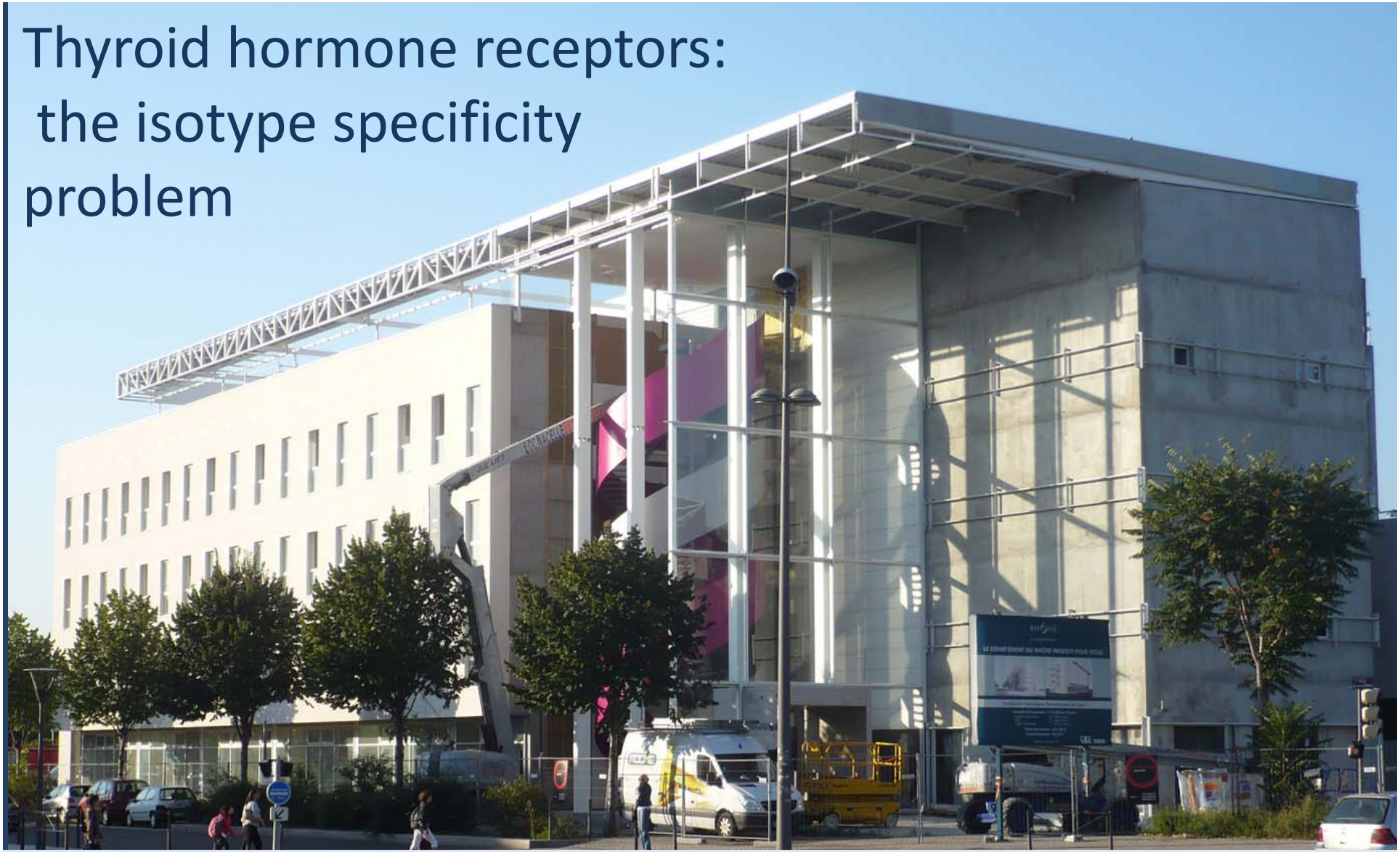
Thyroid hormone receptors: the isotype specificity problem



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PRESENTATION FROM THE 83rd ANNUAL MEETING OF THE AMERICAN THYROID ASSOCIATION, OCTOBER 16-20, 2013 (Frédéric Flamant)

Learning objective:
Finding two good reasons to study TRα1 and TRβ1/2

Hint1) Receptor-selective ligands would be useful.

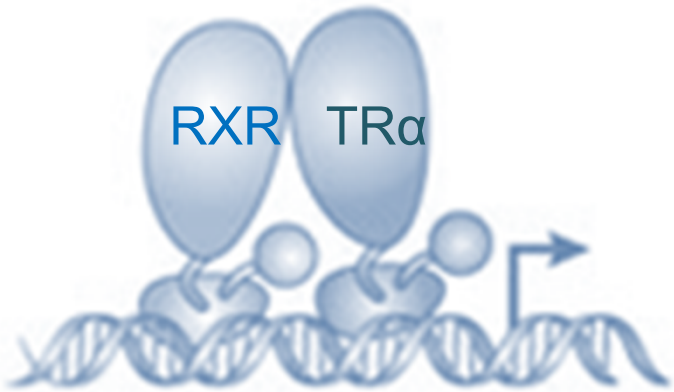
Hint 2) There are now two genetic diseases: RTHα and RTHβ

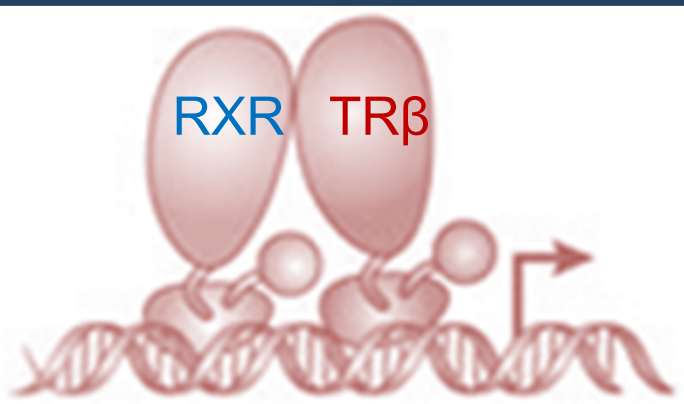
500 My ago: The ancestral TR gene duplication and its consequences.

1) Redundancy: Provides robustness to thyroid hormone signaling

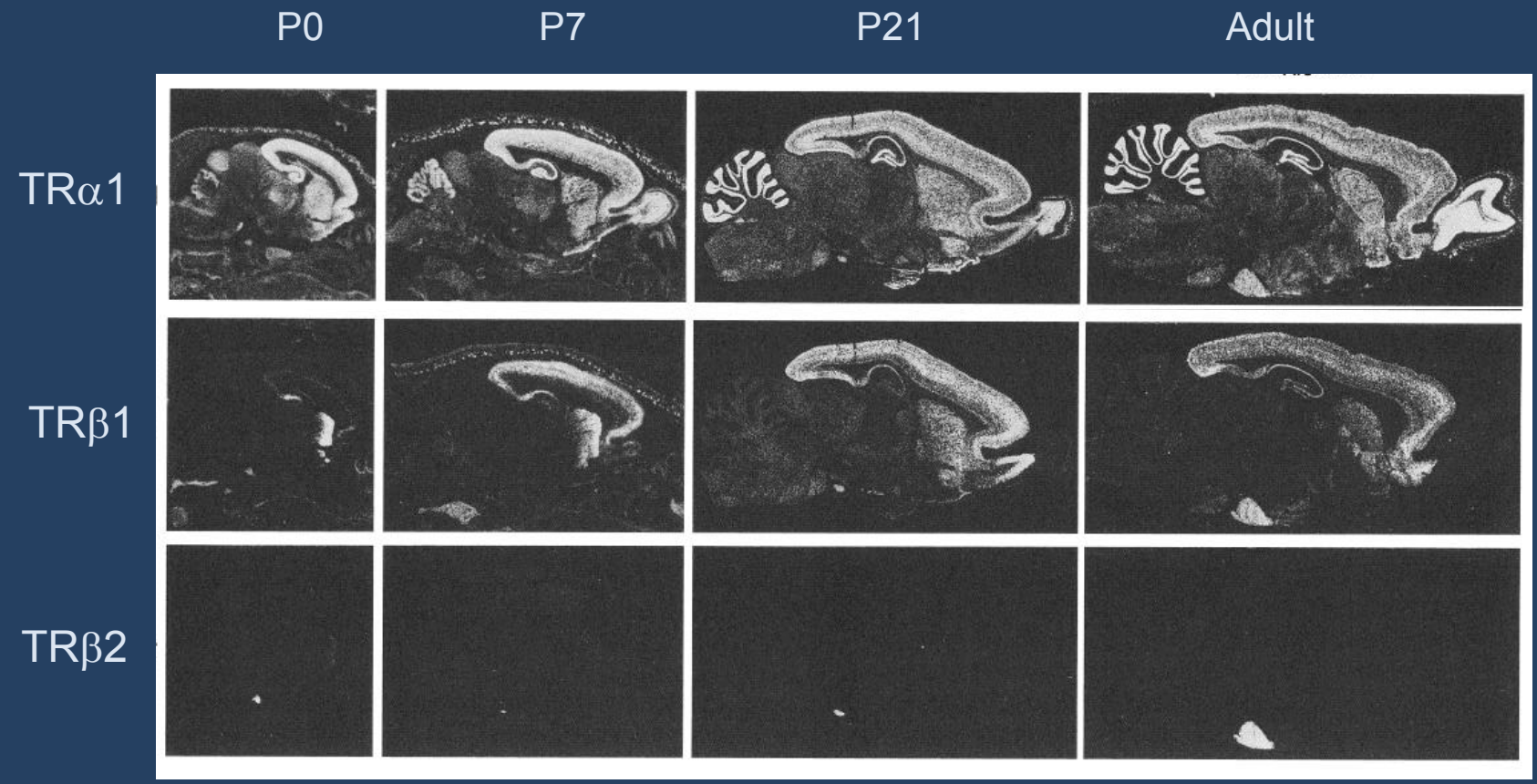
1) Subfunctionalization: changes in expression patterns distributes TR function to two proteins.

2) Neofunctionalization: due to changes in coding sequences one TR can gain new properties.



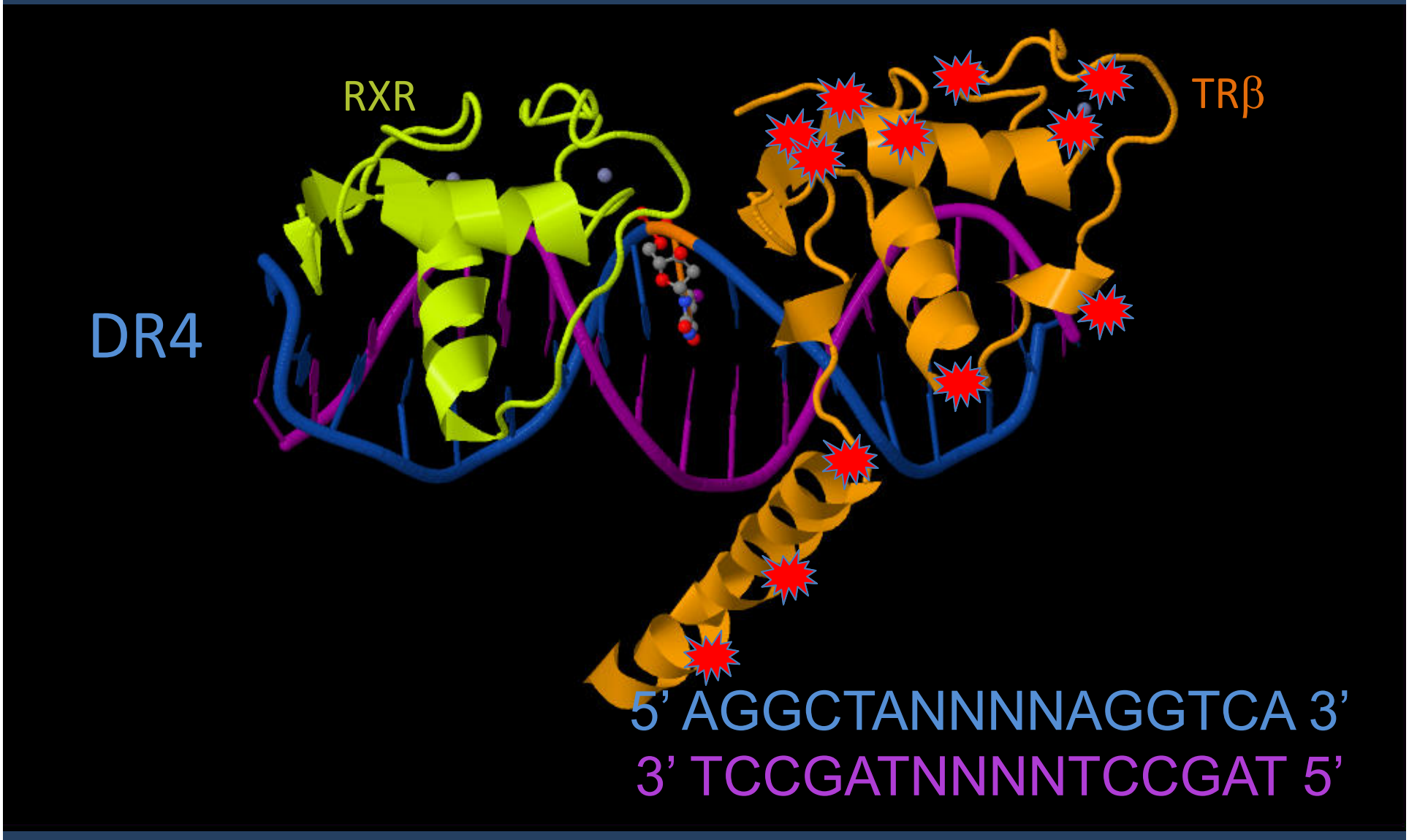


TRα1 is the predominant receptor in brain.



Bradley et al., PNAS 1992

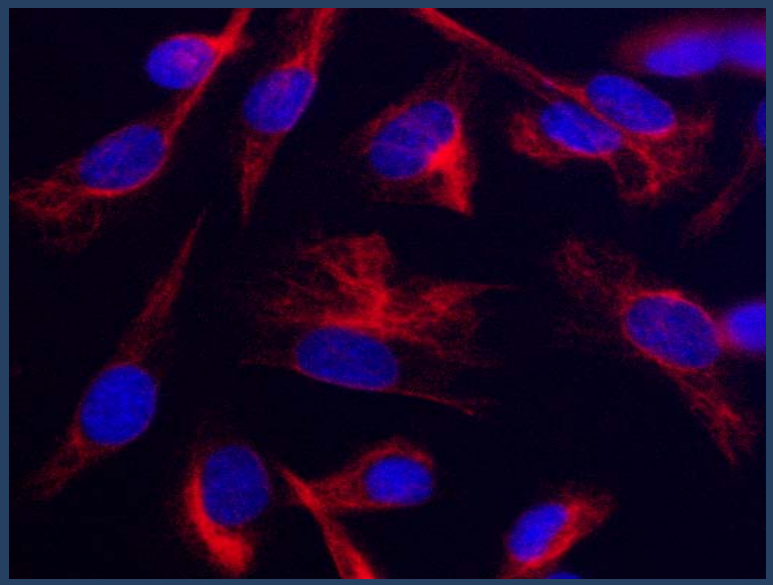
Changes in amino-acids sequence suggest subtle differences in the gene regulation properties of TRα1 and TRβ1/2



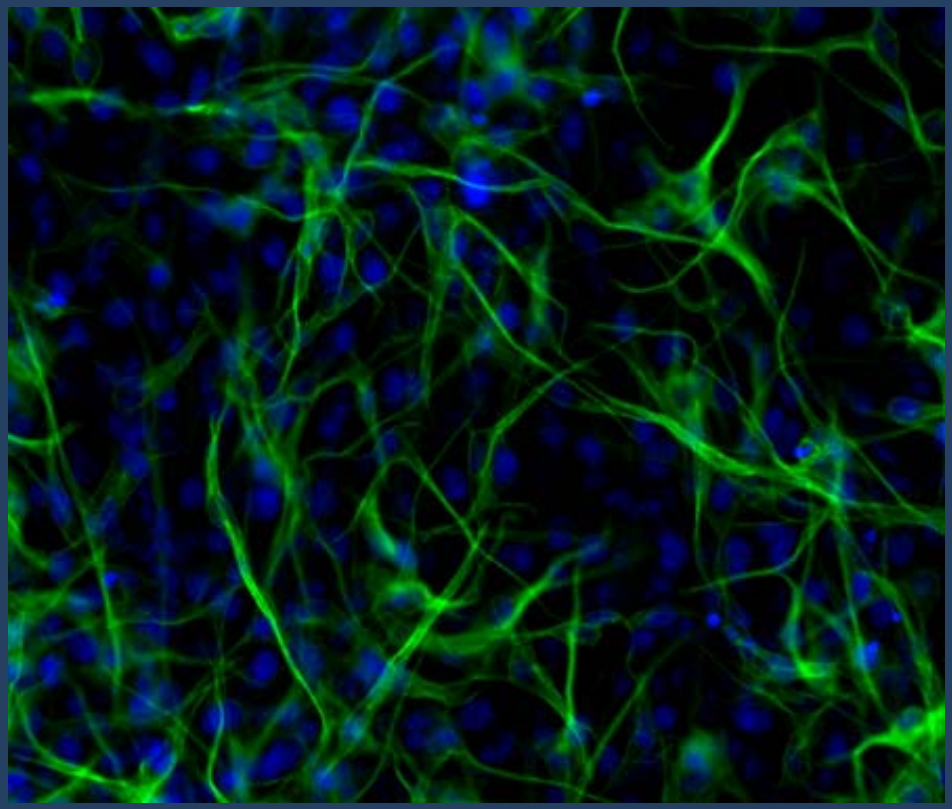
Are the properties of TRα1 and TRβ1 identical?

A global comparative analysis of TRα1 and TRβ1-mediated response to T3 in a neural cell line

C17.2 cells can differentiate into neuronal-like cells after serum deprivation

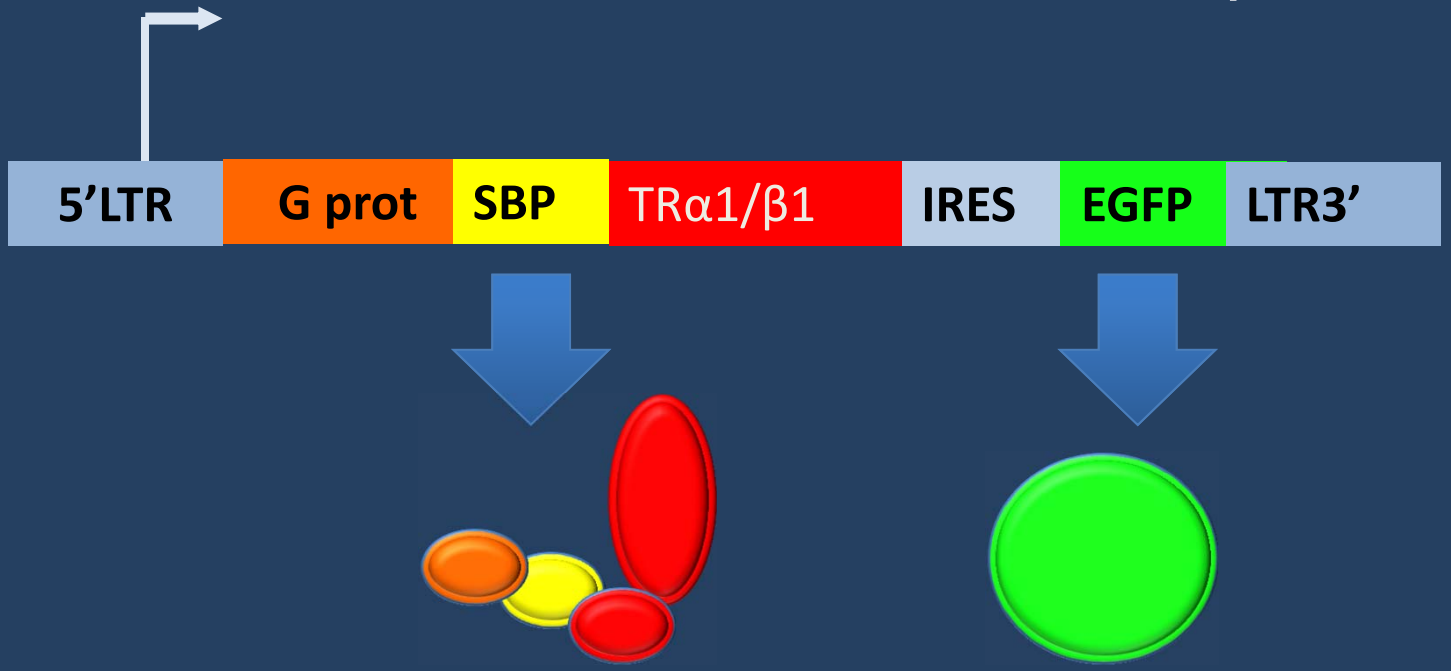


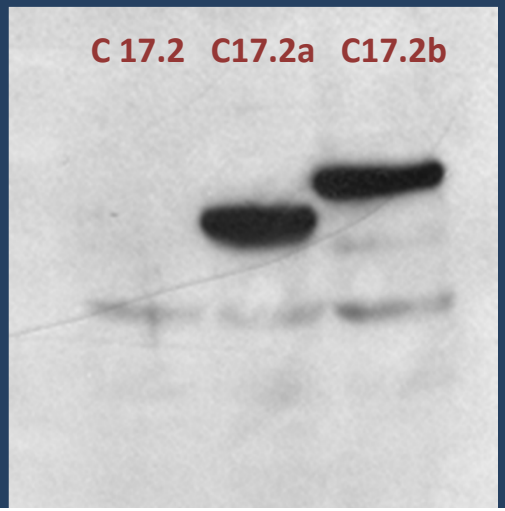
10% serum Nestin Immunostaining



0% Serum Tuj1 immunostaining

Stable expression of tagged receptors GSTRα1 or GSTRβ1 in C17.2 cells = C17.2α and C17.2β cells

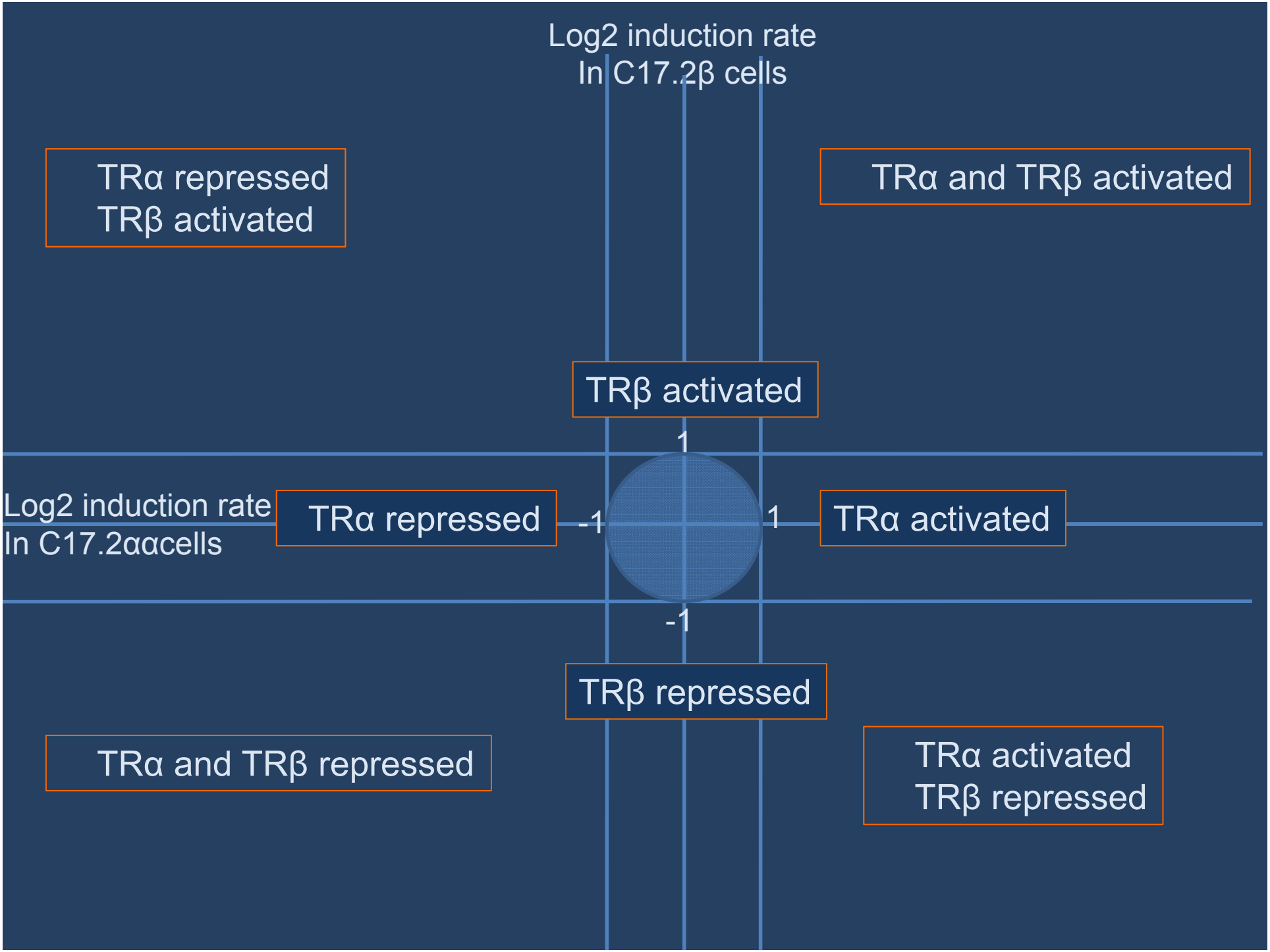




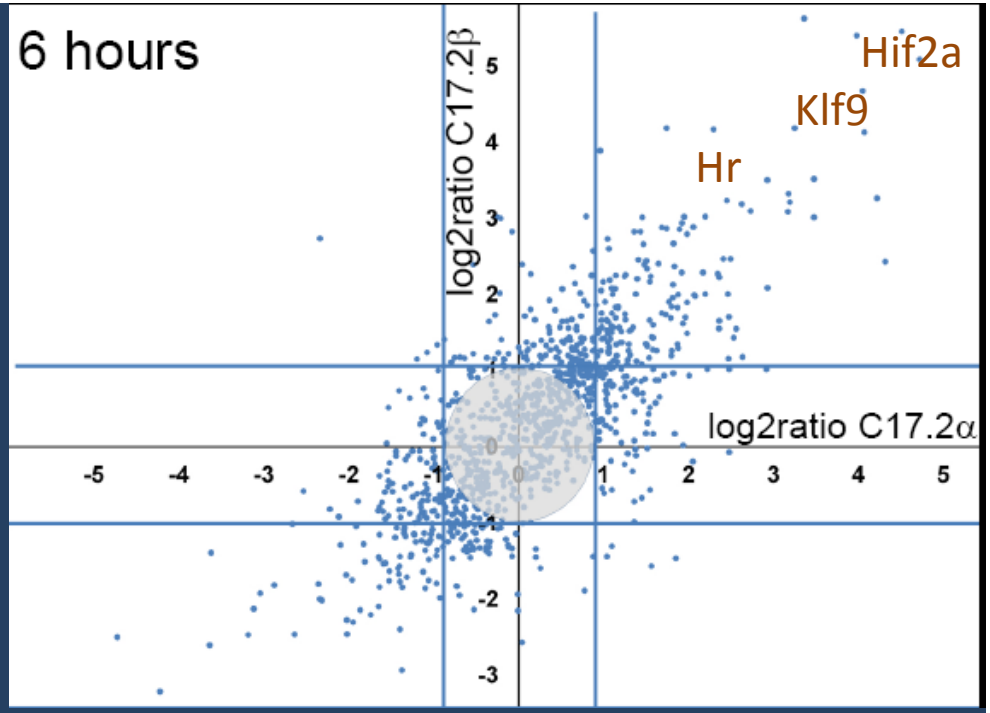
Western Blot
Protein G

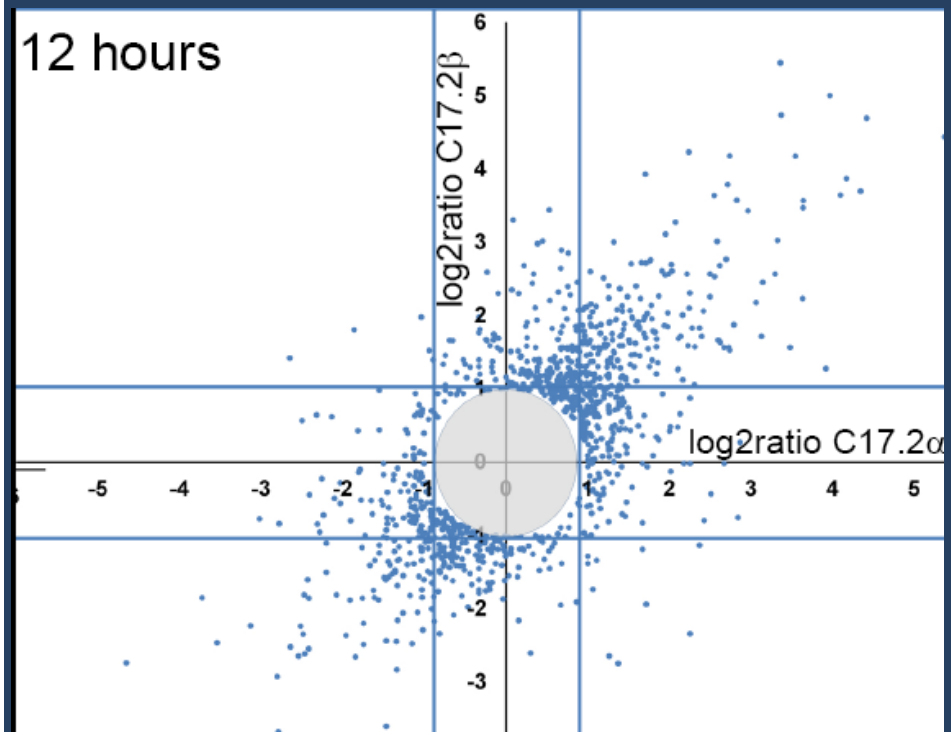
Digital gene expression

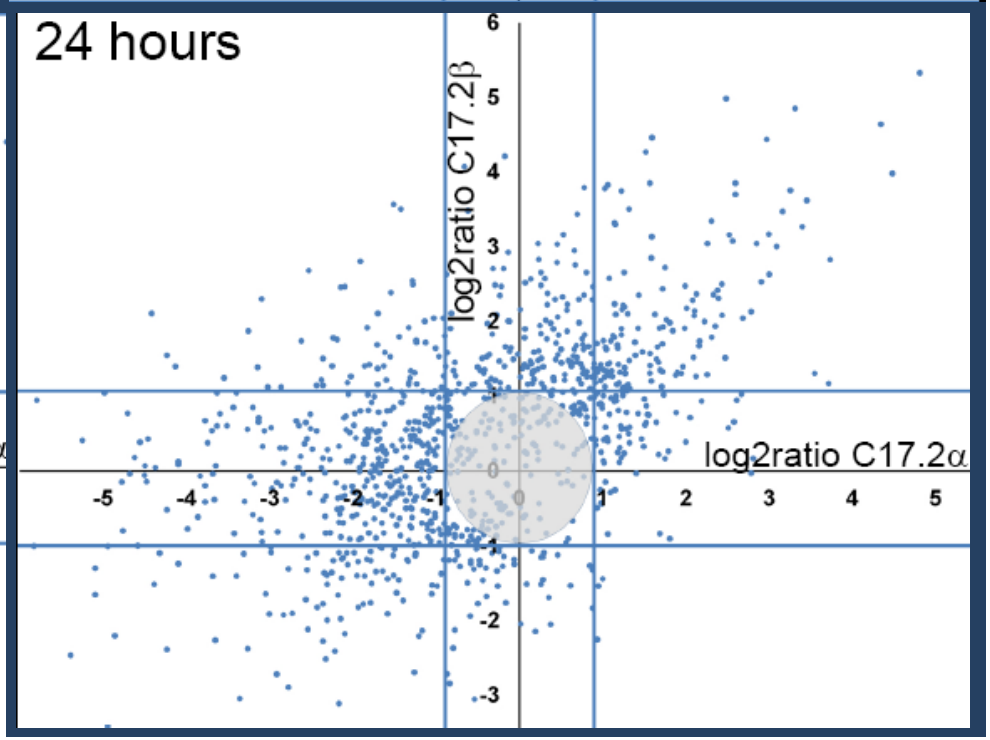
- Treat C17.2a or C17.2b cells with T3
- (10^{-7}M, 6h, 12h, 24h, no serum)
- Extract RNA samples
- Reverse transcribe and prepare cDNA 3'end libraries
- Sequence cDNA libraries
- (>$8x10^6$ reads/sample SOLID sequencing)


Log2 induction rate
In C17.2β cells
TRα repressed
TRβ activated
TRα and TRβ activated
TRβ activated
1
Log2 induction rate
In C17.2ααcells
TRα repressed
-1
1
TRα activated
-1
TRβ repressed
TRα and TRβ repressed
TRα activated
TRβ repressed

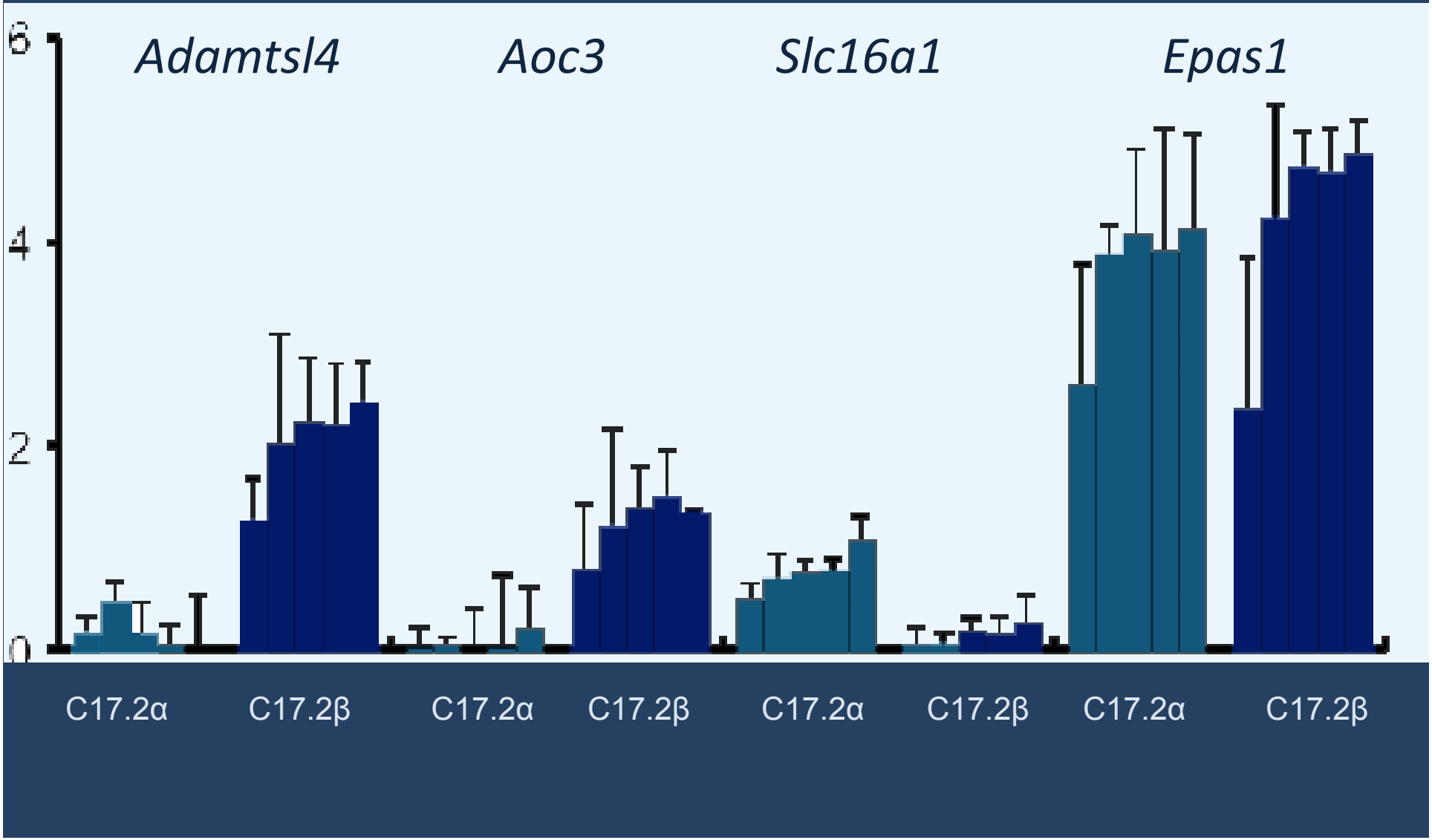
Many genes display receptor-selective response





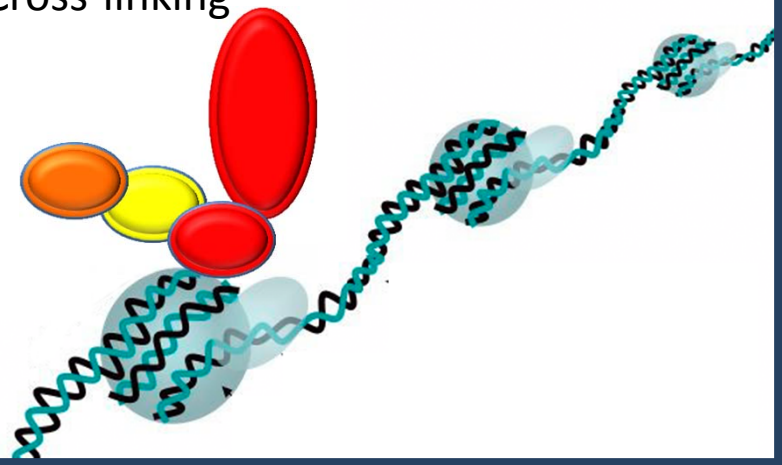


Q-RT-PCR confirmation of TRα1 selective response

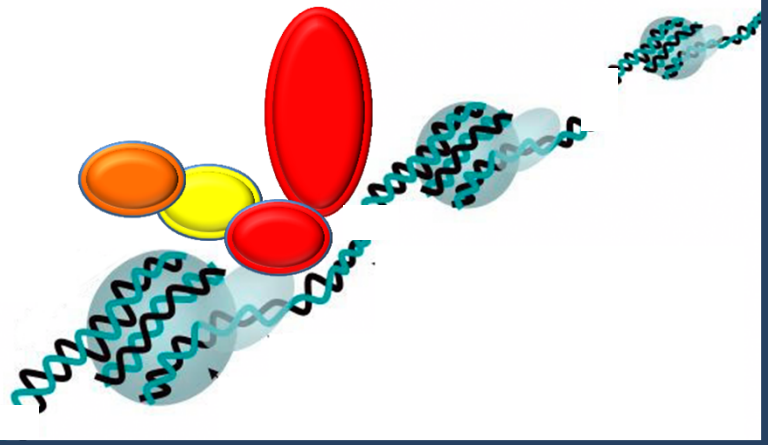


Which genes are direct TR target? Chromatin affinity purification/sequencing: « ChapSeq » in C17.2α and C17.2β cells

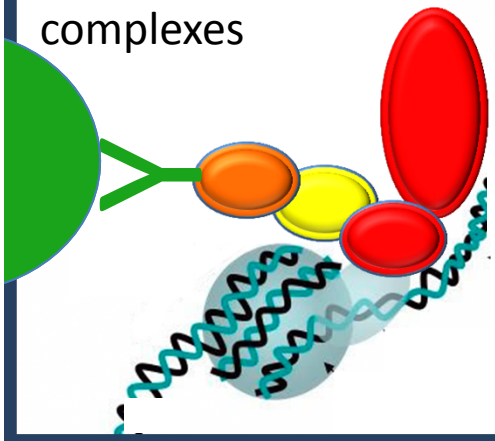
1) Cross-linking



2) DNA breakage



3) IgG affinity purification of TRcontaining complexes



2) Sequence DNA fraction (>10^7 reads)

AGGTCGATCGATCGATGGGACTAGATCG

AGGTCGATCGATCGATGGGACTAGATCG

AGGTCGATCGATCGATGGGACTAGATCG

AGGTCGATCGATCGATGGGACTAGATCG

AGGTCGATCGATCGATGGGACTAGATCG

AGGTCGATCGATCGATGGGACTAGATCG

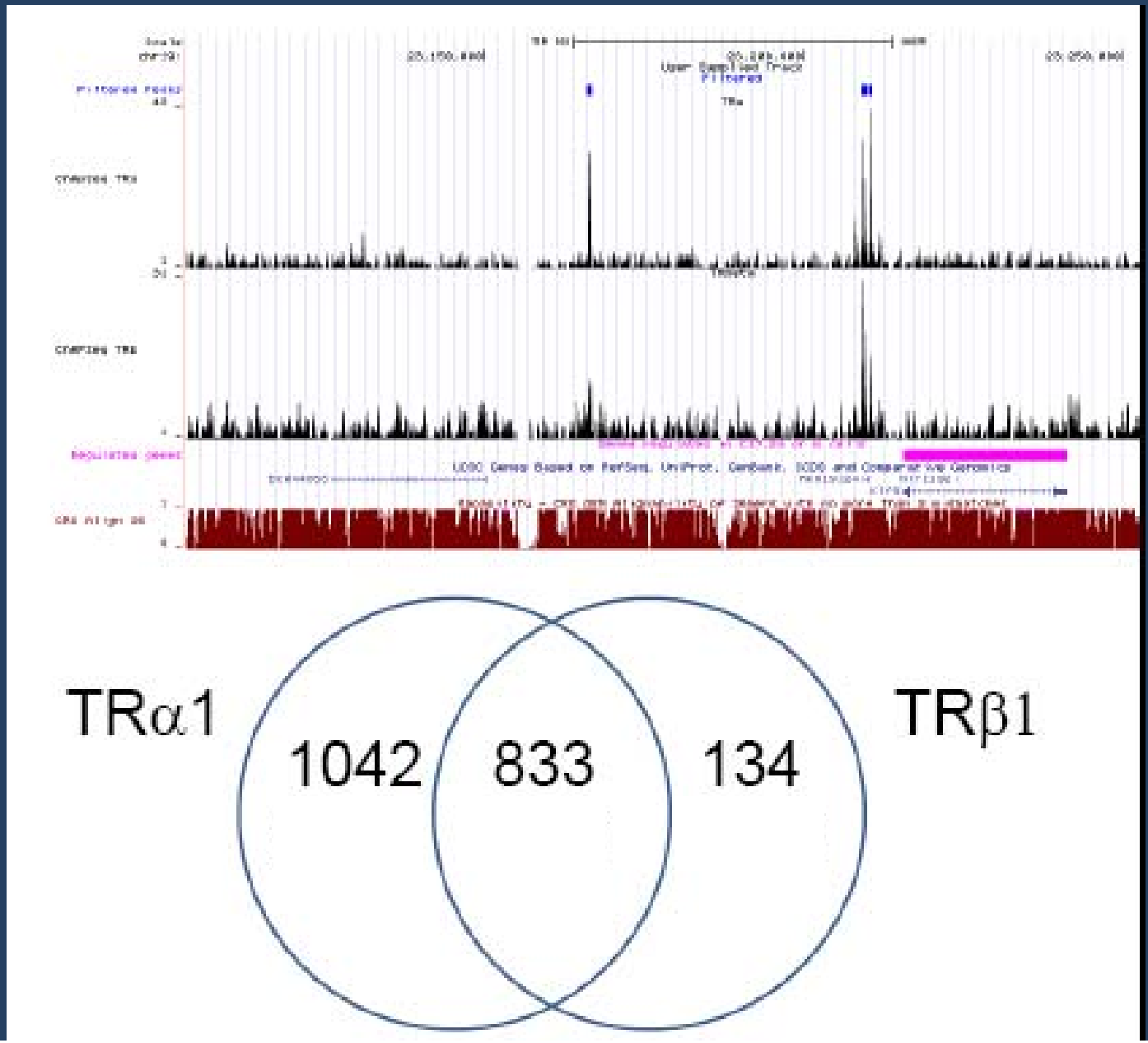
AGGTCGATCGATCGATGGGACTAGATCG

AGGTCGATCGATCGATGGGACTAGATCG

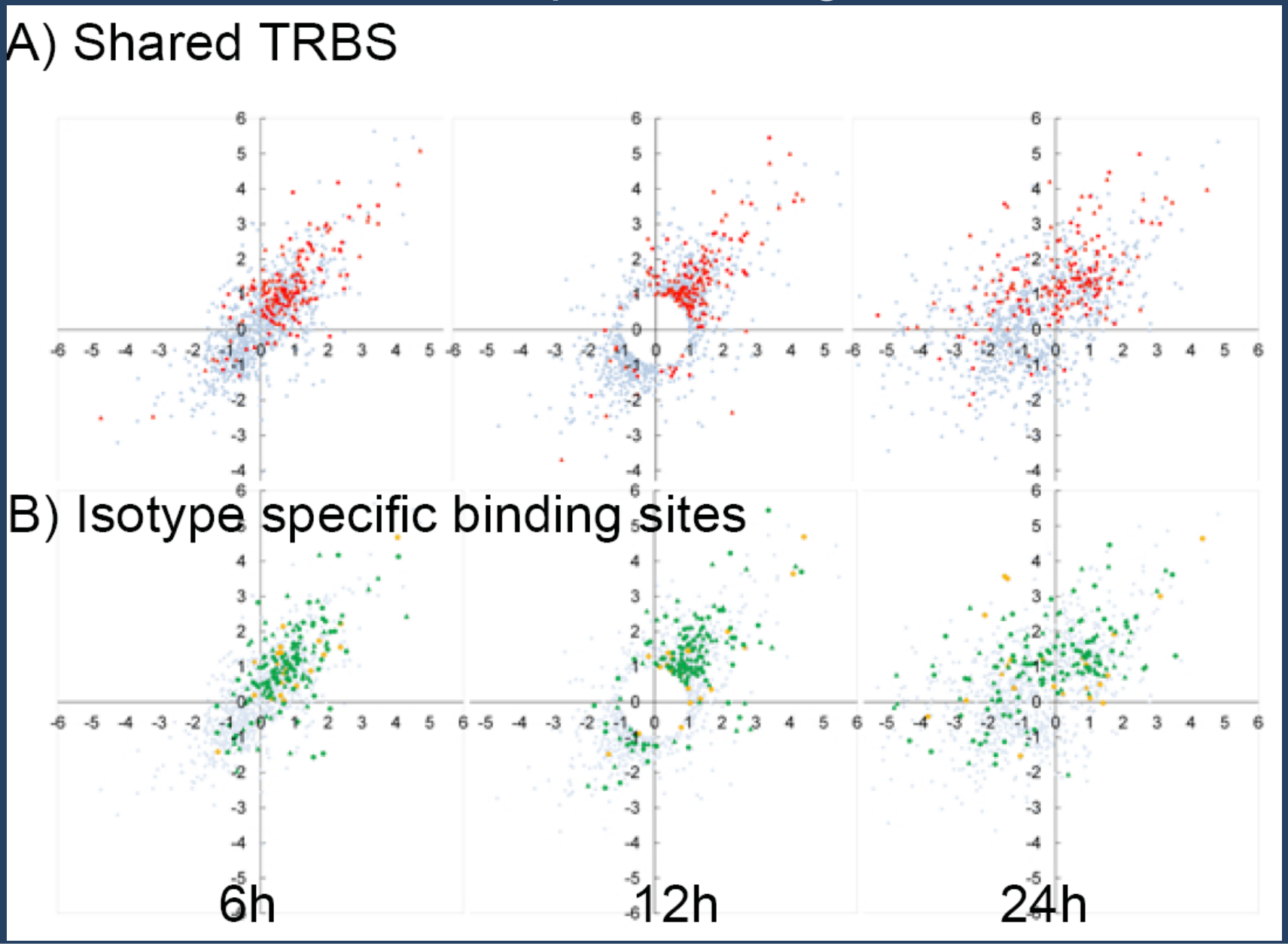
AGGTCGATCGATCGATGGGACTAGATCG

AGGTCGATCGATCGATGGGACTAGATCG

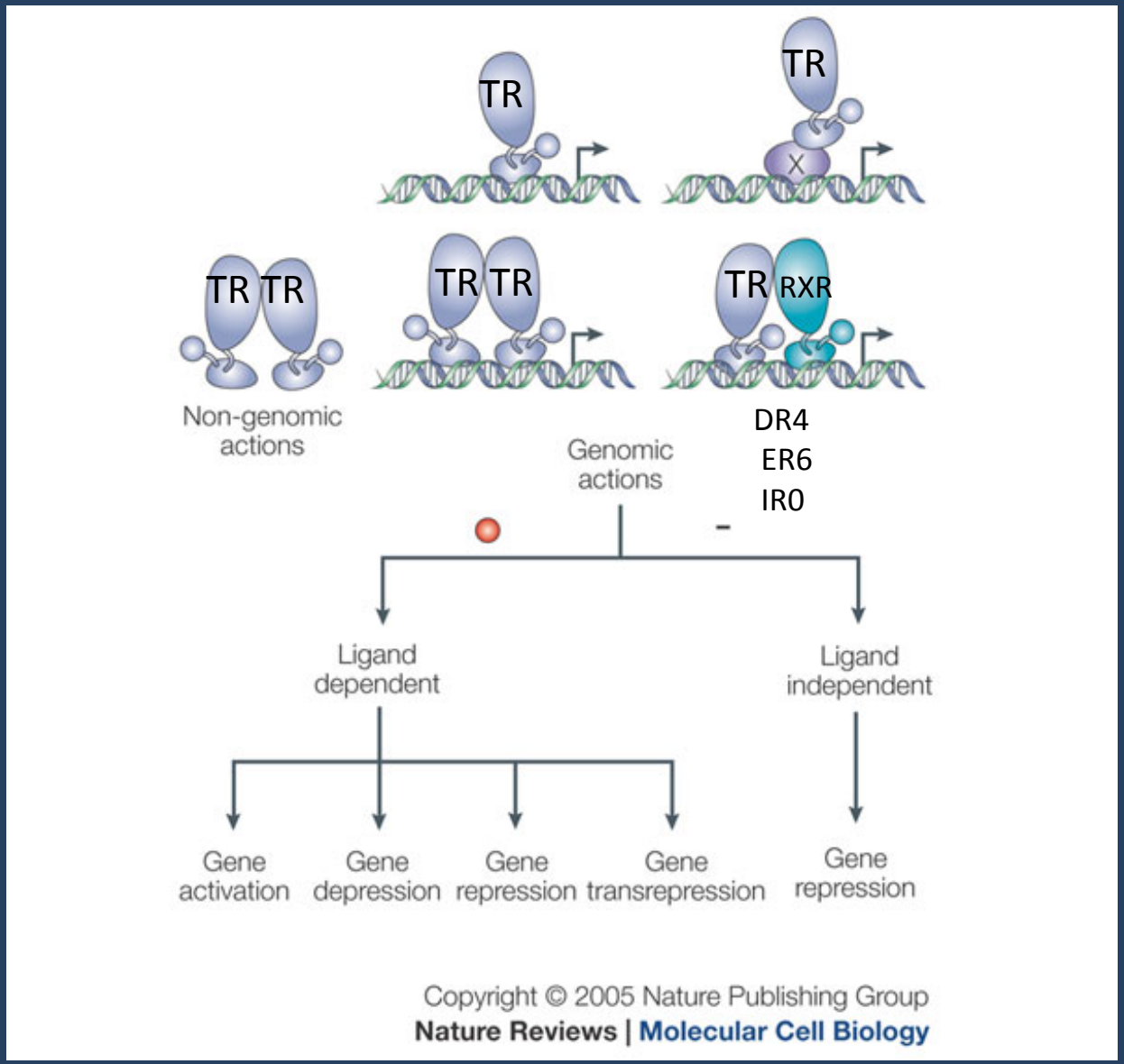
Genome wide analysis reveals the existence of receptor-specific binding sites



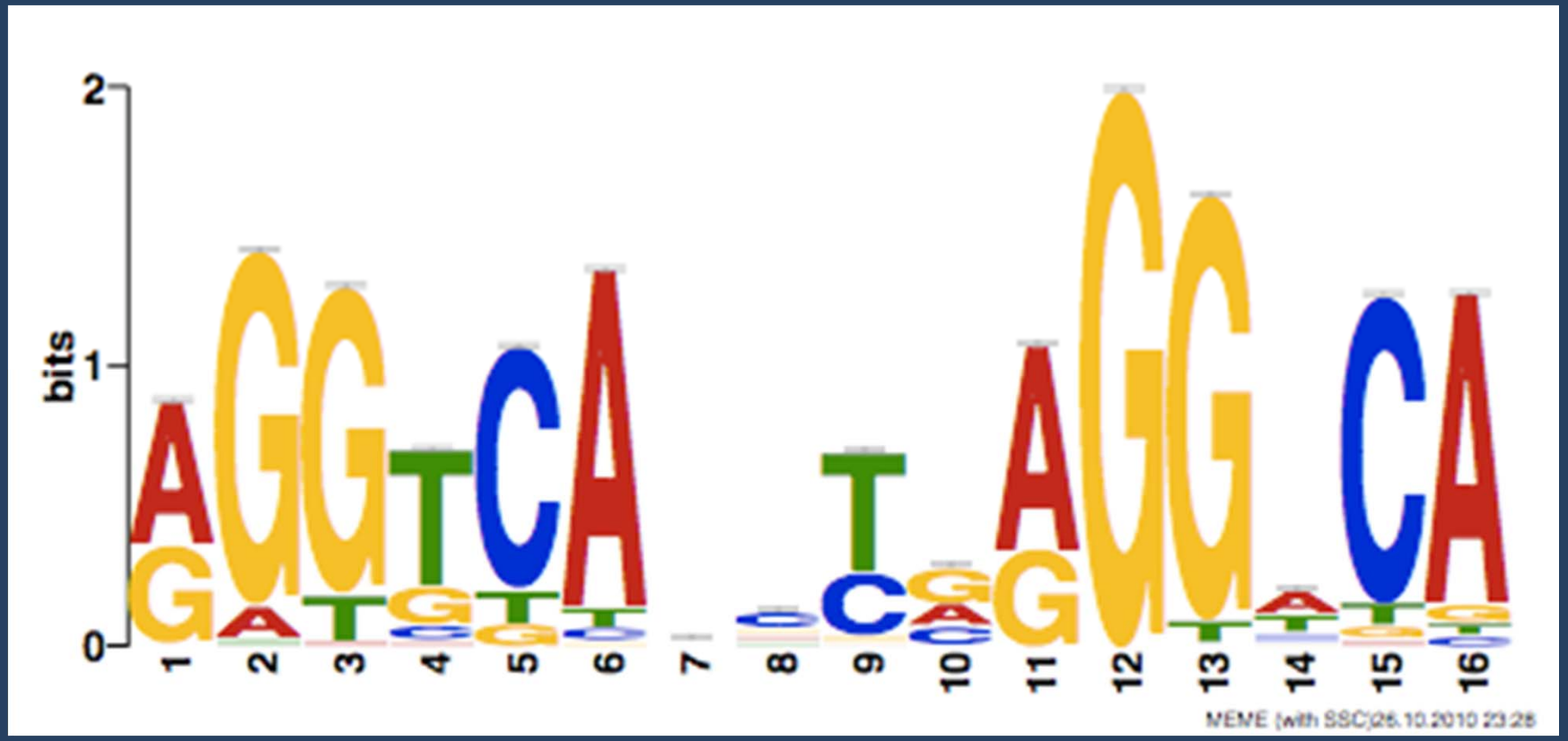
Receptor selective response is not due to differential receptor binding



T3 signaling getting simpler?



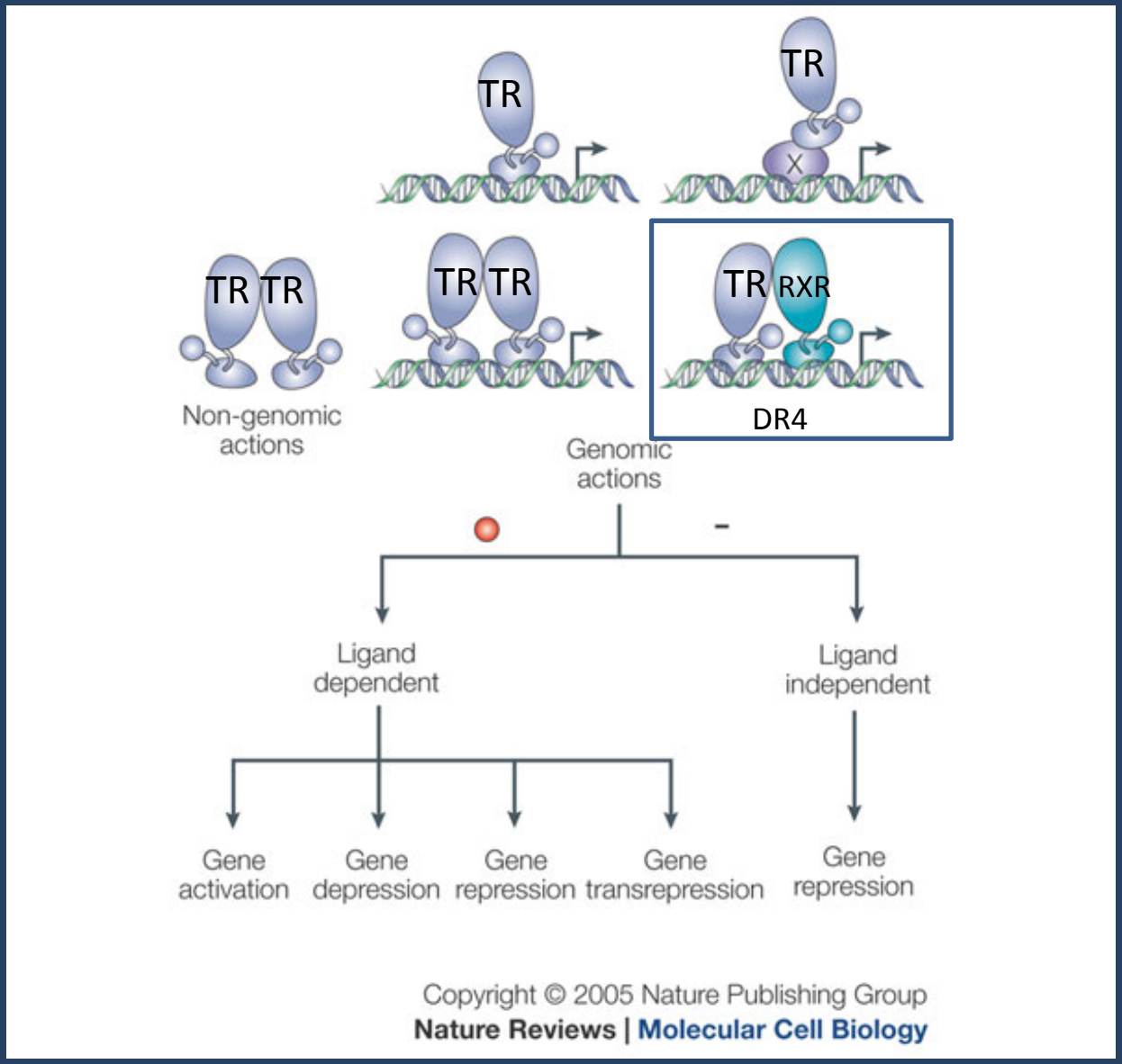
MEME identifies only the DR4 consensus (MEME.org)



DR4 is the only consensus enriched in TR binding sites.

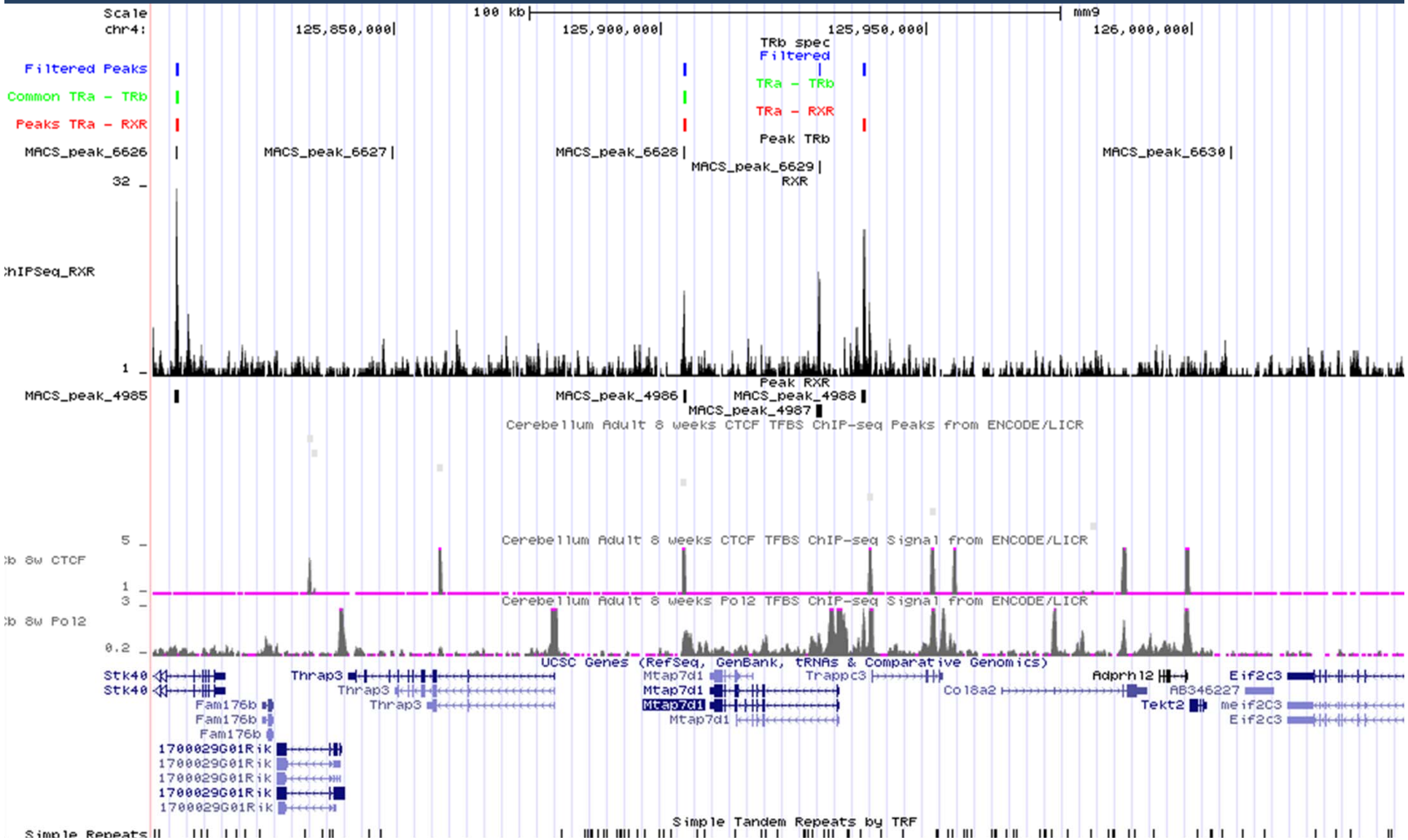
X axis: random sequence y axis: TR binding sites.

Only evidences for DR4

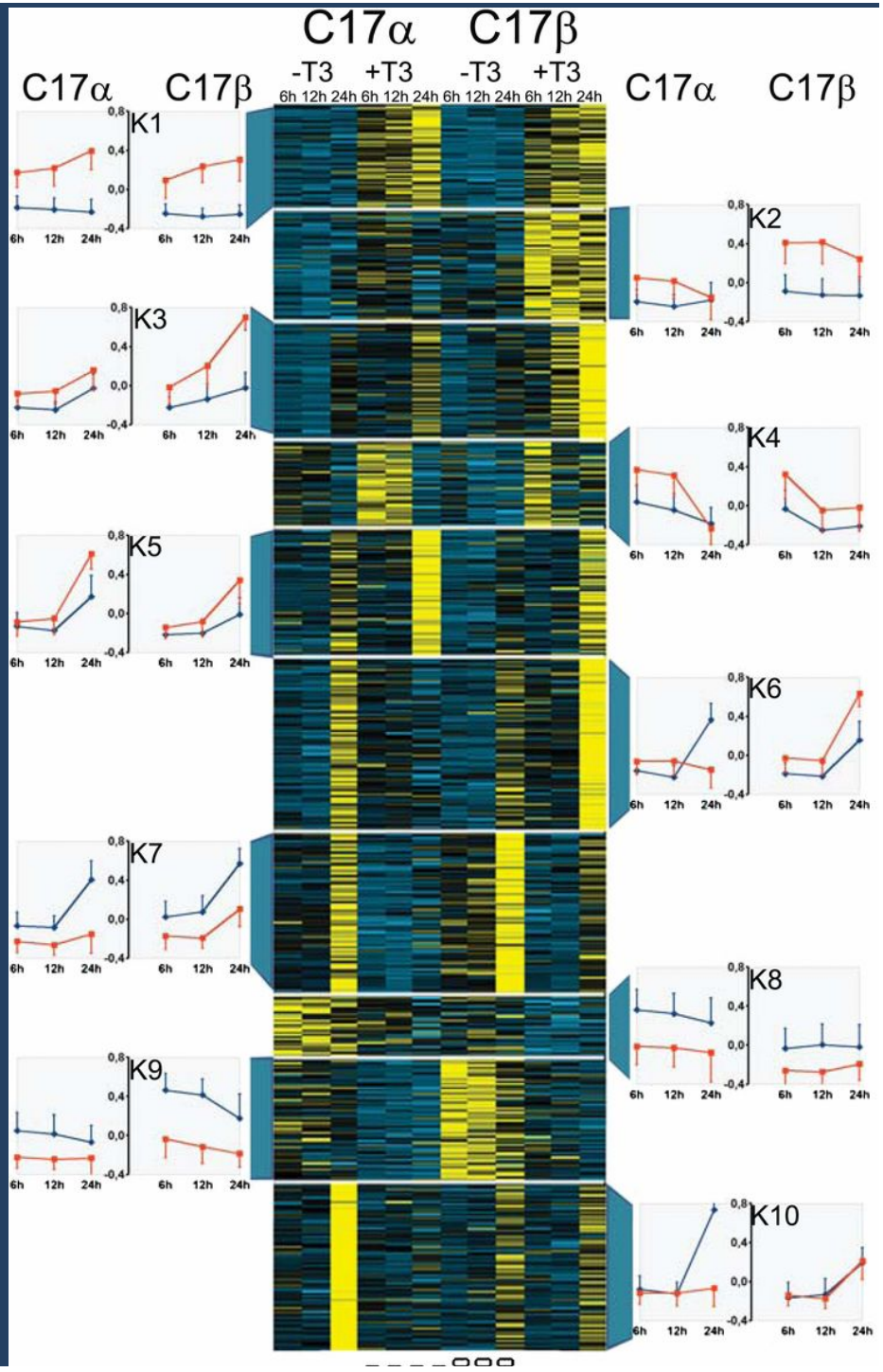


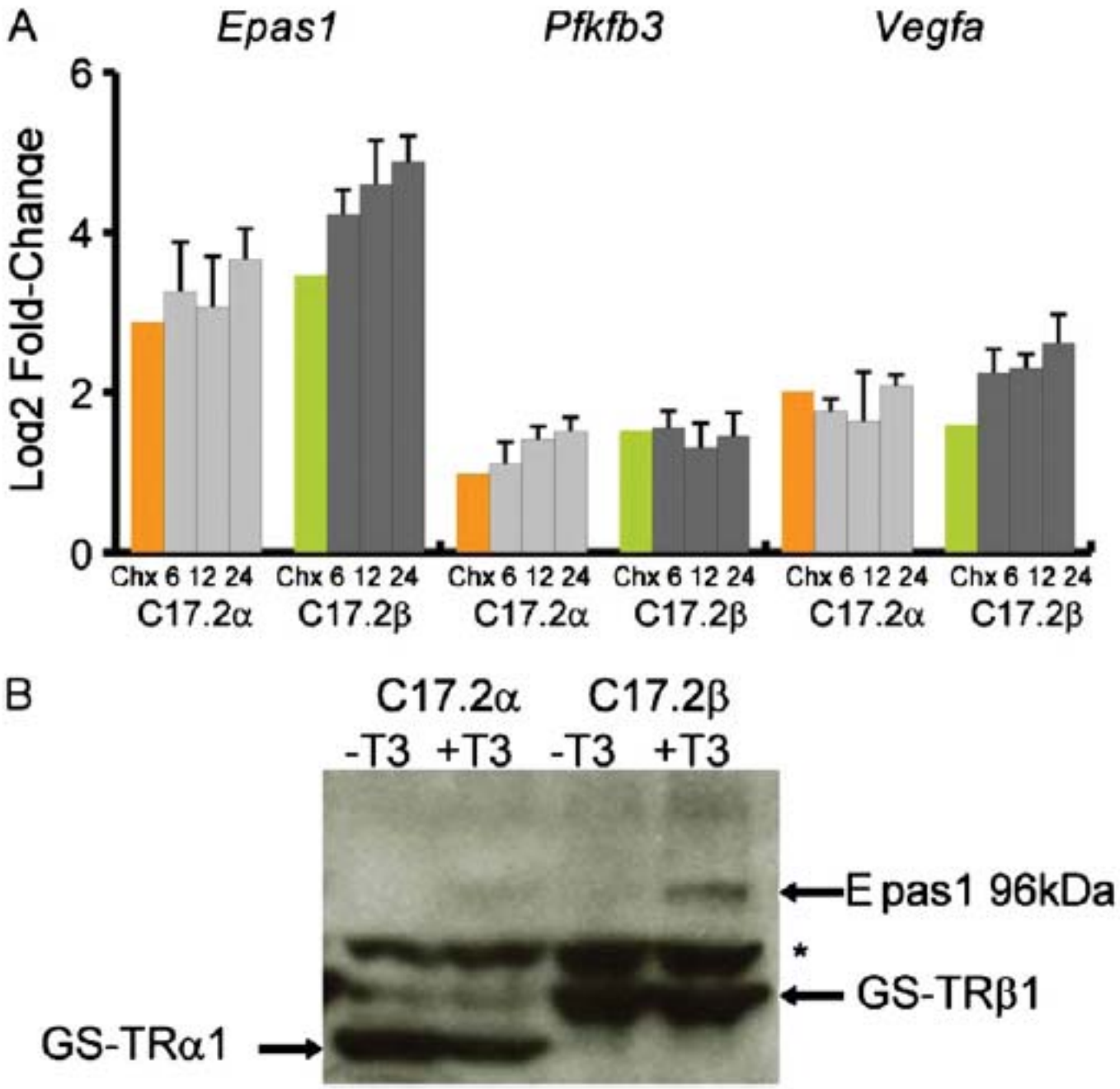
… but still room for alternatives

A recurrent co-occurrence with CTCF occupation

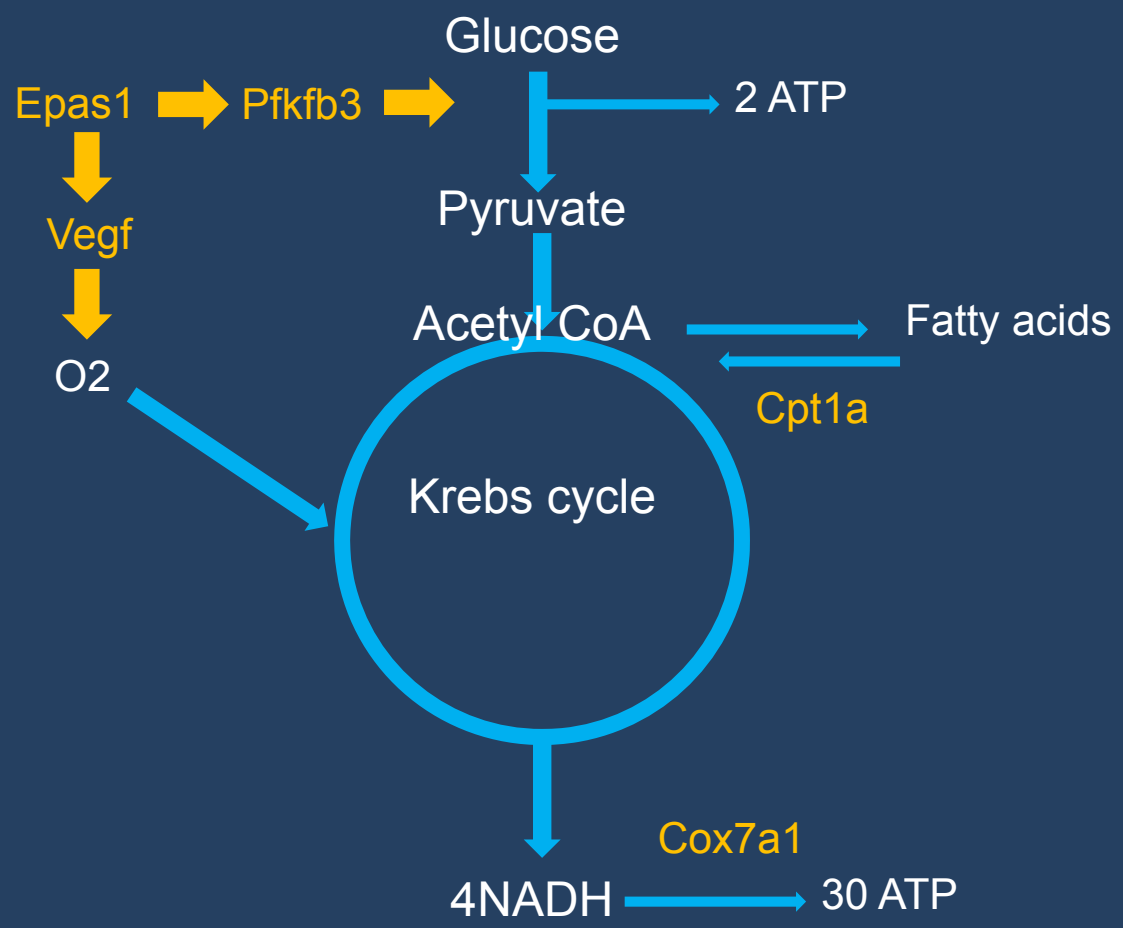


T3 target genes in C17.2: any relevance to neurodevelopment?

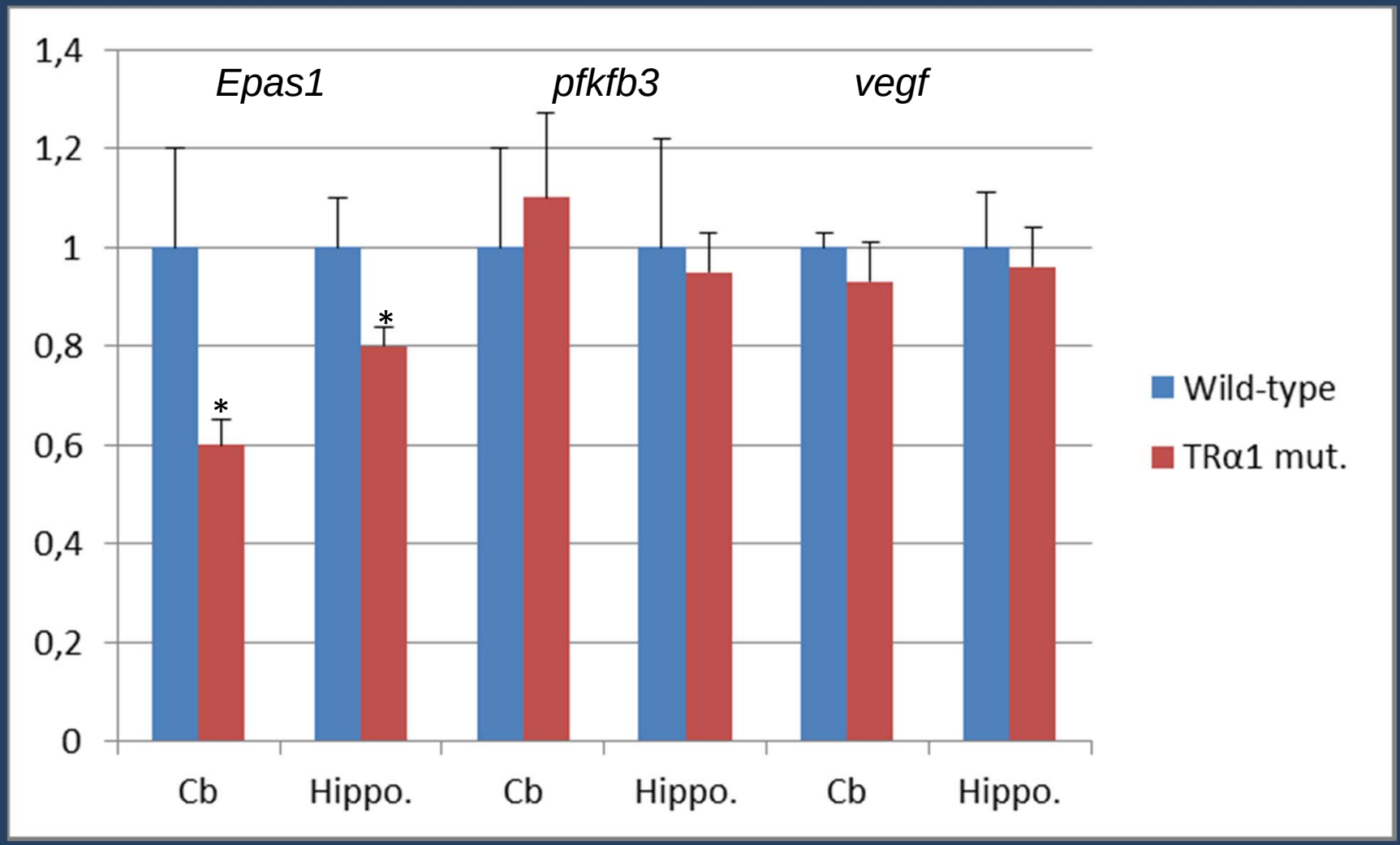



A
Epas1
Pfkfb3
Vegfa
Log2 Fold-Change
6
4
2
0
Chx 6 12 24
C17.2α
C17.2β
B
C17.2α
C17.2β
-T3 +T3 -T3 +T3
Epas1 96kDa
*
GS-TRβ1
GS-TRα1

Hypothetical new connection of T3 with glucose metabolism



A putative regulation of brain sensitivity for hypoxia



Conclusions:

1) Both different expression patterns and different transactivation properties explain the different function of TRα1 and TRβ1

2) Receptor selective response is not explained by selective promoter occupancy

3) TR/RXR/DR4/Coactivator complexes may have allosteric properties.

4) Regulation by TR of the hypoxia-pathway could be of physiological relevance in some situations.

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Nadine Aguilera PBES

CRESCENDO

Agence Nationale de la Recherche GIP ANR

INRA Institut National de la Recherche Agronomique

A sequential intervention of TRα1 and TRβ1 during Purkinje cells differentiation.