The Cardiac Na Channel

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$Na_V1.5$: The Cardiac Sodium Channel

- Voltage gated
- Conducts the inward I_{Na} required for normal cardiac activity
- Encoded by the SCN5A gene
  - Loss-of-function mutation leads to decreased I_{Na}
  - Decreased I_{Na} causes arrhythmias

Sirtuin 1 regulates cardiac electrical activity by deacetylating the cardiac sodium channel

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Received 4 September 2016; accepted 17 January 2017; published online 13 February 2017; doi:10.1038/nm.4284

Sirtuin 1 Deacetylase

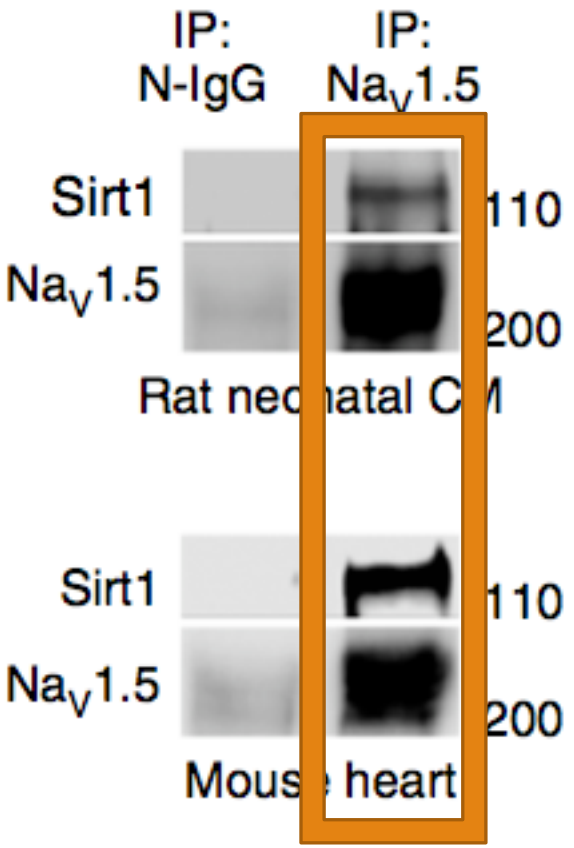
- a.k.a. Sirt1
- Function: deacetylates $Na_V1.5$ at lysine 1479 (i.e. K1479)
- Sirt1 overexpression = decreased ACETYLATION (i.e. increased DEacetylation)
- Sirt1 deficiency = HyperACETYLATION (i.e. decreased DEacetylation)

Research Question

- Known: NAD^+/NADH changes the membrane localization of $Na_V1.5$
- Sirt1 is a NAD^+ dependent deacetylase
- Question:
  - Does Sirt1 regulate I_{Na}, by deacetylating $Na_V1.5$, and if so, does it do this by signaling a change in membrane localization?

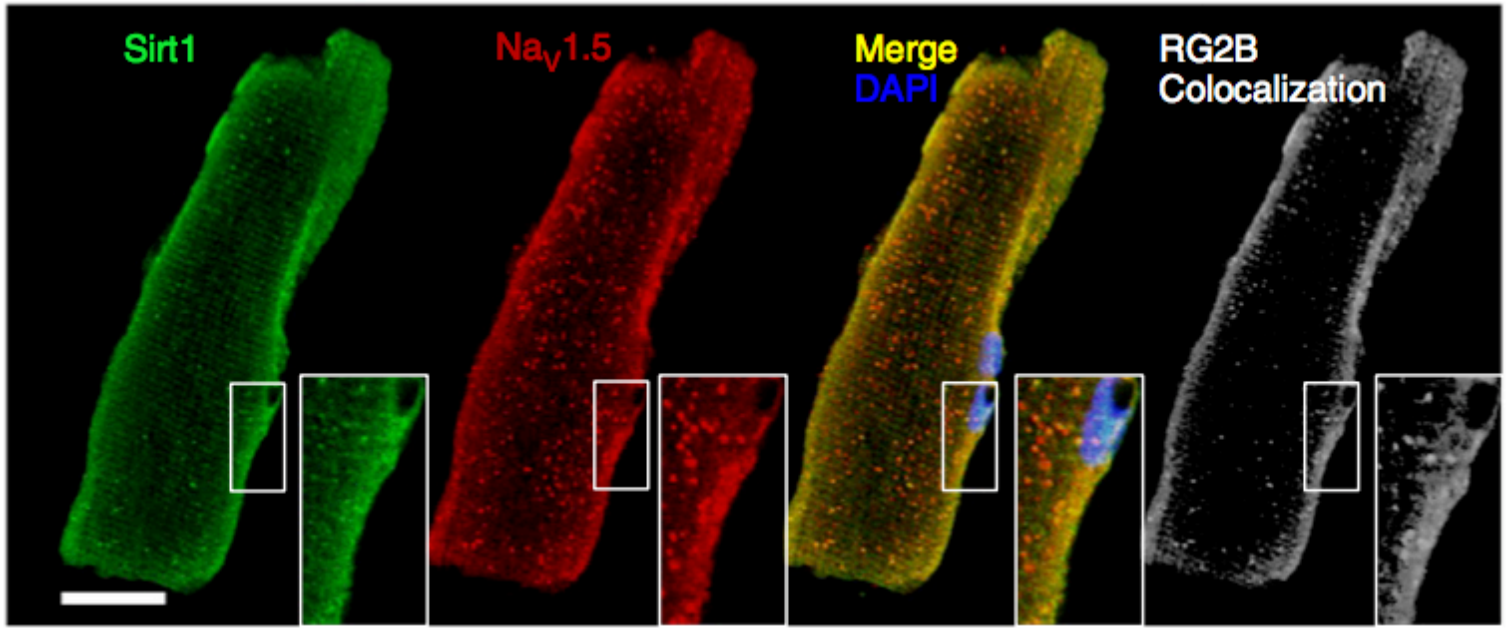
1. Interaction

- Sirt1 must interact with $Na_V1.5$
- Co-precipitation
- Neonatal rat cardiomyocyte and mouse heart

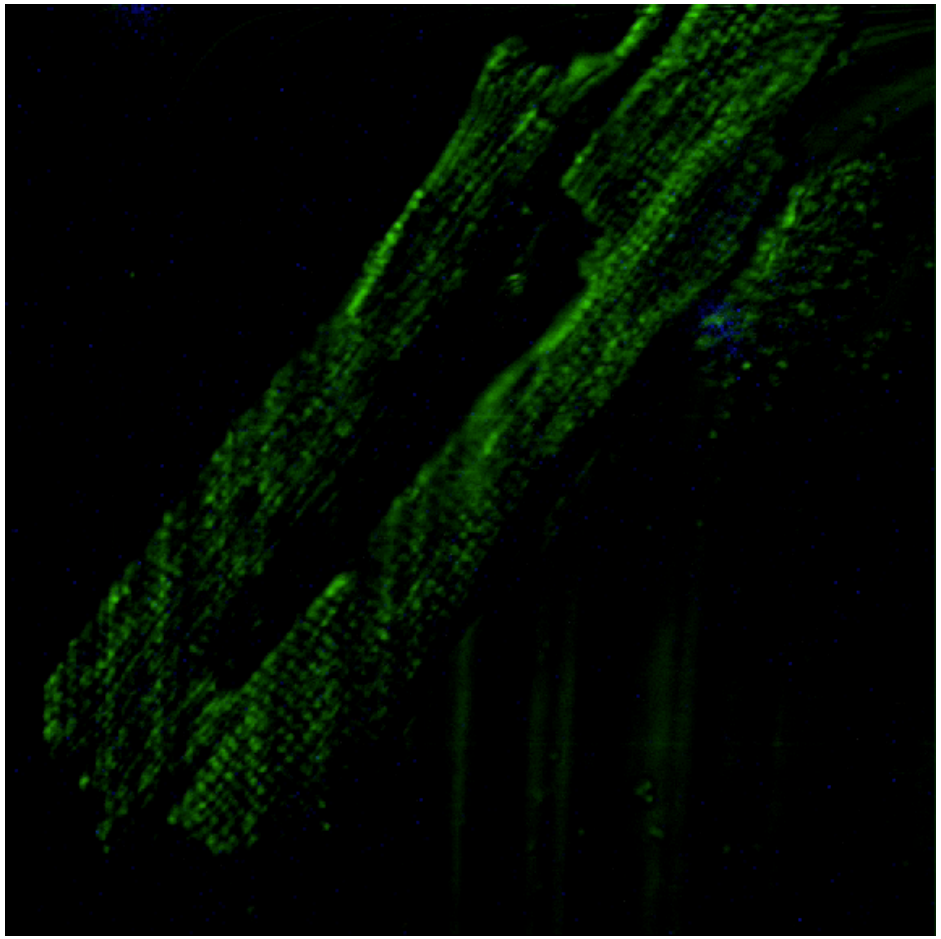


2. Localization

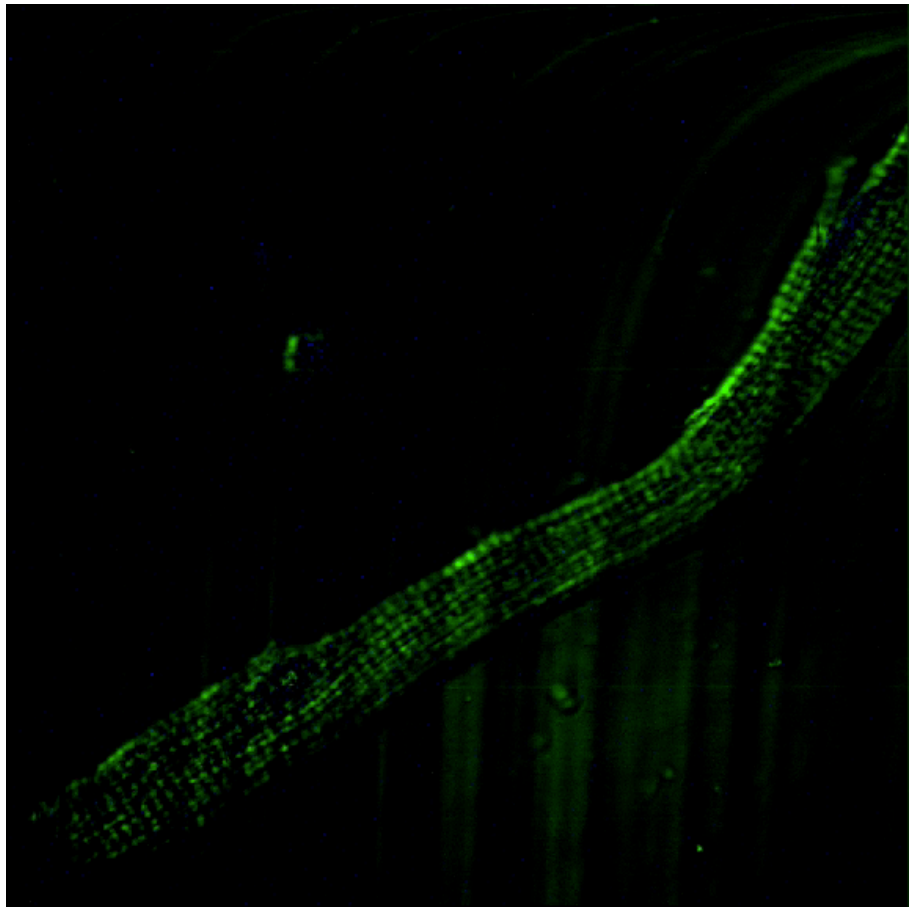
- Both $Na_V1.5$ and Sirt1 localized on the surface of mouse cardiomyocyte



2a. Cell Surface Expression - *Sirt1$^{fl/fl}$*

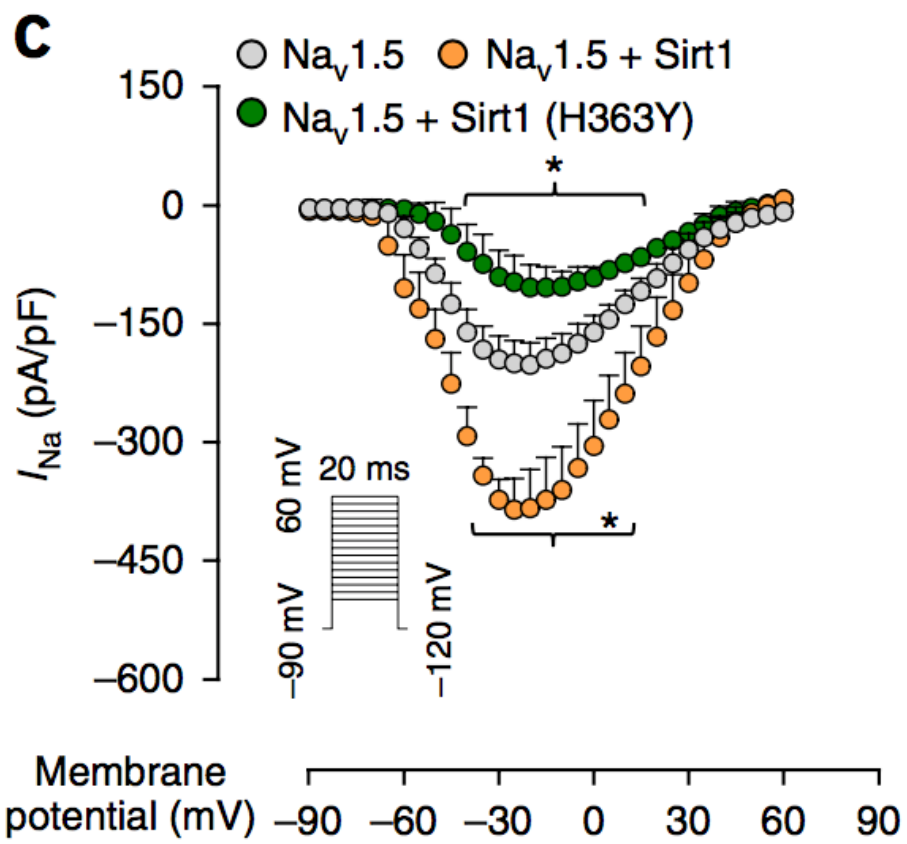


2b. Cell Surface Expression - *cSirt1*$^{-/-}$



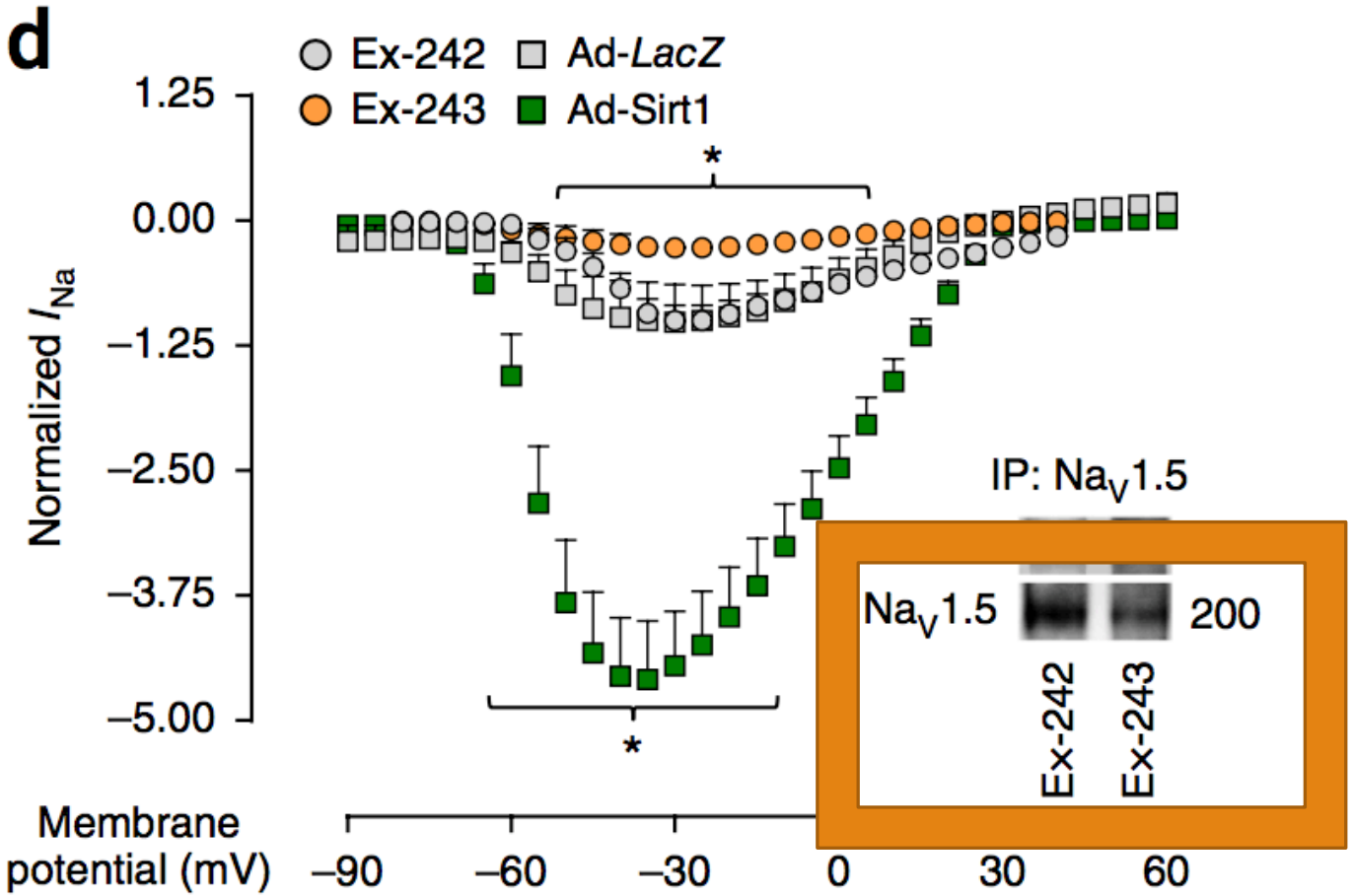
3. Effects on I_{Na}

- Whole cell patch clamp
- Sirt1 (H363Y) = catalytically inactive Sirt1



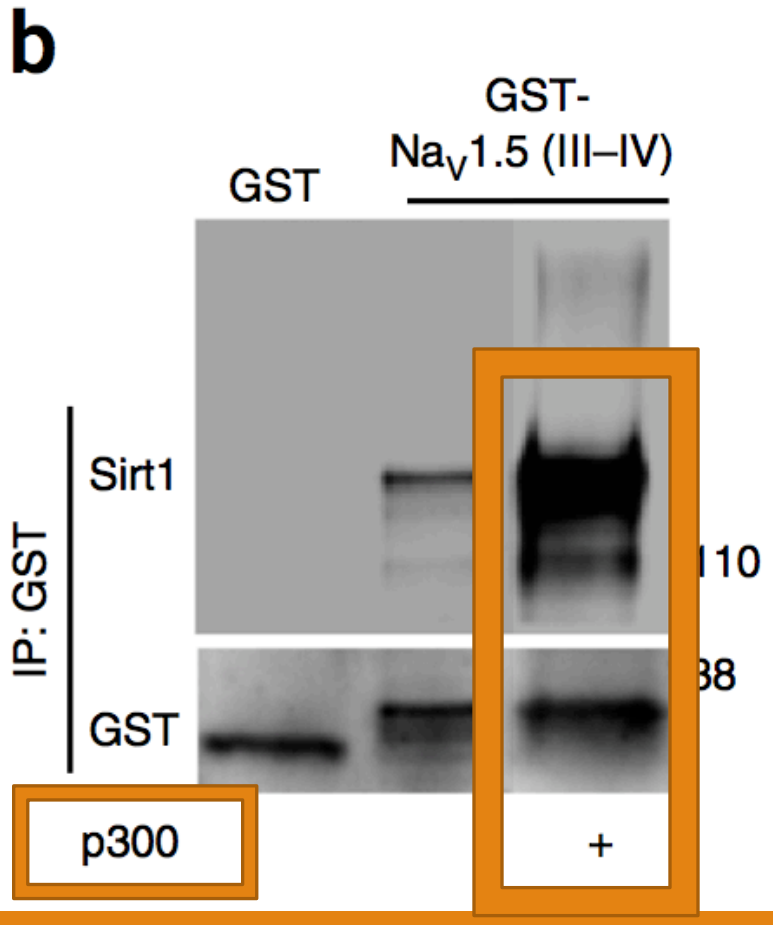
3. Effects on I_{Na}

- Ad-LacZ = control
- Ex-243 = Sirt1 inhibitor
- Ex-242 = inactive Sirt1 inhibitor



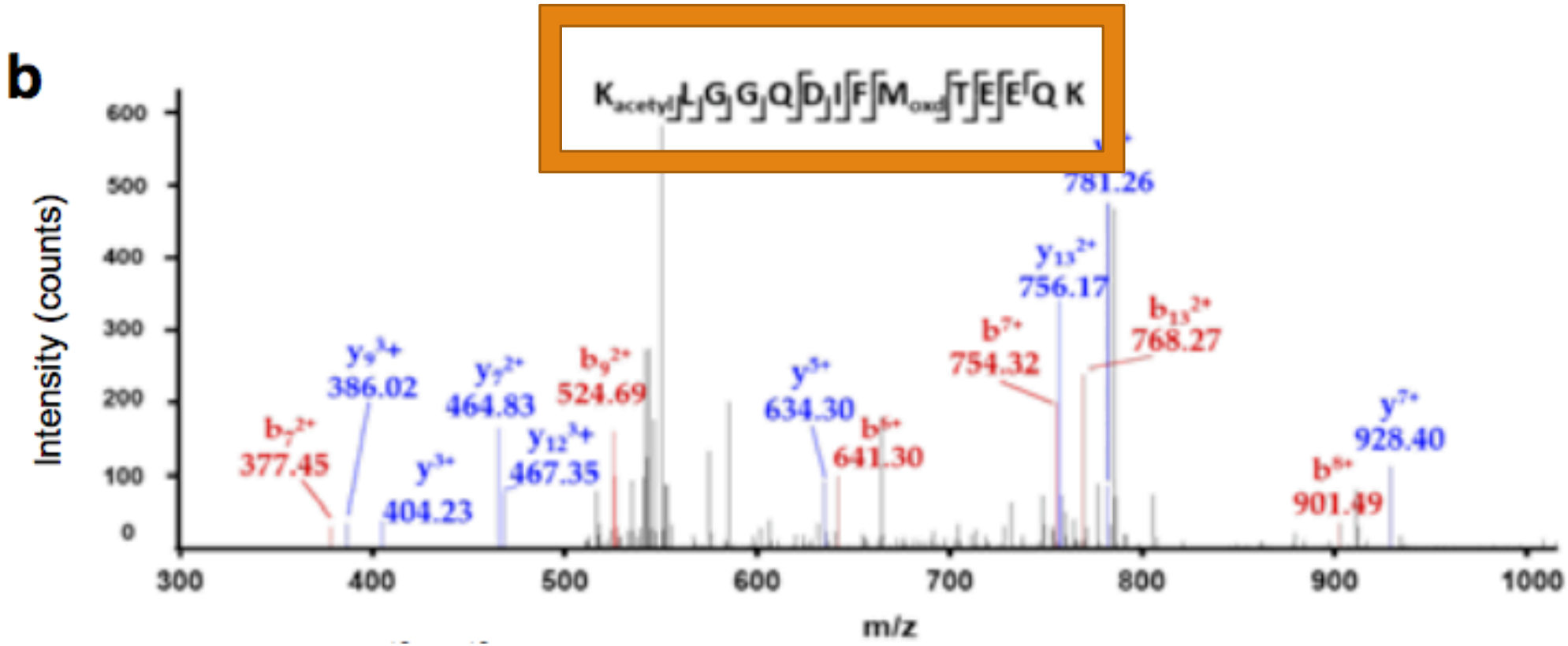
4. Lysine Specific Acetylation

- $Na_V1.5$ III-IV interdomain linker is lysine rich
- Highly conserved in voltage-gated sodium channels
- Lysine deletions and mutations
- Recombinant fusion protein: GST-$Na_V1.5$ (III-IV)



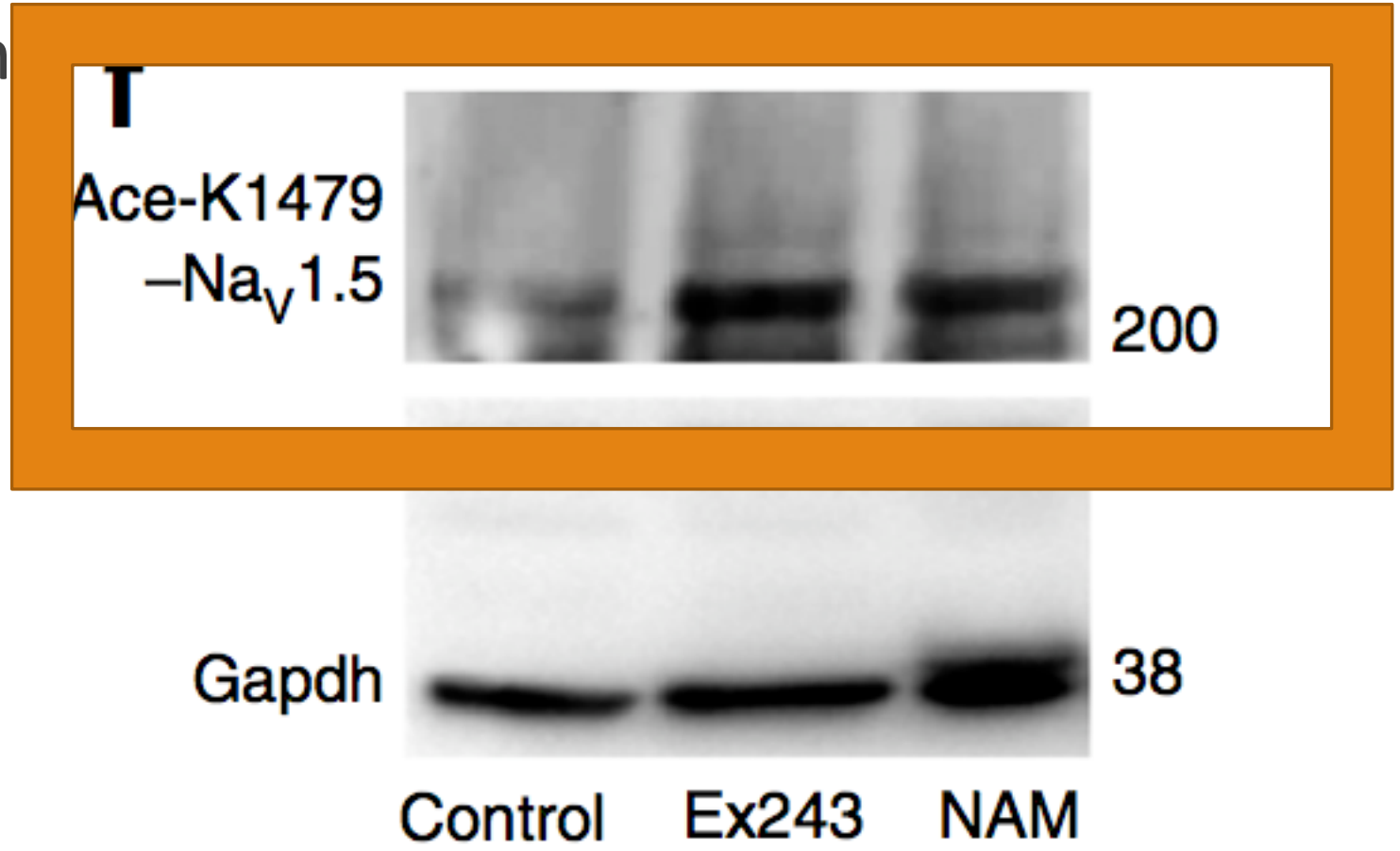
5. Which Lysine?

- MS spectrometry of GST-$Na_V1.5$ (III-IV) acetylated by p300



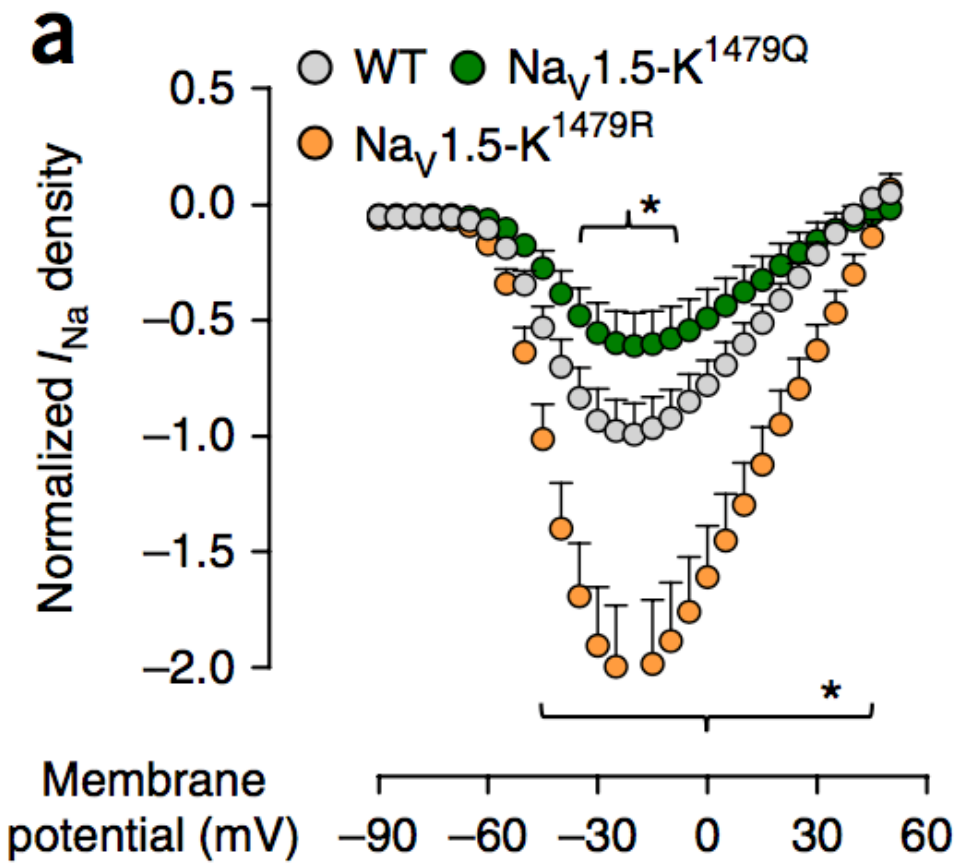
6. K1479

- Control = expression of Sirt1
- Ex-243 = Sirt1 inhibitor
- NAM = drug that inhibits Sirt1



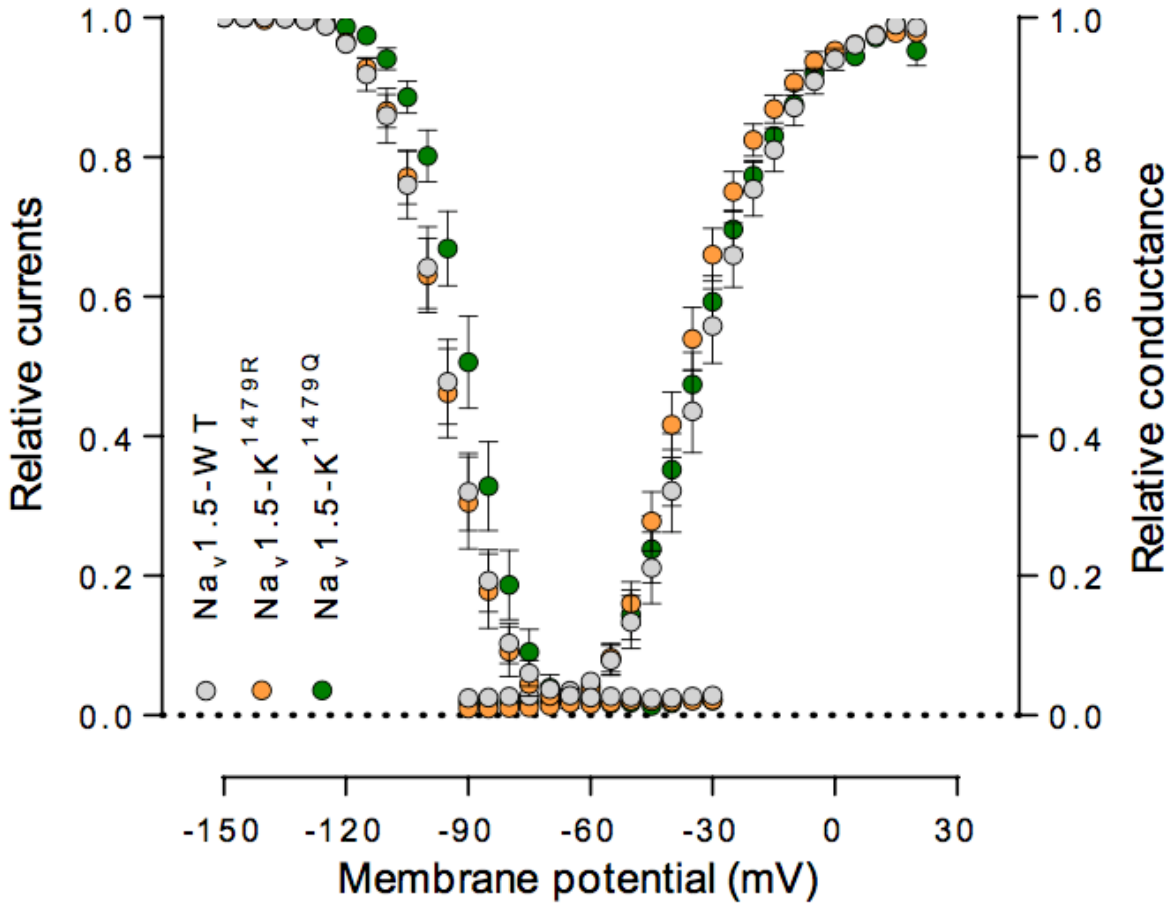
6. K1479

- $Na_V1.5\text{-}K^{1479Q}$ = mutant; constitutively acetylated
- $Na_V1.5\text{-}K^{1479R}$ = mutant; nonacetylatable



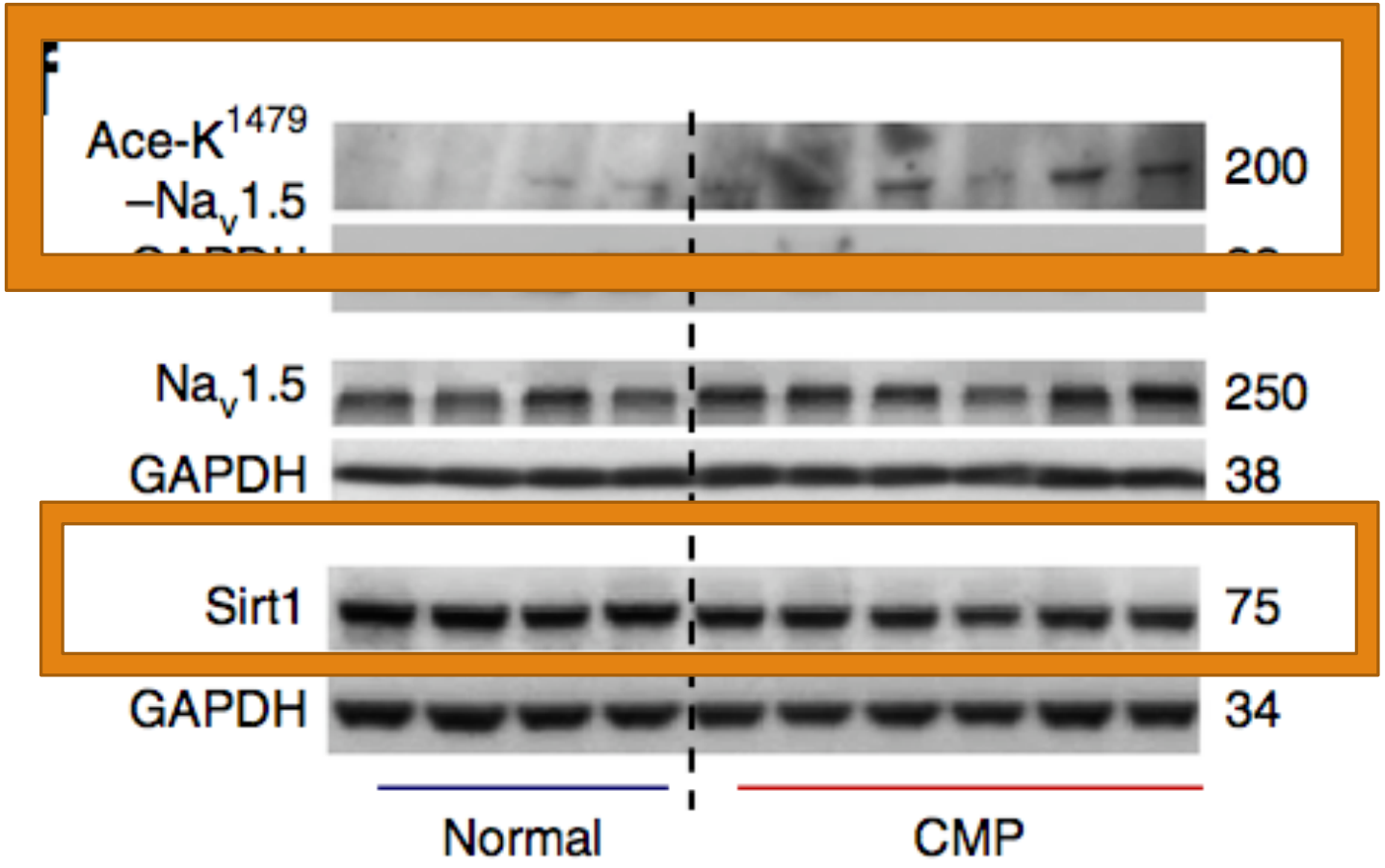
7. How?

- How does I_{Na} increase with deacetylation and decrease with acetylation?
- For $Na_V1.5\text{-}K^{1479Q}$, the steady state inactivation curve is shifted to the right (more positive)



8. Conclusion

- Sirt1 regulates sodium current by deacetylating the sodium cardiac channel on Lysine 1479
- Normal vs cardiomyopathic patient



9. Significance of this Study

- Role of K1479
- Pharmacological and genetic solutions to various arrhythmias
- Possible arrhythmia and heart failure treatments
- For future papers: How does this (de)acetylation “signal” for membrane localization? What’s the mechanism?

References

Vikram, Ajit et al. "Sirtuin 1 Regulates Cardiac Electrical Activity by Deacetylating the Cardiac Sodium Channel." *Nat Med*, vol. 23, no. 3, 2017, pp. 361-367, doi:10.1038/nm.4284

http://www.nature.com/nm/journal/v23/n3/abs/nm.4284.html#supplementary-information.