SUPPLEMENTARY FIGURE LEGENDS

FIG. S1. **No morphological changes in N-WASP-transfected Neuro-2a cells.** Immunostaining of Neuro-2a cells transfected with HA/N-WASP, depicting cellular architecture. Scale bar: 10 μm.

FIG. S2. **Distribution of Gas7 and N-WASP in cortex and cerebellum of adult mice brain.** Cortex (*A*) and cerebellum (*B*) sections were stained with antibodies to Gas7 and N-WASP. Hoechst, MAPII or calbindin staining was used to identify nuclei, neurons and Purkinje cells (Pk), respectively. The superimposed images show combined Gas7 (green) and N-WASP (red) staining, with regions of co-localization in yellow. Bottom right image of each set shows the Boxed area in the previous image magnified 20X. *A*, both Gas7 and N-WASP are expressed in cortical neurons; however, little subcellular co-localization of Gas7 and N-WASP can be detected. *B*, localization of Gas7 in the cerebellum is quite distinct from N-WASP in Purkinje cells. Scale bar: 100 μm (5 μm in magnified area). ML, molecular layer; GL, granular layer.

FIG. S3. **Actin architecture in Gas7-truncate-transfected Neuro-2a cells.** Immunostaining of Neuro-2a cells transfected with Gas7 variants (Gas7/Myc, Gas7ΔWW/Myc, Gas7ΔFCH/Myc, or Gas7Δcoiled/Myc) to represent the distribution of actin in the cells. Gas7-transfected cells have membrane protrusions composed of actin filaments, whereas the Gas7-truncate transfected cells do not. Scale bar: 10 μm.

FIG. S4. **Microtubule architecture in Gas7-truncate-transfected Neuro-2a cells.** Immunostaining of Neuro-2a cells transfected with Gas7 truncates (Gas7/Myc, Gas7ΔWW/Myc, Gas7ΔFCH/Myc, or Gas7Δcoiled/Myc) showing the distribution of microtubules. Gas7-induced membrane protrusions in Neuro-2a cells are not a result of microtubule reorganization, and Gas7 truncates did not induce microtubule reorganization in Neuro-2a cells. Scale bar: 10 μm.

FIG. S5. **Morphology of WW2/EGFP-transfected Neuro-2a cells.** Immunostaining of Neuro-2a cells transfected with WW2/EGFP to examine morphological changes. The WW2/EGFP–transfected Neuro-2a cells did not show any membrane protrusions. Scale bar: 10 μm.

FIG. S6. **Gas7-*cb* is expressed mainly in the nucleus of cells.** Immunostaining of Neuro-2a cells transfected with mouse Gas7-*cb* showing the localization of Gas7-*cb*. Hoechst staining and phase-contrast images show the nucleus and general morphology, respectively. The superimposed images show

combined Gas7-*cb* (red) and Hoechst (green) staining, the regions of co-localization being yellow. Scale bar: 5 μm.

FIG. S7. **Gas7 induces membrane invagination in the COS-7 cells.** Gas7/EGFP was transiently expressed in COS-7 cells and time-lapse recorded by Olympus DeltaVision microscopy (*A*), or immunostained for α-tubulin (*B,* upper panel), actin (*B,* middle panel) and compared with membrane staining (*B,* lower panel), showing the cytoskeleton and membranes 24 h after transfection. $DiIC_{16}(3)$ (D384, Invitrogen) was used for membrane staining (the procedure was carried out as previously described in ref. 50). The tubular structures induced by Gas7 overexpression were highly co-localized with membrane in COS-7; not with actin or microtubule architecture. Gas7 induces membrane deformation in COS-7. Scale bar: *A*, 6 μm (2 μm in magnified area); *B*, 10 μm (2 μm in magnified area).

FIG. S8. **Co-localization of Gas7 and N-WASP in dendritic spines and synaptic vesicles of hippocampal neurons.** Immunostaining for Gas7 and N-WASP in primary culture of embryonic E16.5 mouse hippocampal neurons at 21 DIV. PSD95 and synaptophysin staining were used to identify dendritic spines and synaptic vesicles, respectively. Gas7 (green) and N-WASP (red) co-localized with PSD95 (arrows, A) and synaptophysin (arrows, B). Scale bar: 10 μm (2 μm in inset).

FIG S1

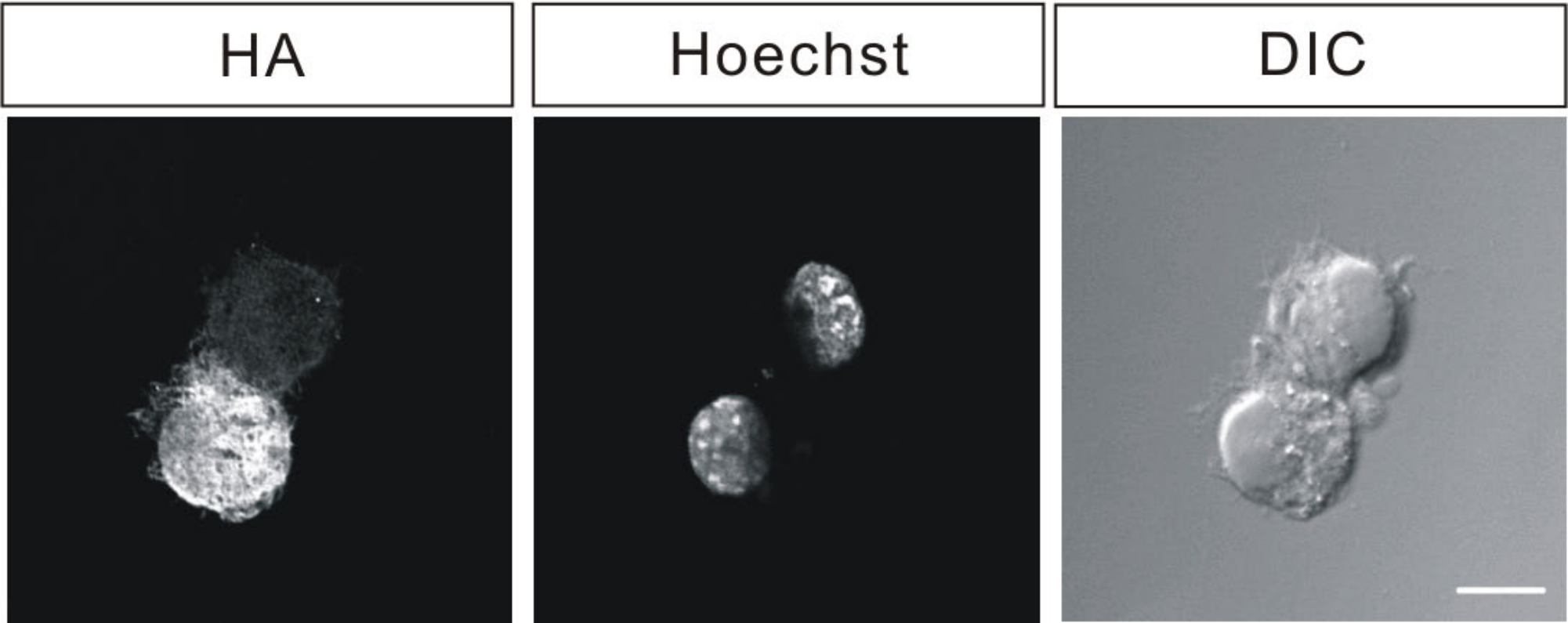


FIG S2

A

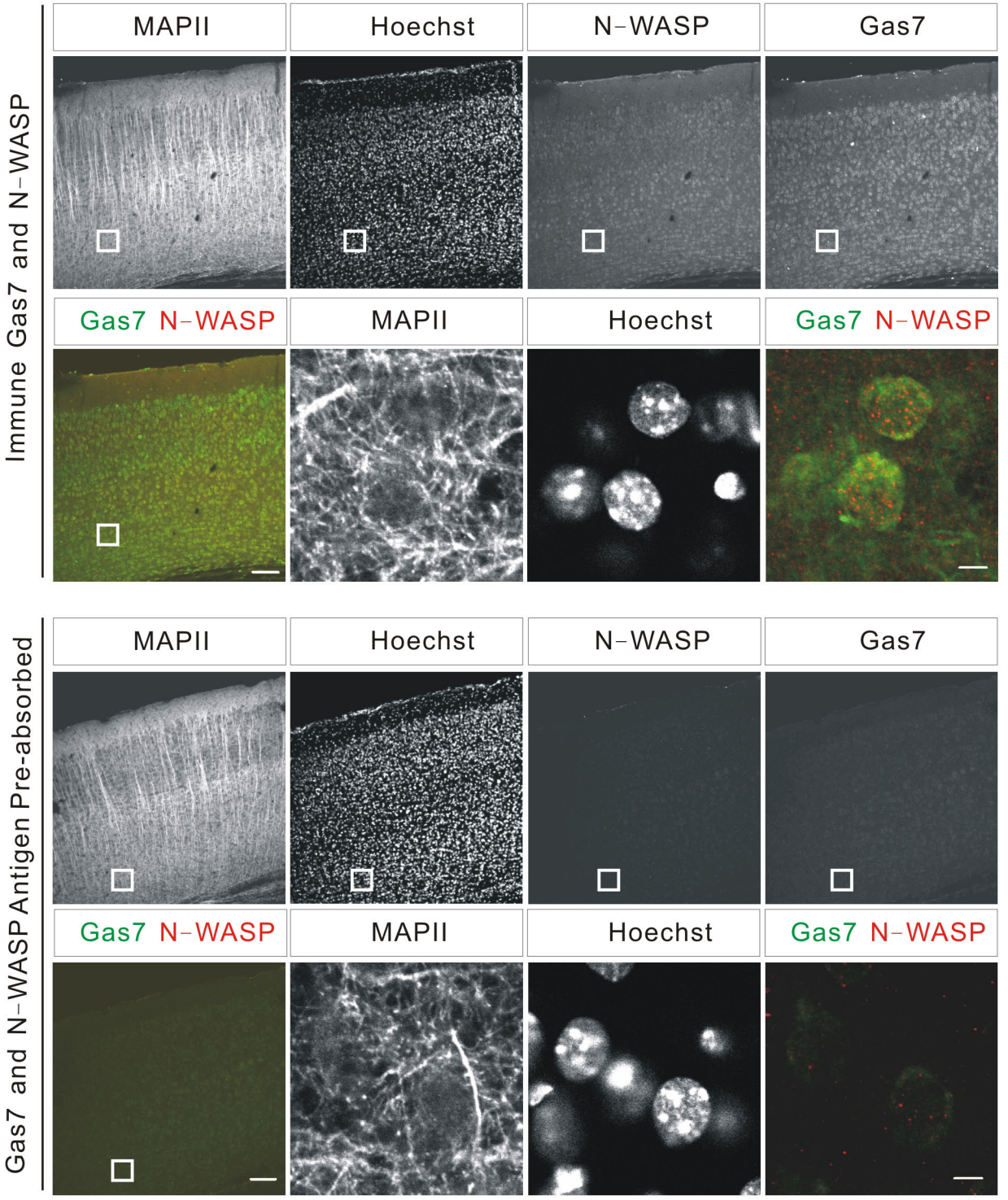

Immune Gas7 and N-WASP
MAPII
Hoechst
N-WASP
Gas7
Gas7 N-WASP
MAPII
Hoechst
Gas7 N-WASP
Gas7 and N-WASP Antigen Pre-absorbed
MAPII
Hoechst
N-WASP
Gas7
Gas7 N-WASP
MAPII
Hoechst
Gas7 N-WASP

FIG S2

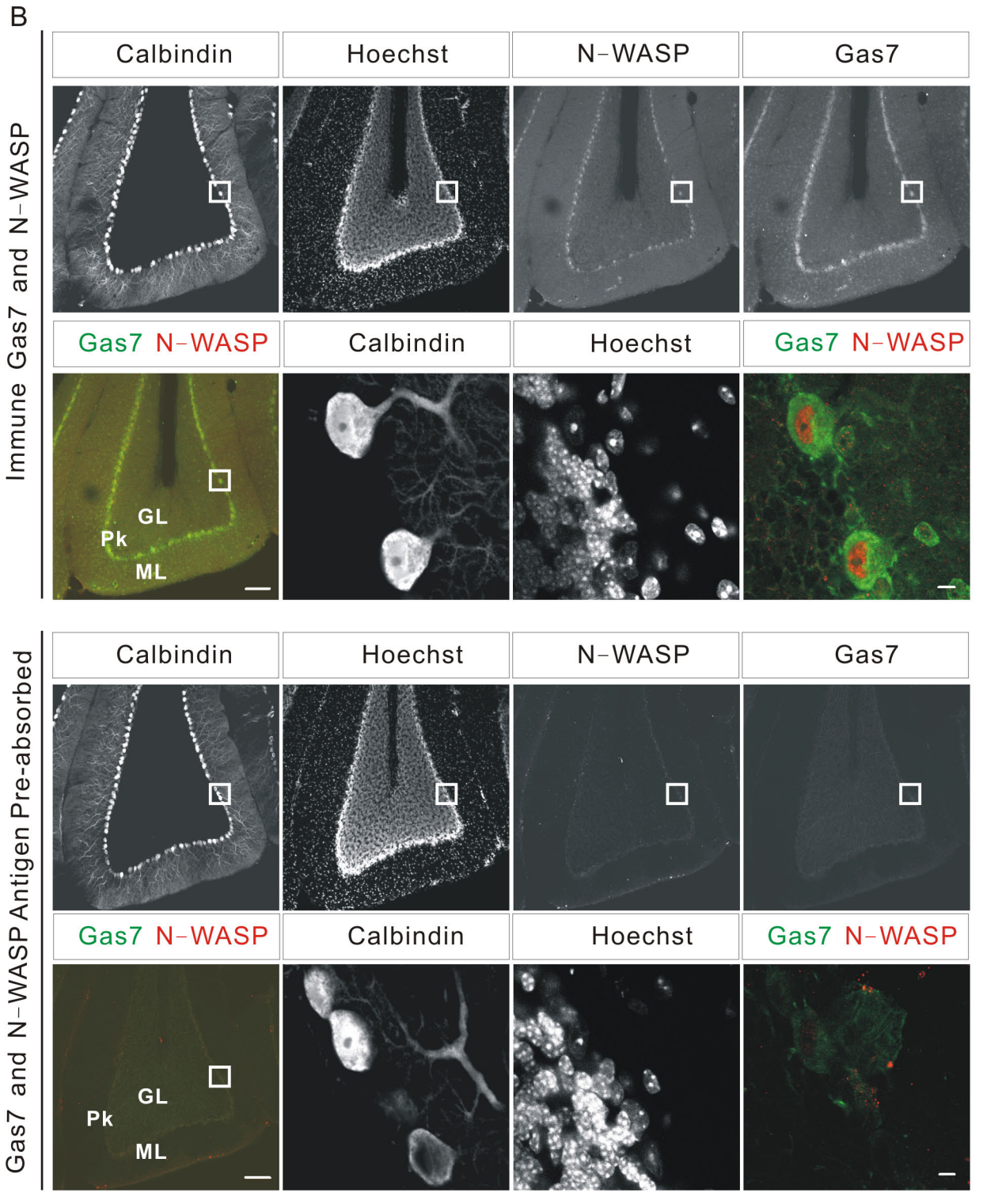

B
Immune Gas7 and N-WASP
Calbindin
Hoechst
N-WASP
Gas7
Gas7 N-WASP
Calbindin
Hoechst
Gas7 N-WASP
GL
Pk
ML
Gas7 and N-WASP Antigen Pre-absorbed
Calbindin
Hoechst
N-WASP
Gas7
Gas7 N-WASP
Calbindin
Hoechst
Gas7 N-WASP
GL
Pk
ML

FIG S3

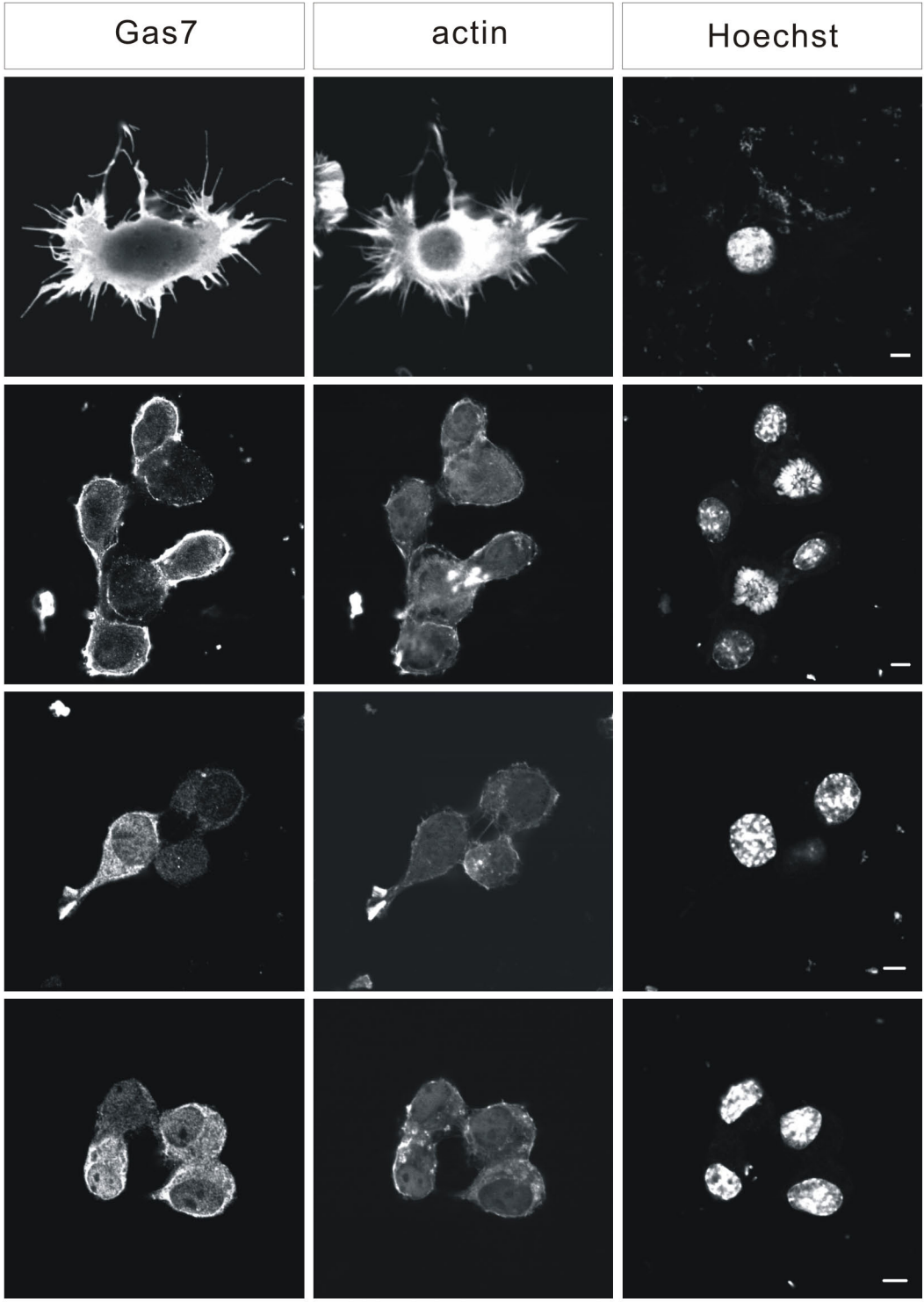


FIG S4

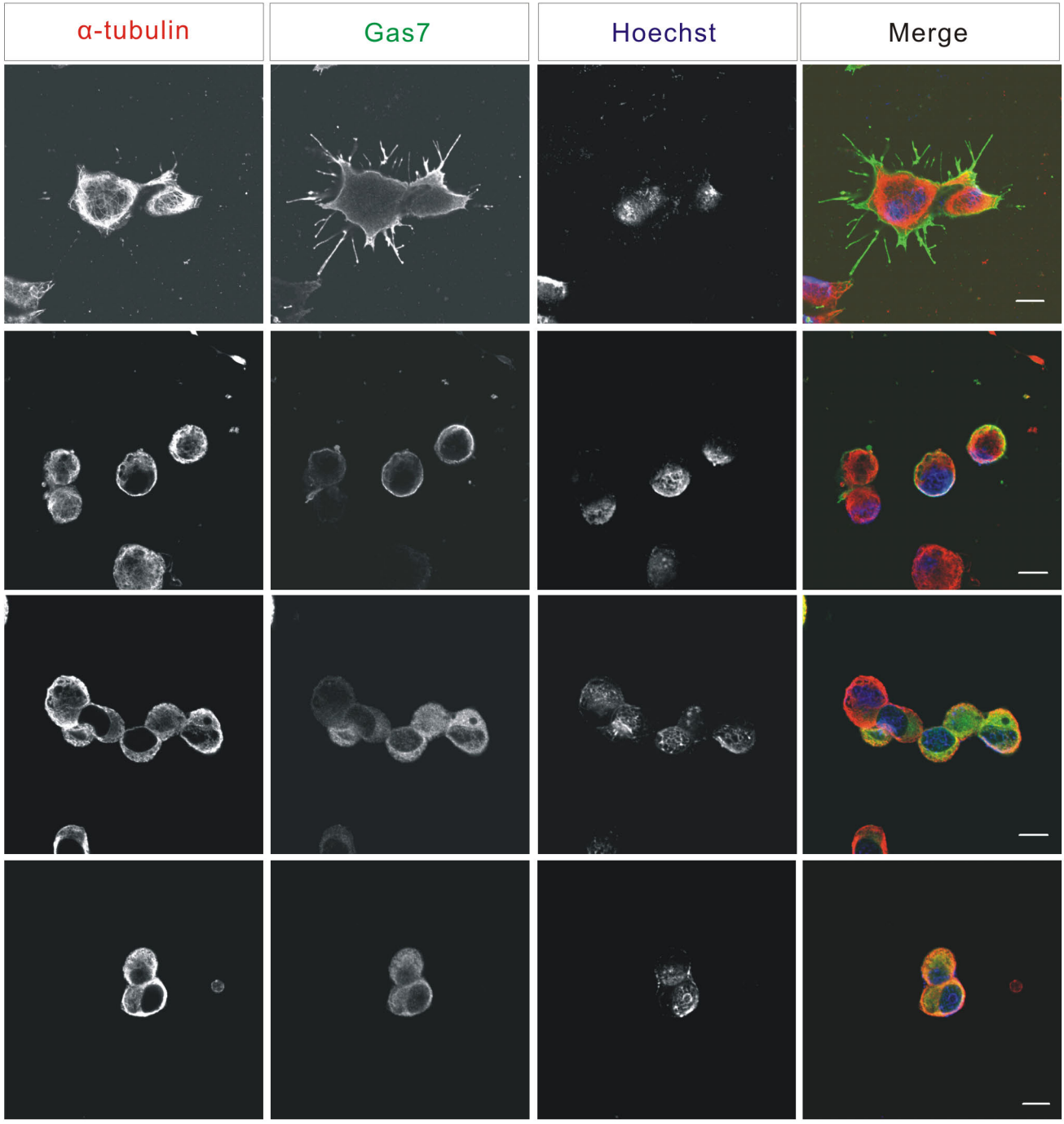

α-tubulin
Gas7
Hoechst
Merge
Gas7
Gas7ΔWW
Gas7ΔFCH
Gas7Δcoiled

FIG S5

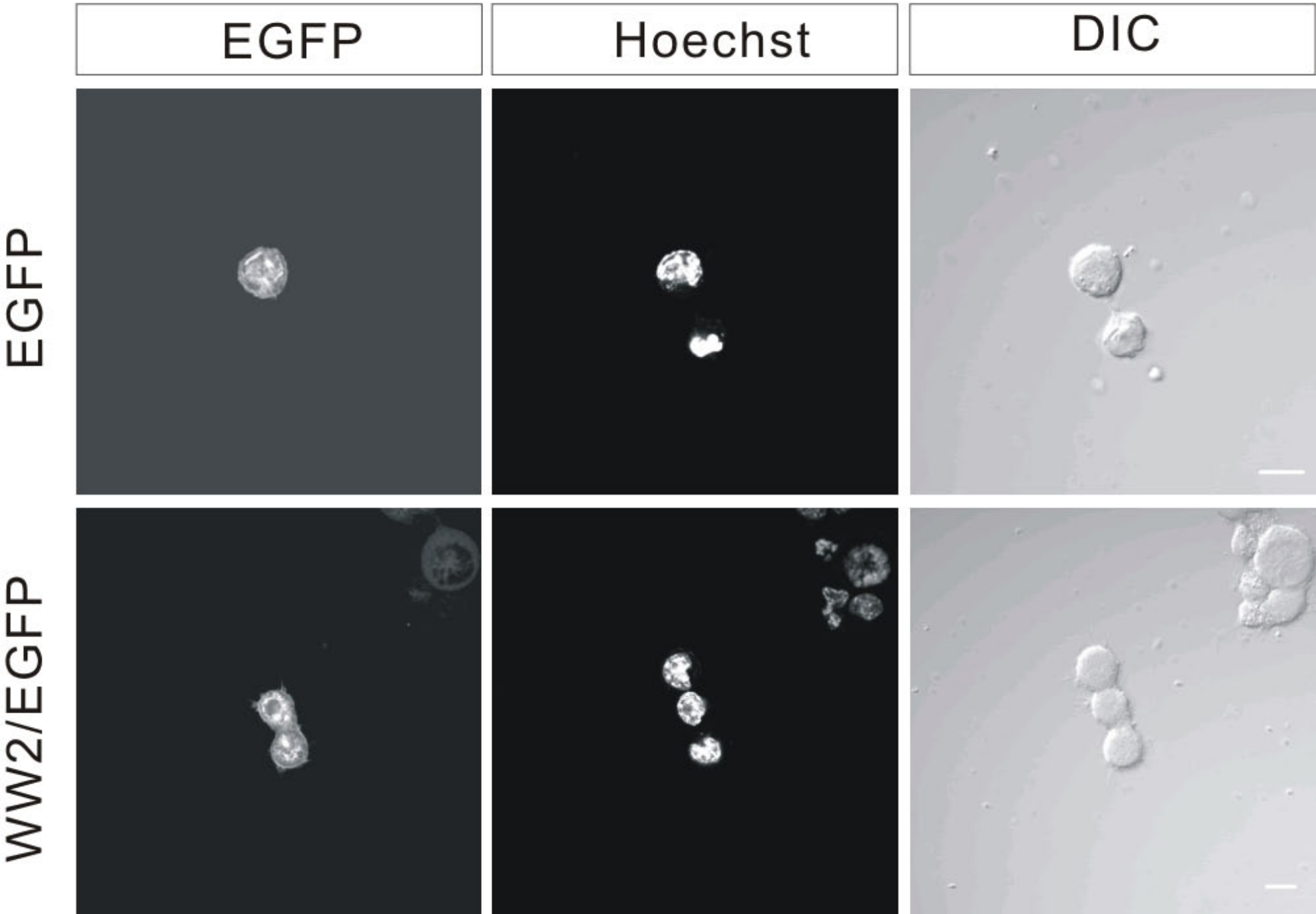


FIG S6

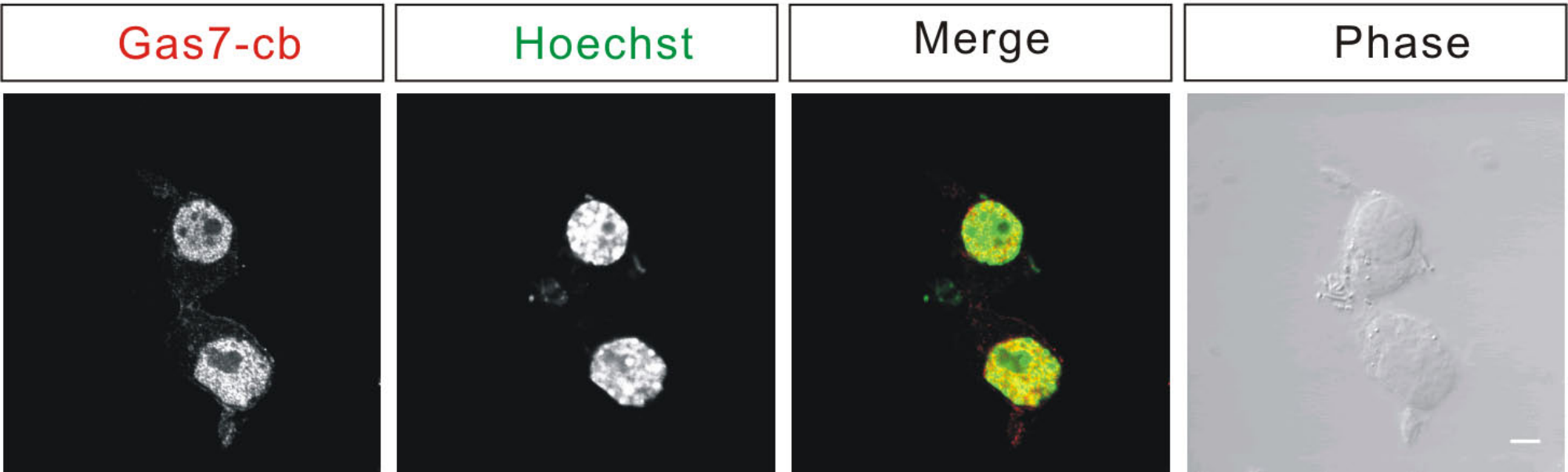


FIG S7

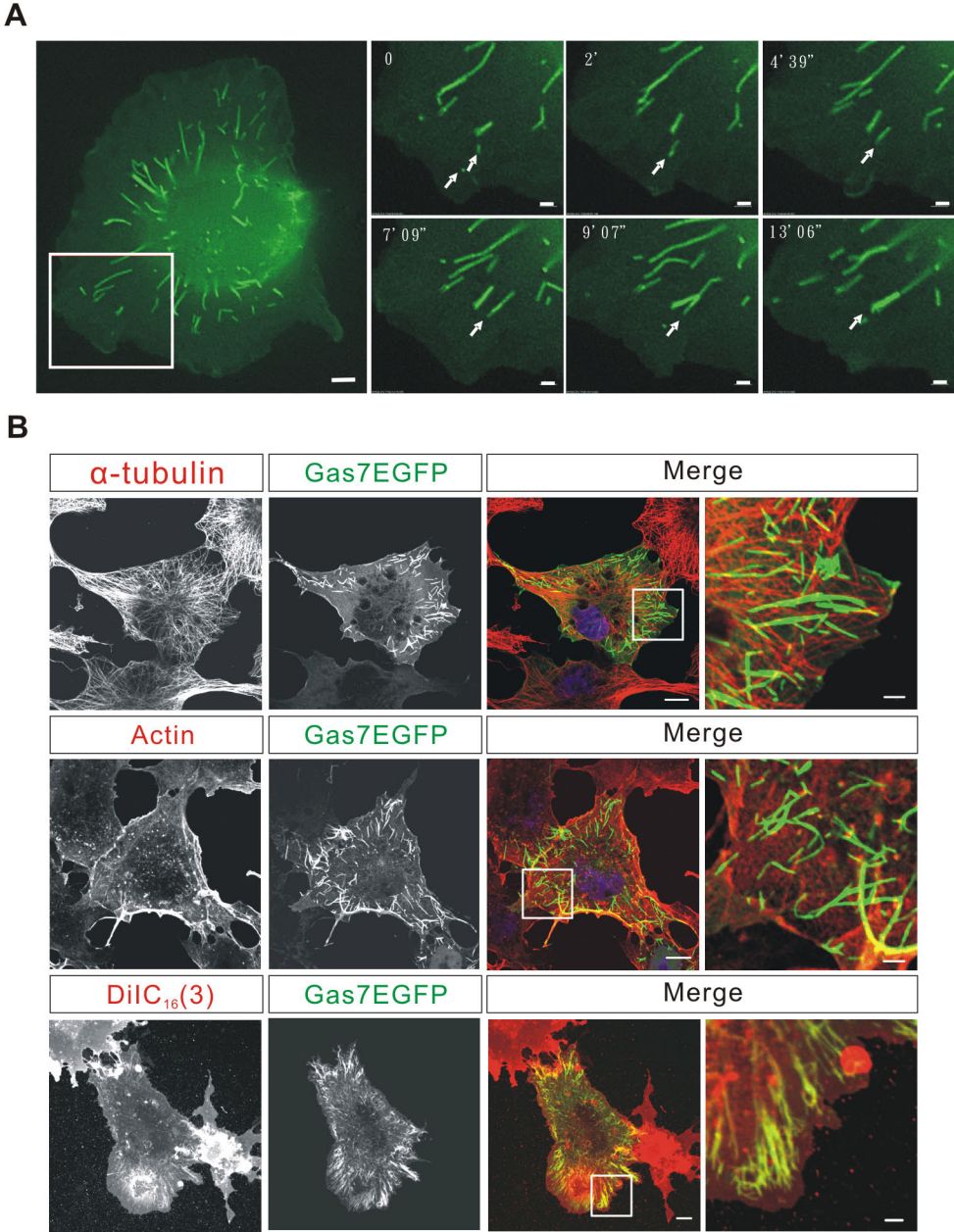
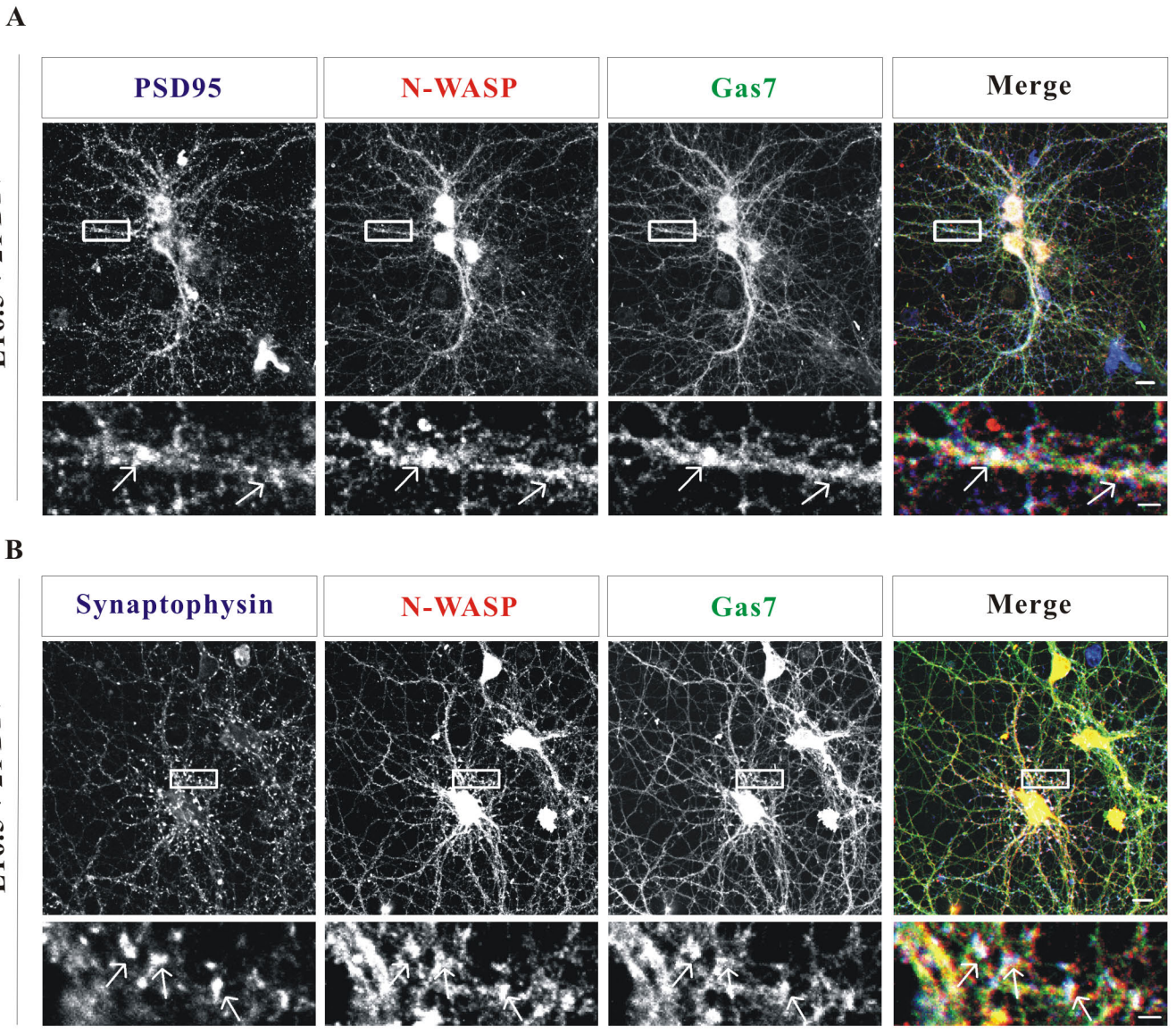

A
0
2'
4' 39"
7' 09"
9' 07"
13' 06"
B
α-tubulin
Gas7EGFP
Merge
Actin
Gas7EGFP
Merge
DiIC16(3)
Gas7EGFP
Merge

FIG S8


A
PSD95
N-WASP
Gas7
Merge
E16.5 + 21 DIV
B
Synaptophysin
N-WASP
Gas7
Merge
E16.5 + 21 DIV