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Supplemental Data

A Restricted Spectrum of Mutations in the *SMAD4* Tumor-Suppressor Gene Underlies Myhre Syndrome

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Table S1. Primer Pairs and Annealing Temperatures Used to Amplify the *SMAD4* Coding Sequence, and Sizes of PCR Products

Exon	Primer Sequence (5′→3′)		Ann. Temp. (°C)	Product Length (bp)
	Forward	Reverse		
1	GTTTTTCACTGTTTCCAAAG	AATTACCCTGTAGTAGCTTG	58	416
2	TGAGTTGGTAGGATTGTGAG	TTGAAACACTATTGAGATCC	58	329
3	GATAGCGTTTATGCTACTTC	TTGTTAATGTTACTGCCTGC	58	282
4	CACTGTAATTGATTTTAGGTG	GACTACACATAAATAAGCAATG	58	386
5+6	GATGACATCTATGAATGTACC	AAAAACAGAAAACAAAGCCC	58	448
7	TTCTTAGACATTGCATAAGC	TAATTCCATTAAAGCCTGTG	58	272
8	GCAAGTGAAAGCCTTATATC	GTACATGGGAAAACATAACC	58	330
9	AGCTATCTTTTGGTTTTATG	CAACAAATAGAGCTTTAAGTC	58	347
10	AATTCTTTTCATGTGAGAGG	ATGCAAACAGGGTCATAGGC	58	445
11	TAGAATGTAGGGAGGATGGG	GGATTGTATTTTGTAGTCCACC	62	338

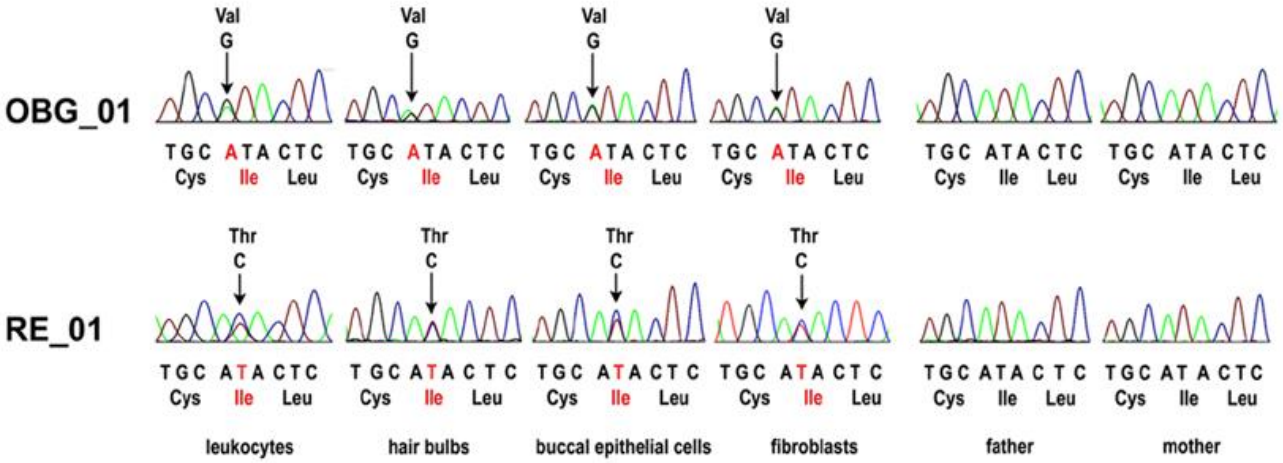

Figure S1. Germline *SMAD4* Mutations Underlie Myhre Syndrome

Electropherograms showing the *de novo* germline origin of the heterozygous c.1498A>G (above) and c.1499T>C (below) missense changes.

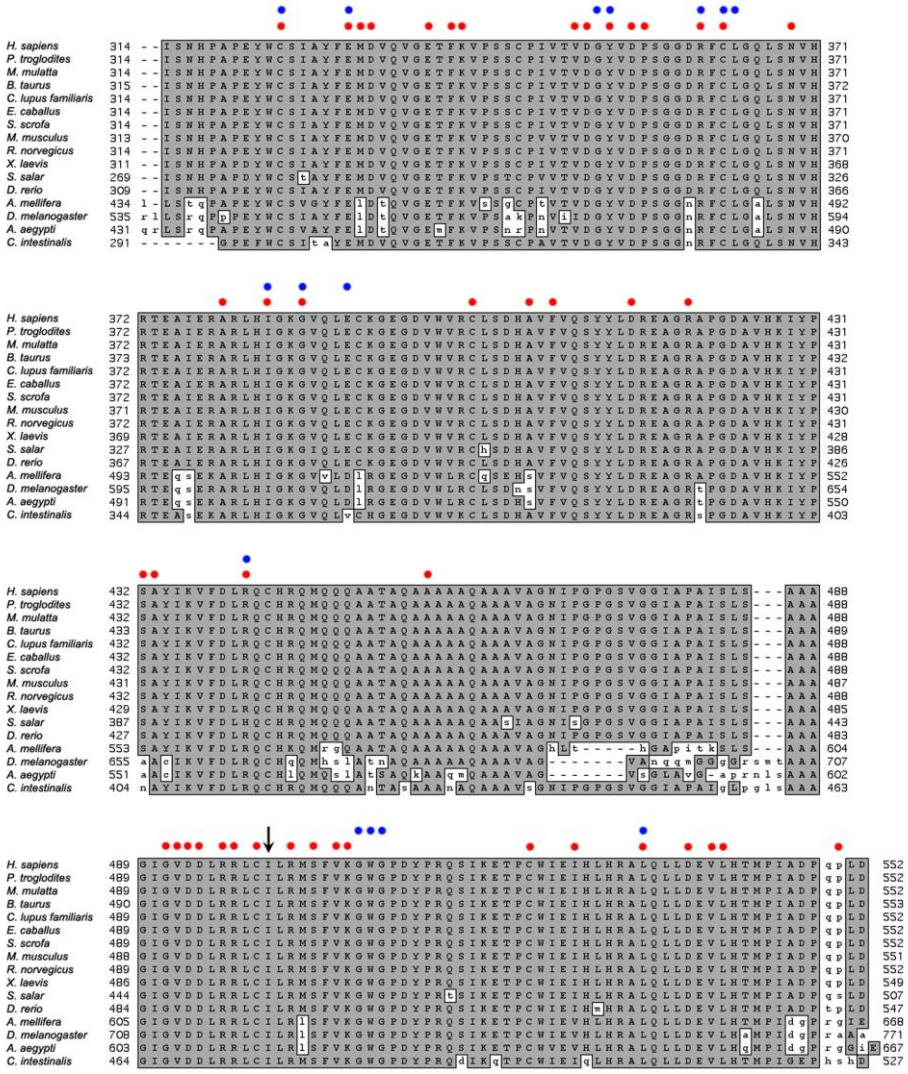

Figure S2. Amino Acid Sequence Conservation of the SMAD4 MH2 Domain (Residues 321 to 530)

Amino acid sequence alignment of SMAD4 orthologs showing conservation of Ile^{500} (arrow) and residues affected by cancer-associated somatic mutations (red circles) and/or germline lesions (blue circles) occurring in juvenile polyposis syndrome (JPS) and JP-hereditary hemorrhagic telangiectasia.

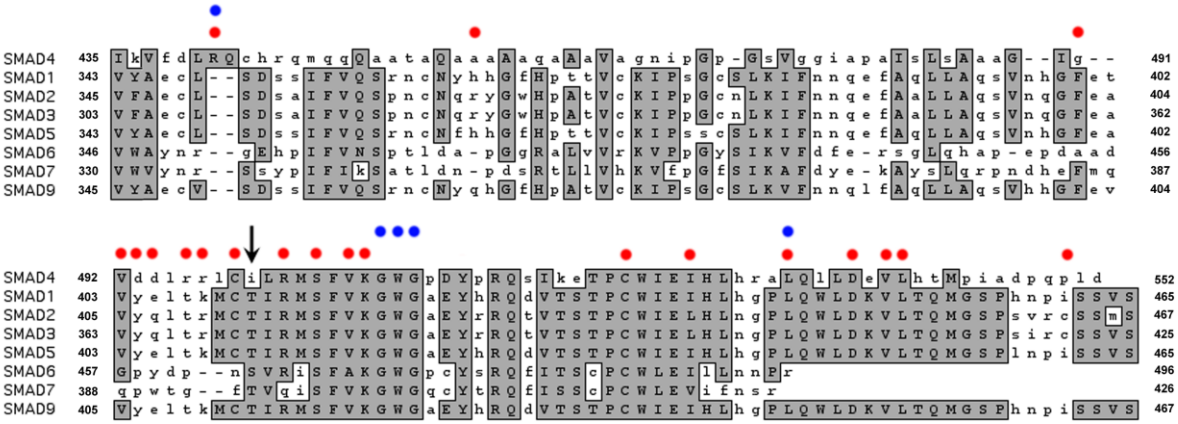

Figure S3. Amino Acid Sequence Conservation of the SMAD4 MH2 Domain Portion Flanking the Residue Mutated in Myhre Syndrome

Partial amino acid sequence alignment of SMAD paralogs showing that Ile^{500} (arrow) is not a conserved residue. In most paralogs, a threonine residue at the corresponding position is observed. Residues affected by cancer-associated somatically acquired lesions (red circles) and/or germline mutations occurring in juvenile polyposis syndrome (JPS) and JP-hereditary hemorrhagic telangiectasia syndrome (blue circles) are shown.

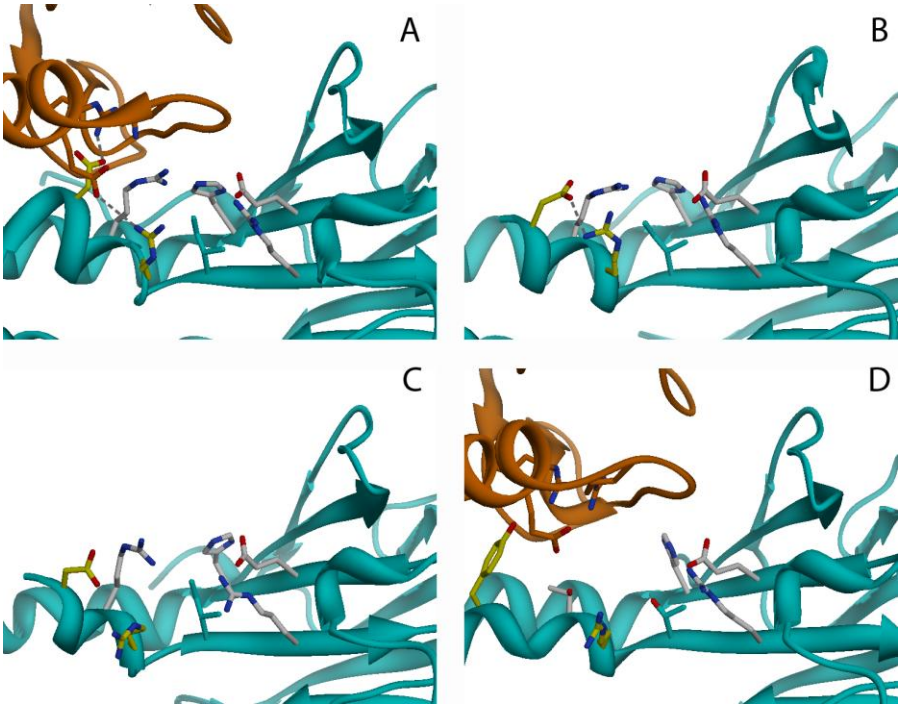

Figure S4. Plasticity of the SMAD4 Asp^{493} and Arg^{497} Side Chains (or Those of Corresponding Residues in R-SMADs) in Different MH2 Domain Structures

In the structures of heterotrimeric complexes (A and D), also one of the SMAD3 interacting subunits (orange) is shown. Asp^{493} and Arg^{497}, Ile^{500}, and the other residues directly interacting with it (*i.e.*, Arg^{496}, Arg^{502}, Glu^{526}, His^{528}) are shown in sticks representations with their C atoms colored in yellow (Asp^{493}, Arg^{497}), cyan (Ile^{500}) or white (all others). In panel D, the evidenced residues are those located in the positions corresponding to the side chains listed above, with the same color code (Tyr^{364}, Arg^{368}, Thr^{371}, Thr^{367}, Arg^{373}, Glu^{397}, His^{399} correspond to Asp^{493}, Arg^{497}, Ile^{500}, Arg^{496}, Arg^{502}, Glu^{526}, His^{528}, respectively). Electrostatic interactions with a minimum distance between charged groups smaller than 4Å are evidenced by dashed grey lines. At the interface of the SMAD4/SMAD3 heterotrimer (pdb code 1U7F, chains B, C) (A), intermolecular electrostatic interactions are present between Asp^{493} and Arg^{287}, and Arg^{497} and Glu^{284}. In the SMAD4 monomeric structure (pdb code 1YGS) (B), an intramolecular ion pair is formed between Asp^{493} and Arg^{497}. SMAD4 structure extracted from its complex with SKI (pdb code 1MR1, chain B) (C). SMAD3/SMAD3 interface in the heterotrimer (pdb code 1U7F, chains C, A) (D). An essentially identical structure is also observed in the SMAD2/SMAD2 interface in the heterotrimer (pdb code 1U7V, chains C, A; not shown). Visualization and analysis of the molecular structure was performed using the program UCSF Chimera.[47]