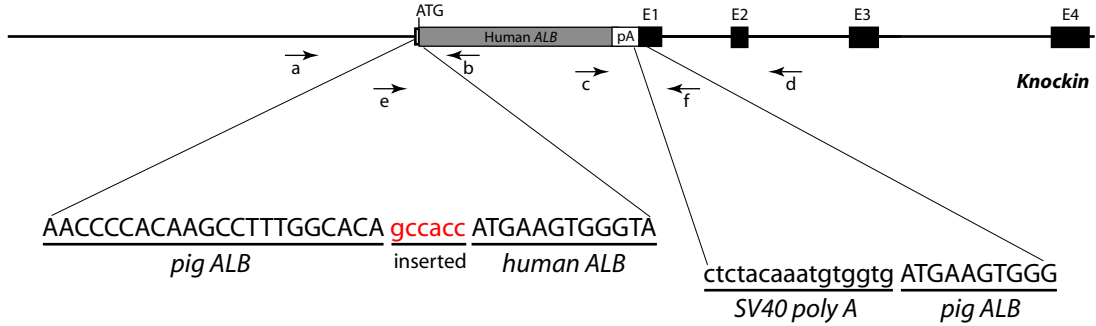


## **Production of Human Albumin in Pigs Through CRISPR/Cas9-Mediated Knockin of Human cDNA into Swine Albumin Locus in the Zygotes**

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and Pumin Zhang<sup>1,4\*</sup>

## **Supplemental Figures**

**a**



**b**

a+b, 1498 bp (green, 5' homology arm; red, inserted sequence; blue, human ALB)

gctgtggaacgccttaaccattgcaccaccagggagcttcatgaaatccatagttcttcatcttttagagcaaaagggcagagagagaatagtc  
 cttcagaggcagagatcagaatttgggggggaaaagtaagGAAGGAAGTTCGAAAGAACATTTTGAAAAATTATCTTTGTTTTAGACATGAA  
 GTTAAATTTATTTTCGAAATTTAAAATAACATTTAAGAAGAGCATGTGACTTTCTTTTTAGTAAAAGCGAGAAAGAAAGAAAAATGTGAAATAGG  
 GATGGAAAGAATATAATGCCTGGTAAAGAAGGCTTAGGATTCTTAAACCCTAATATAATCAGGCTCAGCCTGCCCTGTGTATATCATGATAGA  
 AAAGATTACCACTGCTGCCATTGTGTCCATTTCTTCCCATATCTCCATGAGAGGTACCATACTGCCTCTAAGTACTTTACTCTAGAGCCTGAA  
 TCACCAGTGGGCCAGAGCTGAGGTAAAAATGTAGGACTTCTCAACATCCGTTTTAGTTGTTATGTTGCTATGACTTTTTCTTAGTTTGCATTTG  
 AGACACTGGTTTTTTTGACAGAACAGTTTCAGAAAGATGAAAAGCCATGGAAGCCCTCGAATGCGTGCATGCATGCATGCATGCACACACC  
 CATGTGTATGCATTTTCACTACTTATCAATAAAAGCATACTTGAATTAATAATAGATACAATTCAGAGCTTAGCTCTTTTAAACATGTCTTAT  
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 ACAAAATCGTTTAAACTCTTATGTAATAATTTGATAAGGTGTTTACATAAATTTAATGAATTGACAAGGTCTTGTGGAGAAAAACAGATCCAGACGG  
 CAAACATACGCAAGGGATTTAGTCAAACACATTTTGGCAAATAAATGAAATTTTGTAAATCAGTTGTGAGCCAATGAAATACAAAAATGAGTC  
 TAGTTAATAATCTACAATTATTGGTTAAAGAAGTATATTAGTGTGACTTTCTCTGTTCTGCTACCTTTTCTCTTATCAACCCCAAGCC  
 TTTGGCACAgccaccATGAAGTGGGTAACTTTATTTCCCTTCTTTTCTCTTTAGCTCGGCTTATTCAGGGGTGTGTTTCGTCGAGATGCACA  
 CAAGAGTGAAGTTGCTCATCGGTTTAAAGATTTGGGAGAAGAAAATTTCAAAGCCTTGGTGTGATTGCCTTTGCTCAGTATCTTCAGCAGTGC  
 CATTGGAAGATCATGAAAATTAGTGAATGAAGTAACTGAATTTGCAAAAACATGTGTTGCTGATGAGTCAGCTGAAAATTTGACAAAATCACTT  
 CATACCCTTTTGGAGACAAATTAGTCACAGTTGCAACTCTTCTGTAACCTATGGTGAATGGCTGACTGCT

**c**

c+d, 1314 bp (blue, human ALB; red, SV40 poly A; green, 3' homology arm;)

TCTCTTATTTCCACTTCGGTAGAGGATTTCTAGTTTCTTGTGGGCTAactcgagCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAAC  
 TAGAATGCAGTGAATAAATGCCTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAAGTTAACACACA  
 ACAATTCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTGAATGAAGTGGGT  
 GACTTTTATTTCCCTTCTTTTCTCTTTCAGCTCTGCTTATTCAGGGGTGTGTTTCGTCGAGATACATGTAAGATTCGATTTTTCTATTGTTC  
 ACTTTTATTTTCTAGTAAAAGAAAGTTTCAGTAACTTTGAAATCTTTGATGAATTAAGTTTTCCTTTAGCATTTATTTCTAGACTGTTATGATA  
 TTTTGTATTTGCGAACCTTAGGAAGTTATCTTATTAATATGTTTCTAACATGCTGGGTGAAAAATAGTGAGAATTTGTTAGTACTATAAATTT  
 CTTTTGGCACATGAAGGAGCTTATGTAGAGTGACTTACACTTCACAGAGTGCAGTTGAAGACAGAAATAAAAATTCCAAATTCCTAAAGCCCTAT  
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 CTGCGTTGGTGGAAATATCCTCTATTGGTGTGCTGACagttggcattggccacatgtggctacgggactcttaaat

**a**

sgRNA AAGCCTTTGGCACAATGAAGTGG

Off-target 1 AAGCCATTG CACAATGAAGAAG

Off-target 2 AGACCTTTGGCACAATGAATGAG

Off-target 3 CAGCCTTAGGCACAATTAAGTAG

Off-target 4 AAGCATGTAGCACAATGAAGAAG

**b**

OFF 1F GGAATAAAATGCTTAGAACTGGCT

OFF 1R TTCCAACCTGGCTCAAGACCAT

OFF 2F GACTATCTGAGGTGTGCAGAGG

OFF 2R CTCAATACATCAGGGCACGC

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OFF 3R TCGGGGAGCTTCTGACTTT

OFF 4F CAGGGGAGCACTACCTAGACT

OFF 4R AGGCAGTCCTGAGATCGGAAA

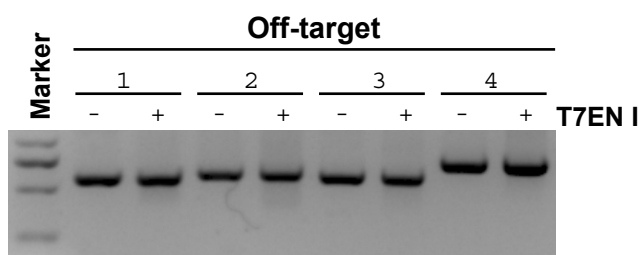
**c**

Figure S3

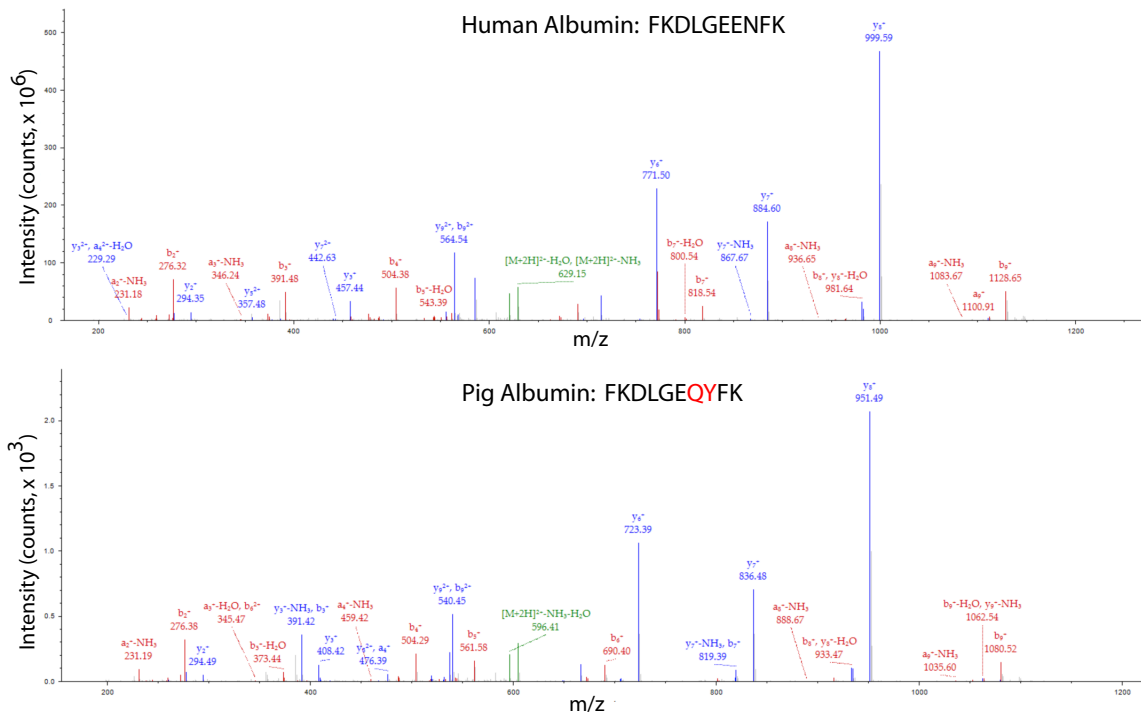
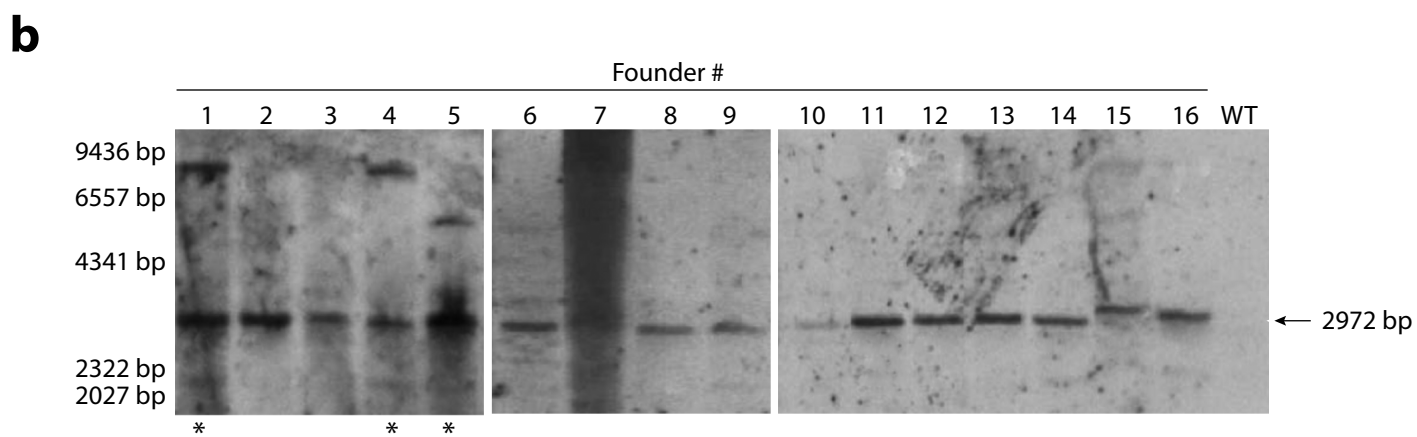
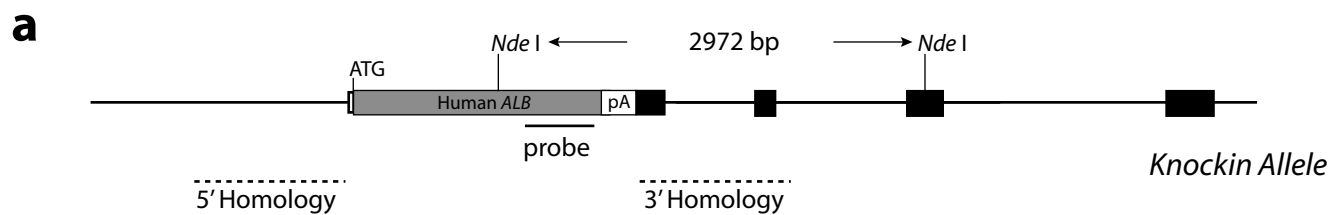


Figure S4



**Figure S1. Sequencing analysis of homologous recombination.** **a.** Illustration of the knockin allele. Sequences of the 5' and 3' junctions of the transgene were shown as well as the relevant pig *ALB* sequence. **b.** Sequence of the PRC product covering the 5' junction between pig *ALB* and the 5' homology arm. **c.** Sequence of the PCR product covering the 3' junction between the 3' homology arm and pig *ALB*.

**Figure S2. Off-target analysis.** **a.** Sequences of the sgRNA and 4 top potential off-target sites. **b.** Primer sequences used to amplify genomic fragments containing the 4 potential off-targets. **c.** T7EN I assay of the PCR fragments containing the 4 sites.

**Figure S3. Mass spectra of human albumin peptide FKDLGEENFK and pig albumin peptide FKDLGEQYFK.**

**Figure S4. Southern blot analysis.** **a.** Diagram of the knockin allele. **b.** Analysis of founder genomic DNA. The genomic DNA was completely digested with *Nde* I. Piglets #1, 4, and 5 (indicated by \*) contain additional insertion of the human *ALB* sequence. The *Nde* I fragment in piglet #15 seemed larger than the predicted 2932 bp the reason for which is unclear at present. The genomic DNA from piglet #7 was degraded.