Supplemental Figure 1

Figure 1C



Figure 3A

| | | MU | TZ5 | | Μ | HH-(| CAL | L4 | KOPN49 | | | | | |
|-------------------------|------|------|------|------|------|------|------|------|--------|------|-----------------|------|--|--|
| Ruxo (1µM) | - | + | - | + | - | + | - | + | - | + | - | + | | |
| Gilt (1µM) | - | - | + | + | - | - | + | + | - | - | + | + | | |
| pCRKL | - | - | | | - | - | - | - | 1 | - | | | | |
| | 1.00 | 0.89 | 0.26 | 0.26 | 1.00 | 0.85 | 0.25 | 0.21 | 1.00 | 0.83 | 0.21 | 0.20 | | |
| CRKL | 1 | - | - | - | - | - | - | I | ł | - | - | - | | |
| | 1.00 | 1.09 | 1.04 | 0.90 | 1.00 | 0.88 | 0.91 | 1.08 | 1.00 | 0.87 | 0.95 | 1.26 | | |
| pSTAT5 | - | | | | - | • | - | | | | 614369 10110 | | | |
| | 1.00 | 0.00 | 0.16 | 0.00 | 1.00 | 0.01 | 0.22 | 0.00 | 1.00 | 0.00 | 0.05 | 0.00 | | |
| STAT5 | - | - | - | - | - | = | - | - | | - | - | - | | |
| | 1.00 | 1.14 | 1.08 | 0.94 | 1.00 | 0.92 | 0.95 | 0.93 | 1.00 | 0.77 | 0.85 | 1.11 | | |
| pERK1/2 | - | - | | | - | - | | - | | - | | - | | |
| | 1.00 | 0.93 | 0.01 | 0.00 | 1.00 | 1.13 | 0.51 | 0.59 | 1.00 | 1.34 | 0.24 | 0.21 | | |
| ERK1/2 | - | - | - | - | - | - | - | - | | - | - | - | | |
| | 1.00 | 1.02 | 1.31 | 1.08 | 1.00 | 0.88 | 0.87 | 0.83 | 1.00 | 0.83 | 1.00 | 1.47 | | |
| PART | | | 0.00 | 0.00 | | | 0.00 | 0.00 | | 4.40 | 0.00 | 0.00 | | |
| | 1.00 | 1.14 | 0.00 | 0.00 | 1.00 | 0.94 | 0.00 | 0.00 | 1.00 | 1.16 | 0.00 | 0.00 | | |
| ANI | | - | - | - | - | - | - | - | - | 8108 | - | 1010 | | |
| 0005/000 | 1.00 | 0.99 | 0.67 | 0.61 | 1.00 | 1.58 | 1.30 | 1.05 | 1.00 | 0.53 | 0.71 | 0.88 | | |
| pS6 ^{S235/236} | | - | | | - | - | | | | - | - | - | | |
| | 1.00 | 0.42 | 0.01 | 0.00 | 1.00 | 0.25 | 0.00 | 0.01 | 1.00 | 0.35 | 0.15 | 0.15 | | |
| pS6 ^{S240/244} | - | - | | - | - | | | - | | - | | | | |
| | 1.00 | 0.81 | 0.27 | 0.64 | 1.00 | 0.75 | 0.57 | 0.55 | 1.00 | 0.40 | 0.20 | 0.19 | | |
| S6 | - | - | - | - | - | - | - | | - | - | - | - | | |
| | 1.00 | 1.05 | 0.96 | 0.74 | 1.00 | 1.19 | 1.02 | 1.13 | 1.00 | 0.77 | 0.89 | 1.15 | | |
| β-actin | - | - | - | - | - | - | - | - | - | - | - | - | | |

Supplemental Figure 1 (continued)

| Figure 3B | | | | | | | | | | | | | | | | | |
|--------------------------|---------|--------|--------|----------|--------------|--------|--------|--------|---------|--------|--------|--------|---------|--------|--------|--------|--|
| | BALL091 | | | | BALL137 | | | | BALL142 | | | | BALL160 | | | | |
| Ruxo (1μM) Gilt (1μM) | - | + - | - + | + + | - | + - | - + | + + | - | + - | - + | + + | - | + - | - + | + + | |
| pCRKL | - | - | - | - | - | - | - | | • | - | - | | - | - | | • | |
| CRKL | 1.00 | 1.15 | 0.26 | 0.15 | 1.00 | 1.45 | 0.14 | 0.00 | 1.00 | 0.68 | 0.08 | 0.03 | 1.00 | 1.00 | 0.22 | 0.01 | |
| | 1.00 | 1.34 | 1.58 | 1.34 | 1.00 | 1.04 | 1.07 | 1.06 | 1.00 | 0.98 | 0.73 | 0.84 | 1.00 | 1.33 | 1.77 | 1.39 | |
| pSIAI5 | 1.00 | 0.00 | 0.00 | 0.00 | NA | NA | NA | NA | 1.00 | 0.02 | 0.16 | 0.00 | NA | NA | NA | NA | |
| STAT5 | - | - | - | = | 6 Barrent | | | | = | = | = | = | = | = | = | = | |
| nFBK1/2 | 1.00 | 1.37 | 1.15 | 1.19 | 1.00 | 1.37 | 1.21 | 1.20 | 1.00 | 1.06 | 0.89 | 0.71 | 1.00 | 1.23 | 1.20 | 1.11 | |
| peritti | 1.00 | 0.76 | 0.34 | 0.20 | 1.00 | 1.17 | 0.41 | 0.30 | 1.00 | 0.66 | 0.17 | 0.17 | 1.00 | 0.97 | 0.52 | 0.30 | |
| ERK1/2 | 1.00 | 1.04 | 1.07 | 1.05 | 1.00 | 0.89 | 0.95 | 1.04 | 1.00 | 0.89 | 0.80 | 0.79 | 1.00 | 1.31 | 1.42 | 1.73 | |
| pS6 ^{S235/236} | - | - | ingi | - opense | - | - | - | | - | - | adate | | - | - | - | - | |
| ~~~ \$240/244 | 1.00 | 0.86 | 0.31 | 0.16 | 1.00 | 0.84 | 0.12 | 0.10 | 1.00 | 0.79 | 0.21 | 0.40 | 1.00 | 0.91 | 0.52 | 0.35 | |
| μoo | 1.00 | 1.14 | 0.46 | 0.32 | 1.00 | 0.73 | 0.18 | 0.19 | 1.00 | 0.23 | 0.20 | 0.24 | 1.00 | 0.92 | 0.70 | 0.48 | |
| S6 | 1 | - | - | - | | | - | | - | - | - | - | - | - | - | - | |
| • | 1.00 | 1.24 | 0.99 | 0.86 | 1.00 | 1.07 | 1.06 | 1.15 | 1.00 | 1.08 | 1.20 | 0.89 | 1.00 | 1.66 | 2.02 | 1.45 | |
| β-actin | - | - | - | - | - | - | - | | | | | | - | - | - | - | |

Figure 5B



Supplemental Figure 1. Quantification of Western Blot signals. Densitometric analysis of western blot signal intensity was performed using ImageJ software. Protein amount of target protein was normalized to that of β -actin in each sample. The amount of the phosphorylated form of target protein was normalized to that of its total protein.



Supplemental Figure 2. Growth curve of MUTZ5 cells in the presence of Ruxolitinib. **(A)** Growth curves were generated at various Ruxolitinib concentrations. Each condition was assessed in triplicate, and data are represented as means ± SD. **(B)** An IC50 value was calculated based on assessment for viability 10 days after Ruxolitinib treatment.

Supplemental Figure 3



Supplemental Figure 3. pCRKL levels upon FLT3i/TKI treatment (1 μ M, 2 h) in MUTZ5 cells. Gilteritinib, but not Quizartinib, significantly reduced pCRKL levels. Dasatinib serves as control.



Supplemental Figure 4. ALL cell engrafment in PB. (A) Representative FACS profile of PB 30 days after transpantation.
(B) Proportions of human ALL cells in PB 30 days after transpantation in each experimental group (related to Figure 4).
(C) Proportions of human ALL cells in PB 30 days after transpantation in each experimental group (related to Figure 5).