SUPPLEMENTARY DATA

THE TMEFF2 TUMOR SUPPRESSOR MODULATES INTEGRIN EXPRESSION, RHOA ACTIVATION AND MIGRATION OF PROSTATE CANCER CELLS

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Contents

SUPPLEMENTARY FIGURE LEGENDS

Supplementary figure 1. A) TMEFF2 but not TMEFF2_ΔGA inhibits cell migration as measured in a wound healing assay. Shown are representative images of cells migrated into the wound after the after the specified times. B) TMEFF2 inhibits RWPE2 cell migration as measured using a Boyden chamber transwell assay. Cells adhering to the bottom of the membrane were fixed, stained with crystal violet and photographed. Shown are representative images of cells after overnight migration.

Supplementary figure 2. Images showing the individual fluorescent channels corresponding to the cells stained with anti-vinculin (green), rhodamine phalloidin (orange), and DAPI (blue). The mergedimage is also shown (left column). See figure 4 from the main text.

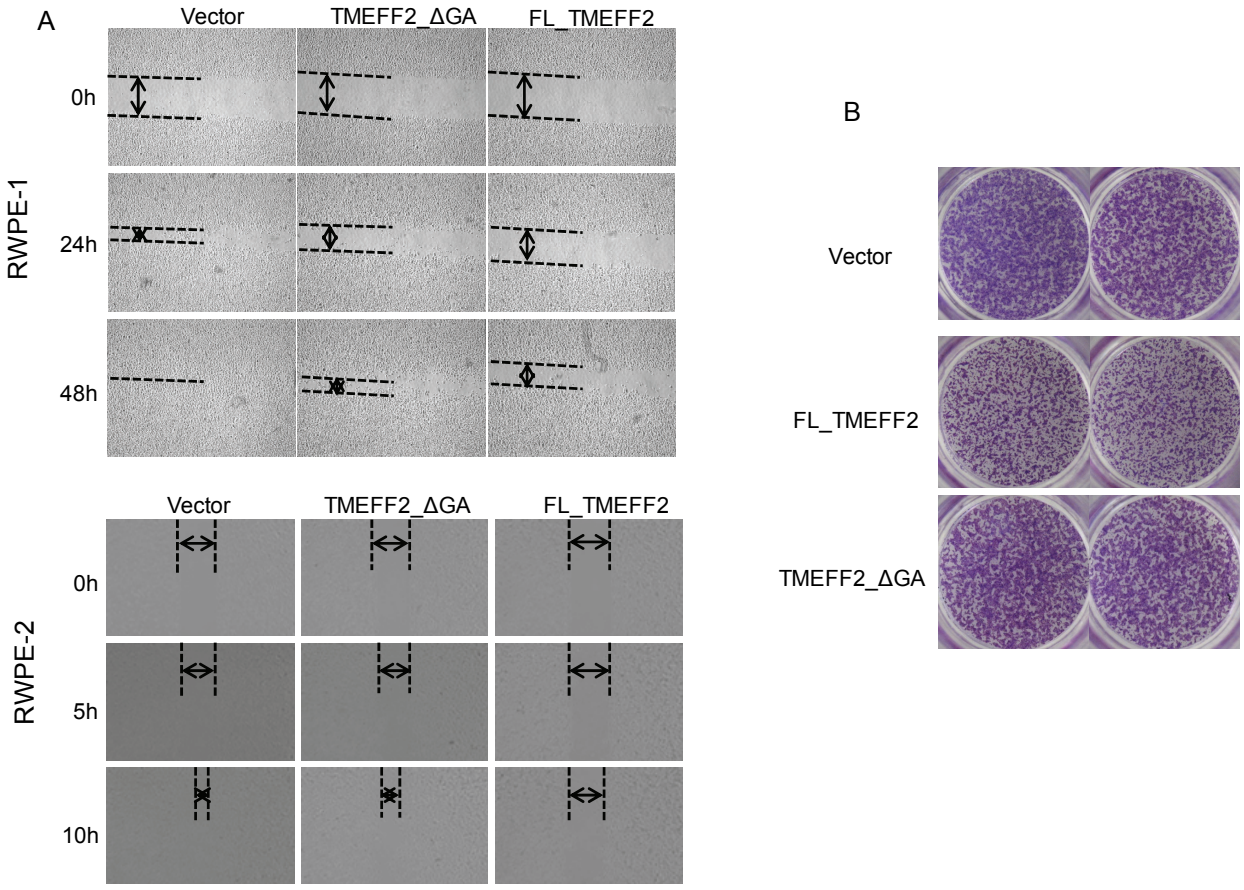
Supplementary figure 3. A) Inhibition of Rho by CT04 blocks RWPE2 cell migration and stress fiber formation. 24 h migration of RWPE2 cells treated with 2 μg/ml CT04 or control medium was measured by wound healing assays using ibidi culture inserts. Data are presented as mean ± SD of two independent experiments (left). Rhodamine phalloidin staining of RWPE2_Vector cells treated with 1 μg/ml CT04 or control medium for 4 h (right). Scale bar, 25 μm. B) Inhibiting Rho activity does not decrease αv or β3 integrin expression. RWPE2 cells were treated with 2 μg/ml CT04 or control medium overnight and total lysates were subjected to immunoblotting analysis with antibodies against the specified integrins. β-tubulin and β-actin were used as loading controls.

Supplementary figure 4. TMEFF2 decreases the expression of integrin genes. The expression of key genes involved in cellular adhesion to the extracellular matrix (ECM) was determined by Human Focal Adhesions RT² Profiler PCR Array using the vector and TMEFF2-expressing RWPE2 cells. Arrows indicate ITGAV and ITGB3 genes have over 2-fold reduction in TMEFF2-expressing cells compared with the vector cells. Fold change in expression of other genes is also indicated.

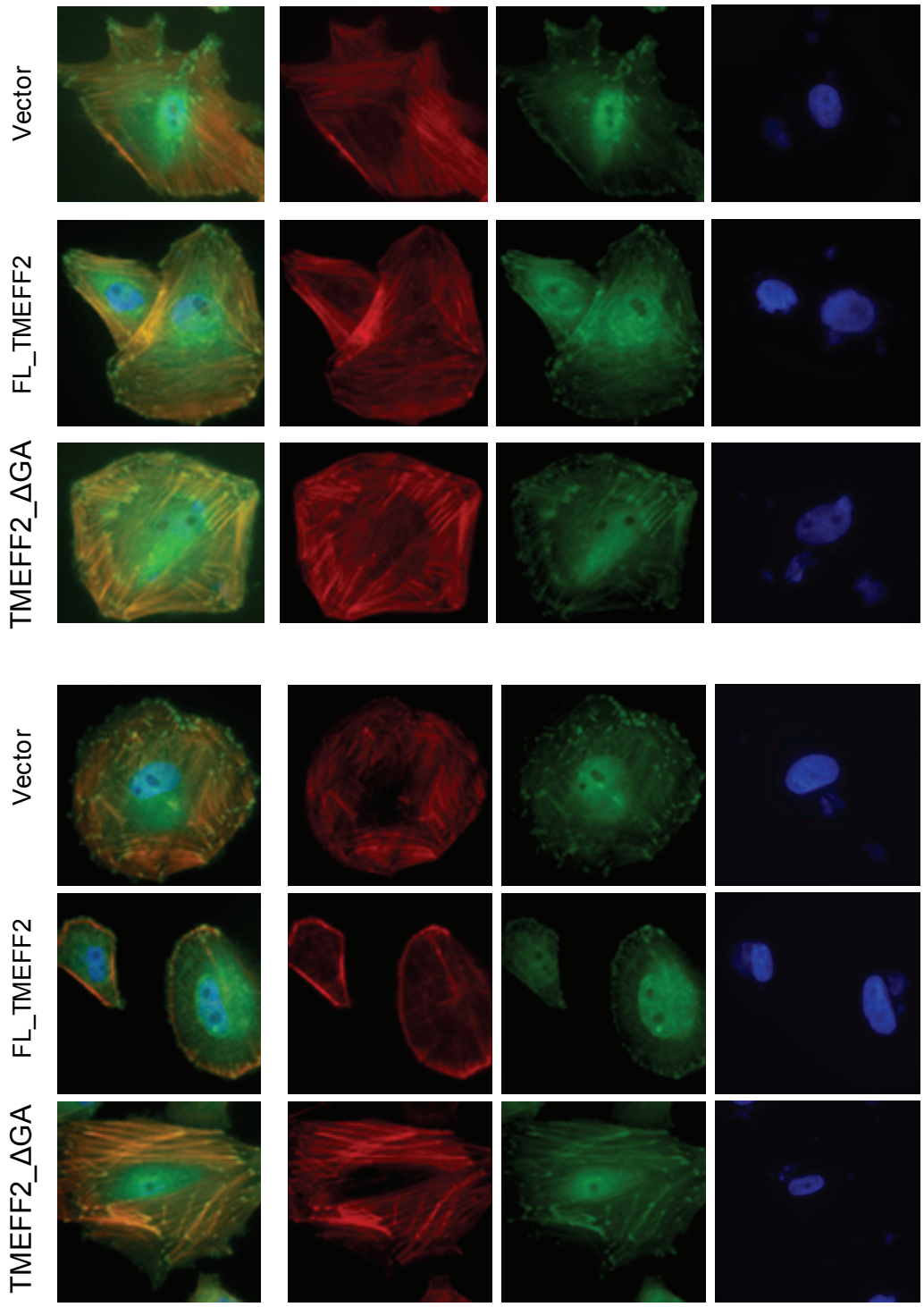
Supplementary figure 5. A) Characterization of the TMEFF2 custom made antibody from SDIX, and B) expression of TMEFF2 and large T-antigen in the TRAMP/TMEFF2 and TRAMP animals. A) The TMEFF2 antibody was tested using the 22Rv1 cell line, which expresses endogenous TMEFF2 (arrow; left lanes) and HEK293T cells expressing recombinant forms of TMEFF2 (right lanes). Knockdown of TMEFF2 using sh_RNA, results in drastic reduction or disappearance of the protein band corresponding to TMEFF2 (left lanes). TMEFF2 can be detected in HEK293T cells expressing a recombinant form of the full length TMEFF2 protein but not a deletion mutant (TMEFF2_ΔFS1) lacking a region that overlaps with the antigenic region used for producing the antibody. Note the presence of a non-specific band (*) with slightly faster mobility than the TMEFF2 protein. The same set of samples was probed with a commercial TMEFF2 antibody from Sigma with similar results. Note that the band corresponding to the TMEFF2_ΔFS1 deletion mutant is detected with the Sigma TMEFF2 antibody since it was produced using a different peptide region. B) Western blot of the ventral (V) and dorsolateral (DLP) lobes from the TRAMP and TRAMP/TMEFF2 transgenic animals and a non-transgenic sibling (C57bl/6) demonstrating the presence of TMEFF2 only in the TRAMP/TMEFF2 animals the T-antigen (T-ag) in the TRAMP and TRAMP/TMEFF2 transgenic animals (arrow). Note the presence of one or more lower cross reacting bands with the T-antigen antibody that also appear in the lobes of the non-transgenic animals (*).

FIGURES

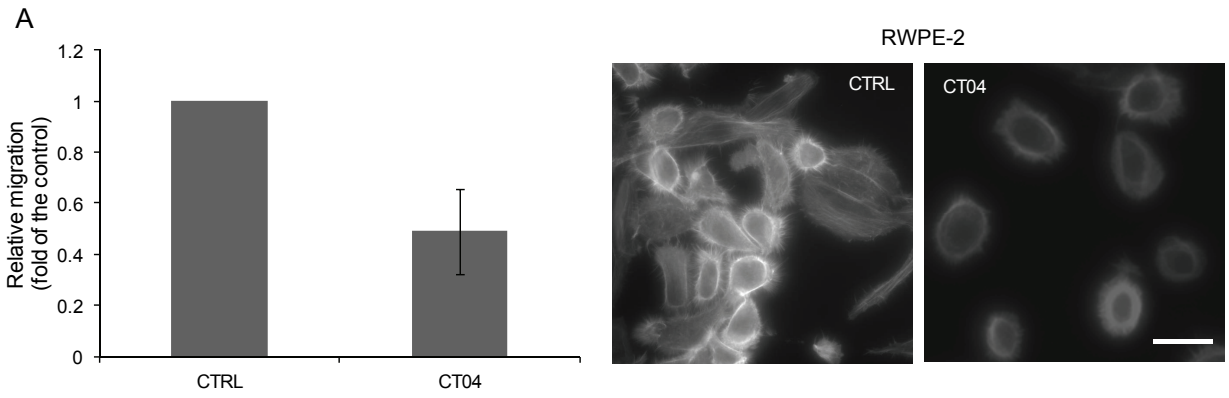
Supplementary Fig. 1

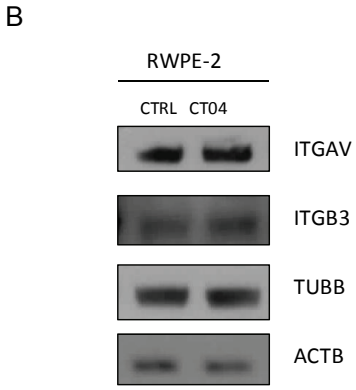


Supplementary Fig. 2

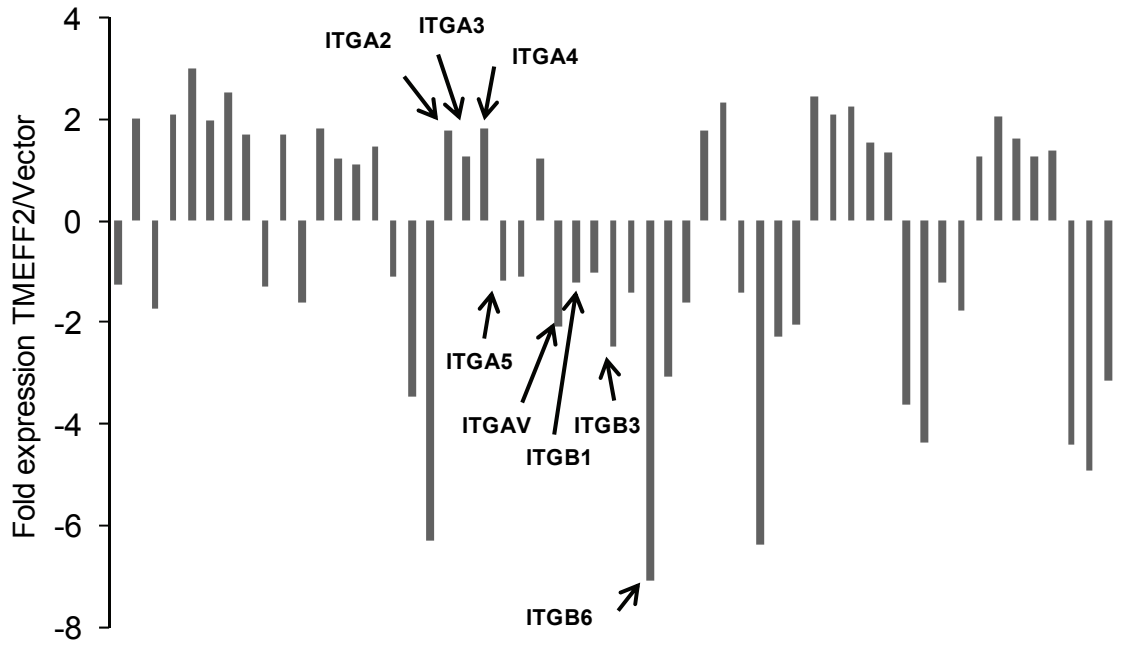


Supplementary Fig. 3





Supplementary Fig. 4



Supplementary Fig. 5