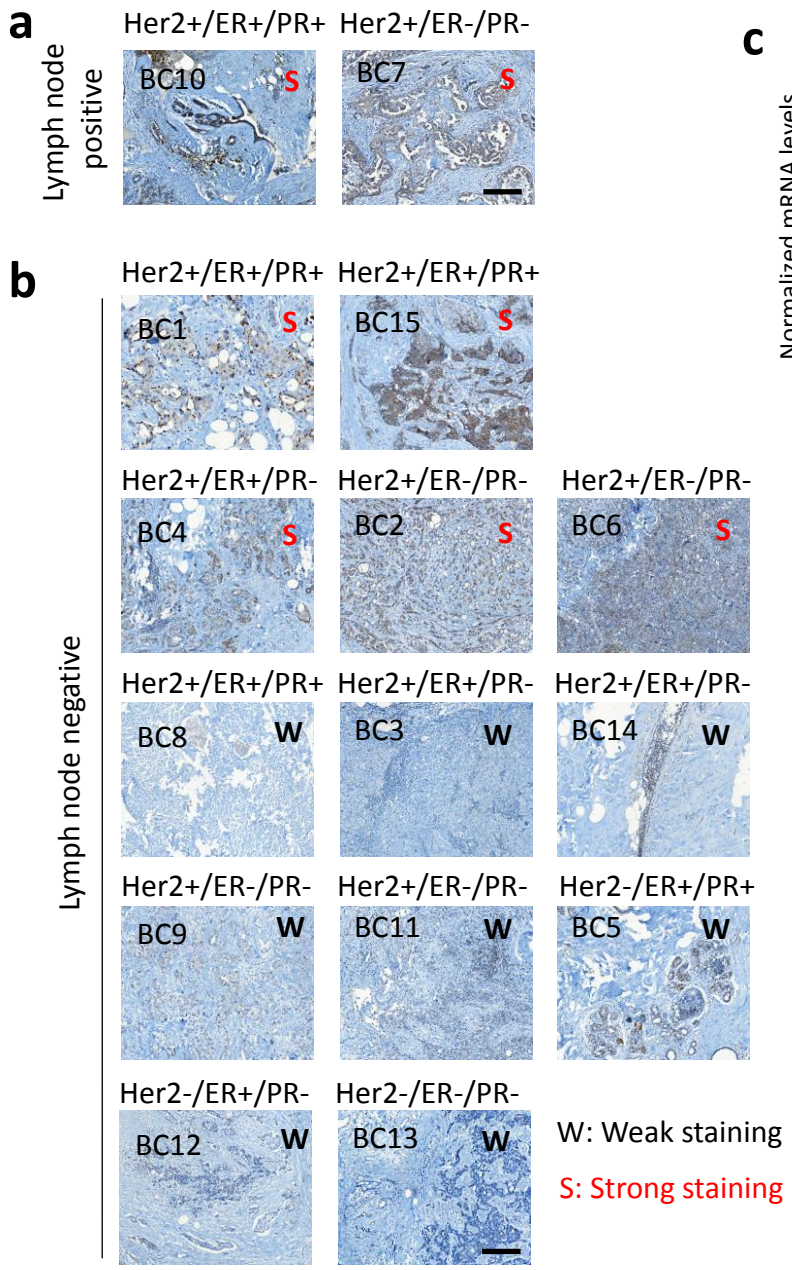
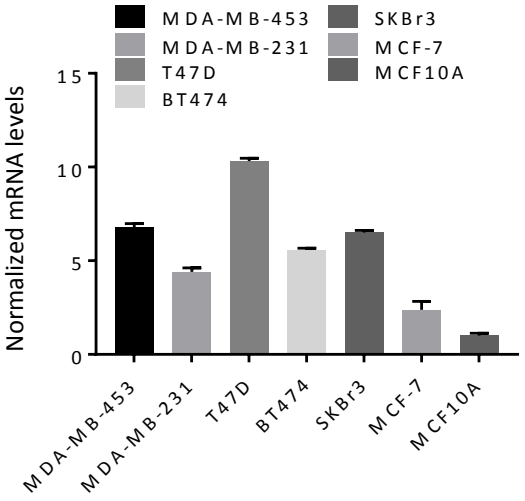
Fig.S1





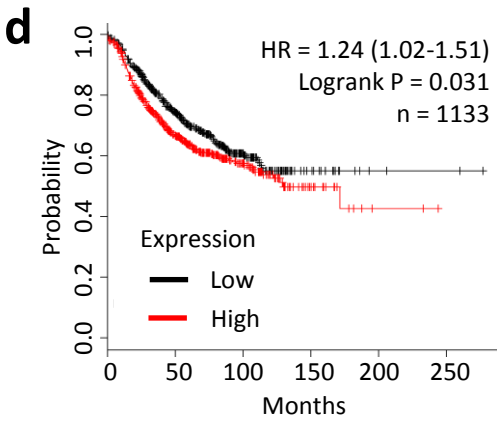


Figure S1. Detection of EGFL6 in tumor tissues by IHC staining and its correlation with survival probability. **a** and **b**, FFPE-fixed tumor tissues from breast cancer patients were stained with anti-EGFL6 antibody (Sigma) and a representative image from each tumor slide is shown. Patient tumor properties including lymph node positivity are grouped in A , for node positive and B, for node negative tumors. Strong (S) or weak (**W)** staining of EGFL6 is indicated on the inside of each images. Scale bar, 100 µm. **c**, Expression of EGFL6 in breast cancer cell was measured by qRT-PCR, n=3, error bars for standard deviation (SD). **d**, Kaplan Meier survival correlation with EGFL6 expression. Median level was used for dividing high vs. low expression groups using a data base for breast cancer patients in https://omictools.com/kaplan-meier-plotter-tool.

Fig.S2

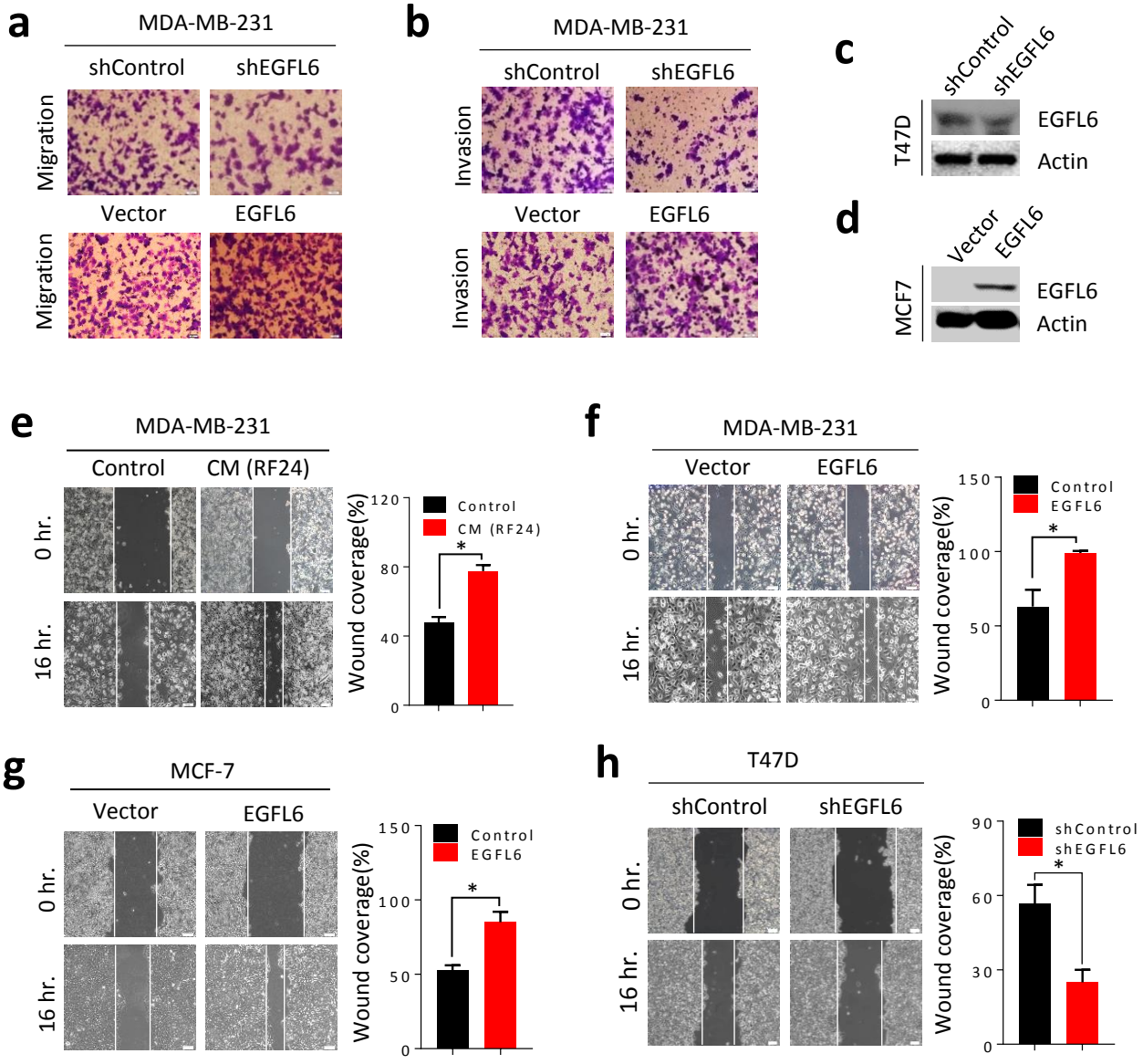


Figure S2. Effects of EGFL6 expression on cancer cell migration. a and **b**, Representative images of migration and invasion of MDA-MB-231/shEGFL6 breast cancer cells in comparison with the parental control cells (shControl). **c**, Western blot detection of EGFL6 in T47D/shEGFL6 cells and control cell lysates. **d**, Western blot detection of EGFL6 in MCF-7/EGFL6 and parental MCF-7 cells. **e**, Representative images and quantitation of the scratched areas covered by cells after 16 hours of culture, in relative to the area (%) scratched at 0 hour in a humidified incubator at 37^{o}C, 5% CO_2 . MDA-MB-231 cells cultured in media containing the conditioned medium (CM-RF24) were compared with the parental (Control) cells for coverage of scratched area. CM-RF24 was collected from 24 hour cultures of RF24 cells. **f, g,** and **h**, Wound healing assays were performed in the same way as in E for the paired cell lines of MDA-MB-231, MCF-7 and T47D with expression or knockdown of EGFL6, respectively.. Representative images and quantitative results of coverage of scratch wound are shown, n=3 *, $p < 0.05$, error bars indicate SD.

Fig.S3

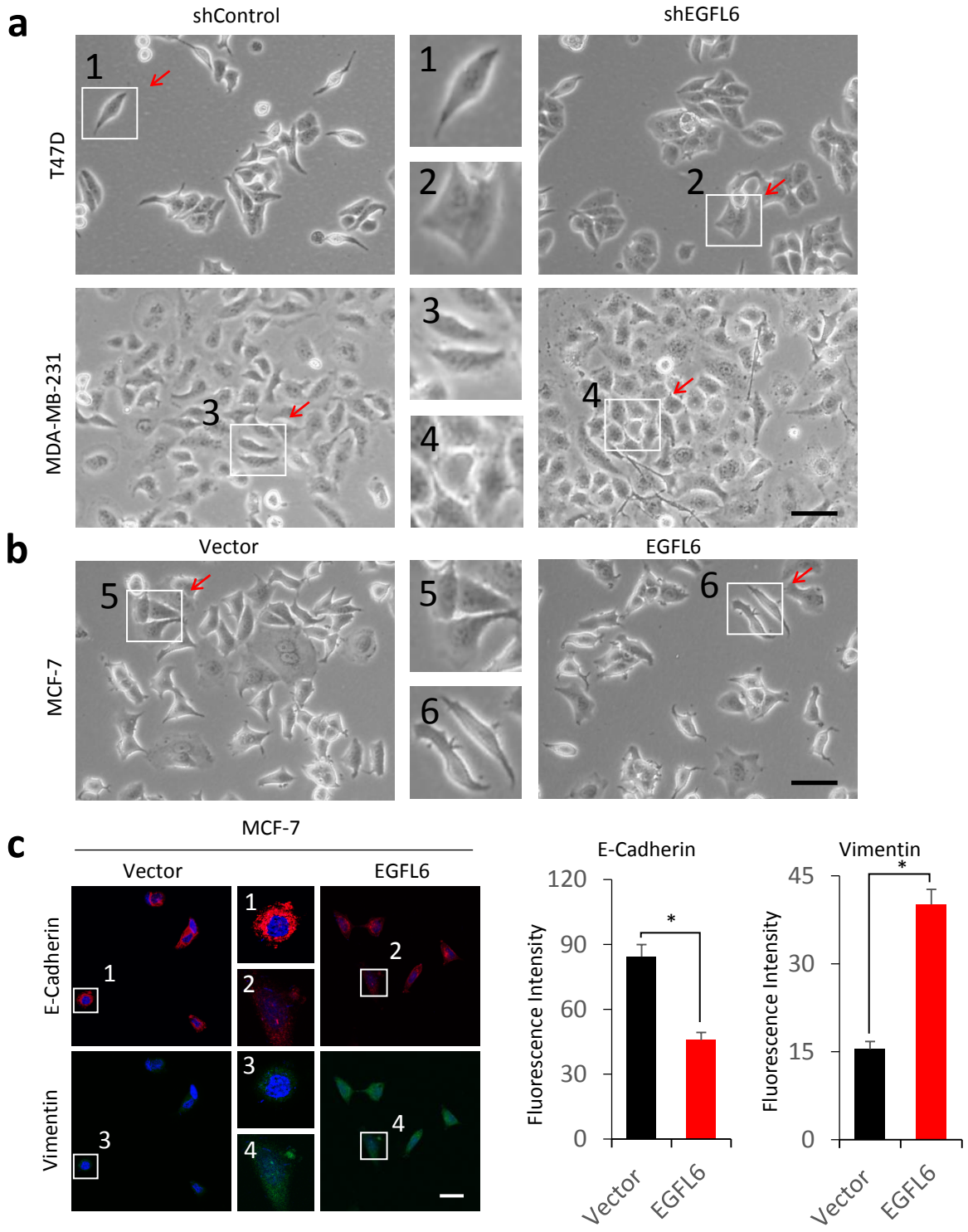

a
shControl
shEGFL6
T47D
MDA-MB-231
1
2
3
4
b
Vector
EGFL6
MCF-7
5
6
c
MCF-7
Vector
EGFL6
E-Cadherin
Vimentin
E-Cadherin
Vimentin
Fluorescence Intensity
120
90
60
30
0
45
30
15
0
*
Vector
EGFL6

Figure S3. EGFL6 expression is associated with EMT. **a,** Morphological images of the paired T47D and T47D/shEGFL6 and MDA-MB-231 and MDA-MB-231/shEGFL6. Scale bar, 40 μm. **b**, Morphological images of MCF-7 in comparison with MCF-7/EGFL6 cells. Scale bar, 40 μm. **c**, Immunofluorescence staining of E-cadherin (Red) and vimentin (Green) in paired MCF-7 and MCF-7/EGFL6 cells. Nuclei were visualized with DRAQ5 staining (blue). Representative images and quantitative data of the staining intensity by image J are shown. $p < 0.05$. Error bar, SD. Scale bar, 20 μm. All experiments were repeated three times, n=3..

Fig.S4

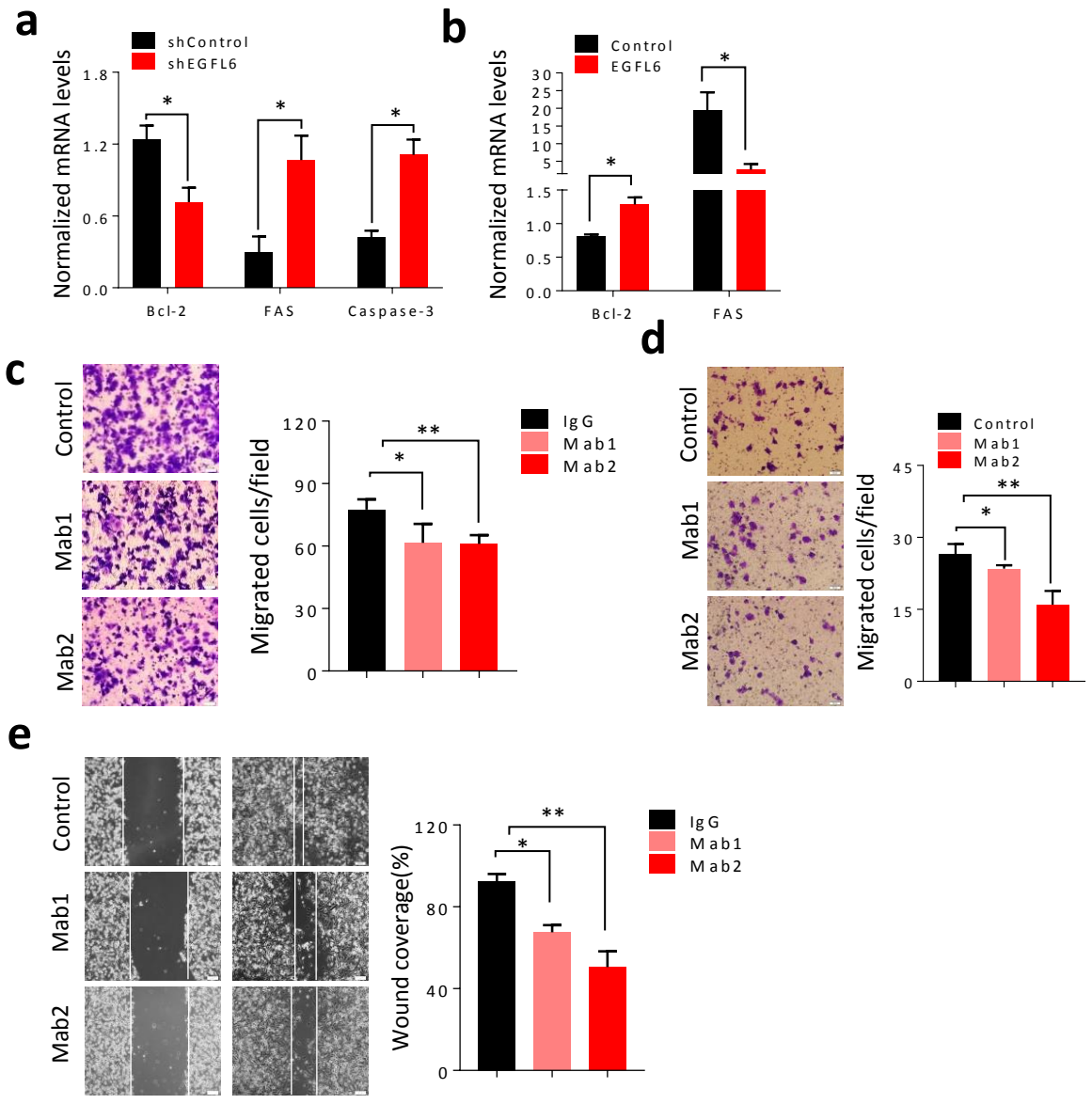


Figure S4. **Anti-EGFL6 antibody showed inhibition of cancer cell migration and knockdown EGFL6 increased cell apoptosis**. **a**, Increased apoptosis markers in EGFL6 knockdown cells (T47D/shEGFL6) in comparison with the parental control (shControl) by qRT-PCR. **b**, Decreased apoptosis markers in MCF-7/EGFL6 cells (EGFL6) in comparison with the parental MCF-7 cells (Control) by qRT-PCR. c, Representative images and quantitative results are shown for MDA-MB-231/EGFL6 cells treated with anti-EGFL6 antibodies Mab1 and Mab2 for 24 hours, at 10μg/ml concentration and isotype IgG was used as a treatment control. **d**, Representative images and quantitative results are shown for T47D cells treated with anti-EGFL6 antibodies Mab1and Mab2 for 24 hours, at 10μg/ml concentration and isotype IgG was used as a treatment control. **e**, Inhibition of cell migration by anti-EGFL6 antibodies Mab1 and Mab2 (10μg/ml) in MDA-MB-231/EGFL6 cells by wound healing assay. Representative images and quantitative results are shown. All experiments were repeated 3 times, *, $p<0.05$, **, $p<0.01$, and error bars indicate standard deviation (SD).

TableS1: Primers for qRT-PCR

E-cadherin-F	GTCTCTCTCACCACCTCCACAG
E-cadherin-R	CTCGGACACTTCCACTCTCTTT
Vimentin-F	GAAGAGAACTTTGCCGTTGAAG
Vimentin-R	GAAGGTGACGAGCCATTTC
Twist-F	GCAAGAAGTCGAGCGAAGAT
Twist-R	GCTCTGCAGCTCCTCGAA
N-cadherin-F	TGCTACTTTCCTTGCTTCTGAC
N-cadherin-R	TAACACTTGAGGGGCATTGTC
Fibronectin F	AGGAAGCCGAGGTTTTAACTG
Fibronectin R	AGGACGCTCATAAGTGTCACC
Snai1-F	TCGGAAGCCTAACTACAGCGA
Snai1-R	AGATGAGCATTGGCAGCGAG
ALCAM-F	TCCTGCCGTCTGCTCTTCT
ALCAM-R	TTCTGAGGTACGTCAAGTCGG
BMI1-F	CGTGTATTGTTCGTTACCTGGA
BMI1-R	TTCAGTAGTGGTCTGGTCTTGT
CD44-F	CTGCCGCTTTGCAGGTGTA
CD44-R	CATTGTGGGCAAGGTGCTATT
ITGB1-F	CCCAGAGGCTCCAAAGATATAAA
ITGB1-R	GCTGTGGTTGGATCTGAGTAA
FGFR2-F	ACTGGAGCCTCATTATGGAAAG
FGFR2-R	AGGTGGTACGTGTGATTGATG
KLF4-F	TCGCCTTGCTGATTGTCTATT
KLF4-R	AATTGGCCGAGATCCTTCTTC
SOX2-F	GAGAGAAAGAAGAGGAGAGAGAAAG
SOX2-R	GCCGCCGATGATTGTTATTATT
NANOG-F	CTCCCTAACAGCTGGGATTTAC
NANOG-R	GACGGCAGCCAAGGTTATTA
OCT4-F	GGAGGAAGCTGACAACAATGA
OCT4-R	CTCTCACTCGGTTCTCGATACT