## Supplementary table

PLB-985 Arf6 WT GFP <sup>-</sup> / 7AAD <sup>-</sup>	PLB-985 Arf6 WT GFP <sup>+</sup> / 7AAD <sup>-</sup>	PLB-985 Arf6 T27N GFP <sup>-</sup> / 7AAD <sup>-</sup>	PLB-985 Arf6 T27N GFP <sup>+</sup> / 7AAD <sup>-</sup>
(10° cens)	(10° cells)	(10° cells)	(10° cells)
-	5.7	10.29	3.47
3.94	3.72	0.67	0.22
2.93	2.35	5.61	2.07
1.1	2.34	3.1	0.9

**Supplementary table 1**. The number of viable cells (7AAD<sup>-</sup>) GFP<sup>+</sup> and GFP<sup>-</sup> PLB-985 cells recovered following sorting after 48h post-transfection.

## **Supplementary figures**



**Supplementary Fig. 1.** Analyses of expression of the GFP constructs by FACS at 48h post-transfection of PLB-985 cells.



flox*/*cre-/-	Arf6 Arf6	Elastase Elastase
flox*/*cre*/-		Elastase cre
flox*/*cre*/*		cre cre
Cre≁	Arf6 Arf6	Elastase Elastase
Cre⁺′-	Arf6	Elastase cre
Cre+/+	Arf6 Arf6	- Cre Cre

**Supplementary Fig. 2.** Generation of PMN-Arf6 conditional KO mice and mouse genotypes used in this study.



**Supplementary Fig. 3.** The N-myristoylated ARF6 inhibitory peptide induces apoptosis and a noncytotoxic concentration of the inhibitory peptide does not inhibit fMLP-induced PMN adhesion. (A) Human PMNs were incubated with the N-myristoylated ARF6 inhibitory peptide and the scrambled peptide for 1h at 37°C and apoptosis was determined as described in Materials and Methods (n=1, triplicate measurements). (**B-D**) Calcein-labeled PMNs were incubated with the inhibitory or the scrambled peptide (1  $\mu$ M) for 1h before stimulation with fMLP (10<sup>-6</sup>M) for 30 min. (**B**) Adhesion to immobilized ICAM-1 (n=2 independent experiments performed in triplicate). (**C**) Adhesion to immobilized fibrinogen (n=3 independent experiments performed in triplicate). (**D**) Adhesion to immobilized fibronectin (n=2 independent experiments performed in triplicate).



**Supplementary Fig. 4.** PMN migration into the air pouches and cell surface expression of the  $\beta 2$  integrins in air pouch PMNs of mice bearing one or two *Cre* transgenes. The amounts of PMNs recruited into the mouse air pouches injected with LPS (**A**) or fMLP (**B**) for 4h were monitored (PBS n=6 mice; LPS: n=30-46 mice; fMLP n=28-36 mice). (**C**) Ly6G<sup>+</sup> cells were gated to evaluate cell surface expression of CD11a (left panel) and CD11b (right panel) in PMNs recruited into the mouse air pouches injected with LPS (**n**=6 mice). (**D**) Cell surface expression of CD11a (left panel) and CD11b (right panel) in PMNs recruited into the mouse air pouches injected with fMLP (n=6-10 mice).