Developing iPSC-derived cellular assays of R155H p97/VCP to assess treatment a rare degenerative disorder

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IBMPFD (Inclusion body myopathy with Paget disease and frontotemporal dementia)

- Affect muscles, bones, and brain.
- Muscle weakness (myopathy) can affect respiratory and heart muscles, leading to life-threatening breathing difficulties.
- Weaken bones, resulting in pain, misshapen bones, fractures.
- Damages parts of the brain that control reasoning, personality, social skills, speech, and language.

http://www.usnon.com/genetics-of-pagets-disease-of-bone-like-disorders-inclusion-body-myopathy-pagets-disease-and-frontotemporal-dementia.htm

p97/Cdc48/VCP

AAA ATPase (ATPase associated with diverse cellular activities)

p97 is essential, highly conserved, and abundant

The first substrate: ubiquitin-β-galactosidase fusion protein*

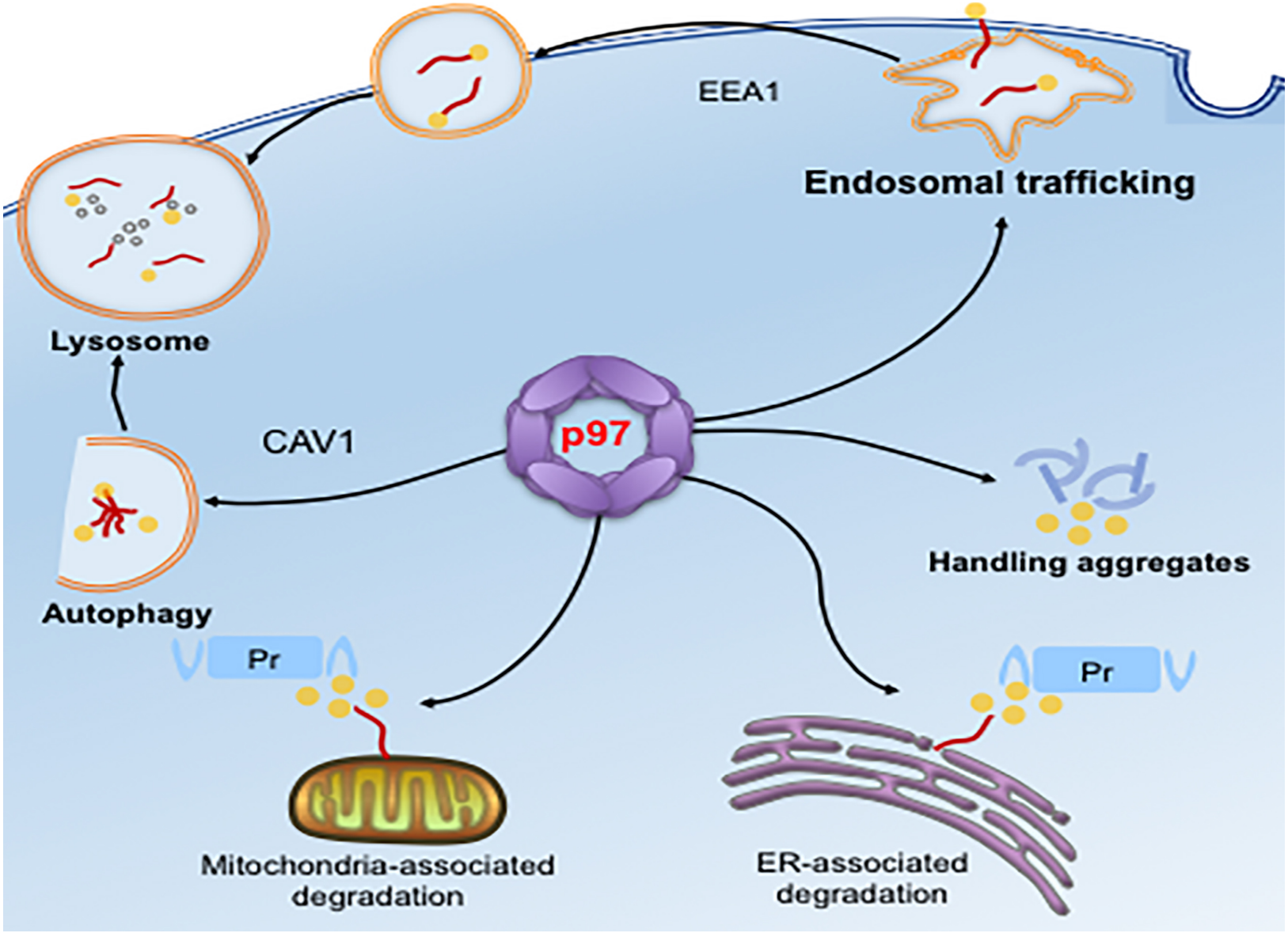
Play a critical role in the degradation of misfolded proteins
endoplasmic reticulum-associated degradation (ERAD)

Other cellular processes including:
Golgi membrane reassembly, membrane transport, myofibril assembly, cell division, formation of protein aggregates, autophagosome maturation

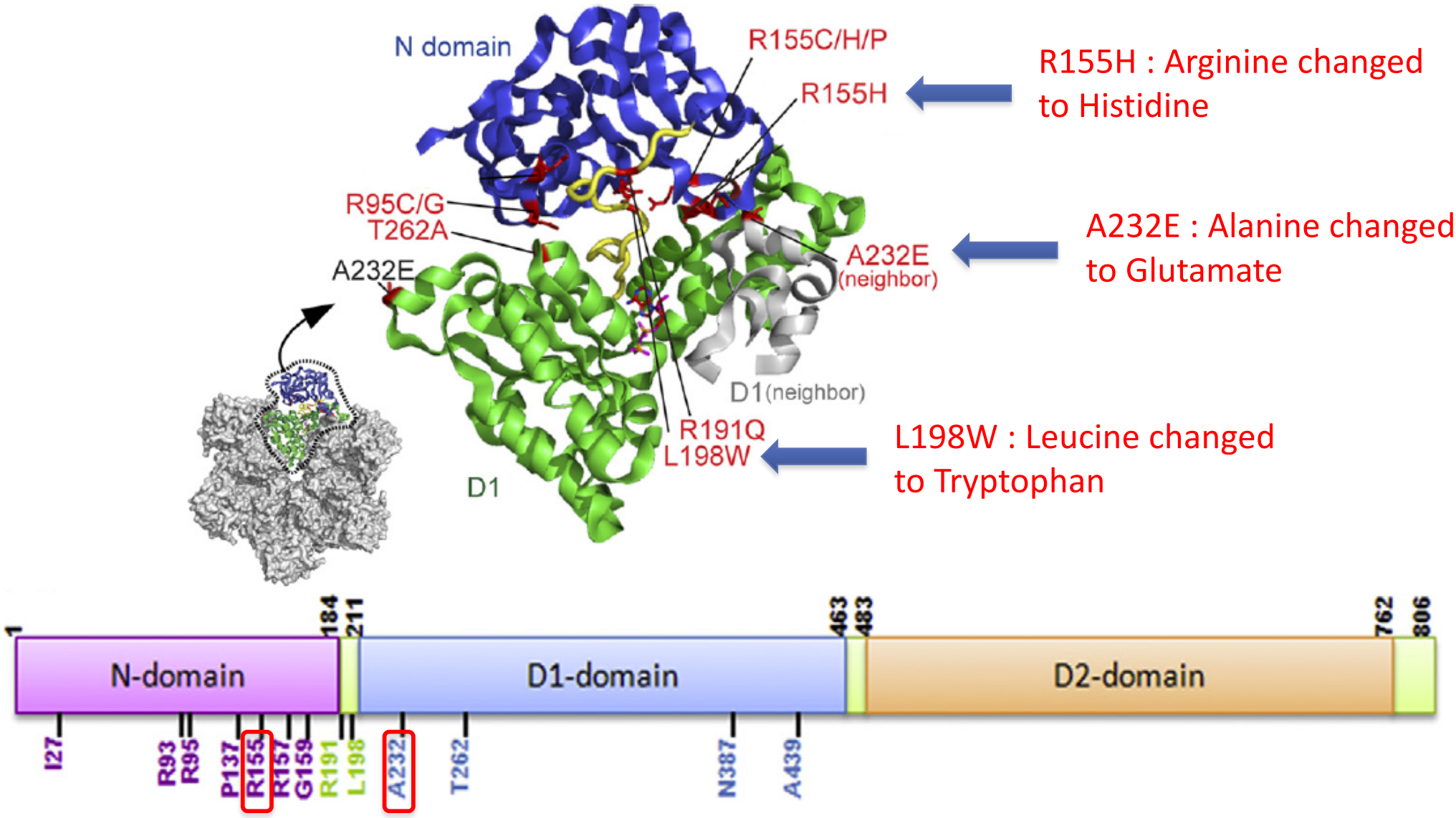
Mutation of p97 causes Inclusion Body Myopathy associated with Paget's disease of bone and Frontotemporal Dementia (IBMPFD) and Amyotrophic Lateral Sclerosis (ALS) know as Lou Gehrig's disease.

*Ghislain et. al., *EMBO J*. (**1996)**, 15, 4884

p97/VCP regulates protein homeostasis



Disease Mutations of p97



Meyer H, Weihl C C. *J. Cell Sci.*, (**2014**), 127,3877.

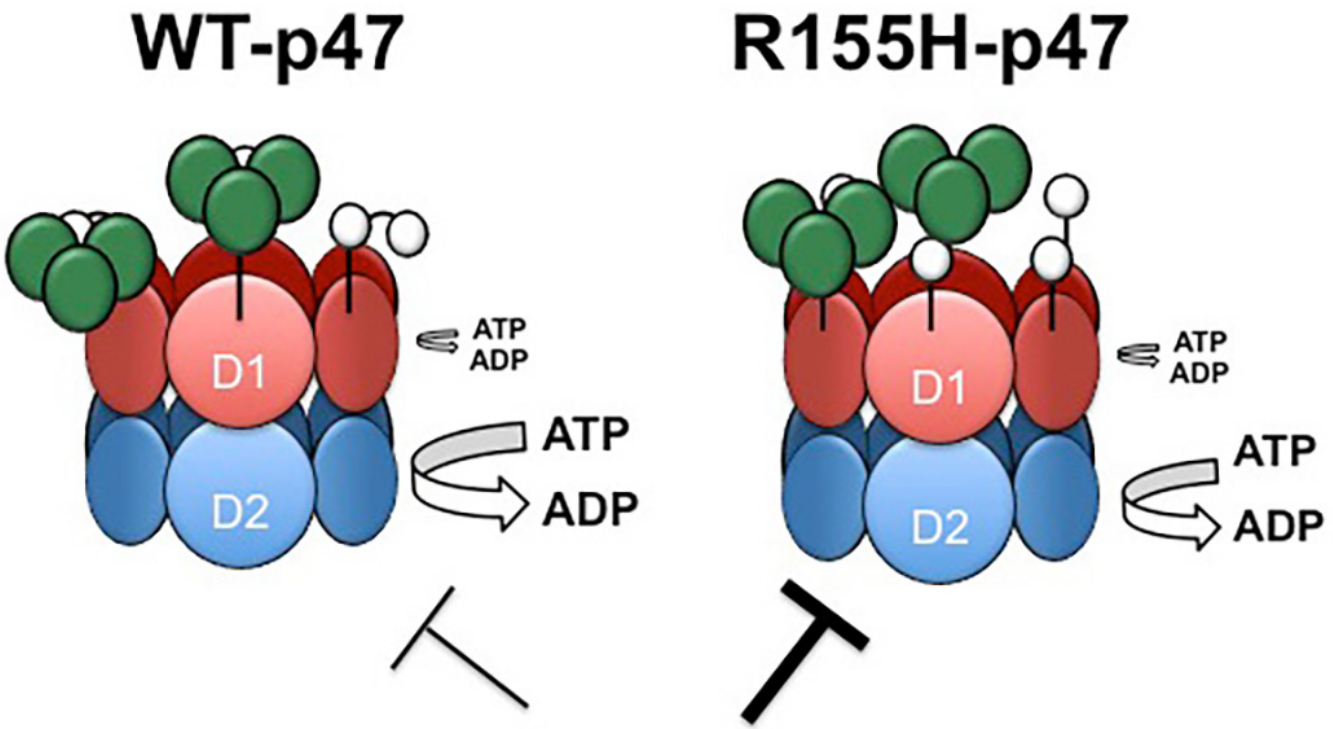
Selective mutant p97 modulator

- **Small molecule inhibitors/activators**

  IBMPFD mutants p97-cofactor complex

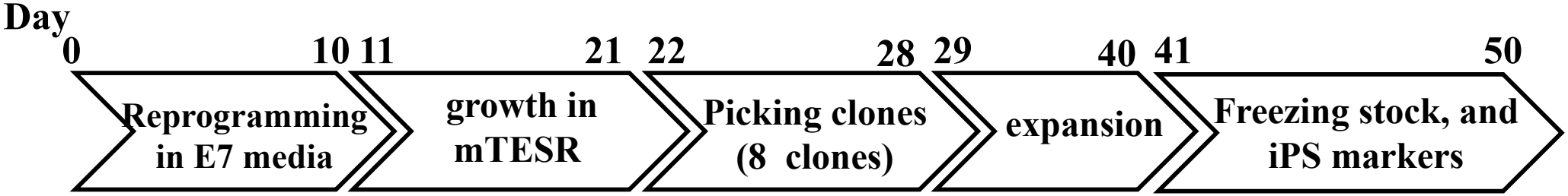
  High throughput screening at NIH/NCATS

  (National Center for Advancing Translational Sciences)

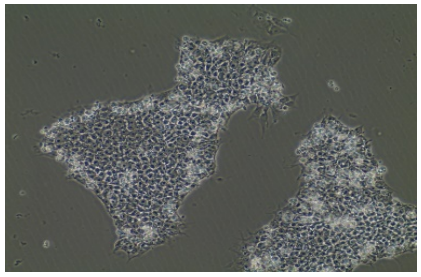


R155H-p47 selective
Selective index:
IC_{50} (WT-p47/R155H-p47) > 10

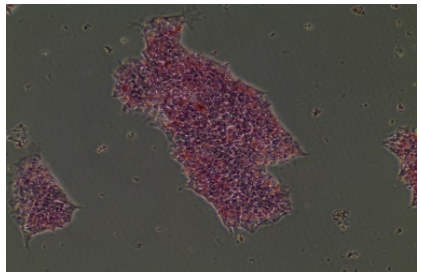
Generation of R155H p97 iPSC from patient's fibroblast



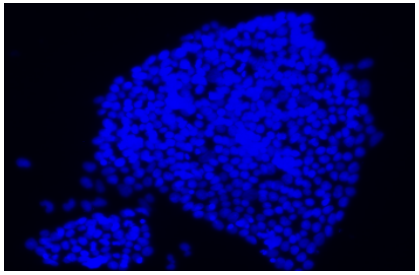
BF



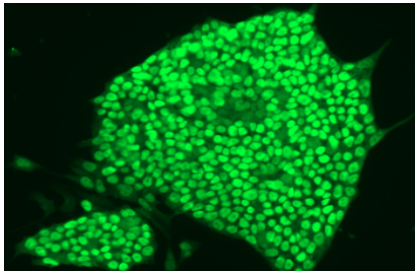
alkaline phosphatase



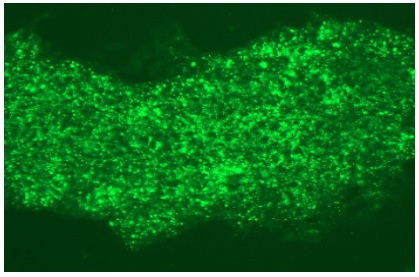
Hoechst



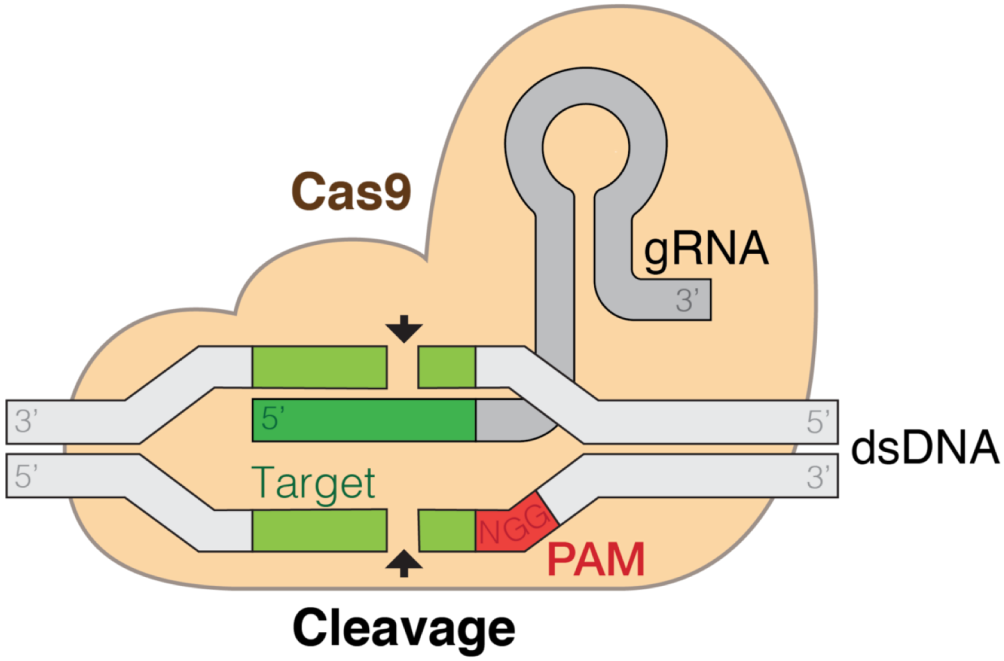
OCT4



SSEA4



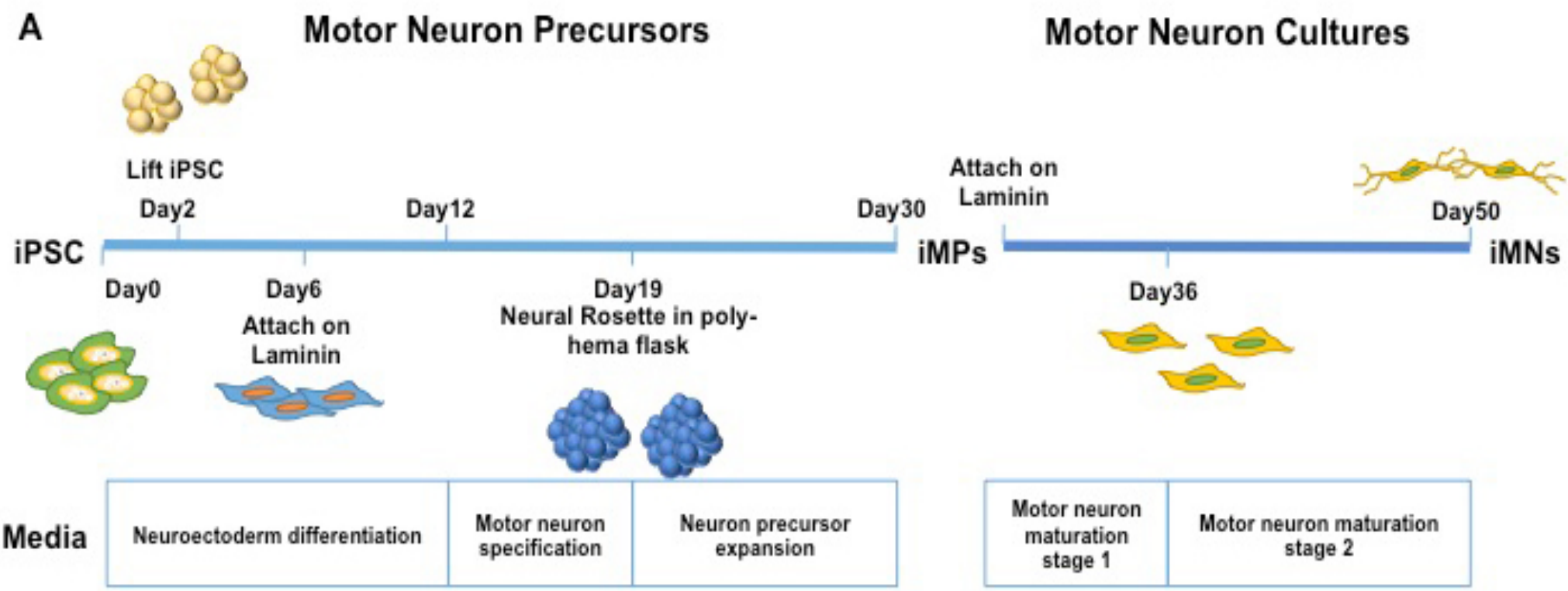
Generation of the isogenic control iPSC lines using CRISPR

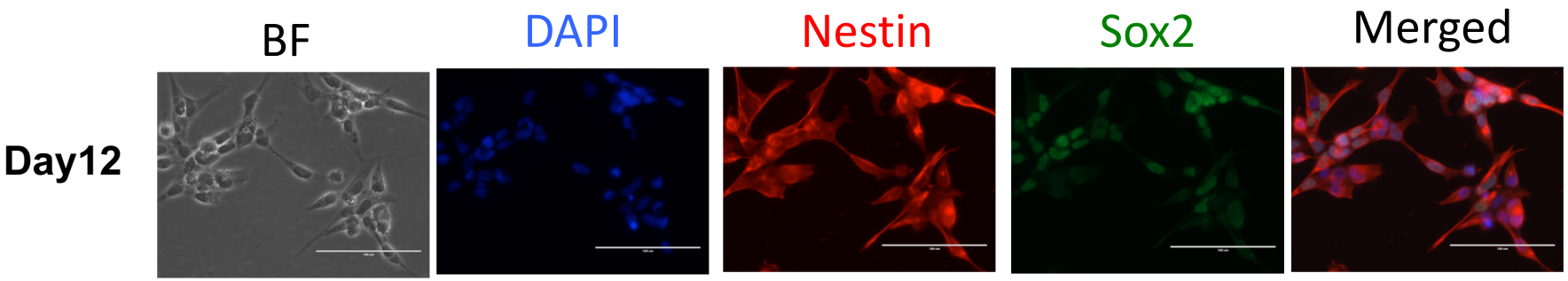


To change R155H +/- to WT (R155H -/-) in the patient derived iPSC cells

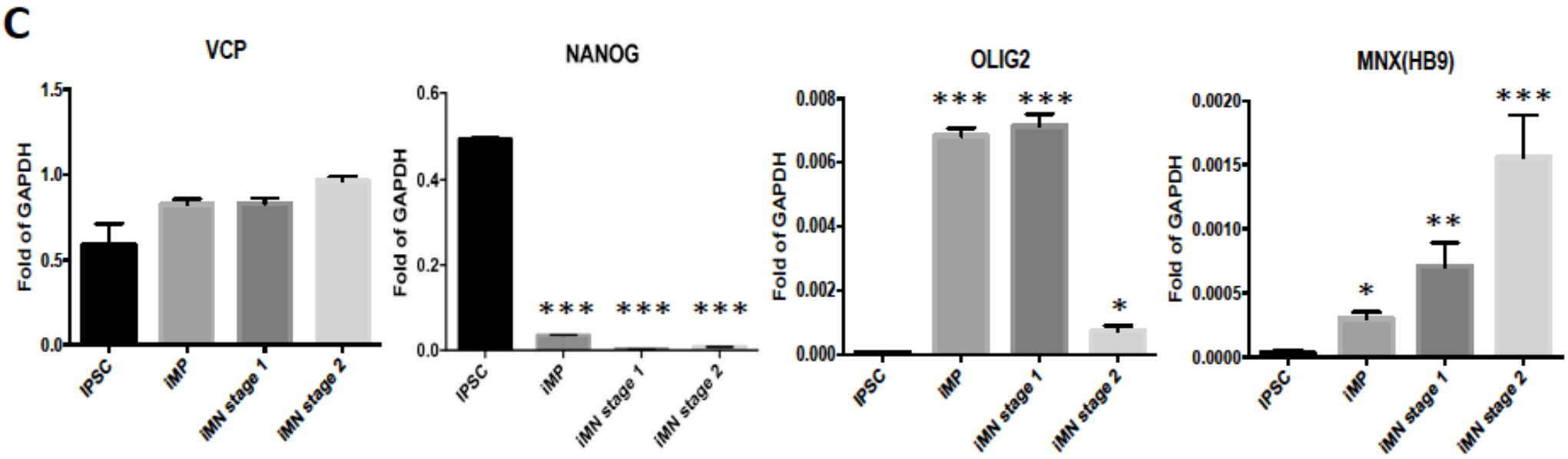
IDT: Alt-R® CRISPR-Cas9 System:
Cationic lipid delivery of CRISPR ribonucleoprotein complex into mammalian cells

iPSC-derived motor neurons





qPCR: expression level of markers for iPSC, iMP, and iMN



One of the R155H lines. We use p97/VCP as a negative control. Data were analyzed using GraphPad Prism software. N=3, T-test * p<0.05, ** p<0.01, *** p<0.001

Acknowledgement

Current lab members

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Staff Research associates: Betty Anderson, Chelsee Sauni

Former lab members

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Staff research associates: Daniel Wong, Derek Moen

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