

## Supplementary Data

### Supplement 1 – Primer sequences

| Genes            | Forward primer (5'→3') | Reverse primer (5'→3')   | Positive control     |
|------------------|------------------------|--------------------------|----------------------|
| <i>ACAN</i>      | CCTCCCCTTCACGTGTA AAA  | GCTCCGCTTCTGTAGTCTGC     | <i>HEK293</i>        |
| <i>Axin2</i>     | CACACCCTTCTCCAATCCAA   | TGGAATTTCTTCCCCACAGA     | <i>HEK293</i>        |
| <i>ETS2</i>      | CAGCGTCACCTACTGCTCTG   | AGTCGTGGTCTTTGGGAGTC     | <i>HepG2</i>         |
| <i>Noggin</i>    | AGGGCTAGAGTTCTCCGAGG   | AGAATGTCTGCGACCACAGC     | <i>Placenta</i>      |
| <i>Wnt3a</i>     | AACTGCACCACCGTCCAC     | AAGGCCGACTCCCTGGTA       | <i>Placenta</i>      |
| <i>GSC</i>       | CCTCCGCGAGGAGAAAAGT    | CGTTCTCCGACTCCTCTGAT     | <i>MCF7</i>          |
| <i>MixL1</i>     | GGTACCCCGACATCCACTT    | GCCTGTTCTGGAACCATACCT    | <i>HepG2</i>         |
| <i>TBX6</i>      | GAACGCGAGAACTGTAAGAGG  | GTGTGTCTCCGCTCCCATAG     | <i>MCF7</i>          |
| <i>Notch</i>     | GCCTCAAGCCCTGAAGAA     | CCTCGAACCAGAACTTCTTG     | <i>HEK293</i>        |
| <i>Twist</i>     | TCTCGGTCTGGAGGATGGAG   | AATGACATCTAGGTCTCCGGC    | <i>HEK293</i>        |
| <i>Slug</i>      | GCCAAACTACAGCGAACTGG   | GGCTGTATGCTCCTGAGCTG     | <i>MDA MB 231</i>    |
| <i>Snail</i>     | GCTGCAGGACTCTAATCCAGA  | ATCTCCGAGGTGGGATG        | <i>HEK293</i>        |
| <i>Brachyury</i> | AACGGCAGGAGGATGTTTC    | GTTACGTA CTCCAGCGGT      | <i>H460</i>          |
| <i>SHH</i>       | CACCCCAATTACAACCCC     | GATGGCCAAAGCGTTCAACT     | <i>MCF7</i>          |
| <i>Gli1</i>      | CCAGCCAGAGAGACCAACAG   | CCCGCTTCTTGGTCAACTT      | <i>HEK293</i>        |
| <i>Gli2</i>      | CACGCTCTCCATGATCTCTG   | CCCCTCTCCTTAAGGTGCTC     | <i>B2B</i>           |
| <i>Gli3</i>      | AGGTGAAAACCGTCAAGGCA   | CTCGAGCCCACTGTTGGAAT     | <i>HEK293</i>        |
| <i>Chordin</i>   | TGAAGGGATTCTATGGCTCAG  | GGCTACCCTTGGTGGTGA       | <i>HEK293</i>        |
| <i>FoxA1</i>     | TAATCATTGCCATCGTGTGC   | ATGTCCAACTGTGGAAGTGC     | <i>HeLa</i>          |
| <i>FoxA2</i>     | GGGAGCGGTGAAGATGGAAG   | TCATGCCGTT CATCCCCAG     | <i>HEK293</i>        |
| <i>Gal-3</i>     | TGTTTGCAATACAAAGCTGGA  | GCAACCTTGAAGTGGTCAGG     | <i>Placenta</i>      |
| <i>BASP1</i>     | TGGATCTCAATGCCAATCCT   | GAGAATGTTTG TCACTCCCAAAA | <i>MCF7</i>          |
| <i>PAX1</i>      | CGAGCATTCACTGAGGATAGG  | CCCACATGAAATGTCAGAGC     | <i>Tonsil tissue</i> |
| <i>PAX9</i>      | GGTGAACGGGTGGAGAAG     | GAGACCATTTGGTGCCTGA      | <i>MCF7</i>          |
| <i>CD24</i>      | GCTTTGGATTTGACATTGCAT  | GCTTGGATCTGGGGTAGAT      | <i>HEK293</i>        |
| <i>HOXA5</i>     | GCGCAAGCTGCACATAAG     | CGGTTGAAGTGGAACCTCT      | <i>HEK293</i>        |
| <i>CCN2</i>      | CCTGCAGGCTAGAGAAGCA    | GATGCACTTTTGCCCTTCT      | <i>Hela</i>          |
| <i>Sox9</i>      | GTACCCGCACTGCACAAC     | TCTCGCTCTCGTTCAGAAGTC    | <i>HEK293</i>        |
| <i>KLF4</i>      | ATCTTTCTCCACGTTCCGCT   | GGAAGTCGCTTCATGTGGGA     | <i>MCF7</i>          |
| <i>Sox2</i>      | ATGGGTTCCGTGGTCAAGTC   | CTGATCATGTCCCGAGGTC      | <i>HEK293</i>        |
| <i>OCT4</i>      | GAAACCCACACTGCAGCAG    | AACCACACTCGGACCACATC     | <i>Placenta</i>      |
| <i>Nanog</i>     | GAGATGCCTCACACGGAGAC   | AGGGCTGTCCTGAATAAGCA     | <i>HepG2</i>         |

Positive controls were determined using the freely available *Human Protein Atlas* (<http://www.proteinatlas.org>)

Supplement 2 – Tabulated overview of expression data

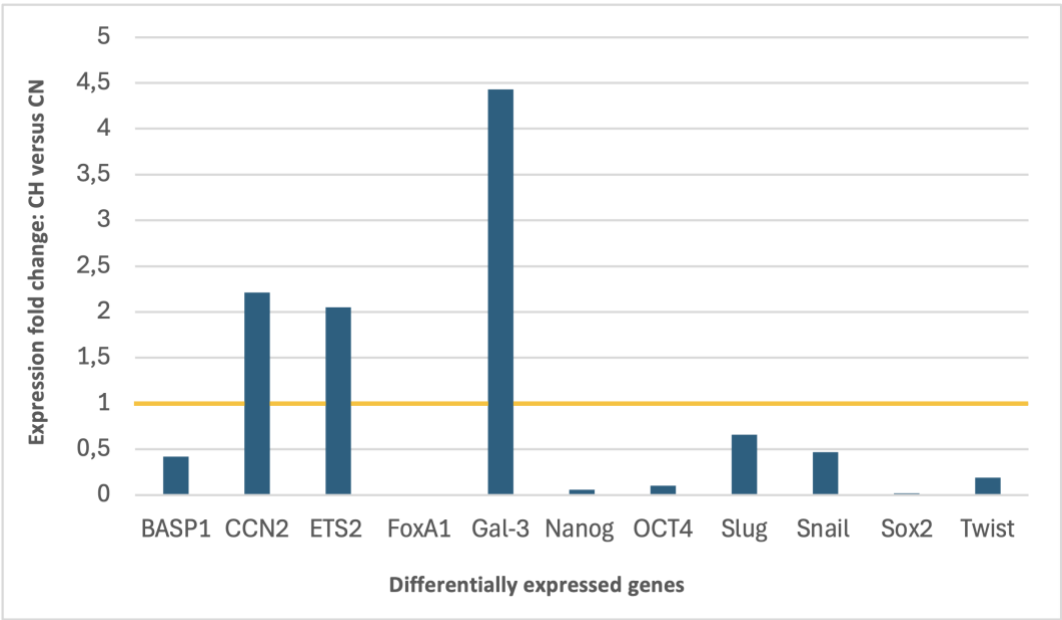
| CH+ only  | CH > CN | CN > CH | CN+ only | CH- and CN- |
|-----------|---------|---------|----------|-------------|
| Chordin   | Gal-3   | Slug    | Pax1     | FoxA2       |
| HOXA5     | ETS2    | Snail   | Axin2    | Gli2        |
| SHH       | CCN2    | BASP1   |          | TBX6        |
| PAX9      |         | Twist   |          | MixL1       |
| Brachyury |         | FoxA1   |          | GSC         |
| CD24      |         | Nanog   |          | Wnt3a       |
| ACAN      |         | Sox2    |          | Noggin      |
| SOX9      |         | OCT4    |          |             |
| Gli1      |         |         |          |             |
| Notch     |         |         |          |             |
| KLF4      |         |         |          |             |
| Gli3      |         |         |          |             |

|        |                 |       |       |       |
|--------|-----------------|-------|-------|-------|
| n = 12 | n = 3<br>n = 11 | n = 8 | n = 2 | n = 7 |
|--------|-----------------|-------|-------|-------|

CH: chordoma, CN: chondrosarcoma

Supplement 3 – Graph of differentially expressed genes



Gene expression in chordomas relative to that in chondrosarcomas. CH: chordoma, CN: chondrosarcoma

Expression fold changes between chordomas and chondrosarcomas were calculated and corrected for primer efficiencies and reference genes, as described. In this figure, the expression fold changes are depicted for genes that are expressed in both chordoma and chondrosarcoma, but at a different level. For example: Galectin-3 is expressed at an almost 4,5-fold higher level in chordoma, as compared to chondrosarcoma.