Supplementary Information:

HDL inhibits ER stress-induced apoptosis of pancreatic β-cells by activation of Smoothened

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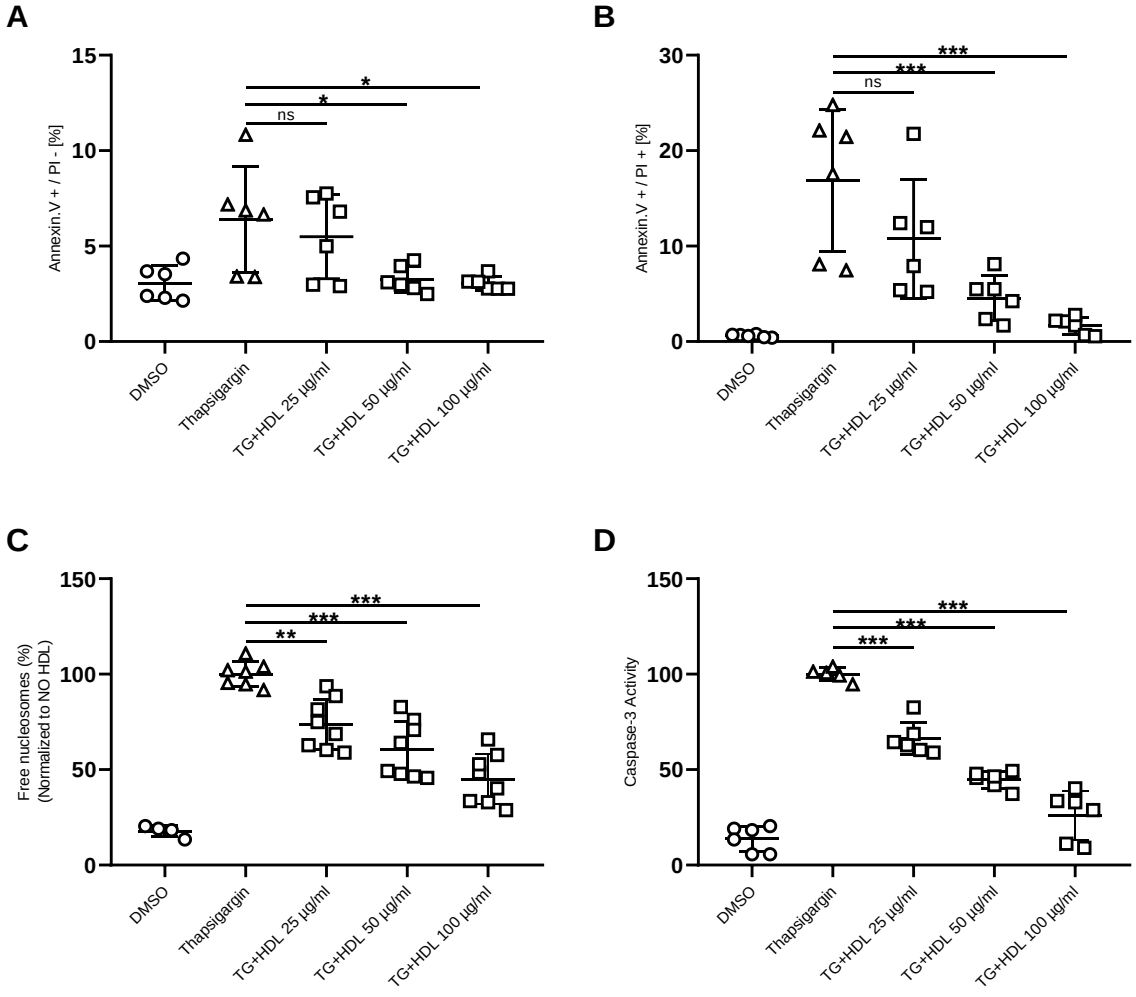
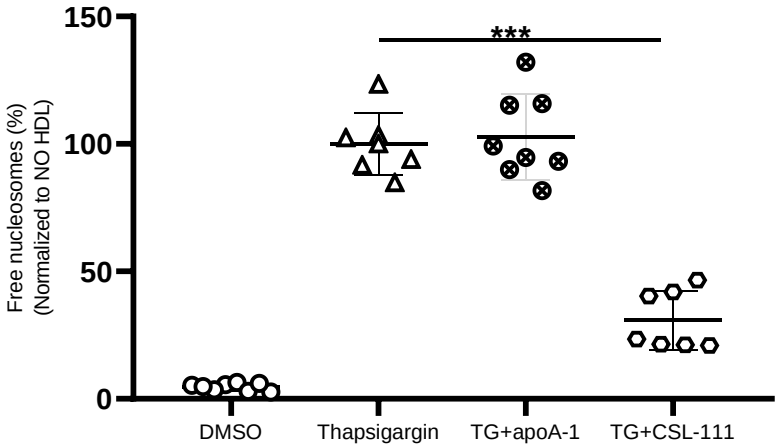
Supplementary Table S1

Sterols and oxysterols	Samples			
	HDL donor1	HDL donor2	LDL donor1	LDL donor2
Lanosterol [µg/mg]	0.37	0.52	1.48	2.72
Lathosterol [µg/mg]	2.18	2.83	10.62	21.66
Desmosterol [µg/mg]	2.47	1.86	7.86	10.21
Cholestanol [µg/mg]	4.70	2.69	12.81	10.95
Cholesterol [mg/mg]	2.82	1.67	10.32	9.89
7α-hydroxycholesterol [ng/mg]	8.36	8.69	68.32	92.32
7ß-hydroxycholesterol [ng/mg]	3.80	2.31	17.27	10.20
24-hydroxycholesterol [ng/mg]	10.12	12.1	30.93	41.04
25-hydroxycholesterol [ng/mg]	0.49	0.38	3.64	3.25
27-hydroxycholesterol [ng/mg]	40.43	25.23	81.05	79.28

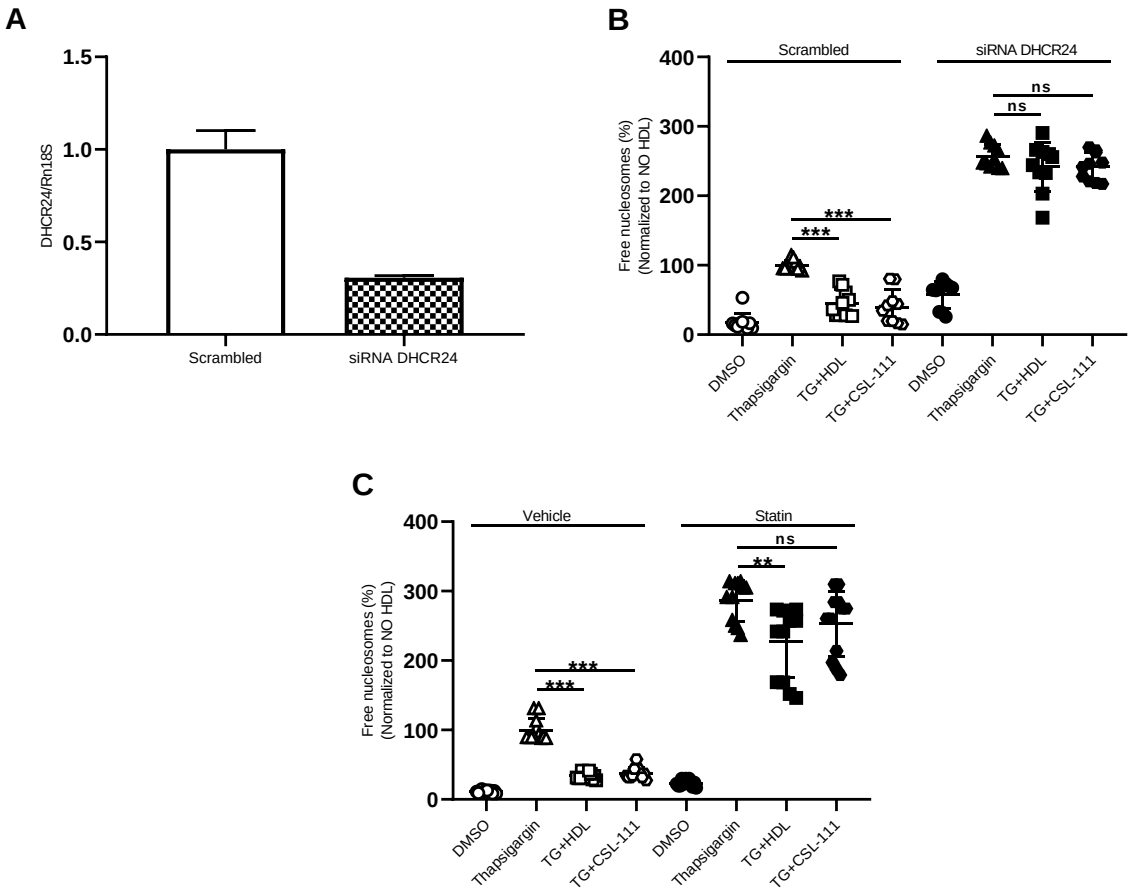
Supplementary Table S1: Concentrations of sterols in HDL and LDL. Sterols were quantified by gas chromatography coupled with mass spectrometry (GC-MS) as described in the methods section. Data represent mass concentrations of each lipid relative mass concentrations of total protein in each lipoprotein.



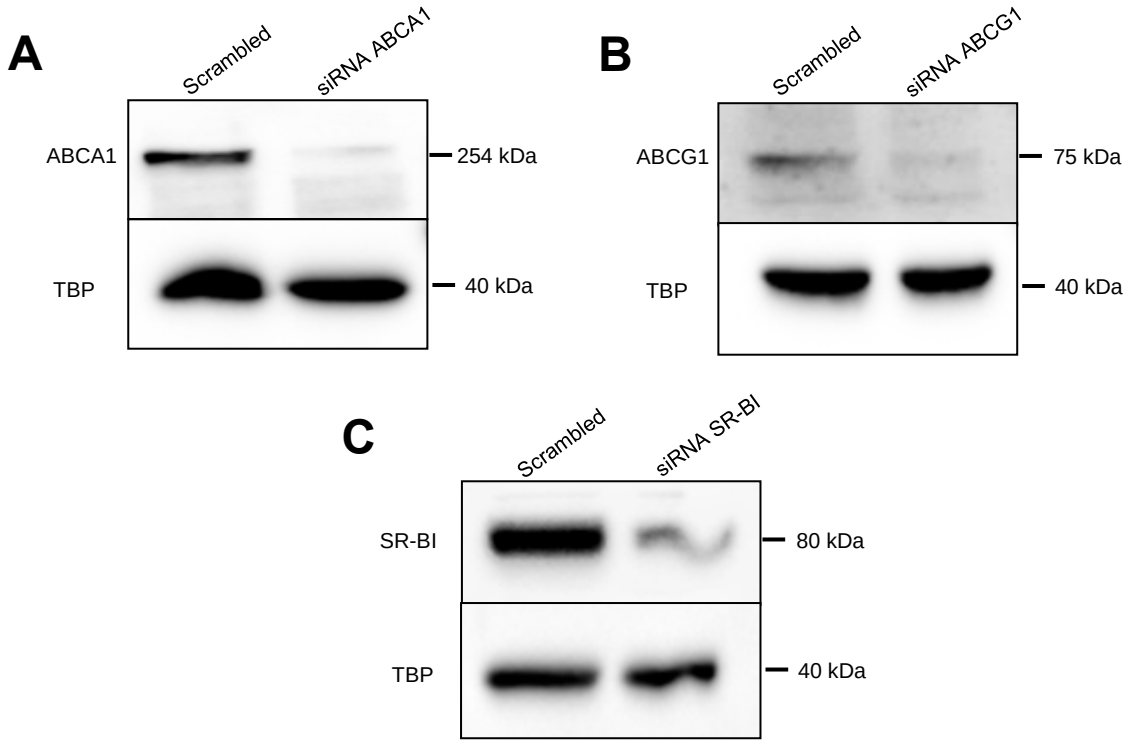
Supplemental Figure S1: HDL inhibits TG-induced apoptosis dose dependently. INS1e cells were treated with 50 nM TG for 16h in the presence or absence of 25, 50 or 100 µg/ml native HDL. Cell death was recorded by Annexin V/PI staining via flow cytometry **(A, B)** or measuring free nucleosomes **(C)** or Caspase-3 activity **(D)**. Early and late apoptosis were defined as Annexin V+/PI- cells **(A)** and Annexin V+/ PI+ **(B),** respectively. Data are presented as mean ± SD of 3 independent experiments, which were analyzed by one-way ANOVA coupled with Tukey's test for multiple comparisons. ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05



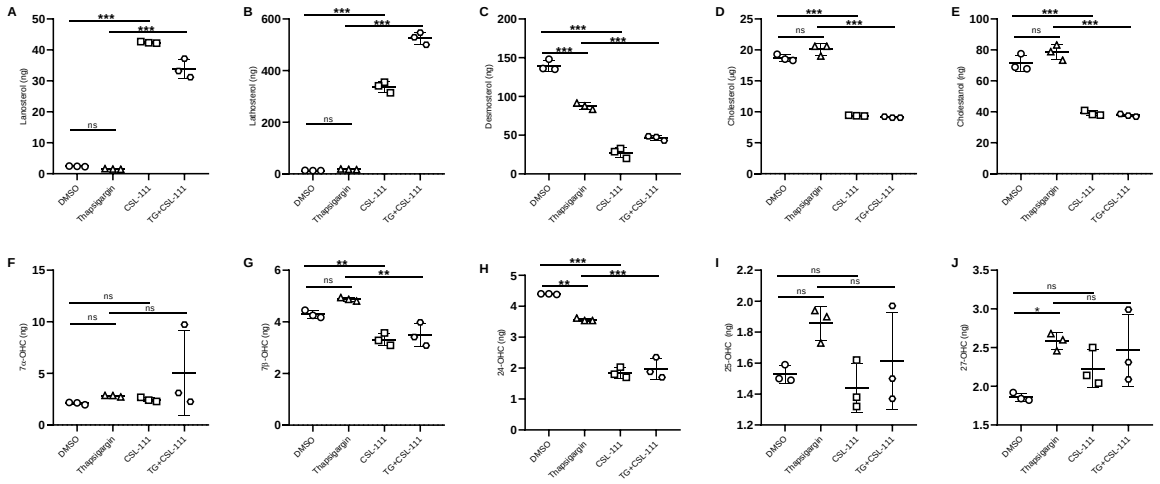
Supplemental Figure S2: Lipid-free apoA-1 does not inhibit TG-induced apoptosis. INS1e cells were treated with 100 nM TG for 4h in the presence or absence of 25 µg/ml lipid-free apoA1 or CSL-111. The cell death was recorded by using the free nucleosomes assay. Data are represented as mean ± SD of 3 independent experiments, which were analyzed by one-way ANOVA coupled with Tukey's test for multiple comparisons. ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05



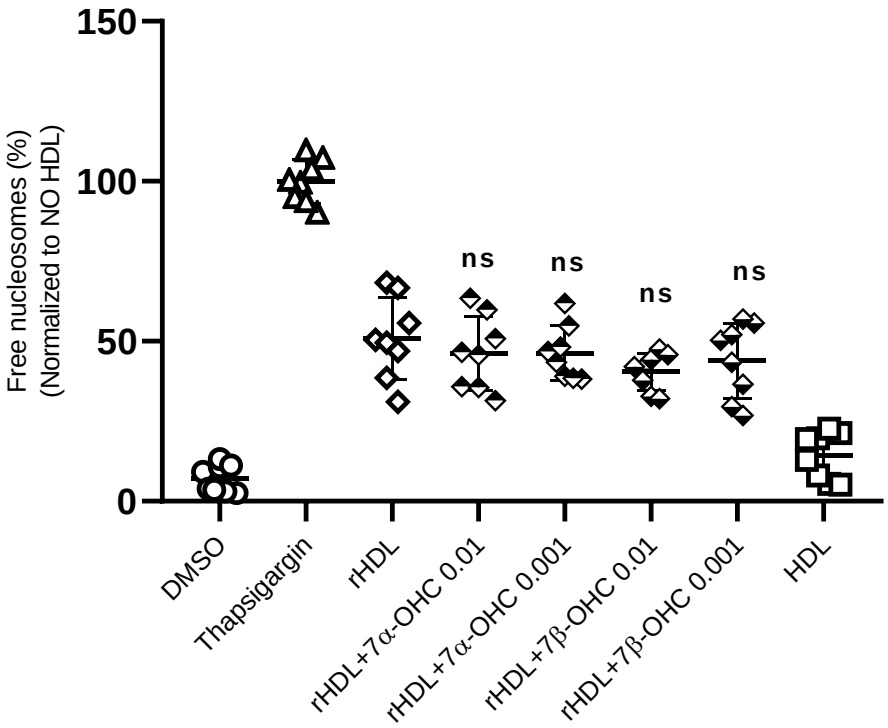
Supplemental Figure S3: The anti-apoptotic activity of HDL is abrogated by interference with cholesterol synthesis. INS1e cells were transfected with specific siRNA against DHCR24 or with non-silencing siRNA (Scrambled). After 48 hours of transfection, DHCR24 mRNA expression was measured by qPCR and normalized to Rn18S **(A)**. The cells were treated with 100 nM TG and incubated with native HDL or CSL-111 for 4h **(B)**. INS1e cells were pre-treated with 1 µM Atorvastatin for 16 hours and with 100 nM in presence and absence of HDL or CSL-111 for 4h **(C)**. The cell death was recorded by using the free nucleosomes assay **(B-C)**. Data shown in figures **B** and **C** , each represent mean ± SD of 3 independent experiments, which were analyzed by one-way ANOVA coupled with Tukey's test for multiple comparisons. ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05



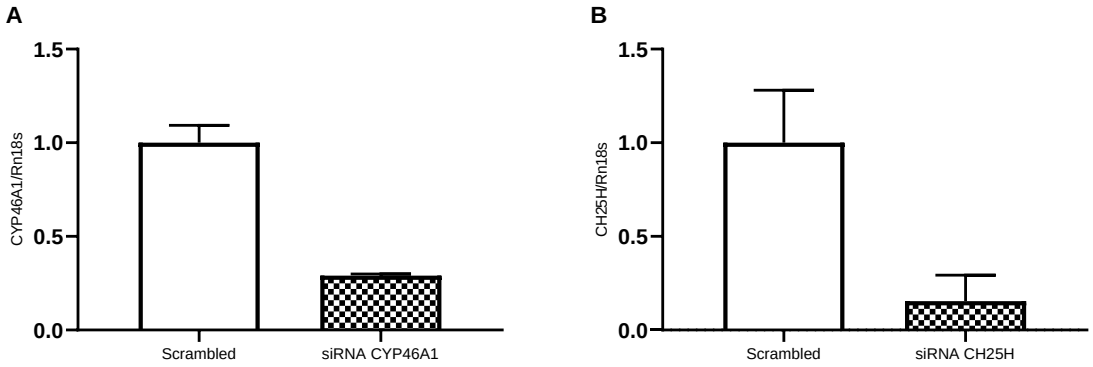
Supplemental Figure S4: Efficiency of RNA interference with ABCA1, ABCG1, or SR-BI. INS1e cells were transfected with specific siRNA against ABCA1 **(A)**, ABCG1 **(B)**, SR-BI **(C)** or with non-silencing siRNA (Scrambled) for 48h. TATA-binding protein (TBP) was used as a loading control. Representative western blots are shown.



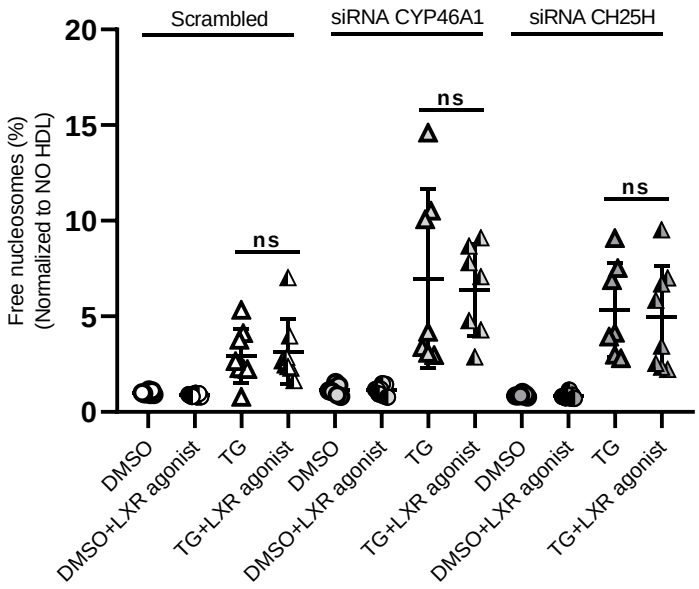
Supplemental Figure S5: Effects of ER stress and HDL on the sterol content of INS1e cells. INS1e cells were treated with 50 nM TG in presence and absence of CSL-111 for 16h and sterols were measured by mass spectrometry from cell pellets. Sterols amounts were normalized to dry pellet amounts. Lanosterol **(A)**, lathosterol **(B)**, desmosterol **(C)**, cholesterol **(D)**, cholestanol **(E)**, 7α-hydroxycholesterol **(F)**, 7β-hydroxycholesterol **(G)**, 24-hydroxycholesterol **(H)**, 25-hydroxycholesterol **(I)** and 27-hydroxycholesterol **(J)**. Data represent means ± S.D. of a triplicate experiment and were analyzed by one-way ANOVA coupled with Tukey's test for multiple comparisons. ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05



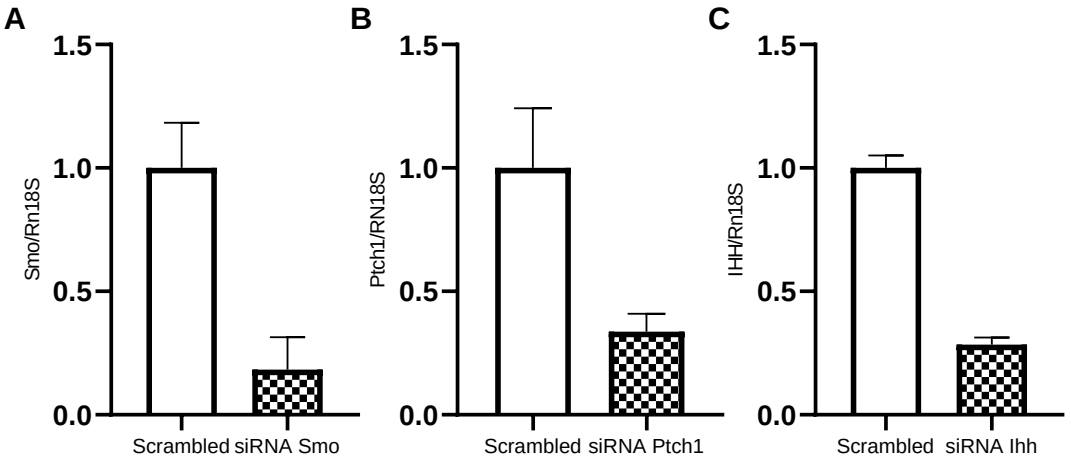
Supplemental Figure S6: Supplementation with 7α-hydroxycholesterol (OHC) or 7β-OHC does not enhance apoptosis inhibition by rHDL. rHDL were supplemented with 7α-OHC or 7β-OHC at molar ratios 0.01 or 0.001relative to apoA-I. INS1e cells were treated with 100 nM TG and incubated with rHDL-/+ oxysterols for 4h. Cell death was recorded by using the free nucleosomes assay. Data are presented as mean ± SD of 3 independent experiments, which were analyzed by one-way ANOVA coupled with Tukey's test for multiple comparisons of rHDL-/+ oxysterols against rHDL.



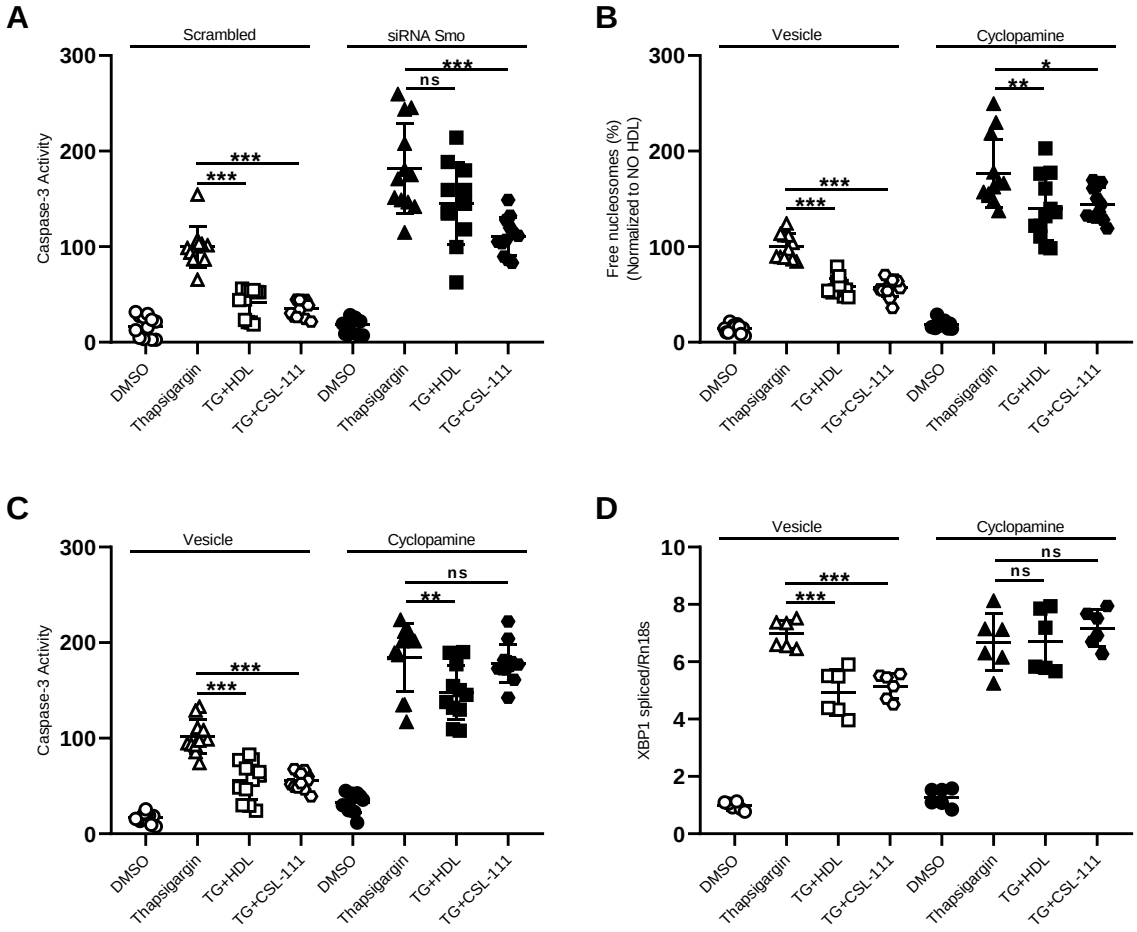
Supplemental Figure S7: Efficiency of RNA interference with CYP46A1 and CH25H. INS1e cells were transfected with specific siRNA against *CYP46A1* **(A)**, *CH25H* **(B),** or with non-silencing siRNA (Scrambled) for 48h. mRNA expressions was normalized to Rn18S.



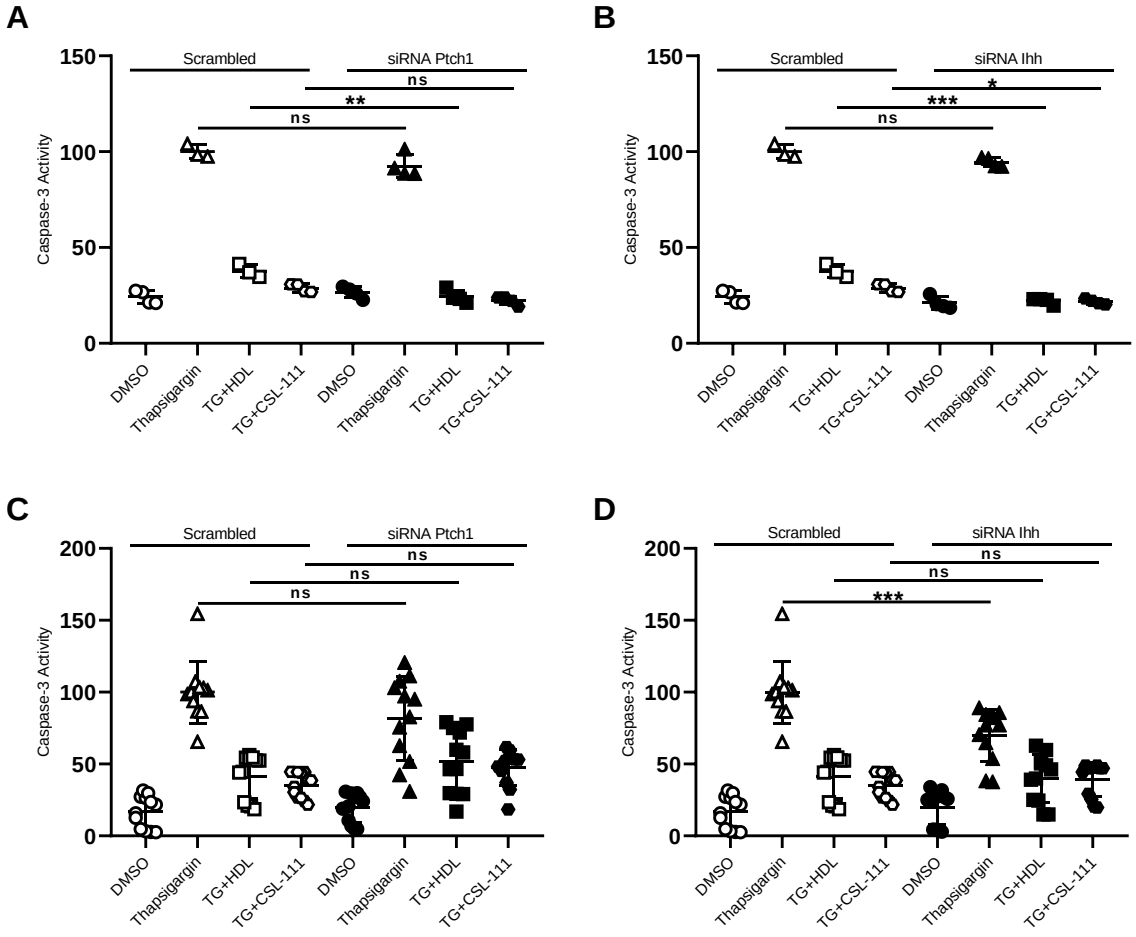
Supplemental Figure S8: LXR activation does not rescue CYP silencing induced cell death. INS1e cells were transfected with specific siRNA against *CYP46A1*, *CH25* or with non-silencing siRNA (Scrambled). After 48 hours of transfection, the cells were treated with 1 µM LXR agonist T0901317 for 16h and then100 nM TG for 4h. The cell death was recorded with the free nucleosomes assay. Data are presented as mean ± SD of 3 independent experiments, which were analyzed by one-way ANOVA coupled with Tukey's test for multiple comparisons in group of Scrambled and knockdown conditions, respectively.



Supplemental Figure S9: Efficiency of RNA interference with *Smo, Ptch1* and *Ihh*. INS1e cells were transfected with specific siRNA against Smo **(A)**, Ptch1 **(B)**, Ihh **(C)** or with non-silencing siRNA (Scrambled) for 48 h. mRNA expression was normalized to Rn18S.

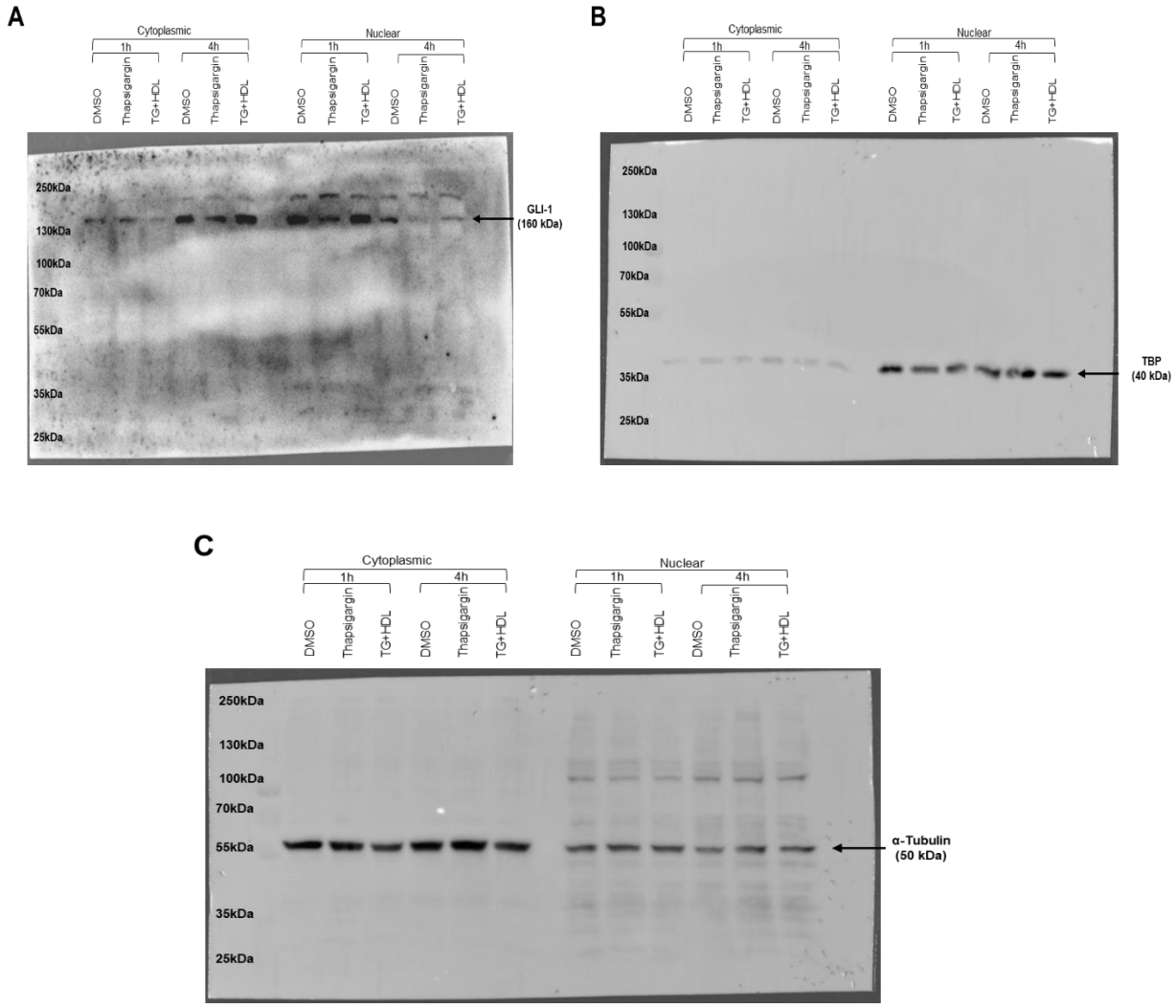


Supplemental Figure S10: Anti-apoptotic effects of HDL depend on Smoothened. INS1e cells were transfected with specific siRNA against *Smo* and non-silencing siRNA (Scrambled) **(A)** or pre-treated with Cyclopamine for 30 min **(B-D).** After 48 hours of transfection or Cyclopamine treatment, the cells were treated with 100 nM TG and incubated with HDL or CSL-111 for 4h. Cell death was recorded by using the free nucleosomes assay **(A-B)** or the Caspase-3 assay **(C)**. XBP1 splicing was measured by qPCR **(D)**. Data are represented as mean ± SD of 3 independent experiments, which were analyzed by one-way ANOVA coupled with Tukey's test for multiple comparisons in group of Scrambled and knockdown conditions **(A)** or in group of vesicle or Cyclopamine conditions **(C-D)**, respectively. ***P≤ 0.001, **P≤ 0.01, *P<0.05



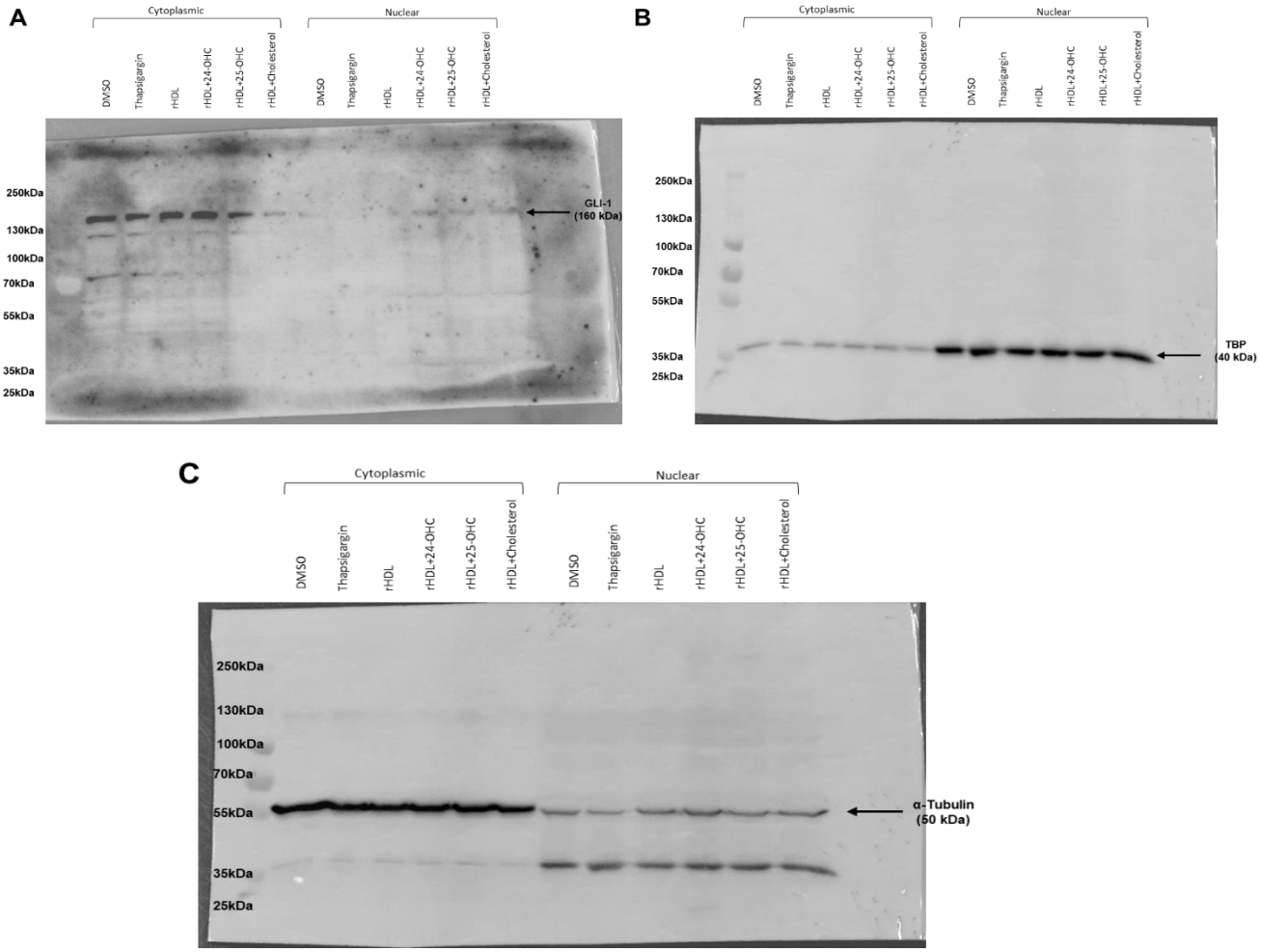
Supplementary figure 11: Anti-apoptotic effects of HDL are independent of Patched1 (*Ptch1*) and Indian Hedgehog (*IHH*). INS1e cells were transfected with specific siRNA against Ptch1 **(A, C)**, Ihh **(B, D)** or non-silencing siRNA (Scrambled). After 48 hours of transfection, the cells were treated with 100 nM TG for 16 h **(A, B)** or 50 nM TG for 4h **(C, D)** in absence or presence of HDL or CSL-111. Cell death was recorded via caspase-3 activity assay. Data are represented as mean ± SD, which were analyzed by one-way ANOVA coupled with Tukey's test for multiple comparisons. ***P≤ 0.001, **P≤ 0.01, *P≤ 0.05

Supplementary Electrophoretic Blot A



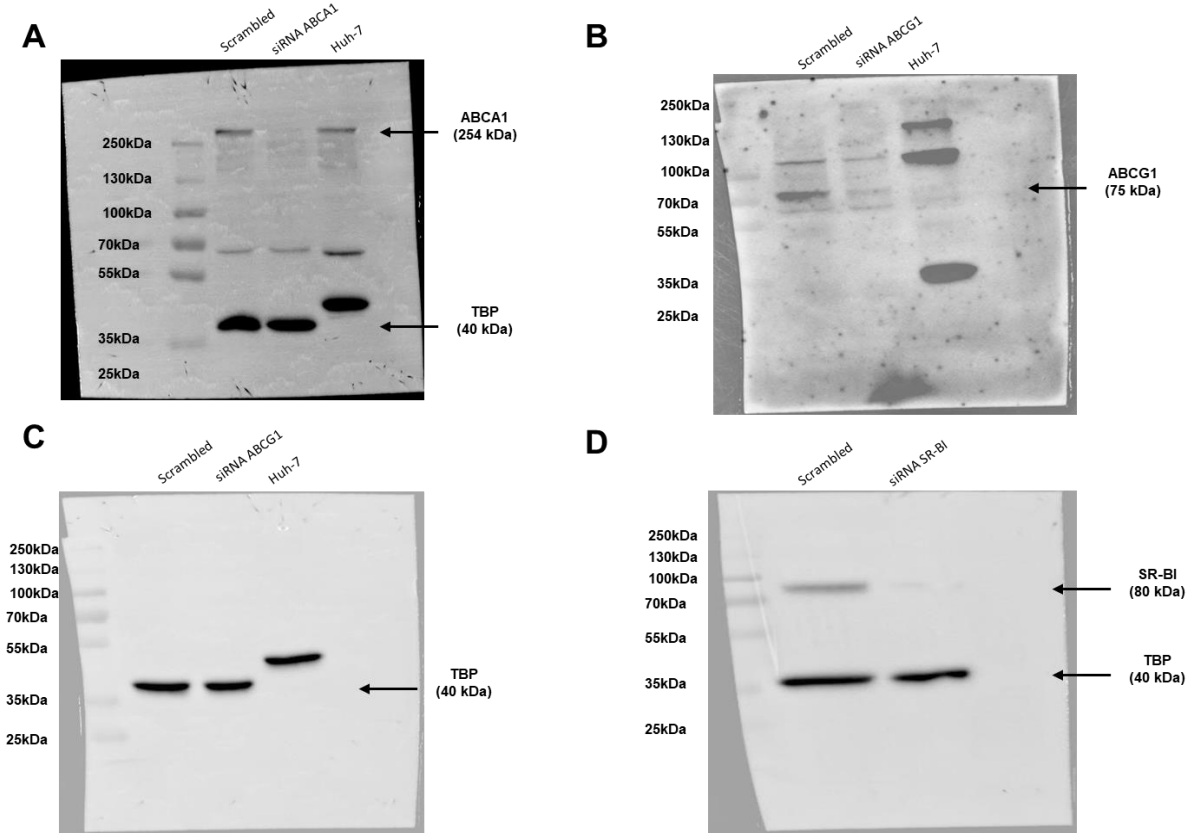
Supplementary Electrophoretic Blot A: Corresponds to the blot shown in Figure 10A. Western blots were probed with anti-GLI-1 (160 kDa) **(A),** anti-TATA binding protein (TBP, 40kDa) (**B),** or anti-α-Tubulin (50kDa) **(C**).

Supplementary Electrophoretic Blot B



Supplementary Electrophoretic Blot B: Corresponds to the blot shown in Figure 10B. Western blots were probed with anti-GLI-1 (160 kDa) **(A)** ,anti-TATA binding protein (TBP, 40kDa) (**B)** or anti-α-Tubulin (50kDa) **(C**).

Supplementary Electrophoretic Blot C



Supplementary Electrophoretic Blot C: Corresponds to the blots shown in Supplementary Figure S4. Western blot in supplementary Figure S4A was probed with anti-ABCA1 (254 kDa) and anti-TATA binding protein (TBP 40 kDa) **(A)** Western blot in supplementary figure S4B was probed with anti-ABCG1 (75 kDa) (**B)** Western blot in supplementary figure S4B was probed with anti-TATA binding protein (TBP 40 kDa) **(C**). Western blot in supplementary figure S4C was probed with anti-SR-BI (80 kDa) and anti-TATA binding protein (TBP 40 kDa) **(D)**