

Supplemental_File

Table S1. Nucleotide sequences for porcine *OPN*

ATGAGAATTGCAGTGATAGCCTTCTGCCTCTGGGGCTTCGCTCTGCCCTTCCAGTTAA
ACAGACTAATTCTGGCAGCTCGGAGGAAAAGCTGCTTTCCAACAAATACACAGATGCT
GTAGCCACATTGCTAAAGCCTGACCCATCTCAGAAGCAGACTTTCCTAGCGCCACAGA
ATACTATTTCTCGGAGGAAACGGACGACTTCAAACAAGAGACCCTGCCAAGCAAGTC
CAACGAAAGCCCTGAGCAAACAGACGATGTGGACGACGACGACGACGAAGACCACG
TGGACAGCAGGGACACGGACTCCGAGGAAGCTGATCACGCTGACGACGCTGACCGAT
CCGACGAGTCTCATCACTCCGATGAATCCGATGAGCTGGTCACCGATTTCCCCACCGAC
ACCCAGCAACCGACGTCACTCCGGCTGTCCCCACGGGAGACCCCAATGATGGCCGC
GGGGATAGTGTGGTCTATGGACTGAGGTCAAATCTAAGAAGTTCCGCAGATCCGAAG
CCCAGCAGCTGGATGCCACAGAGGAAGACCTCACGTACATGTGGAAAGTGAGGAGA
CGGATGGTACCCCCAAGGCCATCCTCGTTGCCAGCGCCTGCACGTGGCTTCTGACTT
GGACAGCCAAGAGAAGGACAGTCAGGAGACGAGTCAGCCGGATGACCGCAGTGTGG
AAACCCGCAGCCAGGAGCAGTCCAAAGAATACACGATCAAGACCTATGATGGGAGCA
ATGAGCATTCCAATGTGATTGAGAGTCAGGAAAATCCCAAAGTCAGCCAAGAATTCCA
CAGCCATGAAGACAAGCTGGTCCCAGACTCTAAGAGCGAAGAAGACAAACACCTGAA
ACTTCGAGTTTCTCATGAATTAGAGAGTGCGTCTTCTGAGATCAAC

Table S2. Nucleotide sequences for porcine 6His-OPN

ATGCATCATCATCATCATATGCTTCCAGTTAAACAGACTAATTCTGGCAGCTCGG
AGGAAAAGCTGCTTTCCAACAAATACACAGATGCTGTAGCCACATTGCTAAAGCCTGA
CCCATCTCAGAAGCAGACTTTCTAGCGCCACAGAATACTATTCCTCGGAGGAAACG
GACGACTTCAAACAAGAGACCCTGCCAAGCAAGTCCAACGAAAGCCCTGAGCAAAC
AGACGATGTGGACGACGACGACGACGAAGACCACGTGGACAGCAGGGACACGGACT
CCGAGGAAGCTGATCACGCTGACGACGCTGACCGATCCGACGAGTCTCATCACTCCGA
TGAATCCGATGAGCTGGTCACCGATTTCCCCACCGACACCCCAGCAACCGACGTCCT
CCGGCTGTCCCCACGGGAGACCCCAATGATGGCCGCGGGGATAGTGTGGTCTATGGAC
TGAGGTCAAATCTAAGAAGTTCCGCAGATCCGAAGCCCAGCAGCTGGATGCCACAG
AGGAAGACCTCACGTCACATGTGGAAAGTGAGGAGACGGATGGTACCCCCAAGGCCA
TCCTCGTTGCCAGCGCCTGCACGTGGCTTCTGACTTGGACAGCCAAGAGAAGGACA
GTCAGGAGACGAGTCAGCCGGATGACCGCAGTGTGGAAACCCGCAGCCAGGAGCAG
TCCAAAGAATACACGATCAAGACCTATGATGGGAGCAATGAGCATTCCAATGTGATTGA
GAGTCAGGAAAATCCCAAAGTCAGCCAAGAATTCCACAGCCATGAAGACAAGCTGGT
CCCAGACTCTAAGAGCGAAGAAGACAAACACCTGAACTTCGAGTTTCTCATGAATTA
GAGAGTGCCTCTTCTGAGATCAAC

Table S3. The alignment of the amino acid sequences for supernatant protein and porcine OPN protein

MRIA**VIAFCLWGFASALPVKQTNSGSSEEKLLSNKYTDAVATLLKPDPSQKQTFL**
APQNTISSEETDDFKQETLPSKSNESPEQTDDVDDDDDEDHVDSRDTDSEEADHA
DDADRSDESHHSDESDELVTDFPTDTPATDVTPAVPTGDPNDGRGDSVVYGLRS
KSKK**FRRSEAQQLDATEEDLTSHVESEETDGTPKAILVAQRLHVASDLDSQEKD**
SQETSQPDDRSVETRSQEQSKEYTIKTYDGSNEHSNVIESQENPKVSQEFHSHED
KLVPDSKSEEDKHLKLRVSHELESASSEIN

Note: Different colours represent different confidence levels (green: peptides with a confidence level $\geq 95\%$; yellow: 95%>peptides with a confidence level $\geq 50\%$; red: 0>peptides with a confidence level $>50\%$; grey: undetected peptide).

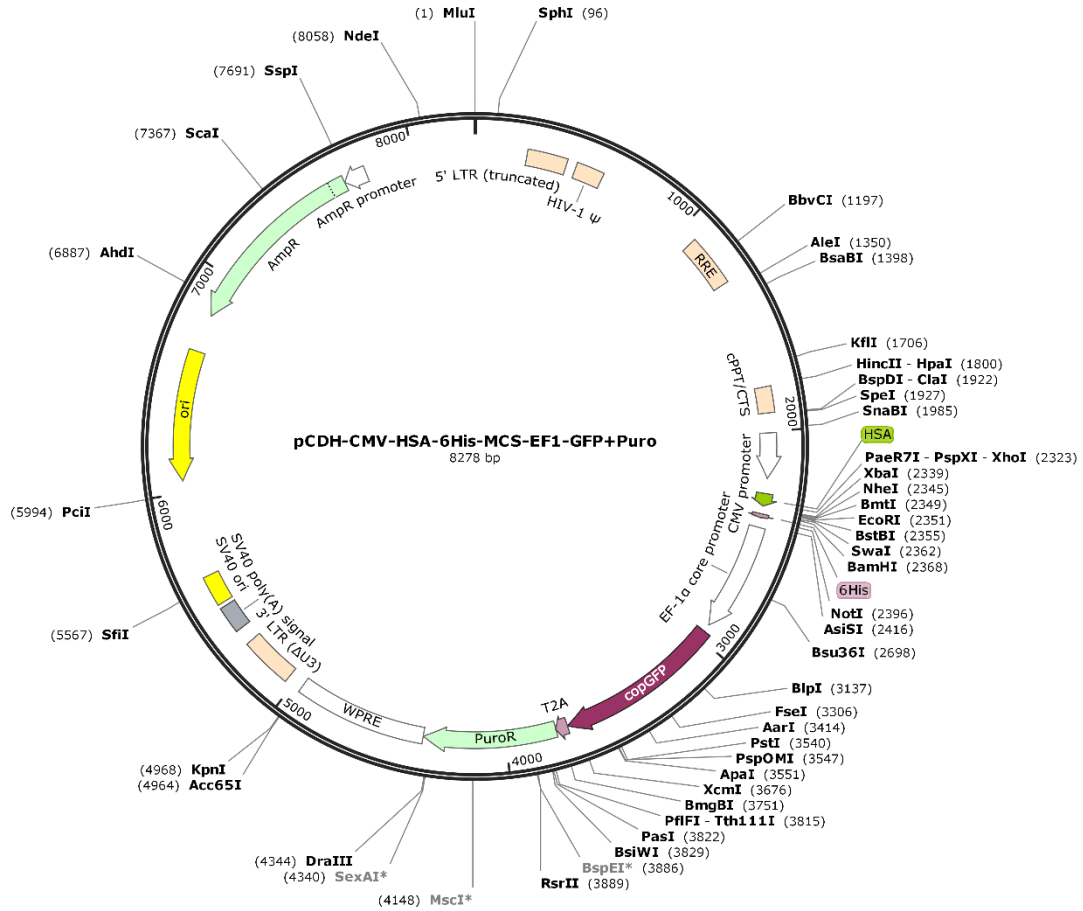


Fig. S1. Map of PCDH-CMV-HSA-MCS-6His-EF1-GFP+Puro vector.

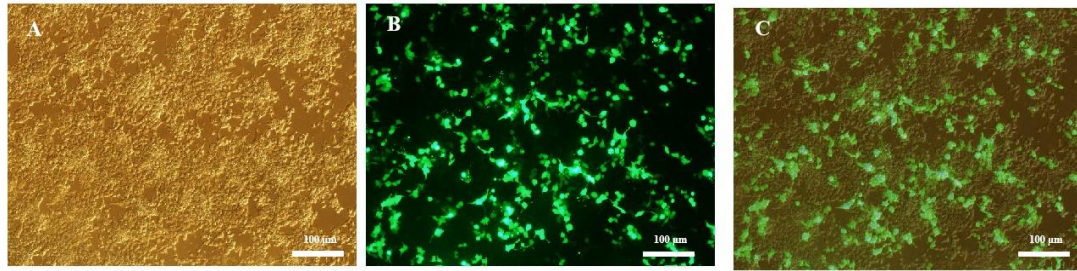


Fig. S2. Production of OPN lentivirus after the co-transfection of lentiviral plasmids and packaging plasmids into 293FT cells.

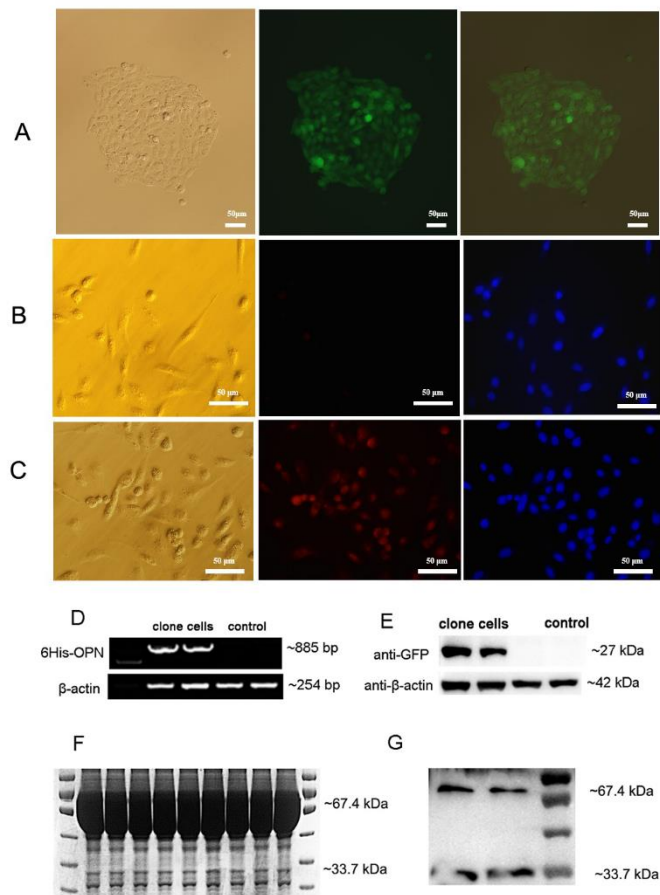


Fig. S3. Production of porcine OPN from the supernatant of single-clone cells. (A) The single clone after virus infection and puro screening. (B and C) IF of the negative control and single-clone cells. (D) RT-PCR of the single-clone cells and negative control. (E) WB of the single-clone cells and negative control. (F) SDS-PAGE of single-clone cell supernatant. (G) WB of the protein powder.

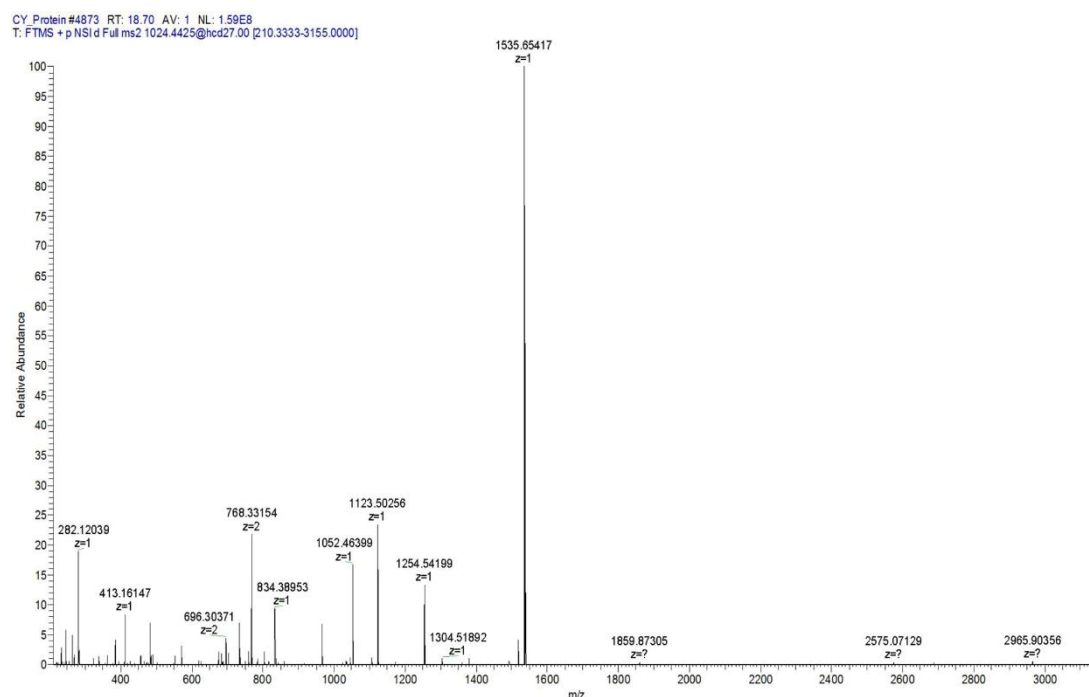


Figure S4: LC-MS/MS results of porcine OPN protein powder. The figure was the detection of peptide fragments and their fragment ions after proteolysis. RT, retention time; AV, average; NL, ionic strength.

Production and purification of porcine OPN protein

The full-length sequence of OPN was successfully amplified from SSCs, with a 909-bp OPN product obtained. The 6His-OPN coding sequence was obtained (885-bp OPN product) and cloned into the PCDH-CMV-HSA-MCS-6His-EF1-GFP+Puro vector (see Figure S1, Additional Files). After co-transfer with three lentivirus packaging plasmids (see Figure S2, Additional Files), the total mRNA of 293FT cells was extracted to measure the level of 6His-OPN mRNA (with untransfected cells used as negative controls). The level of 6His-OPN mRNA in the experimental group was higher than that in the negative control group (Figure S3D).

After CHO cell transfection with lentivirus, IF, RT-PCR, and WB experiments were performed to measure expression (with untransfected cells used as negative controls), the expression level of OPN was higher in single-clone cells than in untransfected cells (Figure S3A, B, C, D, E).

Protein powder (1 mg) was collected for mass spectrometry, which showed that the total protein contained 92% OPN and the predicted isoelectric point is 4.3 (Figure S4, Table 5). The supernatant was analyzed by SDS-PAGE (Figure S3F), and the protein powder was assessed by WB (Figure S3G). The results showed that the supernatant contained porcine OPN with molecular weights of 33.7 kDa and 67.4 kDa.