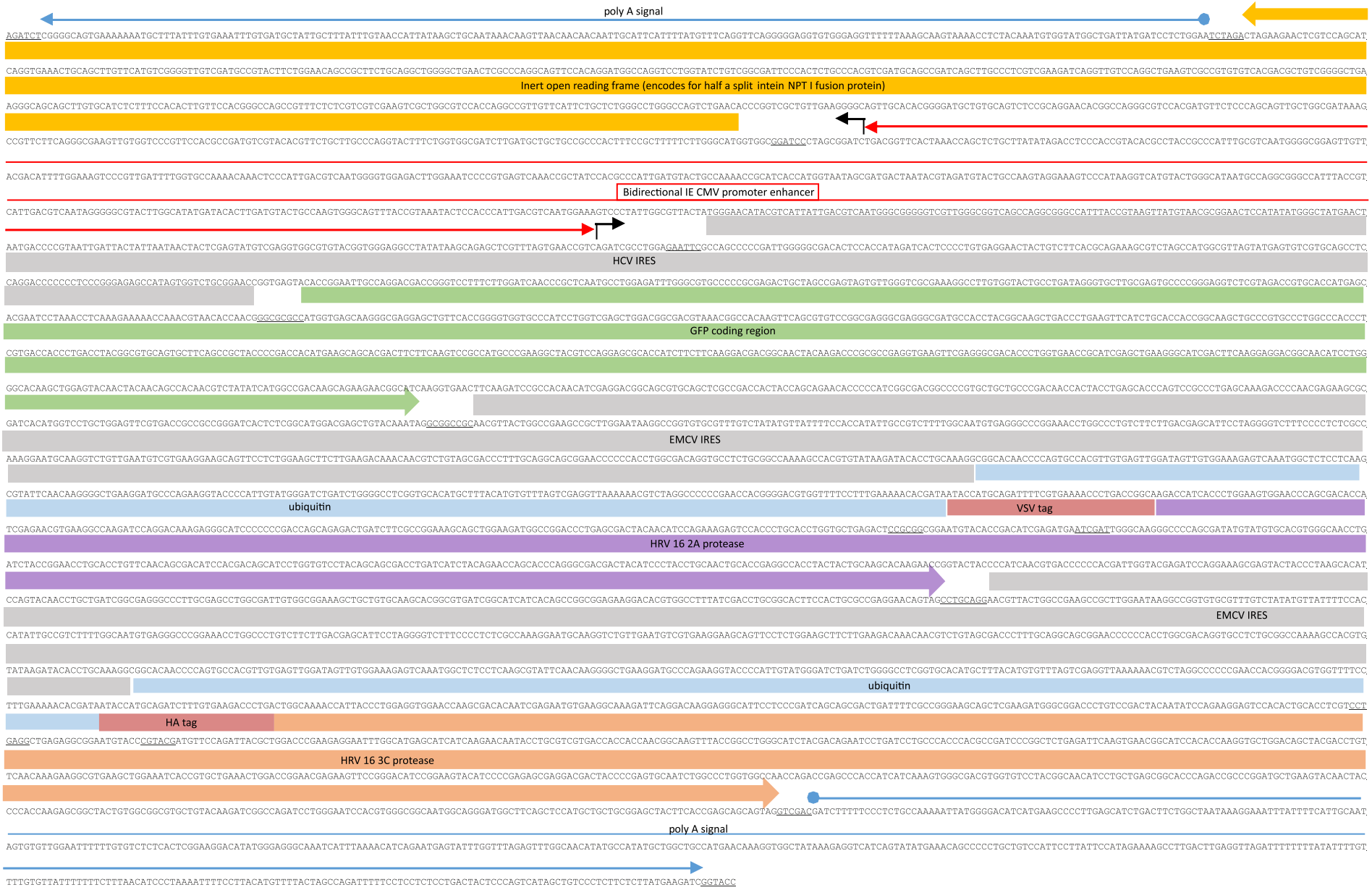

Figure S1. Sequence of the dual promoter mammalian expression cassette used for expression of A16^{TAG}(+/+) mRNA. Sequences of restriction sites used for cloning during the construction of this and subsequent pCIPEP vectors are underlined, with the cassette itself cloned into LITMUS28 via BglII and KpnI sites found at its extreme 5' and 3' ends.

Figure S2. Sequence of HdVwt-Nluc, the Nluc reporter cassette carrying an intron split-HdV ribozyme. Boxed nucleotides are those targeted for synonymous mutagenesis and changed to adenine bases to generate HdVko-Nluc, an equivalent reporter cassette carrying an inactive ribozyme.

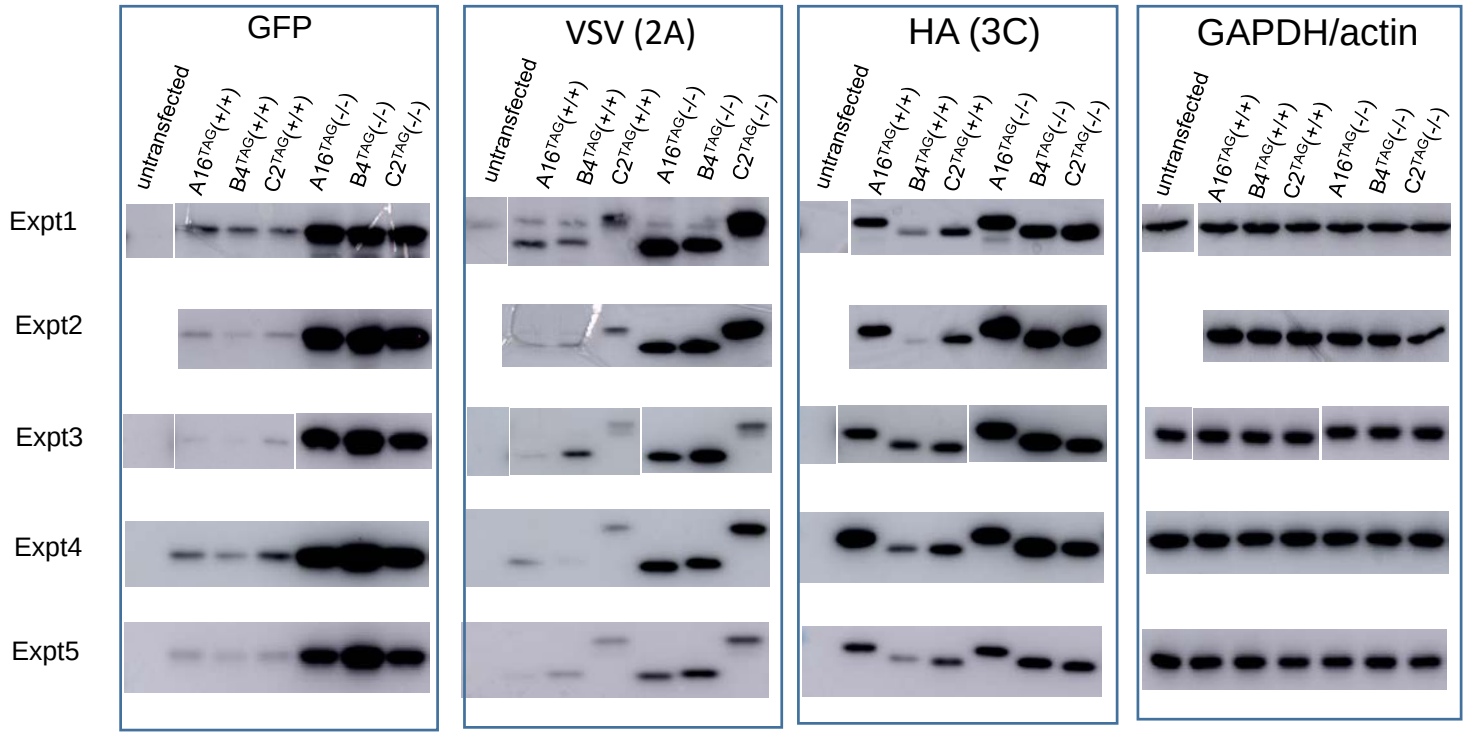

Figure S3. Western blot of cells transfected with the epitope-tagged protease expression vectors encoding the 2A and 3C proteases from A16, B4 and C2. The results from 5 independent transfection experiments are shown. Blots from experiment 1 and 3 have been cropped and edited to re-order the lanes so that they match those of the other blots. Blots from experiment 2 did not include a mock control lane.

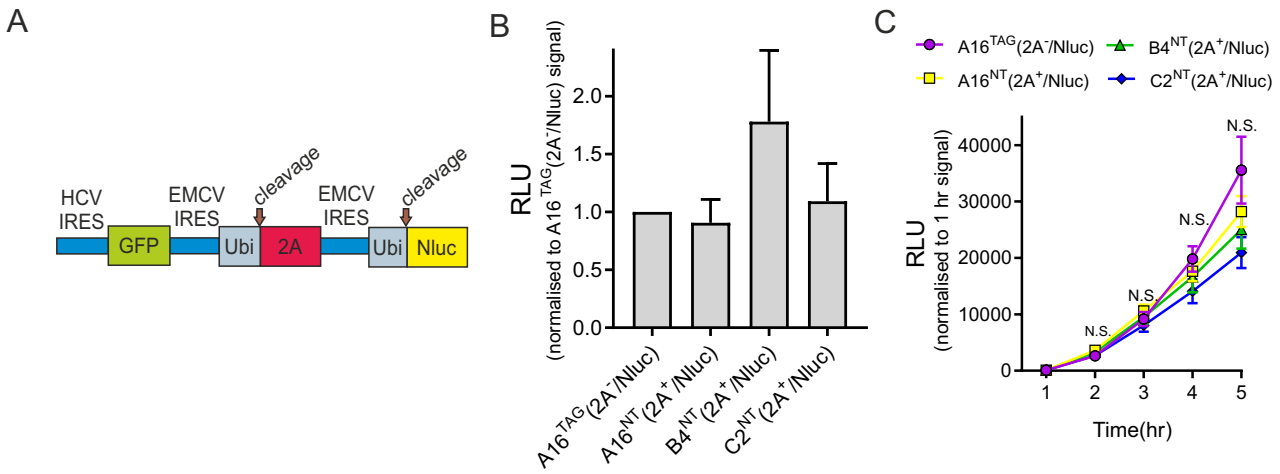

Figure S4. Assessing any early impact that 2A might have on its own EMCV IRES-dependent expression. Cells were electroporated with plasmids that produced a tricistronic mRNA co-expressing GFP, 2A and Nluc. (A) A schematic of the construct design. (B) Data for luciferase values 1 hour post electroporation, normalized to the A16^{TAG}(2A^{-}/Nluc) control expressing an inactive form of 2A. (C) Shows time point values for the different experimental groups after normalizing to the 1 hour transfection values. N.S. = not significant. Data shown represents the mean ± S.E.M.of 5 separate experiments.

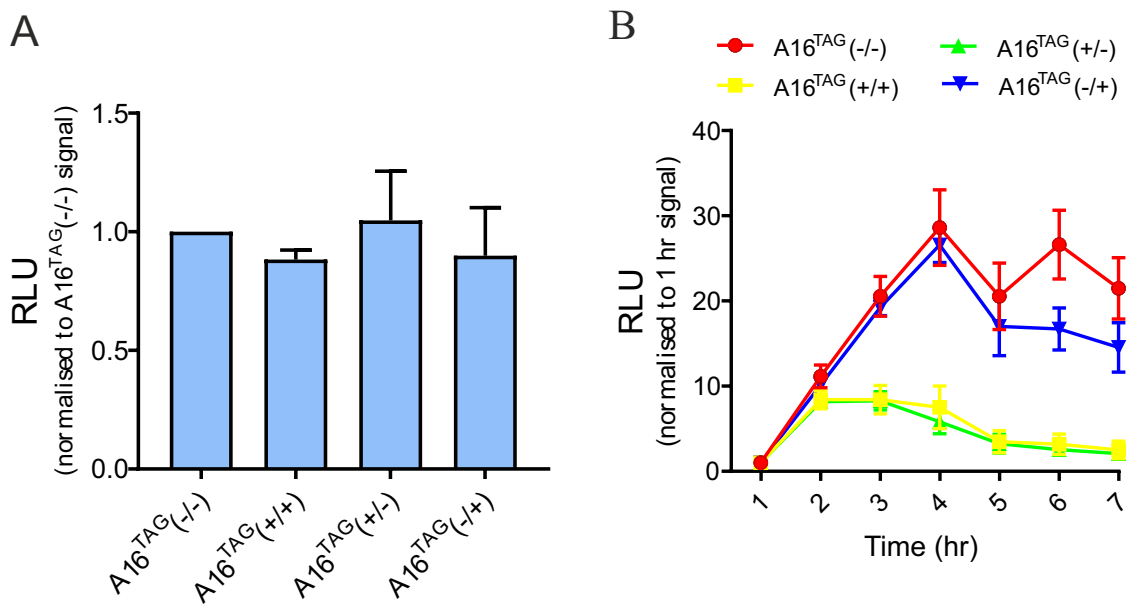

Figure S5. Assessing the individual contribution made by epitope tagged 2A and 3C on the early shutdown of gene expression. Cells were electroporated with vectors co-expressing combinations of active and inactive A16 epitope tagged 2A and 3C proteases as well as the HdV^{WT}Nluc reporter, and luciferase activity monitored over time. (A) Shows the data for luciferase values 1 hour post transfection. (B) Shows time point values for the different experimental groups after normalizing to the 1 hour transfection values. Data shown represents the mean ± S.E.M. of 3 separate experiments.