Title

Deletions in FLT3 juxtamembrane domain define a new class of pathogenic mutations: case report and systematic analysis

Authors

David J. Young, M.D., Ph.D., Bao Nguyen, M.S., Ruiqi Zhu, Li Li, M.D., Ph.D., Mark J. Levis, M.D., Ph.D., Keith W. Pratz, M.D., Amy S. Duffield, M.D., Ph.D., Donald Small, M.D., Ph.D.

Supplemental Tables

Supplemental Table 1. Deletion construct primers

Residue	Forward	Reverse
K568	GTCACAAGTACAAACAATTTAGGTATGAAAGCCAGCTACAG	GCTGGCTTTCATACCTAAATTGTTTGTACTTGTGACAAATTAGCAGGG
Q569	GTCACAAGTACAAAAAGTTTAGGTATGAAAGCCAGCTACAG	GCTGGCTTTCATACCTAAACTTTTTGTACTTGTGACAAATTAGCAGGG
F570	GTCACAAGTACAAAAAGCAAAGGTATGAAAGCCAGCTACAG	GTAGCTGGCTTTCATACCTTTGCTTTTTGTACTTGTGACAAATTAGC
R571	GTCACAAGTACAAAAAGCAATTTTATGAAAGCCAGCTACAGATGG	CTGTAGCTGGCTTTCATAAAATTGCTTTTTGTACTTGTGACAAATTAGC
Y572	GTACAAAAAGCAATTTAGGGAAAGCCAGCTACAGATGG	CTGTAGCTGGCTTTCCCTAAATTGCTTTTTGTACTTGTGAC
E573	GTACAAAAAGCAATTTAGGTATAGCCAGCTACAGATGG	CTGTAGCTGGCTATACCTAAATTGCTTTTTGTACTTGTGAC
S574	GTACAAAAAGCAATTTAGGTATGAACAGCTACAGATGGTACAGGTG	CCTGTACCATCTGTAGCTGTTCATACCTAAATTGCTTTTTGTACTTGTG
Q575	GCAATTTAGGTATGAAAGCCTACAGATGGTACAGGTG	CCTGTACCATCTGTAGGCTTTCATACCTAAATTGCTTTTTGTAC
L576	GCAATTTAGGTATGAAAGCCAGCAGATGGTACAGGTG	CCTGTACCATCTGCTGGCTTTCATACCTAAATTGCTTTTTGTAC
Q577	GCAATTTAGGTATGAAAGCCAGCTAATGGTACAGGTG	CCTGTACCATTAGCTGGCTTTCATACCTAAATTGCTTTTTGTAC
M578	GCCAGCTACAGGTACAGGTGACCGGCTCC	GCCGGTCACCTGTACCTGTAGCTGGCTTTCATACC
V579	GCCAGCTACAGATGCAGGTGACCGGCTCCTCAG	GGAGCCGGTCACCTGCATCTGTAGCTGGCTTTCATACC
Q580	GCCAGCTACAGATGGTAGTGACCGGCTCCTCAGATAATGAGTAC	GGAGCCGGTCACTACCATCTGTAGCTGGCTTTCATACC
V581	GCCAGCTACAGATGGTACAGACCGGCTCCTCAGATAATGAGTAC	GGAGCCGGTCTGTACCATCTGTAGCTGGCTTTCATACC

Supplemental Figure Legends

Supplemental Figure 1. (A) Cells expressing FLT3/Q575Δ were exposed to increasing concentrations of high-dose AG1295 at levels reported to be active against FLT3/ITD. **(B)** Cells expressing either FLT3/ITD or FLT3/Q575Δ were exposed to increasing concentrations of midostaurin for 1 hour. Total cell lysates were collected and subjected to phospho-western analysis for FLT3 autophosphorylation and downstream signaling through STAT5, AKT, and ERK1/2.

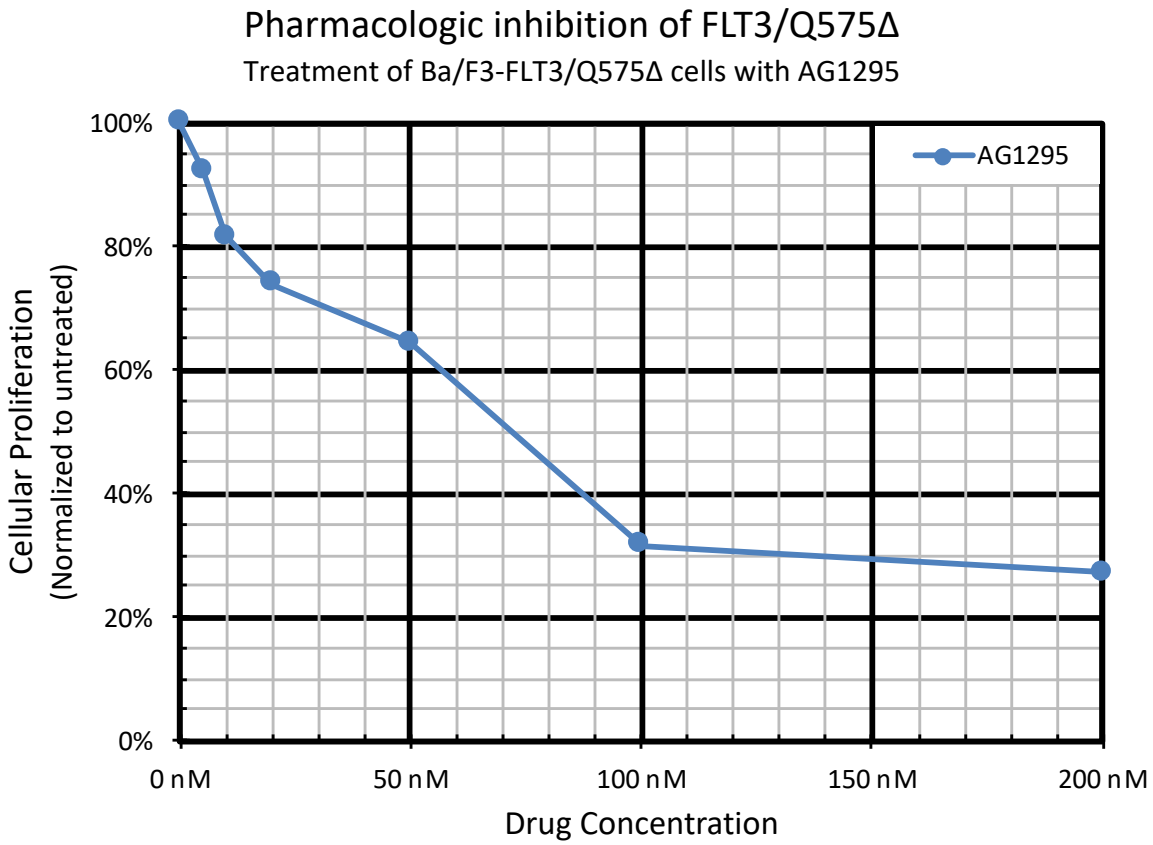
Supplemental Figure 2. (A) Distribution of all FLT3 mutations as reported in COSMIC database. (B) Distribution of silent (synonymous) mutations of FLT3 as reported in COSMIC database. Results of permutation analysis (1×10^6 resamplings) are indicated for two different clusters of silent mutations. The different protein domains are indicated by the colored boxes. Data are from COSMIC query performed on August 9, 2017.

Supplemental Figure 3. Growth of cells expressing different FLT3 constructs as measured by MTT assay. Cells were measured at 2, 3, 4, and 5 days post-plating. Growth in the absence of rm IL-3 was normalized to growth in its presence and plotted as a function of time from plating. Highlighted are key FLT3 constructs (FLT3/ITD and FLT3/WT) as well as the four constructs (Y572Δ, E573Δ, S574Δ, and Q575Δ) that demonstrated growth over background.

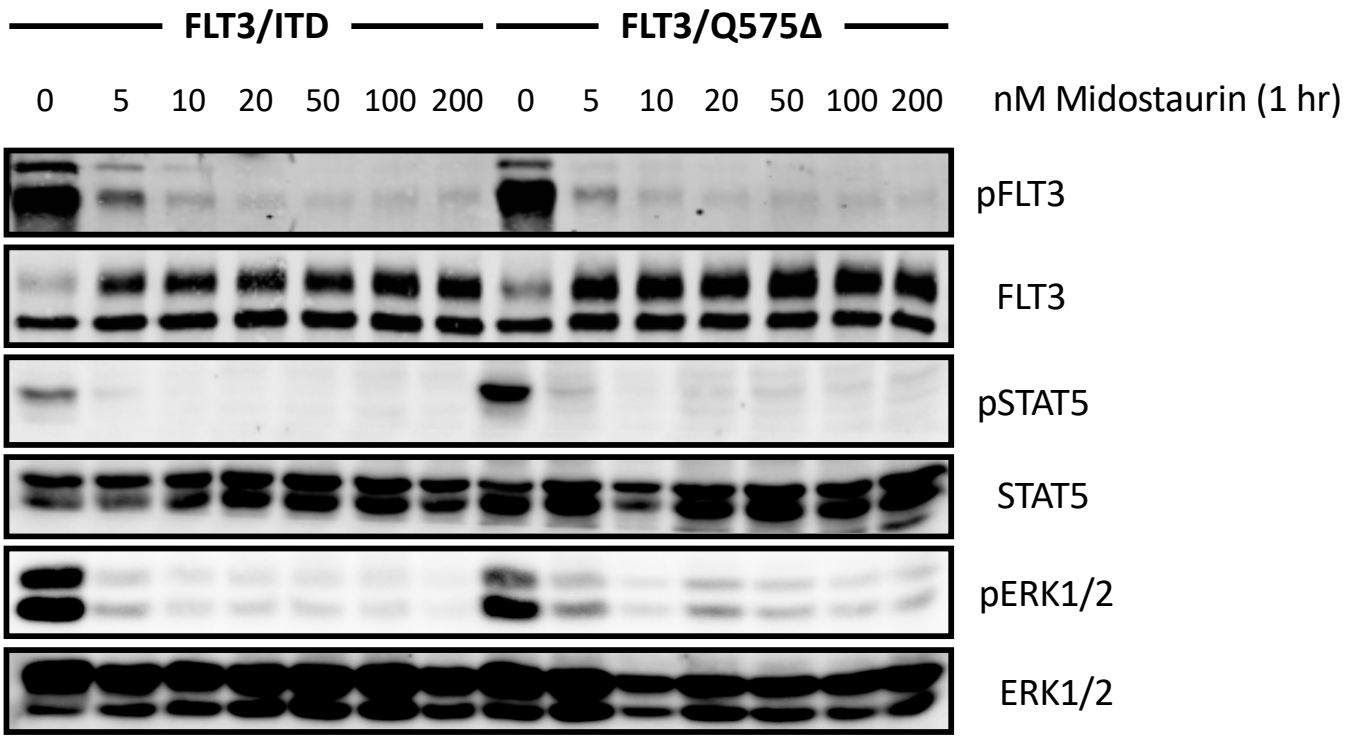
Supplemental Figure 4. Spontaneous apoptosis in the absence of IL-3 was measured. Cells were grown overnight in rm IL-3 and then washed and replated without cytokine. After 48 hours, apoptosis was measured flow-cytometry using Annexin V staining.

Supplemental Figure 1

A

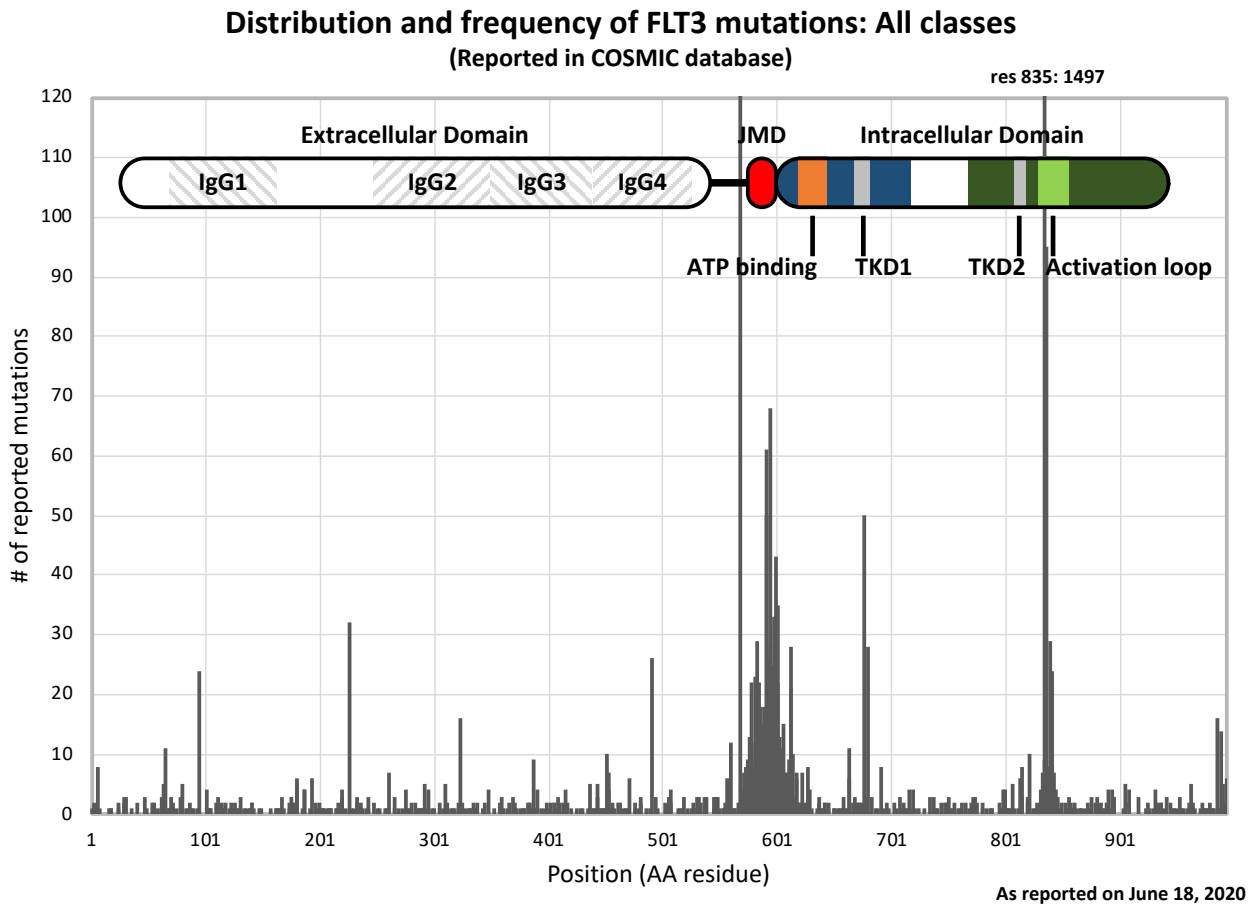


B



Supplemental Figure 2

A



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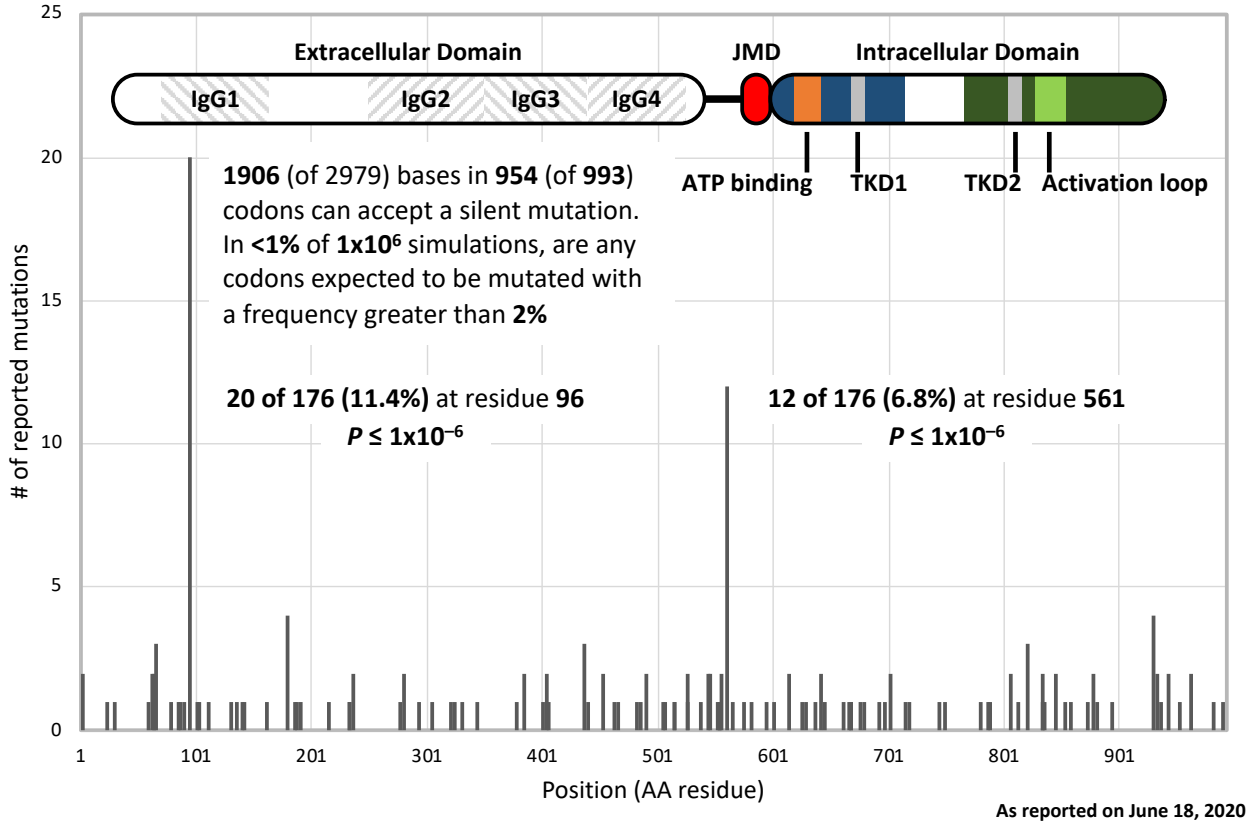
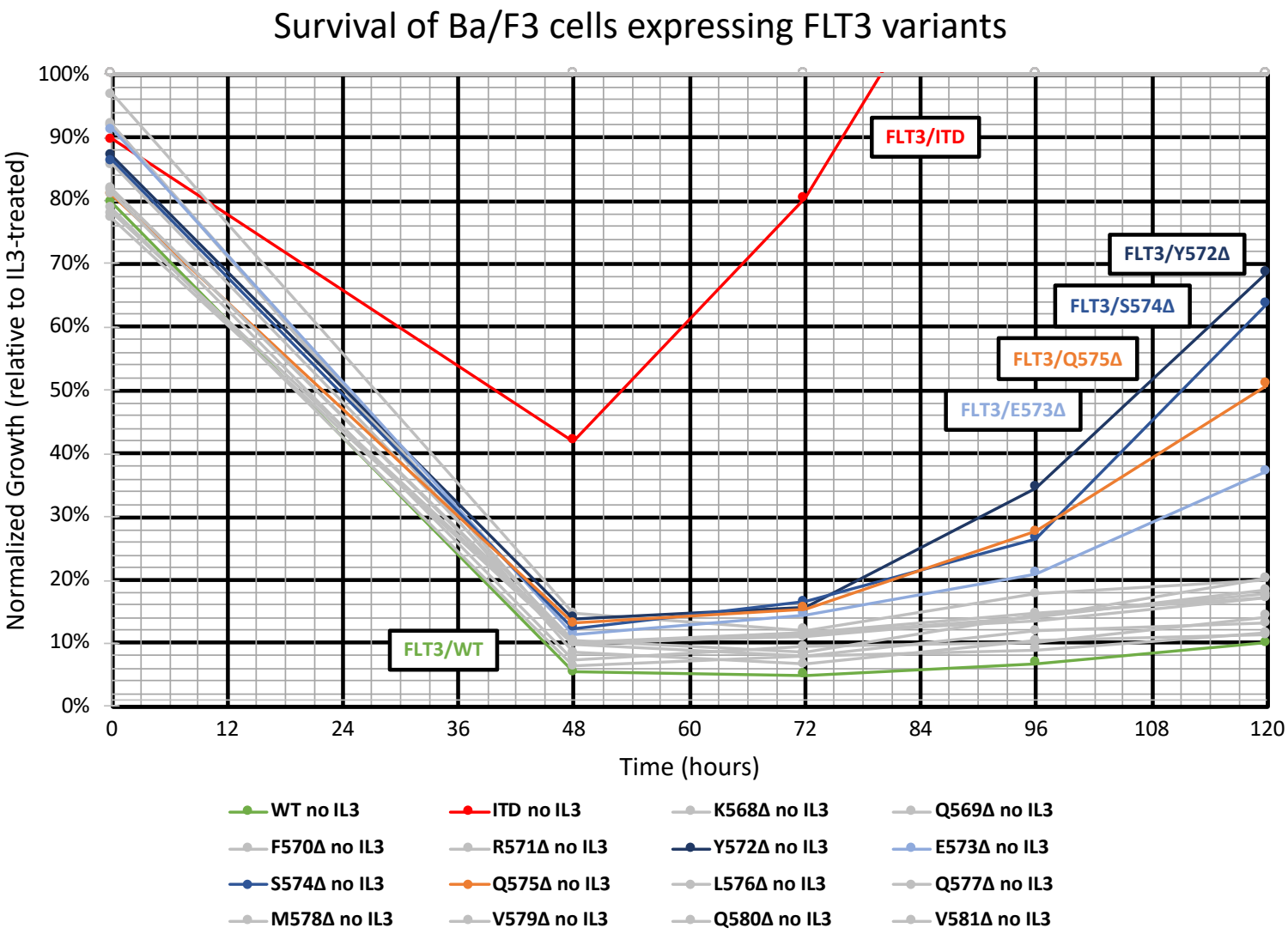


Supplemental Figure 3



Supplemental Figure 4