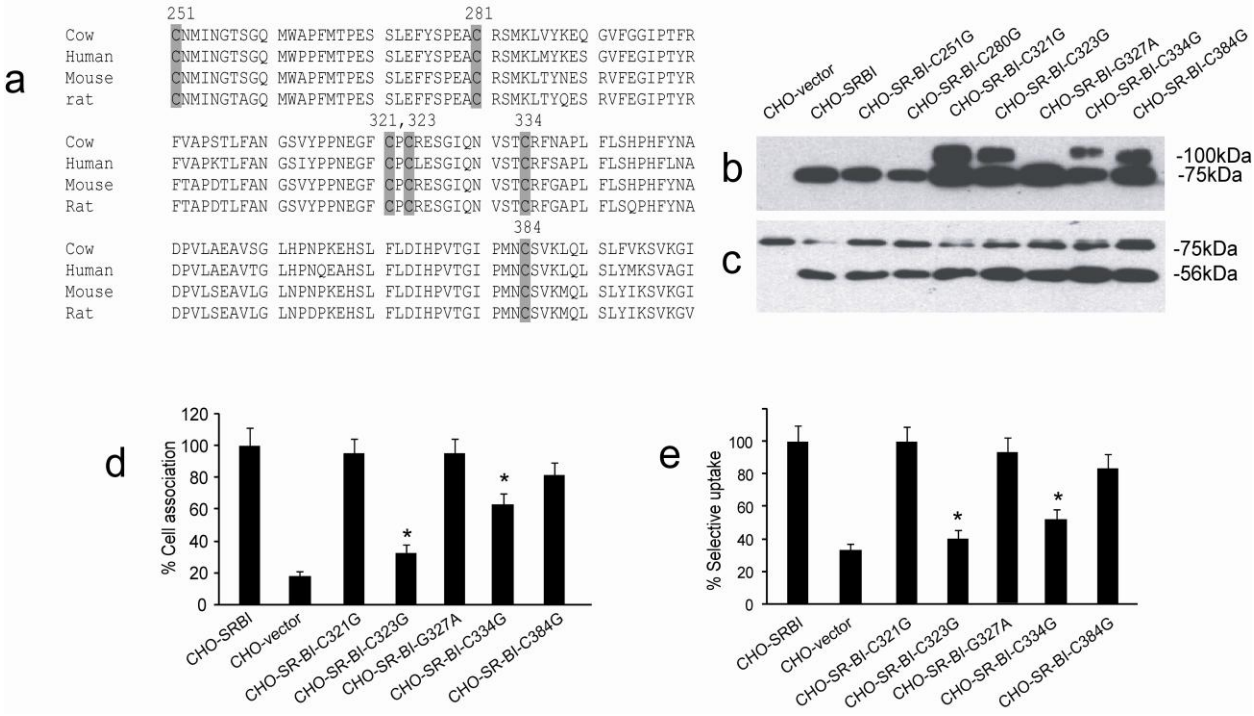
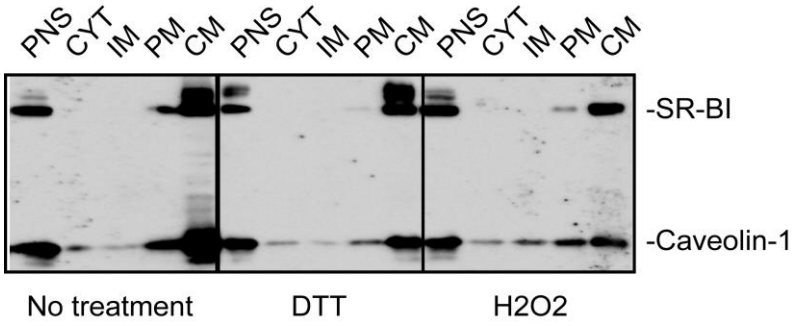
Supplemental materials:

Supplement Figure I. The conserved cysteinyl residues on extracellular loop of SR-BI and their HDL binding and cholesteryl ester uptake - In addition to C323, the extracellular loop of SR-BI contains 5 highly conserved cysteinyl residues (Supp Fig. I**a**). We generated CHO cells stably expressing C251G, C280G, C321G, C334G and C384G (Supp Fig. I**b**). We also generated CHO cells stably expressing G327A mutant and used the cells as non-cysteine mutant control. Among these mutations, C251G and C280G and G327A expressed as a single band at 75kDa as wild type SR-BI did, and C321G, C334G and C384G displayed two bands at 75 kDa and 100kDa as C323G did (Supp Fig. I**b**). Treatment of the cells with deglycosylation enzymes demonstrated that the 100kDa band is highly glycosylated SR-BI (Supp Fig. I**c**). We examined the HDL binding and cholesteryl ester uptake activity of the mutants. A327G had full HDL binding and cholesteryl ester uptake activity (Supp Fig. I**d** and I**e**). For glycosylated mutants, C321G had full HDL binding and cholesteryl ester uptake activity, C384G had most HDL binding and cholesteryl ester uptake activity, and C334G markedly lost HDL binding and cholesteryl ester uptake activity (Supp Fig. I**d** and I**e**). Of note, C321G and C384G mutant SR-BI displayed the highly glycosylated band at 100kDa as C323G mutant SR-BI did, but they still have most HDL binding and cholesterol ester uptake activity, indicating that extra glycosylation of SR-BI may not affect SR-BI-mediated HDL binding and cholesteryl ester uptake.



Supp Fig. I. The conserved cysteinyl residues on extracellular loop of SR-BI and their HDL binding and cholesteryl ester uptake. a, Conserved cysteines on the extracellular loop of SR-BI. SR-BI Sequence fragments from cow, human, mouse and rat are shown. The conserved cysteinyl residues on the extracellular loop of SR-BI are highlighted. **b,** Western blot analysis of cell lysates from CHO cells stably expressing C251G, C280G, C321G, A327G, C334G and C384G (**c**) Deglycosylation analysis. Cell lysates listed above were deglycosylated and applied to western blot. **d** and **e**, HDL binding and selective uptake assays. The data were normalized to expression of the mutant protein relative to wild type SR-BI. $p < 0.05$ vs wild type SR-BI.

Supplement Figure II. Effect of redox reagents on subcellular location of SR-BI. Treatment of CHO-SR-BI cells with 1μM DTT moderately increased SR-BI caveolae distribution and treatment of CHO-SR-BI cells with 1μM H2O2 slightly decreased SR-BI caveolae distribution (based on CM/PNS ratio).



Supp Fig. II. Effect of redox reagents on subcellular targeting of SR-BI. CHO-SR-BI cells were treated with 1μM DTT or 1μM H2O2 for 60 min. The subcellular fractions were isolated with Opti-Prep method and the SR-BI in each fraction was analyzed by Western blot. Post-nuclear supernatant (PNS), cytosol (CYT), intracellular membrane (IM), plasma membrane (PM) and caveolae membrane (CM) fractions.