

## **Supplemental Methods**

### **Manufacturing of phospholipid liposomes**

Synthetic 1-palmitoyl-2-oleoyl (PO) phosphatidylserine (POPS) (PS), phosphatidylethanolamine (POPE) (PE), phosphatidylglycerol (POPG) (PG), phosphatidylcholine (POPC) (PC), 1,2-dioleoyl (DO) PC, bovine liver L- $\alpha$ -PI were obtained from Avanti Polar Lipids (Alabaster, AL). Phospholipid vesicles (liposomes) were prepared by evaporating the chloroform from the desired phospholipid mixture at defined ratios using nitrogen gas and placed in a vacuum overnight to obtain a dried lipid film in the glass tubes. Large multi-lamellar vesicles are formed by gentle swirling in Hepes buffered Saline (10 mM HEPES pH 7.4 and 140 mM NaCl) until all the dried lipid suspension is re-hydrated. Incubation on a bench top incubator at 37°C for 3-5 minutes helps in re-hydration. Then, small unilamellar vesicles were prepared by sonication for 7-10 minutes on ice with one-minute gap intervals between the shocks. The residual large vesicles were removed by filtering using 45-micron filters. For the experiments performed in this report, lipids are mixed in the following ratios: DOPC:POPC (80:20 mol%) (PC), DOPC:POPE (80:20 mol%) (PE), DOPC:POPG (80:20 mol%) (PG), DOPC:PI (80:20 mol%) (PI), DOPC:POPS (80:20 mol%) (PS (20%)), POPS:DOPC (50:50 mol%) (PS (50%)), and POPS:DOPC (80:20 mol%) (PS (80%)).

### **Surface plasmon resonance (SPR) analysis**

The interaction of CD300a with different phospholipids was measured using BIAcore 3000 system (Biacore Inc.). In our analysis, two strategies were employed: either the fusion proteins CD300a-Ig and LAIR-1-Ig were immobilized on a CM5 sensor (Biacore,

GE Healthcare) and liposomes were carried in the flow or the liposomes were coupled to the L1 sensor (Biacore, GE Healthcare) and the proteins were carried in flow. In the first scenario, the proteins were immobilized on the CM5 sensor by amine coupling. A mixture of N-Hydroxysuccinimide (NHS) and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was injected to activate the sensor and next, the fusion proteins at 10 µg/ml in acetate buffer pH 4.5 (Biacore, GE Healthcare) were injected onto the sensor for immobilization. Finally, injecting ethanolamine deactivated the excess of amine reactive groups. Of the four cells of the CM5 sensor only three were used as buffer control, LAIR1-Ig (1000 RU) and CD300a-Ig (1000 RU). The binding of different liposomes at 1mM concentration was tested. In the second case, liposomes were captured onto the L1 sensor chip. Initially, the new sensor chip was cleaned with three 1-minute injections of regeneration solution (50mM NaOH:Isopropanol at 2:3 ratio) at a flow rate of 10 µl/min. Then, 1 mM concentrated liposomes for 10 minutes at a flow rate of 2 µl/min were injected over all flow cells. After that, running buffer (10mM HEPES pH 7.4, 140mM NaCl and 2.5mM CaCl<sub>2</sub>) at a flow rate of 150 µl/min for 2-3 minutes was injected. Then, at 10 µl/min flow rate 100mM NaOH was injected twice for 1 minute and we waited until the base line was stabilized. Finally, the chip was blocked by injecting 0.1 mg/ml BSA for one minute. Then, the different Ig fusion proteins were injected at 10 µg/ml (or other concentrations) to study their binding to liposomes.

### **Blocking experiments of CD300a-Ig to liposomes using SPR**

PS containing liposomes (PS (80%)) were immobilized onto a L1 sensor and the binding of CD300a-Ig was confirmed. Then, the bound CD300a-Ig was dissociated from the

immobilized PS containing liposomes by using 2.5M NaCl plus 5mM EDTA. After the dissociation of CD300a-Ig, AnnexinV was injected to demonstrate the binding of AnnexinV to PS containing liposomes. After that, extensive washes with running buffer were performed to remove the unbound Annexin V. Next, the binding of CD300a-Ig was determined again and the no rise on the resonance units (RU) indicated that Annexin V bound to the immobilized PS containing liposomes blocked the binding of CD300a-Ig to them.

PE containing liposomes (PE) were immobilized onto a L1 sensor and the binding of CD300a was confirmed. Then, the bound CD300a-Ig was dissociated from the immobilized PE containing liposomes by using 2.5M NaCl plus 5mM EDTA. After the dissociation of CD300a-Ig, duramycin was injected to demonstrate the binding of duramycin to PE containing liposomes. After that, extensive washes with running buffer were performed to remove the unbound duramycin. Next, the binding of CD300a-Ig was determined again and the small rise on the resonance units (RU) indicated that duramycin bound to the immobilized PE containing liposomes blocked the binding of CD300a-Ig to them.

### **Structural modeling**

To obtain a structural model of CD300a having a possible phospholipid-binding conformation we modeled the entire CD300a model using SWISSMODEL in project mode with the TIM-4-phospholipid complex (3BIB) as a template. The coordinates of the ligand-free CD300a (2Q87) structure were superimposed onto the model using the align feature of Pymol (DeLano Scientific). The coordinates of the hypothetical model of

CD300a corresponding to the phospholipid-binding site of TIM-4 were swapped into the CD300a structure to give a CD300a model having a binding site conformation similar to TIM-4.

### **Sedimentation assay**

The PC, PS (80%), PS (20%) and PE containing liposomes (0.5 mM) were incubated with 25 µg/ml of CD300a-Ig (or LAIR-1-Ig) protein for 45 minutes at RT in binding buffer (10mM HEPES pH 7.4, 140mM NaCl and 2.5 mM CaCl<sub>2</sub>). The reactions (total volume of 200 µl) were then centrifuged for 45 minutes at 100,000 X g in a Beckman TL-100 rotor at 4°C. The supernatants were precipitated with 10% trichloroacetic acid, and the total protein from the supernatants and pellet fractions was then visualized by 4-12% SDS-PAGE and stained using Gel Code Blue Safe Stain (ThermoScientific).

### **Enzyme linked immuno-sorbent assay (ELISA)**

The amino phospholipids (PE and PS) were diluted in 100% methanol at a concentration of 5 µg/ml, and then 100 µl of lipids were added to each well of a 96-well maxisorp plate and air-dried. The wells were washed once with washing buffer (PBS + 0.05% Tween-20) and blocked with 2% BSA for 2 hours at RT. After blocking, the wells were washed 3 times and incubated with different concentration of Ig fusion proteins for 2 hours at RT diluted in binding buffer (10mM HEPES pH 7.4, 140mM NaCl and 2.5 mM CaCl<sub>2</sub>). The plate was washed thoroughly and incubated with anti-human Ig (Fcγ specific) antibody coupled to horseradish peroxidase HRP (Jackson Immuno Research) for one hour at RT. The peroxidase activity was analyzed by using TMB substrate (ImmunoPure TMB

Substrate Kit-Pierce) and the absorbance was measured at 450 nm in a spectrophotometer.

### **Reporter cell assay**

The reporter cells BWZ.36 stably transfected with LAIR-1/CD3 $\zeta$  construct were previously described.<sup>1</sup> The reporter cell line BWZ.36 was stably transfected by electroporation with the new CD300a/CD3 $\zeta$  construct. In this system, when the BWZ.36 CD300a/CD3 $\zeta$  expressing cell line encounters the CD300a ligand, the ITAM of CD3 $\zeta$  gets phosphorylated and, as a consequence, cytoplasmic NFAT proteins translocate to the nucleus and bind tandem NFAT binding sites in the promoter of the *LacZ* gene. This induces  $\beta$ -galactosidase expression, whose level of induction can be quantitated through its enzyme activity. In the reporter cell assay,  $1 \times 10^5$  of reporter cells were plated in a 24-well plate that were either left uncoated or coated with isotype control, anti-CD300a mAb at 5  $\mu$ g/ml concentration, and also lipids (PC, PS and PE) at 5  $\mu$ g/ml that were air-dried. After 36-48 hours of incubation the cells were harvested and the  $\beta$ -galactosidase activity was determined by enhanced  $\beta$ -gal assay kit (CPRG; Gelantis, Inc.) as previously described<sup>1</sup>.

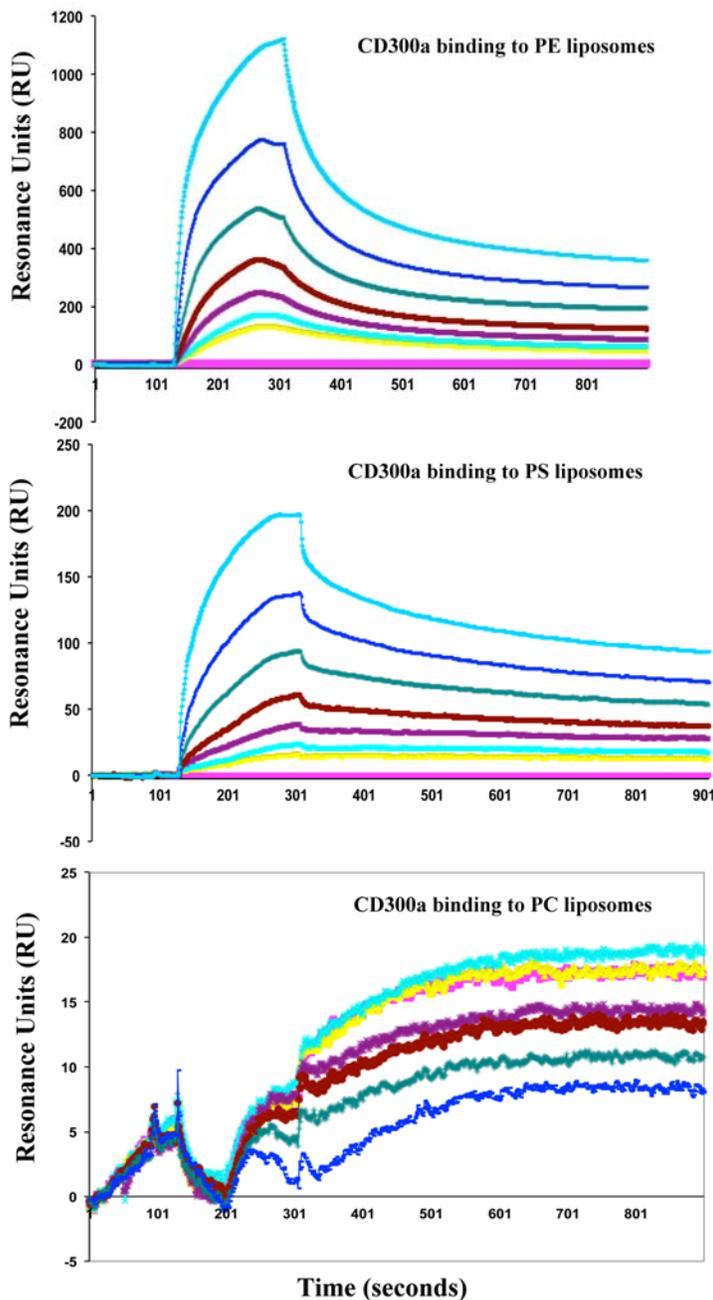
### **Software**

The FACS analysis data were analyzed using FlowJo software (Tree Star.). The graphical representations were made using GraphPad Prism (Version 5). BIACORE 3000 Control Software and BIAevaluation Software were used to analyze the SPR experiments.

## Reference

1. Tang X, Narayanan S, Peruzzi G, et al. A single residue, arginine 65, is critical for the functional interaction of leukocyte-associated inhibitory receptor-1 with collagens. *J Immunol.* 2009;182(9):5446-5452.

## Supplementary Figure 1



### Sensograms of CD300a-Ig binding to liposomes:

Different concentrations of CD300a-Ig ranging from 800 nM to 12.5 nM were used to observe the binding to different liposomes immobilized on the L1 biosensor chip.

The X-axis of each line graph represents the length of time in seconds that the protein has passed through and the Y-axis determines the resonance units (RU) values after subtracting from the negative flow cell. Note that the scale for the Y-axis is different in the three sensograms. The negative control LAIR1-Ig did not bind to any of the liposomes (not shown).