

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	C CGCAGCCTTC ACAAATGGGT CCCTGAGATG TCGGGTCAGC CACAGGTGCT CAGCGCCGTG CCGATTCTTG CAGCGACTCC CAACAGTGTG GAAACCTTGA CGCTCGGAAG TGTTTACCA GGGACTCTAC AGCCCAGTCG GTGTCACAGA GTCGCGGACAA GGCTAAGAAC GTCGCTGAGG GTTGTACAC CTGGAACT
101	C CGCTGAAAGG AAAACACACAC CAGCTCAAG TACCTGTGG GAGAGAAGAG GAGCAGGCGA GTTGCATCA CACAGAACGG CAGGTATGAG GCTGGAGGG GCGACTTTC TTGTGTGTG GTCAAGATTC ATGGACCAAC CTCCTTCTC CTCGTCGTC CAACCGTAGT GTGTCITGCC GTCAGTACT CGACCCCTCC
201	C CCCTCGGATC AACTCATCA TTCTAGAGA TGGGGCACCA AGGCCAGGA GGGGAGGTGA ACTGATAAGG CTACACAGAT GGAAAGCAGG CATCACAGGC GGGAGCCTAG TTGAGTAAGT AAAGATCTCT ACCCCGGTGTG TCCGGTCTC CCTCCCTACT TGACTATTC GATGTGTCTA CCTTCGTCG CCATAGTGTCCG
	KpnI ~~~~~
301	C AAACTGTG TCCCGAAAAA TTATGTGTG GATGTCTAA CCCTGGTAC CTCAGAATAA GGGGGTATTC GGAGACACTT GTAATTCCAG CACTTTGGAA TTTGTACACA GGGGCGTITTT AATACACAA CTACAGGAATT GGGAACCAAG GAGICITATA CCCCTATAAG CCTCTGTGGA CATTAAAGGTG GTGAAAACCT
	BamHI ~~~~~
401	G GCGCAAGACA GGTGGATCCC CTGAGGTCA GATTTGAGA CCAGCGTGC CAAACATGATG AAACCCCGTC TCTACTAAAA TGCAAAATT CCGGTCTGTG CCACTGTAGG GACTTCAGTC CTAAAGCTCT GTGTCGACCC GTTGTACTAC ITTGGGGCGAG ATATGATTTT ACCTTTTAA
501	A AGCTGCGT GTGGCAGAT GCCTATAAAC CCAGCTACTC GGGAGGTGA AGCAGGAGAA TCGCTGAAC CCAGGGAGCA GAGGTGCGAG TGAGCCGAGA TCGGACCGCA ACACCGCTCA CGGATATTG GTGCGATGAG CTCCTCTCT ACAGCGACTT GGTCTCCCT CTCCACAGTC ACTCGCTCT
601	T TGCGCCACT GCACTCCAGC CTGGGCAGA GAGCGAGACT CTGTCCTAA CAATAACTAA ATACATCAAT TAATTAATTA AGTAATATT ATTTCAT AACCGCGTCA CGTGGAGTCG GACCCGCTGT CTGGCTCTGA GACAGAGTT GTTATTGAT TATGTAGTTA ATTAATTAATTA TAAAGAGGTA
701	C CTGGCAAAA TTGTCATGG GCTCTGACAG GCTGCAAGAA AACCTCTAA GGACAGATTC TTGCTCTAGT GACCTAGTC CAGGGCTTTC ACAGCTAGTA GACCGTTTT ACAAGTAAAC CGAGACTGTC CGACGTCIT TTGGAGTAACTT CCGTCTAGG AACCGGAATCA CTGGATCAAG GTCCCGAAAC TGCGATCAT
801	C CAGAGGGTCT GGCCTGTCAC ATTCTCTTTT TTCTCTTTT TTGAGAGACA GGGTCTCGT CTATAGCCA GGCTGGAGTG CAGTGGTGAAG GTACACGGTTC GTCCTCCAGA CGGACAGCTG TAAGAAAAAA AAGAAGAAAA AAAGCTCTGT CCCAGAGCGA GATAICGGGT CGCACCTCAC GICACCCACTC CAGTGCCAAG
901	A AACCGCAGATT CGAACTCTG GGCCTCAAACAA ATCTCTCTAC CTACAGTCT TAAATTTAAAT TGAACAAAC CTGTCATTA CGTITGAAGGA CAATTCCAA TTGCGTCAA GCTTGAGGAC CGGAGTTGT TAGGAGAGTG GATGTACAGA ATTATTTTA ACTTGATTG GACACAGTTA GCAACTTCCT GTAAAGGTT
1001	G ATTCTCTAG ACAATTGTTA CAATAATGT AACATTAT TCACAGCCCA CAATAGCTAA GCAGGTGGAAC GTATACACA CCTCCCCCTTG TTAAACTCTG CTAAGAGTC TTGTTAACAT GTTATTACAG TTGTTAAATA AGTGTGGGT GTTATCGATT CGATATGTG TGAGGGAAAC AAATTGAGAC
	Bbel ~~~~~
1101	A ACAACACCT CACCAAGGAA GTTCTATGTC CTCTTTCTT TTTTTAAGA CGGAGCTCA CTCTGGCGCC CAGGGCTGGAG TGCACTGACA GCTCACTGCA TGTGTTGGA GTGGTCTCTA CAAGATACAG GAGAAAAAGA AAAAATCT GCCTCAGAGT GAGACCGCGG GTCCGACCTC ACGTCACTGT CGAGTGACGT
	XbaI ~~~~~
1201	G CCGCCGACT CCCGGGITCA AGCAATCTC CTGCCCCAGC CTCCCAAGTA GCTGGGATTA CAGGCACACC TGCTACCATG TCCGGCTAAT GTTGTATT CGGAGGCTGA GGGCCCAAGT TCGTTAAGAG GACGGGTCAT CGACCCCTAA GTCCGTGTGG ACGATGGTAC AGGCCGATTA CAAACATAAA

1301 TCACTATAGA TGGGTTCCAC CATGTTGGC AGGCTGGTCT CAAACTCTG ACCTCCGCTG ATCTGCCGC CTGGCCCTCC CAAAGTGCTG GGATTACAGG  
 AGTCATATCT ACCCAAGGTG GTACAACCGG TCCGACCCAGA GTTGGAGGAC TGAGCGGGG GAACCGGAGG GTTCAAGGAC CCTAATGTCC  
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 ACATTCGGTGC ACGTGGGTCC GTAAGAAAAG AAGAAAACCA AAAAACATCTC TGTCACCAAAGA TGATAATGGG TCCGACCTCA CGTCACCGTA CTAGTGTGGA  
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 1601 TATTTCAAGT AGAGATGGGA TTCAACATG TTGCGCCAGC TTGCTCGAA CTCCGTACCT CTGGCGATCA GCCCACCTTG GCTTCCAAA GTGCTGGGAT  
 ATAAAGATCA ITCCTACCTA AAAGTGTAC AACCGGCTGG ACCAGAGCTT GAGGACTGGA GACCGCTAGT CGGGGAGAC CGGAGGGTT CACGACCTA  
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 CAGAGTTCAC ITTGAGGGTG GAGTCGGAGG ACTCATCGAC CCTGATGTC ACACGTTGAGT GTACGGACCC GATTAAGAAA AAAAAAAA TAAAGAATT  
 1901 AACCTTTT AGAGATGGCA TCTCACTACA TTGCTAGG TGTTCTCGAA CTCTTAGGT CAAACGATCC TCTCGCTCA GACTTCAAA GTGCTGGGAT  
 TTGGAAAGA TCTCTACCGT AGAGTGTAGT AACGGATCCG ACCAGAGCTT GAGGATCCG GTTGTAGG AGGACGAGT CTGAAGGTT CACGACCTA

HindIII

2001 GACAGGTGTG GGGCCACCAAG CCCGGTCTCT ATGTCATCT TTCTGATTAA AATAAATAAA TAAAGTTAC CACCCATG GCTCAAGGC CAAACCTCT  
 CTGTCACAC CGGGTGGTCC GGGCAGAGA TACAGGTAGA AAGACTAAAT TTATTTATT ATTTCATG GTGGGAGTAC CGAGGTTCG GTTCTGAAGA

XbaI

2101 AGCTTCTGCA ACACCAAA **EC** GTGCTGACAA GCCAGGAGGC ACTGACTGCT GACCGTGAGT CGCTGTCCTC CCCTCGCTTA AGGGGTCTCC CGGGAAAATG  
 TCGAAGACGT TGTTGTTT **CG** CAGGACTGTT CGGTCTCCG TGACTGACGA CTGGCAGTCA GCGACAGAAG GGGAGCAGAT TCCCCAGAGG GCCCTTTCAC

XbaI

2201 GCAACAGCCG GTCCCGGGGG CCAGGGGCCA CTCGACAGCC CAGGAAGCAGC GGCGCATGAA GCTACCCGGG CCGAAGGGCA AATCCGGCC CGGGCTGCC  
 CGTGTGCGC CAGGGCCCCC GGTTCCGGT GAGCTGCGG GTCTCTCGG CCCGTTACCT CGATGGCGG GGCTCTCCCT TTAGGGCGG GCGCGAGCG

BpuI

2301 CCCGGCGCTT TGGATCTGGG CCCGGCGCT GCGCCCCCCTA CCTGGACTCC GCGGCCGGCC CCGGCCCTCC CACGGCCCGG CGGGCCCTGC CCCTGGAGAC  
 GGGCCGCGGA ACCTAGACCC GGGCCGCGA CGCGGGGGT GGACCTGAGG CGCGGGCCGG GGCGGGAGGG GTGCCGGGGC GGCGGGAGAC GGGACCTCTG

XbaI

2401 CTGAGGGCGC CCTCTCCCG GGGAACTGCG ACCCGGAATC CTGGCTCTCG CCCTGTCGGG CTGCTGAGT GCGGGGACGG TGCCCAGCGC TGCGCACCA  
 GACTCCCGC GGAGGAGGGC CCTCTGACGC TGCGCTTAG GACCGGAGCG GGGACAGCGC GAGGCACTCA CGGCGCTCC ACGGGTGCGG AGCCGTGGG

BpuI

2501 GCGCCGGAGG CAGGACCGAG GAAGGGGACCC GGGCGGACCC CCAACCACTC CCGCTGCTG CACCGCGAGC AGCAGCGCCCG AGCTCACCCC GCCATTGGTG  
 CGGGGCTCCG GTCTGGCTC CTICCGCTGG CCCGCTCTGGG GTTGGTGTAG GGCACAGAC TGCGCGCTCG TCAGTCGGG CGGTAACCAC

BpuI

2601 TTCCCGCGC CTGCAAGGGC CGGGGGGAAG TGCCCTGGCT CACTTCGGT CAGAGGCCAC GCGCCCGGA CGGGCGGTGC AGGTACGAAA AGCCGGGGCG  
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NcoI

2701 GGGATTCCAG GAGTCGTTG GACCAAGGGG GGGAGGGGG CCAGCGGGC GCAGGAGACT AGGGGAGCTG AGCCATGGGC TTGGGGGAGA CGGGGGCGG  
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XbaI

2801 GAGCTCAAG GAGACAGAA GAGCAGGGTC GGGGGCTCGA GGGAGACAG GAGAGCGGG CGGGGGTTT GGGAGAGAGA CAAGGAGAGC GGGGCGGAGG  
 CTCGAGCTC CTCTGGCTT CTCTGGCCAG CCCCGAGCTT CCTCTGCCCC GGCCCCAAA CCTCTCTCT CGTCTCTCG CGCCCGCTCC

BsmBI

2901 CTTGGGGAG ACCAGGAGTA AATCACAAA ATTACTTIG GAAACCGTG CTCTCGAG AACAGATCA GTGTCGGAC AAAGTACAGC GGCCTGGTGT  
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BpuI

3001 GAAACTAACG CACAACCCCA TTACCTCTG CTGACCTGCC TCCGCCACCA GGACCCAGTC TCAGTAAAT GGGAAAGGT CCTCTGGCTC CTCGAAGGC  
 CTTTGATTCG GGGTTGGGT AAATGGGACA GACTGGACG AGGGGGGGT CCTGGTGTAG AGTCACCTTA CCTTCTCCAA GGGACAGGA GGACCTCC

BpuI

3101 CGTCGATGA GGATGTTGCT CACTCTCTA GTGCCCGCT TCTCAAGCCT CTAGGGAAAC ATACAGACAG CGAGGCTGAG GGGCTCCAC CCCACGGCAG  
 GCAAGCTACT CCTACACCGA GTGAAAGT CACGGACGG AGAGCTGGGA GATCCCTTG TATGCTGTC CGTCCGACTC CGGGAGGGTG GGGTGGCGTC

BpuI

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BpuI

3301 GTTACGACTC CTGCTTATC GCAAGGACAG AGGGCTTCT GTATCCCTAG GTTCTCTGGC TTGATGTACT GGAGCAATCA GATCACACGG CGGCTGGAG  
 CAATCTGAGG GACCGGAATAG CGTCTCTGTC TCCCGAAAGA CATAGGGATC CAAAGAACGG AACTACATGA CCTCTGGTACT CTAGTGTGCC GCGCAACCTC

BpuI

3401 AGTGGATGCA AGGTTTATC AGTGGGATTA GGCCTCAGCA GATGGGGGG CCAGAAGGC GTGGGAGTGG GAAGGGTATT TTCCCTGGGA GTCGGGCTGC  
 TCACTCAGT TCCAAACATC TACCTTAAT CGGGAGCTG CTACCCCTCG GGTCTCTGGT CAACCTCAC CTTCCATAA AAGGGGACCT CAGCCCCAGG

AatII

3501 TGAGCAGCTT GGGCTCTCT CTGACCCGCC CTGCCAAACT CATGGTCTCA TCAGTTGATG GCGCTGCTGGT GCGCTGTTGTT GCGCTCTCTG  
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AatII

3601 ACGTCCAGCG CCGTGTGCT CTGCCCCACTA TAAAAAACCA CTGGGGTTT TTATAGGC CAGGACGGGG **ACGTGGCGGG** CTGGGAAAT GCAACATTG  
 TCGAGGTGCG CGAACACAGA GACGGGTGTG ATTTTGGT GACCCCAAA AAATATCGT GTCTGCCCC **TGACACCGCC** GAGCCCTTTA CGTGTAAAC

AatII

3701 GCGAAAAAAA CAGAAATGGC TGTCTCACC CAGGCTCTG GGCACAGGCC TGGGTGCGA GCGCTGCGCA GAGACACCC CCTCTCTAC CCACGACTC  
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3901 CAGAAAAGTT GTTCTCGGTGGGCGAGTGG GCTCAACGCCTT GTATCTCGAG CACTTTGGGA GGCTGAGGTGG GTGCTGATCCG TTGAGCTCAG GAGTCGTGA
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4001 CCAGCCTGGG CAACGTGACA AAACCCCATC TCTACTAAAA ATACAGAAAT TAGCTGGGTG TGCGGGCTCA TGCTGTGGT CCCAGCTATG TGGGATGCTG
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4101 AGGCAGGAGG ATGGCTTCAG CCAGGGAGGC GGAGGTTACA GTGAACCGCG ATCACGCCAC TCTCCCTCAG CCTGGGCAAC AGAGCAGGAT CTGCTCAA
TCCGCTCTCC TACCGAAGTC GGTCCTCCCG CCTCCAAATG CACTTGGCGC TAGTGGGTG AGAGGAGGTC GGACCCGGTG TCTCGTCTTA GAACAGAGTT
4201 AATAAAATAAA GAAAGTTGG TCCTGGCCCTT CCAGCATTTC TCCAGGGTCC TTTTTCAGC AGGGACCTGG CGGAGGCATC GCCTCTGGAC
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4501 ACAGAGGAGC CAGGTCTCCAC TAGACTGAAA GAAGTCTCTG TTAACTCCAAA GGCTGACCTT GAGGACCCACA GGATAAACCTG CGTGTGTTAAT TTGGTGCCTA
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4601 TCAGGCACGG GGACTGTGTG TCCCTACTGT GTGCCCTATAA GCAGGGGGTA CTCGGGGGTA GACGGCAGGAG CAAGACCCAGC CTCTGAAAG GTGATGTGT
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4701 CCTCTGCACCC TGAGTACTGA CTTGTGGAGA AGGAAGGTAG CGCCGCCCTC TGCTCTGCTT GATCACCCCT GTGACCCCT GGTTCTCTGT TCTACCCAG
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**MTH-1 Coding sequence**

5001 ATGGGGCGCTT CCAGGCTCTA TACCCCTGGT CTGGTCTCTG AGCCTCAGCG AGTTCTCTG
TACCCGGGGA GGTCGGAGAT ATGGGACAC GACCAAGGAGC TCGGAGTCG TCAAGAGGAC

Figure 1 Human MTH-1 CDS 5'UTR(chromosome 7, CDS upstream 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.