Assessing tumor-driven perturbations during hematopoiesis in a melanoma mouse model through single-cell multiomic analysis

Using the BD FACSMelody™ Cell Sorter to simultaneously isolate four rare cell populations

Features

- BD FACSMelody™ Cell Sorter enables simultaneous purification of four rare murine hematopoietic stem and progenitor cell (HSPC) populations, saving precious samples and reducing sort time prior to the single-cell multiomic workflow
- Investigate multiple samples with an integrated workflow featuring the BD® Mouse Immune Single-Cell Multiplexing Kit
- Assess changes in surface protein and whole transcriptome expression simultaneously in single hematopoietic cells in tumor-burdened or healthy mice

Hematopoiesis is a highly coordinated process in which hematopoietic stem cells give rise to multilineage blood cells through successive series of increasingly lineage restricted intermediate progenitors (Figure 1). Under steady state conditions during adult life, HSPCs are mainly localized in the bone marrow (BM) niche; however, under pathophysiological conditions such as cancer, extramedullary hematopoiesis can occur in organs like the spleen (SP).

Tumor progression is often associated with an expansion of immunosuppressive myeloid cells including myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs). These cells are known to modulate immune responses that enhance cancer cell stemness, invasion and immune escape. As the tumor progresses, it may interfere with normal BM hematopoiesis by skewing the generation of myeloid cells with tumor promoting functions and induce splenic extramedullary hematopoiesis to support continuous replenishment. Thus, it is important to decipher the mechanisms of tumor-driven perturbations in hematopoiesis.

BD

In this study, we used a melanoma mouse model to study tumor-driven perturbations during hematopoiesis. We sorted four different HSPC populations from the BM of healthy (control) mice and the BM and SP of tumor-burdened mice using the BD FACSMelody Cell Sorter. Sorted cells were then captured by the BD Rhapsody™ Single-Cell Analysis System for simultaneous single-cell whole transcriptome analysis (WTA) and surface protein analysis to identify changes in these populations.

Figure 1

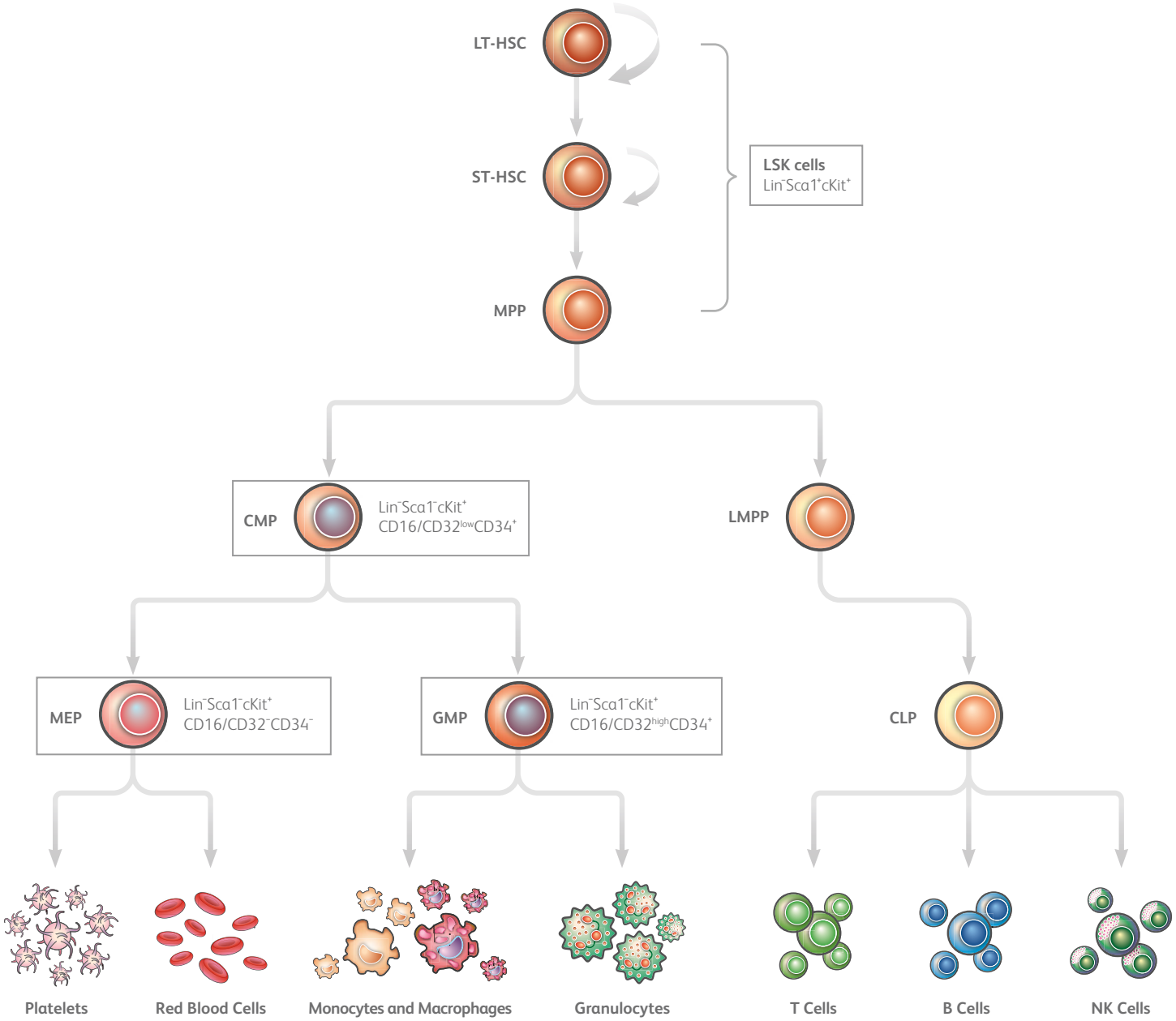

Figure 1. An overview of hematopoiesis in adult mouse bone marrow

Hematopoietic stem cells (HSCs) can be separated into long-term (LT-HSC) and short-term (ST-HSC) classes based on their repopulating capacity. HSCs differentiate into transient multipotent progenitors (MPPs) that give rise to cells with restricted fate, including common myeloid progenitor (CMP) and lymphoid primed multipotent progenitor (LMPP) cells. Myeloid committed CMPs give rise to megakaryocyte erythroid progenitor (MEP) and granulocyte macrophage progenitor (GMP) cells capable of generating terminally differentiated cells, including red blood cells (RBCs), monocytes/macrophages, granulocytes and platelets. The figure also shows phenotypic markers used in this experiment to sort the indicated populations (boxes) for downstream single-cell multiomic analysis.

Using a melanoma mouse model, the first step in the integrated workflow (Figure 2) was to harvest cells from the BM and SP of tumor-burdened mice or BM of healthy mice and then perform magnetic enrichment for lineage negative (Lin$^-$) cells. Next, the Lin$^-$ cells from each tissue were tagged with unique sample tags from the BD Mouse Immune Single-Cell Multiplexing Kit (SMK) to enable pooling of sorted cell populations prior to single-cell capture. The sample tagged cells were then fluorescently labeled with a 6-color panel (Table 1) for cell sorting and costained with a 36-plex BD® AbSeq Reagents (Table 2).

Figure 2

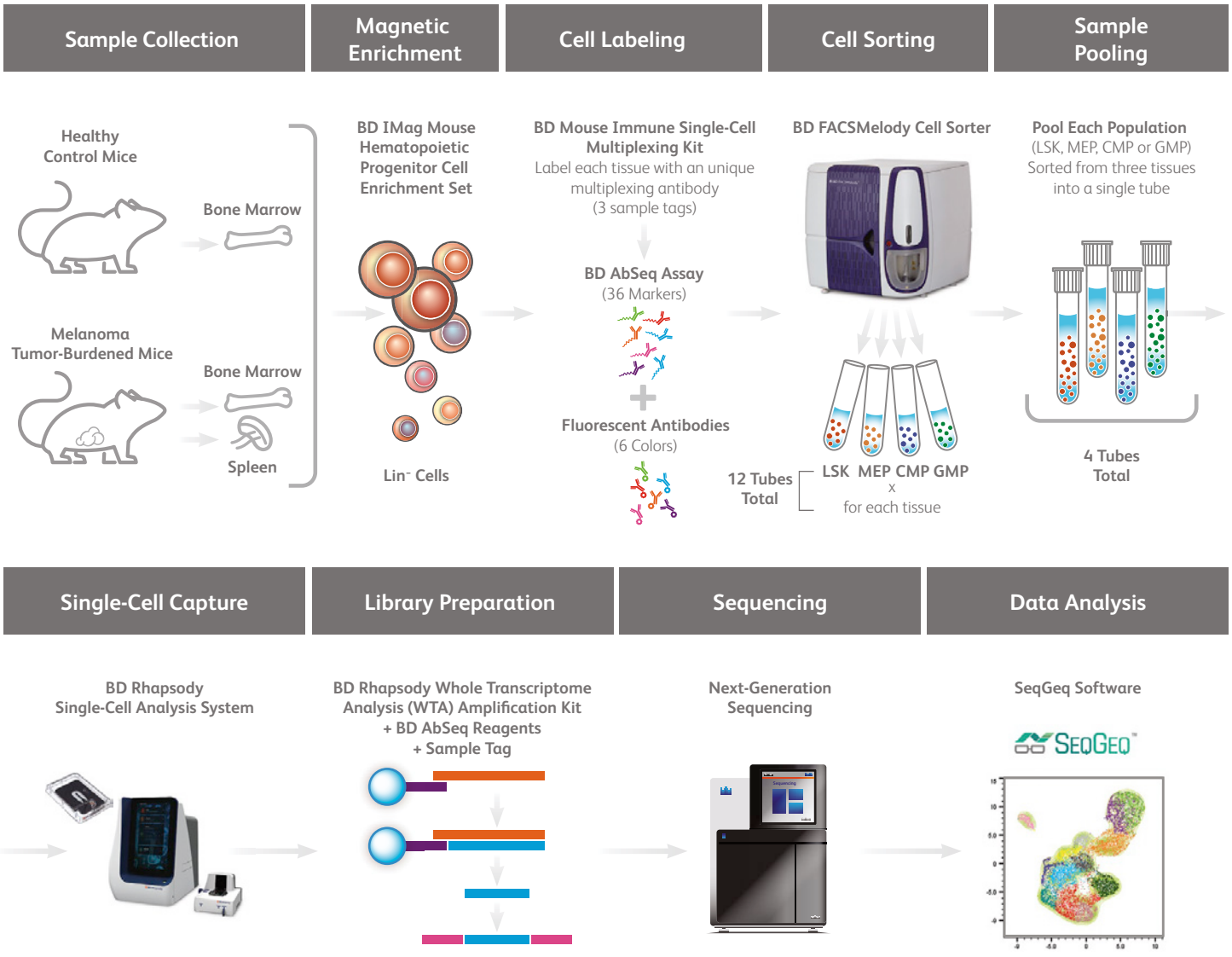

Figure 2. Experimental overview and single-cell analysis workflow

Three to four week-old C57BL/6 mice were injected subcutaneously with either B16-F10 melanoma cells (tumor-burdened mice, n=4) or control media (healthy mice, n=4). BM and SP tissues were harvested after 21 days. Cells were mechanically separated and red blood cells were lysed with BD Pharm Lyse™ Lysing Buffer. Samples were stained with BD Pharmingen™ APC Rat Anti-Mouse CD16/CD32 (Fcγ III/II Receptor) Antibody and magnetically enriched for lineage negative (Lin$^-$) cells using the BD IMag™ Mouse Hematopoietic Progenitor Cell Enrichment Set supplemented with biotinylated antibodies for CD2, CD5 and CD19. Enriched Lin$^-$ cells from tumor-burdened BM and SP and healthy BM were individually stained with unique DNA-barcoded antibodies from the BD Mouse Immune Single-Cell Multiplexing Kit and then stained with a 6-color antibody fluorochrome panel and a 36-plex BD AbSeq Panel. Four different populations from each tissue were sorted simultaneously using the BD FACSMelody Cell Sorter with 4-way sorting capability (12 tubes total). Each one of the four populations from each tissue was pooled into a single tube (4 tubes total). Next, tubes were pooled and then loaded separately onto four cartridges using the BD Rhapsody Single-Cell Analysis System for single-cell capture. After sample retrieval, the BD AbSeq, Sample Tag and mRNA (BD Rhapsody WTA) Libraries were prepared for sequencing. The sequencing results from ~31,000 cells were analyzed using SeqGeq™ v1.6 Software.

Table 1. 6-color fluorochrome labeled antibody panel for fluorescence activated cell sorting

Cell Sorting Panel	
Marker	Dye
Live/Dead	7-AAD
Lineage	PerCP-Cy™5.5
CD127	PE-Cy™7
cKit (CD117)	BB515
Sca1	BV421
CD16/CD32	APC
CD34	PE

7-AAD and PerCP-Cy5.5 are detected in the same channel

Table 2. 36-Plex BD AbSeq Reagents for downstream single-cell surface protein expression analysis

36-Plex BD AbSeq Reagents			
CD9	CD44	CD62L	CD196*
CD11a	CD45RA	CD84	CD197
CD24	CD47	CD103	CD229*
CD27*	CD48	CD105	CD244.2*
CD29	CD49b	CD106	CD370
CD31	CD49d	CD150*	CD90.2
CD38*	CD49e	CD183*	CD93
CD41	CD49f	CD184	CXCR2
CD43	CD61	CD185*	Ly-108

*Custom BD AbSeq Reagents

The 6-color fluorochrome labeled antibody panel enabled separation of the HPSC populations into LSK, CMP, MEP and GMP (Figure 3A). Using a 4-way sorting strategy, the BD FACSMelody Cell Sorter was used to simultaneously purify the four populations of interest from either tumor-burdened BM or SP or healthy BM. The SP from tumor-burdened mice showed increased percentages of HSPCs compared to the SP of healthy mice consistent with the presence of extramedullary hematopoiesis under tumor-burden (Figure 3B). Cells were not sorted from the SP of healthy mice due to insufficient cell numbers.

Figure 3A

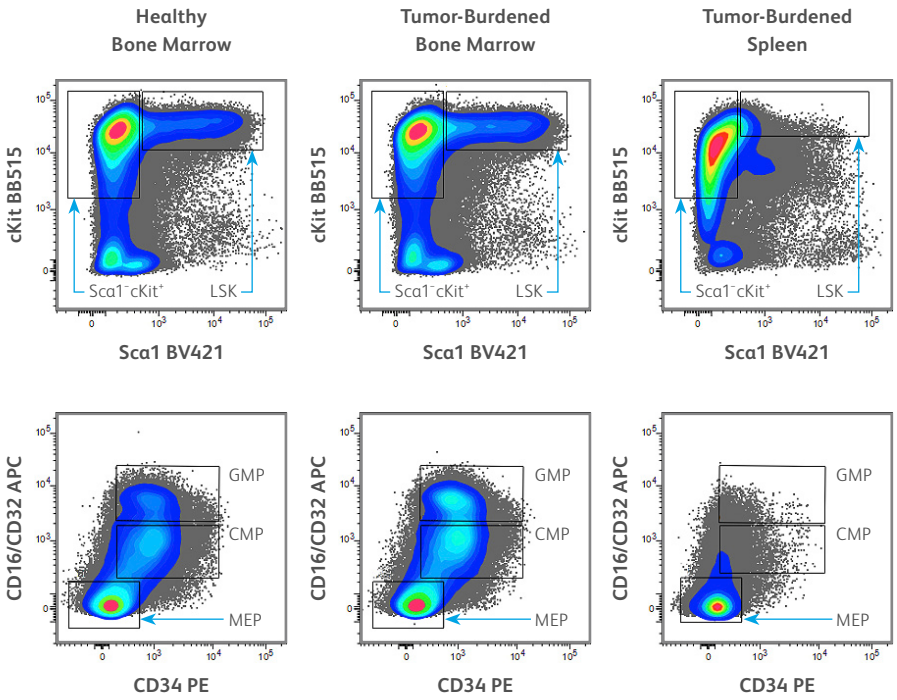

Figure 3B

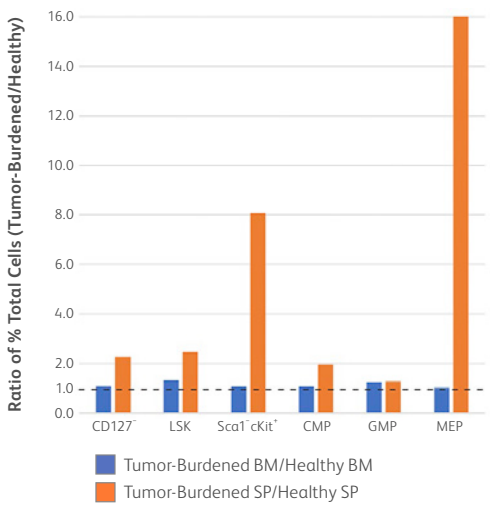

Figure 3. Gating strategy for 4-way sorting using the BD FACSMelody Cell Sorter

The BD FACSMelody Cell Sorter was used to purify HSPC subsets from BM and SP. Cells were first gated for Lin⁻ and live cells, followed by doublet cell discrimination and gating for CD127⁻ cells to exclude lymphoid progenitor cells (not shown). **A**. LSK hematopoietic stem cells were gated as Lin⁻ Sca1⁻cKit⁺ within the CD127⁻ population (top row). Hematopoietic progenitor cell populations MEP, CMP and GMP were identified within the Sca1⁻cKit⁺ gate based on CD16/CD32 and CD34 expression (bottom row). **B**. HSPC ratios between tumor-burdened and healthy tissues (BM or SP).

The unique barcodes present on each sample tag were used to deconvolute each tissue bioinformatically in SeqGeq v1.6 Software during analysis. The dimensionality reduction algorithm TriMap, a plug-in for SeqGeq Software, was used to visualize the sorted populations from the three tissues (healthy BM, tumor-burdened BM and tumor-burdened SP) in an embedded space approximating the higher data structure (Figure 4). Individual sorted populations from the BM of healthy and tumor-burdened mice showed similarities in distribution in the TriMap space. The matching populations from the tumor-burdened SP showed divergent distribution in the same TriMap space compared to the BM of healthy and tumor-burdened mice.

Figure 4A

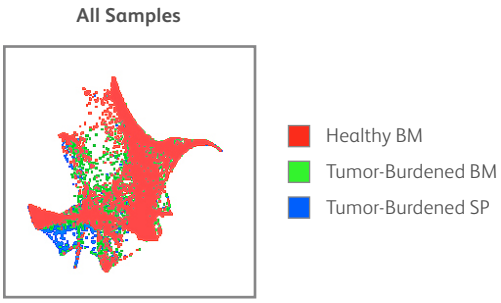

Figure 4B

Figure 4. Unsupervised analysis of sorted hematopoietic stem and progenitor cell populations

TriMap visualization displays single-cell data. A total of ~31,000 cells are shown for all samples. **A.** Overlay of tissues identified by unique sample tags. **B.** Individual sorted populations are displayed.

We initially sought to determine the immunophenotypic and molecular signature of each of the four sorted populations from healthy BM. To do so, we performed differential expression analyses and found proteins and mRNA transcripts that were upregulated in each of the four populations from healthy BM (Figure 5). Consistent with earlier findings in the literature, several proteins including CD43, CD49b and mRNA for *Hlf, Fosb* were upregulated in LSK cells. CMP cells showed upregulation of *Irf8* marking myeloid lineage commitment. Also consistent with literature *Car1/2* and *Mpo* enabled discrimination of megakaryocyte-erythroid and granulocyte-macrophage committed cells, respectively.

Figure 5A

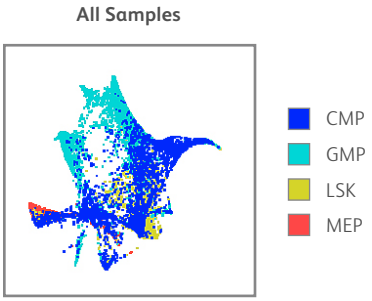

Figure 5B

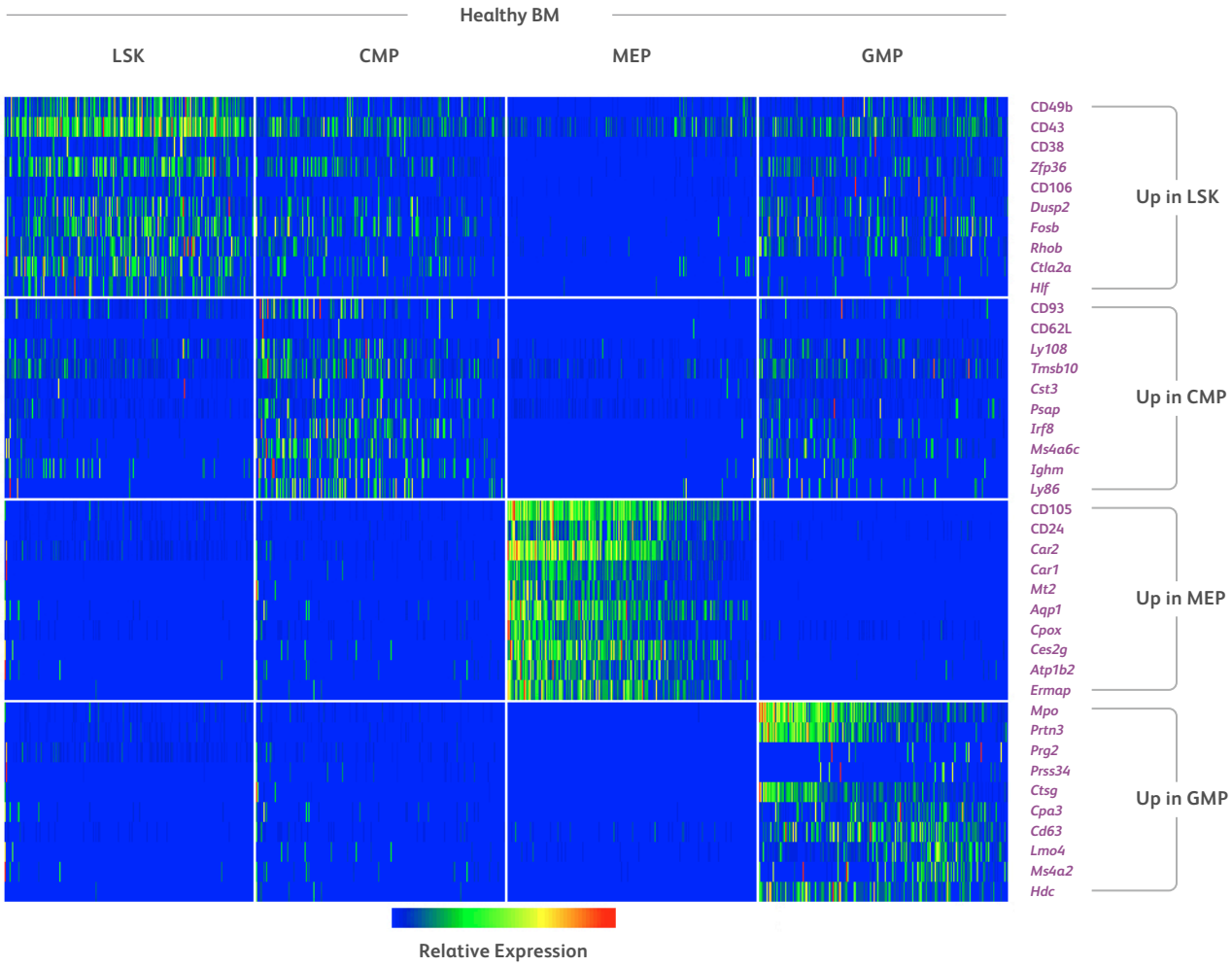

Figure 5. Single-cell protein and whole transcriptome analysis reveal differential expression during normal hematopoiesis

A. TriMap plot showing overlay of CMP, GMP, LSK and MEP populations from healthy BM. **B.** Single-cell heat maps show the top ten differentially expressed proteins and mRNA transcripts (italic) for each sorted population compared to the remaining populations. A total of 200 representative cells from each sorted population are shown in the heatmaps.

In order to gain more insights into tumor-driven perturbations during myelopoiesis, we investigated the differential expression of proteins and mRNA transcripts in CMP cells sorted from the BM of healthy and tumor-burdened mice and the SP of tumor-burdened mice (Figure 6). Consistent with the TriMap visualization, the differences between the BM of healthy and the BM of tumor-burdened mice were less prominent than the differences between the SP of tumor-burdened mice and the BM of healthy or tumor-burdened mice. A discrete subset of cells expressing *Ccl4* was almost exclusively observed in healthy BM CMPs. *Gata2*, which has been shown to play a critical role in proliferation and survival of HSCs, was upregulated in CMP cells from the SP of tumor-burdened mice. CMP cells from the tumor-burdened SP also showed upregulation of CD47 protein and *Itga4* compared to the BM from tumor-burdened mice. Earlier studies have shown that these markers are involved in HSPC trafficking and homing. Thus, these results may suggest that the differences in CMP are due to an effect of selective recruitment of circulating HSPCs to SP and/or to the tumor-conditioned splenic niche. Further studies are needed to dissect the functional outcomes and cellular mechanisms associated with these changes.

Figure 6

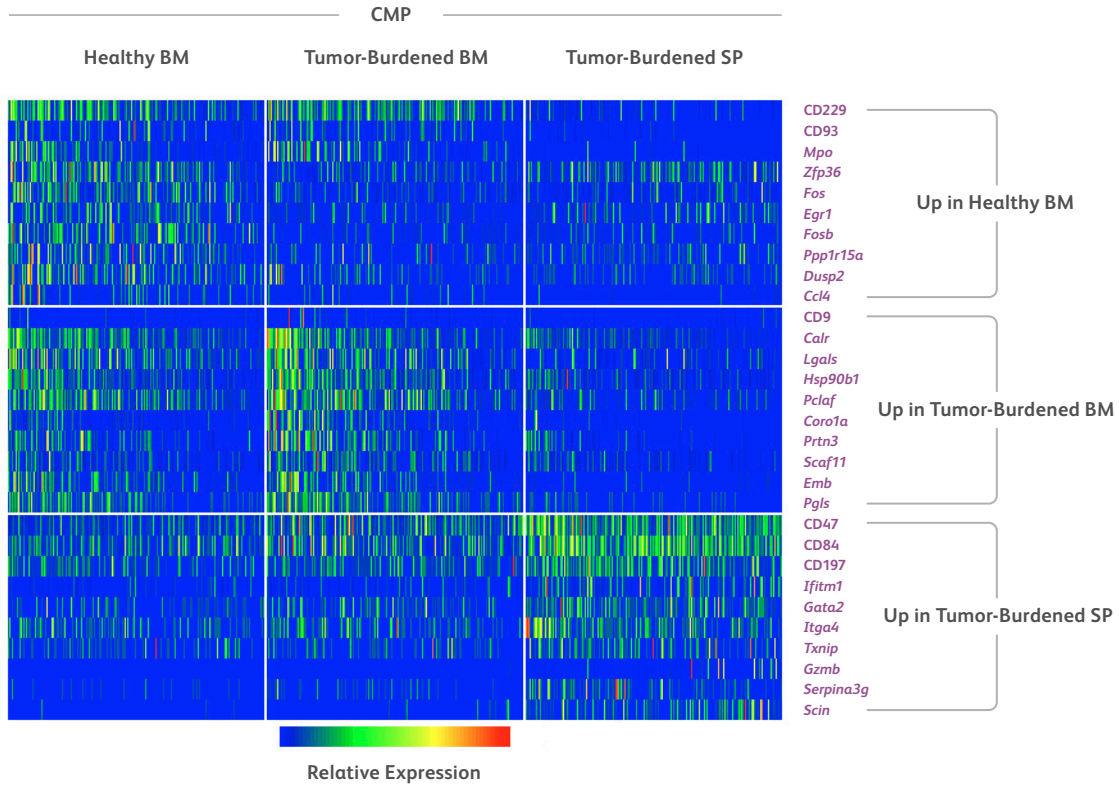

Figure 6. Single-cell protein and whole transcriptome analyses reveal differential expression during myelopoiesis in healthy and tumor-burdened mice

Single-cell heat maps show the top ten upregulated proteins and mRNA transcripts (italic) detected in CMP cells from the BM of healthy mice and the BM and SP of tumor-burdened mice compared to the remaining populations. A total of 200 representative cells from each sorted population are shown in the heatmaps.

In summary, the data showed the ability to simultaneously sort four rare cell populations using the BD FACSMelody Cell Sorter. This ability is critical when needing to preserve precious samples and to minimize sort time. Furthermore, our data demonstrate the power of a comprehensive workflow integrating the BD FACSMelody Cell Sorter and BD Rhapsody Single-Cell Analysis System for a deep characterization of rare cell types and the potential discovery of signatures associated with altered cellular processes.

Ordering information

Systems and software	
Description	
BD FACSMelody™ Cell Sorter	
BD Rhapsody™ Single-Cell Analysis System	
SeqGeq™ v1.6 Software	

Reagents	
Description	Cat. No.
BD Pharmingen™ 7-AAD	559925
BD Pharmingen™ PerCP-Cy™5.5 Mouse Lineage Antibody Cocktail, with Isotype Control	561317
BD Pharmingen™ PE-Cy™7 Rat Anti-Mouse CD127	560733
BD Horizon™ BB515 Rat Anti-Mouse CD117 (cKit)	564481
BD Horizon™ BV421 Rat Anti-Mouse Ly-6A/E (ScaI)	562729
BD Pharmingen™ APC Rat Anti-Mouse CD16/CD32 (Fcγ III/II Receptor)	558636
BD Pharmingen™ PE Rat Anti-Mouse CD34	551387
BD IMag™ Mouse Hematopoietic Progenitor (Stem) Cell Enrichment Set - DM	558451
BD Pharmingen™ Biotin Rat Anti-Mouse CD2	553110
BD Pharmingen™ Biotin Rat Anti-Mouse CD5	553019
BD Pharmingen™ Biotin Rat Anti-Mouse CD19	553784
BD® AbSeq Assay	Contact Your BD Sales Representative
BD® Single-Cell Multiplexing Kit	
BD Rhapsody™ Whole Transcriptome Analysis Amplification Kit	

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

23-22919-00

BD Life Sciences, San Jose, CA, 95131, USA

bdbiosciences.com