Dear editor,

Thank you for your letter on Aug 31, 2015 regarding our manuscript entitled “Hypoxia-inducible factor-1 modulates the up-regulation of the human mutT homologue protein MTH-1 in colorectal cancer” (Manuscript ID: 19933) that was submitted to your journal for publication. We are very pleased to be asked to submit a revision and are delighted to know that the opinion of the referees was positive. We found the comments and suggestions of the reviewers to be extremely helpful. We have revised the manuscript according to the comments from the reviewers and the editorial board. Please find our uploaded revision and response on separate sheets in which we have carefully addressed the issues raised by the reviewer. We believe the comments and suggestions have significantly improved the quality of manuscript and made it publishable. Below is a detailed explanation of these changes requested by reviewers. Please let us know if you have any questions. Thank you.

Best regards.

Yours sincerely,

Hua Yang, M.D., Ph.D.

List of responses

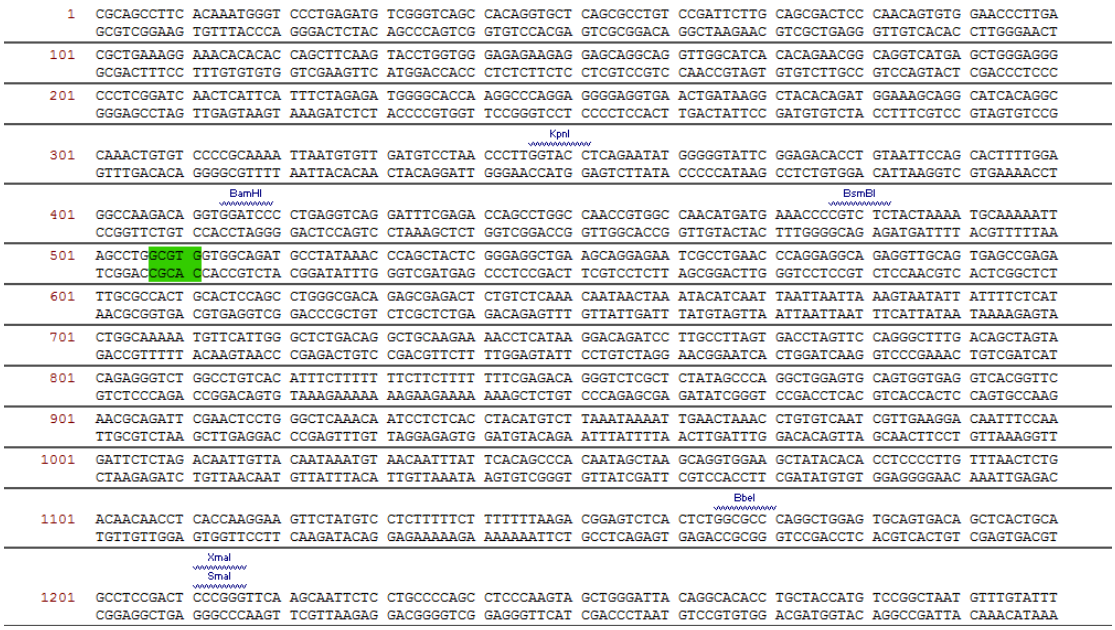
Reviewer #1:

This is an interesting work demonstrating the upregulation of MTH-1 expression in CRC cells via HIF-1α in response to hypoxic stress. The authors applied hypoxic conditions and performed siRNA experiments to show the association between HIF-1α and MTH-1. However, it is unknown whether there is a direct regulation of MTH-1 by HIF-1α or this is mediated by other mediators. It would be thus interesting to perform a ChIP analysis to look for direct HIF-1α binding to the MTH-1 promoter, or at least whether there is a HRE region in the gene promoter. Also, it would be interesting to assess at the clinical level whether IHC expression of MTH-1 is associated with worse prognosis or response to chemotherapy, particularly in advanced stage patients.

Response: Thank you for this great comment. ChIP assay is indeed a powerful and versatile technique used for probing protein-DNA interactions within the natural chromatin context of the cell. This assay can be used to determine whether HIF-1α is associated with MTH-1 promoter. We

would like to perform this analysis to look for direct HIF-1α binding sites of the MTH-1 promoter in the nearly future. Interestingly, according to the reviewer's comment, we searched the core HRE sequence ([A/G]CGTG) in the upstream of MTH-1 coding sequence. Nine putative HIF-1α binding sites ([A/G]CGTG; green box in Fig. 1) were identified in the region of −5000/-1 relative to human MTH-1 promoter (Fig. 1). These results indicate that the sequence covering the consensus HIF-1α binding site may be involved in the up-regulation of MTH-1 gene transcription by HIF-1α.

Just as the reviewer said, it would be interesting to assess at the clinical level whether IHC expression of MTH-1 is associated with worse prognosis. Given the duration of disease and prognosis, we would like to investigate these issues in the nearly future.



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1501  GACTGCAGCC ACAGCCTCTG GGGCTCAAGT GAACCTCCCA CCTCAGCCTC CTGAGTAGCT GAGACTACAG GTGTGTGCCA CCACTTCCGG CTAATTTTTC
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1601  TATTTTCAGT AGAGATGGGA TTTCACCATG TTGGCCGAGC TGGTCTCGAA CTCCTGACCT CTGGCGATCA GCCCACCTTG GCCTCCCAAA GTGCTGGGAT
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1701  TACAGGCGTG AACCACCTCA CCCGGCCATT TTTGTTTTGC TATCATCCAG GCTGGAGTTG CAGTGGCACA ATCACAGCTG ATTGCTGCCA TAACCTCTGG
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1801  GTCTCAAGTG AACCTCCCAC CTCAGCCTCC TGAGTAGCTG GGACTACAGG TGTGCACCAC CATGCCTGGG CTAATTTTTT TTTTTTTTTT AATTCTTAAA
      CAGAGTTCAC TTGGAGGGTG GAGTCGGAGG ACTCATCGAC CCTGATGTCC ACACGTGGTG GTACGGACCC GATTAAAAAA AAAAAAAAAA TTAAGAATTT

1901  AACCTTTTTT AGAGATGGCA TCTCACTACA TTGCCTAGGC TGGTCTCGAA CTCCTAGGCT CAAACGATCC TCCTGCCTCA GACTTCCAAA GTGCTGGGAT
      TTGGAAAAAA TCTCTACCGT AGAGTGATGT AACGGATCCG ACCAGAGCTT GAGGATCCGA GTTTGCTAGG AGGACGGAGT CTGAAGGTTT CACGACCCTA

                                                                                                          HindIII
2001  GACAGGTGTG GGCCACCAAG CCCGGTCTCT ATGTCCATCT TTCTGATTTA AATAAATAAA TAAAAGTTAC CACCCTCATG GCTCCAAGGC CAAAGCTTCT
      CTGTCCACAC CCGGTGGTTC GGGCCAGAGA TACAGGTAGA AAGACTAAAT TTATTTATTT ATTTTCAATG GTGGGAGTAC CGAGGTTCCG GTTTCGAAGA

                                                                                                  XmaI
                                                                                                  SmaI
2101  AGCTTCTGCA ACACCAAAGC GTGCTGACAA GCCAGGAGGC ACTGACTGCT GACCGTGAGT CGCTGTCTTC CCCTCGCTTA AGGGGTCTCC CGGGAAAATG
      TCGAAGACGT TGTGGTTTCG CACGACTGTT CGGTCCTCCG TGACTGACGA CTGGCACTCA GCGACAGAAG GGGAGCGAAT TCCCCAGAGG GCCCTTTTAC

                  XmaI                                                                            XmaI
                  SmaI                                                                            SmaI
2201  GCACAAGCCG GTCCCGGGGG CCAGGGCCCA CTCGACAGCC CAGGAAGCGC GGGCAATGGA GCTACCCGGC CCAGAAGCGA AATCCCGCCC CGGGCTCGCC
      CGTGTTCGGC CAGGGCCCCC GGTCCCGGGT GAGCTGTCGG GTCCTTCGCG CCCGTTACCT CGATGGGCCG GGTCTTCGCT TTAGGGCGGG GCCCGAGCGG

      BbeI
2301  CCCGGCGCCT TGGATCTGGG CCCCGGCGCT GCGCCCCCCA CCTGGACTCC GCCGCCGGCC CCGGCCTCCC CACGGCCCCG CCGGCCCTGC CCCTGGAGAC
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                  XmaI
            BbeI  SmaI
2401  CTGAGGGCGC CCTCCTCCCG GGGAACTGCG ACCCGGAATC CTGGCCTCGC CCCTGTCGCG CTCGCTGAGT GCGGGGACGG TGCCCAGCGC TCGGCACCCA
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2501  GCCCCGAGGC CAGGACCGAG GAAGGCGACC GGGCGGACCC CCAACCACTC CCGCTGTCTG CACGCGCAGC AGCAGCGCCC AGCTCACCCC GCCATTGGTG
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2601  TTCCCCGCGC CTGCAGCGCG CCGCCGGAAG TGCCTGGCCT CACTTCCGGT CAGAGGCCAC GCCCCCGGAA GCGGCGGTGC AGGTACGAAA AGCGCGCGCG
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                                                                                                  NcoI
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                  XhoI
2801  GAGCTCGAAG GAGACCAGAA GAGCAGGGTC GGGGGCTCGA GGGAGACGAG GAGAGCGGGG CCGGGGGTTT GGGAGAGAGA CAAGGAGAGC GGGGCGGAGG
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                        BsmBI
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3001  GAAACTAAGC CACAAACCCA TTTACCCTGT CTGACCTGCC TCCGCCACCA GGACCCAGTC TCAGTGAAAT GGGAAAGGTT CCCTTGTCCT CCTCGAAGGG
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3101  CGTGCGATGA GGATGTGGCT CACTTCTTCA GTGCCCCGCT TCTCAAGCCT CTAGGGGAAC ATACAGACAG GCAGGCTGAG GGGCTCCCAC CCCACGGCAG
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3201  TGTCTAGGGG TGAATGTTTG CAGCTCCTGA AGCCCCAGCA GGCATGTGTT AGAGGGTGCT CTTTCAGTTT AGCCTTGTAG GTGGCTTGTG TTAGTCAGCT
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3301  GTTAGACTCC CTGCCTTATC GCAAGGACAG AGGGCTTTCT GTATCCCTAG GTTTCTTGCC TTGATGTACT GGAGCAATCA GATCACACGG CGGCTTGGAG
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3401  AGTGAGTGCA AGGTTTTATG AGTGGAATTA GCCCTCAGCA GATGGGGGAG CCAGAAGGCA GTTGGAGTGG GAAGGTGATT TTCCCCTGGA GTCGGGCTGC
      TCACTCACGT TCCAAAATAC TCACCTTAAT CGGGAGTCGT CTACCCCCTC GGTCTTCCGT CAACCTCACC CTTCCACTAA AAGGGGACCT CAGCCCGACG

                                                                                                  AatII
3501  TGAGCAGCCT GGGCTCTCCT CTGACCGCCC CTGCCAAACT CATGGTTCCA TCAGTTGATG GCCTGCTGGT GCCTGTTGGT GTGCTTTCCA CGTCCTCTTG
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      AatII
3601  ACGTCCAGCG GCTTGTGTCT CTGCCCACTA TAAAAAACCA CTGGGGTTTT TTTATAGGCA CAGGACGGGG ACGTGGCGGG CTCGGGAAAT GCAACATTTG
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3701  GGCAAAAAAA CAGAAATGCC TGTCCTCACC CAGGTCCCTG GGCACAGGCC TGGGTGTGGA GCCCTGGCCA GAGACCACGC CCTCCTCTAC CCAGCACTTC
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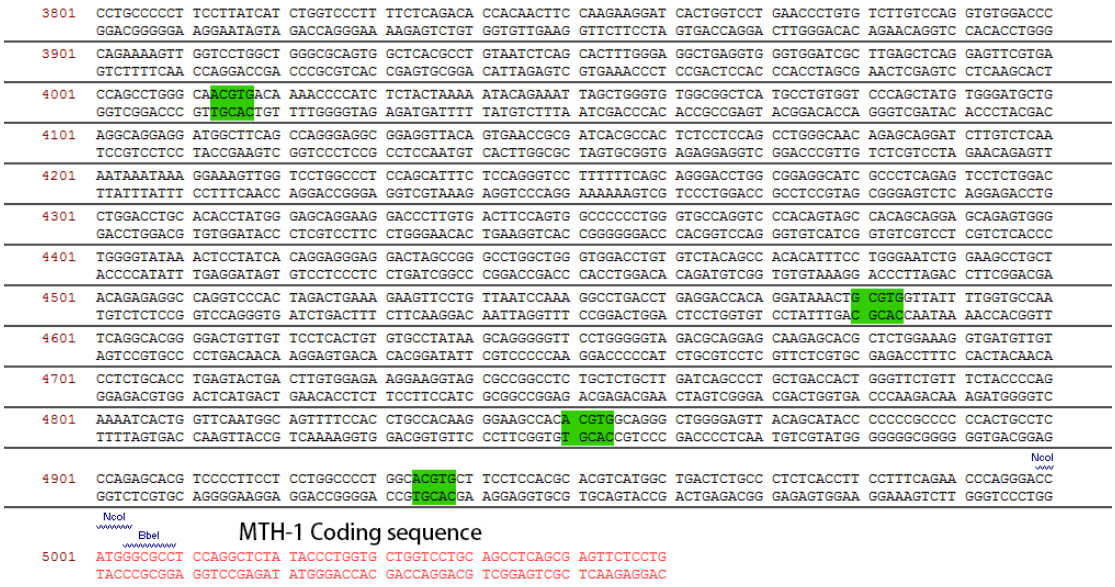

Figure 1 Human MTH-1 CDS 5'UTR(chromosome 7, CDS upsteam 0 to -5000bp)

Reviewer #2:

This is an excellent paper with clear-cut results.

Response: Thank you for this comment.

Reviewer #3:

Very nice work.

Response: Thank you for this comment.