Appendix Figures

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Appendix Figure S1IP: β-cateninIgGInputβ-catenin--

Appendix Figure S1: Specificity of the β -catenin antibody used for affinity purification. Representative β -catenin immunoprecipitation used for LC MS/MS analysis. Non-programmed rabbit IgG was used as a control.

Appendix Figure S2



Appendix Figure S2: Specificity of the β -catenin antibody used for immunofluorescence. EYFP-wild-type- β -catenin (Top panel, full length protein) or EYFP-activated- β -catenin (Bottom panel, aa 1-89 removed) was ectopically expressed in A10 cells then fixed and staining using β -catenin primary antibody (recognizing an epitope in the region surrounding aa 37) and tetramethylrhodamine (TRITC, red) secondary antibody for immunofluorescent analysis. Hoechst 33342 was used for nuclear staining (blue). β -catenin antibody recognizes EYFP-wt β -catenin, but not EYFP-act. β -catenin, which is seen by comparing EYFP and TRITC signals, indicates the specificity of the antibody.

Appendix Figure S3



Appendix Figure S3: Assessment of the co-localization of β -catenin and Plectin. A10 cells were fixed and stained for β -catenin (green), Plectin (red), and nucleus (blue). Co-localization of these proteins was analysed by an RGB line scan. Green arrows indicate un-matched β -catenin and FMRP (green and red lines, respectively) and red arrows indicate matching peaks, demonstrating that the Plectin signal is independent of the β -catenin signal.

Appendix Figure S4



Appendix Figure S4: Assessment of the co-localization of β -catenin and Plectin. A10 cells were treated similarly to (S3) and a set of Z-stack images (240nm interval) was used to render orthogonal projection images. Yellow colour indicates co-localization of Plectin and β -catenin.



Appendix Figure S5: Specific changes in β -catenin localization by GBP-Lifeact. C2C12 myoblasts were transfected with GBP-Lifeact and EYFP- β -catenin and subjected to fluorescent and immunofluorescent analysis, as indicated. The matching peaks of the line scanned RGB signals from F-actin (blue), EYFP- β -catenin (green), and β -catenin (red) demonstrates robust recruitment of EYFP-fusion protein by anchored GBP on the F-actin.



Appendix Figure S6-i: Flag peptide does not interact with neither GBP, EYFP, nor β -catenin. EYFP- β -catenin was co-expressed with Flag-Myocardin 935 (MyoC 935) or Flag-Traf6 with (i) GBP-Lamin B1. The transfected cells were subjected to immunofluorescence analysis with a Flag antibody (AF546, red) and the nucleus was visualized by DNA staining with Hoechst 33342. The micrograph shows EYFP- β -catenin (green) was trapped at anchor sites (nuclear envelope with GBP-Lamin B1 or F-actin with Lifeact-GBP, respectively). Flag-MyoC 935 and Flag-Traf6 were localized at the expected sub-cellular location such as the nucleus for MyoC 935 and cytosol for Traf6. Line scanning of the RGB shows no enrichment of the red signal on either green signal peaks.



Appendix Figure S6-ii: Flag peptide does not interact with neither GBP, EYFP, nor β -catenin. EYFP- β -catenin was co-expressed with Flag-Myocardin 935

(MyoC 935) or Flag-Traf6 with (ii) GBP-Lifeact. The transfected cells were subjected to immunofluorescence analysis with a Flag antibody (AF546, red) and the nucleus was visualized by DNA staining with Hoechst 33342. The micrograph shows EYFP- β -catenin (green) was trapped at anchor sites (nuclear envelope with GBP-Lamin B1 or F-actin with Lifeact-GBP, respectively). Flag-MyoC 935 and Flag-Traf6 were localized at the expected sub-cellular location such as the nucleus for MyoC 935 and cytosol for Traf6. Line scanning of the RGB shows no enrichment of the red signal on either green signal peaks.



Appendix Figure S7: Determination of the conditions for translational inhibition by cycloheximide. A10 cells were transfected with Flag-FMRP and treated with cycloheximide (50 ng/ μ L) or its solvent (DMSO) for the indicated times and subjected to Western blot analysis for expression levels of indicated proteins. Actin was used as a loading control, and dsRed as an indicator for transfection efficiency.