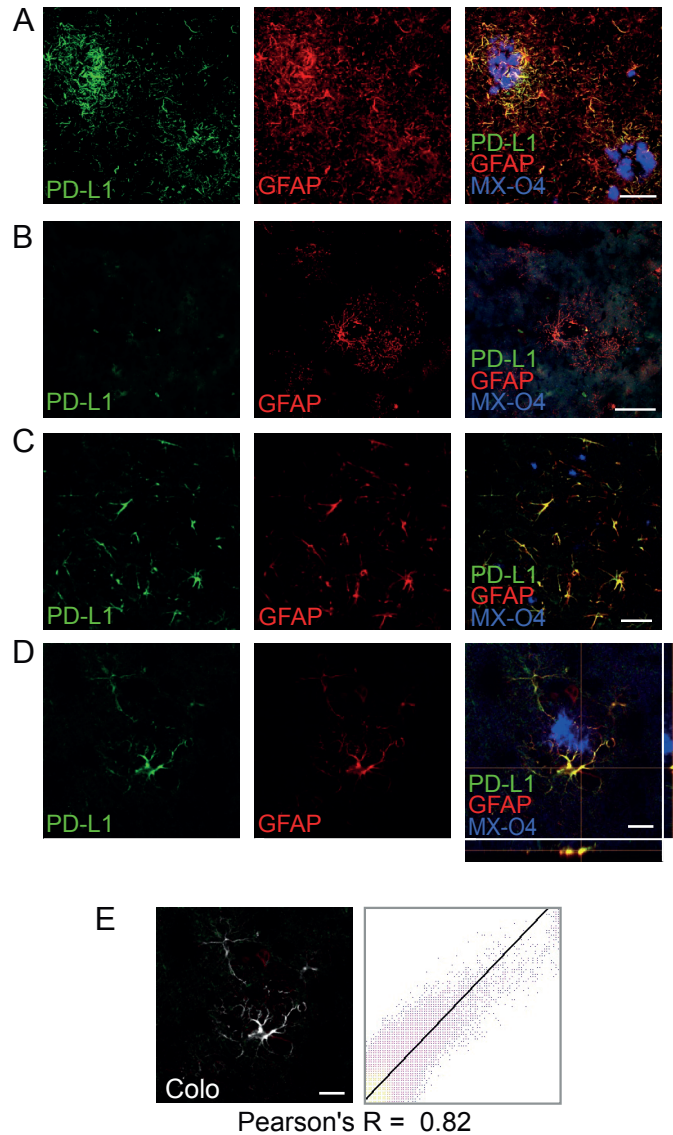


Appendix to

Kummer MP, Ising C, Kummer C, Sarlus H, Griep A, Vieira-Saecker A, Schwartz S, Halle A, Brückner M, Händler K, Schultze JL, Beyer M, Latz E, Heneka MT - Microglial PD-1 modulates neuroinflammation and Alzheimer's disease pathology by astrocytic PD-L1

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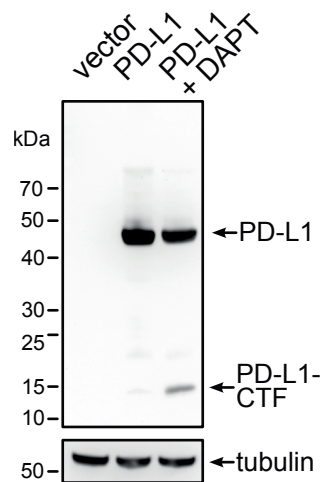
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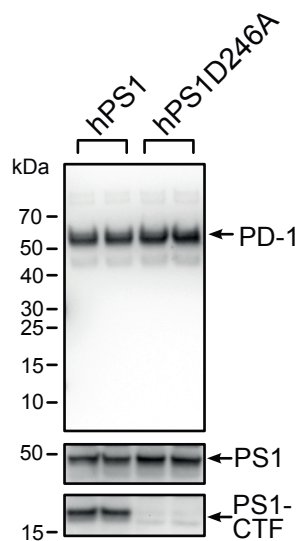
Appendix Figure S1: PD-L1 expression in APP/PS1 mice and AD.

A) Immunohistochemistry of PD-L1 and GFAP in human AD. Amyloid was stained using methoxy-XO4 (bar = 100 μ m). **B)** Immunohistochemistry of PD-L1 and GFAP in human control. Amyloid was stained using methoxy-XO4 (bar = 100 μ m) **C)** Immunohistochemistry of PD-L1 and GFAP in 9-month-old APP/PS1 mouse. Amyloid was stained using methoxy-XO4 (bar = 100 μ m) **D)** Immunohistochemistry of PD-L1 and GFAP in 4-month-old APP/PS1 mouse. Amyloid was stained using methoxy-XO4 (bar = 20 μ m) **E)** Colocalization analysis of D using the ImageJ plugin Colo2 (left panel: colocalized pixels shown in white (Colo), right panel: colocalization analysis including linear regression and the Pearson's correlation coefficient (R) after thresholding).

A - D are also shown in Figure 1 B - E as merged images and in higher magnification.



Appendix Figure S2: PD-L1-Myc expression and DAPT treatment in HEK293 cells
 PD-L1-Myc overexpressing HeLa cells incubated with up to 5 μ M of the γ -secretase inhibitor DAPT for 18 h and cell lysates analyzed using antibody 9E10 against the myc epitope.



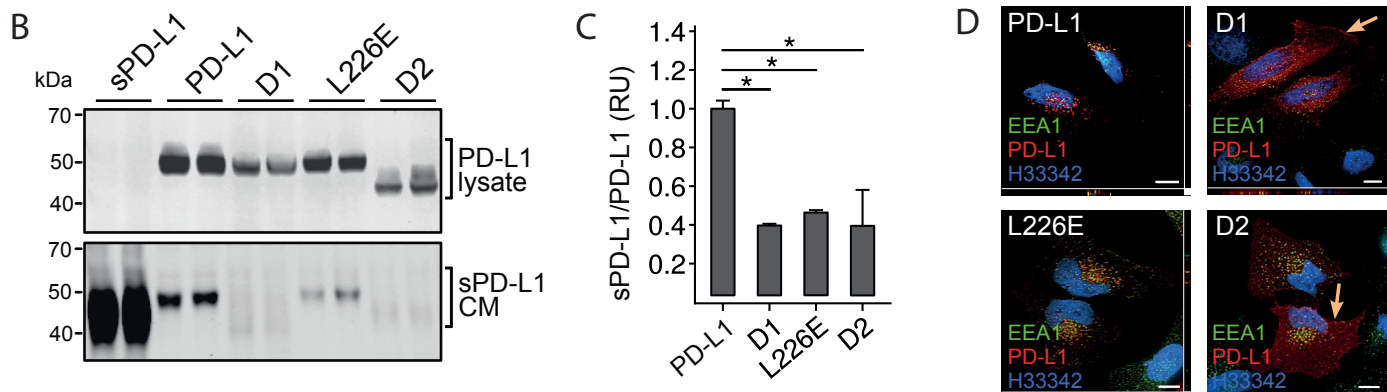
Appendix Figure S3: PD-1 expression in hPS1 and hPS1D246A HEK293 cells
 PD-1-Myc was expressed in HEK 293 cells stably expressing human PS1 or human PS1D246A mutant. The myc epitope was detected in the cell lysates by immunoblotting using antibody 9E10. Detection of presenilin 1 showed the lack of processing of the PS1D246A mutant into the the presenilin 1 c-terminal fragment (PS1-CTF).

A

lumen

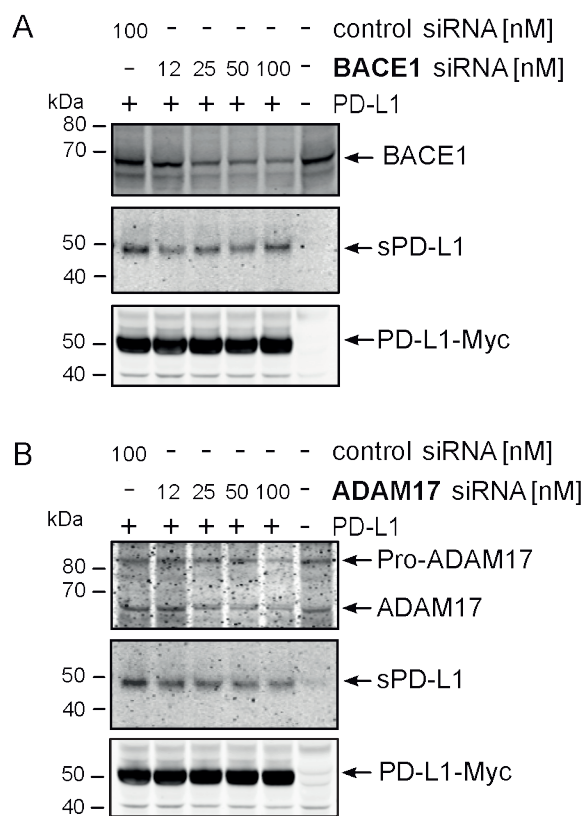
TM

...CTFWRSQPGQNHTAELI**IPEL**PATHPPQNRTHWVLLGS... PD-L1
 ...CTFWRSQPGQNHTAELI**IPEL**PATHPP sPD-L1
 ...CTFWRSQPGQNHTAE-----PATHPPQNRTHWVLLGS... D1
 ...CTFWRSQ-----ATHPPQNRTHWVLLGS... D2
 ...CTFWRSQPGQNHTAELI**IPEE**PATHPPQNRTHWVLLGS... L226E



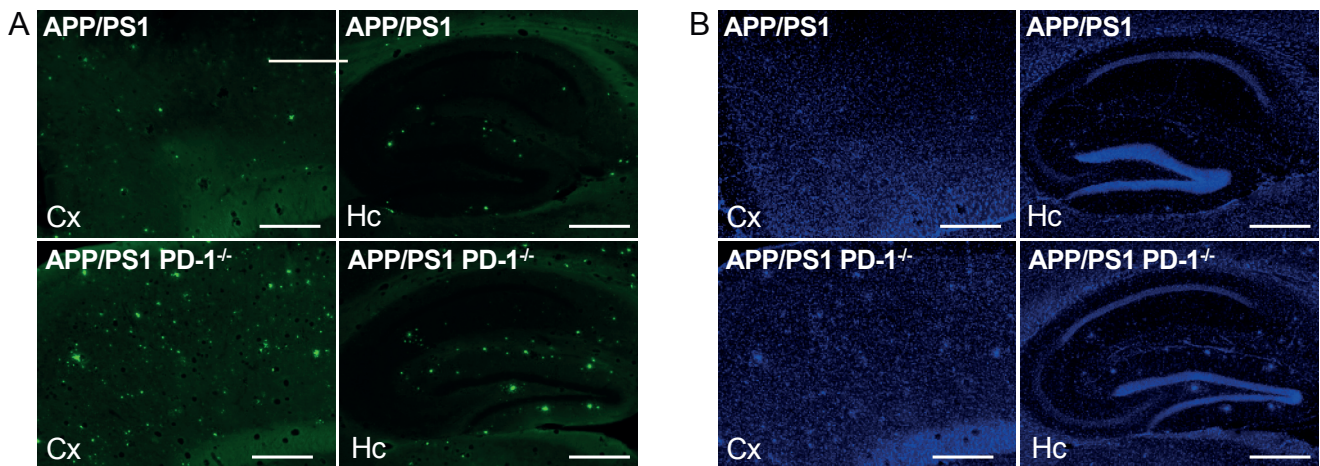
Appendix Figure S4: PD-L1 juxtamembrane mutants

A) Depiction of PD-L1 mutations that were generated to interfere with the juxtamembrane cleavage of PD-L1 at the luminal side (bold letters indicate the predicted transmembrane domain, dashes indicate deleted amino acids, underlining amino acid changes; sPD-L secretory PD-L1; TM, transmembrane domain). **B)** Expression of sPD-L1, PD-L1-myc and three juxtamembrane mutants of PD-L1 in HeLa cells. Lysates and conditioned media (CM) were immunoblotted using antibody E1L3N. **C)** Evaluation of two independent experiments of B (n=2, mean +SEM of a duplicate experiment, one-way ANOVA (F = 17.43, df = 3, p=0.00011), Tukey's HSD, * p<0.05). **D)** Immunocytochemistry of EEA1, PD-L1-myc and the three PD-L1 mutants in HeLa cells (bar=10 μ m).

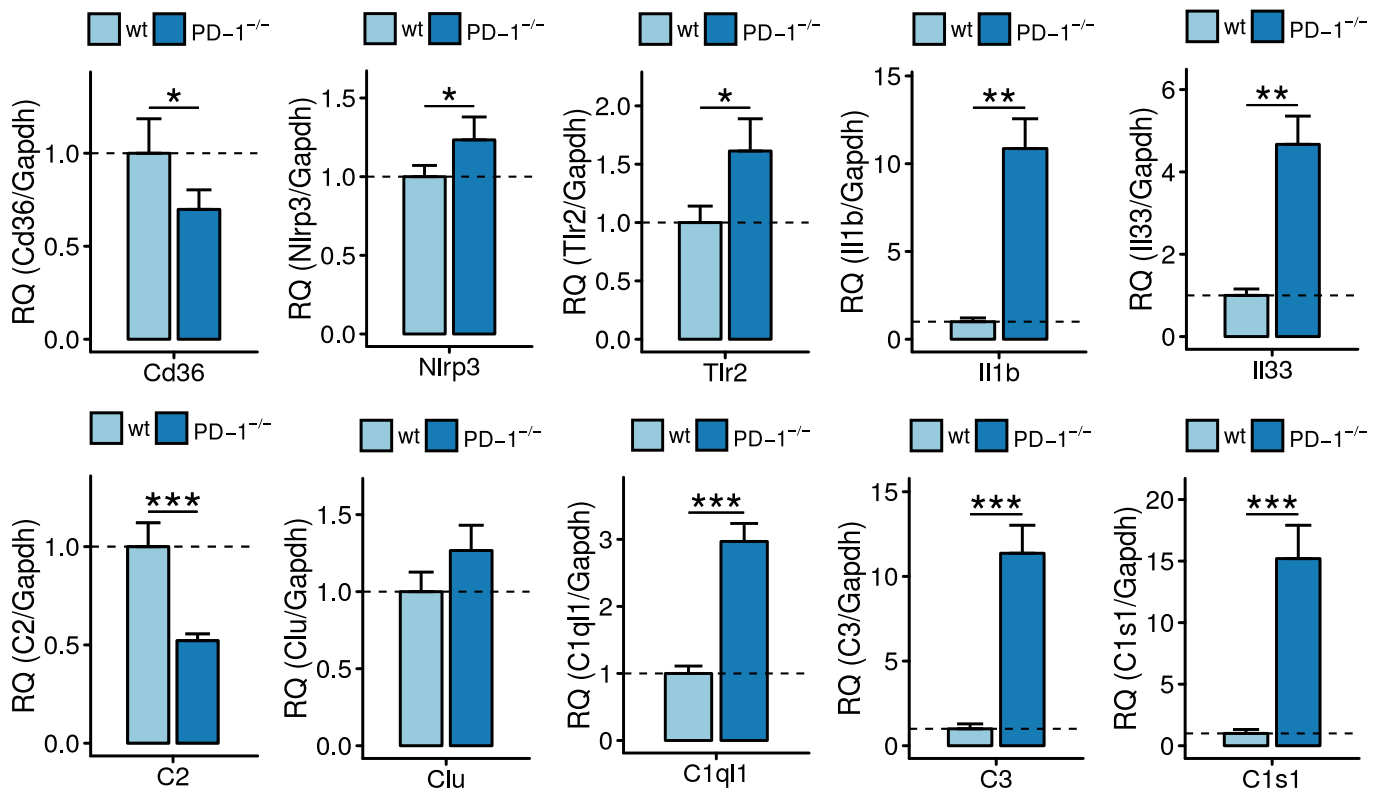


Appendix Figure S5: Secretion of PD-L1 after siRNA knock-down of candidate sheddases in HEK293 cells

Cells were transfected with siRNA targeted against **A)** BACE1 and **B)** ADAM17 or control siRNA that does not have any significant homology with human sequences. Cells were transfected with PD-L1-Myc and the secretion of sPD-L1 in the conditioned medium was detected using antibody AF1019 against the ectodomain of PD-L1.



Appendix Figure S6: Thioflavin T histochemistry of APP/PS1 and APP/PS1 PD-1^{-/-} mice
A) Thioflavin T histochemistry of cortical and hippocampal sections (bar = 500 μm) **B)** Hoechst 33342 histochemistry of cortical and hippocampal sections (bar = 500 μm).



Appendix Figure S7: Gene expression analysis of wild type and PD-1^{-/-} microglia by quantitative PCR

Gene expression in microglia from wild type or PD-1^{-/-} mice normalized to Gapdh expression (mean fold expression +/-SEM of triplicate measurement, Student t-test, * p<0.05, ** p<0.01, *** p<0.001)

ID	Pathway	adjusted p-value	q-value
mmu04610	Complement and coagulation cascades	1.78E-05	1.55E-05
mmu04060	Cytokine-cytokine receptor interaction	1.78E-05	1.55E-05
mmu04015	Rap1 signaling pathway	1.04E-04	9.06E-05
mmu05150	Staphylococcus aureus infection	1.79E-04	1.57E-04
mmu04668	TNF signaling pathway	7.24E-04	6.33E-04

Appendix Table S1

Gene	adjusted p-value	t	DF
Cd36	p=0.00036	4.2	1
Nlrp3	p=0.0018	4.99	1
Tlr2	p=0.00021	6.27	1
Il33	p=0.0016	5.05	1
Il1b	p=6.93E-05	6.94	1

Appendix Table S2

Gene	adjusted p-value	t	DF
C1s1	2.1E-07	11.2	1
C2	2.4E-05	7.68	1
C5ar1	2.45E-05	7.66	1
Itgam	4.33E-05	7.3	1
C3	5.23E-05	7.12	1
C1ra	0.000753	5.51	1
Serping1	0.004841	4.45	1
C1s2	0.01986	3.57	1
C4b	0.02842	3.34	1
Clu	0.02991	3.31	1
C1rb	0.03016	3.31	1
C1ql1	0.03610	3.19	1

Appendix Table S3