



Pancreatic tumor microenvironment modulation by EphB4-ephrinB2 inhibition and radiation

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INTRODUCTION

- The highly immunosuppressive tumor microenvironment of pancreatic ductal adenocarcinoma (PDAC) is a driving factor of treatment resistance.
- A potent immunological adjuvant is radiation therapy (RT).
- RT, however, has also been shown to induce infiltration of immunosuppressive populations and can contribute to the formation of fibrotic stroma, which can contribute to tumor progression.
- To gain benefit from the immunogenic effects of radiation and obtain a durable tumor response, it must be rationally combined with targets aimed at mitigating the influx of immunosuppressive cells and fibrosis (Oweida, A., et al. 2017)
- One such target is ephrinB2, which is overexpressed in PDAC and correlates negatively with prognosis (Lu, Z., et al. 2012)

METHODS

- Animal Model:** Patient derived xenografts (PDX) PANC272 and a mouse pancreata derived cell line (FC1242) were injected subcutaneously into the right flank of athymic nude mice and C57Bl/6 mice respectively.
- Treatment:** Mice (n=5-7/group) were randomized to receive antibody B11, PBS, RT and combinations thereof. Treatment was started 3 days before RT. The dose of RT was 10Gy unless otherwise specified.
- Mechanistic studies:** Mice received the same treatments but tumors were harvested after 72 hours and processed for flow cytometric analysis of CD4 and CD8 populations.
- Multiplex Immunofluorescence:** Staining was performed using IHC and primary antibodies

RESULTS

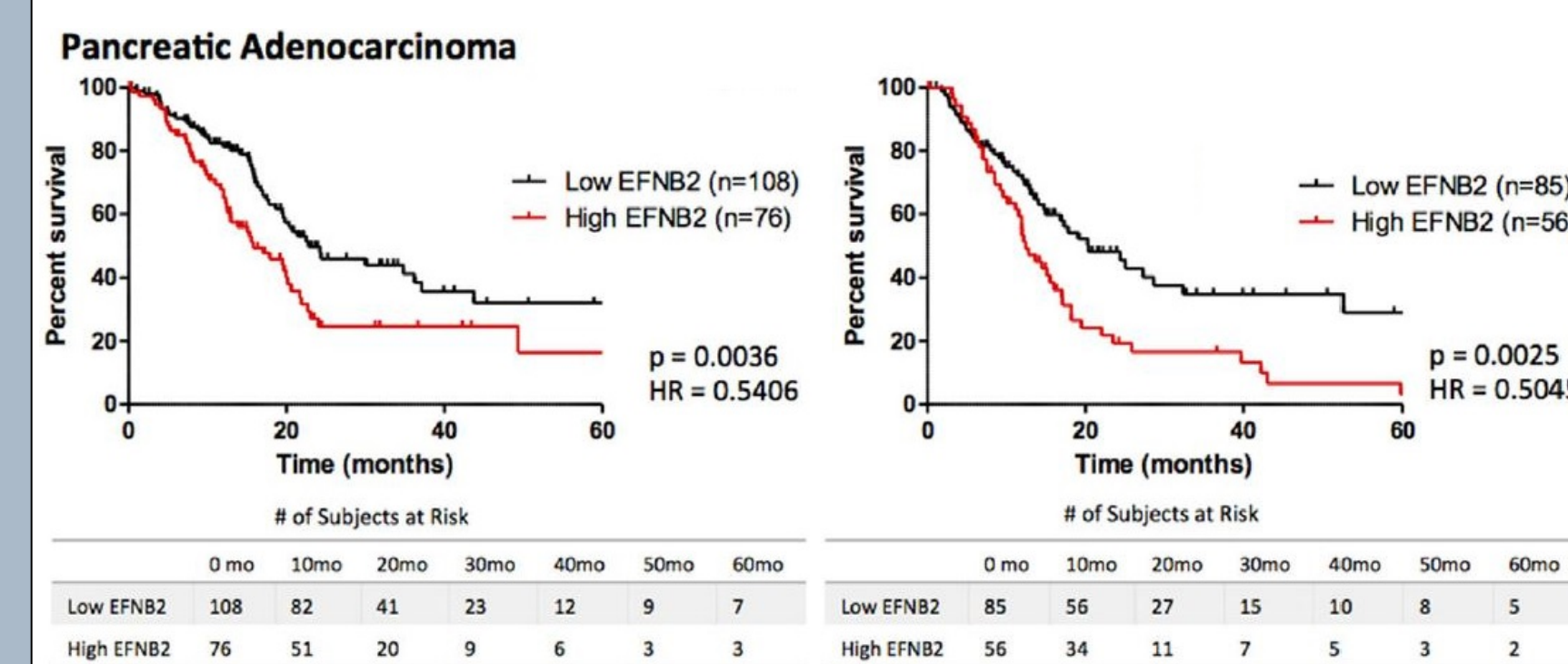


Figure 1 – High ephrinB2 expression correlates with a worse prognosis in PDAC

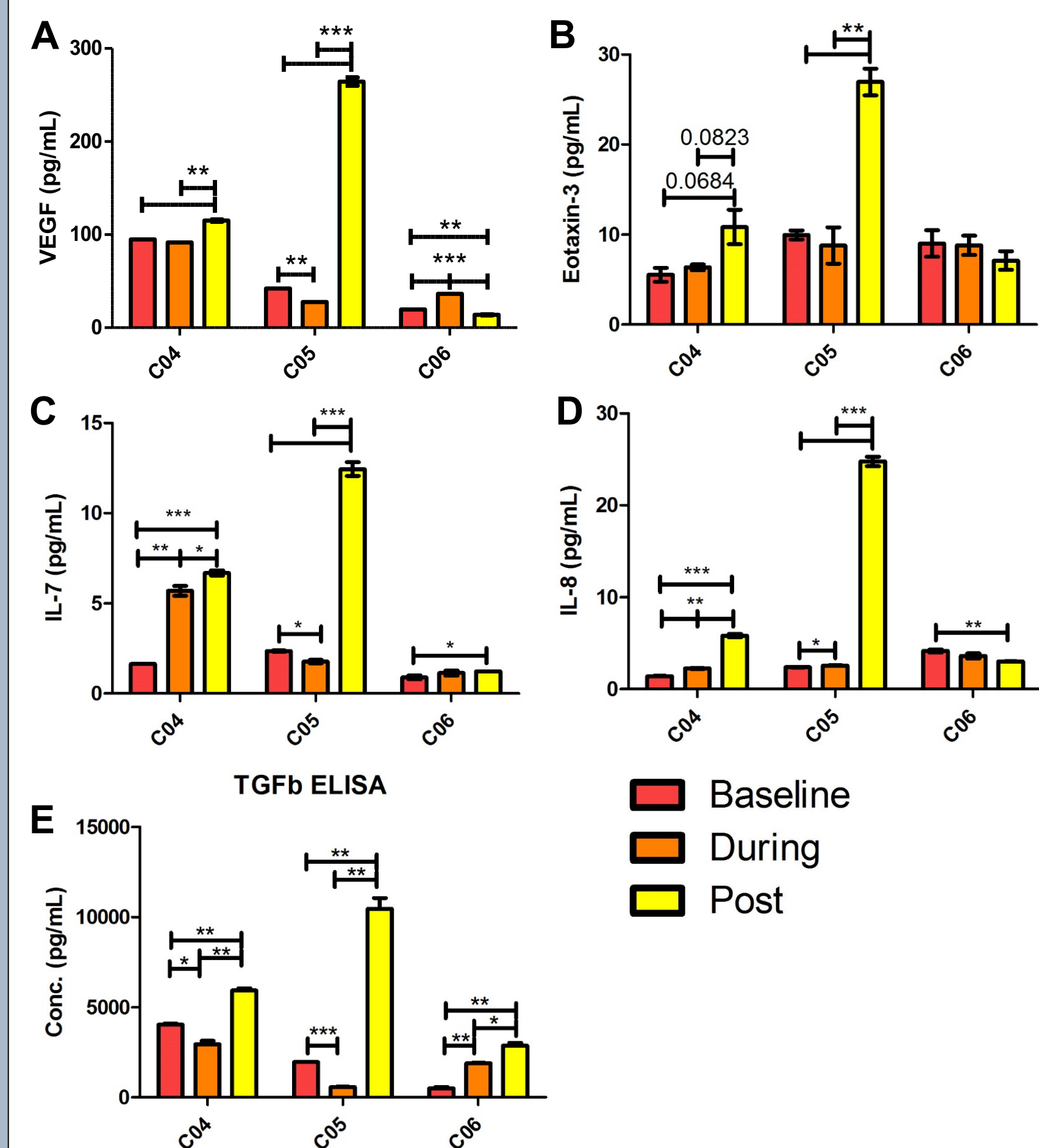


Figure 2 – Circulating factors taken characterized from patients with PDAC at baseline, during, and post radiation

RESULTS

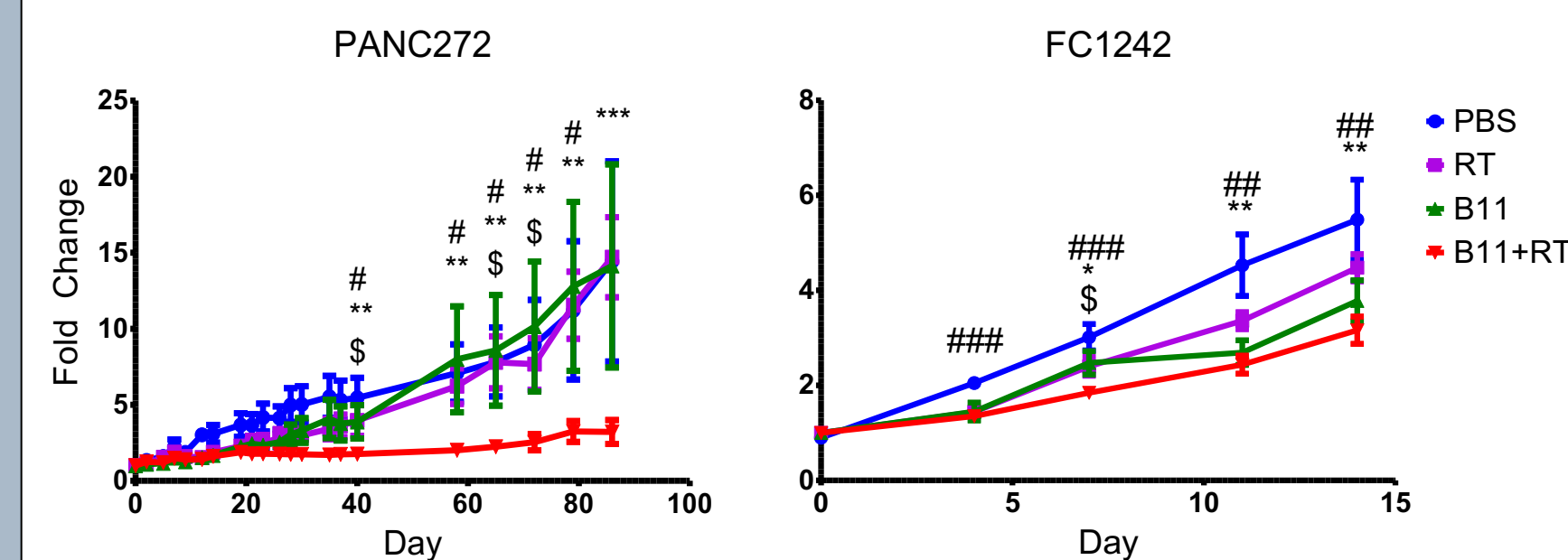


Figure 3 – EphB4-ephrinB2 inhibitor (B11) adds to the effect of RT in PDAC. Fold change of tumors over time for PANC272 and FC1242

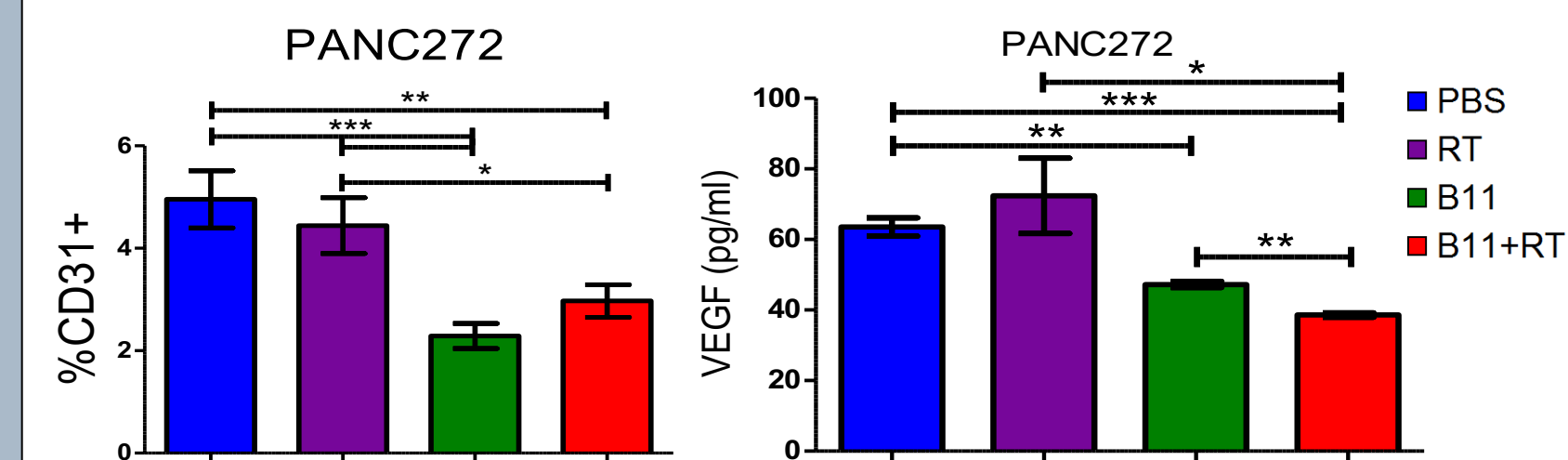


Figure 4 – Inhibition of EphB4-ephrinB2 interaction decreases blood vessel density and circulating VEGF, with accompanying data from patients

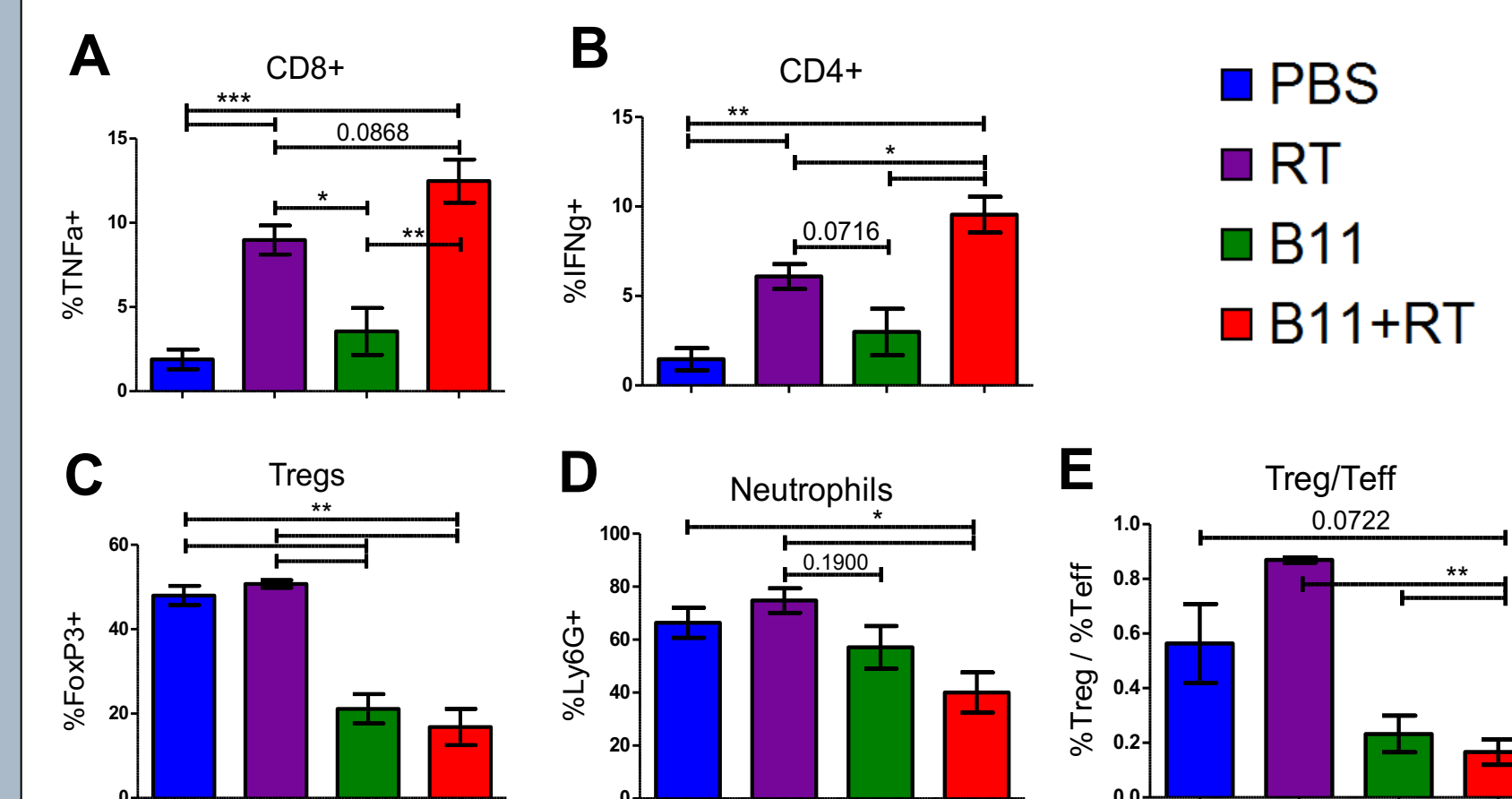


Figure 5 – B11 combination with RT enhances cytotoxic CD4 and CD8 T-cell activation and decreases regulatory T-cell (Treg) and neutrophil infiltration

RESULTS

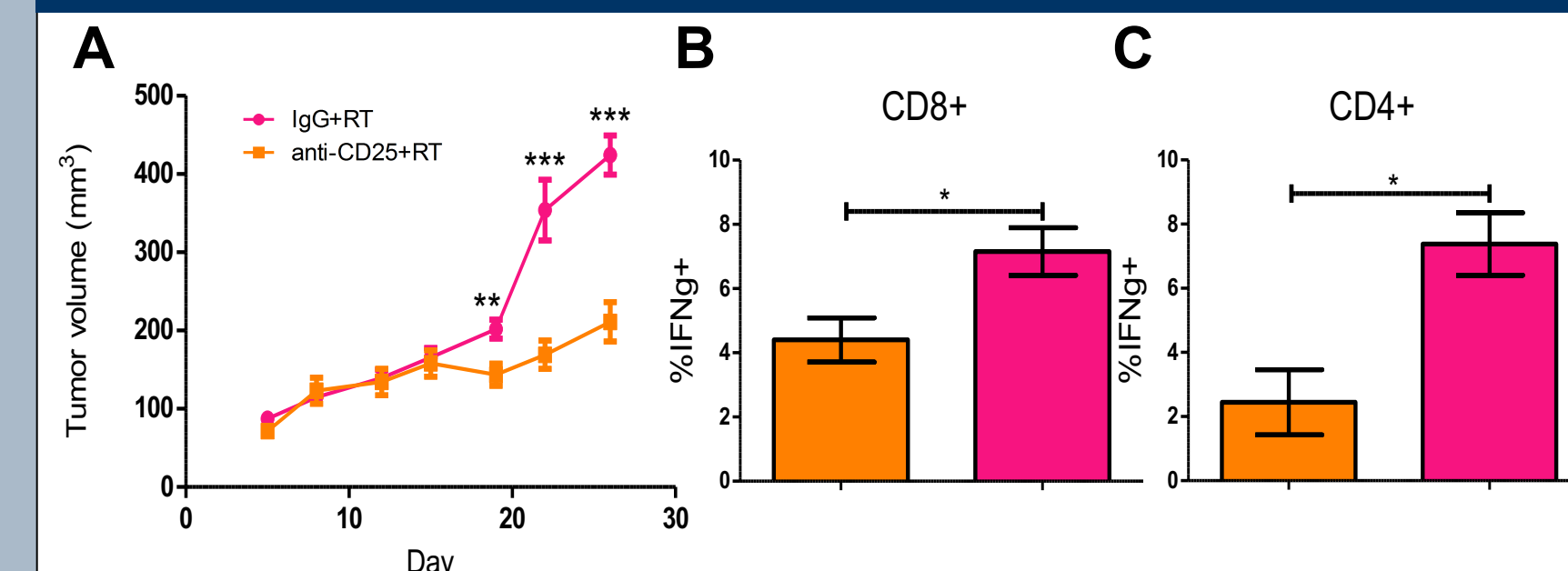


Figure 6 – Treg depletion in combination with RT leads to significantly increased tumor control and is accompanied by higher levels of Teff activation

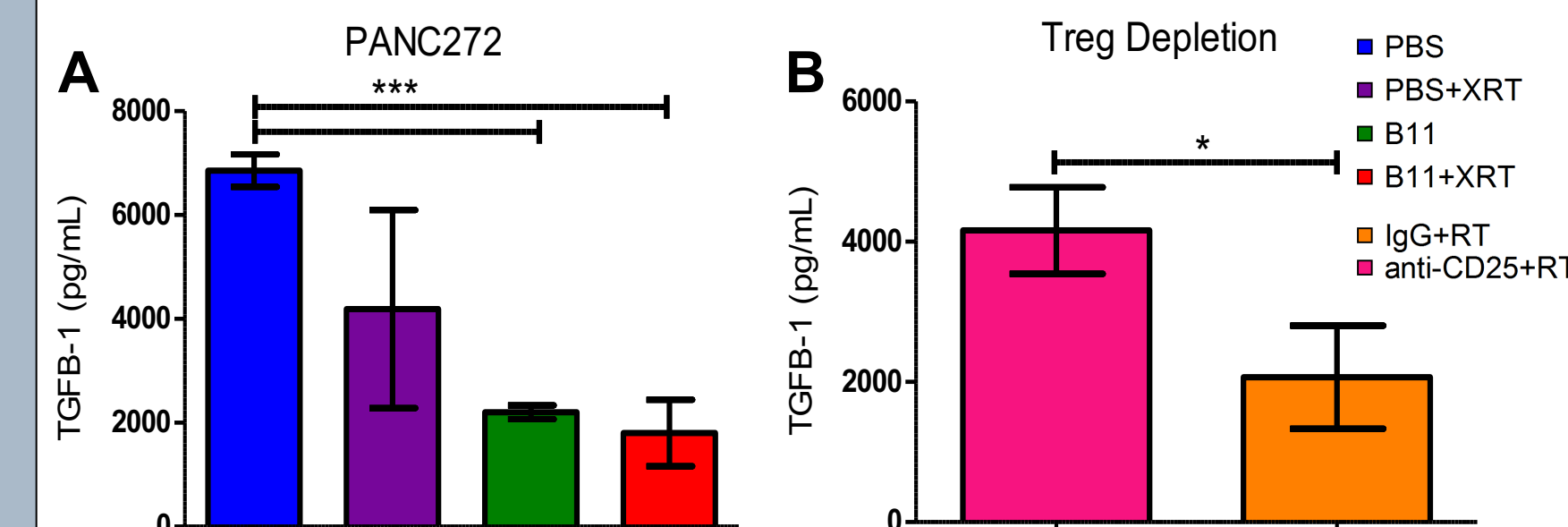


Figure 7 – Plasma TGFβ-1, a major regulator of Treg activation and proliferation, is significantly reduced in B11 treated mice

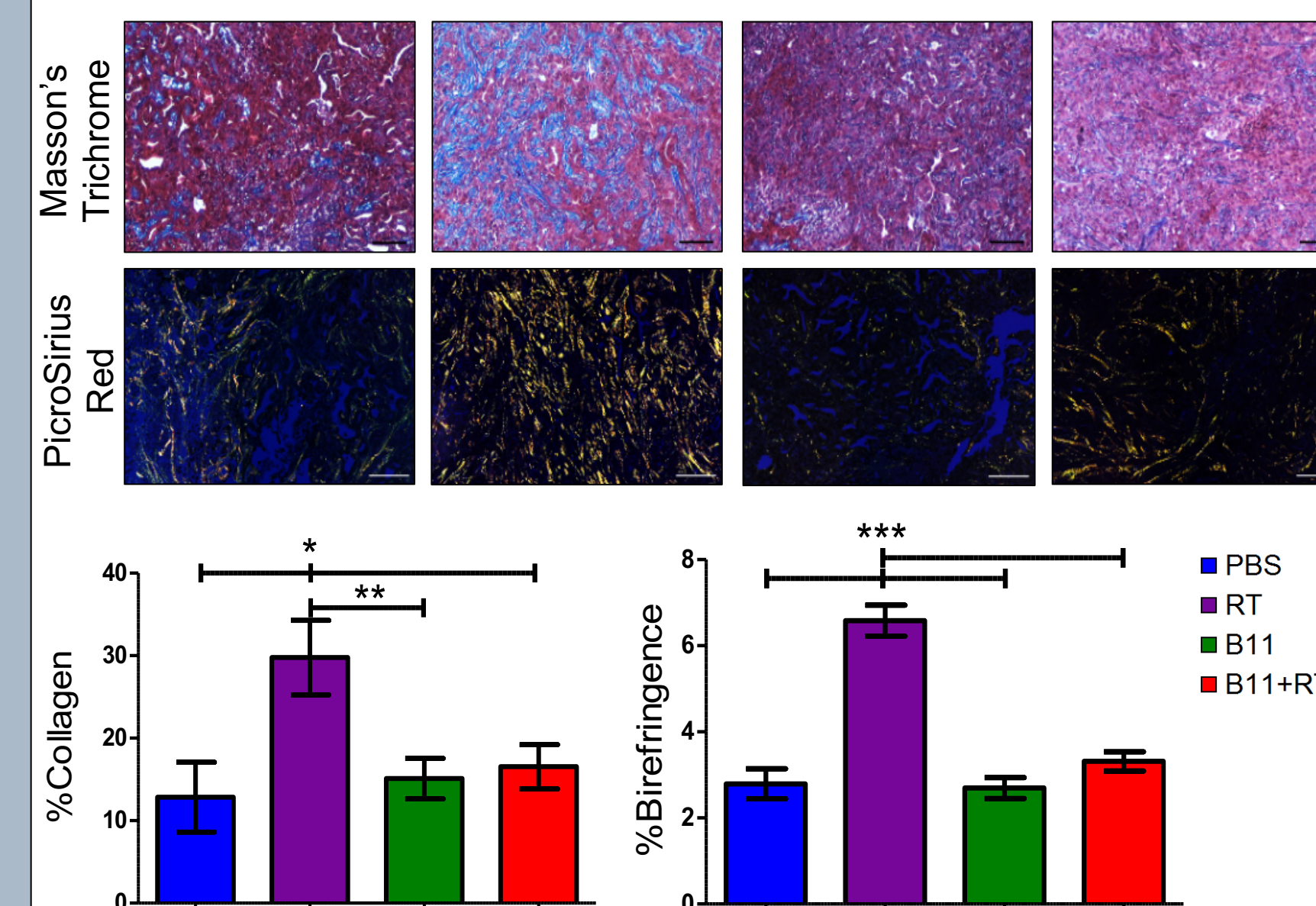


Figure 8 – FC1242 models show increased pro-fibrotic cytokines after RT which appears to be mitigated by EphB4-ephrinB2 inhibition

CONCLUSIONS

- Our study provides strong evidence of Tregs contributing to PDAC resistance to RT and a potential method for reduction in Tregs as well as fibrosis, two areas of clinical importance in PDAC, via EFNB2-EphB4 blockade.
- The effects of targeting EFNB2-EphB4 are not limited to Tregs and fibrosis, but also include reduced angiogenesis and neutrophil infiltration and increased Teff activation.
- The ability to target multiple tumorigenic pathways is a strength of targeting this signaling pair.
- Our findings are supported by clinical data and have translational potential to enhance the therapeutic efficacy of RT in patients with PDAC.
- Future work is aimed at understanding the mechanisms of fibrosis within the tumor microenvironment.

REFERENCES

- Lu, Z., et al., Overexpression of The B-type Eph and ephrin genes correlates with progression and pain in human pancreatic cancer. *Oncol Lett*, 2012. 3(6): p.1207-1212.
- Oweida, A., et al. Ionizing radiation sensitizes tumors to PD-L1 immune checkpoint blockade in orthotopic murine head and neck squamous cell carcinoma. *Oncoimmunology*. 2017; 6: e1356153

ACKNOWLEDGEMENTS

Paul Sandoval Cancer Research Scholarship, Wings of Hope for Pancreatic Research, Paul Calabresi Career Development Award for Clinical Oncology (K12), RSNA grant (#RSD1713), Cancer League of Colorado and Marisco Fund