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Supplemental Data

Mutations Preventing Regulated Exon Skipping in *MET* Cause Osteofibrous Dysplasia

Mary J. Gray, Peter Kannu, Swarkar Sharma, Christine Neyt, Dongping Zhang, Nandina Paria, Philip B. Daniel, Heather Whetstone, Hans-Georg Sprenger, Philipp Hammerschmidt, Angela Weng, Lucie Dupuis, Rebekah Jobling, Roberto Mendoza-Londono, Michael Dray, Peiqiang Su, Megan J. Wilson, Raj P. Kapur, Edward F. McCarthy, Benjamin A. Alman, Andrew Howard, Gino R. Somers, Christian R. Marshall, Simon Manners, Adrienne M. Flanagan, Karl E. Rathjen, Lori A. Karol, Haemish Crawford, David M. Markie, Jonathan J. Rios, Carol A. Wise, and Stephen P. Robertson

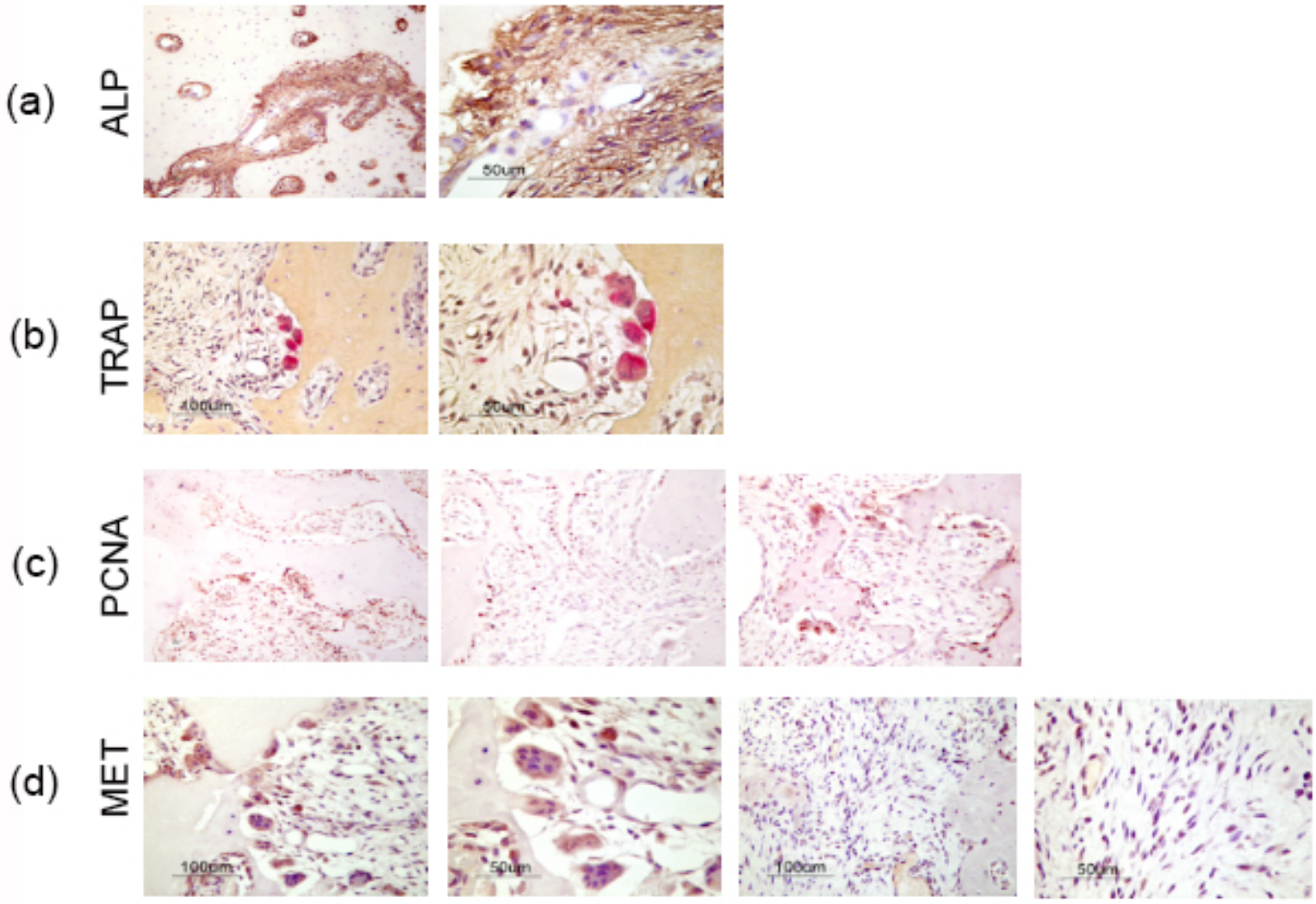

Figure S1. Additional histological data from OFD lesional tissue from individuals with mutations in *MET* (II:3, III;8).
(a) Staining for alkaline phosphatase (ALP). Strong staining of peripheral zones with a relative paucity noted in the most central aspects of lesions.(b) Staining for tartrate resistant acid phosphatase (TRAP). Osteoclasts are observed at the mineralising interface at the periphery of the lesions.(c) Proliferating cell nuclear antigen (PCNA) staining. The most mitotically active cells are those located at the periphery of the lesions. The central spindle-shaped cells show little evidence of proliferative activity.(d) Staining for MET. MET is widespread throughout the lesions with osteoblastic and osteoclastic cells at the mineralising surface of lesions demonstrating pronounced positivity.

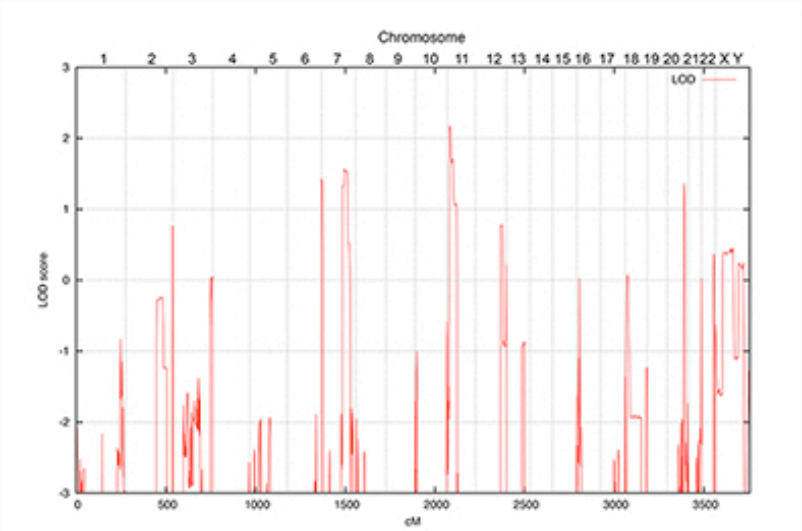

Figure S2. Linkage results from family 1.. LOD plot of linkage analysis of family 1 under a model with penetrance set at 0.9 for the disorder. This analysis resulted in the identification of several regions of inconclusive linkage. With the penetrance set to 1.0 the maximal LOD score for the chromosome 11 region increased to 2.16 and the chromosome 7 and 20 regions disappeared below 0. These maximal LOD scores were too low for the theoretical solving power inherent in this pedigree structure The existance of non-penetrant individuals was tested for by serially excluding two unaffected individuals from the pedigree at a time and recomputing linkage. Linkage signals were maximised if either IV:7 or III:13 are assigned as non-penetrant for the disease Removal of either person moves the LOD score toward significance.If case IV:7 is assigned as non-penetrant, then it is the chromosome 7 haplotype that is linked (LOD 3.0) with the disease whereas if III:13 is designated non-penetrant the chromosome 11 locus contains the disease-linked haplotype (LOD 3.0). The chromosome 20 peak diminished under all permutations.

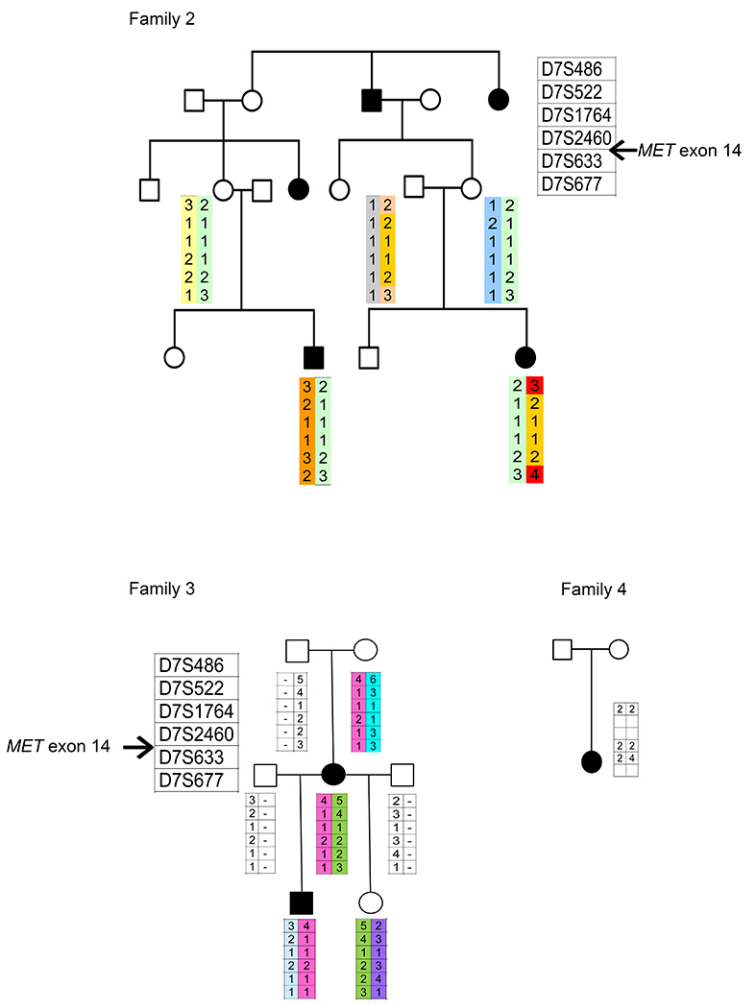

Figure S3. Haplotype analysis of families 2 and 3. Both family 2 and family 3 were shown to segregate the same splice site mutation (c.3082+1G>T) in *MET*. Microsatellites flanking *MET* were genotyped to examine if the mutations in both families were carried on the same haplotype (suggesting a common origin) or arose independantly. Inferred haplotypes are colour coded, indicating that this mutation arose independently on different haplotypes in both kindreds. Family 4 also shared the same mutation but DNA quality precluded analysis to conclusively prove a third independently-arising mutational event.

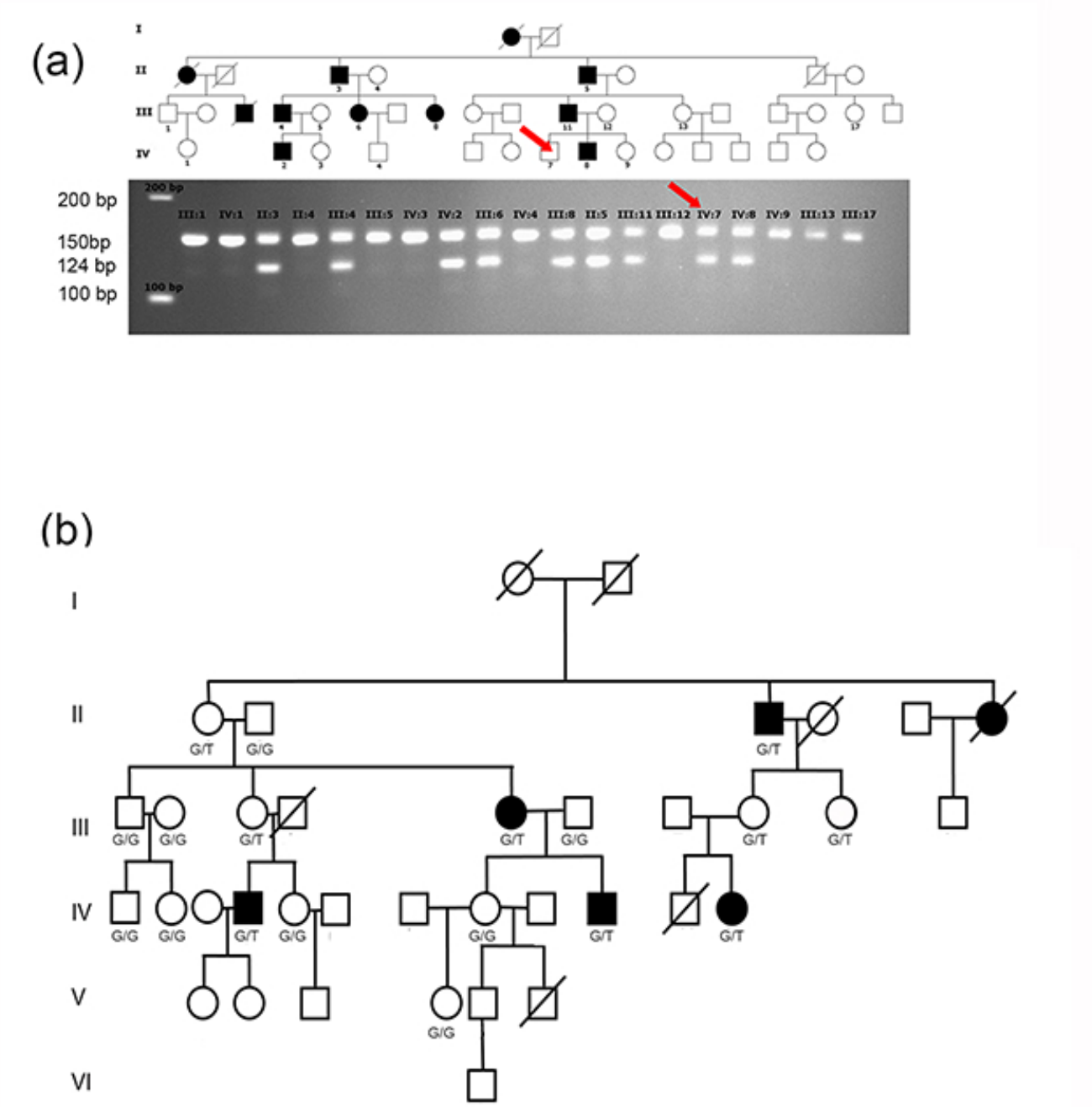

Figure S4. Segregation of mutations in families 1 and 2.
(a) Pedigree of family 1 with filled shapes indicating a clinically evident phenotype. Depicted underneath the pedigree is an agarose gel of PCR amplified products flanking the 26 bp region that is deleted on the mutant allele. The wildtype allele produces a 150 bp product with the deleted allele producing a 124 bp product. The left hand lane depicts 100 and 200 bp markers. The red arrow highlights the position of the non-penetrant individual in the family together with their PCR genotype, showing that they have inherited the mutant allele.(b) Segregation of the c.3082+1G>T mutation in family 2. The genotypes for indicated individuals were obtained by direct Sanger sequencing.

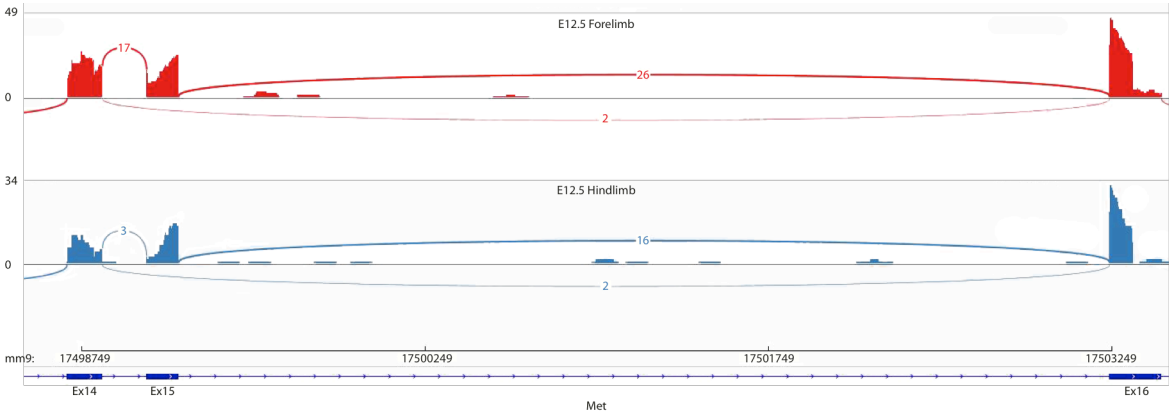

Figure S5. Sashimi plot showing regulated splice-exclusion of *Met* exon 14 in developing mouse limb bud. Embryonic day E12.5 mouse forelimb and hindlimb buds were dissected and RNA analyzed by RNA-sequencing. Low-level expression of $Met^{\Delta 15}$ is evident in both hindlimb and forelimb samples. The number of split-reads spanning multiple exons is shown. Two independent reads were detected in each instance spanning exons 14 and 16 with splice exclusion of exon 15.

Table S1.
Variant discovery after target capture and massively parallel sequencing in three individuals from Family 1.

	III:8	IV:2	IV:8
Annotated monoallelic variants	**2397**	**1817**	**1052**
Novel monoallelic variants	**238**	**190**	**167**
Novel non-synonymous variants	**6**	**6**	**4**

Table S2.
Variant sharing between the three sequenced individuals from Family 1.

	Total
Total Variants (all individuals)	**12120**
Total variants* shared by 3 individuals	**68**
Variants* shared by all 3 individuals as heterozygotes	**13**
Coding variants* shared by all 3 individuals	**1**

*not represented in dbSNP131, 1000-genomes

Table S3
Exome sequencing statistics for the four individuals from family 2.

	IV-12	III-7	IV-10	IV-4
Affection status	affected	unaffected	affected	affected
Reads in target region	140448630	129649374	161173350	130192780
Bases on target at 10X (%)	76.14	75.03	76.53	74.46
Bases on target at 5X (%)	81	80.19	81.26	79.72
Bases on target region at 1X (%)	88.61	88.26	88.77	88.01
Avg fold enrichment in targets	101.86	93.80	116.54	94.21
Total variants (SNVs and indels)	45218	44742	44874	43355

Table S4
Primer sequences for RT-PCR

Gene	**Primer**	**Sequence (5' to 3')**
PPIA	fwd	TGCCTTCTTTCACCTTCCCA
PPIA	rev	GTCCTGGCATCTTGTCCATG
Rsp29	fwd	CCGACTCGTTCCTTTCTCCT
Rsp29	rev	GCACATGTTCAGCCCGTATT
Col1a1	fwd	CCAGGTCCTAAGGGTGACAG
Col1a1	rev	AATGGGACCAGTCAGACCAC
OSX	fwd	CTCGGTTCTCTCCATCTGCC
OSX	rev	TCTTTGTGCCTCCTTTCCCC
Runx2	fwd	ATCCCCATCCATCCACTCCA
Runx2	rev	GAACTGCCTGGGGTCTGAAA
AlkP	fwd	AACCCAGACACAAGCATTCC
AlkP	rev	CGGGCTCAAAGAGACCTAAG
Rankl	fwd	ACACACTACCTGACTCCTGC
Rankl	rev	TCCAACCATGAGCCTTCCAT

Table S5
Markers used for Haplotyping

Marker	**Genomic coordinates (GRCh38)**
D7S486	chr7:115894762 -115894903
D7S522	chr7:116072642 -116072858
D7S1764	chr7:116396232 -116396481
D7S2460	chr7:116407988- 116408180
MET exon 14	chr7:116771850 -116771995
D7S633	chr7:117010785 -117010954
D7S677	chr7:117139414 -117139692

MET extends from chr7:116672400 – 116798500