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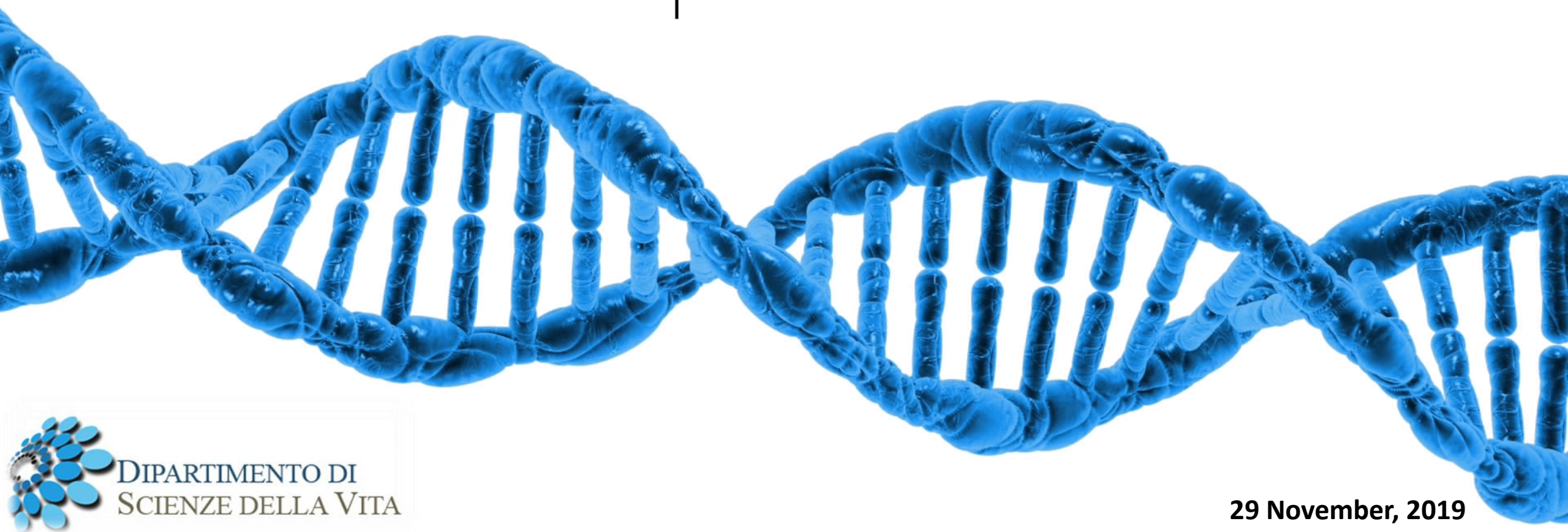
Ultraconserved regions (UCRs) and lncRNAs



DIPARTIMENTO DI SCIENZE DELLA VITA

29 November, 2019

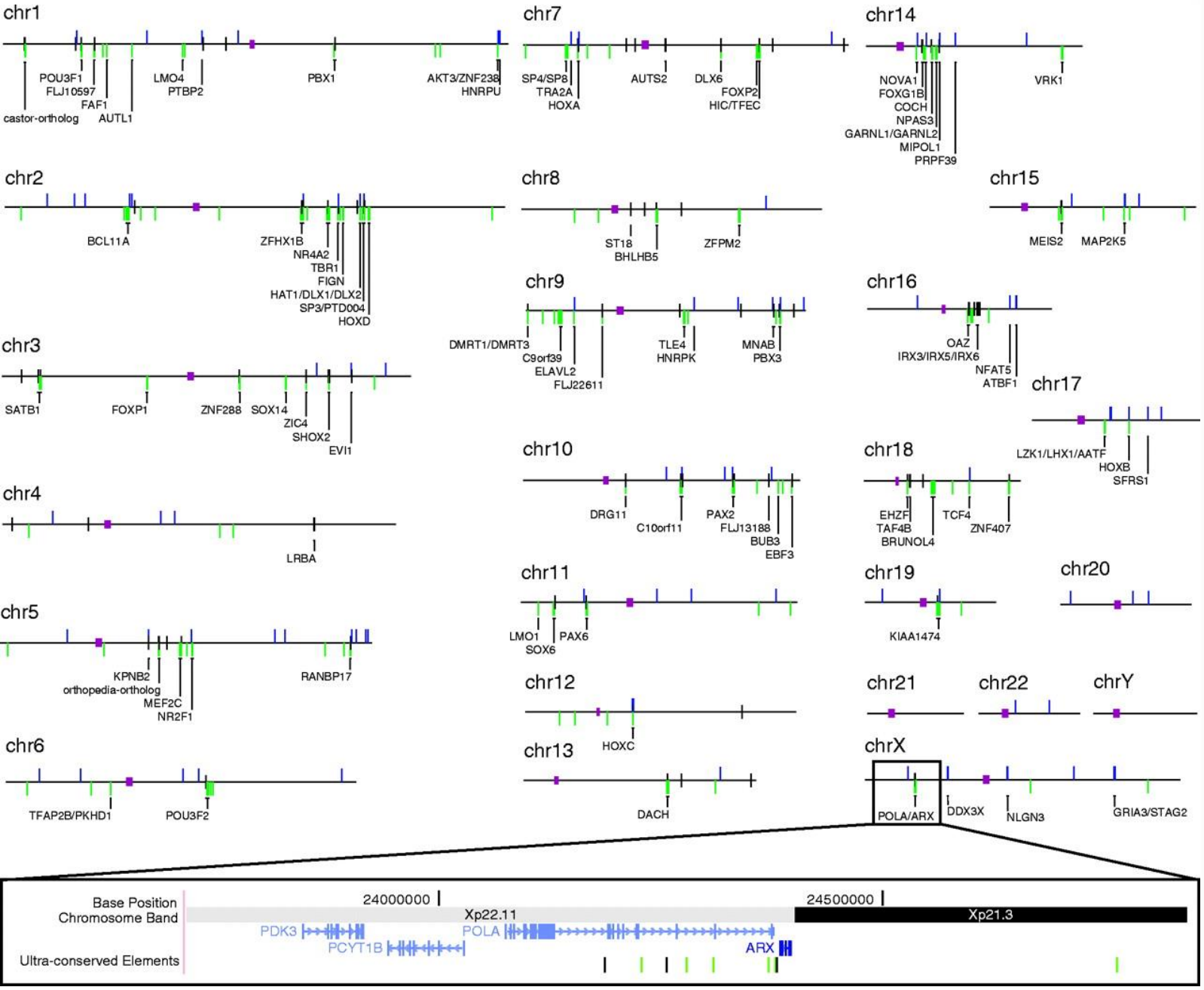
LncRNAs and the importance of evolutionary conservation

In silico studies underscored how homology in secondary structure is more important than primary sequence changes in lncRNA functionality!

lncRNA	Conservation	Function roles
AK028326	Poor conserved	Self-renewal
AK141205	Conserved	Self-renewal
Braveheart	Not conserved	Cardiovascular differentiation
DIGIT	Conserved	Meso-endoderm differentiation
Evx1as	Conserved	Mesoderm differentiation
GAS5	Poor conserved	Self-renewal
Hotair	Poor conserved	Self-renewal Cell proliferation
LincPRESS1	Poor conserved	Pluripotency Cell cycle regulation
LincRNA1592-1552	Poor conserved	Pluripotency
Lin-RoR	Poor conserved	Pluripotency Self-renewal
Meg3	Conserved	Pluripotency Reprogramming
Meteor	Conserved	Mesoderm specification
Neat1	Conserved	Differentiation
Oct4P4	Poor conserved	Self-renewal Cell proliferation
Pnky	Conserved	Neuronal differentiation
TERRA	Conserved	Pluripotency
TUNA	Conserved	Self-renewal Neural differentiation

Fico, Annalisa, et al. "Long non-coding RNA in stem cell pluripotency and lineage commitment: functions and evolutionary conservation." *Cellular and Molecular Life Sciences* 76.8 (2019): 1459-1471

Ultraconserved elements (UCEs): a close look at evolutionary conservation



The extreme conservation could be due to the absence of annotated **transposons** near UCEs

Transposon-free regions coincide with the chromatin bivalent domains, which mark key regulatory genes in embryo development and ESC pluripotency.

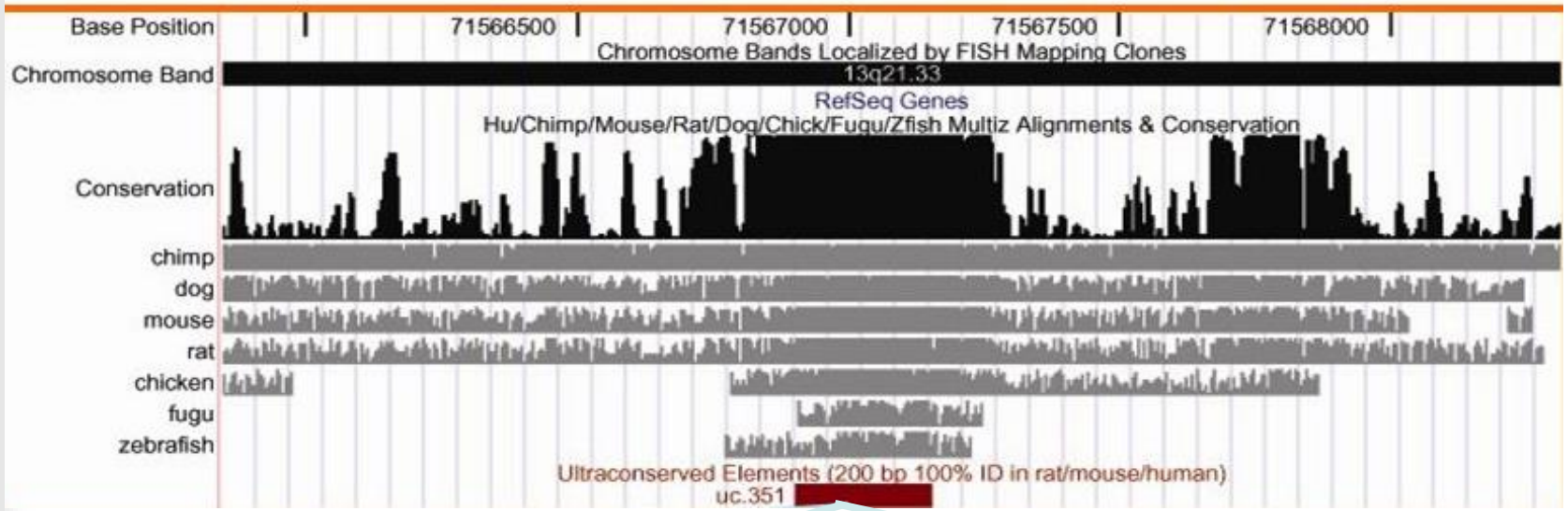
Bejerano, Gill, et al. "Ultraconserved elements in the human genome." *Science* 304.5675 (2004): 1321-1325.

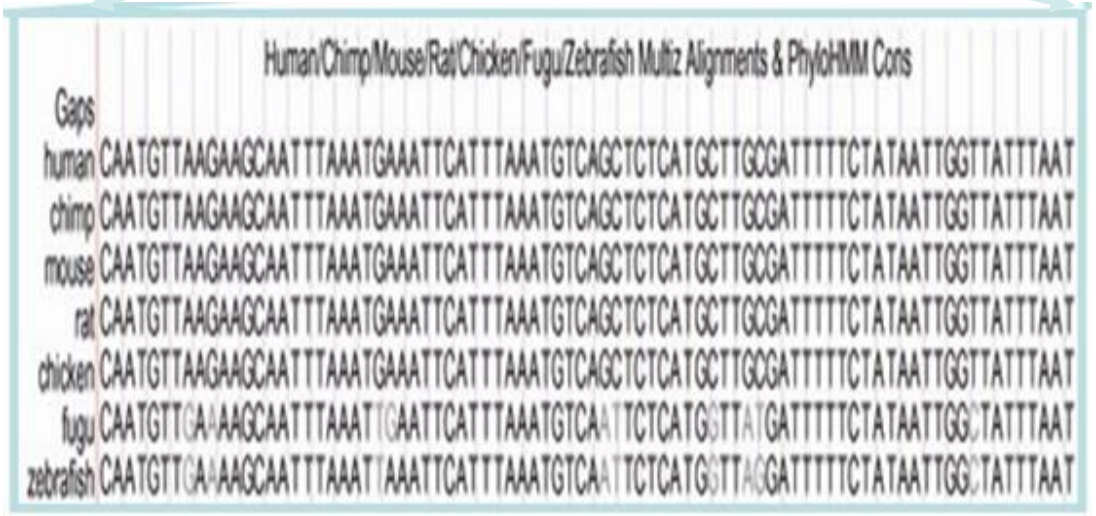
Definition and genomic environment of UCRs

A bit of history

- Genome-scale computational analysis retrieved 3583 human/mouse/ pufferfish UCRs.
- Median UCR length was 125 bp
- Present in **introns**, in **dense clusters around a group of genes** or in "**gene deserts**"

→ **Strong association between locations of UCRs and genes encoding transcription factors.**





Bejerano, Gill, et al. "Ultraconserved elements in the human genome." *Science* 304.5675 (2004): 1321-1325.

UCRs are strongly associated with DNA-binding proteins

Domain description	*INTERPRO ID*	*Fisher test P value*	*Corrected P value*
HTH_lambrepressr	IPR000047	6.40E-20	5.36E-17
Homeobox	IPR001356	1.60E-12	1.34E-09
Antennapedia	IPR001827	1.37E-10	1.15E-07
Paired_box	IPR001523	2.39E-05	2.00E-02
HLH_basic	IPR001092	2.40E-05	2.01E-02
POU_domain	IPR000327	3.06E-05	2.56E-02
Homeo_OAR	IPR003654	3.08E-05	2.58E-02
TF_Fork_head	IPR001766	6.15E-05	5.15E-02
Znf_C4steroid	IPR001628	7.45E-05	6.23E-02
Hormone_rec_lig	IPR000536	1.06E-04	8.86E-02
HMG_12_box	IPR000910	1.81E-04	1.51E-01
Stdhrmn_receptor	IPR001723	2.63E-04	2.20E-01
COUP_TF	IPR003068	7.62E-04	6.38E-01
LIM	IPR001781	1.10E-03	9.18E-01
RtnoidX_receptor	IPR000003	1.28E-03	1.07E+00
FN_III	IPR003961	2.57E-03	2.15E+00

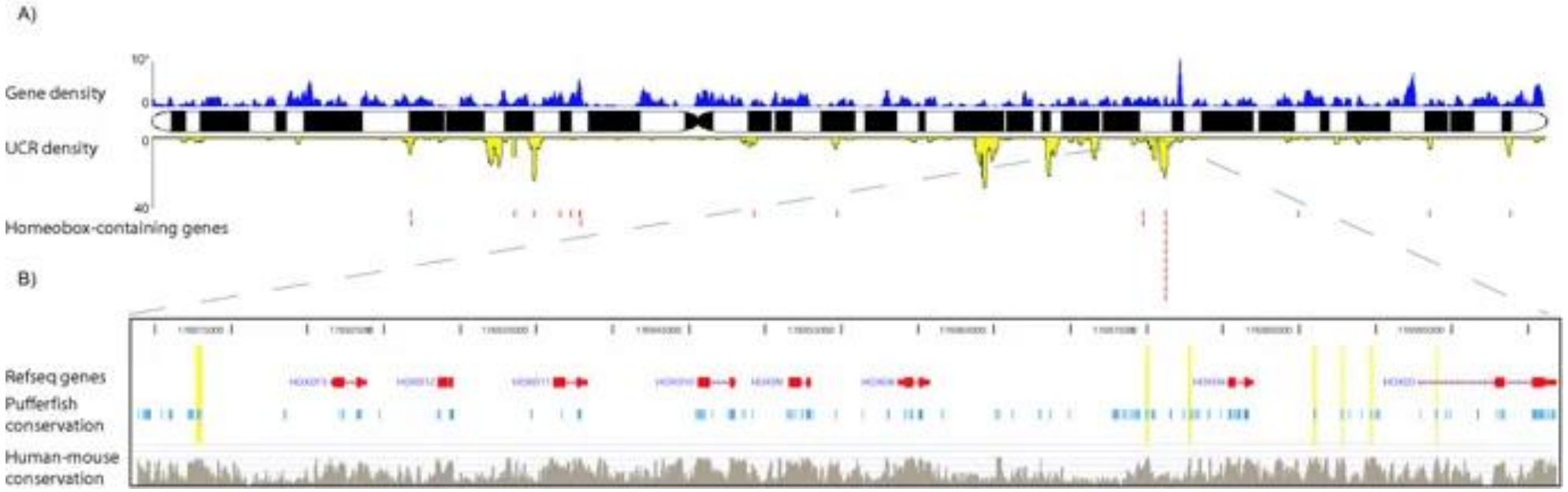
- *Bonferroni-corrected* and uncorrected *Fisher Exact Test p-values* are shown for the 16 most over-represented domains

- **Genomic neighborhoods** of UCRs: **30%** of all homeodomain-encoding genes have an UCR within 8 kbp, and **55%** have one within 100 kb.

Sandelin, Albin, et al. "Arrays of ultraconserved non-coding regions span the loci of key developmental genes in vertebrate genomes." *BMC genomics* 5.1 (2004): 99.

→UCRs are spatially associated with genes encoding regulatory proteins.

UCRs clusters encompass the entire gene loci of key developmental genes

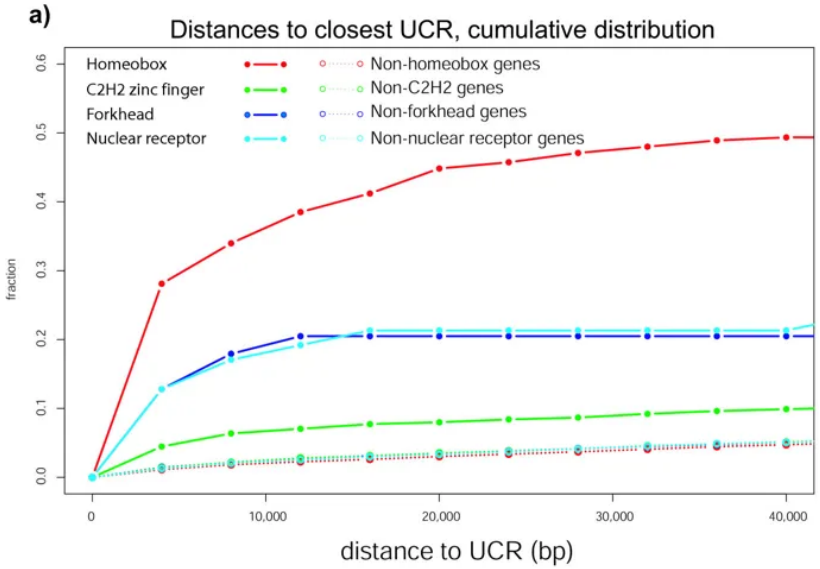


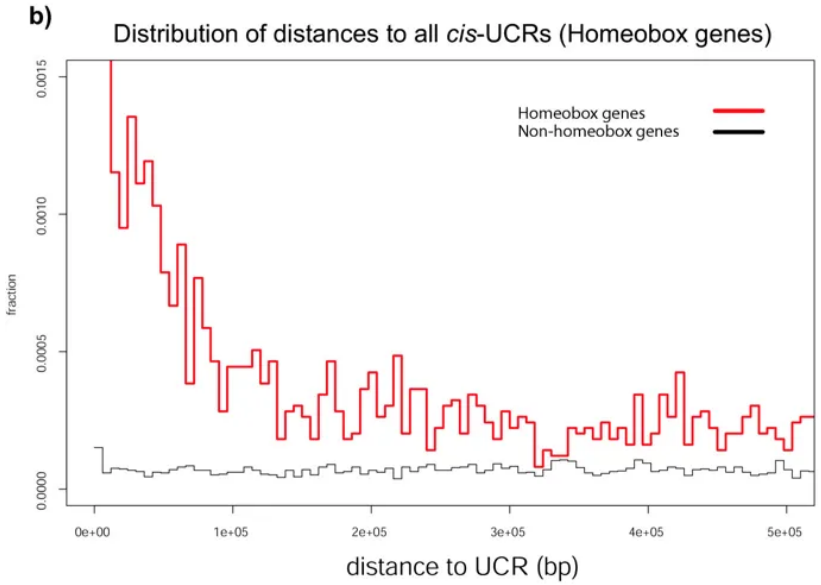
Sandelin, Albin, et al. "Arrays of ultraconserved non-coding regions span the loci of key developmental genes in vertebrate genomes." *BMC genomics* 5.1 (2004): 99.

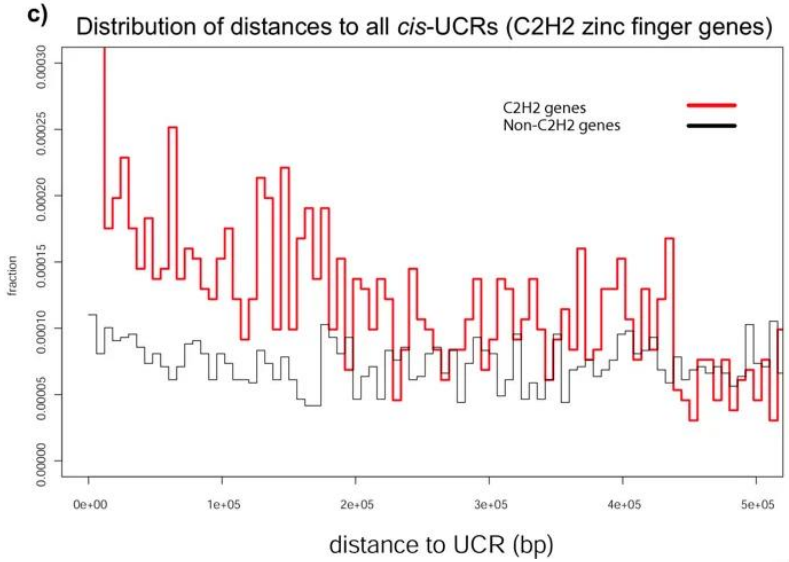
- Visual inspection reveals tendency of UCRs to occur in **large clusters**
- Positions of **homebox-domain containing genes** coincide with local maxima of UCR density.
- The **HoxD** cluster coincides with one of the larger UCR density peaks and is associated with nine UCRs

→ There is no observed correlation between regions of high gene density and UCRs.

UCRs clusters encompass the entire gene loci of key developmental genes





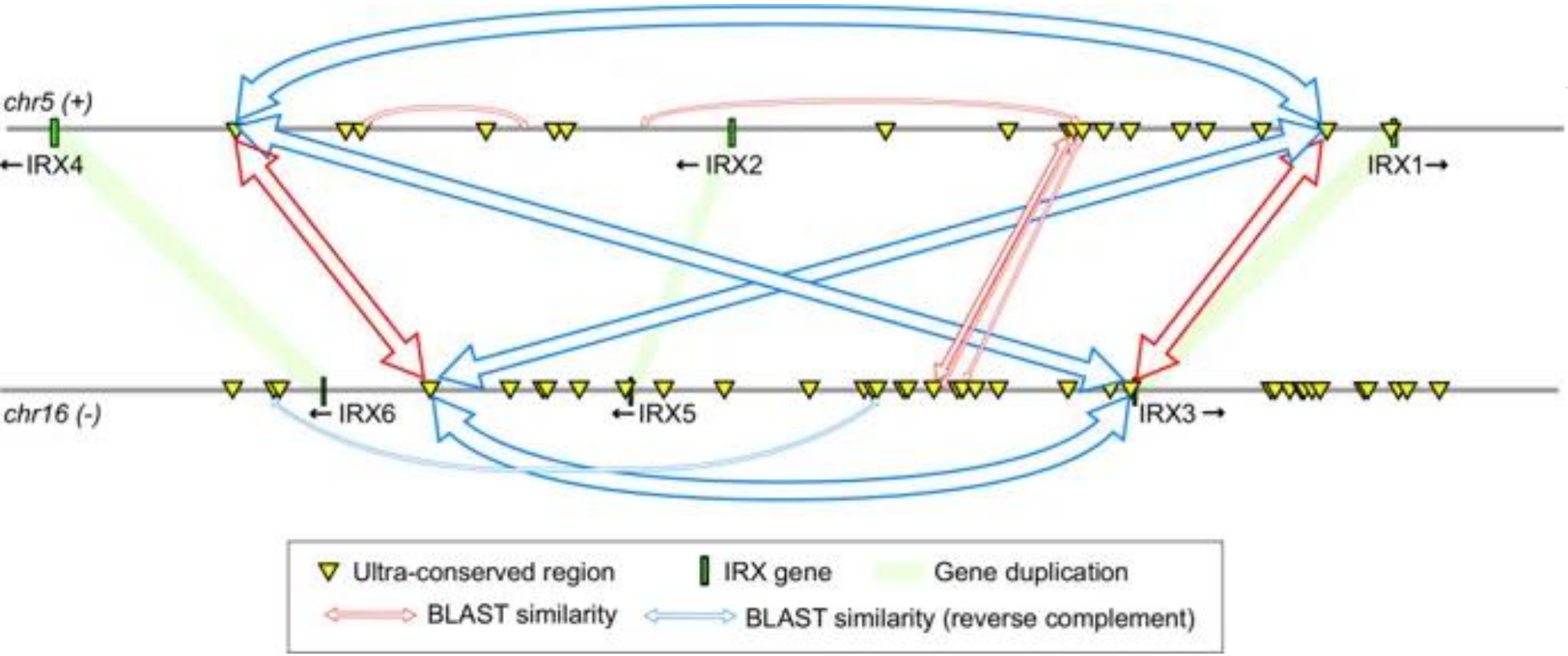


Sandelin, Albin, et al. "Arrays of ultraconserved non-coding regions span the loci of key developmental genes in vertebrate genomes." *BMC genomics* 5.1 (2004): 99.

- Many of the UCRs are adjacent to **homeobox protein-encoding genes** (**Figure 1a**).
- Over-representation of UCRs near homeobox genes extends up to **300 kbp**(**Figure 1b**).
- UCRs near **C2H2 zinc finger genes**, with over-representation of UCRs extending up to **150 kbp** (**Figure 1c**).

→Large clusters of UCRs can span regions of several hundred kilobases around inferred target genes

Rare duplications of UCRs across evolution



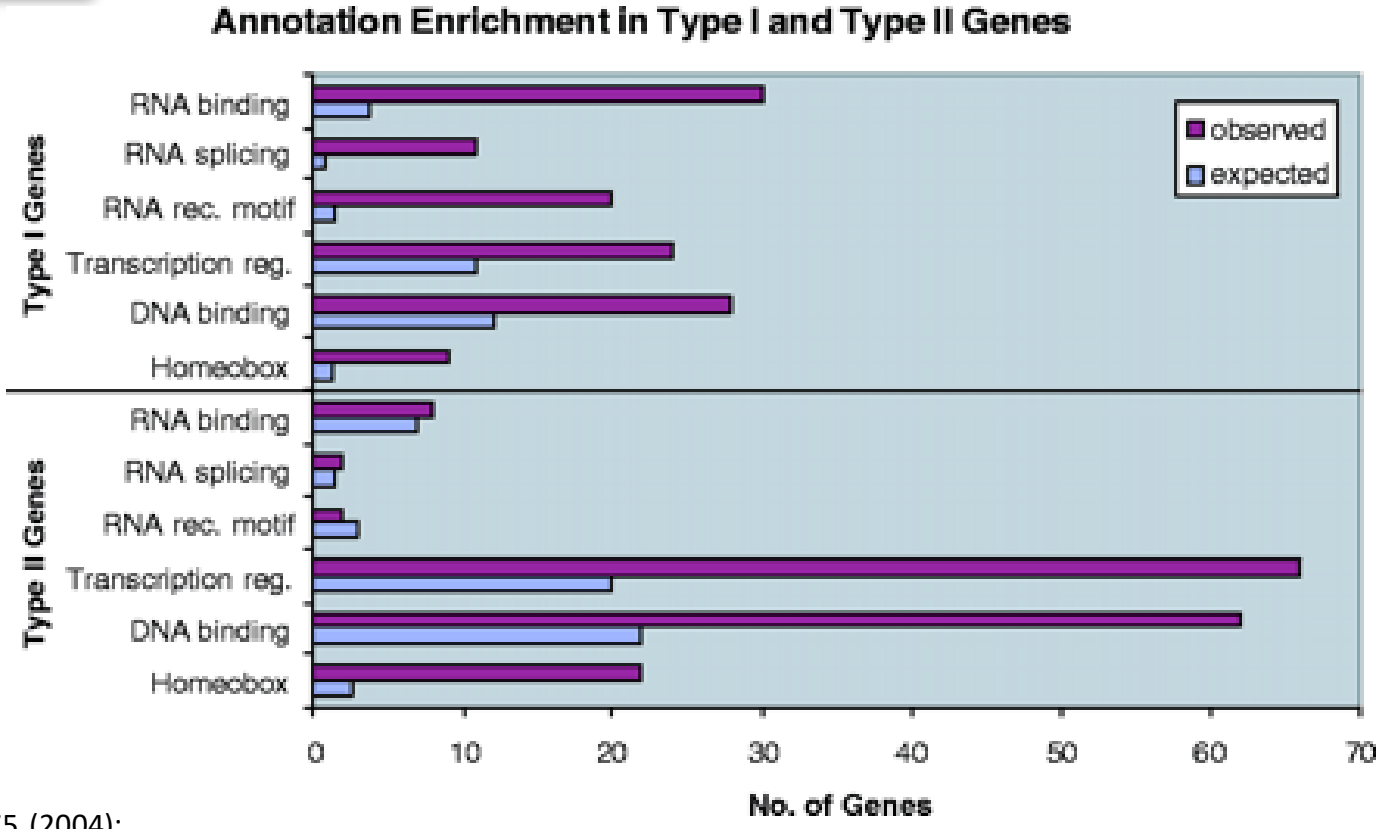
- Five sets of duplicated UCRs adjacent to corresponding duplicated genes.
- Duplicated UCRs are present in the introns of **SOX5** and **SOX6**.
- Similarly positioned arrays of UCRs are present in the four intergenic regions between the **IRX genes**.

Sandelin, Albin, et al. "Arrays of ultraconserved non-coding regions span the loci of key developmental genes in vertebrate genomes." *BMC genomics* 5.1 (2004): 99.

Great majority of UCRs show no similarity between the clusters within the species → the exception is the set of four UCRs highly similar in both cluster position and nucleotide sequence.

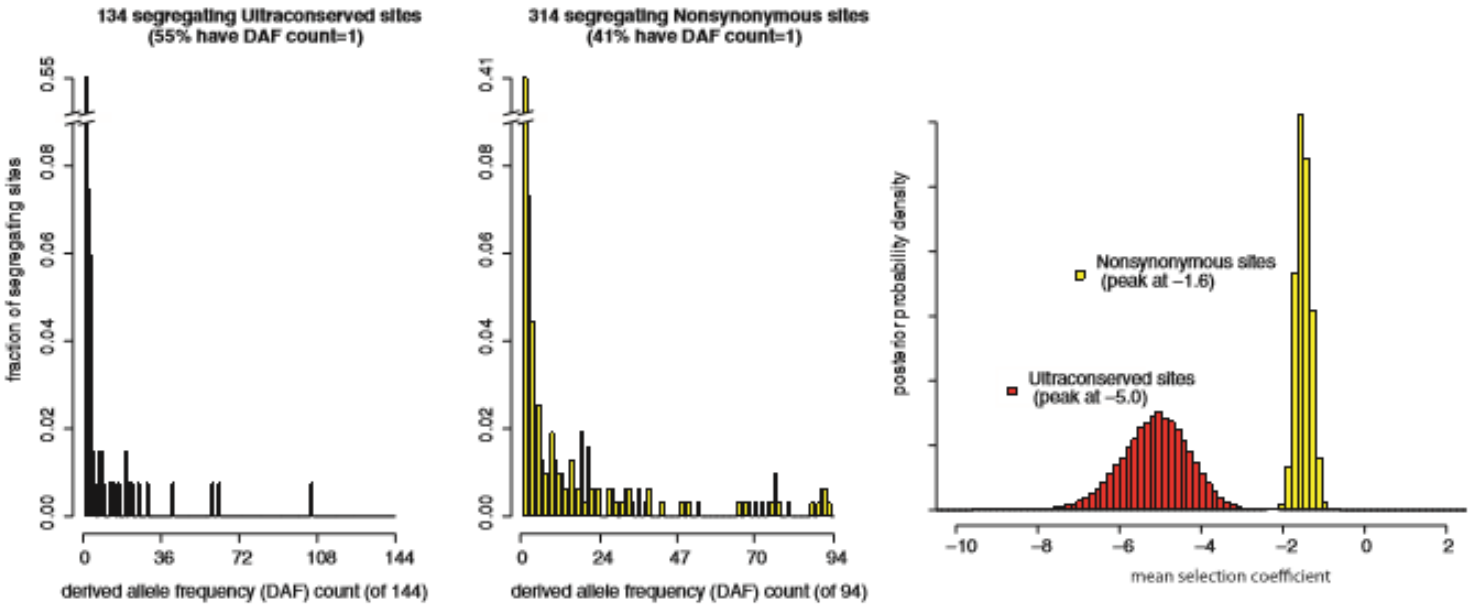
UCRs occur in arrays of highly conserved regulatory elements

- Clusters co-localized with genes encoding proteins for **regulation of development, differentiation and malignancies**
- UCRs fall into multiple functional categories: **enhancers of transcription, regulators of chromatin structure, unknown genes for noncoding transcripts**.
- **Active mechanisms** resulting in the decrease of mutational frequency in UCRs, or **negative pressure** consistent with evolutionary selection against such mutations.



Bejerano, Gill, et al. "Ultraconserved elements in the human genome." *Science* 304.5675 (2004): 1321-1325.

Human Genome UCR Are Ultraselected



Katzman, Sol, et al. "Human genome ultraconserved elements are ultraselected." *Science* 317.5840 (2007): 915-915.

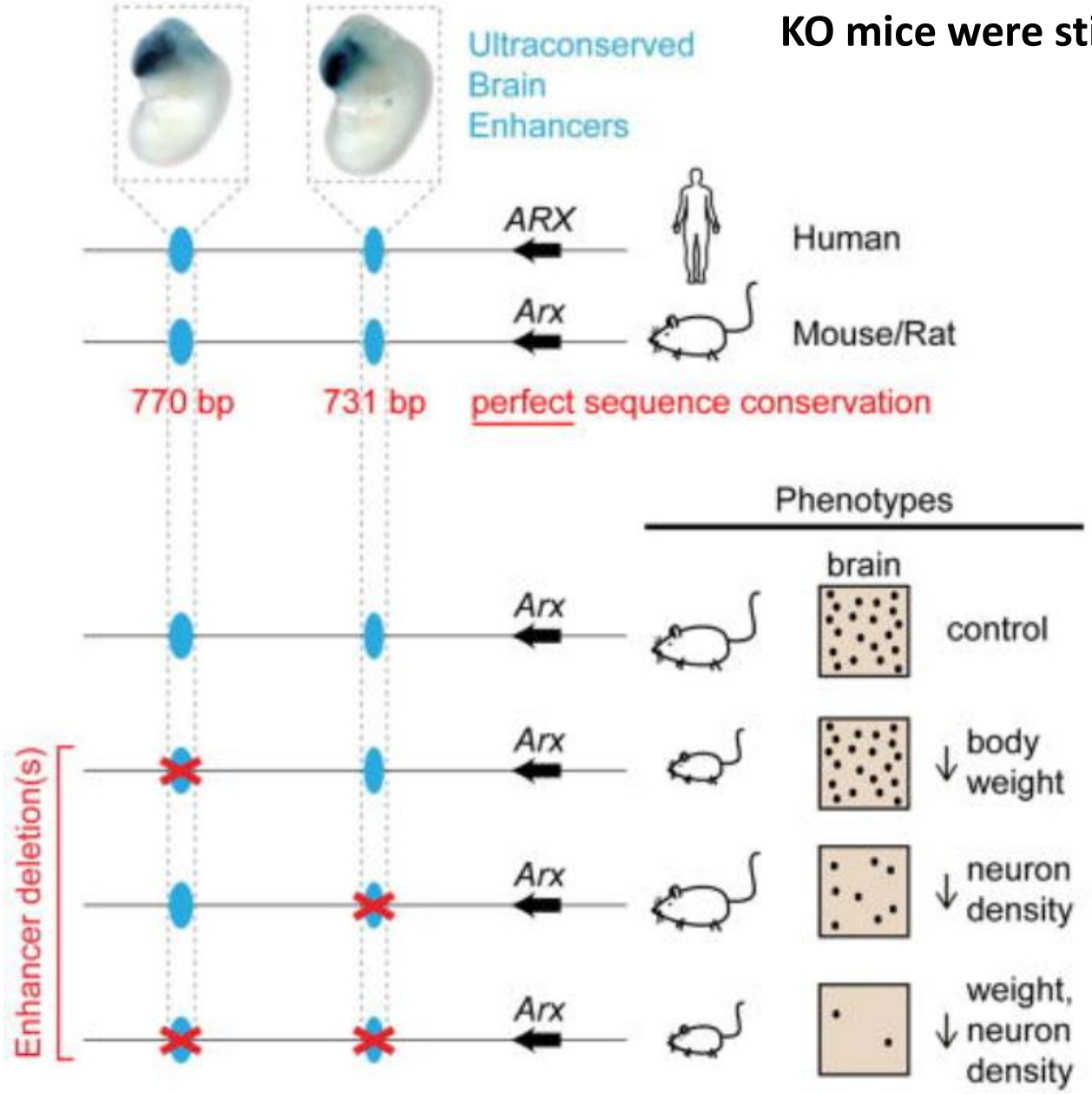
- **DAF** spectrum for the segregating SNPs in the UCRs→ DNA sequences in **72** individuals spanning **315 of UCRs** → 134 segregating sites discovered **(figure1)**
- Comparison with DAF of **314** segregating nonsynonymous sites in **211** genes obtained from **47** individuals **(figure2)**

- only 3% of the segregating UCRs exhibit DAFs of more than 25%, compared with 14% of the segregating nonsynonymous sites
- The posterior distributions indicate that the UCRs have a mean selection coefficient **3X** that of nonsynonymous segregating sites **(figure 3)**

→Selection in the vertebrate-specific ultraconserved noncoding regions is much stronger

Ultraconserved enhancers

KO mice were still viable and fertile



- Ultraconserved loci are normally located near key developmental coding genes
- UCEs can be transcribed and act as enhancers at specific developmental stages in a tissue-specific manner

UCEs have an important role in neural development

Dickel DE et al (2018) Ultraconserved enhancers are required for normal development. Cell 172(3):491–499 e15

Transcribed UCEs (T-UCEs)

- A total of 962 T-UCEs have been annotated
- Expressed in a tissue-specific manner
- Preferentially located in the cytoplasm
- Main molecular mechanism: ‘’decoy’’ function

Functional activities still remain largely unexplored!

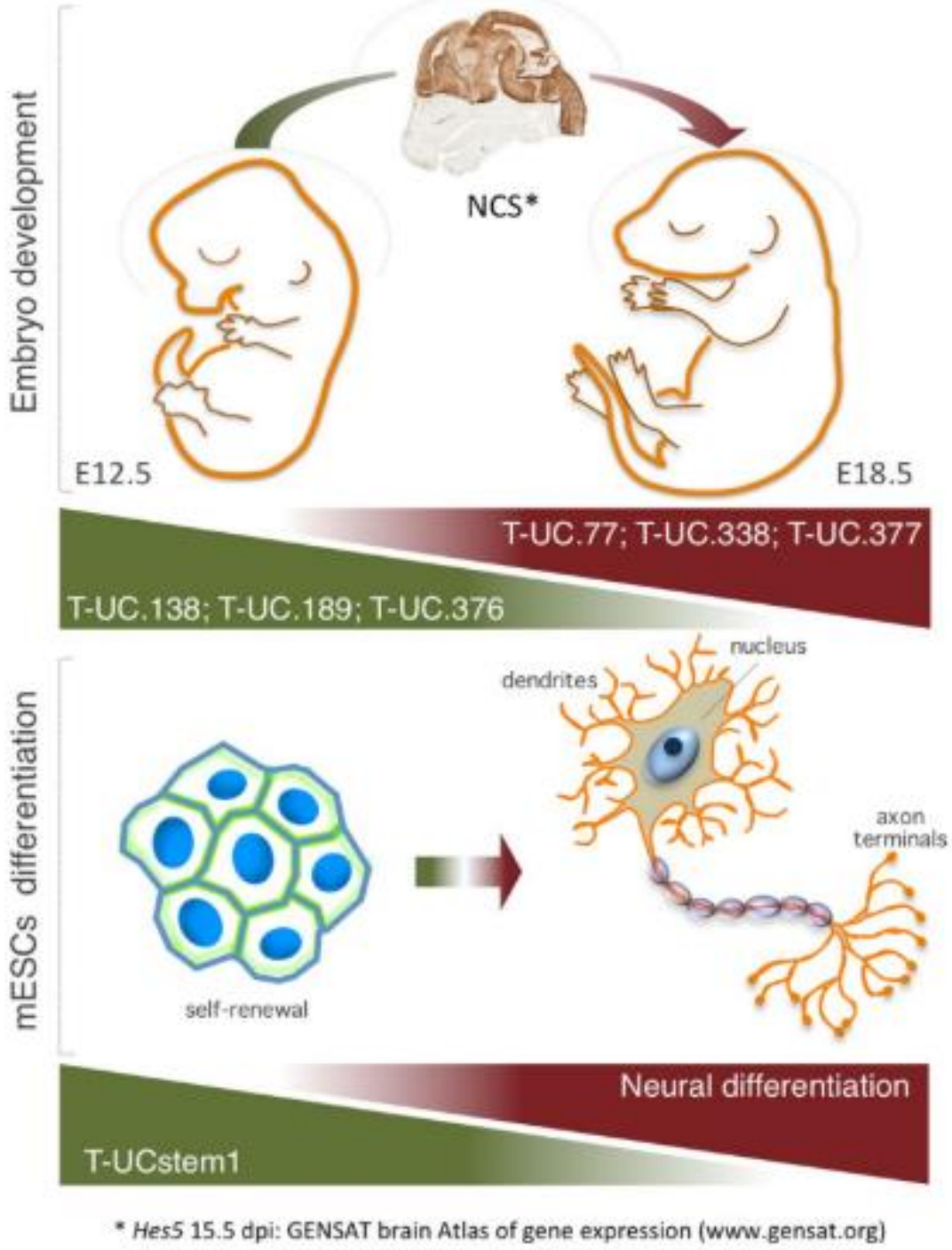
T-UCEs are important during the early stages of development, and in stem cell biology.

The physiological role of this specific class of lncRNAs and their mechanism(s) of action is only recently emerging

Role of T-UCEs during embryonic development

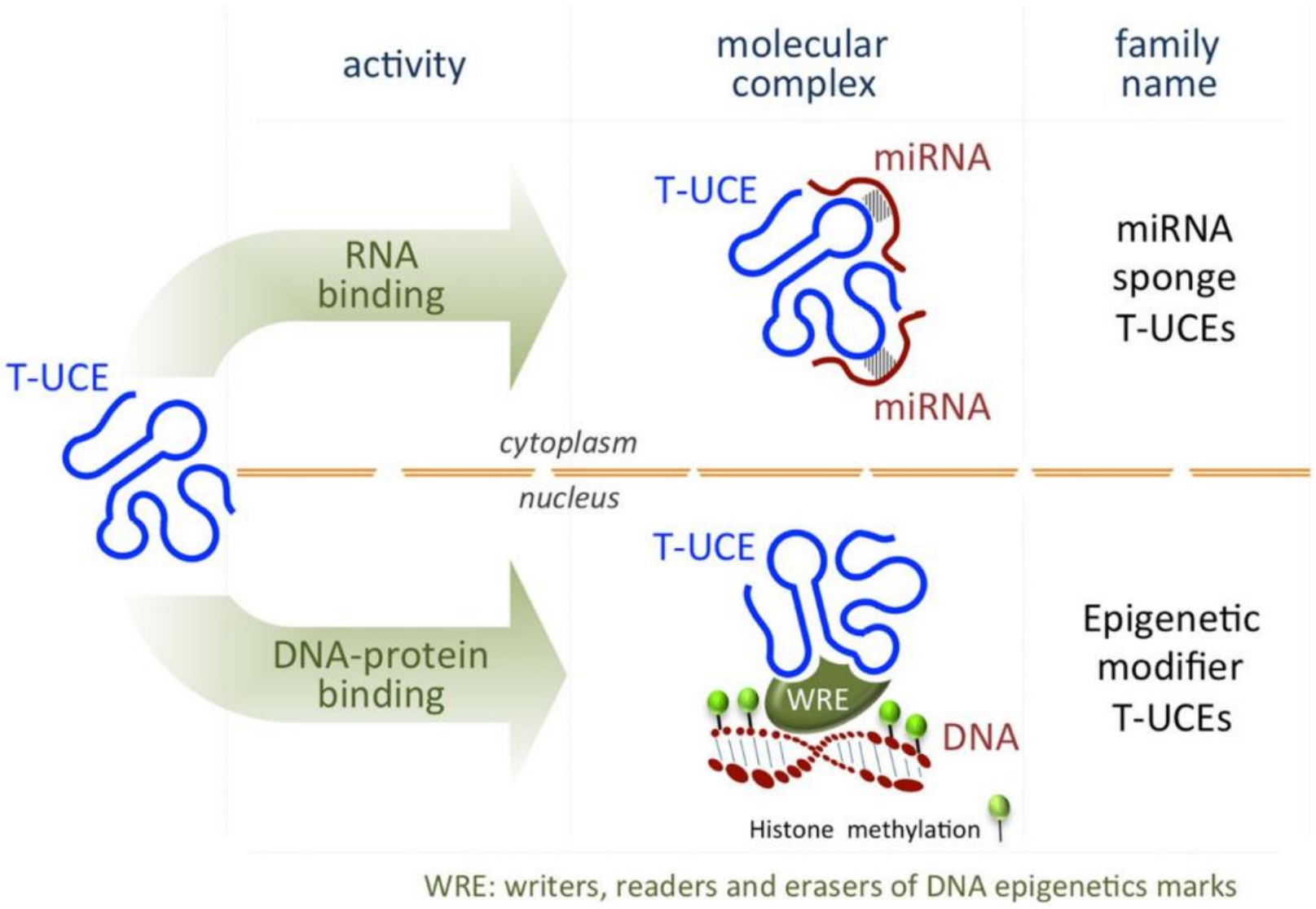
- The majority of UCEs are transcribed into single-stranded transcripts with cell-specific localization.
- T-UCEs are differentially expressed in both time and space

- T-Ucstem1 expression decreases during ESC neural differentiation
- Some T-UCEs remain expressed in adult brain, functioning in homeostasis in the cerebral cortex



Fico, Annalisa, et al. "Long non-coding RNA in stem cell pluripotency and lineage commitment: functions and evolutionary conservation." *Cellular and Molecular Life Sciences* 76.8 (2019): 1459-1471

T-UCE-mediated regulation of ESC self-renewal and differentiation



WRE: writers, readers and erasers of DNA epigenetics marks

The dual role of T-UCE:

- Nuclear T-UCE directly interact with PRC2
- In the cytosol T-UCE act as a sponge

T-UCEs are involved in maintaining pluripotency and silencing developmental genes.

Fico, Annalisa, et al. "Long non-coding RNA in stem cell pluripotency and lineage commitment: functions and evolutionary conservation." *Cellular and Molecular Life Sciences* 76.8 (2019): 1459-1471

Stem Cell Reports
Article

An Ultraconserved Element Containing lncRNA Preserves Transcriptional Dynamics and Maintains ESC Self-Renewal

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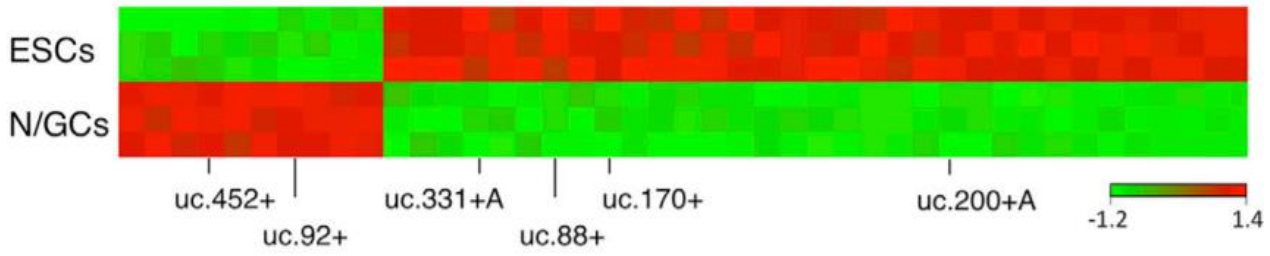
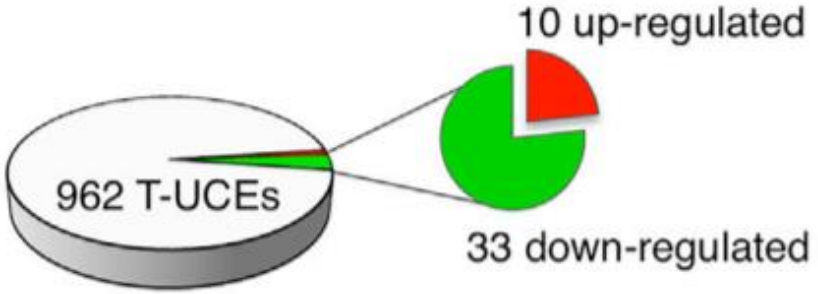
https://doi.org/10.1016/j.stemcr.2018.01.014

Genome-wide profiling reveals T-UCEs differentially expressed during ESC Neural Differentiation

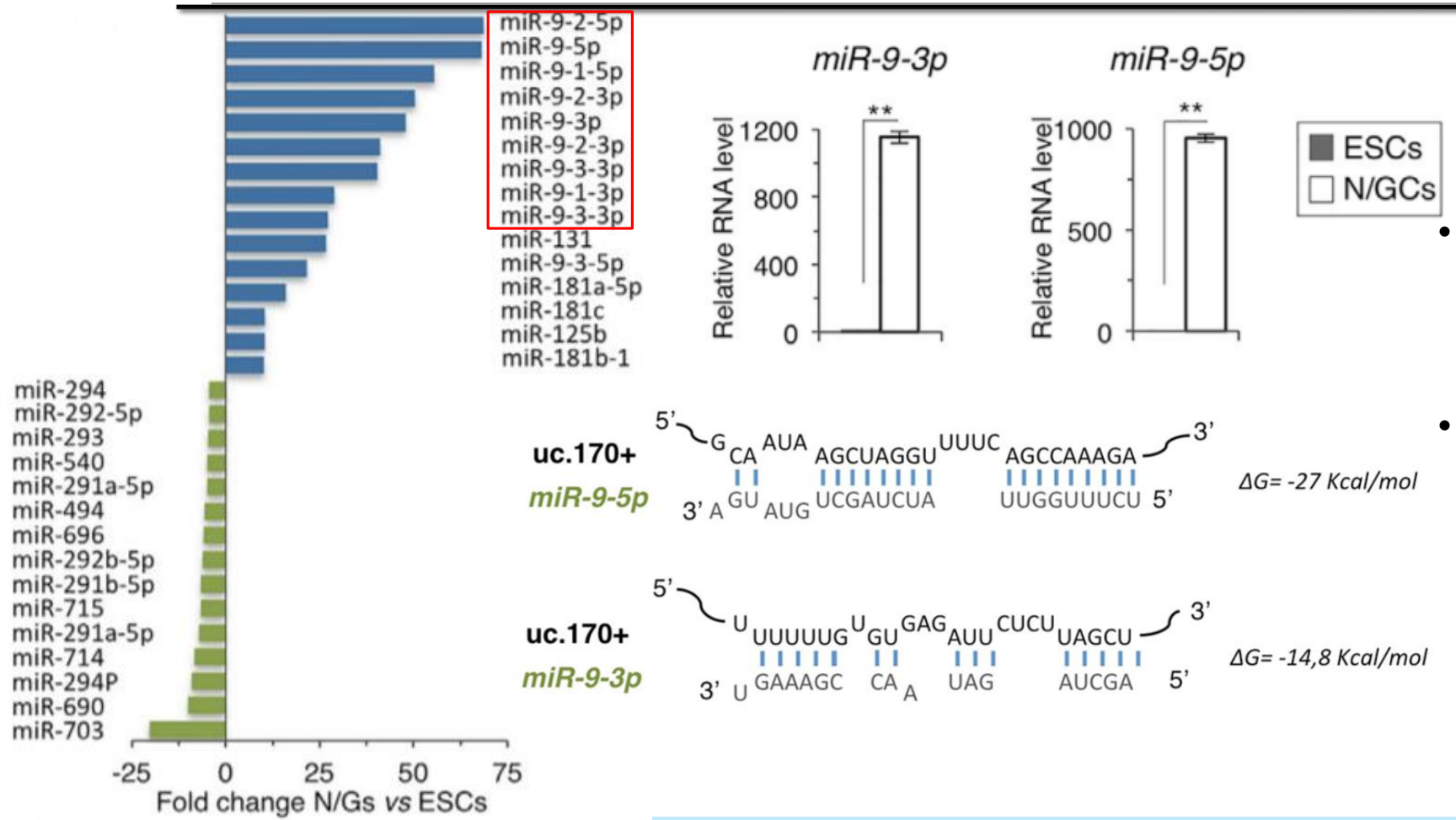
To investigate the role of UCEs in self-renewal/differentiation, they first searched for T-UCEs differentially expressed in undifferentiated versus differentiated ESCs.





- Out of the 962 T-UCEs, only 43 are differentially expressed
- 77% of these are downregulated

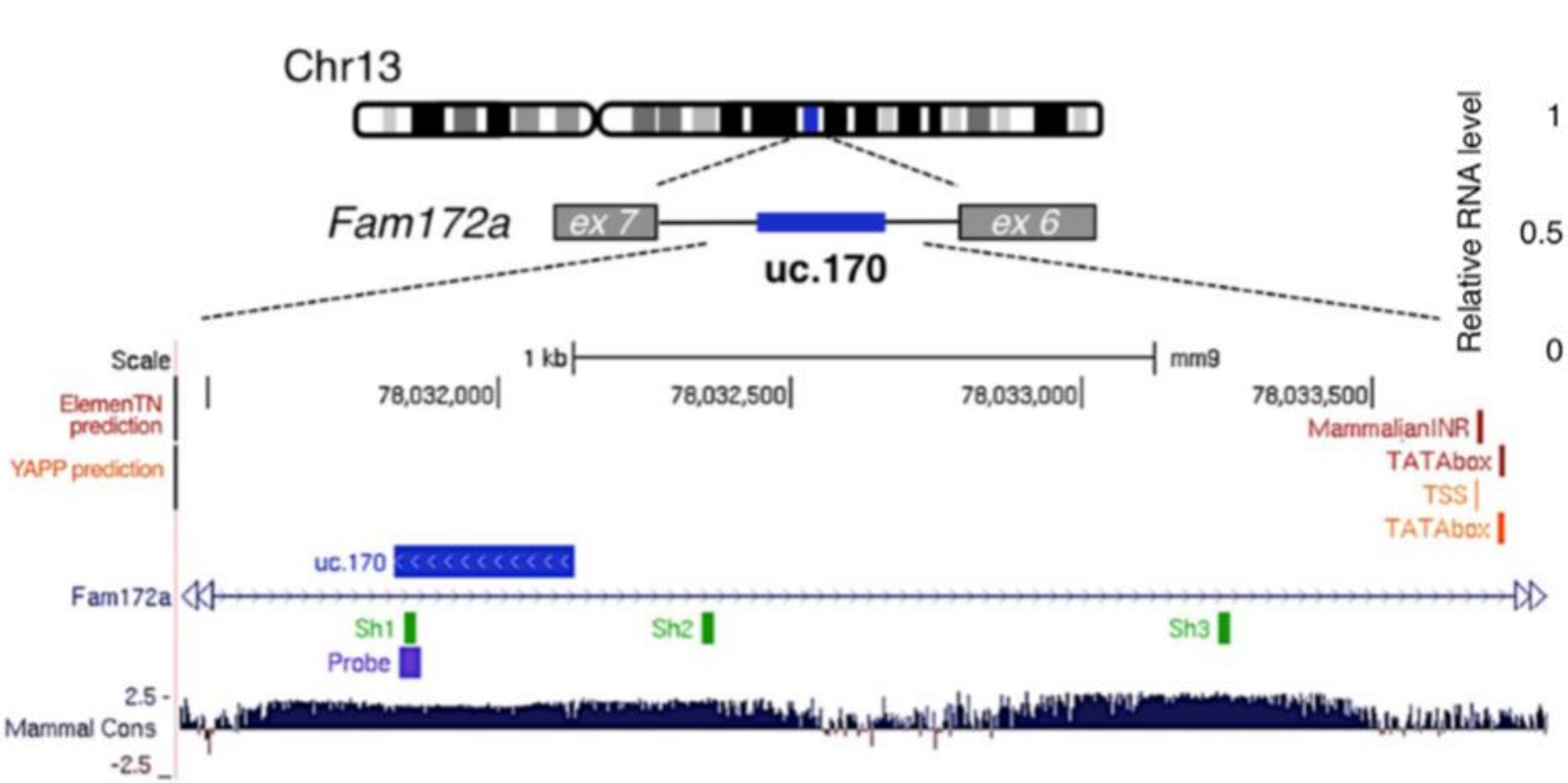
Genome-wide profiling reveals miRNAs differentially expressed during ESC Neural Differentiation

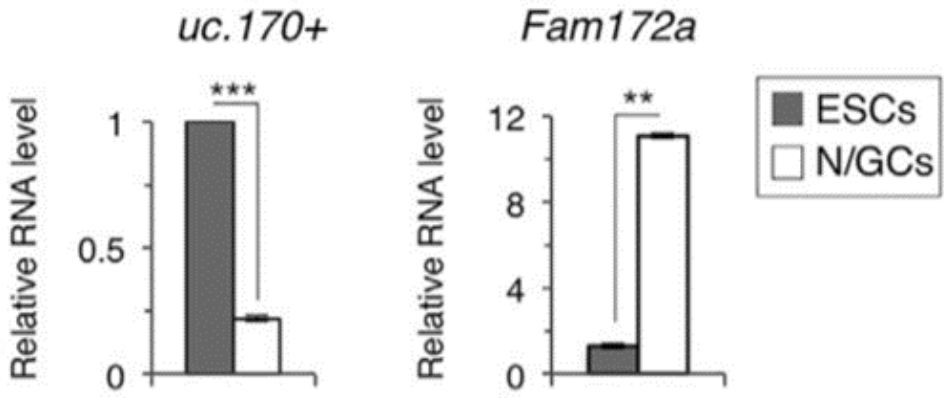


- About 150 miRNAs resulted differentially expressed
- miR-9 was the most upregulated miRNA

They hypothesized that T-UCEs showed a negative correlation with miR-9

Genome-wide profiling reveals T-UCEs differentially expressed during ESC Neural Differentiation

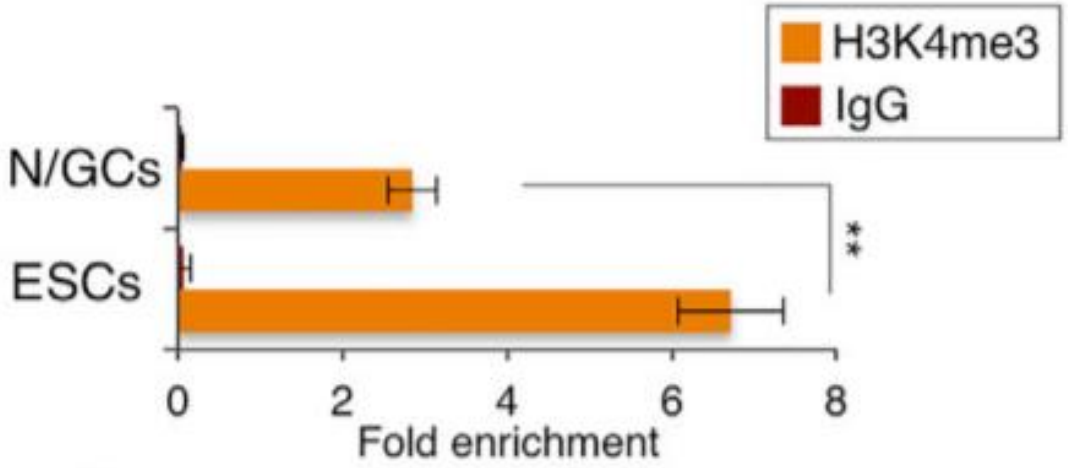


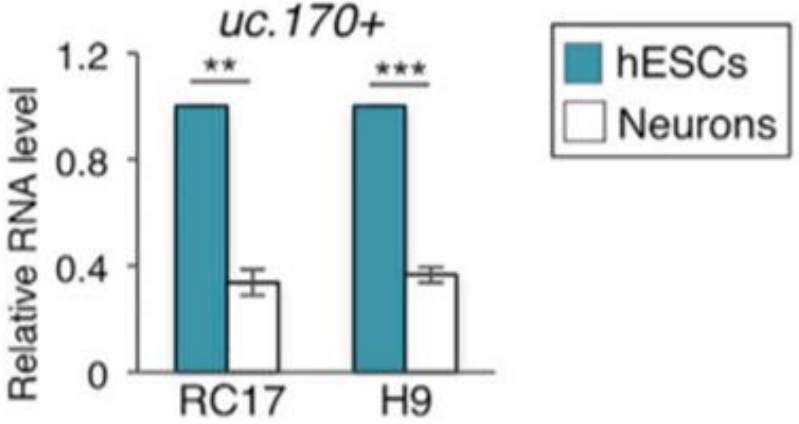


- Uc.170+ and Fam172a transcripts showed opposite expression profiles

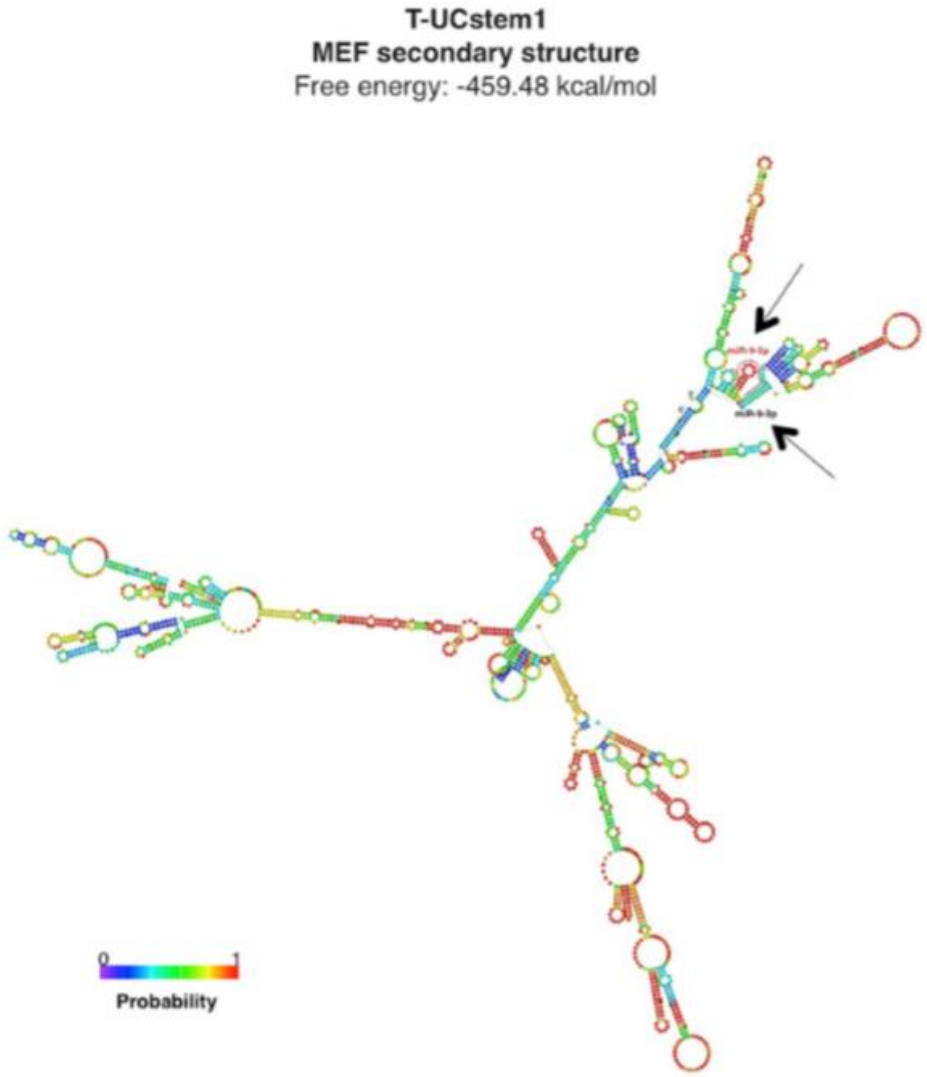
Genome-wide profiling reveals T-UCEs differentially expressed during ESC Neural Differentiation

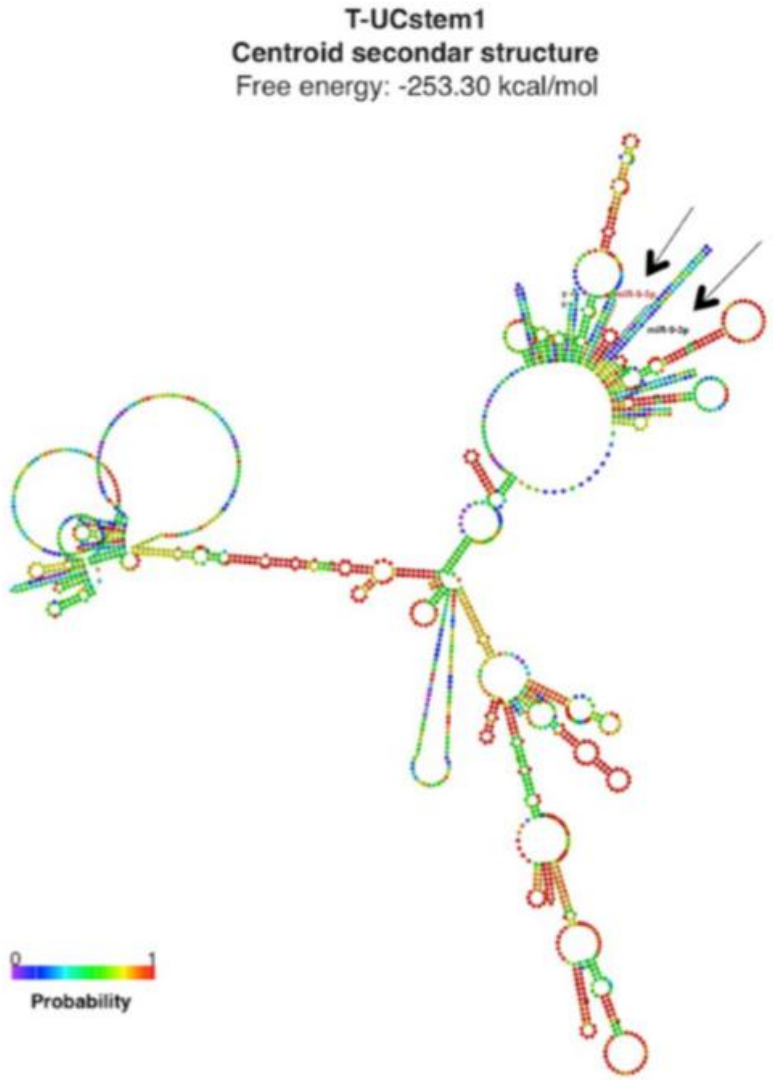
Undifferentiated ESCs show an enrichment of H3K4me3





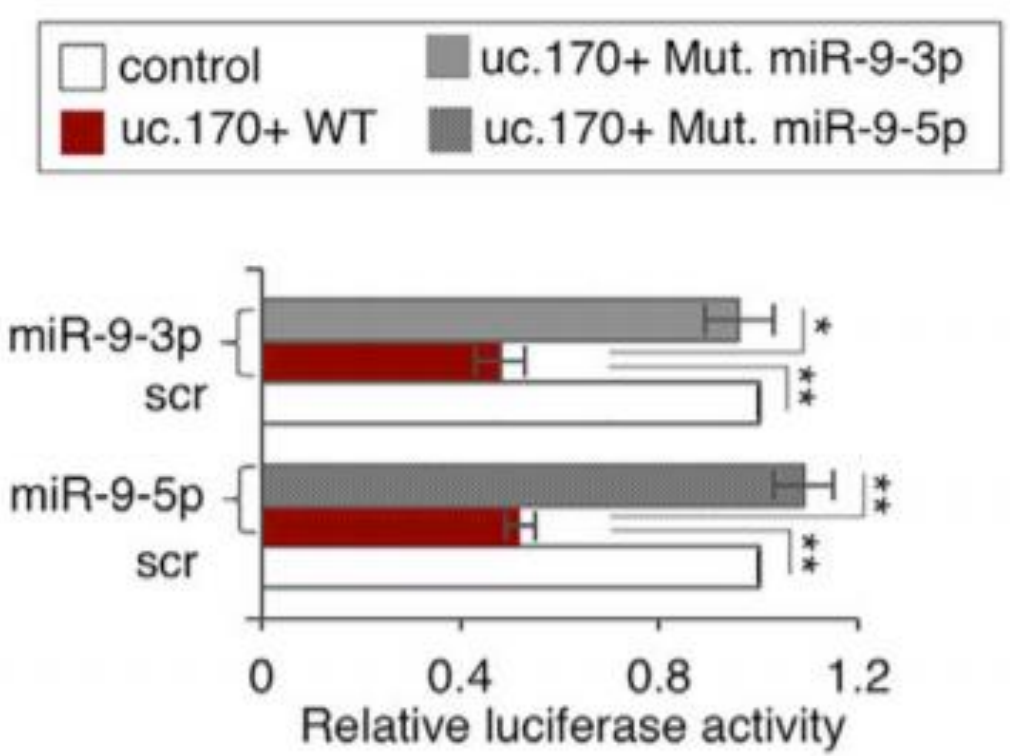
T-UCstem1 was expressed also in hESCs and it was downregulated upon neuronal differentiation

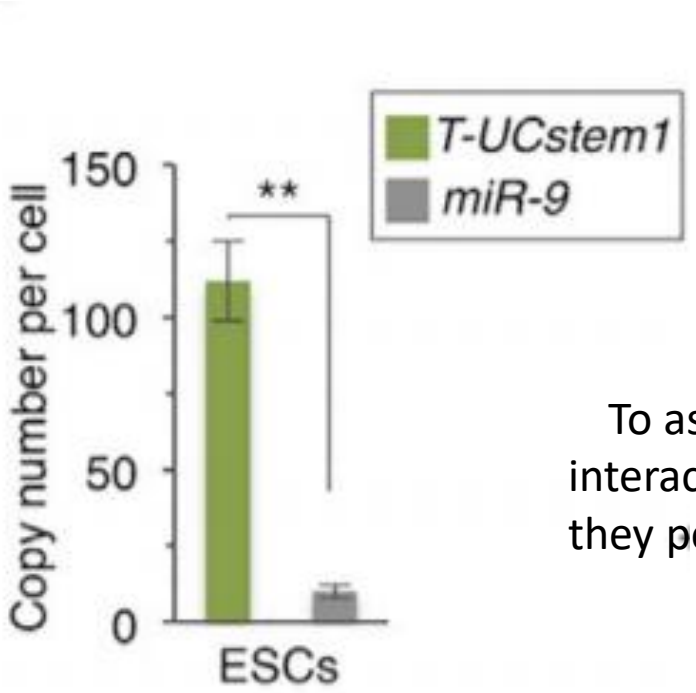




Secondary structure prediction

Direct and functional interaction of T-UCstem1 and miR-9



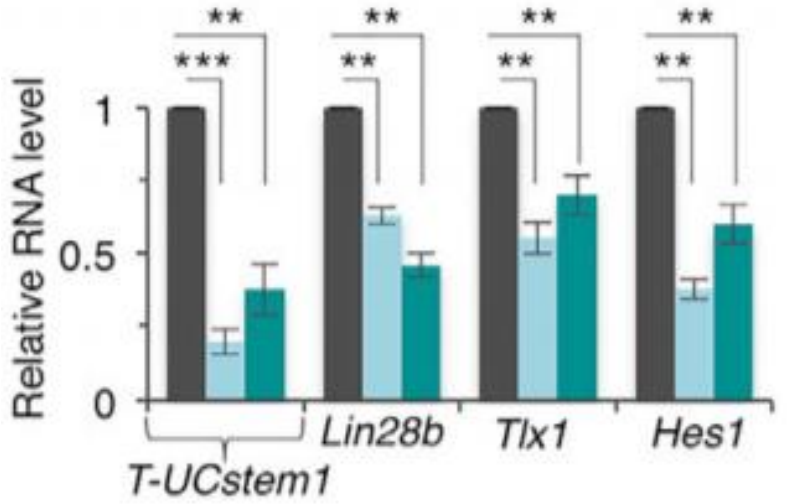


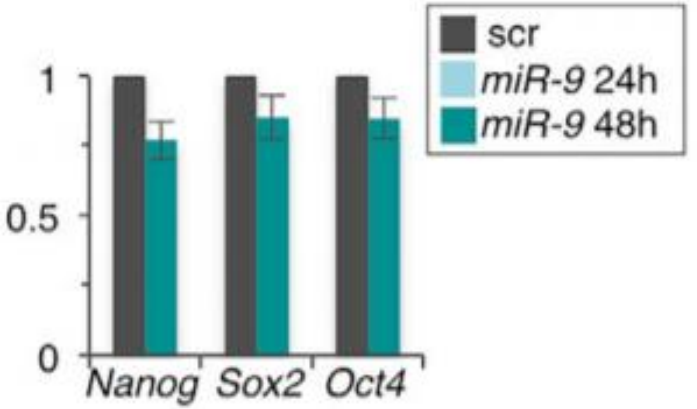
To assess whether there is a functional interaction between T-UCstem1 and miR-9 they performed a luciferase assay in **293FT**

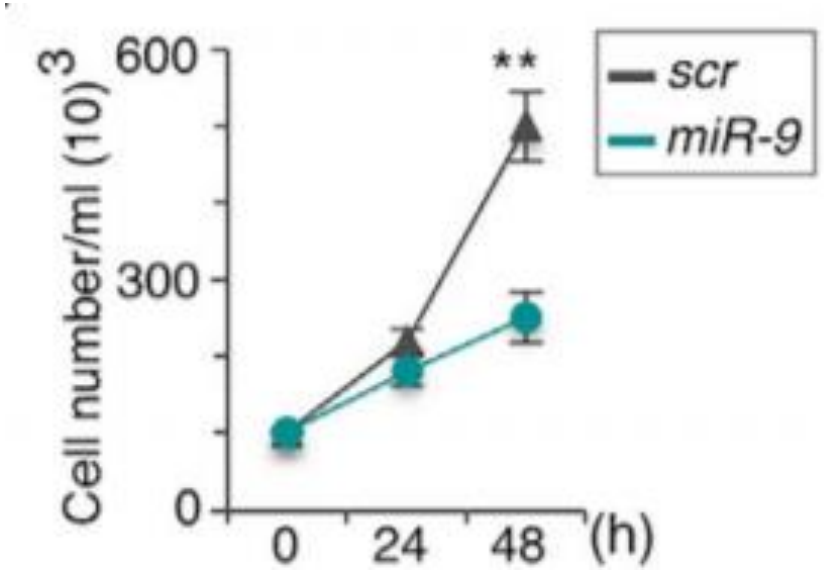
Thus support the idea that T-UCstem1 may be able to function as a sponge for miR-9

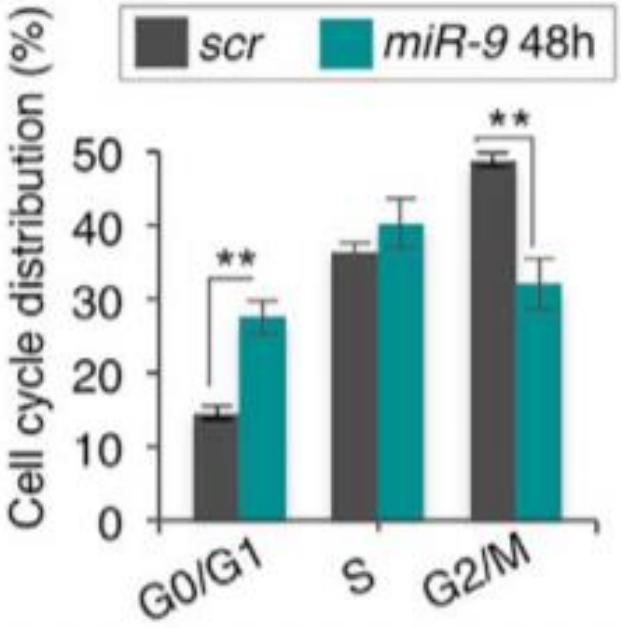
Direct and functional interaction of T-UCstem1 and miR-9

- They transfected ESCs with miR-9-3p/5p and assessed the expression of both T-UCstem1 and the miR-9 targets
- The expression of pluripotency genes was comparable
- Proliferation was reduced in miR-9 compared with scrambled-transfected ESCs
- Cell-cycle distribution analysis of miR-9-transfected ESCs showed a significant G1-phase accumulation



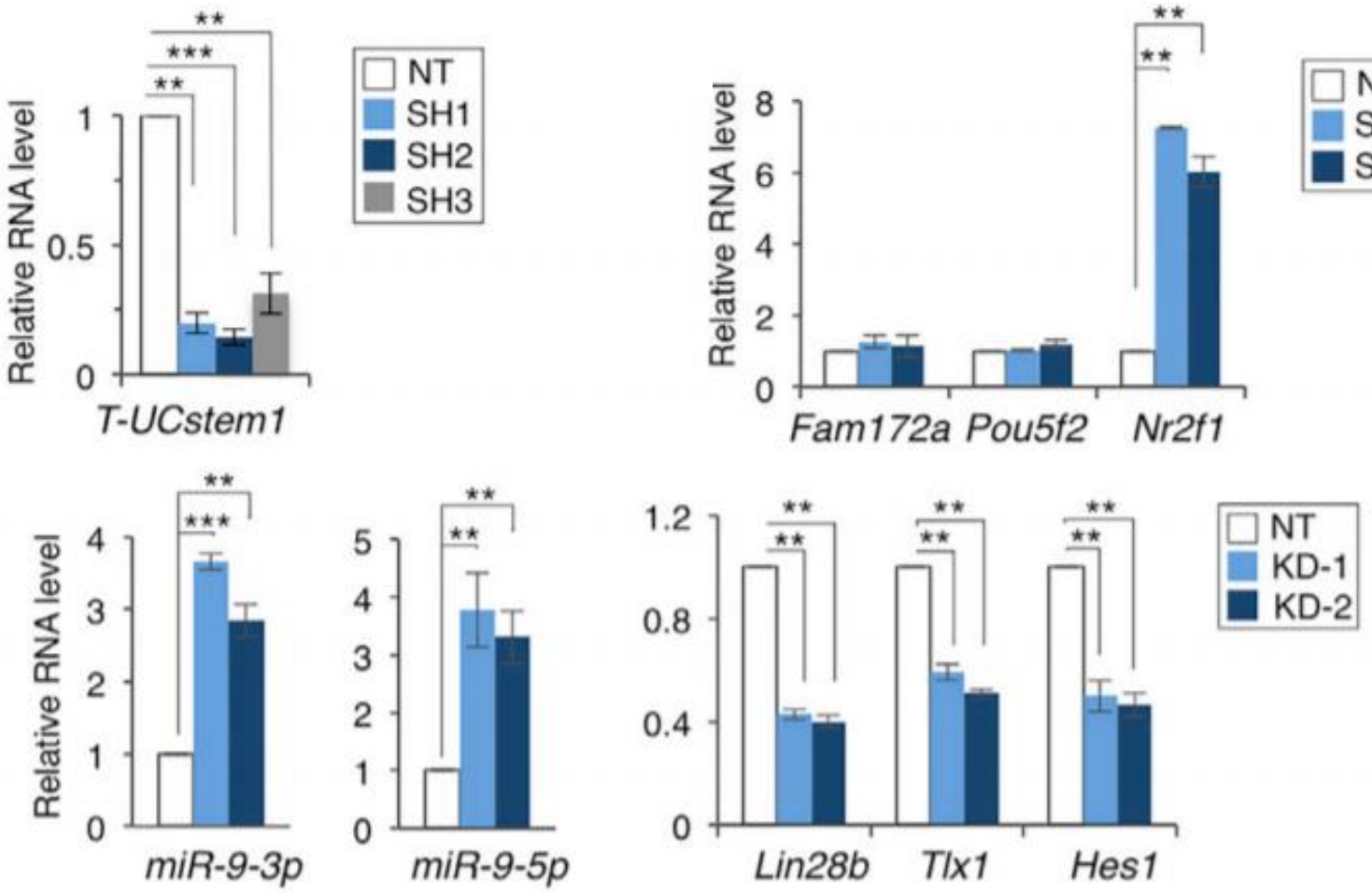






Increased miR-9 cellular levels affect ESC proliferation

Direct and functional interaction of T-UCstem1 and miR-9



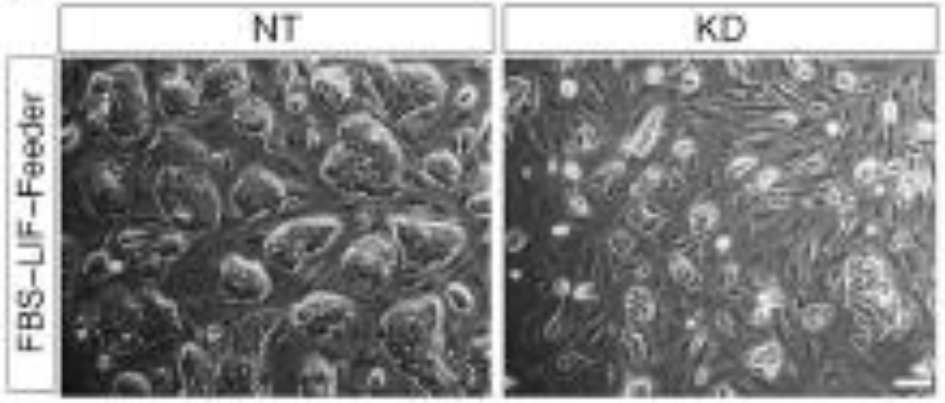
- shRNAs markedly reduced T-UCstem1 expression
- T-UCstem1 does not regulate the host gene mRNA levels
- A significant and consistent increase of both miR-9 mature forms upon T-UCstem1 KD
- Downregulation of the miR-9 targets Lin28b, Tlx1, and Hes1

These findings provide evidence of a functional interplay between T-UCstem1 and miR-9 in ESCs

T-UCstem1 Controls ESC Proliferation by Modulating miR-9 Intracellular Levels

*Analysis of molecular and cellular features of **T-UCstem1 KD ESCs***

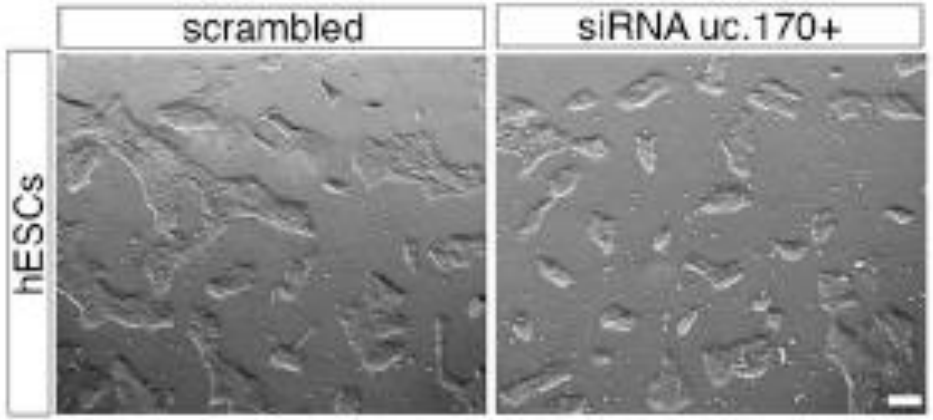
T-UCstem1 KD mESCs



T-UCstem1 KD mESCs Colonies:

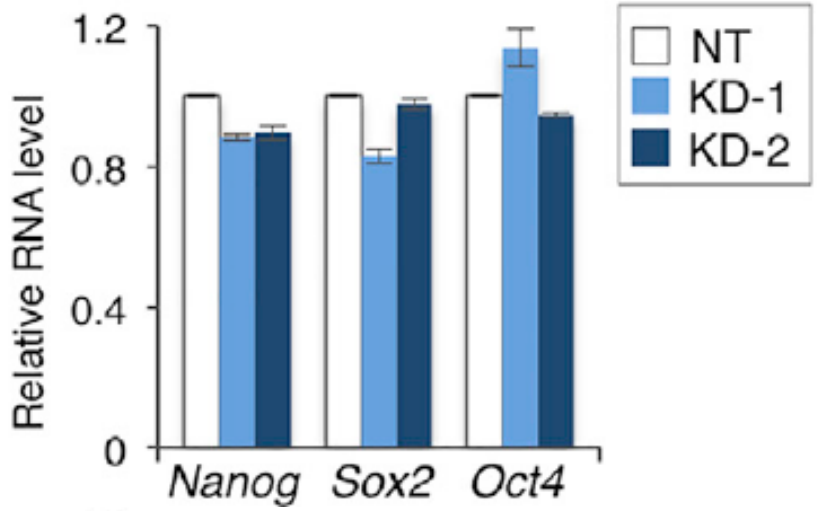
- Flat
- Disorganized
- Smaller

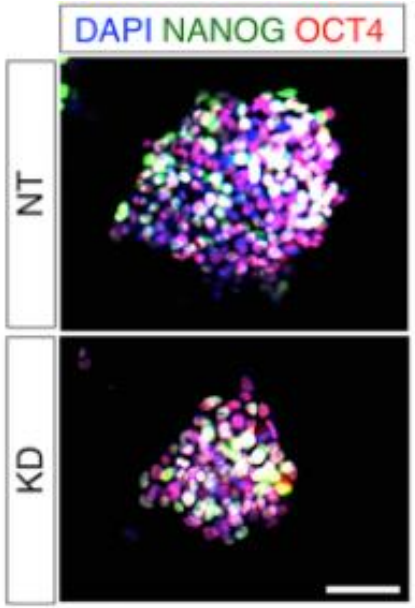
T-UCstem1 KD hESCs

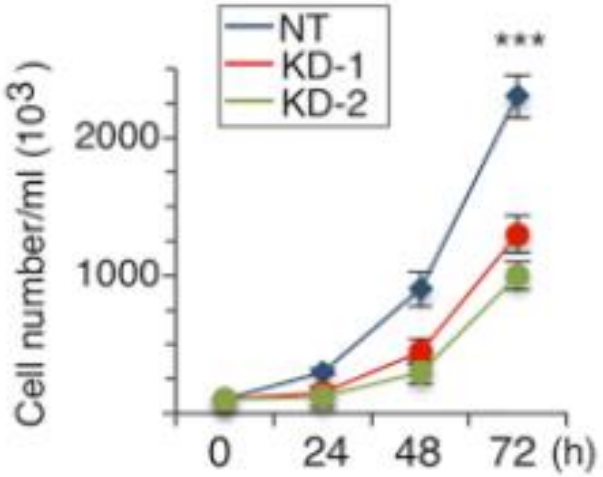


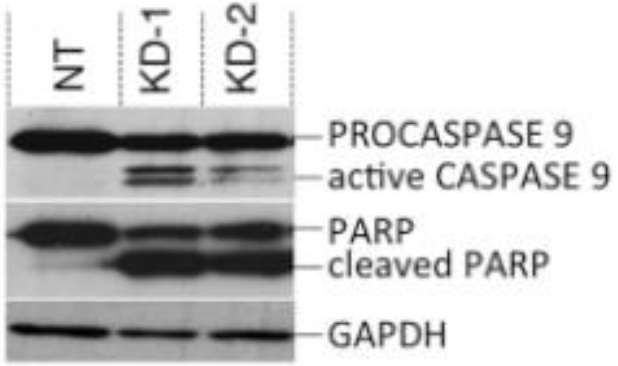
Suggesting a conserved role of T-UCstems1

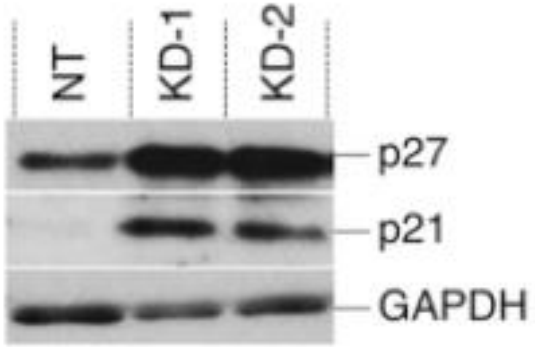
T-UCstem1 Controls ESC Proliferation by Modulating miR-9 Intracellular Levels







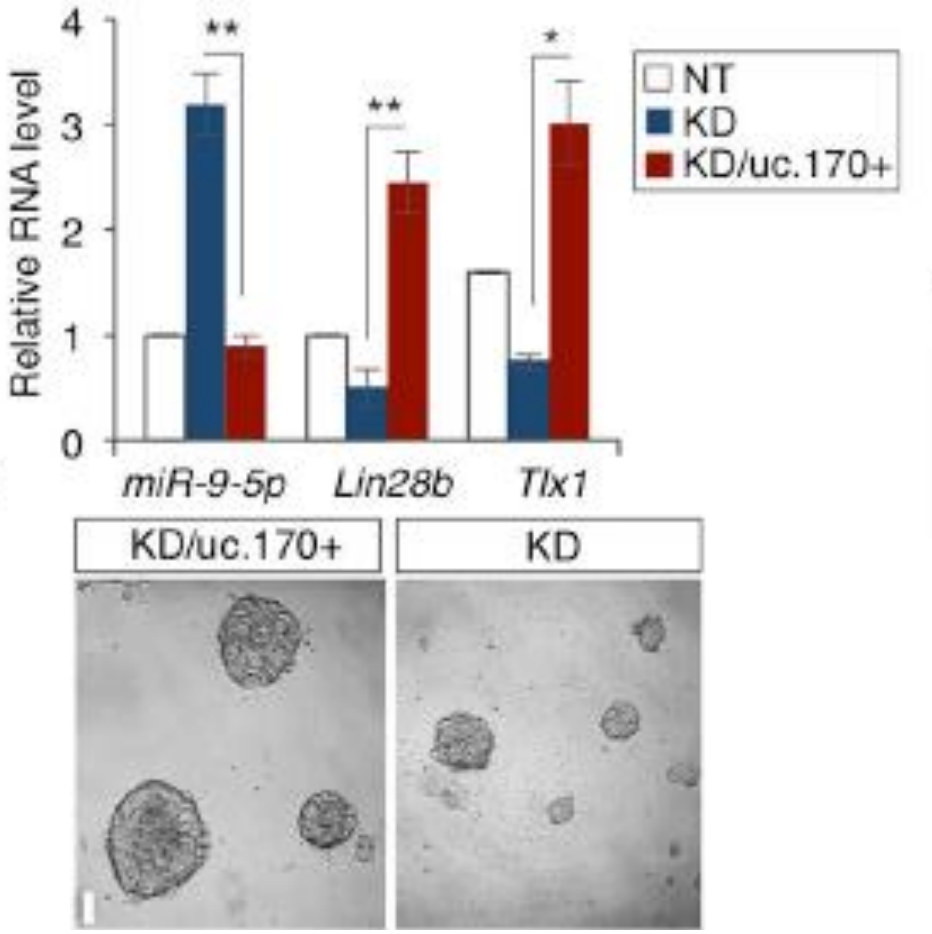


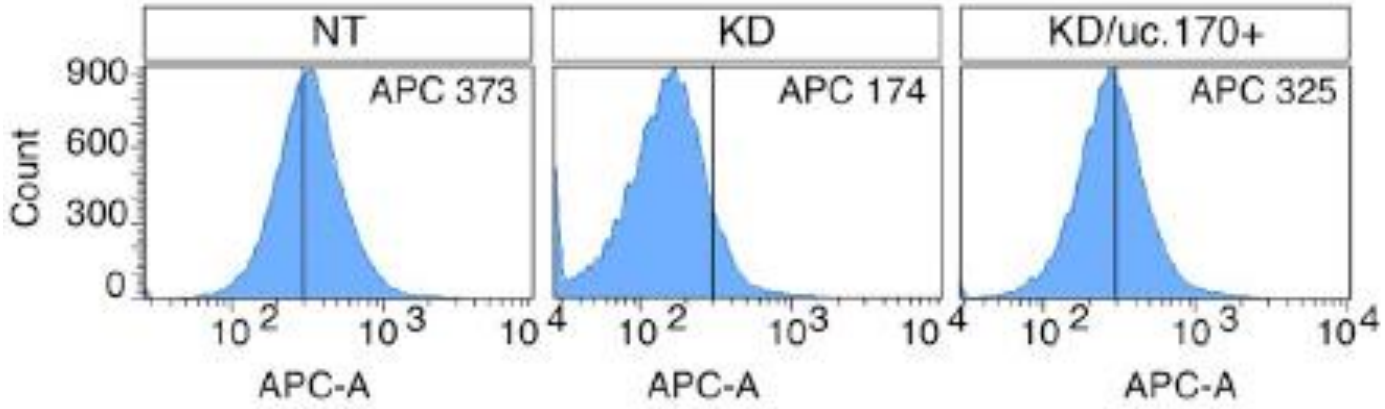


T-UCstem1 KD ESCs showed:

- Comparable levels of *Oct4-Sox2-Nanog;*
- Reduced proliferation rate;
- Reduced cell viability;
- Up-regulation of cell-cycle inhibitors **p27** and **p21**.

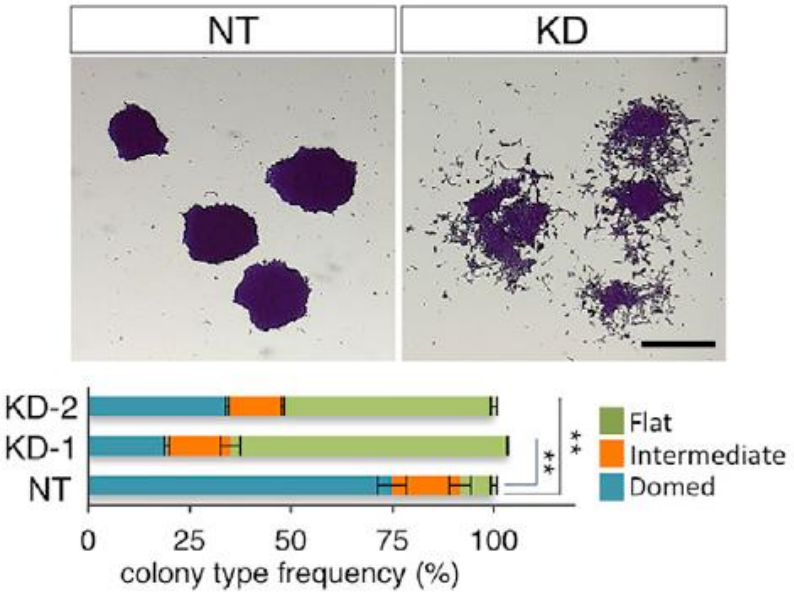
T-UCstem1 Controls ESC Proliferation by Modulating miR-9 Intracellular Levels



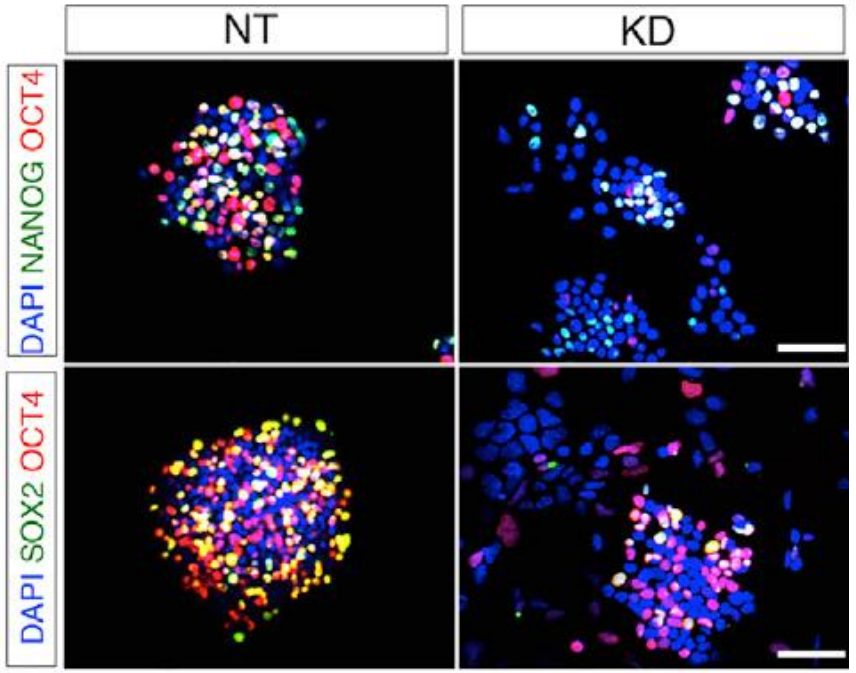


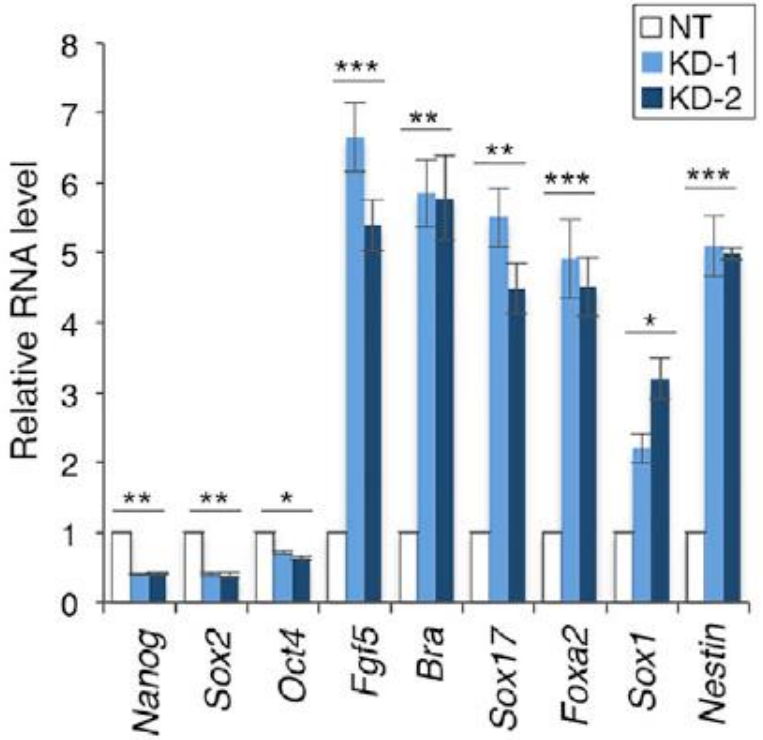
These data demonstrate that a T-UCstem1/miR-9 axis controls cell-cycle progression in ESCs.

T-UCstem1 Preserves ESC Self-Renewal Properties *In Vitro* and *In Vivo*



In less-permissive culture conditions (low density without feeders) **FBS/Lif,** T-UCstem1 KD ESCs rapidly exit pluripotency and undergo differentiation.

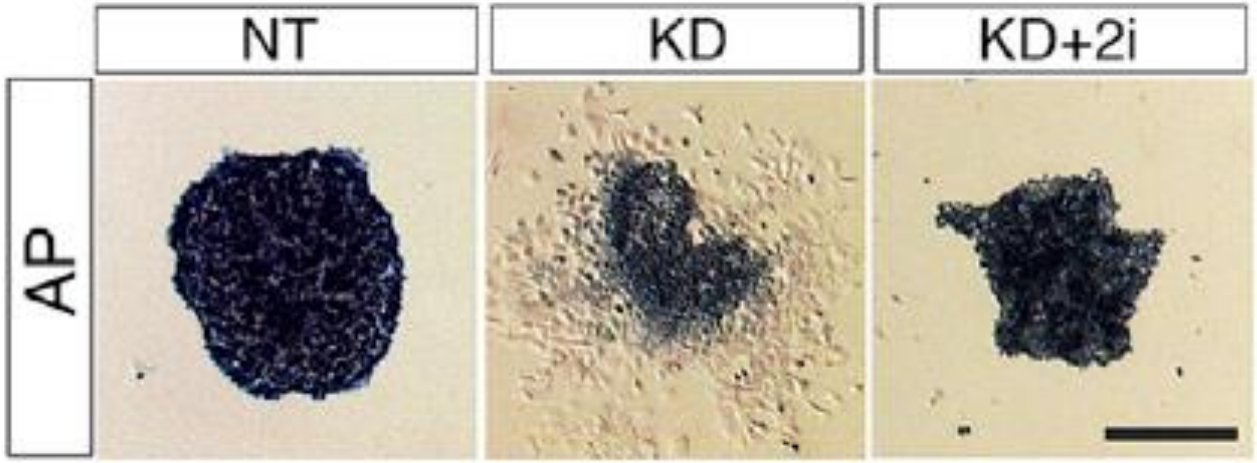


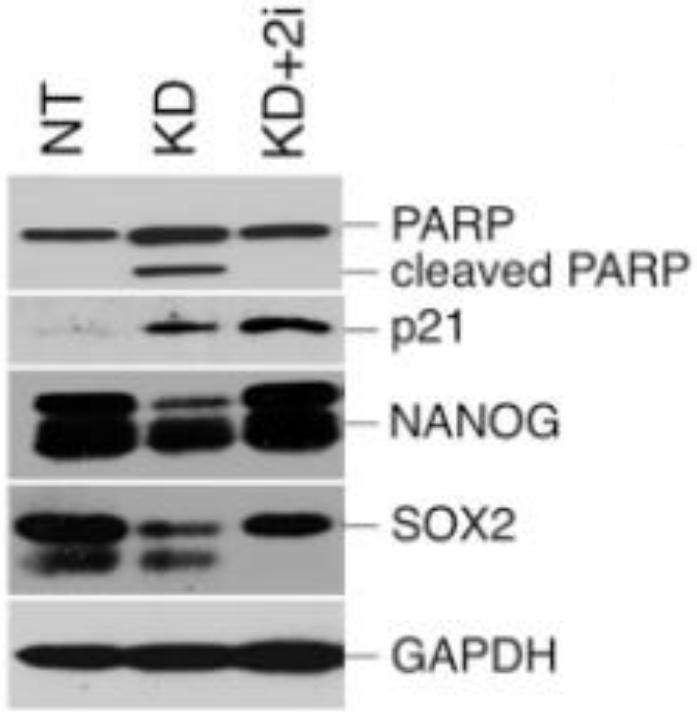


These findings provide evidence of a crucial role of T-UCstem1 in preserving ESC self-renewal and proliferation without affecting pluripotency.

T-UCstem1 Preserves ESC Self-Renewal Properties *In Vitro* and *In Vivo*

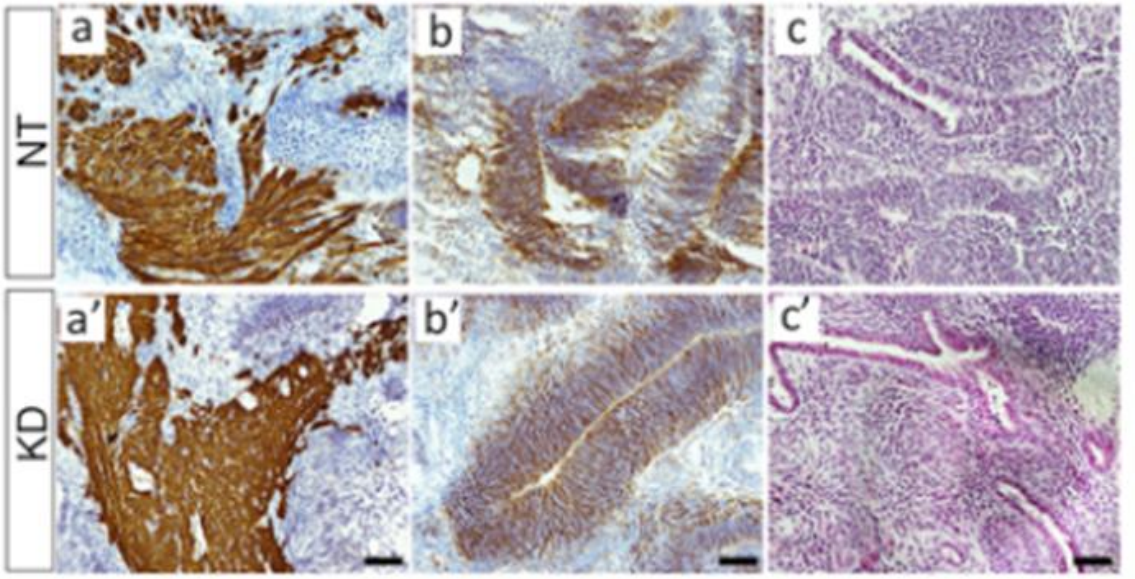
WT phenotype is rescued in 2i culture condition





Thus suggesting that different mechanisms control T-UCstem1-dependent regulation of ESC proliferation and self-renewal.

T-UCstem1 Preserves ESC Self-Renewal Properties *In Vitro* and *In Vivo*

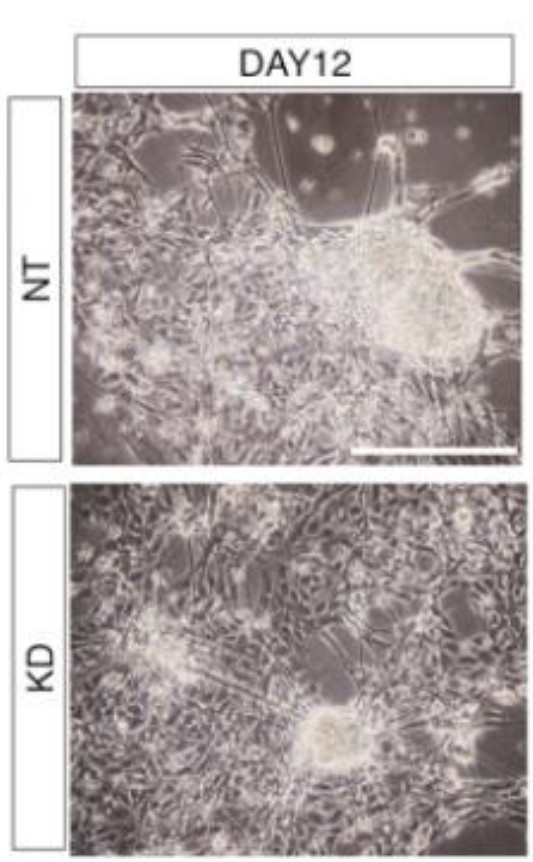


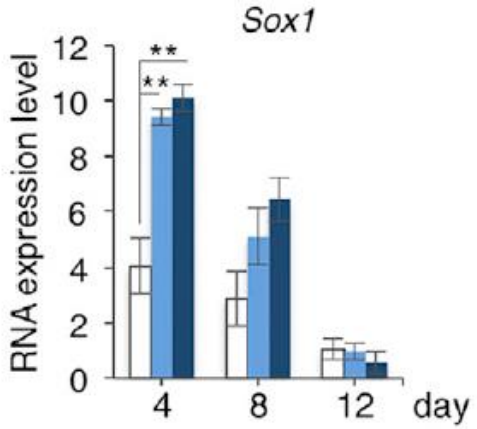
T-UCstem1 KD ESCs generated teratomas smaller in size compared with control, but not different in histological composition.

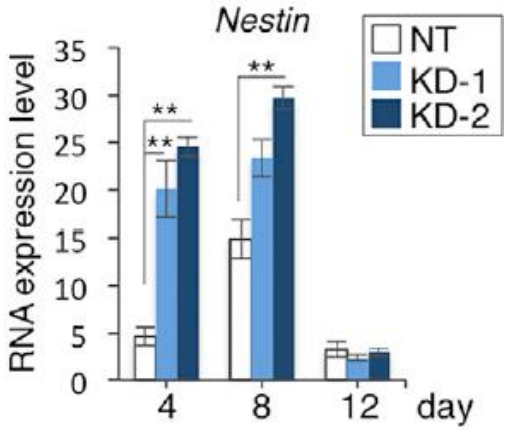
T-UCstem1 KD ESCs maintain pluripotency, but not proliferation rate

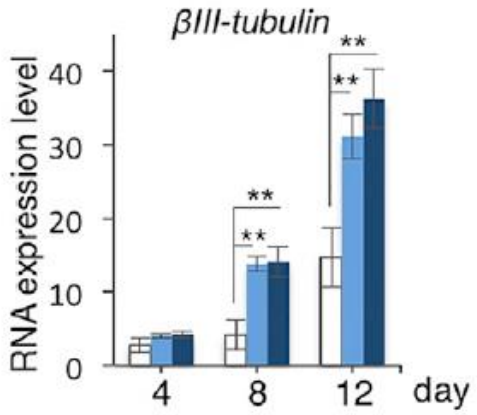
T-UCstem1 Silencing Accelerates and Enhances ESC Differentiation

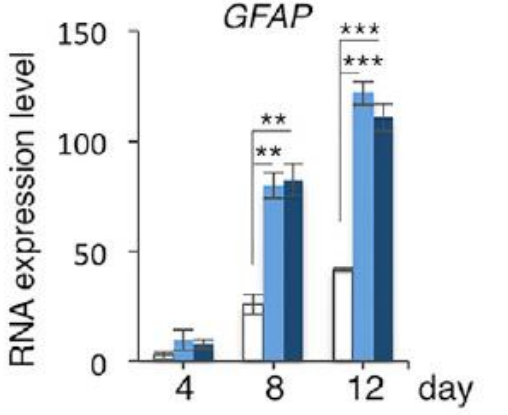
Analysis of ESC neural differentiation

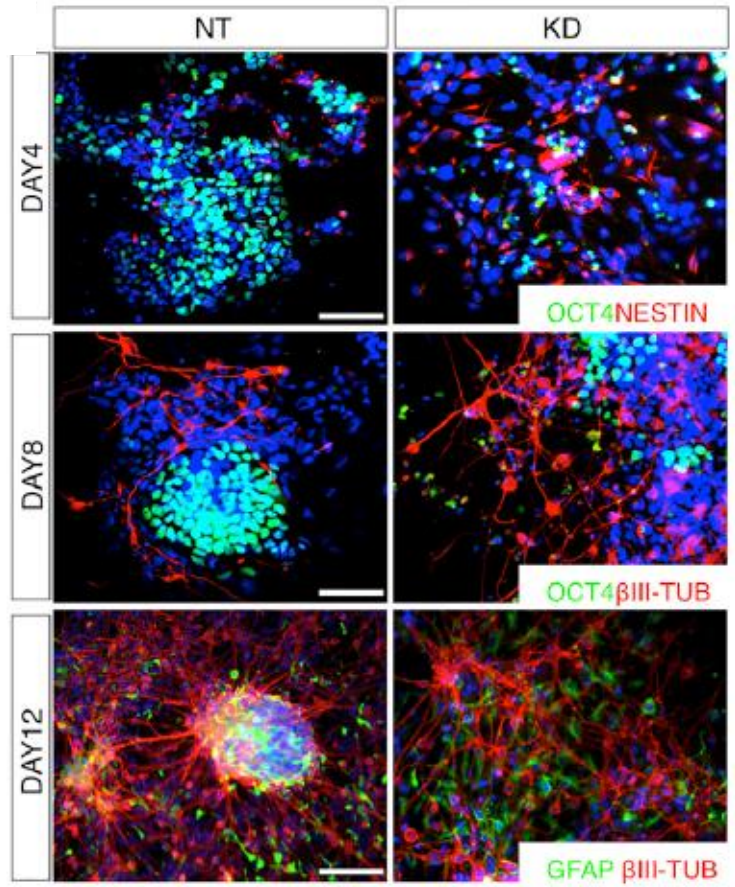








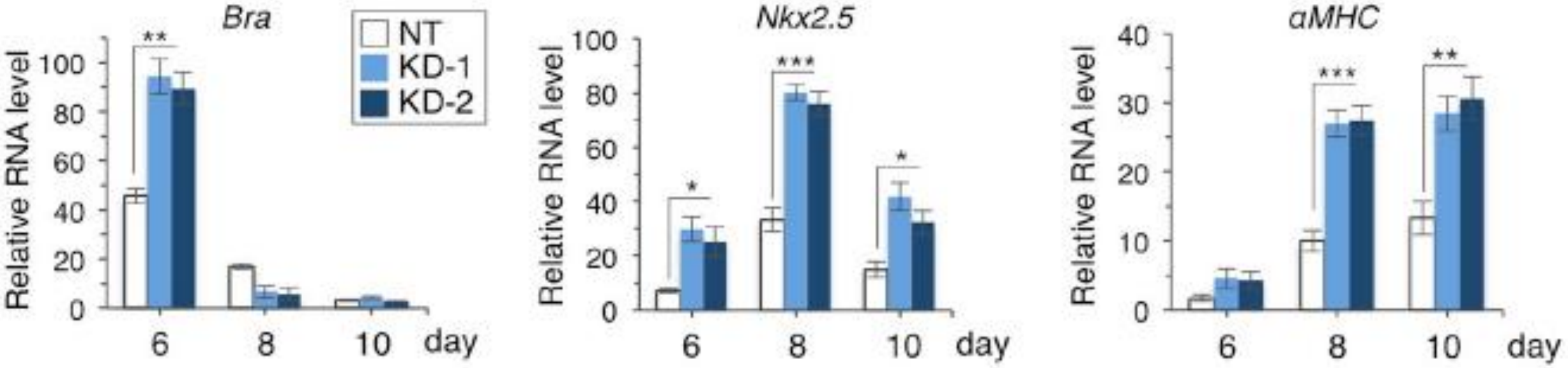




ESC differentiation was accelerated and was more efficient upon T-UC stem1 KD ESCs

T-UCstem1 Silencing Accelerates and Enhances ESC Differentiation

Analysis of ESC cardiac differentiation

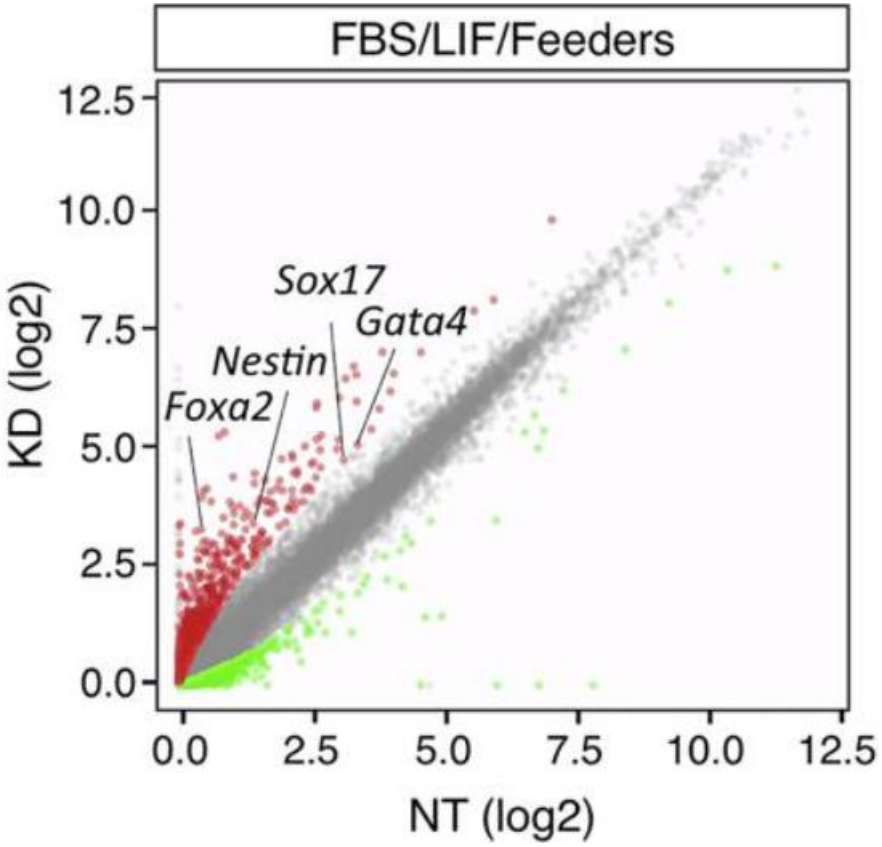


Suggesting that also cardiac specification and differentiation were enhanced and accelerated

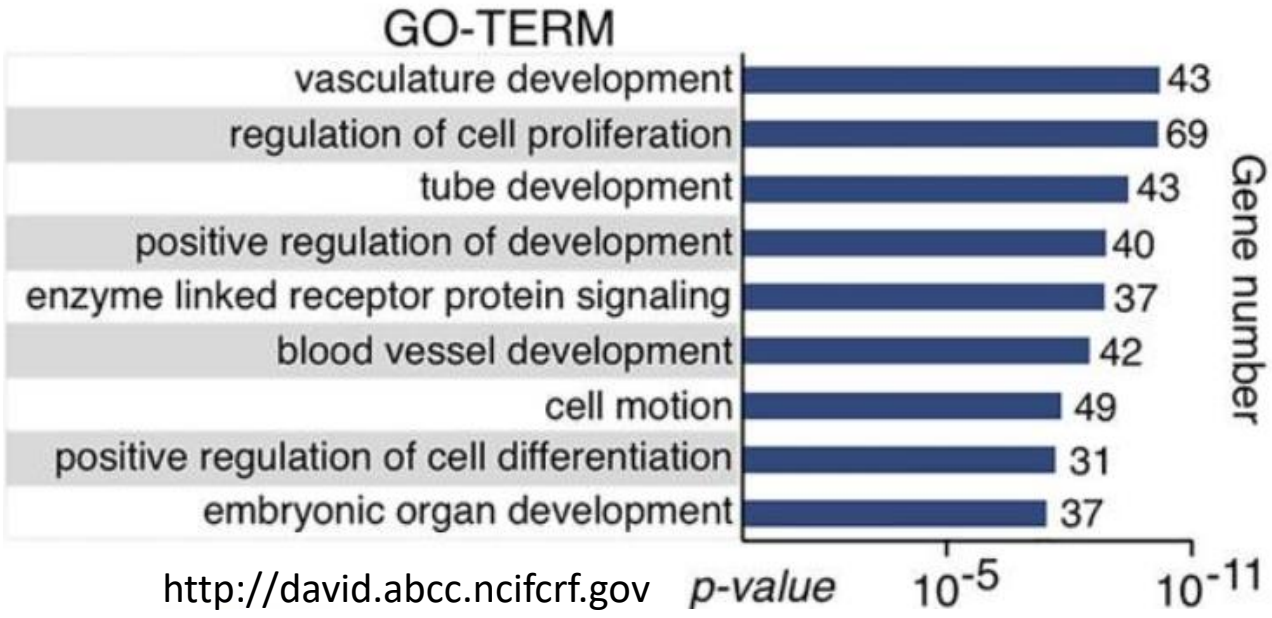
All these results indicate that T-UCstem1 is required to regulate ESC differentiation

T-UCstem1 Preserves the Transcriptional Dynamics of ESCs by Stabilizing PRC2 Complex

*Comparison of **RNA sequencing (RNA-seq)** transcriptome profiling between **T-UCstem1 KD** and **Control ESCs***

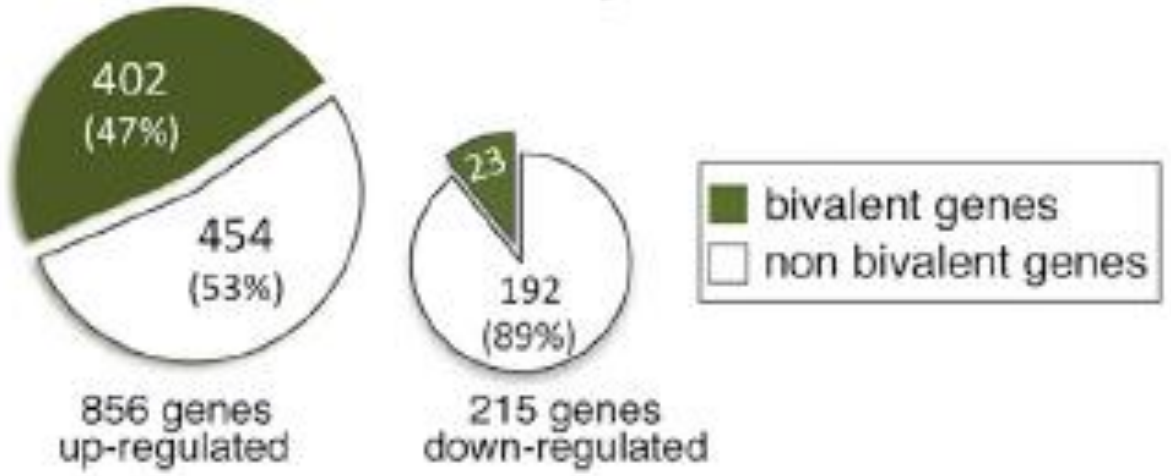


Scatterplot of RNA-seq data shows more then 1,000 differentially expressed genes in **T-UCstem1 KD (KD)** versus **Control (NT) ESCs.**



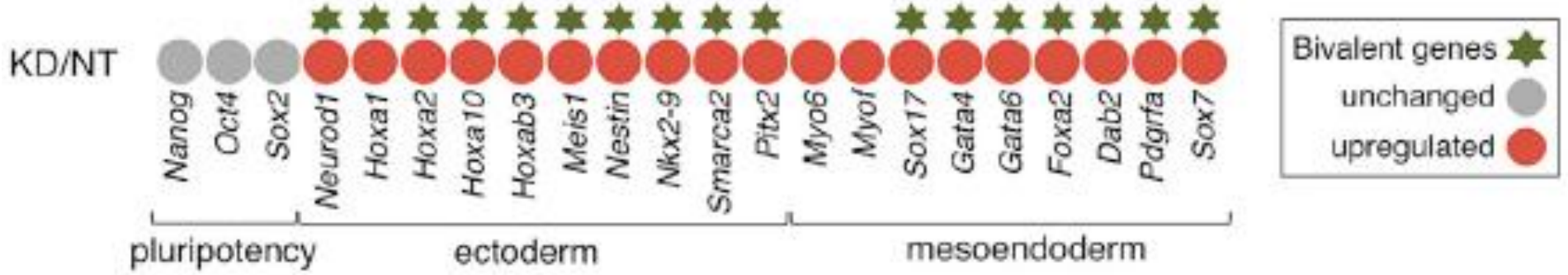
http://david.abcc.ncifcrf.gov

T-UCstem1 Preserves the Transcriptional Dynamics of ESCs by Stabilizing PRC2 Complex



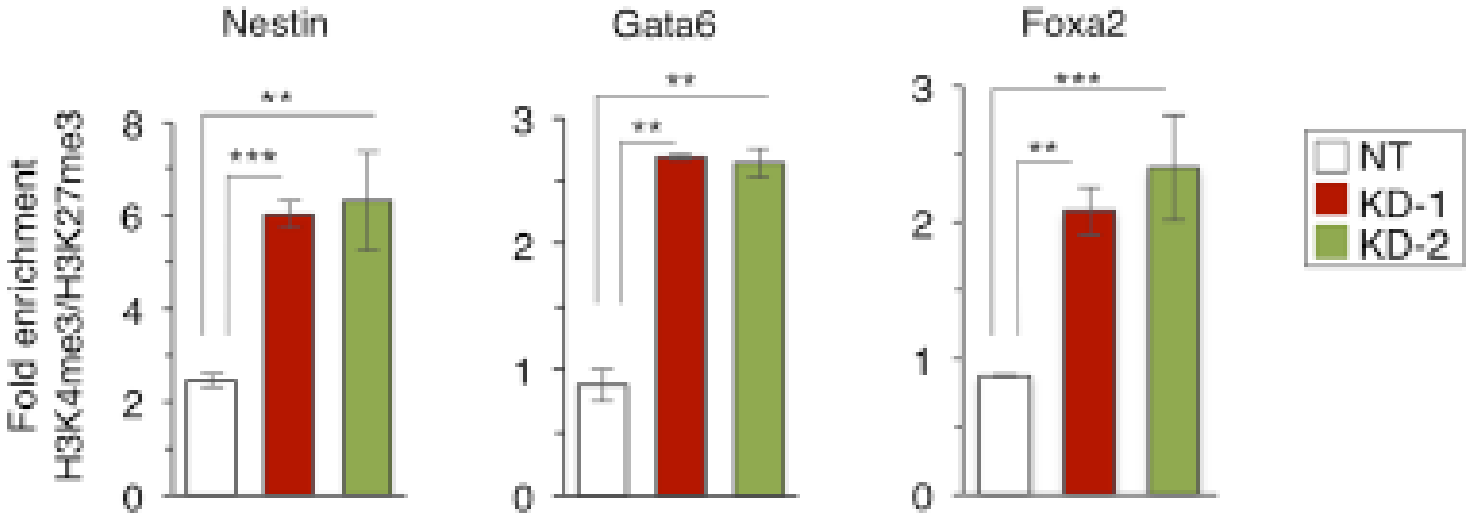
≈70% of genes were **upregulated**

≈50% were associated with bivalent chromatin domains.

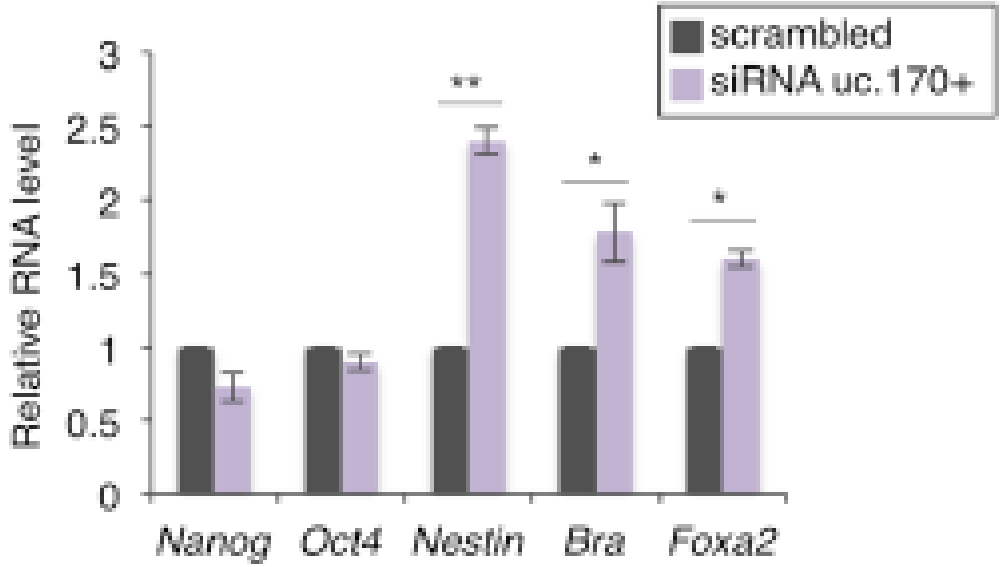


T-UCstem1 Preserves the Transcriptional Dynamics of ESCs by Stabilizing PRC2 Complex

*Analysis the status of **H3K4me3** and **H3K27me3** at the bivalent domains of **Nestin**, **Foxa2**, and **Gata6** genes in **T-UCstem1 KD** and **Control ESCs** by **ChIP analysis***



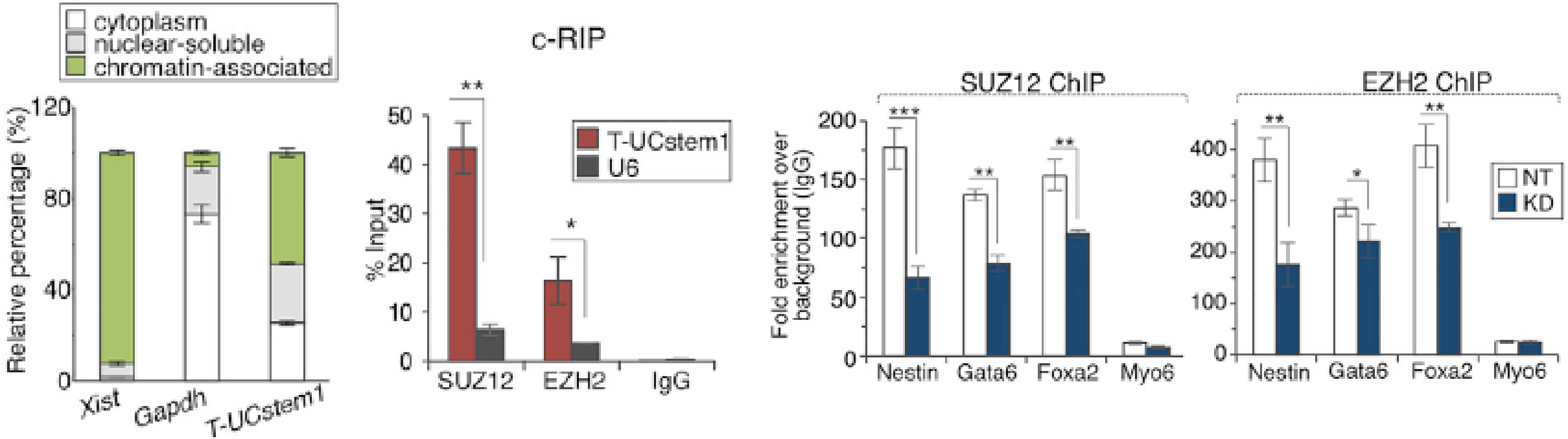
H3K4me3/H3K27me3 ratio significantly increased in T-UCstem1 KD ESCs at the promoter of these representative bivalent genes of the three germ layers.



T-UCstem1 function may be conserved in humans

T-UCstem1 Preserves the Transcriptional Dynamics of ESCs by Stabilizing PRC2 Complex

*Demonstration that T-UCstem1 could directly interact with the **PRC2** and regulates **bivalent gene expression***



These data point to a key role of T-UCstem1 in maintaining ESC transcriptional identity by protecting the epigenetic status of key developmental regulatory genes, stabilizing PRC2 on their bivalent domains.

Conclusions

T-UCstem1 exerts a dual function in ESCs:

1. it controls ESC proliferation by regulating miR-9/Lin28b cellular levels in the cytoplasm;
2. it maintains ESC transcriptional dynamics and self-renewal through PRC2 stabilization in the nucleus without effect on pluripotency.

Thanks for listening!

…and we apologize for boredom!!!