

Arteriovenous malformation MAP2K1-mutant endothelial cells exhibit dysregulated vasculogenesis *in-vitro* and *in-vivo*

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BACKGROUND

Arteriovenous malformation (AVM) is a fast-flow vascular anomaly characterized by abnormal arterial to venous shunts causing ischemia, ulceration, pain, and bleeding. Extracranial AVMs contain a somatic mutation in the MAP2K1 gene. The purpose of this study was to: (1) create a stable MAP2K1 mutant endothelial cell (EC) line, (2) determine how the MAP2K1 mutation affects EC behavior, and (3) create *in-vitro* and *in-vivo* models to test pharmacotherapy.

METHODS

Utilizing CRISPR-Cas9, the MAP2K1 p.K57N mutation was introduced into immortalized arterial endothelial cells (teloHAECs, MUT=p.K57N mutated cells; pWT=control cells). Expression of mutant transcript and elevated phosphorylated ERK (P-ERK) was tested using digital droplet PCR (ddPCR) and Western Blot. Bulk-RNAseq and differential gene expression analysis was conducted comparing mutant and control cells.

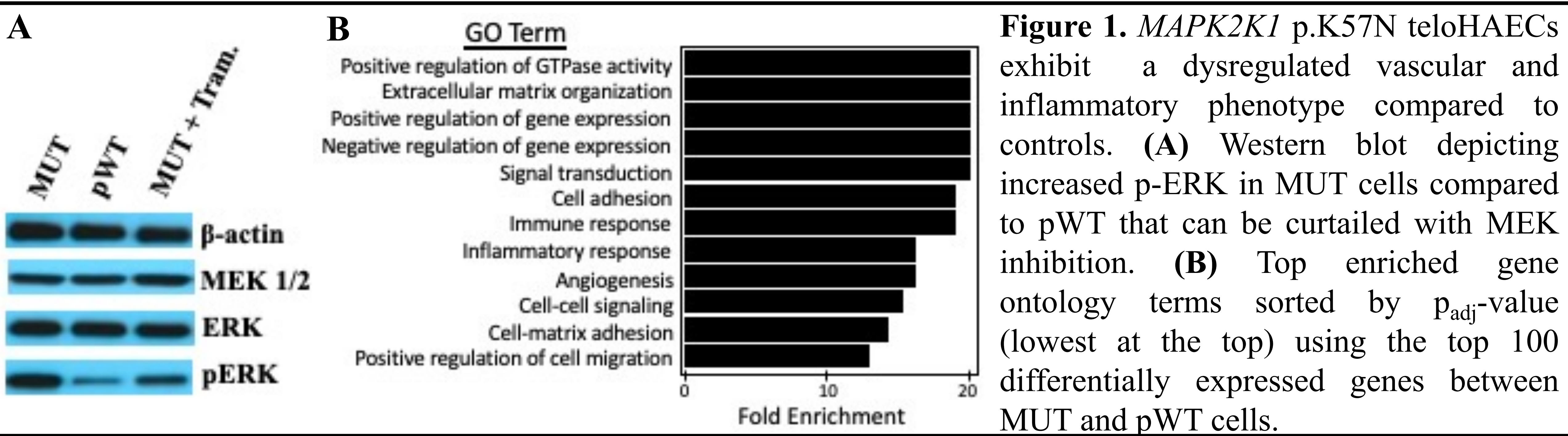


Figure 1. MAPK2K1 p.K57N teloHAECs exhibit a dysregulated vascular and inflammatory phenotype compared to controls. (A) Western blot depicting increased p-ERK in MUT cells compared to pWT that can be curtailed with MEK inhibition. (B) Top enriched gene ontology terms sorted by p_{adj} -value (lowest at the top) using the top 100 differentially expressed genes between MUT and pWT cells.

RESULTS

Protein analysis showed increased P-ERK in mutant cells, confirming the activating effects of the MAP2K1 mutation (Figure 1A). Bulk-RNAseq showed increased vasculogenic and inflammatory gene expression between mutant and control cell lines, including a key regulator of vessel formation angiopoietin-2 (Figure 1B). Murine implants with mutant ECs displayed malformed and dilated vasculature (Figure 2). Baseline Angiopoietin-2 protein levels are upregulated in p.K57N mutant compared to control cells (Figure 3A). MEK inhibition with Trametinib curtailed the secretion of Angiopoietin-2 in mutant cells and reduced expression to near pWT control cellular levels (Figure 3B).

CONCLUSIONS

The MAP2K1 mutation in immortalized ECs activates RAS/MEK signaling, stimulates vasculogenic gene expression, and forms abnormal vascularized lesions *in-vivo*. These models provide insight into the pathophysiology of human AVM, enable preclinical testing of novel drug therapies, and support the efficacy of Trametinib for the treatment of AVM. This provides insight into the pathophysiology of human AVM and a model for understanding the effects of targeted drug therapies.

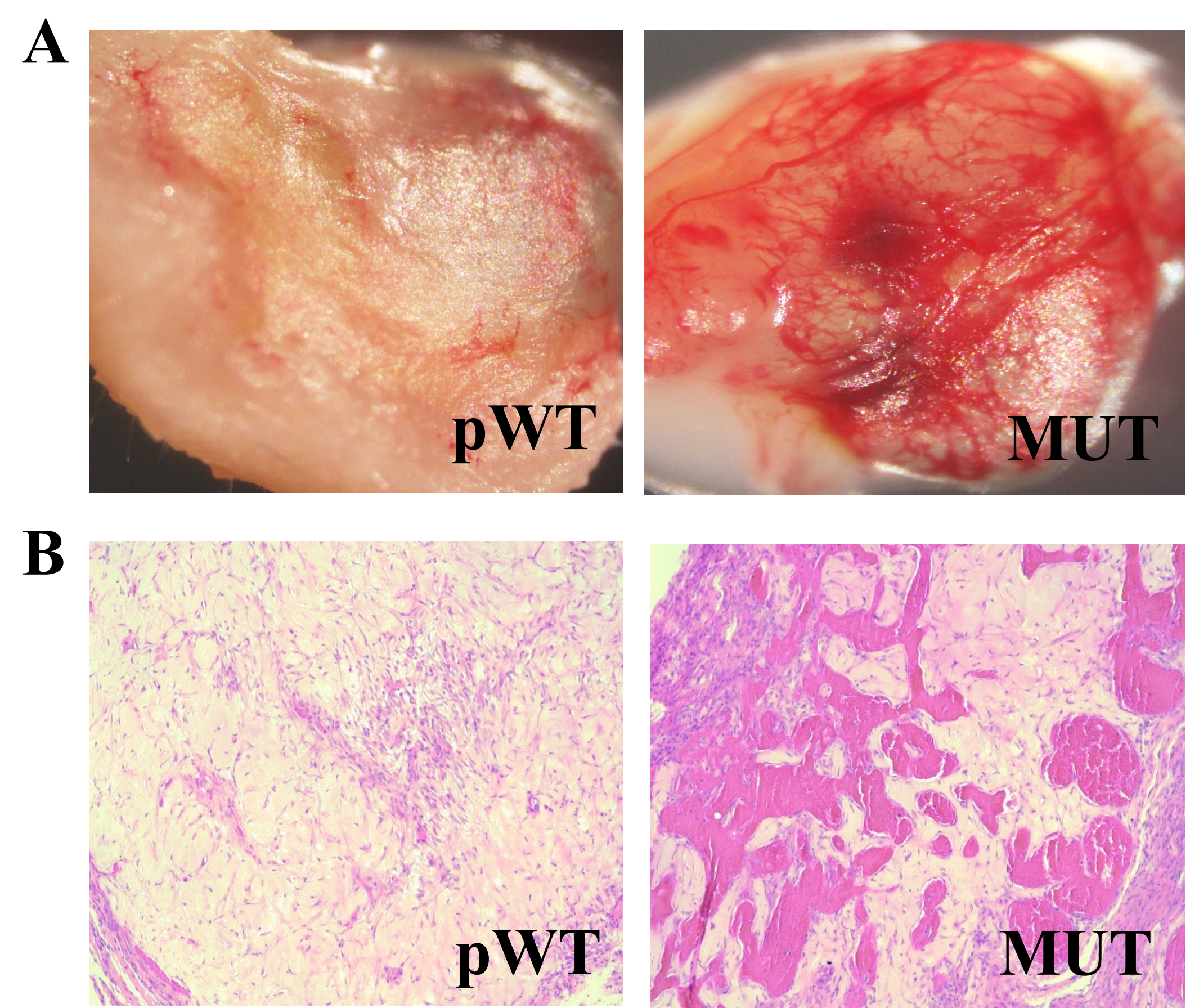


Figure 2. Nude mouse implants (n=4 for each genotype, extracted at t=10 days) following injection with MAP2K1 p.K57N mutant (MUT) and pseudo-wildtype (pWT) engineered teloHAECs. (A) Gross images of cellular implants in nude mice show greater vascularization of mutant implants. (B) Implant histopathology (H&E stain at 10x magnification) exhibits increased and malformed vessels in mutant implants.

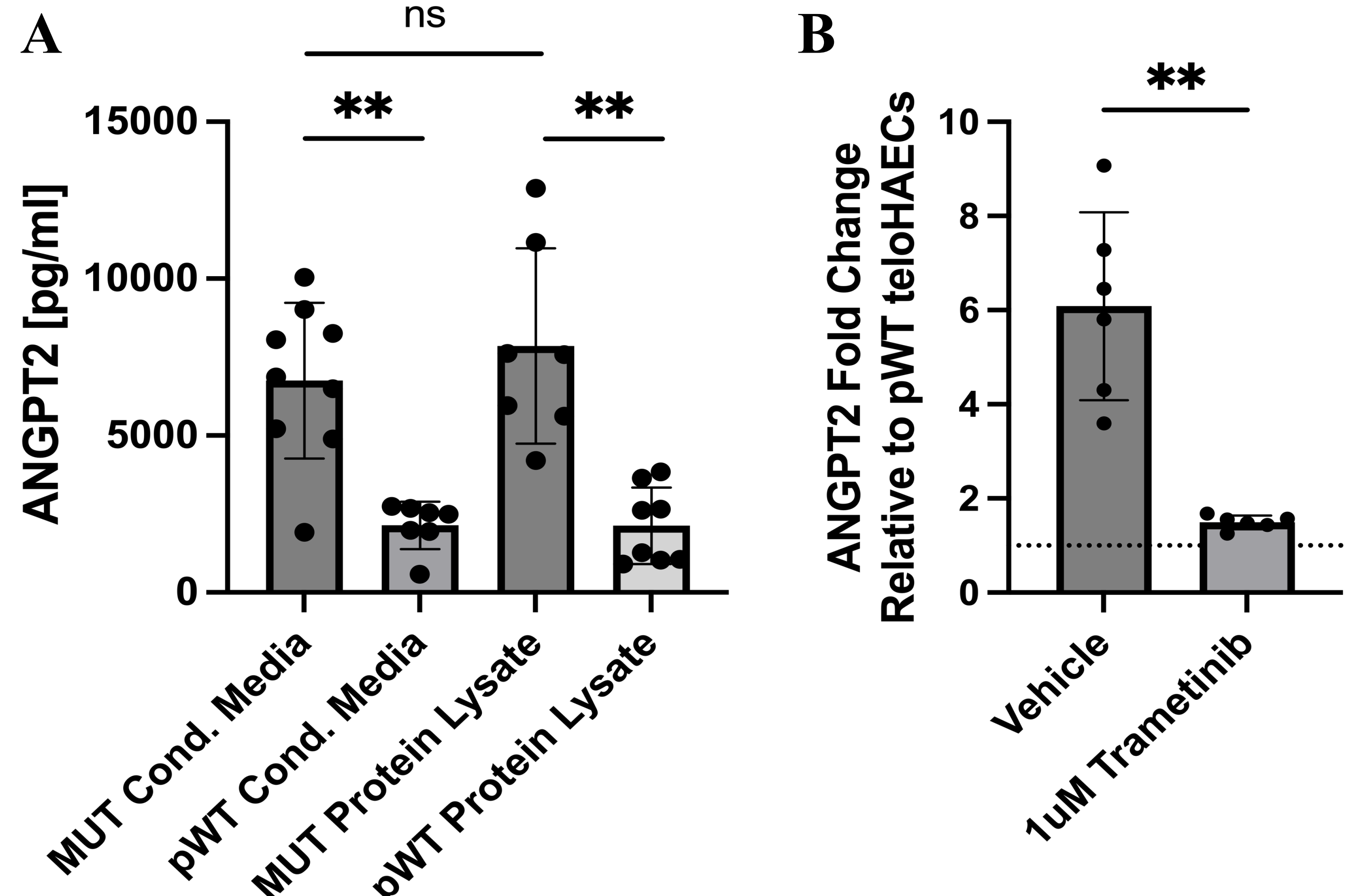


Figure 3. MAPK2K1 p.K57N mutation drives increased production and secretion of ANGPT2 that can be perturbed with MEK inhibition (A) ANGPT2 in both conditioned media and cellular lysates is increased in p.K57N teloHAECs compared to pWT cells (time = 18hrs, n=9 for conditioned media, n=7 for cellular lysates). (B) Significant reduction of extracellular ANGPT2 levels in p.K57N teloHAECs treated with 1uM of Trametinib for 18 hours relative to pWT teloHAECs (n=6), dotted line represents normalized ANGPT2 for pWT control cells. **p<0.001 calculated using Welch's t-tests.

