Supplemental Figure 1. LeBlanc et al., Casp6 in AD and mice brains.

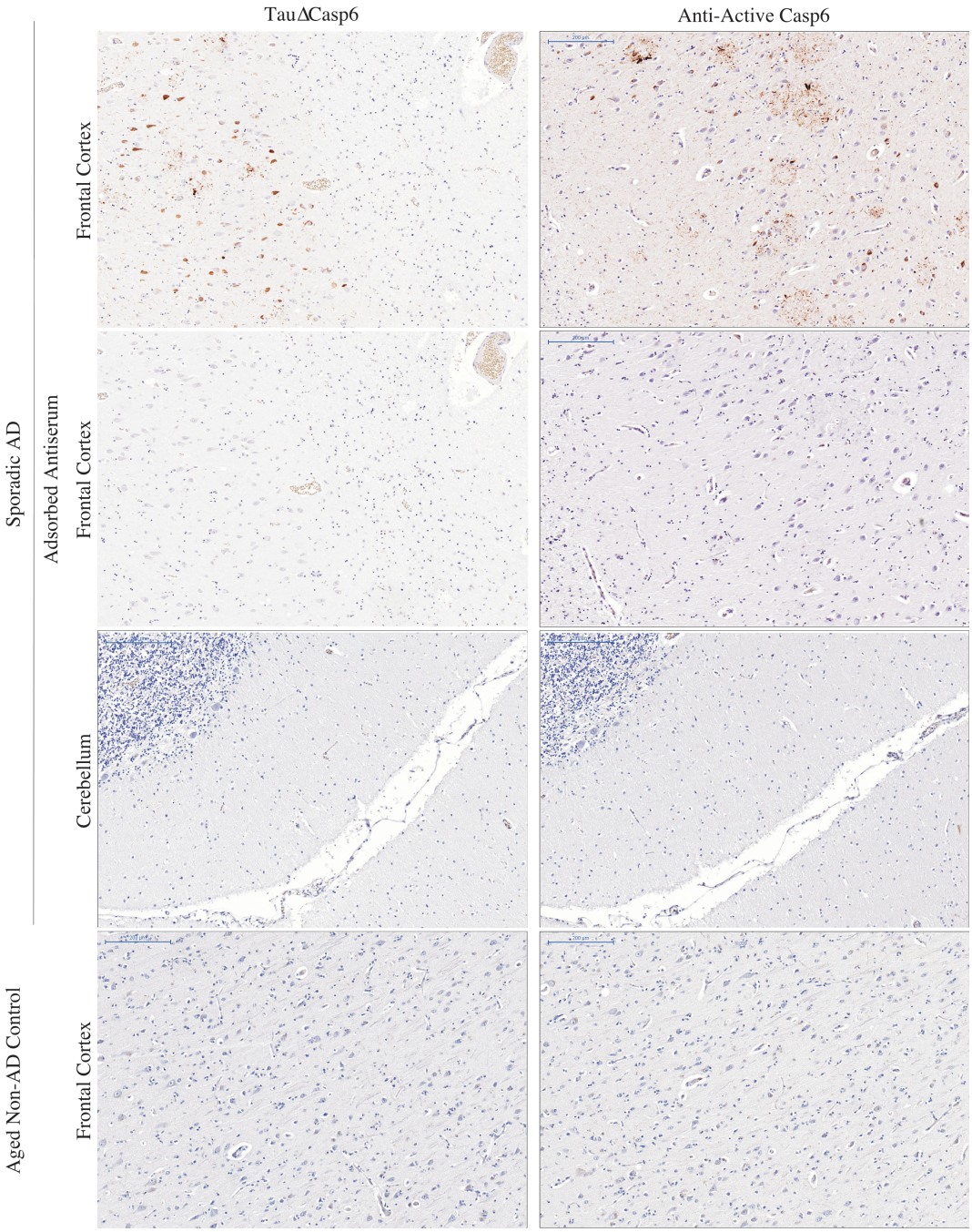

Figure 1S*: *Specificity of anti-Casp6 and TauΔCasp6 antisera. Immunohistochemical staining of human brain tissue sections from a sporadic AD and a non-AD aged matched control. Results show that the positive staining for the TauΔCasp6 and anti-active Casp6 are completely adsorbed with recombinant TauΔCasp6 and Casp6, respectively. The two antisera do not stain cerebellum from AD brains or non-AD control.

Supplemental Figure 2. LeBlanc et al., Casp6 in AD and mice brains.

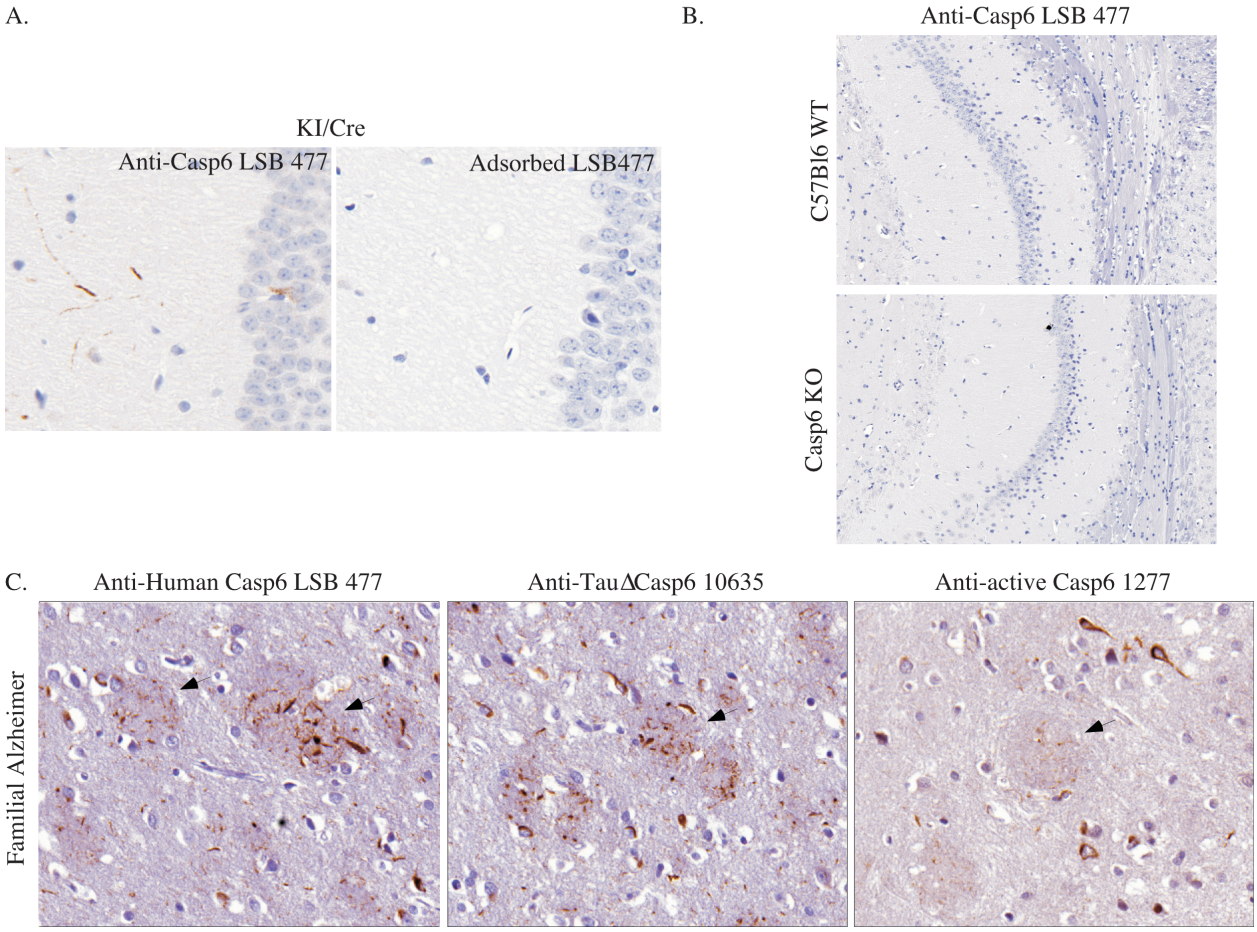

Figure 2S: **Specificity of anti-human Casp6 LSB477 antibody.** Immunohistochemical staining of mouse brains with anti-human Casp6 antibody LSB 477. **A.** Comparison of consecutive sections of a KI/Cre mouse brain with LSB 477 without or with adsorption on recombinant Casp6. **B.** Absence of anti-human casp6 immunoreactivity in the CA1 of WT and Casp6 null mice (from JAX). **C.** Anti-human Casp6 LSB477 compared to anti-TauΔCasp6 and anti-active Casp6 immunostaining in a familial AD case. The LSB477 anti-human Casp6 immunostains the pathology observed with anti-active casp6 and TauΔcasp6 neoepitope antisera [1].

Supplemental Figure 3. LeBlanc et al., Casp6 in AD and mice brains.

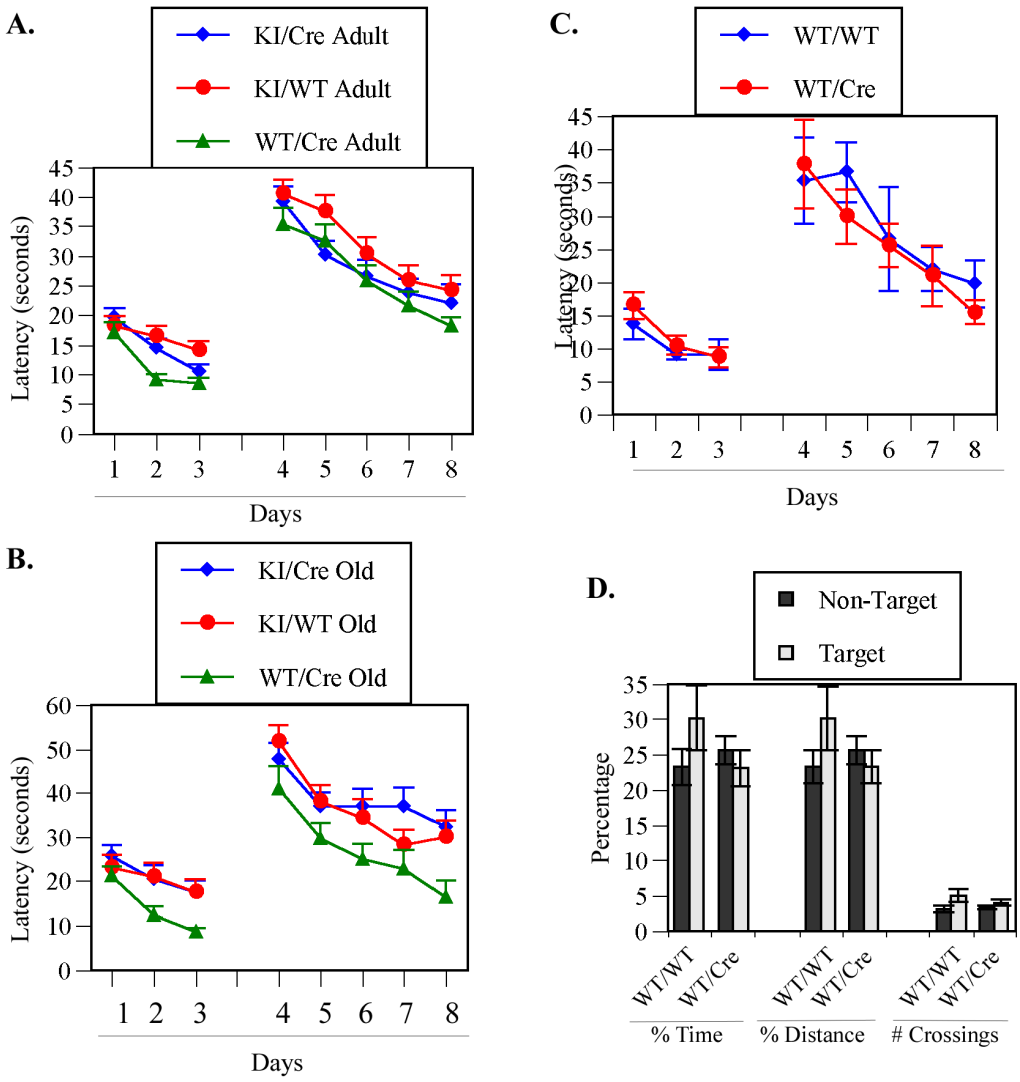

Figure 3S: Acquisition of platform location in the Morris water Maze. **A.** Latency (seconds) of adult mice (≤15 months) to reach visible (day 1-3) and hidden platform (days 4 to 8). Numbers of mice tested are 55 adult (< 15 months old; range of 3.7 to 14.3 months) KI/Cre, 62 adults (range 3.7 to 16.4 months) KI/WT, and 41 adults (range of 3.2 to 13 month old) WT/Cre. **B.** Latency (seconds) of adult mice (≥15 months) to reach visible (day 1-3) and hidden platform (days 4 to 8). Numbers of old mice tested are 36 old (> 15 month old; range of 15.6 to 17.8 months) KI/Cre, 31 old (range of 15.6 to 17. 7 months) KI/WT, and 16 old (range of 16.4 to 19.6

months) WT/Cre. **C.** Latency in seconds for WT/WT and WT/Cre as described in A and B. Numbers of mice tested are n=8 for WT/Wt and n=14 for WT/Cre. **D.** Comparison of WT/Cre mice in water maze with WT/WT littermate controls on the probe day (no platform) of the Morris water maze. The study was done with two groups of male mice at two different times. Group 1 (12-17 months) contained 6 WT/Cre and 5 WT/WT littermates and group 2 (16.67-17.70 months) had 5 WT/WT and 10 WT/Cre littermates. The WT/WT showed a statistically significant preference for the target quadrant compared to the non-target quadrants ($*p \leq 0.05$) while the WT/Cre showed less preference for the target quadrant than the non-target quadrants ($*p \leq 0.01$).

Supplemental Figure 4S. LeBlanc et al., Casp6 in AD and mice brains.

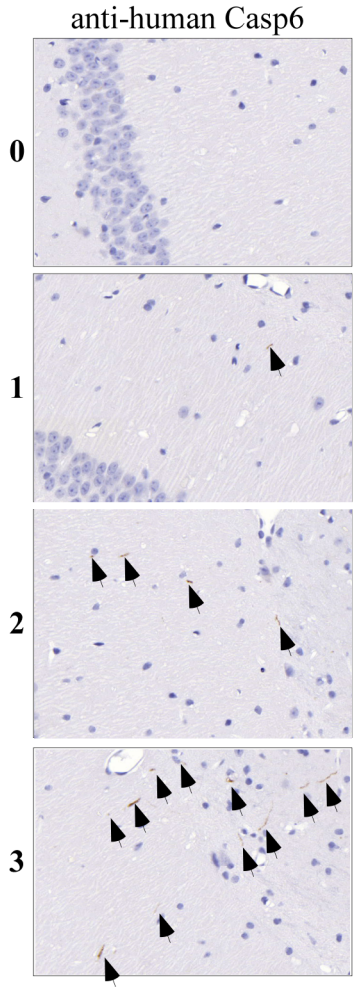

Figure 4S. Example of the abundance of anti-human Casp6 LSB477 immunostaining in mouse hippocampal tissue sections. Casp6 semi-quantitative immunoreactivity score of 0 means absent immunoreactivity, 1 means rare, 2 means frequent, and 3 means abundant immunoreactive neurites. Arrows indicate immunopositive neurites.

Supplemental Figure 5S. LeBlanc et al., Casp6 in AD and mice brains.

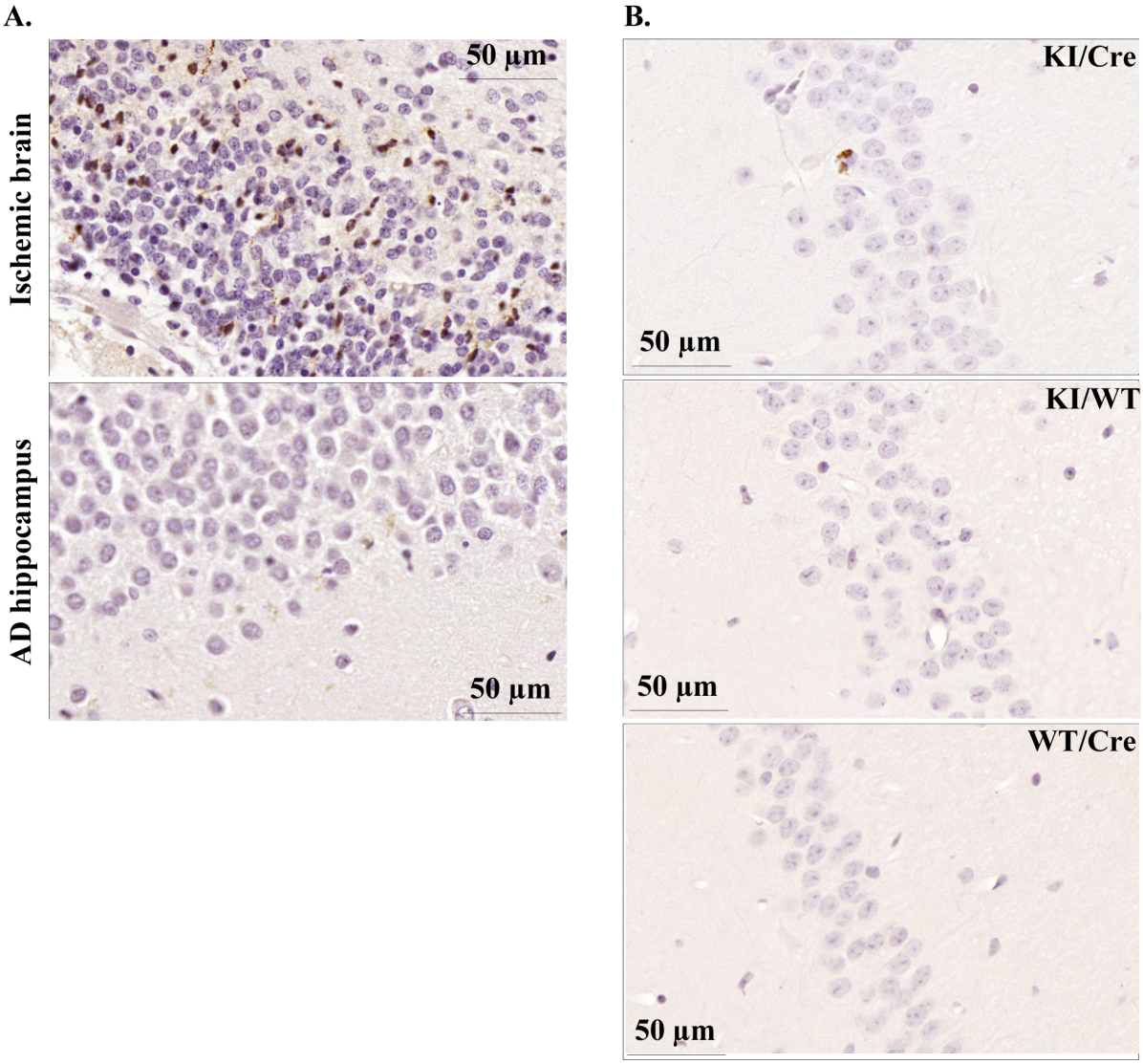

Figure 5S. Immunohistochemistry against active Caspase-3. **A.** Ischemic fetal brain serves as a positive control for the anti-active Casp3 immunoreactivity while AD hippocampal neurons remain negative. **B.** Three sections each of the hippocampus of three independent mice (aged 4 month, 9 month and 20 month old) with the KI/Cre, KI/WT, and WT/Cre genotypes were stained with the anti-active Caspase-3 antibody. The results showed only one active Casp3-positive positive cell in two sections in the 9 month case of the KI/Cre cases, but large amounts of immunopositive neurons in the fetal ischemic brain. There were no immunostaining of the 4 and

20 month old KI/Cre cases, of any of the KI/WT and WT/Cre mice cases, and in the AD brain tissue sections.

Reference for supplemental:

1. Albrecht S, Bogdanovic N, Ghetti B, Winblad B, LeBlanc AC. Caspase-6 activation in familial Alzheimer disease brains carrying amyloid precursor protein or presenilin I or presenilin II mutations. *J Neuropathol Exp Neurol* 2009, **68**(12)**:** 1282-1293.