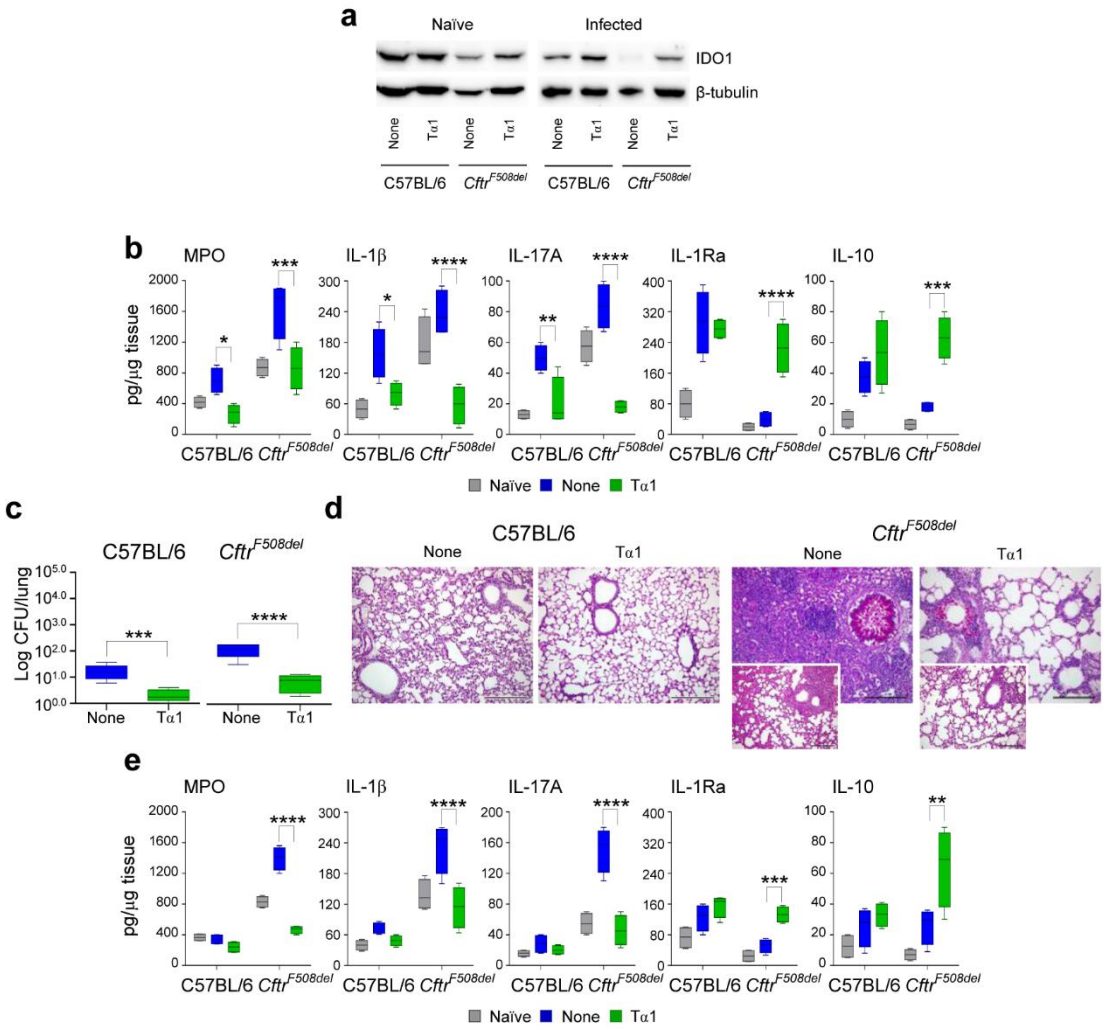
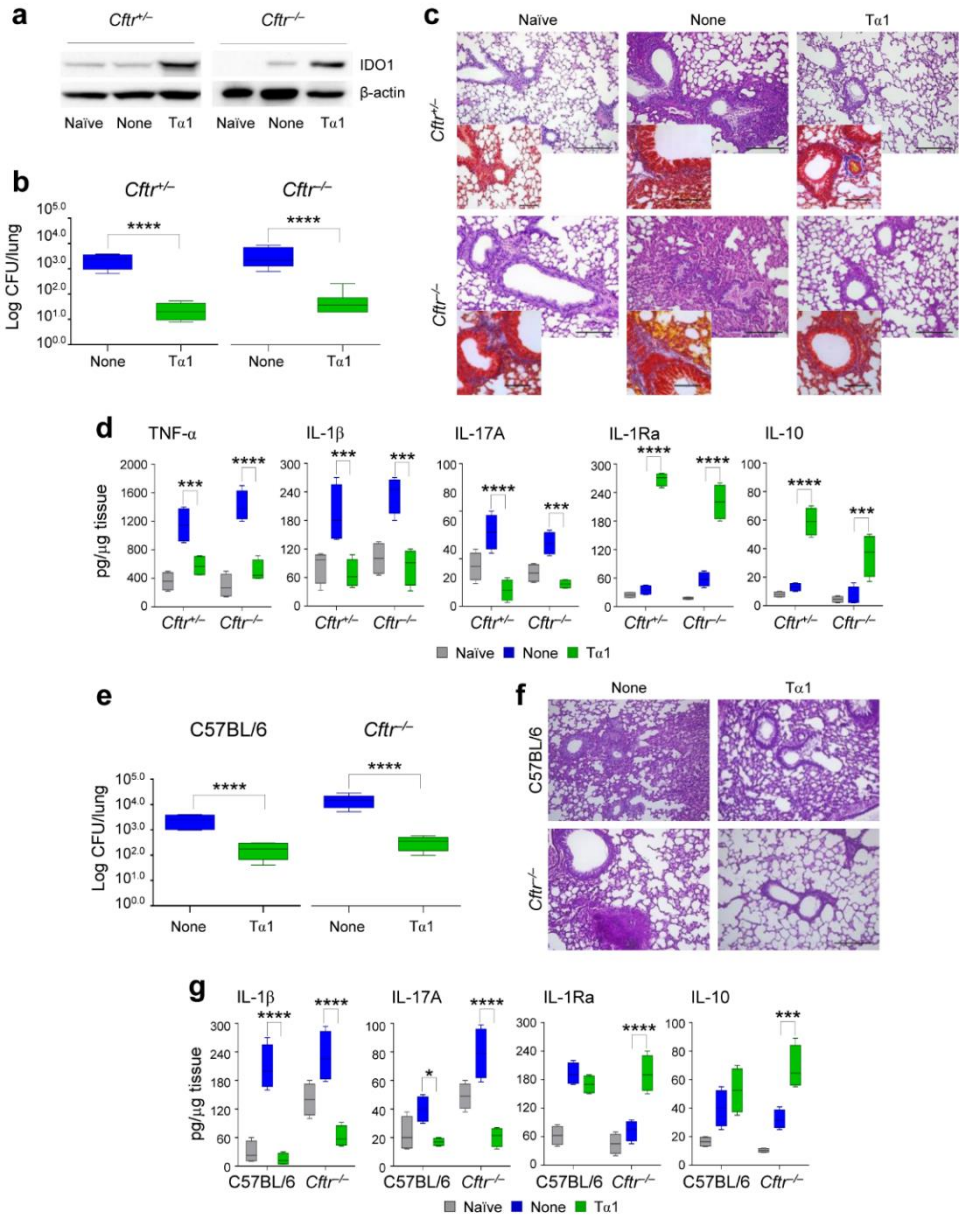
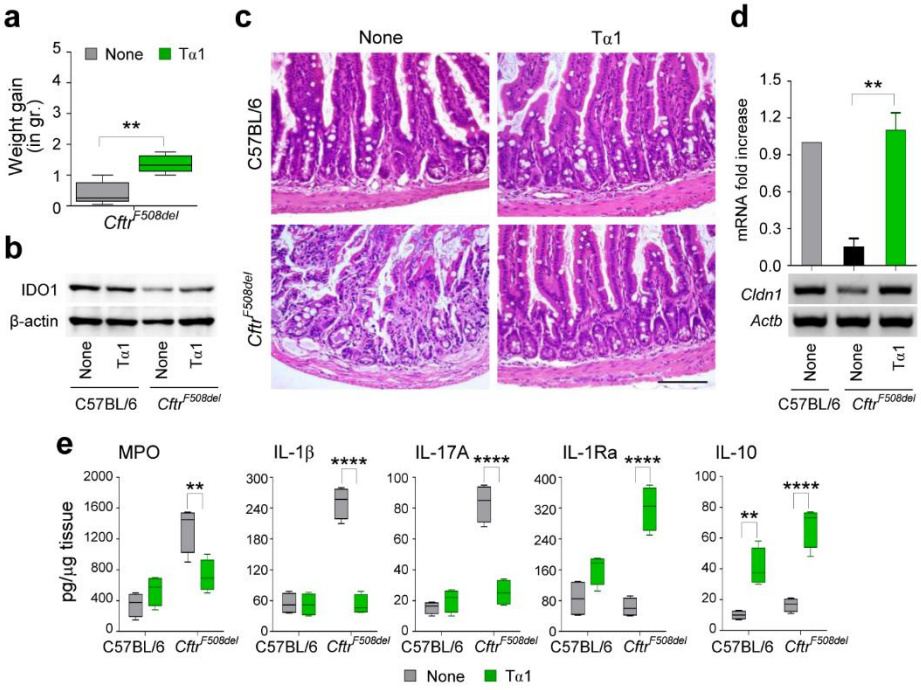


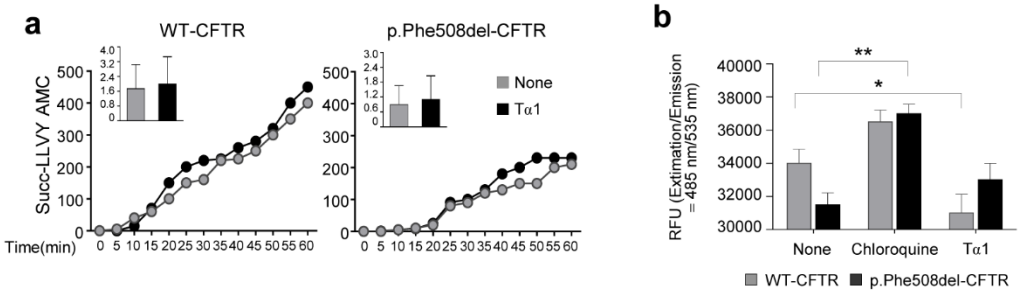
Supplementary Figure 1 Tα1 limits the inflammatory response in vivo. C57BL/6 or *F508del-Cftr* homozygous (*Cftr*^{F508del}) mice were infected intranasally with live *A. fumigatus* conidia and treated intranasally with 200 μg/kg of Tα1 or the scrambled polypeptide daily for 6 days concomitantly with the infection (**a, b**) or starting 7 days post infection (dpi) (**c-e**). (**a**) Representative immunoblot of IDO1 protein (n = 3) in lung lysates and (**b, e**) cytokine production in lung homogenates done at 7 or 14 dpi respectively. (**c**) Fungal growth, expressed as $\log_{10}$ colony-forming units (CFU) and (**d**) lung histology (PAS staining; scale bar, 200 μm) evaluated at 14 dpi. In the insets, lung of naïve mice treated with Tα1 daily for 6 days. (n = 5 images per mouse). Data, mean values ± SD, are presented as box-and-whisker plots; bars represent maximal and minimal values. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, Tα1-treated *vs* scrambled peptide-treated (None) mice, Two-way ANOVA, Tukey's post test. Naïve, uninfected mice. Data are representative (**a, d**) or pooled (**b, c** and **e**) from three independent experiments with six mice/group.



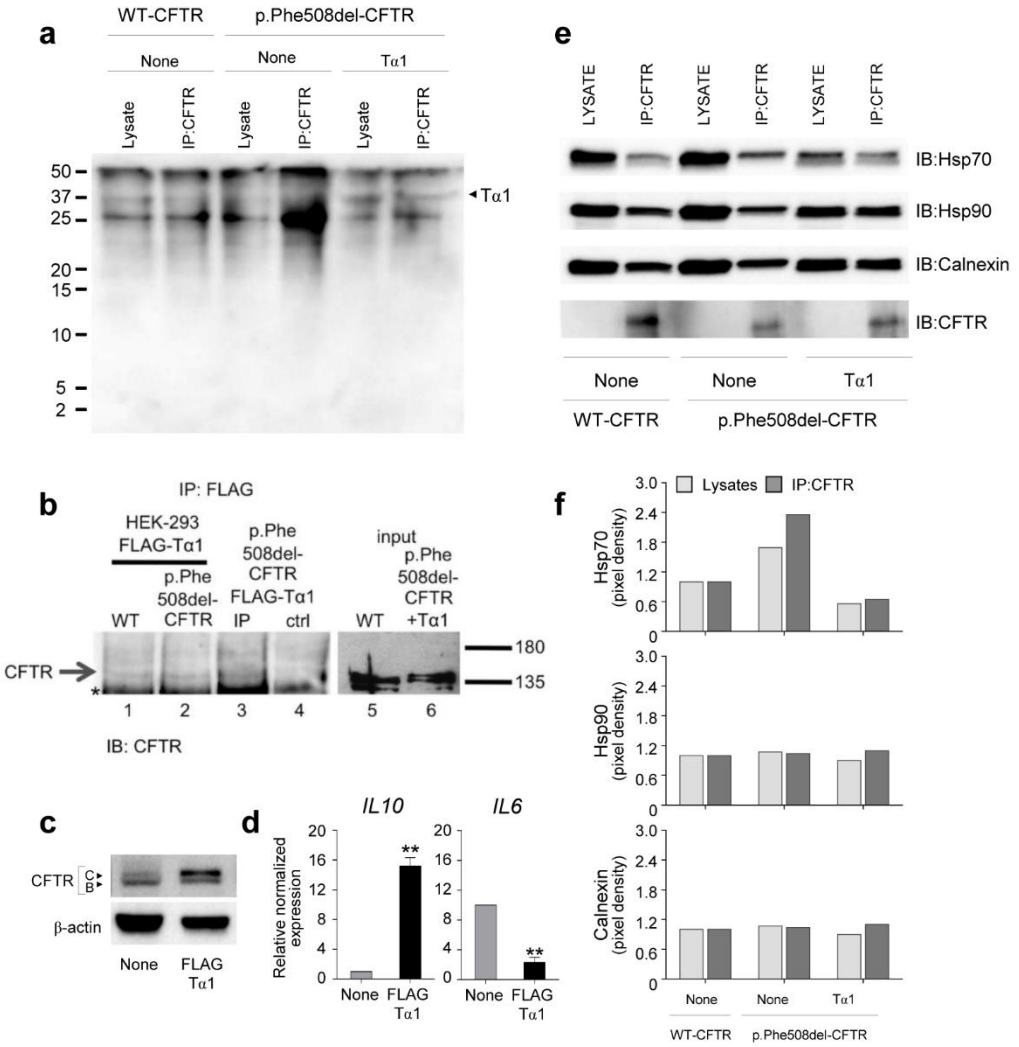
Supplementary Figure 2 Tα1 alleviates inflammation independently of CFTR. *Cftr*$^{+/-}$ and *Cftr*$^{-/-}$ (n = 18) were infected intranasally with live *A. fumigatus* conidia and treated with 200 μg/kg of Tα1 or the scrambled polypeptide intraperitoneally daily for 6 days. (**a**) Representative immunoblot of IDO1 protein (n = 3) in lung lysates. (**b**) Fungal growth, expressed as $\log_{10}$ colony-forming units (CFU); (**c**) Lung histology PAS staining or Masson's trichrome in the inset, scale bar 100 μm) (n = 5 images per mouse). (**d**) Cytokine production in lung homogenates (ELISA assays were done at 7 days post-infection). C57BL6 or *Cftr*$^{-/-}$ mice were given intranasal instillation of 3x10^7 *P. aeruginosa* CFU/mice, treated with Tα1 or the scrambled polypeptide intraperitoneally daily for 6 days and assessed for (**e**) bacterial growth (log CFU), (**f**) lung histology (PAS staining, scale bar 200 μm), (n = 5 images per mouse) and (**g**) cytokine production in lung homogenates at 7 days post-infection). Data, mean values ± SD, are presented as box-and-whisker plots; bars represent maximal and minimal values. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001, Tα1-treated *vs* scrambled peptide-treated (None) mice, Two-way ANOVA, Tukey's post test. Data are representative (**a, c, f**) or pooled (**b, d, e, g**) from three independent experiments with six mice/group.



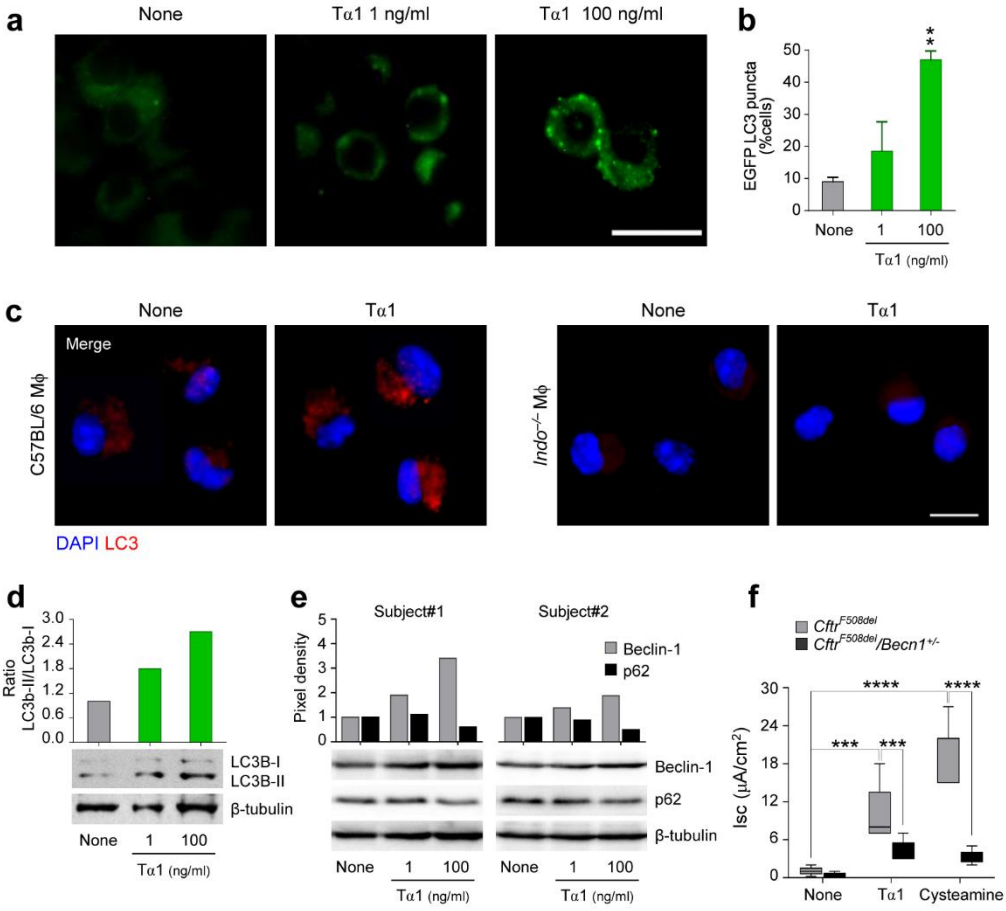
Supplementary Figure 3 Beneficial effects of Tα1 in the gastrointestinal tract. C57BL/6 or *F508del-Cftr* homozygous (*Cftr*^{F508del}) mice were treated with 200 μg/kg of Tα1 or the scrambled polypeptide intranasally daily for 6 days. (**a**) Distribution of weight variation of *Cftr*^{F508del} mice. (**b**) Representative immunoblot of IDO1 protein (n = 3) in small intestine lysates. (**c**) Small intestine histology H&E staining, scale bar 100 μm. (n = 5 images per mouse). (**d**) *Claudin 1 (Cldn1)* gene expression (n = 3) on the small intestine. (**e**) Cytokine production in small intestine homogenates. Data, mean values ± SD, are presented as box-and-whisker plots; bars represent maximal and minimal values. **P<0.01, ****P<0.0001, Tα1-treated *vs* scrambled peptide-treated (None) mice, Two-way ANOVA, Bonferroni post test. Data are representative (**b-d**) or pooled (**a**, **e**) from three independent experiments with six mice/group.



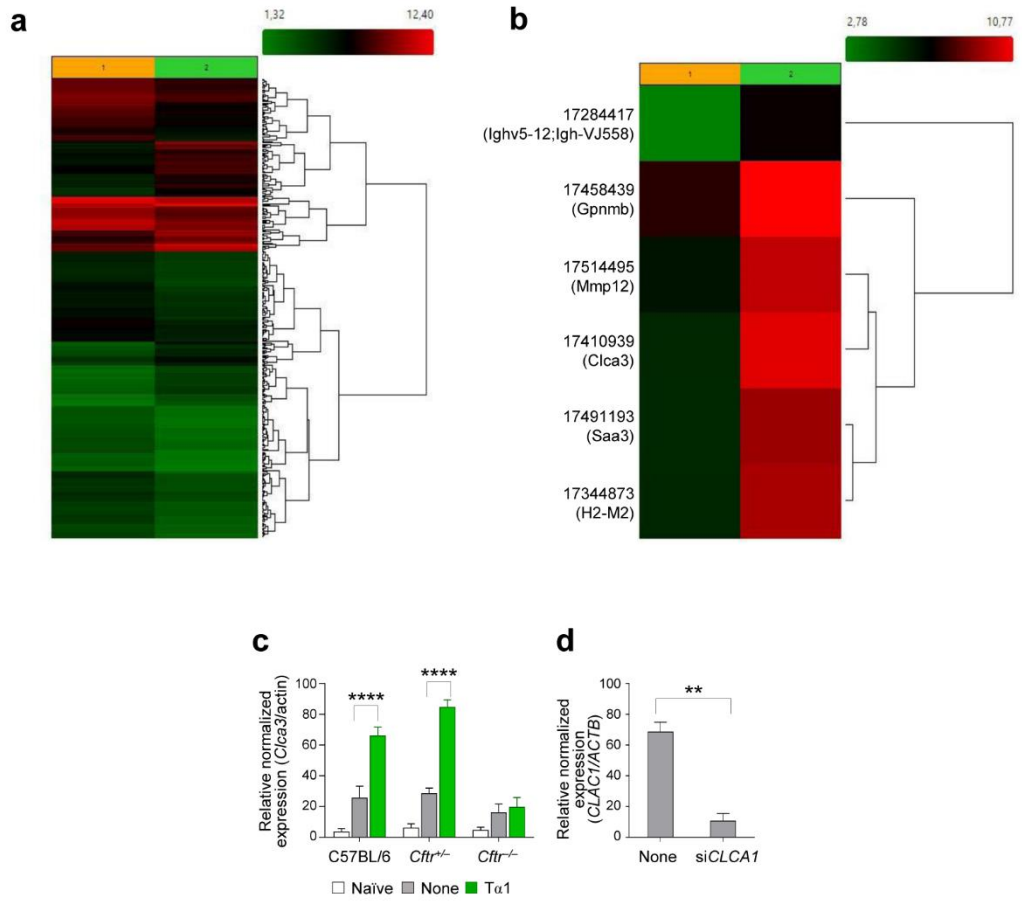
Supplementary Figure 4 Tα1 does not inhibit the proteasomal or lysosomal degradation pathway. CFBE41o-cells or cells transfected with WT-CFTR were treated with Tα1 (100 ng/ml) for 2 h at 37°C and assessed for (**a**) proteasome activity by quantification of the amount of pmols of proteolytic activity. Shown in the insets the relative slope with error bars representing the mean ± SD. (**b**) Lysosome functional activity. For cathepsin activity cells were stained with NBD-PZ (4-nitro-7-1-piperazinyl-2,1,3-benzoxadiazole). Lysosome staining intensity was measured by using an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Data, mean ± SD, are from three experiments. **P*<0.05, ***P*<0.01, treated *vs* untreated (none) cells, two-way ANOVA, Tukey's post test.



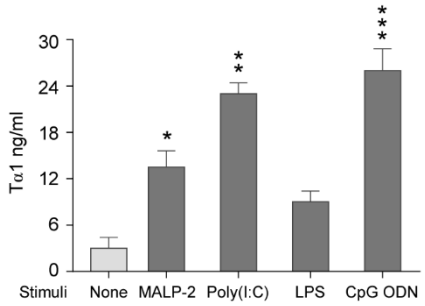
Supplementary Figure 5 Tα1 physically associates with p.Phe508del-CFTR and interferes with molecular chaperones. (**a**) Representative immunoblot (*n* = 3) of cell lysates and immunoprecipitated CFTR from WT- or p.Phe508del-CFTR-transfected CFBE41o- cells treated with 100 ng/ml Tα1 at 37°C for 2 h. Lysates and the immunoprecipitated complex were analysed by immunoblotting using an anti-Tα1 antibody. (**b**) HEK293 (lines 1 and 2) and p.Phe508del-CFTR-transfected CFBE41o- cells (line 3) were transfected with a plasmid expressing FLAG-Tα1. Lysates were immunoprecipitated (*n* = 3) with anti-FLAG antibody bound resin; the FLAG-Tα1-resin was then incubated in the presence of lysates from WT (line 1) or p.Phe508del CFTR-(line 2)-transfected CFBE41o. The remaining proteins bound to the resin after elution with the flag peptide were loaded as negative control (line 4). Samples were resolved by immunoblotting and probed with anti-CFTR antibody. Total lysates from WT- (line 5) or p.Phe508del-CFTR-transfected CFBE41o- cells treated with Tα1 (line 6) were used as input. The asterisk indicates a non-specific band. (**c**) CFTR immunoblot (*n* = 3) and (**d**) *IL10* and *IL6* gene expression in p.Phe508del-CFTR-CFBE41o-cells transfected with empty (None) or FLAG-Tα1-expressing plasmid. Arrows indicate B and C (mature) forms of CFTR. Immunoblots are representative of three independent experiments. Data, mean ± SD. ***P*<0.01, transfected *vs* empty (none) cells. (**e**) Immunoblots with anti-Hsp70, anti-Hsp90 and anti-Calnexin antibody (*n* = 3) on immunoprecipitated CFTR from WT- or p.Phe508del-CFTR-transfected CFBE41o- cells treated as in (**a**). (**f**) Bands were quantified by densitometry and expressed as pixel density. None, vehicle-treated cells.



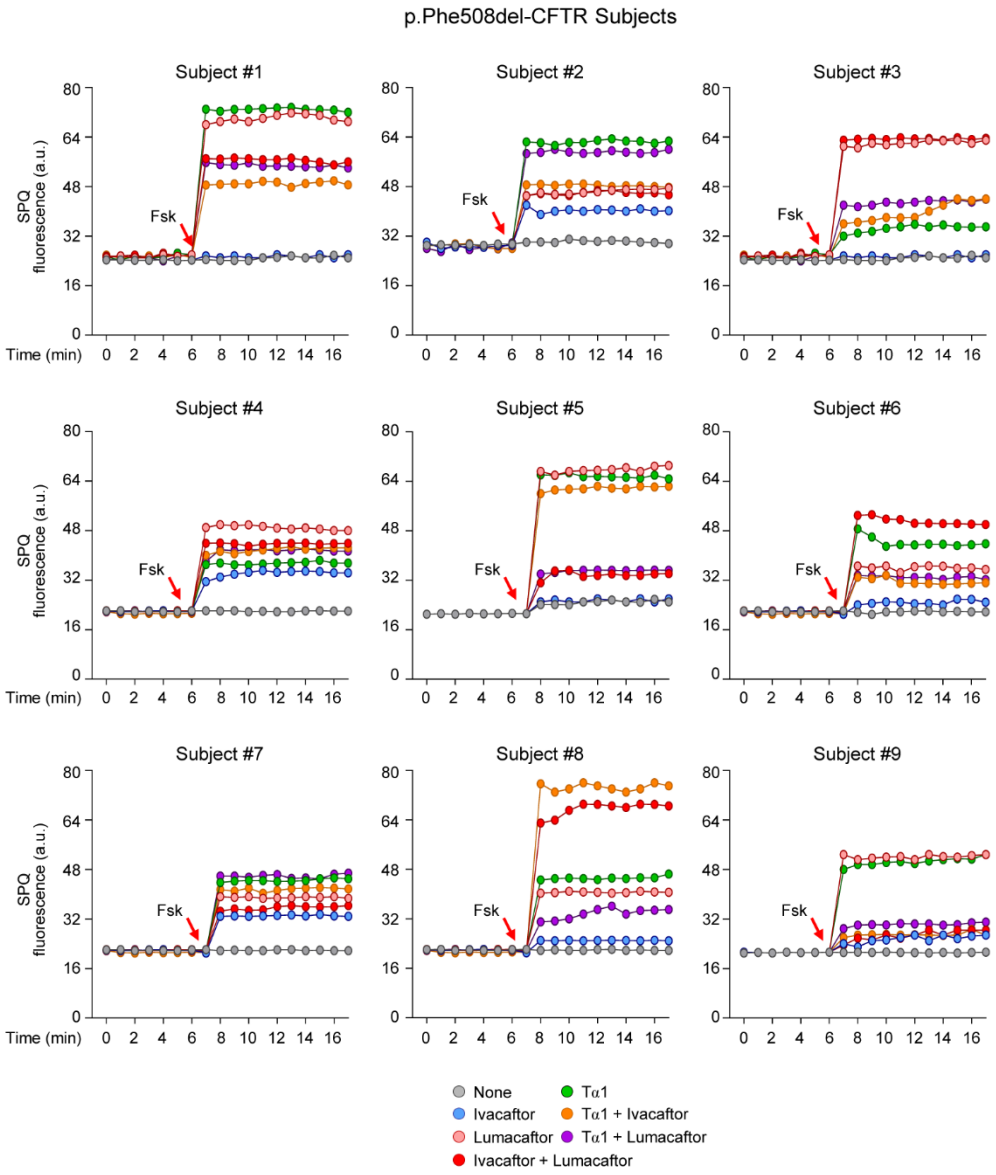
Supplementary Figure 6 Tα1 induces autophagy in HBE cells from subjects with CF. (**a**) Fluorescence images of EGFP-LC3 transiently transfected RAW264.7 exposed to 1 or 100 ng/ml of Tα1 for 4 h. Scale bar, 100 μm (n = 5 images per treatment). (**b**) Number of RAW264.7 cells with EGFP-LC3 punctae. (**c**) Fluorescence image of DAPI-LC3 in purified lung macrophages from C57BL/6 and *Indo*$^{-/-}$ mice stimulated with Tα1 for 4 h (n = 5 images per treatment). (**d**) Cell lysates of EGFP-LC3-trasfected RAW246.7 cells probed with LC3B antibody (*n* = 3). (**e**) HBE cells from subjects with CF with the p.Phe508del mutation were exposed to Tα1 as above before immunoblotting with Beclin-1 and SQSTM1/p62 antibody (*n* = 3). Normalization was performed by probing the membrane with β-tubulin. (**f**) *Cftr*^{F508del}*/Becn1*$^{+/-}$ mice (*n* = 6 mice per group from two independent experiments) were treated with 200 μg/kg of Tα1 intraperitoneally daily for 6 days. Cl^- secretion was measured by means of forskolin-induced increase of the chloride current (Isc ($\mu A/cm^2$)) in ex-vivo ileum mounted in Ussing chambers. Cysteamine was used as positive control. Data, mean ± SD, are representative (**a, c, d, e**) or pooled (**b**, **f**) from three independent experiments. ***P*<0.01, ****P*<0.001, *****P*<0.0001, Tα1-treated *vs* untreated (None) cells, two-way ANOVA, Bonferroni post test.



Supplementary Figure 7 DNA arrays in vivo. Hierarchical clustering (fold change >2,6 or <-2) of all genes (**a**) and of 6 most upregulated genes (**b**) in the lungs of C57BL6 mice (*n* = 3 per group from two independent experiments) infected intranasally with *A. fumigatus* and treated with 200 ng/ml of Tα1 or the scrambled peptide intraperitoneally daily for 6 days. Each row represented a single gene; the two column represent treated *vs* untreated mice. The expression levels of the genes are shown by the color scale: red represents a target gene with high Ct value while green represents a target gene with low Ct value. Algorithm used: One-Way Between-Subject ANOVA (unpaired). (**c**) C57BL/6, *Cftr*$^{-/-}$ and *Cftr*$^{+/-}$ mice were infected intranasally with *A. fumigatus* and assessed for *Clca3* (the murine ortholog of the human *CLCA1*) gene expression in the lungs at 7 days post-infection. (**d**) Validation of siRNA of *CLCA1* in CFBE41o-cells. Data are pooled from three experiments with six mice/group and presented as mean ± SD for all bar graphs. ***P*<0.01, *****P*<0.0001, Tα1-treated *vs* the scrambled peptide (None)-treated mice, siRNA-treated *vs* untreated (scrambled siRNA, None) cells, two-way ANOVA, Bonferroni post test and Student's t test.



Supplementary Figure 8 Production of Tα1 by murine epithelial cells upon TLR stimulation. Lung epithelial cells were stimulated with MALP-2, Poly(I:C), ultrapure LPS from *Salmonella minnesota* Re 595 and CpG ODN (all at 10 µg/ml) for 18 h before Tα1 determination in supernatant by ELISA. Data are pooled from three independent experiments and presented as mean ± SD. **P*<0.05, ***P*<0.01, ****P*<0.001, stimulated *vs* non-stimulated (None) cells, One-way ANOVA, Bonferroni post test.



Supplementary Figure 9 Tα1 rescues p.Phe508del-CFTR activity in HBE cells from subjects with CF. Assessment of iodide efflux by SPQ fluorescence assay upon stimulation with forskolin (Fsk) in HBE cells from 9 subjects with the p.Phe508del mutation treated with Tα1 100 ng/ml alone or in combination with 1 μM ivacaftor or 3 μM lumacaftor for 24 h at 37°C. Treated *vs* untreated (None) cells and ivacaftor alone or combined with Tα1 or lumacaftor. For statistical analysis, see **Supplementary Fig**. **10**. Data are pooled of four (subjects #1-3) or three (subjects #4-9) experiments.

a

		Transferrin Receptor	LAMP1
None	Pearson's coefficient	**0.438**	**0.367**
	Overlap coefficient	0.471	0.401
	Manders' coefficient Primary antibody/TLR9	0.444	0.856
	Manders' coefficient TLR9/Primary antibody	0.973	0.704
Tα1	Pearson's coefficient	0.233	0.441
	Overlap coefficient	0.292	0.5
	Manders' coefficient Primary antibody/TLR9	0.609	0.655
	Manders' coefficient TLR9/Primary antibody	0.94	0.856
Tα1 + DNAse	Pearson's coefficient	0.691	**0.884**
	Overlap coefficient	0.744	**0.9**
	Manders' coefficient Primary antibody/TLR9	0.912	**0.96**
	Manders' coefficient TLR9/Primary antibody	0.972	**0.993**

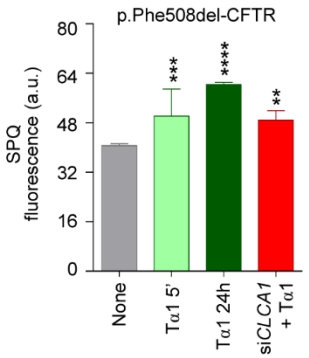
b

p.Phe508del-CFTR		Rab5	Rab7	Rab9
None	Pearson's coefficient	0.706	0.7	0.477
	Overlap coefficient	0.798	0.801	0.544
	Manders' coefficient CFTR/Primary antibodies	1.0	1.0	5.13
	Manders' coefficient Primary antibodies/CFTR	0.87	0.827	1.0
Tα1	Pearson's coefficient	0.512	0.259	0.661
	Overlap coefficient	0.634	0.317	0.745
	Manders' coefficient CFTR/Primary antibodies	0.561	0.179	1.0
	Manders' coefficient Primary antibodies/CFTR	0.99	0.99	0.867

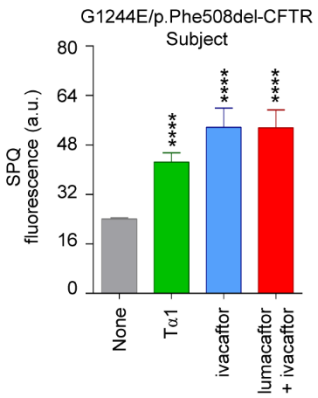
c

p.Phe508del-CFTR		Rab5	Rab9
None	Pearson's coefficient	0.77	0.485
	Overlap coefficient	0.84	0.49
	Manders' coefficient USP36/Primary antibodies	0.844	0.998
	Manders' coefficient Primary antibodies/USP36	0.999	0.175
Tα1	Pearson's coefficient	0.39	0.643
	Overlap coefficient	0.441	0.635
	Manders' coefficient USP36/Primary antibodies	0.176	1.0
	Manders' coefficient Primary antibodies/USP36	1.0	0.348

d



e



Supplementary Figure 10 The co-localization program ImageJ with the JACoP Plugin was used to quantify the degree of overlap by calculating the co-localization coefficients (Pearson's correlation coefficient, Overlap coefficient according to Manders and the Overlap coefficients). The co-localization analysis was done for Fig.1a (panel **a**), Fig. 2k (panel **b**), Fig. 3g (panel **c**). Numbers refer to co-localization coefficients to quantify the degree of overlap. Statistical analyses related to Fig. 4e,f (panels **d, e**). ***P*<0.01, ****P*<0.001, *****P*<0.0001, treated *vs* untreated (None), one-way ANOVA, Bonferroni post test.