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Supplementary Information

Direct Inhibition of the First PDZ Domain of ZO-1 by Glycyrrhizin is a Possible Mechanism of Tight Junction Opening of Caco-2 Cells.

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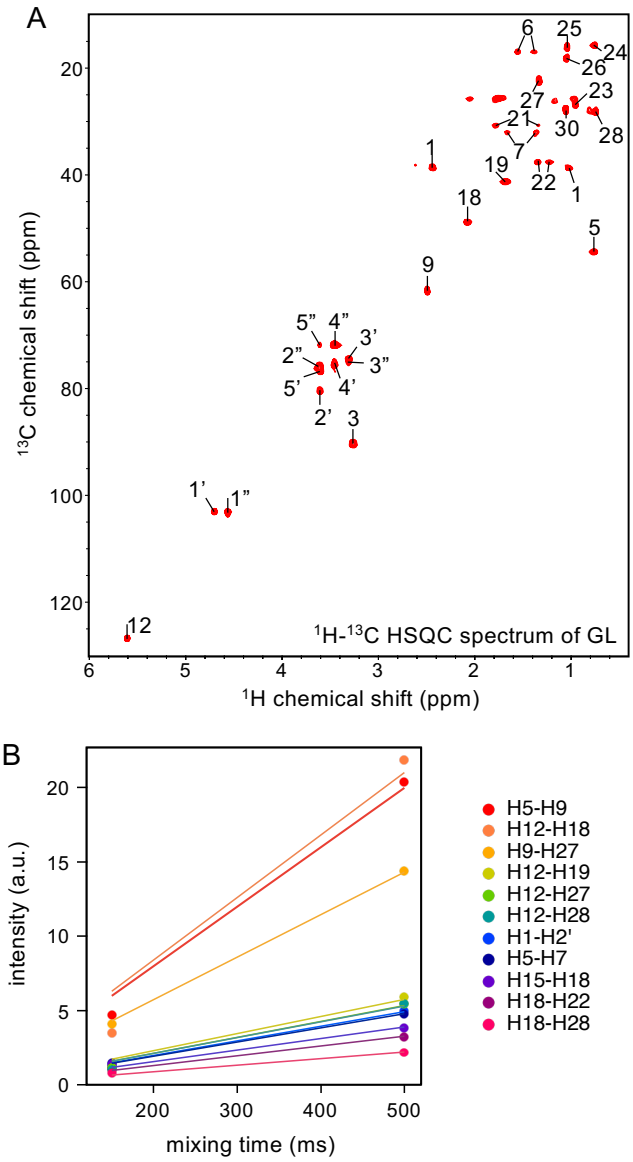

Figure S1

NMR analysis of the ^{1}H-^{15}N HSQC spectra revealed the binding of ZO-1(PDZ1) to GL.

A. ^{1}H-^{13}C HSQC spectrum of GL. The number of carbon bonded to hydrogen is indicated. The chemical formula is shown in the main Figure 2C.

B. The horizontal axis is the mixing time for ^{1}H-^{1}H NOESY and the vertical axis is the NOE peak intensity. H5-H9, shown as a thick red line, was used as the reference.

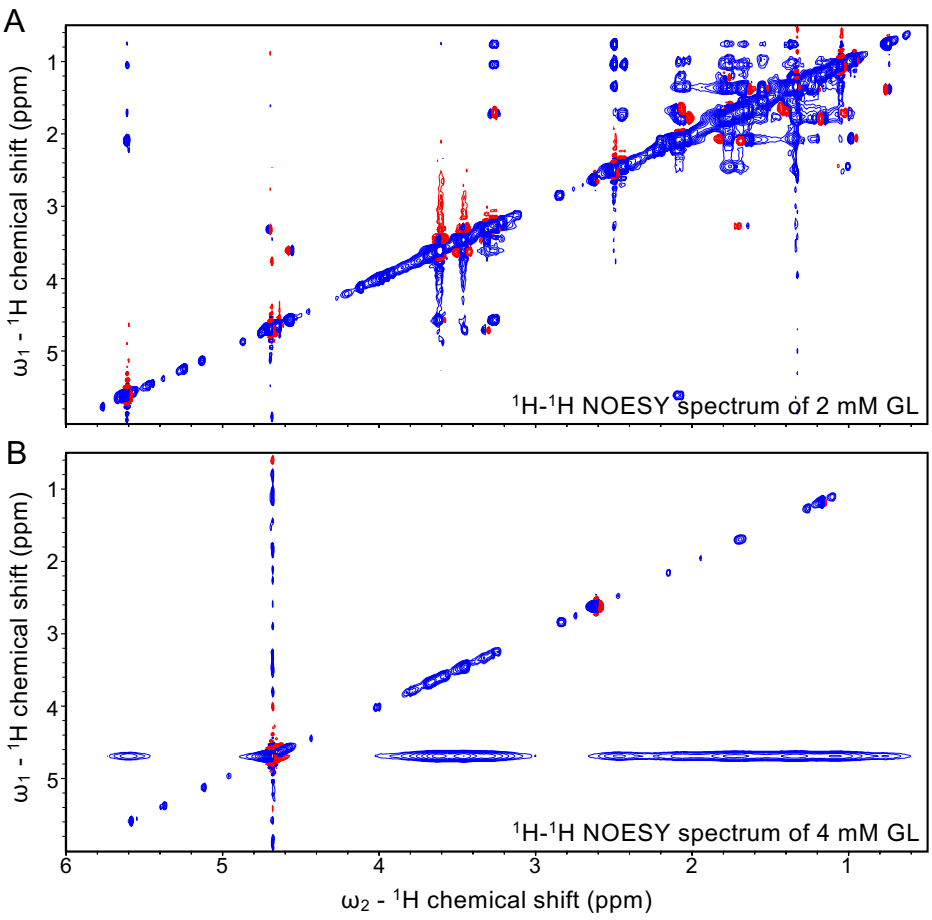

Figure S2

GL forms micelles at high concentrations.

A, B. ^{1}H-^{1}H NOESY spectra of GL at concentrations of 2 mM (**A**) and 4 mM (**B**); blue and red are the same and opposite sign as the diagonal peak, respectively.

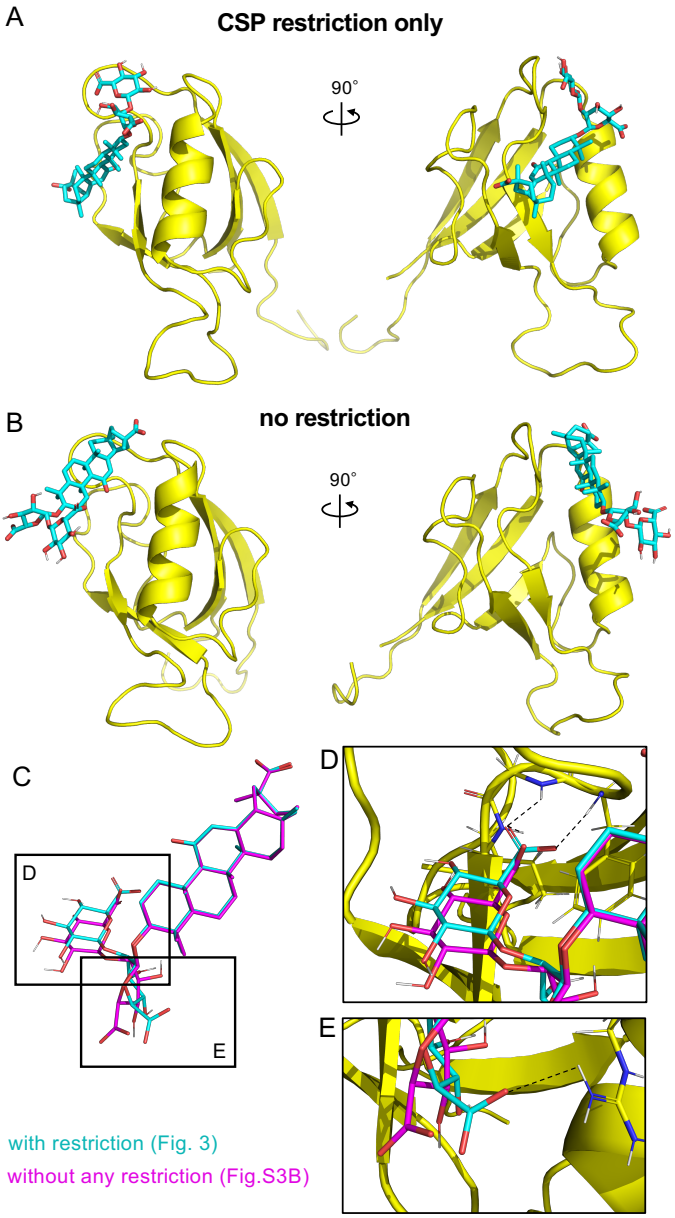

Figure S3

The HADDOCK structure with the highest score without distance restrictions.

A. Only the CSP result from the NMR titration was added as a restriction to the calculations. The aglycone part is located away from the PDZ domain. ZO-1(PDZ1) is colored in yellow and GL in cyan.

B. No restriction was used for calculations. The glucuronate part is away from the PDZ domain.

C. Overlay of docking structures with (cyan) and without (magenta) restriction.

D, E. Enlarged view of the box regions is shown in C with ZO-1(1-PDZ1) (yellow) together. The dotted lines show hydrogen bonds. In the case of docking without restriction, the carbonyl group of GL is oriented in a direction where no hydrogen bond can be formed with ZO-1(PDZ1).

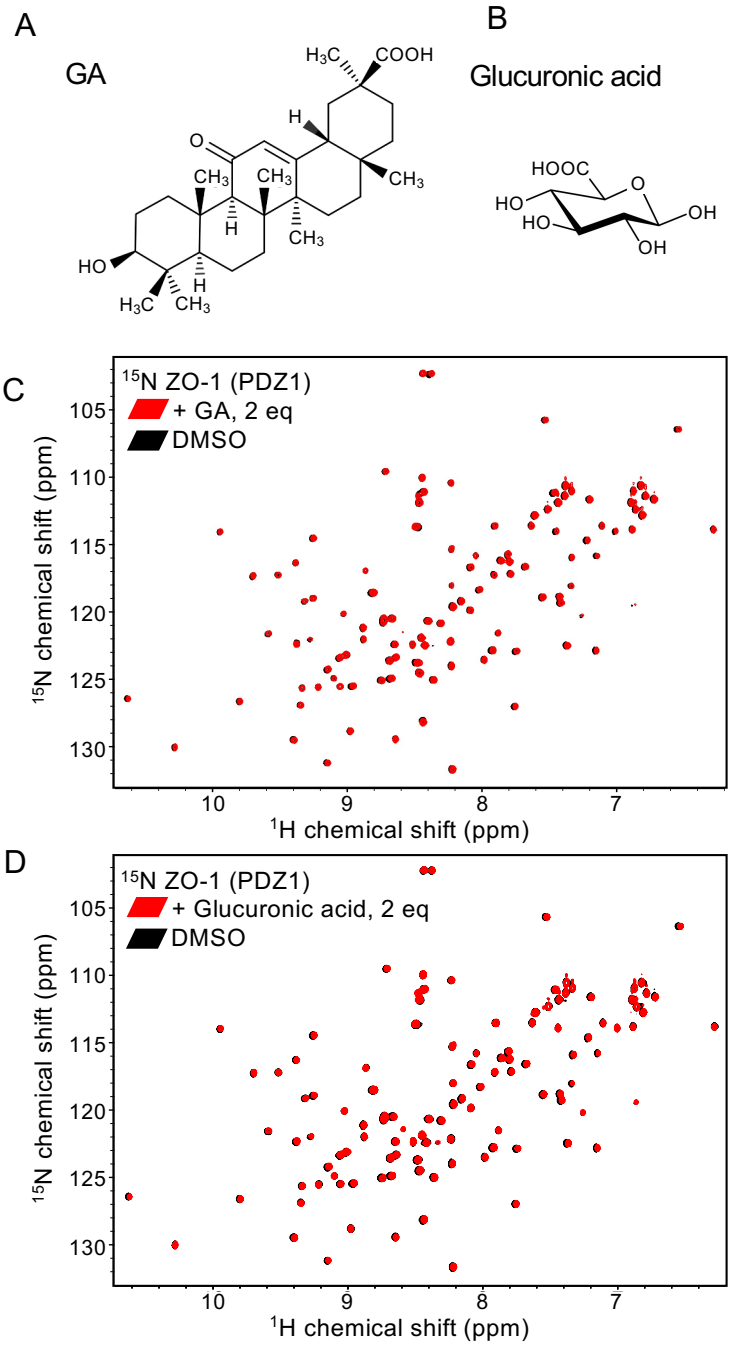

Figure S4

No binding of ZO-1(PDZ1) to GA or glucuronic acid was detected.

A. The chemical formula of GA.

B. The chemical formula of glucuronic acid.

C. HSQC spectra of ZO-1(-PDZ1) with and without two equivalents of GA.

D. HSQC spectra of ZO-1(-PDZ1) with and without two equivalents of glucuronic acid.

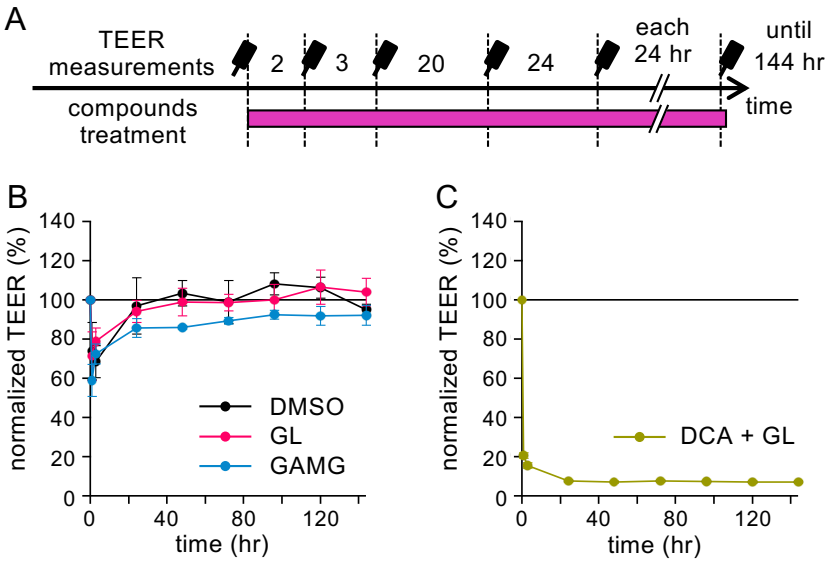

Figure S5

TEER changes in Caco-2 cells.

A. Experimental procedures. Pink arrows indicate the administrations of GL or DMSO. Details of the timing of TEER measurement compound administration are described in Experimental procedures.

B, C. Time course of TEER normalized to 100% of TEER before the initial administration. The effect of GL administered extracellularly was not significantly different from DMSO (**B**) and continued administration of DCA weakened cells (**C**).

atoms	coefficient	distance (Å)
H12-H18	42038.55	2.38
H5-H9	39963.17	2.40
H9-H27	28631.79	2.54
H12-H19	11484.73	2.95
H12-H27	10627.50	2.99
H12-H28	10585.28	2.99
H1-H2'	9840.13	3.03
H5-H7	9581.74	3.04
H15-H18	7789.93	3.15
H18-H22	6503.35	3.25
H18-H28	4419.91	3.46

Table S1

The slope of the fitted line and the calculated distance between the hydrogen atoms. This was given as restriction for the calculation in the HADDOCK program.

Restriction	glycyrrhizin	+	–	–
	mZO1-PDZ1	+	+	–
HADDOCK Score (a.u.)		-47.8 +/- 1.8	-51.2 +/- 1.1	-38.7 +/- 1.6
Cluster Size		5	5	4
RMSD from the overall lowest-energy structure		0.1 +/- 0.1	0.2 +/- 0.1	0.6 +/- 0.1
Van der Waals energy		-37.2 +/- 2.2	-45.1 +/- 1.2	-21.2 +/- 0.4
Electrostatic energy		-136.2 +/- 16.7	-108.4 +/- 4.0	-166.8 +/- 3.0
Desolvation energy		3.0 +/- 0.6	4.6 +/- 0.4	3.7 +/- 0.5
Restraints violation energy		0.4 +/- 0.2	1.1 +/- 0.7	0.0 +/- 0.0
Buried Surface Area		784.6 +/- 17.0	866.1 +/- 4.9	518.0 +/- 12.8
Z-Score		-1.5	-2.2	-1.6
Figure		**3**	**S3A**	**S3B**

Table S2

The obtained parameters in the calculation of the HADDOCK simulation.