Supplementary Figures

Supplementary Figure 6. Trypsin cleavage pattern of G\textsubscript{\alpha}i1 and G\textsubscript{\alpha}i1-122\textsubscript{Rluc}. Membranes from HEK293T cells expressing G\textsubscript{\alpha}i1 or G\textsubscript{\alpha}i1-122\textsubscript{Rluc} were pre-incubated or not with GDP and AlF\textsubscript{4}\textsuperscript{-} and then treated with TPCK trypsin for the indicated period of time, size-fractioned on 10% acrylamide gel and immunoblotted with an anti-G\textsubscript{\alpha}i1-antibody. (a), Trypsin-proteolytic pattern of wild-type G\textsubscript{\alpha}i1. In the absence of trypsin (two left lanes), the expected native 41 kDa G\textsubscript{\alpha}i1 protein is detected. In the presence of trypsin alone, no additional bands were detected since cleavage of inactive G\textsubscript{\alpha}i1 generates peptide fragments that are too small to be detected. Addition of AlF\textsubscript{4}\textsuperscript{-} in the presence of trypsin generated a stable fragment of 39 kDa (2 kDa smaller than the full length G\textsubscript{\alpha}i1) that can be distinguished just under the band of the mature protein. The apparition of such a proteolysis resistant fragment is taken as a reflection of the conformational changes associated with G protein activation\textsuperscript{1}. (b) Trypsin-proteolytic pattern of G\textsubscript{\alpha}i1-122\textsubscript{Rluc}. In the absence of trypsin (two left lanes) a major band of 76 kDa corresponding to the G\textsubscript{\alpha}i1-122\textsubscript{Rluc} fusion is detected. AlF\textsubscript{4}\textsuperscript{-} treatment in the presence of trypsin led to the apparition of 29 kDa that is exactly 2kDa smaller than a 31kDa band that was generated upon trypsin treatment in the absence of AlF\textsubscript{4}\textsuperscript{-}. Although we cannot definitely identify the nature of the bands generated from the fusion construct (the numerous potential Arg/Lys trypsin cleavage sites within Rluc complicating the analysis) the data clearly indicate that AlF\textsubscript{4}\textsuperscript{-} binding affected the proteolysis in a manner consistent with a gain of trypsin resistance, indicative of activation of the G\textsubscript{\alpha}i1-122\textsubscript{Rluc}. The partial proteolytic cleavage patterns observed for the native 41 kDa G\textsubscript{\alpha}i1 and G\textsubscript{\alpha}i1-122\textsubscript{Rluc} is not surprising given that the trypsin treatment was carried out in membrane preparations\textsuperscript{1}.