

Supplementary Figure S1

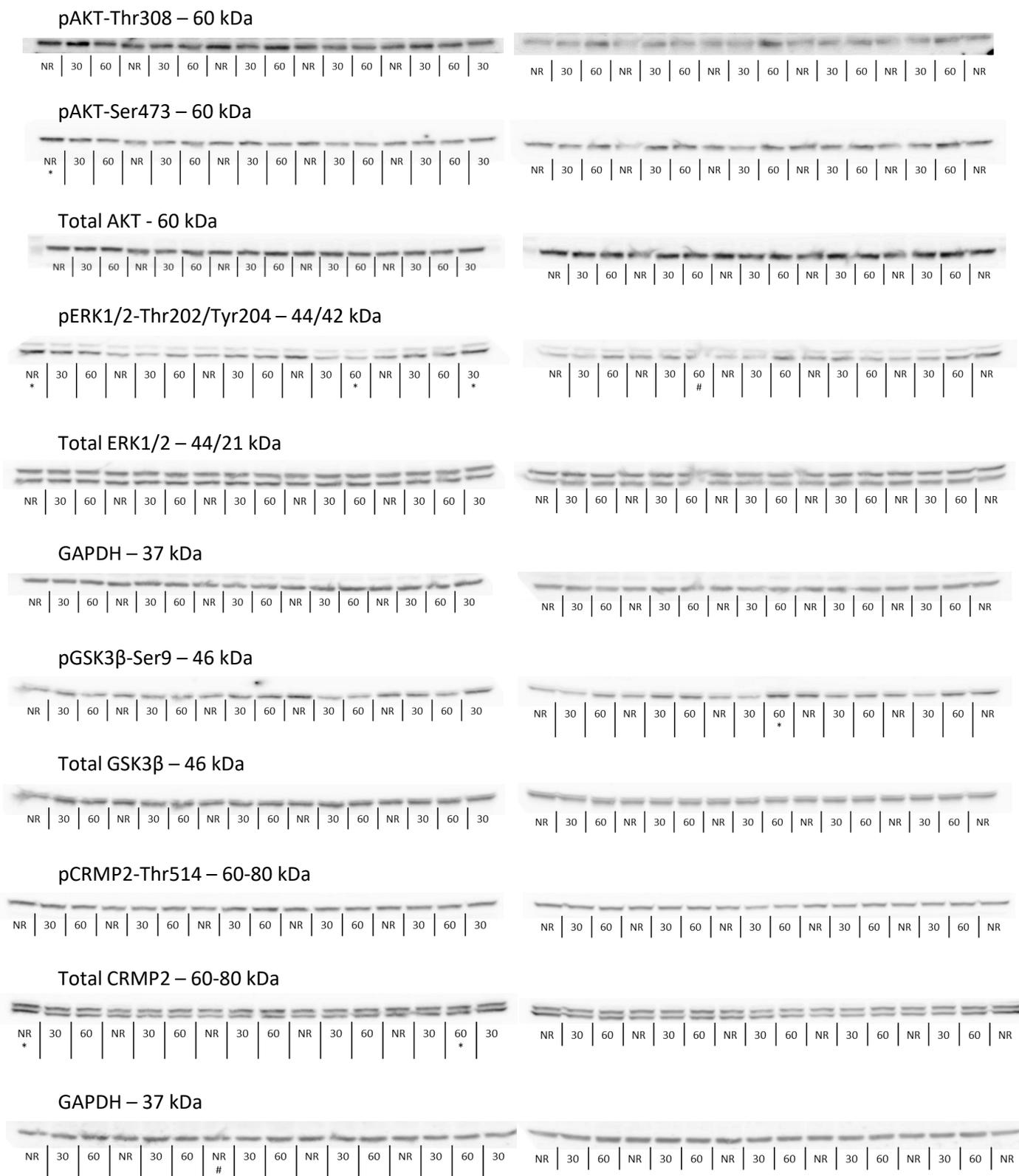


Figure S1. Western-blot visualization of phospho-protein and total-protein levels in the mPFC (Experiment 1). The tissues were collected 30 min (30) or 60 min (60) after alcohol memory retrieval, or without a prior retrieval procedure (NR). Phospho-protein levels of AKT (Thr308, Ser473), GSK3β (Ser9), CRMP2 (Thr514) and ERK1/2 (Thr202/Tyr204) were normalized to the total protein immunoreactivity. Total protein levels of CRMP2 were normalized to GAPDH. AKT and ERK1/2 antibodies were blotted on the same set of membranes, and therefore share the GAPDH levels. GSK3β and CRMP2 were blotted on a different set of membranes. * Samples that were identified as outliers to their experimental group ($z > 2$) and were therefore removed from the analysis. # Distorted bands that were removed from the analysis.

Supplementary Figure S2

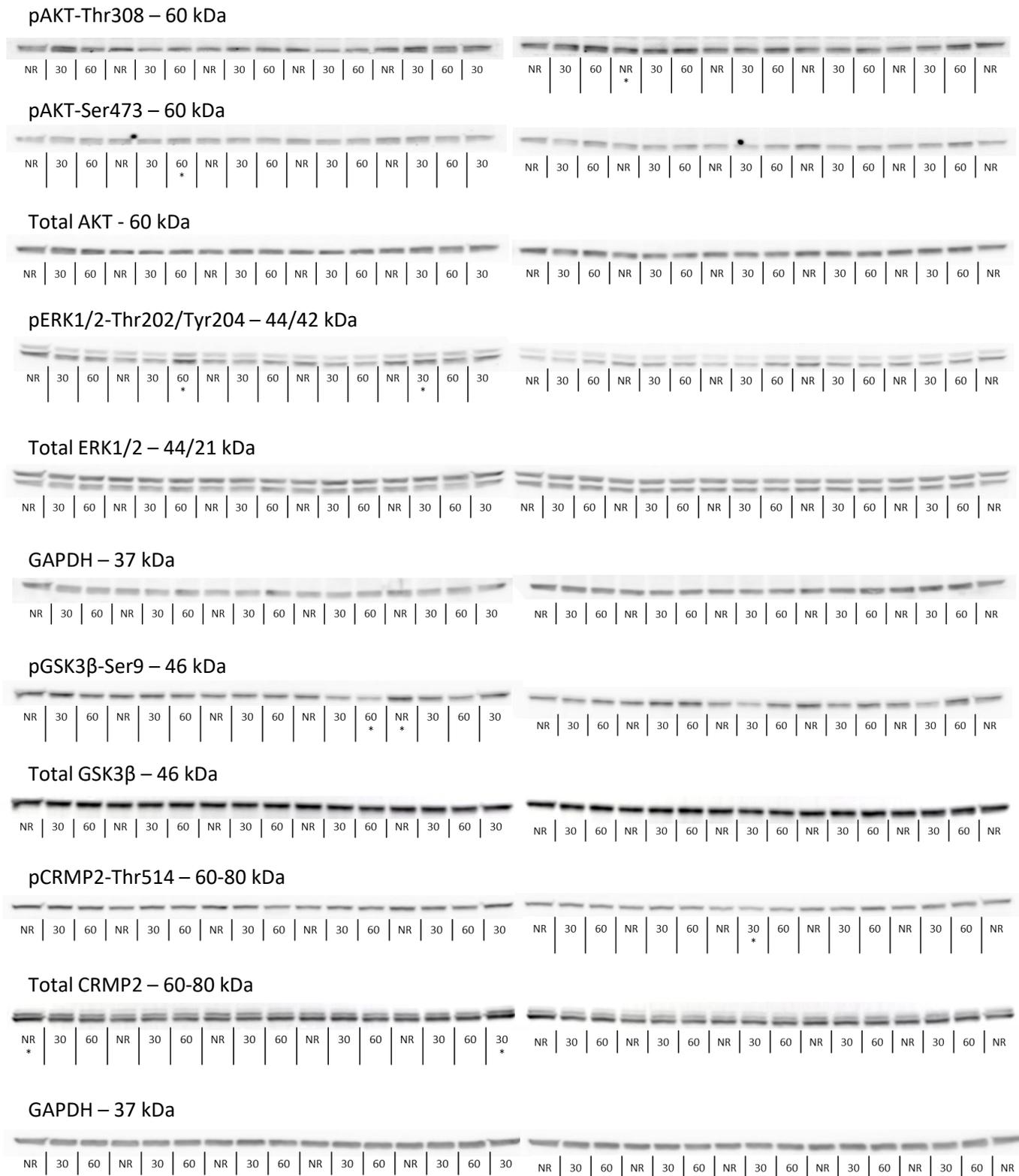


Figure S2. Western-blot visualization of phospho-protein and total-protein levels in the NAc (Experiment 1). The tissues were collected 30 min (30) or 60 min (60) after alcohol memory retrieval, or without a prior retrieval procedure (NR). Phospho-protein levels of AKT (Thr308, Ser473), GSK3 β (Ser9), CRMP2 (Thr514) and ERK1/2 (Thr202/Tyr204) were normalized to the total protein immunoreactivity. Total protein levels of CRMP2 were normalized to GAPDH. AKT and ERK1/2 antibodies were blotted on the same set of membranes, and therefore share the GAPDH levels. GSK3 β and CRMP2 were blotted on a different set of membranes. * Samples that were identified as outliers to their experimental group ($z > 2$) and were therefore removed from the analysis.

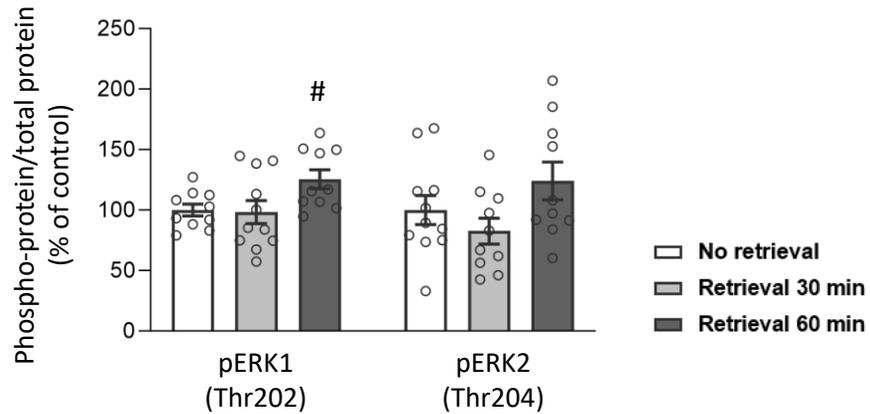
Supplementary Figure S3



Figure S3. Western-blot visualization of phospho-protein and total-protein levels in the amygdala (Experiment 1). The tissues were collected 30 min (30) or 60 min (60) after alcohol memory retrieval, or without a prior retrieval procedure (NR). Phospho-protein levels of AKT (Thr308, Ser473), GSK3 β (Ser9), CRMP2 (Thr514) and ERK1/2 (Thr202/Tyr204) were normalized to the total protein immunoreactivity. Total protein levels of CRMP2 were normalized to GAPDH. AKT and ERK1/2 antibodies were blotted on the same set of membranes, and therefore share the GAPDH levels. GSK3 β and CRMP2 were blotted on a different set of membranes. * Samples that were identified as outliers to their experimental group ($z > 2$) and were therefore removed from the analysis.

Supplementary Figure S5

A



B

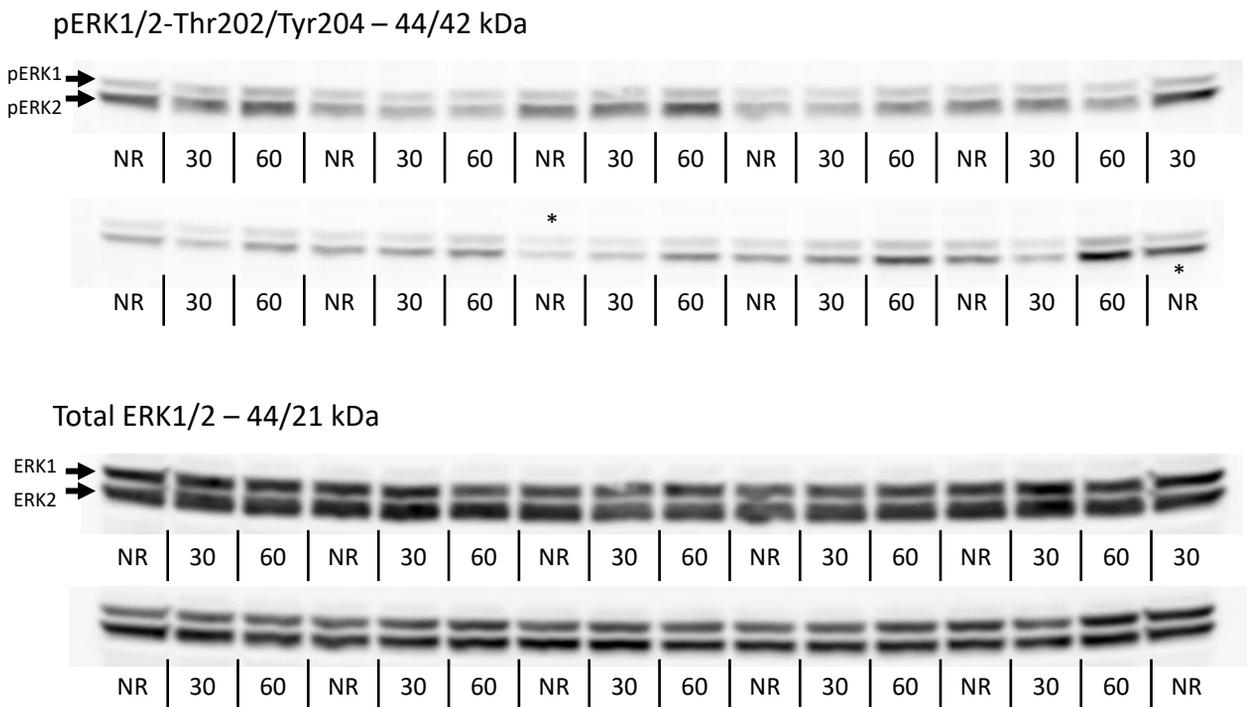
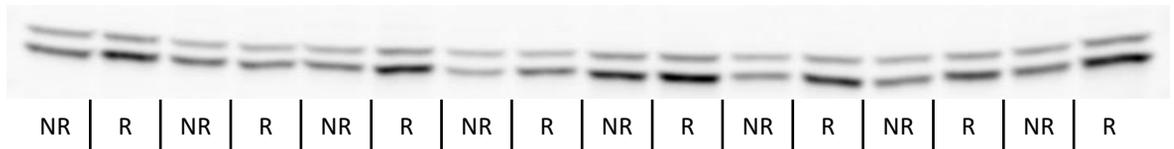


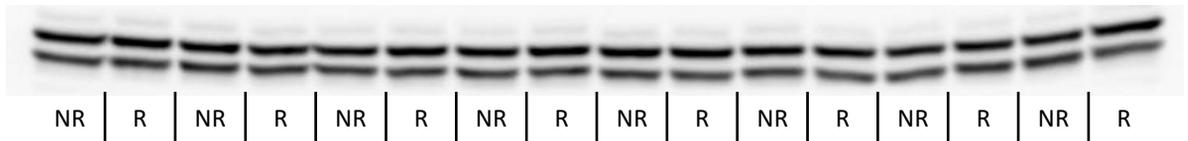
Figure S5. Alcohol memory retrieval increases ERK1 phosphorylation in the amygdala (Experiment 1). **A.** Separate analyses of ERK1 and ERK2 Phosphorylation levels in amygdala samples collected 30 and 60 min after alcohol memory retrieval. Phospho-protein levels of extracellular regulated kinase1/2 (ERK1/2) (Thr202, Tyr204) were normalized to the total protein immunoreactivity. Increased phosphorylation of ERK1 was observed 60 min after the retrieval (one-way ANOVA: pERK1: $F(2,28)=3.75$, $p=0.036$; Dunnett post hoc: No-retrieval vs. retrieval 30-min, $p=0.982$, No-retrieval vs. retrieval 60-min, $p=0.057$; pERK2: $F(2,28)=2.56$, $p=0.096$). Bar graphs represent mean \pm SEM of the percent of change from the control No retrieval group. $n=10-11$ (4-5 males, 5-6 females). # $p = 0.057$ **B.** Western-blot visualization of phospho-protein and total-protein levels. * Samples that were identified as outliers to their experimental group ($z>2$) and were therefore removed from the analysis.

Supplementary Figure S6

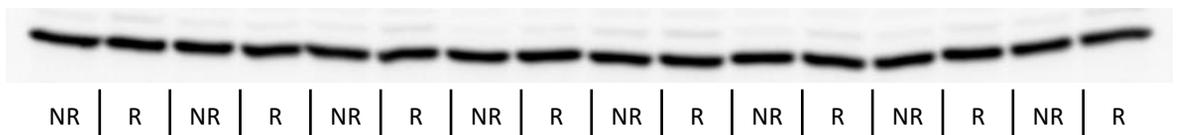
pERK1/2-Thr202/Tyr204 – 44/42 kDa



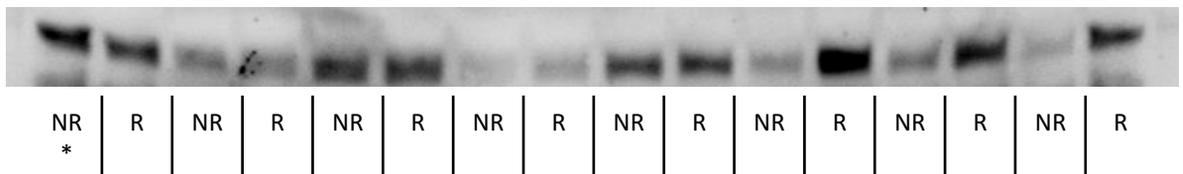
Total ERK1/2 – 44/21 kDa



GAPDH – 37 kDa



pCREB-Ser133 – 43 kDa



Total CREB-Ser133 – 43 kDa



GAPDH – 37 kDa



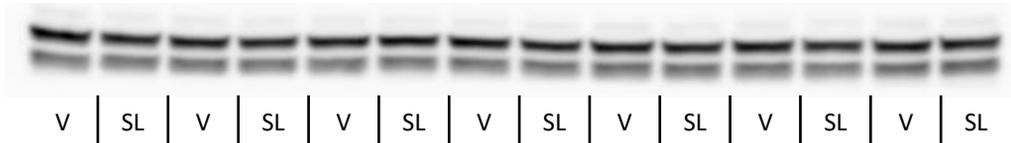
Figure S6. Western-blot visualization of phospho-protein and total-protein levels in the amygdala (Experiment 4). The tissues were collected two hours after alcohol memory retrieval (R), or without a prior retrieval procedure (NR). Phospho-protein levels of ERK1/2 (Thr202/Tyr204) and CREB (Ser133) were normalized to the total protein immunoreactivity. ERK1/2 antibodies were blotted on one membrane and CREB antibodies were blotted on a different set of membrane. * Sample that was identified as an outlier to its experimental group ($z > 2$) and was therefore removed from the analysis

Supplementary Figure S7

pERK1/2-Thr202/Tyr204 – 44/42 kDa



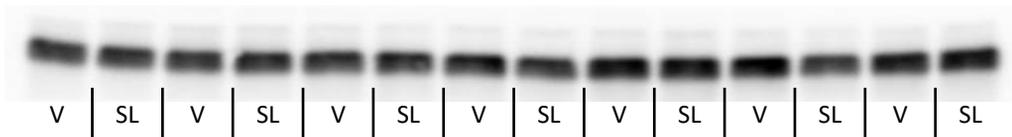
Total ERK1/2 – 44/21 kDa



pS6 – 32 kDa



Total S6 – 32 kDa



GAPDH – 37 kDa

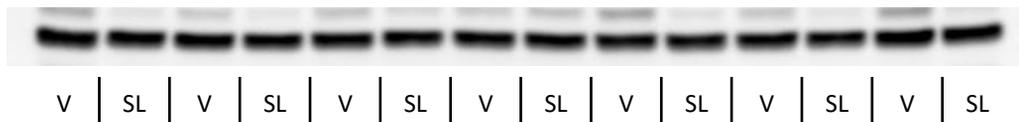


Figure S7. Western blot visualization of phospho-protein and total-protein levels in the amygdala (Experiment 5). The tissues were collected 90 min after injection of vehicle (V) or the MERK1/2 inhibitor SL-327 (50 mg/kg, i.p.) (SL). Phospho-protein levels of ERK1/2 (Thr202/Tyr204) and S6 (Ser235/236) were normalized to the total protein immunoreactivity. * Sample that was identified as an outlier to its experimental group ($z > 2$) and was therefore removed from the analysis.

Experiment 1

Tissues were collected 30 min (30) or 60 min (60) after alcohol memory retrieval, or without a prior retrieval procedure (NR).

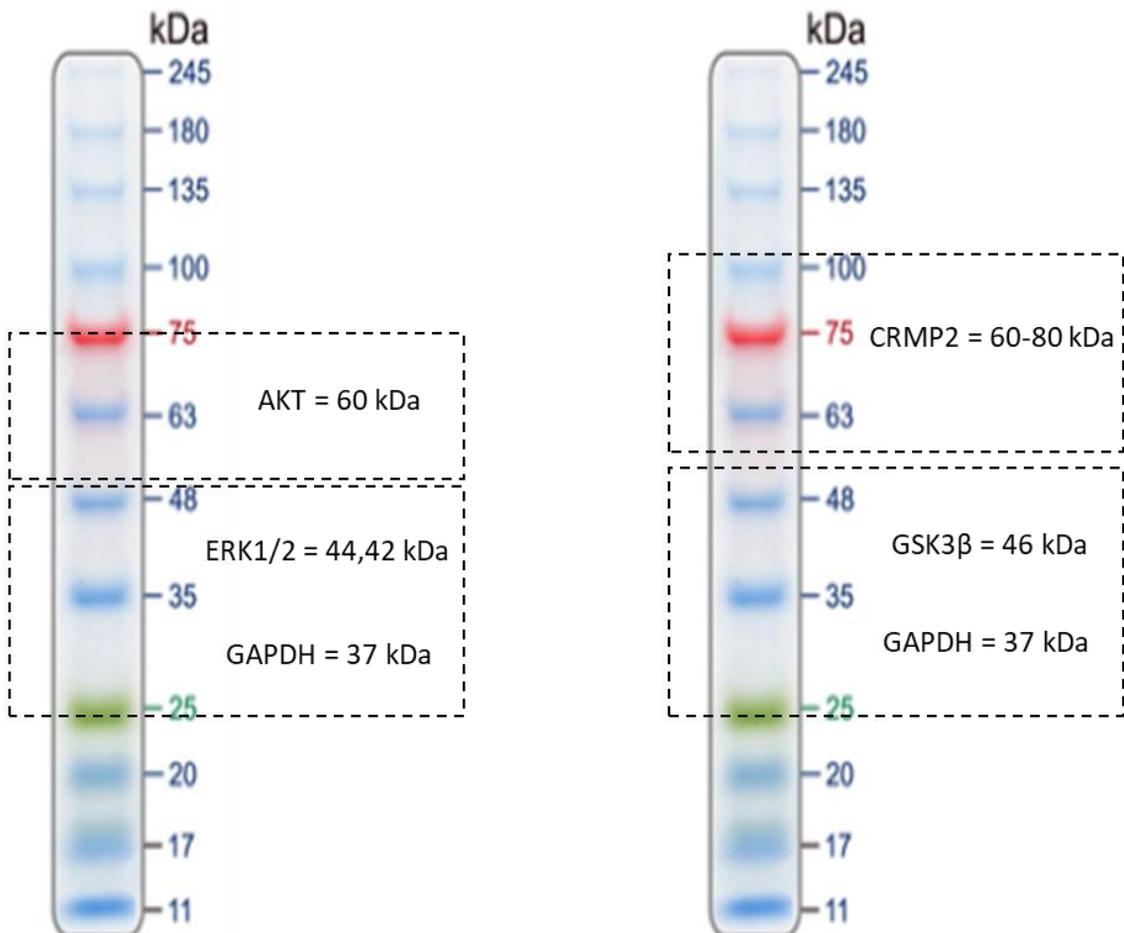
Due to the large number of samples, the samples were distributed across two gels / membranes.

AKT and ERK1/2 antibodies were blotted on the same set of membranes, and therefore share the GAPDH levels. GSK3 β and CRMP2 were blotted on a different set of membranes.

The membranes were cut to segments prior to the blotting process, as illustrated below, therefore images of the complete membranes are not available.

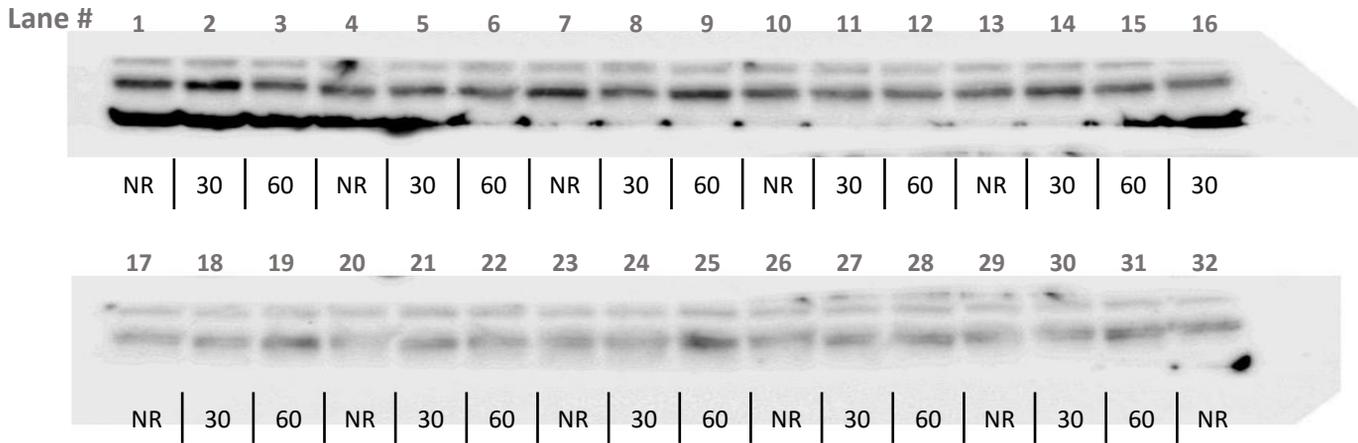
* Samples that were identified as outliers to their experimental group ($z > 2$), and were therefore removed from the analysis.

Distorted bands that were removed from the analysis.

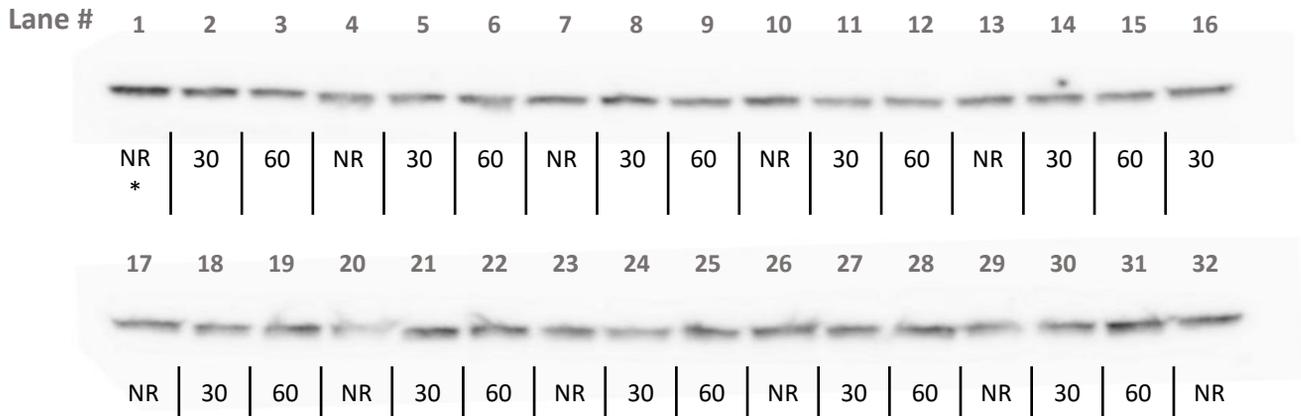


mPFC

pAKT-Thr308 – 60 kDa (Figures 1B and S1)



pAKT-Ser473 – 60 kDa (Figures 1B and S1)



Total AKT - 60 kDa (Figures 1B and S1)

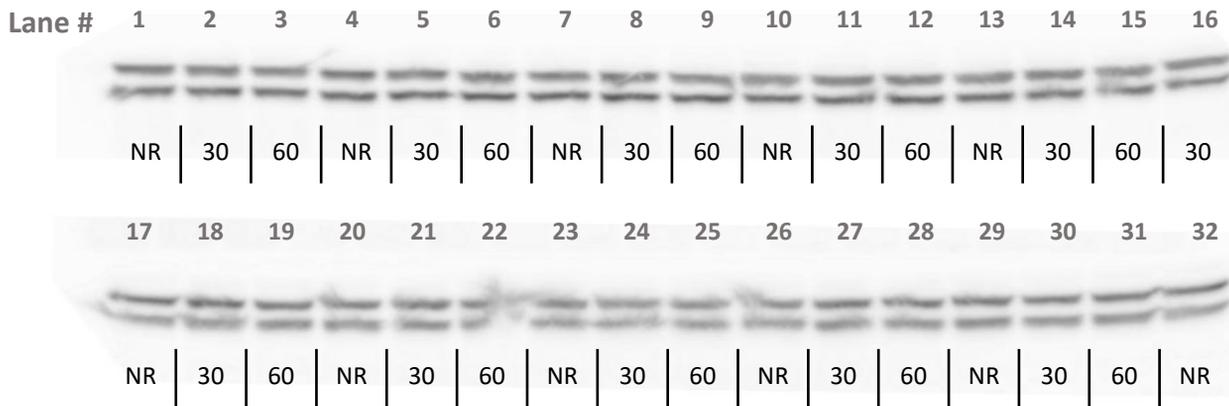


mPFC

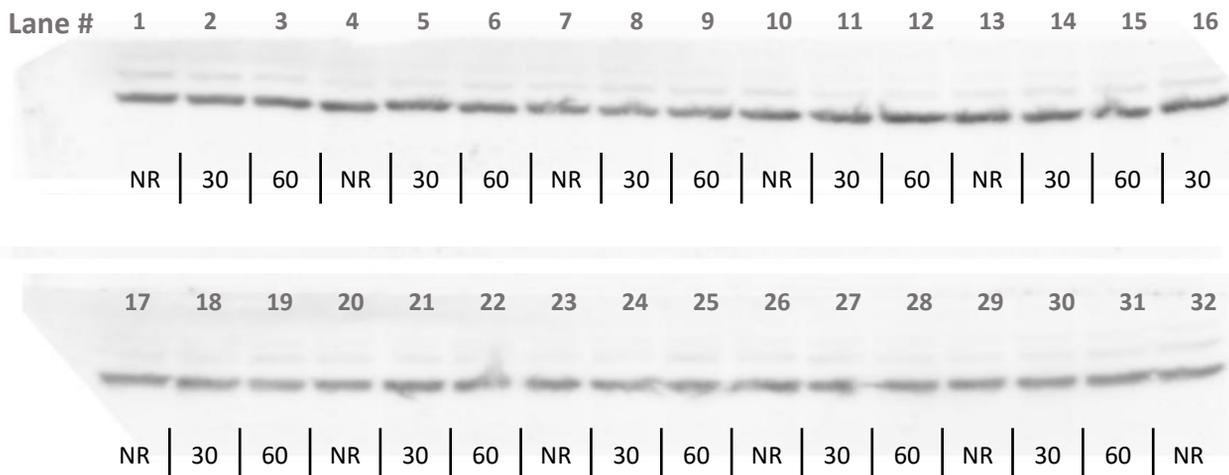
pERK1/2-Thr202/Tyr204 – 44/42 kDa (Figures 4B and S1)



Total ERK1/2 – 44/21 kDa (Figures 4B and S1)

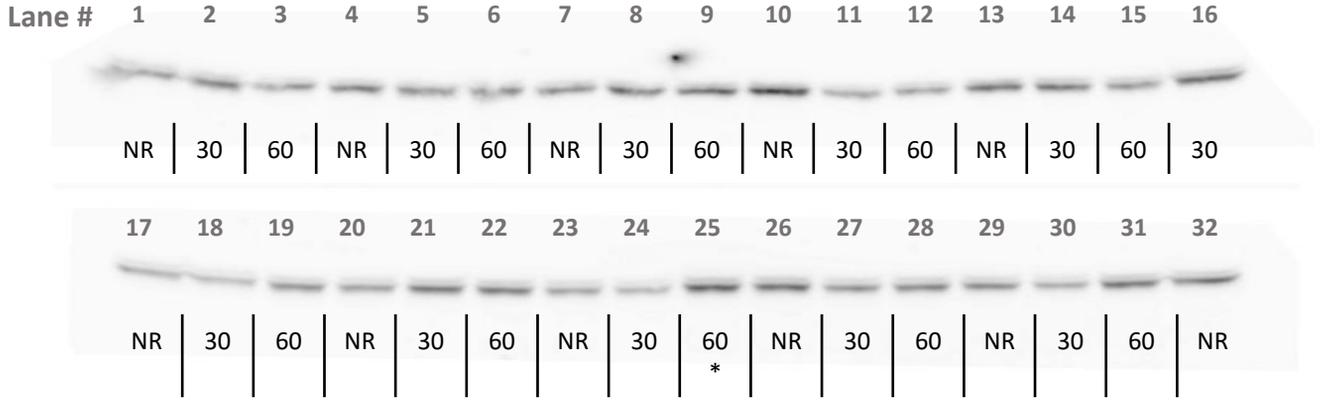


GAPDH – 37 kDa (Figure S1, not in the main figure)

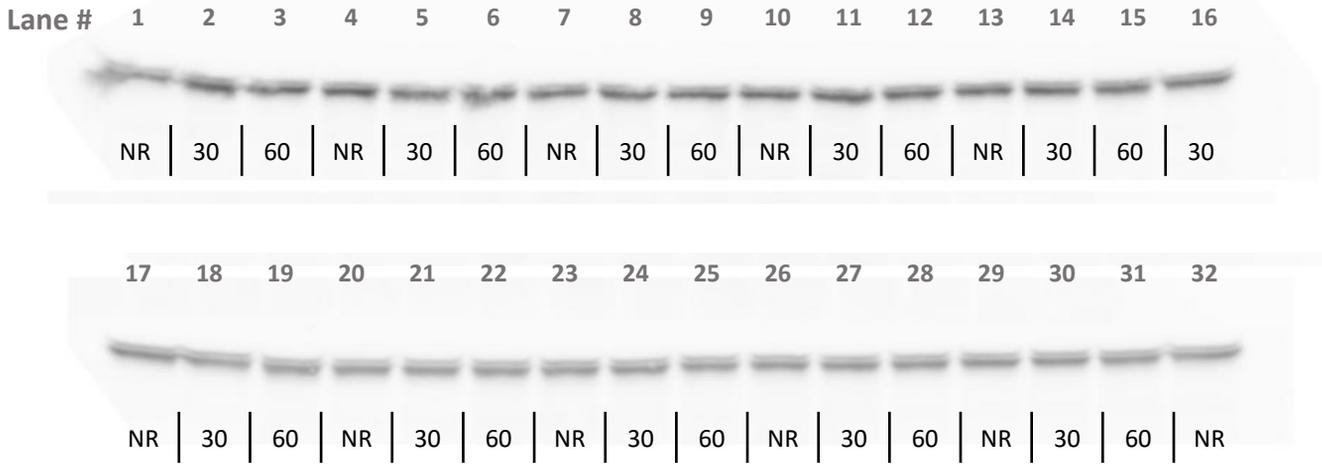


mPFC

pGSK3 β -Ser9 – 46 kDa (Figures 1B and S1)

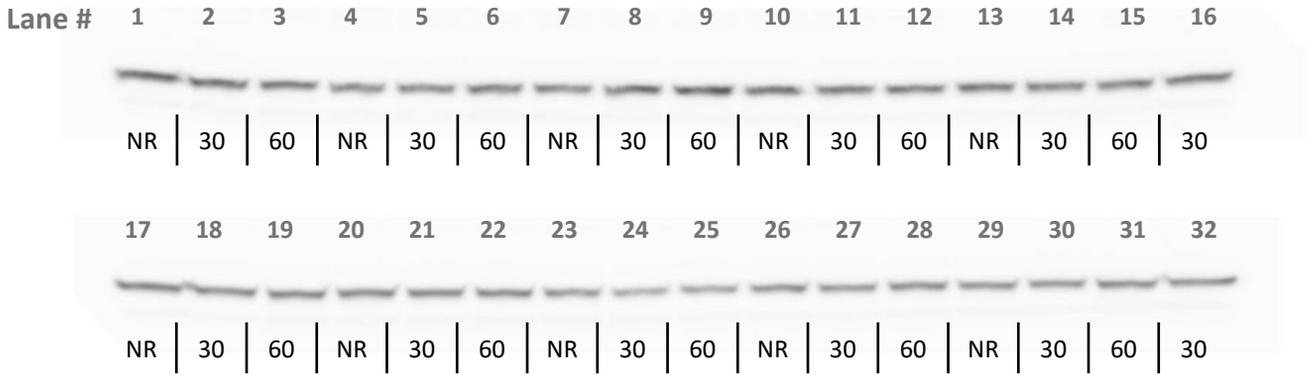


Total GSK3 β – 46 kDa (Figures 1B and S1)

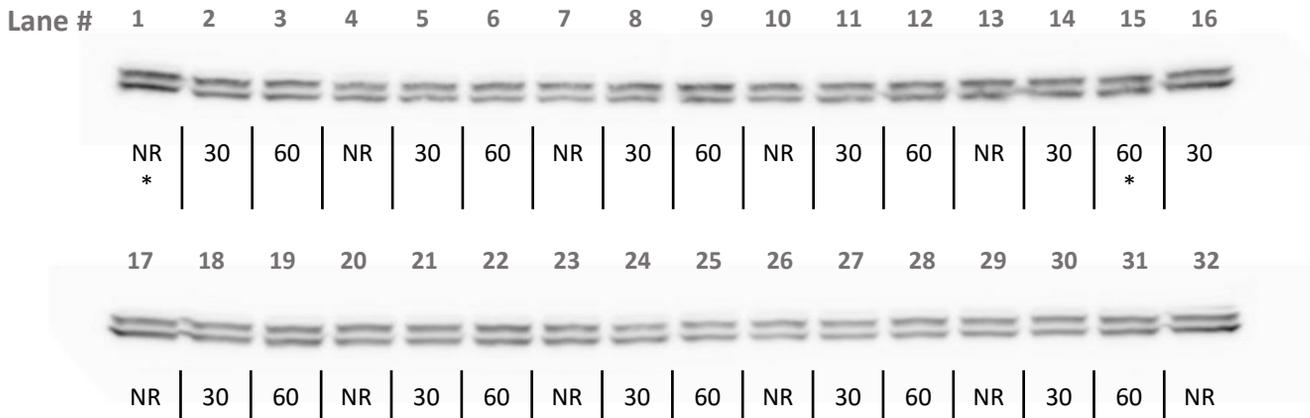


mPFC

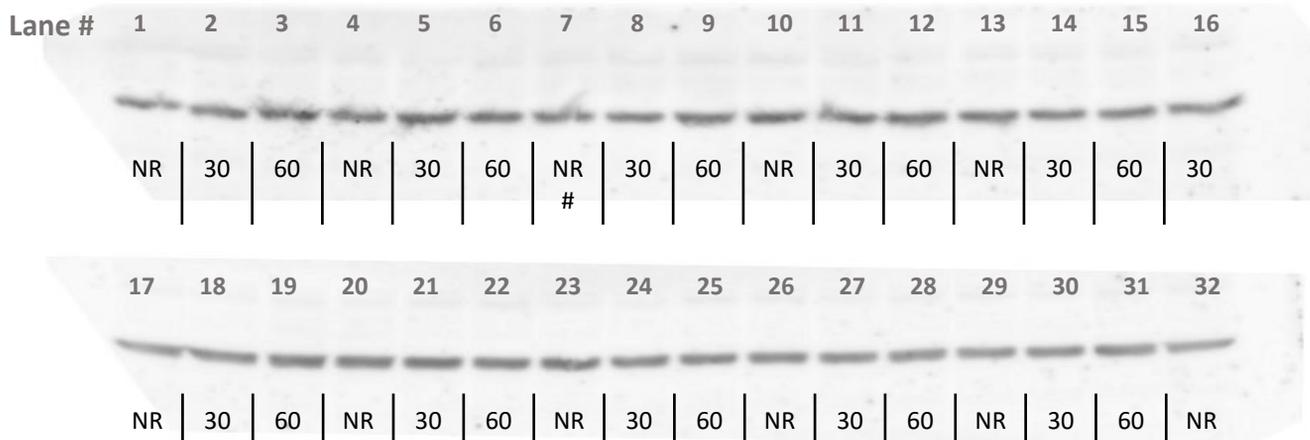
pCRMP2-Thr514 – 60-80 kDa (Figures 1B and S1)



Total CRMP2 – 60-80 kDa (Figures 1C and S1)

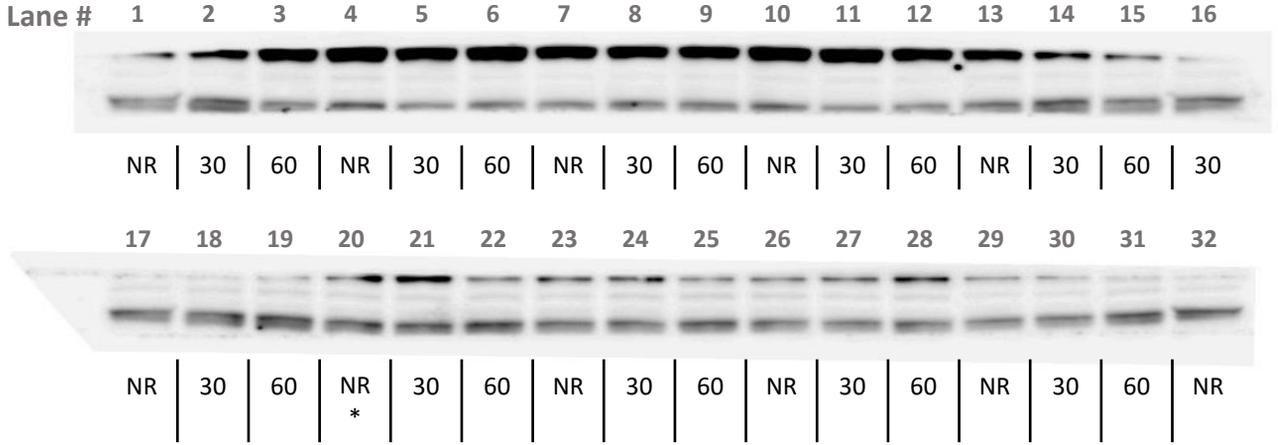


GAPDH – 37 kDa (Figures 1C and S1)

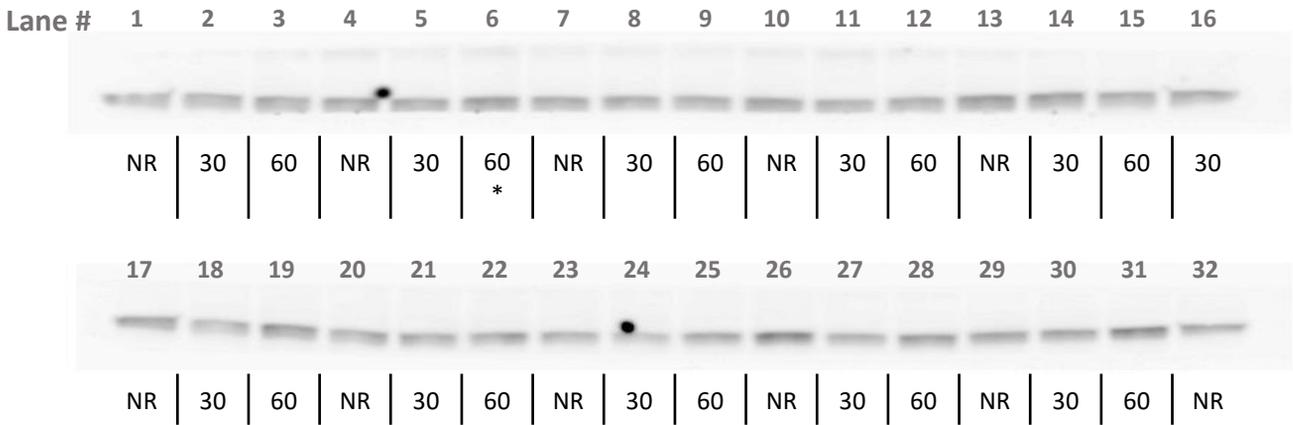


NAC

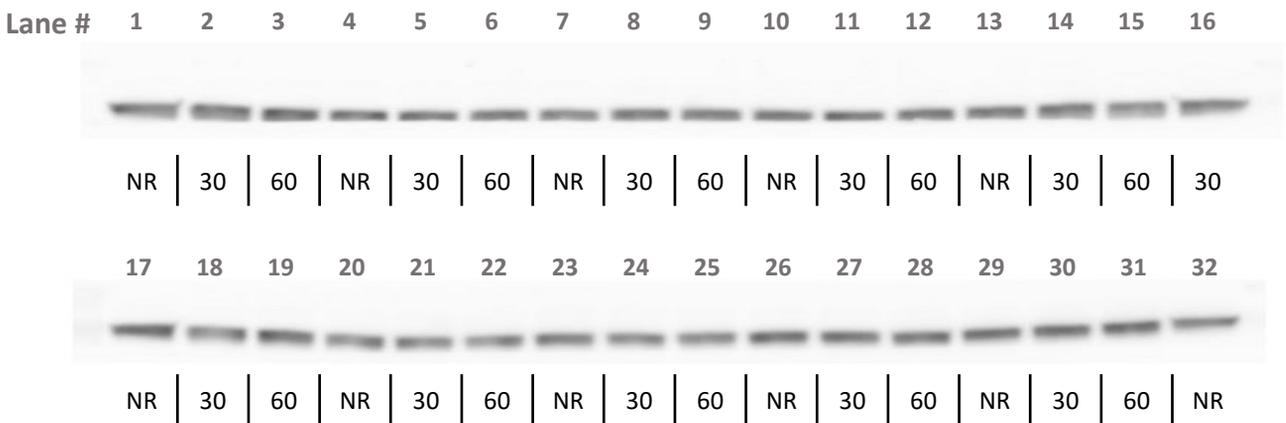
pAKT-Thr308 – 60 kDa (Figures 1D and S2)



pAKT-Ser473 – 60 kDa (Figures 1D and S2)

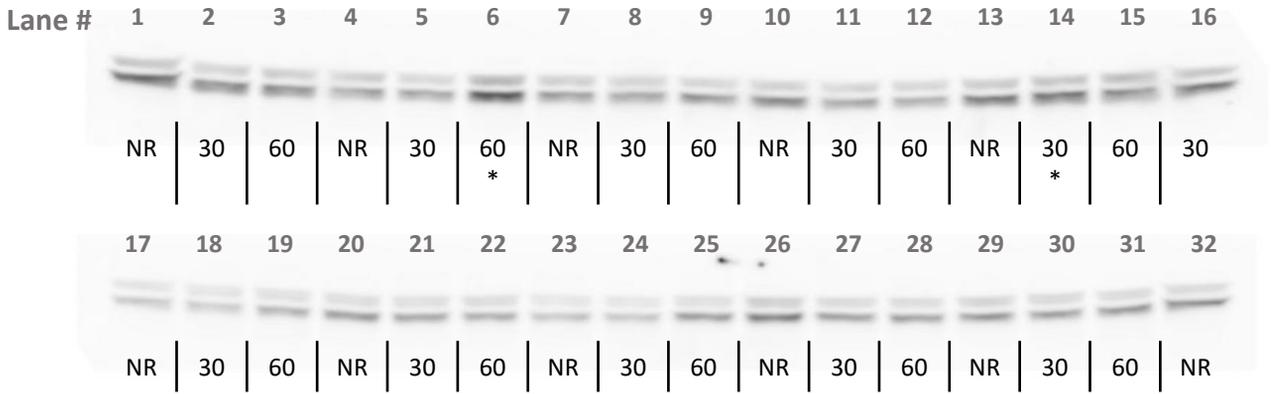


Total AKT - 60 kDa (Figures 1D and S2)

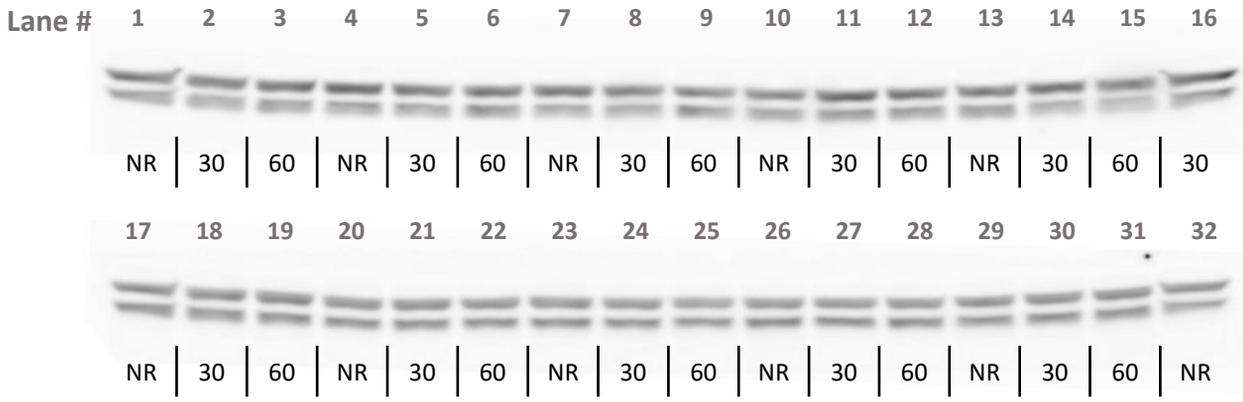


NAC

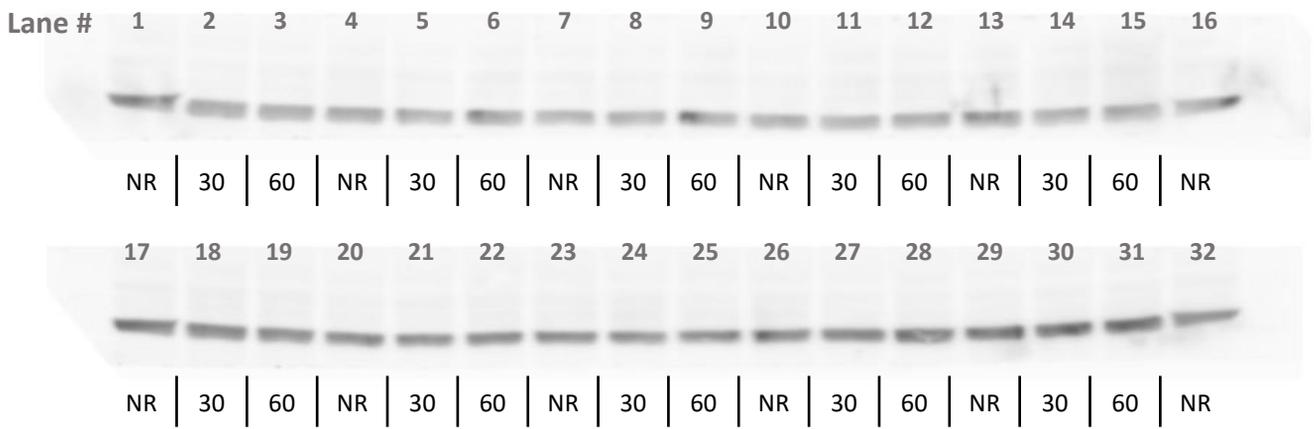
pERK1/2-Thr202/Tyr204 – 44/42 kDa (Figures 4B and S2)



Total ERK1/2 – 44/21 kDa (Figures 4B and S2)

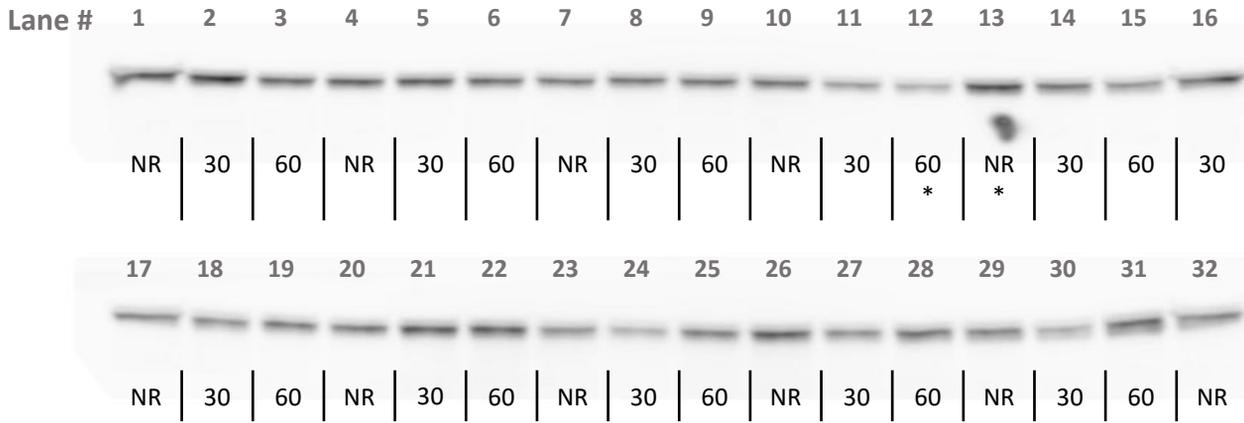


GAPDH – 37 kDa (Figure S2, not in the main figure)



NAC

pGSK3 β -Ser9 – 46 kDa (Figures 1D and S2)

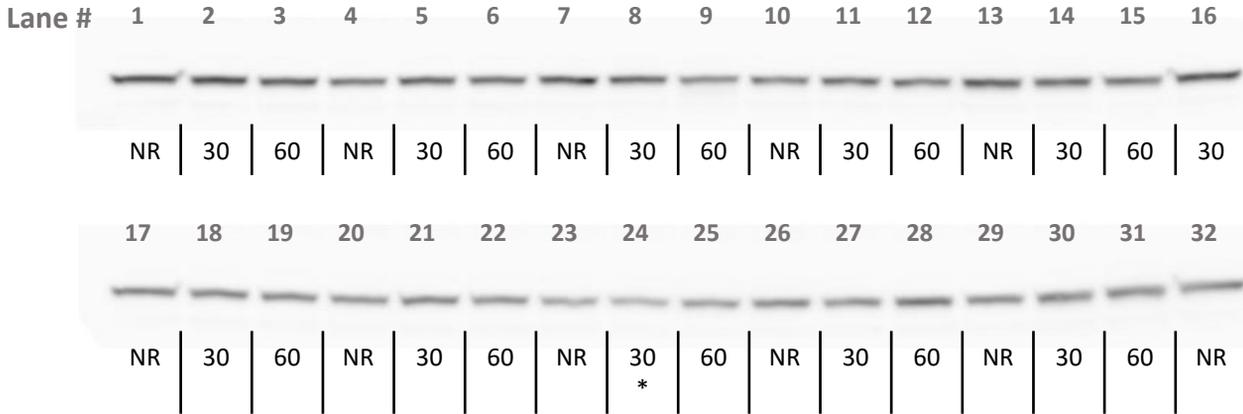


Total GSK3 β – 46 kDa (Figures 1D and S2)

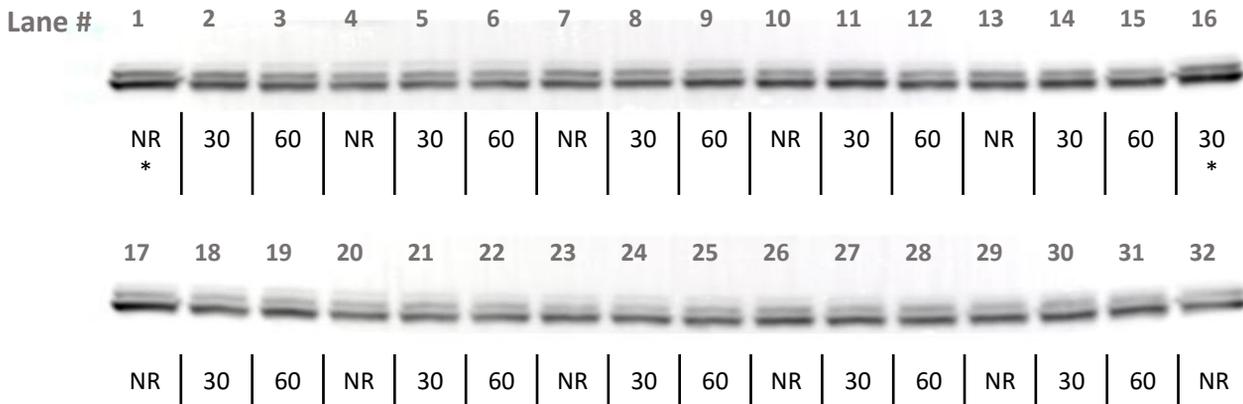


NAC

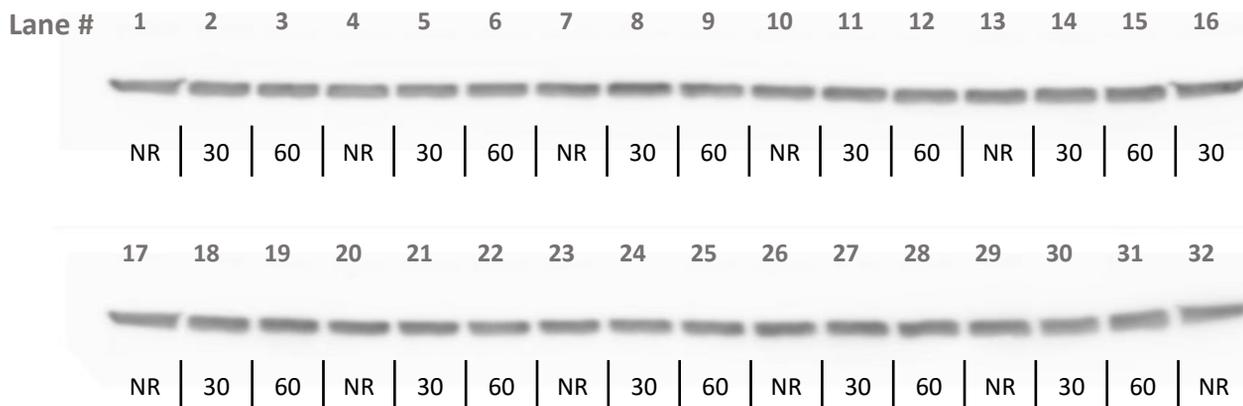
pCRMP2-Thr514 – 60-80 kDa (Figures 1D and S2)



Total CRMP2 – 60-80 kDa (Figures 1E and S2)

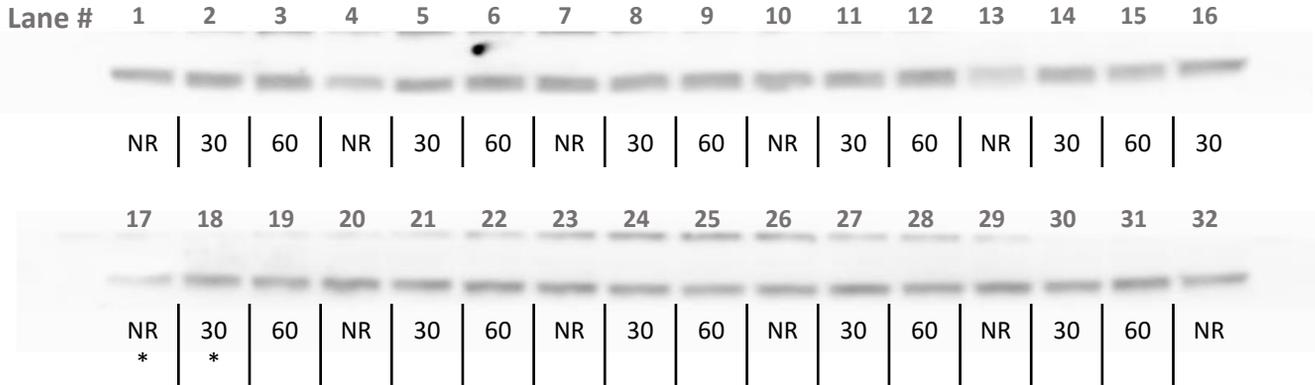


GAPDH – 37 kDa (Figures 1E and S2)

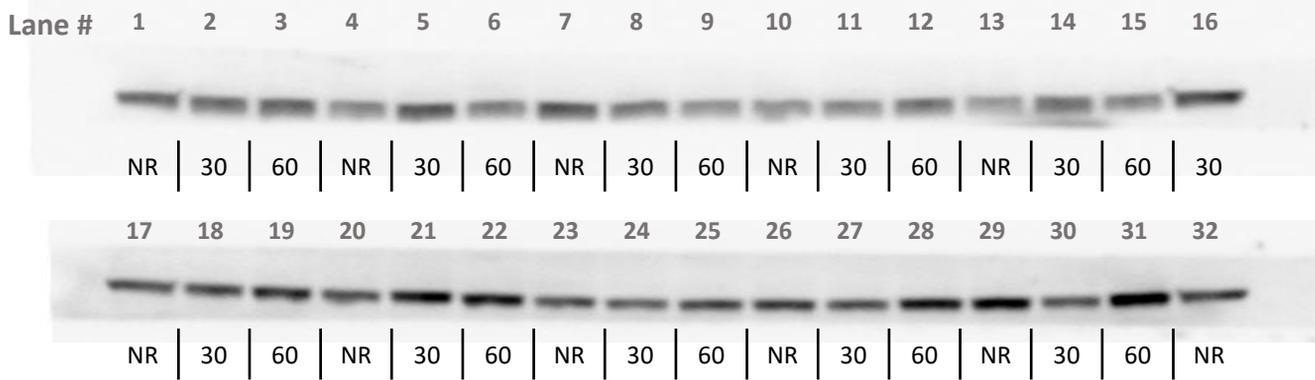


Amygdala

pAKT-Thr308 – 60 kDa (Figures 1F and S3)



pAKT-Ser473 – 60 kDa (Figures 1F and S3)

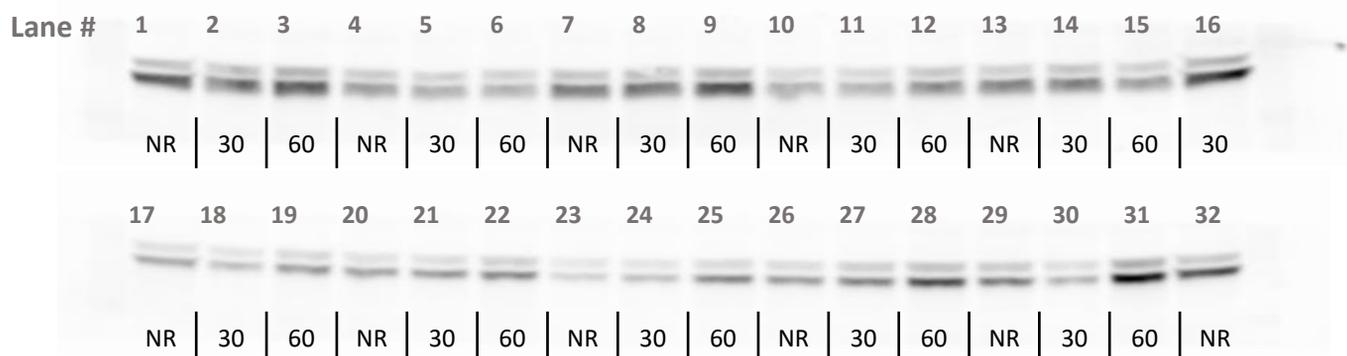


Total AKT - 60 kDa (Figures 1F and S3)



Amygdala

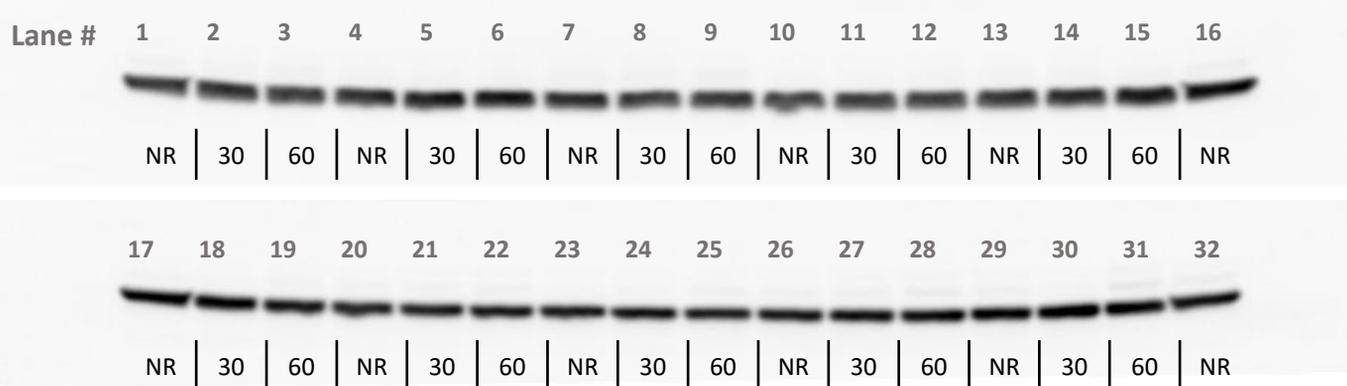
pERK1/2-Thr202/Tyr204 – 44/42 kDa (Figures 4B, S3 and S5)



Total ERK1/2 – 44/21 kDa (Figures 4B, S3 and S5)



GAPDH – 37 kDa (Figure S3, not in the main figure)



Amygdala

pGSK3 β -Ser9 – 46 kDa (Figures 1F and S3)

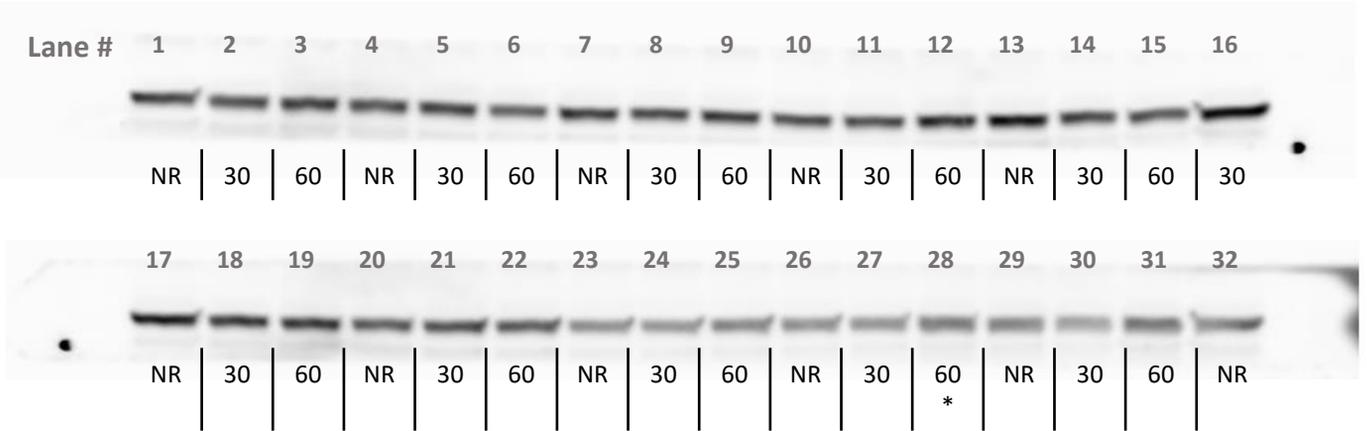


Total GSK3 β – 46 kDa (Figures 1F and S3)



Amygdala

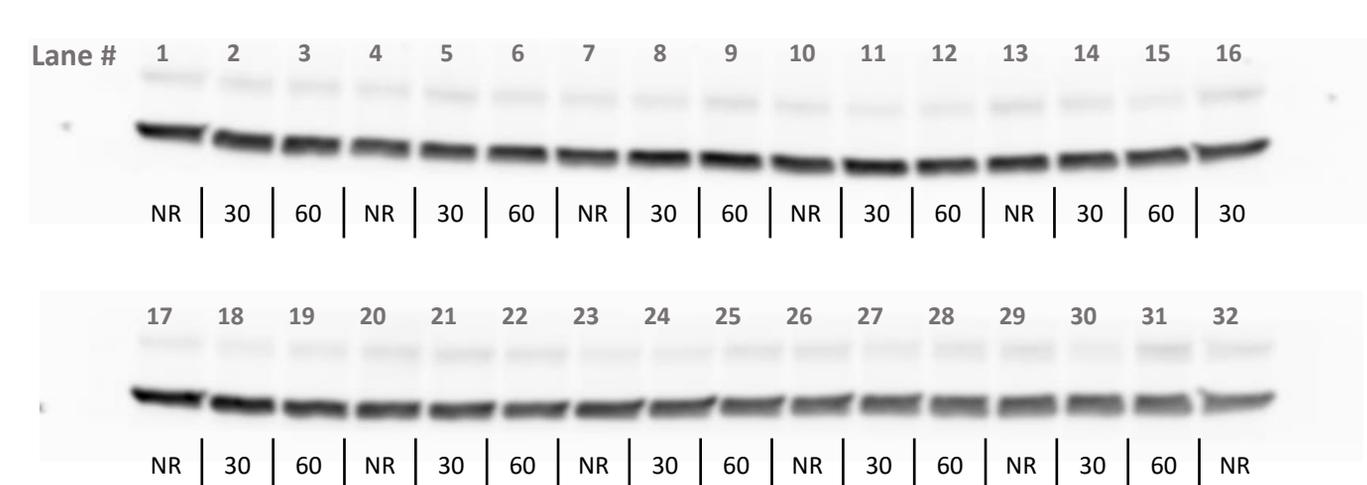
pCRMP2-Thr514 – 60-80 kDa (Figures 1F and S3)



Total CRMP2 – 60-80 kDa (Figures 1G and S3)

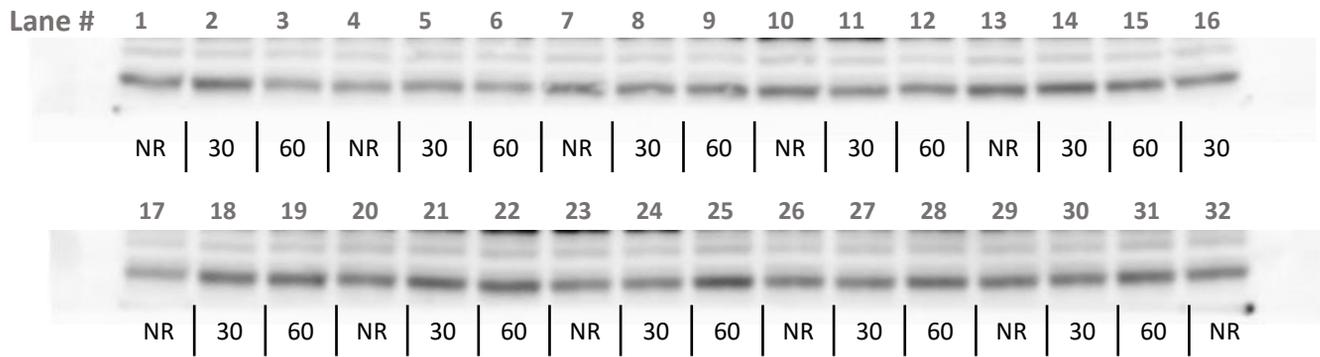


GAPDH – 37 kDa (Figures 1G and S3)

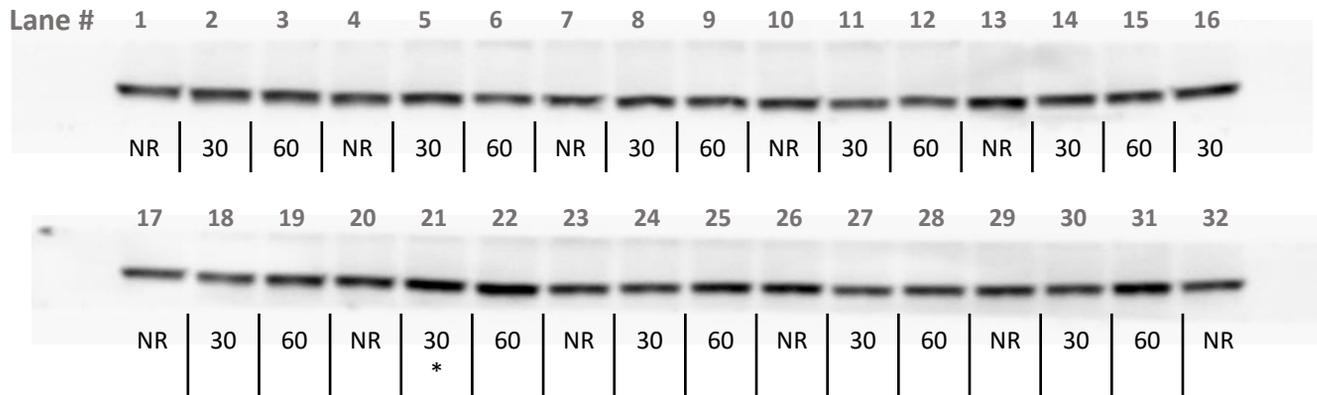


OFC

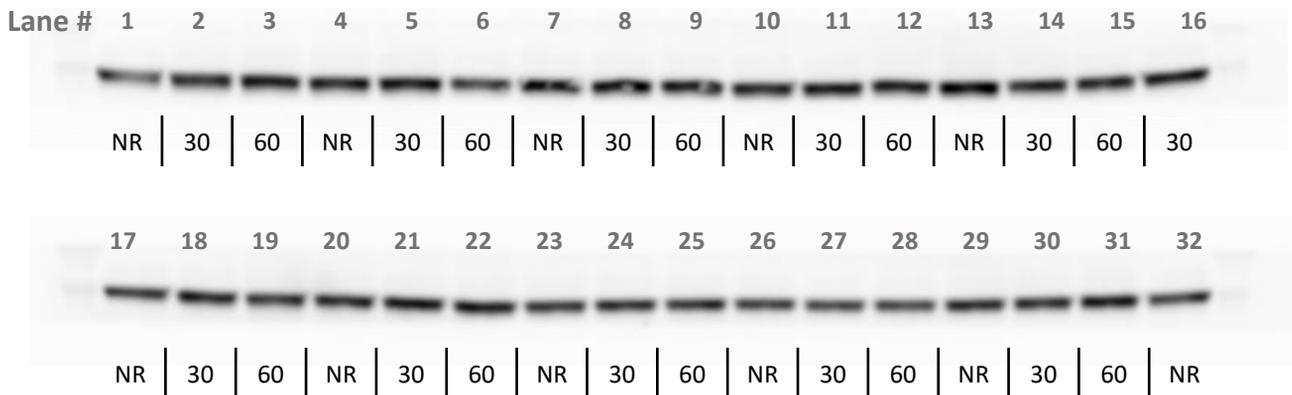
pAKT-Thr308 – 60 kDa (Figure S4)



pAKT-Ser473 – 60 kDa (Figure S4)



Total AKT - 60 kDa (Figure S4)

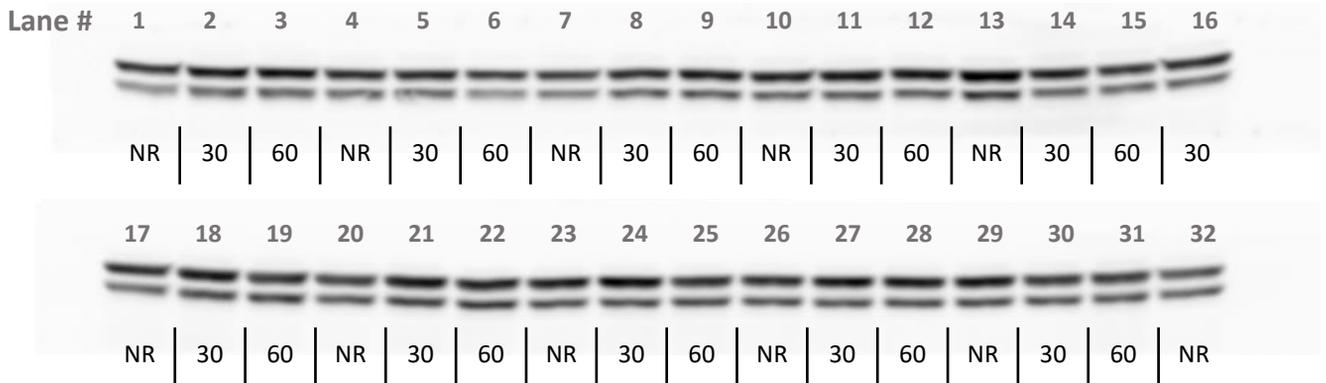


OFC

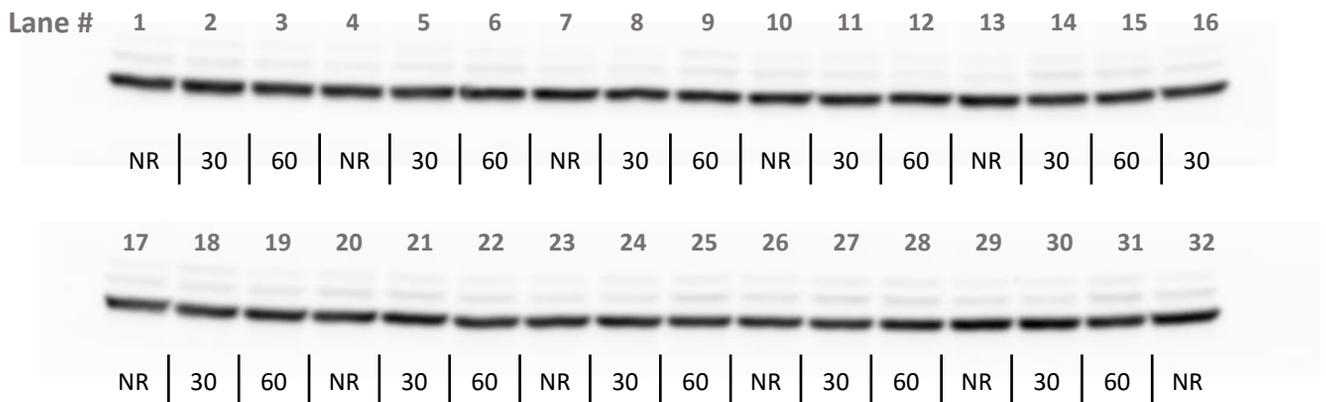
pERK1/2-Thr202/Tyr204 – 44/42 kDa (Figure S4)



Total ERK1/2 – 44/21 kDa (Figure S4)

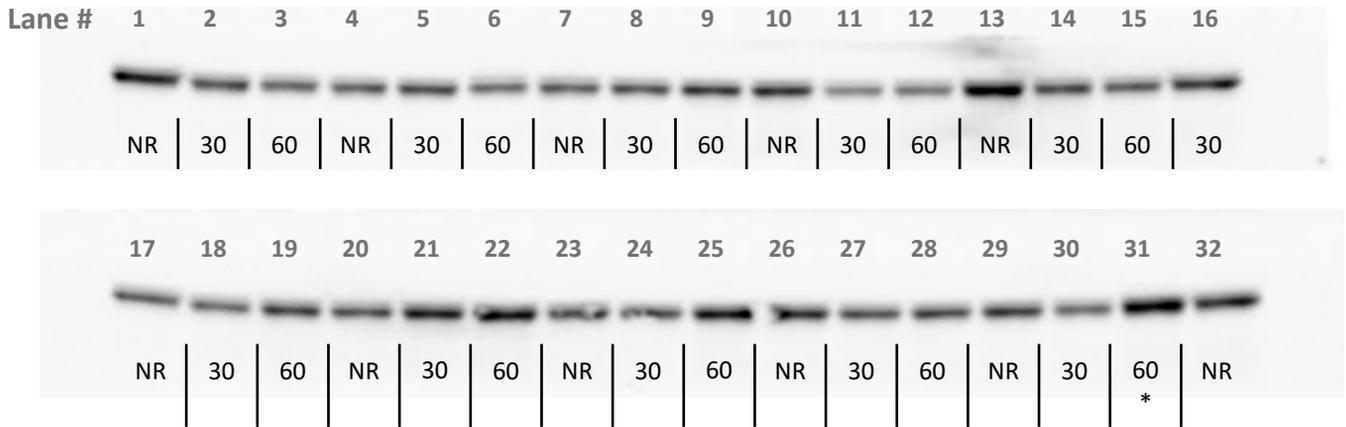


GAPDH – 37 kDa (Figure S4)

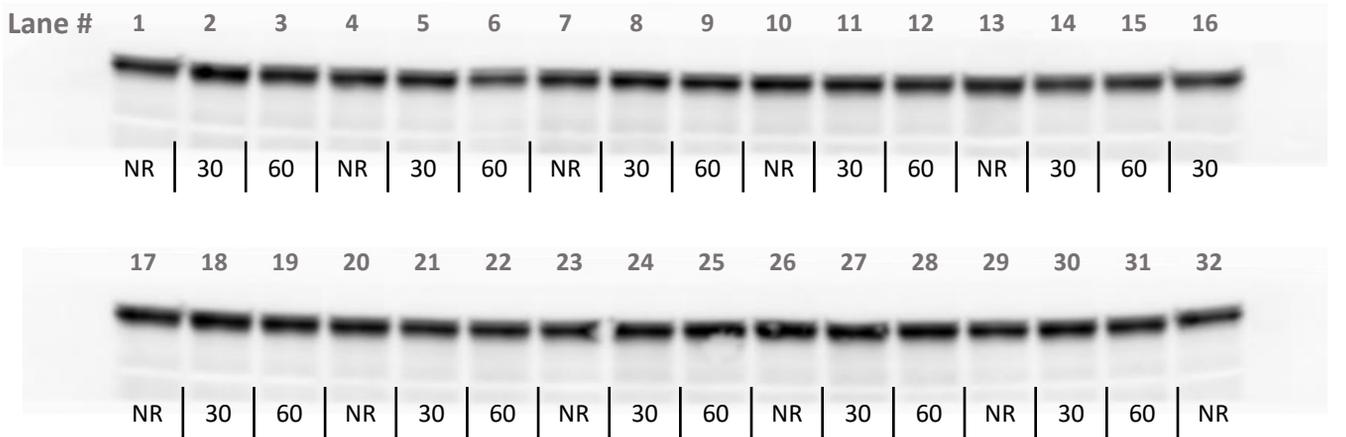


OFC

pGSK3 β -Ser9 – 46 kDa (Figure S4)



Total GSK3 β – 46 kDa (Figure S4)

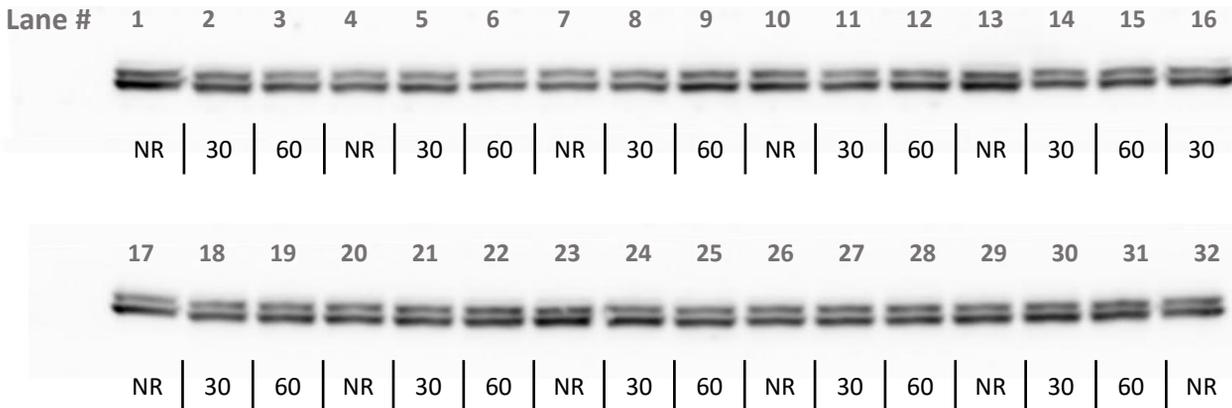


OFC

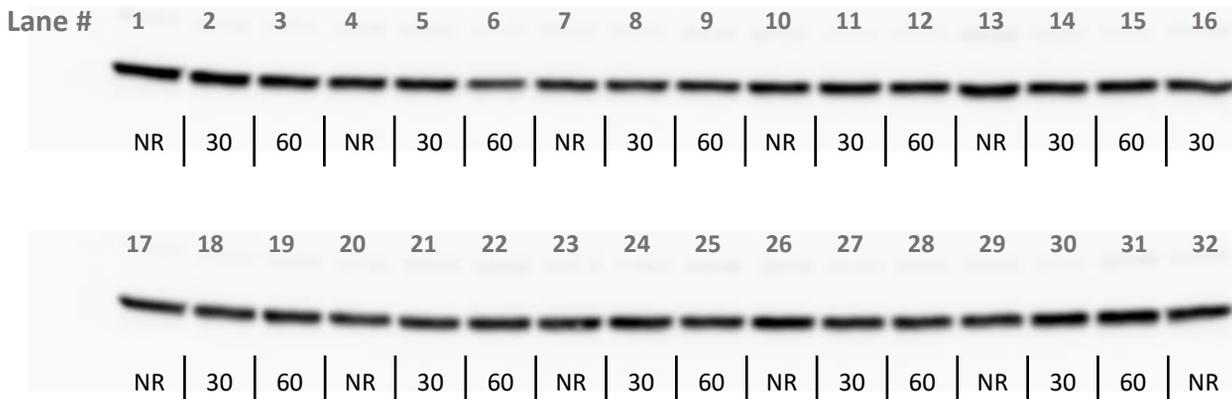
pCRMP2-Thr514 – 60-80 kDa (Figure S4)



Total CRMP2 – 60-80 kDa (Figure S4)



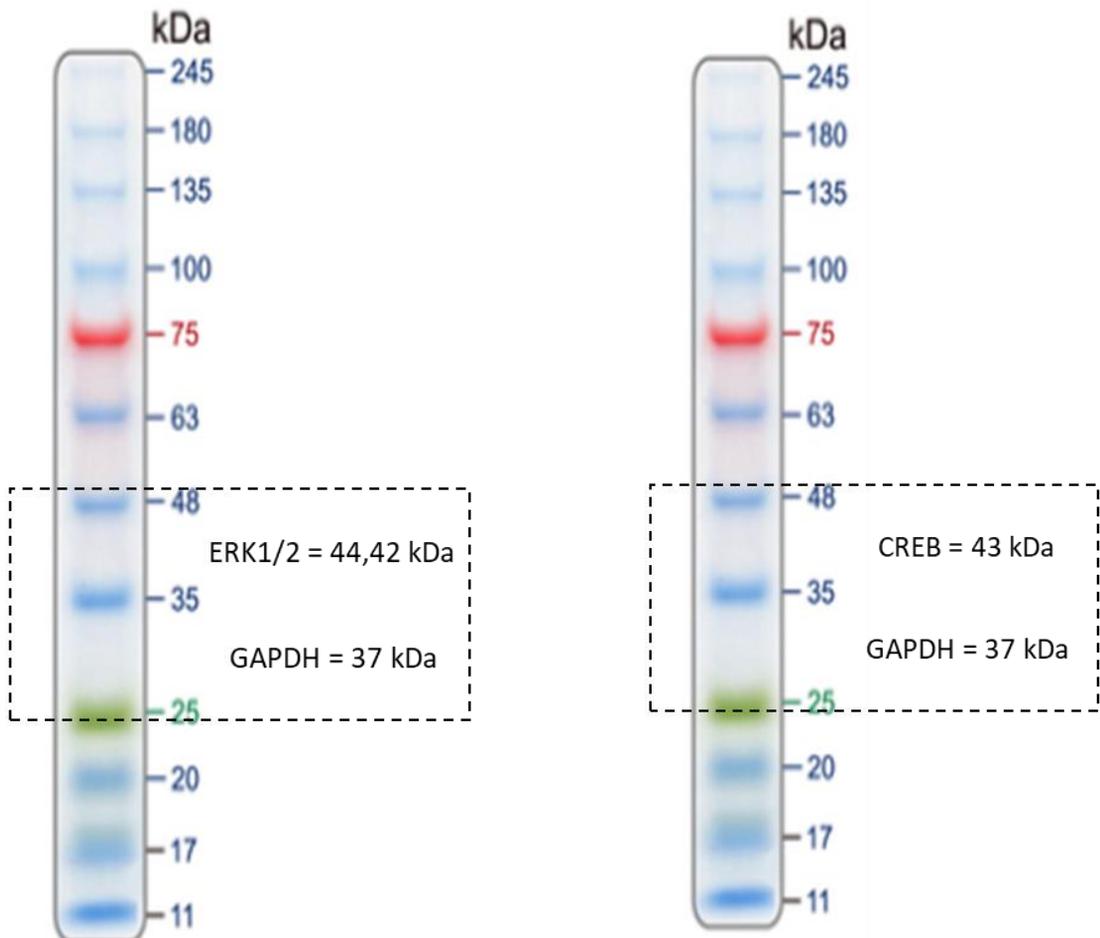
GAPDH – 37 kDa (Figure S4)



Experiment 4

Amygdala tissues were collected two hours after alcohol memory retrieval (R), or without a prior retrieval procedure (NR).

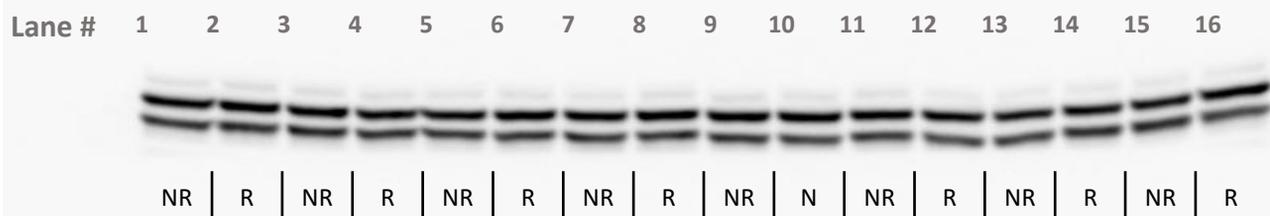
The membrane was cut prior to the blotting process, as illustrated below, therefore image of the complete membrane is not available.



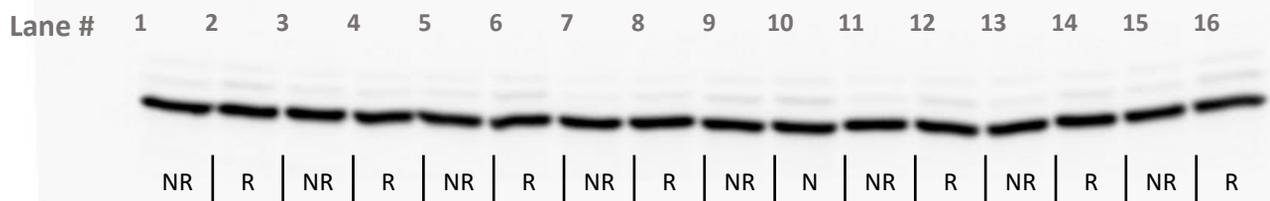
pERK1/2-Thr202/Tyr204 – 44/42 kDa (Figures 4C and S6)



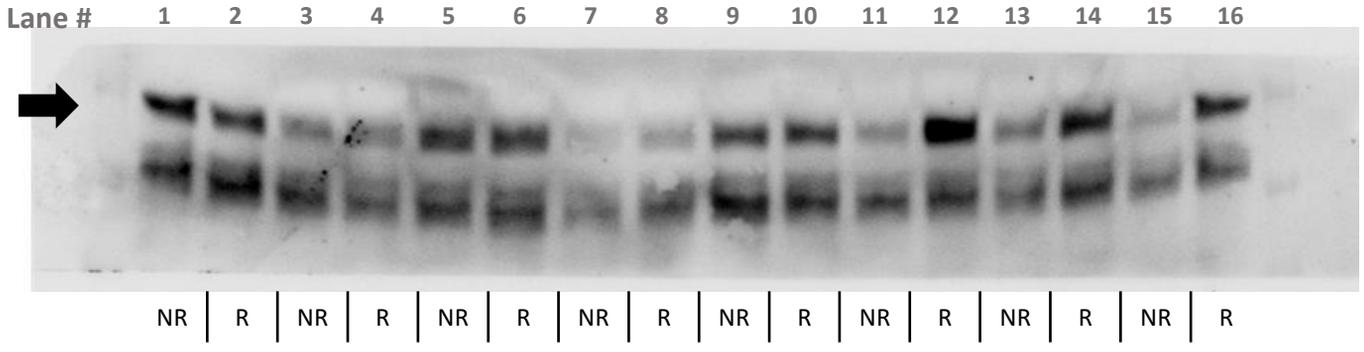
Total ERK1/2 – 44/21 kDa pERK1/2-Thr202/Tyr204 – 44/42 kDa (Figures 4C and S6)



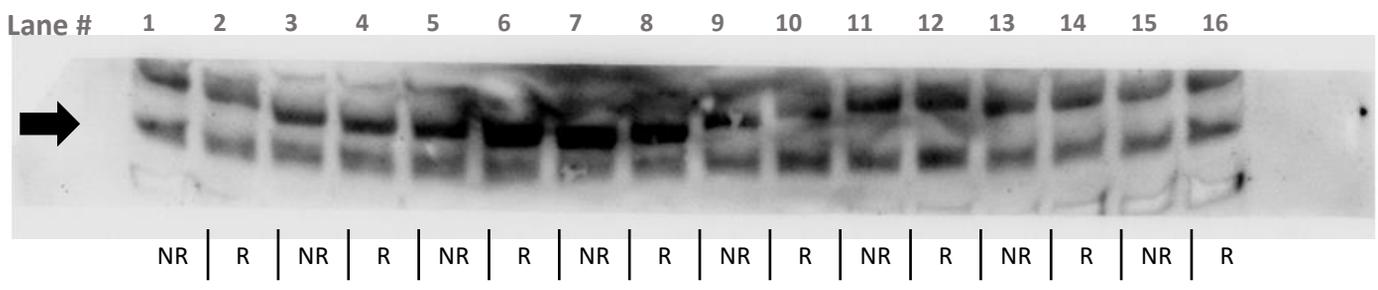
GAPDH – 37 kDa (Figure S6, not in the main figure)



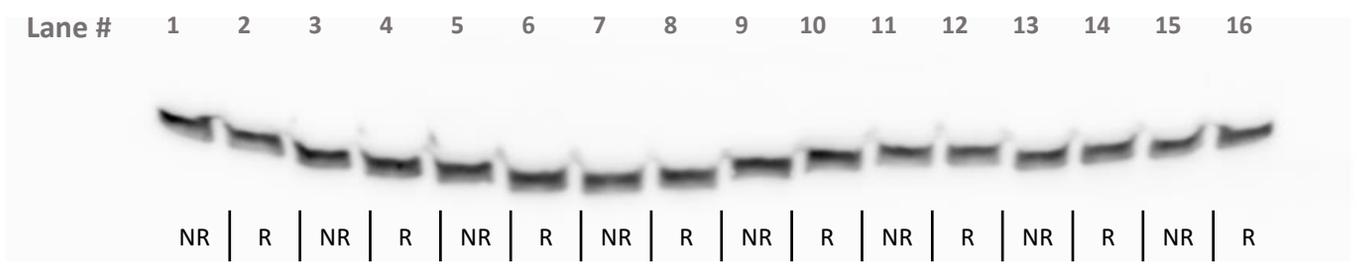
pCREB-Ser133 – 43 kDa (Figures 4C and S6)



Total CREB-Ser133 – 43 kDa (Figures 4C and S6)



GAPDH – 37 kDa (Figure S6, not in the main figure)

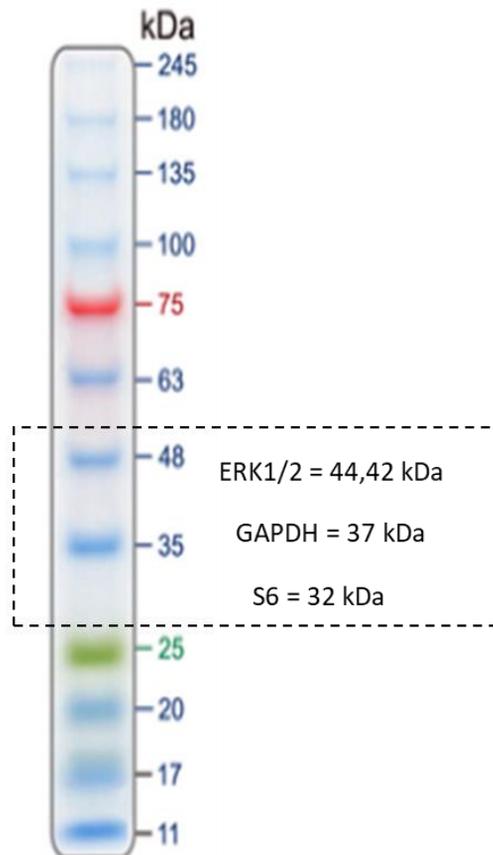


Experiment 5

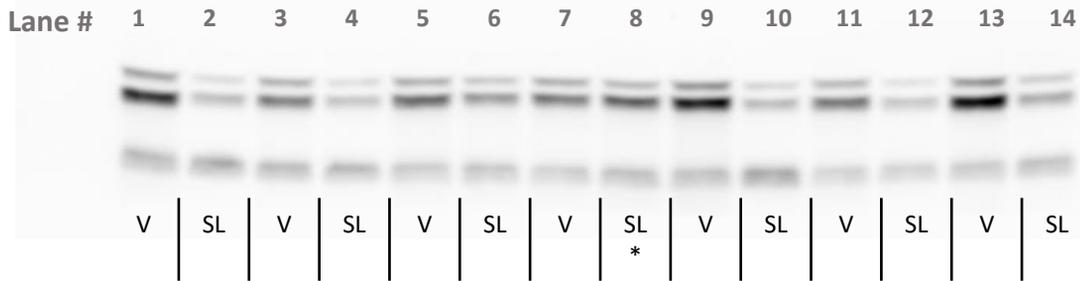
Amygdala tissues were collected 90 min after injection of vehicle (V) or the MERK1/2 inhibitor SL-327 (SL).

The membrane was cut prior to the blotting process, as illustrated below, therefore image of the complete membrane is not available.

* Sample that was identified as an outlier to its experimental group ($z > 2$) and was therefore removed from the analysis.



pERK1/2-Thr202/Tyr204 – 44/42 kDa (Figures 6 and S7)



Total ERK1/2 – 44/21 kDa (Figures 6 and S7)



pS6 – 32 kDa (Figures 6 and S7)



Total S6 – 32 kDa (Figures 6 and S7)



GAPDH – 37 kDa (Figure S7, not in the main figure)

