# Supporting Information

## Chu et al. 10.1073/pnas.1323226111

### SI Materials and Methods

**Cell Culture.** Human breast cancer cell line MCF7 was cultured in DMEM/F12 containing 10% (vol/vol) FBS (Sigma), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco). Human kidney fibroblast HEK 293T and breast cancer cell line MDA-MB-231 were cultured in DMEM (Gibco) containing 10% (vol/vol) FBS (Sigma), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco). All cells were cultured at 37 °C with 5% (vol/vol) $CO_2$.

**Knockdown by siRNA.** Briefly, cells at 10% confluence in six-well plates were transfected with 20 nM of siRNA by Lipofectamine 2000 (Invitrogen) for 3 d. Cells were then harvested for further use. The siRNAs used are Scramble siRNA (Invitrogen), siOGT#1 (sense, 5′-UAAUCAUUUCAAUAACUGCUUCUGC-3′, and antisense, 5′-GCAGAAGCAGUUAUUGAAAUGAUUA-3′) and siOGT#2 (sense, 5′-AUACGAUGGCAUCUUCUGGUAACCC-3′, and antisense, 5′-GGGUUACCAGAAGAUGCCAUCGUAU-3′) (Invitrogen).

**Western Blotting and Histone Extraction.** Western blotting was performed as previously described (1). To prepare total cell lysates, cells were lysed in total cell lysis buffer [50 mM Hepes (pH 7.4), 5 mM EDTA, 1% Triton X-100] and incubated on ice for 10 min. Histone extraction was prepared as previously described (1). Briefly, cells were harvested and incubated with 0.2 N $H_2SO_4$ for 30 min at 4 °C. After centrifugation the supernatants were collected and subjected to trichloroacetic acid precipitation. The precipitants were washed with cold acetone and air dried. The dried proteins were dissolved in $dH_2O$, and the concentrations were determined for further use. For quantification, band intensities were measured by ImageJ (2). Normalization was done by dividing FLAG signal by β-tubulin signal. All results are presented as mean ± SD ($^*P < 0.05$, $n = 3$).

**Antibodies and Reagents.** The antibodies used for immunoblotting were O-linked *N*-acetylglucosamine (GlcNAc) transferase (OGT) (sc32921, Santa Cruz Biotechnology), O-GlcNAc (ab2739, RL-2 clone, Abcam), O-GlcNAc (O7764, CTD110.6 clone, Sigma), α-tubulin (T5168, Sigma), H3K27me2 (ab24684, Abcam), H3H27me3 (ab6002, Abcam), H3K9me1 (ab9045, Abcam), H3K9me2 (ab1220, Abcam), H3K9me3 (ab8898, Abcam), H3 (ab1791, Abcam), H3K4me1 (ab8895, Abcam), H3K4me2 (ab7766, Abcam), H3K4me3 (ab1012, Abcam), H3K36me1 (ab9048, Abcam), H3K36me2 (ab9049, Abcam), H3K36me3 (ab9050, Abcam), H3R17me2 (ab8284, Abcam), H3K79me1 (ab2886, Abcam), H3K79me2 (ab3594, Abcam), H3K79me3 (ab2621, Abcam), H3R26me2 (07-215, Millipore), enhancer of zeste homolog 2 (EZH2) (612666, BD), UTX (A303-374A, Bethyl), JMJD3 (07-1534, Millipore), Lamin B (sc2617, Santa Cruz Biotechnology), SUZ12 (sc271325, Santa Cruz Biotechnology), EED (ab4469, Abcam), RbAp46/48 (ab1765, Abcam), actin (mab1501, Millipore), β-tubulin (mab5562, Millipore), FLAG (F3165, Sigma), and V5 (V8127, Sigma). Cycloheximide, O-(2-acetamido-2-deoxy-d-glucopyranosylidene)amino-N-phenylcarbamate, O-GlcNAz, and phosphine-FLAG were purchased from Sigma.

**Plasmids.** Human full-length OGT (NP_858058.1) was cloned from 293T cDNA into pcDNA3.1-V5-HIS (Invitrogen). Human full-length EZH2 (NP_001190176.1) was cloned from 293T cDNA into pcDNA3-FLAG. Resistant OGT and EZH2-S75A were constructed using a site-directed mutagenesis method as described by the manufacturer (Finnzyme). The primers used for construction of the resistant OGT plasmid against siOGT siRNA are as follows: forward primer, 5′-CTA TGA ATA CTA TTG CGG AGG CGG TGA TCG AGA TGA TCA ACC GAG GAC AGA T-3′ and reverse primer, 5′-ATC TGT CCT CGG TTG ATC ATC TCG ATC ACC GCC TCC GCA ATA GTA TTC ATA G-3′.

**Quantitative Real-Time RT-PCR.** Quantitative real-time RT-PCR (RT-qPCR) was performed as previously described (3). Briefly, cells were harvested, and total RNAs were purified by RNeasy RNA purification kit as described by the manufacturer (Invitrogen). cDNAs were synthesized by reverse transcriptase SuperScript II (Invitrogen) and analyzed by real-time PCR using LightCycler 480 SYBR Green I Master (04 887 352 001, Roche). The Ct values were normalized to S26 rRNA expression. The primers used for RT-qPCR are listed in Table S1.

**Immunoprecipitation and Coimmunoprecipitation.** For immunoprecipitation (IP), total cell lysates from 293T cells or nuclear extract from MCF7 nuclei were prepared using RIPA-IP buffer [50 mM Tris·HCl (pH 8.0), 150 mM NaCl, 1% IGEPALCA-630, 0.5% sodium deoxycholate, 0.1% SDS, and 1 mM DTT]. For co-IP, total cell lysates from 293T cells or nuclear extract from MCF7 nuclei were prepared using IP buffer [20 mM Hepes (pH 7.9), 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, and 0.5% IGEPALCA-630]. After preclear with protein A beads, lysates were incubated with anti-FLAG, anti-V5, anti-EZH2, or control IgG overnight at 4 °C. The immune complexes were incubated with protein A beads for 1 h and washed with the same buffer six times. The samples were eluted by sample buffer, followed by Western blot or SDS/PAGE.

**Microarray.** MCF7 transfected with scramble siRNA, siOGT, or siEZH2 for 3 d were harvested and total RNA purified by RNeasy RNA purification kit as described by the manufacturer (Invitrogen), followed by microarray experiments according to the protocols provided by the manufacturer (3′ IVT Express Kit; Affymetrix) at the Affymetrix Gene Expression Service Laboratory at the Genomics Research Center of Academia Sinica. The biotinylated cRNAs were hybridized to the Affymetrix HG-U133 plus 2.0 whole genome array (Affymetrix). After staining, the array was scanned, and images were acquired with a GeneChIP Scanner 3000 (Affymetrix). Data were analyzed with GeneSpring GX11 software (Agilent).


1. Chu CS, et al. (2011) Protein kinase A-mediated serine 35 phosphorylation dissociates histone H1.4 from mitotic chromosome. *J Biol Chem* 286(41):35843–35851.
2. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9(7):671–675.
3. Shi-Chen Ou D, et al. (2011) Transcriptional activation of endoplasmic reticulum chaperone GRP78 by HCMV IE1-72 protein. *Cell Res* 21(4):642–653.

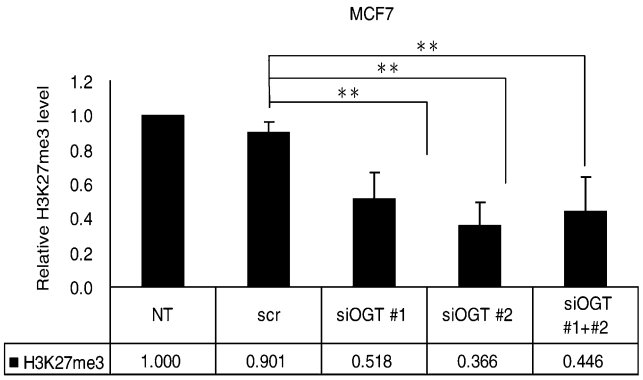



**Fig. S1.** H3K27me3 level drops ~50% by OGT depletion. Histones purified from MCF7 cells mock transfected (NT), transfected with scramble RNA (scr), or two different siOGT (#1 and #2) separately or together were subjected to Western blot using H3 or H3K27me3 Ab. Band intensities were measured by ImageJ. Normalization was done by dividing the H3K27me3 signal by H3 signal. *P* values were measured by Student's *t* test. The results are presented as mean ± SD. $*P < 0.05$, $n = 3$.

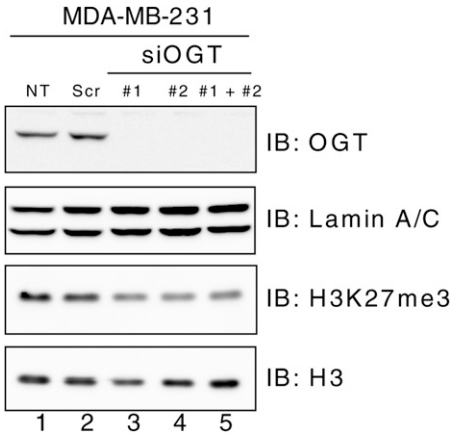



**Fig. S2.** Depletion of OGT down-regulates H3K27 trimethylation in MDA-MB 231 cells. Total cell lysates or histone purified from MCF7 transfected with indicated siRNA were subjected to Western blot using indicated Abs.

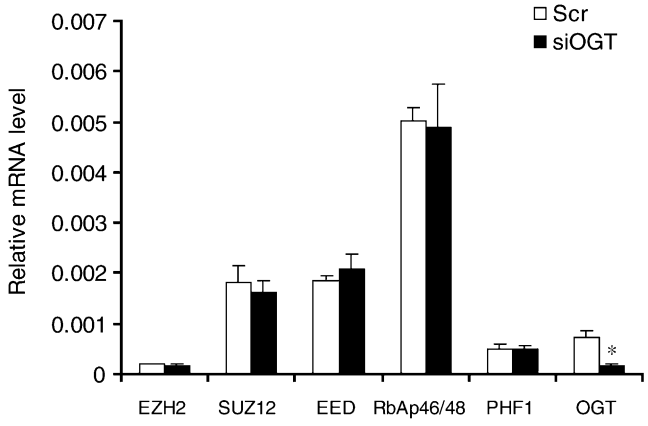



**Fig. S3.** Depletion of OGT does not affect the mRNA levels of polycomb repressive complex 2 subunits. Total RNA purified from MCF7 cells transfected with scramble or siOGT were subjected to real-time RT-PCR using primer pairs for specific transcripts. mRNA level of each gene was normalized to 18s rRNA. *P* values were measured by Student's *t* test. The results are presented as mean ± SD. $*P < 0.05$, $n = 3$.

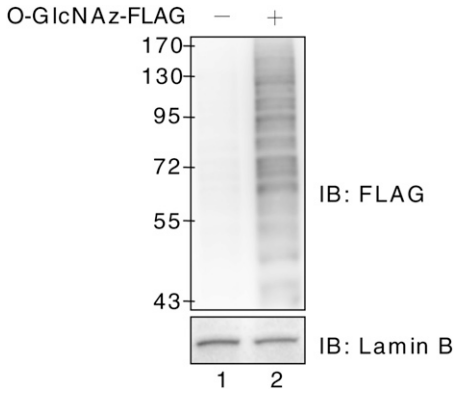
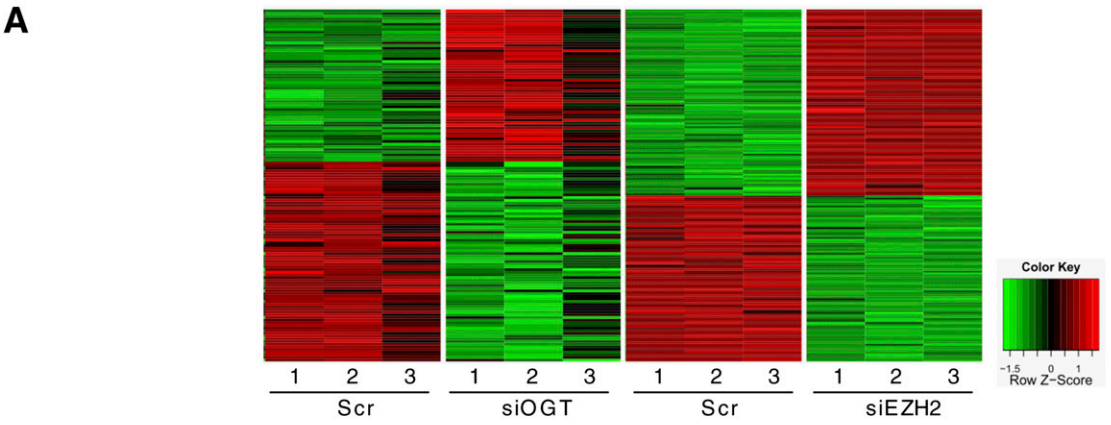



**Fig. S4.** Successful metabolic labeling of O-GlcNAz-FLAG in vivo. Total cell lysates from MCF7 treated with or without O-GlcNAz for 16 h were incubated with phosphine-FLAG overnight. Lysates were subjected to Western blot using FLAG Ab or lamin B Ab.



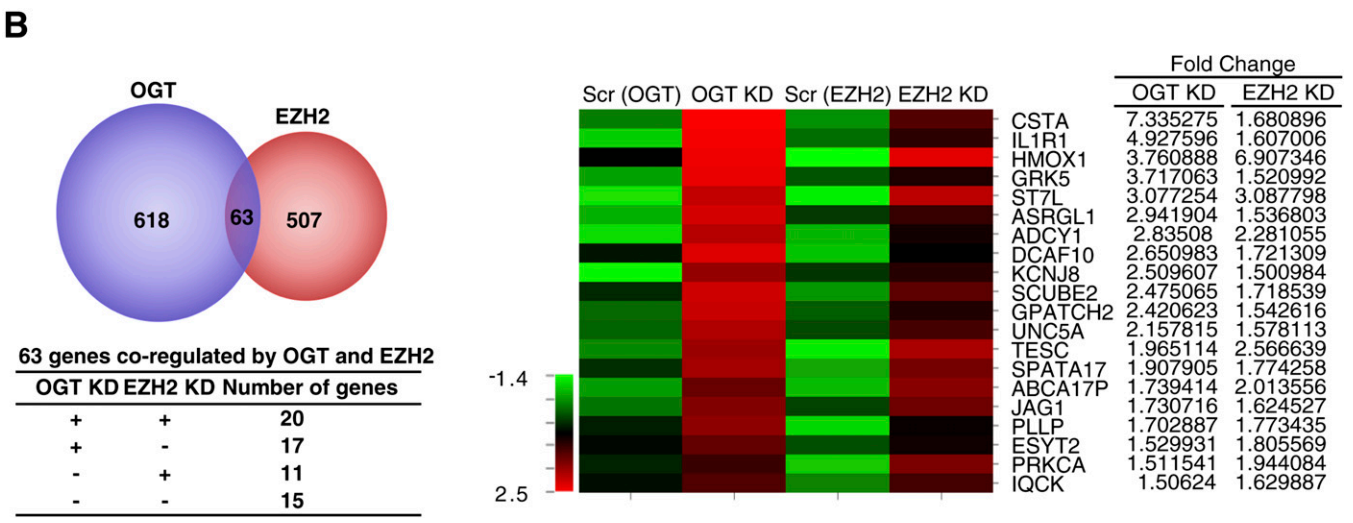




**Fig. S5.** OGT-EZH2 axis suppresses specific tumor suppressor gene expression. (*A*) Heatmap of gene expression in MCF7 cells depleted of OGT or EZH2 by siRNA. (*B*) Venn diagram analysis shows the genes coregulated by EZH2 and OGT. (*Left*) Blue and red circles indicate the genes whose expression was altered when OGT or EZH2, respectively, was depleted from MCF7. (*Center*) "+" indicates up-regulation and "-" indicates down-regulation. (*Right*) Heatmap of the 20 genes up-regulated when OGT and EZH2 were both depleted.

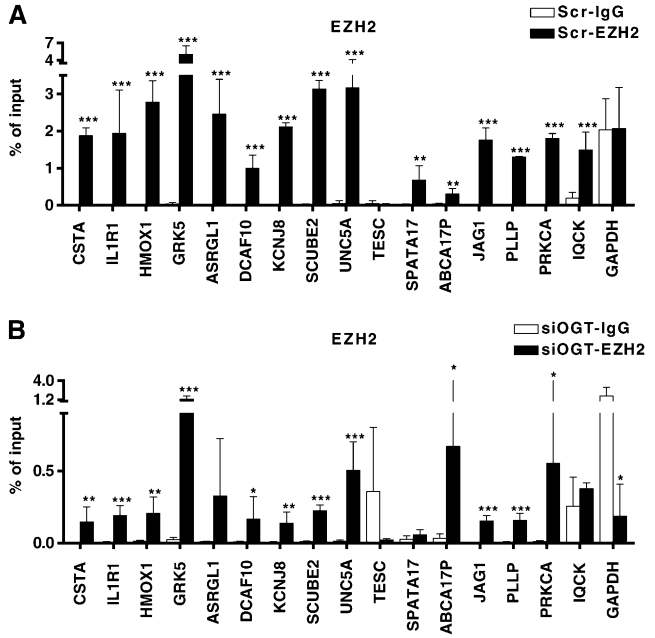



**Fig. S6.** OGT-dependent EZH2 occupancy at the promoter regions of the 16 genes in Fig. 5. MCF7 cells with scramble siRNA (*A*) or siOGT (*B*) were subjected to ChIP with control IgG (white bar) or EZH2 Ab (black bar). GAPDH gene was used as a negative control. *P* values were measured by Student's *t* test. The results are presented as mean ± SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $n = 3$.

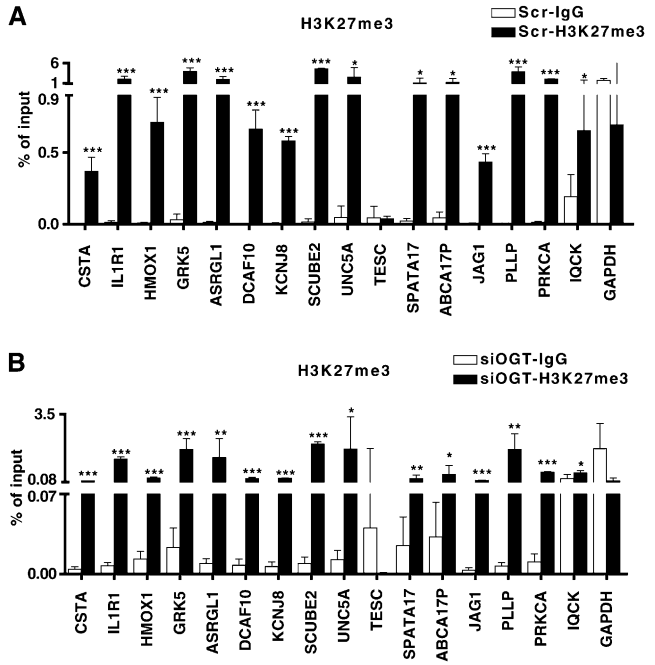



**Fig. S7.** OGT-dependent H3K27me3 enrichment at the promoter regions of the 16 genes in Fig. 5. MCF7 cells with scramble siRNA (*A*) or siOGT (*B*) were subjected to ChIP with control IgG (white bar) or H3K27me3 Ab (black bar). GAPDH gene was used as a negative control. *P* values were measured by Student's *t* test. The results are presented as mean ± SD. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $n = 3$.

**Table S1. Primers for quantitative RT-PCR**

| Gene | Direction | Sequence (5′ to 3′) |
|---|---|---|
| *OGT* | Forward | ACCAGAAGATGCCATCG |
| | Reverse | TGAATATTAGGTTCTCCTACTGCT |
| *EZH2* | Forward | TAATGTGCTGGAATCAAAGGATAC |
| | Reverse | GCTTCATCTTTATTGGTGTTTGAC |
| *Suz12* | Forward | CCATGCAGGAAATGGAAGAATGTC |
| | Reverse | CTGTCCAACGAAGAGTGAACTGC |
| *EED* | Forward | GACGAGAACAGCAATCCAGACC |
| | Reverse | TCCTTCCAGGTGCATTTGGCGT |
| S18 rRNA | Forward | CCTGCGGCTTAATTTGACTC |
| | Reverse | ATGCCAGAGTCTCGTTCGTT |
| *RBBP4* | Forward | AGACTTGCGTCTCCGTGGACA |
| | Reverse | CCTCCTTTGGAACGGCACTGAT |
| *PHF1* | Forward | GGTCCAGTTTGAGGATGATTCGC |
| | Reverse | ATGGCGACACTTCTCACAGCTG |
| *ABCA17P* | Forward | CATGTACGCCAACAAGCTGGTC |
| | Reverse | GAACTTGCTCTTGAGGTGCTGG |
| *ADCY1* | Forward | GTTCGATGAATTAGCCACGG |
| | Reverse | TGATGGTATCAATCATGTCGAGT |
| *ASRGL1* | Forward | GCTGGAGGTTATGCCGACAATG |
| | Reverse | ATAGGTCCGCAGCCTCTTCTAC |
| *DACF10* | Forward | GGTCCTAGAGTTTCTGGCTCAC |
| | Reverse | GCAGTTTCCGTGGTCACTCTCA |
| *ESYT2* | Forward | TGGAACCGTTGATTGGAGATATG |
| | Reverse | TCGTCAGTCCTGTCCAGTTA |
| *GPATCH2* | Forward | AGTTGTGCCCTGGTGGGAAAAG |
| | Reverse | CATGAAGGCGACTGAGTCTAGC |
| *GRK5* | Forward | GGCAGTATCGAGTGCTAGG |
| | Reverse | CGCTTGCAGGCATACATT |
| *IL1R1* | Forward | GGAGGCTGATAAATGCAAGG |
| | Reverse | GGTTAAGAGGACAGGGACG |
| *IQCK* | Forward | TCACGTACACCCTGTCCTCAAG |
| | Reverse | TTCGCTTGGTGAAGCAGGCTAG |
| *KCNJ8* | Forward | GGAGAAAAGTGGTTTGGAGTCCA |
| | Reverse | CCAAAGGGCATTCCTCTGTCATC |
| *PLLP* | Forward | GGTGATGTTCGTCGCTGTCTTC |
| | Reverse | AGAGAACGGTGGCGCTGATGTT |
| *SPATA17* | Forward | GCATTACCTCCTCAGCACAAAGC |
| | Reverse | GCTGGTGGAGTCCTTCTGCTTA |
| *ST7L* | Forward | ACAGAATTTGTCAGAATGTAAGGTATG |
| | Reverse | TTACCAGTCACCCAAGTGTATC |
| *TESC* | Forward | CTGAGTGGAGATCAGCCTACCA |
| | Reverse | CTGTTGTCGAAGAAGGCACGAAC |
| *HMOX1* | Forward | CCAGGCAGAGAATGCTGAGTTC |
| | Reverse | AAGACTGGGCTCTCCTTGTTGC |
| *CSTA* | Forward | AAACTCAAGTTGTTGCTGGAACAAA |
| | Reverse | TTTGTCAACCTGGTATCCAGTAAG |
| *JAG1* | Forward | TGCTACAACCGTGCCAGTGACT |
| | Reverse | TCAGGTGTGTCGTTGGAAGCCA |
| *PRKCA* | Forward | GCCTATGGCGTCCTGTTGTATG |
| | Reverse | GAAACAGCCTCCTTGGACAAGG |
| *SCUBE2* | Forward | GTGTGCTGTCAACAATGGAGGC |
| | Reverse | ATTGCGGGTCTGGCACTCATCA |
| *UNC5A* | Forward | TCTACCTCACGCTGCACAAGCC |
| | Reverse | ACAGTGGTCCATAGCCAGGATG |
| *GAPDH* | Forward | TGGTCTCCTCTGACTTCAACA |
| | Reverse | TGTCATACCAGGAAATGAGCTT |

**Table S2. Primers used for ChIP real-time PCR**

| Gene | Direction | Sequence (5′ to 3′) |
|---|---|---|
| *ABCA17P* | Forward | GGTGCCCCCTGCATGTC |
| | Reverse | GTGCGAACCGCGTACTC |
| *ADCY1* | Forward | GCCAAGAGGGTGGGTTC |
| | Reverse | CCGCGTGACCTCGAGTA |
| *ASRGL1* | Forward | CAGGAGATCAATCACAGTTTGC |
| | Reverse | CAACAAGAGTGAAACTCCGAAT |
| *DACF10* | Forward | AAGAGTGGCATGTCACCTA |
| | Reverse | TGAGTATGTACTGGTGTCTCCACTTTA |
| *ESYT2* | Forward | CAACCTTGCACACTGGCTA |
| | Reverse | GTCCCTGTTTGCGGTCA |
| *GPATCH2* | Forward | TAGGGTAATCGTTAAATCGTTAATTGGA |
| | Reverse | GTCAGGAAGAGAAGGATCGC |
| *GRK5* | Forward | CGAGCCTGCTAGTACCAC |
| | Reverse | TCTCTGTCACTCCATTCTCCT |
| *IL1R1* | Forward | CCTTGCTCTCTCTCCTGC |
| | Reverse | GATTTAATTGACTTACAGCTCCGC |
| *IQCK* | Forward | CCTGGAGAGTCAAGGGAATAAC |
| | Reverse | CTGAGCAGGACACTGGAAAT |
| *KCNJ8* | Forward | ATGAAGTAGCCCAGTTATCTACC |
| | Reverse | AACTTTCTGACTCCAAGTTCAC |
| *PLLP* | Forward | GCGTAGAAAGCGCCTGG |
| | Reverse | CAGGCCGTGGTAGCTTC |
| *SPATA17* | Forward | GGAGCAACCATTAGTTCTCCG |
| | Reverse | TATTTGTCTCTGTCTCTACTGCGA |
| *ST7L* | Forward | TGGCAGCGTGTTTCGATT |
| | Reverse | TCGGCTGTCGTGGAGATA |
| *TESC* | Forward | GTGGGTGTGTTCACCTCAG |
| | Reverse | ATAGCTCTATGGAGAACAACAGTAA |
| *HMOX1* | Forward | TGAGGAGGCAAGCAGTC |
| | Reverse | GTGGGCAACATCAGGAACTTA |
| *CSTA* | Forward | GACGTTTGATGCTTGTTAGGAC |
| | Reverse | TACATGGAATTTGGAAGAAAGAGGG |
| *JAG1* | Forward | ACGATCCCTTCCAAGTACC |
| | Reverse | GGGCGGCGTAGAAGAAC |
| *PRKCA* | Forward | GAGTAGGAAGCGCGAGC |
| | Reverse | GGAGAGTCGGGCTGGTG |
| *SCUBE2* | Forward | GGAGTGATGGTGGGTGTAT |
| | Reverse | GAACAGGTACACAGGGTCTT |
| *UNC5A* | Forward | CACTCCGGGTTTCAGACT |
| | Reverse | GAATCCCTCTGGGCGATG |
| *GAPDH* | Forward | GGGTTCCTATAAATACGGACTGC |
| | Reverse | GGGTTCCTATAAATACGGACTGC |