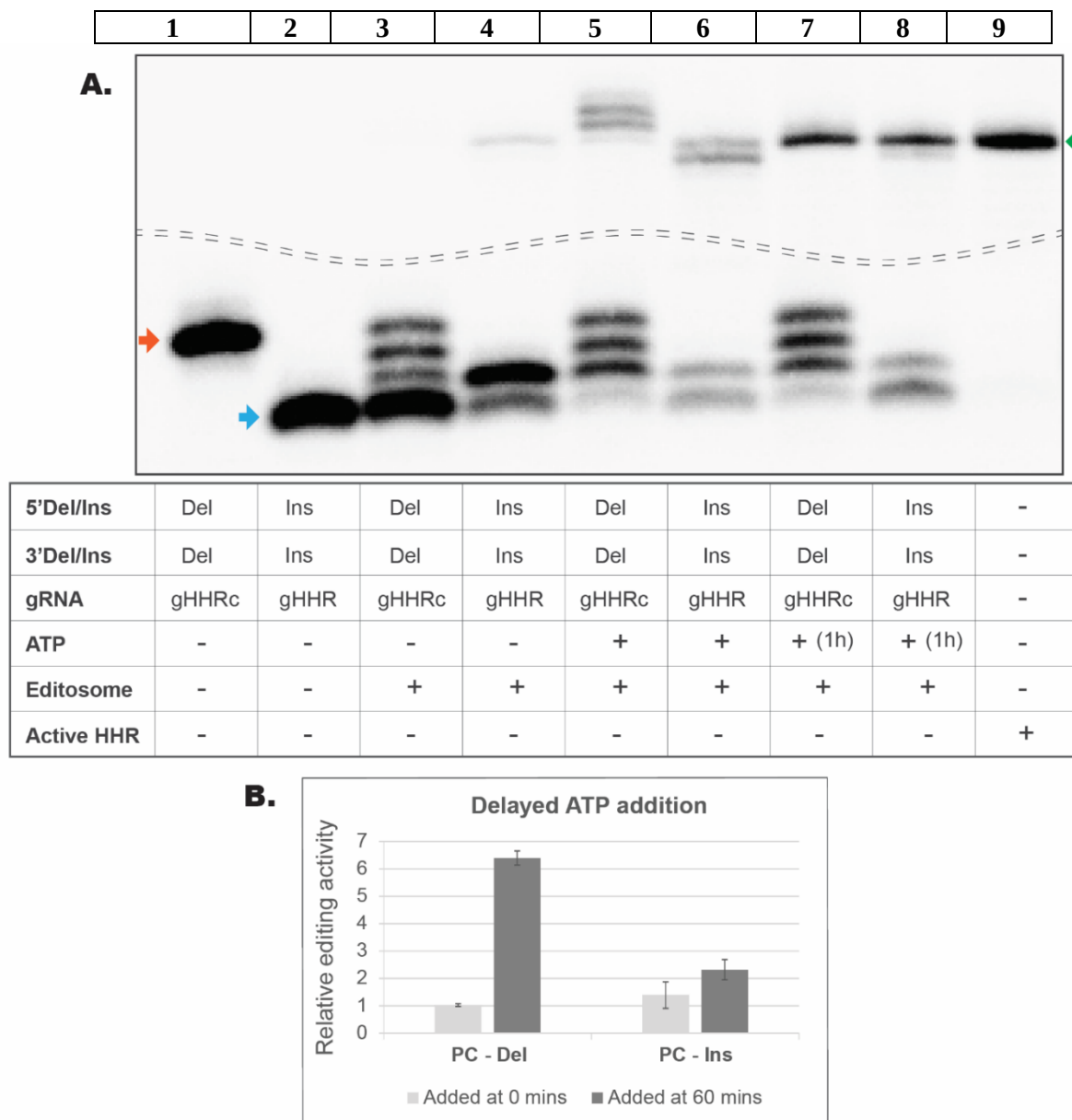




Supplemental Figure S5. Effect of delayed ATP addition on precleaved Insertion and deletion editing.

The impact of adding ATP to precleaved insertion and deletion editing reactions 1 hr post reaction outset was investigated using radiolabeled RNA substrates (A) and FRET-based assays (B). (A) In the absence of editosome (lane 1, 2), [5'Del] and [5'Ins] were not modified (shown by red and blue arrows, respectively). Editing in reactions lacking ATP, only contained ExoUase and TUTase activity on [5'Del] and [5'Ins], respectively (lane 3, 4). A significant difference can be observed among edited products when ATP is added to the reaction at the beginning (lanes 5, 6) compared to an addition 1 hr post reaction outset (lanes 7, 8). Lane 9 contains active HHR as a size control (shown by a green arrow). (B) Addition of ATP one hour after starting the reaction resulted in more edited HHR (measured by FRET cleavage activity) in both precleaved deletion and insertion assays. Relative editing activity is measured in arbitrary units.