## SUPPLEMENTAL MATERIALS AND METHODS

### Strains, media and growth conditions

*Aspergillus fumigatus (AFU)* strains were cultivated at 37°C on complete medium (2g peptone and 1g yeast extract per litre). Strains used in this study are listed in Table S1. Different *AFU* Wt isolates were obtained from the Division of Hygiene and Medical Microbiology, Medical University of Innsbruck.

### Human plasma and serum preparation for fungal growth assay

Patient serum samples were taken from the ALLIVE trial [1]. Hemolytic serum samples were excluded from further analysis.

Human plasma was obtained from the bloodbank of the MUI from healthy donors.

Both, plasma and serum were heat-inactivated at 56°C for 30min to inactivate complement and centrifuged at maximum speed for 5 min at room temperature. Supernatants were transferred to a new tube.

Plasma samples were aliquoted and spiked with $Fe_2(SO_4)_3$ (Sigma) to obtain the following $Fe^{3+}$concentrations: 0µM, 5µM, 10µM, 20µM, 50µM, 75µM and 100µM. Samples were stored at -20°C.

For the fungal growth assay, plasma or serum samples were diluted to 10% with RPMI 1640 (Lonza®) and sterile filtered (0,2µM syringe filter) before being inoculated with *AFU* spores.

### Fungal growth assay

For fungal growth assays, sterile filtered RPMI containing 10% serum or plasma was inoculated with $5x10^4$ *AFU* spores/mL. If not otherwise indicated, assays were carried out in a 96-well plate (Falcon) in a final volume of 200µL. Plates were incubated for up to 72h at 37°C. Fungal growth was assessed using a microscope (Olympus IX70) and photographed (Progress Scan CF).

As indicated, the following supplements were added to fungal cultures: 100µM deferoxamine (Sigma, Germany), 200µM deferasirox (Cayman Chemicals, USA), 2mg/mL human holo-/ apo-Tf (both Sigma, Germany). All cultures containing supplements were compared to vehicle control cultures containing equivalent amounts of DMSO or water, respectively.

**Measurement of iron parameters and ~~NTBI~~ eLPI**

Plasma iron and Tf-saturation in iron-spiked human plasma samples were measured with a Hitachi cobas 8000 modular analyzer (Roche).

eLPI concentrations in undiluted human serum and plasma specimens were determined by the FeROS eLPI kit according to the according to the manufacturer's protocol (Aferrix Tel Aviv, Israel). In general, FeROS eLPI kit ~~th~~is a fluorescence-based assay intended for semiquantitative detection of overt and cryptic redox active forms of non-transferrin-bound iron ~~NTBI (Aferrix Tel Aviv, Israel)~~. In detail, the amount of reactive oxygen species (ROS) formation, which is triggered by a reducing agent, is determined via fluorescence of a probe (Dihydrorhodamine), which only fluoresces if oxidized by ROS. eLPI concentrations are presented in arbitrary units [AU].

**Monitoring of holo-Tf conversion into apo-Tf via Urea PAGE and electrophoresis**

The assay was performed in 6-well plates (Corning). 2mL RPMI without phenol red (Lonza) supplemented with 5µM $Fe^{3+}$ and 2mg/mL human apo-Tf was inoculated with $5x10^4$ *AFU* spores/mL. RPMI containing apo- and holo-Tf, respectively, were used as controls. Plates were incubated at 37°C. After 12h, 24h, 48h and 72h 50µL of supernatant was harvested and used for further apo-TF: holo-TF determination.

For detection of apo- and holo-Tf via Urea PAGE, *AFU* culture supernatants were loaded onto a 6 M-Urea-containing 6% polyacrylamide gel as described previously [2]. Tf bands were visualized by Coomassie Blue staining.

For spectrophotometric determination of apo- and holo-Tf, absorbance of culture supernatant at 280 and 460 nm were measured in a microplate reader (Infinite M200pro, Tecan, Männedorf, Switzerland).

Corrected absorbance values were obtained by subtracting absorbance of pure phenol red-free RPMI from sample absorbance.

**Statistics**

Statistical analysis was performed with R. All parameters measured in patients were provided by Wermke et al. [1]. Statistical analysis was performed by P.V. and T.P. Throughout the study, all authors had access to primary clinical trial data. Correlations of fungal outgrowth probability with clinical parameters (e.g. serum eLPI, ferritin, transferrin saturation) were assessed using multiparameter logistic regression (generalized linear models with a logit link function). The models included the effect of the individual enabling analysis of longitudinal data and the effect of the treatment phase (baseline – day 7 conditioning coded as conditioning phase and transplant – day 100 follow-up coded as follow-up phase). Risk of developing fungal outgrowth is presented as odds ratio (OR) with 95% confidence intervals and p values (OR>1). . p values $<0.05$ are considered significant.

## SUPPLEMENTAL REFERENCES


1. Wermke M, Eckoldt J, Götze KS, et al. Enhanced labile plasma iron and outcome in acute myeloid leukaemia and myelodysplastic syndrome after allogeneic haemopoietic cell transplantation (ALLIVE): a prospective, multicentre, observational trial. Lancet Haematol **2018**; 5:e201–e210.

2. Evans RW, Williams J. The electrophoresis of transferrins in urea/polyacrylamide gels. Biochem J **1980**; 189:541–6.

3. Schrettl M, Bignell E, Kragl C, et al. Siderophore biosynthesis but not reductive iron assimilation is essential for Aspergillus fumigatus virulence. J Exp Med **2004**; 200:1213–9.

4. Schrettl M, Bignell E, Kragl C, et al. Distinct roles for intra- and extracellular siderophores during Aspergillus fumigatus infection. PLoS Pathog **2007**; 3:1195–207.

**SUPPLEMENTAL TABLES**

**Table S1.**

Fungal strains used in this study.

| Strain | Genotype | Reference |
|---|---|---|
| ATCC46645 | Wt | American Type Culture Collection |
| *ΔsidA* | ATCC46645, DsidA::hph | [3] |
| *ΔsidF* | ATCC46645, DsidF::hph | [4] |

**Table S2.**

Patient characteristics

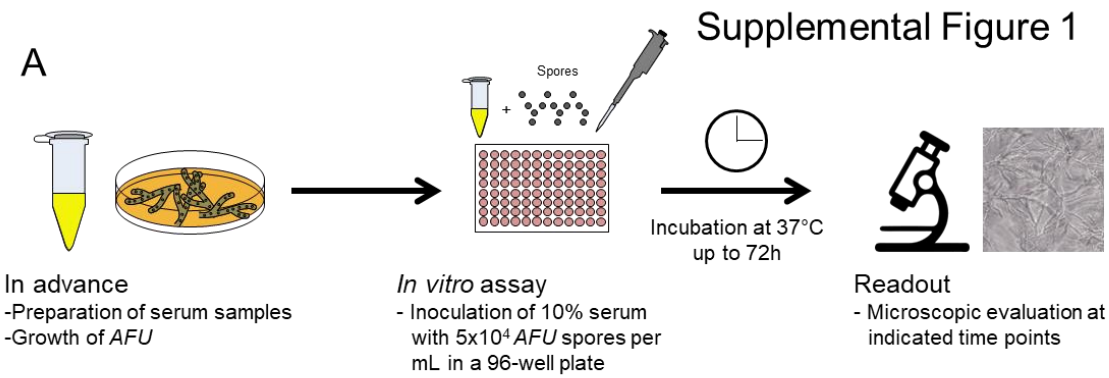
| | |
|---|---|
| **Age** (years) | 61 (53 – 68) |
| **Sex** | |
| Male | 15 (51.7%) |
| Female | 14 (48.3%) |
| **Disease** | |
| MDS | 7 (24.1%) |
| AML | 22 (75.9%) |
| **Disease stage** | |
| First complete remission | 15 (51.7%) |
| Other | 14 (48.3%) |

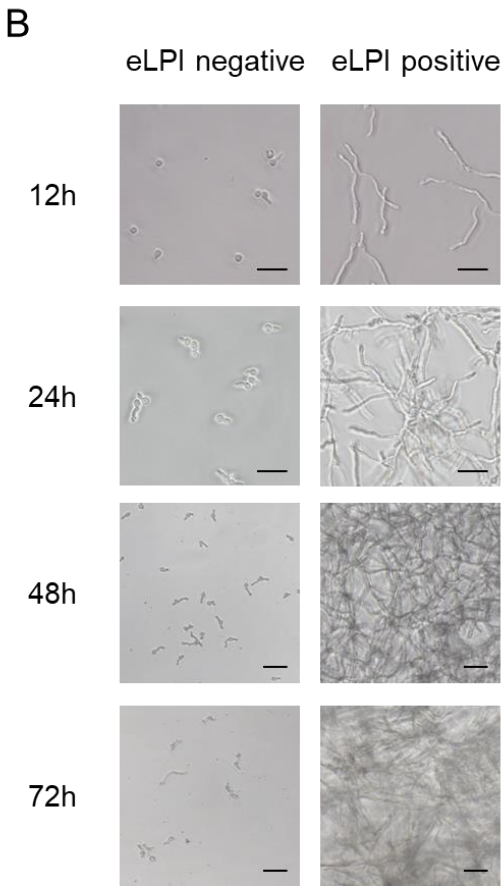
| | |
|---|---|
| **Time from diagnosis to allogeneic HSCT** (months) | 6 (3.5 – 11.5) |
| **Cytogenetics (AML)*** | |
| Intermediate | 16 (72.7%) |
| Adverse | 6 (27.3%) |
| **IPSS risk (MDS)** | |
| Low or intermediate – 1 | 4 (57.1%) |
| Intermediate – 2 or high | 3 (42.9) |
| **HSCT comorbidity index** | |
| 0 | 13 (44.8%) |
| 1 - 2 | 8 (27.6%) |
| ≥ 3 | 8 (27.6%) |
| **Conditioning** | |
| Reduced intensity | 24, (82.8%) |
| Conventional intensity | 5 (17.2%) |
| **Total body irradiation** | |
| Yes | 3 (10.3%) |
| No | 26 (89.7%) |
| **Donor** | |

| | |
|---|---|
| Matched unrelated donor | 26 (89.7%) |
| Matched related donor | 3 (10.3%) |
| **HLA-match** | |
| 10/10 | 24 (82.8 %) |
| 9/10 | 5 (17.2%) |
| **Graft source** | |
| Bone marrow | 1 (3.4%) |
| Peripheral blood stem cells | 28 (96.6%) |
| **In vivo T-cell depletion** | |
| Yes | 1 (3.4%) |
| No | 28 (96.6%) |
| **Immunosuppression** | |
| Ciclosporin or tacrolimus (monotherapy) n, (%) | 2 (6.9%) |
| Ciclosporin or tacrolimus plus mycophenolate-mofetil or mycophenolic acid | 4 (13.8%) |
| Ciclosporin or tacrolimus plus methotrexate | 22 (75.9%) |
| Ciclosporin or tacrolimus plus other | 1 (3.4%) |

Data are given as median (IQR) or n, (%). MDS = myelodysplastic syndrome, AML = acute myeloid leukemia, HSCT = hematopoietic stem cell transplantation, IPSS = International Prognostic Scoring, *according to recommendations of the European Leukemia Network

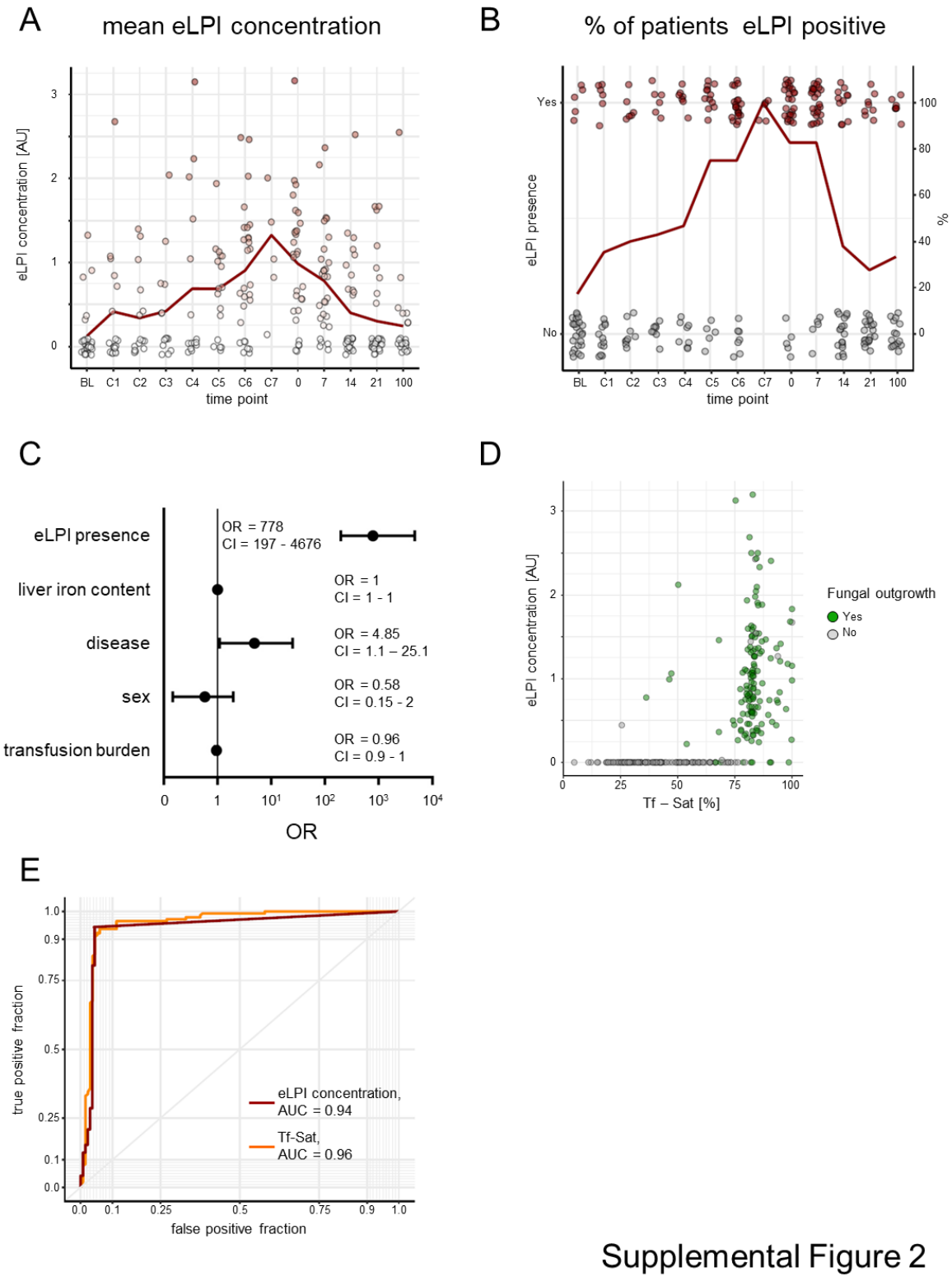
**SUPPLEMENTAL FIGURES**




**Supplemental Figure 1.** (A) Patient serum samples were prepared as described in "Materials and Methods". *Aspergillus fumigatus (AFU)* strains were cultivated at 37°C on complete medium agar plates and spores harvested. Complement-depleted human serum samples were diluted to 10% with RPMI and inoculated with $5\times10^4$/mL *AFU* spores in a 96-well plate in a final volume of 200 µL. Plates were incubated for up to 72h at 37 °C. Fungal outgrowth was assessed by microscopy at indicated time points. (B) Time course of *AFU* outgrowth depending on the presence of enhanced labile plasma iron (eLPI).

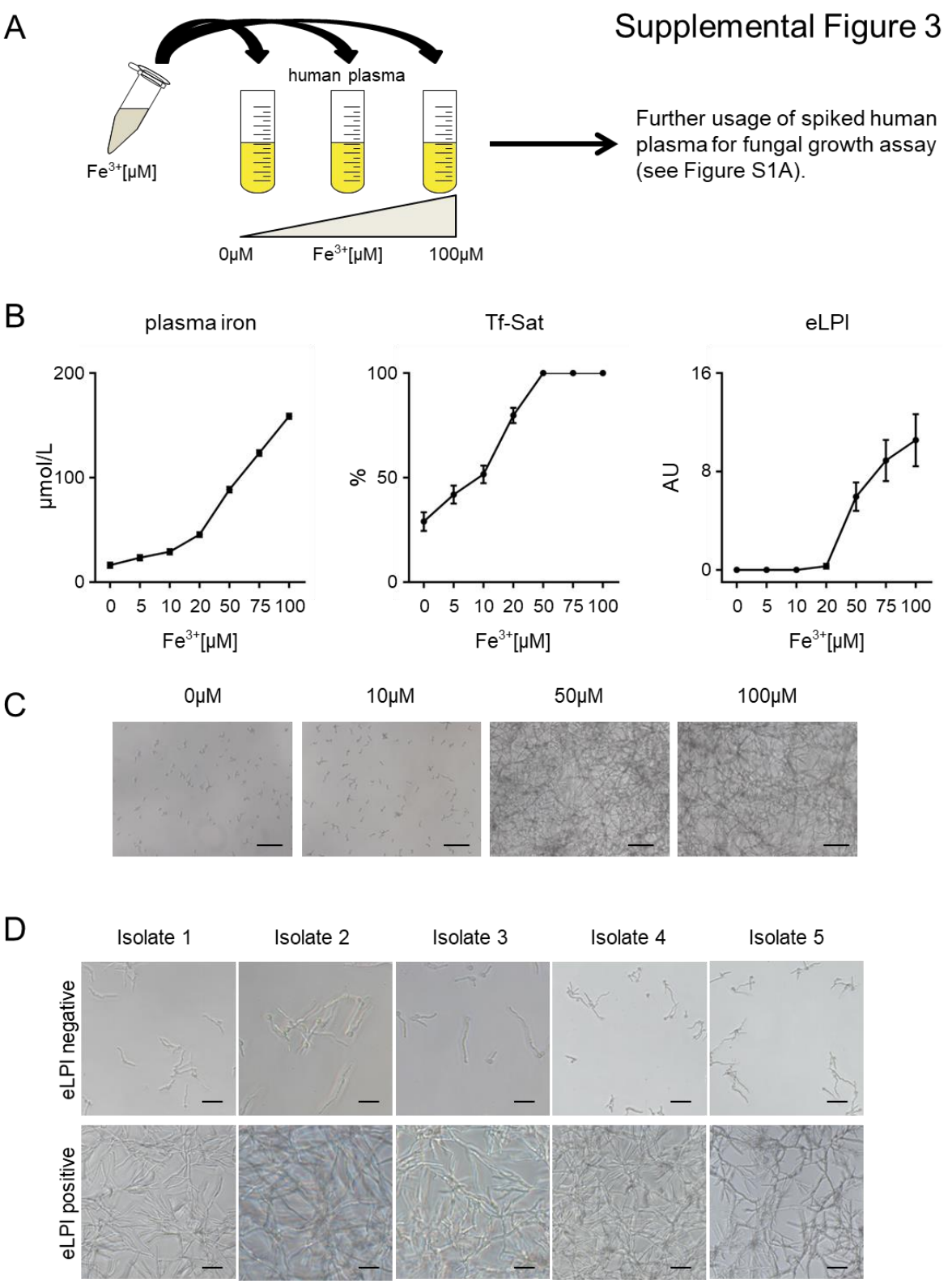
Representative microscopy images of fungal cultures at indicated time points (12h, 24h, 48h and 72h) after inoculation of RPMI containing 10% ferric iron-spiked human plasma with *AFU* spores $5\times10^4$ spores/mL are shown (original magnification at 12h and 24h ×40, bars represent 15 μm; original magnification at 48h and 72h ×20, bars represent 30 μm).




Supplemental Figure 2

**Supplemental Figure 2.** (A) Enhanced labile plasma iron (eLPI) values over time of herein analyzed patient cohort. Each dot represents a measured serum eLPI concentration [arbitrary units, AU] at the indicated time point of one patient during the course of allogeneic hematopoietic stem cell

transplantation (HSCT; BL=baseline; C1-7=conditioning days 1-7; 0=day of transplantation; 7-100=day 7-100 after transplantation). Each dot represents eLPI concentration for one serum sample at a certain time point. The red line represents mean serum eLPI concentration at a particular time point. (B) Percentage of patients developing eLPI during the consecutive phases of HSCT. Each dot represents serum eLPI positivity or negativity (red: positive, gray: negative, threshold: 0.2 AU) for a single sample at the indicated time point during the course of allo-HSCT (BL=baseline; C1-7=Conditioning day 1-7; 0=day of transplantation; 7-100=day 7-100 after transplantation). The red line represents percentage of eLPI-positive serum samples at a particular time point. (C) Relative probability of *Aspergillus fumigatus (AFU)* outgrowth as a function of serum eLPI presence considering clinical confounders. The graph depicts odds ratios (OR) ± 95% confidence interval (CI) for probability of fungal outgrowth. Statistical significance was assessed using a multiparameter logistic regression model for the outgrowth response variable (no fungal growth vs. fungal outgrowth). (D) Graph plotting the relation of transferrin saturation (Tf-Sat), eLPI concentrations and fungal outgrowth. Each dot represents a Tf-Sat value and eLPI concentration for one serum sample. Green color indicates that *AFU* outgrowth was observed, a grey dot stands for no outgrowth. (E) Receiver-operator curves (ROC) for sensitivity and specificity of threshold for Tf-Sat and serum eLPI of analyzed serum samples as predictors of fungal outgrowth. The optimal sensitivity:specificity tradeoff is reached at 0.2 [AU] eLPI and 75.2% Tf-Sat. Area under the curve (AUC) for both markers is indicated.




**Supplemental Figure 3.** (A) Ferric iron ($Fe^{3+}$) spiking of human plasma. Complement-depleted plasma samples obtained from six healthy humans were supplemented with increasing concentrations of $Fe^{3+}$ (0µM-100µM) to saturate transferrin (Tf) and provoke the appearance of enhanced labile plasma iron (eLPI). Subsequently, these ferric iron-spiked plasma samples were used for our fungal assay, as shown

in Supplementary Figure 1 and as described in “Materials and Methods”. (B) Measurement of plasma iron, Tf-Sat and eLPI in iron-spiked human plasma samples. Points represent mean values ± standard error of the mean. (C) Representative microscopy images of fungal cultures taken 48h after *Aspergillus fumigatus (AFU)* spores ($5x10^4$ spores/mL) were seeded in RPMI containing 10% iron-spiked plasma (original magnification ×20, bars represent 30 µm). (D) Nine *AFU* wild-type strains, isolated from patients suffering from invasive aspergillosis were tested for fungal outgrowth in vitro in RPMI containing 10% iron-spiked plasma samples with/without eLPI present. Microscopic images were taken after 48h of culture. Five representative *AFU* isolates are shown (original magnification ×20, bars represent 30µM).