

## LEGENDS TO SUPPLEMENTAL FIGURES

Fig S1. Localization of endogenous and exogenous Fhod3 in cultured rat cardiomyocytes. (A) Neonatal rat cardiomyocytes were double stained with antibodies against Fhod3 (red) and  $\alpha$ -actinin (green). For endogenous Fhod3 staining, the anti-Fhod3(C-20) (upper panels) and the anti-Fhod3(873–974) (lower panels) polyclonal antibodies were used. Scale bar, 10  $\mu$ m. (B and C) Neonatal rat cardiomyocytes transfected with the adenovirus encoding HA-tagged Fhod3 were stained with the antibodies against anti-HA (red) and  $\alpha$ -actinin (green) in (B) or with the antibodies against anti-HA (red) and  $\alpha$ -actinin (green), and with phalloidin (blue) in (C). Scale bars, 10  $\mu$ m.

Fig S2. Effect of exogenous expression of Fhod3 in rat cardiomyocytes. (A and B) Cardiomyocytes transfected with the adenovirus encoding HA-tagged Fhod3 (A) or Fhod3S (B) were fixed and stained with the antibodies against anti-HA (red) and  $\alpha$ -actinin (green). Scale bars, 10  $\mu$ m. (C) Cardiomyocytes transfected with the adenovirus encoding HA-tagged Fhod3S were fixed and stained with the antibodies against anti-HA (red) and  $\alpha$ -actinin (green), and with phalloidin (blue). Scale bars, 10  $\mu$ m.

Fig S3. Fhod3 knock down disrupts sarcomere assembly in rat cardiomyocytes. Cardiomyocytes were transfected with Fhod3-specific siRNAs #1 and #4, and cultured for 48 hours. Cells were fixed and double stained with the antibodies against anti-Fhod3(650–802) (red) and  $\alpha$ -actinin (green). Scale bar, 10  $\mu$ m.

Fig S4. Cardiomyocytes were sequentially transfected with the adenovirus encoding the wild-type or mutant Fhod3 proteins and with Fhod3 siRNA #4 and cultured for 48 hours. Cells were fixed and double stained with the antibodies against anti-HA (red) and  $\alpha$ -actinin (green), and with phalloidin (blue). Scale bar, 10  $\mu$ m.

Fig S5. SDS-PAGE analysis of purified proteins that were used in an actin polymerization assay. Fhod3- $\Delta$ N(wt) (0.3  $\mu$ g), Fhod3- $\Delta$ N(I1127A) (0.4  $\mu$ g), and mDia1-FH1FH2 (1.2  $\mu$ g) were subjected to 10% SDS-PAGE and stained with *Coomassie Brilliant Blue*. Positions for marker proteins are indicated in kDa.

Fig. S1 (Taniguchi *et al.*)

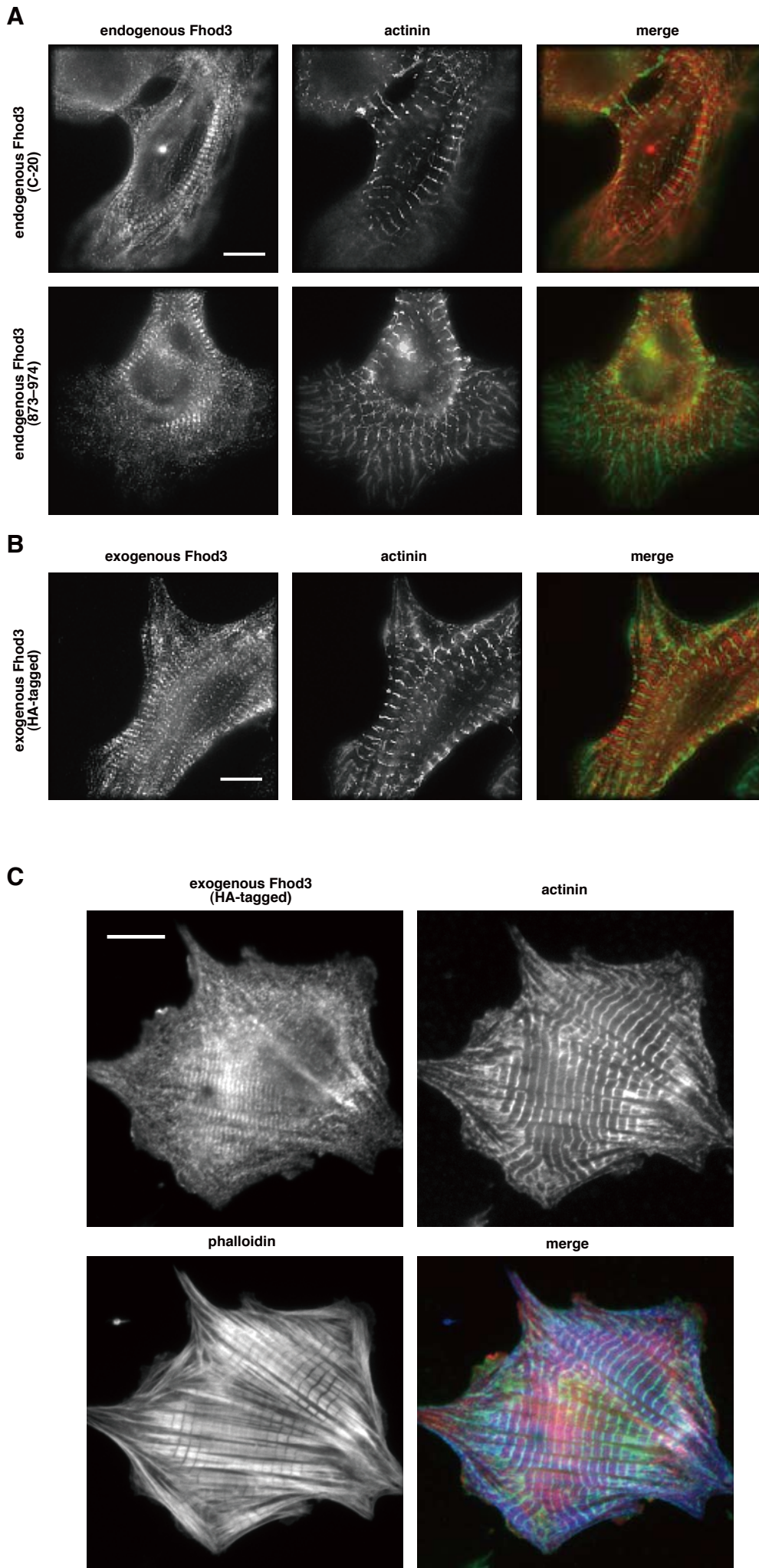


Fig. S2 (Taniguchi *et al.*)

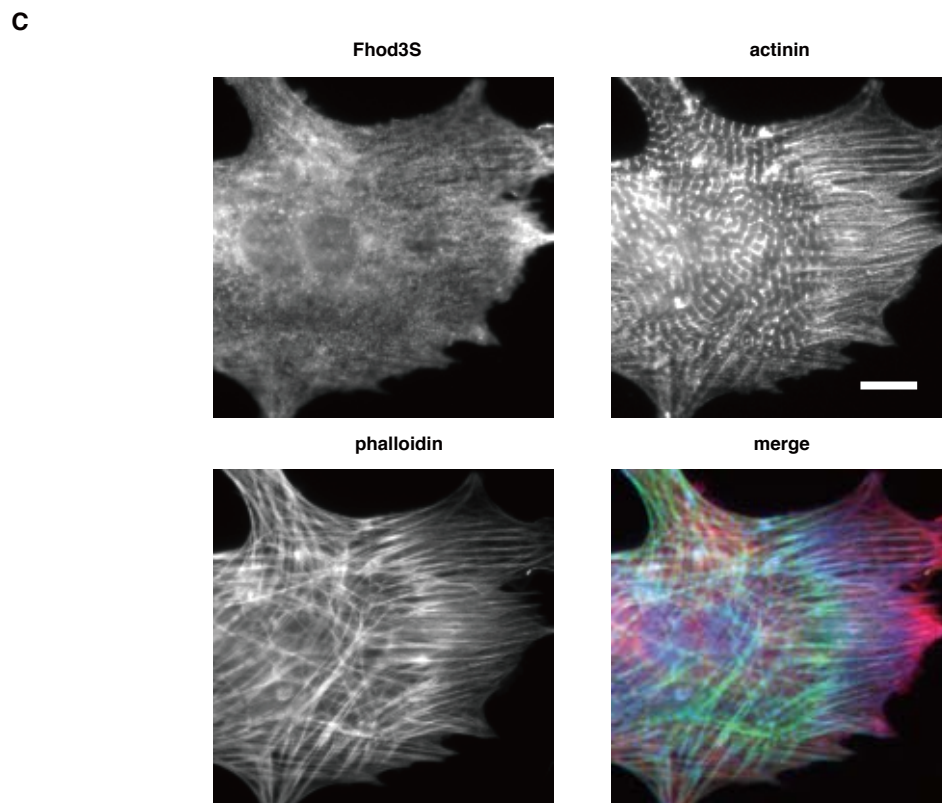
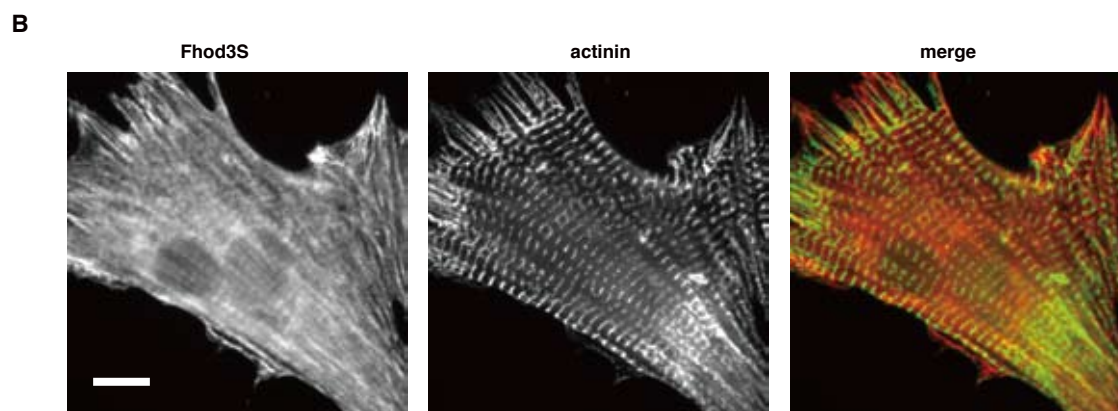
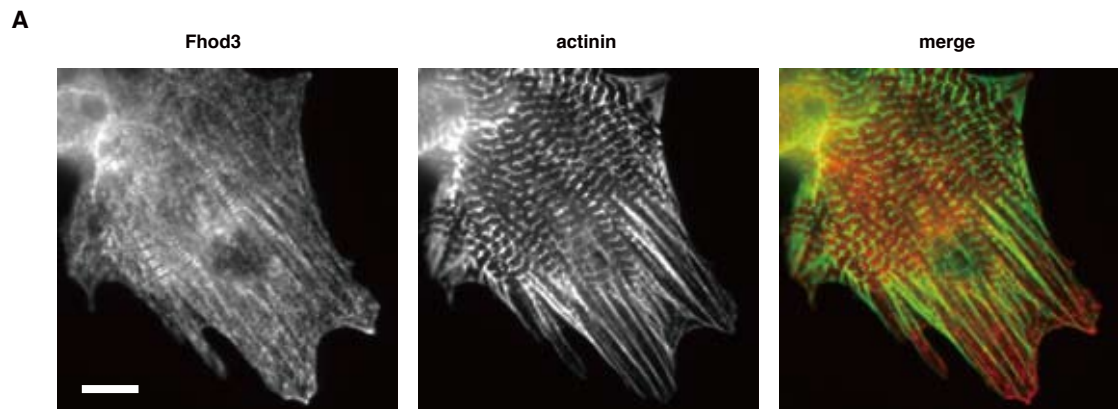


Fig. S3 (Taniguchi *et al.*)

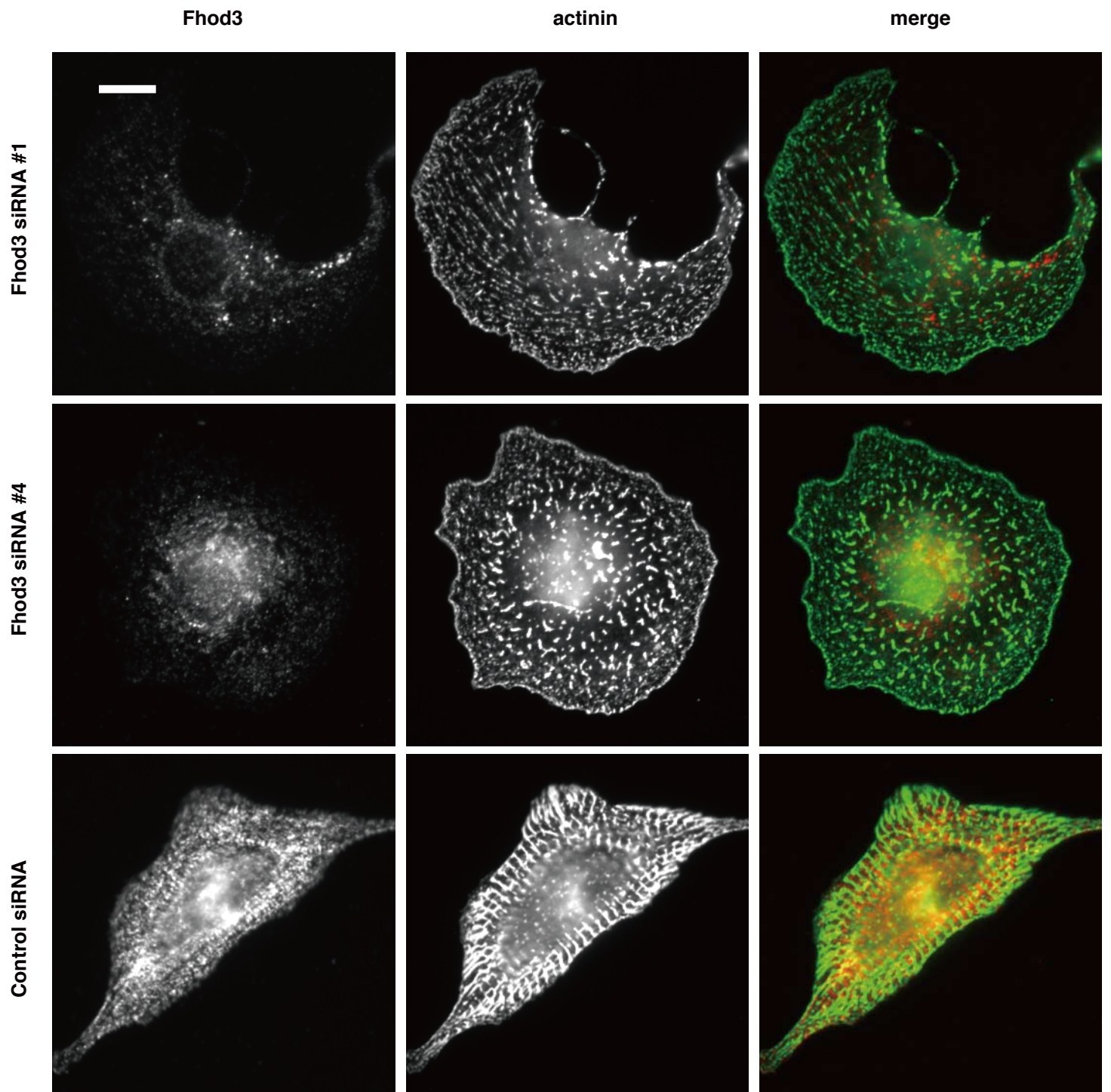


Fig. S4 (Taniguchi *et al.*)

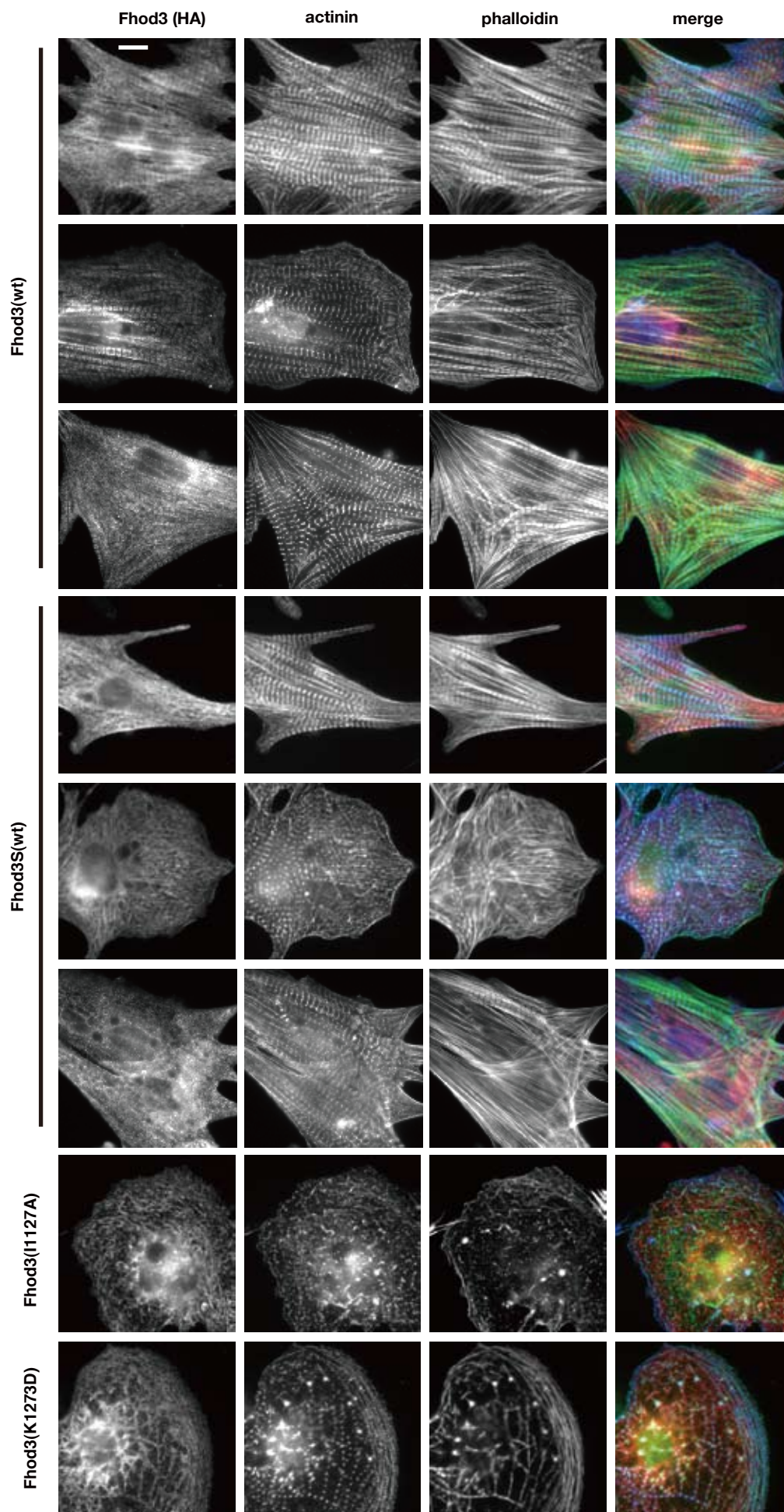


Fig. S5 (Taniguchi *et al.*)

