**Supplemental Material**

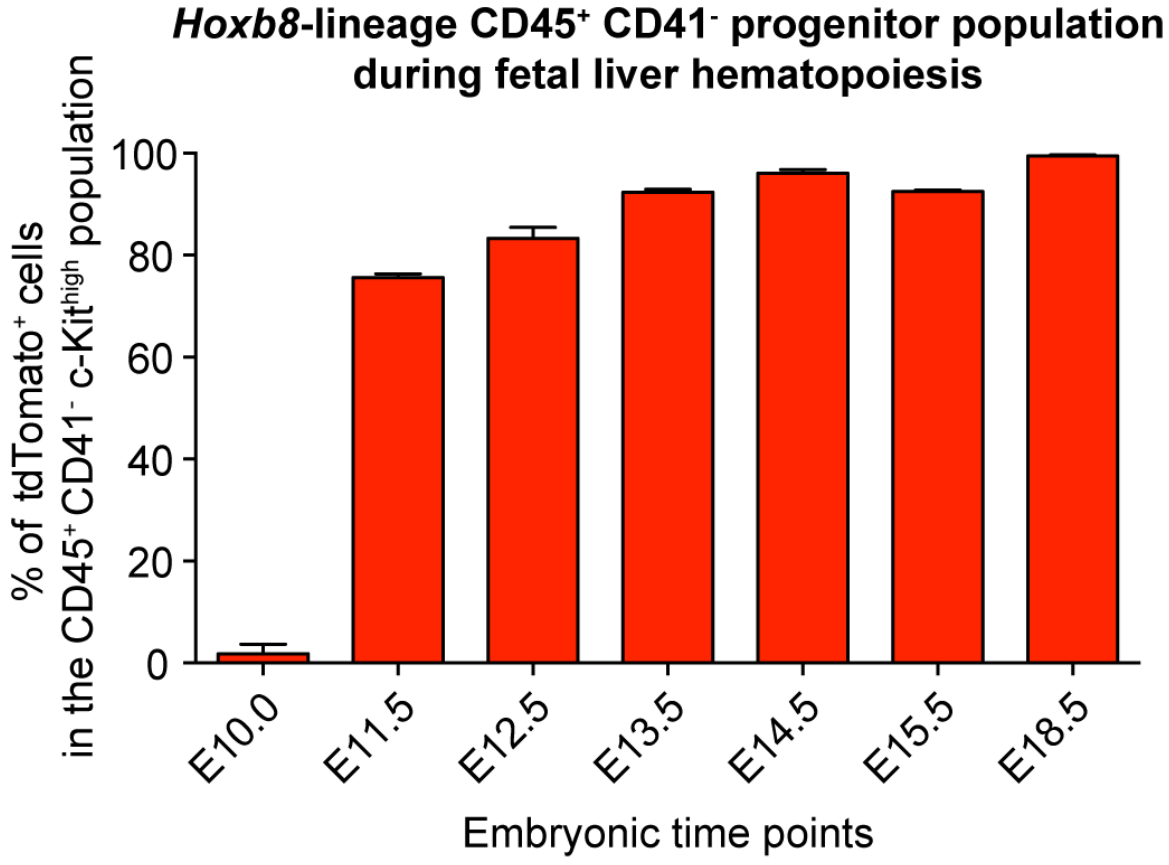


**Figure S1. *Hoxb8*-lineage progenitors in fetal liver hematopoiesis.** Related to Figure 3. Graph showing percentage of $CD45^+$ $CD41^-$ c-$Kit^{high}$ $tdTomato^+$ cells isolated from fetal liver through embryonic development. n=3 biological replicates per time point, bars represent the mean ± SEM.

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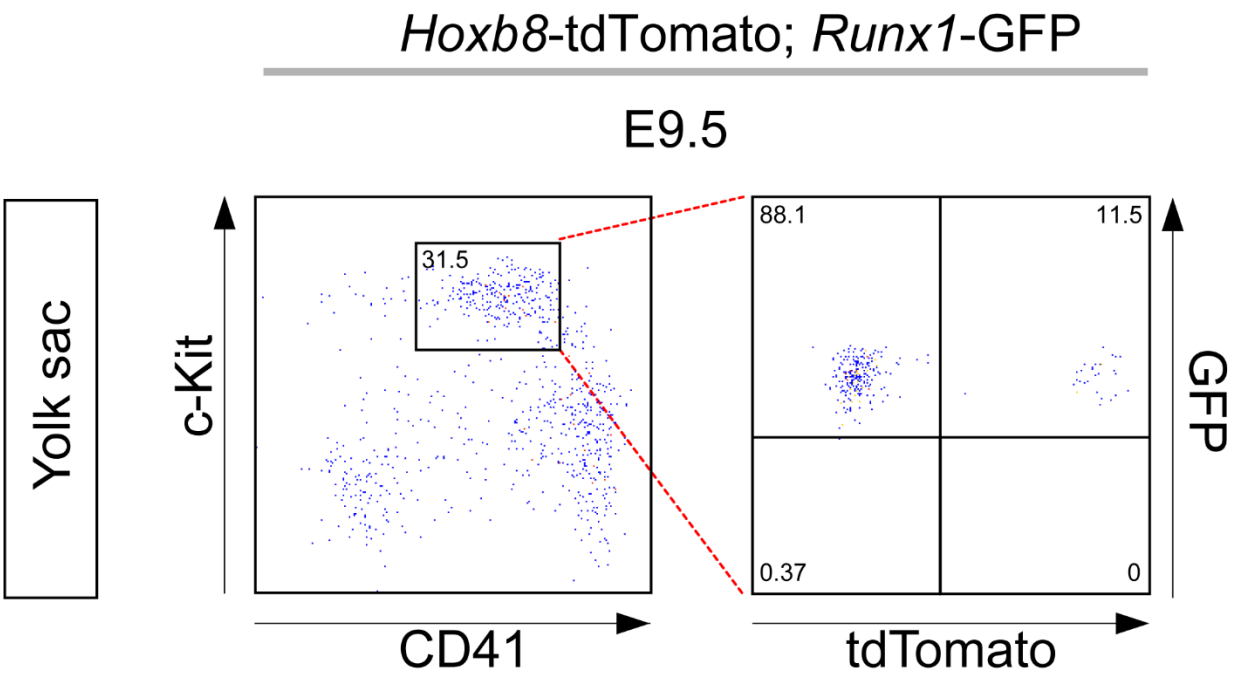


**Figure S2. *Hoxb8*-lineage progenitors comprises of ~9% of the *Runx1* cell population in the yolk sac.** Related to Figure 3. FACS profile of CD41$^{+}$ c-Kit$^{high}$ cells gated for GFP and tdTomato from the yolk sac of E9.5 *Runx1*$^{GFP/+}$; *Hoxb8*$^{IRES\text{-}Cre/+}$; *ROSA26*$^{CAG\text{-}LSL\text{-}tdTomato/+}$ embryos. n=3 pooled biological replicates.

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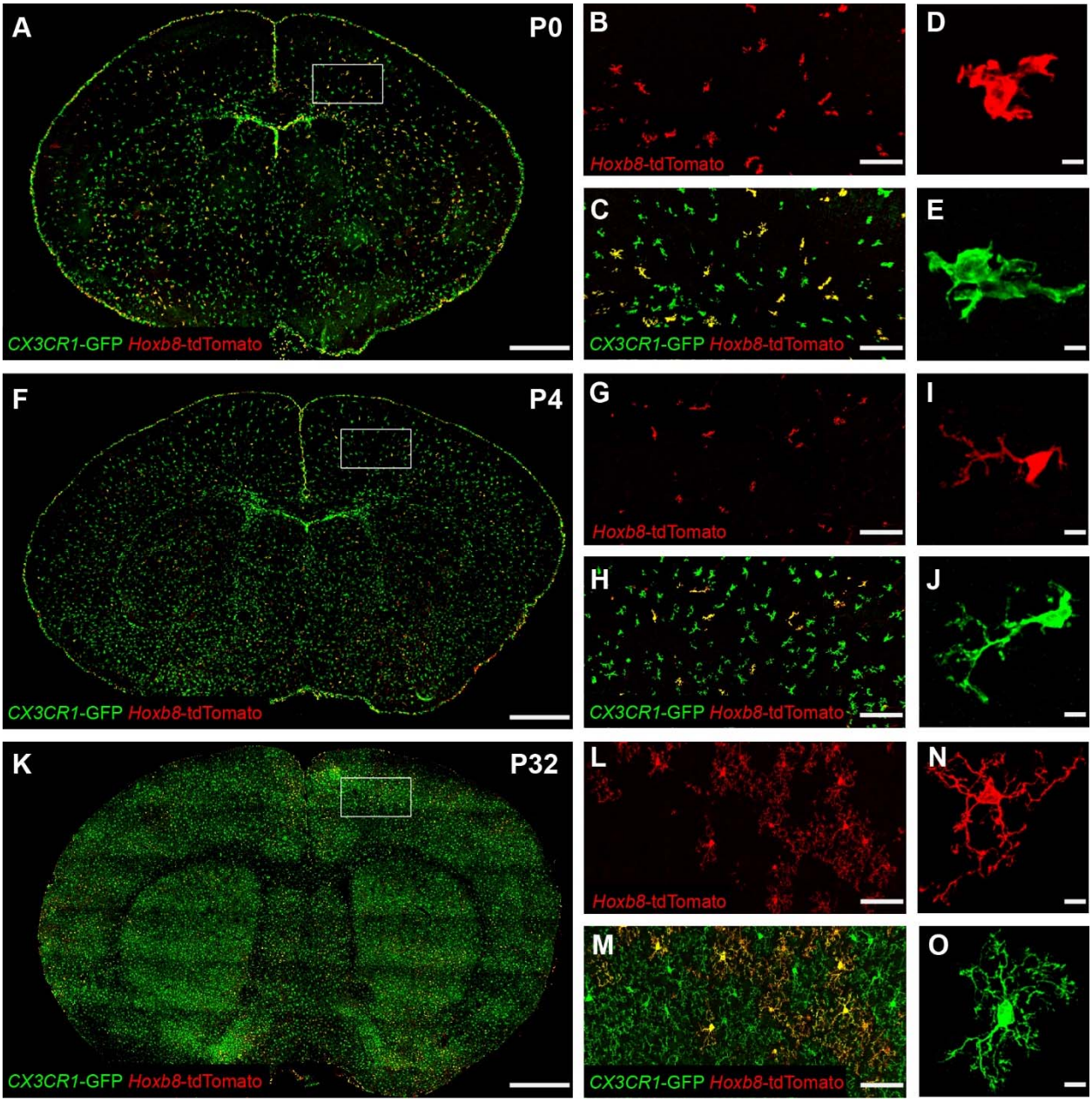


**Figure S3. Postnatal development of *Hoxb8*- and non-*Hoxb8*-microglia.** Related to Figure 2. Representative coronal sections from P0 (A, scale bar: 200 µm), P4 (F, scale bar: 300 µm) and P32 (K, scale bar: 500 µm) brains showing the increasing density (B-C, G-H, L-M, scale bars: 50 µm) and complexity of ramified processes (D-E, I-J, N-O, scale bars: 20 µm) at the respective time-points.

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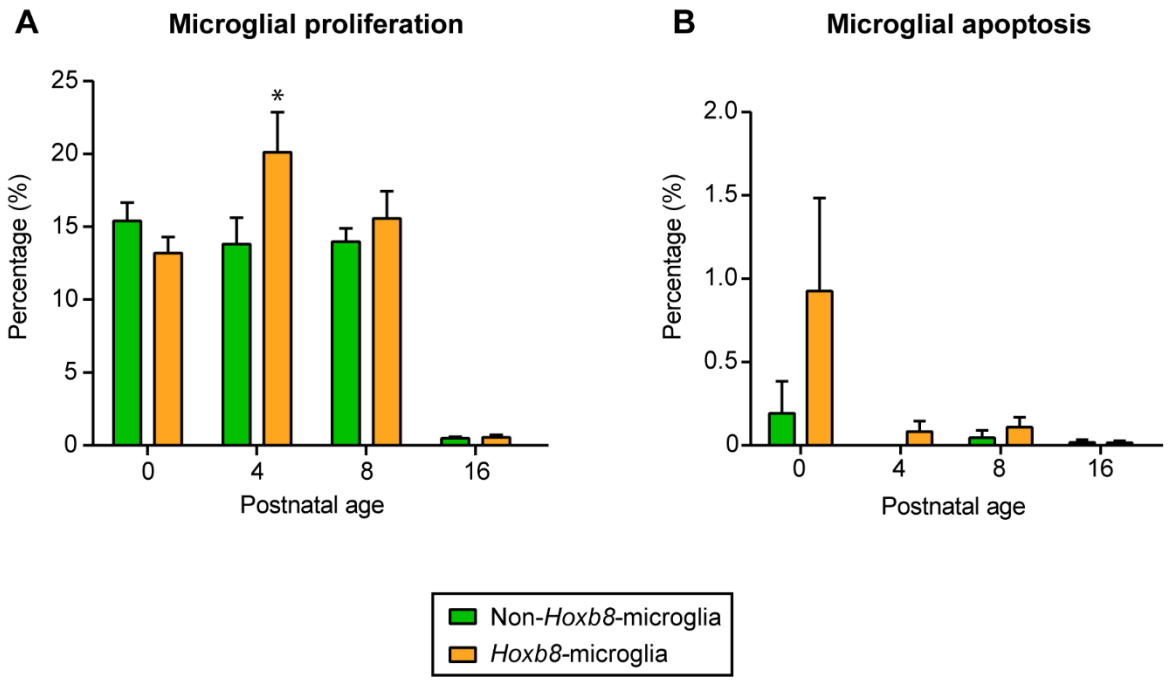


**Figure S4. Developmental dynamics of *Hoxb8*- and non-*Hoxb8*-microglia.** Related to Figure 2. (A,B) Comparison of proliferation (A) and apoptosis (B) between the two microglia populations during early postnatal development. The bars represent proliferating/apoptotic microglia as a percentage of their own specific population. n=3, * $p<0.05$, bars represent the mean ± SEM.

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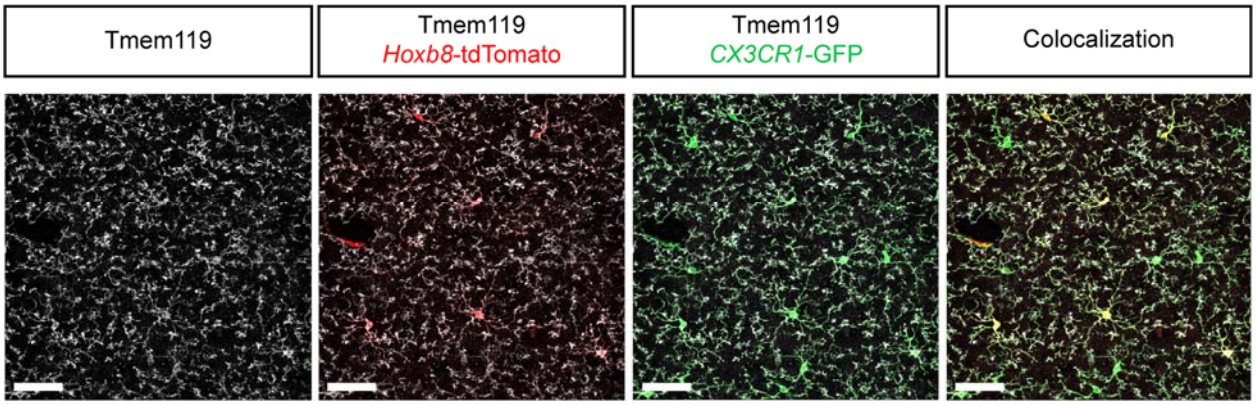


**Figure S5. Tmem119 expression.** Related to Table S2. Both *Hoxb8*- and non-*Hoxb8*-microglia show expression of the transmembrane protein 119 (Tmem119) on their ramified processes. Scale bars: 40 µm.

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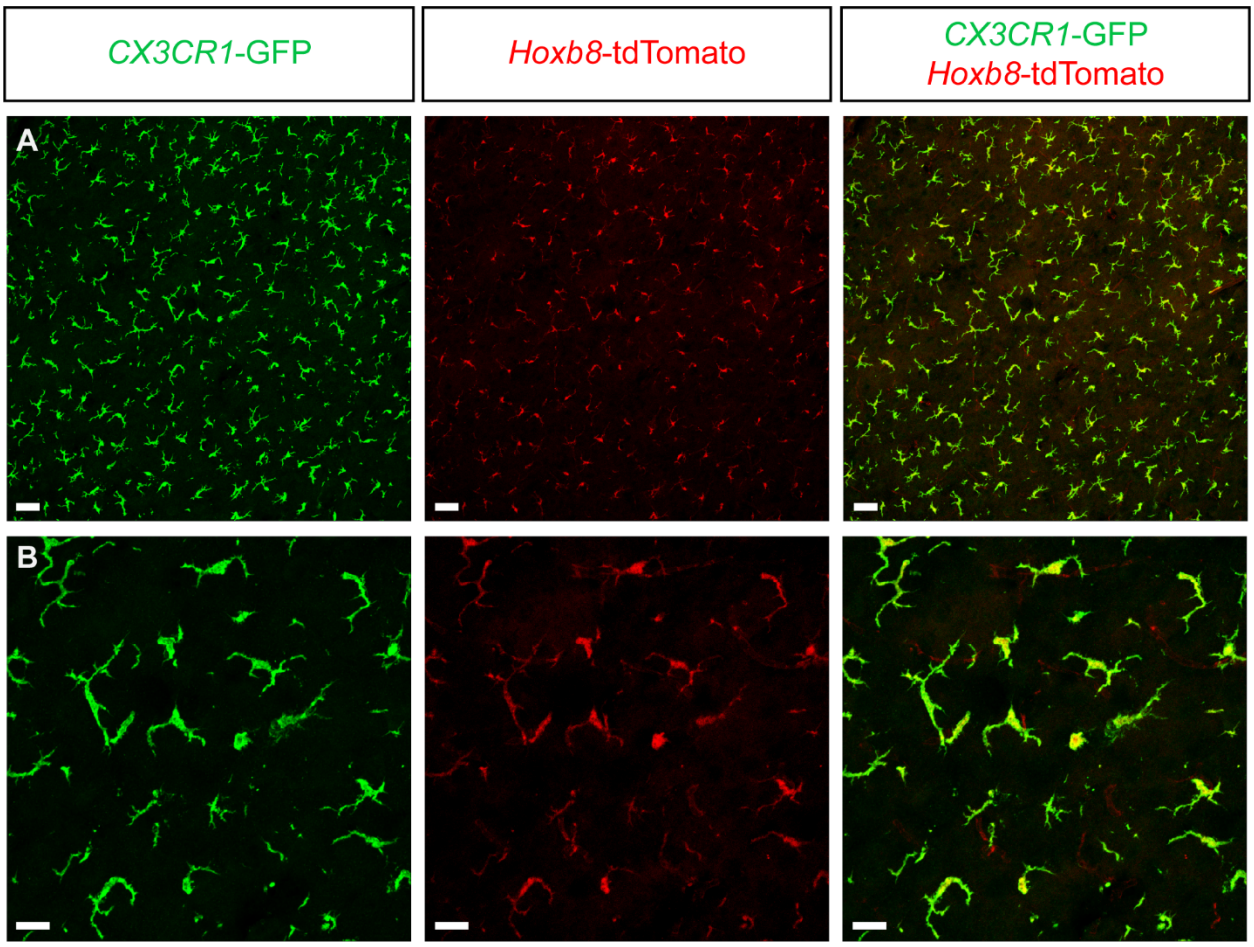


**Figure S6. E12.5 *Hoxb8*-lineage fetal liver hematopoietic progenitors differentiate and repopulate the brain parenchyma in *Csf1r* null mice.** Related to Figure 6. Note that although thousands of microglial cells have engrafted by P17, these cells have not fully matured. Scale bars: (A) 50 μm, (B) 20 μm.

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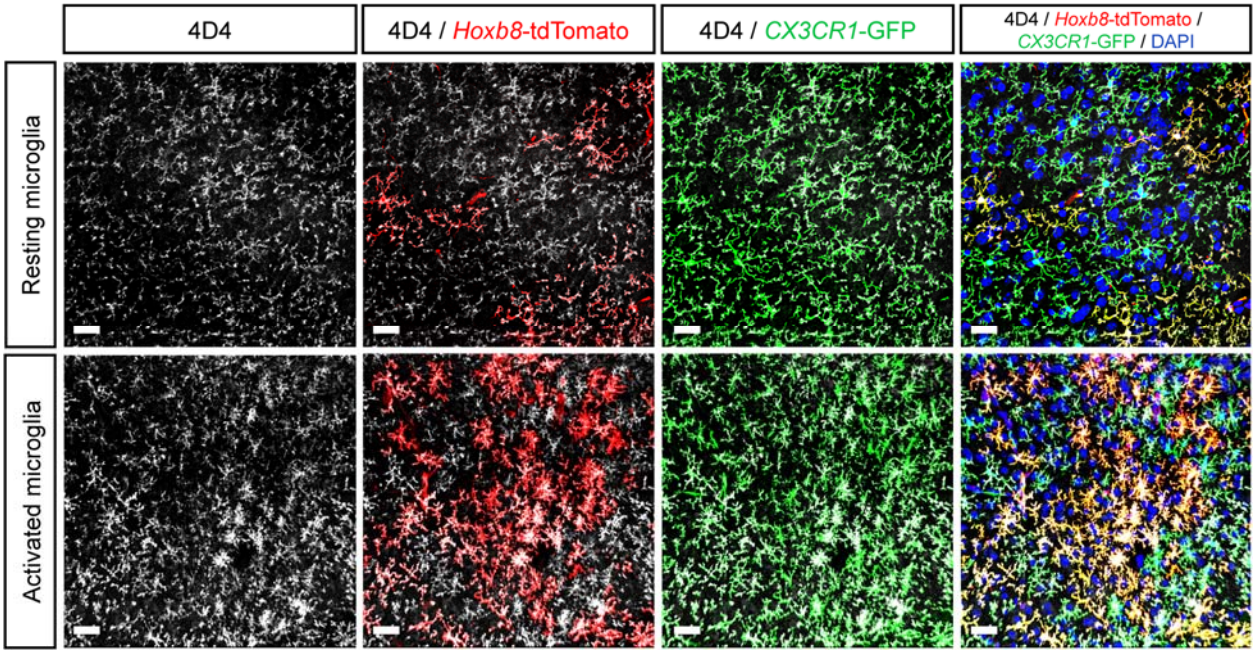


**Figure S7. Resident brain microglia are predominantly present at the site of injury.** Related to Figure 7. The 4D4 antibody allows the distinction of resident brain microglia from peripheral macrophages. Majority of the $GFP^+$ and $GFP^+$ $tdTomato^+$ cells around the site of injury are expressing 4D4 showing that they are resident brain microglia. Scale bars: 30 µm.

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**Table S1. Differentially expressed genes in *Hoxb8*-microglia compared to non-*Hoxb8*-microglia.** Related to Figure 5. RNASTAR analysis revealed 21 genes that were differentially expressed in *Hoxb8*-microglia.

| Ensembl # | Fold Difference | p value | Common Gene Name |
|---|---|---|---|
| ENSMUSG00000027009 | 4.580620809 | <0.0001 | Itga4 |
| ENSMUSG00000030214 | 4.246659468 | <0.0001 | Plbd1 |
| ENSMUSG00000034641 | 3.995970369 | <0.0001 | Cd300ld |
| ENSMUSG00000047735 | 2.772480567 | 0.00122 | Samd9l |
| ENSMUSG00000049037 | 2.954023796 | 0.00145 | Clec4a1 |
| ENSMUSG00000070354 | -2.505748451 | 0.00396 | Gm21975 |
| ENSMUSG00000049103 | 2.306712444 | 0.00754 | Ccr2 |
| ENSMUSG00000073421 | 2.712053997 | 0.00754 | H2-Ab1 |
| ENSMUSG00000026715 | 2.607464549 | 0.00868 | Serpinc1 |
| ENSMUSG00000026832 | 2.744409633 | 0.00868 | Cytip |
| ENSMUSG00000021886 | 2.555949808 | 0.01377 | Gpr65 |
| ENSMUSG00000040711 | 2.607410017 | 0.01377 | Sh3pxd2b |
| ENSMUSG00000104052 | -2.622239415 | 0.01377 | Gm38125 |
| ENSMUSG00000105472 | -2.59562299 | 0.01586 | RP23-200E11.1 |
| ENSMUSG00000064138 | -1.62989463 | 0.01688 | Fam172a |
| ENSMUSG00000051906 | 2.230894994 | 0.04398 | Cd209f |
| ENSMUSG00000009687 | 2.382666625 | 0.05744 | Fxyd5 |
| ENSMUSG00000053063 | 2.122713235 | 0.07959 | Clec12a |
| ENSMUSG00000022550 | -2.284415883 | 0.09254 | Adck5 |
| ENSMUSG00000025150 | 2.291474247 | 0.09957 | Cbr2 |
| ENSMUSG00000037849 | 2.259747087 | 0.09957 | Gm4955 |

**Table S2. Gene expression profiles of *Hoxb8*- and non-*Hoxb8*-microglia.** Related to Figure 5.

Click here to Download Table S2

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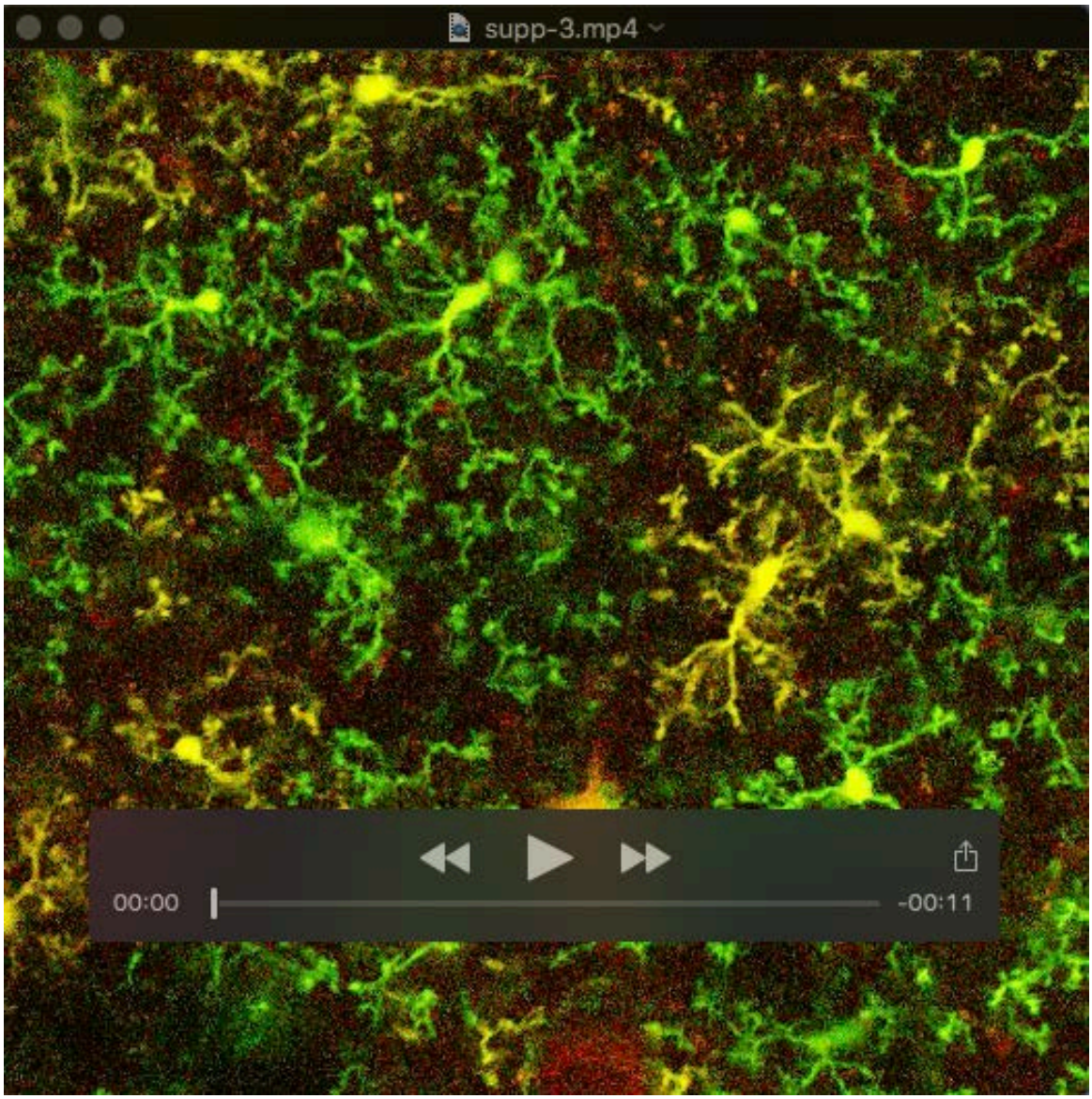


**Movie S1. Microglial activation response to focal laser ablation.** Related to Figure 7. Time-lapse recording of microglial response to damage induced by focused laser ablation in brain cortex, observed by *in vivo* multiphoton imaging. *Hoxb8*-microglia (yellow) and non-*Hoxb8*-microglia (green) exhibit very similar behavior in the event of an injury in their surrounding environment.

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