Addendum

TRANSPARENT PROCESS OPEN ACCESS

EMBO Molecular Medicine

Disease modeling of a mutation in α-actinin 2 guides clinical therapy in hypertrophic cardiomyopathy

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Addendum to: *EMBO Mol Med* (2019) 11: e11115. DOI 10.15252/emmm.201911115 | Published online 3 November 2019

The authors recently contacted the journal to inform the editor of changes they discovered in the isogenic control (HCM_{rep}) clone derived from the hypertrophic cardiomyopathy (HCM)-specific hiPSC clone.

After the initial publication, the authors performed a new genomic sequencing of the HCM and HCM_{rep} hiPSC clones, which revealed in the HCM_{rep} an additional point mutation (T>C, blue in

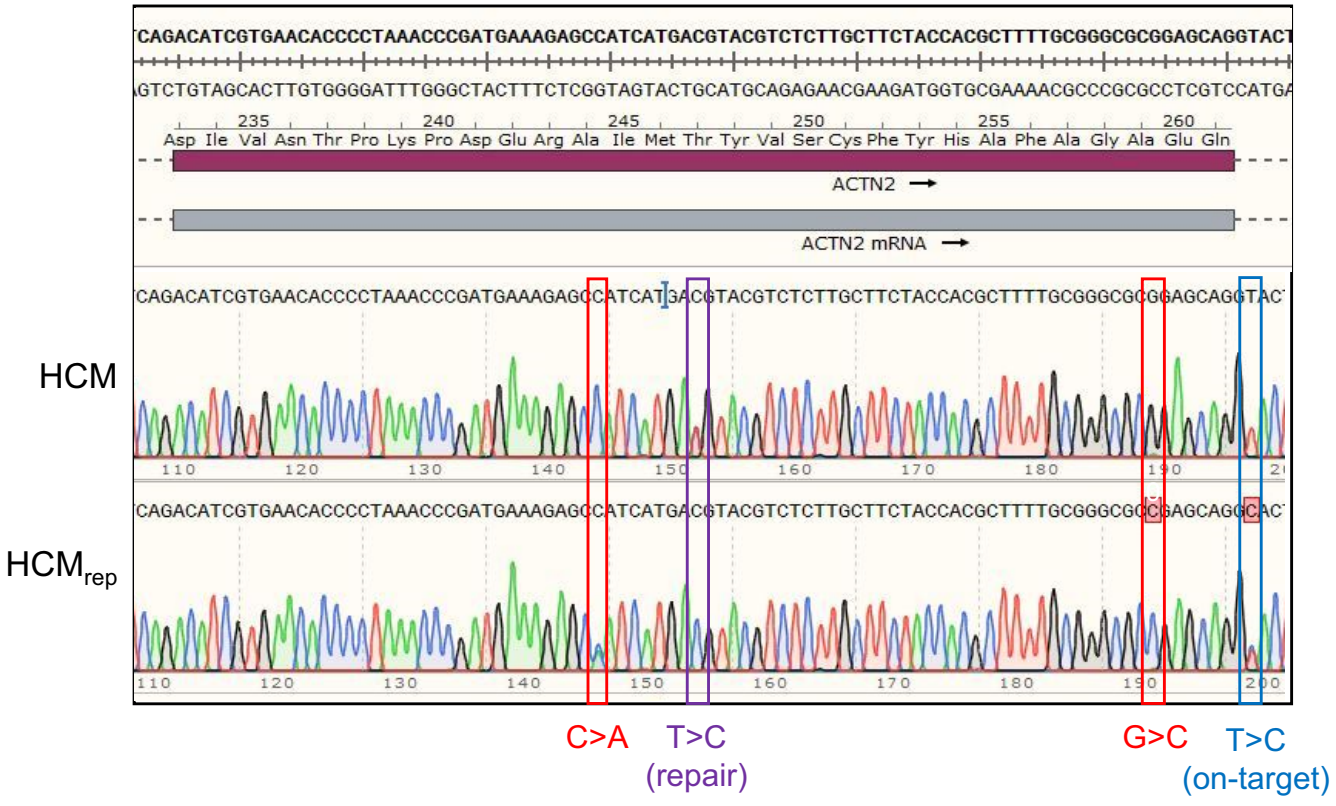

Figure 1. Validation of the HCM and HCM_{rep} hiPSC lines.

Sequencing of the HCM and HCM_{rep} hiPSC clones. The HCM_{rep} clone revealed on the +strand the repaired mutation (T>C (repair), violet), the 2 silent mutations from the repair template (C>A and G>C, red), and the on-target defect (T>C (on-target), blue). Note that the SnapGene software sometimes underlined the presence of a mutation with a red square at the nucleotide site, but sometimes did not.

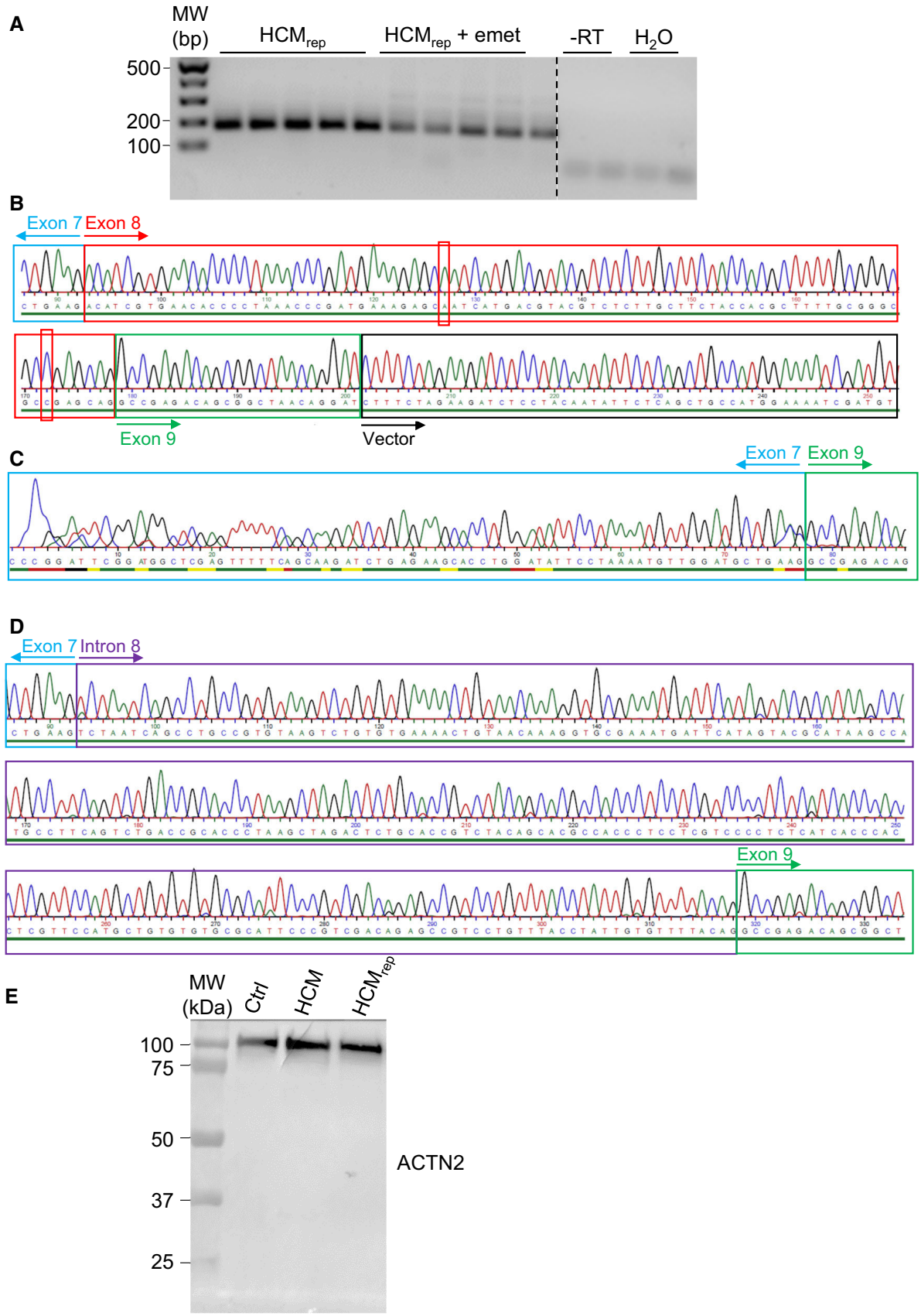

Figure 2.

Figure 2. Evaluation of mRNAs and proteins from HCM_{rep} hiPSC-derived cardiomyocytes.

HCM_{rep} hiPSC-derived cardiomyocytes were cultured for 5 days and treated or not with emetine (600 μg/ml for 4 h).

A Agarose gel electrophoresis of RT–PCR performed between *ACTN2* exons 7 and 9.

B Sanger sequencing of wild-type RT–PCR subclone containing the two silent mutations (C>A and G>C, red) in exon 8.

C Sanger sequencing of RT–PCR mutant-1 subclone deleted of exon 8.

D Sanger sequencing of RT–PCR mutant-2 subclone deleted of exon 8 and retaining 226 bp of intron 8.

E Crude protein fractions from control (Ctrl), HCM, and HCM_{rep} hiPSC cardiomyocytes were processed by Western blot for ACTN2 on an 8% SDS–PAGE. Abbreviations: emet, emetine; −RT, without reverse transcriptase.

Fig 1) next to the CRISPR/Cas9-mediated repair of the HCM gene variant (T>C, violet). This heterozygous mutation (c.697+2T>C) is an on-target artifact of CRISPR/Cas9 gene editing and is located in the donor splice site of *ACTN2* intron 8.

The authors evaluated whether this additional c.697+2T>C transition may affect the mRNA and protein pattern in cultured HCM_{rep} hiPSC-derived cardiomyocytes (Fig 2). RT–PCR revealed additional bands after treatment with emetine, which blocks translation and prevents the degradation of nonsense mRNAs (Fig 2A). Sequencing of emetine-treated RT–PCR subclones revealed wild-type mRNA (Fig 2B) in 72% of cases and two nonsense mRNAs (Fig 2C and D) in 28% of cases. Both mutant mRNAs give rise to a frameshift and a premature termination codon, leading to C-terminal-truncated proteins. Western blot for ACTN2 revealed only one band without any truncated protein at the estimated molecular weight of about 30 kDa in HCM_{rep} hiPSC-derived cardiomyocytes (Fig 2E).

These data showed that HCM_{rep} hiPSC cardiomyocytes exhibit only wild-type *ACTN2* mRNA in the absence of emetine and only the wild-type full-length ACTN2 protein. This addendum indicates that the original data are not affected by this newly discovered Cas9-mediated on-target mutation.