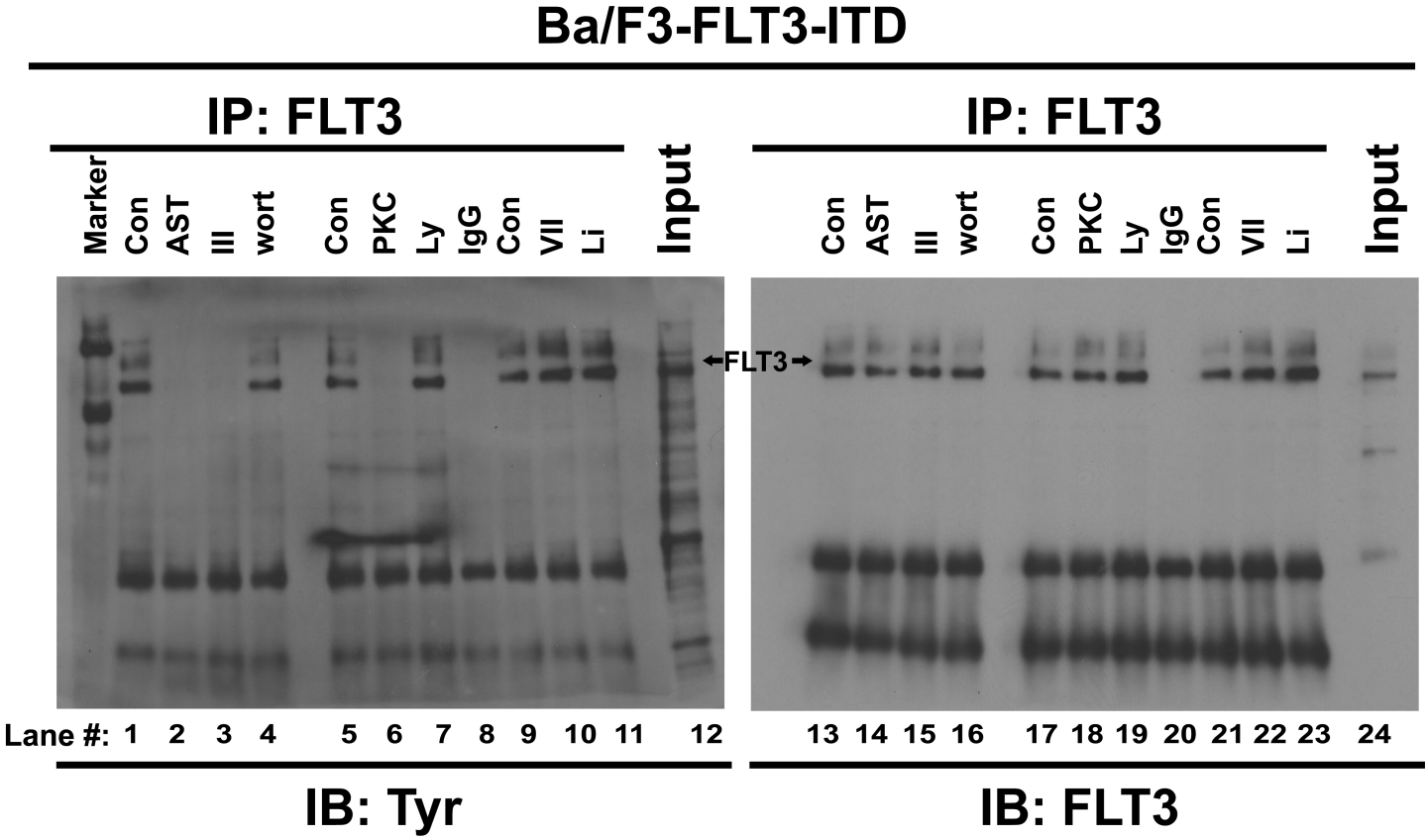
Supplementary Figure Legend

BaF3-FLT3-ITD cells were treated with the inhibitors of FLT3 kinase [0.05 μM PKC412, 0.1 μM AST 487 (AST) and inhibitor III (III)], the inhibitors of the PI3K/AKT pathway [20 μM Ly294002 (Ly) and 0.2 μM Wortmannin (Wort)], and the inhibitors of GSK-3β (10 mM LiCl, 0.5 μM inhibitor VII), or DMSO (Con) for 30 minutes to one hour. The treated cells were harvested and lysed in NP-40 lysis buffer for 30 minutes. Immunoprecipitations were performed using 500 μg of total protein from each treatment as starting materials following a protocol suggested by Santo Cruz biotechnology. Briefly, the reaction volumes were equalized to 500 μl using NP-40 lysis buffer. The samples were incubated with 1μg of the FLT3 antibody for one hour, followed by addition of 25 μl of the protein A/G beads (Santa Cruz Biotechnology). The immunoprecipitation was carried out with rotation at 4˚C overnight. After washing, samples were boiled in SDS-PAGE sample buffer and fractionated by electrophoresis in 8 % polyacrylamide gels and subjected to immunoblotting analysis by first probed with anti tyrosine phosphospecific antibody (pTyr, left panel), the membrane was then stripped and reprobed with anti FLT3 antibody (FLT3, right panel).

Sup Figure


Ba/F3-FLT3-ITD
IP: FLT3
IP: FLT3
Marker
Con
AST
III
wort
Con
PKC
Ly
IgG
Con
VII
Li
Input
Con
AST
III
wort
Con
PKC
Ly
IgG
Con
VII
Li
Input
FLT3
Lane #: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
IB: Tyr
IB: FLT3