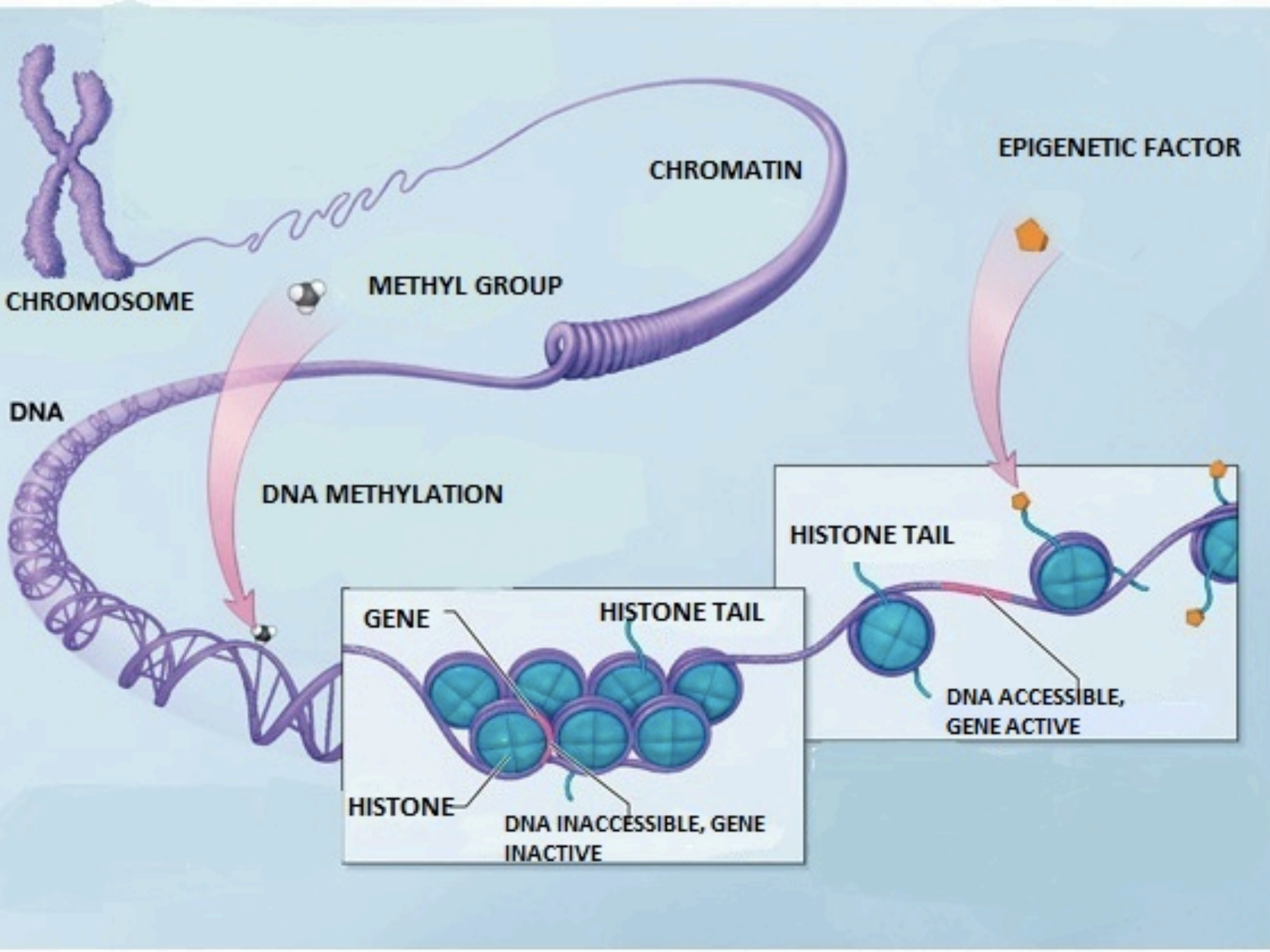


A fluorescence microscopy image of neurons. The nuclei are stained blue, and the cytoplasm and processes are stained red/purple. The neurons are distributed across the field of view, with some showing more extensive branching than others.

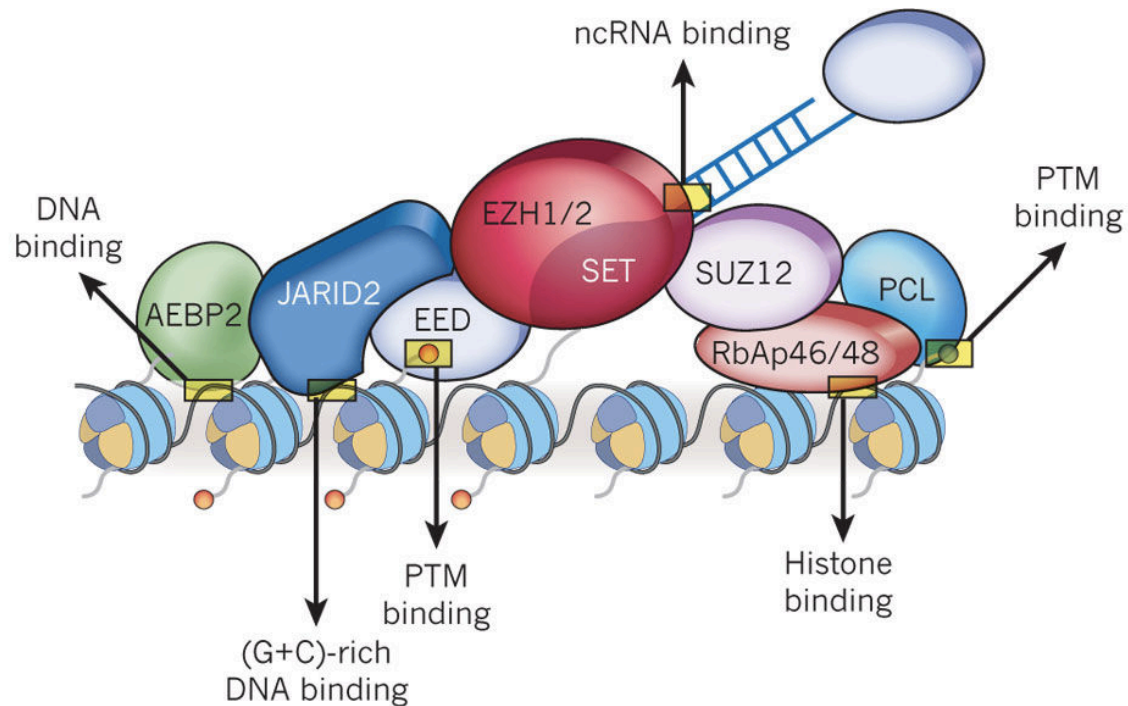
# Ybx1 regulates early neural development

Jenny Loome

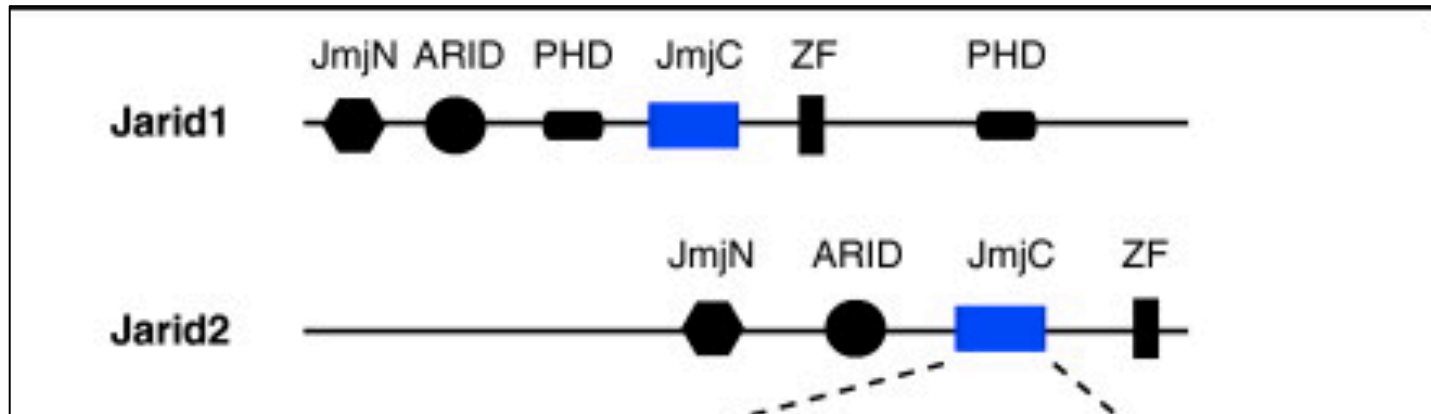


# What is PRC2?

- Polycomb repressive complex 2
- Broad gene regulator (repressor?) in pluripotent cells
- Histone 3 Lysine 27 methyl transferase complex

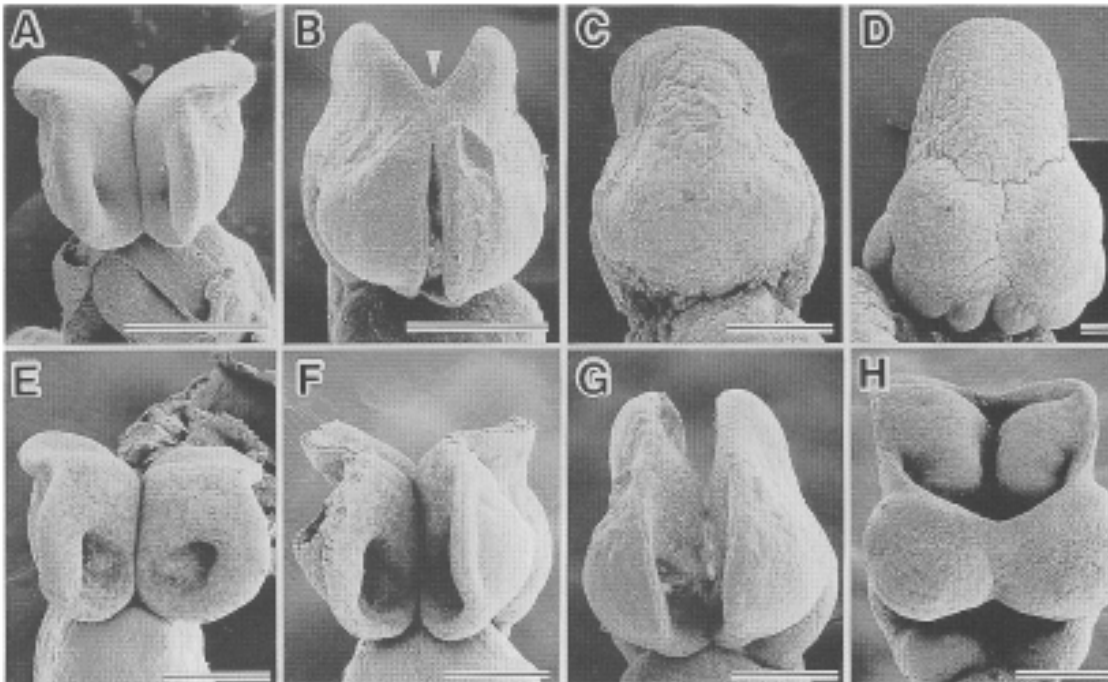
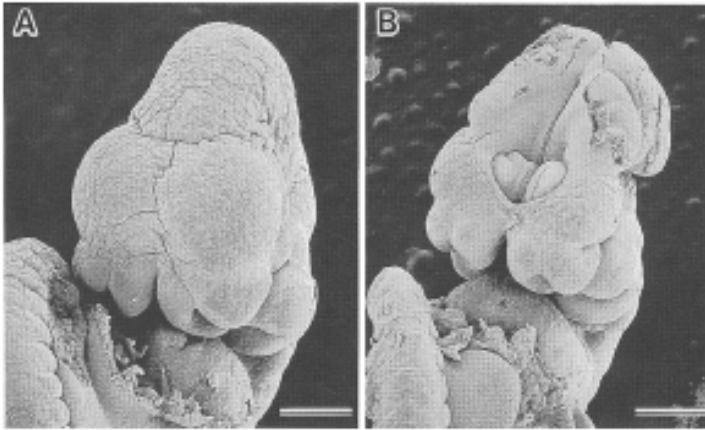


# What is JARID2?



- Member of the Jumonji family of demethylases
  - Binds DNA but catalytically inactive
- Associated with the PRC2 complex
- Can inhibit or promote PRC2 activity

JARID2 gene trap  
prevents neural tube  
closure in midbrain and  
is embryonic lethal

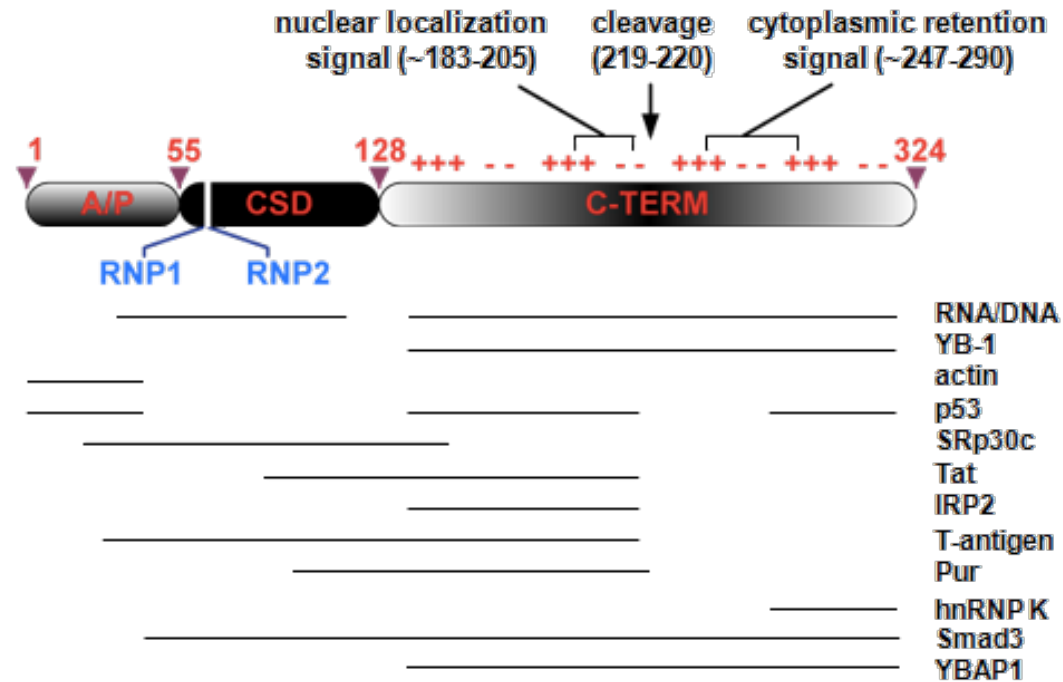


# IP-MS of JARID2 IP

Proteins	peptide #, IgG		spectral count, IgG		peptide #, JARID2		spectral count, JARID2		p value	
	IP1	IP2	IP1	IP2	IP1	IP2	IP1	IP2	IP1	IP2
JARID2	0	0	0	0	14	27	47	63	1e-15	2e-20
SUZ12	0	0	0	0	6	24	23	53	2e-8	2e-17
EZH2	0	0	0	0	6	16	13	22	3e-5	6e-8
EED	0	0	0	0	5	6	13	19	4e-5	5e-7
AEBP2	0	0	0	0	9	3	6	15	6e-3	9e-6
RBBP4	0	0	0	0	6	8	13	12	3e-5	8e-5
YBX1	0	0	0	0	7	4	35	15	6e-6	8e-6

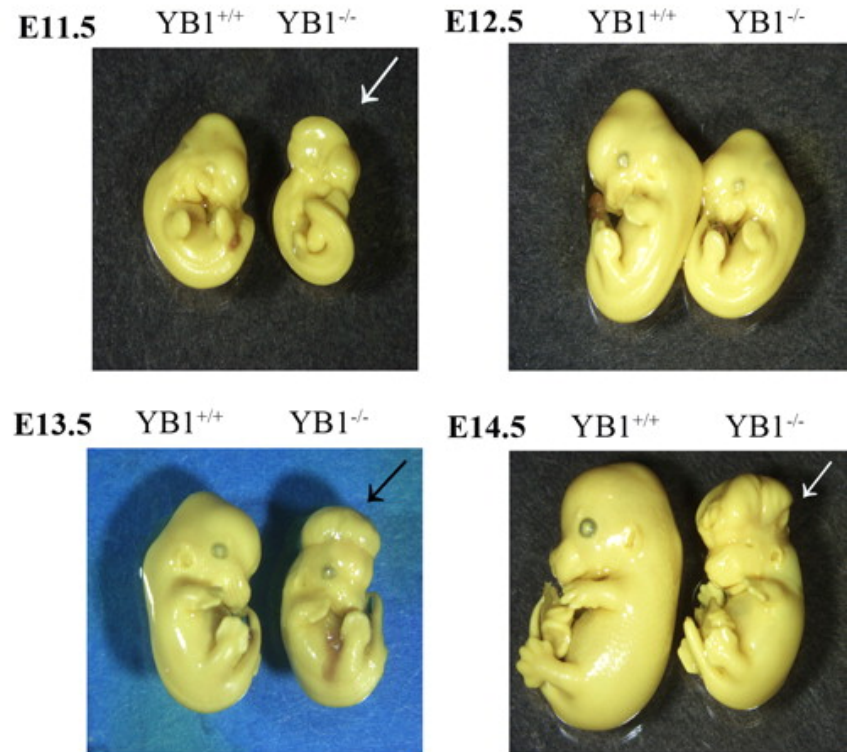
# YBX1 has many functions

- DNA and RNA binding protein
- Cold-shock domain
- Functions in nucleus and cytoplasm
- Regulates DNA replication, transcription, mRNA splicing, mRNA stability, translation



# Ybx1 KO in mice

- Exencephaly
- Embryonic lethality
- Slow MEF growth
  - Explanation for exencephaly?



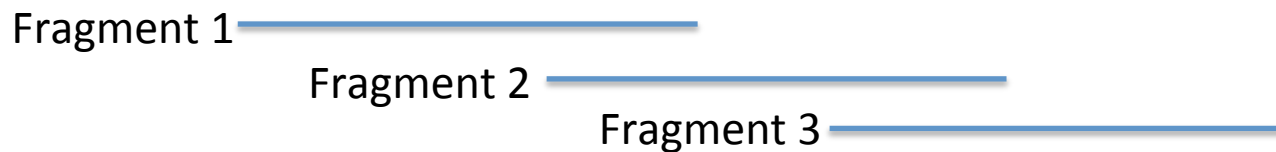
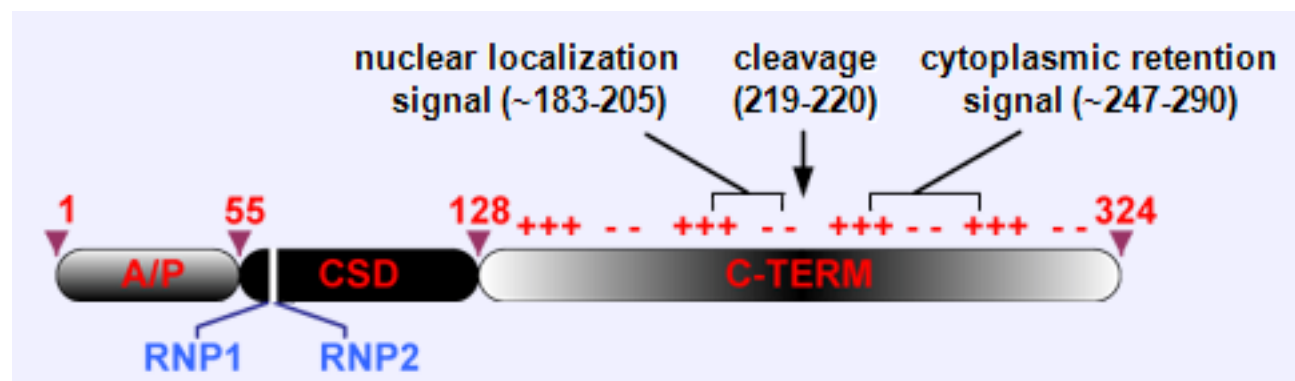
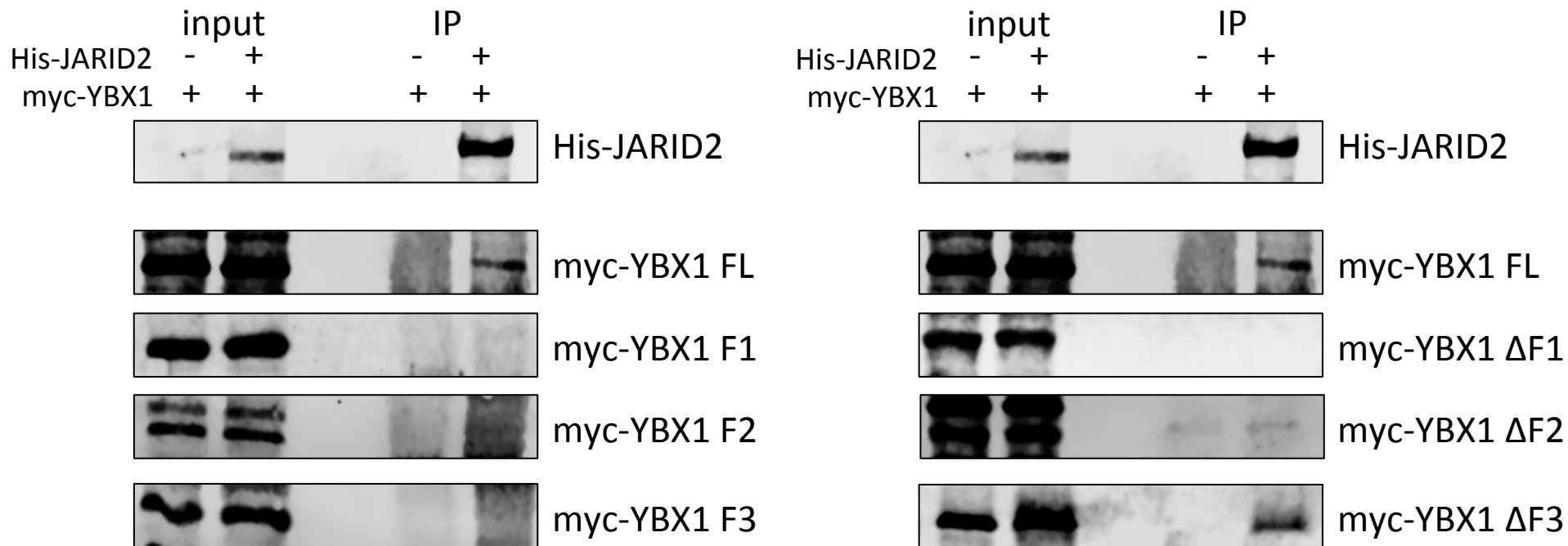


# How do JARID2 and YBX1 interact in early development?

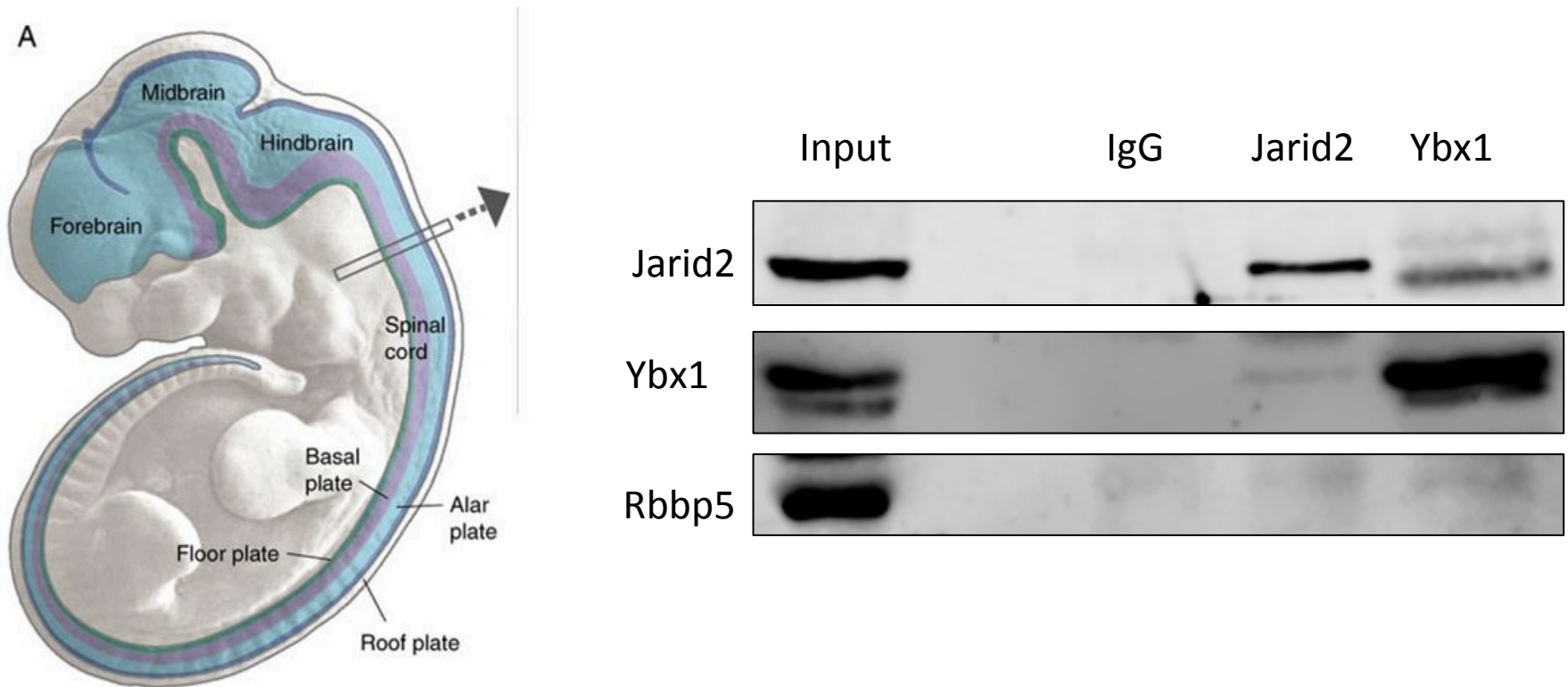
- Do they bind directly or indirectly?
- Why does loss of Ybx1 cause exencephaly?
  - Hypothesis: YBX1 KO causes slower epithelial growth and faster neural growth
    - Mediated at least in part by interaction with PRC2

# Outline

- Biochemical characterization YBX1/JARID2 interaction
  - Recombinant
  - Endogenous
- Effect of Ybx1 loss on mNPC
- Differentiation of wild-type and Ybx1-null mouse NPCs



# Jarid2 and Ybx1 interact in the mouse neural tube



# Neurosphere assay for NPC proliferation

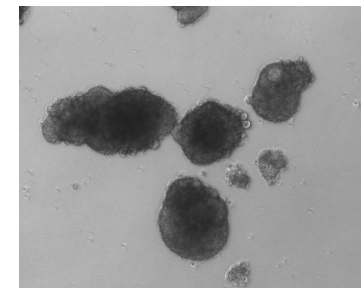
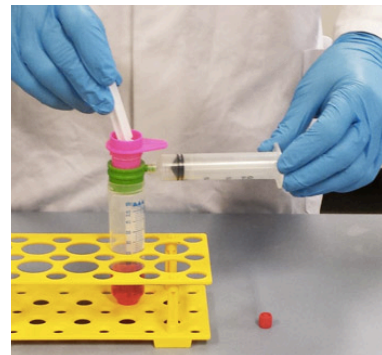
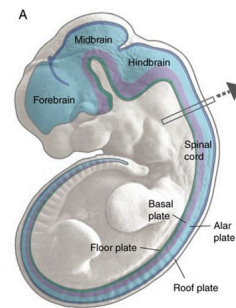
Harvest mouse embryos (Day E12-14)

Dissect the neural tubes

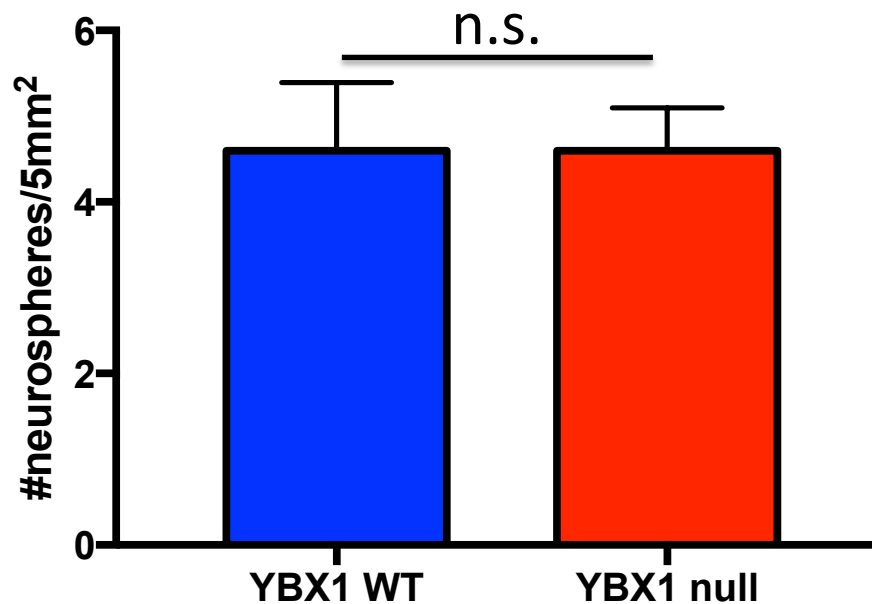
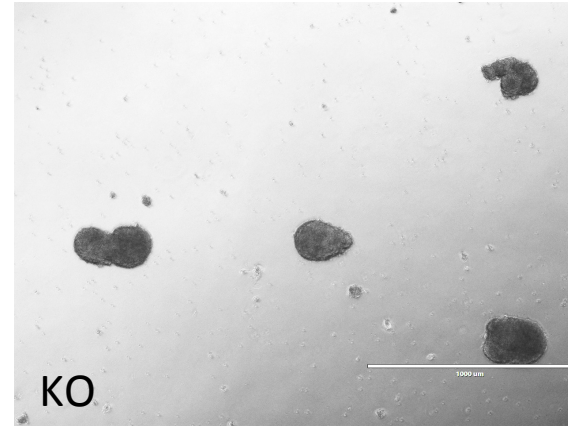
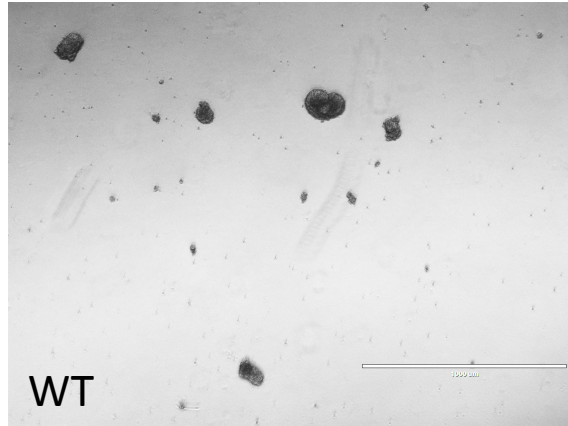
Use collagenase and trypsin to break down tissue

Filter to single cells

Culture in low-attachment plates



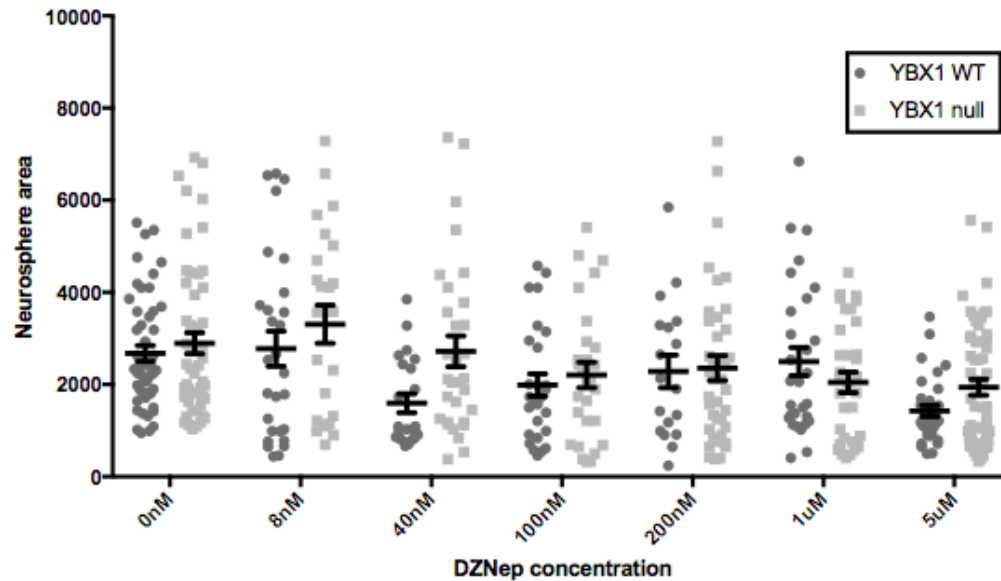
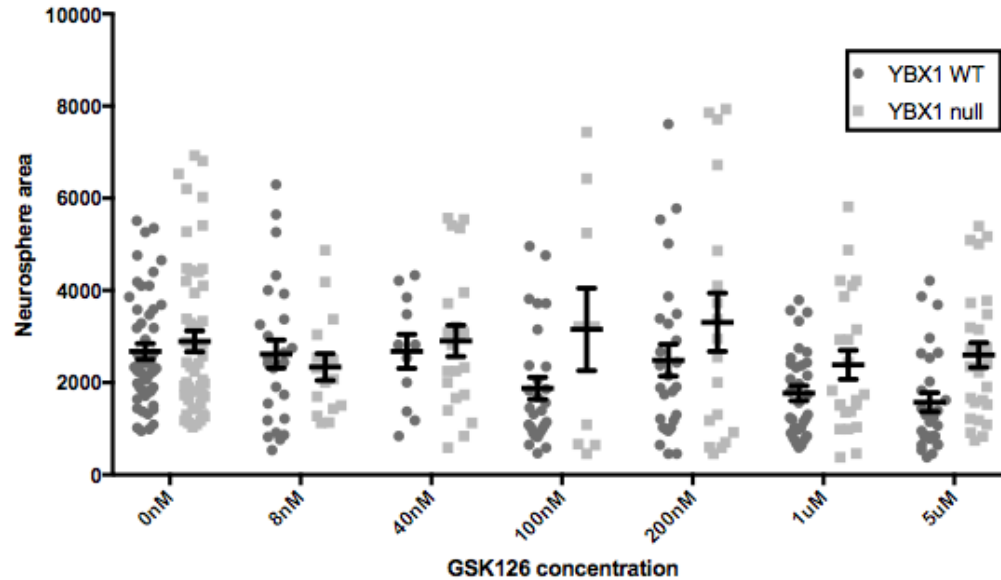
# No difference in number of neurospheres



Day 7  
n=2



# Neurosphere growth in EZH2 inhibitors

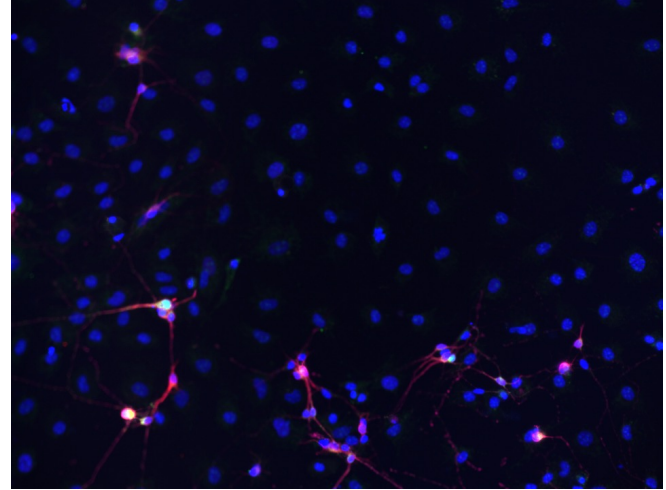
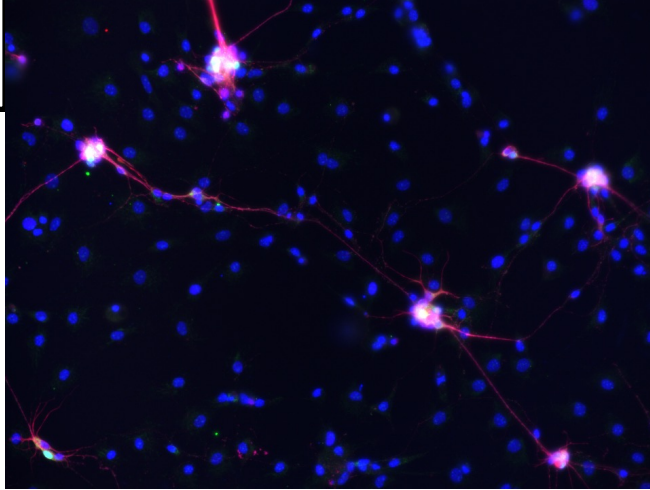




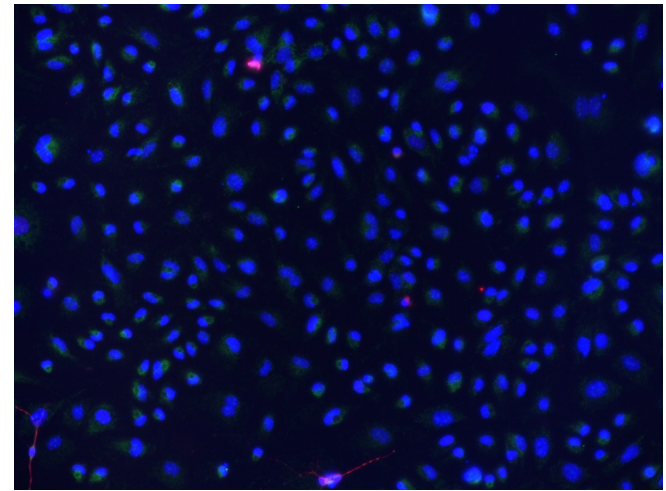
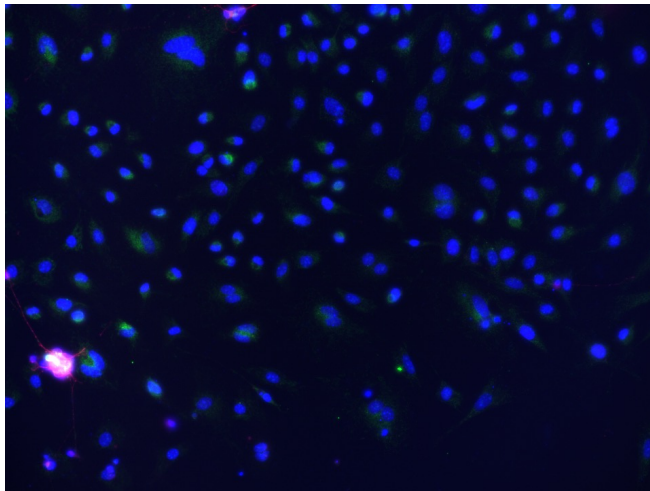
# 7 day NPC differentiation assay

DAPI (DNA)  
TBR1 (neuron)  
MAP2-red  
(Neuron)  
Tuj1-magenta

Ybx1 +/-



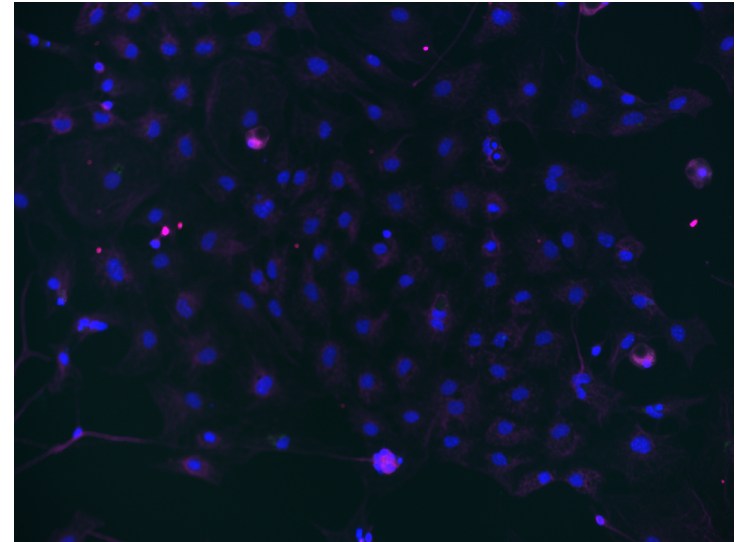
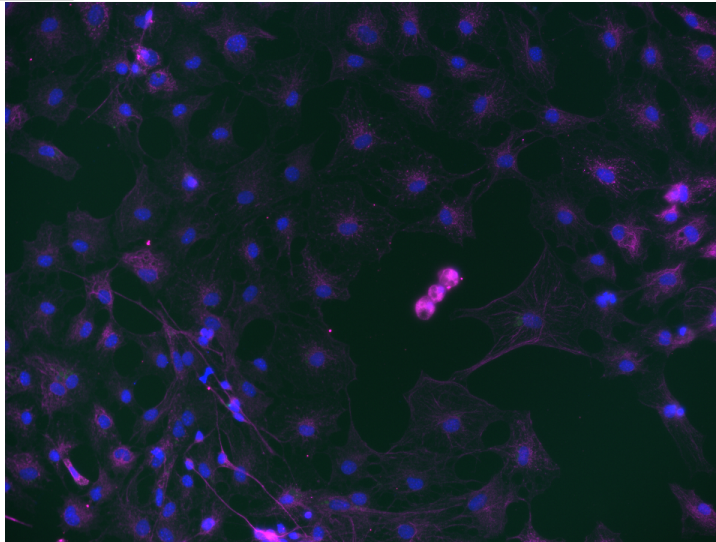
Ybx1 -/-



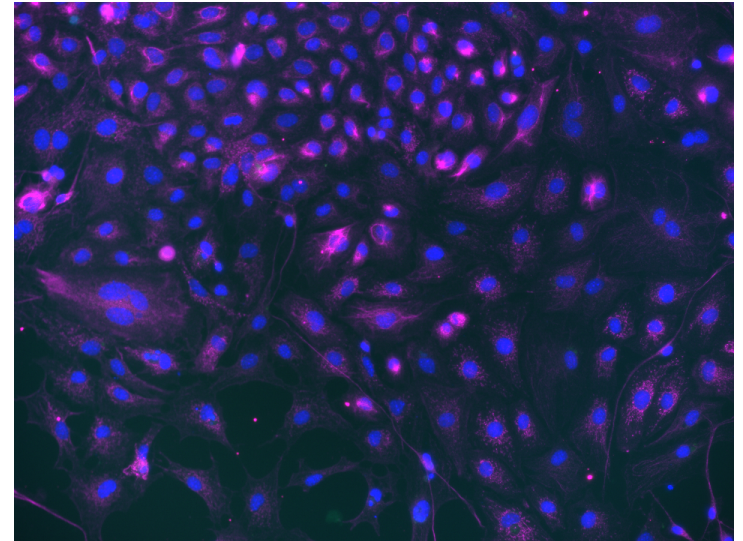
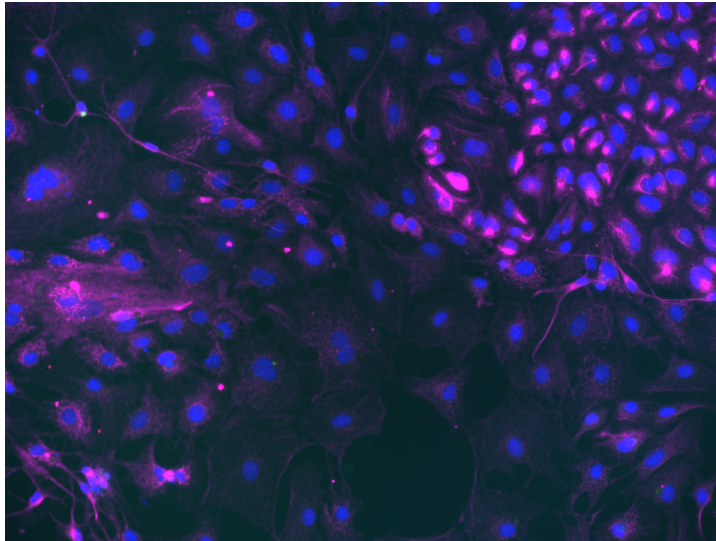
DAPI-blue  
CNPase-  
(oligodendrocyte)

# 7 day NPC differentiation assay

Ybx1 +/-



Ybx1 -/-



# Conclusions

- Recombinant YBX1 and JARID2 physically bind
- Ybx1 interacts with the PRC2 in the mouse neural tube
- Loss of Ybx1 causes hyperproliferation of mouse NPCs
- Ybx1 null NPCs differentiate into neurons less readily than wild types

# Future directions

- Does Ezh2 inhibition affect phenotype of Ybx1 null NPCs?
- Is the molecular influence of Ybx1 mediated through PRC2 in NPCs?
  - Use Ybx1 truncation to prevent nuclear localization and binding to PRC2
- How do Jarid2 KO phenotypes compare the Ybx1 KO?

# Acknowledgments

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- ALSAC
- NIH
- Proteomics Core
  - Vishu Pagala
  - Junmin Peng
- Imaging Core
- Flow Cytometry and Cell Sorting
- Tim Ley



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