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Article

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A humanized minipig model for the toxicological testing of therapeutic recombinant antibodies

In the format provided by the authors and unedited

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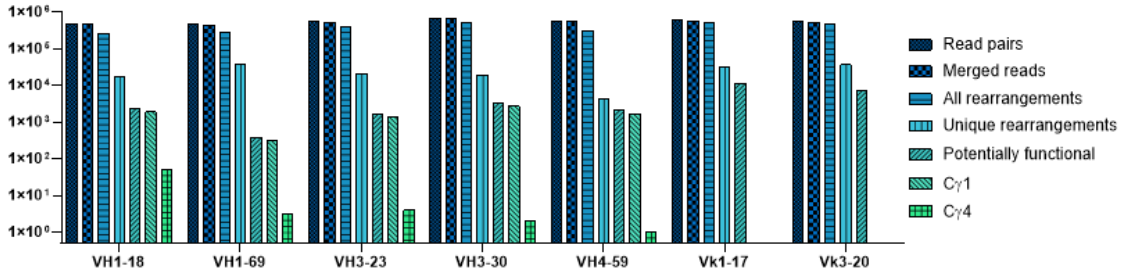

b

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>p9    GTFSSYAISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCA  KN           YWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
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>p180  GTFSSYAISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCA  KD           YFDYWGQGTLVTVSS(4)ASIKGPSVFPLAPSSKSTSGGTAAL
>p181  GTFSSYAISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCA  KD           YDYWGQGTLVTVSS(4)ASTKSPSVFPLAPSSKSTSGGTAAL
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>p267  GTFSSYAISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCA  ANLGY       YFDYWGQGTLVTVSS(4)ASTKGPSVFPLAPCSRSTSESTAAL

VH4-59: KGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR  N(D)N       JH              Cγ
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>s63   KGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR DLGIG      YWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p79   KGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCA  SN          WGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p80   KGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR DRGI     DYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p81   KGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR DQGI     DYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p82   KGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR DLVI     DYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p182  KGLEWIGYIYYSGSTNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAR DLVI     DYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL

VH3-30:  ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK  N(D)N          JH              Cγ
>p1    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCPK EGR            GQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p2    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK            DAFDIWGQGTMVTVSS(3)ASTKGPSVFPLAPSSKSTSGGTAAL
>p3    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK DPY        YFDYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p4    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA  NMVRE       FDYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
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>p3138  ADSVKGRFTISRDDSKNTLYLQMNSLRVEDTAVYFCA  REGDDRAYYSA WFDPWGQGTLVTVSS(4)ASTKGPSVFPLAPCSRSTSESTAAL
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>p2     ADSVKGRFTISRDNSKNTLYLQINSLRAEDTAVYYCAK DRG            FDYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p4     ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK LY           YFDYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p5     ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCA  ITGV           DYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p6     ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK APP            FDIWGQGTMVTVSS(3)ASTKGPSVFPLAPSSKSTSGGTAAL
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>p8     ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK D              YWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
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>p11    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAV      AN         YYYYMDVWGKGTTVTVSS(6)ASTKGPSVFPLAPSSKSTSGGTAAL
>p12    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK DYYGSGSYYN YYYYMDVWGKGTTVTVSS(6)ASTKGPSVFPLAPSSKSTSGGTAAL D3-9
>p14    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK SLDP           WGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
>p15    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK DFG          DAFDIWGQGTMVTVSS(3)ASTKGPSVFPLAPSSKSTSGGTAAL
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>p22    ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK DLD            YFDYWGQGTLVTVSS(4)ASTKGPSVFPLAPSSKSTSGGTAAL
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>p1565  ADSVKGRFTISRDNAKNTLFLQMNSLRAEDTAVYYCA  RFSSESPDYF    YGLDVWGQGTMVTVSS(6)ASTKGPSVFPLAPCSRSTSESTAAL
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>p96   GISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCA  SDHWGL YFDLWGRGTLVTVSS(2)ASTKGPSVFPLAPSSKSTSGGTAAL
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>p48   ARCDIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQSGVPSRFSGSGSGTEFTLTISSLQPEDVATYYCLQHNSYP HKSQ   LTFGGGTKVEIK(4)
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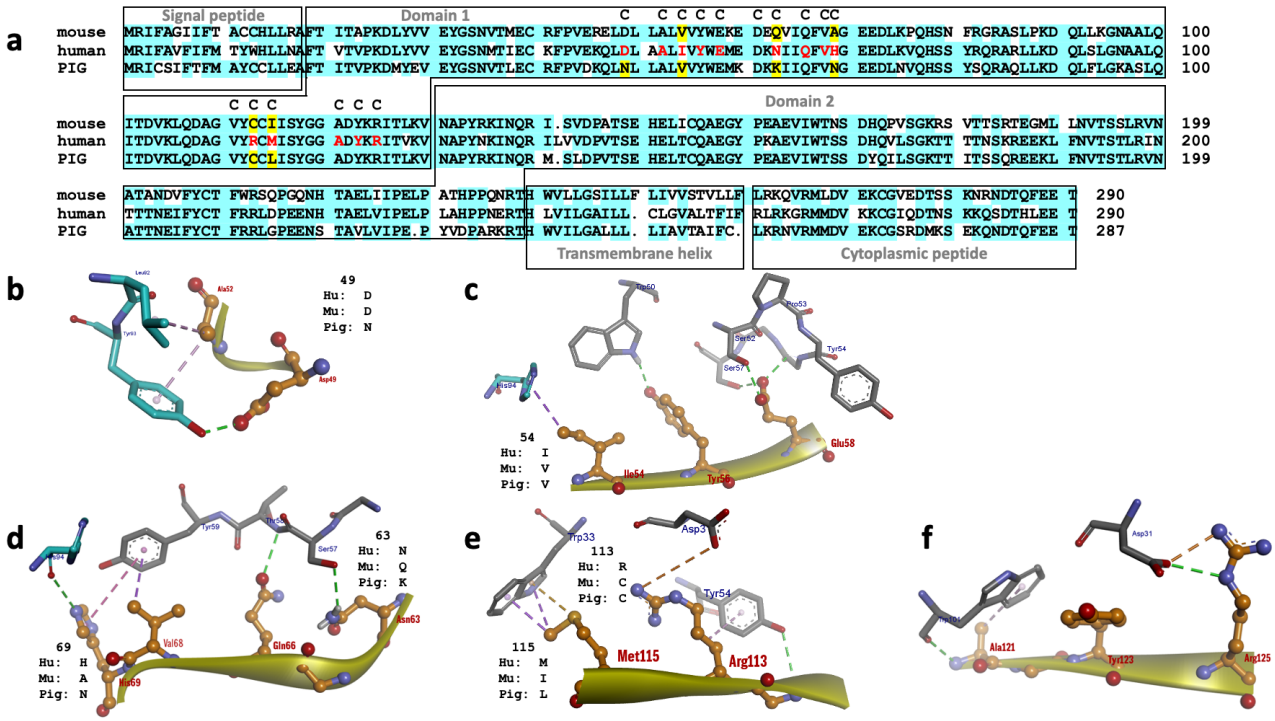
Supplementary Fig. 1 l V(D)J sequencing. **a**, Graphical representation of a total of 3.95 million read pairs obtained from V(D)J sequencing displayed for each PCR product. Read pairs represent the number of paired end reads obtained from the sequencer. After merging (merged reads), the reads were selected based on the presence of the forward and reverse primer sequences which represent all rearrangements. The number of unique amino acid sequences after translating are depicted as unique rearrangements. These sequences were further filtered for the presence of the primer including 5 expected adjacent amino acids in the correct reading frame and the absence of stop codons to obtain potentially functional rearrangements. The isotype of the potentially functional rearrangements was determined by the presence of the protein tag SSKSTSGG (Cγ1) or CSRSTSES (Cγ4). In total, Cγ4 switch variants are very rare, summing up to an average of 0,76% of all functional transgenic VH gene rearrangements found. **b,** Selection of the most prominent sequences of potentially functional rearrangements of the transgenic human Ig genes found in the transgenic minipigs are shown as amino acid sequences. Rare Cγ4 switch variants are also included. The sequence of the transgenic V genes is depicted (in blue) above rearranged sequences, indicating the position of the amino acids corresponding to N(D)N, J and C regions. Where identified, D gene segments are underlined and named right to the sequence. The numbers in parenthesis in VH and Vk sequences indicate the corresponding J element used. Amino acid variants resulting from point mutations are shown in red. The Cγ4 region of switch variants is in brown colour. Same V gene sequences of Cγ4 switch variants are also often associated with Cγ1.



Supplementary Fig. 2 l Sequence and structural mapping of the different interactions of PD-L1 epitope amino acid side-chains with atezolizumab paratope residues. **a,** Sequence alignment of PD-L1 orthologs, human, mouse, and pig. Epitope residues are marked with a C (contact residues); an amino acid 1-letter code is marked in red when the contact is occurring exclusively via the side-chain atoms (amino acids with backbone contact only are kept in black). **b-f,** Molecular models of PD-L1 interaction with atezolizumab. Fourteen interacting side-chains show their contact with paratope residues. The corresponding residue position of PD-L1 interacting with atezolizumab is depicted for human (Hu) mouse (Mu) and pig (Pig) amino acids. Pig PD-L1 residues are indicated in the ball and stick mode (orange carbon atoms) and atezolizumab residues are represented in sticks (grey carbon atoms for the heavy variable region and cyan carbon atoms for the light variable region). PD-L1 and atezolizumab VH and VL are numbered consecutively starting from the first N-terminal residue.

Supplementary Table. 1 l List of Ab compounds.

Compound tested	Specificity	Clinical immunogenicity	Immunogenicity in huIgG1 tg mouse model*	Cross-reactivity mouse / pig
bevacizumab (Avastin)	VEGF	0,63 %	0/10 (8/10)	no[41] / yes[42]
daratumumab (Darzalex)	CD38	0 %	0/10 (4/10)	no / no
atezolizumab (Tecentriq)	PD-L1	30 %	10/10 (10/10)	yes / yes
cergutuzumab amunaleukin	CEA / IL2R	79 %	7/10 (7/10)[20]	yes (IL2R) / yes (IL2R)

*Given as the number of ADA-positive mice of 10 mice immunised in each group. Corresponding numbers for wild type mice are in parenthesis. Unpublished data, manuscript in preparation.

Supplementary Table. 2 l List of primers.

PCR amplification target	Primer name	Sequence 5'->3'
IGH-γ1-γ4 (transgene presence)	IGH_F IGH_R	CTGTCCTACAGTCCTCAGGAC GTGGCACTCATTTACCCGGAG
IGK (transgene presence)	IGK_F IGK_R	TTGTGTGCCTGCTGAAT GCCGCGTGTGAGTAGGTATA
BS (transgene presence)	BS_F BS_R	ATGGCCAAGCCTTTGTCTC GGCGAAGAACTCCAGCATGA
IGH-γ1-γ4 (ddPCR)	dIGH_F dIGH_R	CACCCAGTGCGAGACGAC CTCCTCCTCCCTCGCTCT
IGK (ddPCR)	dIGK_F dIGK_R	GCTATGTAGAAGAGGCAGCT ACCACCTTTCCACCATTACAA
GAPDH (ddPCR)	ddGAPDH_F ddGAPDH_R	CTCAACGACCACTTCGTCAA CCCTGTTGCTGTAGCCAAAT
First VH PCR (RNA sequencing)	Vh1-69_L Vh4-59_L Vh3-30_L Vh3-23_L Vh1-18_L Cg1_4-rev_long	TGGCAGCAGCTACAGGTGTC GCTCCCAGATGGGTCCTGT CGTTGCTCTTTTAAGAGGTGTCC GCTATTTTAAAAGGTGTCCAGTGTG CAGCAACAGGTGCCCACT GGTGTTGCTGGGCTTGTG
Nested VH CR (RNA sequencing)	VH1-18_A VH1-69_A VH3-23/30_A VH4-59_A Cg1_4-PCR_A	ATGGTATCAGCTGGGTGCGAC AGGCACCTTCAGCAGCTATGC GCAGACTCCGTGAAGGGCCG AAGGGACTGGAGTGGATTGGG AGTAGTCCTTGACCAGGCAGC
VK PCR (RNA sequencing)	Vk1-17_A Vk3-20_A Ck-PCR_A	GCCAGGTGTGACATCCAGATG AGGCACCCTGTCTTTGTCTCC CAGATGGTGCAGCCACAGTTC