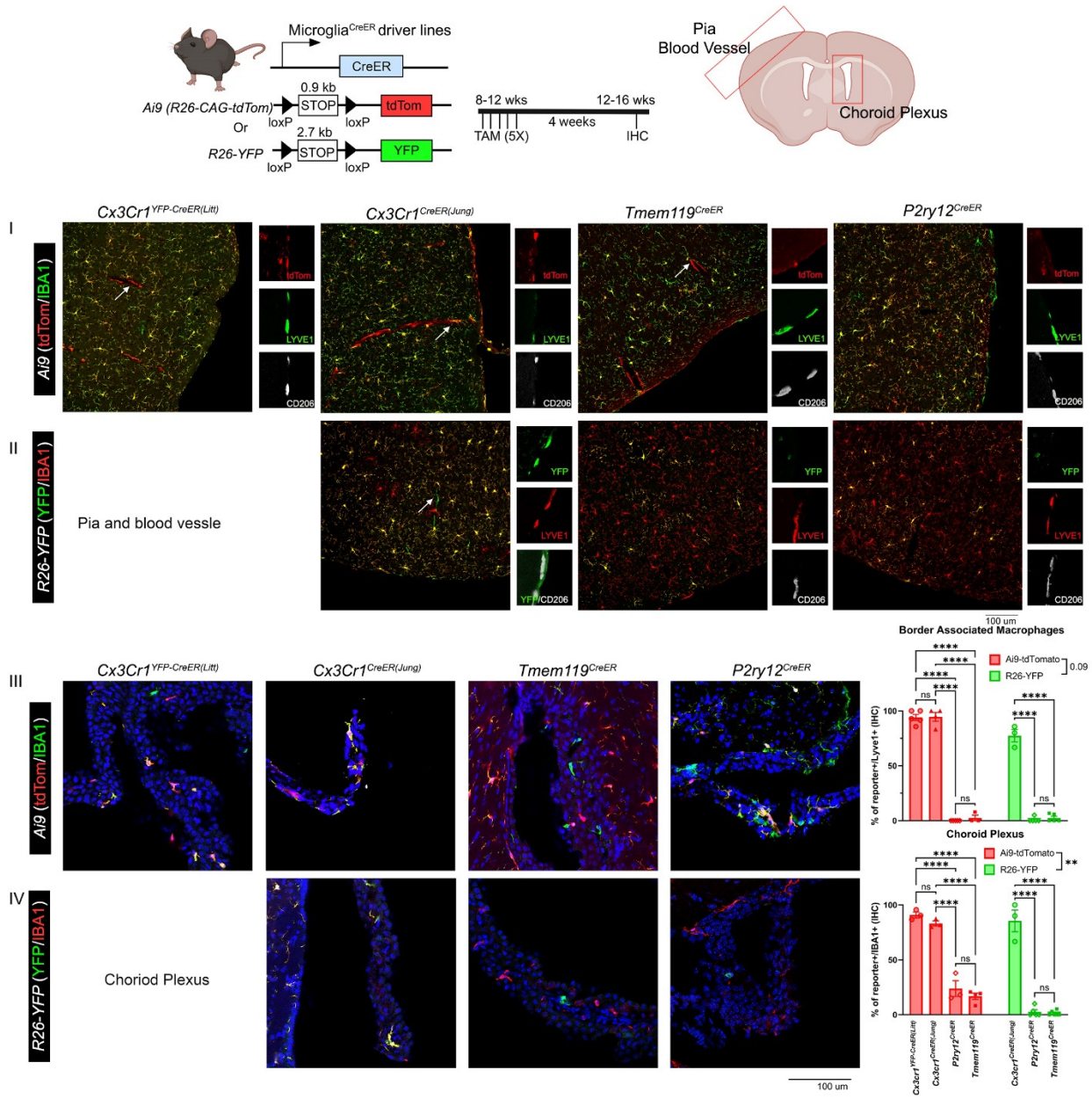
Cell Reports, Volume *43*

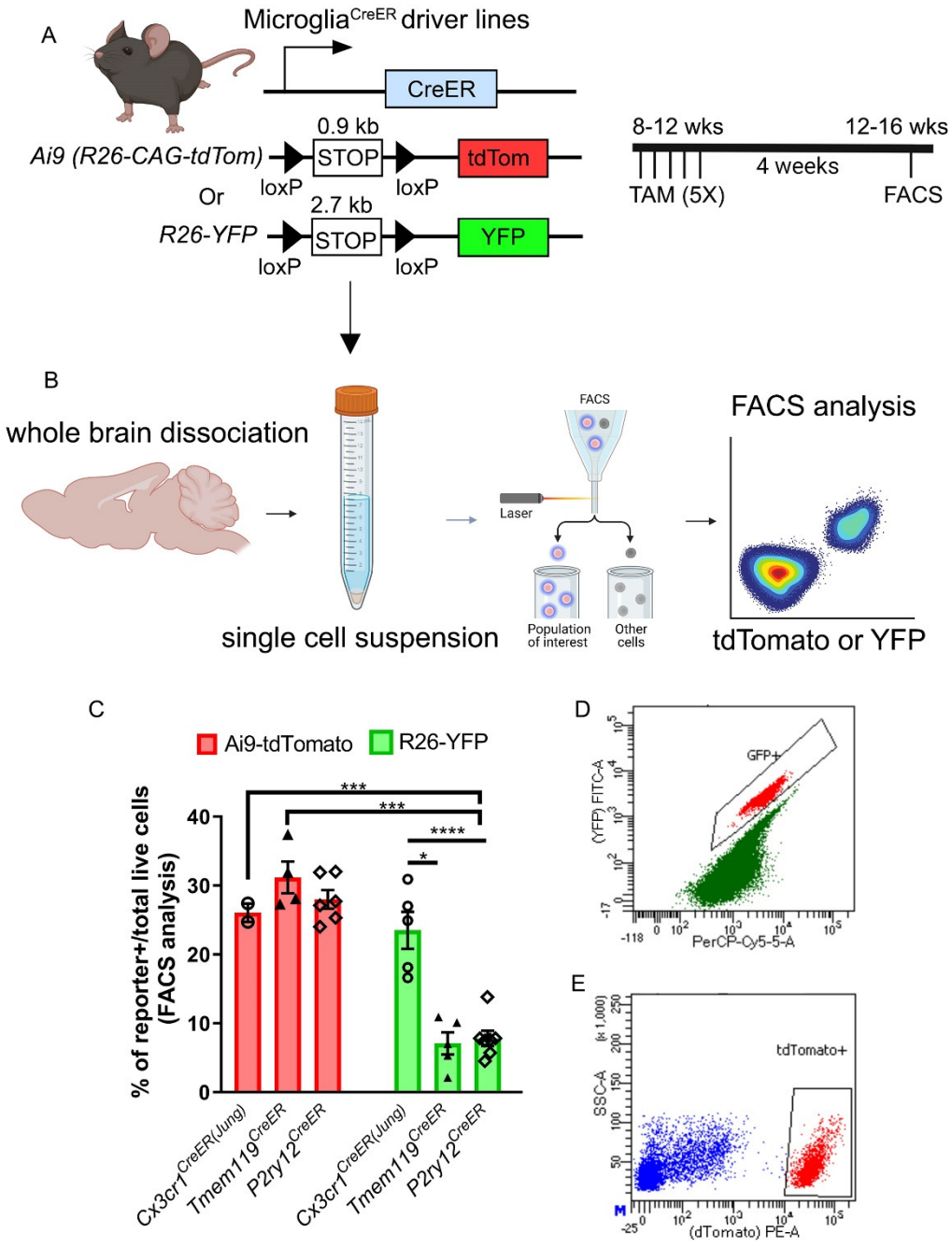
Supplemental information

A comparative evaluation of the strengths and potential caveats of the microglial inducible CreER mouse models

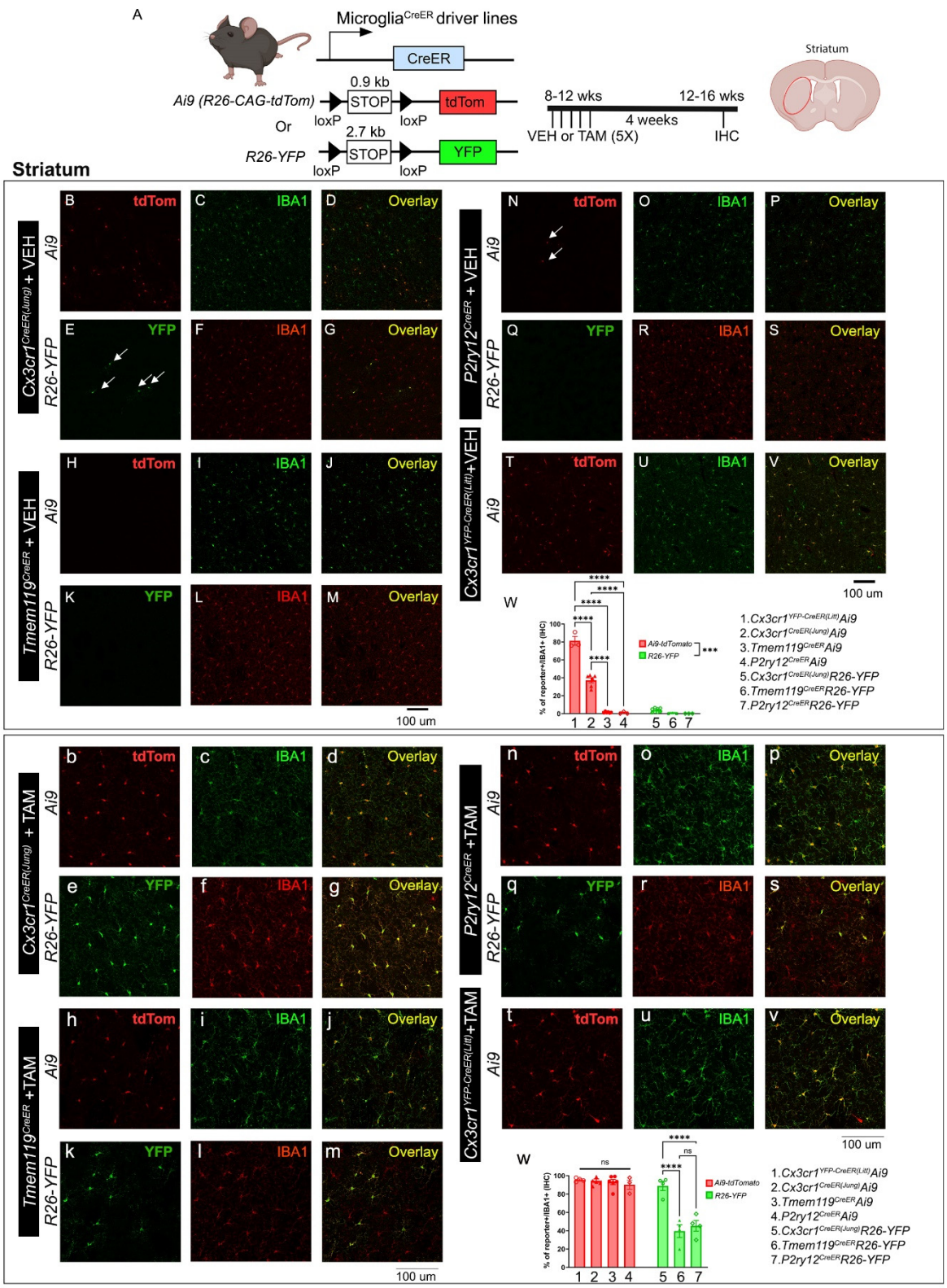
Alicia M. Bedolla, Gabriel L. McKinsey, Kierra Ware, Nicolas Santander, Thomas D. Arnold, and Yu Luo



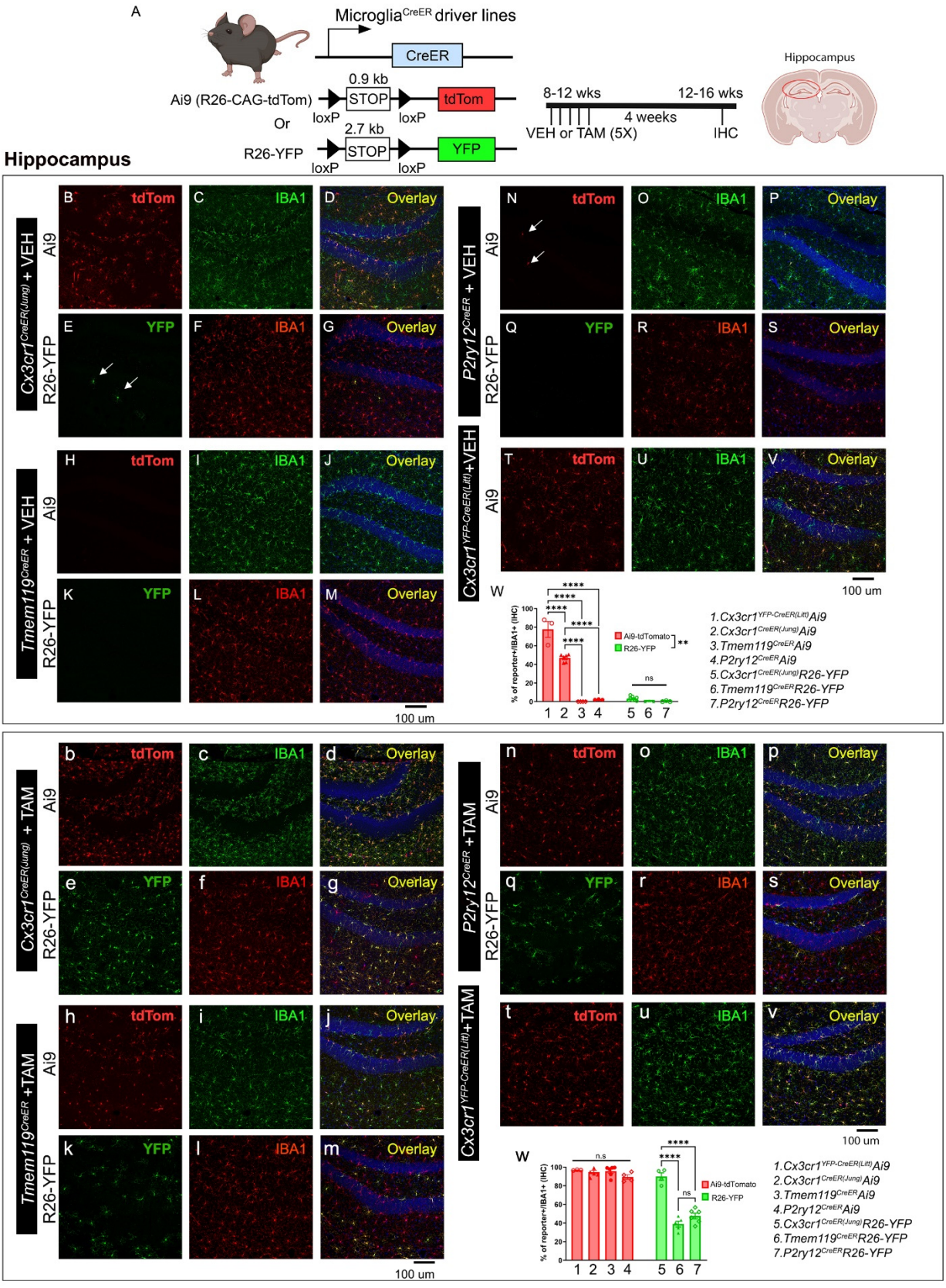
Supplementary Figure 1. Border Associated macrophage (BAMs, visualized by LYVE1 or CD206 immunoreactivity, small panels) labeling in the four different *CreER* driver lines using either the *Ai9 (tdTomato)* or *R26-YFP* reporter mouse lines. Related to Figure 1. The experimental timeline is shown on top of the panel. Representative images from each *CreER* driver and *Ai 9* (I) or *R26-YFP* (II) reporter line in pia and blood vessel associated macrophages and choroid plexus associated macrophages (III *Ai9- tdTomato*) or (IV *R26-YFP*) reporter. Quantification shown on the right. mean±SEM, **p < 0.01 and **** p < 0.0001, for Two-way ANOVA analysis, Tukey post-hoc pair-wise analysis. *Ai9* vs *R26-YFP* is compared as a factor (**, p<0.01). Scale bar = 100µm.



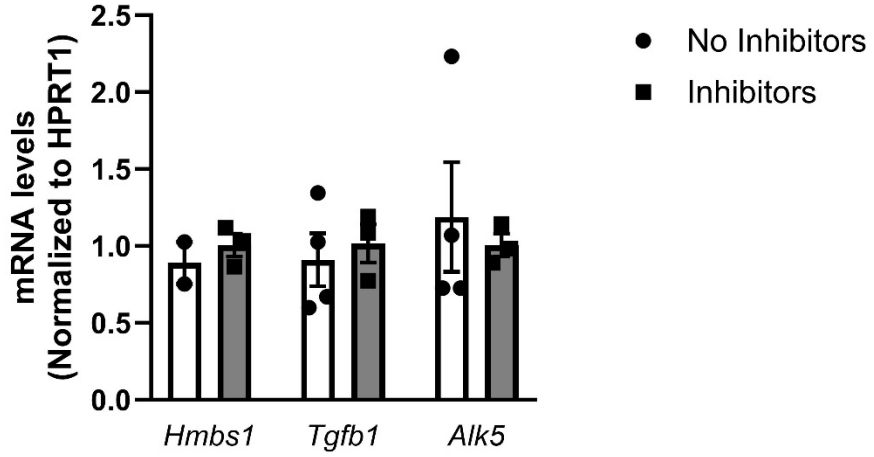
Supplementary Figure 2. FACS analysis of reporter-positive cells in total brain single cell resuspension confirms the reporter recombination efficiency differences among the three investigated *CreER* lines. Related to Figure 1. tdTomato^{+} cell percentage from all three different lines show similar high-efficiency recombination and the YFP^{+} cells show higher recombination efficiency in the *Cx3cr1*$^{CreER(Jung)}$*-R26-YFP* mice but significantly lower recombination efficiency in the *P2ry12*^{CreER}*-R26-YFP* mice and *Tmem119*^{CreER}*-R26-YFP* line. Each data point represents data from one animal. mean±SEM *p < 0.05, ***p < 0.001, and ****p<0.0001 for Two-way ANOVA analysis, Tukey post-hoc pairwise analysis. Data were combined from 2-3 independent cohorts of mice for each line.


A
Microglia CreER driver lines
CreER
Ai9 (R26-CAG-tdTom)
0.9 kb
STOP
tdTom
loxP
loxP
Or
R26-YFP
2.7 kb
STOP
YFP
loxP
loxP
8-12 wks
12-16 wks
4 weeks
VEH or TAM (5X)
IHC
Striatum
Striatum
Cx3cr1 CreER(Jung) + VEH
Ai9
R26-YFP
Tmem119 CreER + VEH
P2ry12 CreER + VEH
Cx3cr1 YFP-CreER(Litt) +VEH
tdTom
YFP
IBA1
Overlay
100 um
W
% of reporter+/IBA1+ (IHC)
Ai9-tdTomato
R26-YFP
1.Cx3cr1 YFP-CreER(Litt) Ai9
2.Cx3cr1 CreER(Jung) Ai9
3.Tmem119 CreER Ai9
4.P2ry12 CreER Ai9
5.Cx3cr1 CreER(Jung) R26-YFP
6.Tmem119 CreER R26-YFP
7.P2ry12 CreER R26-YFP
Cx3cr1 CreER(Jung) + TAM
Tmem119 CreER +TAM
P2ry12 CreER +TAM
Cx3cr1 YFP-CreER(Litt) +TAM
ns

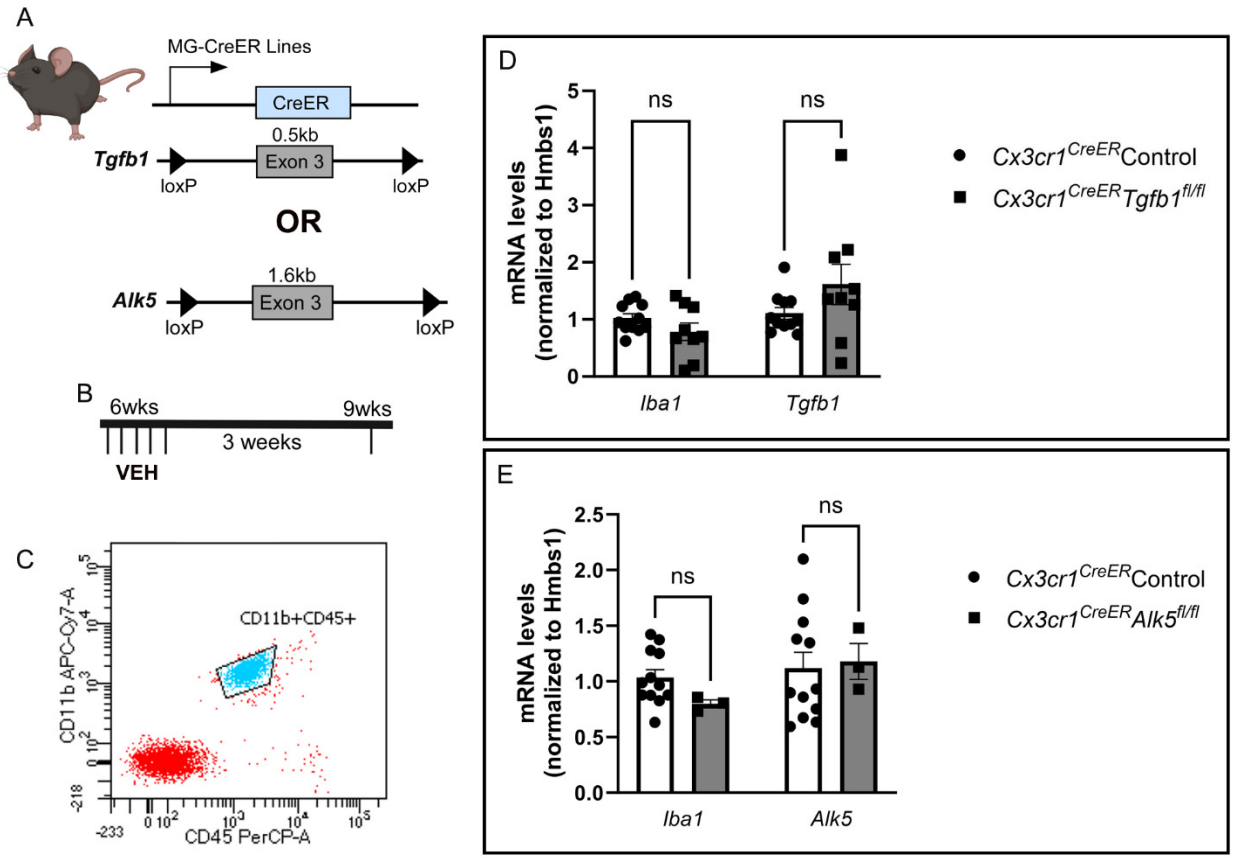
Supplementary Figure 3. Evaluation of the TAM-independent leakiness and the efficiency of TAM-dependent cre recombination in the four different *CreER* driver lines using either the *Ai9* (tdTomato) or *R26-YFP* reporter mouse lines in the striatum. Related to Figure 1. The experimental timeline is shown in panel (A). Representative images from each cre driver and reporter line (B-V for VEH treatment and b-v for TAM treatment). Cre driver and the reporter line are indicated on the left side of the panels. Quantification of reporter^{+} cells in the IBA1^{+} populations in the brain is shown in panel W (for VEH treatment) and w (for TAM treatment). Representative images are taken from the striatal region which reflects the general and homogenous trend in the whole parenchyma. Each data point represents the average of 1 animal (the average for each animal is obtained by quantifying multiple brain sections at similar anatomical locations) and the average for each animal was used as a single data point for statistical analysis. mean±SEM **$p < 0.01$ and ***$p < 0.001$, for Two-way ANOVA analysis, Tukey post-hoc pairwise analysis. *Ai9* vs *R26-YFP* is significantly different as a factor ($p<0.001$). Data were combined from 2 independent cohorts of mice. Scale bar: 100 μm. Compared to the two *Cx3cr1*^{CreER} lines (Littman and Jung), *Tmem119*^{CreER} and *P2ry12*^{CreER} show less leakiness in the absence of TAM but a decreased recombination efficiency and mosaic recombination in microglia.


A
Microglia CreER driver lines
CreER
Ai9 (R26-CAG-tdTom)
0.9 kb
STOP
tdTom
loxP
loxP
Or
R26-YFP
2.7 kb
STOP
YFP
loxP
loxP
8-12 wks
12-16 wks
4 weeks
VEH or TAM (5X)
IHC
Hippocampus
Hippocampus
Cx3cr1 CreER(Jung) + VEH
Ai9
R26-YFP
P2ry12 CreER + VEH
Tmem119 CreER + VEH
Cx3cr1 YFP-CreER(Litt) +VEH
tdTom
YFP
IBA1
Overlay
100 um
W
% of reporter+/IBA1+ (IHC)
Ai9-tdTomato
R26-YFP
ns
1 2 3 4 5 6 7
1.Cx3cr1 YFP-CreER(Litt) Ai9
2.Cx3cr1 CreER(Jung) Ai9
3.Tmem119 CreER Ai9
4.P2ry12 CreER Ai9
5.Cx3cr1 CreER(Jung) R26-YFP
6.Tmem119 CreER R26-YFP
7.P2ry12 CreER R26-YFP
Cx3cr1 CreER(Jung) + TAM
Tmem119 CreER +TAM
P2ry12 CreER +TAM
Cx3cr1 YFP-CreER(Litt) +TAM
n.s

Supplementary Figure 4. Evaluation of the TAM-independent leakiness and the efficiency of TAM-dependent cre recombination in the four different *CreER* driver lines using either the *Ai9* (tdTomato) or *R26-YFP* reporter mouse lines in the hippocampus. Related to Figure 1. The experimental timeline is shown in panel (A). Representative images from each cre driver and reporter line (B-V for VEH treatment and b-v for TAM treatment). Cre driver and the reporter line are indicated on the left side of the panels. Quantification of reporter^{+} cells in the IBA1^{+} populations in the brain is shown in panel W (for VEH treatment) and w (for TAM treatment). Representative images are taken from the hippocampal region which reflects the general and homogenous trend in the whole parenchyma. Each data point represents the average of 1 animal (the average for each animal is obtained by quantifying multiple brain sections at similar anatomical locations) and the average for each animal was used as a single data point for statistical analysis. mean±SEM **p < 0.01 and ***p < 0.001, for Two-way ANOVA analysis, Tukey post-hoc pairwise analysis. *Ai9* vs *R26-YFP* is significantly different as a factor (p<0.001). Data were combined from 2 independent cohorts of mice. Scale bar: 100 μm. Compared to the two *Cx3cr1*^{CreER} lines (Littman and Jung), *Tmem119*^{CreER} and *P2ry12*^{CreER} show less leakiness in the absence of TAM but a decreased recombination efficiency and mosaic recombination in microglia.



Supplementary Fig 5. The addition of transcriptional and translational inhibitors during the cell isolation process does not affect the expression level of *tgfb1* or *Alk5* mRNA in sorted microglia. Related to Figure 3. Microglia were sorted either in the presence or absence of inhibitors and the mRNA levels for 2 housekeeping genes (*Hprt1* and *Hmbs1*), *Tgfb1* and *Alk5* were analyzed with qRT-PCR. mean±SEM. P>0.05, Student's t-test between two groups for all genes.



Supplemental Figure 6: qRT-PCR analysis of FACS sorted brain myeloid cells ($CD11b^{+}/CD45^{int}$) from *Cx3cr1*$^{CreER(Jung)}$ driver line to examine tamoxifen-independent gene recombination of the floxed target genes. Related to Figure 3. Using a small (*Tgfb1*, 0.5kb) and a large (*Alk5*, 1.6kb) floxed region mouse lines, we did not observe any tamoxifen-independent reduction in mRNA levels in either the floxed *Tgfb1*$^{fl/fl}$ or the *Alk5*$^{fl/fl}$ in the total sorted microglia in the absence of TAM treatment in *Cx3cr1*$^{CreER(Jung)}$ *Tgfb1*$^{fl/fl}$ or the *Cx3cr1*$^{CreER(Jung)}$ *Alk5*$^{fl/fl}$ mice. mean±SEM. P>0.05, Student's t-test for all genes.

Table 1. Double reporter labeling of *P2ry12^{CreER}(mut/WT) Ai9-R26-YFP* mice after TAM				
Cell Condition	Mouse 1	Mouse 2	Mouse 3	Average
% of total tdTomato$^+$	77.77778	88.09524	81.69014	82.52105
% of total YFP$^+$	45.29915	35.71429	43.66197	41.55847
% of YFP$^+$:tdTomato$^-$	22.22222	11.90476	18.30986	17.47895
% of YFP$^-$:tdTomato$^+$	54.70085	64.28571	56.33803	58.44153
% of YFP$^+$:tdTomato$^+$	23.07692	23.80952	25.35211	24.07952
*All percentages are calculated by dividing the number of each category of cells by the total reporter positive cells from each animal.				

Supplemental Table 1. Quantification of the FACS analysis of the reporter$^+$ microglia in whole brain single cell suspension prepared from the *P2ry12$^{CreER(mut/WT)}$ Ai9-R26-YFP* mice after TAM treatment.