**SUPPLEMENTAL MATERIAL**

# Targeted deletion of hepatocyte Abca1 increases plasma HDL reverse cholesterol transport via the LDL receptor

Alexander C. Bashore, PhD[1], Mingxia Liu, PhD[1], Chia-Chi C. Key, PhD[1], Elena Boudyguina, PharmD[1], Xianfeng Wang, PhD[1], Caitlin M. Carroll, BA[3], Janet K. Sawyer, MS[1], Adam E. Mullick, PhD[2], Richard G. Lee, PhD[2], Shannon L. Macauley, PhD[3], John S. Parks, PhD[1]

[1]Department of Internal Medicine, Section on Molecular Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157

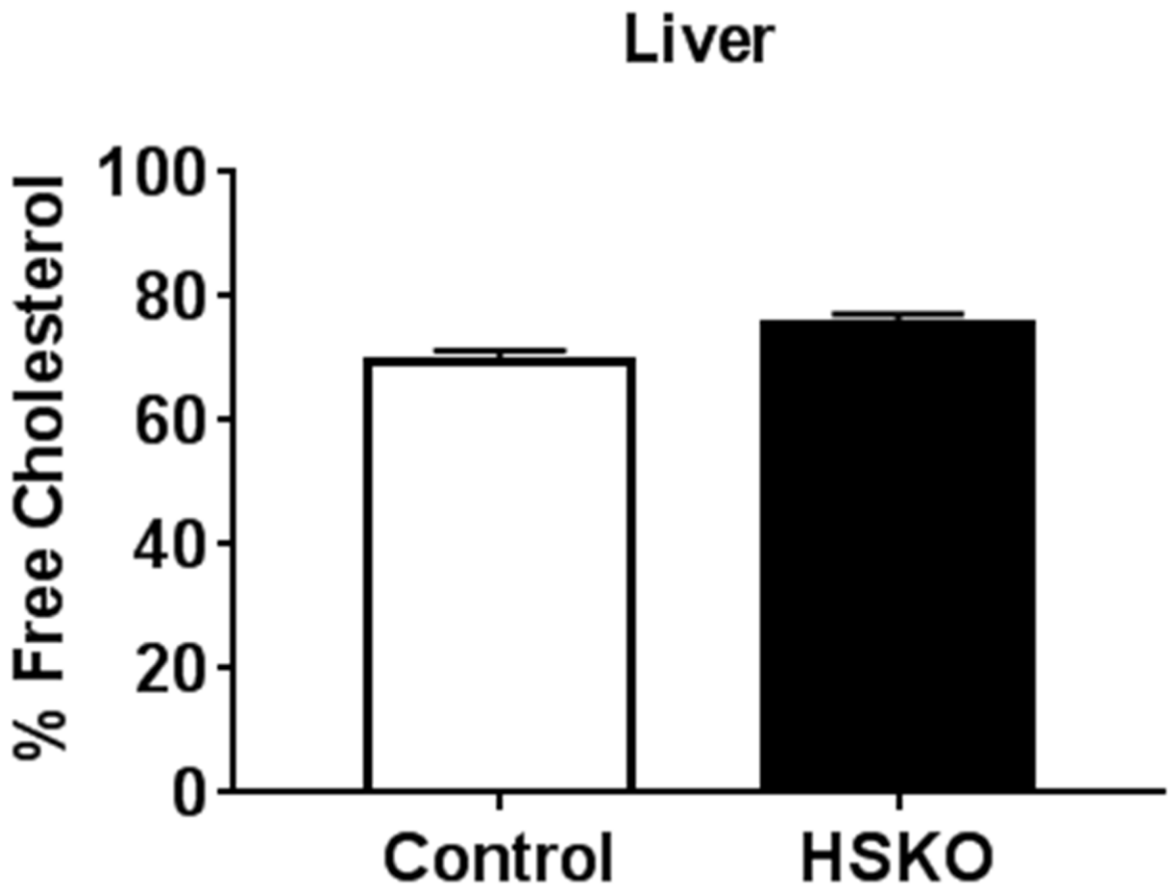
[2]Cardiovascular Group, Antisense Drug Discovery, Ionis Pharmaceuticals, Inc., Carlsbad, CA 92010, USA.

[3] Department of Internal Medicine, Section on Gerontology and Geriatric Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27157

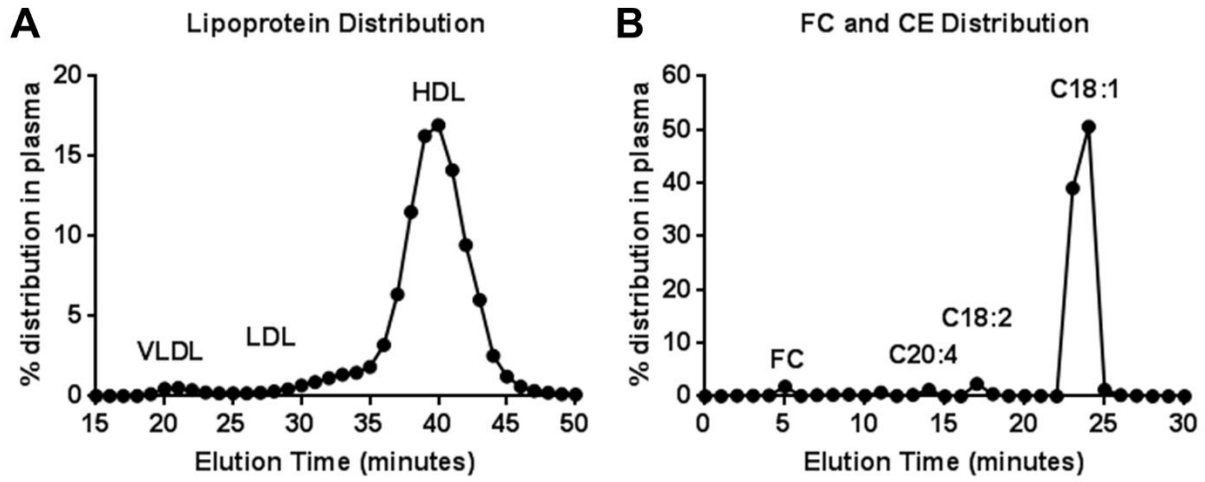
Short title: Liver Abca1 and HDL reverse cholesterol transport

[#]To whom correspondence should be addressed:

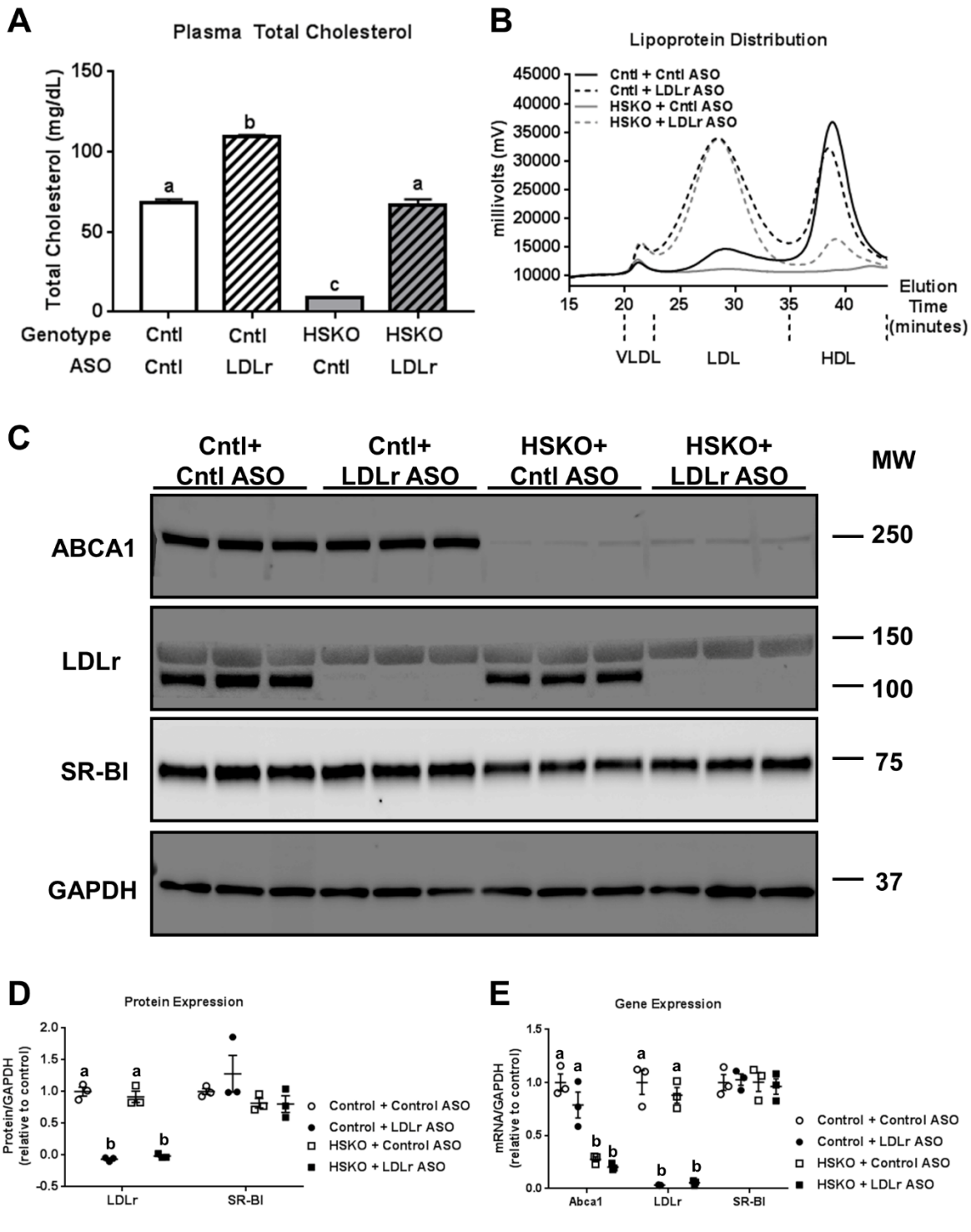
Dr. John S. Parks, Department of Internal Medicine/Section of Molecular Medicine, Wake Forest School of Medicine, Medical Center Blvd., Winston-Salem, NC 27157, USA. E-mail: jparks@wakehealth.edu Tel: +1 336 716 2145; Fax: +1 336 716 6279




**Supplemental Figure I.** Hydrolysis of $^{3}$H-CO human HDL tracer to $^{3}$H-FC in liver. Percentage $^{3}$H-FC in liver 24 hours following $^{3}$H-CO human HDL injection into control or HSKO mice was determined after lipid extraction of liver, thin-layer chromatographic separation of FC and CE, and quantification of radiolabel by liquid scintillation counting. Approximately 70% of the plasma HDL-derived radioactivity was FC in both genotypes of mice, indicating significant hepatic hydrolysis of $^{3}$H-CO to $^{3}$H-FC. Data are mean ± SEM; n=5/genotype.

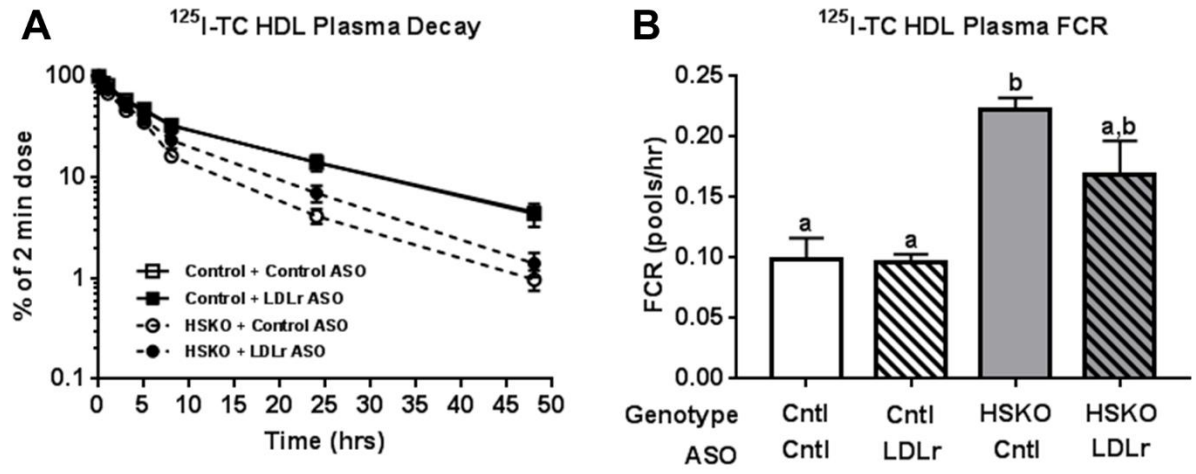



**Supplemental Figure II.** $^{3}$H-CO ($^{3}$H-C18:1) HDL stability in plasma. $^{3}$H-CO radiolabeled human HDL was incubated with plasma at 37°C for 2 min up to 24 hours. Plasma was then fractioned by FPLC to determine $^{3}$H lipoprotein distribution (**A**). The plasma samples were then lipid extracted and $^{3}$H-FC and $^{3}$H CE fatty acyl distribution was analyzed by reverse phase HPLC (**B**). Only data from the 24-hour incubation are shown. Nearly all (95%) of the $^{3}$H-CO HDL tracer eluted in the HDL region of the FPLC column. After a 24-hour incubation of the $^{3}$H-CO HDL tracer with plasma at 37ºC, 94% of the radiolabel eluted in HDL region, suggesting minimal transfer of radiolabel to other plasma lipoproteins (A). In the 2-min incubation, 96.5% of the HDL radiolabel eluted in the CO peak compared with 89.4% in the 24-hour time point, indicating minimal hydrolysis of the $^{3}$H-CO to $^{3}$H-FC over the 24-hour plasma incubation.

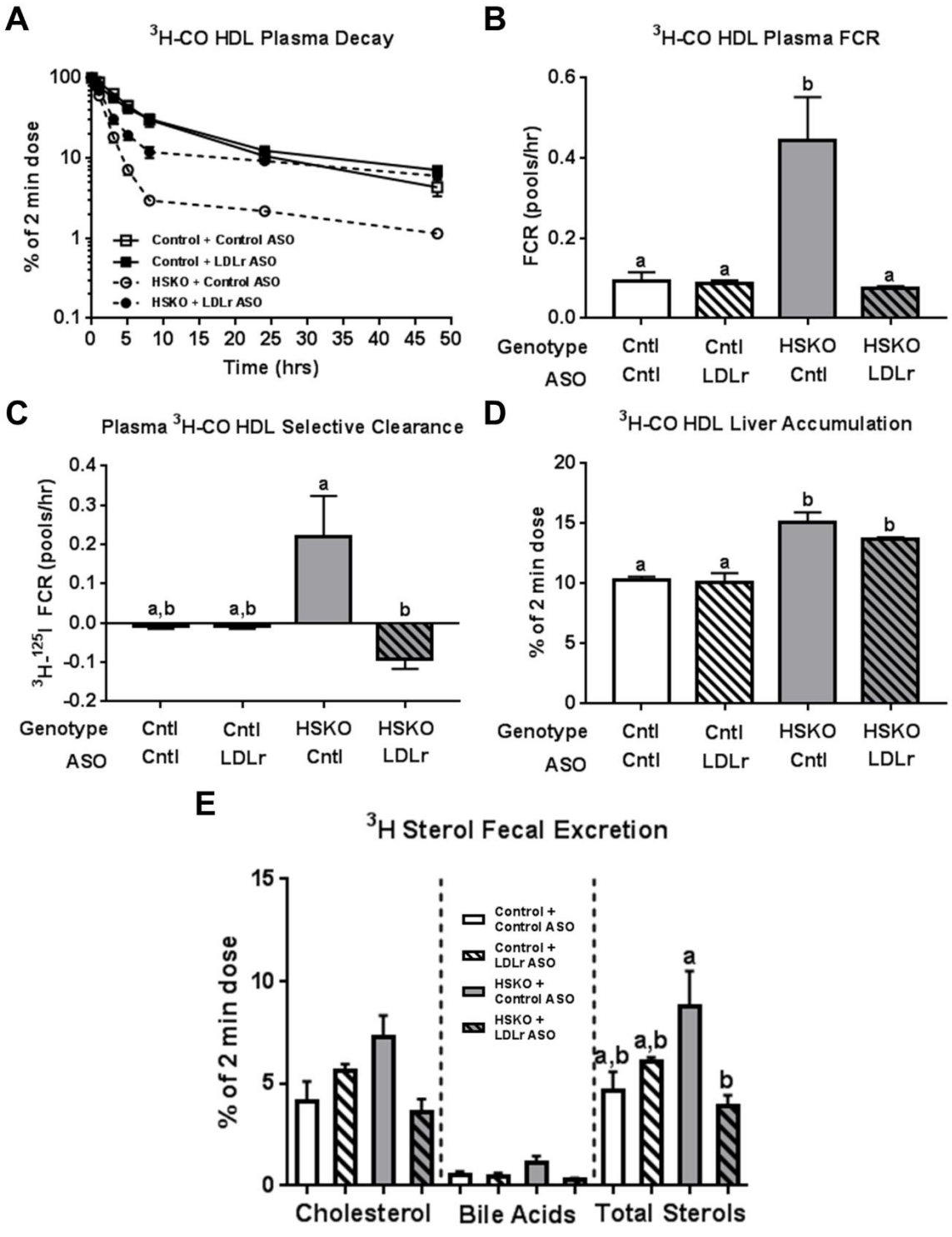



**Supplemental Figure III.** LDLr ASO treatment increases plasma LDL-C and HDL-C in HSKO mice. Efficiency of LDLr silencing was monitored by quantifying total plasma cholesterol (**A**) and

plasma lipoprotein cholesterol distribution by FPLC (mV signal from online cholesterol analyzer) (**B**) following 4 weeks of ASO treatment. Whole liver lysates were immunoblotted for Abca1, LDLr, and SR-BI (**C**) and blots were quantified (**D**) by calculating fold change in protein/GAPDH ratio relative to control mice treated with control ASO. Real-time PCR was performed to analyze gene expression for Abca1, LDLr, and SR-BI and the mRNA/GAPDH ratio relative to control mice treated with control ASO was plotted (**E**). Data are mean ± SEM; n=3 for each group. Groups with different letters are statistically different. Protein and gene expression data are from recipient mice used in mouse HDL tracer studies (Supplemental Figures IV and V).




**Supplemental Figure IV.** Effect of LDLr ASO treatment on *in vivo* catabolism of $^{125}$I-TC labeled mouse HDL. $^{125}$I-TC labeled HDL was injected intravenously in control and HSKO treated with either a control or LDLr targeting ASO. Periodic blood samples were taken over 48 hours to analyze plasma decay (**A**) and FCR (**B**). Data are mean ± SEM. Groups with different superscripts are statistically different ($p<0.05$), n=3 for each group. Control turnover curves in panel A are identical and SEM in nearly all points falls within the symbol.




**Supplemental Figure V.** Effect of LDLr ASO treatment on *in vivo* catabolism of $^{3}$H-CO radiolabeled mouse HDL. $^{3}$H-CO radiolabeled HDL was injected intravenously in control and HSKO mice treated with either a control or LDLr targeting ASO for 4 weeks. Periodic blood

samples were taken over 48 hours to analyze plasma decay (**A**), plasma FCR (**B**), and plasma HDL $^{3}$H-CO selective clearance, using data from Supplemental Figure IVB for $^{125}$I-TC FCR (**C**). At the termination of the study, tissues were harvested to quantify liver accumulation (**D**) and fecal excretion (**E**) of the tracer. Data are mean ± SEM. Groups with different superscripts are statistically different ($p<0.05$), n=3 for each group.