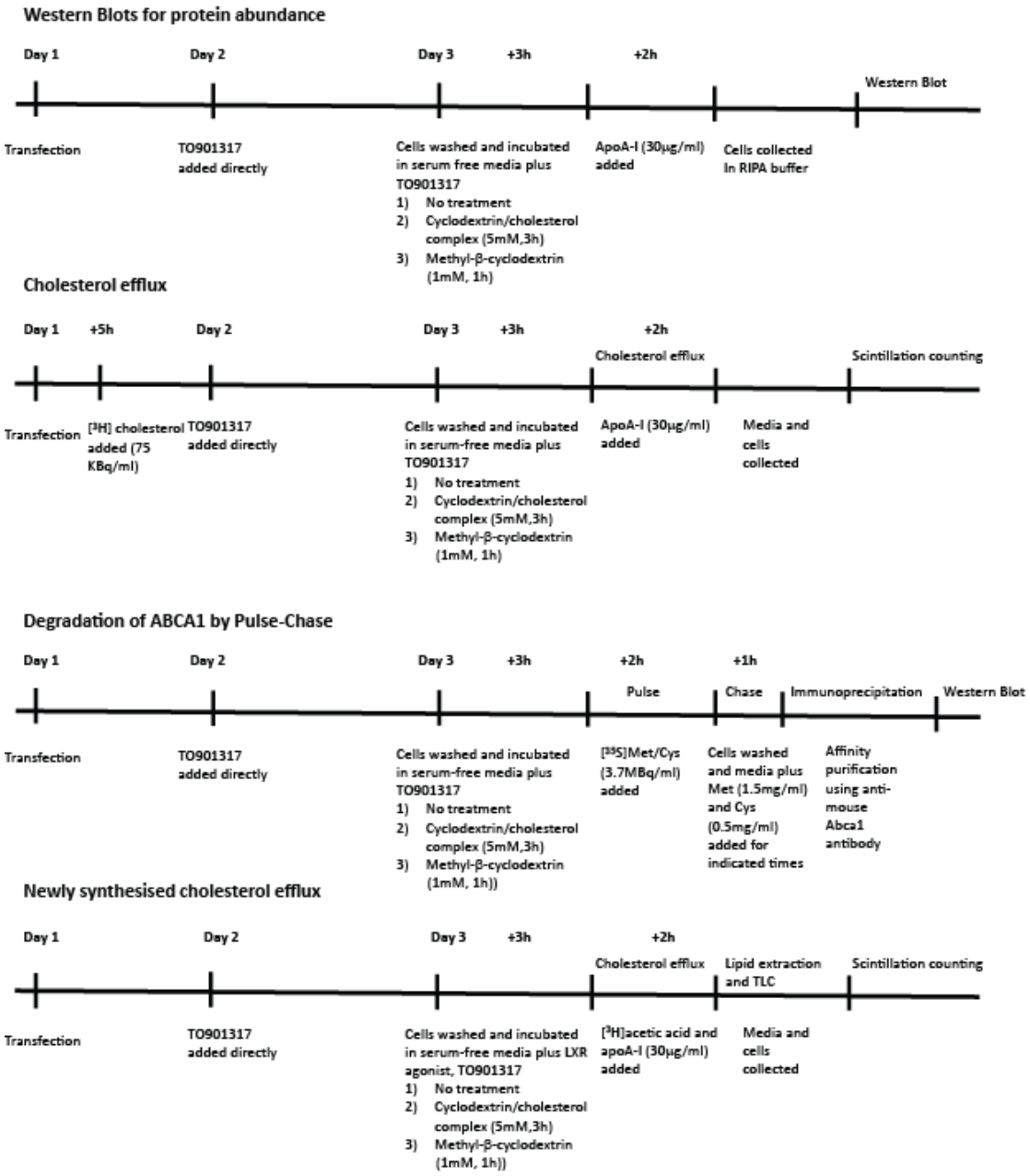
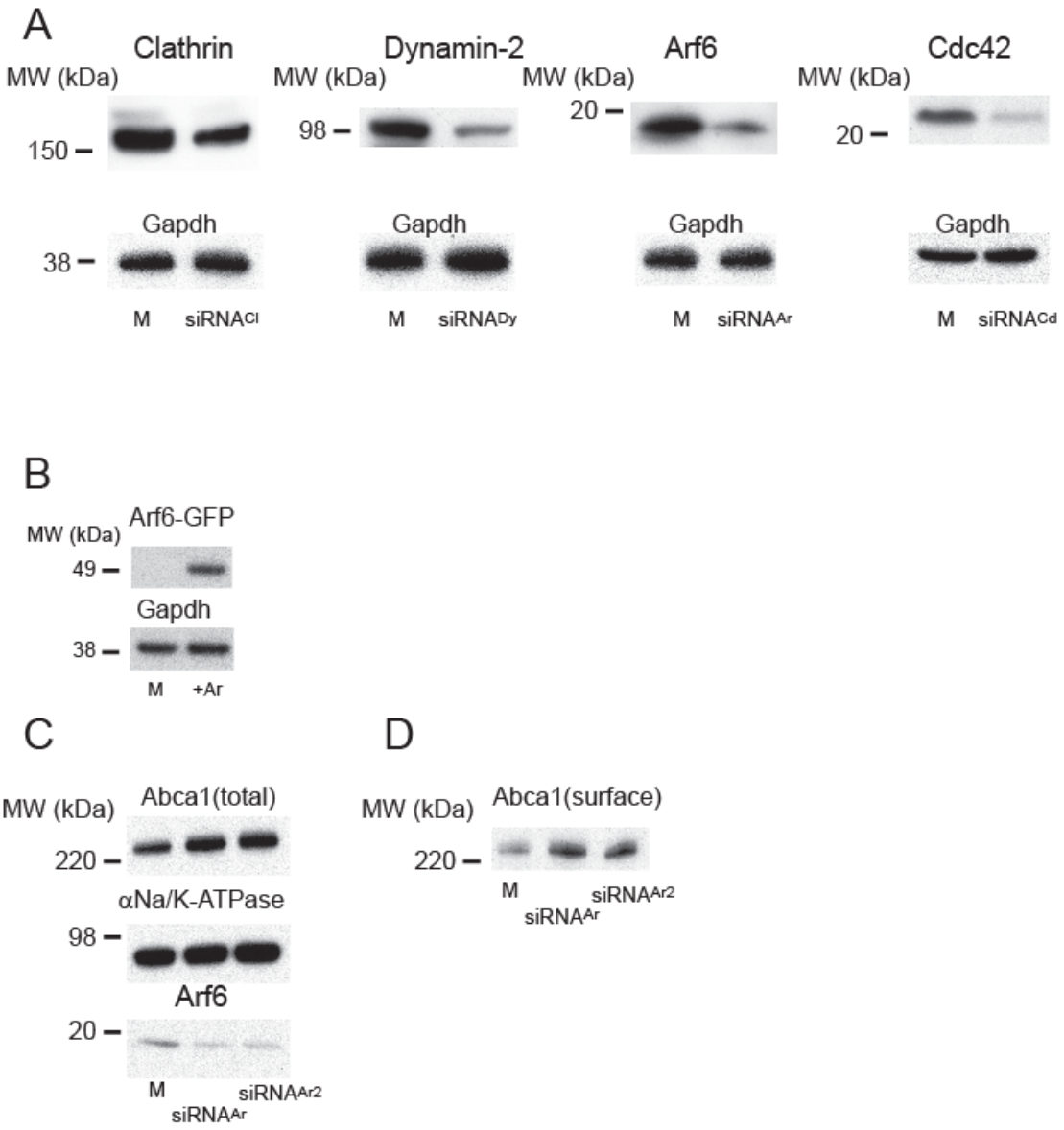
Small GTPase ARF6 Regulates Endocytic Pathway Leading to Degradation of ATP Binding Cassette Transporter A1

Nigora Mukhamedova, Anh Hoang, Huanhuan L. Cui, Irena Carmichael, Ying Fu, Michael Bukrinsky, Dmitri Sviridov

Supplement Material:



Supplemental Figure I: Schematic representation of the experimental design



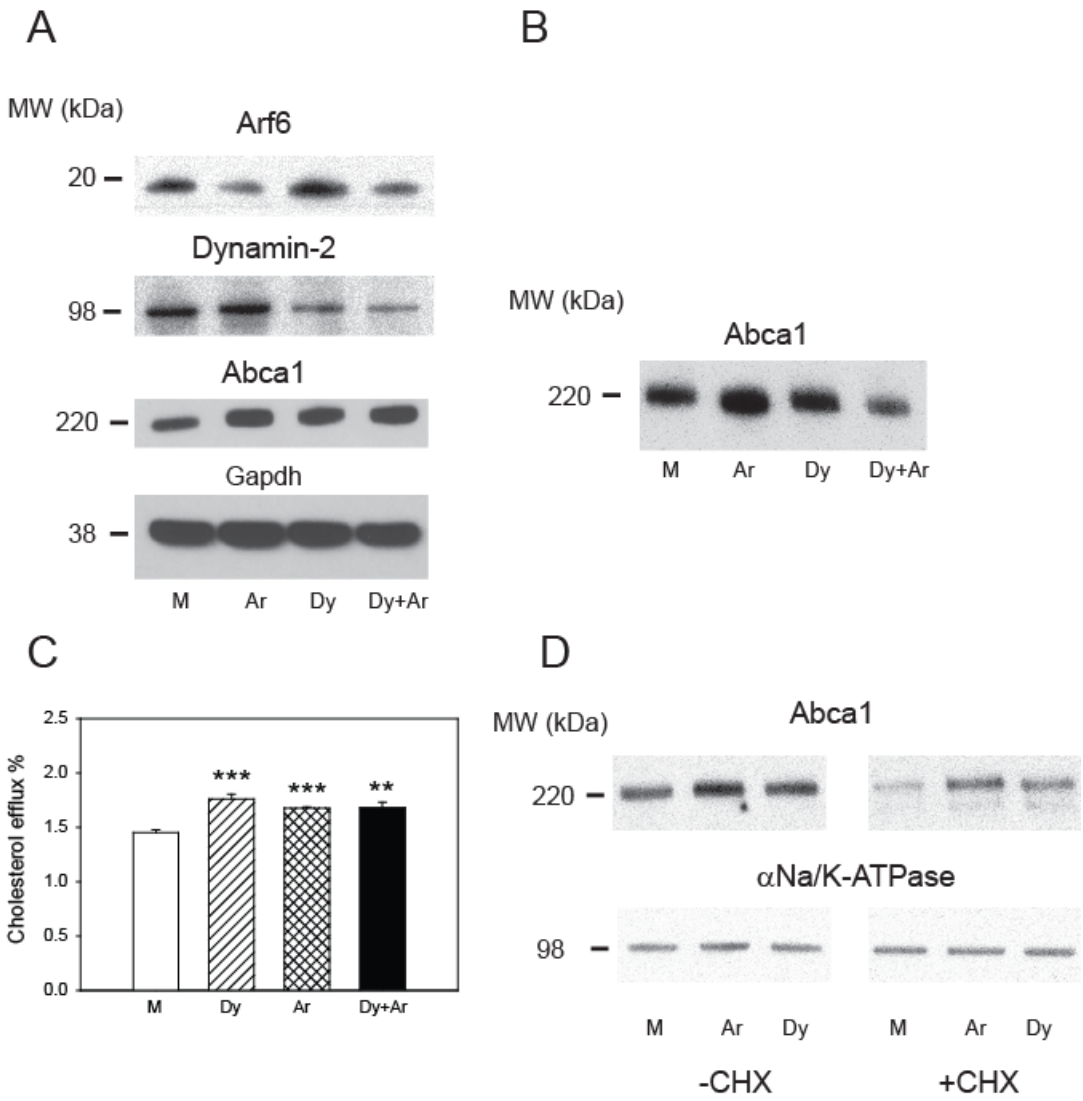
Supplemental Figure II: Silencing and overexpression of elements of endocytic pathways

A – The abundance of clathrin (Cl), dynamin-2 (Dy), Arf6 (Ar) or Cdc42 (Cd) in mock-transfected cells (M) and cells where these proteins were silenced by transfection with corresponding siRNA (siRNA).

B – The abundance of Arf6 in cells transiently transfected with Arf6 (+Ar) or mock (M) plasmid.

C - The abundance of Arf6 and total Abca1 in cells after transfection with original ($siRNA^{Ar}$) or alternative ($siRNA^{Ar2}$) siRNA.

D - The abundance of cell-surface Abca1 in cells after transfection with original ($siRNA^{Ar}$) or alternative ($siRNA^{Ar2}$) siRNA.



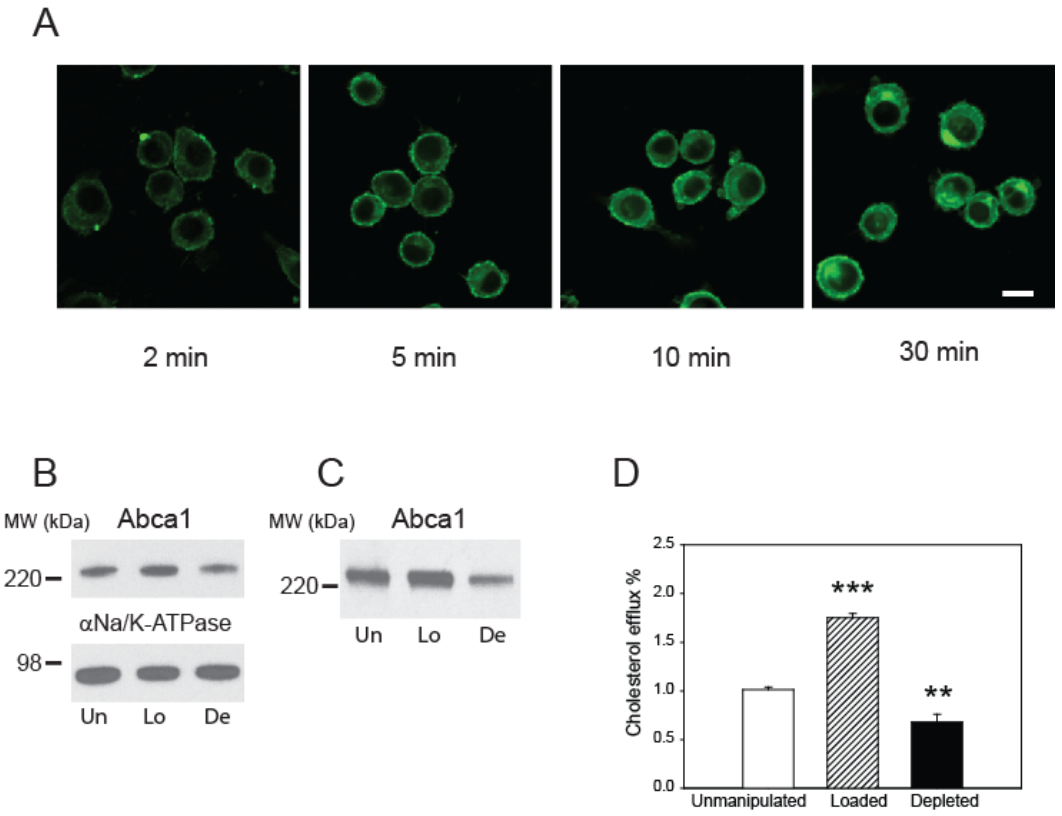
Supplemental Figure III. The effect of co-silencing of Arf6 and Dynamin-2 on ABCA1 abundance and functionality (A-C). The effect of cycloheximide on regulation of Abca1 abundance by Arf6 and Dynamin-2 (D).

A – The abundance of Arf6, Dynamin-2 and total Abca1 in cells with silenced Arf6 (Ar), dynamin-2 (Dy) or both dynamin-2 and Arf6.

B – The abundance of cell-surface Abca1 in cells with silenced Arf6 (Ar), dynamin-2 (Dy) or both dynamin-2 and Arf6.

C - Cholesterol efflux to apoA-I (final concentration 30 μg/ml) from cells with silenced Arf6 (Ar), dynamin-2 (Dy) or both dynamin-2 and Arf6. Means ± SEM of quadruplicate determinations are shown. **p< 0.01, ***p<0.001.

D - The abundance of total Abca1 in cells with silenced Arf6 (Ar) or dynamin-2 (Dy) in the presence or absence of cycloheximide (CHX, 5 μg/ml).



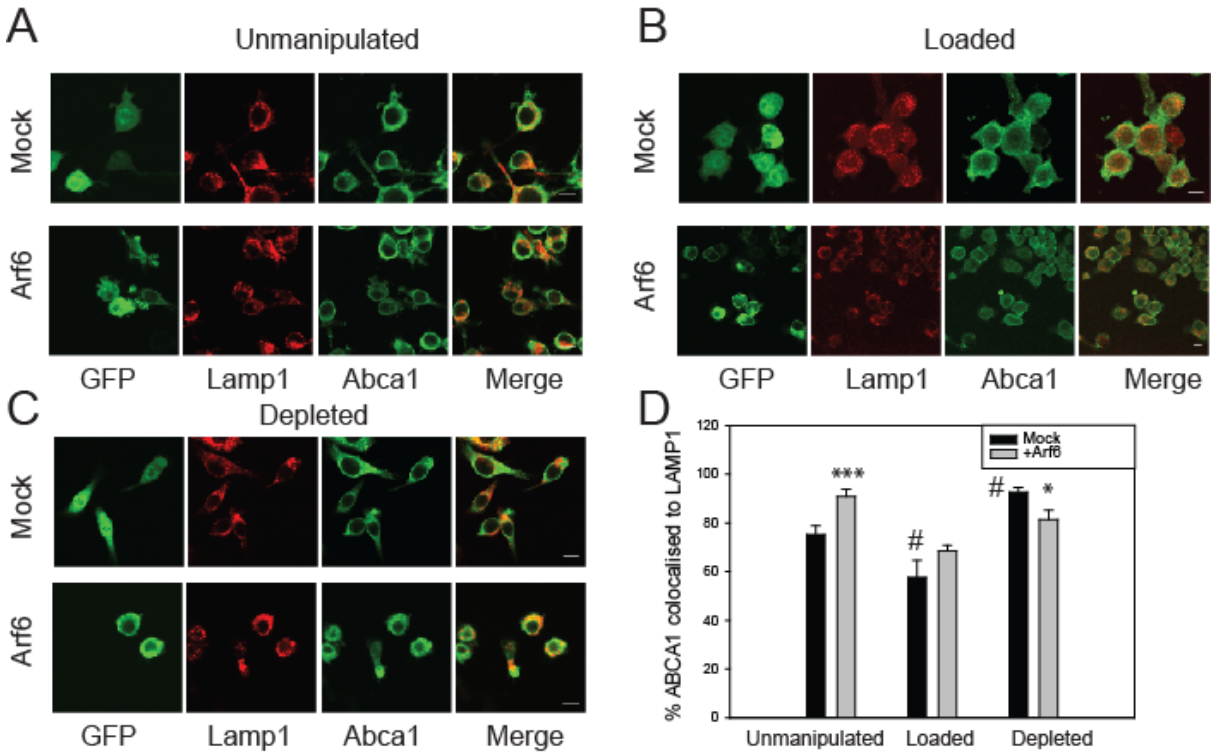
Supplemental Figure IV. The effects of cholesterol-loading and cholesterol- depletion on intracellular cholesterol distribution, Abca1 abundance and cholesterol efflux.

A – Time-course of cellular distribution of cholesterol after loading of cells with methyl-β-cyclodextrin/BODIPY-cholesterol complex. Bar 10 μm.

B, C - The abundance of total (B) and cell-surface (C) Abca1 in cells after cholesterol-loading (Lo) or cholesterol-depletion (De) as compared to cells with unmanipulated cholesterol content (Un).

D – Cholesterol efflux from cells after cholesterol-loading or cholesterol-depletion as compared to cells with unmanipulated cholesterol content.

E - The abundance of lipid rafts in cells with unmanipulated cholesterol content and cholesterol-depleted cells. Bar 10 μm.



Supplemental Figure V: The effect of overexpression of Arf6 on co-localization of Abca1 and Lamp1.

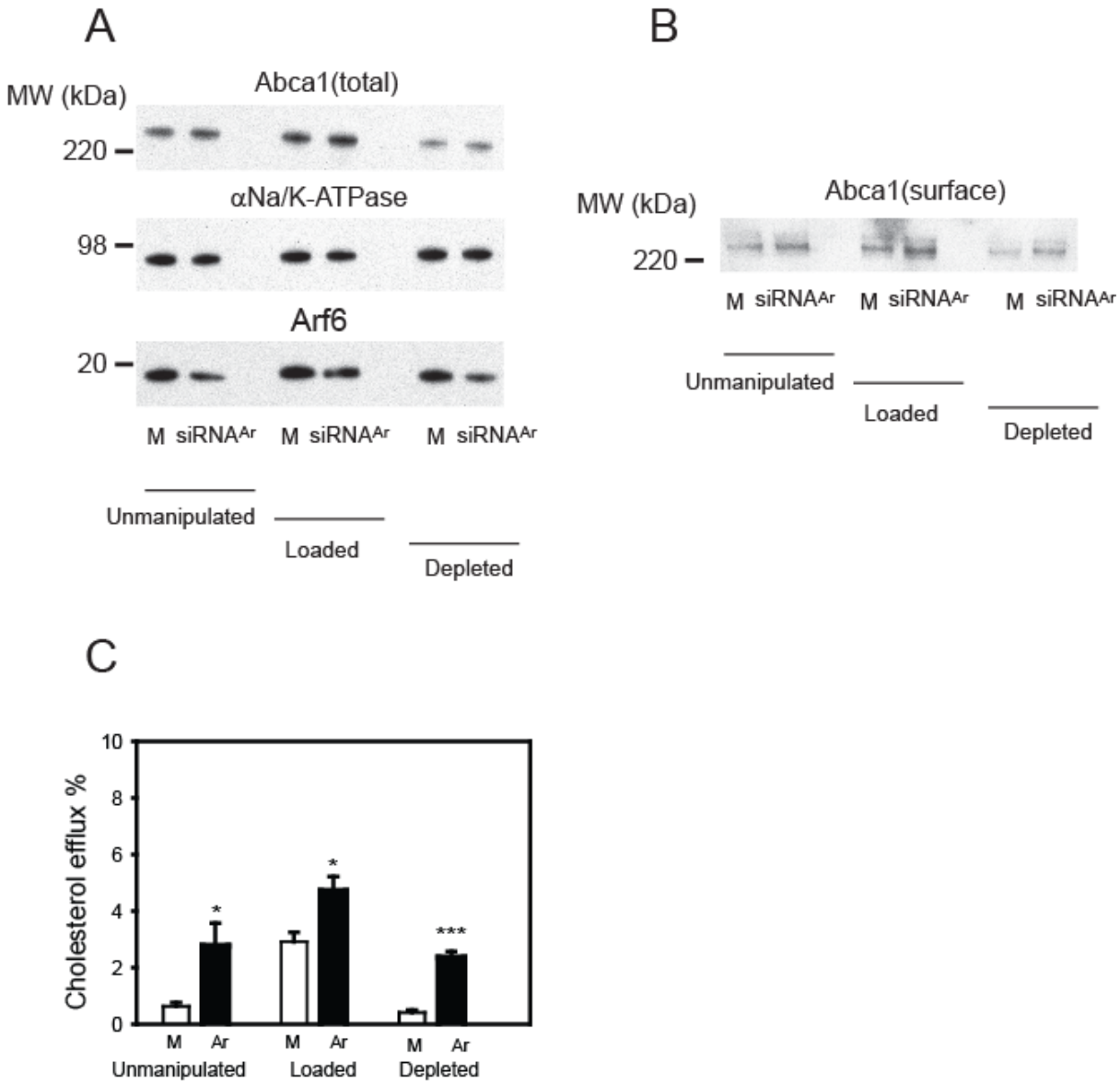
A – Confocal microscopy analysis of co-localization of Abca1 and Lamp1 in cells with unmanipulated cholesterol content transiently transfected with Arf6 or mock plasmid. Left column shows cells expressing GFP (transfected cells); colour for detection of Abca1 was changed to green for visualization of co-localization. Bar 10 μm.

B – Confocal microscopy analysis of co-localization of Abca1 and Lamp1 in cholesterol-loaded cells transiently transfected with Arf6 or mock plasmid. Left column shows cells expressing GFP (transfected cells); colour for detection of Abca1 was changed to green for visualization of co-localization. Bar 10 μm.

C – Confocal microscopy analysis of co-localization of Abca1 and Lamp1 in cholesterol-depleted cells transiently transfected with Arf6 or mock plasmid. Left column shows cells expressing GFP (transfected cells); colour for detection of Abca1 was changed to green for visualization of co-localization. Bar 10 μm.

D - Quantitation of co-localization of Abca1 and Lamp1 from images shown in F-H (Means ± SEM for 50-70 cells for each bar).

*$p<0.05$, ***$p<0.001$ (*versus* mock transfected cells with the same treatment); $^{\#}p<0.01$ (*versus* unmanipulated mock transfected cells)



Supplemental Figure VI. The effect of silencing of Arf6 on the abundance of Abca1 and cholesterol efflux form bone marrow derived macrophages.

A - The abundance of Arf6 and total Abca1 in BMDM with unmanipulated cholesterol content or after cholesterol-loading or cholesterol-depletion, and after transfection with siRNA^{Ar}.

B - The abundance of cell-surface Abca1 in BMDM with unmanipulated cholesterol content or after cholesterol-loading or cholesterol-depletion, and after transfection with siRNA^{Ar}.

C – Cholesterol efflux from BMDM with unmanipulated cholesterol content or after cholesterol-loading or cholesterol-depletion, and after transfection with siRNA^{Ar}.