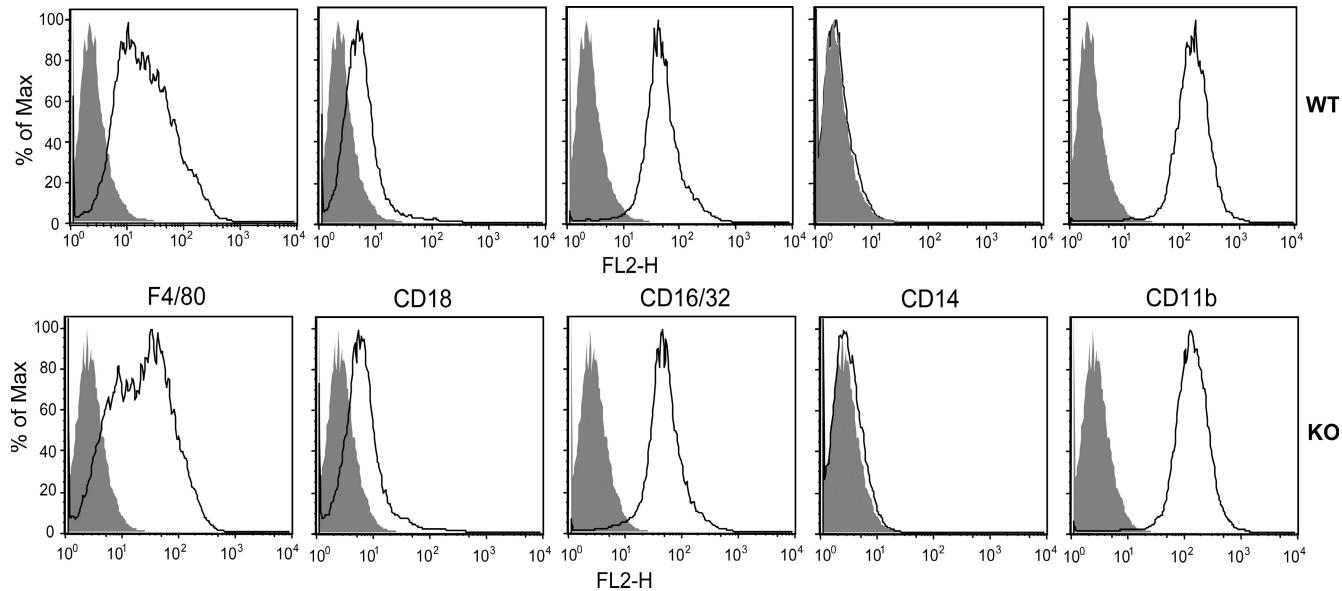
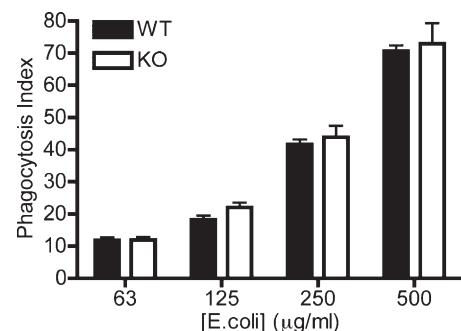


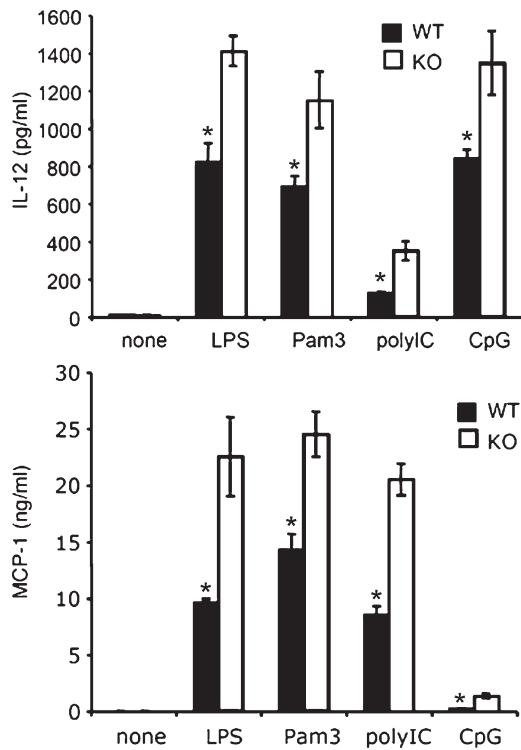
## SUPPLEMENTAL MATERIAL

Yin et al., <http://www.jem.org/cgi/content/full/jem.20091568/DC1>

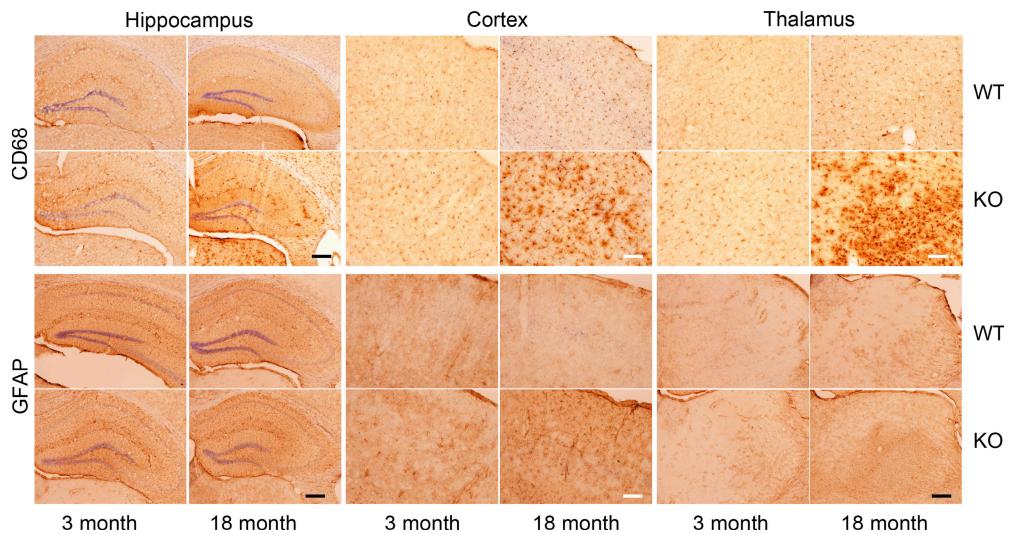
**Figure S1. Comparable surface marker expression of WT and PGRN-deficient BMDMs.** Unstimulated mature BMDMs from WT and PGRN-deficient (KO) mice were stained for F4/80, CD18, CD16/32, CD14, and CD11b, followed by flow cytometric analysis. Shaded histograms represent isotype controls; open histograms represent cell-surface expression.



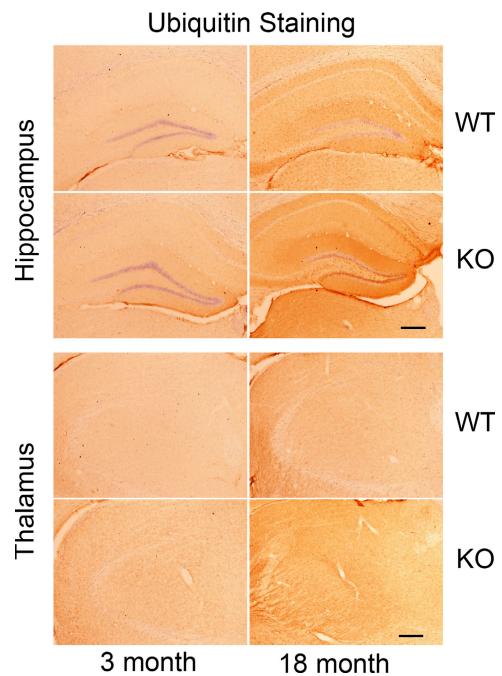
**Figure S2. Comparable phagocytic capacities between WT and PGRN-deficient macrophages.** BMDMs from 2-mo-old WT and PGRN-deficient (KO) mice were infected with the indicated concentrations of FITC-conjugated *E. coli*. Phagocytosis was measured after 30 min as the fluorescence intensity associated within cells but insensitive to Trypan blue and expressed as phagocytosis index. Results are means  $\pm$  SEM from one out of three similar experiments using independent pairs of age- and sex-matched mice.



**Figure S3. Enhanced production of IL-12 and MCP-1 of PGRN-deficient macrophages in response to TLR ligands in vitro.** Mature BMDMs from 2-mo-old WT and PGRN-deficient (KO) mice were stimulated with 100 ng/ml LPS, 0.1 µg/ml Pam3, 1 µg/ml poly I:C, or 1 µg/ml of bacterial DNA CpG, and the conditioned medium was collected 24 h later. IL-12 (top) and MCP-1 (bottom) contents in the medium were determined by ELISA. Results are means ± SEM for triplicates from one out of three similar experiments. \*, P < 0.05 using the Student's t test.



**Figure S4. Augmented age-dependent activation of microglia and astrocytes in the brains of PGRN-deficient mice.** Hippocampal, cortical, and thalamic sections from 3- or 18-mo-old WT and PGRN-deficient (KO) mice ( $n = 6$ ) were immunostained using antibody against CD68 or GFAP, as in Fig. 5. Bars: (black) 200 µm; (white) 80 µm.



**Figure S5. Enhanced age-dependent ubiquitination of the hippocampus and thalamus of PGRN-deficient mice.** Hippocampal and thalamic sections from 3- or 18-mo-old WT and PGRN-deficient (KO) mice ( $n = 6$ ) were immunostained using antibody against ubiquitin, as in Fig. 7. Bars, 200  $\mu$ m.

**Table S1.** Young PGRN-deficient mice show no morphological, hematologic, or biochemical abnormalities

| Criteria   | Mice  |       |        |        |        |        |        |       |       |       |       |       |
|------------|-------|-------|--------|--------|--------|--------|--------|-------|-------|-------|-------|-------|
|            | 1709  | 1710  | 1711   | 1712   | 1713   | 1714   | 1715   | 1716  | 1717  | 1718  | 1719  | 1720  |
| Number     |       |       |        |        |        |        |        |       |       |       |       |       |
| Genotype   | WT    | WT    | WT     | WT     | KO     | KO     | KO     | KO    | KO    | KO    | KO    | KO    |
| Gender     | Male  | Male  | Female | Female | Female | Female | Female | Male  | Male  | Male  | Male  | Male  |
| ALP        | 99    | 94    | 174    | 178    | 127    | 97     | 140    | 116   | 122   | 91    | 91    | 94    |
| ALT        | 18    | 31    | 48     | 407    | 64     | 79     | 32     | 45    | 132   | 29    | 27    | 89    |
| AST        | 74    | 123   | 172    | 1,498  | 848    | 335    | 205    | 196   | 188   | 113   | 100   | 284   |
| GGT        | 0     | 0     | 0      | 0      | 0      | 0      | 0      | 0     | 0     | 0     | 0     | 0     |
| Albumin    | 3     | 3.2   | 3.5    | 3.4    | 3.4    | 3.6    | 3.5    | 3.3   | 3.3   | 3.1   | 3.3   | 2.9   |
| Tot Prot   | 5.2   | 5.7   | 5.9    | 6.1    | 5.7    | 5.8    | 5.7    | 5.3   | 5.5   | 5.4   | 5.6   | 5     |
| Globulin   | 2.2   | 2.5   | 2.4    | 2.7    | 2.3    | 2.2    | 2.2    | 2     | 2.2   | 2.3   | 2.3   | 2.1   |
| Tot Bili   | 0.2   | 0.2   | 0.3    | 0.5    | 0.4    | 0.3    | 0.3    | 0.2   | 0.2   | 0.2   | 0.3   | 0.2   |
| BUN        | 16    | 23    | 28     | 32     | 24     | 24     | 26     | 30    | 34    | 23    | 23    | 27    |
| Creat      | 0.2   | 0.2   | 0.2    | 0.2    | 0.2    | 0.2    | 0.2    | 0.2   | 0.2   | 0.2   | 0.2   | 0.2   |
| Cholest    | 127   | 157   | 97     | 85     | 75     | 91     | 80     | 93    | 95    | 135   | 150   | 105   |
| Glucose    | 180   | 218   | 209    | 275    | 171    | 196    | 216    | 205   | 242   | 223   | 266   | 272   |
| Calcium    | 9.7   | 9.9   | 10     | 9.6    | 9.2    | 9.6    | 9.6    | 9.8   | 10.1  | 9.7   | 9.8   | 9.8   |
| Phos       | 10.2  | 9.1   | 10     | 14.5   | 10.3   | 10.3   | 10.2   | 9.9   | 11    | 9.3   | 10.4  | 10.8  |
| TCO2       | 34    | 22    | 17     | 9      | 18     | 18     | 19     | 21    | 26    | 27    | 20    | 24    |
| Chloride   | 108   | 108   | 114    | 111    | 111    | 110    | 108    | 110   | 109   | 111   | 108   | 111   |
| K          | 7.5   | 6.9   | 2.1    | 11.7   | 7.5    | 7.2    | 7.2    | 7     | 8.7   | 8.2   | 8     | 8.8   |
| Na         | 156   | 152   | 150    | 144    | 150    | 152    | 148    | 154   | 151   | 154   | 152   | 151   |
| A/G ratio  | 1.4   | 1.3   | 1.5    | 1.3    | 1.5    | 1.6    | 1.6    | 1.7   | 1.5   | 1.3   | 1.4   | 1.4   |
| B/C ratio  | 80    | 115   | 140    | 160    | 120    | 120    | 130    | 150   | 170   | 115   | 76.7  | 135   |
| Na/K ratio | 20.8  | 22    | 71.4   | 12.3   | 20     | 21.1   | 20.6   | 22    | 17.4  | 18.8  | 19    | 17.2  |
| Osmol      | 319.8 | 315.9 | 304.5  | 316.3  | 311    | 315.6  | 310    | 321.6 | 322.6 | 322.3 | 320.6 | 322   |
| Anion gap  | 21.5  | 28.9  | 21.2   | 35.7   | 28.5   | 31.2   | 28.2   | 30    | 24.7  | 24.2  | 32    | 24.8  |
| WBC        | 3.86  | 2.8   | 3.38   | 2.4    | 3.5    | 1.4    | 3.2    | 3.4   | 2.4   | 2.4   | 2.8   | 1.4   |
| RBC        | 9.12  | 9     | 9.4    | 9.22   | 9.42   | 8.84   | 8.98   | 8.96  | 9.2   | 9.04  | 9.4   | 8.74  |
| Hemoglobin | 14.52 | 14.32 | 15.1   | 14.92  | 14.92  | 14.06  | 15.12  | 14.42 | 14.56 | 14.78 | 15.4  | 14.42 |
| Hematocrit | 56.6  | 54.8  | 56.8   | 55.6   | 56.8   | 54.2   | 56     | 54    | 55.2  | 54.6  | 56.6  | 52.8  |
| MCV        | 62.1  | 60.9  | 60.6   | 60.2   | 60.4   | 61.3   | 62.3   | 60.4  | 60.1  | 60.5  | 60.2  | 60.3  |
| MCHV       | 25.7  | 26.1  | 26.5   | 26.9   | 26.2   | 26     | 27     | 26.7  | 26.4  | 27    | 26.6  | 27.3  |
| Neut (%)   | 12    | 14    | 10     | 18     | 4      | 24     | 6      | 24    | 18    | 24    | 18    | 26    |
| Lymp (%)   | 84    | 82    | 80     | 78     | 90     | 70     | 89     | 76    | 78    | 74    | 80    | 70    |
| Mono (%)   | 2     | 2     | 4      | 4      | 0      | 2      | 4      | 0     | 2     | 2     | 0     | 4     |
| Eos (%)    | 2     | 2     | 6      | 0      | 6      | 4      | 1      | 0     | 2     | 0     | 2     | 0     |
| Basos (%)  | 0     | 0     | 0      | 0      | 0      | 0      | 0      | 0     | 0     | 0     | 0     | 0     |
| ABS neut   | 463   | 392   | 338    | 432    | 140    | 336    | 192    | 816   | 432   | 576   | 504   | 364   |
| ABS lymp   | 3,242 | 2,296 | 2,704  | 1,872  | 3,150  | 980    | 2,848  | 2,584 | 1,872 | 1,776 | 2,240 | 980   |
| ABS mono   | 77    | 56    | 135    | 96     | 0      | 28     | 128    | 0     | 48    | 48    | 0     | 56    |
| ABS eos    | 77    | 56    | 203    | 0      | 210    | 56     | 32     | 0     | 48    | 0     | 56    | 0     |
| AVS baso   | 0     | 0     | 0      | 0      | 0      | 0      | 0      | 0     | 0     | 0     | 0     | 0     |
| Platelets  | 1,078 | 1,392 | 1,024  | 640    | 994    | 878    | 1,014  | 1,230 | 1,284 | 1,326 | 1,304 | 1,058 |
| Body wt    | 28.81 | 25.3  | 19.06  | 17.66  | 18.27  | 19.48  | 18.74  | 24.43 | 25.92 | 24.11 | 24.84 | 25.44 |
| Liver wt   | 1.35  | 1.34  | 0.96   | 0.87   | 0.95   | 1.14   | 1.03   | 1.47  | 1.55  | 1.24  | 1.39  | 1.45  |
| Splenic wt | 0.13  | 0.08  | 0.07   | 0.09   | 0.1    | 0.08   | 0.08   | 0.07  | 0.08  | 0.08  | 0.07  | 0.01  |
| Lw/bw      | 0.05  | 0.053 | 0.05   | 0.05   | 0.052  | 0.06   | 0.055  | 0.06  | 0.06  | 0.05  | 0.056 | 0.057 |
| Sw/bw      | 0.004 | 0.003 | 0.0035 | 0.005  | 0.0055 | 0.004  | 0.004  | 0.003 | 0.003 | 0.003 | 0.003 | 0.003 |

2-mo-old WT ( $n = 4$ ) and PGRN-deficient ( $n = 8$ ) mice were subjected to evaluations on complete necropsy, including body and selected organ weights, complete blood count, chemistry profiles, and histopathologic survey. The data from individual mice are shown.