ONLINE DATA SUPPLEMENT

Myeloid but not epithelial tissue factor exerts protective anti-inflammatory properties in acid aspiration-induced acute lung injury

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Running head: Myeloid TF exerts anti-inflammatory effects in ALI

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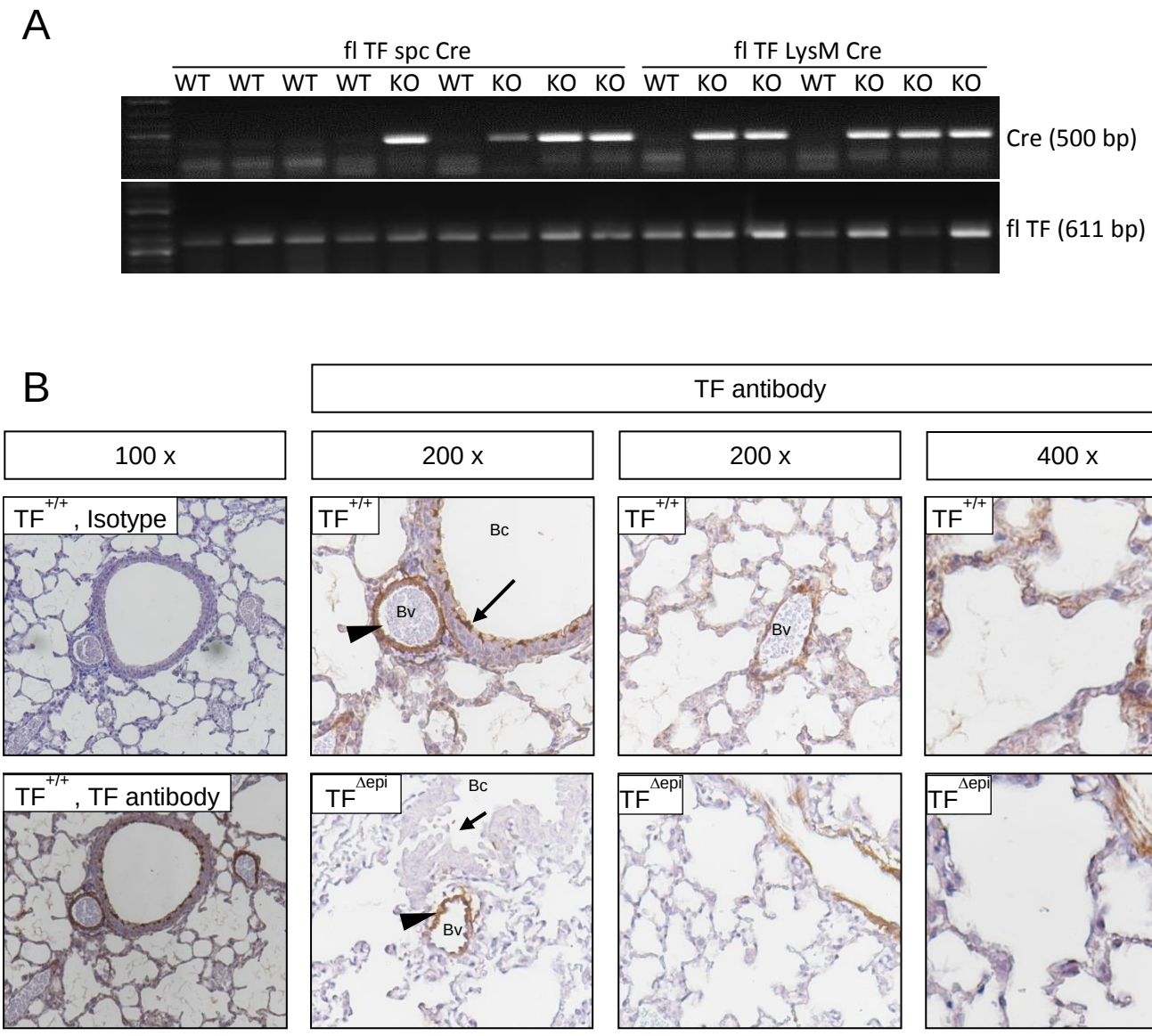
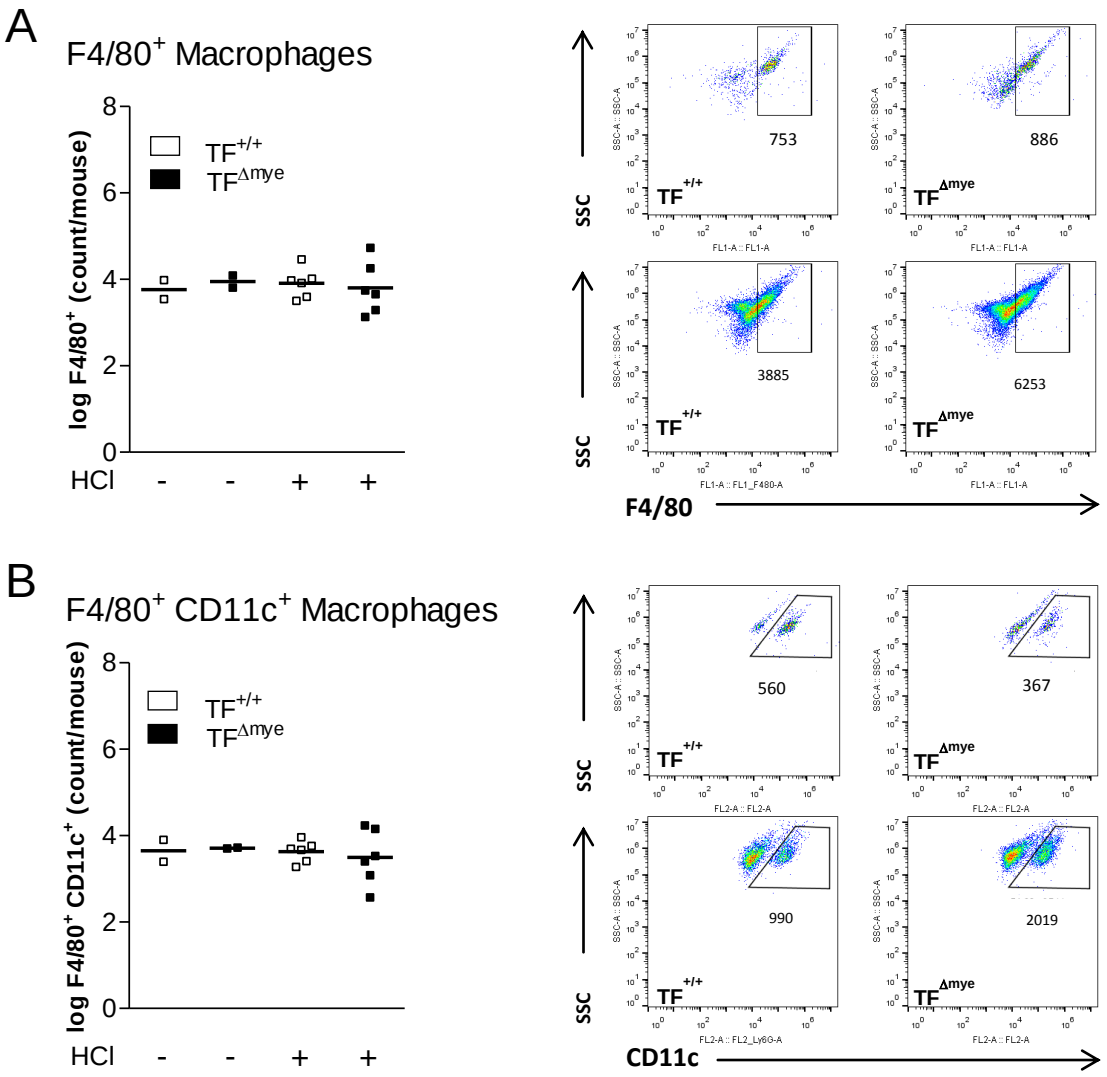
Supplementary figures

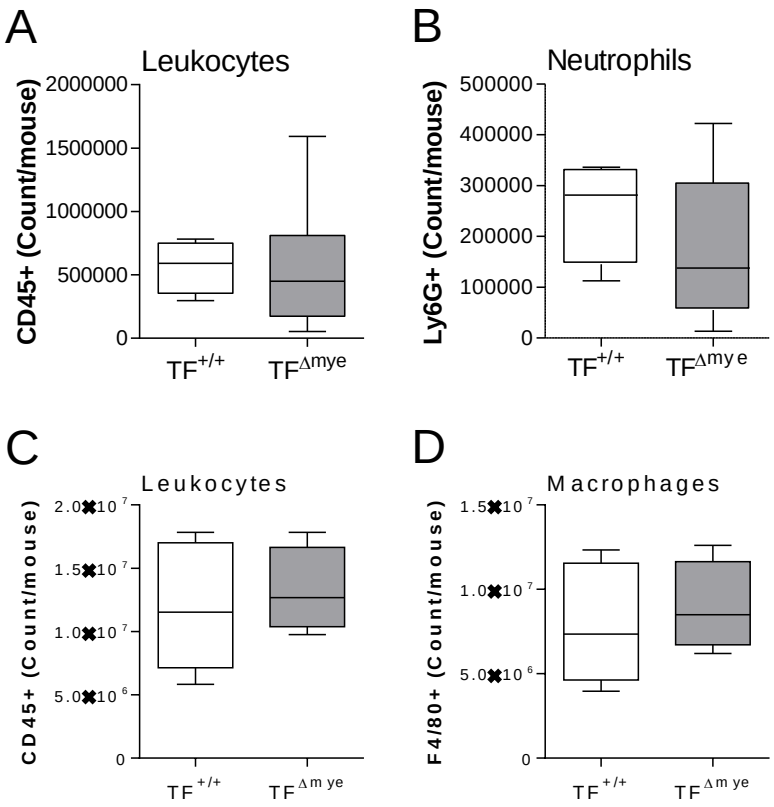
Supplement figure I



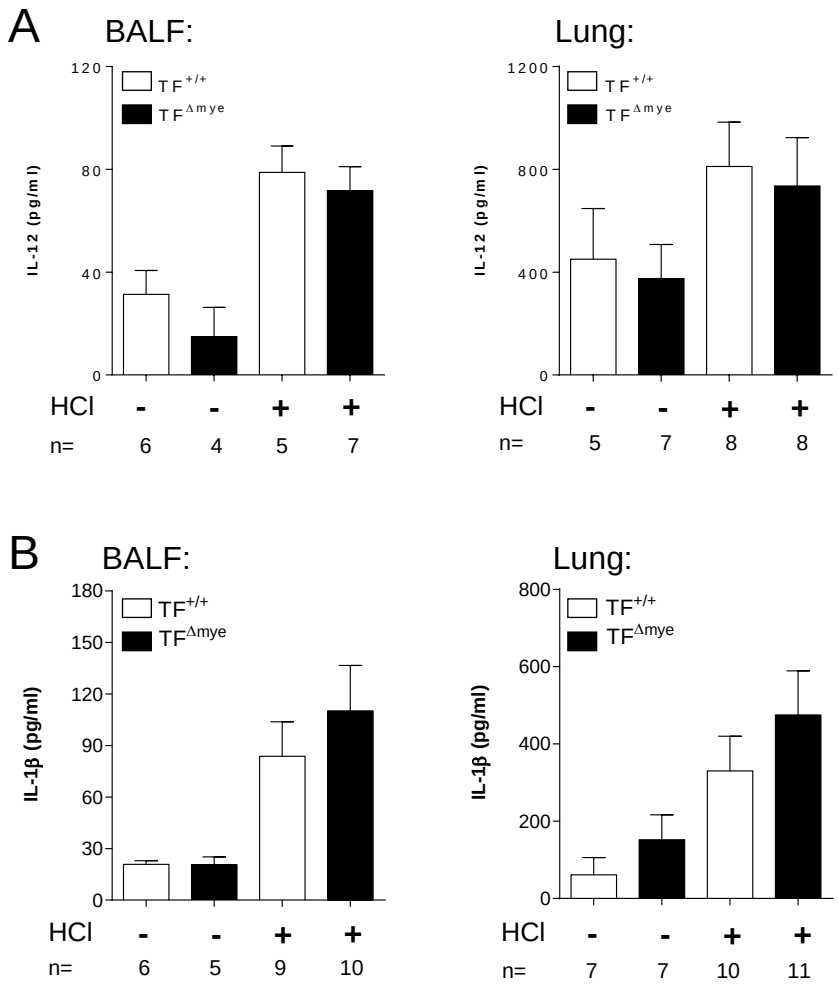
Supplement figure II



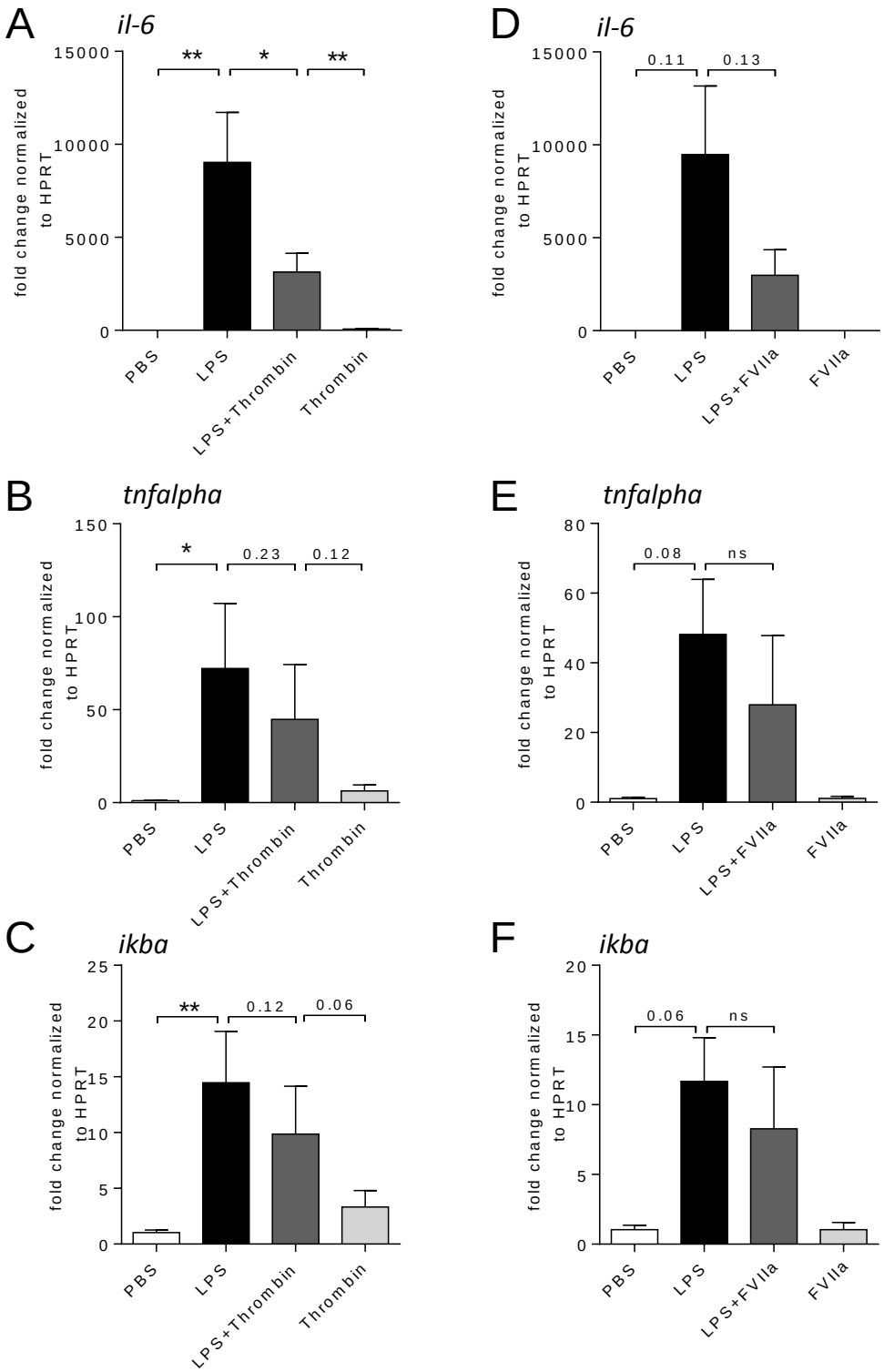
Supplement figure III



Supplement figure IV



Supplement figure V



Supplementary figure legend

Supplemental Figure I: Genotyping data of myeloid and airway epithelial TF-deficient mice. (A) Analysis of genomic DNA of *TF* and *cre* alleles of tissue from $TF^{\Delta epi}$ and $TF^{\Delta mye}$ mice and respective $TF^{+/+}$ littermates by PCR was performed. (B) Histological assessment of naive lungs of wild-type ($TF^{+/+}$) and $TF^{\Delta epi}$ mice. First picture in upper panel shows isotype control staining and in the lower panel the TF antibody staining (brown). Second till fourth pictures shows $TF^{+/+}$ mice in the upper panel and $TF^{\Delta epi}$ mice in the lower panel stained for TF. Magnifications are from 100 x to 400 x as indicated, arrow head indicates endothelial cells, arrow indicates epithelial cells, blood vessel (Bv), bronchus (Bc).

Supplemental Figure II: Macrophage recruitment into the lung 8h post acid-induced acute lung injury (ALI). Accumulation of macrophage subpopulations ($CD45^+$ $F4/80^+$ (A) and $CD45^+$ $F4/80^+$ $CD11c^+$ (B)) in the broncho-alveolar lavage fluid of $TF^{+/+}$ and $TF^{\Delta mye}$ mice was analyzed by flow cytometry, $n_{control}=2$; $n_{HCl}=6$. (A, B) Representative flow cytometry blots were given. Upper panel control mice, lower level HCl-treated mice. For statistical analysis unpaired Student's t-test was performed. Representative flow cytometry blots are given.

Supplemental Figure III: Myeloid TF does not influence leukocyte recruitment during sterile peritonitis. (A, B) Extravasation of leukocytes ($CD45^+$, A) and neutrophils ($CD45^+$ $Ly6G^+$ $F4/80^-$, B) was evaluated by flow cytometry 4 hours post thioglycollate intraperitoneal injection, $n_{TF+/+}=4$; $n_{TF\Delta mye}=8$. (C,D) Extravasation of leukocytes (C) and macrophages ($CD45^+$ $F4/80^+$, D) 72 hours post treatment, $n_{TF+/+}=5$; $n_{TF\Delta mye}=4$. For statistical analysis unpaired Student's t-test was performed.

Supplemental Figure IV: No significant changes in IL-12 and IL-1β levels between myeloid TF and wildtype littermates 8h post acid-induced ALI. Concentrations of (A) IL-12 and (B) IL-1β in broncho-alveolar lavage fluid (BALF) or whole lung tissue were measured by ELISA. For statistical analysis unpaired Student's t-test was performed.

Supplemental Figure V: Effect of thrombin and FVIIa on the inflammatory response of macrophages. Bone marrow cells were isolated and differentiated to bone marrow-derived macrophages by GM-CSF (10 ng/ml) and then stimulated with LPS (10 ng/ml), thrombin (0.66 U/ml) and FVIIa (8 ng/ml) for 3 hours as indicated. (A, D) IL-6, (B, E) TNF-α and (C, F) IκBα mRNA levels were determined by qRT-PCR and depicted as fold PBS control. One-way ANOVA with Tukey's multiple comparisons test was applied for statistical analysis; (A-C) n=5, (D-F) n=3. *p<0.05, **p<0.01, n.s. not significant.

Supplementary methods

Thioglycollate-elicited sterile inflammation

2 ml 4% thioglycollate medium (BD Becton, Dickinson and Company, New Jersey, USA) were injected intraperitoneally and after 4 hours or 72 hours a peritoneal lavage with 8ml PBS was performed. The recollected lavage fluid was centrifuged at 1000 xg for 5 min at room temperature and the cell pellet resupended in 0.5 ml PBS and stained for flow cytometry.

BMDM stimulation

BMDM isolation is described in the method section of the manuscript. BMDMs were stimulated with thrombin (0.66 U/ml, Technoclone, Vienna, Austria) and human FVIIa (8 ng/ml, Novo Nordisk, Vienna, Austria) for 3 hours.

Flow cytometry

Additionally used antibody: α-mouse CD11c-PE (1:80, BioLegend, London, UK).

qPCR

The following primers were applied: HPRT forward: 5'-CGCAGTCCCAGCGTCGTG-3', reverse: 5'-CCATCTCCTTCATGACATCTCGAG-3'; IL-6 forward: 5'-CAAGTCGGAGGCTTAATTACACATG-3', reverse:5'-ATTGCCATTGCACAACTCTTTTCT-3'; TF forward: 5'-CAGTTCATGGAGACGGAGAC-3', reverse: 5'-CAACCACGTTCAGTTTTCTACC-3'; TNF-α forward: 5'- CCACCACGCTCTTCTGTCTAC-3', reverse: 5'- AGGGTCTGGGCCATAGAACT-3'; EGR1 forward: 5'-AGCGAACAACCCTATGAGC-3', reverse: 5'- AGGCCACTGACTAGGCTGAA-3'; IκBα forward: 5'- GAAGCCGCTGACCATGGAA-3', reverse: 5'-GATCACAGCCAAGTGGAGTGGA-3', STAT1 forward: 5'- GCTGCCTATGATGTCTCGTTT-3', reverse: 5'- TGGACATCTGTACGGGATCTT-3'