Sulf2a controls Shh-dependent neural fate specification in the developing spinal cord

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Supplementary Figure S1. Efficiency and specificity of Sulf2aMOsplice-mediated knockdown.

a: Schematic representation of sulf2aMOsplice target sequence overlapping sulf2a exon 11.
b: Knockdown efficiency was controlled by RT-PCR at 24 hpf in embryos injected with ctrlMO or sulf2aMOsplice with primers p1 and p2 indicated on the scheme in a. A PCR product of 221 bp evidences sulf2a splicing while 2430 bp sized PCR product testifies the presence of unspliced sulf2a pre-mRNA forms. PCR reactions were controlled on RNA extracts (-RT).
c-e: Side views of 48 hpf embryos. Detection (c-e) and quantification (f) of OPC by immunodetection of Sox10 (red) and GFP (green) in Tg(olig2:GFP) embryos injected with ctrlMO (c, n=11), sulf2aMOsplice (d, n=12) or sulf2aMOsplice +mRNA (e, n=13).
Datasets were compared using Mann-Whitney’s test (two-tailed).
Data are presented as mean number of cells per embryo +/- s.d (** p<0.01, ns: not significant).
Supplementary Figure S2. Generation of sulf2a mutation by CRISPR/Cas9 technology.
a: Schematic representation of target sequence in sulf2a gene. The sequence targeted by CRISPR/Cas9-mutagenesis is indicated by a yellow arrowhead in exon 11. The wild type (wt) CRISPR target sequence is underlined and the mutant allele, consisting of a 16 nucleotide deletion/9 nucleotide insertion, is shown in italicand dashes. This mutation is predicted to produce a 519 amino acid protein truncated within the hydrophilic domain. b: Lateral views of 24 hpf (top panels) and 72 hpf (bottom panels) embryos showing detection of sulf2a mRNA by whole-mount in situ hybridisation in wt (left panels) and sulf2a−/− (right panels) embryos. Note the decreased sulf2a expression in mutant embryos (n=10 at 24 hpf and n=8 at 72 hpf) compared to wt siblings (n=7 at 24 hpf and n=8 at 72 hpf).
Supplementary Figure 3: *Sulf2a* depletion impairs motor neuron production

a-c: Detection (a-c) and quantification (d) of MNs by immunodetection of Islet1/2 (green) in Tg(olig2;DsRed) embryos (red) injected with ctrlMO (n=22), sulf2aMO\(^{ATG}\) (n=13) or sulf2aMO\(^{splice}\) (n=18) from two independent experiments. Datasets were compared with Mann Whitney’s test (two-tailed). Data are presented as mean number of cells per embryo± s.d (**p< 0.01, **** p<0.0001).
Supplementary Figure 4: Sulf2a does not regulate shh expression
Side views (a, c, e, g) and transverse sections (b, d, f, h) of 24 hpf (a-d) and 36 hpf (e-h) embryos. Detection of shh mRNA in embryos injected with ctrlMO (a, b, e, f) or sulf2aMO splice (c, d, g, h).