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Supplemental information

Mapping antibody footprints using binding profiles

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Supplementary Information

Figure S1. Characteristics of the human monoclonal antibodies used in this study. *Related to Figure 1 and Table S1.* (A) Venn diagram of the binding profile of each of the mAbs based on previous studies (Table 2), (B) HA Head vs. HA stalk antibodies (C) Antigenic binding sites (D) antibody function: Neut+: neutralizing, Neut-: non-neutralizing, HAI+: Hemagglutinin Inhibition activity.

Figure S2. Comparing mAb subtype specificities to their known specificities. *Related to Figure 2.* Comparison of the subtype specificity of all 23 mAbs using AM and ELISA. Black bars denote the overall binding breadth of each mAb to the A/H1N1, A/H3N2 and B subtypes as measured using our influenza AM. mAb specificities based on previous studies are presented by the gray boxes.

Figure S3. Inferring antigenic cartography based on mAb binding antibody profiles. *Related to Figure 3.* The mAb binding profiles across HA strains were used to compute Euclidean distance between influenza strains and monoclonal antibodies using the Euclidean metric. Dendrograms were computed using the complete-linkage algorithm (A) Euclidean distance matrix of 35 influenza HAs from different subtypes computed over the binding profile of all 23 mAbs to each strain. The top colorbar represents the antigenic group of each strain (group 1: H1N1, H5N1, and H9N2; group 2: H3N2 and profiles to 35 HA strains (**Table S1**)). (C-D) Antigenic cartography maps based on Euclidean distance were computed using the Ramacs antigenic cartography package⁶⁴. (C) Antigenic cartography of 8 H1N1 strains based on the binding profiles of 23 H1 mAbs. Influenza strains were divided into 3 known antigenic groups, annotated by color: PR8 lineage, SI06 and BRIS07 lineage and pH1N1 lineage. (D) Antigenic cartography of 23 influenza mAbs based on their binding profiles to 36 influenza A HA proteins from both human and avian subtypes (Table 2 and S2). The shape of each mAb represents its known binding profile to H1 and H3 subtypes based on AM binding profiles. The Color of each mAb represents its epitope specificity. PR8-Puerto Rico 1934, SI06-Solomon Islands 2006, BRIS07-Brisbane 2007, B Vic - B Victoria lineage, B Yam- B Yamagata lineage.

Figure S4. Antigenic cartography of both antigens and mAbs. *Related to Figure 3.* Antigenic cartography maps based on cosine similarity were computed jointly for 8 H1N1 strains (orange circles) and for 23 influenza mAbs based on their binding profiles. The shape of each mAb represents its known binding profile to H1 and H3 subtypes, and colors represent the epitope specificity of each mAb.

Figure S5. Geometric mean distances from the initial patch identified by mAb patch. *Related to Figure 5.* (A-D) Box plots of geometric mean distance from the epitope patch created by the 15 top ranked positions comparing positions within the known epitope to non-epitope positions based on solved structures for each of the mAbs. GMD-Geometric mean distance. p-value legend: ns:p <= 1, *:0.01<p<= 0.05, **:0.001<p<=0.01, ***:0.0001<p<=0.001, ****: p<=0.00001.

Figure S6. Identifying mAb epitope using Z-Dock Docking tool. *Related to Table 1.* For Each mAb with solved structure we used Z-Dock server, docking prediction tool for protein-protein interactions. (A-B) Head mAbs 045-09 2B05 and 047-09 4G02, (C-D) Stalk mAbs CR-9114 and FI6, solved structure in green/pink and Z-Dock top scored prediction in cyan/yellow.

Table S1: Human monoclonal antibodies used in this study. *Related to Figure 1,2 and 3 and Figure S1, S2 and S3.*

mAb	Binding strains	Binding region	Binding site	Function
008-10 5G04	H1, H3	Head	Ca1	HAI+
SFV019-2A02	H1	Head	Ca1	HAI+
045-09 2B05	H1	Head	Lateral Patch	HAI+
047-09 4G02	H1, H3, B	Head	Lateral Patch	HAI+
SFV015-2F04	H1	Head	Lateral Patch	HAI+
SFV009-3D04	H1	Stalk	Anchor Stalk	Neut+
029-09 4A01	H1	Stalk	Central Stalk	Neut+
030-09 2B03	H1	Stalk	Central Stalk	Neut+
045-09 2B06	H1, H3, B	Stalk	Central Stalk	Neut+
047-09 4E01	H1, H3, B	Stalk	Central Stalk	Neut-
051-09 4B02	H1	Stalk	Central Stalk	Neut+

051-09 5A02	H1	Stalk	Central Stalk	Neut+
CR-9114	H1, H3, B	Stalk	Central Stalk	Neut+
FI6	H1, H3	Stalk	Central Stalk	Neut+
SC1000-3D04	H1, B	Stalk	Central Stalk	Neut+
SC1009-3B05	H1	Stalk	Central Stalk	Neut+
SC70-1F02	H1, H3	Stalk	Central Stalk	Neut+
SC70-5B03	H1	Stalk	Central Stalk	Neut+
SFV005-2G02	H1, H3, B	Stalk	Central Stalk	Neut+
030-09 1E05	H1, H3, B	Stalk	Stalk	Neut-
047-09 1G05	H1	Stalk	Stalk	Neut-
051-09 4A03	H1, H3	Stalk	Stalk	Neut+
051-09 5A01	H1	Stalk	Stalk	Neut-

Table S2: Additional avian influenza strains spotted on our influenza antigen microarray related to Table 2 and Figure 3 and S3.

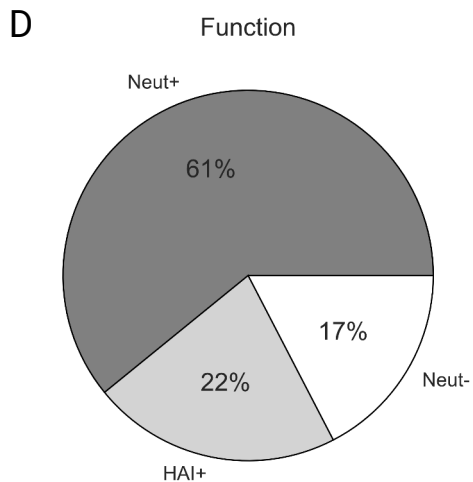
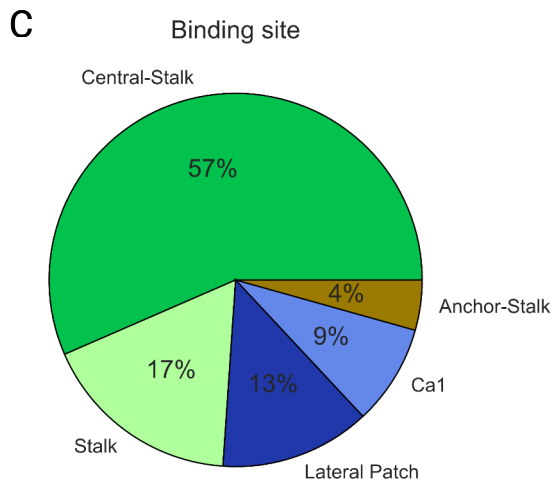
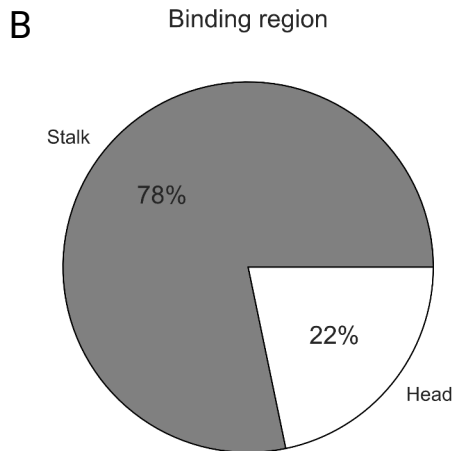
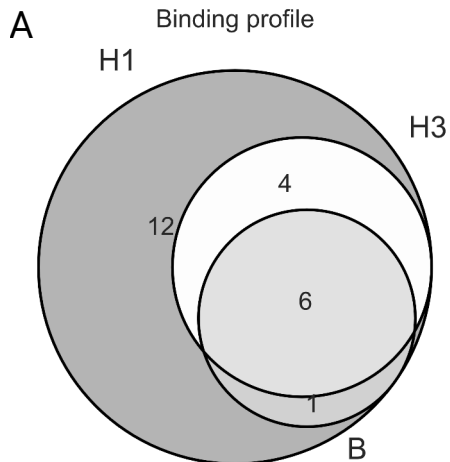
Virus name	Subtype	Protein	Source	Cat#	Accession#
A/Japan/305/1957	H2N2	rHA	Sino	11088-V08H	AAA43185.1
A/mallard/Ohio/657/2002	H4N6	rHA	Sino	11714-V08H	ABI47995.1

A/chicken/Hong Kong/17/1977	H6N4	rHA	Sino	40027-V08H	CAC84244.1
A/New York/107/2003	H7N2	rHA	IRR	FR-69	N/A
A/Netherlands/219/2003	H7N7	rHA	Sino	11082-V08B	AAR02640.1
A/pintail duck/Alberta/114/1979	H8N4	rHA	Sino	11722-V08H	ABB87729.1
A/mallard/Minnesota/Sg-00194/2007	H10N3	rHA	Sino	40184-V08B	ACT84107.1
A/mallard/Alberta/294/1977	H11N9	rHA	Sino	11704-V08H	ABB87228.1

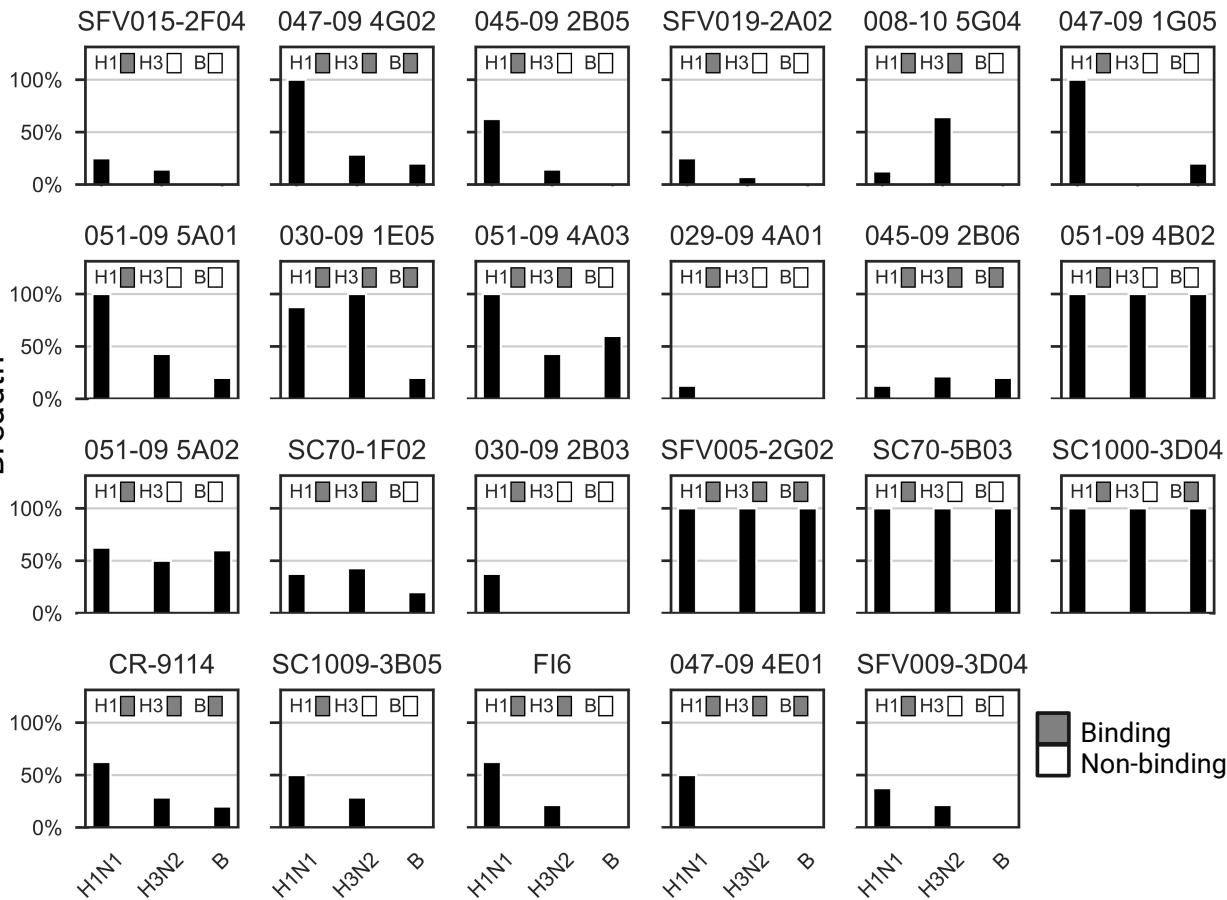
Table S3: Influenza strains spotted on our influenza antigen microarray included in the multiple sequence alignment used by mAb-Patch. Related to Figure 5.

Virus name	Subtype	Protein	Source	Cat#	Accession#
A/South Carolina/1/1918	H1N1	HA	IRR	FR-692	AAD17229.1
A/WSN/1933	H1N1	HA	Sino	11692-V08H	ACF54598.1
A/Puerto Rico/8/1934	H1N1	HA	Sino	11684-V08H	ABD77675.1

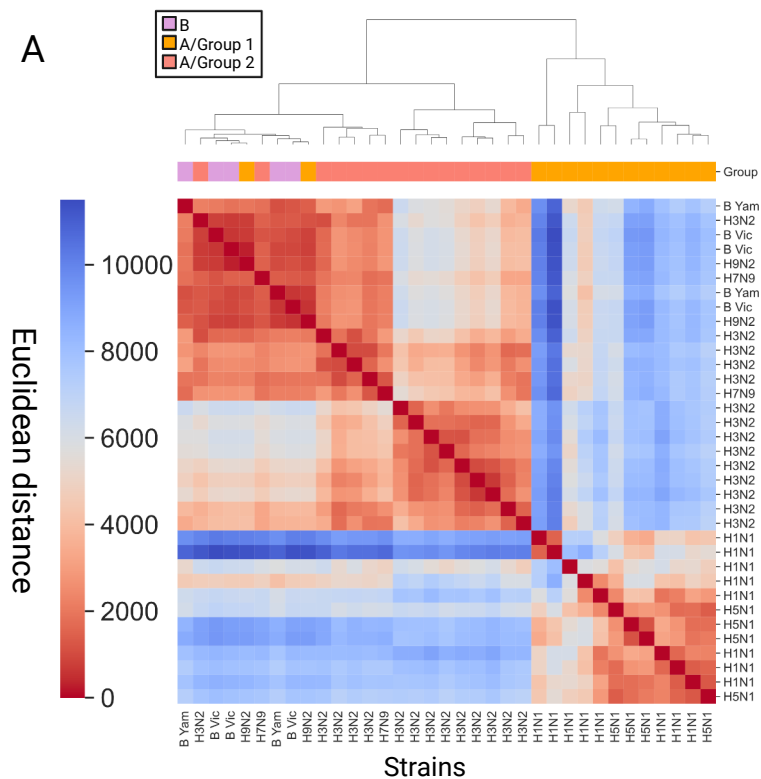
A/Solomon Islands/3/2006	H1N1	HA	Sino	11708-V08H	ABU99109.1
A/Brisbane/59/2007	H1N1	HA	Sino	11052-V08H	ACA28844.1
A/California/07/2009	H1N1	HA	Sino	11085-V08H	ACP41953.1
A/Michigan/45/2015	H1N1	HA	Sino	40567-V08H1	APC60198.1
A/Brisbane/02/2018	H1N1	HA	Native Antigens	custom_EPI1440504	EPI1440504
A/Hong Kong/483/1997	H5N1	HA	Sino	11689-V08H	AAC32099.1
A/Anhui/1/2005	H5N1	HA	Sino	11048-V08H1	ABD28180.1
A/Indonesia/5/2005	H5N1	HA	Sino	11060-V08B	ABW06108.1
A/Bar-headed goose/Qingi/14/2008	H5N1	HA	Sino	11059-V08B1	ACL28277.1
A/Guineafowl/Hong Kong/WF10/1999	H9N2	HA	Native Antigens	FLU-H9N2-HA-100	AOT22363.1
A/Hong Kong/1073/1999	H9N2	HA	Sino	11229-V08H	NP_859037.1



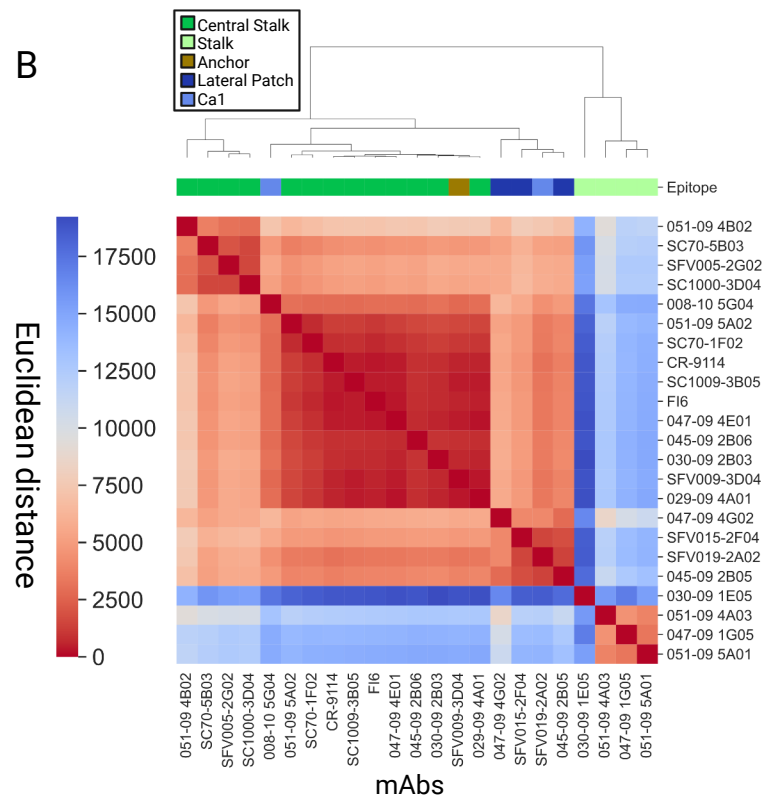
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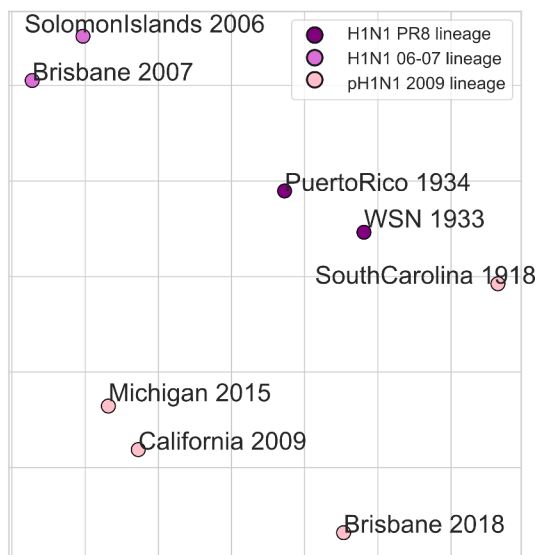
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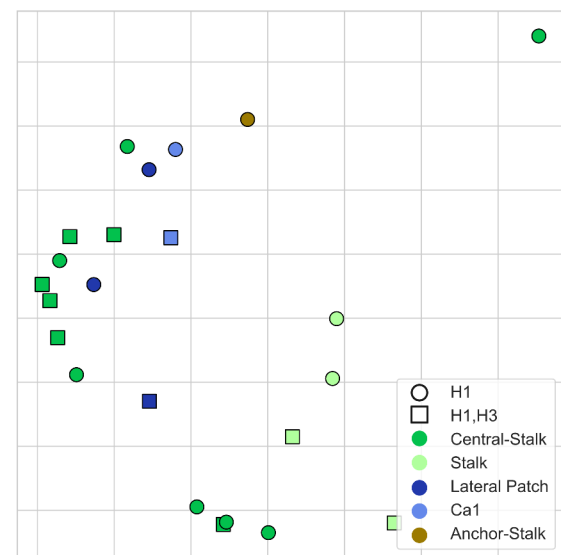
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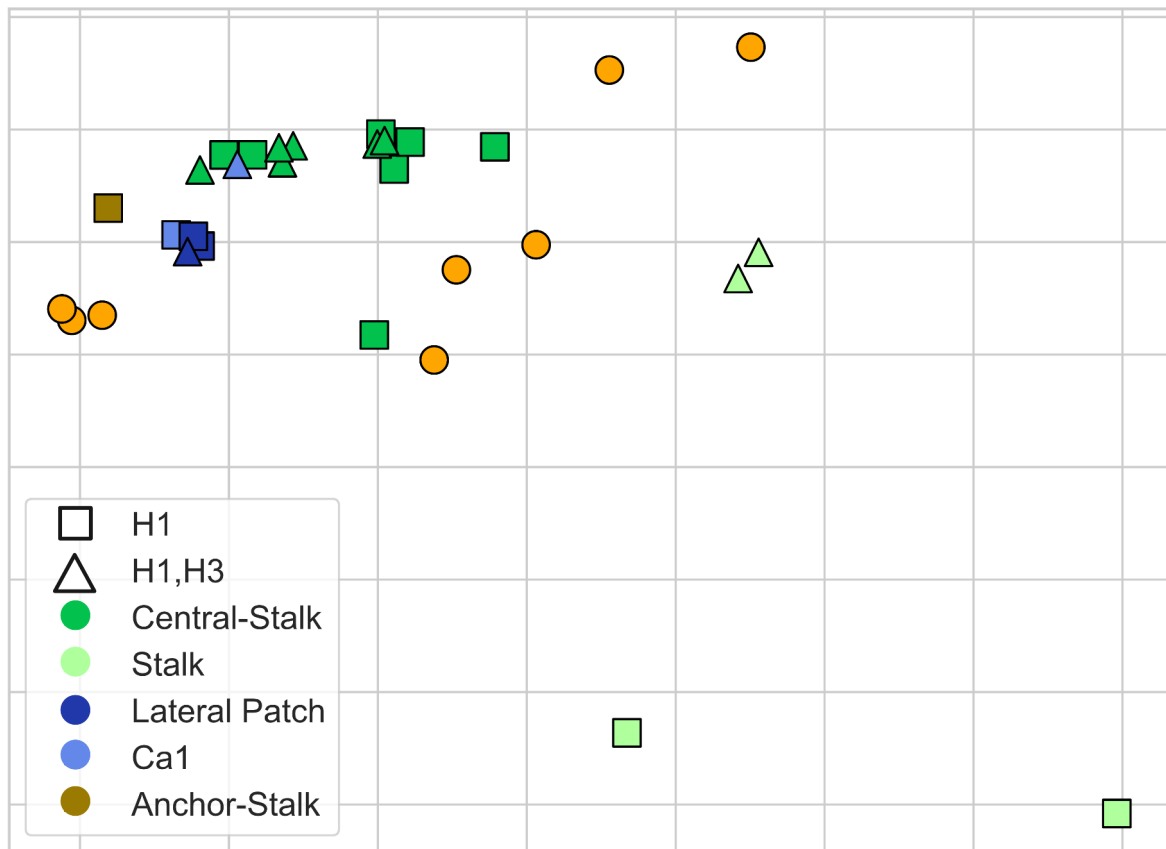


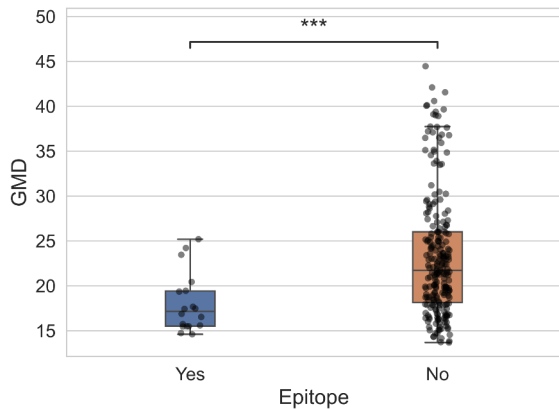
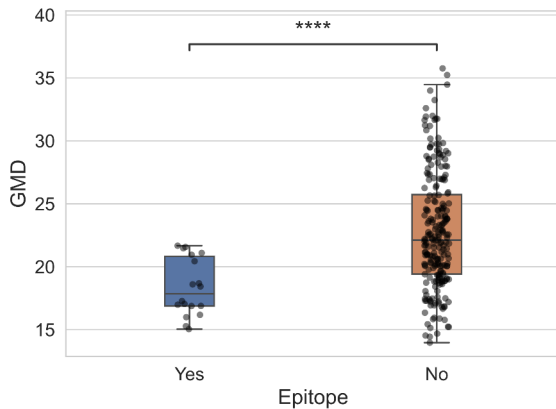
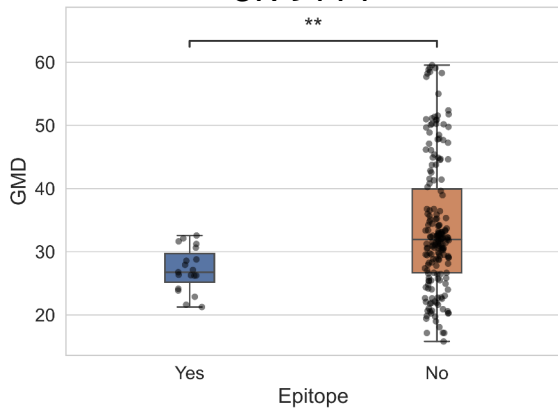
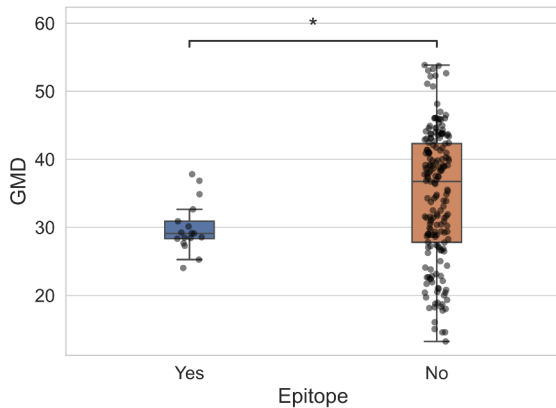
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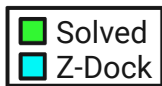


D





A 047-09 4G02**B** 045-09 2B05**C** CR-9114**D** FI6



A



045-09 2B05 - 7MEM

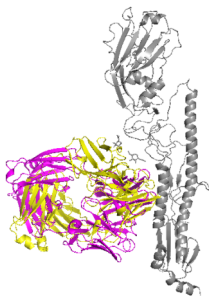
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047-09 4G02 - 5W6G

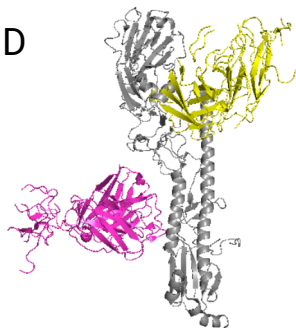


C



CR-9114 - 5CJS

D



FI6 - 3ZTN