**Figure 5. Requirement for endogenous apelin for inhibition of VEGF- or histamine-induced vascular edema.** (**A,B**) Vessel permeability was assessed by the Miles assay. Apelin-deficient (KO) and wt (WT) mice (**A**) or K14-apelin Tg (Tg) and wt (WT) mice (**B**) were injected with 15 μL of PBS in the presence or absence of rhVEGF165 (100ng/ml) or histamine (100μM) intradermally. Thirty min after injection, the inner side of the dorsal skin was inspected. Representative images of the vascular leakage are presented in the upper panels. Bottom panels show the quantification of extravasated Evans blue dye content eluted from dissected skin (mean ±SEM, n=10). **P*<0.05.

**Figure 6. Apelin inhibits VEGF mediated hyperpemeability** (**A**) Effect of apelin on VE-cadherin-mediated endothelial junctions. VE-cadherin expression was studied in HUVEC monolayers. HUVECs cultured with or without pretreatment with apelin for 10 minutes were stimulated with VEGF (20ng/ml) for 10 minutes. F-actin (red) and VE–cadherin (VE-cad.; green) staining is shown. Arrowheads indicate the gaps between adjacent ECs and lack of VE-cadherin. Nuclei were labeled with TOPRO-3 (TOP.; blue). (**B**) Suppression of VEGF-mediated VE-cadherin internalization by apelin. HUVEC cell surface VE-cadherin labeled by anti-VE-cadherin antibody and stimulated with VEGF (20ng/ml) in the presence (apelin) or absence (control) of apelin (100ng/ml). VE-cadherin internalization was visualized in fixed cells using secondary fluorescent antibodies (green). To confirm the internalization of VE-cadherin, cell surface bound VE-cadherin antibodies were removed by a mild acid wash before fixation (right two lines of images). Nuclei were labeled with TOPRO-3 (blue). (**C**) The internalization of endogenous VE-cadherin [in the right two lines of (**B**)] was quantified by the fluorescence intensity of secondary antibodies per cell. **P*<0.01. (**D**) Western blot analysis of cell surface and cytoplasmic VE-cadherin protein on HUVECs that had been stimulated with VEGF (20 ng/ml) and/or apelin (100 ng/ml) for 20 min. The purity of cytoplasmic protein was confirmed by the lack of expression of the cell surface protein

N-cadherin. GAPDH expression was analyzed as a control for an unrelated intracellular protein.

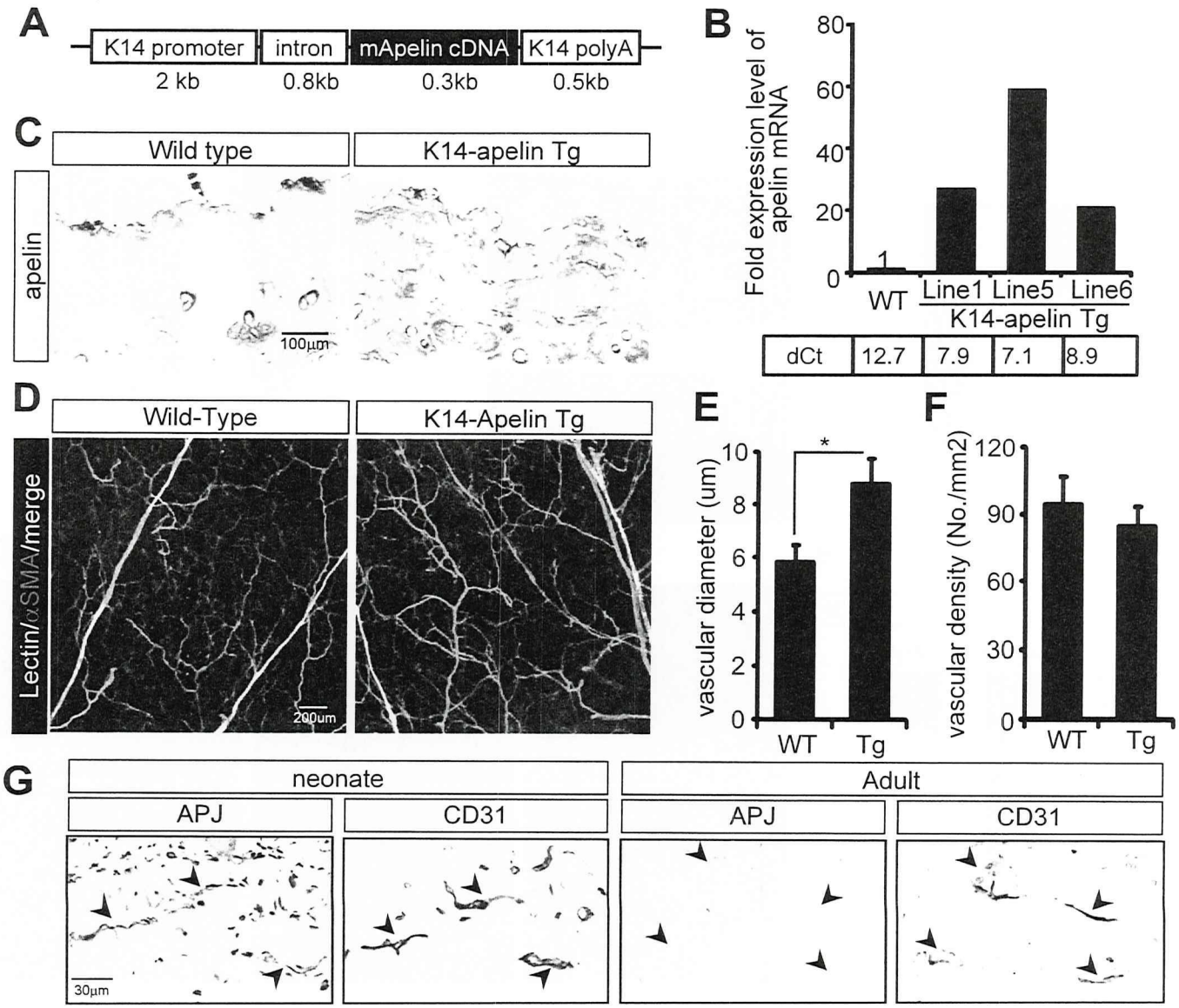


Figure 1 Kidoya H. et al.

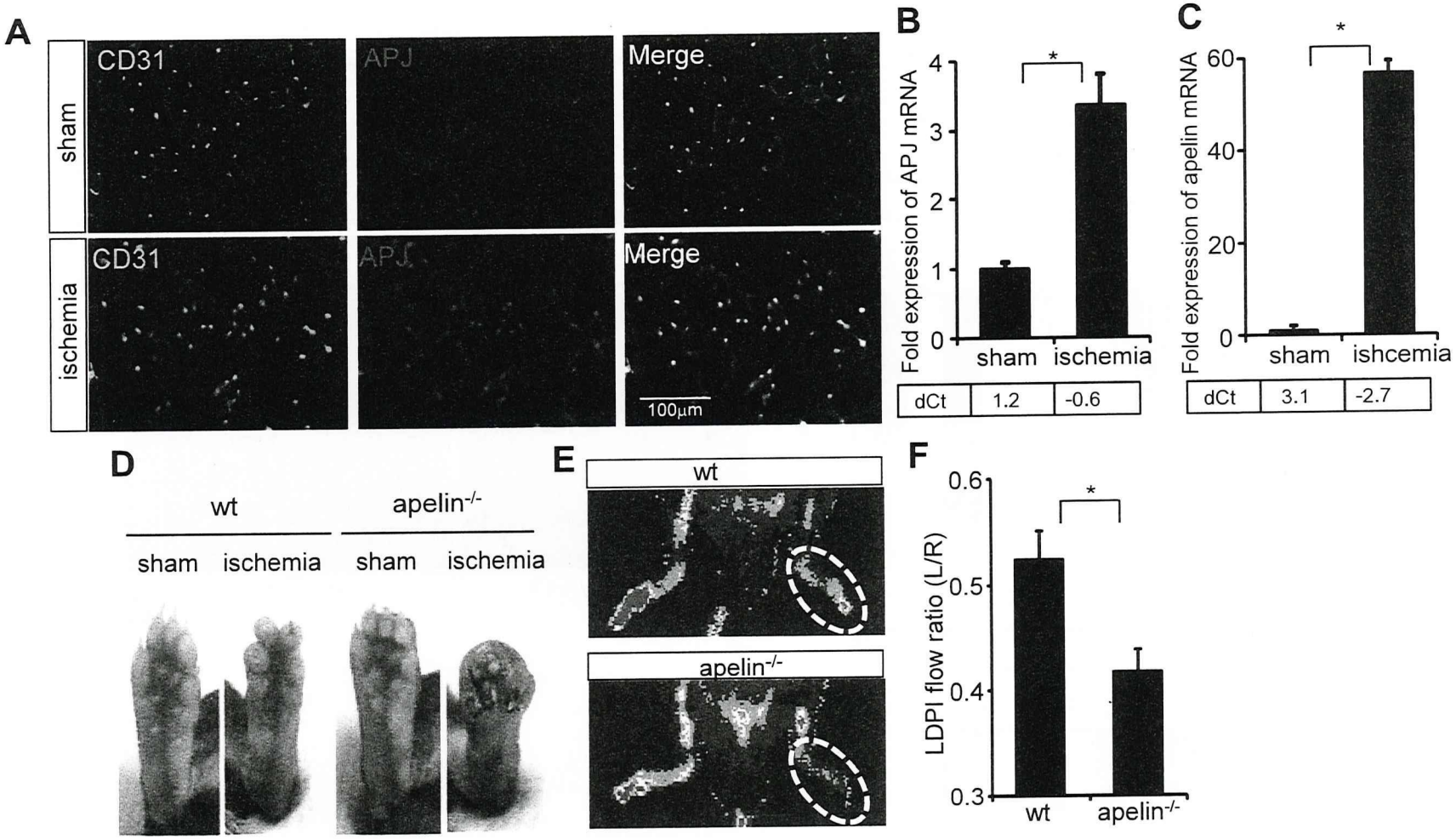


Figure 2 Kidoya H. et al.

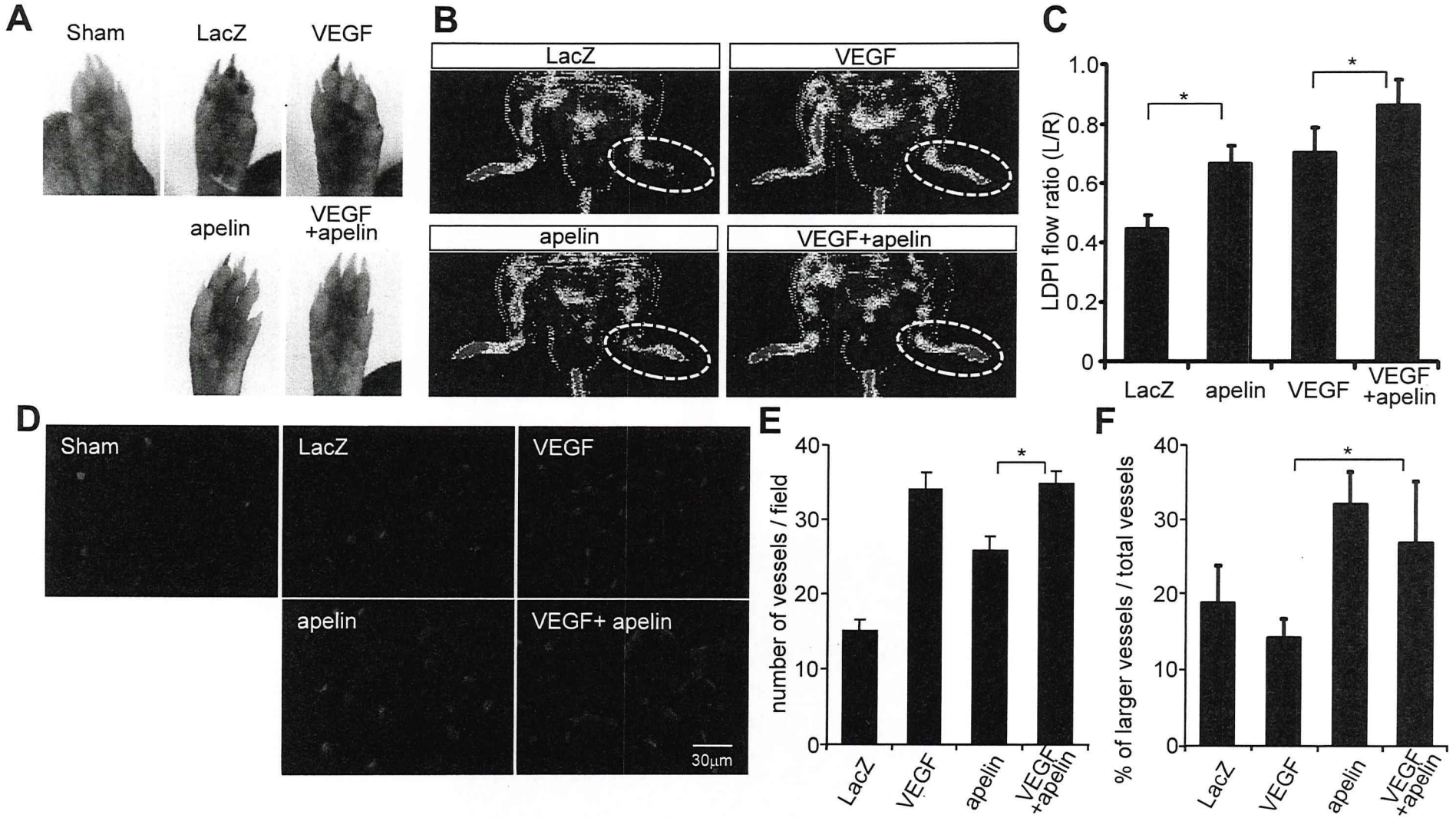


Figure 3 Kidoya H. et al.

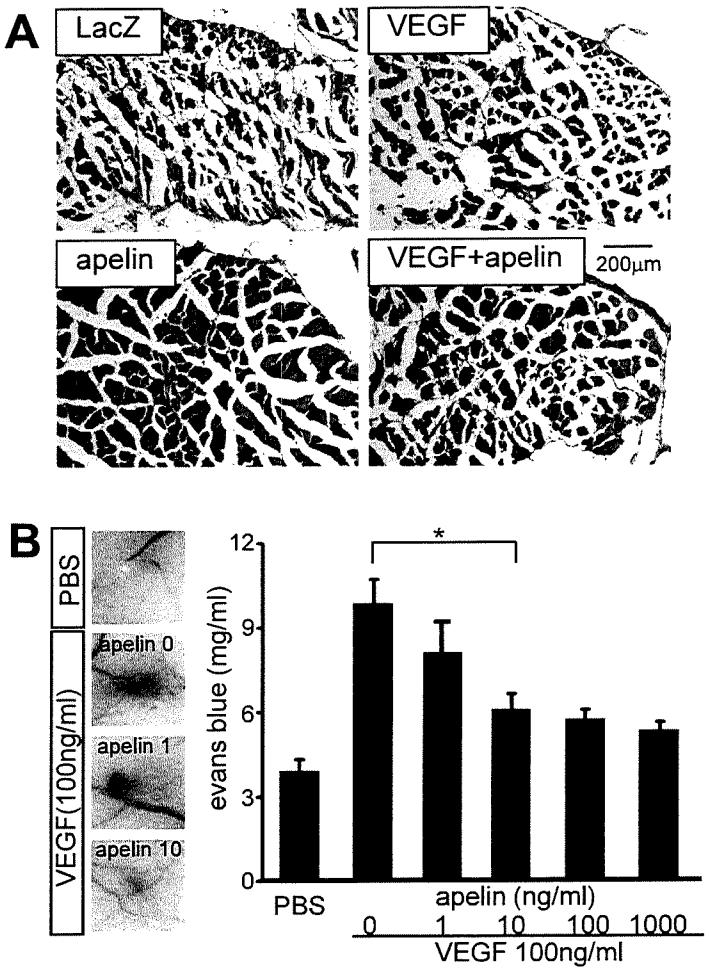


Figure 4 Kidoya H. et al.

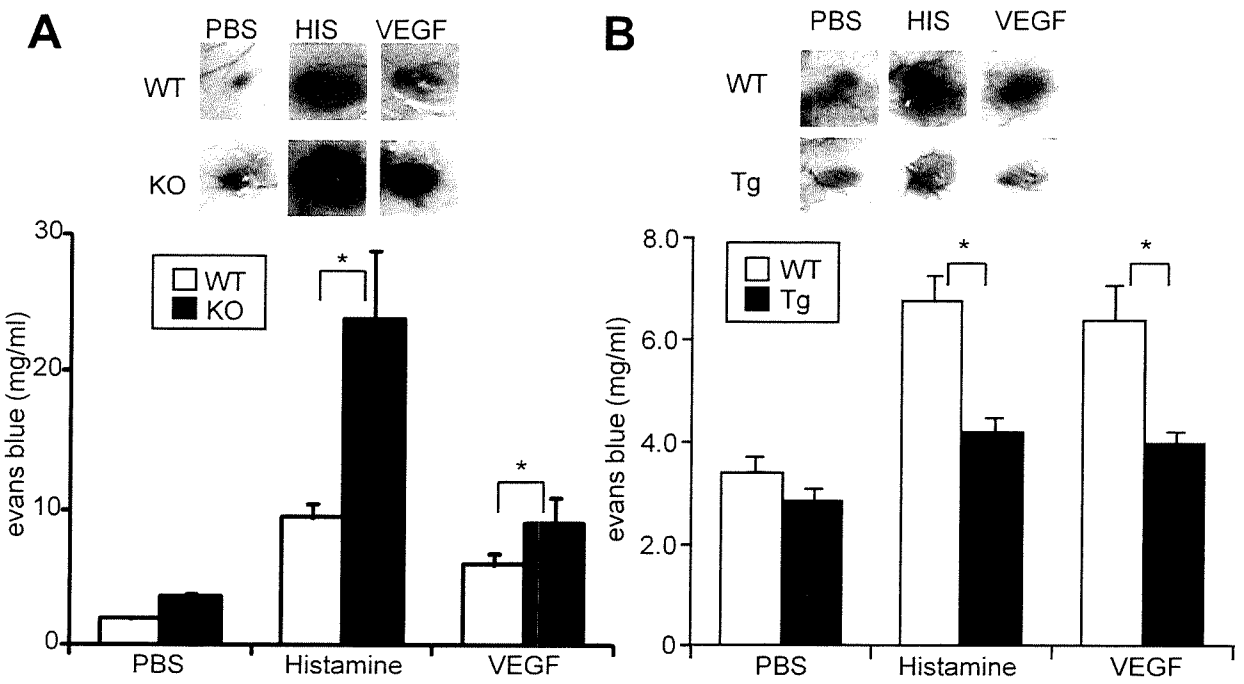


Figure 5 Kidoya H. et al.

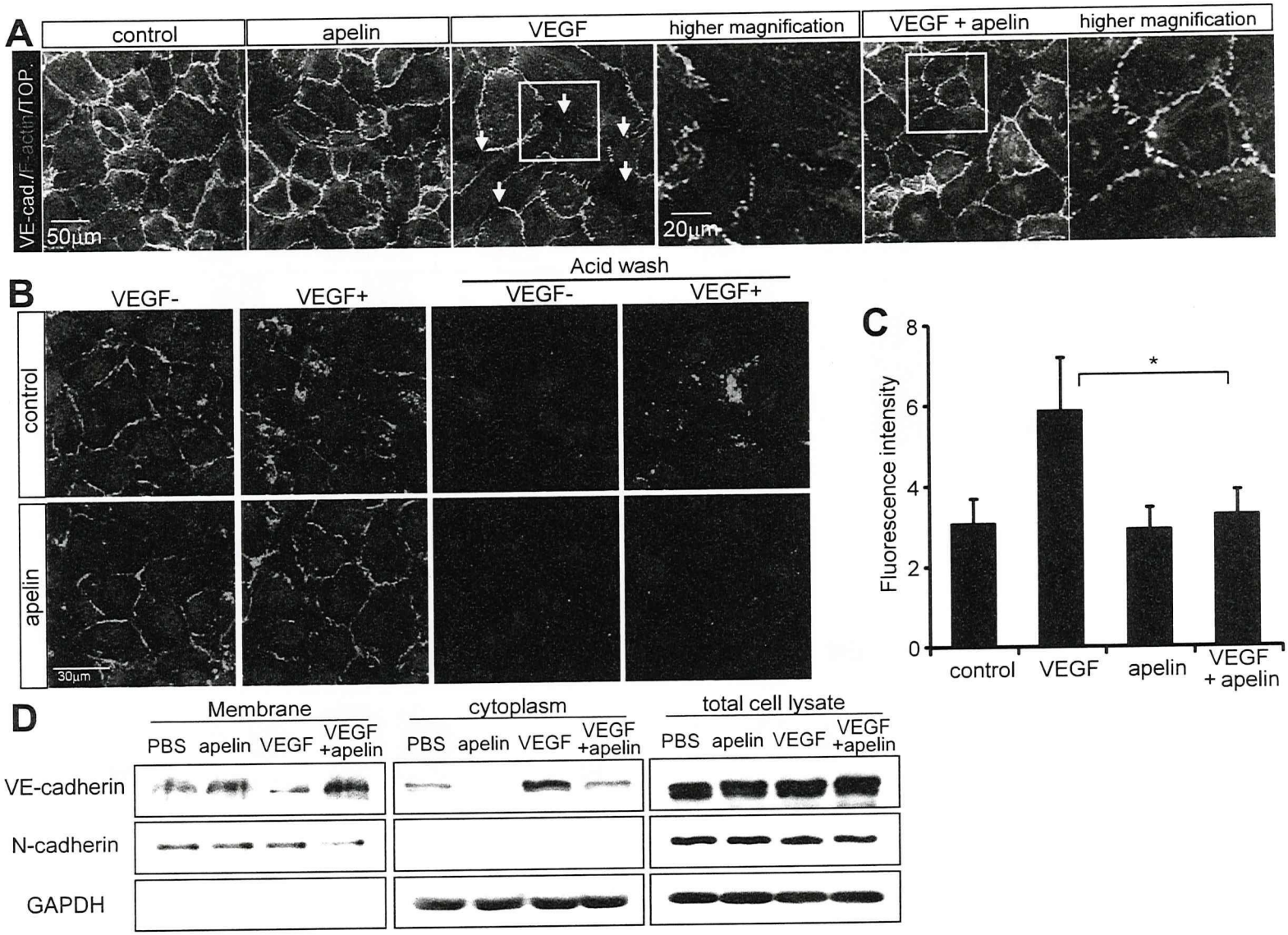


Figure 6 Kidoya H. et al.