Extended and Supplementary data:

High-efficiency base editing enables multiplex inactivation of retroviral and immunologic barriers in porcine xenotransplantation

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This PDF file includes:

Extended Data Figures

Figure 1. Generation of PERV knockout pigs.

Figure 2. Generation and characterization of multiplex Xenoantigen and *GHR* null pigs.

Figure 3. Characterization of multiplex Xenoantigen and *GHR* null pigs.

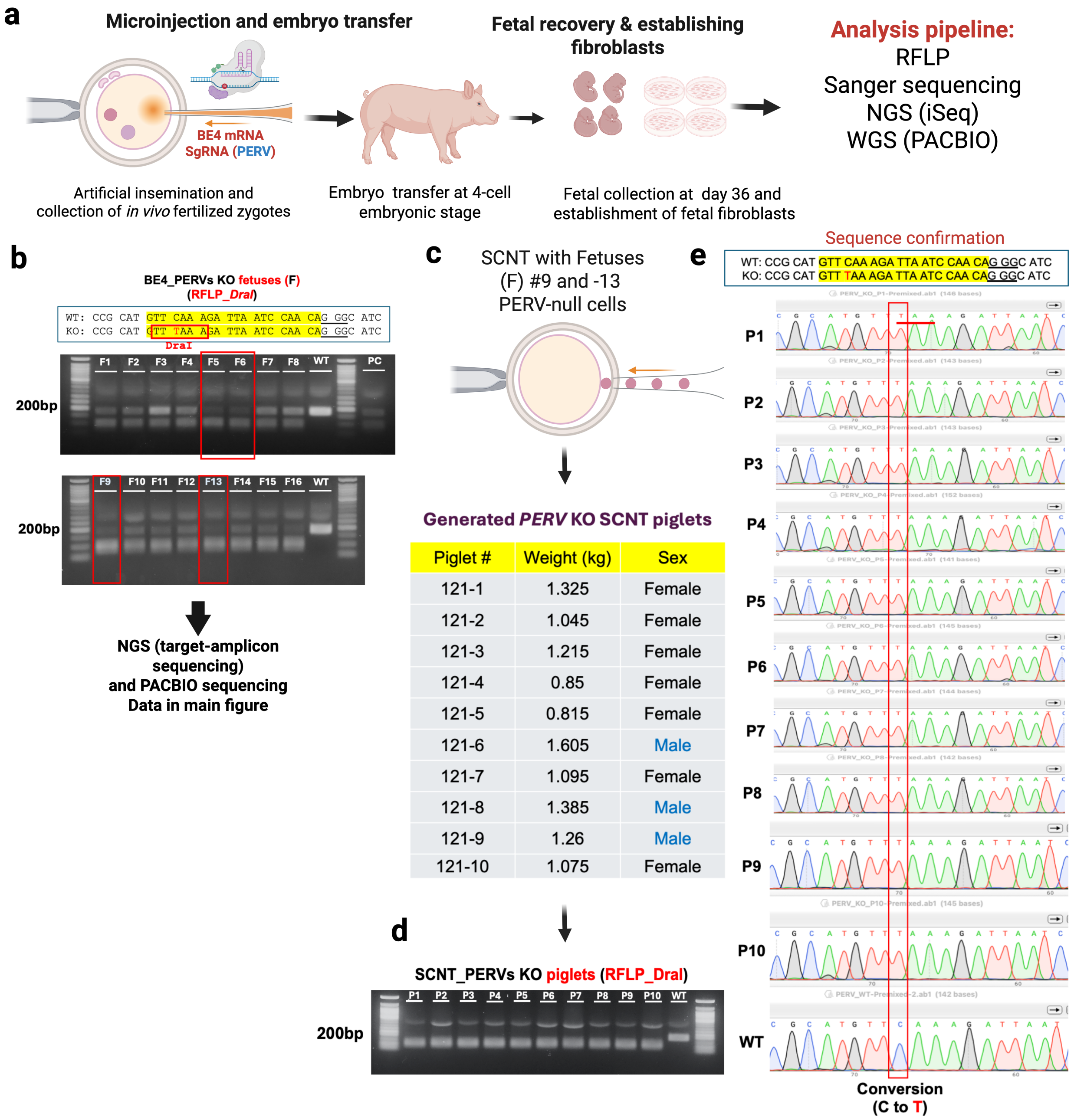
Supplementary Data

1. PAC-Bio high-fidelity WGS reads for fetus #9 and -13 used for generating PERV-null piglets.

2. Pathology report of human blood perfusion of Xenoantigen null and WT livers

Supplementary Tables

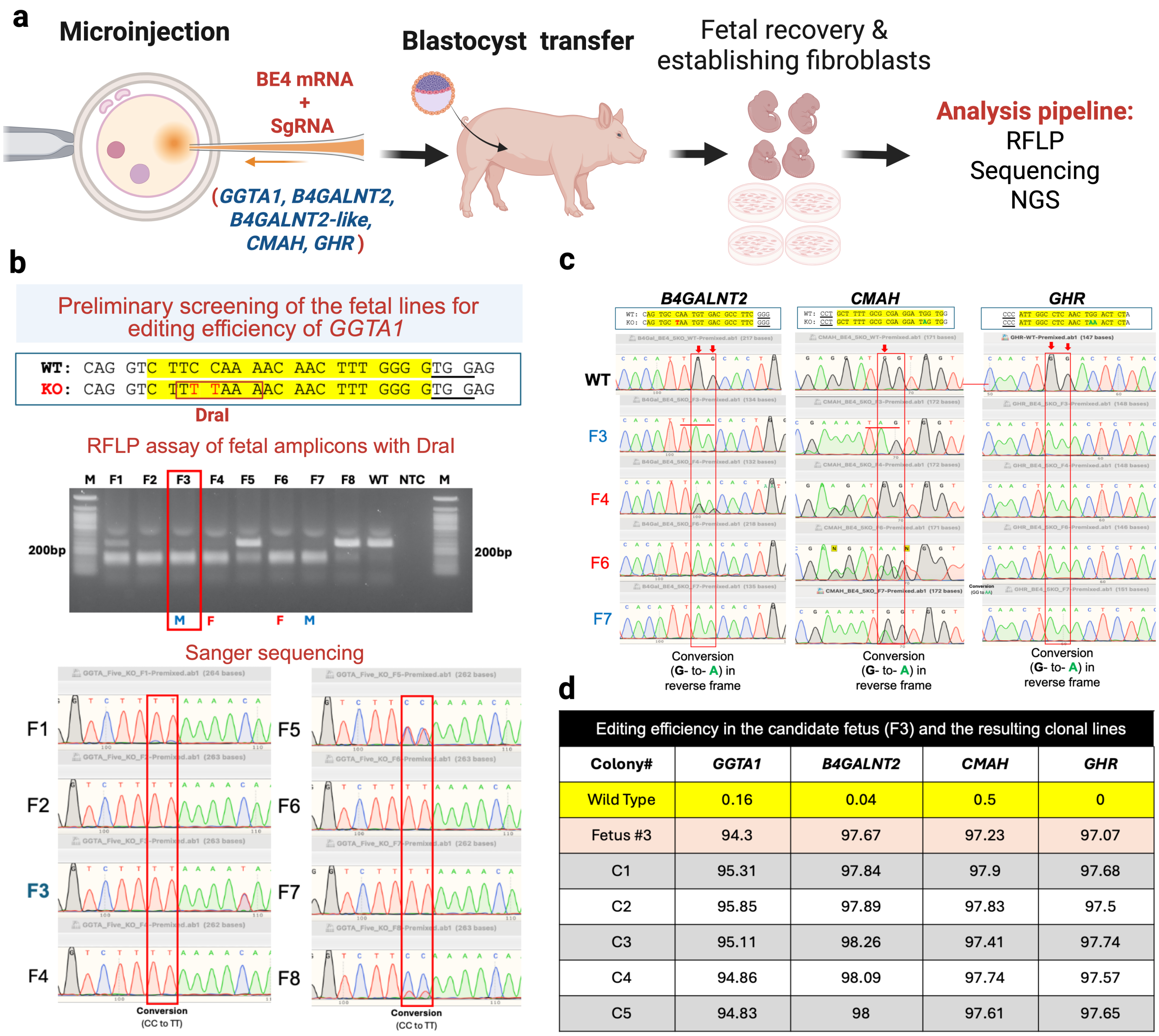
Table S1. Single Guide (sg) RNA used in the manuscript.
Table S2. Primers used
Table S3. Reference Target amplicon sequence of target genes
Table S4. Antibodies and lectins used in the study for immunohistochemistry and FACS analysis



Generated *PERV* KO SCNT piglets

Piglet #	Weight (kg)	Sex
121-1	1.325	Female
121-2	1.045	Female
121-3	1.215	Female
121-4	0.85	Female
121-5	0.815	Female
121-6	1.605	Male
121-7	1.095	Female
121-8	1.385	Male
121-9	1.26	Male
121-10	1.075	Female

Extended Data Fig.1. Generation of PERV knockout pigs. A) Schematic overview of the strategy used to generate PERV knockout pigs. *In vivo*-derived embryos were microinjected with BE4 base-editing reagents targeting PERV loci and transferred into recipient females. Fetuses were recovered at embryonic day 36, and porcine fetal fibroblasts (PFFs) were established from individual fetuses. **B)** Restriction fragment length polymorphism (RFLP) analysis using *DraI*, generated by C-to-T conversion within the editing window, demonstrated high-efficiency editing in fetuses #9 and #13, which was subsequently validated by PacBio HiFi sequencing. **C)** PFFs derived from fetuses #9 and #13 were used as donor cells for somatic cell nuclear transfer (SCNT) to generate PERV-null pigs. **D–E)** RFLP and Sanger sequencing analyses confirmed complete C-to-T conversion of PERV loci in the resulting piglets.

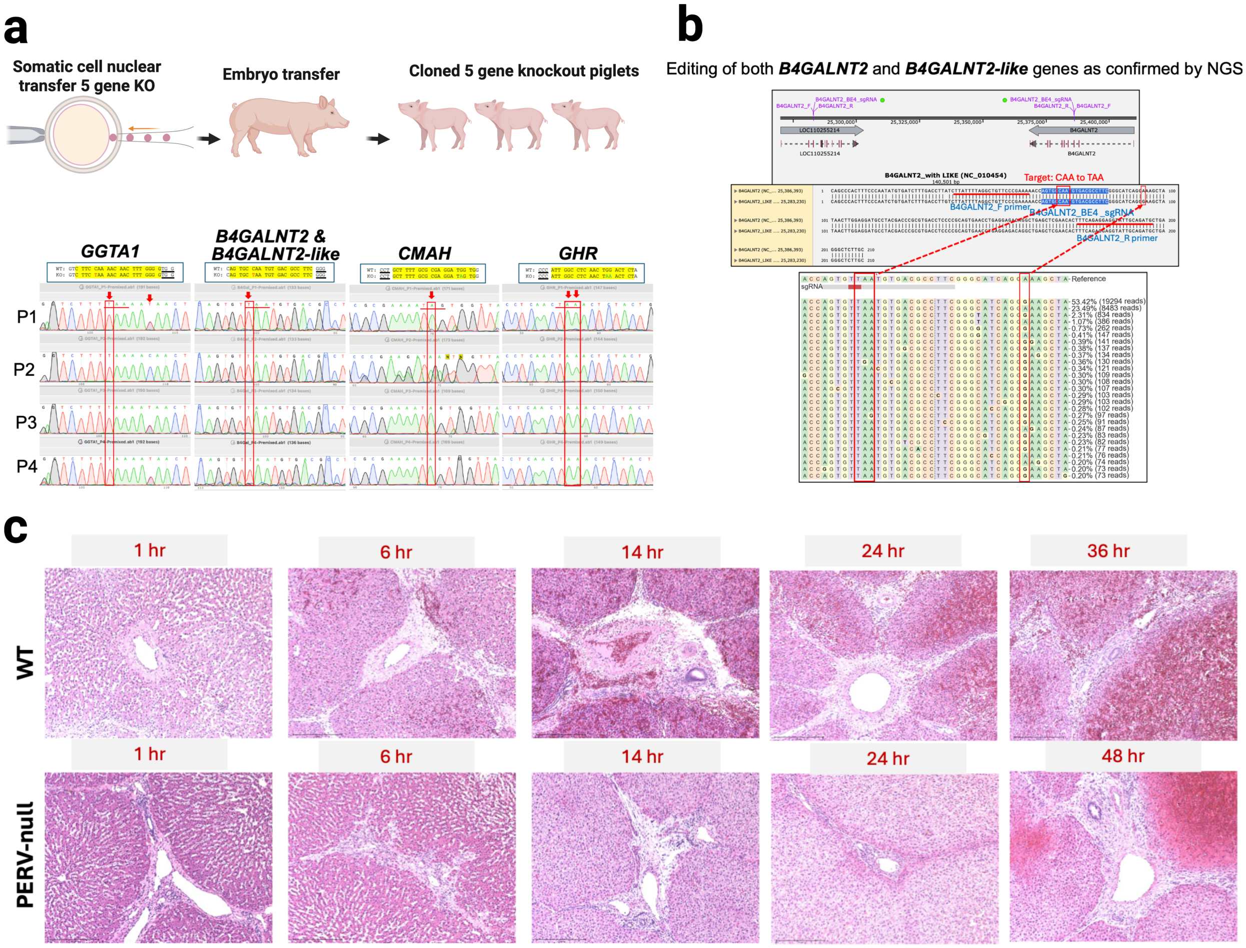


Editing efficiency in the candidate fetus (F3) and the resulting clonal lines

Colony#	*GGTA1*	*B4GALNT2*	*CMAH*	*GHR*
Wild Type	0.16	0.04	0.5	0
Fetus #3	94.3	97.67	97.23	97.07
C1	95.31	97.84	97.9	97.68
C2	95.85	97.89	97.83	97.5
C3	95.11	98.26	97.41	97.74
C4	94.86	98.09	97.74	97.57
C5	94.83	98	97.61	97.65

Extended Data Fig. 2. Generation of multiplex xenoantigen- and *GHR*-null fetal lines for production of five-gene knockout pigs.

a) Schematic overview of the strategy used to generate multiplex xenoantigen knockout pigs. *In vitro* fertilized (IVF) embryos were microinjected with BE4 base-editing reagents targeting *GGTA1, B4GALNT2, CMAH,* and *GHR*. Following embryo transfer, fetuses were recovered at embryonic day 75, and porcine fetal fibroblasts (PFFs) were established from individual fetuses; **b)** Restriction fragment length polymorphism (RFLP) analysis using *DraI*, generated by C-to-T conversion within the editing window, demonstrated efficient editing of *GGTA1* in fetuses #3, #4, and #6, which was further validated by Sanger sequencing; **c)** Sanger sequencing confirmed high-efficiency editing of *B4GALNT2, CMAH,* and *GHR* in fetus #3; **d)** PFFs derived from fetus #3 were clonally expanded, and targeted amplicon sequencing confirmed high-efficiency editing across all targeted loci in individual clonal cell lines.



Extended Data Fig. 3. Characterization of multiplex xenoantigen- and *GHR*-null fetal lines for production of five-gene knockout pigs. a) PFFs derived from colony # 3 were used as donor cells for somatic cell nuclear transfer (SCNT) to generate xenoantigen- and *GHR*-null piglets (n = 4). Sanger sequencing and targeted next-generation sequencing (NGS) confirmed that piglets #3 and #4 were homozygous null at all targeted loci; **b**) The sgRNA designed for *B4GALNT2* also targets the *B4GALNT2-like* locus. A single-nucleotide polymorphism (SNP) located downstream of the sgRNA target site (dotted box) distinguished *B4GALNT2* from *B4GALNT2-like* and confirmed efficient editing at both loci based on NGS read analysis; **c**) Representative histological images of liver biopsies from WT and 5 gene KO pigs on different days and at different times during perfusion. Scale bar: 275 μM.

Supplementary data 1. PAC-Bio high-fidelity WGS reads for fetus #9 and -13 used for generating PERV-null offspring.

BLASTN 2.9.0+ Blast was performed as outlined in Altschul et al., with a
Matrix: blastn matrix 1 -3
Gap Penalties: Existence: 5, Extension: 2

Reference: Stephen F. Altschul, Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, David J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acids Research*, Volume 25, Issue 17, 1 September 1997, Pages 3389–3402.

Summary of PAC-BIO sequencing and Blast results				
Fetus	**# of PERV hits**	**Database**	**# of sequences**	**Total base pairs**
9	36	f9_final.p_ctg.fasta	3,943 sequences	2,596,137,665
13	43	f13_final.p_ctg.fasta	3,025 sequences	2,610,651,477

Fetus #9

```
Query= AY056035.1 Porcine endogenous retrovirus PCR amplicon

Length=26
                                                                 Score      E
Sequences producing significant alignments:                     (Bits)    Value
ctg/p/l/000425/0                                                 44.1      0.001
ctg/p/l/000399/0                                                 44.1      0.001
ctg/p/l/000389/0                                                 44.1      0.001
ctg/p/l/000366/0                                                 44.1      0.001
ctg/p/l/002928/0                                                 44.1      0.001
ctg/p/l/002643/0                                                 44.1      0.001
ctg/p/l/009548/0                                                 44.1      0.001
ctg/p/l/005979/0                                                 44.1      0.001
ctg/p/l/005710/0                                                 44.1      0.001
ctg/p/l/000072/0                                                 44.1      0.001
ctg/p/l/000051/0                                                 44.1      0.001
ctg/p/l/000050/0                                                 44.1      0.001
ctg/p/l/000226/0                                                 44.1      0.001
ctg/p/l/000336/0                                                 44.1      0.001
ctg/p/l/000321/0                                                 44.1      0.001
ctg/p/l/000295/0                                                 44.1      0.001
ctg/p/l/000269/0                                                 44.1      0.001
ctg/p/l/000182/0                                                 44.1      0.001
ctg/p/l/000177/0                                                 44.1      0.001
ctg/p/l/000173/0                                                 44.1      0.001
ctg/p/l/000138/0                                                 44.1      0.001
ctg/p/l/000133/0                                                 44.1      0.001
ctg/p/l/000013/0                                                 44.1      0.001
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ctg/p/l/002318/0                                                 44.1      0.001
ctg/p/l/001040/0                                                 44.1      0.001
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ctg/p/l/000833/0                                                 44.1      0.001
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ctg/p/l/000632/0                                                 44.1      0.001
ctg/p/l/000605/0                                                 44.1      0.001
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ctg/p/l/000524/0                                                 40.1      0.019
ctg/p/l/000054/0                                                 38.2      0.076
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ctg/p/l/000175/0                                                 34.2      1.2
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ctg/p/l/000391/0                                                   32.2       4.7
ctg/p/l/000362/0                                                   32.2       4.7
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ctg/p/l/000062/0                                                   32.2       4.7
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ctg/p/l/000335/0                                                   32.2       4.7
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ctg/p/l/000279/0                                                   32.2       4.7
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ctg/p/l/000157/0                                                   32.2       4.7
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ctg/p/l/000684/0                                                   32.2       4.7
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ctg/p/l/000545/0                                                   32.2       4.7


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 Strand=Plus/Minus
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                |||||| |||||||||||||||||||
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 Strand=Plus/Minus
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               |||||| |||||||||||||||||||
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Query  3       TGTTCAAAGATTAATCCAACAGGG  26
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Potential off-target #1: No PAM motif. No C-to-T conversion

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Sbjct  4840885  AAAGATTAATCCAACAGGG  4840867

>ctg/p/1/000180/0
Length=3309818
 Score = 36.2 bits (18),  Expect = 0.30
 Identities = 24/26 (92%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| ||| |||||||||||||||
Sbjct  2241381  CATGTTTAAAAATTAATCCAACAGGG  2241356
```

Potential off-target #2:
No full-length guide match.
No “C” in the editing window.
No modification.

```
>ctg/p/1/000175/0
Length=3335648
 Score = 34.2 bits (17),  Expect = 1.2
 Identities = 17/17 (100%), Gaps = 0/17 (0%)
 Strand=Plus/Minus
Query  8        AAAGATTAATCCAACAG  24
                |||||||||||||||||
Sbjct  3288290  AAAGATTAATCCAACAG  3288274
```

Other potential off-targets:
No full-length guide match.
No “C” in the editing window. No modification.

```
>ctg/p/l/000200/0
Length=3037360
 Score = 34.2 bits (17),  Expect = 1.2
 Identities = 17/17 (100%), Gaps = 0/17 (0%)
 Strand=Plus/Plus
Query  6        TCAAAGATTAATCCAAC  22
                |||||||||||||||||
Sbjct  1634838  TCAAAGATTAATCCAAC  1634854

>ctg/p/l/001076/0
Length=594514
 Score = 34.2 bits (17),  Expect = 1.2
 Identities = 17/17 (100%), Gaps = 0/17 (0%)
 Strand=Plus/Plus
Query  8       AAAGATTAATCCAACAG  24
               |||||||||||||||||
Sbjct  571034  AAAGATTAATCCAACAG  571050

>ctg/p/l/000422/0
Length=1311407
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  5       TTCAAAGATTAATCCA  20
               ||||||||||||||||
Sbjct  865275  TTCAAAGATTAATCCA  865260

>ctg/p/l/000391/0
Length=1850532
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAA  16
                ||||||||||||||||
Sbjct  1830679  CATGTTCAAAGATTAA  1830694

>ctg/p/l/000362/0
Length=1991486
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAA  16
                ||||||||||||||||
Sbjct  1806648  CATGTTCAAAGATTAA  1806663

>ctg/p/l/000083/0
Length=5135226
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAA  16
               ||||||||||||||||
Sbjct  635934  CATGTTCAAAGATTAA  635949

>ctg/p/l/000074/0
Length=5458418
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  5        TTCAAAGATTAATCCA  20
                ||||||||||||||||
Sbjct  4188262  TTCAAAGATTAATCCA  4188277
```

Other potential off-targets: No full-length guide match. No modification.

```
>ctg/p/l/000062/0
Length=5849719
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  1235238  ATGTTCAAAGATTAAT  1235223

>ctg/p/l/000060/0
Length=5947253
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  2       ATGTTCAAAGATTAAT  17
               ||||||||||||||||
Sbjct  427083  ATGTTCAAAGATTAAT  427098

>ctg/p/l/000335/0
Length=2086631
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAA  16
                ||||||||||||||||
Sbjct  1157591  CATGTTCAAAGATTAA  1157576
```

Other potential off-targets: No full-length guide match. No modification.

```
>ctg/p/l/000302/0
Length=2312585
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 19/20 (95%), Gaps = 0/20 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAATCCA  20
               |||||||||||||| |||||
Sbjct  146399  CATGTTCAAAGATTTATCCA  146380
```

Not a perfect match. No PAM motif. The A-to-T variant is likely a SNP.

```
>ctg/p/l/000279/0
Length=2445595
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAA  16
                ||||||||||||||||
Sbjct  1044370  CATGTTCAAAGATTAA  1044355

>ctg/p/l/000265/0
Length=2514779
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  2044770  ATGTTCAAAGATTAAT  2044755

>ctg/p/l/000157/0
Length=3535457
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  6        TCAAAGATTAATCCAA  21
                ||||||||||||||||
Sbjct  1466445  TCAAAGATTAATCCAA  1466460
```

Other potential off-targets: No full-length guide match. No modification.

```
>ctg/p/l/000142/0
Length=3770831
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  6        TCAAAGATTAATCCAA  21
                ||||||||||||||||
Sbjct  3564532  TCAAAGATTAATCCAA  3564517

>ctg/p/l/000202/0
Length=3013145
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  7        CAAAGATTAATCCAAC  22
                ||||||||||||||||
Sbjct  1622308  CAAAGATTAATCCAAC  1622293

>ctg/p/l/000196/0
Length=3124558
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  1256755  ATGTTCAAAGATTAAT  1256770

>ctg/p/l/000018/0
Length=9509432
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  7522265  ATGTTCAAAGATTAAT  7522250

>ctg/p/l/001423/0
Length=345759
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAA  16
               ||||||||||||||||
Sbjct  219172  CATGTTCAAAGATTAA  219157

>ctg/p/l/000714/0
Length=466072
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  6       TCAAAGATTAATCCAA  21
               ||||||||||||||||
Sbjct  437812  TCAAAGATTAATCCAA  437797

>ctg/p/l/000936/0
Length=712211
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  6       TCAAAGATTAATCCAA  21
               ||||||||||||||||
Sbjct  565900  TCAAAGATTAATCCAA  565885
```

Other potential off-targets: No full-length guide match. No modification.

```
>ctg/p/1/000004/0
Length=14599509
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  2         ATGTTCAAAGATTAAT  17
                 ||||||||||||||||
Sbjct  14458520  ATGTTCAAAGATTAAT  14458505

>ctg/p/1/000684/0
Length=1025105
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAA  16
               ||||||||||||||||
Sbjct  763577  CATGTTCAAAGATTAA  763562

>ctg/p/1/000603/0
Length=1181885
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  3       TGTTCAAAGATTAATC  18
               ||||||||||||||||
Sbjct  678227  TGTTCAAAGATTAATC  678242

>ctg/p/1/000545/0
Length=1313736
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  8        AAAGATTAATCCAACA  23
                ||||||||||||||||
Sbjct  1021717  AAAGATTAATCCAACA  1021702
```

Other potential off-targets: No full-length guide match. No modification.

F13 fetus

```
                                                              Score      E
Sequences producing significant alignments:                  (Bits)    Value

ctg/p/l/000279/0                                               44.1       0.001
ctg/p/l/000255/0                                               44.1       0.001
ctg/p/l/000233/0                                               44.1       0.001
ctg/p/l/000231/0                                               44.1       0.001
ctg/p/l/002483/0                                               44.1       0.001
ctg/p/l/000184/0                                               44.1       0.001
ctg/p/l/000182/0                                               44.1       0.001
ctg/p/l/000155/0                                               44.1       0.001
ctg/p/l/004762/0                                               44.1       0.001
ctg/p/l/004391/0                                               44.1       0.001
ctg/p/l/003920/0                                               44.1       0.001
ctg/p/l/000023/0                                               44.1       0.001
ctg/p/l/000020/0                                               44.1       0.001
ctg/p/l/000018/0                                               44.1       0.001
ctg/p/l/000044/0                                               44.1       0.001
ctg/p/l/000041/0                                               44.1       0.001
ctg/p/l/000064/0                                               44.1       0.001
ctg/p/l/000051/0                                               44.1       0.001
ctg/p/l/000071/0                                               44.1       0.001
ctg/p/l/000083/0                                               44.1       0.001
ctg/p/l/000120/0                                               44.1       0.001
ctg/p/l/000133/0                                               44.1       0.001
ctg/p/l/000011/0                                               44.1       0.001
ctg/p/l/000807/0                                               44.1       0.001
ctg/p/l/000730/0                                               44.1       0.001
ctg/p/l/001207/0                                               44.1       0.001
ctg/p/l/001050/0                                               44.1       0.001
ctg/p/l/000002/0                                               44.1       0.001
ctg/p/l/000001/0                                               44.1       0.001
ctg/p/c/006551/0                                               44.1       0.001
ctg/p/l/000005/0                                               44.1       0.001
ctg/p/l/000004/0                                               44.1       0.001
ctg/p/l/000404/0                                               44.1       0.001
ctg/p/l/000391/0                                               44.1       0.001
ctg/p/l/000366/0                                               44.1       0.001
ctg/p/l/000363/0                                               44.1       0.001
ctg/p/l/000347/0                                               44.1       0.001
ctg/p/l/000329/0                                               44.1       0.001
ctg/p/l/000584/0                                               44.1       0.001
ctg/p/l/000532/0                                               44.1       0.001
ctg/p/l/000019/0                                               40.1       0.019
ctg/p/l/000006/0                                               40.1       0.019
ctg/p/l/000077/0                                               36.2       0.30
ctg/p/l/000042/0                                               34.2       1.2
ctg/p/l/000065/0                                               34.2       1.2
ctg/p/l/000283/0                                               32.2       4.7
ctg/p/l/000229/0                                               32.2       4.7
ctg/p/l/000157/0                                               32.2       4.7
ctg/p/l/000022/0                                               32.2       4.7
ctg/p/l/000035/0                                               32.2       4.7
ctg/p/l/000034/0                                               32.2       4.7
ctg/p/l/000031/0                                               32.2       4.7
ctg/p/l/000030/0                                               32.2       4.7
ctg/p/l/000029/0                                               32.2       4.7
ctg/p/l/000048/0                                               32.2       4.7
ctg/p/l/000045/0                                               32.2       4.7
ctg/p/l/000061/0                                               32.2       4.7
ctg/p/l/000078/0                                               32.2       4.7
ctg/p/l/000076/0                                               32.2       4.7
```

```
ctg/p/l/000070/0                                                     32.2       4.7
ctg/p/l/000110/0                                                     32.2       4.7
ctg/p/l/000142/0                                                     32.2       4.7
ctg/p/l/000132/0                                                     32.2       4.7
ctg/p/l/000015/0                                                     32.2       4.7
ctg/p/l/000813/0                                                     32.2       4.7
ctg/p/l/000790/0                                                     32.2       4.7
ctg/p/l/000848/0                                                     32.2       4.7
ctg/p/l/001105/0                                                     32.2       4.7
ctg/p/l/000309/0                                                     32.2       4.7



>ctg/p/l/000279/0
Length=2076092
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  831728  CATGTTTAAAGATTAATCCAACAGGG  831703

>ctg/p/l/000255/0
Length=2270825
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  804448  CATGTTTAAAGATTAATCCAACAGGG  804423

>ctg/p/l/000233/0
Length=2548522
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plu
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  1579861  CATGTTTAAAGATTAATCCAACAGGG  1579886

>ctg/p/l/000231/0
Length=2570186
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  1957360  CATGTTTAAAGATTAATCCAACAGGG  1957335

>ctg/p/l/002483/0
Length=188985
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  106180  CATGTTTAAAGATTAATCCAACAGGG  106205
```

```
>ctg/p/l/000184/0
Length=1401273
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               ||||||||||||||||| ||||||||
Sbjct  501381  CATGTTCAAAGATTAATTCAACAGGG  501406

>ctg/p/l/000182/0
Length=2691948
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  122531  CATGTTTAAAGATTAATCCAACAGGG  122506

>ctg/p/l/000155/0
Length=4230312
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  1460714  CATGTTTAAAGATTAATCCAACAGGG  1460739

>ctg/p/l/004762/0
Length=65350
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1      CATGTTCAAAGATTAATCCAACAGGG  26
              |||||| |||||||||||||||||||
Sbjct  37088  CATGTTTAAAGATTAATCCAACAGGG  37113

>ctg/p/l/004391/0
Length=75958
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1      CATGTTCAAAGATTAATCCAACAGGG  26
              |||||| |||||||||||||||||||
Sbjct  19221  CATGTTTAAAGATTAATCCAACAGGG  19196

>ctg/p/l/003920/0
Length=94414
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1      CATGTTCAAAGATTAATCCAACAGGG  26
              |||||| |||||||||||||||||||
Sbjct  23552  CATGTTTAAAGATTAATCCAACAGGG  23527

>ctg/p/l/000023/0
Length=16067452
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1         CATGTTCAAAGATTAATCCAACAGGG  26
                 |||||| |||||||||||||||||||
Sbjct  15518781  CATGTTTAAAGATTAATCCAACAGGG  15518756
```

```
>ctg/p/1/000020/0
Length=16569276
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1         CATGTTCAAAGATTAATCCAACAGGG  26
                 |||||| |||||||||||||||||||
Sbjct  13616027  CATGTTTAAAGATTAATCCAACAGGG  13616052

>ctg/p/1/000018/0
Length=16872726
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  2431351  CATGTTTAAAGATTAATCCAACAGGG  2431376

>ctg/p/1/000044/0
Length=11815907
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  1337526  CATGTTTAAAGATTAATCCAACAGGG  1337551

>ctg/p/1/000041/0
Length=12196511
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  3036322  CATGTTTAAAGATTAATCCAACAGGG  3036297

>ctg/p/1/000064/0
Length=9759816
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  8728548  CATGTTTAAAGATTAATCCAACAGGG  8728573

>ctg/p/1/000051/0
Length=10883686
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1     CATGTTCAAAGATTAATCCAACAGGG  26
             |||||| |||||||||||||||||||
Sbjct  1470  CATGTTTAAAGATTAATCCAACAGGG  1445

>ctg/p/1/000071/0
Length=9192088
Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  7656027  CATGTTTAAAGATTAATCCAACAGGG  7656002
```

```
>ctg/p/l/000083/0
Length=8167830
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  5958573  CATGTTTAAAGATTAATCCAACAGGG  5958548

>ctg/p/l/000120/0
Length=5777834
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  502253  CATGTTTAAAGATTAATCCAACAGGG  502278

>ctg/p/l/000133/0
Length=5224621
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  2116020  CATGTTTAAAGATTAATCCAACAGGG  2115995

 Score = 36.2 bits (18),  Expect = 0.30
 Identities = 24/26 (92%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||| ||||
Sbjct  4972239  CATGTTTAAAGATTAATCCAATAGGG  4972264

>ctg/p/l/000011/0
Length=19930340
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  3474675  CATGTTTAAAGATTAATCCAACAGGG  3474650

>ctg/p/l/000807/0
Length=707054
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1      CATGTTCAAAGATTAATCCAACAGGG  26
              |||||| |||||||||||||||||||
Sbjct  26630  CATGTTTAAAGATTAATCCAACAGGG  26605

>ctg/p/l/000730/0
Length=719974
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  387047  CATGTTTAAAGATTAATCCAACAGGG  387072
```

```
>ctg/p/l/001207/0
Length=199370
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  140369  CATGTTTAAAGATTAATCCAACAGGG  140394

>ctg/p/l/001050/0
Length=539755
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  255862  CATGTTTAAAGATTAATCCAACAGGG  255887

>ctg/p/l/000002/0
Length=35377810
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  4908303  CATGTTTAAAGATTAATCCAACAGGG  4908278

>ctg/p/l/000001/0
Length=37491508
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  1973189  CATGTTTAAAGATTAATCCAACAGGG  1973164

>ctg/p/c/006551/0
Length=19057
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1      CATGTTCAAAGATTAATCCAACAGGG  26
              |||||| |||||||||||||||||||
Sbjct  13278  CATGTTTAAAGATTAATCCAACAGGG  13303

>ctg/p/l/000005/0
Length=26117305
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1         CATGTTCAAAGATTAATCCAACAGGG  26
                 |||||| |||||||||||||||||||
Sbjct  17659527  CATGTTTAAAGATTAATCCAACAGGG  17659502

 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1         CATGTTCAAAGATTAATCCAACAGGG  26
                 |||||| |||||||||||||||||||
Sbjct  22383597  CATGTTTAAAGATTAATCCAACAGGG  22383572
```

```
>ctg/p/l/000004/0
Length=26849317
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1    CATGTTCAAAGATTAATCCAACAGGG  26
            |||||| |||||||||||||||||||
Sbjct  824  CATGTTTAAAGATTAATCCAACAGGG  799

>ctg/p/l/000404/0
Length=1365740
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  1161961  CATGTTTAAAGATTAATCCAACAGGG  1161986

>ctg/p/l/000391/0
Length=1085046
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  666155  CATGTTTAAAGATTAATCCAACAGGG  666180

>ctg/p/l/000366/0
Length=1505255
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  235855  CATGTTTAAAGATTAATCCAACAGGG  235880

>ctg/p/l/000363/0
Length=1511906
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| |||||||||||||||||||
Sbjct  1049604  CATGTTTAAAGATTAATCCAACAGGG  1049579

>ctg/p/l/000347/0
Length=1587001
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  834128  CATGTTTAAAGATTAATCCAACAGGG  834153

>ctg/p/l/000329/0
Length=529183
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               ||||| ||||||||||||||||||||
Sbjct  387733  CATGTCCAAAGATTAATCCAACAGGG  387708
```

Unmodified allele: Full-length guide match. “C” in the editing window is unmodified. The atypical T-to-C modification could be a potential sequencing error

```
>ctg/p/l/000584/0
Length=776203
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  558936  CATGTTTAAAGATTAATCCAACAGGG  558911

>ctg/p/l/000532/0
Length=1043252
 Score = 44.1 bits (22),  Expect = 0.001
 Identities = 25/26 (96%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCAACAGGG  26
               |||||| |||||||||||||||||||
Sbjct  699998  CATGTTTAAAGATTAATCCAACAGGG  700023

>ctg/p/l/000019/0
Length=16594298
 Score = 40.1 bits (20),  Expect = 0.019
 Identities = 23/24 (96%), Gaps = 0/24 (0%)
 Strand=Plus/Plus
Query  3        TGTTCAAAGATTAATCCAACAGGG  26
                |||| |||||||||||||||||||
Sbjct  8997607  TGTTTAAAGATTAATCCAACAGGG  8997630

>ctg/p/l/000006/0
Length=24590259
 Score = 40.1 bits (20),  Expect = 0.019
 Identities = 20/20 (100%), Gaps = 0/20 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAATCCA  20
               ||||||||||||||||||||
Sbjct  879191  CATGTTCAAAGATTAATCCA  879210
```

Other potential off-target: No PAM-motif. No modification.

```
>ctg/p/l/000077/0
Length=8648054
 Score = 36.2 bits (18),  Expect = 0.30
 Identities = 24/26 (92%), Gaps = 0/26 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAATCCAACAGGG  26
                |||||| ||| |||||||||||||||
Sbjct  6435571  CATGTTTAAAAATTAATCCAACAGGG  6435596

>ctg/p/l/000042/0
Length=12060338
 Score = 34.2 bits (17),  Expect = 1.2
 Identities = 17/17 (100%), Gaps = 0/17 (0%)
 Strand=Plus/Minus
Query  6         TCAAAGATTAATCCAAC  22
                 |||||||||||||||||
Sbjct  10989117  TCAAAGATTAATCCAAC  10989101

>ctg/p/l/000065/0
Length=9713824
 Score = 34.2 bits (17),  Expect = 1.2
 Identities = 17/17 (100%), Gaps = 0/17 (0%)
 Strand=Plus/Plus
Query  8        AAAGATTAATCCAACAG  24
                |||||||||||||||||
Sbjct  6179604  AAAGATTAATCCAACAG  6179620
```

Other potential off-targets: No full-length guide match. No modification.

```
>ctg/p/l/000283/0
Length=2069269
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  6       TCAAAGATTAATCCAA  21
               ||||||||||||||||
Sbjct  433581  TCAAAGATTAATCCAA  433566
```

```
>ctg/p/l/000229/0
Length=2491534
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 19/20 (95%), Gaps = 0/20 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAATCCA  20
                |||||||||||||| |||||
Sbjct  2165739  CATGTTCAAAGATTTATCCA  2165758
```

No PAM motif. No C-to-T conversion in the window. The A-to-T variation is likely a SNP

```
>ctg/p/l/000157/0
Length=4177983
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAA  16
                ||||||||||||||||
Sbjct  2614161  CATGTTCAAAGATTAA  2614146
```

```
>ctg/p/l/000022/0
Length=16386320
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  6         TCAAAGATTAATCCAA  21
                 ||||||||||||||||
Sbjct  14219288  TCAAAGATTAATCCAA  14219303
```

```
>ctg/p/l/000035/0
Length=13100527
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAA  16
                ||||||||||||||||
Sbjct  5939357  CATGTTCAAAGATTAA  5939342
```

```
>ctg/p/l/000034/0
Length=13195684
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  1091512  ATGTTCAAAGATTAAT  1091527
```

```
>ctg/p/l/000031/0
Length=13501503
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  2         ATGTTCAAAGATTAAT  17
                 ||||||||||||||||
Sbjct  13401945  ATGTTCAAAGATTAAT  13401930
```

Other potential off-targets: No full-length guide match. No modification.

```
>ctg/p/l/000030/0
Length=13865225
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  3         TGTTCAAAGATTAATC  18
                 ||||||||||||||||
Sbjct  13527081  TGTTCAAAGATTAATC  13527066

>ctg/p/l/000029/0
Length=14498594
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1        CATGTTCAAAGATTAA  16
                ||||||||||||||||
Sbjct  2520849  CATGTTCAAAGATTAA  2520834

>ctg/p/l/000048/0
Length=11255473
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  2421392  ATGTTCAAAGATTAAT  2421407

>ctg/p/l/000045/0
Length=11433674
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  5        TTCAAAGATTAATCCA  20
                ||||||||||||||||
Sbjct  6274869  TTCAAAGATTAATCCA  6274854

>ctg/p/l/000061/0
Length=9943486
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  2      ATGTTCAAAGATTAAT  17
              ||||||||||||||||
Sbjct  90264  ATGTTCAAAGATTAAT  90279

>ctg/p/l/000078/0
Length=8605118
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  3792361  ATGTTCAAAGATTAAT  3792346


>ctg/p/l/000076/0
Length=8651189
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1       CATGTTCAAAGATTAA  16
               ||||||||||||||||
Sbjct  910171  CATGTTCAAAGATTAA  910156
```

Other potential off-targets: No full-length guide match. No modification.

```
>ctg/p/l/000070/0
Length=9202715
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  7888261  ATGTTCAAAGATTAAT  7888246

>ctg/p/l/000110/0
Length=5881360
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  1        CATGTTCAAAGATTAA  16
                ||||||||||||||||
Sbjct  2066902  CATGTTCAAAGATTAA  2066917

>ctg/p/l/000142/0
Length=4923165
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  6        TCAAAGATTAATCCAA  21
                ||||||||||||||||
Sbjct  2834137  TCAAAGATTAATCCAA  2834122

>ctg/p/l/000132/0
Length=5238981
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  6        TCAAAGATTAATCCAA  21
                ||||||||||||||||
Sbjct  3462179  TCAAAGATTAATCCAA  3462164

>ctg/p/l/000015/0
Length=18788804
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  2        ATGTTCAAAGATTAAT  17
                ||||||||||||||||
Sbjct  4715006  ATGTTCAAAGATTAAT  4714991

>ctg/p/l/000813/0
Length=700004
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  8       AAAGATTAATCCAACA  23
               ||||||||||||||||
Sbjct  448872  AAAGATTAATCCAACA  448887
>ctg/p/l/000790/0
Length=724722
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  1       CATGTTCAAAGATTAA  16
               ||||||||||||||||
Sbjct  291390  CATGTTCAAAGATTAA  291405
```

Other potential off-targets: No full-length guide match. No modification.

```
>ctg/p/l/000848/0
Length=295309
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Plus
Query  2       ATGTTCAAAGATTAAT  17
               ||||||||||||||||
Sbjct  212421  ATGTTCAAAGATTAAT  212436

>ctg/p/l/001105/0
Length=507962
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  1     CATGTTCAAAGATTAA  16
             ||||||||||||||||
Sbjct  7763  CATGTTCAAAGATTAA  7748

>ctg/p/l/000309/0
Length=1824814
 Score = 32.2 bits (16),  Expect = 4.7
 Identities = 16/16 (100%), Gaps = 0/16 (0%)
 Strand=Plus/Minus
Query  7        CAAAGATTAATCCAAC  22
                ||||||||||||||||
Sbjct  1398515  CAAAGATTAATCCAAC  1398500
```

Other potential off-targets: No full-length guide match. No modification.

Supplementary data #2

Pathology report

Specimen identification
Specimen name: Wild type and genetically modified XENOpig livers
Specimen IDs: 007, 009 and 010 (Wild type pigs perfused with human blood); 005 and 008 (XENOpigs perfused with human blood)
Case number: Pathology report

SPECIMEN LABEL(s): *Ex vivo* perfused liver

PATIENT HISTORY: All pigs were physiologically healthy at the time of euthanasia and liver perfusion.

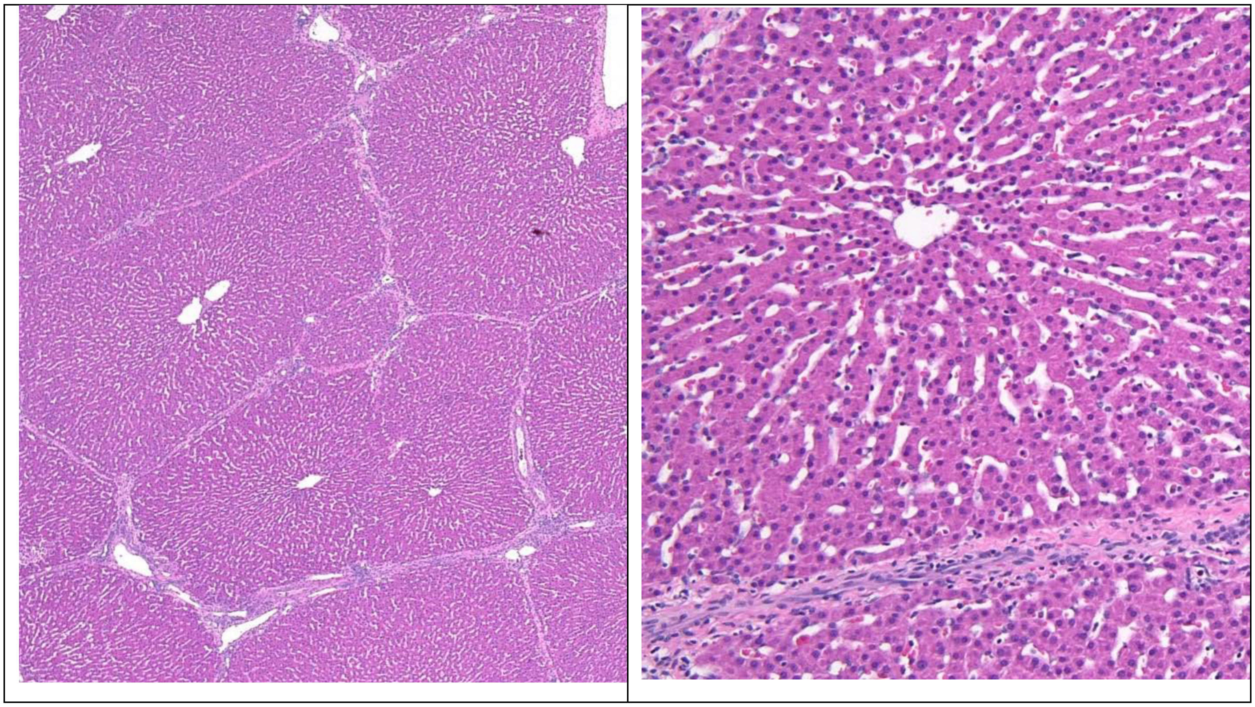
DIAGNOSIS: As seen below, the wild type pigs perfused with human blood exhibited signs of rejection evident by congestion at 6 hrs post-perfusion. The congestion was marked by 14 hr timepoint, and the sporadic congestion became patchy leading to necrosis of the liver samples. By 36 hrs, the congestion and liver necrosis was marked. The lesions in Animals # 007, 009 and 010 were more severe, acute, and exhibited necrotizing features with vascular thromboses, and severe sub-massive to massive necrosis in all liver specimens. Conversely, Animals 005 and 008 displayed multifocal areas of survival, albeit existence of degenerate hepatocytes, which actively produced significant amount of bile.

Overall, the microscopic lesions observed in Animals# 007, 009 and 010 and Animal 005 and 008 were distinguishable. Histological parameters were markedly better at 24 hrs when compared to the wild type pigs, with the first signs of congestion appearing at 36 hrs and marked patchy congestion/ necrosis evident at 48 hrs of age.

Animals 007, 009 and 010

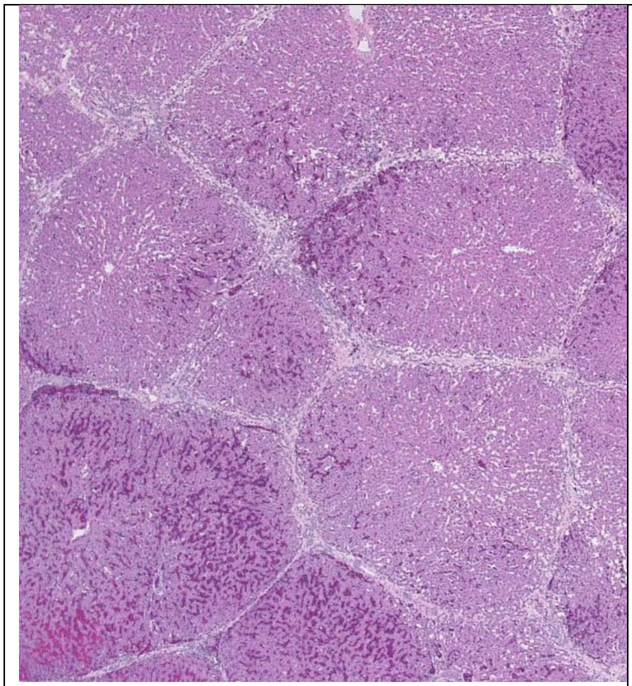
1hr 14m post-surgery (Day 1)

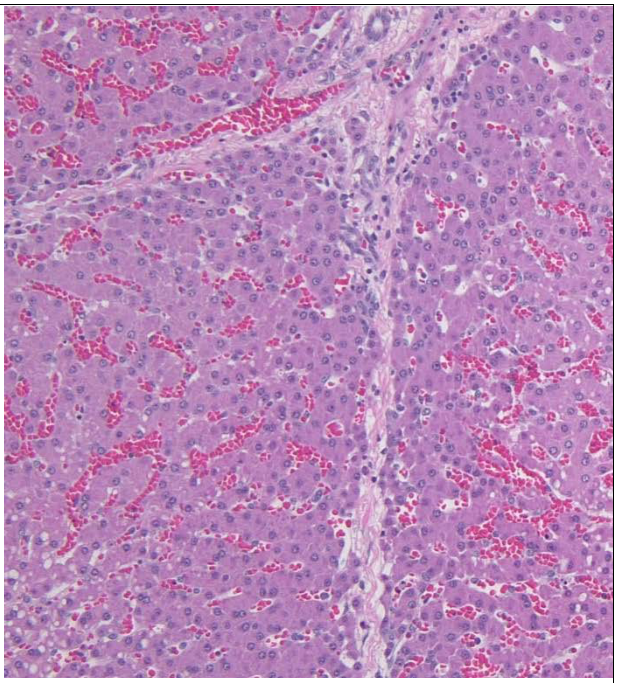
No significant lesion was found.

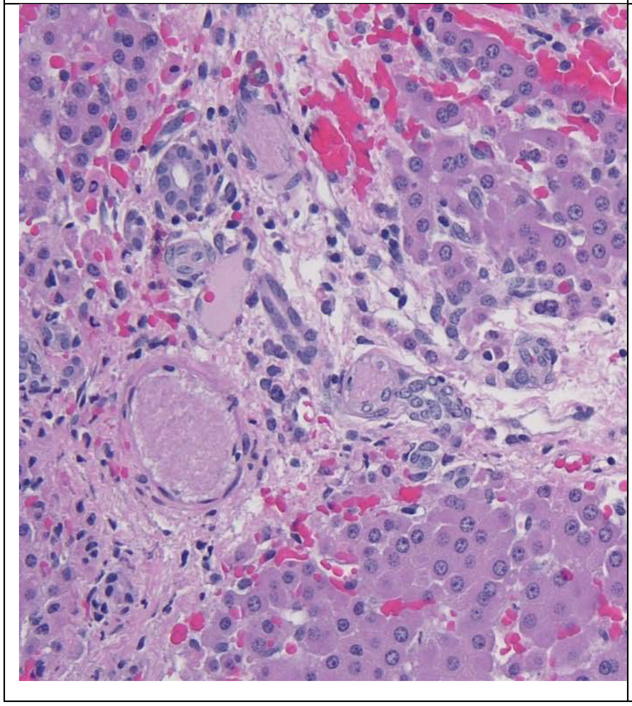


5hr 51m post-surgery (Day 1)

There were multifocal lobular congestion, hepatocellular vacuolar degeneration, and mild coagulative/ischemic necrosis. Several blood vessels (mostly veins) were thrombosed. Few neutrophils and lymphocytes were scattered in the portal and periportal areas. This was the earliest time point showing the pathologic changes associated with xenotransplantation in this animal.

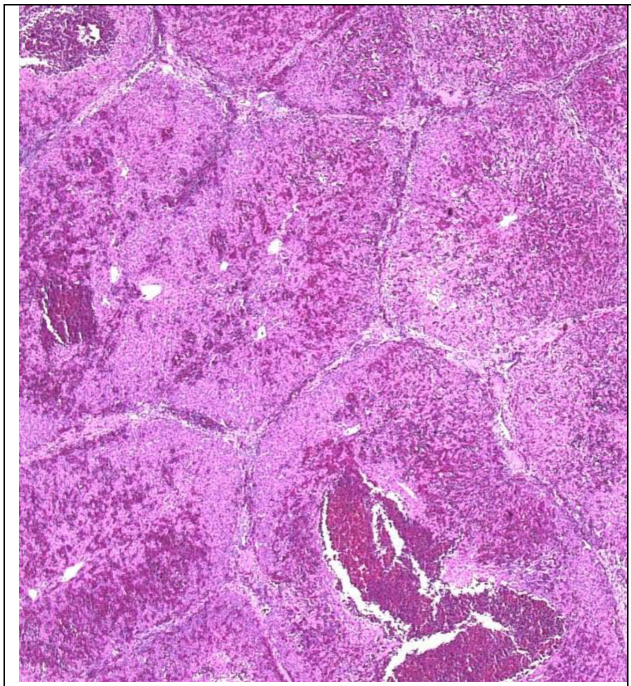


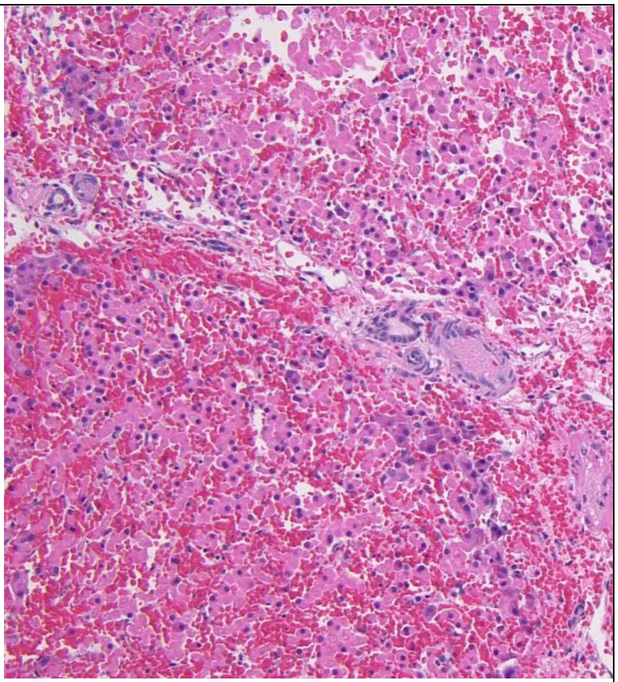


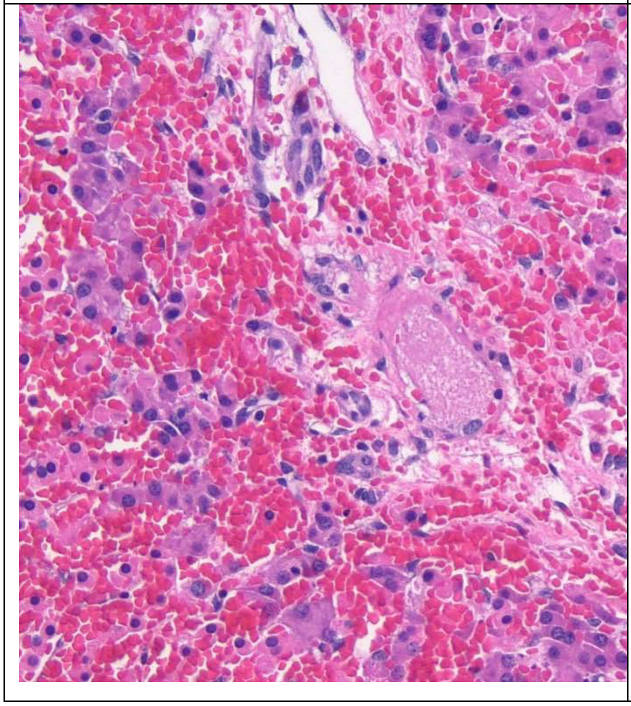


6hr 5m post-surgery (Day 2)

There was diffuse severe submassive hepatocellular coagulative/ischemic necrosis and loss. The hepatic cords were dissociated. Many blood vessels were thrombosed, and the endothelial cells were plump.

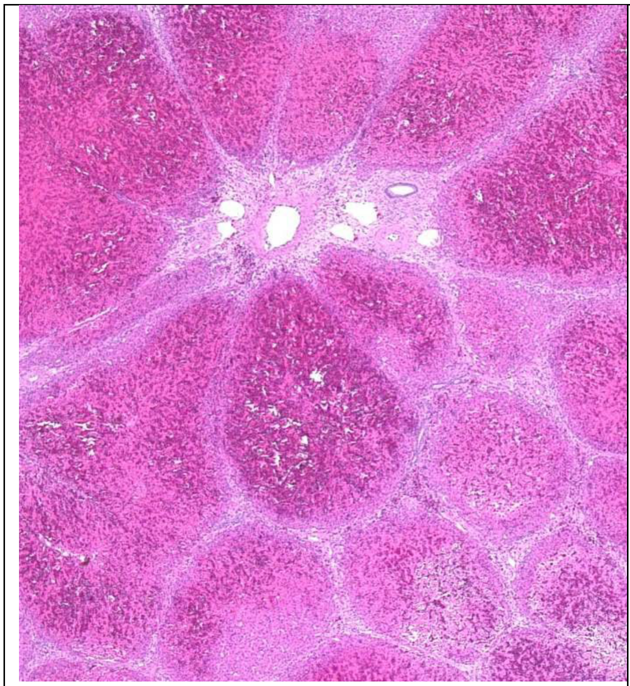


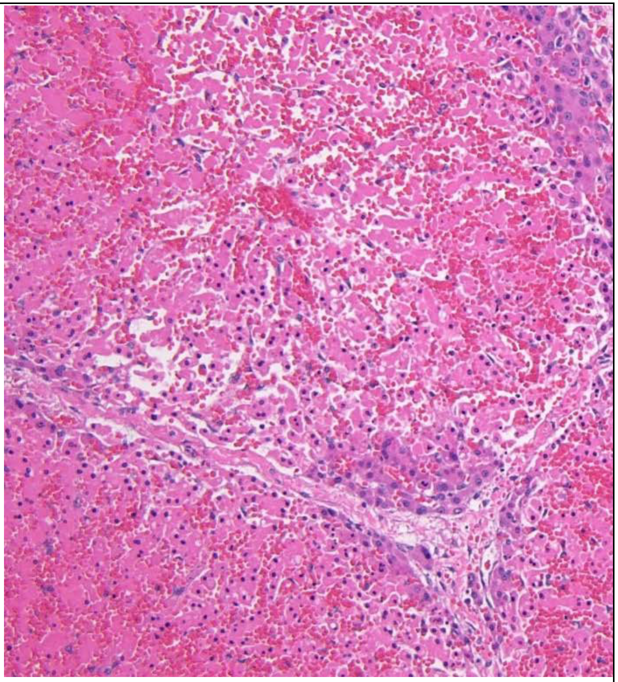


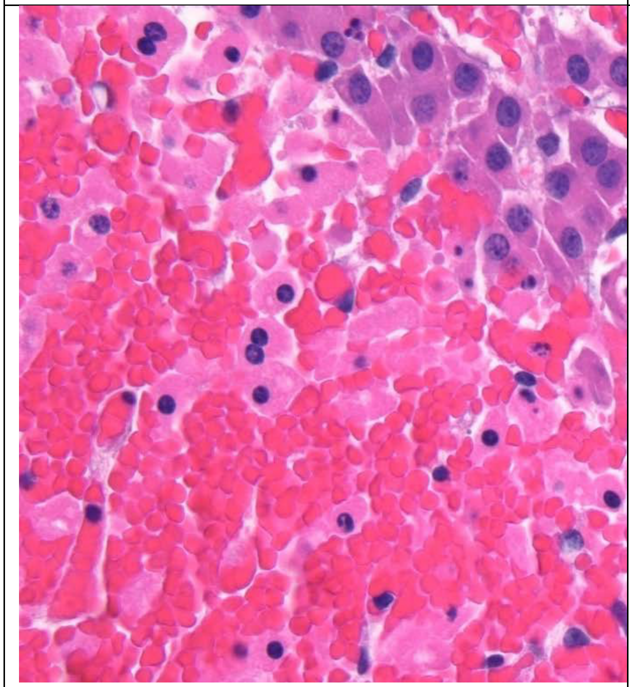


<u>42hr 20m post-surgery (Day 3)</u>

There was diffuse severe sub-massive to massive hepatocellular coagulative/ischemic necrosis and loss. Due to the hepatocellular loss, the hepatic lobules were smaller than normal. The portal areas and lobular septa were expanded with hemorrhage, edema, and inflammation. The blood vessels were thrombosed. The inflammatory cells were neutrophils and lymphocytes.



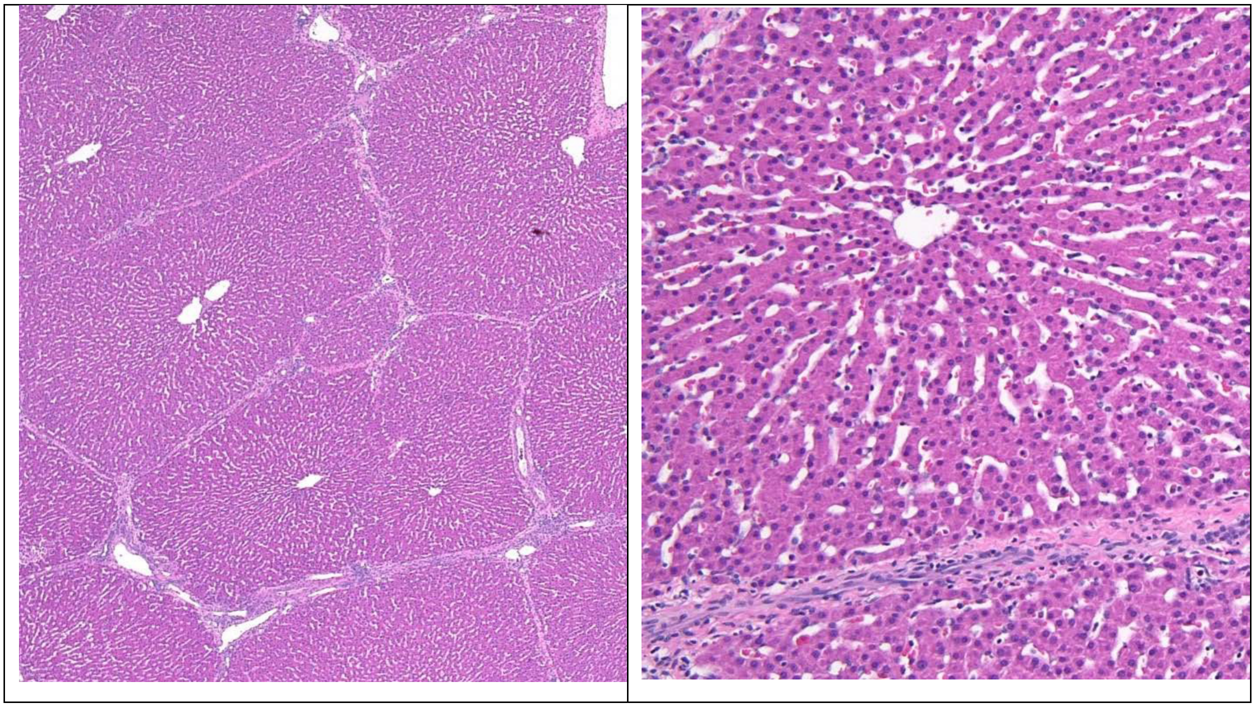




Animals 005 and 008

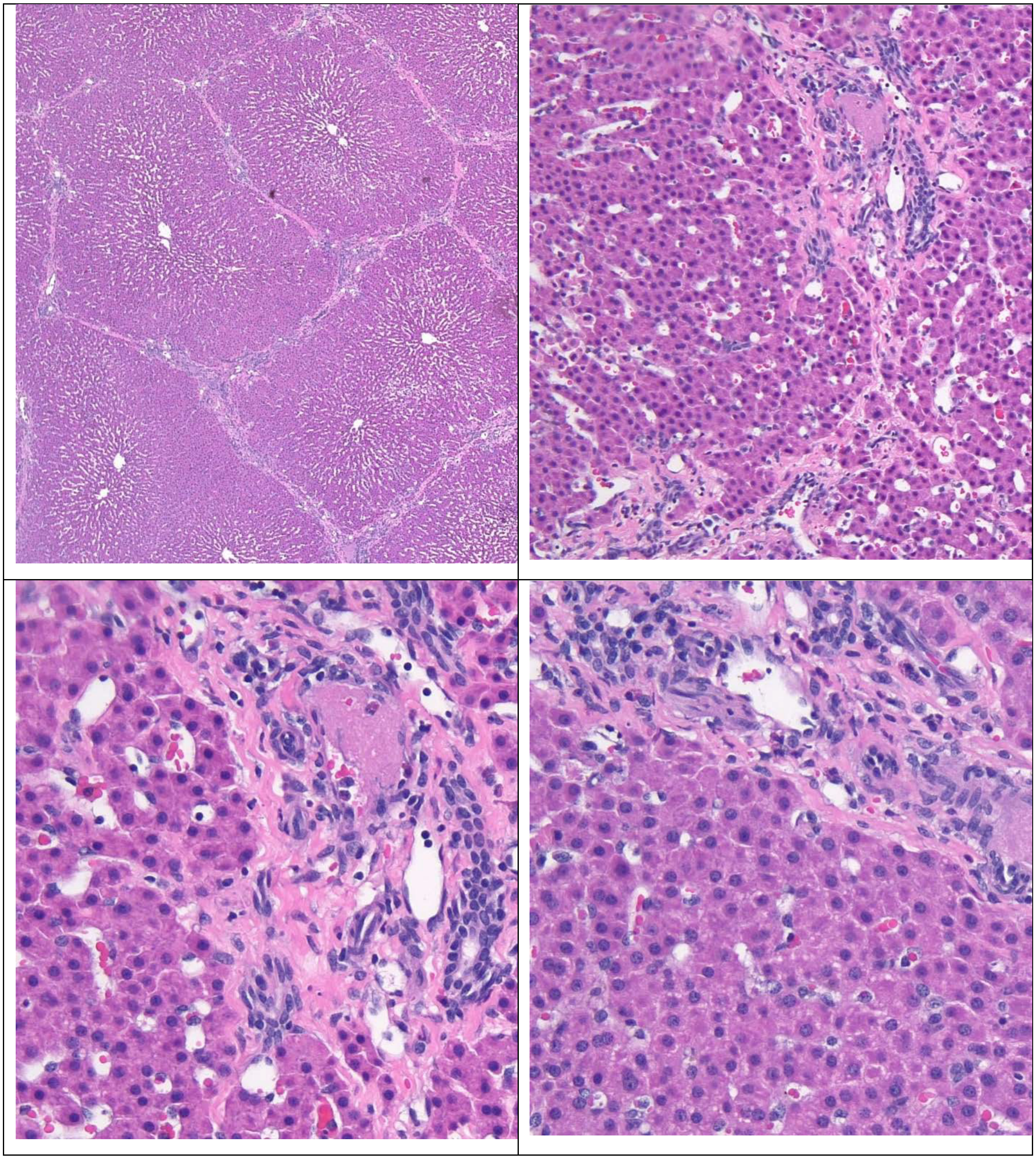
<u>2hr 58m post-surgery (Day 1)</u>

No significant microscopic lesion was noted.



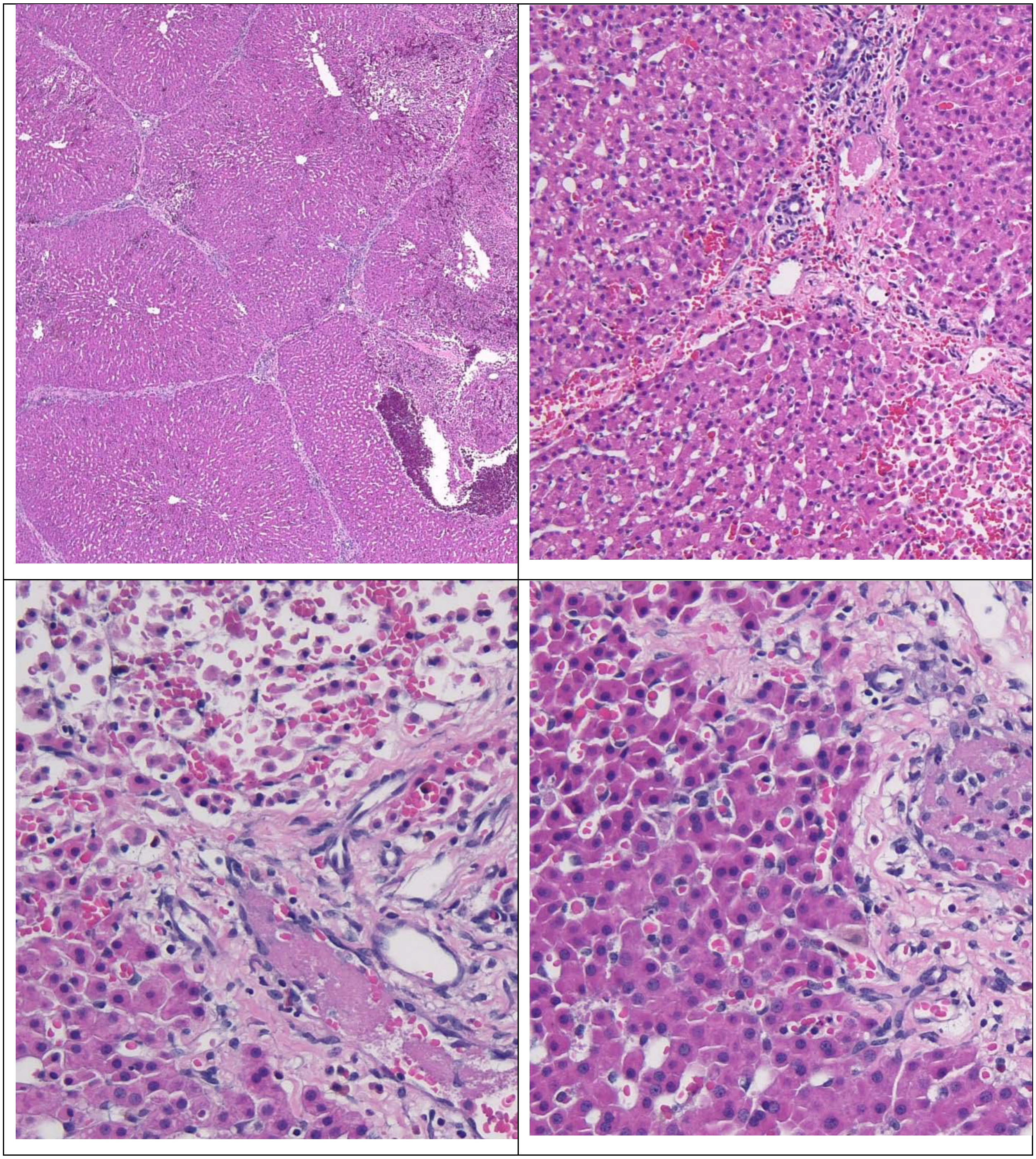
5hr 36m post-surgery (Day 1)

There was multifocal mild centrilobular hepatocellular vacuolar degeneration and multifocal random mild hepatocellular degeneration and coagulative necrosis. Several blood vessels were thrombosed. A few inflammatory cells including neutrophils and lymphocytes infiltrate the portal areas. This was the earliest time point showing microscopic lesions.



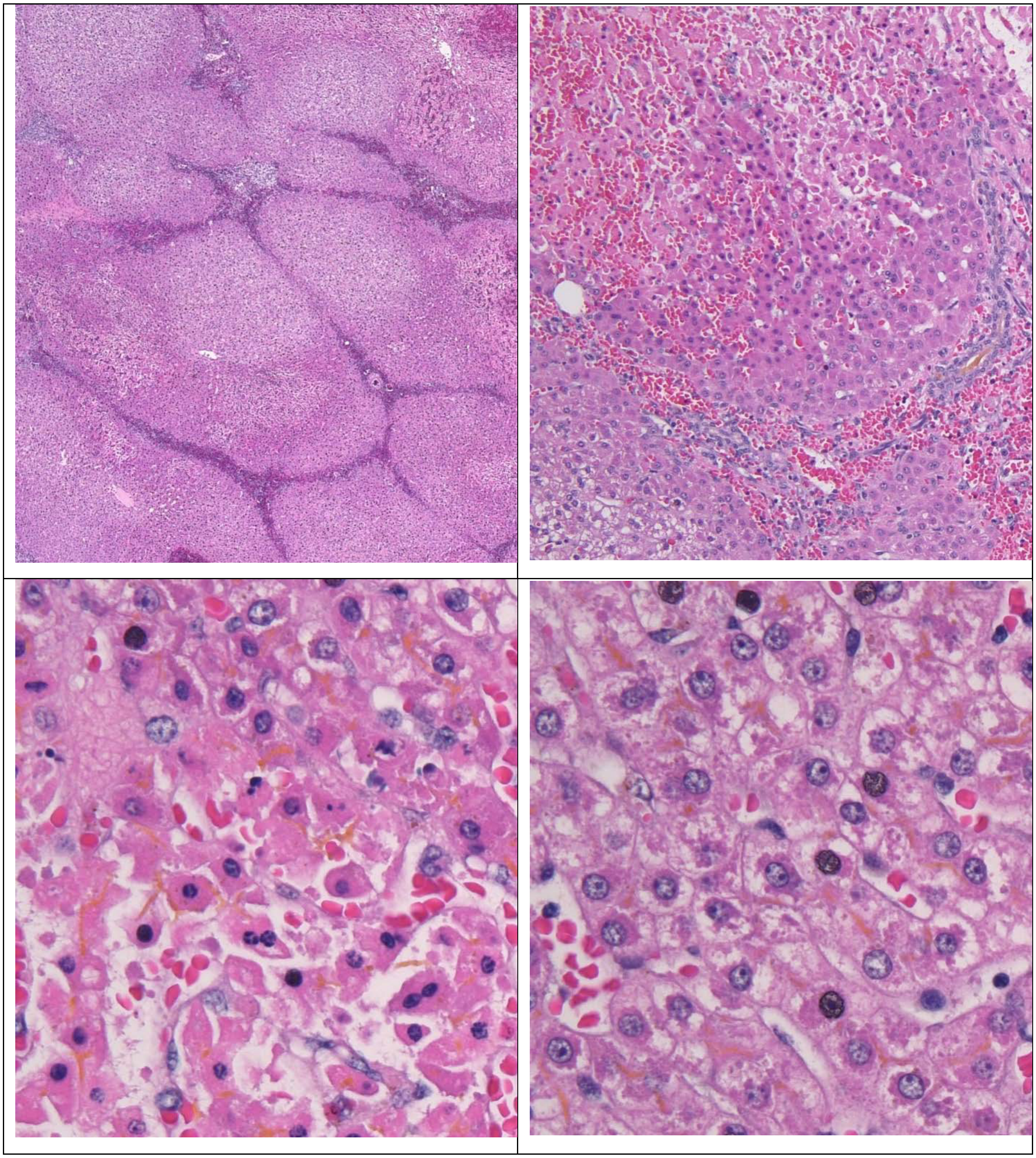
14hr 06m post-surgery (Day 2)

There was mild multifocal centrilobular to midzonal hepatocellular coagulative/ischemic necrosis with hemorrhage. The blood vessels, often veins, were commonly thrombosed with plump endothelial cells. The portal areas had multifocal mild inflammation with neutrophils, eosinophils, and lymphocytes.



46hr 51m post-surgery (Day 3)

There was multifocal mild to moderate centrilobular to midzonal hepatocellular coagulative/ischemic necrosis with hemorrhage. The blood vessels were commonly thrombosed. In the pictures of the lower row, the hepatocytes were either necrotic with pyknosis or degenerate with cytoplasmic vacuoles and yellow brown pigment granules (bile). The bile canaliculi were prominent with bile plugging.



Specimen ID: Wild type pig livers (007, 009 and 010 perfused with human blood)

007 3/25 16:05	Minimal sinusoidal dilatation
007 3/25 16:45	Minimal sinusoidal dilatation and congestion
007 3/25 17:19	Minimal sinusoidal dilatation and congestion
007 3/25 21:56	Focal zone 3 congestion and hepatocyte drop out/ necrosis Minimal sinusoidal dilatation/ congestion
007 3/26 6:05	Zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis
007 3/26 16:46	zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis
007 3/27 8:25	Zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis to areas of Near total hemorrhagic necrosis
007 3/27 8:55	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis to areas of Near total hemorrhagic necrosis

Slide label	Histology findings
009 05/13 17:18	Minimal sinusoidal dilatation
009 05/13 18:33	Minimal sinusoidal dilatation and congestion
009 05/13 20:25	Minimal sinusoidal dilatation and congestion
009 05/13 23:59	Focal zone 3 congestion and hepatocyte drop out/ necrosis Minimal sinusoidal dilatation/ congestion
009 05/14 06:30	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis
009 05/14 17:17	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis to areas of Near total hemorrhagic necrosis C4d -negative on representative block

009 05/15 17:25	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis to areas of Near total hemorrhagic necrosis

Slide label	Histology findings
010 05/20 17:25	Minimal sinusoidal dilatation
010 05/20 17:57	Minimal sinusoidal dilatation and congestion
010 05/20 19:13	Minimal sinusoidal dilatation and congestion
010 05/20 21:10	Focal zone 3 congestion and hepatocyte drop out/ necrosis Minimal sinusoidal dilatation/ congestion
010 05/21 17:45	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis/microthrombosis/ karyorhexis
010 05/21 22:35	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis to areas of Near total hemorrhagic necrosis/major thrombotic plaques
010 05/21 23:58	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ major thrombotic plaques/ necrosis to areas of Near total hemorrhagic necrosis

Specimen ID: XenoPig liver (005 perfused with human blood)

005 2/26 17:00	Minimal sinusoidal dilatation
005 2/26 18:10	Minimal sinusoidal dilatation
005 2/27 7:15	Sporadic patchy congestion
005 2/28 9:10	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis to Near total hemorrhagic necrosis (Marked zone 3 congestion and almost complete liver cell dropout) Some ductular cholestasis Few veins with clots

Specimen ID: XenoPig liver (008 perfused with human blood)

008 4/1 16:24	Minimal sinusoidal dilatation
008 4/1 17:12	Minimal sinusoidal dilatation
008 4/1 18:12	Minimal sinusoidal dilatation
008 4/1 19.22	Minimal sinusoidal dilatation
008 4/1 22:00	Minimal sinusoidal dilatation
008 4/2 6:30	Minimal sinusoidal dilatation
008 4/2 17:20	Sporadic patchy congestion
008 4/3 15:15	Sporadic patchy congestion
008 4/3 16:09	Patchy zone 3 congestion
008 4/3 16:13	Patchy zone 3 congestion/ hemorrhage and hepatocyte dropout/ necrosis

Supplementary Table S1: Single Guide (sg) RNA used in the manuscript

sgRNA	**Sequence + PAM**
PERV	*GTTCAAAGATTAATCCAACAGGG*
GGTA1	*CTTCCAAAACAACTTTGGGGTGG*
B4GalNT2	*AGTGCCAATGTGACGCCTTCGGG*
CMAH	*CACCATCCTCGCGCAAAAGCAGG*
GHR	*AGAGTCCAGTTGAGGCCAATGGG*

Supplementary Table S2: Primers used in the study

Primer Name	**Sequence**	**Size**
PERV_F	*GCCAGTGCTACACCAGTATCAG*	176 bp
PERV_R	*ACTGGTCGATAATCATTGGTCC*	
GGTA1_F	*AGATCAAGTCCGAGAAGAGGTG*	286 bp
GGTA1_R	*ATGGCTGCGTGGTAATAAAAAT*	
B4GALNT2_F	*TTATTTTAGGCTGTTCCCGAAA*	159 bp
B4GALNT2_R	*CATCTGCAATACCTCCTCTGAA*	
CMAH_F	*ATCACGTACCTTACTCACGCCT*	200 bp
CMAH_R	*CTTCCTTACCTCAGGTGGTCTG*	
GHR_F	*ATTCCATTAATACCAAGCGGTG*	174 bp
GHR_R	*ATCCCTTCTGAACATCTGCATT*	

Supplementary Table S3: Reference Target amplicon sequence of target genes

Target Gene (size)	**Amplicon sequence for NGS**
PERV (176bp)	gccagtgctacaccagtatcagtcagacagtaccccttgagtagagaggctcgagaaggaatttggccgcatgttcaaagattaatccaacagggcatcctagctcctgtccaatccccttggaatactcccctgctaccggttaggaagcctgggaccaatgattatcgaccagt
GGTA1 (286bp)	agatcaagtccgagaagaggtggcaagacatcagcatgatgcgcatgaagaccatcggggagcacatcctggcccacatccagcacgaggtggacttcctcttctgcatggacgtggatcaggtcttccaaaacaactttggggtggagaccctgggccagtcggtggctcagctacaggcctggtggtacaaggcacatcctgacgagttcacctacgagaggcggaaggagtccgcagcctacattccgtttggccaggggattttattaccacgcagccat
B4GALNT2 (159bp)	ttattttaggctgttcccgaaaaaccagtgccaatgtgacgccttcgggcatcaggaaagctataacttggaggatgcctacgacccgcgtgacctccccgcagtgaacctgaggagacaggctgagctcgaacactttcagaggaggtattgcagatg
CMAH (200bp)	atcacgtaccttactcacgcctgcatggacctcaagctgggagacaagaggatggtgttcgatccttggttaatcggtcctgcttttgcgcgaggatggtggttactacacgagcctccatctgattggctggagaggctgagccgcgcagatttaatttacatcagtcacatgcactcagaccacctgaggtaaggaag
GHR (174bp)	attccattaataccaagcggtgtctatgtgctgattctctgttgaaatccacagtgcaaccggatccacccattggcctcaactggactctactgaatatcagtttaacagggattcatgcagatatccaagtgagatgggaaccacctcccaatgcagatgttcagaagggat

Supplementary Table S4. Antibodies and lectins used in the study for immunohistochemistry

Applications	Gene	Antibody name	Source	Catalog #	Conjugate	Host/Isotype	Dilution
ICC	*GGTA1*	Primary: α-Gal Epitope (Galα1-3Galβ1-4GlcNAc-R) monoclonal antibody (M86) α-Gal Epitope (Galα1-3Galβ1-4GlcNAc-R) monoclonal antibody (M86)	Enzo Life Sciences	ALX-801-090		Mouse	1 in 100
		Secondary: Goat Anti Mouse Cy5 647	Thermo Fisher	A28181		Goat	1 in 500
DBA lectin fluorescence staining	*B4GALNT2*	Dolichos Biflorus (Horse Gram) Agglutinin (DBA), fluorescein (FITC)	Thermo Fisher	L32474	FITC		1:200 (10 μg/mL); 1 in 250
ICC	*CMAH*	Primary: Purified anti-Neu5Gc Antibody	Biolegend	146903		Chicken	1 in 500
		Secondary: Goat Anti chicken Cy5 647	Thermo Fisher	A28181		Goat	