

Supporting Information for

High Specificity of Germline Antibodies Targeting Hapten: A Universally Intrinsic Feature

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1. Reagents, materials and apparatus

The 96-well polystyrene high bind stripwell microplates with clear bottom for ELISA were purchased from Corning (Corning, NY, USA). The microplate reader was obtained from PerkinElmer (Waltham, MA, USA).

2. Buffers

The common buffer solutions used in the experiment are listed:

(1) Coating buffer (0.05 mol/L, carbonate buffer, pH 9.6);

(2) Blocking buffer (10% skim milk powder (w/v));

(3) Washing buffer (PBST, 0.01 mol/L PBS buffer with 0.05% Tween-20 (v/v), pH 7.2);

(4) Phosphate buffer solution (PBS buffer, 0.01 mol/L, pH 7.4);

(5) Antibody dilution buffer (PBS buffer with 0.2% albumin, (w/v));

(6) Goat anti-mouse IgG (HRP labeled) dilution buffer (PBS buffer containing 5% albumin (w/v));

(7) 3,3',5,5'-Tetramethylbenzidine (TMB) Two-Component Substrate solution (TMB/H₂O₂)

(8) Stopping reagent (2 M H₂SO₄).

42 **3. Supporting Figures and Tables**

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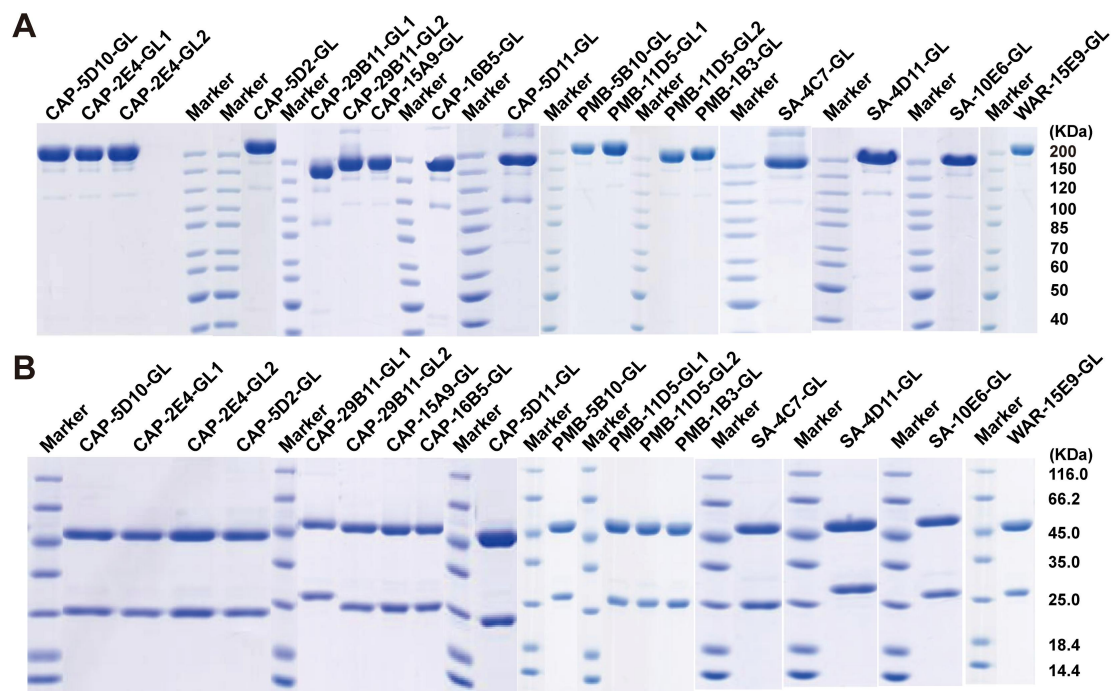


Figure S1. Non-reduced SDS-PAGE (A) and reduced SDS-PAGE (B) analyses of germline antibodies. The SDS-PAGE of ANG-3B3-GL and TLF-3B4-GL were reported in a previous work (*1*).

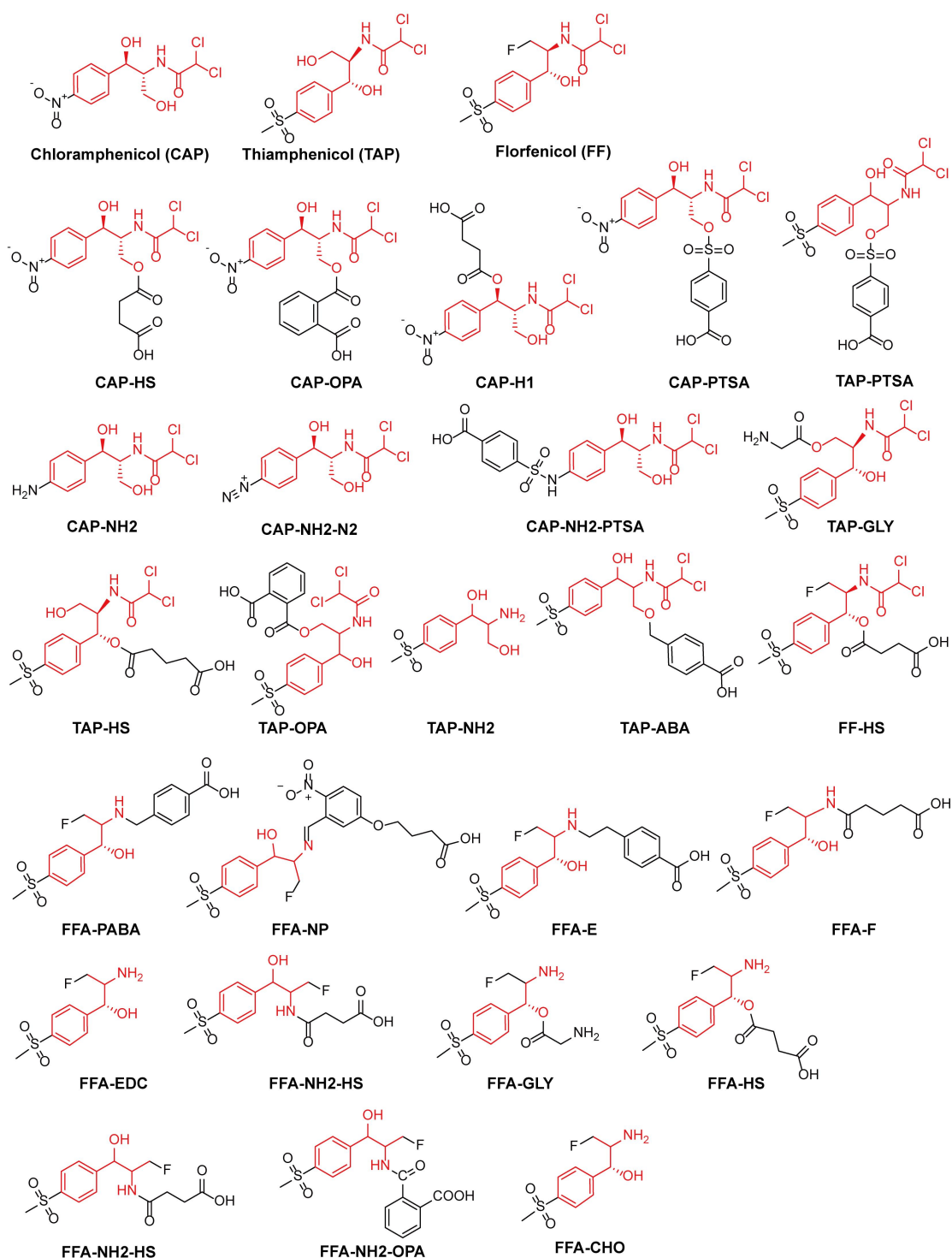


Figure S2. Chemical structures of chloramphenicol (CAP)-class drugs, including CAP, thiamphenicol (TAP), and florfenicol (FF), as well as their corresponding structurally related hapten derivatives shown in Figure 1A. The shared structure is highlighted in red.

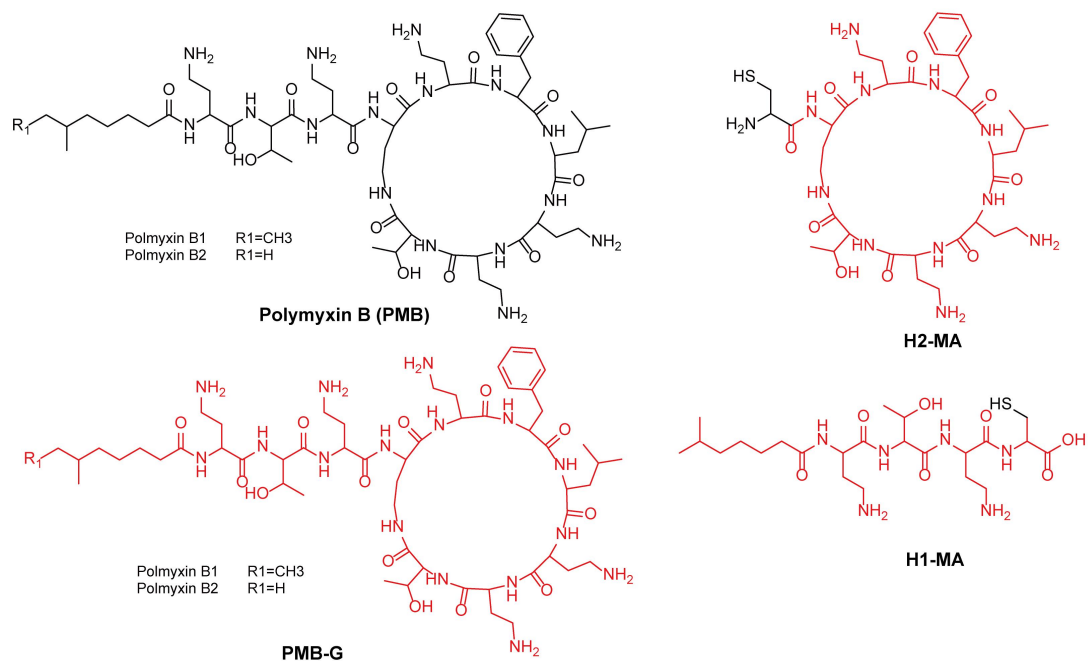


Figure S3. Chemical structures of polymyxin B (PMB), and s their structurally related haptens shown in Figure 1B. The shared structure is highlighted in red.

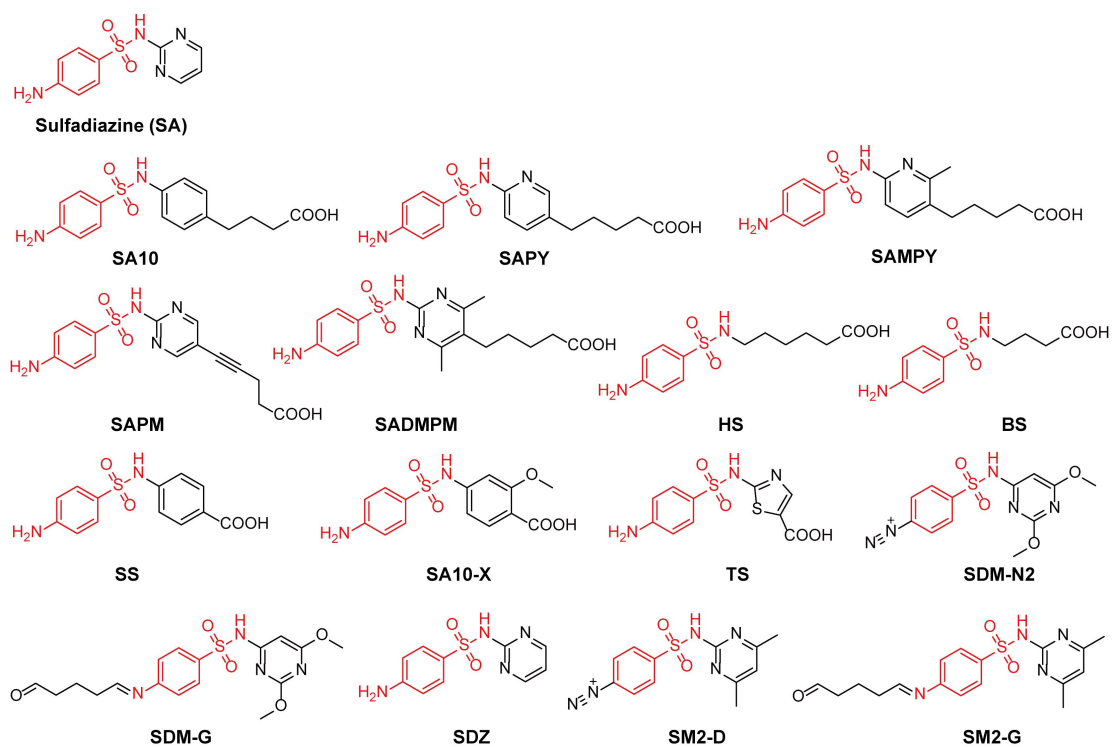


Figure S4. Chemical structures of sulfadiazine (SA), and their 15 structurally related haptens shown in Figure 1C. The shared structure is highlighted in red.

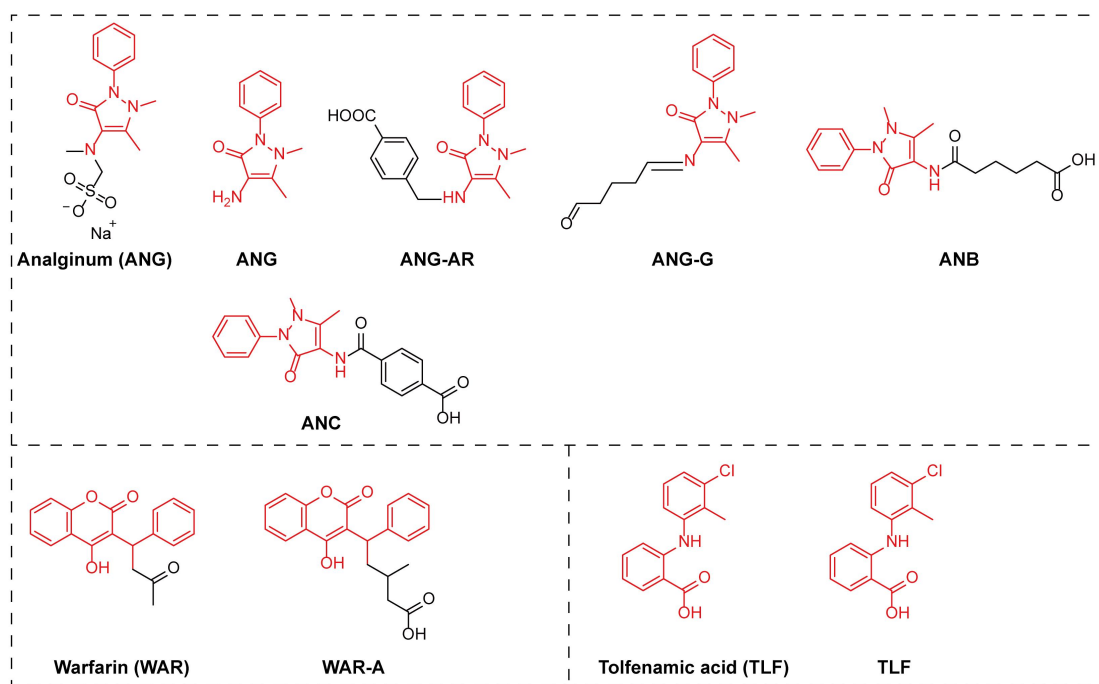


Figure S5. Chemical structures of analginum (ANG), warfarin (WAR), and tolafenamic acid (TLF), as well as their structurally related haptens shown in Figure 1D–1F. The shared structure is highlighted in red.

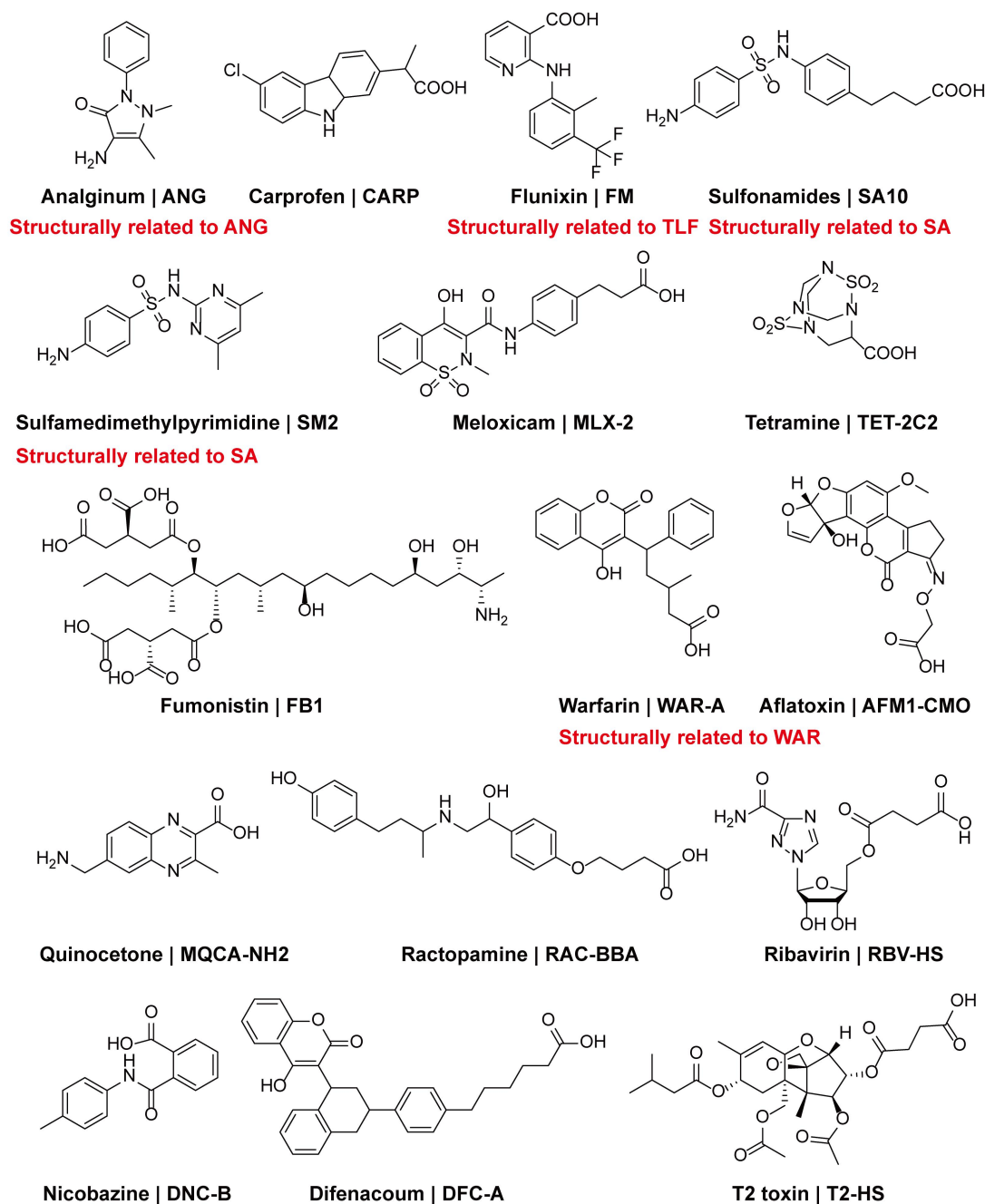
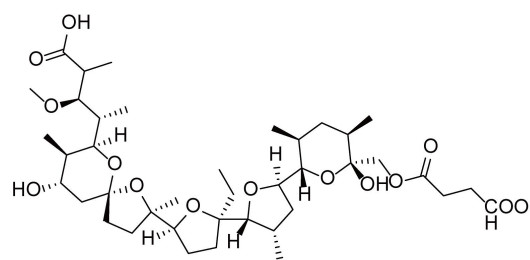
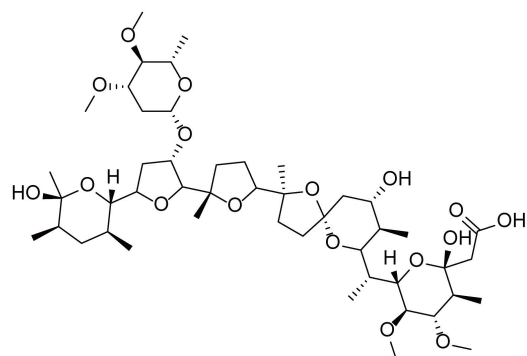
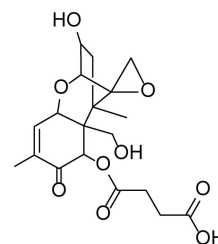
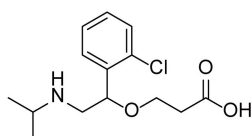
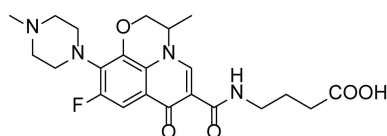
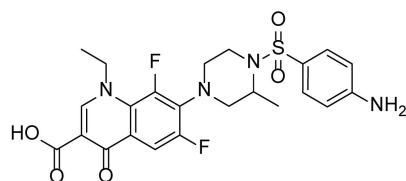
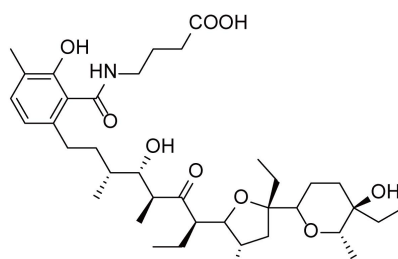
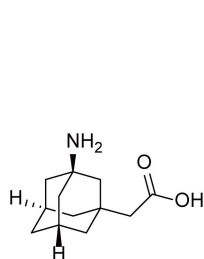
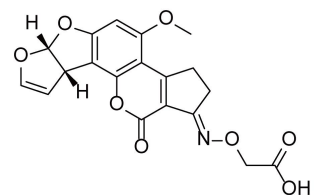
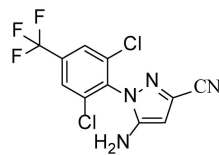
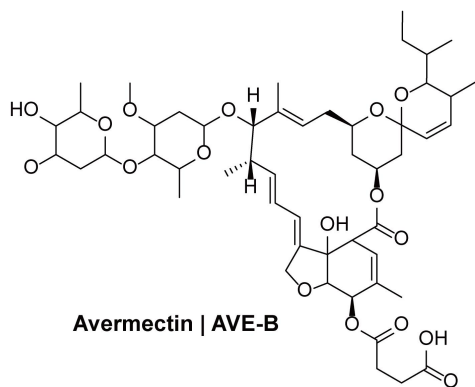


Figure S6. Chemical structures of haptens in the small-molecule library, encompassing a broad range of chemical classes. This hapten library including common veterinary and human antimicrobials (e.g., sulfonamides, quinolones, tetracyclines), illicit additives (e.g., melamine, ractopamine), mycotoxins (e.g., aflatoxin, deoxynivalenol), environmental contaminants (e.g., polychlorinated biphenyls, bisphenol A), rodenticides (e.g., warfarin, brodifacoum), anti-inflammatory agents (e.g., anandamide, tolfenamic acid), and immunosuppressants (e.g., rapamycin). The structural related hapten have been marked in red text. Related to Figure 1G.



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80 **Figure S6. (continued)**

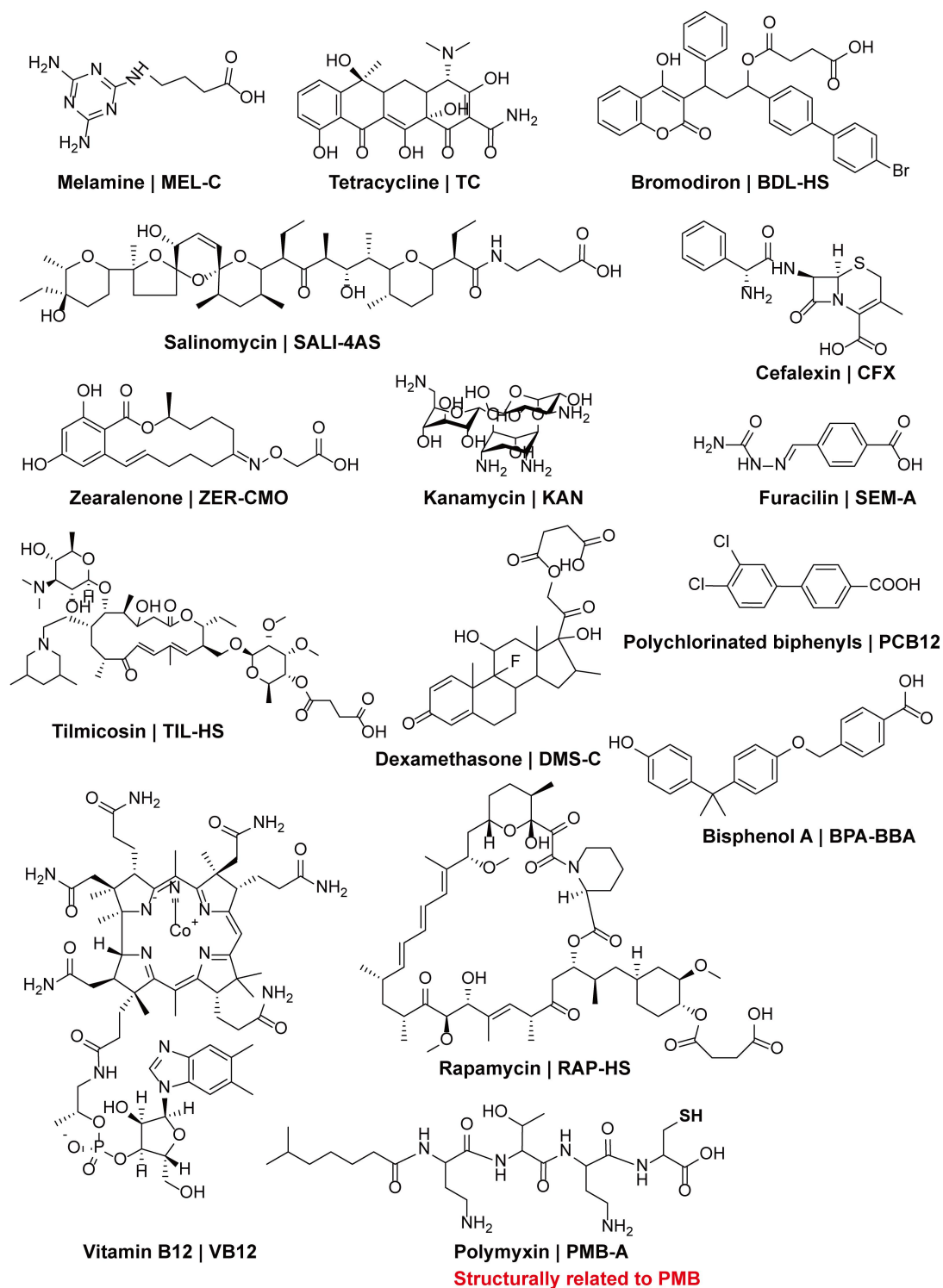


Figure S6. (continued)

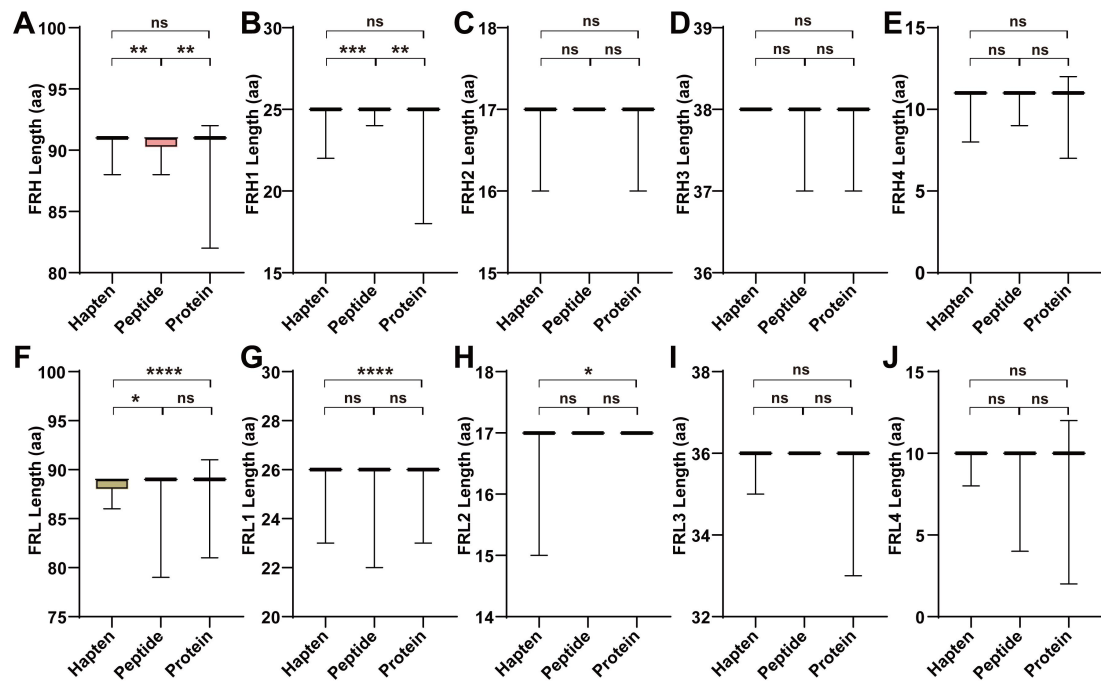


Figure S7. The sequence length analysis of FR region between hapten, peptide and protein targeting germline antibodies.

91 **Table S1.** Small molecules and haptens of mAbs used in this study.

Small molecules	Haptens	Antibodies
Chloramphenicol (CAP)	CAP-HS	CAP-5D10
		CAP-6D8
		CAP-8G3
		CAP-2E4
		CAP-1B12
		CAP-5D2
		CAP-5D11
		CAP-29B11
		CAP-15A9
		CAP-16B5
Polymyxin B (PMB)	H1-MA	PMB-5B10
		PMB-11D5
		PMB-13G6
	H2-MA	PMB-1B3
Sulfadiazine (SA)	TS	SA-4C7
	SA10	SA-4D11
	SAPM	SA-10E6
Warfarin (WAR)	WAR-A	WAR-15E9
Analginum (ANG)	ANG-2	ANG-3B3
		ANG-1H2
		ANG-20A2
Tolfenamic acid (TLF)	TLF	TLF-3B4

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95 **Table S2.** The germline gene usage of 23 mAbs.

Target	Antibody	Germline gene usage					Germline antibody
		IGHV	IGHD	IGHJ	IGKV	IGKJ	
CAP	CAP-5D10	IGHV8-8	IGHD1-1	IGHJ2	IGKV1-117	IGKJ1	CAP-5D10-GL
	CAP-6D8	IGHV8-8	IGHD1-1	IGHJ2	IGKV1-117	IGKJ1	
	CAP-8G3	IGHV8-8	IGHD1-1	IGHJ2	IGKV1-117	IGKJ1	
	CAP-2E4	IGHV8-8	IGHD1-3	IGHJ4	IGKV1-117	IGKJ2	CAP-2E4-GL1
	CAP-1B12	IGHV8-8	IGHD1-3	IGHJ4	IGKV1-117	IGKJ2	CAP-2E4-GL2
	CAP-5D2	IGHV8-8	IGHD2-3	IGHJ4	IGKV1-117	IGKJ2	CAP-5D2-GL
	CAP-29B11	IGHV8-8	IGHD2-12	IGHJ2	IGKV1-117	IGKJ1	CAP-29B11-GL1 CAP-29B11-GL2
	CAP-5D11	IGHV1-87	IGHD2-5	IGHJ3	IGKV3-12	IGKJ5	CAP-5D11-GL
	CAP-15A9	IGHV9-3-1	IGHD1-2	IGHJ2	IGKV1-135	IGKJ1	CAP-15A9-GL
	CAP-16B5	IGHV4-1	IGHD3-2	IGHJ3	IGKV14-111	IGKJ5	CAP-16B5-GL
PMB	PMB-5B10	IGHV1-7	IGHD5-5	IGHJ3	IGKV17-127	IGKJ2	CAP-5B10-GL
	PMB-11D5	IGHV1-14	IGHD3-1	IGHJ2	IGKV17-127	IGKJ2	PMB-11D5-GL1 PMB-11D5-GL2
	PMB-13G6	IGHV1-14	IGHD3-1	IGHJ2	IGKV17-127	IGKJ2	PMB-1B3-GL
	PMB-1B3	IGHV6-6	IGHD1-1	IGHJ2	IGKV4-61	IGKJ1	
	PMB-10D5	IGHV6-6	IGHD1-1	IGHJ2	IGKV4-61	IGKJ1	
SA	SA-4C7	IGHV3-1	IGHD1-1	IGHJ4	IGKV1-135	IGKJ1	SA-4C7-GL
	SA-4D11	IGHV1-87	IGHD1-1	IGHJ4	IGKV3-5	IGKJ1	SA-4D11-GL
	SA-10E6	IGHV5-6	IGHD2-12	IGHJ2	IGKV14-111	IGKJ1	SA-10E6-GL
WAR	WAR-15E9	IGHV1-22	IGHD2-14	IGHJ4	IGKV1-117	IGKJ1	WAR-15E9-GL
ANG	ANG-3B3	IGHV9-3-1	IGHD1-2	IGHJ2	IGKV1-135	IGKJ1	ANG-3B3-GL
	ANG-1H2	IGHV9-3-1	IGHD1-2	IGHJ2	IGKV1-135	IGKJ1	
	ANG-20A2	IGHV9-3-1	IGHD1-2	IGHJ2	IGKV1-135	IGKJ1	
TLF	TLF-3B4	IGHV4-1	IGHD3-2	IGHJ3	IGKV14-111	IGKJ5	TLF-3B4-GL

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98 **Table S3.** The categories of residues used in this study.

Category	Residues
Tiny	Ala, Cys, Gly, Ser, Thr
Small	Ala, Asn, Asp, Cys, Gly, Pro, Ser, Thr, Val
Aliphatic	Ala, Ile, Leu, Val
Aromatic	Phe, His, Trp, Tyr
Non-polar	Ala, Cys, Phe, Gly, Ile, Leu, Met, Pro, Val, Trp, Tyr
Polar	Asp, Glu, His, Lys, Asn, Gln, Arg, Ser, Thr
Charged	Asn, Asp, Gln, Glu, His, Lys, Arg
Basic	His, Lys, Arg
Acidic	Asn, Asp, Gln, Glu

99 The 20 standard amino acids, with their full English names, three-letter abbreviations, and
100 single-letter codes, are as follows: Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N),
101 Aspartic acid (Asp, D), Cysteine (Cys, C), Glutamine (Gln, Q), Glutamic acid (Glu, E), Glycine
102 (Gly, G), Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine
103 (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S), Threonine (Thr, T),
104 Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V).

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Table S4. Top 10 most frequently used IGHV germline genes in the hapten, peptide, along with their CDR residue composition and CDR length.

Groups	IGHV	Frequency	CDR residue composition		CDR length	
			CDRH1	CDRH2	CDRH1	CDRH2
Hapten	IGHV5-6-5	8.4%	GFTFSSYA	ISSGGST	8	7
	IGHV3-2	5.8%	GYSITSGYD	ISYSVAS	9	7
	IGHV1-76	4.0%	GYTFTDYY	IYPGSGNT	8	8
	IGHV1-9	4.0%	GYTFTGYW	ILPGSGST	8	8
	IGHV9-3-1	4.0%	GYTFTNYG	INTYTGEF	8	8
	IGHV1-18	3.6%	GYTFTDYN	INPNNGGT	8	8
	IGHV3-8	3.6%	GYSITSDY	ISYSGST	8	7
	IGHV14-3	3.3%	GFNIKNTY	IDPANGNT	8	8
	IGHV1-87	2.9%	GYTFTSYW	IYPGDGDT	8	8
	IGHV5-6-2	2.9%	GFTFSSYY	INSNGGST	8	8
Peptide	IGHV5-9-3	36.5%	GFTFSSYA	ISSGGSYT	8	8
	IGHV5-17	19.2%	GFTFSDYG	ISSGSSTI	8	8
	IGHV5-9	11.5%	GFTFSSYT	ISGGGGNT	8	8
	IGHV4-1	9.6%	GIDFSRYW	INPDSSTI	8	8
	IGHV5-6-3	3.8%	GFTFSSYG	INSNGGST	8	8
	IGHV10-3	1.9%	GFTFNTYA	IRSKSSNYAT	8	10
	IGHV1-18	1.9%	GYTFTDYN	INPNNGGT	8	8
	IGHV1-19	1.9%	GYTFTDYY	INPYNGGT	8	8
	IGHV14-3	1.9%	GFNIKNTY	IDPANGNT	8	8
	IGHV1-47-32	1.9%	GYTFTSYW	IHPDSET	8	8
Protein	IGHV5-17	14.3%	GFTFSDYG	ISSGSSTI	8	8
	IGHV14-3	7.5%	GFNIKNTY	IDPANGNT	8	8
	IGHV1S81	6.0%	GYTFTSYW	INPSNGRT	8	8
	IGHV1-26	4.4%	GYTFTDYY	INPNNGGT	8	8
	IGHV4-1	4.2%	GIDFSRYW	INPDSSTI	8	8
	IGHV1-9	2.9%	GYTFTGYW	ILPGSGST	8	8
	IGHV2-6-7	2.9%	GFSLTGYG	IWGDGST	8	7
	IGHV3S1	2.8%	GDSITSDY	ISYSGST	8	7
	IGHV1S136	1.8%	GYTFTDYV	IYPYNDGT	8	8
	IGHV1-82	1.8%	GYAFSSSW	IYPGDGDT	8	8

Table S5. Top 10 most frequently used IGKV/IGLV germline genes in the hapten, peptide, and protein groups, along with their CDR residue composition and CDR length.

Groups	IGKV/IGLV	Frequency	CDR residue composition		CDR length	
			CDRL1	CDRL2	CDRL1	CDRL2
Hapten	IGKV1-117	15.8%	QSIVHSNGNTY	KVS	11	3
	IGLV1	10.7%	TGAVTTSNY	GTN	9	3
	IGKV1-110	9.9%	QSLVHSNGNTY	KVS	11	3
	IGKV1-133	5.1%	QSLLYSNGKTY	LVS	11	3
	IGKV6-15	3.7%	QNVGTN	SAS	6	3
	IGKV9-124	3.7%	QEISGY	AAS	6	3
	IGKV3-12	3.3%	KSVSTSGYSY	LAS	10	3
	IGKV2-137	2.9%	KSLLSNGNTY	RMS	11	3
	IGKV4-79	2.9%	SSVSSSY	STS	7	3
	IGLV2	2.6%	TGAVTTSNY	GTS	9	3
Peptide	IGKV11-125	37.3%	QGTSIN	GAS	6	3
	IGKV10-94	19.6%	QGISNY	YTS	6	3
	IGLV1	7.8%	TGAVTTSNY	GTN	9	3
	IGKV13-85	5.9%	DHINNW	GAT	6	3
	IGKV6-15	5.9%	QNVGTN	SAS	6	3
	IGKV1-110	3.9%	QSLVHSNGNTY	KVS	11	3
	IGKV1-133	3.9%	QSLLYSNGKTY	LVS	11	3
	IGKV18-36	3.9%	ESVGSY	SAS	6	3
	IGKV1-117	2.0%	QSIVHSNGNTY	KVS	11	3
	IGKV12-44	2.0%	ENIYSY	NAK	6	3
Protein	IGKV2-137	14.5%	KSLLSNGNTY	RMS	11	3
	IGKV5-48	7.3%	QSIGTS	YAS	6	3
	IGKV4-59	6.1%	SSVSY	DTS	5	3
	IGKV5-43	4.7%	QSISSN	YAS	6	3
	IGKV10-96	3.9%	QDISNY	YTS	6	3
	IGKV14-100	3.9%	QGISSN	HGT	6	3
	IGKV8-30	3.5%	QSLLYSSNQKNY	WAS	12	3
	IGKV12-41	2.9%	GNIHNY	NAK	6	3
	IGKV3-5	2.8%	ESVDSYGNSF	RAS	10	3
	IGKV1-117	2.5%	QSIVHSNGNTY	KVS	11	3

Table S6. Top 5 most frequently used IGHV-IGKV/IGLV germline genes in the hapten, peptide, and protein groups, along with their the secondary-structure occupancies.

Groups	IGHV	IGKV/IGLV	Frequency	Helix	Sheet	Coil
Hapten	All	All	100%	5.6%	51.4%	43.0%
	IGHV1-76	IGKV1-133	3.7%	6.2%	49.7%	44.1%
	IGHV5-6-5	IGKV1-117	3.3%	4.1%	51.4%	44.5%
	IGHV9-3-1	IGKV1-110	2.2%	6.5%	50.4%	43.1%
	IGHV1-72	IGLV1	1.9%	6.5%	53.9%	39.5%
	IGHV3-8	IGKV4-79	1.9%	4.8%	53.8%	41.4%
Peptide	All	All	100%	5.0%	51.1%	43.9%
	IGHV5-9-3	IGKV11-125	29.4%	4.3%	50.6%	45.0%
	IGHV5-9	IGKV10-94	11.8%	4.1%	50.9%	45.0%
	IGHV5-17	IGKV11-125	7.8%	4.5%	51.0%	44.5%
	IGHV4-1	IGKV6-15	5.9%	6.6%	49.5%	43.9%
	IGHV5-9-3	IGKV13-85	5.9%	3.8%	52.1%	44.1%
Protein	All	All	100%	5.6%	51.0%	43.4%
	IGHV5-17	IGKV2-137	14.3%	4.6%	48.3%	47.1%
	IGHV1S81	IGKV5-48	5.9%	6.5%	51.3%	42.2%
	IGHV14-3	IGKV14-100	3.8%	5.4%	51.7%	42.9%
	IGHV4-1	IGKV4-59	3.7%	6.3%	52.5%	41.3%
	IGHV3S1	IGKV5-43	2.8%	4.9%	50.7%	44.4%

121 **Table S7.** Top 5 most frequently used IGHV-IGKV/IGLV germline genes in the hapten, peptide, and protein groups, along
122 with their pLDDT values.

Groups	IGHV	IGKV/IGLV	Frequency	Fv	VH	VL	CDRs	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
Hapten	All	All	100%	96.0	95.4	96.6	92.5	95.3	96.0	84.0	95.8	96.3	94.0
	IGHV1-76	IGKV1-133	3.7%	94.8	93.3	96.4	87.7	93.2	95.9	71.5	93.9	95.7	94.4
	IGHV5-6-5	IGKV1-117	3.3%	96.5	96.1	96.9	92.9	95.6	93.0	86.2	95.1	97.2	95.9
	IGHV9-3-1	IGKV1-110	2.2%	96.6	96.2	97.0	94.2	96.5	96.5	87.3	96.1	97.4	96.1
	IGHV1-72	IGLV1	1.9%	94.8	94.3	95.4	90.4	95.2	95.7	81.1	95.3	94.2	88.6
	IGHV3-8	IGKV4-79	1.9%	93.3	93.6	93.1	86.1	92.6	93.0	81.3	90.9	94.0	76.0
Peptide	All	All	100%	95.7	95.2	96.3	90.4	95.3	95.3	81.0	92.2	95.1	93.7
	IGHV5-9-3	IGKV11-125	29.4%	95.8	95.5	96.1	89.1	95.9	94.7	79.2	85.7	93.8	93.2
	IGHV5-9	IGKV10-94	11.8%	95.6	94.6	96.8	88.9	94.2	93.9	76.5	96.4	95.1	94.7
	IGHV5-17	IGKV11-125	7.8%	95.2	94.3	96.4	87.3	95.0	94.5	75.8	88.4	93.1	94.4
	IGHV4-1	IGKV6-15	5.9%	95.7	94.7	96.8	91.5	95.1	95.9	80.2	97.3	95.9	95.2
	IGHV5-9-3	IGKV13-85	5.9%	96.2	95.4	97.2	90.9	97.8	95.8	83.1	94.8	95.9	95.5
Protein	All	All	100%	96.3	95.8	96.8	93.1	96.0	96.4	86.2	95.8	96.7	94.4
	IGHV5-17	IGKV2-137	14.3%	96.1	95.4	96.8	91.1	94.7	95.9	78.6	95.2	96.0	96.3
	IGHV1S81	IGKV5-48	5.9%	97.1	96.8	97.3	96.5	97.5	97.7	94.0	97.6	98.3	96.5
	IGHV14-3	IGKV14-100	3.8%	96.2	95.6	96.9	92.9	95.2	97.1	83.0	97.5	95.9	96.5
	IGHV4-1	IGKV4-59	3.7%	95.3	95.0	95.6	89.3	95.9	96.4	81.1	92.4	93.7	86.9
	IGHV3S1	IGKV5-43	2.8%	96.8	96.4	97.1	95.3	95.8	97.0	88.7	98.0	98.0	96.9

127 **Table S8.** Top 5 most frequently used IGHV-IGKV/IGLV germline genes in the hapten, peptide, and protein groups, along
128 with their SASA.

Groups	IGHV	IGKV/IGLV	Frequency	Fv	VH	VL	CDRs	CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3
Hapten	All	All	100%	10458.8	5473.7	4985.1	2209.1	395.5	394.0	483.6	519.0	129.1	287.9
	IGHV1-76	IGKV1-133	3.7%	10693.3	5700.6	4992.7	2374.5	382.1	315.3	670.8	677.0	102.8	226.5
	IGHV5-6-5	IGKV1-117	3.3%	10510.5	5407.1	5103.4	2218.7	381.0	308.8	542.7	594.9	142.5	248.7
	IGHV9-3-1	IGKV1-110	2.2%	10662.0	5507.3	5154.7	2259.4	375.2	490.9	415.1	601.7	142.3	234.2
	IGHV1-72	IGLV1	1.9%	10381.0	5424.4	4956.6	2237.1	392.8	316.0	476.7	523.1	114.5	414.0
	IGHV3-8	IGKV4-79	1.9%	10198.6	5435.9	4762.7	2165.6	373.3	345.7	557.9	379.3	101.2	408.3
Peptide	All	All	100%	10578.8	5640.2	4938.6	2303.0	377.3	416.1	683.7	412.5	107.4	306.0
	IGHV5-9-3	IGKV11-125	29.4%	10543.6	5621.9	4921.7	2219.4	353.8	444.9	679.0	335.8	79.8	326.0
	IGHV5-9	IGKV10-94	11.8%	10498.7	5607.9	4890.8	2251.4	334.4	350.8	769.4	345.1	136.3	315.5
	IGHV5-17	IGKV11-125	7.8%	10931.3	5969.8	4961.5	2469.9	351.8	383.6	1035.1	352.3	83.2	263.9
	IGHV4-1	IGKV6-15	5.9%	10557.7	5657.7	4900.0	2203.9	459.7	406.4	606.8	348.9	89.7	292.4
	IGHV5-9-3	IGKV13-85	5.9%	10813.5	6179.3	4634.3	2704.3	388.3	426.2	1221.7	327.3	100.7	240.2
Protein	All	All	100%	10452.7	5523.4	4929.2	2206.8	382.4	393.7	551.5	456.2	126.8	296.1
	IGHV5-17	IGKV2-137	14.3%	10591.2	5549.2	5042.0	2470.3	309.3	378.9	718.0	653.2	148.5	262.3
	IGHV1S81	IGKV5-48	5.9%	10346.3	5504.7	4841.5	2004.4	417.4	400.9	473.3	302.6	120.8	289.4
	IGHV14-3	IGKV14-100	3.8%	10161.6	5529.9	4631.8	2056.8	443.0	371.8	548.1	297.6	139.6	256.7
	IGHV4-1	IGKV4-59	3.7%	10329.7	5656.2	4673.6	2175.8	472.2	405.7	581.1	278.5	98.1	340.2
	IGHV3S1	IGKV5-43	2.8%	10103.1	5293.3	4809.7	1751.3	380.7	327.5	321.0	329.6	126.2	266.2

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Table S9. Top 5 most frequently used IGHV-IGKV/IGLV germline genes in the hapten, peptide, and protein groups, along with their Fv SPs.

Groups	IGHV	IGKV/IGLV	Frequency	Total SPs		Hydrophobic SPs		Negative SPs		Positive SPs	
				Number	Area	Number	Area	Number	Area	Number	Area
Hapten	All	All	100%	21.8	1262.1	5.0	398.2	9.7	487.4	7.0	376.6
	IGHV1-76	IGKV1-133	3.7%	24.9	1494.0	7.1	512.0	5.8	337.0	12.0	645.0
	IGHV5-6-5	IGKV1-117	3.3%	22.0	1325.6	5.7	501.1	6.3	330.0	10.0	494.4
	IGHV9-3-1	IGKV1-110	2.2%	24.5	1411.7	6.2	458.3	8.7	448.3	9.7	505.0
	IGHV1-72	IGLV1	1.9%	22.0	1352.0	5.4	380.0	6.8	362.0	9.8	610.0
	IGHV3-8	IGKV4-79	1.9%	16.8	1048.0	2.4	276.0	6.2	412.0	8.2	360.0
Peptide	All	All	100%	23.8	1385.2	5.9	462.7	10.4	495.4	7.5	427.1
	IGHV5-9-3	IGKV11-125	29.4%	24.3	1448.7	6.9	550.7	7.7	460.0	9.8	438.0
	IGHV5-9	IGKV10-94	11.8%	24.8	1498.3	4.8	453.3	6.5	331.7	13.5	713.3
	IGHV5-17	IGKV11-125	7.8%	26.3	1617.5	8.3	567.5	10.3	682.5	7.8	367.5
	IGHV4-1	IGKV6-15	5.9%	25.3	1333.3	7.0	423.3	7.3	426.7	11.0	483.3
	IGHV5-9-3	IGKV13-85	5.9%	21.7	1120.0	3.7	270.0	5.7	286.7	12.3	563.3
Protein	All	All	100%	20.8	1214.3	4.2	347.9	9.2	467.9	7.3	398.4
	IGHV5-17	IGKV2-137	14.3%	20.0	1106.2	4.9	357.7	7.2	389.2	7.8	359.3
	IGHV1S81	IGKV5-48	5.9%	20.6	1209.7	4.2	322.7	7.0	349.5	9.4	537.5
	IGHV14-3	IGKV14-100	3.8%	21.4	1264.2	4.2	280.9	10.6	605.1	6.6	378.2
	IGHV4-1	IGKV4-59	3.7%	22.2	1215.6	5.7	400.2	5.5	310.7	10.9	504.7
	IGHV3S1	IGKV5-43	2.8%	16.4	1071.2	3.7	377.9	6.2	396.7	6.5	296.7

Table S10. Top 5 most frequently used IGHV-IGKV/IGLV germline genes in the hapten, peptide, and protein groups, along with their CDR SPs.

Groups	IGHV	IGKV/IGLV	Frequency	Total SPs		Hydrophobic SPs		Negative SPs		Positive SPs	
				Number	Area	Number	Area	Number	Area	Number	Area
Hapten	All	All	100%	7.1	480.9	2.0	194.6	3.7	203.2	1.4	83.1
	IGHV1-76	IGKV1-133	3.7%	9.5	604.0	3.5	256.0	4.9	296.0	1.1	52.0
	IGHV5-6-5	IGKV1-117	3.3%	4.6	382.2	1.7	225.6	2.1	116.7	0.8	40.0
	IGHV9-3-1	IGKV1-110	2.2%	8.0	533.3	3.2	241.7	2.3	163.3	2.5	128.3
	IGHV1-72	IGLV1	1.9%	8.2	650.0	1.6	176.0	5.6	430.0	1.0	44.0
	IGHV3-8	IGKV4-79	1.9%	4.6	484.0	1.6	232.0	1.6	82.0	1.4	170.0
Peptide	All	All	100%	7.9	536.3	2.4	236.3	3.5	183.5	2.0	116.5
	IGHV5-9-3	IGKV11-125	29.4%	7.3	542.7	2.7	301.3	2.5	116.7	2.1	124.7
	IGHV5-9	IGKV10-94	11.8%	8.8	615.0	2.2	228.3	5.8	350.0	0.8	36.7
	IGHV5-17	IGKV11-125	7.8%	9.3	705.0	3.8	305.0	1.8	75.0	3.8	325.0
	IGHV4-1	IGKV6-15	5.9%	6.3	350.0	2.0	123.3	2.7	110.0	1.7	116.7
	IGHV5-9-3	IGKV13-85	5.9%	7.7	440.0	2.0	146.7	3.7	193.3	2.0	100.0
Protein	All	All	100%	7.6	507.8	1.8	185.8	3.8	209.8	2.0	112.2
	IGHV5-17	IGKV2-137	14.3%	7.4	458.7	3.1	237.7	3.0	142.0	1.3	79.0
	IGHV1S81	IGKV5-48	5.9%	8.1	569.2	1.3	160.9	4.9	319.1	2.0	89.2
	IGHV14-3	IGKV14-100	3.8%	7.8	477.8	1.3	97.8	2.9	180.0	3.6	200.0
	IGHV4-1	IGKV4-59	3.7%	7.3	430.7	1.7	177.0	4.5	207.4	1.0	46.3
	IGHV3S1	IGKV5-43	2.8%	4.6	376.7	1.8	242.1	2.3	110.3	0.4	24.2

4. References

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