

Supplementary Material

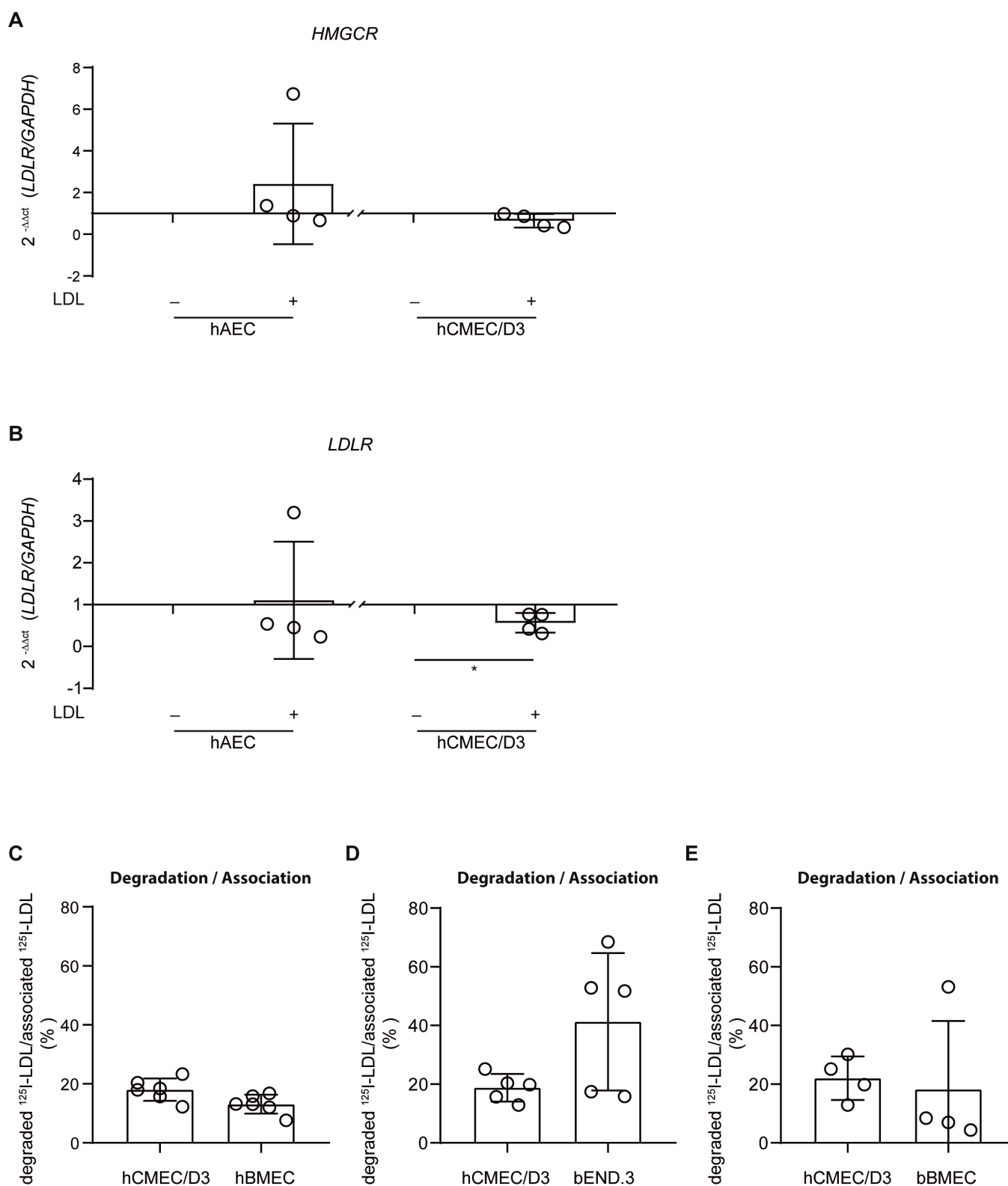


Figure S1. LDL degradation by brain endothelial cells is conserved in primary cells and between species. Confluent hAEC and hCMEC/D3 were incubated with 50 $\mu\text{g/mL}$ of LDL in culture media with 1% FBS. After 24 h, the transcript levels of *HMGCR* (A) and *LDLR* (B) were measured using RT-qPCR. The ratio of ^{125}I -LDL degradation per ^{125}I -LDL association was measured as described in Figure 1 in confluent human primary (hBMEC) (C), mice line (bEND.3) (D) and bovine primary

(bBMEC) (E) brain endothelial cells and compared to hCMEC/D3. Points in graphs represent individual experiments (biological replicates, $n = 4-6$), bars represent the mean and error bars \pm SD. * $p = 0.05$.

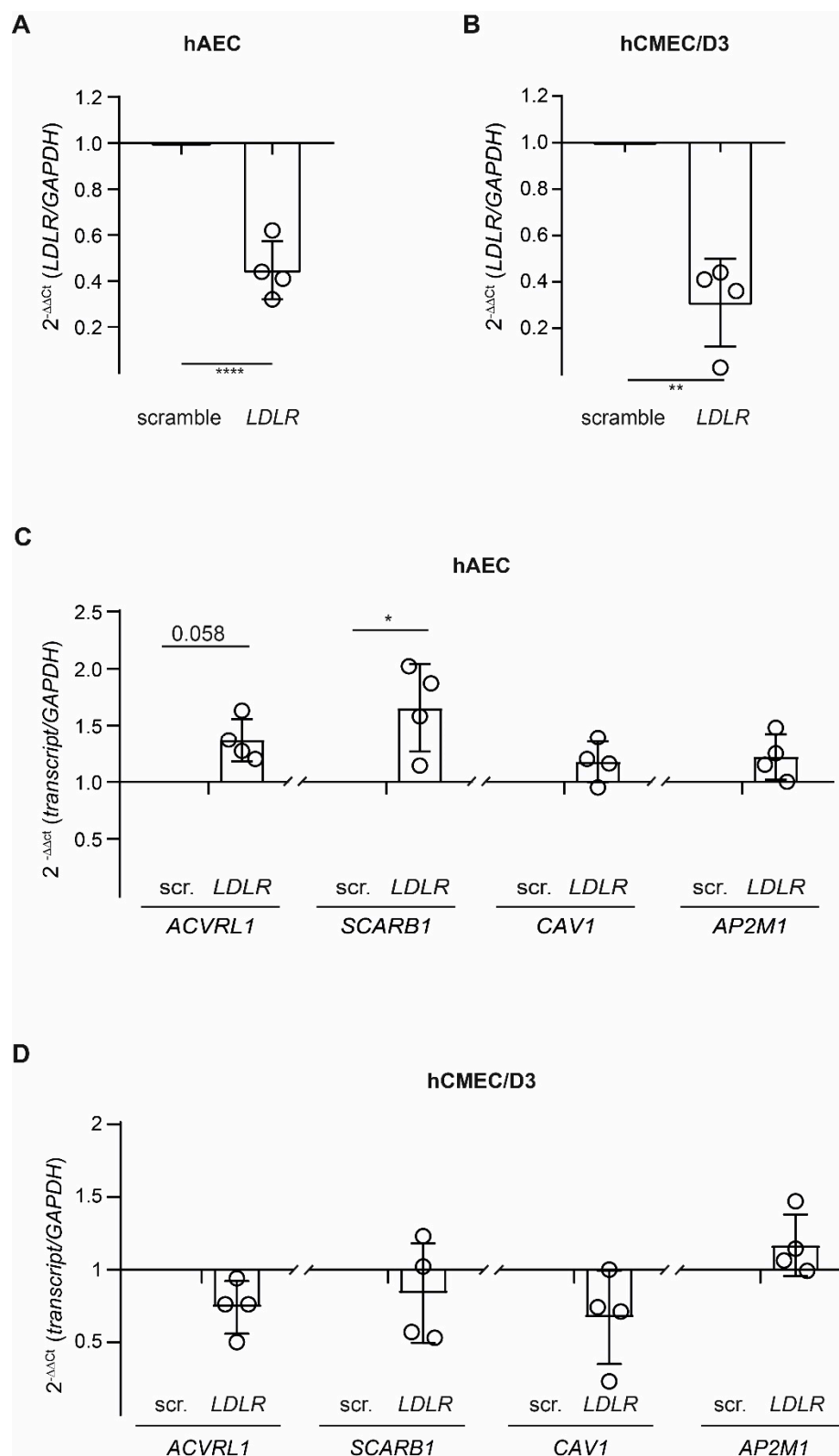


Figure S2. siRNA interference against *LDLR* reduces *LDLR* expression but does not decrease the expression of *SCARB1*, *ACVRL1*, *CAV1* or *AP2M1*. Seventy-two hours after knocking down *LDLR* in hAEC (**A**) or hCMEC/D3 (**B**) using siRNA, *LDLR* transcript level was measured by RT-qPCR, normalized to the housekeeping gene *GAPDH* and compared to non-targeting scramble siRNA

treated cells. The transcript levels of *ACVRL1*, *SCARB1*, *CAV1* and *AP2M1* after knocking down LDLR in hAEC (C) or hCMEC/D3 (D) were measured using RT-qPCR as above. Points in graphs represent individual experiments (biological replicates, $n = 4$), bars represent the mean and error bars \pm SD. * $p = 0.05$, ** $p = 0.01$, and **** $p = 0.0001$, Scr: Scramble.

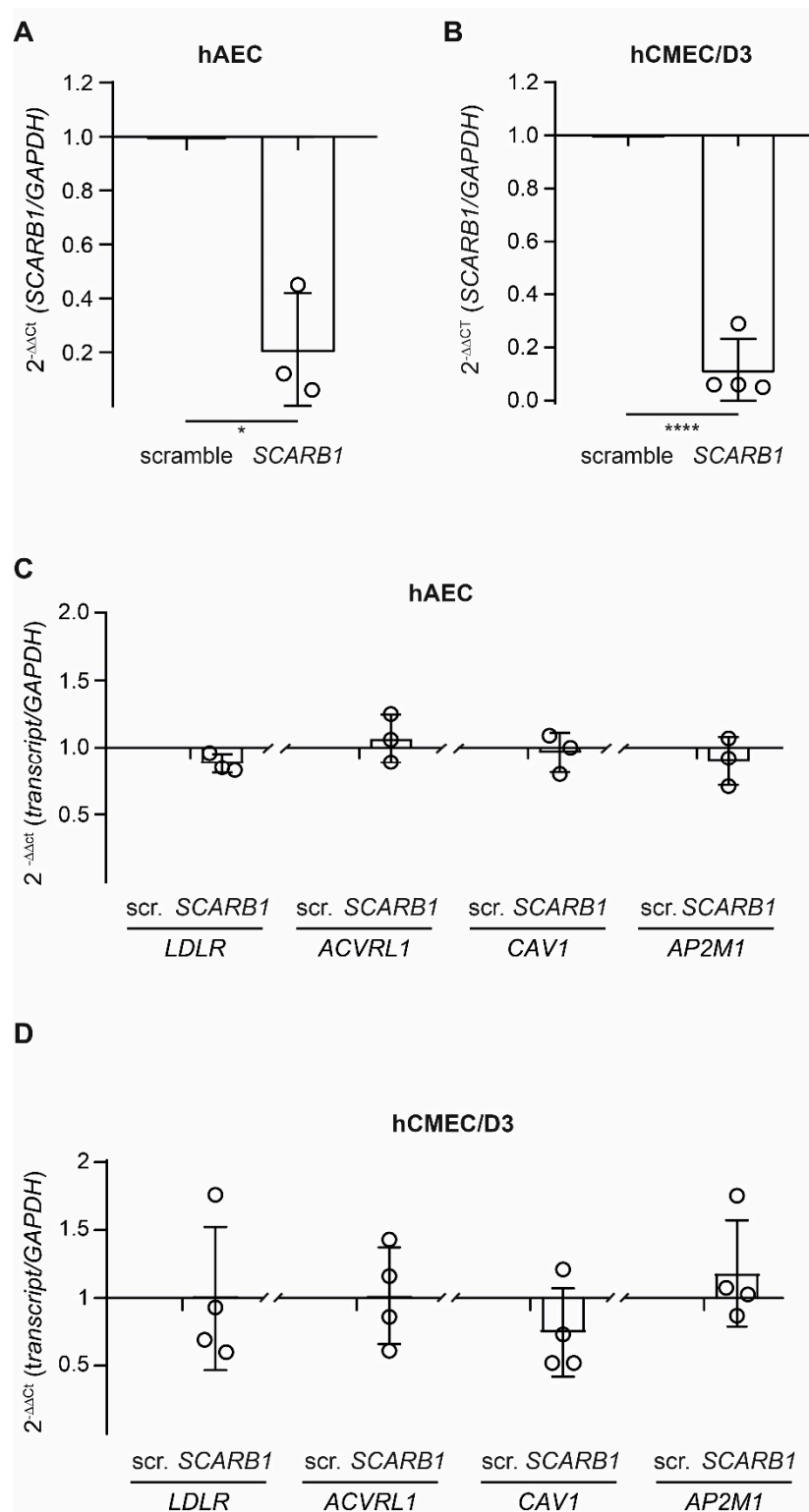


Figure S3. siRNA interference against *SCARB1* reduces *SCARB1* expression but does not alter the expression of *LDLR*, *ACVRL1*, *CAV1* or *AP2M1*. Seventy-two hours after knocking down SR-BI in hAEC (A) or hCMEC/D3 (B) using siRNA, *SCARB1* transcript level was measured by RT-qPCR, normalized to the housekeeping gene *GAPDH* and compared to non-targeting scramble siRNA

treated cells. The transcript levels of *LDLR*, *ACVRL1*, *CAV1* and *AP2M1* after knocking down SR-BI in hAEC (C) or hCMEC/D3 (D) were measured using RT-qPCR as above. Points in graphs represent individual experiments (biological replicates, $n = 3-4$), bars represent the mean and error bars \pm SD. * $p = 0.05$, and **** $p = 0.0001$, Scr: Scramble.

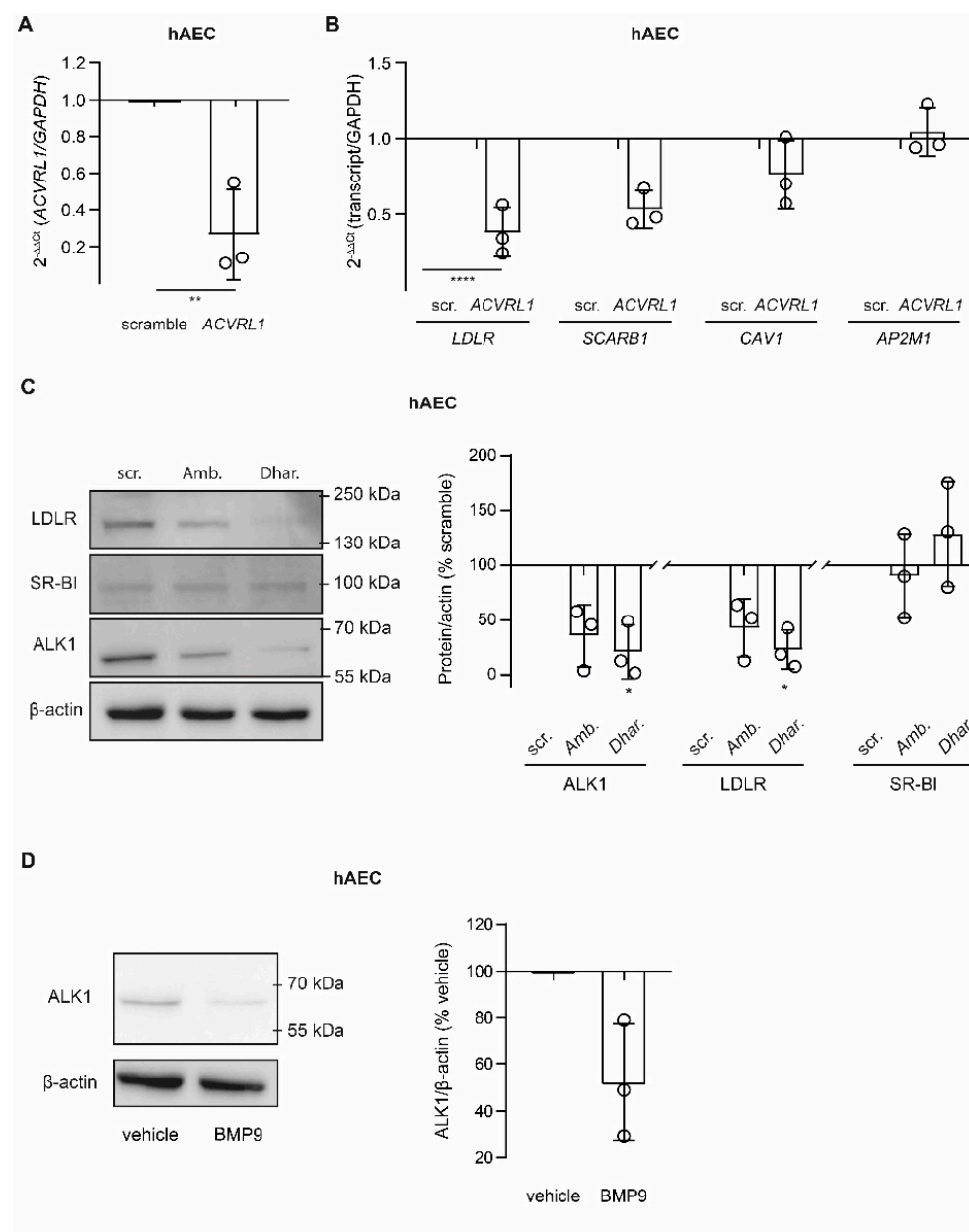


Figure S4. siRNA interference against *ACVRL1* reduces both *ACVRL1* and *LDLR* levels in hAEC. (A) Seventy-two hours after siRNA interference against *ACVRL1* in hAEC, mRNA was isolated and *ACVRL1* transcript level was measured by RT-qPCR, normalized to the housekeeping gene *GAPDH* and compared to non-targeting scramble siRNA treated cells. (B) The transcript levels of *LDLR*, *SCARB1*, *CAV1* and *AP2M1* after knocking down *ACVRL1* in hAEC were measured using RT-qPCR as above. (C) Seventy-two hours after knocking down *ACVRL1* using two vendors siRNA, *LDLR*, *SR-BI* and *ALK1* protein expression was measured using Western blot and normalized to the expression of β -actin. (D) Confluent hAEC were treated with 10 ng/mL of BMP9. After 2 hours, proteins were isolated and measured using Western blot. *ALK1* expression was quantified using ImageJ 2.0.0-rc 65/1.52a and normalized over β -actin. Points in graphs represent individual experiments (biological replicates, $n = 3$), bars represent the mean and error bars \pm SD. * $p = 0.05$, ** $p = 0.01$, and **** $p = 0.0001$ Scr: Scramble, Amb: (Ambion), Dhar: Dharmacon.

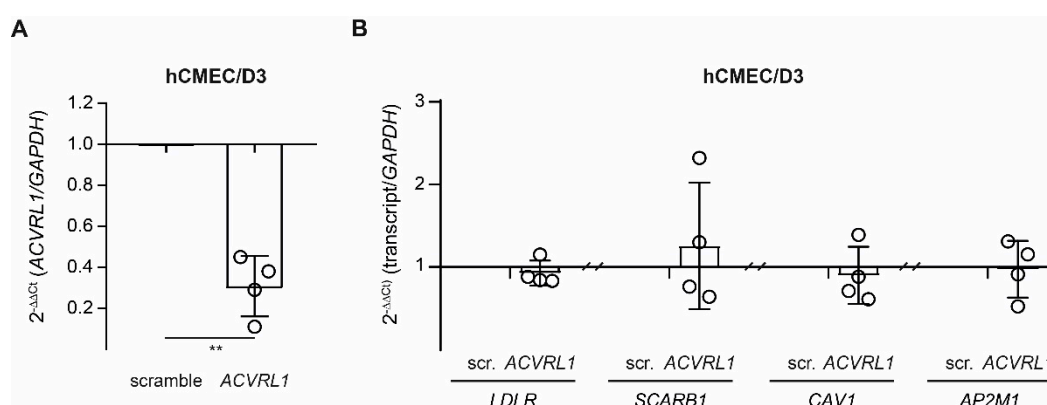


Figure S5. siRNA interference against *ACVRL1* reduces *ACVRL1* in hCMEC/D3. **(A)** seventy-two hours after siRNA interference against *ACVRL1* in hCMEC/D3, *ACVRL1* transcript level was measured as above. **(B)** The transcript levels of *LDLR*, *SCARB1*, *CAV1* and *AP2M1* after knocking down *ACVRL1* in hCMEC/D3 were measured using RT-qPCR as above. Points in graphs represent individual experiments (biological replicates, $n = 4$), bars represent the mean and error bars \pm SD. ** $p = 0.01$, Scr: Scramble.

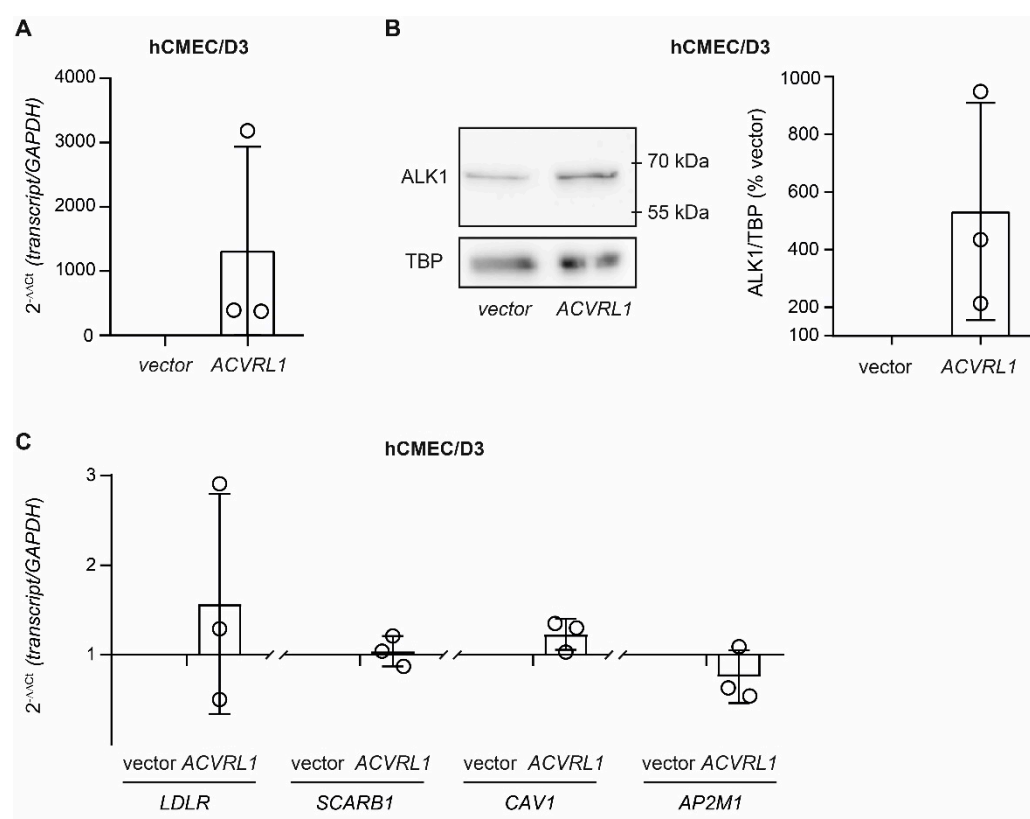


Figure S6. ALK1 transcript and protein expression are enhanced in hCMEC/D3 overexpressing *ACVRL1*. **(A)** hCMEC/D3 were transfected with a plasmid encoding for *ACVRL1* CDS. Several passages after transfection (2–6) and in the presence of the selection antibiotic G418, *ACVRL1* transcript expression was analyzed by RT-qPCR, normalized to the housekeeping gene *GAPDH* and compared to empty vector only. **(B)** ALK1 protein expression in transfected cells was measured by Western blot and normalized to TATA binding protein (TBP). Intensity of bands was measured by densitometry using ImageJ 2.0.0-rc 65/1.52a. **(C)** The transcript levels of *LDLR*, *SCARB1*, *CAV1* and *AP2M1* were measured using RT-qPCR as above. Points in graphs represent individual experiments (biological replicates, $n = 3$), bars represent the mean and error bars \pm SD, Scr: Scramble.

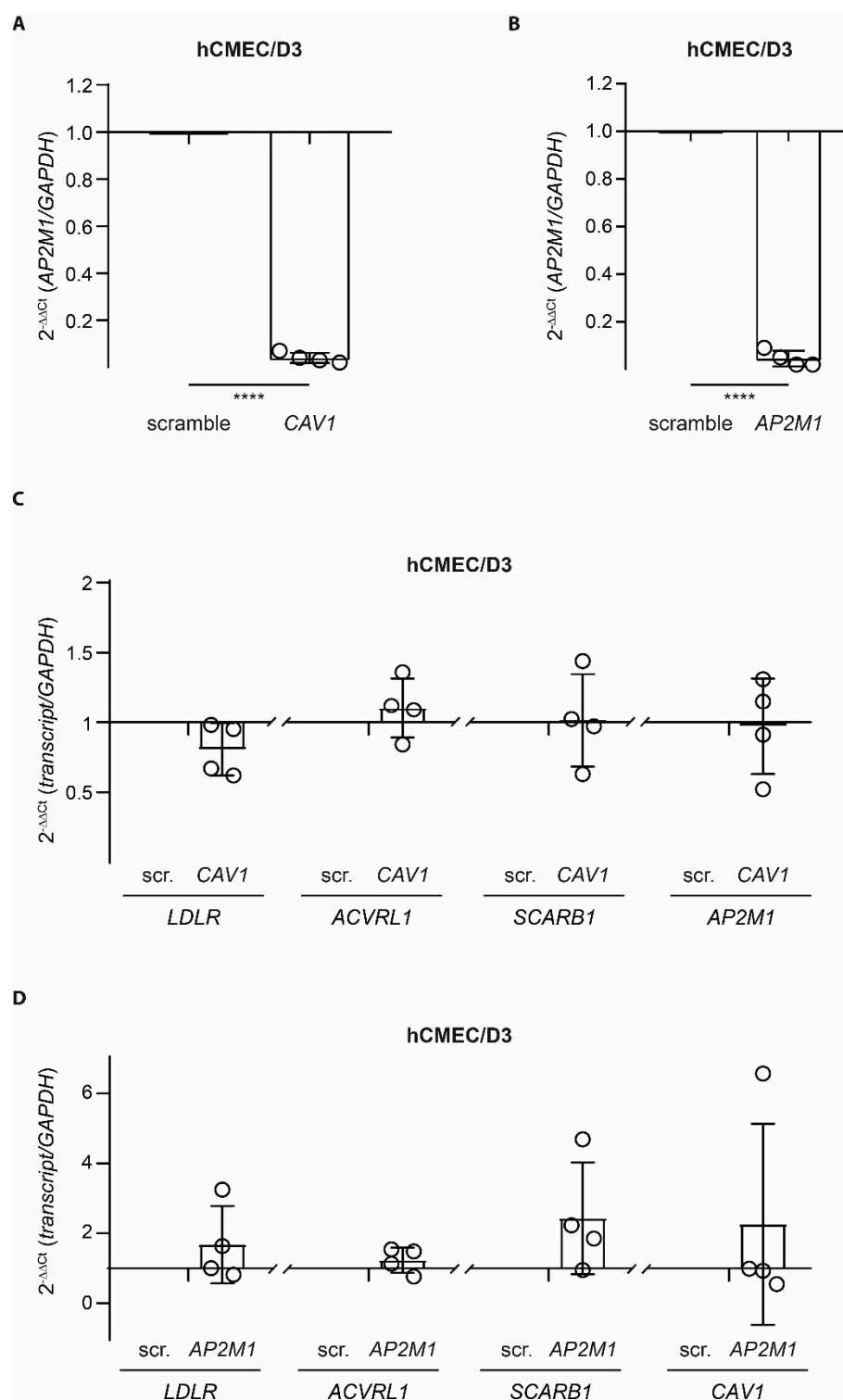


Figure S7. siRNA interference against CAV1 or AP2M1 reduces their respective transcripts in hCMEC/D3. Seventy-two hours after siRNA interference against CAV1 (**A**) and AP2M1 (**B**) in hCMEC/D3, mRNA was isolated and transcript levels were measured using RT-qPCR, normalized to the housekeeping gene GAPDH and compared to non-targeting scramble siRNA. (**C**) Transcript level of *LDLR*, *ACVRL1*, *SCARB1* and *AP2M1* were measured using RT-qPCR in CAV1 knockdown hCMEC/D3 as above. (**D**) Transcript levels of *LDLR*, *ACVRL1*, *SCARB1* and *CAV1* were measured using RT-qPCR in AP2M1 knockdown hCMEC/D3 as above. Points in graphs represent individual experiments (biological replicates, $n = 4$), bars represent the mean and error bars \pm SD. **** $p < 0.0001$, Scr: Scramble.