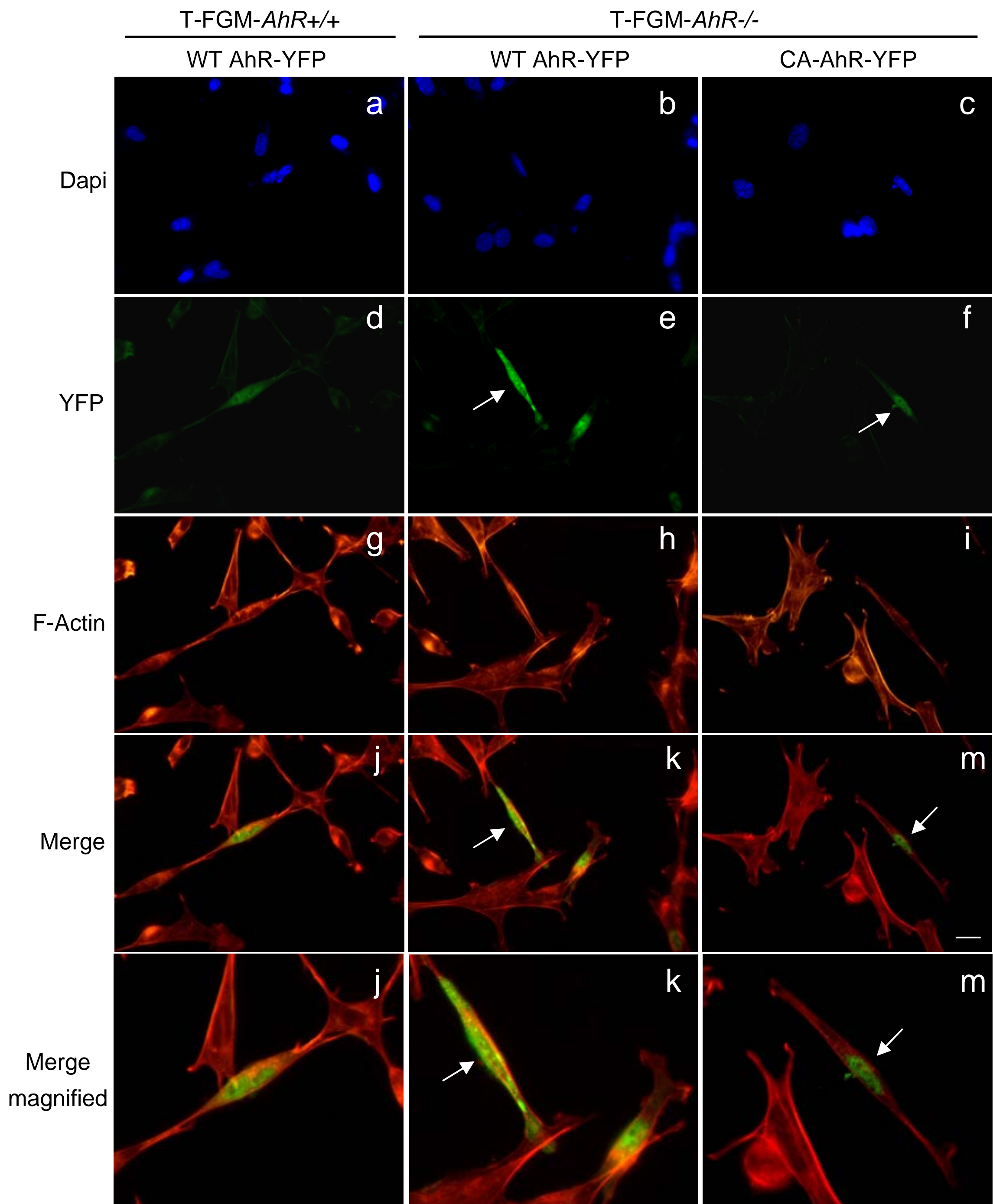
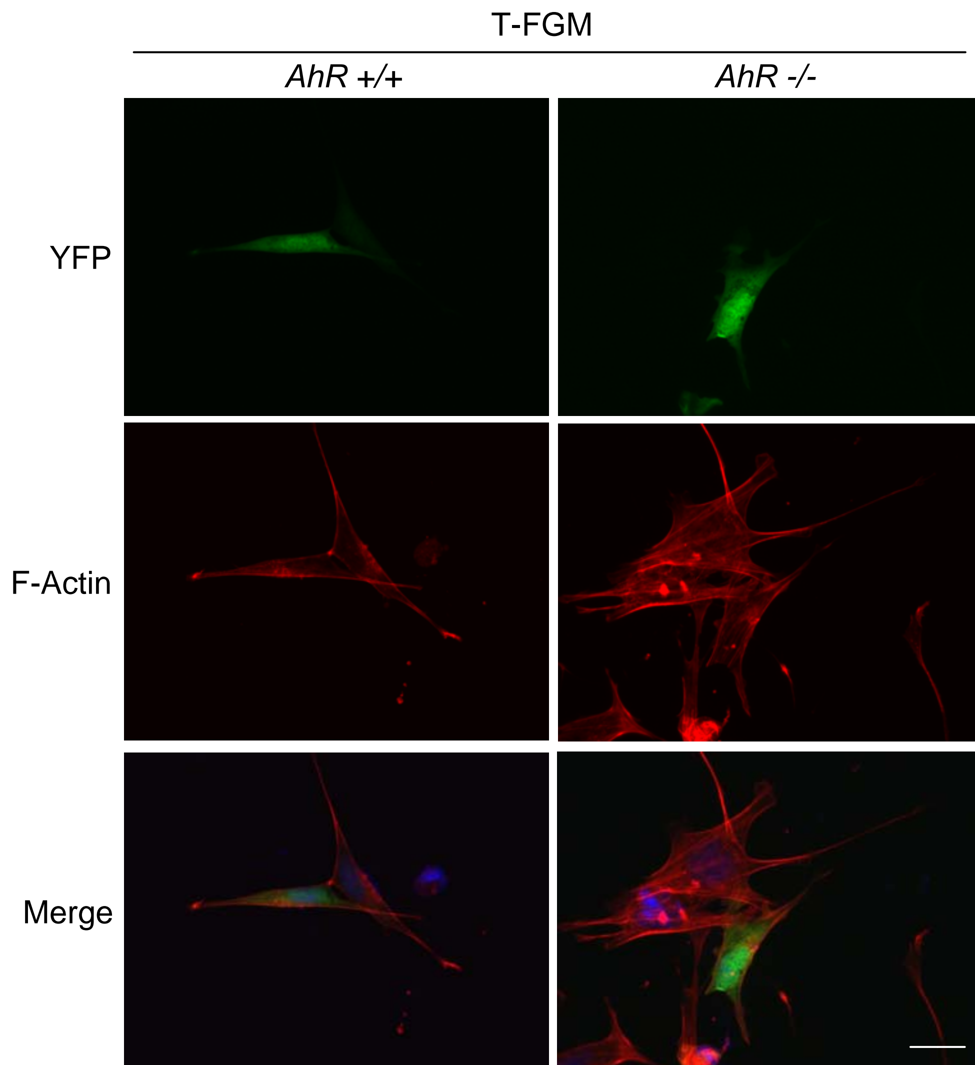
Supplementary Figure 1



Supplementary Figure 1.

CA-AhR is constitutively nuclear in transfected T-FGM. T-FGM *AhR-/-* fibroblasts were transfected with wild type (WT) or constitutively nuclear (CA) AhR fused to the yellow fluorescent protein (YFP). Nuclei were identified by DAPI staining. Cell morphology and stress fibers were visualized by YFP and rhodamin-phalloidin (F-actin) staining, respectively. Calibration bar=10 μm. YFP fluorescence is shown through the green channel to improve visualization of AhR in the merged results

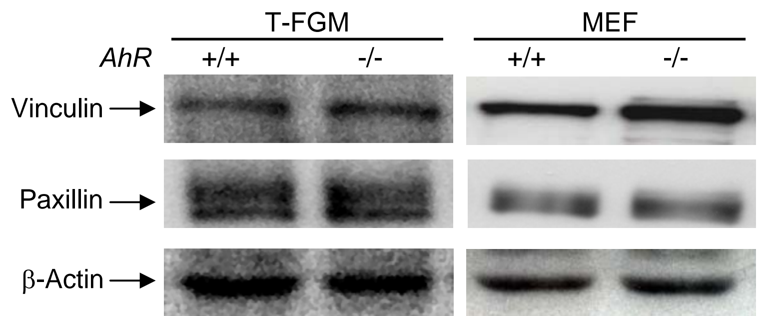
Supplementary Figure 2

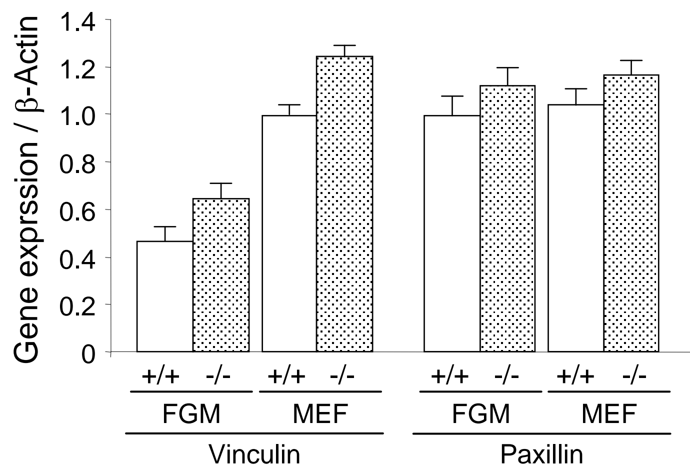


Supplementary Figure 2.

YFP transfection in does not alter the phenotype of T-FGM fibroblasts. T-FGM *AhR+/+* and T-FGM *AhR-/-* fibroblasts were transfected with YFP and cell morphology and stress fibers formation analyzed by YFP and rhodamin-phalloidin (F-Actin) staining, respectively. Calibration bar=10 μm. YFP fluorescence is shown through the green channel to improve visualization of AhR in the merged results

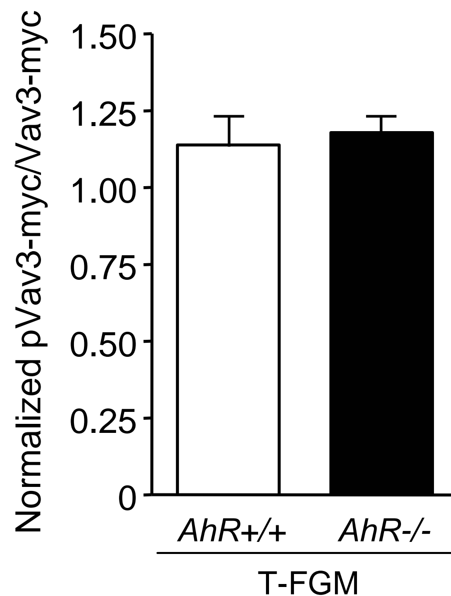
Supplementary Figure 3





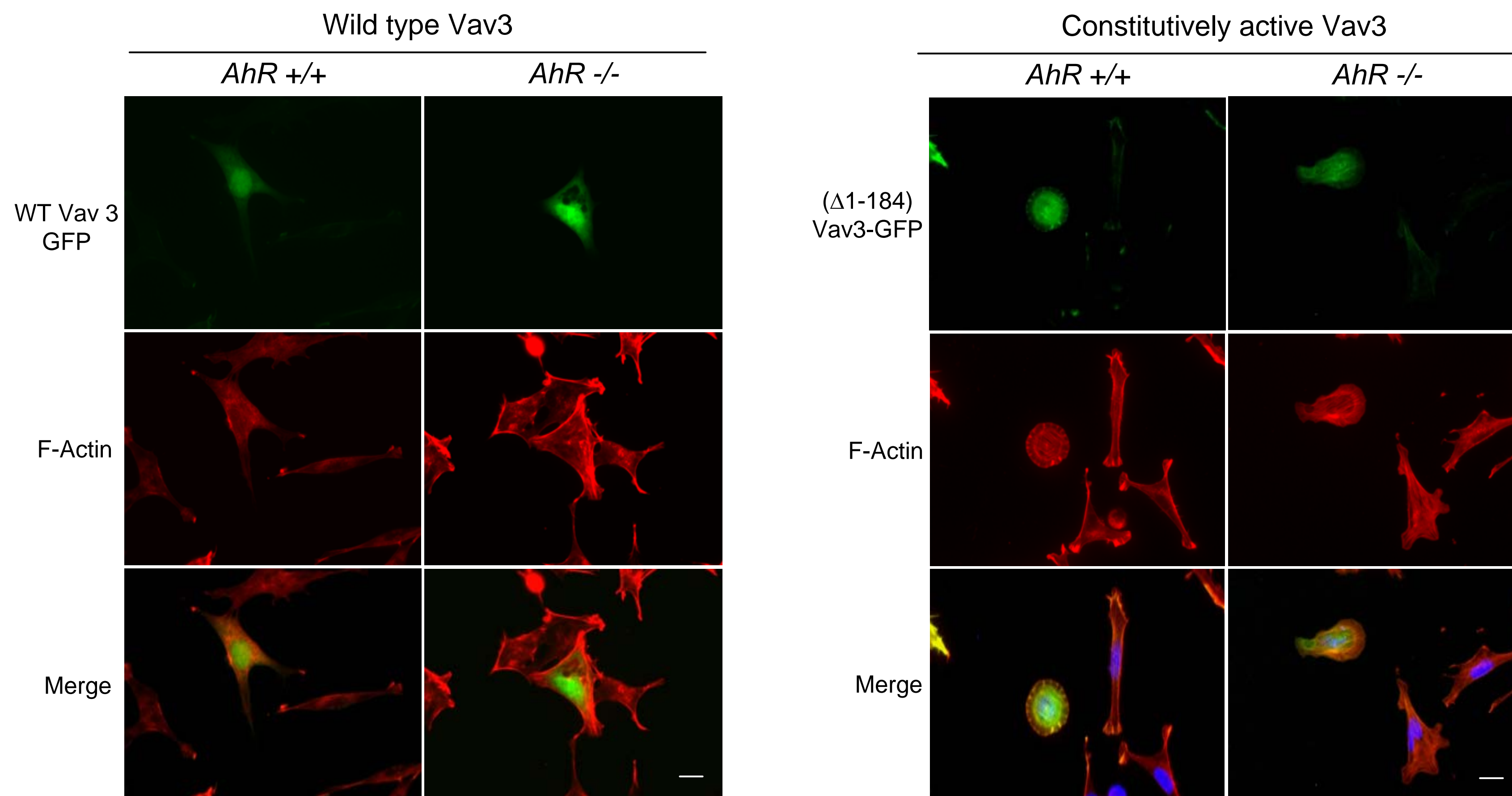
Supplementary Figure 3.

Levels of proteins involved in focal adhesion formation were similar between *AhR+/+* and *AhR-/-* in T-FGM fibroblasts and MEF cells. Western immunoblotting for protein expression was performed using total cell extracts and specific antibodies. β-Actin was used as loading control.



Supplementary Figure 4.

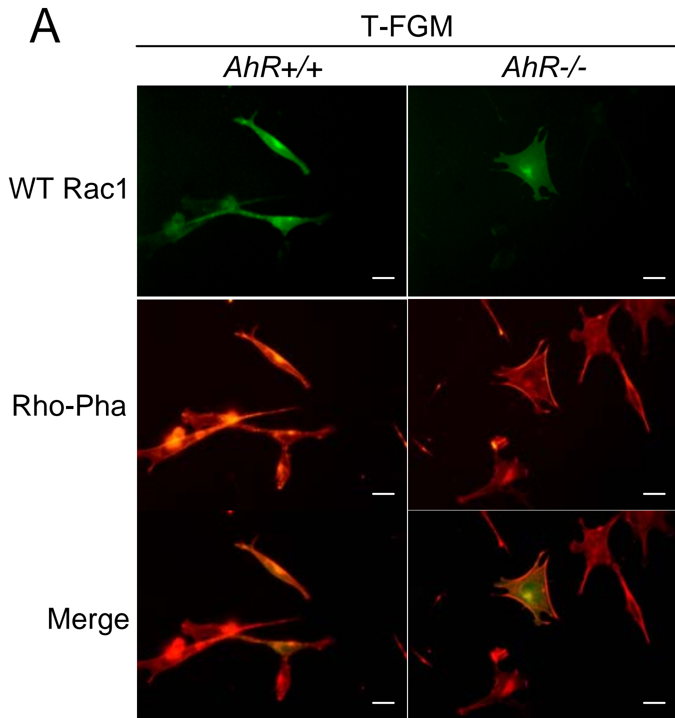
T-FGM *AhR+/+* and T-FGM *AhR-/-* cells have similar ability to phosphorylate Vav3 protein. T-FGM fibroblasts of both genotypes were transiently transfected with a wild type Vav3-myc tagged expression vector. Total cell extracts were prepared and immunoprecipitated with anti-myc-coated Miltenyi-Biotech paramagnetic beads following the instructions of the manufacturer. Immunoprecipitates were analyzed by Western blotting using an antibody against phospho-Tyr (Santa Cruz Biotechnology, PY20) to detect the phosphorylated Vav3-myc fusion protein. Blots were also reprobed with an anti-myc antibody (Santa Cruz Biotechnology, 9E10). The amount of pVav3 was normalized by myc levels and the results plotted for each genotype. The experiment was repeated in two T-FGM cultures with similar results. Data are shown as mean ± S.E.

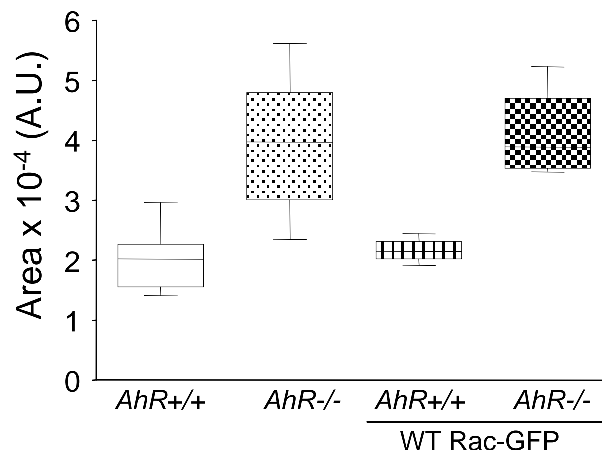


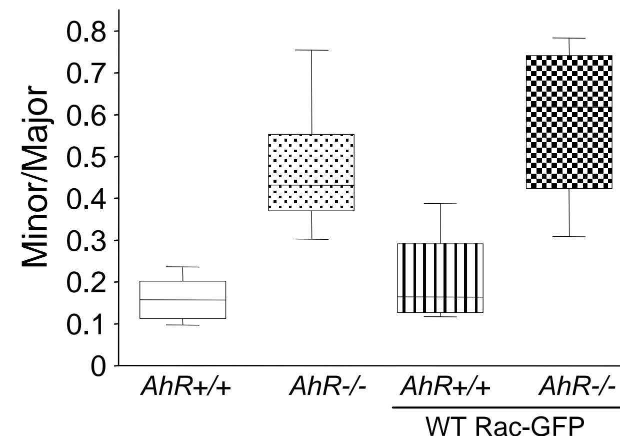
Supplementary Figure 5.

Over-expression of wild type (left panels) or constitutively active (right panels) Vav-3 protein alters the phenotype of T-FGM *AhR+/+* and T-FGM *AhR-/-* fibroblasts to a similar extend, supporting a mechanism in which highly regulated constitutive control of Vav3 expression by the transcriptional activity of AhR has an important role in modulating fibroblast shape and cytoskeleton under physiological conditions. T-FGM-*AhR+/+* and *AhR-/-* cells were transfected with wild type (WT-Vav3) or constitutively active ((Δ1-184) Vav3) Vav3-GFP expression vectors and their morphology analyzed and quantified as indicated in the Methods. Calibration bar=10 μm.

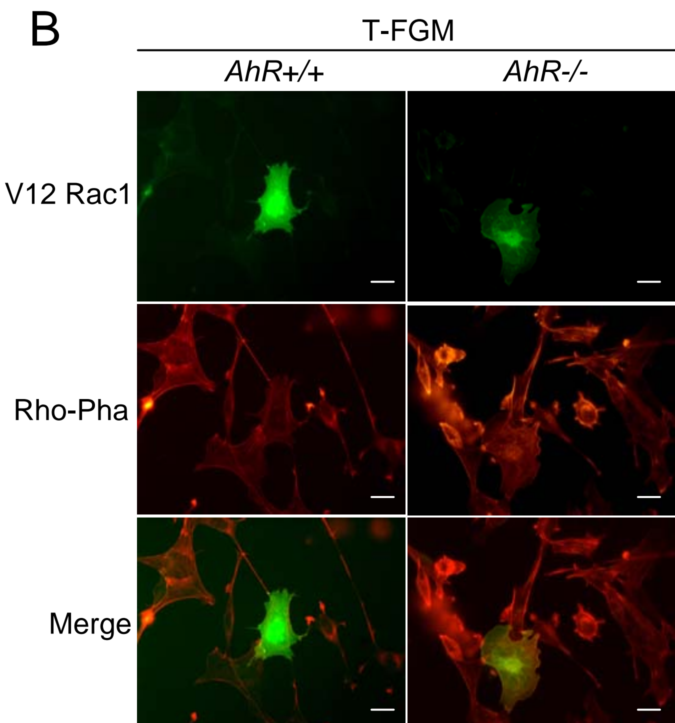
Supplementary Figure 6

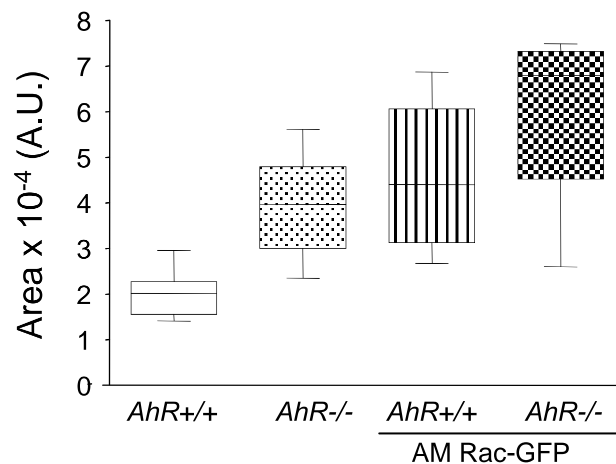


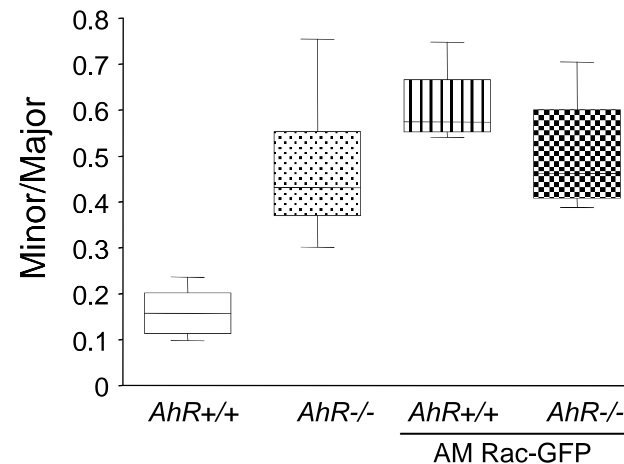




Supplementary Figure 6A. Over-expression of wild type Rac1 does not affect the phenotype of T-FGM *AhR*+/+ and *AhR-/-* cells. Wild type Rac1 was transfected and cells analyzed for changes in morphology. No significant differences were found by measuring cell area and minor/mayor axis ratio. Calibration bar = 10 μm.







Supplementary Figure 6B. Ove-rexpression of constitutively active Rac1 (V12 Rac1) normalizes the phenotype of T-FGM *AhR*+/+ and *AhR-/-* cells. Constitutively active Rac1 (AM Rac-GFP) was transfected and cells analyzed for changes in morphology. AM Rac1 increased cell area and minor/major axis to similar values in *AhR*+/+ and *AhR-/-* cells. Calibration bar = 10 μm.