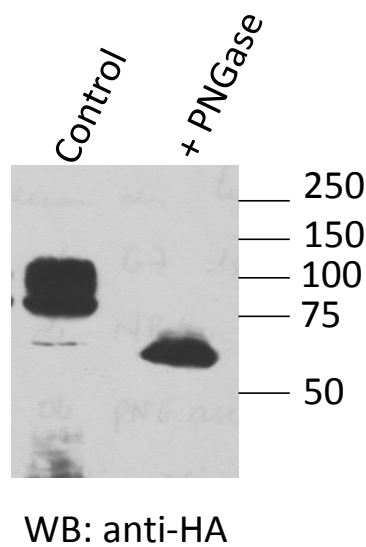


Supplemental information

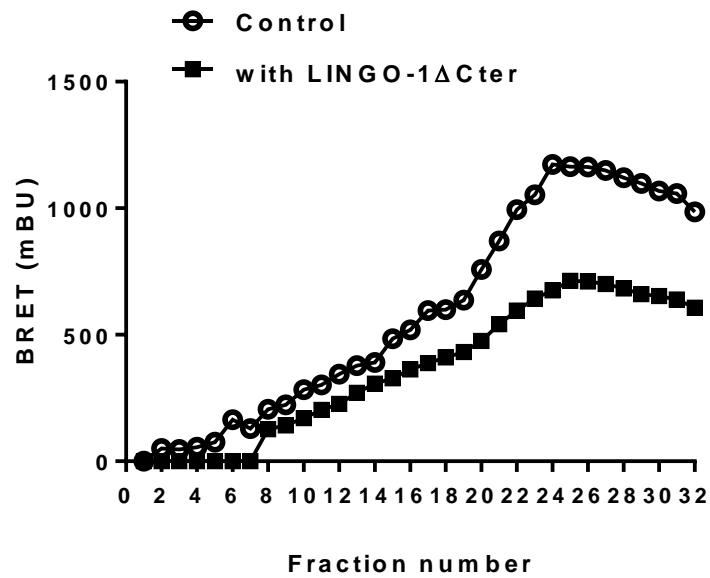
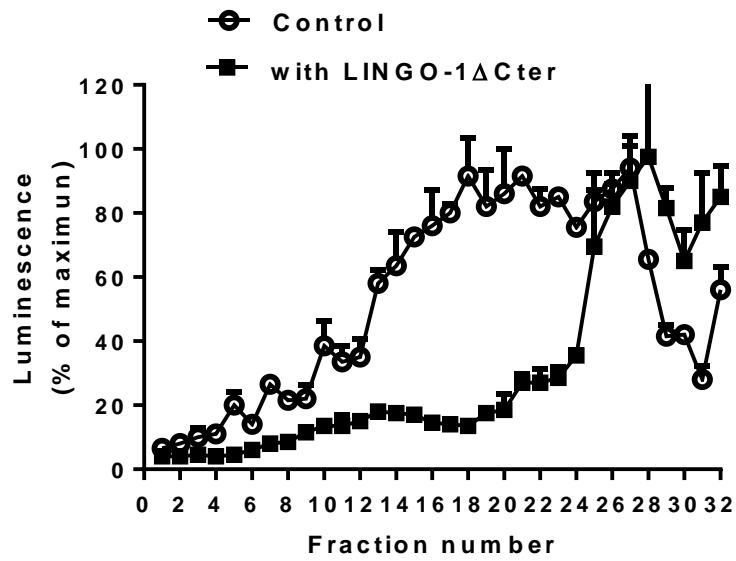
TARGETING THE *CIS*-DIMERIZATION OF LINGO-1 WITH SMALL-MOLECULE AFFECTS ITS DOWNSTREAM SIGNALING

Laetitia Cobret¹, Marie-Ludivine De Tauzia¹, Julien Ferent², Elisabeth Traiffort³,
Imène Hénaoui^{1,4}, Esther Kellenberger^{5,6}, Didier Rognan^{5,6}, Fabienne Godin¹,
Jacques Pantel⁷, Hélène Bénédicti¹ and Séverine Morisset^{1*}

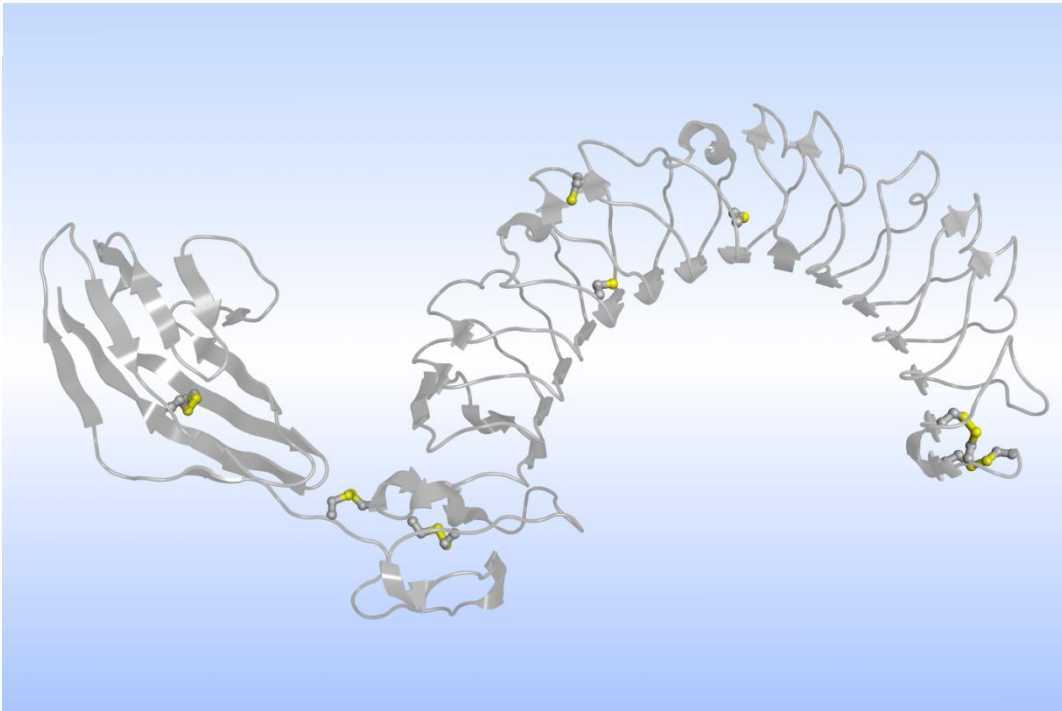
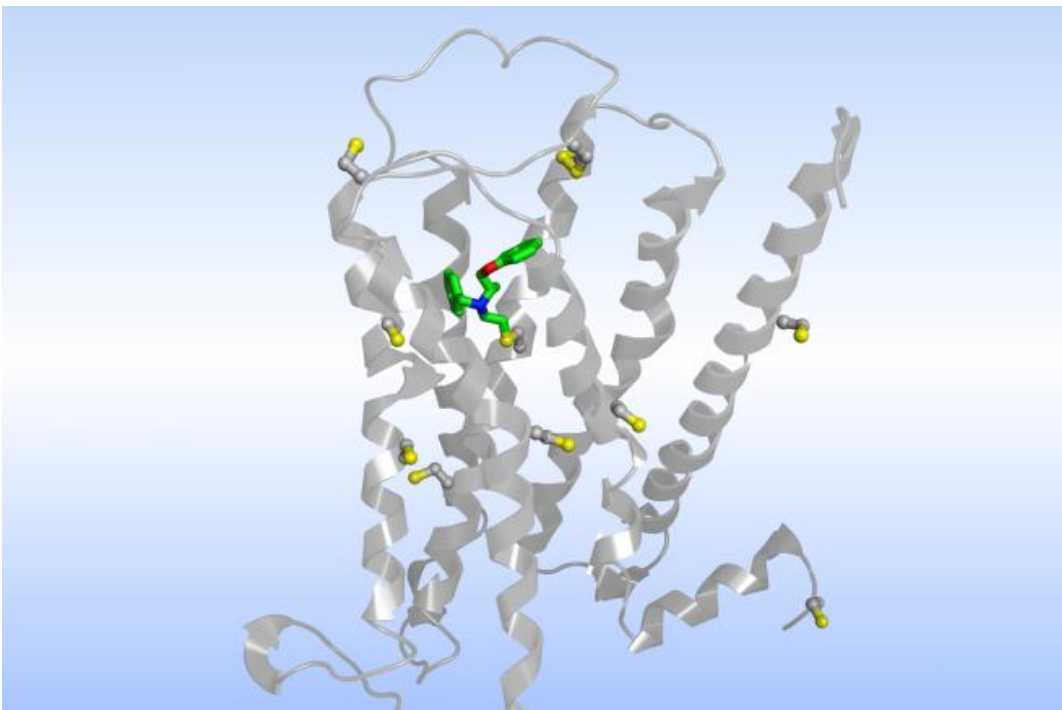


Supplemental Figure 1: Deglycosylation of LINGO-1-HA with Peptide-N-glycosidase (PNGase F). Lysates from HEK-293 cells transiently transfected with the LINGO-HA were incubated in Buffer B (50mM sodium citrate, pH 7.5, 1% Triton, cocktail of protease inhibitor) with or without PNGase F for 2 hours at 37°C. The reaction was stopped by adding loading sample buffer. Samples were separated using 8% SDS-PAGE and analysed by immunoblotting with the anti-BD living colors antibody (BD Biosciences).

Treatment of membranes with PNGase F reduced the 80 kDa and 100 kDa mature forms to about 60 kDa which represented the deglycosylated forms of LINGO-HA. These data demonstrate that recombinant LINGO-1 expressed in HEK-293 cells is N-glycosylated.



Supplemental Figure 2: Effect of LINGO-1ΔCter on the distribution and dimerization of wild type LINGO-1. HEK-293 cells were transiently transfected with LINGO-1-RLuc and LINGO-1-YFP with or without LINGO-1ΔCter. 48 hours after transfection, cells were lysed and subcellular fractions were resolved on a sucrose gradient. Individual fractions were subjected to luminescence and BRET analysis.

A**B**

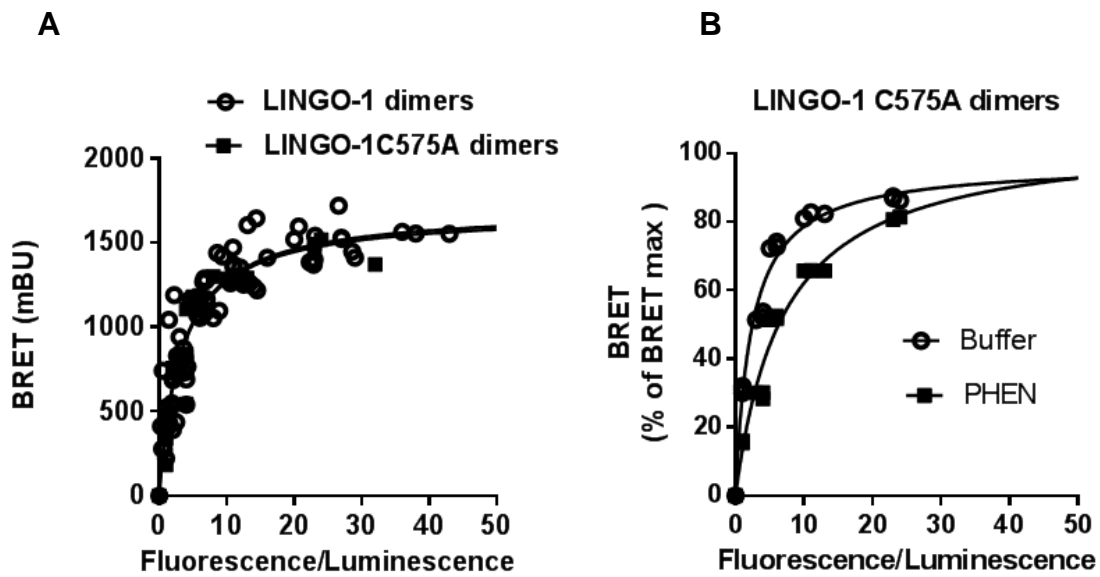
Supplemental Figure 3: Solvent accessibility of cysteine residues in human LINGO-1 and human α_2 adrenergic receptor.

The protein backbone is represented with gray ribbon. Only cysteine residues are shown, their side chain being depicted using CPK-colored ball and sticks

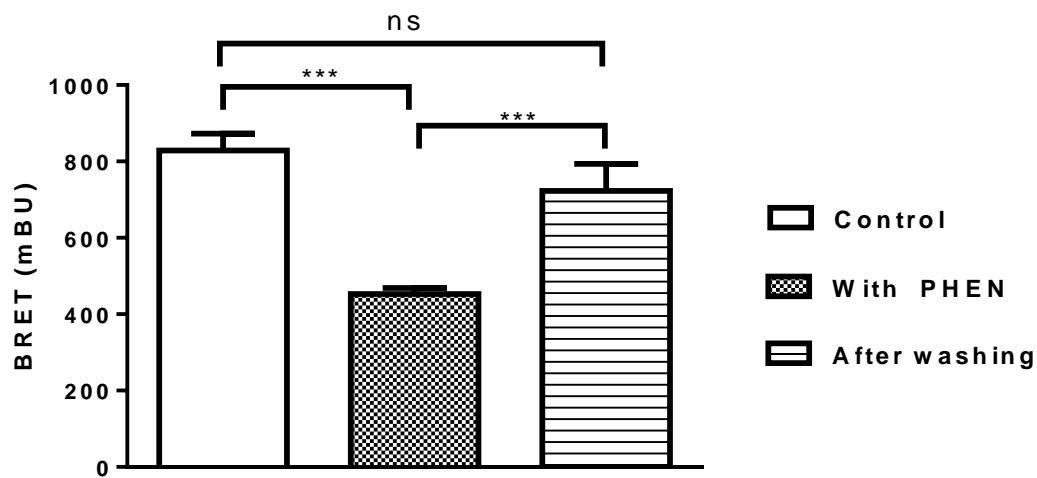
A. The three-dimensional structure of LINGO1 ecto-domain (residues 1-476) was solved by X-ray crystallography (PDB: 2ID5) and revealed a tetrameric organization. [doi: 10.1074/jbc.M607314200] Each protomer contains thirteen cysteine residues, ten engaged in disulfide bridges and the remaining three burying their side chain into the core structure of the leucine rich repeats.

B. The three-dimensional structure of $\alpha 2$ adrenergic receptor was modeled by homology with human $\alpha 2$ adrenergic receptor (PDB: 3P0G).

Details of modeling: The template file was chosen because of sequence similarity between the protein (25.8% identity) as well as shape similarity between PHEN and the ligand bound to transmembrane cavity of the receptor. The model was built using MOE v2011.10 (Chemical Computing Group Inc) based on sequence alignment guided by GPCR constraints. The 3D-structure of PHEN was generated with Corina v3.1 (Molecular Network GmbH). Model of complex between PHEN and $\alpha 2$ adrenergic receptor was obtained from covalent docking of PHEN into the transmembrane cavity (defined in a radius of 10 Å centred on OE1 atom of ASP3.32) using GOLD v5.2 (The Cambridge Crystallographic Data Centre). The best scored pose selected with ASP scoring function well reproduces the binding mode of ligand bound to the template receptor.



Supplemental Figure 4: BRET analysis on LINGO1C575A. The cysteine 575 is located in the transmembrane domain of LINGO-1. In order to investigate its potential role in the action of PHEN, we performed two constructions containing the mutated LINGO fused to Renilla Luciferase (LINGO-1-C575A-RLuc) or with yellow fluorescent protein (LINGO-1-C575A-YFP). BRET saturation curves analysis demonstrates that the mutation does not modify either the ability of LINGO-1 to form dimers (A) nor the ability of PHEN to decrease BRET signal (B).



Supplemental Figure 5: PHEN binds LINGO-1 in a reversible manner. BRET experiments were performed in adherent HEK-293 cells transiently transfected with LINGO-1-RLuc alone or with LINGO-1-RLuc and LINGO-1-YFP. BRET signal was determined in the presence of PHEN immediately before and immediately after extensive washing of the cell culture. After the washing the BRET signal reaches a value non significantly different from the control value. Data are the mean \pm SEM of two independent experiments. *** $p < 0.001$