Targeting USP47 overcomes tyrosine kinase inhibitor resistance and eradicates leukemia stem/progenitor cells in chronic myelogenous leukemia

Supplementary Information

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Supplementary Fig. 1 USP47 is regulated by the RAS/ERK pathway. a The mRNA expression of different USPs in vector (32D^{MIGIR}) and P210^{BCR-ABL} stably transfected 32D (32D^{BCR-ABL}) cells by real-time quantitative PCR (n=3 biologically independent samples per group). Data are mean ± s.d., two-sided Student’s t-test. ***, *p*<0.0001. b-c Primary CML cells, K562, and KBM5^{T315I} cells were treated with indicated inhibitors for 24 hours, and the indicated proteins were detected by Western blot. Source data are provided as a Source Data file.
**Supplementary Fig. 2 USP47 promotes CML cell survival in vivo.** 

a-c USP47 knockdown K562 cells (ShUSP47-2) and control K562 cells (Ctrl ShRNA) (8×10^6) were subcutaneously transplanted into nude mice. Tumor sizes at day 21 are shown (a), and tumor volumes were measured every 4 days (b). USP47 expression was measured by Western blot in tumor cells (c). Data are mean ± s.d., *P* values were analyzed by two-way analysis of variance (ANOVA), ****, *p*<0.0001. 

d The expression of indicated proteins was examined by immunohistochemistry in tumor tissues. Scale bar: 50 μm. Source data are provided as a Source Data file.
Supplementary Fig. 3 Usp47 knockout mice have normal hematopoietic system. 

Number of total white blood cells, red blood cells, granulocytes, lymphocytes, and platelets were analyzed with a blood analyzer in the PB of the *Usp47*+/+ (n=12) and *Usp47*−/− (n=13).
mice. WBC, total number of white blood cells; RBC, total number of red blood cells; PLT, platelets; HGB, hemoglobin. b, c Percentages and number of different blood cells in PB (b) and BM (c) were evaluated. (n=3 biologically independent samples per group) B220, B cells; CD3, T cells; CD4, helper T cells; CD8, cytotoxic T cells; G/M (Gr-1/Mac-1), myeloid cells; Ter119, erythroid cells. Data are mean ± s.d., two-sided Student’s t-test. ns, not significant. Source data are provided as a Source Data file.
Supplementary Fig. 4 *Usp47* knockout does not affect the homing efficacy of CML LSKs but eliminates GFP*+*LSKs in CML mice. a Number of GFP*+*Gr-1*+* cells was measured in PB at different times after BCR-ABL*T315I* retrovirus transplantation (n=6 biologically independent samples per group). Data are mean ± s.d., two-sided Student’s t-test. **, p<0.01; ***, p<0.001;
ns, not significant. **Usp47−/− and Usp47+/+ BM cells were infected with BCR-ABL retrovirus for 48 hours, and the percent of GFP+LSK cells were measured by FACS. The infected BM cells were injected into lethally irradiated Usp47+/+ mice. GFP+LSK cells were monitored by FACS in BM after 18 hours. The homing efficacy was calculated by the ratio of the percentage of GFP+LSK cells ([GFP+LSK] after transplantation 18 h/[GFP+LSK] before transplantation) in BM (n=3 biologically independent samples per group). Data are mean ± s.d., two-sided Student’s t-test. ns, not significant. At 35 days after BCR-ABL<sup>T315I</sup> retrovirus transplantation, the number of GFP+LSKs in BM was examined by FACS (n=3 biologically independent samples per group). Data are mean ± s.d., two-sided Student’s t-test. **, p<0.01. Source data are provided as a Source Data file.
Supplementary Fig. 5 USP47 interacts with and stabilizes YB-1. a The indicated proteins were detected by Western blot in USP47 stably knockdown K562 cells. b K562 cells were treated with P22077 (5 μM) or DMSO for 6 hours first. The cells were then treated with cycloheximide (CHX, 10 μM) for different times; the indicated proteins were determined by Western blot. c The mRNA expression of USP47, YB-1, and POLB in USP47 stably knockdown K562 cells compared to the control cells (n=3 biologically independent samples per group). Data are mean ± s.d., two-sided Student’s t-test. ns, no significant. d The expression of YB-1 in BM mononuclear cells from CML patients (n=41) compared to normal BM CD34+ cells (n=8). Data are mean ± s.d., two-sided Student’s t-test. ns, no significant. Source data are provided as a Source Data file.
Supplementary Fig. 6 YB-1 and USP47 regulate DNA damage repair in CML cells. 

a Time course analysis of the USP47 knockdown-induced DNA damage response by Western blot in KBM5^T315I cells. 

b YB-1, PCNA, and TOPO IIα mRNA levels after YB-1 knockdown at day 7 in KBM5^T315I and K562R cells (n=3 biologically independent samples per group). Data are mean ± s.d., P values were analyzed by one-way analysis of variance (ANOVA). *, p<0.05; **, p<0.01. 

c DNA damage protein expression was measured by Western blot after YB-1 knockdown in the KBM5^T315I cells. 

d In USP47 or YB-1 silenced KBM5^T315I cells, γH2AX foci were detected by immunofluorescence staining. Scale bars = 7.5 μm. 

e γH2AX expression was measured by Western blot after POLB knockdown in the K562 and
KBM5<sup>T315I</sup> cells. f Overexpress USP47 in YB-1 and/or POLB depleted K562 cells, then the indicated proteins were detected by Western blot. g After transfected with USP47 specific shRNA or control shRNA in K562 cells for 48 hours, then the cells were treated with AZD6738 (50 nM or 2500 nM) for 12 hours. Cell viability was measured by CCK-8. (n=3 biologically independent samples per group) Data are mean ± s.d., P values were analyzed by one-way analysis of variance (ANOVA). *, p<0.05; **, p<0.01. Source data are provided as a Source Data file.
Supplementary Fig. 7 P22077 targets USP47 and shows toxic effects on CML cells. 

Protein level of USP47 and YB-1 in primary CML cells (left) and their correlations were shown (middle). Primary CML cells were treated with P22077. The IC50 value was measured. The correlation between USP47 level and IC50 to P22077 was shown (right) (n=3 biologically independent samples per group). **, p<0.01. P value was analyzed by Pearson’s r correlation test, r=0.9733, r²=0.9473. 

b P22077 (30 mg/kg/day) or vehicle were given to mice transplanted with primary CML-R cells by intraperitoneal injection (n=6 biologically independent samples per group) from day 30 to day 45. ***, p<0.001. P value was analyzed by Mantel–Cox-log-rank test. 

c KBM5\textsuperscript{T315I} cells were treated with P22077 (10 μM) with or without MG132 (10 μM) for 8 hours, then proteins were extracted and subjected to Western
The band intensity is shown by histogram (n=3 biologically independent experiments). Data are presented as mean ± s.d. P values were analyzed by one-way analysis of variance (ANOVA), **, p<0.01. d USP7 or USP10 was silenced in KBM5T315I cells with a retroviral transduction system, and the expression of YB-1 protein was examined by Western blot. e USP7 or USP10 stably-knockdown K562 cells were treated with different concentrations of P22077 for 48 hours, the cell viability was measured by CCK8 assay (n=3 biologically independent samples per group). Data are mean ± s.d. P values were analyzed by one-way analysis of variance (ANOVA). *, p<0.05, **, p<0.01. f Wild-type female C57BL/6 mice (6 weeks, n=5 biologically independent samples per group) were treated with P22077 (30 mg/kg) or control solvent for 14 days. The mice were weighed every two days. Source data are provided as a Source Data file.
Supplementary Fig. 8 Gating strategy for human leukemia stem/progenitor cells (CD45+CD34+CD38⁻) in the BM of B-NDG mice. The gating strategy corresponds to Figure 7i.
## Supplementary Table 1 The information of CML patients

| ID     | Age (years) | Gender (F/M) | Imatinib-resistant | 2nd TKIs-resistant |
|--------|-------------|--------------|--------------------|--------------------|
| CML-1  | 52          | F            | Yes                | No                 |
| CML-2  | 60          | M            | No                 | No                 |
| CML-3  | 68          | M            | Yes                | No                 |
| CML-4  | 58          | M            | No                 | No                 |
| CML-5  | 60          | M            | No                 | No                 |
| CML-6  | 57          | M            | No                 | No                 |
| CML-7  | 53          | F            | No                 | No                 |
| CML-8  | 57          | F            | No                 | No                 |
| CML-9  | 59          | M            | No                 | No                 |
| CML-10 | 55          | M            | No                 | No                 |
| CML-11 | 60          | M            | Yes                | Yes                |
| CML-12 | 61          | M            | No                 | No                 |
| CML-13 | 56          | F            | No                 | No                 |
| CML-14 | 57          | F            | No                 | No                 |
| CML-15 | 53          | M            | No                 | No                 |
| CML-16 | 58          | M            | Yes                | Yes                |
| CML-17 | 67          | M            | No                 | No                 |
| CML-18 | 65          | M            | No                 | No                 |
| CML-19 | 65          | M            | No                 | No                 |
| CML-20 | 70          | M            | Yes                | No                 |
| CML-21 | 66          | M            | No                 | No                 |
| CML-22 | 71          | M            | No                 | No                 |
| CML-23 | 70          | M            | No                 | No                 |
| CML-24 | 63          | M            | Yes                | Yes                |
| CML-25 | 59          | M            | No                 | No                 |
| CML-26 | 56          | F            | No                 | No                 |
| CML-27 | 63          | F            | Yes                | No                 |
| CML-28 | 73          | M            | No                 | No                 |
| CML-29 | 68          | M            | No                 | No                 |
| CML-30 | 73          | M            | No                 | No                 |
| CML-31 | 61          | F            | Yes                | No                 |
| CML-32 | 63          | M            | No                 | No                 |
| CML-33 | 59          | M            | No                 | No                 |
| CML-34 | 57          | F            | No                 | No                 |
| CML-35 | 54          | M            | Yes                | No                 |
| CML-36 | 65          | F            | Yes                | No                 |
| CML-37 | 57          | M            | No                 | No                 |
| CML-38 | 64          | M            | No                 | No                 |
| CML-39 | 65          | F            | No                 | No                 |
| CML-40 | 68          | M            | No                 | No                 |
| CML-41 | 73          | M            | No                 | No                 |
Supplementary Table 2. ShRNA target sequences

|                   | USP47 shRNA          |               | BCR-ABL shRNA         |               | YB-1 shRNA         |               | USP7 shRNA         |               | USP10 shRNA        |               | STAT5 shRNA       |               | POLB shRNA        |               |
|-------------------|----------------------|---------------|-----------------------|---------------|-------------------|---------------|-------------------|---------------|-------------------|---------------|------------------|---------------|------------------|---------------|
| USP47-shRNA-1     | GAATCTGTCTTGAAACCAA  |               | BCR-ABL-shRNA         | AGCAGATCGAGACCATCTT  |             |               |               |               |                  |               |                  |               |                  |               |
| USP47-shRNA-2     | GCAATGACTTGCTATTTGAA |               |                       |               | YB-1-shRNA-1     | GGTTCACCCACCTTACTACAT |               |               |                  |               | YB-1-shRNA-2     | GGTCATCGCAACGAAGGTT |               |               |                 |               |
| USP7-shRNA-1      | TGCGAAATCTGCCATGGAA  |               |                       |               | USP7-shRNA-1     | TGCGAAATCTGCCATGGAA  |               |               |                  |               | USP7-shRNA-2     | CTCAGAACCCCTGTGATCAA |               |               |                 |               |
| USP10-shRNA-1     | CTCTCTTTTAGTGCTCTTTT |               |                       |               | USP10-shRNA-1    | CTCTCTTTTAGTGCTCTTTT |               |               |                  |               | USP10-shRNA-2    | CCTATGTGGAAACTAAGTA |               |               |                 |               |
| STAT5-shRNA-1     | GCCATGAATCGCTTCTCTTT |               |                       |               | STAT5-shRNA-1    | GCCATGAATCGCTTCTCTTT |               |               |                  |               | STAT5-shRNA-2    | GCCATGACCTACTCATTAAC |               |               |                 |               |
| POLB shRNA-1      | TACCCACACAAAATAAAGAG |               |                       |               |                  |               | POLB shRNA-1     | TACCCACACAAAATAAAGAG |               |               |                  |               | POLB shRNA-2     | CTGATATCAATTCTCTGTG |               |               |                 |               |
### Supplementary Table 3. Real-time PCR primer sequences for DUBs

| Name   | Forward (5’-3’)          | Reverse (5’-3’)          |
|--------|--------------------------|--------------------------|
| USP1   | AACTGCCATCATATTATACGT    | TTGTGCTCCATTCTTCTTA      |
| USP2   | TGCTGAGACCCCGACATCCT     | TGGGGTCTATCCGGGTAGCTA    |
| USP3   | CTGTTATCGCTGTGATGATG     | CCAAGTCTCTGTAAGTGTCTT    |
| USP4   | GTCTTTGTGAACCTCCTATG     | CAGATTCTTTGCTCATCAT      |
| USP5   | TGGCTATTGGTGTTGGAAG       | GGCAATCTCCAGGTAATT      |
| USP6   | ATGTGGAACCTGAAGGAA       | GCAATGATGTAACCTGTA       |
| USP7   | GCAAGTGCGAGATAGTCGAGGAGG | CCATGGTCTGAGAGGCTCTGAA  |
| USP8   | ACTATACACAATGATGACGGATA  | TGGACTGATGGCTTCTCTC      |
| USP9X  | AGGTGGTGGGATGCTTAT       | GAGGATCTGTTGTATGATAG     |
| USP9Y  | CTCAGTATACACAGAATAAATC   | TCTTCATGCCCCTCTAAA       |
| USP10  | GAATCTGTTCCAAGTTATAC     | CCACCAGTCTTCCTCAA       |
| USP11  | AGTAGAACTGCTGCTTGT       | ATAGAATCGGTATGCTGGA      |
| USP12  | TGCGTATAAGAGTGCAACCT     | GAGTGGCTATGCTATGGA       |
| USP13  | ACGAGCAACGAAATAAATC      | TTCCTCCATCTCAATCAGA      |
| USP14  | AAATGGCTTCCGCGCAGTAT     | TCCACCTTCGCGCAACT       |
| USP15  | GACAGGTATTAGTGATAGA       | GATAGAGATGAAGGAGAG      |
| USP16  | AGAAACCTGAGTTAAGTGATG    | CAATCTTGCTGCTCTCTC      |
| USP17L30 | AAACAAGATTGCCAAGAATG     | TAGAGGACATAGACGAGAG     |
| USP18  | AATGGTTCTGCCTCAATG       | CCAGTGATGTTAGGATT       |
| USP19  | TGGACCTGAGCAAGTTCTC      | ATAGTGGTGGATGACAGCATAG  |
| USP20  | TATGTTGGCTGCGGAGA        | AGGTTCACCGGTCAAGTTGT    |
| USP21  | AGAACCTGAGTTAAGTGATG     | CAATCTTGCTGTCTCCTC      |
| USP22  | GAGAGCAGGATGAATGGA       | AACAGCAACAGGGAATAC      |
| USP24  | TGAGATGCGCAGTATTAGA      | AAGTATCCAGGCAAGTAA      |
| USP25  | TCTCTCTTGAGCTGATATTG     | TTCTGTTGTGCTGTTGAA      |
| USP27X | ATGTTGTAAGGACTATGATATGAC | GAGGTTGAGGCTTGTAAT     |
| USP28  | AATGCTGGAACATTTGGA       | GGAAGATTCAGTAAAGAGAT    |
| USP29  | ATGCTGTTCTCAAGGTAG       | AACTCTTTCTGTTACACAT     |
| USP30  | ACTGATGATGAGGCTTTAG      | TTCCAATGACGAGGTAAT      |
| USP32  | ATGTTGGAACCTGAGAAGA      | GCAATGATGTAACCTGTA      |
| USP34  | GTTTGGACTATGCTAAT        | ACTGAGAAGGATTGATT       |
| Gene  | 1st DNA Segment                      | 2nd DNA Segment                      |
|-------|--------------------------------------|--------------------------------------|
| USP35 | ATTAGCAGGATGATTGAC                   | TGAACCTTCTTAACACGAG                 |
| USP37 | CATCAGTGTGTCAGTCA                    | CTCCAGGTCATTGTAAGTAA                |
| USP38 | CAGCATATTCCTCTCAG                    | ATAGCCAGTCAATCATTC                  |
| USP39 | GGCATCAGTGAAGAAGAA                   | AAGATTAGATATGGAGGAGCAACT            |
| USP40 | CTCTTCTCAGTTATTATACAC                | CAATCTCTTCTTCACTCT                 |
| USP41 | AATGGTTCTGCTCTCAATG                  | CCAGTGTTAGTGGAGTGTGTTGGA           |
| USP42 | ATCAATGATTCACACGATAGTAG              | TTGATGCTCCACACACTTA                 |
| USP43 | CTGCCTCTGCTACTGTTAA                  | GCCACATATGTCCACTAT                 |
| USP44 | CATTCCTCAGTGTTGACTCT                | TGTGTGCTGAAAGTCTCT                |
| USP45 | GCACATATTCCTCAGTCTGTAA               | GCGTGAATAAGTGGAGTCTAAGTA           |
| USP46 | AAGCAAGCAACATCTCGAAT                | CCTGGAACATACATACCATCAAA            |
| USP47 | AGGGTTGTTGATTACTCTGAC               | GCTACTTCTCTTGTTGGAAGGA             |
| USP48 | GCTTTCTACTTCTCTCTGTAA               | ATCAATGATTCACCCCTATT              |
| USP49 | GCTTGTGACCAGTGAAC                   | GAGGTAGTCTGAGATCATTACA             |
| USP50 | GAGATTACACTGAGACAT                  | AGACTTCTCCTTCCTTAGG                |
| USP51 | AACAGCAAGCACATGAAACAC               | CCTGGAACATACATACATCAAA             |
| USP52 | AGGGTTGTTGATTACTCTGAC               | GCTACTTCTCTTGTTGGAAGGA             |
| USP53 | GCTCCTCAGTAACACGATT                 | CTCATTGACAGGAGTAGAAGA              |
| USP54 | ATTCTTCTCCTTCTCTAATAG               | CTTACTCTGTTGATACTGTCT              |
| CYLD  | AGGCTTGGAGATAATGATTGG               | GCAGAATAAGGTTGAGTCTAAGTA           |
| USPL1 | CTCCACATAAGCCTCAGA                  | TCCACCTCAATGCATAGAATAGA            |
| UCHL1 | CAAAGAGTGTAGTCTCTAAAGTGTAA          | GCGTGAATAAGTGGAGTCTCCTAA           |
| UCHL3 | GGCAATTTCCTTCTGATGTAT               | TCTCCCTCTTCTTCTCTGCT              |
| UCHL5 | TTTAATAATGCTTGTGCTACTC              | CGAATATGATGCTCTCTCTAAT            |
| BAP1  | TATCTGTCTGCTTACATG                 | CAATATCATCATCATTCAATAC             |
| ATXN3 | AACATTGCCTGAAATACACT                | TAGTAACTCTCCTCTTCTG                |
| ATXN3L| ACCAATGAGAGAAGATGAAC                | GAAGCAGGAGTTGACACAT               |
| OTUB1 | AGGGATGCTGCTGAGATGAC                | CTTGCGGAGATGAGTACAGA              |
| OTUB2 | GAGGAGGCAAGATGTTCAAGAA              | ACAGTGCCATCTCCTCTCT               |
| OTUD1 | AGATGCTGATGATGATA                   | TAATGAATCATGTTAGACA               |
| OTUD3 | TCTGAAAGACAGCCTGAGAG               | CGAAGCAGGCAATTATTG                |
| OTUD4 | ATCCAAGCAGTCTTATAATCA               | ACTTCTCCTCTCCTACAT                |
| HIN1L | GAAGGATTATTAGGAGGAG                | AGTTACTGTTGAAGAGAGA               |
| OTUD5 | GGACTATCTGATGAAAGAATGC             | TCCGCTTTCTGTTAATGTA               |
| Gene  | Forward Sequence | Reverse Sequence |
|-------|-----------------|------------------|
| OTUD6A | AGTAGCATTGAATCTGTC | ACTCCATTTCTCTCTCT |
| OTUD6B | CAAGAATGACAAGAAGAG | TAACAGCAACAGAATCTA |
| YOD1  | ATACAAGACAGTAAGAA | TATCATCATTAGAGGAGAA |
| A20   | AATGAGATGAGGAGAAG | ATTAGATGAGATGAGTTTG |
| OTUD7A | AGCAATTCTAACAGCAATAAC | GTCTTGCTCTTCTCTTG |
| OTUD7B | AGCAGACACACACAGAATA | TCAGTTCATTTCCACTCCTT |
| TRABID | GACGTATGGCTCTTCTCTT | TGATGACTTTGTATGCTCTATG |
| VCP1P1 | TGGAGTAGTAACCAATTGAGA | TGAAGCCTGAATAGAAGA |
| BRCC3 | GATTACTATGGTCACCTTG | CATCATCCTCATCAATAG |
| COPS5 | ACTCAGATGCTCAATCAG | TGCGGATATTGCTTCTTG |
| COPS6 | CCCTCTTCTGAAGTTGA | GCCTCTCCATTGATTATAT |
| PSMD14 | CAATGCTAATATGATGCTTTA | GATATGAGAATGAAATGAT |
| PSMD7 | AAGAATAGTTGGCTGGTA | CGGAATTAGGACAGTATCT |
| AME7 | GAGTTGAGATTATCCGAATG | AGAGCGTGATATACTTG |
| AME7-LP | ATGGGAAGGTAGAGGAGAAT | TTGATAGGAACAGTGAGT |
| MPND  | GCACGCAACACAAAGTC | TCGGACTAGGAGAAT |
| MYSM1 | ATTGTATTGGACGGATTC | GTTGGATGCTTCTACTG |
| PRPF8 | GGATGAGACTGGGAATGAA | TGGTGTGGAAGATGG |
| EIF3F | TTCCTGATGAGCGCTGGTAA | GGTCATTGATGTTGCTT |
| EIF3H | GATGGACAGATGGATGAA | GACGCTGCTGATACTG |
| JOSD1 | GTGGATTGGAGGCGGAGAG | AGCAGGAGTTCAGAGT |
| JOSD2 | TGAGATCCTGCAAAGAGGT | ATCAGATTGACATCATAG |
| JOSD3 | TTGACAGTCGATGATA | TTCAGTAAATATCTCCTC |
**Supplementary Table 4. Real-time PCR primer sequences**

| Name       | Forward (5’-3’)                  | Reverse (5’-3’)                  |
|------------|----------------------------------|----------------------------------|
| BCR-ABL    | CGGGAGCAGCAGAAGAAGTGT            | CGAAAGGTTGGGGGTCATTTTC           |
| hUSP47     | GCTATGGGACTTGACTCT              | CACTCTCATCATATTCACTATC           |
| hYB-1      | AAGTGATGGAGGCTGGCTGAC           | TTCTTCATTCCGCTCTCTCTC           |
| hPOLB      | GTATTACTGTGGTTCTCTATT           | TGGTGACTCATGGATTTGTC            |
| hGAPDH     | CTTAGCACCCCTGGCCAAG             | TGGTCATGAGTCCTTCCTCAG           |
| mUspl      | GGAACACCGACGATGAAG              | CACCGAGAATCATAATCC              |
| mUsp2      | AGAGACCTGGACTTGAGA              | TGATTGGACACAGCATACA             |
| mUsp3      | TATTCCAAGTCAGTTCCAG             | TCTCATCAAGTTCCCTCTA             |
| mUsp4      | TTGAAGGAGACCTTAATCG            | ACACAGCCTATCCAAATTTC            |
| mUsp6      | GTCCCTACTGCTATGTCT             | CTTGAAGTTGGCTCTATTACA           |
| mUsp7      | TGATGATGATCTGTCTGT             | CAACTGCTGAGGAATATC              |
| mUsp8      | CAAGCAACACGCGGGAATTA           | ACTTGCGGCTCTTGTATTACA           |
| mUsp9x     | GAGAGGATGGCTGAATGGAT            | ACTGTGGTTGGATGAAGGCTAT          |
| mUsp10     | GATGGAAGTCAAAGAAGG             | CACTGGCCTATGTATGATTAGG          |
| mUsp14     | TGGTCTACTGACTTCAA              | GATTTTGATGACCTTAT              |
| mUsp19     | AAGAGGAAGAGAGAAGAGAAG         | AAGACTGAATGACGGCTAT             |
| mUsp36     | GTGTGCTAAGTGAAGAAGAAGAAGA     | AGAGTCAGGAGACTTGAGAT            |
| mUsp47     | TATCCAAAGTGTAGTGAAGAAGAAGA    | ACTCTGCTCATATTCCTACT            |
| mGAPDH     | CTTAGCCCCCCTGGCCAAG           | TGGTCATGAGCCTCTCCTCA            |

h=human, m=mouse