#### Bruton's tyrosine kinase in neutrophils is crucial for host defense against Klebsiella pneumoniae

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(5 Tables and 4 Figures)

# Table S1. Percentage of the lung surface with pneumonia in WT and *Mrp8cre.Btk<sup>fl</sup>/Y* mice after intranasal infection with *K. pneumoniae.*

		24 hours		42 hours	
	WT	Mrp8cre.Btk <sup>fl</sup> /Y	WT	Mrp8cre.Btk <sup>fl</sup> /Y	
pneumonia (%)	0 (10-0)	5 (10-0)	5 (9-1)	10 (10-5)	

n = 6-9 per group per time point. Data are medians and interquartile ranges.

# Table S2. BALF cytokine levels in WT and *Mrp8cre.Btk<sup>fl</sup>/Y* mice after intranasal infection with *K. pneumoniae.*

BALF cytokine	6 h	ours	42 hours	
	WT	Mrp8cre.Btk <sup>fl</sup> /Y	WT	Mrp8cre.Btk <sup>fl</sup> /Y
TNF-α	447 (548-305)	606 (854-433)	420 (639-343)	542 (733-394)
IL-1β	95 (134-77)	97 (112-72)	83 (92-75)	81 (101-62)
IL-6	109 (151-81)	171 (211-142)	150 (262-96)	211 (298-155)

All cytokine levels are pg/ml, n = 7-11 per group per time point. Data are medians and interquartile ranges.

# Table S3. Lung cytokine and chemokine levels in WT and *Btk<sup>-/-</sup>* mice after intranasal infection with *K. pneumoniae.*

Lung	6 hours		24 hours		35 hours	
cytokine	WT	Btk⁻′-	WT	Btk <sup>-/-</sup>	WT	Btk <sup>-/-</sup>
TNF	141 (190-121)	173 (226-163)	1352 (1829-1116)	1548 (2273-530)	1387 (3197-1006)	2391 (2837-1581)
IL-1β	40 (50-20)	43 (80-22)	1467 (1678-1155)	1798 (2220-635)	1276 (1596-667)	2515 (2640-2082)**
IL-6	211 (298-155)	338 (455-279)	1799 (2324-1514)	3948 (5726-1906)	1902 (2911-923)	6675 (10000-6290)***
IL-10	221 (269-200)	145 (239-105)	287 (362-276)	369 (402-319)	220 (223-205)	261 (275-220)
CCL2	627 (939-414)	1185 (1301-987)	6249 (6875-5615)	6709 (8118-5551)	6685 (7319-4935)	7945 (10000-7005)

All cytokine and chemokine levels are pg/ml, n = 8-9 per group per time point. Data are medians and interquartile ranges (\*\* P<0.01, \*\*\* P<0.001).

### Table S4. plasma cytokine and chemokine levels in WT and *Mrp8cre.Btk<sup>fl</sup>/Y* mice after intranasal infection with *K. pneumoniae.*

Plasma	6 hours		24 hours		42 hours	
cytokine and chemokine	WT	Mrp8cre. Btk <sup>fl</sup> /Y	WT	Mrp8cre. Btk <sup>fl</sup> /Y	WT	Mrp8cre. Btk <sup>fl</sup> /Y
TNF-α	5 (6-5)	5 (6-5)	57 (116-23)	184 (267-36)	61 (167-31)	112 (173-88)
IL-10	12 (12-12)	14 (16-11)	13 (16-12)	17 (30-13)	19 (127-14)	22 (37-19)
IL-6	10 (17-6)	4 (4-3)	721 (988-109)	1181 (1584-121)	356 (381-240)	3921 (8046-938)**
CCL2	74 (152-57)	62 (71-59)	658 (2019-263)	2445 (4335-1832)	1824 (4037-299)	1502 (4218-1047)

All cytokine and chemokine levels are pg/ml, n = 6-11 per group per time point. Data are medians and interquartile ranges (\*\* P<0.01).

# Table S5. BALF chemokine levels in WT and *Mrp8cre.Btk<sup>fl</sup>/Y* mice after intranasal infection with *K. pneumoniae.*

BALF chemokine	6 ho	urs	42 hours		
	WT	Mrp8cre.Btk <sup>fl</sup> /Y	WT	Mrp8cre.Btk <sup>fl</sup> /Y	
CXCL1	473 (557-288)	808 (999-506)	391 (517-216)	1188 (1934-442)	
CXCL2	877 (937-719)	902 (942-856)	821 (903-758)	1215 (1419-1004)	
CCL2	<ld< td=""><td><ld< td=""><td>56 (75-31)</td><td>40 (70-35)</td></ld<></td></ld<>	<ld< td=""><td>56 (75-31)</td><td>40 (70-35)</td></ld<>	56 (75-31)	40 (70-35)	

All chemokine levels are pg/ml, LD: limit of detection, n = 7-11 per group per time point. Data are medians and interquartile ranges.



Figure S1 Analysis of cell composition in lung, blood and spleen of WT and *Btk*<sup>-/-</sup> mice

Naïve WT and  $Btk^{-}$  mice were sacrificed (n = 4 per group). Percentage of alveolar macrophages (AMs) and dendritic cells (DCs) in the lung (A), percentage of Ly6C- and Ly6C+ monocytes in blood (B), neutrophil number in blood and spleen (C). Data are represented as scatter dot plots plus median (\*p<0.05).



Figure S2 Btk deficiency does not impair pulmonary inflammatory responses during *K. pneumoniae* pneumonia

WT and  $Btk^{-/-}$  mice were intranasally inoculated with *K. pneumoniae* and euthanized at 6, 24 or 35 hours (n = 8-9 per group). Lung pathology score (A), myeloperoxidase (MPO) levels in lung homogenates (B), percentage of Ly6G positive lung surface was quantified (C). *Mrp8cre.Btkfl/Y* and littermate control (WT) mice were infected intranasally with *K. pneumoniae* and euthanized at 6, 24 or 42 hours (n = 6-11 per group). Lung sections were stained with anti-Ly6G, representative pictures of Ly6G staining at indicated time points (D). Neutrophil numbers in BALF (E). Data are represented as scatter dot plots plus median (\*p<0.05).



# Figure S3 Btk deficiency in neutrophils does not affect neutrophil activation and degranulation during *K. pneumoniae* infection

*Mrp8cre.Btkfl/Y* and WT mice were infected with *K. pneumoniae* via airways and euthanized at 6, 24 or 42 hours (n = 6-11 per group). Surface CD11b expression on BALF neutrophil (A), levels of MPO, elastase and Mrp8/14 in BALF (C), levels of MPO and elastase in plasma (D). Representative pictures of lung sections stained for CitH3 (F) and semi-quantitative scoring of NETs (G). Representative pictures of lung sections stained for  $\gamma$ H2AX (H) and quantification of  $\gamma$ H2AX-positive cells (I). *Mrp8cre.Btkfl/Y* and WT mice *were* infected with *K. pneumoniae* intravenously and euthanized at 24 hours (n = 7-9 per group). Surface CD11b expression on blood neutrophil (B), levels of MPO and elastase in plasma (E). Data are represented as scatter dot plots plus median (\*p<0.05, \*\*p<0.01).



Figure S4 Btk in neutrophil is essential for K. pneumoniae-induced extracellular ROS production Btk<sup>-/-</sup> and WT neutrophils were incubated with live K. pneumoniae (n= 6 per group). Klebsiella bacteria in medium and internalized bacteria (phagocytosis) by PMN after gentamicin treatment are shown as colony forming units (CFU) (A). Analysis of respiratory burst of neutrophils from Btk<sup>-/-</sup> and WT mice using isoluminolchemiluminsence (n= 6 per group). Neutrophils were treated with HBSS+/+, and live K. pneumoniae (MOI10 or 20). Total ROS production during 120 min stimulation was calculated as the total area under the curve (C). Data are represented as scatter dot plots plus median (\*p<0.05, \*\*p<0.01).