

Table S1. Sequences of primers for PCR analysis of hNGF-TG pigs

Gene	Primer sequences (5'-3')	Amplicon length (bp)
β -actin	F: CACTGCCGCATCCTCTTCCT	528
	R: CTCCTGCTTGCTGATCCACATC	
hNGF	F: GAACTCATATTGTACCACGACT	319
	R: CCCCAGAATAGAATGACACC	
EGFP	F: GCCACAAGTTCAGCGTGTCC	584
	R: CATGTGATCGCGCTTCTCGTT	
PB-transposase	F: CAGATGCCTGAGGATGGAC	803
	R: CGTTGTGGCTGTAGATGATGA	

Table S2. Sequences of primers for inverse PCR analysis of transgene integration sites in the genome of hNGF-TG pigs

Primer name	Primer sequences (5'-3')
EcoRV-LA-F	GCATGATTATCTTTTACGTGAC
EcoRV-LA-R	CTGAGATGTCCTAAATGCAC
EcoRV-RA-F	CGACCACTACCAGCAGAACACC
EcoRV-RA-R	CCACCAAGTCCTAGGCCCCAT
BamHI-LA-F	GATGTAGCCCTCAAAGTCTC
BamHI-LA-R	CTTATTATGCTGTCGGGTCA
BamHI-RA-F	CCACTACCAGCAGAACACC
BamHI-RA-R	CTTCAGTGACAACGTCGAGCA
IN-EcoRV-RA-F	CCGTGCCTTCCTTGACCCTG
IN-EcoRV-RA-R	CCACCAAGTCCTAGGCCCCAT

Table S3. Sequences of 11 designed pNGF-targeting sgRNAs

sgRNA name	Sequences (5'-3')
sgRNA1-F	CACCGGGACATTGCTCTCGGTGTG
sgRNA1-R	AAACCACACCGAGAGCAATGTCCC

sgRNA2-F	CACCGATCAGAGTGTAGAACAACA
sgRNA2-R	AAACTGTTGTTCTACACTCTGATC
sgRNA3-F	CACCGTTTAGTCCAGTGGGCTTGG
sgRNA3-R	AAACCCAAGCCCAGTGGACTAAAC
sgRNA4-F	CACCGCTCTTCTGATCGGCGTAC
sgRNA4-R	AAACGTACGCCGATCAGAAGAGC
sgRNA5-F	CACCGCGGTTTATCCGAATCGACA
sgRNA5-R	AAACTGTCGATTTCGGATAAACCGC
sgRNA6-F	CACCGCGTGCTCAGCAGGAAGGCG
sgRNA6-R	AAACCGCCTTCCTGCTGAGCACGC
sgRNA7-F	CACCGTGCGTGCTCAGCAGGAAGG
sgRNA7-R	AAACCCTTCCTGCTGAGCACGCAC
sgRNA8-F	CACCGGCTGGGGCGATAGCCGCC
sgRNA8-R	AAACGGCGGCTATCGCCCCAGCC
sgRNA9-F	CACCGGCTGGGGCGATAGCCGCCA
sgRNA9-R	AAACTGGCGGCTATCGCCCCAGCC
sgRNA10-F	CACCGCAGACCCGCAACATCACTG
sgRNA10-R	AAACCAGTGATGTTGCGGGTCTGC
sgRNA11-F	CACCGGGGCGATAGCCGCCAGGG
sgRNA11-R	AAACCCCTGGCGGCTATCGCCCC

Table S4. Sequences of primers for T7E1 assay

Primer name	Sequences (5'-3')	Amplicon length (bp)
pNGF-E2-1F	AATGGTCAATCCCGGTCCC	632
pNGF-E2-1R	ACCTCCTTGCCCTTGATGTCC	
pNGF-E2-2F	CAAGCGGTCGTCATCCCAC	483
pNGF-E2-2R	ACTATAAATTACCATGCAGTCCT	

Table S5. Sequences of primers for PCR analysis of double-transgenic hNGF-TG/pNGF-KO cells and pigs

Primer name	Sequences (5'-3')	Amplicon length (bp)
P1-F	CTGAGGCTTCAAGACAAGTCCC	WT: 2000,
P1-R	TTTTCAGCCAAAAGCAGACCTCC	hNGF-TG/pNGF-KO: 4900

Table S6. Sequences of expected PCR amplicon for off-target analysis

SgRNA ID no.	Potential off-target sites	Sequences of expected PCR amplicon containing the primers (with underline) and the potential off-target sites (with bold font)
sgRNA 5	mm4__1 258.00 Mbp_1_257997134	<u>GTGTAGGTTGCCAGACCCTCTAATAAACTGGCCCAGAGTCGGGTGAGTCATGGTTGCACCTCCC</u> AAGTCACAGGCTATCCTCACAAGCTGTGCACCTAATTAAGCTCTGATAATCTGTATAAATTAGC ATTTGCTAGTGATAAATATCACGTACCAAGCCTTAAATAATACATTAGCCTTAATTGAATAAATG CATATATTATGGCCTTCTAATGCATATTTGCCATAATGAGTTATCTTTTATGCACAAACAAACAG CAATTCTGACTAATCAGGTGGGGTAATTGTGCTATTAATAATTCCAGGTTTCCTGAGAAAGGTCT CAGTGATGGTCAGATGACAGAAAAGNNNNNNNCATCAACAACAAAACATTATTT CCTTGACTA TTCGGATAAACTTCT TATTTGGGTATTTCACTACCTTTGAACCCCTAGAGTTTTGTAATTAATAT CAACGTCTATACCCCTTTCCCACTCCC
	mm4__4 14.53 Mbp_4_14533954	<u>GGACAGGAAGACCAGCCTTCCACCTGGGCCACTGGGGAGCACCCCAGCCACTACATGGCTGCC</u> GATGTGAAGTCAGTAAGAAGCTTCTCTCTCGTGTTCTATGTCACACCCTCCGTGAACCAGAGGACC TGAACCACCAAGGGAACAAATTCACCATTTCCACTGTAAACCAACAGAAGGAGAAATGCTTGTC ATTTTCTTCTTCTGCTCTTGCCAAGTATCCATCAGAAACCTATTTAATGTGACTATAAGGTCAGA ACAAAAAGAACTAGCAAAAGGTCTATTTGGGGAATACTACATTTTCTTTAAATCCATAAGTTA GCTTCTAACATGCATAAGTT CCGTGAGGATTCGGATAAACAGGAAGTCCAACTTCAAATCCCTT GTCTTTAGGTCAGACCACAGATCTACTGCACAGTATGAGGTCA ATCCAAGGACATCAGGCTGGA
	mm4__4 22.71 Mbp_4_22706127	<u>GAGGGCCCTGGTTTAGCAAA</u> TATCTTTGTACTATTCAATTTCAAGAGTAAAGGGTTTCCTTATAAAA CTCAGAACATAGTTTGAAAACCTTTCTGTGATTGAGATCCTTCTCCTG CCTTGTCATTTTCAGATAA ACCTCTGGTTCTAAAGCACTAATGTT CCTCTAGTGTTCTATTAAGTTAGATGTTTTTCTATAATTTT AAACAAATCTAAGTCAAATGAAAATTTAAAAATATGGATTTCAAACAAACGGTTTAACAAGAAAT

sgRNA 10		GTCTACTCTATTACACTTGTGACAAATCTGGTTAAGACCTCATAAGAATTATATAATTGGCTTCAG AACAGAAACAATTTTTTCATTAATATAACTAGCAAAAATATTTCTATAATCTCTTCTTGCCCCCATAT GAAAATCAGT <u>TAAGGGAAAGCTCAGCTGGC</u>
	mm4__14 55.82 Mbp_14_55822569	<u>TAAAGTGGGAATGGTGCCCCACACCTCCTGAGAACGAGAGTCTGGTGAGTTTGCACCAAGATGAG</u> AACTGCCATTTTAAGAGCAGCTTTCCCCTCCTCAAATACCAGGCTCTCCCTTCCAGAAAGCCCCAG ACTTCCCAGCTTCCTTCTGCTGAGAGATTAATGATCTGCTTCCAGGGGGCCACAGGTGCCCCGGCT GTCCACTTAGATGAGCATAAAAAATCCCCAAAG CAGAAAAGCAACAACACTGAGGGTCTCCTTTG ACACCCAAGGGAAACTGCCCCAGAGGAAAGCCCTGCAGGCCAGGGCACGGGCACAAGTATACGA ACTGAGGCAAAGTGACTGGTATCTAGGATGCGCTGCAAGAAATTCCCTCGTTGGGTCTGTCTTTGTC TGGATCCTGATGTCCACCG
	mm4__1 253.31 Mbp_1_253306534	<u>TGCTTGAATGGATGCATGTGGGGCCAGCAGGACTTGATGGATTAGCCGTAGACTGGGGAACAGG</u> TGTCAAGGATTGTACTCAGGTTTCAGGCTTGTGCACATGGATAGGTCCTGGAGGTGCTGACTGGGT GGGAGAGCAGGTTTGTGGGGACAGACCTA AGTGACGTTGCAGGTCTGGGTCCATGCATTGAGCA GGCTCTTAGGTGTGTTAGGGTTCACAGAAGCCGTTGGAGATGTGATTCTGATGCCTTCTCATTACCC ATCTGTAAAACTTCATTTTCTACTTGTTGAAGGTATGATATGTTTAGAAAAAATAATTTATATTTGCA TATTTTGGATTGCCAACTTGTCTTCTTCAATCAGTTTTCTAGGCCTTTGGGATTGTACCC