Detailed Methods:

Cell lines:

CHO-K1 cell transfectants were selected and maintained in RPMI 1640 and 10% Cosmic Calf Serum (Invitrogen) containing either 400 µg/ml Geneticin® selective antibiotic (G418 Sulfate) for cells transfected with GPI-ICAM-1 and GPI-GM-CSF or with 10 µg/ml Blasticidin (Invitrogen) for cells transfected with GPI-IL-12. Cell pellets of CHO-K1 cells transfected to express GPI-ISMs were lysed with 50 mM Tris-HCl pH 8, 2% n-octyl-β-D-glucopyranoside (A.G. Scientific), 1:100 dilution of Protease Inhibitor Cocktail (Sigma), 5 mM EDTA, 20 mM sodium iodoacetate, and 2 mM PMSF overnight at 4˚C. The lysate was centrifuged at 14,000 rpm for 1 h at 4˚C and used for purification of GPI-APs. Sheep red blood cells (RBCs) were obtained from HemoStat Laboratories.

Antibodies:

Anti-mGM-CSF (A2F17107) secreting hybridoma was a kind gift from Dr. Michael B. Prystowsky, University of Pennsylvania (1) and anti-mICAM-1 (YN1/1.7.4) secreting hybridoma was a kind gift from Fumio Takai, University of British Columbia, Canada. Antibodies were purified from hybridoma supernatant by using a Gammabind plus Sepharose column (GE Healthcare). Purified anti-mIL-12 p40 mAb (C17.8) was obtained from Bio X Cell. Immunoaffinity columns were made by coupling mAbs to CNBr-activated-Sepharose beads (GE Healthcare).

PI-PLC treatment of transfected CHO-K1 cells:

Transfected CHO-K1 cells were washed and resuspended in PBS/EDTA/0.1% ovalbumin to a final concentration of 10 x 10^6 cells/ml. 1U PI-PLC from *Bacillus cereus* (Invitrogen) that

specifically cleaves the GPI-anchor was added to 1 ml of cell suspension and incubated at 37˚C for 45 min. Cells were washed and protein expression was analyzed by flow cytometry.

Incorporation of GPI-ISMs onto sheep RBCs by protein transfer:

To test if the purified GPI-ISMs retained the GPI-anchor and the ability to incorporate onto the lipid bilayer of cell membranes, protein transfer was initially performed on sheep RBCs. GPI-ISMs were incubated with 2 x 10^6 washed sheep RBCs in PBS/0.1% ovalbumin in an end-over-end rotation for 4 h at 37˚C. Unincorporated GPI-ISMs were washed out by centrifugation using FACS buffer (PBS, 5 mM EDTA, 1% CCS). Incorporation was analyzed by flow cytometry.

H5 and H1 VLP preparation:

H5 VLPs and H1 VLPs were made and purified using the recombinant baculovirus (rBV) expression system as previously described (2, 3). In brief, rBVs expressing influenza M1 protein from H1N1 influenza A/PR/8/1934 virus and rBVs expressing influenza HA proteins from H5N1 influenza A/Indonesia/05/05 virus or from H1N1 influenza A/PR/8/1934 virus, were generated, and then co-infected into Sf9 insect-cells to generate H5 or H1 VLPs, respectively. After 2-3 days, infected cell culture supernatants were clarified by centrifugation (6000 rpm, 30 min at 4˚C) and concentrated by the QuixStand hollow fiber based ultrafiltration system (GE Healthcare, Piscataway, NJ). VLPs were purified by sucrose gradient ultracentrifugation with 20% and 60% (wt/vol) layers at 30,000 rpm using a SW32Ti Rotor (Beckman) for 60 min at 4˚C and were collected from the 20%-60% interphase. VLPs were pelleted by ultracentrifugation at 30,000 rpm using a SW32Ti rotor for 30 min at 4˚C. The VLP pellet was resuspended in PBS and stored at

-80˚C until further studies. VLPs were quantified by the Bio-Rad protein assay (Bio-Rad Laboratories, Inc., Hercules, CA) and HA content on the VLPs was approximately 11% of total VLP protein as detected by ELISA (4). Endotoxin levels were tested in the VLP preparations before and after protein transfer using the ToxinSensor™ Chromogenic Limulus Amebocyte Lysate Endotoxin Assay Kit (GenScript).

Physicochemical analysis of protein transfer-modified VLPs:

The size and zeta potential of VLP preparations were measured using the Malvern Zetasizer Nano ZS. Analysis was carried out using 1 ml of 0.2 mg/ml of VLPs at 4°C in either PBS (pH 7.4) for size analysis or 10 mM sodium phosphate buffer (pH 7.4) for zeta potential analysis.

Stability analysis of GPI-GM-CSF incorporated onto VLPs by protein transfer:

GPI-GM-CSF was protein transferred onto H5 VLPs and the resulting GPI-GM-CSF-VLPs were stored at 4˚C. At day 0, 4, 7, 14, 21 and 28, aliquots of protein transferred-VLPs were washed by centrifugation. VLP pellets were resuspended in Leammli sample buffer, boiled and then frozen at -20°C. Western blot and Image J analysis for GM-CSF and VLP-specific proteins were performed to determine GM-CSF:VLP ratios.

Quantitative ELISA:

A direct ELISA was performed to quantify incorporated GPI-GM-CSF. Briefly, 100 µl of 1 µg/ml of GPI-GM-CSF-VLPs was coated onto a 96-well flat bottom ELISA plate in triplicate. As a standard, varying concentrations of recombinant soluble mouse GM-CSF

(Peprotec) were coated on the plate. The plate was blocked with PBS/0.05% Tween/3%BSA, and stained with anti-GM-CSF antibody followed by the horseradish peroxidase (HRP)-conjugated-rabbit-anti-rat-IgG secondary Ab (Thermo Scientific). Tetramethylbenzidine (TMB) (BioLegend) was used to develop color that was then stopped with 2N H_2SO_4 and read at 450 nm.

Electron microscopy analysis of VLPs:

Either unmodified VLPs or VLPs modified by protein transfer with GPI-ICAM-1 were analyzed using transmission electron microscopy at the Emory University Robert P. Apkarian Integrated Electron Microscopy Core facility.

Statistical Analysis:

All data are expressed as mean ± standard error of the mean (SEM). GraphPad Prism 6 was used to perform statistical analysis. Student's t-test, One-way ANOVA or Two-way ANOVA using Tukey's multiple comparisons post-test was used for analysis as described in the figure legends. A $p < 0.05$ value was considered significant.

Supplementary Figure Legends:

Supplemental Figure 1. SDS PAGE of affinity purified GPI-ISMs. GPI-ICAM-1, GPI-GM-CSF, and GPI-IL-12 were purified from transfected CHO-K1 cell lysates by mAb affinity chromatography. Purified GPI-ISMs were subjected to SDS PAGE followed by (**A**) western blot and (**B**) silver staining analysis.

Supplemental Figure 2. Purified GPI-ISMs incorporate onto the surface of sheep RBCs by protein transfer. Purified GPI-ISMs were incubated with sheep RBCs (2×10^6) in PBS with 0.1% ovalbumin for 4 h at 37°C. The RBCs were washed by centrifugation in PBS and then analyzed by flow cytometry.

Supplemental Figure 3. Two GPI-ISMs can incorporate simultaneously onto influenza VLPs by protein transfer. (**A**) H5 VLPs were protein transferred with a constant concentration of GPI-ICAM-1 and increasing concentrations of GPI-IL-12. (**B**) H5 VLPs were protein transferred with a constant concentration of GPI-IL-12 and increasing concentrations of GPI-ICAM-1. The results are a representative of at least three separate experiments.

Supplemental Figure 4. The structural integrity and physicochemical properties of GPI-ISM-protein transferred-VLPs. (**A**) Electron microscopy of H5 VLPs modified by protein transfer. Representative electron microscopy images are depicted of influenza H5 VLPs before (left panel) and after (right panel) protein transfer with GPI-ICAM-1. Scale bar, 100 nm. (**B**) Protein transfer does not affect VLP protein content. H5 VLPs were protein transferred with or without GPI-ICAM-1 at 37˚C and analyzed by SDS PAGE and western blot analysis against ICAM-1 (top) or against H5 VLP by using serum from mice challenged with H5N1 virus (bottom). (**C**) Physicochemical properties of protein transfer-modified-VLPs. H5 VLPs were protein transferred with or without GPI-ICAM-1 at 37˚C for 6 h. The size (left panel; n = 28) and zeta potential (right panel; n = 15) of H5 VLPs before and after protein transfer were measured using the Malvern Zetasizer Nano ZS. (Statistical analysis: (**C**) T-test. * $p < 0.05$, ** $p < 0.01$).

Supplemental Figure 5. Purified GPI-ICAM-1 retains functionality. To test the ability of GPI-ICAM-1 to bind to LFA-1-expressing cells, a plate binding inversion assay was conducted. 100 µl of 100 µg/ml of GPI-ICAM-1 were coated in triplicate onto ELISA plate wells and 250,000 LFA-1-expressing SKW3 cells were allowed to adhere to GPI-ICAM-1. The plate was then inverted in a trough containing PBS/0.1% cosmic calf serum/1 mM $MgCl_2$ for 1 h and the number of bound SKW3 cells was counted using a hemocytometer. SKW3 cells bound to wells coated with GPI-ICAM-1, however binding was significantly inhibited by blocking with an anti-ICAM-1 mAb. The results are a representative of at least three separate experiments. (Statistical analysis: One-Way ANOVA – Tukey's multiple comparisons test, **** $p < 0.0001$, ns – not significantly different).

Supplemental Figure 6. Vaccination with protein transfer-modified GPI-GM-CSF-VLPs that express either 0.029 µg GM-CSF/µg VLPs or 0.089 µg GM-CSF/µg VLPs induces similar levels of homologous and heterologous virus-specific IgG responses. Mice were vaccinated and boosted with 0.5 µg of unmodified VLPs or 0.5 µg of protein transfer-modified-GPI-GM-CSF-VLPs that expressed either 0.029 µg GM-CSF/µg VLPs or 0.089 µg GM-CSF/µg VLPs. Serum was collected from groups of vaccinated mice (n = 5) 6 weeks after boost and 1:5000 dilution of serum was used in an ELISA against coated inactivated homologous H5N1 influenza A/Indonesia/05/2005 virus (left panel) or heterologous H5N1 influenza A/Vietnam/1203/2004 virus (right panel). (Statistical analysis: One-Way ANOVA – Tukey's multiple comparisons test, **** $p < 0.0001$).

References for supplementary text

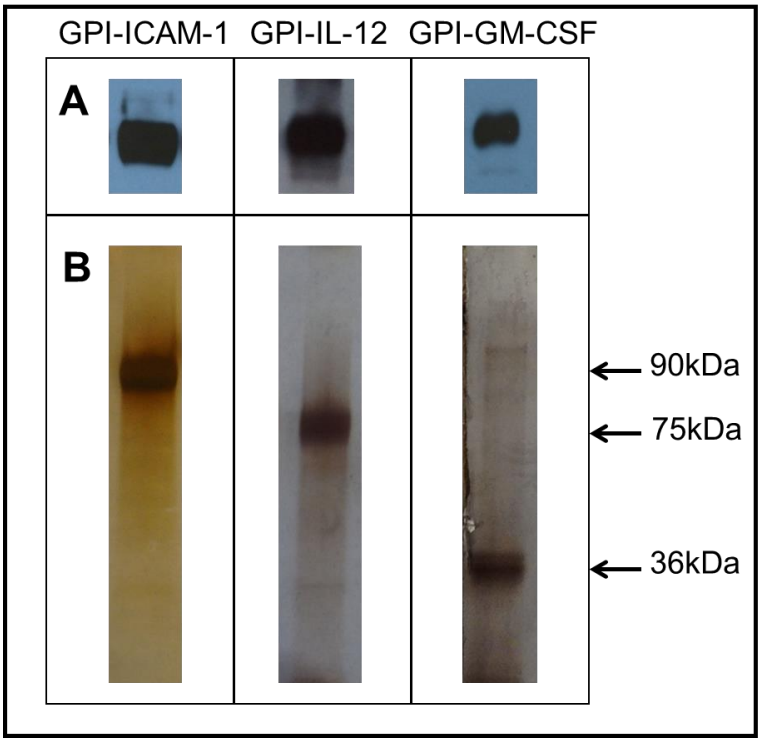
1. Meropol NJ, Kreider BL, Lee VM, Kaushansky K, Prystowsky MB. A neutralizing monoclonal antibody binds to an epitope near the amino terminus of murine granulocyte-macrophage colony-stimulating factor. Hybridoma. 1991;10(4):433-47.

2. Kang SM, Yoo DG, Lipatov AS, Song JM, Davis CT, Quan FS, et al. Induction of long-term protective immune responses by influenza H5N1 virus-like particles. PloS one. 2009;4(3):e4667.

3. Quan FS, Sailaja G, Skountzou I, Huang C, Vzorov A, Compans RW, et al. Immunogenicity of virus-like particles containing modified human immunodeficiency virus envelope proteins. Vaccine. 2007;25(19):3841-50.

4. Song JM, Hossain J, Yoo DG, Lipatov AS, Davis CT, Quan FS, et al. Protective immunity against H5N1 influenza virus by a single dose vaccination with virus-like particles. Virology. 2010;405(1):165-75.

Supplemental Figure 1



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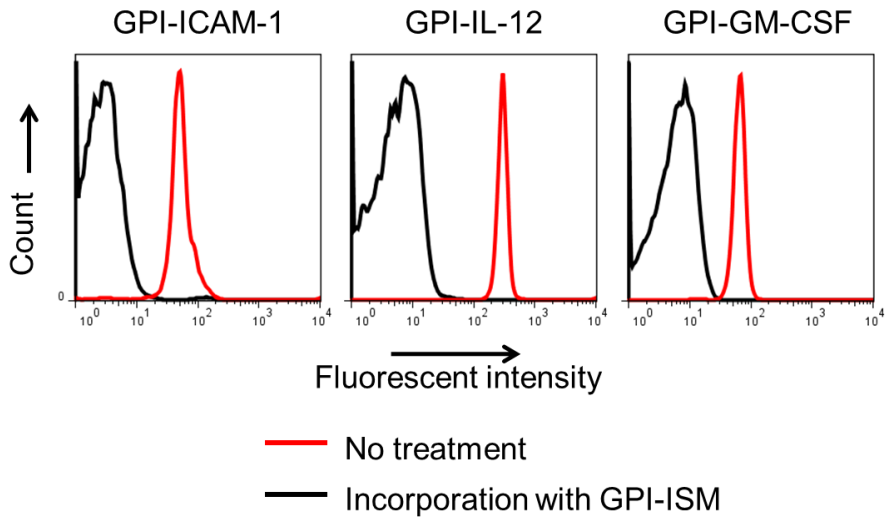

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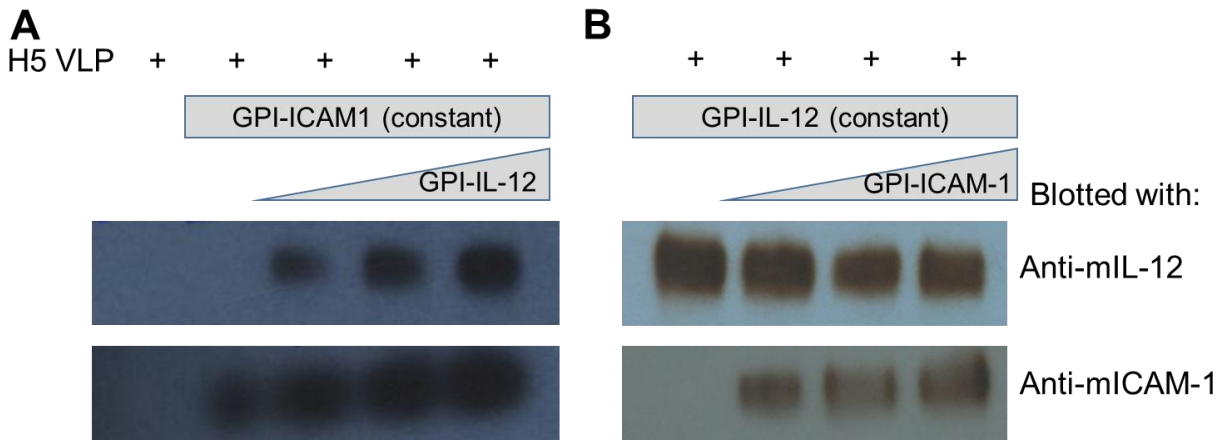

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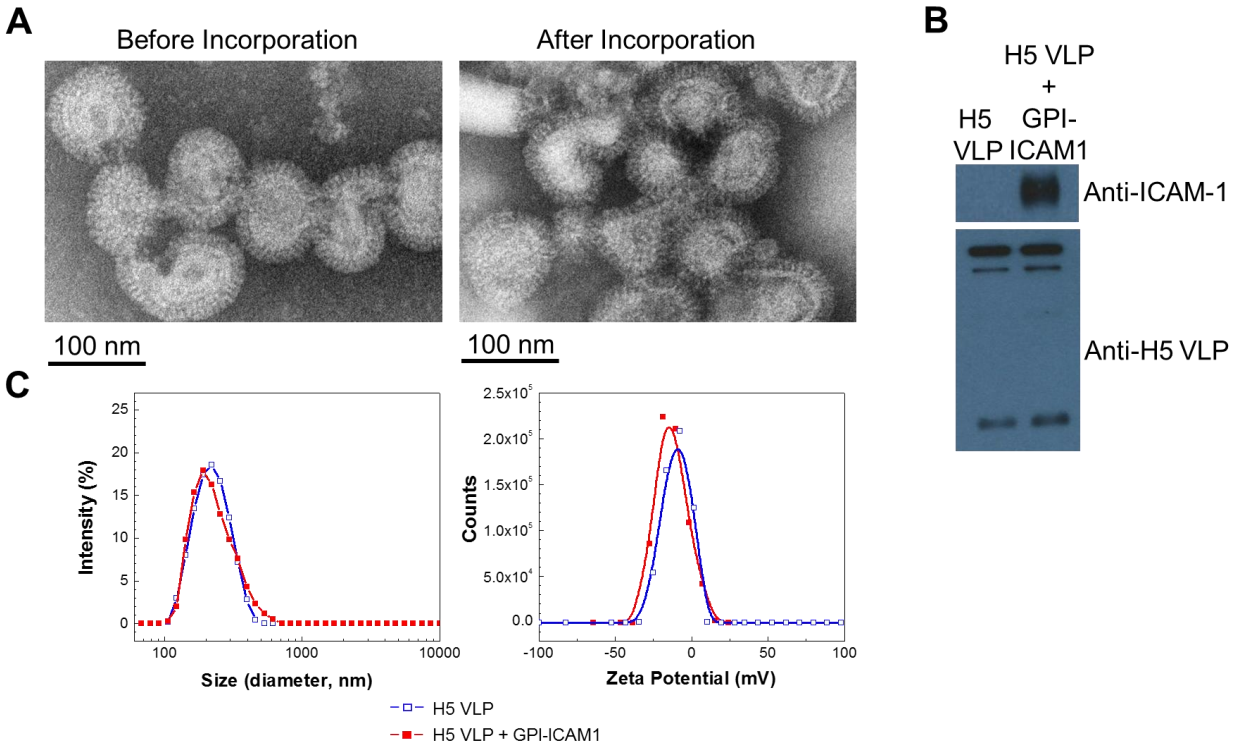

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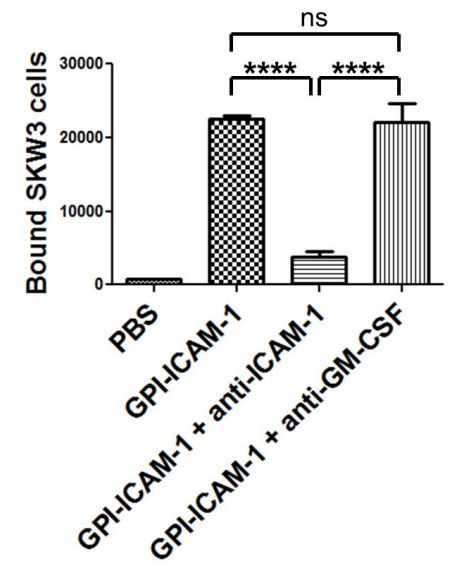

Supplemental Figure 6