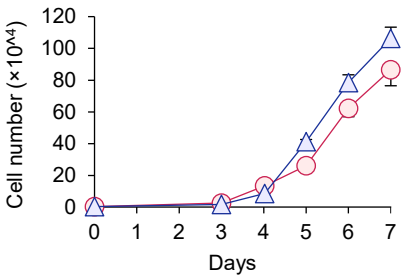
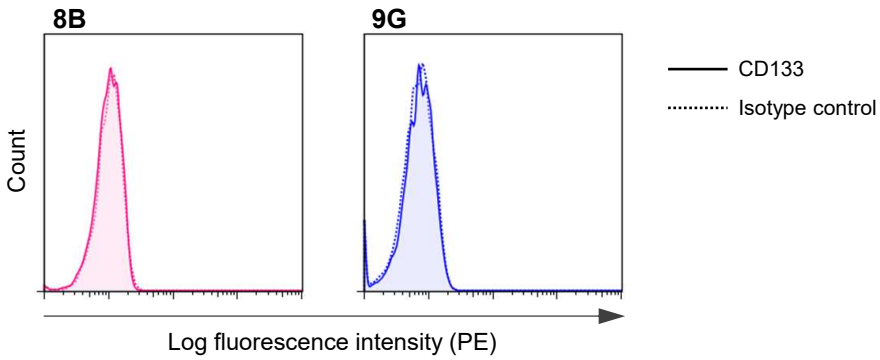
Supplementary Fig. 1



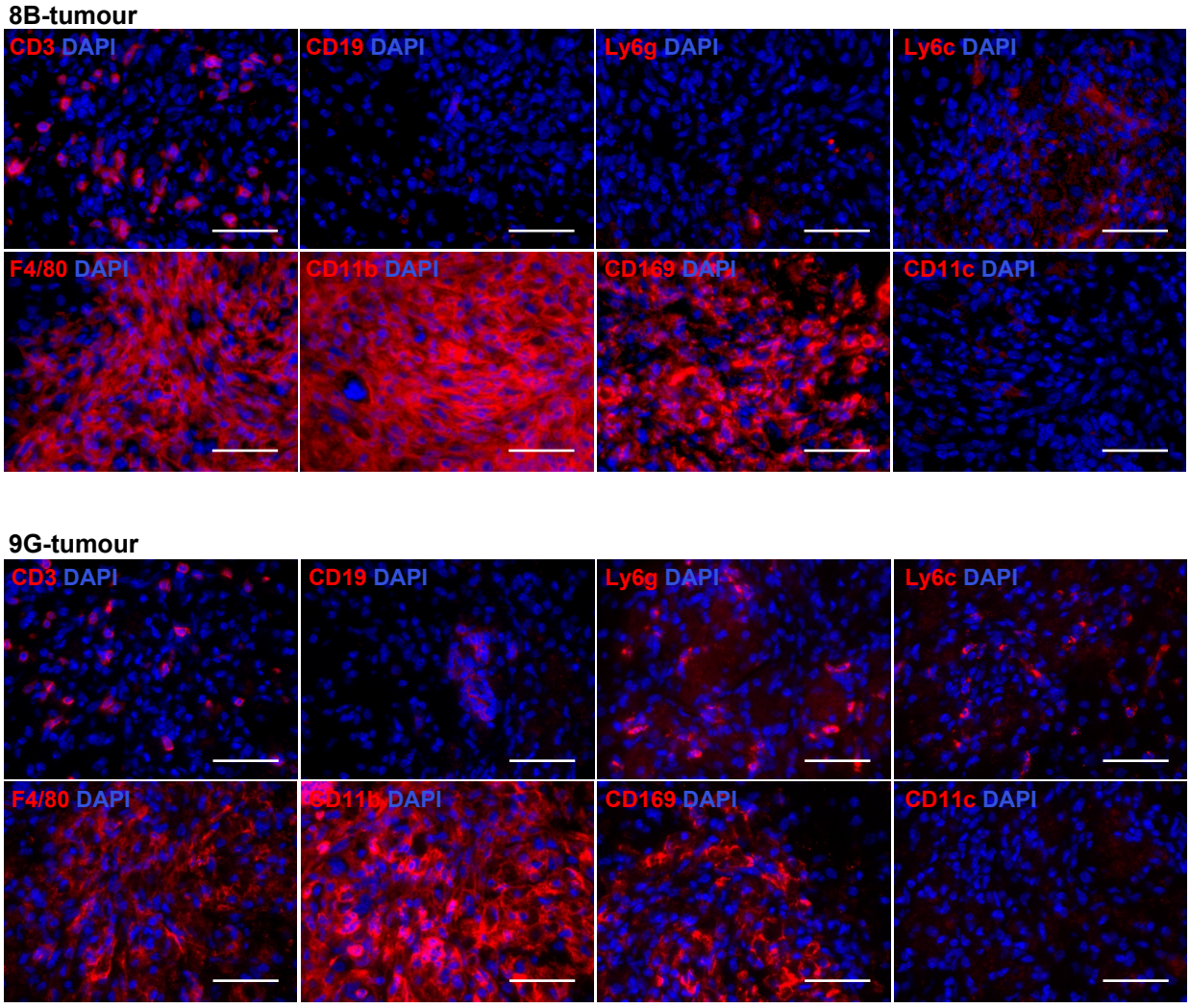
Supplementary Fig. 1 8B and 9G cells proliferated comparably *in vitro*
Ten thousand 8B or 9G cells were seeded into a 96-well plate; the cell number was counted each day. Red circles indicate 8B; blue triangles indicate 9G. Error bars indicate the standard deviation from counts of triplicate wells in a single experiment.

Supplementary Fig. 2



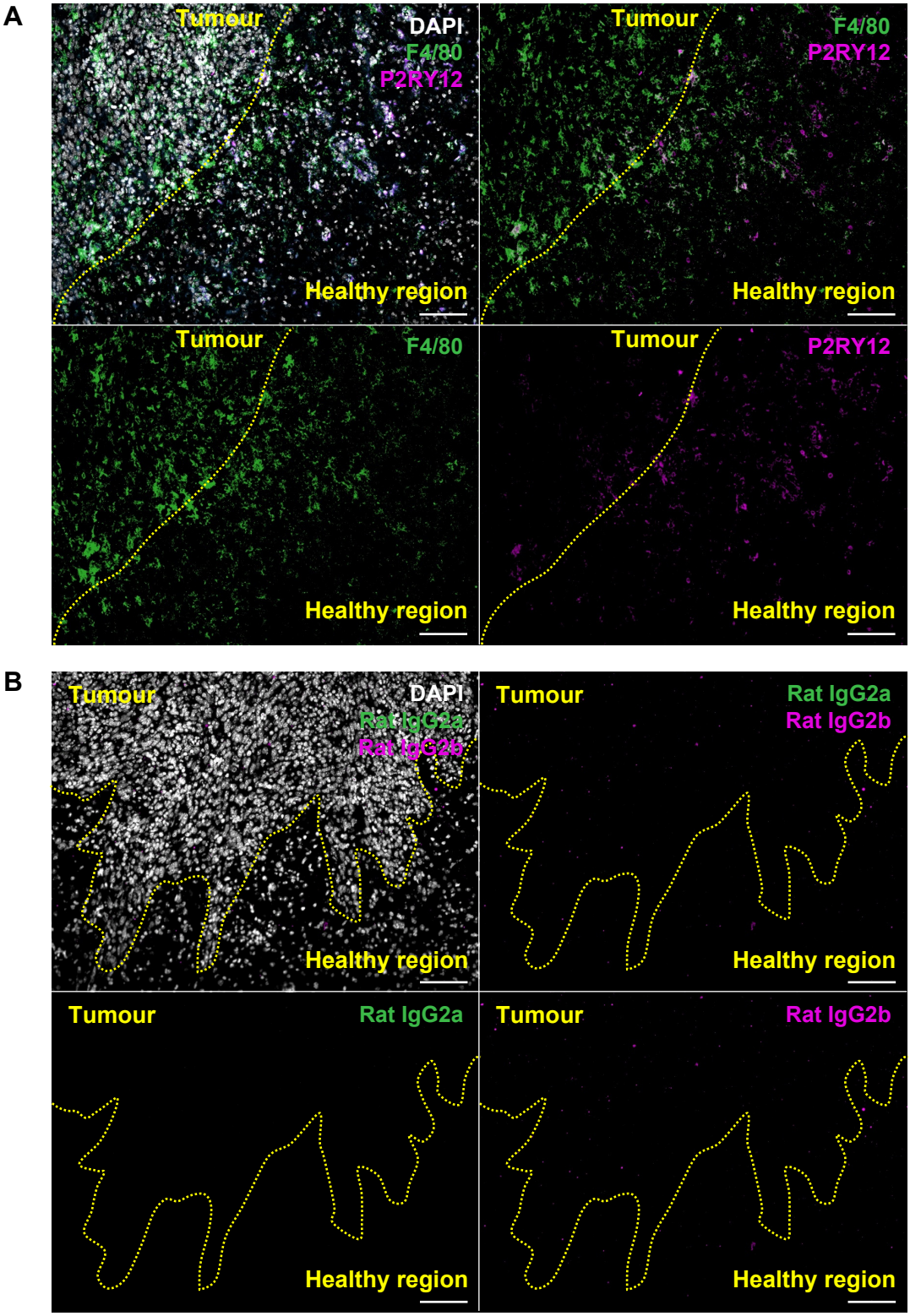
Supplementary Fig. 2 8B and 9G cells did not express CD133
8B and 9G cells were collected by vigorous pipetting without trypsinization and then stained with anti-CD133 monoclonal antibody (315-2C11) or its isotype control antibody (rat IgG2a). Filled histogram showing CD133 staining; dotted line showing staining of isotype control.

Supplementary Fig. 3



Supplementary Fig. 3 Overwhelming infiltration of Mφs in the 8B- and 9G-tumour microenvironment
Representative immunofluorescence images of 8B or 9G tumours in C57BL/6 brain stained with various immune cell markers (red). One thousand 8B or 9G cells were transplanted into the brain. 28 days after inoculation, 8B (upper panel) or 9G (lower panel)-transplanted whole brain tissue was harvested. Infiltrated immune cells were analysed by immunofluorescence staining. Blue represents DAPI staining; red represents each indicated monoclonal antibody staining in the figures. Bars; 50 μm.

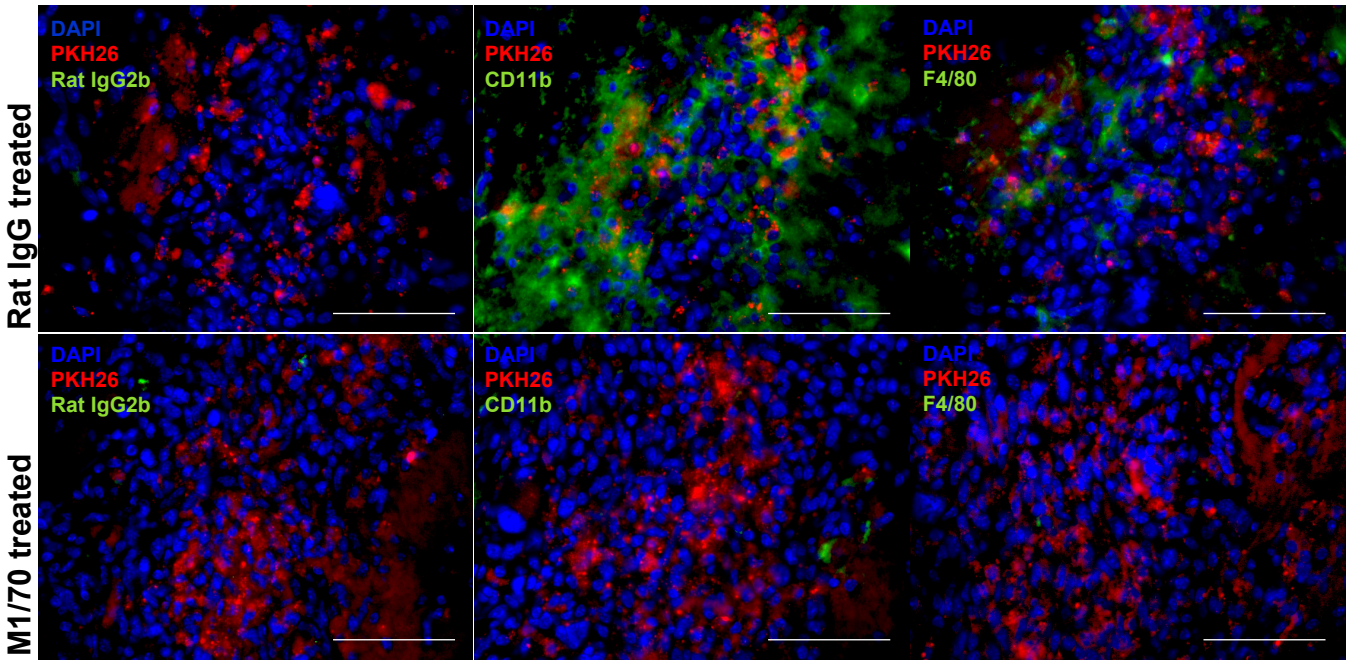
Supplementary Fig. 4



Supplementary Fig. 4 F4/80$^+$ Mφs infiltrated more in glioblastoma tissue in comparison with P2RY12$^+$ microglia

8B glioblastoma brain tissue stained with F4/80 (green) (BM8, Biolegend) and P2RY12 (magenta) (S16007D, Biolegend) (A), or its isotype control antibodies (B). Blue represents DAPI staining. Bars; 100 μm.

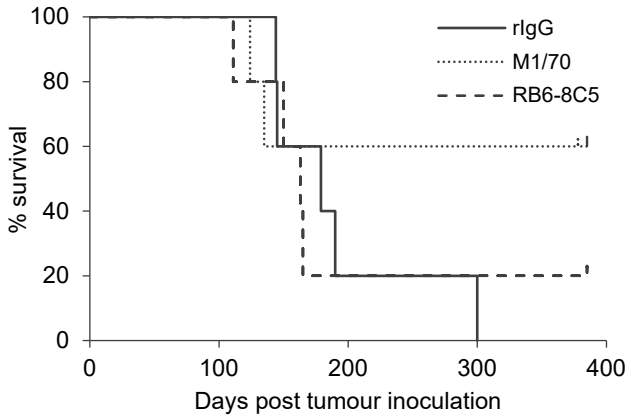
Supplementary Fig. 5



Supplementary Fig. 5 M1/70 antibody administration to mice removed Mφ in tumour
200 μg of each antibody was administered 7 days and 1 day prior to tumor inoculation. On Day 0, 8B cells labeled with PKH26 were introduced into the mouse brain. Brain tissues were harvested 7 days post-inoculation, followed by sectioning and fluorescence immunostaining. Rat IgG treated' and 'M1/70 treated' respectively denote mice treated with the Rat IgG antibody and the M1/70 antibody. Tumour sites in brain tissues were identified by PKH26 fluorescence (red), and the efficacy of Mφ (green) removal was assessed. Blue represents DAPI staining. Bars; 100 μm.

Supplementary Fig. 6

A

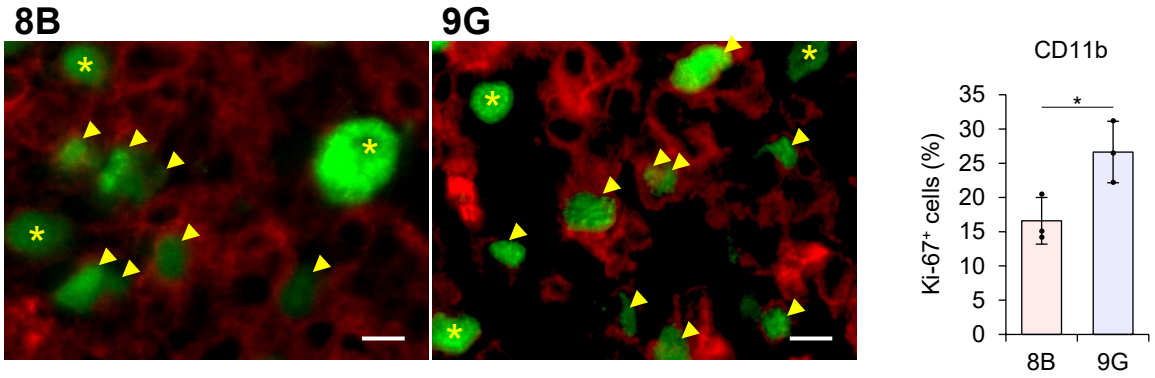


B

		Group		
Result	Frequency % total % column % row	Rat IgG	M1/70	RB6-8C5	Total
Alive		0	3	1	4
		0.00	20.00	6.67	26.27
		0.00	60.00	20.00	
		0.00	75.00	25.00	
Dead		5	2	4	11
		33.33	13.33	26.67	73.33
		100.00	40.00	80.00	
		45.45	18.18	36.36	
Total		5	5	5	15
		33.33	33.33	33.33	

Supplementary Fig. 6 Mφ depletion decreased tumour-occurrence in 8B inoculated mouse
8B cells (1×10^4) were transplanted into the C57BL/6 mouse brain. One day before tumour inoculation, 200 μg of RB6-8C5 antibody (n=5) (large-dotted line), M1/70 antibody (n=5) (small-dotted line) or rat IgG (n=5) (solid line) were intraperitoneally injected. Twenty-one days after tumour inoculation, 100 μg each of the same antibodies were injected again. The mouse survival was observed. **A**, Kaplan-Meier survival curve. **B**, Crosstab analysis.

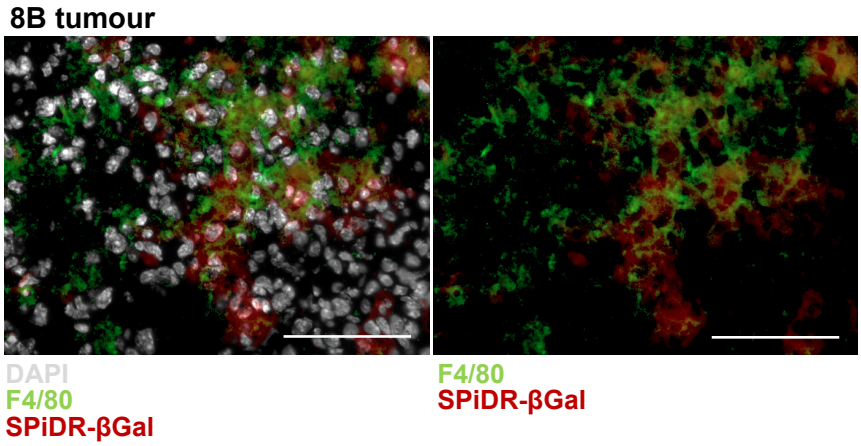
Supplementary Fig. 7



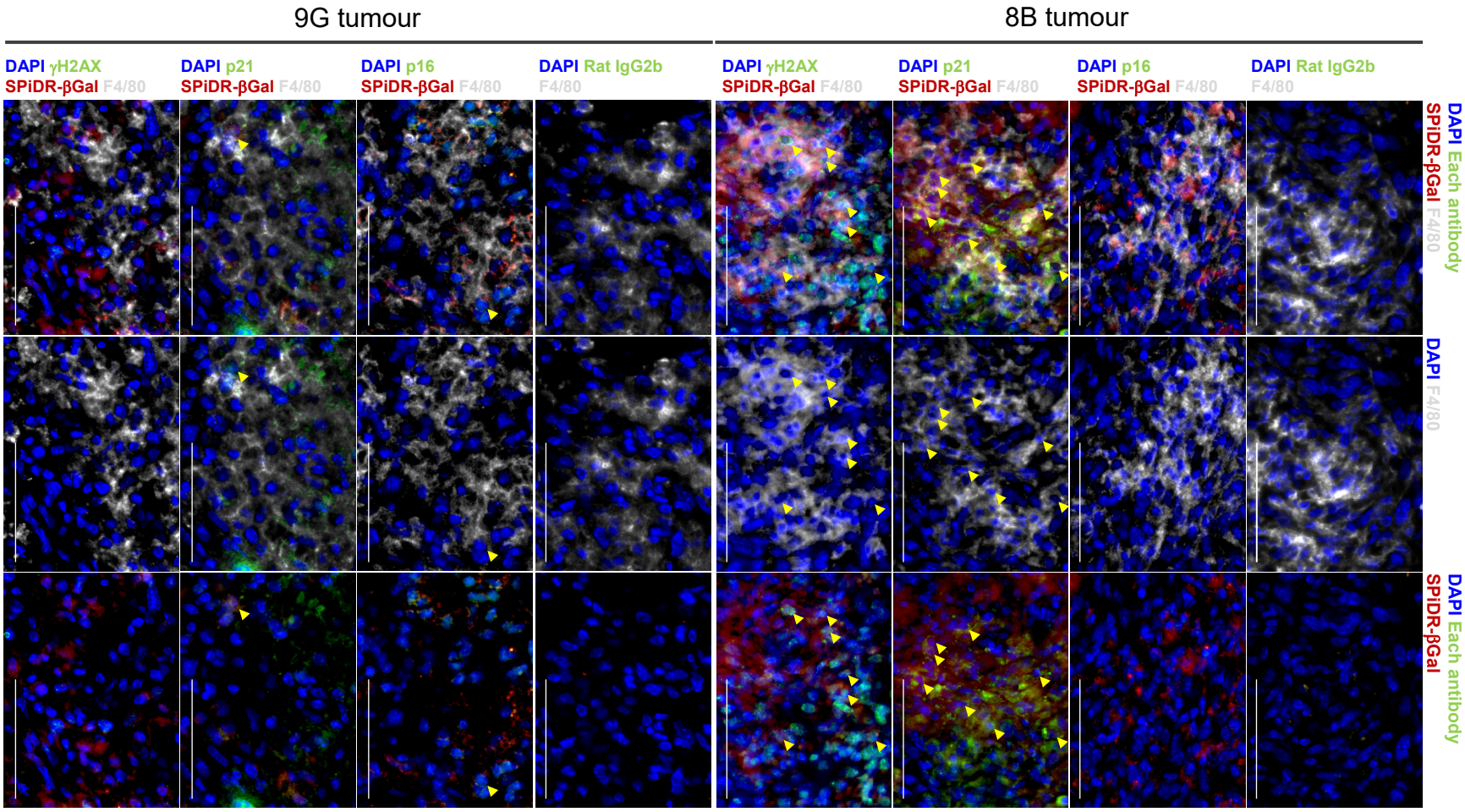
Supplementary Fig. 7 Proportion of Ki-67 positive cells within CD11b positive cells is higher in 9G tumor tissue compared to 8B

1,000 tumor cells were inoculated into C57BL/6 mice brain, and the brain was harvested 3 days later. Fluorescence immunostaining was performed for Ki-67 and CD11b. Red indicates CD11b and green represents Ki-67. In each tumor sample, three fields of view were randomly selected then count the number of Ki-67 positive cells among the CD11b positive population, and the proportion of Ki-67 positive cells was calculated. Yellow arrowheads indicate nuclei that are positive cells for both CD11b and Ki-67. Ki-67 positive nuclei designated by * were excluded from the count as they were identified as tumor cells based on nuclear size and the absence of CD11b. The bars indicate the mean (± SD). Each dot represents the percentage of Ki-67 cells in each view. Bars; 10 μm.

Supplementary Fig. 8



Supplementary Fig. 8 SPiDR-βGal positive F4/80 cells were observed in tumour tissue
SPiDR-βGal staining combined with F4/80 was applied on brain tissue seven days after mice were inoculated with 1000 cells of 8B. Bars: 100 μm.

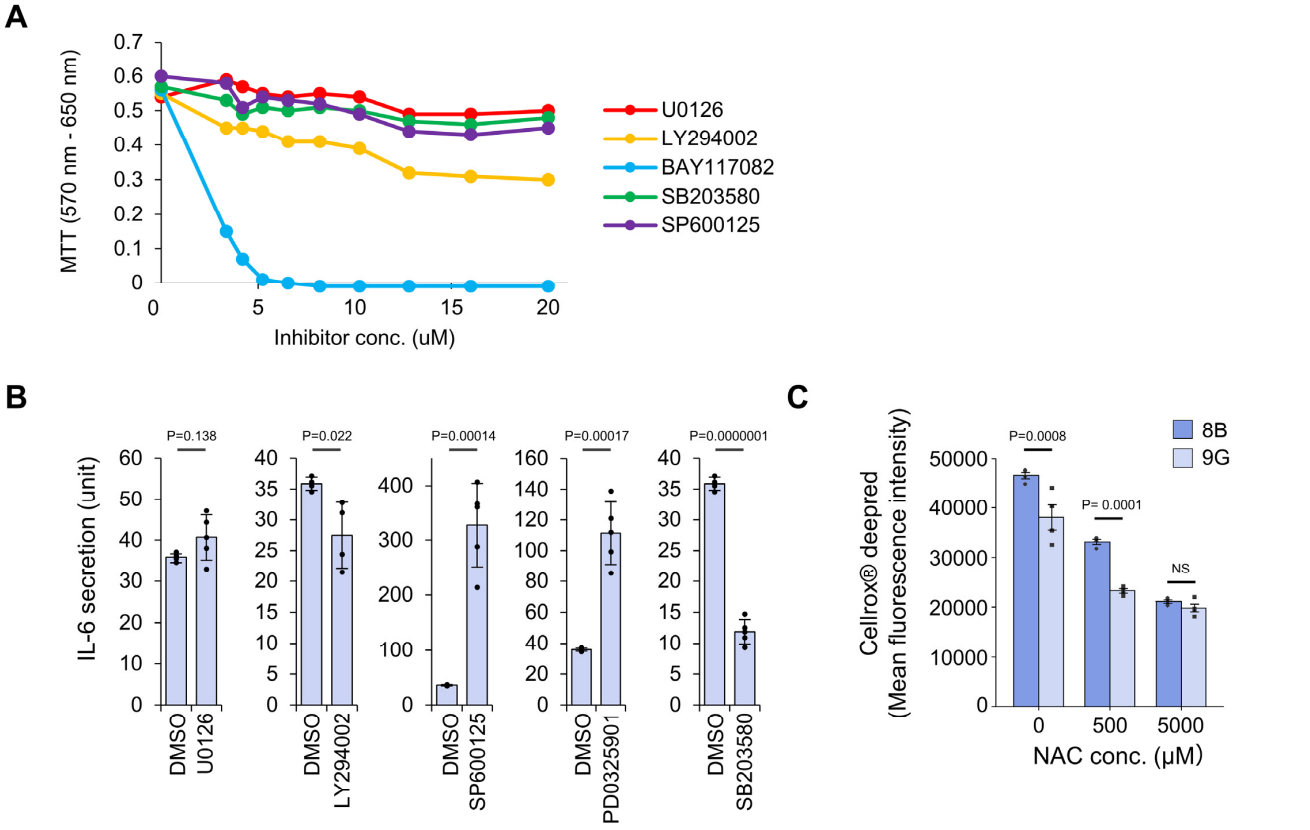


Supplementary Fig. 9

Supplementary Fig. 9
（Continued)

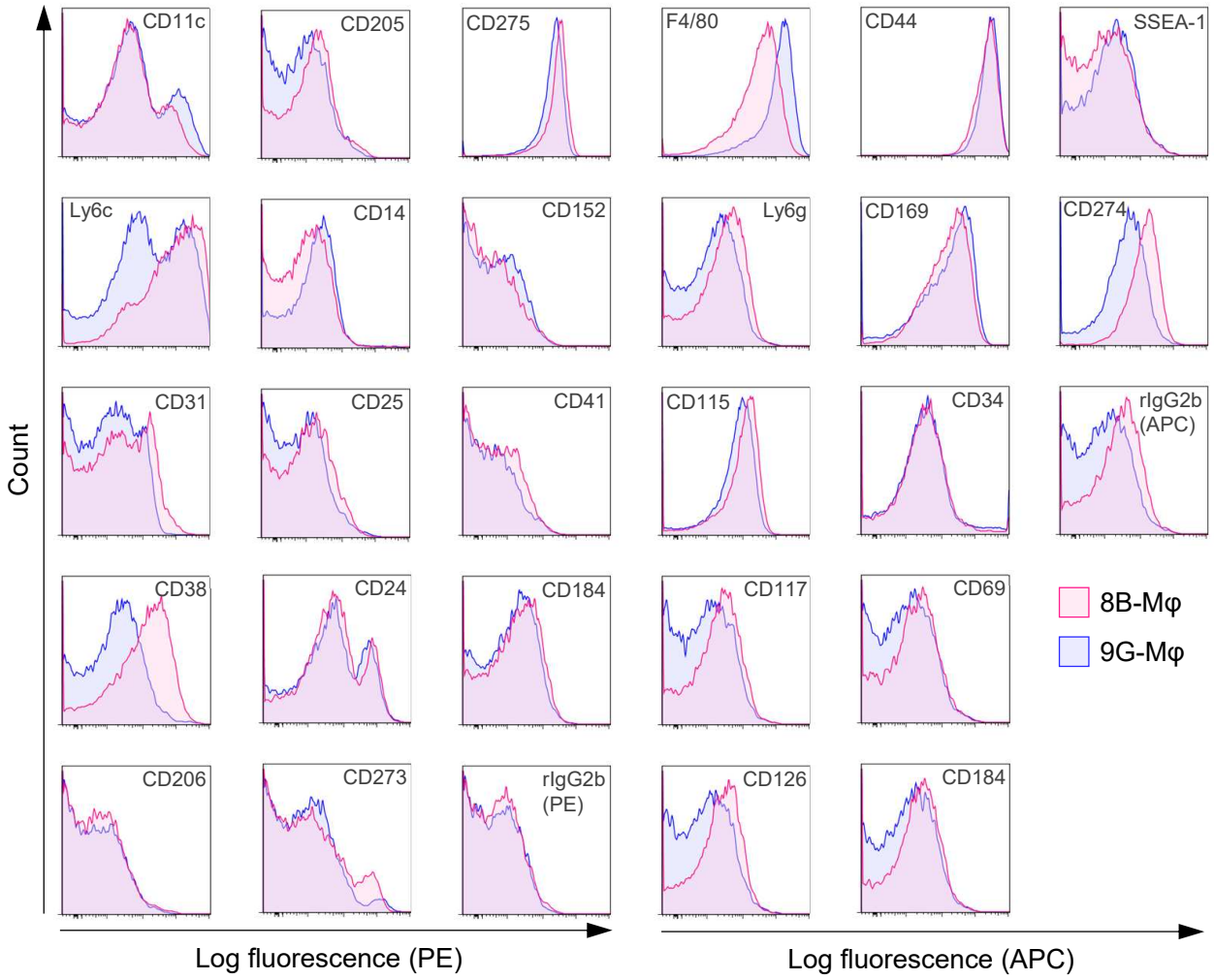
Supplementary Fig. 9 Mφs co-expressing senescence markers were observed in 8B tumors
1,000 of 8B or 9G cells were inoculated into C57BL/6 mice brain, and the brain was harvested 7 days later. Fluorescence immunostaining was performed for $p16^{Ink4a}$, p21, γH2AX, Rabbit IgG (negative control) (green) and F4/80 (white). Blue represents DAPI staining. The yellow arrowheads indicate cells that were co-positive for F4/80, SPiDR-βGal, and either p16, p19, or γH2AX.

Supplementary Fig. 10



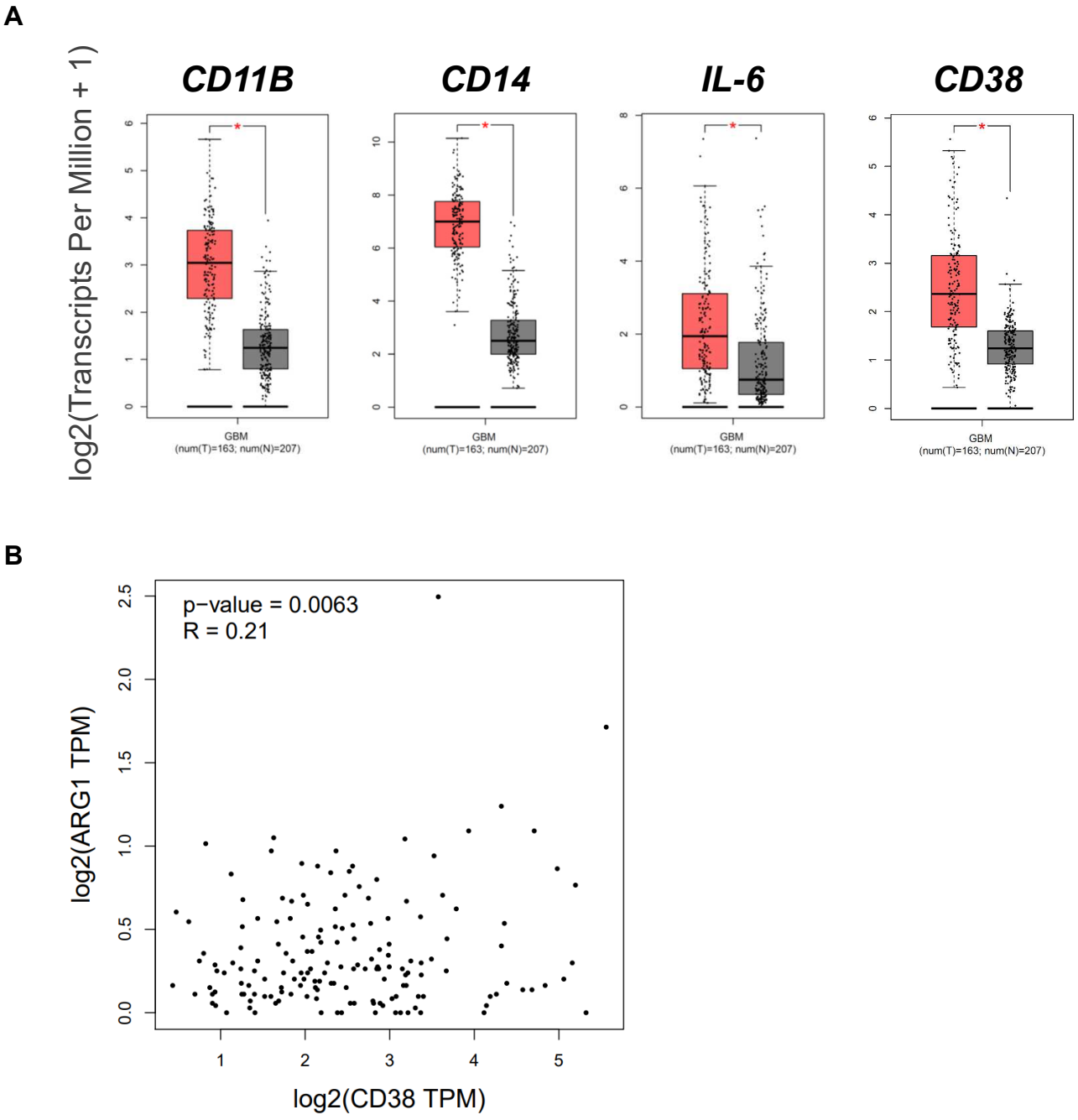
Supplementary Fig. 10 IL-6 secretion in 8B cells mainly controlled by p38 pathway
A, The acceptable concentration of each inhibitor was determined by assessing cell viability with the MTT assay. Various cell signalling pathway inhibitors (0 to 20 μM each) or vehicle (dimethyl sulfoxide) were added to the 8B culture. Twenty-four hours later, the culture medium was discarded; fresh medium, including inhibitors, was added. After another 24 hours, cell viability in each culture condition was also monitored by MTT assay. **B,** To determine which signalling pathway in 8B is important for IL-6 production, various cell signalling pathway inhibitors (20 μM each) or vehicle (dimethyl sulfoxide) were tested as the same described in A. Further, IL-6 accumulation in each supernatant was measured by beads-based cytokine assay system, LEGENDplex™. Bar graphs indicate the average of IL-6 concentration which were normalized by number of live cells. Each dot represents the IL-6 concentration in four wells standardized by the number of viable cells at the time of culture supernatant collection, and the bar graph shows the mean. The number of viable cells was derived from the MTT assay. Error bars indicate the standard deviation from counts of four wells in a single experiment. The values in the graphs represent p-values from Student's *t*-test (unpaired, two-tailed). The experiment was repeated three times and similar results were observed. **C**, ROS production in 8B (indicated in blue bars) or 9G cells (pale blue bars) was analysed. 8B or 9G cells were cultured in glioma medium without *N*-acetylcysteine (NAC) overnight; then, several concentrations of NAC were added and cultured for two hours. Then, 1 μM of Cellrox® deepred were added and cultured for further one hour and ROS levels were analysed by flow cytometry. Error bars indicate the standard deviation from mean fluorescence intensity of Cellrox® deepred in four independent cultures. Each dot represents the mean fluorescence intensity of each independent culture. The values in the graphs represent p-values from Tukey-Kramer HSD test, NS: not significant. The experiment was repeated two times and similar results were observed.

Supplementary Fig. 11



Supplementary Fig. 11 Cell surface marker screening of senescent Mφs
Whole splenocytes were co-cultured with 8B- or 9G-cell supernatant for 7 days. The expression of surface protein indicated in each profile on $CD11b^{+}$ gated cells is shown. The red histogram represents 8B-Mφs; the blue histogram represents 9G-Mφs. PE-anti-Ly6c (HK1.4, Biolegend), -CD31 (390, Biolegend), -CD38 (90, Biolegend), -CD206 (C068C2, Biolegend), -CD205 (HD83, Biolegend), -CD14 (rmC5-3, BD biosciences), -CD25 (3C7, Biolegend), -CD24 (M1/69, Biolegend), -CD273 (TY25, Biolegend), -CD275 (2D3, Biolegend), -CD152 (UC10-4B9, Biolegend), -CD41 (MWReg30, Biolegend), -CD184 (L276F12, Biolegend), PE-Rat IgG2bk, (RTK4530, Biolegend), APC-anti-F4/80 (BM8, Biolegend), -Ly6g (1A8, Biolegend), -CD115 (AFS98, Biolegend), -CD117 (2B8, Biolegend), -CD126 (D7715A7, Biolegend), -CD44 (IM7, Biolegend), -CD169 (3D6.112, Biolegend), -CD34 (MEC14.7, Biolegend), -CD69 (H1.2F3, Biolegend), -CD184 (2B11, eBioscience), -SSEA-1 (MC480, BD biosciences), -CD274 (10F.9G2, Biolegend), and APC-Rat IgG2bk, (RTK4530, Biolegend) were used.

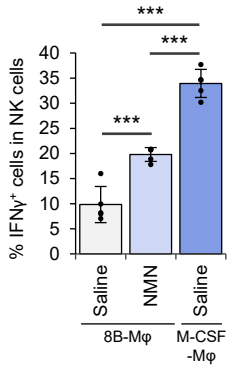
Supplementary Fig. 12



Supplementary Fig. 12 Gene expression analysis of senescence-related genes in human glioblastoma tissues and non-tumour brain tissues

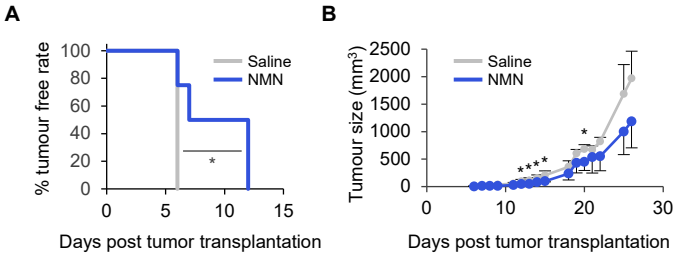
Gene expression in human samples were analysed using GEPIA. A, CD14, IL-6, and CD38 in human glioblastoma tissues (red boxes) and non-tumour brain tissues (black boxes) were shown. The box plots are based on 163 glioblastoma samples and 207 non-tumour brain samples. *p < 0.05, Student's t-test. B, Pairwise Pearson correlation between CD38 and ARG1 in human glioblastoma tissues were analysed using GEPIA.

Supplementary Fig. 13



Supplementary Fig. 13 NK cell activation capability was restored in NMN-treated Mφ
Splenic F4/80-positive Mφ were cultured under conditions with and without NMN. M-CSF induced Mφ was used as a positive control. These Mφ and total spleen cells were co-cultured for two days, and the proportion of IFNγ-positive cells among NK cells was determined by flow cytometry. The bars indicate the mean (± SD). Each dot represents the percentage of IFNγ-positive cells among NK cells in each cultures. ***$p < 0.001$, Tukey-Kramer HSD test.

Supplementary Fig. 14



Supplementary Fig. 14

A, CT26 cells were subcutaneously injected into syngeneic immunocompetent BALB/c mice. NMN or saline was inoculated three times per week until death. Tumour initiation was evaluated using ocular inspection and palpation. The dark blue line indicates NMN-treated mice (n = 4); the grey line indicates saline-treated mice (n = 4). **p < 0.01, log-lank test. **B**, The average tumour size (± SD) of CT26 transplanted mice described in A. The dark blue line indicates NMN-treated mice (n = 4); the grey line indicates saline-treated mice (n = 4). *p < 0.05, Student's t-test.