SUPPORTING MATERIAL

Cholesterol-enriched microdomain formation induced by viral-encoded, membrane active amphipathic peptide

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SUPPORTING FIGURES & LEGENDS

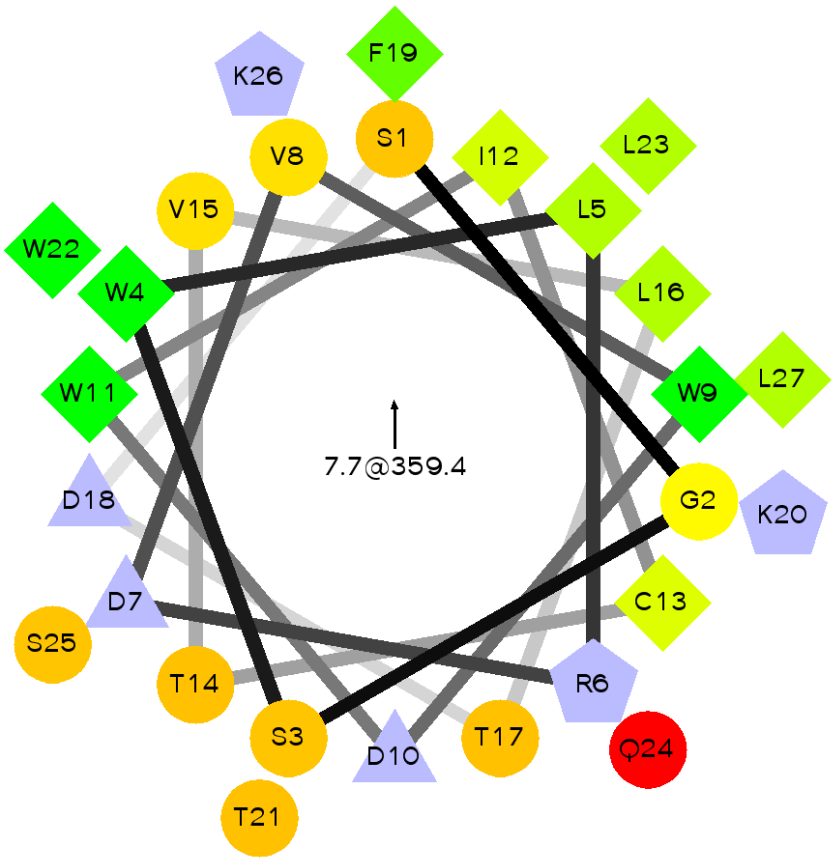

Fig. S1. Helical Wheel Projection of AH Peptide. AH Peptide sequence of amino acids: SGSWLRDVWDWICTVLTDFKTWLQSKL. A helical wheel projection showing hydrophilic residues (circles), hydrophobic residues (diamonds), potentially negatively charged residues (triangles), and potentially positively charged residues (pentagons). Hydrophobicity is color coded: most hydrophobic residue (green), with amount of green decreasing proportionally to the hydrophobicity; zero hydrophobicity (yellow); hydrophilic residues (red); and potentially charged residues (light blue). Projection was produced using a program from http://rzlab.ucr.edu created by Don Armstrong and Raphael Zidovetzki. Version: Id: wheel.pl,v 1.4 2009-10-20 21:23:36 don Exp.

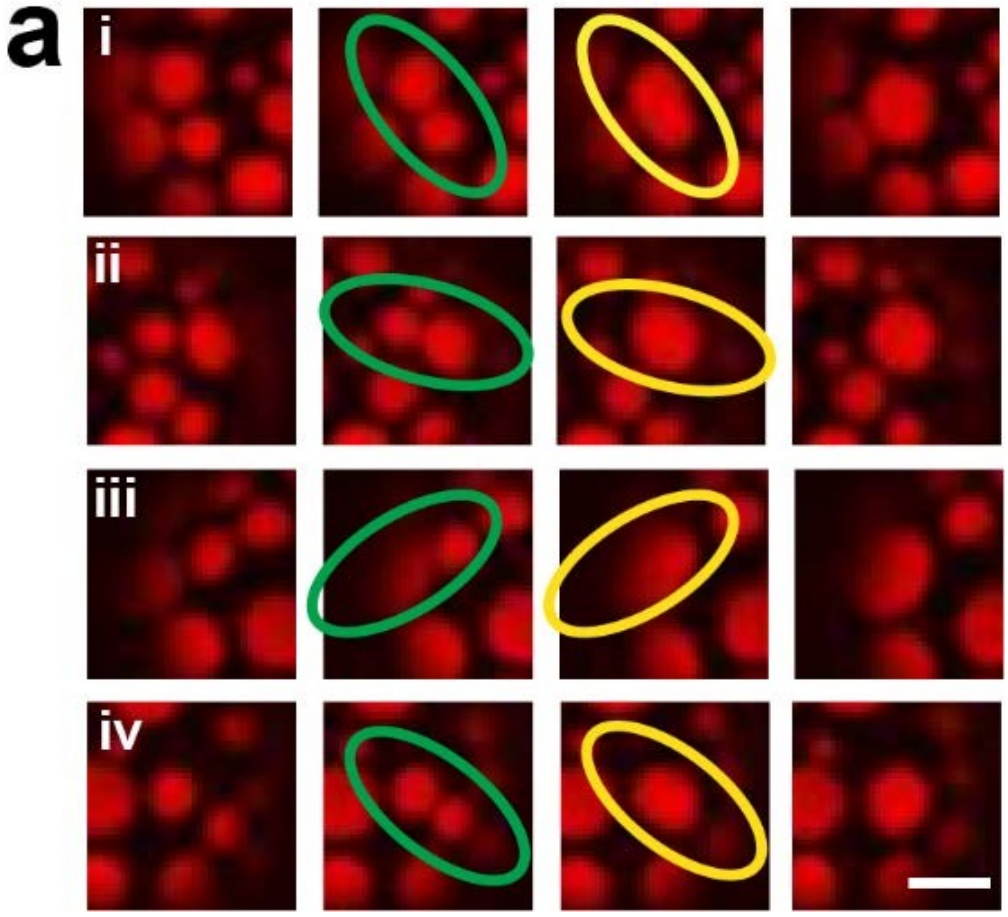

Fig. S2. Peptide-mediated domain coalescence. Selected frames from time-lapse fluorescence images of electroformed GUVs consisting of POPC/ Sphingomyelin/ Cholesterol/ Rho-B DOPE (33/33/33/1) upon incubation with 5 µM AH peptide (See main narrative for additional details). The sequences, i through iv above, illustrate domain growth through domain-domain coalescence at four different instances in a single experiment. Scale Bar, 5 µm.

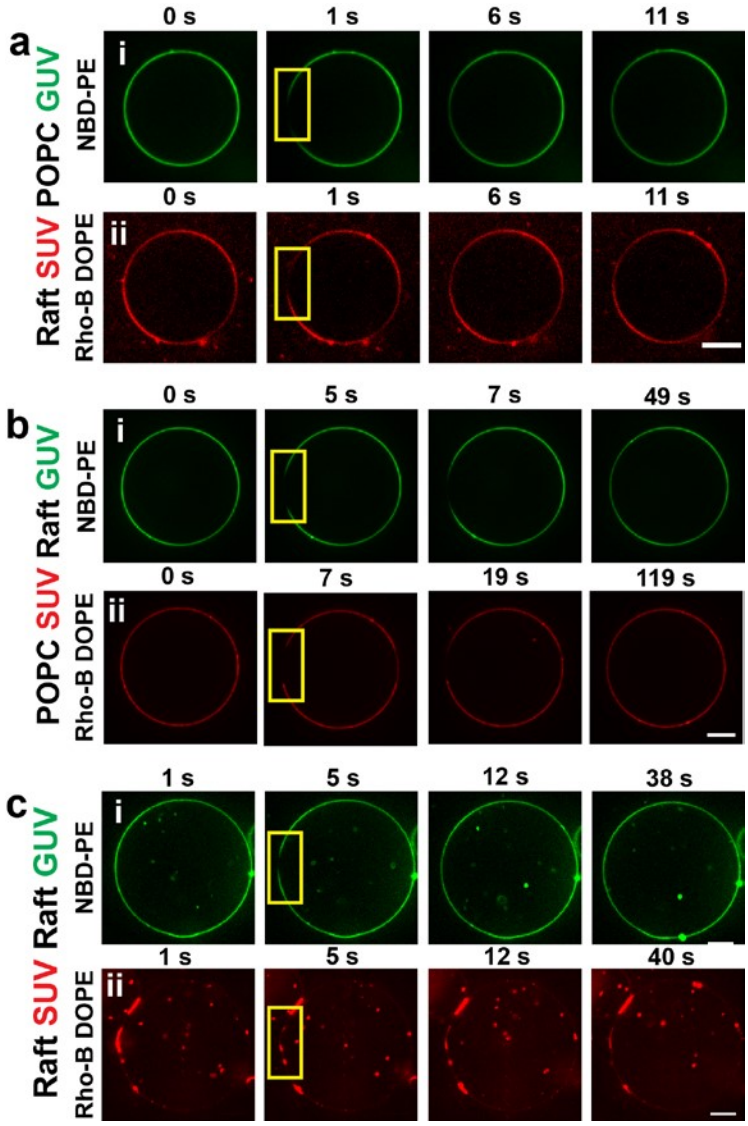

Fig. S3. Competition Assay FRAP Experiments. Selected fluorescence images of FRAP experiments using GUVs and SUVs consisting of either raft [POPC/Sphingomyelin/Cholesterol (33/33/33)] or POPC membranes (as indicated) incubated with 1 uM AH peptide. The remaining three permutations of vesicle size, GUV or SUV, and composition, Raft or POPC, are tested in addition to previously shown GUV/SUV POPC/POPC (see **Fig. 5**). **a,** GUV/SUV POPC/raft (i) bleach NBD-PE, images taken at 0 s, 1 s, 6 s, 11 s (ii) bleach Rho-B DOPE, images taken at 0 s, 1 s, 6 s, 11 s. **b,** GUV/SUV raft/POPC (i) bleach NBD-PE, images taken at 0 s, 5 s, 7s, 49 s (ii) bleach Rho-B DOPE, images taken at 0 s, 7 s, 19 s, 119 s. **c,** GUV/SUV raft/raft (i) bleach NBD-PE with images taken at 1 s, 5 s, 12 s, 38 s (ii) bleach Rho-B DOPE with images taken at 1 s, 5 s,12 s, 40 s. Yellow boxes highlight bleached regions. Scale Bars, 10 µm.

SUPPORTING MOVIE LEGENDS

Movie S1. A representative example (1 of 2) of time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of **POPC/Sphingomyelin/Cholesterol/Rhodamine-B DOPE (33/33/33/1)** containing 300 mM sucrose inside and 300 mM glucose outside duing incubation with **5 μM AH Peptide**. Scale bar = 30 μM.

Movie S2. A representative example (2 of 2) of time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of **POPC/Sphingomyelin/Cholesterol/Rhodamine-B DOPE (33/33/33/1)** containing 300 mM sucrose inside and 300 mM glucose outside duing incubation with **5 μM AH Peptide**. Scale bar = 30 μM.

Movie S3. A time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of **POPC/Sphingomyelin/Cholesterol/DP-EG10-Biotin/Rhodamine-B DOPE (32/32/32/3/1)** containing 300 mM sucrose in the vesicle interior and a mixture of 300 mM glucose (92%) and 1X PBS pH 7.2 (8%) in the exterior bath. After incubation with **2 μM AH** peptide, vesicles were then incubated with **1 μM Oregon Green 488 Neutravidin**, which binds and aids in the visualization of the DP-EG10-Biotin. Scale bar = 20 μM.

Movie S4. A representative time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of Electroformed GUVs consisting of **POPC/Sphingomyelin/Cholesterol/NBD-PE (33/33/33/1)** containing 300 mM sucrose inside and 300 mM glucose outside. Vesicles were incubated with a mixture of 1.5 µM **5-TAMRA-labeled AH peptide** and 1.5 µM AH peptide (1.5 + 1.5 = **3 µM** total). Scale bar = 30 µM.

Movie S5. A typical example (1 of 2) of time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** containing 300 mM sucrose inside and 300 mM glucose outside incubated with **14 µM AH peptide**. Scale bar = 20 µM.

Movie S6. A typical example (2 of 2) of time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** containing 300 mM sucrose inside and 300 mM glucose outside incubated with **14 µM AH peptide**. Scale bar = 20 µM.

Movie S7. A time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** encapsulating 300 mM sucrose inside and 300 mM glucose outside incubated **with 25 µM 5-TAMRA-AH** peptide. Scale bar = 20 µM.

Movie S8. A time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of

POPC/Rhodamine-B DOPE (99/1) containing 300 mM sucrose inside and 300 mM glucose outside incubated with **35 µM 5-TAMRA-AH** peptide. Scale bar = 20 µM.

Movie S9. A time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of **POPC/Sphingomyelin/Cholesterol/Rhodamine-B DOPE (33/33/33/1)** containing 300 mM sucrose inside and 300 mM glucose outside incubated with **4 µM AH peptide**. Vesicle exterior contains 40 µM **2-NBD-derivatized glucose** (2-NBDG) to monitor any membrane leakage. Scale bar = 30 µM.

Movie S10. A time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of electroformed GUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** with 300 mM sucrose inside and 300 mM glucose outside incubated with **10 µM AH peptide**. Vesicle exterior contains 28 µM **NBDderivatized glucose** (2-NBDG) to monitor any membrane leakage. Scale bar = 30 µM.

Movie S11. Competition assay between electroformed GUVs consisting of **POPC/NBD-PE (99/1)** with 300 mM sucrose inside and 100 nm extruded SUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** with 300 mM sucrose inside monitored using a time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy. This is a control experiment in which **no AH peptide**

is introduced. Both GUVs and SUVs were osmotically balanced outside with 300 mM glucose. Scale bar = 30 µM.

Movie S12. A time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of a competition assay between electroformed GUVs consisting of **POPC/NBD-PE (99/1)** with 300 mM sucrose inside and 100 nm extruded SUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** with 300 mM sucrose inside. Prior to incubation with **15 µM AH peptide**, GUVs and SUVs were osmotically balanced outside with 300 mM glucose. Scale bar = 30 µM.

Movie S13. A time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of a competition assay between electroformed GUVs consisting of **POPC/NBD-PE (99/1)** with 300 mM sucrose inside and 100 nm extruded SUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** with 300 mM sucrose inside. Prior to incubation with **500 nM AH peptide**, GUVs and SUVs were osmotically balanced outside with 300 mM glucose. Scale bar = 30 µM.

Movie S14 A time-lapse sequence of fluorescence images, acquired using spinning disk confocal fluorescence microscopy, of a fluorescence recovery after photobleaching (FRAP) experiment of an electroformed GUV consisting of **POPC/NBD-PE (98/2)** with 300 mM sucrose inside and 300 mM glucose outside. This is a control for Movies S15S17 **with no AH peptide and no SUVs added**. Scale bar = 30 µM.

Movie S15. Fluorescence recovery after photobleaching (FRAP) of electroformed GUVs consisting of POPC/NBD-PE (98/2) with 300 mM sucrose inside and 300 mM glucose outside. This is a control for Movies S16 & S17 with **POPC/Rhodamine-B DOPE (991)** 100 nm extruded SUVs in 300 mM sucrose added but with **no AH peptide**. Scale bar = 30 µM.

Movie S16. Fluorescence recovery after photobleaching (FRAP) of electroformed GUVs consisting of **POPC/NBD-PE (98/2)** with 300 mM sucrose inside. The GUV exterior contains 100 nm extruded SUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** with 300 mM sucrose inside. Prior to incubation with **2 µM AH peptide**, GUVs and SUVs are osmotically balanced outside with 300 mM glucose. (**NBD CHANNEL**) Scale bar = 30 µM.

Movie S17. Fluorescence recovery after photobleaching (FRAP) of electroformed GUVs consisting of **POPC/NBD-PE (98/2)** with 300 mM sucrose inside. The GUV exterior contains 100 nm extruded SUVs consisting of **POPC/Rhodamine-B DOPE (99/1)** with 300 mM sucrose inside. Prior to incubation with **2 µM AH peptide**, GUVs and SUVs are osmotically balanced outside with 300 mM glucose. (**RHODAMINE CHANNEL**) Scale bar = 30 µM.