Supplemental Figure 1. Lack of binding to FLT3 homologs and characterization of binding to Fc receptors

A) Summary of Biacore binding data of CLN-049 using human, murine, and rhesus FLT3. B) Summary of Biacore binding data of CLN-049 using recombinant FcyR and FcRn.

| Ligand          | Ka (M⁻¹s⁻¹) | Kd (s⁻¹) | Kₘ (nM) |
|-----------------|-------------|----------|---------|
| Human FLT3      | 1.22E+06    | 3.25E-04 | 0.27    |
| Murine FLT3     | ND          | ND       | >1E+06  |
| Rhesus FLT3     | ND          | ND       | >1E+06  |

| Ligand          | CLN-049     | IgG1 Control | IgG4 Control |
|-----------------|-------------|--------------|--------------|
| FcyRIIA₁₇₅F     | >1E+06      | 4070         | >1E+06       |
| FcyRIIA₁₇₆V     | >1E+06      | 1270         | >1E+06       |
| FcyRIIIB        | >1E+06      | 4760         | >1E+06       |
| FcyRII₂₈₁     | >1E+06      | 12800        | >1E+06       |
| FcyRII₄₂₇₇     | >1E+06      | 11700        | >1E+06       |
| FcyRII₂₈₁     | >1E+06      | 24900        | 58300        |
| FcyRI         | >1E+06      | 3.3          | 1.1          |
| FcRn (pH 6.0)  | 3930        | 2640         | 5500         |
| FcRn (pH 7.4)  | >1E+06      | >1E+06       | >1E+06       |
**Supplemental Figure 2. Lack of sensitivity of CLN-049 to FLT3 mutational status**

A) Summary of FLT3 genotype from evaluated AML cell lines. B) Potency of CLN-049 as measured by cytotoxicity, CD8 activation, and CD4 activation against AML cell lines of indicated FLT3 genotype.
Supplemental Figure 3. FLT3 expression levels on MOLM-13 and Jurkat cells. The indicated cell lines were incubated with PE-labeled 4G8 anti-FLT3 antibody or isotype control and analyzed by flow cytometry.
Supplemental Figure 4. Lack of interference of soluble FLT3L or soluble FLT3 at supraphysiological levels
MOLM-13 cells were labeled with cell proliferation dye and pre-incubated with 10 ng/ml sFLT3L or 150 ng/ml sFLT3L, as indicated, prior to addition of CLN-049 and PBMC at an E:T ratio of 2:1 for 72h. Shown are lysis curves, as flow cytometrically assessed by 7-AAD uptake in MOLM-13 cells.
**Supplemental Figure 5. Flow cytometry gating schema to evaluate in vitro toxicity**

A) Gating schema to identify DC subsets in peripheral blood. B) Representative flow cytometry plots demonstrating that while all evaluable donors had observable pDC and cDC populations on 0h, only ~50% of donors had an observable pDC population after 72h. C) Gating schema to identify CD34+ subsets in BM aspirates.