Supplementary Material

Transcriptional regulation of drug metabolizing CYP enzymes by proinflammatory Wnt5A signaling in human coronary artery endothelial cells

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Supplementary Methods

Supplementary Results

Supplementary Table 1. Genes regulated (≥2 fold up or down) by 4 h Wnt5A treatment in HCAEC. Data are from 3 independent array experiments.

Supplementary Table 2. Substrate compounds of CYP1A1 and CYP1B1 retrieved from cytochrome P450 database SuperCYP (http://bioinformatics.charite.de/supercyp/index.php?site=home, accessed through 'CYP-drug interaction' on 03.08.2020), and SLCO2B1 substrates retrieved from metabolism and transport database Metrabase (http://www-metrabase.ch.cam.ac.uk/, accessed through 'search by protein' on 03.08.2020).

Supplementary Table 3. Genes regulated (≥2 fold up or down) by 4 h Wnt5A/IL-1β combination treatment in HCAEC. Data are from 3 independent array experiments.

Supplementary Table 4. Regulated genes of drug and xenobiotic metabolism pathways significantly *(P<0.05)* enriched in 4 h Wnt5A+IL-1β transcriptome of HCAEC. Data are from 3 independent array experiments.

Supplementary Figure 1. Drug and xenobiotic metabolism pathways most significantly *(P<0.05)* regulated by 4 h IL-1β treatment in HCAEC. Pathways represented as histograms are ranked by the –log value (P value). This figure is generated by reanalysing data published in *Skaria, T., Bachli, E., and Schoedon, G. (2019). Gene Ontology Analysis for Drug Targets of the Whole Genome Transcriptome of Human Vascular Endothelial Cells in Response to Proinflammatory IL-1. Frontiers in Pharmacology 10*. The purpose of reanalysing data from above-said study was to identify the myocardial drug and xenobiotic metabolism pathways regulated by 4 h IL-1β treatment (which was not contained in previous study) that served as an additional positive control for comparing the effects of Wnt5A/ IL-1β combination treatment with sole Wnt5A treatment in HCAEC.

Supplementary Methods

Primary cell culture

HCAEC (Cat. No. CC-2585, Clonetics, Lonza) were cultured in EGM-2MV Single Quots and 5% FBS (Clonetics, Lonza)-enriched EBM-2 medium (Clonetics, Lonza) in a Class 100 HEPA air filtered SteriCult system (Fisher Scientific, Switzerland) as described (Skaria et al., 2017; Skaria et al., 2019). HCAEC used in the present study were tested positively and functionally for CD31 (PECAM-1), CD105 (endoglin), von Willebrand Factor VIII, and acetylated low-density lipoprotein uptake as confirmed by the manufacturer (Lonza, Cell Systems). Masked low-level contamination in cultures were prevented by using antibiotic-free culture medium during the entire study period. Experiments were carried out using HCAEC from passages 3-6 only. HCAEC monolayers were treated with vehicle (sterile pyrogen free 0.1% human serum albumin in 0.9% NaCl) and recombinant human/mouse Wnt5A (250 ng/mL, Cat. No. 645-WN, R&D systems) alone or combined with recombinant human IL-1β (20 U/mL, Cat. No. 200-01B, PeproTech) for 4 h. All pipetting steps were carried out using Biopure ep Dualfilter T.I.P.S. sterile filter tips (Eppendorf) or TipOne aerosol barrier sterile filter pipet tips (USA Scientific).

Whole genome expression profiling and gene ontology analysis

Differential gene expression analysis by competitive two-colour hybridization of cRNA probes onto 4×44K Human Whole Genome Oligonucleotide microarrays (Agilent Technologies) was conducted as described (Skaria et al., 2017; Skaria et al., 2019). Following isolation of total RNA using RNeasy Mini Kit (Qiagen, Basel, Switzerland), RNA was quantified by Nanodrop spectrophotometry. RNA integrity was measured with the Agilent 2100 Bioanalyzer System (Agilent Tech., Basel, Switzerland). Only RNA samples with a RNA integrity number (RIN) >9 were used for the microarray experiments. From each of treated samples, 500 ng of total RNA were subjected to reverse transcription and labelling with Cy3- and Cy5-CTP employing the two-colour Quick Amp Labelling Kit (Agilent Tech., Switzerland) comprising internal control probes and spike in's (labelled Spike A mix with Cy-3 and Spike B mix with Cy-5) to control reaction performance and background normalization of arrays. cRNA fragmentation and hybridization onto the Human GE 4×44K V2 Microarray chips were conducted in accordance with the Quick Amp Labelling protocol (Agilent Techno., version 5.7, 2008). Ozone-induced deterioration of cyanine dyes were avoided by washing then array chips with stabilization and drying solution (Agilent Tech., Switzerland). Scanning, feature extraction and pre-processing in conjunction with data normalization of the microarrays were conducted using the Microarray Scanner and Feature Extraction Software 10.7 (Agilent Tech. Inc.) employing default settings for Agilent 4×44 K two-colour arrays. Spot value normalization was performed with the default linear-lowess normalization. Log ratios and P value Log ratios, calculated based on extensive error model and pixel level statistics computed from the feature and background for each spot, were used to indicate significant differential regulation of genes of the whole transcriptome after linear-lowess normalization and provided in all microarray datasets deposited in NCBI GEO database (available with accession numbers GSE145987, GSE62281 and GSE146691).

The accession GSE145987, presented for the first time in this manuscript, contains 2 independent microarray experiments of HCAEC treated with Wnt5A for 4 h (2 independent replicates). The accession GSE146691, presented for the first time in this manuscript, contains 3 independent microarray experiments of HCAEC treated with Wnt5A/IL-1 combination for 4 h. The accession GSE62281 contains the third independent microarray data of HCAEC treated with Wnt5A for 4 h (third independent replicate). Of these three accession numbers, only the accession GSE62281 is associated with a previous publication (Skaria et al., 2017). However,

the aforesaid previous research article (Skaria et al., 2017) contained analysis/description of the microarray data set of only 8 h Wnt5A alone and 8 h IL-1 alone treatment of HCAEC along with several other experiments addressing the effects of chronic Wnt5A treatment on endothelial barrier function pathway. It has to be noted that though microarray data of single 4 h Wnt5A treatment experiment has been contained in the GSE62281, the aforesaid previous publication (Skaria et al., 2017) contained neither analysis nor description of the 4 h Wnt5A alone or combined Wnt5A/IL-1 microarray data. Furthermore, the aforesaid previous study (Skaria et al., 2017) did not involve any gene ontology analysis for the direct drug/ drug metabolising targets even for the 8 h Wnt5A- or 8 h combined Wnt5A/IL-1-treated whole genome transcriptome of HCAEC.

To identify genes that were consistently regulated ≥ 2-fold in their expression in three independent microarray experiments in each treatment group, the pre-processed microarray data (that underwent linear-lowess normalization and error estimates) were further analysed using GeneSpring GX 9.0 Software (Agilent Tech. Inc.) with default settings for two-colour arrays. Genes regulated ≥2-fold in their expression as found from the second line GeneSpring analysis (Supplementary Tables S1 and S3) were subjected to gene ontology analysis of drug and xenobiotic metabolising enzymes using Metacore GeneGO software version 6.32.69020 (Thomson Reuters, http://portal.genego.com) after setting a P value < 0.05 and FDR cut off (0.05).

References

Skaria, T., Bachli, E., and Schoedon, G. (2017). Wnt5A/Ryk signaling critically affects barrier function in human vascular endothelial cells. *Cell Adh Migr* 11**,** 24-38.

Skaria, T., Bachli, E., and Schoedon, G. (2019). Gene Ontology Analysis for Drug Targets of the Whole Genome Transcriptome of Human Vascular Endothelial Cells in Response to Proinflammatory IL-1. *Frontiers in Pharmacology* 10.

Supplementary Results

Supplementary Table 1. Genes regulated (≥2 fold up or down) by 4 h Wnt5A treatment in HCAEC. Data are from 3 independent array experiments.

Upregulated genes	Downregulated genes
ALDH1L1	A2ML1
ATAD3C	AATK
C1orf172	ABCB5
C2CD4A	ACOT6
C6	ACSM5
CBLN4	AIM2
CEACAM21	ALS2CR12
CELA3A	ANKRD30B
CLCA2	APOL6
CNTN2	ATXN3L
CYP1A1	BCL11A
CYP1B1	BCOR
DDO	BEST1
DNM1L	BHMT
DSCR6	BIN2
EDNRA	C10orf71
EFCAB9	C10orf81
ELK1	C12orf40
ETV3	C12orf54
FAM90A7	C12orf61
FLJ10038	C14orf23
FLJ39080	C14orf86
GSG1	C18orf34
GYPE	C1orf130
HEATR1	C1orf141
HOMER1	C1orf192
HYALP1	C1orf227
IL22RA2	C20orf71
ITGA4	C2orf72
KCNS2	C5orf60
KCTD12	C6orf112
KIR3DL3	C7orf66
KRT24	C7orf72
LOC100130428	C9orf66
LOC100131702	CAGE1
LOC100507699	CCL28
LOC283588	CCNC
LOC440386	CD200R1
MAGEC2	CDH23
MIXL1	CEACAM3
MS4A2	CHI3L1
MYL10	CHRM3
MYO3A	CHRNA4
MYOD1	CLDN19
NEUROD2	CLEC4M
OR6Y1	CLRN2
OR8J1	CMAHP
PADI6	CNKSR2
PGC	CSMD2
POU5F2	CTAGE5
PSG5	DAPL1
PTAR1	DCHS2
PTCD3	DCLK1
PTPRC	DEFB113
RNASE6	DKFZp547J222
ROBO2	DMGDH
S100A14	DNAH17
SCARNA23	DTHD1
SGCD	EDNRB

SHANK2	EIF3E
SHISA2	ENO4
SLA	EPAG
SLC10A7	EPO
SLC16A2	F13A1
SLC24A4	FABP12
SLC39A12	FAM163A
SLC4A5	FBP2
SLCO2B1	FGF16
TBX21	FHAD1
TDRD6	FLJ46120
TLE1	FMN2
TMEM31	FRY
TTC30A	FSTL4
UMODL1	GAFA1
VIT	GATA4
ZNF780A	GCNT3
ZRANB3	GCNT7
	GFAP
	GFRAL
	GNAO1
	GPR6
	GPR77
	GRIP2
	GUCY2C
	HIST1H2BA
	HMCN2
	HNF4A
	HOXD9
	HPGD
	HPSE2
	HSPB7
	IGF2
	IKZF1
	IL16
	IL2
	IL31
	ISL2
	ITGAD
	ITGB6
	KAZN
	KCNV1
	KIAA1875
	KLHL15
	KLK12
	KRTAP19-4
	KRTAP7-1
	LAIR2
	LILRB4
	LIPC
	LOC100128077
	LOC100128164
	LOC100128356
	LOC100128402
	LOC100129072
	LOC100129119
	LOC100129413
	LOC100130157
	LOC100130255
	LOC100130452
	LOC100130701
	LOC100130849
	LOC100131581
	LOC100132147
	LOC100132738
	LOC100144597
	LOC100289333

	LOC100291323 LOC100507634 LOC128322 LOC150568 LOC150935 LOC220980 LOC285370 LOC285778 LOC286190 LOC339862 LOC401433 LOC440117 LOC442028 LOC494558 LOC554201 LOC645591 LOC646034 LOC728084 LOC728192 LOC729177 LOC729444 LOC90834 LOC91149 LPHN3 LRRTM2 MAGEB5 MCHR2 MCTP2 MFSD6L MGC12916 MNDA MUC12 MYBL1 MYOCD NCRNA00264 NCRNA00284 NCRNA00307 NDUFS7 NEB NHLH1 NPIPL3 NT5DC4 NTNG1 ODF2L OPN5 OR4C16 OR4D2 OR4F4 OR6C76 OR9G4 PABPC1P2 PIAS4 PLB1 PLCH1 PLP1 POM121L1P POPDC2 PPP1R1C PRAMEF13 PROZ PRPS1 PSG9 RAD9B RGR RUNX2 SAMD12 SASH1

	SCNN1A SEC14L3 SEMG2 SERPINI2 SFTPB SHCBP1L SLC14A1 SLC3A1 SNAR-C3 SNORA2B SNORA77 SPANXB2 SPINT4 SST ST8SIA6 STAP1 SULT1C2 SYCP2L SYT6 TARP TCEB3C TCTN3 TDRD5 TFAP2D THUMPD2 TIMP1 TPTE2 TRAF3IP3 TRAPPC10 TRIOBP TRPM6 TTTY21 TTTY3 TTTY8 TUBBP5 TXNRD3NB UBASH3A UBE2D3 UBE4B UGT1A4 UGT1A6 USH2A WIPF3 ZBP1 ZCCHC11 ZNF167 ZNF354C ZNF853 ZNF883 ZSCAN5B

Supplementary Table 2. Substrate compounds of CYP1A1 and CYP1B1 retrieved from cytochrome P450 database SuperCYP (http://bioinformatics.charite.de/supercyp/index.php?site=home, accessed through 'CYP-drug interaction' on 03.08.2020), and SLCO2B1 substrates retrieved from metabolism and transport database Metrabase (http://www-metrabase.ch.cam.ac.uk/, accessed through 'search by protein' on 03.08.2020).

Enzyme/ transporter	CYP1A1	CYP1B1	SLCO2B1
Substrates	Acriflavinium-chloride Alum Amiodarone Amodiaquine Benzydamine Capsaicin Chloroquine Cinnarizine Clenbuterol Dacarbazine Daunorubicin Debrisoquine Decamethrin Diclofenac Dronabinol Erythromycin Estradiol Estrone Etacrynic acid Ethinylestradiol Flunarizine Flutamide Fluvastatin Granisetron Haloperidol Ipriflavone Levothyroxine- sodium Melatonin Menadione Mercaptopurine Nicotine Nitrofurantoin Omeprazole Oxaliplatin Paracetamol Perazine Phenacetin Prazosin Procarbazine Progesterone Raltegravir Retigabine Riboflavin (vit B2) Riluzole Tamoxifen Testosterone Theophylline Toremifene Troglitazone Zotepine	Amodiaquine Caffeine Dibutylphthalate Docetaxel Erythromycin Estradiol Estrone Flutamide Hydrogen-peroxide Melatonin Oxaliplatin Procarbazine Progesterone Retinol (vit A) Rosuvastatin Testosterone Theophylline	Dinoprostone Penicillin G Bromosulfophthalein disodium Estrone-3-sulfate Dehydroepiandrosterone sulfate Taurocholic acid Pravastatin Glyburide Pregnenolone sulfate Aliskiren Pemetrexed Fluvastatin Bosentan Etoposide Methanone, [4-[4-(3,4-dihydro-6,7-dimethoxy-2(1H)-isoquinolinyl)-6,7-dimethoxy-2-quinazolinyl]hexahydro-1H-1,4-diazepin-1-yl](3,4-dihydroxy-1-pyrrolidinyl) Rocuronium N-methyl-quinidine Digoxin Pitavastatin Atorvastatin Telmisartan Rosuvastatin CP 671305 M17055 Telmisartan glucuronide Levothyroxine Talinolol Tebipenem pivoxil BDE 47 BDE99 BDE153 Bromosulfophthalein Latanoprost acid Salozinal 3-Pyridinecarboxylic acid, 6-[[(2S)-3-cyclopentyl-1-oxo-2-[4-(trifluoromethyl)-1H-imidazol-1-yl]propyl]amino]- Ethinylestradiol-3-O-sulfate Cerivastatin Montelukast Sulfasalazine Fexofenadine Eltrombopag (betaR,deltaR)-5-cyclopropyl-2-(4fluorophenyl)-4-[[[(3 fluorophenyl)methyl]amino]carbonyl]-beta,delta-dihydroxy-1H-imidazole-1-heptanoic acid Unoprostone carboxylate Ezetimibe glucuronide Iloprost Thromboxane B2

			Scutellarein 6-O-beta-D-glucuronide Scutellarein 7-O-beta-D-glucuronide

Supplementary Table 3. Genes regulated (≥2 fold up or down) by 4 h Wnt5A/IL-1β combination treatment in HCAEC. Data are from 3 independent array experiments.

Upregulated genes	Downregulated genes
ABTB2	A2ML1
ADAM21	ACOT6
ADAMTS9	ACSM5
AGMO	ADRB1
AMOT	AGBL2
AMPD3	AJAP1
APOBEC3G	AKNA
APOL2	ALDOAP2
APOL3	ANK3
APOL4	ANKRD20A2
APOL6	ANKRD20A9P
ARHGAP9	ANKRD23
ARID5B	ANKRD5
ATF3	ANXA11
ATP2C2	AQP9
ATPBD4	ARHGAP18
BARHL1	ARHGEF4
BATF3	BCL11A
BCL2A1	BEST1
BCL6	BIN2
BDKRB2	C10orf99
BHLHE41	C12orf54
BICC1	C13orf15
BIRC3	C14orf23
BMP2	C18orf34
C10orf93	C1orf141
C11orf40	C1orf229
C11orf96	C5orf52
C12orf50	C5orf62
C12orf53	C7orf58
C14orf28	C9orf66
C15orf48	CACNA1G
C19orf29OS	CAGE1
C1orf172	CCDC108
C1QTNF1	CCDC121
C1R	CCDC165
C1S	CCDC48
C22orf33	CCNC
C2CD4A	CDC14B
C2CD4B	CDCA7L
C3	CEACAM7
C3orf52	CES1P1
C6	CH25H
C6orf58	CLEC4GP1
C7orf51	CMAHP
C8orf4	CNIH3
C8orf80	COL22A1
CACNA1B	COX7B2
CAMTA1	CSMD2
CBLN4	CXorf22
CBR3	CXorf51
CCL1	CYP26B1
CCL17	DCHS2
CCL2	DDIT4L
CCL20	DEFB113
CCL3	DLL4
CCL3L3	DNAJB4
CCL4	DNALI1
CCL5	DPP6
CCL7	DSPP
CCL8	E2F2

CCRN4L	E2F8
CD69	EBF1
CD7	EFCAB11
CD70	EHD3
CD83	EIF3E
CDHR3	ENAH
CFB	EPHA5
CHDH	EPN3
CHP2	EVI2B
CHRDL2	FAM124B
CHRNA2	FAM201A
CHST6	FAM26D
CITED4	FAM74A1
CLDN1	FGF12
CLDN14	FGFBP3
CLEC2D	FGFR2
CLEC4A	FILIP1
CNOT4	FLG2
CNTN2	FLJ25328
COL27A1	FLJ41484
CPEB4	FRY
CRIP1	GATA4
CSF1	GFOD1
CSF2	GIMAP7
CSF3	GJA4
CSRP2	GKN2
CTHRC1	GLIPR1L2
CTSL1	GNG2
CX3CL1	GOLGA7B
CXCL1	GPC6
CXCL10	GPR116
CXCL2	H2AFB3
CXCL3	HOXB7
CXCL5	HRCT1
CXCL6	HS3ST1
CXCR7	HS6ST3
CYLD	HTR1D
CYP1A1	HYDIN
CYP1B1	ICA1L
CYP7A1	IGSF6
CYS1	IL16
DCUN1D2	INHBB
DDX58	ISL1
DENND2D	KCNC2
DIO3	KHK
DISC1	KIAA1239
DLG2	KIF2B
DNAJB9	LDB2
DNM1L	LHX9
DRAM1	LILRB4
DSCR6	LOC100128402
DUSP16	LOC100129413
EBI3	LOC100129518
EDNRA	LOC100130849
EFNA1	LOC100131023
EGFL6	LOC100131395
EGOT	LOC100131581
EGR2	LOC100132147
EGR3	LOC100507012
EGR4	LOC100510059
ELOVL7	LOC158376
ENKUR	LOC282980
ERC2	LOC284080
ERO1LB	LOC285954
ETV3	LOC286068
ETV7	LOC286071
F3	LOC286367

FAM101A	LOC340094
FAM122C	LOC400927
FAM129A	LOC400958
FAM129C	LOC401847
FAM150B	LOC440117
FAM201B	LOC441179
FAM71F2	LOC729156
FCGR2A	LOC729444
FCRL2	LOC90834
FER1L6-AS1	LOC91149
FGF18	LRRTM2
FGL1	LYPD1
FILIP1L	MAP7
FLJ25363	MBP
FLJ31104	MCF2L
FOXF1	MCHR2
FOXJ1	MDFI
FSTL3	MEF2C
G0S2	MNDA
GBP1	MTUS1
GBP3	MUC12
GBP4	MYCN
GBP5	NCRNA00284
GCH1	NCRNA00320
GFPT2	NEB
GK	NFIB
GOT1L1	NPIPL3
GPR37L1	NTNG1
GPR68	ODF2L
GRAMD3	OR2K2
GRK1	OR2T12
GRM4	OR5L1
HAS3	OR9G4
HBS1L	OTX1
HCG4B	OVOS2
HERC6	PALMD
HEY1	PAQR4
HGF	PARP15
HIVEP2	PCDH11X
HLA-B	PCDH15
HLA-C	PLAC8
HLA-F	PLB1
HOMER1	PLEKHG4B
ICAM1	PPFIBP2
ICAM4	PRB4
ICAM5	PRICKLE1
ICOSLG	PRO0611
IDO1	PRO1596
IER3	PRO1768
IFI30	PRODH
IFIH1	PRPS1
IFIT5	RASGRP3
IFNGR1	RASSF5
IFNGR2	RASSF9
IL10RA	RD3
IL15	RESP18
IL15RA	RFX4
IL18R1	RIN1
IL18RAP	SAMD13
IL1A	SEC14L3
IL1B	SEMG2
IL22RA2	SERTAD4
IL27	SFTPB
IL36G	SGCD
IL6	SGK223
IL8	SH3BP4
INHBA	SH3TC2

IQCA1	SHE
IRAK2	SIRPD
IRF1	SKOR1
IRX2	SLC22A2
ISG20	SLC4A10
ITGA1	SMAD6
ITGA4	SNAP91
JAM2	SNTG1
KCNE4	SOX18
KCNN2	SPANXN3
KDM6B	SPIC
KIAA0247	SRRM3
KIAA1644	ST8SIA4
KIRREL2	ST8SIA6
KLF6	SULT1C2
KLK3	SYNPO2
KRT24	TBX1
KRTAP3-2	TEX15
KYNU	THBD
LAD1	TMC1
LAMB3	TMEM100
LAMC2	TPD52L1
LIF	TRIM36
LITAF	TTN
LOC100128126	TTTY21
LOC100128262	TUBB1
LOC100128371	TUBBP5
LOC100128869	WDFY4
LOC100129104	WWTR1
LOC100131733	XKR4
LOC100507410	YIF1B
LOC148145	ZCCHC11
LOC283352	ZDHHC15
LOC283922	ZNF30
LOC284263	ZNF365
LOC284570	ZNF367
LOC285972	ZNF395
LOC393076	
LOC440896	
LOC642620	
LOC646999	
LOC728228	
LOC728752	
LTB	
LYPD6	
LYSMD3	
MAGEC2	
MAN1B1	
MAP3K8	
MCM9	
MDGA1	
MIR155HG	
MMP1	
MMP10	
MRGPRX3	
MS4A2	
MSX1	
MTHFD2L	
MTMR7	
MUC4	
MYB	
MYOM1	
N4BP2L1	
NAB1	
NAMPT	
NCOA7	
NEURL3	

NFKB1 NFKBIA NFKBID NFKBIZ NINJ1 NIPAL4 NKAIN1 NKD2 NKX3-1 NNMT NOD2 NPTX1 NR4A3 NR6A1 NRIP1 NTN1 NUAK2 NUP62CL NUPL1 OR10H1 OR11H12 OR1F1 OR2M2 OR5M11 OR5P2 OSGIN2 OXTR P2RY6 PARD3 PARP14 PDE4DIP PDE5A PDGFRA PDLIM4 PDPR PDZD2 PEG10 PELI1 PGR PITPNC1 PITX2 PLA1A PLA2G4A PLCB4 POU5F2 PPAP2B PRINS PRRX1 PSMD5 PTGFR PTGS2 PTPRC PTX3 RAB11FIP4 RASGRP1 RASSF3 RC3H1 RCAN1 RCSD1 REL RELB RFPL4B RGS16 RGS2 RIPK2 RLBP1 RND1	

RNF157 RRAD RSPO3 RTP4 RUNX1 S100A3 SAMD4A SAMD9L SAT1 SAV1 SDC4 SEC24A SELE SEMA3C SERPINA3 SERPINB13 SERPINB2 SERPINE2 SGPP2 SHANK2 SLC10A7 SLC12A7 SLC19A2 SLC22A24 SLC2A6 SLC30A3 SLC41A2 SLC44A3 SLC6A4 SLC7A2 SLC8A3 SLC9A4 SLCO4A1 SLCO5A1 SNORA60 SNX3 SOCS1 SOD2 SOX3 SPINLW1 SPON2 SQRDL SRD5A2 SSU72 ST6GAL1 ST8SIA2 STAT5A STC2 STON2 SYT9 TAP1 TAS2R39 TCHH TFAP2A TGFB3 THSD7B TIAM2 TIFA TLR2 TM4SF4 TMCO2 TMEM106A TMEM146 TMEM217 TMEM37 TMEM71 TMEM72	

TNF TNFAIP2 TNFAIP3 TNFAIP6 TNFAIP8 TNFRSF11B TNFRSF9 TNFSF10 TNFSF15 TNFSF9 TNIP1 TNIP3 TPPP TRAF1 TRIM36 TSLP TTC30A TTC39A UBD UBR4 UGCG UMODL1 UPP1 VCAM1 VEGFA VIPR2 VSTM1 WNT7B WTAP ZC3H12A ZC3H12C ZDHHC23 ZFR2 ZNF785	

Supplementary Table 4. Regulated genes of drug and xenobiotic metabolism pathways significantly *(P<0.05)* enriched in 4 h Wnt5A+IL-1β transcriptome of HCAEC. Data are from 3 independent array experiments.

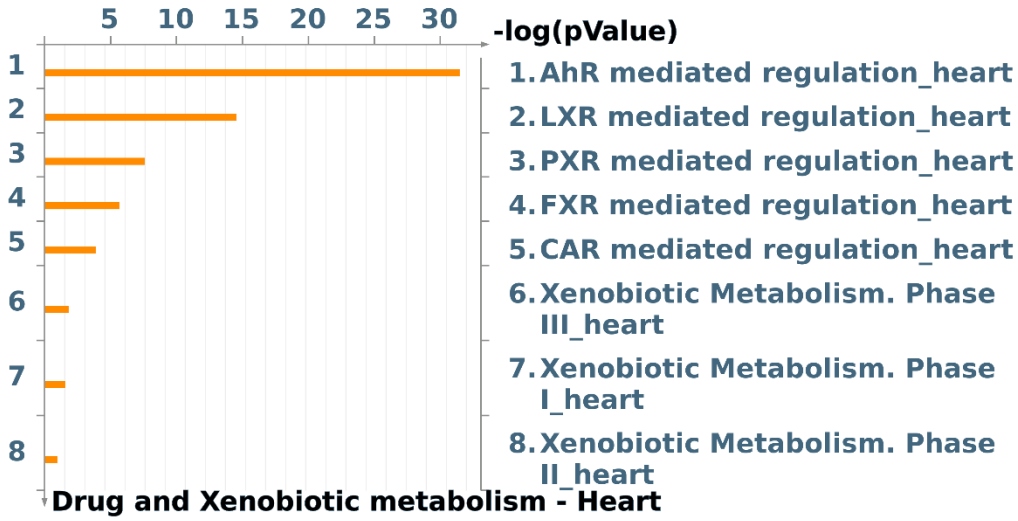
Gene symbol	Protein name	Class	Regulation
†ABTB2[a]	Ankyrin repeat and BTB/POZ domain containing protein 2	Generic binding protein	Up
†AMPD3[a]	AMP deaminase 3	Generic enzyme	Up
†AQP9[e]	Aquaporin-9	Generic channel	Down
†BCL2A1 [a]	Bcl-2-related protein A1	Generic binding protein	Up
†BDKRB2 [a,b,c]	B2 bradykinin receptor	GPCR	Up
†C1R[b]	Complement C1r subcomponent	Generic protease	Up
†C1S[b]	Complement C1s subcomponent	Generic protease	Up
†C3[b]	Complement C3	Generic binding protein	Up
†CACNA1B [a]	Voltage-dependent N-type calcium channel subunit alpha-1B	Voltage-gated ion-channel	Up
CCL1 [a]	C-C motif chemokine 1	Receptor ligand	Up
†CCL2 [a,c]	C-C motif chemokine 2	Receptor ligand	Up
†CCL20 [a]	C-C motif chemokine 20	Receptor ligand	Up
†CCL4 [a]	C-C motif chemokine 4	Receptor ligand	Up
†CCL5 [a]	C-C motif chemokine 5	Receptor ligand	Up
CCL7 [a]	C-C motif chemokine 7	Receptor ligand	Up
†CCL8[b]	C-C motif chemokine 8	Receptor ligand	Up
†CFB[b]	Complement factor B	Generic protease	Up
COX7B2 [a,b]	Cytochrome c oxidase subunit 7B2, mitochondrial	Generic enzyme	Down
CSF2[b]	Granulocyte-macrophage colony-stimulating factor	Receptor ligand	Up
†CSF3[b]	Granulocyte-macrophage colony-stimulating factor	Receptor ligand	Up
†CTSL[b]	Cathepsin L1	Generic protease	Up
†CXCL10 [b]	C-X-C motif chemokine 10	Receptor ligand	Up
*†CYP1A1 [a,c,f,g]	Cytochrome P450 1A1	Generic enzyme	Up
≠†CYP7A1 [b,c,d,f]	Cytochrome P450 7A1	Generic enzyme	Up
†CYP1B1 [a,b,g]	Cytochrome P450 1B1	Generic enzyme	Up
DCHS2 [a]	Protocadherin-23	Generic binding protein	Down
†DNALI1[b]	Axonemal dynein light intermediate polypeptide 1	Generic binding protein	Down
EBI3 [a]	Interleukin-27 subunit beta	Receptor ligand	Up
*†EDNRA [a,b,c]	Endothelin-1 receptor	GPCR	Up
†EFNA1[b]	Ephrin-A1	Receptor ligand	Up
≠†EPHA5[c]	Ephrin type-A receptor 5	Receptor with enzyme activity	Down
†FCGR2A [a]	Low affinity immunoglobulin gamma Fc region receptor II-a	Generic receptor	Up
†FILIP1L[b]	Filamin A-interacting protein 1-like	Generic protein	Up
†GBP1 [a,b]	Guanylate-binding protein 1	Generic binding protein	Up
†GCH1 [a]	GTP cyclohydrolase 1	Generic enzyme	Up
≠†GNG2 [a,b]	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	G beta/gamma	Down
†GPR37L1 [a,b,c]	G-protein coupled receptor 37-like 1	GPCR	Up
≠†HOXB7 [a]	Homeobox protein Hox-B7	Transcription factor	Down
†ICAM1 [a,c,d]	Intercellular adhesion molecule 1	Generic receptor	Up
†IDO1 [a,b]	Indoleamine 2,3-dioxygenase 1	Generic enzyme	Up
≠†IFNGR1 [a]	Interferon gamma receptor 1	Generic receptor	Up
≠†IFNGR2 [a]	Interferon gamma receptor 2	Generic receptor	Up
†IL1B [a,b,c,d]	Interleukin-1 beta	Receptor ligand	Up
†IL6 [a,b,c,d]	Interleukin-6	Receptor ligand	Up
IL10RA[b]	Interleukin-10 receptor subunit alpha	Generic receptor	Up
†IRF1 [a]	Interferon regulatory factor 1	Transcription factor	Up
†ITGA4 [a]	Integrin alpha-4	Generic receptor	Up
†KDM6B[b]	Lysine-specific demethylase 6B	Generic enzyme	Up
†KLF6 [a]	Krueppel-like factor 6	Transcription factor	Up
≠KLK3[b,f]	Prostate-specific antigen	Generic protease	Up
†LAMB3[c]	Laminin subunit beta-3	Receptor ligand	Up
†LAMC2[c]	Laminin subunit gamma-2	Receptor ligand	Up

†LILRB4[b]	Leukocyte immunoglobulin-like receptor subfamily B member 4	Generic receptor	Down
†MS4A2[b]	High affinity immunoglobulin epsilon receptor subunit beta	Generic receptor	Up
†MTMR7 [a]	Myotubularin-related protein 7	Protein phosphatase	Up
MYB[c]	Transcriptional activator Myb	Transcription factor	Up
†NAB1 [a]	NGFI-A-binding protein 1	Transcription factor	Up
†NCOA7 [a]	Nuclear receptor coactivator 7	Generic binding protein	Up
†NFIB [a]	Nuclear factor 1 B-type	Transcription factor	Down
†NFKB1 [a]	Nuclear factor NF-kappa-B p105 subunit	Transcription factor	Up
†NINJ1 [a,b]	Ninjurin-1	Generic binding protein	Up
†NNMT[b,h]	Nicotinamide N-methyltransferase	Generic enzyme	Up
†NR4A3[b]	Nuclear receptor subfamily 4 group A member 3	Transcription factor	Up
†NTN1 [a]	Netrin-1	Receptor ligand	Up
OR10H1[c]	Olfactory receptor 10H1	GPCR	Up
OR1F1[c]	Olfactory receptor 1F1	GPCR	Up
OR2K2[c]	Olfactory receptor 2K2	GPCR	Down
OR2M2[c]	Olfactory receptor 2M2	GPCR	Up
OR5L1[c]	Olfactory receptor 5L1	GPCR	Down
OR5M11[c]	Olfactory receptor 5M11	GPCR	Up
OR5P2[c]	Olfactory receptor 5P2	GPCR	Up
*OR9G4[c]	Olfactory receptor 9G4	GPCR	Down
OXTR [a,b,c]	Oxytocin receptor	GPCR	Up
†PDE5A [a]	cGMP-specific 3',5'-cyclic phosphodiesterase	Generic enzyme	Up
†PDGFRA [a,b,d]	Platelet-derived growth factor receptor alpha	Receptor with enzyme activity	Up
†PDLIM4[b]	PDZ and LIM domain protein 4	Generic binding protein	Up
†PEG10 [a]	Retrotransposon-derived protein PEG10	Generic binding protein	Up
†PGR [a]	Progesterone receptor	Transcription factor	Up
≠†PLA2G4A [a,b,g]	Cytosolic phospholipase A2	Generic phospholipase	Up
†PTGS2 [a,b,d]	Prostaglandin G/H synthase 2	Generic enzyme	Up
†REL [a]	Proto-oncogene c-Rel	Transcription factor	Up
†RELB [a]	Transcription factor RelB	Transcription factor	Up
≠RESP18 [a]	Regulated endocrine-specific protein 18	Generic protein	Down
†SAT1[b]	Diamine acetyltransferase 1	Generic enzyme	Up
†SDC4 [a,b]	Syndecan-4	Generic receptor	Up
SLC22A2[e]	Solute carrier family 22 member 2	Transporter	Down
SLC22A24[e]	Solute carrier family 22 member 24	Transporter	Up
SLC6A4[e]	Sodium-dependent serotonin transporter	Transporter	Up
†SLC7A2[e]	Cationic amino acid transporter 2	Transporter	Up
†SLCO4A1[e]	Solute carrier organic anion transporter family member 4A1	Transporter	Up
†SOCS1 [a]	Suppressor of cytokine signaling 1	Generic binding protein	Up
†SOD2 [a]	Superoxide dismutase [Mn], mitochondrial	Generic enzyme	Up
†STAT5A [a]	Signal transducer and activator of transcription 5A	Transcription factor	Up
†STC2 [a]	Stanniocalcin-2	Generic binding protein	Up
†SYT9 [a]	Synaptotagmin-9	Generic receptor	Up
TFAP2A[d]	Transcription factor AP-2-alpha	Transcription factor	Up
≠†TGFB3 [a,b]	Transforming growth factor beta-3 proprotein	Receptor ligand	Up
†TLR2 [a]	Toll-like receptor 2	Generic receptor	Up
†TNF [a,b,c,d]	Tumor necrosis factor	Receptor ligand	Up
†TNFAIP2[b]	Tumor necrosis factor alpha-induced protein 2	Generic protein	Up
≠†TNFRSF9[b]	Tumor necrosis factor receptor superfamily member 9	Generic receptor	Up
†TNFSF10[f]	Tumor necrosis factor ligand superfamily member 10	Receptor ligand	Up
†VCAM1 [a]	Vascular cell adhesion protein 1	Generic receptor	Up
≠†VEGFA[b]	Vascular endothelial growth factor A	Receptor ligand	Up
WNT7B [a]	Protein Wnt-7b	Receptor ligand	Up

	WW domain-containing transcription regulator protein 1		

Genes regulated in AhR mediated regulation_heart[a]
Genes regulated in LXR mediated regulation_heart[b]
Genes regulated in PXR mediated regulation_heart[c]
Genes regulated in FXR mediated regulation_heart[d]
Genes regulated in Xenobiotic Metabolism. Phase III_heart[e]
Genes regulated in CAR mediated regulation_heart[f]
Genes regulated in Xenobiotic Metabolism. Phase I_heart[g]
Genes regulated in Xenobiotic Metabolism. Phase II_heart[h]
*similarly regulated across Wnt5A, Wnt5A+IL-1, and IL-1[$] transcriptomes
≠regulated only in Wnt5A+IL-1 but not in Wnt5A or IL-1[$] transcriptomes
[†]Protein expression verified in normal human myocardium as shown in The Human Protein Atlas database (accessed on 03.08.2020)
[$]Genes of Drug and xenobiotic metabolism pathways most significantly ($P<0.05$) regulated by 4 h IL-1β treatment in HCAEC (Refer Supplementary Figure 1). Genes were identified by reanalysing data published in *Skaria, T., Bachli, E., and Schoedon, G. (2019). Gene Ontology Analysis for Drug Targets of the Whole Genome Transcriptome of Human Vascular Endothelial Cells in Response to Proinflammatory IL-1. Frontiers in Pharmacology 10*. The purpose of reanalysing data from above-said study was to identify the genes of myocardial drug and xenobiotic metabolism pathways regulated by 4 h IL-1β treatment (which was not contained in previous study) that served as an additional positive control for comparing the effects of Wnt5A/ IL-1β combination treatment with sole Wnt5A treatment in HCAEC.

Supplementary Figure 1



Supplementary Figure 1. Drug and xenobiotic metabolism pathways most significantly *(P<0.05)* regulated by 4 h IL-1β treatment in HCAEC. Pathways represented as histograms are ranked by the –log value (P value). This figure is generated by reanalysing data published in *Skaria, T., Bachli, E., and Schoedon, G. (2019). Gene Ontology Analysis for Drug Targets of the Whole Genome Transcriptome of Human Vascular Endothelial Cells in Response to Proinflammatory IL-1. Frontiers in Pharmacology 10*. The purpose of reanalysing data from above-said study was to identify the myocardial drug and xenobiotic metabolism pathways regulated by 4 h IL-1β treatment (which was not contained in previous study) that served as an additional positive control for comparing the effects of Wnt5A/ IL-1β combination treatment with sole Wnt5A treatment in HCAEC.