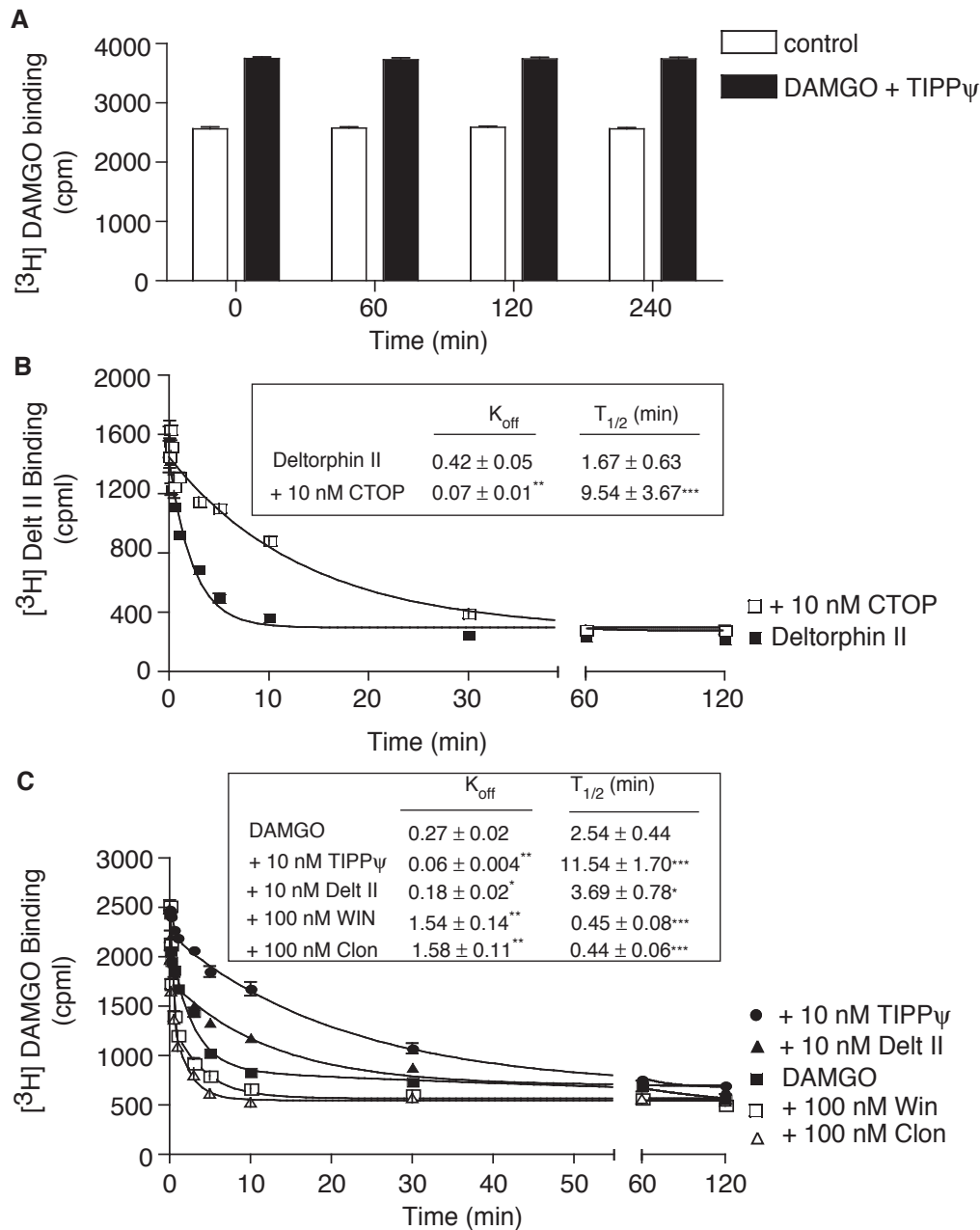


**Title:** G-protein coupled receptor heteromerization: A role in allosteric modulation of ligand-mediated receptor binding  
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**Supplemental Figure 3.** Dissociation kinetics of radiolabeled ligand at  $\mu$ OR- $\delta$ OR heteromers. **(A)** SK-N-SH whole cells endogenously expressing  $\mu$ OR and  $\delta$ OR in a ratio of 2:1 were incubated with 10 nM [ $^3$ H]DAMGO in the absence or presence of 10 nM of TIPP $\psi$  for 1 h at 37°C. The plates were then kept on ice for different time intervals and bound radioactivity was measured as described in methods. **(B)** SK-N-SH whole cells endogenously expressing  $\mu$ OR and  $\delta$ OR in a ratio of 2:1 were incubated with 6 nM [ $^3$ H]Deltorphin II for 1 h at 37°C. The supernatant was removed, the plates were kept on ice, and cells were incubated with cold 1  $\mu$ M Deltorphin II in the absence or presence of 10 nM of the  $\mu$ OR antagonist, CTOP, for different time intervals (0-120 min) as described in Materials and Methods. **(C)** The effect of TIPP $\psi$ , Deltorphin II (10 nM) clonidine or WIN (100 nM) on the dissociation of [ $^3$ H]DAMGO bound to  $\mu$ OR- $\delta$ OR in the presence of 1  $\mu$ M DAMGO was determined in SK-N-SH cells and ligand binding determined as described in Materials and Methods. Data analyzed using Prism version 4.0 represent Mean  $\pm$  SEM (n=3). \*p<0.05; \*\*p<0.01, Dunnett's test