

Supplemental information for “Defining short linear motif binding determinants by phage-based multiplexed deep mutational scanning” by Benz et al.

Figure S1. ROC analysis to benchmark the DMS by phage analysis PSSMs created from the by input library normalized DMS-BM selection data, compared to PSSMs generated from peptide instances curated in the ELM database.

Figure S2. Heat map and PSSM representation of DMS analysis results generated through selections against the DMS-BM library.

Figure S3. Binding titration curves as detected by FP of FITC-labeled probe peptides.

Figure S4. Affinity determinations through fluorescence polarization-based competition experiments.

Figure S5. AlphaFold3 model of the TLN1 PTB-TPTE2 complex. Larger version of Fig. 1K.

Figure S6. Heat map and PSSM representation of DMS analysis results generated through selections against the DMS-BM library.

Figure S7. AlphaFold3 model of the complex of NSP9 and the NOTCH4 A1603V/P1613V peptide (QGVWLGAVEPWEPL) overlayed with the model of the AXIN (IQEQGFGFPLDLGAS).

Figure S8. Correlation between NGS counts and affinities.

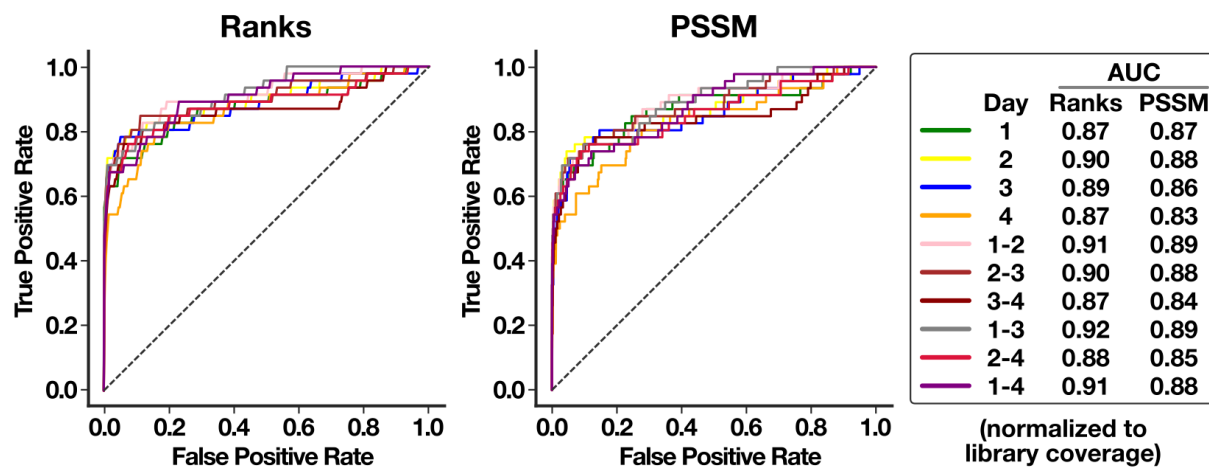


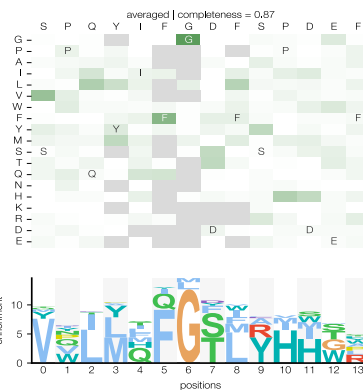
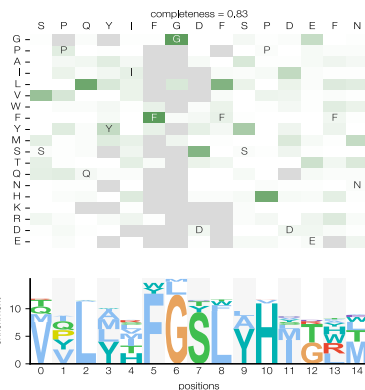
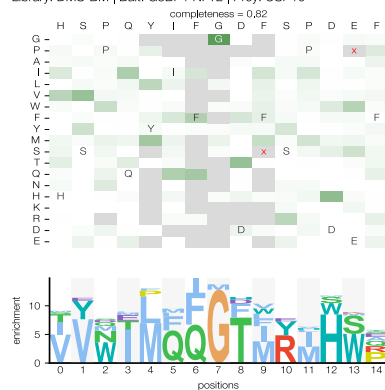
Figure S1. ROC analysis to benchmark the DMS by phage analysis PSSMs created from the by input library normalized DMS-BM selection data, compared to PSSMs generated from peptide instances curated in the ELM database.

Figure S2.

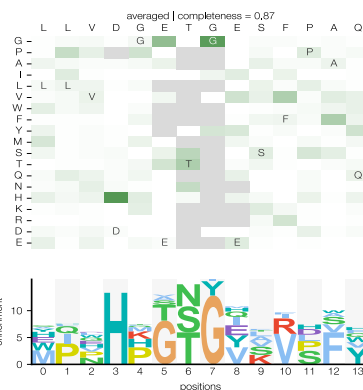
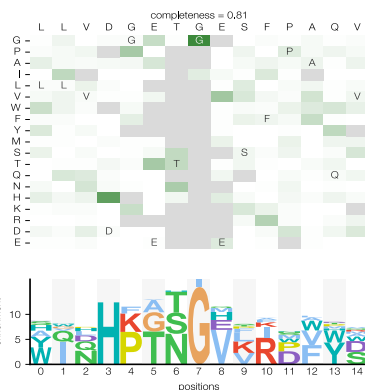
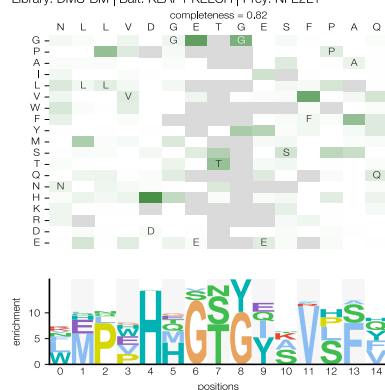


Fig. S2. Continued

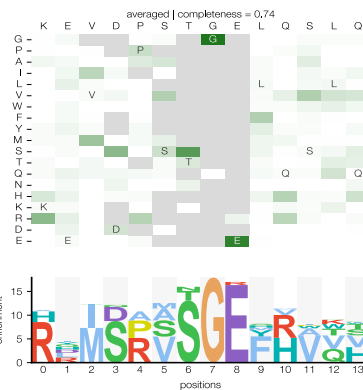
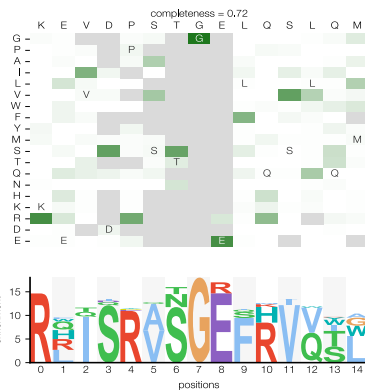
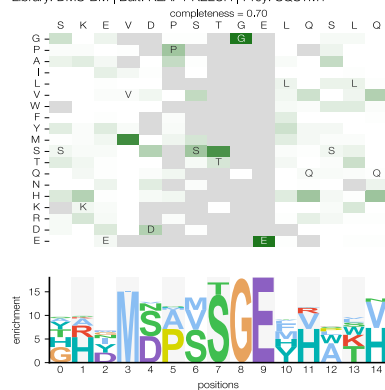
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Library: DMS-BM | Bait: KEAP1 KELCH | Prey: NFE2L1



Library: DMS-BM | Bait: KEAP1 KELCH | Prey: SQSTM1



Library: DMS-BM | Bait: MDM2 SWB | Prey: KIAA1671

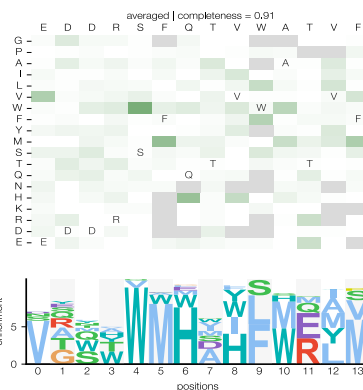
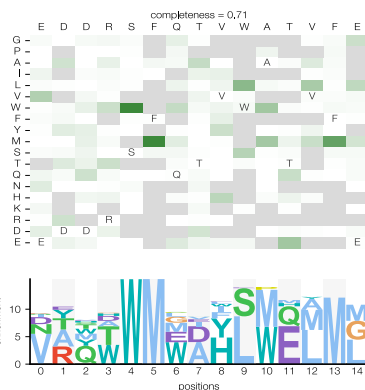
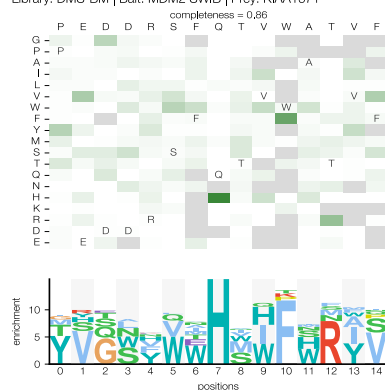
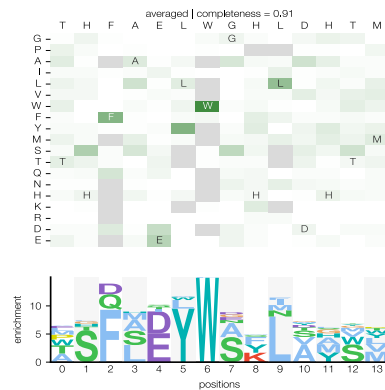
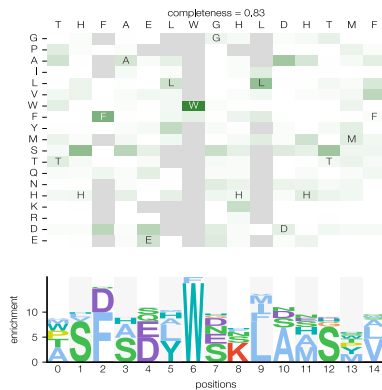
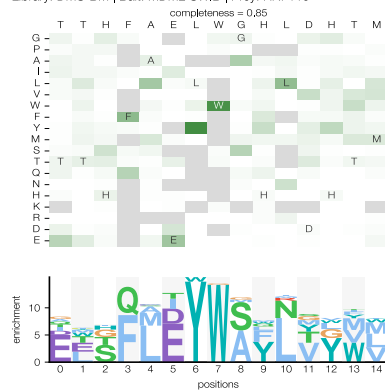
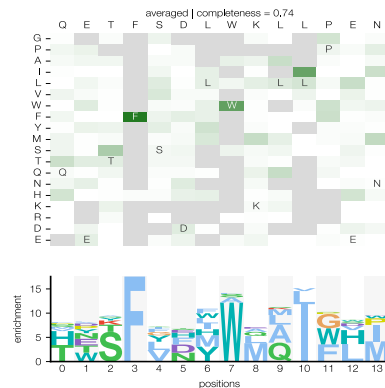
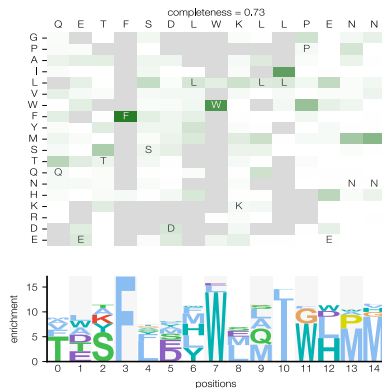
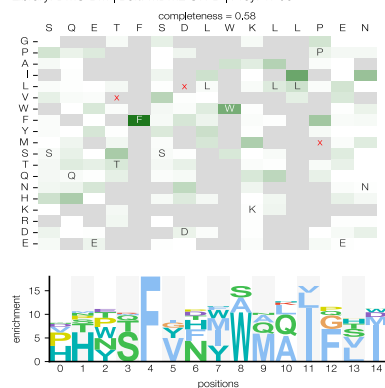


Fig. S2. Continued

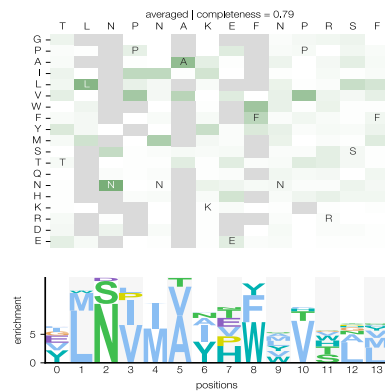
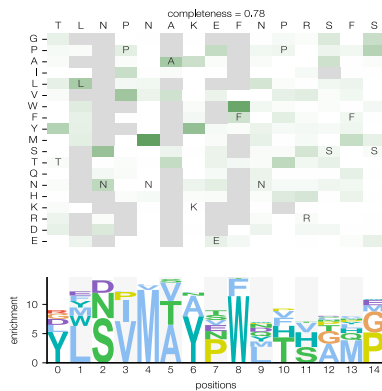
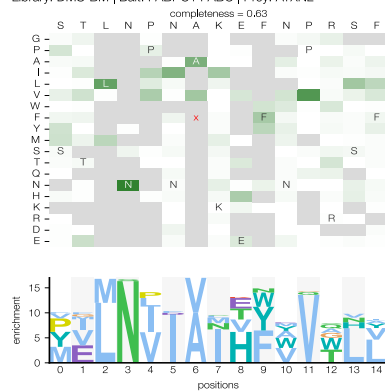
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Library: DMS-BM | Bait: MDM2 SWIB | Prey: TP53



Library: DMS-BM | Bait: PABPC1 PABC | Prey: ATXN2



Library: DMS-BM | Bait: PABPC1 PABC | Prey: PAIP1

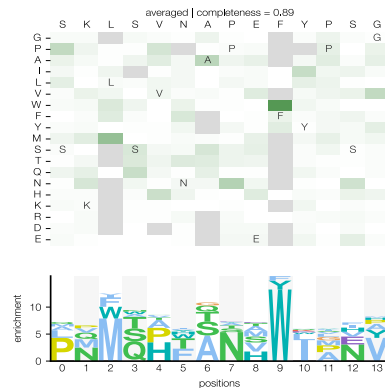
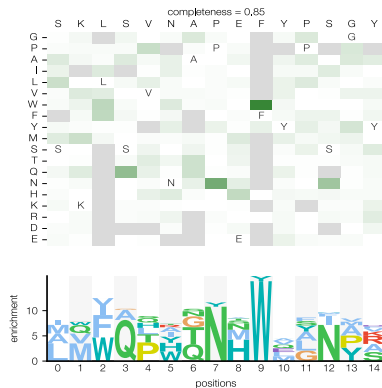
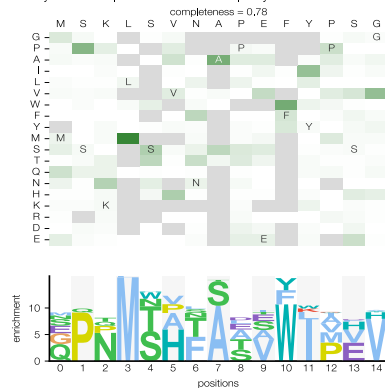
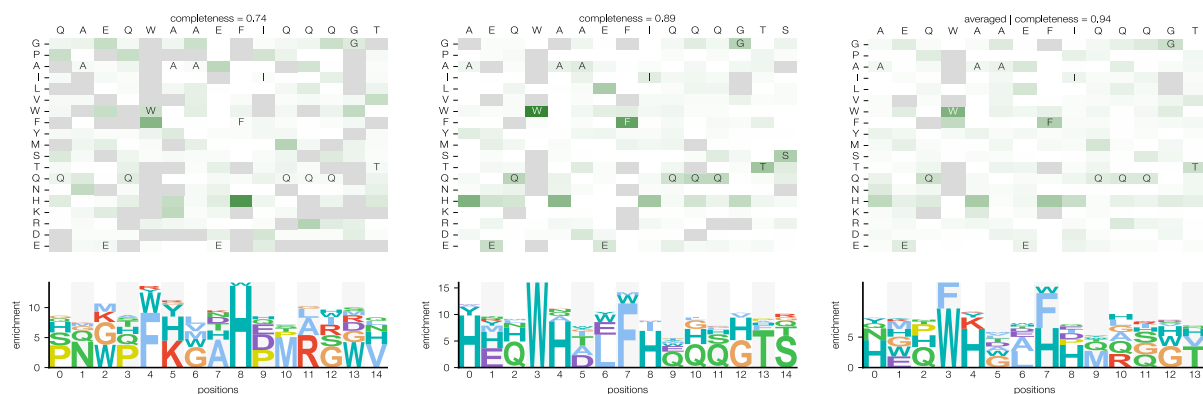
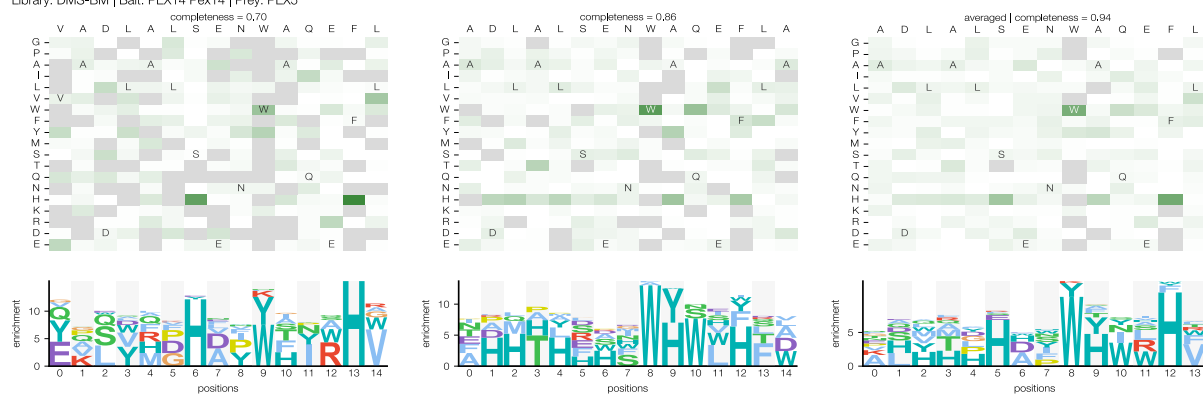
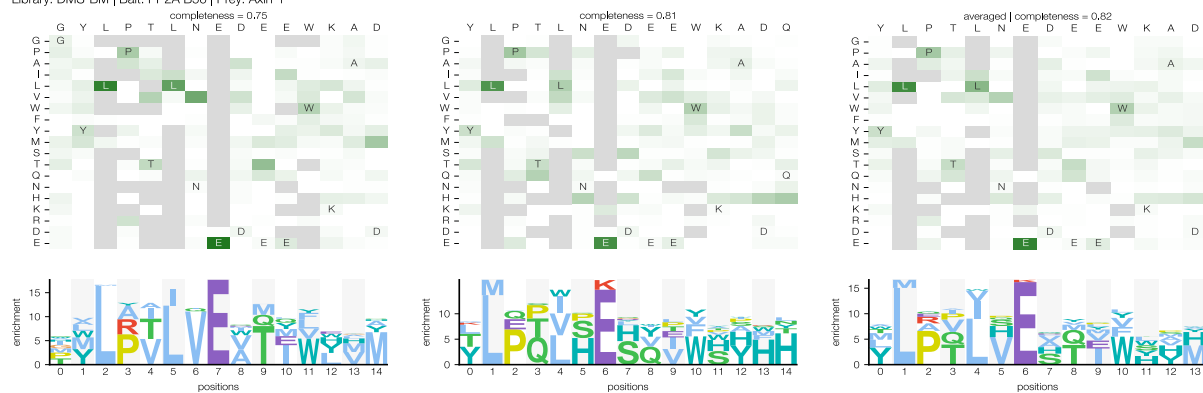


Fig. S2. Continued

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Library: DMS-BM | Bait: PP2A B56 | Prey: Axin-1



Library: DMS-BM | Bait: PP2A B56 | Prey: CDCA2

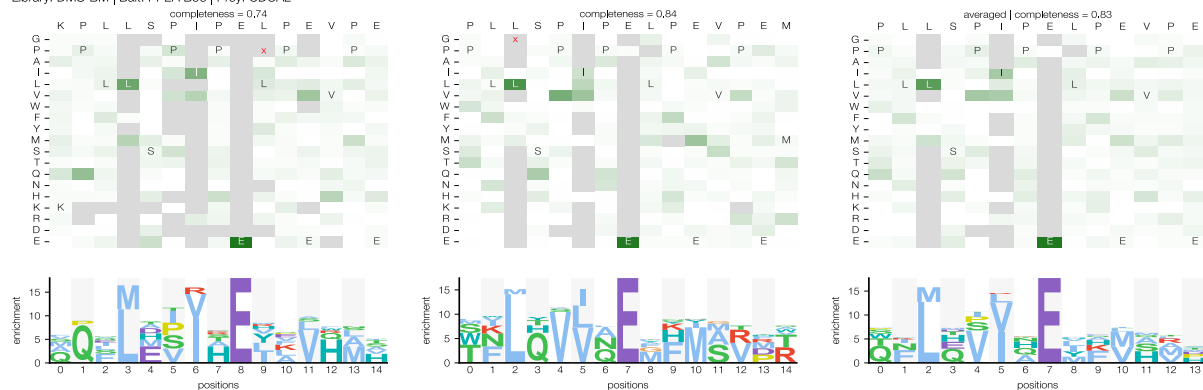
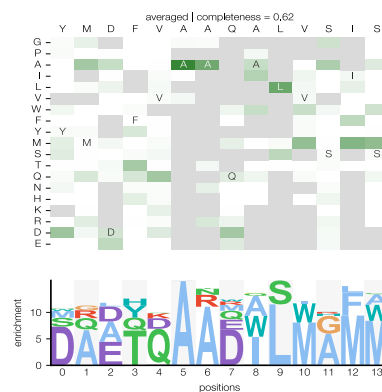
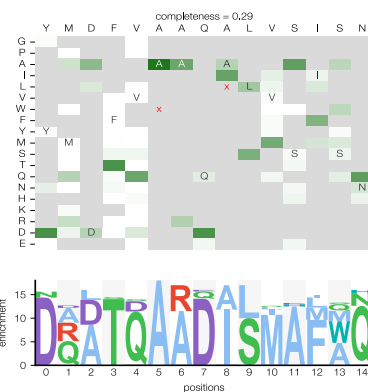
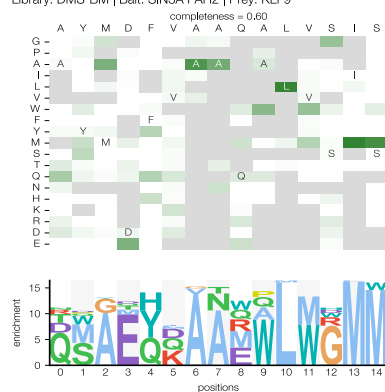
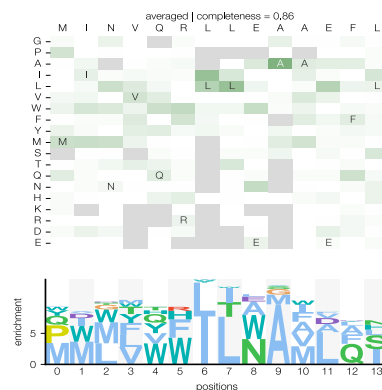
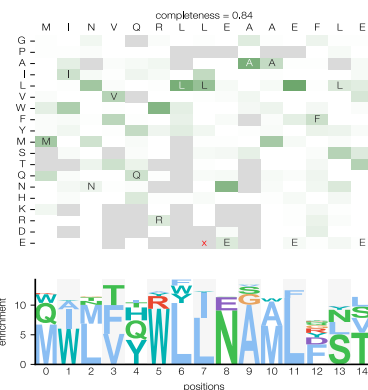
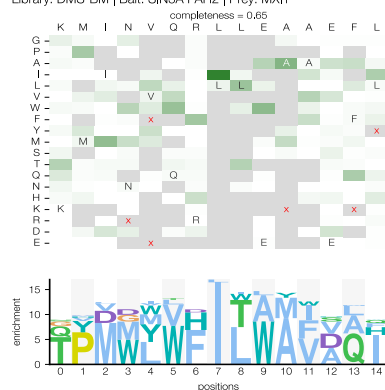


Fig. S2. Continued

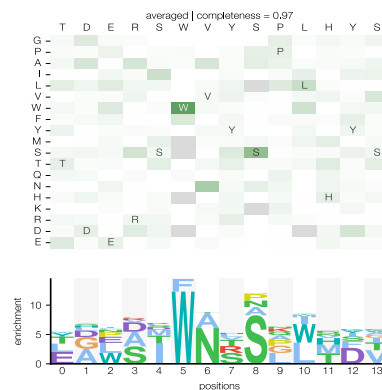
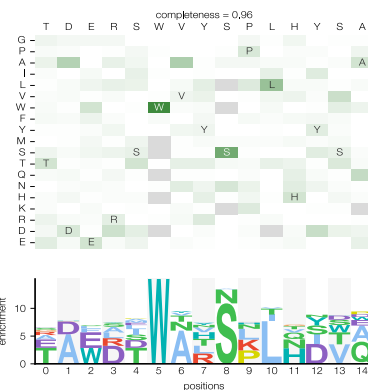
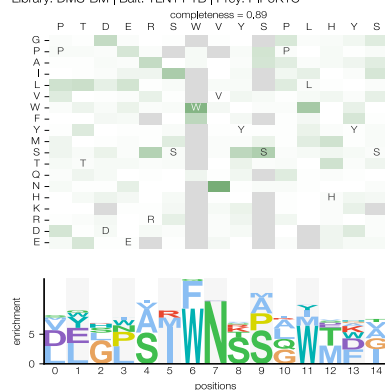
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Library: DMS-BM | Bait: SIN3A PAH2 | Prey: MX1



Library: DMS-BM | Bait: TLN1 PTB | Prey: PIP5K1C



Library: DMS-BM | Bait: TLN1 PTB | Prey: TPST2

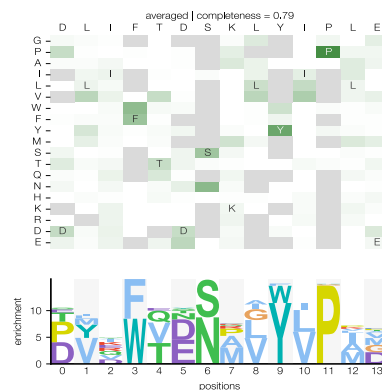
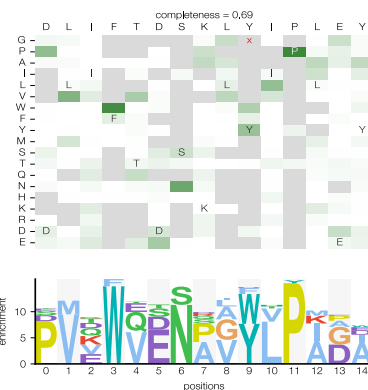
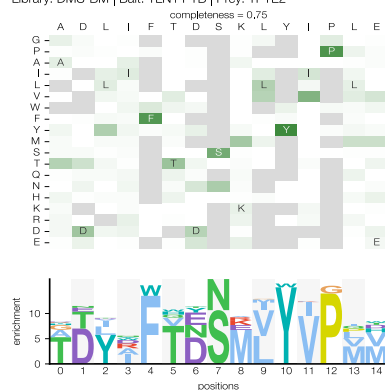
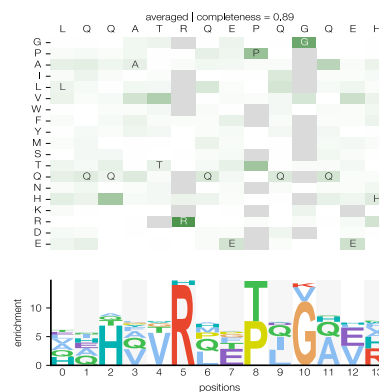
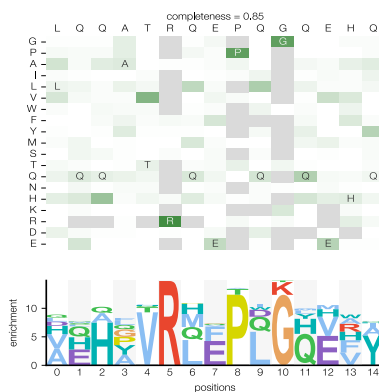
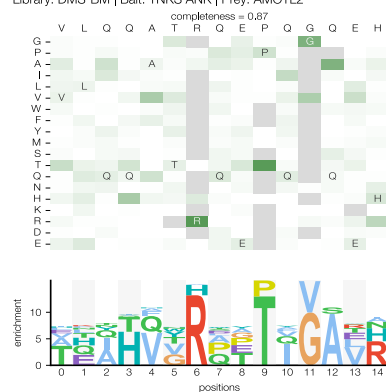
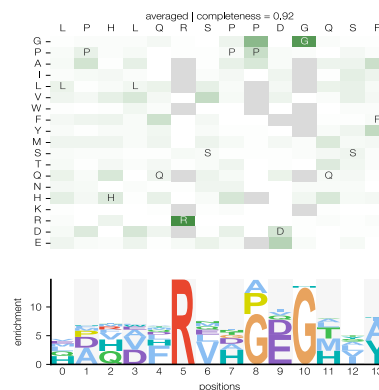
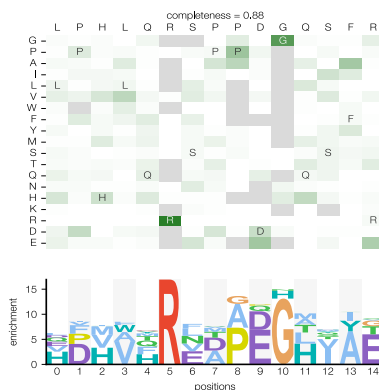
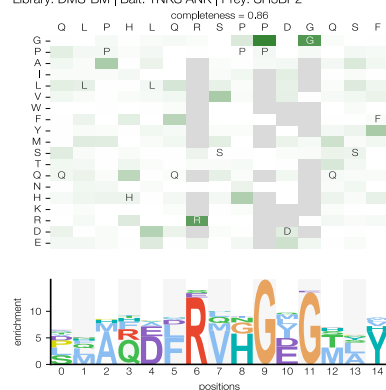


Fig. S2. Continued

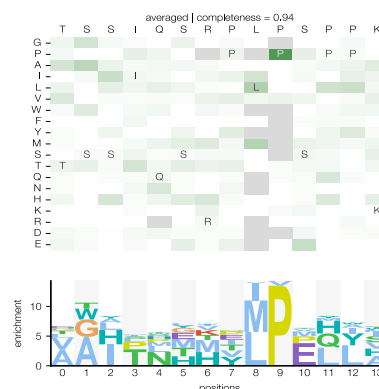
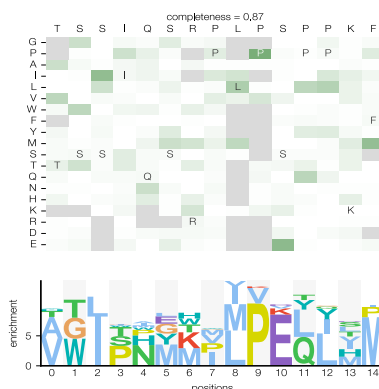
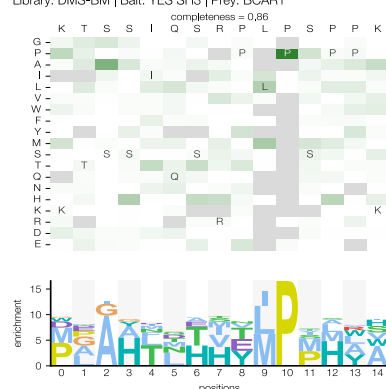
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Library: DMS-BM | Bait: TNKS ANK | Prey: SH3BP2



Library: DMS-BM | Bait: YES SH3 | Prey: BCAR1



Library: DMS-BM | Bait: YES SH3 | Prey: CBL

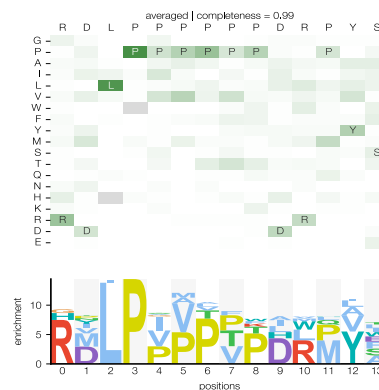
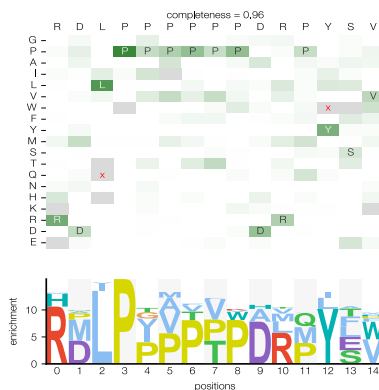
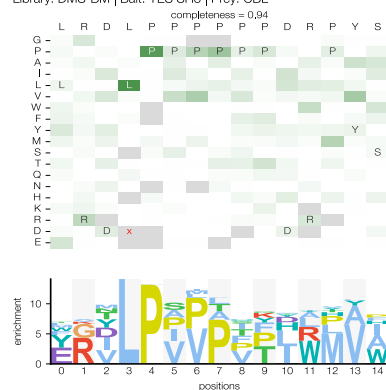


Figure S2. Heat map and PSSM representation of DMS analysis results generated through selections against the DMS-BM library. Each parental peptide is represented twice (left and middle) in overlapping registry. The averaged results are showed to the right.

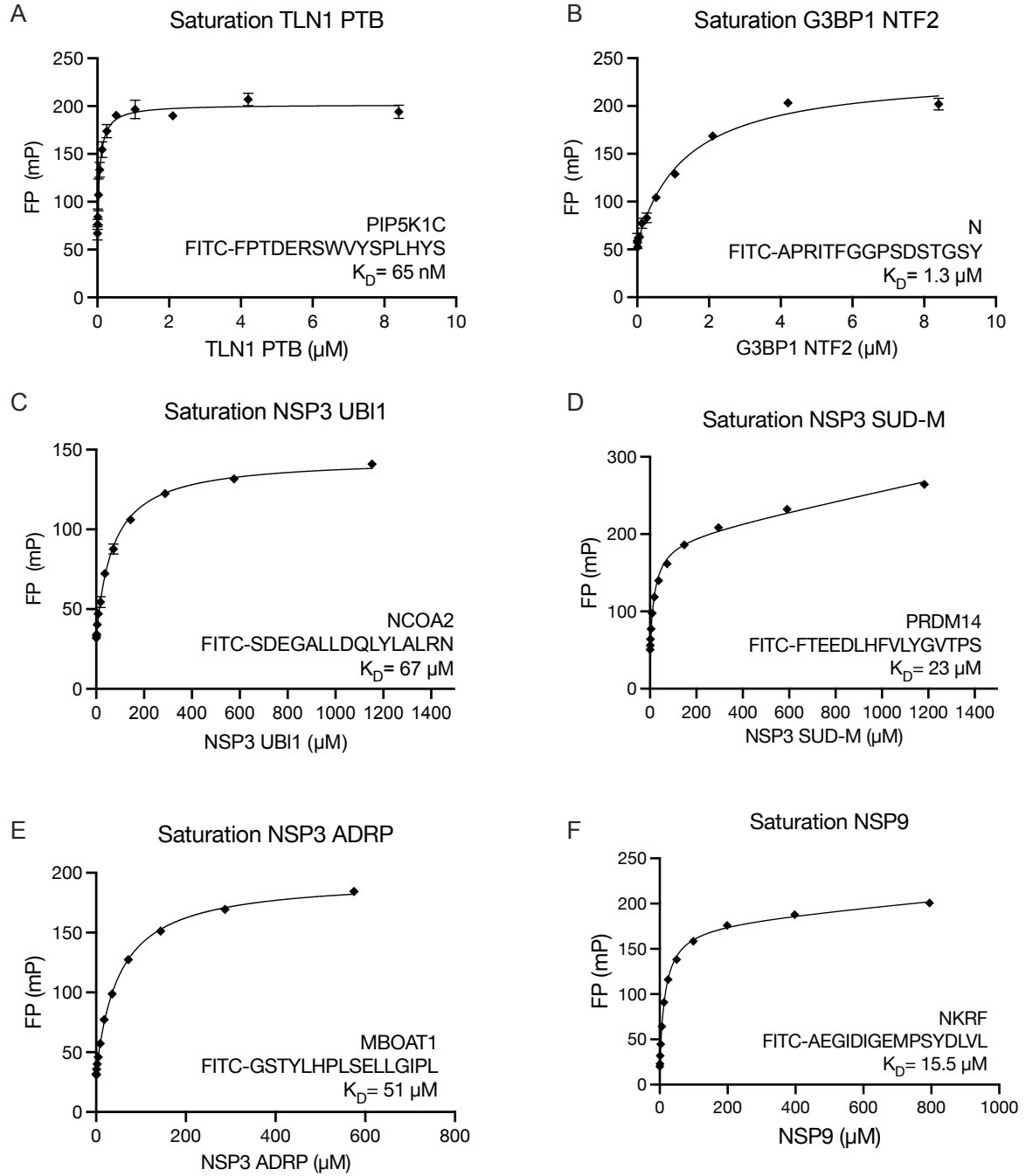


Figure S3. Binding titration curves as detected by FP of FITC-labeled probe peptides (indicated) binding to A) TLN1 PTB, B) G3BP1 NTF2, C) NSP3 UBI1, D) NSP3 SUD-M, E) NSP3 ADRP, and F) NSP9.

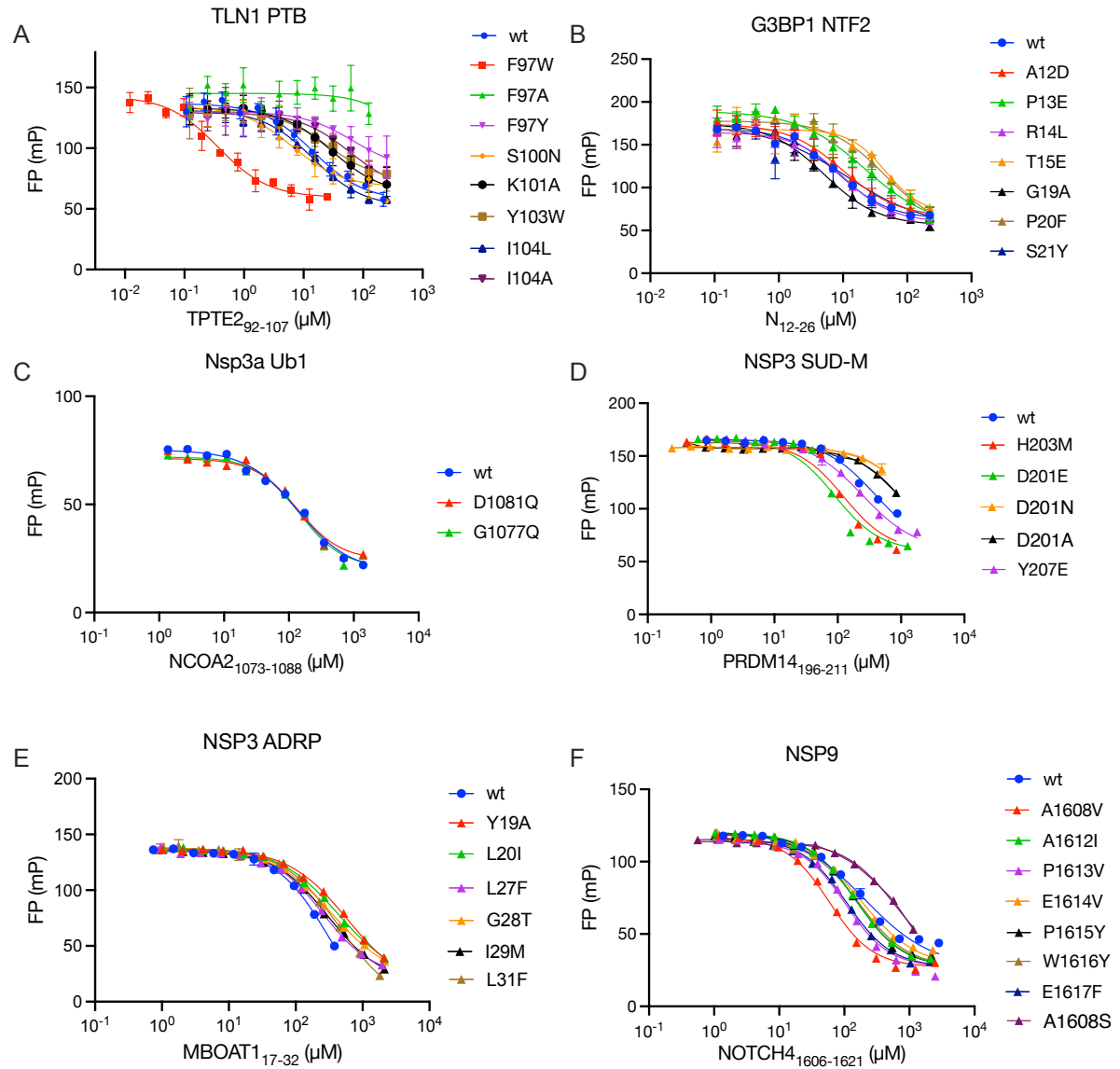


Figure S4. Affinity determinations through fluorescence polarization-based competition experiments of wild-type and mutant TLN1 PTB, B) G3BP1 NTF2, C) NSP3 UB11, D) NSP3 SUD-M, E) NSP3 ADRP, and F) NSP9.

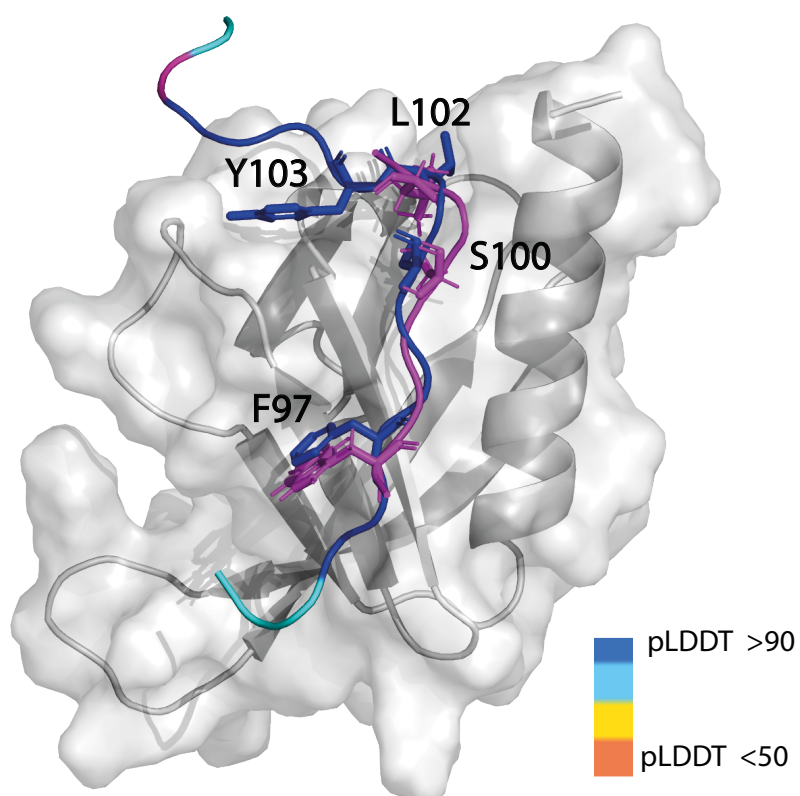


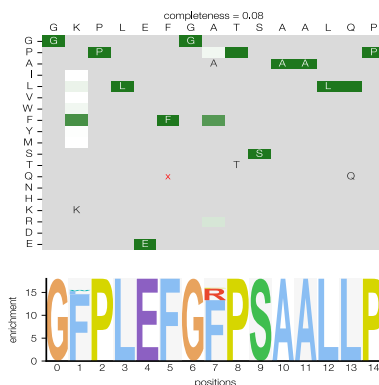
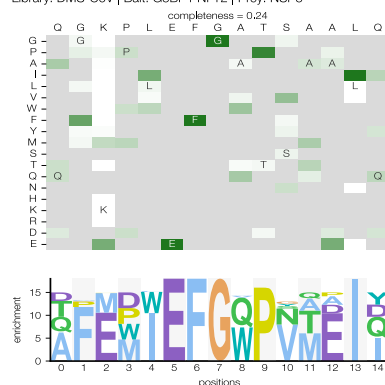
Figure S5. AlphaFold3 model of the TLN1 PTB-TPTE2 complex overlaid with the previously solved NMR structure of TLN1 PTB in complex with PIP5K1C (PDB id 2G35; peptide in magenta). The TPTE2 peptide is colored according to the pLDDT score, which shows the high confidence of the model (dark blue very high confidence pLDDT > 90; light blue pLDDT 90 > pLDDT > 70). The figure is a larger version of Fig. 2K.

Figure S6.

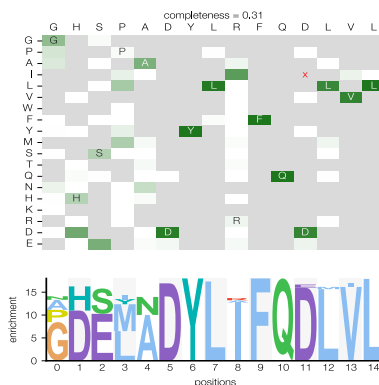
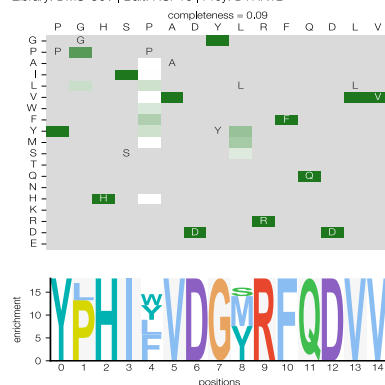


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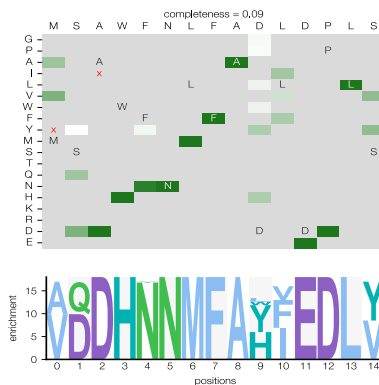
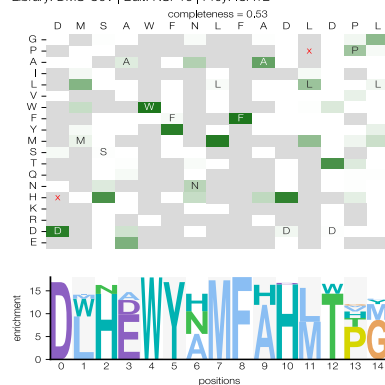
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Library: DMS-CoV | Bait: NSP16 | Prey: DYRK1B



Library: DMS-CoV | Bait: NSP16 | Prey: ICA1L



Library: DMS-CoV | Bait: NSP3 ADRP | Prey: AZIN2

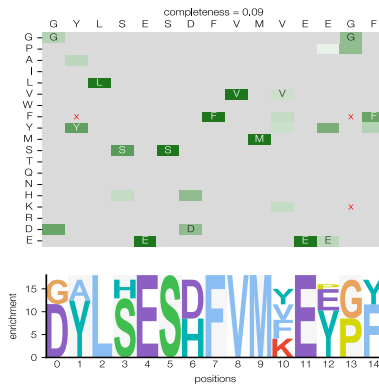
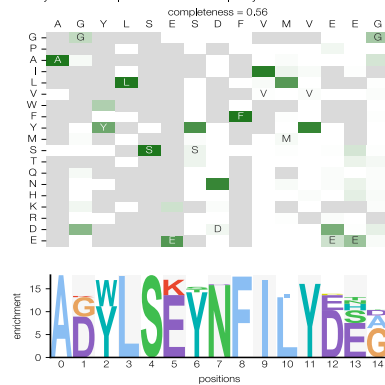
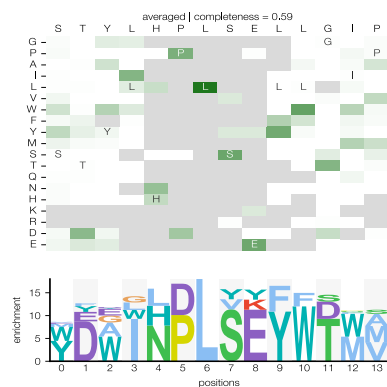
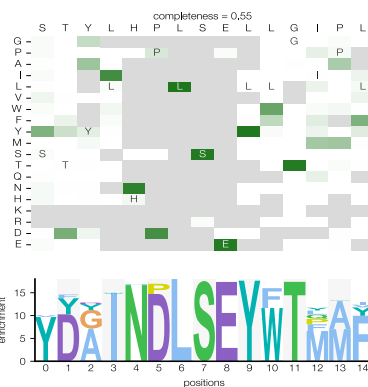
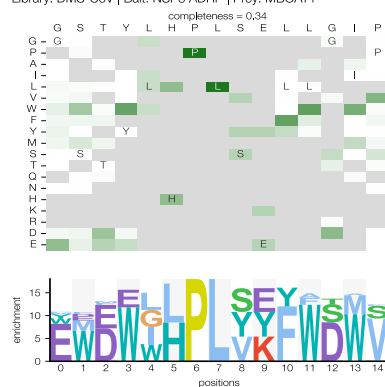
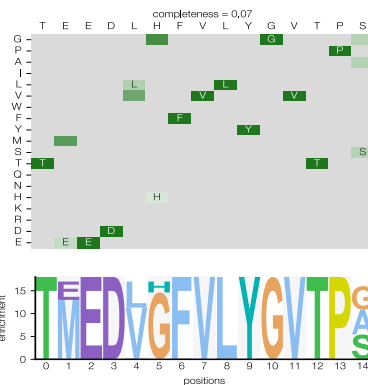
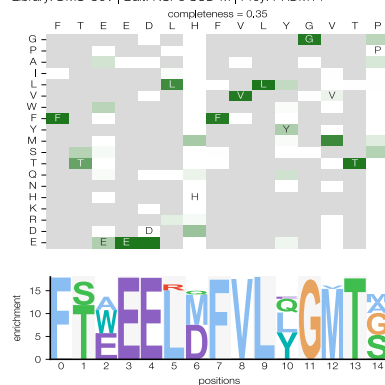


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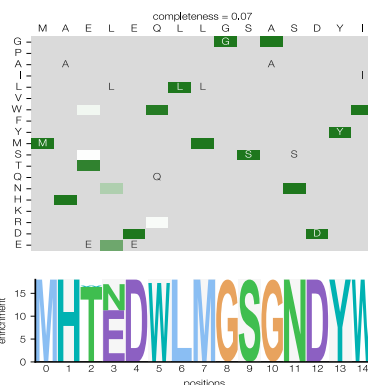
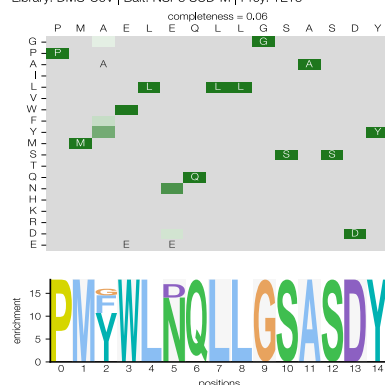
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Library: DMS-CoV | Bait: NSP3 SUD-M | Prey: PRDM14



Library: DMS-CoV | Bait: NSP3 SUD-M | Prey: TET3



Library: DMS-CoV | Bait: NSP3 UBI1 | Prey: NCOA2

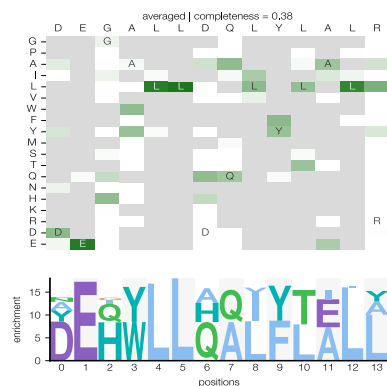
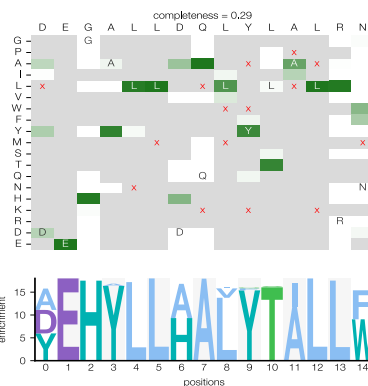
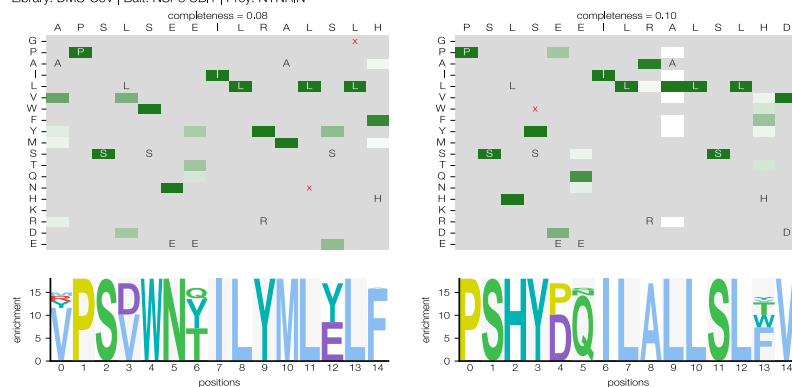
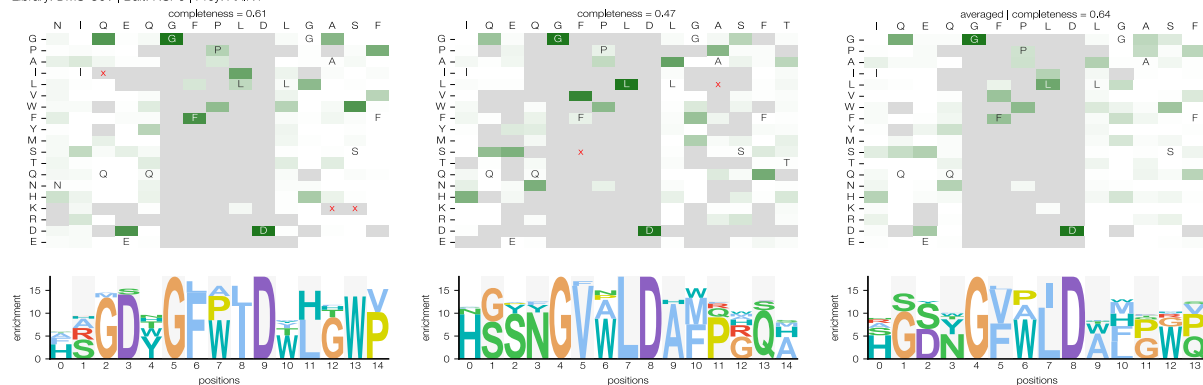


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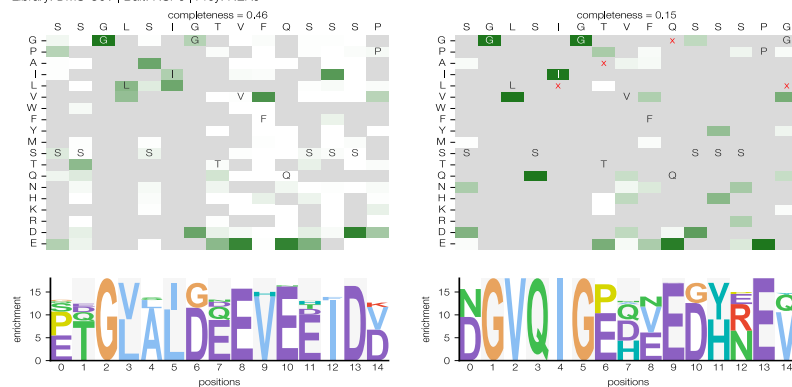
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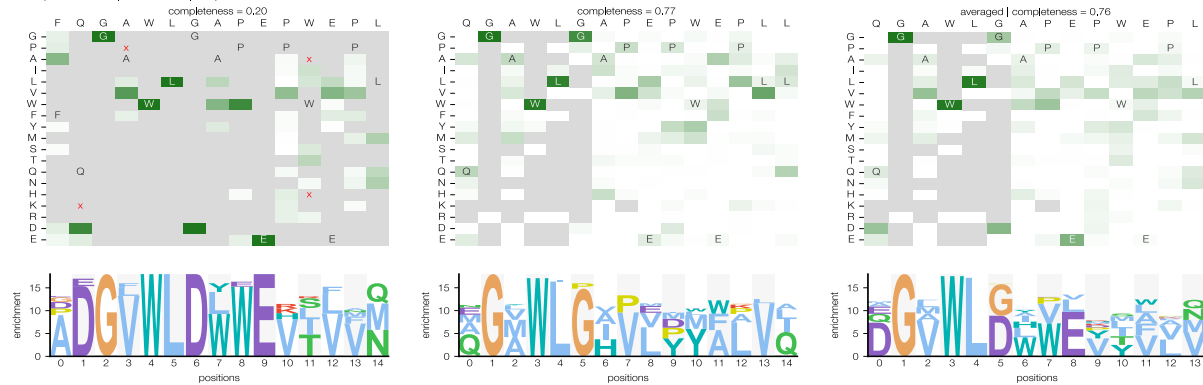
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Library: DMS-CoV | Bait: NSP9 | Prey: NOTCH4



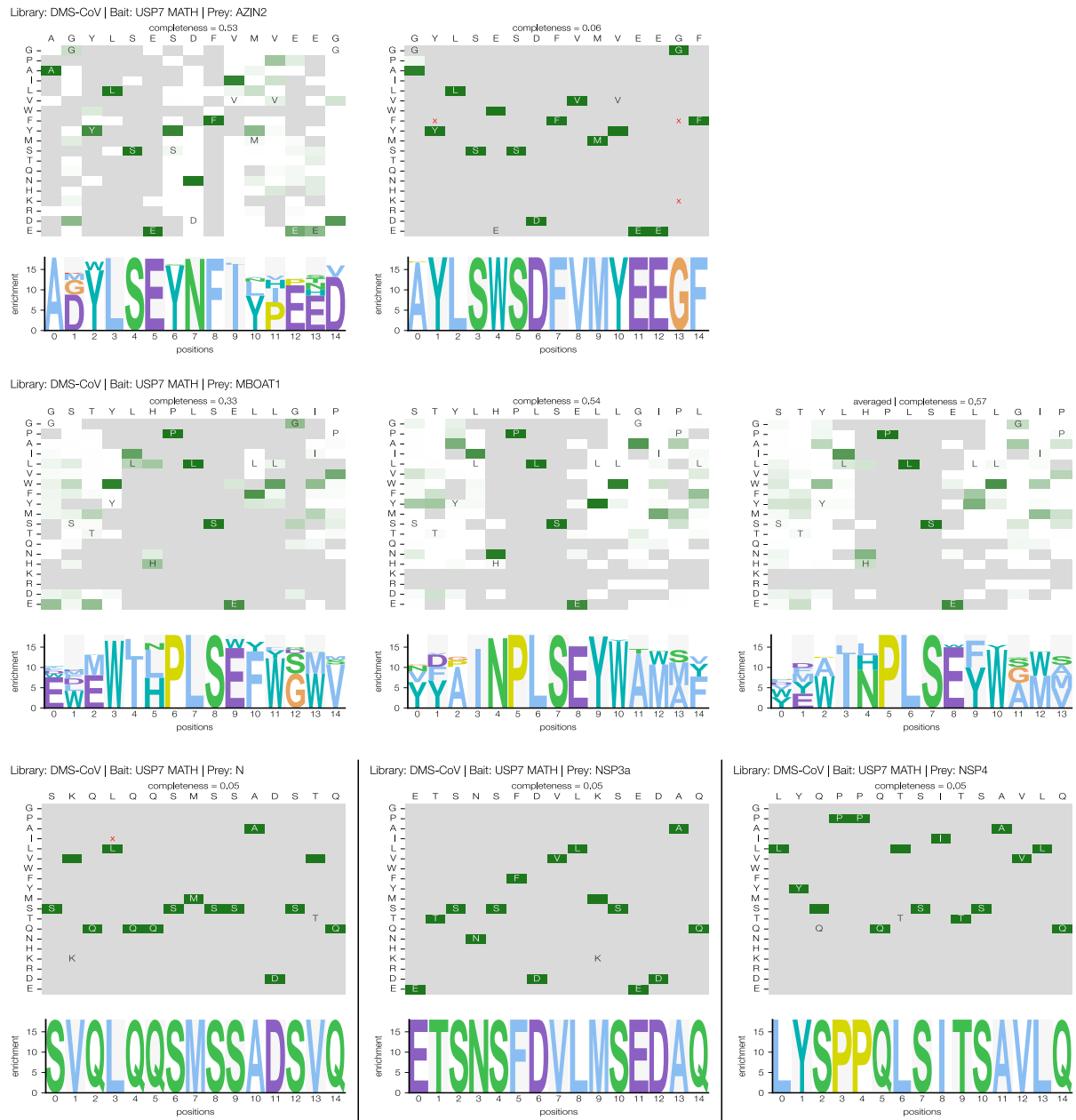


Figure S6. Heat map and PSSM representation of DMS analysis results generated through selections against the DMS-CoV library. Each parental peptide is represented twice (left and middle) in overlapping registry. The averaged results are showed to the right when available.

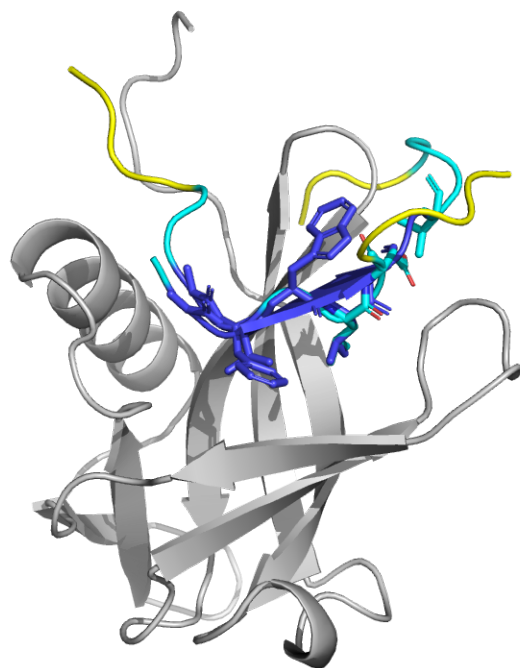


Figure S7. AlphaFold3 model of the complex of NSP9 and the NOTCH4 A1603V/P1613V peptide (QGVWLGAVERPWEPL) overlaid with the model of the AXIN (IQEQGFGLDLGAS). Peptide coloring is according to the pLLDT score (deep blue = high confidence). Generated using PyMol.

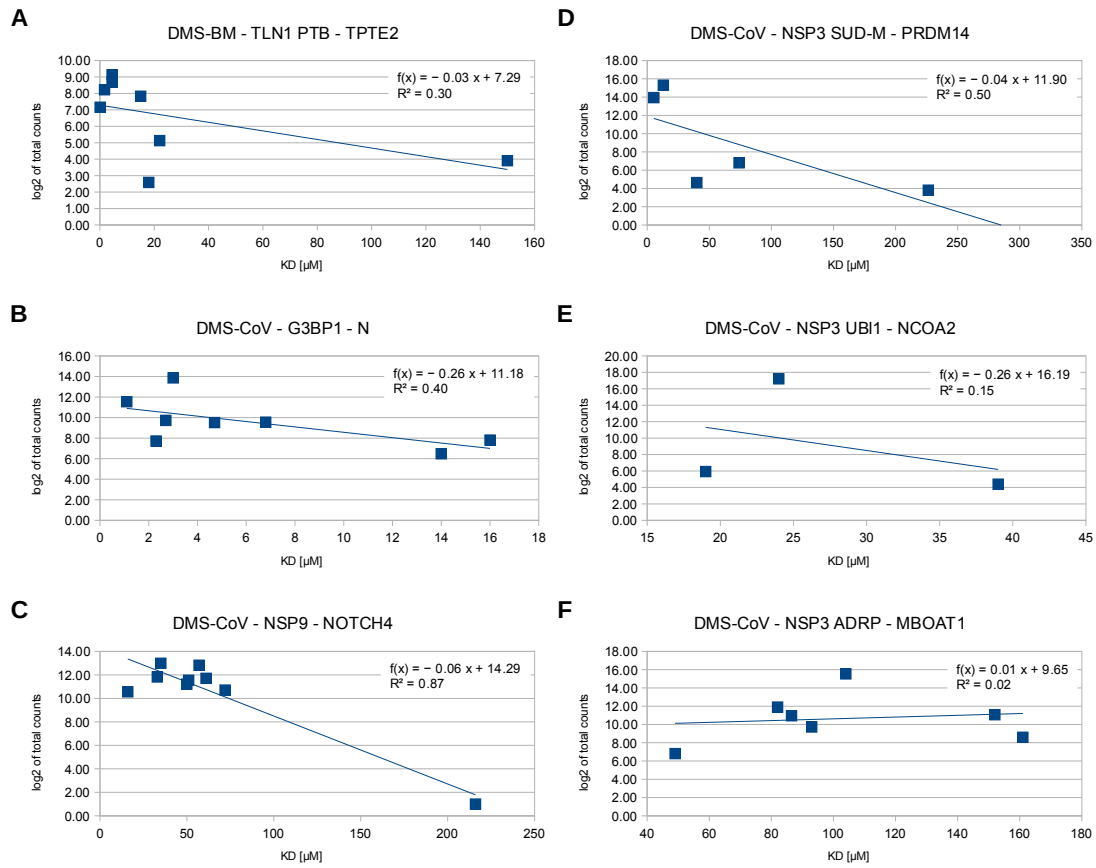


Figure S8. Correlation between NGS counts and affinities. The log₂ of the total NGS counts of a peptide variant observed in selection against a given bait plotted against the measured affinities. Data is shown for (A) TLN1 PTB domain and the TPTE₉₂₋₁₀₇ peptide, (B) G3BP1 NTF and the N₁₂₋₂₆ peptide, (C) NSP9 and the NOTCH4₁₆₀₆₋₁₆₂₁ peptide, (D) NSP3 SUD-M and the PRDM14₁₉₆₋₂₁₁ peptide, (E) NSP3 UBI and the NCOA2₁₀₇₃₋₁₀₈₈ peptide, and (F) NSP3 and the MBOAT1₁₇₋₃₂ peptide.