Inhibition of Lp-PLA$_2$ reduces complex coronary atherosclerotic plaque development

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Supplementary methods and tables

Supplementary Table 1. Arterial PC composition by mass spectrometry

	No DM/HC	**DM/HC Control**	**DM/HC Darapladib**
	(Values normalized to % total PC; mean ± sem)		
PC	97.30 ± 0.30	92.35 ± 0.76*	94.36 ± 0.36#
lysoPC	2.40 ± 0.23	7.33 ± 0.75*	5.31 ± 0.37#
oxPC	0.30 ± 0.08	0.32 ± 0.06	0.33 ± 0.04

Total lipid extracts from iliac arteries harvested at study end were analyzed by mass spectrometry. All results were calculated relative to the total concentration of PC within each sample. *P < 0.01 no DM/HC versus DM/HC control; #P < 0.05 DM/HC control versus darapladib (n = 3 no DM/HC, 16 DM/HC control, 18 DM/HC treated).

Supplementary Table 2. Influence of darapladib on coronary artery gene expression

Data ranked according to the degree of upregulation induced by DM/HC

Gene Description	Human Gene Symbol	Human Entrez GeneID	Upregulation with DM/HC (fold)	Influence of Darapladip	
				% Change	*P* value
Matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	MMP9	4318	69.8	-72	0.090
CD4 antigen (p55)	CD4	920	25.1	-30	0.103
Interleukin 1 receptor antagonist	IL1RN	3557	21.1	-56	0.019
cytochrome b-245, beta polypeptide (chronic granulomatous disease), gp91phox	CYBB	1536	19.4	-55	0.024
CD48 antigen (B-cell membrane protein)	CD48	962	19.2	-53	0.068
apolipoprotein E	APOE	348	17.4	-47	0.061
chitinase 3-like 1 (cartilage glycoprotein-39)	CHI3L1	1116	17.3	-57	0.076
CD68 antigen	CD68	968	16.7	-48	0.040
cathepsin S	CTSS	1520	16.0	-53	0.020
phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	PLA2G7 (Lp-PLA2)	7941	14.6	-55	0.012

integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	ITGB2	3689	14.3	-54	0.029
chemokine (C-C motif) receptor 1	CCR1	1230	11.0	-48	0.192
N-acetylneuraminate pyruvate lyase (dihydrodipicolinate synthase)	NPL	80896	10.7	-42	0.048
neutrophil cytosolic factor 1 (p47phox)	NCF1	653361	10.4	-54	0.025
heparan sulphate (glucosamine) 3-O-sulfotransferase 2	HS3ST2	9956	9.8	-55	0.162
ATP-binding cassette, sub-family A (ABC1), member 1	ABCA1	19	9.1	-22	0.128
scavenger receptor class B, member 1	SCARB1	949	7.3	-34	0.150
ecotropic viral integration site 2B	EVI2B	2124	7.2	-41	0.043
Fas ligand (TNF superfamily, member 6)	FASLG	356	7.2	-40	0.066
bridging integrator 2	BIN2	51411	7.1	-53	0.009
chemokine (C-C motif) ligand 5	CCL5	6352	7.1	-39	0.046
hypothetical protein FLJ22457	DENND2D	79961	6.9	-45	0.034
adrenergic, beta-3-, receptor	ADRB3	155	6.8	-21	0.549
plasminogen activator, urokinase receptor	PLAUR	5329	6.7	-39	0.026
heme oxygenase (decycling) 1	HMOX1	3162	6.4	-44	0.033
CD40 ligand (TNF superfamily, member 5,	CD40LG	959	6.4	-13	0.709

hyper-IgM syndrome)					
uncoupling protein 2 (mitochondrial, proton carrier)	UCP2	7351	6.1	-53	0.030
interleukin 2 receptor, alpha	IL2RA	3559	5.9	-29	0.399
platelet-activating factor receptor	PTAFR	5724	5.8	-49	0.009
GM2 ganglioside activator	GM2A	2760	5.4	-50	0.016
vascular cell adhesion molecule 1	VCAM1	7412	5.3	-7	0.680
leukocyte-associated Ig-like receptor 1	LAIR1	3903	4.8	-42	0.016
CD163 antigen	CD163	9332	4.8	-34	0.141
chemokine (C-C motif) ligand 2	CCL2	6347	4.7	-45	0.084
interleukin 1, beta	IL1B	3553	4.7	-39	0.157
CD36 antigen (collagen type I receptor, thrombospondin receptor)	CD36	948	4.5	-17	0.412
ADAM-like, decysin 1	ADAMDEC1	27299	4.4	26	0.621
interleukin 18 (interferon-gamma-inducing factor)	IL18	3606	4.1	-14	0.515
chemokine (C-X-C motif) receptor 3	CXCR3	2833	4.0	-68	0.015
interleukin 8	IL8	3576	3.8	-21	0.655
Peroxisome proliferative activated receptor, gamma	PPARG	5468	3.8	-24	0.357
ecotropic viral integration site 2A	EVI2A	2123	3.7	-39	0.041

arachidonate 5-lipoxygenase	ALOX5	240	3.6	-14	0.469
Major histocompatibility complex, class II, DM alpha	HLA-DMA	3108	3.6	-38	0.007
transmembrane 7 superfamily member 1 (upregulated in kidney)	TM7SF1	7107	3.5	2	0.947
intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	ICAM1	3383	3.5	-26	0.122
CD83 antigen (activated B lymphocytes, immunoglobulin superfamily)	CD83	9308	3.4	-39	0.139
bone morphogenetic protein 2	BMP2	650	3.3	15	0.682
chemokine (C-X-C motif) ligand 16	CXCL16	58191	3.3	-29	0.053
renal tumor antigen	RAGE	5891	3.2	-19	0.627
solute carrier family 27 (fatty acid transporter), member 4	SLC27A4	10999	3.2	-39	0.047
β-arrestin 2	ARRB2	409	3.0	-47	0.004
chemokine (C-C motif) ligand 4	CCL4	6351	3.0	-35	0.153
major facilitator superfamily domain containing 1	MFSD1	64747	3.0	-32	0.080
integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide)	ITGAM	3684	3.0	-33	0.169
CCAAT/enhancer binding protein (C/EBP), alpha	CEBPA	1050	2.9	-27	0.202
fatty acid synthase	FASN	2194	2.8	-52	0.147

Peroxisome proliferative activated receptor, delta	PPARD	5467	2.8	-37	0.123
chemokine (C-X3-C motif) receptor 1	CX3CR1	1524	2.6	-32	0.425
interleukin 6 (interferon, beta 2)	IL6	3569	2.5	-26	0.341
Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	PTGS1	5742	2.5	-6	0.879
chemokine (C-C motif) ligand 3	CCL3	6348	2.4	-38	0.198
platelet/endothelial cell adhesion molecule (CD31 antigen)	PECAM1	5175	2.3	27	0.455
insulin receptor substrate 1	IRS1	3667	2.2	10	0.819
endothelin receptor type A	EDNRA	1909	2.2	18	0.634
tumor necrosis factor (TNF superfamily, member 2)	TNF	7124	2.1	-2	0.930
chemokine (C-C motif) receptor 2	CCR2	1231	1.9	-86	0.026
basigin (OK blood group)	BSG	682	1.7	9	0.776
mesenchyme homeo box 2 (growth arrest-specific homeo box)	MEOX2	4223	1.6	5	0.926
tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)	TIMP1	7076	1.6	-47	0.105
early growth response 1	EGR1	1958	1.6	-26	0.694
transforming growth factor, beta 1 (Camurati-Engelmann disease)	TGFB1	7040	1.6	-30	0.426

Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	PTGS2	5743	1.6	38	0.485
adiponectin, C1Q and collagen domain containing	ADIPOQ	9370	1.5	56	0.620
CD97 antigen	CD97	976	1.5	-4	0.927
cystatin A (stefin A)	CSTA	1475	1.4	-213	0.178
adrenergic, beta-1-, receptor	ADRB1	153	1.3	131	0.602
insulin-like growth factor 1 receptor	IGF1R	3480	1.2	-30	0.842
chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	CXCL12	6387	1.2	17	0.886
leptin (obesity homolog, mouse)	LEP	3952	1.2	-11	0.961
Peroxisome proliferative activated receptor, alpha	PPARA	5465	1.1	107	0.889
Complement factor D (adipsin) (PREPROADIPSIN ML)	CFD	1675	1.1	72	0.825
CD34 antigen	CD34	947	1.1	128	0.825
Smoothelin	SMTN	6525	0.8	-19	0.886
matrix Gla protein	MGP	4256	0.8	-49	0.586
Actin, alpha 2, smooth muscle, aorta	ACTA2	59	0.8	-40	0.559
serine (or cysteine) proteinase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	PAI-1	5054	0.7	-74	0.358

For myeloperoxidase no measurable transcripts were found. A statistically significant effect of darapladib is highlighted in greens.

Supplementary Table 3 TAQMAN sequences for all genes studied

Human Gene Symbol	Forward	Reverse	Probe
ABCA1	AAGGTTGGTGAGTGGGCGAT	ATGGCTGTTGAGAGCTTGCG	TCCATACTTCACGAGGCCCAGTTTCCG
ACTA2	AATGGGACAAAAAGACAGCTACGT	CGTGTTCTATTGGGTACTTCAAGGT	CGAAGCCCAAAGCAA AAGAGGAATCCT
ADAMDEC1	ACTGAGCACCTCCTGGGTCC	GGCCACAGAATCCCGTTCAT	CCAAAAGCCCTCAGAT CATGGAACACTGC
ADIPOQ	ACTAGCCCTGCCCAGTCTCG	GGTACCGTTGTGGCCAGGAT	CACCGAGAAGCCTGG AGCACTACTGCC
ADRB1	GGACGTCGGTGGACGTACTG	CAGCAGGCTCTGGTAGCGAA	CGGCCAGTATCGAGA CCCTGTGTGTCA
ADRB3	GGGCACAGAGTAGGGAACCG	CTGGTGCACCTTCGTTCCTG	CCAGGCCTCTTTTGCT TCAACCCCAAG
ALOX5	TGTGCTTCCCTGAGAACATCAA	CCGTCGTCCCGGTAGAAGT	CATGGACAGCAAAGA AGACATTCCCACC
APOE	AGATGCGTGACGAGCTGGAG	CCGCATGTCTTCCACCAGAG	CAACTGGCTGCCCTG CTCCTCCACTT
ARBB2	GCTCAGCGACAACCGAGAGA	AGGACACGAGGATTCCCAGC	CTTGTTGGCACCCTCC TTCACGATGGT
BIN2	CTACAGCAGCGAGTGGGACG	CGCTGAACTGGGCGACATAG	CTGACCAGGCCTTGA GGACCATGGAAA
BMP2	CCCACGGAGGAGTTTATCACC	TGGAGTTGGCTGTTGCAGGT	TCCAAAGTCTCCTGTG TCTGTTCCCGA
BSG	GGGCCACCTAATGTCAAGGC	AAGGAGTCCGACTTGCAGGC	CTCCCCCTCGGTAGC GTGCTCTGACTT
CCL2	CTCCCACACCGAAGCTTGAA	AAGGACCTGGGTGCAGAAGG	AGCATGAAGGTCTCTG CAGCCCTCCTG
CCL3	CTCAGAGCTCCCAGGCCACT	CTCCAAGCCCGGTGTCATCT	ATGTATTCCTGGACCC AGGCGTCCTCG
CCL4	CTCTCCTCCGCCAAGACCAT	CTGAGAGCGCTGGAGAGCAG	TGACTGTCCTCTCCCT CCTGGTCCTGG
CCL5	TCCATGGCAGCAGTCGTCTT	TCAGGCTCAAGGCTTCCTCC	AGAAAGAACCGCCAG GTGTGTGCCAAC
CCR1	CTTTTGGACCCCCTACAATCTG	CTGATCGGTGAACAGGGATTC	CTGTGTTTGTTTCTGC TTTCCA
CCR2	GACCAAGCCATGCAGGTGAC	GTTTGGCAATGTGCTTTCGG	TGACGCACTGCTGCAT CAACCCTATCA
CD163	CCCTCCTTGTGGGATTGTCC	TCTGAGCACGTCACAGCAGC	CCAGATCCTGGGGCC ACAGTGACTGT

CD34	GCTTGGGCTGCTCTTTCTCC	GCCACTGAGGCAGAGGATGA	TCTCCAAGCCATGCCT CTTGACTGTGC
CD36	ACAGAGTTCGTTATCTAGCCAAGGA	CCATTGGGCTGTAGGAAAGAGA	AATATAACCCAGGACC CTGAGACCCACACA
CD4	AGATCTCGCAGAGCTGCCGT	AGGCGAGAGGTCAACTCGGT	CATGACCGCCCAGAA TCTTGGTCTGGT
CD40LG	TCATAGCCAGCCTCTGCCTG	ACTCGAAGACTCCGCCCAAG	TAAGATTCTCTCCGAC CCGCTTGGGGA
CD48	AAAGTGGCGCATTGCACATC	ACAGGGTCAAGCACCGTCAG	TACGGGTGTCCCCATT CACCTTCAGGA
CD68	TGACATGACGTTGGCTGTGC	GTCACCGTGAAGGATGGCAG	CTTCTTAGGGGCCCT GCTGGGACTCCT
CD83	AAGCCCTTGGAGCCAGTGAC	TCGTGCTTCTGTCCAGTGGG	TTTTTCCCGAGTCGAA GGCTCC
CD97	CATTGCCCAGCTCTTCGTGT	TAGGACAGGACCCAGCTCCG	TGCACCTGGGTCTTC GGCCTGTTACTC
CEBPA	AGCGCAACGTGGAGACTCA	GTTCGCGGCTCAGTTGTTC	CGGTCATTGTC ACTGGTCAGCT CCA
CFD	AAGCTGCAGGTTCTCCTGGG	TGGTGTCAGGCTGGCTGTCT	CTCGAAGCGCCTGTA CGACGTGCTC
CHI3L1	AAGTCGGTGCCACCATTCCT	CTCTGTTCCAGGCAGGGCTT	TGGACTGGACCTAGC CTGGATCTCCCC
CSTA	TCCTGCCCAGAGAAGATGGA	CCTGGATTTCTGGAGTGGCA	TCTGGCTTCAGTTAAG CCCCCAGGCAT
CTSS	CCACCTTGGATCGTCACTGG	TTCCCATCGAATGCTCCAGA	TTCTTTTCCCAGATGA GACGCCGTGCT
CX3CR1	TGAGGAACCAGCCACCAACT	CTCTCAACAGCACCAATTAAGCAT	TCCCAAGCTTCAACTT CCCCACAGC
CXCL12	TGCCGATTCTTTGAGAGCCA	CTGAAGGGCACAGTTCGGAG	CGTTGCCAGAGCCAA CATCAAGCATCT
CXCL16	GAACATCAGCCCTGGTGCC	CTGACGTGACTGCTCCTGCC	TTGGTCATCATTTTCC TTCTCACCGGA
CXCR3	CTGTCTGCTCTTTGCGCTCC	GCTGTACTGGCAGTGGGTGG	CATCTTCCTATCGGCC CACCCTGATGA
CYBB	CATGCCTTCGAGTGGTTTGC	GGCAAAGTGATTGGCCTGTG	ACCCAGATGCAGGAG AGGAACAACGCC
DENND2D	ACATCAAGCGGGAGGCAAAT	ACAAATCGACGGTTGGCCTT	AAGGCCACTTCCAAGA ACGGGCCTTCT
EDNRA	CTGATAGCCAGCCTTGCCCT	GCCCAGCCAGCAGCTTAAAT	TGGAGACCTCATCTAT GTGGTCATTGATCTCC C

EGR1	GCAGTGACCACCTCACCACC	GCCGCAAGTGGATCTTGGTA	CTTGCGTTCATCGCTC CTGGCAAACTT
EVI2A	GGCAAGCGTCAGCCTAGAAG	TAGGTTGGGCCCTGTGACCT	CTGGCAAGCAGTGGT CTATGGCCTGCT
EVI2B	ATTCTGCAGGTGGAGGTGGC	CCAGGACCATTCCAACAATGA	TTCCTCCTGACTCCCA TCAAGACCTCA
FASLG	TCCCTCTGGAATGGGAAGACA	CATTGATCACAAGGCTGCCC	TTCACCCCAGAGACCA AGGCAATTCCA
FASN	GCACGAACACAGACGGTTCC	TACAGGGAGCGGATGAGCTG	AAGGAGCAAGGTGTG ACCTTCCCCTCG
GAPDH	CATGACCACAGTCCATGCCA	AGCTCAGGGATGACCTTGCC	ACCCAGAAGACTGTG GATGGCCCCTCT
GM2A	TCAAAGCCGGCACCTACTCA	AGGGAGATCTTGGCACAGCC	CTGCCTCAACTGGAG CTACCTGGCTGG
HLA-DMA	ACCCATCGCTGAGGTGTTCA	CCCGATTCCTTCCACAGGAG	CACTGACGGTGACCT GGGAGCATCACT
HMOX1	AACGCCACCAAGTTCAAGCA	GTTGAGCAGGAAGGCGGTCT	ACCGCTCCCGAATGA ACACTCTGGAGA
HS3ST2	GGCACCCCTGGGCAAT	GCAATTTCCCAGGACCTCTTATG	CCAGATGGCTTCCCCT TGGC
ICAM1	AAGTGGGCACACCCGTTTCT	GGAGGGAGTCGCCATTATGC	CCTTGGATGGACTGTT CCCAGCCTCAG
IGF1R	CTGCCCGACAGACACTCAGG	TGTCTCTCGTCGAAGCTGGC	CCTGGGCCGTTCTCA GCCTTGTGT
IL18	TCATACGAAATCTGAACGACCAAGT	CTGAGGTGCATTATCTGAACAGTCA	TCAGGCATATCCTCAA ACACGGCTTGA
IL1B	CCTCCTCTCAGAGCCCATCC	AGAAGCTGGGTGCCAAGGAC	CAGCCTGGTGGAAAC CATGGCACACTA
IL1RN	GTTACCTAATCTCTTTCCTCCTTTTCC	TGCATCCTGCAAGGTCTCTT	TCAGAGACTGCCTGC CACCCCTTG
IL2RA	CTGGGAAAGCCGAGGAAAC	TGGCAATAACGTTGAGAAGATAAGC	CCACTGAGCACCTACT GTATGCGGGCT
IL6	TGTCGAGGCTGTGCAGATTAGTA	TGGTGGCTTTGTCTGGATTCT	AGCACTGATCCAGAC CCTGAGGCAAA
IL8	CAGCCCGTGTCAACATGACT	CACAGAGAGCTGCAGAAAGCA	AAAACTGCCAAGAAG GCAACA
IRS1	TGCTGGCATAGGCGCTTAAA	AAGGACTTCAAACAGCGCCC	CCTCCTCATCAGCCTC TGGGCAACAGT
ITGAM	ATCGCTGTCTGCCAGAAAATC	GAGGGTGACCTTGAGTTCTTCCT	AGTGTGACATCCCATC CTTCGGCATC
ITGB2	CTGCTCAGGGCTCTCAACGA	GACGAAGGGAAGCACCGTCT	TGTCCACGAAAGACC CAAAGCCGATG
LAIR1	AATCTCTCCAGGACGGGCAC	AGACAGAGCGCGAAGCACAG	CCATGGCCCAGCCCC TGCAG

LEP	CTGGCTGCTGAAGCCTTGAA	CGCACAGGCTCTCTTCTCCA	AGCTCAGGTTTCTTCC CCCGACTGTGG
MEOX2	CTCGTCTTGCATAATCGCGG	CAGAGCTTGGTGCTGCTGCT	CATCACCATCACCACC ATCACCACCAG
MFSD1	TGAACCTCATGGGATGGCTG	TCCAAGTAAGCAAGGGCCAA	TGGTCACACAACCCTT GGGGTCACACT
MGP	TTTATATCGCCACAGCAGAGATG	CGCAAGCTTCCCGGTTTAA	CGAAAGCCCAAGAGA GAATCCGAGAACTC
MMP9	CGCTCAATTTCACTCGCGTAT	CTCCGTGCTCCTTAACACCAA	CCCAACGCCGACATC GTTATCCA
MPO	CTCTGCTGCTGAGCCAGGTC	AACAACCGCTCCCGAATCTC	CCAAGCTGAATCGCC AGAACCAAATCG
NCF1	TTCCTGGTGAAGTGGCAGGA	GGATGATCCTGTTCTCCGGG	CGCCGGTAGACCAGC TTCTCAGACAGG
NPL	TCCTGAAGGAGGTGGCTGCT	AGCCCTTGGAAGGTGGGAAT	AATCCCATCCAACAAC TCTTCCGCACG
PAI-1	CAGGATAGGAGCGCAGGCTT	GTGGCAGACAGCGCAAGAGT	ACAGCAACGTGACTG GAACGCAAATCA
PECAM1	CCAGGTGCTGTTCTATAAGGATGA	CGGGCTTCGGAAATGAAAT	TCACAACGTCTCCTCC ACGAAGAACACAG
PLA2G7 (Lp-PLA2)	GGCACCTTCCTGCGTTTGTA	GGGATCCAGAGGGTGTCAGA	TATCCGTCCCAAGATG GTGACCC
PLAUR	CCTCCAAATGGCTTGCAGTG	TGCCTGTGGCTTCCAGACAT	CGGAACATCCATGAGT GCTGTTCCCCT
PPARA	AGTTCAATGCGCTGGAGCTG	CATGCACGATACCCTCCTGC	CTCCGCAGCAAATGAT AGCAGCCACAA
PPARD	CGCTGTCAAGTTCAATGCC	GACACGTTCATGAGGCCTGG	TTCATCGCAGCCATCA TTCTGTGTGGA
PPARG	ATGACAGCGACCTGGCGATA	CGATGGGCTTCACATTCAGC	TCATTATTCTCAGTGG AGACCGCCCACG
PPIA	ATGTGCCAGGGTGGTGACTT	ATGCCAGGACCCGTATGCTT	CCATAGATGGACTTGC CACCAGTGCCA
PTAFR	GCAGCATGCCCGTACTCATC	CTTGACCTCTGCATTGCGCT	CTTCTGCAACCTGGTC ATCATCCGCAC
PTGS1	CAGGGCGCCAACCTCAT	TCTTGAAGAACTGGTGGGTGAA	TTTGCCTTCTTTGCCC AACA
PTGS2	TGTATCCTCCGACAGCCAAAG	GCGGAGGTGTTCAGGAGTGT	CACTCAAGTCGAGATG ATCTACCCGCCT
RAGE	CCGGAGCGTCTGTTCTAAGC	TAGAAGCCGTCGGTGAGCAG	CACACAGAGTACGTCT CCACCCGCTGG
RPL27	GTACTCTTTCTTCCTTTCCGCTGTAT	ACCTTCCCGGGTTTCATGA	CTTGCCCATTTCAGCA GCGACCACT
RPL32	GCGCAGCGAAGAAAATGAA	AGGATACTAGCTGGGTGCTTAATCTG	TGAGCCACAACTGGA ACTCCTGTAT

SCARB1	GCAGGTTCAATGCACCCTTG	GGTGACCGGATGAATGTCCA	AGGCAGTGTCTGGCC TCCACCCTAACA
SLC27A4	CCGCATCCTGTCCTTTGTGT	CCCGGATCAGCTCCATAGTGT	ATCCGGCTGGTCCGT GTCAACG
SMTN	CGTCCCAACAGTGGCTCAA	GCTCTTGTTCCTGTGGCTTCA	CACCTCACACCGTTCC AGCCTGTGT
TGFB1	GCCCACTTGGGATCGATTAAA	CCGGTAACGTCCGAAGGAAG	CCCAACACACGGAGA CCCAGTCCTCTC
TIMP1	TCACAGGCTCTGACAAGGGC	GGACTGCCAGGTGCACATTC	TCCAGAGCCGCCACC TTGCCTG
TM7SF1	CTCCCGAGCCTGCTACAACC	ACCACGCCAAACACCACGTA	CGACTGGTACAACGT GTCAGACCAGGCA
TNF	CAAGGACTCAGATCATCGTCTCA	CGGCTTTGACATTGGCTACA	CCTCAGATAAGCCCGT CGCCCA
UCP2	AGTTCTCCGTTGCCTCTCATG	GCCAGGCTTCCCACAGTGT	TTTGTCCCCATGGAGT TGACCCCA
VCAM1	TCGTCGTGATCTTTGGAGCC	GCAAGTCAGGGAAGCGGAGT	TCTGCGCAATCATTTT GTCTTCAGGGA

Supplementary methods.

Animal model. We measured blood glucose levels daily for the first week after induction of diabetes, followed by weekly checks or daily, in cases in which the serum glucose level was >350 mg dl^{-1}. Insulin was administered based on glucose levels: 350-400 mg dl^{-1}: 11 units of long-acting insulin s.q., 401-450: 16 units, 451-500 22 units, >500 mg dl^{-1}: 25 units. The average units of administered insulin per animal over the duration of the study did not differ between groups (control- 798 ± 581, treated 682 ± 493, p=0.52). No animal suffered ketoacidosis.

To achieve hypercholesterolemia we generally administered the animals a diet consisting of 0.5% cholesterol, 5% lard and 0.4% sodium cholate. In those animals that did not achieve a serum cholesterol level over 400 mg dl^{-1} the diet was changed to one consisting of 2.0% cholesterol, 20% lard and 1.5% sodium cholate (both diets from Animal Specialties). During the entire study 4 animals in the DM/HC control group received the higher cholesterol diet (1 throughout the study period, 1 for a total of 45 days, 1 for 30 days and 1 for 15 days) while 2 animals in the treatment group received the higher diet (1 for the entire study and 1 for 15 days). If the serum cholesterol level exceeded 800 mg dl^{-1}.the diet was adjusted by adding normal pig chow to the 0.5% cholesterol diet. If, however, the cholesterol level remained above 800 mg dl^{-1}.for 2 consecutive months, the animal was removed from the study and excluded from analysis. A number of animals, in both groups, exhibited cholesterol levels exceeding 800 mg dl^{-1}

transiently so that the mean cholesterol level approached or exceeded 800 mg dl^{-1} at individual time points.

We collected blood samples at baseline and at monthly intervals for analysis of plasma Lp-PLA_2 activity from the jugular vein after the area around the manubrium sterni was shaved and cleaned with Nolvosan^{tm}. Following sedation with a single IM injection of 20 mg/kg of ketamine, 0.06 mg kg^{-1} of meditomidine, and 0.06 mg kg^{-1} of atropine anesthesia was maintained with 1-3% isoflurane. Blood was collected into EDTA-containing Vacutainer tubes (Kendal Tyco Health).

We also collected blood (100ml) for PBMC isolation in tubes containing 15% EDTA (Kendal Tyco Health). Equal volume of PBS (without Ca^{++} and Mg^{++}, Invitrogen Carlsbad, CA) was added and mixed well by gentle pipetting. Ficoll-Plaque Plus (15 ml) (Invitrogen Carlsbad, CA) was added in 50 ml conical tubes. The diluted blood sample was carefully layered over Ficoll-Plaque Plus. Samples were centrifuged at 600-740g for 20 min at room temperature. Following centrifugation, the mononuclear cell layer was transferred into a new tube, PBS was added (3x volume) and the solution was mixed well with a pipette. Samples were centrifuged at 740-980g for 10 min (4°C), supernatants were removed and the cells re-suspended in PBS. Cells were then separated into three 15 ml tubes (culturing, RNA isolation, protein isolation). Samples were centrifuged at 118g for 5 min (RT or at 4 °C) and supernatant was removed. Cells were then placed in Tri-reagent (Invitrogen) for RNA isolation. Approximately 5-6% of mononuclear cells were monocytes.

The mean body weights at study entry were: DM/HC control 30.6 ± 1.0 kg (*n*=17), DM/HC darapladib treated 29.5 ± 0.9 kg (*n*=20) and non DM/non HC control 37.7 ± 3.8 kg (*n*=3). At the end of the study the weights increased in the DM/HC control group to 50.9 ± 3.7 kg (a 66.5% increase in weight), DM/HC darapladib treated 62.5 ± 4.5 kg (111.6%) and non-DM/non-HC control 109.6 ± 10.0 kg (191.0%). Although treated animals exhibited greater weight gain than DM/HC control animals, the increase did not achieve statistical significance.

Histological evaluation. We stained and subsequently analyzed left anterior descending coronary arterial sections with Movat's pentachrome. Four to eight sections, each 5 mm in length, were evaluated per artery. Each section was immediately placed in formaldehyde with the distal edge marked. Only sections cut perpendicular to the plane of the lumen and without side branches were used for analysis. We evaluated on average 5.1 sections in the control group and 5.8 sections in the treated group. Morphometric analysis of the left anterior descending artery was performed to determine the lumen area, and areas subtended by the internal elastic lamina (IEL) and external elastic lamina (EEL). Medial area was calculated as the EEL minus –the IEL area and the plaque area was calculated as the IEL-lumen area. Morphometric analysis was performed using a NIH imaging software system. The results presented reflect the average

intimal area of all the evaluated sections in each artery. In addition the arteries were evaluated for fibrous cap thickness and lesions were classified using the modified AHA/Virmani score.[7] All analyses were performed by investigators blinded to the study group.

We determined destruction of the tunica media using the following 0–4 scale:
0 - no medial destruction
1 - internal elastic lamina disrupted but no overt medial destruction
2 - destruction of <50% of the medial thickness,
3 - destruction of >50% of the medial thickness with the EEL intact
4 - destruction of >50% of the medial thickness with the EEL disrupted.

Immunohistochemistry. Following deparaffinization, rehydration and heat-induced antigen retrieval we blocked sections with 3% hydrogen peroxide, avidin/biotin and protein block (DakoCytomation). A goat polyclonal cathepsin-S antibody (Santa Cruz Biotechnology Inc) at a concentration of 3 μg ml^{-1} was applied for 30 min. Omission of the primary antibody and an isotype matched IgG (Southern Biotechnology) was used as negative controls. The primary antibody was labeled with an anti-goat biotinylated secondary antibody (Jackson ImmunoResearch) diluted 1:200 and streptavidin-hrp (Dako Cytomation). Staining was detected with 3, 3 diaminobenzidine (DAB) and counterstained with hematoxylin. Slides were dehydrated, cleared and coverslipped. The intimal area and Cathepsin S positive (CS+) area of left anterior descending arteries (LADs) from treated and untreated animals was determined by morphometric analysis using Image-Pro Plus (MediaCybernetics).

For immunofluorescence, we prepared the slides as described above. For antigen retrieval, slides were incubated with proteinase K (DakoCytomation) for 20 min at room temperature followed by extensive rinsing with 1 x PBS. Slides were blocked for 90 min at room temperature with 10% total serum (for triple stain, 3.3% of serum from the species in which the respective primary antibodies were raised). Primary Antibodies were added to the slides (anti-Smooth Muscle α-actin, [Sigma-Aldrich], anti collagen I/III [Abd-Serotec], anti-Cathepsin S [Santa Cruz Biotechnology]) and incubated at 4 ^{o}C for 16 hrs. Slides were rinsed with 1 x PBS. Respective labeled secondary antibodies (AMCA, Texas Red, and FITC) were added to the slides and incubated for 120 minutes at room temperature. Slides were rinsed extensively with 1 x PBS and mounted with “Vectashield” hard set mounting media (Vector Laboratories).

For light microscopic images we used a Leica DM4000 B microscope utilizing the 5x, and 40x Leica objectives. Imaging was performed with a Leica DC 480 camera. Image-pro Plus 6.2 software package was used for processing and saving images.

The fluorescent microscopy images were obtained utilizing the same microscope making use of the 10x, and 20x objectives. Images were obtained by a Leica

DFC340 FX camera utilizing EL 6000 fluorescent source. Image-pro Plus 6.2 software was used for processing and saving images.

Measurement of cholesterol and lipoprotein Lp-PLA$_2$ activity within lipoprotein fractions. We measured lipoprotein fractions for cholesterol and Lp-PLA$_2$ as follows: cholesterol determination was performed using Infinity Cholesterol reagent (Thermo Corp cat #TR13421/2350-250) per manufacturer's instructions. Briefly, 150 µl of cholesterol reagent was added to 50 µl of fraction and incubated for 15 min at 37 °C. Samples are read in a fluorescence plate reader at 590 nm. To determine Lp-PLA$_2$ activity within the fractions we incubated 150 µl of the fraction with 40 µl assay buffer (20 mM HEPES, 150 mM NaCl, PH 7.4) and 10 µl of 1 mM [^{3}H]-PAF at 37 °C for 5 min.

Measurement of arterial Lp-PLA$_2$ activity and phospholipids. Frozen iliac sections were ground into small pieces using a mortar and pestle on dry ice, insuring that the samples remained frozen. Approximately 100 mg of frozen homogenate was weighed out as accurately as possible. Arterial samples were homogenized using a Polytron after addition of 1 ml buffer (mmol/L: Tris50 [pH 8], CHAPS 10, EGTA 2, EDTA 2, and 1 mg ml^{-1} of each of the protease inhibitors leupeptin, antipain, and pepstatin-A) per 100 mg of tissue. Samples were kept cool at all times. Homogenates were then centrifuged for 10 minutes at 4 °C at 335g prior to transferring supernatants to fresh tubes. The supernatant was diluted to 2 mg protein ml^{-1} with homogenization buffer prior to analysis for Lp-PLA$_2$ activity. To define the contribution of Lp-PLA$_2$ to the total aortic "PAF-AH" activity, we assayed the supernatant of the iliac homogenate in the presence or absence of a saturating concentration of the potent and selective Lp-PLA$_2$ inhibitor SB480848 (i.e., 1 µM final concentration from DMSO stock). In brief, 25 µl of supernatant (50 µg protein/tube) was added to tubes containing 165 µl buffer (20 mM HEPES, 150 mM NaCl, PH 7.4 ± SB480848) and 10 µl of 1 mM [^{3}H]-PAF and incubated at 37 °C for 30 mins.

For mass spectrometry we added approximately 100 mg of frozen ground iliac artery powder to 2 ml chloroform and 1 ml methanol, together with internal standards dimyristoyl phosphatidylcholine (40 nmoles), lysoheptadecoyl phosphatidylcholine (4 nmoles) and butylated hydroxytoluene (50 µg ml^{-1}). Samples were then homogenized with an Ultraturrex homogenizer for two minutes, with cooling in an ice bath. After further addition of 3 ml methanol and 1.6 ml (0.9% w:v) NaCl, extracts were vigorously shaken to obtain a single phase. Chloroform (2 ml) and water (2 ml) were then added with further shaking to form two phases which were then separated by centrifugation at 400g for ten min. The lower chloroform layer was decanted, dried under nitrogen gas and the dried extracts were stored under nitrogen at -80^{o}C until analysis.

In order to perform electrospray ionisation mass spectrometry we dissolved dried lipid extracts in solvent (methanol:chloroform:water 70:20:10 v:v, 50 µl) and injected 10 µl into a mobile phase of the same solvent delivered by a Waters 1525 HPLC at 10 µl min^{-1} into the electrospray ionization interface of a Quattro

Micro triple quadrupole mass spectrometer (both Waters). Collision gas-induced dissociation of all protonated PC, oxidized PC and LPC species generated a diagnostic phosphorylcholine fragment of m/z=184. Consequently, diagnostic precursor scans of m/z=184 were used to quantify PC species. We collected data in continuum mode and spectra were averaged over the eluting samples peak to provide the data for analysis. We obtained mass spectrometric data at unit resolution and processed using MassLynx software (Micromass). After conversion to centroid format, ion intensities were corrected for the ^{13}C effect and the progressive decreasing formation of the diagnostic ion with increasing molecular mass. Concentrations of PC and oxPC species were calculated with reference to the dimyristoyl PC internal standard and lyso PC species by reference to the lysoheptadecoyl PC standard as previously described.[16]

Concentrations of 27 identified PC species were calculated with reference to the dimyristoyl PC internal standard; fractional compositional values are reported for the 16 most abundant PC species, but all 27 species were used for calculation of total PC concentration. Similarly, concentrations of the 10 identified lyso PC species were calculated with reference to the lysohepatadecoyl PC standard. Ion peaks with m/z values characteristic of oxPC were identified in the mass range m/z 594-666 and quantified with reference to the dimyristoyl PC internal standard, as their extraction and ionisation efficiencies were comparable to unmodified PC species. These ion peaks most likely represent oxPC species where the modifed fatty acyl esterified at the *sn*-2 position has undergone non-enzymatic cleavage to generate shorter chain aldehyde and carboxyl substituted moieties. We made no attempt to determine structural compositions and, as the diagnostic precursor scan of m/z 184 recognizes all lipid molecules containing the phosphocholine headgroup, these ion peaks very probably contain multiple individual molecular species. Consequently, this analysis provides an assessment of the maximum contribution of oxidised species to the overall PC composition. The presence of oxPC species at these nominal masses was confirmed by exact mass analysis on an LTQ Orbitrap XL mass spectrometer (ThermoFisher) as described previously[16].

Gene expression using quantitative real-time PCR. For quantification of RNA we arrayed the RNA equivalent of 10 ng mRNA per well into 384-well plates using a Biomek FX robot (Beckman Coulter) and quantitative RT-PCR was carried out using a 7900HT Sequence Detector System (Applied Biosystems) in a 5 µl reaction volume. TaqMan Universal PCR Master Mix 2X (Applied Biosystems) and universal PCR conditions recommended by the manufacturer were followed.

We analyzed the Taqman gene expression data based on normalized expression values, using scaled geometric mean of the selected reference genes for the normalization factor calculations. The raw Taqman intensity levels for the coronary tissue samples were normalized against the intensities of three housekeeper genes (cyclophilin, RPL27 and RPL32) having self-consistent

results. The PBMC expression normalization also used three reference genes (cyclophilin and RPL27, plus BSG, a gene with similar stability in this study). This PBMC normalization did not use RPL32 expression levels as the PBMC samples showed significant RPL32 changes during the DM/HC induction period (week zero to week four).