

Supplemental Figure S1

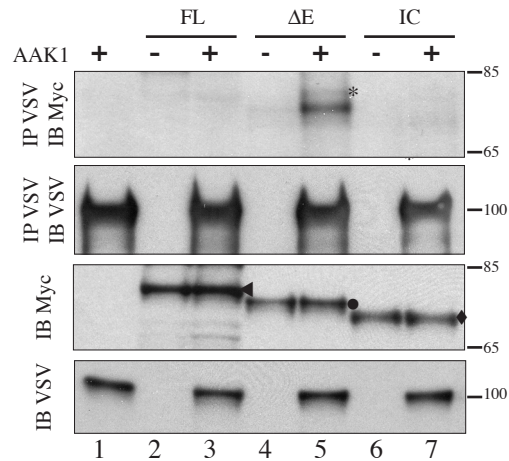


Figure S1 (in association with Figure 1A): AAK1 directly interacts with the activated form of membrane-anchored Notch

293T cells were transiently transfected with plasmids encoding different Notch constructs with (+) or without (-) VSV-tagged AAK1. The following amounts of plasmids were transfected to achieve the same levels of Notch expression: FL (◀, lanes 2 and 3, 5 μg transfected), ΔE (●, lane 4, 3 μg and lane 5, 1 μg (in order to achieve identical levels of expression, as the presence of AAK1 increases the level of ΔE)) and IC (◆, lanes 6 and 7, 4 μg). Cells were lysed 48h after transfection and lysates were analysed by western blotting (3-8% SDS-PAGE) either directly for Notch (IB Myc) and AAK1 (IB VSV), or after immunoprecipitation with AAK1 (IP VSV) for either Notch (IB Myc) or AAK1 (IP VSV). The band observed for FL corresponds to the p120 furin-matured product of full-length Notch; all Notch constructs used here are deleted of their C-terminal domain. Only the ΔE form of Notch can be coimmunoprecipitated with AAK1 (lane 5). An asterisk indicates a slower-migrating form of ΔE.