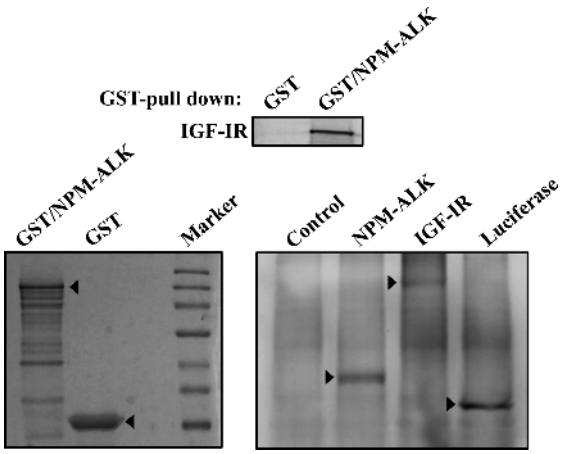


Figure W1. Physical association between NPM-ALK and IGF-IR. GST pull-down assay was performed by incubating recombinant GST–NPM-ALK with *in vitro* translated IGF-IR. GST pull-down of the complex was followed by WB using anti–IGF-IR antibody (upper panel). The bottom left panel shows the recombinant GST–NPM-ALK (arrowheads). The bottom right panel shows *in vitro* translated IGF-IR protein (arrowheads). Luciferase and NPM-ALK were included as positive controls (arrowheads).

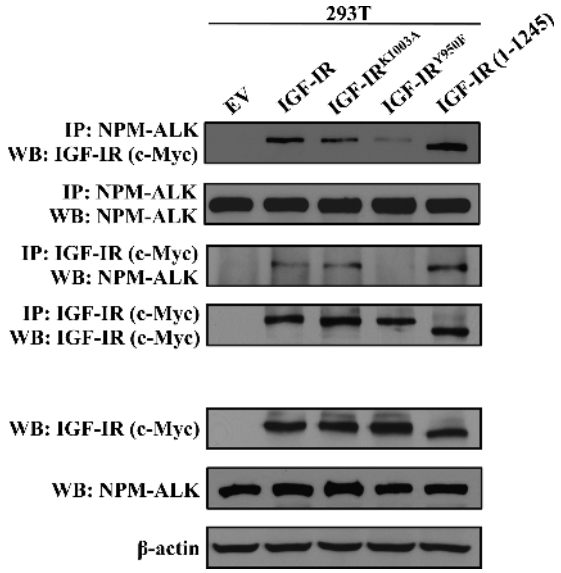


Figure W2. Tyr^{950} of IGF-IR is the site of its association with NPM-ALK. Transfection studies in 293T cells showed that the association of IGF-IR with NPM-ALK was remarkably decreased when the Tyr^{950} residue of IGF-IR was mutated to phenylalanine. The IGF-IR (1–1250) mutant lacks the C terminus segment. The levels of expression of NPM-ALK and IGF-IR and its different mutants are illustrated in the lower panel.

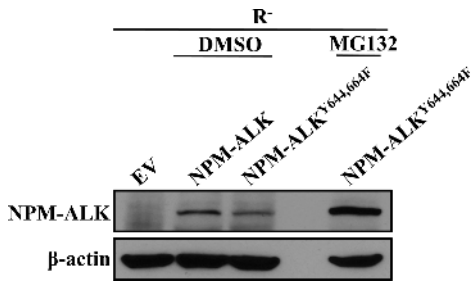
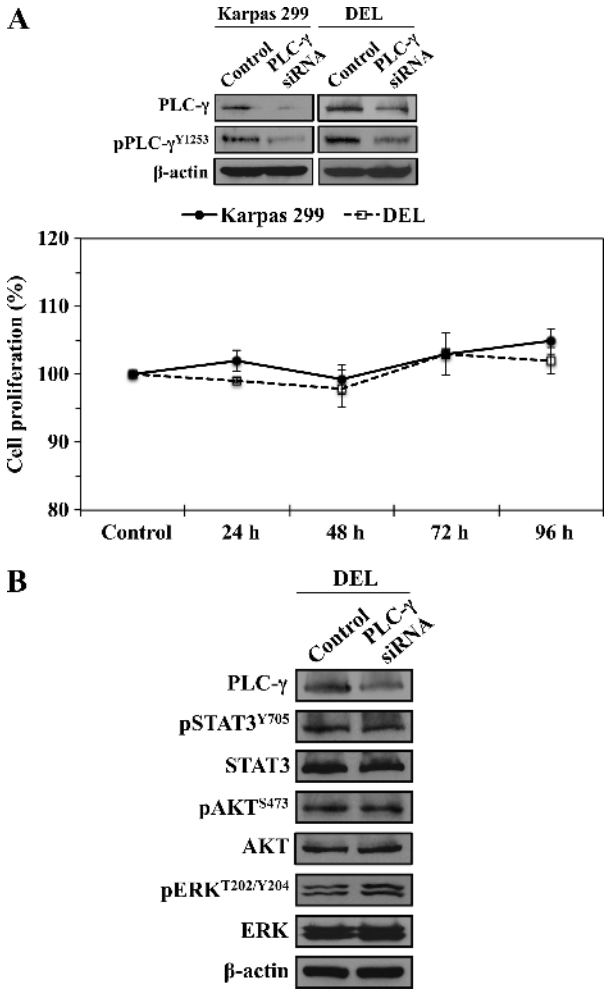


Figure W3. Effect of MG132 on NPM-ALK^{Y644,664F} expression. Treatment of R$^-$ cells with MG132 significantly increased the levels of transfected NPM-ALK^{Y644,664F} protein, which suggests that NPM-ALK^{Y644,664F} expression is regulated by the protein degradation pathway. β-Actin shows equal protein loading.



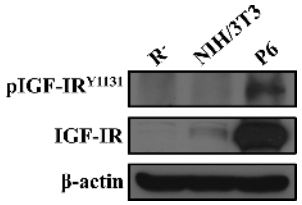


Figure W4. Effects of specific targeting of PLC-γ on NPM-ALK^{+} T cell lymphoma cells. (A) Specific targeting of PLC-γ by siRNA decreased its basal levels in Karpas 299 and DEL cell lines, which was also associated with down-regulation of pPLC-γ^{Y1253} (upper panel). However, treatment with PLC-γ siRNA for up to 96 hours did not affect the proliferation of Karpas 299 and DEL cells (lower panel). β-Actin shows equal protein loading. (B) Treatment of DEL cell line with PLC-γ siRNA did not affect the phosphorylation levels of STAT3, AKT, or ERK. β-Actin shows equal protein loading. Similar results were obtained in Karpas 299 cells (data not shown).

Figure W5. Expression of IGF-IR in NIH/3T3 cells. WB showed that the NIH/3T3 cell line expresses small levels of IGF-IR protein. In addition, pIGF-IR^{Y1131} is not present in these cells. Lysates from R^{-} and P6 cells were used as negative and positive controls, respectively, for the expression of IGF-IR/pIGF-IR^{Y1131}. β-Actin supported equal loading of the proteins.

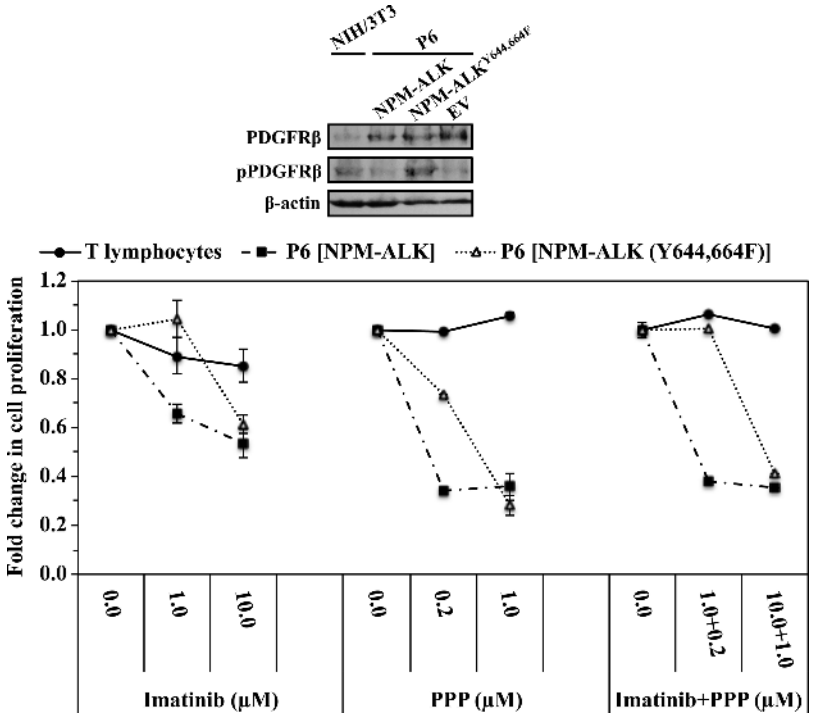


Figure W6. Effects of inhibition of IGF-IR and PDGFRβ on the proliferation of P6 cells stably transfected with NPM-ALK or NPM-ALK^{Y644,664F}. PDGFR and pPDGFR^{Y1021} are expressed in P6 cells stably transfected with EV, NPM-ALK, or NPM-ALK^{Y644,664F} (upper panel). The expression of pPDGFR^{Y1021} is higher in P6 cells transfected with NPM-ALK^{Y644,664F}, suggesting that increased phosphorylation of PDGFR might be due to the lack of association and interactions between NPM-ALK and IGF-IR. NIH/3T3 cells were used as a positive control. At 48 hours, targeting IGF-IR using PPP, PDGFR using imatinib mesylate or PPP and IGF-IR by combining the two inhibitors induced a concentration-dependent decrease in the viability of P6 cells transfected with NPM-ALK or NPM-ALK^{Y644,664F} (lower panel). However, the two types of P6 cells were more sensitive to the effects of PPP compared with imatinib (PPP: $P < .0001$ for both NPM-ALK and NPM-ALK^{Y644,664F}; imatinib: $P < .001$ for NPM-ALK and $P = .001$ for NPM-ALK^{Y644,664F}). In addition, the effects of PPP alone appeared to be almost comparable to the effects of PPP and imatinib combined together ($P = .0003$ for NPM-ALK and $P = .0002$ for NPM-ALKY^{644,664F}). It is possible that these results were because P6 cells express higher levels of IGF-IR/pIGF-IR than PDGFR/pPDGFR. At low concentrations, P6 cells transfected with NPM-ALK were more sensitive to the effects of the different treatment regimens than P6 cells transfected with NPM-ALK^{Y644,664F}. At higher concentrations, the two types of P6 cells demonstrated similar responses. Further studies are required to explore these observations. Normal human T cells were used as a negative control for the effects of PPP and imatinib. The concentrations of PPP and imatinib used to treat P6 cells were based on previously published data related to these inhibitors in mouse fibroblasts [32,64].