

Appendix Contents:

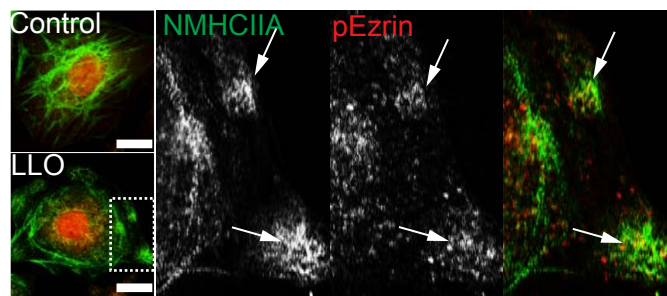
Appendix Figure S1

Appendix Figure S2

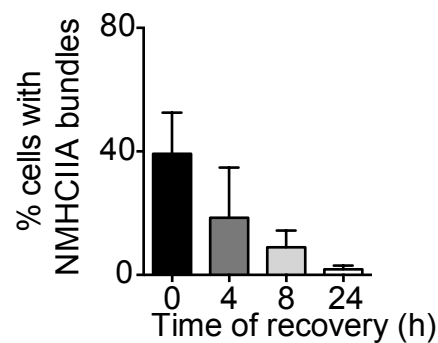
Appendix Figure S3

Appendix Figure S1 - NMHCIIA bundles contain pEzrin and progressively disassemble after LLO washout

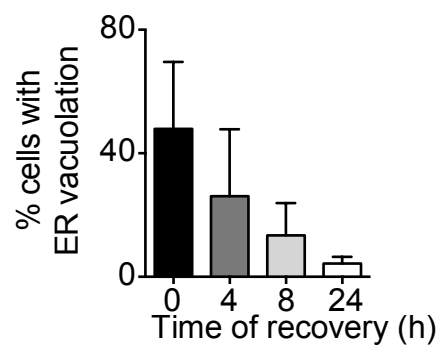
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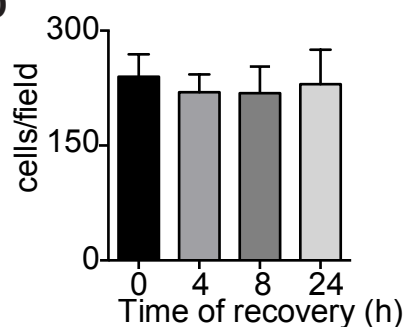
B



C



D

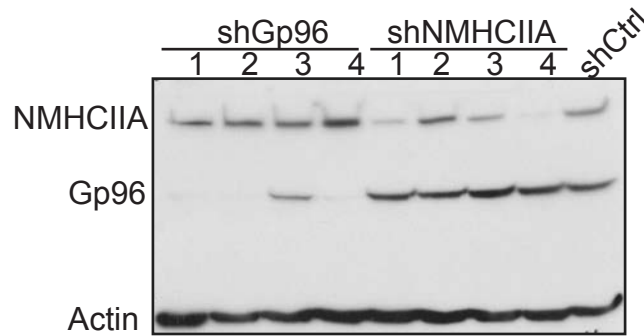


Appendix Figure S1 - NMHCIIA bundles contain pEzrin and progressively disassemble after LLO washout

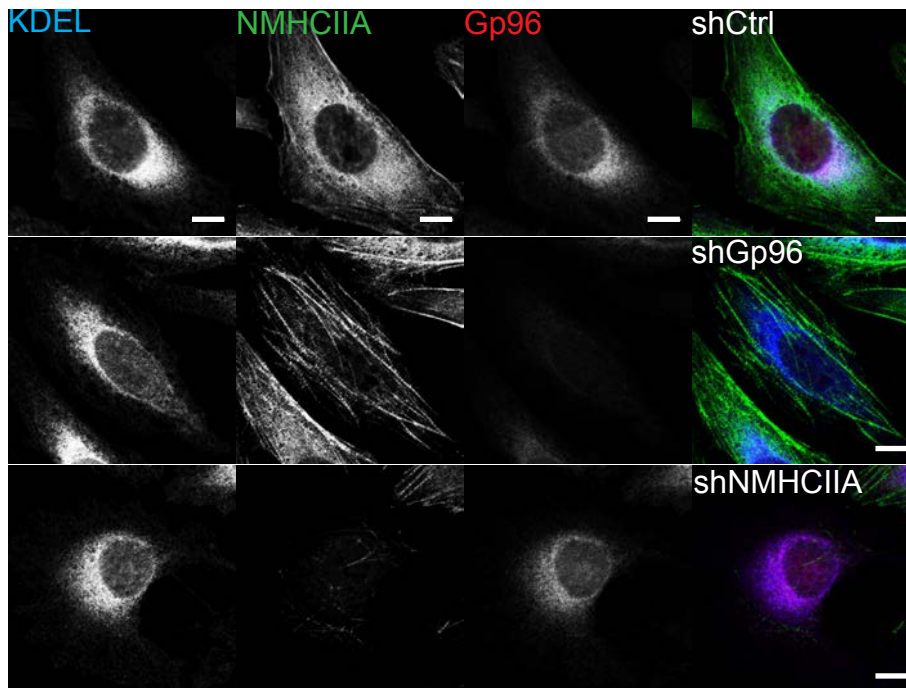
(A) Confocal microscopy images of HeLa cells left untreated (Control) or treated with LLO (0.5 nM 15 min). Cells were immunolabelled for NMHCIIA (green) and pEzrin (red). Scale bar - 10 μ m. Insets show NMHCIIA bundles and arrows indicate the association between NMHCIIA and pEzrin. **(B-D)** Quantification of the % of cells showing **(B)** NMHCIIA-bundles and **(C)** ER vacuolation at different time points following treatment with 0.1 nM LLO for 15 min and washout. **(D)** Number of cells per microscope field at different time points following LLO washout. Values are mean \pm SEM ($n \geq 3$).

Appendix Figure S2 - Depletion of Gp96 and NMHCIIA in HeLa cells. Gp96 and NMHCIIA control actomyosin remodelling during LLO treatment and modulate cell shape

A



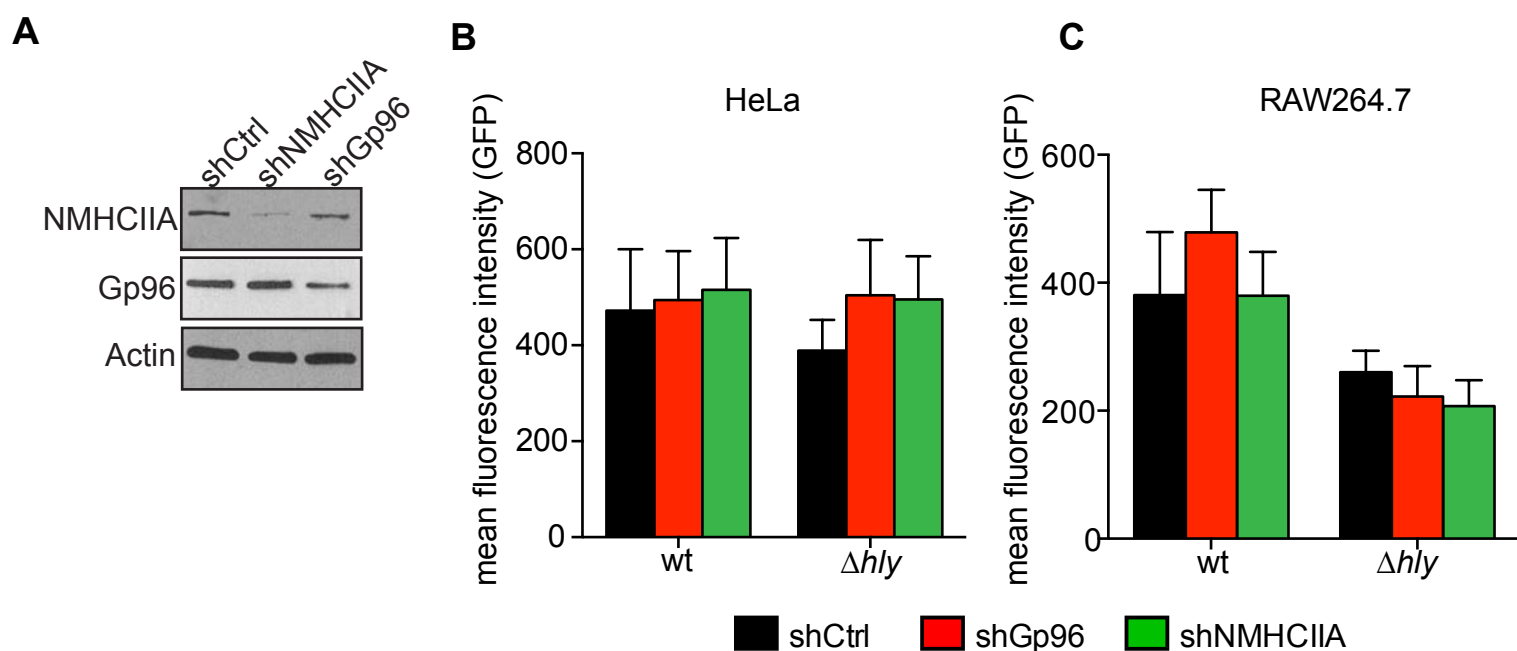
B



Appendix Figure S2 - Depletion of Gp96 and NMHCIIA in HeLa cells

(A) Immunoblots of NMHCIIA, Gp96 and actin levels in HeLa cells stably expressing shRNA control oligonucleotides (shCtrl) or oligonucleotides targeting the expression of Gp96 (shGp96) or NMHCIIA (shNMHCIIA). The three independent cell lines showing most efficient protein depletion (shGp96 #1, #2, #4 and shNMHCIIA #1, #3, #4) were used in all subsequent experiments. **(B)** Confocal images of shControl, shGp96 or shNMHCIIA HeLa cells immunolabelled for KDEL-proteins (blue), NMHCIIA (green) and Gp96 (Red). Scale bar - 10 μ m.

Appendix Figure S3 - Depletion of Gp96 and NMHCIIA from RAW264.7 macrophages and intracellular bacterial burden displayed by infected cells analysed



Appendix Figure S3 - Depletion of Gp96 and NMHCIIA from RAW264.7 macrophages and intracellular bacterial burden displayed by infected cells analysed

(A) Immunoblots of NMHCIIA, Gp96 and actin levels in RAW264.7 macrophages stably expressing shRNA control oligonucleotides (shCtrl) or oligonucleotides targeting the expression of Gp96 (shGp96) or NMHCIIA (shNMHCIIA). **(B, C)** Flow cytometry analysis of the GFP geometric mean fluorescence intensity of **(B)** HeLa cells or **(C)** RAW264.7 macrophages expressing control shRNA oligonucleotides (shCtrl) or shRNA oligonucleotides targeting the expression of Gp96 (shGp96, red bars) or NMHCIIA (shNMHCIIA, green bars) infected with GFP-expressing wt or Δhly *Lm*. At least 5,000 up to 10,000 GFP positive cells were analysed for each sample per experiment. Values are mean \pm SEM ($n \geq 3$).