Minami *et al.*

SUPPLEMENTARY DATA for

Progranulin Protects against Amyloid β Deposition and Toxicity in Alzheimer's Disease Mouse Models

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Supplementary Figures 1–10
Supplementary Tables 1–2

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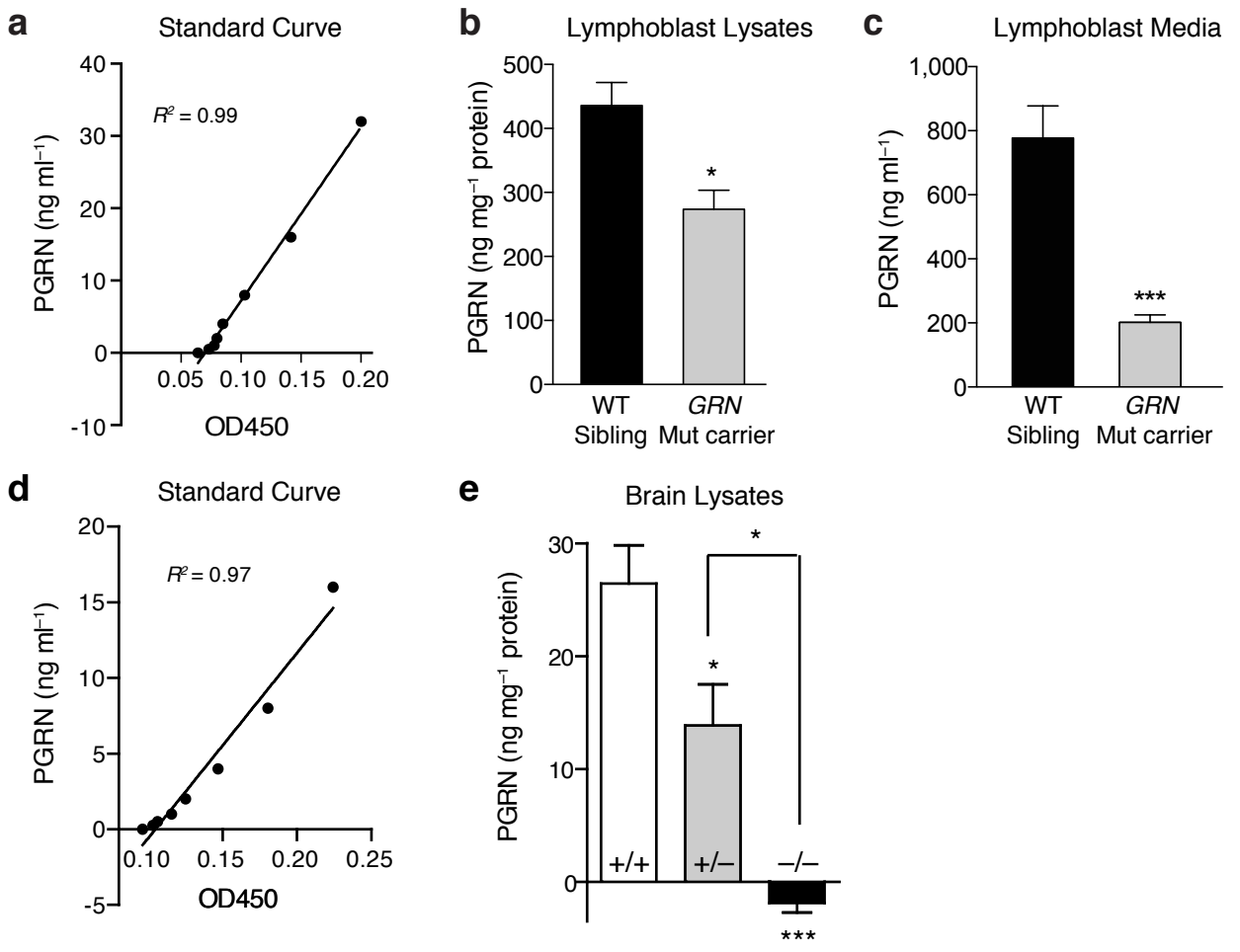


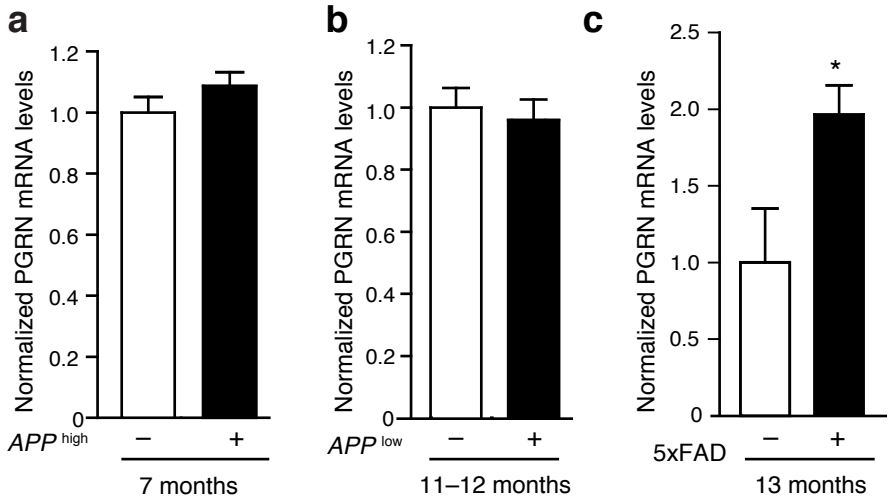
Supplementary Figure 1. Human and mouse PGRN quantification by ELISA

(**a**) Standard curve with recombinant human PGRN, showing a linear range of values between 0–32 ng ml^{-1}. $R^2 = 0.99$.

(**b**–**c**) Dilution-adjusted final concentration of PGRN protein levels from lysates (**b**) or media (**c**) of lymphoblasts derived from *GRN* mutation carriers and their sibling controls ($n = 15$ from 5 independent subjects, *, $p < 0.05$, ***, $p < 0.001$ by linear mixed model).

(**d**) Standard curve with recombinant mouse PGRN, showing a linear range of values between 0–16 ng ml^{-1}. $R^2 = 0.97$.

(**e**) Dilution-adjusted final concentration of PGRN protein levels from wildtype, heterozygous, and *GRN* knockout mice. ($n = 4$ each, *, $p < 0.05$, ***, $p < 0.001$ by one-way ANOVA).

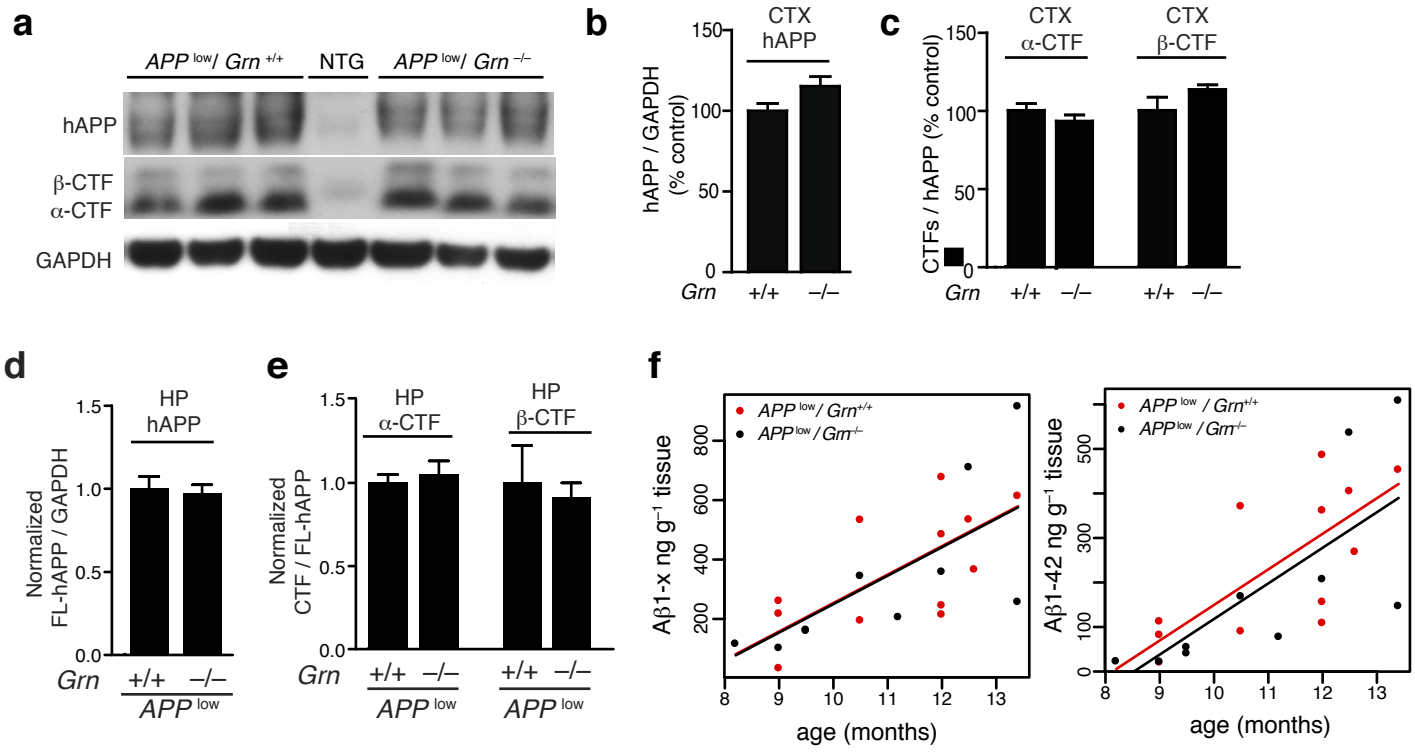


Supplementary Figure 2. Transcriptional regulation of PGRN in AD mouse models

(**a**) Levels of PGRN mRNA in 7-month-old *APP* ^{high}-J20 mice. $n = 10$, $n = 8$, unpaired student's t-test.

(**b**) Levels of PGRN mRNA in 11–12-month-old *APP* ^{low}-J9 mice. $n = 10$, $n = 9$, unpaired student's t-test.

(**c**) Levels of PGRN mRNA in 5xFAD mice and non-transgenic controls. $n = 3$, $n = 6$, *, $p < 0.05$, unpaired student's t-test.



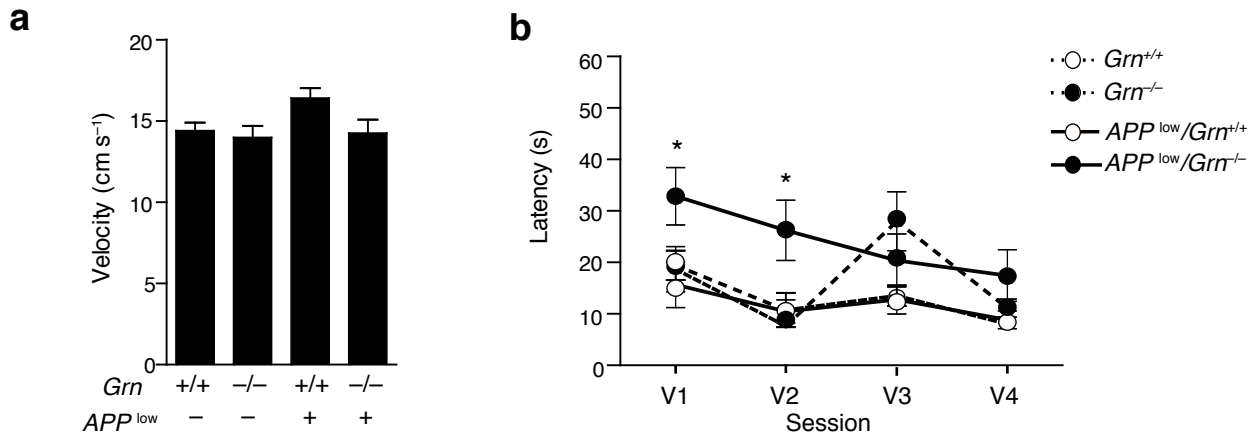
Supplementary Figure 3. APP processing is not altered in *APP* ^{low}/*Grn*$^{-/-}$ mice

(**a**–**e**) Effects of PGRN deficiency on hAPP processing. (a) Western blot analyses of hAPP metabolic fragments in cortical lysates of 9–13-month-old *APP* ^{low}/*Grn*$^{+/+}$ and *APP* ^{low}/*Grn*$^{-/-}$ mice.

(**b**, **c**) Quantification of levels of hAPP (**b**) and α-CTF and β-CTF (**c**) in the cortex of 9–13-month-old *APP* ^{low}/*Grn*$^{+/+}$ and *APP* ^{low}/*Grn*$^{-/-}$ mice. Values were not significantly different by unpaired student's t-test. n = 8–12.Values are mean ± SEM.

(**d**, **e**) Quantification of levels of hAPP (**d**) and β-CTF and α-CTF (**e**) in the hippocampus of 9–13-month-old *APP*^{low}/*Grn*$^{+/+}$ and *APP*^{low}/*Grn*$^{-/-}$ mice. Values were not significantly different by unpaired student's t-test. n = 12, 10. Values are mean ± SEM.

(**f**) Left: linear regression of Aβ1-x (ng g^{-1} tissue) against both age and genotype (as a dummy variable). The fitted model was given by: Aβ1-x = (-707.78) + (95.77) x age + (3.84) x genotype (dummy). The age-adjusted Aβ1-x in 9–13-month-old *APP* ^{low}/*Grn*$^{+/+}$ mice (dummy = 0) did not differ from that in *APP* ^{low}/*Grn*$^{-/-}$ mice (dummy = 1), since the β2 coefficient (3.84) is not significantly different from 0 (P = 0.96). Right: Linear regression of Aβ1-42 against both age and genotype (as a dummy variable). The fitted model was given by: Aβ1-42 = (-681.21) + (79.93) x age + (31.47) x genotype (dummy). The age-adjusted Aβ1-42 in *APP* ^{low}/*Grn*$^{+/+}$ mice (dummy = 0) did not differ from that in *APP*^{low}/*Grn*$^{-/-}$ mice (dummy = 1), since the β2 coefficient (31, 47) is not significantly different from 0 (P = 0.59). n = 12, 10.

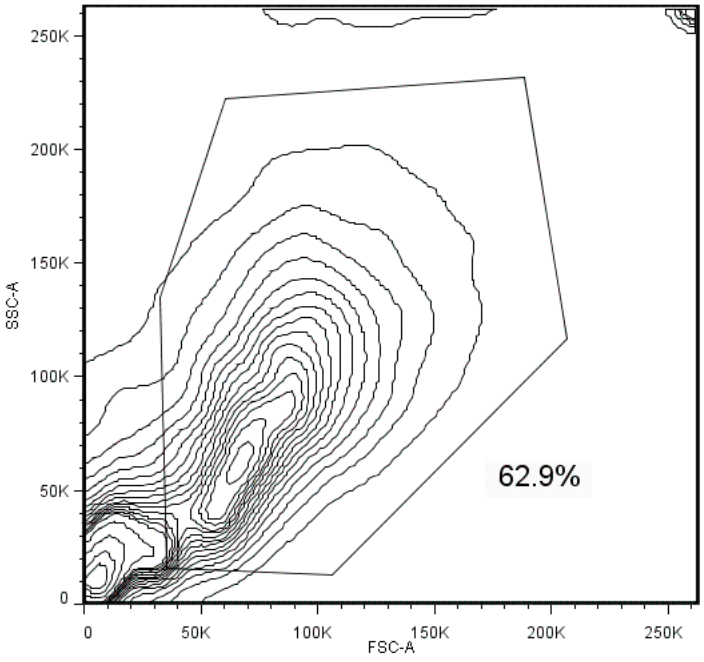


Supplementary Figure 4. PGRN deficiency does not affect swim speed or performance in the visible platform Morris water maze

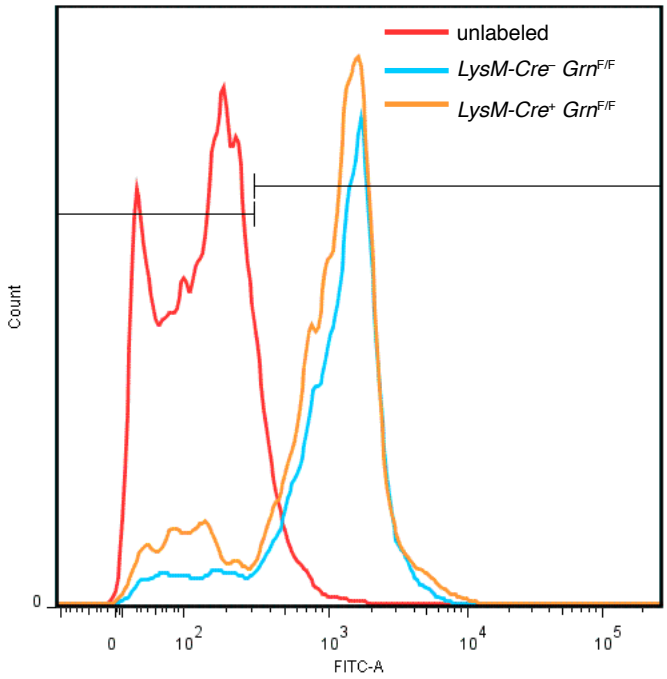
(**a**) Swim speed (cm s^{-1}) of *Grn*$^{+/+}$, *Grn*$^{-/-}$, *APP* ^{low}*/Grn*$^{+/+}$ and *APP* ^{low}*/Grn*$^{-/-}$ mice was not significantly different, two-way ANOVA, $n = 13$, $n = 12$, $n = 12$, $n = 11$.

(**b**) Latency to locate the visible platform was measured after the probe trial was completed. There was no significant effect of genotype on latency for the visible platform trials by mixed effects model with linear regression. There was a significant difference between *APP* ^{low}*/Grn*$^{-/-}$ mice and all other groups on sessions 1 and 2, two-way ANOVA with *Bonferroni* post-hoc test. $n = 13$, $n = 12$, $n = 12$, $n = 11$. *, $P < 0.05$.

a



b



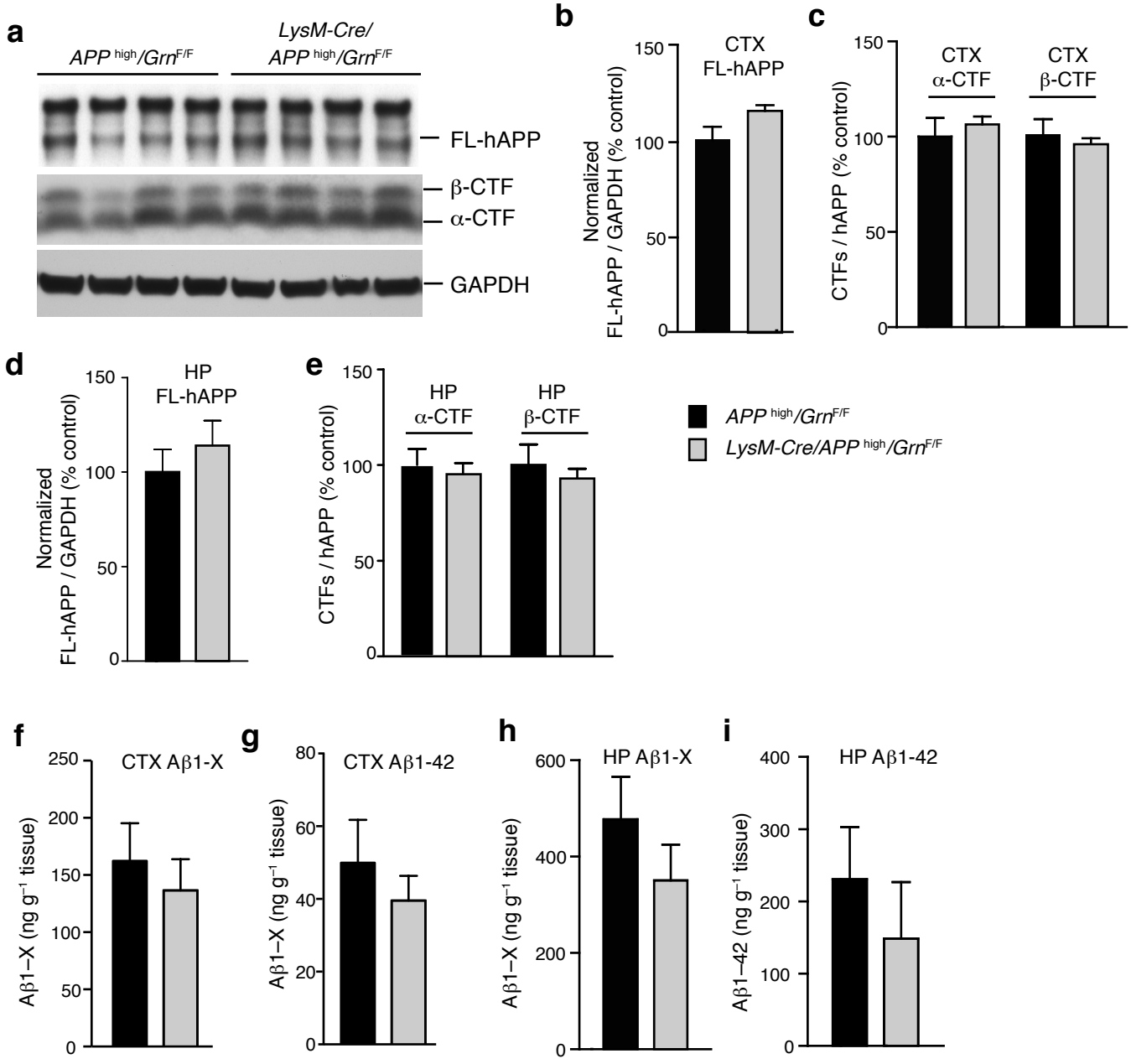
c

unstained	12.3%
LysM-Cre⁻ Grn$^{F/F}$	84.1%
LysM-Cre⁺ Grn$^{F/F}$	89.8%

Supplementary Figure 5. Purity of adult microglial isolation

(**a**) Representative result from flow cytometry, gated on live cells, of isolated adult mouse microglia. 63% of cells were gated for analysis of CD11b expression.

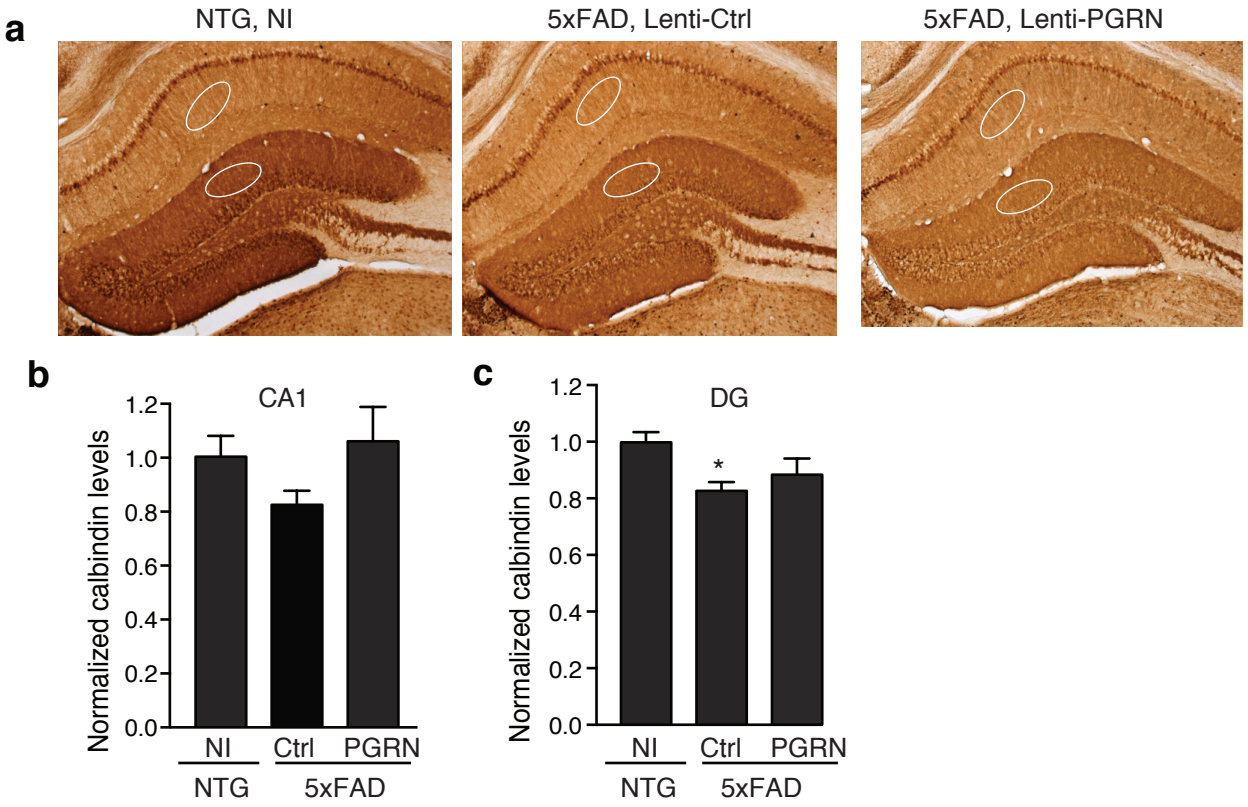
(**b**) CD11b-FITC expression from unlabeled control cells, CD11b-FITC-labeled *LysM-Cre⁻/Grn*$^{F/F}$ cells, and CD11b-FITC-labeled *LysM-Cre⁺/Grn*$^{F/F}$ cells. 84% of *LysM-Cre⁻/Grn*$^{F/F}$ and 90% of *LysM-Cre⁺/Grn*$^{F/F}$ cells were CD11b-FITC positive of the parental gate. 4 mice were pooled for each condition.



Supplementary Figure 6. Microglial PGRN deficiency does not affect APP processing

(**a**–**e**) Western blot analysis of full-length human APP (FL-hAPP), α-CTF, and β-CTFs from the cortex (**a**–**c**) and hippocampus (**d**–**e**) of 4-month-old *APP* high*/Grn*F/F and *LysM-Cre/APP* high*/Grn*F/F mice. *n* = 8, *n* = 6 for both cortex (CTX) and hippocampus (HP).

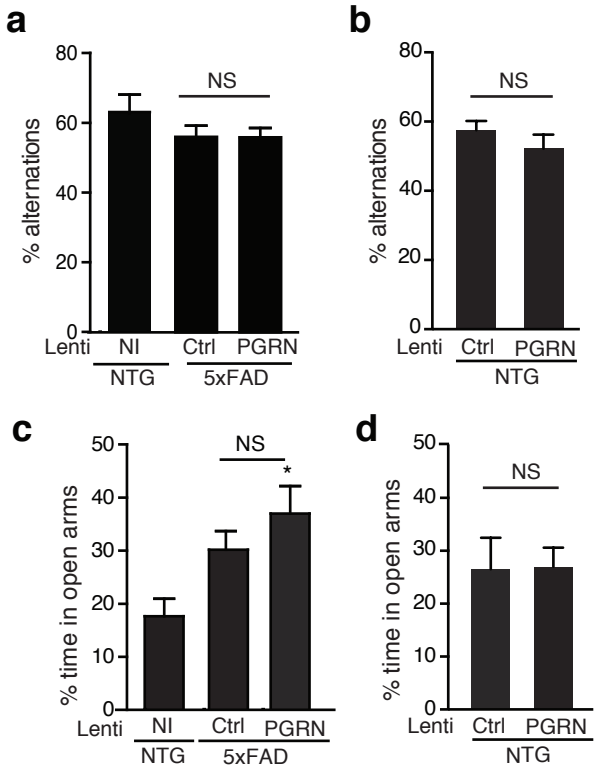
(**f**–**i**) ELISA measurement of Aβ1-X (**f**, **h**) and Aβ1-42 (**g**, **i**) levels in the CTX (**f**, **g**) or HP (**h**, **i**) of 4-month-old *APP* high*/Grn*F/F and *LysM-Cre/APP* high*/Grn*F/F mice. *n* = 8, *n* = 5 (CTX); *n* = 8, *n* = 6 (HP). No significant differences were detected in Aβ, APP, or APP fragments, unpaired student's t-test.



Supplementary Figure 7. Hippocampal PGRN overexpression does not affect calbindin levels in 5xFAD mice

(**a**) Representative images of calbindin immunostaining in the hippocampus of non-injected non-transgenic mice (NTG, NI) or 5xFAD mice injected with Lenti-Ctrl or Lenti-PGRN. Ovals indicate the locations for quantification.

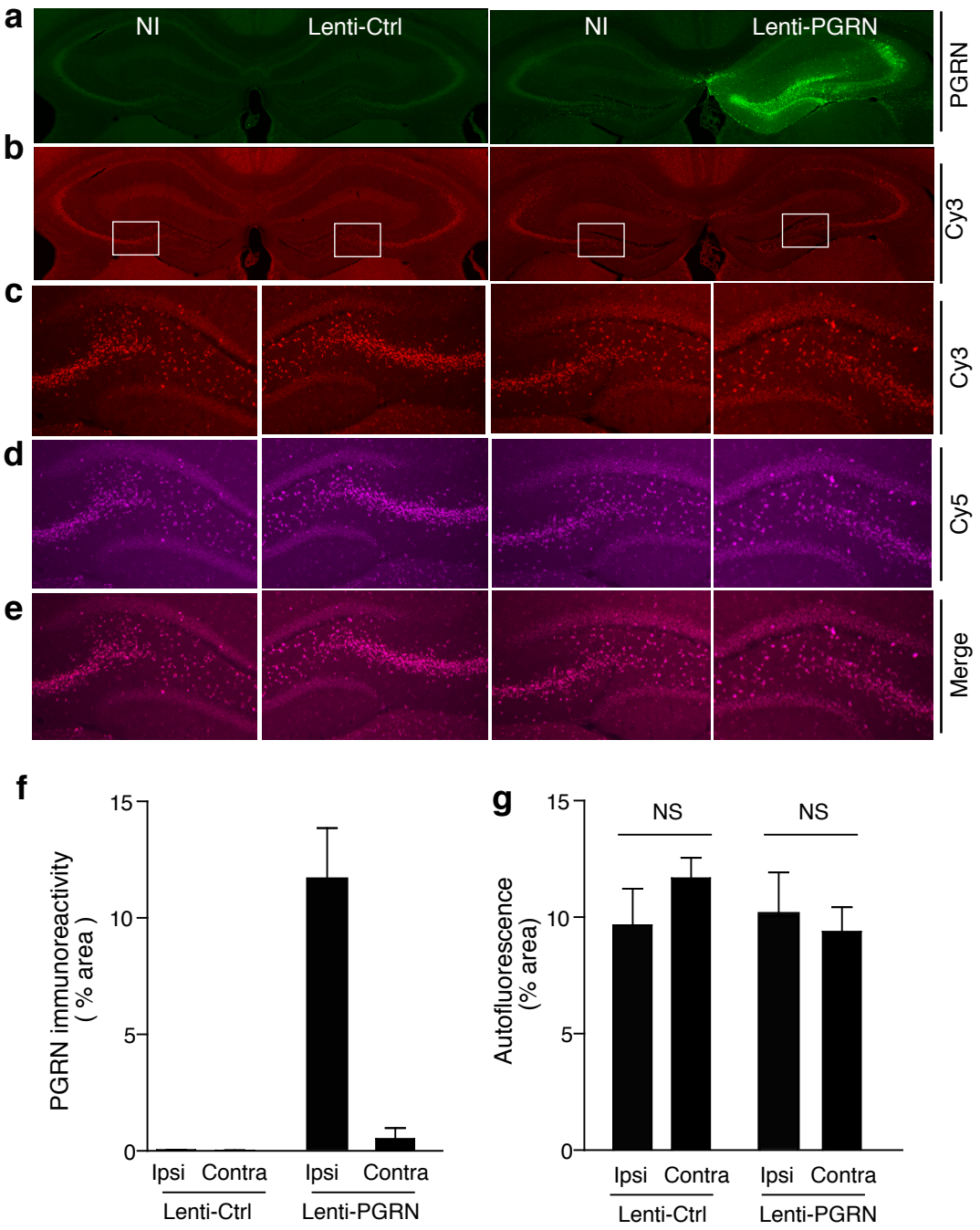
(**b**) Quantification of calbindin immunoreactivity in the CA1 (**b**) or DG (**c**) of non-injected non-transgenic mice (NTG, NI) or 5xFAD mice injected with Lenti-Ctrl or Lenti-PGRN. Values represent means of 2–3 sections per mouse, $n = 6, 8, 8$ mice/group, *, $P < 0.05$, one-way ANOVA, *Tukey-kramer* post-hoc analyses.



Supplementary Figure 8. Hippocampal PGRN overexpression does not affect non-hippocampus-dependent behaviors in 5xFAD mice

(**a**–**b**) 5xFAD mice (**a**) and NTG controls (**b**) injected with Lenti-Ctrl or Lenti-PGRN were tested for spontaneous alternation in the small y-maze. $n = 9$, $n = 12$, $n = 13$ (a), $n = 6$, $n = 7$ (**b**). NS, not significant by one-way ANOVA (**a**) or unpaired student's t-test (**b**).

(**c**–**d**) 5xFAD mice (**c**) and NTG controls (**d**) injected with Lenti-Ctrl or Lenti-PGRN were tested for anxiety in the elevated plus maze. $n = 9$, $n = 12$, $n = 13$ (**c**), $n = 6$, $n = 7$ (**d**). *, $P < 0.05$, one-way ANOVA *Tukey-Kramer* post-hoc analyses (NTG, NI vs. hAPP, Lenti-PGRN). NS, not significant by one-way ANOVA (**c**) or unpaired student's t-test (**d**).



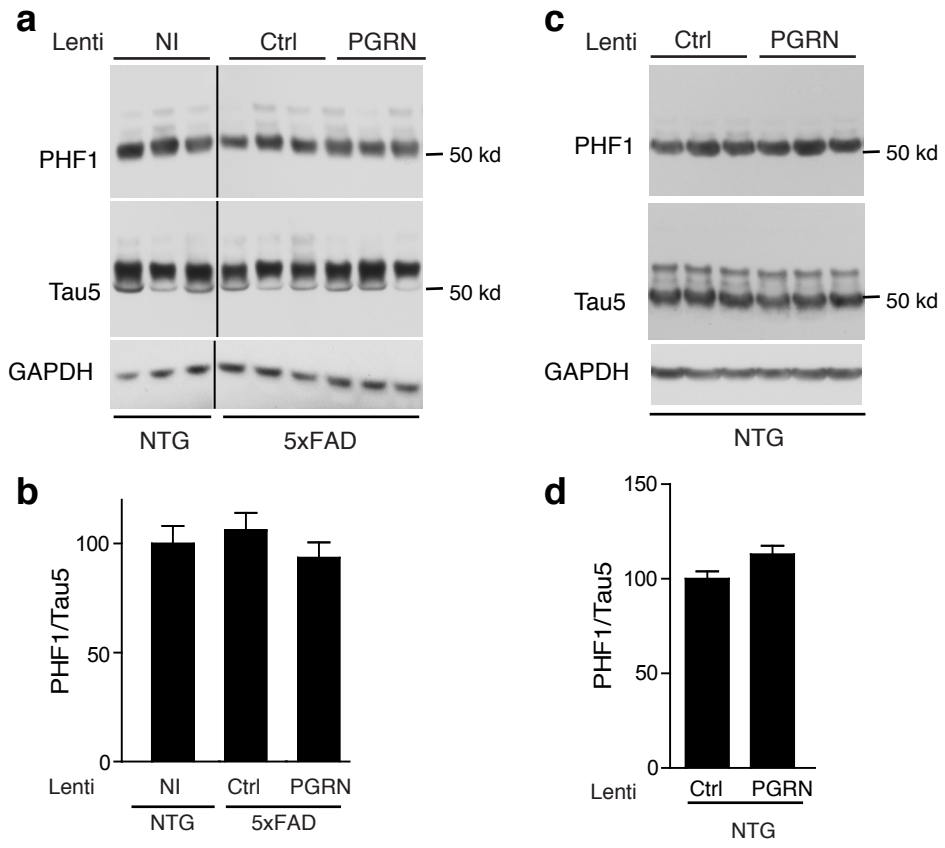
Supplementary Figure 9. PGRN overexpression does not affect the accumulation of autofluorescent material in aged non-transgenic mice

(**a**–**b**) Representative images of PGRN immunostaining (**a**) and Cy3 autofluorescence (**b**) in 16–20 month-old non-transgenic mice injected unilaterally with either Lenti-Ctrl (left panels) or Lenti-PGRN (right panels).

(**c**–**e**) Magnification of the insets of autofluorescent signal in the DG, visualized in the Cy3 (**c**) or Cy5 (**d**) channel. The complete colocalization of Cy3 and Cy5 (**e**) confirms autofluorescent signal.

(**f**) Quantification of PGRN immunoreactivity (% of area) in non-transgenic mice injected with Lenti-Ctrl or Lenti-PGRN. *n* = 9/treatment.

(**g**) Quantification of Cy3 autofluorescent signal from non-transgenic mice injected with Lenti-Ctrl or Lenti-PGRN. *n* = 9/treatment. NS, not significant.



Supplementary Figure 10. PGRN overexpression does not affect PHF1-positive phospho-tau levels

(**a**, **c**) Representative Western blot of hippocampal lysates from non-infected (NI) non-transgenic (NTG) brains or 5xFAD or NTG brains injected with either Lenti-Ctrl or Lenti-PGRN. PHF1 antibody (top) detects phospho-tau (Ser396 and Ser404) and Tau5 antibody (middle) detects total tau. Levels of GAPDH served as loading controls.

(**b**, **d**) Quantification of Western blots for phospho-tau from 5xFAD mice (**b**) or NTG mice (**d**). $n = 8$, $n = 8$, $n = 8$ (**b**); $n = 6$, $n = 6$ (**d**).

Supplementary Table-1

Summary of Human Brain Samples Used to Quantify PGRN Levels[1]

Case#	Age[2]	CDR	PMI[3] (min)	SEX	Plaque Counts (# plaques/mm^2)	NP[4]
82	86	0	280	F	0	ND
230	96	0	195	F	0	ND
546	80	0	285	F	0	ND
580	93	0	1140	F	0	ND
684	84	0	1110	F	0	ND
900	79	0	410	F	0	ND
978	70	0	1230	M	0	ND
1004	75	0	300	M	0	ND
1039	85	0	315	M	0	ND
1229	91	0	300	F	0	ND
1409	88	0	537	F	0	ND
611	87	5	90	F	3.04	AD
73	94	5	170	M	4.1	AD
992	88	5	195	F	6.0	AD
1203	93	5	180	F	6.4	AD
607	103	5	235	F	7.6	AD
247	73	5	440	F	8.4	AD
736	94	5	260	F	9.2	AD
399	96	5	180	M	9.7	AD
649	84	5	435	F	10.8	AD
38	84	5	715	M	11.2	AD
505	94	5	198	F	12	AD
350	88	5	210	F	13.2	AD

Notes:

1. Human brain samples (Bm-22, *superior temporal gyrus*) were obtained from Dr. Vahram Haroutunian at the Mount Sinai School of Medicine, New York City, New York, USA
2. The average age of non-demented (ND) samples is 84.3; the average age of AD samples is: 89.8. $p > 0.05$.
3. PMI: Post-mortem Interval in minutes
4. NP: Neuropathological diagnosis

Supplementary Table-2. qRT-PCR primer sequences

TNFα	S	CATCAGTTCTATGGCCCAGA
	AS	TGCTCCTCCACTTGGTGGTT
IL1α	S	CACCTTACACCTACCAGAGTGATTTG
	AS	TGTTGCAGGTCATTTAACCAAGTG
IL1β	S	GAATCTATACCTGTCCTGTG
	AS	ATCTTGTTGAAGACAAACCG
iNOS	S	AGGCTCCTCACGCTTGGGTCTTG
	AS	CTGCGGGGAGCCATTTTGGTG
IL4	S	TGCGAAGCACCTTGGAAGCCC
	AS	GGGACGCCATGCACGGAGATG
TGFβ	S	AACCCCCATTGCTGTCCCGTG
	AS	GCGCTGAATCGAAAGCCCTGT
VEGF1	S	GCTGCACCCACGACAGAAGGA
	AS	TCGTTACAGCAGCCTGCACAGC
CD206	S	ACAGACAGGAGGACTGCGTGGTT
	AS	CCGGTACTACAGCATGGCTTTGTG
COX2	S	GTACCGCAAACGCTTCTCCCTG
	AS	CCTCCAAAGGTGCTCGGCTTCCA
Arg1	S	AGCCGCTGGAACCCAGAGAGA
	AS	TGGACCTCTGCCACCACACCA
GAPDH	S	GGGAAGCCCATCACCATCTT
	AS	GCCTTCTCCATGGTGGTGAA
PGRN	S	GTGTTGTGAGGATCACATTC
	AS	CTATGACCTTCTTCATCCAG