**Supplementary information**

The new and recurrent FLT3 juxtamembrane deletion mutation shows a dominant negative effect on the wild-type FLT3 receptor

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**Supplemental Tables:**

- Supplemental Table S1: Overview about available material and performed analyses.

**Supplemental Figures:**

- Supplemental Figure S1: Schematic representation of the juxtamembrane deletion mutant FLT3 receptor in comparison to the WT receptor.
- Supplemental Figure S2: Cell surface expression of FLT3 constructs.
- Supplemental Figure S3: Binding capacity for biotinylated FLT3 ligand.
- Supplemental Figure S4: Full-length immuno blots of figure 1 and 2.
- Supplemental Figure S5: Dominant negative effect of FLT3 p.Q569Vfs*2 and its heterodimerisation with FLT3 WT

**Figure S1**

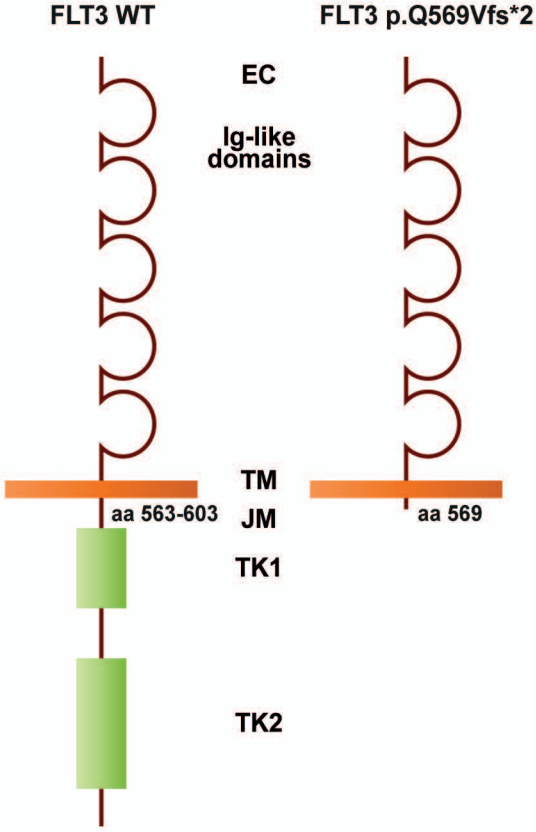



**Figure S1: Schematic representation of the juxtamembrane deletion mutant FLT3 receptor in comparison to the FLT3 WT receptor.** The mutated receptor lacks a large proportion of the juxtamembrane domain as well as other intracellular domains, especially the tyrosine kinase domains 1/2.
aa: amino acid, EC: extracellular domain, Ig-lke domain: Immunglobuline-like domain, TM: transmembrane domain, JM: juxtamembrane domain, TK1/2: tyrosine kinase domain 1/2.

**Figure S2**

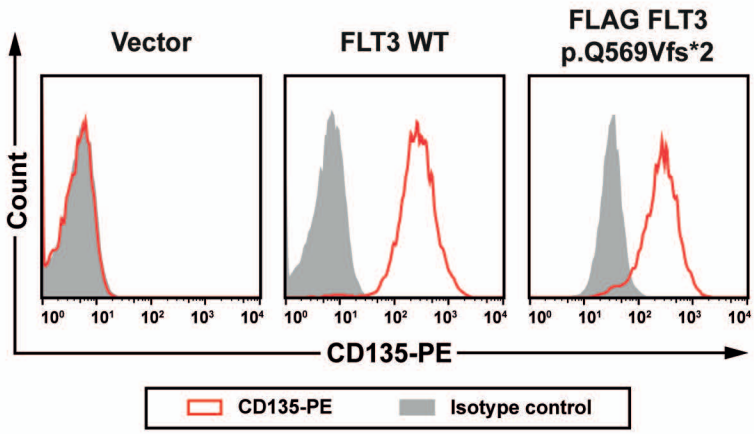



**Figure S2: Cell surface expression of the FLT3 constructs.** Ba/F3 cells stably expressing the indicated constructs were stained with CD135 antibody (red) or isotype control (grey) and analyzed by flow cytometry. One representative example is shown.

**Figure S3**

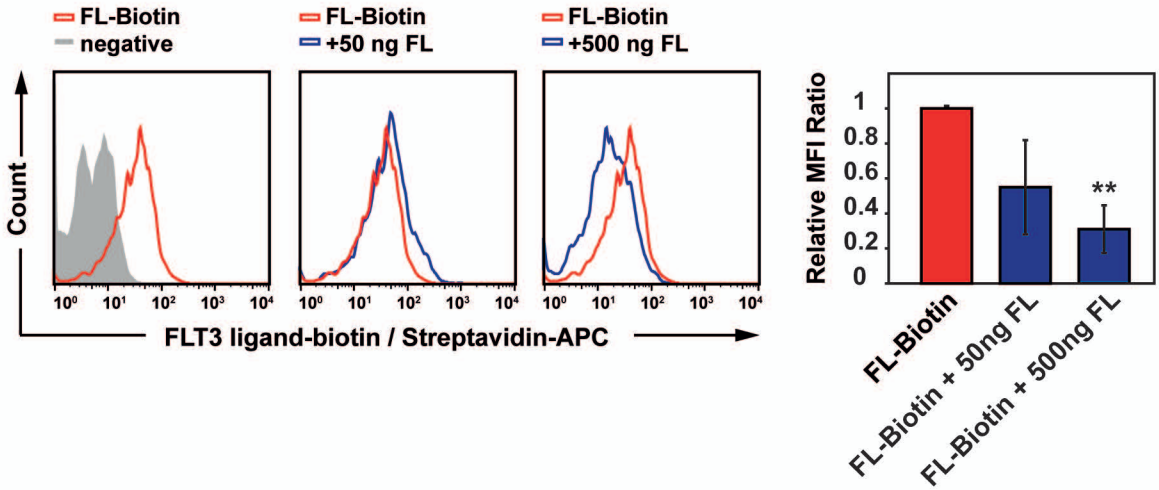
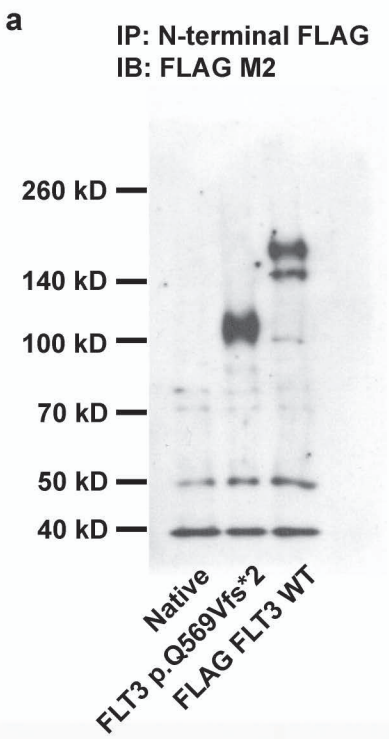
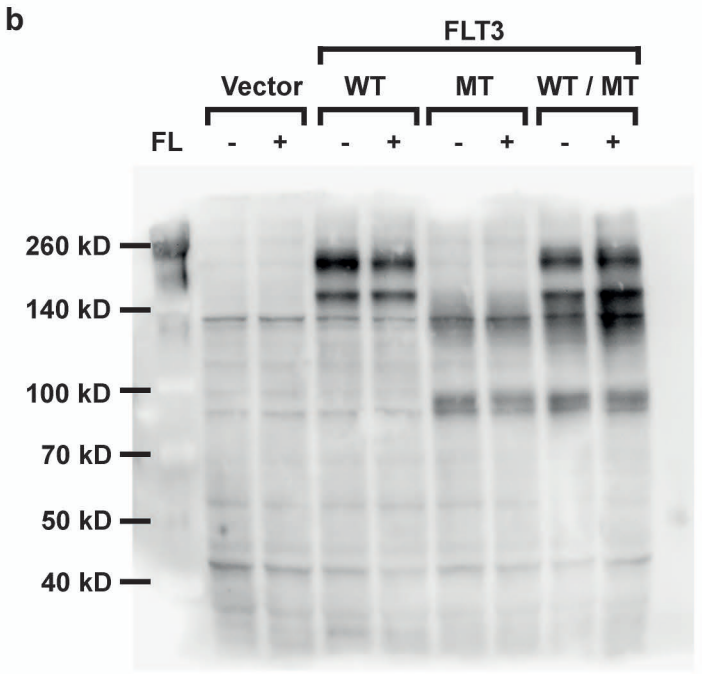
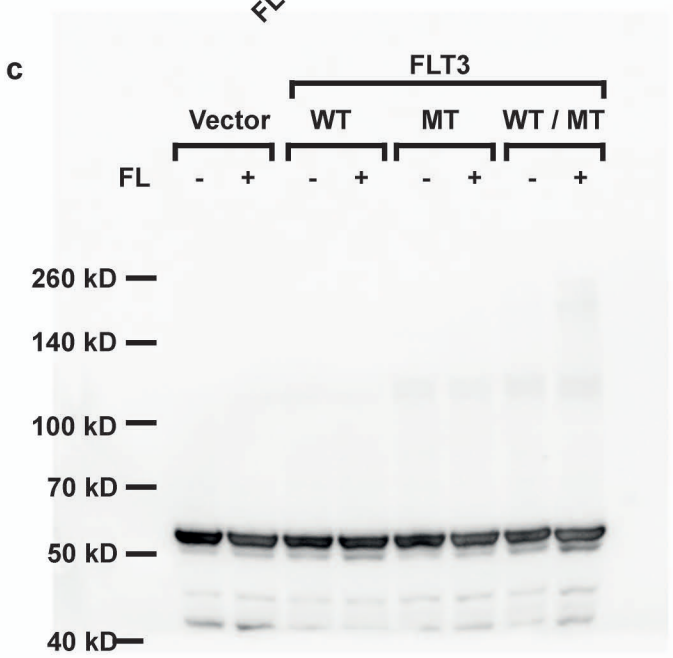


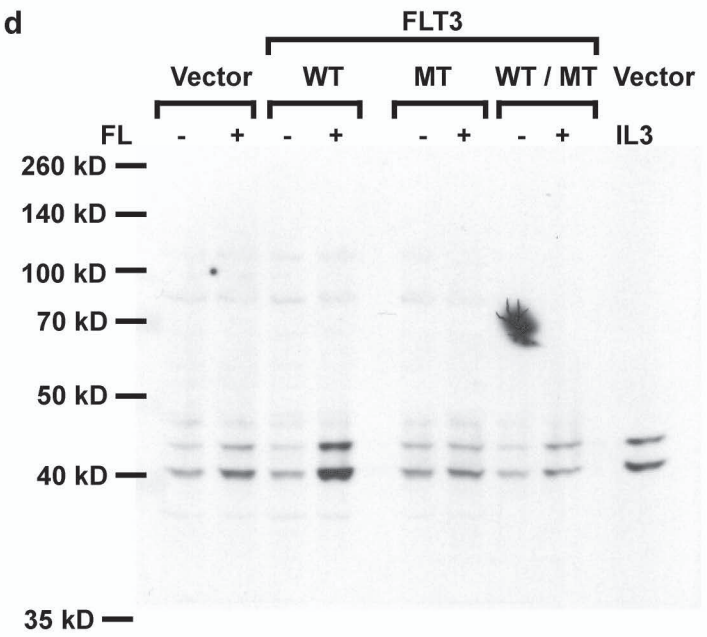

**Figure S3: Binding capacity for biotinylated FLT3 ligand.** Ba/F3 cells stably expressing FLT3 p.Q569Vfs*2 were incubated with biotinylated human FLT3 ligand (FL). Receptor bound biotinylated FL was detected with streptavidin-APC using flow cytometry. As a negative control biotinylated soybean trypsin inhibitor was used. Different amounts of unbiotinylated FL (50 ng; 500 ng) were added to determine the staining specificity. One representative example is shown (left). Binding of biotinylted FL to FLT3 p.Q569Vfs*2 without addition of unbiotinylated FL is set as 1. Shown are mean values of the relative mean fluorescence intensity ratios (MFI) ± SEM of at least three independent experiments (right); ** $p<0.01$.

**Figure S4**



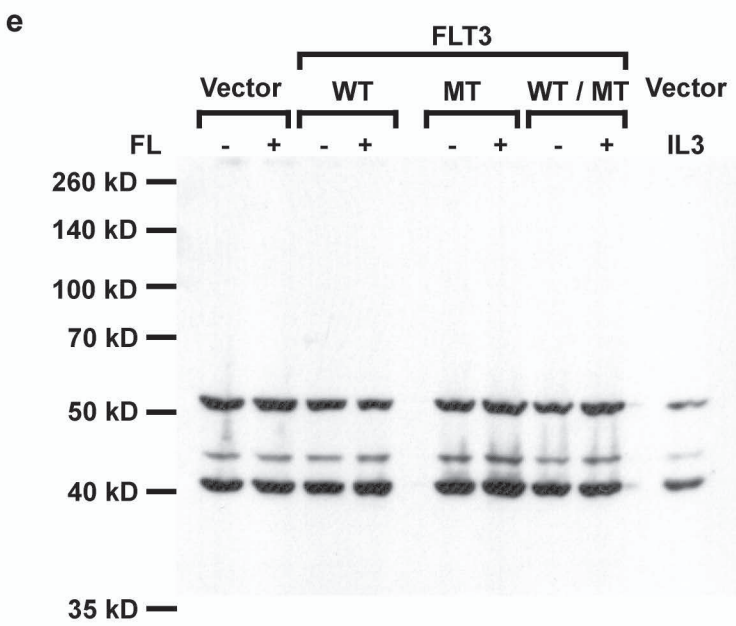




**Figure S4: Full-lenght immuno blots of Figure 1 and 2.** (a) Phoenix Eco cells were transfected with indicated constructs and expression analysed by immuno blot. It is the flull-lenght immuno blot of Figure 1c. (b) Ba/F3 cells stably expressing the indicated constructs were analyzed by immuno blot. After cell lysis the FLT3 protein was detected with an FLT3 antibody. It is the full-length immuno blot of Figure 1d. (c) $\alpha$-Tubulin was detected in the whole cell lysates of stably transduced Ba/F3 cells as loading control. It is the full-lenth immuno blot of Figure 1d. (d) Full-length immuno blot of Figure 2c. Phosphorylation of ERK was analyzed of stably transduced Ba/F3 cells. (e) First ERK and then $\alpha$-Tubulin were analyzed on the blot. It is the full-length immuno blot of Figure 2c.
(MT = FLT3 p.Q569Vfs*2)

**Figure S5**

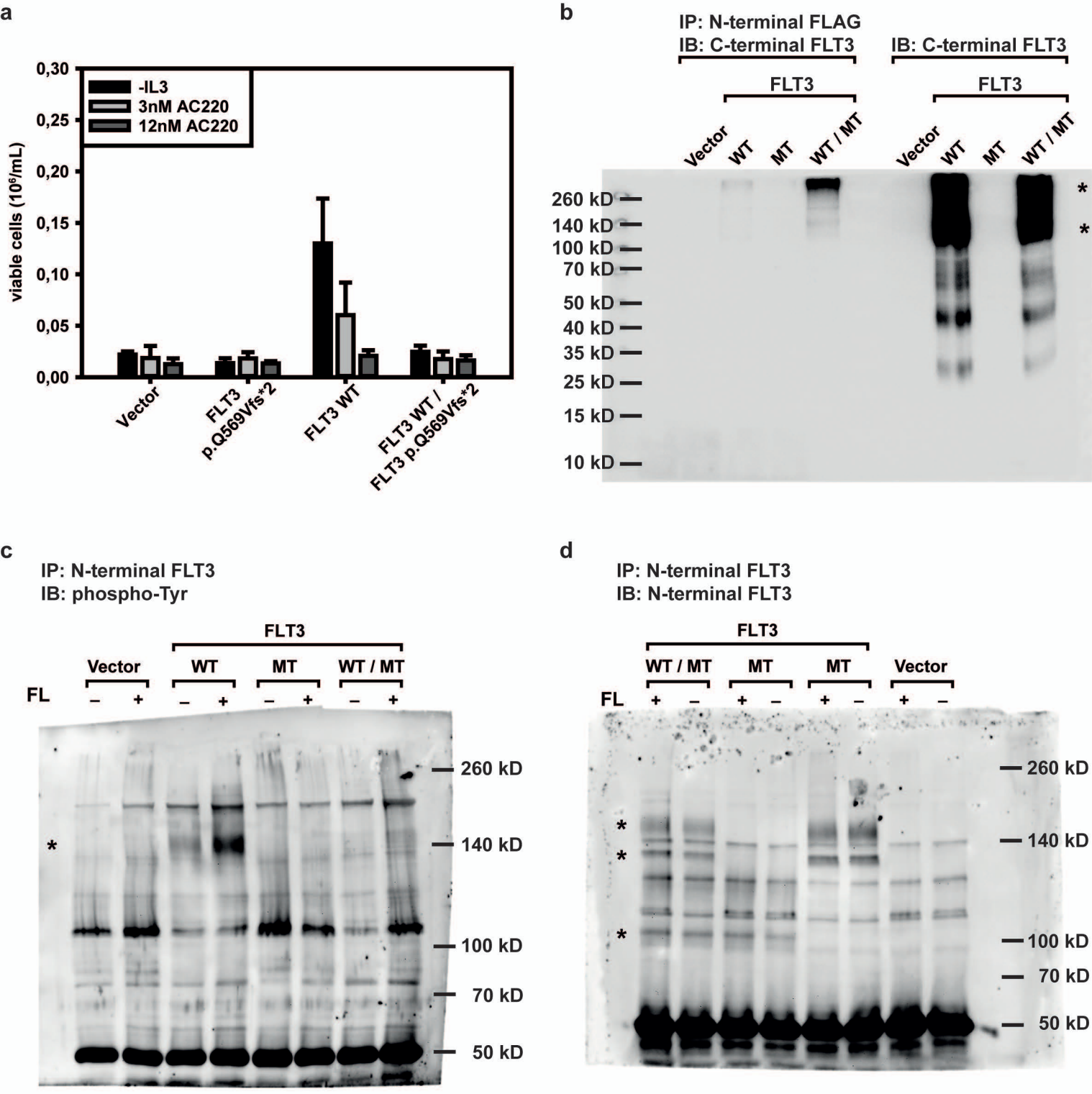



**Figure S5: Dominant negative effect of FLT3 p.Q569Vfs*2 and its heterodimerisation with FLT3 WT.** (a) Ba/F3 cells stably expressing the indicated constructs were seeded at a density of $4\times10^4$/mL in the abscence of cytokines and indicated concentrations of AC220. Viable cells were counted by trypan blue exclusion after 72 hours. Shown are mean values ± SEM of three independent experiments. (b) HEK 293T were transfected with indicated consructs. After lysis FLT3 p.Q569Vfs*2 was immunoprecipitated via its FLAG-tag from whole cell lysates. SDS-gel was run without reducing agents and in MOPS buffer. After blotting the FLT3 WT was detected with a C-terminal FLT3 antibody (* indicating FLT3 dimer and monomer; MT = FLT3 p.Q569Vfs*2). (c,d) From whole cell lysates of stable Ba/F3 cells the FLT3 protein was immunoprecipitated with an N-terminal FLT3 antibody (SF1.340) (* indicating FLT3 specific bands; MT = FLT3 p.Q569Vfs*2). After blotting the phosphorylation status of FLT3 was detected by a pTyr antibody (c) and total FLT3 protein with a different N-terminal FLT3 antibody (4B12) (d).