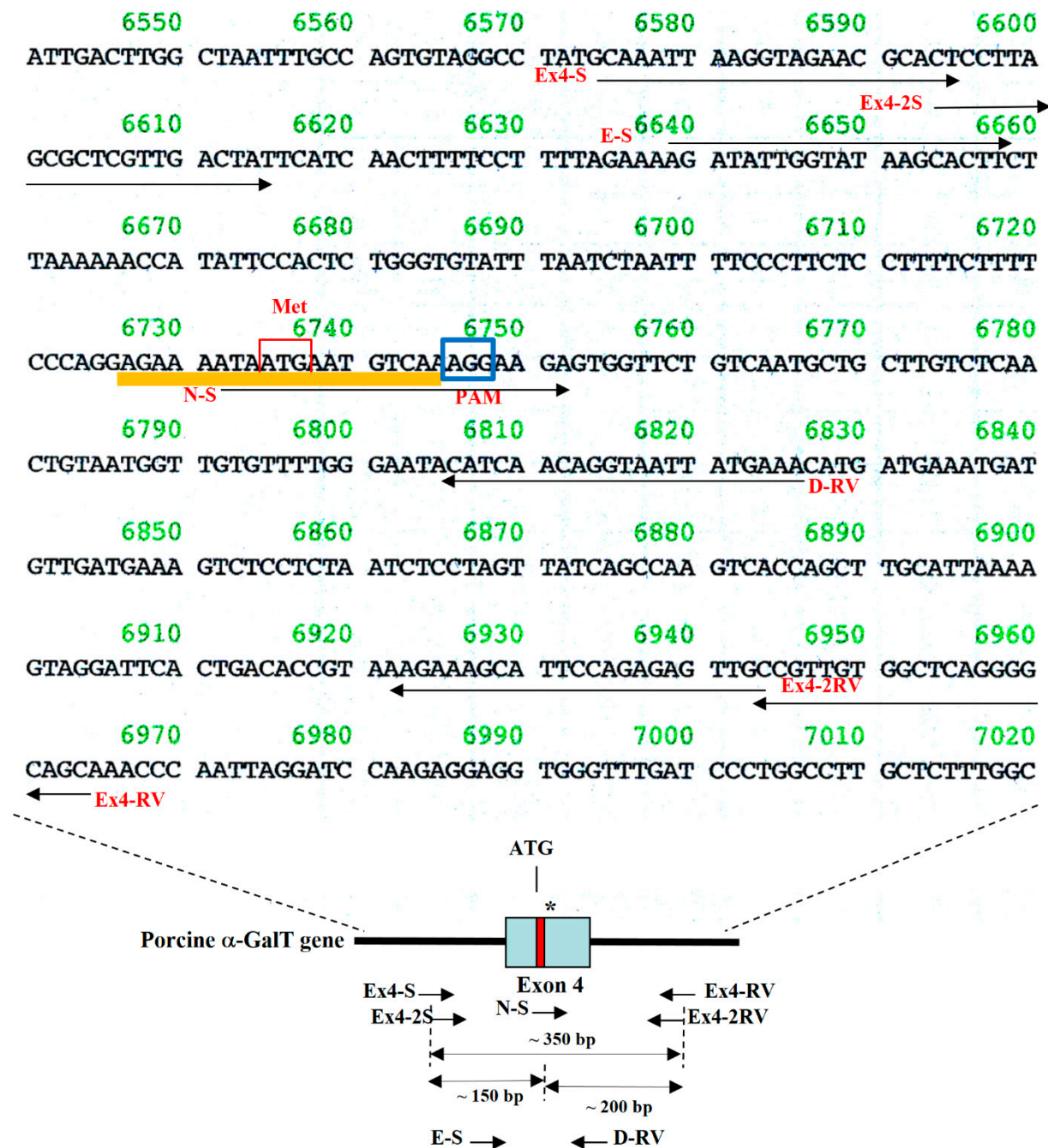


## Supplementary Information



**Figure S1.** Genomic sequence spanning exon 4 of porcine *GGAT1*. The translation initiation site, ATG, is shown in a red box. The PAM (protospacer adjacent motif) site is shown in a blue box. The target sequence recognized by gRNA#3 is shown underlined in orange. Primers used for PCR and sequencing are indicated by arrows underneath the sequence and in a schematic representation of the exon 4 of *GGAT1*. Asterisks indicate the mutated portion of the exon 4 of *GGAT1*.

**Table S1.** Primer sets used for PCR.

| Primer         | Sequence (5'–3')      | Reference |
|----------------|-----------------------|-----------|
| <i>N-S</i>     | ATAATGAATGTCAAAGGAAGA | –         |
| <i>D-RV</i>    | TTTCATAATTACCTGTTGATG | –         |
| <i>E-S</i>     | AGATATTGGTATAAGCACTTC | –         |
| <i>Ex4-S</i>   | GCAAATTAAGGTAGAACGCA  | [27]      |
| <i>Ex4-2S</i>  | CTCCTTAGCGCTCGTTGGCT  | [27]      |
| <i>Ex4-RV</i>  | GCTGCCCTGAGCCACAACG   | [27]      |
| <i>Ex4-2RV</i> | GCAACTCTCTGGAATGCTTT  | [27]      |
| <i>1-S</i>     | AGAGGGTAACATTTGAGTAGA | –         |
| <i>1-2S</i>    | TAACATTTGAGTAGAGACCTT | –         |
| <i>1-RV</i>    | TTCAAAATCTATGTTACTGGT | –         |
| <i>1-2RV</i>   | ATCTATGTTACTGGTTCATGA | –         |
| <i>3-S</i>     | CCTACACCACAGCCACAGCAA | –         |
| <i>3-2S</i>    | CCACAGCCACAGCAACATGGG | –         |
| <i>3-RV</i>    | GGAGGGAGGCTGCAGTAGAAA | –         |
| <i>3-2RV</i>   | AGGCTGCAGTAGAAATAGGAA | –         |
| <i>4-S</i>     | TAACCCACTGCACTGAGCTGG | –         |
| <i>4-2S</i>    | ACTGCACTGAGCTGGGGACTG | –         |
| <i>4-RV</i>    | ATGTGTTCAATAAATATAAGA | –         |
| <i>4-2RV</i>   | TCAATAAATATAAGATGAATA | –         |

**Table S2.** List of candidate genes that potentially cause off-target mutations.

| Sequence                                 | Score | Mismatches  | Locus            |
|--|-------|-------------|------------------|
| (1) GAAAAAATAATGAATGACAAGAG <sup>1</sup> | 4.6   | 2MMs [3:17] | chr1: –243831704 |
| (2) GAGAAAAAATAATGTCATGGG <sup>2</sup>   | 4.2   | 2MMs [8:20] | chr940: +158850  |
| (3) GATAAAATAATGAATGTAAAAGG <sup>3</sup> | 2.6   | 2MMs [3:18] | chr9: –126332949 |

<sup>1</sup> The gRNA#3 used here exhibits similarity to *Sus scrofa* breed mixed chromosome 1, Sscrofa10.2 zinc finger protein GLIS3 (Sequence ID: ref|NC\_010443.4| Length: 315321322 Number of Matches: 3076), as shown below.

```
Query 1          GAAAAAATAATGAATGACAAGAG 23
                |||
Sbjct 243831731 GAAAAAATAATGAATGACAAGAG 243831709
```

<sup>2</sup> The gRNA#3 used here also exhibits similarity to *Sus scrofa* breed mixed unplaced genomic scaffold, Sscrofa10.2 ChrUScaf1915 (Sequence ID: ref|NW\_003538807.1| Length: 253766 Number of Matches: 4), as shown below.

```
Query 1          GAGAAAAAATAATGTCATGGG 23
                |||
Sbjct 158852     GAGAAAAAATAATGTCATGGG 158874
```

<sup>3</sup> The gRNA#3 used here also exhibits similarity to *Sus scrofa* breed mixed chromosome 9, Sscrofa10.2 dynamin-3-like (Sequence ID: ref|NC\_010451.3| Length: 153670197 Number of Matches: 1149), as shown below.

```
Query 1          GATAAAATAATGAATGTAAAAGG 23
                |||
Sbjct 126332976 GATAAAATAATGAATGTAAAAGG 126332954
```