Supplemental Figure 1

A) Heatmap of CD34+ normalized basal signaling across different conditions.

B) Heatmap showing expression levels of various markers (CD34, CD14, CD19, CD3, CD45, CD61, CD15) across different cell lines (NBM44, sAML1, sAML7, NBM 43, MF 9, MF 23, MF 26, MF 29, MF 20, MF 21, MF 16, MF 20, MF 16, MF 9, MF 7, sAML 1, sAML 7) under different treatments (Run 1, Run 2, Run 3).

C) Heatmap showing the effect of different treatments (Pev, TNFα, Pev + TNFα) on gene expression levels of pCREB across different cell lines (NBM44, sAML1, sAML7) under different conditions.
**Supplemental Fig. 2**

**A**

|     | NBM31 | LRS5 PB |
|-----|-------|---------|
| CD34| ![Image](image1) | ![Image](image2) |
| CD14| ![Image](image3) | ![Image](image4) |

**B**

|     | UT | Rux | Pev | Rux + Pev |
|-----|----|-----|-----|-----------|
| TNFα| ![Image](image5) | ![Image](image6) | ![Image](image7) | ![Image](image8) |
| MIP-1β| ![Image](image9) | ![Image](image10) | ![Image](image11) | ![Image](image12) |
| IL-6| ![Image](image13) | ![Image](image14) | ![Image](image15) | ![Image](image16) |
| IL-8| ![Image](image17) | ![Image](image18) | ![Image](image19) | ![Image](image20) |
| IL-10| ![Image](image21) | ![Image](image22) | ![Image](image23) | ![Image](image24) |

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Further detailed analysis can be found in the main text of the paper.
| Patient Alias | Diagnosis      | JAK2/MPL/CALR mutation | VAF (%) |
|--------------|----------------|------------------------|---------|
| MF2          | MF post ET     | JAK2                   | 36%     |
| MF9          | MF post PV     | JAK2                   | 37%     |
| MF15         | MF post ET     | JAK2                   | 57%     |
| MF16         | MF post PV     | JAK2                   | 73%     |
| MF20         | PMF            | JAK2                   | 96%     |
| MF23         | MF post ET     | JAK2                   | 43%     |
| MF26         | MF post PV     | JAK2                   | 81%     |
| MF27         | MF post ET     | JAK2                   | 94%     |
| MF36         | PMF            | JAK2                   | 88%     |
| sAML1        | AML post MF    | JAK2                   | 56%     |
| sAML7        | AML post MF    | JAK2                   | 95%     |
| MF530675     | PMF            | MPL                    | N/A     |
| MF700869     | MF post ET     | CALR                   | N/A     |
Supplemental figure legends

Supplemental Figure 1. Mass cytometry analysis of primary samples treated with pevonedistat.

A) Heatmap of mass cytometry analysis of CD34+ cells from primary patient samples at basal conditions. Three mass cytometry runs were conducted and signals from CD34+ cells from primary MF/sAML patient samples were normalized to CD34+ cells from normal bone marrow (NBM32 for run 1, NBM43 for run 2, and NBM44 for run 3).
B) Identification of cell populations by cell-surface markers in two NBM donor samples and three MF/sAML patients.
C) pCREB signals in NBM43, NBM44, MF9, sAML1 and sAML7 patient samples after treatment with 1 μM pevonedistat for 1 hour, 20 ng/mL TNFα for 15 minutes, or in combination.

Supplemental Figure 2. viSNE plots of drug-treated primary patient samples after intracellular cytokine mass cytometry.

A) Identification of cell populations by cell-surface markers in normal bone marrow donor (NBM31), normal peripheral blood (LSR5 PB) and two MF (MF2, MF26) samples.
B) Intracellular cytokine signals after treatment with 5 μM ruxolitinib for 4 hours, 1 μM pevonedistat for 4 hours, or in combination.

Supplemental Figure 3. Intracellular cytokine mass cytometry analysis of CD14+ cells.

A) Mass cytometry showing reduced TNFα (upper panel) and IL-6 (bottom panel) in monocytes from primary patient samples treated with 5 μM ruxolitinib, 1 μM pevonedistat, or their combination. Cells were treated for 4 hours.
B) Intracellular cytokines of CD14+ cells from normal bone marrow (NBM31), normal peripheral blood (LSR5 PB) and two MF (MF2, MF26) samples analyzed by mass cytometry at basal (left; normalized to NBM31) and after drug treatment (right). Cells from patient samples were treated with 5 μM ruxolitinib for 4 hours, 1 μM pevonedistat for 4 hours, or in combination. Signals from each patient sample per treatment condition were normalized to its basal signal.

Supplemental Figure 4. Pevonedistat treatment of mice transplanted with Ba/F3-MPL W515L cells.

A) Flow cytometry validation of GFP+ Ba/F3 cells expressing MPL W515L relative to parental Ba/F3 cells.
B) Percentage of GFP+ cells (transplanted Ba/F3-MPLW515L cells) in the peripheral blood of BALB/c mice. Each week mice were treated 5 days in a row, followed by 2 untreated days with vehicle (n = 10) or pevonedistat (n = 10). Blood collected on date of sacrifice for vehicle-treated mice or day 19 for pevonedistat-treated mice. **** P < 0.0001; by Student’s t-test.
C) Kaplan-Meier survival plot of BALB/c mice transplanted with Ba/F3-MPLW515L cells treated with vehicle (n = 10) or pevonedistat (n=10).

D, E, F) % of GFP+ cells in the bone marrow (D), and spleen weights (E) and liver weights (F) of BALB/c mice transplanted with Ba/F3-MPLW515L cells and treated with vehicle (n
= 10) or pevonedistat (n=10). Dashed grey lines representative of healthy, normal organ weights. **** $P < 0.0001$; by Student’s t-test

G) Body weights of mice treated with vehicle or pevonedistat at indicated days.
H) Representative images showing sizes of excised spleens and livers from vehicle or pevonedistat-treated mice.

Supplemental Table legends.
Supplemental Table 1. Clinical characteristics of patient samples.