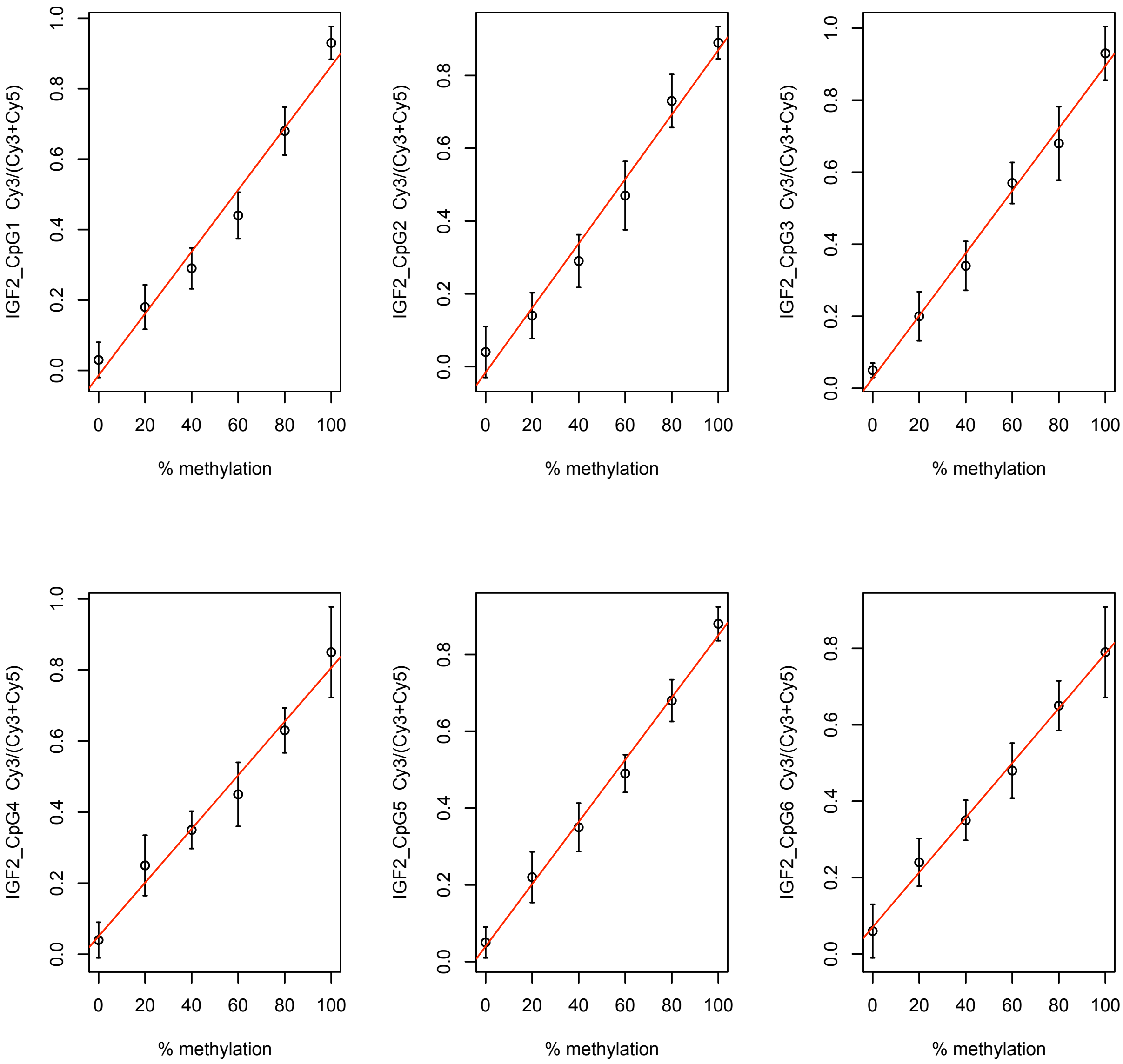
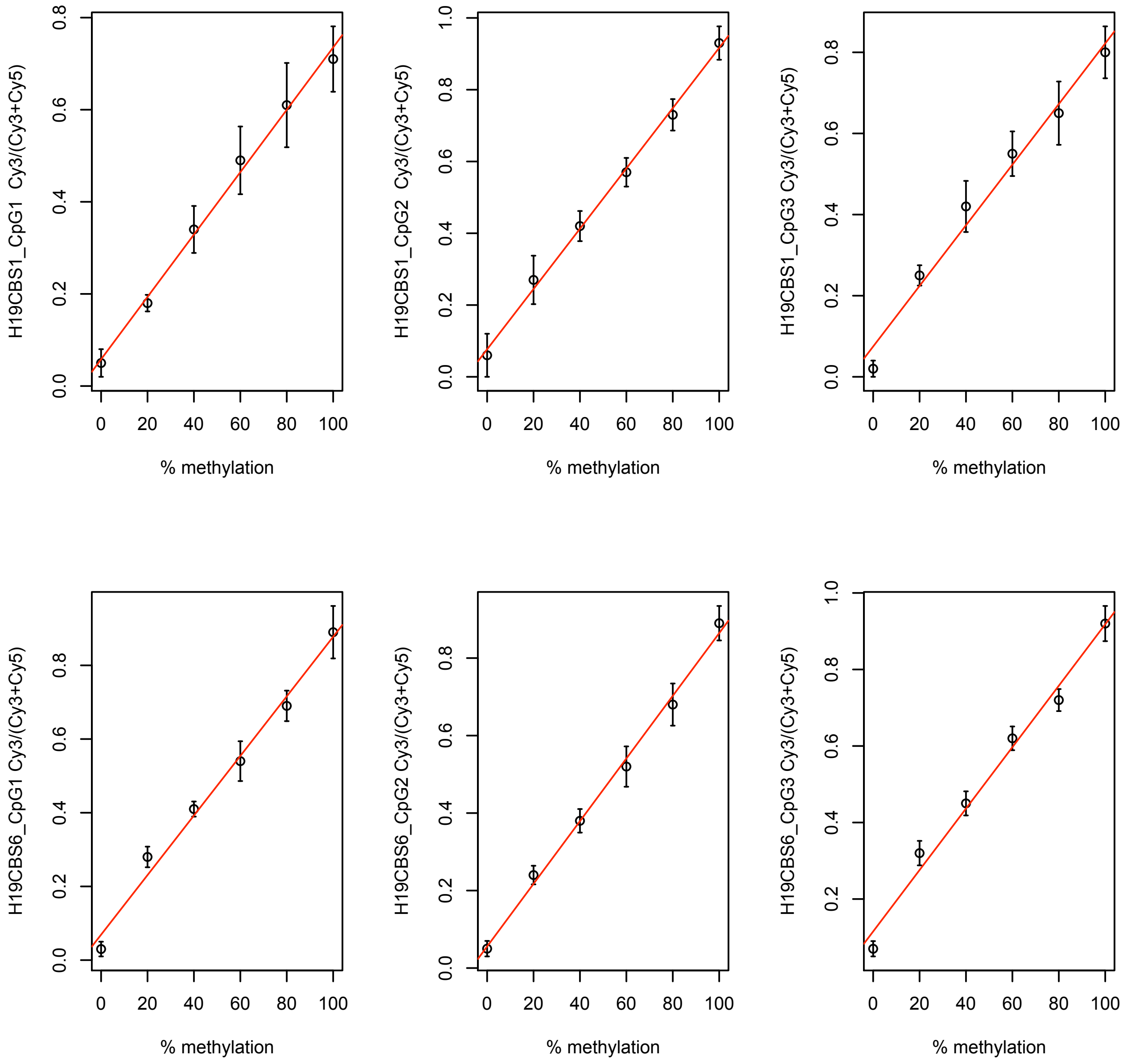
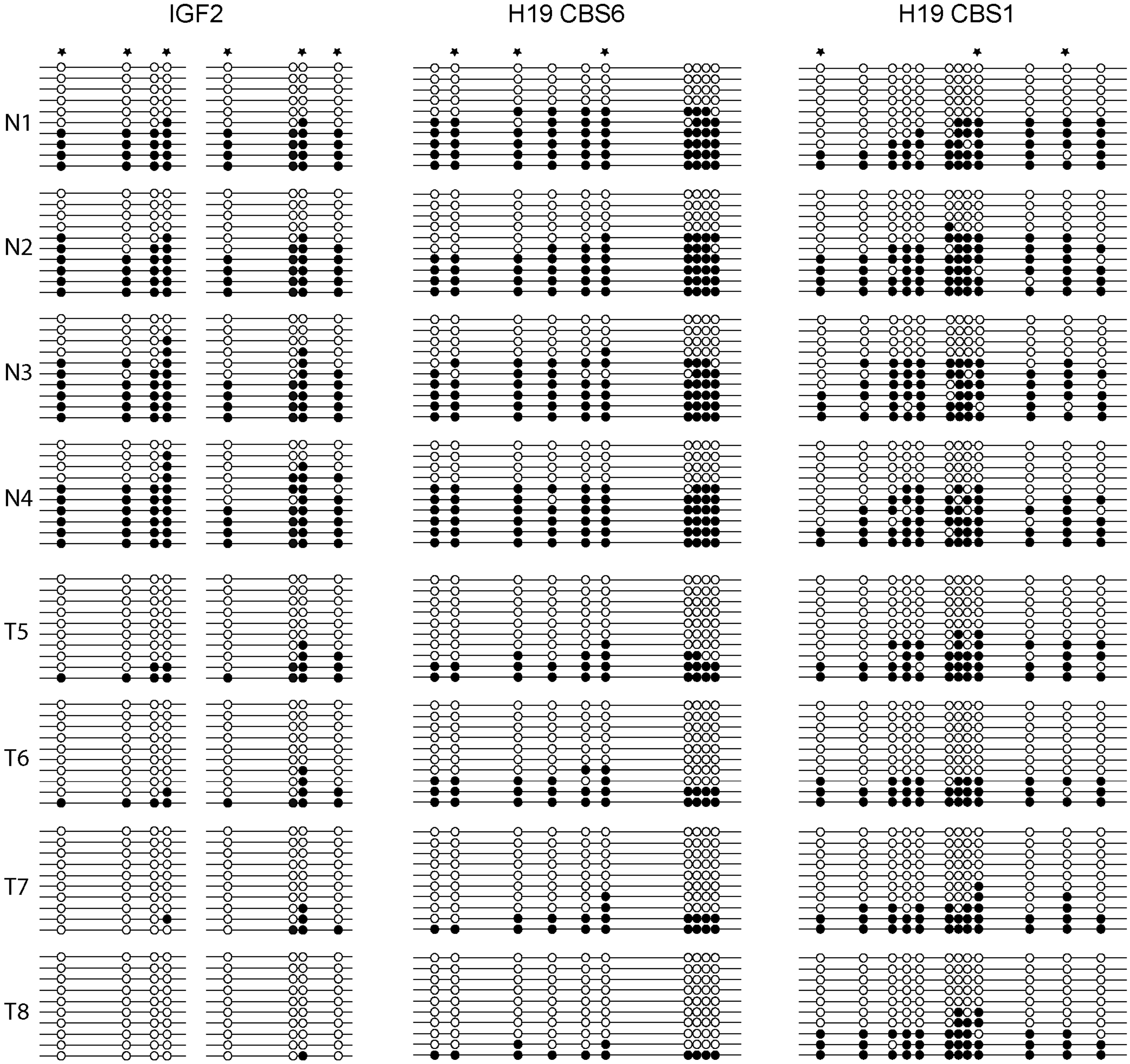


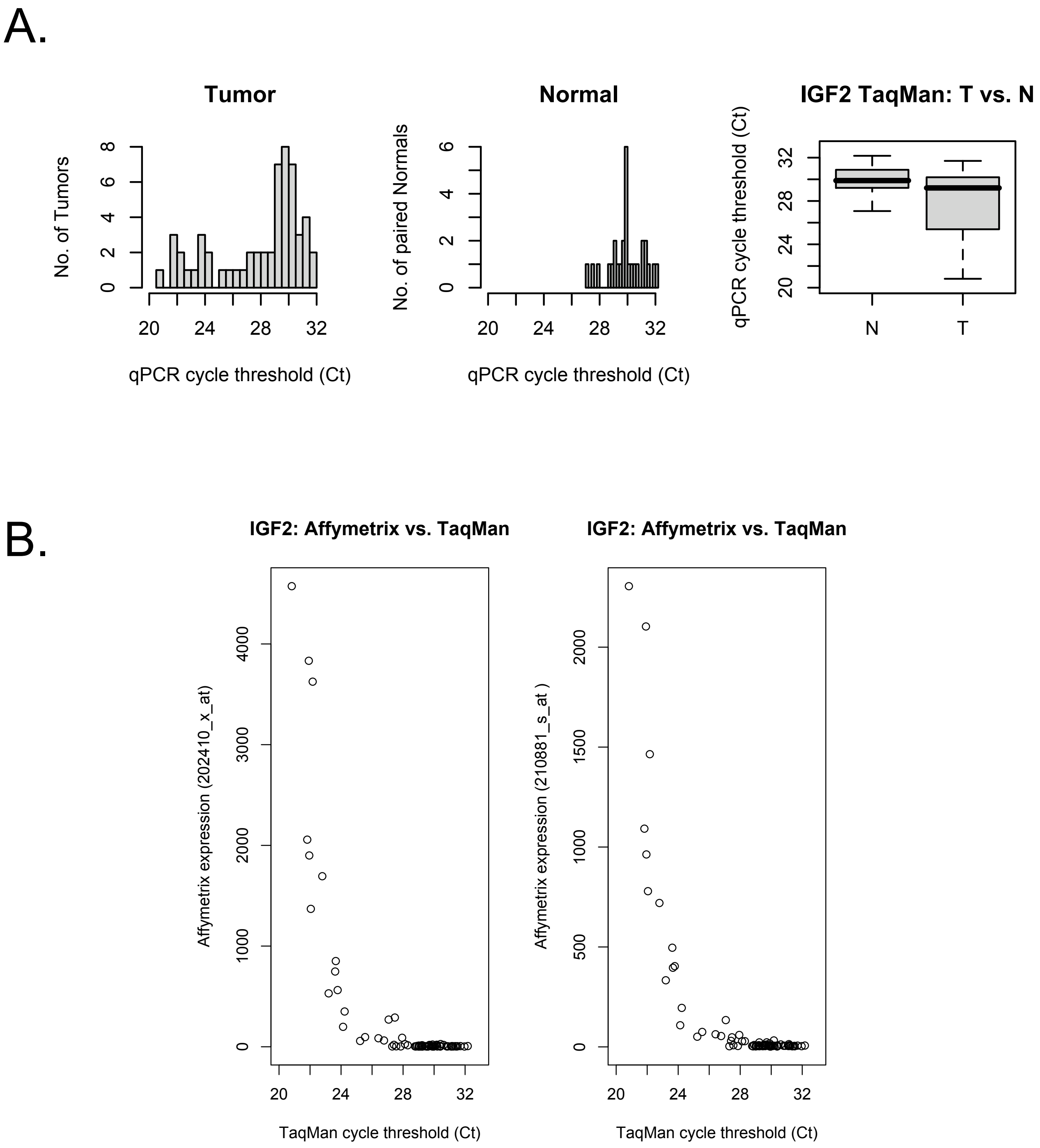
Supplementary Figure 1: The calibration curves of cytosine methylation levels. A total of 36 LDR primers were designed to interrogate the methylation status of 12 individual CpG dinucleotide sites at IGF2 and H19 DMRs. Synthetic DNA templates of methylated and unmethylated sequences were mixed in various ratios and followed by bisulfite/PCR/LDR/-Universal Array assay treatment. Six, three and three cytosines were analyzed at each of the IGF2, H19CBS1 and H19CBS6 loci. Each experiment was repeated at least three times.



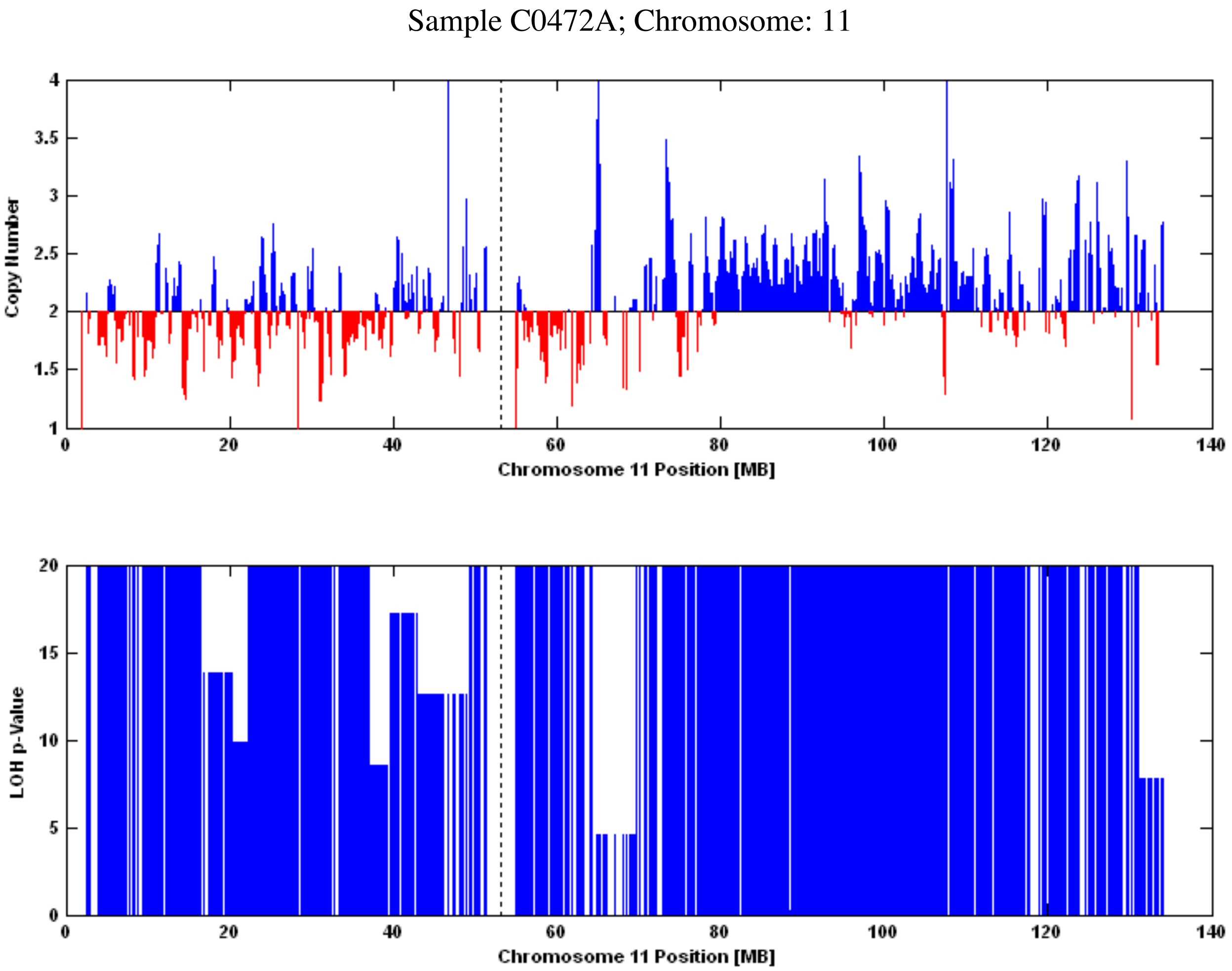
Supplementary Figure 1: (continue)



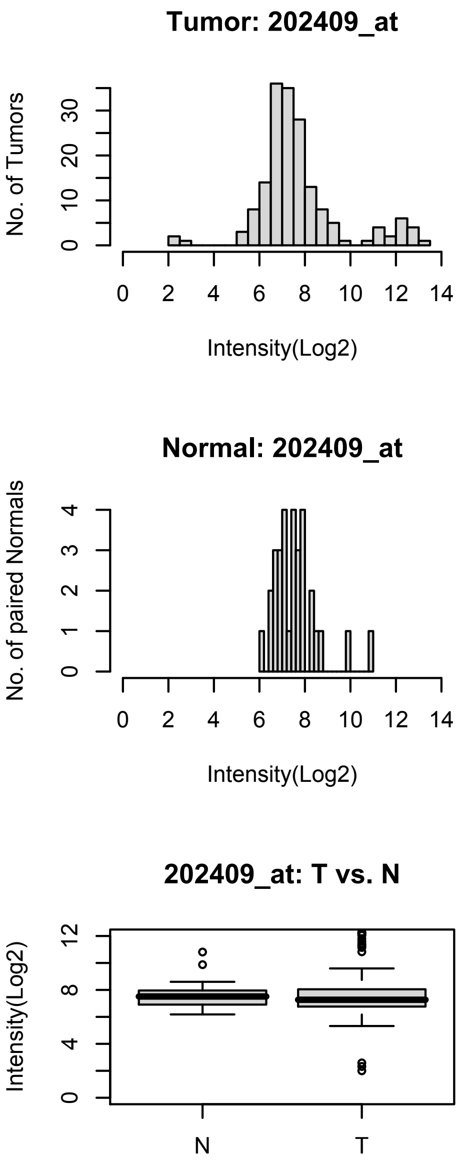
Supplementary Figure 2: Bisulfite sequencing of eight genomic DNA samples extracted from normal (N) and cancerous (T) colorectal tissues. Genomic DNAs were treated with sodium bisulfite, PCR amplified and subcloned before sequencing. Ten clones were picked and analyzed for each DNA sample. Each line represents an individual sequencing result in a given sample. Solid and open circles indicate methylated and unmethylated cytosines at CG dinucleotides, respectively. The cytosines analyzed by bisulfite/PCR/LDR/Universal array were indicated with stars.



Supplementary Figure 3: Quantitative real-time RT-PCR (TaqMan) was performed in 61 primary colorectal tumors and 29 matched normal colonic tissues to determine IGF2 RNA levels. TaqMan primer-probe set HS01005963_m1 (Applied Biosystems, CA) spanning IGF2 exon 3-4 boundary was used for the experiment. (A) The distributions of IGF2 RNA levels in CRC tumor and normal tissues. Gene expression is shown in qPCR cycle threshold (Ct) value. The increased IGF2 expression is tumor-specific ($p = 0.0073$). (B) The correlations of IGF2 expression levels in 61 colorectal tumors measured by Affymetrix HG-U133A2 array and TaqMan assay. The gene expression levels determined by Affymetrix probe sets (202410_x_at and 210881_s_at) were consistently identified using real-time RT-PCR assay ($p<0.0001$).



Supplementary Figure 4: An example of copy neutral LOH or UPD at the IGF2 (11p15) locus in a colorectal tumor.

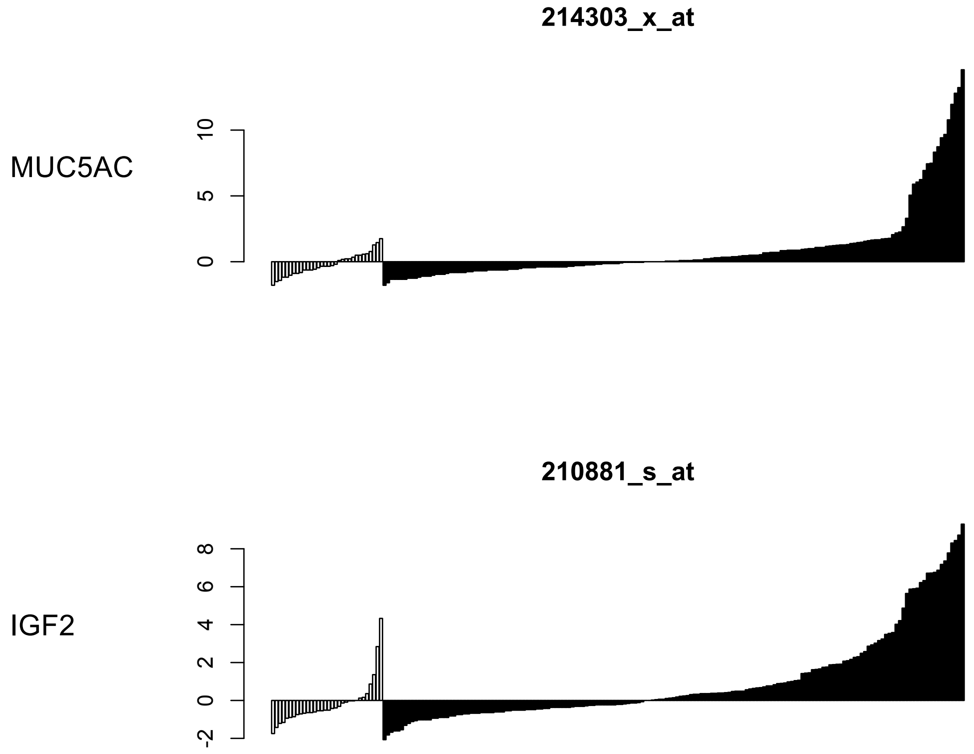


Tumor: 202409_at

Normal: 202409_at

202409_at: T vs. N

Supplementary Figure 5: The distribution of IGF2 expression levels in 167 primary CRC and 32 matched normal colonic tissues. The gene expression levels were measured using HG-U133A2 array probe set 202409_at. No tumor-specific differential IGF2 expression (p = 0.566) was observed.



Supplementary Figure 6: IGF2 was the first candidate pair identified by COPA. The gene expression data of 167 primary CRC and 32 normal colorectal tissues were employed in the study. White and black colors represent the gene expression levels of normal and tumor samples, respectively.

Supplementary Table 1

PCR primers (methylation)	Sequences
IGF2_1 RP	CGCTGCCAACTACCGCACATCGGGGTTTATTTTTTTAGGAAGTATAGTTAT
IGF2_1 FP	CGCTGCCAACTACCGCACATCACCCTTCTATTAAACAAACTACCCTATTC
IGF2_2 RP	CGCTGCCAACTACCGCACATCTGTGGGAGGAGGTGGATTTTAGT
IGF2_2 FP	CGCTGCCAACTACCGCACATCCCTCAAAATACCTAAAACACTCACCTC
H19_CBS1 RPb	CGCTGCCAACTACCGCACATCGTATAGGTATTTTTGGAGGTTTTTTATTTAGT
H19_CBS1 FPc	CGCTGCCAACTACCGCACATCCGCATAAATATCTATCTCTAACAACCCTC
H19_CBS6 RP	CGCTGCCAACTACCGCACATCTTGAGGGTTGGGGAGATGAGATAT
H19_CBS6 FP	CGCTGCCAACTACCGCACATCCCAAAAAAAACTTAAACTATAATATATAAACCTACA

LDR primers (methylation)	Sequences
IGF2_1 49C	cy3TTTTTTAGGAAGTATAGTTACGTCGTTTTTTATTGGTTTC
IGF2_1 49T	cy5GTTTATTTTTTTAGGAAGTATAGTTATGTTGTTTTTTATTGGTTTT
IGF2_1 148C	cy3GGTGTTTGGAGGTGGAGGAGGTTTTTATATTTC
IGF2_1 148T	cy5GGTGTTTGGAGGTGGAGGAGGTTTTTATATTTT
IGF2_1 177C	cy3TTTCGTGTTATGATTTTTTAAGGCGGGTTATTC
IGF2_1 177T	cy5TTTTTATATTTCGTGTTATGATTTTTTAAGGTGGGTTATTT
IGF2_1 49Com(Z3)	pGTTAAGTAGAGTTGTGTGTTTAGTGGTTTTTGTTGGGGCCGTAAGCCCGTATGGCAGATCAA
IGF2_1 148Com(Z4)	pGTGTTATGATTTTTTAAGGTGGGTTATTCGTGTGTATTTATGGCCGTGCTGGGGACAAGTCAA
IGF2_1 177Com(Z5)	pGTGTGTATTTTTTATTTTTAGTGTTAGGTAGAAGTTTATTTTTATTTAGGTTGCAACGGGCTGGTCAACGTCAA
IGF2_2 46C	cy3GGAGGTGGATTTTAGTTTTTAGTTTTAGGGTTTTGAATC
IGF2_2 46T	cy5GGAGGTGGATTTTAGTTTTTAGTTTTAGGGTTTTGAATT
IGF2_2 156C	cy3TGTTGTGGTTTGTGGTTTAGGCGATTTTAGC
IGF2_2 156T	cy5GTTGTTGTGGTTTGTGGTTTAGGCGATTTTAGT
IGF2_2 205C	cy3TTAGAATTGAGGTTGGTAGTTAGTTTTAGTTTTAGTTTTAATTGC
IGF2_2 205T	cy5TTTTAGAATTGAGGTTGGTAGTTAGTTTTAGTTTTAGTTTTAATTGT
IGF2_2 46Com(Z6)	pGTTGTTAGTTTAGTTTTTTGTTTAGTTTGTTTTATAGTTTGAGTTTTAGTAGCATCATGGGGGAAAGCTTCGTCAA
IGF2_2 156Com(Z7)	pGTTTTTAGAATTGAGGTTGGTAGTTAGTTTTAGTTTTAGTTTTAATTGCCGTTTGCGGTCGTCCTTGCTCAA
IGF2_2 205Com(Z8)	pGAGGTAGAGAGGTGAGTGTTTTAGGTATTTTGAGGGATTGCCGCACCGTCCGTCATCTCAA

Supplementary Table 1 (continue)

<u>LDR primers</u> (methylation)	<u>Sequences</u>
H19 CBS6 79C	Cy3TGGATGATGGGGATTTCGGTTTTAGC
H19 CBS6 79T	Cy5TTTTTGGATGATGGGGATTTTGGTTTTAGT
H19 CBS6 132C	Cy3TTGGTAGGTATAGAAATTGGGGGTTTTTGC
H19 CBS6 132T	Cy5TTTTGGTAGGTATAGAAATTGGGGGTTTTTGT
H19 CBS6 194C	Cy3GTTTTTTTTTTTGTTTTATTATTCGGATGGTATAGAATC
H19 CBS6 194T	Cy5ATGTTTTTTTTTTTGTTTTATTATTTGGATGGTATAGAATT
H19 CBS6 79Com(Z12)	pGTGAGGTTTTTTTTTATAGGGTTTTGGTAGGTATAGAAAGACGCACCGCAACAGGCTGTCAA
H19 CBS6 132Com(Z13)	pGTAGTATATGGGTATTTGTGGATGTTTTTTTTTTTGTTCATCGCTGCAAGTACCGCACTCAA
H19 CBS6 194Com(Z14)	pGGTTGTAAGTGTGGATTTAAAAGTGGTTGCGGGCTGGGACGTGCAGACCGTTCAA
H19 CBS1 57C	Cy3GTTTTTTATTTAGTTTTGGATGTTAGTTTTATTAAGGGC
H19 CBS1 152C	Cy3GGAATTAGAAGTGGTCGCGCGGC
H19 CBS1 213C	Cy3TCGAGTATGTTTGGTTGGGGTTTATTTATAGAAAC
H19 CBS1 57T	Cy5GGTTTTTTATTTAGTTTTGGATGTTAGTTTTATTAAGGGT
H19 CBS1 152T	Cy5CGTGTGGAATTAGAAGTGGTCGTGTGGT
H19 CBS1 213T	Cy5GTTTGAGTATGTTTGGTTGGGGTTTATTTATAGAAAT
H19 CBS1 57Com(Z33)	pGGTTTATTTTGTTGATTTTATTAAGGGAGGTTCGCGATTTCGACTCAAGCGGCTCTTT
H19 CBS1 152Com(Z34)	pGGTAGTGTAGGTTTATATATTATAGTTTGAGTATGTTTGGTTGGCGCAATGGTAGGTGAGCAAGCAGA
H19 CBS1 213Com(Z35)	pGTTTTAGGTTTTTTAGGTTAGGTGTTGTATTGGTTTTCGTCCCCGTTACCTAGGCGATCAGA

<u>RT-PCR / LDR primers</u>	<u>Sequences</u>
IGF2_820_For	CCCCCACAACAACCCTCTTAAA
IGF2_820_Rev	GGTCGTGCCAATTACATTTCATTTG
IGF2_820_Com	CCCCAGAAATCACAGGTGGGCACGTCGC
Vic_IGF2_820_G	VIC-ATAATGAGTCCCTGAACCAGCAAAGAGAAAAGAAGGG
Ned_IGF2_820_A	NED-TGAGTCCCTGAACCAGCAAAGAGAAAAGAAGGA