Supplemental information

Fig. S1 Correlation between peptidolytic activities of different proteasome preparations from different organisms. Each dot represents results for one of the 5920 peptides tested. (**a**) Mtb20SOG vs Mtb20SWT WT proteasomes; (**b**) Mtb20SOG vs Rhod20S; (**c**) Mtb20SOG vs bov20S; (**d**) Rhod20S vs bov20S. RFU, relative fluorescence units. The plots were generated with Prism (GraphPad Software, Inc. San Diego, CA).

Fig. S2 Most and least active substrates for bov20S proteasomes attributable to **(a)** β 5, **(b)** β 1, and **(c)** β 2 subunits. For β 5, the P1 amino acids appeared to be a most important determinant than the P3 amino acids, and the P2 amino acids the least important, as the hydrophobic L is the most favored with P3 aromatic amino acids Y/W/F, whereas the hydrophilic P1 N is the least favored. For β 1, P2 amino acids appeared to be a more important determinant than P3, as the P2 aromatic F/Y/W are the most favored, whereas the P2 basic R and small hydrophobic amino acids are the least favored. For β 2, the P3 amino acids appeared to be a more important determinant than the P2 amino acids, as the basic R/K are most favored, whereas the acidic E/D are the least favored. X-axis, Acetyl-P3P2P1-AMC; Y-axis, RFU.

Fig. S3 Correlation between k_{cat}/K_M values of Mtb20SOG and Mtb20SWT for individual substrates. The R^2 0.82 matches the R^2 0.78 of the correlation of substrate high throughput screening results between Mtb20SOG and Mtb20SWT.

Figure S1

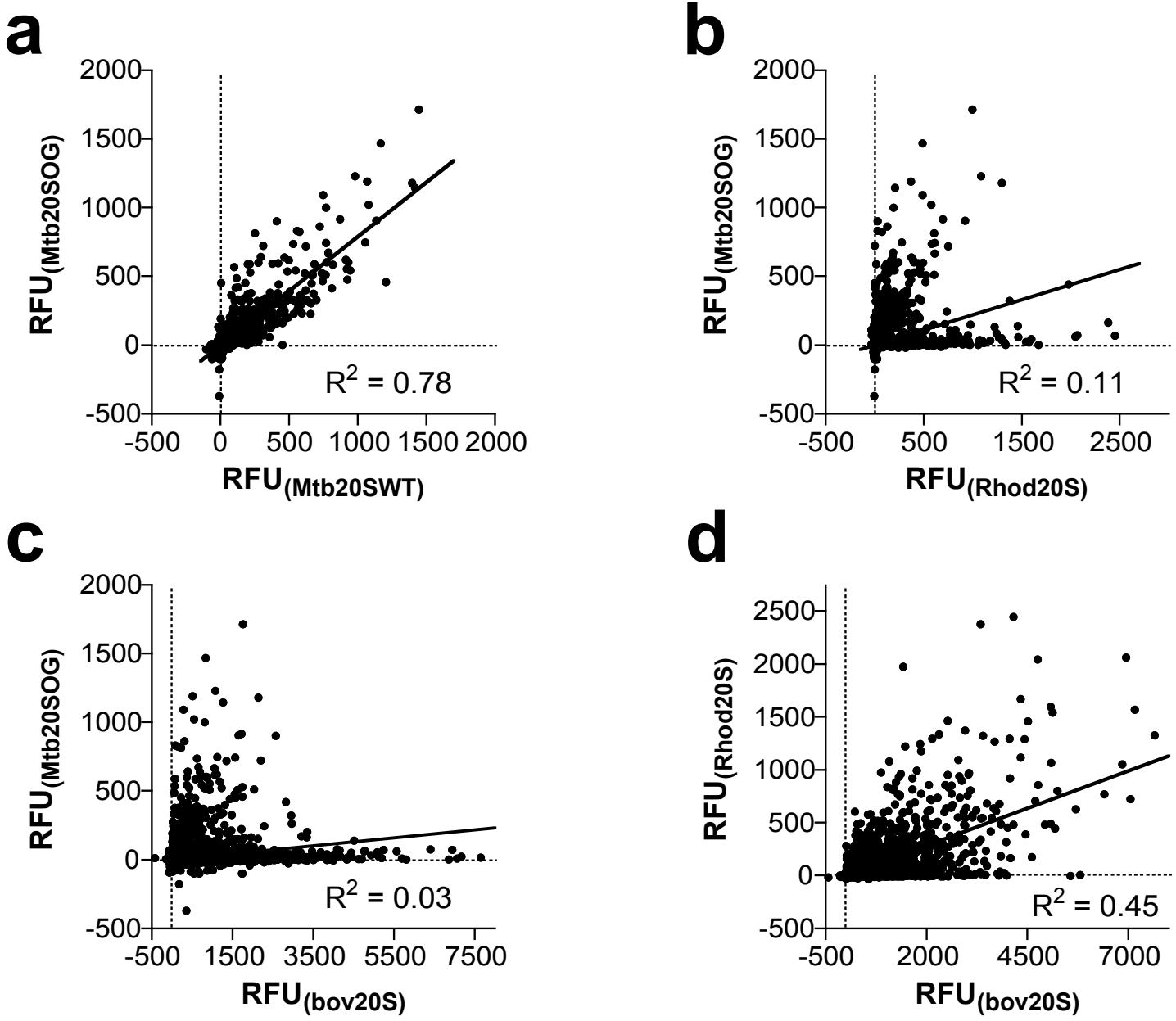

a
2000
1500
1000
500
0
-500
-500 0 500 1000 1500 2000
RFU(Mtb20SOG)
RFU(Mtb20SWT)
R2 = 0.78
b
2000
1500
1000
500
0
-500
-500 500 1500 2500
RFU(Mtb20SOG)
RFU(Rhod20S)
R2 = 0.11
c
2000
1500
1000
500
0
-500
-500 1500 3500 5500 7500
RFU(Mtb20SOG)
RFU(bov20S)
R2 = 0.03
d
2500
2000
1500
1000
500
0
-500
-500 2000 4500 7000
RFU(Rhod20S)
RFU(bov20S)
R2 = 0.45

Fig. S2

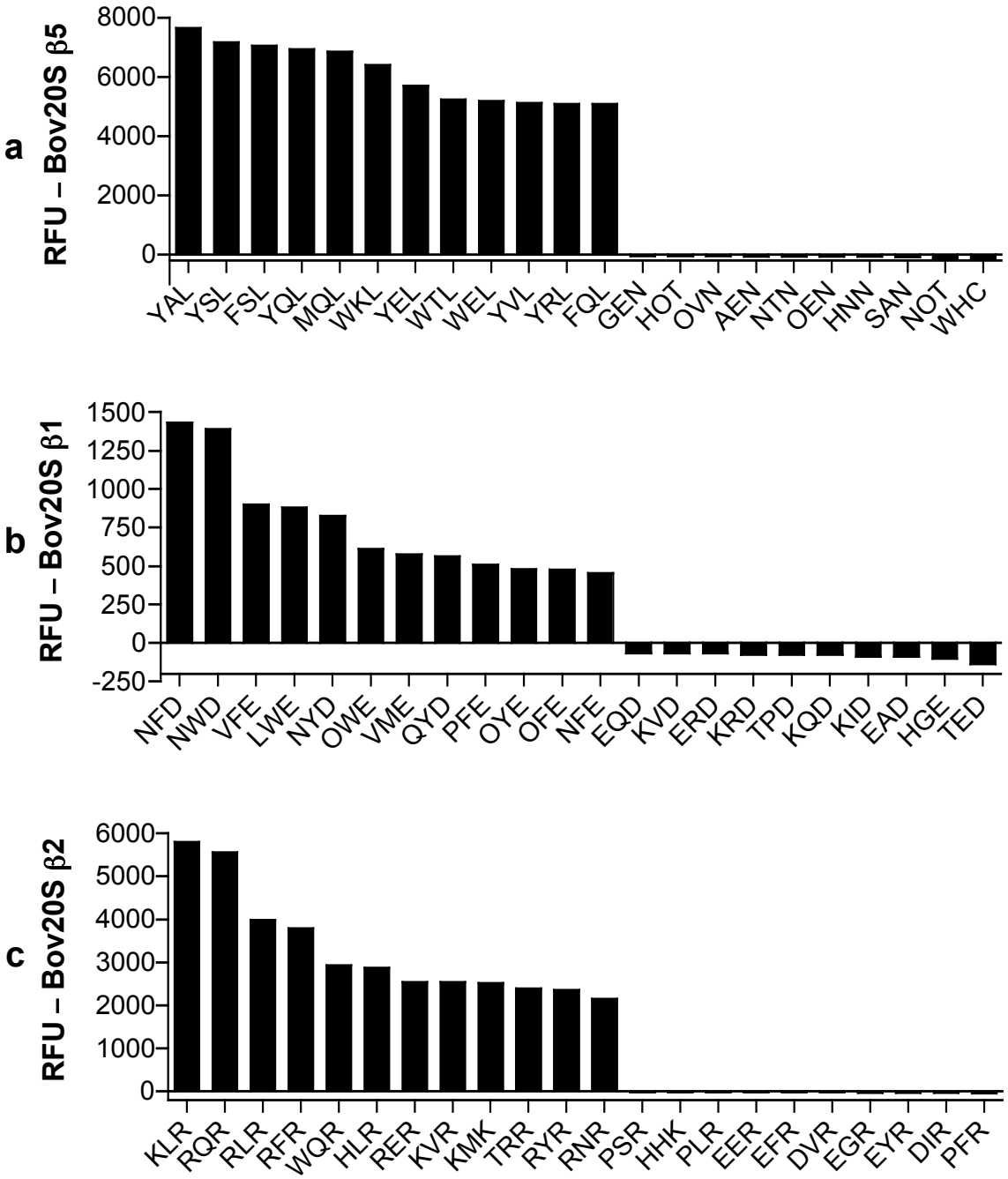
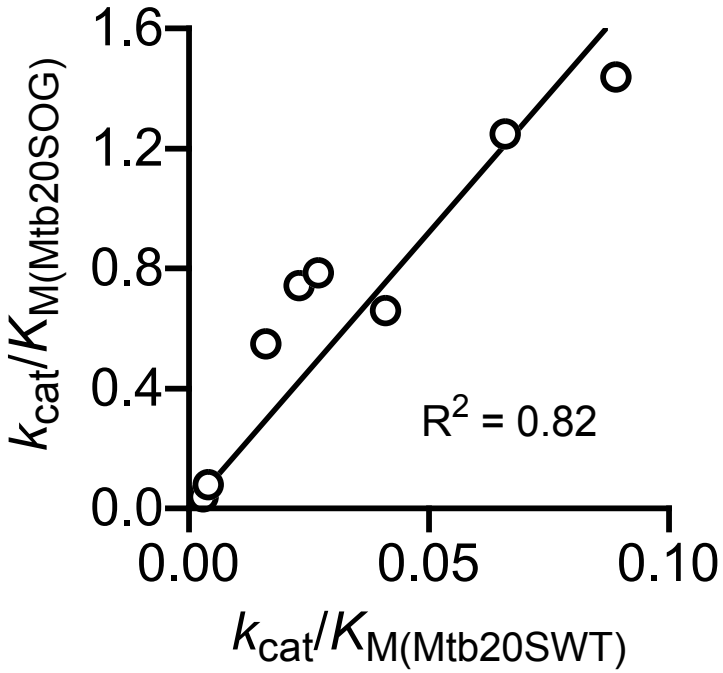

a
RFU – Bov20S β5
8000
6000
4000
2000
0
YAL YSL FSL YQL MQL WKL YEL WTL WEL YVL YRL FQL GEN HOT OVN AEN NTN OEN HNN SAN NOT WHC
b
RFU – Bov20S β1
1500
1250
1000
750
500
250
0
-250
NFD NWD VFE LWE NYD OWE VME QYD PFE OYE OFE NFE EQD KVD ERD KRD TPD KQD KID EAD HGE TED
c
RFU – Bov20S β2
6000
5000
4000
3000
2000
1000
0
KLR RQR RLR RFR WQR HLR RER KVR KMK TRR RYR RNR PSR HHK PLR EER EFR DVR EGR EYR DIR PFR

Fig. S3


1.6
1.2
0.8
0.4
0.0
0.00
0.05
0.10
$k_{cat}/K_{M(Mtb20SOG)}$
$k_{cat}/K_{M(Mtb20SWT)}$
$R^2 = 0.82$