[1]Supplementary Material

Supplementary Tables

Supplemental Table S1. Antibodies for magnetic bead sorting, immunoblotting and immunofluorescence (IF)

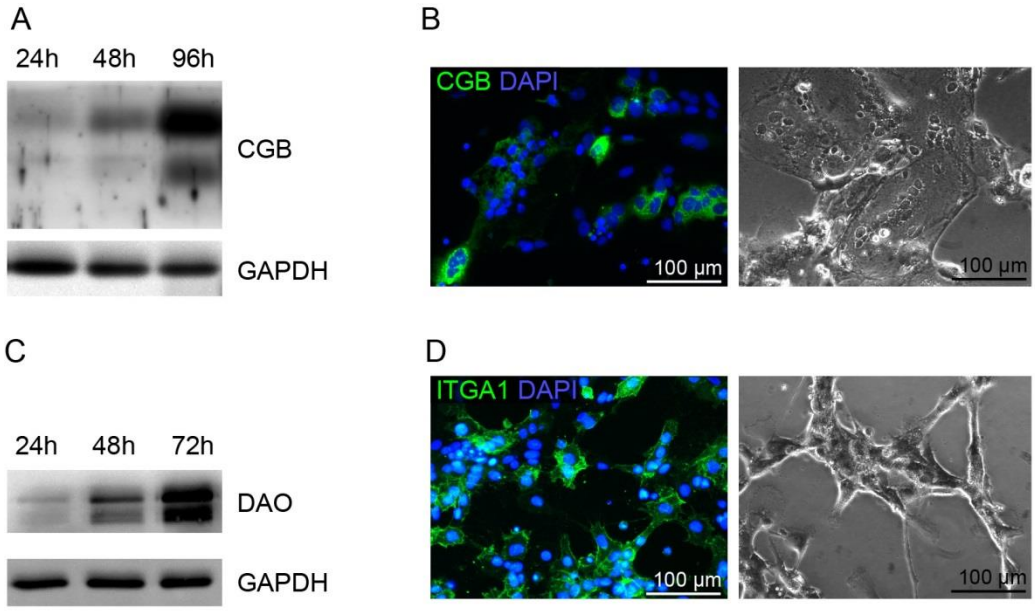
antibody	company	product #	lot #	dilution immunoblot	dilution IF
ABCA1	Abcam	ab18180	GR130756-1	1:1000	1:100
CGB	DAKO	A0231	-	1:1000	1:100
CK7	Dako	M7018	20028070	-	1:100
DAO	Sigma	HPA031033	-	1:1000	-
EGFR	Cell Signaling	4267S	11	1:1000	-
GAPDH	Cell Signaling	2118	8	1:1000	-
HLAG	EXBIO	11-291-M001	527350	1:100	-
HMGCR	Sigma-Aldrich	AMAB90619	02949	1:500	
HSD3B1	Sigma-Aldrich	HPA043264	A115246	1:1000	1:100
ITGA1	Millipore	MAB1973	-	-	1:100
LRP1	Abcam	ab92544	GR208758-1	1:1000	-
SR-BI	BD Transduction Laboratories	610882	T40262160652	1:500	-

[1]Supplementary material to Vondra et al, Cholesterol metabolism in trophoblasts

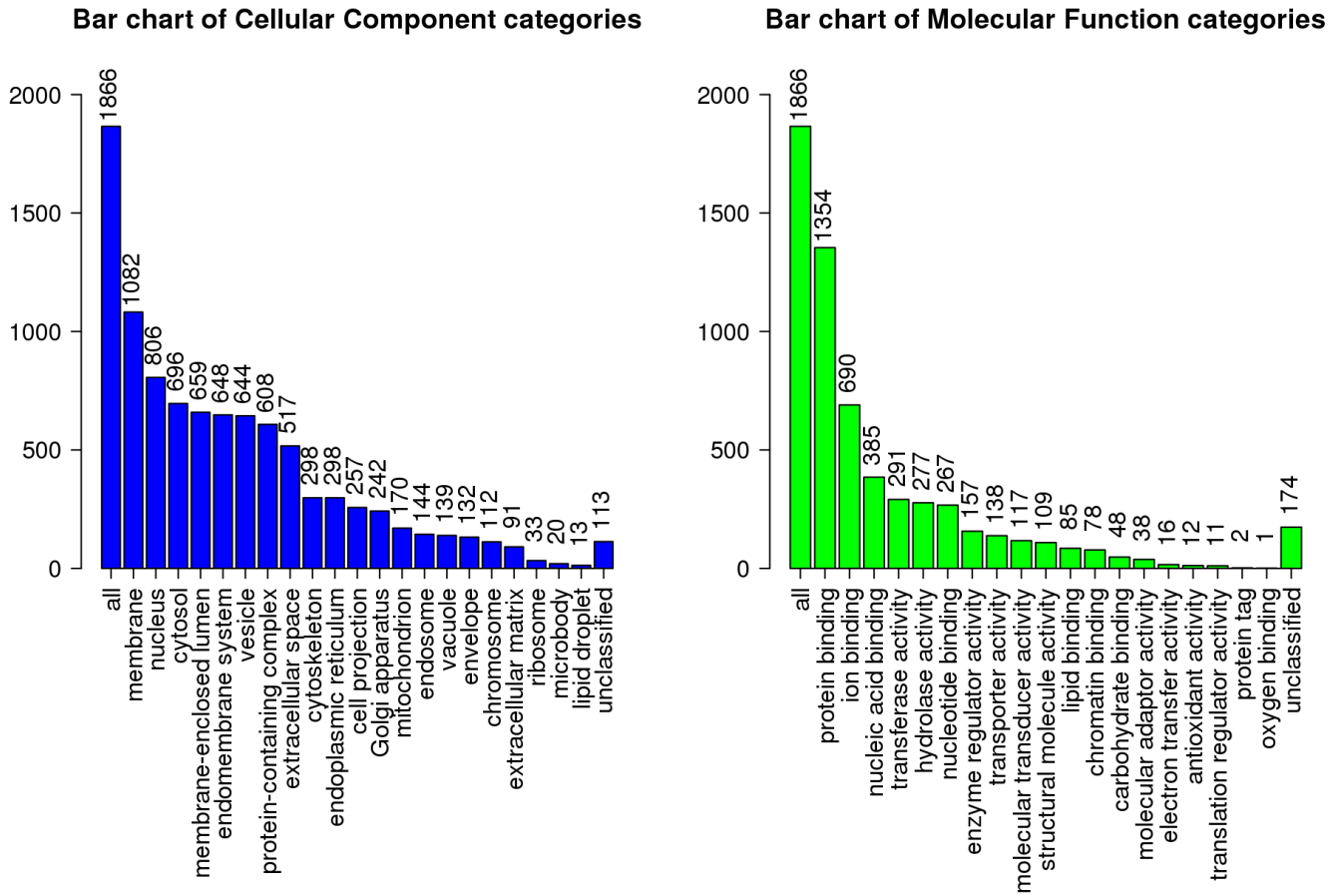
Supplemental Table S2. Taqman primer IDs. Assays were purchased from Thermo Fisher Scientific (Waltham, MA, US).

primer	official gene name	taqman ID
GAPDH	GAPDH	Hs99999905_m1
LDLR	LDLR	Hs00181192_m1
SR-BI	SCARB1	Hs00969821_m1
LRP1	LRP1	Hs00233856_m1
HMGCR	HMGCR	Hs00168352_m1
SREBP2	SREBF2	Hs01081748_m1
LXRβ	NR1H2	Hs01027215_g1
LXRα	NR1H3	Hs00172885_m1
ABCA1	ABCA1	Hs00194045_m1
IDOL	MYLIP	Hs00203131_m1
CYP11A1	CYP11A1	Hs00167984_m1
HSD3B1	HSD3B1	Hs04194787_g1

Supplementary Figures

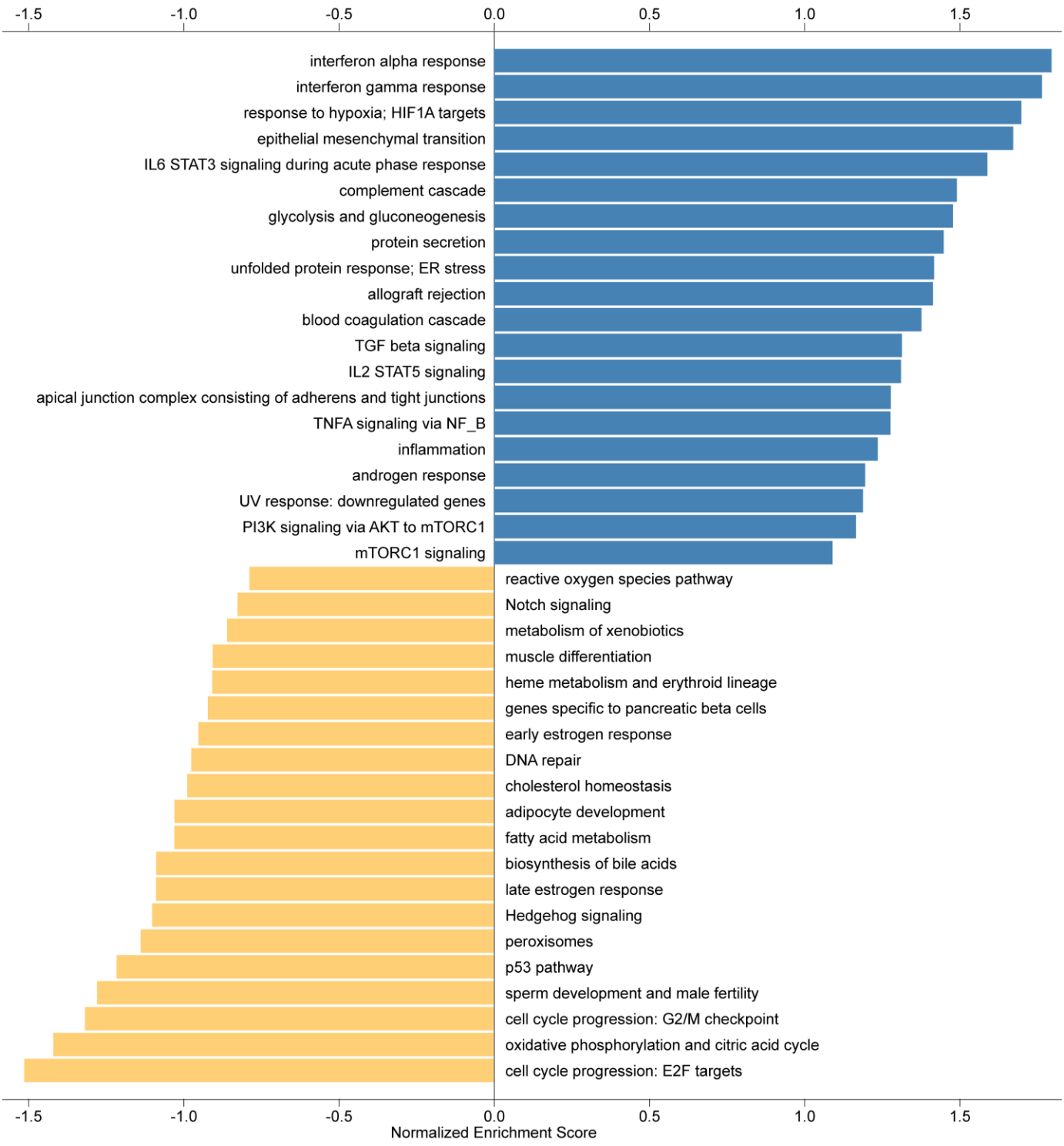


Supplemental Figure S1. Phenotypic characterization of extravillous trophoblast (EVT) and syncytiotrophoblast (STB) cultures. Representative analyses of STB (A, B) and EVT (C, D) cultures from human first trimester placenta are shown. CGB: chorionic gonadotropin; DAO: diamine oxidase; ITGA1: integrin subunit alpha 1



Supplemental Figure S2. Gene ontology (GO) enrichment analysis of RNA-seq data.

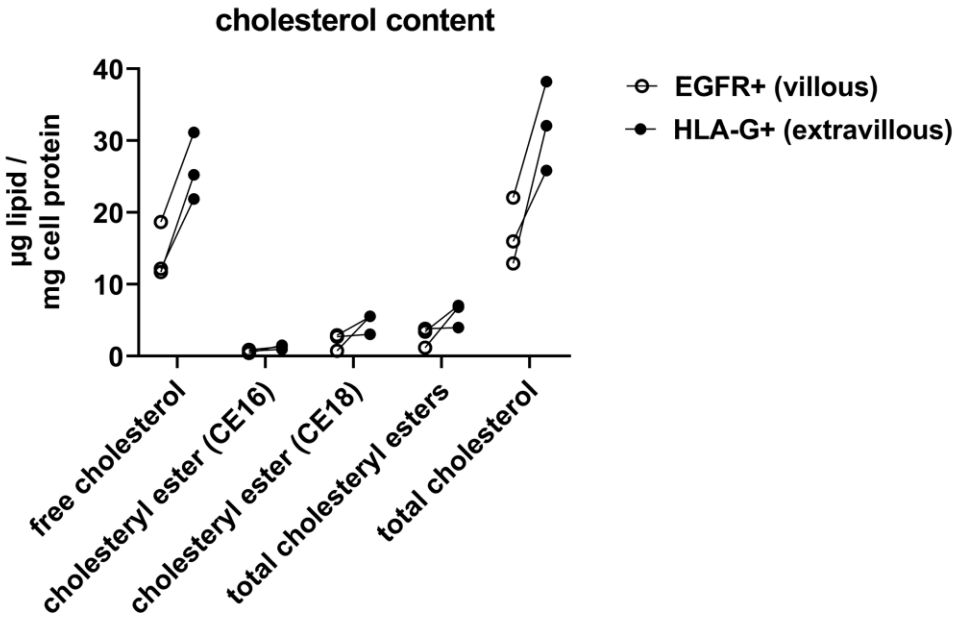
Primary human trophoblasts were isolated from first term placenta tissue and separated into EGFR+ vCTBs and HLAG+ EVTs. RNA was isolated and quantified by RNA-seq. Differentially expressed genes were analyzed according to their gene ontology (GO) annotations. The y-axis represents the number absolute frequency of differentially expressed genes in the respective category.



Supplemental Figure S3. Gene set-enrichment analysis (GSEA) of RNA-seq data

Primary human trophoblasts were isolated from first term placenta tissue and separated into EGFR+ vCTBs and HLAG+ EVTs.

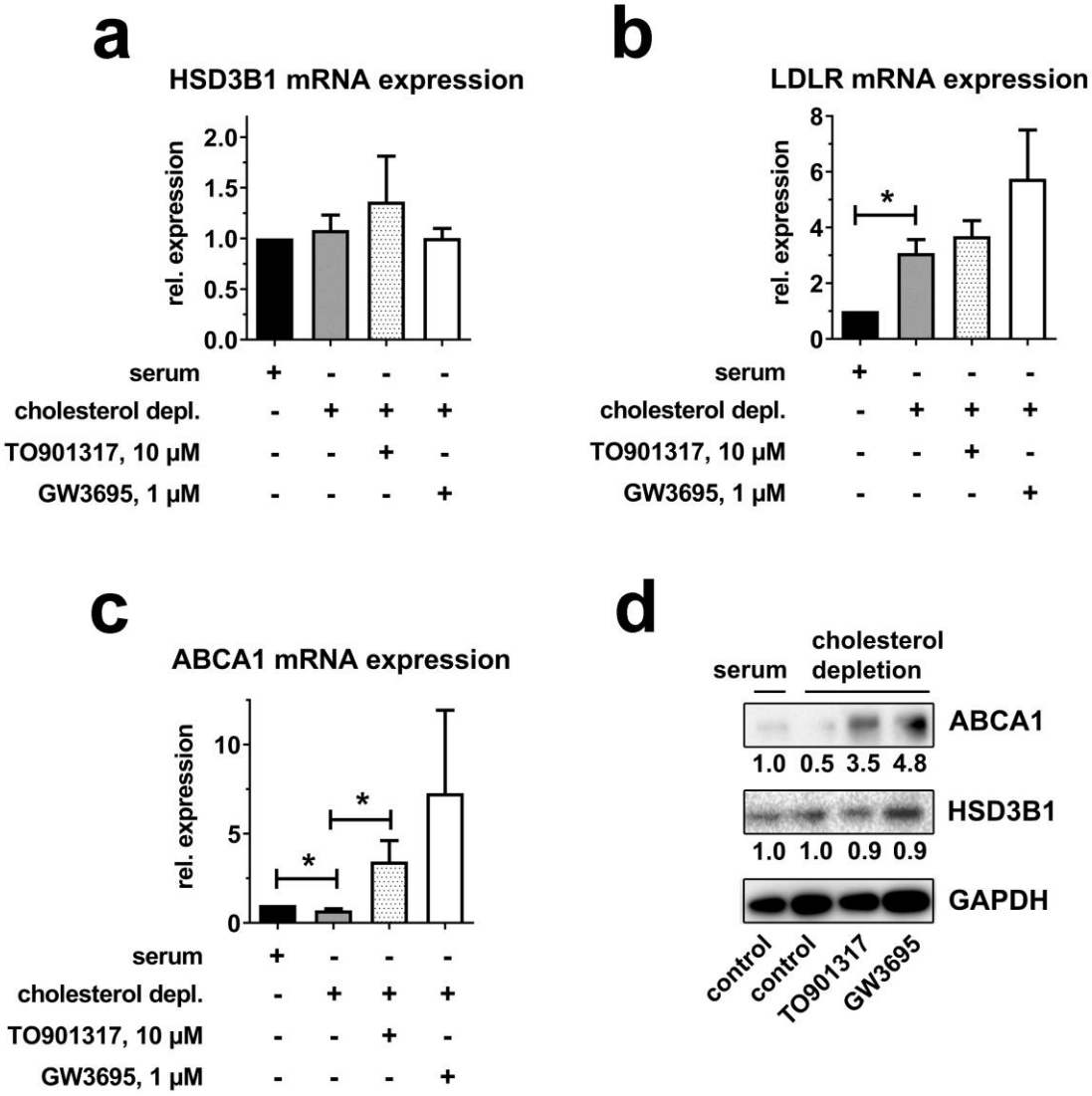
RNA was isolated and quantified by RNA-seq. Data were analyzed by GSEA using Hallmark gene sets as database. Positive enrichment scores indicate enrichment in EVTs whereas negative enrichment scores indicate enrichment of the respective pathway in vCTBs.



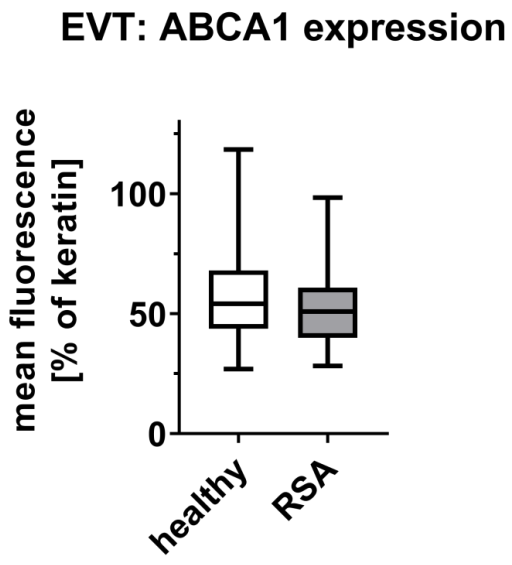
Supplemental Figure S4. Extravillous trophoblasts display increased cholesterol content

This figure shows an alternative graphical representation of the data presented in Figure 2 in the main manuscript.

Primary human trophoblasts were isolated from first trimester placenta tissue, separated into EGFR+ vCTBs and HLAG+ EVTs and analyzed by gas chromatography. Total cholesteryl ester content is split into CE16 and CE18 (cholesterol esterified to a fatty acid containing 16 and 18 C-atoms, respectively). Total cholesterol is calculated as the sum of free cholesterol and total cholesteryl esters. Each of the connected data points show the difference in cholesterol content between vCTBs and EVTs, derived from the very same placenta sample and isolation procedure. In total, three independent trophoblast isolations and gas chromatography analyses were performed. vCTB: villous cytotrophoblast; EVT: extravillous trophoblast.



Supplemental Figure S5 – HSD3B1 expression is not regulated by cholesterol depletion or LXR-activation. Extravillous trophoblasts (EVTs) were isolated from first trimester human placenta and cultivated either in media containing 10% FBS or in cholesterol depletion media (10% lipoprotein-deficient serum instead of FBS, 5 µM lovastatin, 100 µM mevalonate) with or without LXR agonists (10 µM TO901317 or 1 µM GW3695) for 24 hrs. mRNA was isolated followed by qRT-PCR and expression was normalized to TATA box binding protein (TBP) expression. Data are derived from n=3 independent trophoblast isolations (a-c). Equally treated cells were analyzed by immunoblot and quantification of n=2 independent trophoblast isolations are shown (d). Error bars represent SDs.



Supplemental Figure S6 – ABCA1 expression in extravillous trophoblasts (EVTs) of patients with repeated idiopathic spontaneous abortions (RSA). Decidual tissue section from RSA (n = 23) and age-matched healthy controls (n = 17) were double-stained with ABCA1 and cytokeratin 7 to identify trophoblasts. ABCA1 was quantified in CK7-positive EVTs. Data were quantified from 5 randomized fields per tissue section. Error bars represent SDs.