**Supplemental methods**

**Single cell DNA sequencing (scDNA-seq)**

*Libraries*

T-cells were depleted for some samples with a *JAK2*V617F mutation of low allele burden (i.e. <20%) using a FACS sorting of CD3-negative cells (FACS Melody, BD) to enrich the sample for tumoral cells. After this optional step, cells were washed by spinning at 300g for 10’ and were then resuspended in Tapestri cell buffer for a final concentration of 3500 cells /µL. A total of 120 000 cells were loaded in a Tapestri microfluidics cartridge for single cell encapsulation followed by cell lysis and barcoding. Barcoded samples were then processed for amplification using a custom panel designed for covering all known mutations of the selected samples and also classical hotspots in myeloid genes (*ASXL1, DNMT3A, CALR, EZH2, IDH1, IDH2, JAK2, KRAS, MPL, NRAS, RUNX1, SF3B1, SRSF2, TET2, TP53* and *U2AF1*). Libraries were checked for quality by migration on a Bioanalyser DNA1000 chip (Agilent) and were quantified using a fluorometric method (Qubit, ThermoFisher). Finally, libraries were normalized and pooled for a 2x150pb sequencing on an Illumina NovaSeq for a theorical number of 52 million reads per sample. In addition, an aliquot of the cells used for scDNA-seq library was frozen for *JAK2*V617F quantification (as detailed below).

*Bioinformatics*

FASTQ files were first analyzed by Tapestri pipeline (v2.0.2) for reads trimming and alignment across hg19 genome. Reads were assigned to single cell by combining the cell barcodes. Then, variant calling in each cell was performed with GATKv3.7 and variants and quality scores data for all cells were computed into a loom file for secondary analysis with Tapestri Insight software. The following filters were applied to remove low-confidence cells and variants: (i) removing genotype in cell with quality <30 and/or read depth<30, (ii) removing cells with <50% of genotypes present, (iii) removing genotype in cell with alternate allele frequency <30% and (iv) removing variants mutated in less than 20 cells. Then, remaining variants were filtered by annotation of databases (COSMIC, gnomAD) and visualization of BAM files on IGV for removing known polymorphisms and sequencing artefacts. Distinct clones were derived from the selected clones and were reviewed for allele frequency of mutations, genotyping quality scores and read depth to remove artefact due to allele drop out (ADO) that corresponds to a non-amplification of one of the two allele during the single-cell PCR. The review of clones was made blinded of the genotyping of colonies results. Finally, data about clones were exported from Tapestri Insight and we used a custom R script to generate plots.

**Genotyping of colonies**

*Liquid culture*

Prior to FACS sorting, cells were stained with anti-CD34 Pacific Blue (clone 581, Biolegend) and anti-Lineage FITC cocktail (anti-CD3, CD14, CD16, CD19, CD20 and CD56). Then, cells were washed and resuspended in a FITC marker for viability (SytoxGreen, ThermoFisher). Single live CD34-positive and Lin-negative cells were sorted into 96-wells plate with 100µL of culture media StemSpam SFEMII (Stemcell Technologies) with human cytokines: SCF, IL3, IL6, IL9, IL11, TPO, G-CSF (20ng/mL of each), GM-CSF (50ng/mL) and EPO (3U/mL). Between two to five 96-wells plates were sorted depending on the % of CD34-positive cells available. Cells were cultured for 14 days, and 50µL of fresh media was added at day 8 of culture. In addition, between 400 to 2000 CD34-positive cells were sorted into a vial and frozen for further quantification of *JAK2*V617F mutation.

*Culture termination and DNA extraction*

After 14 days of culture, colonies grown in methylcellulose were picked individually and transferred into chelex solution for DNA extraction. Liquid cultures of single CD34+ cells were performed in flat-bed 96 well plates and colonies obtained after 14 days were transferred using a 12-channel pipet into V-bottom 96-well PCR plates. The plates were centrifuged, the supernatant was removed, and 35µL of Chelex100 5% + 0.1% Triton solution was added to the pellets for DNA extraction. The plates containing cells in chelex solution were heated in a thermocycler for 15’ at 56°C followed by 8’ at 99°C and centrifuged for 10 minutes at 1'000 g. The supernatant containing the DNA was transferred into a new plate and used for analyses.

*Genotyping of colonies*

For genotyping of colonies, the *JAK2*V617F status was determined by an allele-specific PCR with primers labelled followed by capillary electrophoresis on an ABI3130 instrument. For additional mutations, we used fragment analysis for insertion or deletion and BigDye Sanger sequencing for single nucleotide variation. The strategy was to first genotype all colonies for *JAK2*V617F mutation and then to genotype around 40 colonies for other somatic mutations. At least 3 informative colonies with the same mutational profile was required for determination of a distinct clone. If these 40 colonies were not sufficient for reconstructing the clonal architecture (order of acquisition not retrieved or mutation not detected) we extended the number of colonies studied.

**Digital PCR for *JAK2*V617F quantification**

Frozen cells used both for scDNA-seq and liquid culture were used for quantification of JAK2V617F mutation. Briefly, DNA was extracted using QIAmp DNA micro kit (Qiagen) and then loaded on a chip with 20'000 partitions for an allele specific PCR using probes with FAM marker for wild type *JAK2* and VIC for JAK2*V617F*. After PCR, the chip was analyzed on a QuantStudio3D (ThermoFisher) and the absolute quantification of the allele burden was calculated from the number of positive wells for each fluorophore.

**Statistics**

For unsupervised clustering, a Factor Analysis of Mixed Data (FAMD) was performed followed by a clustering based on the ward distance (R package FactoMineR).

Parameters integrated in the FAMD were:

* VAF of *JAK2*V617F mutation (determined on granulocytes) (numerical variable)
* Number of additional mutations (numerical variable)
* Gene with the higher VAF among all additional mutations (categorial variable)
* If a somatic mutation was acquired before *JAK2*V617F (Yes/No, categorial variable)
* VAF of the mutation acquired before *JAK2*V617F (numerical variable)
* Number of mutations acquired after *JAK2*V617F (numerical variable)
* If the *JAK2*V617F mutation evolved to homozygosity (Yes/No, categorial variable)
* If a branching evolution occurred in the clone with the driver mutation (Yes/No, categorial variable)
* If an additional mutation occurring after *JAK2*V617F evolved to homozygosity (Yes/No, categorial variable)
* If an additional mutation occurred in a separated clone from the clone with the driver mutation (Yes/No, categorial variable)

FAMD is a multivariate dimension reduction technique designed for dataset with both quantitative (e.g. number of mutations, size of clones…) and categorical data (e.g. order of acquisition of JAK2 mutation: ‘before/after/separated clone’…). The aim of such approach is to summarize the variability of the dataset with a minimum of variable. Each dimension of the FAMD is a linear combination of all variables and can be considered as a variable explaining the dataset variability. We did the FAMD with 5 dimensions for representing the data because these 5 dimensions explained most of the inter-individual variability and we plotted the individuals on the 2 first axis (Figure 4). Then we applied a hierarchical classification which combines the individuals sequentially using the Ward’s minimum variance methodology. We selected 4 groups by inspecting the dendrogram produced by hierarchical clustering which is the best compromise between the loss of intra-cluster variability and the gain of inter-cluster variability (see below).

