## Supplemental methods:

### Flow cytometric analysis of cell surface proteins

Leukocytes were isolated using Immunoprep Reagent System (Beckman Coulter) or Ficoll-Paque separation. Cells were stained sequentially with a mouse-anti-human Mer antibody or mouse IgG1 isotype control, a PE-conjugated donkey-anti-mouse secondary antibody, and anti-human CD19 and anti-human CD45 fluorophore-conjugated antibodies. The following cell surface antibodies were used for flow cytometric analysis: mouse-anti-human Mer PE (FAB8912P), mouse IgG1 isotype control (MAB002) anti-mouse Mer conjugated with FITC (R&D Systems); CD19 PE-Cy7 (557835), B220 APC (553092), mouse CD43 PE (553271), biotinylated mouse CD25 (553069) and streptavidin PE-Cy7(557598) (BD Biosciences); CD45 PE-Texas Red (IM2710U; Beckman Coulter); donkey-anti-mouse APC (715-136-150), donkey-anti-mouse PE (715-116-150) (Jackson ImmunoResearch). The mouse-anti-human Mer monoclonal antibody (Mer 590) used for analysis of prospective patient samples and *in vitro* studies was produced by the Graham laboratory as previously described.[1] Fluorescence was measured using a FC-500 flow cytometer (Beckman Coulter) for patient samples or a Gallios flow cytometer (Beckman Coulter) for cell lines, both with CXP data analysis software (Beckman Coulter). The following antibodies were used to assess hematopoietic progenitors: Sca-1 (Ly-6A/E)-FITC (eBioscience) CD127-PerCP-Cy5.5 (eBioscience), CD34-eFluor660 (eBioscience), CD13/32 clone 93, (eBioscience), c-kit-PE-Cy7 (Biolegend), Flk2/Flt3-PE (BD Biosciences) and Lineage antibody cocktail-V450 (BD Biosciences). Single cell suspensions of bone marrow were run on a Gallios flow cytometer (Beckman Coulter) and analyzed using FlowJo version X (Tree Star, Inc.).

**Immunoblot Analysis**

Whole cell lysates were prepared in a buffer containing 50mM HEPES (pH 7.5), 150mM NaCl, 10mM EDTA, 10% (v/v) glycerol, 1% (v/v) Triton X-100, 1mM sodium orthovanadate, 0.1mM sodium molybdate, and Complete Mini protease inhibitors (Roche Diagnostics). Lysates were incubated on ice for 15-20 minutes and soluble protein supernatant was collected after centrifugation. The following antibodies were used for immunoblot analysis according to manufacturer recommendations: anti-human Mer (Epitomics Inc.); anti-Actin (sc-1616, Santa Cruz Biotechnology); anti-tubulin (#2125S), anti-phospho-p44/42 MAPK (ERK1/2, Thr202/Tyr204, #9106), anti-p44/42 MAPK (#9102), anti-phospho-p38 MAPK (Thr180/Tyr182, #9211S), anti-p38 MAPK (#9217), anti-phospho-AKT (Ser473, #9271L), anti-AKT (#9272), anti-phospho-mTOR (Ser2448, #2971), anti-mTOR (#2972) anti-PARP (#9542), anti-Caspase 3 (#9665), anti-Caspase 8 (#9746S), anti-Bax (#5023), anti-Bcl-XL (#2762), anti-Puma (#4976) (Cell Signaling Technology); anti-NOXA (AB5761), anti-PI3K (05-217) (Millipore); goat-anti-mouse IgG-HRP, goat-anti-rabbit IgG-HRP (BioRad). Proteins were visualized by horseradish peroxidase chemiluminescence detection (Perkin-Elmer).

**Real-Time quantitative RT-PCR**

Diluted RNA (100 ng or 1 ng) was used as a template for real time RT-PCR in the presence of primer pairs homologous to Mer and 18s rRNA, respectively. Oligonucleotides and probes were designed using Primer Express software (Applied Biosystems). Reactions were performed in a final volume of 25 µl containing 1× TaqMan Universal PCR Master Mix, 10 U Anti-RNase (Fisher Scientific), 6.25 U MMLV-RT (Ambion), 50 nM 18S rRNA probe (5′-

TGCTGGCACCAGACTTGCCCTC) or 200 nM Mer probe (5′-CAGGCCTGACGGAGATGGCGG), and optimized concentrations of gene-specific primers. Probes were conjugated to VIC or 6FAM reporter dyes, respectively, and TAMRA quencher dye (Applied Biosystems). Primer sequences and concentrations used are as follows: Mer forward 5′-CCCCCTCCGTGCTAACTGTT, 300 nM; Mer reverse 5′-TGTGGGCCTCACAACTGAAG, 900 nM; 18S rRNA forward 5′-CGGCTACCACATCCAAGGAA, 50 nM; 18S rRNA reverse 5′GCTGGAATTACCGCGGCT, 50nM. Three independent experiments were performed in triplicate and mean threshold cycle values were used for all calculations. To confirm the absence of DNA contamination, control reactions were performed in duplicate for all samples in the absence of reverse transcriptase.

**Interaction models**

Interactions between Mer knockdown and chemotherapeutic agents were assessed using the Bliss independence model (reviewed in (1)). The frequency of affect (Fa) expected for an additive interaction between Mer inhibition and chemotherapeutic agents was calculated using the following formula: $Fa(1+2) = Fa1 + (1-Fa1) * Fa2$ where Fa1 and Fa2 are the effects of shRNA-mediated Mer inhibition (Fa1) and treatment with chemotherapy (Fa2). Statistically significant differences ($p < 0.05$) were determined using the two-tailed student's paired t test. All statistical analyses were performed using Graphpad Prism v4 software (Graphpad Software, Inc., La Jolla, CA).

**Supplemental Table S1.** Evaluation of newly diagnosed B-ALL patient samples.

| | | Mer Status | | |
|---|---|---|---|---|
| **Sample ID** | **Cytogenetics** | **Western** | **Flow Cytometry** | **RT-PCR** |
| 611729 | t(1;19) | + | + | + |
| 608225 | t(1;19) | + | + | IS |
| 607913 | t(1;19) | + | + | IS |
| 612296 | t(1;19) | + | + | + |
| 609399 | t(1;19) | + | + | IS |
| 611758 | t(1;19) | + | + | + |
| 609396 | t(1;19) | + | + | IS |
| 612373 | t(1;19) | + | + | + |
| POG 1999-00102 | t(1;19) | + | + | IS |
| POG 1999-02042 | t(1;19) | IS | + | IS |
| POG 1999-01862 | t(1;19) | + | + | IS |
| POG 1999-00342 | t(1;19) | + | + | IS |
| POG 1999-00182 | t(1;19) | + | + | IS |
| 5091/3534 | t(1;19) | + | IS | IS |
| 5565/3401 | t(1;19) | + | IS | IS |
| 5587/2189 | t(1;19) | + | IS | IS |
| 6034/3797 | MLL infant | - | - | - |
| 6123/3892 | MLL not infant | - | IS | IS |
| 6052/3821 | t(12;21) | - | - | - |
| 6568 | t(12;21) | - | - | - |
| 6117/3816 | hyperdiploid | - | - | - |
| 6121/3890 | hyperdiploid | IS | - | IS |
| 6155 | hyperdiploid | IS | - | IS |
| 6702 | hyperdiploid | - | - | IS |
| 6514 | hyperdiploid | - | - | IS |
| 6542 | hyperdiploid | - | - | - |
| 6043/3809 | hyperdiploid | IS | - | IS |
| 6650 | normal | + | + | + |

t(1,19) = translocation which results in expression of the fusion protein E2A-PBX1
t(12;21) = translocation which results in expression of the fusion protein TEL-AML
MLL = rearrangement involving the mixed-lineage leukemia protein
IS = insufficient sample

**Supplemental Table S2.** Prospective flow cytometric analysis of pediatric patients with t(1;19) pre-B ALL

| Sample ID | Age (years), Sex | Mer % by flow cytometry | Recurrent Disease |
|---|---|---|---|
| 203 | 1, F | **98.9%** | No |
| 268 | 10, M | **91.4%** | No |
| 313 | 2, M | **85.5%** | No |
| 99 | 2, F | **80.6%** | No |
| 317 | 2, F | **40.6%** | No |
| 228 | 2, M | **18.7%** | No |
| 261 | 3, M | **12.5%** | Yes |
| 1 | 12, F | 8.0% | No |
| 32 | 10, M | 0.7% | No |

**Supplemental Table S3**. **Inhibition of Mer results in synergistic induction of leukemia cell death in combination with cytotoxic chemotherapies in B-ALL cells.** Cultures of the 697 (top panel) and REH (bottom panel) shMer cell lines were exposed to the indicated concentrations of chemotherapy for 48 hours and apoptotic and dead cells were identified by flow cytometry after staining with YoPro-1-iodide and propidium iodide dyes [see Figure 5]. The percent of apoptotic and dead cells relative to cultures treated with vehicle only were determined. The expected frequency of affected (Fa) for an additive interaction between Mer inhibition and treatment of chemotherapy was determined using the Bliss additivity model[2] and is shown (Additive). Statistically significant (two-tailed student's paired t-test p-value) increases in the Fa mediated by chemotherapeutics in combination relative to the values expected for an additive interaction indicate synergy. Mean values and standard errors were derived from at least three independent experiments.

| 697 | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Chemotherapy** | **Frequency of Affected (Fa)** | | | | | **p value** | **n** |
| | **shMer1** | **shMer4** | **ChemoTx** | **Additive** | **Combination** | | |
| Methotrexate | 0.007 +/- 0.021 | - | 0.061+/-0.031 | 0.069 +/- 0.023 | 0.361 +/- 0.076 | 0.0040 | 5 |
| | - | 0.039+/- 0.018 | | 0.099 +/- 0.014 | 0.350+/- 0.062 | 0.0120 | 5 |
| Dexamethasone | 0.022 +/- 0.016 | - | 0.111 +/- 0.015 | 0.130 +/- 0.022 | 0.395 +/- 0.035 | 0.0006 | 7 |
| | - | 0.051+/- 0.023 | | 0.157 +/- 0.020 | 0.192 +/- 0.019 | 0.0211 | 7 |
| Vincristine | 0.019+/-0.011 | - | 0.043+/-0.017 | 0.061+/-0.014 | 0.299+/-0.088 | 0.0219 | 6 |
| | - | 0.089+/-0.013 | | 0.128+/-0.019 | 0.501+/-0.087 | 0.0034 | 6 |
| L-Asparaginase | 0.006+/-0.006 | - | 0.099 +/- 0.014 | 0.104 +/- 0.016 | 0.281 +/- 0.044 | 0.0197 | 5 |
| | - | 0.075 +/- 0.013 | | 0.166 +/- 0.022 | 0.286 +/- 0.037 | 0.0299 | 5 |

| REH | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Chemotherapy** | **Frequency of Affected (Fa)** | | | | | **p value** | **n** |
| | **shMer1** | **shMer4** | **ChemoTx** | **Additive** | **Combination** | | |
| Methotrexate | 0.028 +/- 0.011 | - | 0.294+/-0.060 | 0.315 +/- 0.055 | 0.536 +/- 0.050 | 0.0138 | 6 |
| | - | 0.000+/- 0.009 | | 0.815 +/- 0.038 | 0.898 +/- 0.029 | 0.0092 | 5 |
| Dexamethasone | 0.043 +/- 0.009 | - | 0.018 +/- 0.011 | 0.060 +/- 0.019 | 0.042 +/- 0.011 | 0.2798 | 3 |
| | - | 0.000+/- 0.008 | | 0.017 +/- 0.020 | 0.000 +/- 0.007 | 0.3640 | 3 |
| Vincristine | 0.029+/-0.008 | - | 0.073+/-0.036 | 0.010+/-0.033 | 0.400+/-0.091 | 0.0308 | 5 |
| | - | 0.007+/-0.009 | | 0.079+/-0.038 | 0.742+/-0.093 | 0.0013 | 5 |
| L-Asparaginase | 0.040+/-0.011 | - | 0.026 +/- 0.006 | 0.065 +/- 0.015 | 0.192 +/- 0.004 | 0.0054 | 7 |
| | - | 0.014 +/- 0.004 | | 0.040 +/- 0.004 | 0.058 +/- 0.012 | 0.1288 | 7 |

## Supplemental Figure Legend

**Figure S1. Mer expression does not correlate with E2A-PBX1 status in human B-cell precursor ALL cell lines.**
Whole cell lysates of human B-ALL cell lines were subjected to western blotting with anti-Mer (~180 kD) antibody. Anti-actin (~43 kD) western blots are presented as a loading control. Cytogenetic and relapse information is indicated.

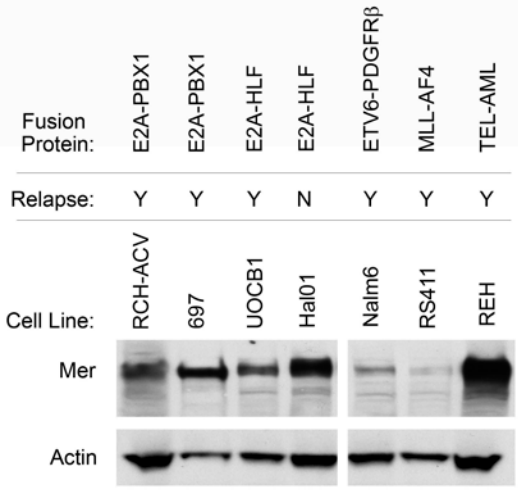
**Figure S2. Mer knockdown in the E2A-PBX1$^{+}$ human B-ALL cell line, 697 and E2A-PBX$^{-}$ B-ALL cell line, REH was confirmed by flow cytometry.** Wild-type cells were infected with lentiviral particles containing one of two independent shRNA constructs targeting Mer (shMer1, shMer4) or GFP (shControl) as a non-silencing control. Mean fluorescence intensity (MFI) of shControl and shMer knockdown 697(A) and REH (B) cells was calculated by subtracting the MFI of cells stained with secondary antibody from the MFI of cells stained with anti-Mer primary and PE secondary antibodies. Using these adjusted MFI values, Mer expression relative to wild-type 697 or REH cells was determined. Three independent measurements, mean values, and standard errors are displayed.

**References:**

1. Rogers, A. E.; Le, J. P.; Sather, S.; Pernu, B. M.; Graham, D. K.; Pierce, A. M.; Keating, A. K., Mer receptor tyrosine kinase inhibition impedes glioblastoma multiforme migration and alters cellular morphology. *Oncogene* **2011**.

2. Berenbaum, M. C., What is synergy? *Pharmacological reviews* **1989,** *41* (2), 93-141.

## Supplemental Figure S1




## Supplemental Figure S2