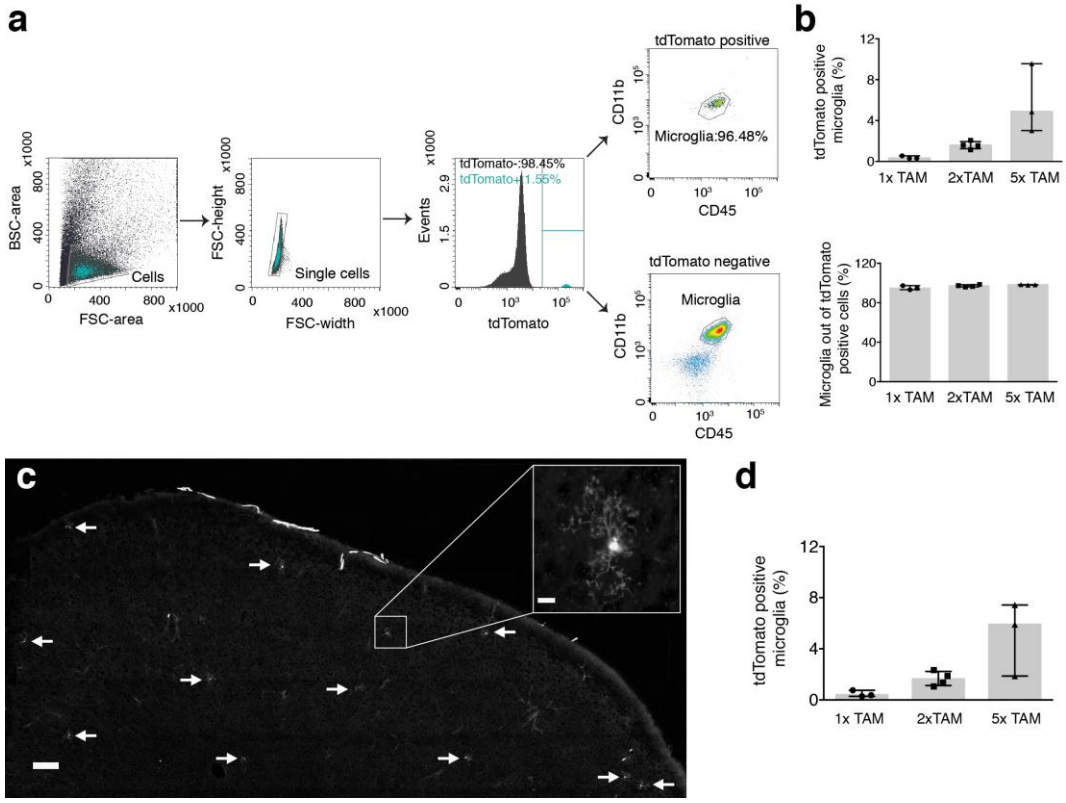
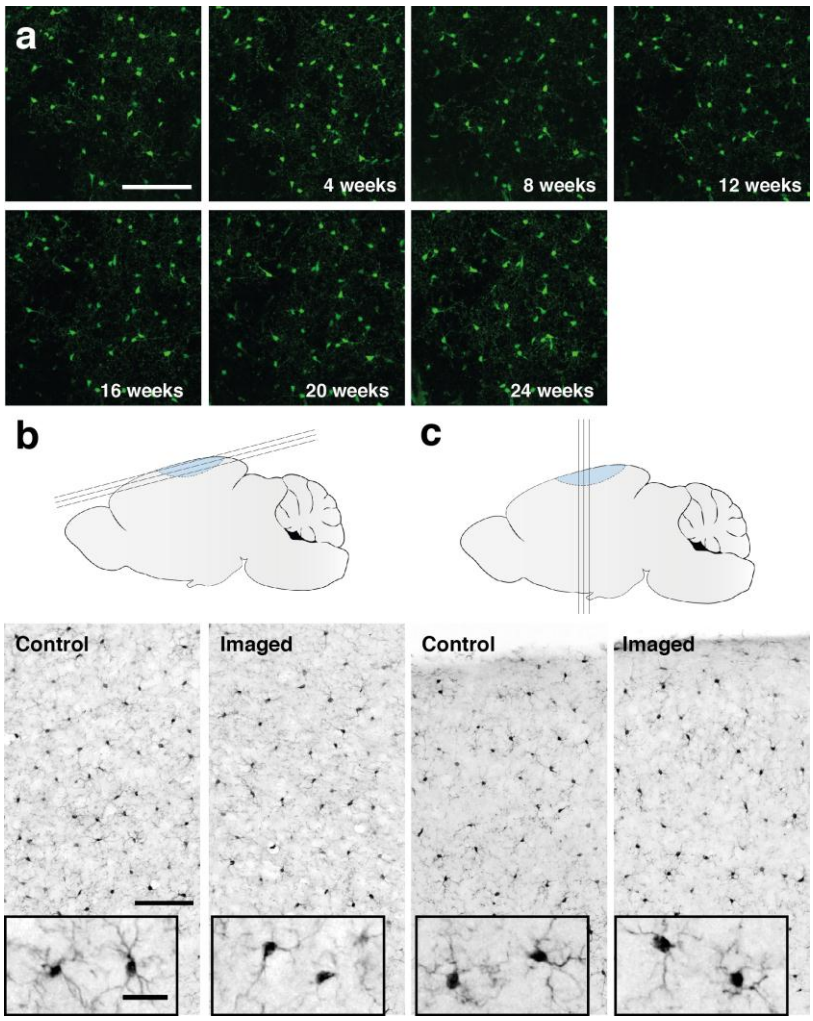


Supplementary Figure 1

Genetic labeling of microglia

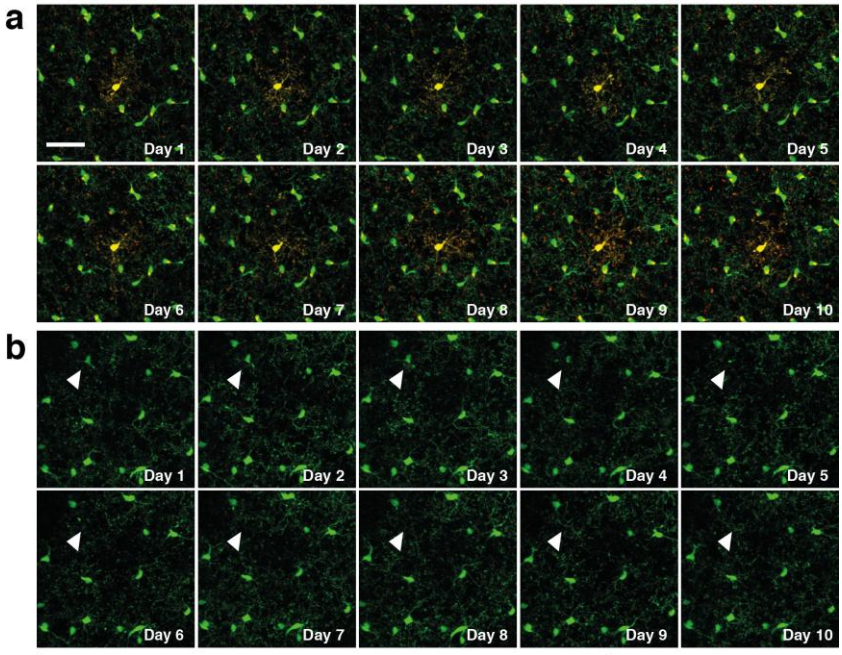
Male and female 2-3 month-old CreERT2;R26-tdTomato mice or CreERT2;R26-tdTomato;Iba1-eGFP transgenic mice were treated with 1x, 2x (48 h apart), or 5x (each 24 h apart) Tamoxifen (TAM; 100 µg per gram bodyweight) and analyzed 4 weeks later. One hemisphere was used for flow cytometry (only the neocortex was used, see methods for details), the second hemisphere was used for histology. (**a**) Exemplary gating strategy of an animal injected with 2xTAM. Cells were gated based on forward (FSC) and backward scatter (BSC). FSC-width and height was used to determine single cells. 1.55% of single cells were tdTomato positive, of which the vast majority (approximately 97%) were microglia, identified as the $CD11b^{high}$ and $CD45^{low}$ cell population by flow cytometry. tdTomato-positive microglia were not distinguishable from tdTomato-negative microglia in size and granularity, as indicated by their similar position in the FSC/BSC blot (tdTomato-positive cells colour-coded in green) and were distributed across the microglial population based on CD11b and CD45 intensity. (**b**) Flow cytometric analysis of the neocortex revealed a dose-dependent increase in the induction of tdTomato-positive cells, of which the vast majority were microglia (n=3, 4, 3 mice per group, for 1x, 2x, and 5x TAM, respectively). (**c**) Histological analysis of tdTomato-positive microglia (arrows) revealed an apparent random distribution throughout the neocortex (shown in the frontal cortex). Morphologically, tdTomato-labeled microglial cells revealed all aspects of mature microglia (insert). Scale bars 100 µm and 10 µm for the insert. Some perivascular macrophages also showed recombination, but were clearly distinguishable from microglia due to their elongated shape and lack of processes. (**d**) The total number of tdTomato-positive cells per neocortex was estimated and related to the total number of Iba-1-positive microglia in the neocortex (the latter was assessed in **Supplementary Figure 4** and the mean number of 880,000 was used for the calculation). In accordance with the flow cytometry analysis, a dose-dependent and comparable increase was found (n=3, 4, 3 mice per group, for 1x, 2x, and 5x TAM, respectively).



Supplementary Figure 2

Long-term repetitive *in vivo* imaging does not cause microglial activation

(**a**) There was no noticeable activation of microglial cells in any of the *in vivo* imaged areas. Shown are in vivo images (z-projections of imaged volumes) of a triple-transgenic CD11b-CreERT2;R26-tdTomato;Iba1-eGFP mouse after an imaging period of 24 weeks (6 months). Scale bar is 100 µm. (**b, c**) Consistently, postmortem immunohistochemical analysis of the triple-transgenic CD11b-CreERT2;R26-tdTomato;Iba1-eGFP animals after the 24 week-imaging period using an Iba1-antibody did not reveal any signs of microglial activation, regardless of whether the cortical tissue was cut horizontally (b) or coronally (c), when compared to a non-imaged control animal of the same age. Blue area indicates the location of the cranial window. Three imaged and three control mice were analysed. Scale bars are 100 µm and 20 µm for the insert.



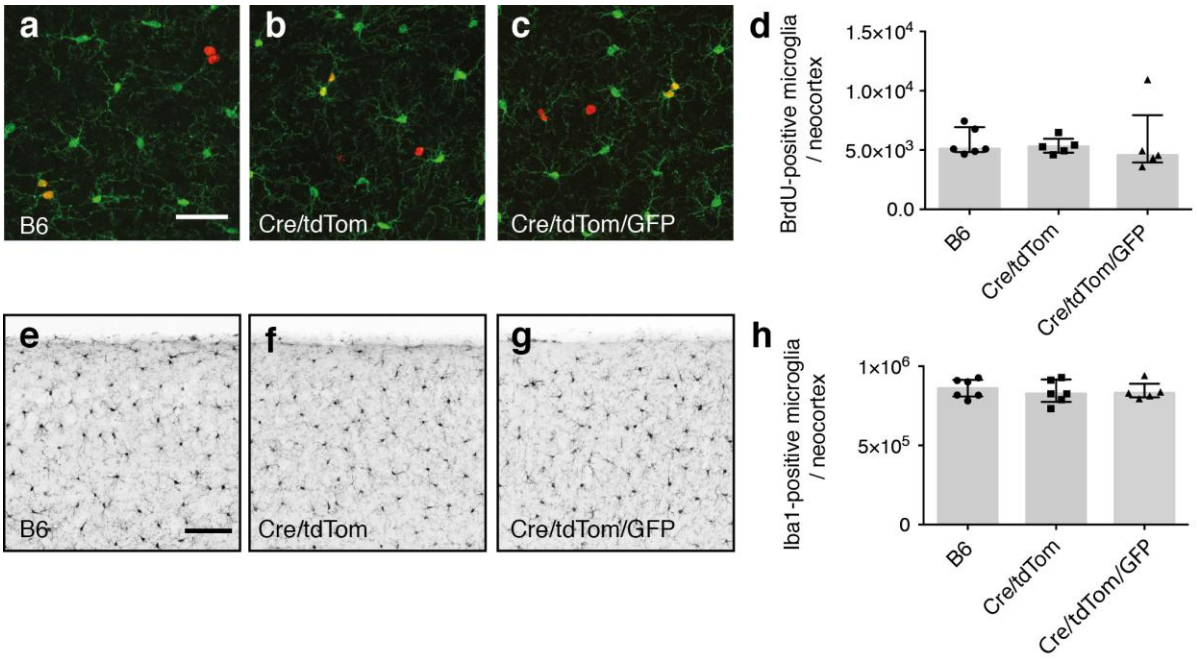
c

	Tracked microglia /mouse	New	Lost
tdTomato microglia	5.8 ± 1.0 (100%)	0	0
Iba1-eGFP microglia	136 ± 20 (100%)	2.33 ± 1.20 (1.72%)	1.67 ± 0.67 (1.23%)

Supplementary Figure 3

Daily imaging of neocortical microglial cells rules out rapid cell turnover

To study potential rapid replacement of microglial cells that would have been unrecognized in our long-term biweekly imaging protocol (see Fig. 1 and 2), three CD11b-CreERT2;R26-tdTomato;Iba1-eGFP mice (13 months of age; one male, two females) and two CD11b-CreERT2;R26-tdTomato mice (13 months of age; two males) were imaged daily for 10 consecutive days. (**a, b**) Shown are z-projections of imaged volumes of CD11b-CreERT2;R26-tdTomato;Iba1-eGFP mice where recombined microglia appeared yellow while surrounding microglia were green. An example of a recombined yellow microglial cell with surrounding green cells that are all stable over the imaging period of ten days is shown in (a). An example with a lost green microglial cell on day 5 is shown in (b). Scale bar is 40 µm. Note that in contrast to the long-term biweekly imaging (Fig. 2), the daily imaging also allowed us to track the Iba1-eGFP positive cells (green) since movement from day-to-day was minimal. (**c**) Average number of tdTomato-positive and Iba1-eGFP-positive cells tracked per mouse, the number of newly appearing cells (New) and the number of lost cells (Lost) during the imaging period. Given is the mean ± SEM of n=5 mice. The analysis and tracking was done as described for the long-term analysis in Figure 3. Occasionally, additional structures, which were connected to microglial processes, were observed. In two cases, these resembled a cell body in shape and size, but were identified as being formed by distinct microglial processes reminiscent of recently described extruded exophers albeit in *C. elegans* (Melentijevic, I. *et al.* C. elegans neurons jettison protein aggregates and mitochondria under neurotoxic stress. *Nature* **542**, 367–371, 2017), whereas in three other instances, their composition was ambiguous. Though rapid splitting and consecutive death of the daughter cell in these cases cannot be ruled out, these observations were excluded from the analysis. Moreover, they do not change the lifetime estimate of the microglia.



Supplementary Figure 4

No difference was observed in total microglial numbers or the number of BrdU-labeled microglia in the different mouse lines used in this study

To exclude differences in microglial number or microglial proliferation based on BrdU incorporation, 7-month-old C57BL/6J mice, CD11b-CreERT2;R26-tdTomato;Iba1-eGFP (Cre/tdTom/GFP) mice and CD11b-CreERT2;R26-tdTomato (Cre/tdTom) mice of mixed gender were selected (n=5-6 per group). The selected age corresponds to the mid-time of the biweekly imaging period (4 months to 10 months) that was used for quantification in Figure 3. Mice were intraperitoneally injected on five consecutive days with BrdU and then analysed three days later. (**a-c**) Immunofluorescence double staining for Iba1 (green) and BrdU (red). Scale bar is 50 µm. (**d**) Quantification of double-labeled (yellow) cells (Iba1/BrdU) in the neocortex revealed no differences in BrdU-labeled microglia among the three groups (Kruskal-Wallis, $\chi^2_{(2)} = 2.19$, $p>0.05$); dataset was checked for outliers using Graphpad Prism's ROUT method and an outlier value was excluded from the Cre/tdTom dataset. (**e-g**) Immunohistochemical staining of the neocortex using an Iba1 antibody for the three genotypes. Scale bar is 100 µm. (**h**) Stereological analysis of total neocortical microglia revealed no difference in microglial number between C57BL/6J control mice and the double- and triple-transgenic animals used in this study (Kruskal-Wallis, $\chi^2_{(2)} = 0.10$, $p>0.05$).

Supplementary Table 1 | Numbers of tracked microglia in individual mice

	Gender	Age	Positions	Tracked microglia
Young				
Mouse 1	m	4	4	4
Mouse 2	m	4	2	4
Mouse 3	f	3.6	3	4
Mouse 4	m	3.6	2	3
Mouse 5	f	4	4	6
Mouse 6	f	5.1	5	7
Mouse 7	m	3.6	4	5
Mouse 8	m	3.5	4	5
Mouse 9	f	3.2	3	7
Mouse 10	f	3.5	3	6
Mouse 11	m	3.7	2	3
Mouse 12	f	3.7	4	4
Mouse 13	m	4	2	4
Mouse 14	m	3.5	4	4
Mouse 15	f	3.5	5	7
Mouse 16	f	3.5	4	5
Mouse 17	m	4.1	7	9
Mouse 18	m	4.1	5	10
Mouse 19	f	3.6	1	1
Mouse 20	m	3.6	2	2
Mouse 21	f	4.8	2	4
Mouse 22	m	4.6	1	1
Mouse 23	f	3.7	3	5
Mouse 24	f	3.7	3	3
Mouse 25	f	4.8	4	5
Mouse 26	f	4.1	4	5
Mouse 27	m	4.1	5	9
Total number of tracked microglia				132
Adult				
Mouse 1	f	9.8	5	10
Mouse 2	m	9.8	3	4
Mouse 3	f	9.6	4	10
Mouse 4	f	9.7	6	14
Mouse 5	m	9.6	5	7
Mouse 6	f	9.7	3	8
Total number of tracked microglia				53

Young (3.2 – 5.1 month old) and adult (9.6 - 9.8 month old) mice were imaged. The number of imaging positions per mouse is indicated and ranged from 1-7. Within these imaging positions 1 – 14 tdTomato-positive microglia could be tracked.

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Supplementary Table 2 | Numbers of tracked microglia in individual APPPS1 mice

	Gender	Age	Positions	Tracked microglia	
				Non-plaque associated	Plaque associated
Mouse 1	f	4.5	1	2	1
Mouse 2	m	4.5	3	5	2
Mouse 3	f	3.6	1	1	0
Mouse 4	m	3.5	3	8	4
Mouse 5	m	3.5	5	10	1
Mouse 6	f	3.6	1	1	0
Mouse 7	f	3.7	5	7	1
Mouse 8	f	3.7	3	4	0
Mouse 9	m	4.8	4	7	0
Total number of tracked microglia				45	9

Young (3.5 – 4.8 month-old) triple transgenic CD11b-CreERT2/R26-tdTomato/APPPS1 mice were imaged. The number of imaging positions, that allowed tracking of microglia throughout the entire imaging period per mouse is indicated and ranged from 1-5. Within these imaging positions 1 – 10 tdTomato-positive microglia could be tracked. The number of microglia attached and not attached to Aβ amyloid plaques that were tracked are indiacted per mouse. For details and summary of the findings see Figure 4.

Nature Neuroscience: doi:10.1038/nn.4631