bEnd.3 cells as the responses to the FFAR4 agonist EPA were similar to AH-7614. Alternatively, AH-7614 and EPA may be stimulating a receptor other than FFAR4 to mediate these anti-inflammatory effects (although here is no evidence for this in the literature).

After evaluating the data it became apparent that the change in protein expression did not align with our original hypothesis. For the time course experiment, the decrease in IκB after 24 hours of EPA treatment suggests EPA stimulated an inflammatory response and should have increased VCAM-1. However, there was no change in VCAM-1 expression. Previous work in this laboratory evaluated the impact of EPA on Cx43 expression and only observed a decreased in Cx43 after 48 hours [52]. As Cx43 expression is increased by inflammation through the NF-κB pathway [16,53], the decrease in Cx43 suggests the reduction in inflammation to EPA might have occurred at a later time than we evaluated (i.e. 48 hours). However, AH-7614 and EPA decreased VCAM-1 after 24 hours in later experiments (figure 8). The issue with our initial data (figure 5) may be the challenges associated with time course experiments. This likely increased the variability in the data along with the degradation in samples making it difficult to find significance.

The majority of this discussion has revolved around the decreased inflammatory response after 24 hours. However, we also observed an increased inflammatory response after 1.5 hours (figure 7). This observation is not consistent with the reported anti-inflammatory effects of the FFAR4. However, previous studies evaluating FFAR4 responses used later times (6-48 hours) [6,9,42,44,46,93–95]. Only 3 studies addressed FFAR4 responses at an earlier time (4 hours) [36–38] and the earliest evaluated pERK, showing a rapid peak after 2.5 minutes [90] that would precede any changes in IκB and/or pIKK as phosphorylation of ERK activates the NF-κB pathway (figure 3) [9]. Responses to FFAR4 (a $G\alpha_q$ receptor) may initially activate the NF-κB pathway enough to