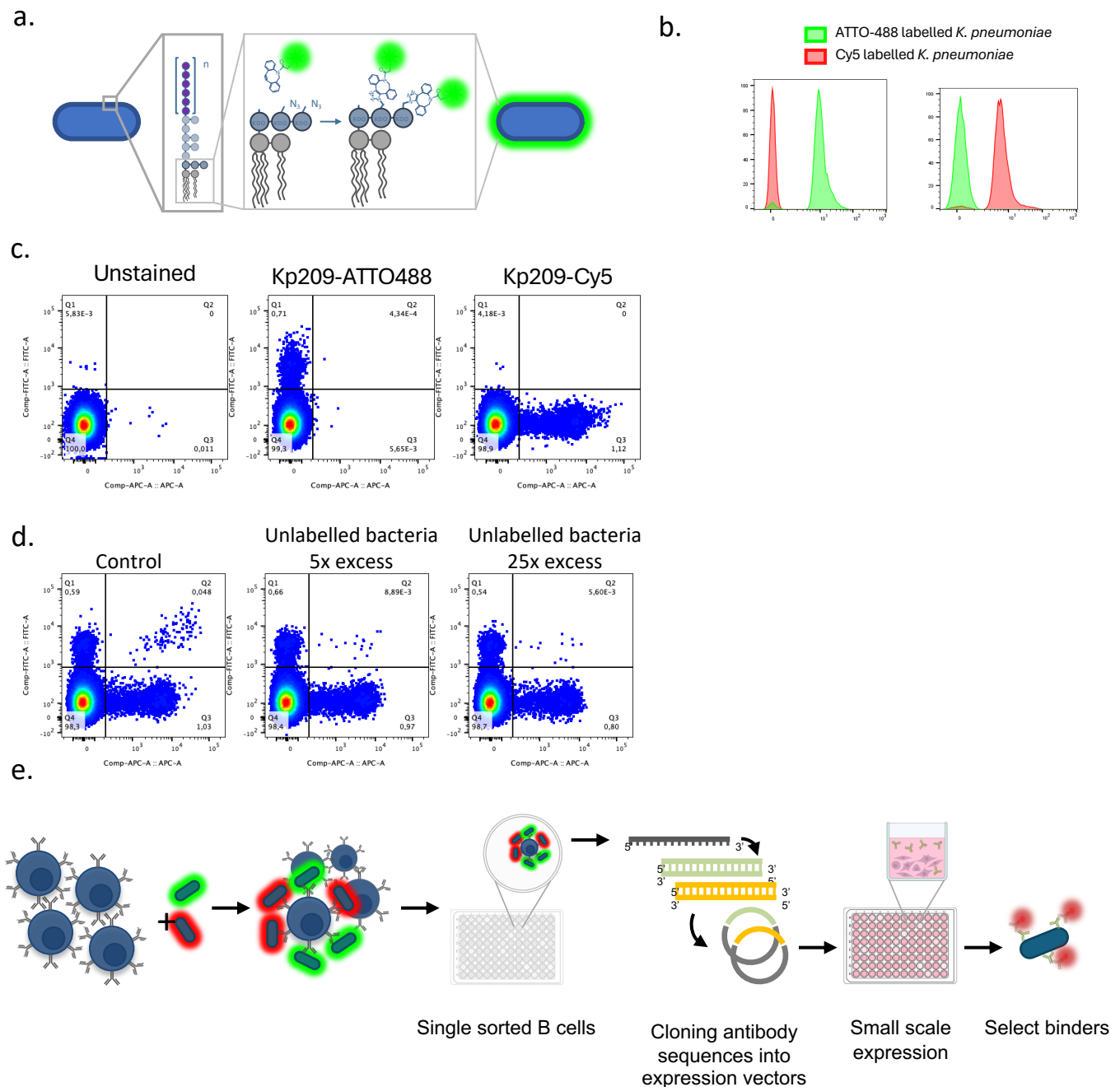
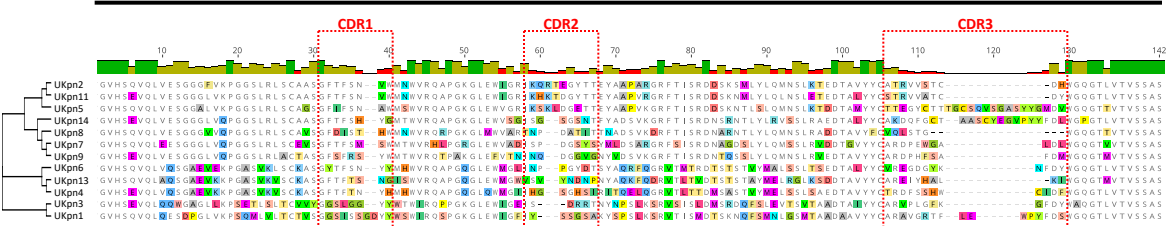




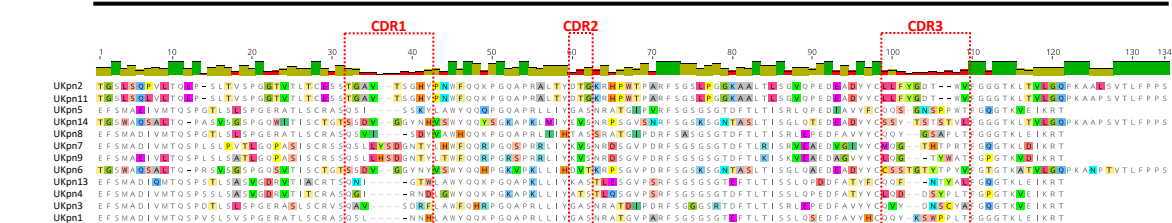
**Figure S1 |Optimization of B cell staining with *KpnO2*.** **(a)** Cartoon depicting fluorescent labeling of the outer membrane of *K. pneumoniae* via click-chemistry. *K. pneumoniae* was cultured overnight in the presence of KDO-azide (2  $\mu$ M), which was metabolically incorporated into the LPS core. Cyclooctyne-labelled fluorophores (50  $\mu$ M) were added, which “clicked” to the azide handles. **(b).** Fluorescent labeling of *KpnO2* with Cy5 or ATTO-488 was assessed via flow cytometry. Fluorescent intensity (gMFI) and number of events normalized to mode are depicted on the X- and Y-axis, respectively. **(c)** Human B cell isolated from healthy donors were incubated with *KpnO2*-ATTO-488 or *KpnO2*-Cy5 (MOI of 0.5 per fluorophore). B cells (CD19<sup>+</sup>) that bound *KpnO2*-ATTO-488 or *KpnO2*-Cy5 were detected. **(d)** Human B cell isolated from healthy donors were incubated with *KpnO2*-ATTO-488 and *KpnO2*-Cy5 (MOI of 0.5 per fluorophore) with an excess of unlabeled *KpnO2*. B cells (CD19<sup>+</sup>) that bound both *KpnO2*-ATTO-488 and *KpnO2*-Cy5 were detected. **(e)** Cartoon depicting the process of *K. pneumoniae*-specific antibody identification. B cells were stained with differently fluorescently labelled *K. pneumoniae*, and B cells double positive for both fluorescent markers were single cell sorted. The variable regions of the heavy and light chain (VH and VL, respectively) were amplified by RT-PCR and cloned into IgG1 expression vectors. Antibodies were recombinantly expressed and tested for antibody binding to *K. pneumoniae*. **(b)** Fluorescent intensity (gMFI) and number of events normalized to mode are depicted on the X- and Y-axis, respectively.

## a. Alignment VH

### Alignment VH

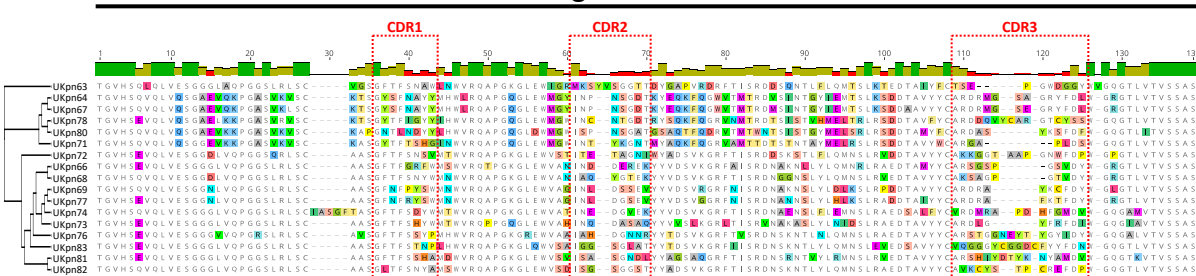


### Alignment VL

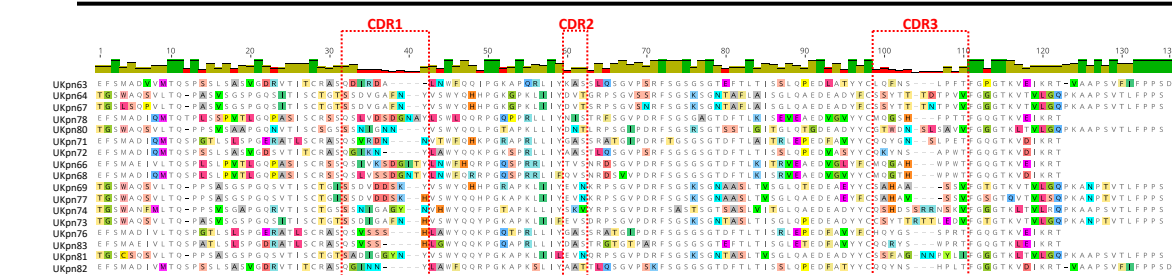


## b. Alignment VH

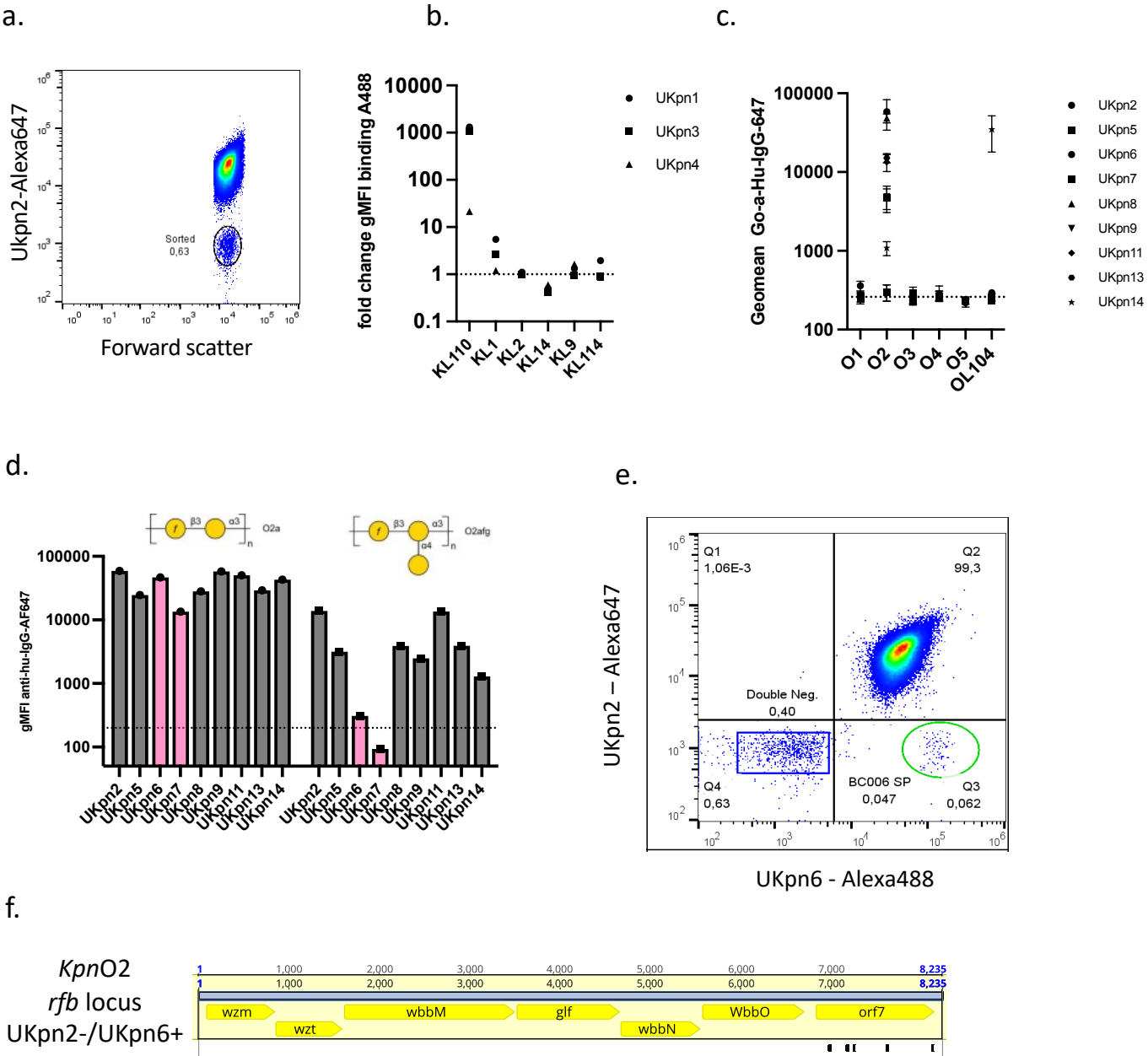
### Alignment VH



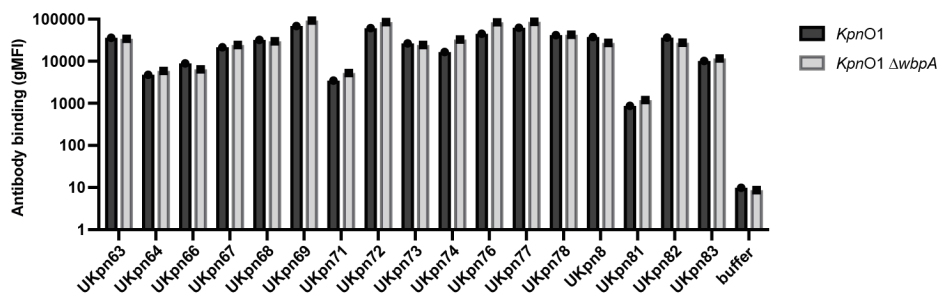
### Alignment VL



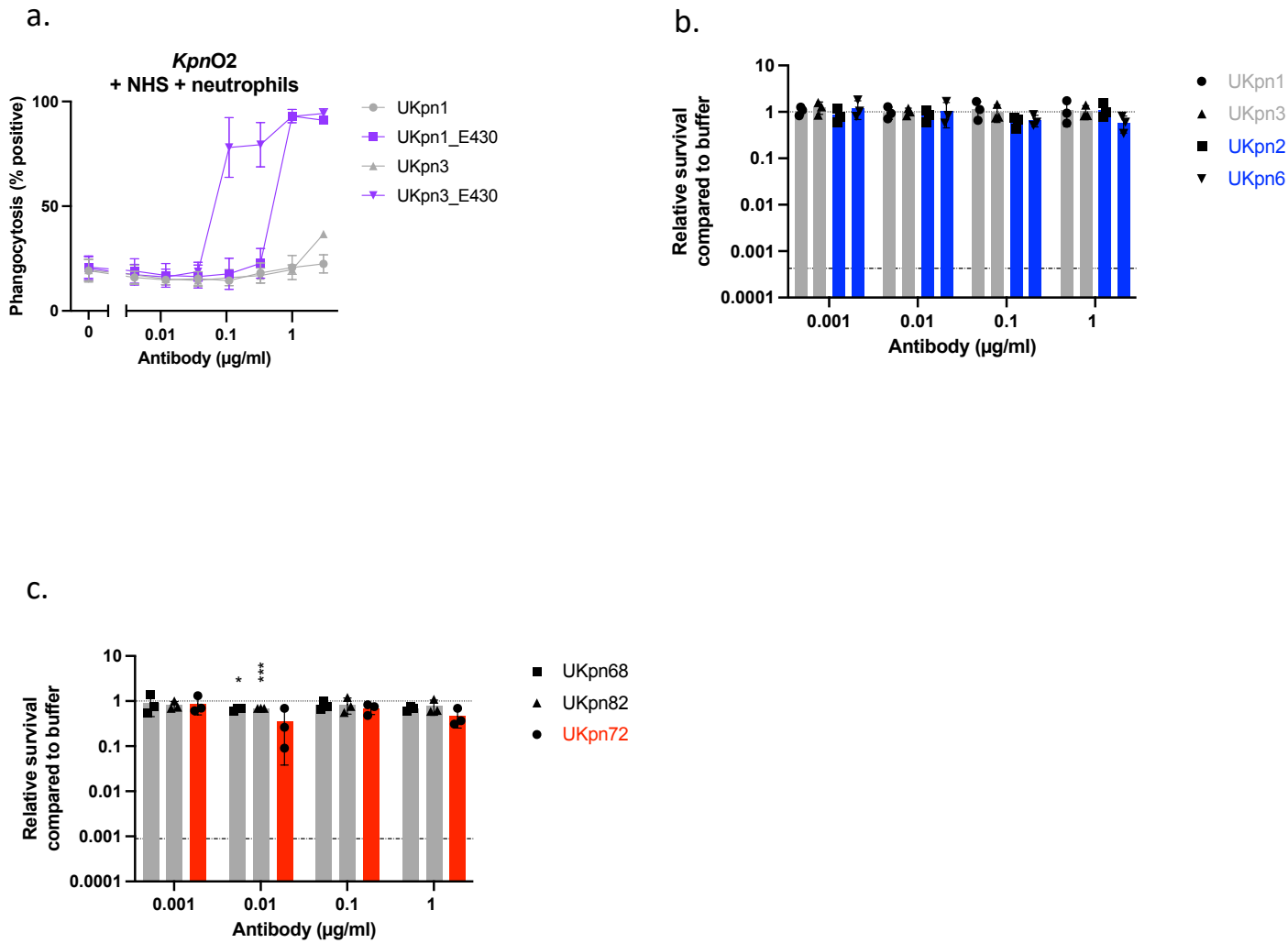
**Figure S2 | VH/VL sequences of 29 anti-*Kpn* mAbs. (a&b)** Alignments of the variable regions of the heavy and light chains (VH and VL, respectively) of the unique *KpnO2* (a) and *KpnO1* (b) binding antibodies. Amino acid sequence alignment was generated using Genious R9 (v9.1.6). Location of the CDR regions determined using IgBlast web tool (Release 1.20.0)



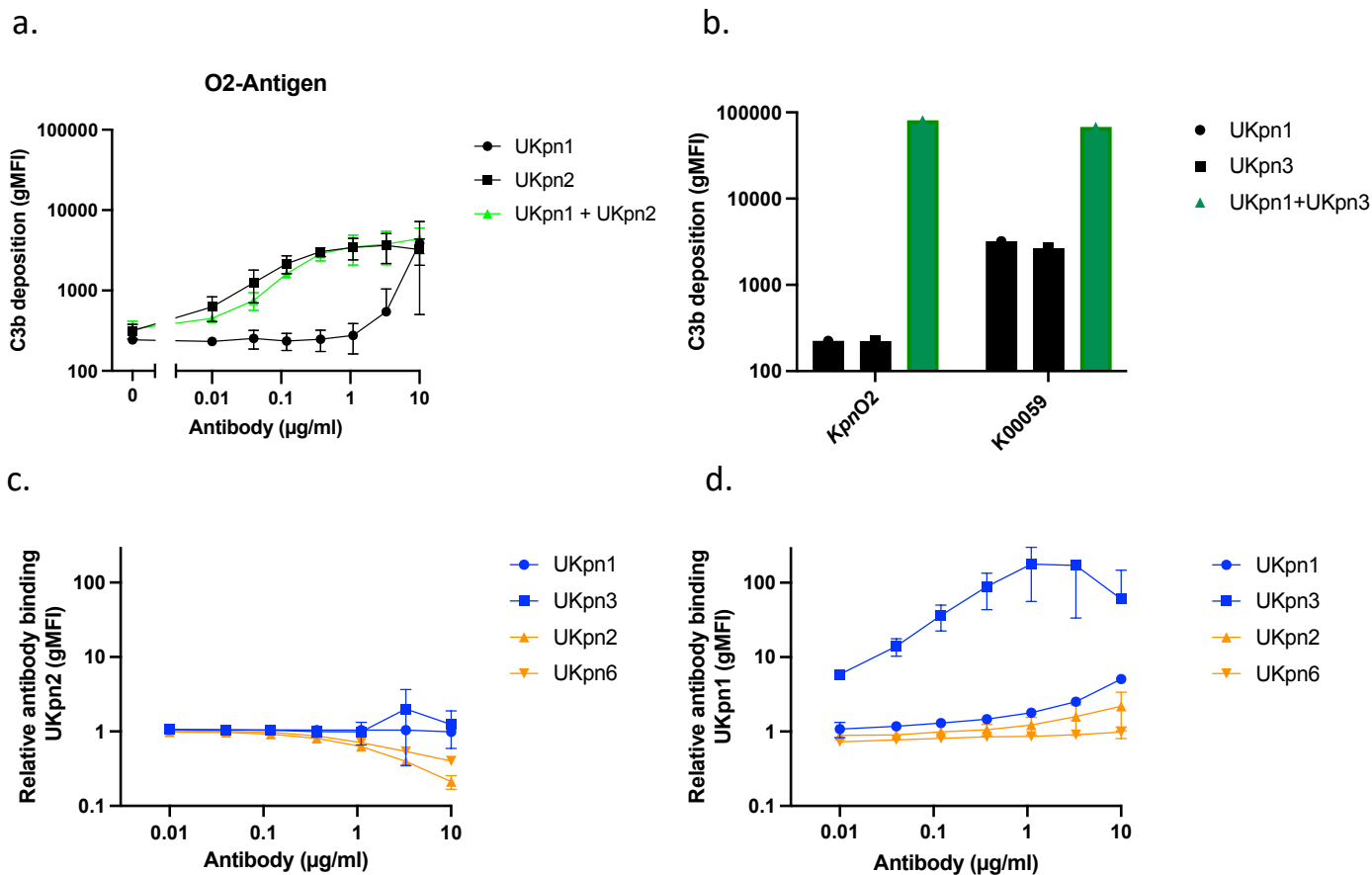
**Figure S3 | Binding of anti-*KpnO2* mAbs to different *Kpn* isolates.** (a) To identify the antigenic targets of the anti-*KpnO2* antibodies, a transposon library of *KpnO2R* (isogenic variant of *KpnO2*<sup>34</sup>) was incubated with fluorescently labelled UKpn2, and antibody binding was detected using flow cytometry. Bacteria that were not bound by the antibodies were single cell sorted. (b) Binding of UKpn1, UKpn3 and UKpn4 to clinical isolates expressing various capsule types. IgG1 binding was detected by flow cytometry using anti-hu-IgG-AF488. Flow cytometry data are represented as fold change of gMFI values of bacterial populations over the background. (c) Binding of UKpn2, UKpn5-9, UKpn11, UKpn13 and UKpn14 to clinical isolates expressing various O-antigen types. Data represent mean  $\pm$  standard deviation of three independent experiments. (d) Binding of UKpn2, UKpn5-9, UKpn11, UKpn13 and UKpn14 to clinical isolates expressing either the O2a- (UM-05-B-#1) or O2afg-antigen (MC-04166) (d). Bacteria were incubated with 1  $\mu$ g/ml IgG1, and binding was detected by flow cytometry using anti-hu-IgG-AF647. Flow cytometry data are represented by gMFI values of bacterial populations. (e) A transposon library of *KpnO2R* was incubated with UKpn2-AF647 and UKpn6-AF488, and antibody binding was detected using flow cytometry. Bacteria that were bound by UKpn6-AF488 but not by UKpn2-AF647 were single cell sorted (green gate). (f) The barcodes of single cell sorted transposon mutants were sequenced to determine the location of the transposon inserts. Mutants that were longer bound by UKpn6-AF488 but not by UKpn2-AF647 had transposon insertions in *orf7*.



**Figure S4 | mAbs directed against *KpnO1* do not target the capsule.** Anti-*KpnO1* IgG1 binding to the capsule deletion mutant of *KpnO1* ( $\Delta wbpA$ ). Bacteria were incubated with 1  $\mu\text{g}/\text{ml}$  IgG1, and binding was detected by flow cytometry using anti-hu-IgG-AF647. Flow cytometry data are represented by gMFI values of bacterial populations.



**Figure S5 | Antibodies targeting *Klebsiella* induce phagocytosis and killing by neutrophils.** (a) Antibody-dependent phagocytosis of *K. pneumoniae* *KpnO2*. GFP expressing bacteria were incubated in the presence of antibody and 3%  $\Delta$ NHS (*KpnO2*) as a source of complement for 15 minutes at 37°C. Isolated human neutrophils were allowed to phagocytose opsonized bacteria for 15 minutes at 37°C. Afterwards, the percentage of GFP-positive neutrophils was determined by flow cytometry. (b,c) *K. pneumoniae* *KpnO2* (b) or *KpnO1* (c) at  $2 \times 10^5$  bacteria/ml were incubated with antibodies and 8%  $\Delta$ NHS (*KpnO2*) or 1%  $\Delta$ NHS (*KpnO1*) for 3 hours at 37°C. The number of CFU/ml was calculated and shown as relative survival by dividing by the no antibody control. (a-c) Data represent mean  $\pm$  SD of three independent experiments. (a,b) Data were analyzed by one-way ANOVA with Dunnett multiple comparison analysis compared to without mAb control. Significant differences are indicated, \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .



**Figure S6 | Combination of capsule antibodies boosts antibody binding.** **(a)** Antibody-dependent C3b deposition on *KpnO2* pre-incubated with UKpn1, UKpn2 or a combination of both. After antibody binding, bacteria were incubated in 3% normal human serum (NHS) as a complement source. C3b-deposition was detected using anti-hu-C3b-AF647 by flow cytometry. **(b)** Same setup as in **(a)** with strains *KpnO2* and K00059 at a fixed antibody concentration of 1  $\mu\text{g/ml}$ . Data represent the gMFI values of bacterial populations. **(c-d)** *KpnO2* was incubated with **(c)** 0.1  $\mu\text{g/ml}$  directly labelled anti-O2-antigen UKpn2-AF488 or **(d)** anti-capsule UKpn1-AF647. **(b)** A concentration range of antibodies UKpn1, UKpn2, UKpn3 or UKpn6 was added to the labeled antibodies. UKpn2-AF488 and UKpn1-AF647 binding was detected by flow cytometry. **(a,b)** In the conditions where antibodies were combined, both antibodies were added in the same concentration. The total antibody concentration in the assay is the same for single or combination of antibodies. The relative antibody binding was calculated by dividing gMFI by fluorescent antibody only condition. **(a,c,d)** Data represent mean  $\pm$  SD of three independent experiments. **(b)** Data represent data obtained from a single experiment.

| Name                                      | O-type | K-type  | Selection marker | Reference                               |
|-------------------------------------------|--------|---------|------------------|-----------------------------------------|
| KpnO2 (Kp209)                             | O2a    | KL110   |                  | Janssen et al., 2020 <sup>43</sup>      |
| KpnO2 ΔwbbO                               | -      | KL110   | Gentamycin       | This study                              |
| KpnO2 ΔwbaP                               | O2a    | -       | Gentamycin       | This study                              |
| KpnO2 pULTRA-gfp                          | O2a    | KL110   | Kanamycin        | This study                              |
| KpnO2 pULTRA-lux                          |        |         | Gentamycin       | This study                              |
| KpnO2R (Kp209_CSTR)<br>transposon library | O2a    | KL110   | Kanamycin        | Van der Lans et al., 2023 <sup>34</sup> |
| KpnO1<br>(UM-05-A#1)                      | O1     | KL114   |                  | Verschuren et al., unpublished          |
| KpnO1 ΔwbbO                               | -      | KL114   | Gentamycin       | This study                              |
| KpnO1 ΔwbbY                               | O2a    | KL114   | Gentamycin       | This study                              |
| KpnO1 ΔwbaP                               | O1     | -       | Gentamycin       | This study                              |
| KpnO1 pULTRA-gfp                          | O1     | KL114   | Kanamycin        | This study                              |
| KpnO1 pULTRA-lux                          | O1     | KL114   | Gentamycin       | This study                              |
| UM-2A#3.1                                 | O1     | KL20    |                  | Verschuren et al., unpublished          |
| UM13B#1                                   | O1     | KL9     |                  | Verschuren et al., unpublished          |
| MB-17667                                  | O1     | KL30-D1 |                  | Van den Bunt et al., 2020 <sup>53</sup> |
| JS367*                                    | O2afg  | KL9     |                  | This study                              |
| MA-25932                                  | O3b    | KL60    |                  | Van den Bunt et al., 2020 <sup>53</sup> |
| MA-49950                                  | O4     | KL17    |                  | Van den Bunt et al., 2020 <sup>53</sup> |
| MA-44575                                  | O5     | KL10    |                  | Van den Bunt et al., 2020 <sup>53</sup> |
| MA-18898                                  | OL104  | KL14    |                  | Van den Bunt et al., 2020 <sup>53</sup> |
| K00059                                    | O3a    | KL110   |                  | Van den Bunt et al., 2020 <sup>53</sup> |
| UM-05-B-#1                                | O2a    | KL114   |                  | Verschuren et al., unpublished          |
| MC-04166                                  | O2afg  | KL136   |                  | Van den Bunt et al., 2020 <sup>53</sup> |
| N0008                                     | O3a    | KL1     |                  | This study                              |
| MB-16059                                  | O3b    | KL2     |                  | Van den Bunt et al., 2020 <sup>53</sup> |

**Table S1 | Strains used in this study**

|                     |                                                                 |
|---------------------|-----------------------------------------------------------------|
| RT-PCR              |                                                                 |
|                     |                                                                 |
| Heavy IgG           | Sequence                                                        |
| H1_For_VH1          | ACAGGTGCCCCACTCCCAGGTGCAG                                       |
| H1_For_VH3          | AAGGTGTCCAGTGTGARGTGCAG                                         |
| H1_For_VH4/6        | CCCAGATGGGTCTGTCCCAGGTGCAG                                      |
| H1_For_VH5          | CAAGGAGTCTGTTCCGAGGTGCAG                                        |
| H1_Rev_int_CH1      | GTTCGGGGAAGTAGTCCTTGAC                                          |
|                     |                                                                 |
| Kappa               |                                                                 |
| K1_For_Vk1/2        | ATGAGGSTCCCYGCTCAGCTGCTGG                                       |
| K1_For_Vk3          | CTCTTCCTCCTGCTACTCTGGCTCCAG                                     |
| K1_For_Vk4          | ATTTCTCTGTTGCTCTGGATCTCTG                                       |
| K1_For_Pan_Vk       | ATGACCCAGWCTCCABYCWCCTG                                         |
| K1_Rev_Ck543        | GTTTCTCGTAGTCTGCTTTGCTCA                                        |
| K1_Rev_Ck494        | GTGCTGTCCTTGCTGTCTGCT                                           |
|                     |                                                                 |
| Lambda              |                                                                 |
| λ1_For_Vλ1          | GGTCCTGGGCCCCAGTCTGTGCTG                                        |
| λ1_For_Vλ2          | GGTCCTGGGCCCCAGTCTGCCCTG                                        |
| λ1_For_Vλ3          | GCTCTGTGACCTCCTATGAGCTG                                         |
| λ1_For_Vλ4/5        | GGTCTCTCTCSCAGCYTGTCTG                                          |
| λ1_For_Vλ6          | GTTCTTGGGCCAATTTTATGCTG                                         |
| λ1_For_Vλ7          | GGTCCAATTCYCAGGCTGTGGTG                                         |
| λ1_For_Vλ8          | GAGTGGATTCTCAGACTGTGGTG                                         |
| λ1_Rev_Cλ           | CACCAGTGTGGCCTTGTTGGCTTG                                        |
|                     |                                                                 |
|                     |                                                                 |
| 2nd PCR             |                                                                 |
|                     |                                                                 |
| Heavy IgG           |                                                                 |
| H2_For_AgeIC_VH1/5  | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCCGAGGTGCAGCTGGTGCAG            |
| H2_For_AgeIC_VH3    | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCTGAGGTGCAGCTGGTGGAG            |
| H2_For_AgeIC_VH3-23 | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCTGAGGTGCAGCTGTTGGAG            |
| H2_For_AgeIC_VH4    | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCCCAGGTGCAGCTGCAGGAG            |
| H2_For_AgeIC_VH4-34 | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCCCAGGTGCAGCTACAGCAGTG          |
| H2_For_AgeIC_VH1-18 | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCCCAGGTTCACTGGTGCAG             |
| H2_For_AgeIC_VH1-24 | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCCCAGGTCCAGCTGGTACAG            |
| H2_For_AgeIC_VH3-33 | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCTCAGGTGCAGCTGGTGGAG            |
| H2_For_AgeIC_VH3-9  | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCTGAAGTGCAGCTGGTGGAG            |
| H2_For_AgeIC_VH4-39 | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCCCAGCTGCAGCTGCAGGAG            |
| H2_For_AgeIC_VH6-1  | GTTTCTGGTGGCTACTGCAACCGGTGTACATTCCCAGGTACAGCTGCAGCAG            |
| H2_Rev_JH1/2/4/5    | GATGGGCCCTTGGTGCTAGCTGAGGAGACGGTGAC <u>CAG</u>                  |
| H2_Rev_JH3          | GATGGGCCCTTGGTGCTAGCTGAAGAGACGGTGAC <u>CAITG</u>                |
| H2_Rev_JH6          | GATGGGCCCTTGGTGCTAGCTGAGGAGACGGTGACCGTG                         |
|                     |                                                                 |
| Kappa               |                                                                 |
| K2_For_Vk1-5        | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGACATCCAGATGACCCAGTC  |
| K2_For_Vk1-9        | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGACATCCAGTTGACCCAGTCT |
| K2_For_Vk1D-43      | GTAATCAGCACTTGCCTGGAATTCTCTATGGCTGCCATCCGGATGACCCAGTC           |
| K2_For_Vk2-24       | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGATATTGTGATGACCCAGAC  |
| K2_For_Vk2-28       | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGATATTGTGATGACTCAGTC  |
| K2_For_Vk2-30       | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGATGTTGTGATGACTCAGTC  |
| K2_For_Vk3-11       | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGAAATTGTGTTGACACAGTC  |
| K2_For_Vk3-15       | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGAAATAGTGATGACGCAGTC  |
| K2_For_Vk3-20       | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGAAATTGTGTTGACGCAGTCT |
| K2_For_Vk4-1        | GTAATCAGCACTTGCCTG <u>GAATTC</u> TCTATGGCTGACATCGTGATGACCCAGTC  |
| K2_Rev_Jk1/4        | GATGGTGCAGCCACCGTACGTTTGATYTCCACCTTGGTC                         |
| K2_Rev_Jk2          | GATGGTGCAGCCACCGTACGTTTGATCTCCAGCTTGGTC                         |
| K2_Rev_Jk3          | GATGGTGCAGCCACCGTACGTTTGATATCCACTTTGGTC                         |
| K2_Rev_Jk5          | GATGGTGCAGCCACCGTACGTTTAATCTCCAGTCGTGTC                         |
|                     |                                                                 |
| Lambda              |                                                                 |
| λ2_For_Vλ1          | GTTTCTGGTGGCTACTGCT <u>ACCGGT</u> TCCTGGGCCCAGTCTGTGCTGACKCAG   |
| λ2_For_Vλ2          | GTTTCTGGTGGCTACTGCT <u>ACCGGT</u> TCCTGGGCCCAGTCTGCCCTGACTCAG   |
| λ2_For_Vλ3          | GTTTCTGGTGGCTACTGCTACCGGTTCTGTGACCTCCTATGAGCTGACWCAG            |
| λ2_For_Vλ4/5        | GTTTCTGGTGGCTACTGCTACCGGTTCTCTCTCSCAGCYTGTGCTGACTCA             |
| λ2_For_Vλ6          | GTTTCTGGTGGCTACTGCT <u>ACCGGT</u> TCTTGGGCCAATTTTATGCTGACTCAG   |
| λ2_For_Vλ7/8        | GTTTCTGGTGGCTACTGCT <u>ACCGGT</u> TCCAATTCYACAGCTGTGGTGACYCAG   |
| λ2_Rev_Cλ           | GTTGGCTTGAAGCTCCTCACTCAGGGYGGGAACAGAGTG                         |

**Table S2| RT-PCR primers**