

VEGF-A ELISA

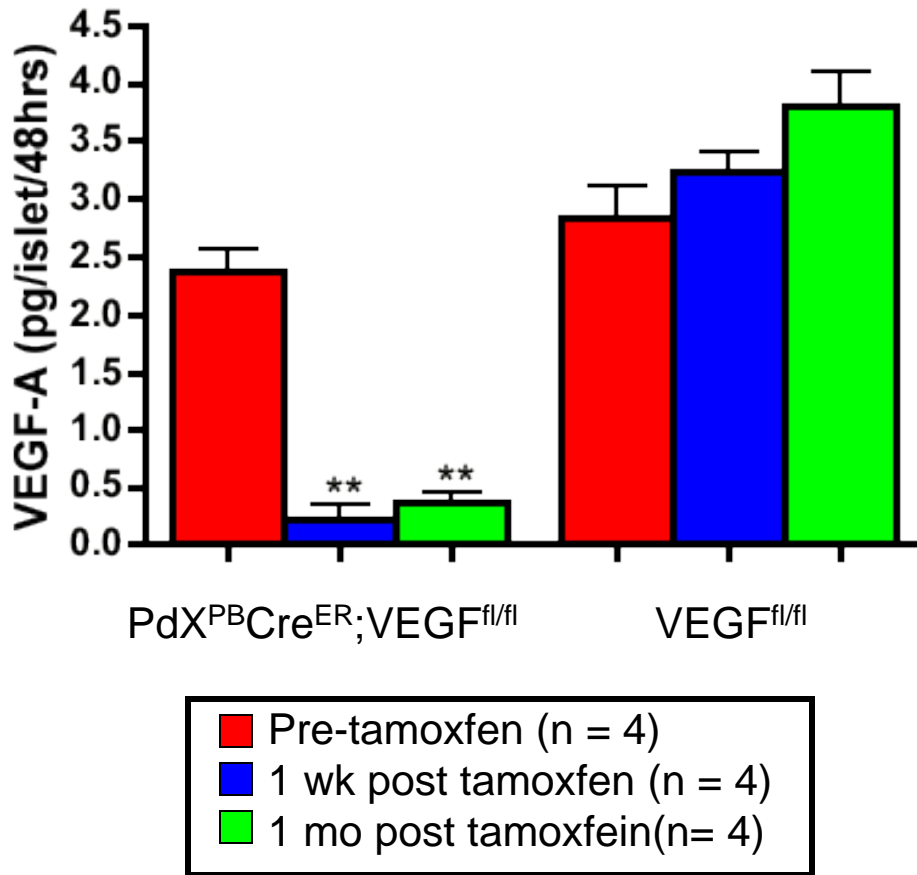


Figure 9. VEGF-A expression is significantly reduced in Pdx^{PB}Cre^{ER};VEGF^{fl/fl} islets after administration of 3 doses of 8mg tamoxifen. Islets were isolated from adult Pdx^{PB}Cre^{ER};VEGF^{fl/fl} and VEGF^{fl/fl} mice (n=4 mice) at the indicated time points before and after tamoxifen treatment. Isolated islets were cultured for 48 hours at 37° C in aliquots of 70 islets per 500ul of media. Islets and culture media (450 ul) were subsequently collected and stored at -80° C. VEGF-A levels in the culture media samples were then measured using an VEGF-A ELISA (n=3 wells). ** p-value < 0.0001 by t-test

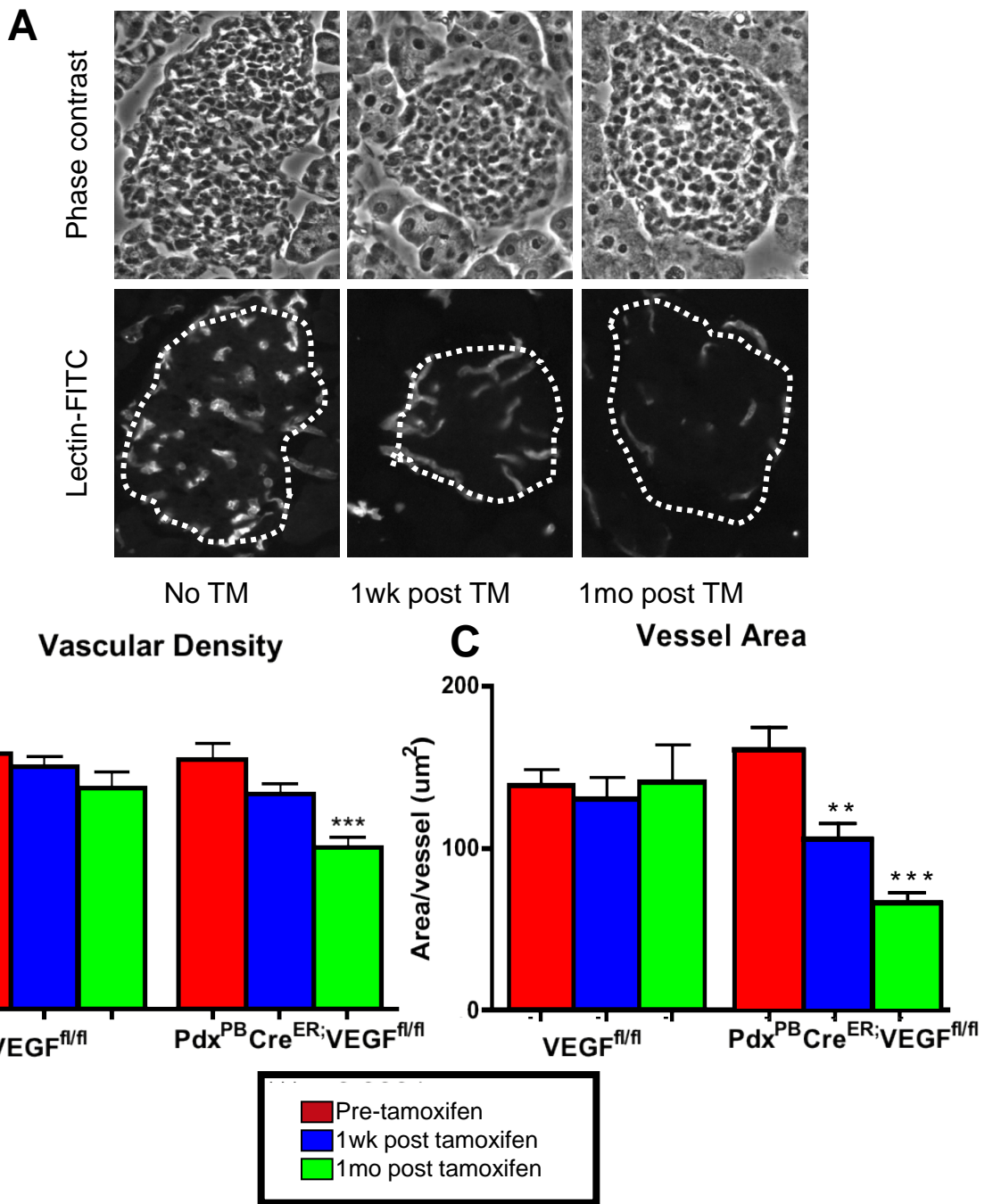


Figure 10. Decreased VEGF-A expression reduces vascular density in Pdx^{PB}Cre^{ER};VEGF^{fl/fl} islets. (A) Mice were infused with tomato-lectin to label the functional vasculature. Sections of the pancreas from Pdx^{PB}Cre^{ER};VEGF^{fl/fl} mice were imaged to assess the lectin labeled vessels at the indicated time points. Phase contrast was used to determine the islet perimeters. All images are at 20X. (B) The vascular density of VEGF^{fl/fl} islets (n=15) was unchanged 1 week (wk) and 1 month (mo) after tamoxifen. However, the vascular density of tm treated Pdx^{PB}Cre^{ER};VEGF^{fl/fl} islets was decreased 1mo post tam (n=20) when compared to -tm islets (n=12), there was no significant change in islets 1 wk after tm (n=31). (C) Measurements of area per vessel of Pdx^{PB}Cre^{ER};VEGF^{fl/fl} islets showed a significant decrease at 1wk (n=32) and 1mo (n=25) post tm when compared to islets from mice not treated with tamoxifen (n=20). No change was observed in VEGF^{fl/fl} islets (n=30). **p-value= 0.0031 and ***p-value≤0.0001

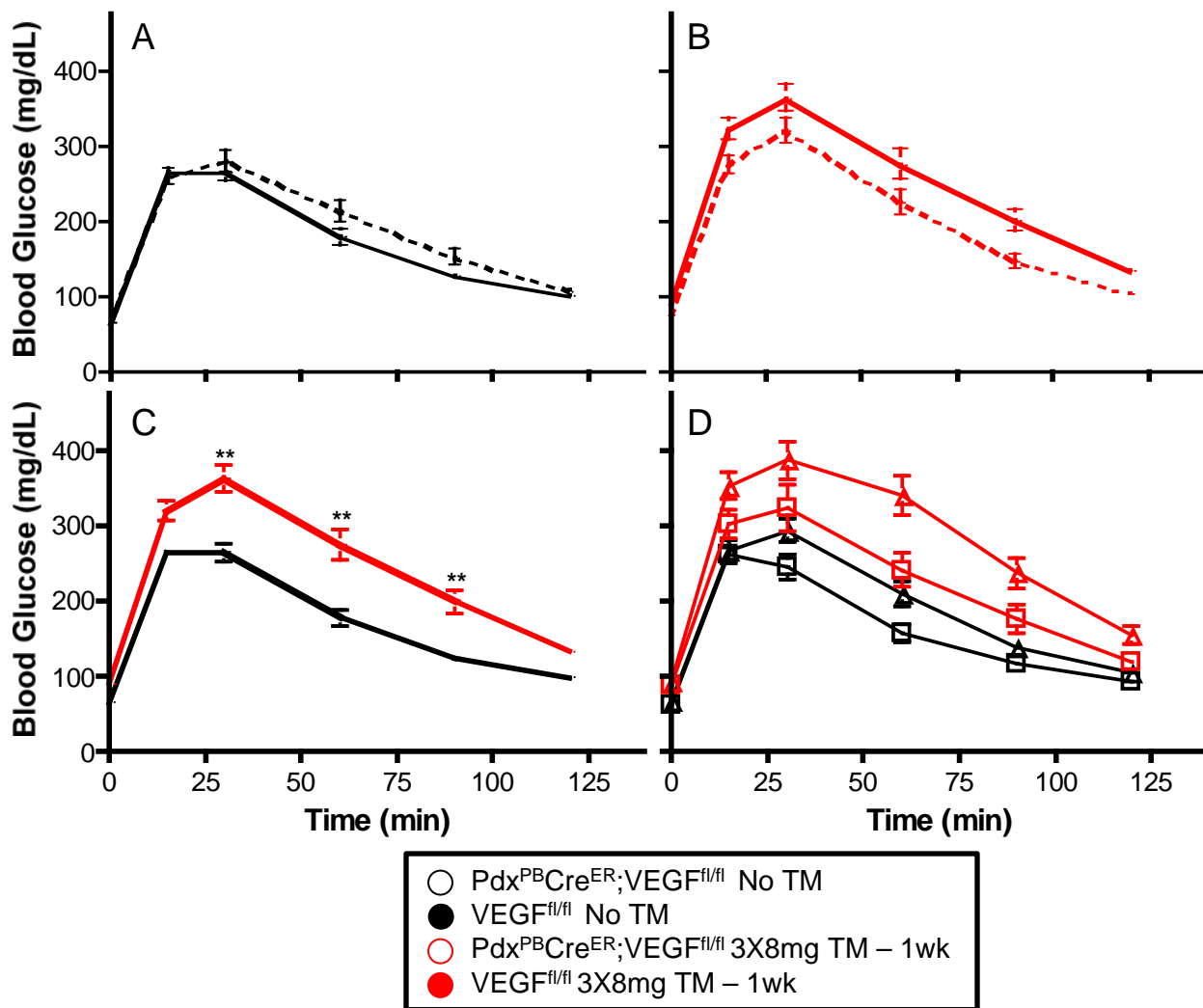


Figure 11. Glucose Tolerance changes in $Pdx^{PB}Cre^{ER};VEGF^{fl/fl}$ mice post tamoxifen treatment . (A) All $Pdx^{PB}Cre^{ER};VEGF^{fl/fl}$ (- ○-) and $VEGF^{fl/fl}$ (- ●-) mice underwent glucose tolerance testing (GTT) prior to tamoxifen injection. (B) One week after 3X8mg of tamoxifen, both $Pdx^{PB}Cre^{ER};VEGF^{fl/fl}$ (- ○-) and $VEGF^{fl/fl}$ (- ●-) mice, underwent another GTT. (C) There was a significant decrease in glucose clearance in the $Pdx^{PB}Cre^{ER};VEGF^{fl/fl}$ mice after receiving tamoxifen (red) when compared to their GTTs before tamoxifen (black). (D) This was still observed with the GTTs of male (△) and female (□) mice were separated and compared **p-value= 0.003 by unpaired t-test.

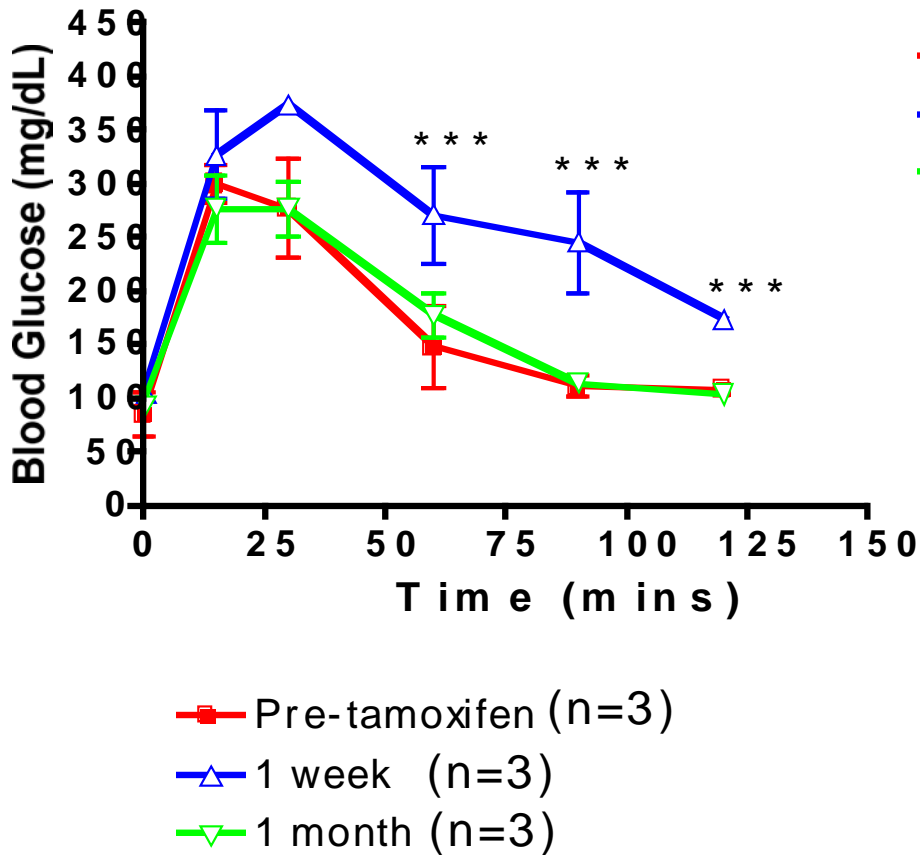


Figure 12. Summary of Glucose Tolerance tests in $Pdx^{PB}Cre^{ER};VEGF^{fl/fl}$. Glucose Tolerance Tests in $Pdx^{PB}Cre^{ER};VEGF^{fl/fl}$ Mice Return to 'Normal' 1 Month Post Tamoxifen Treatment This graph shows the GTT results for three $Pdx^{PB}Cre^{ER};VEGF^{fl/fl}$ mice that were tested at all three time points. Prior to tamoxifen treatment these mice had normal glucose clearance (-■-). However, at 1 week post tamoxifen treatment their GTTs results showed a delay in glucose clearance (-△-). This delay in glucose clearance was no longer observed in the tamoxifen treated $Pdx^{PB}Cre^{ER};VEGF^{fl/fl}$ mice at 1 month (-▽-) ***p-value= 0.003 by unpaired t-test.