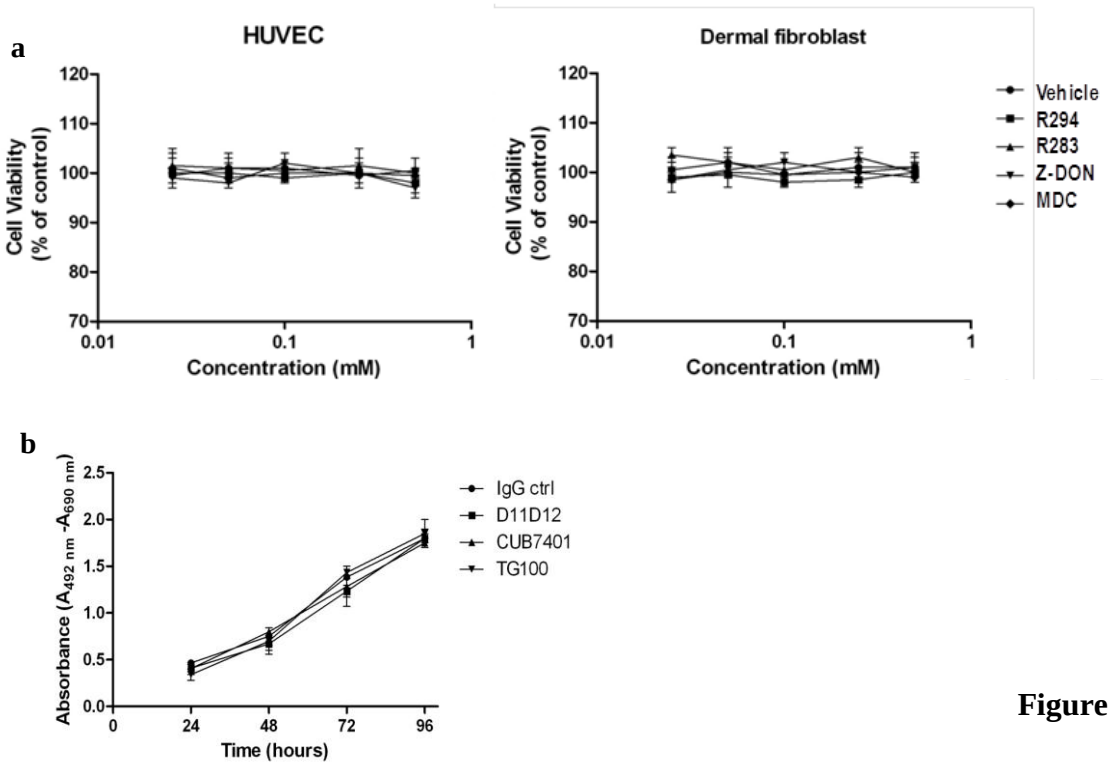

Figure S1

Supplementary Figure S1. (a) Determination of the cytotoxicity of TG2 inhibitors R294, R283, Z-DON and MDC at the concentrations of 0.05, 0.075, 0.1, 0.25, 0.5 and 0.75mM on HUVECs cultured in endothelial cell growth medium or dermal fibroblasts cultured in DMEM medium containing 10% FBS over a 72 h period. The XTT assay was used to measure cell viability. Vehicle of the inhibitor (DMSO 0.01%) was used as the control treatment. Data represent mean values ± S.D. form 3 separate experiments. **(b)** Proliferation of HUVECs in response TG2 comercial antibodies CUB7401 and TG100, using the XTT assay as in **(a)**. An IgG-matched isotype was used as control.

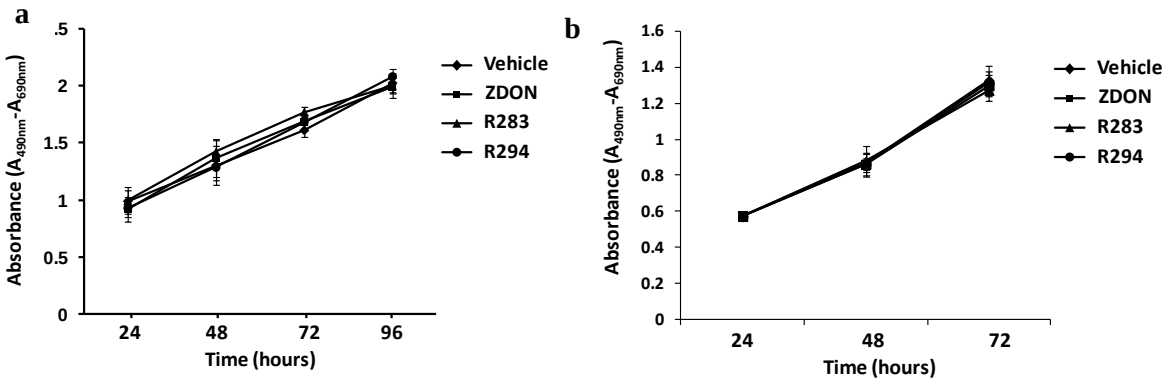

Figure S2

Supplementary Figure S2. (a and **b)** Determination of the cytotoxicity of TG2 inhibitors R294 (100μM), R283 (100μM) and Z-DON (50μM) on HUVECs **(a)** or dermal fibroblasts **(b)** at different time courses via XTT assay. Vehicle of the inhibitors (DMSO 0.01%) was used as the control treatment. Data represent mean values ± S.D. from 3 separate experiments.

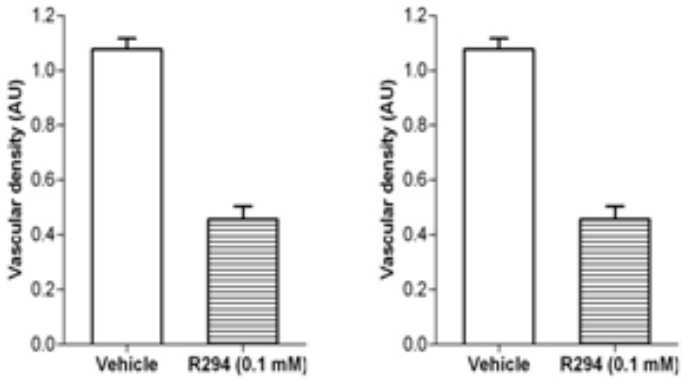

Figure S3

Supplementary Figure S3. Tubule outgrowth of explants from the rat aorta in Matrigel in response to R294 at the different concentrations indicated. Controls are untreated or treated with vehicle alone (DMSO 0.01%). Graphs correspond to the quantification of the vascular density and maximal outgrowth (arbitrary units). Tubules were quantified by computer assisted image analysis. Values shown equal mean values ± S.D. from 4 separate experiments.

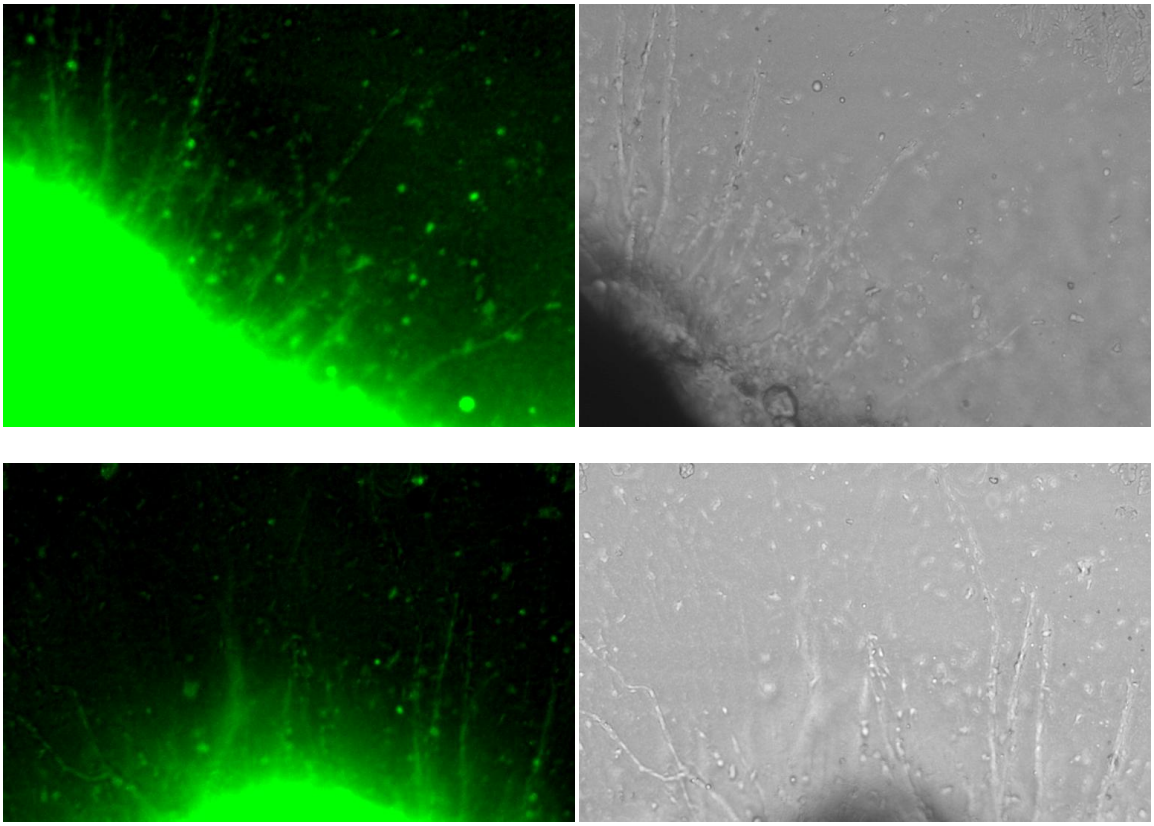

Figure S4

Supplementary Figure S4. Endothelial cell staining in the thin layer collagen aorta ring assay using CD31 as the endothelial cell marker. Immunofluorescence staining was performed to detect the presence of CD31 antigen in the tube structures in the thin layer aorta ring assay by using a specific anti-CD31 primary antibody and an FITC-conjugated secondary antibody. The fluorescence signals were detected using an epifluorescence microscope.

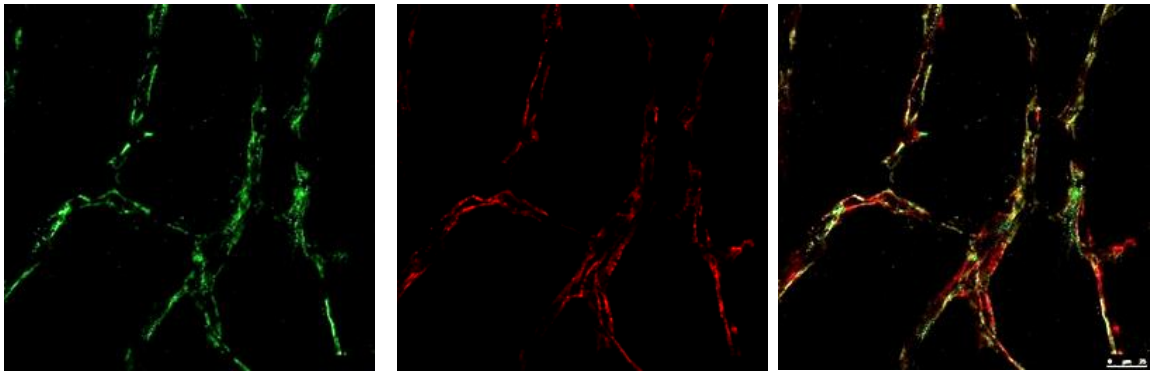

Figure S5

Supplementary Figure S5. Detection of TG2 in the HUVEC co-culture assay. Immunofluorescence staining was performed to detect the presence of TG2 by using its specific antibody Cub7402 (green), while CD31 was used as the endothelial marker (red) as introduced in Materials and Methods.

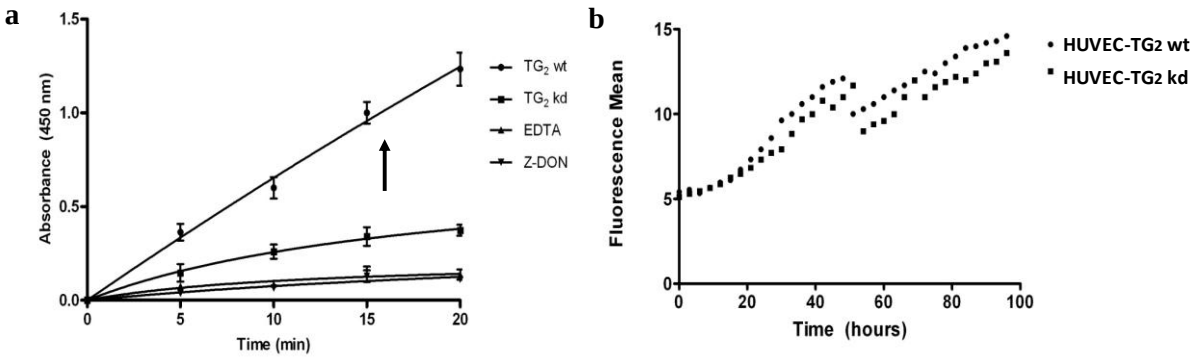

Figure S6

Supplementary Figure S6. (a) Crosslinking activity of cellular TG2 in lysates from HUVEC-TG2wt and -TG2kd, measured as the ability of extracts to incorporate biotin-pepT26, a biotinylated preferred first substrate of the isoenzyme, into the immobilised second substrate spermine as described in the Materials and Methods (CovTest). The TG2-mediated formation of the isopeptide product was revealed with the couple-enzymatic reaction of HRP-labelled streptavidin, using the TMB substrate. Data represent the mean ± S.D. absorbance values of three replicates. **(b)** Proliferation of HUVECs bearing either the GFP-scrambled vector (HUVEC-TG2wt) or the GFP-TG2 shRNA (HUVEC-TG2kd), indicated as fluorescence over time. Cells were seeded in 96 well-plates and incubated in the IncuCyte ESSEN instruments for 96 h. Fluorescence was registered in two different fields per well, every 3 h. The inflection in the curve (black arrow) matches with a replacement of fresh medium at 48 h. The average of two series is given in the plot.

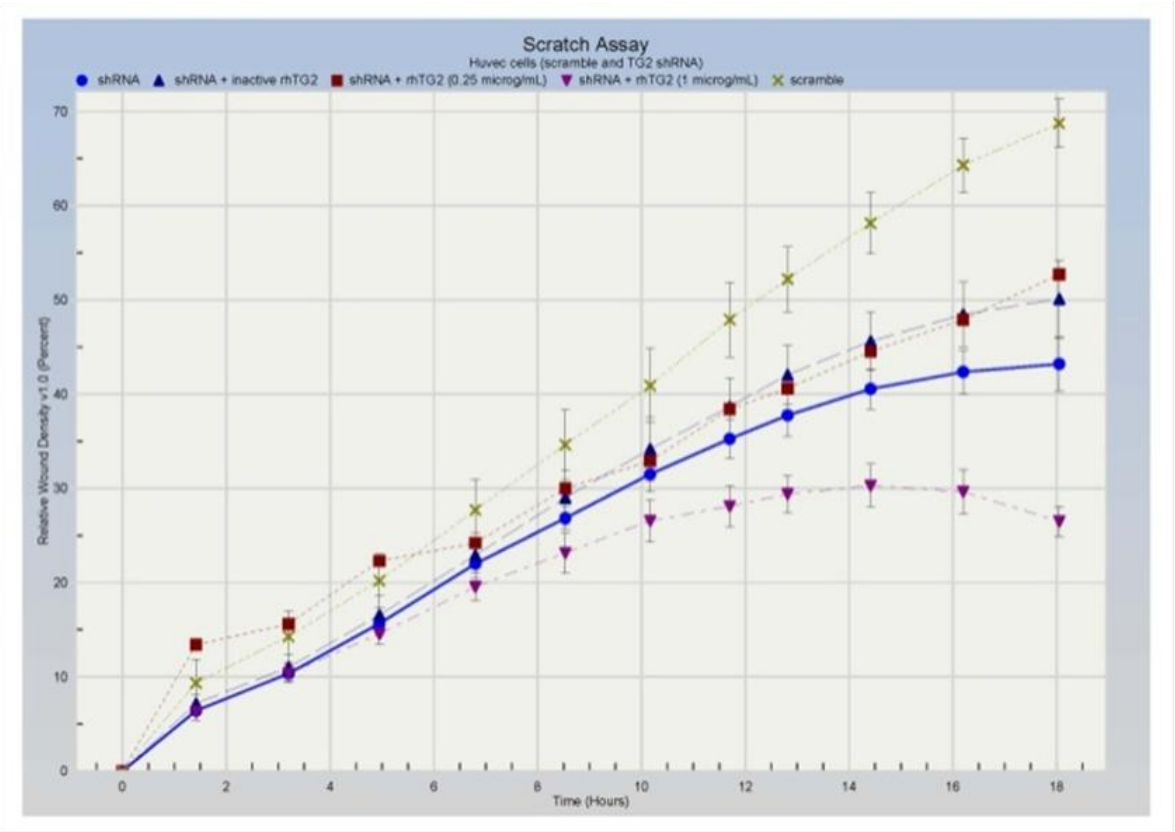

Figure S7

Supplementary Figure S7. Migration ability of HUVEC-TG2wt and HUVEC-TG2kd on fibronectin. Scratch assay. Migration of HUVEC-TG2wts and HUVEC-TG2kd on fibronectin, in the absence or presence of exogenously added rhTG2 at different concentrations, as indicated in the figure. The control is the enzymatically inactivated rhTG2**.** Graph illustrates the quantitative analysis of the relative wound density in scratch assays as in shown in **Figure 4e**.