Supplementary Materials: A SOX2 Reporter System Identifies Gastric Cancer Stem-Like Cells Sensitive to Monensin

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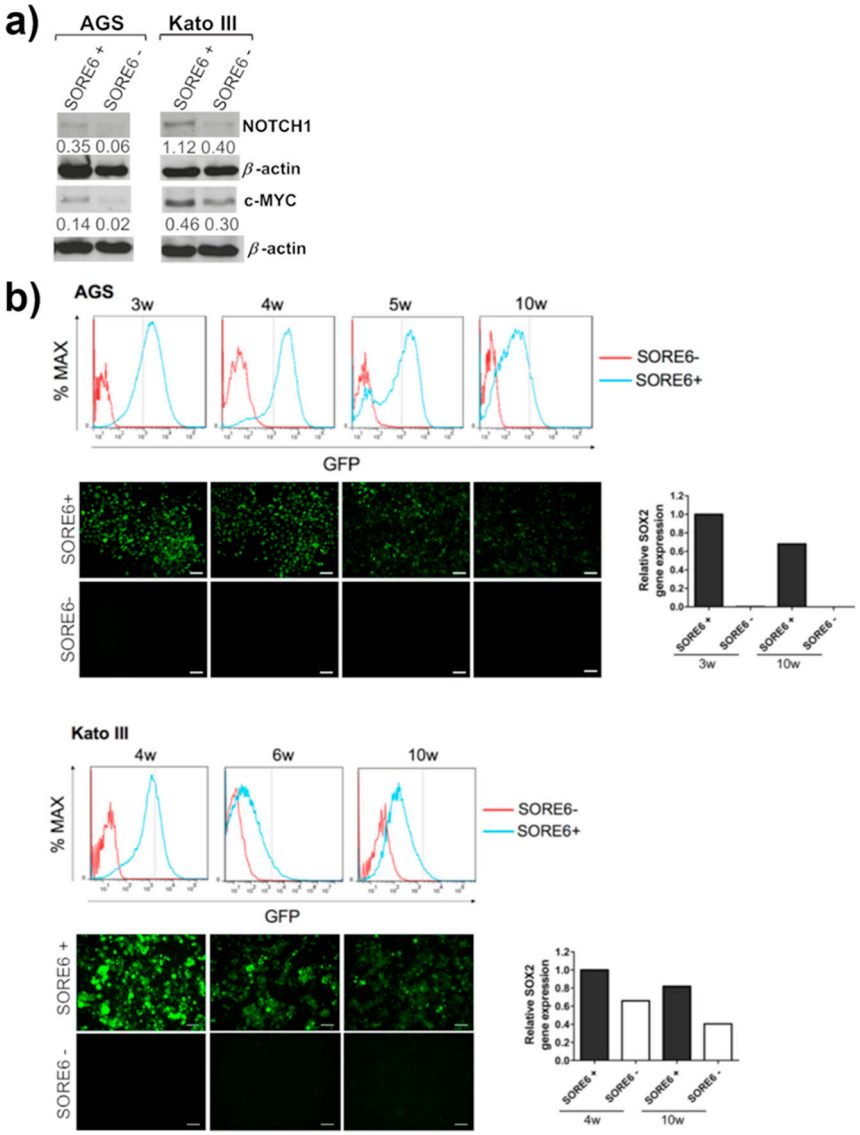


Figure S1. SORE6+ cells are enriched in NOTCH1 and c-MYC and give rise to SORE6− cells in both gastric cancer cell lines. **a**) Western blot exhibiting NOTCH1 and c-MYC expression in SORE6+ and SORE6− cells from both cell lines. b-actin was used as an internal control. Numbers below every western blot picture represent semi-quantitative analysis of each line (intensity ratio: "gene of interest"/b-actin). Pictures show cropped areas of western blots, the whole images are included in the Supplementary Materials. **b**) FACS plot of AGS SORE6+ cells and AGS SORE6− cells 3, 4, 5 and 10 weeks (w) after sorting followed by fluorescence images of the respective populations at those timepoints, scale bar = 100 mm. SORE6+ cells can regenerate to SORE6− cells while SORE6− cells are always GFP negative. Followed by the analysis of SOX2 expression – intensity ratio - (by western blot) in both SORE6 subpopulations at the initial and final time-point. Results are mean ± SD. Significant differences

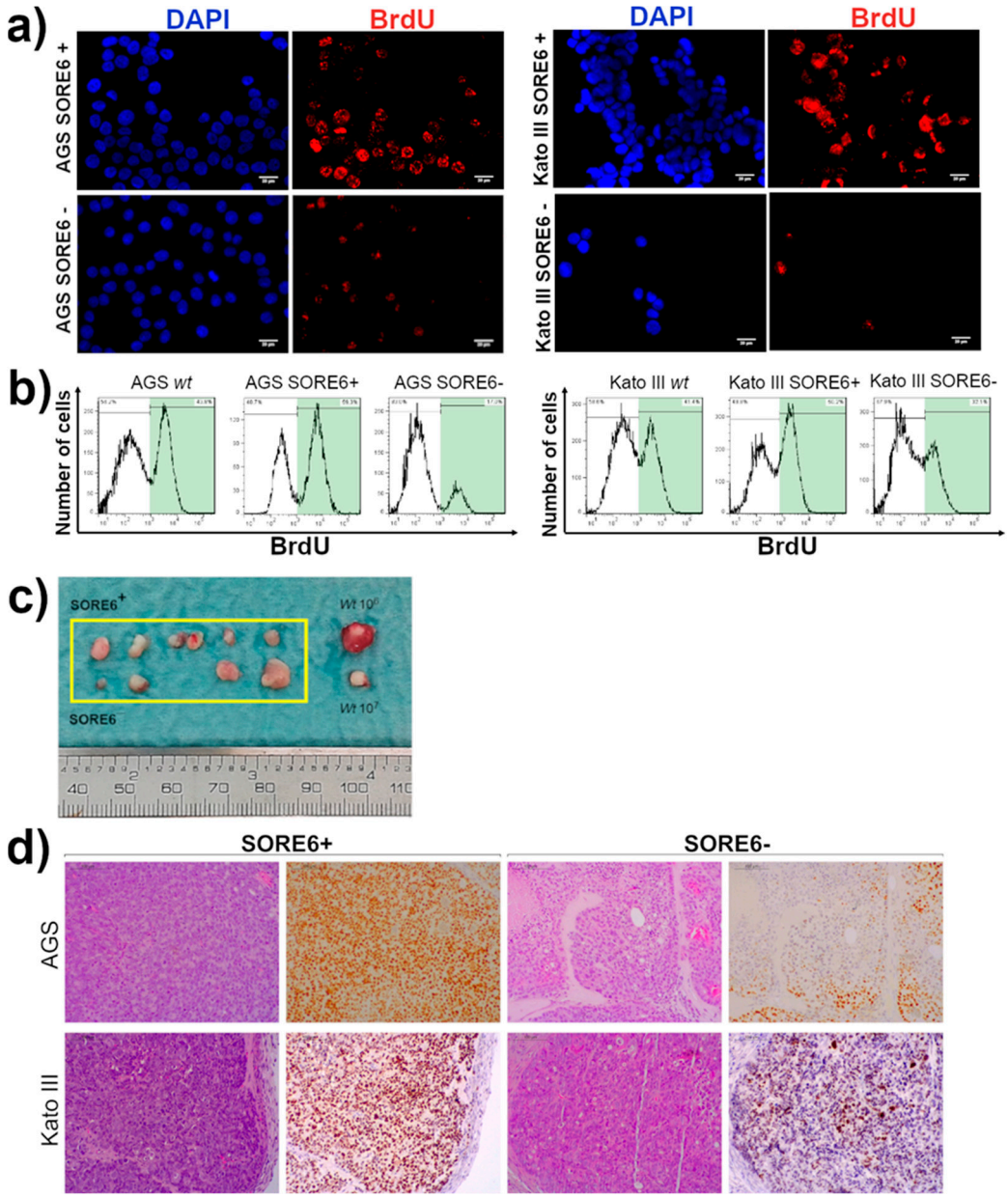


Figure S2. SORE6+ cells have a higher incorporation of BrdU compared to the SORE6− cells and an enhanced ability to form tumors in vivo. **a**) Immunofluorescence channels displaying the DAPI and the BrdU staining in AGS and Kato III SORE6 subpopulations. Scale bar = 100 mm. **b**) FACS plot of AGS wt, SORE6+ and SORE6− cells and Kato III wt, SORE6+ and SORE6− cells showing the proliferative activity of the cells. **c**) Representative picture of the tumors obtained (yellow rectangle) after inoculation of six mice with AGS SORE6+ cells (six tumors; above) or AGS SORE6− cells (four tumors; below). **d**) H&E staining and respective immunohistochemical staining for SOX2 from the tumors originated from SORE6+ and SORE6− cells from both cell lines. All tumors exhibited necrotic regions of variable extension. Magnification, 200 ×, scale bar = 100 mm.

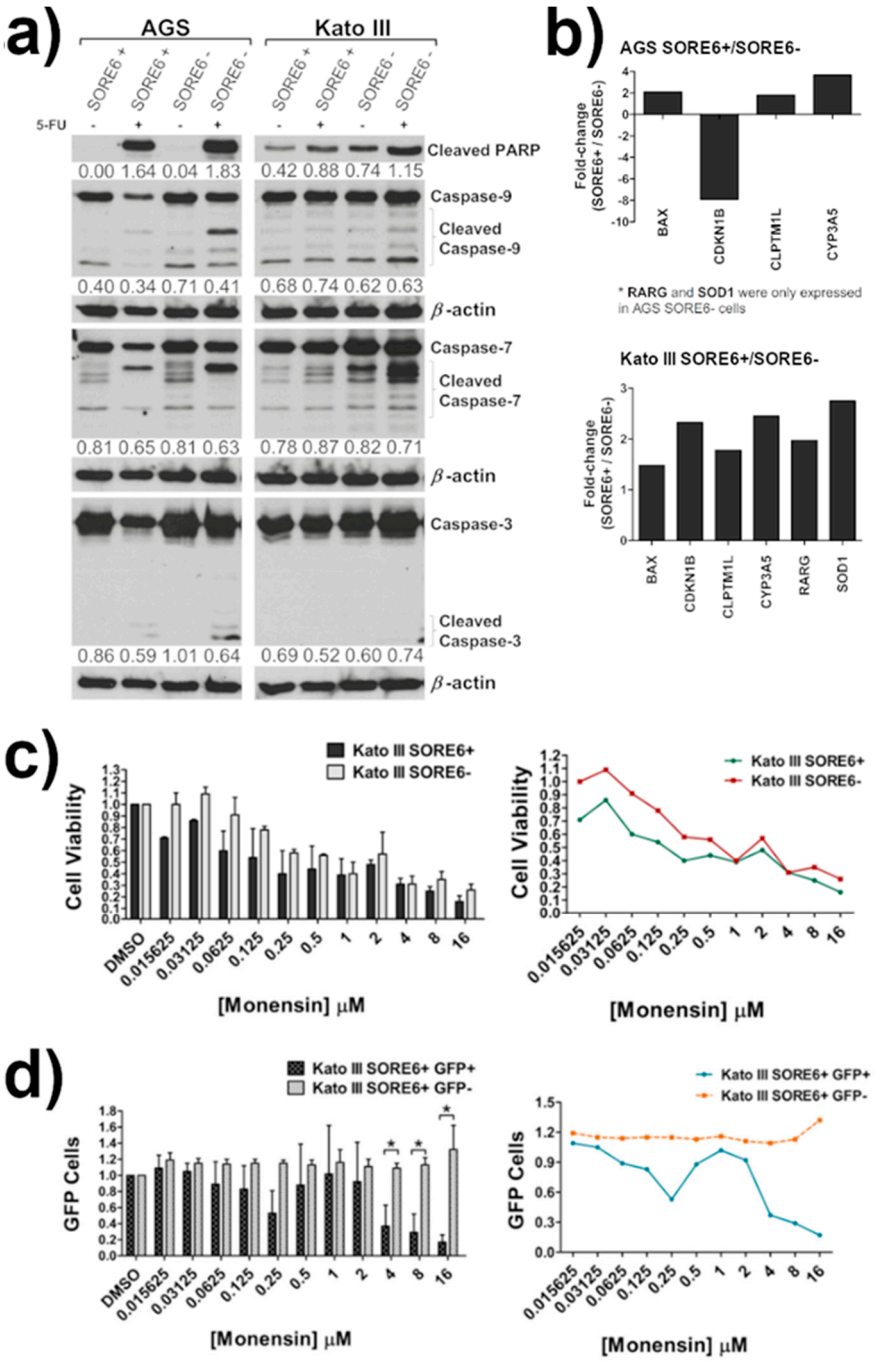
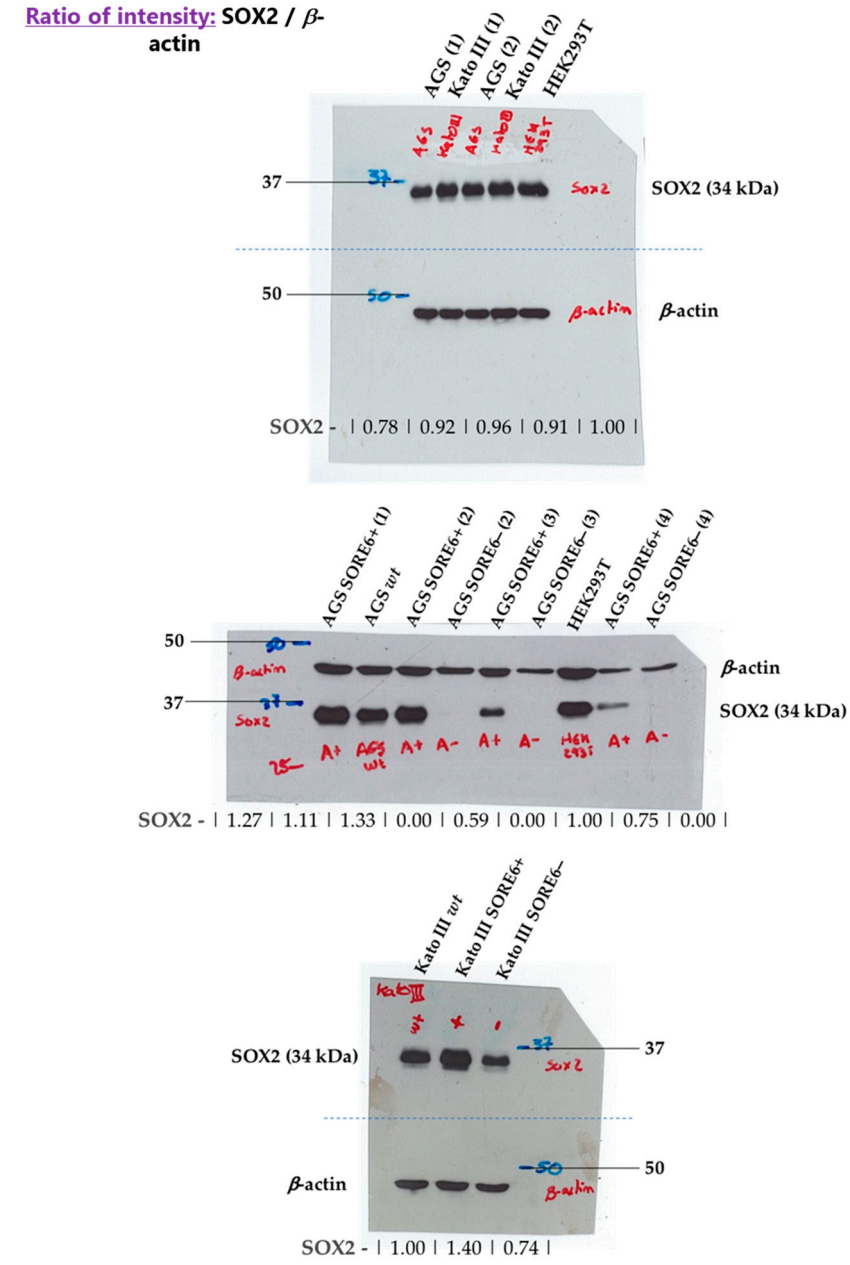


Figure S3. SORE6− cells are more sensitive to chemotherapeutic drugs and Kato III SORE6+ cells are more sensitive to monensin than SORE6− cells. a) Western blot showing cleaved PARP, Caspases-9, −7, −3 and cleaved Caspase-9, −7 and −3 expression in SORE6+ and SORE6− cells from both cell lines after incubation for 48 h with 5-FU. b-actin was used as an internal control. Numbers below every western blot picture represent semi-quantitative analysis of each line (intensity ratio: "gene of interest"/b-actin). Pictures show cropped areas of western blots, the whole images are included in the Figure S4) Validation, through RT-PCR, in AGS and Kato III SORE6 subpopulations of the most relevant genes that showed a significant fold change, up- or down-regulation, ($p \leq 0.05$) in AGS SORE6+ cells compared to AGS SORE6− cells. c) Kato III SORE6+ and SORE6− cells viability results after 48h treatment with monensin, normalized for the DMSO. d) Number of GFP+ and GFP- cells in the Kato III SORE6+ population after 48h treatment with monensin, normalized for the DMSO. Results are mean ± SD of three independent experiments. Significant differences (* $p \leq 0.05$). .

Full unedited gels for Figure 2 a) and d)



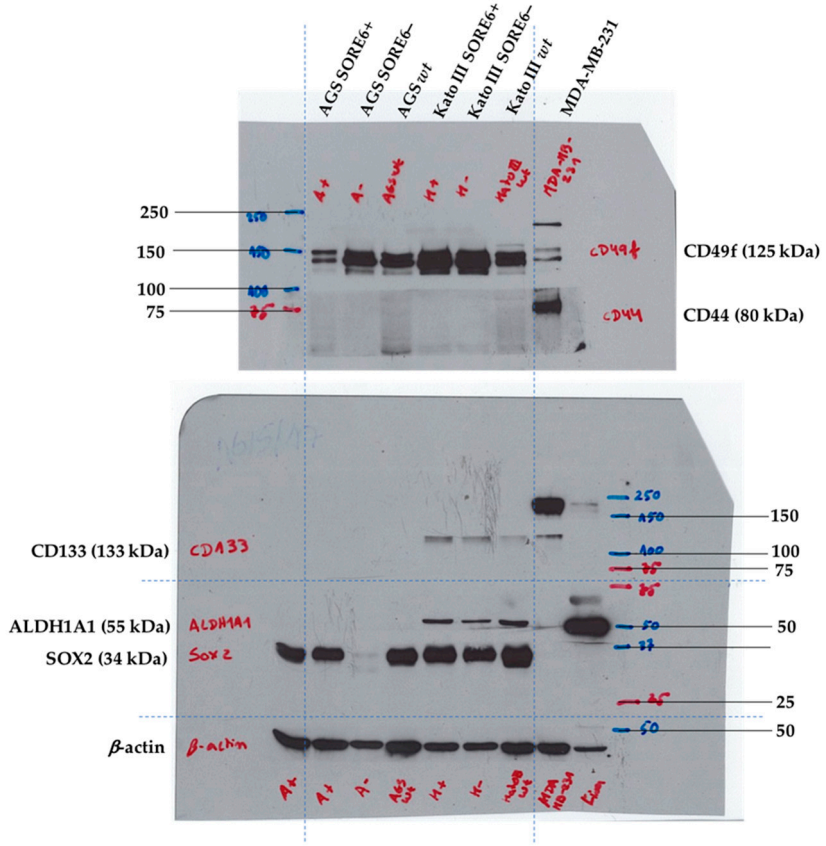
Full unedited gels for Figure 2 e)

Ratio of intensity: CD49f / β-actin

Ratio of intensity: CD44 / β-actin

Ratio of intensity: CD133 / β-actin

Ratio of intensity: ALDH1A1 / β-actin



CD49f - | 01.80 | 5.38 | 3.05 | 6.84 | 7.38 | 3.47 | 1.00 |

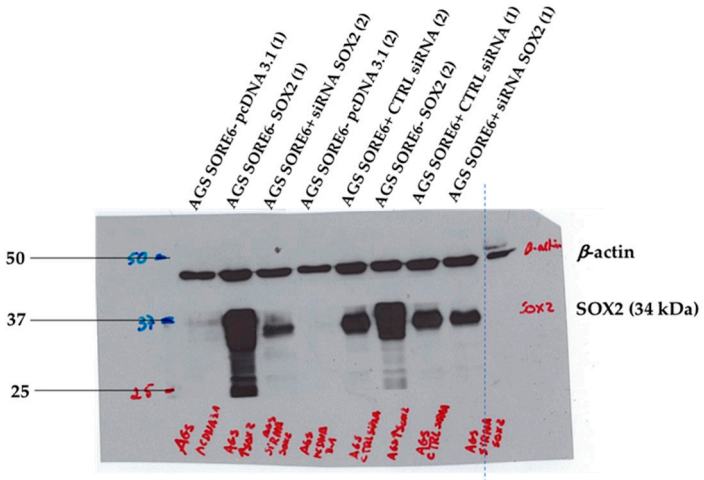
CD44 - | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 |

CD133 - | 0.00 | 0.00 | 0.00 | 1.67 | 1.15 | 0.61 | 1.00 |

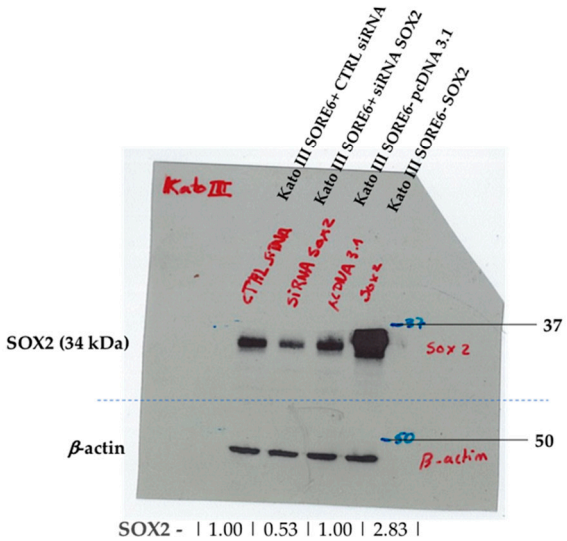
ALDH1A1 - | 0.00 | 0.00 | 0.00 | 1.09 | 0.65 | 1.00 | 0.00 |

Full unedited gels for Figure 5 a) and b)

Ratio of intensity: SOX2 / β-actin



SOX2 - | 1.00 | 76.21 | _ | _ | _ | _ | 1.00 | 0.75 |

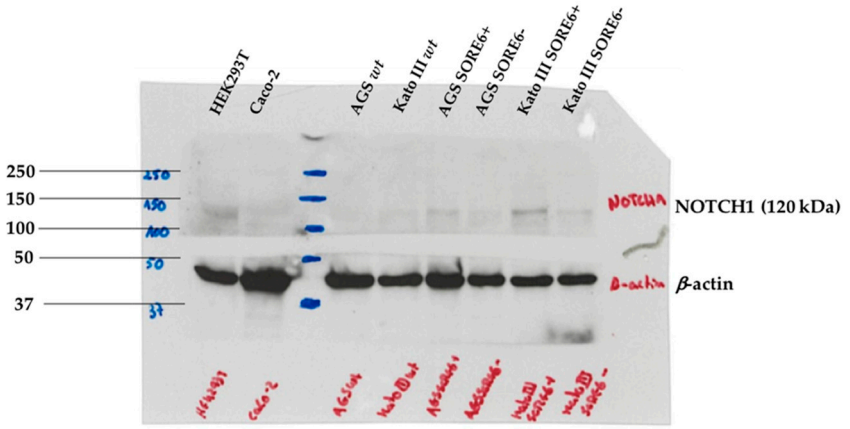


SOX2 - | 1.00 | 0.53 | 1.00 | 2.83 |

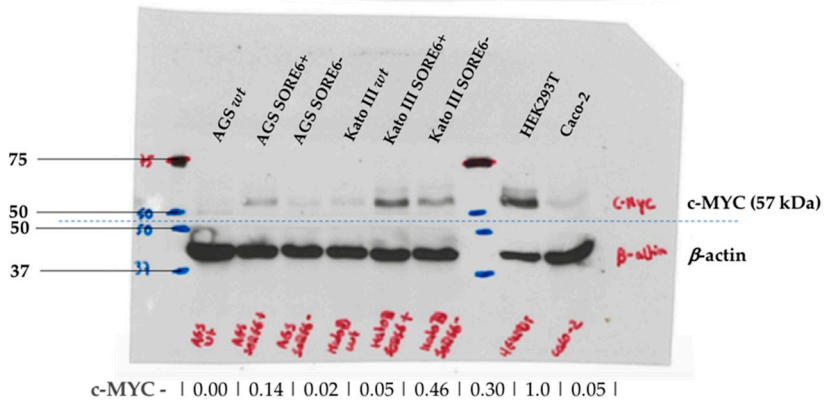
Full unedited gels for Supplementary Figure 1 a)

Ratio of intensity: NOTCH1 / β-actin

Ratio of intensity: c-MYC / β-actin



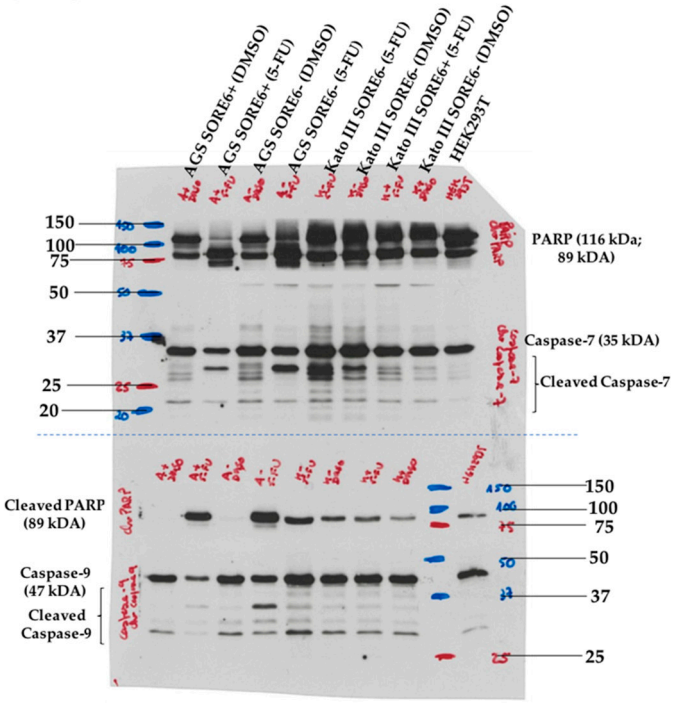
NOTCH1 - | 1.00 | 0.27 | 0.03 | 0.16 | 0.35 | 0.06 | 1.12 | 0.40 |



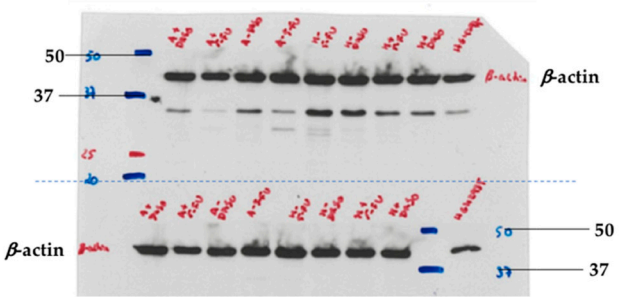
c-MYC - | 0.00 | 0.14 | 0.02 | 0.05 | 0.46 | 0.30 | 1.0 | 0.05 |

Full unedited gels for Supplementary Figure 3 a)

Ratio of intensity: Caspase-7 / β-actin
Ratio of intensity: Cleaved PARP / β-actin
Ratio of intensity: Caspase-9 / β-actin



Caspase-7 -	0.81	0.65	0.81	0.63	0.78	0.87	0.82	0.71	1.00
Cleaved PARP -	0.00	1.64	0.04	1.83	0.42	0.88	0.74	1.15	1.00
Caspase-9 -	0.40	0.34	0.71	0.41	0.68	0.74	0.62	0.63	1.00



Full unedited gels for Supplementary Figure 3 a)

Ratio of intensity: Caspase-3 / β-actin

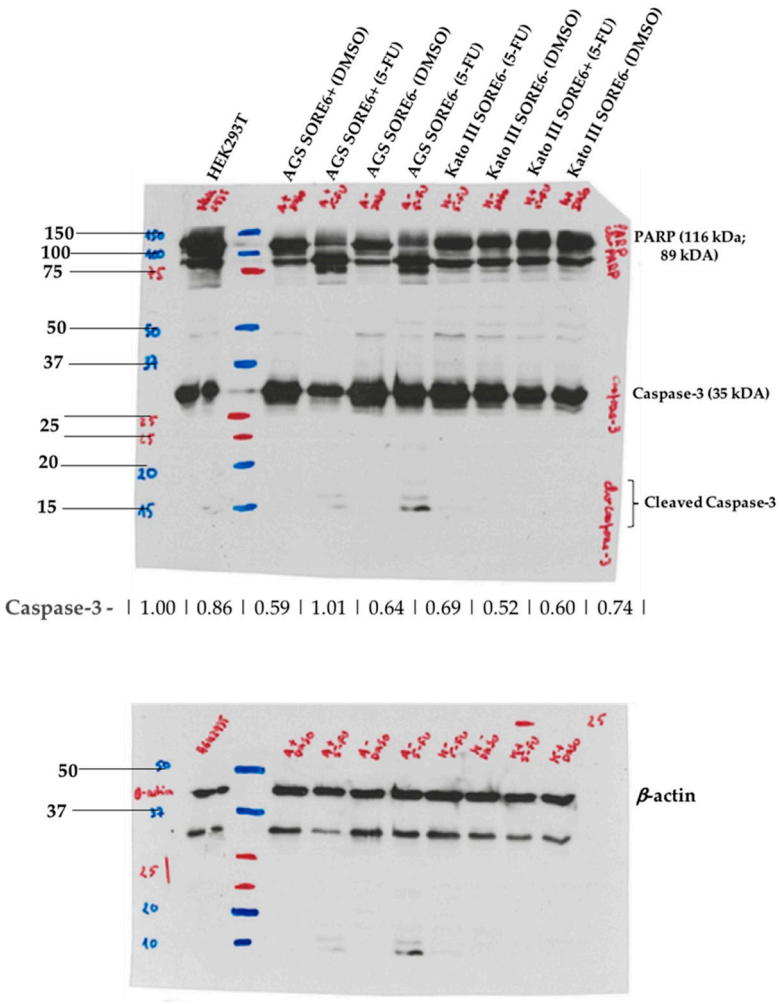


Figure S4. Uncropped western blots images corresponding to all the figures in the manuscript.