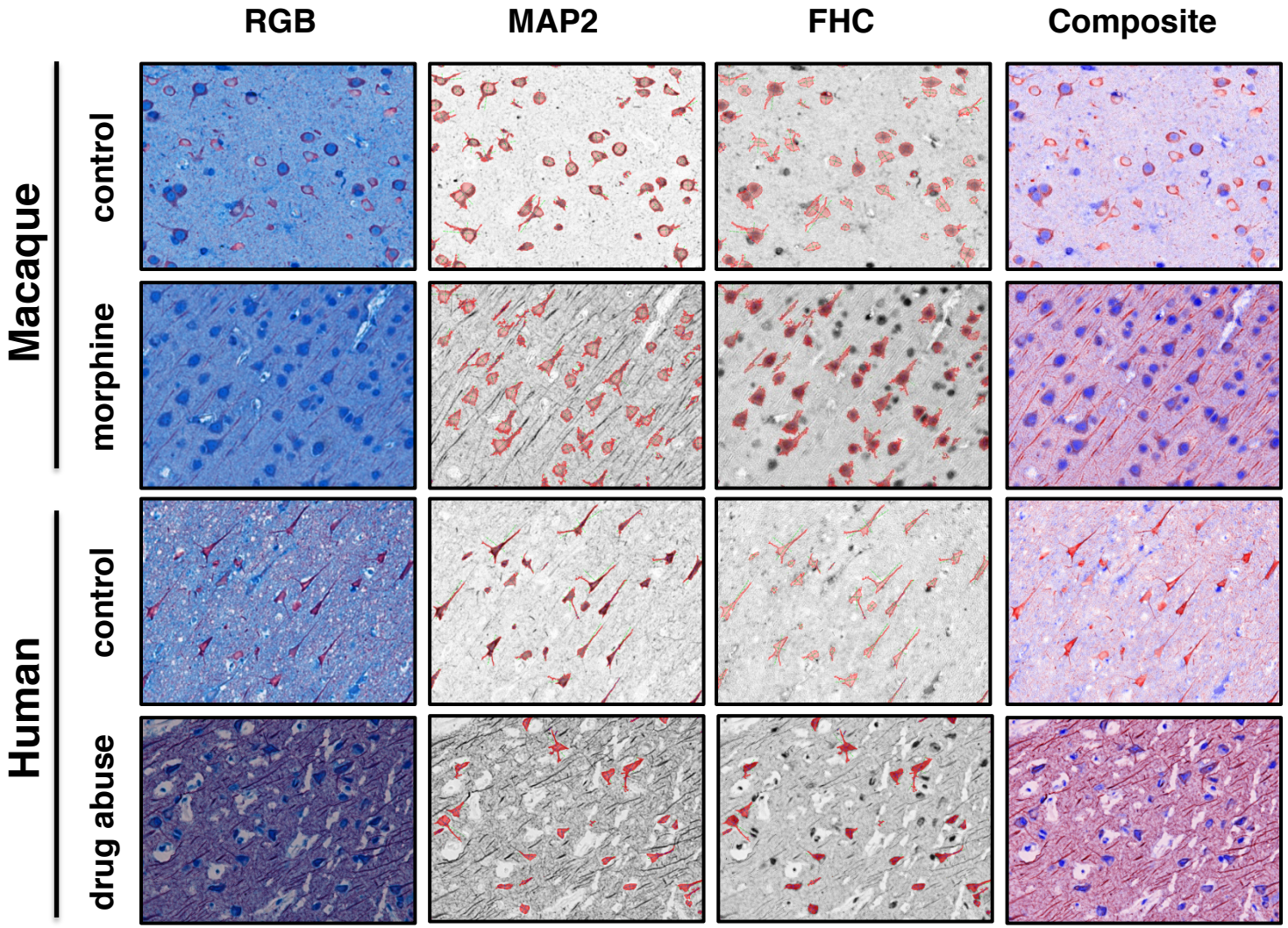
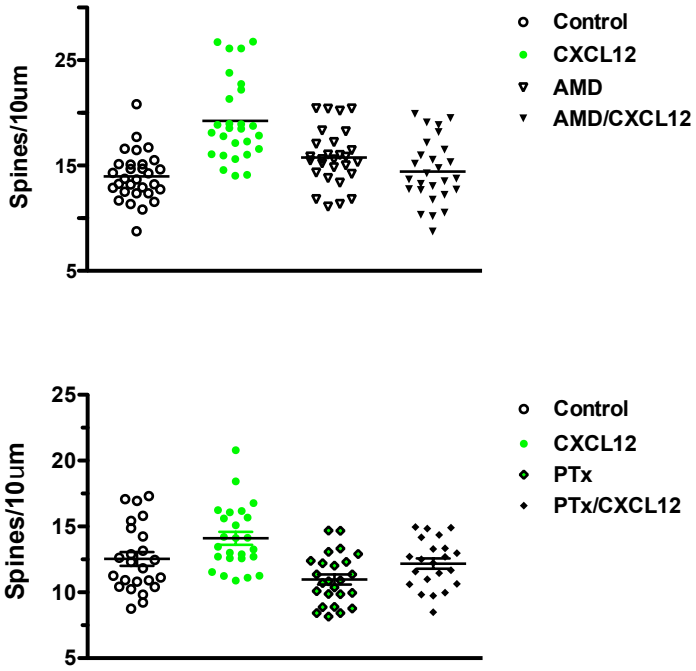
# Supplementary Figure 1



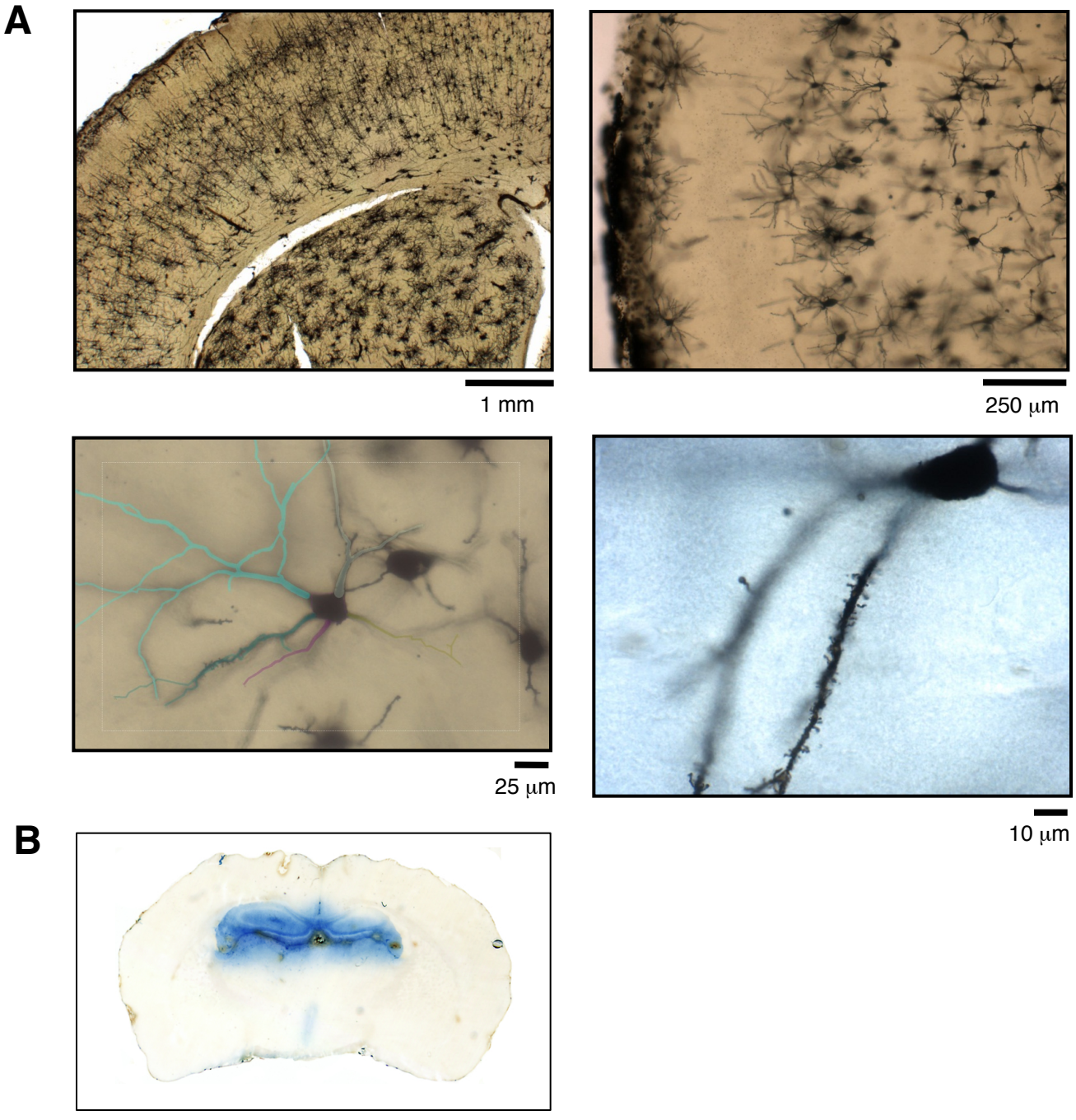

**Supplementary Figure 1** - Semi-quantitative measure of FHC protein within MAP2+ neurons using multispectral imaging. Individual cortical neurons are digitally identified and isolated from frontal cortex tissue using the optical density (OD) of the MAP2:NovaRed complex. The neuronal map is superimposed over the corresponding OD of the FHC:VectorBlue spectrum. Optical densities of both MAP2 and FHC are combined in a pseudocolored composite image. Both the macaque morphine treatment group and the human drug use group show greater FHC protein expression within cortical neurons than the respective control groups. (RGB: red green blue image, MAP2: optical density image of MAP2:NovaRed conjugated chromogen, FHC: optical density of FHC:Vector:Blue conjugated chromogen. Composite: pseudocolored image of MAP2 (red) and FHC (blue) optical densities. All Images at 40X Magnification.

# Supplementary Figure 2




**Supplementary Figure 2** - CXCR4 antagonist AMD3100 and pertussis toxin inhibit the effect of CXCL12 on dendritic spine density. Rat cortical neurons cultured for 21 days were pre-treated with either AMD3100 (100ng/ml; added 20 min before addition of CXCL12) or Pertussis Toxin (PTx, 100ng/ml; 18hrs before addition of CXCL12) and then exposed to CXCL12 (20nM, 3 hours). Data reported in green are significantly different than controls ($P<0.05$; One way ANOVA followed by Dunnett test).

# Supplementary Figure 3




**Supplementary Figure 3** - Technical approaches for in vivo experiments. (**A**) Methods for *in vivo* dendritic spine analysis. Rat brains were stained using a Golgi stain kit, as described in the methods. This system selectively impregnates a subset of neurons, in a likely random manner. Individual neurons from layer II/III of the prefrontal cortex were reconstructed in their entirety, first by tracing the dendrites, then by indicating individual dendritic spines. This process creates a 3-dimensional map of each neuron, including fine morphological details. (**B**) Stereotaxic brain injections target the ventricular system. In order to confirm the correct targeting of our ICV injections, we injected 5 μL of 2% Evans blue dye into the left lateral ventricle and verified distribution of the dye.

# Supplementary Table 1

| Total Patients | Drug Use | HIV Status | HAND (MSK Score) | |
|---|---|---|---|---|
| *N*=51 | **DU-** *N*=33 | **HIV-** *N*=14 | | |
| | | **HIV+** *N*=19 | **0-0.5** | *N*=6 |
| | | | **1-2** | *N*=12 |
| | | | **3-4** | *N*=1 |
| | **DU+** *N*=18 | **HIV-** *N*=7 | | |
| | | **HIV+** *N*=11 | **0-0.5** | *N*=6 |
| | | | **1-2** | *N*=5 |
| | | | **3-4** | *N*=0 |

**Supplementary Table 1** - Human tissue cohort. Postmortem human tissue was collected from three sources: the National NeuroAIDS Tissue Consortium, National Development and Research Institutes (NDRI), and the Drexel University Department of Pathology. Patient samples were grouped based on illicit drug use (all including opiate use) and HIV status. Neurocognitive impairment was also quantified for each patient, using the Memorial Sloan Kettering (MSK) scale. This scale ranges from 0 to 4, where 0=no cognitive impairment, 0.5=sub-clinical impairment, 1=mild impairment, 2=moderate impairment, 3=severe impairment, and 4=profound impairment.

# Supplementary Table 2

| | Group | MSK | Race | Sex | Age | CD4 count (cells/mm$^3$) | CSF VL (copies/mL) | Plasma VL (copies/mL) |
|---|---|---|---|---|---|---|---|---|
| **1** | Control | Normal | W/Cau | M | 66 | | - | - |
| **2** | Control | Normal | W/Cau | F | 73 | | - | - |
| **3** | Control | Normal | W/Cau | F | 61 | | - | - |
| **4** | Control | Normal | W/Cau | M | 44 | | - | - |
| **5** | Control | Normal | W/Cau | M | 49 | | - | - |
| **6** | Control | Normal | W/Cau | M | 65 | | - | - |
| **7** | Control | Normal | W/Cau | M | 63 | | - | - |
| **8** | Control | Normal | W/Cau | M | 43 | | - | - |
| **9** | Control | Normal | W/Cau | M | 52 | | - | - |
| **10** | Control | Normal | Black/AA | F | 30 | | - | - |
| **11** | Control | Normal | Unknown | M | 64 | | - | - |
| **12** | Control | Normal | Black/AA | F | 61 | | - | - |
| **13** | Control | Normal | W/Cau | F | 54 | | - | - |
| **14** | Control | Normal | Black/AA | M | 56 | | - | - |
| **15** | DU+ | Normal | W/Cau | M | 54 | | - | - |
| **16** | DU+ | Normal | W/Cau | M | 61 | | - | - |
| **17** | DU+ | Normal | Black/AA | M | 57 | | - | - |
| **18** | DU+ | Normal | Hispanic | M | 47 | | - | - |
| **19** | DU+ | Normal | Hispanic | M | 47 | | - | - |
| **20** | DU+ | Normal | Hispanic | F | 47 | | - | - |
| **21** | DU+ | Normal | Unknown | F | 68 | | - | - |
| **22** | HIV+ | Normal | W/Cau | M | 44 | 147 | | 50 |
| **23** | HIV+ | Normal | W/Cau | M | 34 | | | |
| **24** | HIV+ | Normal | W/Cau | M | 38 | | | |
| **25** | HIV+ | Sub-clin. | W/Cau | M | 64 | 61 | 7,484 | 75 |
| **26** | HIV+ | Sub-clin. | Black/AA | M | 44 | 234 | 618 | 16,909 |
| **27** | HIV+ | Sub-clin. | W/Cauc | M | 59 | | | |
| **28** | HIV+ | Mild | W/Cau | M | 50 | 3 | <50 | 750,000 |
| **29** | HIV+ | Mild | W/Cau | M | 36 | 27 | 670 | 18,985 |
| **30** | HIV+ | Mild | Black/AA | F | 46 | 36 | 1,543 | 380,189 |
| **31** | HIV+ | Mild | Other | M | 45 | 26 | 161 | 316,227 |
| **32** | HIV+ | Mild | W/Cau | M | 36 | | | |
| **33** | HIV+ | Moderate | W/Cau | F | 34 | 14 | 76 | 15,906 |
| **34** | HIV+ | Moderate | W/Cau | M | 42 | 2 | 1,222,799 | 287,582 |
| **35** | HIV+ | Moderate | Other | F | 35 | 360 | 96 | 436,515 |
| **36** | HIV+ | Moderate | Black/AA | F | 34 | | | |
| **37** | HIV+ | Moderate | W/Cau | M | 34 | | | |
| **38** | HIV+ | Moderate | Black/AA | F | 34 | | | |
| **39** | HIV+ | Moderate | Black/AA | M | 37 | | | |
| **40** | HIV+ | Profound | W/Cau | M | 38 | 43 | 1,237,903 | 1,843 |
| **41** | DU+HIV+ | Normal | W/Cau | F | 30 | 407 | | 187 |
| **42** | DU+HIV+ | Normal | Hispanic | F | 46 | 88 | 1,305 | 74,294 |
| **43** | DU+HIV+ | Normal | W/Cau | M | 46 | 3 | 201 | 779,000 |
| **44** | DU+HIV+ | Normal | W/Cau | M | 37 | | | |
| **45** | DU+HIV+ | Sub-clin. | Other | F | 44 | 66 | 249 | 750,000 |
| **46** | DU+HIV+ | Sub-clin. | W/Cau | M | 56 | | | |
| **47** | DU+HIV+ | Mild | W/Cau | M | 54 | 211 | | 400 |
| **48** | DU+HIV+ | Mild | Other | F | 51 | | | |
| **49** | DU+HIV+ | Mild | W/Cau | M | 32 | 49 | 3,140,694 | 972,503 |
| **50** | DU+HIV+ | Mild | W/Cau | M | 39 | | | |
| **51** | DU+HIV+ | Moderate | W/Cau | M | 43 | 44 | <50 | 65 |

**Supplementary Table 2** - Human subject demographics, disease group, and clinical data at time of death**:** Patients 1 to 21 are HIV negative; patients 1 to 14 and 22 to 40 did not abuse drugs; all DU but 2 (marked by asterisk in table) abused opiates; other substances of abuse were: cocaine (13/18), alcohol (13/18), cannabis (9/18), stimulants (7/18); sedatives and hallucinogens (6/18).

# Supplementary Table 3

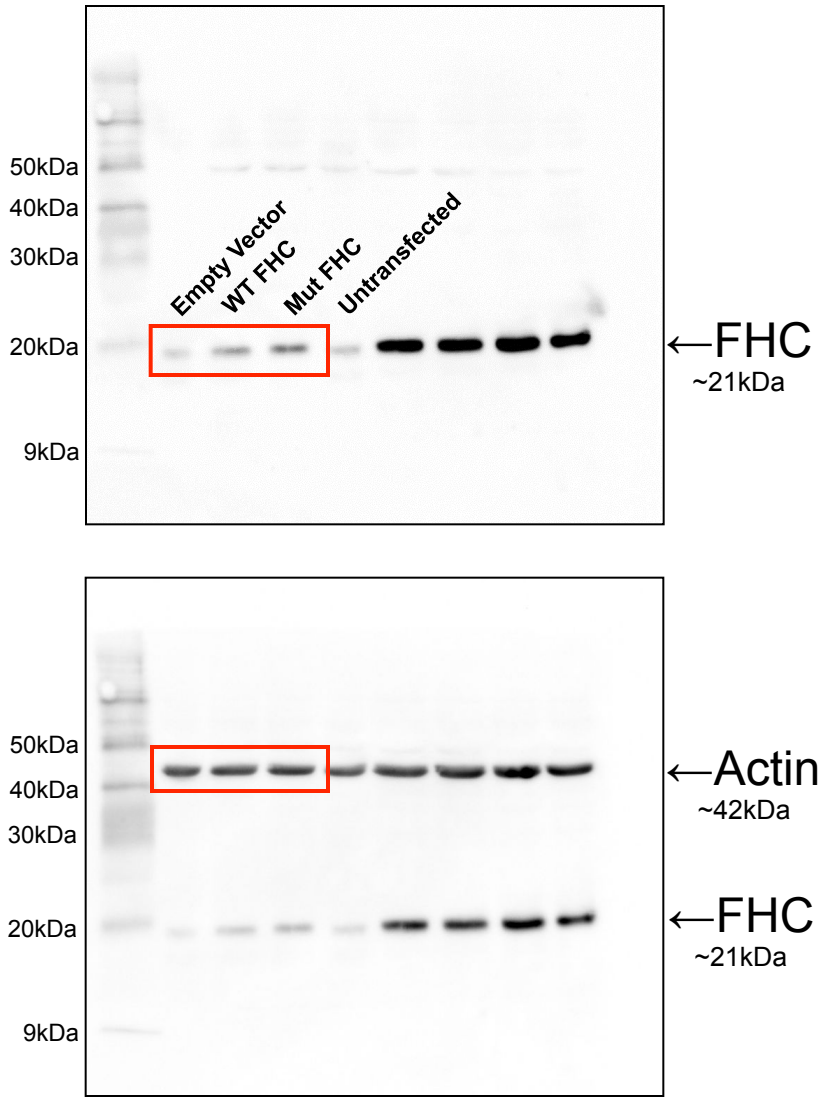
| | ID | Sex | Age | SIV | Morphine | Weight at necropsy (kg) |
|---|---|---|---|---|---|---|
| **Control** | CL14 | F | 10 | - | - | 5.2 |
| | DP27 | F | 9 | - | - | 7.2 |
| | FE82 | F | 7 | - | - | 8.6 |
| | FM11 | M | 8 | - | - | 10.2 |
| | FV39 | M | 6 | - | - | 10.9 |
| | GA15 | M | 6 | - | - | 12.2 |
| | HM63 | M | 3 | - | - | 3.8 |
| | HN64 | M | 3 | - | - | 4.9 |
| | HP24 | M | 3 | - | - | 3.5 |
| | IP37 | F | 16 | - | - | 8.7 |
| | IP43 | F | 6 | - | - | 5.4 |
| **Morphine** | 4684 | F | 5 | - | + | 6.3 |
| | 4690 | M | 3 | - | + | 5.1 |
| | 4697 | M | 3 | - | + | 4.4 |
| **SIV** | 4678 | F | 4 | + | - | 3.6 |
| | 4679 | F | 3 | + | - | 5.6 |
| | 4680 | F | 5 | + | - | 4.9 |
| | 4688 | M | 3 | + | - | 4.9 |
| **Morphine/SIV** | 4692 | F | 3 | + | + | 4.2 |
| | 4693 | F | 3 | + | + | 2.9 |

**Supplementary Table 3** - Non-human primate tissue cohort. Prefrontal cortex was obtained from rhesus macaques that underwent 4 types of treatment: Control (*n*=11), Morphine (*n*=3), SIV (*n*=4), and Morphine + SIV (*n*=2). Animals were randomly assigned to treatment groups, without regard to sex, age, or weight.

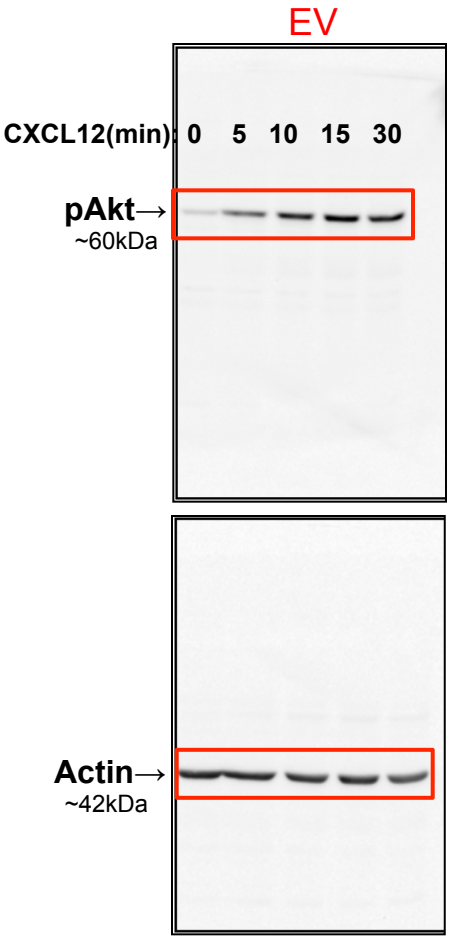
| | Resting membrane potential (mV) | Input Resistance (MΩ) | Spike threshold (mV) | Peak spike amplitude (mV) | Afterhyperpolarization (mV) | Spike half-width (ms) | 20-80% rise time (ms) |
|---|---|---|---|---|---|---|---|
| Vehicle $_{AMD}$ | -79.6±1.0 | 149.3±13.3 | -41.8±1.5 | 75.2±3.3 | -12.1±1.3 | 1.2±0.06 | 0.28±0.02 |
| AMD3100 | -76.9±2.3 | 138.3±9.5 | -38.4±3.0 | 72.6±5.3 | -12.8±1.5 | 1.3±0.12 | 0.31±0.02 |
| Vehicle $_{CXCL12}$ | -75.82±1.9 | 124.2±1.3.6 | -40.4±1.1 | 72.4±1.8 | -12.7±1.0 | 1.2±0.9 | 0.28±0.01 |
| CXCL12 | -76.1±2.3 | 121.8±13.2 | -42.9±2.3 | 74.0±2.8 | -12.0±0.9 | 1.2±0.07 | 0.29±0.01 |

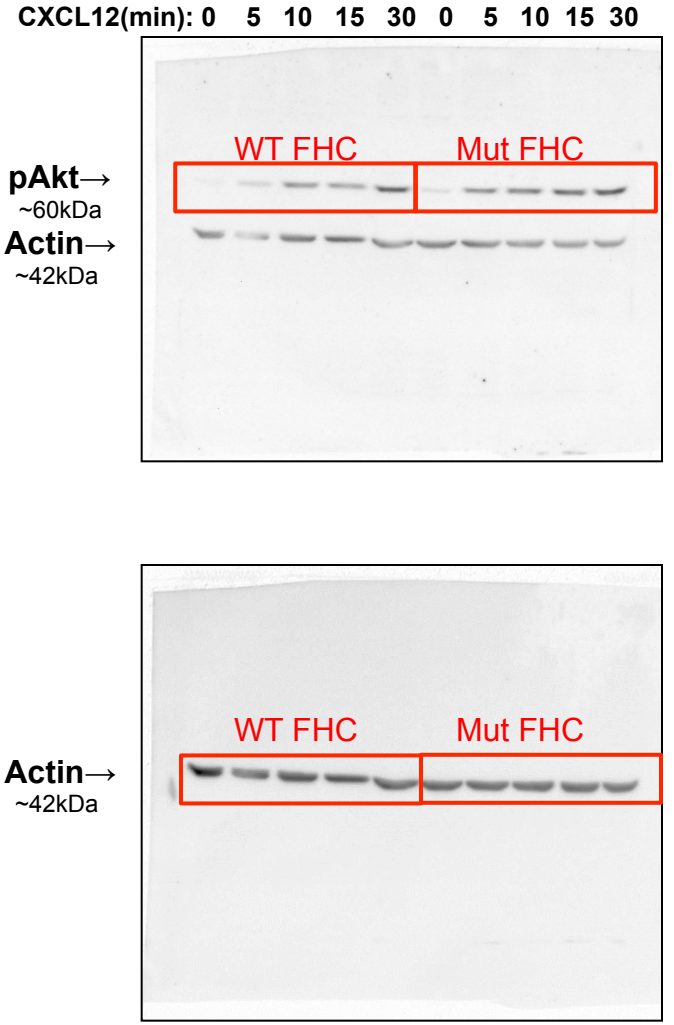
**Supplementary Table 4**. Physiological properties of layer 2/3 pyramidal neurons exposed to CXCL12, AMD3100 or their respective vehicles. There were no significant differences between drug and vehicle conditions (all $p>0.05$).

# Full unedited gel for figure 3a



# Full unedited gels for figure 3b (pAkt)



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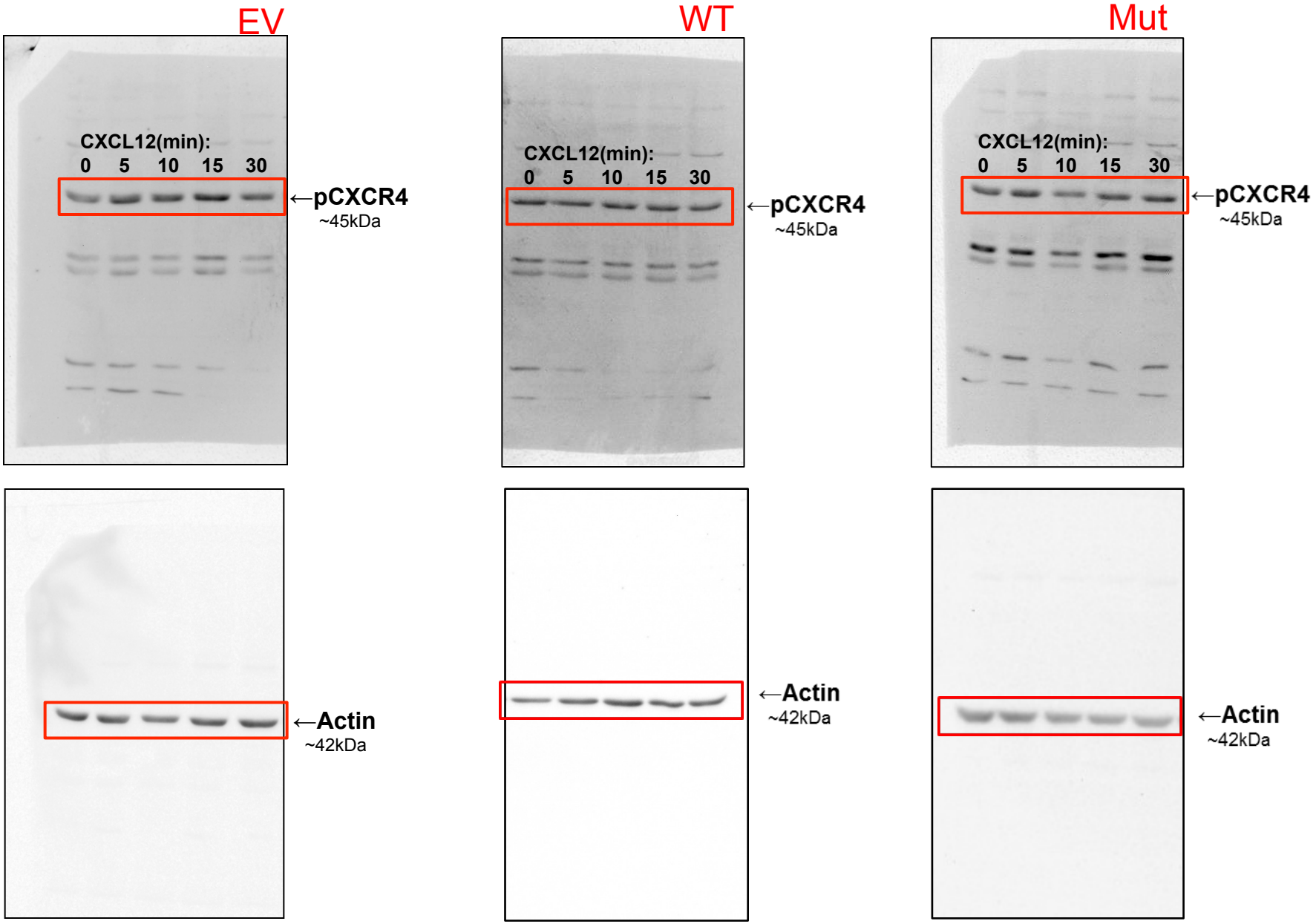

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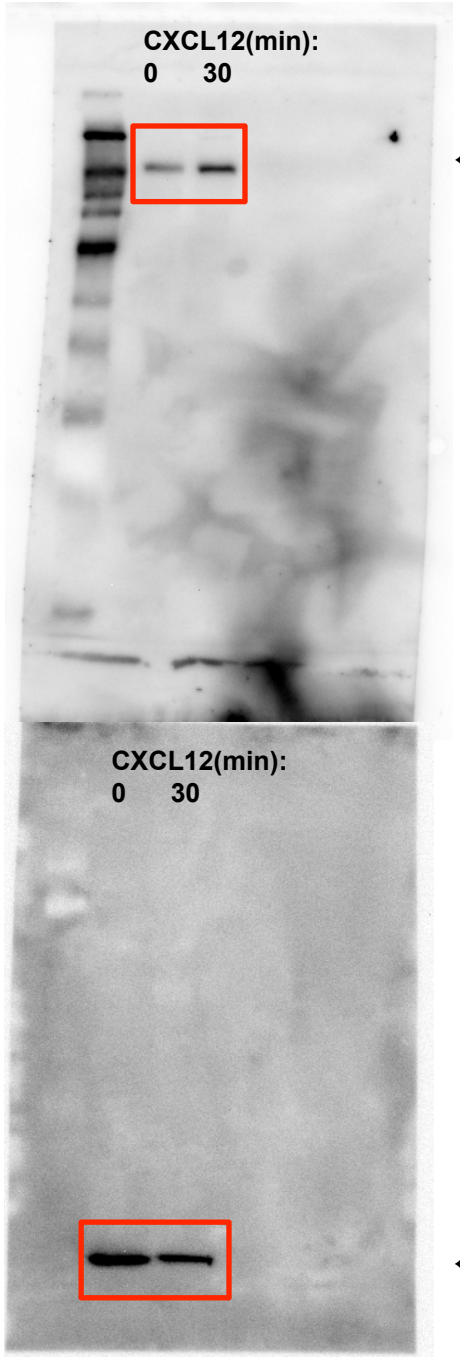

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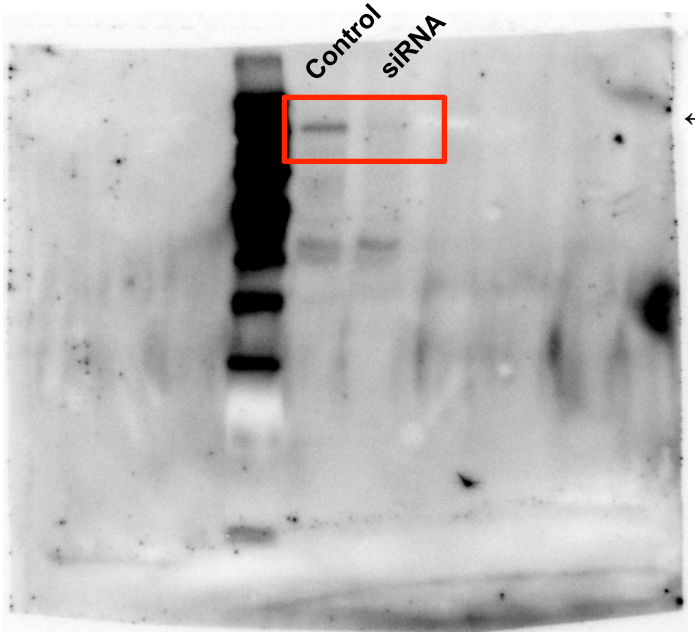
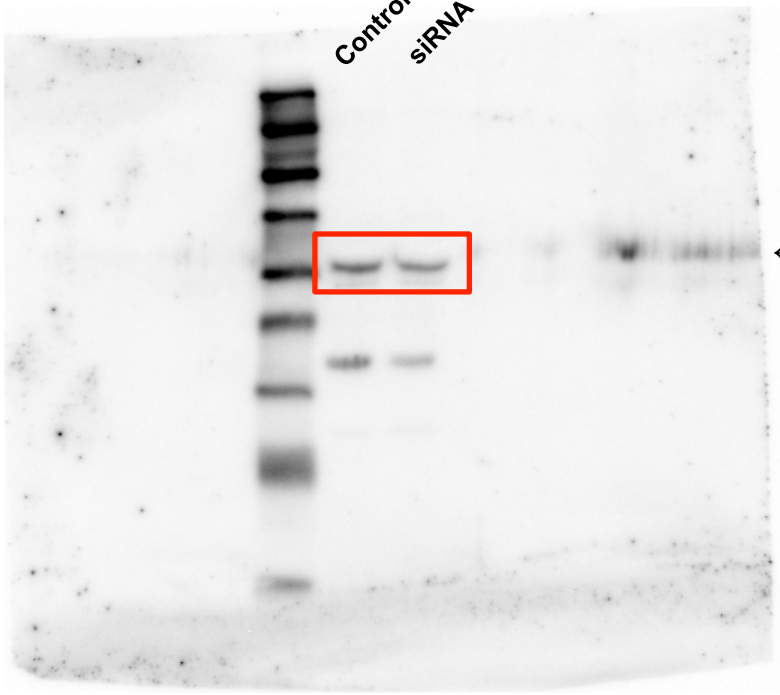
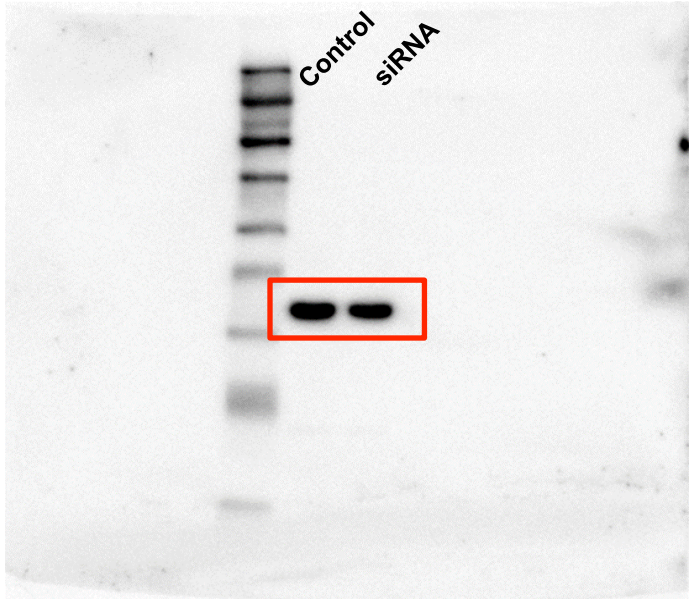



←HDAC4
~140kDa

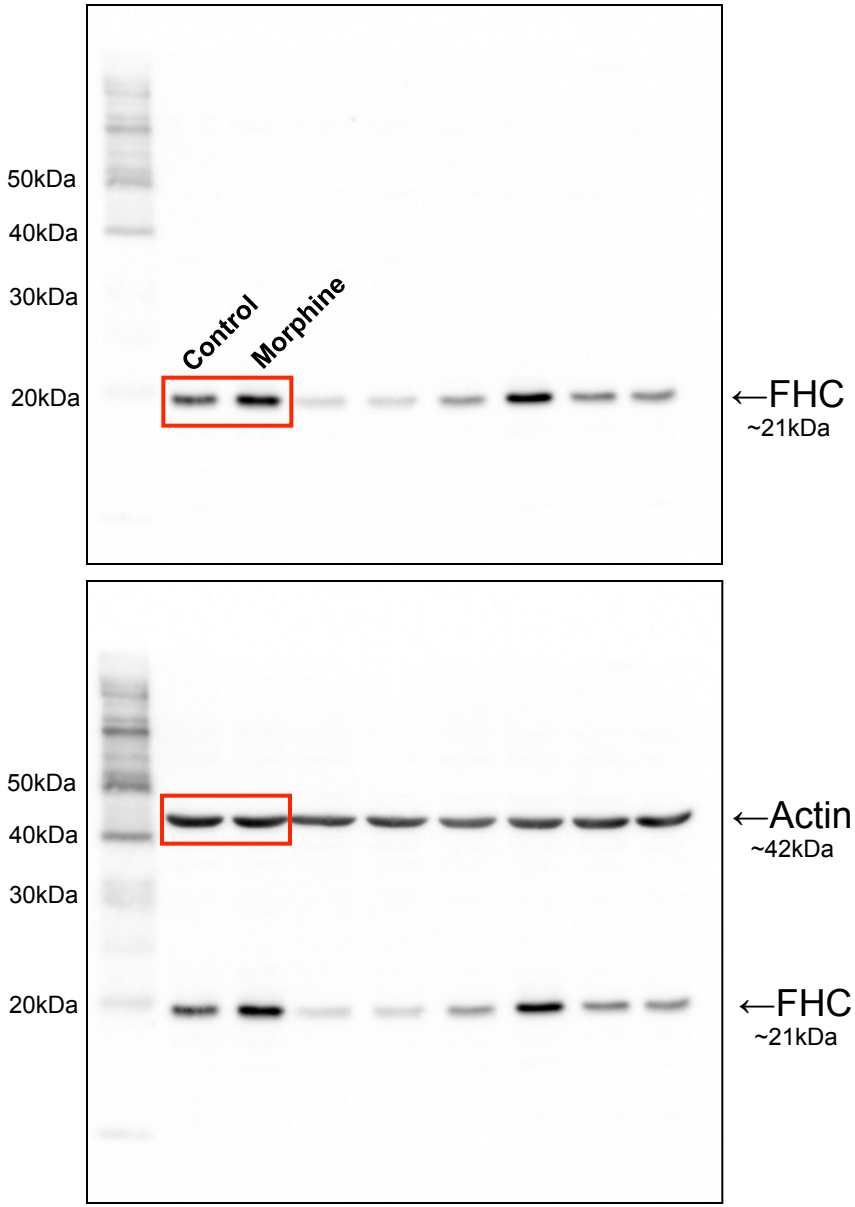



←HDAC1
~62kDa




←Actin
~42kDa

# Full unedited gel for figure 6a



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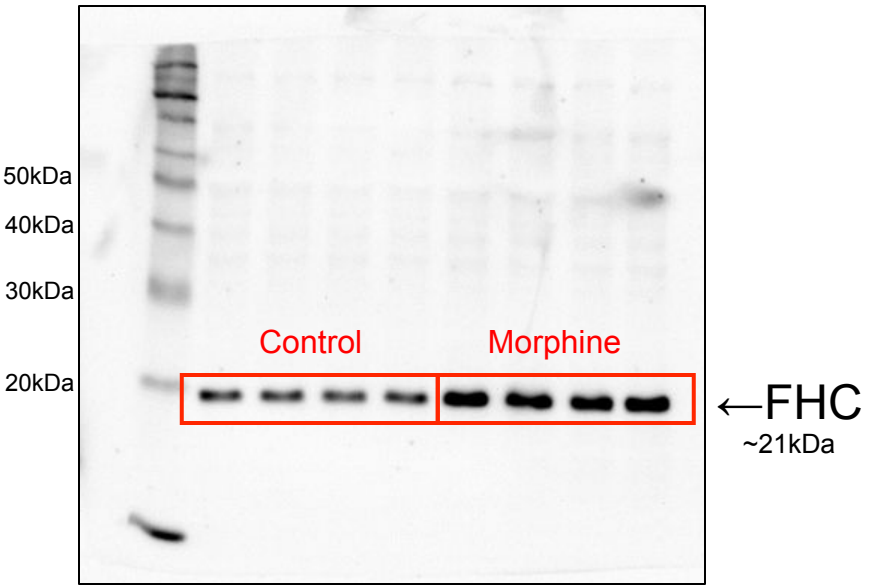


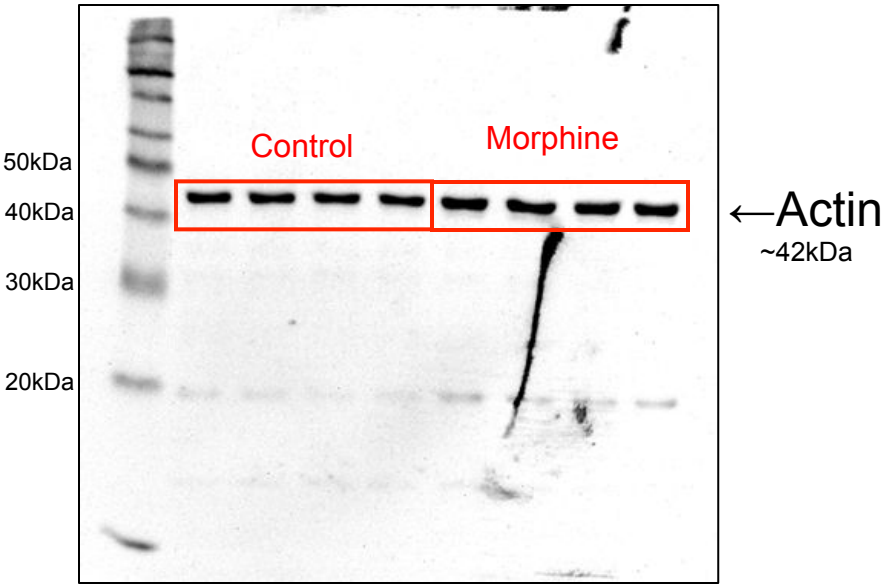

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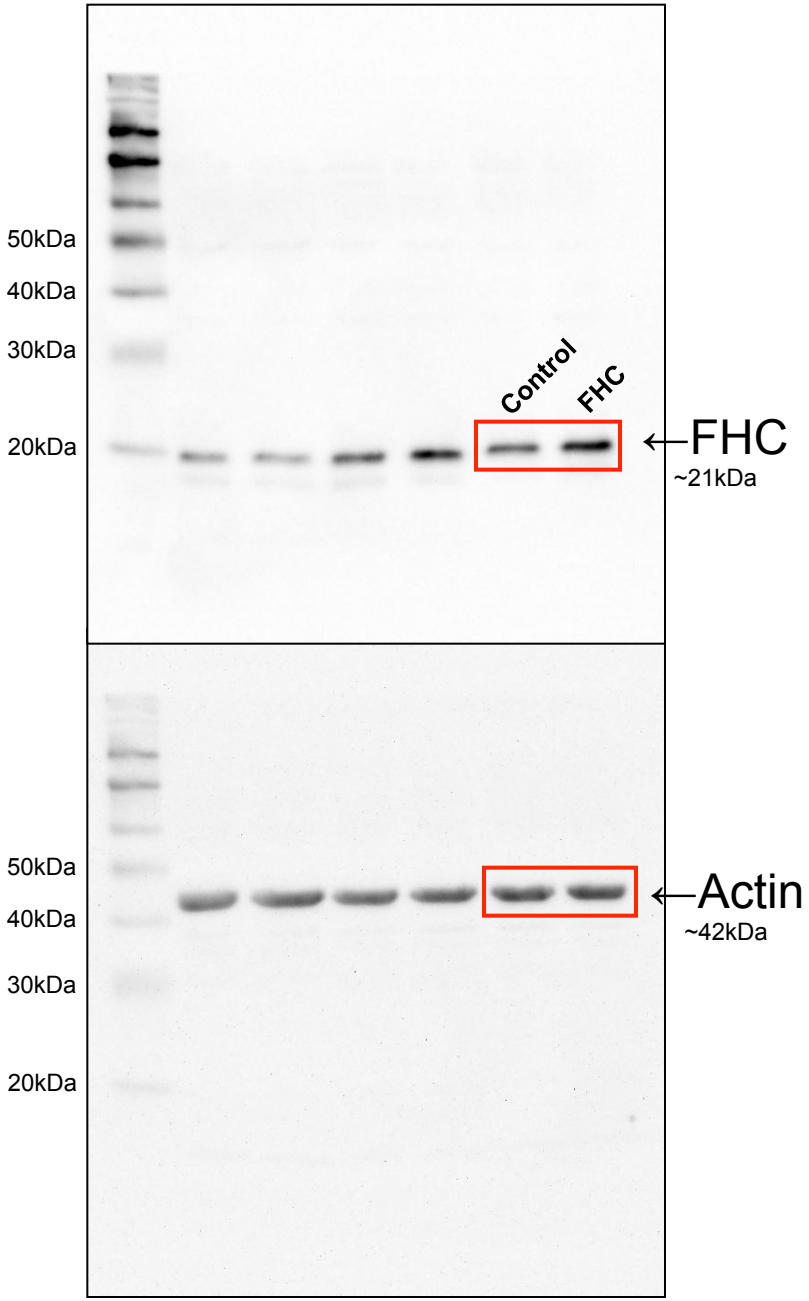

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