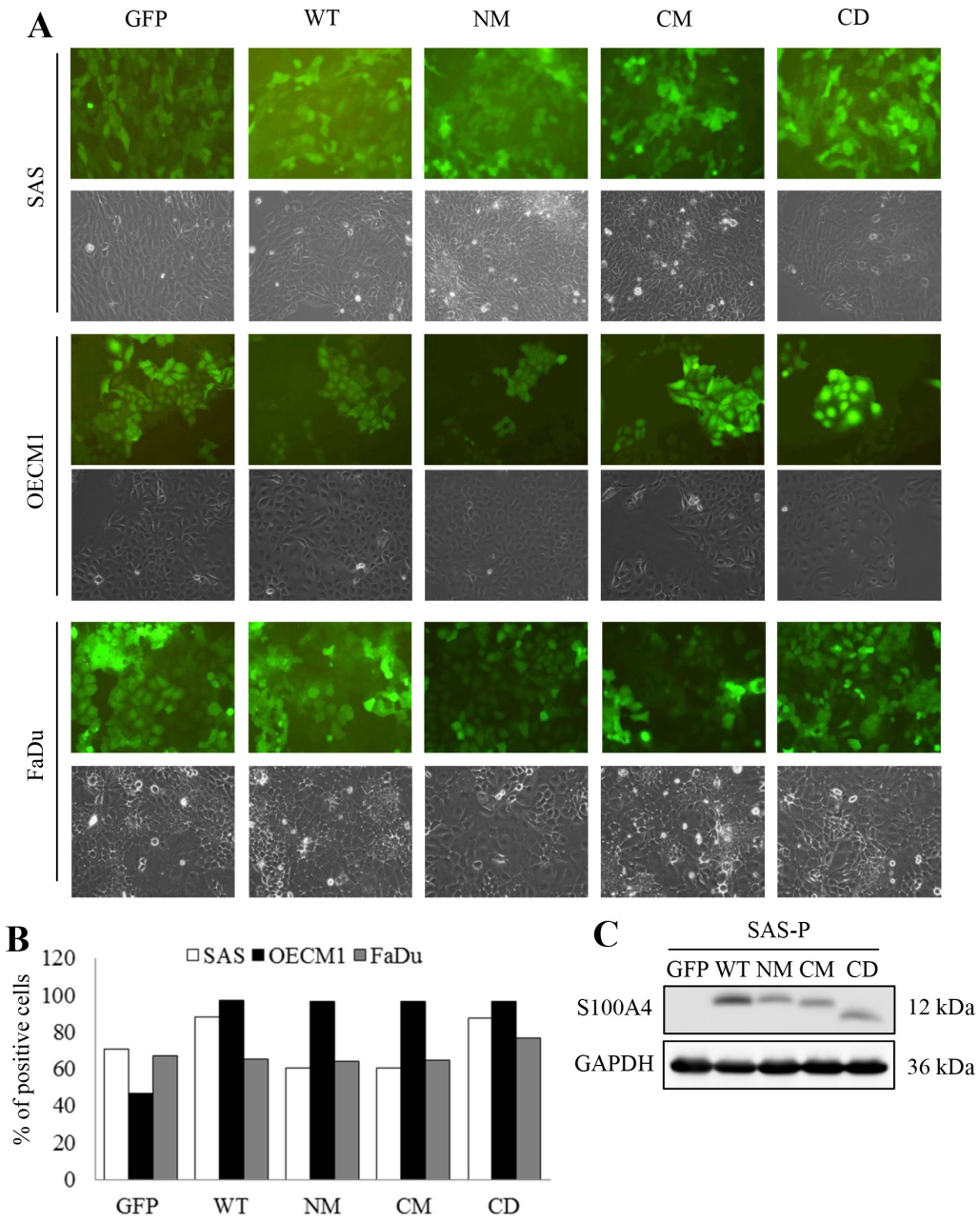
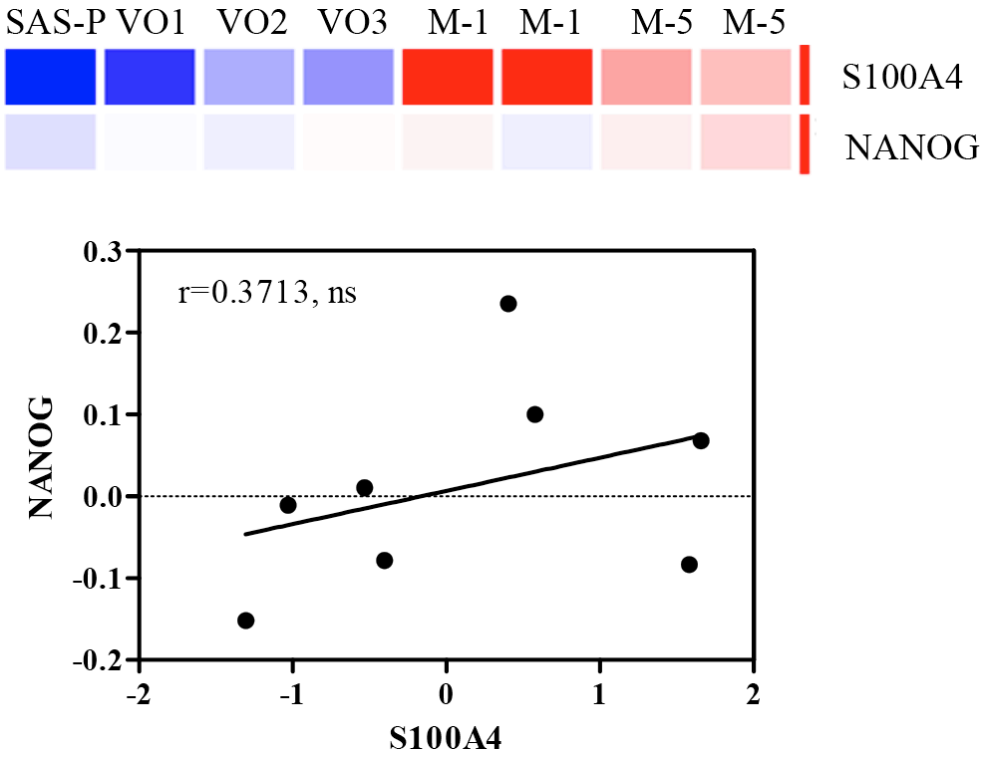
Attenuation of cancer-initiating cells stemness properties by abrogating S100A4 calcium binding ability in head and neck cancers

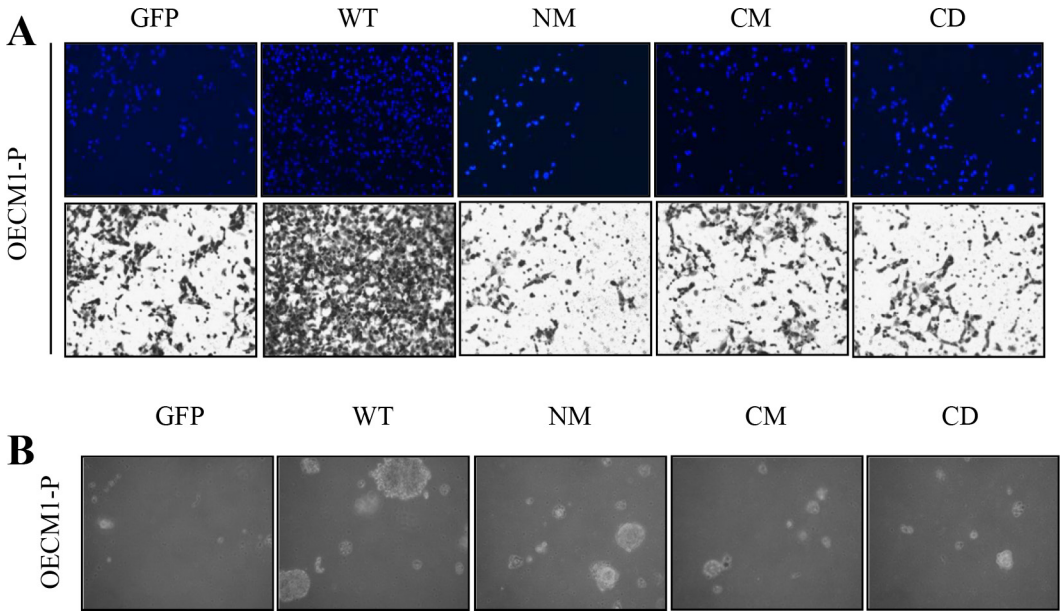
Supplementary Materials



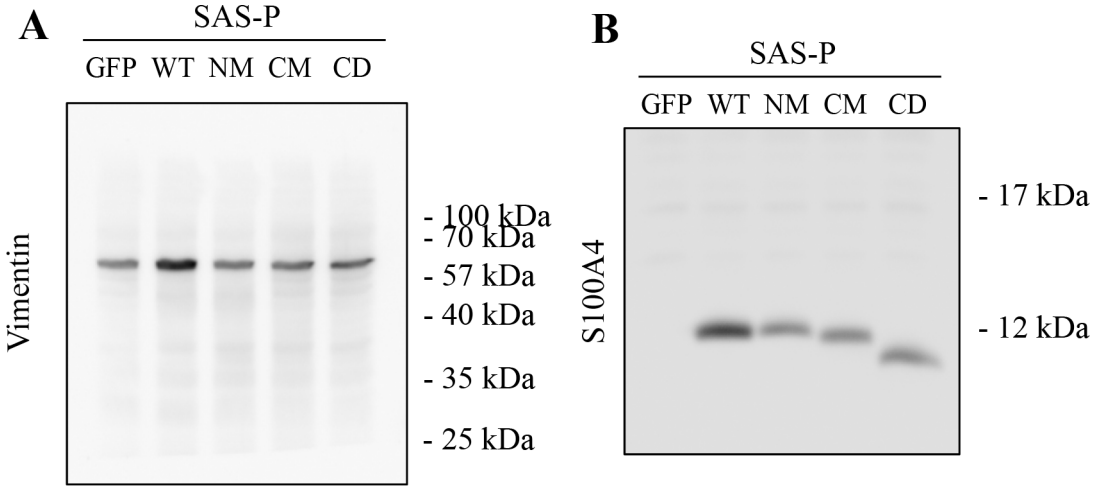
Supplementary Figure S1: Establishment of wild-type and mutants S100A4 overexpressing HNSCCs. (**A**) HNSCC cells, SAS, OECM1 and FaDu were infected with lentivirus co-expressing GFP and S100A4. (**B**) The percentage of GFP positive HNSCC cells after virus transduction. (**C**) Protein level of S100A4 and GAPDH in SAS-parental (P) was determined by immunoblot analyses.



Supplementary Figure S2: The gene correlation of S100A4 and Nanog of SAS derived *in vivo* cell lines. The heat maps of the S100A4 and Nanog of SAS derived in vivo cell lines. Red and blue determine as high and low expression levels, respectively (top panel). Expression correlation analysis of S100A4 (X-axis) and Nanog (Y-axis) (bottom panel).



Supplementary Figure S3: The mutants S100A4 reduce the tumorigenic capacity of OECM1 cells. (**A**) The migration ability of OECM1-P cell lines was examined as described in materials and methods. (**B**) Anchorage-independent growth of S100A4-overexpressing OECM1-P cell lines was analyzed.



Supplementary Figure S4: The specificity data of antibodies. The immunoblotting analyses of Vimentin (**A**) and S100A4 (**B**) were showed. The proper molecular markers were labeled.

A

Primary antibodies	Catalog number	Commercial company	Reference
Actin	MA5-15739	ThermoFisher	[1, 2]
CK18	#4548	Cell Signaling Technology	[3, 4]
E-cadherin	610182	BD Biosciences	[5-7]
GAPDH	MAB374	EMD Millipore	[8, 9]
Glut3	ab15311	abcam	[10-12]
GRP78	610978	BD Biosciences	[13-15]
Nanog	AB9220	EMD Millipore	[16-18]
p-p53 ser15	#9284	Cell Signaling Technology	[19, 20]
p53	#9282	Cell Signaling Technology	[21, 22]
S100A4	A5114	Dako	[23, 24]
Vimentin	550513	BD Biosciences	[23, 25]

B Reference

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Supplementary Figure S5: The antibodies list. The list of the primary antibodies used in the study (**A**) and references of primary antibodies (**B**) were showed.