Flow cytometry
Platelet activation and α-granules secretions were assessed by FACS analysis using activated $\alpha_{IIb}\beta_3$ and P-selectin surface expression as described earlier.[1] The activated platelets were incubated with either PE-conjugated anti–P-selectin antibody or PE-conjugated anti-$\alpha_{IIb}\beta_3$ antibody (5μL/10^6 cells) for 15 minutes. To analyze glycoprotein VI (GPVI) and $\alpha_{IIb}\beta_3$ integrin expression, platelets were incubated with FITC-labeled anti-GPVI or anti-$\alpha_{IIb}\beta_3$ antibody for 15min. Platelets were defined by their forward- and side-scatter characteristics. Data for 10^4 platelets were acquired using a FACS Calibur instrument (Becton Dickinson, San Jose, CA) and analyzed using the manufacturer's CellQuest software program. To assess cellular free cholesterol platelets were fixed with 1% paraformaldehyde for 30min, incubated for 15 minutes with filipin (50 μg/mL in PBS) and analyzed immediately by flow cytometry.[2]

Western blotting
Gel-filtered platelets (1×10^8/mL) were lysed in a lysis buffer, equal amounts of protein from both WT and SR-BI$^{-/-}$ platelets were separated by SDS-PAGE on a 12% gel and transferred to PVDF membranes. Blots were blocked and probed with antibodies for PAR-3, PAR-4, P2Y1, P2Y12, or SR-BI. Equal loading was confirmed by reprobing the stripped membranes with the β-actin antibody.

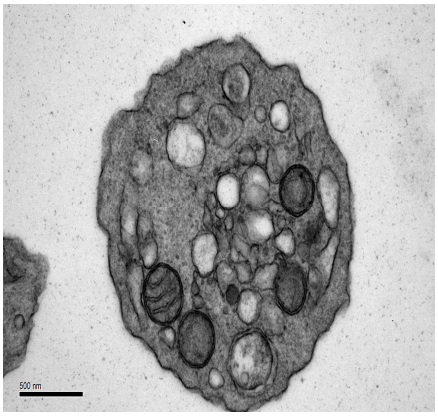
Electron microscopy
One volume of PRP from chimeric WT mice with WT bone marrow(BM) or SR-BI$^{-/-}$ BM were added to 5 volumes of 0.1% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 40 minutes. The samples were then centrifuged to get platelet film and the platelet film was fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 1–2 hours. The samples were then postfixed with 1% osmium tetroxide for 60 minutes at 4° C. After en bloc staining and dehydration with ethanol , the samples were embedded with eponate 12 medium (tell Pella Inc) .Ultra thin sections of 85nm were cut with diamond knife, stained with uranyl acetate and lead citrate, and then observed with a PhilpsCM12 electron microscope operated at 60 kv.

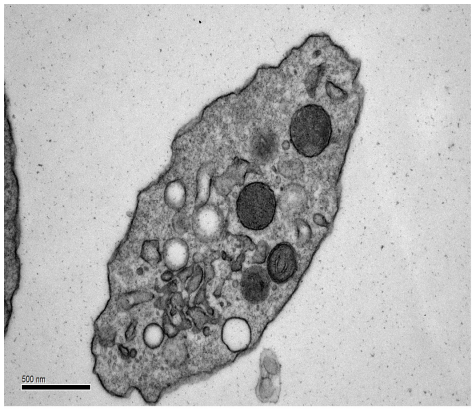
REFERENCES

1. Podrez EA, Byzova TV, Febbraio M, et al. Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. *Nat Med*. 2007;13:1086–1095.
2. Dole VS, Matuskova J, Vasile E, et al. Thrombocytopenia and platelet abnormalities in high-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol*. 2008;28:1111–1116.

Figure S1. Ultrastructure of platelets
(A,B) Platelets from chimeric WT mice with SR-BI$^{-/-}$ BM or WT BM were visualized using standard transmission electron microscope. Scale bar = 500nm. (A) platelets from WT (SR-BI$^{-/-}$ BM), (B) platelets from WT (WT BM).

A **B**





WT (KO BM) WT (WT BM)