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

Simon Grelet, Laura A. Link, Breege Howley, Clémence Obellianne, Viswanathan Palanisamy, Vamsi K. Gangaraju, J. Alan Diehl and Philip H. Howe

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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.