

Original Western Blots (pre-cut)

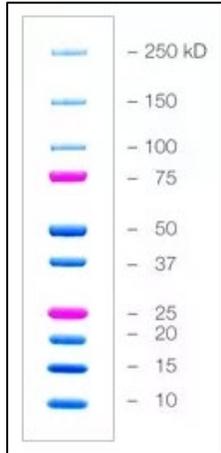
Alcohol Use Disorder Associated Gene *FNDC4* Alters
Glutamatergic and GABAergic Neurogenesis

Zhu X, *et al.* 2025

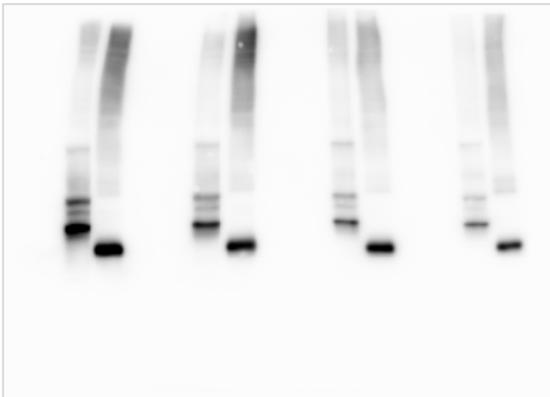
Introduction

1. All Western blot pictures were obtained by using a Bio-Rad ChemiDoc Touch Imaging System.
2. A Bio-Rad **Precision Plus Protein Dual Color Standards** (cat#: [1610374](#), see image for molecular weights of markers) was used for all blots in this study.
3. On a single blot (PVDF membrane), **protein bands** were visualized by Chemiluminescence, and **prestained protein markers (standards)** were visualized by Colormetric picture.
4. Chemiluminescence and Colormetric pictures were **Merged** to show the sizes of bands (see examples below).
5. All pre-cut blots shown in subsequent slides are Merged pictures with **red-dashed boxes** to indicate cut blots that are presented in the Figures. (see pictures below for examples: red-dashed box corresponding to **Figure 2B**)

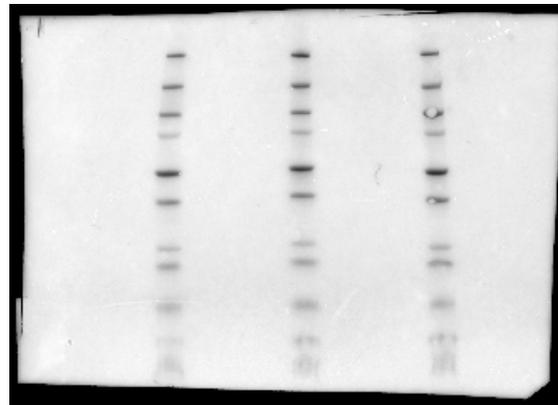
Bio-Rad
(cat#: [1610374](#))



Protein Blot
(Chemiluminescence)



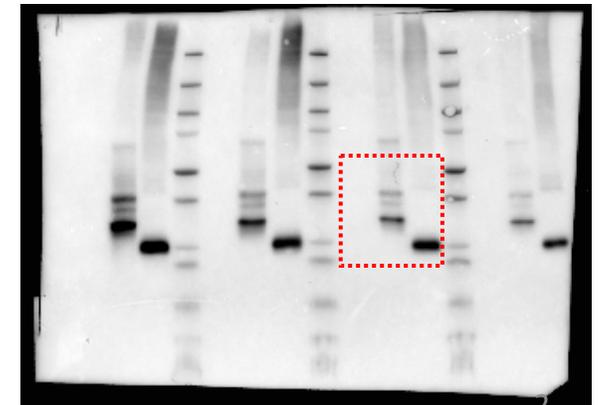
Prestained Markers
(Colormetric)



+

=

Merged



Full unedited blot for **Figure 2B** & **Supplemental Figure S3A**

Figure 2B

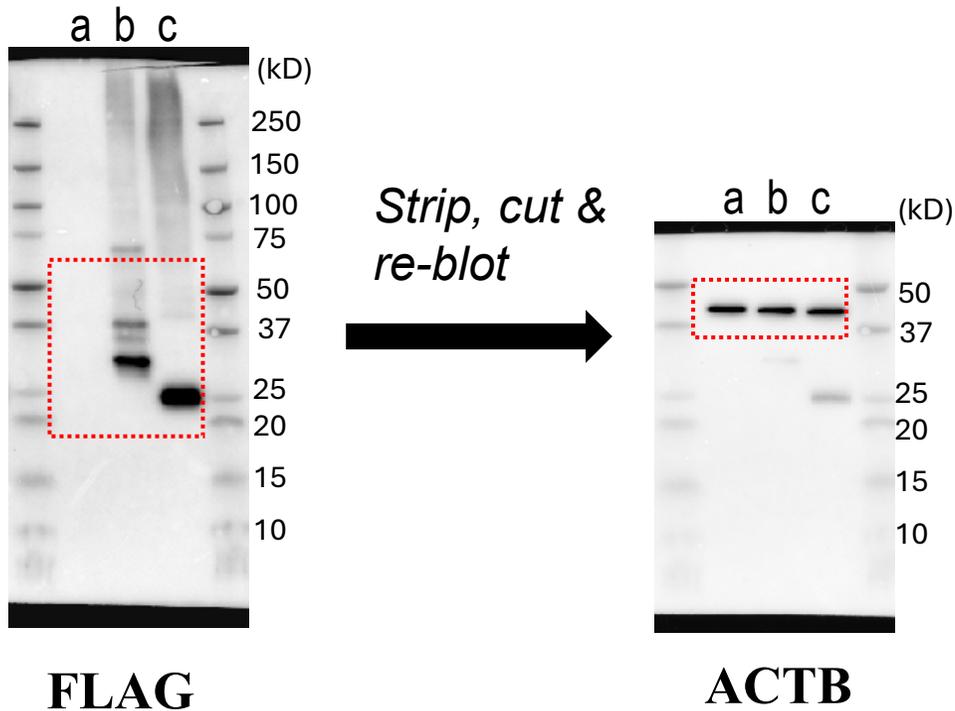
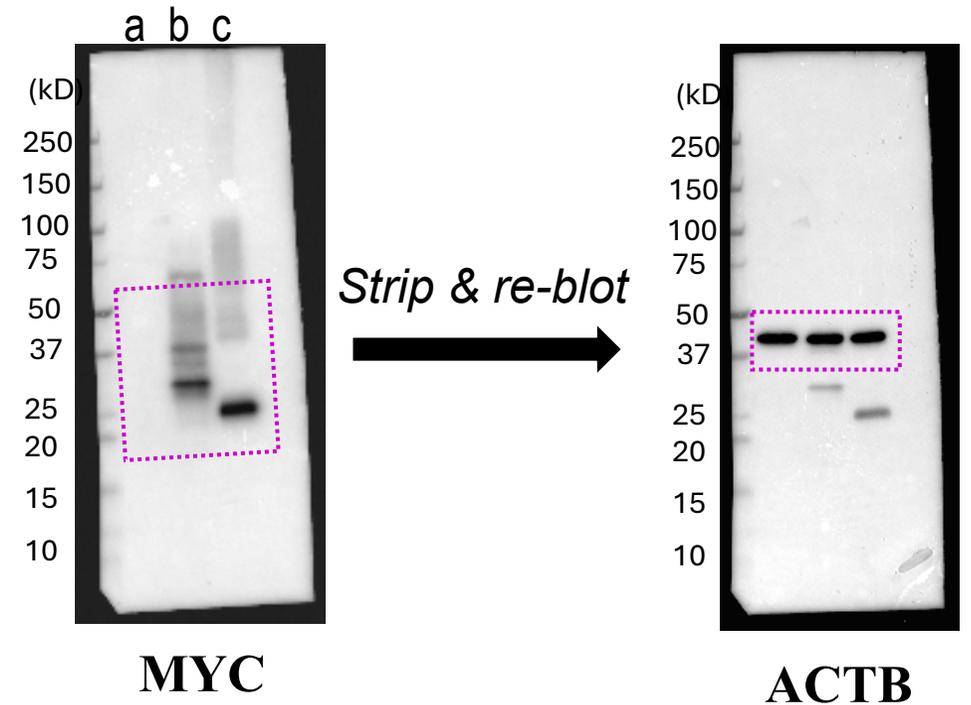


Figure S3A

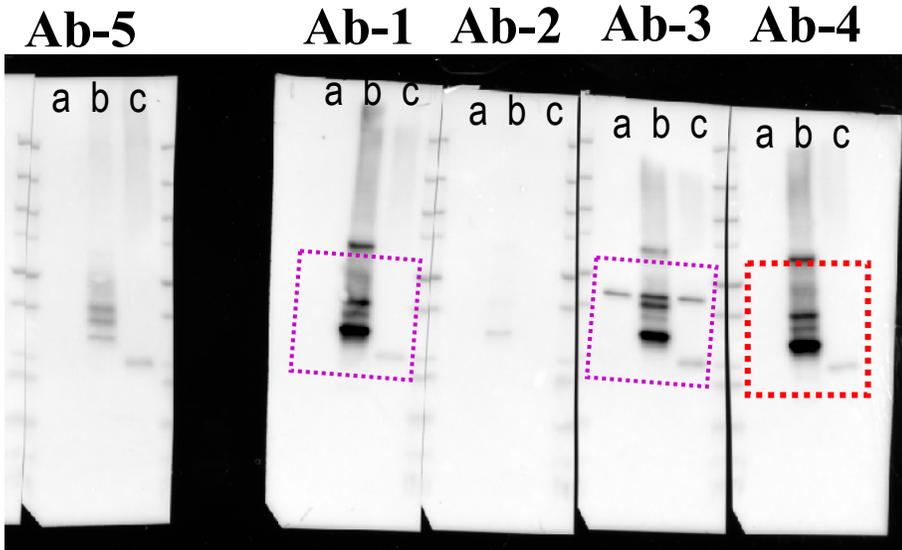


Sample info:

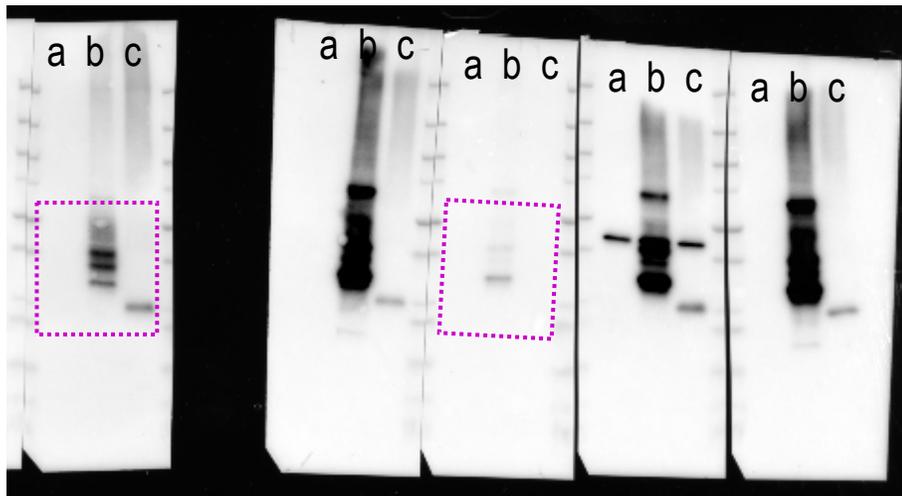
- a: Empty vector (EV)
- b: Canonical FNDC4 over-expression (MYC-FLAG-tagged)
- c: **Truncated** FNDC4 over-expression (MYC-FLAG-tagged)

Full unedited blot for **Figure 2C** & **Supplemental Figure S3B**

FNDC4 Antibodies (Ab)*

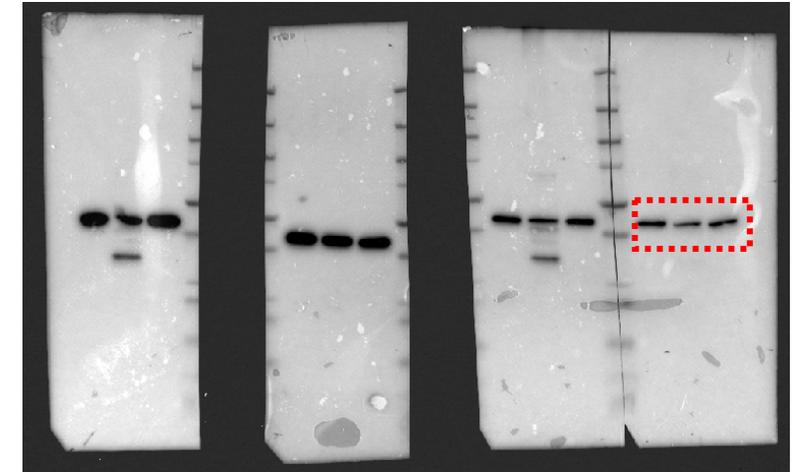


↓ *longer exposure*



Strip & re-blot
→

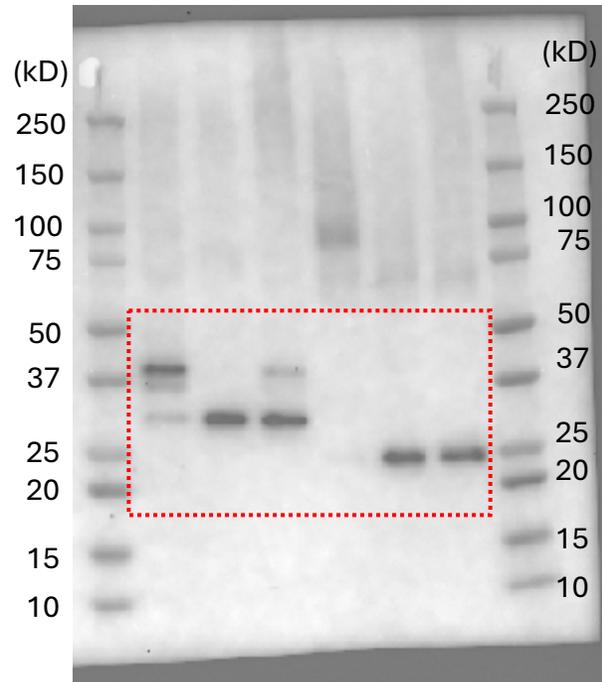
ACTB



* FNDC4 Ab info:

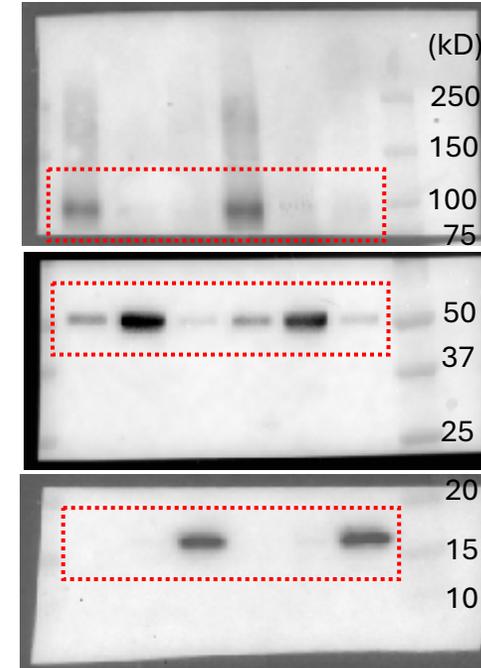
Ab#	Source	Catalog number	Clone ID	Antigen amino acids (aa)
Ab-1	OriGene	TA505462	OTI3B11	aa1 – aa234
Ab-2	ThermoFisher	PA5-62486	N/A	aa194 – aa234
Ab-3	OriGene	TA505463S	OTI1B7	aa1 – aa234
Ab-4	OriGene	TA505459	OTI3E10	aa1 – aa234
Ab-5	Abnova	H00064838-M01	7F9	aa45 – aa154

Full unedited blot for **Figure 2D**



FLAG

Strip, cut & re-blot

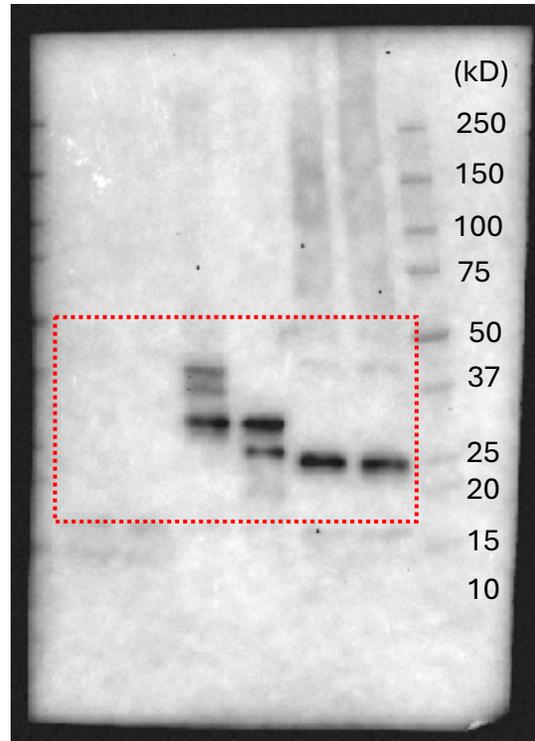


ATP1A1

α -Tubulin

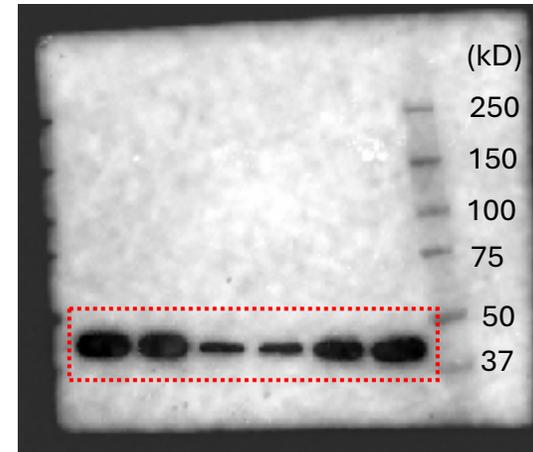
Histone H3

Full unedited blot for **Figure 2E**



FLAG

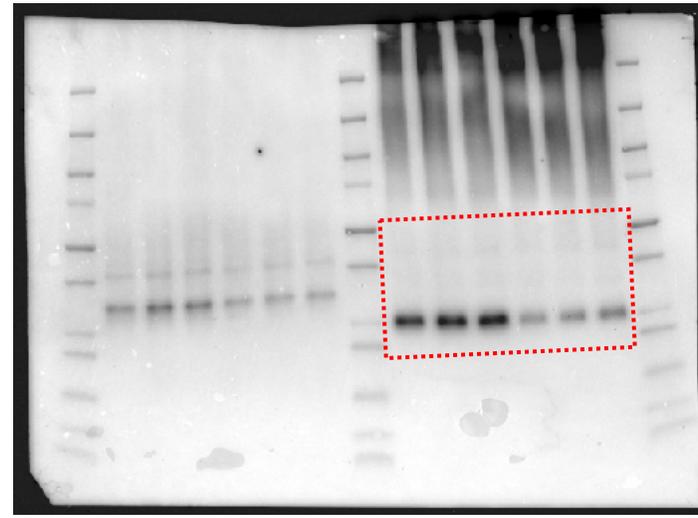
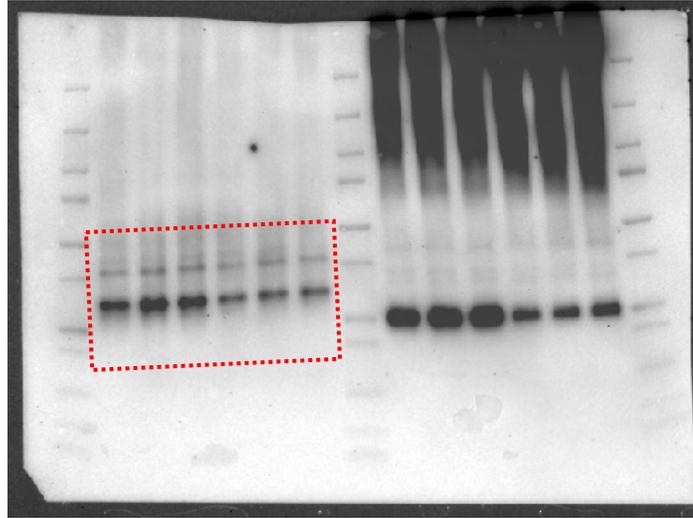
Strip, cut & re-blot



ACTB

Full unedited blot for **Figure 2F**

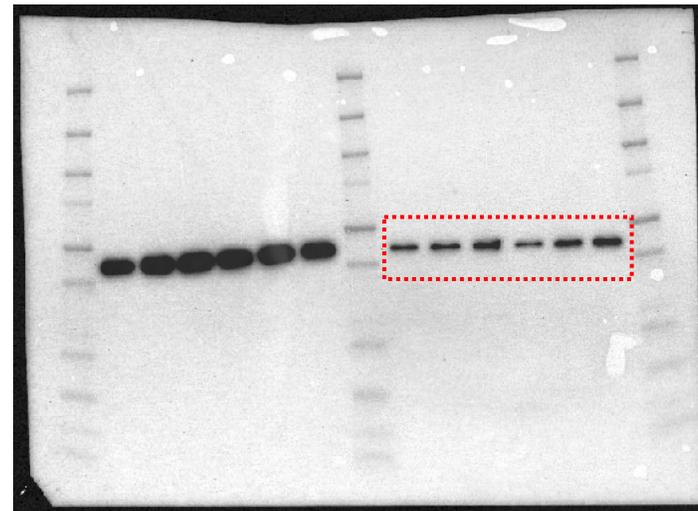
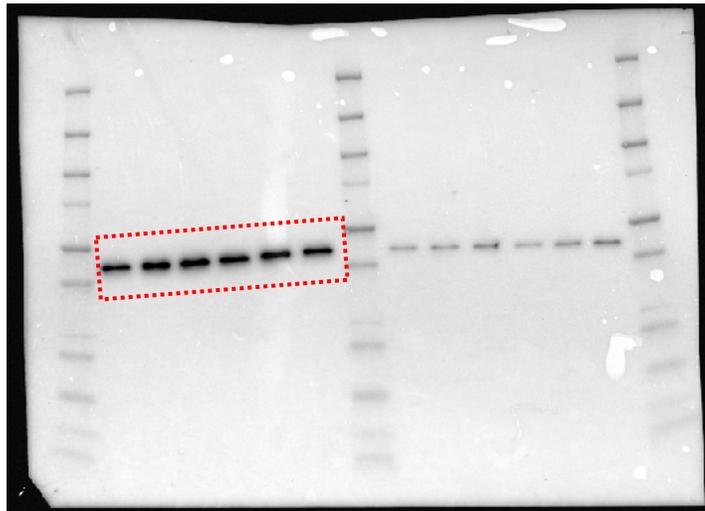
FLAG



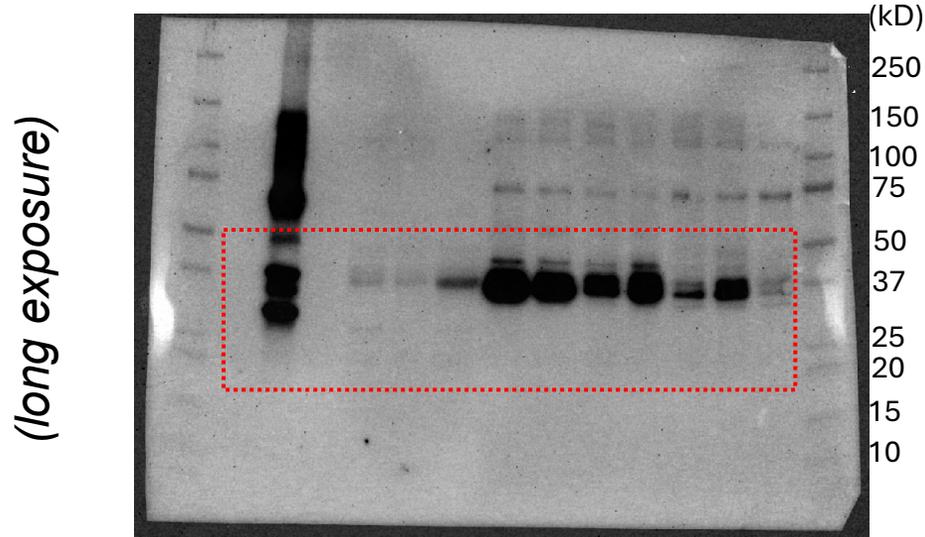
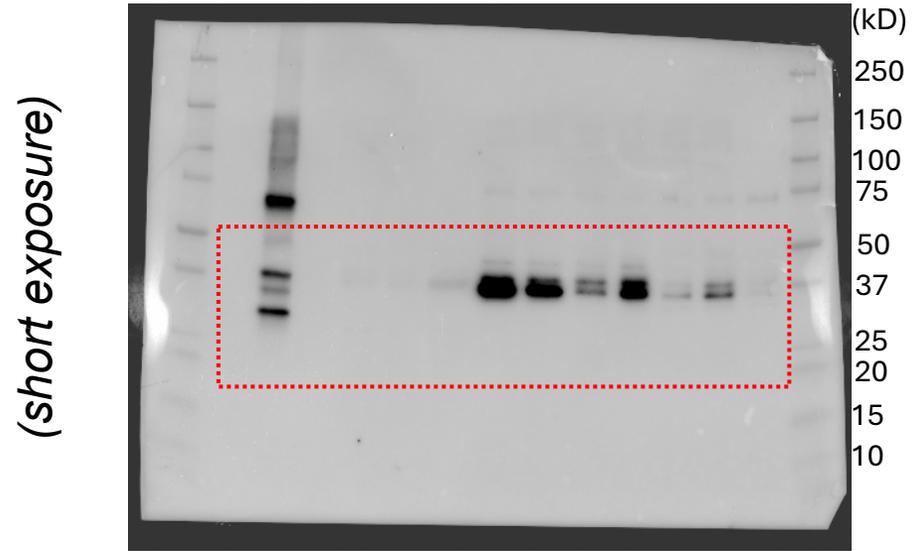
↓ *Strip & re-blot*

↓ *Strip & re-blot*

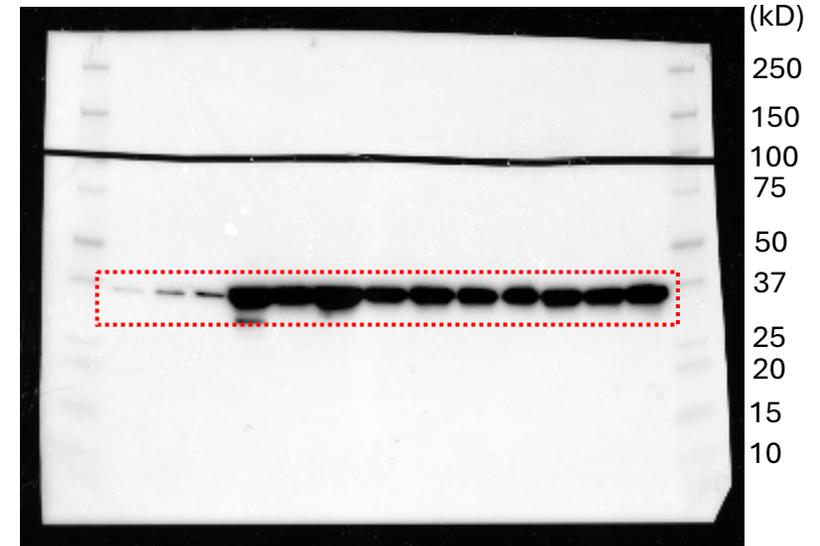
ACTB



Full unedited blot for Figure 2H



Strip, cut & re-blot

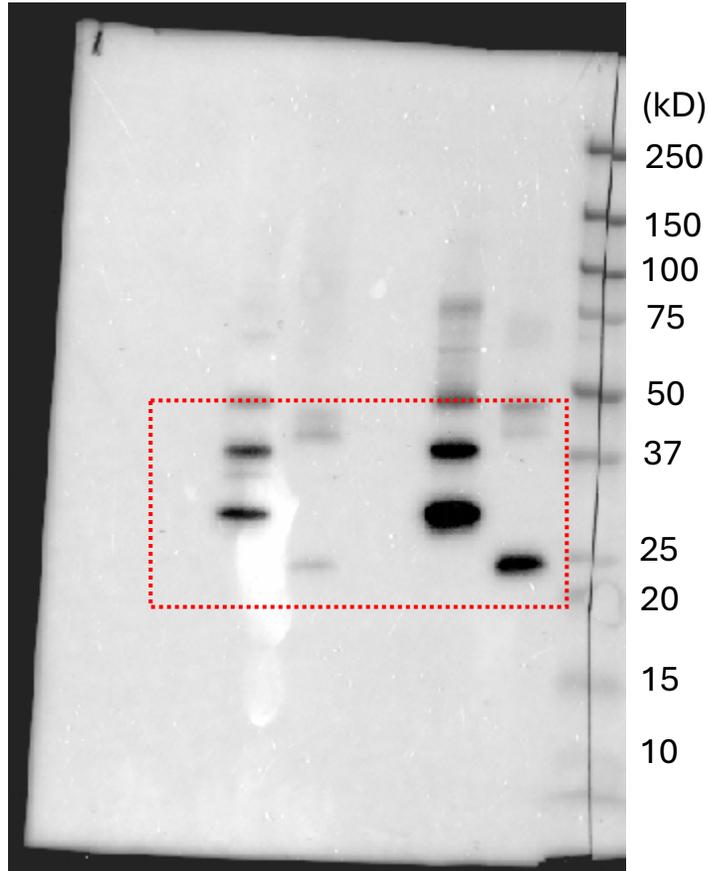


GAPDH

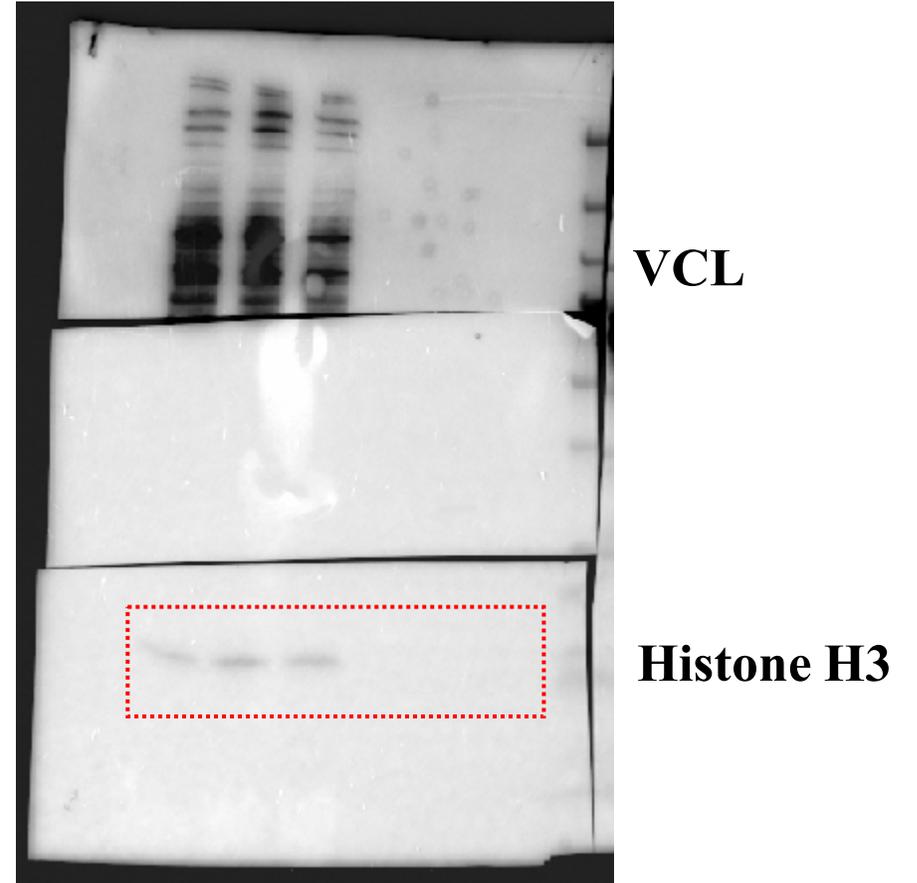
FND C4 (Ab-4; OTI3E10)

Full unedited blot for **Figure 3C** (part 1)

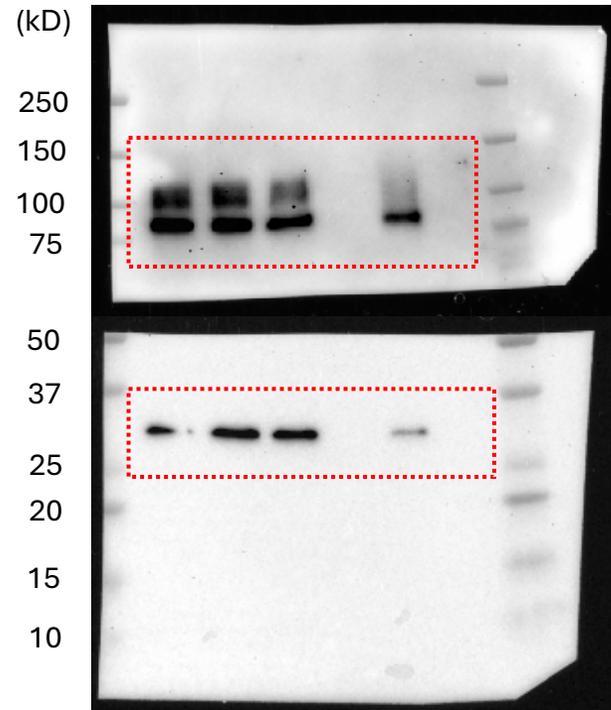
MYC



Strip, cut & re-blot

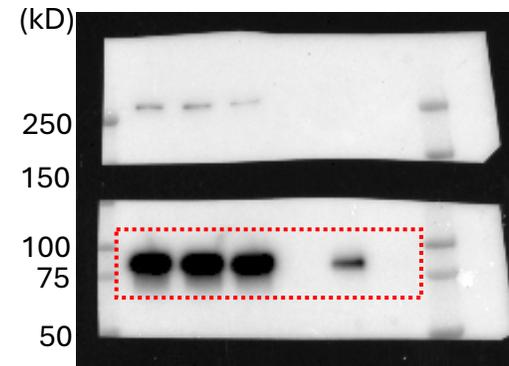


Full unedited blot for **Figure 3C** (part 2)



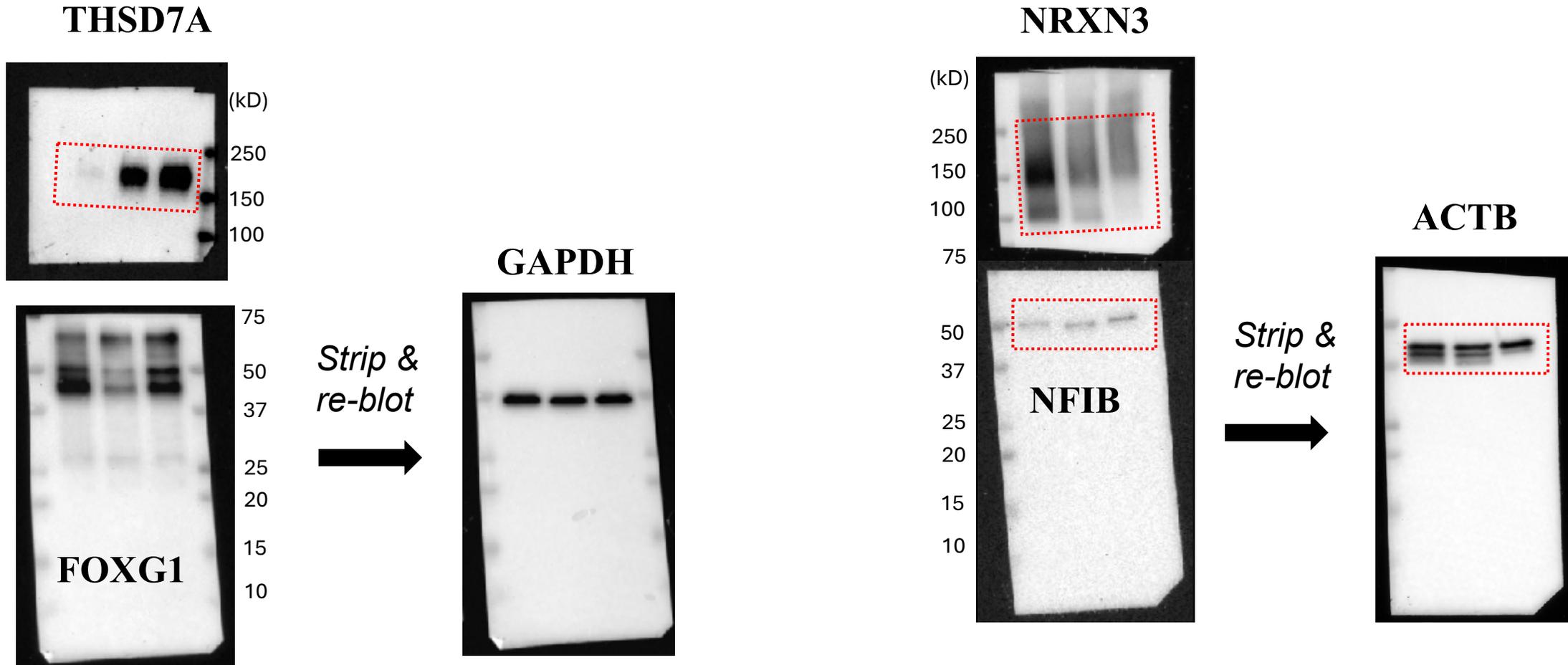
ITGB1

ANXA5



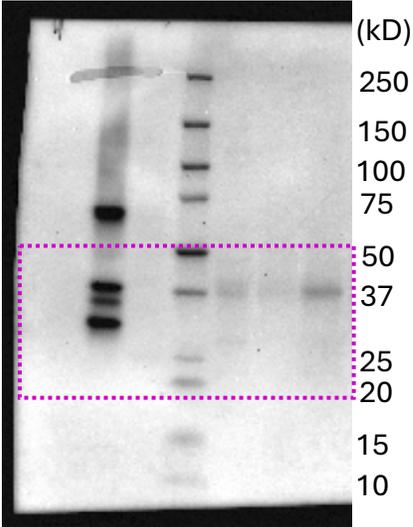
HSP90B1

Full unedited blot for **Figure 6H**



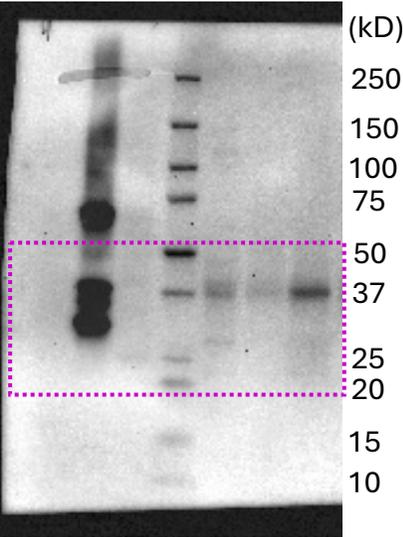
Full unedited blot for **Supplemental Figure S3D**

(short exposure)

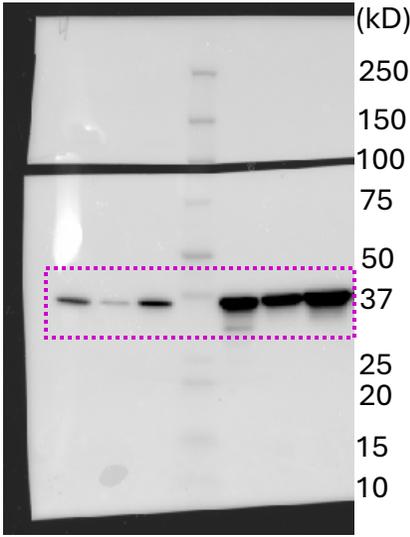


FNDC4 (Ab-4)

(long exposure)



Strip, cut & re-blot



GAPDH

Blots in Supplementary *Fig. S3E*

