Supplemental Material

Table S1. Differentially expressed genes in RhoA DN (T19N)-overexpressing vs. RhoA CA (Q63L)-overexpressing HUVEC. See Excel file.

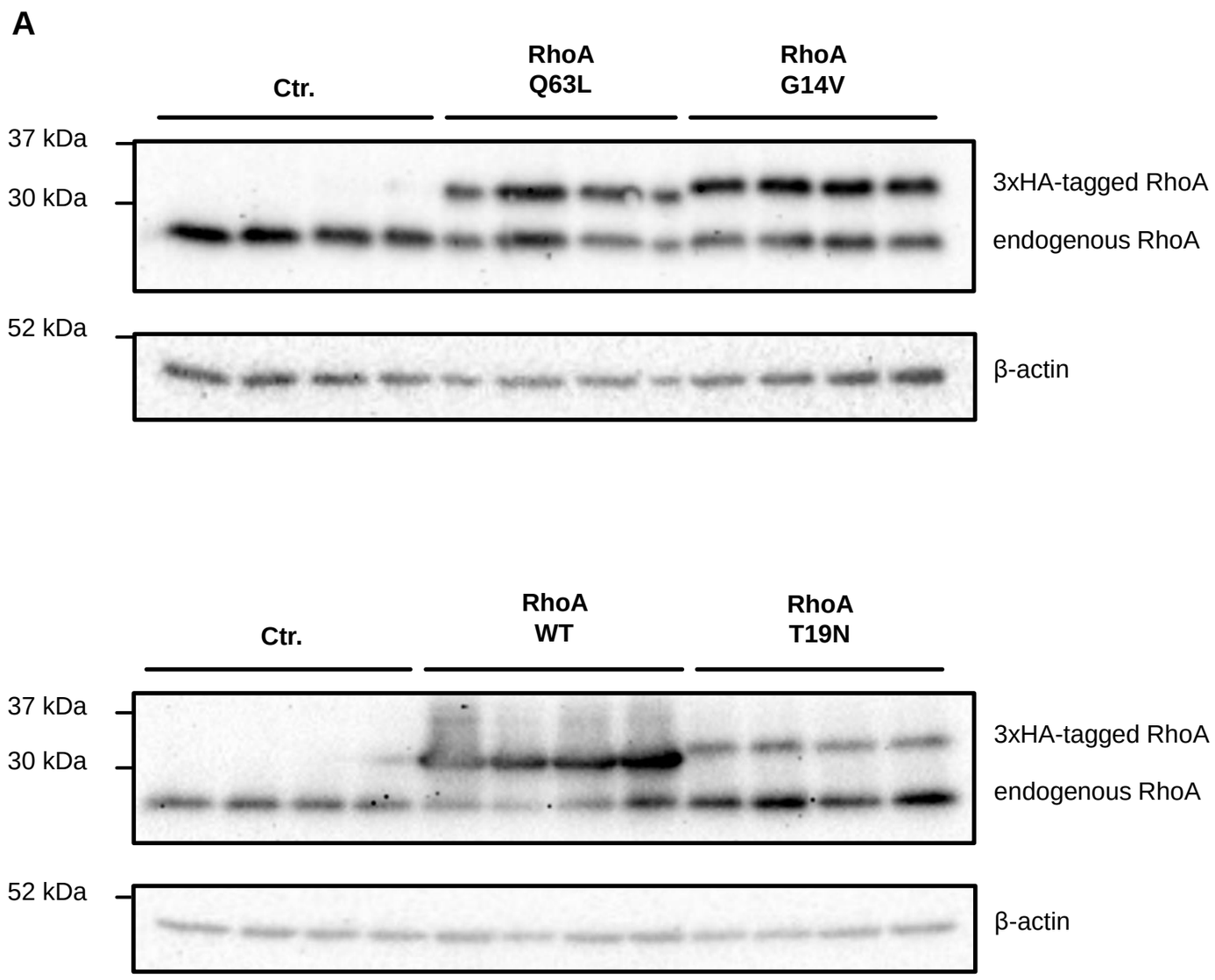

Figure S1. A) Western blots show RhoA protein levels of control-transduced HUVEC (Ctr) compared with HUVEC overexpressing constitutively active (RhoA Q63L or G14V; upper panel), wild-type, or dominant-negative (RhoA WT or T19N; lower panel) variants of RhoA. The bands for the endogenously expressed and exogenously introduced RhoA can be distinguished based on the size differences caused by the 3xHA tag of the lentivirally expressed RhoA constructs. **B)** Total RhoA expression was normalized to actin levels and set 100% for control-transduced HUVEC. For all constructs, significant overexpression of total RhoA (endogenous + 3xHA-labeled RhoA) was observed between 1.5-fold (G14V) and 3.1-fold (WT) compared with control-transduced HUVEC (endogenously expressed RhoA only). *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$ compared to control-transduced HUVEC; (n=4). C) Overexpression of dominant-negative RhoA significantly increased the expression of endogenous RhoA, while overexpression of constitutively active RhoA (G14V, but not Q63L) significantly reduced the expression of endogenous RhoA compared to control-transduced cells. ** = $p<0.01$, * = $p<0.05$ compared to control-transduced HUVEC; (n=4).

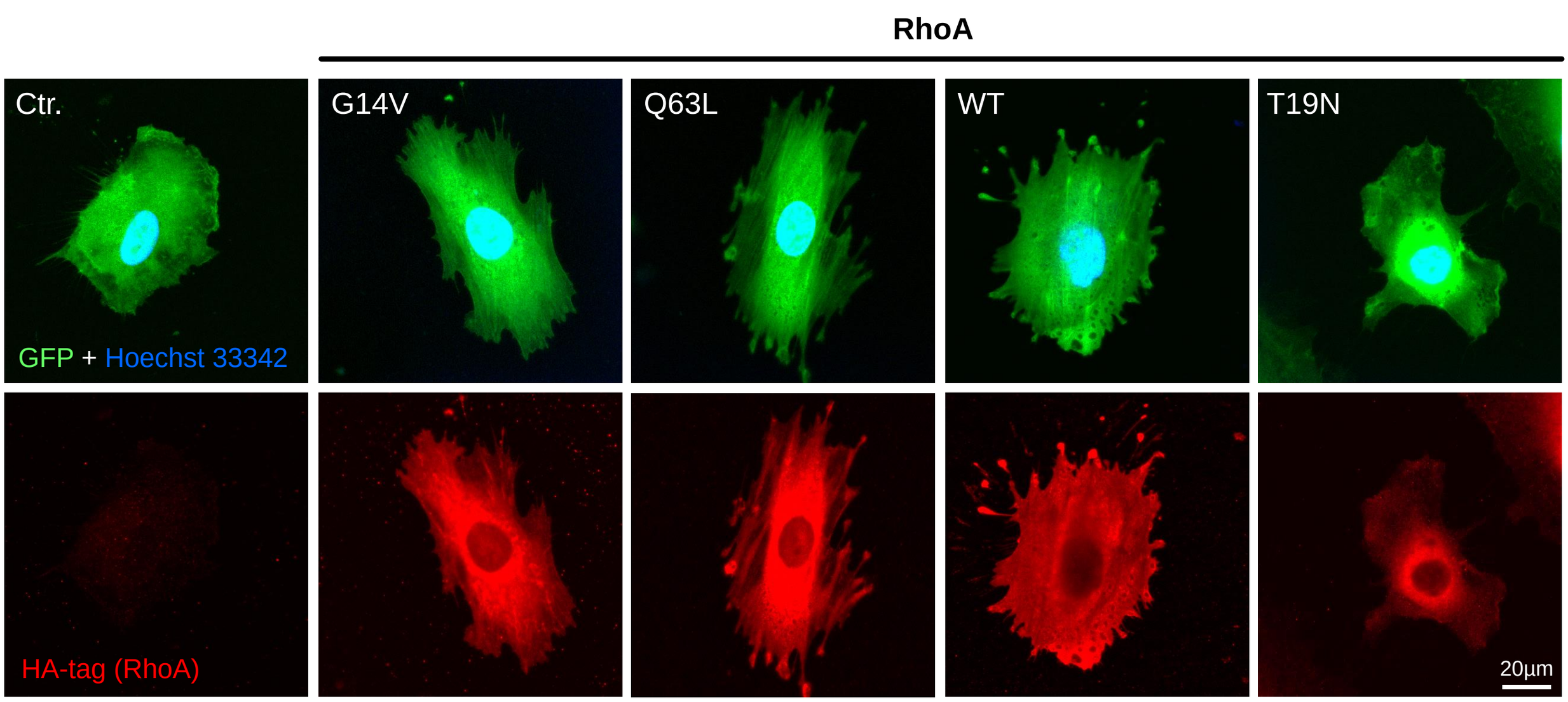

Figure S2. Expression and localization of RhoA variants. A) HUVEC transduced with lentiviral particles leading to overexpression of G14V, Q63L, WT, and T19N variants of RhoA, or Ctr.-transduced HUVEC expressing the reporter GFP (upper panel). RhoA was detected with an antibody directed against the N-terminal HA tag of RhoA variants (lower panel). In constitutively active (G14V, Q63L) and wild-type RhoA-transduced HUVEC, RhoA is localized over the entire cell surface except for the nucleus, likely due to the association of active RhoA with the plasma membrane of flat-growing HUVEC. In contrast, inactive dominant-negative RhoA (T19N) is predominantly localized in perinuclear regions of the cell. As expected, no HA expression is detectable in control cells.

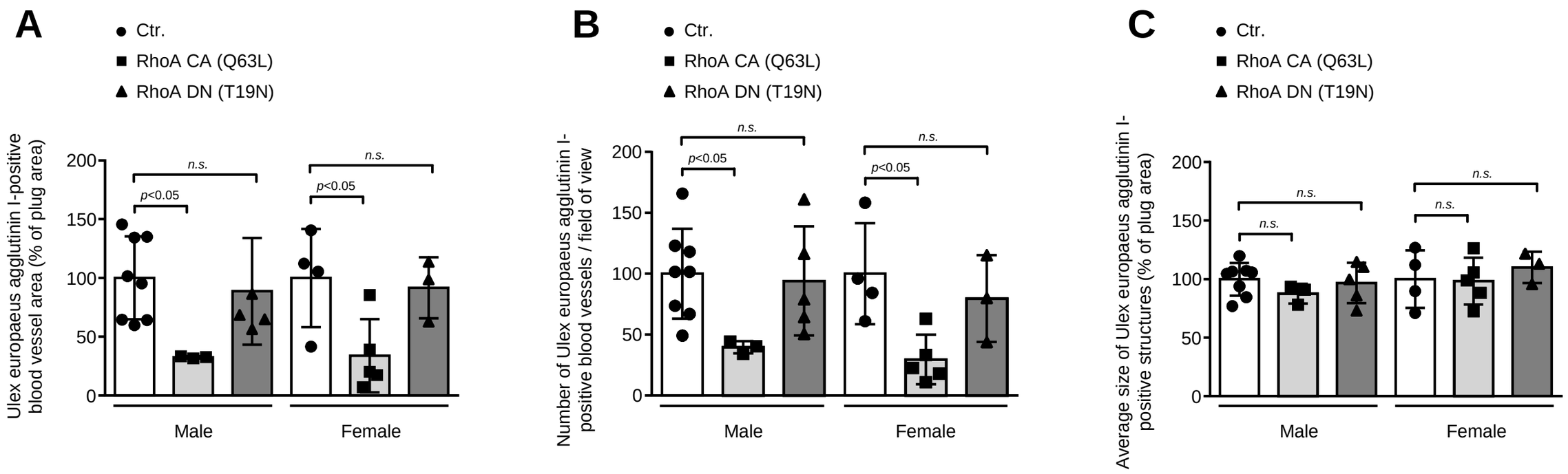

Figure S3. Impact of RhoA on Ulex europaeus agglutinin I (UEA I)- and murine hemoglobin-positive humanoid neovessel formation in male and female NSG™ mice *in vivo*. A-C) Overexpression of constitutively active (Q63L) RhoA in HUVEC reduces VEGF- and bFGF-induced blood vessel formation *in vivo* as compared to control-transduced HUVEC, whereas overexpression of dominant-negative RhoA (T19N) has no significant effect (n=3-8 plugs). Sex-specific analyses indicate inhibitory effects of RhoA Q63L overexpression in HUVEC implanted in both male and female mice. Blood vessel growth was analysed by quantification of the UEA I-positive blood vessel area (A). Moreover, the number of UEA I-positive blood vessels per high-power field (B) as well as the average size of these vessels (C) were determined. D-F) In addition, as a surrogate for the connection of intra-plug blood vessels to the functional blood circulation of the murine host, murine hemoglobin in association with neovessel structures was determined by histomorphometric immunofluorescence analysis after antibody labelling of mouse hemoglobin subunit alpha. Overexpression of constitutively active (Q63L) RhoA in HUVEC reduces VEGF- and bFGF-induced murine hemoglobin-positive blood vessel formation *in vivo* as compared to control-transduced HUVEC, whereas overexpression of dominant-negative RhoA (T19N) has no significant effect (n=3-5 plugs). Sex-specific analyses indicate inhibitory effects of RhoA Q63L overexpression on HUVEC-induced neovessel formation both in male and female mice. Blood vessel growth was analysed by quantification of the hemoglobin-positive blood vessel area (D). Moreover, the number of hemoglobin-positive blood vessels per high-power field (E) as well as the average size of these vessels (F) were determined. Data are shown as mean±SD. *n.s.* = non significant.

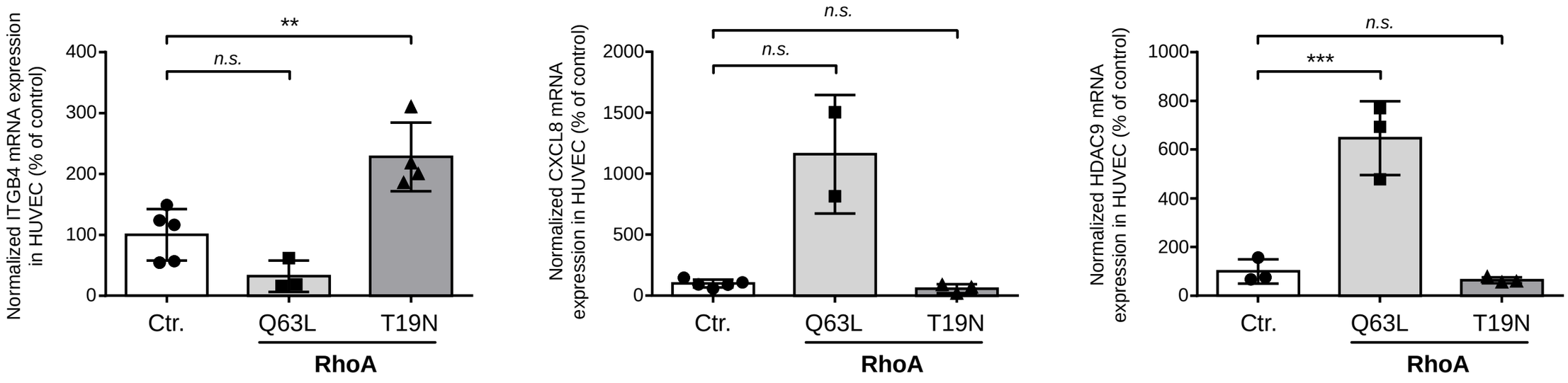

Figure S4. A-H) Validation of RNA-Seq results of selected differentially expressed genes using qRT-PCR. ** $p<0.01$ / *** $p<0.001$ vs. control-transduced HUVEC (n=2-5). *n.s.* = non significant.