Supplementary Materials for

MiR-146a encapsulated liposomes reduce vascular inflammatory responses through decrease of ICAM-1 expression, macrophage activation, and foam cell formation

Donald Ho,†[1] Tyler Lynd,†[2] Claire Jun,[3] Juhee Shin,[4] Reid Millican,[5] Benjamin K. Estep,[2] Jun Chen,[2] Xixi Zhang,[2] Brigitta Brott,[6] Dong-Woon Kim,[4] Jennifer Sherwood,[#5] and Patrick Hwang[#5]

[1] Department of Pediatric Dentistry, University of Alabama at Birmingham, AL, 35294, USA

[2] Department of Biomedical Engineering, University of Alabama at Birmingham, AL, 35294, USA

[3] School of Arts and Sciences, University of Pennsylvania, PA, 19104, USA

[4] Department of Anatomy and Cell Biology, Brain Research Institute, Chungnam National University College of Medicine, Daejeon, 35015, Republic of Korea

[5] Endomimetics, LLC, Birmingham, AL, 35242, USA E-mail: patrick@endomimetics.com, jasherwood@endomimetics.com.

[6] Department of Medicine and Division of Cardiovascular Disease, University of Alabama at Birmingham, AL, 35233, USA

Correspondence: patrick@endomimetics.com, jasherwood@endomimetics.com

This file includes:

Figure S1.1 Freeze-thaw effect on liposome without sucrose.

Figure S1.2 Long term analysis of liposome size distribution.

Figure S1.3 Size distribution analysis of miR-146a encapsulated liposomes following freeze thaw.

Figure S2. Analytic gating strategy of flow cytometry for miR-146a transfected cells.

Figure S3. Analysis of TRAF6 and IRAK1 production in LPS treated- and LPS and free miR-146a treated differentiated U937 cells.

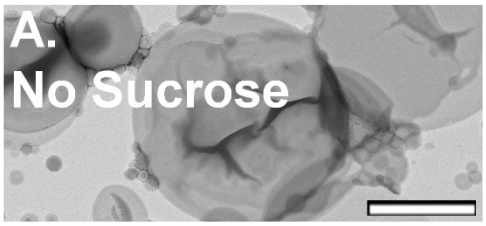
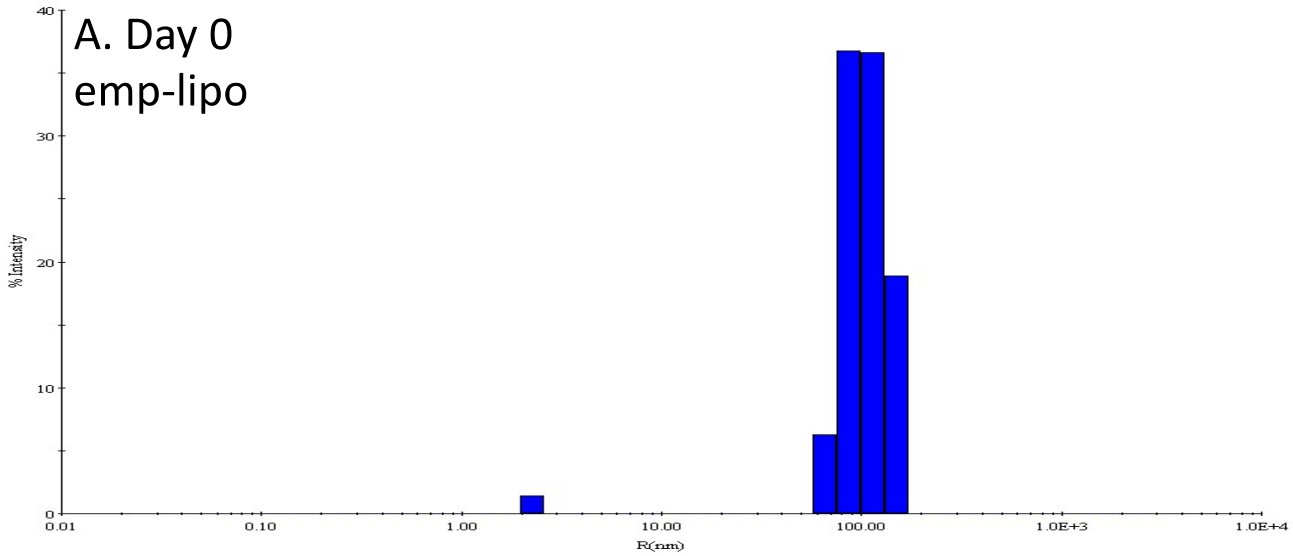
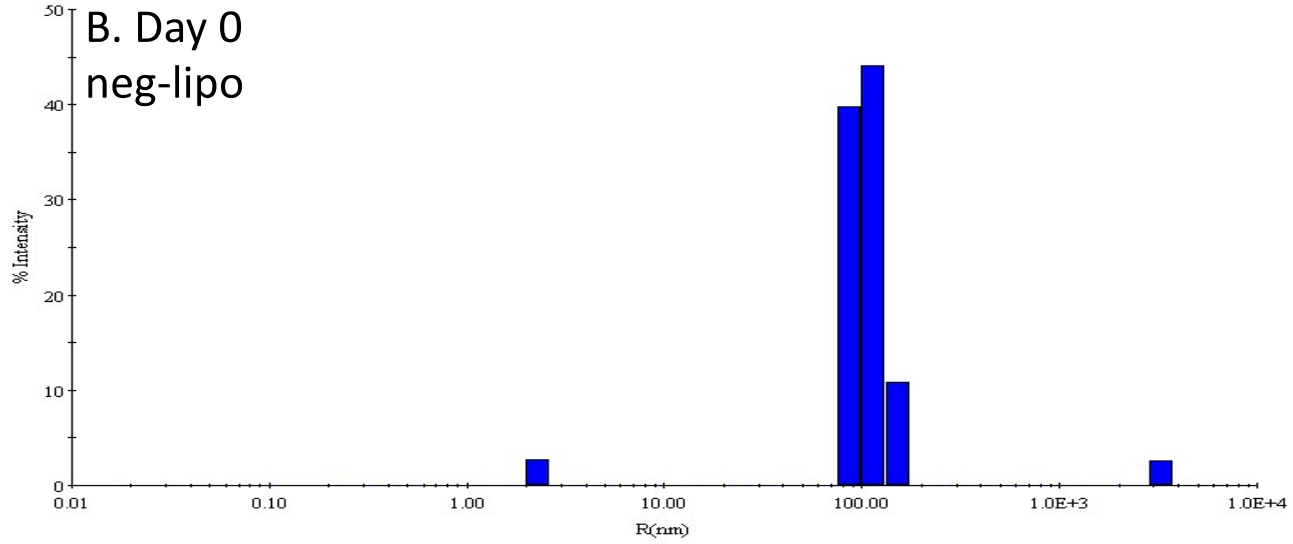
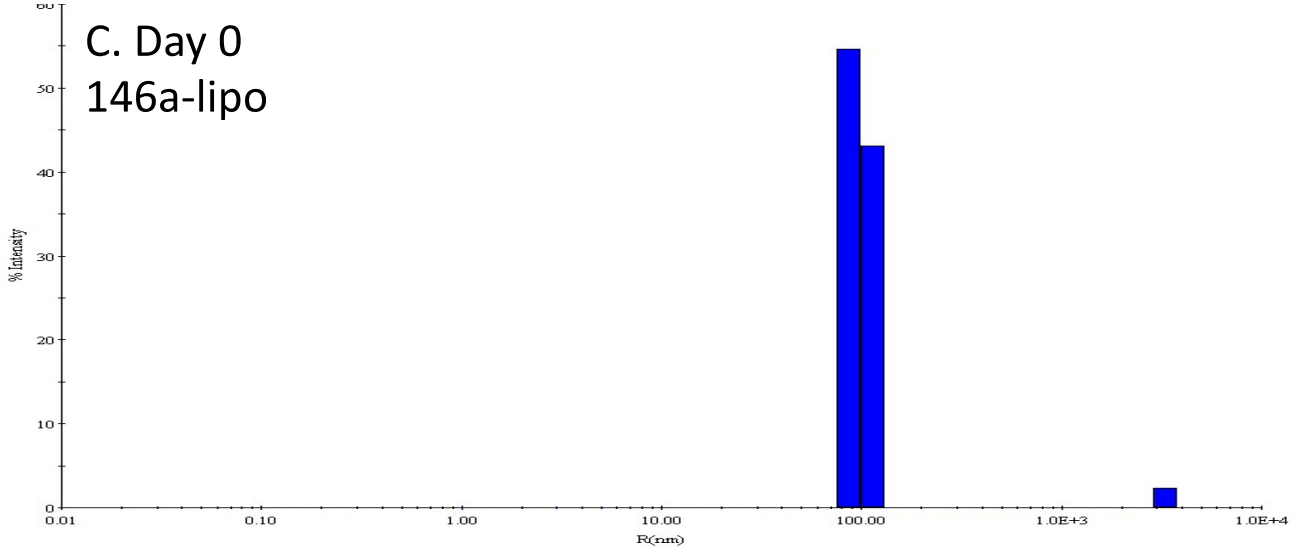
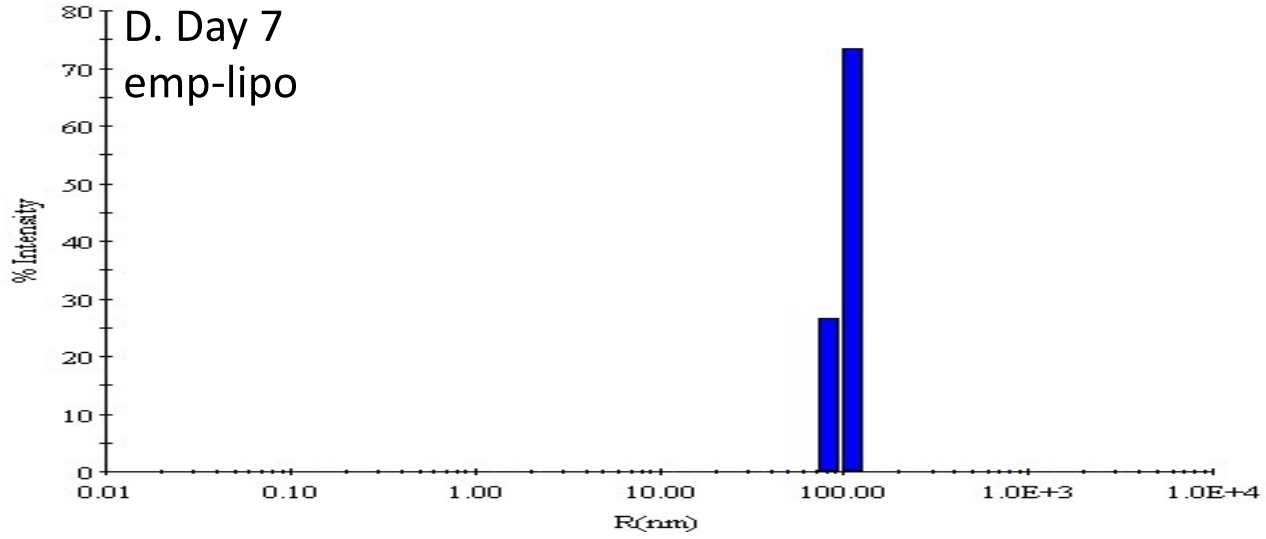


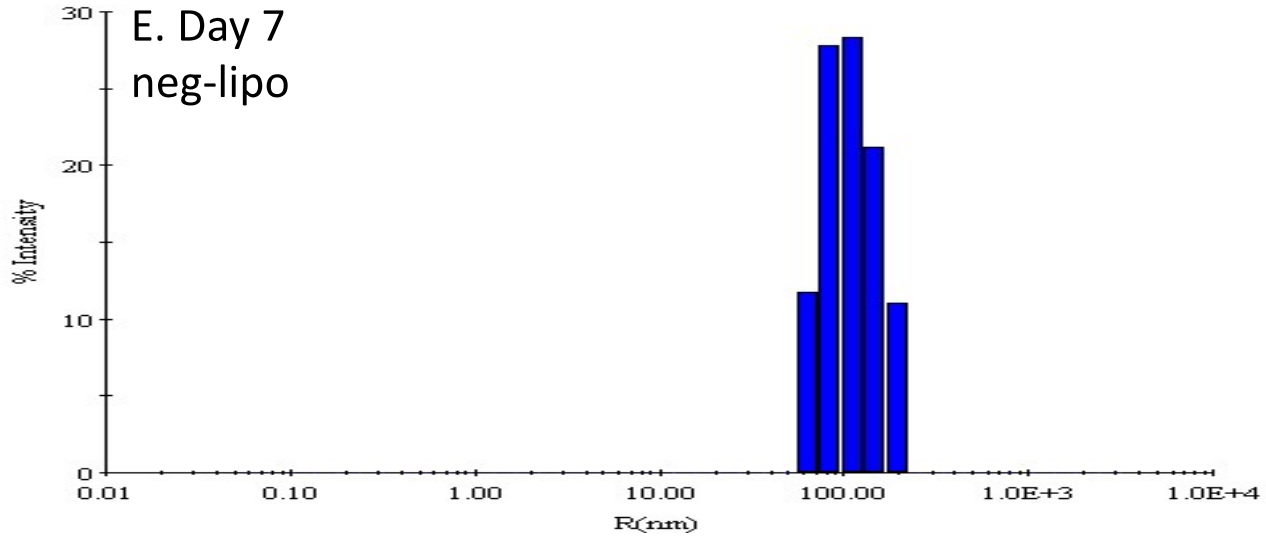
Figure S1.1. Freeze-thaw effect on liposome without sucrose A.) Representative TEM image of liposome frozen at -80 °C without sucrose after freeze thaw. Scale bar is 1 µm.

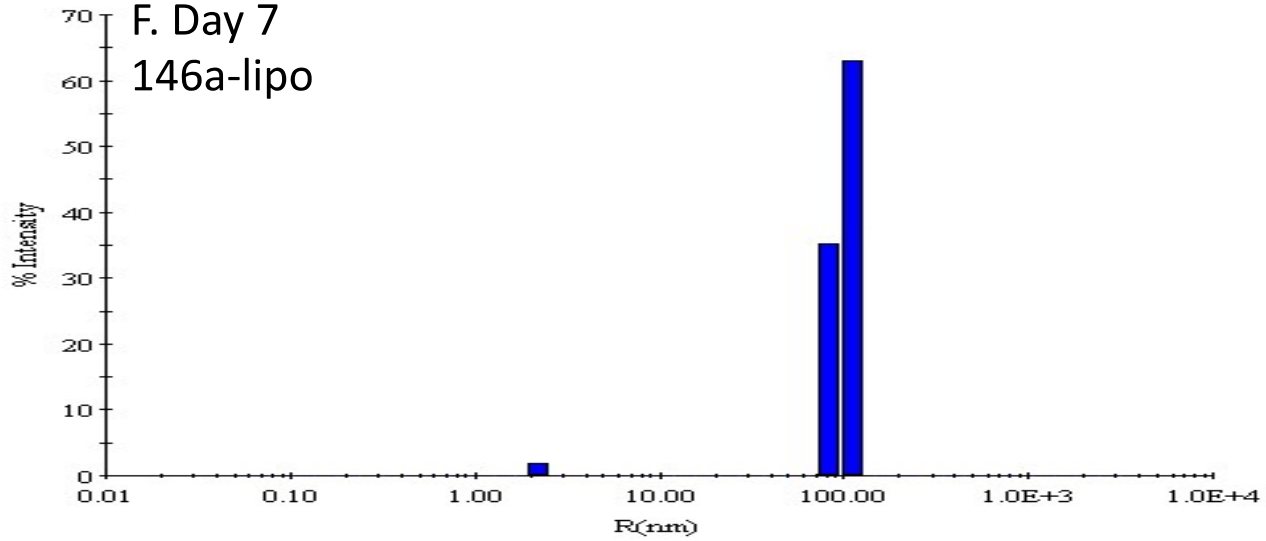

A. Day 0
emp-lipo
% Intensity
R(nm)

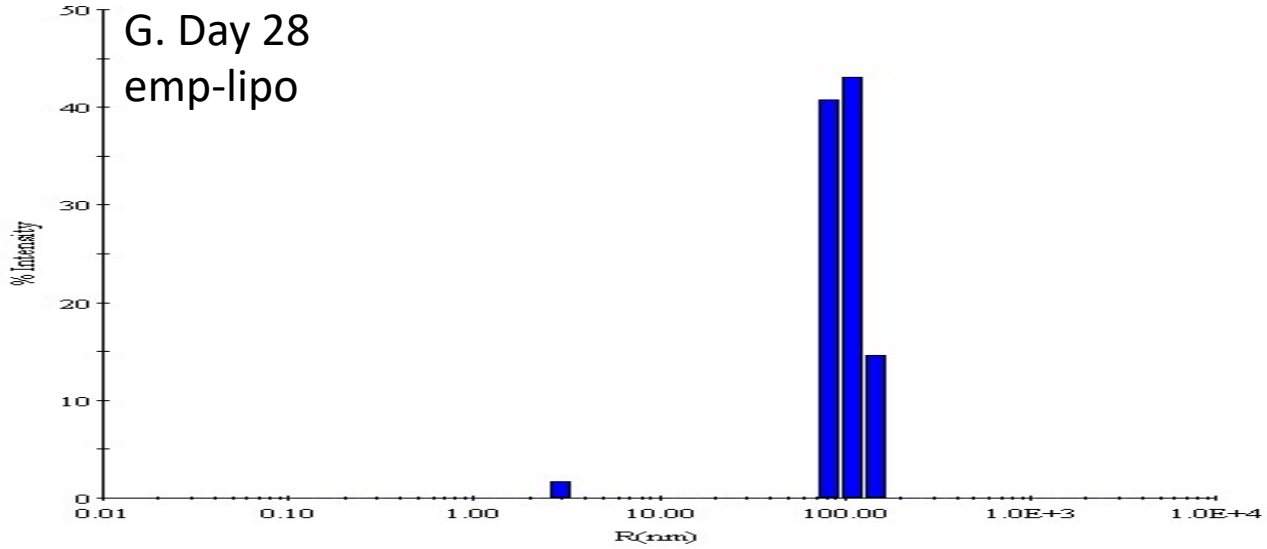

B. Day 0
neg-lipo
% Intensity
R(nm)

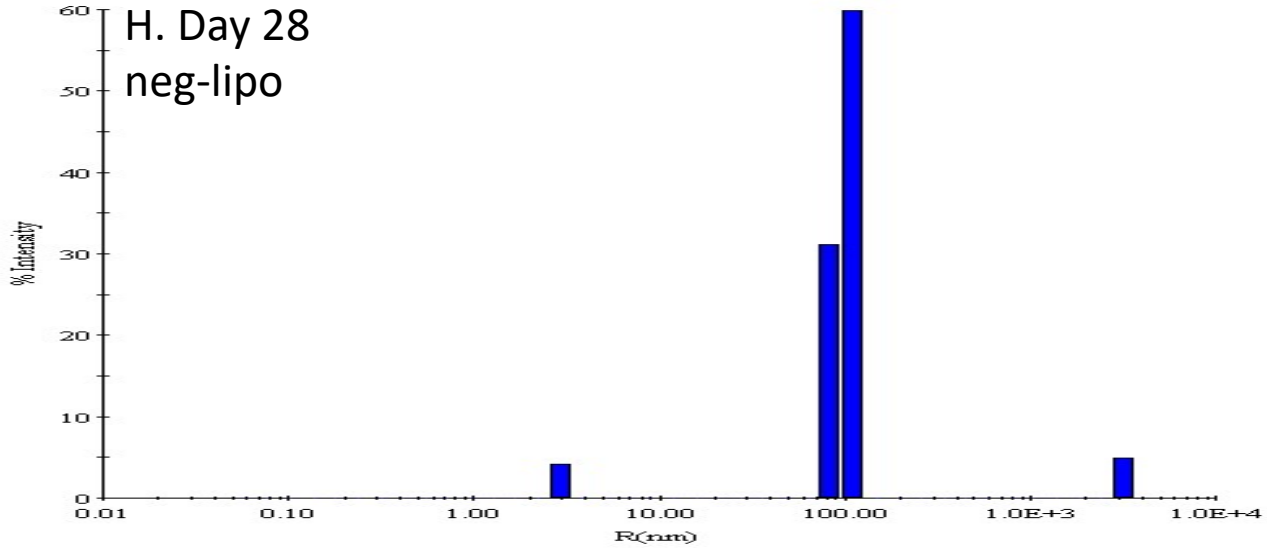

C. Day 0
146a-lipo
% Intensity
R(nm)

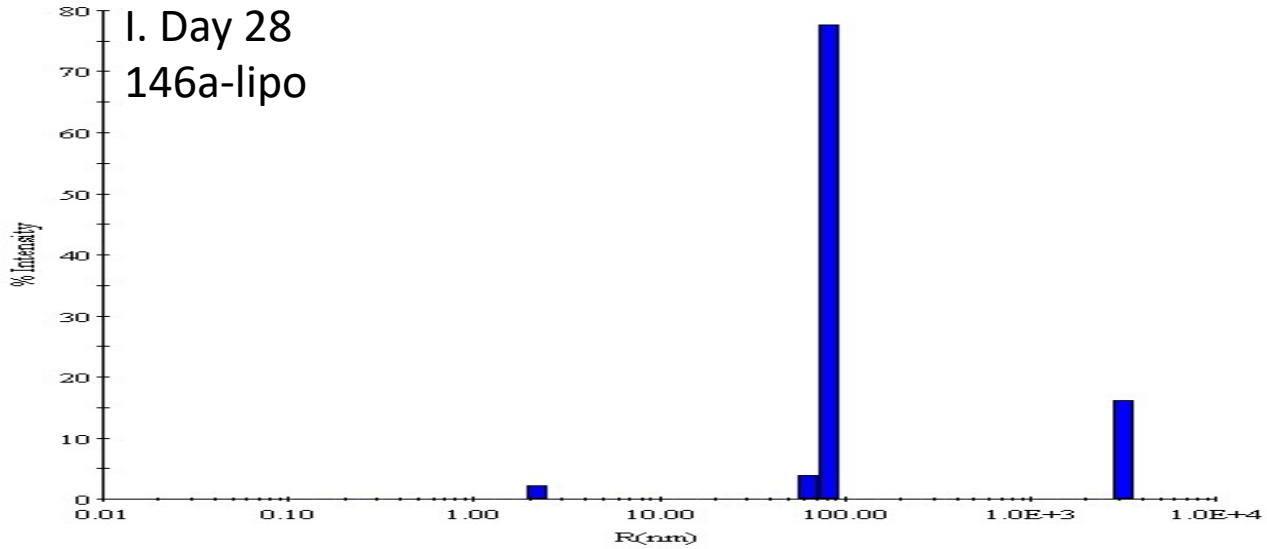

D. Day 7
emp-lipo
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


E. Day 7
neg-lipo
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


F. Day 7
146a-lipo
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


G. Day 28
emp-lipo
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


H. Day 28
neg-lipo
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


I. Day 28
146a-lipo
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4

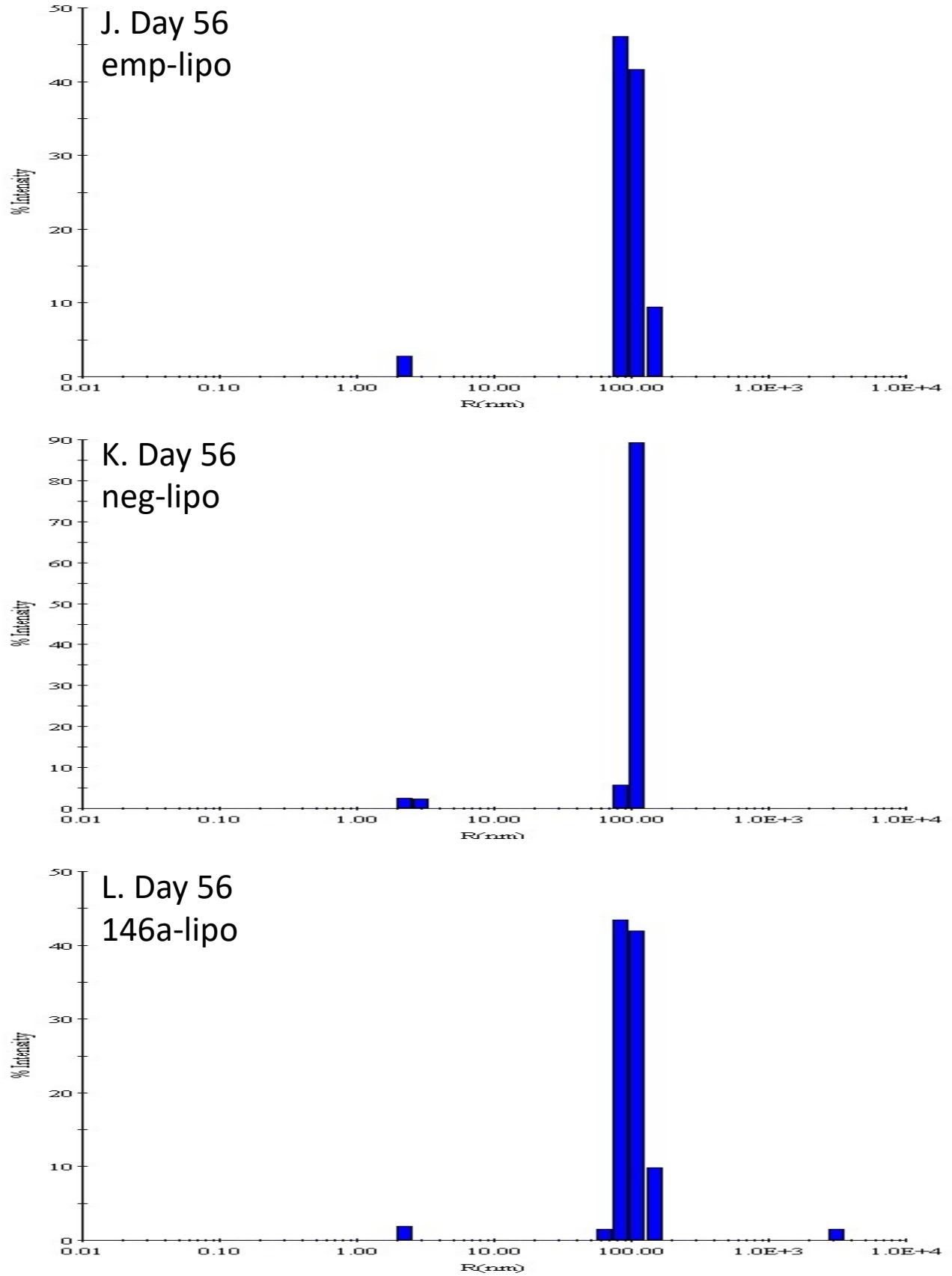
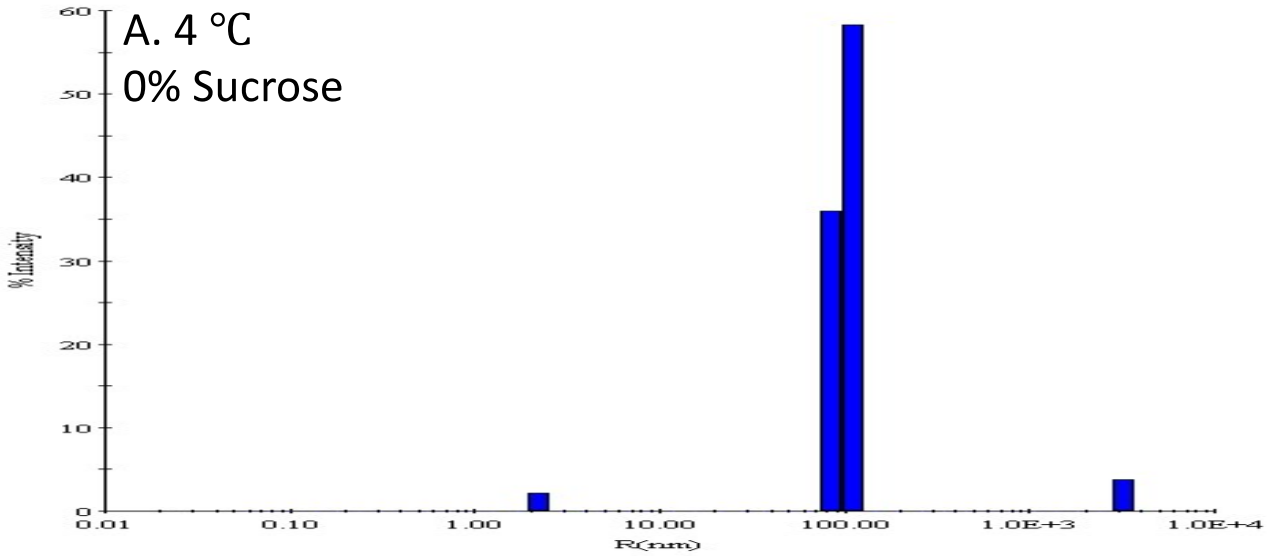
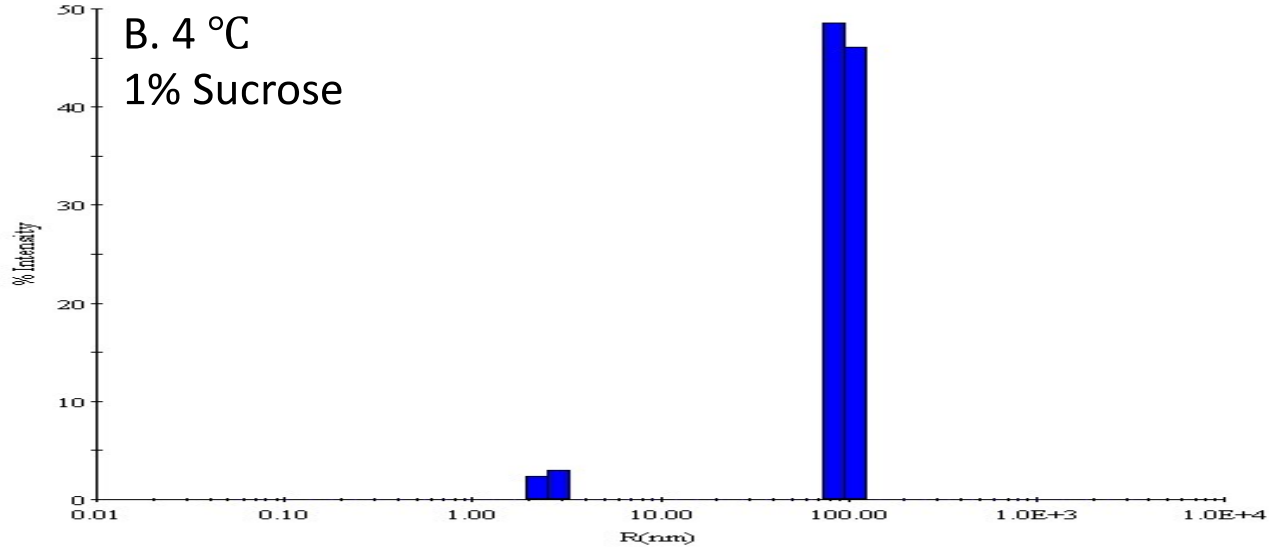
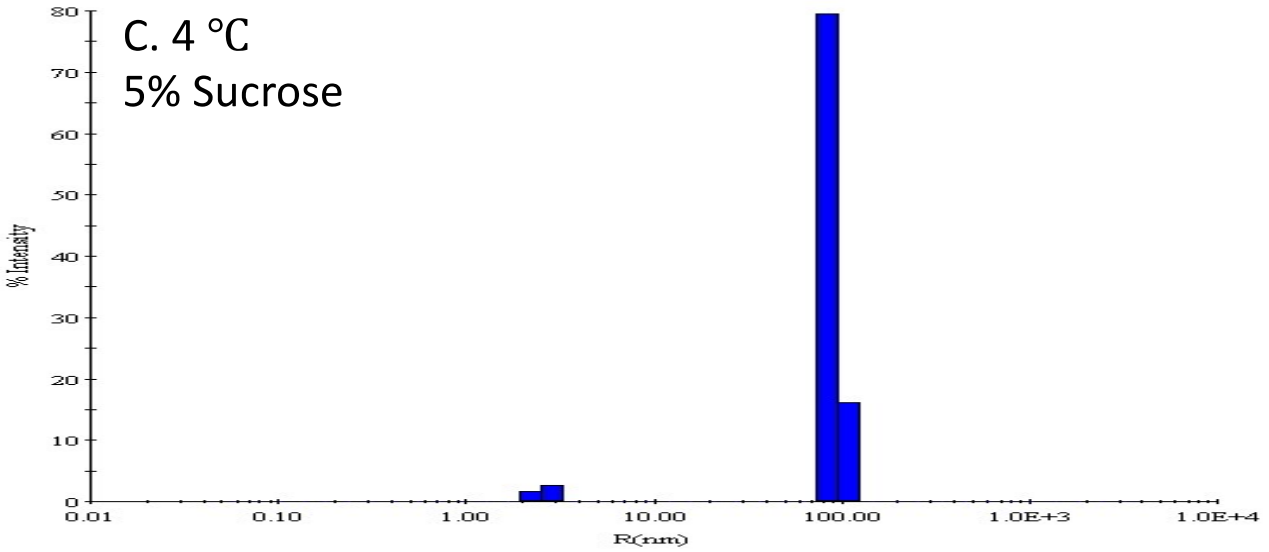
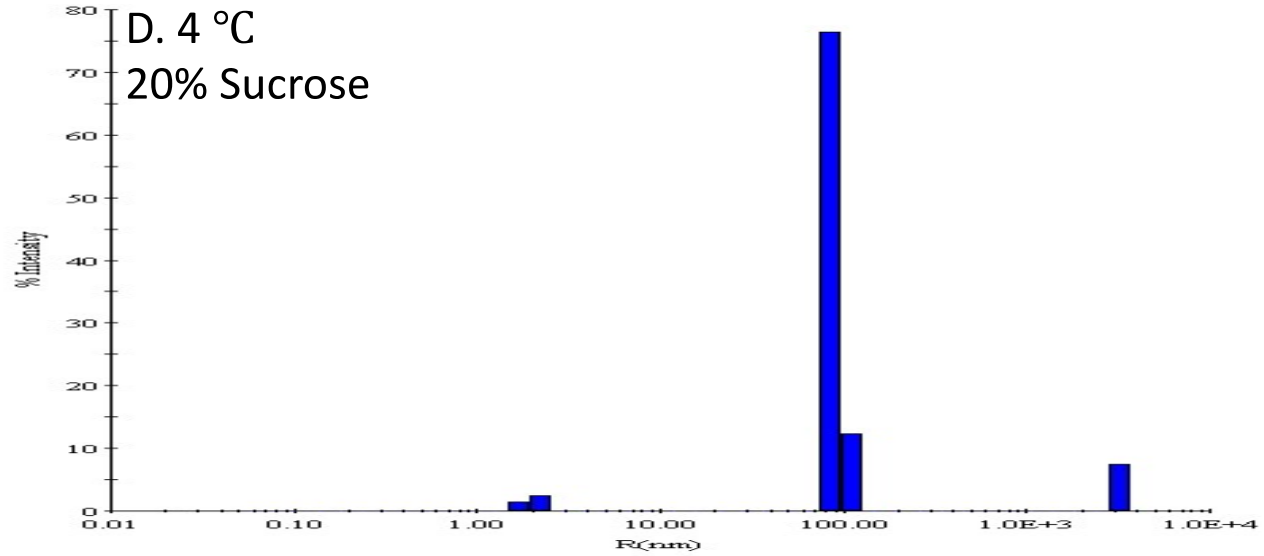


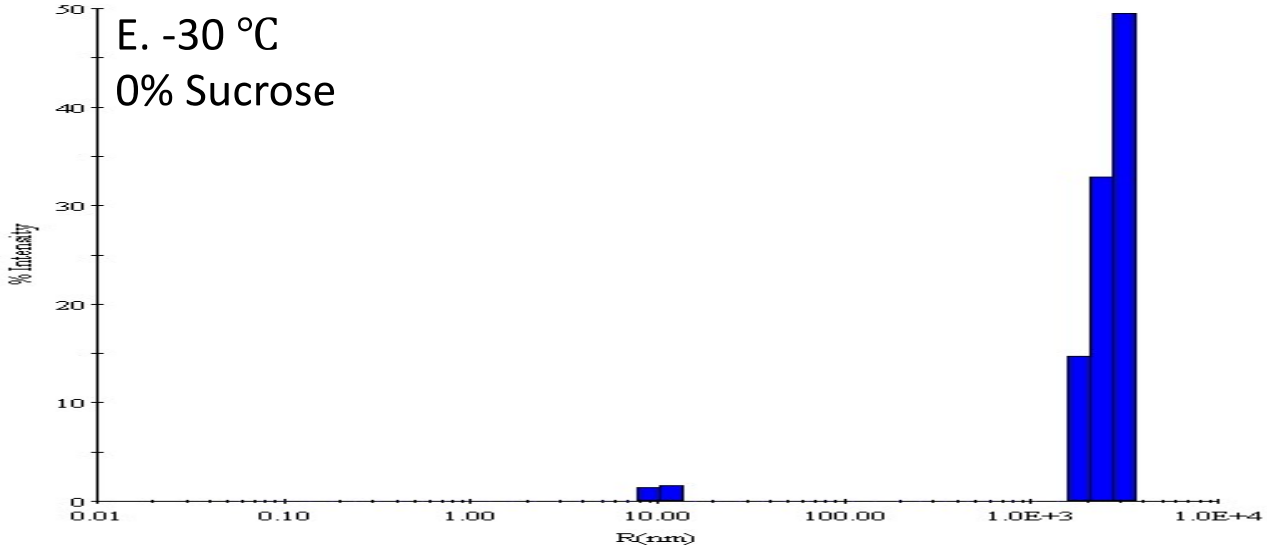
Figure S1.2. Long term analysis of liposome size distribution. Over the course of 56 days, the size distribution of emp-lipo, neg-lipo, and 146a-lipo was analyzed using DLS. **A.-C.**) Size distribution of respective liposomes at day 0. **D.-F.**) Size distribution of respective liposomes at day 7. **G.-I.)** Size distribution of respective liposomes at day 28. **J.-L.)** Size distribution of respective liposomes at day 56.

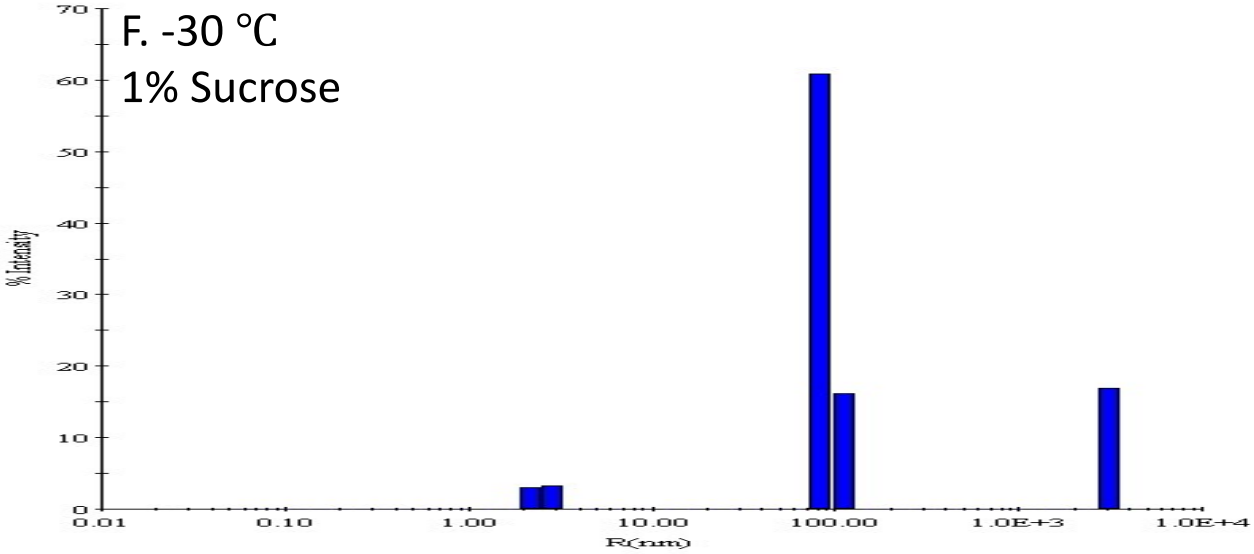

A. 4 °C
0% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4

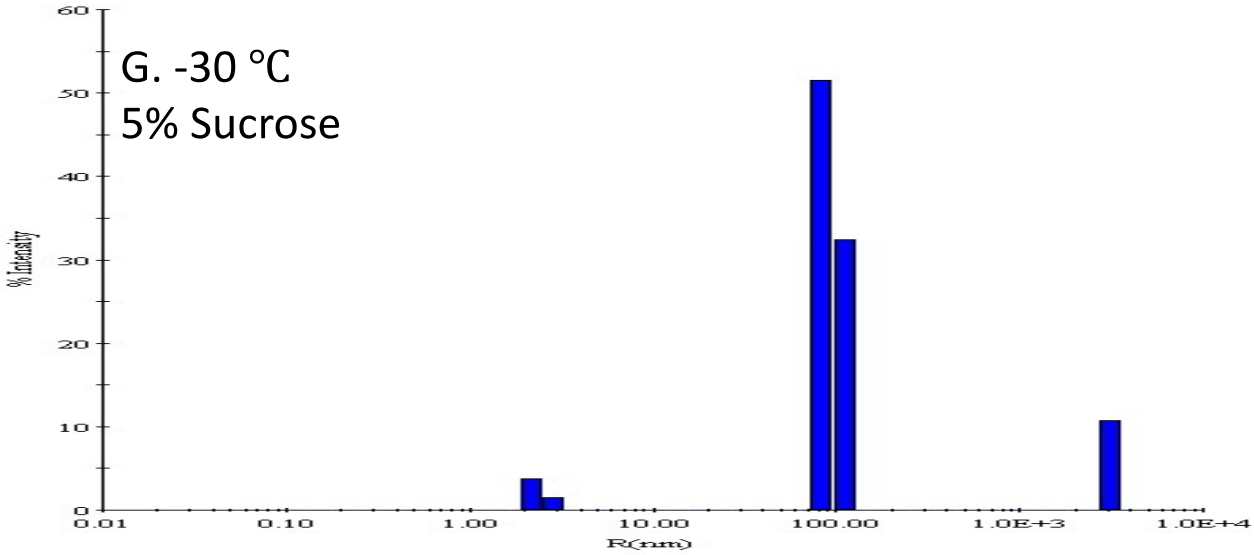

B. 4 °C
1% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4

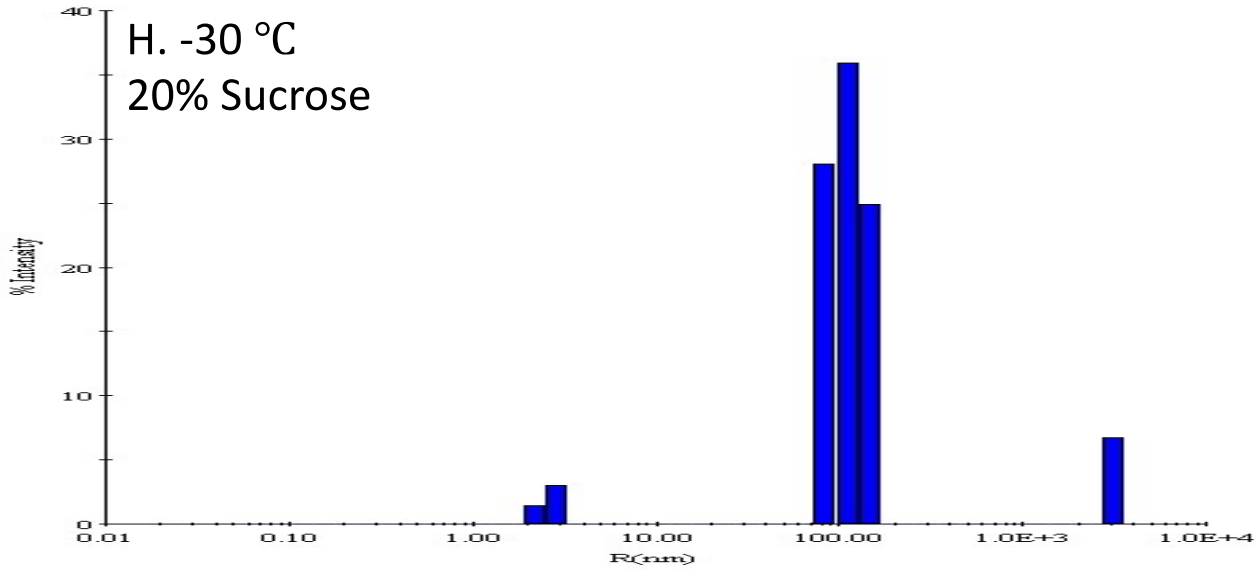

C. 4 °C
5% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4

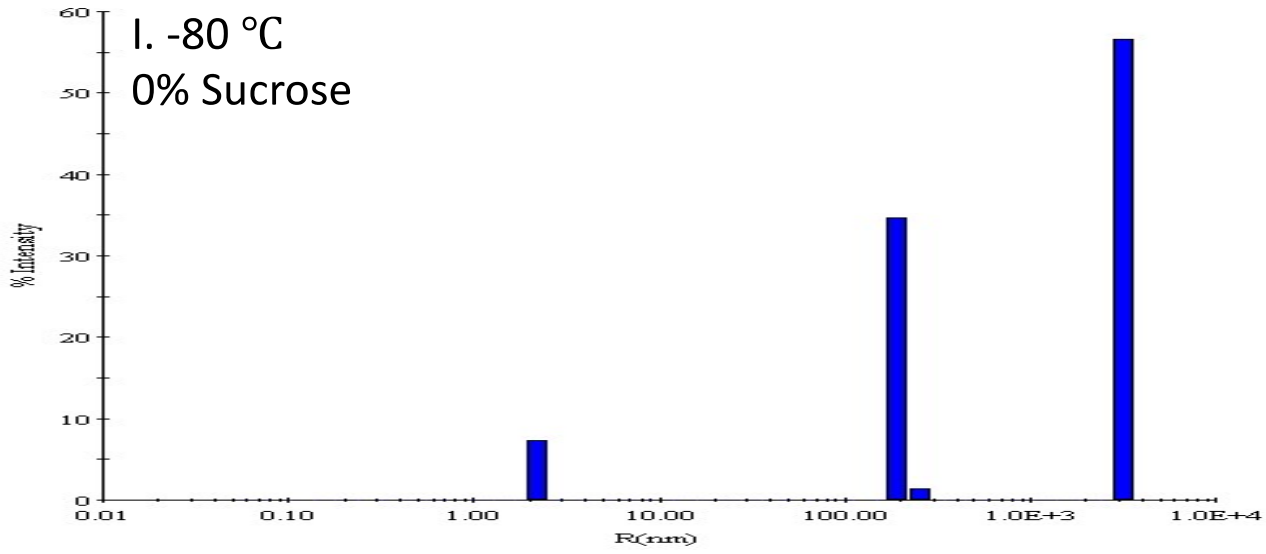

D. 4 ℃
20% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


E. -30 ℃
0% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


F. -30 ℃
1% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


G. -30 ℃
5% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


H. -30 ℃
20% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4


I. -80 ℃
0% Sucrose
% Intensity
R(nm)
0.01
0.10
1.00
10.00
100.00
1.0E+3
1.0E+4

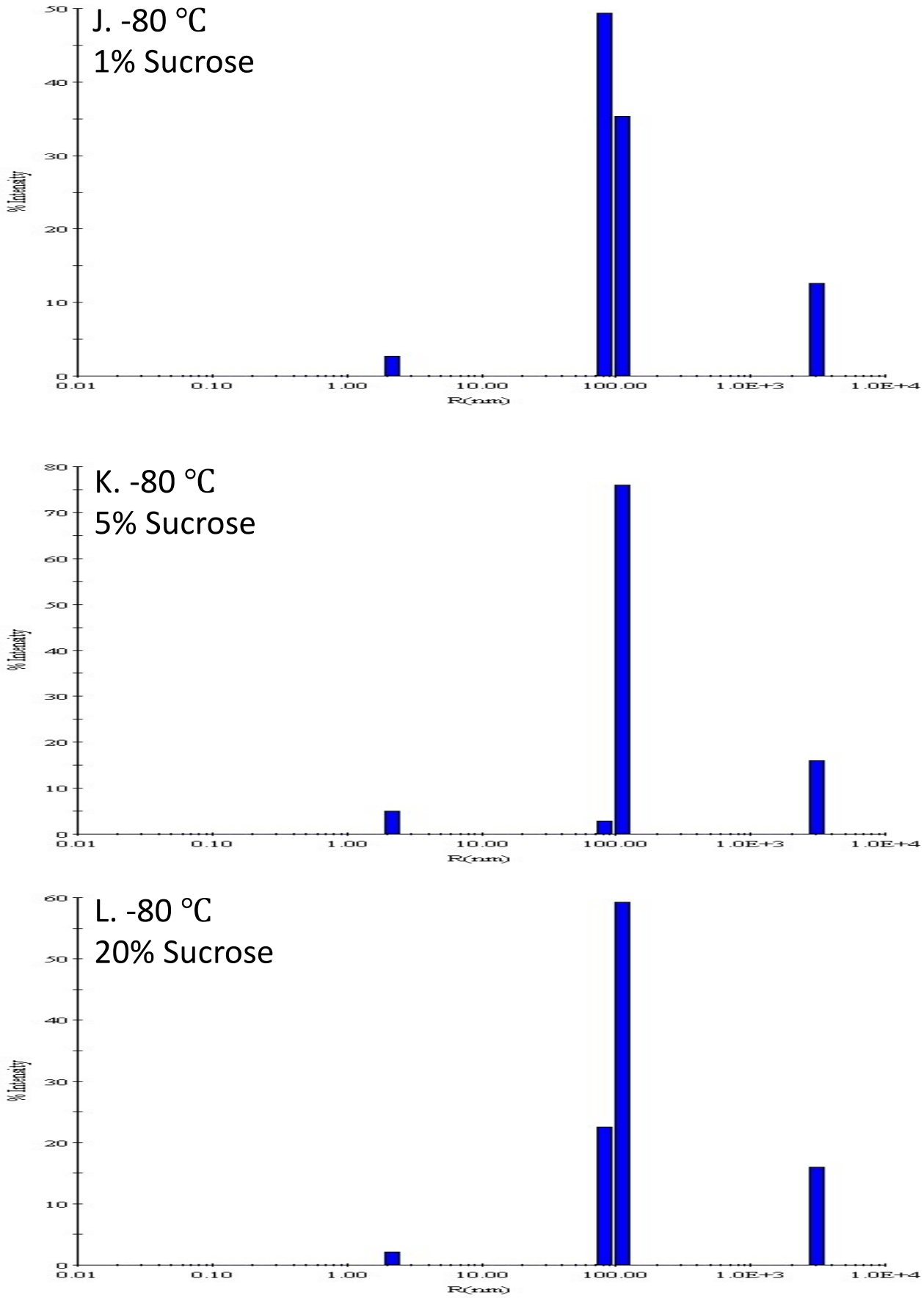


Figure S1.3. Size distribution analysis of miR-146a encapsulated liposomes following freeze thaw. **A.-D.)** Size distribution at 4 °C with 0, 1, 5, and 20% sucrose respectively. **E.-H.)** Size distribution at -30 °C with 0, 1, 5, and 20% sucrose respectively. **I.-L.)** Size distribution at -80 °C with 0, 1, 5, and 20% sucrose respectively.

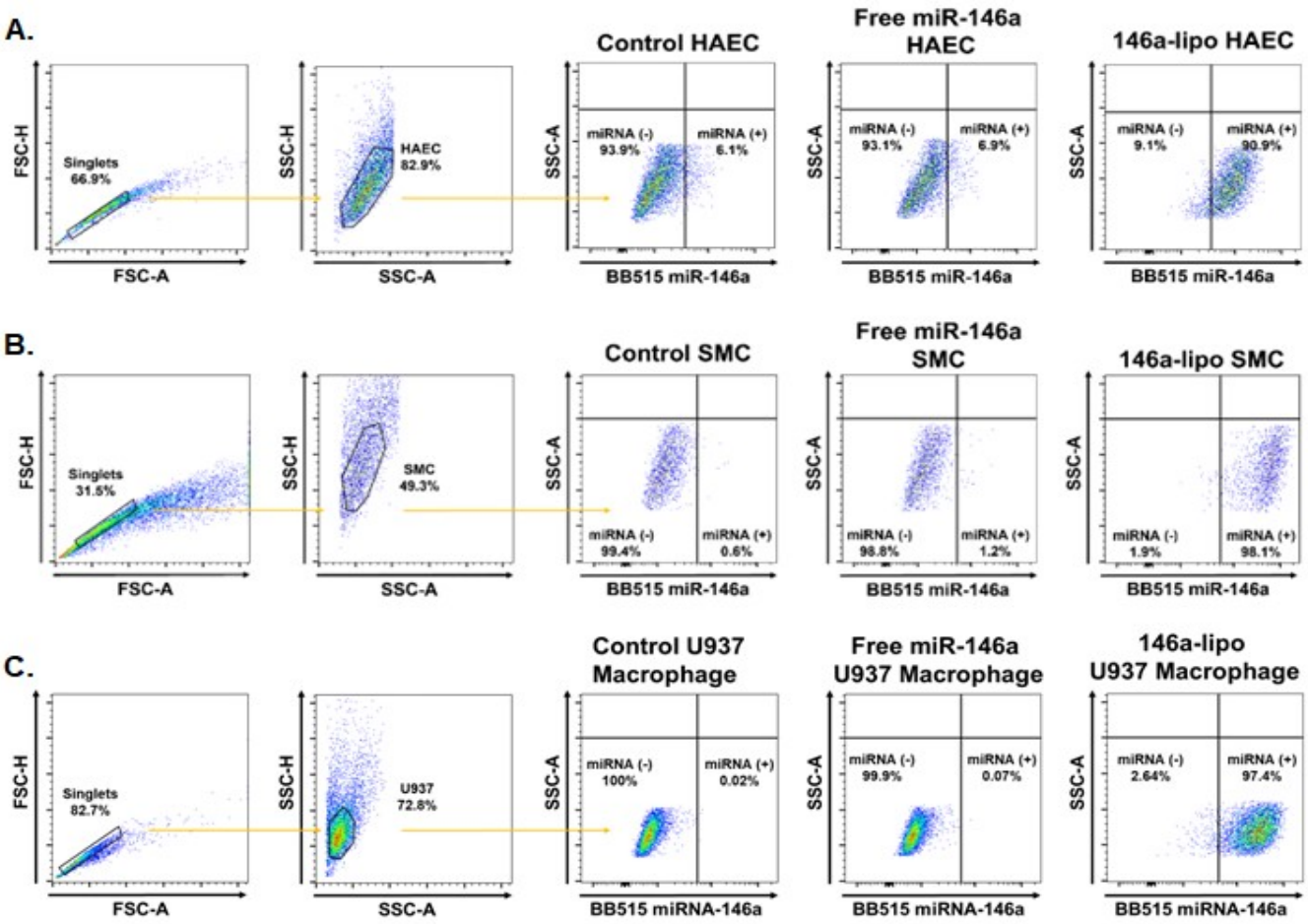


Figure S2. Analytic gating strategy of flow cytometry for miR-146a transfected cells. After transfection/treatment of free miR-146a and miR-146a encapsulated liposomes to **A.)** human aortic endothelial cells (HAECs), **B.)** human aortic smooth muscle cells (SMCs), and **C.)** PMA-differentiated U937 cells, and cells were detached with trypsin, washed twice with PBS, and fixed before running flow cytometry on a BD FACSymphony. Controls/cells without treatment were used as a negative control. Data was then analyzed and gated in FlowJo 10 as shown above. The frequency of the miR-146a+ transfected cells after doublet discrimination were then evaluated and are presented in Table 2. BB515 wavelengths: 490/515nm excitation/emission.

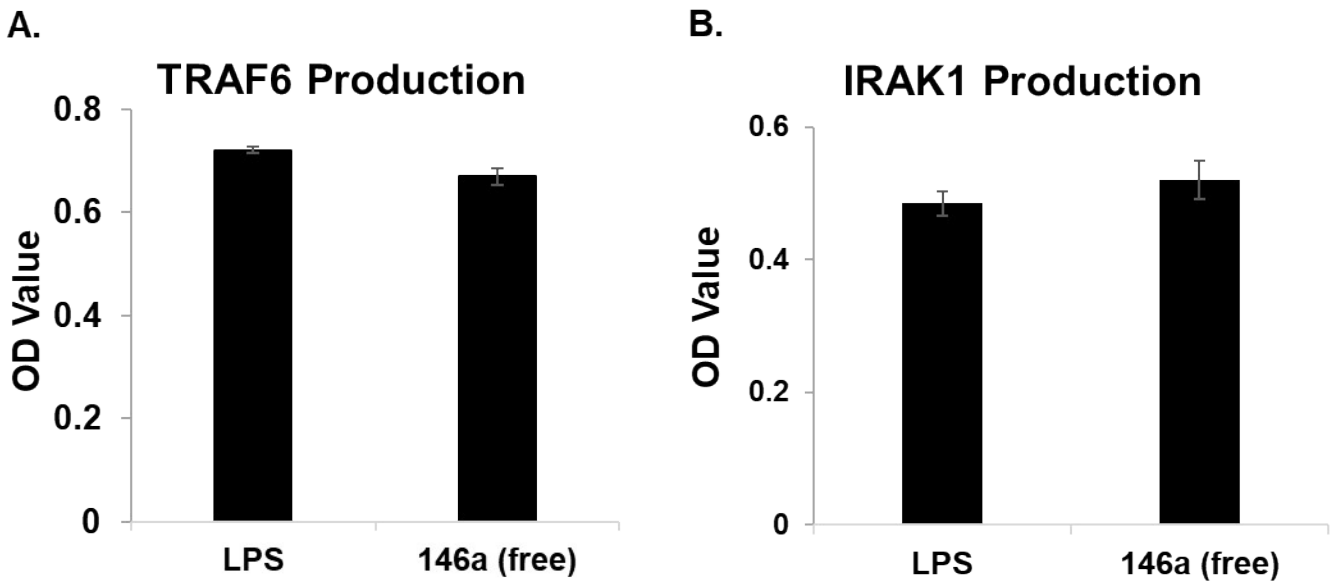


Figure S3. Analysis of TRAF6 and IRAK1 production in LPS treated- and LPS & free miR-146a treated differentiated U937 cells.