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MEETING ABSTRACT

A3.47

Dissecting the functions of multiple interactions of STAC3 in skeletal muscle excitation–contraction coupling

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Background: The adaptor protein STAC3, previously identified as an essential component of the excitation–contraction (EC) coupling machinery, was reported to have three distinct functions: (1) it facilitates the membrane expression of $Ca_V1.1$; (2) it is crucial for the function of $Ca_V1.1$ as a voltage sensor and as a calcium channel; (3) it is essential for the conformational coupling between $Ca_V1.1$ and the RyR1. We and others have previously identified two distinct interactions that STAC3 establishes with $Ca_V1.1$, one between the SH3-1 domain of STAC3 and the II–III loop of $Ca_V1.1$, and one between the C1-linker region of STAC3 and the proximal C-terminus of $Ca_V1.1$. However, which of these interactions is responsible for each function is still elusive.

Methods: Using the CRISPR/Cas9 method, we generated a double STAC3/$Ca_V1.1$ KO skeletal muscle cell line. As previously reported in mouse KO myotubes, in the newly generated cell line, $Ca_V1.1$ currents are negligible and EC coupling fails unless STAC3 expression is rescued. In order to determine which $Ca_V1.1$/STAC3 interaction is responsible for each function of STAC3, we are going to reconstitute STAC3 KO myotubes with STAC3 fragments that contain the domain responsible for that particular interaction.

Results and discussion: Ongoing recordings of $Ca_V1.1$ currents in the presence of either STAC3 fragment indicate that the interaction one between the C1-linker region of STAC3 and the proximal C-terminus of $Ca_V1.1$ is essential for the functionality of $Ca_V1.1$ as a calcium channel, whereas the one between the SH3-1 domain of STAC3 and the II–III loop loop of $Ca_V1.1$ is dispensable.

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Keywords: excitation–contraction coupling – STAC3 – $Ca_V1.1$ channels

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