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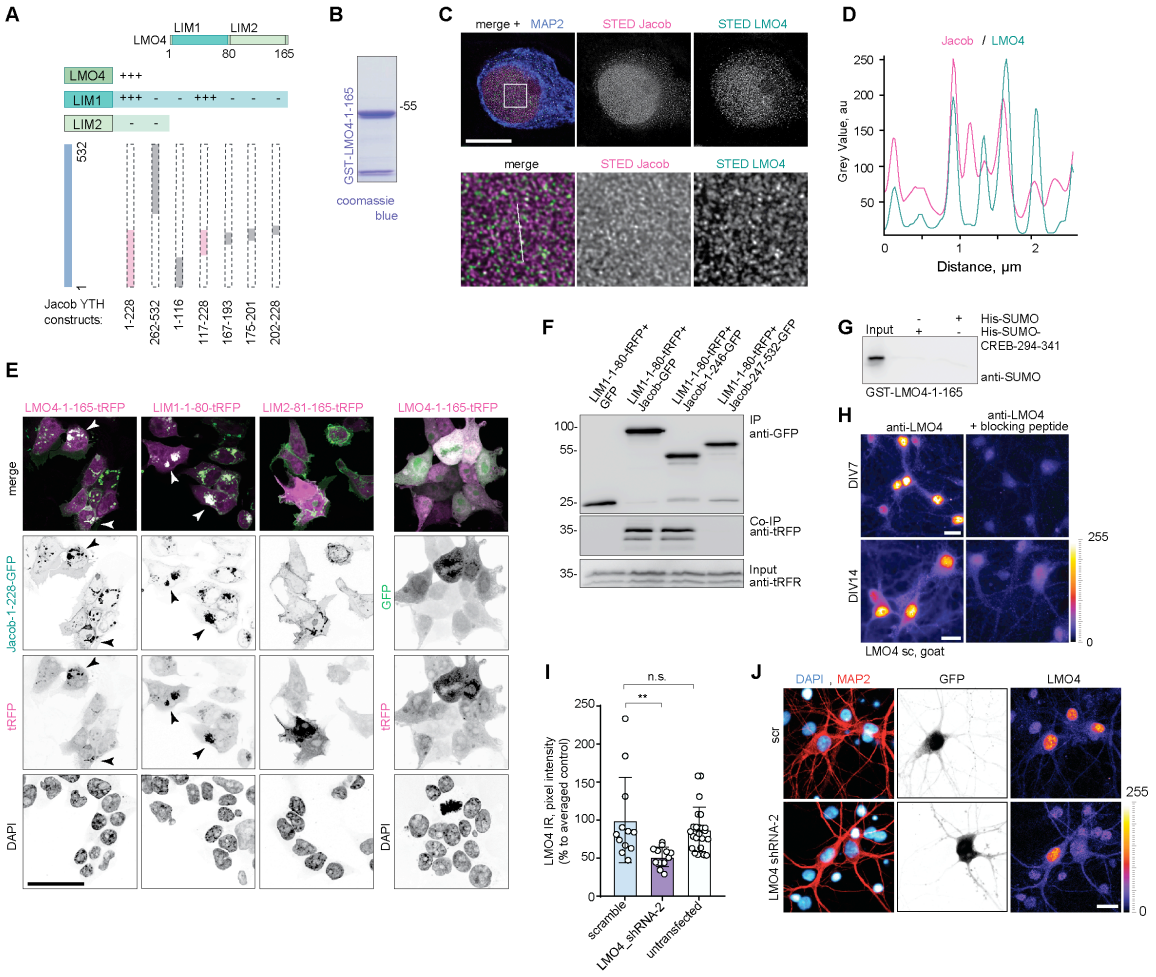


Appendix Figure S1. Confirmation of the LMO4-Jacob interaction, LMO4 antibody specificity and quantification of LMO4 knockdown efficiency, confirmation of Jacob-PP1 interaction, protein purity and loading controls for pull down experiments and studies on the interaction and network activity depending upon Jacob S180 phosphorylation.

(A) Jacob-117-228 interacts with the LIM1 domain of LMO4 in Y2H. (+++) indicates a strong interaction while (–) indicates no interaction. Evaluation was based on the number of colonies growing in triple drop-out media.

(B) Coomassie blue stained gel showing purity of GST-LMO4 used in the pull-down experiments.

(C, D) Super-resolution STED imaging revealed association of Jacob with LMO4 in the nucleus **(C).** DIV16 primary hippocampal neurons stained with antibodies against MAP2, LMO4, pan-Jacob. Upper panel - scale bar:10 µm, lower panel (inserts 5 µm x5 µm – denoted by the white square). **(D)** Line profiles indicate relative intensities for deconvolved STED channels along a 2.5 µm line.

(E) Jacob-1-228-GFP co-recruits the LIM1 (1-80), but not LIM2 (81-165) domain of LMO4. Confocal images of HEK293T cells co-transfected either with Jacob-1-228-GFP or GFP together with LMO4 constructs. Arrows indicate co-recruitment. Scale bar: 40 µm.

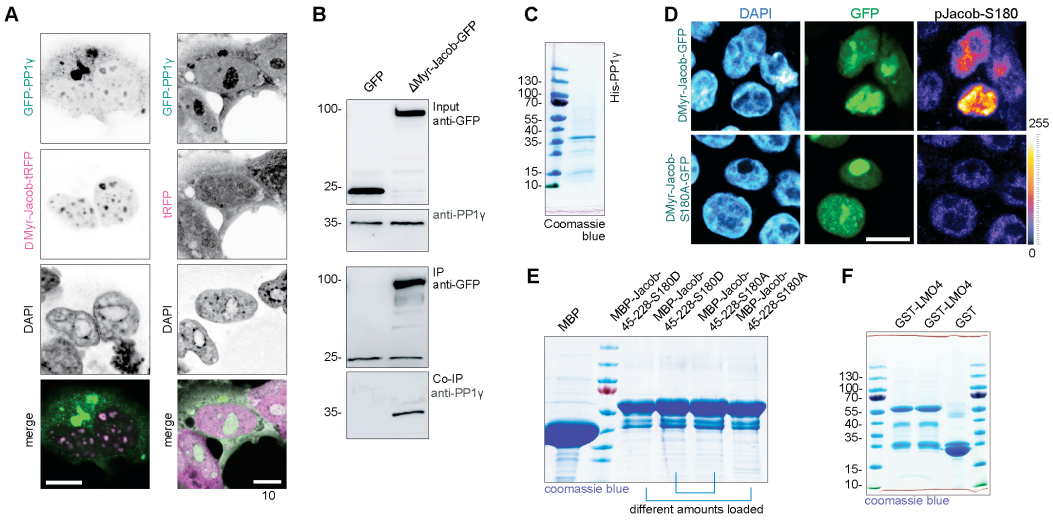
(F) LIM1-1-80-tRFP co-immunoprecipitate with Jacob-1-246-GFP, but not Jacob-247-532-GFP from HEK293T cell extracts.

(G) Pull-down experiments revealed no interaction between His-SUMO-CREB-294-341 with GST-LMO4.

(H) A goat LMO4 antibody was used to stain LMO4 in hippocampal neurons (DIV 7 and DIV 14) in the presence or absence of the blocking peptide. Scale bar: 15 µm.

(I) Nuclear LMO4 staining intensity was downregulated in neurons expressing shRNA targeting LMO4 mRNA compared to scrambled-transfected (scr shRNA) or non-transfected cells. Data represented as mean ± SEM. n=12-23 nuclei. **p<0,021 by Kruskal-Wallis test followed by Dunn's multiple comparison test.

(J) Representative, confocal images of hippocampal neurons transfected with shRNA targeting LMO4 or scrambled control. Reduction of nuclear LMO4 level was confirmed in immunocytochemistry with the goat anti-LMO4 antibody. Scale bar:10 µm.



Appendix Figure S2. Jacob interacts with PP1γ.

(A) Confocal images of HEK293T cells overexpressing GFP-PP1γ together with either ΔMyr-Jacob-tagRFP or tRFP control revealed nuclear co-clustering of both proteins. Scale bar: 20 μm.

(B) ΔMyr-Jacob-GFP but not GFP co-immunoprecipitate with endogenous PP1γ from HEK293T cells extracts.

(C) Coomassie blue stained gel showing purity of commercially available His-PP1γ.

(D) Nuclear Jacob (ΔMyr-Jacob-GFP), but not nuclear phosphodeficient mutant (ΔMyr-Jacob-S180A-GFP) expressed in HEK293T cells is phosphorylated. Confocal images of HEK293T cells overexpressing Jacob and immunostained with anti-pS180Jacob antibodies. Scale bar: 10 μm.

(E) Image of gels stained with coomassie blue showing the purity of bacterially produced Jacob mutants used for pull-down assays.

(F) Images of gels stained with coomassie blue showing inputs for bacterially produced GST-LMO4 coupled to beads used for pull-down assay with Jacob and PP1γ.