

Cappato *et al.*

Supplementary information

Supplementary Table 1. List of the oligonucleotides used in this work.

Name	Sequence
Oligonucleotide for <i>LMX1B</i> mutation screening	
LMX1b-ex1FnewATG	GCGTCCCATGGATATAGCAA
LMX1b-ex1R	CCCCAACGCCCGTGCCACA
LMX1b-ex2Fnew	TTTCGCCCTGTGCGCTACAG
LMX1b-ex2Rnew	GCTTACTGTTGGTAGTCTTG
LMX1b-ex3F	TTCTGAGCACCGCCAACAC
LMX1b-ex3R	CCCGTCCCTGAGGCCTG
LMX1b-ex4F	GGAGCCACGGCAGGTGTCAAC
LMX1b-ex6R	CAGCCTCTGCCCCAGCTCAC
LMX1b-ex7F	TGCCTGGGGAGTCCCAAGG
LMX1b-ex8Rnew	GCGTCCGTGCGCCTGGCTG
Oligonucleotide for <i>LMX1B</i> cDNA analysis	
LMX1B-2FcDNA	CAGTGCGCGGCGTGTCTAGCA
LMX1Bex1f-R_H	AAGCTTTTGCTATATCCATGGGACGC
LMX1B-274F	CCGGGACTGACAAGCAGGTGAC
LMX1B-274Flu	CCGGGACTGACAAGCAGGTGACAGAGGAG
LMX1B-274R	CCTGCTTGTCAGTCCCGG
LMX1B-ex2RcDNA	TGCTGACACGCCGCGCACTG
LMX1B-ex3RcDNA	CTCCTTGAGCACGAATTCGTC
LMX1B-ex4RcDNA	CTTCATGTCCCCATCTTCATCC
GEL2F LMX1B	CAGAGCCCCTACGGCAGCAG
LMX1B-3RSNP	GAATTCGTCGCCCTTGCGTAGCTGCCGC
LMX1BflagR	GGTCTTTGTAATCGGAGGCG
Oligonucleotide for the analysis of the basal promoter and the 5'UTR of <i>LMX1B</i>	
LMX1B-1495F_K	GGTACCTCACTGCAAACCCTAGGAG
LMX1B-PROM3F	CTCCTCGTAGCGCTGGAGC
PromLMX1B-635F	ACGGCACTATTTGACGTGGAG
PromLMX1B-791F	GGGTGGAGCAGAGGGATTG
PromLMX1B-791R	CAAATCCCTCTGCTCCACCC
Oligonucleotide for <i>used for mutagenesis</i>	
LMX1B mutins F	GGCGATCGTGGCTAGCGGCGACGCGC
LMX1B mutins R	GCGCGTCGCCGCTAGCCACGATCGCC
LMX1B 3F RS2277158	TCTGCTGCTGCGTGTGTGAGCGGCAGCTAC
LMX1B 3R RS2277158	GTAGCTGCCGCTCACACACGCAGCAGA
226MutF	GCGCGCGTGGCTGCAAATGTCCGGGA
226MutR	TCCCGGACATTTGCAGCCACGCGCGC
LMX1B -174F	CCCGCCCGCACGATGCCGGGG
LMX1B -174R	CCCGGCATCGTGCGGGCGGG

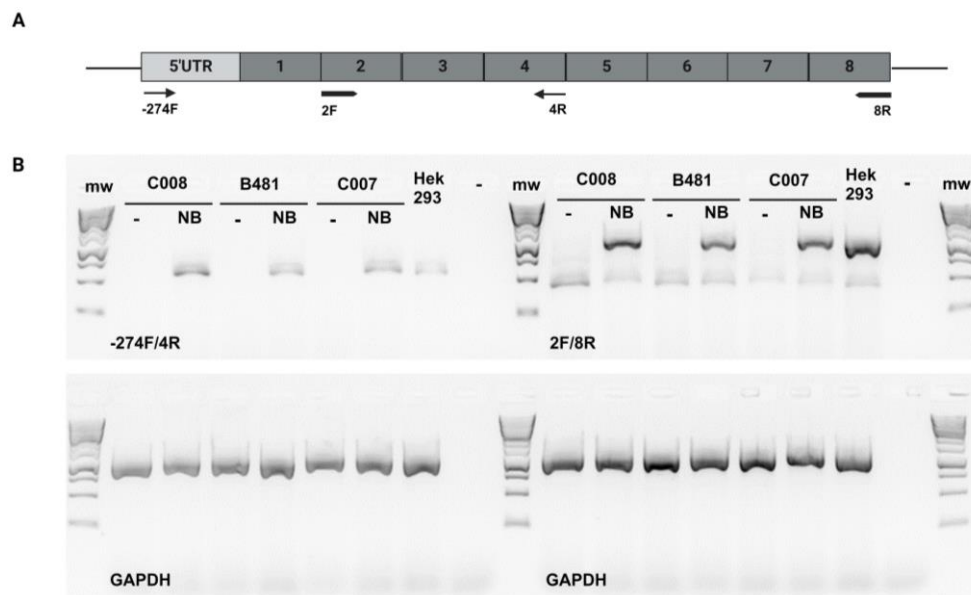
Supplementary Table 2. Variants scoring according to ACMG* criteria.

ID sequence (MANE SELECT)	Variant position	Variant classification	Scoring
NM_001174147.2	c.-174C>T	VUS	PM2, PP1, BP4
NM_001174147.2	c.-226G>A	VUS	PM2, PP1, BP4

VUS, Variant of Uncertain Significance

*Richards S, et al. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30.

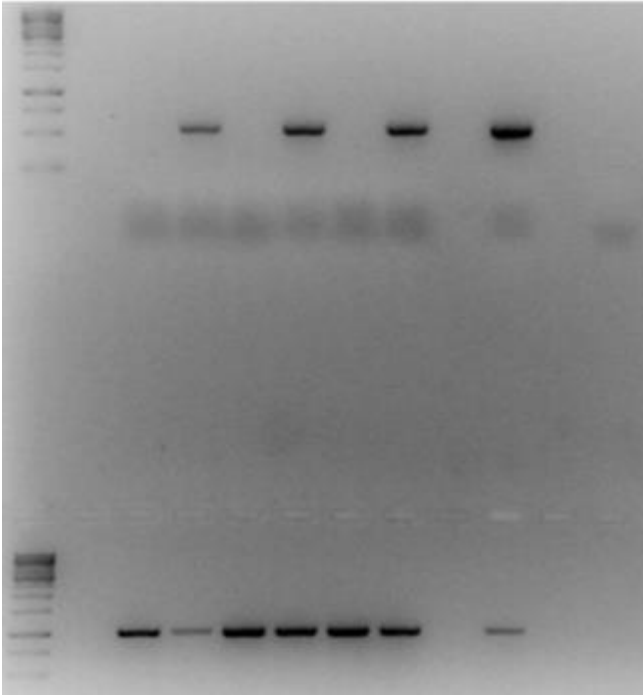
Supplementary Figure 1



Supplementary Figure 1. Treatment by Sodium Butyrate induces *LMX1B* gene expression in lymphoblasts. Lymphoblastoid cell lines derived from B481 proband, his affected father (C007) and unaffected mother (C008) were treated 24 hours with 10mM Sodium butyrate (NB). Whole cDNA was obtained after retro-transcription of 2 μ g of RNA the *LMX1B* cDNA was amplified by PCR in two fragments to cover the whole coding sequence. The bands corresponding to *LMX1B* were detectable only in cells treated with NB and in the Hek-293, used as a positive control. The housekeeping gene *GAPDH* was used to check the cDNA synthesis. Image created with BioRender.com.

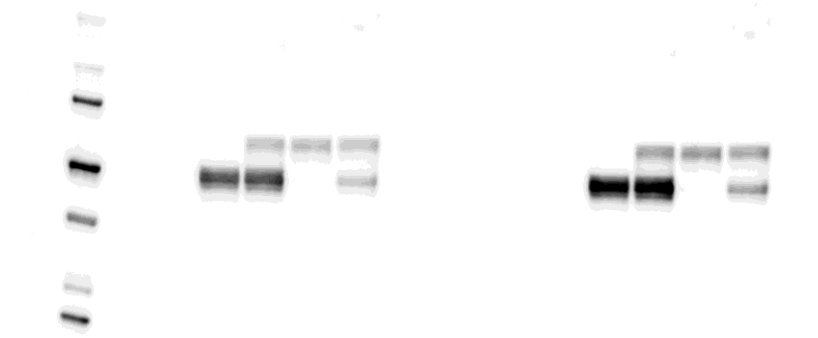
Supplementary Figure 2. Uncropped images of blots and gels. (a) Uncropped gel images for Figure 3a. (b) Uncropped blot images for Figure 4d; (c) Uncropped gel images for Figure 5a; (d) Uncropped blot images for Figure 6b; (e) Uncropped gel images for 5a Supplementary Figure 1b.

(a) Uncropped gel images for Figure 3a. Upper lanes RT-PCR specific for *LMX1B*; bottom lanes RT-PCR for housekeeping *GAPDH*. 1Kb molecular weight marker.

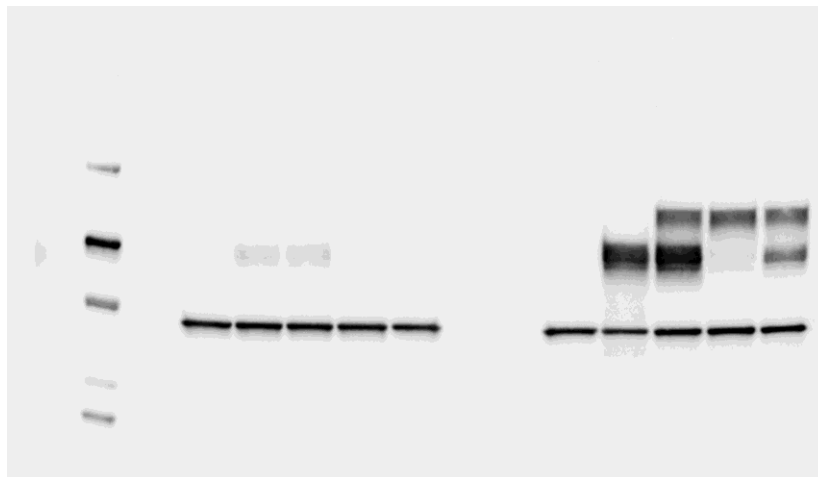


(b) Uncropped blot images for Figure 4d

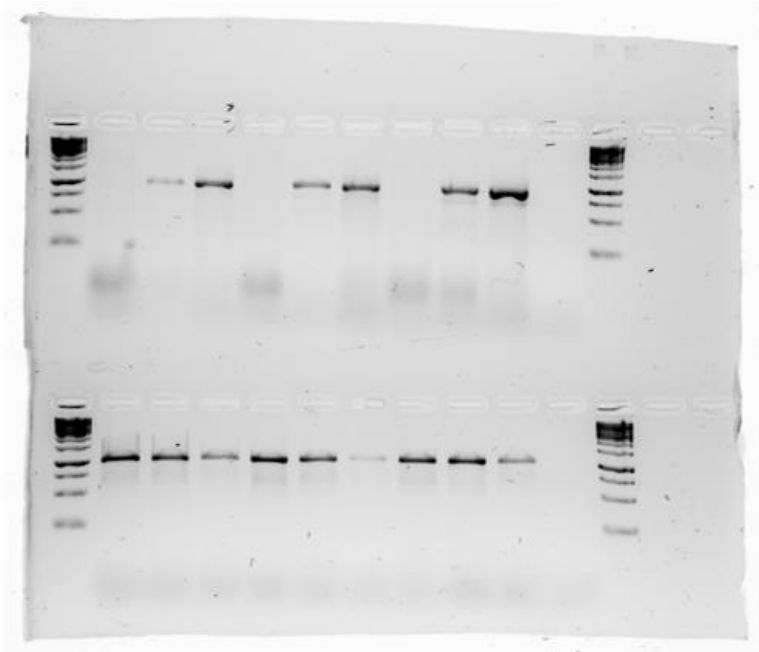
WB anti-LMX1B (left panel) and anti-FLAG (right panel). Membrane was cut.



Corresponding anti-GAPDH WB after membrane stripping. The anti-FLAG primary antibodies and the anti-GAPDH are both anti-mouse and are recognized by the HRP conjugated anti-Mouse secondary antibody.

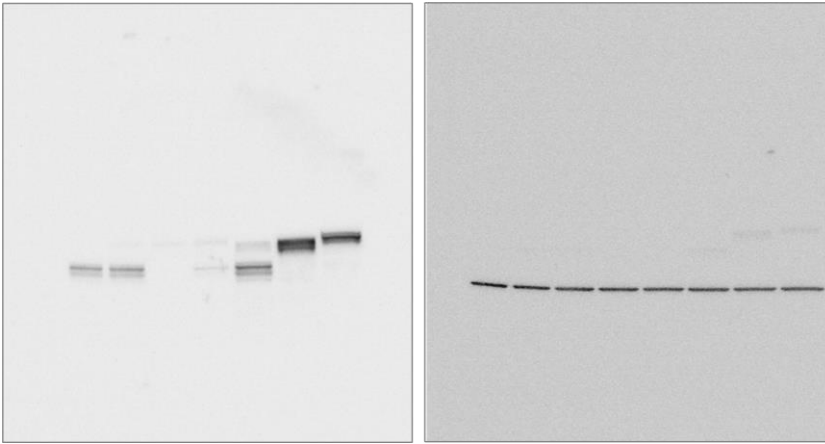


(c) Uncropped gel images for Figure 5a. Upper lanes RT-PCR specific for *LMX1B*; bottom lanes RT-PCR for housekeeping *GAPDH*. 1Kb molecular weight marker.

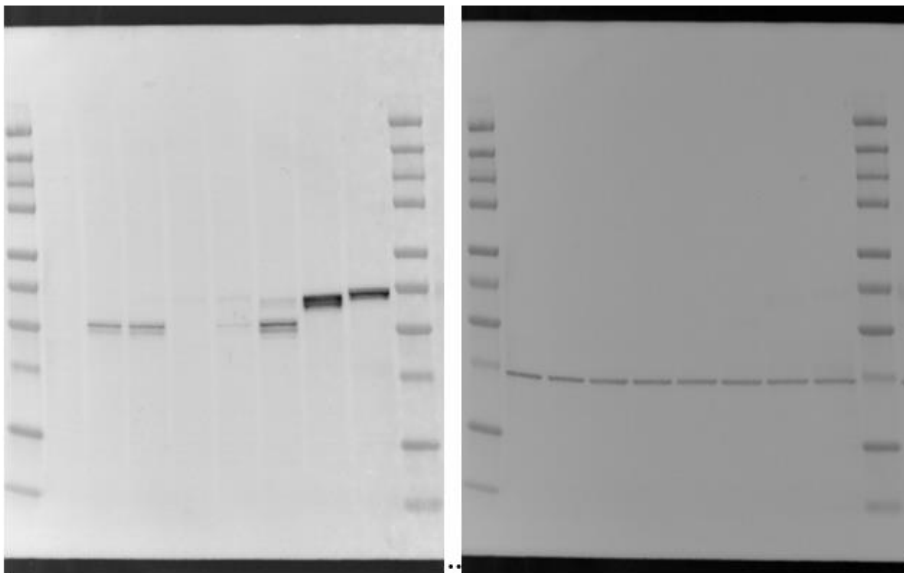


(d) Uncropped blot images for Figure 6b.

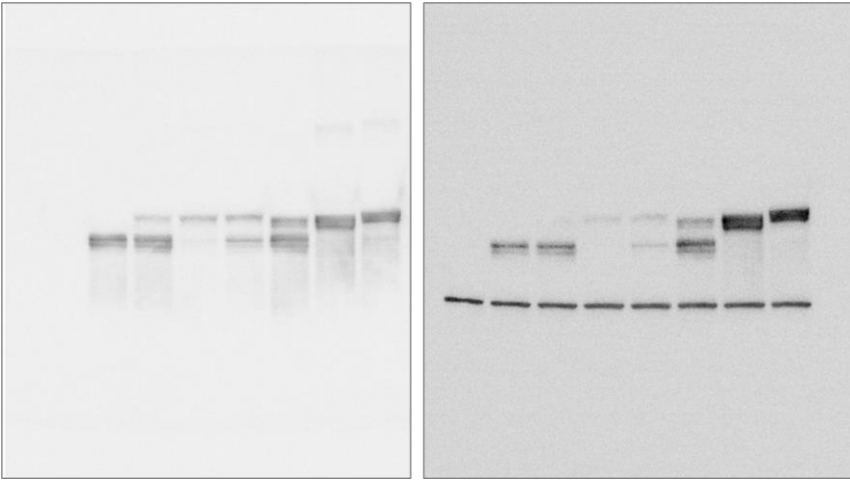
Uncropped gel images for Figure 6b WB anti-LMX1B (left panel) and the corresponding anti-GAPDH WB after membrane stripping (right panel)



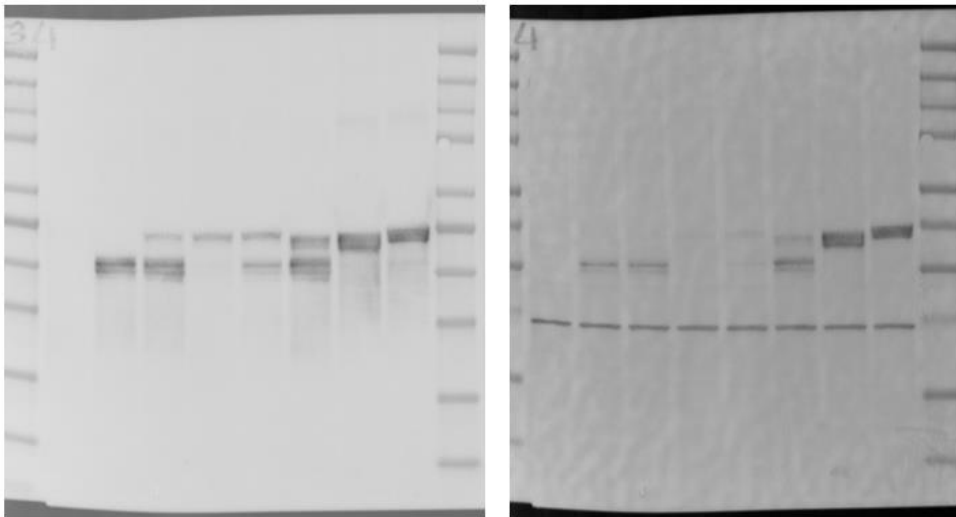
Composite images of the same blot images and their marker images, merged with the Uvitec software according to good laboratory practice protocol.



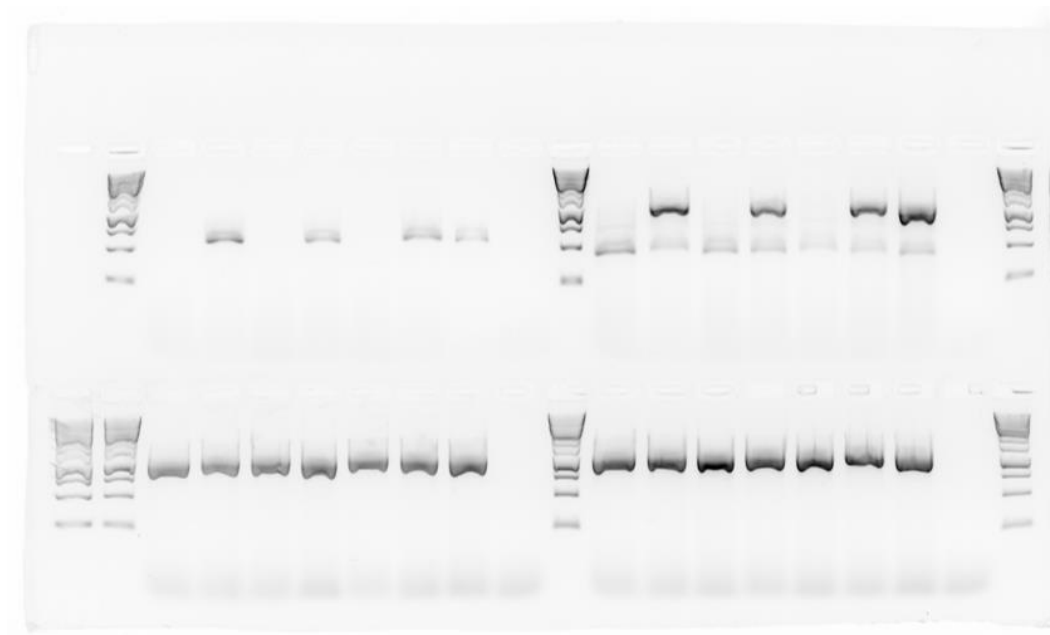
Uncropped gel images for Figure 6b WB anti-FLAG (left panel) and the corresponding anti-GAPDH WB after membrane stripping (right panel); the anti-FLAG primary antibodies and the anti-GADPH are both anti-mouse and are recognized by the HRP conjugated anti-Mouse secondary antibody.



Composite images of the same blot images and their marker images, merged with the dedicated Uvitec software according to good laboratory practice protocol.



(e) Uncropped gel images for 5a Supplementary Figure 1b. Upper lanes RT-PCR specific for *LMX1B*; bottom lanes RT-PCR for housekeeping *GAPDH*. 1Kb molecular weight marker.



Supplementary Figure 2. Uncropped images of blots and gels. (a) Uncropped gel images for Figure 3a. (b) Uncropped blot images for Figure 4d; (c) Uncropped gel images for Figure 5a; (d) Uncropped blot images for Figure 6b; (e) Uncropped gel images for 5a Supplementary Figure 1b.