# Tumour-Associated Macrophages Exhibit Anti-Tumoural Properties in Sonic Hedgehog Medulloblastoma

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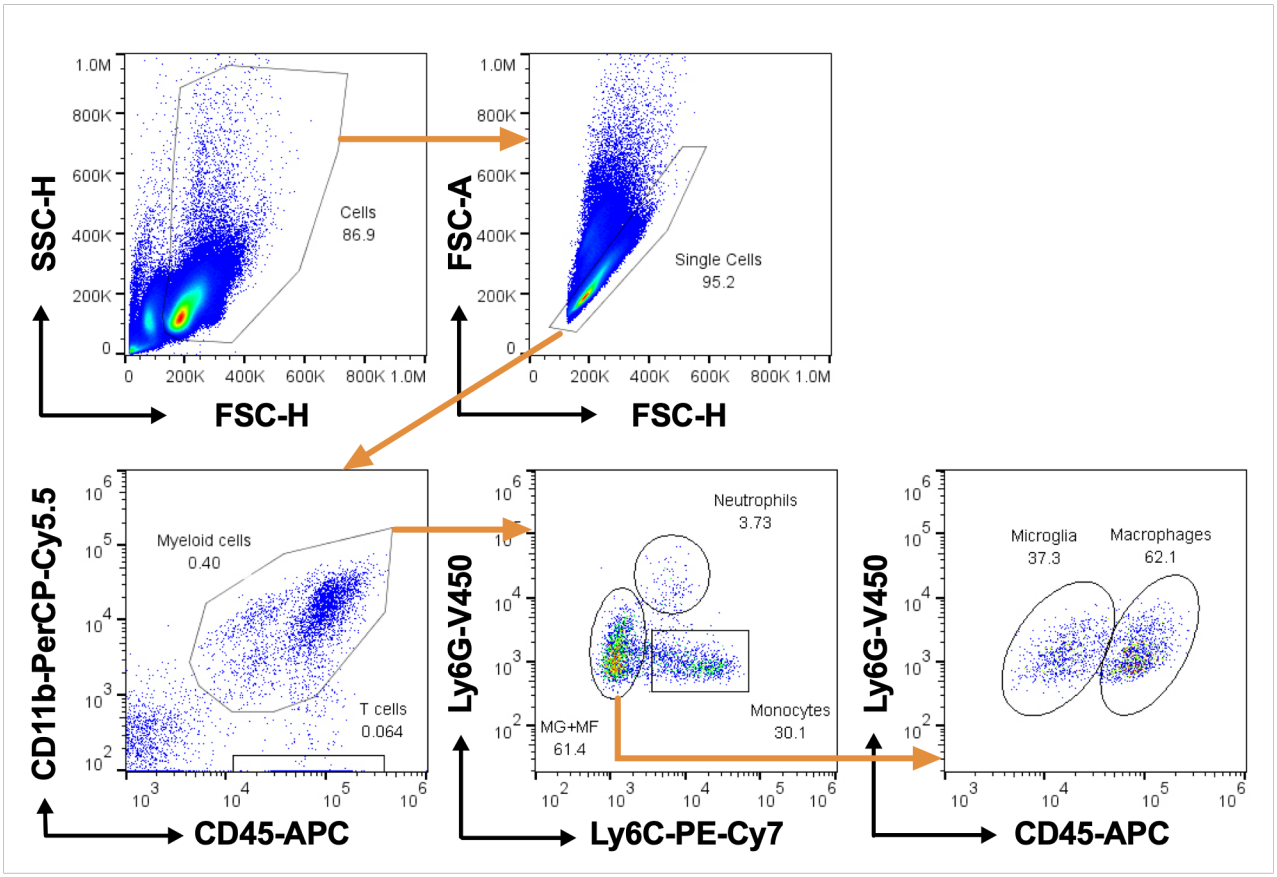
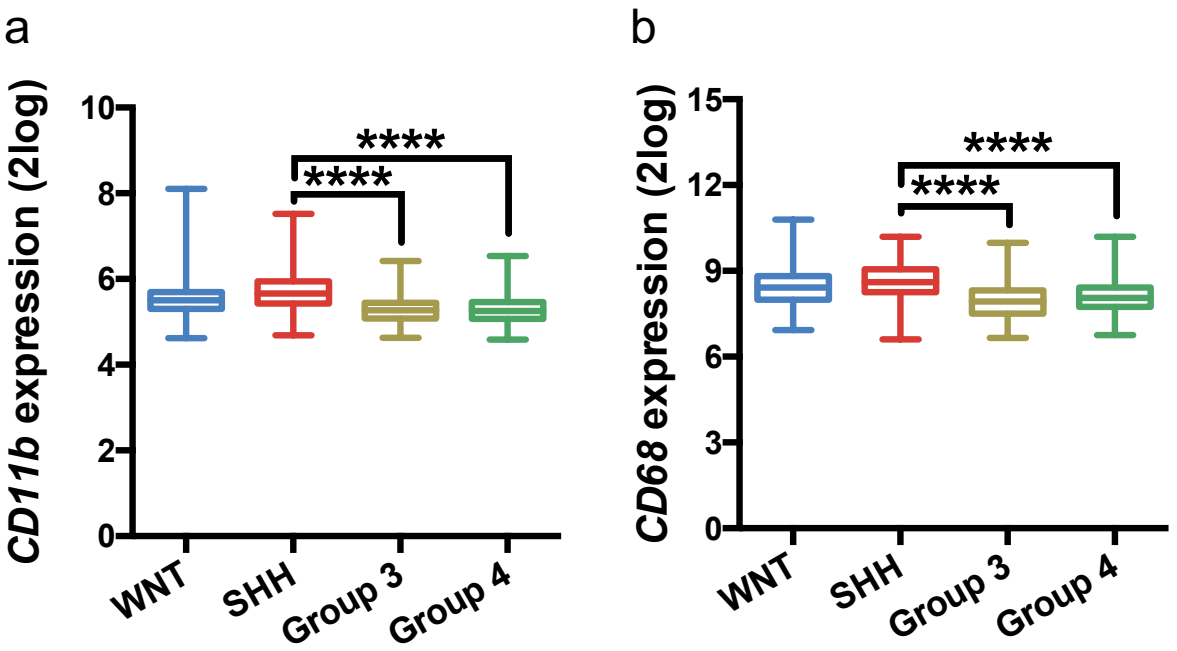
Supplementary Information

Supplementary Figure 1.



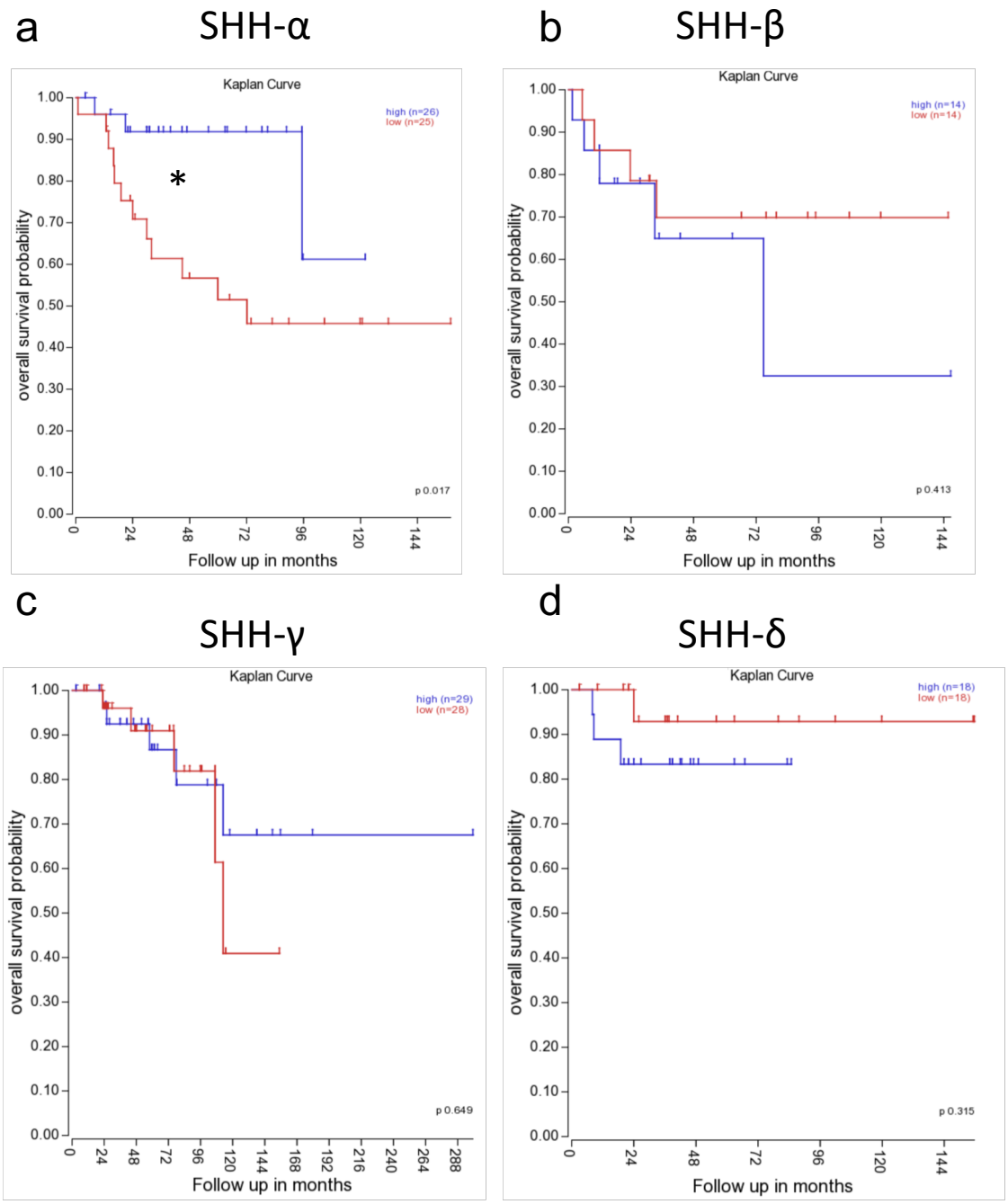

**Supplementary Figure 1. Gating strategy to separate immune cells populations.** After selection of cell population, we separated single cells, followed by selection of CD11b and CD45 positive cells. $CD45^{+}CD11b^{-}$ cells were considered as lymphoid cells. $CD45^{+}$ $CD11b^{+}$ cells were a mix of bone marrow-derived myeloid cells and microglia. We separated them by Ly6G and Ly6C expression – $Ly6G^{+}$ cells are neutrophils, $Ly6C^{+}$ cells are freshly infiltrated monocytes. Ly6C low cells were separated into $CD45^{low}$ (microglia) and $CD45^{hi}$ (macrophage) cells.

Supplementary Figure 2.



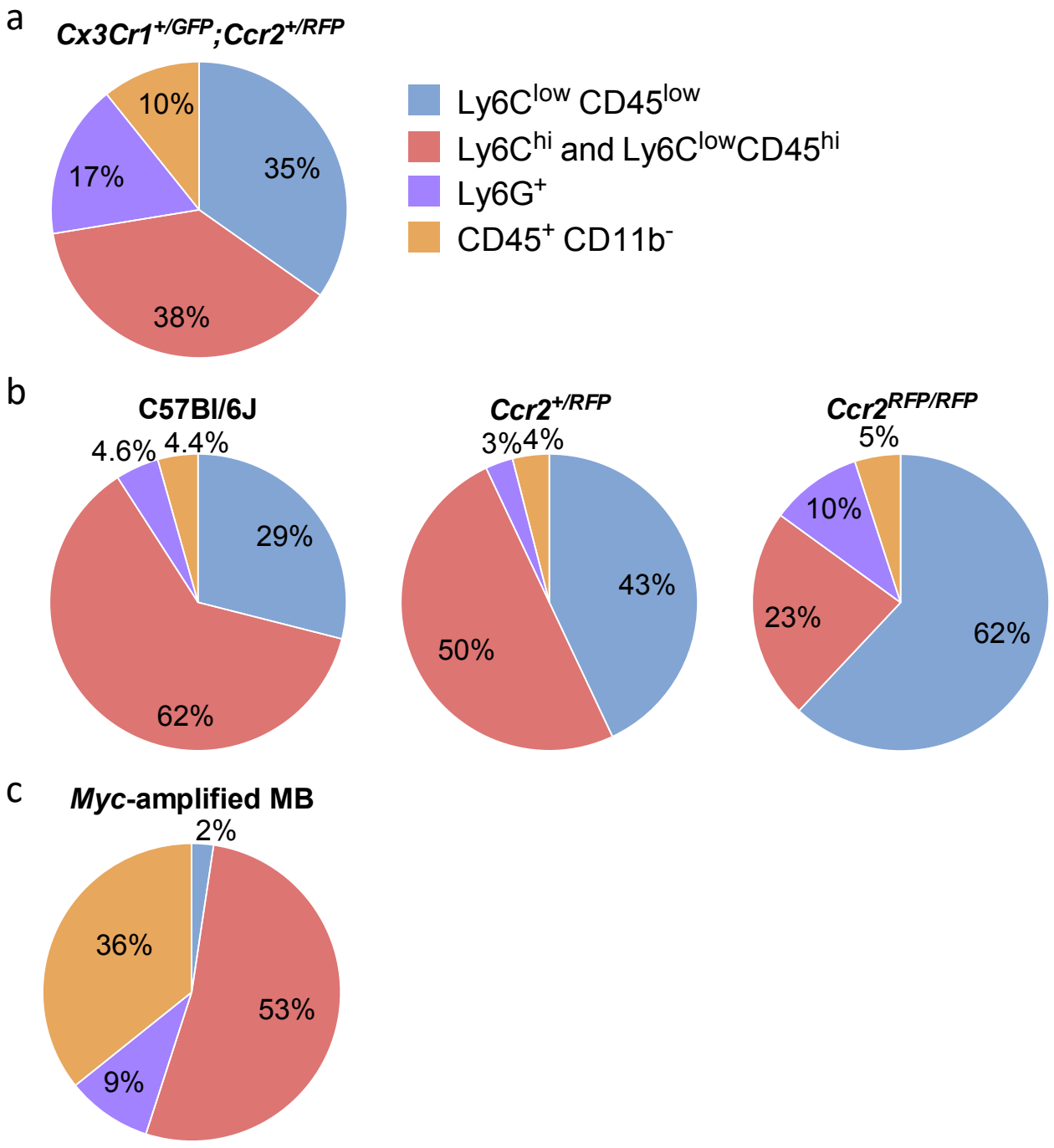

**Supplementary Figure 2. SHH medulloblastoma has pronounced activation of macrophage-associated genes.** Microarray data analysis of human patients MB cohort for macrophage-related genes *CD11b* (a), *CD68* (b) from previously published study[15]. WNT – Wingless, SHH – Sonic Hedgehog, N=70 (WNT), N=223 (SHH), N=144 (Group 3), N=326 (group 4). One way ANOVA with multiple comparisons. ****$P$<0.0001 (F= 65.45 (a), 54.94 (b)).

Supplementary Figure 3.



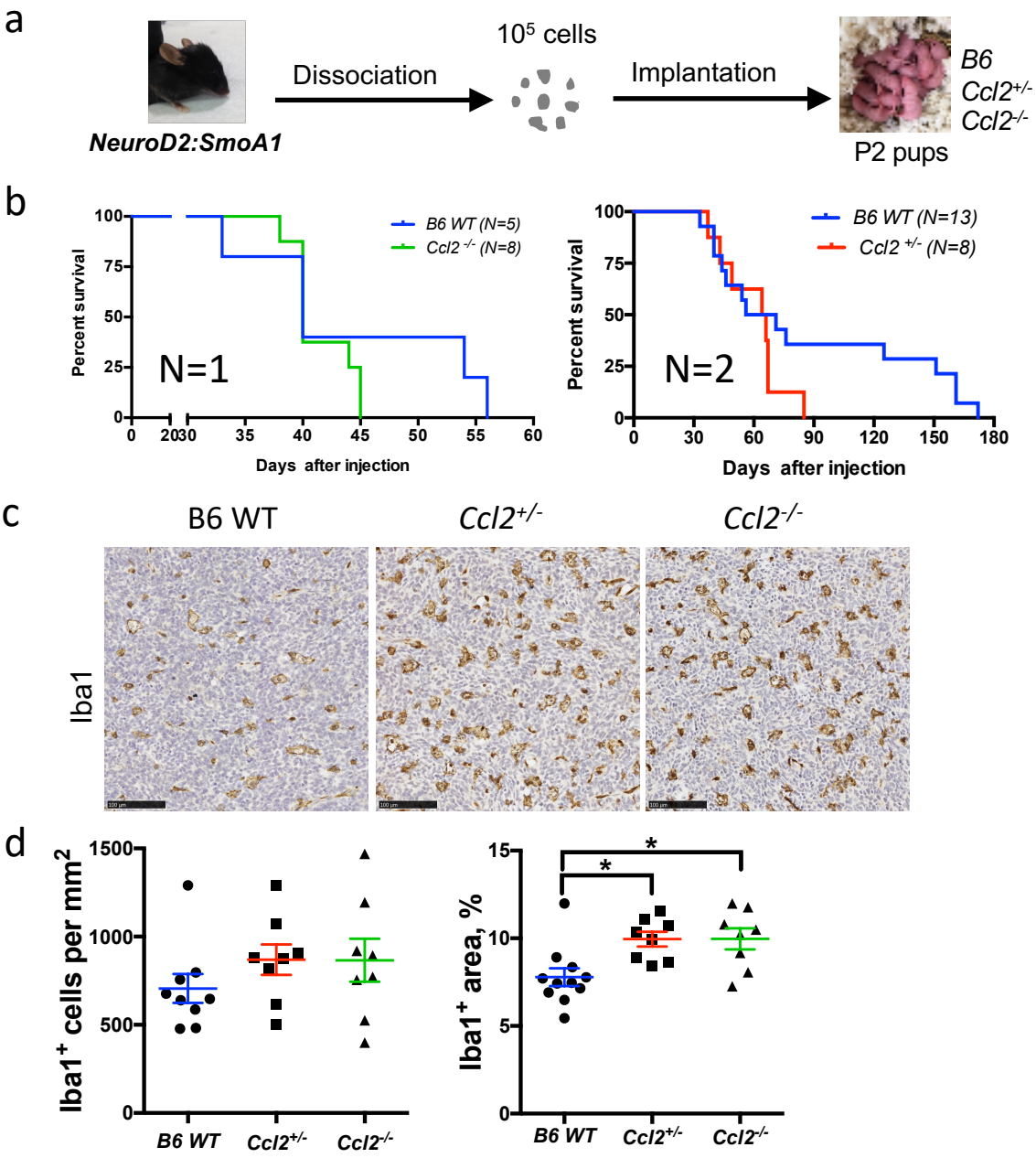

**Supplementary Figure 3. SHH medulloblastoma α subtype patients have the highest survival benefit from presence of macrophages.** Kaplan curves for MB patients separated by SHH subgroup and differentiated by macrophage gene *AIF1* expression. Log-rank Mantel-Cox test, **P*<0.05.

Supplementary Figure 4.



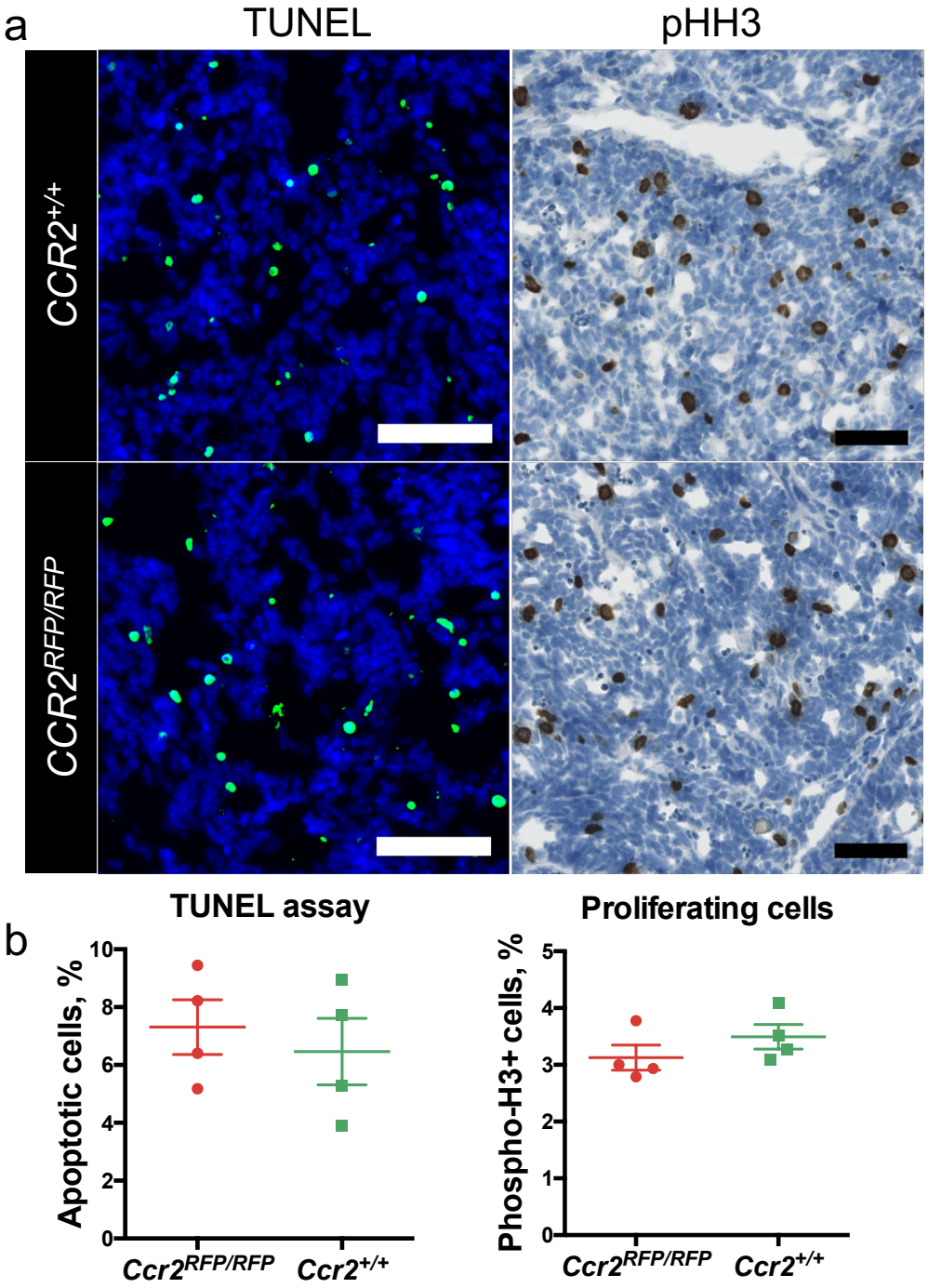

**Supplementary Figure 4. Deletion of *Ccr2* from tumour microenvironment decreases infiltration of macrophages in orthotopic implantation model.** A) Immune cell composition of *NeuroD2:SmoA1* tumours orthotopically implanted in *Ccr2*$^{+/RFP}$*Cx3cr1*$^{+/GFP}$ mice for immune cells tracing, N=5. B) *NeuroD2:SmoA1* tumors orthotopically implanted in C57Bl/6J, N=9; *Ccr2*$^{+/RFP}$, N=9; *Ccr2*$^{RFP/RFP}$, N=7. C) *Myc*-amplified MB (group 3) model implanted in B6 mice (N=4).

Supplementary Figure 5.



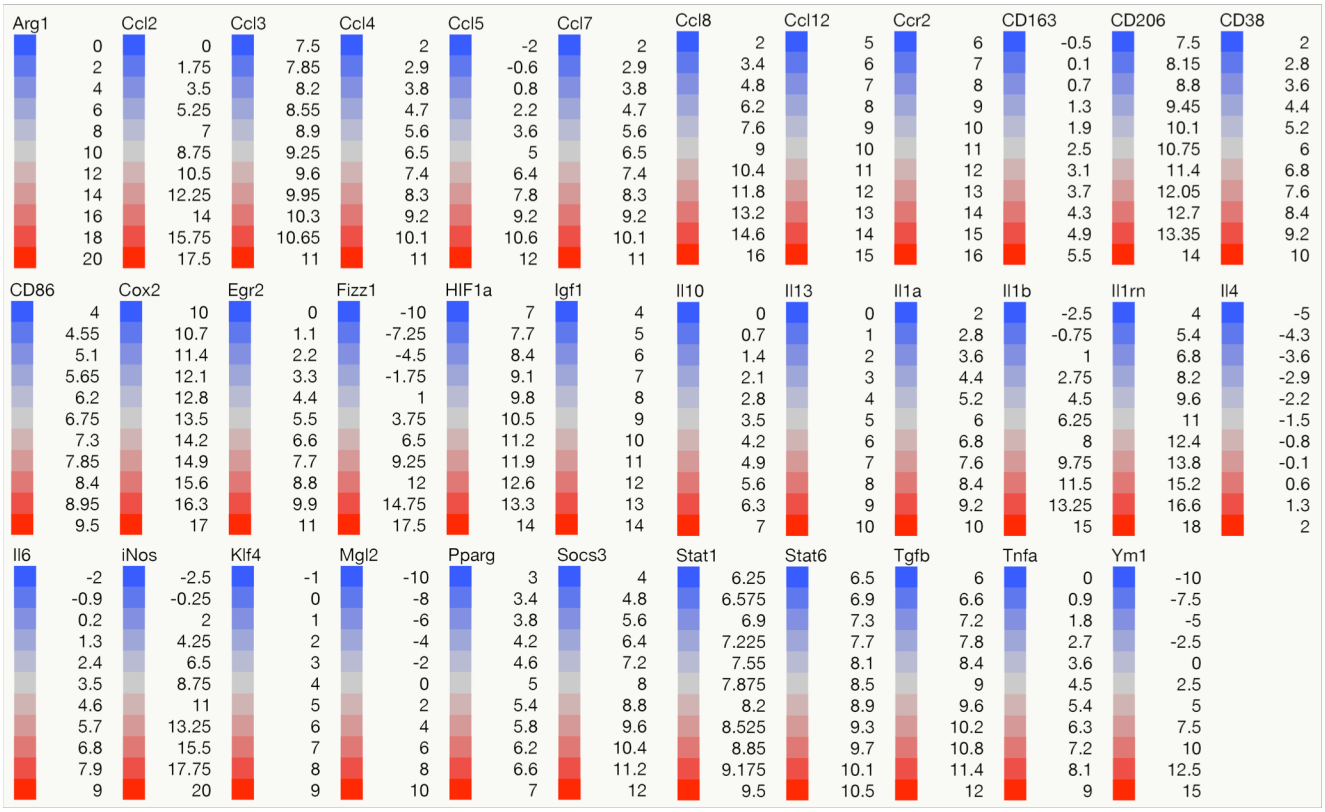

**Supplementary Figure 5. Generation of MB tumours in *Ccl2*$^{-/-}$ mice does not decrease number of TAMs.** A) Schematic of animal experiments. *NeuroD2:SmoA1* tumour tissue was extracted, dissociated into a single cell suspension and orthotopically injected into P2 pups of indicated genotypes. B) Kaplan-Meier graphs of genetically modified mice injected with *SmoA1* tumours, *Ccl2*$^{-/-}$ (N=8, green), *Ccl2*$^{+/-}$ (N=8, red), and *Ccl2*$^{+/+}$ (N=13, blue), N represents indicated number of independent tumour injection experiments. C) Representative IHC images for TAM marker Iba1 in generated tumours. D) Quantification of Iba1+ cells number and area in tumours at the endpoint, N=9 for *Ccl2*$^{+/+}$, N=8 for *Ccl2*$^{+/-}$ and *Ccl2*$^{-/-}$, Mean ± S.E.M. One-way ANOVA, **P*<0.05.

Supplementary Figure 6.



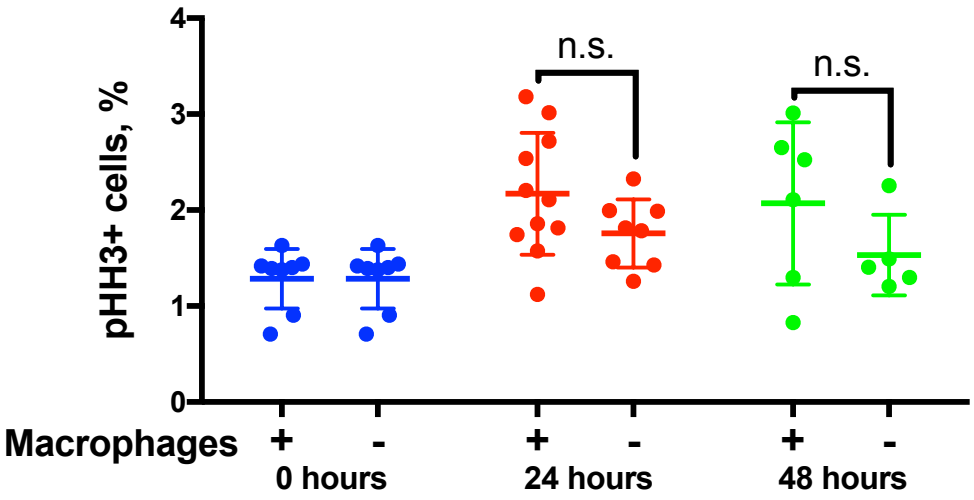

**Supplementary Figure 6. Deletion of *Ccr2* from tumour microenvironment does not affect apoptosis or proliferation levels at the endpoint.** A) TUNEL assay and phosphohistone-H3 immunohistochemistry analyses of tumours generated in *Ccr2*$^{+/+}$ and *Ccr2*$^{RFP/RFP}$ mice. Scale bars represents 50 μm; data are representative of at least four independent experiments. B) Quantification of TUNEL or phosphohistone-H3 positive cells in (A). Each data point is an average value of at least five images analyzed. Mean ± S.E.M. Mann-Whitney U test, P>0.05.

Supplementary Figure 7.




**Supplementary Figure 7.** Expression range (*log2* transformed) legends for microarray image shown in Fig. 5b.

Supplementary Figure 8.




**Supplementary Figure 8. Macrophages presence does not affect tumour cells proliferation.** Tumour slices were exposed to cultured differentiated macrophages for up to 48 hours. Phopho histone-H3 IHC staining was quantified using whole slide scanning and image analysis as described in methods section. Mean ± S.E.M., Mann-Whitney U test, n.s. *P*>0.05.

Supplementary Table 1. List of PCR primers used in current work.

| Target name | Forward primer 5'-3' | Reverse primer 5'-3' |
|---|---|---|
| ***Ccl2*** | CCCACTCACCTGCTGCTACT | TCTGGACCCATTCCTTCTTG |
| ***Actb*** | GGCTGTATTCCCCTCCATCG | CCAGTTGGTAACAATGCCATGT |
| ***SOCS3*** | TACTGAGCCGACCTCTCTC | AGCTGGGTCACTTTCTCATA |
| ***COX2*** | CCGTCCCCTCACTAGGACTT | ATCCTGGTCGGTTTGATGCT |
| ***CCL5*** | TGCCCACGTCAAGGAGTATTTC | AACCCACTTCTTCTCTGGGTTG |
| ***STAT1*** | TGGTGAAATTGCAAGAGCTG | CAGACTTCCGTTGGTGGATT |
| ***HIF1a*** | GGGTACAAGAAACCACCCAT | GAGGCTGTGTCGACTGAGAA |
| ***CCL3*** | ACTGCCTGCTGCTTCTCCTACA | AGGAAAATGACACCTGGCTGG |
| ***CCL4*** | ACCATGAAGCTCTGCGTGTC | CCATTGGTGCTGAGAACCCT |
| ***Cnn3*** | CATGACAGCCTATGGGACTC | CTCCCGAGGGTAGTCGTCTG |
| ***Il1a*** | AGTCAACTCATTGGCGCTTGA | AGAGAGATGGTCAATGGCAGA |
| ***CD38*** | GAAGACTACGCCCCACTTGT | ATGGGCCAGGTGTTTGGATT |
| ***IL10*** | GCTCTTACTGACTGGCATGAG | CGCAGCTCTAGGAGCATGTG |
| ***CD163*** | TGCTGTCACTAACGCTCCTG | TCATTCATGCTCCAGCCGTT |
| ***PPARg*** | GCCCTTTGGTGACTTTATGGA | GCAGCAGGTTGTCTTGGATG |
| ***STAT6*** | ACCTGTCCATTCGCTCACTG | CTCTGGAGTAGGAAGGGGCT |
| ***KLF4*** | GTGCCCCGACTAACCGTTG | GTCGTTGAACTCCTCGGTCT |
| ***IL4*** | AGATGGATGTGCCAAACGTCCTCA | AATATGCGAAGCACCTTGGAAGCC |
| ***IL13*** | GGCAGCATGGTATGGAGTGT | CTTGCGGTTACAGAGGCCAT |
| ***IGF1*** | AGACAGGCATTGTGGATGAG | TGAGTCTTGGGCATGTCAGT |
| ***EGR2*** | TGCTAGCCCTTTCCGTTGA | TCTTTTCCGCTGTCCTCGAT |
| ***MGL2*** | GATAACTGGCATGGACATATG | TTTCTAATCACCATAACACATTC |
| ***Il1b*** | CAGGCTCCGAGATGAACAAC | GGTGGAGAGCTTTCAGCTCATAT |
| ***iNOS*** | CCCTTCAATGGTTGGTACATGG | ACATTGATCTCCGTGACAGCC |
| ***Arg1*** | GGAATCTGCATGGGCAACCTGT | AGGGTCTACGTCTCGCAAGCCA |
| ***Fizz1*** | TCCCAGTGAATACTGATGAGA | CCACTCTGGATCTCCCAAGA |
| ***Ym1*** | CAGGTCTGGCAATTCTTCTGAA | GTCTTGCTCATGTGTGTAAGTGA |
| ***Il6*** | GCCTTCTTGGGACTGATGCT | AGTCTCCTCTCCGGACTTGTG |
| ***TNFa*** | AAGCCTGTAGCCCACGTCGTA | GGCACCACTAGTTGGTTGTCTTTG |
| ***CD86*** | TTGTGTGTGTTCTGGAAACGGAG | AACTTAGAGGCTGTGTTGCTGGG |
| ***TGFb*** | CTTCAATACGTCAGACATTCGGG | GTAACGCCAGGAATTGTTGCTA |
| ***CD206*** | GGCAGGATCTTGGCAACCTAGTA | CCTTTCTTCCGACTCTTCACCC |
| ***Ccr2*** | TGTGATTGACAAGCACTTAGACC | TGGAGAGATACCTTCGGAACTT |