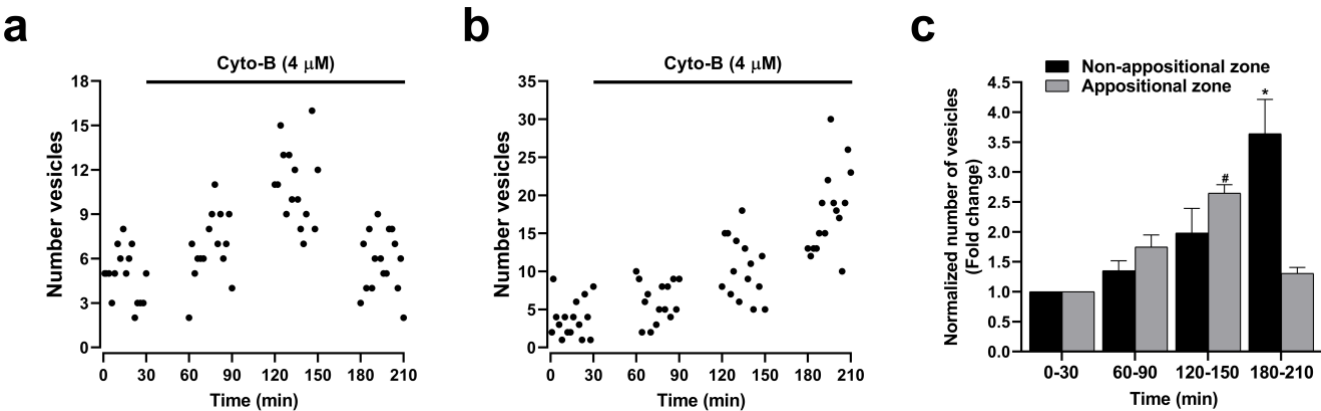


**Supplementary Figure 1.** Inhibition of RhoA activity or its synthesis significantly reduces the size of Cx43 and Cx26 GJCs plaques. HeLa-Cx43 and HeLa-Cx26 transfected with empty vector or RhoAWT or RhoAV14 or RhoAN19 or siRNA RhoA or siRNA UnR constructs (green) were fixed 24 h after transfection and immunolabeling with anti-Cx43 (a) or anti-Cx26 (b) (both shown in red). Quantification of the effect of different constructs on plaque formation at cell-cell borders is shown for Cx43 (c) and Cx26 (d). Data are presented as mean  $\pm$  SEM (n= 20 cell pairs per condition). All treatments were compared to the control condition (Untransfected) (\*p<0.05). Scale bar: 10  $\mu$ m.



**Supplementary Figure 2.** Cyto-B increases the number of vesicles in the non-appositional zone in HeLa-Cx26GFP cells. Cells were treated with Cyto-B (4 $\mu$ M) for up to 210 min. Representative graphs show the quantification of the number of vesicles in the appositional zone (a) and the non-appositional zone (b) in HeLa-Cx26GFP cells. The graph shows the fold change in the number of vesicles for the evaluated appositional and non-appositional plasma membrane (c). Non-appositional zone (black bars) time intervals (60-90, 120-150 and 180-210 min) were compared to the control condition (0-30 min). Data are presented as mean  $\pm$  SEM (n = 3) \*p<0.05. Appositional zone (gray bars) time intervals (60-90, 120-150 and 180-210 min) were compared to the control condition (0-30 min). Data are presented as mean  $\pm$  SEM (n = 3) #p<0.05.