Title: Increased levels of NETosis in Myeloproliferative Neoplasms are not linked to thrombotic events

Running title: NETosis in MPN

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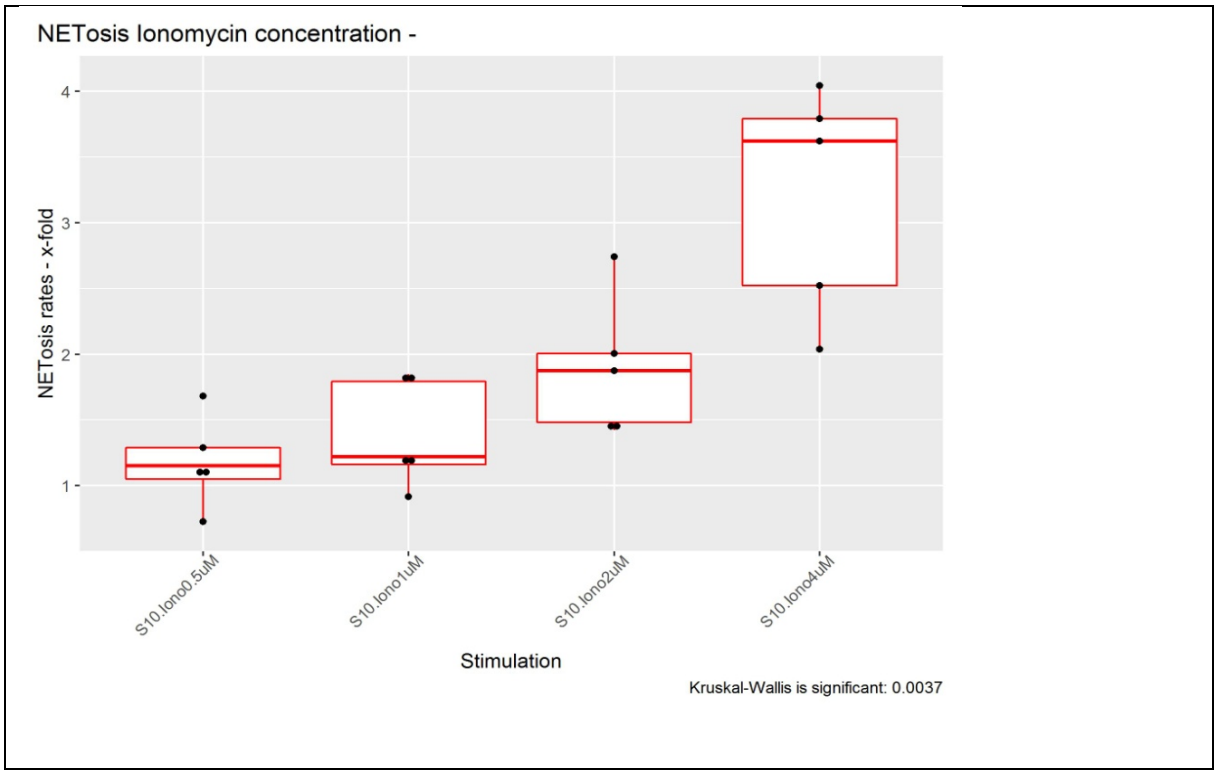
Supplemental table 1

Total number of healthy donors	28
Gender [F/M]	15/13
Age [median, years]	59,9
Thomboenbolic events	1
Bleeding events	1
Pts with CVRF	6
Prophylactic Treatment	
Anticoagulation	3
Antiplatelet	1

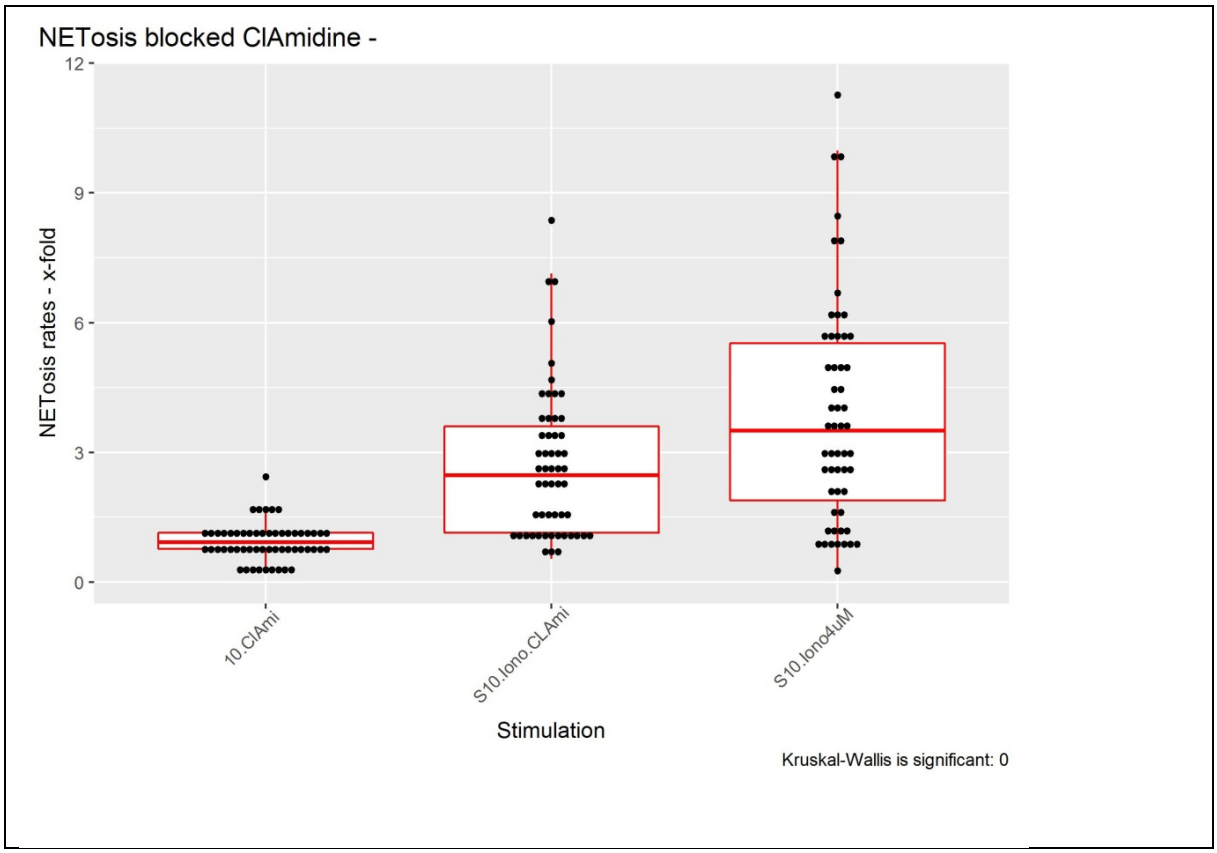
Supplemental Figures

Supplemental Figure 1



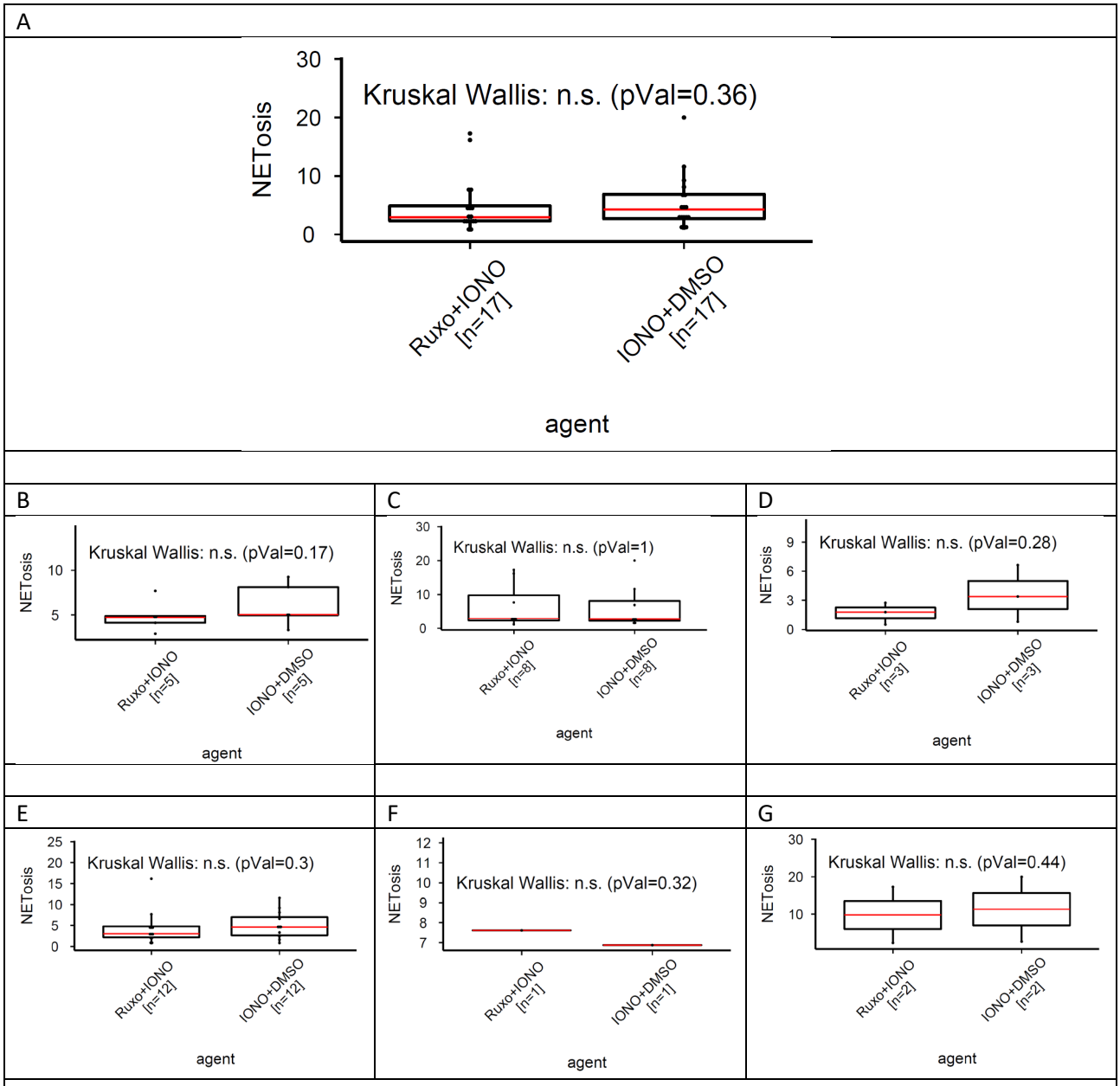
Dose-dependency illustrated by NETosis rates shown according to increasing ionomycin concentrations. Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements.

Supplemental Figure 2



Blocking of NETosis by Cl-amidine. Pretreatment of neutrophils with 200nM Cl-amidine prior to NETosis induction by 4uM ionomycin resulted in decreased NETosis rates. Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements.

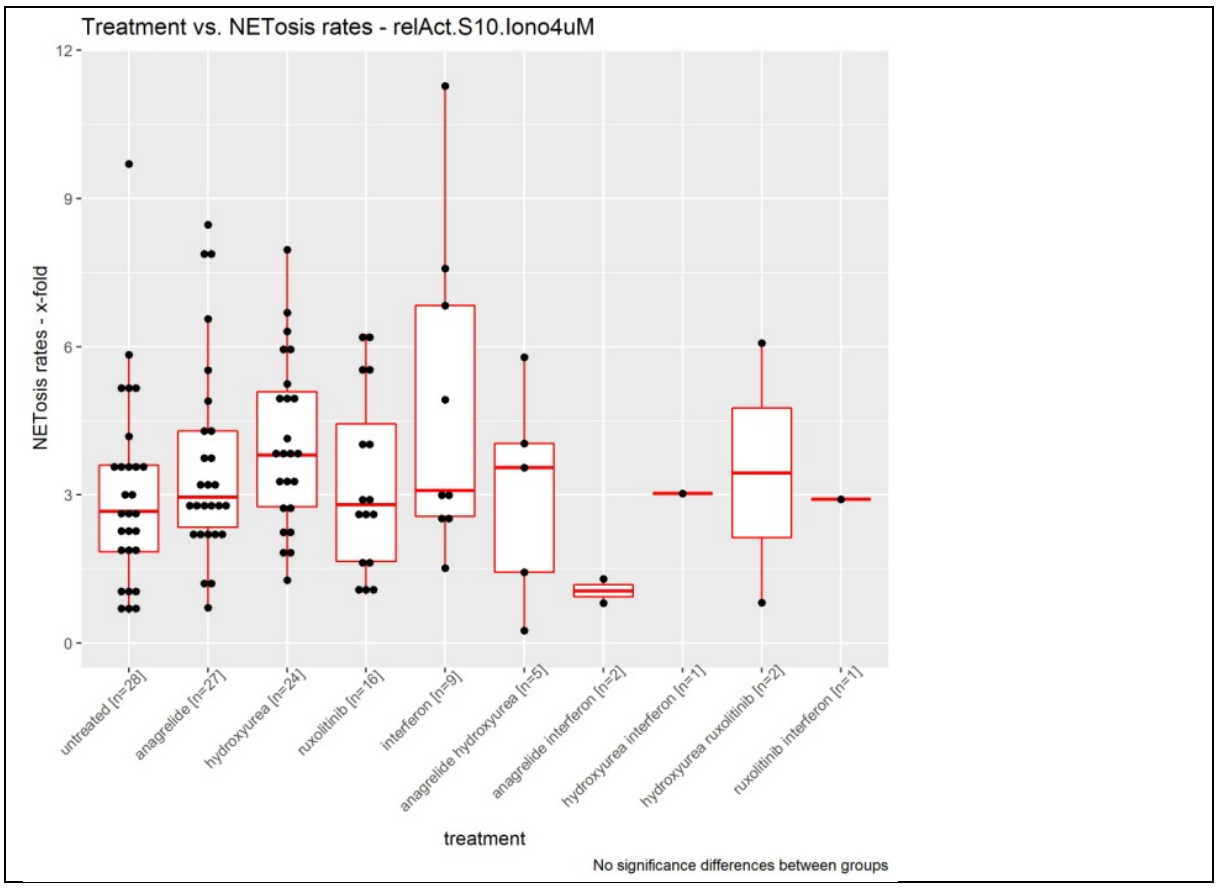
Supplemental Figure 3



Ruxolitinib pre-treatment. In-vitro induced NETosis rates after pretreatment with Ruxolitinib (final concentration 300nM) or vehicle for 2.5h prior to NETosis induction by Ionomycin (final 4µM) for additional 2.5h

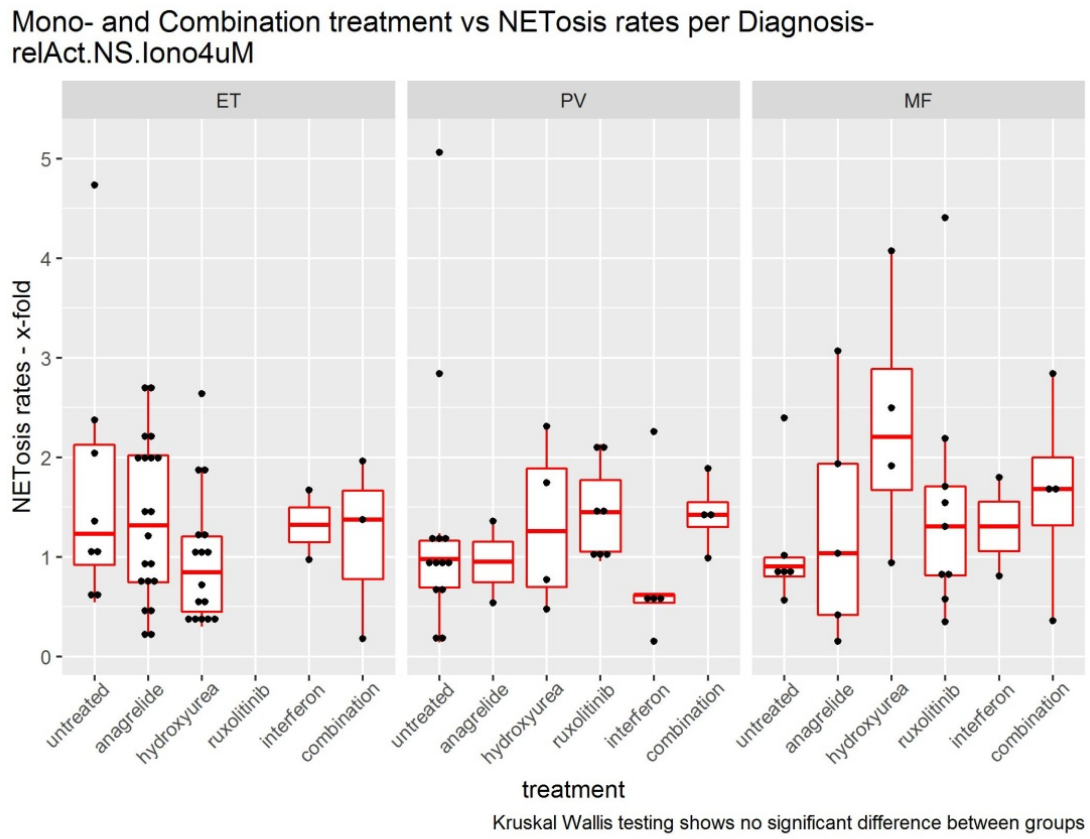
A) Comparison in primary neutrophils from 17 MPN patients. B-D) Subanalysis of MPN subgroups: (B) PV patients n=5, (C) ET patients n=8, (D) PMF patients (n=3). E-G) Subanalysis according to mutational status: (E) JAK2-mutated(n=12), (F) triple-negativ (n=2), (G) MPL-mutated subgroup (n=2)

Supplemental Figure 4



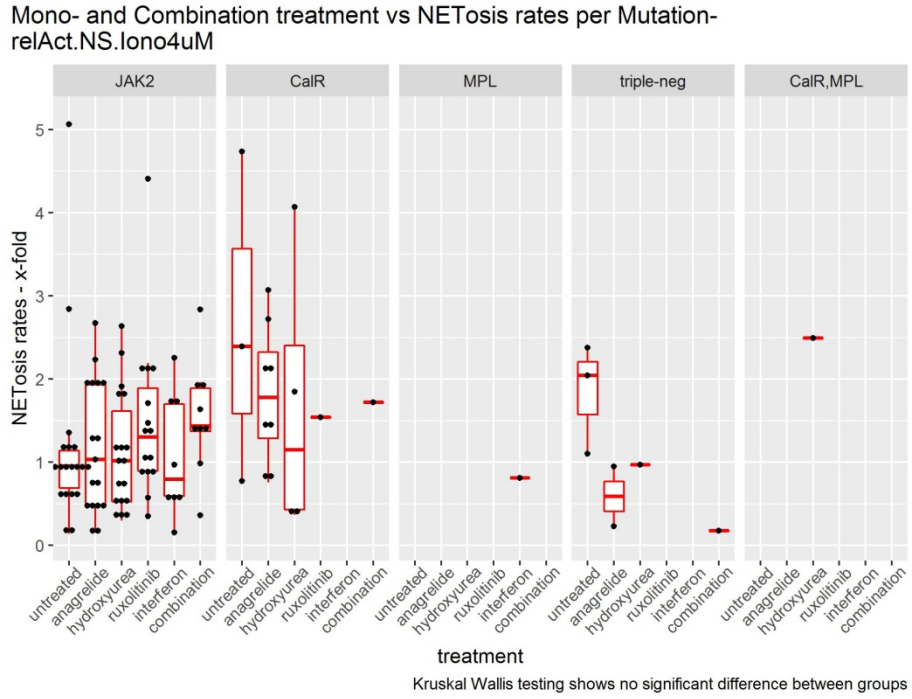
Induced NETosis rates in MPN patients determined by ELISA shown by treatment: untreated n=28, anagrelide n=27, hydroxyurea n= 24, Ruxolitinib n= 16, interferon= 9, anagrelide/hydroxyurea n=5. Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements. Treatment groups did not significantly differ. P= NS (Kruskal Wallis Test)

Supplemental Figure 5



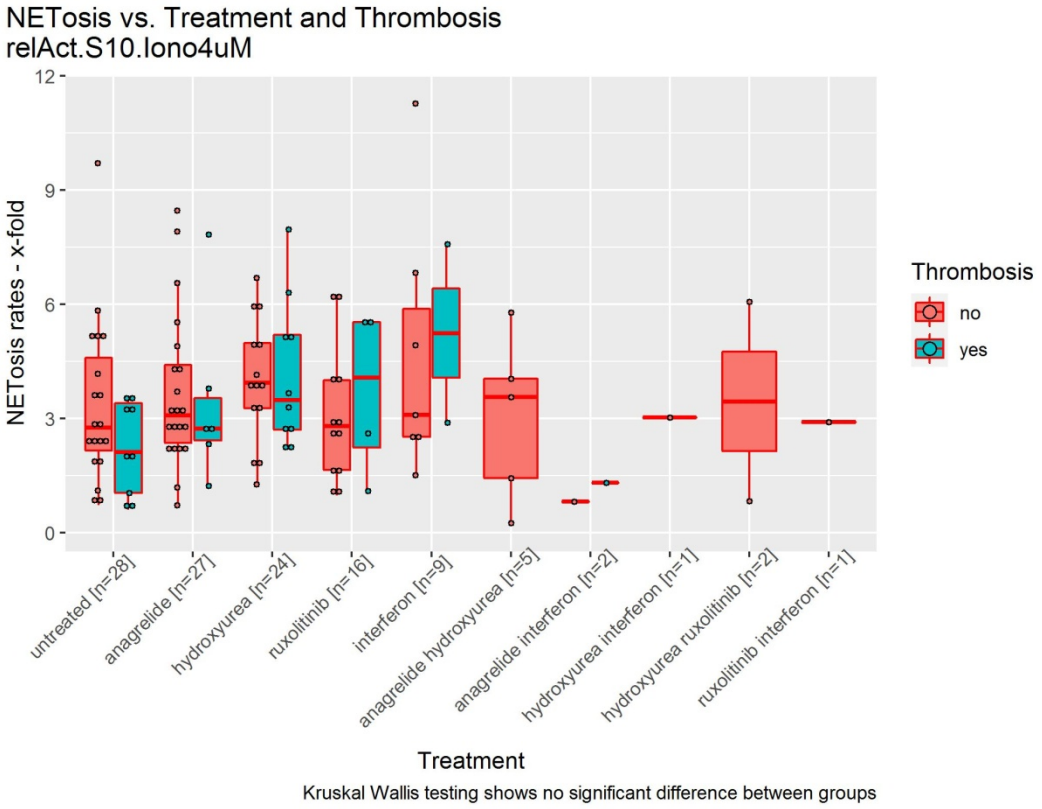
Induced NETosis rates in MPN sub-entities according to treatment: Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements.

Supplemental Figure 6



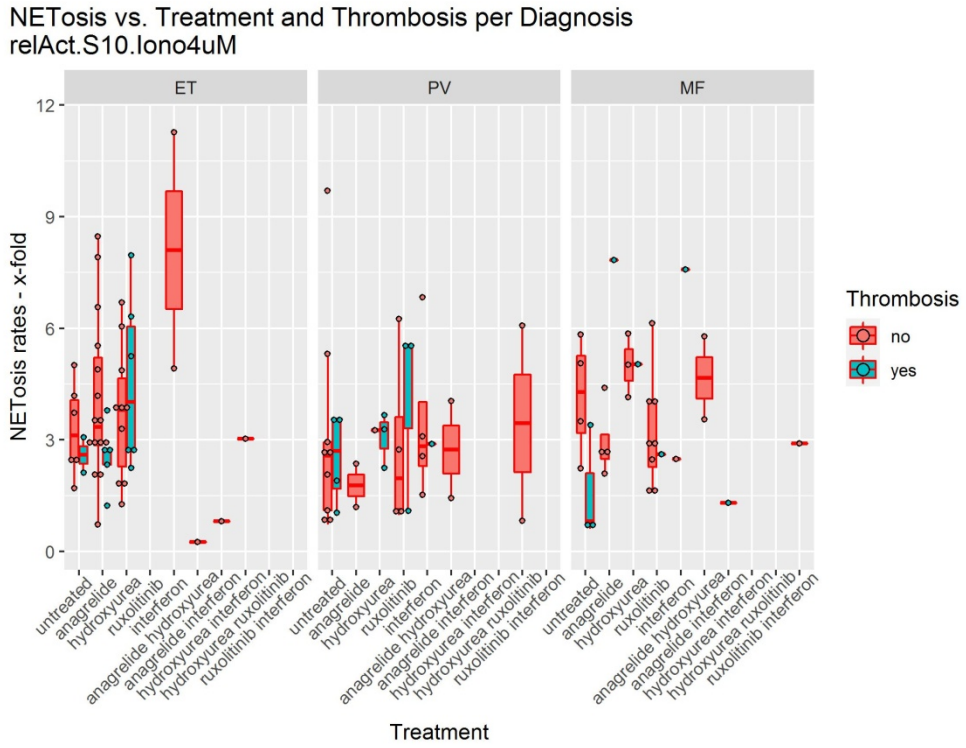
Induced NETosis rates in MPN subentities according to mutational status: Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements. Overall treatment groups CalR-mutated patients (n=16) demonstrated higher NETosis rates than observed in patients carrying a JAK2 mutation.

Supplemental Figure 7



Induced NETosis rates shown by treatment: groups and history of thrombotic events. Untreated n=28, anagrelide n=27, hydroxyurea n= 24, Ruxolitinib n= 16, interferon= 9, anagrelide/hydroxyurea n=5. Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements.

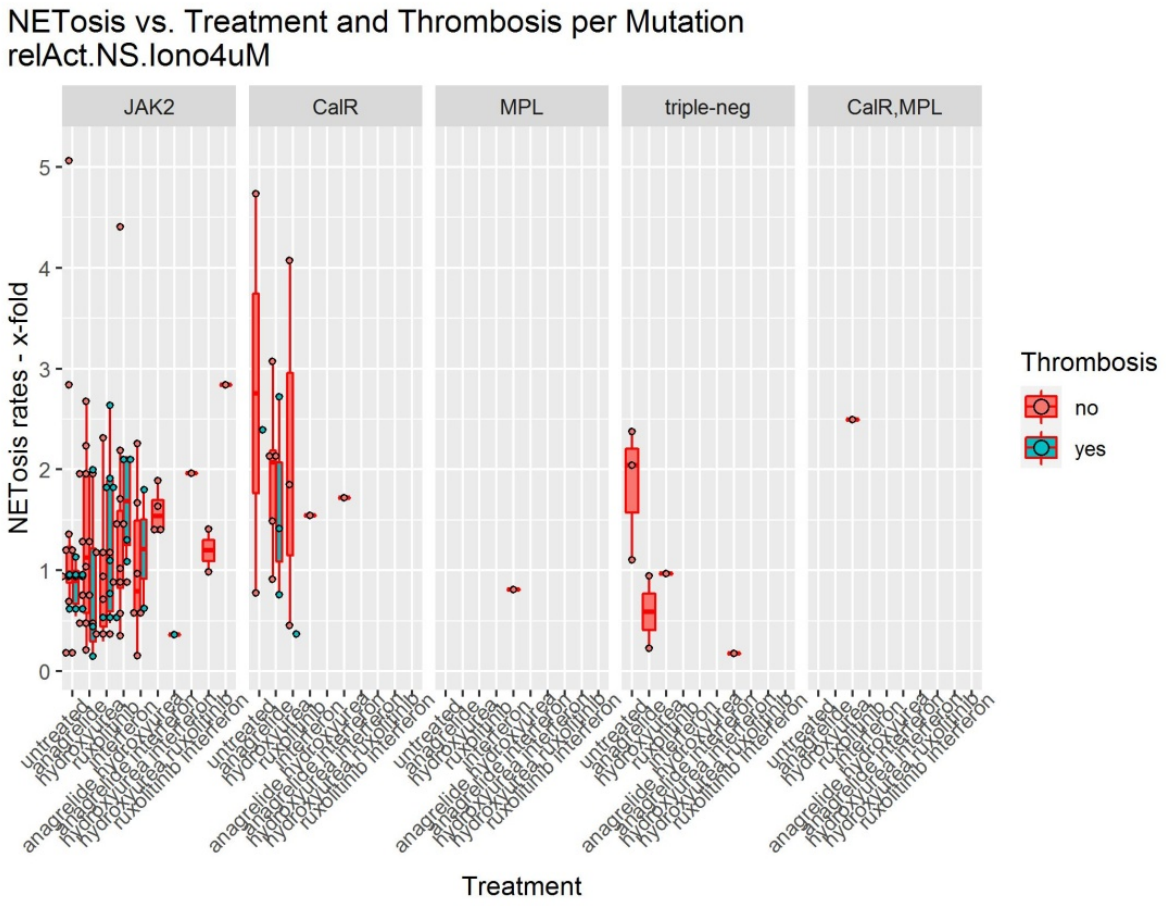
Supplemental Figure 8



Induced NETosis rates shown by treatment:groups, subentity and history of thrombotic events. untreated n=28, anagrelide n=27, hydroxyurea n= 24, Ruxolitinib n= 16, interferon= 9, anagrelide/hydroxyurea n=5). Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements.

In ET patients with thrombotic events NETosis rates were lower in untreated and anagrelide treated patients than in patients who had a history of thrombosis. In the hydroxyurea subgroup there appeared to be no difference. In contrast, in the PV-subgroup untreated patients with a thrombotic event had slightly higher NETosis rates. In the, however smaller, group of Ruxolitinib treated PV-patient this tendency of higher NETosis rates in patients with a history of a thrombotic event was more pronounced. The largest difference in NETosis rates between patients with and without a thrombotic event was observed in the subgroup of untreated MF patients.

Supplemental Figure 9

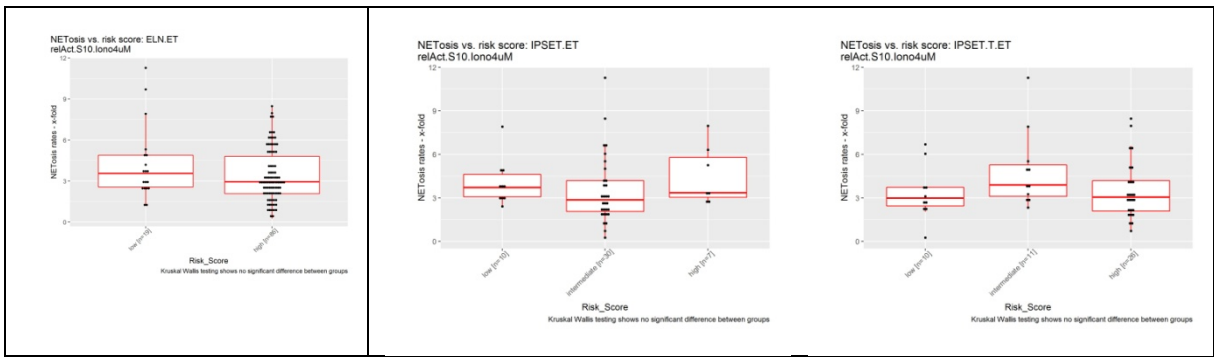


Induced NETosis rates shown by treatment:groups, mutational status and history of thrombotic events. Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements.

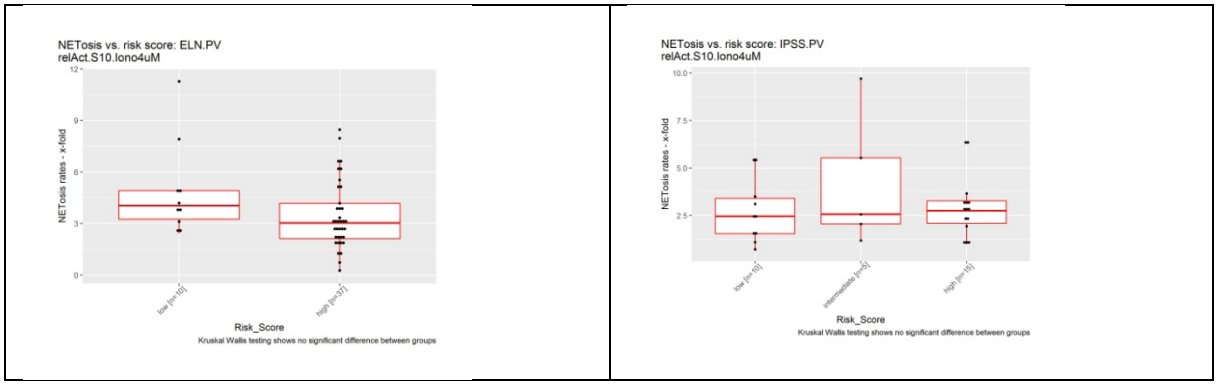
Analyzing treatment groups according to mutational status and history of thrombotic events, patients carrying a JAK2- or a CalR-Mutation and who had a thrombotic event showed in both groups, untreated and anagrelide-treated, lower NETosis rates than patients without events. In contrast, patients receiving hydroxyurea, Ruxolitinib or interferon who had a thrombotic events demonstrated slightly higher NETosis rates in the JAK2 mutated subgroup (no events in CalR-mutated subgroup for hydroxyurea, Ruxolitinib or interferon.

Supplemental Figure 10

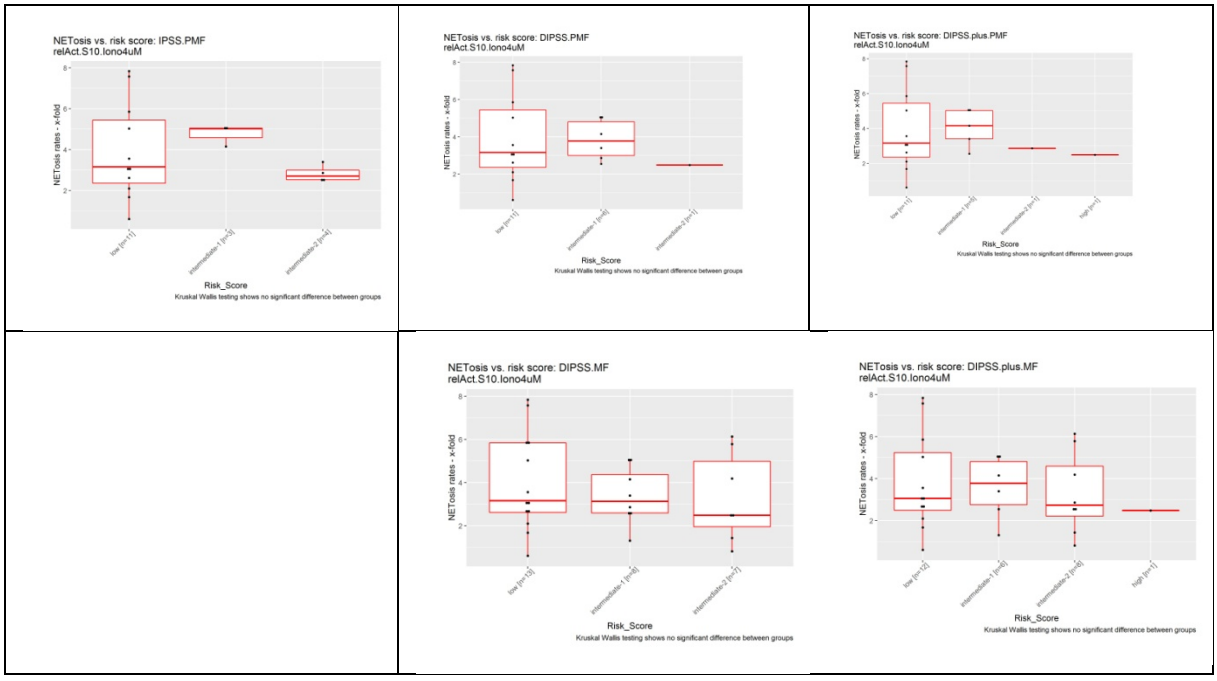
Panel A)



Panel B)



Panel C)



Induced NETosis rates shown by MPN risk scores, Panel A) ELN-ET, IPSET, IPSET-thrombosis, Panel B) ELN- PV and IPSS, Panel C) : IPSS, DIPSS, DIPSS plus. Data presented as x-fold of vehicle controls. Boxplots show the 25%- and 75%-quartiles and the median, overlayed dot plots indicates individual measurements.