



# The neuroblastoma specific lincRNA NESPR controls noradrenergic cell identity

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Ghent University

Keystone symposium , Whistler Conference Centre

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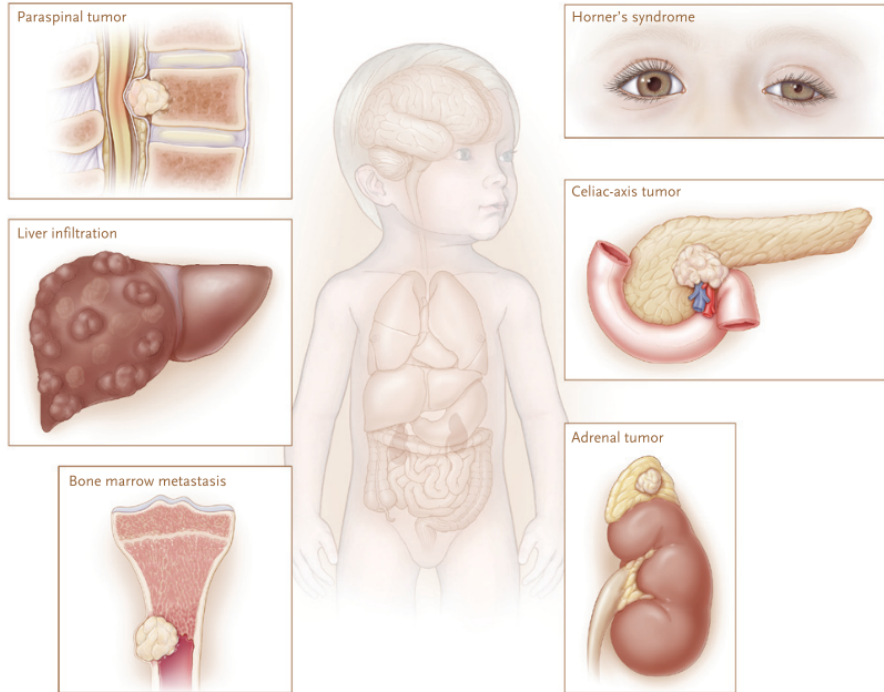
CENTRUM MEDISCHE  
GENETICA GENT



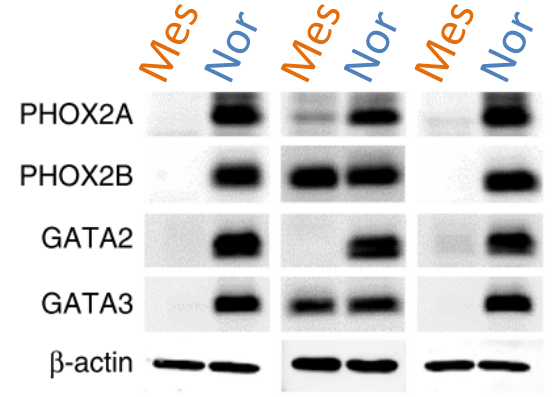
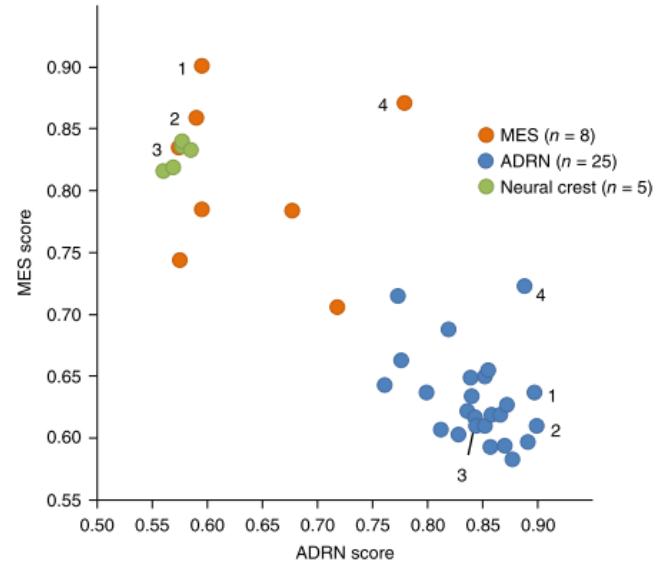
CRIG

tegen Kanker

# NEUROBLASTOMA: A PEDIATRIC CANCER (OR TWO ?)



(Maris, NEJM, 2010)



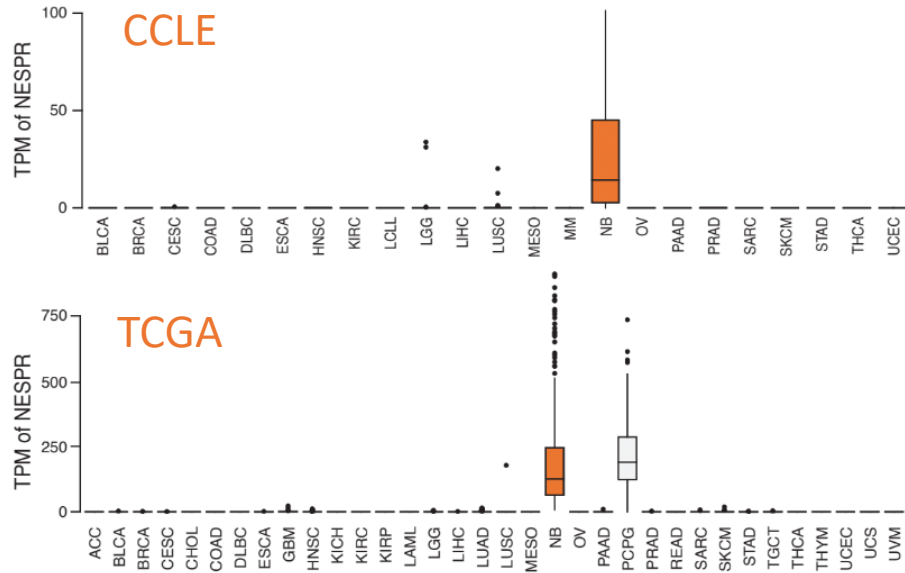
(Van Groningen, Nat. Gen., 2017)

- Median age at diagnosis : 17 Months
- Most common in ages under 1 year
- 10.2 cases per million of children under 15
- No significant progress in high-risk patient treatment

Mesenchymal : JUN, FOS...  
Noradrenergic : PHOX2B, GATA3, HAND2, ISL1...

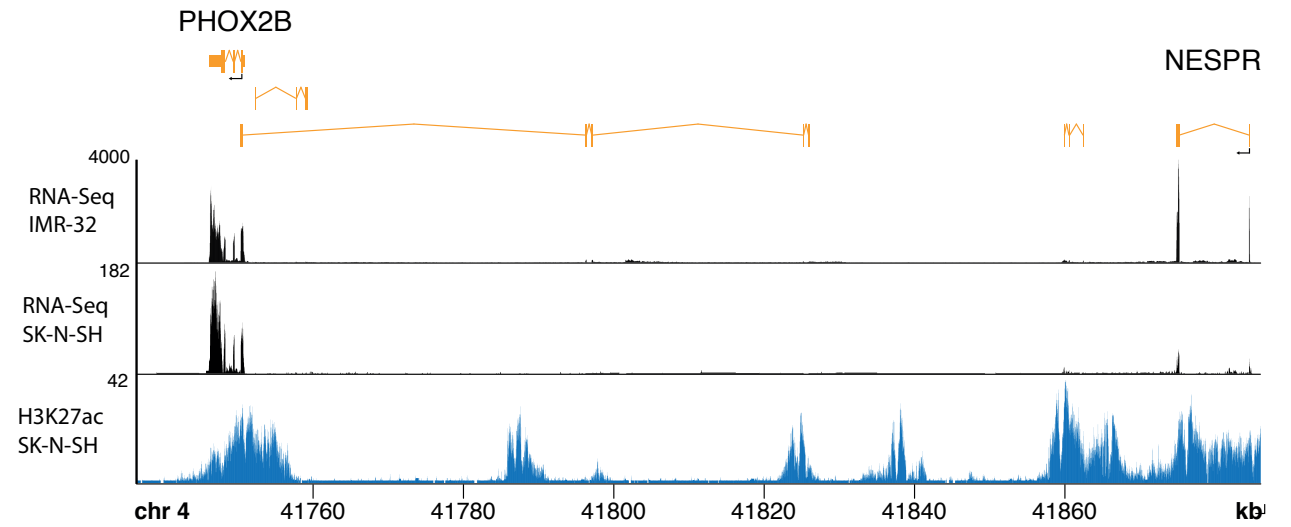
# NESPR IS NEUROBLASTOMA-SPECIFIC & TRANSCRIBED FROM PHOX2B SE

## NESPR expression across tumors



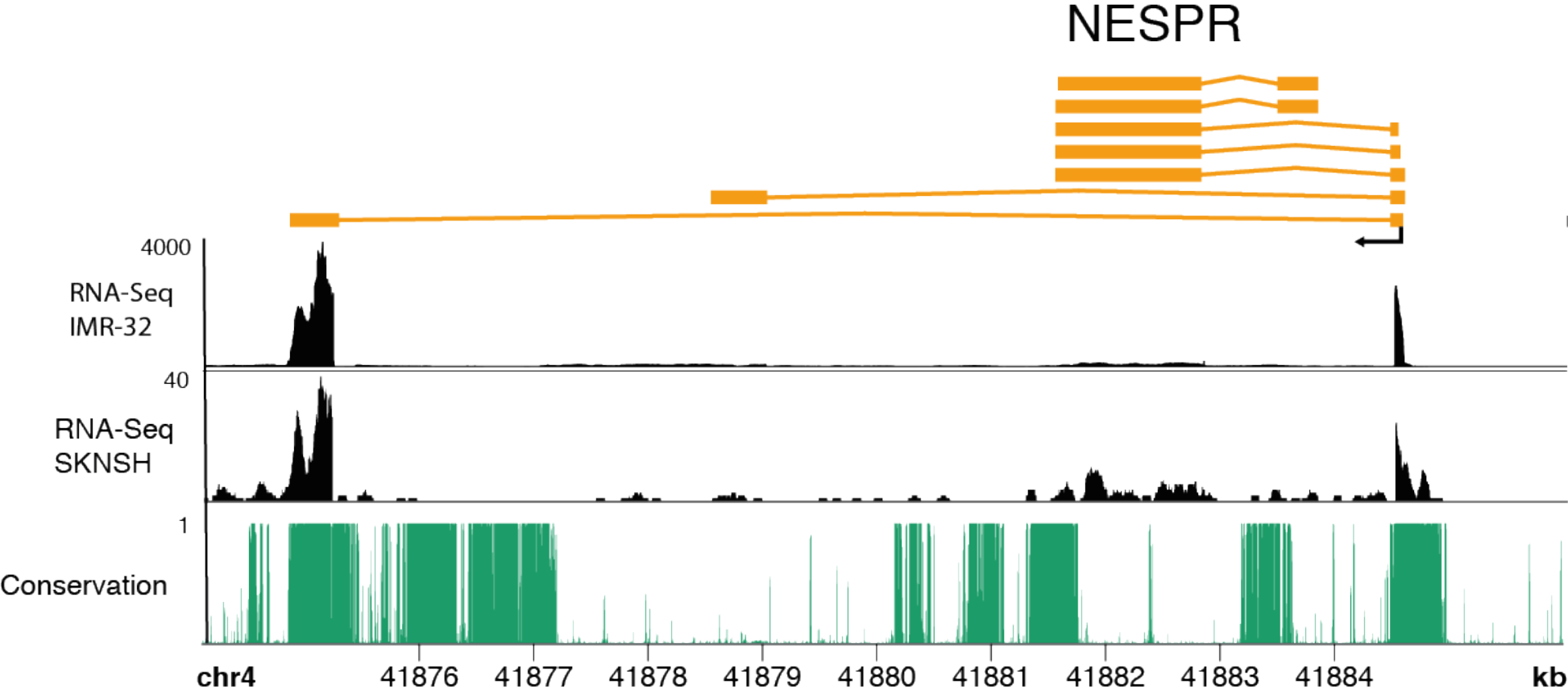
NESPR is NB-specific

## NESPR genomic neighbourhood

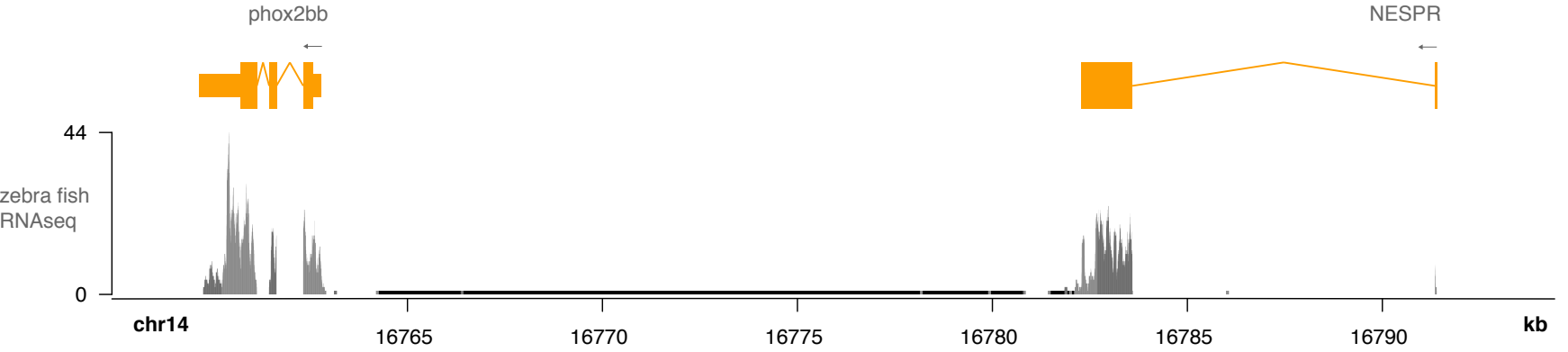
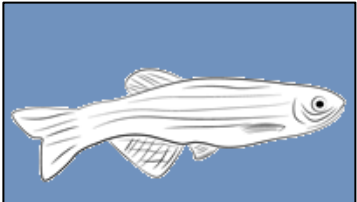
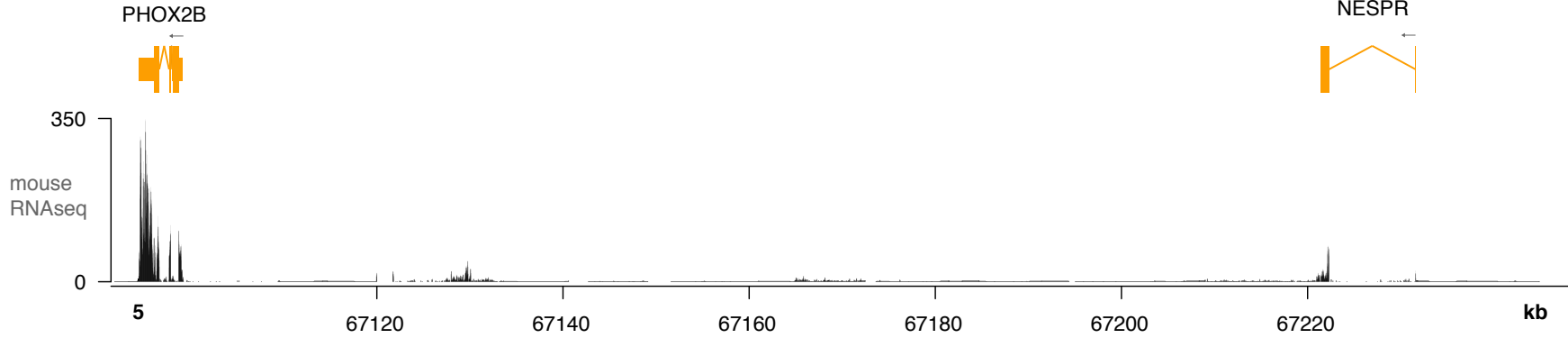


NESPR arises from the PHOX2B super-enhancer region

# NESPR IS EFFICIENTLY SPLICED AND HIGHLY CONSERVED IN MAMMALS

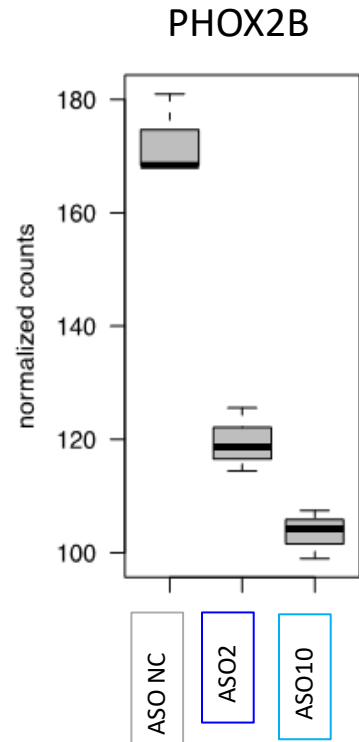
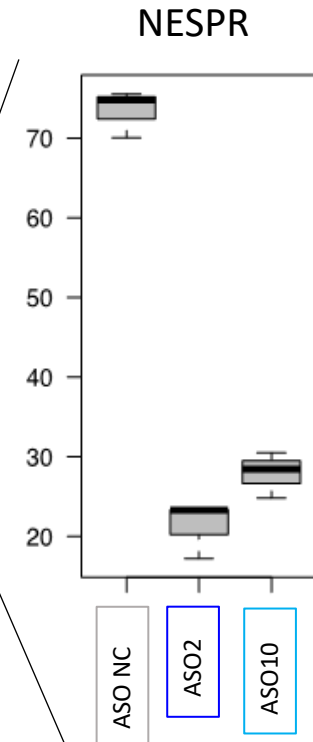
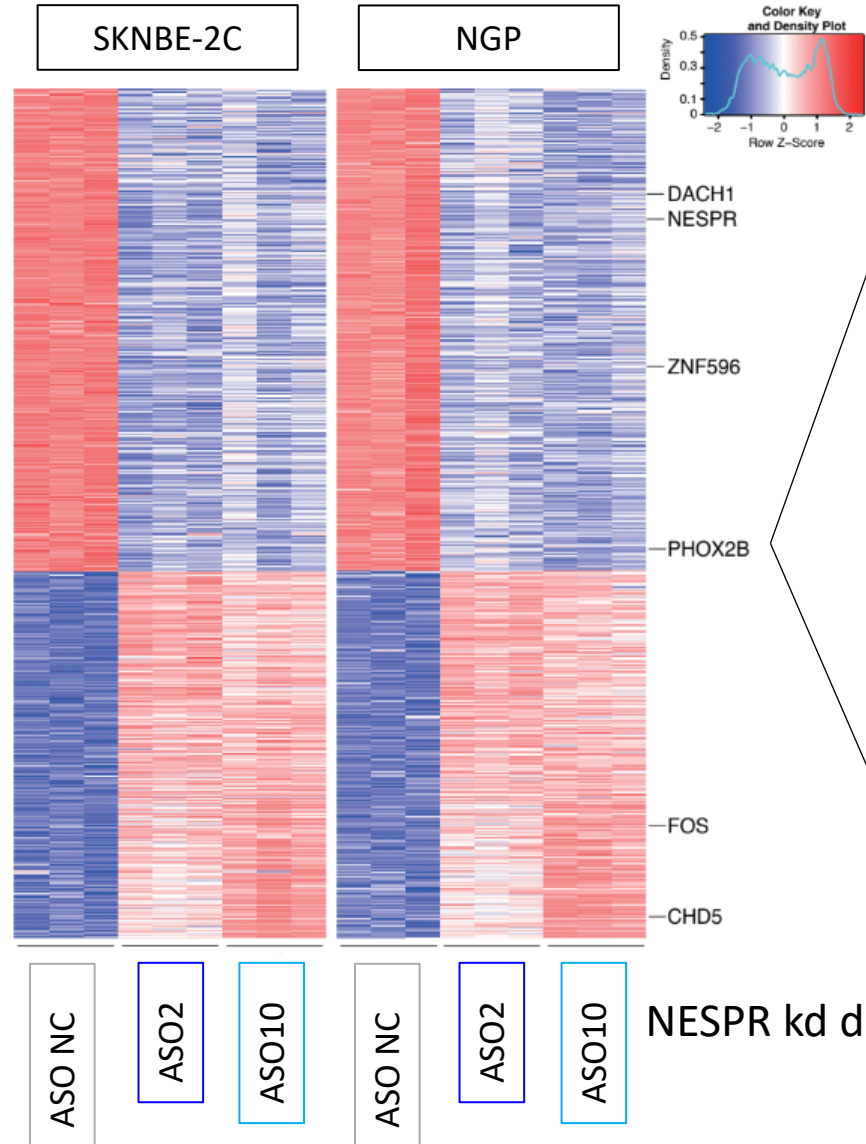


# NESPR ORTHOLOGS IN MURINE AND ZEBRAFISH NB TUMORS

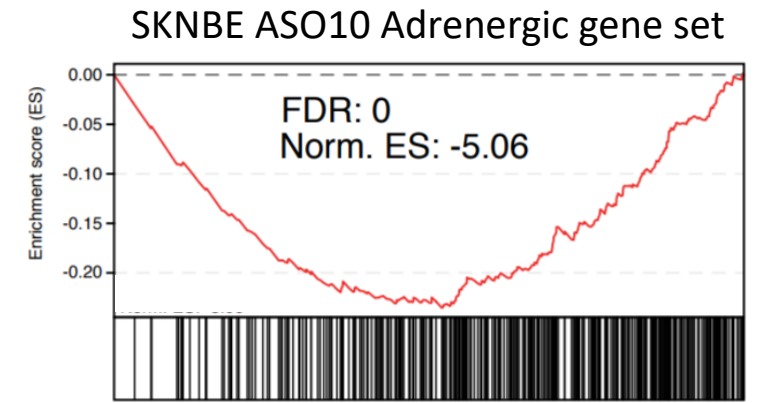
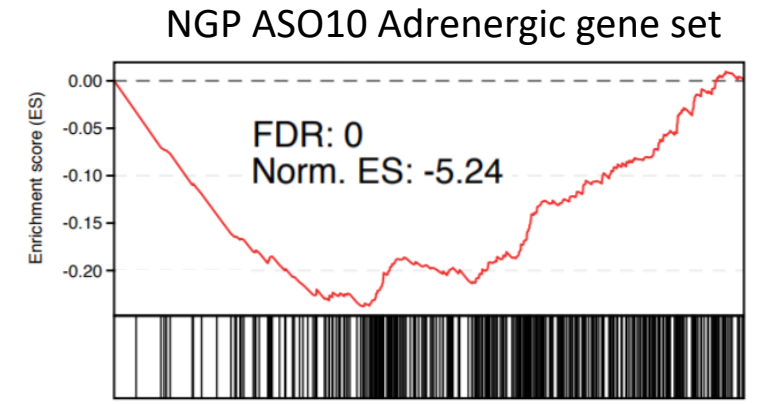


# NESPR PARTICIPATES IN NORADRENERGIC IDENTITY REGULATION

NESPR knock-down in NB cell lines

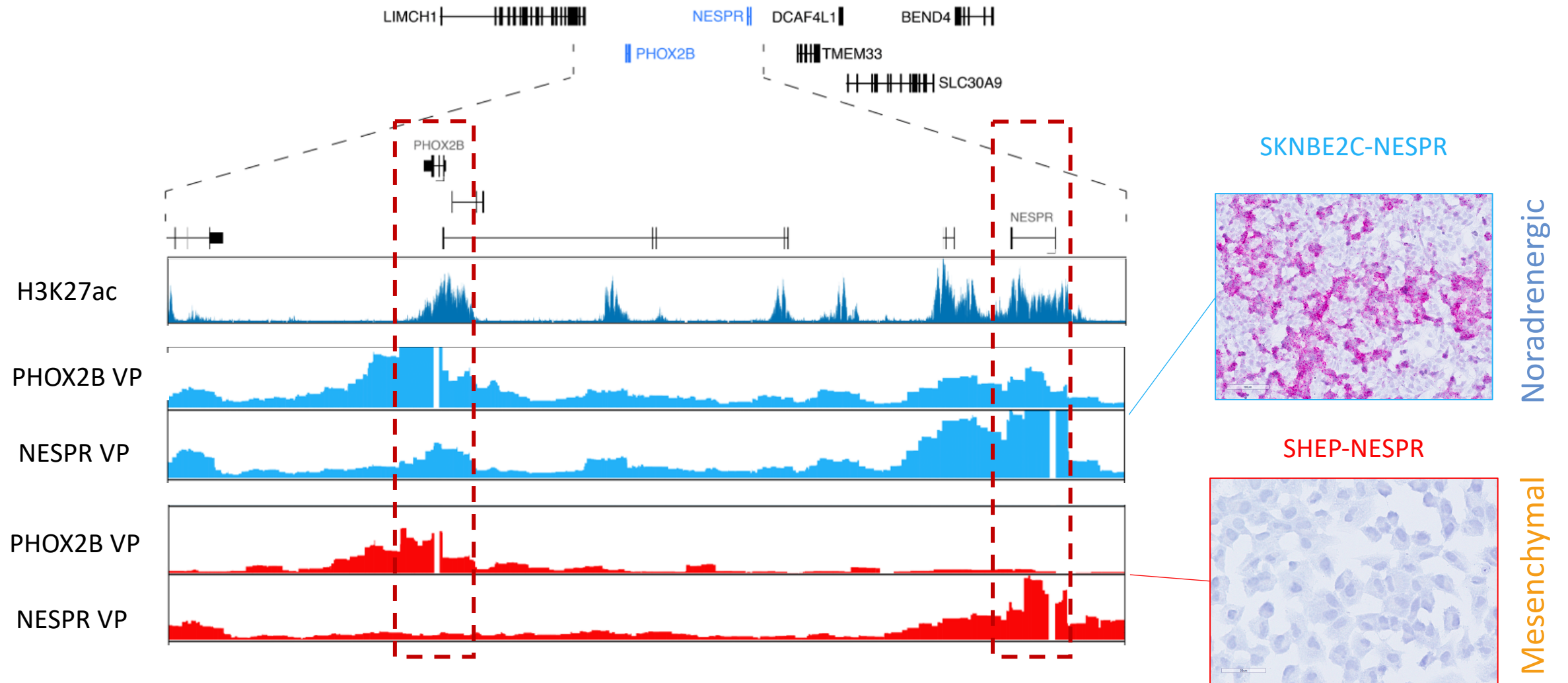


GSEA



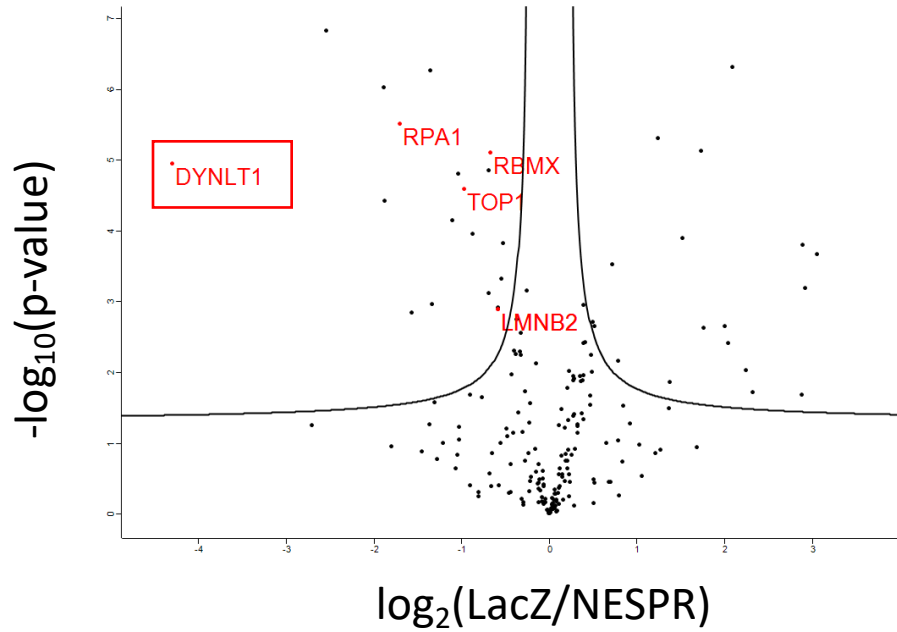
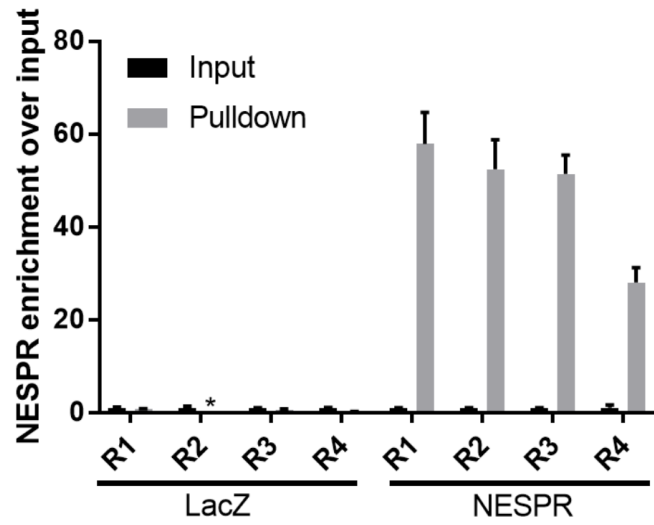
NESPR kd disrupts expression of Tfs composing the noradrenergic core regulatory circuitry (CRC)

# PHOX2B & NESPR REGION IN NORADRENERGIC VS. MESENCHYMAL CELLS

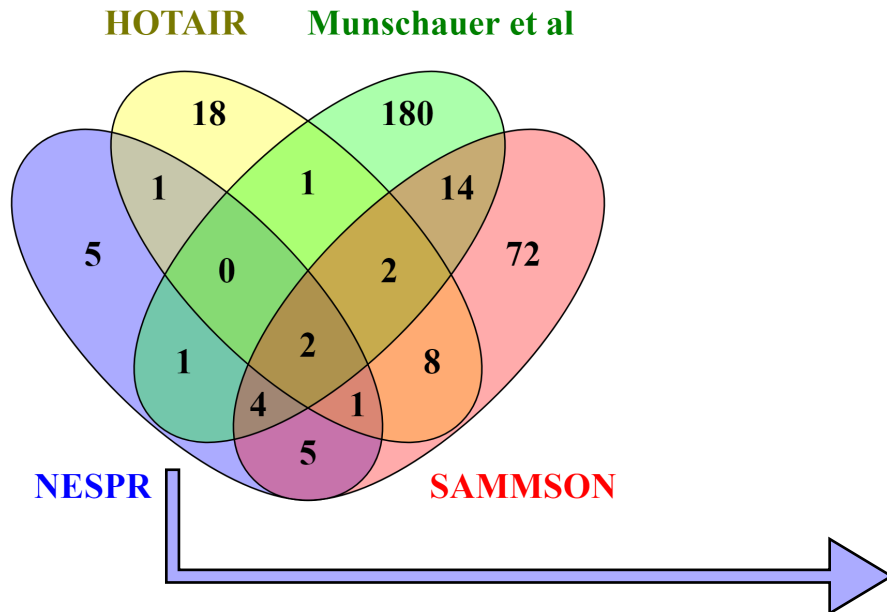


- NESPR & PHOX2B loci are joined by chromatin looping in noradrenergic cells
- Mesenchymal cells do not exhibit looping and do not express NESPR

# NESPR ASSOCIATED PROTEIN ARE CORRELATED TO NORADRENERGIC GS



Louis "ChIRP" Delhaye

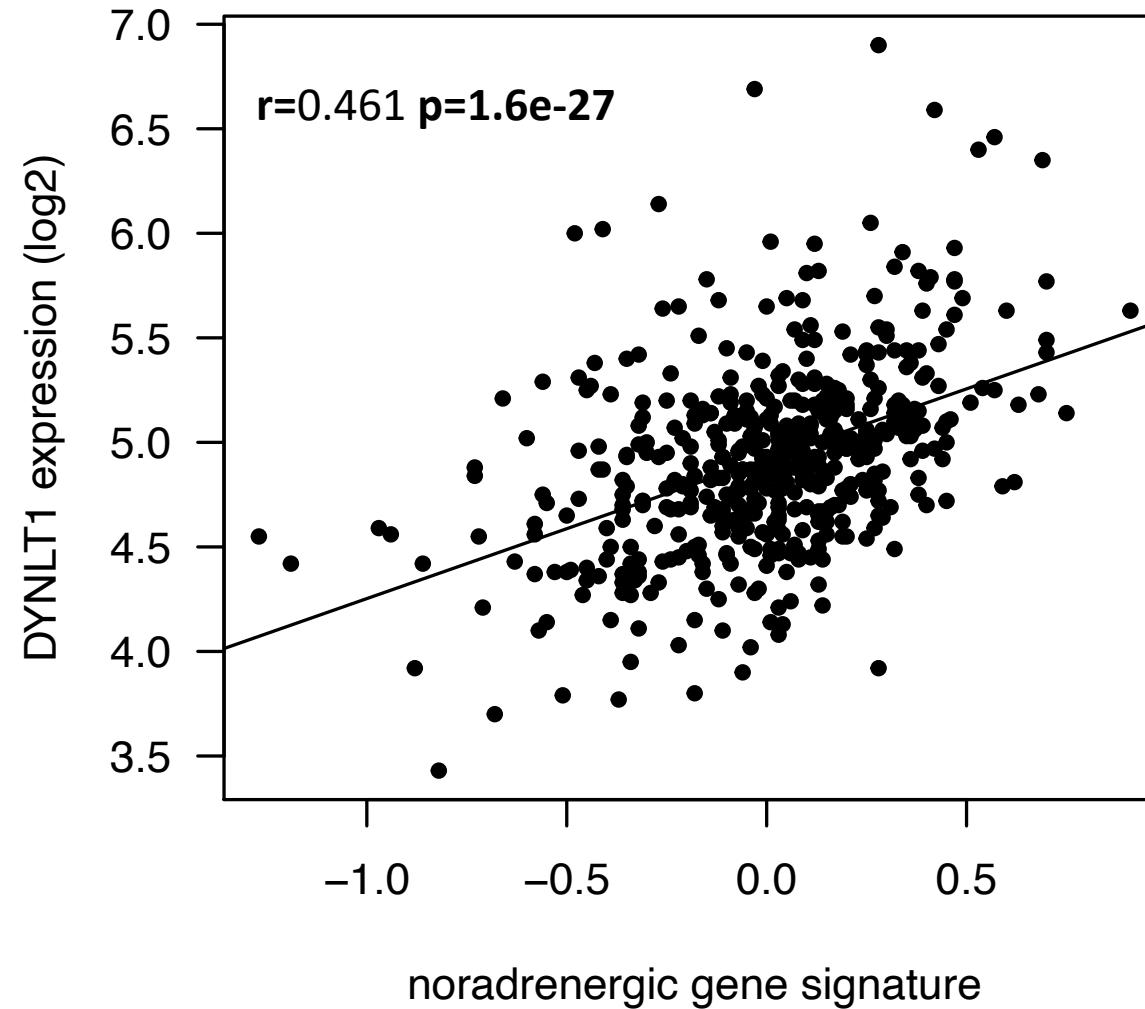


Difference	Name	RNA-dependent complexes (R-Deep)
-1.706149578	RPA1	yes
-0.673391342	RBMX;RBMXL1	yes
-4.298970222	DYNLT1	NA
-0.977371216	TOP1	yes, GATA2D2A-B
-0.585682392	LMNB2	NO

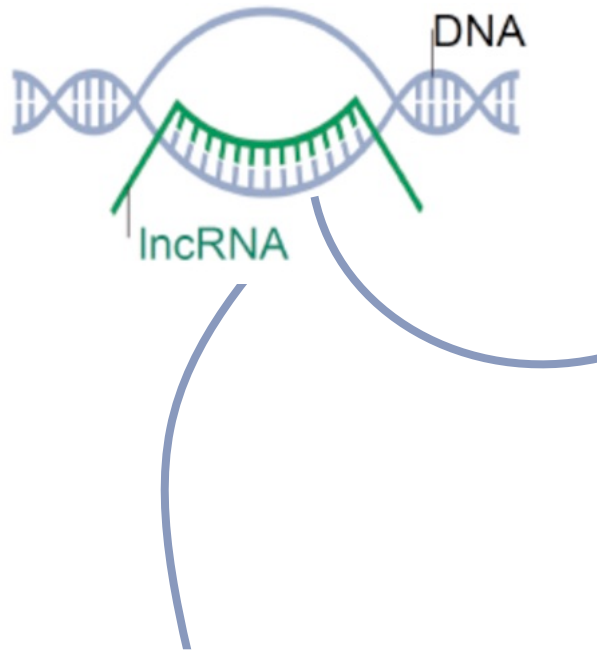


# NESPR ASSOCIATED PROTEIN ARE CORRELATED TO NORADRENERGIC GS

## neuroblastoma tumors



# NESPR BINDING SITES OVERLAP WITH NORADRENERGIC CRC TFS



HOMER Motif analysis in NESPR-captured DNA

**CTAATTGC**  
**TAA** **GGG**

- ISL1 motif
- 1010 peaks (32.13%) pvalue =  $1.10^{-22}$

**AGATAACA**  
**TAA** **GGG**

- GATA3 motif
- 790 peaks (25.14%) pvalue =  $1.10^{-12}$



Louis "ChIRP" Delhaye

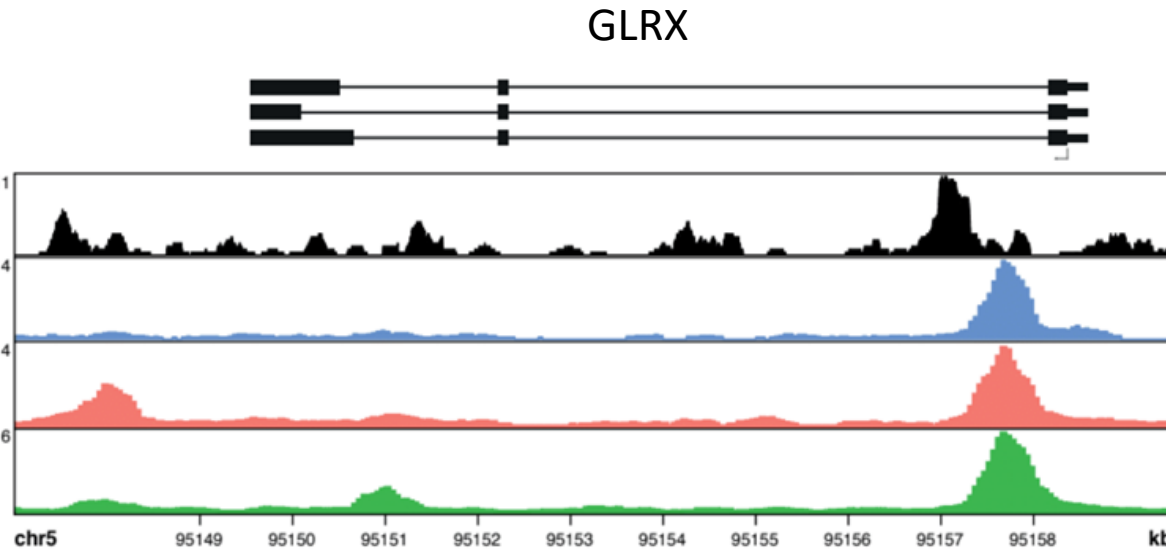


NESPR ChIRP-Seq

PHOX2B ChIP-Seq

GATA3 ChIP-Seq

ISL1 ChIP-Seq



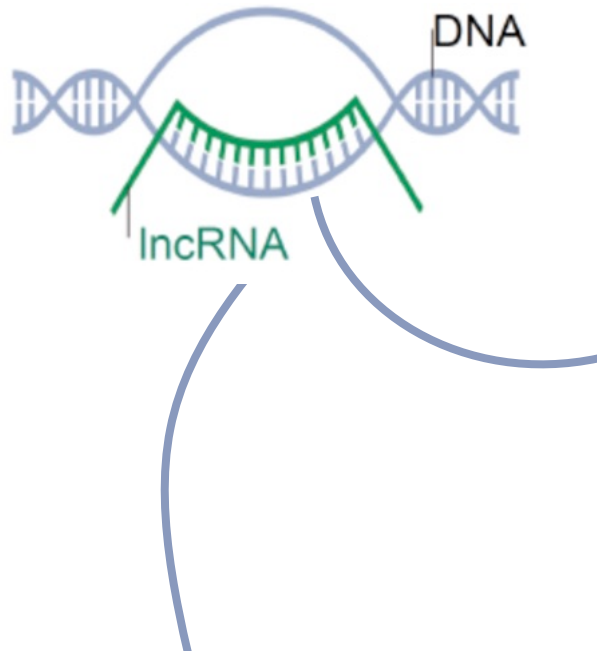
PHOX2B : NESPR 232

GATA3 : NESPR 169

ISL1 : NESPR 268

84

# NESPR BINDING SITES OVERLAP WITH NORADRENERGIC CRC TFS



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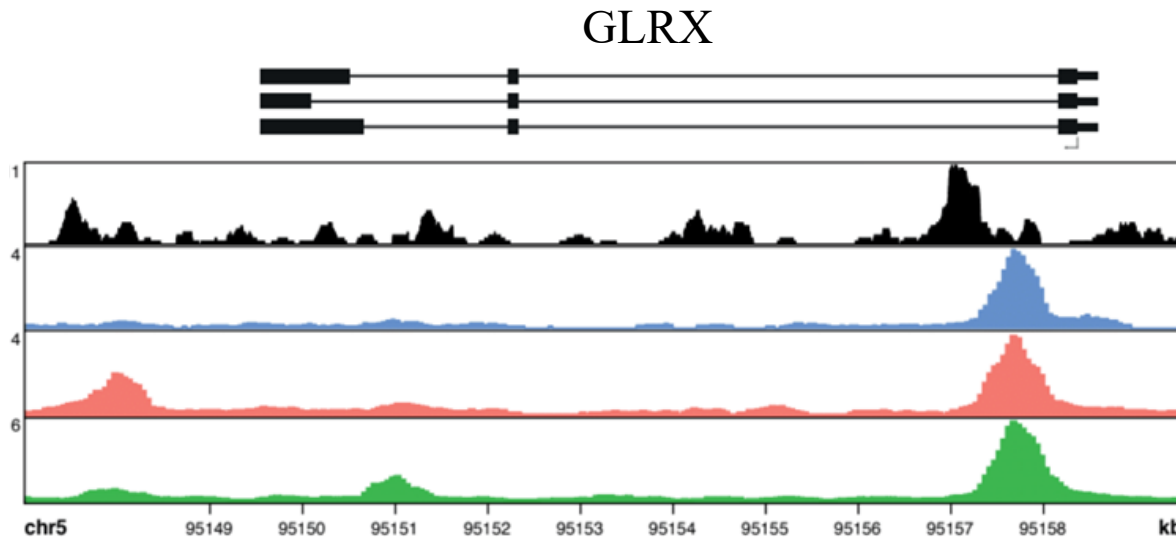


NESPR ChIRP-Seq

PHOX2B ChIP-Seq

GATA3 ChIP-Seq

ISL1 ChIP-Seq



PHOX2B : NESPR 232

GATA3 : NESPR 169

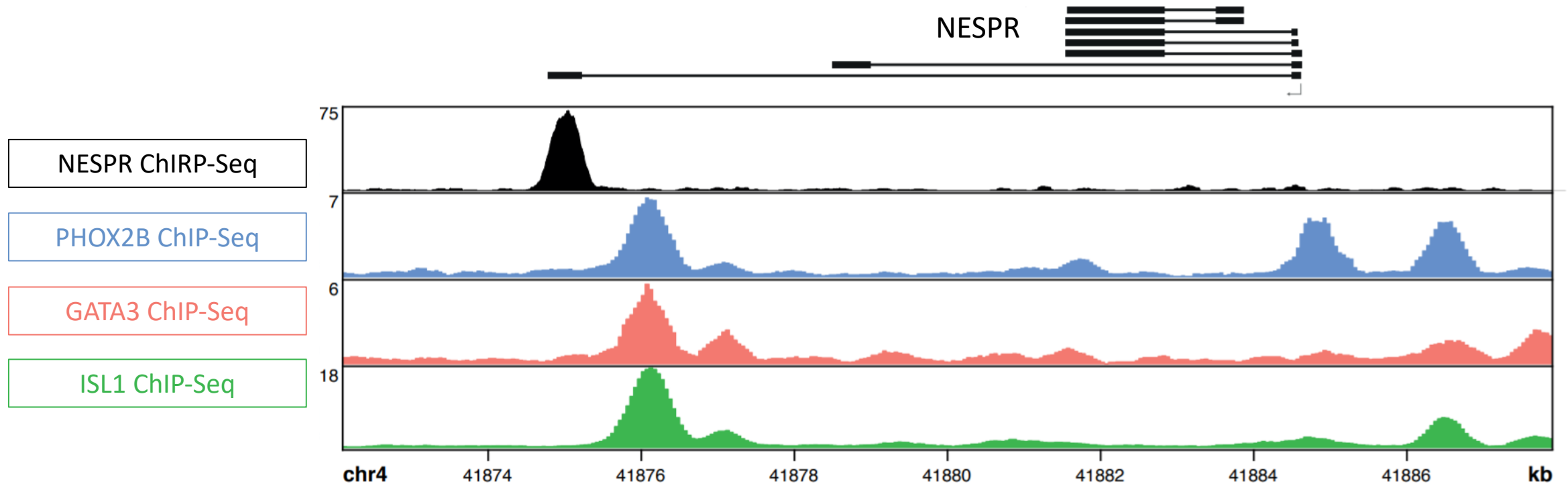
ISL1 : NESPR 268



# NORADRENERGIC CRC TFS BIND NESPR LOCI

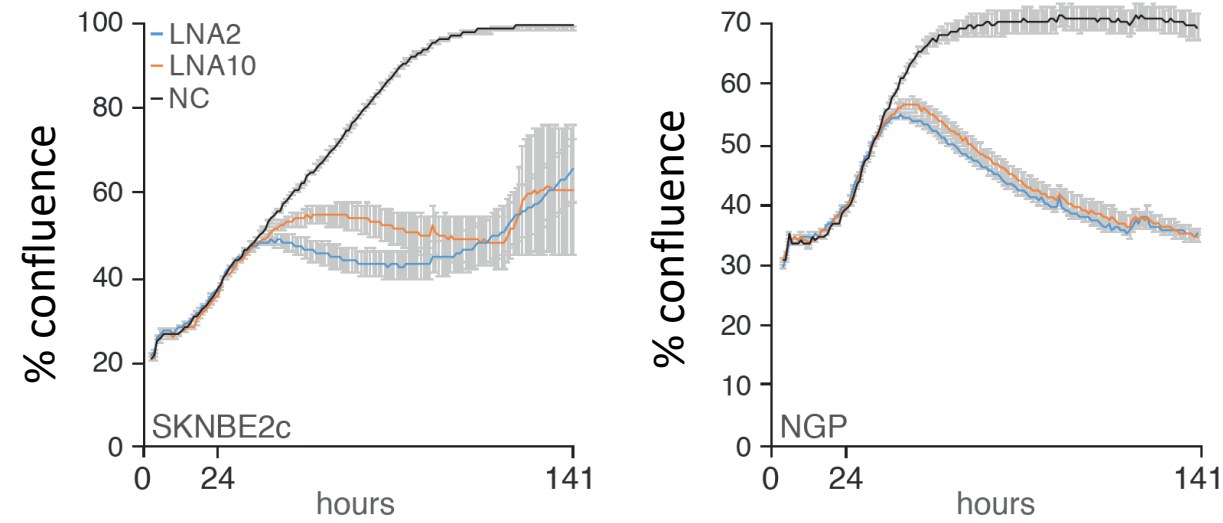


Louis "ChIRP" Delhaye

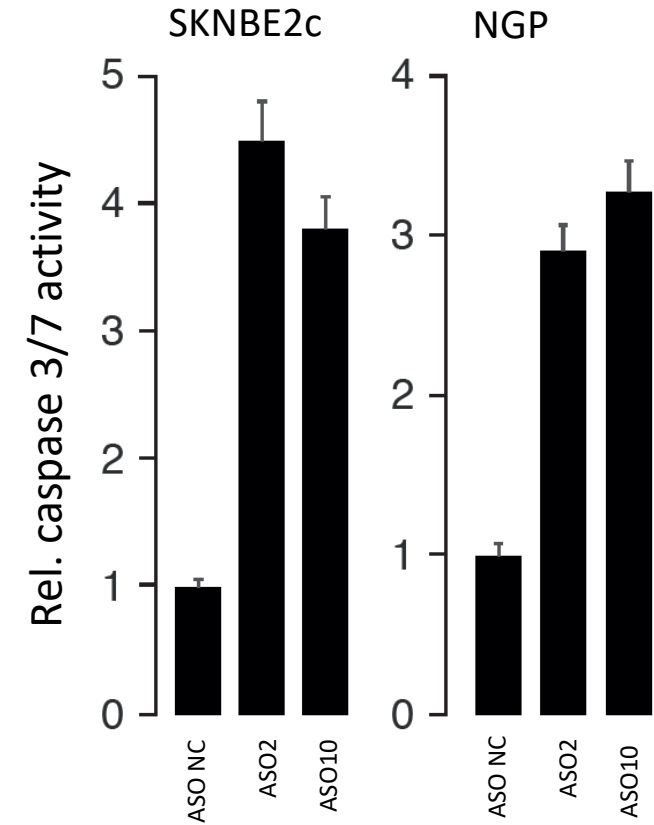


# NESPR KNOCK-DOWN **IMPAIRS** NEUROBLASTOMA **CELL PROLIFERATION**

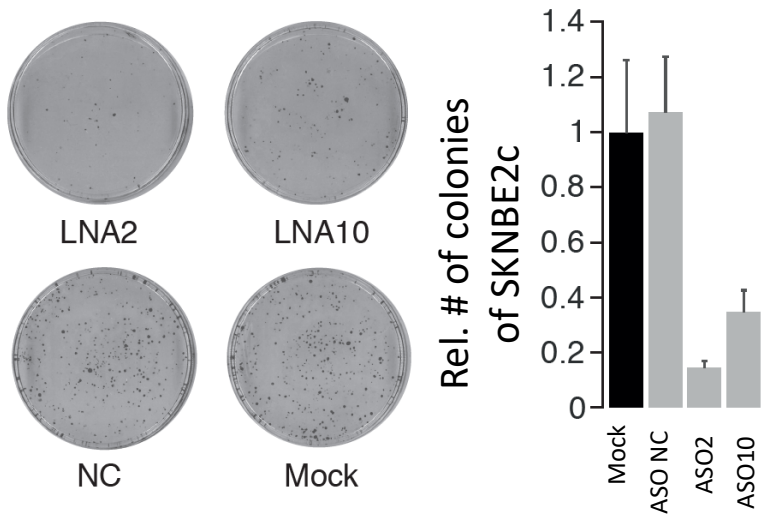
## Proliferation

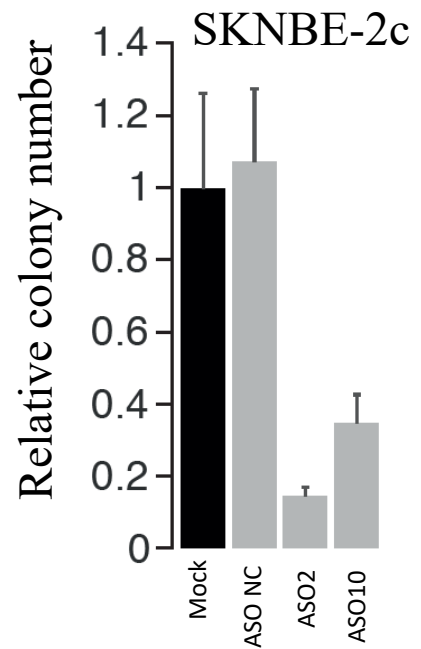
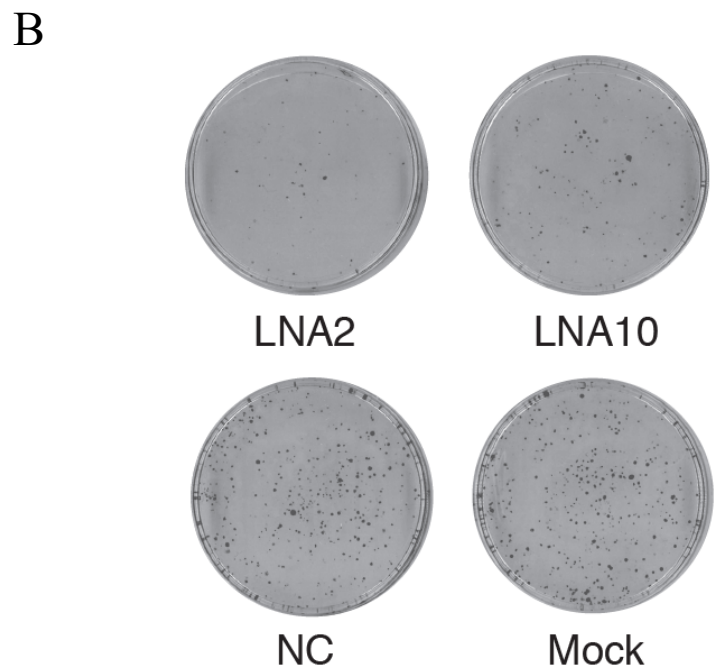
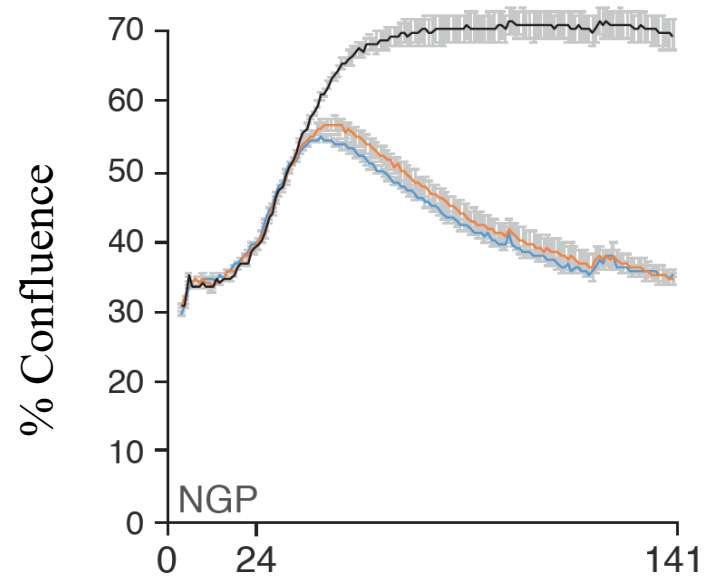
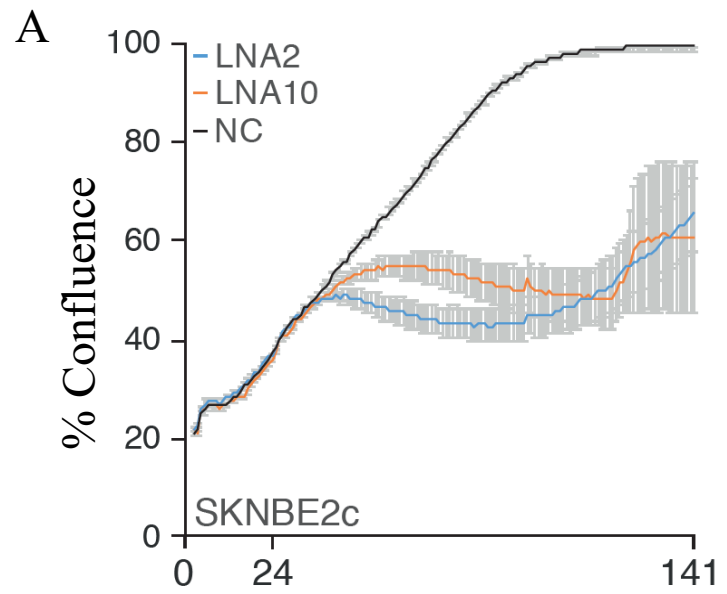


## Apoptosis



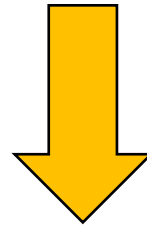
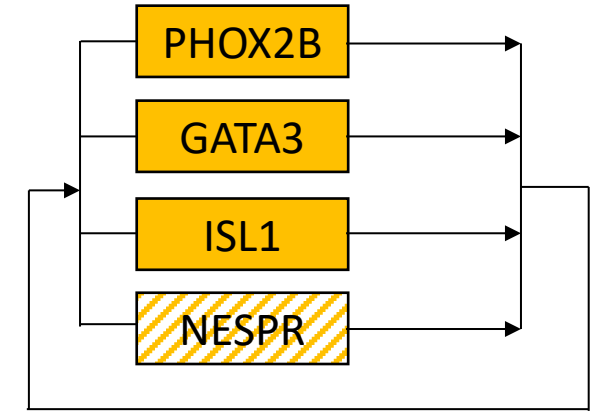
## Colony formation assay





# CONCLUSIONS & NEXT STEPS

1. NESPR shows neuroblastoma-specific expression
2. NESPR regulates PHOX2B and other members of the noradrenergic CRC
3. Noradrenergic TFs and NESPR binding sites overlap
4. Data indicates NESPR behaves as a novel node in the noradrenergic CRC
5. Biological processes are driven by signal transduction networks (STNs)...*which should include lncRNAs*



- A. Investigate NESPR's role at binding sites (ChIP)
- B. Validate/investigate interaction function(s) (RIP, kd, rescue)
- C. Verify trans-effect by exogenous expression (Oex)



# Acknowledgements



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