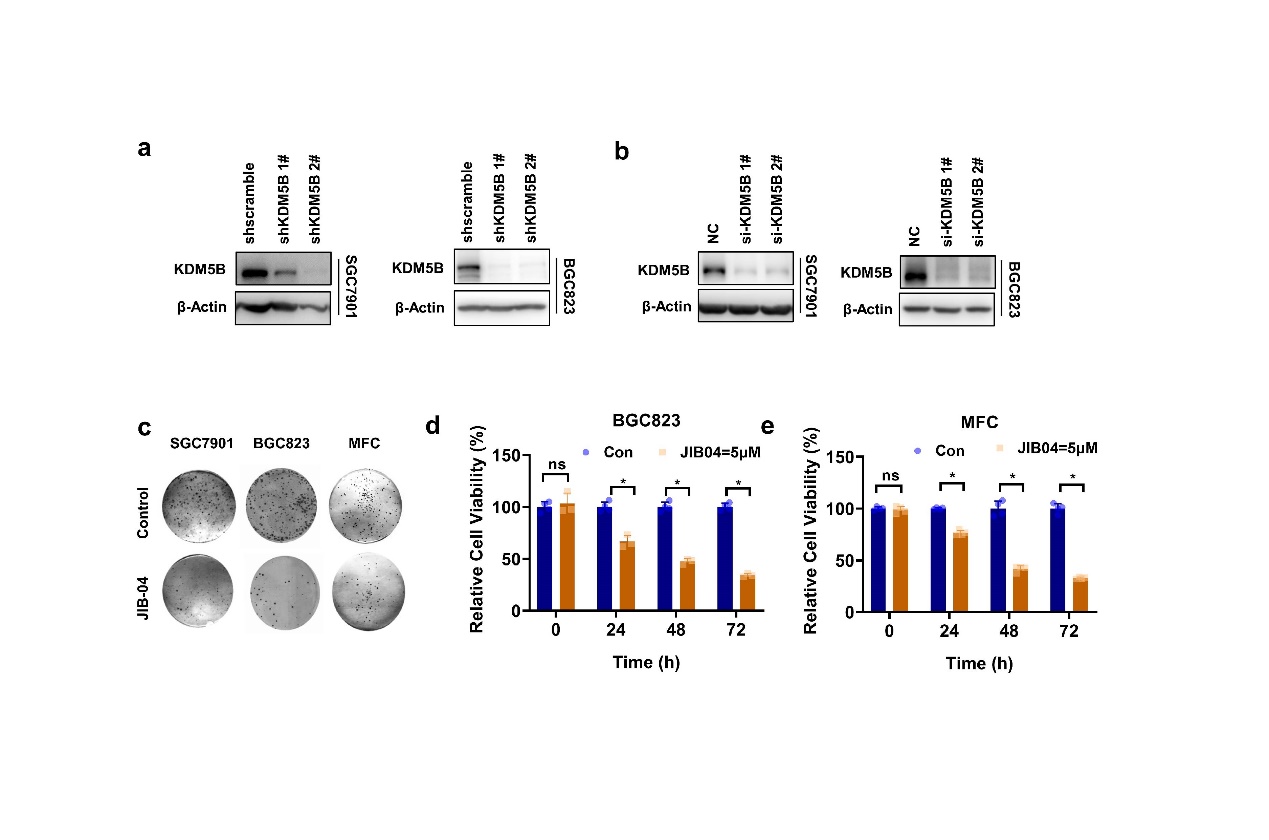
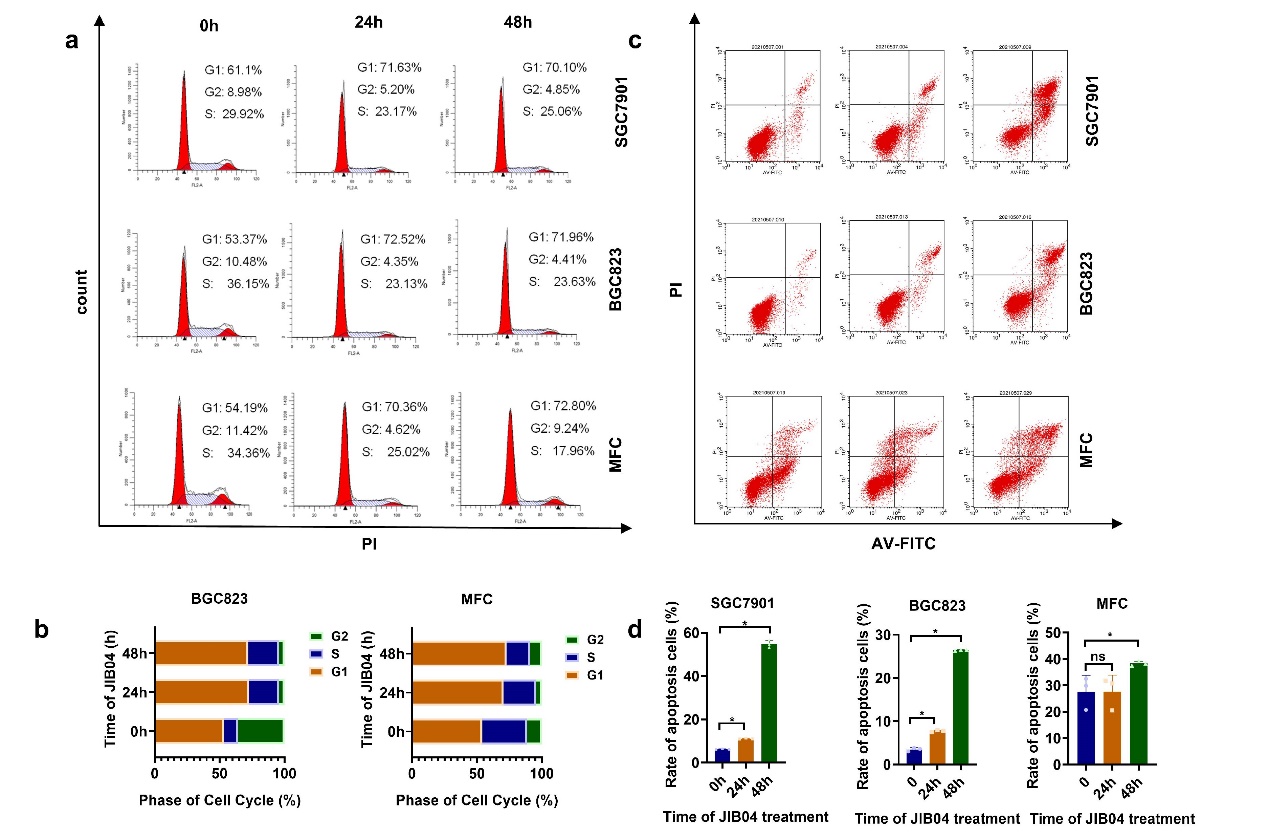
**Supplementary Figures**



**Figure. S1**

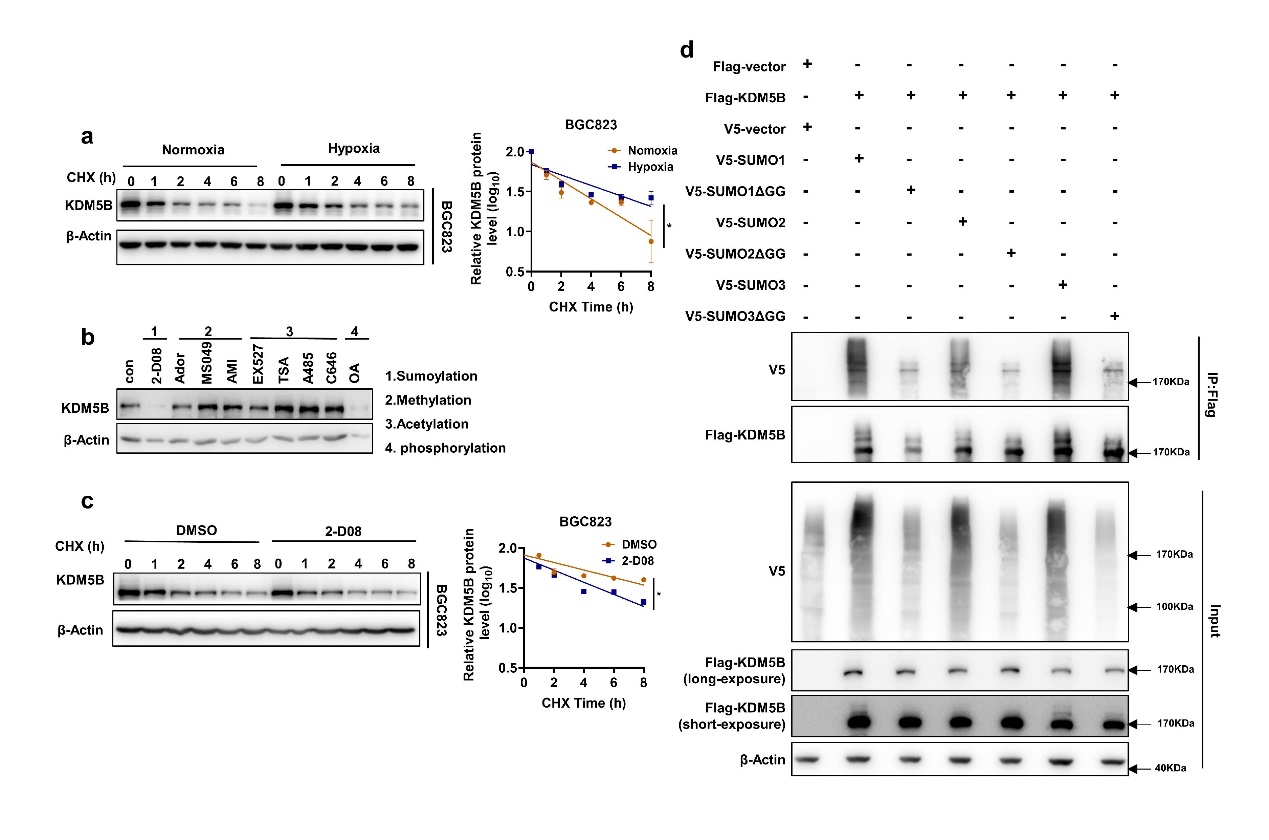
**KDM5B was upregulated to promote cell proliferation in gastric cancer a-b** The knocking down of KDM5B by shRNA or siRNA in SGC7901 and BGC823 cells was verified by Western blotting. **c** Colony formation of SGC7901, BGC823 and MFC cells treated with JIB04 (1 μM). **d, e** Relative cell viability of BGC823 and MFC cells at 24h, 48h and 72h after treatment of JIB04 (5 μM) were determined by CCK8 assay (*mean ± SD, n = 3, one-way ANOVA, \*p<0.05*).

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**Figure. S2**

**KDM5B inhibition induced G1/S arrest and apoptosis**

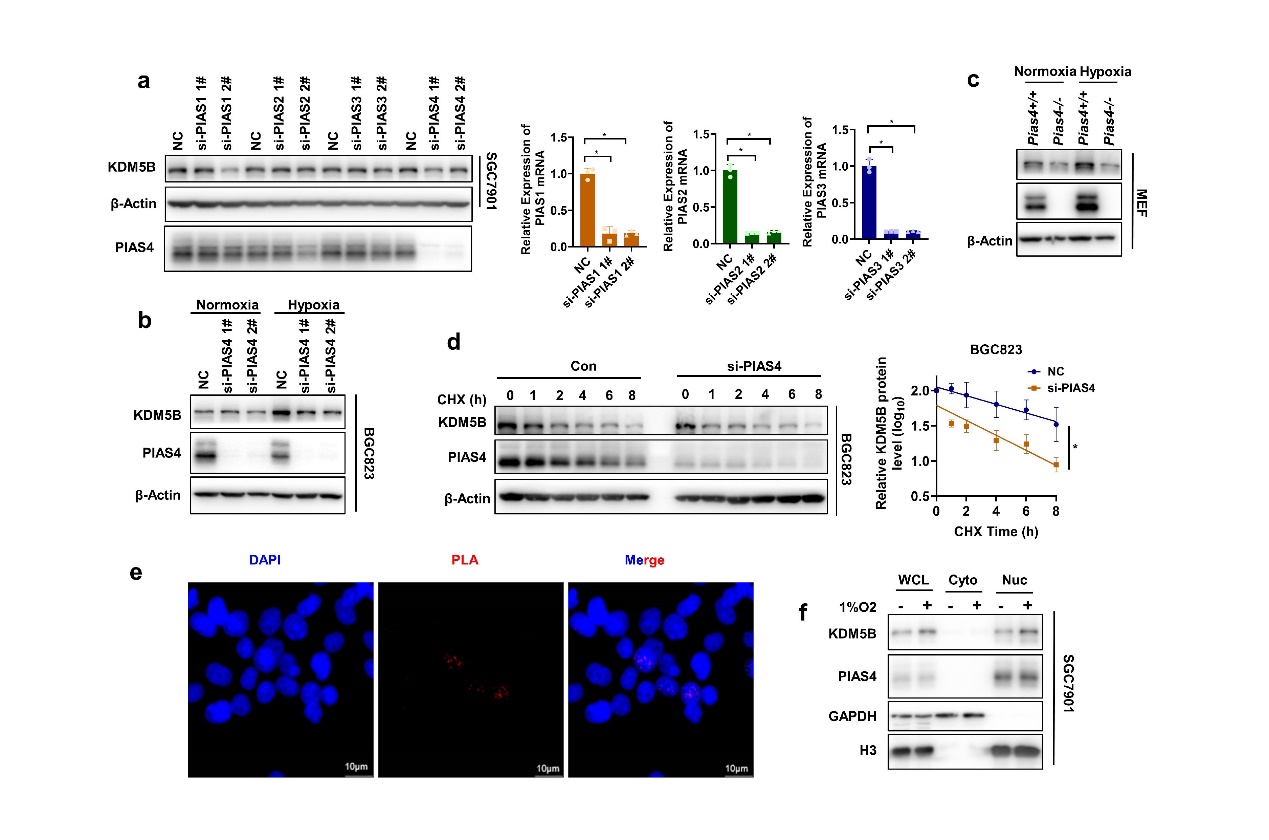
**a, b** Flow cytometry analysis of cell cycle in SGC7901, BGC823 and MFC cells before and after treatment of JIB04 at 24 and 48 hours. **c, d** Flow cytometry analysis of cell apoptosis in SGC7901 cells before and after treatment of JIB04 (5μM) at 24h and 48h hours.

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**Figure. S4**

**Hypoxia induced SUMO3-dependent SUMOylation and subsequent stabilization of KDM5B**

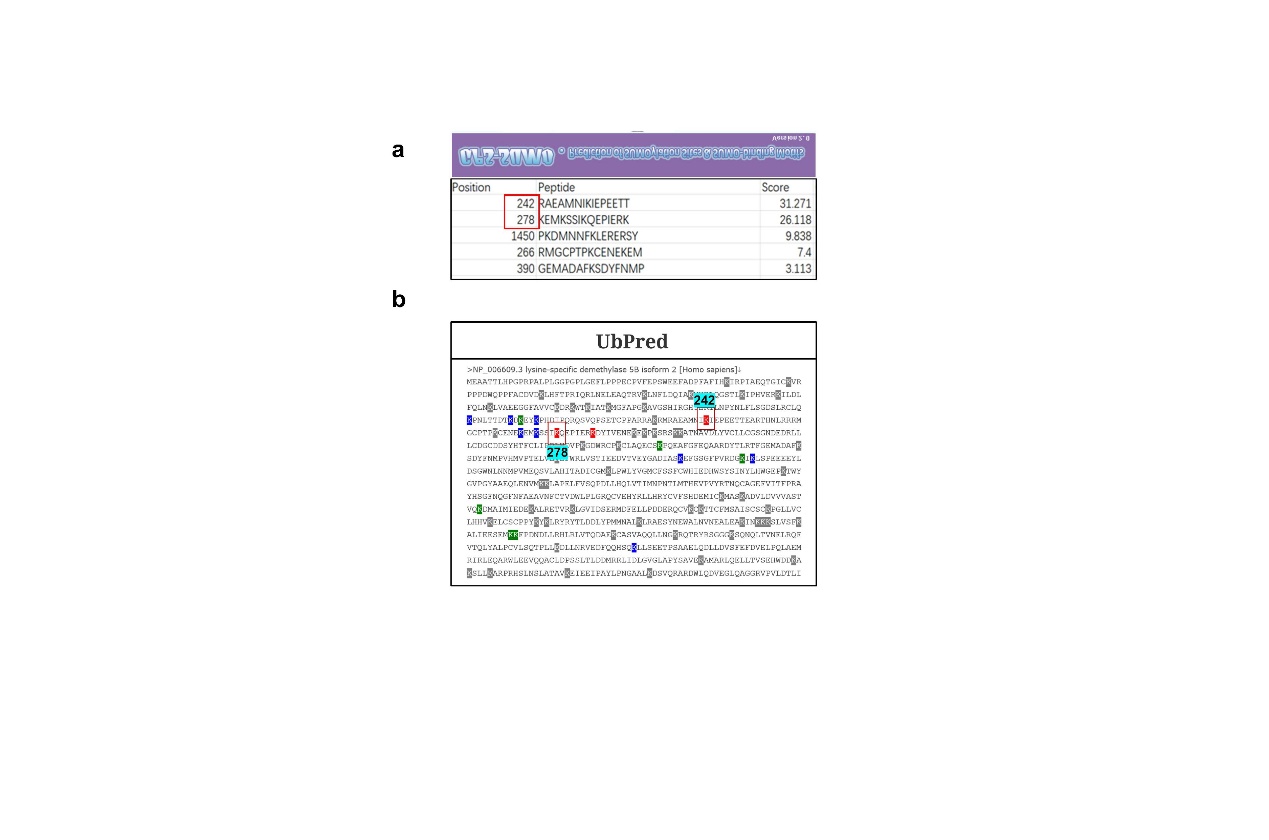
**a** The half-life of KDM5B protein in BGC823 cells under normoxia and hypoxia (1%O2) was determined by CHX assay. The relative KDM5B protein expression was quantified by ImageJ (*mean ±* *SD, n = 3,* ANCOVA analysis*, \*p<0.05*). **b** KDM5B expression in SGC7901 cells treated with various inhibitors as indicated，2-D08 (200μM), ADOX (20μM), MS049 (10μM), AMI-1 (10μM), EX527 (100nM), TSA (1μM), A485 (10nM), C646 (10μM), and okadaicacid (OA) (10nM) for 24h were determined by Western blotting. **c** The half-life of KDM5B protein in BGC823 cells under hypoxia (1%O2) with 2-D08 (200μM) or DMSO treatment was determined by CHX assay. The relative KDM5B protein expression was quantified by ImageJ (*mean ± SD, n = 3,* ANCOVA analysis*, \*p<0.05*). **d** HEK293T co-transfected with indicated plasmids for 48h under normoxia, and the SUMOylation assay with Flag antibody was performed followed by western blot analysis.

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**Figure. S5**

**PIAS4 was the SUMO E3 ligase for hypoxia-induced KDM5B SUMOylation.**

**a** The protein expression of KDM5B was determined by Western blotting with or without the PIAS1/2/3/4 knocking down by siRNA with 1%O2 treatment for 6h. **b** The protein expression of KDM5B in BGC823 under normoxia and 1%O2 condition for 6h with or without PIAS4 knockdown by siRNA was analyzed by western blotting. **c** Western blotting analysis of the protein expression of KDM5B in *Pias4*+/+ and *Pias4-/-* MEF under normoxia and 1%O2.for 6h. **d** BGC823 was transiently transfected with PIAS4 siRNA for 48h later with cycloheximide (CHX) and 1% O2 treatment for the indicated times, and then the half-life of KDM5B was analyzed by western blotting. **e** PLA assay was performed withing Flag and PIAS4 antibody after 293T co-transfected with Flag-KDM5B and HA-PIAS4 for 48h. The red PLA spots in the nucleus indicated a positive PLA signal, suggesting interactions between the two proteins. **f** The nuclear and cytosol fractions were isolated using the Nuclear-Cytosol Extraction Kit (Thermo, USA), then the distribution of KDM5B and PIAS4 was analyzed by western blotting (H3 and GAPDH were used as marker of nuclear and cytosol fraction separately).



**Figure. S6**

**PIAS4-mediated KDM5B SUMOylation prevents it from ubiquitination-dependent proteasomal degradation**

**a** The SUMOylation modification sites of KDM5B were predicted by GPS-SUMO based on the SUMO modification consensus motif. K242 and K278 were the top 2 sites which were the most potential sites modified by SUMOylation. **b** The ubiquitination sites of KDM5B were predicted by UbPred. K242 and K278 were in the red highlight, means the strongest possibility be ubiquitinated at these 2 sites.

**Supplementary Tables**

**Table. S1**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence** | **supplier** |
| siKDM5B-1# | GAGGGCAUUAUGAACGAAUTT  AUUCGUUCAUAAUGCCCUCTT | Gene Pharma Company (Shanghai, China) |
| siKDM5B-2# | GGAGACUAGUAAGCACUAUTT  AUAGUGCUUACUAGUCUCCTT | Gene Pharma Company (Shanghai, China) |
| siPIAS1-1# | GGAACUAAAGCAAAUGGUUTT  AACCAUUUGCUUUAGUUCCTT | Gene Pharma Company (Shanghai, China) |
| siPIAS1-2# | GCAGCCUGGUUUCUUCCAATT  UUGGAAGAAACCAGGCUGCTT | Gene Pharma Company (Shanghai, China) |
| siPIAS2-1# | CCCUGCGGUUCAGAUUAAATT  UUUAAUCUGAACCGCAGGGTT | Gene Pharma Company (Shanghai, China) |
| siPIAS2-2# | GCCUAUGAAAGUCUAAUAUTT  AUAUUAGACUUUCAUAGGCTT | Gene Pharma Company (Shanghai, China) |
| siPIAS3-1# | GCAAGUGCAGCAGAUUCUUTT  AAGAAUCUGCUGCACUUGCTT | Gene Pharma Company (Shanghai, China) |
| siPIAS3-2# | CCCUUUAUCUACAGAUGAATT  UUCAUCUGUAGAUAAAGGGTT | Gene Pharma Company (Shanghai, China) |
| siPIAS4-1# | GCUGAAGCCCACCGAAUUATT  UAAUUCGGUGGGCUUCAGCTT | Gene Pharma Company (Shanghai, China) |
| SiPIAS4-2# | GCUCUACGGAAAGUACUUATT  UAAGUACUUUCCGUAGAGCTT | Gene Pharma Company (Shanghai, China) |

**siRNA sequences used for knockingdown**

**Table. S2**

**Primer sequences used for clone**

|  |  |
| --- | --- |
| PIAS4 **Δ**RING-C337S | F:5'-CTCTGCACGGGAGGGCACCGAGAG-3'  R:5'-CTCTCGGTGCCCTCCCGTGCAGAG-3' |
| PIAS4 **Δ**RING-C342S | F:5'-GCAGGTGTGCGGAGGTCTCTGCACGG-3'  R:5'-CCGTGCAGAGACCTCCGCACACCTGC-3' |
| PIAS4 **Δ**RING-H344A | F:5’-TGCAGAGACCTCCGCAGCCCTGCAGTGCTTTG-3’  R:5'-CAAAGCACTGCAGGGCTGCGGAGGTCTCTGCA-3' |
| PIAS4 **Δ**RING-C347S | F:5'-ACACAGCATCAAAGGACTGCAGGGCTGCG-3'  R5’-CGCAGCCCTGCAGTCCTTTGATGCTGTGT-3’ |
| PIAS4-W363A | F:5'-CACACGGGGCACATCGCGGTGGGCTTCTTCTC-3'  R:5'-GAGAAGAAGCCCACCGCGATGTGCCCCGTGTG-3' |

**Table. S3**

**Primer sequences used for qPCR**

|  |  |
| --- | --- |
| HUMAN-P21 | F:5’-CTGGAGACTCTCAGGGTCGAAA-3’  R:5’-GATTAGGGCTTCCTCTTGGAGAA-3’ |
| HUMAN-ZBTB17 | F:5’-TGTAACCCCTCCCTCCAAGC-3’  R:5’-GTCTAGCACACAGCTCTGAACG-3’ |
| HUMAN-CDC45 | F:5’-CTTGAAGTTCCCGCCTATGAAG-3’  R:5’-GCATGGTTTGCTCCACTATCTC-3’ |
| HUMAN-TP53 | F:5’-CCTGGTCCTCTGACTGCTCT-3’  R:5’-GTGTAGGAGCTGCTGGTGCA-3’ |
| HUAMN-ABL1 | F:5’-AGGAGCTCTCATGGGTGAACA-3’  R:5’-GTTCTCCCCTACCAGGCAGTT-3’ |
| HUMAN-PLK1 | F:5’-AAAGAGATCCCGGAGGTCCTA-3’  R:5’-GGCTGCGGTGAATGGATATTTC-3’ |
| HUMAN-CDC20 | F:5’-GCACAGTTCGCGTTCGAGA-3’  R:5’-CTGGATTTGCCAGGAGTTCGG-3’ |
| HUMAN-KDM5B | F:5’-AATCAAACTGAGCCACCCCA-3’  R:5’-CAGTCCACCTCATCTCCTTCTG-3’ |
| HUMAN-ACTINB | F:5’-ACTCTTCCAGCCTTCCTTCC-3’  R:5’-CGTCATACTCCTGCTTGCTG-3’ |
| HUMAN-p21-chip-p1 | F:5’-AGTGTGGCCAAAGGATCTGA-3’  R:5’-ACTGAGATTTGCAGCAGACAC-3’ |
| HUMAN-p21-chip-p2 | F:5’-GCCCATTAATATTATAGGTCTGGC-3’  R:5’-CAATGCTGGC CTCGAAG-3’ |
| HUMAN-p21-chip-p3 | F:5’-GCCTCTCTTCAAACATTGTACAAG-3’  R:5’-TCTATGAGAGTCCTTGTGGGC-3’ |
| HUMAN-p21-chip-p4 | F:5’-GGAGTCTCACTCTGTCACCCA-3’  R:5’-AATATGGTG AAACCCCGTC TC-3’ |
| HUMAN-p21-chip-p5 | F:5’-AGACGGGGTTTCACCATATTG-3’  R:5’-GATTACAGGCATGCACCACC-3’ |
| HUMAN-p21-chip-p6 | F:5’-ATGGTGGTGCATGCCTGTA-3’  R:5’-TCAGCTTTCAGAGGAATTCACC-3’ |
| HUMAN-p21-chip-p7 | F:5’-TGAAGGTGAATTCCTCTGAAAGC-3’  R:5’-GACAAGAGTGCCCAGTCCAG-3’ |
| HUMAN-p21-chip-p8 | F:5’-CTGGACTGGGCACTCTTGTC-3’  R:5’-GACAAAATAGCCACCAGCCTC-3’ |