



**Supplementary information, Fig. S3  $\alpha$ -MHC K1897R mutation reduces the interaction between  $\alpha$ -MHC and Titin.**

**a** Schematic of sarcomere in mice myocardial tissue. The intercepts showing the structure of the half A-band in sarcomere. **b** Overview of Titin structure. Ig domain was painted in dark red, fn3

domain was painted in light red. Underneath, the location of the fn3-fragments (I106-108, A77-78, A80-82 & A84-86) in Titin was indicated. **c** IP showed diminished interaction between  $\alpha$ -MHC and Titin resulting from the mutation of  $\alpha$ -MHC K1897R in HEK293T cells. Anti-Myc magnetic beads were added in per immunoprecipitated samples, followed by detection of His-Titin fragments (I106-108, A77-78, A80-82 & A84-86) and  $\alpha$ -MHC K1897 Lactyl Lysine. **d** Schematic showed diminished interaction between  $\alpha$ -MHC and Titin resulting from mutation of the  $\alpha$ -MHC K1897 site. **e-l** Interaction between  $\alpha$ -MHC and Titin under physiological conditions and Ang II treatment conditions were determined by IP analysis. (**e-h**) HEK293T cells were transfected with plasmids expressing Myc- $\alpha$ -MHC WT, Myc- $\alpha$ -MHC K1249R, Myc- $\alpha$ -MHC K1533R, Myc- $\alpha$ -MHC K1897R and transfected with plasmids expressing fn3-fragments His-Titin I106-108 (**e**), His-Titin A77-78 (**f**), His-Titin A80-82 (**g**), His-Titin A84-86 (**h**). Equal amounts of lysates were prepared for IP with Anti-Myc magnetic beads, followed by detection of His. (**i-l**) HEK293T cells were transfected with plasmids expressing Myc- $\alpha$ -MHC WT, Myc- $\alpha$ -MHC K1249R, Myc- $\alpha$ -MHC K1533R, Myc- $\alpha$ -MHC K1897R and transfected with plasmids expressing fn3-fragments His-Titin I106-108 (**i**), His-Titin A77-78 (**j**), His-Titin A80-82 (**k**), His-Titin A84-86 (**l**) without or with Ang II treatment. Equal amounts of lysates were prepared for IP with anti-Myc magnetic beads, followed by detection of His.