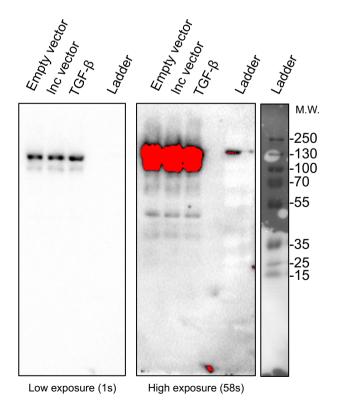
Addendum: A regulated *PNUTS* mRNA to IncRNA splice switch mediates EMT and tumour progression

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Nature Cell Biology 19, 1105-1115 (2017); published online 19 July 2017; corrected after print 13 November 2017.

In this Addendum, the authors include western blot data using a C-terminal *PNUTS* antibody. This is important in that an annotation of the alternative spliced form of *PNUTS*, denoted in the UCSC genome browser (https://genome.ucsc.edu/), depicts it as a non-coding RNA. However, downstream of the alternative splice site is an alternative AUG located in frame in the *PNUTS* ORF at position 1039. The potential for a protein product of ~61 kDa being generated from this AUG was examined experimentally using a C-terminal raised antibody to *PNUTS* to exclude the possibility that the N-terminal deletion of the splice isoform was not the reason that the predicted 61-kDa protein was not detected in cells using an N-terminal generated antibody. The results presented here confirm our previous results using the N-terminal *PNUTS* antibody and originally presented in Supplementary Fig. 2b of the Article; namely, that this predicted ~61-kDa product is not detectable in cells under the conditions used, even under conditions of overexpression.

Figure: lncRNA-PNUTS does not encode for a N-terminal truncated-protein product. The result of a western blot analysis of PNUTS protein expression in CaCo-2 cells upon transient lncRNA-PNUTS expression (3 days) or TGFß treatment (1 day) is shown. The C-terminal antibody used was EPR11706 (Abcam: Ab173285; clone PPP1R10; 1/1000 dilution) raised against the C-terminal region of the PNUTS protein (amino acids 550–650). The western blot protocol and extracts used in this experiment were identical to those described in Supplementary Fig. 2 of the original Article.



Anti-PPP1R10 antibody [EPR11706]