NOTCH1 signaling in oral squamous cell carcinoma via a TEL2/SERPINE1 axis

SUPPLEMENTARY MATERIALS

Supplementary Figure 1: Expression of NOTCH, SERPINE1 and ETV7 in OSCC lines. (A) RT-qPCR showing relative expression of NOTCH 1, 2, 3 and 4 in SJG lines and OK, n = 3. (B) RT-qPCR analysis showing levels of SERPINE1 mRNA in SJG lines, n = 3. (C) RT-qPCR analysis of levels of ETS1, ETS2, ETV6 and ETV7 mRNA in SJG lines and OK, n = 3. Data represent mean ± SD.
**Supplementary Table 1: STR profiles of SJG lines and validation of NOTCH1 mutations by Sanger sequencing.** See Supplementary Table 1

**Supplementary Table 2: Microarray data for SJG6 Blank and SJG+NICD cells.** See Supplementary Table 2

**Supplementary Table 3: KEGG pathway analysis**

| Pathway Name                                                      | Corrected $p$ value | log value     |
|------------------------------------------------------------------|---------------------|---------------|
| ECM-receptor interaction                                         | 4.61E-05            | 4.336299075   |
| Focal adhesion                                                   | 0.0002              | 3.698970004   |
| Notch signaling pathway                                          | 0.0008              | 3.096910013   |
| Regulation of actin cytoskeleton                                 | 0.0011              | 2.958607315   |
| Metabolic pathways                                              | 0.0037              | 2.431798276   |
| p53 signaling pathway                                           | 0.0037              | 2.431798276   |
| Insulin signaling pathway                                        | 0.0037              | 2.431798276   |
| Tight junction                                                   | 0.0098              | 2.008773924   |
| Hypertrophic cardiomyopathy (HCM)                               | 0.0102              | 1.991399828   |
| Tyrosine metabolism                                             | 0.0105              | 1.978810701   |
| Fatty acid metabolism                                           | 0.0119              | 1.924453039   |
| Leukocyte trans endothelial migration                            | 0.0131              | 1.882728704   |
| Viral myocarditis                                                | 0.0161              | 1.793174124   |
| Cell cycle                                                       | 0.0168              | 1.774690718   |
| Toll-like receptor signaling pathway                             | 0.0197              | 1.705533774   |
| Axon guidance                                                    | 0.0197              | 1.705533774   |
| Amoebiasis                                                       | 0.0228              | 1.642065153   |
| Other glycan degradation                                         | 0.0228              | 1.642065153   |
| Protein digestion and absorption                                | 0.0228              | 1.642065153   |
| Progesterone-mediated oocyte maturation                          | 0.0292              | 1.534617149   |
| Apoptosis                                                        | 0.0292              | 1.534617149   |
| Vascular smooth muscle contraction                               | 0.0292              | 1.534617149   |
| alpha-Linolenic acid metabolism                                 | 0.0296              | 1.528708289   |
| Dilated cardiomyopathy                                          | 0.0306              | 1.514278574   |
| Fat digestion and absorption                                    | 0.0358              | 1.446116973   |
| Other types of O-glycan biosynthesis                            | 0.0358              | 1.446116973   |
| Spliceosome                                                      | 0.0358              | 1.446116973   |
| Fatty acid elongation in mitochondria                            | 0.0358              | 1.446116973   |
| Fc gamma R-mediated phagocytosis                                | 0.0358              | 1.446116973   |
| Bacterial invasion of epithelial cells                           | 0.0358              | 1.446116973   |
| Purine metabolism                                               | 0.0358              | 1.446116973   |
| MAPK signaling pathway                                           | 0.0358              | 1.446116973   |
| Adipocytokine signaling pathway                                 | 0.0362              | 1.441291429   |
| Pathway                                                                 | P-value  | q-value             |
|-------------------------------------------------------------------------|----------|---------------------|
| Arrhythmogenic right ventricular cardiomyopathy (ARVC)                   | 0.0362   | 1.441291429         |
| Toxoplasmosis                                                           | 0.0362   | 1.441291429         |
| Type II diabetes mellitus                                               | 0.0362   | 1.441291429         |
| Cell adhesion molecules (CAMs)                                          | 0.0437   | 1.359518563         |
| Phosphatidylinositol signaling system                                   | 0.0495   | 1.305394801         |
| Pathways in cancer                                                      | 0.0502   | 1.299296283         |
| Butanoate metabolism                                                   | 0.0502   | 1.299296283         |
| Citrate cycle (TCA cycle)                                               | 0.053    | 1.27572413          |
| Pathogenic Escherichia coli infection                                   | 0.053    | 1.27572413          |
| Huntington's disease                                                    | 0.055    | 1.259637311         |
| Arachidonic acid metabolism                                            | 0.0604   | 1.218963061         |
| Lysosome                                                                | 0.0605   | 1.218244625         |
| Ether lipid metabolism                                                  | 0.0715   | 1.145693958         |
| Osteoclast differentiation                                              | 0.0741   | 1.130181792         |
| GnRH signaling pathway                                                  | 0.0882   | 1.054531415         |
| Pyruvate metabolism                                                    | 0.0882   | 1.054531415         |
| Ubiquitin mediated proteolysis                                          | 0.0882   | 1.054531415         |
| PPAR signaling pathway                                                 | 0.089    | 1.050609993         |
| Long-term depression                                                    | 0.089    | 1.050609993         |
| RIG-I-like receptor signaling pathway                                   | 0.0912   | 1.040005162         |
| Leishmaniosis                                                           | 0.0936   | 1.028724151         |
| Chronic myeloid leukaemia                                              | 0.0961   | 1.017276612         |
| Lysine degradation                                                      | 0.0987   | 1.005682847         |
| Go Accession | GO Term                                                                 | p-value   | Corrected p-value | Count in Selection | % Count in Selection | Count in Total | % Count in Total |
|--------------|--------------------------------------------------------------------------|-----------|-------------------|--------------------|----------------------|----------------|------------------|
| GO:0031428   | box C/D snoRNP complex                                                   | 4.14E-10  | 9.78E-06          | 5                  | 4.9504952            | 9              | 0.046066437      |
| GO:0070761   | pre-snoRNP complex small nucleolar ribonucleoprotein complex            | 2.57E-09  | 3.04E-05          | 5                  | 4.9504952            | 12             | 0.061421916      |
| GO:0005732   | snoRNA binding                                                          | 4.87E-08  | 3.83E-04          | 5                  | 4.9504952            | 20             | 0.10236986       |
| GO:1902904   | negative regulation of fibril organization                              | 8.21E-08  | 4.84E-04          | 5                  | 4.9504952            | 22             | 0.112606846      |
| GO:1902903   | regulation of fibril organization                                        | 1.34E-07  | 5.28E-04          | 3                  | 2.970297             | 3              | 0.015355479      |
| GO:0030308   | negative regulation of cell growth                                      | 1.89E-06  | 0.006361553       | 8                  | 7.920792             | 161            | 0.82407737       |
| GO:0005615   | extracellular space                                                     | 2.58E-06  | 0.00695763        | 21                 | 20.79208             | 1303           | 6.6693964        |
| GO:0000974   | Prp19 complex                                                           | 2.65E-06  | 0.00695763        | 3                  | 2.970297             | 6              | 0.030710958      |
| GO:1903054   | negative regulation of extracellular matrix organization                | 4.62E-06  | 0.010325498       | 3                  | 2.970297             | 7              | 0.03582945       |
| GO:0044421   | extracellular region part                                               | 4.81E-06  | 0.010325498       | 39                 | 38.61386             | 3765           | 19.271126        |
| GO:0042026   | protein refolding                                                       | 6.60E-06  | 0.012991914       | 4                  | 3.960396             | 24             | 0.12284383       |
| GO:0005576   | extracellular region                                                    | 7.22E-06  | 0.013113523       | 44                 | 43.564358            | 4606           | 23.575779        |
| GO:0051346   | negative regulation of hydrolase activity                               | 8.69E-06  | 0.014658065       | 11                 | 10.891098            | 409            | 2.0934637        |
| GO:0044452   | nucleolar part                                                          | 1.13E-05  | 0.016684284       | 5                  | 4.9504952            | 57             | 0.2917541        |
| GO:0008201   | heparin binding                                                         | 1.13E-05  | 0.016684284       | 7                  | 6.930693             | 147            | 0.75241846       |
| GO:0005539   | glycosaminoglycan binding                                                | 1.29E-05  | 0.01796228        | 8                  | 7.920792             | 209            | 1.0697651        |
| GO:0071675   | regulation of mononuclear cell migration                                | 2.15E-05  | 0.028164886       | 3                  | 2.970297             | 11             | 0.056303423      |
| GO:0030336   | negative regulation of cell migration                                   | 2.89E-05  | 0.032474402       | 7                  | 6.930693             | 170            | 0.87014383       |
| GO:0031932   | TORC2 complex                                                           | 2.85E-05  | 0.032474402       | 3                  | 2.970297             | 12             | 0.061421916      |
| GO:2000506   | negative regulation of energy homeostasis                               | 2.65E-05  | 0.032474402       | 2                  | 1.980198             | 2              | 0.010236986      |
| GO:0045926   | negative regulation of growth                                           | 3.10E-05  | 0.032977507       | 8                  | 7.920792             | 236            | 1.2079644        |
| GO:0002688   | regulation of leukocyte chemotaxis                                       | 3.32E-05  | 0.032977507       | 5                  | 4.9504952            | 71             | 0.363413         |
| GO:2000146   | negative regulation of cell motility                                    | 3.35E-05  | 0.032977507       | 7                  | 6.930693             | 174            | 0.8906178        |
| GO:0051085   | chaperone mediated protein folding requiring cofactor                    | 3.69E-05  | 0.034886237       | 3                  | 2.970297             | 13             | 0.06654041       |
| GO:0061202   | gamma-aminobutyric acid transport vesicle membrane                       | 4.68E-05  | 0.04095736        | 3                  | 2.970297             | 14             | 0.0716589        |
| GO:0061200 | clathrin-sculpted gamma-aminobutyric acid transport vesicle | 4.68E-05 | 0.04095736 | 3 | 2.970297 | 14 | 0.0716589 |
| GO:0038201 | TOR complex negative regulation of cellular component movement | 5.83E-05 | 0.04918293 | 3 | 2.970297 | 15 | 0.0767774 |
| GO:0051271 | negative regulation of locomotion sterol desaturase activity | 6.06E-05 | 0.04933887 | 7 | 6.930693 | 191 | 0.97763216 |
| GO:0040013 | C-5 sterol desaturase activity | 6.69E-05 | 0.05261740 | 7 | 6.930693 | 194 | 0.9929877 |
| GO:0000248 | negative regulation of ATP biosynthetic process regulation of extracellular matrix organization sulfur compound binding | 7.91E-05 | 0.05661090 | 2 | 1.980198 | 3 | 0.015355479 |
| GO:0002040 | sprouting angiogenesis positive regulation of chemotaxis defense response to virus | 7.91E-05 | 0.05661090 | 2 | 1.980198 | 3 | 0.015355479 |
| GO:2001170 | regulation of cell growth clathrin-sculpted vesicle | 8.65E-05 | 0.05994675 | 3 | 2.970297 | 17 | 0.087014385 |
| GO:1903053 | regulation of cell growth clathrin-sculpted vesicle | 8.89E-05 | 0.05994675 | 7 | 6.930693 | 203 | 1.0390542 |
| GO:0001558 | regulation of cell growth clathrin-sculpted vesicle | 1.02E-04 | 0.06426427 | 9 | 8.910892 | 359 | 1.8375391 |
| GO:0060198 | clathrin-sculpted vesicle | 1.03E-04 | 0.06426427 | 3 | 2.970297 | 18 | 0.092132874 |
| GO:0002040 | sprouting angiogenesis positive regulation of chemotaxis defense response to virus | 1.01E-04 | 0.06426427 | 4 | 3.960396 | 47 | 0.24056917 |
| GO:0050921 | positive regulation of chemotaxis defense response to virus | 1.21E-04 | 0.07151497 | 5 | 4.9504952 | 93 | 0.47601986 |
| GO:0051607 | negative regulation of cellular metabolic process regulation of ATP biosynthetic process | 1.20E-04 | 0.07151497 | 6 | 5.940594 | 148 | 0.757537 |
| GO:0023023 | MHC protein complex binding regulation of cell migration | 1.43E-04 | 0.07709776 | 3 | 2.970297 | 20 | 0.10236986 |
| GO:0030334 | MHC class II protein complex binding negative regulation of cellular metabolic process | 1.41E-04 | 0.07709776 | 11 | 10.891089 | 556 | 2.8458822 |
| GO:0023026 | MHC class II protein complex binding negative regulation of cellular metabolic process | 1.43E-04 | 0.07709776 | 3 | 2.970297 | 20 | 0.10236986 |
| GO:0031324 | regulation of ATP biosynthetic process | 1.44E-04 | 0.07709776 | 24 | 23.762377 | 2104 | 10.769309 |

**Supplementary Table 5: GSEA molecular functions.** See Supplementary Table 5
**Supplementary Table 6: List of TaqMan® probes**

| TaqMan probes | Company/Source | Assay ID          |
|----------------|----------------|-------------------|
| NOTCH1         | Thermofisher   | Hs01062014_m1     |
| SERPINE1       | Thermofisher   | Hs00167155_m1     |
| ETS1           | Thermofisher   | Hs00428293_m1     |
| ETS2           | Thermofisher   | Hs00232009_m1     |
| ETV3           | Thermofisher   | Hs01051028_g1     |
| ETV5           | Thermofisher   | Hs00927557_m1     |
| ETV6           | Thermofisher   | Hs00231101_m1     |
| ETV7           | Thermofisher   | Hs00903229_m1     |
| ERG            | Thermofisher   | Hs01554629_m1     |
| CTCF           | Thermofisher   | Hs00902016_m1     |
| 18S            | Thermofisher   | Hs03003631_g1     |
| TBP            | Thermofisher   | Hs00427620_m1     |

**Supplementary Table 7: List of primary and secondary antibodies used for immunofluorescence and Western blot**

| Antibody                  | Company/Source        | Catalog number/Reference | Host species | Dilution IF or (WB) |
|---------------------------|-----------------------|--------------------------|--------------|---------------------|
| Phalloidin AF488          | Thermofisher          | A12379                   | NA           | 1/2000              |
| Phalloidin AF555          | Thermofisher          | A34055                   | NA           | 1/2000              |
| Phalloidin AF647          | Thermofisher          | A22297                   | NA           | 1/2000              |
| anti-Cleaved NOTCH1       | Cell Signaling        | D3B8 #4147               | Rabbit       | 1/100               |
| anti-SERPINE1 (1D5)       | Novus Biologicals     | NBP2-37532               | Mouse        | 1/100, 1/250 (WB)   |
| anti-ETV7                 | Atlas Antibodies      | HPA029033                | Rabbit       | 1/100               |
| anti-Involucrin           | In-house              | Sy7 Hudson D, et al., Hybridoma (1992), 11(3):367-379 | Mouse        | 1/500               |
| anti-Mouse AF555          | Life Technologies     | A31570                   | Mouse        | 1/300               |
| anti-Mouse AF647          | Life Technologies     | A31571                   | Mouse        | 1/300               |
| anti-Mouse AF488          | Life Technologies     | A11001                   | Mouse        | 1/300               |
| anti-Rabbit AF488         | Life Technologies     | A11008                   | Rabbit       | 1/300               |
| anti-Rabbit AF555         | Life Technologies     | A21428                   | Rabbit       | 1/300               |
| anti-Rabbit AF647         | Life Technologies     | A21244                   | Rabbit       | 1/300               |
| anti-Vinculin (7F9)       | Santa Cruz Biotechnology | sc-73614             | Mouse        | 1/10000 (WB)        |
| anti-Mouse HRP            | Jackson Lab           | 715-035-151              | Mouse        | 1/10000 (WB)        |

Footnote: NA-Not applicable; IF- Immunostainings of cells or tissue; WB- Western blotting.