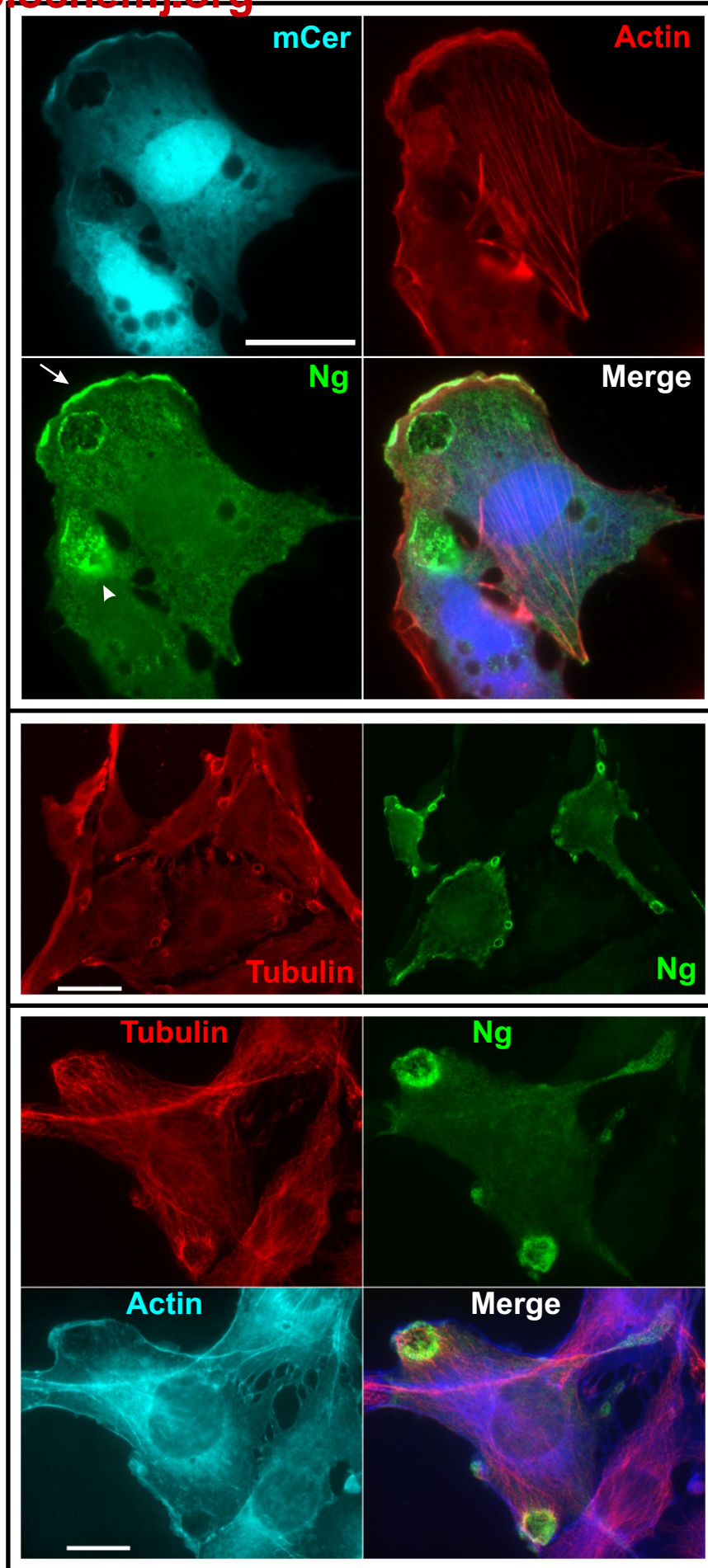


Supplementary Figure 1

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## Supplementary Figure 1

**Characterization of plasma membrane ring-shaped structures (PMRSSs). (Upper panel)** NIH-3T3 cells transfected with Ng and mCerulean were processed for IF using the cool method. Note the big crater and plasma membrane patch in neighbouring cell (arrowhead) that are labelled by Ng but not by phalloidin (Actin). Diminished mCerulean labelling at these regions suggest plasma membrane depressions. The protruding edge of the lamellipodium, however, is visibly labelled by both Ng and actin (arrow). **(Intermediate panel)** Ng transfected cells that were counterstained with anti-tubulin antibodies showed several Ng-labelled PMRSSs where tubulin also accumulates. Note the localization of PMRSSs at the cell periphery and the presence of tubulin-labelled PMRSSs in cells with no Ng expression. **(Lower panel)** Detail of a Ng-expressing cell that features two big Ng-labelled craters that showed tubulin but not actin colocalization. Bars represent 25  $\mu\text{m}$ .

Ng-labelled PMRSSs are common at peripheral and dynamic areas of transfected cells. They resemble craters open at the dorsal plasma membrane. It is not unusual to observe discrete areas of the plasma membrane displaying intense Ng labelling (upper panel, arrowhead) that might be related to the formation or disassembly of PMRSSs. In cells co-expressing Ng and mCerulean (a brighter variant of the cyan fluorescent protein, CFP), the fluorescent protein was absent from the craters, suggesting that PMRSSs really correspond to plasma membrane depressions. Phalloidin labelled membrane ruffles and edges of lamellipodia, where Ng is often seen, but did not label PMRSSs. The absence of actin at these structures suggests that they could represent plasma membrane areas that have been detached from the cortical actin cytoskeleton, such as membrane blebs. Tubulin colocalizes with Ng and is also concentrated at PMRSSs (mid-panel). Tubulin antibodies labelled PMRSSs in many cells, with and without Ng expression, showing that PMRSSs are not induced by Ng expression but normally present in many cells. 1  $\mu\text{M}$  Nocodazol (10 min) lead to rapid microtubule disassembly, disappearance of PMRSSs and redistribution of Ng in multiple spots or miniblebs along the plasma membrane (not shown). 10 mM methyl- $\beta$ -cyclodextrin (30 min) to deplete cells of cholesterol or 1  $\mu\text{M}$  cytochalasin D (30 min) that prevents actin polymerization, did not affect the number or morphology of Ng-labelled PMRSSs.