Supplementary Information

Activation of mechanosensitive ion channel TRPV4 normalizes tumor vasculature and improves cancer therapy

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Supplementary Figure Legends

Supplementary Fig.1. A) Representative traces showing increases in the cytosolic calcium in response to a selective TRPV4 agonist, 4-α-PDD (arrow) in Fluo-4 loaded in normal and tumor endothelial cells. Arrow denotes the time when the cells were stimulated with the TRPV4 agonist. B) Quantitative analysis of the relative changes in the cytosolic calcium influx in NEC and TEC.

Supplementary Fig.2. A) Immunohistochemical analysis showing increased vessel density in tumors (21 days) from TRPV4 knockout mice (KO) compared to wild type mice (WT). Frozen sections of tumors (10 μm thickness) were stained with CD31 (green) to identify tumor micro vessels. B) Quantitative analysis of vessel density in WT and KO tumors.

Supplementary Fig.3. Tumor bearing mice were injected with TRITC-dextran (3000 MW; red) via tail vein, fixed and sections were immunostained with CD31 (green). Note the increased dextran fluorescence (arrows) in the tumor tissue in TRPV4KO mice compared to wild type indicating leaky vessels.

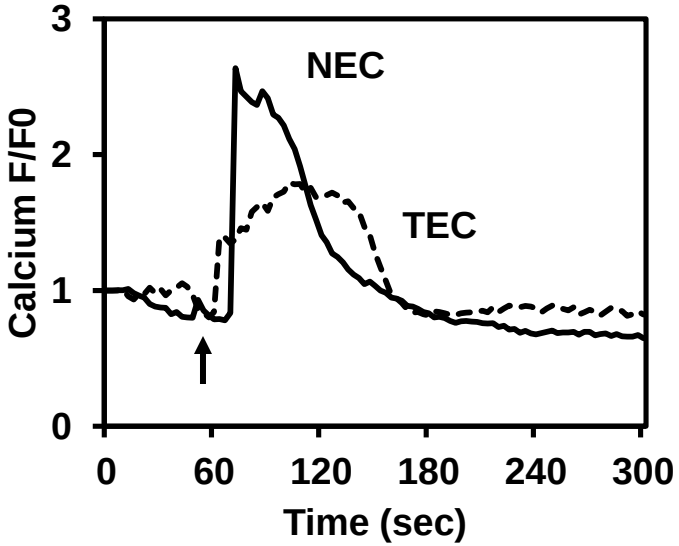
Supplementary Fig.4. A) Phase contrast and fluorescence images of TEC transfected with TRPV4-EGFP B) Representative traces showing increases in the cytosolic calcium in response to a selective TRPV4 agonist, GSK1016790A in Fluo-4 loaded TEC overexpressing TRPV4-EGFP or EGFP alone. Arrow denotes the time when the cells were stimulated with the TRPV4 agonist. C) Quantitative analysis of the relative changes in the GSK1016790A-induced cytosolic calcium influx in TEC expressing TRPV4-EGFP or EGFP alone.

Supplementary Fig.5. NEC and TEC were allowed to spread for 4 h at 37ºC and later shifted on to a microscope stage and random cell migration was recorded every 10 min using time lapse microscopy. Quantification of cell migration as measured by marking the centroid of migrating cells overtime. Note: TEC migration is 4 fold higher than NEC.

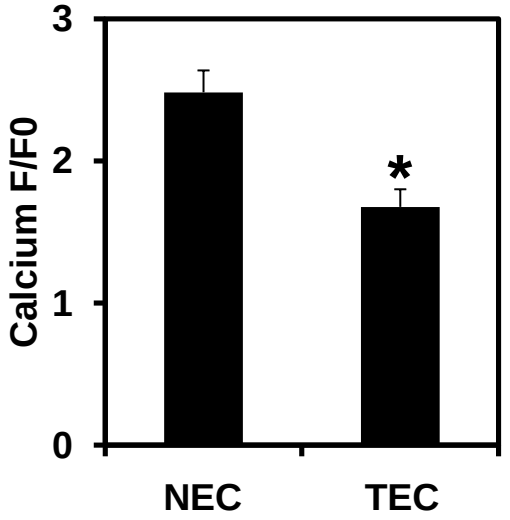
Supplementary Fig.6. Cells (TEC and TEC+TRPV4) were allowed to form tubes on a Matrigel (2D) as described in Fig.4 A. Images were obtained and tube length was measured using NIH Image J. Quantification of tube length revealed almost 10 fold increase in tube formation in TEC + TRPV4 cells compared to TEC alone.

Supplementary Fig.7. Representative Western blot showing the levels of active-Rho and total Rho for TEC and TEC treated with TRPV4 activator GSK1016790A. Rho activity was measured using the Rhotekin-RBD binding assay. Densitometry analysis of changes in Rho activity which was quantified by normalizing the levels of active Rho with that of total Rho (* $p < 0.05$). Note: TEC exhibit high basal Rho activity compared to NEC which was reduced by TRPV4 activation with GSK.

A

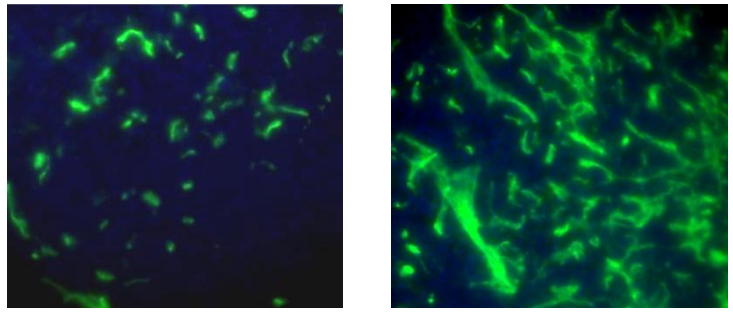


B



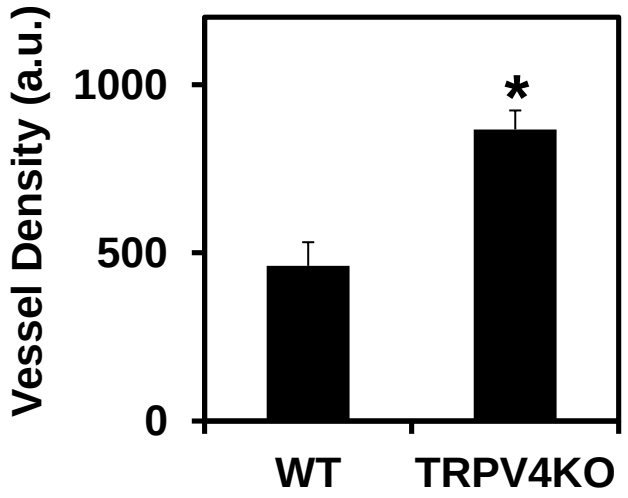
Adapala, Suppl Fig.1

A

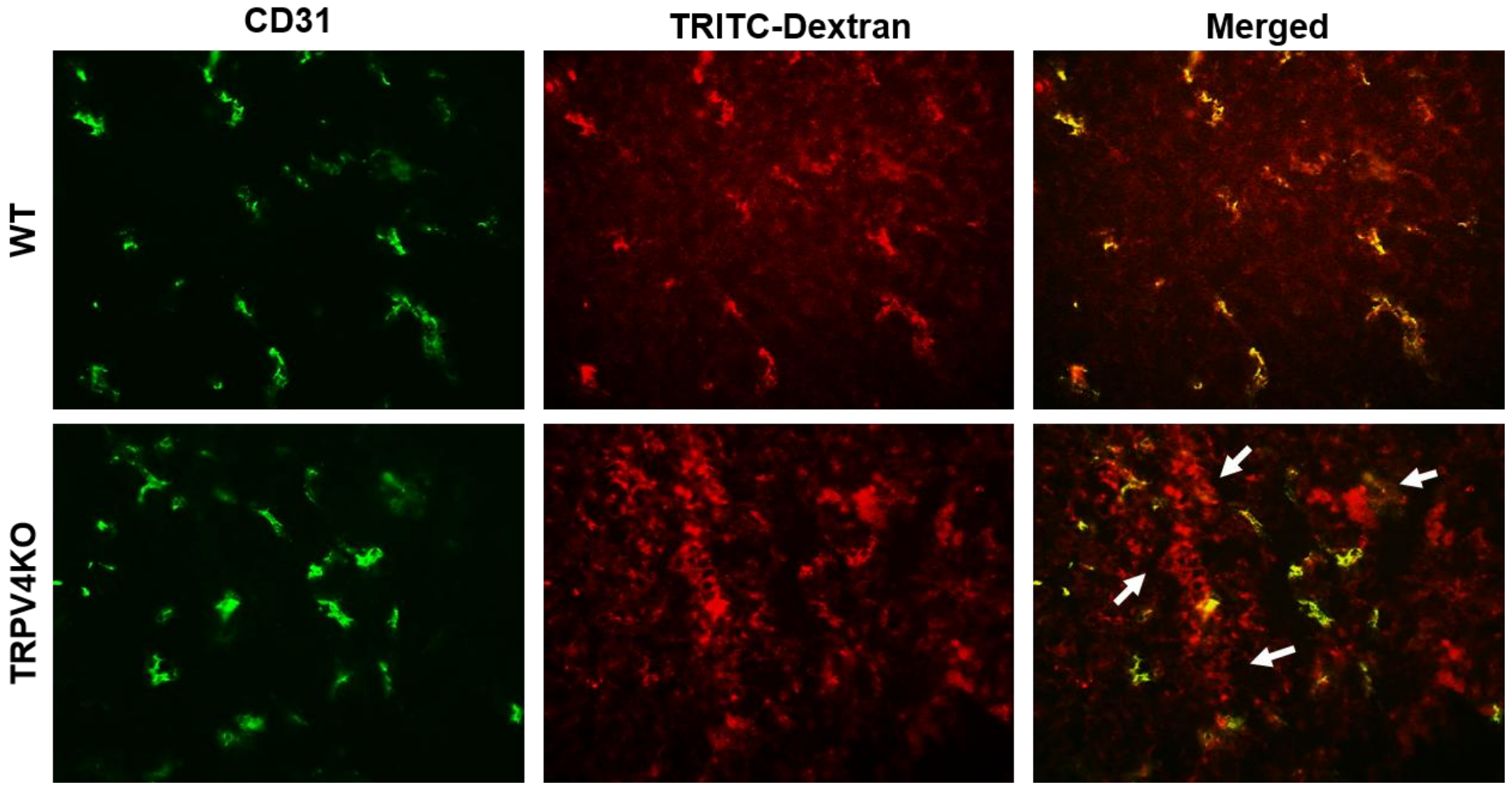


WT TRPV4KO

B

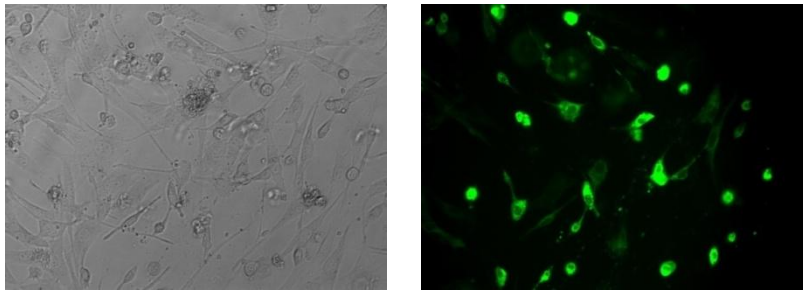


Adapala, Suppl Fig.2



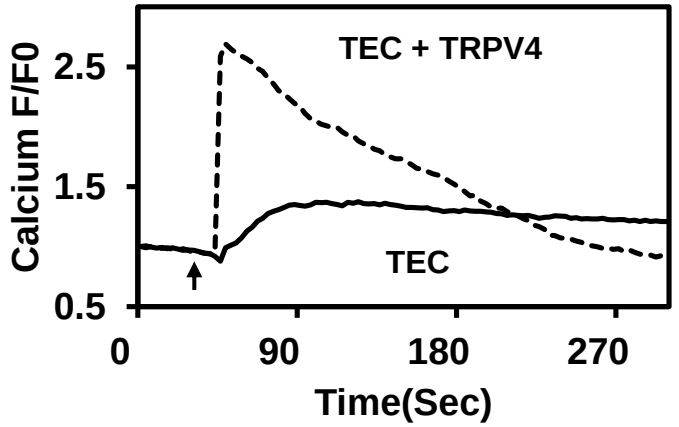
Adapala, Suppl Fig.3

A

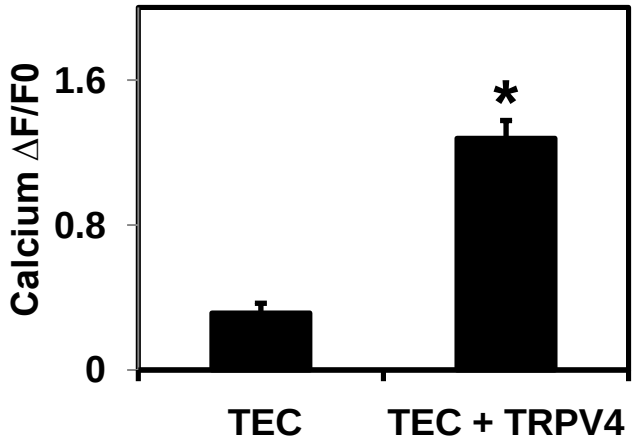


Phase EGFP

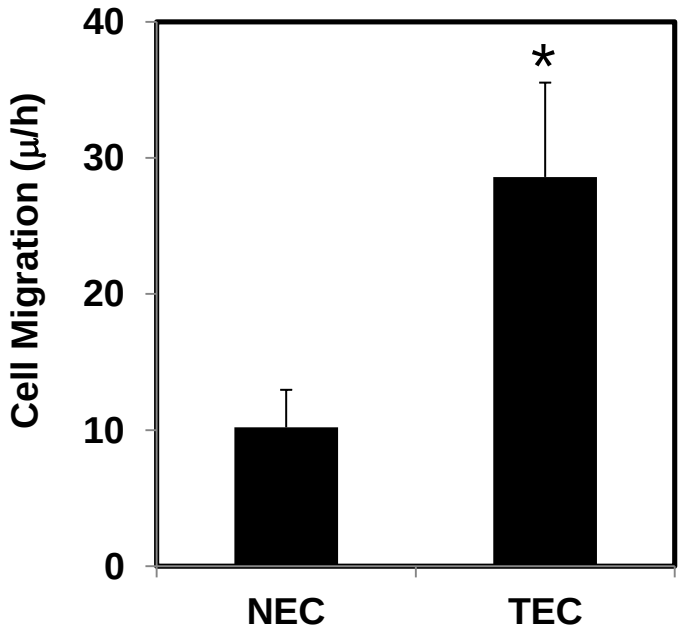
B



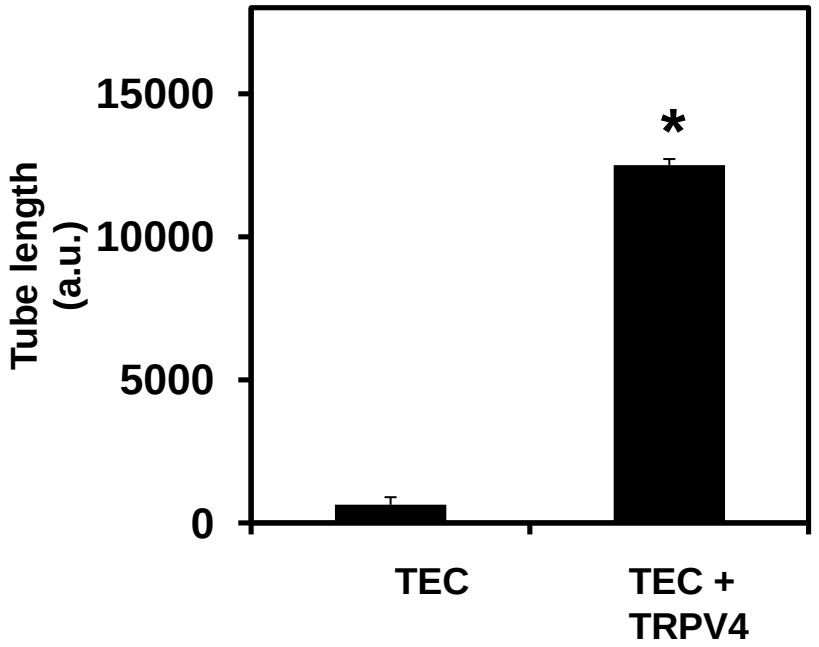
C



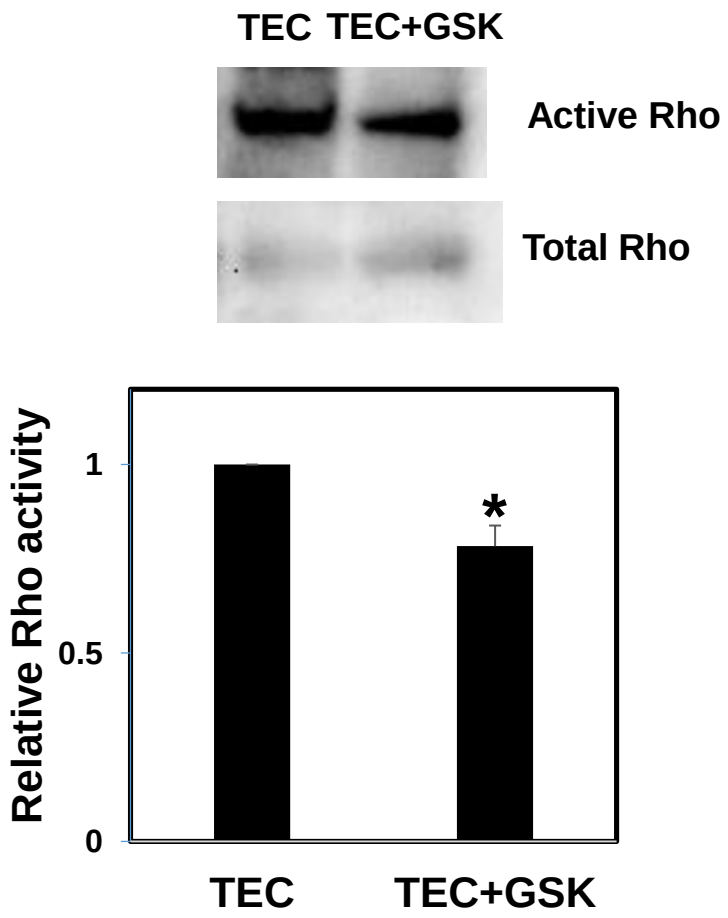
Adapala, Suppl Fig.4



Adapala, Suppl Fig.5



Adapala, Suppl Fig.6



Adapala, Suppl Fig.7