Supporting Information for:

Solution NMR Approaches for Establishing Specificity of Weak Heterodimerization of Membrane Proteins

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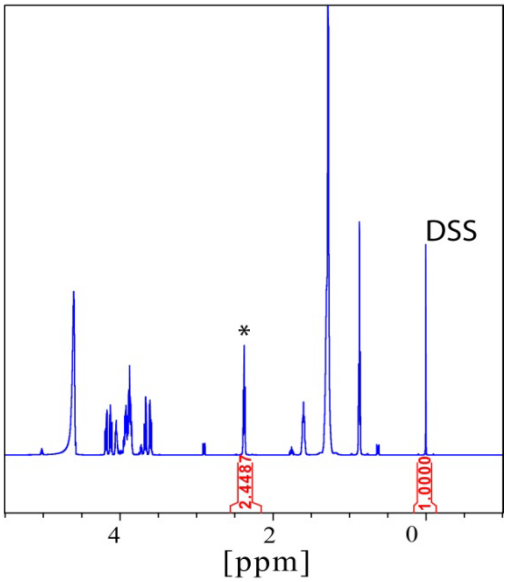
Contents: Complete reference 8 and supporting Figures 1-5.

Complete Reference 8:

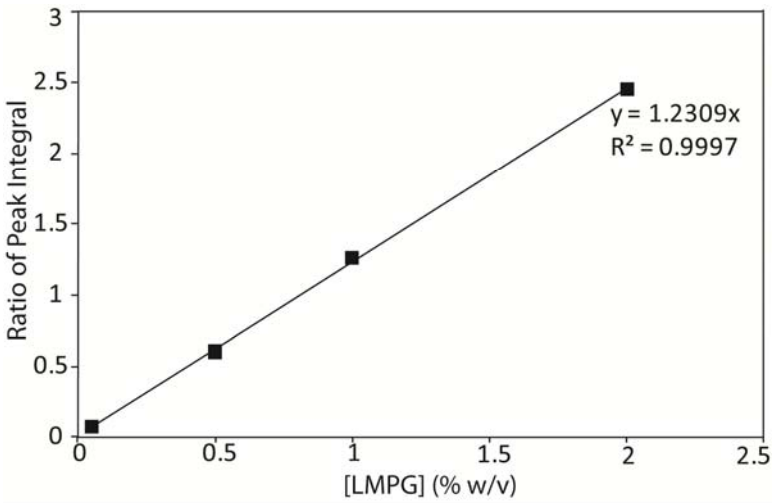
Vetrivel, K. S.; Zhang, X. L.; Meckler, X.; Cheng, H. P.; Lee, S. H.; Gong, P.; Lopes, K. O.; Chen, Y.; Iwata, N.; Yin, K. J.; Lee, J. M.; Parent, A. T.; Saido, T. C.; Li, Y. M.; Sisodia, S. S.; Thinakaran, G. *J Biol Chem* 2008, *283*, 19489-19498.

Supporting Figure 1. Determination of detergent concentration using 1D ^{1}H NMR. **A.** 1D ^{1}H NMR spectrum of standard sample containing 2% (w/v) LMPG and 5 mM DSS. The integral for the peak marked with an asterisk from LMPG was compared to the integral from the DSS peak (representing exactly 5.0 mM) as the basis for determining the total LMPG concentration. **B.** Calibration plot from 4 standard samples (0% LMPG, 0.5% LMPG, 1% LMPG and 2% LMPG, all with 5 mM DSS). Here y represents the ratio of peak integrals for peaks marked in panel A. R^2 represents the accuracy of linear regression on experimental data of the standard samples. A perfect fit is obtained when R^2=1.

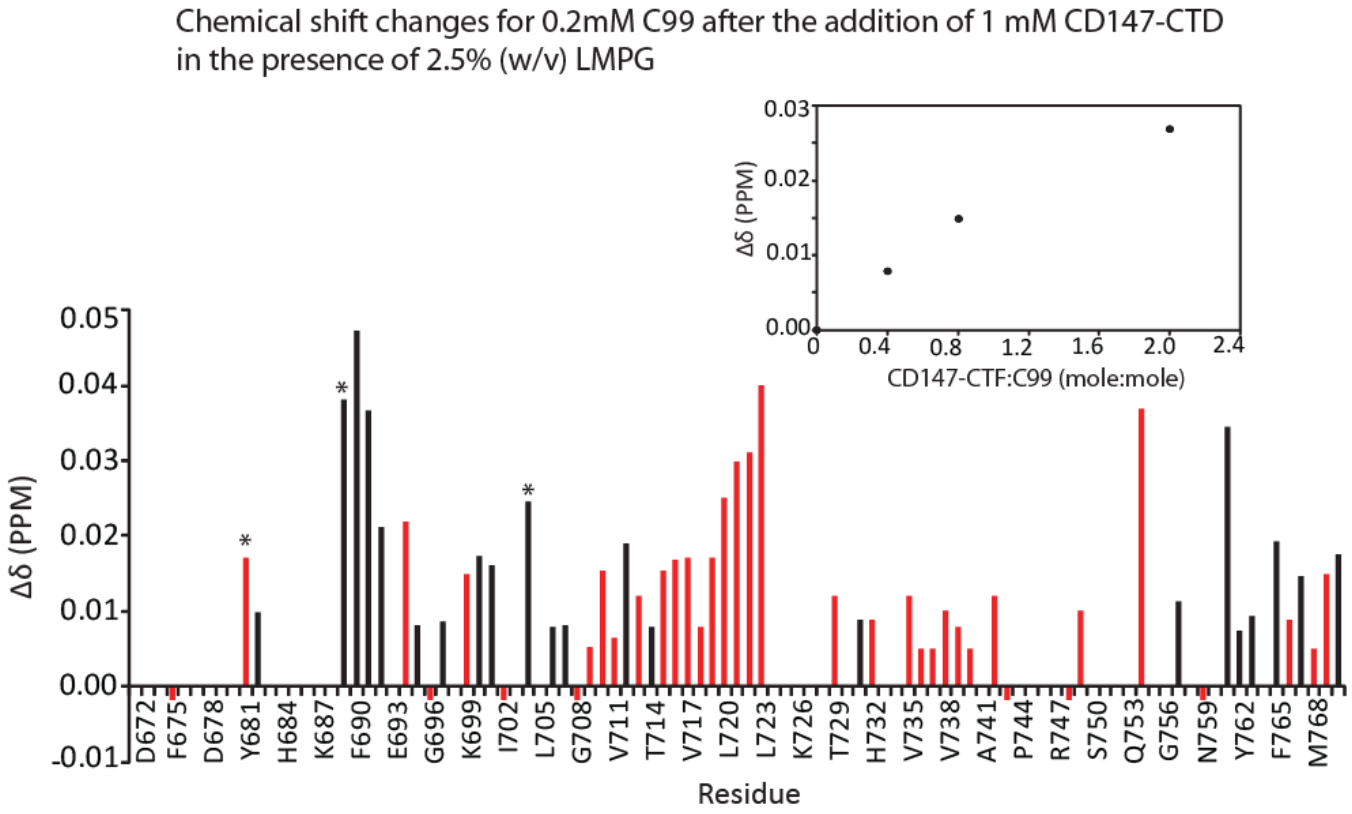
A



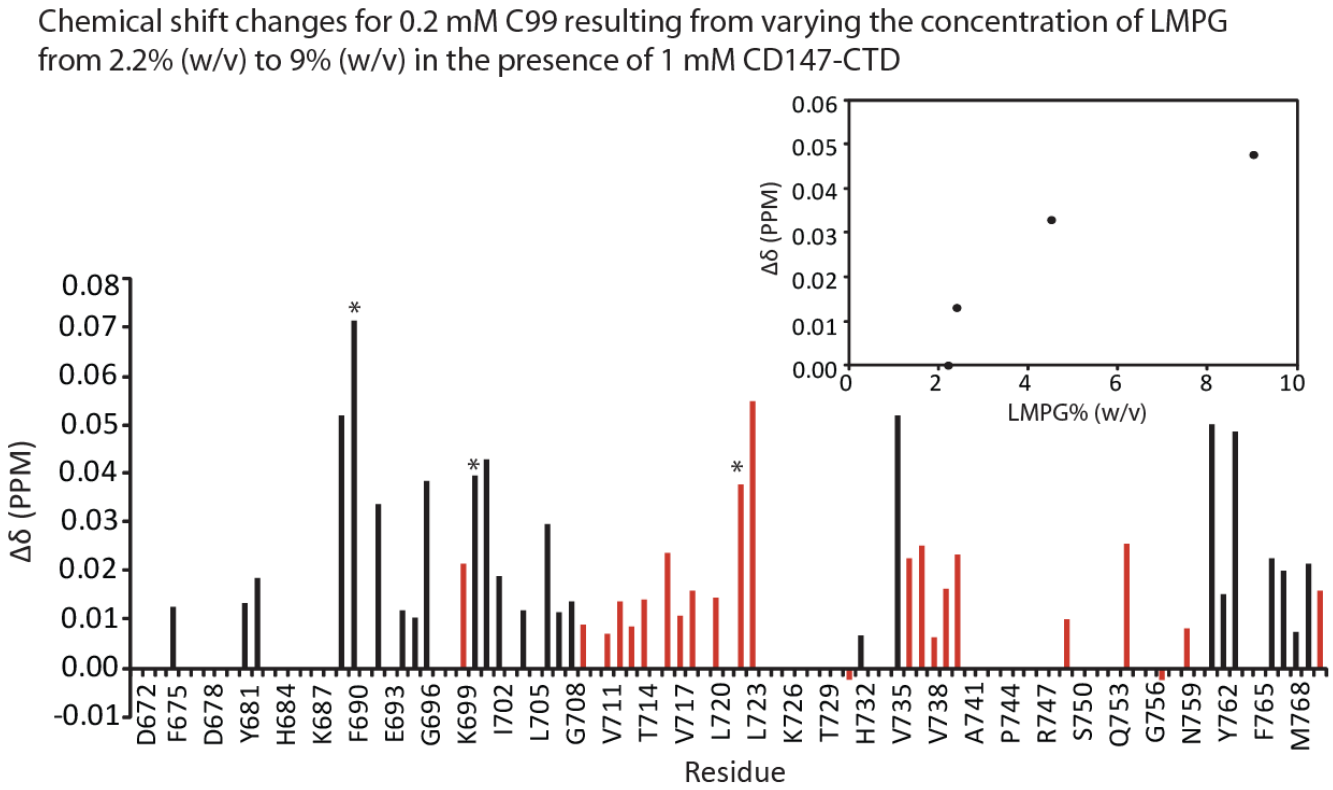
B



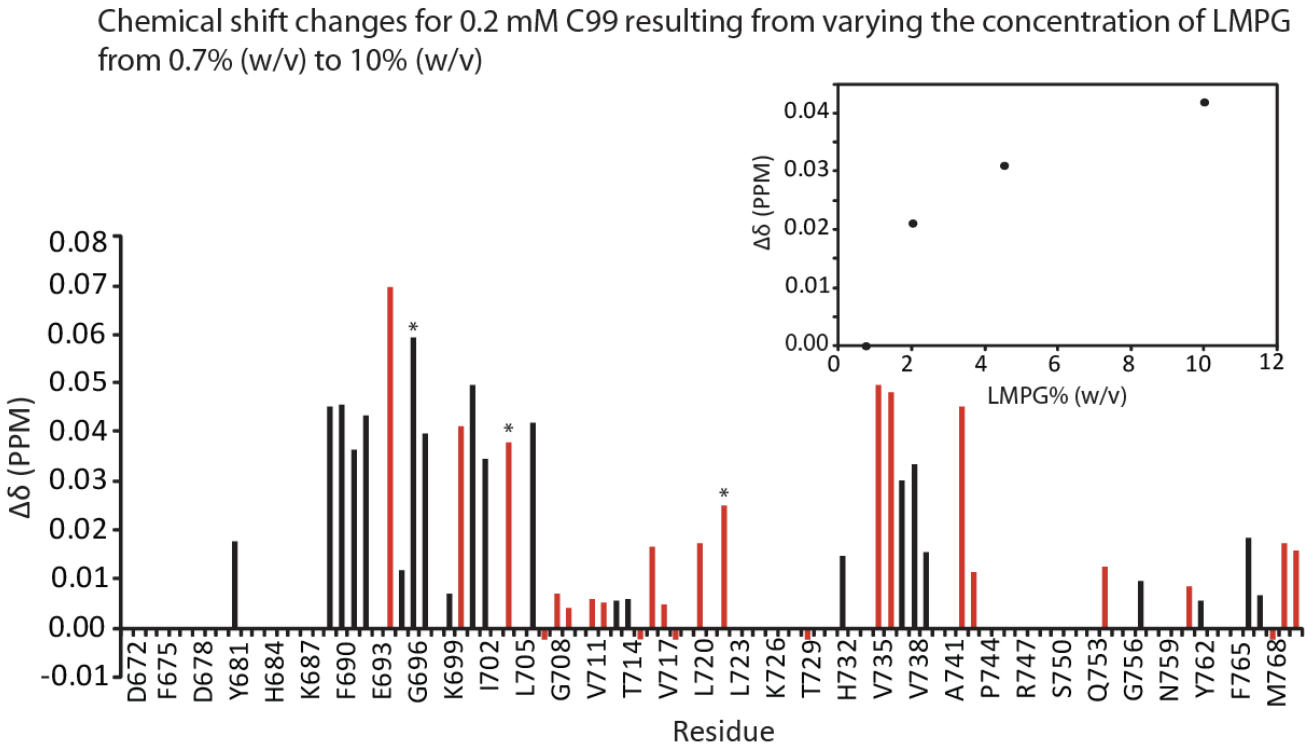
Supporting Figure 2. TROSY NMR peak ^{1}H chemical shift changes for 0.2 mM ^{15}N-C99 upon the addition of a 5 molar excess of unlabeled CD147-CTD (see Figure 4A in paper). Samples contained 2.5% (w/v) LMPG. The reported chemical shift changes were calculated as a composite of changes in both ^{1}H and ^{15}N dimensions using the equation $\Delta\delta = \sqrt{(\Delta\delta_H)^2 + (\Delta\delta_N/5)^2}$. The residues exhibiting no chemical shift changes were represented by a red bar with small negative value of chemical shift changes. The residues exhibiting positive shifts in the ^{1}H dimension were colored in black. The residues with negative shifts in the ^{1}H dimension were colored in red. Residues with no data represent unresolved or unassigned peaks. **Inset:** The average |Δδ| values for the three C99 peaks Y681, V689, G704 (see asterix) were plotted as a function of the mole % of CD147-CTD.



Supporting Figure 3. TROSY NMR peak ^{1}H chemical shift changes for 0.2 mM ^{15}N-C99 with 1 mM ^{14}N-CD147-CTD as the LMPG concentration was varied from from 2.2% (w/v) to 10% (w/v). The reported chemical shift changes were calculated as a composite of changes in both ^{1}H and ^{15}N dimensions using the equation $\Delta\delta = \sqrt{(\Delta\delta_H)^2 + (\Delta\delta_N/5)^2}$. The residues exhibiting no chemical shift changes were represented by a red bar with small negative value of chemical shift changes. The residues exhibiting positive shifts in the ^{1}H dimension were colored in black. The residues with negative shifts in the ^{1}H dimension were colored in red. Residues with no data represent unresolved or unassigned peaks. Inset: The average |Δδ| values for the three C99 peaks F690, G700, M722 (see asterix) were plotted as a function of the concentration (% w/v) of LMPG.

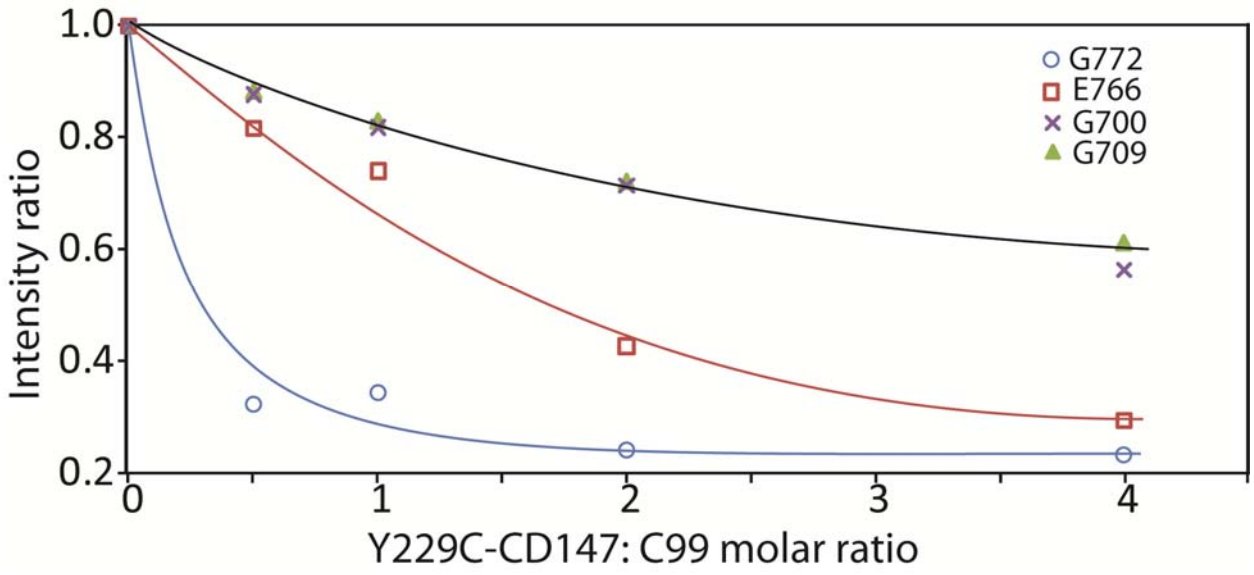


Supporting Figure 4. TROSY NMR peak ^{1}H chemical shift changes for 0.2 mM ^{15}N-C99 as the LMPG concentration was varied from 0.7% (w/v) to 10% (w/v). The reported chemical shift changes were calculated as a composite of changes in both the ^{1}H and ^{15}N dimensions using the equation $\Delta\delta = \sqrt{(\Delta\delta_H)^2 + (\Delta\delta_N/5)^2}$. The residues exhibiting no chemical shift changes were represented by a red bar with small negative value of chemical shift changes. The residues exhibiting positive shifts in the ^{1}H dimension were colored in black. The residues with negative shifts in the ^{1}H dimension were colored in red. Residues with no data represent unresolved or unassigned peaks. Inset: The average |Δδ| values for the three C99 peaks G696, G704, M722 (see asterix) were plotted as a function of the concentration (w/v) of LMPG.



Supporting Figure 5. Effects of nitroxide free radical-induced paramagnetic relaxation enhancement on peaks from selective residues of 0.2 mM U-^{15}N-C99 when this protein was titrated either by spin-labeled CD147-CTD or by spin-labeled KCNE1 (negative control). All experiments were carried out using a 600 MHz Bruker Avance NMR spectrometer at 318°K. **A.** Variation of selected ^{1}H,^{15}N-TROSY peaks intensities when U-^{15}N-C99 was titrated with nitroxide-labeled Y229C-CD147. Peaks from C99 residues E766 (red square) and G772 (blue diamond) were observed to experience dramatic relaxation enhancement from the nitroxide located near the membrane/cytosol interface on CD147-CTD, which indicates proximity between the spin label on CD147-CTD and these sites. C99 peaks from residues located near the interface on the other side of the membrane (G700, G709) broadened only to about the same extent as observed when a negative control titration was carried out (next panel). **B.** TROSY peak intensities when C99 was subjected to a negative control titration by nitroxide-labeled S64C-KCNE1. Peaks from residues distributed throughout C99 were observed to undergo very similar degrees of PRE-induced line broadening.

A



B