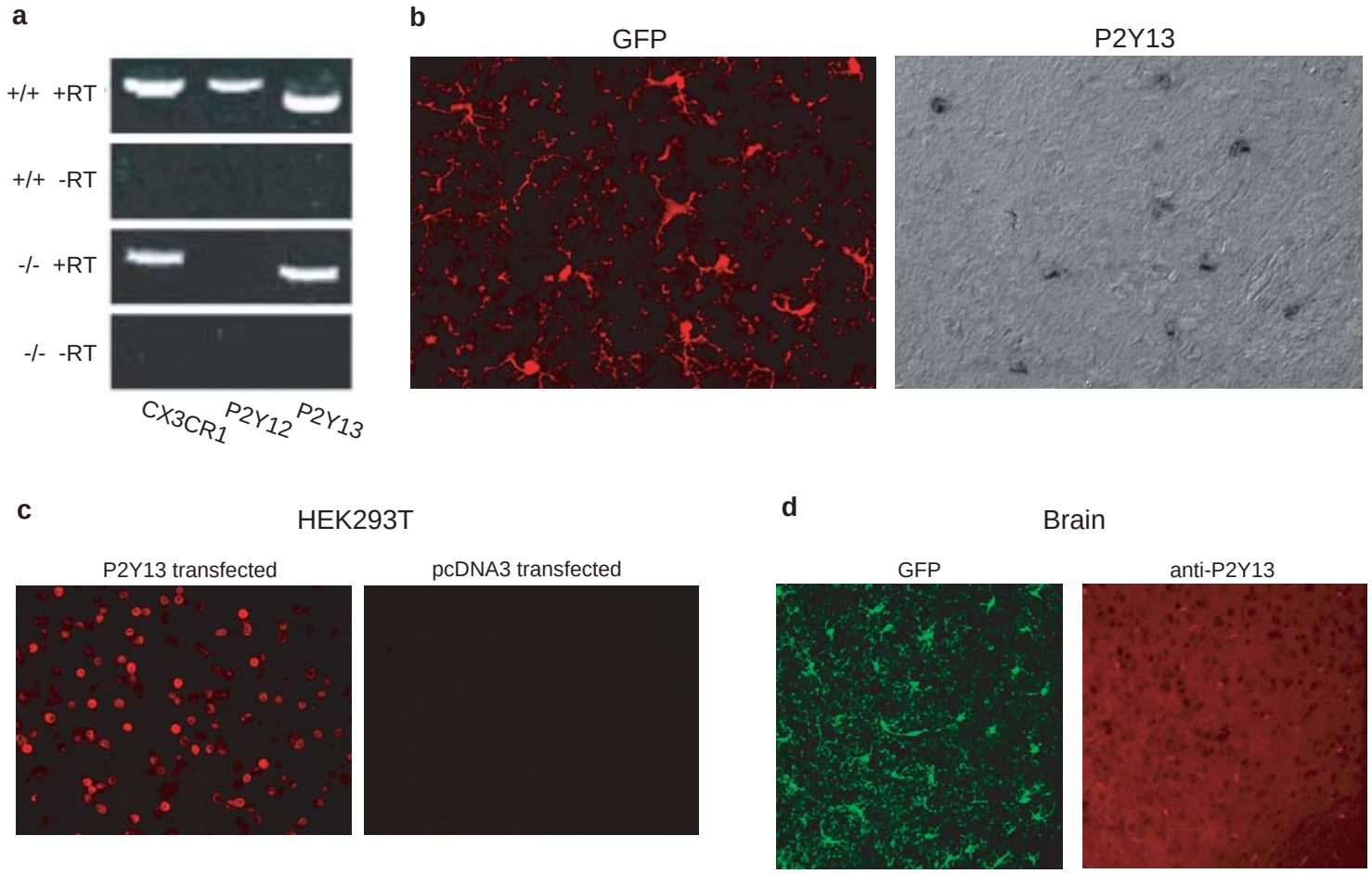

Supplementary Fig. 3. Microglia express P2Y13 transcripts, but not detectable receptor protein. (**a**) RT-PCR products were produced with primer pairs designed to specifically amplify P2Y12 or P2Y13 coding regions (or CX3CR1 as a positive control) using total RNA from microglia cultured from wild-type (+/+) or P2Y12-deficient mice. Amplification of RNA-derived reverse transcripts was confirmed by lack of signal in the absence of reverse transcriptase (RT). We also performed quantitative RT-PCR to ascertain whether P2Y13 mRNA expression is markedly altered in P2Y12-deficient mice, reflecting either a compensatory increase or a decrease due to the close proximity (~5 kilobases) of the P2Y13 gene to the targeted locus. We found a slight reduction (~2-fold) in P2Y13 signal amplified from brain tissue of P2Y12-deficient mice compared to wild-type littermates. (**b**) In situ hybridization of brain sections from P2ry12+/+Cx3cr1+/GFP mice using a P2Y13-specific anti-sense probe revealed weak, but detectable signals in GFP-positive microglia. (**c**) Transiently transfected HEK923T cells expressing mouse P2Y13 cDNA show immunoreactivity with anti-P2Y13 antibody, whereas vector (pcDNA3)-transfected controls do not. (**d**) Brain sections from P2ry12+/+Cx3cr1+/GFP mice lacked detectable immunoreactivity when stained with anti-P2Y13 antibody. GFP fluorescence shows location of microglia. Scale bars = 50 µm.

All procedures were performed as described in the Methods. The following primers pairs were used for PCR analysis:
P2Y12: 5'-CCTCAGCCAATACCACCTTCTCCCC-3' and 5' CGCTTGGTTCGCCACCTTCTTGTCCCTT-3'
P2Y13: 5'-GGGACACTCGGATGACACAGCTGC-3' and 5'-GCCAGAAAGAGAGTTGCTTCTTTAGCAATAAACAGC-3'
CX3CR1: 5'-TTCACGTTCGGTCTGGTGGG-3' and 5'-GGTTCCTAGTGGAGCTAGGG-3'

Anti-P2Y13 receptor polyclonal antibody was generated by immunizing rabbit with a synthetic peptide corresponding to the mouse P2Y13 C-terminus (NH2-Cys-Thr-Ala-Gly-Ser-Ser-Glu-Asp-His-His-Ser-Ser-Gln-Thr-Asp-Asn-Ile-Thr-Leu-Ala-OH; Anaspec, Inc). Antibody was affinity purified using a Sulfolink coupling gel (Pierce) to immobilize the antigenic peptide.