      | 5.6978 | 4.9888 | 1.2616 | 1.0097 | 1.1792 | 1.4547 |
| ABCG2                                                      | 3.1930 | 2.9834 | 1.3857 | 1.0247 | 1.6881 | 1.9427 |
| cKIT                                                       | 4.1073 | 2.4193 | 1.5513 | 0.5305 | 0.8123 | 1.1454 |
| TWIST                                                      | 1.8778 | 1.0831 | 0.9848 | 0.7418 | 1.6121 | 1.3219 |
| SOX2                                                       | 0.5401 | 0.5294 | 1.1952 | 1.0390 | 0.6420 | 0.8253 |
| SNAIL                                                      | 1.0095 | 0.8613 | 0.9997 | 1.2009 | 0.8371 | 0.9815 |
| aZINTG                                                     | 0.8926 | 0.9239 | 1.4272 | 1.2698 | 0.6310 | 0.8630 |
| eCAD                                                       | 1.2990 | 1.2040 | 0.9186 | 0.8662 | 0.5652 | 0.5403 |
| OCTA4                                                      | 0.8121 | 0.9045 | 1.1952 | 1.0390 | 0.6420 | 0.8253 |

| LNCaP 72h, 7d and 14d, standard culture conditions | Gene | LNCaP N1 72h | LNCaP NP8 72h | LNCaP N1 7d | LNCaP NP8 7d | LNCaP N1 14d | LNCaP NP8 14d |
|---------------------------------------------------|------|--------------|--------------|------------|------------|------------|------------|
| IGFbp5                                           | 1.8900 | 2.2500 | 3.5751 | 3.9866 | 4.1834 | 3.3764 |
| CXC4R                                            | 1.7670 | 5.9920 | 2.1765 | 2.6681 | 1.4397 | 0.7113 |
| CD133                                            | 2.6760 | 2.7260 | 2.3072 | 1.6234 | 1.4714 | 1.4912 |
| ABCG2                                            | 1.1603 | 1.3966 | 1.9175 | 1.5901 | 2.3979 | 2.1487 |
| cKIT                                             | 2.8350 | 5.5840 | 1.2993 | 0.8689 | 1.6469 | 1.6869 |
| TWIST                                            | 0.9152 | 2.6900 | 1.7854 | 1.0589 | 1.3391 | 1.1578 |
| SOX2                                             | 0.8750 | 1.0120 | 1.4483 | 1.1010 | 1.1517 | 1.1462 |
| SNAIL                                            | 1.0270 | 0.9818 | 1.3688 | 1.0457 | 1.3214 | 1.2266 |
| aZINTG                                           | 1.0286 | 1.0515 | 1.2359 | 0.8815 | 1.0596 | 0.9837 |
| OCTA4                                            | 0.8593 | 1.0960 | 1.1738 | 1.0739 | 1.0244 | 0.8205 |

| MCF7 72h, 5d and 14d, standard culture conditions | Gene | MCF7 N1 72h | MCF7 NP8 72h | MCF7 N1 5d | MCF7 NP8 5d | MCF7 N1 14d | MCF7 NP8 14d |
|---------------------------------------------------|------|--------------|--------------|------------|------------|------------|------------|
| ALDH1 A1                                         | 1.2409 | 0.6121 | 3.8762 | 2.8082 | 1.4950 | 4.5079 |
| MN9                                              | 1.1842 | 1.2337 | 2.2267 | 1.1864 | 2.7468 | 3.0160 |
| CD133                                            | 2.4281 | 1.5013 | 0.9907 | 1.2304 | 2.2773 | 2.4706 |
| cMYC                                             | 2.0999 | 2.0243 | 1.1345 | 0.8447 | 2.0327 | 2.0158 |
| ABCG2                                            | 0.7743 | 0.6056 | 1.4176 | 1.0405 | 2.2876 | 1.9762 |
| CD44                                             | 3.3247 | 1.9783 | 1.0844 | 0.7895 | 1.9733 | 2.3349 |
| TWIST                                            | 2.1662 | 2.3250 | 2.2362 | 2.0539 | 1.6965 | 1.1801 |
| cKIT                                             | 0.4302 | 0.4718 | 1.6855 | 1.1173 | 0.9240 | 2.8926 |
| BCL2                                             | 0.7197 | 0.7360 | 1.3943 | 1.2490 | 1.5370 | 1.6252 |
| OCTA4                                            | 1.1835 | 1.0563 | 1.1465 | 0.9528 | 1.7328 | 1.8650 |
| b1INTG                                           | 1.0701 | 0.6346 | 1.3064 | 0.9719 | 1.8270 | 1.6572 |
| SOX2                                             | 0.7802 | 0.6200 | 1.1944 | 1.0254 | 1.5174 | 1.7138 |
| SNAIL                                            | 1.4500 | 1.5178 | 1.5473 | 1.2574 | 1.5501 | 1.6842 |
| a2INTG                                           | 1.3808 | 1.1861 | 0.9523 | 0.8390 | 1.2932 | 1.4472 |

| Du145 7d, standard culture conditions | Gene | Du145 N1 7d | Du145 NP8 7d |
|-------------------------------------|------|-------------|-------------|
| TWIST                               | 6.7377 | 11.0187 |
| ALDH1                               | 1.7287 | 2.6154 |
| CD133                               | 2.0506 | 2.2199 |
| BCL2                                | 1.3931 | 1.7695 |
| CXC4R                               | 1.4474 | 1.5499 |
| a2INTG                              | 1.1054 | 1.2346 |
| eCAD                                | 1.3261 | 1.2591 |
| IGFbp5                              | 0.8385 | 0.8401 |
| OCTA4                               | 0.6945 | 1.0696 |
| SOX2                                | 1.0895 | 1.2348 |
| SNAIL                               | 0.9779 | 1.0592 |