



Supplementary Materials

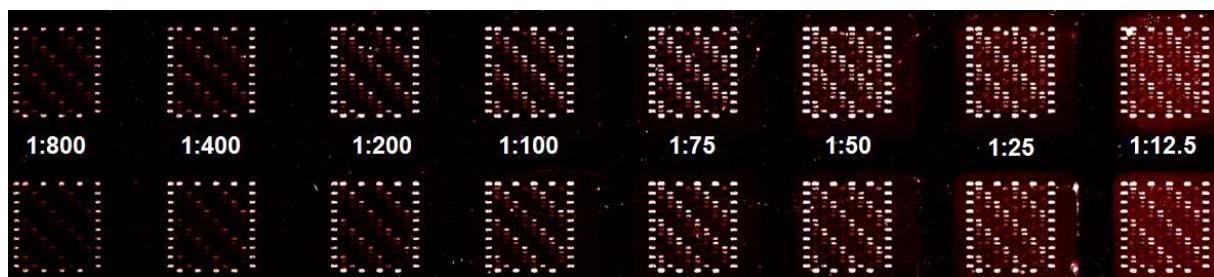
# Serological Number for Characterization of Circulating Antibodies

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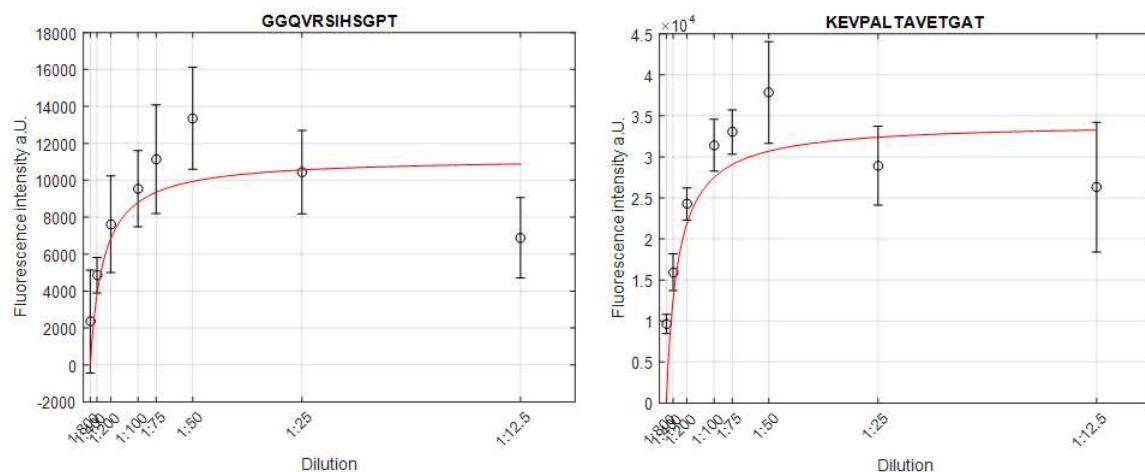
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## S1. Immunostaining of Peptide Arrays at Different Serum Dilutions

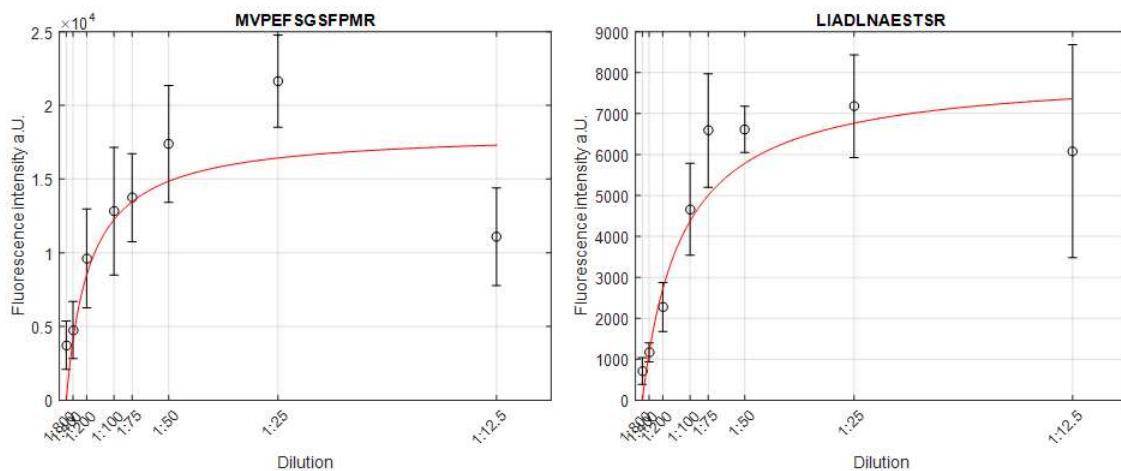
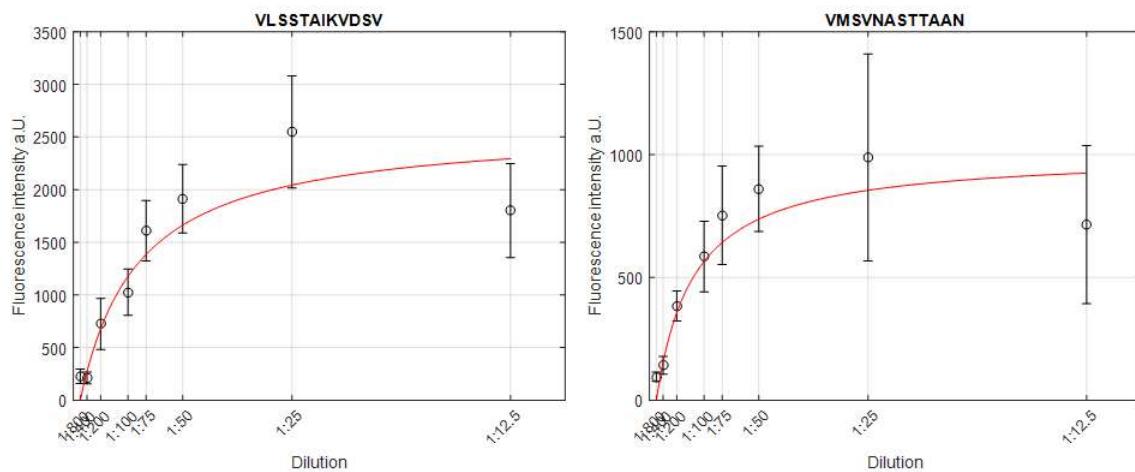
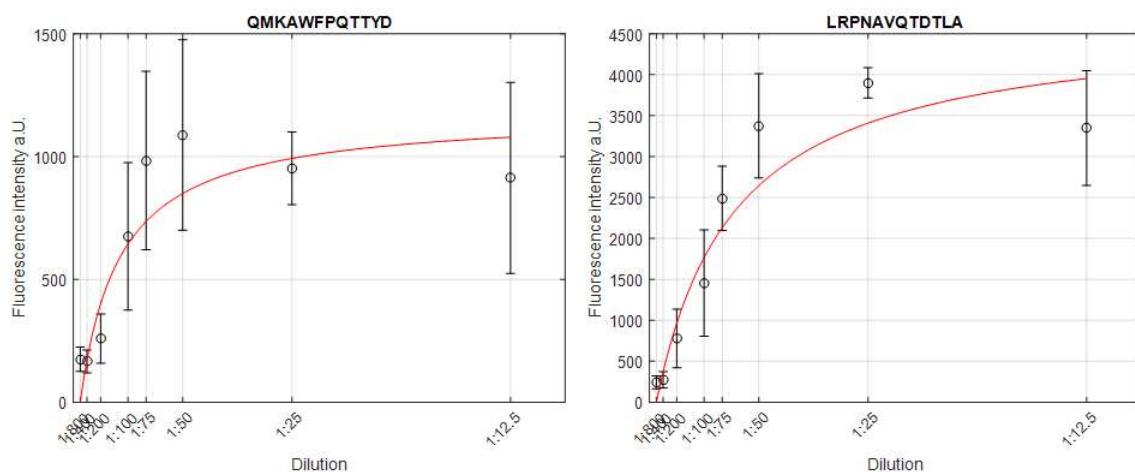


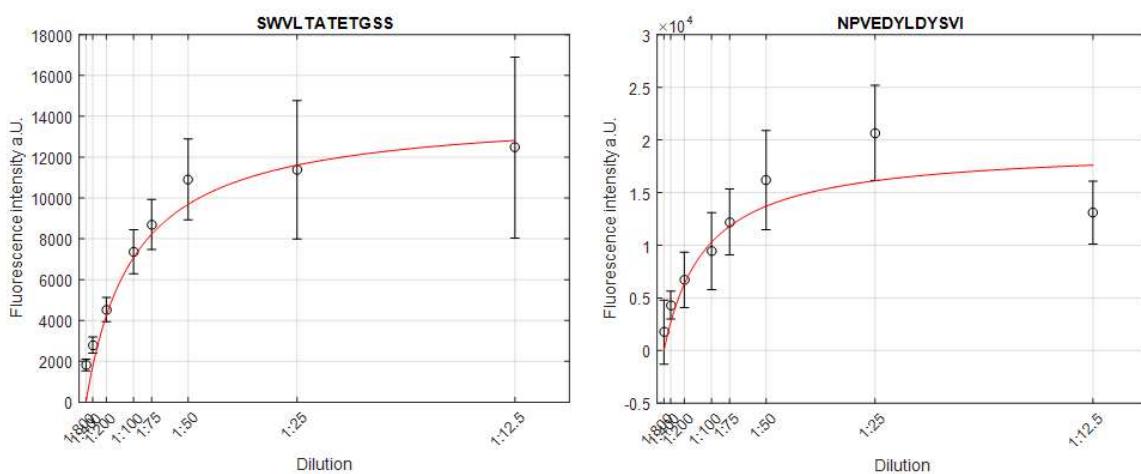
**Figure 1.** Fluorescent image of the peptide arrays at different dilutions. The white spots in the frames represent reference signals.

## S2. Fluorescent Signals of the Peptides from Table 1 versus the Dilution (Circles) and Their Fitting with a Saturation Curve (Red Line) According to Equation (7)

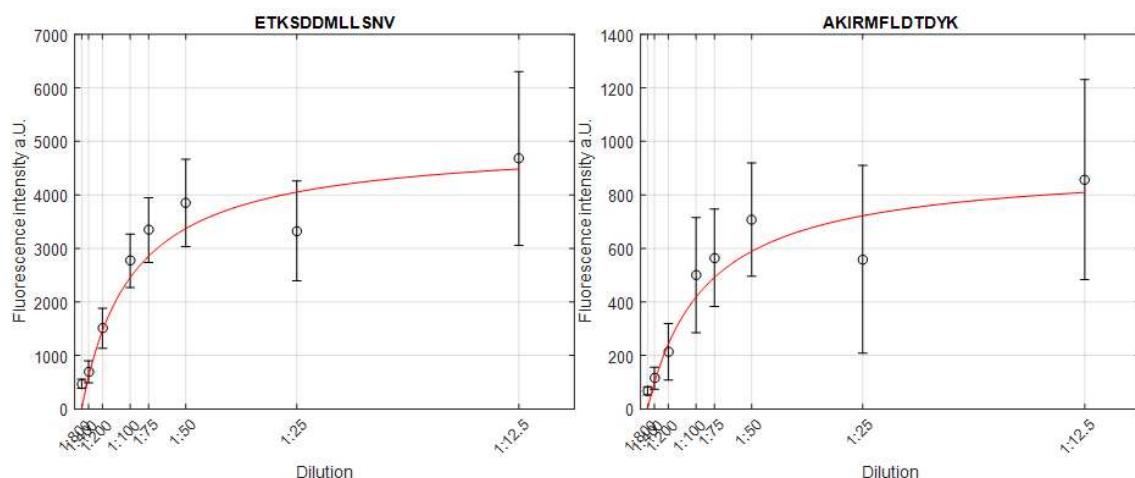


**Figure 2.** Saturation curves of peptides GGQVRSIHSQPT and KEVPALTAVETGAT.

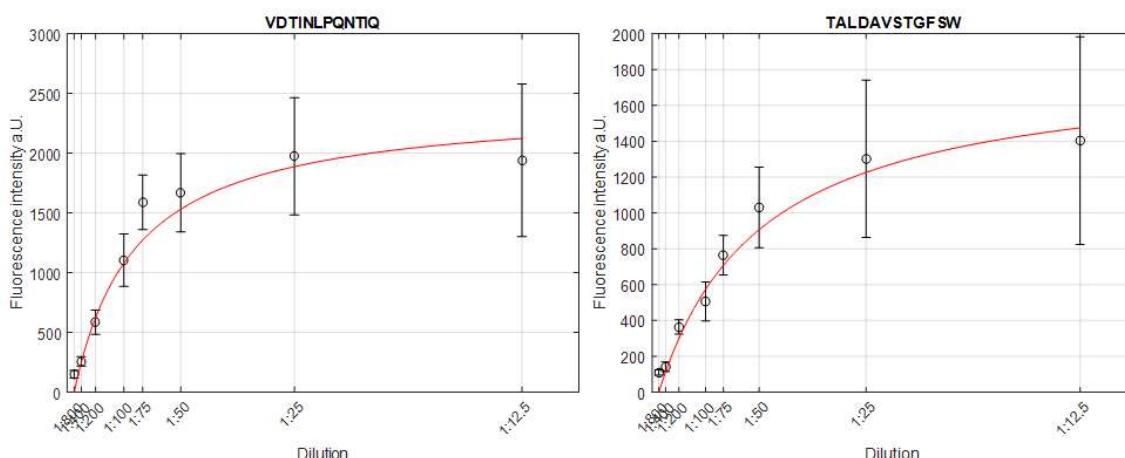
**Figure 3.** Saturation curves of peptides MVPEFSGSFFPMR and LIADLNAESTSR.**Figure 4.** Saturation curves of peptides VLSSTAIKVDSV and VMSVNASTTAAN.**Figure 5.** Saturation curves of peptides QMKAWFPQTTYD and LRPNAVQTDTLA.



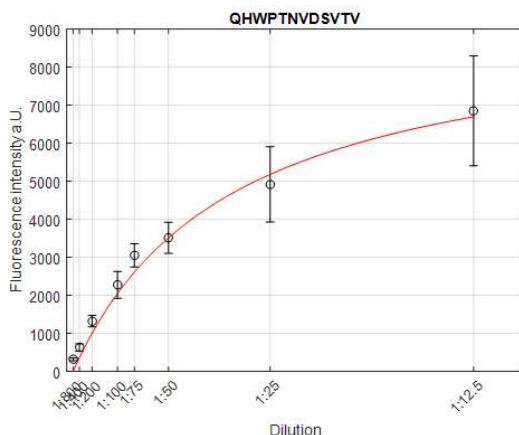
**Figure 6.** Saturation curves of peptides SWVLTATETGSS and NPVEDYLDYSVI.



**Figure 7.** Saturation curves of peptides ETKSDDMILLSNV and AKIRMFLDTDYK.



**Figure 8.** Saturation curves of peptides VDTINLPQNTIQ and TALDAVSTGFSW.



**Figure 9.** Saturation curves of peptide QHWPTNVDSVTV.

### S3. MATLAB Code to Approximate Fluorescent Signals for Peptides (Circles in S2 with a Saturation Curve (Red Line in S2) and to Calculate the S-number

MATLAB Function [Result] uses the table with a fluorescent signals at different dilutions from different slides “Tabelle, Slidenr” to generate the saturation curves and corresponding *S*-numbers.

```
% 1) Import data as table
% 2) Specify variables for dilution used (x_labels), position of x_labels (x_ticks) and slide number
(Slidenr)
function [Result]=SerumPlot(x_labels, x_ticks, Table, Slidenr)
% Aqcuire the dimension of the dataset „Table“
List_Dilution=sort(unique(Table.Dilution),'descend');
Count_Dilution=size(List_Dilution,1);
List_Peptide=sort(unique(Table.Peptide));
Count_Peptide=size(List_Peptide, 1);
% Generate model for fitting according to Fobs=(Fmax*C)/(S+C)
g = fittype( @(c, S, x) c*x./(S+x) );
opts = fitoptions( g );
opts.Display = 'Off';
% Generate matrix for data storage
MedianInt=nan(Count_Dilution, Count_Peptide);
Result(1,:){='ID','Peptide', 'Intensity', 'Fmax', 'S', 'rs'};
% Data fitting and plotting
for iPep=1:Count_Peptide
figure(iPep);
% Define datasets for plotting (x, y),
% calculate standard deviation for each peptide and each dilution per slide
for iDil=1:Count_Dilution
MedianInt(iDil,iPep)= median(Table.RedForegroundMedian(Table.Dilution==List_Dilution(iDil) &
strcmp(Table.Peptide, List_Peptide(iPep)),:));
MedianStdev(iDil,iPep)= std(Table.RedForegroundMedian(Table.Dilution==List_Dilution(iDil) &
strcmp(Table.Peptide, List_Peptide(iPep)),:));
end
% Plotting
YData=MedianInt(:,iPep);
XData=x_ticks;
ZErr=MedianStdev(:,iPep);
errorbar(XData, YData, ZErr, 'ok');
```

```
hold on;
Fmax=max(YData);
Fmin=min(YData);
Xmax=max(XData);
%Define boundaries for fitting. Adjust if necessary
opts.Lower = [0 0];%[Fmin 1];
opts.Upper = [Inf Inf];%[Fmax 1];
opts.StartPoint= [1 1];
lb= [-Inf, -Inf, 0, 0];
ub= [Inf, Inf, 2*Fmax, 2*Xmax];
% Fitting
[fitresult, gof] = fit( XData, YData, g, opts );
plot(linspace(min(XData),max(XData),1000),feval(fitresult,linspace(0,Xmax,1000)),'r')
hold off;
cf=coeffvalues(fitresult);
H=List_Peptide(iPep);
grid on;
% Annotations to the plot
xlabel('Dilution');
set(gca, 'XTick', XData, 'XTickLabelRotation', 45, 'XTickLabel', x_labels)
ylabel('Fluorescence intensity a.U.');
Title_Seq=([H{1}]);
title([ Title_Seq ]);
hold off;
% Save figure
filenamebp=(['Fit_', H{1},Slidenr,'.fig']);
savefig(filenamebp);
hold off;
% Save fit coefficients to 'Result'
Result(iPep,:){iPep, H{1}, 'Intensity', cf(1), cf(2), gof.rsquare};
end
end
```



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