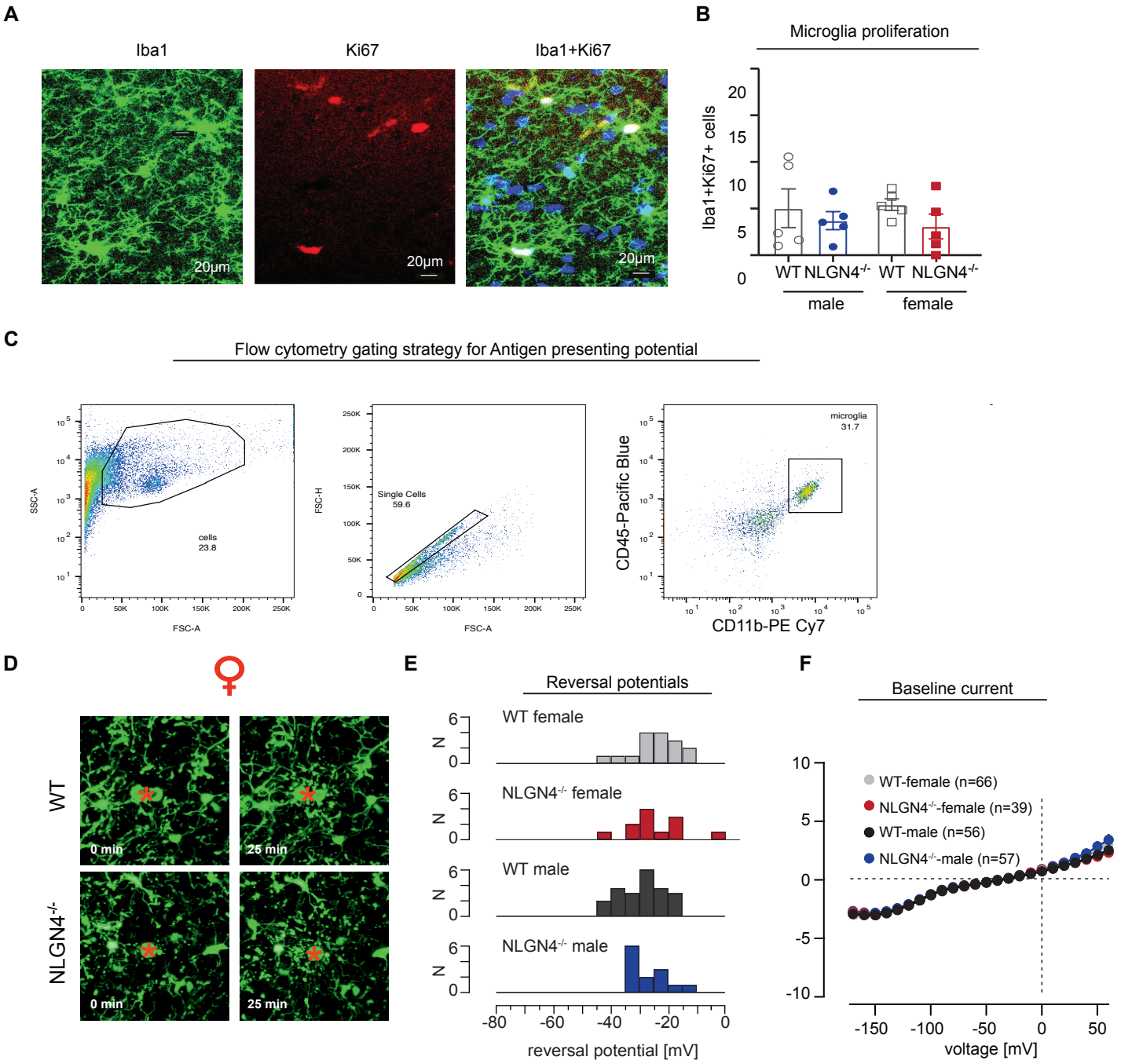

Supplementary Figure 1. Proliferation of microglia cells and FACS gating strategies.

(A) Iba1 (green) and Ki67 (red), as a proliferation marker, were stained in hippocampal slices (scale bar 20µm). **(B)** Quantification of Iba1/Ki67- positive cells revealed no difference between WT and NLGN4$^{-/-}$ male and female in CA3 of hippocampal brain slices. We performed 2 independent experiments. (2-way ANOVA following Bonferroni's multiple comparisons test male and female (WT) n=5 (NLGN4$^{-/-}$) n=5). **(C)** Gating strategy for MHCI, MHCII and CD54 expression. After the single cell gating, microglia cells were defined as CD45+CD11b+ population. Expression analysis was done by measuring mean fluorescence intensity of CD45+CD11b+. **(D)** Representative pictures of laser-induced process movement of EGFP-positive microglia in the hippocampus of acute brain slices from 13-week-old female WT and NLGN4$^{-/-}$ mice. Asterisk indicates laser lesion location. **(E)** Distribution of the reversal potentials (indicative of the membrane potentials) of microglia from 13-week-old female and male WT or NLGN4$^{-/-}$ animals (number of cells: female WT=66, female NLGN4$^{-/-}$ =39, male WT=56, male NLGN4$^{-/-}$=57). We performed 3 independent experiments. **(F)** Average current density-voltage relationships of microglia from 13-week-old female and male WT or NLGN4$^{-/-}$ animals obtained from voltage pulses 3 revealed no significant changes (2-way ANOVA followed by Bonferroni's multiple comparisons, male WT vs. NLGN4$^{-/-}$: p>0.9999, female WT vs. NLGN4$^{-/-}$: p>0.9999; number of cells: female WT=66, female NLGN4$^{-/-}$ =39, male WT=56, male NLGN4$^{-/-}$ =57). We performed 3 independent experiments. All experiments were performed using mice on a C57Bl6/J background.

Supplementary Discussion on the analysis of electrophysiological recordings of microglia:

Here, we explain the experimental design and the determination of the required sample size for 10 µM ATP application in order to rationalize our statistical approach.

1. **Estimating the effect size:**

ATP-evoked (10 µM) specific outward conductance between +20mV to +60 mV (Gout) in hippocampal microglia (WT) were expected to be in the range of 30 pS/pF with a (rather high) SD of 10 pS/pF. This value was estimated from previous experiments and studies. For NLGN4$^{-/-}$ microglia, we want to test for effects that minimally reduce our readout by 50%, thus, the estimated Gout for NLGN4$^{-/-}$ is 15 pS/pF (SD still at 10 pS/pF). The estimated parameters above correspond to an effect size of approx. 0.7, as determined by Gpower software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower).

2. **Estimating the sample size:**

If we use an ANOVA test and want to use an alpha of 0.05 and a beta of 0.95, we need a total sample size of 36 in all groups. We have three independent groups (WT, NLGN4$^{-/-}$, NLGN4$^{-/-}$-E2), and we thus need at least 12 independent samples per group. (As a comparison: for SD values of 15 pS/pF, we get an effect size of only ~0.5 a sample size increases to 21/group).

3. **What is an independent sample in electrophysiology?**

During revision of the current paper, a discussion emerged whether membrane currents recorded from different microglial cells in the same mouse are dependent or independent. Does the sample size of 12 (see previous point) corresponds to 12 patch-clamped microglial cells or to (pooled recordings of microglia from) 12 different animals. Classically, this question would be clearly answered by electrophysiologists from all over the world by: Membrane currents from individual cells are individual samples. However, in order to consider potential inter-individual or technical (slice and buffer preparation) variances, these cells should be distributed among a reasonable number of different animals/preparations. In compliance to the 3-R principles, the number of animals is typically between 3 and 5 mice (depending on the outcome and success rate of the person performing patch clamp experiments).

Do we inflate the sample size when we measure microglia from the same mouse?

We therefore tested for the dependency of membrane recordings from different microglia from specific individuals. The findings are presented in a data set below that was obtained from patch clamp recordings on male WT microglia (13-week-old mice; Fig. 6C) in the current study. The following table summarizes the ATP-evoked (10 µM) specific outward conductance's between +20mV to +60 mV (Gout) in hippocampal microglia from male WT mice (in pS/pF):

animal 1	animal 2	animal 3	animal 4	animal 5
16.58	7.72	34.65	21.45	18.29
24.17	20.30	22.39	16.83	11.80
21.30	17.78	75.82	15.36	40.15
			21.36	60.62
			14.81	50.05
			33.45	

We thus must deal with a nominal variable (mouse number) and a metric variable (Gout). Correlation of a nominal and a metric variables cannot be statistically tested. However, if microglia membrane currents derived from microglia from the same individual were dependent, one would expect that the per-mouse-standard deviation (SD) is always smaller than the SD of all recordings together (the total SD of all 20 samples from 5 mice is 17.4). This is not the case for the reported data (SD):

animal 1	animal 2	animal 3	animal 4	animal 5
3.8	6.7	28.0	7.0	20.7

We therefore argue that microglia patch clamp recordings can and should be accounted as independent statistical samples, regardless of their origin from the same or from different individuals at the same genetic background, age and sex. This also means that a one-way ANOVA using pooled microglia from all mice is sufficient to test for differences between mouse groups.