

**SUPPLEMENTAL TABLE AND FIGURES**

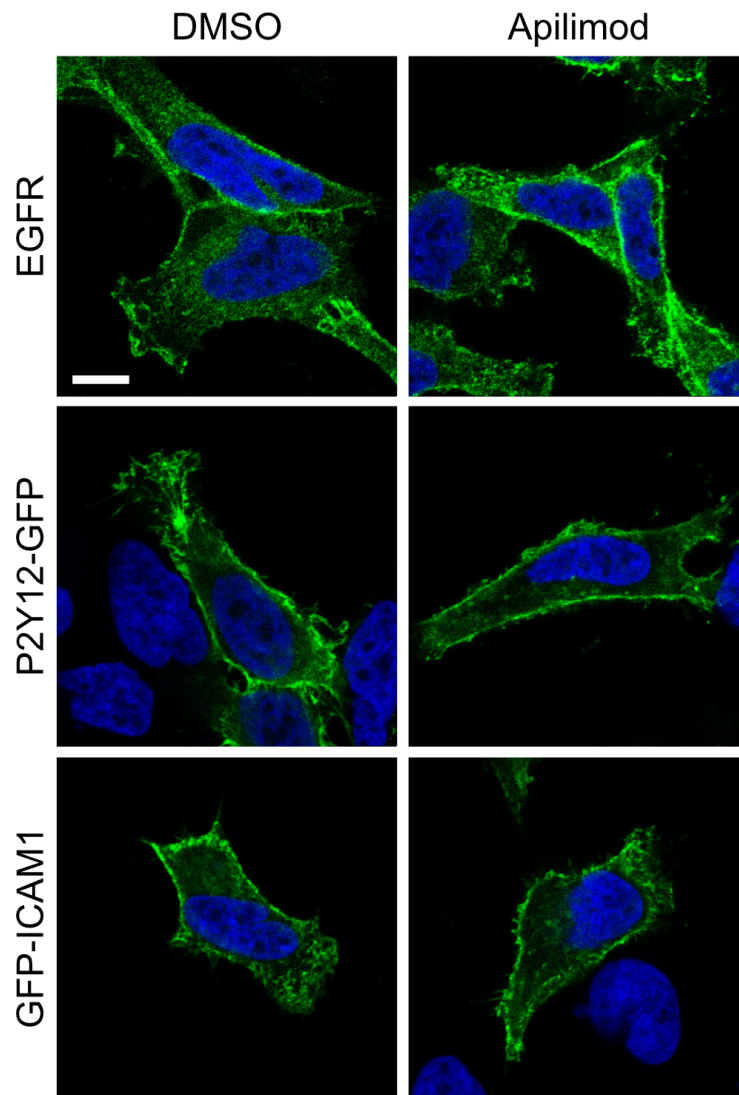
**Supplementary Table S1. qRT-PCR primers used in this study**

<b>Genes</b>	<b>Primer Sequence (forward)</b>	<b>Primer Sequence (reverse)</b>
Gapdh	5'-TGGCAAAGTGGAGATTGTTGCC-3'	5'-AAGATGGTGATGGGCTTCCCG-3'
Rplpo	5'-TGACATCGTCTTTAAACCCCG-3'	5'-TGTCTGCTCCCACAATGAAG-3'
Hprt	5'-TGAAAGACTTGCTCGAGATGTCAT-3'	5'-TCCAGCAGGTCAGCAAAGAA-3'
Rsp29	5'-GTCTGATCCGCAAATACGGG-3'	5'-AGCCTATGTCCATCGCGTACT-3'
$\beta$ -Mhc	5'-AGGGCGACCTCAACGAGAT-3'	5'-CAGCAGACTCTGGAGGCTCT-3'
$\alpha$ -Sma	5'-GTCCCAGACATCAGGGAGTAA-3'	5'-TCGGATACTTCAGCGTCAGGA-3'
Bnp	5'-GACAGCTCTTGAAGGACC-3'	5'-GTACCGATCCGGTCTAT-3'
Anp	5'-ATCACCTGGGCTTCTTC-3'	5'-CCTCCTGGGCTGTTATCTT-3'
Col1 (heart)	5'-CCTGCCTGCTTCGTGTAA-3'	5'-GGTCACGTTCAAGTTGGTCA-3'
Col1 (liver)	5'-TGCTTTCTGCCCCGGAAG-3'	5'-GGGATGCCATCTCGTCCA-3'
Col3	5'-TCCTGAAGATGTCCTTGATGTACAG-3'	5'-TTCAGAGACTTCTTTACATTGCCATT-3'
Fibronectin 1	5'-GGTGTAGCACAACTTCCAATTACG-3'	5'-GGAATTTCCGCCTCGAGTCT-3'
$\alpha$ -skeletal actin	5'-TACCACCGGCATCGTGTTG-3'	5'-CCAGGTCCAGACGCATGAT-3'
Ctgf	5'-CTCCACCCGAGTTACCAATG-3'	5'-TGGCGATTTTAGGTGTCCG-3'
Periostin	5'-AAGAGATGGTCACTTCACGC-3'	5'-GCACTGGAGGGTATTTAGGATG-3'
Il-6 (heart)	5'-CAAAGCCAGAGTCCTTCAGAG-3'	5'-GTCCTTAGCCACTCCTTCTG-3'
Il-6 (liver)	5'-GCCCACCAAGAACGATAGTCA-3'	5'-CAAGAAGGCAACTGGATGGAA-3'
Ccl2	5'-GCAGTTAACGCCCCACTCA-3'	5'-CCAGCCTACTCATTGGGATCA-3'
F4/80	5'-TGACAACCAGACGGCTTGTG-3'	5'-GCAGGCGAGGAAAAGATAGTGT-3'
Tnf- $\alpha$ (heart)	5'-CTTCTGTCTACTGAACTTCGGG-3'	5'-CAGGCTTGTCACCTCGAATTTTG-3'
Tnf- $\alpha$ (liver)	5'-TGGGACAGTGACCTGGACTGT-3'	5'-TTCGGAAAGCCCATTTGAGT-3'
Tgf $\beta$	5'-GAGCCCGAAGCGGACTACTA-3'	5'-CACTGCTTCCCGAATGTCTGA-3'
Mmp9	5'-TGAGTCCGGCAGACAATCCT-3'	5'-CGCCCTGGATCTCAGCAATA-3'





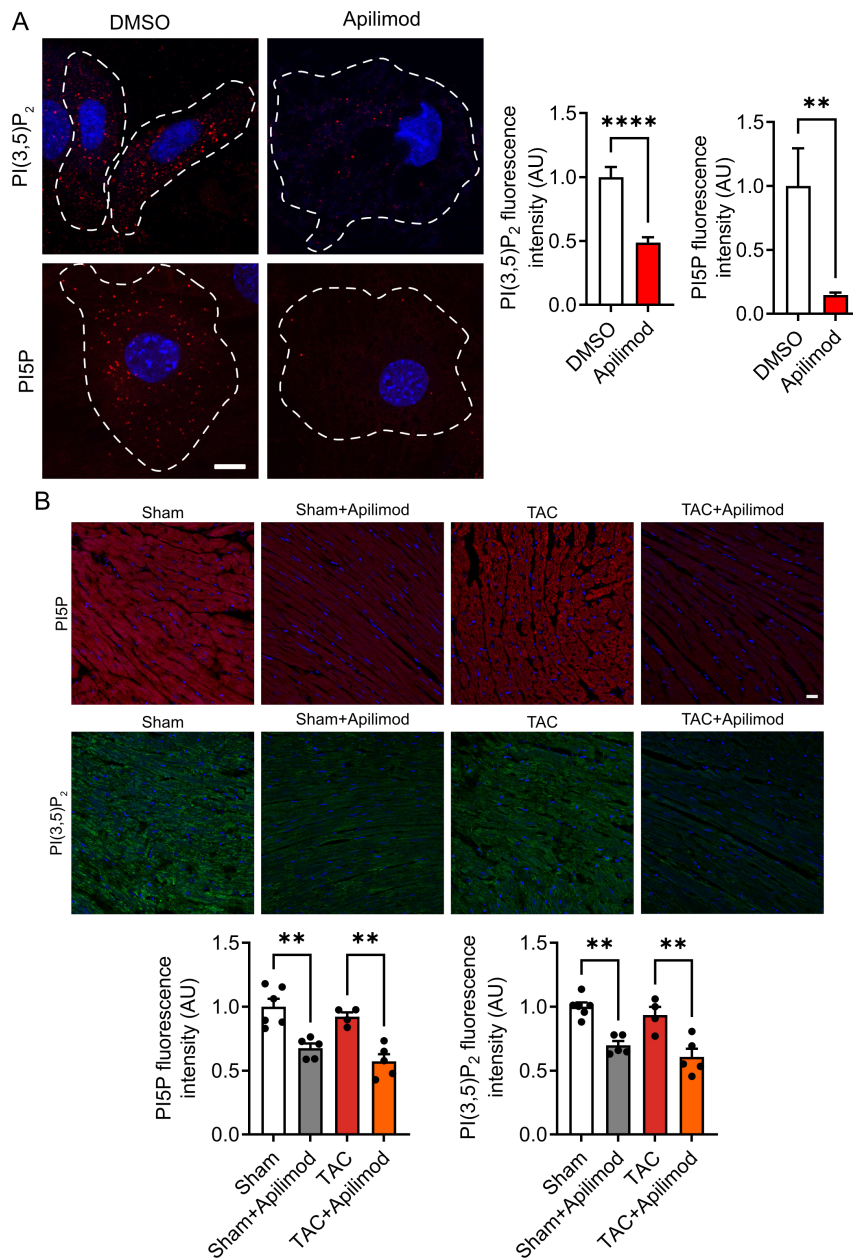




**Figure S2. Apilimod does not alter EGFR, P2Y12 and ICAM1 steady-state localization.**

(A) HeLa cells were serum-starved and treated with Apilimod or vehicle only (DMSO), fixed and stained for endogenous EGFR or overexpressed P2Y12-GFP or GFP-ICAM1 (shown in green). Nuclei were stained with DAPI (in blue). Bar is 10  $\mu$ m.



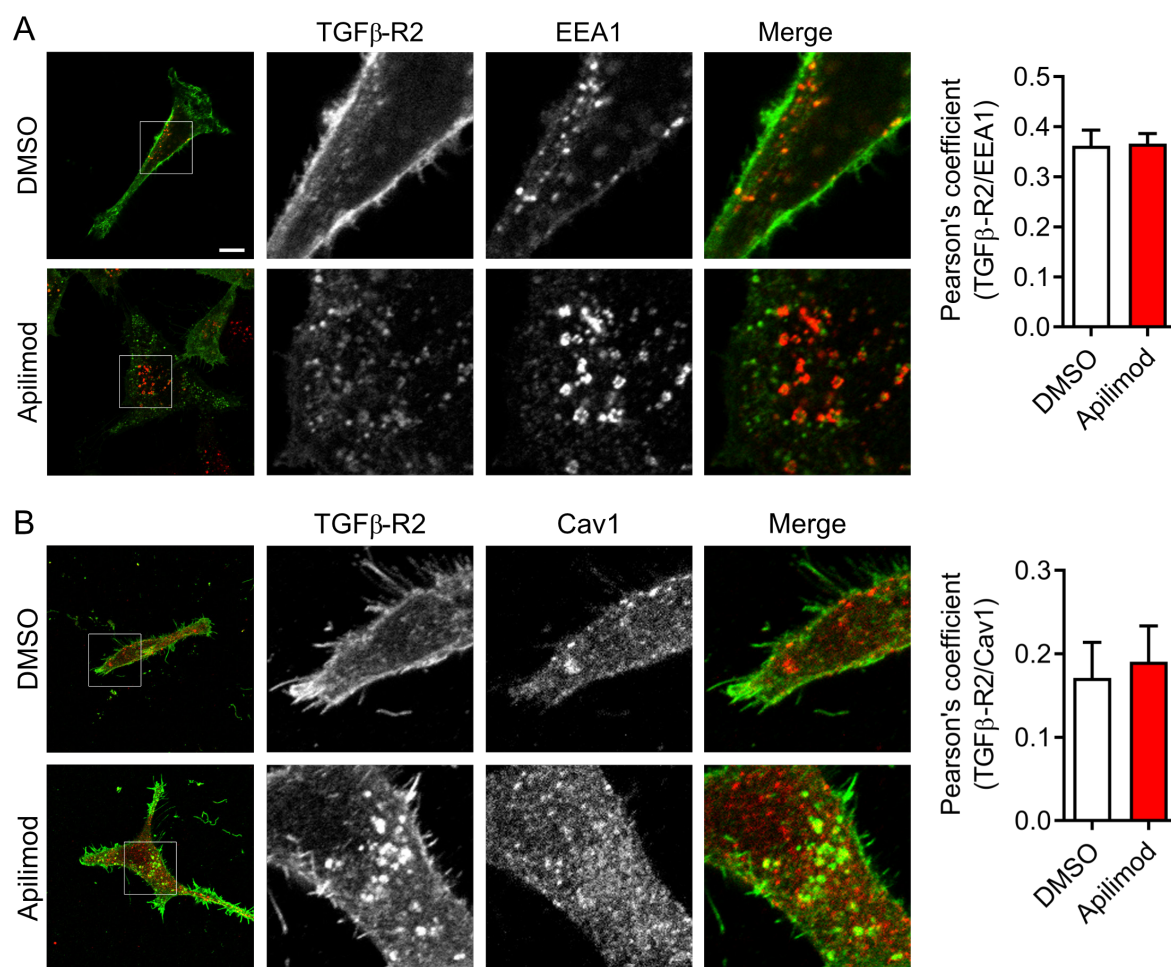


**Figure S3. Apilimod inhibits PIKfyve activity in vivo and in vitro.**

**(A)** Primary cardiac fibroblasts were treated with Apilimod (100 nM) or with DMSO alone for 48 h, fixed and stained for PI5P or PI(3,5)P<sub>2</sub> (shown in red). Nuclei were stained with DAPI (in blue). Bar is 10  $\mu$ m. Quantifications are shown on the right,  $n = 9-16$  cells. Student's t-test (unpaired, two-tailed), \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ .

**(B)** Cardiac cryosections from mice treated as indicated were immunostained for PI5P (shown in red) or PI(3,5)P<sub>2</sub> (shown in green). Nuclei were stained with DAPI (in blue). Bar is 20  $\mu$ m. Quantifications shown on the right are from 4-6 mice per group. ANOVA followed by Bonferroni's post-hoc test, \*\* $p < 0.01$ .

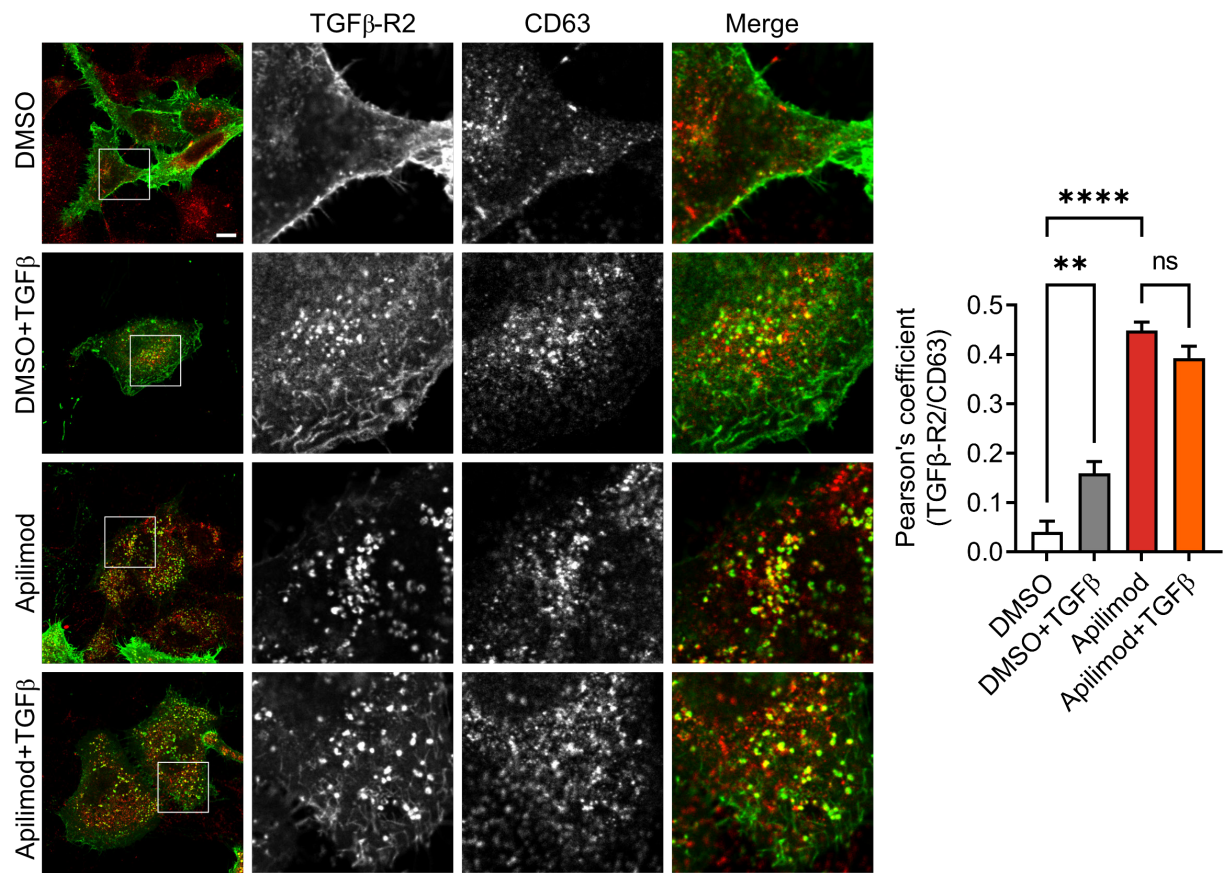




**Figure S4. Internalized TGF $\beta$ -R2 does not colocalized with EEA1 or Cav1 in Apilimod-treated cells.**

**(A-B)** Serum-starved HeLa cells expressing flag-TGF $\beta$ -R2 were treated with Apilimod or vehicle only (DMSO), fixed and stained with an anti-flag antibody (green) and anti-EEA1 (A) or anti-Cav1 (B) antibodies (red). Bar is 10  $\mu$ m. Right panels show the quantification of colocalization between flag-TGF $\beta$ -R2 and the indicated markers.

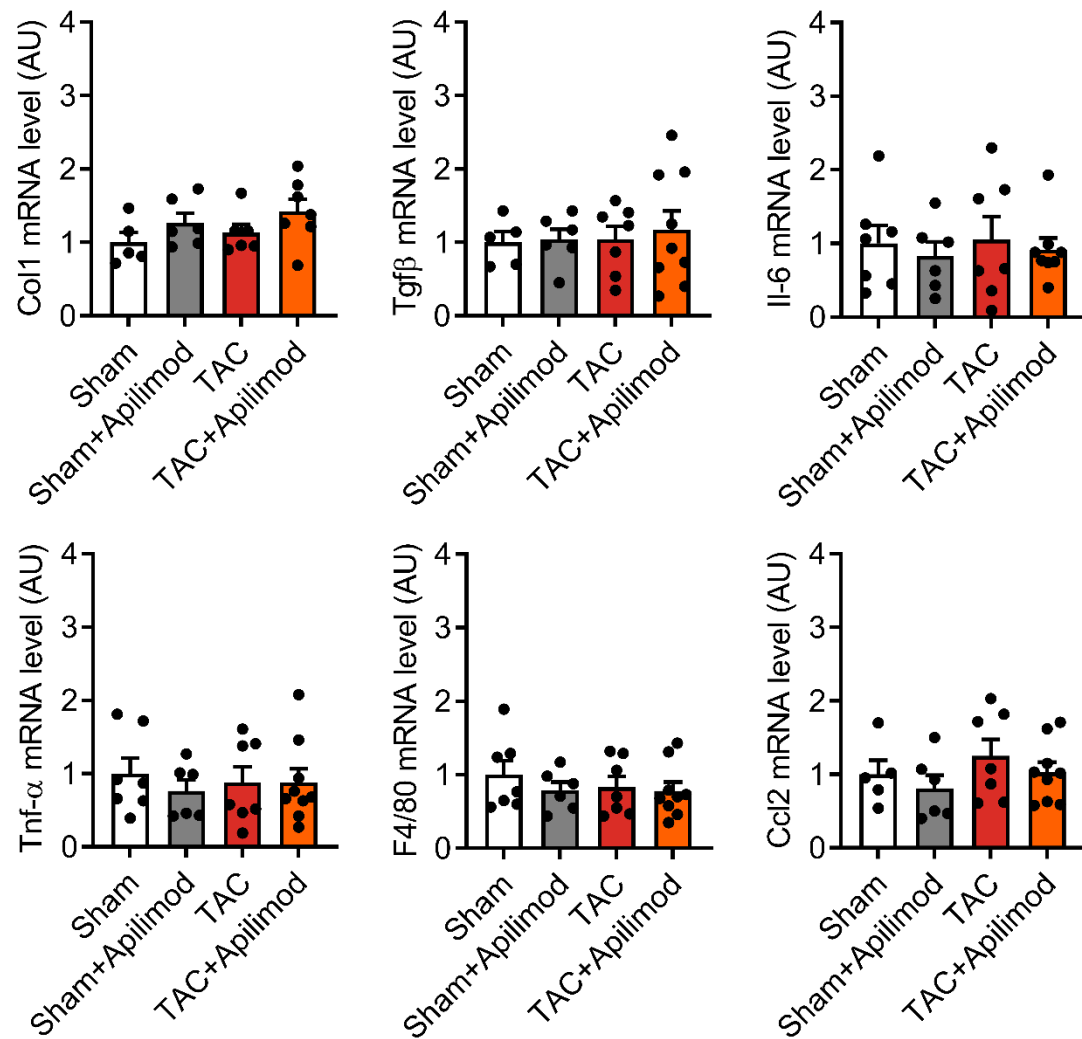




**Figure S5. TGFβ-R2 colocalization with CD63 in Apilimod and TGFβ treated cells.**

Serum-starved HeLa cells expressing flag-TGFβ-R2 were treated with Apilimod or DMSO as indicated, stimulated with TGFβ, fixed and stained with anti-flag (in green) and anti-CD63 (in red) antibodies. Bar is 10 μm. Quantification of colocalization between TGFβ-R2 and CD63 is shown on the right. n = 36-53 cells across 3 independent experiments. ANOVA followed by Bonferroni's post-hoc test, \*\*p < 0.01; \*\*\*\*p < 0.0001; ns, non-significant.





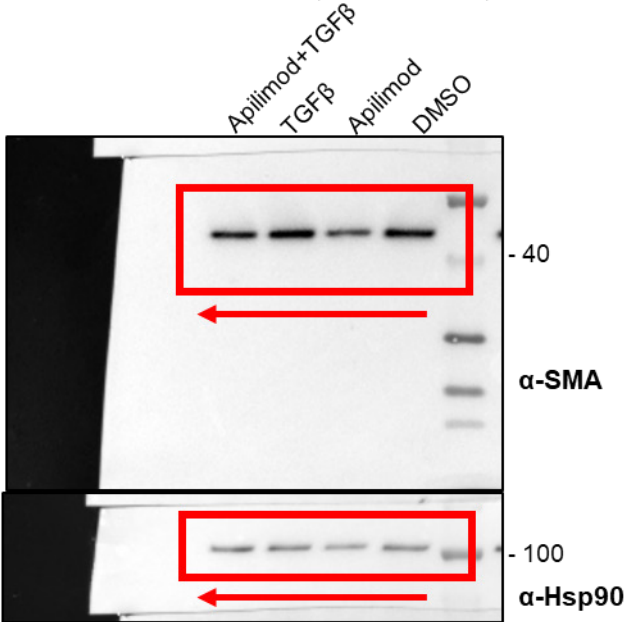
**Figure S6. Fibrotic and inflammatory status of mice liver.**

Expression levels of collagen 1 (Col1), Tgfβ, Il-6, Tnf-α, F4/80 and Ccl2 were measured by qRT-PCR in hepatic tissues of mice treated as indicated. n = 5-9 mice per group.

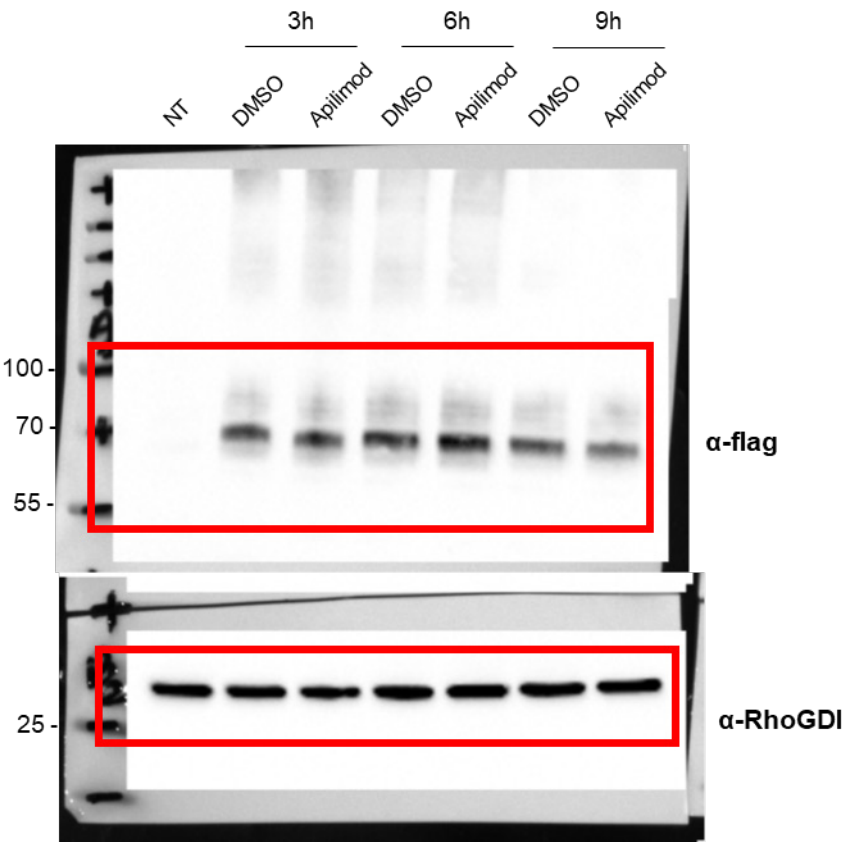


FULL UNEDITED GELS

Full unedited gel for Figure 2G



Full unedited gel for Figure 5B





Full unedited gel for Figure 6C

