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Sessile alveolar macrophages communicate with alveolar epithelium to modulate immunity.

Westphalen K, Gusarova GA et al

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Background

- Bacterial constituents activate macrophages via Toll like receptor 4, causing secretion of proinflammatory cytokines
- Release of cytokines leads to recruitment of neutrophils
- Neutrophils may injure alveolar tissue
- Mechanism of tissue protection through modulation of immune response is not fully discovered

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Macrophages

- Monocytes develop from myeloid progenitor cells
- Monocytes migrate into tissues and differentiate into macrophages
- Main task is the production of cytokines and chemokines (e.g. IL-1β, TNF-α, IL-6, IL-8, IL-12) phagocytosis and production and release of reactive oxygen species ROS

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Toll-like receptors

Toll-like receptor	Ligand
TLR-1:TLR-2 heterodimer	Lipomannans, Lipoproteins Cell-wall β-glucans, lipoteichoic acids, Zymosan
TLR-2:TLR-6 heterodimer	Ligands of TLR-1:TLR-2 heterodimer
TLR-3	Double-stranded RNA
TLR-4	LPS, Lipoteichoic acids
TLR-5	Flagellin
TLR-7	Single-stranded RNA
TLR-8	Single-stranded RNA
TLR-9	DNA with unmethylated CpG
TLR-10	Unknown

K Murphy. Janeway´s Immunobiology. 8th ed. New York: Garland Science 2012,p.86

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Knock-out Mouse

- Alveolar macrophages (AMs) express CD11c
- CD 11c-cre mice + Rosa26-LSL-EYFP mice -> enhanced yellow fluorescence protein (YFP) in $CD11c^{+}$ cells
- To distinguish Dendritic cells microinjections with AM Marker Siglec F[13] and MHC-II-Ab were given
- Live confocal microscopy of isolated mouse lungs
- Alveolar Dye-injections only marked AMs in lumen
- YFP^{+} - $MHC\text{-}II^{low}$ cells from BAL did not induce T-cell proliferation in antigen-presenting assays

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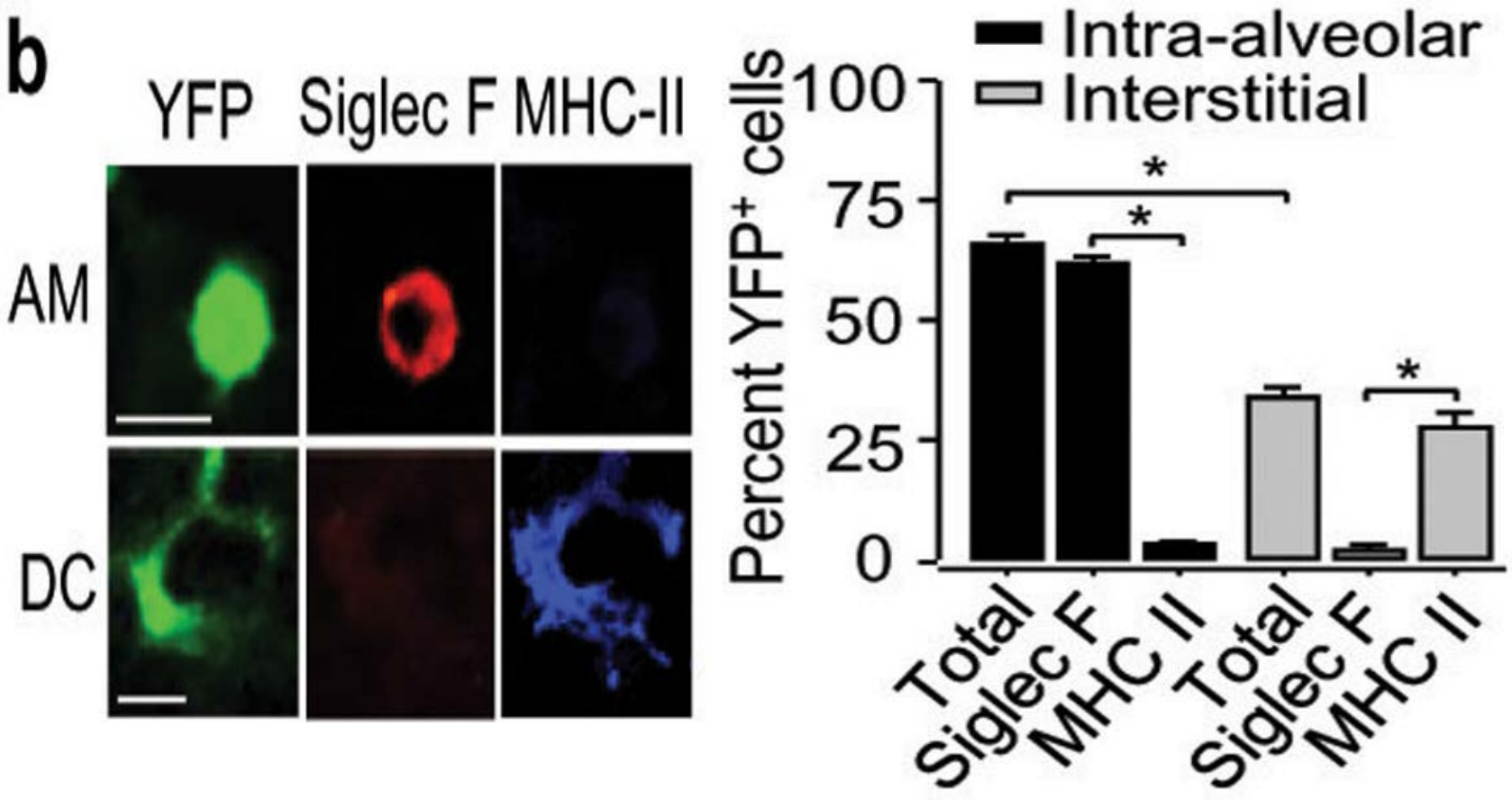

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- 1 AM/ every 3 alveoli
- Remained sessile within AM diameter
- Imaging studies for 4h
- Even after BAL,microinjection of S.aureus and buffer

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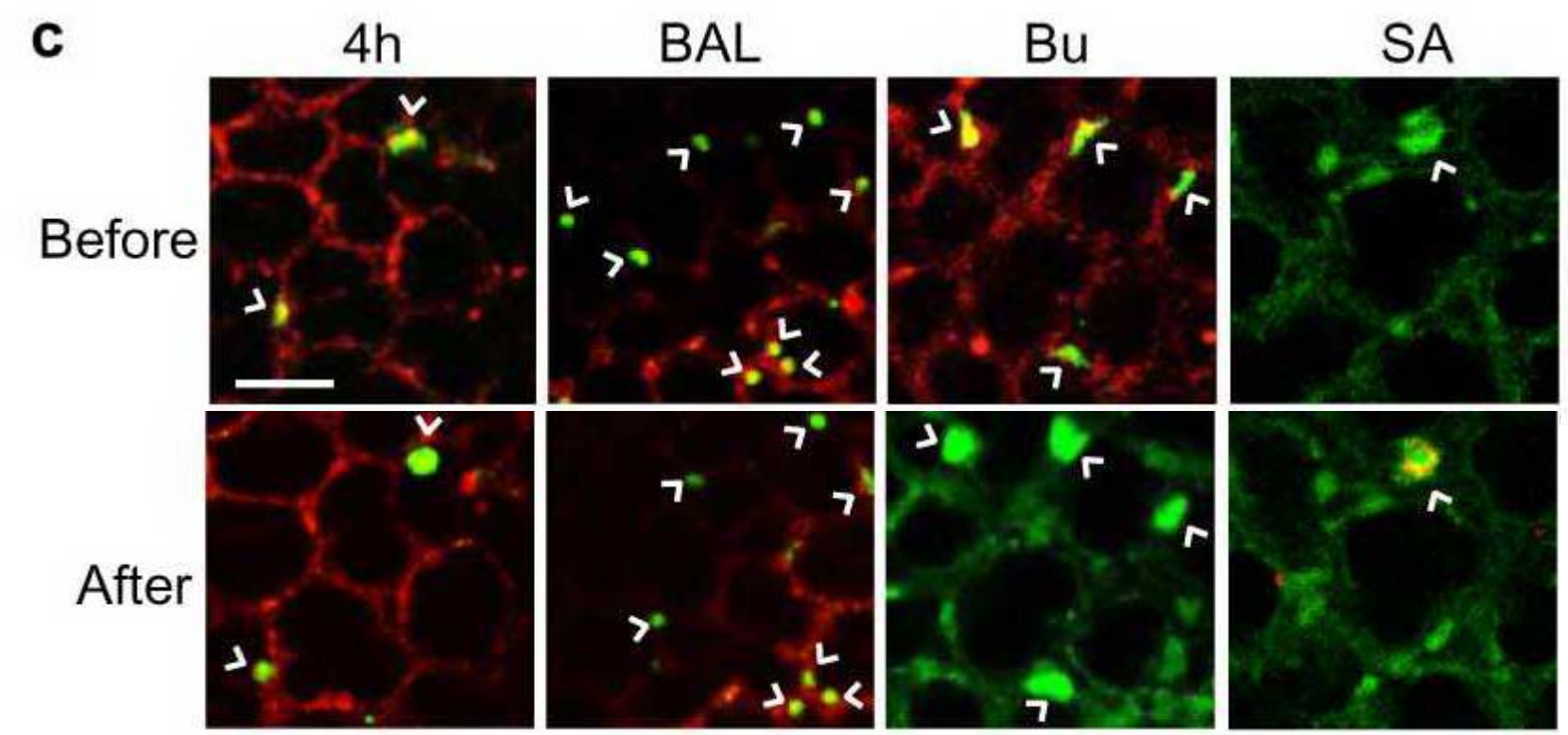
Discussion



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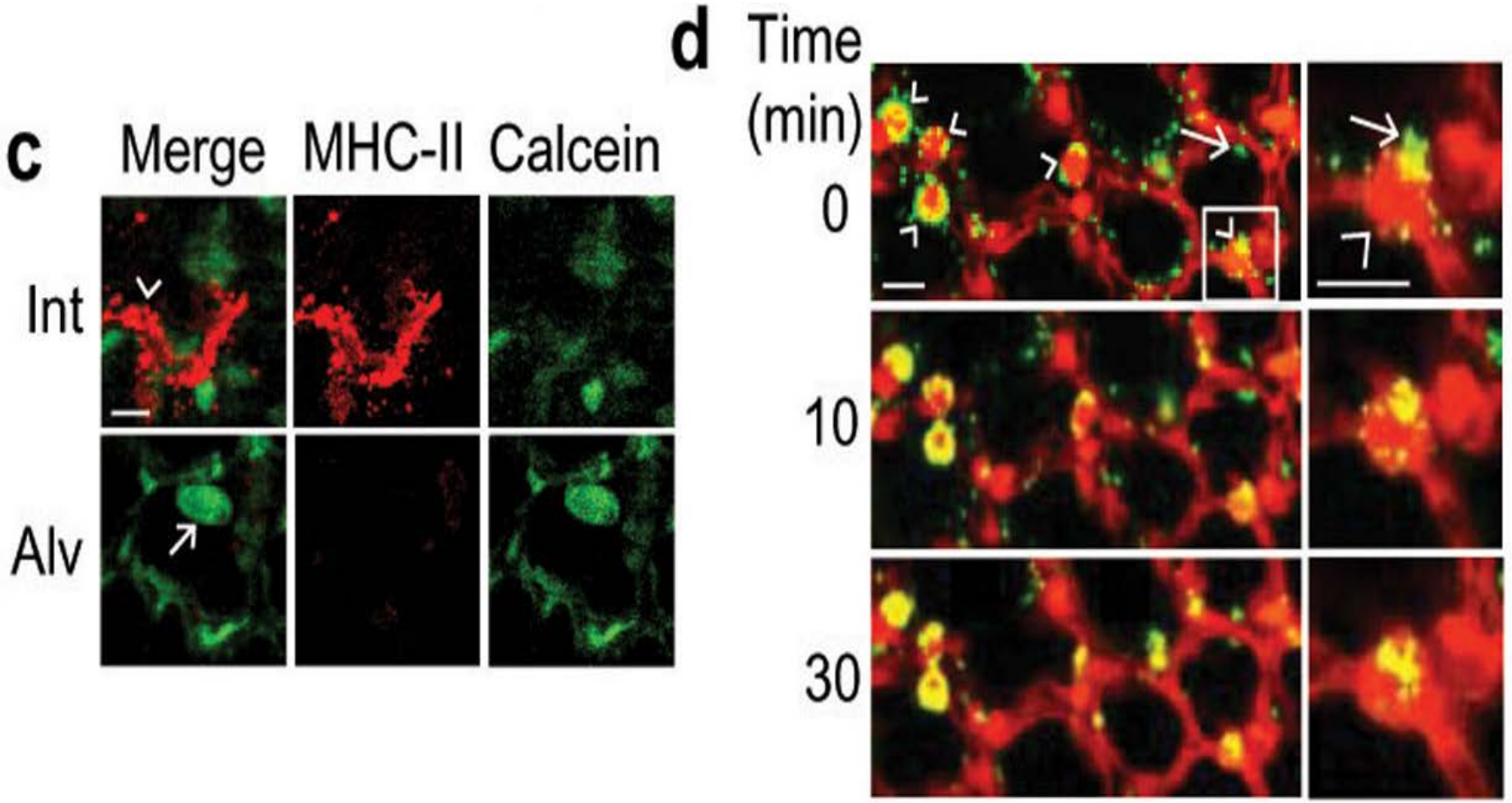

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- AMs can form Gap junctions with alveolar epithelium using connexin 43 (Cx43)
- Photolytic uncaging to enhance cytosolic Ca^{2+}
- Fluorescence recovery after photobleaching (FRAP)
- Uncaging-induced Ca^{2+} waves moved from 40% of AMs to epithelium
- Cx43 correlated with the FRAP
- GAP 26/27 inhibitor of Cx43-based GJCs and hemichannels stops the Ca^{2+} waves

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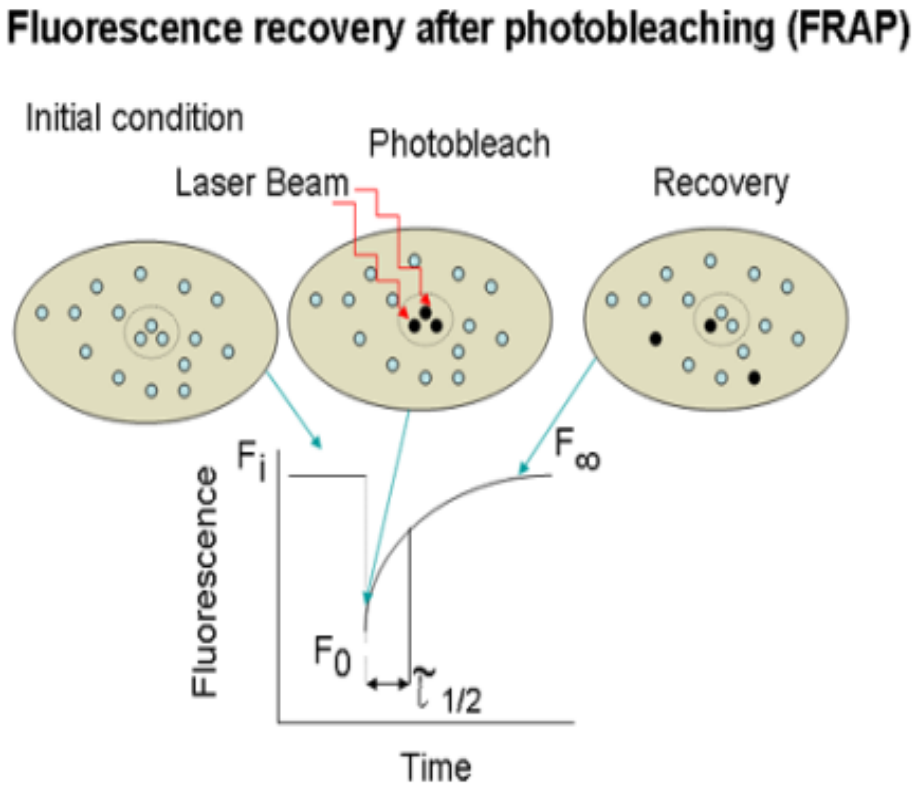
FRAP



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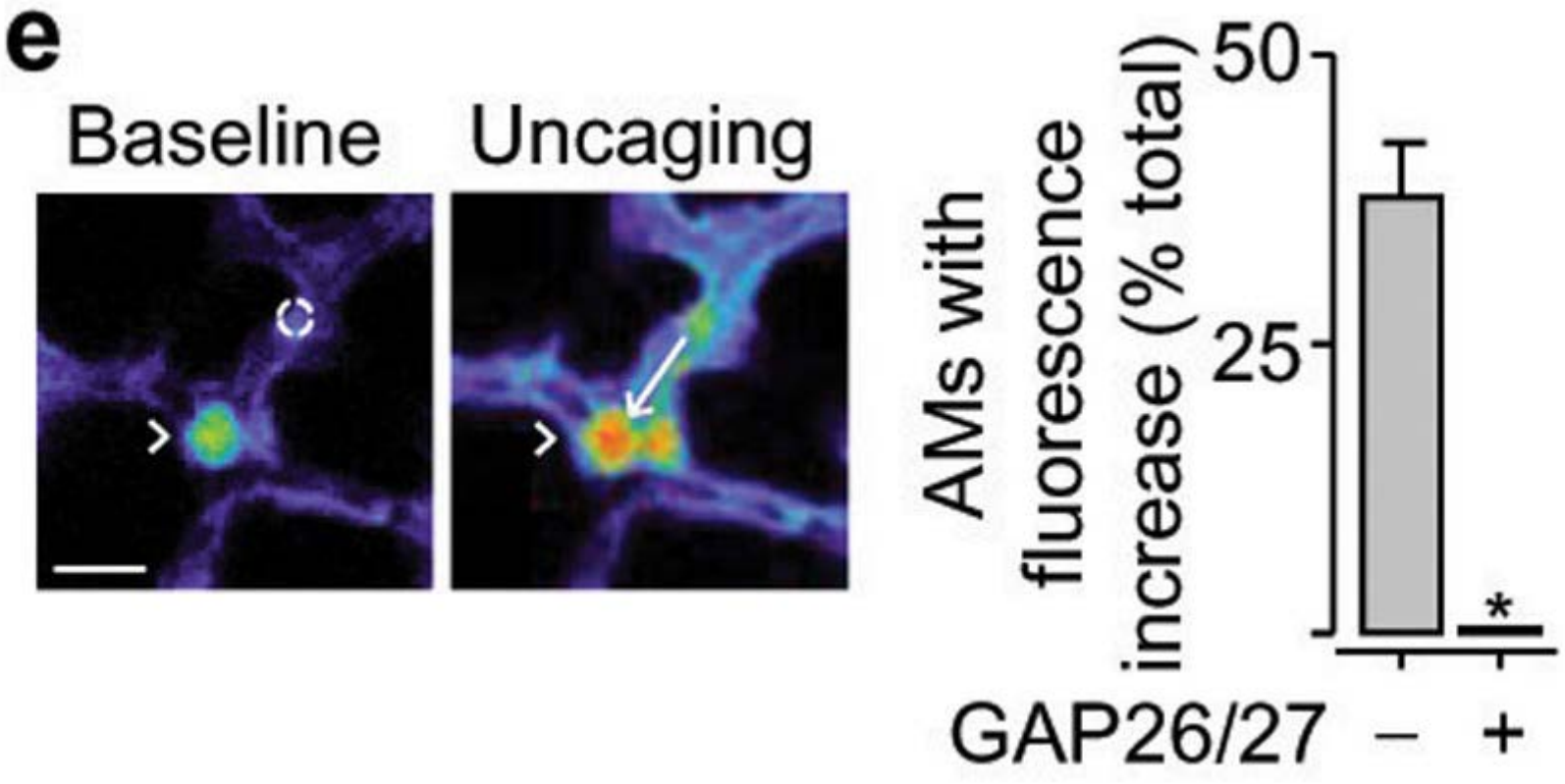

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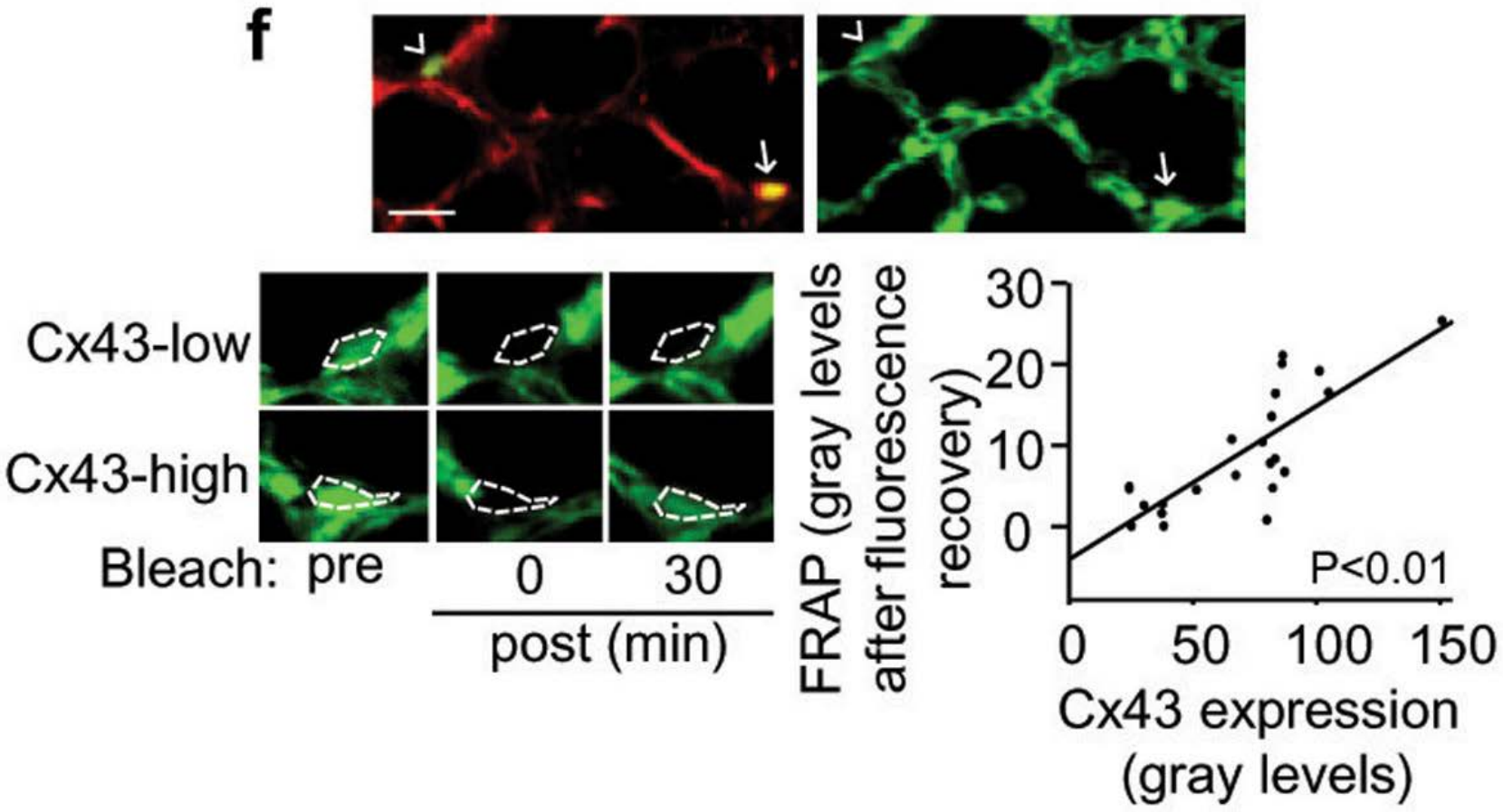

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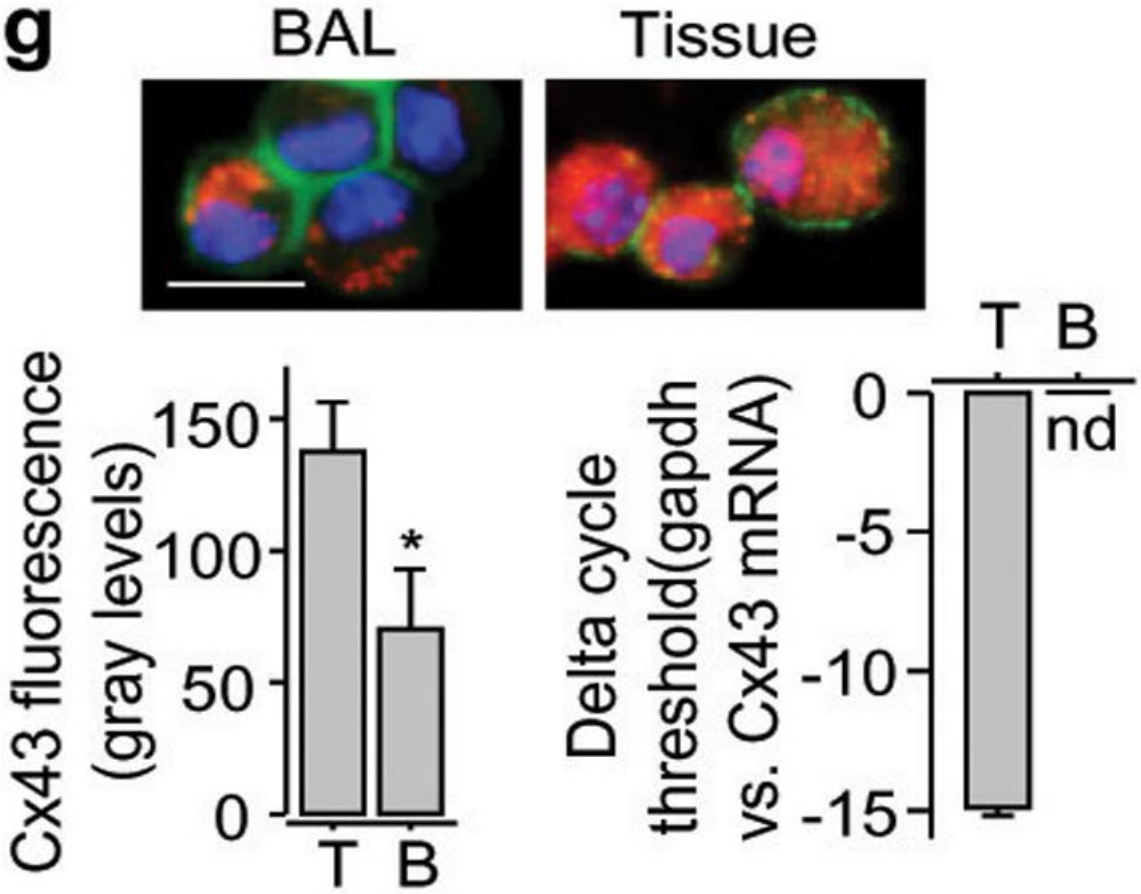

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Lung injury

- For simulation of injury intranasal LPS or PBS were instilled
- Obtained lung 1h, 4h and 24h post LPS instillation
- Fluorescent LPS confirms LPS entry in AMs

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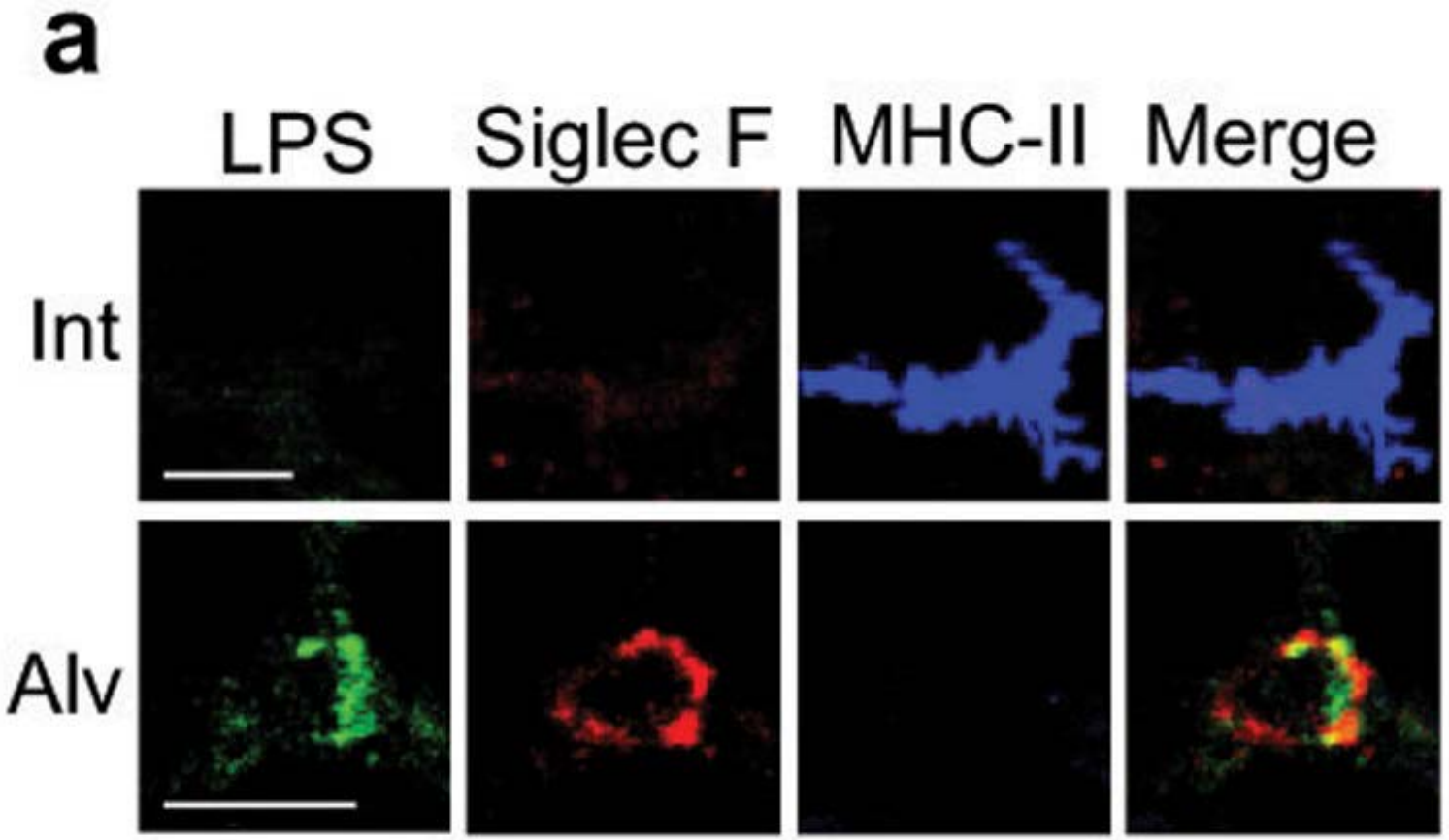

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- AMs of LPS-stimulated lungs showed synchronous Ca^{2+} waves appearing every 10-20min
- Spikes last for 10-15sec
- Initial appearance after 4h and increasing within 24h

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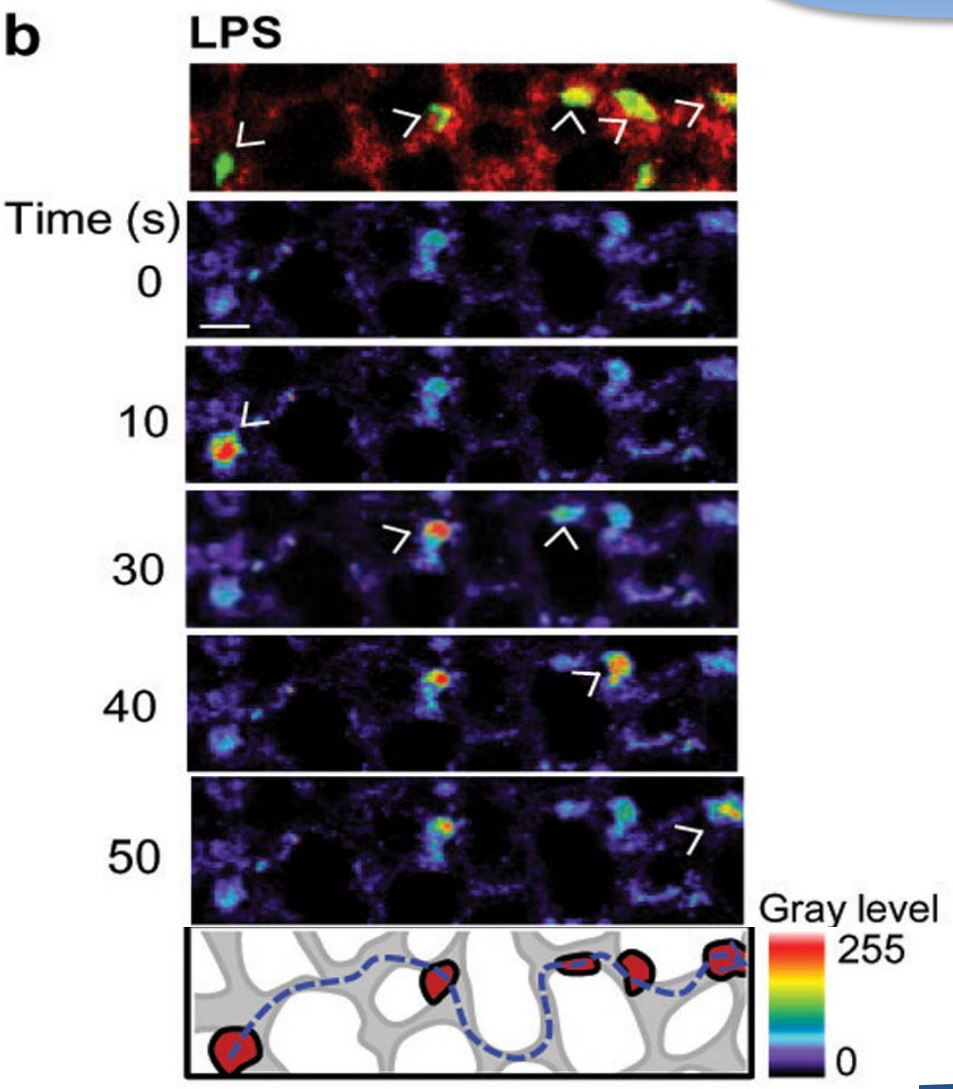

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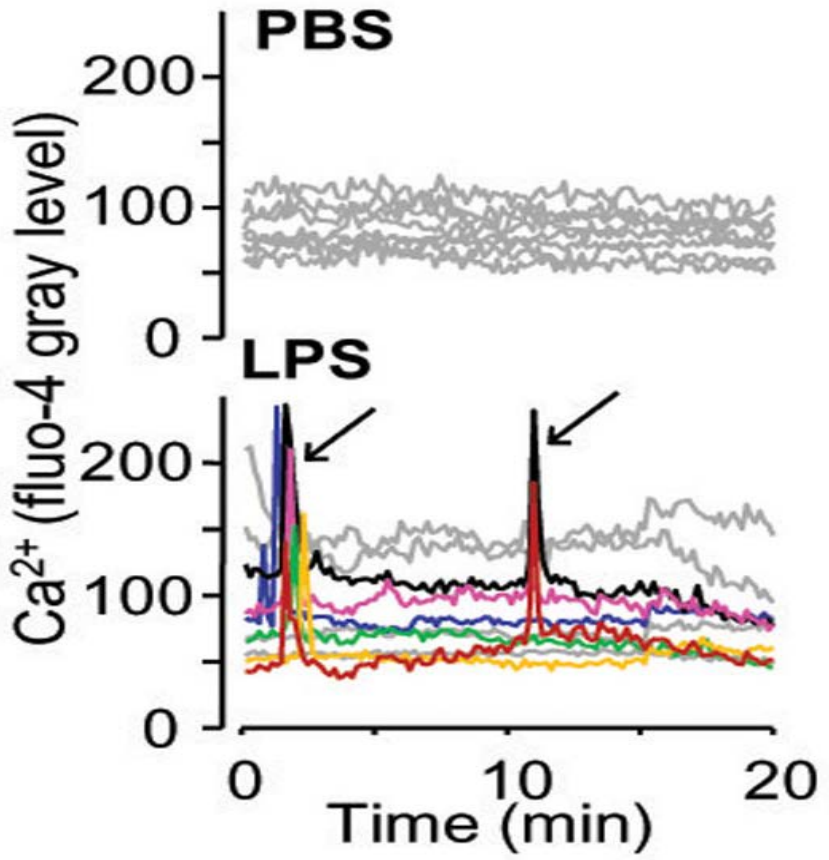

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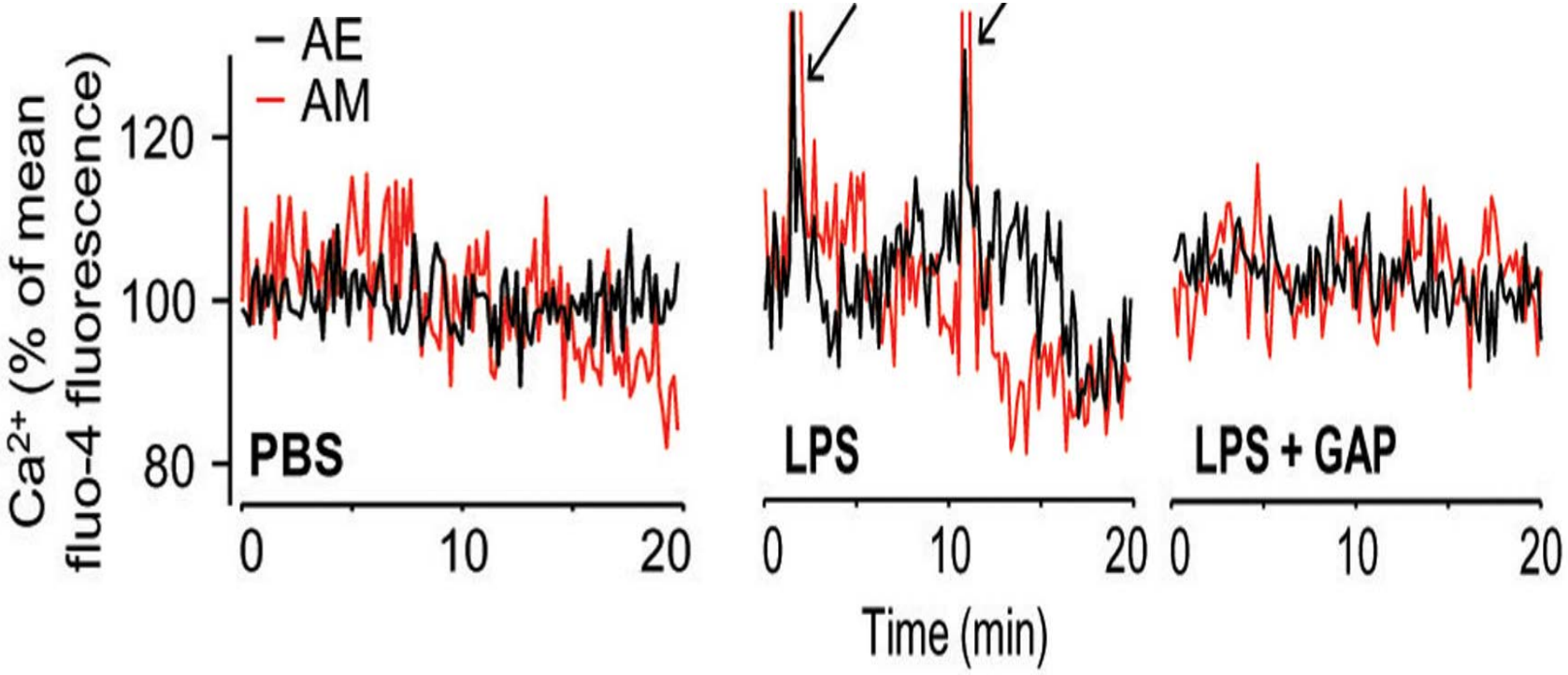

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- Depletion of extracellular Ca^{2+} had no inhibiting effect on the Ca^{2+} spikes (source for spikes is intracellular Ca^{2+})
- PPADS did not influence the spikes -> connexin hemichannels do not induce spike intercommunication
- Sessile AMs Communicate through epithelium

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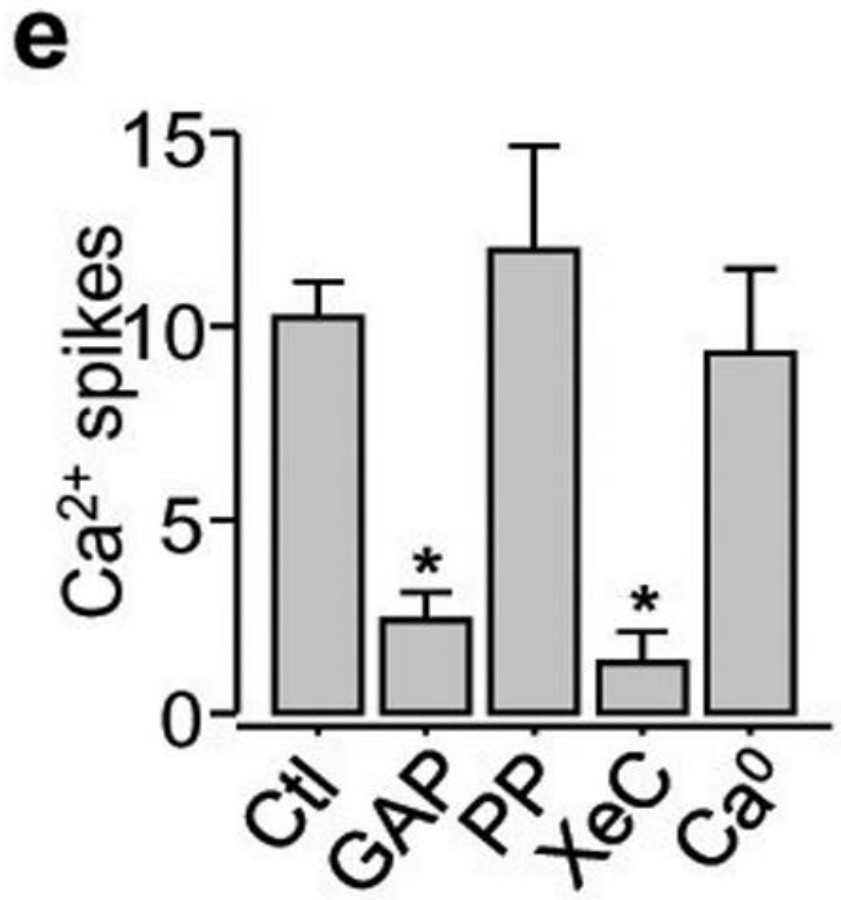

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- Myeloid differentiation factor 88 (MyD88) and TIR-domain-containing adapter-inducing interferon-β (TRIF)

- In CD11cMyD88−/− mice Ca^{2+} spikes were missing and alveolar neutrophil entry at 24h was decreased

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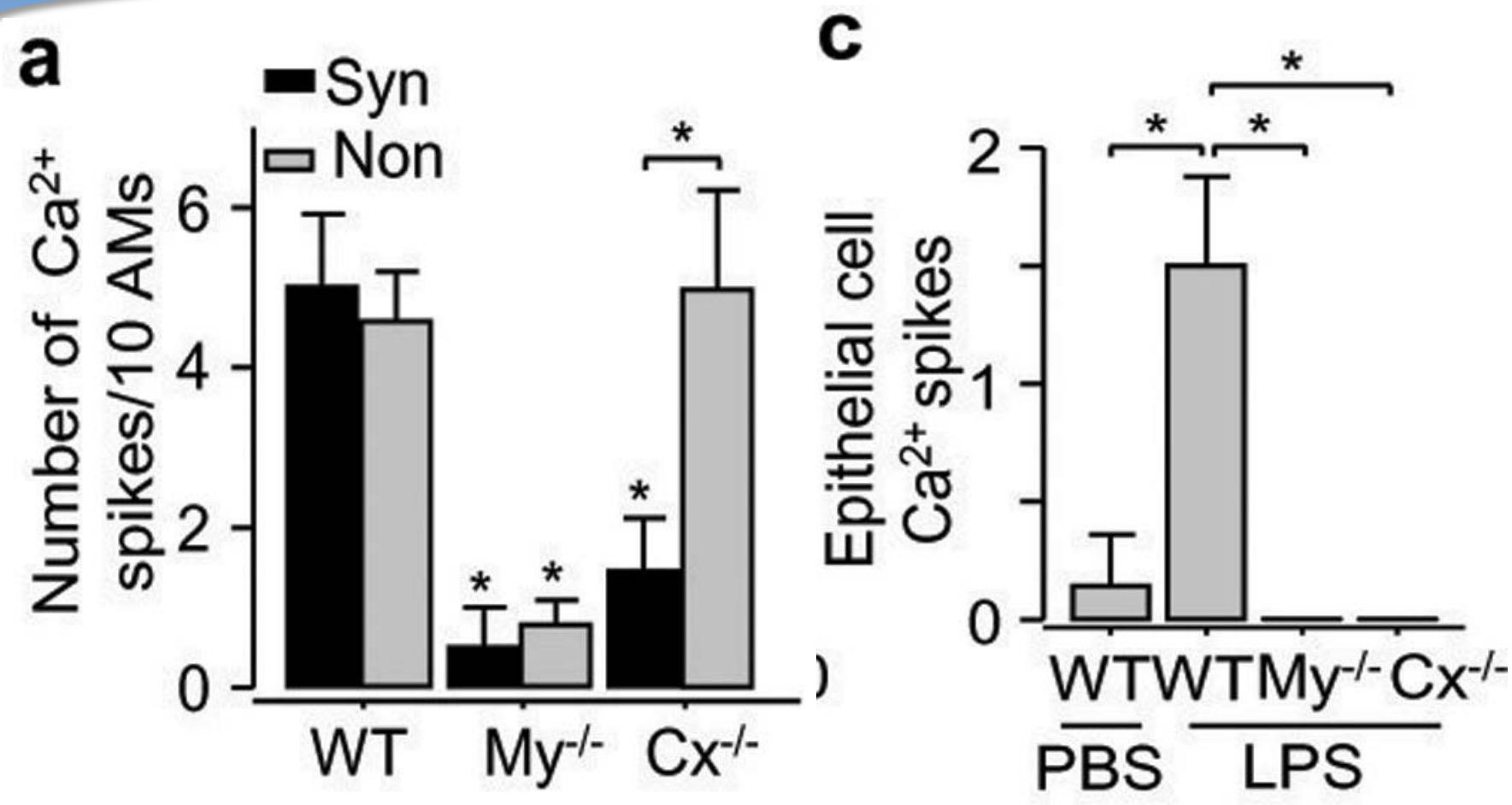

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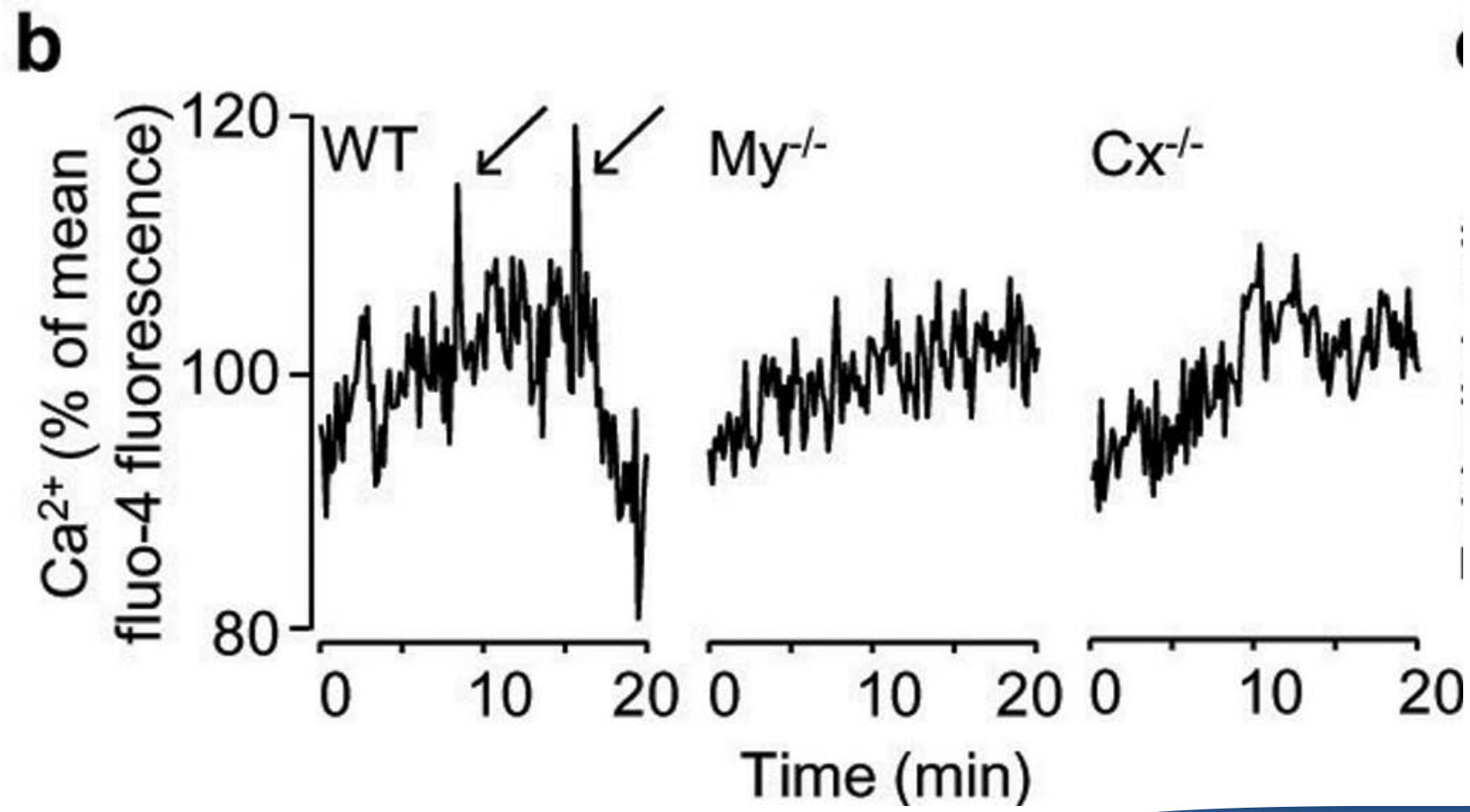

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- CD11cCx43$^{-/-}$ mice displayed increased alveolar neutrophil recruitment and BAL-leukocyte count
- This may indicate enhanced lung inflammation
- Cx43$^{floxed/floxed}$ mice as littermate control
- BAL from CD11cCx43$^{-/-}$ mice comprised more proinflammatory cytokines and the mice showed a higher mortality

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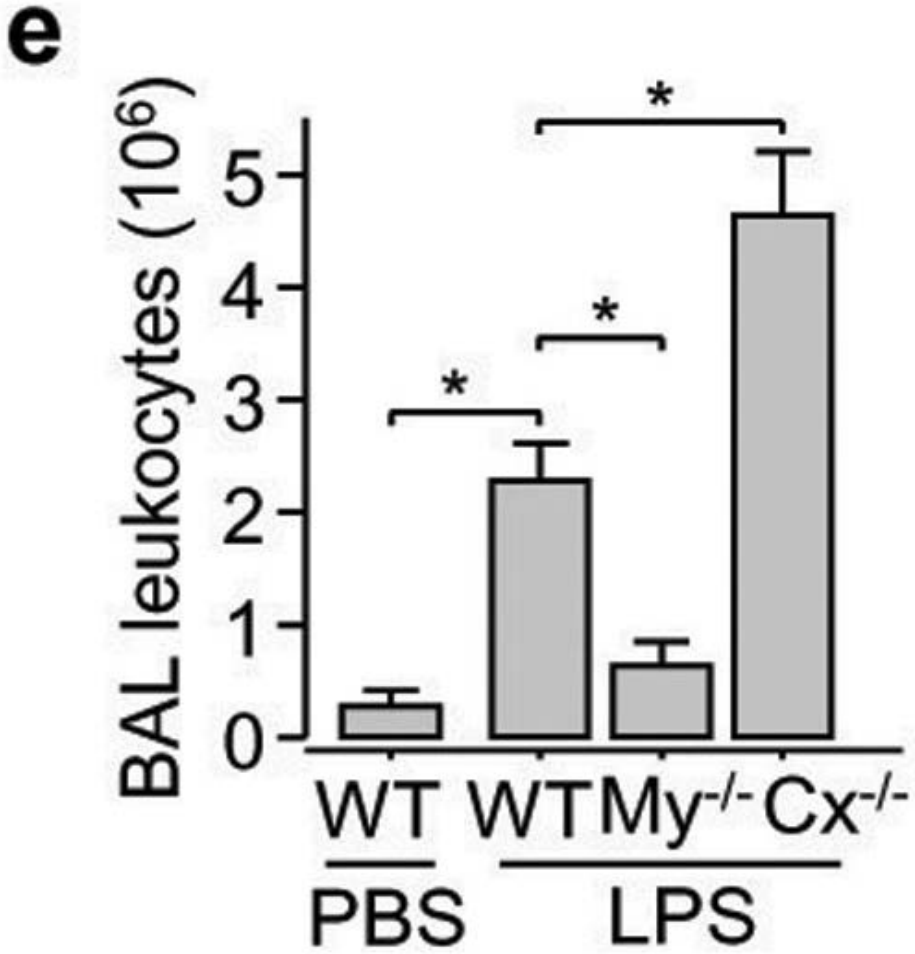

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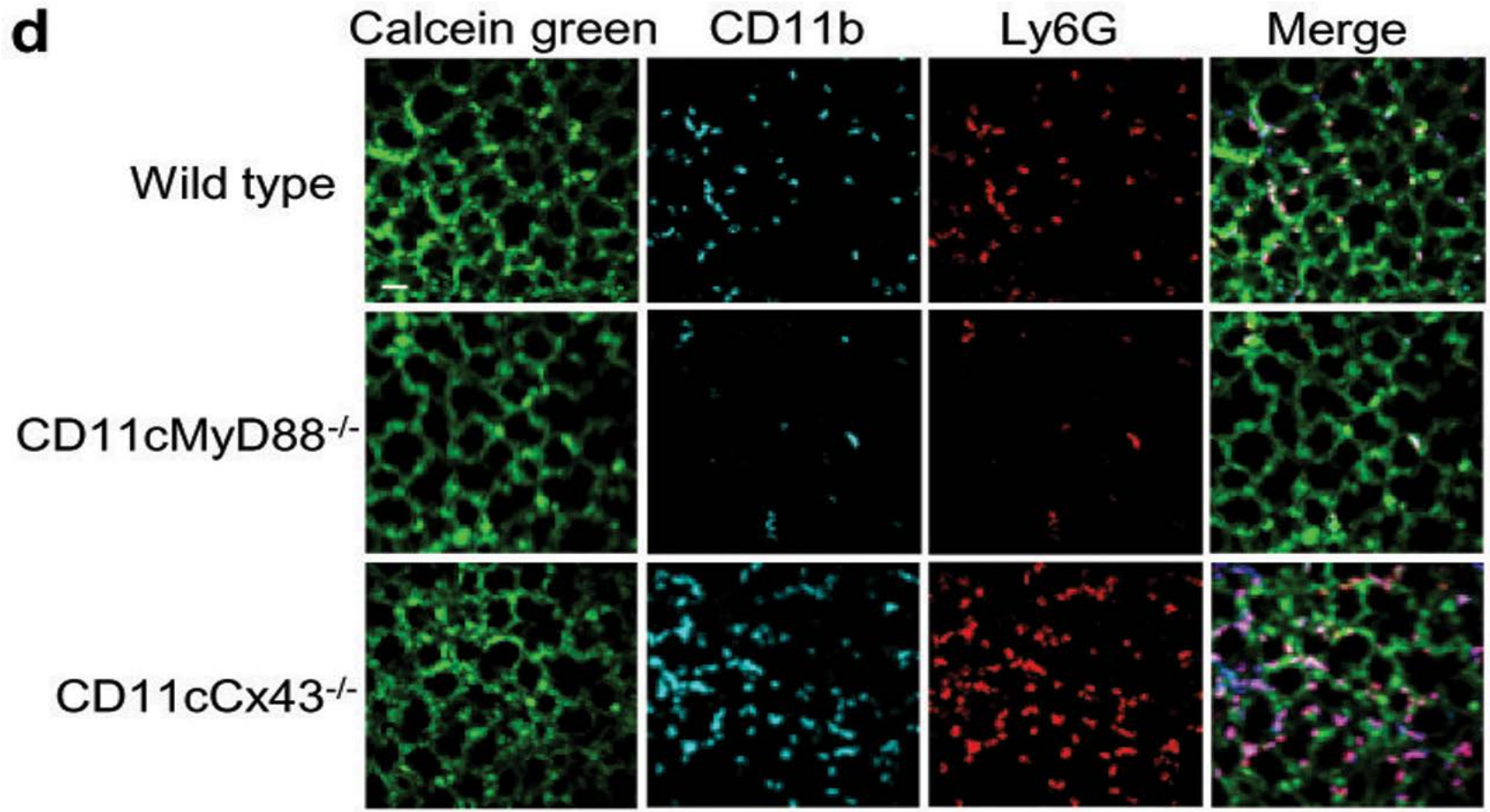

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- LPS caused elevated degradation of IκBα and translocation of NFκB in CD11cCx43$^{-/-}$ mice
- Enhanced intracellular Ca^{2+} leads ti activation of Ca^{2+} /calmodulin-dependent kinase kinase (CAMKK) and the Akt kinase (pro-survival)
- LPS induced Akt phosphorylation in WT, but not in CD11cCx43$^{-/-}$ mice and BAPTA-AM treatment (intracellular Ca^{2+} chelator)
- SPC-Cx43 $^{-/-}$ mice -> loss of Cx43 in alveolar epithelium

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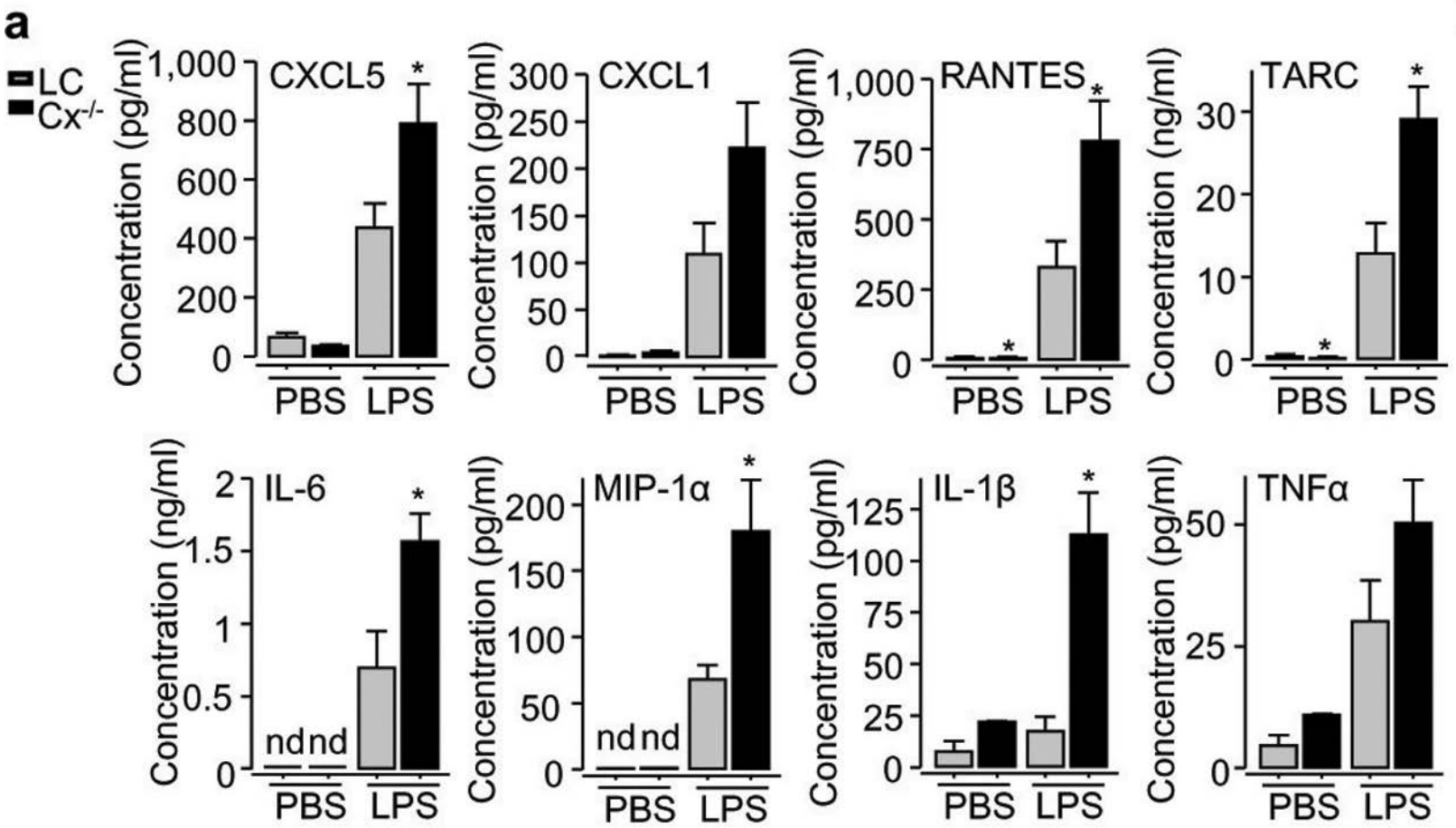

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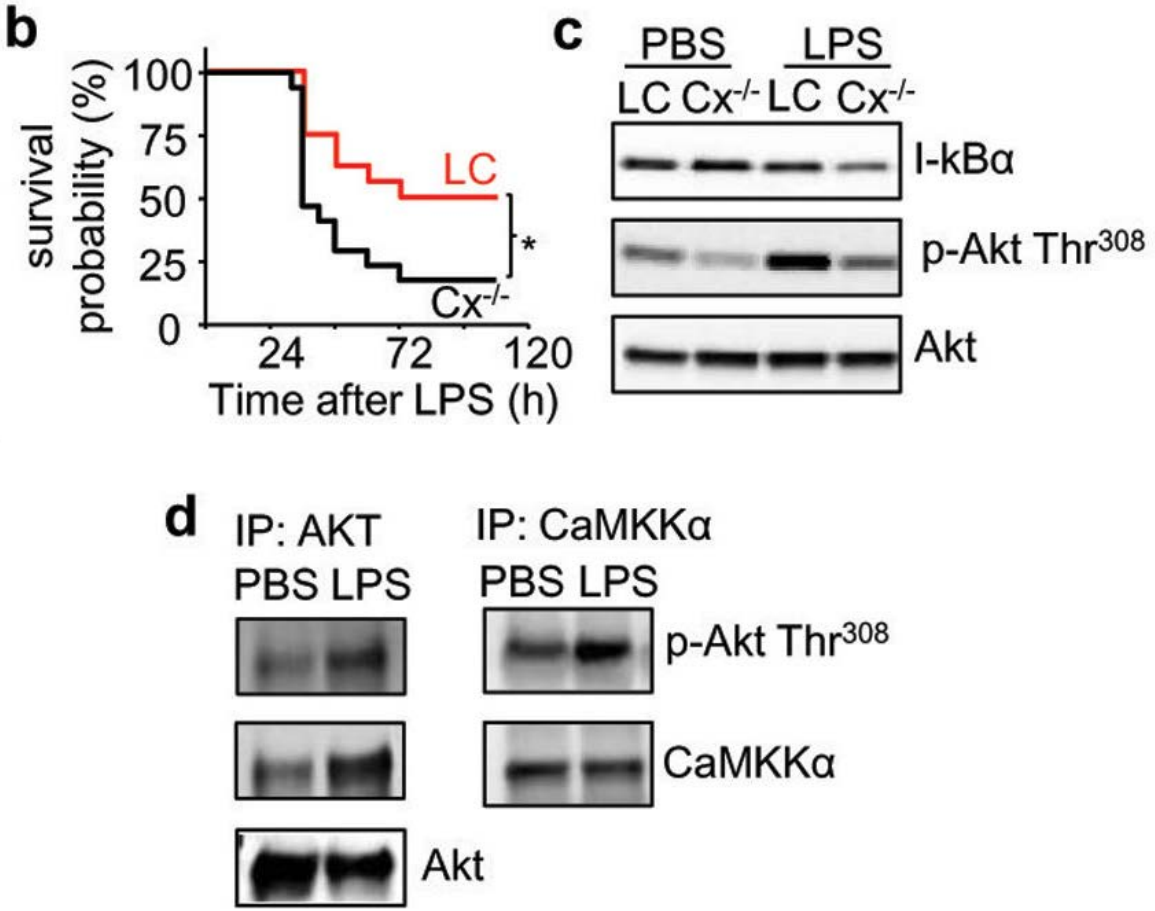

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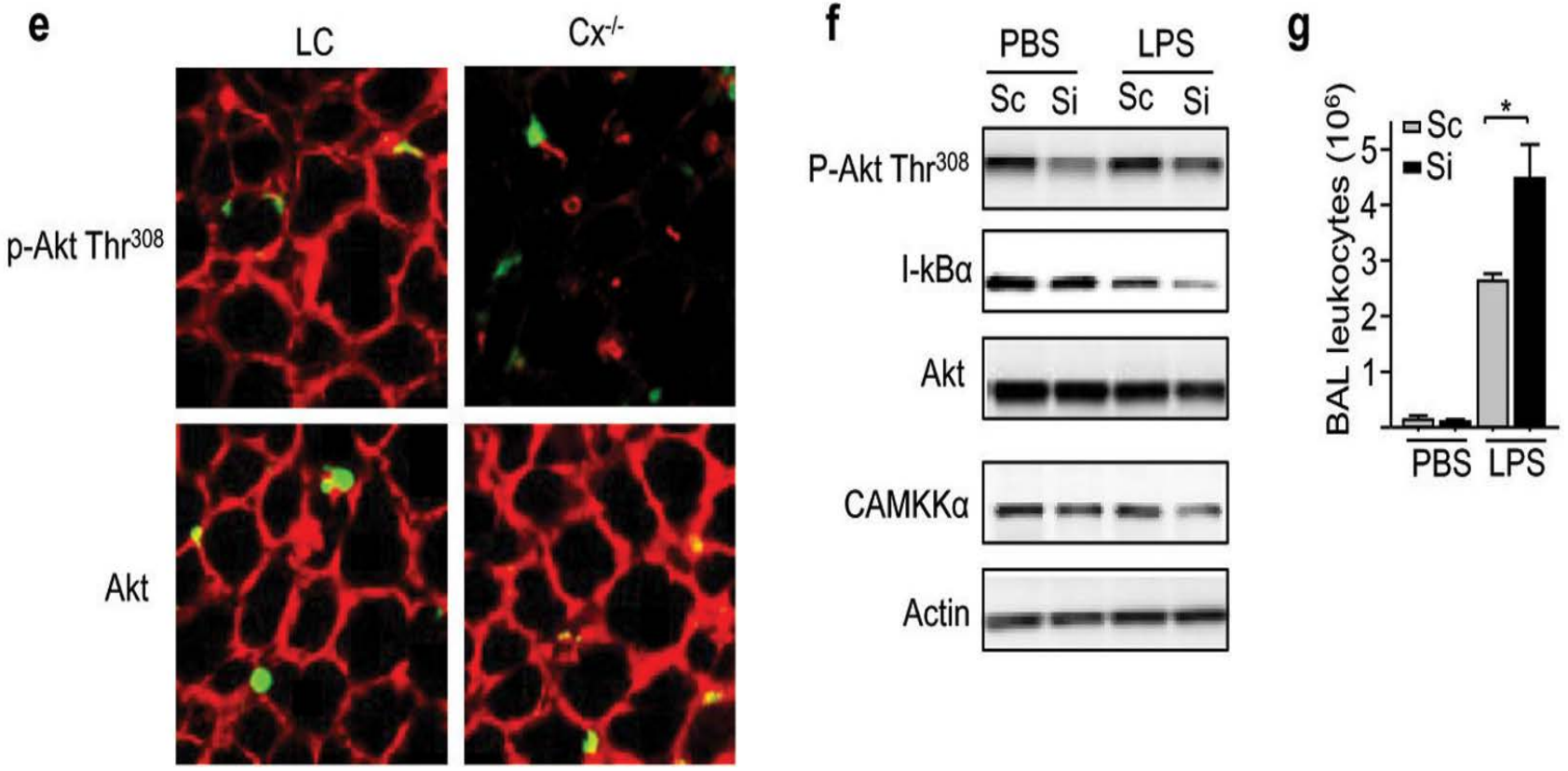

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Discussion

- $Cx43^{high}$ AMs may play an important role in syncytial communication to diminish lung inflammation
- Further studies are necessary to fully understand the role of Ca^{2+} - regulatory mechanism
- Cx43 expression and GJCs in AMs may be a possible target for drug delivery in inflammatory lung disease

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Thank you for your attention!

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