



### Supplementary Figure 6 Processing of APPL.sd

APPL.sd is an APPL construct with a small deletion within the ECD. In contrast to wildtype APPL, this mutant protein has been proposed to not undergo proteolytic processing which under normal conditions would result in the release of the ECD from APPL. The suggestion that APPL.sd is secretion defective was based on western blot analysis with an antibody directed against the ECD of APPL. However, these studies were not accompanied by studies with an antibody specific for the ICD of *Drosophila* APPL. In the course of another project, analysing the processing of APP-family members, we have raised an antibody against the ICD of APPL (A. Loewer, R. Paro, G. Merdes, in preparation). This enabled us to characterize the processing of APPL in more detail and to directly test for the formation of CTFs from APPL.sd. Western blot analysis from transgenic *Drosophila* expressing APPL and APPL.sd in the compound eye by the use of *GMR-GAL4* revealed the presence of CTFs in a significant higher amount in APPL.sd flies in comparison to APPL, indicating that APPL.sd is processed normally. Since the overexpressed protein level of APPL has to be at least six-fold higher than APPL.sd for the induction of still weaker PNS phenotypes, the higher amount of CTFs should also not be considered responsible for the phenotype induction in APPL.sd expressing flies. The situation is further complicated by a difference in molecular weight of the CTFs generated from APPL.sd and APPL. Whereas the majority of CTFs generated from wildtype or APPL overexpressing flies have a molecular weight of appr. 12 kD, the CTFs from APPL.sd are 3-4 kd smaller. This could be caused by the 33 aa which are missing in the sd-construct suggesting that the cleavage of APPL occurs sequence specifically N-terminal to the sd-deletion. APPL.s expressing flies, *wt* flies and the *appl*<sup>d</sup> mutant served as controls for the specificity of our antibody.