The American Journal of Human Genetics, Volume *90*

Supplemental Data

Primate Genome Gain and Loss: A Bone Dysplasia, Muscular Dystrophy, Bone Cancer Syndrome Resulting from Mutated Retroviral-Derived MTAP Transcripts

Olga Camacho-Vanegas, Sandra Catalina Camacho, Jacob Till, Irene Miranda-Lorenzo, Esteban Terzo, Maria Celeste Ramirez, Vern Schramm, Grace Cordovano, Giles Watts, Sarju Mehta, Virginia Kimonis, Benjamin Hoch, Keith D. Philibert, Carsten A. Raabe, David F. Bishop, Marc J. Glucksman, and John A. Martignetti

Figure S1. Amino Acid Sequence of MTAP Splice Variants

Exons 1-7 are shown in black typeface and MTA-coordinating amino acids are underlined. Exon 9S - red; 9L - purple, 10 - blue (two different reading frames are used); 11 - green. Arrows above exons depict the positions of translational stop codons.

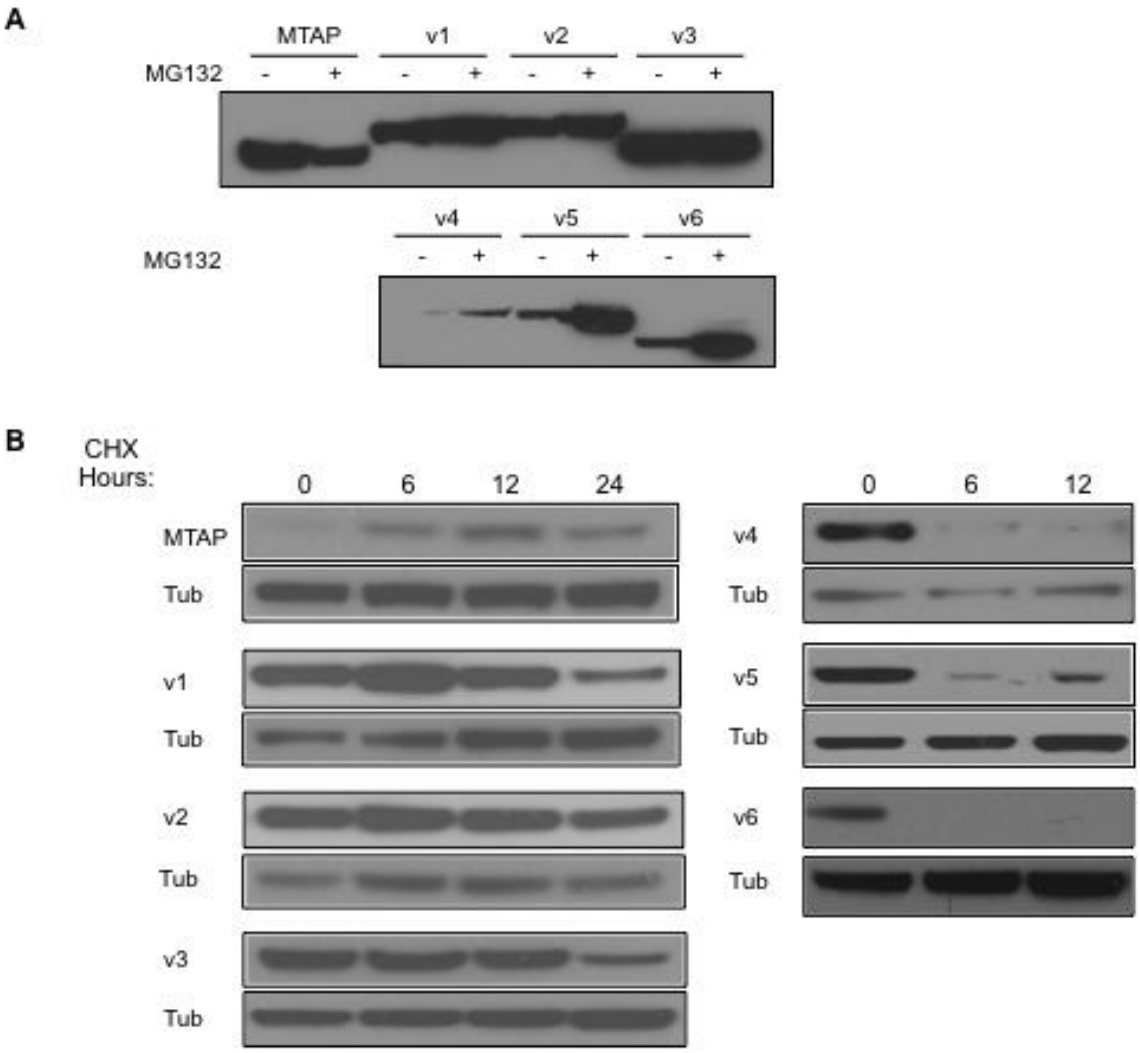

Figure S2. Protein Stability of MTAP Splice Variants

(A) Transiently transfected MCF7 cells expressing each MTAP isoform were treated with MG132 (5mM) for 4 hours.

(B) Transiently transfected MCF7 cells were exposed to cyclohexamide (10μM) for 6, 12 and 24 hours. Archetype MTAP: MTAP

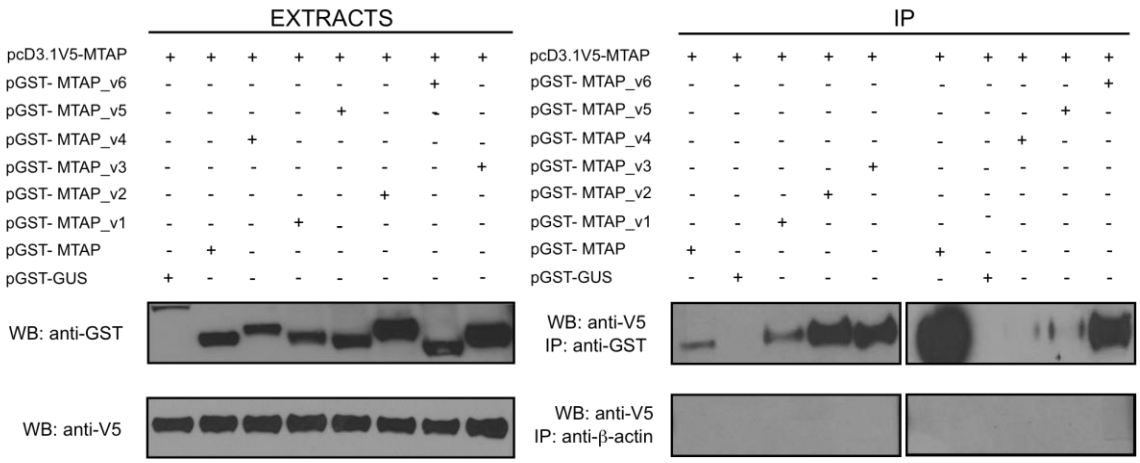

Figure S3. Coimmunoprecipitation Studies Showing Interaction between Archetype MTAP and All MTAP Splice Variants

Combinations of differentially-tagged (-V5 and -GST) isoforms of MTAP were co-expressed in PC3M cells and co-immunoprecipitated. Immunoprecipitation was performed with anti-GST and anti-β-actin as control. Lysates (left panel). Co-immunoprecipitates: MTAP, MTAP_v1, v2, v4, v5 and v6 (right panel). Archetype MTAP: MTAP

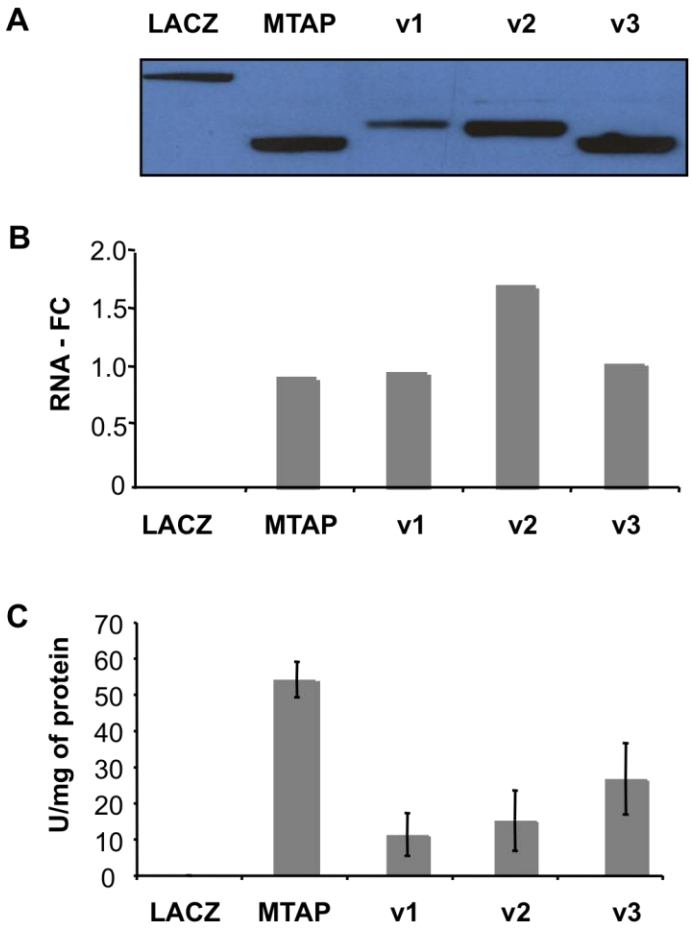

Figure S4. **Determination of MTAP Activity on Cell Extracts from MCF7 Stably Expressing MTAP_v1, v2, and v3 V5-Tagged Fusion Proteins**

(A) Western-blot showing protein expression.

(B) Quantitative real-time PCR results using primers that recognize all variants.

(C) Intracellular MTAP activity levels.

The error bars represent the averages of three independent experiments.

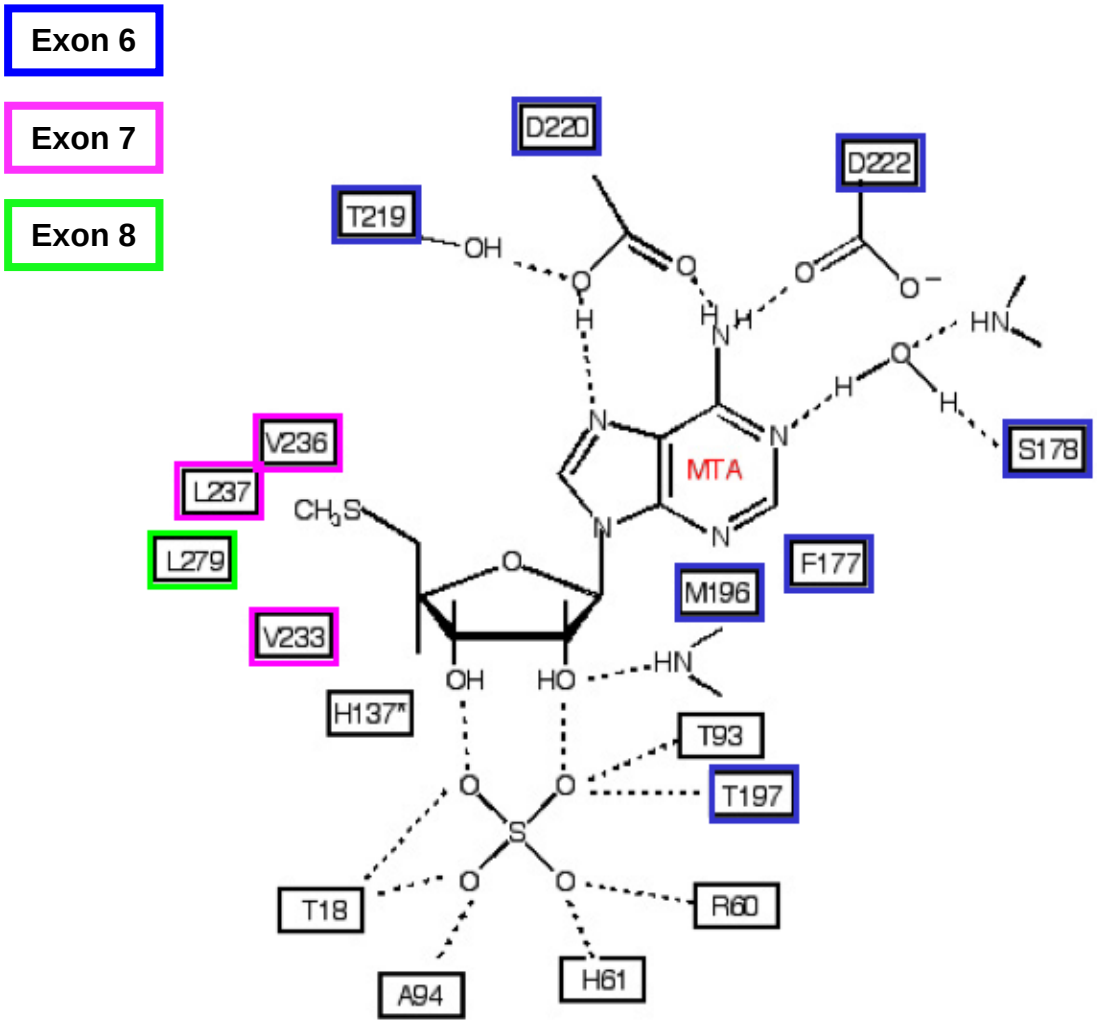

Figure S5. Amino Acids Coordinating to MTA

Note that exon 8 contributes only one amino acid in this structure.

Table S1. DNA and cDNA Amplifying Primers

MICROSATELLITE PRIMERS	5´ 3´
D9SB3 Forward	CGGAATCAACCTAAATGTAG
D9SB3 Reverse	CAGTTCTGTTCACATTGCTG
AL624 Forward	CACCAAAGTGAGTGTGCCAT
AL624 Reverse	GCTTTACCAGCTATCTGGGT
AL882 Forward	CATCACAGGACATAGCAGC
AL882 Reverse	TGAGGCTTGATGATGGTAG
GENOMIC PRIMERS	
gExon 1 Forward	GAACGCGGGAATTCGATTTGG
gExon 1 Reverse	TGCAAGCGTACCCTCTCCTC
gExon 2 Forward	GGCTTGGCAGGAATGGAG
gExon 2 Reverse	TCAAGCCAGCAAGGAGAAATG
gExon 3 Forward	GAGTCCTGTTGTGGTTGAA
gExon 3 Reverse	CAATGTAAGGGGCAAGAGCA
gExon 4 Forward	ACTCTAGGAGAAAACAGTTGG
gExon 4 Reverse	ACCAGCTACAATAGCCTAAAG C
gExon 5 Forward	AGATAAAGTTGACTCACCAGC
gExon 5 Reverse	CCAATTAACCAAATGCCAGAT G
gExon 6 Forward	AGTTGTGCATGTGCTAGTATG
gExon 6 Reverse	ACACCCTATTGGCACCCAGA
gExon 7 Forward	GCAGTGGAATTTTAAGTTCTAGTA
gExon 7 Reverse	GGCATAAAATACATGAGGACCAA
gExon 8 Forward	GTATGTTTCCTGCGTCCTCA
gExon 8 Reverse	CATACTCTTTGATAGGCAAGGG
gExon 9 Forward	AAGTTCCAGATGATCCTCAG
gExon 9 Reverse	CAGTCTTCTGTAACTGCTTC
CDNA PRIMERS	
Exon 1 Forward	GCACTGCTCACTCCCGCGCA
Exon 5 Forward	CAGAGGAGTGTGCCATATTC
Exon 6 Forward	GAGGGACCTCGTTTTAGCTC
Exon 8 Reverse	CATACTCTTTGATAGGCAAGGG
Exon 11 Reverse	ACTATAGGATGTCATGATTT
Exon 6F1	GACAGATTATGACTGCTGGAAGGAG
REAL TIME PCR PRIMERS	
MTAP- Forward	CGGTGGACCGGGTCTTAAAGAC
MTAP-wt Reverse	ATGTCTTGGTAATAAAACAGAAAACTGG

MTAP_v1 Forward	ACCCTCCATAACCTGAAGATG
MTAP_v1 Reverse	CTGAAAGGCACTTCTTACCTC
MTAP_v2 Forward	CTCCATAACCTGAAGATGATCA
MTAP_v2 Reverse	GACAGGGGCAGGATTTCAC
MTAP_v3 Forward	ACCCTCCATAACCTGAAGGTA
MTAP_v3 Reverse	GGATGTCATGATTTCATGCAAAT
MTAP_v4 Forward	AGCACGAGGAAGCAATGATC
MTAP_v4 Reverse	GCTGAAAGGCACTTCTTACCT
MTAP_v5 Forward	AGCACGAGGAAGCAATGATC
MTAP_v5 Reverse	CCAGCCAGGTCTAAGGGAG
MTAP_v6 Forward	AGGAGCACGAGGAAGCAGTA
MTAP_v6 Reverse	GGATGTCATGATTTCATGCAAAT
MTAP-All Forward	ACCACCACCGCCGTGAAG
MTAP-All Reverse	CTTGCAAGGAGGACGCAATC
Actin Forward	CAATGACCCCTTCATTGACC
Actin Reverse	GATCTCGCTCCTGGAAGATG
HPRT Forward	TATTGTAATTGACCAGTCAACAG
HPRT Reverse	GGTCCTTTTCACCAGCAAG
PLASMID PRIMERS	
V5: for MTAP	ATGTCTTGGTAATAAAACAGAAAACTGG
V5: for v1-v2	TGATTTCATGCAAATATATGTTG
V5: for v3-v4	TGGCGGGAGCTGAAAGGCACTTC
Intron 5-SalI Forward	ACGCGTCGACGTCGGCTGGGGAAACAGGATTATGTGA
Intron 8-NheI reverse	CTAGCTAGCTAGATGTAGAGTTCCCAGCAC
Intron 8-SpeI Forward	GGACTAGTTCCAGCCTGATGATTATAACCAATG
Intron 9-XhoI Reverse	CTAGCTAGCTAGTTCCATTATCATTTTGCCTTTTG
Exon 9-XhoI Forward	CCGCTCGAGCGGCAACAGAGCGAGAGT CTTC
Intron 11-SacII Reverse	TCCCCGCGGGGAACCACAACCTATGAGATGCT

Underlining in the PCR primers above indicates the introduced sequence including restriction sites.