

# The evolution of the regulatory landscape of *FOXP2*: comparative characterization of two functional enhancers in extant vertebrates and extinct hominins

ANTONIO BENÍTEZ-BURRACO<sup>1,2</sup>, RAÚL TORRES-RUIZ<sup>3</sup>, MARTA MARTÍNEZ-LAGE<sup>3</sup>,

SANDRA RODRÍGUEZ-PERALES<sup>3</sup>, AND PALOMA GARCÍA-BELLIDO<sup>2,4</sup>

1. Department of Philology, University of Huelva, Huelva, Spain ✉ [antonio.benitez@dfesp.uhu.es](mailto:antonio.benitez@dfesp.uhu.es)

2. Faculty of Modern Languages, University of Oxford, Oxford, United Kingdom

3. Molecular Cytogenetics Group. Centro Nacional Investigaciones Oncológicas (CNIO), Madrid, Spain.

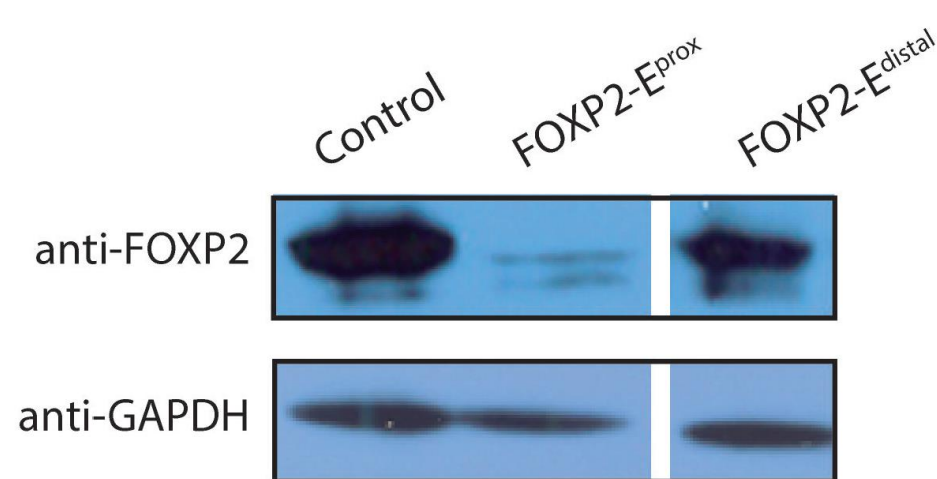
4. Faculty of Linguistics, Philology and Phonetics, University of Oxford, Oxford, United Kingdom.



## ① AIM

Shedding light on possible regulatory differences of two enhancers of *FOXP2*

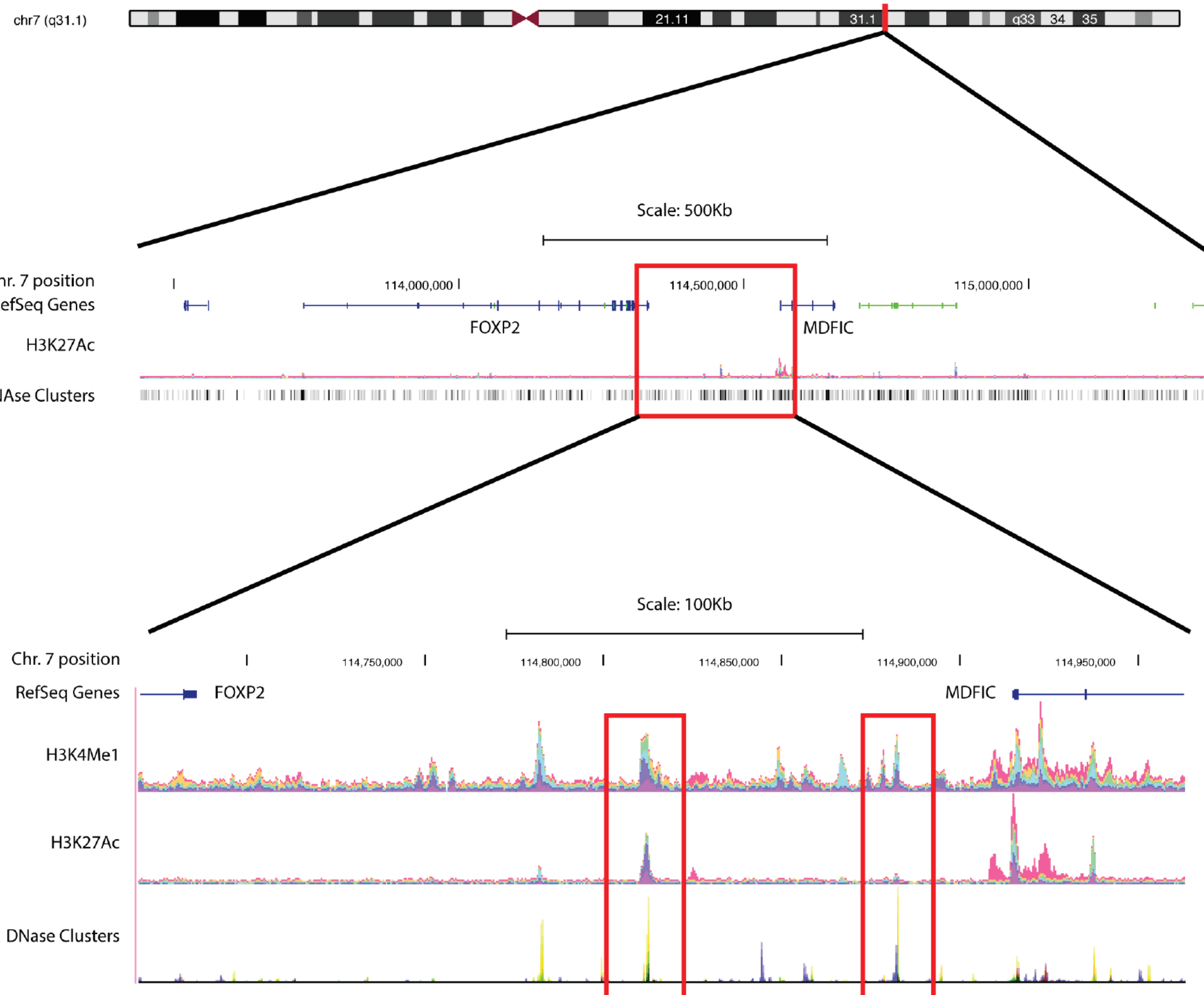
- Located downstream the gene
- Their deletion by CRISPR/Cas9 in a neuronal cell line downregulates *FOXP2* and decreases *FOXP2* protein levels.



## ② HOW

*In silico* analysis

- Looking for sequence differences with species in which vocalization has been studied in the context of language evolution (songbirds, cetaceans, bats, primates, rodents)
- Looking for sequence differences with extinct hominins (Neanderthals and Denisovans)
- Putting the focus on potential binding factors as revealed by ChIP-seq



**FOXP2**

- Involved in language development
- Involved in sensorimotor learning
- No differences with extinct hominins in the coding region
- Expected differences in expression levels/patterns (e.g. binding site for POU3F2)

### Proximal enhancer

**Positions diverse in Denisovans**

Position (hg19)	Position in enhancer	Human	Denisovan
114458274	1402	A	G
114459196	2324	A	G
114459947	3075	C	T
114460158	3286	T	G
114461593	4721	A	T
114462035	5163	A	G

**Positions diverse in Neanderthals**

Position (hg19)	Position in enhancer	Human	Neanderthal
114459001	2129	G	T
114460158	3286	T	G
114460788	3916	A	G
114461593	4721	A	T
114462035	5163	A	G

Transcription Factor ChIP-seq (161 factors) from ENCODE with Factorbook Motifs

### Distal enhancer

**Position diverse in Denisovan**

No diverse position

**Position diverse in Neanderthals**

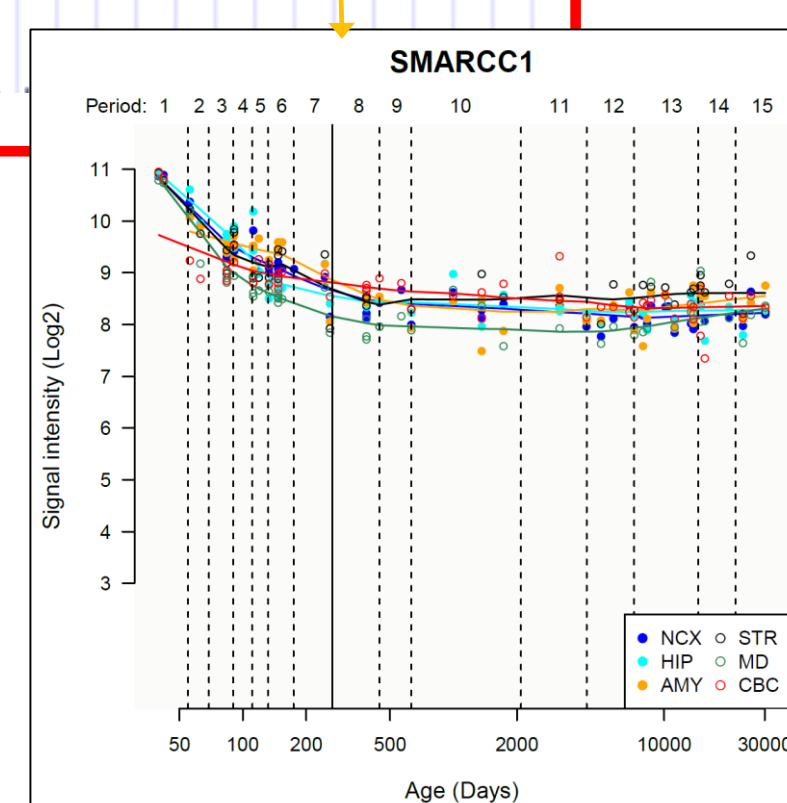
Position (hg19)	Position in enhancer	Human	Neanderthal
114541580	211	A	G
114542390	1021	G	C

Transcription Factor ChIP-seq (161 factors) from ENCODE with Factorbook Motifs

## ③ RESULTS

Enhancer 1 (proximal to *FOXP2*) is worth analyzing in detail, because:

- It exhibits a stronger enhancer activity
- It is conserved in primates and cetaceans, but not in rodents or songbirds
- It shows sequence differences with both Neanderthals and Denisovans
- One change might affect the binding site for one transcriptional factor involved in brain development



HBT Human Brain Transcriptome

### SMARCC1

- Part of the large ATP-dependent chromatin remodeling complex SNF/SWI
- Involved in forebrain development
- Modulates the expression of Pax6 target genes
- Involved in vitamin D-coupled transcription regulation (*Foxp2* expression in the brain is significantly altered by vitamin D deficiency)

### FUNDING

This research was supported by funds from The University of Oxford/John Fell OUP Research (Grant 121/435 to Paloma Garcia-Bellido), the Spanish National Research and Development Plan, Instituto de Salud Carlos III, and FEDER (FIS project no. PI14/01884 to Sandra Rodriguez-Perales), and the Spanish Ministry of Economy and Competitiveness (grant numbers FFI2014-61888-EXP and FFI2016-78034-C2-2-P to Antonio Benítez-Burraco).

### ACKNOWLEDGEMENT

We thank Carles Lalueza-Fox and Pere Gelabert Xirinachs (UPF, Spain) for providing the sequences of the two enhancers in Neanderthals and Denisovans