

Hematopoietic Somatic Mosaicism is Associated with an Increased Risk of Postoperative Atrial Fibrillation

Short title: Hematopoietic mosaicism predisposes to postoperative atrial fibrillation

Supplementary materials

Post-operative outcomes

All patients underwent cardiac ECG monitoring until discharge. POAF was defined as an episode of AF lasting at least 30 seconds within the 7 days following surgery. The need for catecholamine support after surgery was recorded.

Peri-operative AKI was defined by an increase in serum creatinine within 72 hours after AVR and graded using the International Kidney Disease: Improving Global Outcomes classification (KDIGO) criteria as follows: grade 1: post-operative serum creatinine 1.5–1.9x baseline or an increase in serum creatinine ≥ 26.5 $\mu\text{mol/L}$; grade 2: post-operative serum creatinine 2.0–2.9x baseline; and grade 3: post-operative serum creatinine 3x baseline or increase in serum creatinine to ≥ 353.6 $\mu\text{mol/L}$ or initiation of renal replacement therapy.

Type 5 myocardial infarction (MI) was defined by the association on the days following surgery of high concentrations of high sensitivity Troponin T >10 x 99th percentile upper reference limit for fourth generation Troponin T with new pathological Q waves, left bundle branch block, or abnormal left ventricular wall motion on transthoracic echocardiogram at discharge¹. To assess the surgery-induced inflammatory response, the plasma CRP peak was measured within 72 hours after surgery.

Sample size calculation and selection process

An expected difference of 30% in HSM prevalence was assumed between the two groups (20% for patients without POAF and 50% for patients with POAF). The estimation of HSM prevalence was based on previous HSM screening methods close to definition 1. No bibliographic background allowed the estimation of HSM prevalence using definitions based on larger panels and lower VAF thresholds. As definition 1 was the most stringent, a higher HSM prevalence was expected for the other 3 definitions. Assuming a strength of association for definition 2, 3 and 4 between POAF and HSM at least as high as the association found with definition 1, with an alpha risk of 5% and a statistical power of 90%, at least 51 patients per group were required. To maximize a potential effect of HSM on POAF avoiding bias related to confounding variables, 52 POAF positive and 52 POAF negative patients displaying comparable distributions for age, BMI, diabetes, were selected (details in the “cohort and pre-operative clinical assessment” section and Figure S1). In order to reach comparable baseline characteristics between POAF and SR groups, the following process was applied: 2 blinded investigators performed a pairing for each POAF patient using the following stepwise process: (i) for each POAF patients, all SR patients yielding a ± 5 year similar age were screened, (ii) among the screened SR patients yielding ± 5 year similar age, a screening for same diabetes status was performed, (iii) among the screened SR patients presenting ± 5 year similar age and the same diabetic status, a screening for same gender was performed and (iv) among the remaining patients, the patient presenting the closest BMI was paired. In case unavailable pairing for patients at one step, the next step was performed. The pairing reports performed by the 2 investigators were then compared. In case of mismatch between the 2 pairing reports, a consensus was reached with a third investigator. The investigators performing the pairing process were blinded for the non-prespecified variables.

DNA sequencing

DNA extraction, library preparation and sequencing

DNA from buffy coat samples was extracted with QIAamp DNA Micro Kit (Qiagen) following the manufacturer's instructions and submitted to the Integrated Genomics Operation (IGO) at the Memorial Sloan-Kettering Cancer Center (MSKCC) for quality and quantity analysis, library preparation and sequencing. DNA quality was measured with TapeStation 2200 and all samples had a DNA Integrity Number (DIN) >6. DNA quantity was measured with PicoGreen. For each sample, ~200ng of genomic DNA was used for library construction using the KAPA Hyper Prep Kit (Kapa Biosystems KK8504) with 8 cycles of PCR. After sample barcoding, 2.5 ng-1 µg of each library were pooled and captured by hybridization with baits specific to either the HemePACT (Integrated Mutation Profiling of Actionable Cancer Targets related to Hematological Malignancies) assay, designed to capture all protein-coding exons and select introns of 576 (2.88Mb) commonly implicated oncogenes, tumor suppressor genes² (Table S2). Capture pools were sequenced on the HiSeq 4000, using the HiSeq 3000/4000 SBS Kit (Illumina) for PE100 reads. Samples were sequenced to a mean depth of coverage of 1175x. For detailed information on the sample quality control checks used to avoid potential sample and/or barcode mix-ups and contamination from external DNA, see².

Mutation data analysis

The data processing pipeline for detecting variants in Illumina HiSeq data was as follows. First the FASTQ files were processed to remove any adapter sequences at the end of the reads using cutadapt (v1.6). The files were then mapped using the BWA mapper (bwa mem v0.7.12). After mapping the SAM files were sorted and read group tags added using the PICARD tools. After sorting in coordinate order the BAM's were processed with PICARD MarkDuplicates. The marked BAM files were then processed using the GATK toolkit (v 3.2)

according to the best practices for tumor normal pairs. They were first realigned using ABRA (v 0.92) and then the base quality values were recalibrated with the BaseQRecalibrator. Somatic variants were then called in the processed BAMs using MuTect (v1.1.7). The full pipeline is available at https://github.com/soccin/BIC-variants_pipeline and the post-processing code at <https://github.com/soccin/Variant-PostProcess>.

To identify somatic variants and eliminate germline variants, all samples were run against a Frozen-Pool of 10 random genomes. Additionally, to reduce the risk of SNP contamination, mutations with a MAF (minor allelic frequency) cutoff of 0.01 using the gnomAD database and variants with 35% variant allelic frequency (VAF) were excluded.

Single Nucleotide Variations (SNVs) mutations [Missense, Nonsense, Splice Site, Splice Regions] that were supported by at least 4 or more mutant reads, with a VAF >1% and with coverage of 50x or more were selected.

Somatic variants were classified according to their pathogenicity as follows: variants were classified as “deleterious” when the single nucleotide variant (SNV) was predicted to affect protein function as determined by the PolyPhen-2 software (possibly and probably damaging), and the SIFT software (deleterious). Variants were classified as “pathogenic” if reported as pathogenic/likely pathogenic by ClinVar ³ and/or oncogenic/predicted oncogenic/likely oncogenic by OncoKb ⁴. These databases report pathogenicity in cancer and other diseases, based on supporting evidence from curated literature.

Mass cytometry

Preparation of PBMC and myocardial samples

PBMC were obtained from whole blood using CPT mononuclear cell preparation tubes with sodium heparin (Becton Dickinson) according to manufacturer’s instructions. The Atrial tissue samples were obtained from the right atrial appendage before aortic cross-clamping and cardioplegia. The tissue was immediately cleaned, cut and digested at 37°C for 45 min in

0.1% collagenase I (in DMEM F12 medium). Digested tissue was flushed using a 19G needle, then centrifuged (10 min at 1400rpm and 4°C). The pellet was resuspended in PBS and filtered through 40 µM mesh. A second centrifugation was performed (10min at 1400rpm and 4°C) and the supernatant was removed. Lysis of red blood cells was then performed using a red blood cell lysis buffer (155 mM NH₄Cl, 10 mM NaHCO₃, 127 mM EDTA). A last centrifugation was performed to isolate leukocyte-containing fraction (1400rpm, 4°C). PBMC and cells from myocardial samples were then counted and the pellet was frozen in 90% FCS and 10% DMDS.

Antibodies

Unless otherwise specified, all the antibodies were from Fluidigm and purchased as conjugates (Table S9). Six antibodies were labeled respectively with ¹⁰³Rh, ¹¹³In, ¹¹⁵In, ¹⁴⁰Ce, ¹⁵⁰Nd and ¹⁵⁷Gd from Innovachem using the X8 polymer supplied in Maxpar Labeling Kits (Fluidigm®) according to manufacturer's protocol. Anti-CD8 antibody was labeled with MCP9 polymer supplied in Maxpar labeling kits (Fluidigm®) according to manufacturer's protocol. Anti-Perforin antibody was labeled with 139La Lightning-Link Metal kit (Expedeon®). Anti-CD1c and anti-CADM1 antibodies were labeled using ¹⁹⁵Pt and ¹⁹⁶Pt Cisplatin (BuyIsotope®) accordingly to the protocol published by Mei et al. ⁵.

Cell staining

After thawing, PBMC and the leukocyte fractions from atrial tissues (4x10⁴ to 1.4.10⁶ cells) were stained for viability (10⁷ cells/ml) in calcium magnesium-free phosphate buffered saline (PBS) added with 1 mM Cisplatin (Fluidigm®) for 5 min at RT. Reaction was quenched with MaxPar Cell Staining Buffer (CSB, Fluidigm®). After centrifugation (400 g, 5 min, RT), cells were resuspended in CSB at a concentration of 3x10⁷ cells/mL and incubated (RT, 10 min) with Fc Block (1/100, Becton Dickinson®). The 12 antibodies used for which epitope binding was previously reported by manufacturers to be sensitive to fixation (Table S9) were

added for an additional 30 min incubation at RT. Stained cells were then washed twice before barcoding accordingly to the manufacturer's protocol (Fluidigm®).

Two pools of the 17 PBMC samples (one pool with 10 samples and one pool with 7 samples) and two pools of the 17 atrial samples (one pool with 10 samples and one pool with 7 samples), from HSM mutations carriers or non-carriers, were processed separately for barcoding together with one aliquot of a control PBMC sample from a single healthy donor in each pool in order to assess batch effects. Cells were incubated in MaxPar Fix I buffer for 10 min at RT, washed twice in Barcode Perm Buffer, resuspended in 800 µL Barcode Perm Buffer and incubated with the appropriate Palladium barcode for 30 min at RT. After centrifugation (600g, 5 min, RT), cells were washed twice with Maxpar CSB and stained for 30 min RT with surface antibodies directed against epitopes that were not reported by manufacturers to be sensitive to fixation (Table S9) added with Fc Block reagent (1/100, Becton Dickinson®). After two washes with CSB, cells were fixed and permeabilized with eBioscience™ FOXP3/Transcription Factor Staining Buffer St protocol B. After 30 min of incubation at RT with 1 ml of Fixation/Permeabilization working solution, cells were washed twice with 6 ml of 1x Permeabilization Buffer, incubated 30 min at RT with intracellular antibodies mix (Table S9), and washed twice with 2 ml of 1x Permeabilization Buffer. Pellets were resuspended in 1 ml Maxpar Fix and Perm buffer added with 125 mM ^{191/193}Ir intercalator for one hour at RT and washed twice in Maxpar CSB and twice in Maxpar Water. Cells were resuspended in Maxpar Water at 5x10⁵ cells/mL with 1:10 volume of Four-Element Calibration Beads (Fluidigm) and analyzed on a Helios mass cytometer instrument (Fluidigm®).

Data corresponding to the 2 barcoded pools of 17 PBMCs samples and the 2 barcoded pools of 17 atrial samples (added with one control PBMC aliquot) were acquired during 28 periods (10 runs for each PBMC pools, 6 runs for the first pool of atrial samples and 2 runs for the

second pool of atrial samples) of maximum 150 minutes at a maximum rate of 500 events/sec, with a tuning protocol (mass resolution, mass calibration, dual count calibration, and quality control test) performed between each period of data acquisition.

For the compensation matrix calculation, Comp Beads (Becton Dickinson®) were single stained in CSB with 1µg of each antibody used in the panel for 30 minutes at RT except for ¹⁹⁶Pt -labelled anti-CADM1 antibody, which is a chicken IgY, that is not captured by Comp Beads. It was replaced by an anti-CD8 (clone SK1, Mouse IgG1_κ) conjugated with the same batch of ¹⁹⁶Pt. After two washes with CSB and two washes with Maxpar Water, beads were mixed and acquired as a single tube at a maximum rate of 500 events/sec.

Data processing

Data were downstream processed for normalization using calibration beads signals and debarcoded following the manufacturer's instructions.

Batch effect normalization

The data acquired during the 28 periods of acquisition were thereafter considered as 8 independent batches in order to compensate for possible signal drift over duration of acquisition of a single barcoded pool. The control PBMC sample included in each barcoded run was gated on nucleated - single - biological - non beads - CD45⁺ live events (Figure S4) and used as an anchor for application of the CytofBatchAdjust R code published by Schuyler et al. ⁶. Briefly, each channel was tested for adjustment using the percentile method at 0.75pc, 0.80pc, 0.85pc, 0.90pc, 0.95pc and/or 0.99pc and channels adjustments as well as scaling factors were checked visually for each anchor sample to determine the best fitted percentile adjustment of each channel as illustrated in Figure S4. After this pre-tuning, final adjustments were performed accordingly (Figure S4), and batch-adjusted FCS files from each single tissue were concatenated to reconstitute original samples.

Spillover compensation

The FCS file from mixed single stained beads was imputed in CATALYST R ⁷ using the NNLS method. The output compensation matrix (Figure S4) was applied to all the files and compensated FCS files were edited.

Data analysis.

Debarcoded - batch normalized – concatenated - compensated FCS files were gated on nucleated - single - biological - non beads - CD45⁺ live events (Figure S4) and processed for phenotype analysis using R 4.0.0 and a modified version of the Cytokit package including UMAP computation using the uwot package (<http://github.com/i-cyto/cytokitlab>). Dimension reduction was performed using the Umap algorithm and the output 2-dimensions map was used for density-based clustering using the Cluster X algorithm. Illustrations were edited using the Cytokit ShinyAPP browser and Cytobank®. For comparison of identified clusters in HSM carriers versus non-HSM carriers, a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli was applied, assuming all clusters to be samples from a population with the same scatter using threshold of 5% for significant FDR.

RNAseq

Classical CD14⁺⁺CD16⁻ monocytes sorting

PBMCs were sorted into an Influx sorter (Becton Dickinson®) equipped with a 86 µm nozzle and tuned at a pressure of 24.6 psi and a frequency of 48.25 kHz. Sample fluid pressure was adjusted to reach an event rate of 10 000 events/second. Samples were stained 15 min at RT in PBS with Zombie UV (1/100, Biolegend), rinsed twice with PBS, and stained with Pacific Blue anti-human CD16 (clone 3G8, 1/800), BV786 anti-human CD15 (clone HI98, 1/100), FITC anti-human CD14 (clone 61D3, 1/200), PE anti-human CD66b (clone G10F5, 1/100), PE-CF594 anti-human CD3 (clone UCHT1, 1/200), PE-Cy7 anti-human CD19 (clone HIB19, 1/500), AF700 anti-human CD56 (clone HCD6, 1/200) and APC-eF780 anti-human HLA-DR

clone (LN3, 1/200). After two rinses in PBS – 0.5% BSA, cells were directly run on the cell sorter. The gating strategy used to sort classical monocytes is presented in Figure S5.

RNAseq analysis

Preoperative sorted classical monocytes

Sequencing reads were mapped to the human RefSeq reference library yielding an average of 9.5 million reads per patient. RefSeq counts were collapsed to gene symbols, taking the highest-expressing isoform when there were multiple sequence-to-gene mappings. The top 10,000 genes were selected for gene expression analysis. The DESeq2 R package⁸ was used to normalize counts and calculate differential expression between patients carrying HSM mutations and non-carriers, using sex as a covariate (design = ~sex + HSM). The Benjamini-Hochberg method was used to calculate false discovery rates (FDR). The log₂ of DESeq2-normalized counts was used for further analysis, such as principal components analysis (PCA) and pathway analysis. We tested differential expression of GO:Biological Process gene annotation terms⁹ from MSigDB¹⁰; only terms which had at least 75% of member genes in the top-expressing 10,000 genes were considered, which limited terms to those most relevant for monocytes, leaving a list of 1,302 terms. To test differential expression of GO:BP terms, we used a consensus of three methods: 1) Fisher's exact test for overrepresentation with the top 500 differentially-expressed genes (FDR ≈ 0.11); 2) Gene Set Enrichment Analysis (Subramanian et al. 2005) on non-directional p-value ranking of genes with exponent weight $p = 0$, equivalent to a Kolmogorov-Smirnov test; and 3) calculating the variance explained (r^2) by HSM status for each gene in a term, the mean of which acted as a test statistic, and comparing this to a null distribution calculated by permuting group labels 10,000 times. A consensus p-value was formed by z-score averaging the p-values of the three methods. For plotting, some top-ranking terms were combined if their Cohen's kappa > 0.3 (inspired by gProfiler¹¹).

Pre and postoperative sorted classical monocytes

Classical CD14⁺⁺CD16⁻ monocytes were sorted from PBMC samples and total RNA extracted using the Direct-Zol RNA Microprep kit with on-column DNaseI treatment (Zymo research). Whole transcriptome amplification and cDNA synthesis were performed with the Ovation RNA-seq V2 kit (NuGEN) following the manufacturer's instructions. Sequencing libraries were further prepared using the Ovation Ultralow V2 DNA-seq Library preparation kit. Libraries were pooled and sequenced on a HiSeq4000 (Illumina) to obtain 20 million single-end 50bp reads.

To assess the impact of surgery on classical monocyte activation, the transcriptome signature of sorted classical monocytes collected the day before surgery and at 24 hours after surgery was assessed according to HSM status in a subgroup of 6 patients (3 non-HSM and 3 HSM patients) (Figure S3). DESeq2 was used to analyze differential expression between pre- and post-surgery in a paired manner (in DESeq2, "design = ~patient + surgery"). The 500 genes most differentially regulated by surgery were used as input to test for enrichment of GO and Hallmark gene sets of size ≤ 500 , gathered from the MSigDB database.

Euclidian distances and PCA were computed using the top 10,000 most highly expressing genes in the dataset, on log₂ expression values (computed using the DESeq2 rlog function on DESeq2-normalized counts) (Figure S3).

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Supplementary Tables.

Table S1. Pre-operative characteristics of the POAF and SR patients selected from the POMI-AF cohort yielding comparable distribution for age, BMI, diabetic status and gender

	Total cohort (n=133)	Total SR (n = 81)	Control SR (n=52)	POAF (n = 52)	p*	p**
Age, years	68±9	67±10	70±9	71±8	0.048	0.57
Women, n (%)	47 (35)	30 (37)	20 (38)	17 (33)	0.31	0.68
BMI (kg/m ²)	28 [24 ; 32]	28 [25 ; 32]	28 [24 ; 31]	28 [24 ; 31]	1	0.79
Obese, n (%)	49 (35)	31 (36)	18 (35)	18 (35)	0.90	1
History of diabetes, n (%)	48 (36)	33 (41)	15 (29)	15 (29)	0.11	1
History of AF, n (%)	0	0	0	0	1	1
Hypertension, n (%)	91 (68)	58 (72)	36 (69)	33 (63)	0.20	0.53
LVEF (%)	63 [56; 68]	61 [56;70]	64 [58;71]	64 [57;67]	0.85	0.46
LA area (cm ²)	23±6	22±6	21±5	22±5	0.85	0.3
Transaortic Vmax (m/s)	4.4 [4.10 ; 4.76]	4.4 [4.1 ; 4.7]	4.4 [4.1 ; 4.7]	4.4 [4.02 ; 4.8]	0.48	0.37
Aortic valve area (cm ²)	0.70±0.2	0.68±0.17	0.68±0.18	0.75±0.22	0.06	0.11
Indexed aortic valve area (cm ² /m ²)	0.36 [0.3 ; 0.42]	0.35 [0.29 ; 0.41]	0.35 [0.29 ; 0.39]	0.39 [0.29 ; 0.45]	0.16	0.25
Pre-operative serum creatinin (mg/l)	9 [7 ; 11]	8.5 [7 ; 10]	8 [7 ; 10]	9 [7 ; 11]	0.16	0.38
Pre-operative MDRD clearance (ml/min/1,73 m ²)	84±25	86±25	86±23	82±23	0.42	0.76
Pre-operative CRP (mg/l)	3 [3;4]	3 [3;3]	3 [3;4]	3 [3;5]	0.20	0.14
Amiodarone, n (%)	0	0	0	0	1	1
Betablockers, n (%)	46 (35)	29 (36)	20 (38)	17 (33)	0.72	0.53
Statin, n (%)	78 (59)	51 (63)	33 (63)	27 (52)	0.26	0.23

ACE inhibitors, n (%)	45 (34)	26 (32)	20 (38)	19 (37)	0.63	0.84
Combined CABG, n (%)	37 (28)	23 (28)	14 (27)	14 (27)	0.6	1
Extracorporeal circulation duration (min)	73 [61 ; 95]	70 [60 ; 93]	70 [58 ; 92]	79 [64 ; 101]	0.3	0.15

Variables are given as median [1st quartile-3rd quartile], mean \pm SD or number (percentage). BMI: body mass index. LA area: left atrial area. LVEF: left ventricular ejection fraction.

Table S2. Panel of screened genes (HemePACT panel)

ABL1	CRKL	GNAS	MAP2K1	PLK2	SMARCD1
ACTG1	CRLF2	GNB1	MAP2K2	PMAIP1	SMC1A
ACVR1	CSDE1	GPS2	MAP2K4	PMS1	SMC3
AGO2	CSF1R	GREM1	MAP3K1	PMS2	SMG1
AKT1	CSF3R	GRIN2A	MAP3K13	PNRC1	SMO
AKT2	CTCF	GSK3B	MAP3K14	POLD1	SMYD3
AKT3	CTLA4	GTF2I	MAPK1	POLE	SOCS1
ALK	CTNNB1	H3F3A	MAPK3	POT1	SOS1
ALOX12B	CUL3	H3F3B	MAPKAP1	PPARG	SOX17
AMER1	CUX1	H3F3C	MAX	PPM1D	SOX2
ANKRD11	CXCR4	HDAC1	MCL1	PPP2R1A	SOX9
APC	CYLD	HDAC4	MDC1	PPP4R2	SP140
AR	CYSLTR2	HDAC7	MDM2	PPP6C	SPEN
ARAF	DAXX	HGF	MDM4	PRDM1	SPOP
ARHGEF28	DCUN1D1	HIF1A	MED12	PRDM14	SPRED1
ARID1A	DDR2	HIRA	MEF2B	PREX2	SRC
ARID1B	DDX3X	HIST1H1B	MEN1	PRKAR1A	SRSF2
ARID2	DICER1	HIST1H1C	MET	PRKCI	STAG1
ARID3A	DIS3	HIST1H1D	MGA	PRKD1	STAG2
ARID3B	DNAJB1	HIST1H1E	MGAM	PTCH1	STAT3
ARID3C	DNMT1	HIST1H2AC	MITF	PTEN	STAT5A
ARID4A	DNMT3A	HIST1H2AG	MLH1	PTP4A1	STAT5B
ARID4B	DNMT3B	HIST1H2AL	MLL	PTPN1	STAT6
ARID5A	DOT1L	HIST1H2AM	MOB3B	PTPN11	STK11
ARID5B	DROSHA	HIST1H2BC	MPEG1	PTPN2	STK19
ASXL1	DTX1	HIST1H2BD	MPL	PTPRD	STK40
ASXL2	DUSP22	HIST1H2BG	MRE11A	PTPRS	SUFU
ATM	DUSP4	HIST1H2BJ	MSH2	PTPRT	SUZ12
ATP6AP1	E2F3	HIST1H2BK	MSH3	RAB35	SYK
ATP6V1B2	EED	HIST1H2BO	MSH6	RAC1	TAP1
ATR	EGFL7	HIST1H3A	MSI1	RAC2	TAP2
ATRX	EGFR	HIST1H3B	MSI2	RAD21	TBL1XR1
ATXN2	EGR1	HIST1H3C	MST1	RAD50	TBX3
AURKA	EIF1AX	HIST1H3D	MST1R	RAD51	TCEB1
AURKB	EIF4A2	HIST1H3E	MTOR	RAD51B	TCF3
AXIN1	EIF4E	HIST1H3F	MUTYH	RAD51C	TCF7L2
AXIN2	ELF3	HIST1H3G	MYC	RAD51D	TEK
AXL	EP300	HIST1H3H	MYCL1	RAD52	TERT
B2M	EP400	HIST1H3I	MYCN	RAD54L	TET1
BABAM1	EPAS1	HIST1H3J	MYD88	RAF1	TET2
BACH2	EPCAM	HIST2H3C	MYOD1	RARA	TET3
BAP1	EPHA3	HIST2H3D	NBN	RASA1	TGFBR1

BARD1	EPHA5	HIST3H3	NCOA3	RB1	TGFBR2
BBC3	EPHA7	HLA-A	NCOR1	RBM10	TMEM127
BCL10	EPHB1	HLA-B	NCOR2	RECQL	TMPRSS2
BCL11B	ERBB2	HNF1A	NCSTN	RECQL4	TNFAIP3
BCL2	ERBB3	HOXB13	NEGR1	REL	TNFRSF14
BCL2L1	ERBB4	HRAS	NF1	RET	TOP1
BCL2L11	ERCC2	ICOSLG	NF2	RFWD2	TP53
BCL6	ERCC3	ID3	NFE2	RHEB	TP53BP1
BCOR	ERCC4	IDH1	NFE2L2	RHOA	TP63
BCORL1	ERCC5	IDH2	NFKBIA	RICTOR	TRAF2
BCR	ERF	IFNGR1	NKX2-1	RIT1	TRAF3
BIRC3	ERG	IGF1	NKX3-1	RNF43	TRAF5
BLM	ERRFI1	IGF1R	NOTCH1	ROBO1	TRAF7
BMPR1A	ESCO2	IGF2	NOTCH2	ROS1	TSC1
BRAF	ESR1	IKBKE	NOTCH3	RPS6KA4	TSC2
BRCA1	ETNK1	IKZF1	NOTCH4	RPS6KB2	TSHR
BRCA2	ETV1	IKZF3	NPM1	RPTOR	TYK2
BRD4	ETV6	IL10	NRAS	RRAGC	U2AF1
BRIP1	EZH1	IL7R	NSD1	RRAS	U2AF2
BTG1	EZH2	INHA	NT5C2	RRAS2	UBR5
BTK	FAM175A	INHBA	NTHL1	RTEL1	UPF1
CALR	FAM46C	INPP4A	NTRK1	RUNX1	VAV1
CARD11	FAM58A	INPP4B	NTRK2	RUNX1T1	VAV2
CARM1	FANCA	INPPL1	NTRK3	RXRA	VEGFA
CASP8	FANCC	INSR	NUF2	RYBP	VHL
CBFB	FANCD2	IRF1	NUP93	SAMHD1	VTCN1
CBL	FAS	IRF4	P2RY8	SDHA	WHSC1
CCND1	FAT1	IRF8	PAK1	SDHAF2	WHSC1L1
CCND2	FBXO11	IRS1	PAK7	SDHB	WT1
CCND3	FBXW7	IRS2	PALB2	SDHC	WWTR1
CCNE1	FGF19	JAK1	PARK2	SDHD	XBP1
CD274	FGF3	JAK2	PARP1	SESN1	XIAP
CD276	FGF4	JAK3	PAX5	SESN2	XPO1
CD28	FGFR1	JARID2	PBRM1	SESN3	XRCC2
CD58	FGFR2	JUN	PCBP1	SETBP1	YAP1
CD79A	FGFR3	KDM5A	PDCD1	SETD1A	YES1
CD79B	FGFR4	KDM5C	PDCD1LG2	SETD1B	ZBTB7A
CDC42	FH	KDM6A	PDGFRA	SETD2	ZFHX3
CDC73	FLCN	KDR	PDGFRB	SETD3	ZRSR2
CDH1	FLT1	KEAP1	PDPK1	SETD4	
CDK12	FLT3	KIT	PDS5B	SETD5	
CDK4	FLT4	KLF4	PGR	SETD6	
CDK6	FOXA1	KMT2A	PHF6	SETD7	

CDK8	FOXL2	KMT2B	PHOX2B	SETDB1	
CDKN1A	FOXO1	KMT2C	PIGA	SETDB2	
CDKN1B	FOXP1	KMT2D	PIK3C2G	SF3B1	
CDKN2A	FUBP1	KMT5A	PIK3C3	SGK1	
CDKN2B	FURIN	KNSTRN	PIK3CA	SH2B3	
CDKN2C	FYN	KRAS	PIK3CB	SH2D1A	
CEBPA	GATA1	KSR2	PIK3CD	SHOC2	
CENPA	GATA2	LATS1	PIK3CG	SHQ1	
CHEK1	GATA3	LATS2	PIK3R1	SLX4	
CHEK2	GLI1	LCK	PIK3R2	SMAD2	
CIC	GNA11	LMO1	PIK3R3	SMAD3	
CIITA	GNA12	LTB	PIM1	SMAD4	
CRBN	GNA13	LYN	PLCG1	SMARCA4	
CREBBP	GNAQ	MALT1	PLCG2	SMARCB1	

Table S3. Conventional CHIP panel

ABL1	DNMT3B	KIT	PRPF40B	U2AF2
ASXL1	EED	KDM6A	PRPF8	WHSC1
ATM	EGFR	KRAS	PTEN	WT1
BCL11B	EP300	KMT2D	PTPN11	WPO1
BCOR	ETV6	LUC7L2	RAD21	ZRSR2
BCORL1	EZH2	MAP2K1	RET	
BRAF	FANCL	MEF2B	RIT1	
BRCC3	FBXW7	MPL	RPL10	
CALR	FLT3	MYD88	RUNX1	
CBL	GATA1	NF1	SETBP1	
CBLB	GATA2	NOTCH1	SETD2	
CD79B	GATA3	NOTCH2	SF1	
CEBPA	GNAS	NOTCH3	SF3A1	
CNOT3	GNB1	NPM1	SF3B1	
CREBBP	IDH1	NRAS	SH2B3	
CRLF2	IDH2	NT5C2	SMC1A	
CSF1R	IKZF1	PAX5	SMC3	
CSF3R	IKZF2	PDGFRA	SRSF2	
CTCF	IKZF3	PDS5B	STAG2	
CTNNB1	IL7R	PHF6	STAT3	
CUX1	JAK1	PIGA	TET2	
CXCR4	JAK2	PIK3CA	TLR2	
DNMT3A	JAK3	PIM1	TP53	

Table S4. Called somatic variants

Patient ID	Hugo Symbol	Chromosome	HGVSc	HGVSp Short	Start Position	Variant Classification	Variant Type	Variant allelic frequency (%)	mutation_effect OncoKb	oncogenic/ OncoKb	ClinVar	SIFT	PolyPhen
78	ASXL1	20	c.1186C>T	p.Q396*	3102187	Nonsense_Mutation	SNP	21,961	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
78	XPO1	2	c.591-2A>T	p.X197_splice	61726050	Splice_Site	SNP	8,257	Unknown	NA	NA	NA	NA
84	ASXL1	20	c.2077C>T	p.R693*	31022592	Nonsense_Mutation	SNP	12,850	Likely Loss-of-function	Likely Oncogenic	Pathogenic	NA	NA
84	ESCO2	8	c.112C>A	p.Q38K	27633937	Missense_Mutation	SNP	1,559	Unknown	NA	NA	tolerated(0.31)	benign(0.01)
86	MGA	15	c.6428_6429 insTTATTA CTGGCTT TTTC	p.G2144 Yfs*41	42042231	Frame_Shift_Ins	INS	10,938	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
87	EED	11	c.1200A>T	p.K400N	85989441	Missense_Mutation	SNP	2,385	Unknown	NA	NA	tolerated(0.05)	benign(0.022)
87	HIST1H2AG	6	c.48G>C	p.K16N	27100898	Missense_Mutation	SNP	1,517	Unknown	NA	NA	deleterious_low_confidence(0.01)	unknown(0)
87	ROS1	6	c.3366C>A	p.C1122*	117683781	Nonsense_Mutation	SNP	1,536	Unknown	NA	NA	NA	NA
93	GATA3	10	c.1172C>G	p.S391W	8115823	Missense_Mutation	SNP	3,149	Unknown	NA	NA	deleterious(0.01)	probably_damaging(0.997)
94	TET2	4	c.2862G>A	p.W954*	106157961	Nonsense_Mutation	SNP	4,641	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
94	MED12	X	c.5005G>C	p.D1669H	70355083	Missense_Mutation	SNP	1,996	Unknown	NA	NA	NA	possibly_damaging(0.902)
96	ATXN2	12	c.534_538 delGCAAC	p.Q179A fs*69	112036781	Frame_Shift_Del	DEL	21,667	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
105	CARD11	7	c.2872C>T	p.L958F	2953068	Missense_Mutation	SNP	1,349	Unknown	NA	NA	tolerated(0.18)	benign(0.063)
105	HIST1H1E	6	c.524A>G	p.K175R	26157142	Missense_Mutation	SNP	1,036	Unknown	NA	NA	deleterious(0.02)	unknown(0)
105	NOTCH2	1	c.7G>T	p.A3S	120612014	Missense_Mutation	SNP	13,757	Unknown	NA	Benign	tolerated_low_confidence(0.5)	unknown(0)
105	NOTCH2	1	c.8C>T	p.A3V	120612013	Missense_Mutation	SNP	13,904	Unknown	NA	NA	tolerated_low_confidence(0.57)	unknown(0)
106	NF1	17	c.1720delA	p.S574A	29548	Frame_Shift_Del	DEL	9,124	Likely	Likely	NA	NA	NA

				fs*12	946	Del			Loss-of-function	Oncogenic			
106	FBXO1	2	c.98C>A	p.P33Q	48132762	Missense_Mutation	SNP	26,163	Unknown	NA	NA	tolerated_low_confidence(0.48)	unknown(0)
108	AR	X	c.176A>T	p.Q59L	66765164	Missense_Mutation	SNP	30,204	Unknown	NA	NA	deleterious_low_confidence(0.04)	unknown(0)
108	DNMT3A	2	c.2309C>T	p.S770L	25463184	Missense_Mutation	SNP	1,189	Likely Loss-of-function	Likely Oncogenic	Likely pathogenic	deleterious(0.01)	probably_damaging(0.996)
108	SF3B1	2	c.1988C>T	p.T663I	198267369	Missense_Mutation	SNP	1,226	Unknown	NA	NA	deleterious(0)	probably_damaging(1)
108	SMC1A	X	c.2132G>A	p.R711Q	53432008	Missense_Mutation	SNP	1,258	Unknown	NA	NA	deleterious(0)	probably_damaging(0.997)
111	SF3B1	2	c.2098A>G	p.K700E	198266834	Missense_Mutation	SNP	8,742	Switch-of-function	Likely Oncogenic	Likely pathogenic	deleterious(0)	probably_damaging(0.96)
141	ARID2	12	c.1277C>T	p.T426M	46231437	Missense_Mutation	SNP	1,645	Unknown	NA	NA	deleterious(0)	probably_damaging(0.975)
156	SMAD4	18	c.-127-2A>T		48573288	Splice_Site	SNP	11,194	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
156	NT5C2	10	c.1681G>T	p.E561*	104849434	Nonsense_Mutation	SNP	1,105	Unknown	NA	NA	NA	NA
162	DNMT3A	2	c.1111G>T	p.E371*	25469931	Nonsense_Mutation	SNP	3,720	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
162	ZRSR2	X	c.1167C>G	p.Y389*	15841083	Nonsense_Mutation	SNP	26,432	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
164	CUX1	7	c.1382C>T	p.A461V	101917519	Missense_Mutation	SNP	1,282	Unknown	NA	NA	tolerated(0.06)	benign(0.197)
170	TET2	4	c.3798T>G	p.N1266K	106164930	Missense_Mutation	SNP	7,832	Likely Loss-of-function	Likely Oncogenic	NA	deleterious(0)	probably_damaging(1)
170	CTCF	16	c.244G>A	p.V82M	67644979	Missense_Mutation	SNP	3,990	Unknown	NA	NA	tolerated_low_confidence(0.14)	possibly_damaging(0.556)
172	TET2	4	c.1455_1456 insT	p.N486*	106156554	Frame_Shift_Ins	INS	15,922	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
173	SMAD4	18	c.-127-2A>T		48573288	Splice_Site	SNP	7,471	Likely Loss-of-function	Likely Oncogenic	NA (germ)	NA	NA
173	SMAD4	18	c.-127-1G>T		48573289	Splice_Site	SNP	8,092	Likely Loss-of-function	Likely Oncogenic	NA (germ)	NA	NA
180	CUX1	7	c.3569G>A	p.R1190Q	101877434	Missense_Mutation	SNP	7,858	Unknown	NA	NA	deleterious(0)	unknown(0)

180	DNMT3A	2	c.2525A>G	p.Q842R	25458648	Missense_Mutation	SNP	1,288	Unknown	NA	NA	deleterious(0.03)	probably_damaging(0.985)
180	DNMT3A	2	c.977G>A	p.R326H	25470497	Missense_Mutation	SNP	3,679	Unknown	NA	NA	deleterious(0)	probably_damaging(1)
180	SMARCA4	19	c.3683T>C	p.V1228A	11144102	Missense_Mutation	SNP	1,230	Unknown	NA	NA	NA	benign(0.349)
22	CHEK2	22	c.671G>A	p.R224H	29121015	Missense_Mutation	SNP	4,604	Unknown	NA	Uncertain significance	tolerated(0.51)	benign(0.016)
22	ATM	11	c.4577C>T	p.P1526L	108163486	Missense_Mutation	SNP	1,392	Unknown	NA	NA	deleterious(0.01)	possibly_damaging(0.493)
22	DNMT3A	2	c.1898C>T	p.P633L	25466805	Missense_Mutation	SNP	2,669	Unknown	NA	NA	deleterious(0)	possibly_damaging(0.635)
22	DNMT3A	2	c.2185C>T	p.R729W	25463308	Missense_Mutation	SNP	3,955	Likely Neutral	Likely Neutral	NA/ONCO mutation is likely neutral.	deleterious(0)	probably_damaging(0.999)
22	SMG1	16	c.7865A>G	p.E2622G	18847447	Missense_Mutation	SNP	1,533	Unknown	NA	NA	deleterious(0)	probably_damaging(0.979)
22	PDS5B	13	c.3059G>T	p.C1020F	33332227	Missense_Mutation	SNP	17,105	Unknown	NA	NA	deleterious(0)	benign(0.014)
22	SMC3	10	c.334G>A	p.D112N	112337656	Missense_Mutation	SNP	3,625	Unknown	NA	NA	tolerated(0.52)	benign(0.233)
22	TET2	4	c.4792T>A	p.Y1598N	106196459	Missense_Mutation	SNP	2,555	Unknown	NA	NA	deleterious(0.03)	benign(0.432)
22	UBR5	8	c.7725G>A	p.M2575I	103274260	Missense_Mutation	SNP	2,551	Unknown	NA	NA	tolerated(0.61)	possibly_damaging(0.469)
193	MOB3B	9	c.478C>T	p.R160W	27359175	Missense_Mutation	SNP	28,950	Unknown	NA	NA	deleterious(0)	probably_damaging(1)
193	XPO1	2	c.591-2A>T	p.X197_splice	61726050	Splice_Site	SNP	5,747	Unknown	NA	NA	NA	NA
194	RAD21	8	c.514delA	p.R172Efs*12	117869680	Frame_Shift_Del	DEL	12,342	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
199	FLT1	13	c.547C>T	p.R183C	29008324	Missense_Mutation	SNP	3,885	Unknown	NA	NA	deleterious(0.03)	probably_damaging(0.932)
199	GNAS	20	c.1462G>A	p.A488T	57429782	Missense_Mutation	SNP	6,048	Unknown	NA	not provided	tolerated_low_confidence(0.1)	unknown(0)
203	FGFR1	8	c.489T>A	p.D163E	38285922	Missense_Mutation	SNP	2,077	Unknown	NA	NA	tolerated(0.26)	benign(0.011)
203	FGFR1	8	c.490G>T	p.D164Y	38285921	Missense_Mutation	SNP	2,080	Unknown	NA	NA	tolerated(0.08)	possibly_damaging(0.694)
204	ARID1B	6	c.995G>C	p.G332A	157100058	Missense_Mutation	SNP	3,241	Unknown	NA	NA	NA	unknown(0)
204	CDK8	13	c.1129C>G	p.Q377E	26975621	Missense_Mutation	SNP	2,273	Unknown	NA	NA	tolerated(0.7)	benign(0.011)
204	CDK8	13	c.1130A>G	p.Q377R	26975622	Missense_Mutation	SNP	2,283	Unknown	NA	NA	tolerated(0.32)	benign(0.025)
24	DNMT3A	2	c.977G>T	p.R326L	25470497	Missense_Mutation	SNP	1,381	Unknown	NA	NA	deleterious(0)	probably_damaging(1)

24	DNMT3A	2	c.2174-2A>G	p.X725_splice	25463321	Splice_Site	SNP	5,917	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
214	ATM	11	c.9022C>T	p.R3008C	108236086	Missense_Mutation	SNP	6,802	Loss-of-function	Oncogenic	Pathogenic/Likely pathogenic	deleterious(0)	probably_damaging(0.988)
214	SETD1B	12	c.23A>C	p.H8P	122242666	Missense_Mutation	SNP	28,613	Unknown	NA	NA (may be germline)	NA	possibly_damaging(0.703)
215	CASP8	2	c.-77_35del GTTCTCCT CC...TTATTGA		202122878	Missense_Mutation	DEL	28,583	Unknown	NA	NA	NA	NA
215	TET2	4	c.5736T>A	p.H1912Q	106197403	Missense_Mutation	SNP	1,195	Unknown	NA	NA	deleterious(0)	probably_damaging(0.999)
215	PDS5B	13	c.3070G>T	p.V1024F	33332238	Missense_Mutation	SNP	8,871	Unknown	NA	NA	deleterious(0.01)	benign(0.009)
25	CUX1	7	c.4364G>A	p.S1455N	101892135	Missense_Mutation	SNP	1,873	Unknown	NA	NA	deleterious(0.01)	unknown(0)
235	SMARCA4	19	c.2527G>T	p.A843S	11130288	Missense_Mutation	SNP	1,008	Unknown	NA	NA	NA	benign(0.052)
253	CSF3R	1	c.2234G>A	p.C745Y	36932316	Missense_Mutation	SNP	2,363	Unknown	NA	NA	tolerated(0.21)	benign(0.012)
253	ROBO1	3	c.1900G>A	p.V634M	78717099	Missense_Mutation	SNP	1,220	Unknown	NA	NA	deleterious(0)	probably_damaging(0.996)
265	BLM	15	c.3206C>A	p.T1069K	91337583	Missense_Mutation	SNP	6,250	Unknown	NA	NA	tolerated(1)	benign(0.003)
265	CCND1	11	c.839A>T	p.E280V	69466001	Missense_Mutation	SNP	1,202	Unknown	NA	NA	tolerated(0.06)	probably_damaging(0.966)
265	NFE2	12	c.424C>T	p.P142S	54686856	Missense_Mutation	SNP	4,825	Unknown	NA	NA	deleterious(0.04)	possibly_damaging(0.766)
265	TET2	4	c.287G>A	p.R96H	106155386	Missense_Mutation	SNP	4,130	Unknown	NA	NA	tolerated(0.09)	probably_damaging(0.999)
269	NCOR2	12	c.1532C>A	p.P511H	124887058	Missense_Mutation	SNP	1,818	Unknown	NA	NA	NA	unknown(0)
POMI-AF2-0002	ATM	11	c.5250G>A	p.W1750*	108172447	Nonsense_Mutation	SNP	2,297	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
POMI-AF2-0002	TYK2	19	c.22A>G	p.M8V	10489061	Missense_Mutation	SNP	1,004	Unknown	NA	NA	tolerated(0.19)	benign(0)
POMI-AF2-0005	APC	5	c.317G>A	p.R106H	112102982	Missense_Mutation	SNP	1,003	Unknown	NA	Conflicting interpretations of pathogenicity Likely benign;Uncertain significance	NA	benign(0.006)

POMI- -AF2- 0005	MGA	15	c.7295A>T	p.E2432 V	42052 624	Missense_Mu tation	SNP	1,842	Unknown	NA	NA	deleterious(0)	probably_dama ging(0.998)
POMI- -AF2- 0005	SETD1 B	12	c.23A>C	p.H8P	12224 2666	Missense_Mu tation	SNP	32,06 3	Unknown	NA	NA (may be germline)	NA	possibly_dama ging(0.703)
POMI- -AF2- 0007	ATR	3	c.1692T>A	p.D564E	14227 8133	Missense_Mu tation	SNP	4,147	Unknown	NA	NA	tolerated_low_conf dence(0.29)	benign(0.01)
POMI- -AF2- 0007	DNMT 3A	2	c.2644C>T	p.R882C	25457 243	Missense_Mu tation	SNP	1,345	Likely Loss-of- function	Likely Oncogenic	Pathogenic/Li kely pathogenic	deleterious(0.02)	probably_dama ging(0.997)
POMI- -AF2- 0007	TET2	4	c.5618T>C	p.I1873T	10619 7285	Missense_Mu tation	SNP	1,064	Likely Loss-of- function	Likely Oncogenic	NA	deleterious(0)	probably_dama ging(1)
POMI- -AF2- 0017	FGFR3	4	c.1864G>A	p.A622T	18077 99	Missense_Mu tation	SNP	3,440	Unknown	NA	NA	deleterious(0.01)	probably_dama ging(0.999)
POMI- -AF2- 0021	KDM6 A	X	c.1924-2A>T	p.X642_ splice	44928 822	Splice_Site	SNP	5,797	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0021	GNA12	7	c.563G>A	p.R188Q	27730 98	Missense_Mu tation	SNP	2,801	Unknown	NA	NA	deleterious(0.02)	benign(0.358)
POMI- -AF2- 0021	KDM5 A	12	c.1453G>T	p.E485*	44344 4	Nonsense_M utation	SNP	1,279	Unknown	NA	NA	NA	NA
30	DNMT 3A	2	c.976C>A	p.R326S	25470 498	Missense_Mu tation	SNP	2,465	Unknown	NA	NA	deleterious(0)	probably_dama ging(1)
POMI- -AF2- 0024	CBL	11	c.443+1G>A	p.X148_ splice	11910 3406	Splice_Site	SNP	1,566	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0024	KMT2 C	7	c.5053G>T	p.A1685 S	15188 2672	Missense_Mu tation	SNP	20,98 8	Loss-of- function	Likely Oncogenic	NA	NA	probably_dama ging(0.996)
POMI- -AF2- 0024	MAP2 K4	17	c.823A>G	p.I275V	12028 620	Missense_Mu tation	SNP	1,786	Unknown	NA	NA	deleterious(0)	probably_dama ging(0.94)
POMI- -AF2- 0024	STAT5 B	17	c.1994A>T	p.Y665F	40359 659	Missense_Mu tation	SNP	2,632	Unknown	NA	NA	tolerated(0.16)	possibly_dama ging(0.498)
POMI- -AF2- 0025	CTCF	16	c.1808G>A	p.R603H	67663 407	Missense_Mu tation	SNP	1,287	Unknown	NA	NA	tolerated(0.06)	benign(0.288)
POMI- -AF2- 0025	EGFR	7	c.1567T>A	p.C523S	55229 260	Missense_Mu tation	SNP	4,651	Unknown	NA	NA	deleterious(0)	probably_dama ging(0.998)

POMI- -AF2- 0028	RUNX 1	21	c.848A>C	p.Q283P	36171 717	Missense_Mu tation	SNP	1,548	Unknown	NA	NA	tolerated(0.13)	benign(0.02)
POMI- -AF2- 0029	EP400	12	c.7214G>A	p.R2405 H	13253 4881	Missense_Mu tation	SNP	1,481	Unknown	NA	NA	NA	unknown(0)
POMI- -AF2- 0029	FBXO1 1	2	c.98C>A	p.P33Q	48132 762	Missense_Mu tation	SNP	26,84 0	Unknown	NA	NA	tolerated_low_conf idence(0.48)	unknown(0)
POMI- -AF2- 0029	SMG1	16	c.3737C>A	p.T1246 N	18872 057	Missense_Mu tation	SNP	1,813	Unknown	NA	NA	tolerated(0.49)	benign(0.045)
POMI- -AF2- 0032	NCOR 2	12	c.6500G>A	p.S2167 N	12481 9075	Missense_Mu tation	SNP	1,648	Unknown	NA	NA	NA	possibly_dama ging(0.669)
POMI- -AF2- 0032	SDHA	5	c.1414G>A	p.E472K	23669 6	Missense_Mu tation	SNP	3,340	Unknown	NA	Conflicting interpretations of pathogenicity Likely benign(2);Un certain significance(3)	tolerated_low_conf idence(0.23)	benign(0.001)
POMI- -AF2- 0032	SETD1 B	12	c.23A>C	p.H8P	12224 2666	Missense_Mu tation	SNP	31,91 9	Unknown	NA	NA (may be germline)	NA	possibly_dama ging(0.703)
POMI- -AF2- 0033	DNMT 3A	2	c.2058C>G	p.D686E	25464 455	Missense_Mu tation	SNP	1,072	Unknown	NA	NA	deleterious(0)	probably_dama ging(0.998)
POMI- -AF2- 0033	SDHA	5	c.1915C>G	p.L639V	25645 5	Missense_Mu tation	SNP	1,600	Unknown	Predicted Oncogenic	Uncertain significance	tolerated_low_conf idence(0.06)	benign(0.06)
POMI- -AF2- 0034	DNMT 3A	2	c.2114T>G	p.I705S	25463 568	Missense_Mu tation	SNP	1,795	Unknown	NA	NA	deleterious(0)	probably_dama ging(0.991)
POMI- -AF2- 0034	KMT2 B	19	c.5636G>A	p.G1879 D	36223 007	Missense_Mu tation	SNP	1,602	Unknown	NA	NA	NA	probably_dama ging(0.992)
POMI- -AF2- 0042	FBXO1 1	2	c.152A>C	p.Q51P	48132 708	Missense_Mu tation	SNP	2,715	Unknown	NA	NA	tolerated_low_conf idence(0.22)	unknown(0)
POMI- -AF2- 0042	ATM	11	c.1259T>G	p.I420R	10812 1451	Missense_Mu tation	SNP	3,361	Unknown	NA	NA	deleterious(0)	possibly_dama ging(0.786)
POMI- -AF2- 0042	ATM	11	c.2921+1G>A	p.X974_ splice	10814 1874	Splice_Site	SNP	5,105	Likely Loss-of- function	Likely Oncogenic	Pathogenic/Li kely pathogenic	NA	NA

POMI-AF2-0042	AXL	19	c.1964A>G	p.D655G	41759541	Missense_Mutation	SNP	25,900	Unknown	NA	NA	deleterious(0)	probably_damaging(1)
POMI-AF2-0042	SF3B1	2	c.2098A>G	p.K700E	198266834	Missense_Mutation	SNP	5,737	Switch-of-function	Likely Oncogenic	Likely pathogenic	deleterious(0)	probably_damaging(0.96)
POMI-AF2-0042	SETD8	12	c.179C>T	p.P60L	123875223	Missense_Mutation	SNP	12,500		NA	NA	deleterious_low_confidence(0)	benign(0.123)
POMI-AF2-0048	HIST1H2BJ	6	c.38A>G	p.K13R	27100492	Missense_Mutation	SNP	1,047	Unknown	NA	NA	deleterious_low_confidence(0.04)	benign(0.031)
POMI-AF2-0048	DNMT3A	2	c.2390A>G	p.N797S	25462017	Missense_Mutation	SNP	1,379	Unknown	NA	NA	deleterious(0)	probably_damaging(0.973)
POMI-AF2-0052	AR	X	c.173A>T	p.Q58L	66765161	Missense_Mutation	SNP	8,661	Unknown	NA	Uncertain significance	deleterious_low_confidence(0.01)	unknown(0)
POMI-AF2-0052	AXIN1	16	c.1463G>C	p.G488A	348043	Missense_Mutation	SNP	25,052	Unknown	NA	NA	tolerated(0.07)	probably_damaging(0.968)
POMI-AF2-0052	CREBBP	16	c.1810C>G	p.L604V	3830746	Missense_Mutation	SNP	1,724	Unknown	NA	NA	NA	probably_damaging(1)
POMI-AF2-0052	TET2	4	c.4075C>T	p.R1359C	106190797	Missense_Mutation	SNP	1,338	Likely Loss-of-function	Likely Oncogenic	NA	deleterious(0.01)	possibly_damaging(0.841)
POMI-AF2-0062	AR	X	c.176A>T	p.Q59L	66765164	Missense_Mutation	SNP	10,101	Unknown	NA	NA	deleterious_low_confidence(0.04)	unknown(0)
POMI-AF2-0057	CDK8	13	c.116A>G	p.K39R	26828894	Missense_Mutation	SNP	1,331	Unknown	NA	NA	tolerated(1)	benign(0.015)
POMI-AF2-0057	DNMT3A	2	c.2159G>T	p.R720L	25463523	Missense_Mutation	SNP	1,099	Unknown	NA	NA	deleterious(0)	probably_damaging(0.988)
POMI-AF2-0057	STAG2	X	c.3706-1G>A	p.X1236_splice	123229221	Splice_Site	SNP	1,891	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA
POMI-AF2-0057	MGAM	7	c.2885A>T	p.E962V	141752173	Missense_Mutation	SNP	4,987	Unknown	NA	NA	deleterious(0)	benign(0.086)
POMI-AF2-0057	SYK	9	c.1160A>G	p.K387R	93637110	Missense_Mutation	SNP	1,069	Unknown	NA	NA	tolerated(0.07)	benign(0.111)
POMI-AF2-0065	ATM	11	c.7513A>T	p.K2505*	108201146	Nonsense_Mutation	SNP	1,094	Likely Loss-of-function	Likely Oncogenic	NA	NA	NA

POMI- -AF2- 0066	DNMT 3A	2	c.2640G>C	p.M880I	25457 247	Missense_Mu tation	SNP	2,853	Likely Loss-of- function	Likely Oncogenic	NA	tolerated(0.09)	probably_dama ging(0.963)
POMI- -AF2- 0066	NT5C2	10	c.1681G>T	p.E561*	10484 9434	Nonsense_M utation	SNP	1,262	Unknown	NA	NA	NA	NA
POMI- -AF2- 0066	RET	10	c.1051G>A	p.V351I	43602 007	Missense_Mu tation	SNP	4,934	Unknown	NA	Uncertain significance	tolerated(0.37)	benign(0.042)
POMI- -AF2- 0070	DNMT 3A	2	c.2644C>T	p.R882C	25457 243	Missense_Mu tation	SNP	1,754	Likely Loss-of- function	Likely Oncogenic	Pathogenic/Li kely pathogenic	deleterious(0.02)	probably_dama ging(0.997)
POMI- -AF2- 0071	CIC	19	c.1464+1G>A	p.X488_ splice	42794 104	Splice_Site	SNP	1,475	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0071	NF1	17	c.2294G>A	p.R765H	29554 278	Missense_Mu tation	SNP	4,202	Unknown	NA	Conflicting interpretations of pathogenicity Likely benign;Uncert ain significance	deleterious(0.01)	possibly_dama ging(0.482)
POMI- -AF2- 0074	ASXL1	20	c.1210C>T	p.R404*	31021 211	Nonsense_M utation	SNP	2,912	Likely Loss-of- function	Likely Oncogenic	Pathogenic	NA	NA
POMI- -AF2- 0074	TSC2	16	c.5069-47_5068+27del CAGGAAAGGTAGGGCCGG GTGGGGCCCTGCAG TG	p.X1690_ splice	21379 25	Splice_Site	DEL	33,887	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMi- -AF2- 0077	ASXL2	2	c.341G>A	p.S114N	26022 316	Missense_Mu tation	SNP	1,248	Unknown	NA	NA	tolerated(0.49)	possibly_dama ging(0.727)
POMi- -AF2- 0077	KMT2 C	7	c.2521C>T	p.R841 W	15194 4998	Missense_Mu tation	SNP	9,271	Unknown	NA	NA	NA	probably_dama ging(1)
POMi- -AF2- 0077	MYC	8	c.745A>G	p.S249G	12875 1208	Missense_Mu tation	SNP	2,067	Unknown	NA	NA	tolerated(0.26)	benign(0.204)
POMi- -AF2- 0077	TET2	4	c.4354C>T	p.R1452 *	10619 3892	Nonsense_M utation	SNP	4,834	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMi- -AF2- 0077	TET2	4	c.3571C>T	p.Q1191 *	10616 4061	Nonsense_M utation	SNP	4,683	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 2	MED1	X	c.5285A>G	p.K1762 R	70356 390	Missense_Mu tation	SNP	1,049	Unknown	NA	NA	NA	benign(0.196)

0076													
POMI- -AF2- 0076	NOTC H2	1	c.7G>T	p.A3S	12061 2014	Missense_Mu tation	SNP	14,42 3	Unknown	NA	Benign	tolerated_low_confidence(0.5)	unknown(0)
POMI- -AF2- 0076	NOTC H2	1	c.8C>T	p.A3V	12061 2013	Missense_Mu tation	SNP	14,97 6	Unknown	NA	NA	tolerated_low_confidence(0.57)	unknown(0)
POMI- -AF2- 0076	PDS5B	13	c.2525_2526 insAATG TATTTA ATGGATG CTTTT	p.N842K fs*11	33316 778	Frame_Shift_ Ins	INS	13,63 6	Unknown	NA	NA	NA	NA
POMI- -AF2- 0076	PIK3C 2G	12	c.648_649ins AAGTCACT	p.W217 Kfs*12	18435 663	Frame_Shift_ Ins	INS	15,09 4	Unknown	NA	NA	NA	NA
POMI- -AF2- 0076	ATM	11	c.7231_7235 delGAAAA	p.E2411 Qfs*16	10819 9889	Frame_Shift_ Del	DEL	14,89 4	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0076	FANC D2	3	c.3462_3463ins CAAACA AATGCAATAGTATCCTATG TGTCACAGATAT	p.I1155Q fs*30	10128 944	Frame_Shift_ Ins	INS	17,50 0	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0076	SYK	9	c.1160A>G	p.K387R	93637 110	Missense_Mu tation	SNP	1,072	Unknown	NA	NA	tolerated(0.07)	benign(0.111)
POMI- -AF2- 0080	TET2	4	c.5075_5076dup AA	p.Y1693 Nfs*3	10619 6740	Frame_Shift_ Ins	INS	7,646	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0092	SMAD 4	18	c.-127-2A>T		48573 288	Splice_Site	SNP	12,82 1	Likely Loss-of- function	Likely Oncogenic	NA (germ)	NA	NA
POMI- -AF2- 0091	DNMT 3A	2	c.2481C>G	p.F827L	25458 692	Missense_Mu tation	SNP	2,284	Unknown	NA	NA	tolerated(0.33)	probably_dama ging(0.947)
POMI- -AF2- 0091	TET2	4	c.3851C>T	p.S1284 F	10618 0823	Missense_Mu tation	SNP	1,897	Likely Loss-of- function	Likely Oncogenic	NA	deleterious(0)	probably_dama ging(0.982)
43	ALK	2	c.1918G>A	p.G640R	29498 088	Missense_Mu tation	SNP	2,113	Unknown	NA	Uncertain significance	deleterious(0.03)	benign(0.064)
43	DUSP2 2	6	c.169G>A	p.D57N	33514 4	Missense_Mu tation	SNP	19,40 9	Unknown	NA	NA	deleterious(0.01)	possibly_dama ging(0.726)
43	HIST1 H3B	6	c.370G>C	p.D124H	26031 919	Missense_Mu tation	SNP	2,244	Unknown	NA	NA	deleterious_low_co nfidence(0.02)	possibly_dama ging(0.826)
43	TERT	5	c.1129C>T	p.R377C	12938 72	Missense_Mu tation	SNP	2,041	Unknown	NA	NA	tolerated(0.12)	benign(0.009)
POMI- -AF2- 1	NCOR 1	17	c.3686G>A	p.R1229 Q	15976 868	Missense_Mu tation	SNP	33,33 3	Unknown	NA	NA	NA	probably_dama ging(0.99)

0102													
POMI- -AF2- 0102	KMT2 C	7	c.5053G>T	p.A1685 S	15188 2672	Missense_Mu tation	SNP	28,00 0	Loss-of- function	Likely Oncogenic	NA	NA	probably_dama ging(0.996)
POMI- -AF2- 0102	TET2	4	c.1924C>T	p.Q642*	10615 7023	Nonsense_M utation	SNP	1,630	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0107	ARID1 B	6	c.820G>A	p.A274T	15709 9883	Missense_Mu tation	SNP	1,205	Unknown	NA	NA	NA	unknown(0)
POMI- -AF2- 0107	JAK3	19	c.983T>C	p.L328S	17952 450	Missense_Mu tation	SNP	1,653	Unknown	NA	NA	tolerated(0.07)	benign(0.346)
POMI- -AF2- 0107	RRAG C	1	c.1005G>C	p.L335F	39311 654	Missense_Mu tation	SNP	8,834	Unknown	NA	NA	deleterious(0)	possibly_dama ging(0.557)
POMI- -AF2- 0112	DNMT 3A	2	c.2635A>G	p.N879D	25457 252	Missense_Mu tation	SNP	1,064	Unknown	NA	NA	deleterious(0.04)	probably_dama ging(0.994)
POMI- -AF2- 0112	DNMT 3A	2	c.2645G>A	p.R882H	25457 242	Missense_Mu tation	SNP	1,314	Likely Loss-of- function	Likely Oncogenic	Conflicting interpretations of pathogenicity Pathogenic(2) ;Uncertain significance(1)	deleterious(0)	probably_dama ging(0.993)
POMI- -AF2- 0113	NT5C2	10	c.1678G>A	p.E560K	10484 9437	Missense_Mu tation	SNP	1,420	Unknown	NA	NA	tolerated_low_conf dence(0.4)	unknown(0)
POMI- -AF2- 0117	KMT2 C	7	c.2807A>T	p.E936V	15192 7369	Missense_Mu tation	SNP	11,97 9	Unknown	NA	NA	NA	probably_dama ging(0.943)
POMI- -AF2- 0121	FLT1	13	c.1138T>A	p.S380T	29002 027	Missense_Mu tation	SNP	1,418	Unknown	NA	NA	tolerated(0.41)	possibly_dama ging(0.842)
POMI- -AF2- 0121	SETD1 B	12	c.23A>C	p.H8P	12224 2666	Missense_Mu tation	SNP	28,57 1	Unknown	NA	NA (may be germline)	NA	possibly_dama ging(0.703)
POMI- -AF2- 0121	GNAS	20	c.2530C>A	p.R844S	57484 420	Missense_Mu tation	SNP	2,225	Likely Gain-of- function	Likely Oncogenic	Pathogenic/Li kely pathogenic	deleterious(0.01)	probably_dama ging(1)
POMI- -AF2- 0122	NT5C2	10	c.1681G>T	p.E561*	10484 9434	Nonsense_M utation	SNP	1,249	Unknown	NA	NA	NA	NA
POMI- -AF2-	CASP8	2	c.-77_35delG TTCTCCT		20212 2878	Missense_Mu tation	DEL	30,70 9	Unknown	NA	NA	NA	NA

0128			...AGCCAGGGTGGTTATTG A										
POMI- -AF2- 0128	GNB1	1	c.169A>G	p.K57E	17472 29	Missense_Mu tation	SNP	2,389	Gain-of- function	Oncogenic	NA	deleterious(0)	probably_dama ging(0.992)
POMI- -AF2- 0128	TET2	4	c.689dupT	p.S231V fs*23	10615 5787	Frame_Shift_ Ins	INS	12,45 5	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0132	BCL2	18	c.619C>T	p.R207 W	60795 959	Missense_Mu tation	SNP	1,060	Unknown	NA	NA	deleterious(0.05)	benign(0.003)
POMI- -AF2- 0132	DNMT 3A	2	c.852delC	p.Y284*	25470 909	Frame_Shift_ Del	DEL	32,67 8	Likely Loss-of- function	Likely Oncogenic	Likely benign	NA	NA
POMI- -AF2- 0132	MDM4	1	c.1100C>T	p.S367L	20451 8437	Missense_Mu tation	SNP	2,709	Unknown	NA	NA	deleterious(0)	probably_dama ging(0.972)
POMI- -AF2- 0146	DNMT 3A	2	c.2645G>T	p.R882L	25457 242	Missense_Mu tation	SNP	2,749	Likely Loss-of- function	Likely Oncogenic	Conflicting interpretations of pathogenicity Pathogenic(2) ;Uncertain significance(1)	deleterious(0)	probably_dama ging(0.991)
POMI- -AF2- 0151	FGFR3	4	c.58G>A	p.A20T	17957 19	Missense_Mu tation	SNP	1,506	Unknown	NA	NA	tolerated_low_conf dence(0.43)	unknown(0)
52	EPHA5	4	c.2557C>T	p.R853*	66213 873	Nonsense_M utation	SNP	1,000	Unknown	NA	NA	NA	NA
52	PLCG2	16	c.2497G>A	p.E833K	81960 766	Missense_Mu tation	SNP	1,070	Unknown	NA	Uncertain significance	tolerated(0.31)	benign(0.08)
52	KMT2 D	12	c.11164C>T	p.Q3722 *	49427 324	Nonsense_M utation	SNP	1,483	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
POMI- -AF2- 0156	DNMT 3A	2	c.1903C>T	p.R635 W	25466 800	Missense_Mu tation	SNP	3,065	Likely Loss-of- function	Likely Oncogenic	Conflicting interpretations of pathogenicity Likely pathogenic(2) ;Uncertain significance(1)	deleterious(0)	possibly_dama ging(0.854)
POMI- -AF2- 0158	DUSP2 2	6	c.427G>A	p.V143I	34826 6	Missense_Mu tation	SNP	32,52 3	Unknown	NA	NA	tolerated(0.06)	benign(0.106)

POMI- -AF2- 0158	DNMT 3A	2	c.976C>T	p.R326C	25470 498	Missense_Mu tation	SNP	7,670	Unknown	NA	NA	deleterious(0)	probably_dama ging(1)
POMI- -AF2- 0158	DNMT 3A	2	c.2081A>C	p.H694P	25464 432	Missense_Mu tation	SNP	1,608	Unknown	NA	NA	deleterious(0)	possibly_dama ging(0.784)
POMI- -AF2- 0158	WHSC 1	4	c.928-5_928delCCCAGC	p.X310_ splice	19198 63	Splice_Site	DEL	10,04 3	Unknown	NA	NA	NA	NA
POMI- -AF2- 0161	NOTC H3	19	c.2099C>T	p.A700V	15296 343	Missense_Mu tation	SNP	1,619	Unknown	NA	NA	tolerated(0.26)	probably_dama ging(0.911)
POMI- -AF2- 0159	DUSP2 2	6	c.181C>T	p.Q61*	33515 6	Nonsense_M utation	SNP	20,79 7	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
61	SPEN	1	c.3856G>A	p.G1286 R	16256 591	Missense_Mu tation	SNP	2,035	Unknown	NA	NA	NA	possibly_dama ging(0.71)
68	RPTO R	17	c.40G>T	p.G14W	78519 469	Missense_Mu tation	SNP	1,208	Unknown	NA	NA	tolerated(0.08)	benign(0.319)
68	ASXL1	20	c.3727C>T	p.Q1243 *	31024 242	Nonsense_M utation	SNP	1,752	Likely Loss-of- function	Likely Oncogenic	NA	NA	NA
5	AURK A	20	c.71G>A	p.R24H	54961 561	Missense_Mu tation	SNP	11,85 0	Unknown	NA	NA	deleterious(0)	benign(0.249)
5	FLT4	5	c.2912G>A	p.R971Q	18004 5859	Missense_Mu tation	SNP	1,794	Unknown	NA	NA	tolerated(0.53)	benign(0.015)
5	SDHA	5	c.1414G>A	p.E472K	23669 6	Missense_Mu tation	SNP	3,117	Unknown	NA	Conflicting interpretations of pathogenicity Likely benign(2);Un certain significance(3)	tolerated_low_conf idence(0.23)	benign(0.001)
5	ATM	11	c.8180T>C	p.V2727 A	10820 6600	Missense_Mu tation	SNP	3,491	Unknown	NA	Uncertain significance	deleterious(0)	probably_dama ging(0.973)
7	HIST1 H2BJ	6	c.38A>G	p.K13R	27100 492	Missense_Mu tation	SNP	1,942	Unknown	NA	NA	deleterious_low_co nfidence(0.04)	benign(0.031)

DEL: Deletion HGVS: Human Genome Variation Society notation in the cDNA; HGVSp: HGVS, Human Genome Variation Society notation in the protein; INS: Insertion; SNP: Single Nucleotide Polymorphism; VAF: Variant Allele Frequency

Table S5. Pre-operative baseline characteristics according to HSM status

	Total (n = 104)	Non-HSM (n = 41)	HSM (n = 63)	P
Age, years	70 [66 ; 75]	68 [62 ; 71]	73 [68 ; 77]	<0.001
Women, n (%)	37 (36)	19 (46)	18 (29)	0.06
BMI (kg/m ²)	28 [24 ; 31]	28 [24 ; 32]	28 [24 ; 30]	0.15
Obese, n (%)	36 (35)	18 (44)	18 (29)	0.11
History of diabetes mellitus, n (%)	30 (29)	11 (26)	19 (30)	0.71
LVEF (%)	65 [58 ; 70]	65 [56 ; 70]	64 [60 ; 68]	0.76
LA area (cm ²)	22±5	22±5	22±5	0.71
Transaortic Vmax (m/s)	4.4 [4.06 ; 4.77]	4.4 [4.1 ; 4.6]	4.4 [4.1 ; 4.5]	0.83
Mean transaortic gradient (mmHg)	49.4 [40 ; 59.8]	49 [40 ; 56]	50 [40 ; 61]	0.38
Aortic valve area (cm ²)	0.69 [0.57 ; 0.85]	0.68 [0.6 ; 0.85]	0.71 [0.56 ; 0.84]	0.75
Indexed aortic valve area (cm ² /m ²)	0.36 [0.29; 0.44]	0.34 [0.30; 0.42]	0.39 [0.30; 0.44]	0.68
Pre-operative creatinin (mg/l)	9 [7 ; 10]	8 [7 ; 9]	9 [7 ; 11]	0.13
Pre-operative MDRD clearance (ml/min/1,73)	86±23	89±21	82±23	0.09
Pre-operative CRP (mg/l)	3 [3; 4]	3 [3; 3]	3 [3; 5]	0.45
Pre-operative leukocyte count (cells/ mm ³)	7410 [6240; 8875]	6760 [5995; 8027]	7705 [6755; 9645]	0.009
Pre-operative monocyte count (cells/mm ³)	600 [500 ; 700]	500 [500 ; 650]	600 [525 ; 800]	0.004
Pre-operative lymphocyte count (cells/mm ³)	1700 [1300; 2100]	1700 [1300; 2150]	1700 [1300; 2175]	0.74
Pre-operative neutrophils count (cells/mm ³)	4800 [4100; 6300]	4600 [3650; 5450]	5100 [4300; 6700]	0.08
Pre-operative eosinophil count (cells/mm ³)	100 [100; 200]	100 [100; 200]	100 [100; 200]	0.47
Pre-operative basophil count (cells/mm ³)	0 [0; 100]	0 [0; 100]	0 [0; 100]	0.72

Variables are given as median [1st quartile-3rd quartile], mean ±SD or number (percentage). BMI: body mass index. LA area: left atrial area. LVEF: left ventricular ejection fraction. Hematopoietic somatic mosaicism (HSM) as defined by the HemePACT panel with VAF upper than 1% screening method.

Table S6. Post-operative outcomes according to HSM status

	Non-HSM (n = 41)	HSM (n = 63)	Unadjusted p-value	Univariate OR (CI 95%)	Adjusted p-value
Post-operative atrial fibrillation, n (%)	13 (32)	39 (62)	0.0026	3.5 (1.49 to 7.7)	0.0031
Post-operative acute kidney injury, n (%)	7 (17)	13 (20)	0.65		
Post-operative type 5 MI, n (%)	4 (10)	18 (29)	0.02	3.7 (1.18 to 10.7)	NS
Post-operative troponin peak (ng/l)	290 [255; 443]	379 [297; 652]	0.005		NS
Catecholamine use \geq 24hours, n (%)	5 (12)	19 (30)	0.053		0.055
Length of hospital stay (days)	11 [9; 13.3]	12 [10; 14]	0.053		NS
Intra-hospital death, n (%)	0	1 (2)	0.42		
Re-intervention, n (%)	1 (2)	6 (9)	0.32		
Cardiac Tamponade, n (%)	0	2 (3)	0.68		

Variables are given as median [1st quartile-3rd quartile], mean \pm sd or number (percentage). Adjusted P-value is provided for post-operative outcomes significantly associated with HSM in univariable analysis ($p < 0.10$). Backward multiple regression model was performed for Post-operative CRP peak, post-operative troponin peak and days under catecholamine and included age, BMI, diabetes, and extracorporeal circulation duration as covariates. For Post-operative atrial fibrillation and type V myocardial infarction, a backward logistic regression model was performed and included age, BMI, diabetes, and extracorporeal circulation duration as covariates. The residuals normality were assessed for post-operative CRP peak (Shapiro-Wilk test, $p = 0.12$). BMI: body mass index. Hematopoietic somatic mosaicism (HSM) as defined by the HemePACT panel with VAF upper than 1% screening method. MI: myocardial infarction. NR: variables not retained by the model. POAF: postoperative atrial fibrillation.

Table S7. Backward logistic regression for POAF

Variable	Univariate OR (CI 95%)	Unadjusted p-value	Multivariate OR (CI 95%)	Adjusted p-value
Age	1.01 (0.97 to 1.06)	0.57	NR	NR
BMI	1.01 (0.94 to 1.09)	0.67	NR	NR
Diabetes	1 (0.43 to 2.34)	1	NR	NR
Extracorporeal circulation duration	1.01 (0.99 to 1.02)	0.61	NR	NR
HSM	3.5 (1.49 to 7.7)	0.0026	3.5 (1.5 to 8.04)	0.0031

Backward logistic regression model was performed. NR: variables not retained by the model. BMI: body mass index. HSM: Hematopoietic somatic mosaicism as defined by the HemePACT panel with VAF upper than 1% screening method.

Table S8. Backward multiple regression for postoperative CRP peak

Variable	Coefficient	SD	r _{partial}	Adjusted p-value
Age	-2.05	0.96	-0.22	0.0349
BMI	NR	NR	NR	NR
Diabetes	24.04	16.9	0.15	0.16
POAF	40	16.02	0.25	0.01
Extracorporeal circulation duration	NR	NR	NR	NR
HSM	48.9	17	0.28	0.0061

Backward multiple regression model was performed for Post-operative CRP peak and included age, BMI, diabetes, POAF, extracorporeal circulation duration and HSM as covariates. The residuals normality were assessed for post-operative CRP peak (Shapiro-Wilk test, $p=0.12$). BMI: body mass index. HSM: Hematopoietic somatic mosaicism as defined by the HemePACT panel with VAF upper than 1% screening method.

Table S9. Leukocyte markers used for mass cytometry analysis

Target	Clone	Antibody Isotype	Metal Tag	Hand coupling/polymer	Concentration	Antibody Supplier	Antibody reference	Antibody lot
CD45	HI30	Mouse IgG1, κ	89Y		1 µg/100µL	Fluidigm	3089003B	1151903
CD282	W15145C	Mouse IgG2a, κ	103Rh	Maxpar X8	2 µg/100µL	Biolegend	392302	B250232
Pan-CEA	TET2	Mouse g G2bka	113In	Maxpar X8	2 µg/100µL	Cliniscience	sc-59875	12806
Connexin 43	578618	Mouse IgG2A	115In	Maxpar X8	2 µg/100µL	R&D System	MAB7737	CGTK0118061
CD8	SK1	Mouse IgG1, κ	116Cd	Maxpar MCP9	2 µg/100µL	Biolegend	344727	B265197
Perforin	dG9	Mouse IgG2b, κ	139La	LightningKit	2 µg/100µL	Biolegend	308102	B267038
CCR2	K036C2	Mouse IgG2a, κ	140Ce	Maxpar X8	2 µg/100µL	Biolegend	357202	B240747
CD196/CCR6	G034E3	Mouse IgG2b, κ	141Pr		1 µg/100µL	Fluidigm	3141003A	3471805
CD19	HIB19	Mouse IgG1, κ	142Nd		1 µg/100µL	Fluidigm	3142001B	381907
CD127	A019D5	Mouse IgG1, κ	143Nd		1 µg/100µL	Fluidigm	3143012B	2501815
CD38	HIT2	Mouse IgG1, κ	144Nd		1 µg/100µL	Fluidigm	3144014B	2991817
CD163	GHI/61	Mouse IgG1, κ	145Nd		1 µg/100µL	Fluidigm	3145010B	2351602
IgD	IA6-2	Mouse BALB/c IgG2a, κ	146Nd		1 µg/100µL	Fluidigm	3146005B	671816
CD20	2H7	Mouse IgG2b, κ	147Sm		1 µg/100µL	Fluidigm	3147001B	2601808
CD34	581	Mouse IgG1, κ	148Nd		1 µg/100µL	Fluidigm	3148001B	1841814
CD194	L291H4	Mouse IgG1, κ	149Sm		1 µg/100µL	Fluidigm	3149029A	2991816
CD64	10.1	Mouse IgG1, κ	150Nd	Maxpar X8	2 µg/100µL	Biolegend	305029	B245146
CD123	6H6	Mouse IgG1	151Eu		1 µg/100µL	Fluidigm	3151001B	2431808
TCRgd	11F2	Mouse BALB/c IgG1	152Sm		1 µg/100µL	Fluidigm	3152008B	441915
CD185	RF8B2	LOU/M IgG2b, κ	153Eu		1 µg/100µL	Fluidigm	3153020B	1621806
CD3	UCHT1	Mouse IgG1, κ	154Sm		1 µg/100µL	Fluidigm	3154003B	71917
CD27	L128	Mouse BALB/c IgG ₁	155Gd		1 µg/100µL	Fluidigm	3155001B	1731805
CD183	G025H7	Mouse IgG1, κ	156Gd		1 µg/100µL	Fluidigm	3156004B	2991818
Fox P3	259D/C7	Mouse BALB/c IgG ₁	157Gd	Maxpar X8	2 µg/100µL	BD	560044	817636

CD33	WM53	Mouse IgG1, κ	158Gd		1 µg/100µL	Fluidigm	3158001B	1031707
CD11c	Bu15	Mouse IgG1, κ	159Tb		1 µg/100µL	Fluidigm	3159001B	2991809
CD28	CD28.2	Mouse IgG1, κ	160Gd		1 µg/100µL	Fluidigm	3160003B	3181807
CD26	BA5b	Mouse IgG2a, κ	161Dy		1 µg/100µL	Fluidigm	3161015B	1531522
CD66b	80H3	Mouse / IgG1	162Dy		1 µg/100µL	Fluidigm	3162023B	651902
CD172a	SE5A5	Mouse IgG1, κ	163Dy		1 µg/100µL	Fluidigm	3163017B	2471516
CD161	HP-3G10	Mouse IgG1, κ	164Dy		1 µg/100µL	Fluidigm	3164009B	2991815
CD45RO	UCHL1	Mouse IgG2a, κ	165Ho		1 µg/100µL	Fluidigm	3165011B	241813
CD24	ML5	Mouse IgG2a, κ	166Er		1 µg/100µL	Fluidigm	3166007B	1501811
CD197	G043H7	Mouse IgG2a, κ	167Er		1 µg/100µL	Fluidigm	3167009A	71919
CD206	15-2	Mouse IgG1, κ	168Er		1 µg/100µL	Fluidigm	3168008B	2431803
CD25	2A3	Mouse BALB/c IgG ₁ , κ	169Tm		1 µg/100µL	Fluidigm	3169003B	2881804
CD45RA	HI100	Mouse IgG2b, κ	170Er		1 µg/100µL	Fluidigm	3170010B	3521705
Granzyme B	GB11	Mouse IgG1, κ	171Yb		1 µg/100µL	Fluidigm	3171002B	1521802
CX3CR1	2A9-1	Rat IgG2b, κ	172Yb		1 µg/100µL	Fluidigm	3172017B	1731807
HLA-DR	L243	Mouse IgG2a, κ	173Yb		1 µg/100µL	Fluidigm	3173005B	1731803
CD4	SK3	Mouse IgG1, κ	174Yb		1 µg/100µL	Fluidigm	3174004B	1571804
CD14	M5E2	Mouse IgG2a, κ	175Lu		1 µg/100µL	Fluidigm	3175015B	2991814
CD56	NCAM16.2	Mouse BALB/c IgG _{2b} , κ	176Yb		1 µg/100µL	Fluidigm	3176008B	2151801
CD1c	L161	Mouse IgG1, κ	195Pt	Cisplatin	2 µg/100µL	Biolegend	331502	
CADM1	30	Chicken IgY	196Pt	Cisplatin	2 µg/100µL	Cliniscience	CM004-3	
CD16	3G8	Mouse IgG1, κ	209		1 µg/100µL	Fluidigm	3209002B	3041801

Table S10. Presets used for unsupervised clustering

Markers used for dimension reduction and clustering algorithms		
CD45	CD123	CD197
CD282	TCRgd	CD206
Connexin 43	CD185	CD25
CD8	CD3	CD45RA
CCR2	CD27	Granzyme B
CD196/CCR6	CD183	CX3CR1
CD19	CD33	HLA-DR
CD127	CD11c	CD4
CD38	CD28	CD14
CD163	CD26	CD56
IgD	CD66b	CD1c
CD20	CD172a	CADM1
CD34	CD161	CD16
CD194	CD45RO	
CD64	CD24	
Umap parameters		
n_neighbors	1	100
learning_rate	1	10
init	spca	spca
n_epochs	500	500
pcg_rand	TRUE	TRUE
scale	colrange	colrange
min_dist	20	20
spread	20	20
nn_method	fnn	annoy
n-tree	-	100
ClusterX parameters		
gaussian	TRUE	TRUE
alpha	0.0001	0.01
detectHalos	FALSE	TRUE
SVMhalos	FALSE	TRUE

Table S11. Baseline characteristics of the 17 patients selected for leukocyte mass cytometry experiment according to HSM status

Variable	Total	Non-HSM (n=6)	HSM (n=11)	p-value
Age (years)	66±8	65±8	67±8	0.63
Male, n(%)	12 (71)	4 (66)	8 (73)	0.77
BMI (kg/m ²)	26±2,5	27,7±2	26,5±,3	0.35
Diabetes, n(%)	5 (29)	3 (50)	2 (20)	0.41
Preoperative MDRD clearance (ml/min/1.73)	88±21	89±19	87±22	0.86
LVEF (%)	61±8	57±9	63±8	0.17

Variables are given as median [1st quartile-3rd quartile], mean ±SD or number (percentage). BMI: body mass index. LA area: left atrial area. LVEF: left ventricular ejection fraction.

Table S12. HSM mutations in the patients selected for leukocyte mass cytometry experiment

Patient ID	Mutated gene	VAF (%)	Type of mutation
1	DNMT3A	1.75	Missense
2	DNMT3A	32.6	Frame shift deletion
3	DNMT3A DNMT3A	1.06 1.31	Missense Missense
4	DNMT3A TET2	2.28 1.89	Missense Missense
5	TET2	7.65	Frame shift insertion
6	TET2	1.63	Nonsense
7	ATM	1.09	Nonsense
8	NF1 CIC	4.2 1.48	Nonsense Splice site
9	ASXL1 TSC2	2.9 33.8	Nonsense Splice site
10	SMAD4	12.8	Splice site
11	DUSP22	20.79	Nonsense

VAF: Variant allelic frequency

Table S13. Baseline characteristics of patients whose preoperative sorted classical monocytes were analyzed by RNA-seq

Variable	Total (n=33)	Non-HSM (n=13)	HSM (n=20)	p-value
Age (years)	73 [64 ; 77]	70 [62 ; 77]	73 [66 ; 77]	0.36
Male, n(%)	27 (84)	10 (77)	17 (35)	0.89
BMI (kg/m ²)	28.5±3.6	26.8±3.5	29.4±2.4	0.093
Diabetes, n(%)	0	0	0	1
Preoperative MDRD clearance (ml/min/1.73 m ²)	86±19	84±13.9	87±22.7	0.64
LVEF (%)	63±7.3	64±9.9	62±4.9	0.59

Variables are given as median [1st quartile-3rd quartile], mean ±SD or number (percentage). BMI: body mass index. LA area: left atrial area. LVEF: left ventricular ejection fraction.

Table S14. HSM mutations in patients selected for the preoperative RNA sequencing experiment

Patient ID	Mutated gene	VAF (%)	Type of mutation	Sorted classical monocytes count
1	DNMT3A ZRSR2	3.72 26.4	Nonsense Nonsense	678998
2	DNMT3A	1.07	Missense	144057
3	DNMT3A	1.38	Missense	155791
4	DNMT3A	1.75	Missense	44851
5	DNMT3A SF3B1 SMC1A	1.18 1.23 1.26	Missense Missense Missense	90127
6	TET2	7.83	Missense	15876
7	TET2	15.9	Frame shift insertion	235467
8	TET2	1.19	Missense	269427
9	ATM	3.49	Missense	67769
10	HIST1H3B DUSP22	2.24 19.4	Missense Missense	178587
11	ASXL1	21.96	Nonsense	131460
12	MGA	10.94	Frame shift insertion	70241
13	ATXN2	21.66	Frame shift deletion	47126
14	NF1	9.12	Frame shift deletion	331664
15	SF3B1	8.74	Missense	125774
16	RAD21	12.34	Frame shift deletion	39019
17	ROBO1	1.22	Missense	40427
18	FGFR3	3.44	Missense	28789
19	KDM6A	5.79	Splice site	59894
20	EGFR	4.65	Missense	341758

VAF: Variant allelic frequency

Figure S1. Flow chart of the cohort

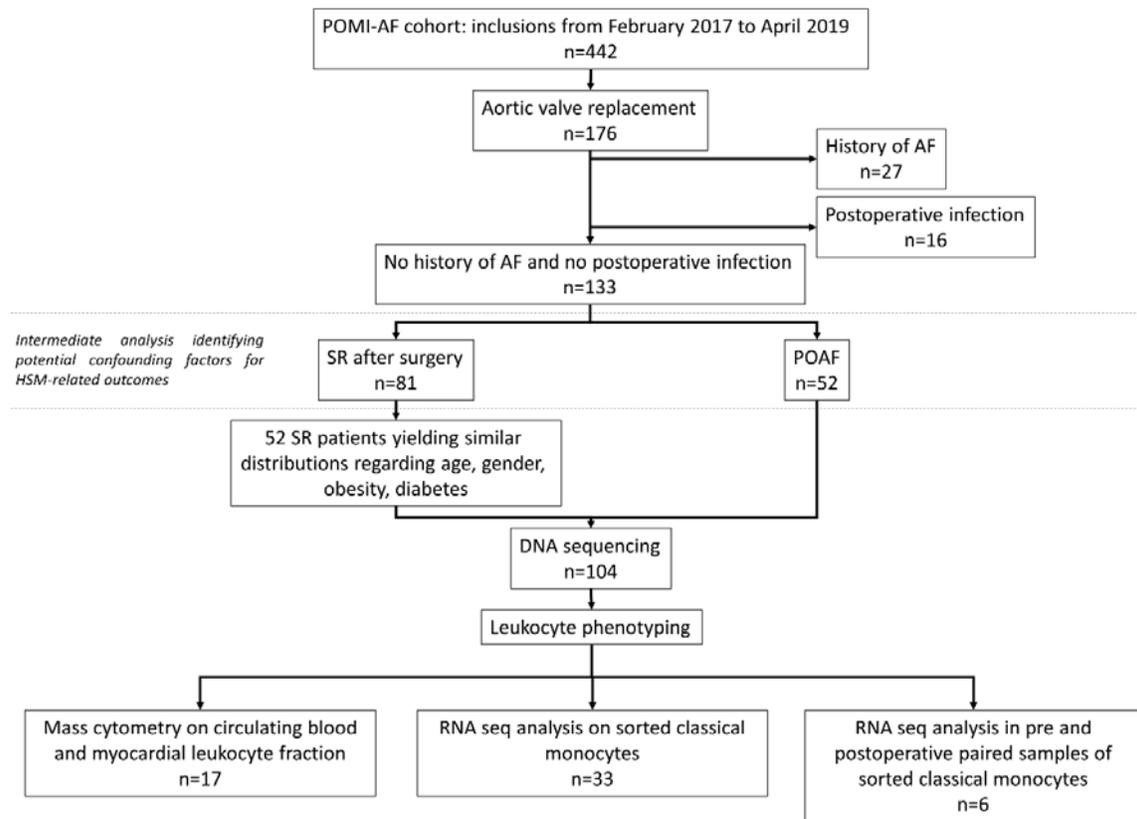


Figure S2. Distribution of HSM mutations:

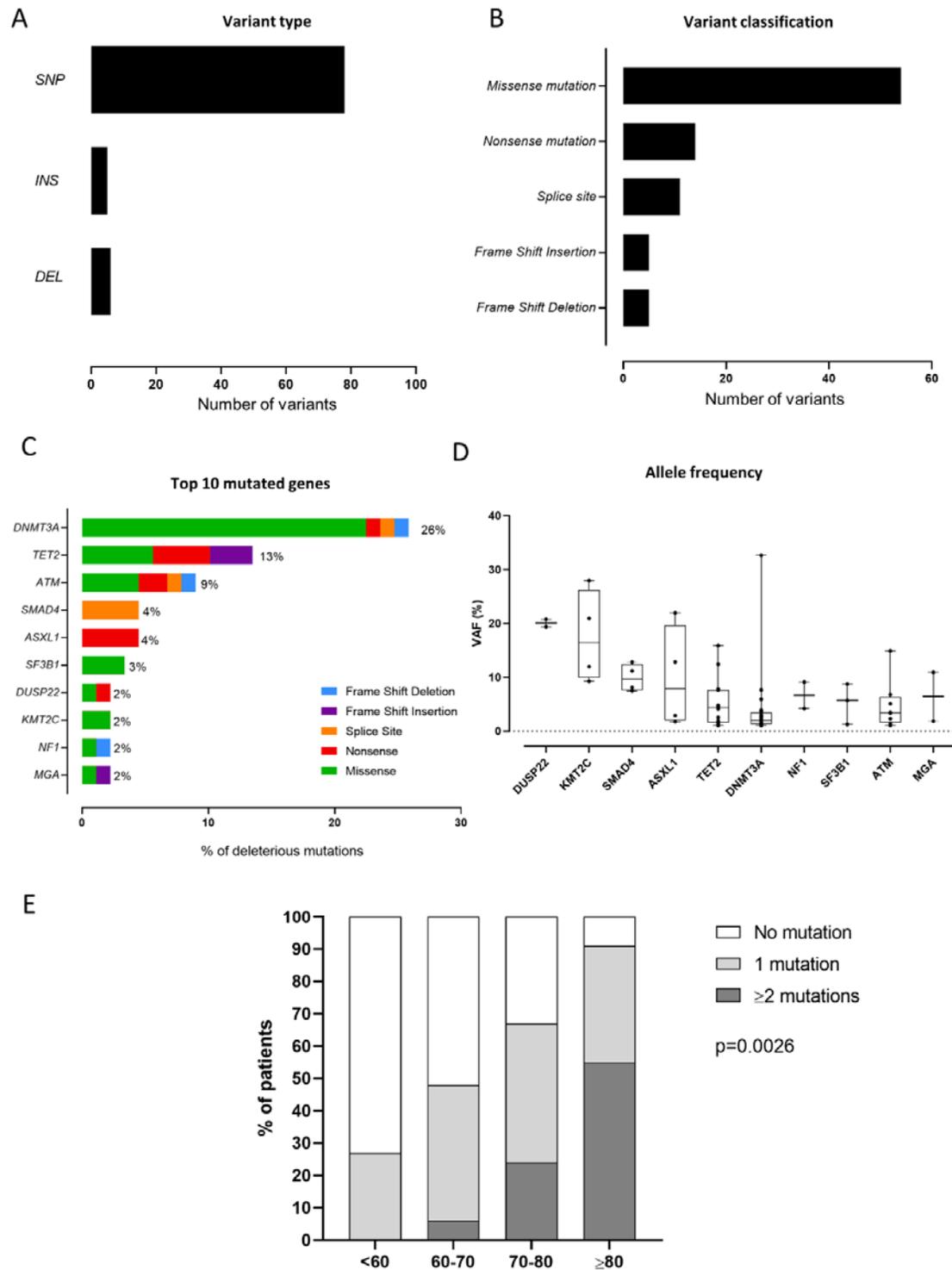


Figure S2. HSM prevalence and mutations in the aortic valve replacement (AVR) cohort. **A.** Variants subtypes: SNP: single nucleotide polymorphism INS: insertion DEL: deletion. **B.** Variant

classification for observed mutations. **C.** Top 10 mutated genes and proportion of each gene regarding all deleterious mutations. **D.** Distribution of variant allele frequency (VAF) of the top 10 mutated genes. **E.** Prevalence and number of mutations per patient according to age. P value was computed using Chi-square test.

Figure S3. RNAseq analysis of pre and post operative sorted classical monocytes according to HSM status

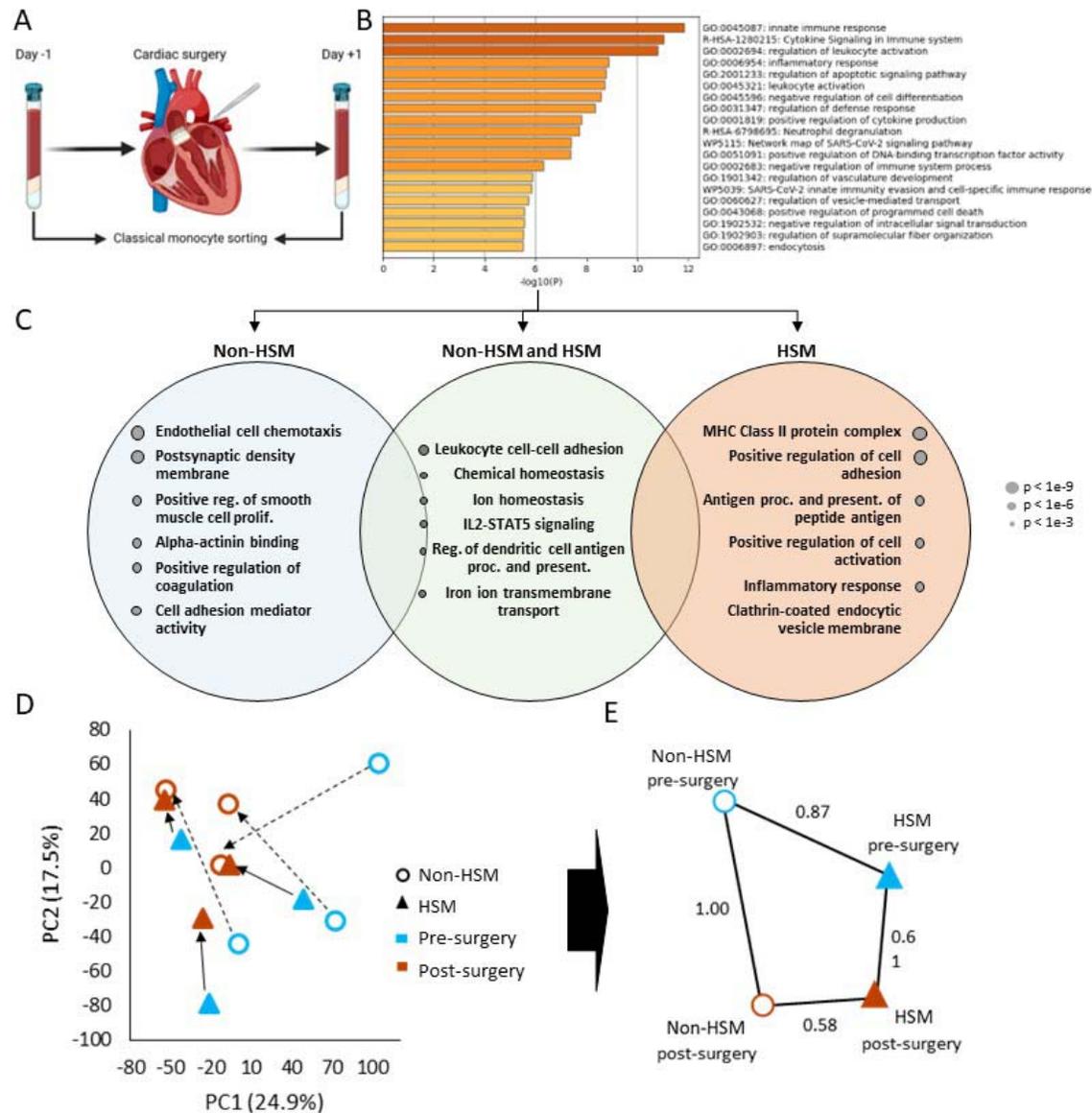


Figure S3. **A.** PBMCs samples were collected the day before and at 24 hours after surgery in 6 patients (3 non-HSM and 3 HSM carriers). Classical monocytes CD14⁺⁺CD16⁻ were sorted and a paired RNAseq analysis was performed. Among HSM carriers, 2 patients presented deleterious and/or pathogenic variants in DNMT3A and 1 patient presented deleterious and/or pathogenic variants in ATM, AXL and SF3B1 **B.** Enrichment analysis of surgery-induced differential gene expression in all patients. Top-ranking GO and Hallmark genes. **C.** Enrichment analysis of surgery-induced differential gene expression. The top-ranking GO and Hallmark genesets are displayed for each category. Genesets which were differentially expressed in HSM but not ($p > 0.05$) in non-HSM patients are

displayed, and vice versa. Genesets which were differentially expressed ($p < 0.01$) in both HSM and non-HSM patients are also shown. **D.** Principal components plot of \log_2 expression values. Arrows connect each patient's pre- and post-surgery points. **E.** Euclidian distances between the centroids of each group. Post-surgery expression profiles become more similar to each other. Distances between pre-surgery to post-surgery are smaller in HSM patients than in patients without HSM ($p = 0.022$).

Figure S4. Mass cytometry data pre-processing

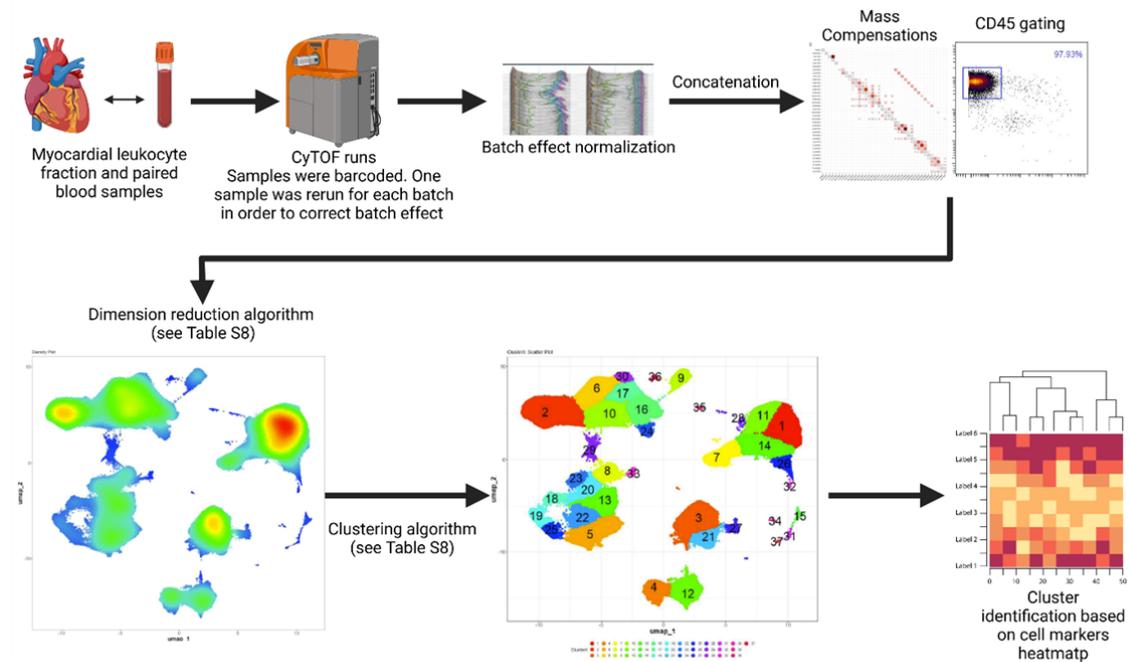


Figure S4. Mass cytometry data pre-processing. Paired myocardial and blood samples were collected from patients undergoing cardiac surgery and who underwent DNA sequencing. Each sample was barcoded and samples were acquired in bulk with one sample was rerun for each CyTOF run in order to correct batch effect. A gating on $CD45^+$ live cells was then performed. A dimension reduction algorithm was then applied and the clustering algorithm ClusterX was applied on the entire data set. Cells clusters identification was then performed according to Heatmap based on cell markers associated with each cluster.

Figure S5. Gating strategy for classical monocytes sorting

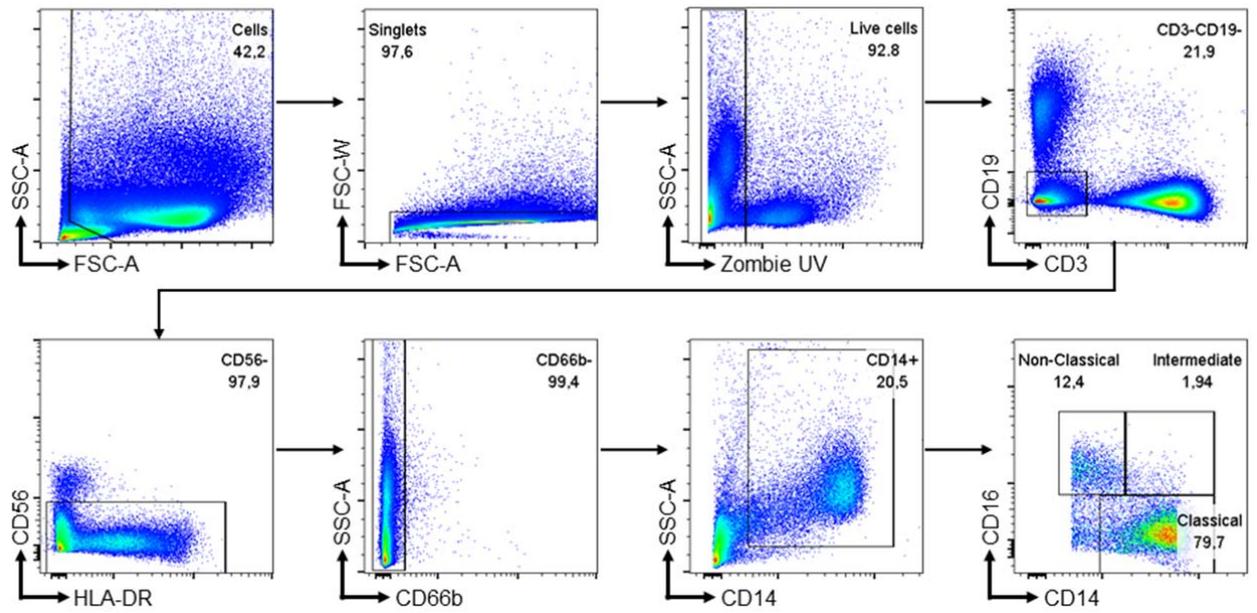


Figure S5. Gating strategy for classical monocyte sorting.