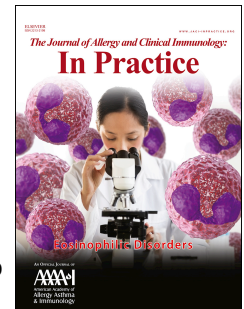


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Serum soluble OX40 as a diagnostic and prognostic biomarker for DIHS/DRESS

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Original article

Title: Serum soluble OX40 as a diagnostic and prognostic biomarker for DIHS/DRESS

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29

Abstract

Background: Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a severe adverse drug reaction commonly associated with the reactivation of human herpesvirus 6 (HHV-6). There are currently no adequate biomarkers for the early diagnosis and detection of DIHS/DRESS. Notably, OX40 (CD134) plays an important role in allergic inflammation and functions as a cellular receptor for HHV-6 entry. We previously reported that the membrane-bound form of OX40 in CD4⁺ T cells was upregulated in DIHS/DRESS.

Objective: We sought to investigate the clinical significance of serum soluble OX40 (sOX40) in DIHS/DRESS.

Methods: Serum sOX40 levels in patients with DIHS/DRESS ($n = 39$), maculopapular exanthema/erythema multiforme ($n = 17$), Stevens-Johnson syndrome/toxic epidermal necrolysis ($n = 13$), autoimmune bullous diseases ($n = 5$), and in healthy volunteers ($n = 5$) were examined by the enzyme-linked immunosorbent assay. Copy numbers of HHV-6, HHV-7, and CMV in peripheral blood mononuclear cells were quantified using real-time PCR.

Results: Serum sOX40 levels in patients with DIHS/DRESS in the acute stage were elevated in parallel with high OX40 expression on CD4⁺ T cells. Serum sOX40 levels significantly positively correlated with disease severity and serum levels of thymus and

49 activation-regulated chemokine, interleukin-5, and interleukin-10. HHV-6-positive patients
50 presented with higher sOX40 levels than HHV-6-negative patients, and serum sOX40 levels
51 correlated with HHV-6 DNA loads.

52 **Conclusion:** Serum sOX40 level can be a useful diagnostic marker for DIHS/DRESS that
53 reflects the disease severity. Elevated serum sOX40 levels also predict HHV-6 reactivation
54 in patients with DIHS/DRESS.

55

Highlights

What is already known about this topic?

Drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms (DIHS/DRESS) is a severe drug reaction commonly associated with human herpesvirus 6 (HHV-6) reactivation. OX40 is involved in both Th2 type inflammatory response and HHV-6 replication.

What does this article add to our knowledge?

Serum sOX40 levels in patients with DIHS/DRESS in acute stage were elevated and significantly positively correlated with disease severity. In patients with DIHS/DRESS with HHV-6 reactivation, serum sOX40 levels correlated with peak HHV-6 viral loads.

How does this study impact current management guidelines?

Serum sOX40 level can be a useful diagnostic and prognostic marker for DIHS/DRESS. Elevated level of serum sOX40 can also predict the extent of HHV-6 reactivation.

Key words: CD134, DIHS, DRESS, HHV-6, OX40, thymus and activation-regulated chemokine

75 **Abbreviations used**

76 CMV: Cytomegalovirus

77 DIHS: Drug-induced hypersensitivity syndrome

78 DRESS: Drug reaction with eosinophilia and systemic symptoms

79 EM: Erythema multiforme

80 HHV: Human herpesvirus

81 MPE: Maculopapular exanthema

82 PBMC: Peripheral blood mononuclear cell

83 SJS: Stevens-Johnson syndrome

84 sOX40: Soluble OX40

85 TARC: Thymus and activation-regulated chemokine

86 TEN: Toxic epidermal necrolysis

87

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Introduction

Drug-induced hypersensitivity syndrome (DIHS)/drug reaction with eosinophilia and systemic symptoms (DRESS) is a severe adverse drug reaction characterized by fever, generalized maculopapular erythema, liver dysfunction, and hematological abnormalities,¹ with a mortality rate of 5–10%.^{2, 3} These symptoms occur 2–6 weeks after the administration of a specific drug, such as carbamazepine, allopurinol, or salazosulfapyridine. In the case of antiepileptic drugs, it occurs in approximately 1 in 3,000 drug exposures.⁴ DIHS/DRESS is usually associated with the reactivation of human herpesviruses (HHVs), including HHV-6 and HHV-7, and cytomegalovirus (CMV).^{5, 6} In particular, HHV-6 reactivation is detected in the vast majority of cases and is associated with the severity of DIHS/DRESS.^{7, 8} Systemic corticosteroids are the main treatment for DIHS/DRESS, although the optimal dose has not been established.^{9, 10} Because the clinical manifestation in the early stage of DIHS/DRESS can resemble other, more common drug-induced reactions, the diagnosis is often delayed, leading to multiple organ failure and death.^{2, 3} It is therefore necessary to identify early diagnostic markers for this condition.

HHV-6 latently infects monocytes/macrophages, whereas during the proliferation phase, it preferentially infects activated CD4⁺ T cells to produce many copies of the viral genome.¹¹ OX40, also known as CD134, is a member of the tumor necrosis factor receptor superfamily. OX40 is expressed predominantly on activated lymphocytes, particularly CD4⁺

108 T cells, and functions as a cellular receptor for HHV-6 entry.¹² The OX40 ligand (OX40L) is
109 expressed on antigen-presenting cells and activated T cells.¹³ The OX40-OX40L interaction
110 is required for the generation of the long-term memory response, optimal T cell activation,
111 and Th2 differentiation.¹⁴⁻¹⁶ We have recently shown that OX40 and OX40L are upregulated
112 in CD4⁺ T cells and peripheral blood mononuclear cells (PBMCs), respectively, in patients
113 with DIHS/DRESS, suggesting that the OX40-OX40L interaction may be involved in the
114 activation of CD4⁺ T cells and initiation of DIHS/DRESS.^{17, 18}

115 In a manner similar to other members of the tumor necrosis factor receptor superfamily, a
116 soluble form of OX40 (sOX40) may be produced by OX40 shedding and/or alternative
117 splicing.¹⁹ sOX40 has anti-inflammatory effects, antagonistic to those of the membrane-
118 bound form of OX40L, and it inhibits the inflammatory response, mimicking regulatory T cell
119 function.²⁰ It is known that serum sOX40 levels are abnormal in various autoimmune and
120 inflammatory diseases, such as systemic sclerosis,²¹ rheumatoid arthritis,²² and atopic
121 dermatitis,²³ although the role of sOX40 in these pathologies is unknown. In contrast,
122 sOX40 levels have not been investigated in patients with DIHS/DRESS, despite the fact
123 that sOX40 may be involved in the pathophysiology of DIHS/DRESS, namely in HHV-6
124 reactivation and Th2 type response. In this study, we focused on serum sOX40 levels and
125 performed a retrospective longitudinal study in patients with DIHS/DRESS. Our data
126 suggest that serum sOX40 levels may serve as a biomarker for early diagnosis and severity

127 in DIHS/DRESS.

128

Methods

Blood samples

Blood samples were obtained from patients with DIHS/DRESS ($n = 39$), MPE/EM ($n = 17$), SJS/TEN ($n = 13$), and autoimmune bullous diseases ($n = 5$), as well as from healthy volunteers ($n = 5$), between October 2009 and July 2020 (see Table E1 in the Online Repository). DIHS (including atypical DIHS), DRESS (including possible, probable, and definite DRESS), SJS, overlap SJS-TEN, and TEN were diagnosed based on the previously described criteria.²⁴⁻²⁶ Blood samples in the acute stage were taken on average 7.7 days (range 1–18 days) after the onset of skin eruption in DIHS/DRESS, 4.1 days (range 1–13 days) in MPE/EM, and 7.2 days (range 3–14 days) after the onset of SJS/TEN. Patients who visited our department more than 20 days after the onset on the disease were excluded.

For a retrospective longitudinal study, we collected 79 samples from 22 patients with DIHS/DRESS. These patients had been followed for up to 32.5 ± 2.2 days (range 19–58 days) after the onset, and each patient was tested two to four times during the follow-up.

This study was approved by the Ethics Committee of the Nara Medical University (reference no. 1753). All studies using human materials were performed according to the principles of the Declaration of Helsinki. Informed consent was obtained through the opt-out method because of the retrospective observational nature of this study.

Enzyme-linked immunosorbent assay (ELISA) and chemiluminescent enzyme immunoassay

Serum sOX40 levels were measured with a Human CD134/OX40 ELISA kit (IBL, Gunma, Japan). Serum interleukin-5 (IL-5) and IL-10 levels were evaluated using commercial ELISA kits from R&D Systems (Minneapolis, MN) and MyBioSource (San Diego, CA), respectively. Serum levels of thymus and activation-regulated chemokine (TARC/CCL17) (Sysmex Co., Kobe, Japan), IL-4 (BD Pharmingen, San Diego, CA), and IFN- γ (Thermo Fisher Scientific, Indianapolis, IN) were analyzed by the chemiluminescent enzyme immunoassay according to the manufacturers' protocols.

Flow cytometry

We analyzed the expression of OX40 on CD4⁺ T cells in DIHS/DRESS by a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) and CellQuest software (Becton Dickinson) as previously reported.^{17, 18} PBMCs were isolated from the whole blood by Ficoll density gradient centrifugation (GE Healthcare, Little Chalfont, UK) and stored at -80 °C until use. For staining cell surface of PBMCs, fluorescein isothiocyanate-conjugated anti-OX40 (clone ACT35, 1:10 dilution), phycoerythrin-conjugated anti-CD3 (UCHT1, 1:10), and allophycocyanin-conjugated anti-CD4 (RPA-T4, 1:10) antibodies were used (all from BD

Pharmingen).

Real-time PCR for quantitative analysis of HHV-6, HHV-7, and CMV DNA

We examined HHV-6, HHV-7, and CMV DNA copy numbers in PBMCs using real-time PCR, following the methods described by Watzinger *et al.* with some modifications.²⁷ Peripheral blood was periodically obtained from the acute stage to the remission stage. DNA was extracted using a QIAamp DNA Blood mini-kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. All reactions were set up as singleplex PCRs in a total volume of 25 μ L that contained 12.5 μ L of the TaqMan Fast Advanced Master Mix (Thermo Fischer Scientific), primers at concentrations between 300 and 900 nM, and a 200 nM TaqMan probe (see Table E2 in the Online Repository). The mixtures were amplified on a StepOne real-time PCR system (ABI) using the following cycling parameters: 2 min at 50 $^{\circ}$ C, 20 s at 95 $^{\circ}$ C, and 53 cycles of 1 s at 95 $^{\circ}$ C and 20 s at 60 $^{\circ}$ C.

Scoring severity of DIHS/DRESS

We used the previously described DIHS/DRESS scoring system.²⁸ Clinical history, physical findings, and blood test results essential for scoring were retrospectively retrieved from the medical records.

Statistical analysis

Statistical analyses were performed using EZR software version 1.54 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).²⁹ The Mann-Whitney U test was applied to compare serum sOX40 levels between two independent groups. Correlations between serum sOX40 levels and various parameters, including cytokine levels and the severity score of DIHS/DRESS, were evaluated with the Spearman rank correlation coefficient. The Wilcoxon signed rank test was used to compare sOX40 levels in patients with DIHS/DRESS during acute and late phases. In all tests, effects were considered statistically significant if *P*-value was less than 0.05. Data are presented as the mean $\pm$ standard error of the mean. Receiver operating characteristic curve was plotted using SPSS software version 28.0 (IBM, Armonk, NY). The cut-off point was obtained according to the maximum Youden's index (Sensitivity + Specificity – 1).

Results

Serum sOX40 level is proportional to the fraction of OX40-expressing CD4⁺ T cells in patients with DIHS/DRESS

We investigated the correlation between the percentage of OX40-expressing CD4⁺ T cells and serum sOX40 levels, using blood samples of six patients with DIHS/DRESS collected in the acute and late phases of the disease. In this analysis, we used the flow cytometry data previously described.¹⁷ In all six patients, the percentage of OX40-expressing CD4⁺ T cells in the acute stage was higher than that in the late stage. In each patient, serum sOX40 levels decreased in parallel with the down-regulation of OX40 expression on CD4⁺ T cells over the course of the disease (Figure 1). We confirmed that serum sOX40 reflects the percentage of OX40-expressing CD4⁺ T cells in patients with DIHS/DRESS.

<<FIG 1>>

Elevated serum sOX40 levels in DIHS/DRESS

We examined whether serum sOX40 levels were elevated in DIHS/DRESS and other diseases. Serum sOX40 levels were significantly higher in patients with DIHS/DRESS in the acute stage (646 ± 151 pg/mL) than in healthy controls ($P < 0.001$) or in patients with

MPE/EM (201 ± 110 pg/mL; $P < 0.001$), SJS/TEN (155 ± 95 pg/mL; $P < 0.005$), or autoimmune bullous diseases (41 ± 33 pg/mL; $P < 0.05$) (Figure 2). For the receiver operating characteristic (ROC) curve for DIHS/DRESS versus MPE/EM, an area under the curve (AUC) value of 0.781 (95% CI: 0.638, 0.923) and a cut-off point of 7 pg/mL were obtained; the sensitivity and specificity were 0.872 and 0.647, respectively (Figure E1A). For the ROC curve for DIHS/DRESS versus SJS/TEN, an AUC value of 0.767 (95% CI: 0.608, 0.927) and a cut-off point of 231 pg/mL were found; the sensitivity and specificity were 0.667 and 0.846, respectively (Figure E1B). There was no significant difference in sOX40 levels between the patients with prior systemic steroid treatment ($n = 20$) and without ($n = 19$) (660 ± 164 pg/mL vs. 632 ± 262 pg/mL; $P = 0.415$). These results indicate that sOX40 level increases specifically in patients with DIHS/DRESS independent of the preceding treatment, and that sOX40 levels can be useful in distinguishing DIHS/DRESS from the other disease groups.

<<FIG 2>> <<FIG E1>>

Clinical correlations of serum sOX40 levels in DIHS/DRESS

To determine whether sOX40 is useful as a severity biomarker for DIHS/DRESS, we evaluated the correlations between serum sOX40 levels and clinical severity and laboratory

test findings. Serum sOX40 levels correlated positively with the severity score ($r_s = 0.527$, $P < 0.001$).

Previous studies have shown that serum TARC level is elevated in patients with DIHS/DRESS³⁰ and associated with the severity.³¹ We found that there was positive correlation between sOX40 and TARC ($r_s = 0.597$, $P < 0.0001$). We next examined the correlations between sOX40 and the cytokines that have been reported to be elevated in DIHS/DRESS, such as IL-4, IL-5, and IL-10.³² Serum sOX40 levels correlated positively with IL-5 ($r_s = 0.411$, $P < 0.05$) and IL-10 levels ($r_s = 0.608$, $P < 0.01$) but not with that of IL-4 ($r_s = 0.370$, $P = 0.0575$). Parenthetically, there was no correlation between sOX40 and IFN- γ ($r_s = 0.106$, $P = 0.606$).

Finally, serum sOX40 levels did not correlate with serum levels of creatinine and alkaline phosphatase but correlated with C-reactive protein ($r_s = 0.430$, $P < 0.01$) (Figure 3).

<<FIG 3>>

A longitudinal study of serum sOX40 levels in DIHS/DRESS

Serum levels of sOX40 differ in the acute and chronic phases of some diseases.^{21, 22} To investigate changes in serum sOX40 levels over time, we performed a longitudinal study of sOX40 levels in 22 patients with DIHS/DRESS from whom we could obtain serum samples

over a long period. Serum samples in the acute stage were taken between 2 and 18 days after the onset of DIHS/DRESS (average no. of days, 8.7), whereas serum samples in the late stage were obtained between 19 and 58 days after the onset (average no. of days, 31.6). The interval between the acute stage and the late stage in 22 patients was 23.5 ± 2.2 days (range 13–56 days). There was a statistically significant difference between serum sOX40 levels at the acute and late stages ($P < 0.0005$, Wilcoxon signed rank test) (Figure 4). Of the 22 patients, 15 had elevated serum sOX40 level in the acute phase, which decreased in the late phase. In five patients, serum sOX40 level were below 10 pg/mL throughout the acute and late phases. Serum levels in two patients were increased in the late phase.

<<FIG 4>>

Relationship between the reactivation of HHV-6 and serum sOX40 levels

Because OX40 functions as a cellular receptor for HHV-6 entry, the correlation between HHV-6 amount and serum sOX40 level was analyzed. First, we measured sOX40 levels in patients with and without HHV-6 reactivation. Reactivation of human herpesviruses, namely, HHV-6, HHV-7, or CMV, was detected in all 39 patients with DIHS/DRESS. Of these, HHV-6 reactivation was identified in 33 patients. In 32 of these 33 patients, HHV-6 DNA was

detected in their PBMCs by real-time PCR, and one had elevated serum anti-HHV-6 IgG antibodies. Among the six patients without HHV-6 reactivation, two and four were positive for HHV-7 DNA and CMV DNA, respectively. Although the difference was not statistically significant, HHV-6-positive patients tended to have higher sOX40 levels than HHV-6-negative patients ($P = 0.267$; Figure 5A). Furthermore, there was a positive correlation between sOX40 levels and HHV-6 DNA loads in the 32 HHV-6 DNA positive patients ($r_s = 0.497$, $P < 0.005$) (Figure 5B).

<<FIG 5>>

Kinetics of HHV-6 DNA copy numbers and serum sOX40 levels

The kinetics of HHV-6 DNA copy numbers in PBMCs and serum sOX40 levels for the two representative DIHS/DRESS patients with HHV-6 reactivation are shown in Figure 6. In case 1, serum sOX40 levels peaked at day 5, and HHV-6 reactivation was first observed on day 12. HHV-6 viral loads increased and peaked at day 19. In case 2, sOX40 peaked at day 9, and HHV-6 was first reactivated at day 15. The peak of HHV-6 DNA loads was at day 22. In both cases, at the peak of serum sOX40 levels, HHV-6 had not been reactivated yet. C-reactive protein appeared to decrease in parallel with sOX40.

299 In addition to these two patients, 12 other patients had four measurements of sOX40 during
300 the acute and late phases as shown in Figure E2. In 6 out of the 12 patients shown in
301 Figure E2, when elevated levels of sOX40 levels were observed, HHV-6 had not been
302 reactivated yet (cases 4, 7, 8, 9, 12, and 14)

303

304 <<FIG 6>> <<FIG E2>>

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Discussion

It is often difficult to distinguish DIHS/DRESS from other drug eruptions. Because DIHS/DRESS may cause multiple organ failure and death,^{2, 3} a reliable biomarker of this condition is critically needed. In this study, we observed that serum sOX40 levels were significantly elevated in patients with early phase DIHS/DRESS compared with those in patients with MPE/EM, SJS/TEN, autoimmune bullous disease, or in healthy volunteers. Furthermore, serum sOX40 levels positively correlated with the severity score and C-reactive protein. It should be noted that of the 39 patients with DIHS/DRESS, three patients with extremely elevated serum sOX40 died. We have previously reported that OX40 expressed on CD4⁺ T cells can be a useful biomarker for the diagnosis of DIHS/DRESS.¹⁷ However, the flow cytometry technique is difficult to use in daily clinical practice. In contrast, serum sOX40 level can be easily measured by ELISA and applied to early diagnosis and prediction of the severity of DIHS/DRESS.

Eosinophils and TARC, one of the Th2 chemokines, are elevated in the acute phase of DIHS/DRESS, indicating that DIHS/DRESS is associated with Th2 polarizing conditions.^{2, 30, 33} We previously showed that OX40-expressing CD4⁺ T cells are more abundant in DIHS/DRESS. The OX40-OX40L interaction enhances Th2 differentiation.¹⁴ In this study, we revealed that serum sOX40 levels positively correlated with serum levels of TARC and IL-5, a specific activator of eosinophils,³⁴ suggesting that upregulation of OX40 and

elevated sOX40 levels may induce Th2 polarization in DIHS/DRESS. In addition to Th2 cells, regulatory T (Treg) cells are preferentially increased in the peripheral blood of patients with DIHS/DRESS at the acute stage.³⁵ We have demonstrated previously that about 10% of OX40-expressing CD4⁺ T cells express FoxP3, a Treg cell marker, in the early phase of DIHS/DRESS.¹⁷ In the current study, we revealed a correlation between serum levels of sOX40 and IL-10, an immunosuppressive cytokine. Although it was not possible to establish the relative amounts of sOX40 and IL-10 that originated from Th2 cells, Treg cells, or other cells, the current data suggest that sOX40 might be involved in Treg responses as well as Th2.

Activated CD4⁺ T cells are the preferential target of the fully permissive infection of HHV-6.¹¹ Although T cells are normally non-permissive for HHV-6 infection, they became highly susceptible to such infection when OX40 was overexpressed.¹² Actually, serum sOX40 levels in DIHS/DRESS patients with HHV-6 reactivation tended to be higher than in patients without HHV-6 reactivation, suggesting that OX40 may play an important role in HHV-6 reactivation and proliferation. Besides DIHS, HHV-6 occasionally causes encephalitis upon reactivation after allogeneic hematopoietic stem cell transplantation (allo-HSCT). In patients who underwent allo-HSCT, the upregulation of OX40 expression on T cells coincided with the time of the peak viral load.³⁶ In this study, by analyzing the kinetics of HHV-6 DNA copies in PBMCs and serum sOX40 levels in two DIHS/DRESS patients with HHV-6

reactivation, we found that serum sOX40 levels were elevated before the reactivation of HHV-6. The reason for the difference in HHV-6 viral load and serum sOX40 kinetics between patients who underwent allo-HSCT and patients with DIHS/DRESS remains unclear. However, unlike in the patients who underwent allo-HSCT, it is possible that in patients with DIHS, the administration of causative drugs activates T cells that express OX40, resulting in the stimulation of HHV-6 replication.

This study had some limitations. First, our study was a single-center study with a small sample size. Second, in the DIHS/DRESS group, the period set as acute stage was longer (from 1 to 18 days after the onset) than that in the MPE/EM group (1–13 days) and SJS/TEN group (3–14 days). Third, the characteristics of the patients and healthy volunteers such as age and sex differed between groups. A multi-center study with a larger sample size will be necessary for the confirmation of our findings.

In conclusion, this is the first study to analyze serum sOX40 levels in patients with DIHS/DRESS. Serum sOX40 level can be a useful marker not only for the early diagnosis of DIHS/DRESS but also for the prediction of the severity and presence of HHV-6 reactivation. sOX40 may be involved in both Th2 type inflammatory process and HHV-6 replication. Our study also contributes to better understanding of the pathogenesis of DIHS/DRESS.

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Figure legends

FIGURE 1. Time course of changes in serum sOX40 levels and cell surface expression of OX40 on CD4⁺ T cells in six patients with DIHS/DRESS.

FIGURE 2. Serum sOX40 levels in 39 patients with DIHS/DRESS, 17 patients with MPE/EM, 13 patients with SJS/TEN, 5 patients with autoimmune bullous diseases, and in 5 healthy controls.

FIGURE 3. Correlations between serum sOX40 levels and DIHS/DRESS severity score (A), serum levels of thymus and activation-regulated chemokine (TARC) (B), IL-4 (C), IL-5 (D), IL-10 (E), IFN- γ (F), creatinine (G), alkaline phosphatase (ALP) (H), and C-reactive protein (CRP) (I). Statistical analysis was performed by the Spearman rank correlation test.

FIGURE 4. Elevated serum levels of sOX40 in the acute phase of DIHS/DRESS. Blood samples in the acute phase were taken between 2 and 18 days after the onset of DIHS/DRESS, whereas blood samples in the late phase were obtained between 19 and 58 days after the onset. Statistical analysis was performed using the Wilcoxon signed rank test.

FIGURE 5. Relationship between serum sOX40 levels and reactivation of herpesviruses in patients with DIHS/DRESS. **A**, Serum sOX40 level in the 33 patients with HHV-6 reactivation tended to be higher than in the 6 patients without HHV-6 reactivation ($P = 0.267$). **B**, Correlation between serum sOX40 level and peak HHV-6 DNA copy number in the HHV-6-positive patients with DIHS/DRESS.

505

506 **FIGURE 6.** Time-dependent changes in skin eruption, HHV-6 DNA loads, serum sOX40

507 levels, and serum CRP levels of two representative cases of DIHS/DRESS. In both these

508 cases, at the peak of serum sOX40 levels, HHV-6 had not been reactivated yet.

509

Table E1. Features of patients with DIHS/DRESS, MPE/EM, SJS/TEN, autoimmune bullous disease, and healthy volunteers.

	Age (Mean $\pm$ SD)	Sex	
		Male	Female
DIHS/DRESS ($n = 39$)	57 $\pm$ 19.2	21	18
MPE/EM ($n = 17$)	68 $\pm$ 22.1	6	11
SJS/TEN ($n = 13$)	66 $\pm$ 23.6	3	10
Autoimmune bullous diseases ($n = 5$), including bullous pemphigoid ($n = 2$) pemphigus vulgaris ($n = 2$) pemphigus foliaceus ($n = 1$)	88 $\pm$ 32.4	4	1
Healthy volunteers ($n = 5$)	35 $\pm$ 3.8	3	2

Table E2. Sequence details of PCR primers and probes used

Virus type	Target	Primer type	Oligonucleotide sequence (5'–3')	Nucleotide positions
CMV	MIE protein	Forward primer	AAC TCA GCC TTC CCT AAG ACC A	2414–2435
		Reverse primer	GGG AGC ACT GAG GCA AGT TC	2470–2489
		Probe	CAA TGG CTG CAG TCA GGC CAT GG	2437–2459
HHV-6	DNA polymerase gene	Forward primer	GAA GCA GCA ATC GCA ACA CA	57517–57536
		Reverse primer	ACA ACA TGT AAC TCG GTG TAC GGT	57568–57590
		Probe	AAC CCG TGC GCC GCT CCC	57544–57561
HHV-7	Major capsid protein	Forward primer	CCC AAC TAT TTA CAG TAG GGT TGG TG	84230–84255
		Reverse primer	TTT AGT TCC AGC ACT GCA ATC G	84332–84353
		Probe	CTA TTT TCG GTC TTT CCA ATG CAC GCA (AS)	84258–84284

FIGURE E1. Receiver operating characteristic (ROC) curves for DIHS/DRESS versus MPE/EM and DIHS/DRESS versus SJS/TEN. A, From the ROC curve for DIHS/DRESS versus MPE/EM, an area under the curve (AUC) value of 0.781 (95% CI: 0.638, 0.923) was obtained. From the ROC curve for DIHS/DRESS versus SJS/TEN, an AUC value of 0.767 (95% CI: 0.608, 0.927) was obtained.

FIGURE E2. Relationships between skin eruption, HHV-6 DNA loads, serum sOX40 levels and serum CRP levels in the remaining 12 patients with DIHS/DRESS except for the two patients shown in Figure 6

Figure 1

—●— Serum sOX40
 —□— OX40 expression on CD4⁺ T cells

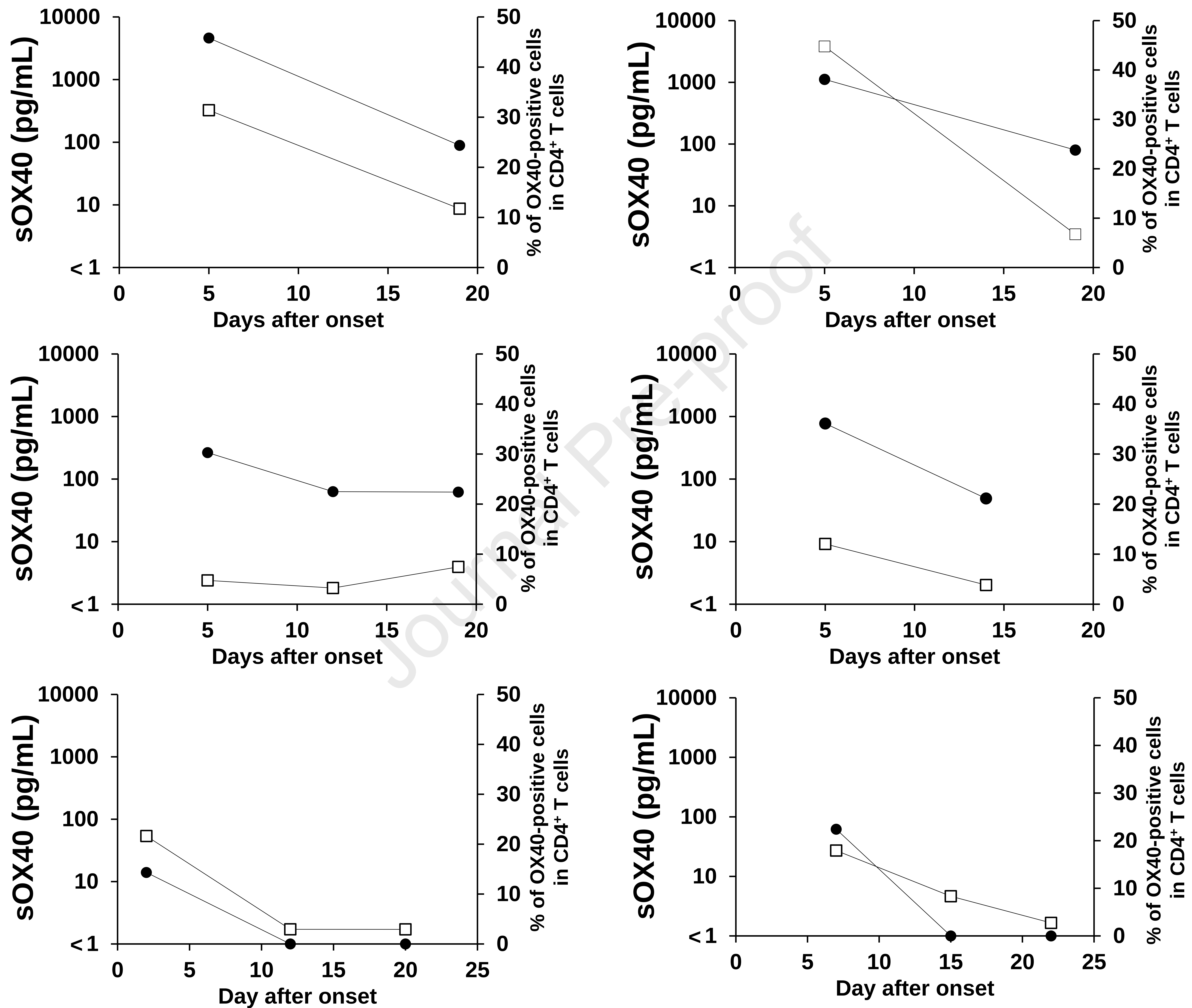


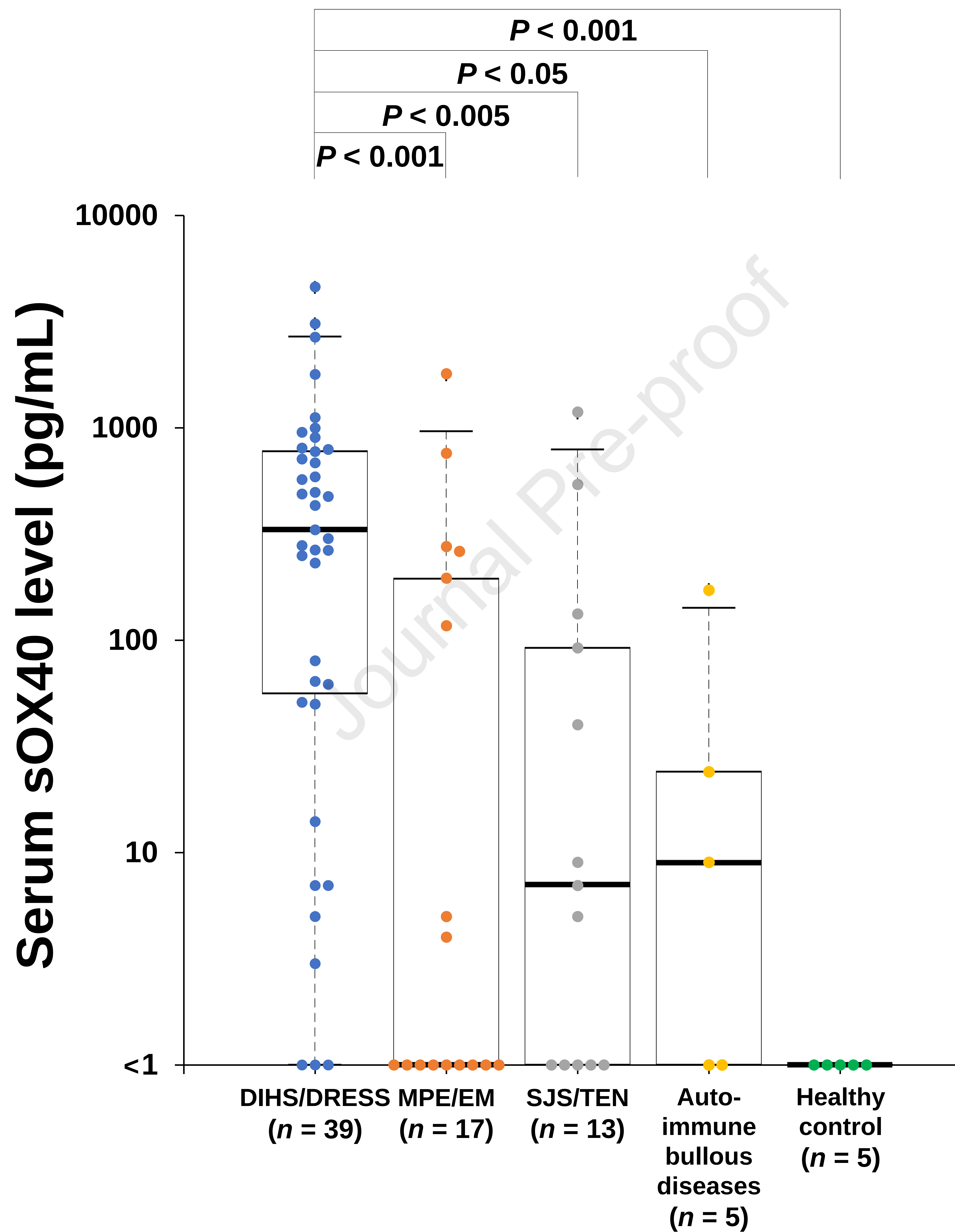
Figure 2

Figure 3

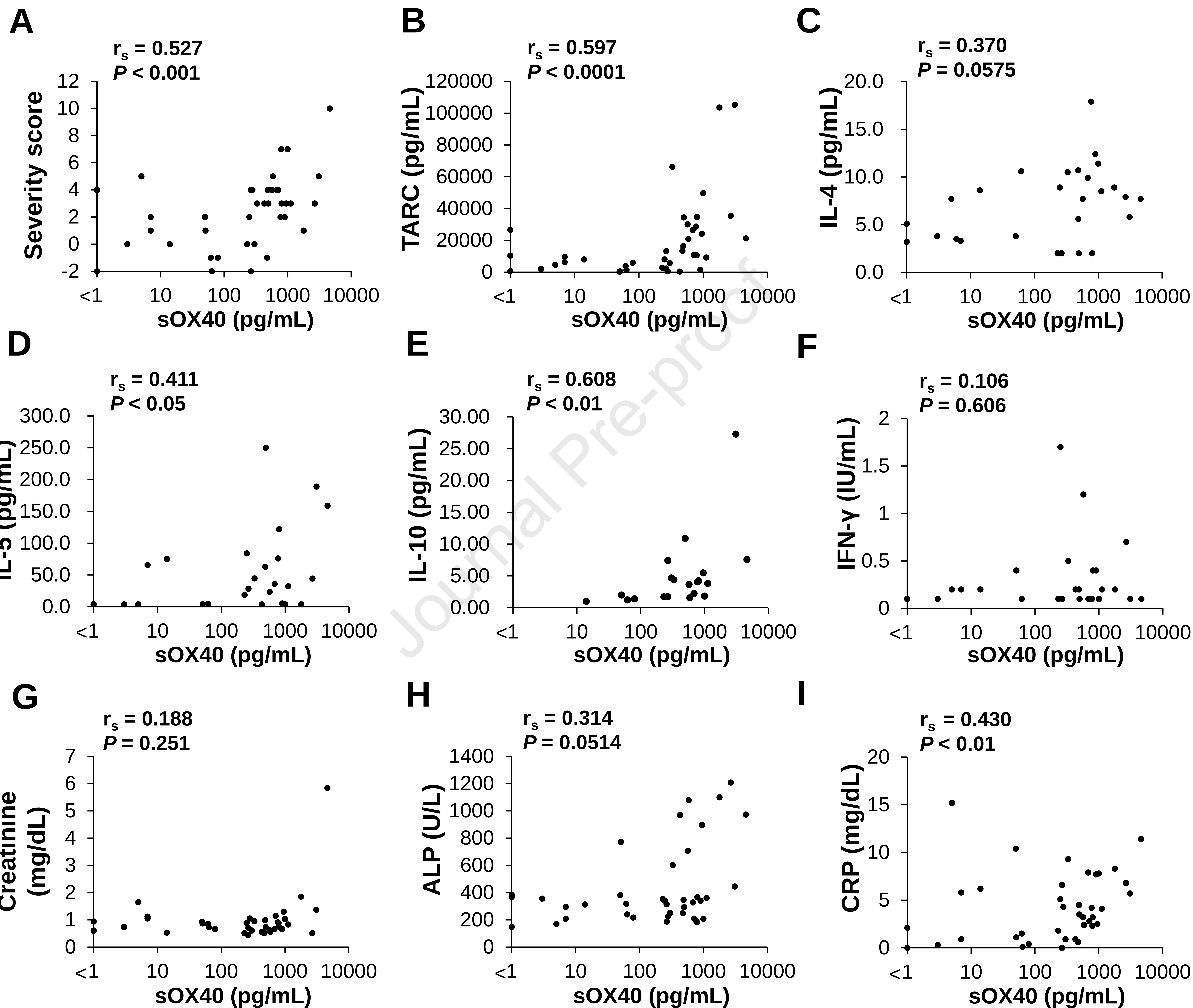


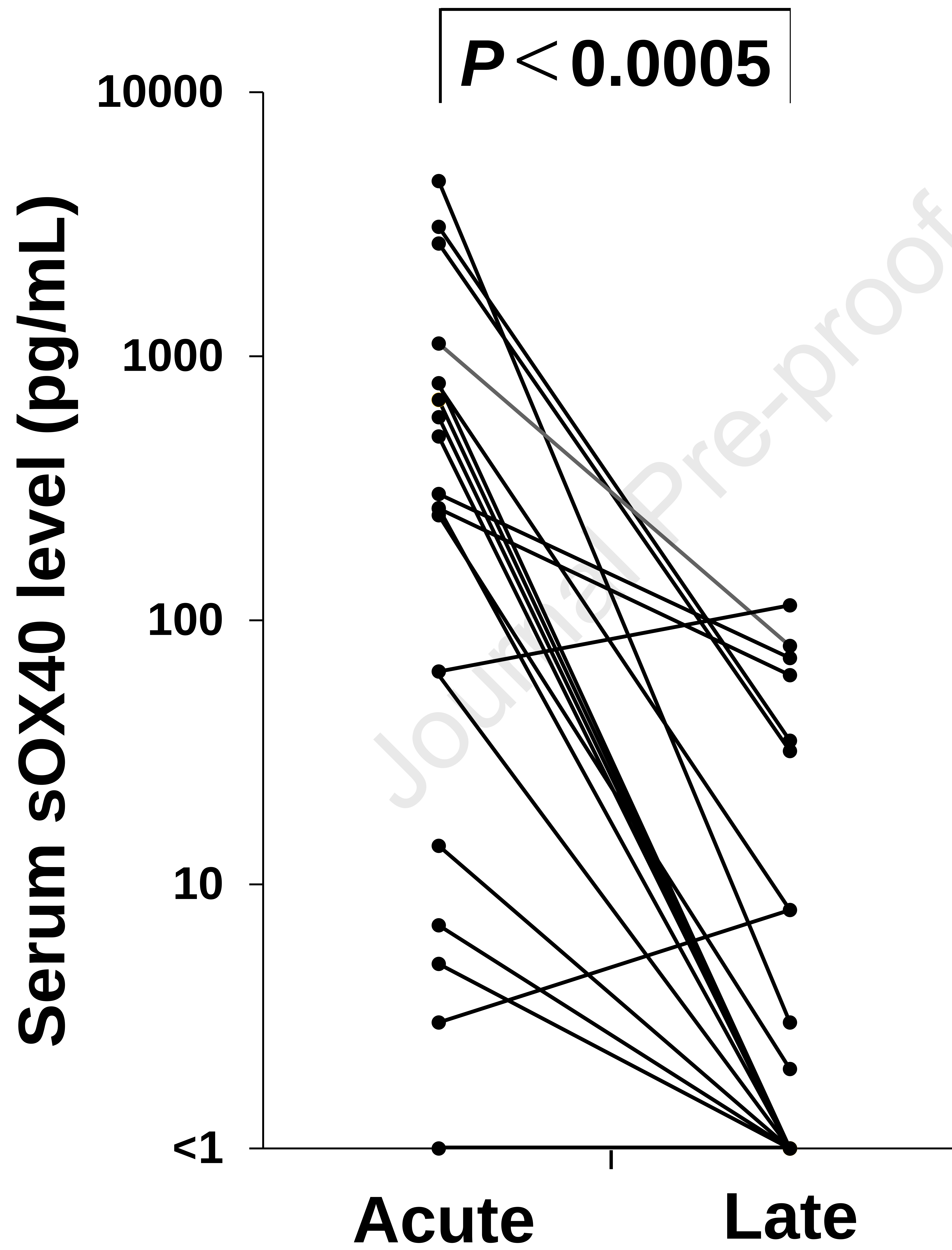
Figure 4

Figure 5

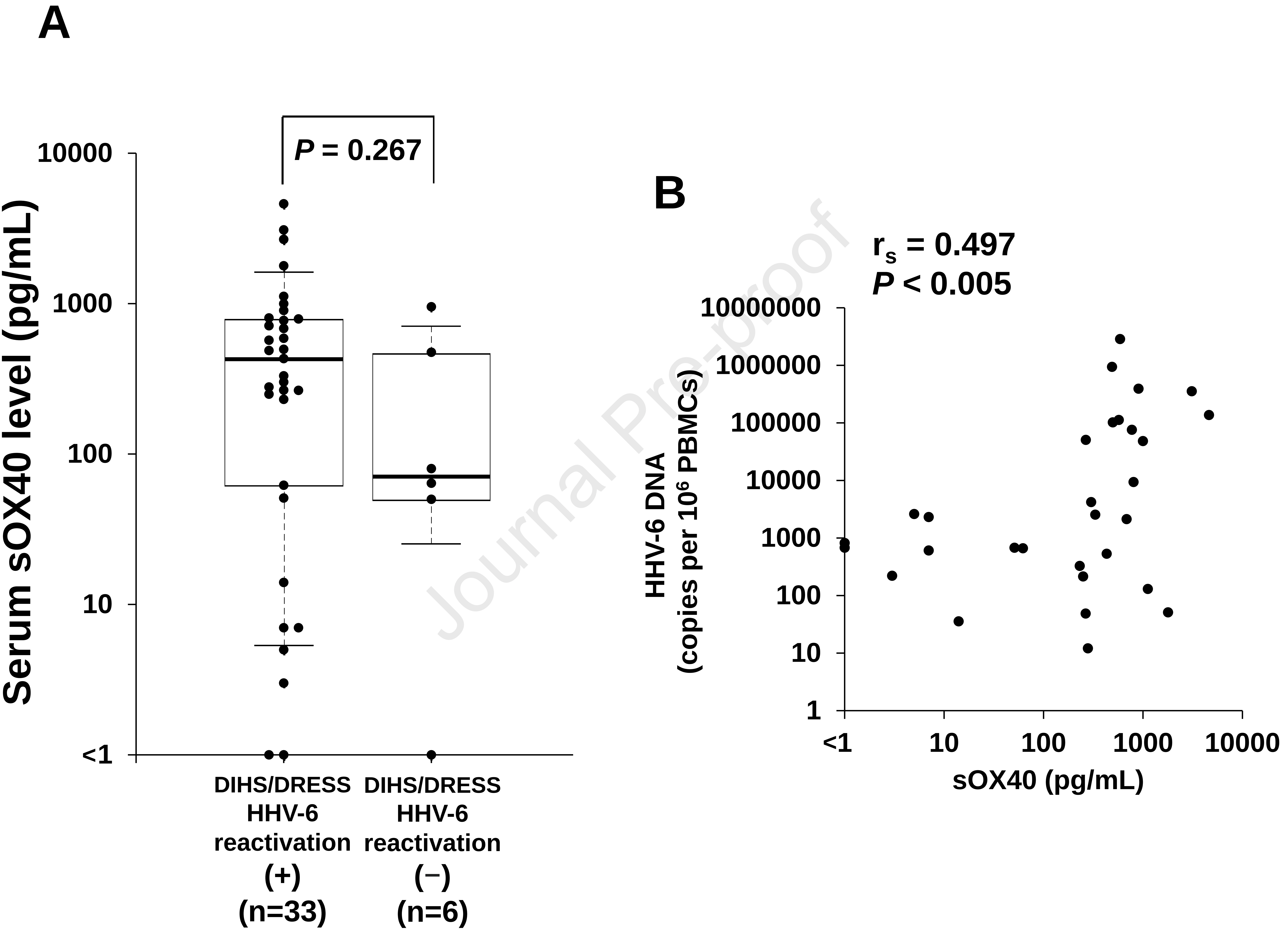


Figure 6

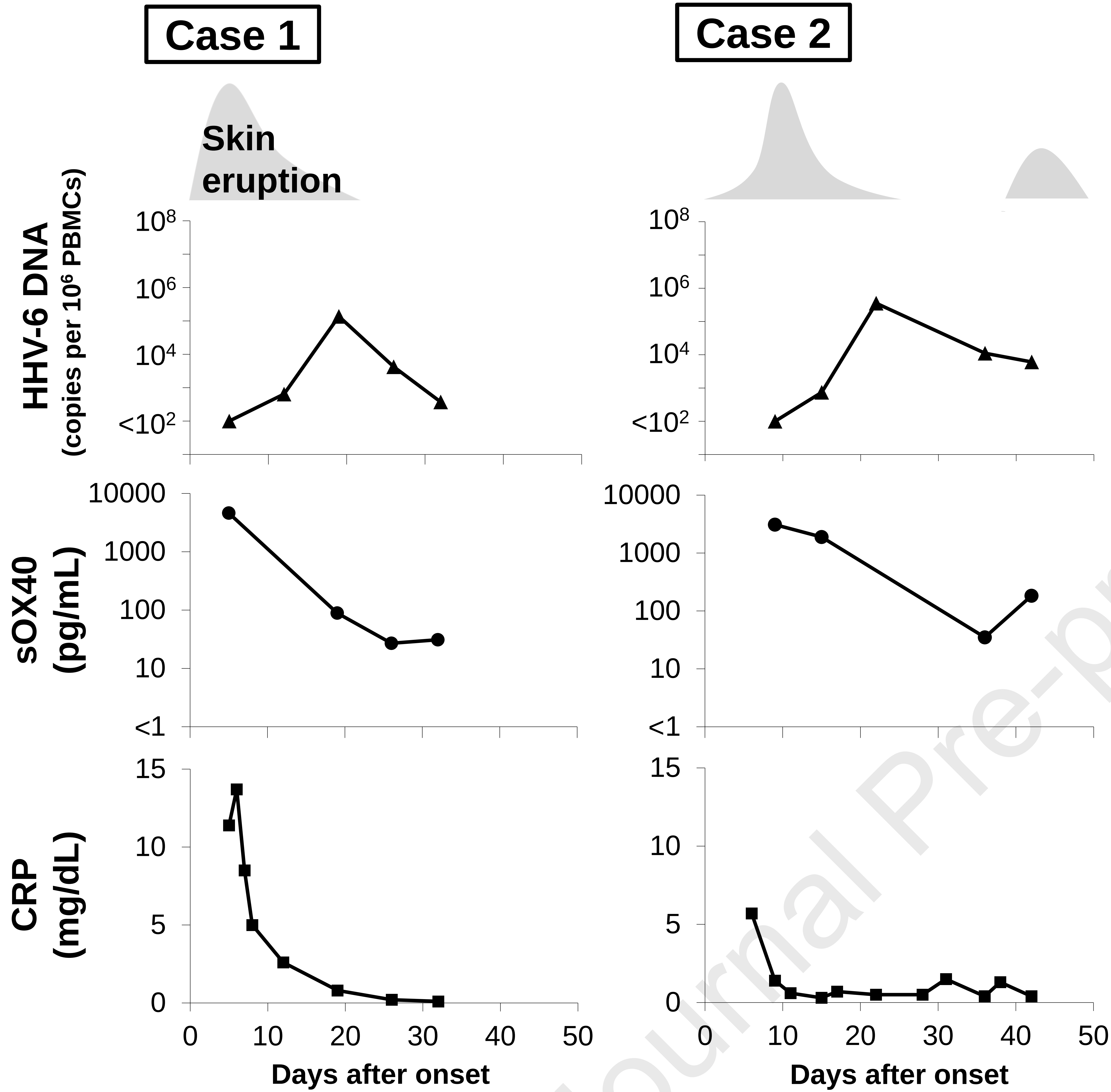


Figure E1

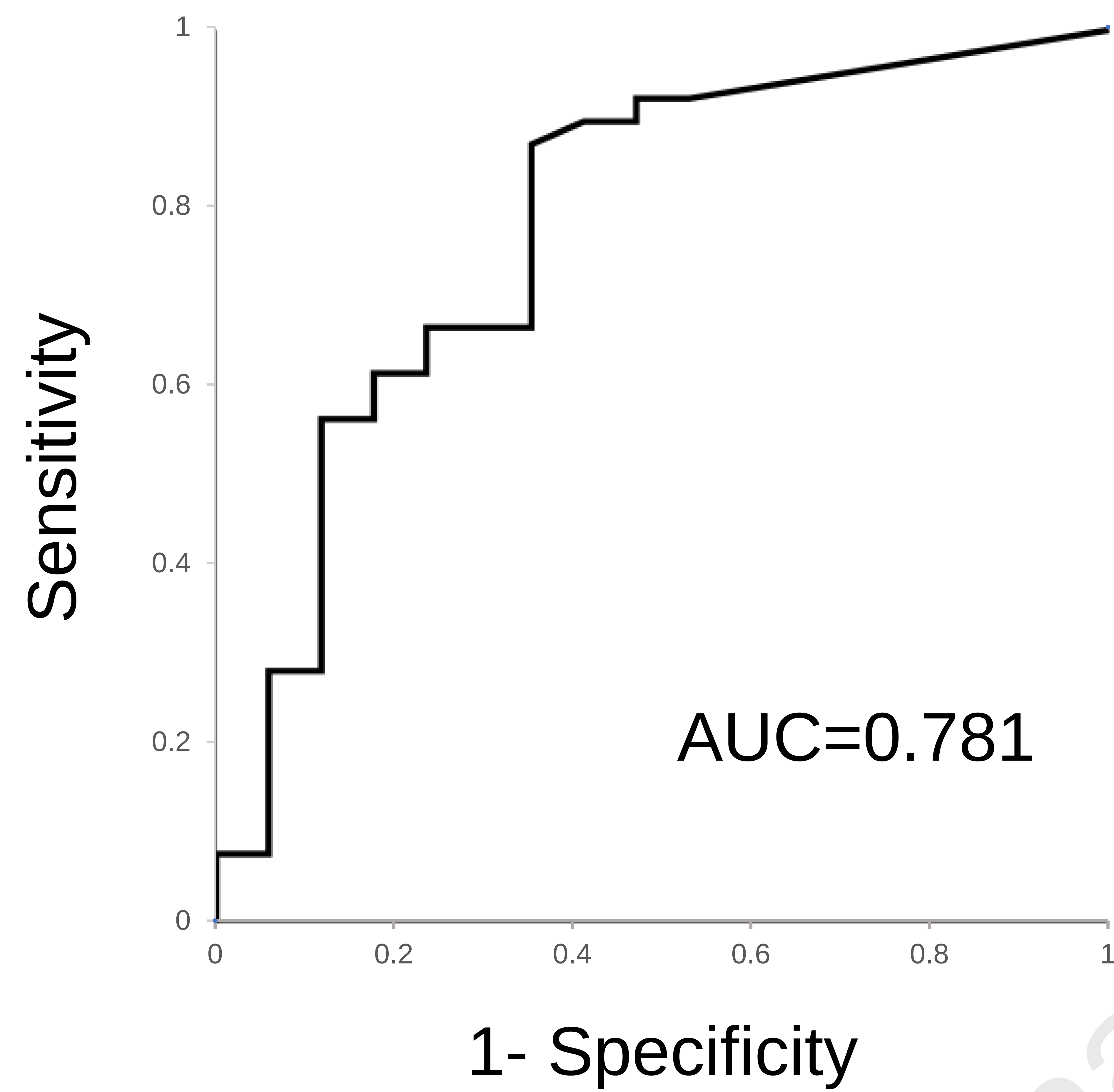
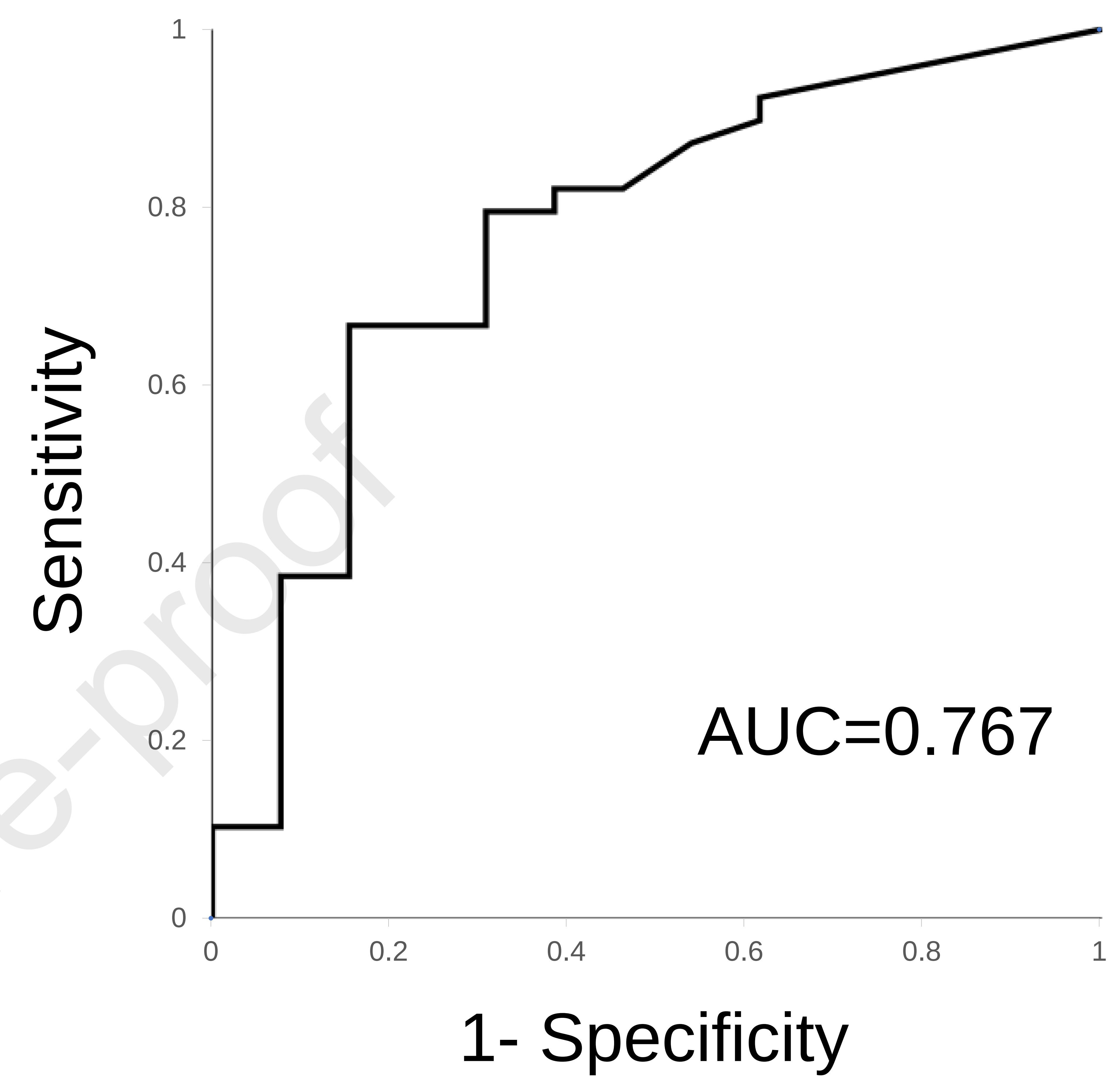
A**B**

Figure E2

