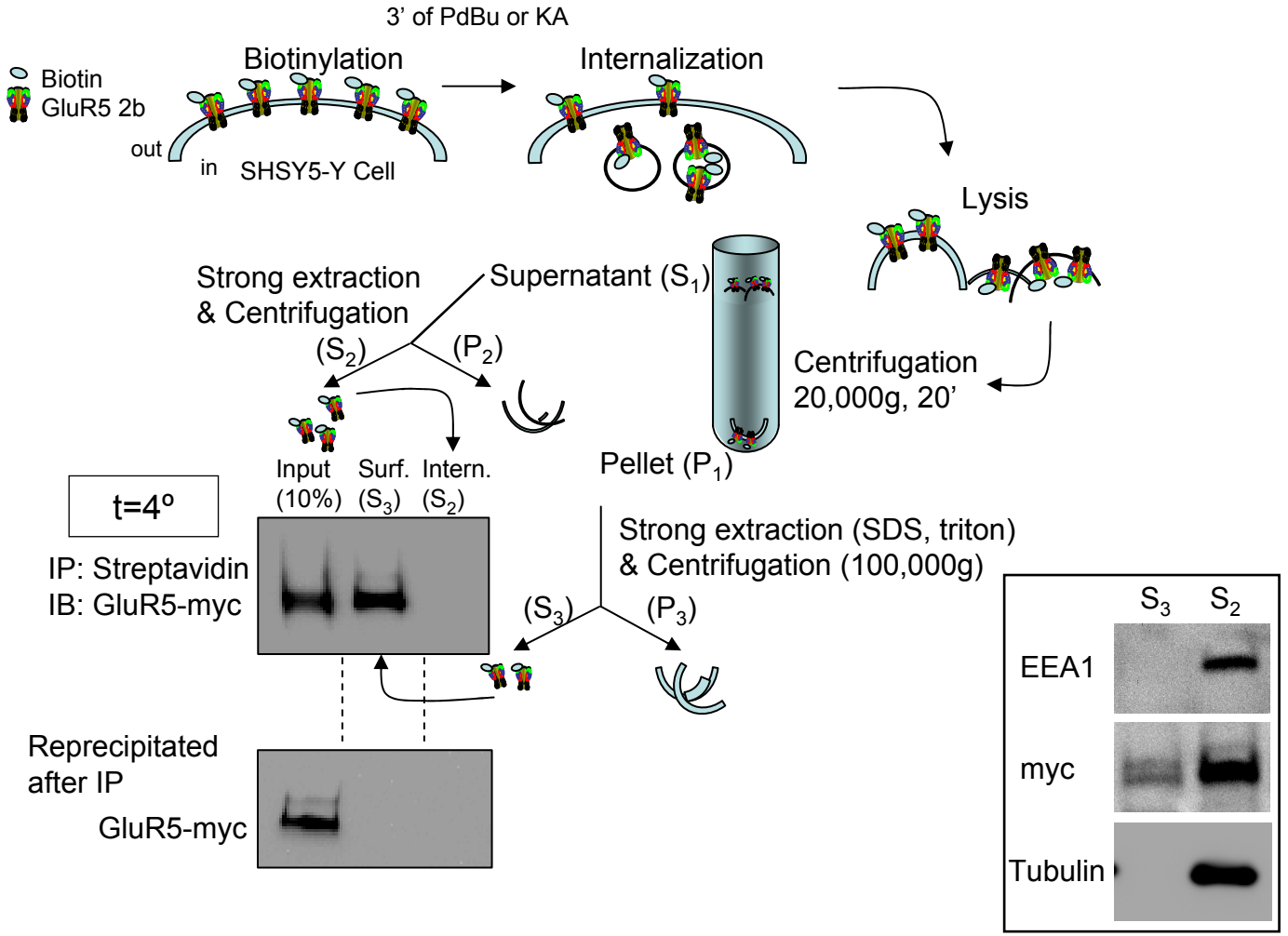

Figure S1. Endocytosed biotinylated receptors were separated from receptors remaining in the membrane by two consecutive centrifugation steps. The first centrifugation separated light (S1) from heavy membranes (P1) whilst the second served to isolated extracted receptors from each membrane fraction (S2 and S3). Several controls were undertaken to assure that this method properly worked. Insets show that membrane biotinylated receptors do not appear at intracellular pools when endocytosis does not proceed (e.g. 4 ºC) (top left blot). To ensure that all the biotinylated protein was precipitated in a single step, the beads were recovered and the remaining of the sample reincubated overnight with streptavidin. As can be seen no biotinylated protein remained after the first immunoprecipitation (bottom left blot). Intracellular proteins, such as EEA1 or tubulin, do not contaminate fractions containing surface receptors ((S3) (blot on the right)