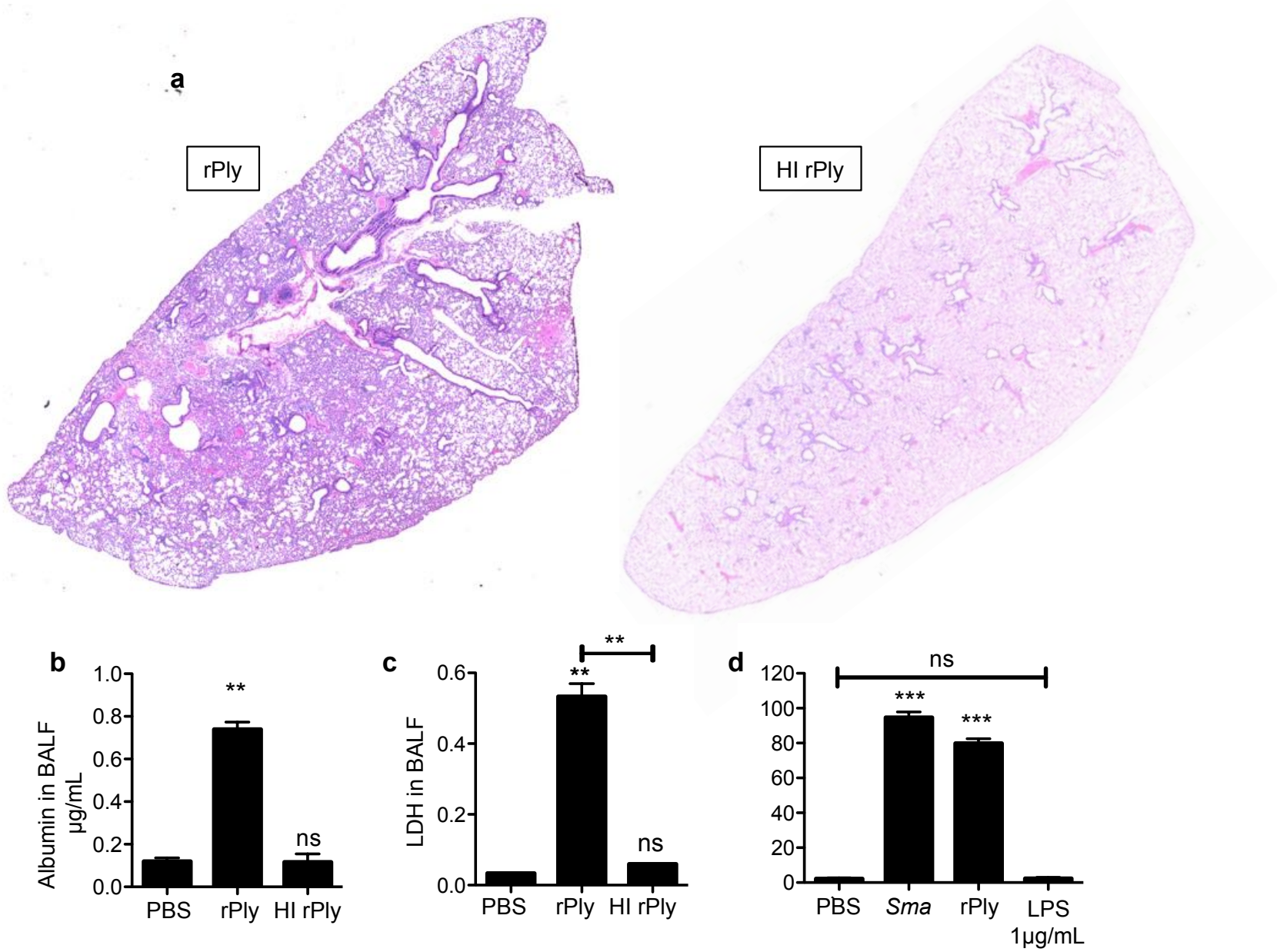

Figure S1: Pneumolysin induces pore-forming toxin dependent tissue damage. C57BL/6 mice (4-6 per cohort) were challenged intratracheally with 0.2 ng of recombinant pneumolysin (rPly) or heat-inactivated rPly (HI-rPly). Challenged mice were sacrificed at 24 hours post-infection for (**a**) pathological evaluation of their lungs and determination of (**b**) albumin and (**c**) lactate dehydrogenase (LDH) levels in BALF. (**d**) LDH release of A549 cells infected with *Sma* or challenged with pneumolysin or LPS 1µg/mL. Mann-Whitney U test was used for two-group comparisons. *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$.

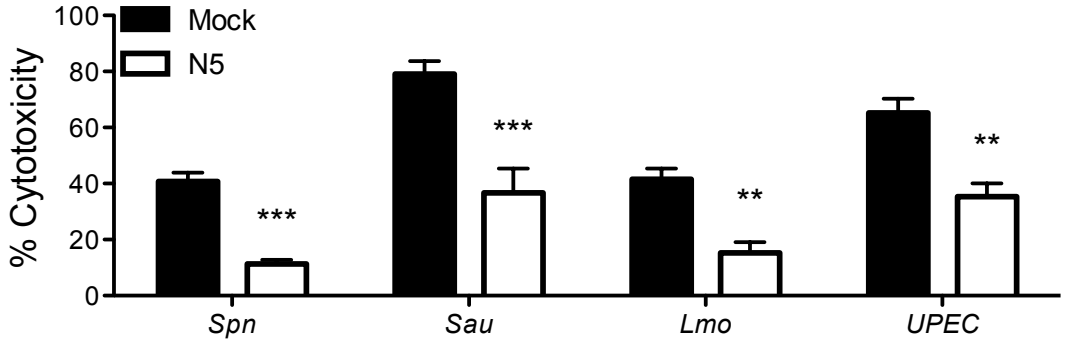

Figure S2: Only PFT-producing pathogens induce RIPK1 dependent necroptosis in respiratory epithelial cells. Percent cytotoxicity of A549 cells pretreated with necrostatin-5 (10μM) and infected with *S. pneumoniae* (*Spn*), *S. aureus* (*Sau*), *Listeria monocytogenes* (*Lmo*) and uropathogenic *E. coli* (*UPEC*). Mann-Whitney U test was used for two-group comparisons. *, $P \leq 0.05$, **, $P \leq 0.01$, ***, $P \leq 0.001$. The mean value for *in vitro* experiments was averaged from >3 separate experiments.

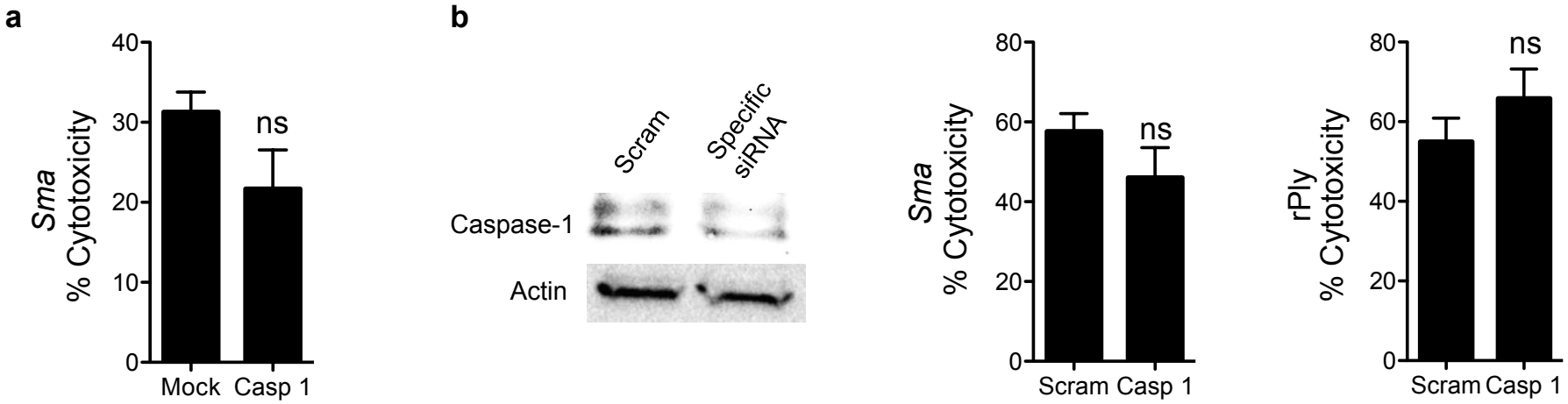

Figure S3: PFT-induced respiratory epithelial cell death is independent of caspase-1 activity. (**a**) Percent cytotoxicity of A549 cells pretreated with caspase-1 inhibitor YVAD-CHO and infected with *S. marcescens*. (**b**) Immunoblots for caspase-1 and corresponding percent cytotoxicity of *Sma* infected A549 cells transfected with siRNA against caspase-1. Mann-Whitney U test was used for two-group comparisons. The mean value for *in vitro* experiments was averaged from >3 separate experiments.

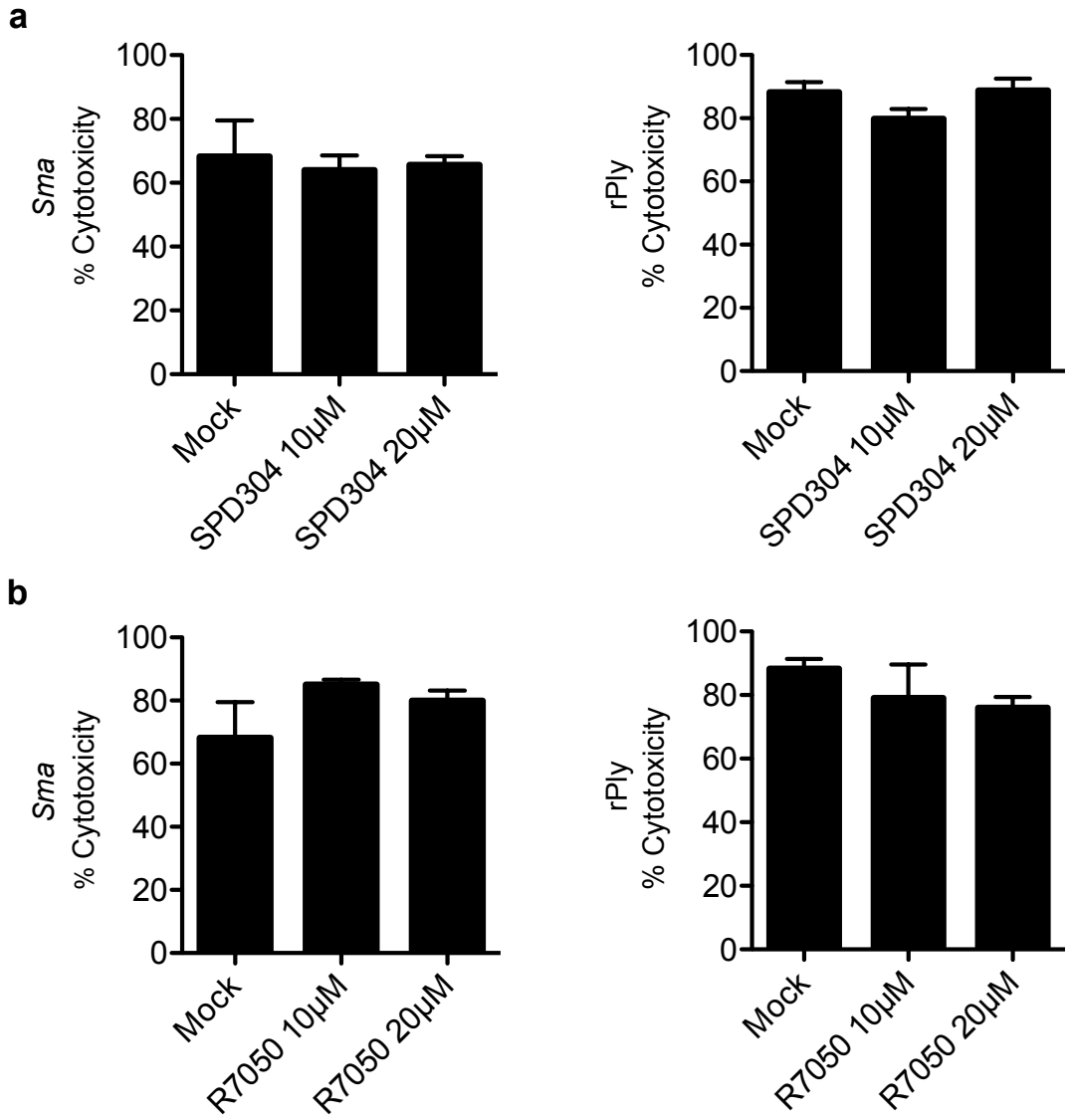

Figure S4: TNF-α or TNFR1 inhibition does not protect against PFT-mediated primary respiratory epithelial cell necroptosis. Percent cytotoxicity of normal human primary epithelial cells infected with *Sma* or challenged with rPly pretreated with (**a**) a small inhibitor of TNF-α (SPD304) or (**b**) a selective inhibitor of TNFR1 (R7050). For multi-group comparisons Dunn's multiple-comparison post-test was used. The mean value for *in vitro* experiments was averaged from >3 separate experiments.

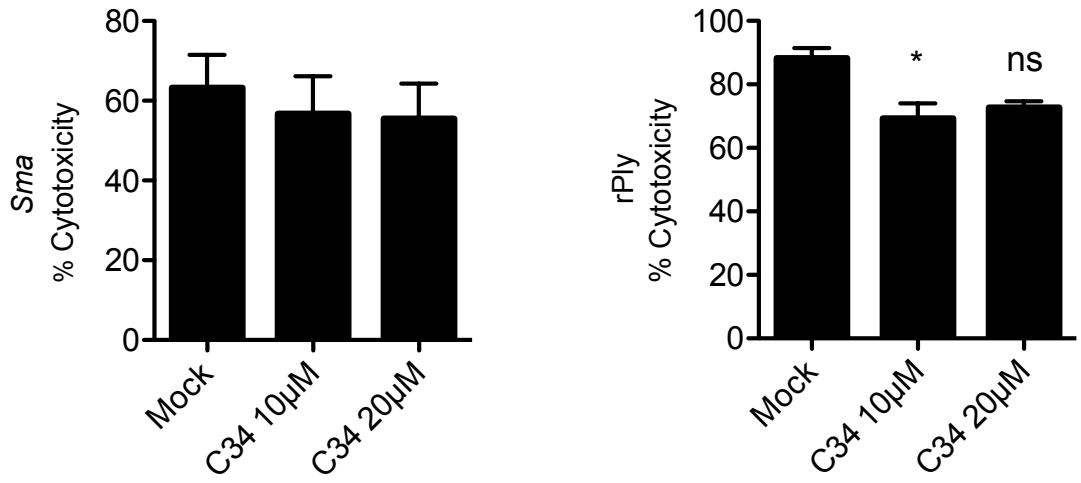

Figure S5: TLR4 inhibition does not protect against PFT-mediated primary respiratory epithelial cell necroptosis. Percent cytotoxicity of normal human primary bronchial epithelial cells infected with *Sma* or challenged with rPly pretreated with a small inhibitor of TLR4 (C34). For multi-group comparisons Dunn's multiple-comparison post-test was used. The mean value for *in vitro* experiments was averaged from >3 separate experiments.

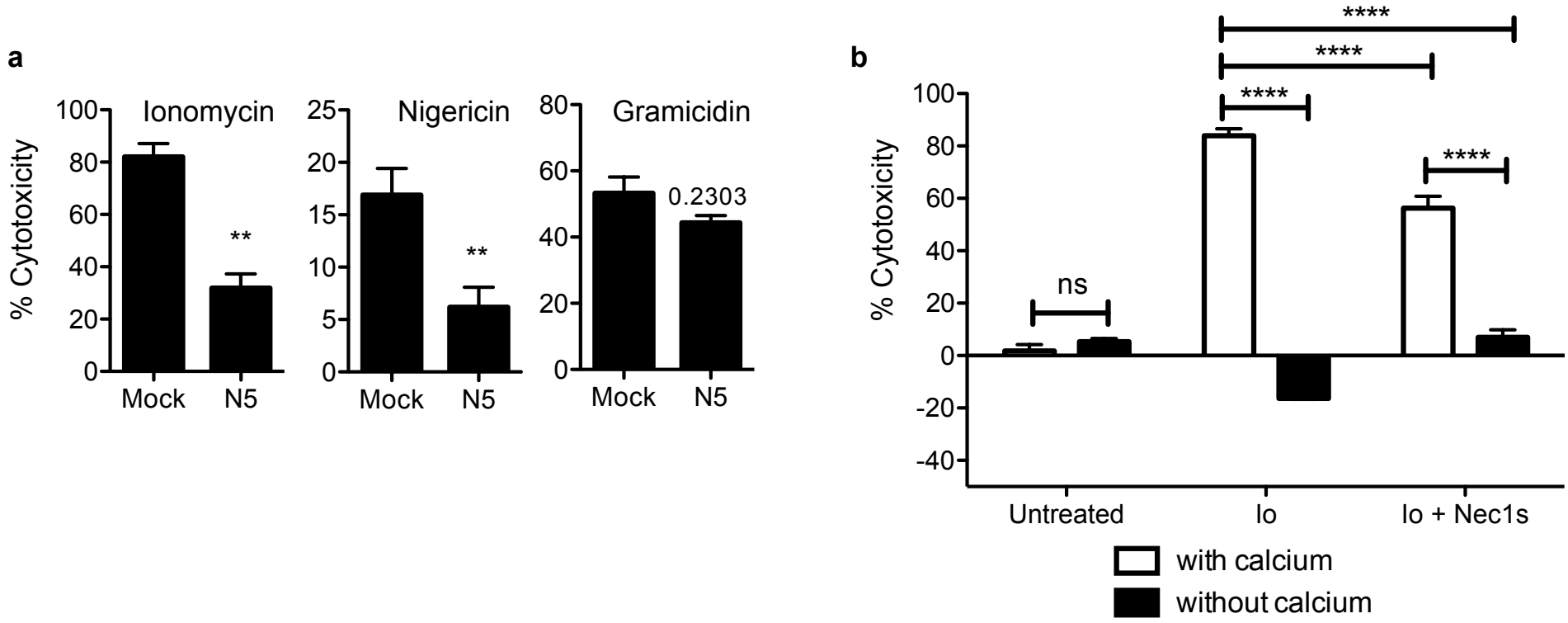

Figure S6: Ion dysregulation initiates PFT-induced necroptosis. (**a**) Percent cytotoxicity of A549 alveolar epithelial cells pre-treated with necrostatin-5 (N5, 100 μM), then challenged with ionomycin (20 μM), nigericin (20 μM) or gramicidin (20 uM). (**b**) Percent cytotoxicity of A549 alveolar epithelial cells challenged with ionomycin (20μM) in presence or absence of calcium in the culture media after pretreatment with necrostatin-1s (Nec1s, 10 μM). Mann-Whitney U test was used for two-group comparisons. *, P ≤ 0.05, **, P ≤ 0.01, ***, P ≤ 0.001, ****, P ≤ 0.0001.

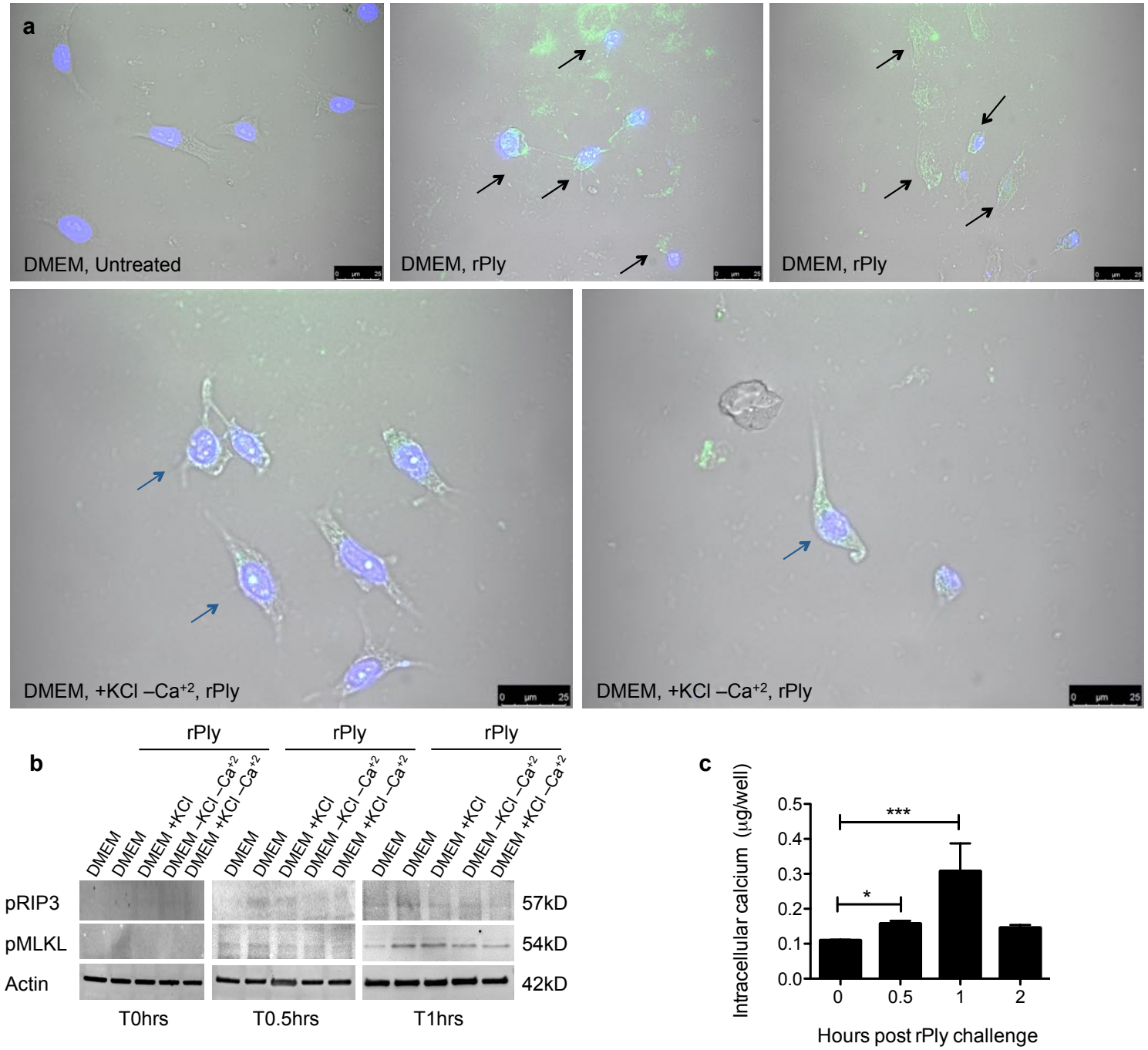

Figure S7: Modulation of Ca^{++} and K^{+} dysregulation reduces activation of necroptosis. (**A**) Immunofluorescent staining of A549 alveolar epithelial cells challenged with rPly in presence of calcium in the culture media or in calcium depleted media supplemented with potassium (KCl 130 mM). Black arrows show membrane disruption, blue arrows show membrane integrity. (**B**) Immunoblots for pMLKL and pRIP3 in lung tissue of A549 alveolar epithelial cells challenged with rPly in presence of calcium in the culture media (DMEM), DMEM supplemented with potassium (KCl 130 mM, DMEM +KCl), calcium depleted media (DMEM –KCl-Ca^{+2}) or calcium depleted media supplemented with potassium (DMEM +KCl-Ca^{+2}). (**C**) Kinetics of intracellular calcium levels (µg/well) in A549 challenge with rPly (0.32 µg/mL). For multi-group comparisons Dunn's multiple-comparison post-test was used. The mean value for *in vitro* experiments was averaged from 3 separate experiments.