

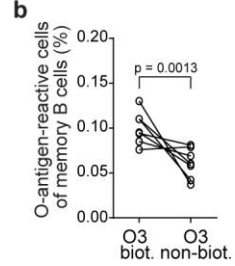
In the format provided by the authors and unedited.

Cross-specificity of protective human antibodies against *Klebsiella pneumoniae* LPS O-antigen

Tim Rollenske^{1,2}, Valeria Szijarto³, Jolanta Lukasiewicz⁴, Luis M. Guachalla⁵, Katarina Stojkovic⁴, Katharina Hartl³, Lukas Stulik³, Simone Kocher², Felix Lasitschka⁵, Mohammed Al-Saedi⁶, Jutta Schröder-Braunstein⁷, Moritz von Frankenberg⁸, Gereon Gaebelin^{9,12}, Peter Hoffmann¹⁰, Sabrina Klein¹¹, Klaus Heeg¹¹, Eszter Nagy³, Gabor Nagy³ and Hedda Wardemann^{1,2*}

¹Max Planck Research Group Molecular Immunology, Max Planck Institute for Infection Biology, Berlin, Germany. ²Division of B Cell Immunology, German Cancer Research Center, Heidelberg, Germany. ³Arsanis Biosciences, Vienna, Austria. ⁴Department of Immunochemistry, Ludwik Hirsfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wroclaw, Poland. ⁵Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany. ⁶Department of General and Transplant Surgery, University Hospital Heidelberg, Heidelberg, Germany. ⁷Institute of Immunology, University Hospital Heidelberg, Heidelberg, Germany. ⁸Department of General, Abdominal and Minimal Invasive Surgery, Hospital Salem, Heidelberg, Germany. ⁹Department of Visceral, Transplantation, Thoracic and Vascular Surgery, University Hospital Leipzig, Leipzig, Germany. ¹⁰Department of Gastroenterology, University Hospital Heidelberg, Heidelberg, Germany. ¹¹Department of Infectious Diseases, Medical Microbiology and Hygiene, University Hospital Heidelberg, Heidelberg, Germany. Present address: ¹²Department of General, Visceral, Vascular and Pediatric Surgery, Saarland University Medical Center, Homburg, Germany. *e-mail: h.wardemann@dkfz.de

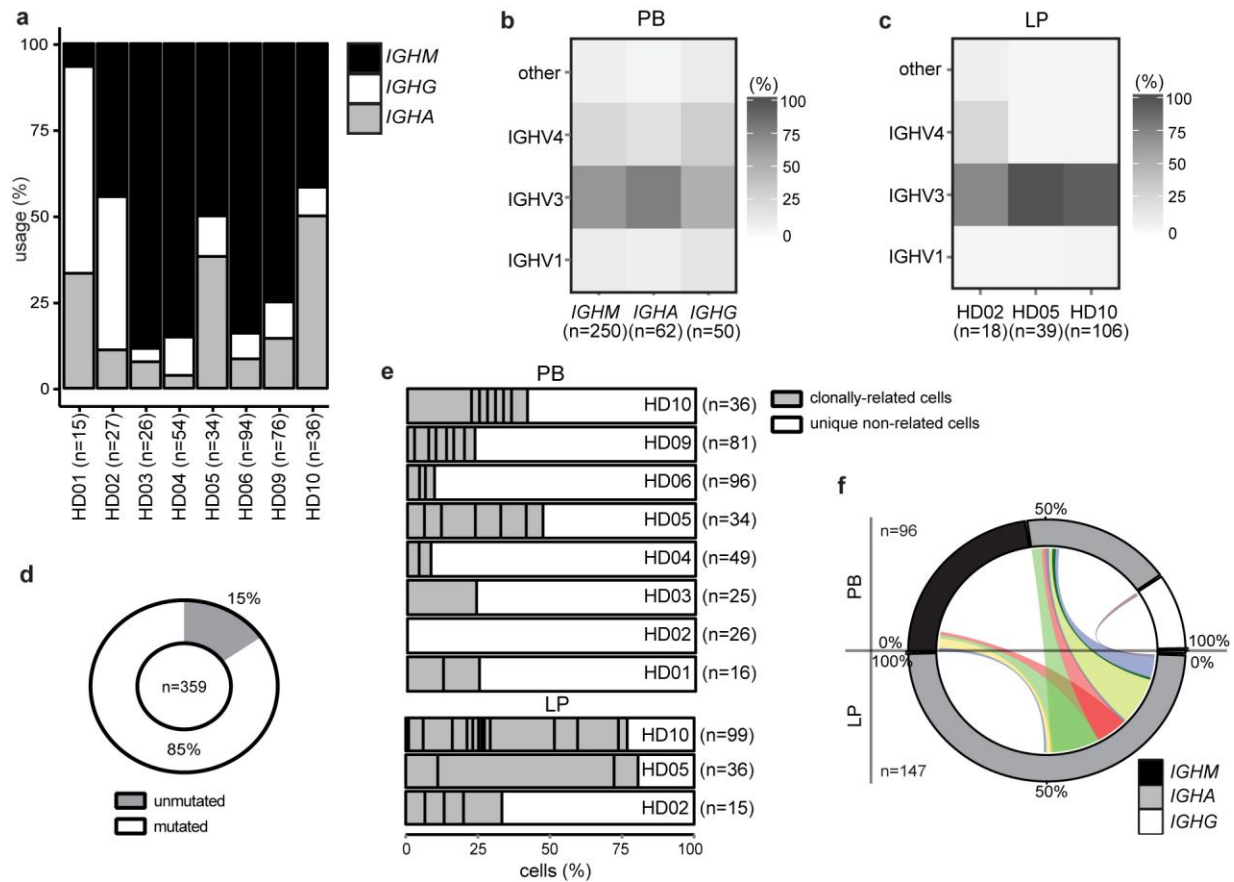
O3 MeP-[2- α Manp-(1-2)- α Manp-(1-2)- α Manp-(1-3)- α Manp-(1-3)- α Manp-(1-3)- α Manp-(1-3)- α Manp-(1-3)- α Manp-(1-CP
CP 3)- β GlcNAc-(1-5)-[H- α Hepp-(1-4)]- α Kdo-PEG_n-biotin



Supplementary Figure 1

Identification of O3-reactive B cells using biotinylated O3 O-antigen

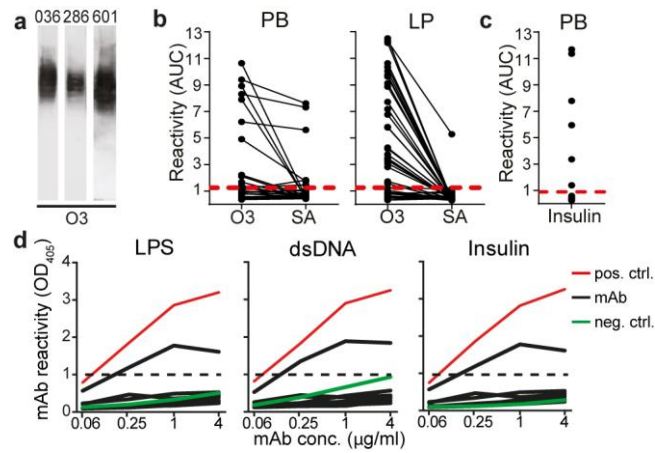
a, Schematic linear structure of biotinylated O3 *K. pneumoniae* O-antigen; repeating unit (outlined), MeP = Methylphosphate; Man = Mannose; KdO = 3-Deoxy-D-manno-oct-2-ulosonic acid; GlcNAc = N-acetylglucosamine; Hep = L-glycero-D-manno-heptose; PEG = Polyethylene glycol. **b**, Percentage of O3-reactive 7-AAD⁻, CD19⁺, CD27⁺ peripheral blood B cells in eight donors compared to the background staining using non-biotinylated O3 (right). p-value was calculated using two-tailed Student's t-test. Data are representative of at least two independent experiments.



Supplementary Figure 2

Immunoglobulin gene characteristics of O3-reactive PB and LP B cells

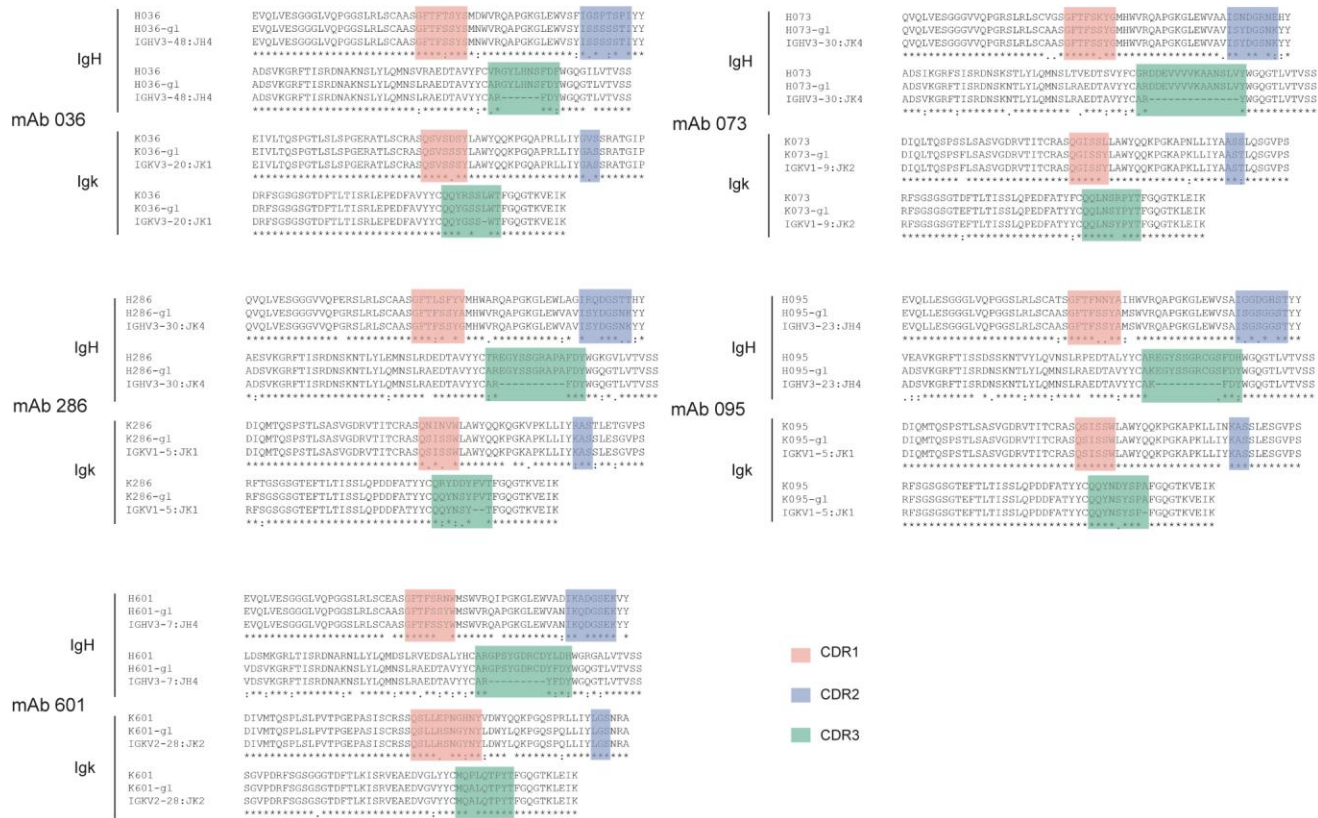
a, *IGH* gene isotype distribution for O3-reactive PB memory B cells isolated by flow cytometric single cell sorting. **b,c**, *IGHV* gene family usage for O3-reactive PB memory B cells of the indicated isotypes from the same donors as shown in panel **a** (**b**) and LP plasmablasts isolated by flow cytometric single cell sorting from the indicated donors (**c**). **d**, Percentage of mutated and unmutated *IGHV* genes from O3-reactive PB memory B cells of the same donors as in panel **a**. **e**, Distribution of cell clusters of clonally related (gray boxes) compared to unique non-related (white) O3-reactive B cells in PB memory B cells or LP plasmablasts from the indicated donors. **f**, Clonal relationship of O3-reactive B cells of the indicated isotypes in PB memory B cells and LP plasmablasts of donors HD02, HD05, and HD10. Colors depict individual B cell clusters. n indicates numbers of analyzed sequences.



Supplementary Figure 3

O3-reactive PB memory B cell and LP plasmablast antibodies bind specifically to O3 O-antigen

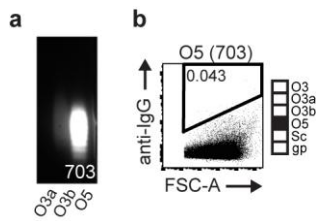
a, Immunoblot with whole O3 LPS for representative mAbs cloned from O3-reactive memory B cells from PB (036, 286) and from LP plasmablasts (601). **b**, O3 and streptavidin reactivity of individual recombinant mAbs from O3-reactive PB memory B cells ($n = 41$) and LP plasmablasts ($n = 41$) based on ELISA area under curve (AUC) measurements. **c**, Insulin ELISA reactivity of O3-reactive PB memory B cell antibodies ($n = 41$) under blocking conditions with 2% BSA. **d**, Polyreactivity ELISA with *E. coli* LPS, dsDNA and insulin under non-blocking conditions without BSA. Representative mAbs compared to the positive and negative control mAbs are shown. Dashed lines indicate negative thresholds (**b-d**). Data are representative data of at least two independent experiments (**a-d**).



Supplementary Figure 4

Germline reversion of mutated O3-reactive antibodies

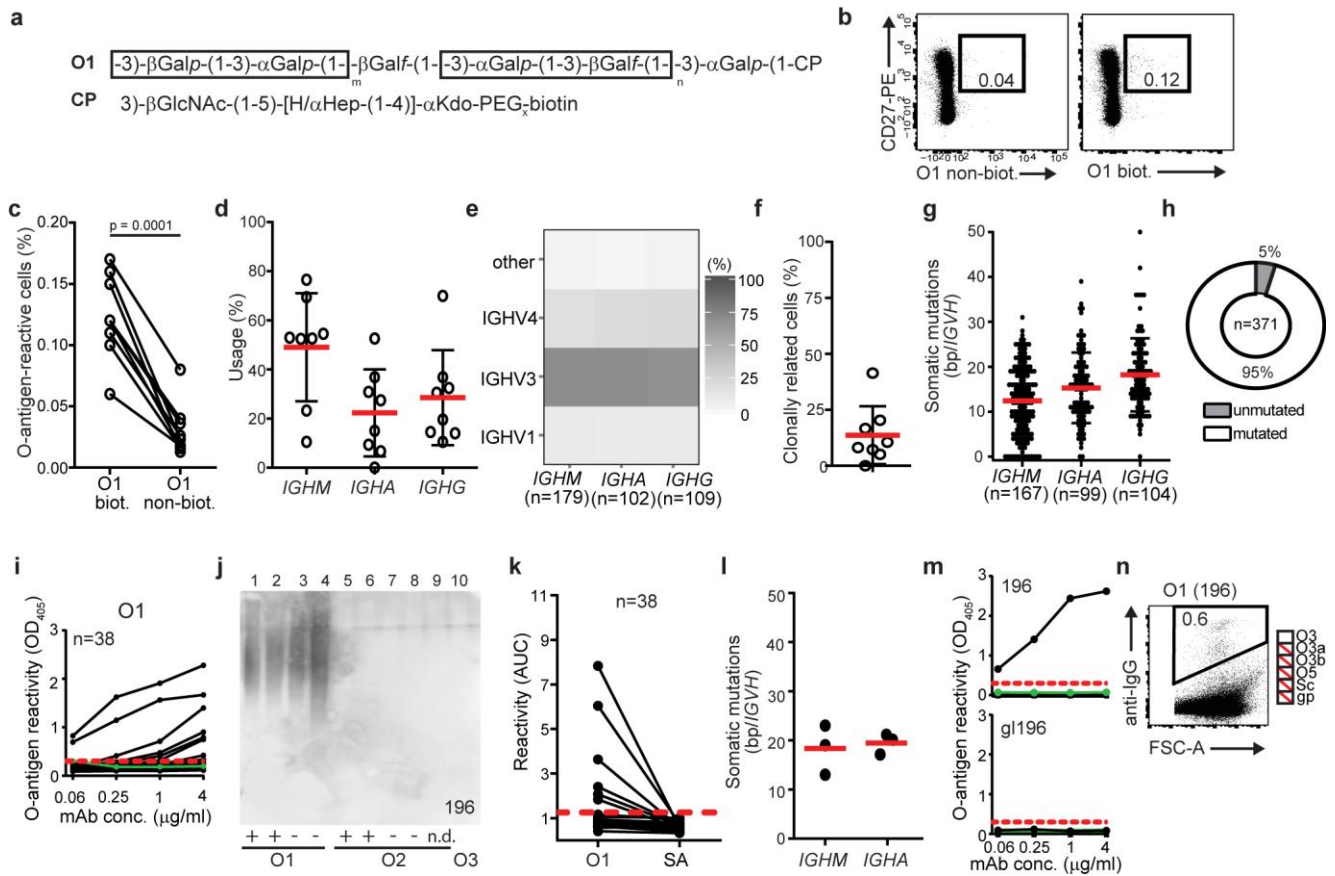
Gene sequence alignment of *IGH* and *IGK* or *IGL* variable regions of the indicated mutated mAbs and their inferred germline counterpart compared to the respective germline V and J gene segments. CDRs are highlighted.



Supplementary Figure 5

O5-specific mAb 703

a, mAb 703 shows specificity for O5 in immunoblot with whole LPS. **b**, Reactivity of mAb 703 with intestinal murine microbes as measured by flow cytometry. Sorting gate and percentage of antibody-bound bacteria is indicated. Boxes show the *K. pneumoniae* O-serotype, *S. cerevisiae*, and gp140 reactivity profile (strong-black; non-reactive-white). Data are representative of two independent experiments.

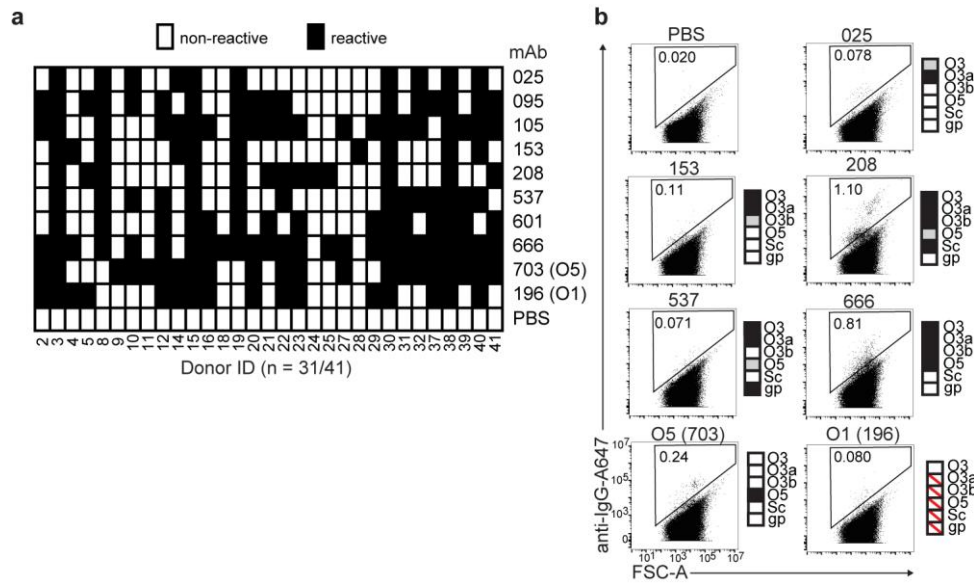


Supplementary Figure 6

Immunoglobulin gene features and antibody characteristics of O1-reactive PB B cells

a, Schematic linear structure of biotinylated O1; repeating units (outlined); Gal = Galactose; KdO = 3-Deoxy-D-manno-oct-2-ulosonic acid; GlcNAc = N-acetylglucosamine; Hep = L-glycero-D-manno-heptose; PEG = Polyethylene glycol. **b**, Flow cytometric analysis of the frequency of 7-AAD⁻, CD19⁺ PB B cells reactive to biotinylated O1 (right) compared to the negative control using non-biotinylated O1 (left) for a representative healthy donor detected by Streptavidin Alexa647 conjugate. Gates and percentages indicate the frequency of O1-reactive cells. **c**, Percentage of O1-reactive 7-AAD⁻, CD19⁺ PB B cells (left) in eight donors compared to the background staining using non-biotinylated O1 (right). p-value was calculated using two-tailed Student's t-test. **d**, *IGH* gene isotype usage for O1-reactive PB memory B cells from the same donors as in panel c. Means (red lines) and SD (black lines) are indicated. **e**, *IGHV* gene family usage for O1-reactive PB memory B cells of the indicated isotypes from the same donors as shown in panel d. n represents the number of analyzed *IGH* sequences per isotype. **f**, Percent of clonally-related O1-reactive B cells per sample in PB memory B cells of the same donors as in panel d. Means (red lines) and SD (black lines) are indicated. **g**, Number of somatic hypermutations in *IGHV* genes of the indicated isotypes from O1-reactive peripheral blood B cells. Data are pooled from the same eight donors as in panel d. Means (red lines) and SD (black lines) are indicated. n represents the number of analyzed *IGH* sequences per isotype. **h**, Percentage of mutated and unmutated *IGHV* genes from O1-reactive PB memory B cells. **i**, O1-reactivity of mAbs cloned from O1-reactive PB memory B cells as measured by ELISA. Green lines indicate the negative

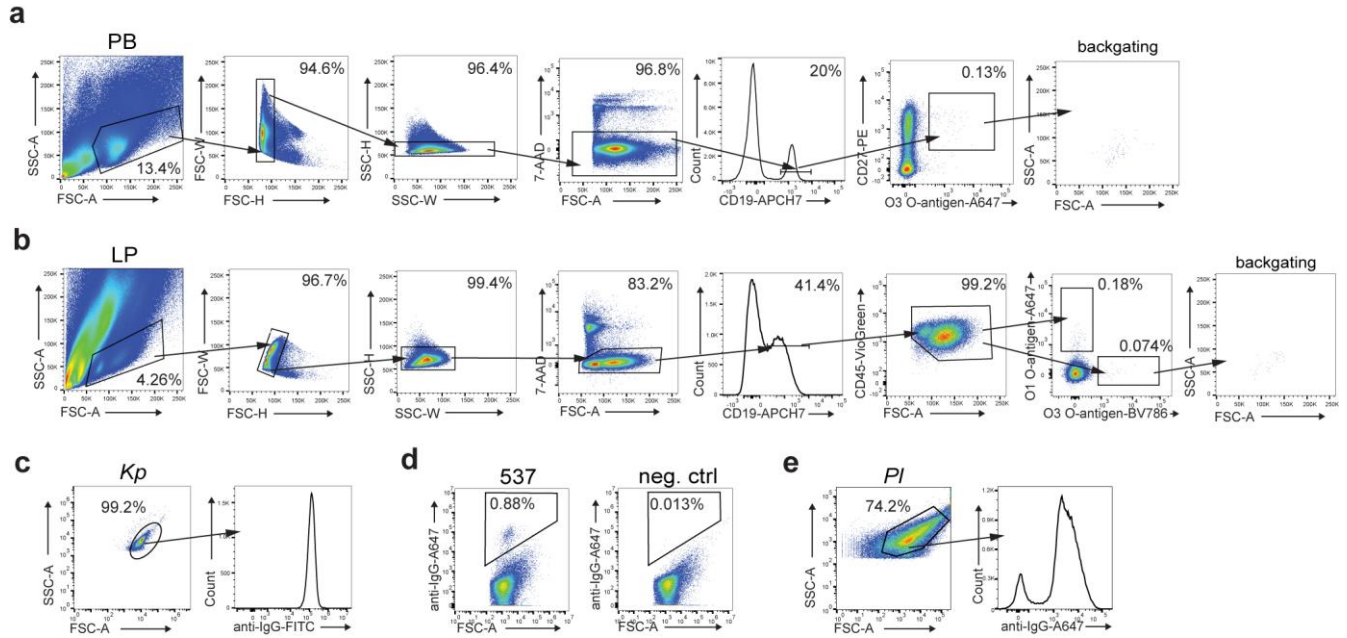
isotype control mAb and red lines the threshold for positivity. n indicates the number of tested mAbs. **j**, Immunoblot reactivity with O1, O2, and O3 LPS for a representative O1-reactive (anti-galactan II) mAb; + and - indicate serotypes expressing the galactan III or the galactan I variant, respectively. **k**, O1 and streptavidin reactivity of individual mAbs from O1-reactive PB memory B cells based on ELISA area under curve (AUC) measurements. **l**, *IGHV* gene somatic hypermutation counts for ELISA O1-specific mAbs (**i**, **k**) of the indicated isotypes (*IGHM*, n = 3; *IGHA*, n = 3). Red lines indicate means. **m**, Anti-O1 ELISA reactivity of the indicated mAb (top) and its predicted germline form (bottom). Green lines indicate reactivity of the negative isotype control mAb. Red lines indicate negative thresholds. **n**, Reactivity of the O1-reactive mAb 196 with murine intestinal microbes as measured by flow cytometry (**Fig. 6a**). Sorting gate and percentage of antibody-bound bacteria is indicated. Boxes show the *K. pneumoniae* O-serotype, *S. cerevisiae*, and gp140 reactivity profile (non-reactive-white). Red diagonal lines indicate non-tested. Data are representative of at least two independent experiments (**b**, **c**, **i** – **k**, **m**, **n**).



Supplementary Figure 7

Cross-specificity of individual *K. pneumoniae* O-antigen reactive antibodies with microbes from human intestine

a,b, Reactivity of the indicated mAbs with intestinal microbes of 31/41 tested human stool samples (**a**) and from a single individual as measured by flow cytometry compared to the PBS negative control (**b**). Sorting gate and percentage of antibody-bound bacteria are indicated. The *K. pneumoniae* O-serotype, *S. cerevisiae*, and gp140 reactivity profiles of each antibody are shown. Data are representative of two independent experiments (**b**).



Supplementary Figure 8

Flow cytometry gating strategy

a-e, Flow cytometry gating strategies for O3-reactive PB memory B cells (**a**; **Fig. 1**) and O1- and O3-reactive LP plasmablasts (**b**; **Fig. 1**) as well as for mAbs with reactivity for *K. pneumoniae* (**c**; **Fig. 3**), a representative human microbiota sample (**d**; **Fig. 6**), and *P. luteola* (**e**; **Fig. 6**).

Supplementary Table 1: Ig gene and reactivity information of O-antigen-reactive PB memory B cell antibodies

Clone	Donor	Cluster	IGHV	IGHD	IGHJ	HSHM	Isotype	IGKV	IGKJ	KSHM	IGLV	IGLJ	LSHM	O3 ELISA	O3 Immunoblot	Streptavidin	Insulin
028	HD09		IGHV3-72*01	IGHD5-24*01	IGHJ4*02	18	IGHM	IGKV1-16*01	IGKJ4*01	8				0	n.d.	0	0
030	HD09		IGHV3-30*18		IGHJ4*02	12	IGHM	IGKV3-20*01	IGKJ1*01	13				0	n.d.	0	0
035	HD09		IGHV4-4*02	IGHD5-12*01	IGHJ4*02	24	IGHA1	IGKV1-5*01	IGKJ3*01	16				0	n.d.	0	0
036	HD09	36	IGHV3-48*04		IGHJ4*02	16	IGHM	IGKV3-20*01	IGKJ1*01	5				1	1	0	0
045	HD09	83	IGHV4-61*08	IGHD2-8*02	IGHJ4*01	40	IGHM				IGLV1-51*01	IGLJ3*01	22	1	1	0	0
047	HD09	83	IGHV4-61*01	IGHD2-8*01	IGHJ4*02	32	IGHM				IGLV1-51*01	IGLJ3*01	12	1	1	0	0
055	HD06		IGHV4-b*01	IGHD2-15*01	IGHJ4*02	2	IGHM	IGKV2-28*01	IGKJ2*01	0				0	smear	0	0
056	HD06		IGHV4-34*01	IGHD3-10*01	IGHJ1*01	20	IGHG3	IGKV3-15*01	IGKJ4*02	11				0	n.d.	1	1
059	HD06		IGHV3-11*05	IGHD5-12*01	IGHJ4*02	11	IGHM	IGKV2-28*01	IGKJ1*01	3				0	n.d.	0	0
060	HD06		IGHV1-18*01	IGHD4-4*01	IGHJ5*02	11	IGHM	IGKV3-20*01	IGKJ1*01	0				0	n.d.	0	0
061	HD06		IGHV5-51*01	IGHD2-15*01	IGHJ4*02	0	IGHM	IGKV3-20*01	IGKJ1*01	0				0	n.d.	0	0
073	HD09		IGHV3-30*03	IGHD2-15*01	IGHJ4*02	26	IGHA1	IGKV1-9*01	IGKJ2*01	9				1	1	0	0
074	HD09	36	IGHV3-48*04	IGHD1-1*01	IGHJ4*02	27	IGHA1	IGKV3-20*01	IGKJ1*01	9				1	1	0	0
075	HD09		IGHV3-11*01		IGHJ3*02	25	IGHA2	IGKV3-20*01	IGKJ1*01	12				0	n.d.	0	0
080	HD09	83	IGHV4-61*01	IGHD2-8*01	IGHJ4*02	33	IGHM				IGLV1-51*01	IGLJ3*01	23	1	smear	1	1
081	HD09		IGHV4-4*02	IGHD3-10*01	IGHJ5*02	5	IGHM	IGKV1-5*01	IGKJ1*01	5				0	n.d.	0	0
093	HD09		IGHV3-23*04	IGHD6-19*01	IGHJ4*02	21	IGHM				IGLV4-69*01	IGLJ3*01	9	1	n.d.	1	1
096	HD09	37	IGHV3-48*04	IGHD6-19*01	IGHJ4*02	24	IGHM				IGLV1-51*01	IGLJ1*01	10	1	smear	1	1
100	HD06		IGHV1-18*01	IGHD3-16*02	IGHJ4*02	4	IGHM				IGLV1-51*01	IGLJ3*01	2	0	n.d.	0	0
104	HD06	46	IGHV3-53*01	IGHD1-26*01	IGHJ4*02	15	IGHM				IGLV1-44*01	IGLJ2*01	7	0	1	0	0
105	HD06		IGHV3-21*01	IGHD2-15*01	IGHJ5*02	6	IGHM	IGKV1-9*01	IGKJ2*01	1				0	n.d.	0	0
113	HD06		IGHV4-b*01	IGHD4-4*01	IGHJ4*02	16	IGHA2	IGKV3-20*01	IGKJ4*01	7				0	1	0	0
114	HD06		IGHV3-23*04	IGHD4-4*01	IGHJ4*02	14	IGHM	IGKV3-20*01	IGKJ4*01	4				0	n.d.	0	0
115	HD06	47	IGHV3-53*01	IGHD1-26*01	IGHJ5*02	25	IGHM				IGLV1-44*01	IGLJ2*01	11	1	1	0	0
118	HD06		IGHV3-72*01	IGHD3-9*01	IGHJ4*02	5	IGHM	IGKV1-16*02	IGKJ4*01	3				0	n.d.	1	0
127	HD09		IGHV1-8*01	IGHD1-7*01	IGHJ4*02	20	IGHG2	IGKV4-1*01	IGKJ1*01	9				0	n.d.	0	0
139	HD09		IGHV4-59*01		IGHJ5*02	50	IGHA1				IGLV2-11*01	IGLJ3*01	4	1	smear	1	1
142	HD09		IGHV4-39*01	IGHD6-19*01	IGHJ1*01	6	IGHM	IGKV3-15*01	IGKJ1*01	0				0	n.d.	0	0
144	HD09	37	IGHV3-48*04	IGHD6-19*01	IGHJ4*02	17	IGHM				IGLV1-51*01	IGLJ1*01	4	1	1	0	0
242	HD06		IGHV3-72*01	IGHD1-1*01	IGHJ4*02	3	IGHM	IGKV1-16*02	IGKJ4*01	1				0	n.d.	0	0
266	HD09		IGHV3-23*04		IGHJ4*02	25	IGHG2	IGKV3-15*01	IGKJ2*01	7				0	n.d.	0	0
278	HD09		IGHV3-9*01	IGHD3-22*01	IGHJ4*02	7	IGHA2	IGKV3-11*01	IGKJ3*01	4				0	n.d.	0	0
286	HD09	24	IGHV3-30*04	IGHD6-19*01	IGHJ4*02	26	IGHA2	IGKV1-5*03	IGKJ1*01	12				1	1	0	0
290	HD06	55	IGHV3-7*02	IGHD5-12*01	IGHJ4*02	28	IGHA1	IGKV1-27*01	IGKJ1*01	8				0	n.d.	0	0
291	HD06		IGHV4-61*08	IGHD6-13*01	IGHJ4*02	10	IGHG2	IGKV3-15*01	IGKJ4*01	4				0	n.d.	0	0
298	HD06		IGHV3-23*04	IGHD1-26*01	IGHJ6*03	15	IGHA1	IGKV1-39*01	IGKJ1*01	11				0	n.d.	0	0
302	HD06	55	IGHV3-7*02	IGHD5-12*01	IGHJ4*02	28	IGHA1	IGKV1-27*01	IGKJ1*01	8				0	n.d.	0	0
339	HD06		IGHV4-4*02	IGHD4-17*01	IGHJ3*02	21	IGHG1	IGKV1-39*01	IGKJ1*01	10				0	n.d.	0	0
368K	HD09		IGHV4-31*03	IGHD6-6*01	IGHJ6*02	17	IGHA1	IGKV1-8*01	IGKJ1*01	0				0	n.d.	0	1
368L	HD09		IGHV4-31*03	IGHD6-6*01	IGHJ6*02	17	IGHA1				IGLV1-51*01	IGLJ3*01	7	0	n.d.	0	0
376	HD09		IGHV3-30*04	IGHD5-12*01	IGHJ4*02	23	IGHG2	IGKV1-33*01	IGKJ3*01	12				0	n.d.	0	0

HD = healthy donor; cluster = clonally related cells; 1 = reactive; 0 = non-reactive

Supplementary Table 2: Ig gene and reactivity information of O-antigen-reactive LP plasmablast antibodies

Clone	Donor	Cluster	IGHV	IGHD	IGHJ	HSJM	Isotype	IGKV	IGKJ	KSHM	IGLV	IGLJ	LSHM	O3 ELISA	O3 Immunoblot	Streptavidin
008L	HD10	15	IGHV3-23*01	IGHD2-8*01	IGHJ4*02	28	IGHA1	IGKV3-20*01	IGKJ2*02	34	IGLV1-40*02	IGLJ2*01	10	0	0	0
009	HD10	25	IGHV3-30*01	IGHD3-10*01	IGHJ4*02	27	IGHA1	IGKV1-5*03	IGKJ2*01	10				1	1	0
014	HD10	63	IGHV3-7*03	IGHD3-10*01	IGHJ4*02	22	IGHA1	IGKV2-28*01	IGKJ2*01	8				1	1	0
025	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	24	IGHA1	IGKV2-30*01	IGKJ1*01	17				1	1	0
029	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	25	IGHA1	IGKV2-30*01	IGKJ1*01	15				1	1	0
062	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	25	IGHA1	IGKV2-30*01	IGKJ1*01	19				1	1	0
095	HD10	19	IGHV3-23*01	IGHD3-22*01	IGHJ4*02	30	IGHA2	IGKV1-5*03	IGKJ1*01	11				1	1	0
103	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	30	IGHA1	IGKV2-30*01	IGKJ1*01	22				1	1	0
105	HD10	63	IGHV3-7*03	IGHD2-21*02	IGHJ4*02	23	IGHA1	IGKV2-28*01	IGKJ2*01	19				1	1	0
111	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	27	IGHA1	IGKV2-30*01	IGKJ1*01	21				1	1	0
113	HD10	19	IGHV3-23*01	IGHD2-15*01	IGHJ4*02	27	IGHA2	IGKV1-5*03	IGKJ1*01	15				0	0	0
131	HD10		IGHV4-4*02	IGHD5-24*01	IGHJ4*02	16	IGHA1	IGKV3-11*01	IGKJ5*01	12				0	0	0
142	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	25	IGHA1	IGKV2-30*01	IGKJ1*01	18				1	1	0
147	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	23	IGHA1	IGKV2-30*01	IGKJ1*01	18				1	1	0
153	HD10		IGHV3-48*02	IGHD2-8*01	IGHJ4*02	27	IGHA2	IGKV3-11*01	IGKJ1*01	20				1	1	0
163	HD10		IGHV3-23*01		IGHJ4*01	37	IGHA1	IGKV2-28*01	IGKJ1*01	6				0	0	0
171	HD10	15	IGHV3-23*01	IGHD2-8*01	IGHJ4*02	24	IGHA2	IGKV2-30*01	IGKJ2*01	29				1	1	0
175	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	27	IGHA1	IGKV2-30*01	IGKJ1*01	20				0	0	0
177	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	35	IGHA1	IGKV2-30*01	IGKJ1*01	13				0	0	0
182	HD10		IGHV3-30*03	IGHD1-7*01	IGHJ2*01	29	IGHA1	IGKV1-5*03	IGKJ2*02	14				1	0	1
183	HD10		IGHV3-23*01	IGHD3-9*01	IGHJ4*02	17	IGHA2	IGKV2-30*01	IGKJ2*01	5				0	0	0
191	HD10	26	IGHV3-30*01	IGHD3-22*01	IGHJ4*02	30	IGHA2	IGKV1-5*03	IGKJ1*01	13				1	1	0
208	HD10		IGHV4-39*05		IGHJ4*02	18	IGHA2				IGLV1-44*01	IGLJ2*01	16	1	1	0
212	HD10	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	26	IGHA1	IGKV2-30*01	IGKJ1*01	17				1	1	0
461	HD10	19	IGHV3-23*01	IGHD6-19*01	IGHJ4*02	31	IGHA1	IGKV1-5*03	IGKJ1*01	13				1	1	0
533L	HD10	16	IGHV3-23*02	IGHD7-27*01	IGHJ3*02	27	IGHA2	IGKV2-30*01	IGKJ1*01	24	IGLV1-51*01	IGLJ3*02	14	0	0	0
537	HD10	19	IGHV3-23*01	IGHD6-19*01	IGHJ4*02	20	IGHA1	IGKV1-5*03	IGKJ1*01	20				1	1	0
547	HD10	64	IGHV3-7*03	IGHD4-17*01	IGHJ1*01	24	IGHA1	IGKV2-28*01	IGKJ2*01	13				1	1	0
552	HD10	63	IGHV3-7*03	IGHD3-16*01	IGHJ4*02	21	IGHA1	IGKV2-28*01	IGKJ2*01	16				1	1	0
555	HD10	64	IGHV3-7*03	IGHD4-17*01	IGHJ1*01	24	IGHA1	IGKV2-28*01	IGKJ2*01	12				1	1	0
583	HD10	20	IGHV3-23*01	IGHD3-10*02	IGHJ4*02	44	IGHA1	IGKV1-39*01	IGKJ1*01	19				0	1	0
598	HD10		IGHV1-69*13	IGHD3-10*01	IGHJ3*01	34	IGHA1	IGKV2-24*01	IGKJ1*01	1				0	0	0
601	HD10	63	IGHV3-7*03	IGHD2-21*02	IGHJ4*02	26	IGHA1	IGKV2-28*01	IGKJ2*01	15				1	1	0
657	HD10	52	IGHV3-64*05	IGHD7-27*01	IGHJ4*02	28	IGHA1	IGKV2-30*01	IGKJ1*01	23				0	0	0
666	HD10	16	IGHV3-23*01	IGHD7-27*01	IGHJ3*01	30	IGHA2	IGKV2-30*02	IGKJ1*01	14				1	1	0
671	HD10	28	IGHV3-30*10		IGHJ4*02	19	IGHA1	IGKV1-17*01	IGKJ3*01	10				0	0	0
694	HD10		IGHV3-23*02		IGHJ3*02	36	IGHA1	IGKV2-30*01	IGKJ1*01	23				1	1	0
702	HD10	27	IGHV3-30*06	IGHD6-19*01	IGHJ4*02	33	IGHA2	IGKV1-5*03	IGKJ1*01	12				1	1	0
703	HD10	16	IGHV3-23*02	IGHD7-27*01	IGHJ3*02	24	IGHA2	IGKV2-30*01	IGKJ1*01	19				0	0	0
713	HD10		IGHV3-23*01	IGHD7-27*01	IGHJ4*02	21	IGHA2	IGKV1-5*03	IGKJ1*01	10	IGLV1-51*02	IGLJ2*01	7	1	0	1
715	HD10	63	IGHV3-7*03	IGHD3-16*01	IGHJ4*02	26	n/a	IGKV1-39*01	IGKJ1*01	14				0	0	0

HD = healthy donor; cluster = clonally related cells; 1 = reactive; 0 = non-reactive

Supplementary Table 3: Ig gene and reactivity information of O-antigen-specific memory B cell and LP plasmablast antibodies

Antibodies				Ig gene features								Antibody reactivity																	
				IGH				IGK/IGL				O-antigen ELISA					Immunoblot			Kp FACS			ELISA	SPR	Polyreactivity ELISA				
Clone	Donor	Tissue	Cluster	IGHV	IGHD	IGHJ	SHM	Isotype	IGKV/IGLV	IGKJ/IGLJ	SHM	O3	EC50 [ug/ml]	O3a	O3b	O5	O3a	O3b	O5	O3	O3a	O3b	O5	ASCA	gp140	dsDNA	E. coli	LPS	Insulin
009	HD10	LP	25	IGHV3-30*01	IGHD3-10*01	IGHJ4*02	27	IGHA1	IGKV1-5*03	IGKJ2*01	10	1	1.2	1	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0
014	HD10	LP	63	IGHV3-7*03	IGHD3-10*01	IGHJ4*02	22	IGHA1	IGKV2-28*01	IGKJ2*01	8	1	7.7	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	1	1	0	0	0	
025	HD10	LP	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	24	IGHA1	IGKV2-30*01	IGKJ1*01	17	1	6.2	1	0	0	1	0	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
029	HD10	LP	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	25	IGHA1	IGKV2-30*01	IGKJ1*01	15	1	1.7	1	0	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
036	HD09	PB	36	IGHV3-48*04		IGHJ4*02	16	IGHM	IGKV3-20*01	IGKJ1*01	5	1	0.1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	
045	HD09	PB	83	IGHV4-61*08	IGHD2-8*02	IGHJ4*01	40	IGHM	IGLV1-51*01	IGLJ3*01	22	1	1.8	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
047	HD09	PB	83	IGHV4-61*01	IGHD2-8*01	IGHJ4*02	32	IGHM	IGLV1-51*01	IGLJ3*01	12	1	3.1	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
062	HD10	LP	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	25	IGHA1	IGKV2-30*01	IGKJ1*01	19	1	2.4	1	0	0	1	1	0	1	1	0	0	0	0	0	0	0	
073	HD09	PB		IGHV3-30*03	IGHD2-15*01	IGHJ4*02	26	IGHA1	IGKV1-9*01	IGKJ2*01	9	1	0.1	1	0	0	1	0	0	n.d.	n.d.	n.d.	n.d.	0	1	0	0	0	
074	HD09	PB	36	IGHV3-48*04	IGHD1-1*01	IGHJ4*02	27	IGHA1	IGKV3-20*01	IGKJ1*01	9	1	0.1	1	0	0	1	0	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
095	HD10	LP	19	IGHV3-23*01	IGHD3-22*01	IGHJ4*02	30	IGHA2	IGKV1-5*03	IGKJ1*01	11	1	3.3	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	
103	HD10	LP	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	30	IGHA1	IGKV2-30*01	IGKJ1*01	22	1	2.1	1	0	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
104	HD06	PB	46	IGHV3-53*01	IGHD1-26*01	IGHJ4*02	15	IGHM	IGLV1-44*01	IGLJ2*01	7	1	>20	1	1	0	1	1	1	n.d.	n.d.	n.d.	n.d.	0	1	0	0	0	
105	HD10	LP	63	IGHV3-7*03	IGHD2-21*02	IGHJ4*02	23	IGHA1	IGKV2-28*01	IGKJ2*01	19	1	0.5	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	1	0	0	0	
111	HD10	LP	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	27	IGHA1	IGKV2-30*01	IGKJ1*01	21	1	6.8	1	0	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
113	HD06	PB		IGHV4-b*01	IGHD4-4*01	IGHJ4*02	16	IGHA2	IGKV3-20*01	IGKJ4*01	7	1	6.6	1	1	1	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
115	HD06	PB	47	IGHV3-53*01	IGHD1-26*01	IGHJ5*02	25	IGHM	IGLV1-44*01	IGLJ2*01	11	1	3.6	1	1	1	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	1	1	1	
142	HD10	LP	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	25	IGHA1	IGKV2-30*01	IGKJ1*01	18	1	2.6	1	0	0	1	0	0	1	1	0	0	0	0	0	0	0	
144	HD09	PB	37	IGHV3-48*04	IGHD6-19*01	IGHJ4*02	17	IGHM	IGLV1-51*01	IGLJ1*01	4	1	6.1	1	0	0	1	0	0	n.d.	n.d.	n.d.	n.d.	0	1	0	0	0	
147	HD10	LP	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	23	IGHA1	IGKV2-30*01	IGKJ1*01	18	1	1.2	1	0	0	1	0	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
153	HD10	LP		IGHV3-48*02	IGHD2-8*01	IGHJ4*02	27	IGHA2	IGKV3-11*01	IGKJ1*01	20	1	0.2	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
171	HD10	LP	15	IGHV3-23*01	IGHD2-8*01	IGHJ4*02	24	IGHA2	IGKV3-20*01	IGKJ2*01	29	1	0.1	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
191	HD10	LP	26	IGHV3-30*01	IGHD3-22*01	IGHJ4*02	30	IGHA2	IGKV1-5*03	IGKJ1*01	13	1	3.3	1	1	1	1	1	1	n.d.	n.d.	n.d.	n.d.	0	1	0	0	0	
208	HD10	LP		IGHV4-39*05		IGHJ4*02	18	IGHA2	IGLV1-44*01	IGLJ2*01	16	1	1.4	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	
212	HD10	LP	52	IGHV3-64*05	IGHD3-9*01	IGHJ4*02	26	IGHA1	IGKV2-30*01	IGKJ1*01	17	1	2.8	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
286	HD09	PB	24	IGHV3-30*04	IGHD6-19*01	IGHJ4*02	26	IGHA2	IGKV1-5*03	IGKJ1*01	12	1	2.1	1	1	1	1	1	1	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
461	HD10	LP	19	IGHV3-23*01	IGHD6-19*01	IGHJ4*02	31	IGHA1	IGKV1-5*03	IGKJ1*01	13	1	2.3	1	0	0	1	0	1	n.d.	n.d.	n.d.	n.d.	0	1	0	0	0	
537	HD10	LP	19	IGHV3-23*01	IGHD6-19*01	IGHJ4*02	20	IGHA1	IGKV1-5*03	IGKJ1*01	20	1	0.8	1	0	1	1	0	1	1	1	0	1	0	1	0	0	0	
547	HD10	LP	64	IGHV3-7*03	IGHD4-17*01	IGHJ1*01	24	IGHA1	IGKV2-28*01	IGKJ2*01	13	1	19.0	1	1	1	1	1	0	n.d.	n.d.	n.d.	n.d.	0	0	0	0	0	
552	HD10	LP	63	IGHV3-7*03	IGHD3-16*01	IGHJ4*02	21	IGHA1	IGKV2-28*01	IGKJ2*01	16	1	0.5	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	1	0	0	0	
555	HD10	LP	64	IGHV3-7*03	IGHD4-17*01	IGHJ1*01	24	IGHA1	IGKV2-28*01	IGKJ2*01	12	1	0.1	1	1	0	1	1	0	n.d.	n.d.	n.d.	n.d.	0	1	0	0	0	
601	HD10	LP	63	IGHV3-7*03	IGHD2-21*02	IGHJ4*02	26	IGHA1	IGKV2-28*01	IGKJ2*01	15	1	0.1	1	1	0	1	1	0	1	1	1	0	0	1	0	0	0	
666	HD10	LP	16	IGHV3-23*01	IGHD7-27*01	IGHJ3*01	30	IGHA2	IGKV2-30*02	IGKJ1*01	14	1	0.2	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0	
694	HD10	LP		IGHV3-23*02		IGHJ3*02	36	IGHA1	IGKV2-30*01	IGKJ1*01	23	1	4.5	1	0	1	1	1	1	1	1	0	1	0	0	0	0	0	
702	HD10	LP	27	IGHV3-30*06	IGHD6-19*01	IGHJ4*02	33	IGHA2	IGKV1-5*03	IGKJ1*01	12	1	3.2	1	1	0	1	1	0	1	1	1	1	0	1	0	0	0	

HD = healthy donor; PB= peripheral blood; LP = lamina propria; cluster =clonally related cells; 1 = reactive; 0 = non-reactive; n.d. = not determined