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Supplementary Materials for

JAK inhibition reduces SARS-CoV-2 liver infectivity and modulates inflammatory responses to reduce morbidity and mortality

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This PDF file includes:

Supplementary Figure 1

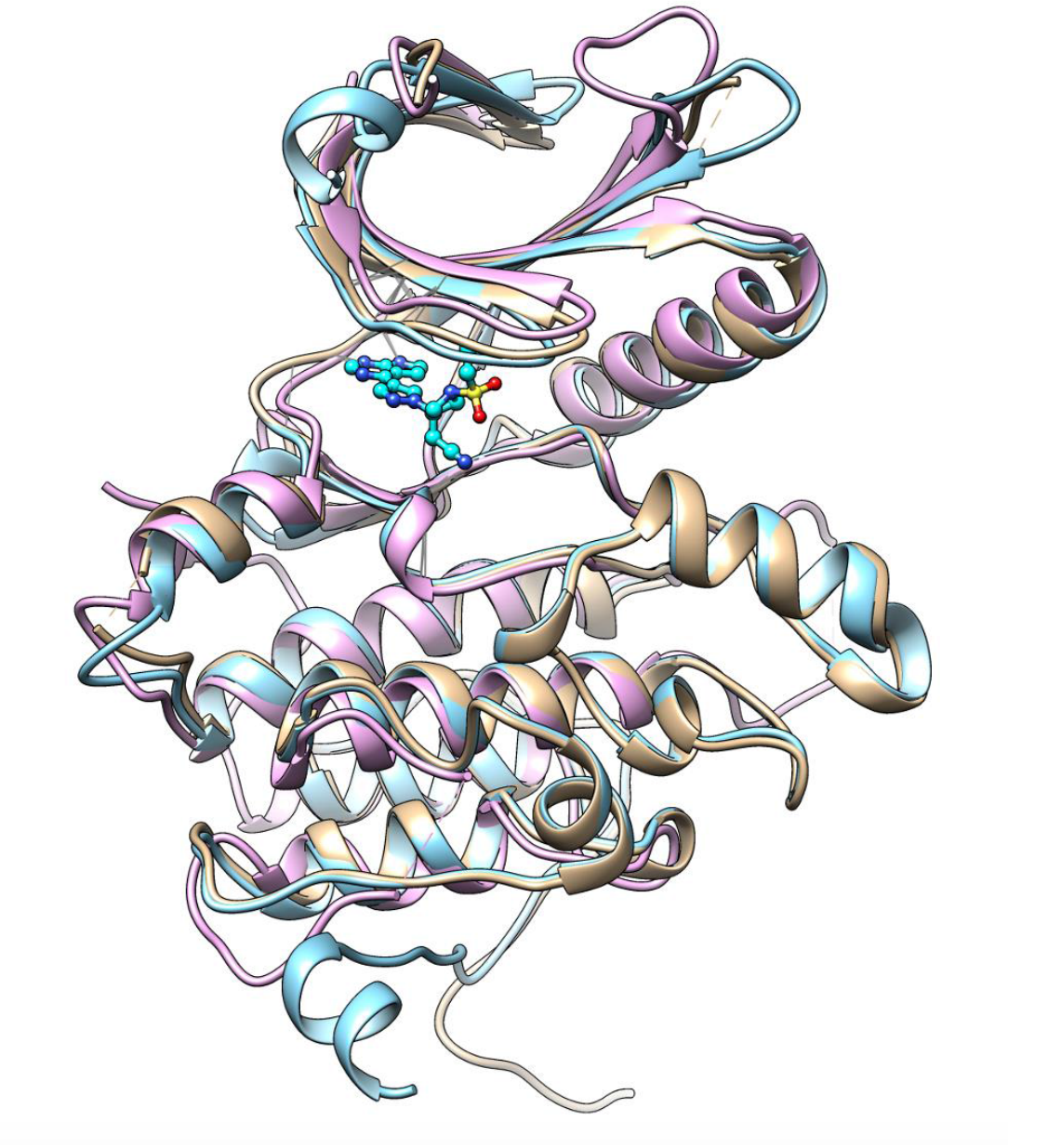

Figure S1. Ribbon diagram overlay of the catalytic domains of BIKE (tan; PDB ID 4w9x), AAK1 (light blue; PDB ID 4wsq), and GAK (light purple; PDB ID 4y8d). This shows baricitinib bound to BIKE (ball-and-stick ligand atom color coding: C-cyan, N-blue, O-red, S-yellow). Pairwise sequence identities and root-mean-square-deviations on alpha Carbon atomic coordinates (r.m.s.d.s) were as follows: BIKE vs. AAK1: sequence identity=75%, r.m.s.d. =1.0Å; BIKE vs. GAK: sequence identity=38%, r.m.s.d.=1.6Å).

Supplementary Figure 2

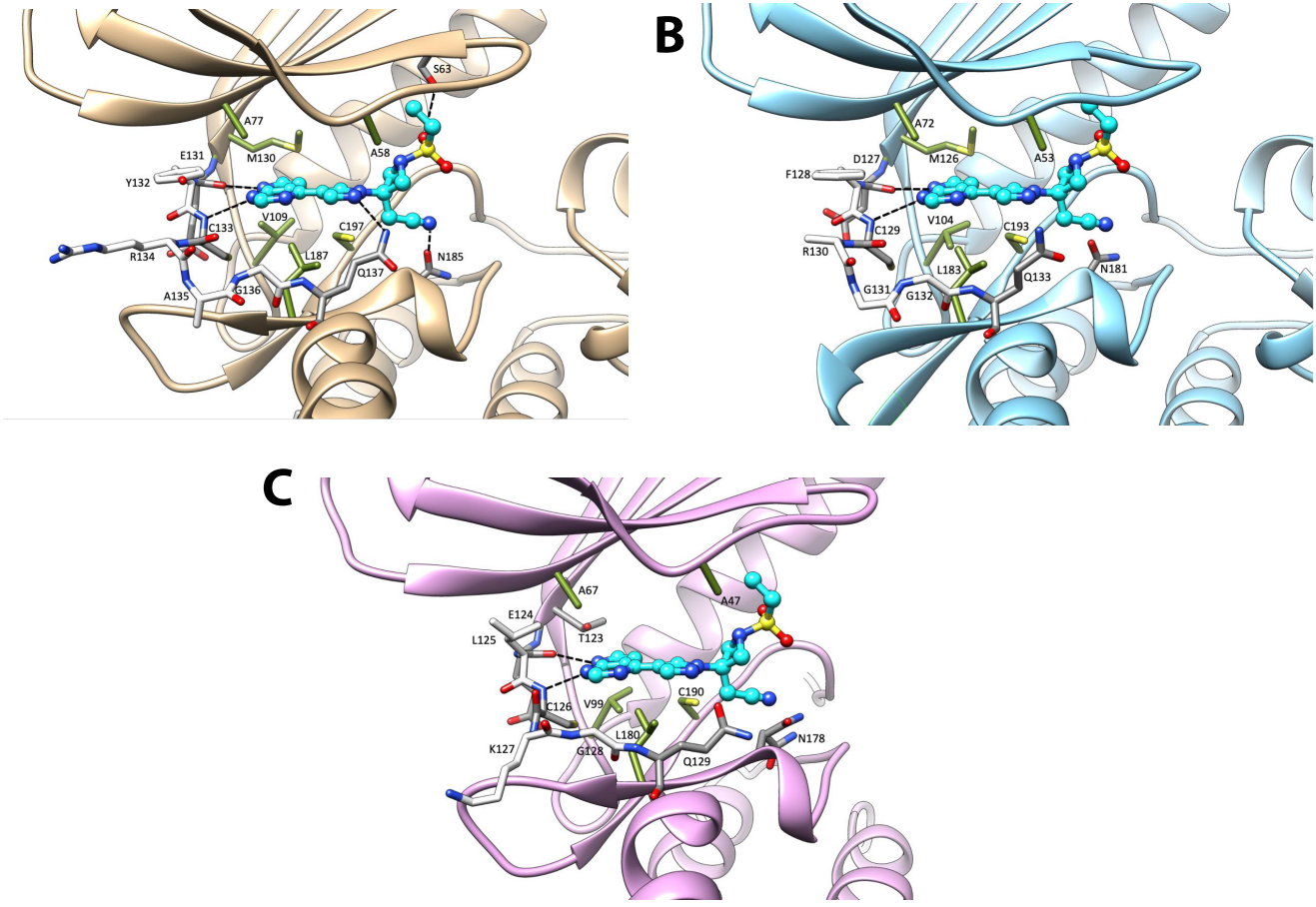

Figure S2. NAK family member enzyme active sites showing experimentally-determined or modeled binding of baricitinib. The same color code as in Figure S1 is used, with labeled stick figure residues (protein atom color coding: C-white, grey, or green; N-blue; O-red; S-yellow) that make direct hydrogen bonds (C- grey; dashed lines) or van der Waals interactions (C-green; <~4Å) with the drug. **A**, BIKE with bound baricitinib. **B**, AAK1 and **C**, GAK, both with superposition docked baricitinib showing predicted hydrogen bonds with the hinge region. The predicted modes of baricitinib binding to AAK1 and GAK are devoid of major steric clashes and consistent with reported Kd values.

Supplementary Figure 3

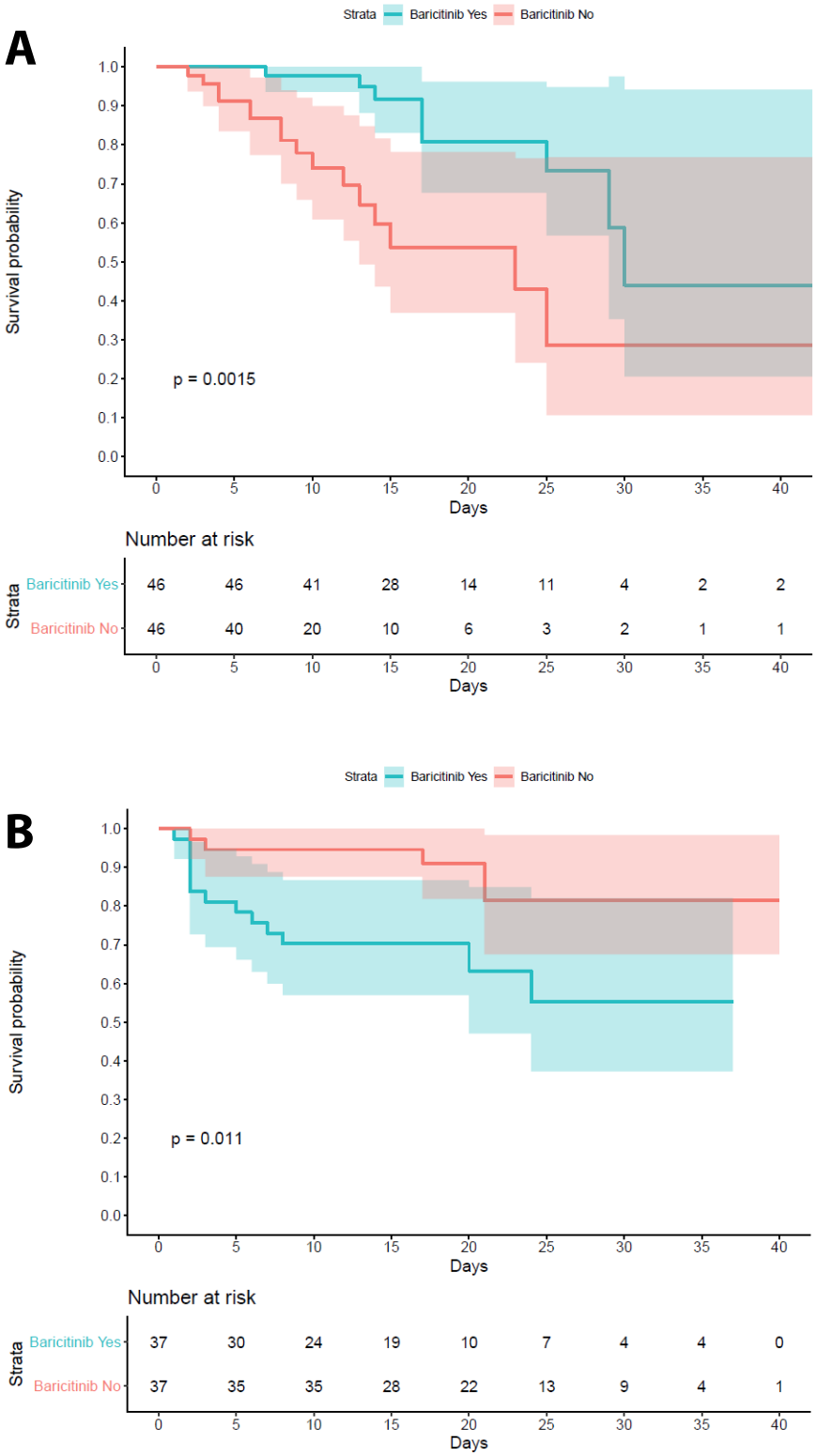

Figure S3. Kaplan-Meier analysis. Unmerged data from the Albacete Hospital (**A**) and University of Pisa (**B**) cohorts with 453 participants (92 on baricitinib and 361 in the control group).

Supplementary Figure 4

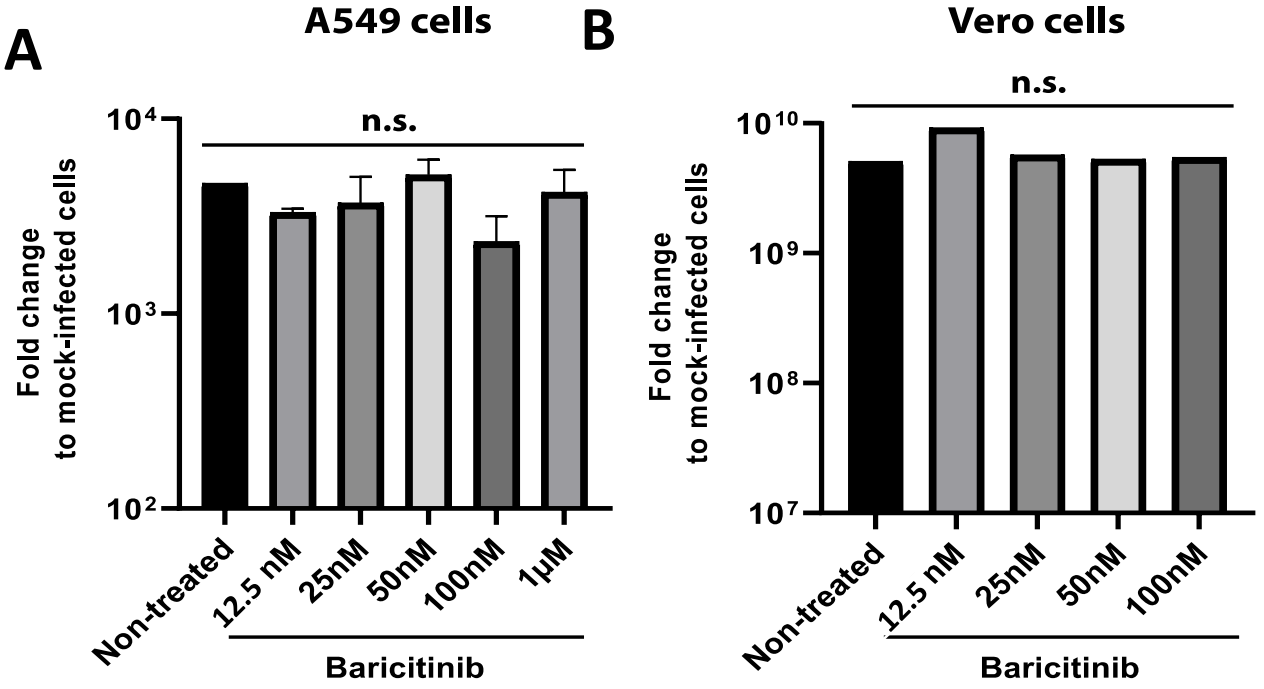

Figure S4. Commonly used cell models of viral infection are not suitable to study baricitinib effects. Baricitinib did not have antiviral effects in A549 human lung epithelial cells (**A**), and monkey kidney cells (**B**).

Table S1. Computation modelling to assess mutational impact on kinase binding. Results of superposition docking of baricitinib with observed point mutant 3D structures of BIKE, AAK1, and GAK.

Protein	Wild-type residue	Observed/predicted interaction between drug and protein	Mutation	Observed mutation frequency	Likelihood of protein loss of function due to impaired structure stability	Likelihood of steric clash between baricitinib and mutant protein
BIKE	Ala58	van der Waals	Ala58→Asp	2.4%	None	Low
		van der Waals	Ala58→Val	<0.1%	None	Low
	Glu131	Backbone H-bond	Glu131→Gln	<0.1%	None	None
		Backbone H-bond	Glu131→Lys	<0.1%	None	None
	Arg134	None	Arg134→Glu	<0.1%	None	None
		None	Arg134→Leu	<0.1%	None	None
	Ala135	None	Ala135→Thr	<0.1%	None	None
AAK1	Ala72	van der Waals	Ala72→Thr	<0.1%	None	Low
	Gly132	None	Gly132→Val	<0.1%	None	None
GAK	Glu124→Val	Backbone H-bond	Glu124→Val	<0.1%	None	None
	Gly128→Glu	None	Gly128→Glu	<0.1%	None	None
	Gln129→Arg	None	Gln129→Arg	<0.1%	None	None

Table S2. Baseline demographic, clinical, and laboratory characteristics of COVID-19 patients treated with baricitinib and/or with standard COVID-19 therapy from the Misericordia Hospital of Grosseto. A comparison with other groups could not be performed. A dose of 4mg daily baricitinib was given for 10-14 days.

Features at baseline	**Patients (n=25)**
Male/female, N (%)	15/10 (60/40)
Age years, median (IQR)	65 (42-89)
Days interval from symptoms onset and therapy commencing	11 (3-27)
Cough, N (%)	9 (36)
Dyspnea, N (%)	10 (40)
Sputum production, N (%)	0 (0)
Headache, N (%)	2 (8)
Diarrhea, N (%)	2 (8)
Ageusia/Anosmia, N (%)	0 (0)
Hypertension, N (%)	9 (36)
Diabetes, N (%)	2 (8)
COPD, N (%)	0 (0)
CVD, N (%)	2 (8)
Malignancy, N (%)	4 (16)
Fever °C (IQR)	37.8 (37.1-39.5)
Respiratory rate N/min	15 (13-20)
SpO2 (%)	96 (85-100)
PaO2/FiO2, median (IQR)	271 (162-408)
SBP mm/Hg, median (IQR)	130 (110-160)
DBP mm/Hg, median (IQR)	75 (60-99)
WBC ($x10^9$/L), median (IQR)	6.13 (3.03-10.52)
Neutrophils ($x10^9$/L), median (IQR)	4.22 (1.22-7.88)
Lymphocytes ($x10^9$/L), median (IQR)	1.16 (0.46-2.65)
Hemoglobin (g/dL), median (IQR)	14.6 (10.07-17)
Platelets ($x10^9$/L), median (IQR)	196 (91-563)
ALT (IU/L), median (IQR)	29 (12-273)
AST (IU/L), median (IQR)	28 (18-147)
Creatinine (mg/dl), median (IQR)	0.94 (0.47-1.94)
Clearance creatinine (ml/min), median (IQR)	78.6 (37.9-152.6)
D-dimer mcg/mL, median (IQR)	0.88 (0.22-56,83)
Fibrinogen mg/dL, median (IQR)	504 (219-901)
Ferritin ng/mL, median (IQR)	1076 (182-2690)
Antibiotic therapy azithromycyn, patient number (%)	22 (88)
Hydroxychloroquine 200mg bd, patient number (%)	18 (72)
Lopinavir 200 mg /ritonavir 50 mg od, patient number (%)	6 (24)
Darunavir 800 mg/colbicistat 150mg od, patient number (%)	3 (12)
Tocilizumab 8 mg/kg, patient number (%)	1 (4)
No ‘anti-COVID’ drugs , patient number (%)	6 (24)