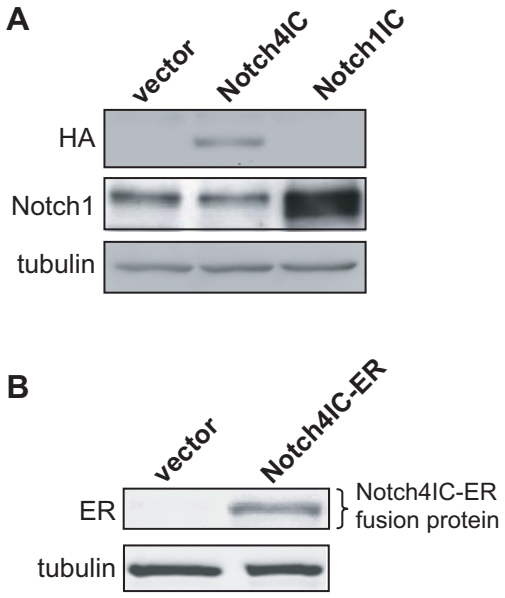
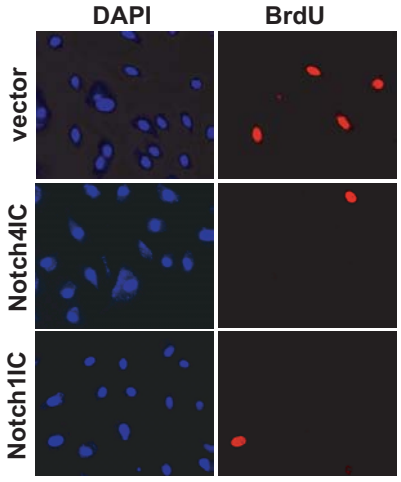
Supplemental material, Figure S1



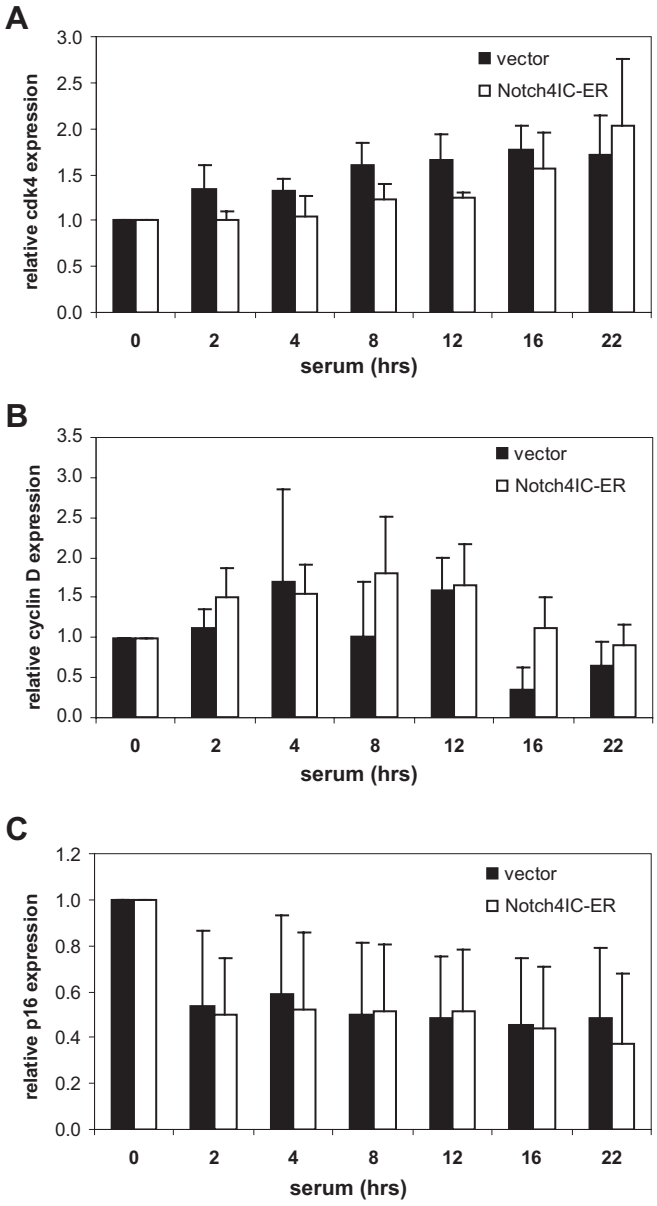
Expression of Notch4IC, Notch1IC or Notch4IC-ER. (**A**) HUVEC transduced with empty vector, Notch4IC or Notch1IC were analyzed by immunoblotting with an antibody against the HA epitope (HA) or Notch1. (**B**) HUVEC-vector and HUVEC-Notch4IC-ER were tested by immunoblotting with an antibody against ER. Immunoblotting for tubulin was performed as a loading control (tubulin).

Supplemental material, Figure S2



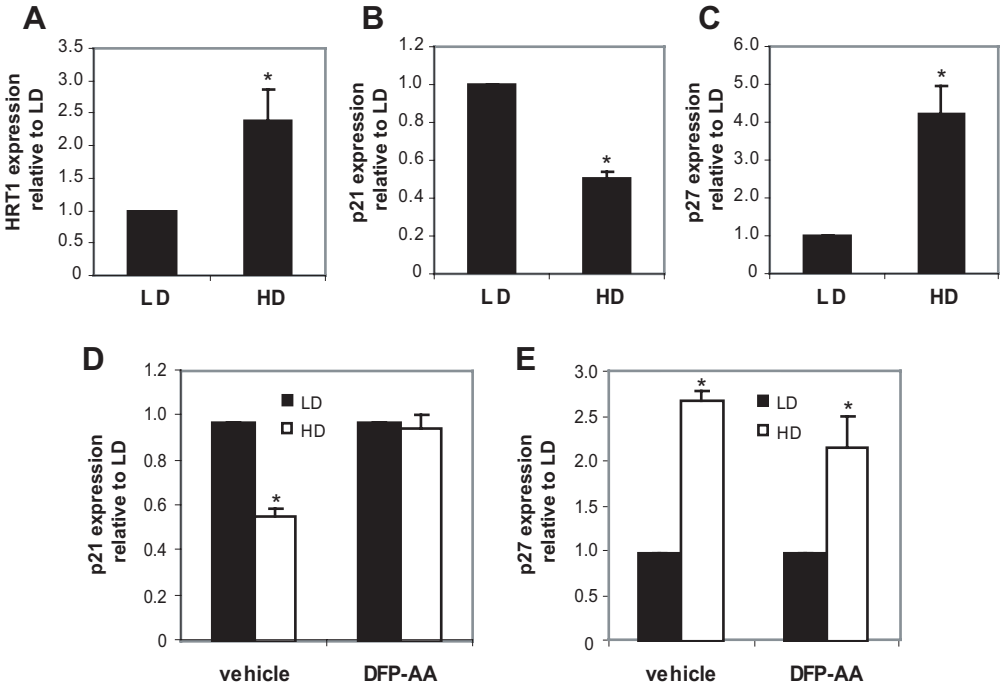
Notch4IC and Notch1IC inhibit S-phase entry in HAEC. HAEC were transduced with MIY (vector), MIY-Notch4IC (Notch4IC) or MIY-Notch1IC (Notch1IC). Asynchronously growing cells were assayed for BrdU incorporation by immunofluorescent staining. The antibody used against BrdU was Alexa Fluor 594 conjugated. DAPI staining was used to define nuclear localization and for total cell counts.

Supplemental material, Figure S3



Relative expression of cdk4, cyclin D and p16^{INK4a} in HUVEC-vector and Notch4IC-ER following serum stimulation. HUVEC transduced with vector or Notch4IC-ER were synchronized in quiescence by serum starvation and induced to re-enter the cell cycle by addition of serum. Cells were treated with 4-OHT to induce Notch4IC-ER nuclear translocation. At the indicated times after serum stimulation cells were assessed by immunoblotting for expression of cdk4 (**A**), cyclin D (**B**) and p16^{INK4a}(**C**). Immunoblots were analyzed by densitometry and normalized for tubulin. Graphs represent the mean ± SEM of three experiments (except for **B** where n = 4) and show no statistically significant differences between vector and Notch4IC-ER.

Supplemental material, Figure S4



Densitometric analysis shows statistically significant Notch-dependent downregulation of $p21^{Cip1}$ following endothelial cell-cell interactions. HUVEC were plated at low (LD, ~ 30% confluent) and high (HD, 100% confluent) density. (**A**) mRNA expression of the Notch target gene, HRT1, was assayed by RT-PCR. Expression of HRT1 and GAPDH were analyzed by densitometry. HRT1 expression was normalized to GAPDH. (**B** and **C**) Immunoblots for $p21^{Cip1}$ and $p27^{Kip1}$, as well as tubulin, were analyzed by densitometry. Quantification of $p21^{Cip1}$ and $p27^{Kip1}$ was normalized to tubulin. (**D** and **E**) HUVEC at LD and HD were treated with the γ-secretase inhibitor, DFP-AA (0.1 μM), or with vehicle alone. Immunoblots for $p21^{Cip1}$ and $p27^{Kip1}$, as well as tubulin, were analyzed by densitometry as in B and C. Graphs represent the mean ± SEM of at least three experiments. * $p < 0.05$ compared to LD.