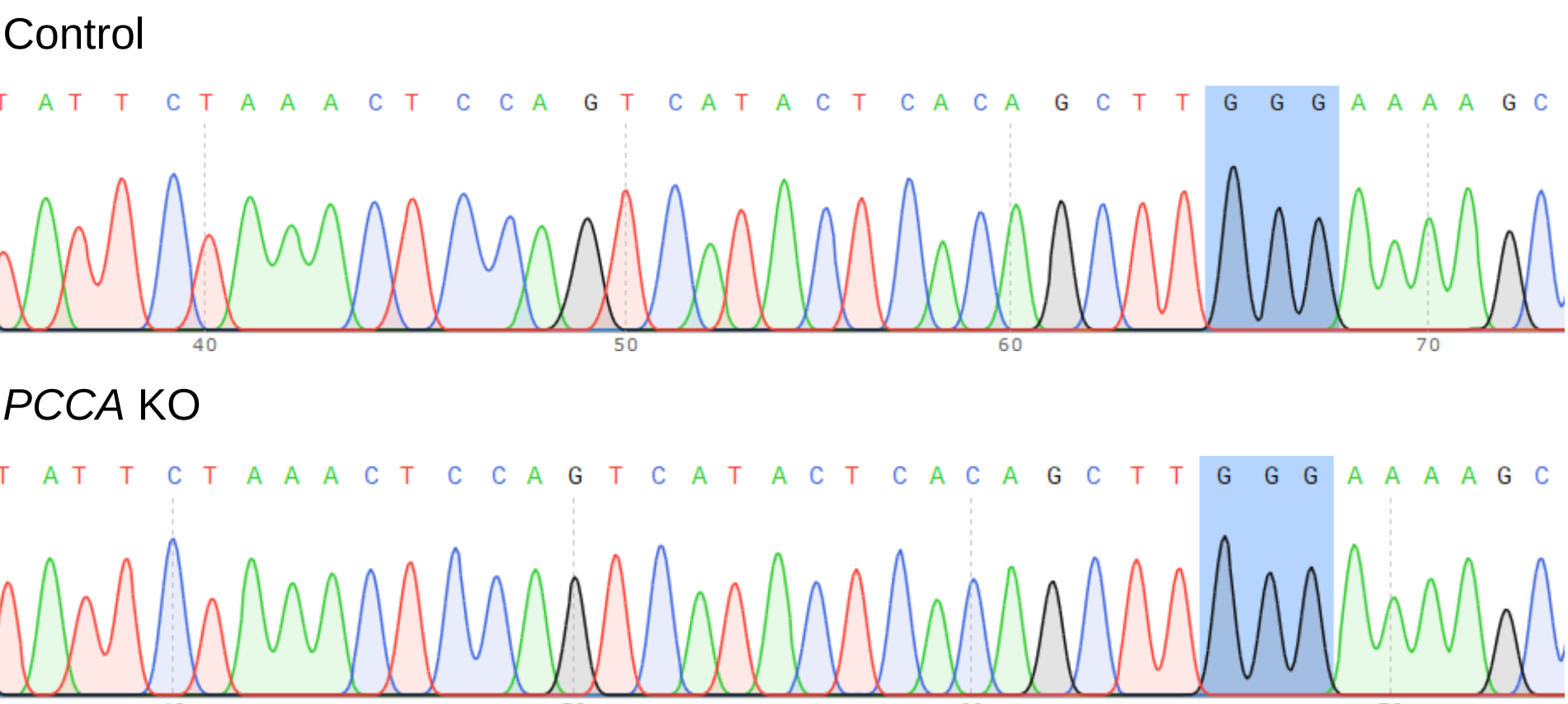


Figure 1S: Evaluation of potential off-target sites by Sanger sequencing.

The off-target sites presented in this work were selected according to score predicted in Breaking-Cas. For each off-target site (OT), the genomic locus or contig is specified according to ENSEMBL database, and its sequence is shown with mismatches highlighted in red and PAM in blue. In all cases the sequence for the unedited iPSC line (control) was compared to the *PCCA* or *PCCB* KO line. The analysis revealed the absence of off-target events within the top four predicted off-target sites for both clones.

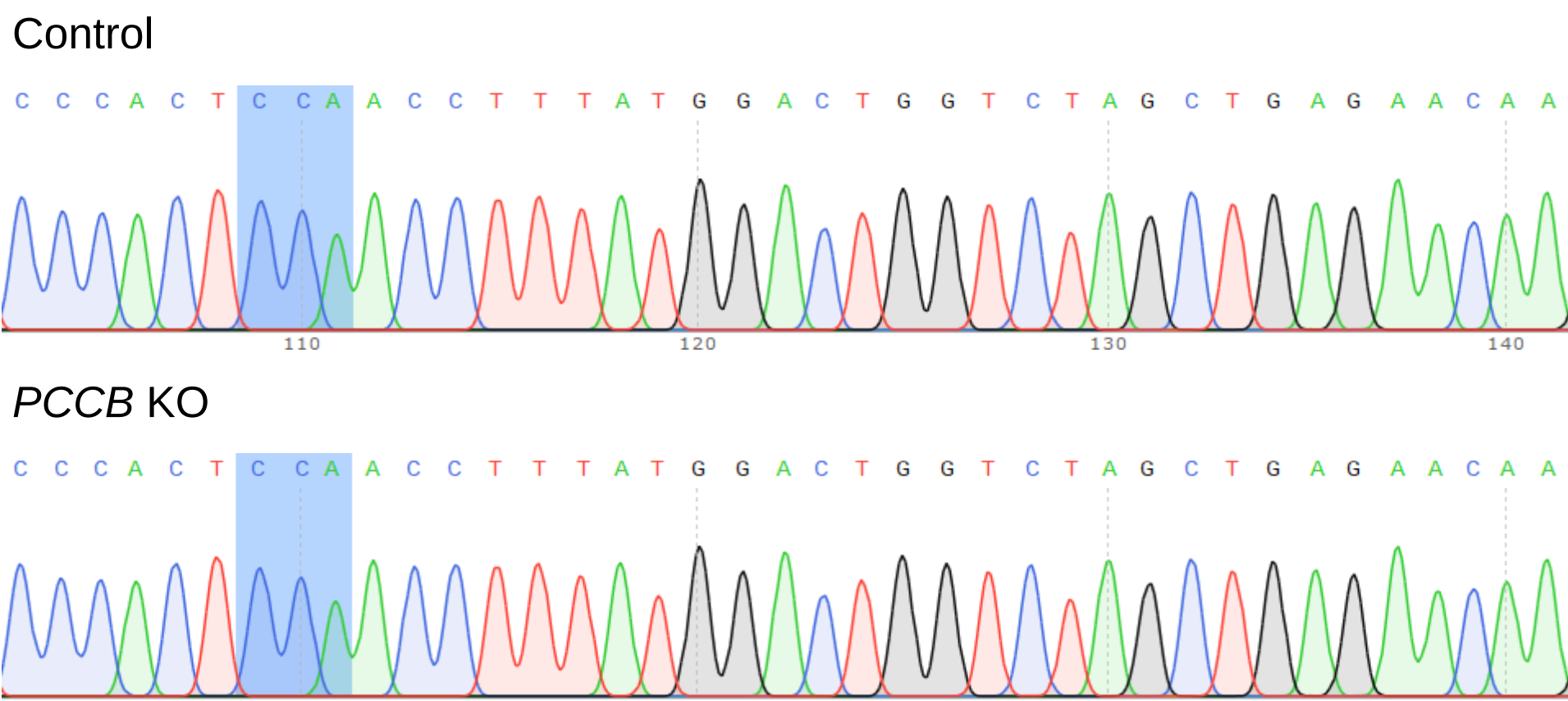
PCCA gRNA target:TTCATTTCAGACTCACAGCTTGGG

OT1: *LINC02869* (chr 1). TCCA**GT**CA**CT**ACTCACAGCTTGGG

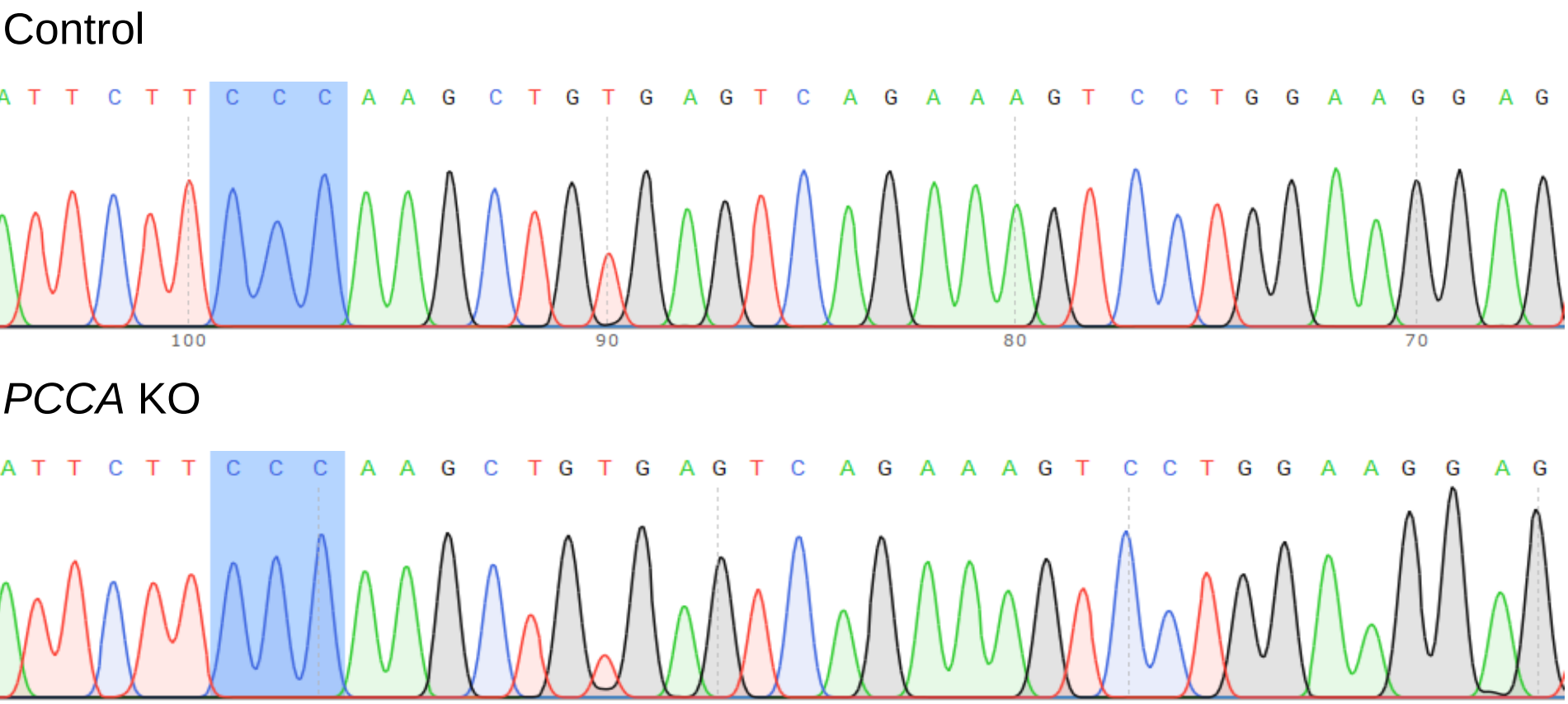


PCCB gRNA target:ATGGACCAGGCCATAACGGTGGG

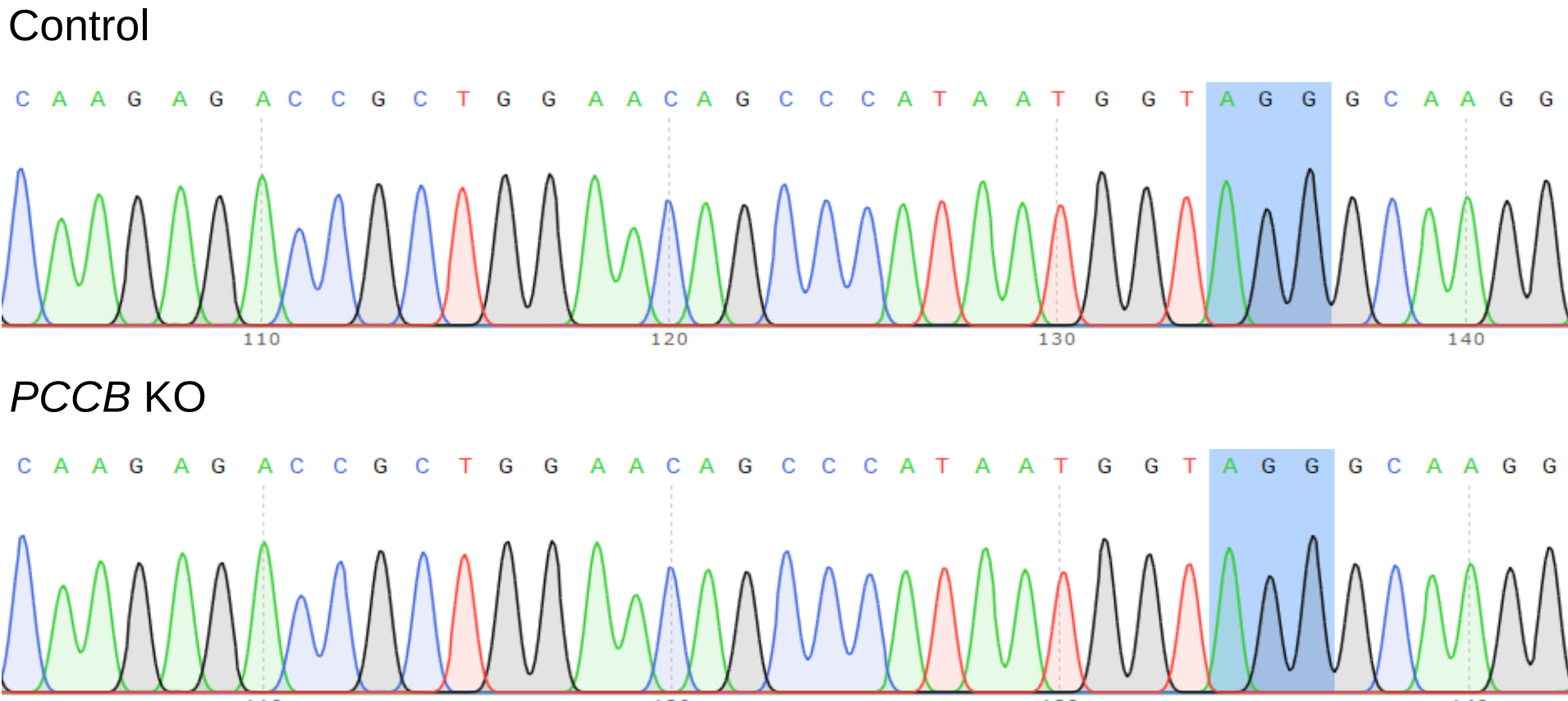
OT1: *SLC7A14-AS1* (chr 3). CCAACCTTTATGG**ACT**GGTCTAG



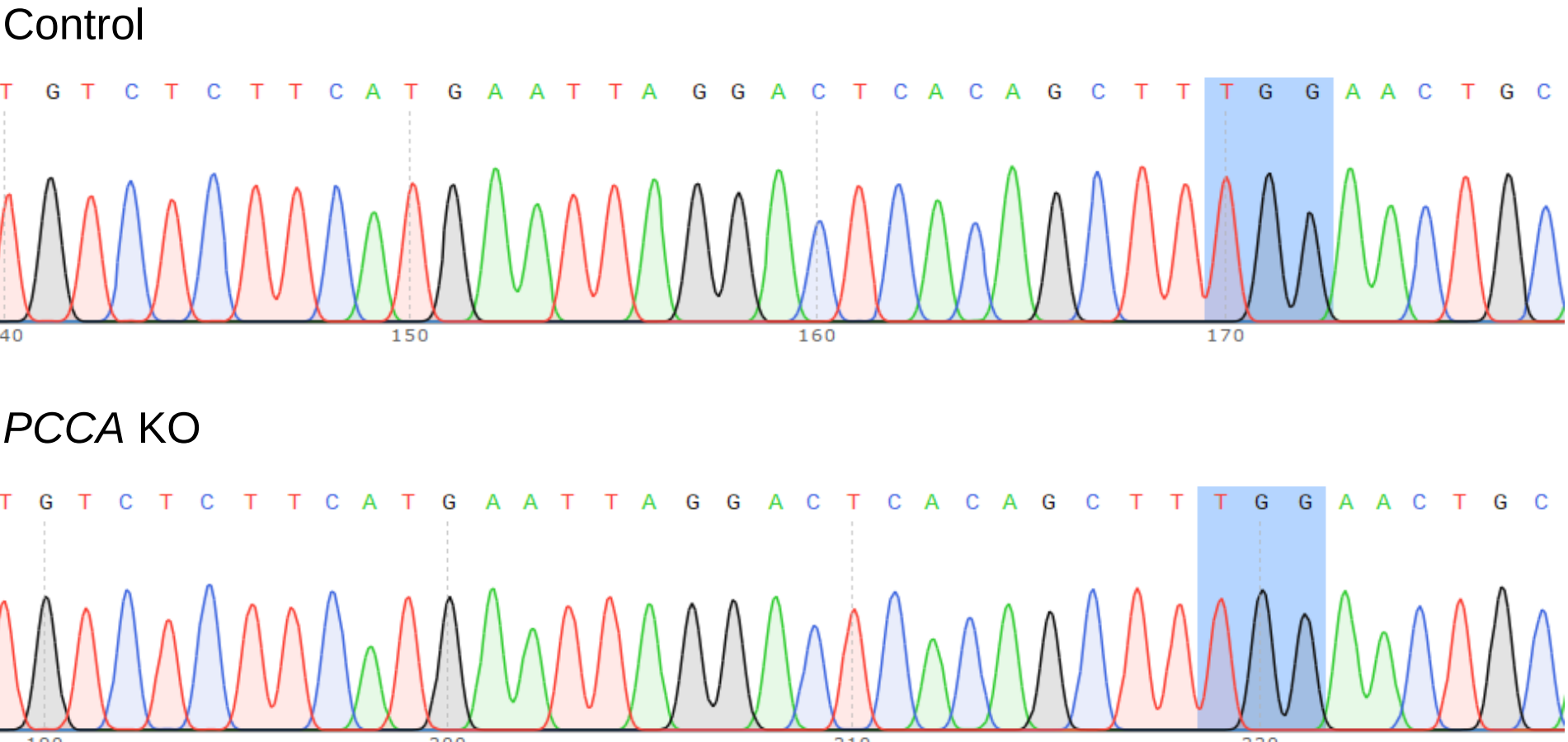
OT2: *STX11* (chr 6). CCCAAGCTGTGAGTC**AGAA**AGTC



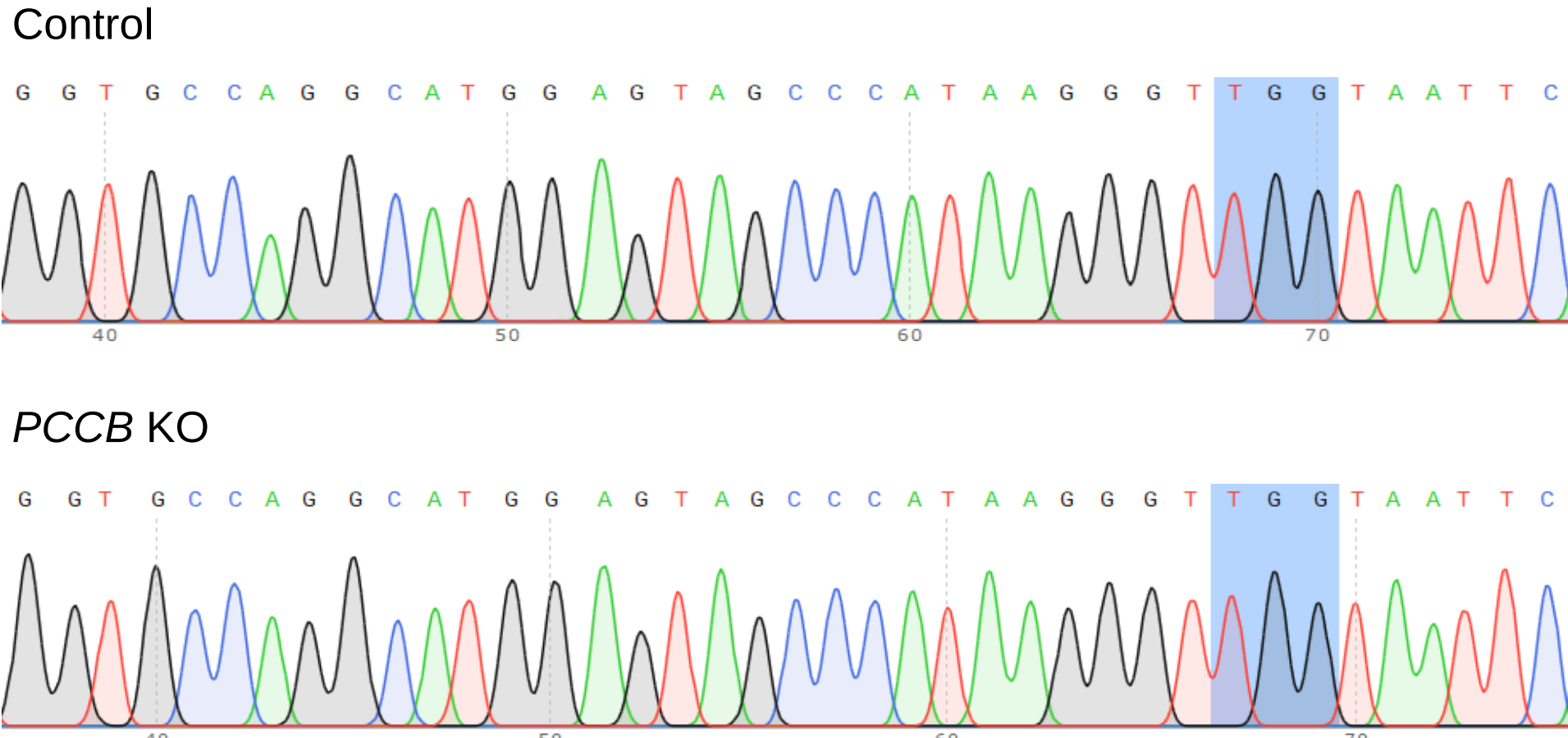
OT2: *AC117526.2* (chr 5). CTGGA**ACAG**CCCATATGGTAGG



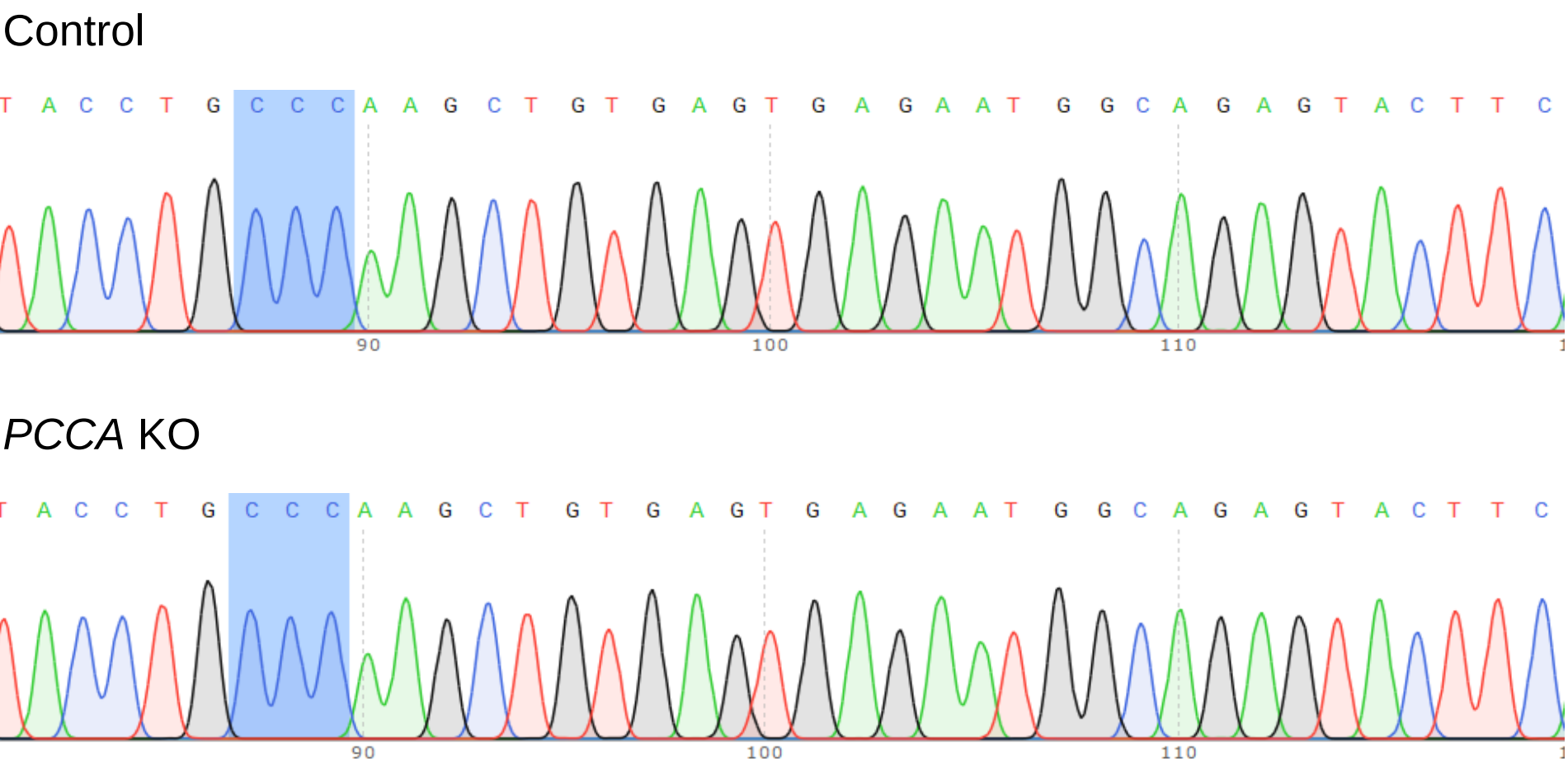
OT3: *ENSG00000250971* (chr 4). TGAATT**AG**GACTCACAGCTTTGG



OT3: *AL034431.16* (chr 20). ATGGAG**TAG**CCCATAA**GG**TTGG



OT4: *AC064870.5* (chr 2). CCCAAGCTGTGAGT**GAGA**ATGGC



OT4: *SLC2A13* (chr 12). CCCACCTTTAT**CCC**CTGGTTCAT

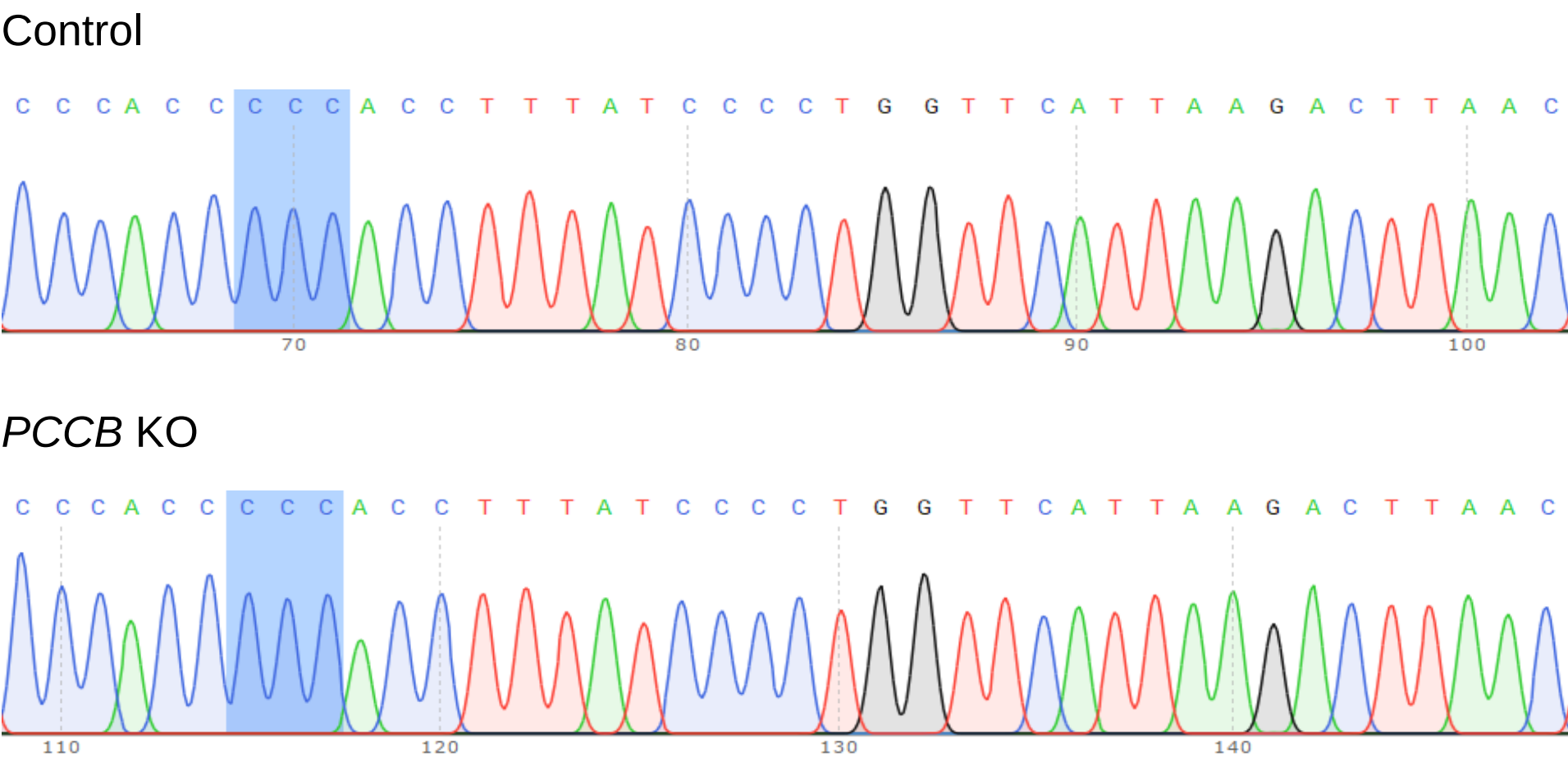


Figure 2S

(A) STR profiling guaranteed the genetic identity between the established iPSC lines and the parental line.
(B) PCR showing negative mycoplasma test in the *PCCA* and *PCCB* KO iPSC lines. A contaminated cell line was used as a positive control (C+). M (DNA Ladder). B (blank).

A.

Marker	Parental wild-type iPSC line		<i>PCCA</i> KO iPSC line		<i>PCCB</i> KO iPSC line	
	Size (Allele)		Size (Allele)		Size (Allele)	
D7S820	268 (9)	280 (12)	268 (9)	280 (12)	268 (9)	280 (12)
CSF1PO	320 (10)	320 (10)	320 (10)	320 (10)	320 (10)	320 (10)
Th01	187 (9.3)	187 (9.3)	187 (9.3)	187 (9.3)	187 (9.3)	187 (9.3)
D13S317	230 (11)	230 (11)	230 (11)	230 (11)	230 (11)	230 (11)
D16S539	277 (11)	277 (11)	277 (11)	277 (11)	277 (11)	277 (11)
vWA	180 (17)	180 (17)	180 (17)	180 (17)	180 (17)	180 (17)
TPOX	231 (8)	231 (8)	231 (8)	231 (8)	231 (8)	231 (8)
D5S818	153 (11)	157 (12)	153 (11)	157 (12)	153 (11)	157 (12)
Amelogenin	108 (X)	108 (X)	108 (X)	108 (X)	108 (X)	108 (X)

B.

