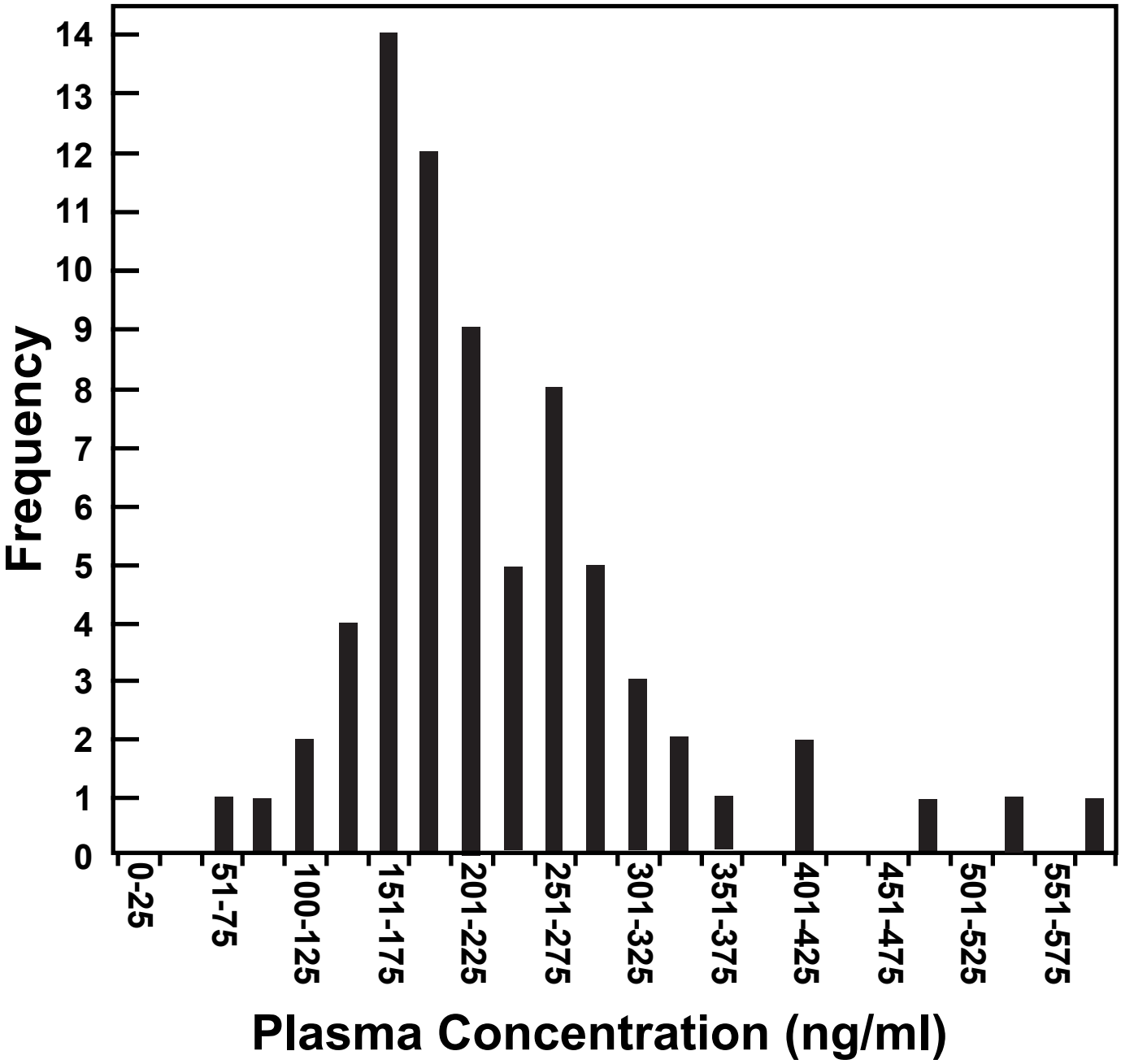
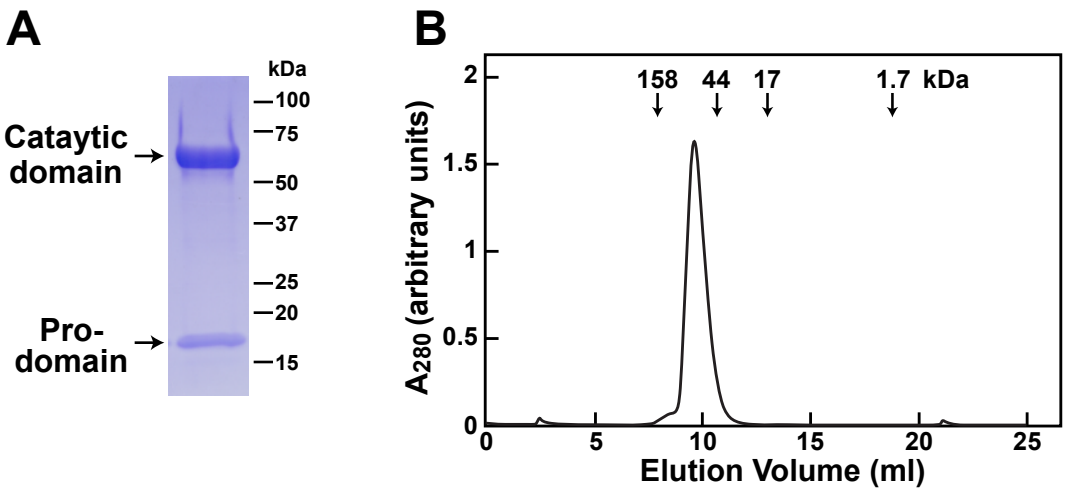
Suppl. Figure 1



Suppl. Figure 2



Supplemental General Methods

Cholesterol, triglyceride, and protein concentrations in plasma were measured as previously described (1). Reagents were obtained from Sigma-Aldrich Chemicals unless stated otherwise. Real-time reverse transcriptase-PCR was performed as described previously (2). *Ldlr*$^{-/-}$ (3), *Pcsk9*$^{-/-}$ (2), *Arh*$^{-/-}$ (4), and *LDLR*$^{h/h}$ (5) mice were produced as described in the indicated reference. Mice that express only human LDLR (*LDLR*$^{h/h}$) and lack ARH were generated by breeding *LDLR*$^{h/h}$ with *Arh*$^{-/-}$ mice.

Cultured Cell Experiments. HepG2 cells (ATCC, HB-8065) were set up at 5 x 10^5 cells/60-mm dish in Medium B on day 0. On day 2, Medium B was replaced with Medium C for 18-24 hours before initiating experiments. Immortalized mouse embryonic fibroblasts (MEFs) were a gift from Joachim Herz (UT Southwestern) (6). MEF cells were set up at 1 x 10^5 cells/60-mm dish in Medium E on day 0. On day 1, medium was replaced with Medium F 18-24 hours before initiating the experiments.

Stable Transfection of Human Embryonic Kidney (HEK) 293S cells with Epitope-tagged PCSK9. HEK 293S cells (7) were cultured in Medium B and transfected with 2 μg of expression plasmid pCMV-hPCSK9-FLAG (8) or pCMV-hPCSK9(D374Y)-FLAG using FuGENE 6 transfection reagent according to manufacturer's instructions (Roche). Plasmid pCMV-hPCSK9(D374Y)-FLAG was produced by site-directed mutagenesis (Stratagene) using the oligonucleotide 5'-GGTGCCTCCAGCTACTGCAGCACCTG-3' and pCMV-hPCSK9-FLAG. Colonies expressing the *neo*-containing plasmid were isolated and expression of PCSK9 was assessed by immunoblot analysis using an anti-FLAG M2 antibody.

Additional Antibodies. Additional antibodies used were the following: IgG-HL1 and IgG-C7, mouse monoclonal antibodies that recognize the human LDLR (9); anti-human transferrin receptor (Zymed Laboratories); horseradish peroxidase-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories); horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham); monoclonal anti-actin mouse ascites (clone AC-10 - Sigma); and Alexa Fluor conjugated goat anti-rabbit and anti-mouse IgGs (Invitrogen). Polyclonal antiserum against the bovine LDLR (Ab 4548), the murine LDLR cytoplasmic tail (Ab 3143), LRP, RAP, ARH, polyclonal antibodies were described previously (8, 10). Rabbit polyclonal IgG recognizing the bovine CI-MPR was the kind gift of Dr. Stuart Kornfeld (Washington University).

References

1. Shimano, H., Horton, J.D., Hammer, R.E., Shimomura, I., Brown, M.S., and Goldstein, J.L. 1996. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J. Clin. Invest.* 98:1575-1584.
2. Rashid, S., Curtis, D.E., Garuti, R., Anderson, N.N., Bashmakov, Y., Ho, Y.K., Hammer, R.E., Moon, Y.-A., and Horton, J.D. 2005. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. *Proc. Natl. Acad. Sci. USA* 102:5374-5379.
3. Ishibashi, S., Brown, M.S., Goldstein, J.L., Gerard, R.D., Hammer, R.E., and Herz, J. 1993. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery [see comments]. *J. Clin. Invest.* 92:883-893.
4. Jones, C., Hammer, R.E., Li, W.-P., Cohen, J.C., Hobbs, H.H., and Herz, J. 2003. Normal sorting but defective endocytosis of the low density lipoprotein receptor in mice with autosomal recessive hypercholesterolemia. *J. Biol. Chem.* 278:29024-29030.
5. Knouff, C., Malloy, S., Wilder, J., Altenburg, M.K., and Maeda, N. 2001. Doubling expression of the low density lipoprotein receptor by truncation of the 3'-untranslated region sequence ameliorates type III hyperlipoproteinemia in mice expressing the human apoe2 isoform. *J Biol Chem* 276:3856-3862.
6. Willnow, T.E., and Herz, J. 1994. Genetic deficiency in low density lipoprotein receptor-related protein confers cellular resistance to Pseudomonas exotoxin A.

Evidence that this protein is required for uptake and degradation of multiple ligands. *J. Cell Sci.* 107:719-726.

7. Reeves, P.J., Thurmond, R.L., and Khorana, H.G. 1996. Structure and function in rhodopsin: high level expression of a synthetic bovine opsin gene and its mutants in stable mammalian cell lines. *Proc. Natl. Acad. Sci. U S A* 93:11487-11492.
8. Park, S.W., Moon, Y.-A., and Horton, J.D. 2004. Post-transcriptional regulation of low density lipoprotein receptor protein by proprotein convertase subtilisin/kexin type 9a in mouse liver. *J. Biol. Chem.* 279:50630-50638.
9. van Driel, I.R., Goldstein, J.L., Sudhof, T.C., and Brown, M.S. 1987. First cysteine-rich repeat in ligand-binding domain of low density lipoprotein receptor binds Ca2+ and monoclonal antibodies, but not lipoproteins. *J. Biol. Chem.* 262:17443-17449.
10. Russell, D.W., Schneider, W.J., Yamamoto, T., Luskey, K.L., Brown, M.S., and Goldstein, J.L. 1984. Domain map of the LDL receptor: sequence homology with the epidermal growth factor precursor. *Cell* 37:577-585.

Supplemental Figure Legends

Suppl. Figure 1. PCSK9 concentrations in plasma from 72 individuals. The cohort consisted of 35 males and 37 females ranging from 21-56 years of age. Plasma was collected in an EDTA tube following an overnight fast. PCSK9 concentrations were measured by ELISA as described in Methods.

Suppl. Figure 2. Purification of secreted PCSK9. **(A)** Coomassie blue staining of purified PCSK9. FLAG-tagged human PCSK9 secreted from HEK 293S cells was purified as described in Methods. Ten μg of purified protein was subjected to SDS-PAGE on a 4-15% gradient gel and protein bands were detected with Coomassie brilliant blue R-250 stain. Arrows indicate the catalytic and prodomains of secreted PCSK9. **(B)** Gel filtration chromatography of purified secreted PCSK9. Protein (100 μg) was loaded onto a Superdex 75 10/300 GL column and chromatographed at a flow rate of 0.4 ml/min. Absorbance at 280 nm was monitored continuously to identify the position of eluted FLAG-tagged PCSK9. Standard molecular weight markers (γ globulin, M_r 158,000; ovalbumin, M_r 44,000; myoglobin, M_r 17,000; vitamin B_{12}, M_r 1,700) were chromatographed on the same column under identical conditions (arrows).

Supplemental Table 1

Relative hepatic gene expression levels pre- and post-parabiosis in wild-type (WT), *Ldlr*$^{-/-}$, and TgPCSK9 (Tg) mice

Parabiotic Pair	Genotype	SREBP-2		LDLR	
		Pre	Post	Pre	Post
1a	WT	1.0	0.7	1.0	1.2
1b	WT	1.0	1.4	1.0	1.5
2a	WT	1.0	0.8	1.0	0.7
2b	WT	1.0	1.0	1.0	0.7
3a	WT	1.0	1.0	1.0	1.9
3b	WT	1.0	1.1	1.0	0.8
4a	WT	1.0	1.2	1.0	0.9
4b	WT	1.0	1.1	1.0	1.2
5a	*Ldlr*$^{-/-}$	1.0	1.1	-	-
5b	WT	1.0	1.1	1.0	0.8
6a	*Ldlr*$^{-/-}$	1.0	0.8	-	-
6b	WT	1.0	1.3	1.0	1.9
7a	*Ldlr*$^{-/-}$	1.0	1.8	-	-
7b	WT	1.0	1.2	1.0	1.1
8a	*Ldlr*$^{-/-}$	1.0	0.9	-	-
8b	WT	1.0	0.9	1.0	1.0
9a	Tg	1.0	0.9	1.0	0.7
9b	WT	1.0	0.6	1.0	0.8
10a	Tg	1.0	0.9	1.0	0.9
10b	WT	1.0	1.8	1.0	1.7
11a	Tg	1.0	1.5	1.0	1.8
11b	WT	1.0	1.5	1.0	1.1
12a	Tg	1.0	1.6	1.0	1.3
12b	WT	1.0	0.7	1.0	1.9