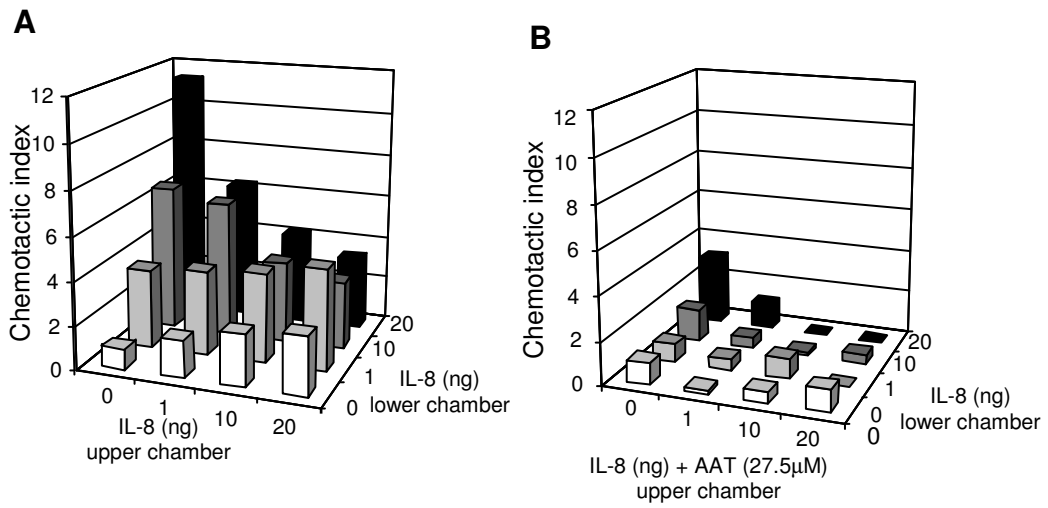
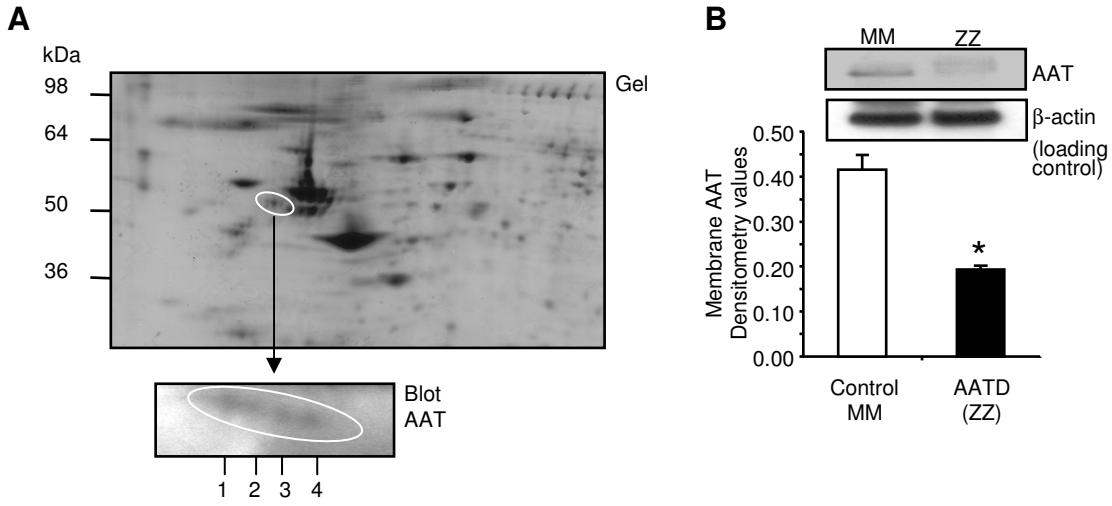


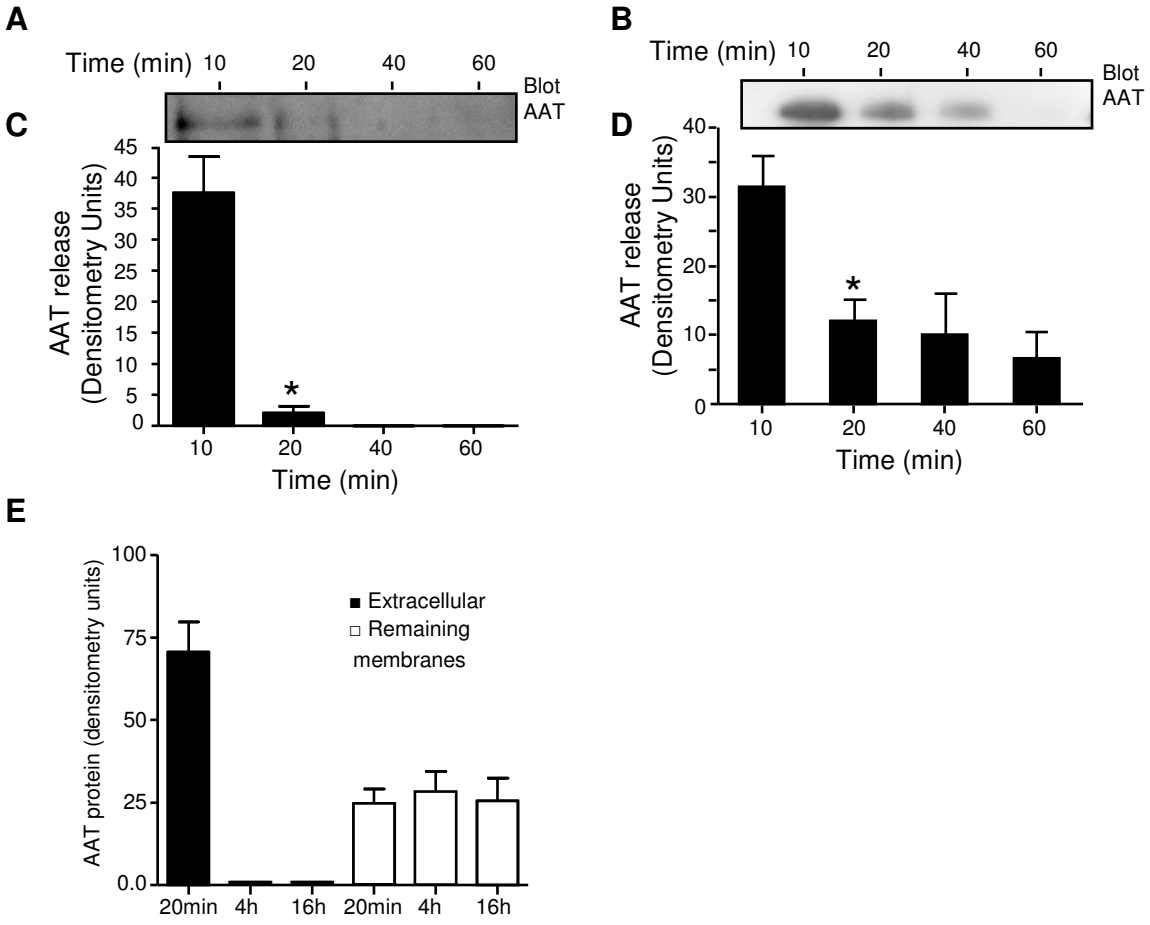

**Supplemental material Figure 1. AAT inhibits neutrophil chemotaxis.**

**A;** Checkerboard analysis revealed that neutrophil migration depended on the presence of an IL-8 (0,1,10 or 20ng / $2x10^7$ cells) gradient across the filter, suggesting IL-8 induced chemotaxis rather than chemokinesis. Data are expressed as fold induction compared with unstimulated cells. **B;** Within this checkerboard analysis, IL-8 (10ng) induced mean chemotactic index of normal (MM) neutrophils was decreased in the presence of exogenous AAT (27.5μM) added to the upper chamber. Results illustrated in panels **A&B** were performed in triplicate on three consecutive days and each measurement is the mean ± S.E.




**Supplemental material Figure 2. Localization and expression of AAT in peripheral blood neutrophils.**

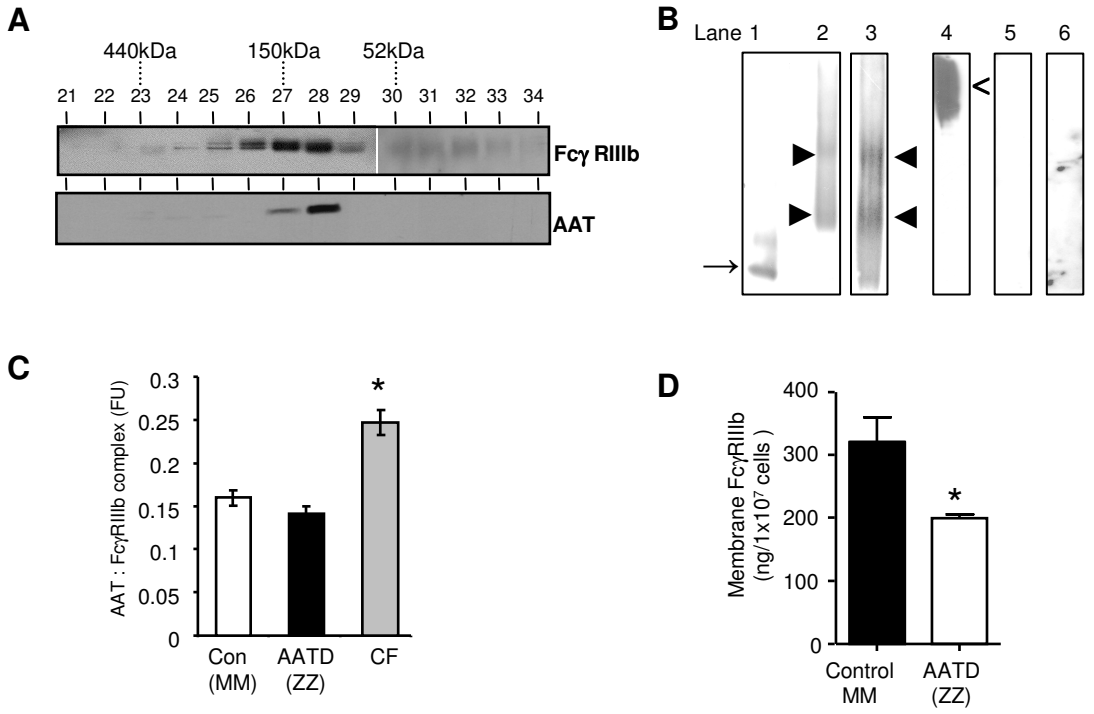
**A;** Coomassie blue–stained 2D SDS-PAGE gel of isolated MM neutrophil membranes (top panel). A Western blot probed with polyclonal anti-AAT revealed four membrane associated AAT isoforms of similar molecular mass (~52 kDa) (lower panel). **B;** MM and ZZ (n=3) neutrophil membrane preparations immunoblotted using goat polyclonal anti-AAT or monoclonal anti-actin as a loading control. AAT densitometry values normalized against actin revealed a significant reduction in the level of AAT on ZZ neutrophils (*P =0.002).




**Supplemental material Figure 3. The maximum release of AAT from neutrophil membranes exposed to IL-8 and TNF-α occurs at 10 minutes.**

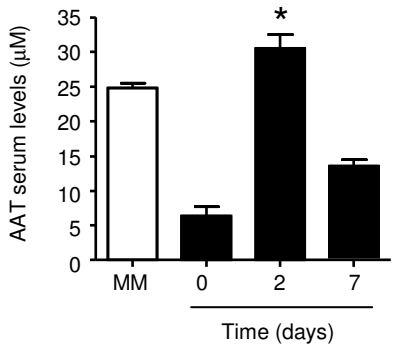
Western blot showing extracellular released AAT (52kDa) following MM neutrophil treatment with; **A;** IL-8 (10ng) or **B;** TNF-α (10ng) for 10, 20 40 or 60 min.

Quantification of the AAT immuno-bands by densitometry revealed IL-8 **(C)** and TNF-α **(D)** caused the maximum release of AAT after 10 min (* $p < 0.05$ between 10 min IL-8 or TNF-α exposure versus 20 min). The illustrated Western blots are a representative result from one of 3 separate experiments. **E;** Quantification of AAT by densitometry of immuno-bands revealed high levels of extracellular AAT in the extracellular milieu after 20 min exposure to IL-8 (10ng). Subsequently neutrophils were cultured for a further 4 or 16 h, with no increase in levels of AAT detected in the extracellular surrounding media or cell membrane fractions.



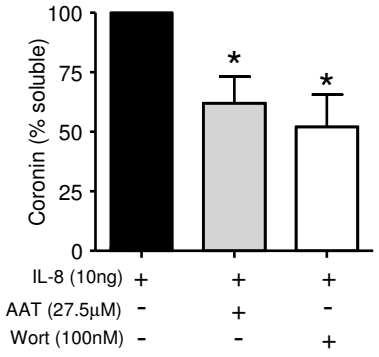

**Supplemental material Figure 4. An evaluation of the AAT:FcγRIIIb complex and expression of FcγRIIIb on MM and ZZ neutrophil membranes .**

**A;** Neutrophil released AAT was chromatographed by gel filtration on Superose 6. By immuno-blotting FcγRIIIb and AAT co-eluted in fraction 28. **B;** Native gel electrophoresis and Western blot analysis of serum purified AAT (Lane 1). AAT ran principally as a single band (→). Altered migration of neutrophil released AAT from MM neutrophils treated with 10ng/ml IL-8 (lane 2) was observed, possibly indicating an AAT:protein complex (►). Western blot analysis of neutrophil released material probed for FcγRIIIb (lane 3). FcgRIIIb migrated similarly to neutrophil released AAT (◄) possibly indicating a FcγRIIIb:AAT protein complex. AAT polymers (lane 4) migrated differently when compared to the monomer form of AAT or neutrophil released AAT (<). Western blot analysis for NE (lane 5) and PR3 (lane 6) were negative, implying that the AAT complex does not contain either protease. **C;** Levels of AAT:FcγRIIIb complex in serum of CF (n=8) individuals was significantly higher than normal MM (Con; n=6) and AATD (n=6) subjects (* $p < 0.05$ CF versus control and ZZ). **D;** Membrane bound FcγRIIIb was quantified by ELISA and found to be significantly lower in isolated resting ZZ-AATD neutrophils compared to MM cells (*P=0.007).



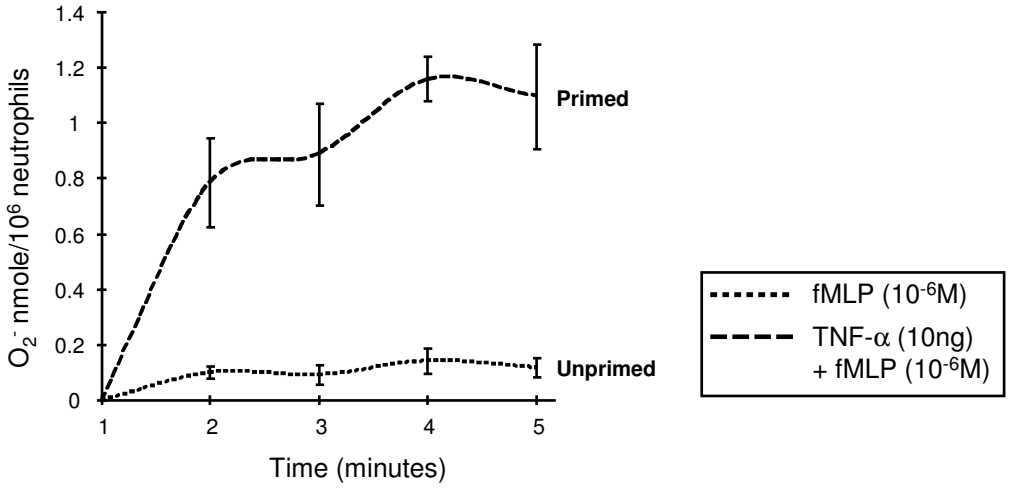

**Supplemental material Figure 5. Serum levels of AAT Pre- and Post-augmentation therapy.**

Levels of AAT in serum of ZZ-AATD individuals (■, n=4), pre- (day 0) and 2 and 7 days post augmentation therapy. Serum levels of AAT on day 2 post treatment were comparable to normal MM controls (□) and significantly higher than day 0 and 7 (*P<0.0001).



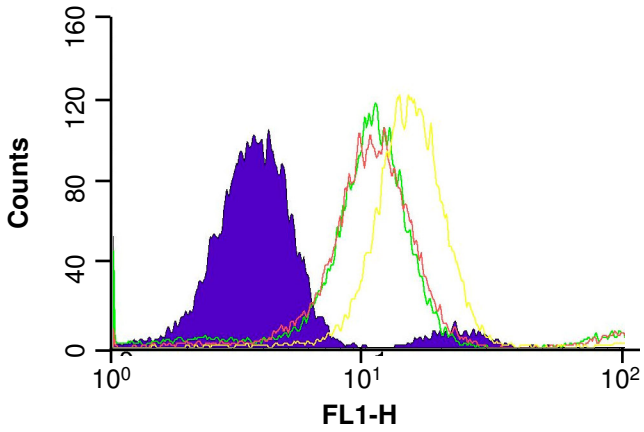

**Supplemental material Figure 6. AAT inhibits solubilization of the actin binding protein coronin.**

Solubility of the F-actin-associated protein coronin-1 is diminished in MM neutrophils treated with either AAT (27.5µM) or wortmannin (100nM) (*P=0.01).




**Supplemental material Figure 7. Time course of superoxide production by TNF-α primed and unprimed cells.**

The rate of superoxide ($O_2^-$) release from neutrophils ($1x10^6$/ml) stimulated by fMLP was significant greater in primed versus un-primed purified neutrophils. Neutrophils were incubated in the absence (un-primed) or presence of TNF-α (10ng) (primed) for 5 minutes. After this incubation cells were added to cytochrome c and stimulated by the addition of $10^{-6}$M fMLP. $O_2^-$ quantification was performed in triplicate on two consecutive days.




**Supplemental material Figure 8. Soluble FcγRIIIb rebinds the ZZ-AATD neutrophil membrane upon sIC stimulation.**

Purified ZZ-AATD neutrophils remained unstimulated (red line) or were stimulated with sIC (10% v/v) in the presence (yellow; mean fluorescence = 12.81 ) or absence (green; mean fluorescence = 17.09) of ZZ serum. FACs analysis are one illustrative result from three independent experiments and show a significant increase in membrane levels of FcγRIIIb in ZZ-AATD stimulated with sIC in the presence of ZZ serum ($P<0.001$). The isotype control antibody is illustrated in purple (filled).