

# **Molecular features and vulnerabilities of recurrent chordomas**

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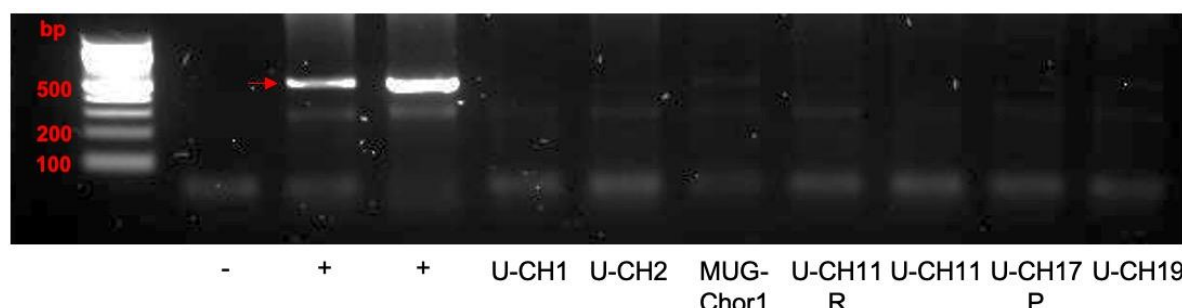
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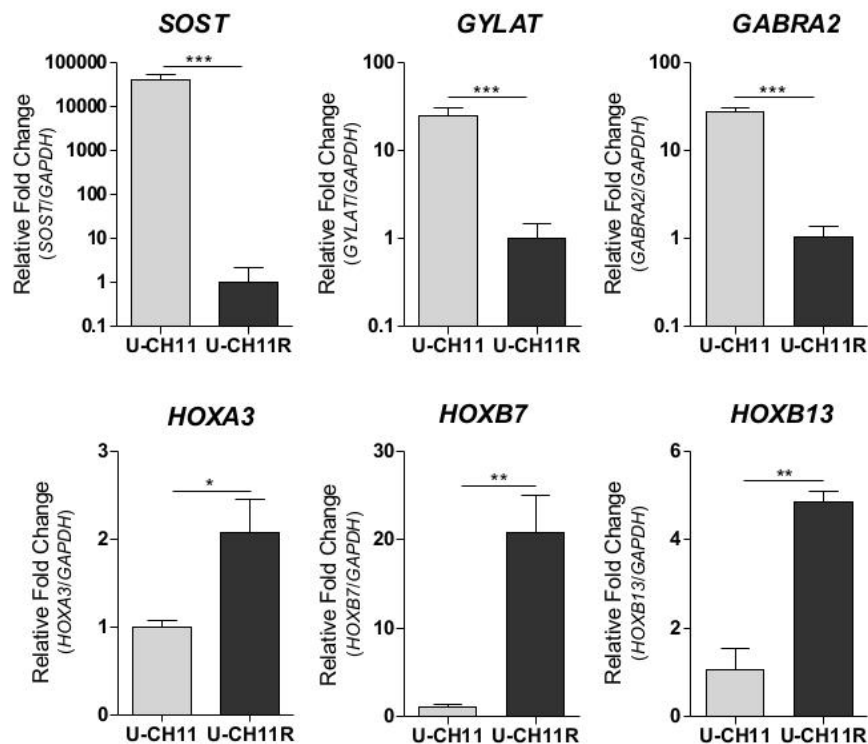
## Additional Information



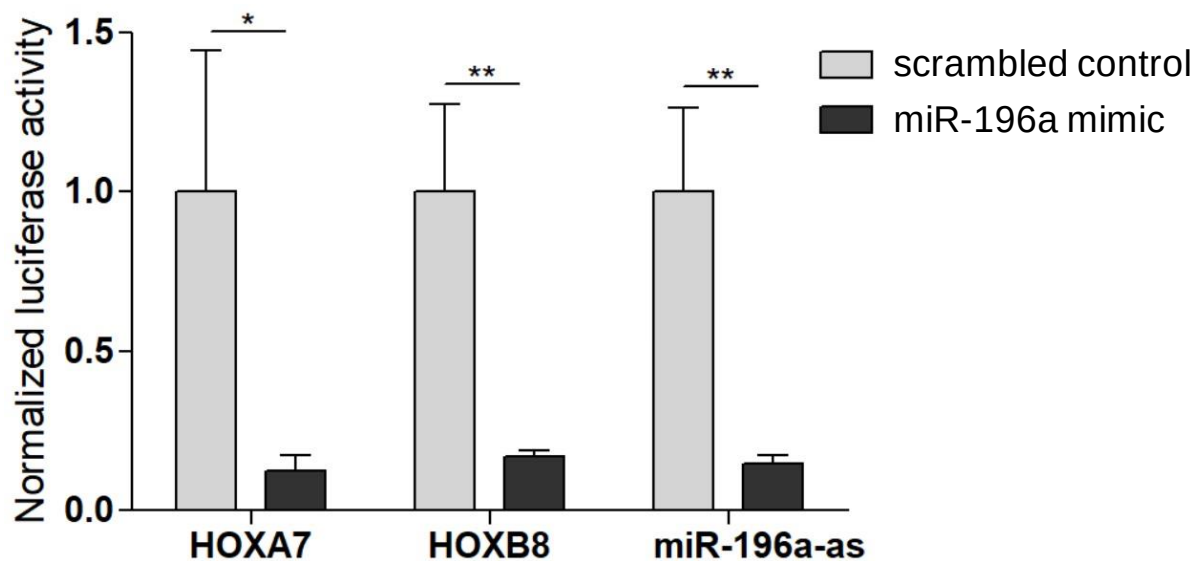
**Additional figure 1: Results of mycoplasma PCR of cell lines included in the analyses.** (-) negative control; (+) two different mycoplasma positive controls showing the expected amplicon (~500bp). All cell lines tested were negative.

|                | HE  | brachyury     | S100-protein | vimentin | pan-cytokeratin | EMA | Ki-67 |
|----------------|-----|---------------|--------------|----------|-----------------|-----|-------|
| primary        |     |               |              |          |                 |     |       |
| relapse        |     |               |              |          |                 |     |       |
|                | HE  | brachyury     | S100-protein | vimentin | pan-cytokeratin | EMA | Ki-67 |
| Primary tissue | NOS | +++ (nuclear) | -/+          | +++      | +++             | ++  | 1%    |
| U-CH11         |     | +++ (nuclear) | -            | ++       | +++             | +   | <1%   |
| Relapse tissue | NOS | +++ (nuclear) | -            | ++       | +++             | ++  | 5%    |
| U-CH11R        |     | +++ (nuclear) | -            | ++       | +++             | +   | 5%    |

**Additional figure 2: Immunohistochemistry (IHC) of the primary and the relapsed sacral chordoma in comparison to the derived cell lines.** IHC results were interpreted as follows: “no immunoreactivity detected” (-), “immunoreactivity ≤ 30%” (+), “immunoreactivity > 30% and < 70%” (++), “immunoreactivity in ≥ 70%” (+++) of the total number of chordoma cells analyzed. HE: hematoxylin/eosin; EMA: epithelial membrane antigen. NOS: not otherwise specified.

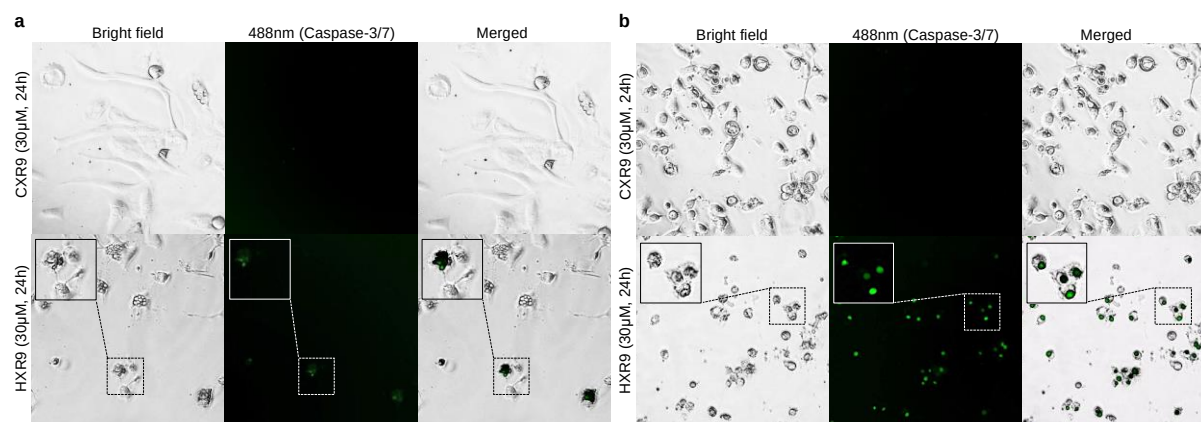


**Additional figure 3: Validation of gene expression differences between U-CH11 and U-CH11R.** Expression levels of selected genes were quantified by qRT-PCR analyses and normalized against *GAPDH*. Experiments were performed in technical triplicates and at least in biological duplicates and Student's t-tests were performed to determine statistical significance (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001).

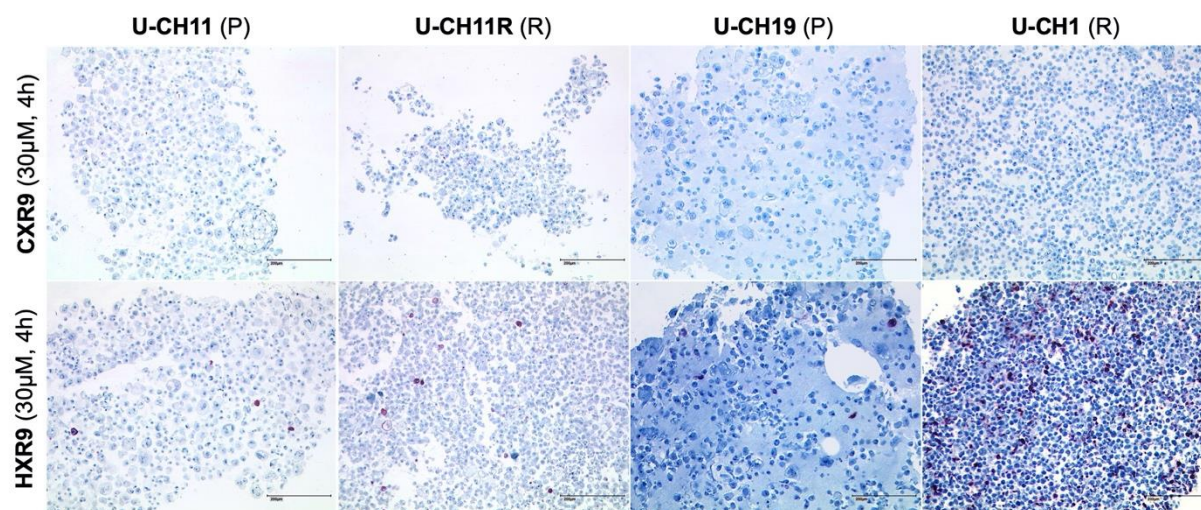


**Additional figure 4: Dual Luciferase reporter assay of miR-196a-5p in HEK293T cells.** Firefly luciferase reporters containing either the complementary site of *HOXA7* and *HOXB8* or the perfect antisense sequence of miR-196a were co-transfected with a miR-196a mimic or a scrambled control, respectively. Firefly luciferase activity was normalized to Renilla luciferase activity. Experiments were performed in technical

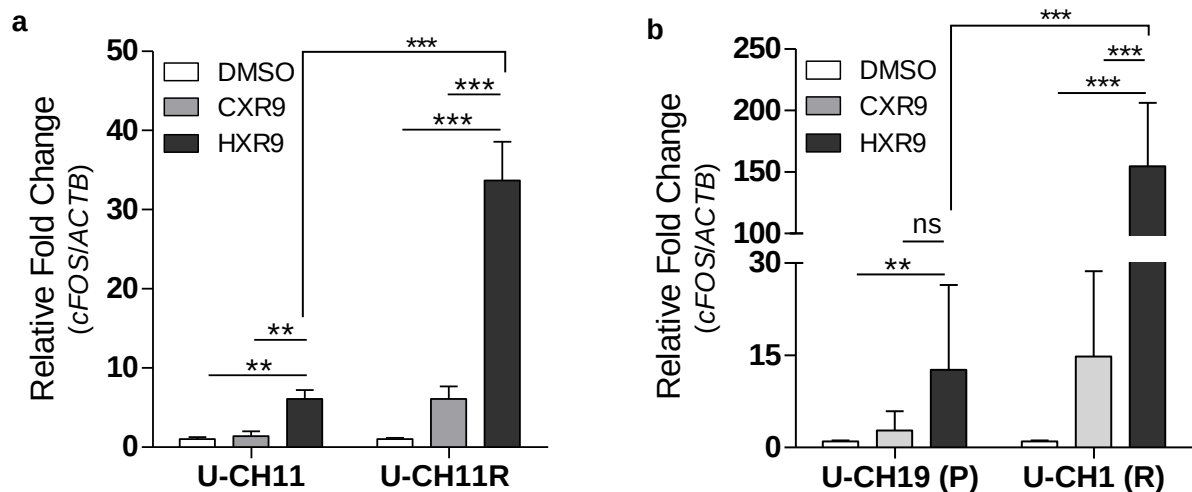
and biological triplicates and Student's t-tests were performed to determine statistical significance (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).



**Additional figure 5: Activation of Caspase-3/7 in U-CH19 and U-CH1.** Activation of cleaved Caspase-3/7 (green channel) in U-CH19 (a) and U-CH1 (b) treated for 24h with 30μM CXR9 or HXR9 investigated by EarlyTox Caspase-3/7 NucView 488 assays.



**Additional figure 6: Induction of apoptosis in HXR9 treated chordoma cell lines assessed by immunocytochemistry of cleaved Caspase-3 (red staining).** Cells were treated for 4h with 30μM HXR9 or the control peptide CXR9. Compared to HXR9 treatment, no cleaved Caspase-3 positivity was observed in response to CXR9.



**Additional figure 7: Expression levels of *cFOS* normalized against *ACTB*.** *CFOS* expression levels in response to treatment with HXR9 or two control compounds (CXR9 and DMSO) in U-CH11 versus U-CH11R (**a**) and U-CH19 compared to U-CH1 (**b**) cell lines quantified by qRT-PCR and normalized against *ACTB*. Gene expression experiments were performed in technical and biological triplicates. Statistical differences were determined by Student's t-tests (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

**Additional table 1:** Antibodies used for immunostainings of chordoma cell lines and tissue

| Antibody                          | Clone      | Company | Dilution |
|-----------------------------------|------------|---------|----------|
| Brachyury                         | EPR18113   | Abcam   | 1:4000   |
| Cytokeratin                       | AE1 + AE3  | Dako    | 1:100    |
| Epithelial Membrane Antigen (EMA) | E29        | Dako    | 1:500    |
| Ki-67                             | MIB-1      | Dako    | 1:200    |
| S100-protein                      | Z0311      | Dako    | 1:1000   |
| Vimentin                          | VIM3B4     | Dako    | 1:300    |
| cleaved-caspase 3                 | polyclonal | Dako    | 1:500    |

**Additional table 2:** STR profiles of U-CH11 and U-CH11R

|         | AMEL | D13S317 | D7S820 | D16S539 | Penta E | TH01  |
|---------|------|---------|--------|---------|---------|-------|
| U-CH11  | X    | 14      | 9 10   | 9 11    | 7 14    | 6 9.3 |
| U-CH11R | X    | 8 14    | (9) 10 | 9 11    | 7 14    | 6 9.3 |

|         | D18S51 | D3S1358 | D8S11779 | TPOX | CSF1PO | Penta D |
|---------|--------|---------|----------|------|--------|---------|
| U-CH11  | 14     | 15      | 13 16    | 8    | 10 11  | 9 12    |
| U-CH11R | 14     | 15 16   | 13 16    | 8    | 10 11  | 9 12    |

**Additional table 8:** Base-level expression of selected genes based on microarray gene expression data. Mean raw intensity values and standard deviations are given.

| GeneSymbol                                       | U-CH11             |          | U-CH11R            |          | U-CH19             |          | U-CH1              |         |
|--------------------------------------------------|--------------------|----------|--------------------|----------|--------------------|----------|--------------------|---------|
|                                                  | mean raw intensity | sd       | mean raw intensity | sd       | mean raw intensity | sd       | mean raw intensity | sd      |
| <b>References</b>                                |                    |          |                    |          |                    |          |                    |         |
| <i>MS4A1</i> (CD20; not expressed)               | 5.00               | 0.42     | 3.08               | 1.84     | 5.65               | 0.78     | 8.52               | 2.22    |
| <i>T</i> (Brachyury; diagnostic chordoma marker) | 2943.89            | 107.91   | 1071.99            | 330.40   | 4701.53            | 394.69   | 2218.19            | 436.73  |
| <i>GAPDH</i> (housekeeping gene)                 | 45973.58           | 16236.78 | 44591.84           | 18072.73 | 154013.26          | 12658.19 | 52720.23           | 8436.82 |
| <i>ACTB</i> ( $\beta$ -Actin; housekeeping gene) | 28015.21           | 7603.27  | 34013.52           | 18369.19 | 32282.21           | 2325.51  | 21732.87           | 4452.30 |
|                                                  |                    |          |                    |          |                    |          |                    |         |
| <i>FOS</i>                                       | 11234.00           | 5180.33  | 965.12             | 201.18   | 1280.55            | 547.23   | 1698.53            | 540.58  |
|                                                  |                    |          |                    |          |                    |          |                    |         |
| <b>Different in U-CH11 vs U-CH11R</b>            |                    |          |                    |          |                    |          |                    |         |
| <i>SOST</i>                                      | 1897.31            | 535.03   | 3.24               | 1.05     |                    |          |                    |         |
| <i>GLYT</i>                                      | 3213.43            | 436.36   | 2.81               | 1.42     |                    |          |                    |         |
| <i>GABRA2</i>                                    | 7896.56            | 5438.50  | 4.90               | 4.14     |                    |          |                    |         |
| <i>HOXA3</i>                                     | 30301.23           | 29563.06 | 136296.54          | 12150.52 |                    |          |                    |         |
| <i>HOXB7</i>                                     | 30.45              | 5.78     | 477.83             | 189.74   |                    |          |                    |         |
| <i>HOXB13</i>                                    | 30.22              | 3.21     | 308.18             | 25.71    |                    |          |                    |         |