

Published in final edited form as:

Science. 2009 July 3; 325(5936): 100–104. doi:10.1126/science.1168974.

LXR Regulates Cholesterol Uptake through Idol-dependent Ubiquitination of the LDL Receptor

Noam Zelcer[§], Cynthia Hong, Rima Boyadjian, and Peter Tontonoz^{*}

Howard Hughes Medical Institute and Department of Pathology and Laboratory Medicine, University of California, Los Angeles, CA 90095, USA.

Abstract

Cellular cholesterol levels reflect a balance between uptake, efflux and endogenous synthesis. Here we show that the sterol-responsive nuclear receptor LXR helps maintain cholesterol homeostasis not only through promotion of cholesterol efflux, but also through suppression of low-density lipoprotein (LDL) uptake. LXR inhibits the LDL receptor (LDLR) pathway through transcriptional induction of Idol (Inducible Degradator of the LDLR), an E3 ubiquitin ligase that triggers ubiquitination of the LDLR on its cytoplasmic domain, thereby targeting it for degradation. LXR ligand reduces, whereas LXR knockout increases, LDLR protein levels *in vivo* in a tissue-selective manner. Idol knockdown in hepatocytes increases LDLR protein levels and promotes LDL uptake. Conversely, adenovirus-mediated expression of Idol in mouse liver promotes LDLR degradation and elevates plasma LDL levels. The LXR-Idol-LDLR axis defines a complementary pathway to sterol response element binding proteins for sterol regulation of cholesterol uptake.

The LDL receptor (LDLR) is central to the maintenance of plasma cholesterol levels (1). Mutations in this receptor are the leading cause of autosomal dominant hypercholesterolemia (ADH), characterized by elevated plasma cholesterol levels, and increased risk of cardiovascular disease (2,3). In line with its pivotal role in cholesterol homeostasis, expression of the LDLR is tightly regulated. Transcription of the *LDLR* gene is coupled to cellular cholesterol levels through the action of the sterol response element binding protein (SREBP) transcription factors (4,5). Enhanced processing of SREBPs to their mature forms when cellular sterol levels decline leads to increased *LDLR* transcription (6). Posttranscriptional regulation of LDLR expression is also a major determinant of lipoprotein metabolism. Genetic studies have identified mutations in the genes encoding the LDLR adaptor protein 1 (*LDLRAP1/ARH*) (7,8) and the SREBP target gene proprotein convertase subtilisin/kexin 9 (*PCSK9*) that result in altered stability, endocytosis, or trafficking of the LDLR (9-13).

The Liver X Receptors (LXRs) are also important transcriptional regulators of cholesterol metabolism. LXR α (NR1H3) and LXR β (NR1H2) are sterol-dependent nuclear receptors activated in response to cellular cholesterol excess (14). LXR target genes such as *ABCA1* and *ABCG1* promote the efflux of cellular cholesterol and help to maintain whole-body sterol homeostasis (15,16). Mice lacking LXRs accumulate sterols in their tissues and manifest accelerated atherosclerosis, whereas synthetic LXR agonists promote reverse cholesterol transport and protect mice against atherosclerosis (17-19). The coordinated regulation of intracellular sterol levels by the LXR and SREBP signaling pathways led us to investigate whether LXRs control the uptake as well as efflux of cholesterol.

*To whom correspondence should be addressed. ptontonoz@mednet.ucla.edu.

[§]Current address: Division of Biopharmaceutics, Leiden/Amsterdam Center for Drug Research, Gorlaeus Laboratories, Leiden University, PO Box 9502, 2300RA Leiden, The Netherlands

We initially tested the ability of LXRs to modulate LDL uptake in HepG2 human liver cells and primary mouse macrophages. Treatment with synthetic LXR ligand (GW3695 or T1317) decreased binding and uptake of BODIPY-labeled LDL (Fig. 1A). The LXR ligands did not induce changes in LDLR mRNA expression (Fig. S1A); however, they decreased LDLR protein levels rapidly and in a dose-dependent manner and this effect was independent of cellular sterol levels (Fig. 1B-D). Levels of ABCA1 protein, an established target of LXR, were reciprocally increased by LXR ligands (Fig. 1B-D). LXR ligands had no effect on LDLR levels in macrophages or mouse embryonic fibroblasts (MEFs) lacking LXR α and LXR β (Fig. 1E and S1B). LXR activation also decreased LDLR protein but not mRNA levels in human SV589 fibroblasts (Fig. S1C,D) (20).

To investigate the link between endogenous LXR ligands and LDLR expression, we used an adenovirus vector encoding oxysterol sulfotransferase (Sult2b1) (21,22). Depletion of oxysterol agonists by Sult2b1 in SV589 cells led to increased LDLR protein, and this effect was reversed by synthetic ligand (Fig. S1E). We further tested the effect of LXR agonists on LDLR produced from a transfected vector (i.e. not subject to endogenous SREBP regulation). In HepG2 cells stably expressing an LDLR-GFP fusion protein, LDLR-GFP expression was localized primarily on the plasma membrane (Fig. 1F). Ligand activation of LXR decreased LDLR-GFP expression and redistributed the protein from the plasma membrane to intracellular compartments.

To investigate the mechanism by which LXR affects the LDLR we examined LXR target genes by transcriptional profiling. We identified a potential mediator, denoted on our array as 9430057C20Rik (Table. S1), that corresponds to a protein originally identified on the basis of its interaction with myosin regulatory light chain (23). We propose that this protein, which has been variably referred to as Mir and Mylip, be renamed Idol (for Inducible Degrader of the LDLR) to reflect its biologic function (see below). Idol contains a band 4.1 and Ezrin/Radixin/Moesin homology (FERM) domain that mediates interactions with cytoplasmic domains of transmembrane proteins (24,25). Unique among FERM domain-containing proteins, Idol also contains a C-terminal RING domain and has been proposed to act as an E3-ubiquitin ligase, although its biological substrate(s) have not been identified (23,26). *Idol* mRNA is widely expressed in mice *in vivo* (Fig. S2A). Exposure of cells to increasing concentrations of LDL induced expression of both *Idol* and *Abca1* mRNA, indicating that their expression is responsive to extracellular cholesterol levels (Fig. S2B). Furthermore, LXR agonists induced *Idol* expression in multiple cells in an LXR-dependent manner, including primary hepatocytes, primary macrophages, MEFs, and HepG2 cells (Fig. 2A and S2C-E). Treatment of mice with GW3965 induced expression of *Idol* in multiple tissues, including spleen, intestine and adrenals (Fig. 2B). Interestingly, only modest regulation of *Idol* by LXR agonist was observed in liver, consistent with the degree of *Abca1* regulation (Fig S2F). *Idol* mRNA levels were also substantially decreased in spleen and liver of *Lxra* β ^{-/-} mice compared to wild-type controls (Fig. S2G).

LXR regulation of *Idol* was not sensitive to cycloheximide, suggesting that it was a direct transcriptional effect (Fig. S3A). It was not secondary to induction of SREBP-1c, because oxysterols that block SREBP processing still induced *Idol* expression (Fig. S3B). LXRs activate target genes by binding to consensus elements (LXREs) in their promoters. We identified an LXRE approximately 2.5 kb upstream of the mouse *Idol* translation start site (Fig. S3C) and generated a reporter construct encompassing this region. Activation by LXR α and GW3965 resulted in a ~4-fold increase in reporter activity that was largely abolished in the absence of a functional LXRE (Fig. S3D). Electromobility shift analysis showed that LXR/RXR bound to wild-type but not mutant versions of the *Idol* LXRE (Fig. S3E).

Given that Idol is a putative E3 ubiquitin ligase, we hypothesized that Idol induction might underlie the ability of LXRs to regulate LDLR abundance. Using a cotransfection system in human HEK293T kidney cells, we found that Idol expression redistributed LDLR-GFP from the plasma membrane to an intracellular compartment (Fig. 2C) and reduced the level of LDLR-GFP protein in a dose-dependent manner (Fig. 2D). Indeed, even levels of Idol protein too low to be detected by our antibody were effective. By contrast, Idol carrying a point mutation (C387A) in the catalytic RING domain (26) had no effect on the LDLR (Fig. 2C and S4A). Idol levels are very low, even when driven from an exogenous vector, suggesting that Idol is an unstable protein. In support of this idea, Idol levels were greatly enhanced when the RING domain was mutated, raising the possibility that Idol might catalyze its own degradation (Fig. S4A).

The effect of Idol on membrane proteins appears to be selective for the LDLR. Levels of transfected LRP1-GFP or APP-GFP proteins, both of which undergo regulated endocytosis similar to the LDLR, were unaffected by Idol expression (Fig. S4A). Similarly, Idol did not influence protein levels of ABCA1-GFP, endogenous transferrin receptor, endogenous myosin regulatory light chain, or the LDLR family members Lrp4 and SorLA (Fig. S4A,B). The very closely related family member ApoER2 was marginally affected by Idol. Adenovirus-mediated expression of Idol reduced LDLR protein levels in primary hepatocytes, HepG2 cells, and McR-H7777 rat hepatocytes (Fig. 2E) and also reduced LDL uptake in MEFs and McR-H7777 cells (Fig. 2F).

To explore the role of endogenous Idol in LDLR regulation, we developed shRNAs targeting Idol (Fig. S5). Idol-specific shRNAs increased LDLR protein levels in MEFs (Fig. 3A) and McR-H7777 cells (Fig. 3B), without affecting *LDLR* or *LXR* or *SREBP2* target mRNAs (Figs. 3C and S5), suggesting that Idol activity is a physiological mechanism for regulating LDLR abundance. In support of this idea, Idol-specific shRNAs increased LDL uptake in both fibroblasts (Fig. 3D) and McR-H7777 cells (Fig. 3E). Finally, the ability of an LXR ligand to reduce LDLR protein levels was diminished by Idol shRNA, implicating Idol in LXR-dependent regulation of the LDLR (Fig. 3F).

Pulse-chase labeling studies showed that Idol did not block *LDLR* mRNA translation or appearance of the immature protein (p), but it completely prevented appearance of the mature (m) glycosylated form (Fig. 4A). Given that mutation of the RING domain inactivates Idol, the most parsimonious explanation for our data is that Idol acts as an E3 ligase to trigger ubiquitination of the LDLR itself, thereby marking it for degradation. We found that ubiquitination of transfected LDLR in 293T cells was dramatically enhanced by expression of active but not mutant Idol (Fig. 4B). Analysis of a series of LDLR receptor mutants that become trapped at various stages of the maturation or recycling pathway revealed that Idol is capable of acting in the ER. LDLR G546D, which is unable to exit the ER (27), could still be degraded by Idol (Fig. 4C). Treatment of cells with brefeldin A, which blocks protein trafficking out of the ER, did not inhibit ubiquitination of the WT LDLR (Fig. S6A) or the G546D mutant (Fig. S6B). Treatment with ammonium chloride, a disruptor of lysosomal pH, inhibited LXR-induced endogenous LDLR degradation (Fig. S6C). These data suggest that Idol can ubiquitinate the precursor LDLR in the ER and that subsequent trafficking to the lysosome is required for degradation. Importantly, we also observed LXR ligand-dependent ubiquitination of endogenous LDLR in primary macrophages (Fig. 4D) and Idol-dependent ubiquitination of endogenous LDLR in primary hepatocytes (Fig. 4E). GW3965 triggered LDLR ubiquitination within 4 hours, consistent with the time course of LDLR degradation (Fig. 4D, cf. Fig. 1D).

We determined the structural requirements for Idol-dependent LDLR degradation using mutational analysis. Idol had no effect on an LDLR lacking the entire 50-amino acid intracellular domain (Fig. S6D). The LDLR intracellular domain contains 3 highly conserved

lysines and one cysteine that could be potential sites for ubiquitination (Fig. 4F) (28). Single mutations of any of these residues, or combined mutation of all three lysine residues, did not prevent Idol from degrading the LDLR (Fig. 4G and S6E). However, superimposing a cysteine mutation on constructs containing two or three mutated lysines rendered the LDLR insensitive to degradation. Additional mutagenesis revealed that either an intact K20 or an intact C29 was required for Idol-mediated degradation (Figs. 4G and S6E). Finally, not only did combined mutation of the K20 and C29 residues block LDLR degradation by Idol, it also blocked ubiquitination (Fig. 4H). Interestingly, the proteasome blocker MG132, despite stabilizing Idol protein, did not stabilize the LDLR, consistent with previous reports that degradation of the LDLR occurs in the lysosome (Fig. 4H) (29,30).

To investigate whether activation of the LXR-Idol pathway affects LDLR expression *in vivo*, we treated mice with GW3965. LXR agonist reduced LDLR protein levels in a tissue-selective manner, concordant with the degree of Idol induction (Fig. 5A, cf. Fig 2C). Whereas prominent effects were observed in intestine and peritoneal macrophages, LXR ligand had minimal effect on Idol mRNA and LDLR protein levels in liver. Remarkably, we observed a reciprocal effect on LDLR protein levels in resident macrophages and intestine when we analyzed mice in which the LXR-Idol pathway is inactive (Fig. 5B). LDLR protein levels were substantially higher in macrophages and intestine of LXR $\alpha\beta$ ^{-/-} mice compared to wild-type controls. LDLR levels were also slightly higher in liver. Thus, gain or loss of LXR-Idol activity affects LDLR expression *in vivo*.

Since Idol was not subject to strong LXR regulation in liver, we employed an alternative strategy to test its function in this tissue. We infected mice with adenoviral vectors encoding β -galactosidase or mouse Idol. Idol expression increased plasma levels of total and unesterified cholesterol, whereas levels of triglycerides, free fatty acids and glucose were not significantly altered (Fig. 5C and S7A). Fractionation of plasma lipoproteins revealed that Idol expression caused a phenotype reminiscent of that exhibited by *Ldlr*^(-/-) mice, with the appearance of a prominent LDL peak not present in the control mice (Fig. 5D and S7B). Plasma apoB protein levels were approximately 3-fold increased in Idol-transduced mice and there was no difference in PCSK9 levels (Fig. S7C,D). Consistent with our *in vitro* results, hepatic expression of LXR and SREBP-2 target genes was not affected by Idol expression (Fig. S7E), but LDLR protein levels were markedly reduced (Fig. 5E). In contrast, transferrin receptor levels were not altered by Idol. Finally, Idol adenovirus had no effect on plasma cholesterol levels (Fig. S8A) or lipoprotein profiles (Fig. S8B) in *Ldlr*^(-/-) mice, demonstrating that Idol requires LDLR expression for these effects.

In summary, we have shown that the sterol-sensitive nuclear receptor LXR regulates LDLR-dependent cholesterol uptake through a pathway independent of the SREBPs. LXR induces expression of Idol, which in turn catalyzes the ubiquitination of the LDLR, thereby targeting it for degradation. These results provide a potential explanation for an earlier observation that an ectopically-expressed LDLR could still be regulated by sterols (31). Identification of the Idol-LDLR pathway fills a gap in our understanding of how LXRs control cholesterol homeostasis. The ability of LXRs to respond to excess cellular cholesterol by promoting efflux through ABC transporters has been extensively documented (14). The LXR-Idol-LDLR pathway provides a mechanism to simultaneously limit LDL cholesterol uptake. Idol and ABCA1 are coordinately regulated by LXR in a cell-type selective manner, consistent with this functional link.

Interestingly, the LXR-Idol pathway appears to be most active in peripheral cells such as macrophages, adrenals and intestine. At the same time, however, Idol is constitutively expressed in liver, and gain or loss of Idol function in cultured hepatocytes regulates LDLR

protein levels and affects LDL uptake. Forced expression of Idol in liver *in vivo* profoundly reduces LDLR levels, further indicating that Idol is capable of degrading LDLR in this tissue.

Whether Idol expression is required for LDLR degradation *in vivo* is a critical question that remains to be addressed. If it is required, then conceivably the Idol pathway could be targeted pharmacologically so as to increase LDLR levels and enhance LDL clearance.

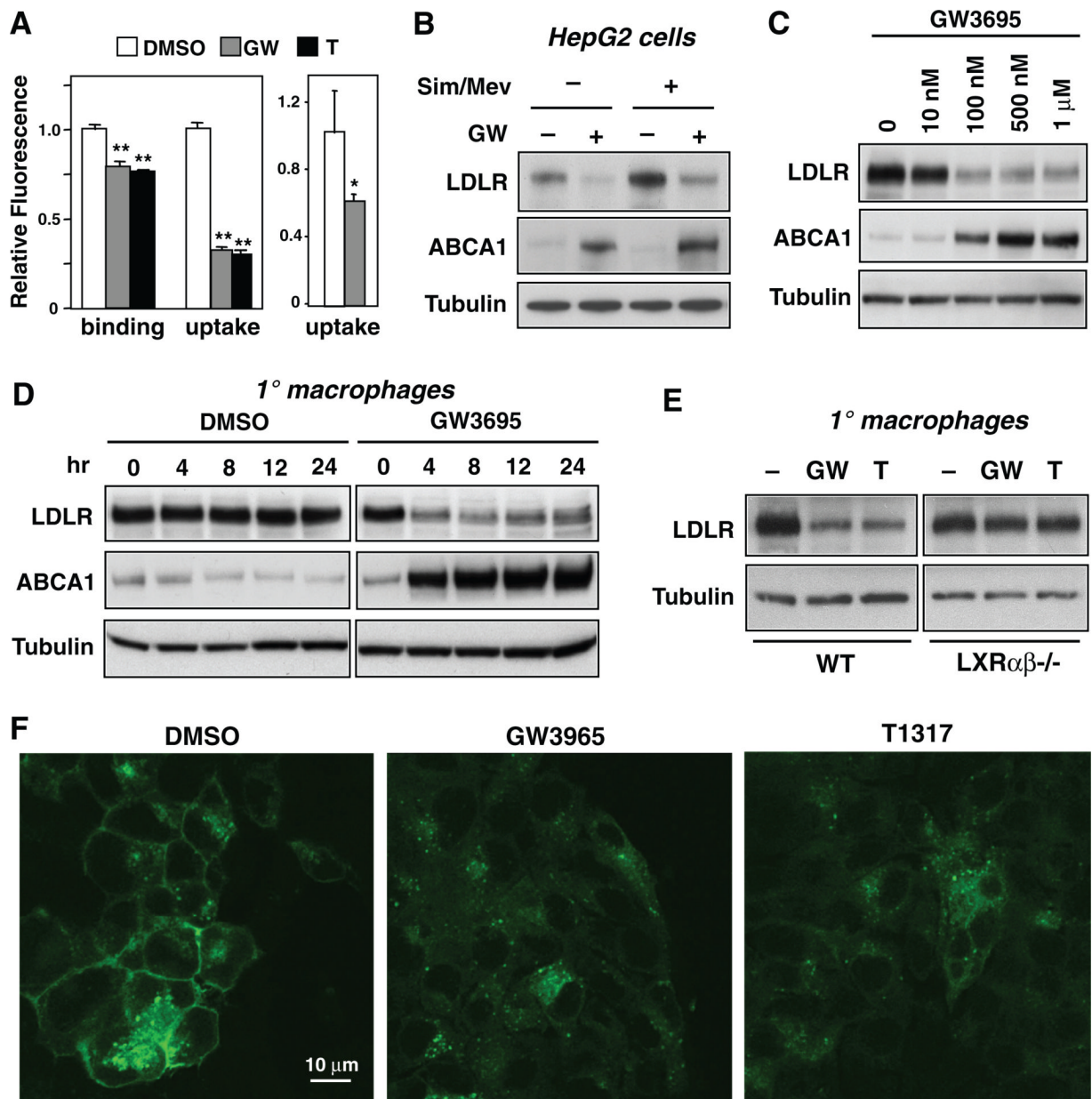
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

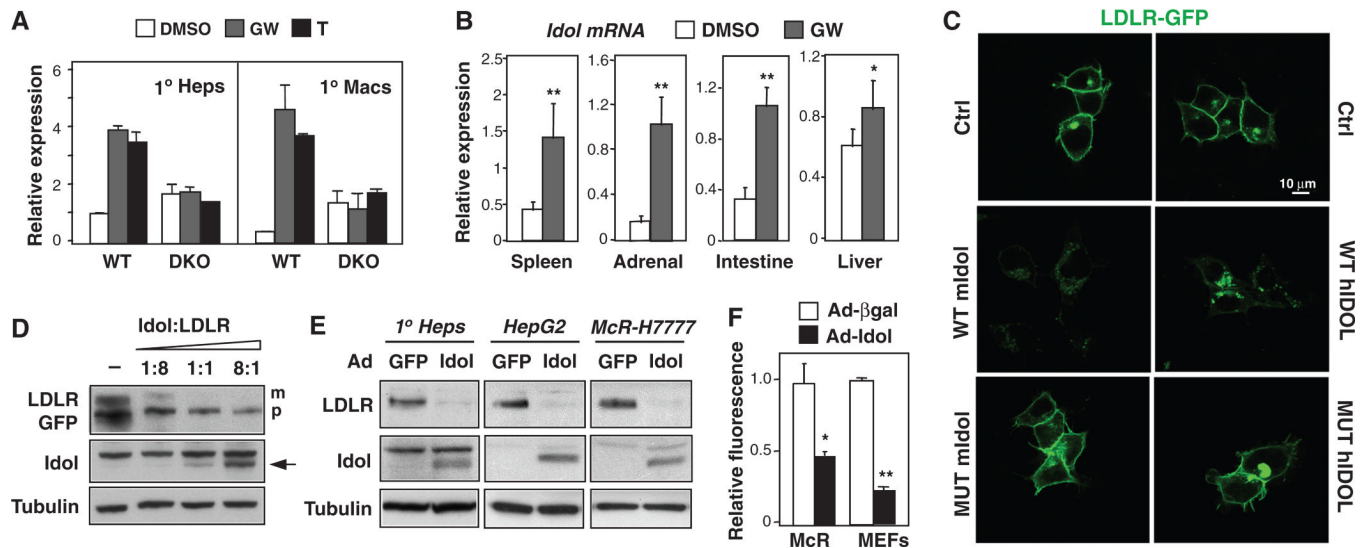
1. Russell DW, et al. Cell Jun;1984 37:577. [PubMed: 6327078]
2. Tolleshaug H, Hobgood KK, Brown MS, Goldstein JL. Cell Mar;1983 32:941. [PubMed: 6299582]
3. Brown MS, Goldstein JL. Science Apr 4;1986 232:34. [PubMed: 3513311]
4. Yokoyama C, et al. Cell Oct 8;1993 75:187. [PubMed: 8402897]
5. Hua X, et al. Proc Natl Acad Sci U S A Dec 15;1993 90:11603. [PubMed: 7903453]
6. Goldstein JL, DeBose-Boyd RA, Brown MS. Cell Jan 13;2006 124:35. [PubMed: 16413480]
7. Garcia CK, et al. Science May 18;2001 292:1394. [PubMed: 11326085]
8. Cohen JC, Kimmel M, Polanski A, Hobbs HH. Curr Opin Lipidol Apr;2003 14:121. [PubMed: 12642779]
9. Abifadel M, et al. Nat Genet Jun;2003 34:154. [PubMed: 12730697]
10. Cohen J, et al. Nat Genet Feb;2005 37:161. [PubMed: 15654334]
11. Seidah NG, et al. Proc Natl Acad Sci U S A Feb 4;2003 100:928. [PubMed: 12552133]
12. Maxwell KN, Breslow JL. Proc Natl Acad Sci U S A May 4;2004 101:7100. [PubMed: 15118091]
13. Park SW, Moon YA, Horton JD. J Biol Chem Nov 26;2004 279:50630. [PubMed: 15385538]
14. Zelcer N, Tontonoz P. J Clin Invest Mar;2006 116:607. [PubMed: 16511593]
15. Repa JJ, et al. Science Sep 1;2000 289:1524. [PubMed: 10968783]
16. Kennedy MA, et al. Cell Metab Feb;2005 1:121. [PubMed: 16054053]
17. Peet DJ, et al. Cell May 29;1998 93:693. [PubMed: 9630215]
18. Tangirala RK, et al. Proc Natl Acad Sci U S A Sep 3;2002 99:11896. [PubMed: 12193651]
19. Joseph SB, et al. Proc Natl Acad Sci U S A May 28;2002 99:7604. [PubMed: 12032330]
20. Adams CM, et al. J Biol Chem Dec 10;2004 279:52772. [PubMed: 15452130]
21. Chen W, Chen G, Head DL, Mangelsdorf DJ, Russell DW. Cell Metab Jan;2007 5:73. [PubMed: 17189208]
22. Bensinger SJ, et al. Cell Jul 11;2008 134:97. [PubMed: 18614014]
23. Olsson PA, Korhonen L, Mercer EA, Lindholm D. J Biol Chem Dec 17;1999 274:36288. [PubMed: 10593918]
24. Anderson RA, Lovrien RE. Nature Feb 16-22;1984 307:655. [PubMed: 6694756]
25. Bretscher A, Edwards K, Fehon RG. Nat Rev Mol Cell Biol Aug;2002 3:586. [PubMed: 12154370]
26. Bornhauser BC, Johansson C, Lindholm D. FEBS Lett Oct 9;2003 553:195. [PubMed: 14550572]
27. Hobbs HH, Russell DW, Brown MS, Goldstein JL. Annu Rev Genet 1990;24:133. [PubMed: 2088165]
28. Cadwell K, Coscoy L. Science Jul 1;2005 309:127. [PubMed: 15994556]
29. Maxwell KN, Fisher EA, Breslow JL. Proc Natl Acad Sci U S A Feb 8;2005 102:2069. [PubMed: 15677715]
30. Zhang DW, et al. J Biol Chem Jun 22;2007 282:18602. [PubMed: 17452316]
31. Sharkey MF, Miyanojara A, Elam RL, Friedmann T, Witztum JL. J Lipid Res Dec;1990 31:2167. [PubMed: 2090710]

32. We thank members of the Tontonoz and Edwards laboratories for fruitful discussions, especially J. Wohlschlegel, P. Edwards and S. Young. We thank P. Tarr for assistance with confocal microscopy, K. Matter and A. Gonzalez for LDLR plasmids and I. Koster for critically reading the manuscript. N.Z was supported by a long-term postdoctoral fellowship from the Human Frontier Science Program Organization. P.T. is an investigator of the Howard Hughes Medical Institute. This work was also supported by NIH grants HL066088, HL090553 and HL030568. UCLA and two of the authors (N.Z., P.T.) have filed a patent related to this work.

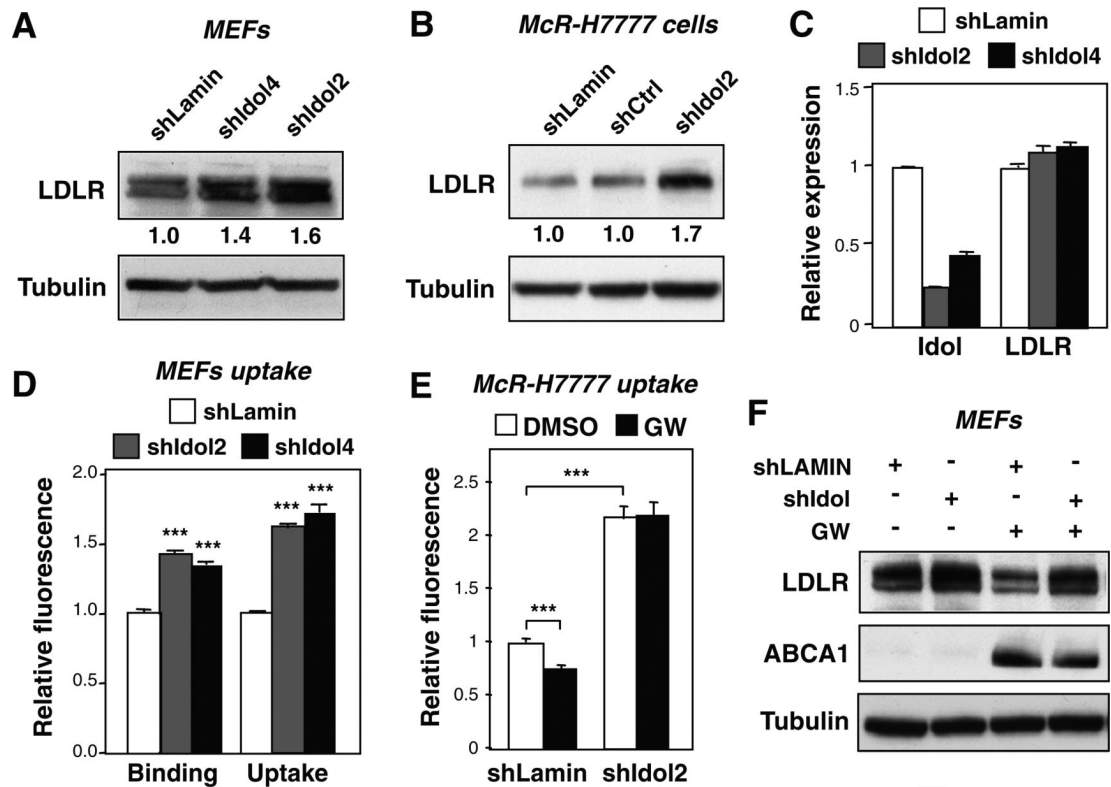
**Figure 1.**

Activation of LXR inhibits LDL uptake through reduction in LDLR protein expression. (A) BODIPY-LDL binding and uptake in HepG2 cells and mouse peritoneal macrophages treated with DMSO or the synthetic LXR ligands GW3965 (GW) and T0901317 (T) ($N=6$). (B) HepG2 cells were pretreated with DMSO or GW (1 μ M) for 8 h and subsequently grown in LPDS, or in sterol depletion medium (LPDS supplemented with 5 μ M simvastatin and 100 μ M mevalonic acid) containing either DMSO or GW for an additional 18 h. (C) Primary mouse peritoneal macrophages were cultured in sterol depletion medium and treated with indicated doses of GW for 8 h. (D) Peritoneal macrophages were cultured in sterol depletion medium and treated with GW (1 μ M) for the indicated time. (E). Peritoneal macrophages from WT or *Lxrαβ*^{-/-} mice were cultured in sterol depletion medium and treated with LXR ligands. (F) Immunofluorescence images of HepG2 cells stably expressing LDLR-GFP treated with DMSO, GW or T (1 μ M) for 72 h. All blots are representative of at least 3 independent

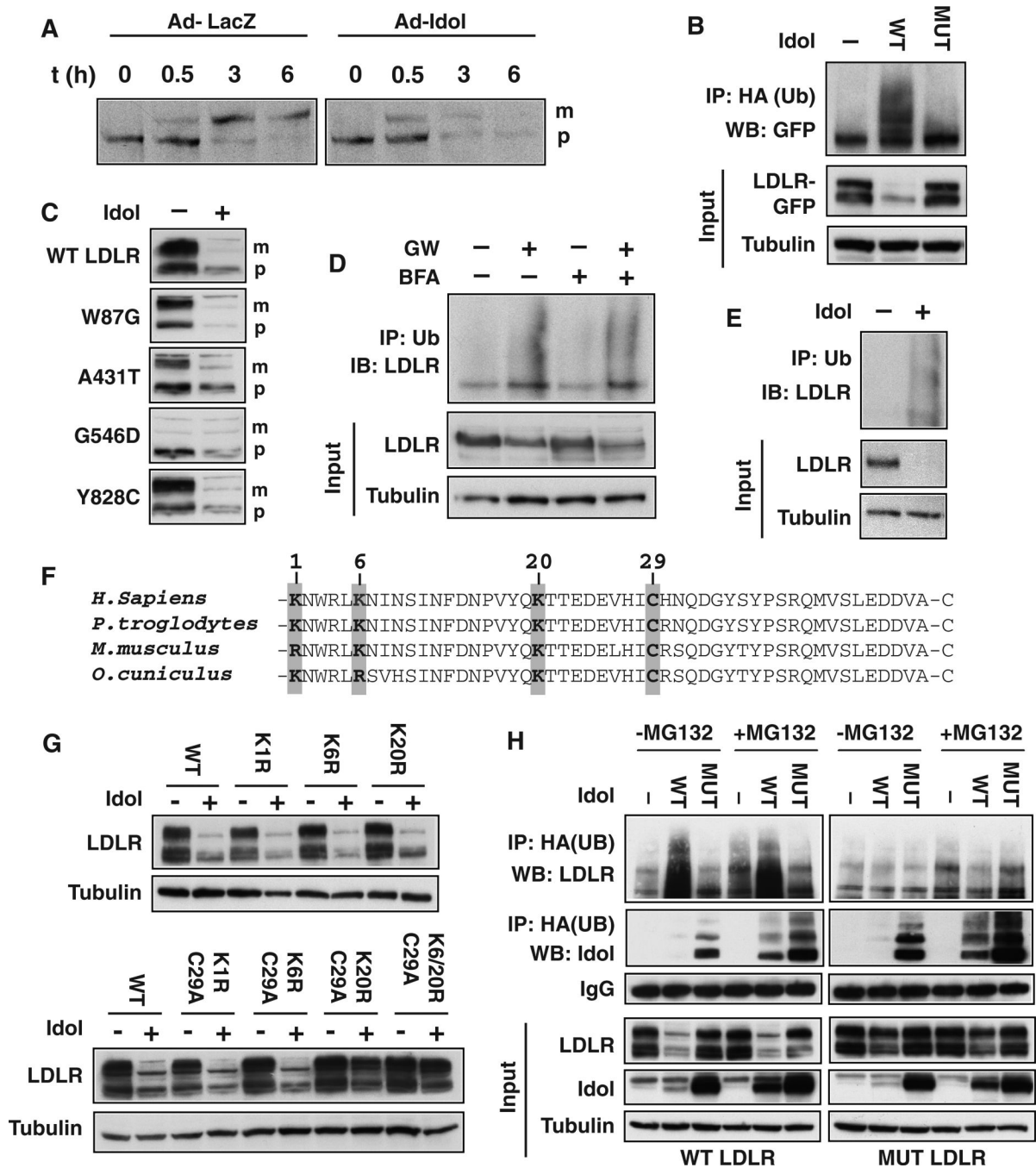
experiments. * $P < 0.05$, ** $P < 0.01$. Error bars in this and all subsequent figures represent the mean \pm SD.

**Figure 2.**

The LXR target gene *Idol* is a regulator of LDLR protein levels. (A) LXR-dependent regulation of *Idol* in primary mouse hepatocytes and peritoneal macrophages following treatment with GW or T (1 μM). (B) Induction of *Idol* mRNA expression in tissues of mice treated with 40 mg/kg GW3956 by oral gavage for 3 days ($N = 6$ per group). Gene expression was measured by realtime PCR. (C) Immunofluorescence images of HEK293 cells cotransfected with LDLR-GFP and either wildtype or RING domain mutant human and mouse *Idol*. (D) Dose-dependent reduction of LDLR-GFP protein in HEK293 cells co-transfected with m*Idol* and LDLR-GFP expression plasmids. Total cell lysates were analyzed by immunoblotting. Arrow indicates the *Idol* protein. (E) Primary hepatocytes, HepG2 cells or McRH7777 cells were cultured in sterol depletion medium and infected with Ad-GFP or Ad-*Idol*. Total cell lysates were analyzed by immunoblotting. (F) BODIPY-LDL uptake in McRH777 (McR) cells and LXRα MEFs following infection with Ad-βgal or Ad-*Idol* ($N = 3$). All blots are representative of at least 3 independent experiments. * $P < 0.05$ ** $P < 0.01$.

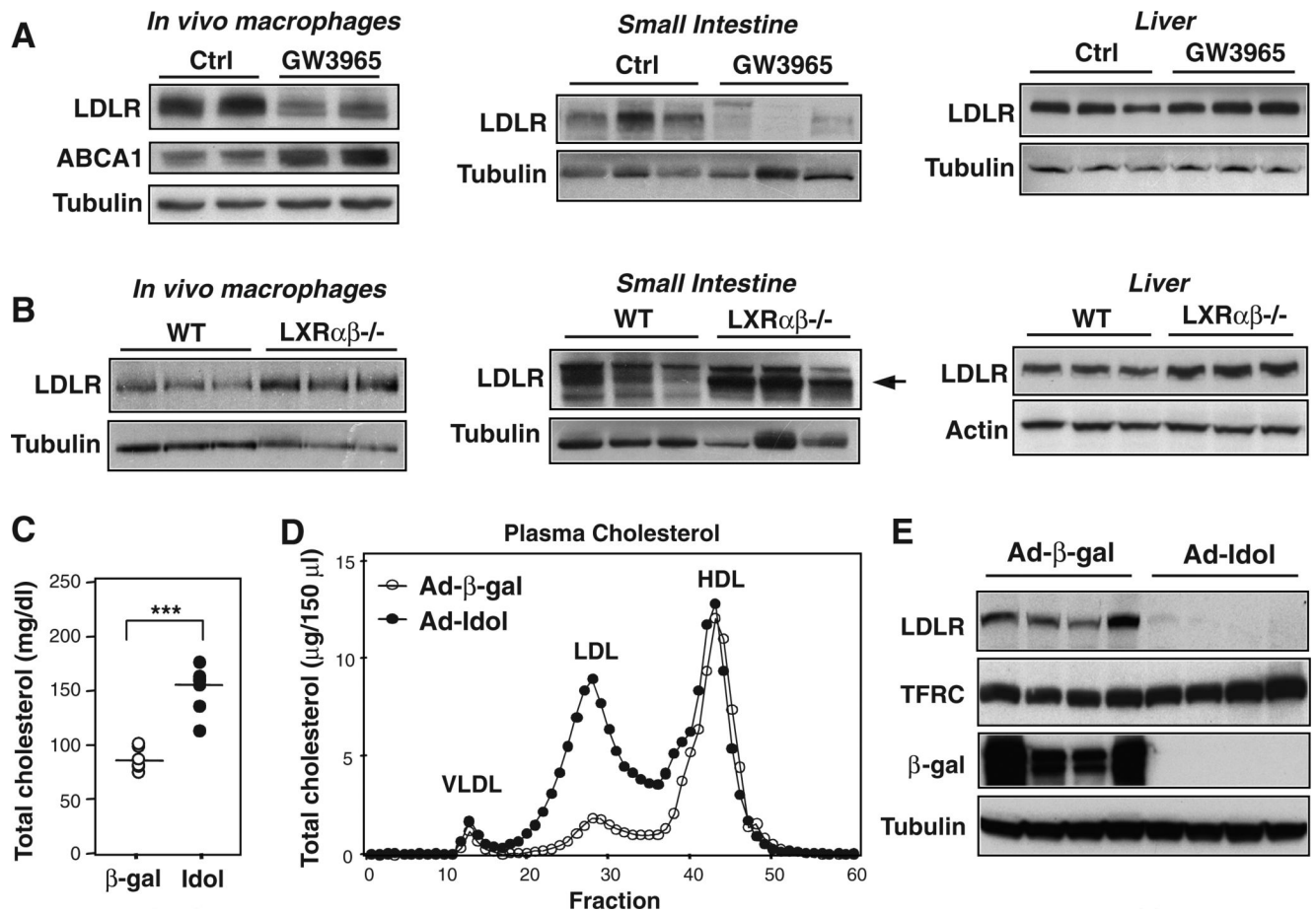
**Figure 3.**

Idol knockdown induces LDLR protein expression and promotes LDL uptake. (A) LXR α MEFs were infected with control (shLamin) or two independent adenoviral Idol shRNA constructs and cultured for in sterol-depletion medium. Cell lysates were analyzed by immunoblotting. (B) Immunoblot analysis of lysates from McRH7777 cells treated as in A. (C) Gene expression was analyzed by realtime PCR in LXR α MEFs treated as in A. ($N=3$). (D) BODIPY-LDL binding and uptake was determined for LXR α MEFs following infection with Ad-shLAMIN, Ad-shIdol2, or Ad-shIdol4 ($N=3$). (E) BODIPY-LDL uptake was determined for McRH7777 cells following infection with Ad-shLAMIN or Ad-shIdol2 followed by treatment with DMSO or GW (1 μ M) as indicated ($N=4$). (F) LXR α MEFs were infected with Ad-shLAMIN or Ad-shIdol2 for 24 h. Subsequently, cells were treated with DMSO or GW followed by culture in sterol depletion medium. All blots are representative of at least 3 independent experiments. *** $P < 0.001$.

**Figure 4.**

Idol reduces LDLR protein expression through ubiquitination of conserved residues in its cytoplasmic domain. (A) 24 h after infection with Ad-LacZ or Ad-Idol HepG2-LDLR-GFP, cells were pulsed with [³⁵S]methionine and [³⁵S]cysteine for 15 min and chased as indicated. Samples were immunoprecipitated at the indicated time points following labeling. (B) HEK293 cells were cotransfected with LDLR-GFP, Idol and HA-ubiquitin expression plasmids. After 36 h, lysates were subjected to immunoprecipitation and immunoblotting. (C) Total HEK293 cell lysates were analyzed by immunoblotting 48 h after cotransfection with Idol and WT or mutant LDLR expression plasmids. (D) Peritoneal macrophages were cultured in sterol depletion medium and treated with 1 μM GW3965 for 4 hours. Total lysates were

immunoprecipitated with anti-ubiquitin then immunoblotted for LDLR. (E) Primary mouse hepatocytes were infected with Ad-GFP or Ad-Idol and cultured in sterol depletion medium. After 24 h lysates were immunoprecipitated with anti-ubiquitin antibody and then immunoblotted for LDLR. (F) Evolutionary conservation of the LDLR intracellular domain. Potential ubiquitination sites are indicated. (G) Immunoblot analysis of HEK293 total cell lysates cotransfected with control or Idol expression plasmids along with the indicated mutated LDLR constructs. Numbering in the LDLR constructs refers to 1F. (H) HEK293 cells were cotransfected with LDLR, mutant LDLR (K6R/K20R/C29A), Idol and HA-Ubiquitin expression plasmids as indicated. Subsequently, cells were treated with vehicle or 25 μ M MG132 for 6 h. Blots are representative of at least 2 independent experiments.

**Figure 5.**

Idol expression regulates LDLR expression and affects plasma cholesterol and LDL levels *in vivo*. (A) C57BL/6 mice were treated for 3 days with 40 mpk GW3965 by oral gavage. Total lysates from resident peritoneal macrophages, small intestine (ileum) and liver were analyzed for protein levels by immunoblotting. Macrophages were isolated from the peritoneal cavity and processed without *in vitro* culture. (B) Total lysates from macrophages, small intestine (ileum) and liver from WT and Lxr $\alpha\beta^{-/-}$ mice were analyzed by immunoblotting. Macrophages were isolated from the peritoneal cavity and processed without *in vitro* culture. (C) Analysis of plasma cholesterol 6 days after transduction of C57BL/6 mice with Ad- β -gal or Ad-Idol. ($N = 8$ mice/group.) (D) Cholesterol content of size-fractionated lipoproteins from mice infected with Ad- β -gal or Ad-Idol. (E) Immunoblot analysis of total liver lysates. Data are representative of at least 2 independent experiments. *** $p < 0.001$.