*Supplementary information for:*

# *Therapeutic potential of Nlrp1 inflammasome, Caspase-1, or Caspase-6 against Alzheimer disease cognitive impairment*

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**Table S1. Behavioral data comparing J20 versus genetically ablated J20, WT versus genetically ablated WT, and J20 and genetically ablated J20 versus WT mice.**

| **Nlrp1, Casp1, Casp6 genetically ablated J20 vs. J20 mice** | | **J20** | **J20/*Nlrp1*$^{-/-}$** | **J20/*Casp1*$^{-/-}$** | **J20/*Casp6*$^{-/-}$** | | **J20/*Nlrp1*$^{+/-}$** | **J20/*Casp1*$^{+/-}$** | **J20/*Casp6*$^{+/-}$** |
|---|---|---|---|---|---|---|---|---|---|
| **NOR** | Mice (*n*) | 15 | 10 | 13 | 11 | | 14 | 11 | 11 |
| | Discrimination index | | ▲ (p<0.0001) | ▲ (p<0.0001) | ▲ (p<0.0001) | | ▲ (p<0.0001) | ▲ (p<0.0001) | ▲ (p<0.0001) |
| | | | | | | | | | |
| **Barnes Maze** | Mice (*n*) | 13 | 10 | 13 | 9 | | 12 | 11 | 10 |
| Learning acquisition | Primary errors | | ▼ (p=0.0017) | ▼ (p=0.02) | ▼ (p=0.03) | | ▼ (p=0.01) | = | = |
| | Primary latency | | = | = | = | | = | = | = |
| Probe test | Primary errors | | = | ▼ (p=0.0016) | ▼ (p=0.03) | | = | = | = |
| | Primary latency | | = | = | = | | = | = | = |
| | Target preference | | no difference | no difference | no difference | | no difference | no difference | no difference |

| **Nlrp1, Casp1, Casp6 genetically ablated WT vs. WT mice** | | **WT** | **WT/*Nlrp1*$^{-/-}$** | **WT/*Casp1*$^{-/-}$** | **WT/*Casp6*$^{-/-}$** | | **WT/*Nlrp1*$^{+/-}$** | **WT/*Casp1*$^{+/-}$** | **WT/*Casp6*$^{+/-}$** |
|---|---|---|---|---|---|---|---|---|---|
| **NOR** | Mice (*n*) | 16 | 13 | 12 | 12 | | 12 | 13 | 11 |
| | Discrimination index | | = | = | = | | = | = | = |
| | | | | | | | | | |
| **Barnes Maze** | Mice (*n*) | 16 | 13 | 12 | 12 | | 12 | 12 | 11 |
| Learning acquisition | Primary errors | | = | = | = | | = | = | = |
| | Primary latency | | = | = | = | | = | = | = |
| Probe test | Primary errors | | = | = | = | | = | = | = |
| | Primary latency | | = | = | = | | = | = | = |
| | Target preference | | no difference | no difference | no difference | | no difference | no difference | no difference |

| **J20 and genetically ablated J20 vs. WT mice** | | **WT** | **J20** | **J20/*Nlrp1*$^{-/-}$** | **J20/*Casp1*$^{-/-}$** | **J20/*Casp6*$^{-/-}$** | | **J20/*Nlrp1*$^{+/-}$** | **J20/*Casp1*$^{+/-}$** | **J20/*Casp6*$^{+/-}$** |
|---|---|---|---|---|---|---|---|---|---|---|
| **NOR** | Mice (*n*) | 16 | 15 | 10 | 13 | 11 | | 14 | 11 | 11 |
| | Discrimination index | | ▼ (p=0.0001) | = | = | = | | = | = | = |
| | | | | | | | | | | |
| **Barnes Maze** | Mice (*n*) | 16 | 13 | 10 | 13 | 9 | | 12 | 11 | 10 |
| Learning acquisition | Primary errors | | ▼ (p=0.03) | = | = | = | | = | = | = |
| | Primary latency | | = | = | = | = | | = | = | = |
| Probe test | Primary errors | | NS p=0.06 | = | = | = | | = | = | = |
| | Primary latency | | = | = | = | = | | = | = | = |
| | Target preference | | impaired | no difference | no difference | no difference | | no difference | no difference | no difference |

Episodic memory was measured with novel object recognition (NOR) while spatial memory was measured with the Barnes maze. Mice numbers are indicated in each group of mice. Upward arrow (▲) represents increased value,

downward arrow (▼) represents decreased value, equal sign (=) represents unchanged value. Significant ($p<0.05$) or non-significant (NS) but close ($0.1>p>0.05$) statistical p values are indicated below or next to arrows.

**Table S2. Iba1$^+$-microglial and GFAP$^+$-astrocyte immunostaining density comparing J20 and genetically ablated J20 versus WT, or genetically ablated J20 versus J20 in the hippocampus and cortex.**

**Hippocampus**

| vs. WT mice | | WT | J20 | J20/*Nlrp1*$^{-/-}$ | J20/*Casp1*$^{-/-}$ | J20/*Casp6*$^{-/-}$ | | J20/*Nlrp1*$^{+/-}$ | J20/*Casp1*$^{+/-}$ | J20/*Casp6*$^{+/-}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **Iba1 vs WT** | Mice (*n*) | 8 | 6 | 5 | 6 | 4 | | 8 | 6 | 6 |
| | Iba1$^+$ cell density | | ▲ ($p<0.0001$) | ▲ ($p=0.0002$) | = | = | | ▲ ($p=0.002$) | = | ▲ ($p=0.035$) |
| **GFAP vs WT** | Mice (*n*) | 6 | 6 | 5 | 6 | 4 | | 6 | 6 | 6 |
| | GFAP$^+$ density | | = | = | = | = | | = | = | = |
| **vs. J20 mice** | | | | | | | | | | |
| **Iba1 vs J20** | Mice (*n*) | 8 | 6 | 5 | 6 | 4 | | 8 | 6 | 6 |
| | Iba1$^+$ cell density | ▼ ($p<0.0001$) | | ▼ ($p=0.0006$) | ▼ ($p<0.0001$) | ▼ ($p<0.0001$) | | ▼ ($p<0.0001$) | ▼ ($p<0.0001$) | ▼ ($p<0.0001$) |
| **GFAP vs J20** | Mice (*n*) | 6 | 6 | 5 | 6 | 4 | | 6 | 6 | 6 |
| | GFAP$^+$ density | = | | = | = | = | | = | = | = |

**Cortex**

| vs. WT mice | | WT | J20 | J20/*Nlrp1*$^{-/-}$ | J20/*Casp1*$^{-/-}$ | J20/*Casp6*$^{-/-}$ | | J20/*Nlrp1*$^{+/-}$ | J20/*Casp1*$^{+/-}$ | J20/*Casp6*$^{+/-}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **Iba1 vs WT** | Mice (*n*) | 8 | 6 | 5 | 6 | 4 | | 8 | 6 | 6 |
| | Iba1$^+$ cell density | | ▲ ($p<0.0001$) | ▲ ($p=0.0001$) | = | = | | ▲ ($p<0.0001$) | = | ▲ ($p=0.01$) |
| **GFAP vs WT** | Mice (*n*) | 6 | 6 | 5 | 6 | 4 | | 6 | 6 | 6 |
| | GFAP$^+$ density | | = | = | ▲ ($p=0.03$) | = | | = | = | = |
| **vs. J20 mice** | | | | | | | | | | |
| **Iba1 vs J20** | Mice (*n*) | 8 | 6 | 5 | 6 | 4 | | 8 | 6 | 6 |
| | Iba1$^+$ cell density | ▼ ($p<0.0001$) | | = | ▼ ($p<0.0001$) | ▼ ($p=0.002$) | | = | ▼ ($p<0.0001$) | ▼ ($p<0.0001$) |
| **GFAP vs J20** | Mice (*n*) | 6 | 6 | 5 | 6 | 4 | | 6 | 6 | 6 |
| | GFAP$^+$ density | = | | = | = | = | | = | = | = |

Immunohistochemical staining density was measured as described in methods. Upward arrow (▲) represents increased value, downward arrow (▼) represents decreased value, equal sign (=) represents unchanged value. Significant ($p<0.05$) statistical p values are indicated.

**Table S3. Immunohistochemical Aβ staining density and ELISA-measured Aβ in the hippocampus and cortex of genetically ablated J20 compared to J20 mice.**

**Hippocampus**

| **Nlrp1, Casp1, Casp6 genetically ablated J20 vs. J20 mice** | | **J20** | ***J20/Nlrp1*$^{-/-}$** | ***J20/Casp1*$^{-/-}$** | ***J20/Casp6*$^{-/-}$** | | ***J20/Nlrp1*$^{+/-}$** | ***J20/Casp1*$^{+/-}$** | ***J20/Casp6*$^{+/-}$** |
|---|---|---|---|---|---|---|---|---|---|
| **Amyloid plaques** | Mice (*n*) | 6 | 5 | 6 | 4 | | 6 | 6 | 6 |
| | Aβ staining density | | ▼ (p=0.001) | ▼ (p=0.001) | ▼ (p=0.007) | | ▼ (p=0.001) | ▼ (p=0.001) | ▼ (p=0.004) |
| | | | | | | | | | |
| **Amyloid protein levels (ELISA)** | Mice (*n*) | 6 | 6 | 6 | 5 | | 6 | 6 | 6 |
| Formic acid soluble amyloid levels | Total Aβ | | ▼ (p<0.0001) | ▼ (p=0.001) | ▼ (p=0.03) | | ▼ (p=0.002) | ▼ (p=0.0001) | ▼ (p=0.0002) |
| | Aβ38/total Aβ | | ▼ (p=0.005) | = | = | | = | ▼ (p=0.007) | = |
| | Aβ40/total Aβ | | ▲ (p<0.0001) | NS p=0.054 | = | | = | ▲ (p=0.0003) | ▲ (p=0.003) |
| | Aβ42/total Aβ | | ▼ (p=0.0005) | NS p=0.062 | = | | = | ▼ (p=0.028) | NS p=0.087 |
| RIPA soluble amyloid levels | Total Aβ | | ▼ (p=0.01) | = | = | | = | = | = |
| | Aβ38/total Aβ | | = | = | = | | = | = | = |
| | Aβ40/total Aβ | | = | = | = | | = | = | = |
| | Aβ42/total Aβ | | = | = | = | | = | = | = |

**Cortex**

| **Nlrp1, Casp1, Casp6 genetically ablated J20 vs. J20 mice** | | **J20** | ***J20/Nlrp1*$^{-/-}$** | ***J20/Casp1*$^{-/-}$** | ***J20/Casp6*$^{-/-}$** | | ***J20/Nlrp1*$^{+/-}$** | ***J20/Casp1*$^{+/-}$** | ***J20/Casp6*$^{+/-}$** |
|---|---|---|---|---|---|---|---|---|---|
| **Amyloid plaques** | Mice (*n*) | 6 | 5 | 6 | 4 | | 6 | 6 | 6 |
| | Aβ staining density | | = | = | = | | = | = | = |
| | | | | | | | | | |
| **Amyloid protein levels (ELISA)** | Mice (*n*) | 6 | 6 | 6 | 5 | | 6 | 6 | 6 |
| Formic acid soluble amyloid levels | Total Aβ | | = | = | = | | = | = | = |
| | Aβ38/total Aβ | | = | = | = | | = | = | = |
| | Aβ40/total Aβ | | = | = | = | | = | = | = |
| | Aβ42/total Aβ | | = | = | = | | = | = | = |
| RIPA soluble amyloid levels | Total Aβ | | = | = | = | | = | = | = |
| | Aβ38/total Aβ | | = | = | = | | = | = | = |
| | Aβ40/total Aβ | | = | = | = | | = | = | = |
| | Aβ42/total Aβ | | = | = | = | | = | = | = |

Immunohistochemical staining density and ELISA were done as described in methods. Upward arrow (▲) represents increased value, downward arrow (▼) represents decreased value, equal sign (=) represents unchanged

value. Significant ($p<0.05$) or non-significant (NS) but close ($0.1>p>0.05$), statistical p values are indicated. NS indicates not significant.

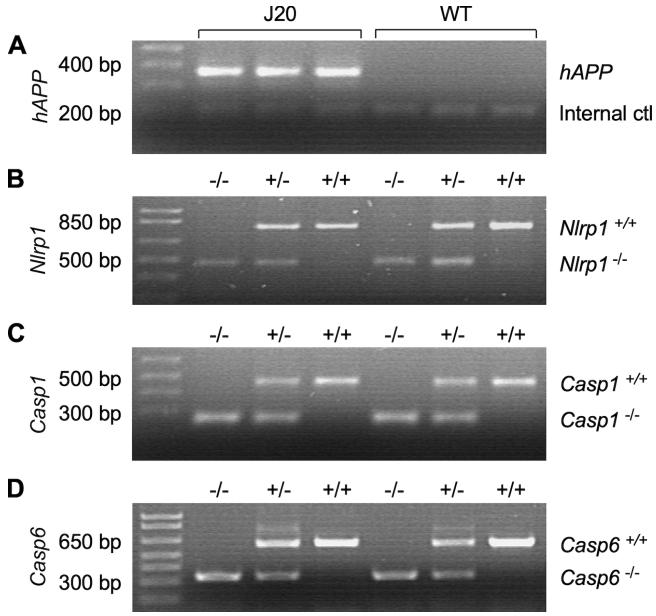


**Fig. S1. *Nlrp1*, *Casp1*, or *Casp6* can be ablated from J20 and WT mice.** Representative PCR of **A** 360 bp *hAPP*$^{Sw/Ind}$ transgene amplicon in J20 mice, **B** 490 bp null and 792 bp WT allele for *Nlrp1*, **C** 300 bp null and 500 bp WT allele for *Casp1*, and **D** 340 bp null and 620 bp WT allele for *Casp6*.

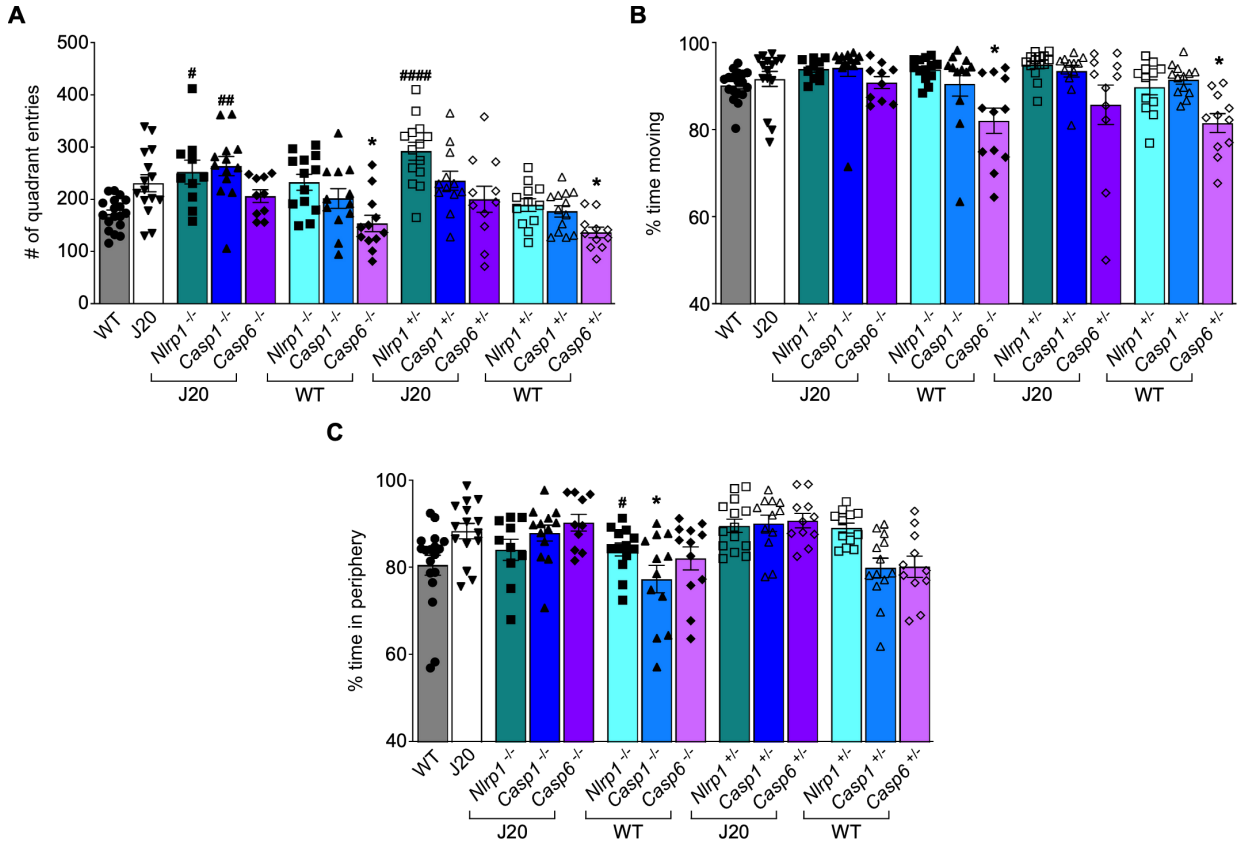


**Fig. S2. *Nlrp1*, *Casp1*, or *Casp6* genetic ablation does not alter locomotor behaviour.** Open field **A** number of quadrant entries [$F = 7.779$, $p < 0.0001$], **B** percentage time moving [$F = 4.745$, $p < 0.0001$], and **C** percentage time in periphery, indicative of thigmotaxis [$F = 4.863$, $p < 0.0001$]. Bars represent mean ± SEM of all mice per group; symbols denote performance of individual mice. $n$ = 18 WT, 15 J20, 10 J20/*Nlrp1*$^{-/-}$, 13 J20/*Casp1*$^{-/-}$, 10 J20/*Casp6*$^{-/-}$, 13 WT/*Nlrp1*$^{-/-}$, 12 WT/*Casp1*$^{-/-}$, 12 WT/*Casp6*$^{-/-}$, 14 J20/*Nlrp1*$^{+/-}$, 12 J20/*Casp1*$^{+/-}$, 11 J20/*Casp6*$^{+/-}$, 12 WT/*Nlrp1*$^{+/-}$, 13 WT/*Casp1*$^{+/-}$, 11 WT/*Casp6*$^{+/-}$ for (**A-C**). One-way ANOVA, Bonferroni's post-hoc compared to WT ($^{\#}$) or J20 (*). $^{\#}$ or * $p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#\#}p < 0.0001$.

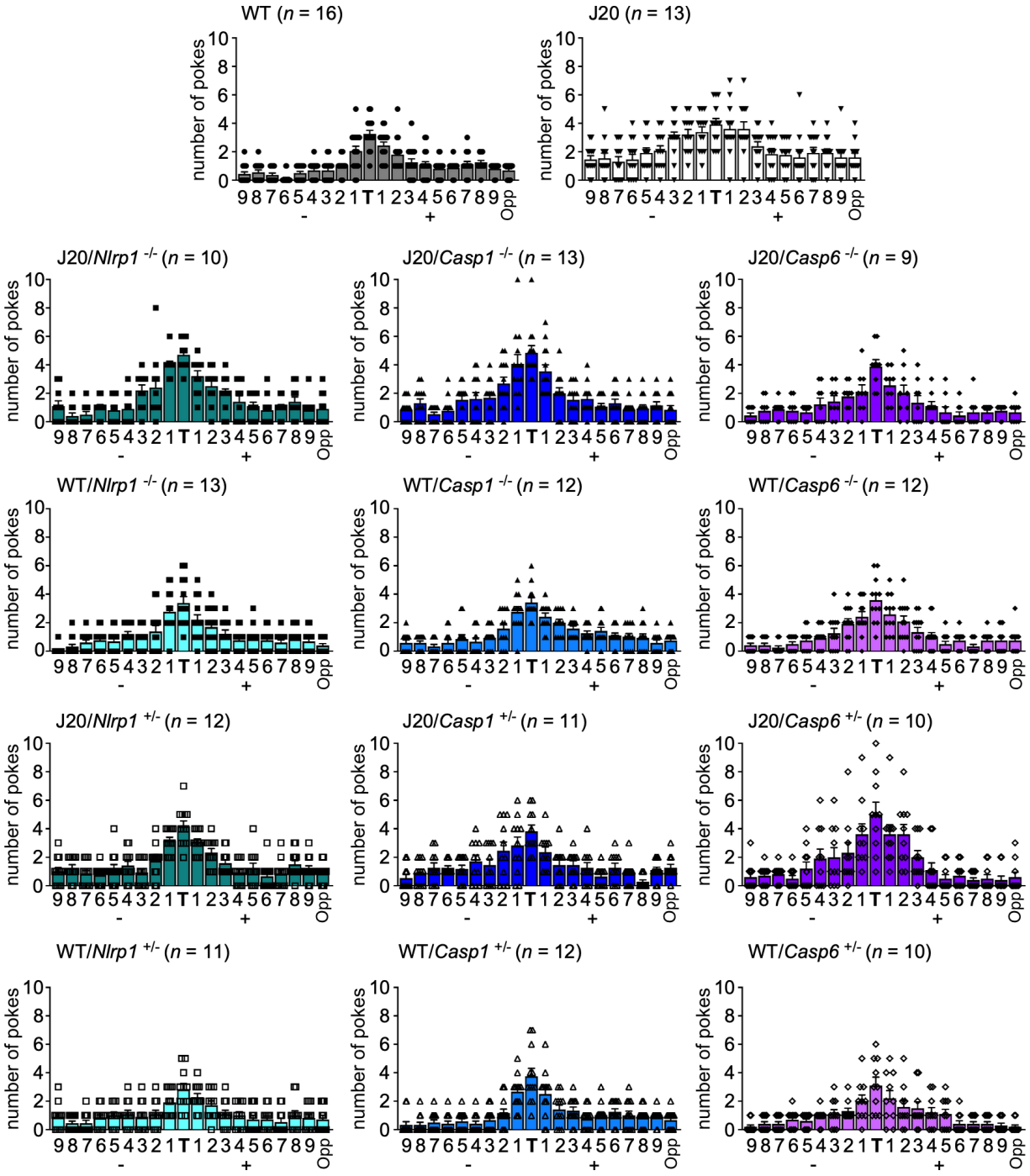


**Fig. S3**. ***Nlrp1*, *Casp1*, or *Casp6* genetic ablation prevents cognitive deficits in J20 mice.** Barnes maze distribution of pokes to each hole during the probe, where (T) indicates target hole. Bars represent mean ± SEM of all mice per group; symbols denote performance of individual mice.

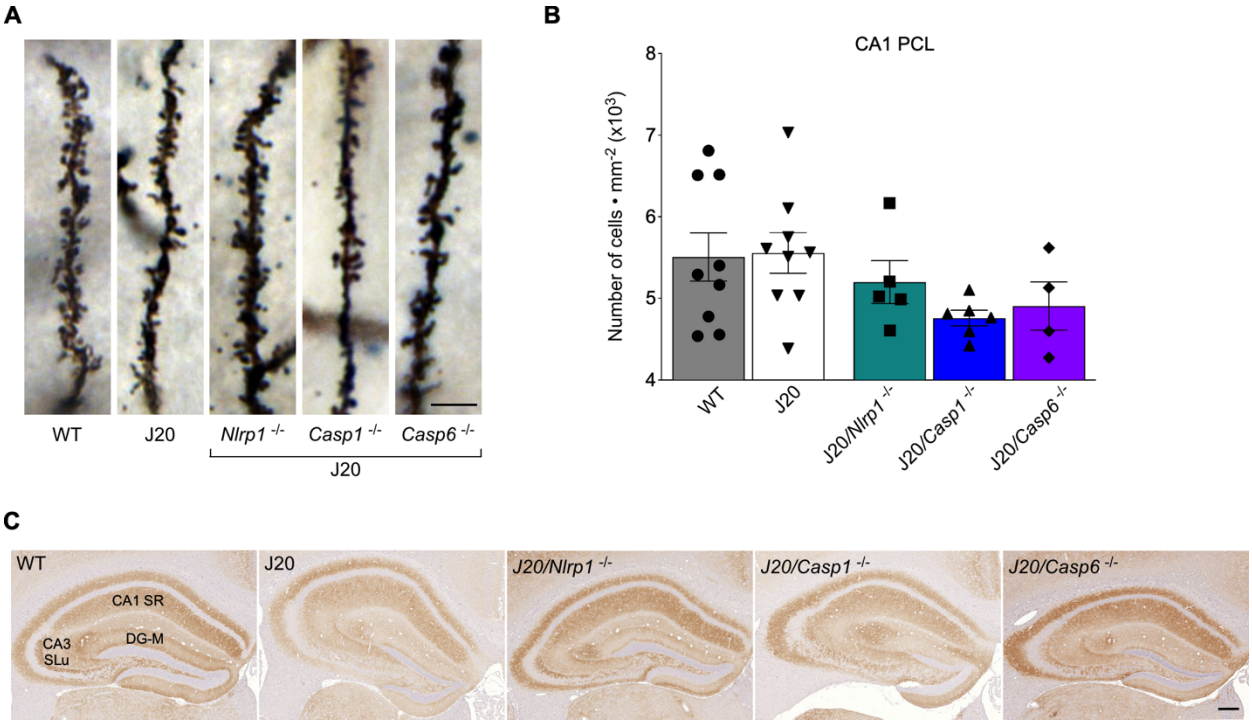


**Fig. S4. Synaptic density alterations after *Nlrp1*, *Casp1*, or *Casp6* genetic ablation in J20 mice. A** Representative Golgi-Cox-stained dendritic spines in the stratum radiatum (SR) of the hippocampal CA1. Scale bar = 5 µm. **B** Cell density in the pyramidal cell layer (PCL) of the hippocampal CA1. **C** Representative synaptophysin staining of the hippocampus. CA1 SR = stratum radiatum of the CA1, CA3 SLu = stratum lucidum of the CA3, DG-M = molecular layer of the dentate gyrus. Scale bar = 200 µm.

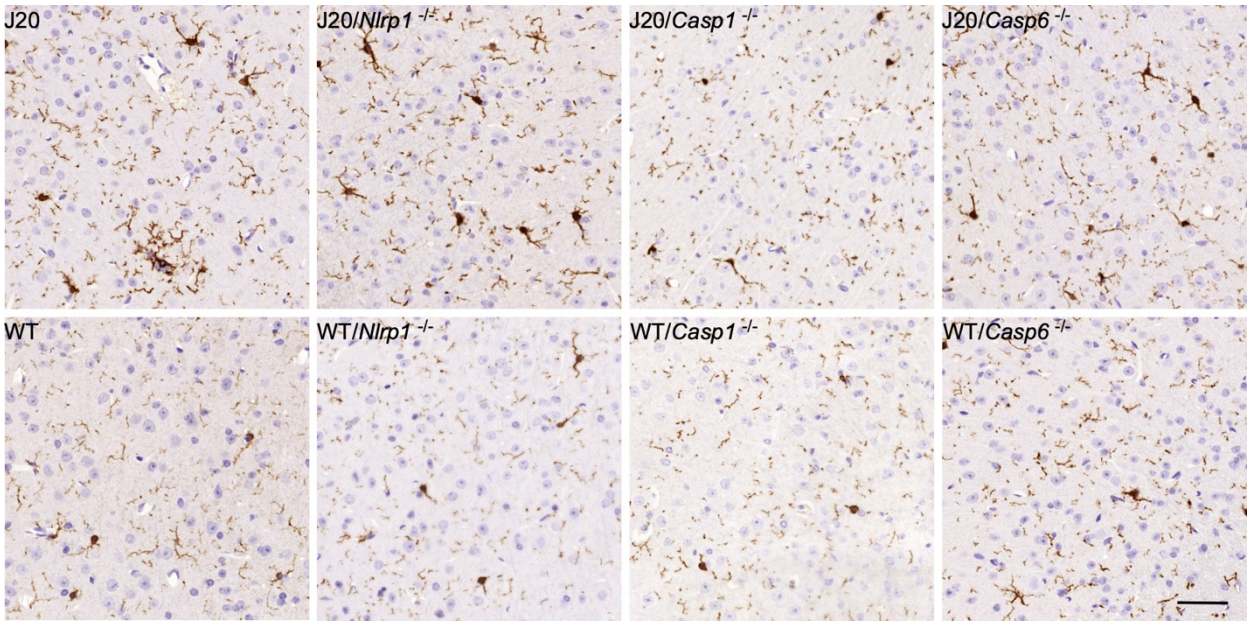


**Fig. S5. *Nlrp1*, *Casp1*, or *Casp6* genetic ablation reduces microglia activation in J20 mice.** Representative micrographs of $Iba1^{+}$ immunostained-microglia in the retrosplenial cortex. Scale bar = 50 μm.

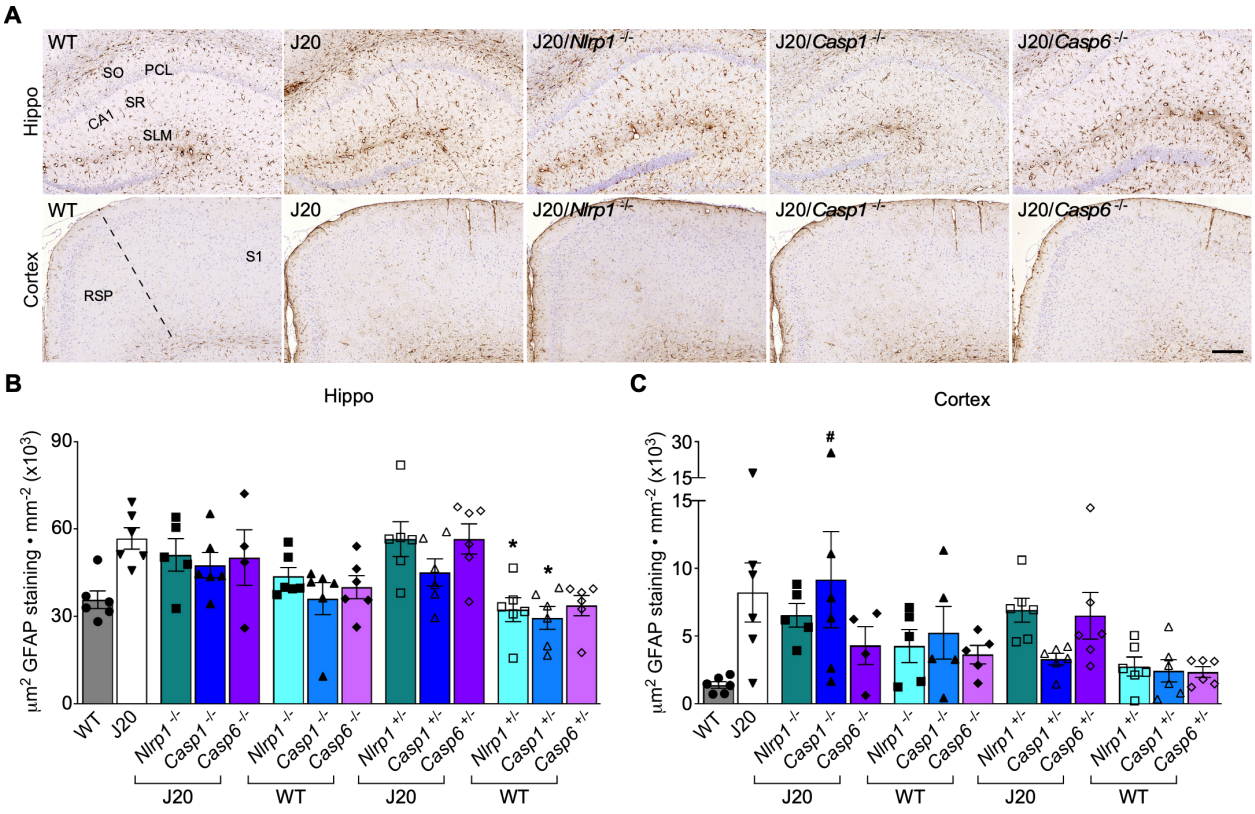


**Fig. S6. *Nlrp1*, *Casp1*, or *Casp6* genetic ablation does not alter GFAP$^+$ astrocytes in J20 mice. A** Representative GFAP$^+$ micrographs showing the stratum oriens (SO), pyramidal cell layer (PCL), SR, and stratum lacunosum-moleculare (SLM) of the hippocampal CA1 (top) and retrosplenial and S1 cortex (bottom). Scale bar = 200 µm. **B** GFAP$^+$ staining density from the SO to the SLM in the hippocampal CA1 [F = 4.317, p = 0.000032]. **C** GFAP$^+$ staining density in the cortical retrosplenial and S1 area [F = 2.572, p = 0.0063]. Bars represent mean ± SEM of all mice per group; symbols denote individual results. *n* = 6 WT, 6 J20, 5 J20/*Nlrp1*$^{-/-}$, 6 J20/*Casp1*$^{-/-}$, 4 J20/*Casp6*$^{-/-}$, 6 WT/*Nlrp1*$^{-/-}$, 6 WT/*Casp1*$^{-/-}$, 6 WT/*Casp6*$^{-/-}$, 6 J20/*Nlrp1*$^{+/-}$, 6 J20/*Casp1*$^{+/-}$, 6 J20/*Casp6*$^{+/-}$, 6 WT/*Nlrp1*$^{+/-}$, 6 WT/*Casp1*$^{+/-}$, 6 WT/*Casp6*$^{+/-}$ for (**B,C**). One-way ANOVA, Bonferroni's post-hoc compared to WT ($^{\#}$) or J20 (*). $^{\#}$ or * $p < 0.05$.

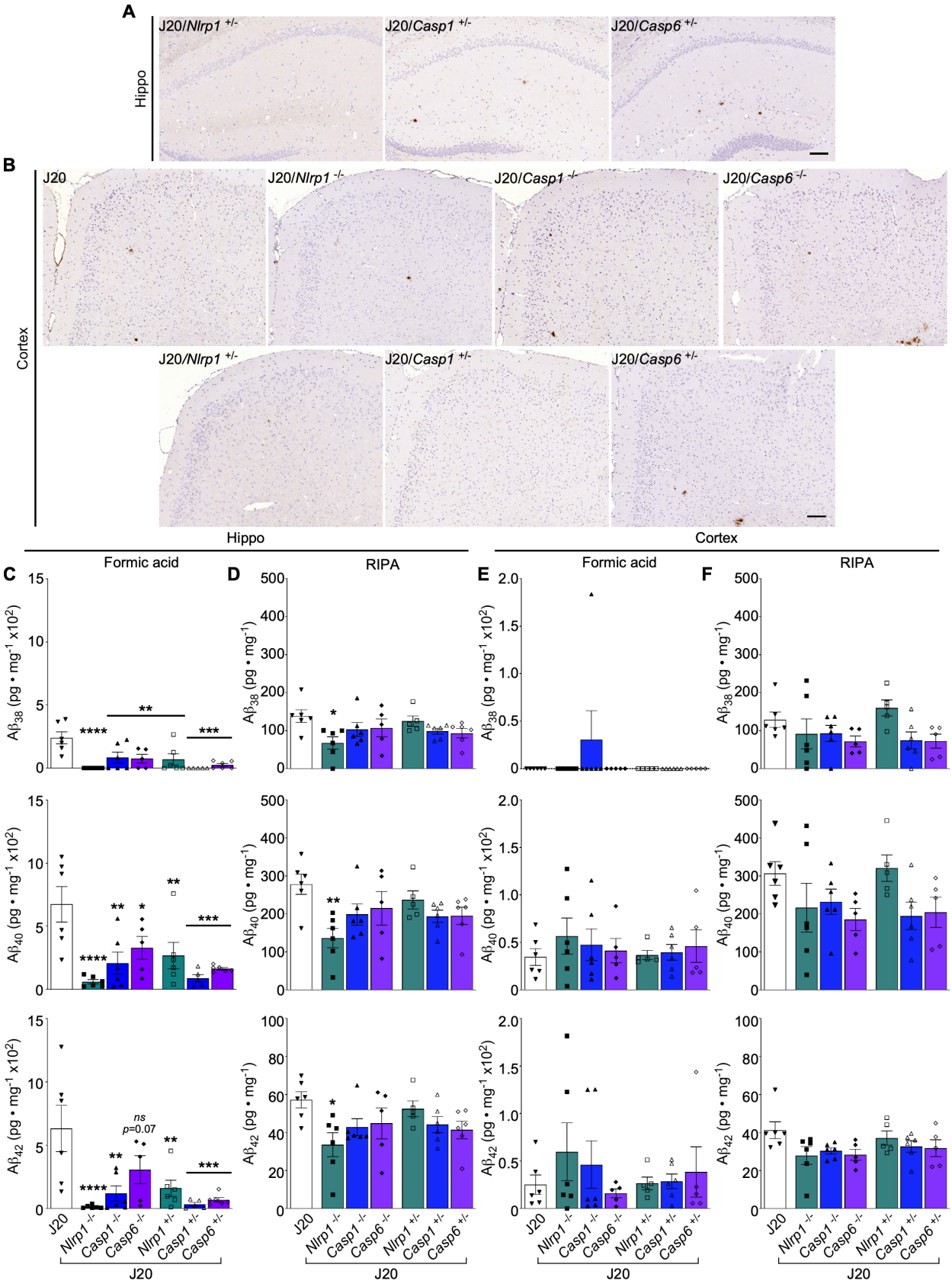
A
Hippo
J20/Nlrp1 +/-
J20/Casp1 +/-
J20/Casp6 +/-
B
Cortex
J20
J20/Nlrp1 -/-
J20/Casp1 -/-
J20/Casp6 -/-
J20/Nlrp1 +/-
J20/Casp1 +/-
J20/Casp6 +/-
Hippo
Cortex
C
Formic acid
D
RIPA
E
Formic acid
F
RIPA
Aβ38 (pg • mg-1 x102)
Aβ40 (pg • mg-1 x102)
Aβ42 (pg • mg-1 x102)
Aβ38 (pg • mg-1)
Aβ40 (pg • mg-1)
Aβ42 (pg • mg-1)
ns
p=0.07
J20
Nlrp1 -/-
Casp1 -/-
Casp6 -/-
Nlrp1 +/-
Casp1 +/-
Casp6 +/-
J20

**Fig. S7. *Nlrp1*, *Casp1*, or *Casp6* genetic ablation prevents Aβ deposition in J20 mice. A-B** Representative Aβ-stained micrographs of the **A** hippocampus and **B** cortical retrosplenial and S1 area. Scale bars = 100 μm. **C** Hippocampal formic acid-soluble $A\beta_{38}$ [F = 6.346, p = 0.0001], $A\beta_{40}$ [F = 6.198, p = 0.0001], and $A\beta_{42}$ [F = 6.166, p = 0.0002] protein levels. **D** Hippocampal RIPA-soluble $A\beta_{38}$, $A\beta_{40}$ [F = 2.733, p = 0.02], and $A\beta_{42}$ protein levels. **E** Cortical formic acid-soluble $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ protein levels. **F** Cortical RIPA-soluble total $A\beta_{38}$, $A\beta_{40}$, and $A\beta_{42}$ protein levels. Bars represent mean ± SEM of all mice per group; symbols denote performance of individual mice. *n* = 6 J20, 6 J20/*Nlrp1*$^{-/-}$, 6 J20/*Casp1*$^{-/-}$, 5 J20/*Casp6*$^{-/-}$, 6 J20/*Nlrp1*$^{+/-}$, 6 J20/*Casp1*$^{+/-}$, 6 J20/*Casp6*$^{+/-}$ for (**C-F**). One-way ANOVA, Dunnett's post-hoc compared to J20. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.