

Pro-angiogenic tumor-stroma interactions in the feline oral squamous cell carcinoma microenvironment

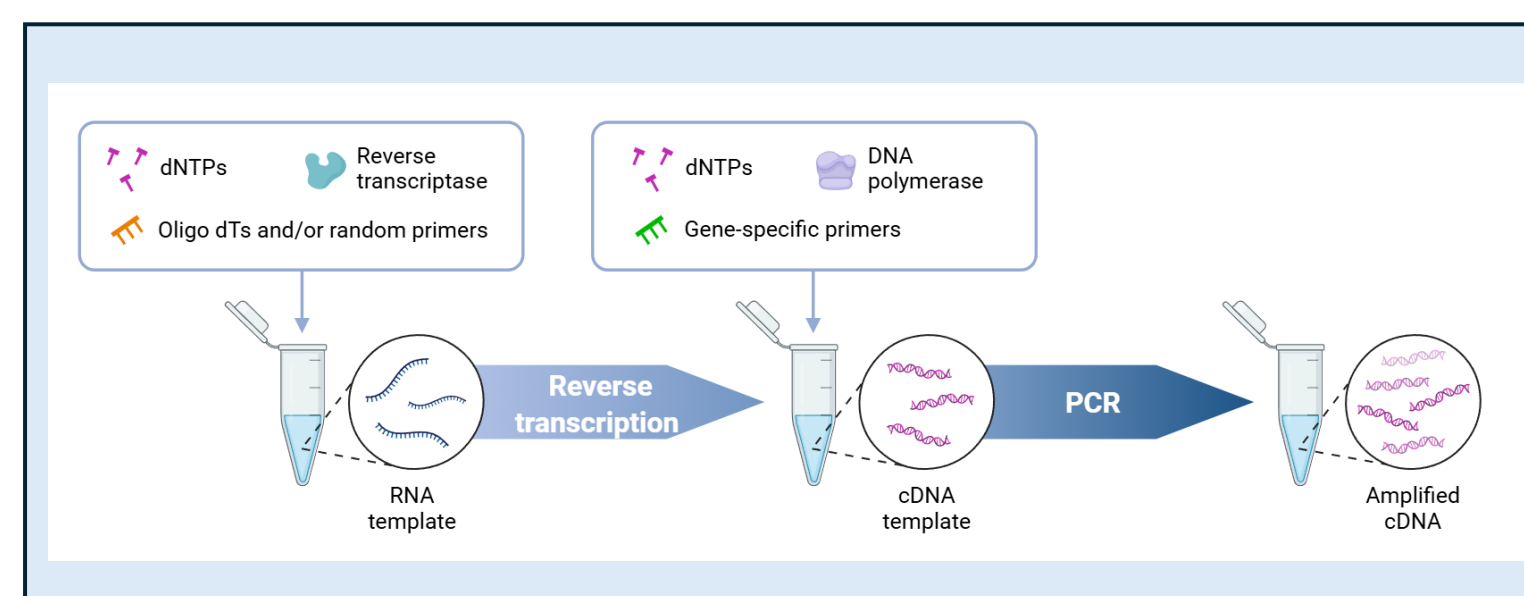
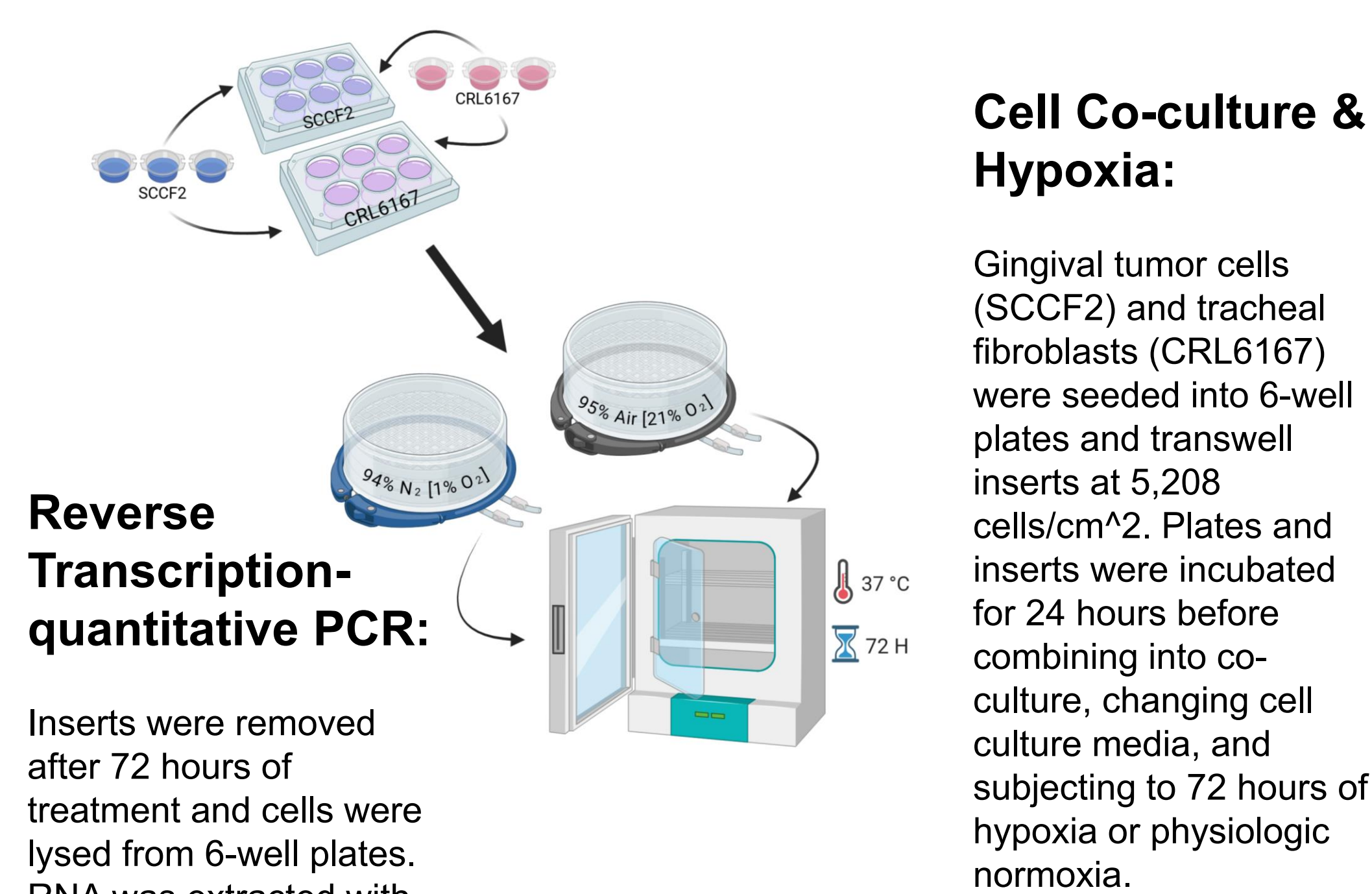
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INTRODUCTION

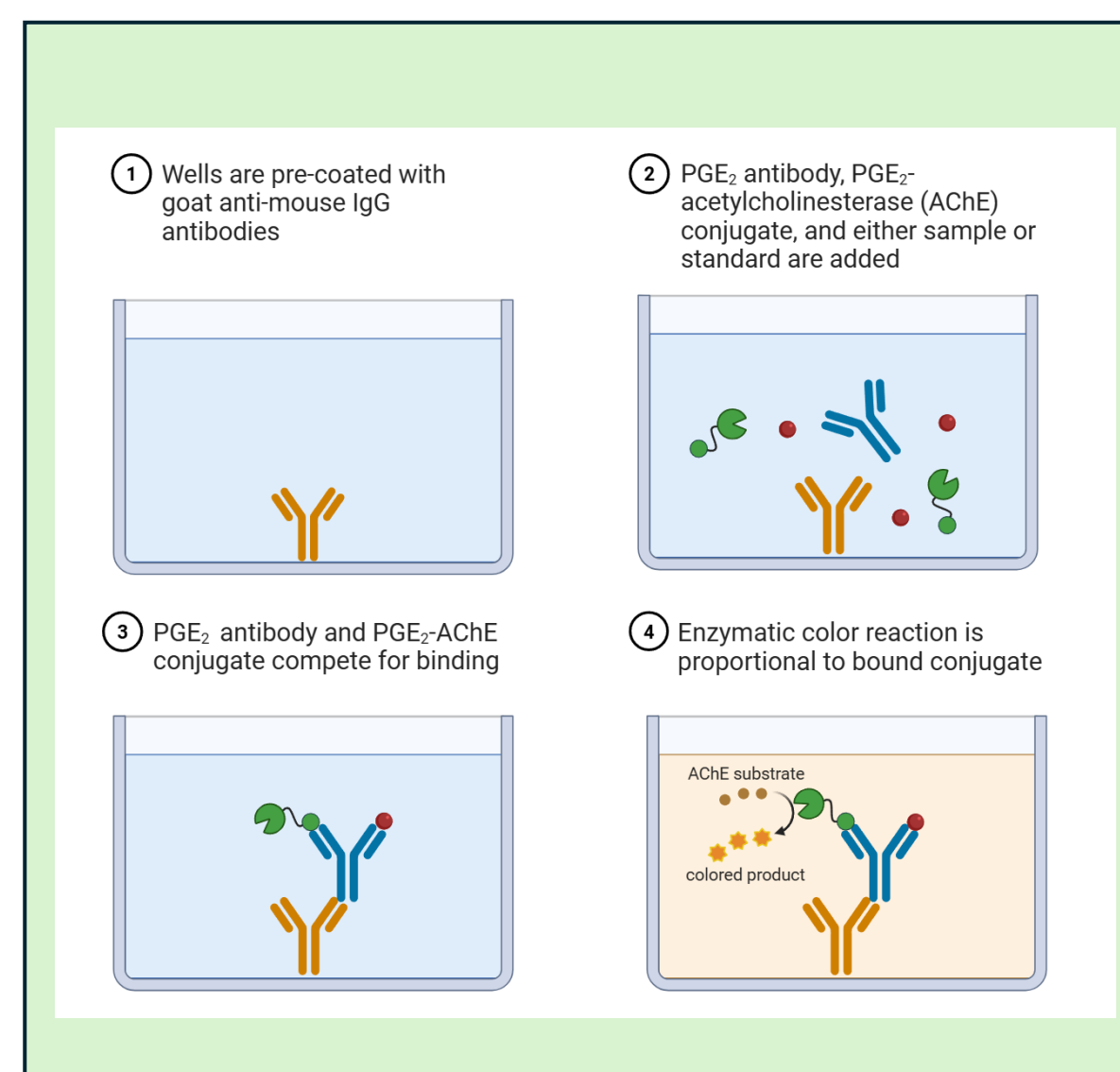
- Feline oral squamous cell carcinoma (FOSCC) has poor survival outcomes due to treatment resistance against chemotherapy and radiation.
- Carcinomas contain both tumor cells and cancer-associated fibroblasts with variable access to oxygen and nutrients as the tumor outgrows local vascular support. (Klobukowska 2016)
- Several studies have found an association between inflammation and hypoxia as drivers of angiogenesis in tumors (Nasry 2018)

METHODS



Competitive ELISA:

Conditioned cell culture media was reserved after 72 hours of treatment for use with a monoclonal prostaglandin E2 ELISA kit (514010; Cayman Chemical, Ann Arbor, MI).



RESULTS

Angiogenic Gene Expression

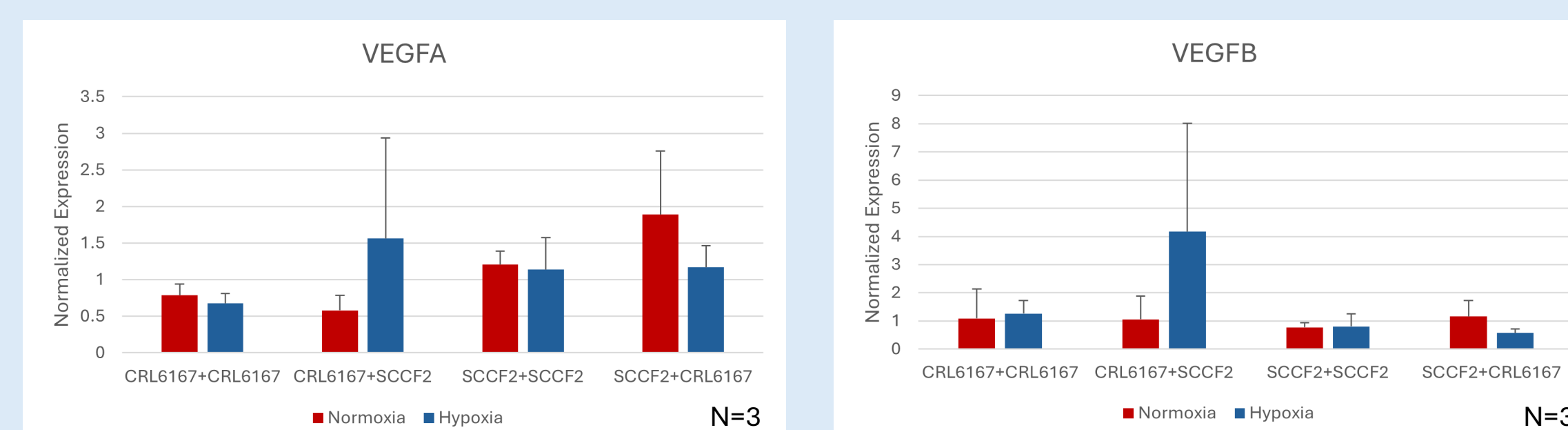


Figure 1.1 & 1.2 No significant differences in VEGFA or VEGFB expression were observed in response to hypoxia or co-culture. Statistics were calculated using a 2-tailed T-test to separately evaluate the effect of hypoxia and the effect of co-culture using GraphPad software.

Lymphangiogenic Gene Expression

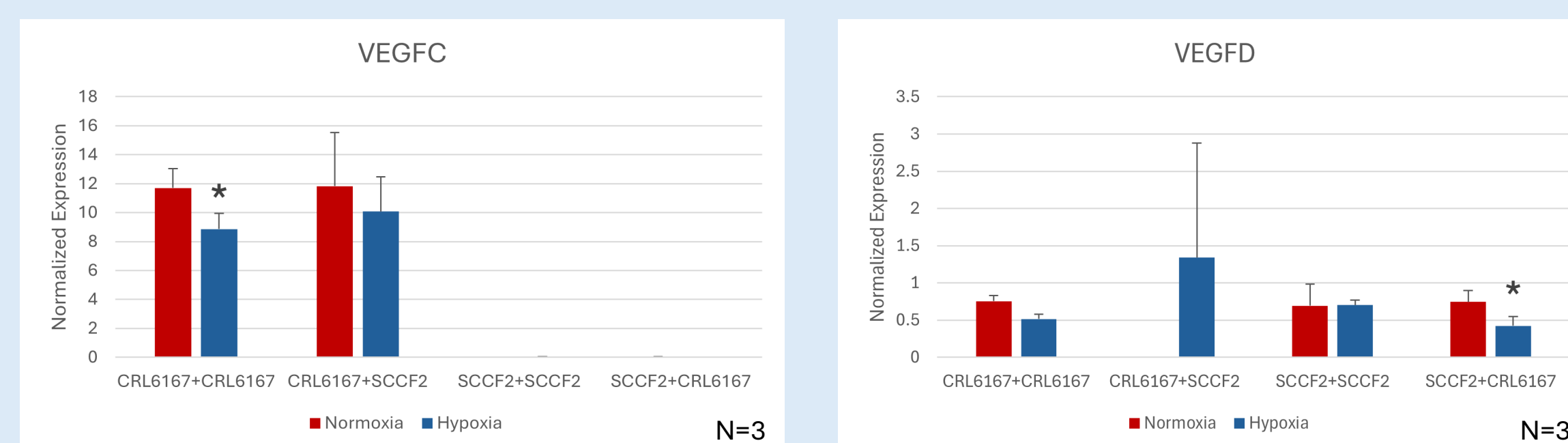


Figure 2.1 & 2.2 VEGFC and VEGFD were the only genes with a statistically significant response to hypoxia in CRL6167 cells grown with fibroblast inserts (p=0.0459) and SCCF2 cells grown with fibroblast inserts (p=0.0457) respectively. There was not a statistically significant change in gene expression in response to co-culture. Statistics were calculated using a 2-tailed T-test to separately evaluate the effect of hypoxia and the effect of co-culture using GraphPad software. Samples that did not consistently exhibit gene expression across all conditions and replications were excluded from analysis.

References

- Klobukowska HJ, Munday JS. High Numbers of Stromal Cancer-Associated Fibroblasts Are Associated With a Shorter Survival Time in Cats With Oral Squamous Cell Carcinoma. *Veterinary Pathology*. 2016;53(6):1124-1130. doi:10.1177/0300985816629713
- Nasry WHS, Wang H, Jones K, Dirksen WP, Rosol TJ, Rodriguez-Lecompte JC, Martin CK. CD147 and Cyclooxygenase Expression in Feline Oral Squamous Cell Carcinoma. *Vet Sci*. 2018 Aug 13;5(3):72. doi: 10.3390/vetsci5030072. PMID: 30104530; PMCID: PMC6163611.

RESULTS

Inflammatory Gene Expression

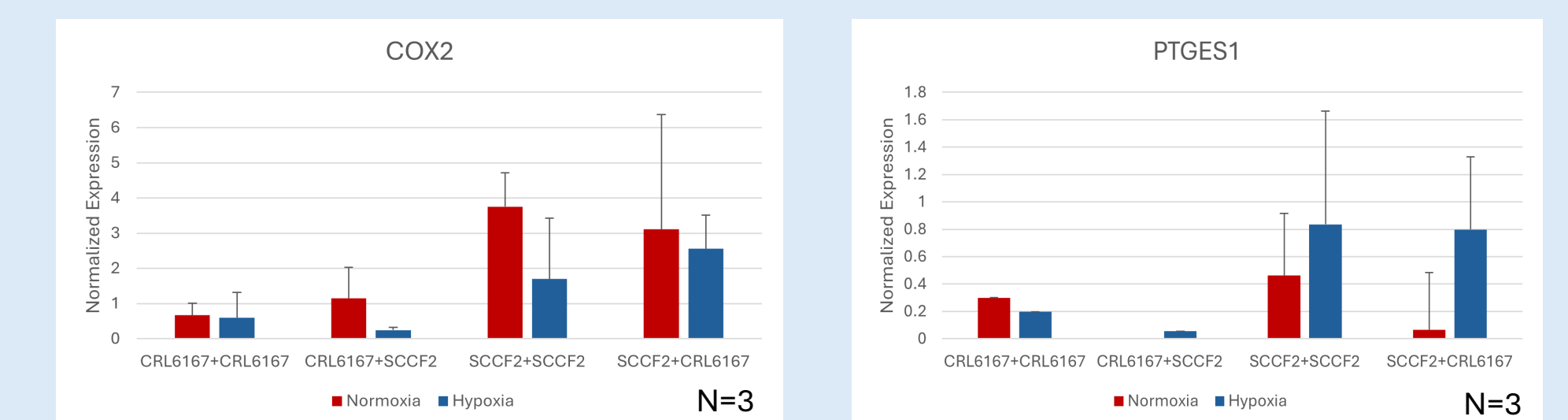


Figure 4.1 No significant differences in COX2 or PTGES1 expression were observed in response to hypoxia or co-culture. Statistics were calculated using a 2-tailed T-test to separately evaluate the effect of hypoxia and the effect of co-culture using GraphPad software. Samples that did not consistently exhibit gene expression across all conditions and replications were excluded from analysis.

Inflammatory PGE₂ Production

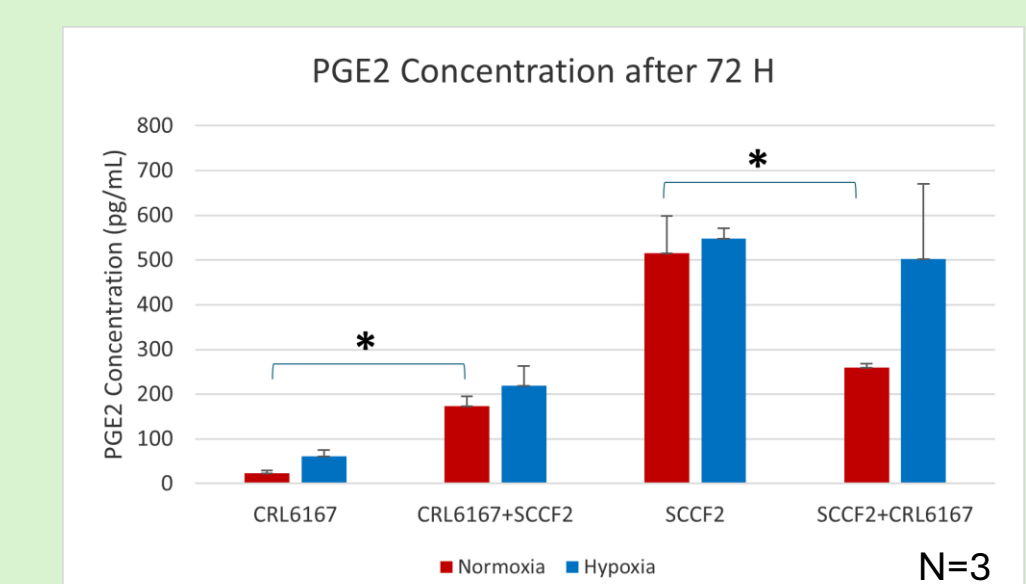


Figure 4.1 PGE₂ concentration showed a statistically significant response to co-culture in both tracheal fibroblasts (p=0.0003) and gingival FOSCC cells (p=0.0059). There was not a statistically significant response to hypoxia. Significance was determined using a 2-tailed T-test to separately evaluate the effect of hypoxia and the effect of co-culture using GraphPad software.

CONCLUSION

- VEGFC and VEGFD were the only genes to show a significant response to hypoxia, but the significance was mild, and the effect was not consistent between cell culture conditions. The relationship between hypoxia-inducible factor 1α and VEGF upregulation have been well reported in current literature. The lack of response in VEGFA, the best studied of the VEGF family, could be an effect of chronic hypoxia.
- Co-culture did not have a significant impact on gene expression but significantly impacted PGE₂ production in tracheal fibroblasts and gingival FOSCC cells. This may indicate that additional genes are responsible for the observed changes.
- The lack of additional significant findings could be due to limited experimental replication or low total RNA expression. Alternatively, pro-angiogenic cell signaling may not occur in a paracrine manner between FOSCC cells and fibroblasts, but it is too early to draw firm conclusions without further replication.

Acknowledgments

Figures created in <https://BioRender.com>

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