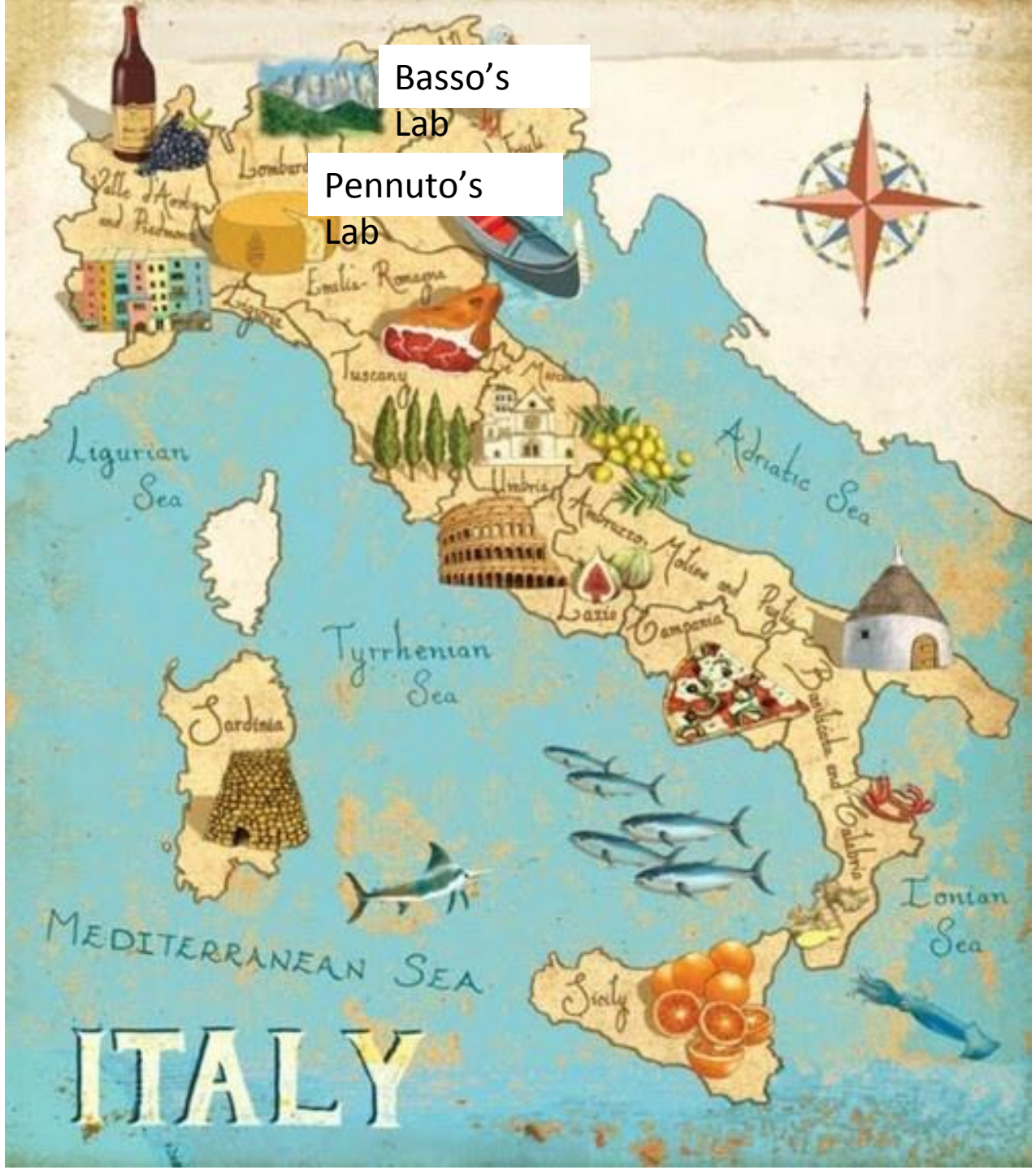
Silencing of Mutant AR Co-activators as a Therapeutic Approach

Manuela Basso, Assistant Professor
University of Trento, Centre for Integrative Biology

UNIVERSITY OF TRENTO - Italy

CIBIO

Trento,
Italy


Basso's Lab
Pennuto's Lab
Ligurian Sea
Tyrrhenian Sea
Adriatic Sea
Ionian Sea
MEDITERRANEAN SEA
ITALY

Italian Meeting with researchers and Scientists

November 2013, Padova

April 18th 2015, Trento

III GIORNATA
Malattia di Kennedy 2016

November 12th 2016, Milan

The fourth meeting is coming soon….

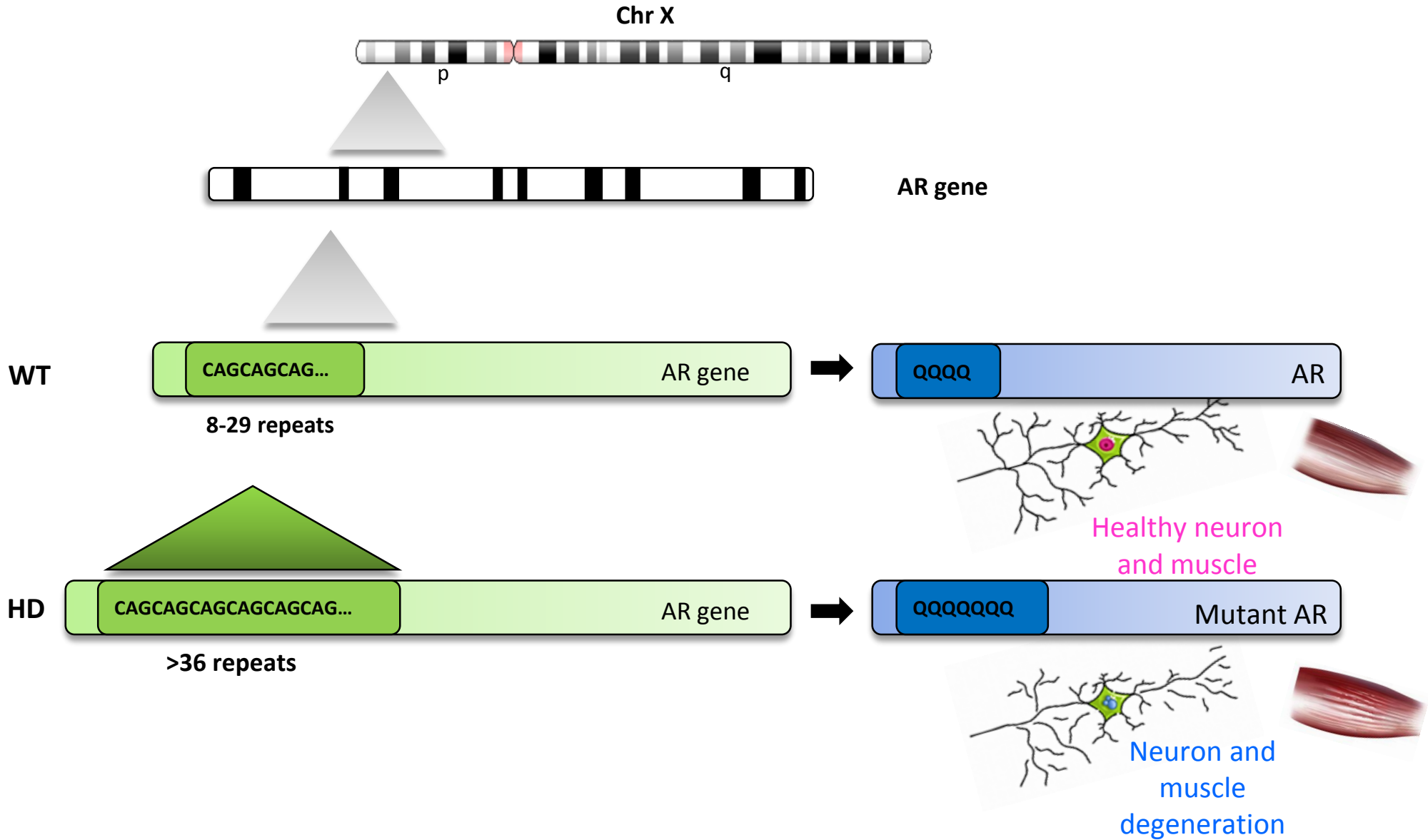
EUROPEAN
NEURO
MUSCULAR
CENTRE

March 27th-29th 2015

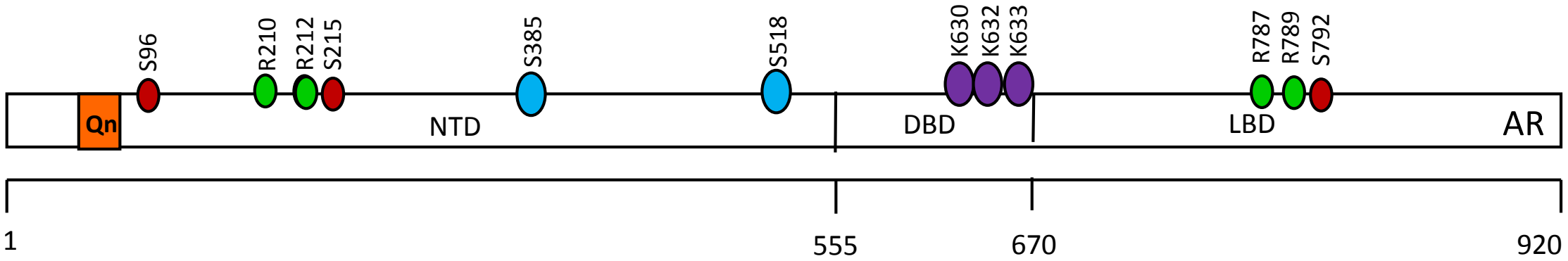
Naarden, The Netherlands

A new one coming up soon

SBMA is caused by expansion of a polyglutamine tract in AR



AR post-translational modifications



Polyglutamine tract

Phosphorylation

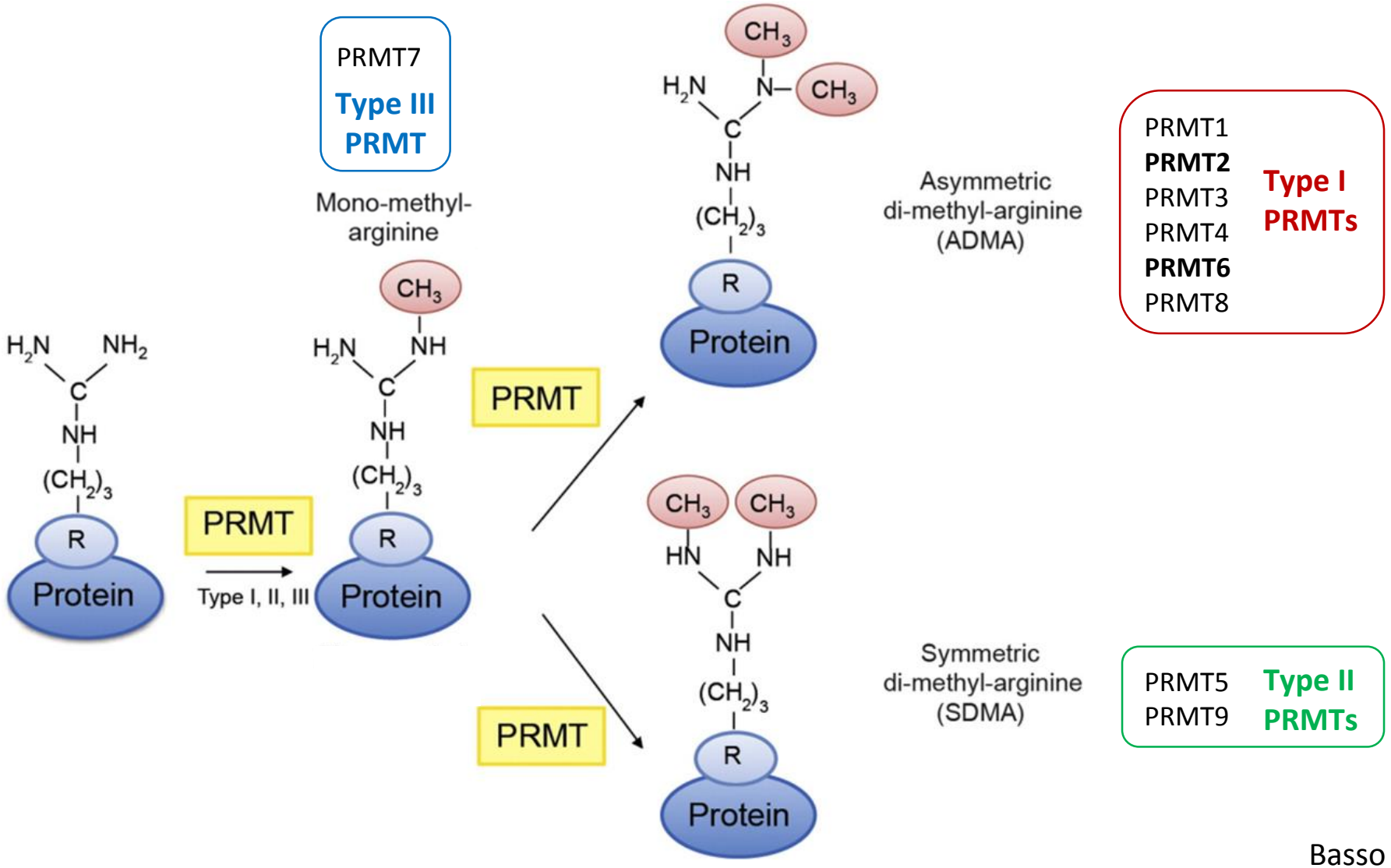
SUMOylation

R methylation

Acetylation

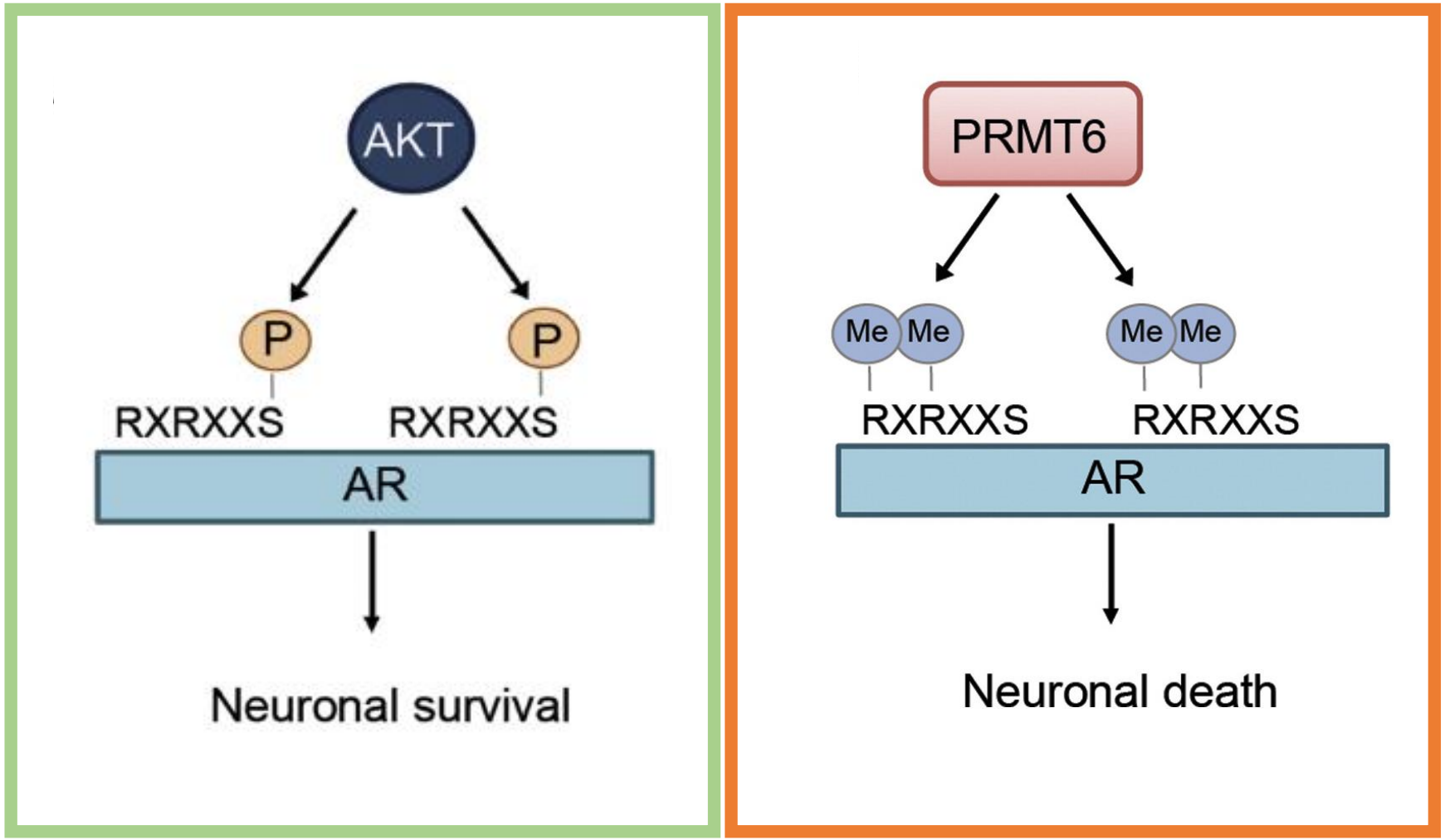
Palazzolo et al., 2007
Scaramuzzino et al., 2015
Montie et al., 2012
Chua et al., 2015

Arginine methylation is catalyzed by Protein Arginine Methyltransferases (PRMTs)



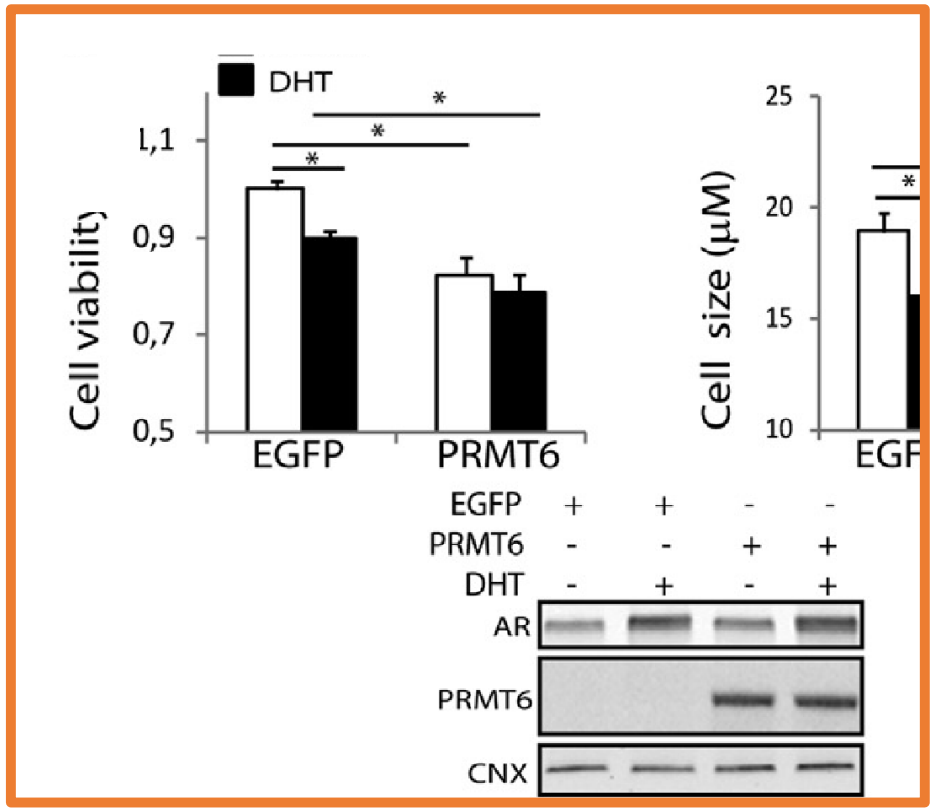
Basso and Pennuto, 2015

Arginine methylation and serine phosphorylation are mutually exclusive and both modulate AR function



Basso & Pennuto, Exp Neurol 2015

Does PRMT6 modify toxicity *in vitro*?



Scaramuzzino et al., 2015

Does PRMT6 modify toxicity *in vivo*?

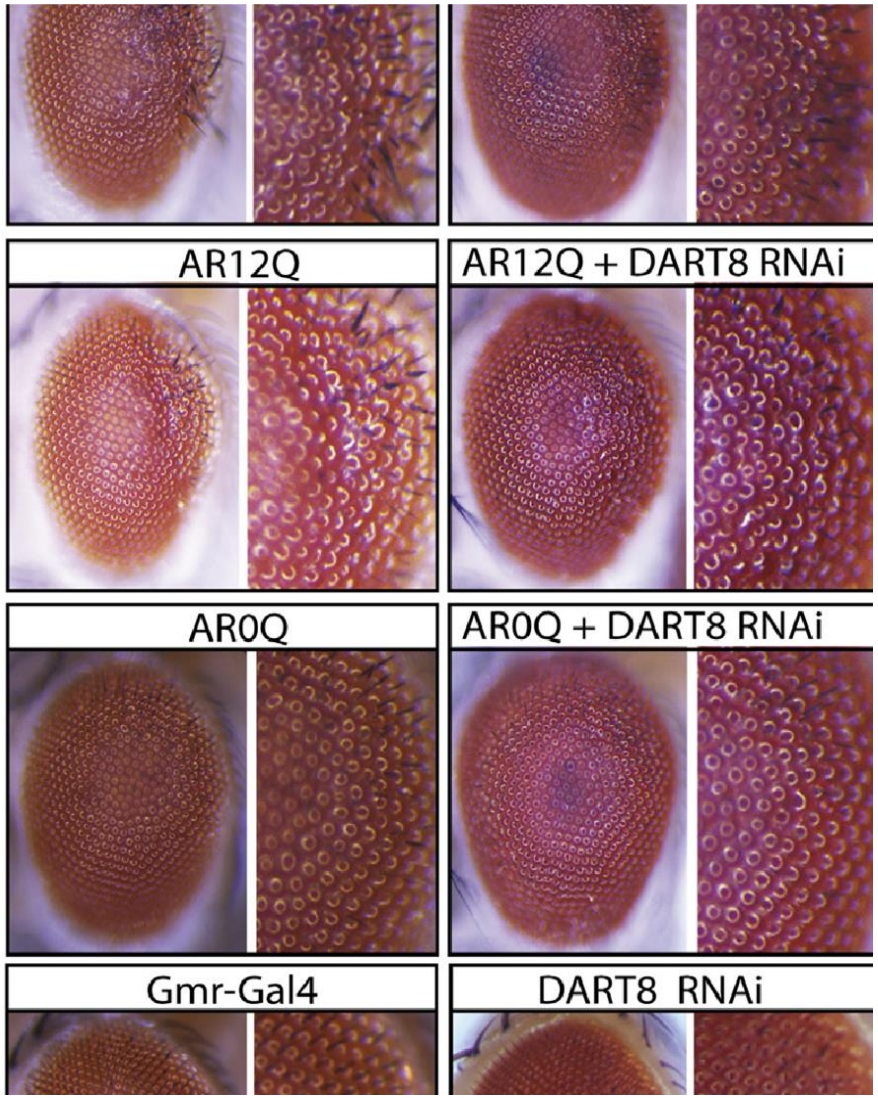
AR **X** **KD PRMT6**

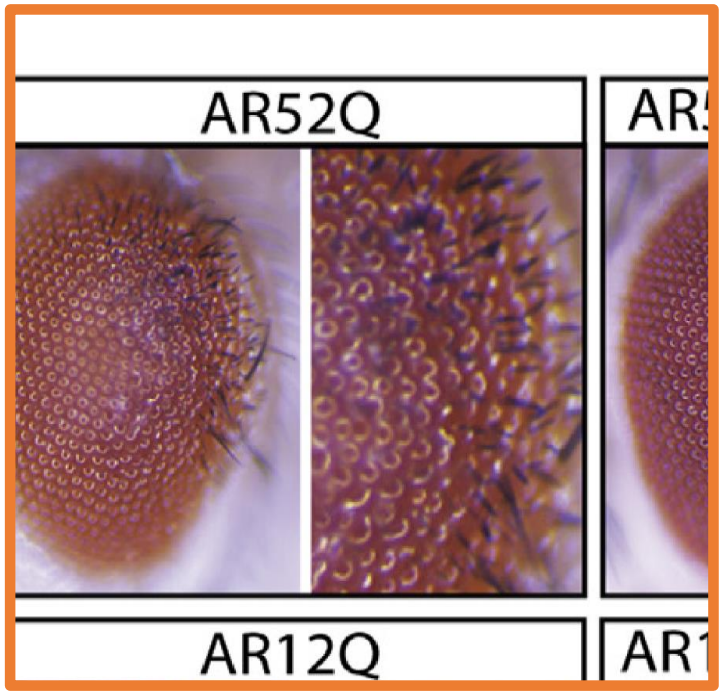
UAS-AR0Q
or
UAS-AR52Q

GMR-Gal4; DART8
silencing

In collaboration with the lab of Udai Pandey, University of Pittsburgh

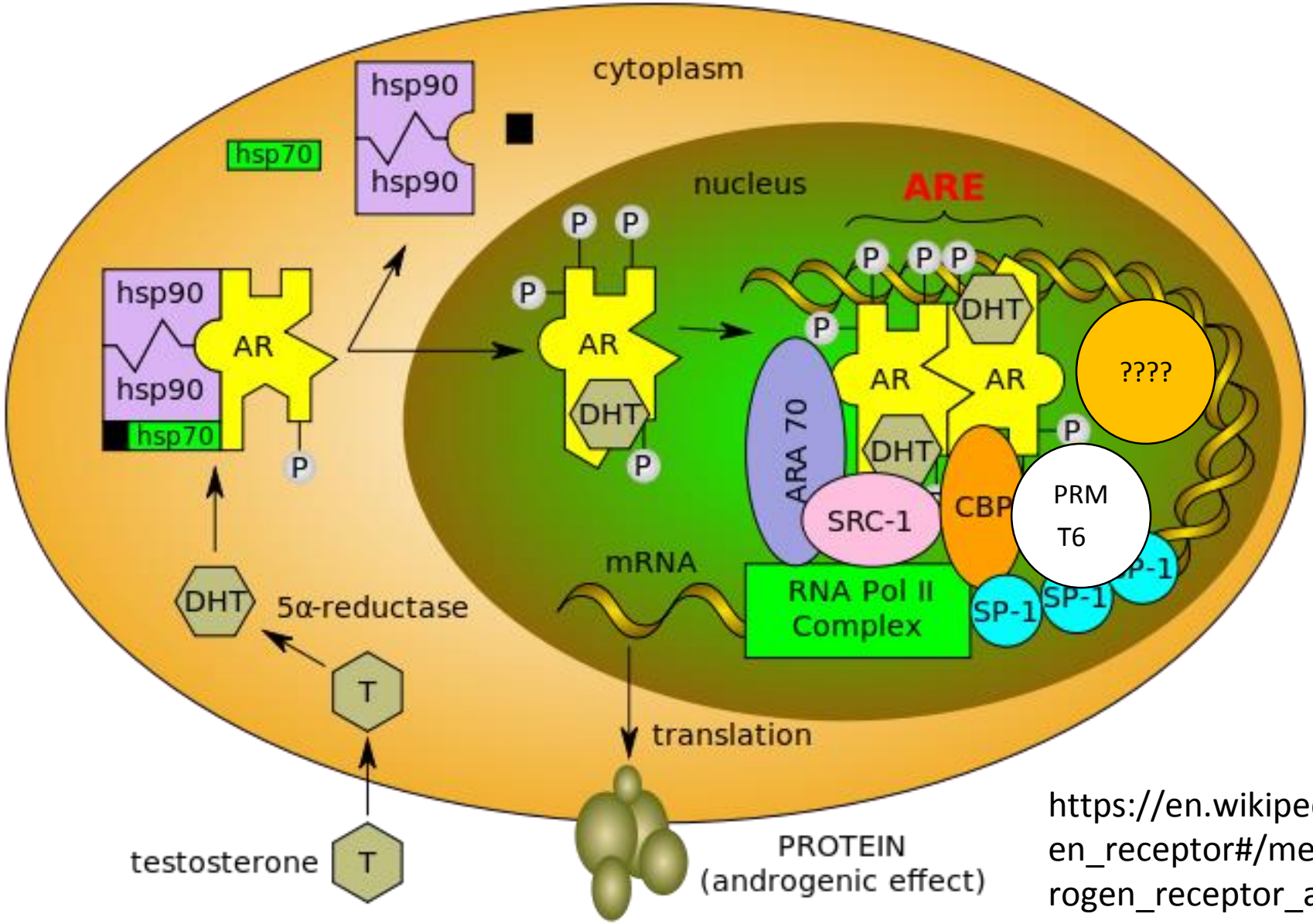
PRMT6 modifies AR toxicity *in vivo*





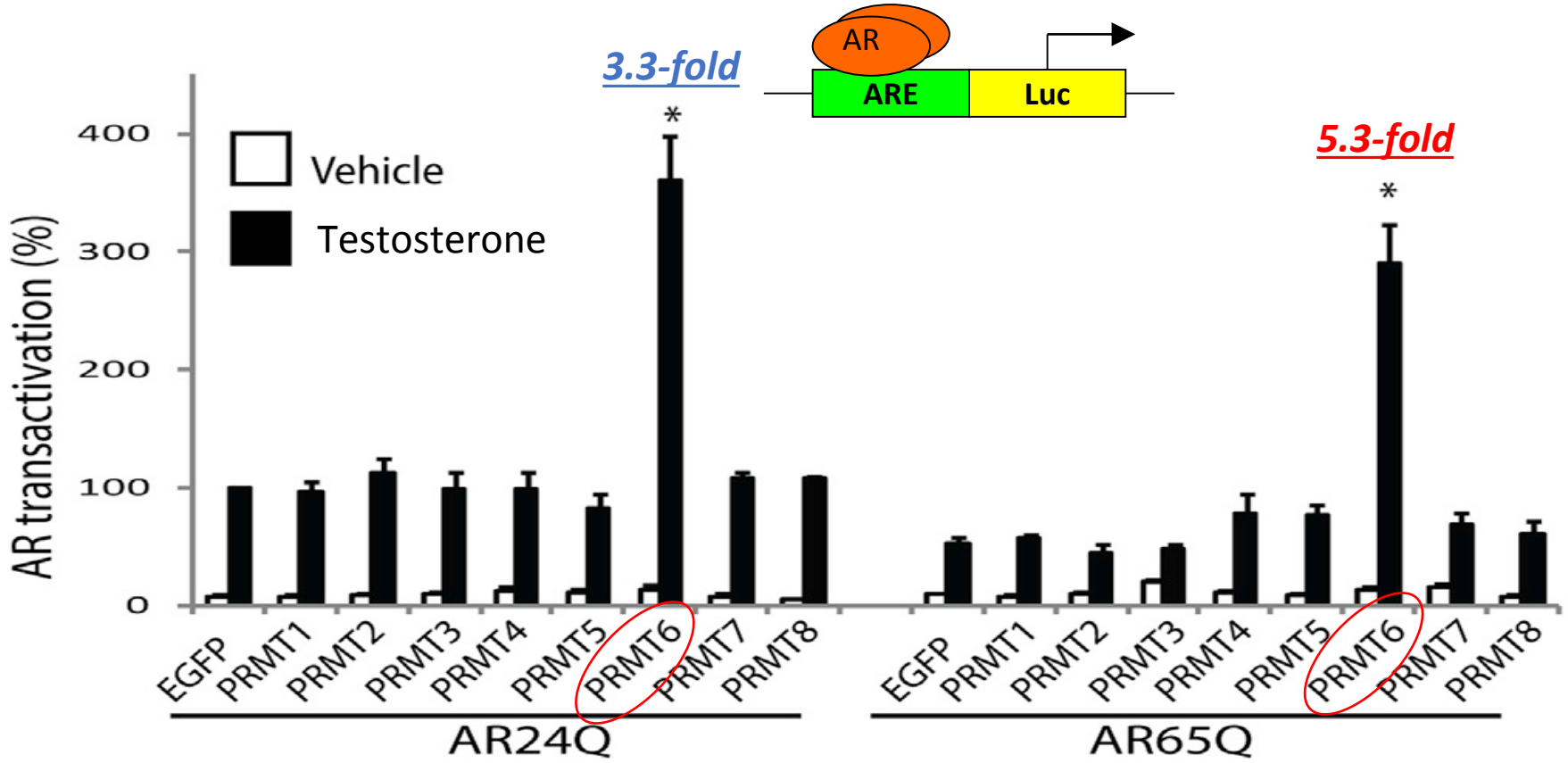
Scaramuzzino et al., 2015

AR is transcription factor



https://en.wikipedia.org/wiki/Androgen_receptor#/media/File:Human_androgen_receptor_and_androgen_binding.svg

PRMT6 specifically transactivates AR



AR transactivation by PRMT6 is enhanced by polyglutamine expansion

Scaramuzzino et al., Neuron 2015

Is it possible to reduce the activity of PRMT6 and preserve the physiological functions of AR?

Hypothesis

SBMA pathogenesis is modified through arginine methylation of polyQ-expanded AR

1. Silence PRMT6 by an artificial microRNA to assess the rescue of polyQ-expanded AR *in vivo*

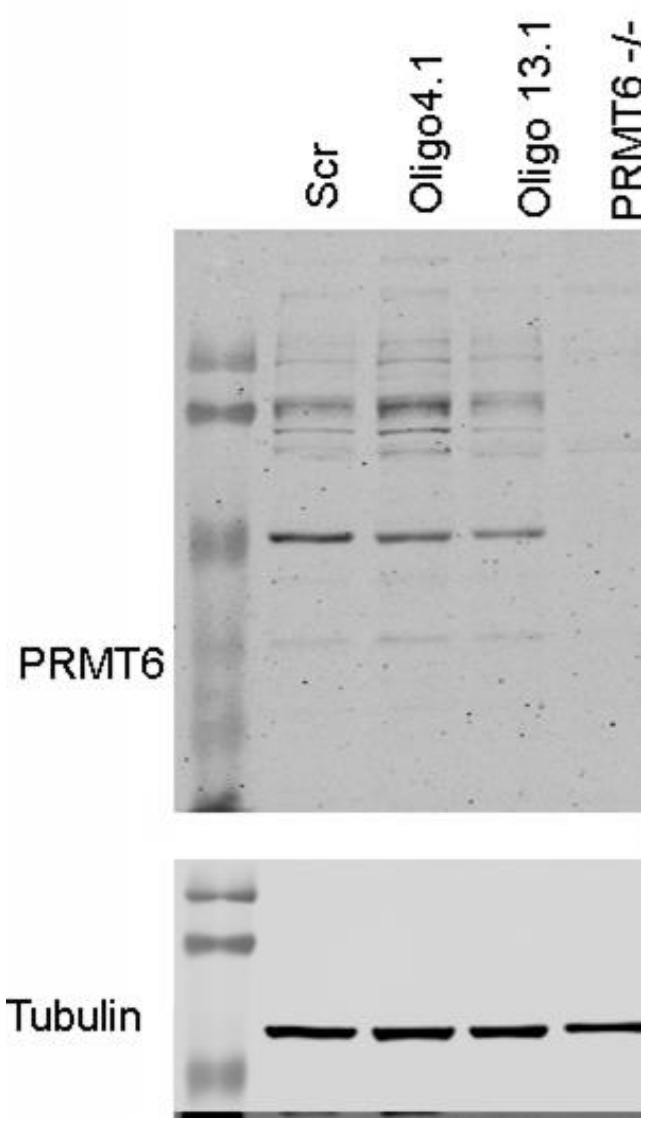
2. Silence PRMT6 by novel selective inhibitors to assess the rescue of polyQ-expanded AR

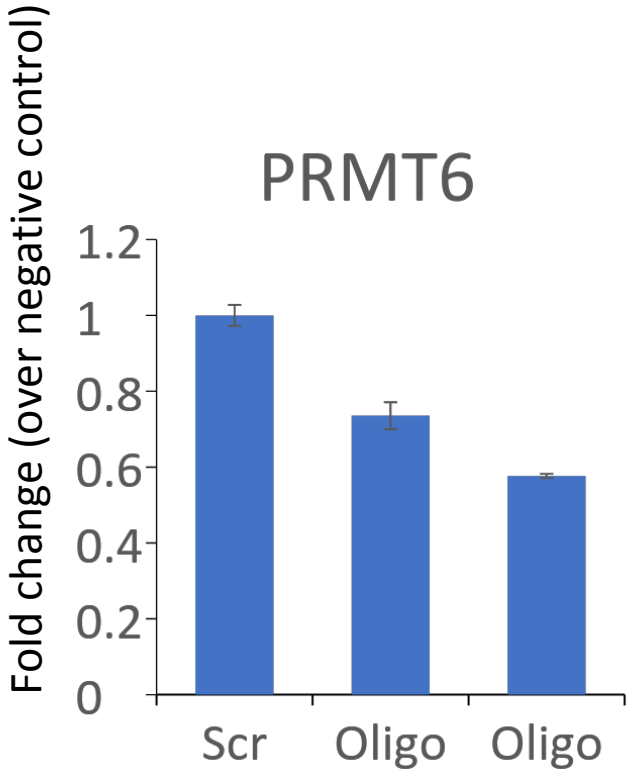
1a

Identify the best artificial miRNA for PRMT6

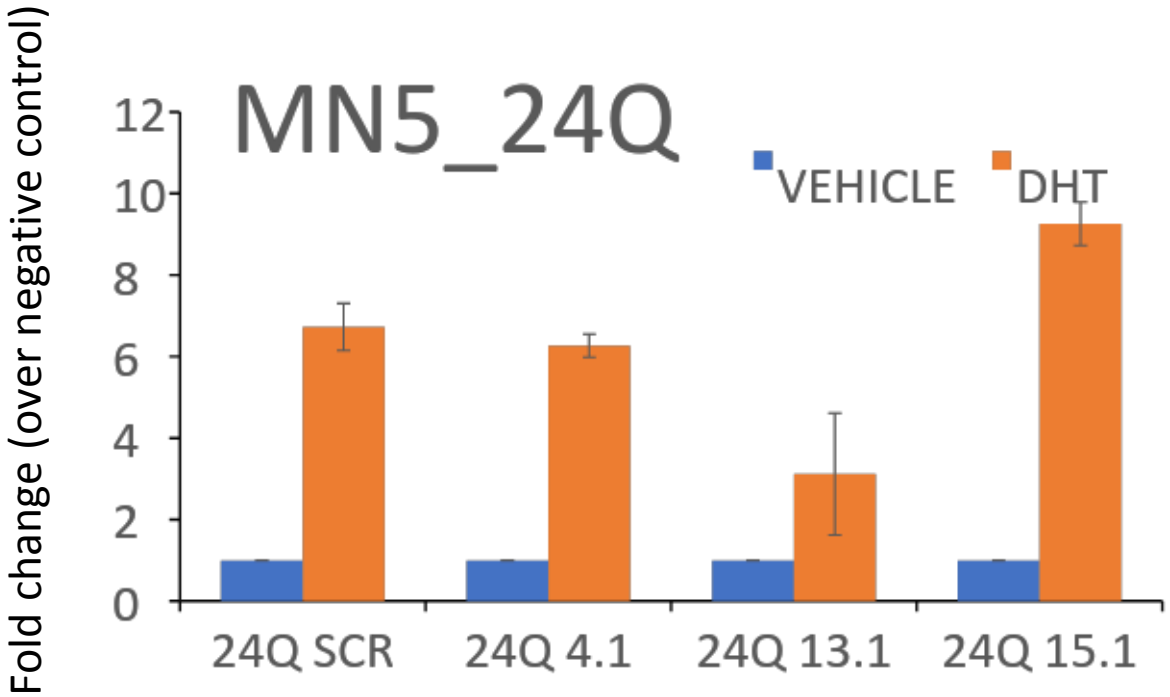
Debasmita Tripathy, postdoctoral

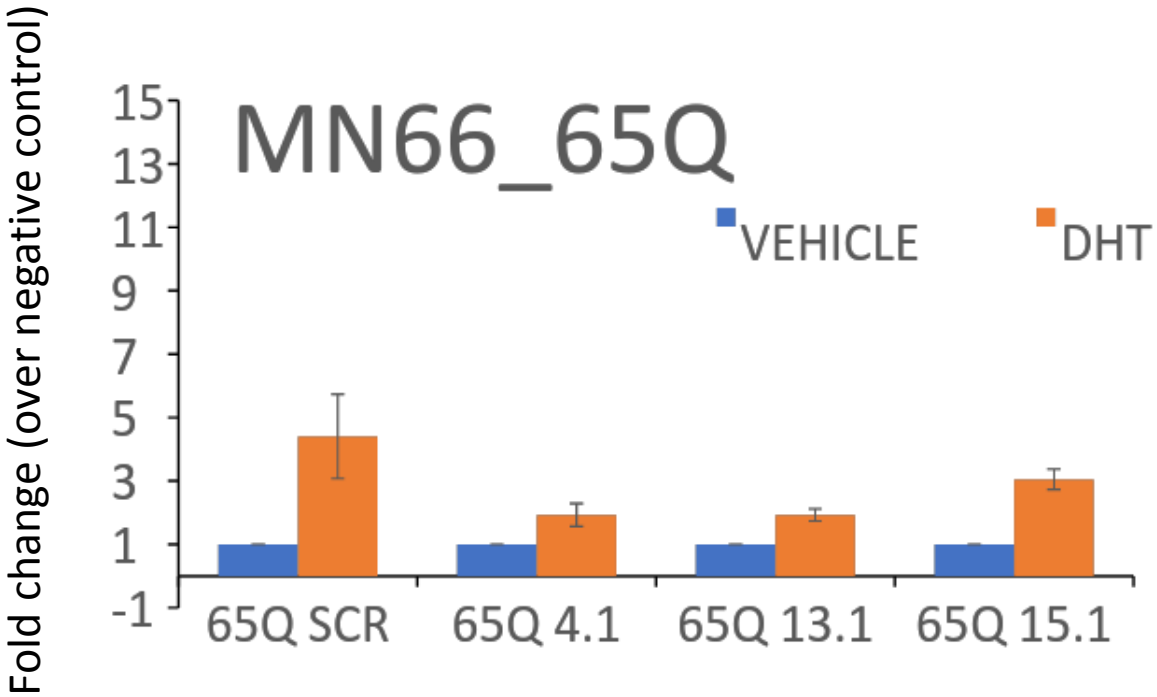
The artificial miRNA 13.1 silences PRMT6 of nearly 50%





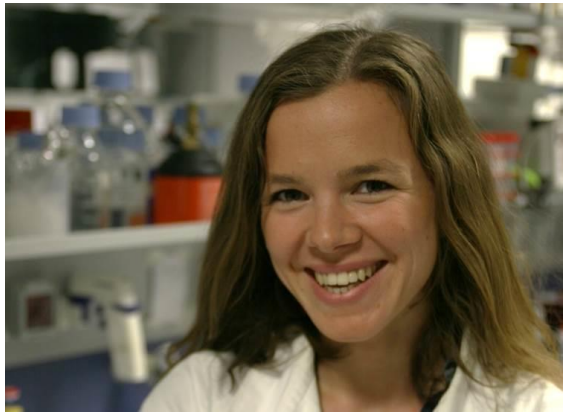
The artificial miRNA 13.1 reduces AR function





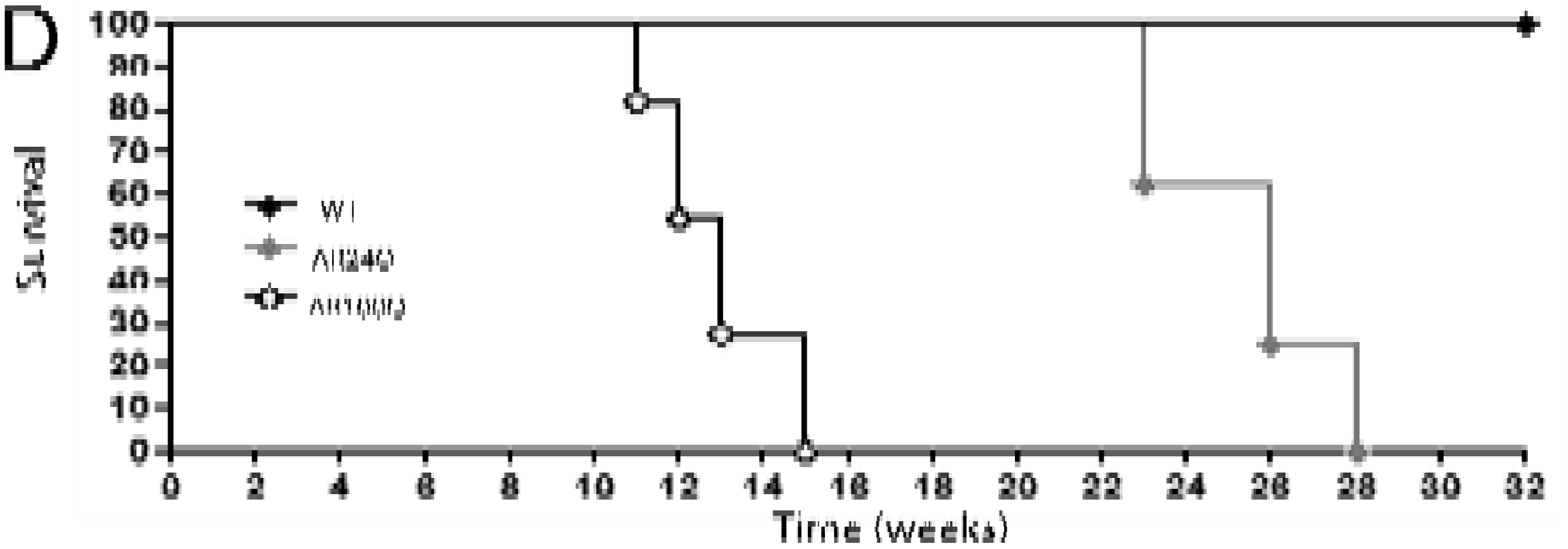
1b

New transgenic mouse model



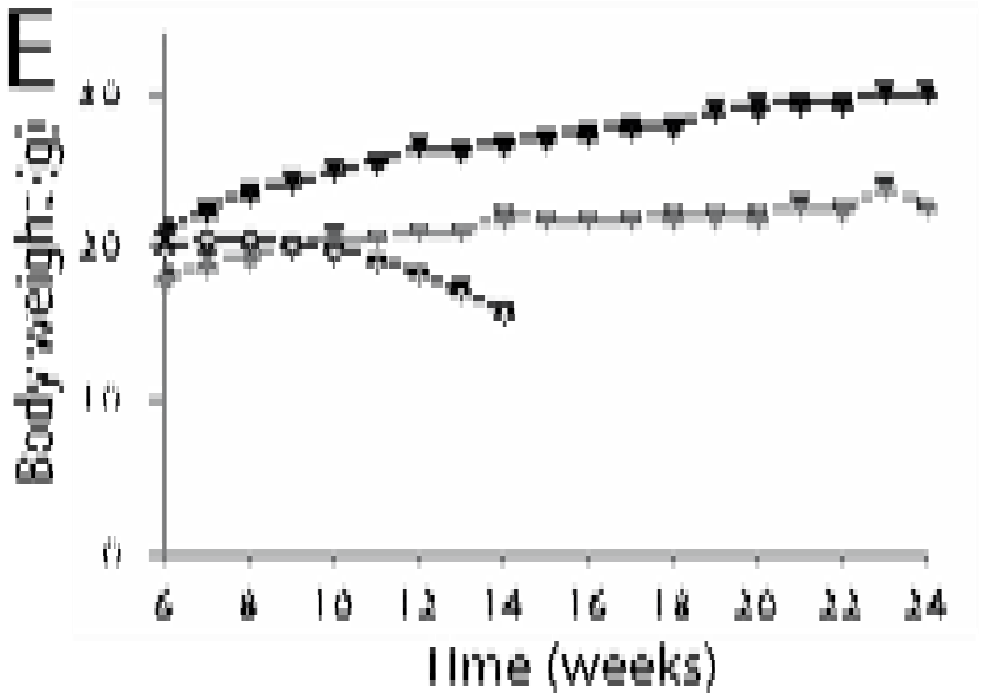
Mathilde Chivet
Pennuto's lab

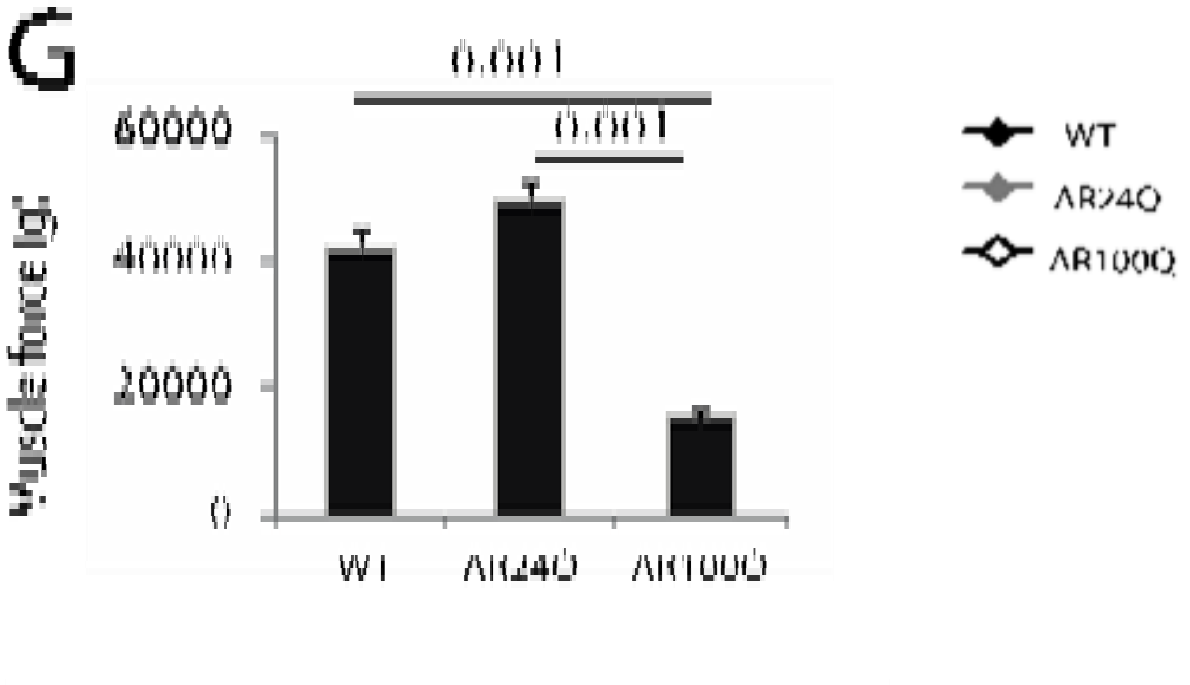
Life span is reduced in mice overexpressing expanded AR



Chivet et al., in preparation

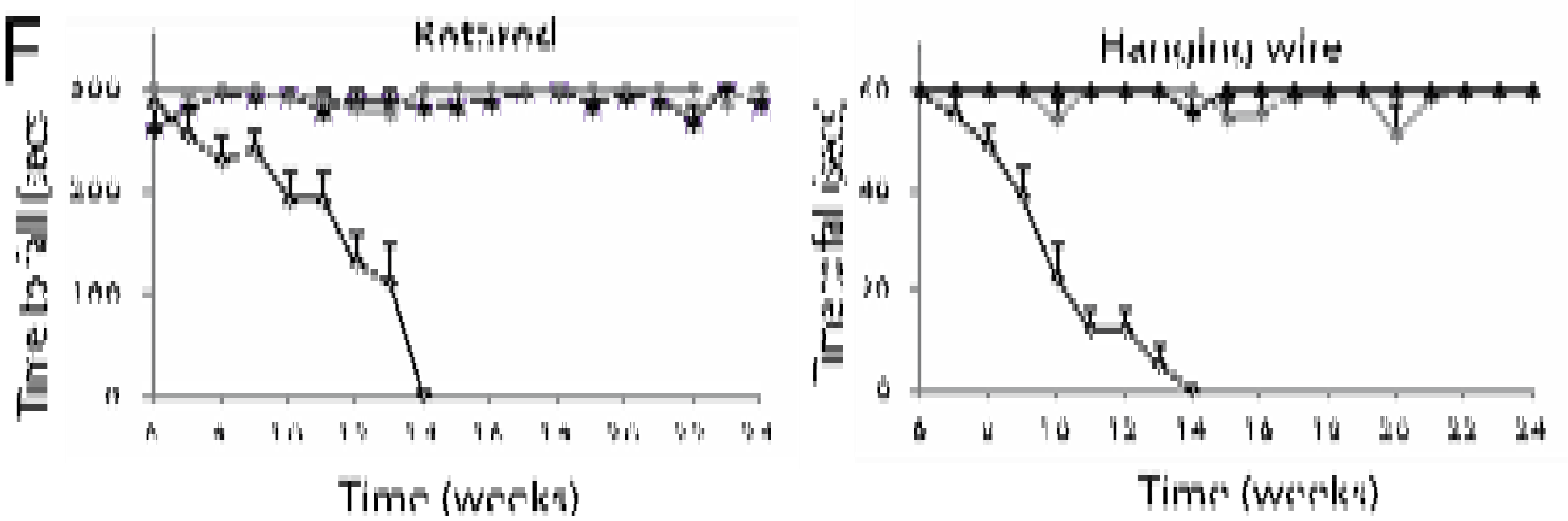
Weight body and strength are reduced in mice overexpressing expanded AR





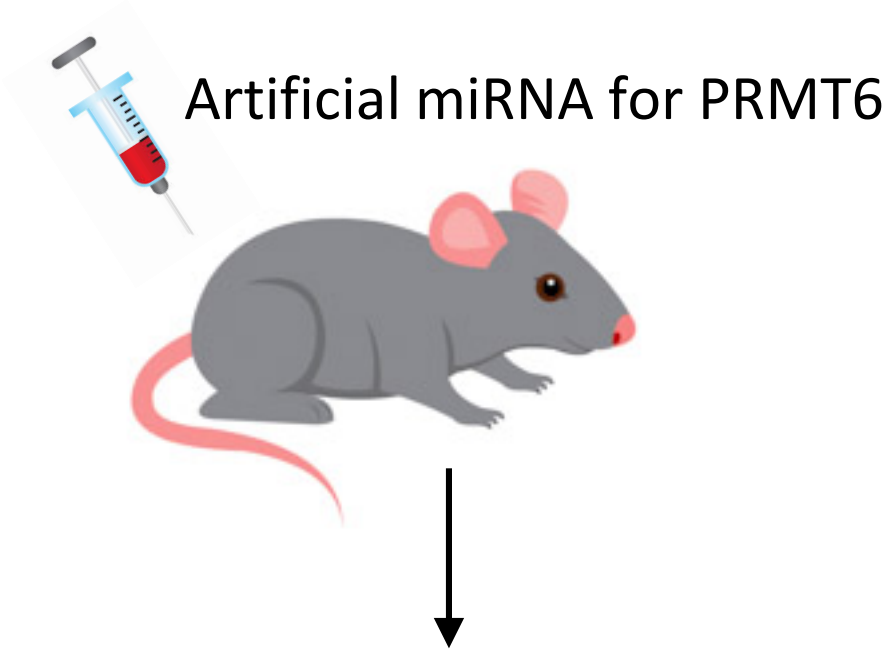
Chivet et al., in preparation

Motor coordination is also reduced in mice overexpressing expanded AR



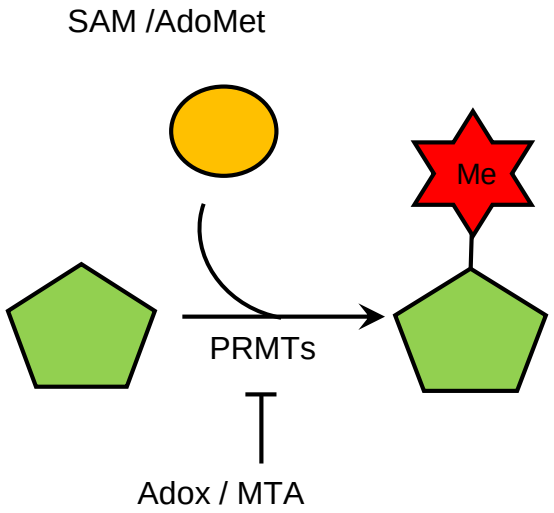
Chivet et al., in preparation

In progress:



In collaboration with Giuseppe Ronzitti, Genethon

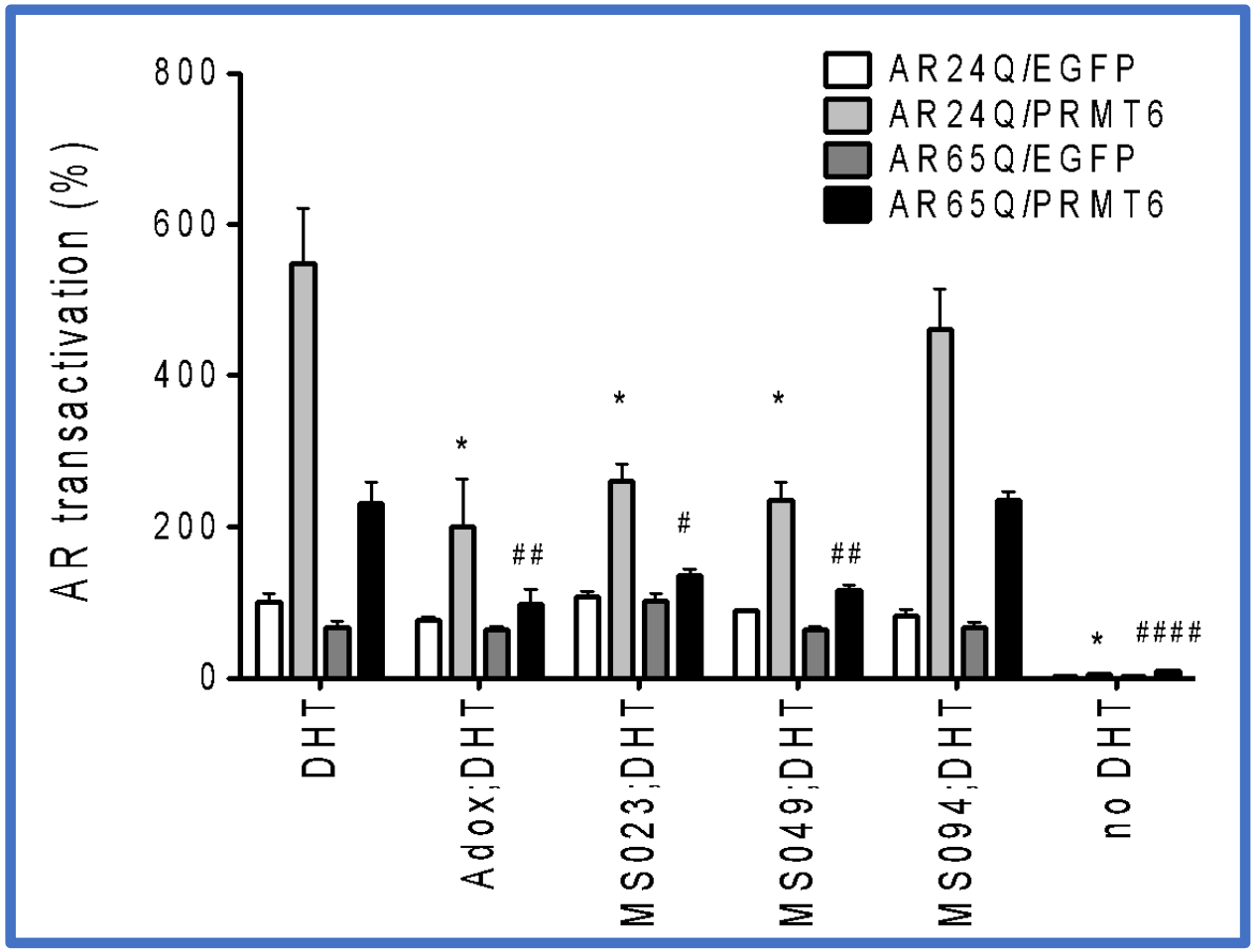
2



Novel pharmacological inhibitors
MS023 and MS049

In collaboration with Masoud Vedadi, University of Toronto

The novel inhibitors reduce AR-dependent transactivation



Where are we going?

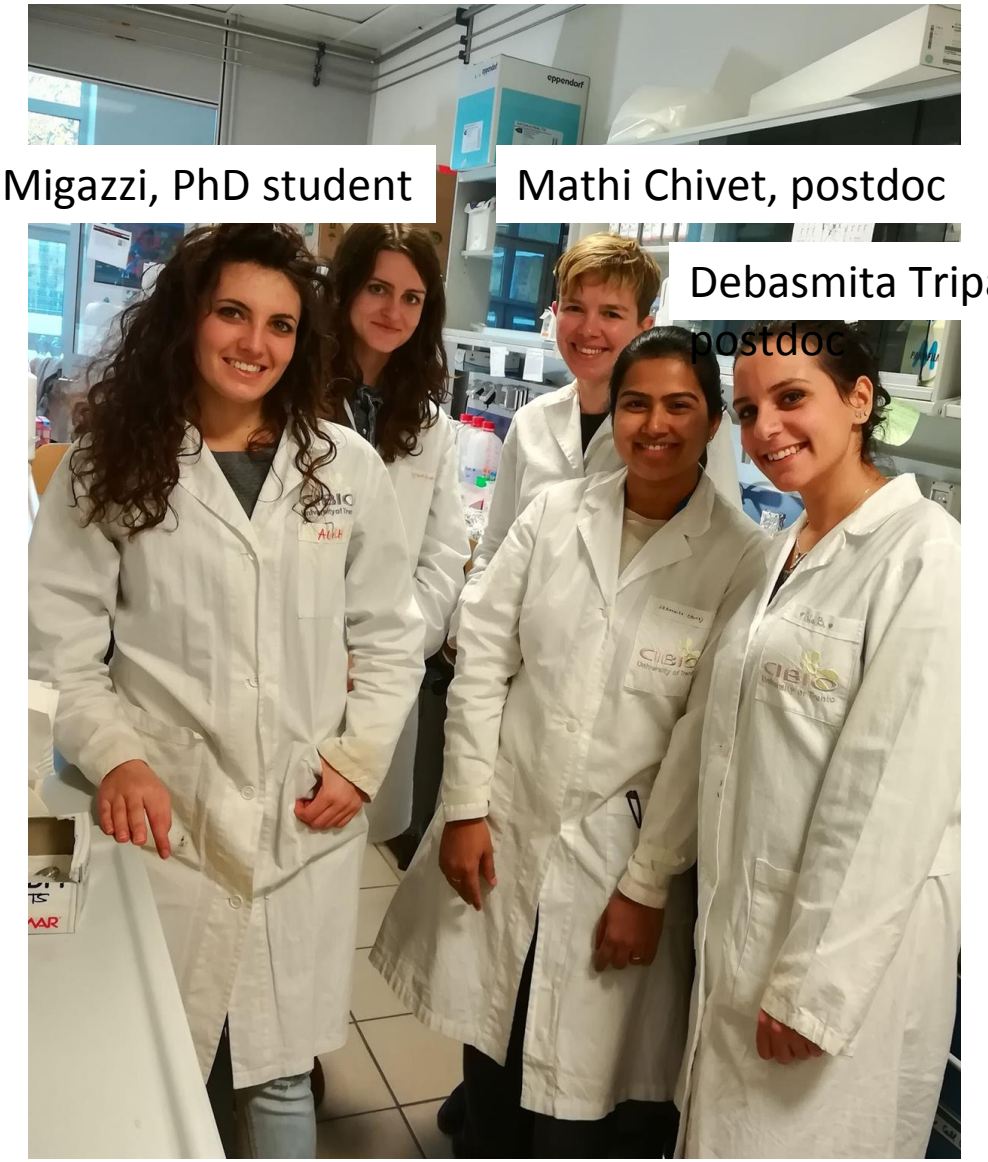
1-Which are other AR coactivators?

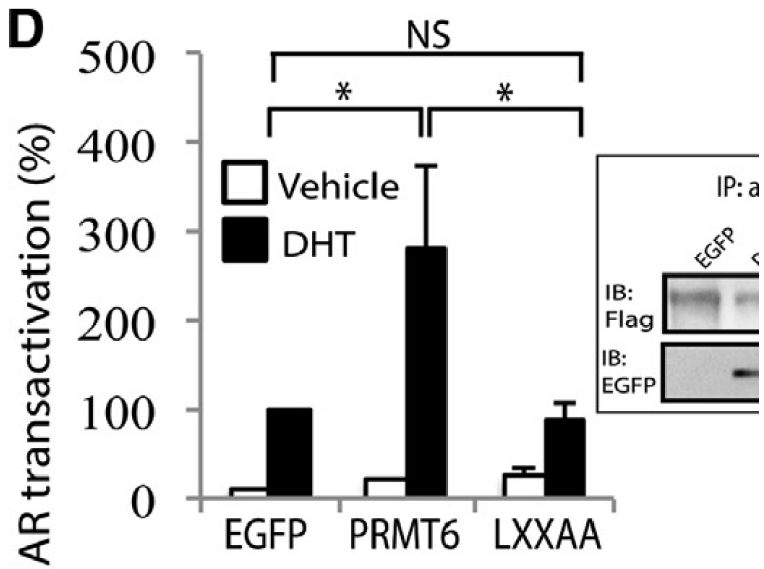
2-Is it feasible to reduce the over-activation of AR to correct its transcriptional activity but maintain its physiological functions?

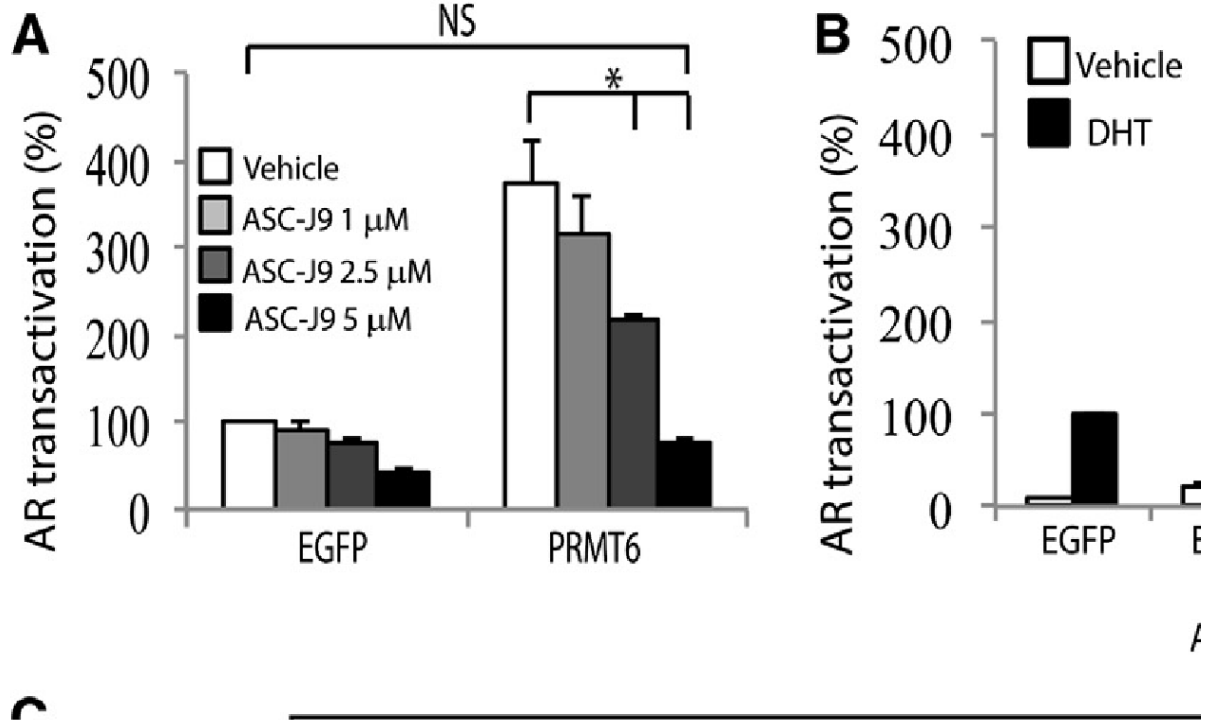
Acknowledgements

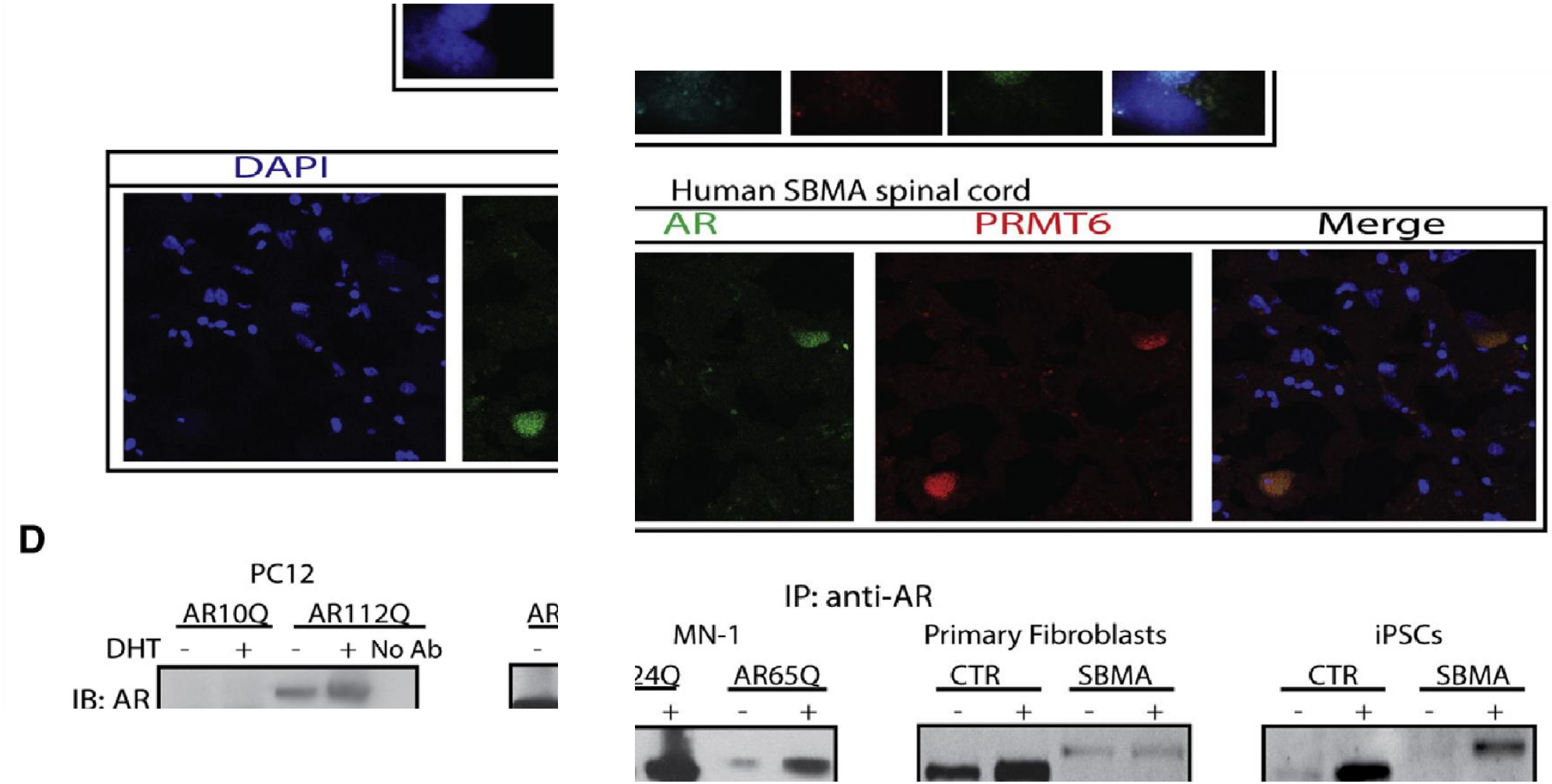
Maria Pennuto's Lab
University of Padova

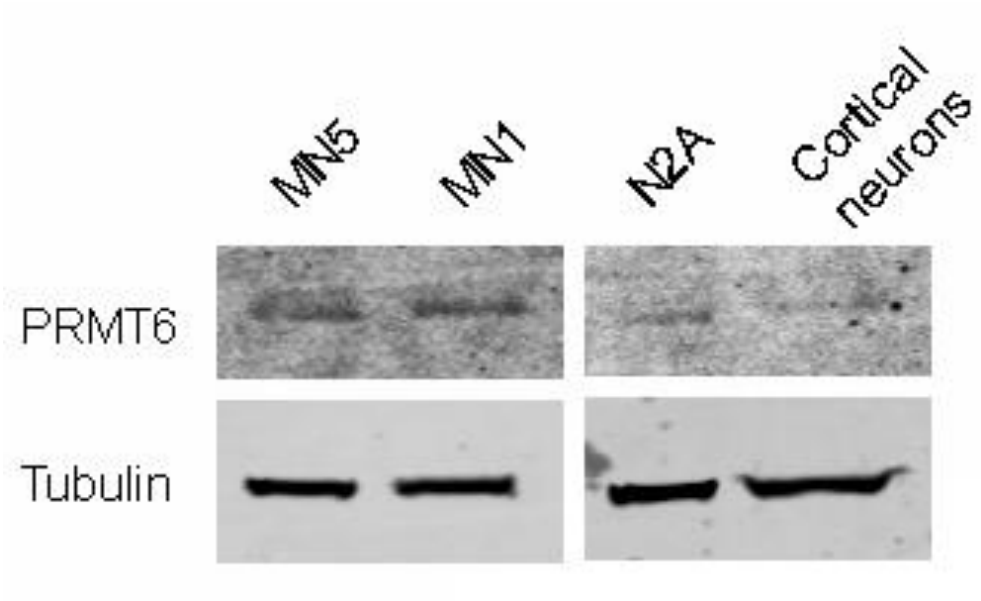
Mathilde Chivet (Postdoctoral fellow)
Carlo Rinaldi's Lab
Oxford University
Giuseppe Ronzitti
Genethon, France

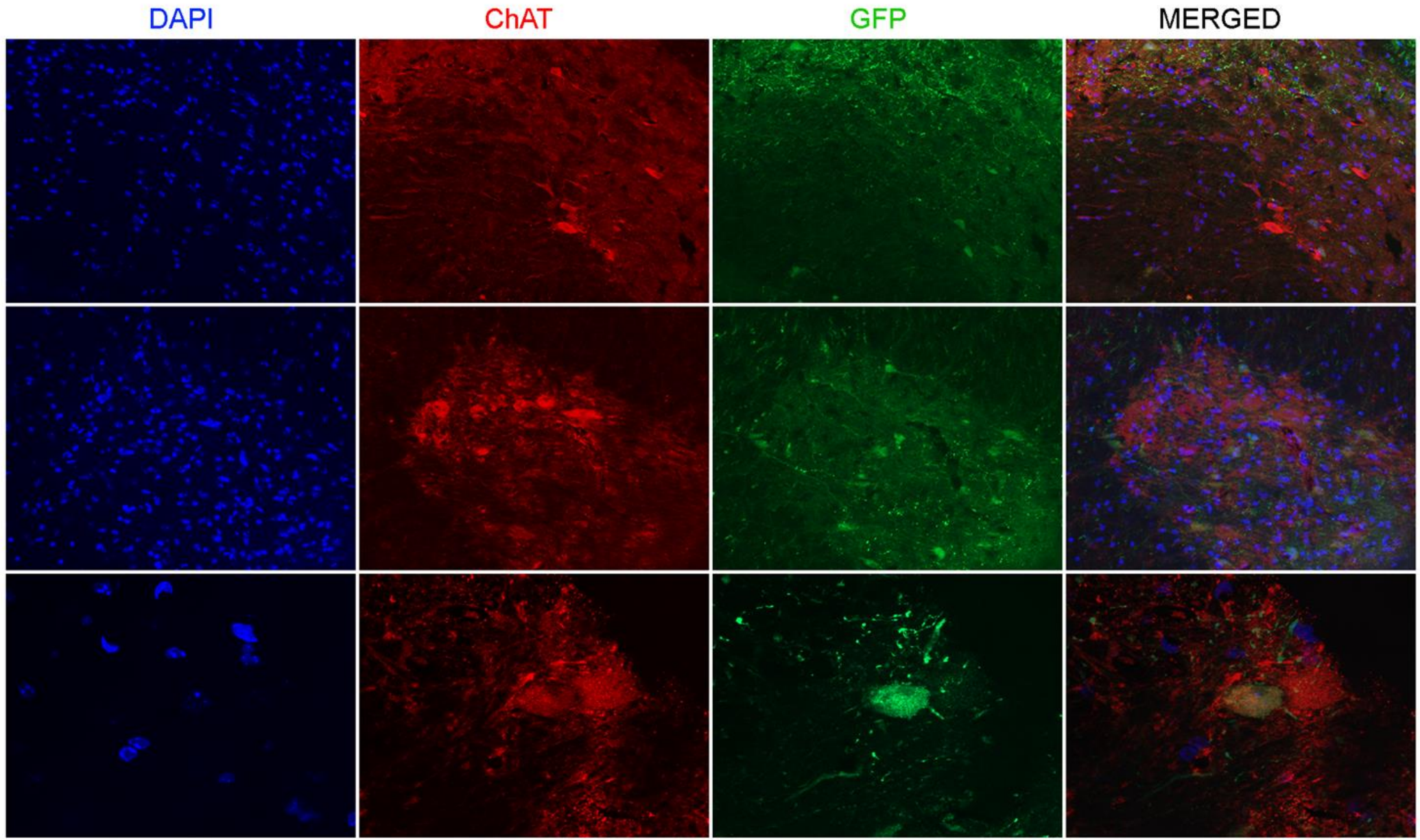


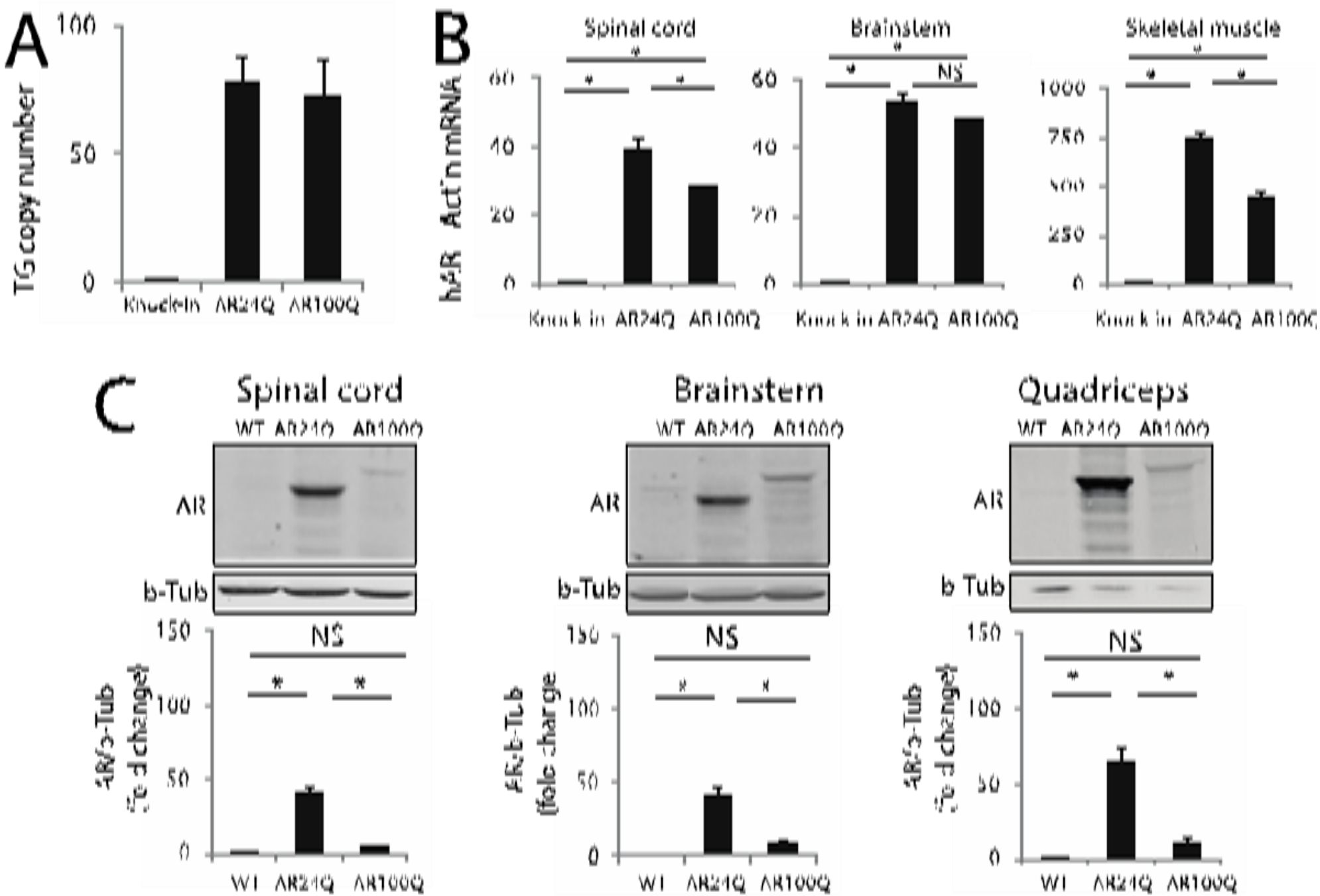

D
NS
*
*
500
400
300
200
100
0
AR transactivation (%)
Vehicle
DHT
EGFP
PRMT6
LXXAA
IP: a
EGFP
IB:
Flag
IB:
EGFP


A
NS
*
500
400
300
200
100
0
AR transactivation (%)
Vehicle
ASC-J9 1 μM
ASC-J9 2.5 μM
ASC-J9 5 μM
EGFP
PRMT6
B
500
400
300
200
100
0
AR transactivation (%)
Vehicle
DHT
EGFP


DAPI
Human SBMA spinal cord
AR
PRMT6
Merge
D
PC12
AR10Q
AR112Q
DHT - + - + No Ab
IB: AR
IP: anti-AR
MN-1
24Q
AR65Q
Primary Fibroblasts
CTR
SBMA
iPSCs
CTR
SBMA


MN5
MN1
N2A
Cortical neurons
PRMT6
Tubulin


DAPI
ChAT
GFP
MERGED



Chivet et al., in preparation