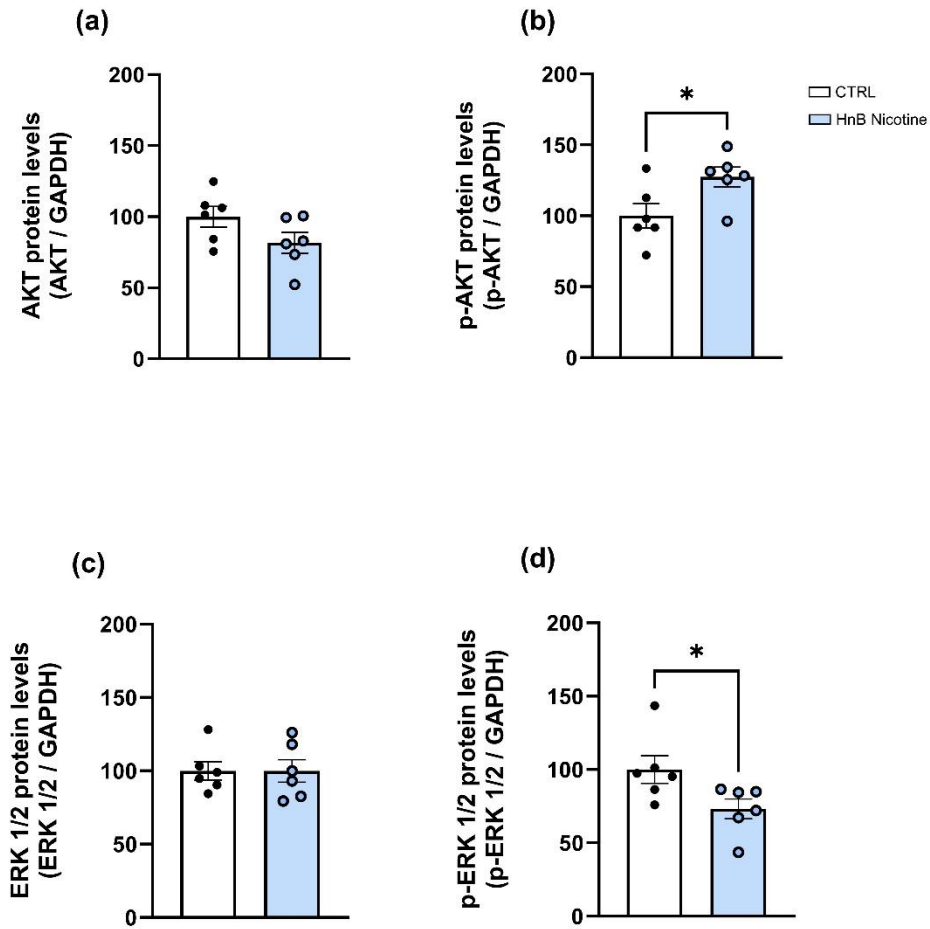
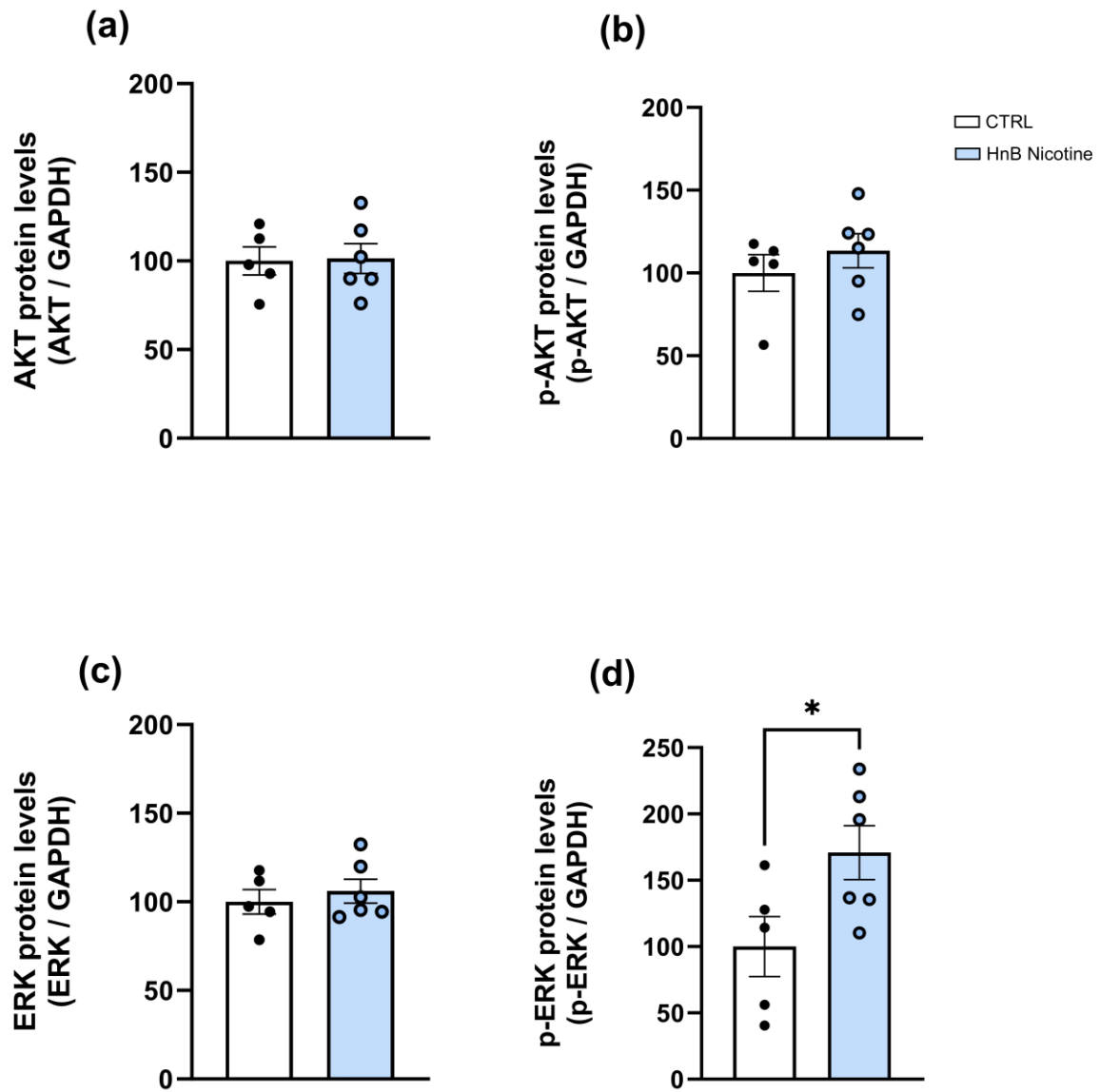


## Supplementary Material



**Figure S1.** Effects of the nicotine exposure via an HnB device on AKT (a), p-AKT (b), ERK 1/2 (c) and p-ERK 1/2 (d) protein levels in the rat VTA. Bars represent the means  $\pm$  SEM; \*  $p < 0.05$ ; two-tailed t-test.



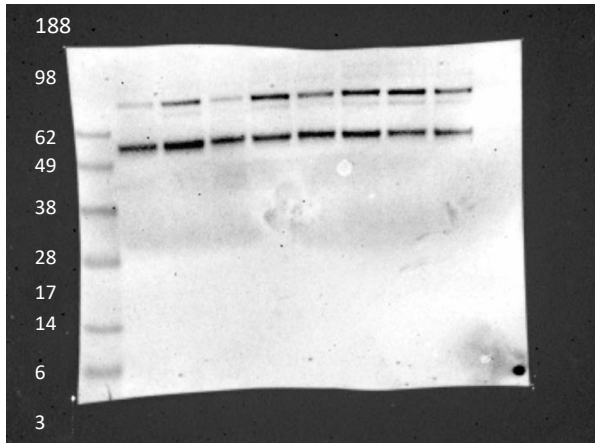
**Figure S2.** Effects of the nicotine exposure via an HnB device on AKT (a), p-AKT (b), ERK 1/2 (c) and p-ERK 1/2 (d) protein levels in the rat NAC. Bars represent the means  $\pm$  SEM; \*  $p < 0.05$ ; two-tailed t-test.

## WESTERN BLOT IMAGES

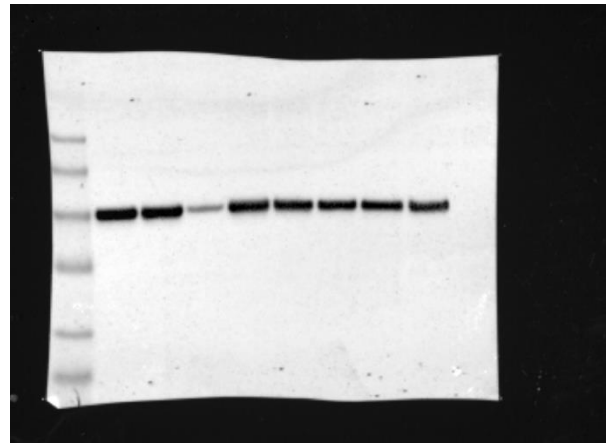
### Nucleus Accumbens

#### GEL A

p-AKT (~ 60 kDa)

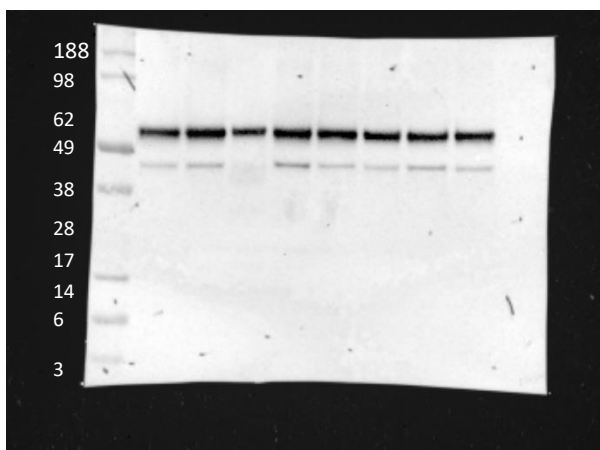


GAPDH (~ 36 kDa)

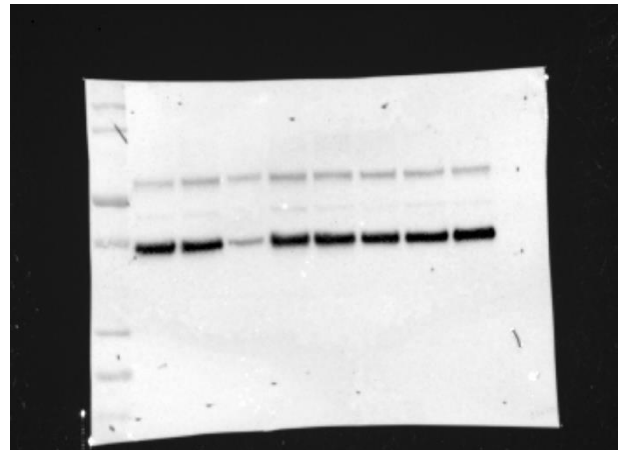


#### GEL A

AKT (~ 60 kDa)



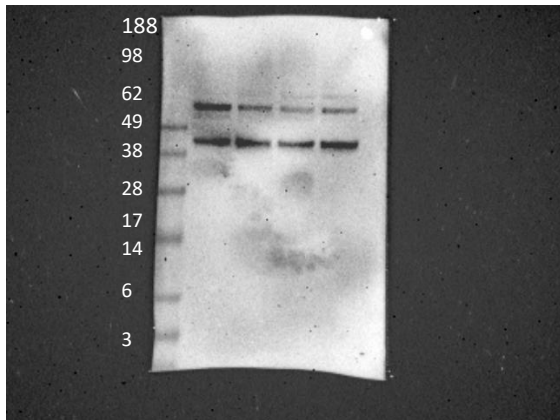
GAPDH (~ 36 kDa)



## *Nucleus Accumbens*

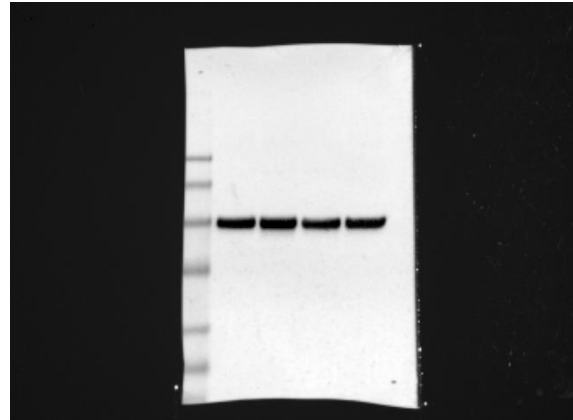
### **GEL B**

**p-AKT (~ 60 kDa)**



5 CTRL 5 HnB 6 CTRL 6 HnB

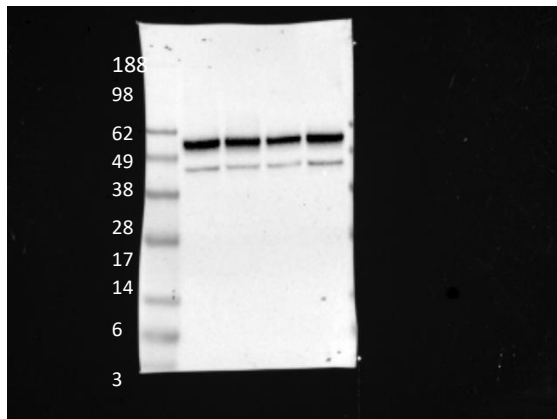
**GAPDH (~ 36 kDa)**



5 CTRL 5 HnB 6 CTRL 6 HnB

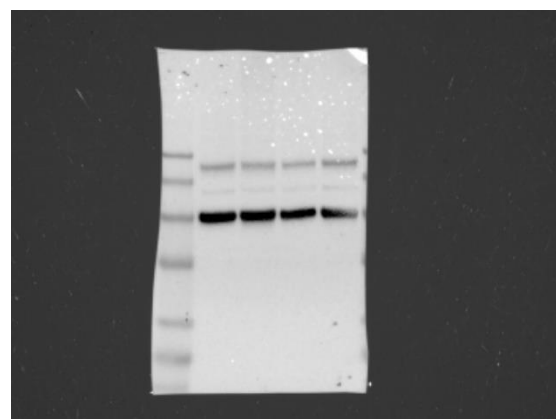
### **GEL B**

**AKT (~ 60 kDa)**



5 CTRL 5 HnB 6 CTRL 6 HnB

**GAPDH (~ 36 kDa)**

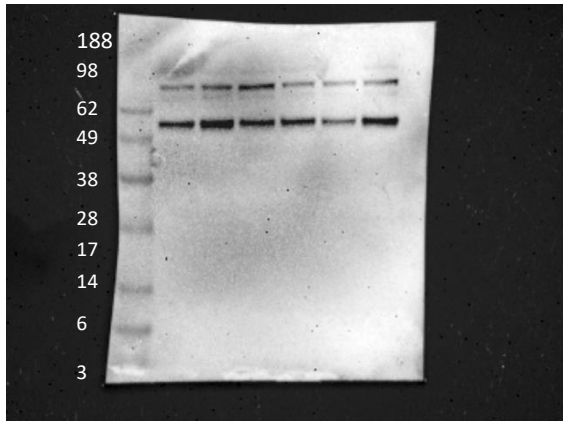


5 CTRL 5 HnB 6 CTRL 6 HnB

## *Nucleus Accumbens*

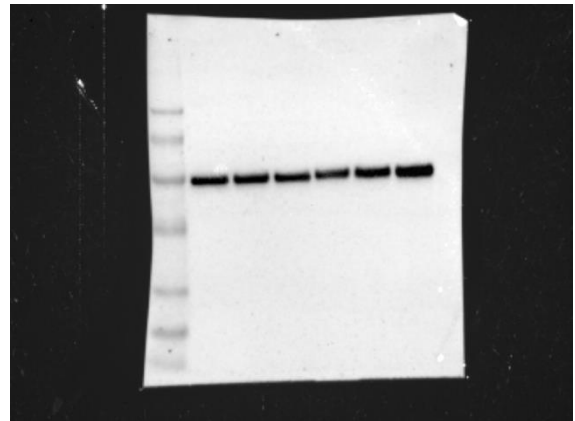
**GEL C**

**p-AKT (~ 60 kDa)**



3 CTRL 1 HnB 4 CTRL 4 HnB 5 CTRL 6 HnB

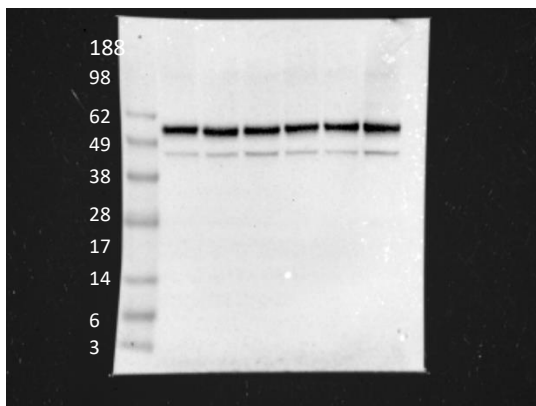
**GAPDH (~ 36 kDa)**



3 CTRL 1 HnB 4 CTRL 4 HnB 5 CTRL 6 HnB

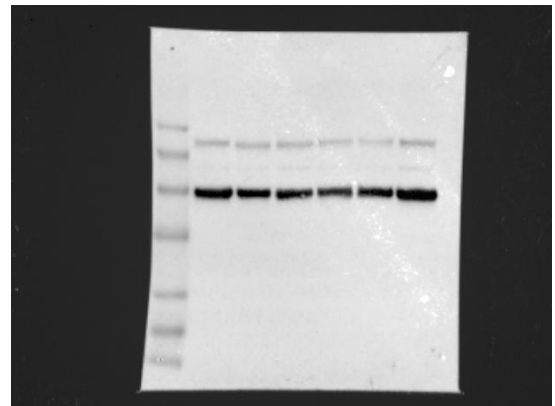
**GEL C**

**AKT (~ 60 kDa)**



3 CTRL 1 HnB 4 CTRL 4 HnB 5 CTRL 6 HnB

**GAPDH (~ 36 kDa)**

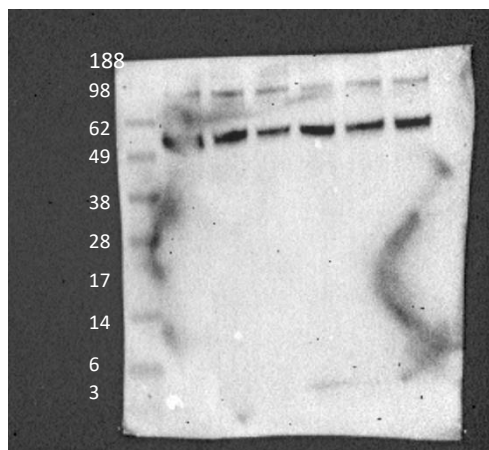


3 CTRL 1 HnB 4 CTRL 4 HnB 5 CTRL 6 HnB

## *Nucleus Accumbens*

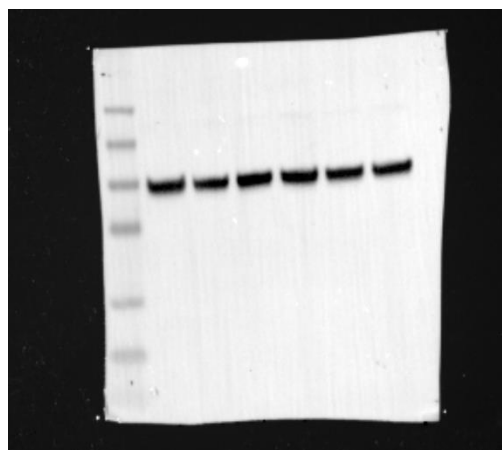
**GEL D**

**p-AKT (~ 60 kDa)**



1 CTRL 6 CTRL 2 HnB 3 HnB 4 HnB 5 HnB

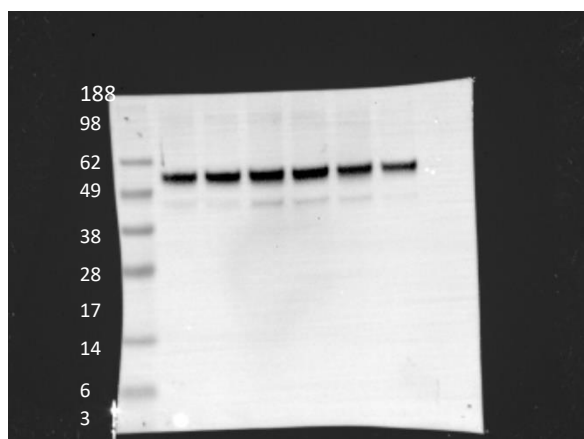
**GAPDH (~ 36 kDa)**



1 CTRL 6 CTRL 2 HnB 3 HnB 4 HnB 5 HnB

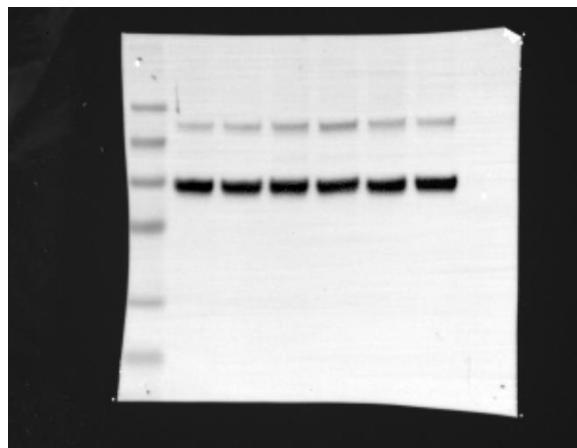
**GEL D**

**AKT (~ 60 kDa)**



1 CTRL 6 CTRL 2 HnB 3 HnB 4 HnB 5 HnB

**GAPDH (~ 36 kDa)**



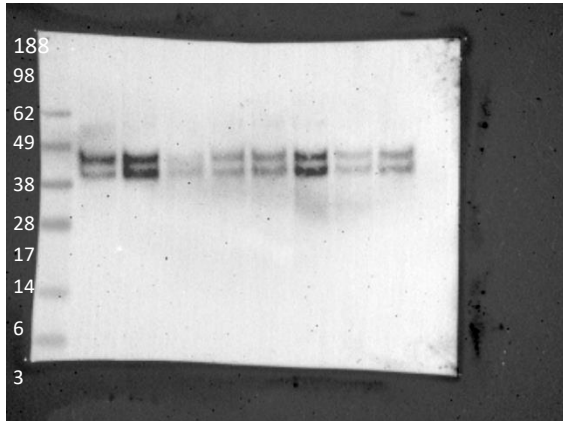
1 CTRL 6 CTRL 2 HnB 3 HnB 4 HnB 5 HnB

## Nucleus Accumbens

**GEL A**

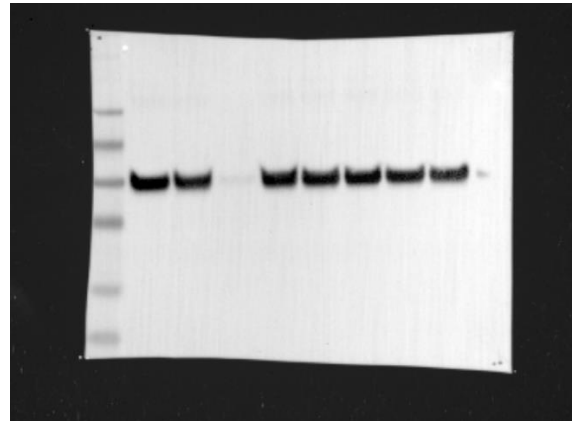
p-ERK 1/2 (~ 42/44 kDa)

GAPDH (~ 36 kDa)



1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL 4 HnB

*Representative bands in figure 4b*



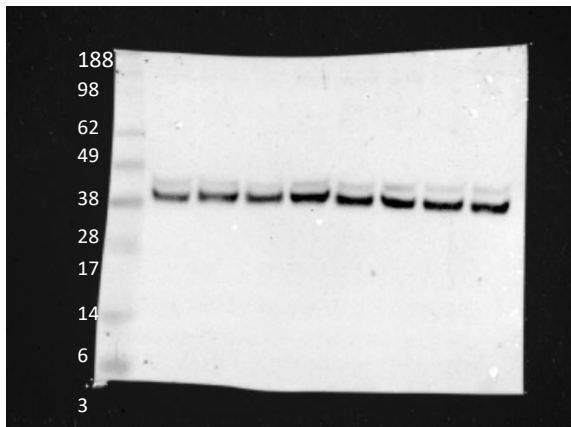
1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL 4 HnB

*Representative bands in figure 4b*

**GEL A**

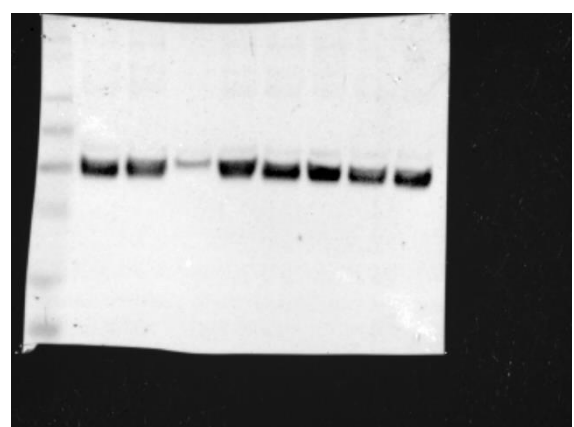
ERK 1/2 (~ 42/44 kDa)

GAPDH (~ 36 kDa)



1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL 4 HnB

*Representative bands in figure 4b*



1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL 4 HnB

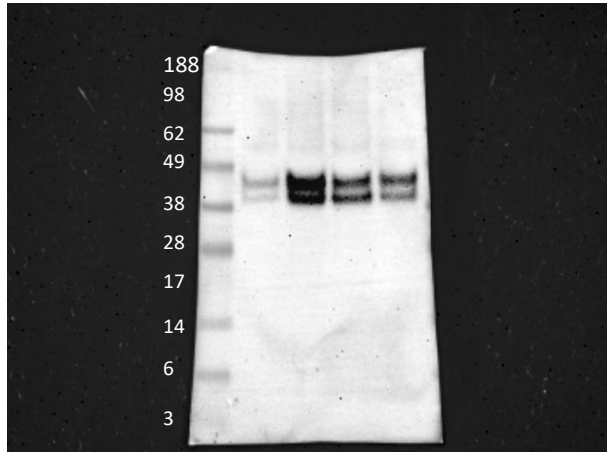
*Representative bands in figure 4b*

## *Nucleus Accumbens*

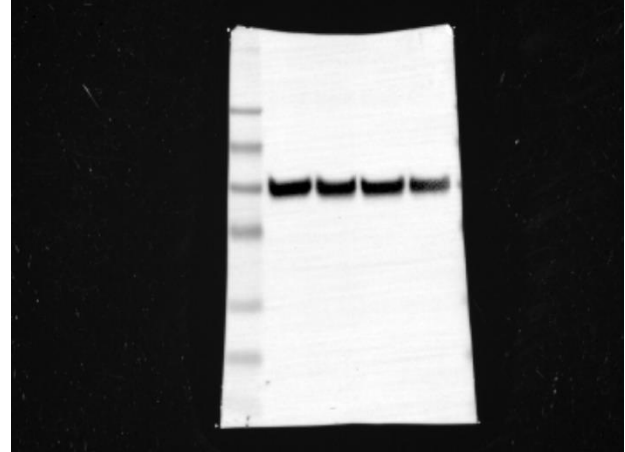
**GEL B**

**p- ERK 1/2** (~ 42/44 kDa)

**GAPDH** (~ 36 kDa)



5 CTRL 5 HnB 6 CTRL 6 HnB

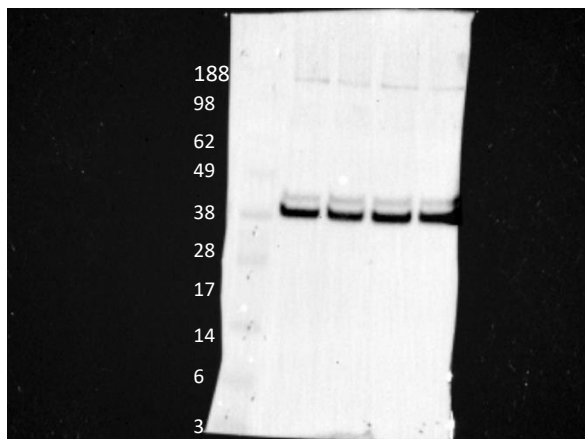


5 CTRL 5 HnB 6 CTRL 6 HnB

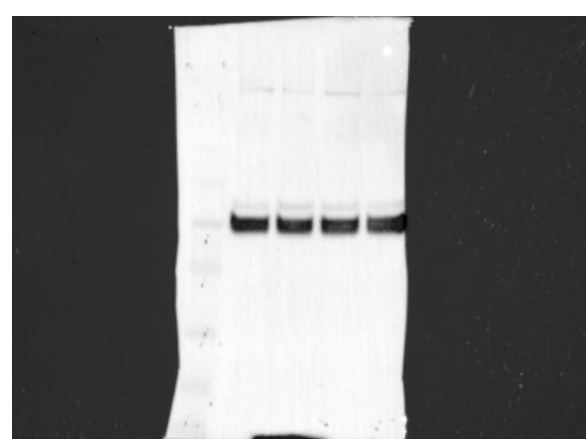
**GEL B**

**ERK 1/2** (~ 42/44 kDa)

**GAPDH** (~ 36 kDa)



5 CTRL 5 HnB 6 CTRL 6 HnB



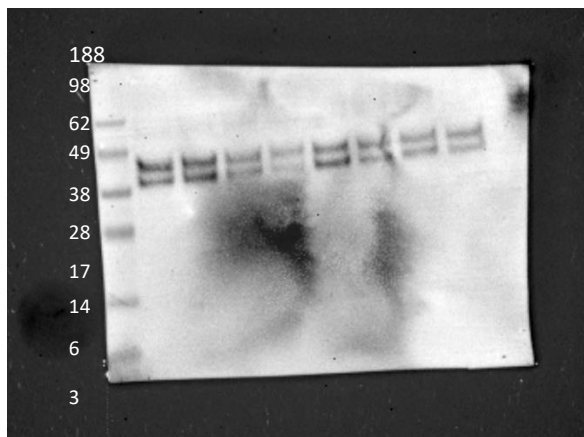
5 CTRL 5 HnB 6 CTRL 6 HnB



## *Nucleus Accumbens*

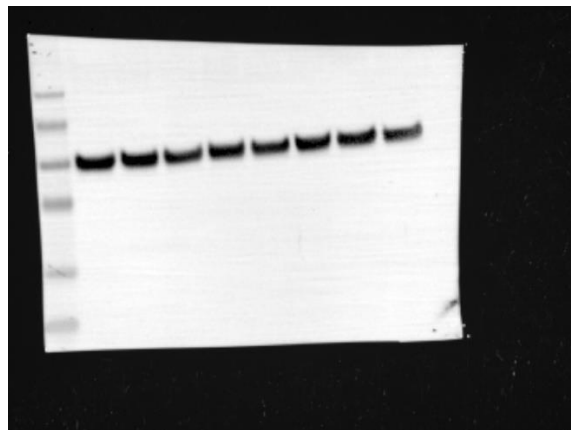
**GEL C**

**p- ERK 1/2 (~ 42/44 kDa)**



1 CTRL 3 CTRL 4 CTRL 5 CTRL 6 CTRL 2 HnB 4 HnB 6 HnB

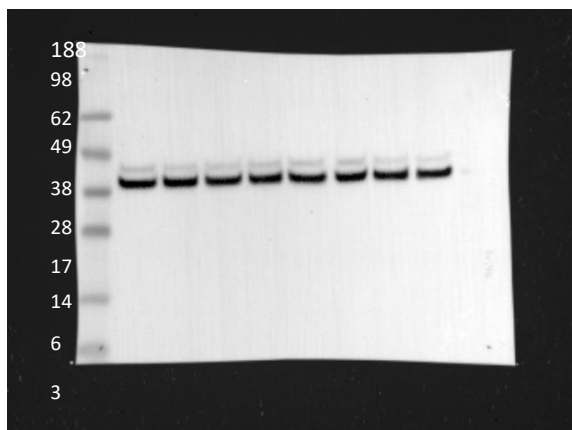
**GAPDH (~ 36 kDa)**



1 CTRL 3 CTRL 4 CTRL 5 CTRL 6 CTRL 2 HnB 4 HnB 6 HnB

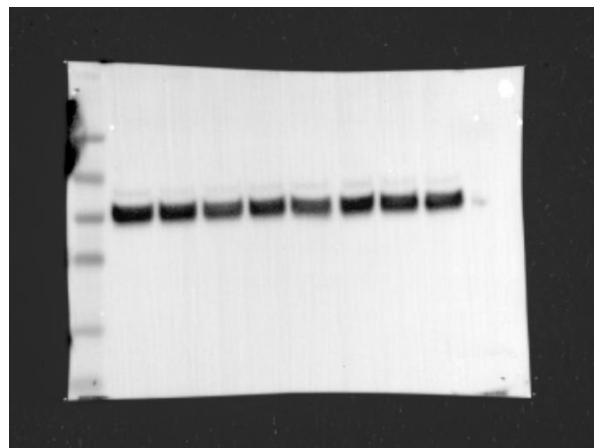
**GEL C**

**ERK 1/2 (~ 42/44 kDa)**



1 CTRL 3 CTRL 4 CTRL 5 CTRL 6 CTRL 2 HnB 4 HnB 6 HnB

**GAPDH (~ 36 kDa)**

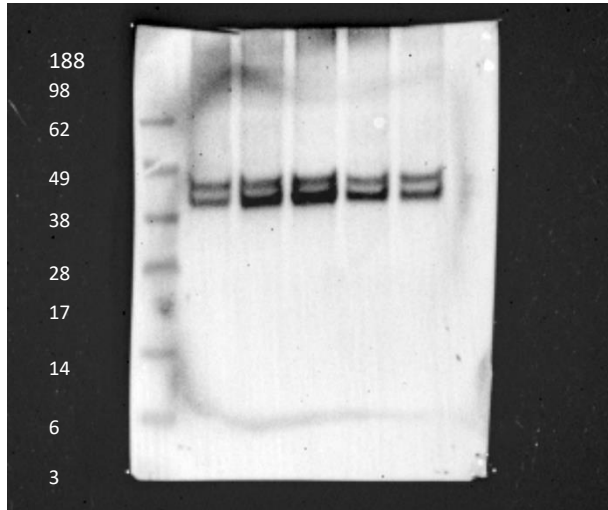


1 CTRL 3 CTRL 4 CTRL 5 CTRL 6 CTRL 2 HnB 4 HnB 6 HnB

## *Nucleus Accumbens*

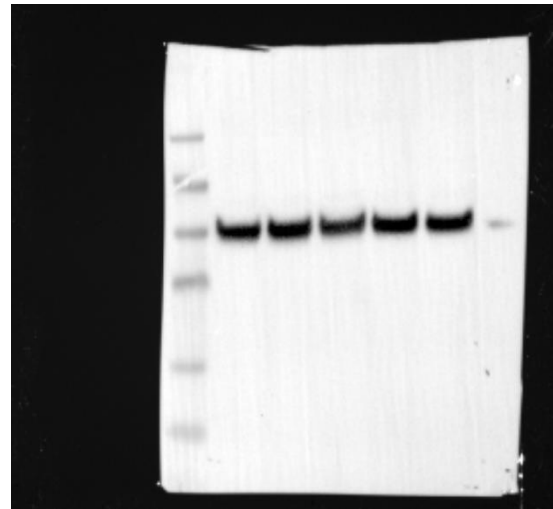
**GEL D**

**p- ERK 1/2 (~ 42/44 kDa)**



1 CTRL 3 CTRL 1 HnB 3 HnB 5 HnB

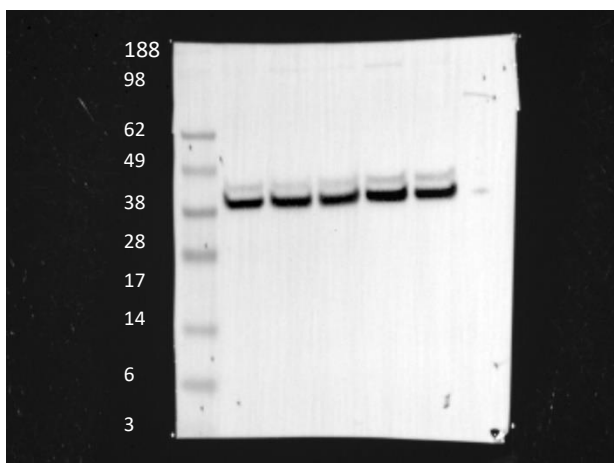
**GAPDH (~ 36 kDa)**



1 CTRL 3 CTRL 1 HnB 3 HnB 5 HnB

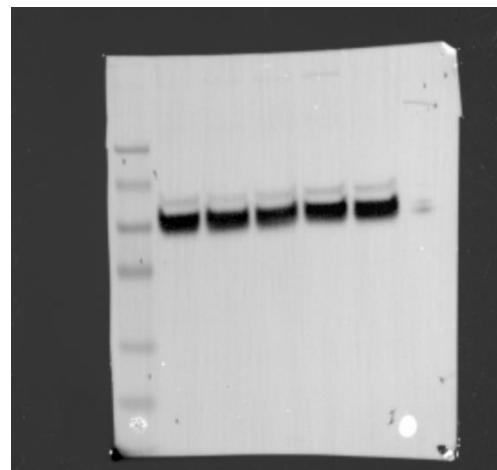
**GEL D**

**ERK 1/2 (~ 42/44 kDa)**



1 CTRL 3 CTRL 1 HnB 3 HnB 5 HnB

**GAPDH (~ 36 kDa)**



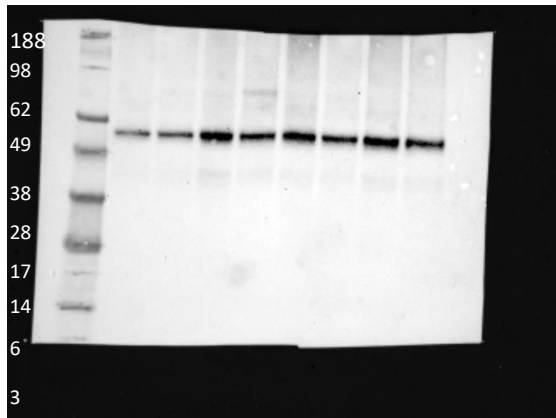
1 CTRL 3 CTRL 1 HnB 3 HnB 5 HnB

## WESTERN BLOT IMAGES

### Ventral Tegmental Area

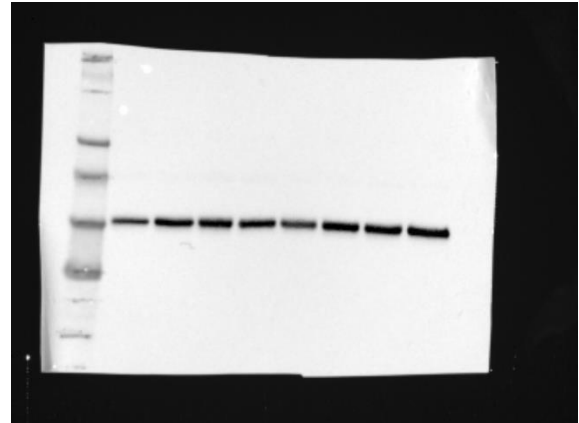
**GEL A**

p- AKT (~ 60 kDa)



1 CTRL 2 CTRL3 CTRL 4 CTRL 1 HnB 2 HnB 3 HnB 4 HnB

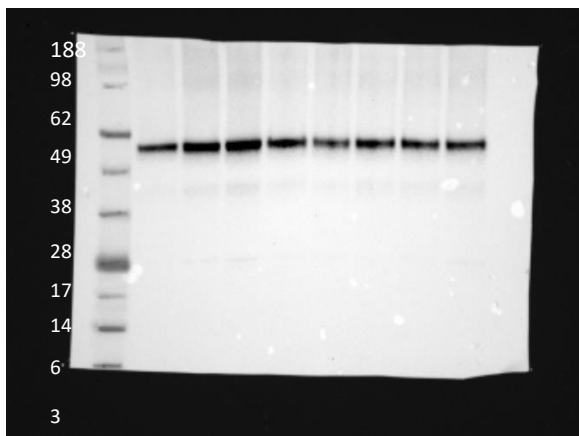
GAPDH (~ 36 kDa)



1 CTRL 2 CTRL 3 CTRL 4 CTRL 1 HnB 2 HnB 3 HnB 4 HnB

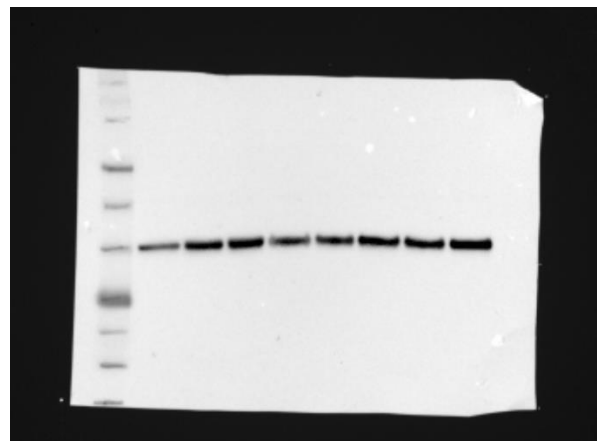
**GEL A**

AKT (~ 60 kDa)



1 CTRL 2 CTRL 3 CTRL 4 CTRL 1 HnB 2 HnB 3 HnB 4 HnB

GAPDH (~ 36 kDa)

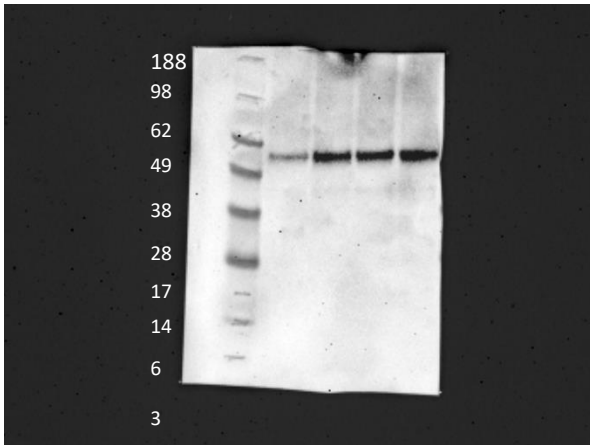


1 CTRL 2 CTRL 3 CTRL 4 CTRL 1 HnB 2 HnB 3 HnB 4 HnB

## ***Ventral Tegmental Area***

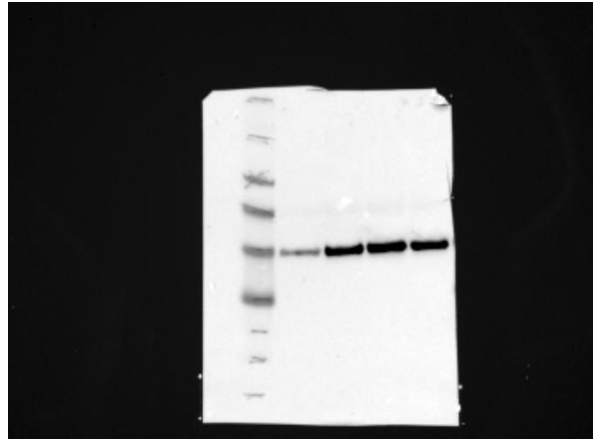
**GEL B**

**p- AKT (~ 60 kDa)**



5 CTRL 5 CTRL 6 HnB 6 HnB

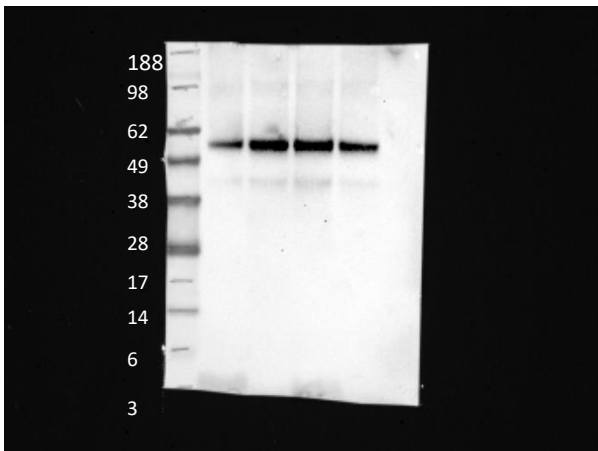
**GAPDH (~ 36 kDa)**



5 CTRL 5 CTRL 6 HnB 6 HnB

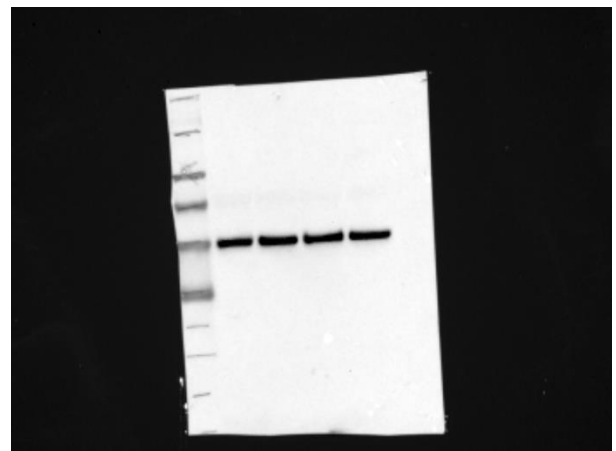
**GEL B**

**AKT (~ 60 kDa)**



5 CTRL 5 CTRL 6 HnB 6 HnB

**GAPDH (~ 36 kDa)**



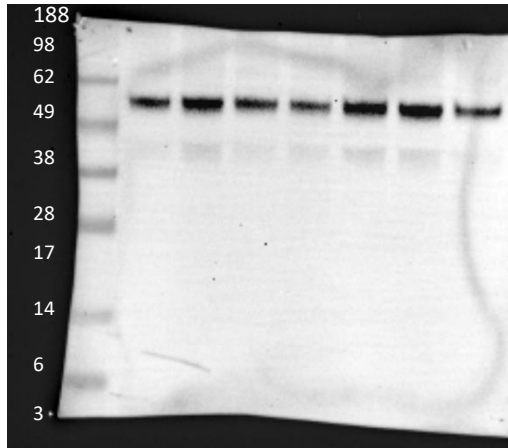
5 CTRL 5 CTRL 6 HnB 6 HnB

## Ventral Tegmental Area

**GEL C**

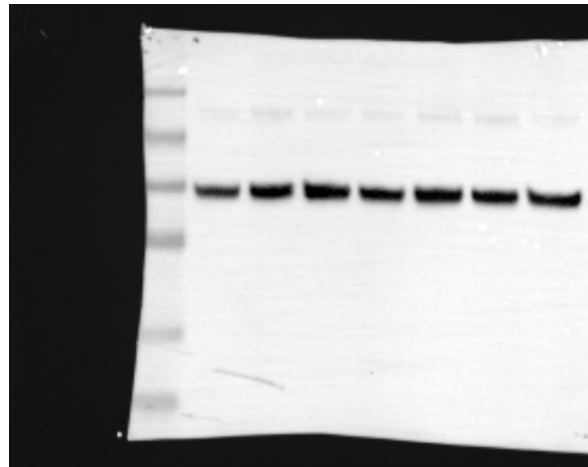
p- AKT (~ 60 kDa)

GAPDH (~ 36 kDa)



1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL

*Representative bands in figure 3a*



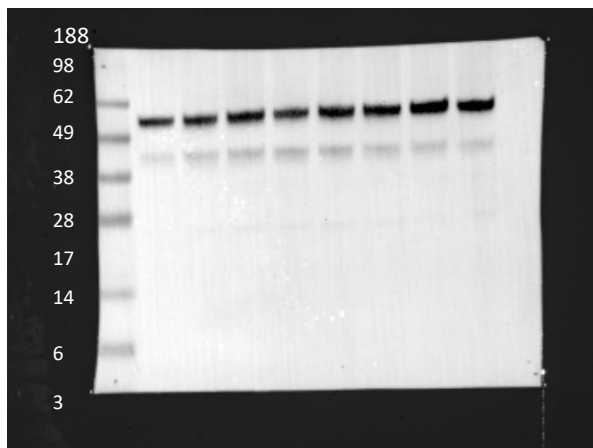
1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL

*Representative bands in figure 3a*

**GEL C**

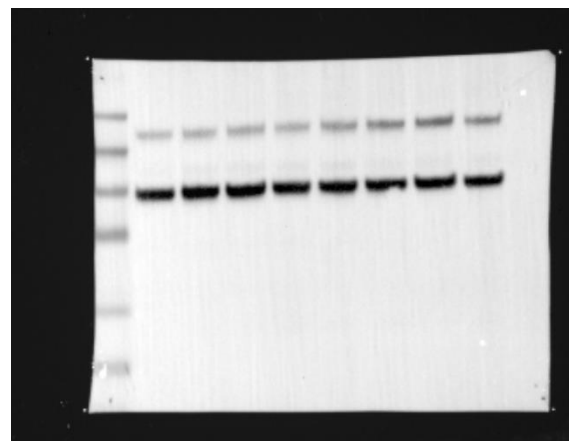
AKT (~ 60 kDa)

GAPDH (~ 36 kDa)



1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL

*Representative bands in figure 3a*



1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL

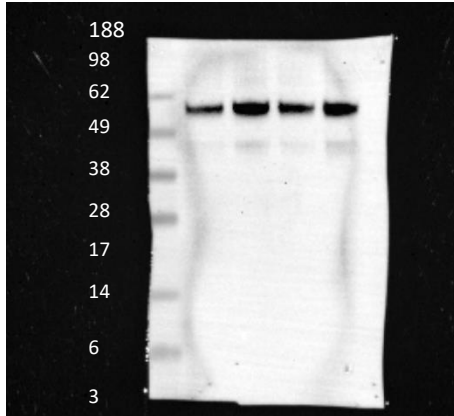
*Representative bands in figure 3a*

## ***Ventral Tegmental Area***

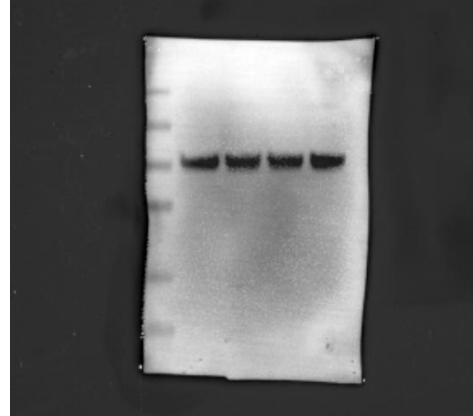
**GEL D**

**p- AKT (~ 60 kDa)**

**GAPDH (~ 36 kDa)**



5 CTRL 5 HnB 6 CTRL 6 HnB

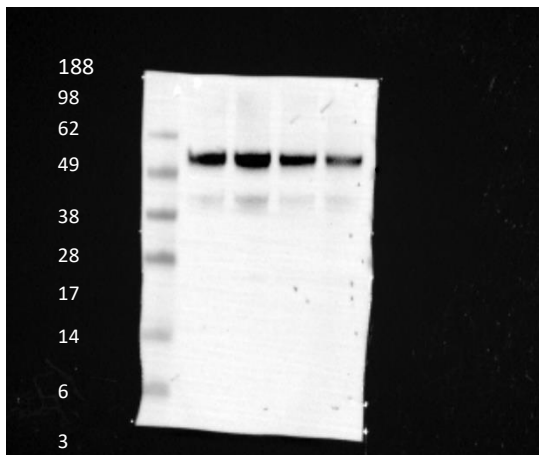


5 CTRL 5 HnB 6 CTRL 6 HnB

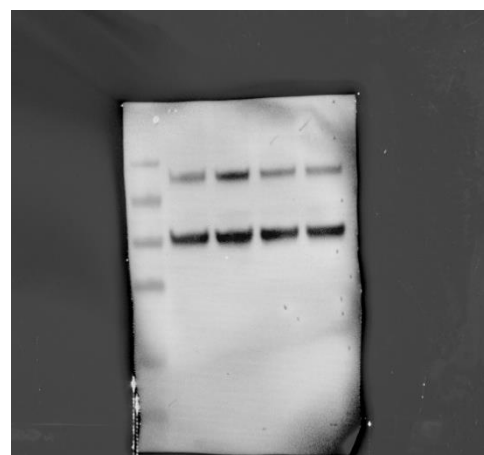
**GEL D**

**AKT (~ 60 kDa)**

**GAPDH (~ 36 kDa)**



5 CTRL 5 HnB 6 CTRL 6 HnB

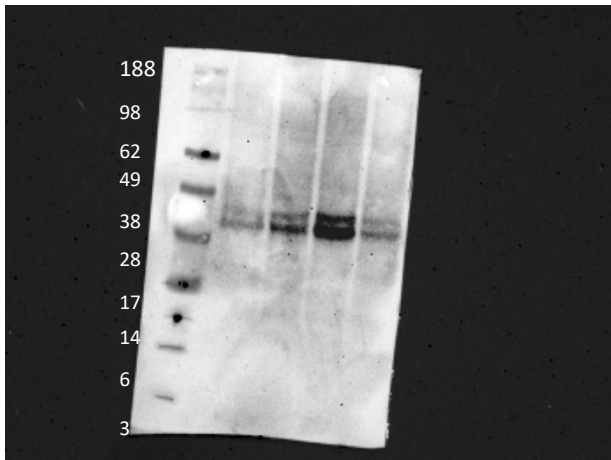


5 CTRL 5 HnB 6 CTRL 6 HnB

***Ventral Tegmental Area***

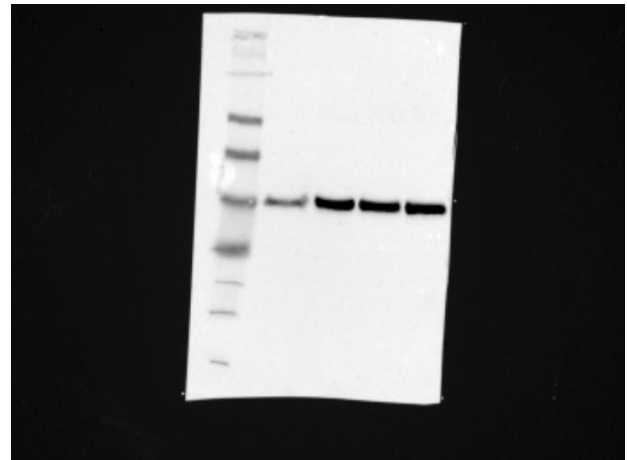
**GEL A**

**p- ERK 1/2 (~ 42/44 kDa)**



**1 CTRL 2 CTRL 1 HnB 2 HnB**

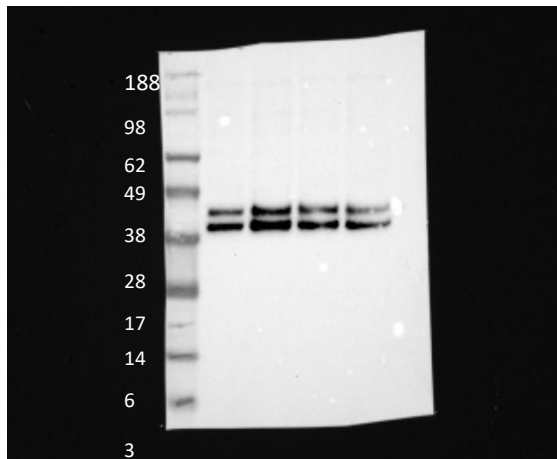
**GAPDH (~ 36 kDa)**



**1 CTRL 2 CTRL 1 HnB 2 HnB**

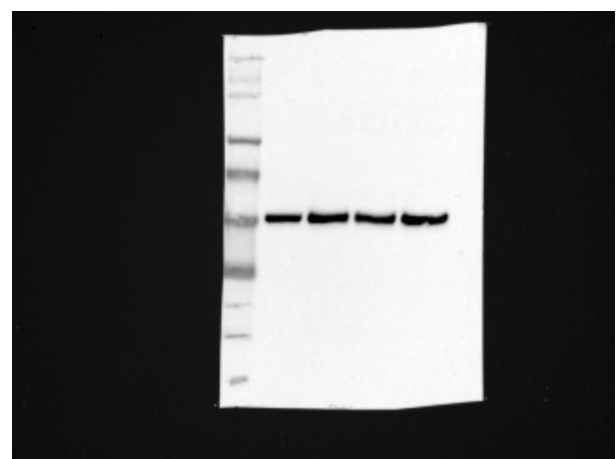
**GEL A**

**ERK 1/2 (~ 42/44 kDa)**



**1 CTRL 2 CTRL 1 HnB 2 HnB**

**GAPDH (~ 36 kDa)**



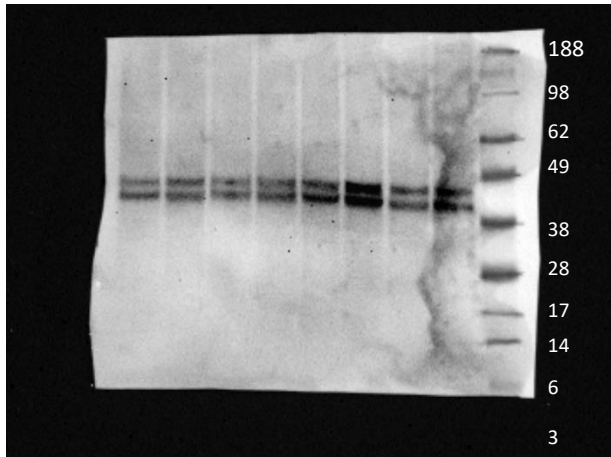
**1 CTRL 2 CTRL 1 HnB 2 HnB**

## ***Ventral Tegmental Area***

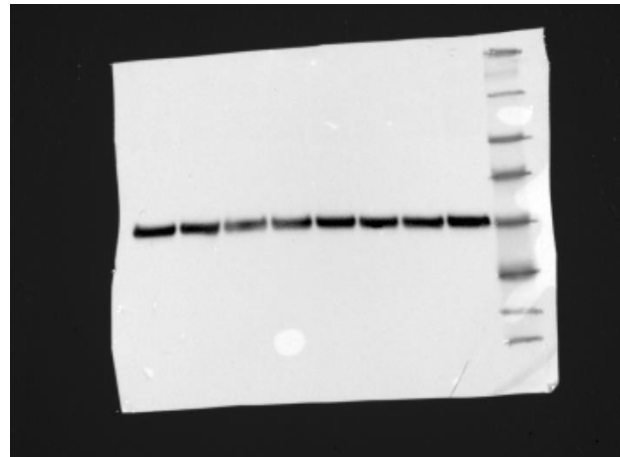
**GEL B**

**p- ERK 1/2 (~ 42/44 kDa)**

**GAPDH (~ 36 kDa)**



6 HnB 5 HnB 4 HnB 3 HnB 6 CTRL 5 CTRL

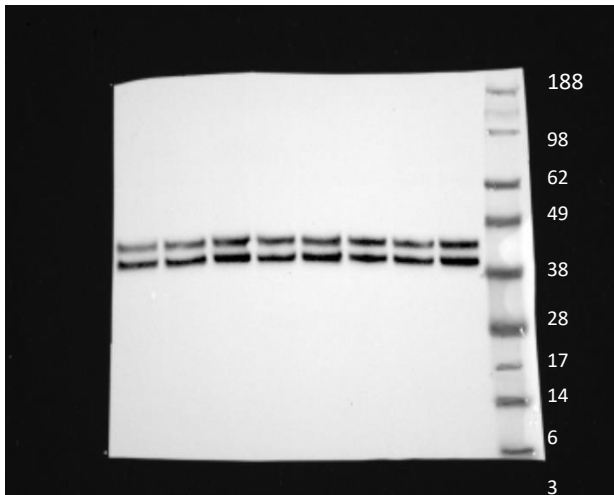


6 HnB 5 HnB 4 HnB 3 HnB 6 CTRL 5 CTRL

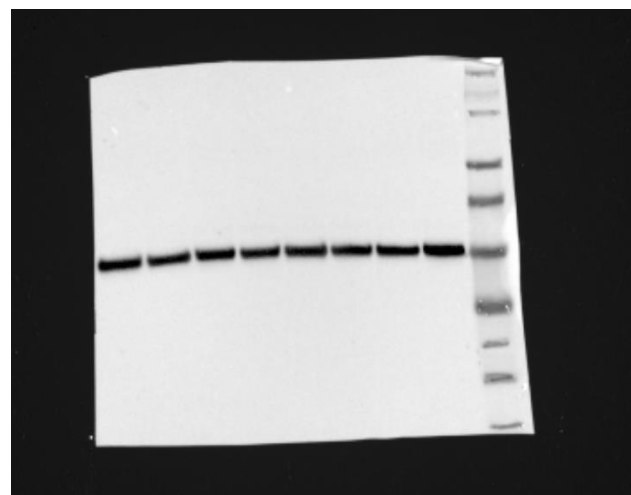
**GEL B**

**ERK 1/2 (~ 42/44 kDa)**

**GAPDH (~ 36 kDa)**



6 HnB 5 HnB 4 HnB 3 HnB 6 CTRL 5 CTRL



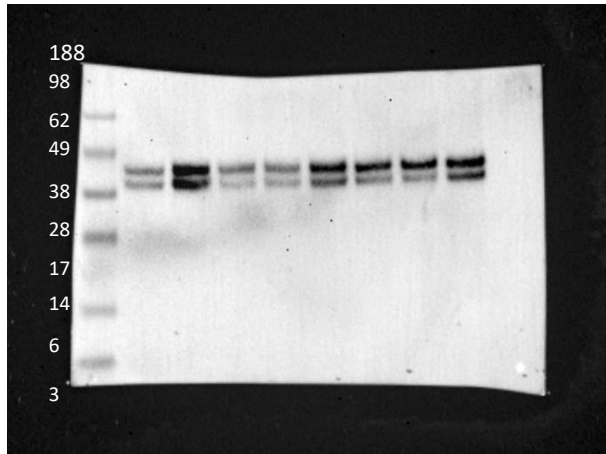
6 HnB 5 HnB 4 HnB 3 HnB 6 CTRL 5 CTRL



## Ventral Tegmental Area

**GEL C**

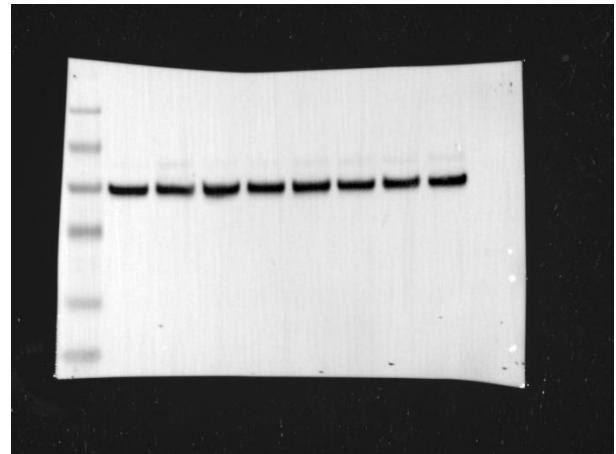
p- ERK 1/2 (~ 42/44 kDa)



1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL 4 HnB

*Representative bands in figure 3b*

GAPDH (~ 36 kDa)

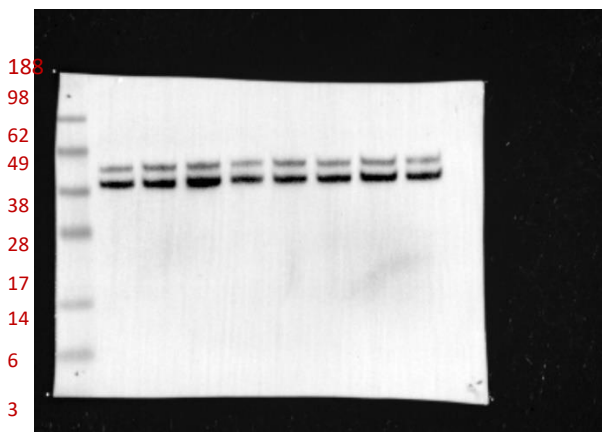


1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL 4 HnB

*Representative bands in figure 3b*

**GEL C**

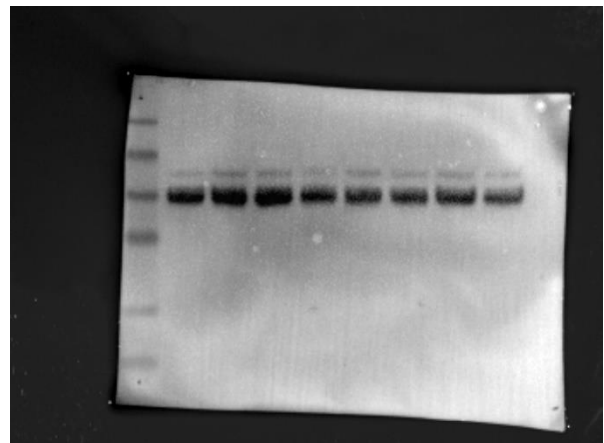
ERK 1/2 (~ 42/44 kDa)



1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL 4 HnB

*Representative bands in figure 3b*

GAPDH (~ 36 kDa)



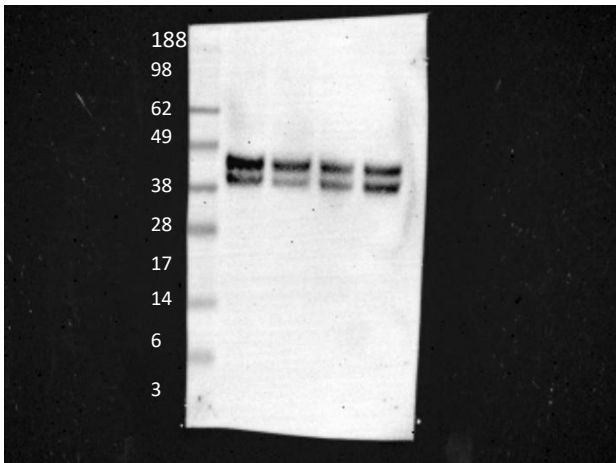
1 CTRL 1 HnB 2 CTRL 2 HnB 3 CTRL 3 HnB 4 CTRL 4 HnB

*Representative bands in figure 3b*

***Ventral Tegmental Area***

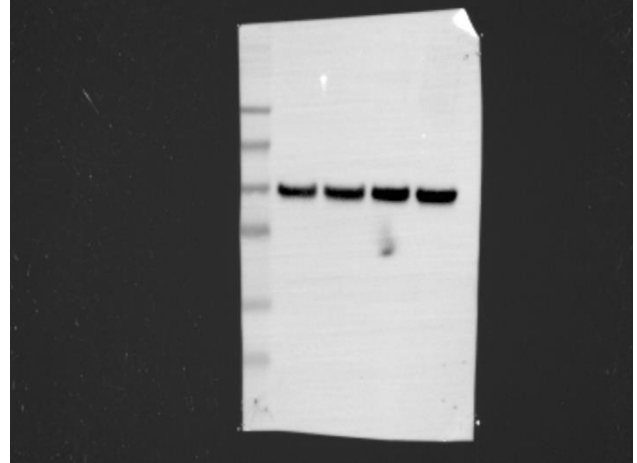
**GEL D**

**p- ERK 1/2 (~ 42/44 kDa)**



**5 CTRL 5 HnB 6 CTRL 6 HnB**

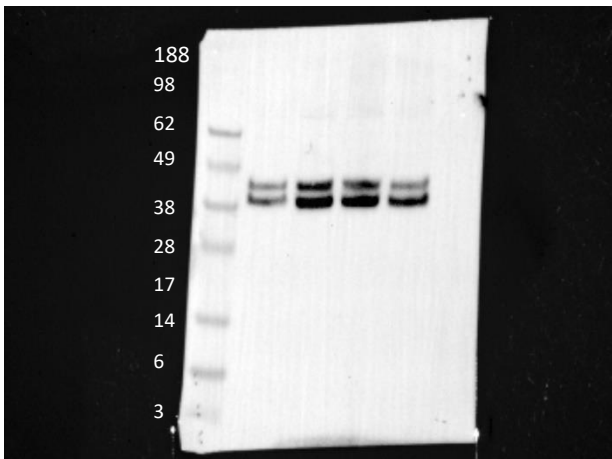
**GAPDH (~ 36 kDa)**



**5 CTRL 5 HnB 6 CTRL 6 HnB**

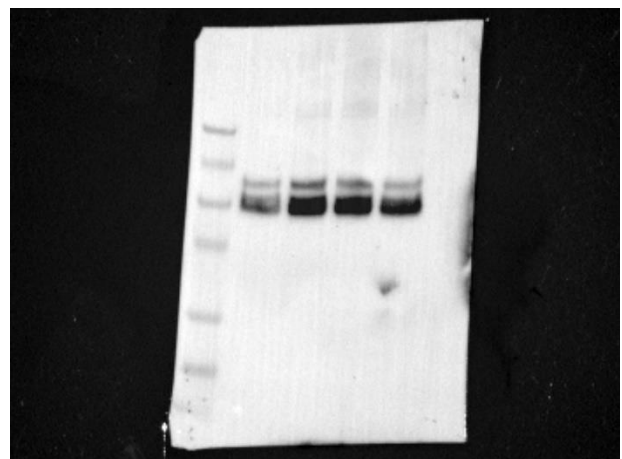
**GEL D**

**ERK 1/2 (~ 42/44 kDa)**



**5 CTRL 5 HnB 6 CTRL 6 HnB**

**GAPDH (~ 36 kDa)**



**5 CTRL 5 HnB 6 CTRL 6 HnB**