The VLDL Receptor Promotes Lipotoxicity and Increases Mortality in Mice Following an Acute Myocardial Infarction

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SUPPLEMENTARY DATA

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SUPPLEMENTAL TABLES

Supplemental Table 1. Hypoxia-induced changes in lipid classes in HL-1 cells

Lipid class	Normoxia	Hypoxia	*P* value
Triglyceride	2.40 ± 0.12	4.00 ± 0.35	0.02
Cholesterol	16.0 ± 0.04	13.7 ± 0.73	0.14
Cholesterol ester	0.20 ± 0.03	0.20 ± 0.02	0.14
Phosphatidyl choline	100 ± 12.8	103 ± 0.15	0.12

Data are expressed as mean ± SEM μg lipid/mg protein (n = 6).

Supplemental Table 2. Hypoxia-induced changes in VLDLr expression in different cell types

Cell type	Species	Fold change	*P* value
Endothelial cells	Human	5.50 ± 1.01	0.0001
Smooth muscle cells	Human	4.80 ± 2.05	0.01
Myotubes	Human	2.30 ± 0.88	0.01
NIH-3T3 (fibroblasts)	Mouse	1.56 ± 0.38	0.01
HL-1 cells	Mouse	3.50 ± 0.23	<0.0001
L6 cells	Mouse	4.62 ± 0.68	0.04

VLDLr gene expression was determined by RT-PCR. Data are expressed as fold change ± SEM between hypoxia and normoxia ($n = 6$).

Supplemental Table 3. Effect of VLDLr knockout on ceramide species in mouse hearts after a sham operation or an experimentally induced myocardial infarction

Lipid class	Lipid name	VLDLr$^{+/+}$ sham	VLDLr$^{-/-}$ sham	FC	VLDLr$^{+/+}$ MI	VLDLr$^{-/-}$ MI	FC
Ceramide	**Cer(d18:0/16:0)**	0,00006±0,00001	0,00005±0,00003	1,0256	0,00006±0,00002	0,00008±0,00006	0,7666
	Cer(d18:0/18:0)	-	0,00004±0,00001	-	0,00004±0,00001	0,00006±0,00000	0,6846
	Cer(d18:0/20:0)	0,00002±0,00000	0,00003±0,00001	0,8093	0,00003±0,00000	0,00004±0,00002	0,6368
	Cer(d18:0/22:0)	-	0,00006±0,00000	-	0,00004±0,00001	0,00004±0,00001	0,9616
	Cer(d18:0/24:0)	0,00002±0,00000	-	-	0,00003±0,00001	0,00004±0,00002	0,7820
	Cer(d18:0/24:1)	0,00002±0,00000	0,00003±0,00002	0,7953	0,00002±0,00000	0,00004±0,00002	0,6669
	Cer(d18:0/24:1)	0,00367±0,00105	0,00337±0,00072	1,0916	0,00526±0,00184	0,00463±0,00276	1,1360
	Cer(d18:1/18:0)	0,0047±0,00086	0,00437±0,00073	1,0761	0,00688±0,00228	0,00507±0,00225	1,3573
	Cer(d18:1/20:0)	0,00675±0,00108	0,00904±0,00352	0,7464	0,01034±0,00434	0,01077±0,0058	0,9604
	Cer(d18:1/22:0)	0,00949±0,00129	0,00911±0,00157	1,0419	0,01204±0,00378	0,01045±0,00519	1,1524
	Cer(d18:1/24:0)	0,00531±0,00059	0,00349±0,00031	1,5207*	0,00638±0,00102	0,00451±0,00128	1,4148
	Cer(d18:1/24:1)	0,00496±0,00093	0,00364±0,00081	1,3628	0,00608±0,00119	0,00456±0,00179	1,3334
	Cer(d18:1/26:0)	0,00015±0,00002	0,00013±0,00003	1,1354	0,00017±0,00003	0,00014±0,00004	1,1957
	Cer(d18:1/26:1)	0,00005±0,00001	0,00004±0,00001	1,1754	0,00005±0,00001	0,00005±0,00002	1,0438
	Sum Cer	0,03514±0,00527	0,0333±0,00642	1,0553	0,04733±0,01438	0,04035±0,01864	1,1730
Glycoceramide	**GlcCer(d18:1/16:0)**	0,00320±0,0005	0,00219±0,00099	1,4590	0,0032±0,00042	0,00286±0,00034	1,1168
	GlcCer(d18:1/18:0)	0,00128±0,0002	0,00103±0,00036	1,2384	0,0013±0,00029	0,00127±0,00028	1,0168
	GlcCer(d18:1/20:0)	0,0026±0,00029	0,00337±0,00191	0,7692	0,00267±0,00033	0,00379±0,00118	0,7038
	GlcCer(d18:1/22:0)	0,00572±0,00049	0,00542±0,00173	1,0556	0,00604±0,00095	0,00639±0,0001	0,9457
	GlcCer(d18:1/24:0)	0,00365±0,0003	0,00215±0,00026	1,6952*	0,00416±0,00046	0,00262±0,0006	1,5883*
	GlcCer(d18:1/24:1)	0,0032±0,00048	0,00216±0,00088	1,4774	0,00353±0,00046	0,00289±0,00075	1,2204
	GlcCer(d18:1/26:0)	-	-	-	0,00018±0,00000	-	-
	Sum GlcCer	0,01963±0,00182	0,01633±0,00606	1,2024	0,02092±0,00247	0,01983±0,00351	1,0552
Lactoceramide	**LacCer(d18:0/16:0)**	0,0003±0,00005	0,00027±0,00006	1,0957	0,00028±0,00008	0,00028±0,00005	0,9951
	LacCer(d18:0/18:0)	0,00028±0,00002	0,00028±0,00002	1,1445	0,00028±0,00005		1,2766
	LacCer(d18:1/16:0)	0,00076±0,00011	0,00076±0,00011	1,2975	0,00092±0,00001	0,00073±0,00017	1,2541
	LacCer(d18:1/18:0)	0,00125±0,00026	0,00105±0,00018	1,1951	0,00166±0,00041	0,0010±0,00014	1,6510*
	LacCer(d18:1/18:0)	0,00522±0,00077	0,00715±0,0023	0,7295	0,00685±0,00128	0,00688±0,00141	0,9948
	LacCer(d18:1/22:0	0,00414±0,00037	0,00441±0,00094	0,9380	0,00520±0,00100	0,00421±0,00111	1,2357
	LacCer(d18:1/24:0)	0,00087±0,00019	0,00052±0,00011	1,6754*	0,00115±0,00022	0,00059±0,00011	1,9422*
	LacCer(d18:1/24:1)	0,00104±0,00022	0,00063±0,00016	1,6554*	0,00113±0,00025	0,0007±0,00017	1,5979
	Sum LacCer	0,01381±0,00168	0,01482±0,0035	0,9316	0,01746±0,00292	0,01448±0,00267	1,2057

Data are expressed as mean ± SD nmol lipid/mg wet weight and fold change (FC) between VLDLr$^{+/+}$ and VLDLr$^{-/-}$ mice ($n = 6$). *$P < 0.05$.

Supplemental Table 4. Echocardiographic data at baseline and after dobutamine stress (1 μg/g body weight) in VLDLr$^{+/+}$ and VLDLr$^{-/-}$ mice

	Rest		Stress	
	VLDLr$^{+/+}$	**VLDLr$^{-/-}$**	**VLDLr$^{+/+}$**	**VLDLr$^{-/-}$**
LVOT/BW (mm/g)	0.043 ± 0.003	0.048 ± 0.004*	-	-
Heart rate (BPM)	422 ± 68	353 ± 50*	474 ± 45	393 ± 48*
LPWd /BW(mm/g)	0.018 ± 0.003	0.024 ± 0.004*	0.022 ± 0.002	0.029 ± 0.004**
LVEDd /BW(mm/g)	0.14 ± 0.01	0.15 ± 0.01*	0.12 ± 0.01	0.13 ± 0.02*
LVESd /BW(mm/g)	0.09 ± 0.02	0.10 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
FS (%)	33.0 ± 8.4	36.1 ± 3.8	59.0 ± 8.1	62.7 ± 4.9
CI (ml/min/g)	1.64 ± 0.41	1.32 ± 0.31	2.06 ± 0.37	1.19 ± 0.39***
RWTh (mm)	0.26 ± 0.05	0.32 ± 0.08	0.37 ± 0.03	0.43 ± 0.07*
DTI s´ (cm/s)	2.15 ± 0.25	2.16 ± 0.22	2.91 ± 0.50	2.61 ± 0.53
DTI e´ (cm/s)	2.75 ± 0.52	2.57 ± 0.38	3.48 ± 0.54	2.81 ± 0.25**

LVOT, left ventricular outflow tract; BW, body weight; LPWd, left posterior wall diameter; LVEDd, left ventricular end-diastolic diameter; LVESd, left ventricular end-systolic diameter; FS, fractional shortening; CI, cardiac index (cardiac output/BW); RWTh, relative wall thickness; DTI s´, Doppler tissue imaging of systolic velocity; DTI e´; Doppler tissue imaging of early diastolic velocity. Data are expressed as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

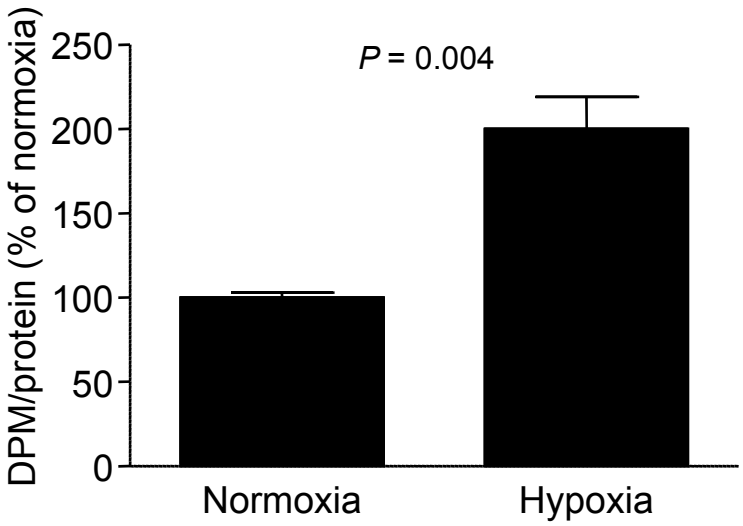
Supplemental Table 5. Characteristics of patients from whom ischemic heart biopsies were obtained and analyzed for VLDLr expression and lipid content

Number	52
Age (years)	67.0 ± 8.7
BMI (kg/m²)	27.6 ± 3.8
Number with diabetes	10
Serum lipids (mmol/l)	
Cholesterol	5.2 ± 1.1
Triglycerides	1.9 ± 0.9
LDL	1.3 ± 0.3
HDL	2.9 ± 0.9
Number taking medication	
Statins	25
Beta blockers	28
Nitrates	12
Calcium antagonists	7
Angiotensin convering enzyme inhibitors	13
Angiotensin II receptor blockers	2
Acetylsalicylic acid (aspirin)	28
Heparin	10
Wafarin	2
Glycoprotein IIB inhibitor	1
Anti arrhythmia drugs	2
Insulin	4
Other	20

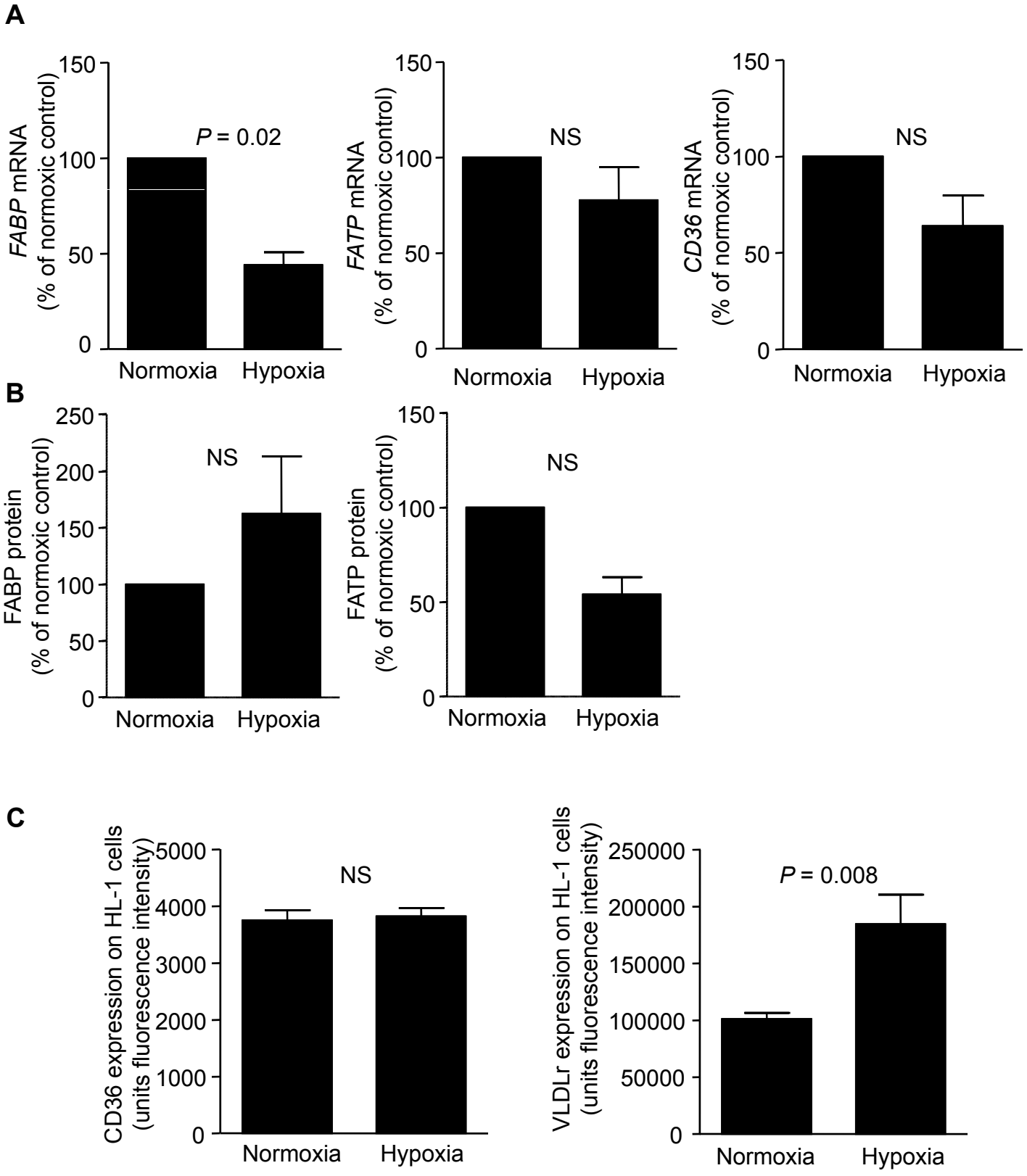
Data are expressed as mean ± SEM

Supplemental Table 6. siRNA sequences used

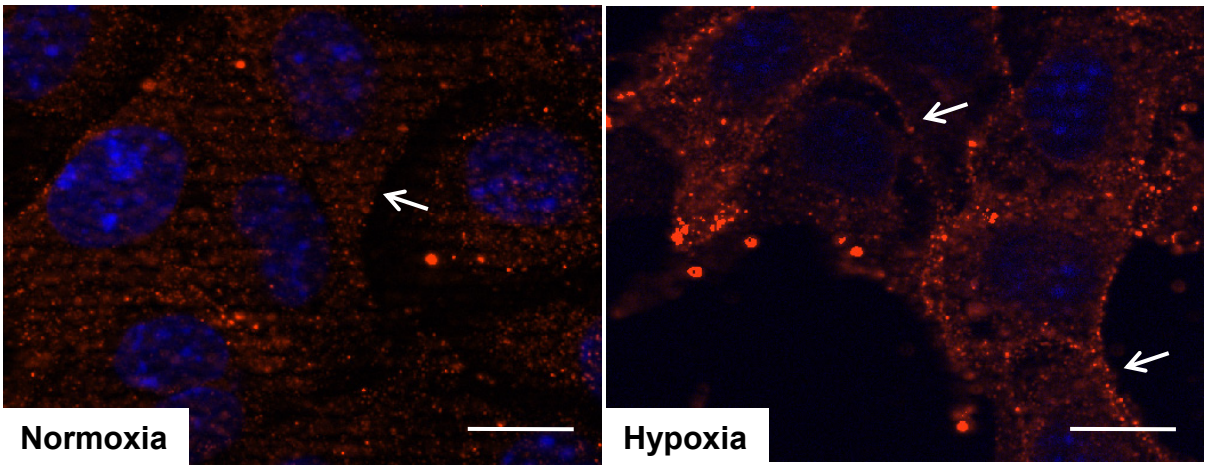
siRNA		5'→ 3'
VLDLr#1	Sense	CGAGAAGAACUGUGUAAAGtt
	Antisense	CUUUACACAGUUUCUCGtc
VLDLr#2	Sense	GAUCUUCACUAAUCGAAGAtt
	Antisense	UCUUCGAUUAGUGAAGAUCag
VLDLr#3	Sense	CCAUAGCUGUGGAUCCGUUtt
	Antisense	AACGGAUCCACAGCUAUGGag
VLDLr#4	Sense	GGAAGAUUGGCCUAGAGAGtt
	Antisense	CUCUCUAGGCCAAUCUUCCtg
HIF-1α#1	Sense	GCUUCUGUUAUGAGGCUCAtt
	Antisense	UGAGCCUCAUAACAGAAGCtt
HIF-1α#2	Sense	CAGCUGACCAGUUACGAUUtt
	Antisense	AAUCGUAACUGGUCAGCUtg
HIF-1α#3	Sense	GCUUUGAUGUGGAUAGCGAtt
	Antisense	UCGCUAUCCACAUCAAAGCaa
HIF-1α#4	Sense	GGAUACAAGCUGCCUUUUUtt
	Antisense	AAAAAGGCAGCUUGUAUCCtc
LPL#1	Sense	GGACGGUAACGGGAAUGUAtt
	Antisense	UACAUUCCCGUUACCGUCCat
LPL#2	Sense	CGAGGUUUCCACAAAUAAAtt
	Antisense	UUUAUUUGUGGAAACCUCGgg
LPL#3	Sense	AGCUCUAUCUUGUUAGUUAtt
	Antisense	UAACUAACAAGAUAGAGCUgg



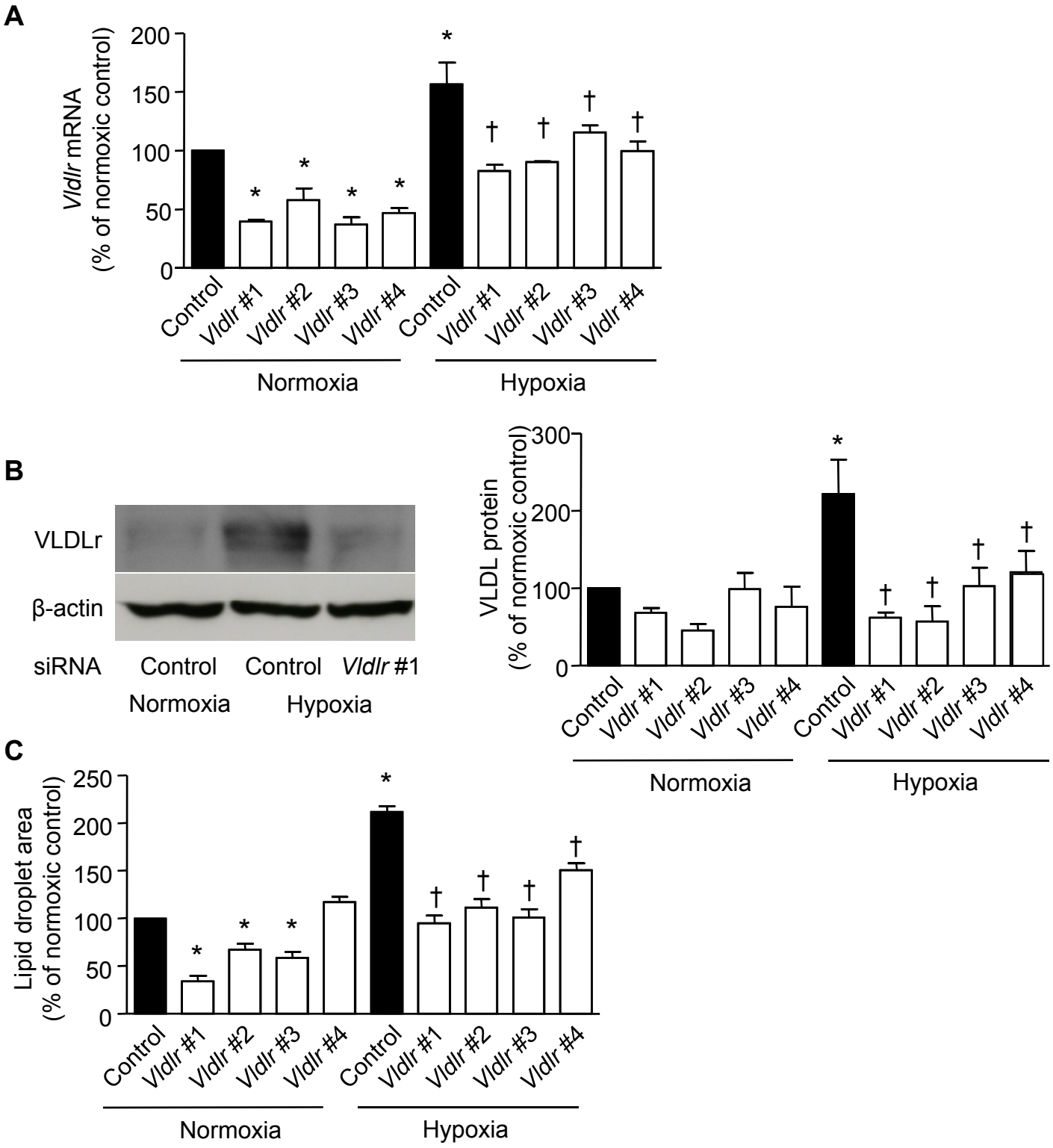
Supplemental Figure 1
Hypoxia increases uptake of fatty acids in HL-1 cells. The recovery of radioactivity was measured in HL-1 cells incubated under normoxic or hypoxic conditions for 35 min in medium containing ^{3}H-oleic acid crosslinked to albumin (n = 6). Results are shown as mean ± SEM.



Supplemental Figure 2
Hypoxia does not increase the expression of fatty acid transporters or surface expression of CD36 in HL-1 cells. (**A**) Real-time quantitative RT-PCR analysis of *FABP*, *FATP* and *CD36* mRNA and (**B**) quantification of immunoblots against FABP and FATB in HL-1 cells incubated in normoxia or hypoxia for 8 h (n = 6). (**C**) Cell surface expression of CD36 and VLDLr (as positive control) on HL-1 cells incubated in normoxia or hypoxia for 8 h (n = 3). Results are shown as mean ± SEM.

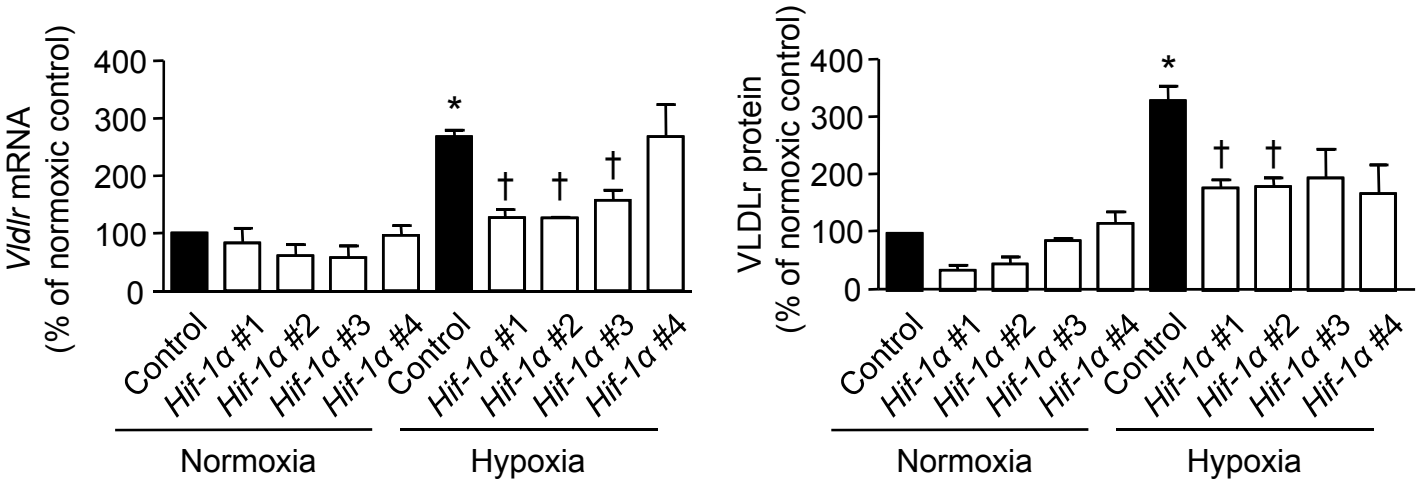


Supplemental Figure 3
Hypoxia increases the expression of VLDLr in HL-1 cells. Immunohistochemistry of VLDLr (red) and nuclei [stained blue with 4,6'-diamidino-2-phenylindole (DAPI)] in HL-1 cells incubated in normoxia or hypoxia for 8 h. Arrows indicate increased membrane VLDLr staining, scale bar represents 10 μm.

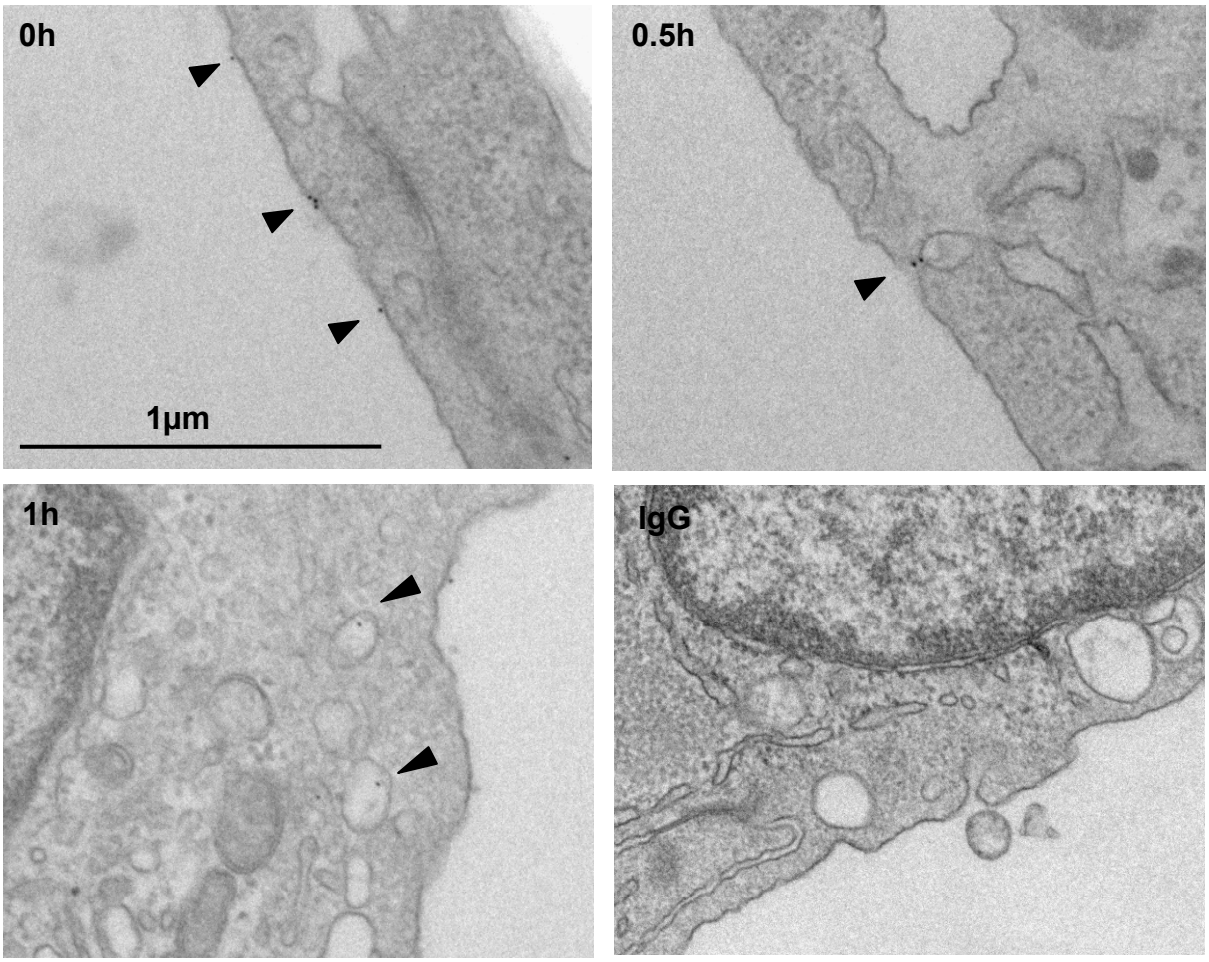


Supplemental Figure 4

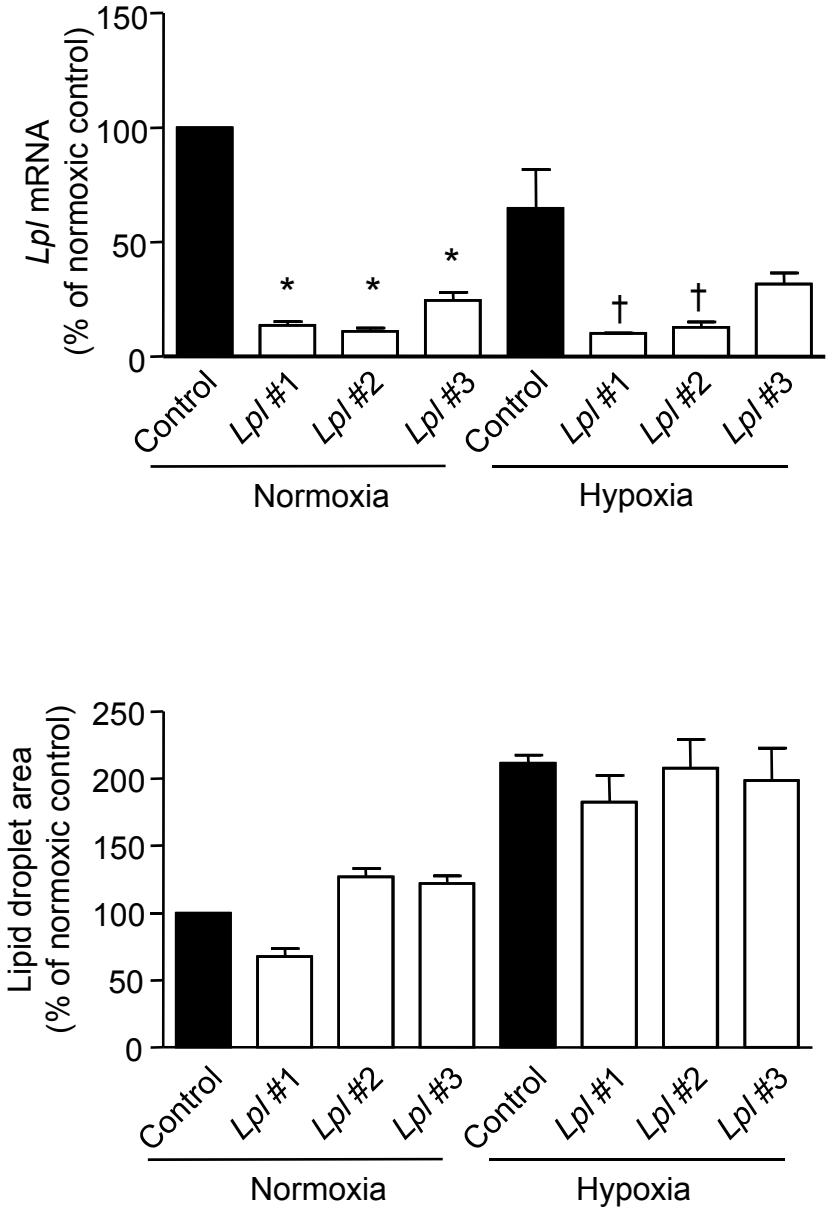
Transfection with *Vldlr* siRNAs reduces the expression of *Vldlr* and the accumulation of lipid droplets in HL-1 cells. (**A**) Real-time quantitative RT-PCR analysis of *Vldlr* mRNA expression in HL-1 cells transfected with the indicated siRNAs and incubated in normoxia or hypoxia for 8 h (n = 6). (**B**) Representative immunoblot and quantification of VLDLr in HL-1 cells transfected with the indicated siRNAs and incubated in normoxia or hypoxia for 8 h (n = 6). (**C**) The total area of Oil Red O-stained lipid droplets in HL-1 cells transfected with the indicated siRNAs and incubated in normoxia or hypoxia for 8 h (n = 6). Results are shown as mean ± SEM. *P < 0.05 vs. normoxic control; †P < 0.05 vs. hypoxic control.



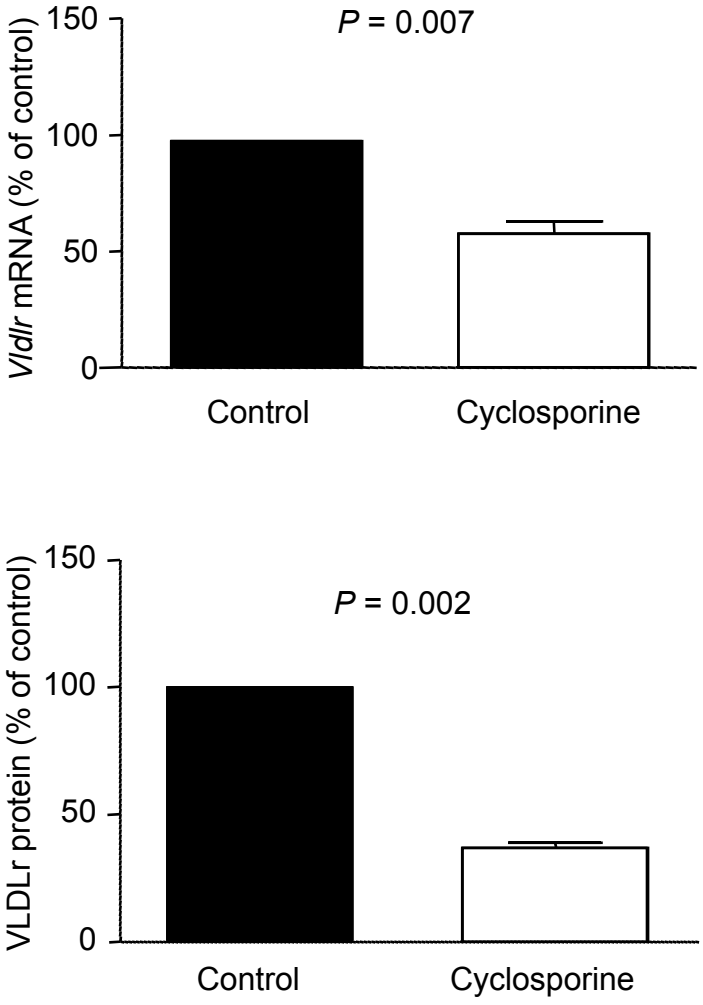
Supplemental Figure 5
Transfection with *HIF-1α* siRNAs reduces the expression of Vldlr. Real-time quantitative RT-PCR analysis of *Vldlr* mRNA (left) and VLDLr protein levels (right) in HL-1 cells transfected with the indicated siRNAs and incubated in normoxia or hypoxia for 8 h (n = 6). Results are shown as mean ± SEM. *P < 0.05 vs. normoxic control; † P < 0.05 vs. hypoxic control.



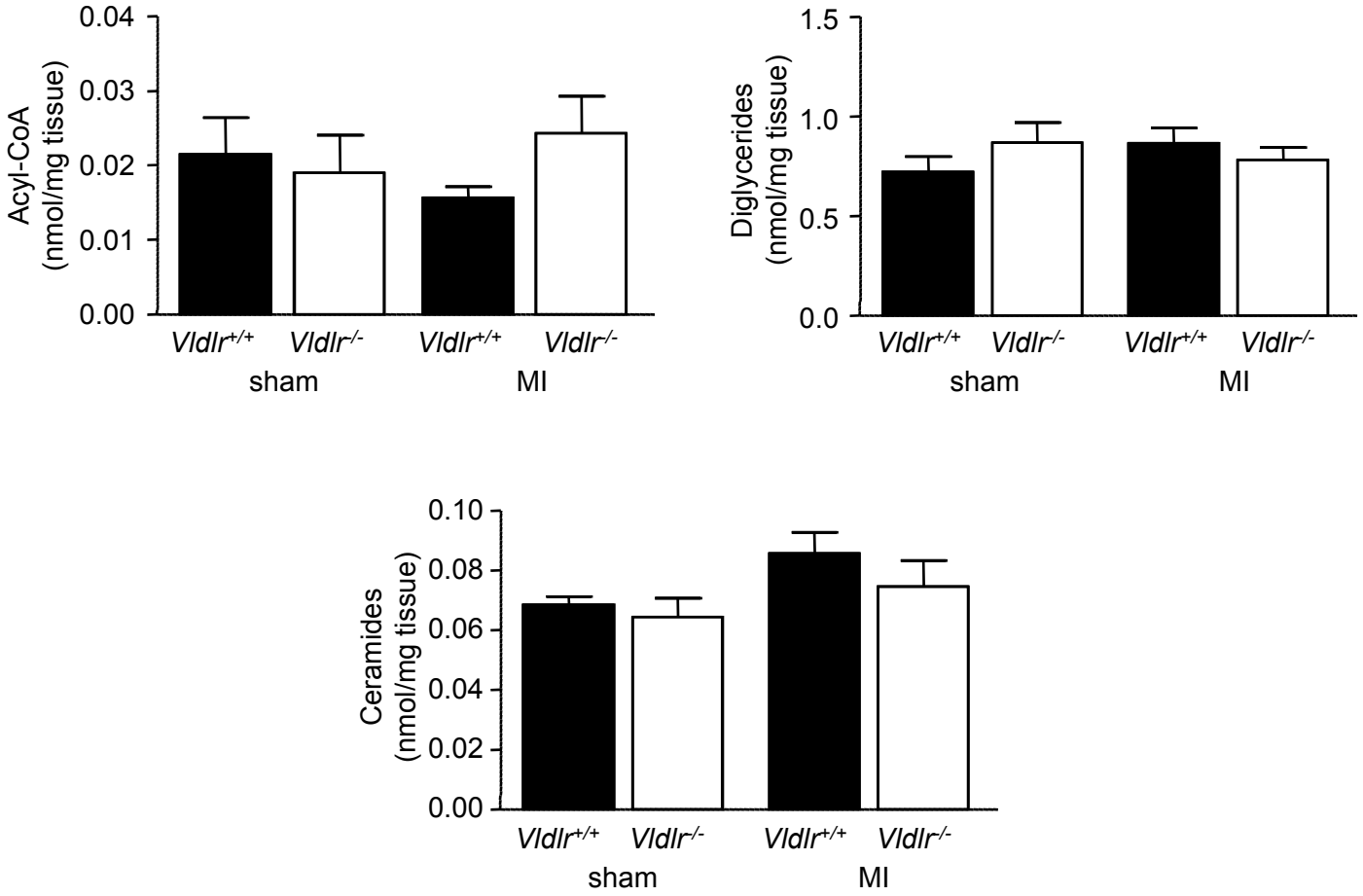
Supplemental Figure 6
The VLDLr is internalized during hypoxia in HL-1 cells. Electron-microscopy of HL-1 cells incubated with gold-labeled antibodies to VLDLr and chased for up to 1 h in hypoxic conditions. Control is incubated with gold-labeled IgG for 1 h in hypoxic conditions. Arrowheads indicate gold-labeled VLDLr, scale bar represents 1 µm.



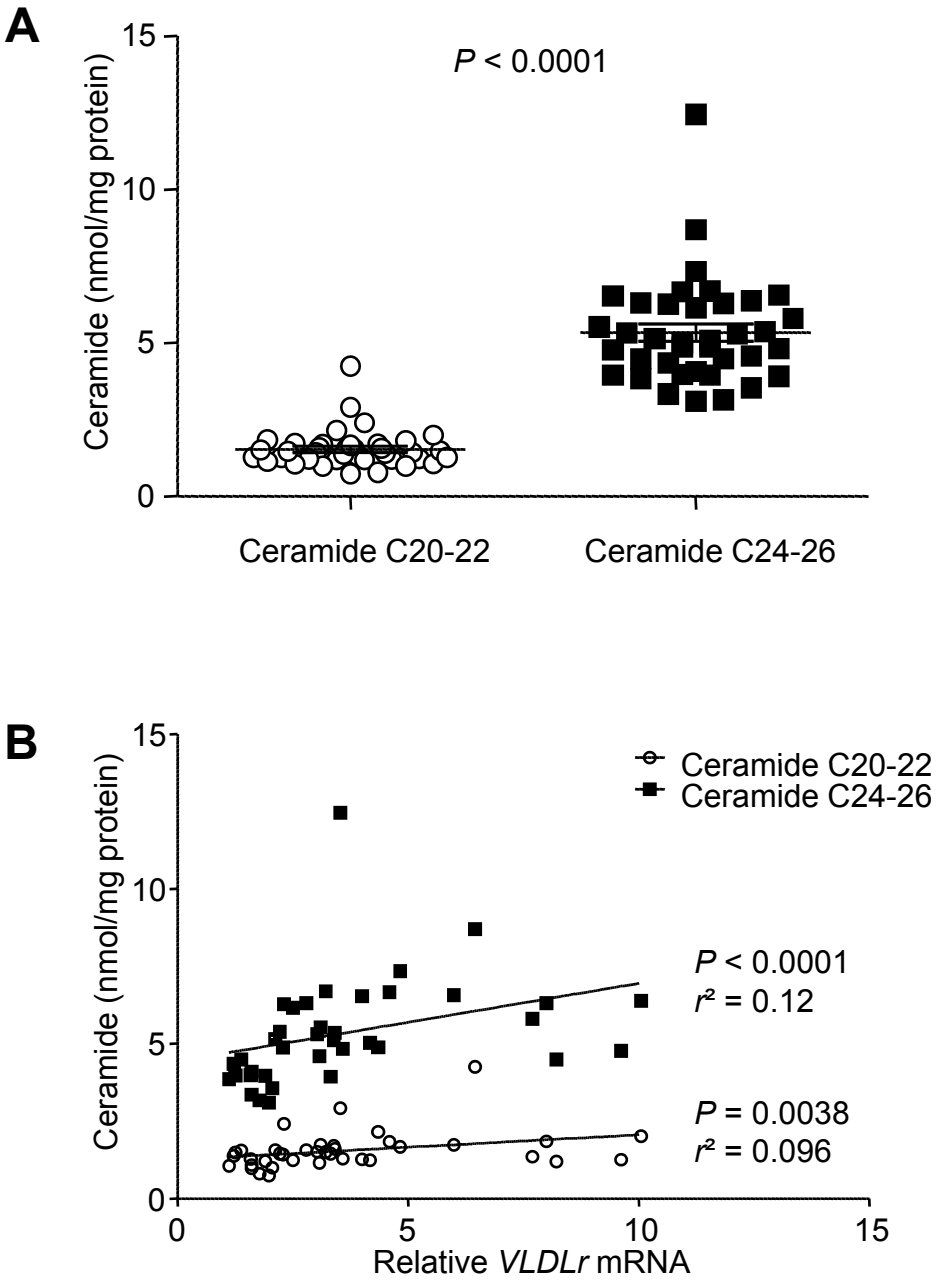
Supplemental Figure 7
Transfection with *Lpl* siRNAs does not reduce the accumulation of lipid droplets in HL-1 cells. Real-time quantitative RT-PCR analysis of *Lpl* mRNA expression (upper) and the total area of Oil Red O-stained lipid droplets (lower) in HL-1 cells transfected with the indicated siRNAs and incubated in normoxia or hypoxia for 8 h (n = 6). Results are shown as mean ± SEM.



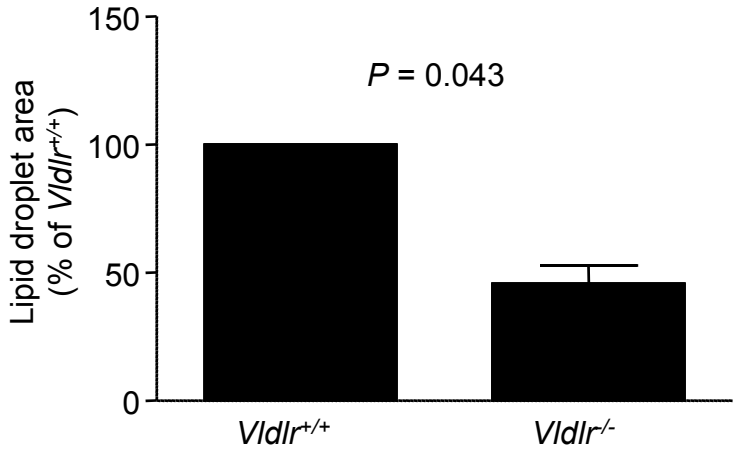
Supplemental Figure 8
Long-term cyclosporine treatment reduces VLDLr expression. Real-time quantitative RT-PCR analysis of *Vldlr* mRNA expression and quantification of immunoblots against VLDLr in HL-1 cells treated with cyclosporine (1 µmol/l) for 1 week (n = 6). Results are shown as mean ± SEM.



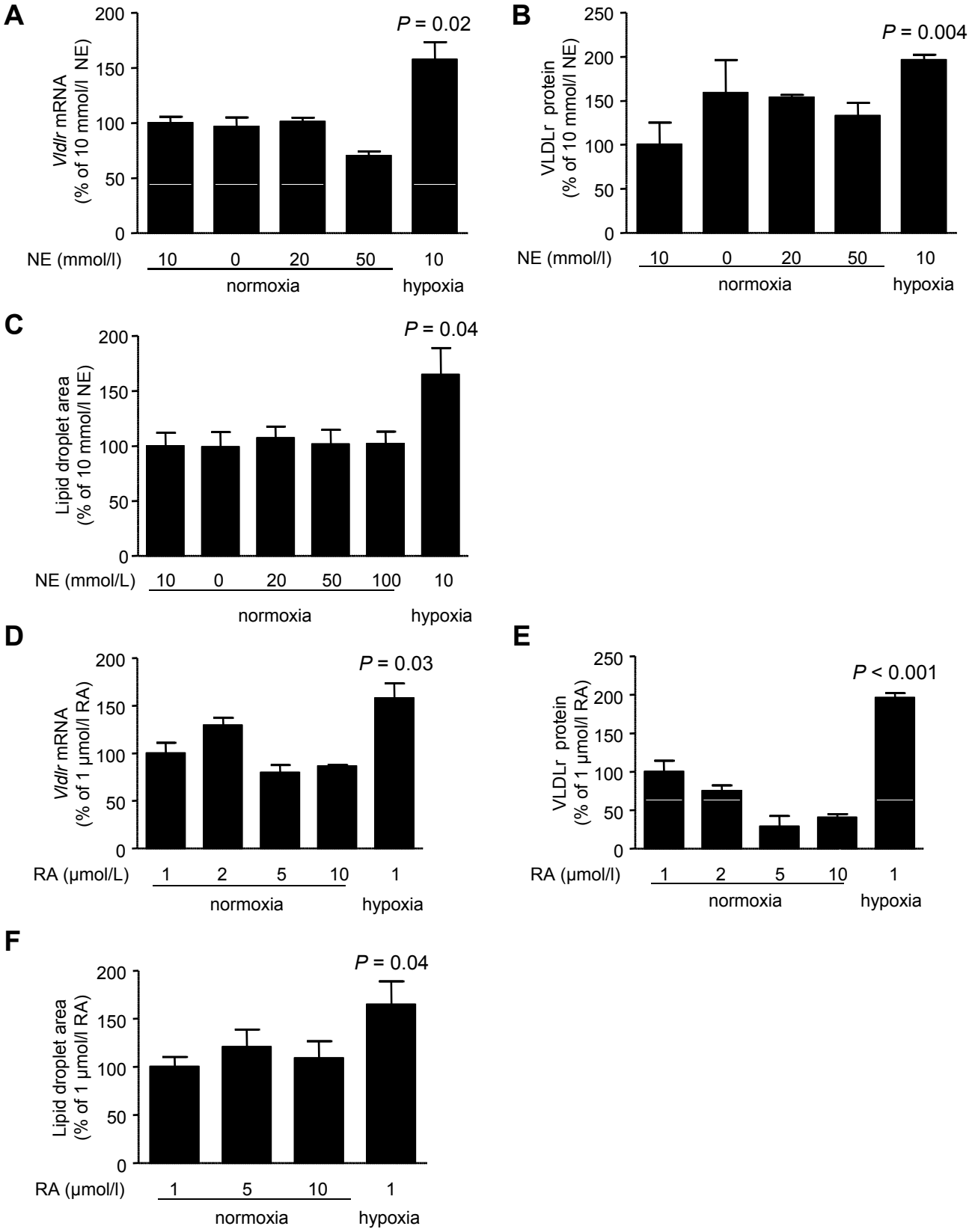
Supplemental Figure 9
VLDLr knockout does not affect total levels of lipid species in ischemic mouse hearts. Total levels of long-chain acyl-CoA, diglycerides and ceramides in hearts from *Vldlr*$^{+/+}$ and *Vldlr*$^{-/-}$ mice 6 h after a sham operation or an experimentally induced myocardial infarction (MI) (*n* = 6). Results are shown as mean ± SEM.



Supplemental Figure 10
C24-26 ceramides are the most abundant ceramide species in ischemic hearts and correlate with *VLDLr* expression. (**A**) The levels of C20-22 and C24-26 ceramides in biopsies from ischemic human hearts. (**B**) The relation between *VLDLr* mRNA expression and levels of C20-24 and C24-26 ceramides in biopsies from ischemic human hearts.

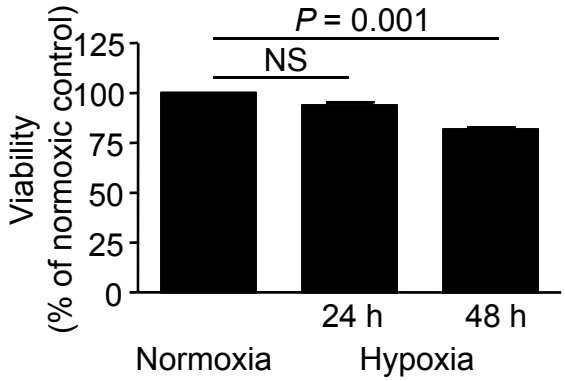


Supplemental Figure 11
The total area of Oil Red O-stained lipid droplets in in hearts from *Vldlr*$^{+/+}$ and *Vldlr*$^{-/-}$ mice 1 week after an experimentally induced myocardial infarction (*n* = 9). Results are shown as mean ± SEM.



Supplemental Figure 12

Neither norepinephrine (NE) nor retinoic acid (RA) affects VLDLr expression or lipid accumulation in HL-1 cells. *Vldlr* mRNA and protein expression and total area of Oil Red O-stained lipid droplets were not affected by incubating HL-1 cells with different concentrations of (**A-C**) NA or (**D-F**) RA for 24 h (n = 9). Results are shown as mean ± SEM.



Supplemental Figure 13
Hypoxia does not affect viability in HL-1 cells. Viability assessed by trypan blue staining in HL-1 cells incubated in normoxia or hypoxia (*n* = 9). Results are shown as mean ± SEM.