

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-1 $\alpha$  in response to hypoxic stress. The authors applied hypoxic conditions and performed siRNA experiments to show the association between HIF-1 $\alpha$  and MTH-1. However, it is unknown whether there is a direct regulation of MTH-1 by HIF-1 $\alpha$  or this is mediated by other mediators. It would be thus interesting to perform a ChIP analysis to look for direct HIF-1 $\alpha$  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-1 $\alpha$  is associated with MTH-1 promoter. We

would like to perform this analysis to look for direct HIF-1 $\alpha$  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-1 $\alpha$  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-1 $\alpha$  binding site may be involved in the up-regulation of MTH-1 gene transcription by HIF-1 $\alpha$ .

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.

1	CGCAGCCTTC	ACAAATGGGT	CCCTGAGATG	TCGGGTCAGC	CACAGGTGCT	CAGCGCCTGT	CCGATTCTTG	CAGCGACTCC	CAACAGTGTG	GAACCCCTGA
	GCGTCGGGAG	TGTTTACCCA	GGGACTCTAC	AGCCCACTGC	GTGTCCACGA	GTGCGCGACA	GGCTAAGAAC	GTGCTGAGG	GTGTGCACAC	CTTGGGAACT
101	CGCTGAAGG	AAACACACAC	CAGCTTCAAG	TACCTGGTGG	GAGAGAAGAG	GAGCAGGCAG	GTGGGCATCA	CACAGAACCG	CAGGTCATGA	GCTGGGAGGG
	GCGACTTTC	TTTGTGTGTG	GTCGAAGTTC	ATGGACCACC	CTCTCTTCTC	CTCGTCCGTC	CAACCGTAGT	GTGTCTTGCC	GTCCAGTACT	CGACCCCTCC
201	CCCTCGGATC	AATCTATTCA	TTTCTAGAGA	TGGGGCACCA	AGGCCCAAGG	GGGGAGGTGA	ACTGATAAGG	CTACACAGAT	GGAAAGCAGG	CATCAGAGGC
	GGGAGGCTAG	TTGAGTAAGT	AAAGATCTCT	ACCCCGTGGT	TCCGGGTCTC	CCCTCCACT	TGACTATTCC	GATGTGTCTA	CCTTTCGTCC	GTAGTGTCCG
301	CAAATGTGT	CCCCGCAAAA	TTAATGTGTT	GATGTCTTAA	CCCTTGGTAC	CTCAGAAATAT	GGGGGTATTG	GGAGACACCT	GTAATTCAG	CACCTTTTGA
	GTTTGACACA	GGGGCGTTTT	AATTACACAA	CTACAGGATT	GGGAACCATG	GAGTCTTATA	CCCCATAAG	CCTCTGTGGA	CATTAAAGTC	GTGAAAACCT
401	GGCCAAGACA	GGTGGATCCC	CTGAGGTCAG	GATTTTCGAGA	CCAGCCTGGC	CAACCGTGGC	CAACATGATG	AAACCCCGTC	TCTACTAAAA	TGCAAAAAAT
	CCGGTCTGT	CCACCTAGGG	GACTCCAGTC	CTAAAGCTCT	GTTCGGACCG	GTTCGGACCG	GTGTACTTAC	TTTGGGGCAG	AGATGATTTT	ACGTTTTTAA
501	AGCCTGCGCT	GTGGCAGAT	GCCTATAAAC	CCAGCTACTC	GGGAGGCTGA	AGCAGGAGAA	TGCGCTGAAC	CCAGGAGGCA	GAGGTTCAG	TGAGCCGAGA
	TCGACCCGCA	GCACCGTCTA	CGGATATTGG	GGTCGATGAG	CCCTCCGACT	TCGTCTCTTT	AGCGGACTTG	GGTCTCCGT	CTCCAACGTC	ACTCGGCTCT
601	TTGGCGCACT	GCACTCCAGC	CTGGGCGACA	GAGCGAGACT	CTGTCTCAAA	CAATAACTAA	ATACATCAAT	TAATTAATTA	AAGTAATATT	ATTTTCTCAT
	AACGCGGTGA	CGTGAGGTGC	GACCCGCTGT	CTCGCTCTGA	GACAGAGTTT	GTTATTGATT	TAITGATGTA	ATTAATTAAT	TTCAATTATA	TAAAGAGATA
701	CTGGCAAAAA	TGTTCAATTGG	GCTCTGACAG	GCTGCAAGAA	AACCTCATAA	GGACAGATCC	TTGCCTTAGT	GACCTAGTTC	CAGGGCTTTG	ACAGCTAGTA
	GACCGTTTTT	ACAAGTAACC	CGAGACTGTC	CGACGTTCTT	TTGGAGTATT	CCTGTCTAGT	AACGGATCA	CTGGATCAAG	GTCCCGAAAC	TGTCGATCAT
801	CAGAGGGTCT	GGCCTGTAC	ATTTCTTTTT	TTCTTCTTTT	TTTCGAGACA	GGGTCTCGCT	CTATAGCCCA	GGCTGGAGTG	CAGTGGTGAG	GTACCGGTTT
	GTCTCCAGA	CCGACAGTGA	TAAAGAAAAA	AAGAAGAAAA	AAAGCTCTGT	CCCAGAGCGA	GATATCGGGT	CCGACCTCAC	GTCAACCTC	CAGTGCCAAAG
901	AACGCGATTT	CGAACTCCTG	GGCTCAAAAC	ATCCTCTCAC	CTACATGTCT	TAAATAAAAT	TGAACATAAC	CTGTGTCAAT	CGTTGAAGGA	CAATTTCCAA
	TTGCGTCTAA	GCTTGAGGAC	CCGAGTTTGT	TAGGAGAGTG	GATGTACAGA	ATTTATTTTA	ACTTGATTTG	GACACAGTTA	GCAACTTCCT	GTTAAAGGTT
1001	GATTCTCTAG	ACAATTGTTA	CAATAAATGT	AACAATTTAT	TCACAGCCCA	CAATAGCTAA	GCAGGTGGAA	GCTATACACA	CCTCCCTTGG	TTTAAGTCTG
	CTAAGAGATC	TGTTAACAAAT	GTTATTTACA	TTGTTAAATA	AGTGTGCGGT	GTTATCGATT	CGTCCACCTT	CGATATGTGT	GGAGGGGAAC	AAATTGAGAC
1101	ACAACAACCT	CACCAAGGAA	GTTCATATGC	CTCTTTTTTT	TTTTTTAAGA	CGGAGTCTCA	CTCTGGCGCC	CAGGCTGGAG	TGCAGTGACA	GCTCACTGCA
	TGTTGTGGA	GTGTTCTGTT	CAAGATACAG	GAGAAAAAGA	AAAAAATTTCT	GCCTCAGAGT	GAGACCGCGG	GTCCGACCTC	ACGTCACTGT	CGAGTGACGT
1201	GCCTCCGACT	CCCGGGTTCA	AGCAATTCTC	CTGCCCCAGC	CTCCCAAGTA	GCTGGGATTA	CAGGCACACC	TGCTACCATG	TCCGGCTAAT	GTGTTGTAAT
	CGGAGGCTGA	GGGCCCCAGT	TCGTTAAGAG	GACGGGGTCT	GAGGGTTTAT	CGACCCCTAAT	GTCCGTGTGG	ACGATGGTAC	AGGCCGATTA	CAAAACATAA

1301	TCAGTATAGA TGGGTTCAC CATGTTGGCC AGGCTGGTCT CAAACTCCTG ACCTCCGCTG ATCTGCCCGC CTTGCCCTCC CAAAGTGCTG GGAATACAGG AGTCATATCT ACCCAAGGTG GTACAACCGG TCCGACCAGA GTTTGAGGAC TGGAGGCGAC TAGACGGGCG GAACCGGAGG GTTTCACGAC CCTAATGTCC
1401	TGTAAGCCAC TGCACCCAGG CATTTCTTTC TTCTTTTGTG TTTTITTAGG ACAGGGTTTT ACTATTACCC AGGCTGGAGT GCAGTGGCAT GATCACAGCT ACATTCGGTG ACGTGGGTCC GTAAAGAAAG AAGAAAAACA AAAAAATCTC TGTCCTCAAA TGATAATGGG TCCGACCTCA CGTACCGGTA CTAGTGTCGA
1501	GACTGCAGCC ACAGCTCTCT GGGCTCAAGT GAACCTCCCA CCTCAGCCTC CTGAGTAGCT GAGACTACAG GTGTGTGCCA CCACTTCCGG CTAATTTTTT CTGACGTCCG TGTCCGAGAC CCGAGTTCA CTGAGGGT GGAGTCGGAG GACTCATCGA CTCTGATGTC CACACACGGT GGTGAAGGCC GATTAAAAAG
1601	TATTTTCAGT AGAGATGGGA TTTCAACATG TTGGCCGAGC TGGTCTCGAA CTCTGACCTT CTGGCGATCA GCCCACCTTG GCTTCCCAAA GTGCTGGGAT ATAAAAAGTA TCTCTACCTT AAAGTGGTAC AACCGGCTCG ACCAGAGCTT GAGGACTGGA GACCGCTAGT CGGGTGGAAAC CGGAGGGTTT CACGACCCTA
1701	TACAGGCGTG AACCACTCA CCGGGCCATT TTTGTTTTGC TATCATCCAG GCTGGAGTTG CAGTGGCACA ATCAGAGCTG ATTGCTGCCA TAACCTCTGG ATGTCGCGAC TTGGTGGAGT GGGCCGTAA AACAACAACG ATAGTAGTCT CGACCTCAAC GTACCGGTGT TAGTGTGAGT TAACGACGGT AITGAGAGCC
1801	GTCTCAAGTG AACCTCCAC CTCAGCCTCC TGAGTAGCTG GGACTACAGG TGTGCACCAC CATGCTCTGG CTAATTTTTT TTTTTTTTTT AATTCTTAAA CAGAGTTTCA TTGGAGGGTG GAGTCGGAGG ACTCATCGAC CCTGATGTCC ACACGTGGTG GTACGGACCC GATTAAAAAA AAAAAAATAA TTAAGAATTT
1901	AACCTTTTTT AGAGATGGCA TCTCACTACA TTGCTAGGCT TGGTCTCGAA CTCTAGGCT CAAACGATCC TCTGCTCA GACTTCCAAA GTGCTGGGAT TTGAAAAAAA TCTTACCGT AGAGTAGTGT AACGGATCCG ACCAGAGCTT GAGGATCCGA GTTTGCTAGG AGGACGGAGT CTGAAGSTTT CACGACCCTA
2001	GACAGTGTG GGCACCAAG CCCGGTCTCT ATGTCCATCT TTCTGATTTA AATAAATAAA TAAAGTTTAC CACCTCATG GTCCTAAGG CAAAGCTTCT CTGTCCACAC CCGGTGGTTC GGGCCAGAGA TACAGGTAGA AAGACTAAAT TTATTTATTT ATTTTCAATG GTGGGAGTAC CGAGTTCTCG GTTTCGAGA
2101	AGCTTCTGCA ACACCAAAAG GTGCTGACAA GCCAGGAGGC ACTGACTGCT GACCGTGAGT CGCTGTCTTC CCTTCGTTA AGGGGTCTCC CGGAAAAATG TCGAAGACGT TGTGGTTTTG CAGGACTGTT CGGTCTCCG TGACTGACGA CTGGCACTCA GCGACAGAAG GGGAGCGAAT TCCCCAGAG GCCCTTTTAC
2201	GCACAAGCCG GTCCCAGGGG CAGGGGCCCA CTCGACAGCC CAGGAAGCGC GGGCAATGGA GCTACCCGGC CCAGAAGCGA AATCCCGCCC CGGGCTCGCC CGTGTCTCGG CAGGGGCCCC GGTCCCGGGT GAGCTGTGCG GTCTTTCGCG CCAATGTACT CGATGGGCGG GGTCTTCGCT TTAGGGCGGG CCGCGAGCGG
2301	CCCGGCGCT TGGATCTGGG CCGCGGCGCT GCGCCCCCA CTTGGACTCC GCGCGCGGCC CCGGCTCTCC CACGGCCCCG CCGGCTCTGC CCTGGAGAC GGGCCCGGGA ACCTAGACCC GGGCCGCGA CCGGGGGGTG GAGCTGAGG CCGCGCGCCG GCGCCGAGGG GTGCCGGGGC GGGCCGGAGC GGGACCTCTG
2401	CTGAGGGCGC CTCTCTCCCG GGAAGTCTGC ACCCGGAATC CTGGCTCTGC CCTGTCTGCG CTCGCTGAGT GCGGGGACGG TGCCACGCGC TCGGCACCCA GACTCCCGCG GGAGGAGGCG CCTTGACGC TGGGCTTAG GACCGGAGCG GGGACAGCGC GAGCGACTCA CGCCCTTGCC ACGGCTGCGG AGCCCTGGGT
2501	GCCCCGAGGC CAGGACCGAG GAAGGCGACC GGGCGGACCC CCAACCACTC CCGCTGCTG CACGCGCAGC AGCAGCGCCC AGCTCACCCC GCCATTGGTG CCGGGCTCGG GTCTCGGCTC CTTCGCTGGG CCGGCTGCTG TCGTCTGCGG TCGAGTGGGG CGGTAAACAC
2601	TTCCCCGCGC CTGACGCGCG CCGCCGGAAG TGCTTGGCTC CACTTCCGCT CAGAGGCCAC GCCCCCGGAA CCGGCGGTGC AGGTACGAAA AGCGCGCGCG AAGGGCGCG GACGTGCGCG GCGGCGCTTC ACGGACCGGA GTGAAGGCCA GTCTCCGGTG CCGGGGGCTT CGCCGCCACG TCAATGCTTT TCGCGCGCGC
2701	GGGATTCAGG GAGTCGTGTT GACCAGGGAG GGGAGCGGG CCAGCGGGGG GCAGGAGACT AGGGGAGCTG AGCCATGGGC TTGGGGGAGA GCGGGGCGCG CCTAAGCTAC CTCGACACCA CTGCTCCCTC CCTTCGCCCC CGTCTCTGTA TCCCTCTGAG TCGGTACCCG AACCCCTCT CGCCCGCGCC
2801	GAGCTCGAAG CAGACCAGAA GAGCAGGGTC GGGGGCTCGA GGGAGACGAG GAGAGCGGGG CCGGGGGTTT GGGAGAGAGA CAAGGAGAGC GGGGCGGAGG CTCGAGCTTC CTCTGTCTCT CTCTGCCAG CCCCCGAGCT CCTCTGCTC CTCTCGCCCC GGGCCCCAAA CCTCTCTCT GTTCTCTCTG CCCCCTCTC
2901	CTTGGGGGAG ACCAGGAGTA AATCACAAAA ATTTACTTTG GAAACCGTGG CTCTTGGCAG AAACAGATCA GTGTGCGGAC AAGTACACG GGCCTGGTGT GAACCCCTCT TGGTCTCAT TTAGTGTITT TAAATGAAC CTTTGGCACC GAAGACCGTC TTTGTCTAGT CACAGCCCTG TTTCATGTGC CCGGACCCAC
3001	GAAACTAAGC CACAAACCCA TTTACCTGT CTGACCTGCC TCCGCAACCA GGAACCACTC TCAAGTAAAT GGGAAAGGTT CCTTGTCTCT CCTCGAAGG CTTTGATTCG GTGTGTTGGT AAATGGGACA GACTGGACGG AGGCGGTGGT CTTGGGTGAG AGTCACTTTA CCTTTTCCAA GGGAAACAGGA GAGACTTCCC
3101	CTGCGATGA GATGTGGCT CACTTCTTCA GTGCCCGCT TCTCAAGCTC CTAGGGGAAC ATACAGACAG GCAGGCTGAG GGGCTCCAC CCGACGCGAG GCGGCTACT CCTACACCGA GTGAAGAAGT CACGGGCGCA AGAGTTCGGA GATCCCCTTG TATGCTGTC GTTCGAGACT CCGGAGGGTG GGGTCCGCTC
3201	TGCTAGGGG TGAATGTTT GAGTCTCTGA AGCCCCAGCA GGCATGTGTT AGAGGGTGTCT CTTTCAGTTT AGCCTTGTAG GTGGCTGTG TTAGTCAGCT ACAGATCCCC ACTTACAAC GTGAGGACT TCGGGGTCTG CCGTACACAA TCTCCACGA GAAAGTCAAA TCGGAACATC CACCGAACAC AATCAGTCGA
3301	GTAGACTCC CTGCTTATC GCAAGGACAG AGGCTTTTCT GTATCCCTAG GTTCTTGGC TTGATGTACT GAGCAATCA GATCACAGCG CGGCTTGGAG CAATCTGAGG GACGGAATAG CGTCTCTGTC TCCGAAAGA CATAGGGATC CAAGAAGCGG AACTACATGA CCTCGTTAGT CTAGTGTGCC GCCGAACCTC
3401	AGTGAAGTCA AGTTTTTATG AGTGAATTA GCCCTCAGCA GATGGGGGAG CCAGAAGGCA GTTGGAGTGG GAAGGTGATT TTCCCTTGGG GTGCGGCTGC TCACTCACGT TCAAAAATAC TCACCTTAAT CGGGAGTCTG CTACCCCTCT GGTCTTCCGT CAACCTCACC CTTCCACTAA AAGGGGACCT CAGCCGACG
3501	TGAGCAGCCT GGGCTCTCT CTGACCGCCC CTGCCAAACT CATGTTTCCA TCAGTTGATG GCCTGCTGGT GCCTGTTGGT GTGCTTTCCA CGTCTCTTGT ACTCTCGGA CCGAGAGGA GACTGGCGGG GACGGTTTGA GTACCAACTAC CGGACGACCA CGGACAACCA CACGAAGGTT GCAGGAGAAC
3601	ACGTCCAGCG GCTTGTGCT CTGCCACTA TAAAAACCA CTGGGGTTTT TTTATAGGCA CAGGACGGGG ACGTGGCGGG CTCGGGAAAT GCAACATTTG TGCAGTCTGC CGAACACAGA GACGGGTGAT ATTTTITGTT GACCCCAAAA AAATATCCGT GTCTGCCCC GCGAGCGCCC GAGCCCTTTA CGTTGTAAAC
3701	GGCAAAAAAA CAGAAATGCC TGTCTCACC CAGGTCCTGT GGCACAGGCG TGGGTGTGGA GCCTGGCCA GAGACACGC CCTCTCTAC CCAGCACTTC CCGTTTTTTT GTCTTTACGG ACAGGAGTGG GTCCAGGGAC CCGTGTCCGG ACCCACACCT CGGGACCGGT CTCTGTGCG GAGAGAGATG GGTCTGAAG

