TMUB1 Inhibits BRL-3A Hepatocyte Proliferation by Interfering with the Binding of CAML to Cyclophilin B through its TM1 Hydrophobic Domain.

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Gene sequence

Tmubl sequence:

Transmembrane and ubiquitin-like domain-containing protein 1, Rattus norvegicus (245 aa)

MALIEGVGDEVTVLFSVLACLLVLALAWVSTHTTESTDPLPQSSGTTTPAQPSEAMTAIDSIREEAPGAESPSLRH
RGPSAQPEPEAGVTASTPPDSPQEPLLLRLKFLNDSEQVARAWPQDTIGSLKRTQFPGREQQVRLIYQGQLLGD
DTQTLGSLHLPPNCVLHCHVSTRVGPPHPPCPPGSEPGPSGLEIGSLLLPLLLLLLLLWYCQIQYRPFFPLTATLGL
AGFTLLLSLLAFAMYRP

TM1 TM2 UL

TMUB1 CDS sequence:

Atggccttgattgaaggcgtaggggatgaggtgactgtccttttttcggtgcttgcctgccttctggtgctggccctcgcctgggtctcaacaca
tacgactgagagtacagatcccctaccacagtcgtcagggaccacaacaccagcacagcccagtgaagccatgacagccattgatagcatc
agagaggaggccccaggagctgagagtcccagcctgaggcacagaggtccatctgcacagccagagcctgaggcaggggtcacagcatc
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ccattggctccttgaaaagaacccagtttccaggccgggaacagcaggttcgactcatctaccaaggccaactgctaggagacgacaccca
gacactgggcagtctccaccttccccccaactgcgttctccactgccacgtgtccacaagagtcggtccccgcatcctccctgcccaccggg
gtcagagcccggcccctccgggctggaaatcggcagccttctgttgcccctgctgcttctgctgctgctcctgctctggtactgccagatccagt
accggccctctttccccctgaccgctaccttgggcctggccggcttcaccctgctcctcagtctcctggcctttgccatgtatcgcccgtag

TM1 (580-642 bp) TM2 (655-717 bp) UL (304-525 bp)

Flag tag coded sequence:

ATGGATTACAAGGATGACGACGATAAG

Synthetic sequences

Flag-Tmubl

ATGGATTACAAGGATGACGACGATAAGatggccttgattgaaggcgtaggggatgaggtgactgtccttttttcggtgcttgcctg
ccttctggtgctggccctcgcctgggtctcaacacatacgactgagagtacagatcccctaccacagtcgtcagggaccacaacaccagcac
agcccagtgaagccatgacagccattgatagcatcagagaggaggccccaggagctgagagtcccagcctgaggcacagaggtccatctg
cacagccagagcctgaggcaggggtcacagcatcaacacctccagactctccacaggaacccctactgctacggttgaaatttctcaatgac
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gagtcggtccccccgcatcctccctgcccaccggggtcagagcccggcccctccgggctggaaatcggcagccttctgttgcccctgctgcttct
gctgctgctcctgctctggtactgccagatccagtaccggcccttctttcccctgaccgctaccttgggcctggccggcttcaccctgctcctca
gtctcctggcctttgccatgtatcgcccgtag

1. Flag-△TM1：knockout 580—642 bp

ATGGATTACAAGGATGACGACGATAAGatggccttgattgaaggcgtaggggatgaggtgactgtccttttttcggtgcttgcctgc
cttctggtgctggccctcgcctgggtctcaacacatacgactgagagtacagatcccctaccacagtcgtcagggaccacaacaccagcaca
gcccagtgaagccatgacagccattgatagcatcagagaggaggccccaggagctgagagtcccagcctgaggcacagaggtccatctgc
acagccagagcctgaggcaggggtcacagcatcaacacctccagactctccacaggaacccttactgctacggttgaaatttctcaatgact
ctgaacaggtggccagggcctggcctcaggacaccattggctccttgaaaagaacccagtttccaggccgggaacagcaggttcgactcat
ctaccaaggccaactgctaggagacgacacccagacactgggcagtctccaccttccccccaactgcgttctccactgccacgtgtccacaa
gagtcggtccccccgcatcctccctgcccaccggggtcagagcccggcccctccgggctggaacagtaccggcccttctttcccctgaccgcta
ccttgggcctggccggcttcaccctgctcctcagtctcctggcctttgccatgtatcgcccgtag

2. Flag-△TM2：knockout 655—717 bp

ATGGATTACAAGGATGACGACGATAAGatggccttgattgaaggcgtaggggatgaggtgactgtccttttttcggtgcttgcctgc
cttctggtgctggccctcgcctgggtctcaacacatacgactgagagtacagatcccctaccacagtcgtcagggaccacaacaccagcaca
gcccagtgaagccatgacagccattgatagcatcagagaggaggccccaggagctgagagtcccagcctgaggcacagaggtccatctgc
acagccagagcctgaggcaggggtcacagcatcaacacctccagactctccacaggaacccttactgctacggttgaaatttctcaatgact
ctgaacaggtggccagggcctggcctcaggacaccattggctccttgaaaagaacccagtttccaggccgggaacagcaggttcgactcat
ctaccaaggccaactgctaggagacgacacccagacactgggcagtctccaccttccccccaactgcgttctccactgccacgtgtccacaa

gagtcggtccccgcatcctccctgcccaccggggtcagagcccggcccctccgggctggaaatcggcagccttctgttgcccctgctgcttct

gctgctgctcctgctctggtactgccagatccagtaccggccctttgccatgtatcgcccgtag

3. **Flag-ΔUL1：knockout 304-453 bp**

ATGGATTACAAGGATGACGACGATAAGatggccttgattgaaggcgtaggggatgaggtgactgtccttttttcggtgcttgcctg

ccttctggtgctggccctcgcctgggtctcaacacatacgactgagagtacagatcccctaccacagtcgtcagggaccacaacaccagcac

agcccagtgaagccatgacagccattgatagcatcagagaggaggccccaggagctgagagtcccagcctgaggcacagaggtccatctg

cacagccagagcctgaggcaggggtcacagcatcaacacctccagactctccacaggaacccacccagacactgggcagtctccaccttcc

ccccaactgcgttctccactgccacgtgtccacaagagtcggtccccgcatcctccctgcccaccggggtcagagcccggcccctccgggct

ggaaatcggcagccttctgttgcccctgctgcttctgctgctgctcctgctctggtactgccagatccagtaccggcccttctttcccctgaccgc

taccttgggcctggccggcttcaccctgctcctcagtctcctggcctttgccatgtatcgcccgtag

4. Flag-△UL2：knockout 376-525 bp

ATGGATTACAAGGATGACGACGATAAGatggccttgattgaaggcgtaggggatgaggtgactgtccttttttcggtgcttgcctg

ccttctggtgctggccctcgcctgggtctcaacacatacgactgagagtacagatcccctaccacagtcgtcagggaccacaacaccagcac

agcccagtgaagccatgacagccattgatagcatcagagaggaggccccaggagctgagagtcccagcctgaggcacagaggtccatctg

cacagccagagcctgaggcaggggtcacagcatcaacacctccagactctccacaggaacccctactgctacggttgaaatttctcaatgac

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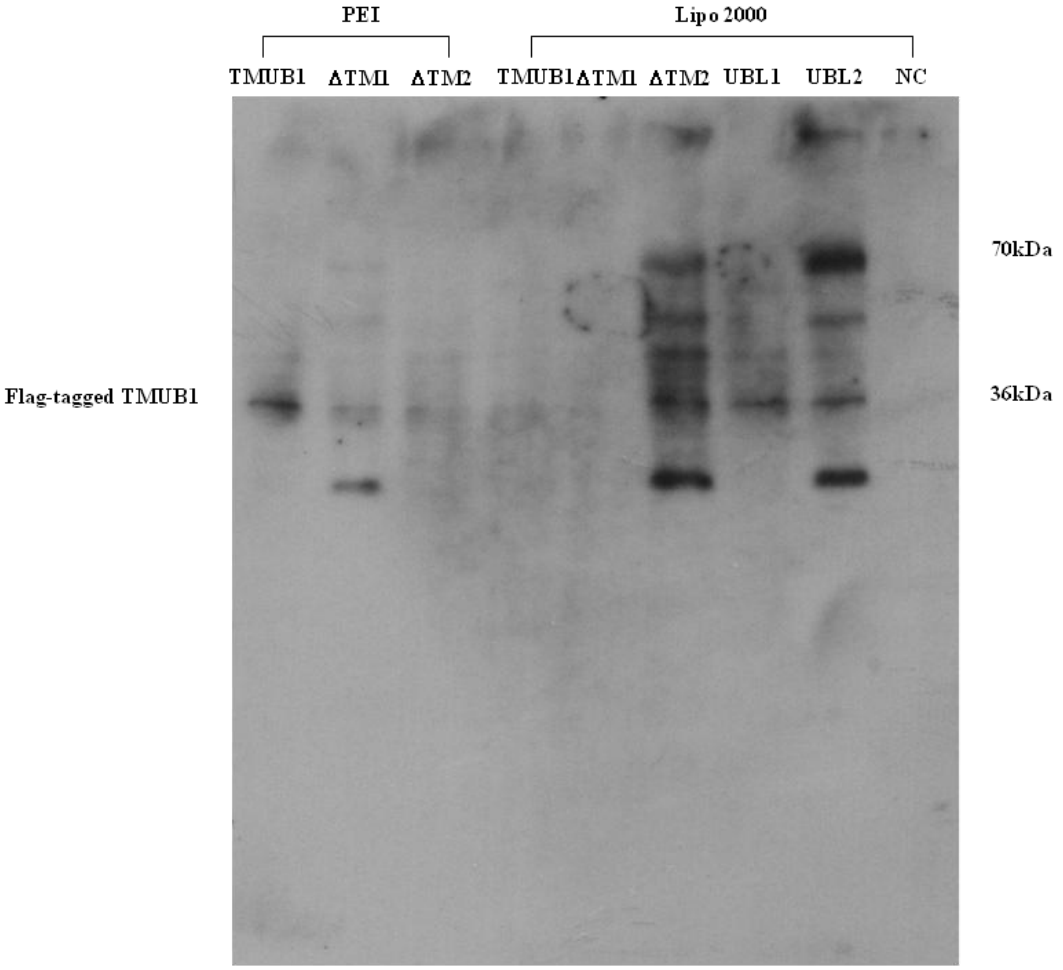


Fig 1. The first plasmid transfection with BRL-3A hepatocytes. PEI and Lipo 2000 was tested repectively. BRL-3A rat hepatocytes were cultured in high-glucose DMEMsupplemented with 10% fetal bovine serum at 37˚C with 5% CO2. When the hepatocytesreached 80-90% confluence, they were passagedand placed in a 100-mm culture dish.After 24 hours, when the cells had reached 40-60% confluence, they were passaged and placed in DMEM without penicillin-streptomycin. A total of 21 μl PEI or 72ulLipoFiterTM was mixed with 979ml DMEM (PEI) or 928ml DMEM (Lipo 2000) in a 100-mm culture dish and was not disturbed for 5 min. The final concentration of the plasmid was 7-8ug (PEI) or 24 μg (Lipo 2000) DNA/1ml DMEM. The two abovementioned solutions were mixed, left undisturbedfor 20 min and added to DMEM in a 100-mm culture dish with a final volume of 12 ml. The cells were cultured at 37˚C with 5% CO2 for 7 hours and were then changed from DMEM to high-glucose DMEM supplemented with 10% fetal bovine serum. After 24 hours, the cells were collected, and protein was extracted for Western blot analysis with an anti-Flag antibody.

In this transfection experiment, we can see flag-tagged TMUB1 was tested in both kinds of transfection solution but with lower transfection efficiency. Hepatocytes suffered from extensive death. We thought it was due to long time incubation with transfection solution. So we operated a scond transfection experiments and decreased the incubation time to 4-6 hours.

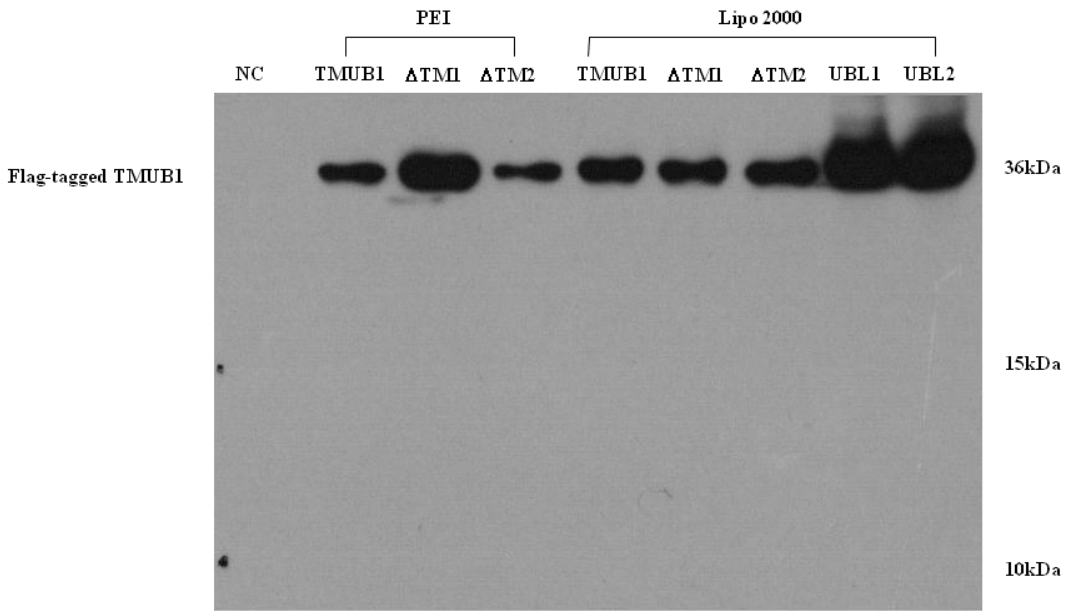


Fig 2. The second plasmid transfection with BRL-3A hepatocytes. PEI and Lipo 2000 was tested repectively. BRL-3A rat hepatocytes were cultured in high-glucose DMEMsupplemented with 10% fetal bovine serum at 37˚C with 5% CO2. When the hepatocytesreached 80-90% confluence, they were passagedand placed in a 100-mm culture dish.After 24 hours, when the cells had reached 40-60% confluence, they were passaged and placed in DMEM without penicillin-streptomycin. A total of 21 μl PEI or 72ulLipoFiterTM was mixed with 979ml DMEM (PEI) or 928ml DMEM (Lipo 2000) in a 100-mm culture dish and was not disturbed for 5 min. The final concentration of the plasmid was 7-8ug (PEI) or 24 μg (Lipo 2000) DNA/1ml DMEM. The two abovementioned solutions were mixed, left undisturbedfor 20 min and added to DMEM in a 100-mm culture dish with a final volume of 12 ml. The cells were cultured at 37˚C with 5% CO2 for 4-6 hours and were then changed from DMEM to high-glucose DMEM supplemented with 10% fetal bovine serum. After 24 hours, the cells were collected, and protein was extracted for Western blot analysis with an anti-Flag antibody.

In this transfection experiment, we can see flag-tagged TMUB1 was tested in both kinds of transfection solution with satified transfection efficiency. Hepatocytes suffered from extensive death. We thought it was due to long time incubation with transfection solution. So we operated a scond transfection experiments and decreased the incubation time to 4-6 hours.

So we choosed lipo 2000 as the transfection solution and incubation time was 4-6 hours.

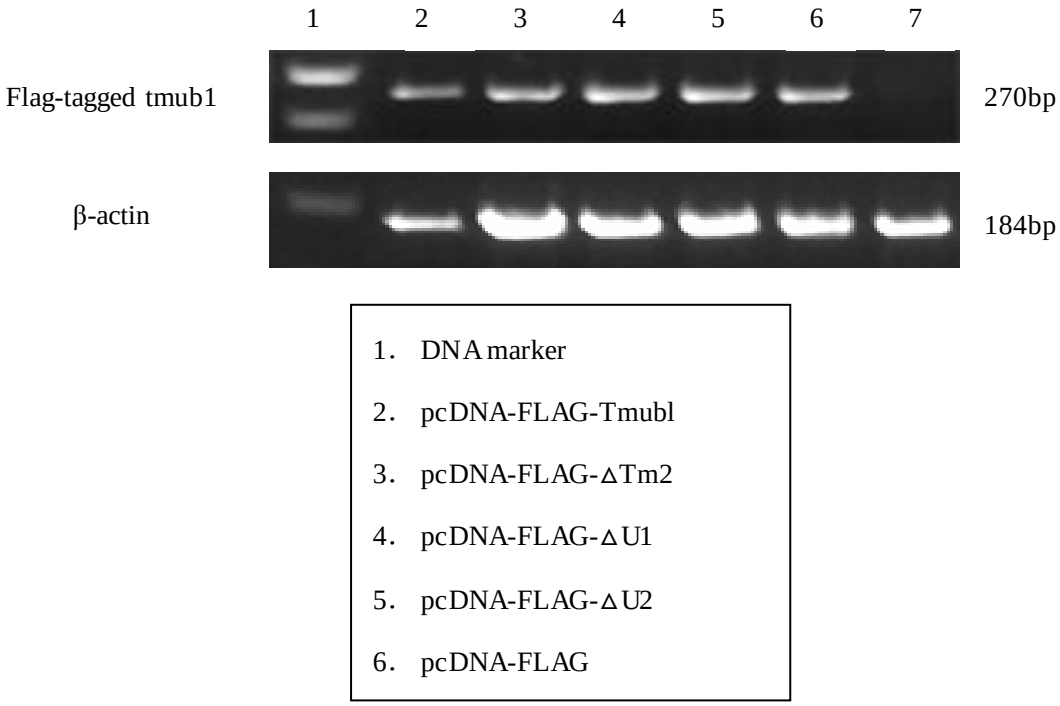
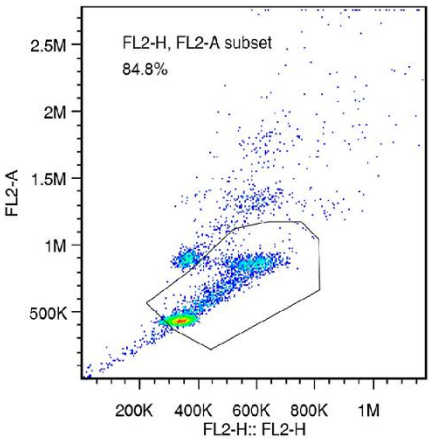
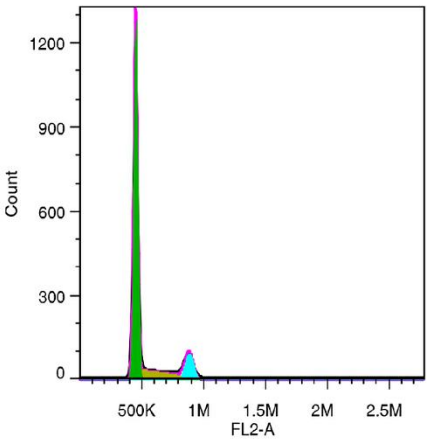


Fig3. PCR test for hypatocytes plasmid transfection. The sense primers are located in the region of Flag and the anti-sense primers are licated in the region of Tmub1. The sense and antisense primers for pcDNA-FLAG-Tmub1 and its mutant amplification are as follows: 5'-AAGGATGACGACGATAAGAT-3' and 5'-GAGGTGTTGATGCTGTGA-3', respectively.



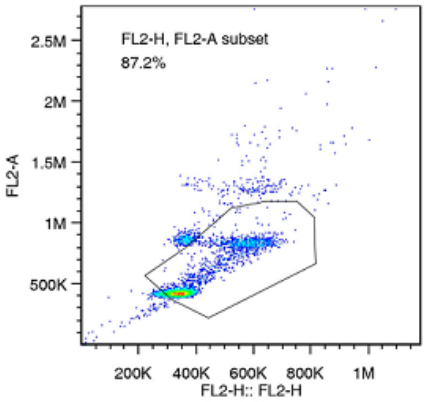
C06 6.fcs
FSC-A, SSC-A subset
8070



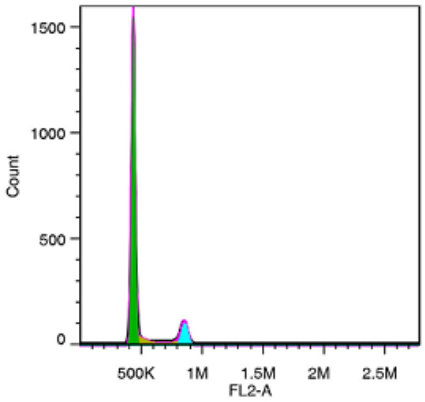
C06 6.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 1.46
G1 = 73.45
S = 15.31
G2 = 11.27
G1(mean) = 2731761.14
G2 (mean) = 5339320.86
G1 cv = 5.21
G2 cv = 5.54
sub-G1 = -1.07
super-G2 = 0.25

6841

Fig4A. Cell cycle tested: G0/G1 phase 0h



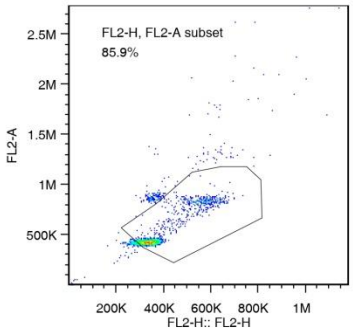
C07 7.fcs
FSC-A, SSC-A subset
7642



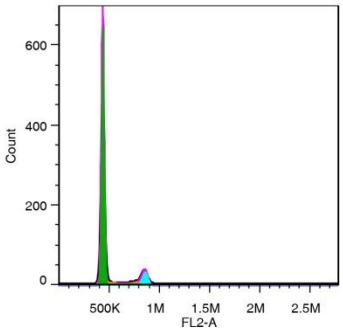
C07 7.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 2.26
G1 = 76.06
S = 13.83
G2 = 10.18
G1(mean) = 2643729.46
G2 (mean) = 5184530.88
G1 cv = 4.44
G2 cv = 4.52
sub-G1 = -1.14
super-G2 = -0.04

6665

Fig4B. Cell cycle tested: G0/G1 phase 2h



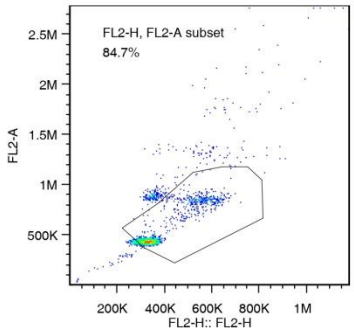
C08 8.fcs
FSC-A, SSC-A subset
3251



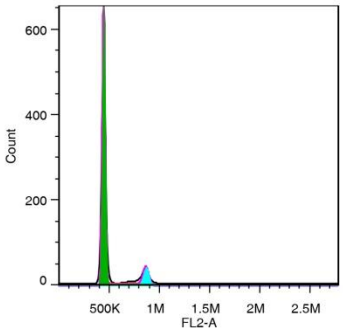
C08 8.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 1.13
G1 = 80.79
S = 10.71
G2 = 8.4
G1(mean) = 2651772.87
G2 (mean) = 5185776.67
G1 cv = 4.56
G2 cv = 4.78
sub-G1 = -0.56
super-G2 = -0.2

2794

Fig4C. Cell cycle tested: G0/G1 phase 4h



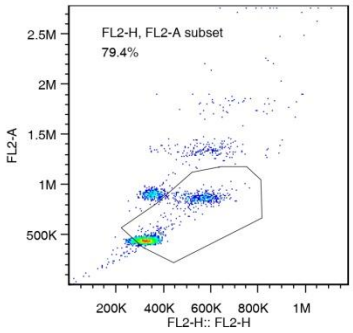
C09 9.fcs
FSC-A, SSC-A subset
3440



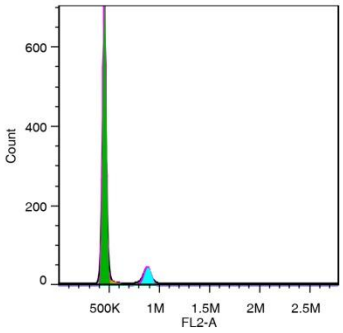
C09 9.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 1.06
G1 = 82.83
S = 7.29
G2 = 9.54
G1(mean) = 2708180.14
G2 (mean) = 5246335.81
G1 cv = 4.82
G2 cv = 4.61
sub-G1 = -0.21
super-G2 = 0.73

2914

Fig4D. Cell cycle tested: G0/G1 phase 6h



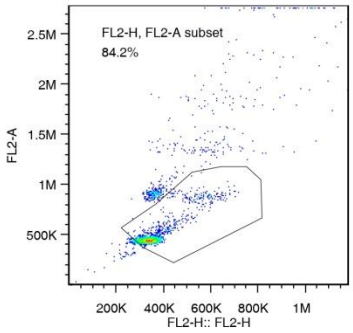
C10 10.fcs
FSC-A, SSC-A subset
4115



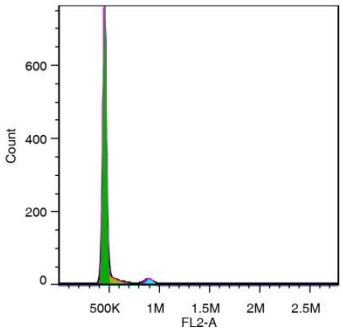
C10 10.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 0.7
G1 = 82.55
S = 6.99
G2 = 10.47
G1(mean) = 2744273.51
G2 (mean) = 5345305.26
G1 cv = 5.07
G2 cv = 5.29
sub-G1 = -0.87
super-G2 = 0.13

3266

Fig4E. Cell cycle tested: G0/G1 phase 8h



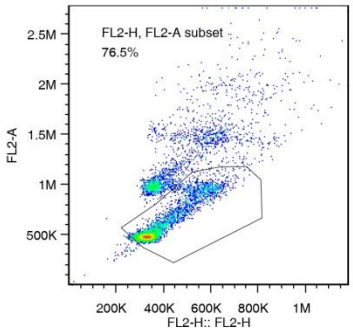
C11 11.fcs
FSC-A, SSC-A subset
3906



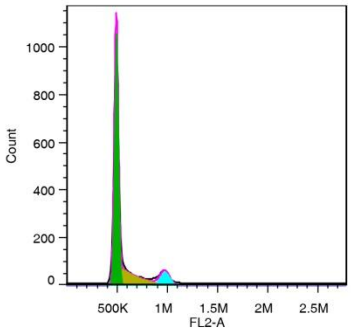
C11 11.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 0.59
G1 = 84.05
S = 12.02
G2 = 3.76
G1(mean) = 2761599.02
G2 (mean) = 5449474.43
G1 cv = 4.82
G2 cv = 5.48
sub-G1 = -0.74
super-G2 = 0.02

3290

Fig4F. Cell cycle tested: G0/G1 phase 10h



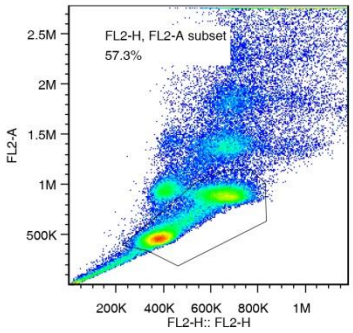
C12 12.fcs
FSC-A, SSC-A subset
9655



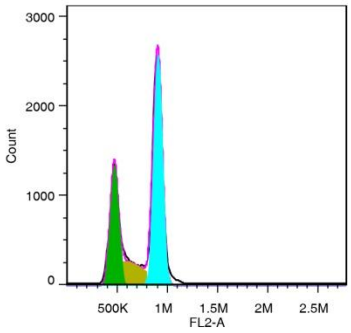
C12 12.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 1.52
G1 = 67.79
S = 22.69
G2 = 8.62
G1(mean) = 2981514.39
G2 (mean) = 5898221.95
G1 cv = 5.67
G2 cv = 6.89
sub-G1 = -0.85
super-G2 = 0.13

7390

Fig4G. Cell cycle tested: G0/G1 phase 12h



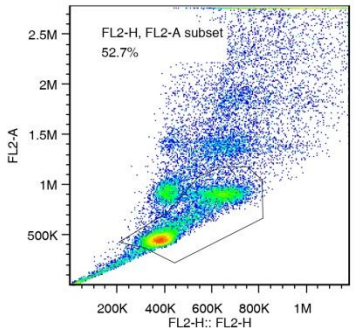
A01 1.fcs
FSC-A, SSC-A subset
87522



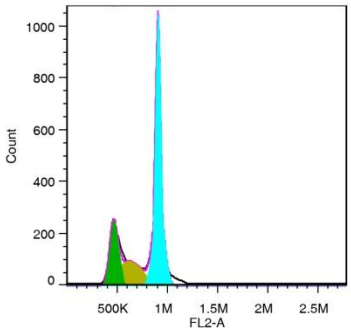
A01 1.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 8.38
G1 = 14.51
S = 5.15
G2 = 78.94
G1(mean) = 2864254.45
G2 (mean) = 5475712.71
G1 cv = 10.63
G2 cv = 7.32
sub-G1 = -0.61
super-G2 = 1.04

50122

Fig4H. Cell cycle tested: G2/M phase 0h



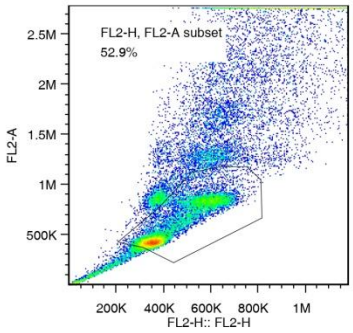
A02 2.fcs
FSC-A, SSC-A subset
28528



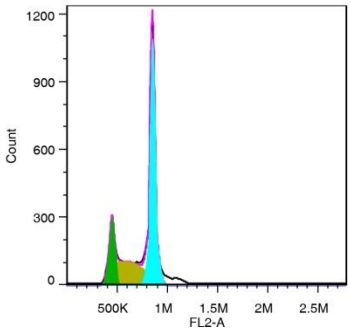
A02 2.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 4.21
G1 = 10.64
S = 6.75
G2 = 79.32
G1(mean) = 2818657.07
G2 (mean) = 5513306.6
G1 cv = 10.88
G2 cv = 8.14
sub-G1 = -0.95
super-G2 = 0.64

15045

Fig4I. Cell cycle tested: G2/M phase 1h



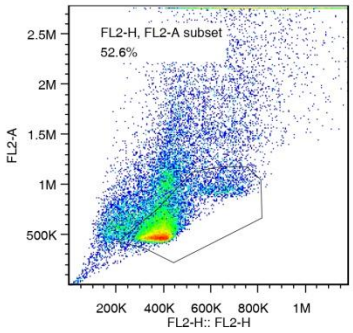
A03.fcs
FSC-A, SSC-A subset
27965



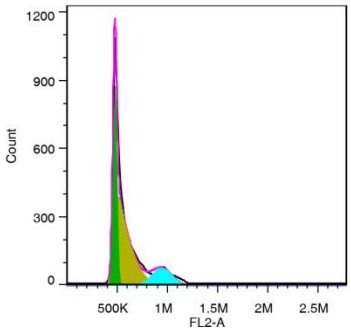
A03.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 3.33
G1 = 7.32
S = 6.14
G2 = 80.57
G1(mean) = 2635887.79
G2 (mean) = 5187244.33
G1 cv = 8.85
G2 cv = 7.28
sub-G1 = -0.99
super-G2 = 2.77

14805

Fig4J. Cell cycle tested: G2/M phase 2h



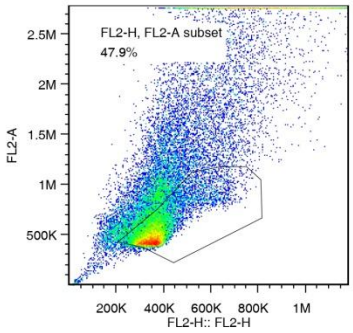
A09 9.fcs
FSC-A, SSC-A subset
23771



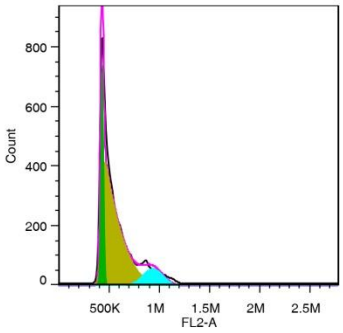
A09 9.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 6.6
G1 = 31.22
S = 53.65
G2 = 14.44
G1(mean) = 2889073.47
G2 (mean) = 5690690
G1 cv = 5.71
G2 cv = 14.99
sub-G1 = -5.05
super-G2 = 0.78

12499

Fig4K. Cell cycle tested: G1/S phase 0h



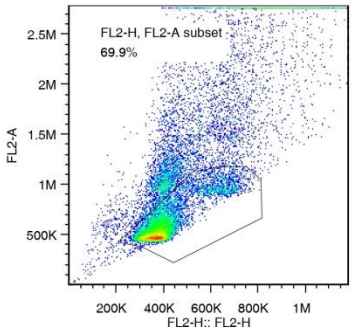
A10 10.fcs
FSC-A, SSC-A subset
26022



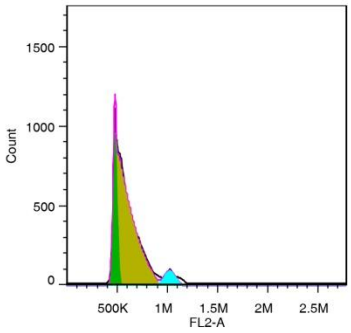
A10 10.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 4.49
G1 = 21.21
S = 64.48
G2 = 10.85
G1(mean) = 2594718
G2 (mean) = 5630670.99
G1 cv = 4.88
G2 cv = 15.44
sub-G1 = -0.65
super-G2 = -0.41

12469

Fig4L. Cell cycle tested: G1/S phase 2h



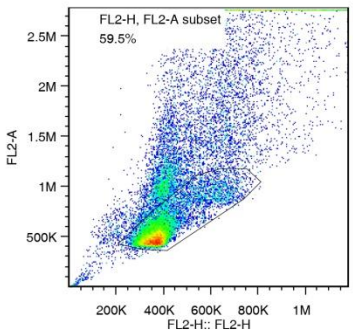
A06 6.fcs
FSC-A, SSC-A subset
20238



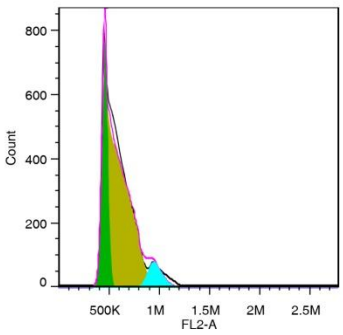
A06 6.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 7.2
G1 = 17.11
S = 64.68
G2 = 10.37
G1(mean) = 2899893.39
G2 (mean) = 5720175
G1 cv = 4.95
G2 cv = 12.03
sub-G1 = -4.07
super-G2 = 1.88

14151

Fig4M. Cell cycle tested: G1/S phase 4h



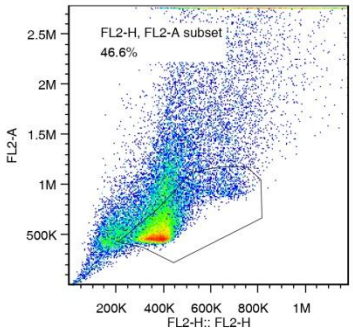
A07 7.fcs
FSC-A, SSC-A subset
22780



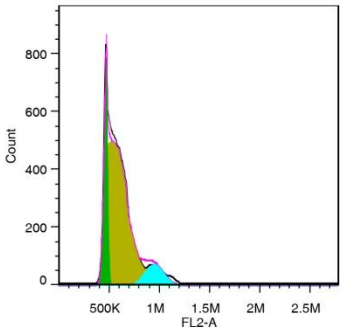
A07 7.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 8.52
G1 = 12.46
S = 61.34
G2 = 10.75
G1(mean) = 2830602
G2 (mean) = 5484292
G1 cv = 9.76
G2 cv = 15.61
sub-G1 = -1.29
super-G2 = 0.8

13558

Fig4N. Cell cycle tested: G1/S phase 6h



A08.fcs
FSC-A, SSC-A subset
25317



A08.fcs
Cell Cycle
Dean-Jett-Fox
RMS = 8.38
G1 = 17.34
S = 66.94
G2 = 13.93
G1(mean) = 2935067.83
G2 (mean) = 5720175
G1 cv = 8.53
G2 cv = 14.43
sub-G1 = 1.8
super-G2 = 0.15

11786

Fig4O. Cell cycle tested: G1/S phase 8h

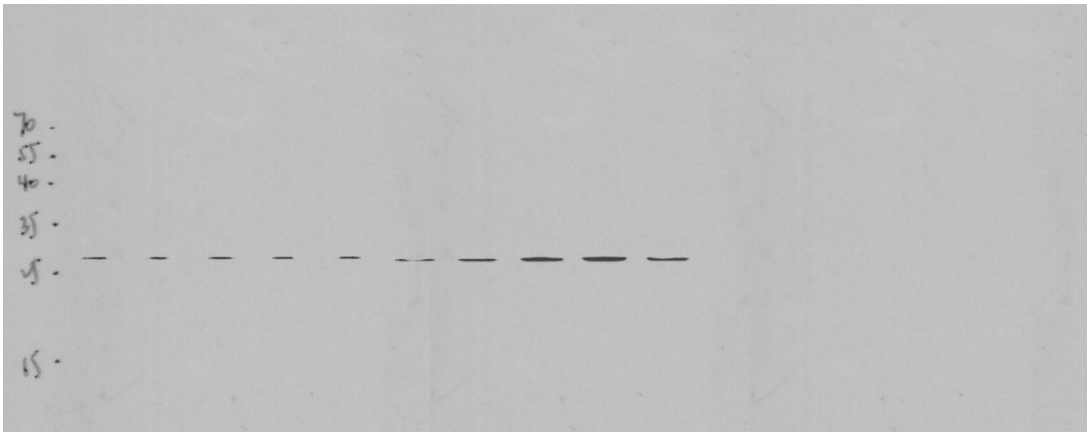


Fig5A. Original gel for TMUB1 expression in sham-operated and operated group

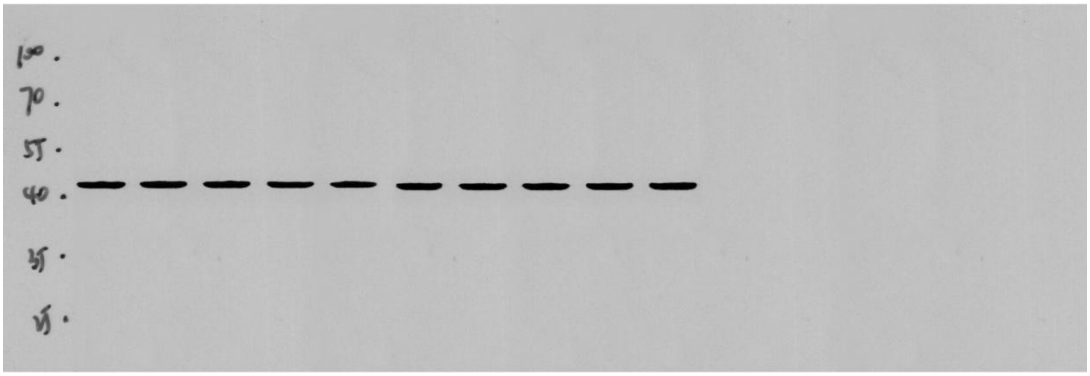


Fig5B. Original gel forβ -actin expression in sham-operated andoperated group

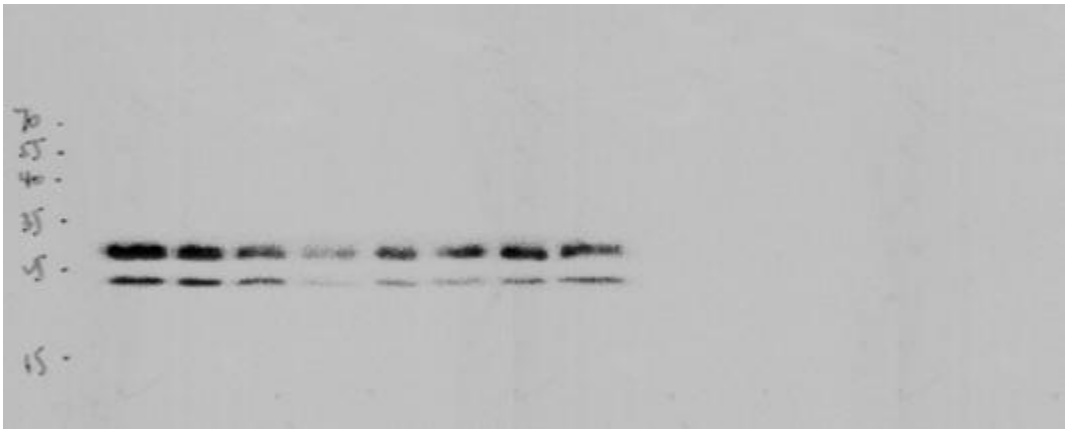


Fig5C. The expression of TMUB1 in the M phase and G1/S phase.

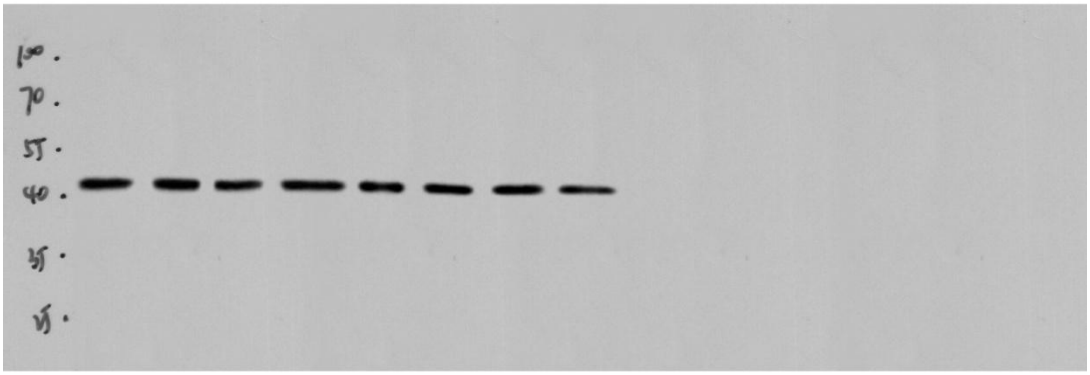


Fig5D. The expression of β -actin in the M phase and G1/S phase.

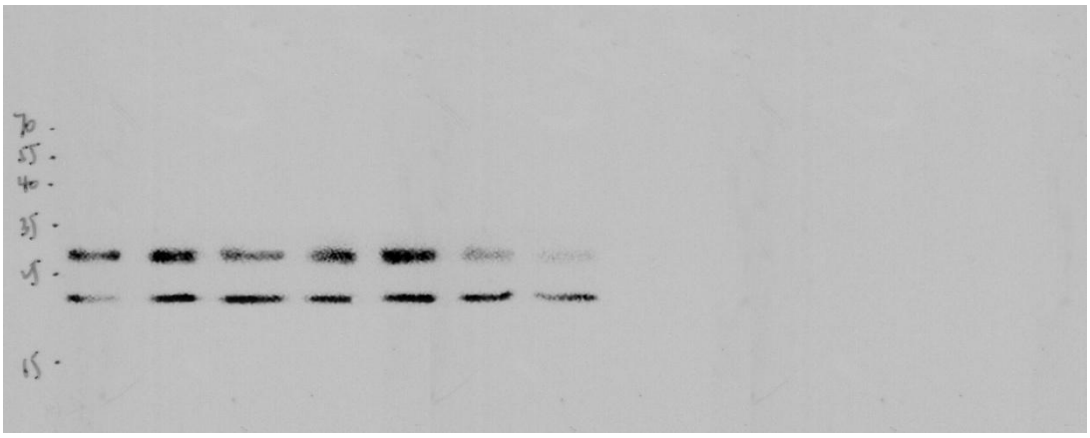


Fig5E. The expression of TMUB1 in the G0/G1 phase.

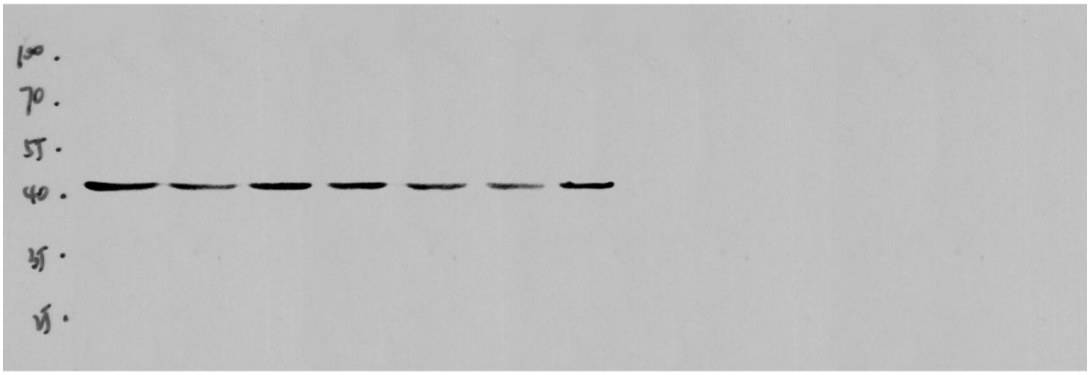


Fig5F. The expression of β -actin in the G0/G1 phase.

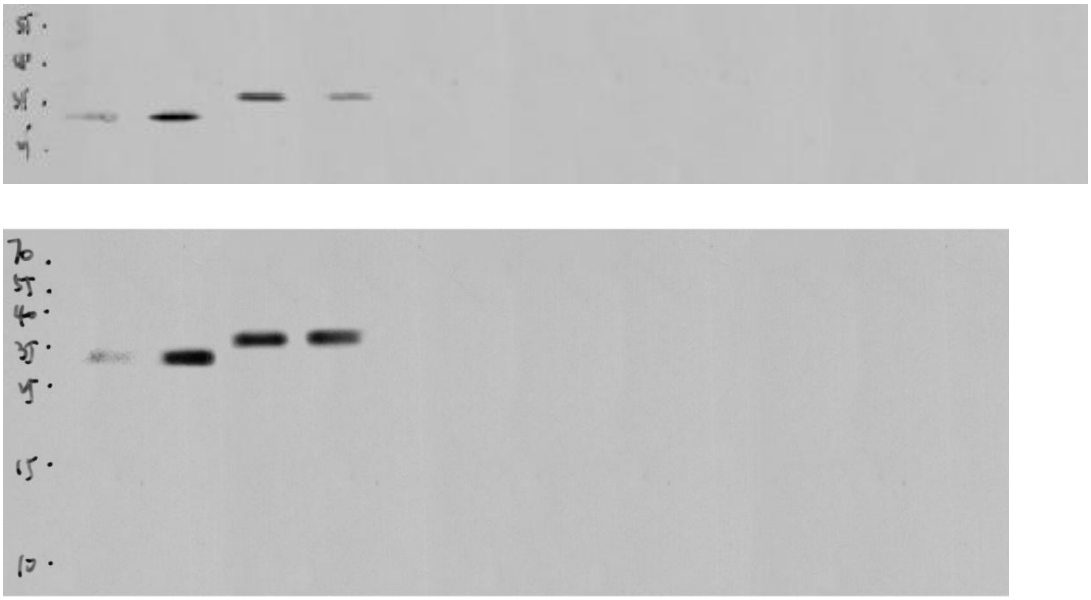


Fig5G. The effects of transfection of full-length TMUB1, its mutants and siRNA were tested by Westernblotting

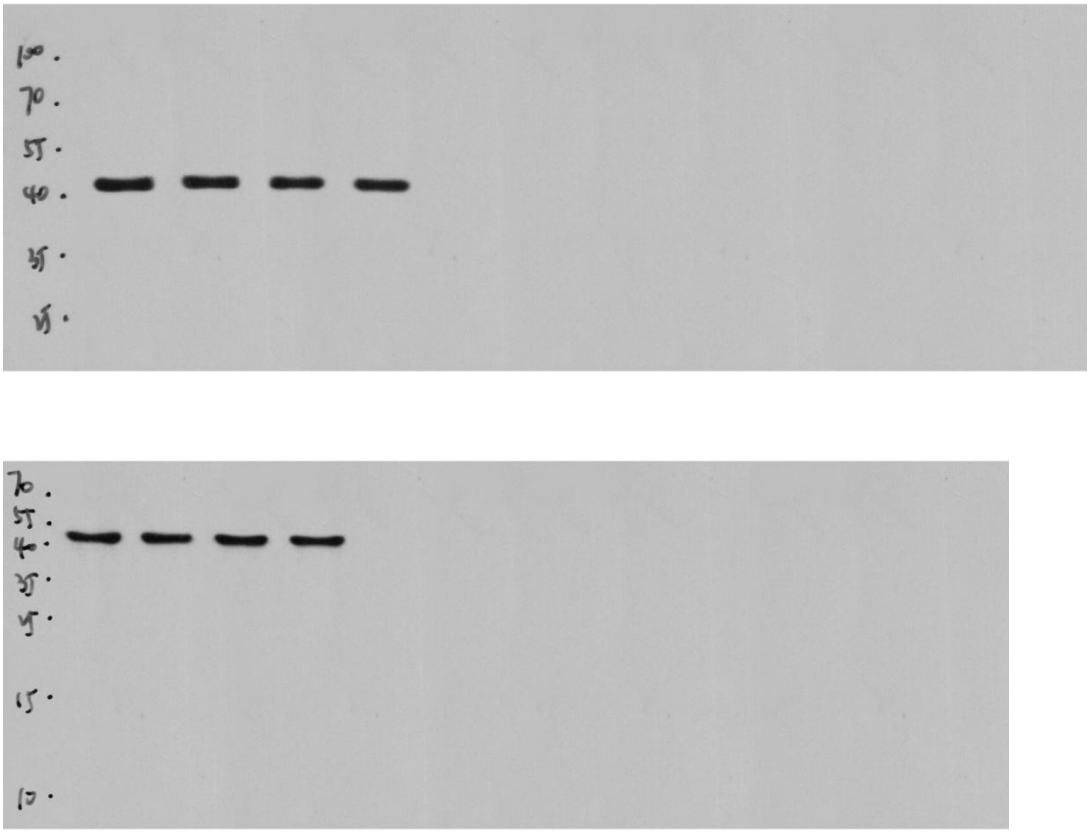


Fig5H. The expression of TMUB1 after transfection of full-length TMUB1, its mutants and siRNA were tested by Westernblotting

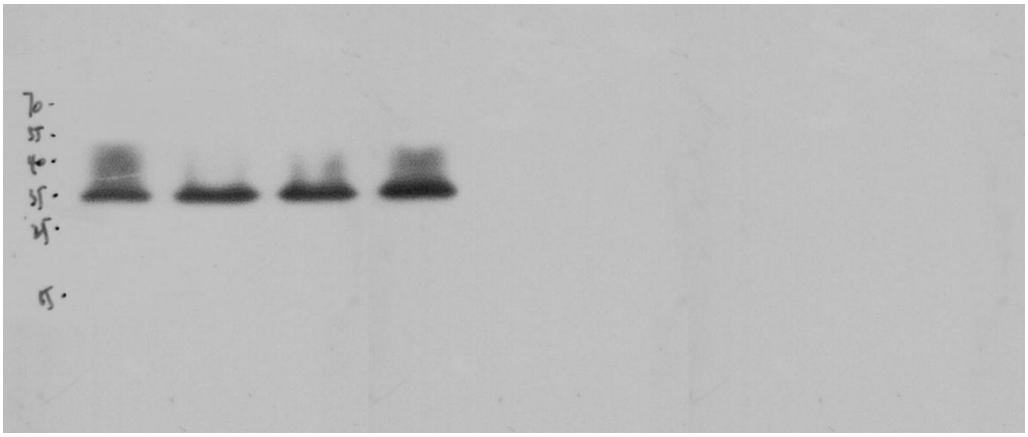


Fig5I. The expression of CAML after transfection of full-length TMUB1, its mutants and siRNA were tested by Westernblotting.

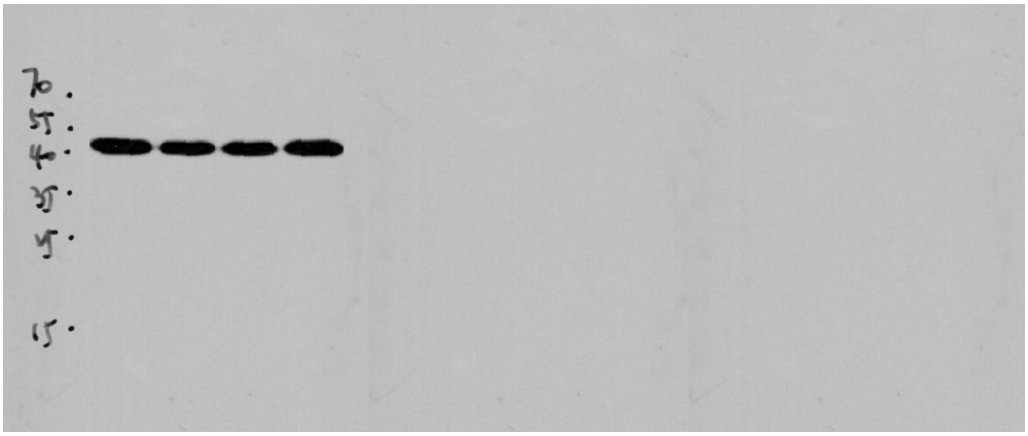


Fig5J. The expression of β -actin after transfection of full-length TMUB1, its mutants and siRNA were tested by Westernblotting.