

# E2F3 promotes cancer growth and is overexpressed through copy number variation in human melanoma

Zhicai Feng,<sup>1</sup> Cheng Peng,<sup>1</sup>  
 Daojiang Li,<sup>2</sup> Danhua  
 Zhang,<sup>3</sup> Xu Li,<sup>1</sup> Fengran  
 Cui,<sup>1</sup> Yanhong Chen,<sup>4</sup>  
 Quanyong He<sup>1</sup>

<sup>1</sup>Department of Burns and Plastic Surgery, The Third Xiangya Hospital of Central South University, Changsha, Hunan, China; <sup>2</sup>Department of Gastrointestinal Surgery, The Third Xiangya Hospital of Central South University, Changsha, Hunan, China; <sup>3</sup>Department of Medical Laboratory, Huakang Hospital, Dazhou, Sichuan, China; <sup>4</sup>Fujian Medical University, Fuzhou, Fujian, China

→ Video abstract



Point your Smartphone at the code above. If you have a QR code reader the video abstract will appear. Or use:  
<http://youtu.be/1BDs6YrXxf8>

Correspondence: Quanyong He  
 Department of Burns and Plastic Surgery,  
 The Third Xiangya Hospital of Central  
 South University, Tongzipo Road,  
 Number 172, Changsha, Hunan, China  
 Tel +86 133 0731 3730  
 Fax +86 731 8863 8888  
 Email qyonghe@126.com

**Introduction:** Melanoma is a malignant tumor that seriously affects patients. The pathogenesis of malignant melanoma is complex, and the cell cycle is closely related to tumor progression. Based on the catalog of cancer somatic mutations, we found that overexpression of the *E2F3* gene ranked first in percentage increase in not only melanoma but also in all human cancer tissues. However, there are few studies on the high expression of *E2F3* and its carcinogenic mechanism in melanoma.

**Methods and results:** We found that *E2F3* showed extensive copy number amplification that was positively correlated with the expression level. Patients with high copy number had a significantly poorer prognosis. We also found that *E2F3* levels were significantly negatively correlated with promoter methylation. However, we showed that the *E2F3* promoter region is hypomethylated, and in normal cells or tumor cells, the methylation level did not correlate with expression. Finally, we knocked down the *E2F3* gene in melanoma cells by shRNA. Colony formation, anchorage-dependent growth, and EdU cell proliferation experiments showed a significant decrease in proliferation. Flow cytometry showed a significant increase in the G0/G1 ratio.

**Conclusion:** It can be speculated that copy number amplification and other mechanisms result in the high expression of *E2F3* in melanoma, which promotes tumor progression by involving the cell cycle. *E2F3* is a good target for the treatment of melanoma.

**Keywords:** *E2F3*, melanoma, mechanism, overexpression, copy number, methylation

## Introduction

Malignant melanoma is the most aggressive malignant tumor in the skin, and there is a clear trend toward familial clustering.<sup>1</sup> Of all patients who die of skin tumors, approximately 80% have malignant melanoma.<sup>2</sup> In 2018, the number of new cases of malignant melanoma in the US was estimated to be 91,270, and the estimated death toll was 9,320.<sup>3</sup>

The cell cycle includes four consecutive phases, from the stationary phase (G0 phase) to the proliferative phases (G1 phase, S phase, G2 phase, and M phase) and back to the stationary phase.<sup>4</sup> The key to developing malignant cells is the destruction of normal cell cycle progression, especially in G1 phase. Subsequently, cell cycle-associated regulatory proteins and signal transduction pathways are altered to allow tumor cells to successfully undergo cell cycle progression, lose their ability to differentiate, and undergo malignant proliferation.<sup>5</sup>

The mechanistic mechanism of melanoma involves the abnormal expression of multiple genes associated with cell cycle. The most important of these genes are those that play an important role in normal cell cycle activity but are overexpressed in cancer, interfering with the cell cycle and promoting the development of tumors. For example, the *USP39* gene is highly expressed in melanoma. Silencing *USP39* can inhibit the proliferation of melanoma cells in vitro and in vivo, induce G0/G1 arrest, and promote apoptosis.

The *STAT3* gene is highly expressed in melanoma, and *STAT3* promotes tumor cell growth by regulating the expression of genes involved in cell survival and proliferation.<sup>6</sup> The *cyclin E* gene is also highly expressed in melanoma. *Cyclin E* interacts with CDK2 to form the cyclin E-CDK2 complex, which is highly expressed in both local and metastatic melanoma.<sup>7</sup>

With the release of relevant cancer data in The Cancer Genome Atlas (TCGA) database, cancer-related research has advanced. In this study, we found in the catalog of cancer somatic mutations (TCGA database) that the *E2F3* gene ranked first with a 28.12% increase in expression in melanoma. However, few studies have examined the role of E2F3 in melanoma. We will explore the mechanisms that lead to elevated E2F3 in melanoma and evaluate its associated carcinogenic properties.

## Methods

### Bioinformatics

The catalog of cancer somatic mutations<sup>8</sup> is the world's greatest source of manual mapping of somatic mutations related to human cancer and is used to assess methylation mutations in human cancer tissues. TCGA melanoma expression level data, copy number data, and methylation data were downloaded from the TCGA website (<https://cancergenome.nih.gov/>). The Oncomine website (<https://www.oncomine.org/resource/login.html>) provided E2F3 expression analysis in the TCGA and gene expression omnibus (GEO) (accession number GSE3189 and GSE7553) databases. In addition, we have provided two supplemental tables on the data used in this article. More details about the raw data can be found in Tables S1 and S2.

### Cell culture

The human malignant melanoma cell lines SK-MEL-28, MuM-2C, A-375, and HaCaT were purchased from American Type Culture Collection, Manassas, VA, USA, and the cells were maintained according to the manufacturer's protocol. The cells were cultured with Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum and antibiotics (100 µg/mL streptomycin and 100 U/mL penicillin). All cells were cultured in a humidified environment at 37°C and 5% CO<sub>2</sub>. The use of the cell lines was approved by the ethics board of Xiangya Hospital of Central South University.

### Lentiviral production and transduction

The shRNA targeting *E2F3* (5'-TTGCGTACTTTAA GTACTAA-3'; shR-*E2F3*; Thermo Fisher Scientific) was designed, and the scramble sequence (5'-TTCTCCGAACG TGTCACGT-3') was used as a lentivirus negative control (NC).

The human malignant melanoma cell line A375 was transfected with *E2F3*-shRNA and NC-shRNA viruses. The transfection efficiency of the recombinant lentivirus was tested by fluorescence microscopy (Olympus Corporation, Tokyo, Japan), and RT-PCR was used to assess the efficiency of knockdown.

### RNA purification and reverse transcription PCR

Total RNA was extracted using TRIZOL reagent (Thermo Fisher Scientific). The reverse transcription reaction was performed using a ReverTra Ace qPCR RT Master Mix with a gDNA remover (Toyobo Co., Ltd., Osaka, Japan). The cDNA was amplified by the KODSYBR<sup>®</sup> qPCR Mix (Toyobo Co., Ltd.) on a LightCycler<sup>®</sup> 480II System (Hoffmann-La Roche, Basel, Switzerland) according to the manufacturer's instructions. The primer sequences for the target gene were as follows: forward primer 5'-TATCCCTAAACCCGCTTCC-3', reverse primer 5'-TTCACAAACGGTCTTCTA-3'.

### DNA purification

DNA was isolated using the Easypure Genomic DNA Kit (TransGen Biotech, Beijing, People's Republic of China) according to the manufacturer's instructions. The concentration of DNA was assessed spectrophotometrically and confirmed by gel electrophoresis, and the DNA was stored at -20°C. The results were normalized to *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* gene expression.

### Analysis of DNA methylation

For bisulfite-polymerase chain reaction, the BSP primer was designed in MethPrimer 2.0.<sup>9</sup> Then, the bisulfite-modified DNA was amplified with forward and reverse primers for target genes (bisulfite sequencing or restriction PCR, BSP). The DNA fragments were purified using the Gene JET Gel Extraction Kit (Thermo Fisher Scientific) and mixed at the appropriate proportions. According to the kit instructions, the second-generation sequencing library was prepared with the VAHTSTM Turbo DNA Library Prep Kit (Vazyme Biotech Co., Ltd., Nanjing, China) for Illumina Kits (Illumina, Inc., San Diego, CA, USA), and sequencing was performed with the Illumina MiSeq high-throughput sequencing platform (Illumina, Inc.). The primers for the bisulfite sequencing were as follows: forward primer 5'-GGATYGTTTTAGGTTAGGGAGT-3', reverse primer 5'-AAAARGAAAACAATAAATTCC-3'.

### Plate colony formation assay

Melanoma cells transfected with *E2F3*-shRNA lentivirus were used to prepare cell suspensions and counted. Two

hundred cells were added to a six-well plate. The cells were further cultured in a cell incubator for 14 days, the cell culture medium was changed every 3 days, and the cell state was observed. Photographs were taken with a fluorescence microscope before the end of the experiment, and the cells were washed twice with PBS. Then, 500  $\mu$ L of Giemsa dye was added to each well, and the cells were stained for 10–20 minutes. The cells were washed three times with ddH<sub>2</sub>O and photographed with a digital camera.

### Anchorage-dependent growth

The cells were suspended in 0.3% agar medium (DMEM containing 10% FBS) and plated on a 0.6% agar matrix layer at a concentration of  $3.0 \times 10^4$  cells/60 mm dish. The cells were cultured in a humidified environment (5% CO<sub>2</sub>) at 37°C. The number of colonies that were 50  $\mu$ m or larger were counted after 2 weeks.

### EdU cell proliferation assay

Lentivirus-transfected melanoma cells in the logarithmic growth phase were seeded in 96-well plates. Then, 100  $\mu$ L of 50  $\mu$ mol/L EdU medium was added per well, and the plates were washed with PBS after 2 hours and fixed. Each well was sequentially supplemented with 100  $\mu$ L of 1 $\times$  Apollo staining reaction solution and 100  $\mu$ L of 1 $\times$  Hoechst 33,342 nuclear staining reaction solution, evaluated by EdU detection and photographed by inverted fluorescence microscopy.

### Cell cycle analysis

Melanoma cells transfected with *E2F3*-shRNA lentivirus were incubated in six-well plates. When the cells reached 80% confluence, the medium was removed, the cells were suspended, and the supernatant was discarded by centrifugation. The cell pellet was washed with cold PBS. Cells were collected by centrifugation and fixed in 75% cold ethanol for 1 hour. Prior to analysis, the cells were washed with PBS again and suspended in a solution containing 10  $\mu$ g/mL RNase A and 50  $\mu$ g/mL propidium iodide. The cell suspension was filtered at a rate of 300 cells/second, and the cell cycle was analyzed by a FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA, USA).

### Statistical analysis

GraphPad Prism 7 software was used to analyze the data. Methylation, copy number variation (CNV), and expression levels between normal and cancer tissues were assessed by a two-tailed unpaired Student's *t*-test, and the error bars in the figure represent the SD or SEM. Spearman's correlation coefficient (*r*) was used to determine the correlation. Receiver operator characteristic curves were constructed based on the

level of *E2F3* gene expression. The data were considered significant at  $P < 0.05$ .

## Results

### High expression of the *E2F3* gene in melanoma

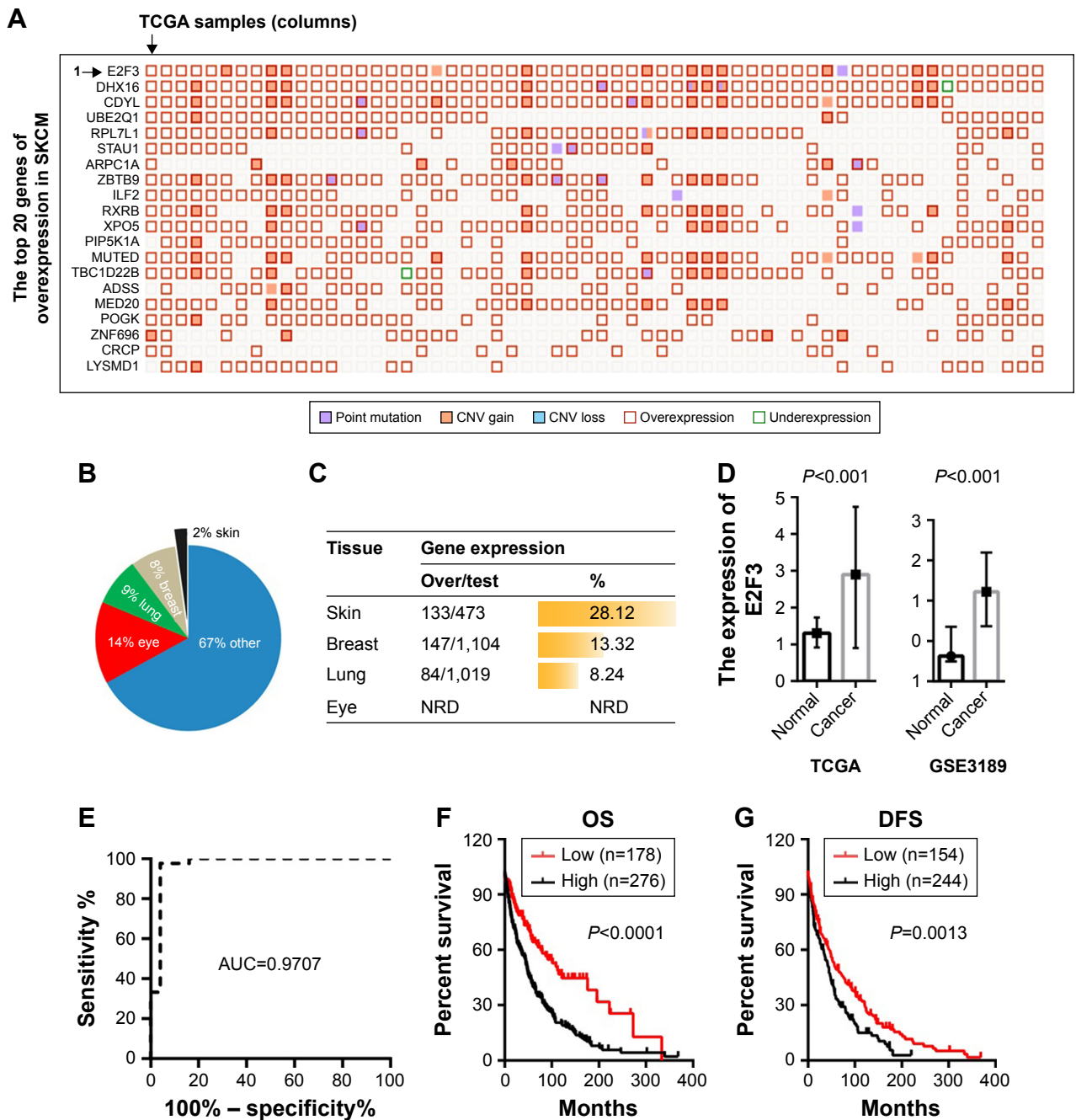
Based on the TCGA data, we found that among the genes with high expression in melanoma, the *E2F3* gene ranked first with an increase of 28.12% (Figure 1A and C). The *E2F3* gene may promote the development of melanoma. It is worth noting that *E2F3*-related research in the skin is extremely rare, while studies in the eye, lung, and breast are more common (Figure 1B). We found that the lung and breast ranked second and third, respectively, while melanoma ranked first (Figure 1C). Therefore, it is necessary to study the relationship between *E2F3* and melanoma. Further study found that *E2F3* gene expression was increased in the TCGA and GEO databases (Figure 1D), and the ROC curve of GSE3189 revealed that *E2F3* can identify melanoma tissue (AUC=0.9709) (Figure 1E). Survival analysis showed that high *E2F3* expression results in decreases in OS and disease-free survival (DFS) of patients (Figure 1F and G).

### The relationship between CNV and *E2F3* expression level

We sought to explore the cause of the high expression of *E2F3*. First, we found that *E2F3* showed extensive copy number amplification (CNA) and high DNA expression in solid cancers (Figure 2A), and amplification was the main cause of high gene expression. Therefore, we hypothesized that the high expression of *E2F3* in melanoma was caused by CNA. The TCGA database showed that the expression *E2F3* level significantly increased in tissues with high copy number compared with tissues with low copy number (Figure 2B). The expression of the *E2F3* gene was significantly positively correlated with CNV level (Figure 2C), showing that CNA is one of the causes of the increase in *E2F3*. More importantly, we found that CNV was significantly associated with clinical prognosis, with significantly lower OS and DFS in patients with high copy number (Figure 2D and E).

### The relationship between DNA methylation and *E2F3* gene expression in the promoter region

In addition to CNA, promoter methylation is another mechanism that controls expression levels that should



**Figure 1** High expression of the *E2F3* gene in melanoma and its influence on prognosis.

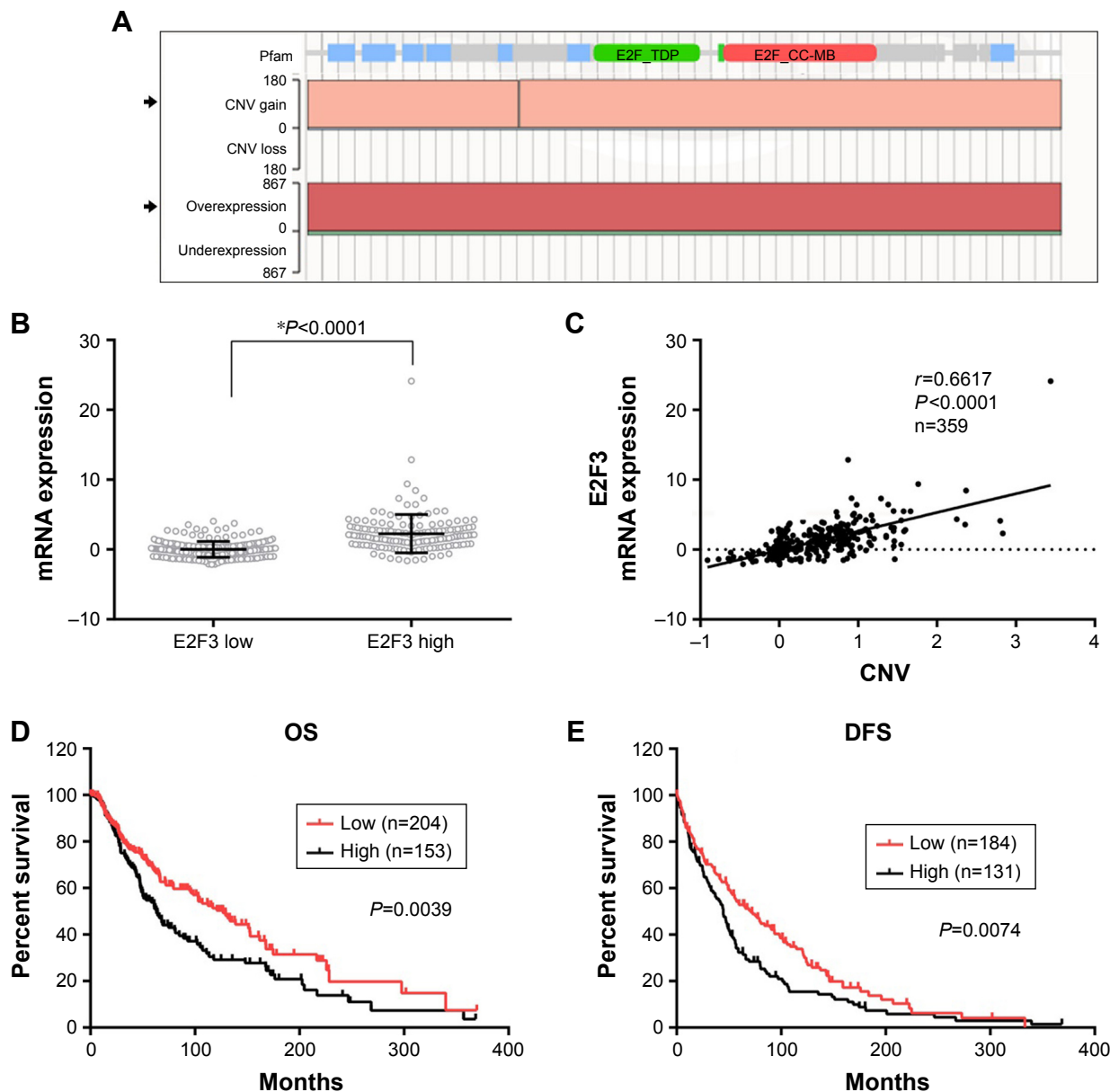
**Notes:** (A) A “Mutation Matrix” plot between genes and samples of melanoma tissue that contains 20 top ranked genes (rows) and TCGA samples (columns) with each box representing a Gene-Sample combination. The *E2F3* gene ranked as the top melanoma-related gene. (B) *E2F3* has been studied in many tissues, including eye, lung, and breast, but studies of the skin are extremely rare. (C) The overexpression percentage (%) is represented as a histogram across different primary tissue types, and the overexpression percentage of the *E2F3* gene in melanoma is the highest. (D) The *E2F3* gene shows an increased expression level in melanoma tissues compared with normal tissues. The left was derived from the TCGA database, and the right was from the GSE3189 (more details can be found at <https://www.oncomine.org>). (E) The ROC curve of GSE3189. (F) The overexpression of the *E2F3* gene affected the OS of 454 melanoma patients. (G) The overexpression of the *E2F3* gene affected the DFS (disease free since initial treatment) of 398 melanoma patients.

**Abbreviations:** CNV, copy number variation; DFS, disease-free survival; NRD, no related data; OS, overall survival; SKCM, skin cutaneous melanoma; TCGA, The Cancer Genome Atlas.

not be overlooked. In investigating whether high *E2F3* expression is regulated by methylation, we found that *E2F3* expression in hypermethylated tissues was obviously lower than that in tissues with low methylation by analyzing the

TCGA database (Figure 3A), and the methylation level was significantly negatively correlated with the *E2F3* expression level (Figure 3B). However, we showed that methylation level did not affect OS or DFS (Figure 3C and D). The





**Figure 2** Copy number variation results in the high expression of the *E2F3* gene and affects prognosis.

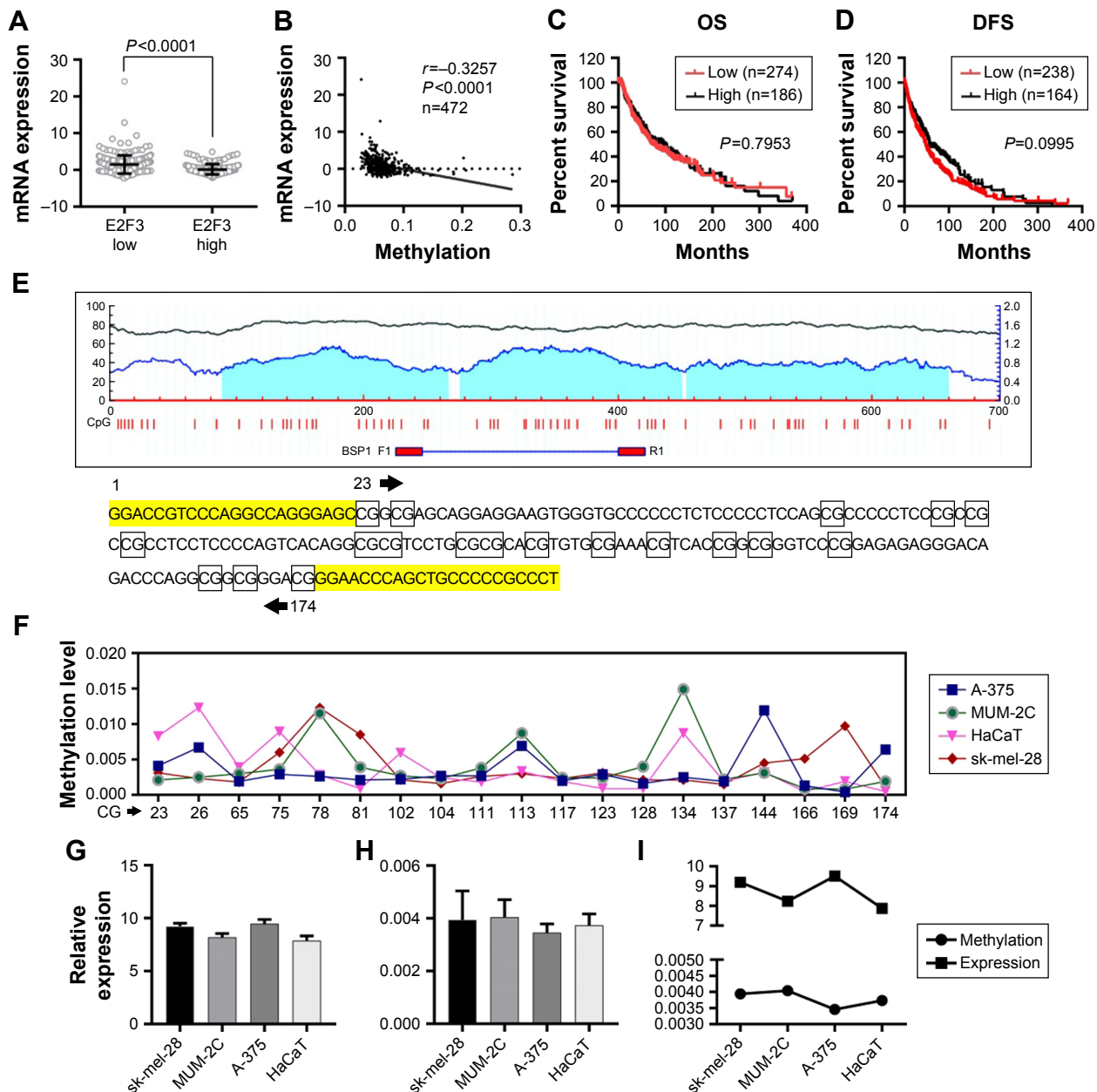
**Notes:** (A) COSMIC shows that *E2F3* variations in solid cancer are dominated by CNV gains and overexpression. (B) The *E2F3* gene shows an increased expression level in patients with high *E2F3* copy number (unpaired t-test,  $*P<0.0001$ ). (C) The expression of the *E2F3* gene is significantly positively correlated with the CNV level. (D) The copy number of the *E2F3* gene affects the OS of 357 melanoma patients. (E) The copy number of the *E2F3* gene affects the DFS of 315 melanoma patients.

**Abbreviations:** CNV, copy number variation; COSMIC, catalog of cancer somatic mutations; DFS, disease-free survival; OS, overall survival.

promoter region of *E2F3* was heavily enriched with CpG islands (Figure 3E), further confirming this trend. The methylation status of 19 CpG sites in the promoter region in four cell lines was analyzed by bisulfite sequencing PCR. The results showed that the promoter region was hypomethylated in both normal cells and malignant tumor cells and that there was no correlation between methylation and expression levels (Figure 3F–I; Tables S3 and S4). These results suggest that methylation may not be the cause of the elevated *E2F3* expression.

## Carcinogenic properties of the *E2F3* gene

Research about whether high *E2F3* expression can promote the progression of melanoma is still lacking. To solve this problem, we successfully knocked down the *E2F3* gene in melanoma cells. Melanoma cells that lacked the *E2F3* gene showed reduced proliferation in colony formation (Figure 4A and B), anchorage-dependent growth (Figure 4C and D), and EdU cell proliferation experiments (Figure 4E and F). These experiments show that *E2F3* can affect cell proliferation. At the same time, we found that the G0/G1 population increased



**Figure 3** DNA methylation does not result in the high expression of the *E2F3* gene or affect prognosis.

**Notes:** (A) The *E2F3* gene shows an increased expression level in patients with hypomethylation. (B) The expression of the *E2F3* gene is significantly inversely correlated with the methylation level. (C and D) The methylation of the *E2F3* gene does not affect OS and DFS. (E) The promoter region of *E2F3* is enriched with CpG islands (more details can be found in MethPrimer at <http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi>). The BSP1 synthesis sequence FIR1 contains 19 CpG sites. (F) Methylation of 19 CpG sites in four cell lines. (G) Relative expression by PCR. (H) Methylation levels in cells. (I) Methylation and expression levels are inconsistent in the four cell lines.

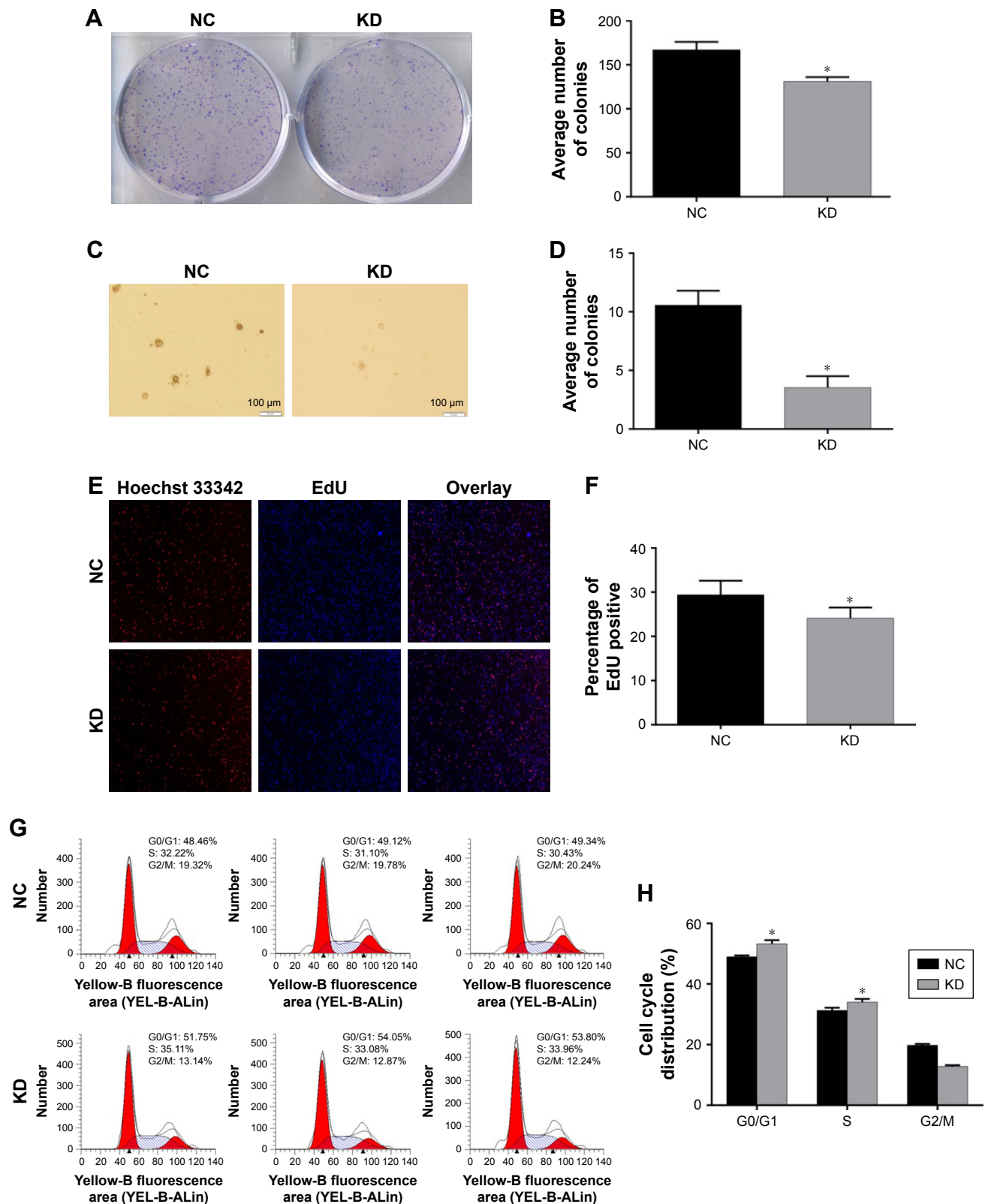
**Abbreviations:** DFS, disease-free survival; OS, overall survival.

significantly after *E2F3* gene knockdown (Figure 4G and H), which indicates that *E2F3* regulates the cell cycle and promotes melanoma.

## Discussion

The *E2F3* gene is located on chromosome 6p22 and is 91.5 kb in length. *E2F3* is a major member of the E2F family and plays an important role in regulating the cell cycle, proliferation, and apoptosis.<sup>10</sup>

Feber et al found *E2F3* gene amplification on chromosome 6p22 in the bladder cancer cell lines TCCSUP and HT1376. Analysis showed that higher clinical stage and pathological grade of bladder cancer corresponded with a higher positive rate of *E2F3* expression.<sup>11</sup> Silven Bilke et al found that approximately 2/3 of prostate cancers showed high expression of *E2F3*. *E2F3* can be used as an independent factor to predict the OS of prostate cancer patients through multivariate analysis.<sup>12</sup> Ren et al found that miR-449a inhibits



**Figure 4** Carcinogenic properties of the *E2F3* gene.

**Notes:** (A) Colony formation of the cells after transient transfection with *E2F3*-shRNA or NC-shRNA. (B) Histogram showing colony formation in the groups. (C) Images of anchorage-dependent growth. (D) The average number of colonies decreased following *E2F3* gene knockdown. (E) The *E2F3* gene affects cell proliferation. Proliferation was assessed using an EdU cell proliferation assay kit (magnification, 100×). (F) The cell proliferation rate decreased following *E2F3* gene knockdown. (G) The cell cycle distribution in melanoma cells transfected with *E2F3*-shRNA or NC-shRNA. (H) Histogram showing the cell cycle distribution (%). All the results are shown as the mean ± SEM, \**P*<0.05.

**Abbreviations:** KD, knockdown; NC, negative control.

the expression of *E2F3*, which decreased when miR-449a was overexpressed in the lung cancer cell lines A549 and 95D. Another study found cell cycle arrest in G1 phase and inhibition of cell proliferation.<sup>13</sup> Libertini et al found that the expression of *E2F3* in laryngeal squamous cell carcinoma was significantly higher than that in normal nasopharyngeal mucosa ( $P < 0.001$ ), and less differentiation correlated with a higher expression level ( $P < 0.05$ ).<sup>14</sup> However, it is worth noting that we found that the expression level of the *E2F3* gene was the highest in melanoma, ranking first among all tissues, but few studies have explored this relationship. Therefore, it is necessary to conduct in-depth studies. We also confirmed that E2F3 can interfere with the cell cycle to promote the development of melanoma.

Many studies have found that CNV is an important factor affecting the prognosis of cancer patients. Specific CNVs can be used to assess prognosis.<sup>15</sup> A meta-analysis showed that *MET* gene amplification is inversely related to the OS of non-small-cell lung cancer patients and increases the risk of death.<sup>16</sup> Liu et al reported that lung cancer patients with four copies of the *mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2)* gene promoter have a shorter median survival and a 47% increased risk of death compared to those with two or three copies, which shows that the *MAPKAPK2* gene can be used as a genetic marker to predict the prognosis of lung cancer.<sup>17</sup> We confirmed that E2F3 was also regulated by this mechanism.

In addition to CNV, promoter methylation has been shown to affect gene expression in numerous studies. For example, Kim et al found that hypomethylation of the *NAT1* gene may increase the transcriptional activation of breast cancer genes. Therefore, the hypomethylated *NAT1* gene plays an important role in the development of breast cancer.<sup>18</sup> Other related studies include those on FGFR (sarcoma),<sup>19</sup> PRAME (leukemia),<sup>20</sup> and IGF2 (colorectal cancer).<sup>21</sup> The promoter hypermethylation of these genes is closely related to the occurrence of the corresponding tumors. In our study, we found that the expression level of the *E2F3* gene was negatively correlated with methylation but did not affect the prognosis of patients. Moreover, there was no correlation between the methylation and expression levels in four cell lines. It can be inferred that methylation may be one of the reasons for the high expression of *E2F3*, but more research needs to be done.

In summary, we discussed the mechanism of the high expression of *E2F3* in melanoma. Finally, we knocked down the *E2F3* gene with lentivirus, which significantly reduced the proliferation of melanoma cells and increased

the proportion of cells in the G0 phase. Therefore, it can be speculated that E2F3 can promote cancer. However, considering the extensive and high expression of *E2F3*, more in-depth research is needed.

## Acknowledgment

This work was supported by the Zensure Leader Plan of the Third Xiangya Hospital of Central South University (grant number 20170302).

## Disclosure

The authors report no conflicts of interest in this work.

## References

- Rastrelli M, Tropea S, Rossi CR, Alaibac M. Melanoma: epidemiology, risk factors, pathogenesis, diagnosis and classification. *In Vivo*. 2014; 28(6):1005–1011.
- Trotter SC, Sroa N, Winkelmann RR, Olencki T, Bechtel M. A Global Review of Melanoma Follow-up Guidelines. *J Clin Aesthet Dermatol*. 2013;6(9):18–26.
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin*. 2018;68(1):7–30.
- Norbury C, Nurse P. Animal cell cycles and their control. *Annu Rev Biochem*. 1992;61:441–470.
- Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature*. 2001;411(6835):366–374.
- Kortylewski M, Jove R, Yu H. Targeting STAT3 affects melanoma on multiple fronts. *Cancer Metastasis Rev*. 2005;24(2):315–327.
- Georgieva J, Sinha P, Schadendorf D. Expression of cyclins and cyclin dependent kinases in human benign and malignant melanocytic lesions. *J Clin Pathol*. 2001;54(3):229–235.
- Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high-resolution. *Nucleic Acids Res*. 2017;45(D1):D777–D783.
- Li LC, Dahiya R. MethPrimer: designing primers for methylation PCRs. *Bioinformatics*. 2002;18(11):1427–1431.
- Miles WO, Tschöp K, Herr A, Ji JY, Dyson NJ. Pumi1 facilitates miRNA regulation of the E2F3 oncogene. *Genes Dev*. 2012;26(4):356–368.
- Feber A, Clark J, Goodwin G, et al. Amplification and overexpression of E2F3 in human bladder cancer. *Oncogene*. 2004;23(8):1627–1630.
- Bilke S, Schwentner R, Yang F, et al. Oncogenic ETS fusions deregulate E2F3 target genes in Ewing sarcoma and prostate cancer. *Genome Res*. 2013;23(11):1797–1809.
- Ren XS, Yin MH, Zhang X, et al. Tumor-suppressive microRNA-449a induces growth arrest and senescence by targeting E2F3 in human lung cancer cells. *Cancer Lett*. 2014;344(2):195–203.
- Libertini SJ, Chen H, Al-Bataina B, et al. The interleukin 6 receptor is a direct transcriptional target of E2F3 in prostate tumor derived cells. *Prostate*. 2012;72(6):649–660.
- Kumaran M, Cass CE, Graham K, et al. Germline copy number variations are associated with breast cancer risk and prognosis. *Sci Rep*. 2017;7(1):14621.
- Ye S, Li J, Hao K, Yan J, Zhou H. The Efficacy and Risk Profile of c-Met inhibitors in Non-small Cell Lung Cancer: a Meta-analysis. *Sci Rep*. 2016;6:35770.
- Liu B, Yang L, Huang B, et al. A functional copy-number variation in MAPKAPK2 predicts risk and prognosis of lung cancer. *Am J Hum Genet*. 2012;91(2):384–390.
- Kim SJ, Kang HS, Chang HL, et al. Promoter hypomethylation of the N-acetyltransferase 1 gene in breast cancer. *Oncol Rep*. 2008;19(3):663–668.



19. Goldstein M, Meller I, Orr-Urtreger A. FGFR1 over-expression in primary rhabdomyosarcoma tumors is associated with hypomethylation of a 5' CpG island and abnormal expression of the AKT1, NOG, and BMP4 genes. *Genes Chromosomes Cancer*. 2007;46(11):1028–1038.
20. Roman-Gomez J, Jimenez-Velasco A, Castillejo JA, et al. Promoter hypermethylation of cancer-related genes: a strong independent prognostic factor in acute lymphoblastic leukemia. *Blood*. 2004;104(8):2492–2498.
21. Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. *Cancer Res*. 2002;62(22):6442–6446.

## Supplementary materials

Table S1 GSE3189

Samples	Name column	E2F3
GSM71671	Normal	215.25
GSM71672	Normal	153.95
GSM71673	Normal	265.3
GSM71674	Normal	199.4
GSM71675	Normal	327.95
GSM71676	Normal	163.7
GSM71677	Normal	203.3
GSM71678	Nevus	319.5
GSM71679	Nevus	283.85
GSM71680	Nevus	369.7
GSM71681	Nevus	225.4
GSM71682	Nevus	199.2
GSM71683	Nevus	270.55
GSM71684	Nevus	295.1
GSM71685	Nevus	304.3
GSM71686	Nevus	326.3
GSM71687	Nevus	290.7
GSM71688	Nevus	306.95
GSM71689	Nevus	372.55
GSM71690	Nevus	184.05
GSM71691	Nevus	365.15
GSM71692	Nevus	369.8
GSM71693	Nevus	294.65
GSM71694	Nevus	367.75
GSM71695	Nevus	642
GSM71696	Melanoma	584.9
GSM71697	Melanoma	669.35
GSM71698	Melanoma	439.55
GSM71699	Melanoma	493.05
GSM71700	Melanoma	417.3
GSM71701	Melanoma	549.8
GSM71702	Melanoma	649.4
GSM71703	Melanoma	558.9
GSM71704	Melanoma	449.25
GSM71705	Melanoma	689.95
GSM71706	Melanoma	713.9
GSM71707	Melanoma	786.1
GSM71708	Melanoma	1,071.75
GSM71709	Melanoma	405.55
GSM71710	Melanoma	686.95
GSM71711	Melanoma	634.85
GSM71712	Melanoma	464.4
GSM71713	Melanoma	624.65
GSM71714	Melanoma	610.55
GSM71715	Melanoma	676.65
GSM71716	Melanoma	810.25
GSM71717	Melanoma	659.05
GSM71718	Melanoma	498.05
GSM71719	Melanoma	604.75
GSM71720	Melanoma	368.95
GSM71721	Melanoma	526.55
GSM71722	Melanoma	511.15
GSM71723	Melanoma	582.1
GSM71724	Melanoma	519.85
GSM71725	Melanoma	740.1

(Continued)

Table S1 (Continued)

Samples	Name column	E2F3
GSM71726	Melanoma	602.05
GSM71727	Melanoma	399.4
GSM71728	Melanoma	435.4
GSM71729	Melanoma	423.1
GSM71730	Melanoma	423
GSM71731	Melanoma	557.65
GSM71732	Melanoma	537.55
GSM71733	Melanoma	536.95
GSM71734	Melanoma	539.95
GSM71735	Melanoma	733.9
GSM71736	Melanoma	780.4
GSM71737	Melanoma	999.3
GSM71738	Melanoma	669.75
GSM71739	Melanoma	563.9
GSM71740	Melanoma	490.6

Table S2 GSE7553

Samples	Name column	E2F3
GSM183222	Melanoma in situ	596.8995
GSM183223	Melanoma in situ	485.8525
GSM183226	Metastatic melanoma	1,043.514
GSM183227	Metastatic melanoma	1,065.36
GSM183228	Metastatic melanoma	1,097.4305
GSM183229	Metastatic melanoma	1,367.071
GSM183230	Metastatic melanoma	904.909
GSM183231	Metastatic melanoma	966.979
GSM183232	Metastatic melanoma	795.534
GSM183233	Metastatic melanoma	1,049.4855
GSM183252	Metastatic melanoma	1,675.07
GSM183253	Metastatic melanoma	1,161.0115
GSM183254	Metastatic melanoma	676.6665
GSM183255	Metastatic melanoma	1,252.875
GSM183256	Metastatic melanoma	1,091.715
GSM183257	Metastatic melanoma	1,446.735
GSM183273	Metastatic melanoma	763.7105
GSM183274	Metastatic melanoma	1,067.1765
GSM183275	Metastatic melanoma	972.223
GSM183276	Metastatic melanoma	984.8085
GSM183277	Metastatic melanoma	935.2895
GSM183278	Metastatic melanoma	1,323.74
GSM183279	Metastatic melanoma	757.3935
GSM183280	Metastatic melanoma	1,052.9955
GSM183281	Metastatic melanoma	970.099
GSM183282	Metastatic melanoma	1,097.2135
GSM183283	Metastatic melanoma	1,349.745
GSM183284	Metastatic melanoma	973.492
GSM183285	Metastatic melanoma	959.895
GSM183286	Metastatic melanoma	967.631
GSM183287	Metastatic melanoma	1,336.92
GSM183288	Metastatic melanoma	918.0085
GSM183289	Metastatic melanoma	1,649.06
GSM183290	Metastatic melanoma	1,127.6965
GSM183291	Metastatic melanoma	1,256.515
GSM183292	Metastatic melanoma	1,410.8
GSM183293	Metastatic melanoma	1,364.66
GSM183294	Metastatic melanoma	3,495.27

(Continued)

**Table S2** (Continued)

Samples	Name column	E2F3
GSM183295	Metastatic melanoma	921.3215
GSM183296	Metastatic melanoma	1,392.965
GSM183297	Metastatic melanoma	582.005
GSM183298	Metastatic melanoma	1,088.088
GSM183299	Normal human epidermal melanocytes	821.5565
GSM183234	Normal skin	289.57
GSM183300	Normal skin	554.652
GSM183301	Normal skin	360.1345
GSM183302	Normal skin	359.8905
GSM183224	Primary melanoma	454.667
GSM183225	Primary melanoma	472.3105
GSM183235	Primary melanoma	2,032.71
GSM183258	Primary melanoma	3,043.785
GSM183259	Primary melanoma	1,404.6755
GSM183260	Primary melanoma	1,513.8725
GSM183261	Primary melanoma	1,540.7
GSM183262	Primary melanoma	797.319
GSM183263	Primary melanoma	1,008.3395
GSM183264	Primary melanoma	733.7925
GSM183265	Primary melanoma	1,466.6135
GSM183266	Primary melanoma	838.3575
GSM183303	Primary melanoma	1,035.0555
GSM183304	Primary melanoma	612.168

**Table S4** E2F3 pair all sample site methylation information table

E2F3 pair methylation position	SK-MEL-28	MUM-2C	A-375	HaCaT
23	0.0031	0.0041	0.0041	0.0033
26	0.0039	0.0045	0.0037	0.0043
65	0.0038	0.003	0.0029	0.0039
75	0.0041	0.0036	0.0029	0.0049
78	0.0033	0.0045	0.0026	0.0038
81	0.0035	0.0039	0.0021	0.0039
102	0.0021	0.0027	0.0022	0.0039
104	0.0036	0.0043	0.0027	0.0024
111	0.0036	0.0038	0.0027	0.0038
113	0.003	0.0047	0.0035	0.0033
117	0.0033	0.0039	0.003	0.0039
123	0.0031	0.0024	0.0032	0.0039
128	0.0051	0.004	0.0036	0.0039
134	0.0021	0.0049	0.0025	0.0037
137	0.0035	0.0042	0.0039	0.0039
144	0.0045	0.0041	0.0042	0.0032
166	0.0051	0.0049	0.0033	0.0035
169	0.0077	0.0042	0.0034	0.0029
174	0.0031	0.0043	0.0034	0.0035

**Table S3** E2F3 gene relative expression of by PCR

Cell name	GAPDH	E2F3	$\Delta Ct$
SK-MEL-28	13.07	22.12	9.05
	12.8	22.11	9.31
	12.93	22.15	9.22
MUM-2C	12.81	20.85	8.04
	12.76	20.97	8.21
	12.47	20.91	8.44
A375	12.43	21.7	9.27
	12.23	21.75	9.52
	12.16	21.91	9.75
HaCaT	13.35	20.99	7.64
	13.31	21.25	7.94
	13.27	21.32	8.05

**OncoTargets and Therapy****Publish your work in this journal**

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic agents and protocols on

Submit your manuscript here: <http://www.dovepress.com/oncotargets-and-therapy-journal>

patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Dovepress