Supplementary Figure 1: SPADE plots of normal bone marrow sample #6. SPADE clustering was performed on all samples (normal and AML) simultaneously to generate a single tree structure for all samples. All of the cell events from each sample were then mapped to the common tree structure. Each node of the SPADE tree is colored for the median expression of the indicated markers from low (blue) to high (red). The size of each node is correlated to the fraction of cells mapping to the node; however, a minimum size was enforced for most nodes to allow visualization of node color. Immunophenotypic grouping of nodes was performed manually on the basis of the median marker expression level of each node, and based on analysis of the relevant biaxial plots (e.g., CD38 vs. CD34).

Supplementary Figure 2: The frequency of cells in the indicated (manually-gated) populations. Colored boxes group immunophenotypic populations. HSPC, hematopoietic stem and progenitor cells; B, blasts (immunophenotypic); Mono, = monocyte lineage cells; Gran, granulocyte lineage; RBC, red blood cell lineage; B-Cell, B cell lineage. Error bars indicate standard errors.

Supplementary Figure 3: Multi-dimensional binning analysis of the $CD34^{+}CD38^{low}$ subset confirmed that distinct karyotype and genotype-specific immunophenotypic patterns are observed in high-dimensional space. Hierarchical grouping of normal and AML samples based on the pairwise correlation of the distribution of cells across the 100 multi-dimensional bins are shown.

Supplementary Figure 4: AML subtypes are characterized by distinct phosphoprotein signaling patterns. Log fold change in phosphoprotein staining relative to normal cells within each gated immunophenotypic population. Measured phosphoproteins are: **A**) pSTAT5, **B**) pMAPKAPK2, **C**) pERK, and **D**) p4E-BP1. Colored boxes group immunophenotypic populations: HSPC, hematopoietic stem and progenitor cells; B, blasts (immunophenotypic); Mono, monocyte lineage cells; Gran, granulocyte lineage; RBC, red blood cell lineage; B-Cell, B cell lineage. Error bars indicate standard errors.

Supplementary Figure 5: viSNE analysis of $CD34^{+}CD38^{low}$ subset demonstrates signaling aberrancy is most pronounced in cells outside the viSNE space occupied by normal cells. Samples were analyzed across 19 surface marker dimensions (as in Figure 5) and then each cell event was colored for the phospho-epitope staining of **A**) pSTAT5 or **B**) pMAPKAPK2. Blue cell events have the lowest levels of staining while red cell events exhibit the highest levels. The AML subtype is indicated for each sample.

Supplementary Figure 6: Median basal intracellular signaling of $CD34^{+}CD38^{low}$ cells from AML samples of each subtype. Median expression level of the indicated markers in the total $CD34^{+}CD38^{low}$ population, each data point represents the median expression level of one patient sample or one of the 14 sample aliquots from the five healthy donors. Each phospho-epitope is indicated.

Supplementary Figure 7: HU treatment does not increase the fraction of apoptotic cells measured by cPARP levels. Negative values reflect the very low cPARP signal that is further reduced by noise subtraction. Colored boxes group immunophenotypic populations. HSPC, hematopoietic stem and progenitor cells; B, blasts (immunophenotypic); Mono, monocyte lineage cells; Gran, granulocyte lineage; RBC, red blood cell lineage; B-Cell, B cell lineage. Error bars indicate standard errors.

Supplementary Figure 8: Histone 3 lysine 9 acetylation decreases with immunophenotypic differentiation in both normal and AML patient samples. Median antibody staining for H3K9ac in cells of the indicated (manually-gated) populations. Error bars indicate standard errors.

Supplementary Figure 9: Gating strategy is shown for each population. Cell events for normal sample #6 are shown. Gates for all samples were applied based on the gating of the normal samples, In almost all cases, the exact same gate boundaries defined by the normal samples was used for all AML samples (without regard to the cell distribution), in rare cases, minimal adjustments were made to separate NK cells from AML cells with bright aberrant CD7 expression. For mature cells of each lineage, a lineage population was first defined (blue box) and then each stage of maturation was sub-gated as shown. Some populations required multiple Boolean gates to achieve a pure cell population. In these cases, only events falling within all of the gates were considered to be in the population.