SUPPLEMENTARY MATERIALS FOR

Bat-specific adaptations in interferon signaling and GBP1 contribute to enhanced viral tolerance

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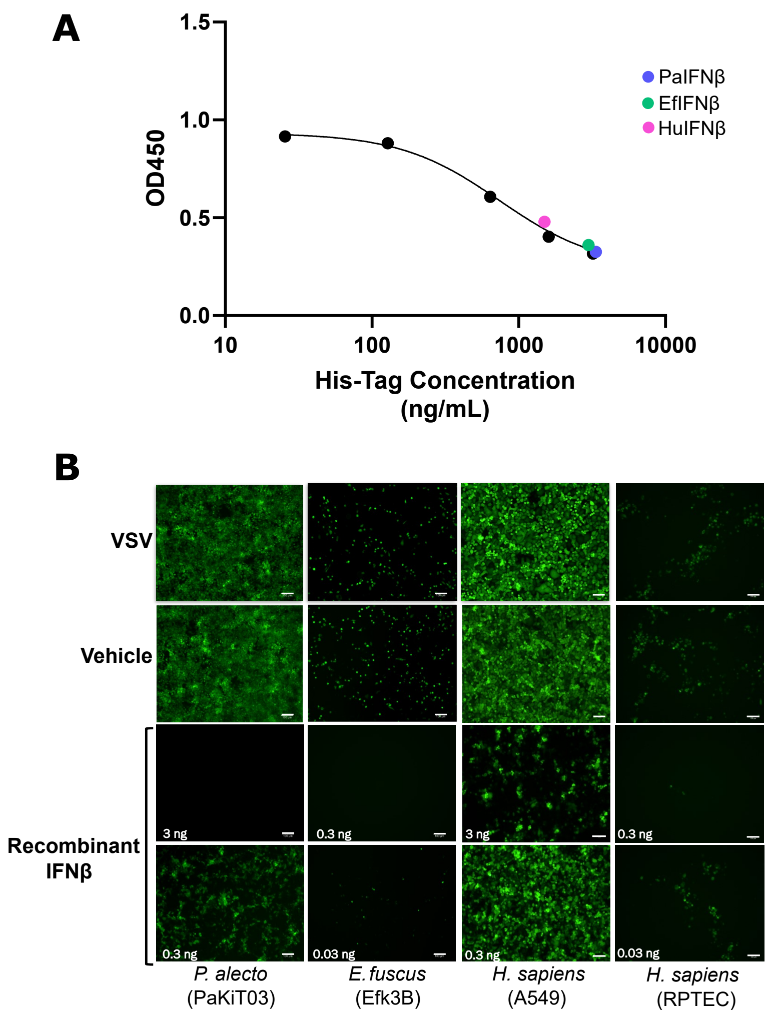
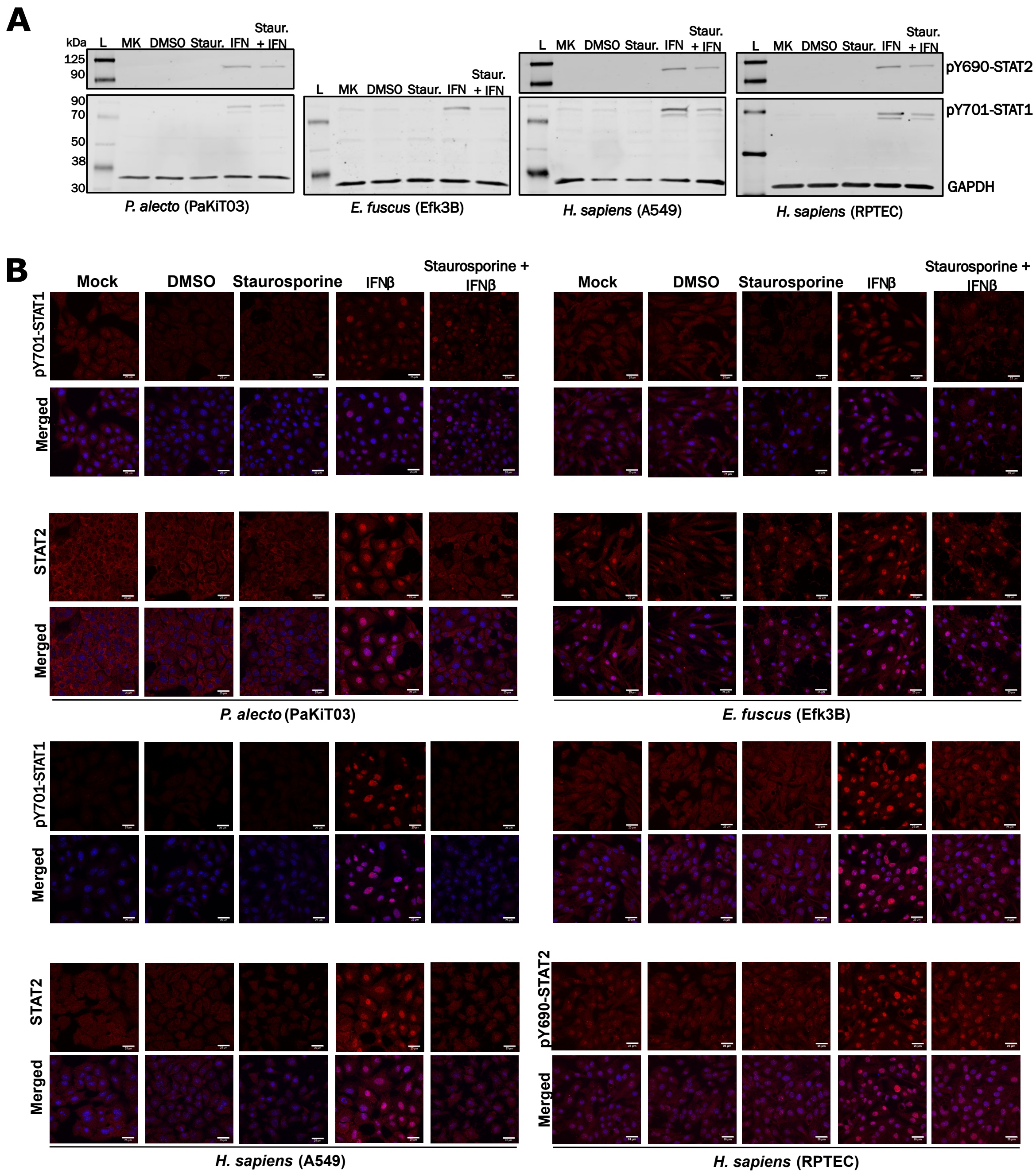
The supplement contains: Supplementary Figures 1 to 17

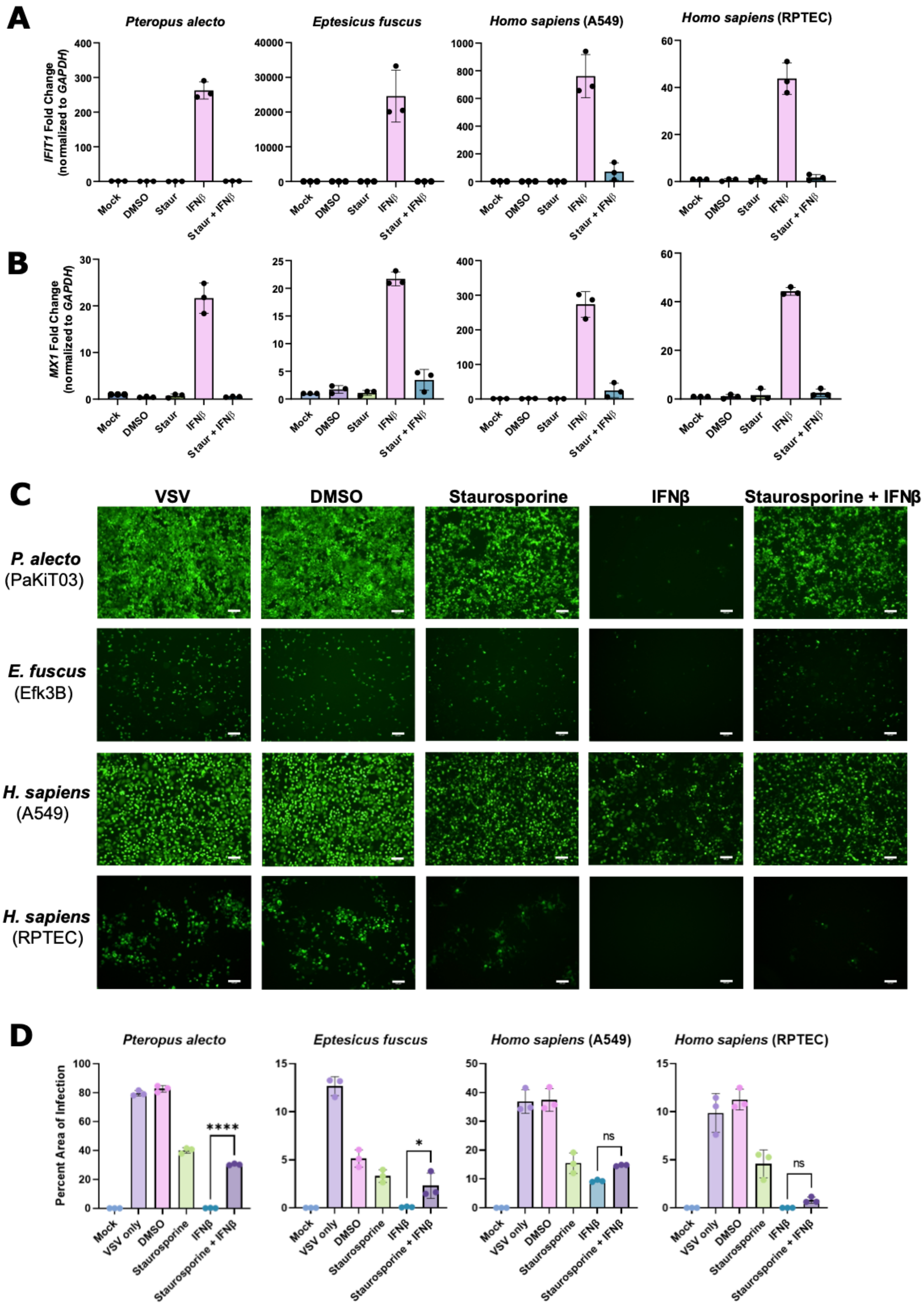
Supplementary Tables S1 to S7 are provided as sheets in a separate Excel file



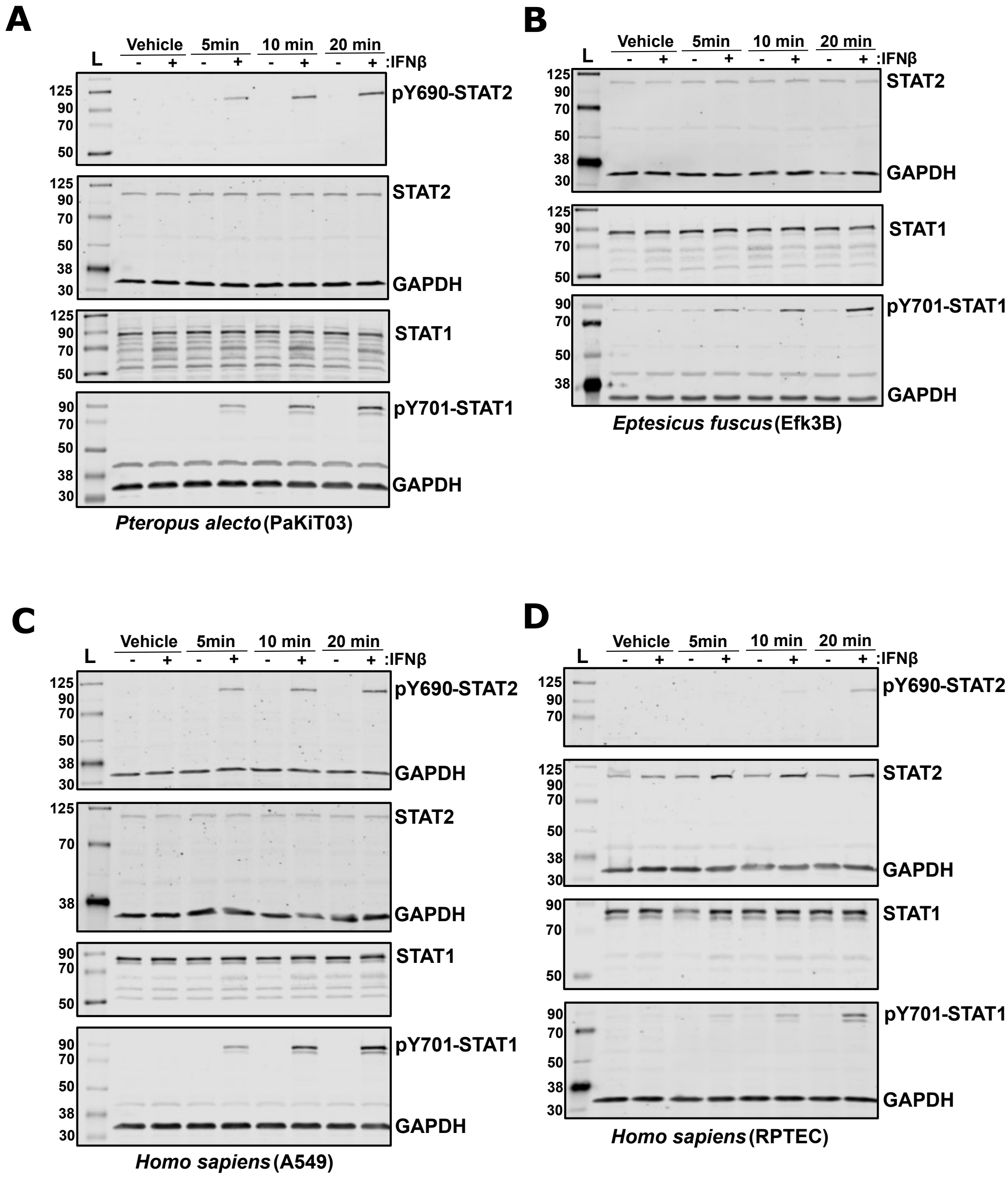
Supplementary Figure 1. Generation of functional IFNβ. (**A**) Stock concentrations for IFNβ expressing a 6x-His tag for *P. alecto, E. fuscus* and humans were determined by a competitive ELISA. Black dots represent standards included in the commercial ELISA. (**B**) *P. alecto* (PaKiT03), *E. fuscus* (Efk3B), and human A549 and primary RPTEC cells were treated with the vehicle control or with serially diluted, species-matched IFNβ for 6 hours prior to infection with VSV-GFP (MOI 0.1). Infection levels were assessed 16 hours post infection using fluorescent microscopy. The last dilution where 100% protection was observed was selected as 1 unit (i.e. 1U) of IFNβ for each species. Scale bars = 100 μM. Image is representative of 3 replicates.



Supplementary Figure 2. Phosphorylation of STAT1 and STAT2 during bat IFNβ signaling. *P. alecto* (PaKiT03), *E. fuscus* (Efk3B), and human (A549, RPTEC) cells were treated with Staurosporine (Staur; 500nM), species-matched IFNβ (10 U/mL), or treated with both Staurosporine and IFNβ for 4 hours. (**A**) Protein lysates from cells were harvested to evaluate the phosphorylation of STAT1 (pY701-STAT1) and STAT2 (pY690-STAT2). GAPDH was used as a loading control. Lane “L” represents molecular weight ladder (kDa). (**B**) Cells were fixed in 100% methanol and stained for pY701-STAT1, pY690-STAT2, STAT2, and/or DAPI. Nuclear translocation was visualized by confocal microscopy. Scale bars = 25 µM. Image is representative of 3 replicates.



Supplementary Figure 3. Phosphorylation is required for IFNβ-induced antiviral response. *P. alecto* (PaKiT03), *E. fuscus* (Efk3B), and human (A549, RPTEC) cells were treated with Staurosporine (Staur; 500nM), species-matched IFNβ (10 U/mL), or treated with both Staurosporine and IFNβ for 4 hours. qRT-PCR was performed to evaluate the upregulation of *IFIT1* (**A**) and *MX1* (**B**) transcripts. (**C**) Following 4 hours of Staurosporine and/or IFNβ treatment, cells were infected with VSV-GFP (MOI 0.1) for 16 hours. Viral replication was visualized using fluorescent microscopy, and (**D**) GFP signal was quantified using ImageJ to determine percent area of infection. Data are represented as mean ± SD, n=3 replicates/time points (Ordinary one-way ANOVA).



Supplementary Figure 4. Full immunoblots for STAT1 and STAT2 phosphorylation. PaKiT03 (**A**), Efk3B (**B**), A549 (**C**), and RPTEC (**D**) cells were treated with vehicle or species matched IFNβ (10 U/mL) for 5, 10, and 20 minutes. Total STAT1, STAT2, and phosphorylated STAT1 (pY701-STAT1) and STAT2 (pY690-STAT2) were assessed by immunoblotting. GAPDH was used as a loading control. Lane "L" represents molecular weight ladder (kDa).

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P. vampyrus        1  MAQWEMLQNLDSPFQDQLHQLYTNSLLPVDIRQYLAVWIEDQNWQEAALGKDDSQANMLFFHFLDQLKYECGRCSQDPEC  80
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R. aegyptiacus     1  MAQWEMLQNLDSPFQDQLHQLYTNSLLPVDIRQYLAVWIEDQNWQEAALGNDDSKANMLFFHFLDQLKYECGRCSQDPEC  80
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D. rotundus        1  MAQWEMLQNLDSPFQDQLHQLYTNSLLPMDIRQYLAVWIEDQNWQEAALGNNNSQANMLFLHFLDQLNYECARCSQDPER  80
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M. lucifugus     160  VKSISHLKDLQDVFLFRYKTQAPVRTSSLDPHQTRQQQILQATLNELDRRRKEVLDASRGLLGRLTTLIELLLPNLEQWK  239
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M. davidii       160  VKAISQLKDLQDVFLFRFKAQGPVRTSPLDPHQTRQQQVLQATLNELDRRRKEVLDASRGLLGRLTTLIELLLPNLEQWK  239
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*Supplementary Figure 5 continues on next page

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P. vampyrus	*240*	VQQQKACIGSREDG--GLELSQLEKWFTDGAKLLFHLRQLLKELKGLSHLVSYHHDPLTTGVDLREAQVTELLQRLLLRA	*317*
P. rodricensis	*240*	VQQQKACIGSREDG--GLELSQLEKWFTDGAKLLFHLRQLLKELKGLSHLVSYHHDPLTTGVDLREAQVTELLQRLLLRA	*317*
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P. kuhlii	*239*	AQQQRTCIGAPAEG--GLELVQLEKWFTDGAQLLFHLRQLLKELKDLS-HVSYDNDRLASGADQQQARATELLRHLLHRA	*315*
H. sapiens	*240*	AQQQKACIRAPIDH--GLE--QLETWFTAGAKLLFHLRQLLKELKGLSCLVSYQDDPLTKGVDLRNAQVTELLQRLLHRA	*315*

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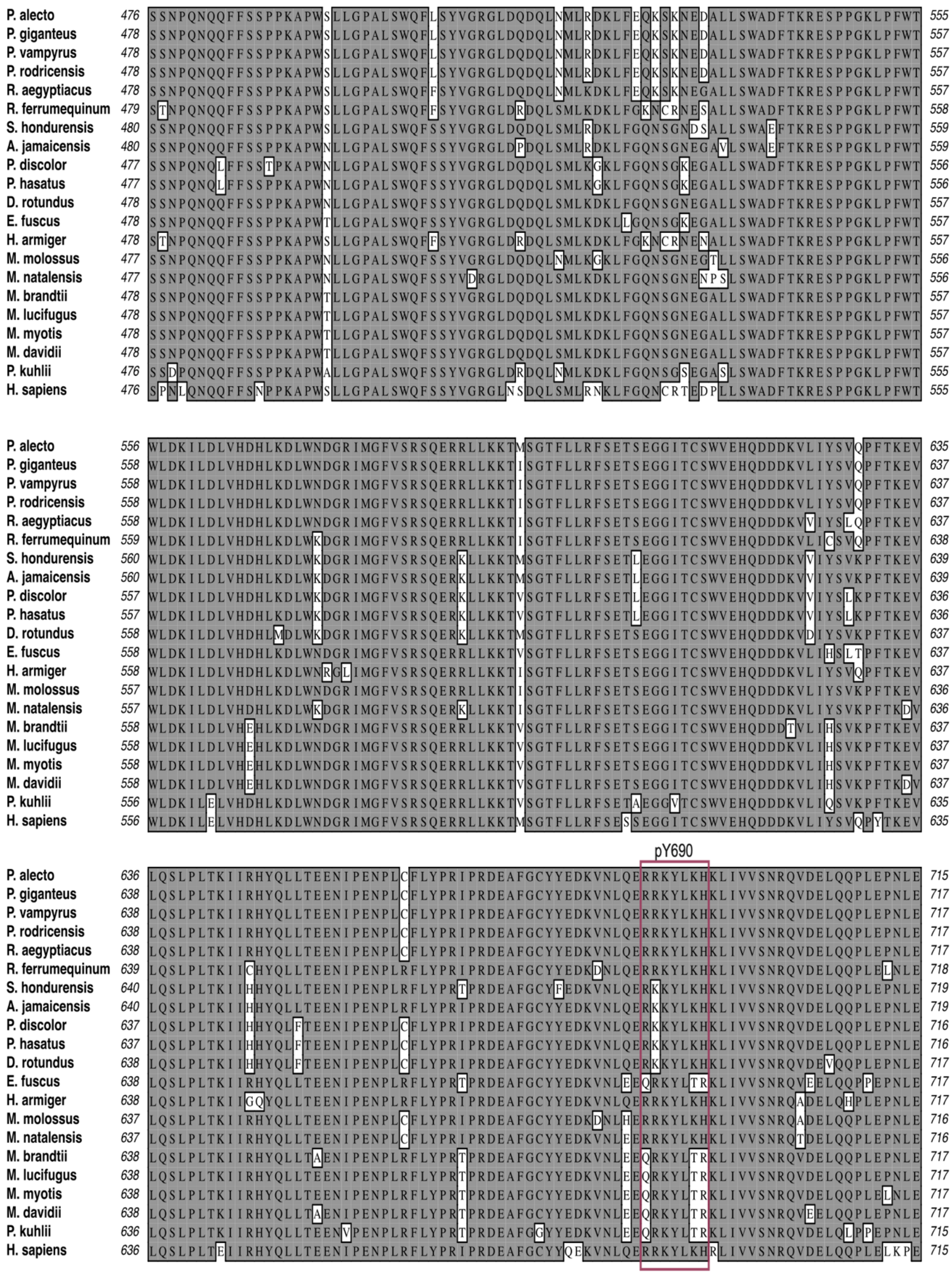
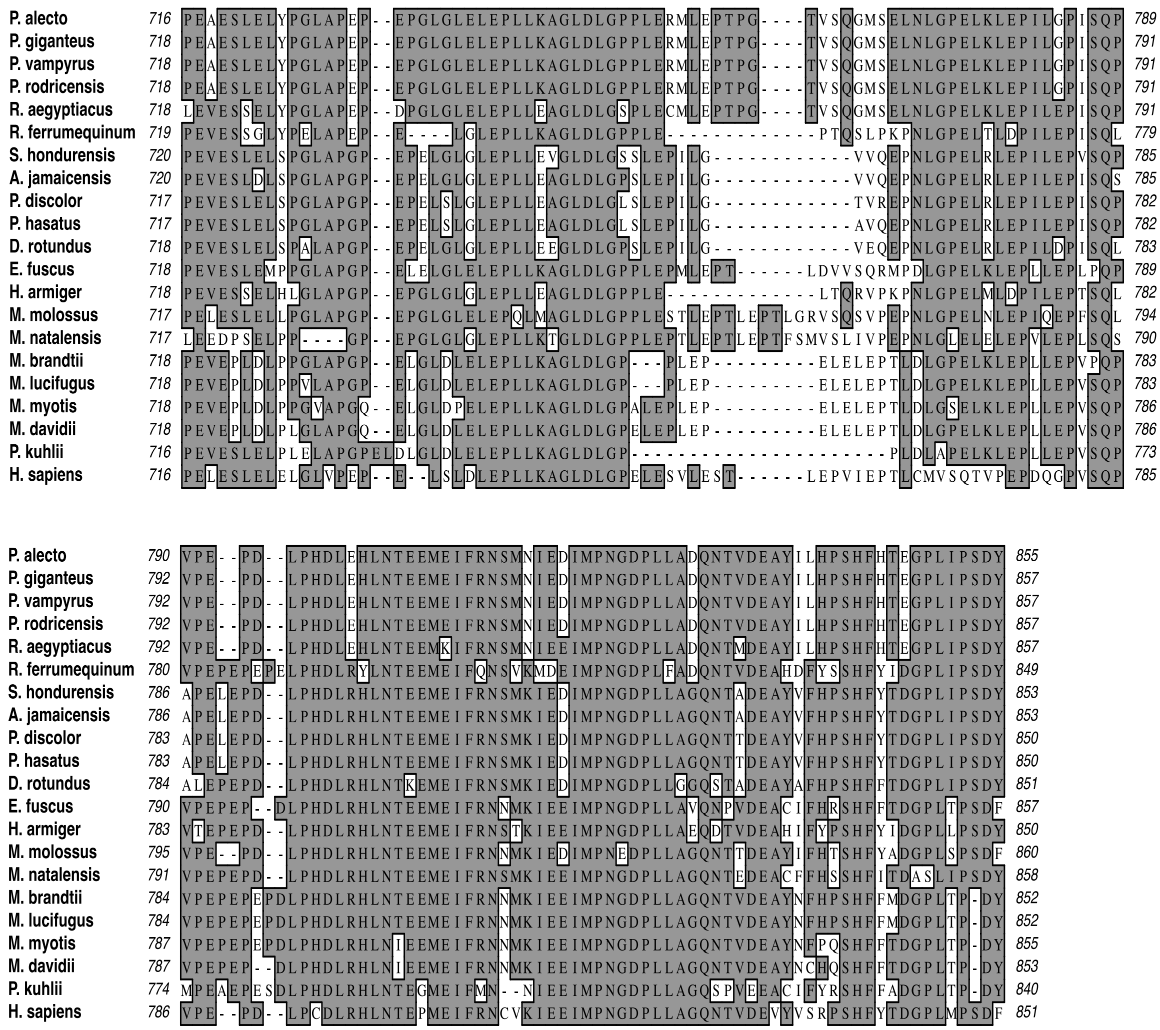
S. hondurensis	*400*	LIWDFRYLTLTEQRSGGSGKGSNKSLLGVTEELHIISFTVRYTYQGLKQELKTDTLPVVIISNTNQLSIAWASVLWFNLL	*479*
A. jamaicensis	*400*	LIWDFRYLTLTEQRSGGSGKGSNKSLLGVTEELHIISFTVKYTYQGLKQELKTDTLPVVIISNTNQLSIAWASVLWFSLL	*479*
P. discolor	*397*	LIWDFRYLTLTEQRSGGSGKGSNKSLLGVTEELHIISFTVKYTYQGLKQELQTDTLPVVITSNTNQLSIAWASVLWFNLL	*476*
P. hasatus	*397*	LIWDFRYLTLTEQRSGGSGKGSNKSLLGVTEELHIISFTVKYTYQGLKQELQTDTLPVVITSNTNQLSIAWASVLWFNLL	*476*
D. rotundus	*398*	LIWDFRYLTLTEQRSGGSGKGTNKSLLGVTEELHIISFTVKYTYQGLKQELQTDTLPVVITSNTNQLSIAWASVLWFNLL	*477*
E. fuscus	*398*	LIWDFGYLTLLEQRSGGSGKGSSKGLLGVTEELHIISFTVQYTYQGLKQELKADTLPVVIISNTNQLSIAWASILWFNLL	*477*
H. armiger	*398*	LIWDFGYLTLMEQRSGGSGKGSNKGLLGVTEELHIISITVKYTYQGLKQELKTDTLPVVIISNMNQLSIAWASVLWFNLL	*477*
M. molossus	*397*	LIWDFGYLSLVEQRSGGSGKGSNKGLLGVTEELHIISFTVKYTYQGLKQELKTDTLPVVIISNMNQLSIAWASILWFNLL	*476*
M. natalensis	*397*	LIWDFGYLTLLEQRSGGSGKGSNKGLLGVTEELHIISFTVKYTYQGLKQELQTDTLPVVIISNVNQLSIAWASILWFNLL	*476*
M. brandtii	*398*	LIWDFGYLTLLEQRSGGSGKGSNKGLLGVTEELHIISFTVRYTYQGLKQELKADTLPVVIISNMNQLSIAWASILWFNLL	*477*
M. lucifugus	*398*	LIWDFGYLTLLEQRSSGSGKGSNKGLLGVTEELHIISFTVRYTYQGLKQELKADTLPVVIISNMNQLSIAWASILWFNLL	*477*
M. myotis	*398*	LIWDFGYLTLLEQRSGGSGKGSNKGLLGVTEELHVISFTVRYTYQGLKQELKADTLPVVIISNMNQLSIAWASILWFSLL	*477*
M. davidii	*398*	LIWDFGYLTLLEQRSGGSGKGSSKGLLGVTEELHIISFTVRYTYQGLKQELKADTLPVVVISNMNQLSIAWASILWFNLL	*477*
P. kuhlii	*396*	LIWDFGYLTLLEQRAGGSGKGSSKGLLGVTEELHIISFTVRYTYQGLKQELKADTLPVVVISNPNQLSIAWASILWFNLL	*475*
H. sapiens	*396*	LIWDFGYLTLVEQRSGGSGKGSNKGPLGVTEELHIISFTVKYTYQGLKQELKTDTLPVVIISNMNQLSIAWASVLWFNLL	*475*

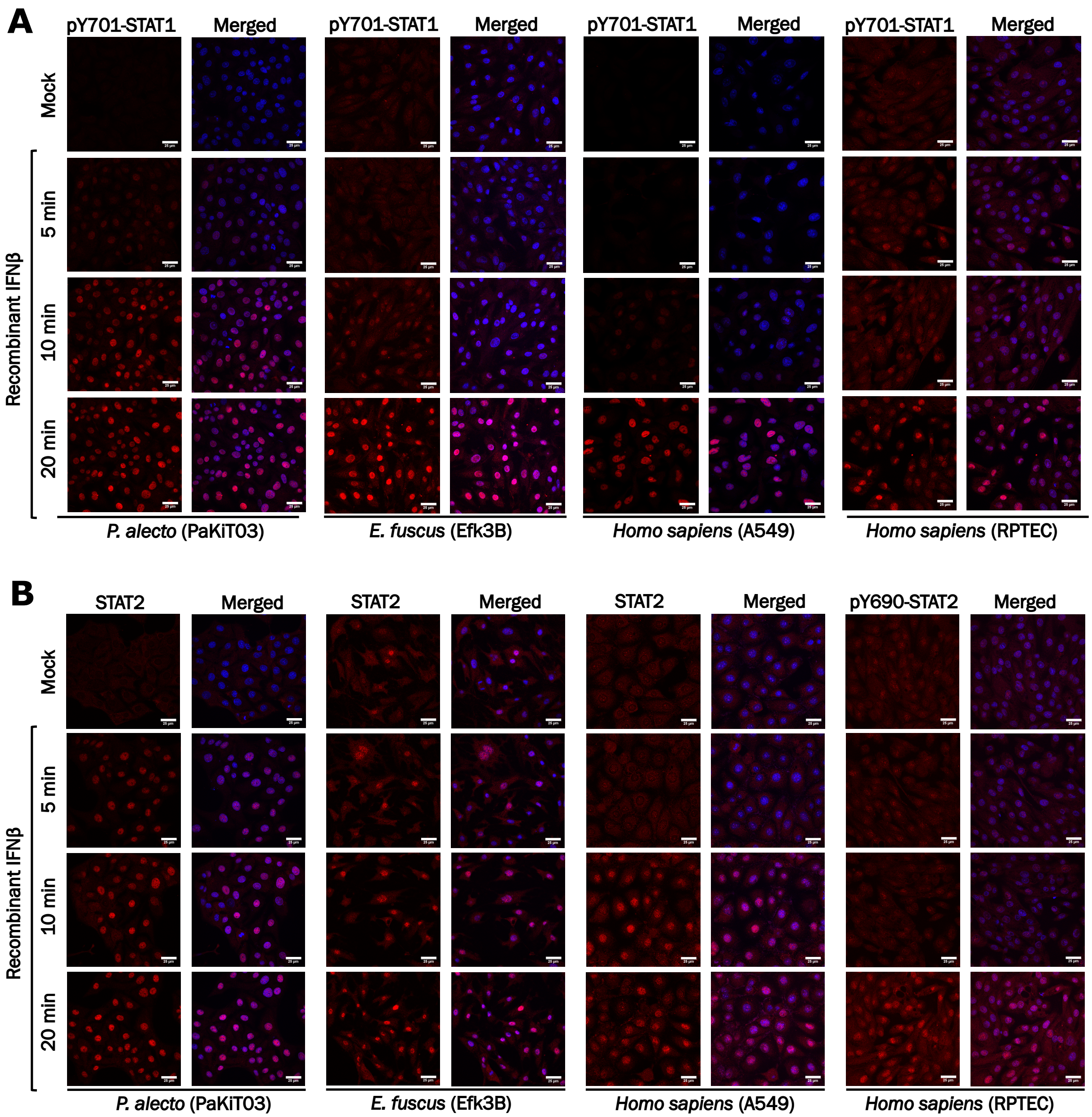
***Supplementary Figure 5 continues on next page**



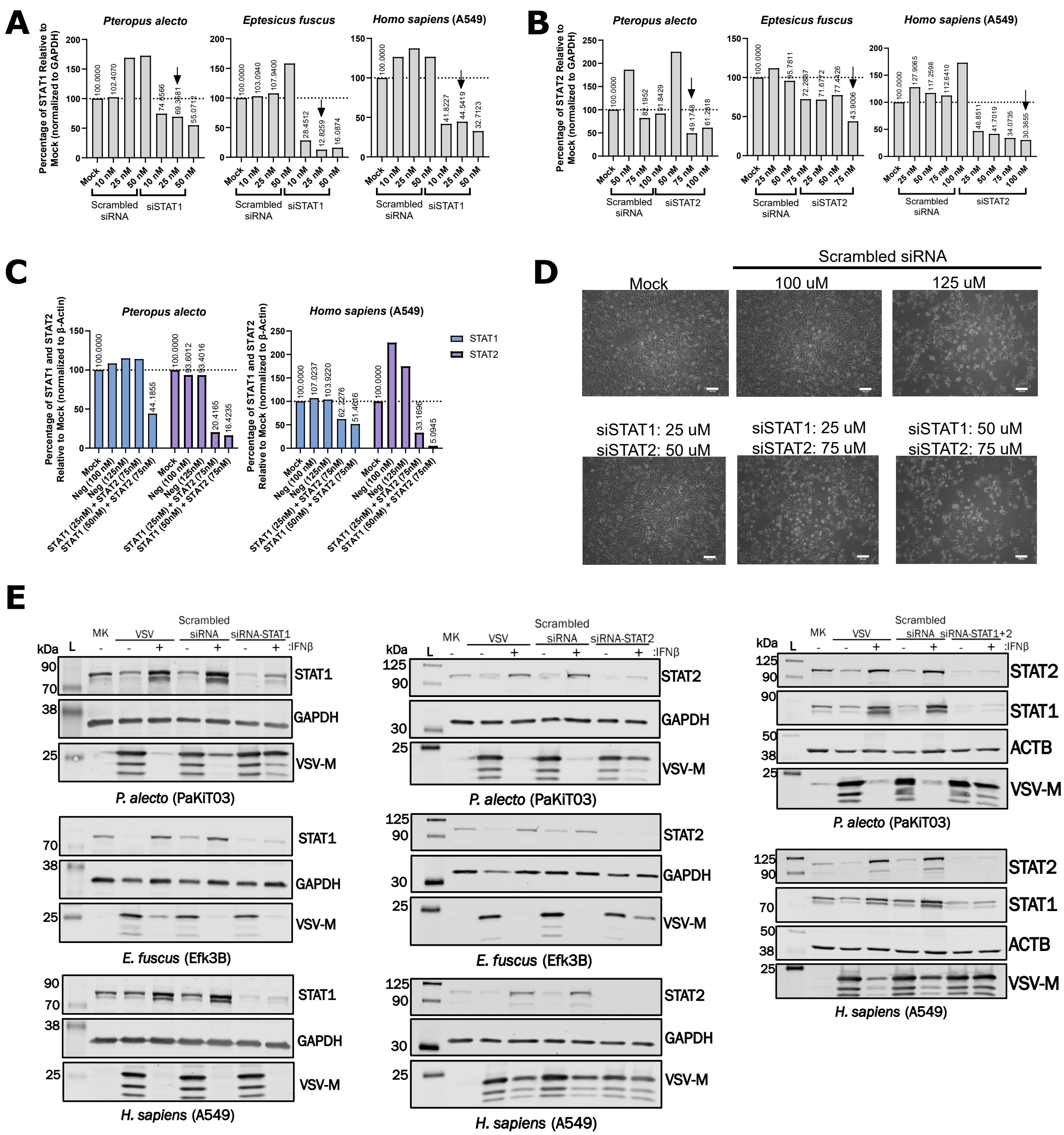
***Supplementary Figure 5 continues on next page**



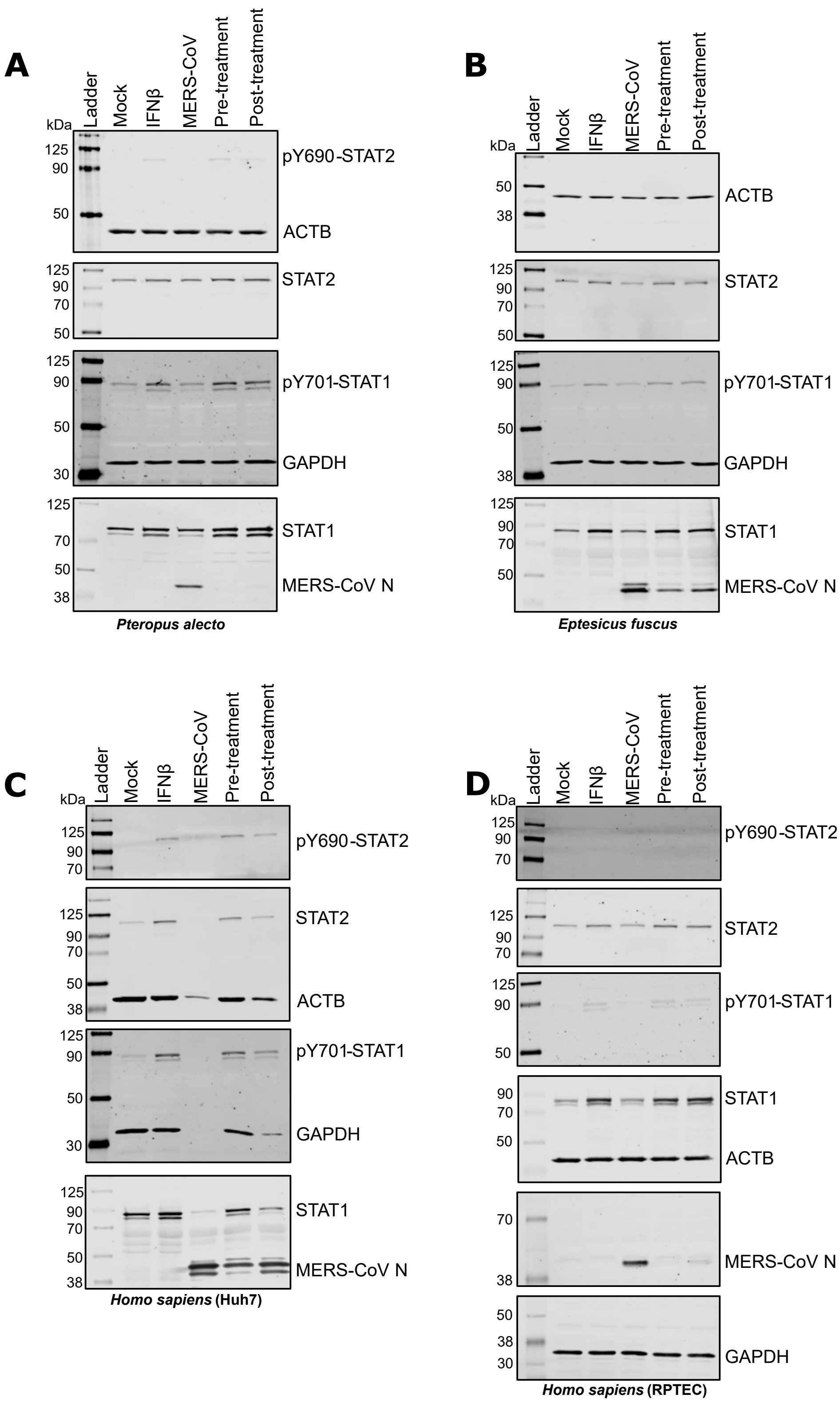
Supplementary Figure 5. Bat STAT2 multiple sequence alignment. Grey boxes indicate identical residues. Purple box highlights region surrounding the tyrosine phosphorylation site in humans. ClustalW alignment was generated using MacVector software. Human sequence was included for comparison.



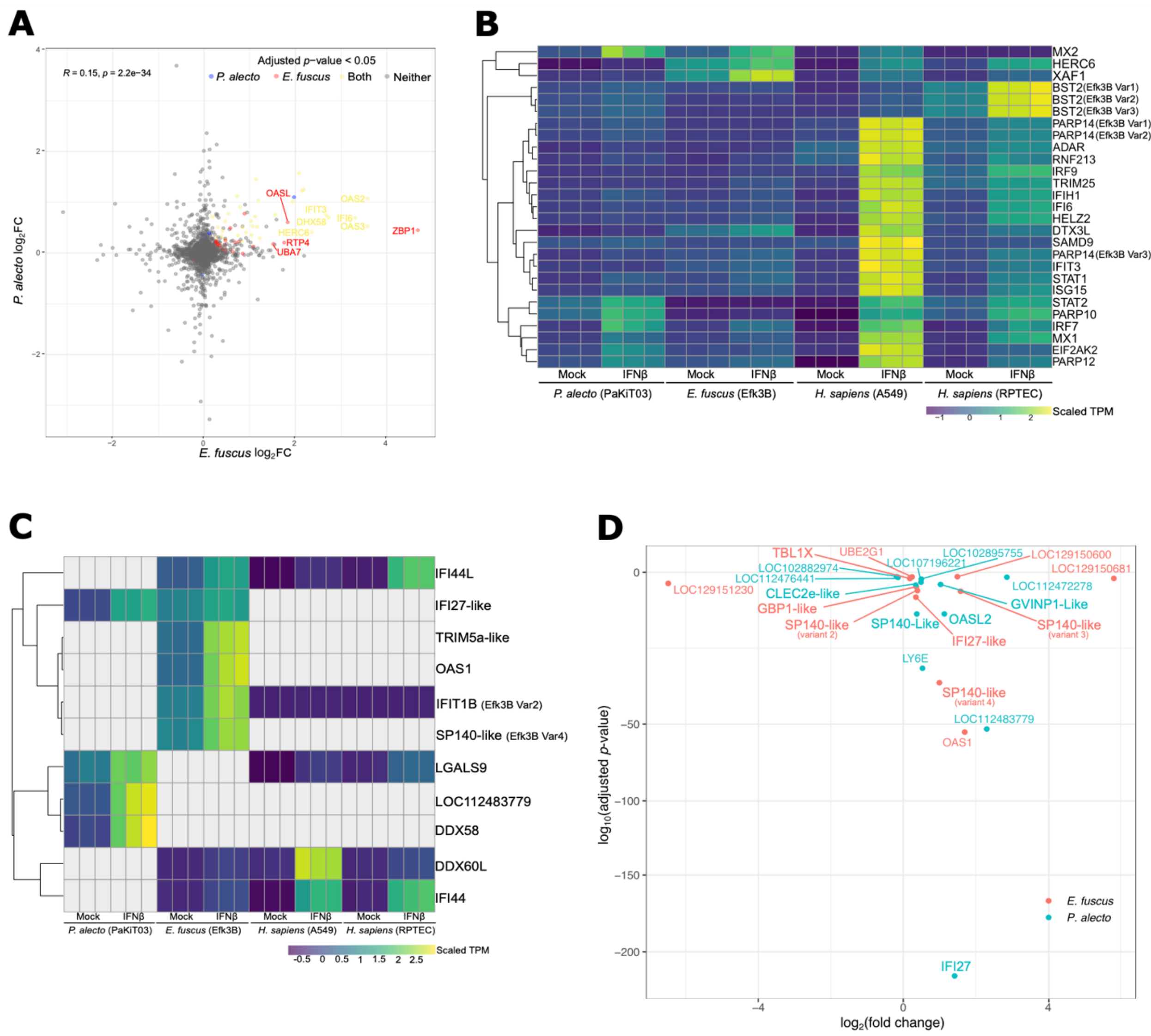
Supplementary Figure 6. Nuclear translocation of STAT1 and STAT2. *P. alecto* (PaKiT03), *E. fuscus* (Efk3B), and human A549 and RPTEC cells were treated for 5, 10, and 20 minutes with species matched IFNβ (10 U/mL) or with the vehicle control. Cells were stained for pY701-STAT1 (**A**), pY690-STAT2 or STAT2 (**B**), and DAPI. Nuclear translocation of stained proteins was visualized by confocal microscopy. Scale bars = 25 μM. Image is representative of 3 replicates.



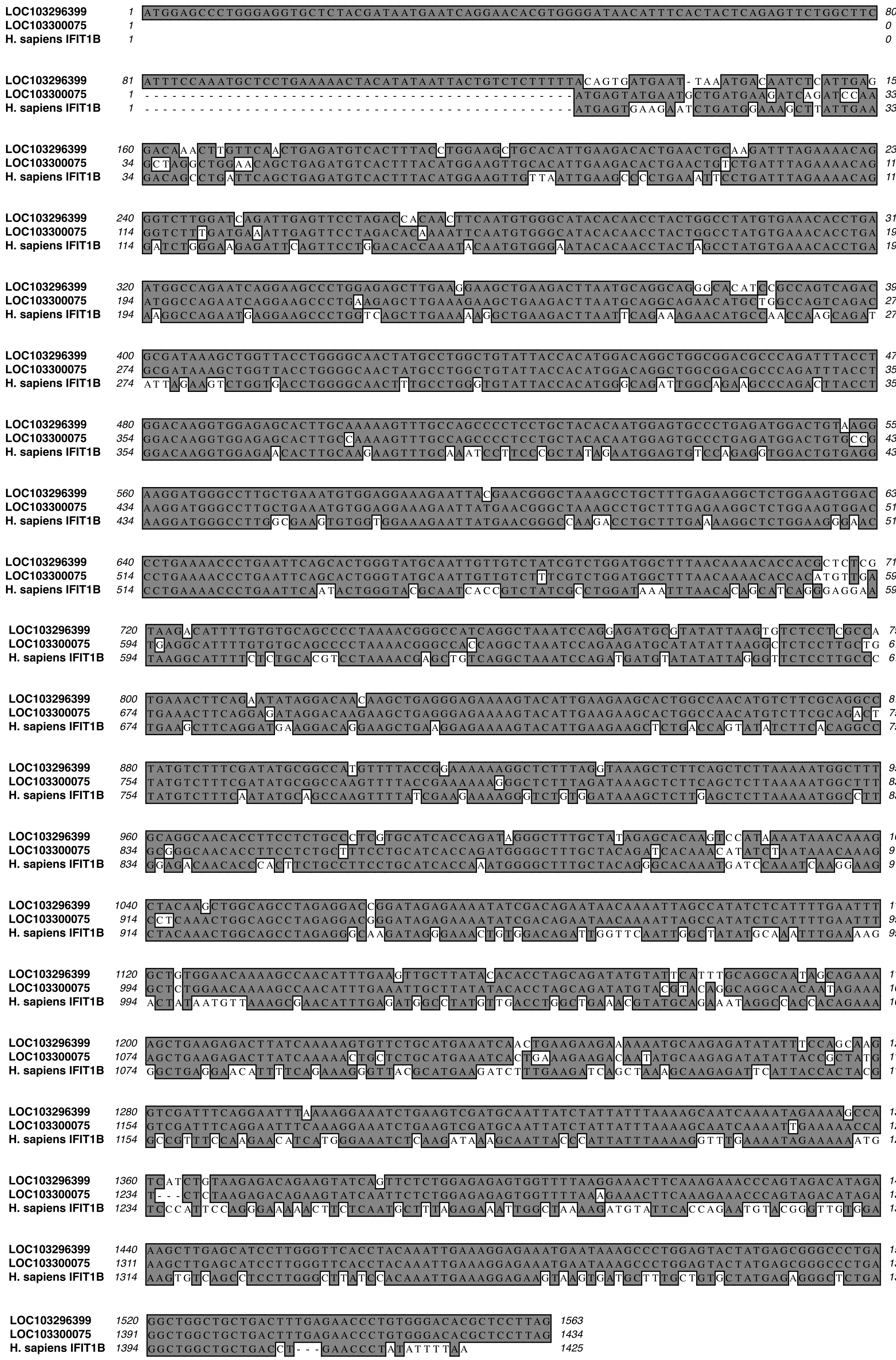
Supplementary Figure 7. siRNA-mediated knockdown of STAT1 and STAT2. *P. alecto* (PaKiT03), *E. fuscus* (Efk3B), and human A549 cells were transfected 4 hours post seeding with various concentrations of siRNA targeting STAT1 (**A**) and STAT2 (**B**) individually, and in combination to achieve a double knockdown (**C**). (**A-C**) Forty-eight hours later, protein lysates were harvested and STAT1, STAT2, GAPDH, and/or β-actin levels were quantified by immunoblotting. (**D**) Brightfield images of EfK3B cells transfected with siSTAT1 and siSTAT2 at concentrations where single knockdowns were achieved. Scale bars = 100 μM. (**E**) PaKiT03, Efk3B, and human A549 cells were transfected with siRNA-STAT1 (25 nM), siRNA-STAT2 (75 nM), a combination of both siRNAs (STAT1, 25nM; STAT2, 75nM), or with the scrambled siRNA (control) 4 hours post seeding cells. After 48 hours, cells were treated with species-matched IFNβ (10 U/mL) for 6 hours, followed by infection with VSV-GFP (MOI 0.1) for 16 hours. Cell lysates were harvested and assessed by immunoblotting for VSV-M, GAPDH, β-actin, STAT1 and/or STAT2. Immunoblots are representative of 3 replicates.



Supplementary Figure 8. Full immunoblots of IFNβ treatment and MERS-CoV infection. PaKiT03 (**A**), Efk3B (**B**), Huh7 (**C**), and RPTEC (**D**) cells were pretreated with species-matched IFNβ for 6 hours prior to infection with MERS-CoV or received IFNβ treatment following infection with MERS-CoV (MOI 0.1) for 48 hours. Protein lysates were harvested and probed for total STAT1 and STAT2, pY701-STAT1, pY690-STAT2, MERS-CoV nucleoprotein (N), GAPDH, and β-actin by immunoblotting.



Supplementary Figure 9. Host transcriptional response to IFNβ stimulation. (**A**) Function enrichment plot for differentially expressed genes (DEGs) in PaKiT03 and EfK3B cells. (**B**) Heatmap indicating the expression levels of the top 27 bat DEGs involved in the IFNβ response in PaKiT03, Efk3B, A549, and RPTEC cells, ranked by *p*-value. *E. fuscus* transcript variants are indicated in brackets (i.e. var1) and are plotted against the transcript levels of a single variant that was detected in PaKiT03, A549, and RPTEC cells. (**C**) Heatmap indicating the expression levels of genes that do not exist in all four cell lines but were significantly differentially expressed in at least one cell line. 'LOC' symbol represents genes that do not have a published symbol and orthologs that have not yet been determined or named. (**D**) Top upregulated genes in PaKiT03 and EfK3B cells, orthologues for which were not clearly identifiable within the human genome or orthologous genes were not upregulated upon IFNβ stimulation of human cells. Paralogs are indicated (i.e. var1). See **Supplementary Table S7** for the representative LOC symbol.


LOC103296399
LOC103300075
H. sapiens IFIT1B

Supplementary Figure 10. Gene alignment of *E. fuscus* and human *IFIT1B*. Grey boxes indicate identical residues. ClustalW alignment was generated using MacVector software. Two uncharacterized genes (LOC103296399 and LOC103300075) were identified to be orthologous to human *IFITB*.

```
P. alecto IFIT1       1 ATGAGTAAGAATGCTGATGAAGATCAGGTCAAAGATAGGCTGGAGCAGCTGAGATGTCACTTTACATGGAAGTTGCTAAT 80
H. sapiens IFIT1      1 ATGAGTACAAATGGTGATGATCATCAGGTCAAGGATAGTCTGGAGCAATTGAGATGTCACTTTACATGGGAGTTATCCAT 80

P. alecto IFIT1     81 CGAAGACACTGAAATTACCGATTTAGAAAACAGGGTTTTCGATGAGATTGAATTCCTAGACATCAAATACAACGTGGGAA 160
H. sapiens IFIT1    81 TGATGACGATGAAATGCCTGATTTAGAAAACAGAGTCTTGGATCAGATTGAATTCCTAGACACCAAATACAGTGTGGGAA 160

P. alecto IFIT1    161 TTCACAACCTACTGGCCTATATAAAGCACCTGAAAGGCCAAAATAAGGAAGCCCTGGAGAGCTTGAAAGAAGCTGAAACC 240
H. sapiens IFIT1   161 TACACAACCTACTAGCCTATGTGAAACACCTGAAAGGCCAGAATGAGGAAGCCCTGAAGAGCTTAAAAGAAGCTGAAAAC 240

P. alecto IFIT1    241 TTAATCCAGCAAGAACGTACCAACCAATCGAACACAAAAAGGCTGGTTACCTGGGGCAACTATGCCTGGCTGTATTACCA 320
H. sapiens IFIT1   241 TTAATGCAGGAAGAACATGACAACCAAGCAAATGTGAGGAGTCTGGTGACCTGGGGCAACTTTGCCTGGATGTATTACCA 320

P. alecto IFIT1    321 CATGGGCAGCCTGGGAGAAGTCCAGATTTATCTGGACAAAGTGGAAAACACTTGCAAGAAGCTTGCCAGTCCCTCCTGCT 400
H. sapiens IFIT1   321 CATGGGCAGACTGGCAGAAGCCCAGACTTACCTGGACAAGGTGGAGAACATTTGCAAGAAGCTTTCAAATCCCTTCCGCT 400

P. alecto IFIT1    401 GTAGACTGGAGTGTCCTGAGATGGACTGTGAGGAAGGATGGGCCTTGCTGAAATGTGGAGGAAAGAATTACGAACGAGCC 480
H. sapiens IFIT1   401 ATAGAATGGAGTGTCCAGAAATAGACTGTGAGGAAGGATGGGCCTTGCTGAAGTGTGGAGGAAAAAATTATGAACGGGCC 480

P. alecto IFIT1    481 AAGGCCTGCTTTGAAAAGGCTCTGGAAGCGGACCCTGAAAACCCTGAATTCAACACTGGGTATGCGATCACCATGTTTCG 560
H. sapiens IFIT1   481 AAGGCCTGCTTTGAAAAGGTGCTTGAAGTGGACCCTGAAAACCCTGAATCCAGCGCTGGGTATGCGATCTCTGCCTATCG 560

P. alecto IFIT1    561 TCTGGATAGCTTTCACAAAGAAACGCAGTTTAAAGAGGCATTTTCTCTACACCCCCTAAAACAGGCCATCAGGCTAAATC 640
H. sapiens IFIT1   561 CCTGGATGGCTTTAAATTAGCCACAAAAAATCACAAGCCATTTTCTTTGCTTCCCCTAAGGCAGGCTGTCCGCTTAAATC 640

P. alecto IFIT1    641 CGGAAGATGCATATATTAAGGCTCTCCTTGGCCTGAAGCTTCAGGATGTAGGAGAACAAGCTGAGGGACAAAAGTACATT 720
H. sapiens IFIT1   641 CAGACAATGGATATATTAAGGTTCTCCTTGCCCTGAAGCTTCAGGATGAAGGACAGGAAGCTGAAGGAGAAAAGTACATT 720

P. alecto IFIT1    721 GAAGAAGCACTGACCAAAATGTCGTCGCAGACCTATGTCTTTCGCTATGCAGCCATGTTTTACCGAAGAAAAGGCTCTGT 800
H. sapiens IFIT1   721 GAAGAAGCTCTAGCCAACATGTCCTCACAGACCTATGTCTTTCGATATGCAGCCAAGTTTTACCGAAGAAAAGGCTCTGT 800

P. alecto IFIT1    801 GGATAAAGCGCTTCAGCTCTTAAAAACTGCTTTGCAGGCAACACCCTCTTCTGCCTTCCTTCATCACCAGATAGGGCTTT 880
H. sapiens IFIT1   801 GGATAAAGCTCTTGAGTTATTAAAAAAGGCCTTGCAGGAAACACCCACTTCTGTCTTACTGCATCACCAGATAGGGCTTT 880

P. alecto IFIT1    881 GCTACAGATCACAAGTTCGTCAAATAAAGAAAACTGCAAAATGGCAGCCTAGAGGACAGGATAGAGAAAATGTCAATAGA 960
H. sapiens IFIT1   881 GCTACAAGGCACAAATGATCCAAATCAAGGAGGCTACAAAAGGGCAGCCTAGAGGGCAGAACAGAGAAAAGCTAGACAAA 960

P. alecto IFIT1    961 ATAACAAGATTAGCCATATTTCATTTTGAATTCACACTGAAACAAAAGCCCACATTTGAGATAGCTTACAAACACCTGGC 1040
H. sapiens IFIT1   961 ATGATAAGATCAGCCATATTTCATTTTGAATCTGCAGTGGAAAAAAAGCCCACATTTGAGGTGGCTCATCTAGACCTGGC 1040

P. alecto IFIT1   1041 AGATATGTACATAGAAGCAGGCAACTATGAAAAAGCTGAAGACACTTATCAAAAAGTGTTACGCCTGAAAAAACTTGACA 1120
H. sapiens IFIT1  1041 AAGAATGTATATAGAAGCAGGCAATCACAGAAAAGCTGAAGAGAATTTTCAAAAATTGTTATGCATGAAACCAGTGGTAG 1120

P. alecto IFIT1   1121 AAGAAGTTCTGCAAGAAATATATTTCTACTATGGCCAATTCCAGGAATTTCAAAGGAAATCTGAAATCGATGCAATTATC 1200
H. sapiens IFIT1  1121 AAGAAACAATGCAAGACATACATTTCCACTATGGTCGGTTTCAGGAATTTCAAAAGAAATCTGACGTCAATGCAATTATC 1200

P. alecto IFIT1   1201 TATTATTTAAAAGCATAAAAAATAGAAAACCAGTCATATTCAAGAGATAAAAGTATCAGTTCTTTGGAGAAATTGGTTTT 1280
H. sapiens IFIT1  1201 CATTATTTAAAAGCTTAAAAAATAGAACAGGCATCATTAACAAGGGATAAAAGTATCAATTCTTTGAAGAAATTGGTTTT 1280

P. alecto IFIT1   1281 AAGGAAACTTCGGAGAAATGGATCAGACATAGAAAGCTTGAGCATCCTCGGGTTCATCTACAAATTGAAAGGAGAAATGA 1360
H. sapiens IFIT1  1281 AAGGAAACTTCGGAGAAAGGCATTAGATCTGGAAAGCTTGAGCCTCCTTGGGTTCGTCTACAAATTGGAAGGAAATATGA 1360

P. alecto IFIT1   1361 ATGAAGCCTTGGAGTATTACGAGCAGGCCCTAAAGCTGGCTGCTGACTTTGAGAGCTTTGTGTGA 1425
H. sapiens IFIT1  1361 ATGAAGCCCTGGAGTACTATGAGCGGGCCCTGAGACTGGCTGCTGACTTTGAGAACTCTGTGAGACAAGGTCCTTAG 1437
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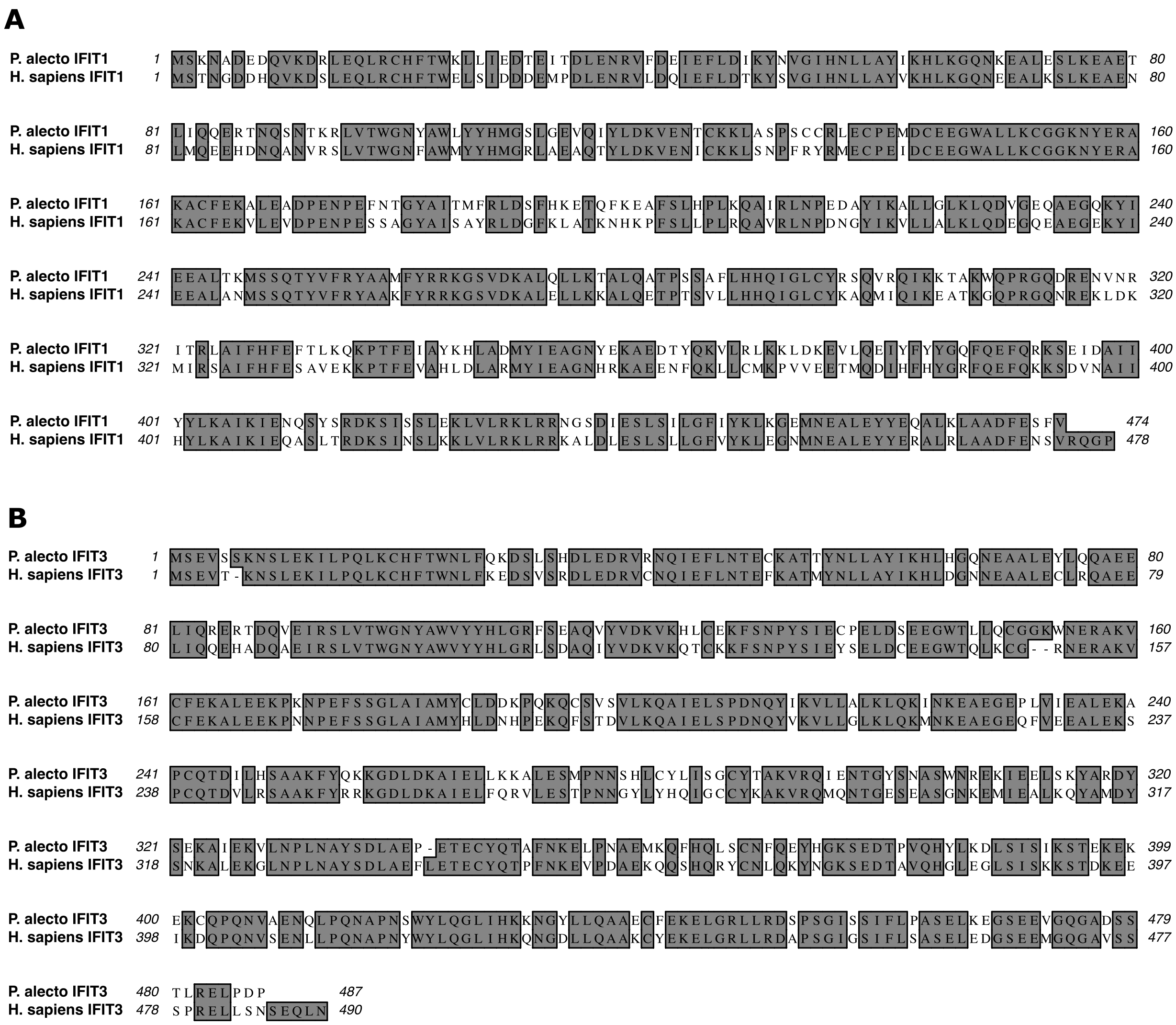
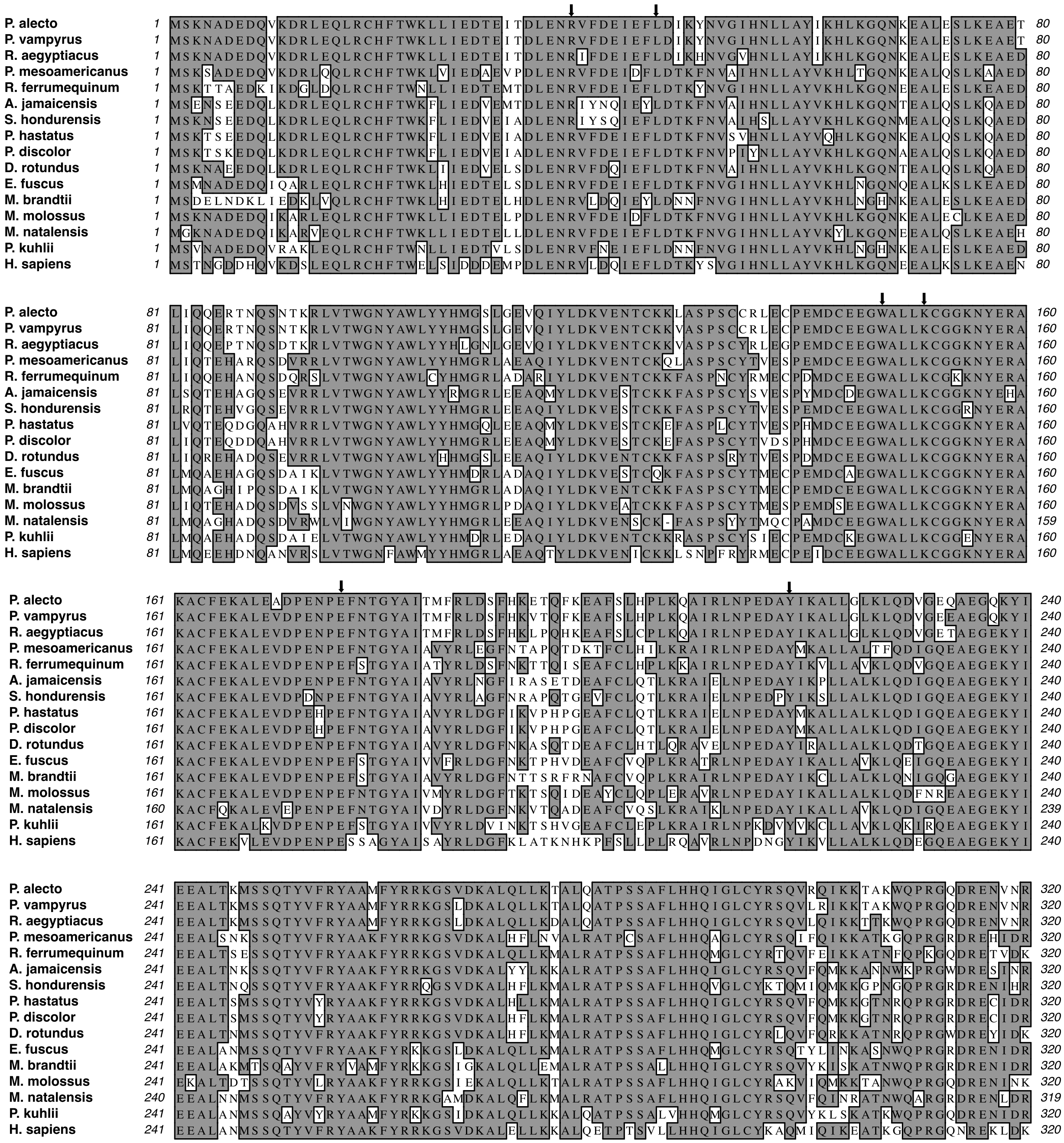
Supplementary Figure 11. Gene alignment of *P. alecto* and human *IFIT1*. Grey boxes indicate identical residues. ClustalW alignment was generated using MacVector software.



Supplementary Figure 12. Amino acid sequence comparison of human and *P. alecto* IFIT1 and IFIT3. Grey boxes indicate identical residues. ClustalW alignment was generated using MacVector software for IFIT1 (**A**) and IFIT3 (**B**).



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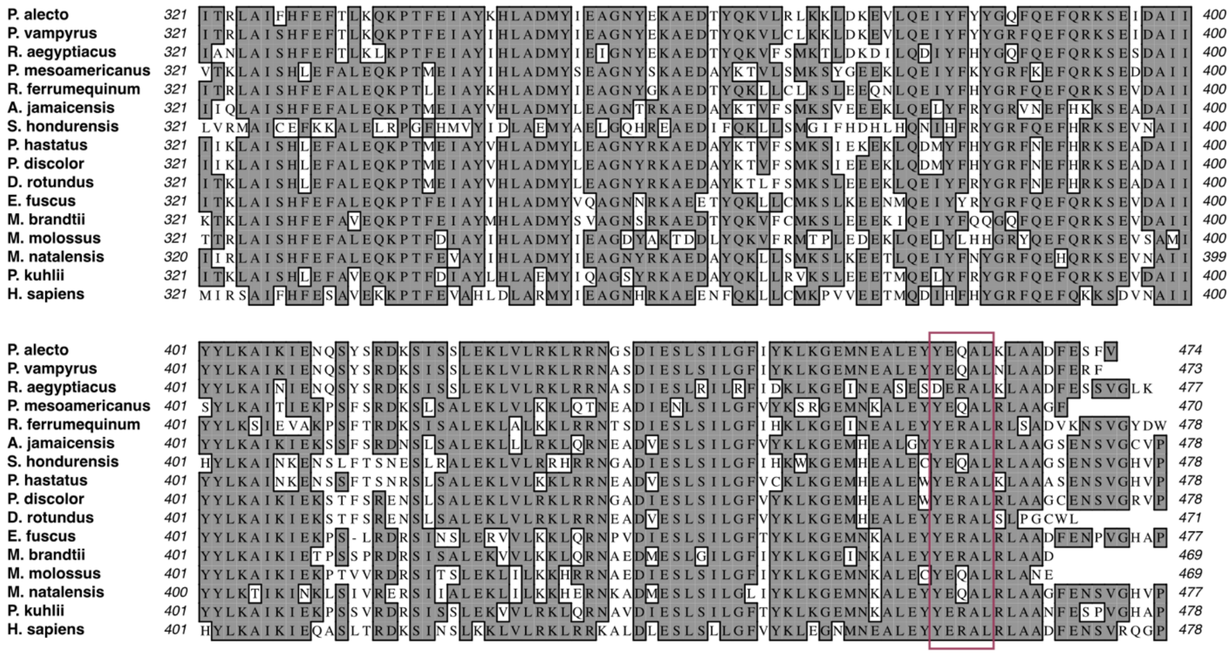
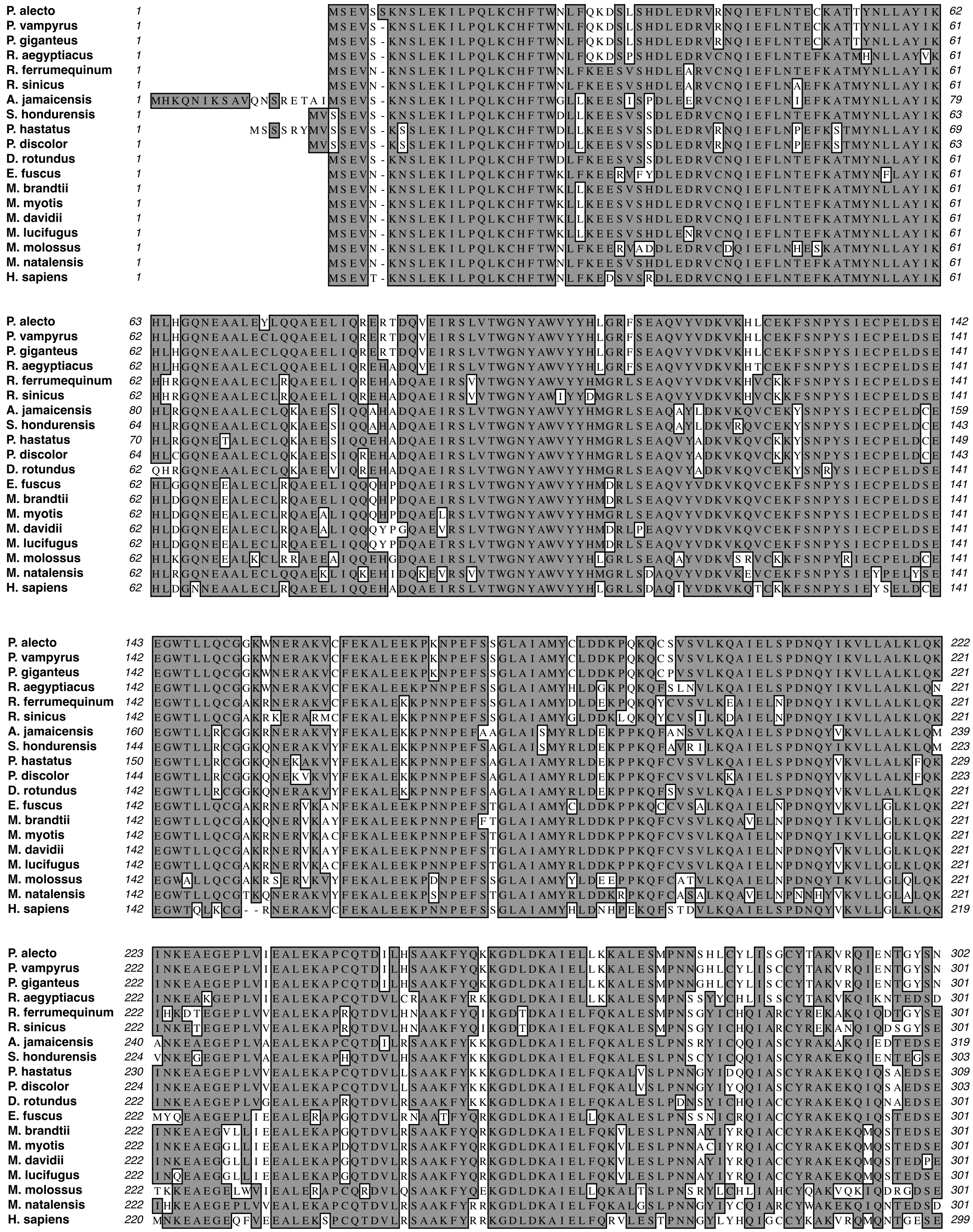
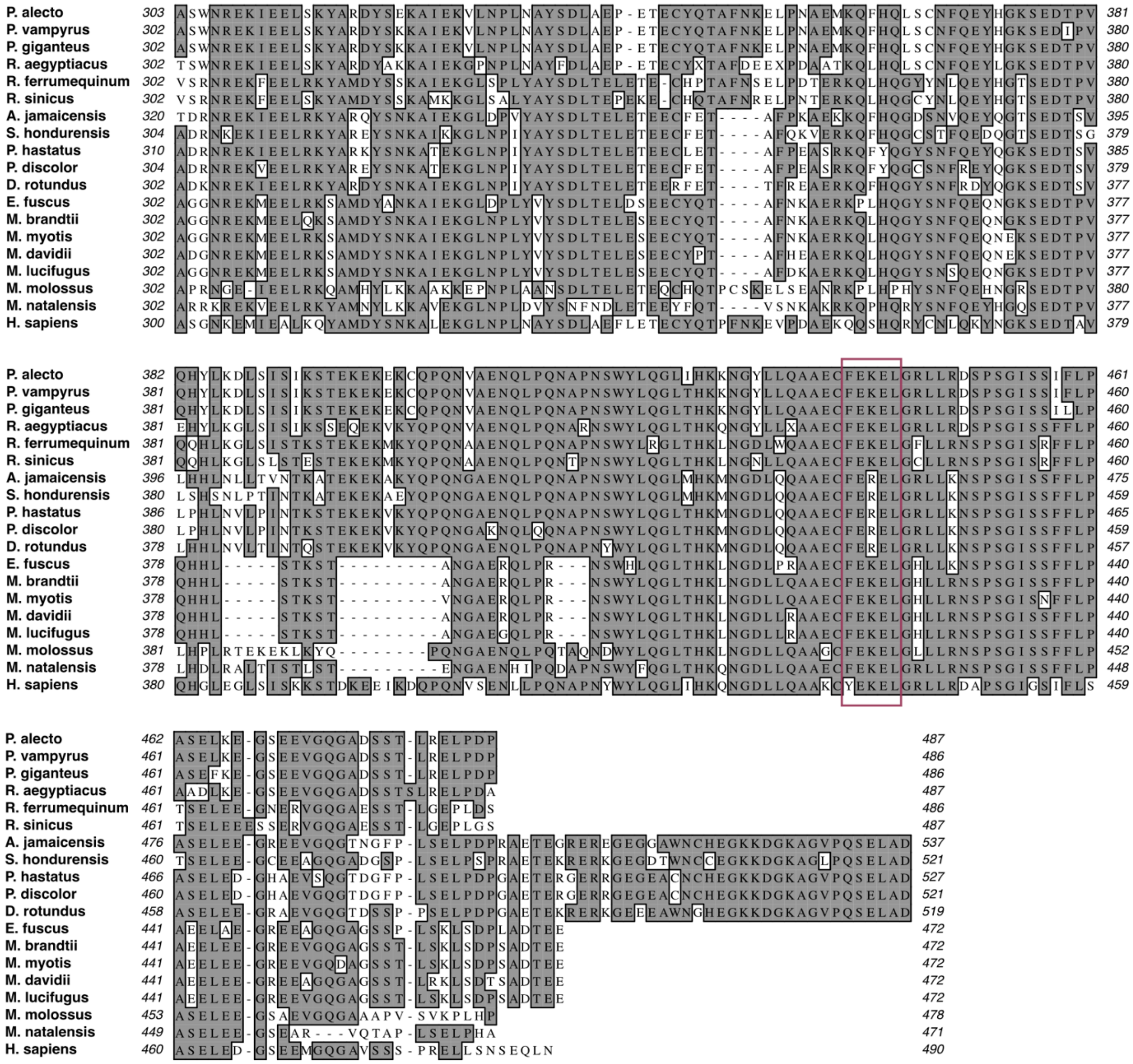


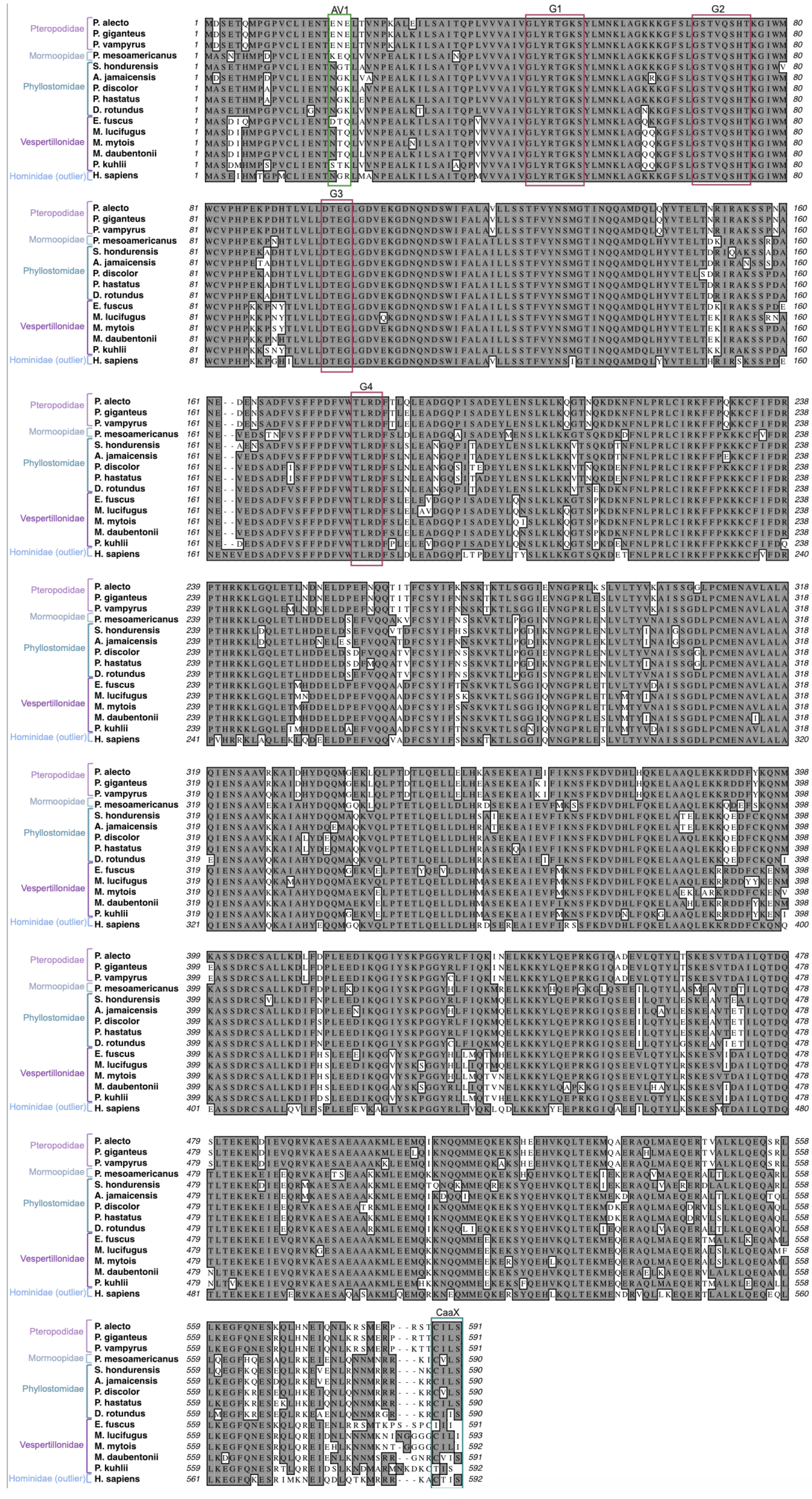
Supplementary Figure 13. Multiple protein sequence alignment for bat IFIT1. Grey boxes indicate identical residues. ClustalW alignment was generated using MacVector software. Human sequence was included as a comparator. Black arrows indicate residues in human IFIT1 that are known to play a role in mRNA binding (R38, L46, W147, K151, E176 and Y218). Purple box highlights IFIT3 binding motif.



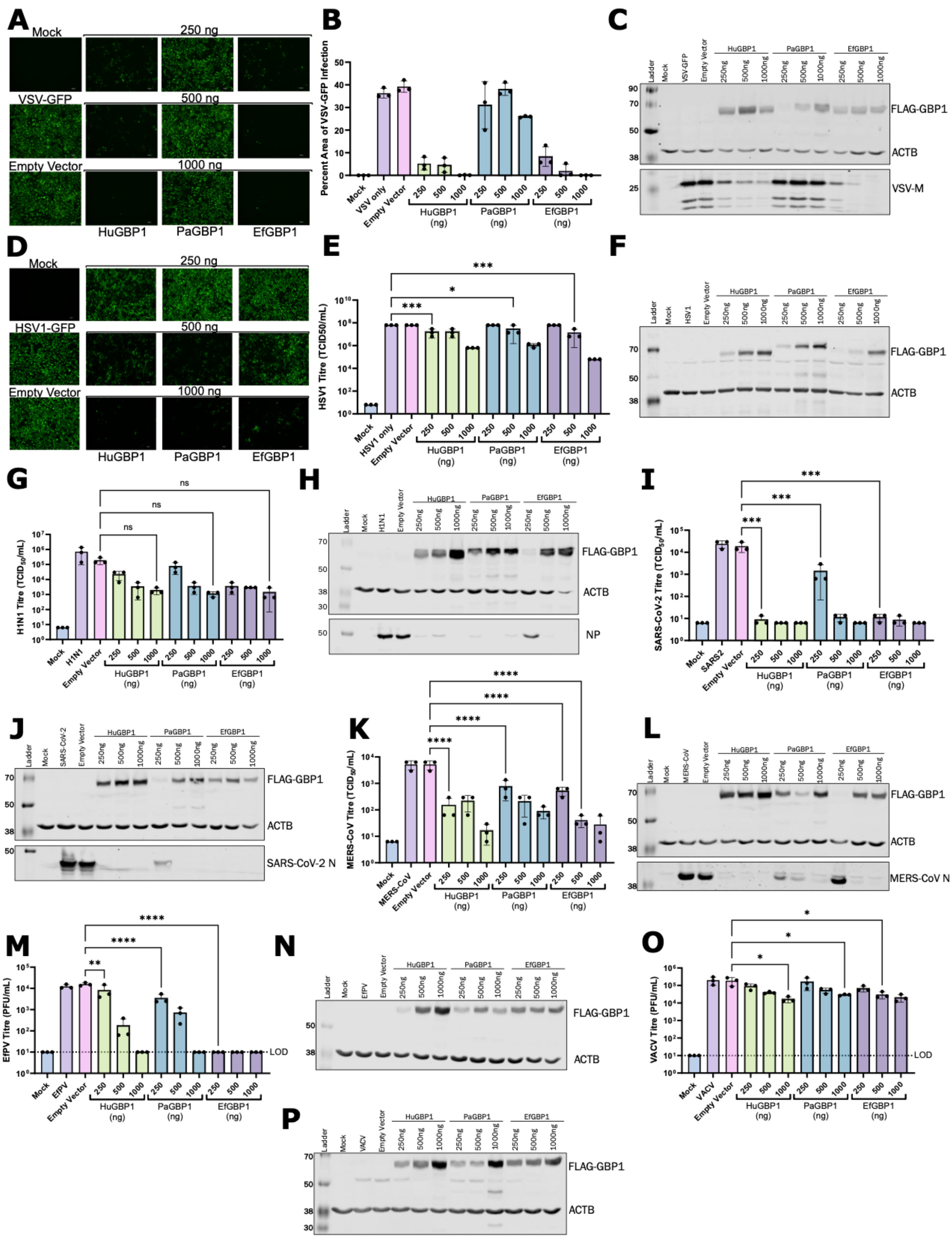
***Supplementary Figure 14 continues on next page**



Supplementary Figure 14. Multiple protein sequence alignment for bat IFIT3. Grey boxes indicate identical residues. ClustalW alignment was generated using MacVector software. Human sequence was included as a comparator. Purple box highlights IFIT1 binding motif.

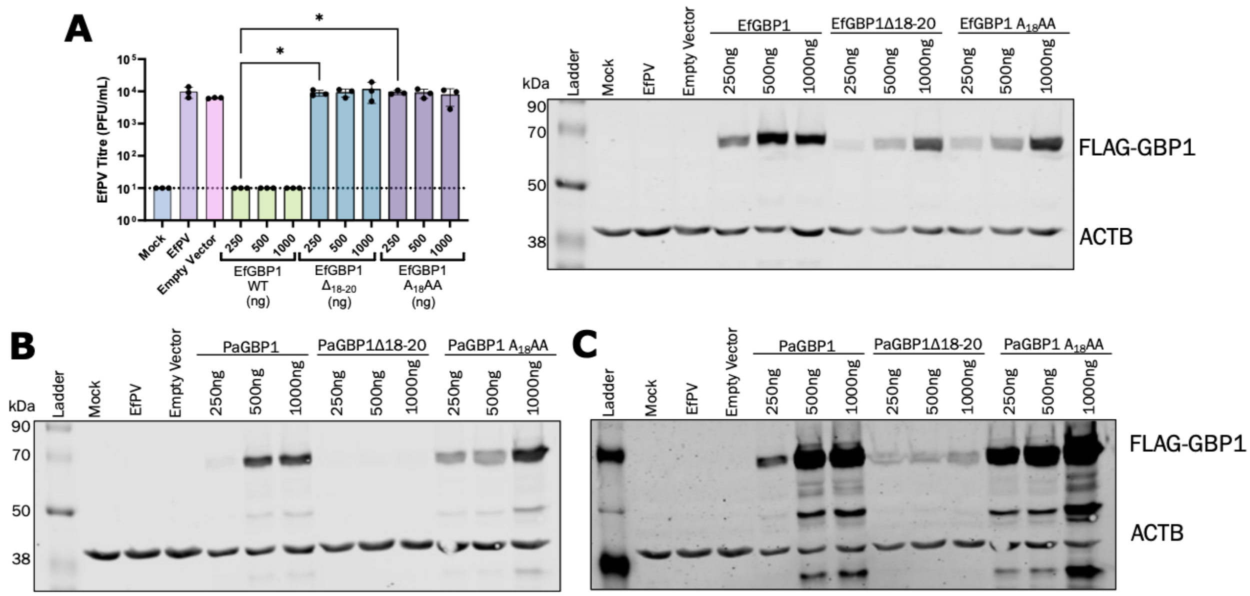

AV1
G1
G2
G3
G4
CaaX
Pteropodidae
Mormoopidae
Phyllostomidae
Vespertilionidae
Hominidae (outlier)
P. alecto
P. giganteus
P. vampyrus
P. mesoamericanus
S. hondurensis
A. jamaicensis
P. discolor
P. hastatus
D. rotundus
E. fuscus
M. lucifugus
M. mytois
M. daubentonii
P. kuhlii
H. sapiens

Supplementary Figure 15. **Multiple protein sequence alignment for bat GBP1.** Grey boxes indicate identical residues. The four conserved motifs withing the GTP-binding domain (G1-G4) and the carboxy-terminal isoprenylation (CaaX) motif are marked in purple and teal boxes, respectively. Newly identified motif that plays a role in the antiviral activity of EfGBP1 against EfPV (AV1) is marked in green. ClustalW alignment was generated using MacVector software. Human sequence was included as a comparator.



A
Mock
250 ng
VSV-GFP
500 ng
Empty Vector
1000 ng
HuGBP1
PaGBP1
EfGBP1
B
Percent Area of VSV-GFP Infection
Mock
VSV only
Empty Vector
250
500
1000
HuGBP1 (ng)
PaGBP1 (ng)
EfGBP1 (ng)
C
Ladder
Mock
VSV-GFP
Empty Vector
HuGBP1
PaGBP1
EfGBP1
250ng
500ng
1000ng
FLAG-GBP1
ACTB
VSV-M
D
Mock
250 ng
HSV1-GFP
500 ng
Empty Vector
1000 ng
HuGBP1
PaGBP1
EfGBP1
E
HSV1 Titre (TCID50/mL)
Mock
HSV1 only
Empty Vector
F
HSV1
FLAG-GBP1
ACTB
G
H1N1 Titre (TCID50/mL)
ns
H1N1
H
NP
I
SARS-CoV-2 Titre (TCID50/mL)
SARS2
J
SARS-CoV-2
SARS-CoV-2 N
K
MERS-CoV Titre (TCID50/mL)
MERS-CoV
L
MERS-CoV N
M
EfPV Titre (PFU/mL)
EfPV
LOD
N
O
VACV Titre (PFU/mL)
VACV
P

Supplementary Figure 16. Antiviral activity of GBP1. A549-ACE2 cells were transfected with 250 to 1000ng of HuGBP1, PaGBP1, or EfGBP1 for 24 hours, followed by infection with VSV-GFP (MOI 0.01) (**A-C**), HSV1- GFP (MOI 0.01) (**D-F**), H1N1-PR8 (MOI 0.01) (**G-H**), SARS-CoV-2 (MOI 0.01) (**I-J**), MERS-CoV (MOI 0.1) (**K-L**), VACV (MOI 0.01) (**M-N**), or EfPV (MOI 0.01) (**O-P**). Immunofluorescent microscopy was performed for VSV-GFP to quantify virus replication. Supernatant for all other viruses was titered by $TCID_{50}$ or plaque assay (n=3; One-way ANOVA, Tukey's multiple comparisons test). Immunoblotting was performed to confirm FLAG-GBP1 expression. β-actin was used as loading control. VSV-GFP, H1N1, SARS-CoV-2, and MERS-CoV infection was further validated by probing for virus-specific proteins in immunoblots.



Supplementary Figure 17. Mutagenesis of GBP1. (**A**) A549-ACE2 cells were transfected with 250 to 1000ng of EfGBP1 wildtype (WT), deletion (Δ_{18-20}), or substitution ($A_{18}AA$) variant for 24 hours, followed by infection with EfPV (MOI 0.01) for 48 hours. Supernatant was titered by plaque assay (n=3; One-way ANOVA, Tukey's multiple comparisons test). Cell lysates were probed for FLAG-GBP1 and ACTB. All samples in Figures 4F, 4G and S17 were processed together and were derived from three independent biological replicates. (**B**) A549-ACE2 cells were transfected with 250 to 1000ng of WT, Δ_{18-20}, or $A_{18}AA$ for PaGBP1 for 24 hours, followed by infection with EfPV (MOI 0.01) for 48 hours. Cell lysates were probed for FLAG-GBP1 and ACTB. (**C**) Overexposed image of (B) to demonstrate reduced expression of PaGBP1(Δ_{18-20}).