

Expanded View Figures

Figure EV1. *STAT3/5* expression correlates with disease clonality.

- A Graphical summary of the literature analysis on genomic CTCL studies performed in the last 10 years including larger cohorts of patients, as described in Table EV3. The number of patients carrying 17q gain, specifically of the region containing the *STAT3/5* genes (17q11.2-17q21.31) is written and depicted in black, in comparison to the total number of patients included and depicted in blue or orange. Blue color depicts Sezary syndrome patients, whereas orange depicts mycosis fungoides patients.
- B–E Spearman correlation analysis using CNA log₂ ratios and the percentage of clonal CD3⁺ cells detected in patients with (B) *TP53*, (C) *MYC*, (D) *STAT1*, and (E) *SOC31*.
- F–H Spearman correlation analysis on expression data extracted from the Oncomine™ Platform as published in Caprini *et al* (2009). Patients with 17q (*STAT3/5*) gains were selected and the percentage of clonal CD3⁺ cells was correlated with the respective *STAT3/5* gene expression for the (F) *STAT3_208991* reporter, (G) *STAT5A_203010* reporter, and (H) *STAT5B_212550* reporter.

Source data are available online for this figure.

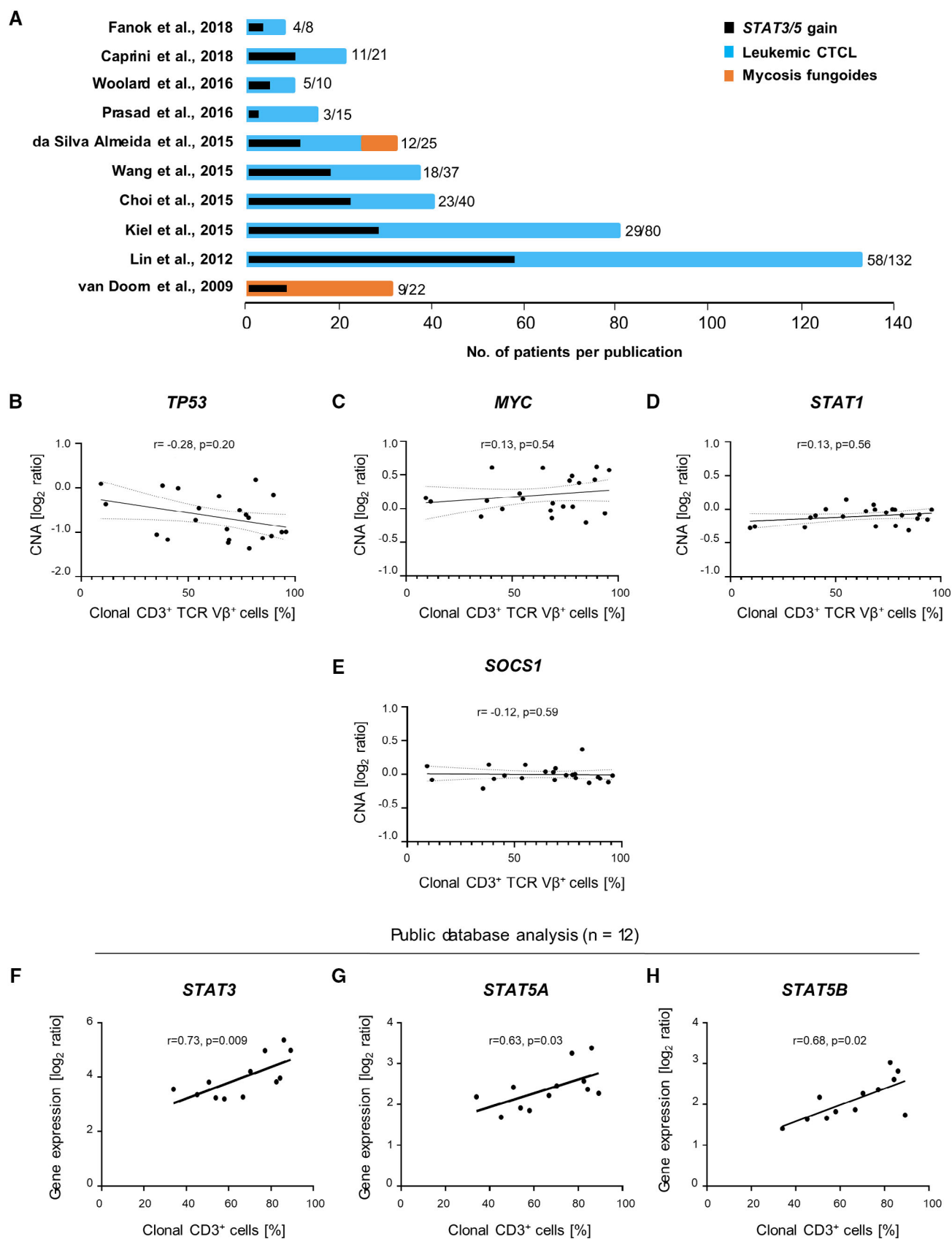


Figure EV1.

Figure EV2. JPX-0750 and IQDMA have distinct mechanisms of action.

- A–D Immunoblot showing total and pY-STAT3/pY-STAT5 levels (i.e., phospho-Tyr (705)-STAT3 and phospho-Tyr (694/699)-STAT5A/B) in (A) Myla cells upon 6 h treatment with JPX-0750 and (B) Hut78 cells upon 24 h treatment with JPX-0750. (C) Myla and (D) Hut78 cells were treated with 24 h IQDMA. HSC70 was used as loading control. The normalized phospho- and total protein levels, quantified by densitometry, are shown below the respective blots. One representative out of three independent experiments is shown.
- E RT-qPCR for STAT5 target genes in the SeAx cell line after 24 h treatment with JPX-0750. Gene expression was normalized to *GAPDH*. Statistical significance was calculated using two-way ANOVA with multiple comparisons. *P*-value: < 0.05 (*), < 0.01 (**), < 0.001 (***), and 0.0001 (****). *P*-value summaries are provided in Appendix Table S1. Error bars represent mean $\pm$ SD. One experiment performed in triplicates is shown.
- F, G Cleaved and total PARP protein levels in Myla cells treated with (F) JPX-0750 for 6 h and (G) IQDMA for 24 h. HSC70 was used as loading control. The normalized total protein levels, quantified by densitometry, are shown below the respective blots. One representative out of two independent experiments is shown.
- H, I Immunoblotting for cleaved caspase 3 levels in SeAx cells after 24 h treatment with (H) JPX-0750 and (I) IQDMA. β -Actin or HSC70 were used as loading controls. The normalized total protein levels, quantified by densitometry, are shown below the respective blots. One representative out of two independent experiments is shown.

Source data are available online for this figure.

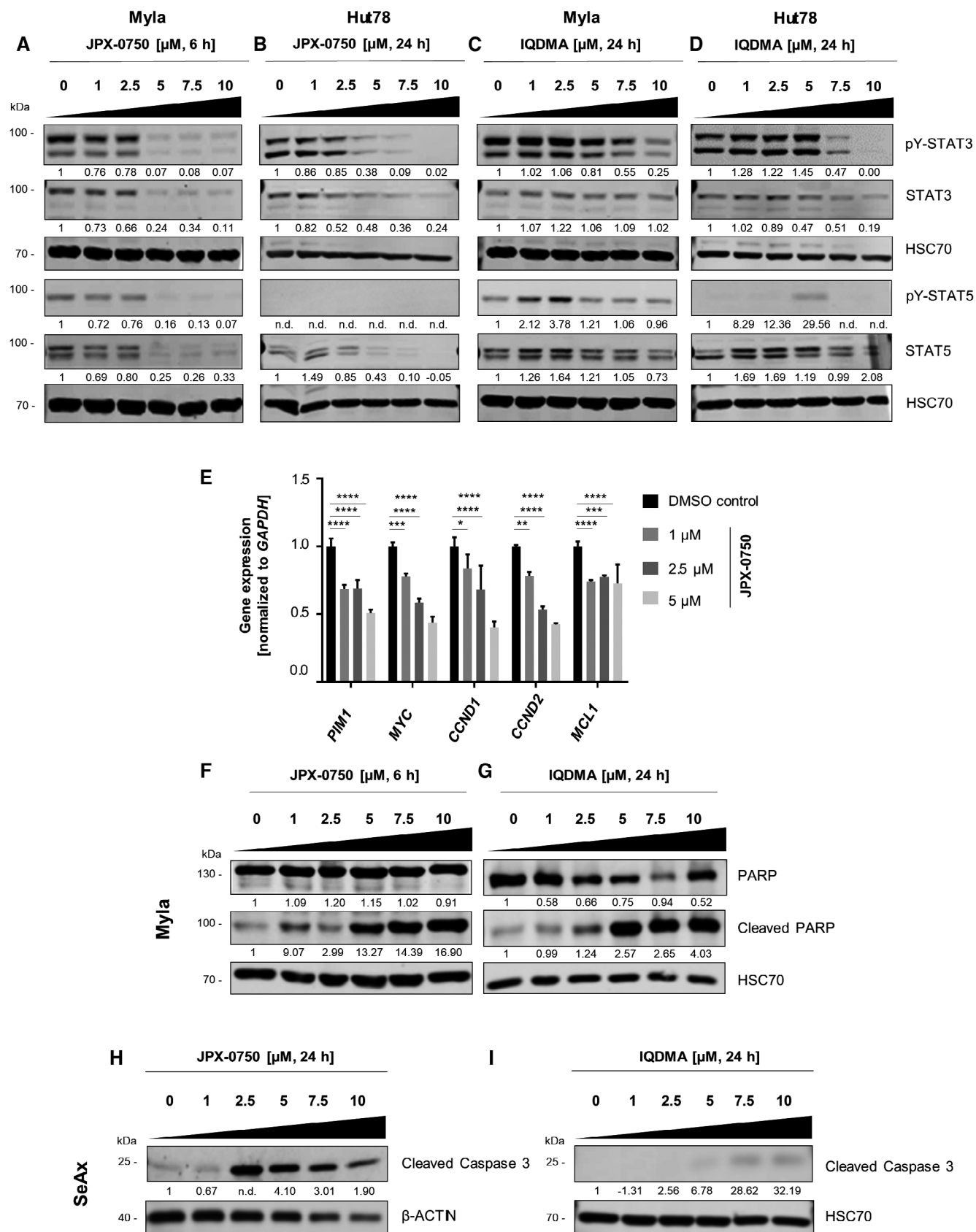


Figure EV3. In-depth analysis of IQDMA shows its multi-kinase inhibitory function.

- A Thermal shift profiles of 2 μ M STAT5B with 100 μ M IQDMA or 100 μ M STAT5-binding peptide (positive control). The local maxima indicate the melt temperature of the protein ($T_m \pm 0.3^\circ\text{C}$). One representative out of two independent experiments performed in technical triplicates is shown.
- B Fluorescence polarization assay using 180 nM STAT5B with 100 μ M IQDMA or 100 μ M peptide (positive control). The positive control exhibits a single-site displacement profile. Error bars represent mean $\pm$ SD. Three independent experiments were performed.
- C IQDMA structure.
- D Subcellular fractions of SeAx cells treated with IQDMA for 18 h and immunoblotted for pY-STAT3 (phospho-Tyr (705)-STAT3) and total STAT3. The cells were collected 30 min after 5 ng/ml IL-2 cytokine addition. α -Tubulin and histone H3 were used as loading controls for cytoplasmic and nuclear fractions, respectively. The normalized levels of phospho- and total STAT3 in the nucleus and cytoplasm, quantified by densitometry, are shown below the respective blots. One experiment was performed.
- E Bar graph showing the cellular pathways that are most affected by IQDMA treatment as determined by network enrichment analysis.
- F Subcellular fractions of SeAx cells treated with FRAX597 for 18 h and immunoblotted for pY-STAT3/pY-STAT5 levels (i.e., phospho-Tyr (705)-STAT3 and phospho-Tyr (694/699)-STAT5A/B) and total STAT3/5 levels. The cells were collected 30 min after 5 ng/ml IL-2 cytokine addition. α -Tubulin and histone H3 were used as loading controls for cytoplasmic and nuclear fractions, respectively. The normalized levels of phospho- and total STAT3/5 in the nucleus and cytoplasm, quantified by densitometry, are shown below the respective blots. One experiment was performed.

Source data are available online for this figure.

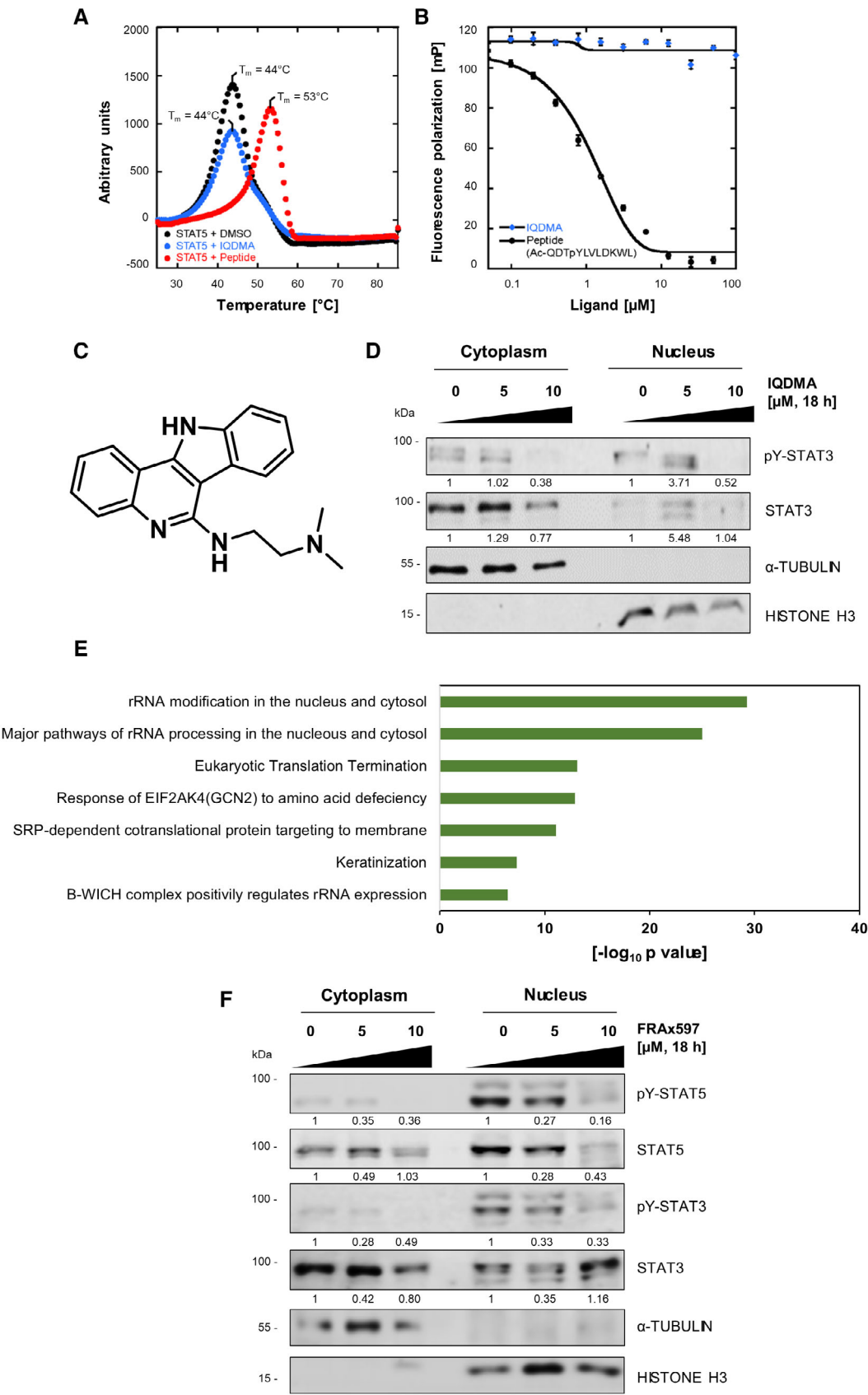


Figure EV3.

	JPX-0750	IQDMA	FRAx597	IC ₅₀ [μM, 48 h]
H1	6.78	16.16	3.10	
H2	13.26	7.99	<100	
H3	6.27	1.73	<100	
H4	11.57	1.58	<100	
H5	0.94	0.68	3.50	
H6	3.51	2.52	<100	
H7	9.50	8.02	1.59	
H8	9.23	8.75	n.d.	
H9	0.80	0.86	0.95	
H10	1.54	0.97	1.64	
H11	1.05	0.88	0.78	
H12	1.22	0.71	1.41	
H13	1.65	0.86	1.37	
H14	0.95	1.00	0.86	
H15	1.28	1.10	1.52	

Figure EV4. JPX-0750, IQDMA, and FRAx597 show varying toxicity to healthy cells.

Heatmap showing IC₅₀ values upon treatment of primary PBMCs isolated from healthy controls with JPX-0750, IQDMA, and FRAx597. One experiment performed in triplicates using CellTiter-Glo viability assays upon 48 h drug treatment is shown. The relative STAT3/5 log₂ ratio value is depicted in gray. Source data are available online for this figure.

Figure EV5. The PAK kinase inhibitor inhibits malignant cell dissemination into the lymph nodes *in vivo*.

A–C Flow cytometric analysis of malignant cell dissemination into (A) lymph nodes, (B) liver, and (C) kidney (Vehicle: *n* = 3, JPX-0750: *n* = 3, IQDMA: *n* = 3; Vehicle: *n* = 2, FRAx597: *n* = 3, with *n* representing the number of analyzed tumors per group) as measured by the percentage of human CD45⁺ cells in the respective organ. Error bars represent mean ± SEM. Statistical significance was calculated using a two-tailed paired *t*-test with Welch's correction. *P*-value: < 0.05 (*).

D–F H&E and IHC analyses of Hut78-derived tumors treated with JPX-0750 (*n* = 3), IQDMA (*n* = 2) or vehicle (*n* = 4), and FRAx597 (*n* = 3) or vehicle (*n* = 2), and stained with (D) H&E and (E) cleaved caspase 3 (CC3) to detect cell death and (F) CD31 vessel marker, with *n* representing the number of analyzed tumors per group. Pictures shown are from contiguous sections. Dotted rectangles indicate magnified areas. Scale bars, 200, 100, and 25 μm. Violin plots show the perimeter of the annotated tumor cell infiltration/expansion region into the intradermal and subcutaneous region of the skin, as well as quantification of the percentage of CC3⁺ cells in the tissue. CD31 staining was quantified as raw counts of vessels with lumen. Statistical significance was calculated using a two-tailed paired *t*-test with Welch's correction. *P*-value: < 0.05 (*), 0.0001 (****). The bold dashed line in the middle of the violin plot denotes the median value, while the thin dotted lines denote the interquartile range.

Source data are available online for this figure.

Myla organ infiltration

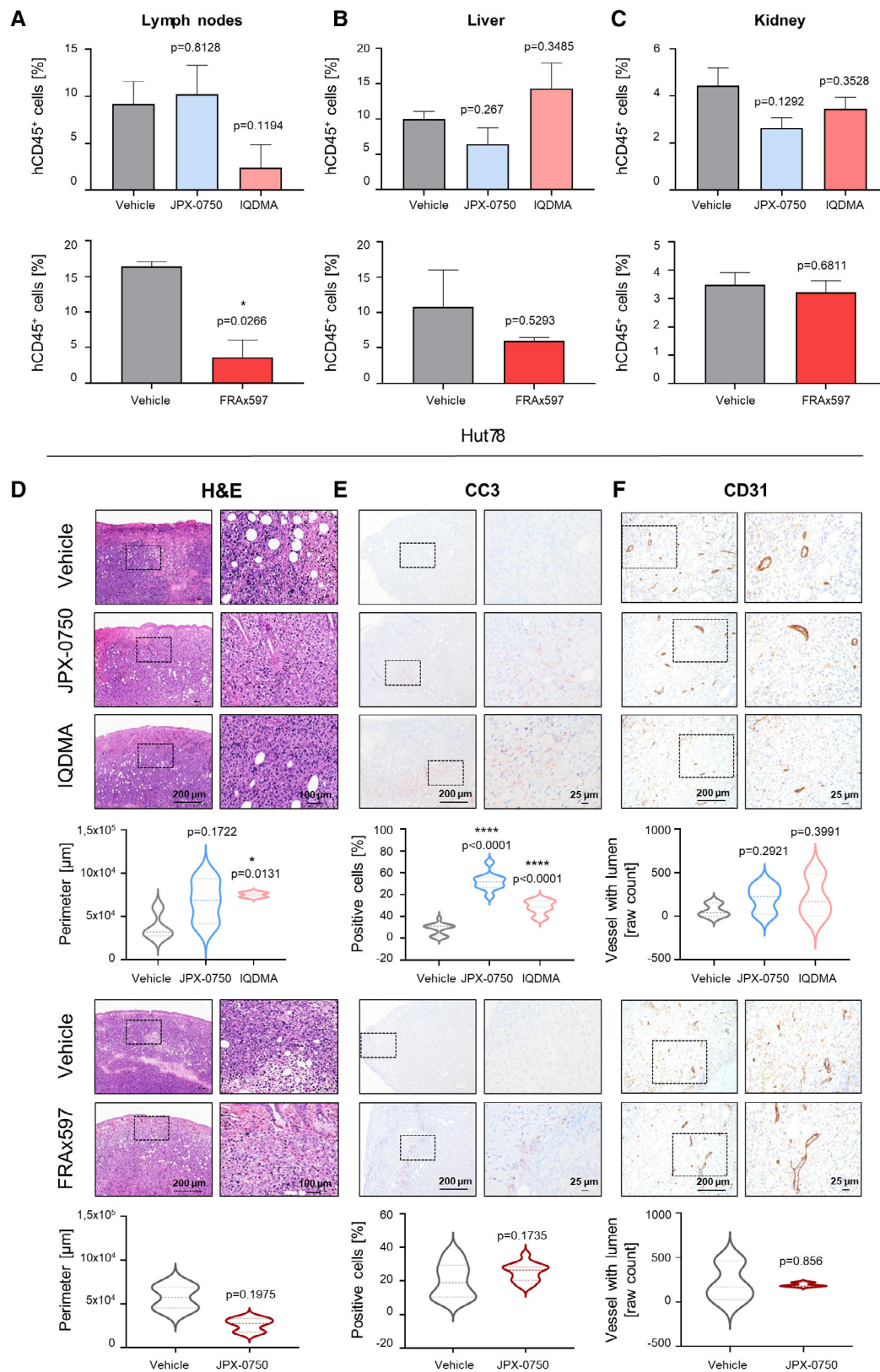


Figure EV5.