

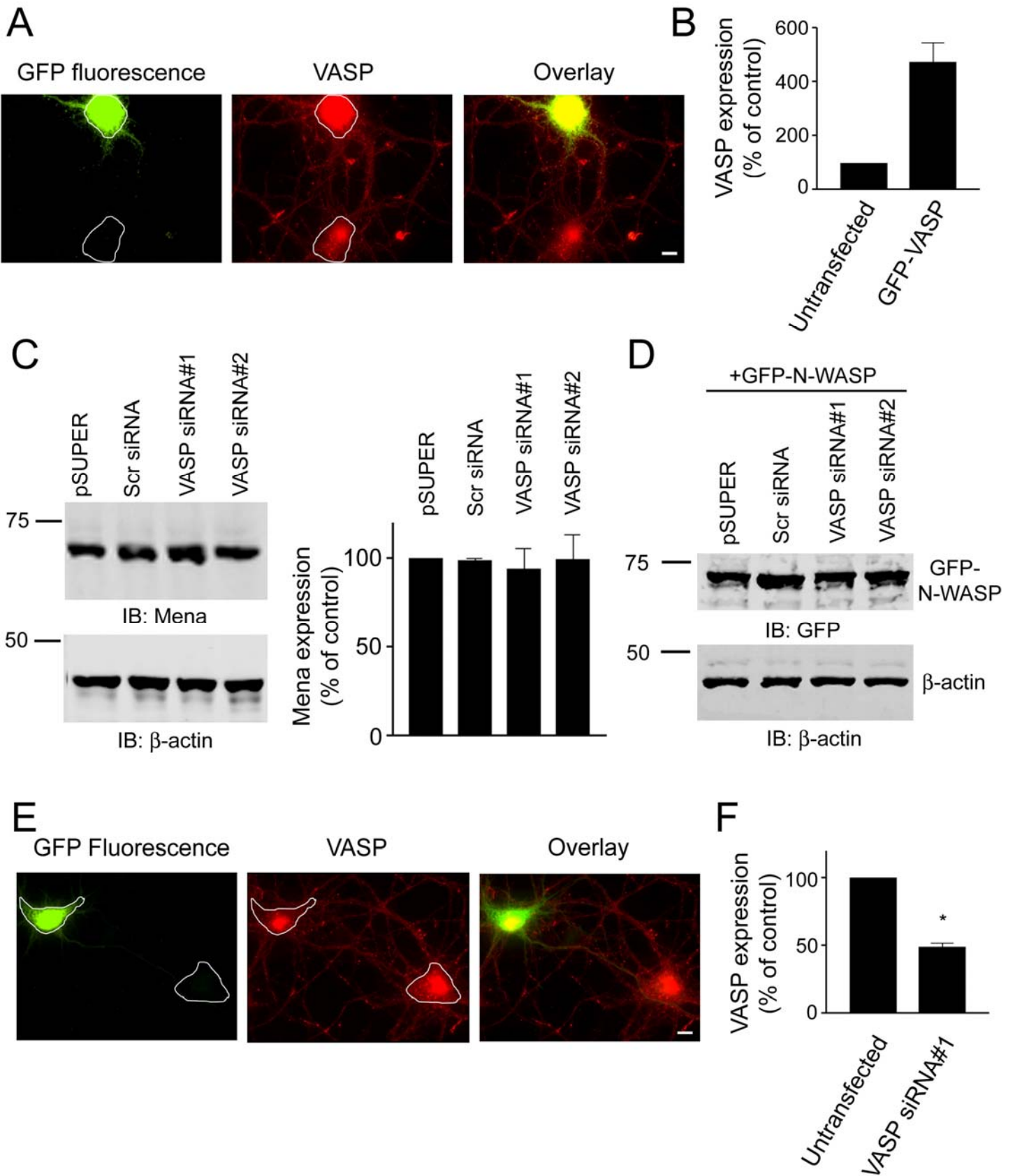
## SUPPLEMENTARY FIGURE LEGENDS

**SUPPLEMENTARY FIGURE 1. VASP siRNA knocks down endogenous expression of the protein in neurons.** *A,E*, Hippocampal neurons were transfected with GFP-VASP (*A*) or GFP and VASP siRNA (*E*) at day 5 in culture, fixed and immunostained for VASP at day 11. The cell body of a transfected and neighboring untransfected neurons are outlined. Bar, 10  $\mu\text{m}$ . *B,F*, The amount of VASP was quantified by measuring the fluorescent intensity in untransfected neurons and transfected neurons expressing GFP-VASP (*B*) or VASP siRNA (*F*). Error bars represent S.E.M. for 24-26 neurons from three independent experiments. *C*, Cell lysates from R2Fs transfected with VASP siRNAs, pSUPER empty vector, or scrambled siRNA (*Scr siRNA*) were immunoblotted for Mena and  $\beta$ -actin (loading control). Quantification of blots from three separate experiments is shown (*right panel*). Error bars represent S.E.M. *D*, HEK-293T cells were co-transfected with GFP-N-WASP and either VASP siRNAs, pSUPER empty vector, or scrambled siRNA. Cell lysates were immunoblotted for GFP and  $\beta$ -actin (loading control).

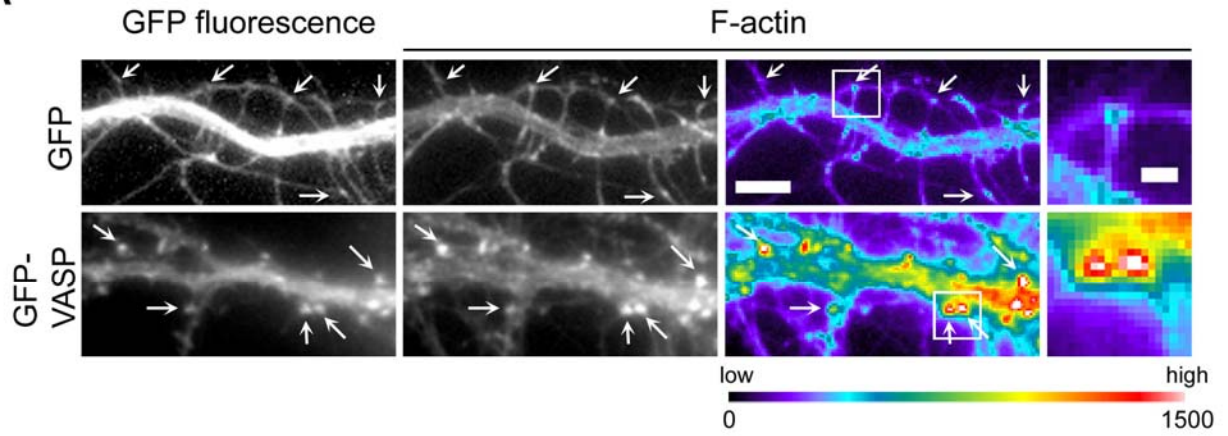
**SUPPLEMENTARY FIGURE 2. VASP regulates actin assembly in spines.** *A*, Pseudo-color coded images from Fig. 4A are shown. *B*, Pseudo-color coding of images from Fig. 4C is shown. Bar, 5  $\mu\text{m}$ . For panels *A* and *B*, higher magnification images of the boxed regions are shown (*far right panels*). Bar, 1  $\mu\text{m}$ .

**SUPPLEMENTARY FIGURE 3. VASP regulates synaptic strength.** *A*, Pseudo-color coding for PSD95 staining from images in Fig. 6A is shown. *B,D*, Images of GFP and GFP-VASP expressing neurons stained for Homer (*B*) and Shank (*D*) are shown in grayscale (*middle panels*) and pseudo-color coding (*right panels*). Bar, 5  $\mu\text{m}$ . Arrows indicate spines. Quantification of the normalized fluorescent intensity in individual spines of neurons stained for Homer (*C*) and Shank (*E*) is shown. Error bars represent S.E.M. for 100 spines from three separate experiments (\*\*,  $p < 0.0001$ ; \*,  $p < 0.04$ ). *F,G*, Pseudo-color coding for sGluR1 from images in Fig. 6C and 7A, respectively, is shown. Bar, 5  $\mu\text{m}$ . For panels *A,B,D,F,G*, higher magnification images of the boxed regions are shown (*far right panels*). Bar, 1  $\mu\text{m}$ .

Supplemental Figure 1



**A**



**B**

