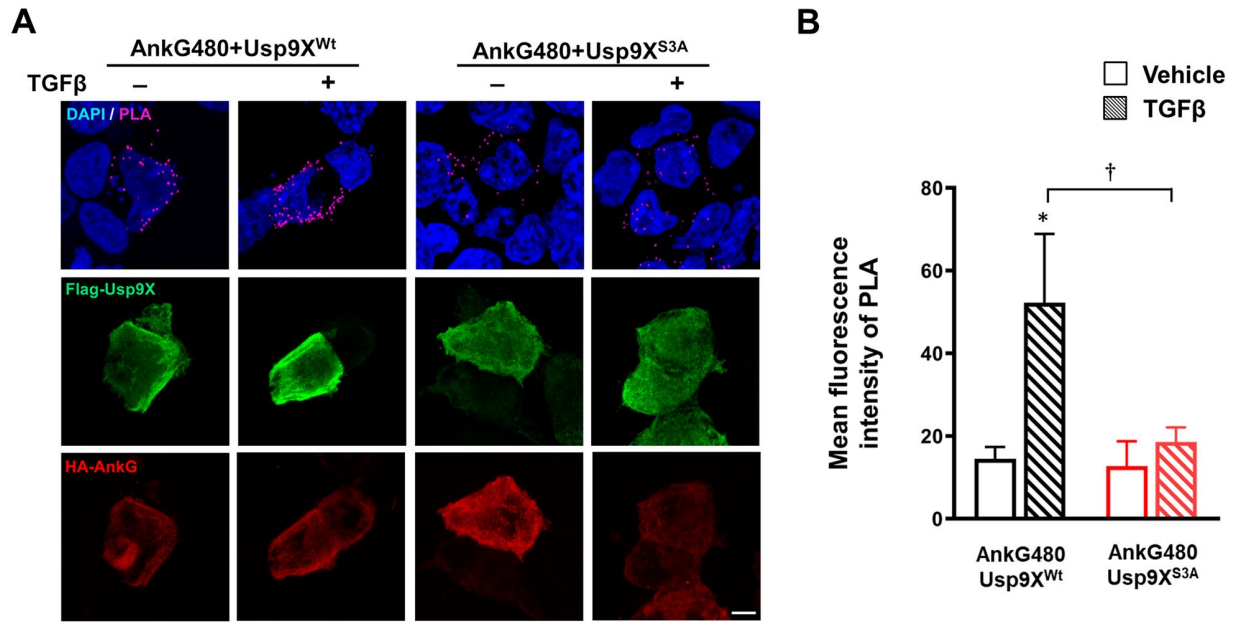


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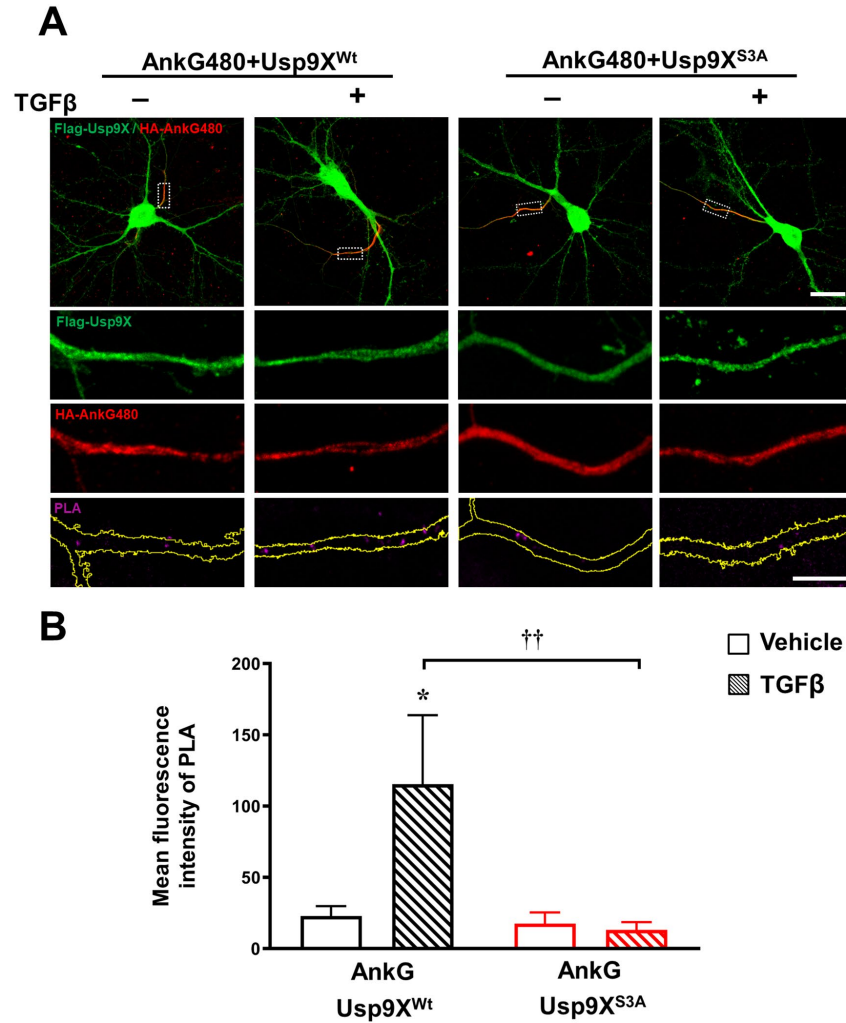
**Supplemental Information**

**TGF- $\beta$ -Induced Phosphorylation of Usp9X  
Stabilizes Ankyrin-G and Regulates  
Dendritic Spine Development and Maintenance**

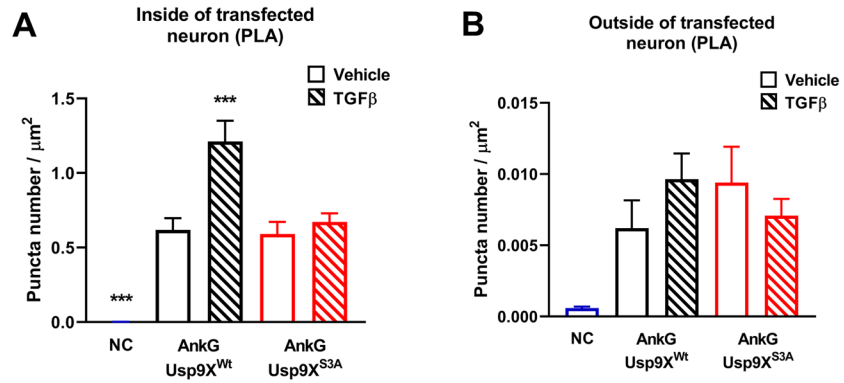
**Sehyoun Yoon, Euan Parnell, and Peter Penzes**



**Figure S1, related to Figure 3. TGFβ-mediated phosphorylation of Usp9X enhances the interaction with ankyrin-G 480.** (A) *In situ* PLA measurement of the interaction between HA-AnkG 480 and Flag-Usp9X<sup>Wt</sup> or Flag-Usp9X<sup>S3A</sup> in neurons. Scale bar, 5 μm. (B) The intensity of PLA signal (n = 12-15 cells for each group). \*P < 0.05; †P < 0.05 by a two-way ANOVA followed by Bonferroni post-tests. All data represent mean ± SEM. TGFβ (-): vehicle; TGFβ (+): 20 ng/ml of TGFβ for 1 hr.



**Figure S2, related to Figure 4. TGFβ-mediated phosphorylation of Usp9X in axon initial segments enhances interaction between ankyrin-G 480 and Usp9X.** (A) Confocal images of primary cortical neurons for detection of interaction between HA-AnkG 480 and Flag-Usp9X<sup>Wt</sup> or Flag-Usp9X<sup>S3A</sup> with PLA. Scale bar, 20 μm (top); 5 μm (bottom). White rectangles indicate the area in the zoomed insets below each image. (B) Bar graph of PLA signal with TGFβ treatment in HA-AnkG 480 and Flag-Usp9X<sup>Wt</sup> or Flag-Usp9X<sup>S3A</sup> expressing cells (n = 7-9 cells of each group). \*P < 0.05; \*\*P < 0.01 by a two-way ANOVA followed by Bonferroni post-tests. All data represent mean ± SEM. TGFβ (-): vehicle; TGFβ (+): 20 ng/ml of TGFβ for 1 h.



**Figure S3, related to Figure 4C. TGF $\beta$ -mediated phosphorylation of Usp9X in dendritic shafts and spines enhances interaction between ankyrin-G and Usp9X. (A-B)** Bar graph of PLA puncta numbers with negative control (blue), vehicle (plain pattern), or TGF $\beta$  (comb pattern) treatment in HA-AnkG and Flag-Usp9X<sup>Wt</sup> (black bars) or Flag-Usp9X<sup>S3A</sup> (red bars) expressing cells (n = 11-15 neurons for each group). \*\*\*P < 0.001 by one-way ANOVA followed by non-parametric statistical analysis. All data are presented as mean  $\pm$  SEM.