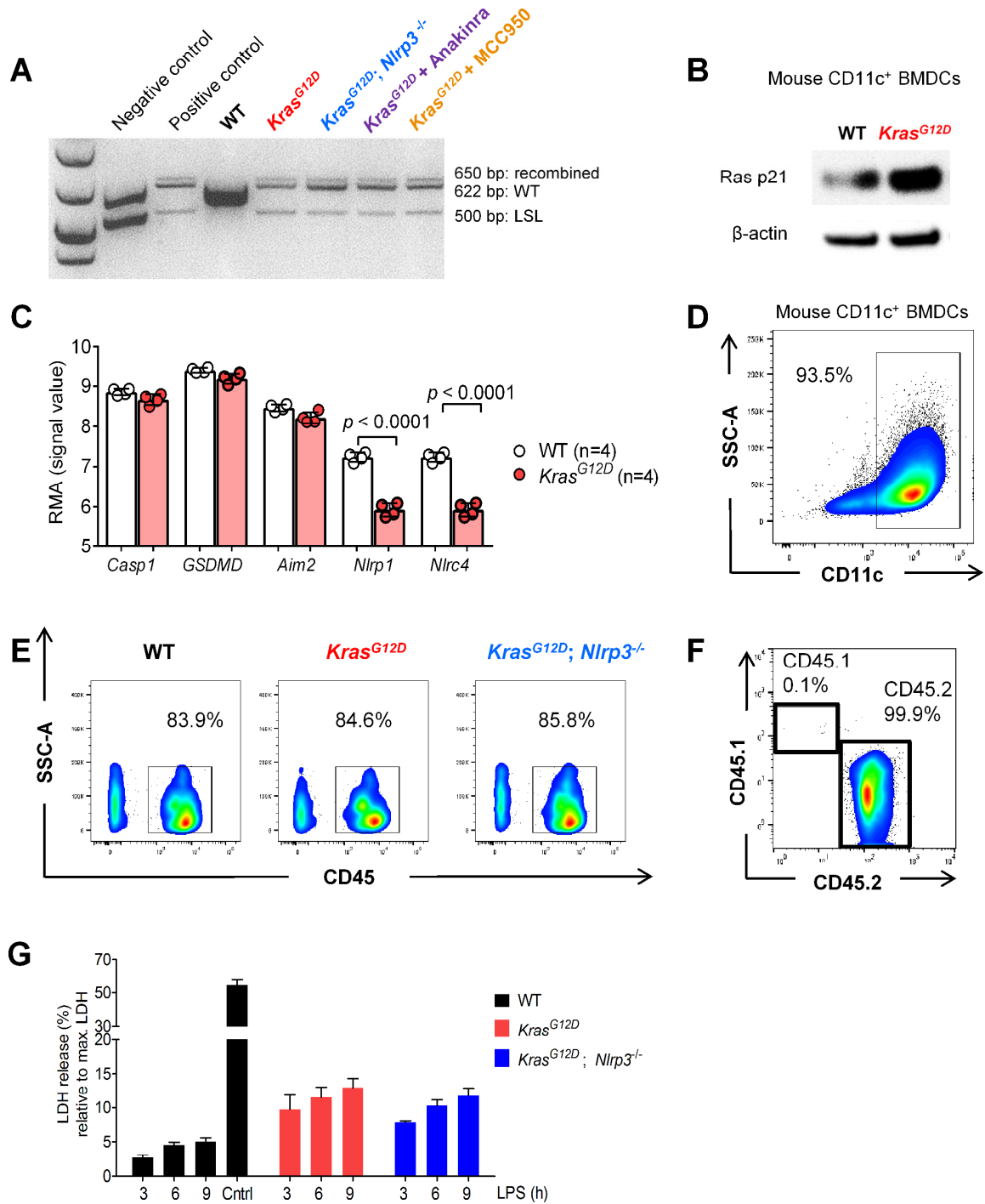


**Oncogenic Kras<sup>G12D</sup> causes myeloproliferation via NLRP3 inflammasome activation**

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**Fig. S1**



**Supplementary Fig. S1 Proof of successful Cre- recombination and engraftment**

**(A)** Proof of successful Cre- recombination in C57BL/6 recipient mice that have undergone transplantation of WT, *Kras<sup>G12D</sup>*, *Kras<sup>G12D</sup>* or *Nlrp3<sup>-/-</sup>*, *Kras<sup>G12D</sup>* BM and that were treated with tamoxifen. The image is representative for three independent experiments.

**(B)** Proof of the increased Ras expression in BMDCs derived from *Kras*<sup>G12D</sup> mice compared to WT littermates, detected by western blot. The image is representative for four independent experiments.

**(C)** Expression of *Casp1*, *GSDMD*, *Aim2*, *Nlrp1* and *Nlrp4* in BMDCs isolated from WT (n=4) or *Kras*<sup>G12D</sup> (n=4) mice following treatment by tamoxifen, as analyzed by microarray. Data are shown as mean ± SEM.

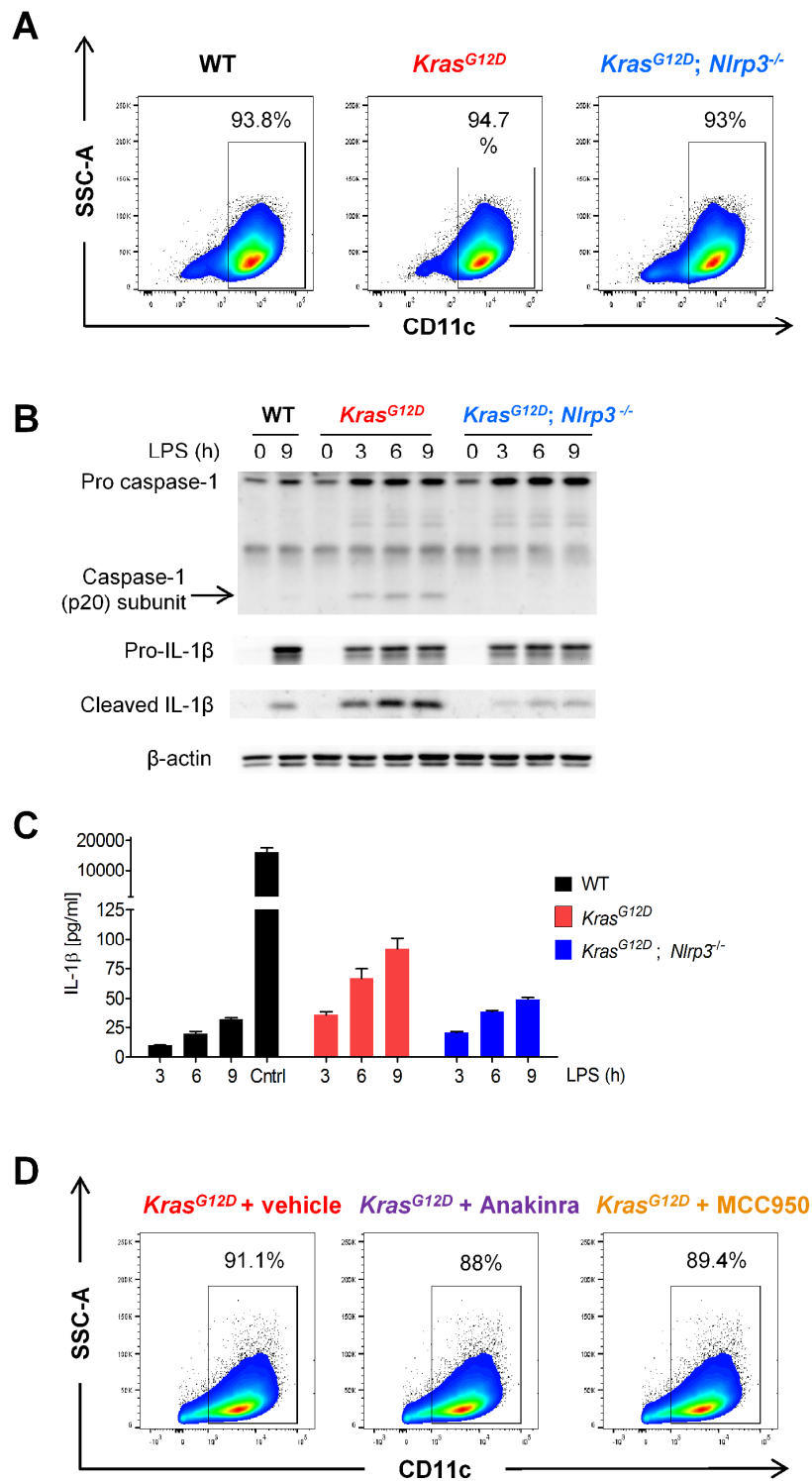
**(D)** Representative flow cytometry plot showing the gating strategy used for CD11c<sup>+</sup> BMDCs generated from WT, *Kras*<sup>G12D</sup> or *Kras*<sup>G12D</sup>; *Nlrp3*<sup>-/-</sup> BM mice.

**(E)** Representative flow cytometry plots showing the frequency of CD45<sup>+</sup> cells in WT, *Kras*<sup>G12D</sup> or *Kras*<sup>G12D</sup>; *Nlrp3*<sup>-/-</sup> BM mice, as quantified 4 weeks post-transplantation and prior to tamoxifen treatment.

**(F)** Representative flow cytometry plot showing the engraftment efficiency in chimera mice following transplantation of WT, *Kras*<sup>G12D</sup> or *Kras*<sup>G12D</sup> and *Nlrp3*<sup>-/-</sup> BM (CD45.2) into lethally irradiated WT recipient mice (CD45.1).

**(G)** The graph displays the levels of LDH release in BMDCs generated from WT, *Kras*<sup>G12D</sup> and *Kras*<sup>G12D</sup>; *Nlrp3*<sup>-/-</sup> BM mice, quantified from cell-free supernatants by colorimetric assay, following LPS-priming for the indicated times. The control sample (Cntrl) was LPS-primed for 4.5 hours in addition to Nigericin. Data points are shown as mean ± SD, and are representative of 3 biological replicates.

**Fig. S2**



**Supplementary Fig. S2** CD11c<sup>+</sup> BMDCs, after isolation of BM from WT, *Kras<sup>G12D</sup>* or *Kras<sup>G12D</sup>; Nlrp3<sup>-/-</sup>* BM mice

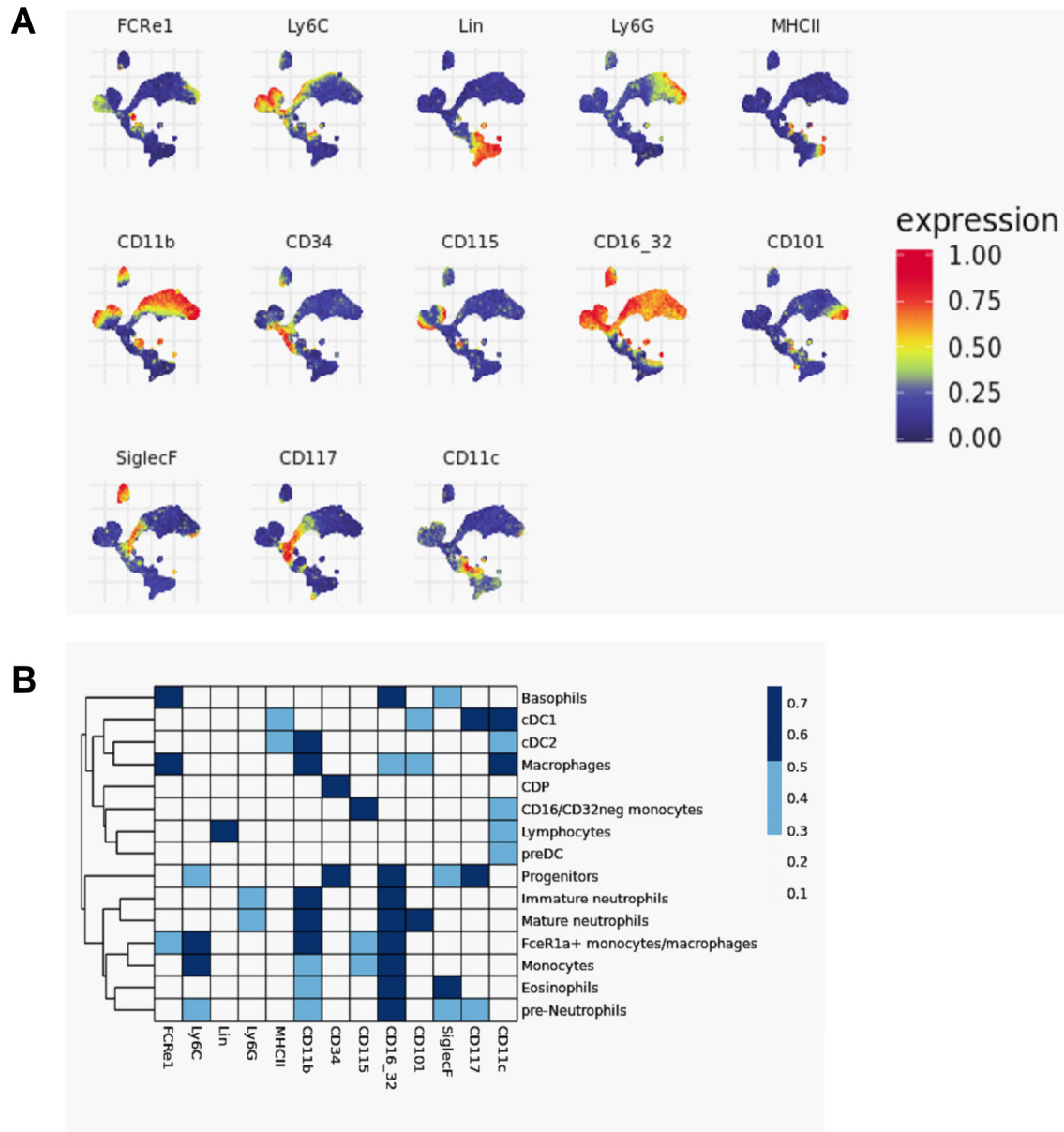
**(A)** Representative flow cytometry plots showing the normal generation of CD11c<sup>+</sup> BMDCs, after isolation of BM from WT, *Kras*<sup>G12D</sup> or *Kras*<sup>G12D</sup>; *Nlrp3*<sup>-/-</sup> recipient mice.

**(B)** Western blot shows the amount of caspase-1 (p20 subunit) and cleaved IL-1 $\beta$  analysed in cell-free supernatants, and amount of pro-IL-1 $\beta$  analysed in cell lysates of BMDCs generated from WT, *Kras*<sup>G12D</sup> or *Kras*<sup>G12D</sup>; *Nlrp3*<sup>-/-</sup> BM mice, following LPS-priming for the indicated times. The blot is representative for three independent experiments.

**(C)** The graph displays the levels of IL-1 $\beta$  in BMDCs generated from WT, *Kras*<sup>G12D</sup> or *Kras*<sup>G12D</sup>; *Nlrp3*<sup>-/-</sup> BM mice, quantified from cell-free supernatants by ELISA, following LPS-priming for the indicated times. The control sample (Cntrl) was LPS-primed for 4.5 hours in addition to Nigericin. Data points are shown as mean  $\pm$  SD, and are representative of 3 biological replicates.

**(D)** Representative flow cytometry plots showing the normal generation of CD11c<sup>+</sup> BMDCs, after isolation of BM from *Kras*<sup>G12D</sup> BM mice treated with vehicle, Anakinra or MCC950.

**Fig. S3**

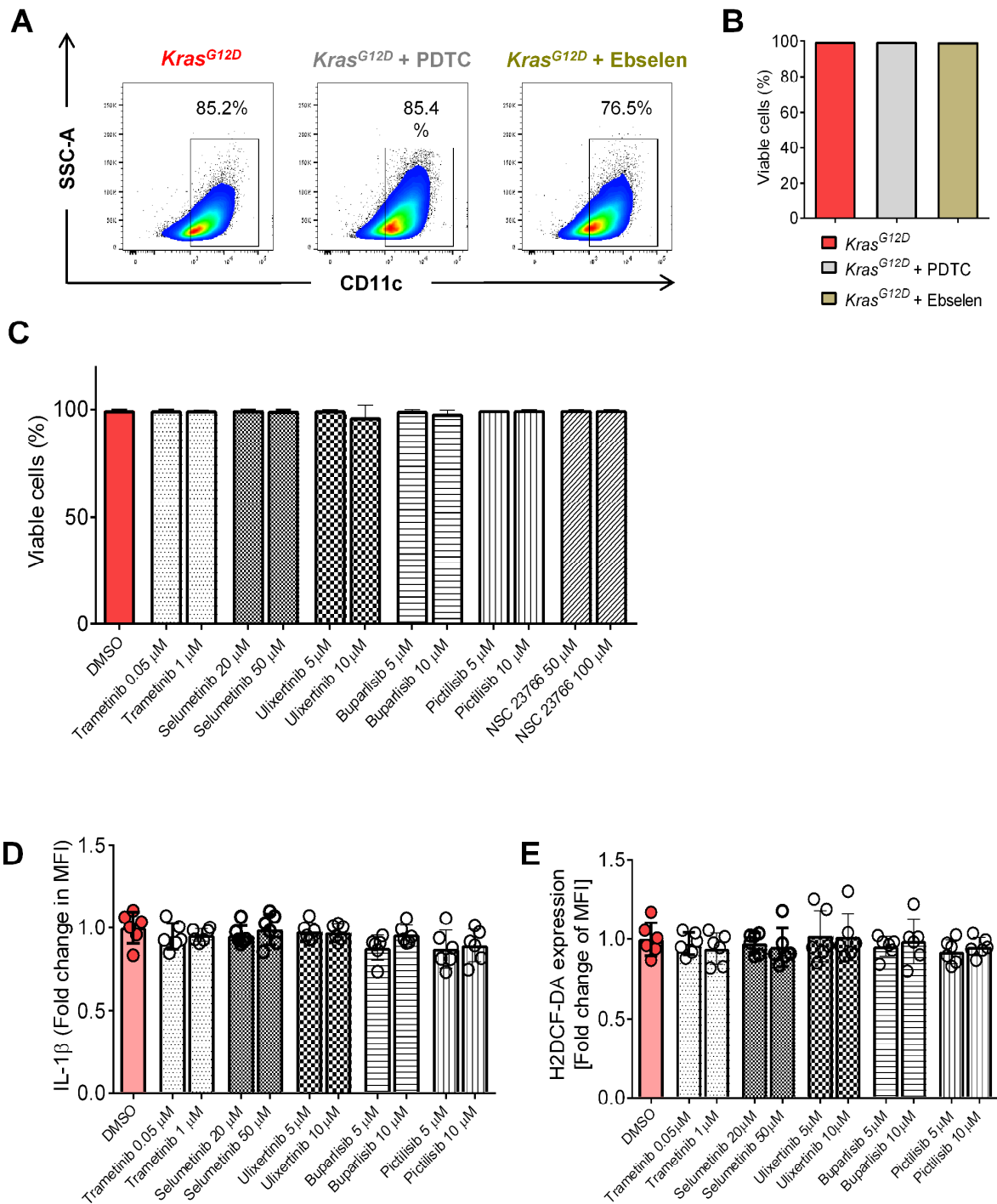


**Supplementary Fig. S3 UMAP plot and heat map for each annotated population after FlowSOM-guided metaclustering.**

**(A)** UMAP plot displaying expression of the indicated markers on stochastically selected BM CD45<sup>+</sup> leukocytes (gated on live/single cells/CD45) from vehicle-treated *Kras*<sup>G12D</sup> (n=4), Anakinra-treated *Kras*<sup>G12D</sup> (n=5) or MCC950-treated *Kras*<sup>G12D</sup> (n=5) BM mice. Lin = lymphocyte lineage cocktail containing anti-CD19, anti-CD3, anti CD49b and anti-TER119.

**(B)** The heat map shows the median marker expression (value range: 0-1) for each annotated population after FlowSOM-guided metaclustering.

**Fig. S4**



**Supplementary Fig. S4 Treatment of *Kras*<sup>G12D</sup> BMDCs with ROS inhibitors or MEK, ERK and PI3K inhibitors**

**(A)** Representative flow cytometry plots showing the normal generation of CD11c<sup>+</sup> BMDCs, after isolation of BM from *Kras*<sup>G12D</sup> BM mice and treatment with DMSO or ROS inhibitors PDTC and Ebselen.

**(B)** The graph shows the percentage of viable cells in CD11c<sup>+</sup> BMDCs generated from *Kras*<sup>G12D</sup> BM mice, after treatment with ROS inhibitors PDTC and Ebselen.

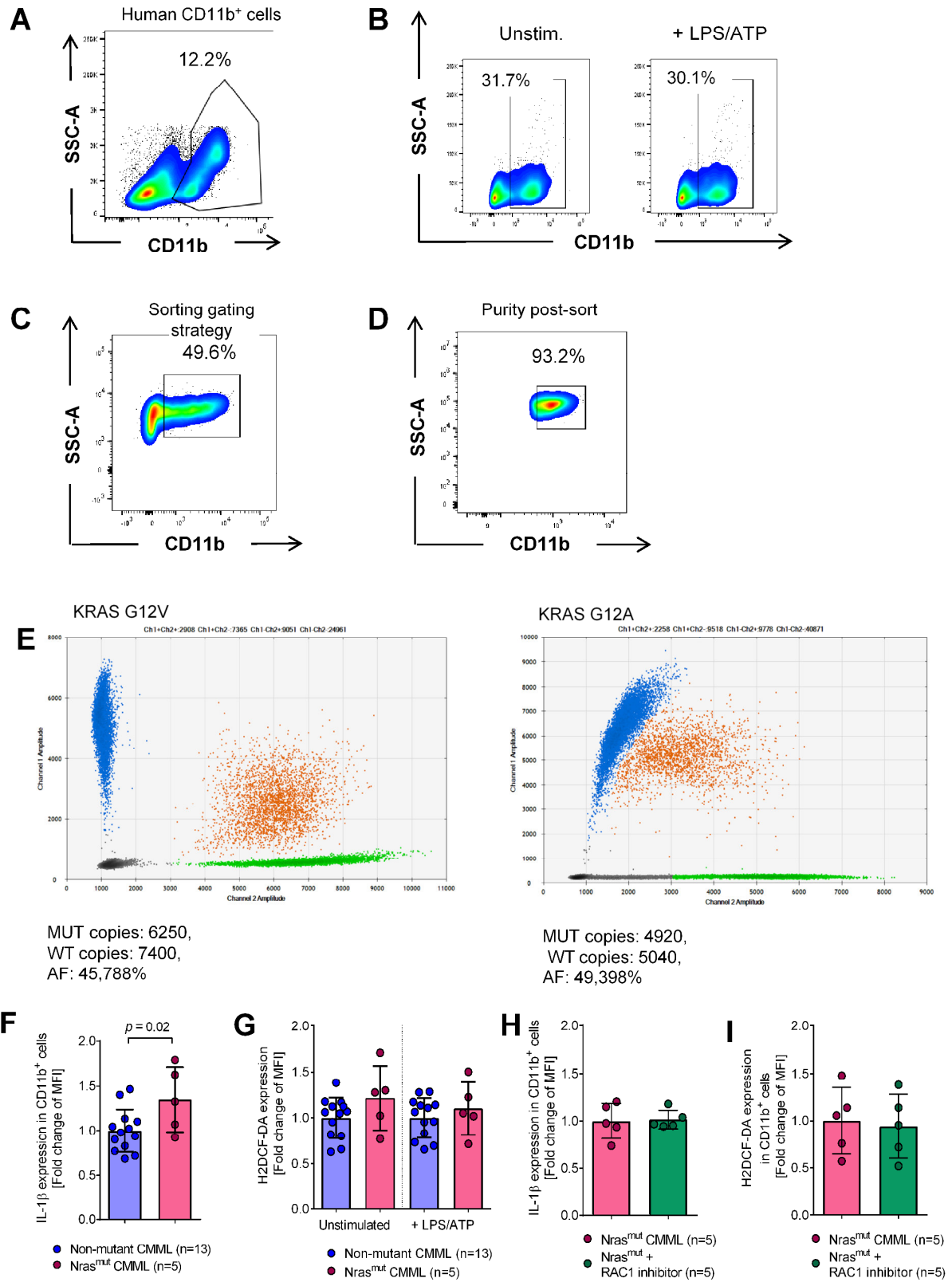
**(C)** The graph shows the percentage of viable cells in CD11c<sup>+</sup> BMDCs generated from *Kras*<sup>G12D</sup> BM mice, after treatment with DMSO, 0.05  $\mu$ M or 1  $\mu$ M of MEK-specific inhibitor Trametinib, 20 or 50  $\mu$ M of MEK-specific inhibitor Selumetinib, 5 or 10  $\mu$ M of ERK-specific inhibitor Ulixertinib, 5 or 10  $\mu$ M of PI3K-specific inhibitor Buparlisib, 5 or 10  $\mu$ M of PI3K-specific inhibitor Pictilisib for 30 hours, 50 or 100  $\mu$ M of RAC1 inhibitor NSC 23766 for 1 hour.

**(D)** The graph shows the fold change of IL-1 $\beta$  expression as measured by flow cytometry in CD11c<sup>+</sup> BMDCs generated from *Kras*<sup>G12D</sup> BM mice, after treatment with DMSO, 0.05  $\mu$ M or 1  $\mu$ M of MEK-specific inhibitor Trametinib, 20 or 50  $\mu$ M of MEK-specific inhibitor Selumetinib, 5 or 10  $\mu$ M of ERK-specific inhibitor Ulixertinib, 5 or 10  $\mu$ M of PI3K-specific inhibitor Buparlisib, 5 or 10  $\mu$ M of PI3K-specific inhibitor Pictilisib for 30 hours, and stimulation with 200 ng/ml LPS and 5 mM ATP, normalized to DMSO-treated *Kras*<sup>G12D</sup> BMDCs.

**(E)** The graph shows the fold change of H2DCF-DA expression as measured by flow cytometry in CD11c<sup>+</sup> BMDCs generated from *Kras*<sup>G12D</sup> BM mice, after treatment with DMSO, 0.05  $\mu$ M or 1  $\mu$ M of MEK-specific inhibitor Trametinib, 20 or 50  $\mu$ M of MEK-specific inhibitor Selumetinib, 5 or 10  $\mu$ M of ERK-specific inhibitor Ulixertinib, 5 or 10  $\mu$ M of PI3K-specific inhibitor Buparlisib, 5 or 10  $\mu$ M of PI3K-specific inhibitor Pictilisib for 30 hours, and stimulation with 200 ng/ml LPS and 5 mM ATP, normalized to DMSO-treated *Kras*<sup>G12D</sup> BMDCs.

All data are shown as mean  $\pm$  SEM.

**Fig. S5**



**Supplementary Fig. S5 Analysis of PBMCs isolated from  $Kras^{mut}$ ,  $Nras^{mut}$  or non-mutant patients**

**(A)** Representative flow cytometry plot showing the gating strategy used for  $CD11b^+$  PBMCs isolated from  $Kras^{mut}$  or non- $Kras^{mut}$  patients.

**(B)** Representative flow cytometry plots showing the gating strategy used for  $CD11b^+$  PBMCs, and confirming no effect on the cells following stimulation with LPS and ATP.

**(C)** Representative flow cytometry plot showing the gating strategy and purity of sorted  $CD11b^+$  cells from  $Kras^{mut}$  patients PBMCs.

**(D)** Representative flow cytometry plot confirming the purity of sorted  $CD11b^+$  cells from  $Kras^{mut}$  patients PBMCs.

**(E)** Representative ddPCR results showing a two-dimensional (2D) scatter plot with the four clusters obtained with mutant and wild-type alleles. The number of events is very high which represents a very high copy number of mutant *Kras* G12V or G12A molecules, respectively in the specimen (blue: droplets contain only mutant template; orange: droplets contain both templates; black: droplets contain no template, green: droplets contain only wild-type template). The plot is representative of 3 independent experiments.

**(F)** The graph displays the fold change of IL-1 $\beta$  expression as measured by flow cytometry in  $CD11b^+$  cells of CMML patients with an *NRAS* mutation ( $Nras^{mut}$ ) (n=5), or without an *NRAS* mutation (non-mutant) (n=13), after stimulation with 200 ng/ml LPS and 5 mM ATP, normalized to non-mutant.

**(G)** The graph displays the fold change of H2DCF-DA expression as measured by flow cytometry in  $CD11b^+$  cells of  $Nras^{mut}$  (n=5) and non-mutant (n=13) CMML patients, normalized to non-mutant in both stimulation conditions.

**(H)** The graph displays the fold change of IL-1 $\beta$  expression as measured by flow cytometry in  $CD11b^+$  cells of CMML patients with an *NRAS* mutation ( $Nras^{mut}$ ) treated with DMSO (n=5) or 100  $\mu$ M of RAC1 inhibitor NSC 23766 (n=5), after stimulation with 200 ng/ml LPS and 5 mM ATP, normalized to DMSO-treated  $Nras^{mut}$ .

**(I)** The graph displays the fold change of H2DCF-DA expression as measured by flow cytometry for in  $CD11b^+$  cells of CMML patients with an *NRAS* mutation ( $Nras^{mut}$ ) treated with DMSO (n=5) or 100  $\mu$ M of RAC1 inhibitor NSC 23766 (n=5), after stimulation with 200 ng/ml LPS and 5 mM ATP, normalized to DMSO-treated  $Nras^{mut}$ .

## Supplementary Tables

Suppl. Table S1: Characteristics of JMML patients

|                          |                                   |
|--------------------------|-----------------------------------|
| Total number of patients | 9                                 |
| Variable                 | <b><u>Median (Range)</u></b>      |
| Age                      | 4 (0.5-7.6)                       |
|                          | <b><u>% (absolute number)</u></b> |
| Gender                   |                                   |
| Female                   | 33.3 (3)                          |
| Male                     | 66.7 (6)                          |
| Diagnosis                |                                   |
| JMML                     | 100 (9)                           |
| <b>KRAS mutation</b>     |                                   |
| Yes                      | 100 (9)                           |

Abbreviations: JMML = Juvenile Myelomonocytic Leukemia

**Suppl. Table S2: Characteristics of AML patients**

|                                 |                                   |
|---------------------------------|-----------------------------------|
| <b>Total number of patients</b> | 33                                |
| <b>Variable</b>                 | <b><u>Median (Range)</u></b>      |
| <b>Age</b>                      | 61 (25-80)                        |
|                                 | <b><u>% (absolute number)</u></b> |
| <b>Gender</b>                   |                                   |
| Female                          | 51.5 (17)                         |
| Male                            | 48.5 (16)                         |
| <b>Diagnosis</b>                |                                   |
| AML                             | 100 (33)                          |
| <b><i>KRAS</i> mutation</b>     |                                   |
| Yes                             | 33.3 (11)                         |
| No                              | 66.7 (22)                         |

Abbreviations: AML = Acute Myeloid Leukemia

**Suppl. Table S3: Characteristics of CMML patients**

|                                 |                                   |
|---------------------------------|-----------------------------------|
| <b>Total number of patients</b> | 23                                |
| <b>Variable</b>                 | <b><u>Median (Range)</u></b>      |
| <b>Age</b>                      | 75 (55-89)                        |
|                                 | <b><u>% (absolute number)</u></b> |
| <b>Gender</b>                   |                                   |
| Female                          | 39.1 (9)                          |
| Male                            | 60.9 (14)                         |
| <b>Diagnosis</b>                |                                   |
| CMML                            | 100 (23)                          |
| <b><i>RAS</i> mutation</b>      |                                   |
| <i>KRAS</i>                     | 21.7 (5)                          |
| <i>NRAS</i>                     | 21.7 (5)                          |
| No <i>RAS</i> mutation          | 56.5 (13)                         |

Abbreviations: CMML = Chronic myelomonocytic leukemia

**Suppl. Table S4: Antibodies for flow cytometry**

| Antibody                             | Clone       | Catalogue number | Flouorochrome  | Vendor        |
|--------------------------------------|-------------|------------------|----------------|---------------|
| Anti-mouse CD11b                     | M1/70       | 557397           | PE             | BD Bioscience |
| Anti-mouse CD11b                     | M1/70       | 564443           | BUV 737        | BD Bioscience |
| Anti-mouse/human CD11b               | M1/70       | 101205           | FITC           | Biolegend     |
| Anti-mouse/human CD11b               | M1/70       | 101224           | PB             | Biolegend     |
| Anti-mouse CD11c                     | N418        | 117306           | FITC           | Biolegend     |
| Anti-mouse CD11c                     | N418        | 117322           | PB             | Biolegend     |
| Anti-mouse CD11c                     | N418        | 35-0114-82       | PE-Cy5.5       | eBioscience   |
| Anti-mouse CD45                      | 30-F11      | 103108           | FITC           | Biolegend     |
| Anti-mouse CD45                      | 30-F11      | 103126           | PB             | Biolegend     |
| Anti-mouse CD45                      | 30-F11      | 565967           | BUV 395        | BD Bioscience |
| Anti-mouse CD45.1                    | A20         | 110706           | FITC           | Biolegend     |
| Anti-mouse CD45.2                    | 104         | 109820           | PB             | Biolegend     |
| Anti-mouse CD45R/B220                | RA3-6B2     | 103247           | BV510          | Biolegend     |
| Anti-mouse CD45R/B220                | RA3-6B2     | 103212           | APC            | Biolegend     |
| Anti-mouse CD90.2                    | 30-H12      | 105326           | PE-Cy7         | Biolegend     |
| Anti-mouse CD90.2                    | 30-H12      | 105307           | PE             | Biolegend     |
| Anti-mouse CD34                      | SA376A4     | 152208           | BV 421         | Biolegend     |
| Anti-mouse CD115                     | AFS98       | 135517           | BV 605         | Biolegend     |
| Anti-mouse CD101                     | Moushi101   | 12-1011-82       | PE             | eBioscience   |
| Anti-mouse CD117                     | 2B8         | 105810           | PE-Cy5         | Biolegend     |
| Anti-mouse CD19                      | eBio1D3     | 17-0193-82       | APC            | eBioscience   |
| Anti-mouse CD3                       | 17A2        | 100236           | APC            | Biolegend     |
| Anti-mouse CD49b                     | DX5         | 108910           | APC            | Biolegend     |
| Anti-mouse CD16/32                   | 93          | 101337           | BV 711         | Biolegend     |
| Anti-mouse Ly-6C                     | AL-21       | 560525           | PerCP Cy5.5    | BD Bioscience |
| Anti-mouse FceRIa                    | MAR-1       | 134329           | AlexaFlour 488 | Biolegend     |
| Anti-mouse Ly6G                      | 1A8         | 565707           | BUV 563        | BD Bioscience |
| Anti-mouse I-A/I-E<br>(MHC class II) | M5/114.15.2 | 750280           | BUV 661        | BD Bioscience |
| Anti-mouse TER119                    | Ter-119     | 17-5921-81       | APC            | eBioscience   |
| Anti-mouse Siglec-F                  | E50-2440    | 562757           | PE-CF594       | BD Bioscience |
| Anti-mouse IL-1 $\beta$ Pro-form     | NJTEN3      | 12-7114-80       | PE             | eBioscience   |
| Anti-human IL-1 $\beta$              | CRM56       | 12-7018-41       | PE             | eBioscience   |

**Abbreviations:**

PB: Pacific blue

FITC: Fluorescein isothiocyanate

PE: Phycoerythrin

APC: Allophycocyanin

-Cy: -Cyanine

BV-: Brilliant Violet

PerCP-: Peridinin-chlorophyll-protein

BUV-: Brilliant Ultraviolet