

Tryptic digestion of Sox2 protein would yield 31 different peptides, out of which, the highlighted peptide has Ser246 (determined using iPEP tool; <http://ipep.moffitt.org/>).

Digestion:Trypsin

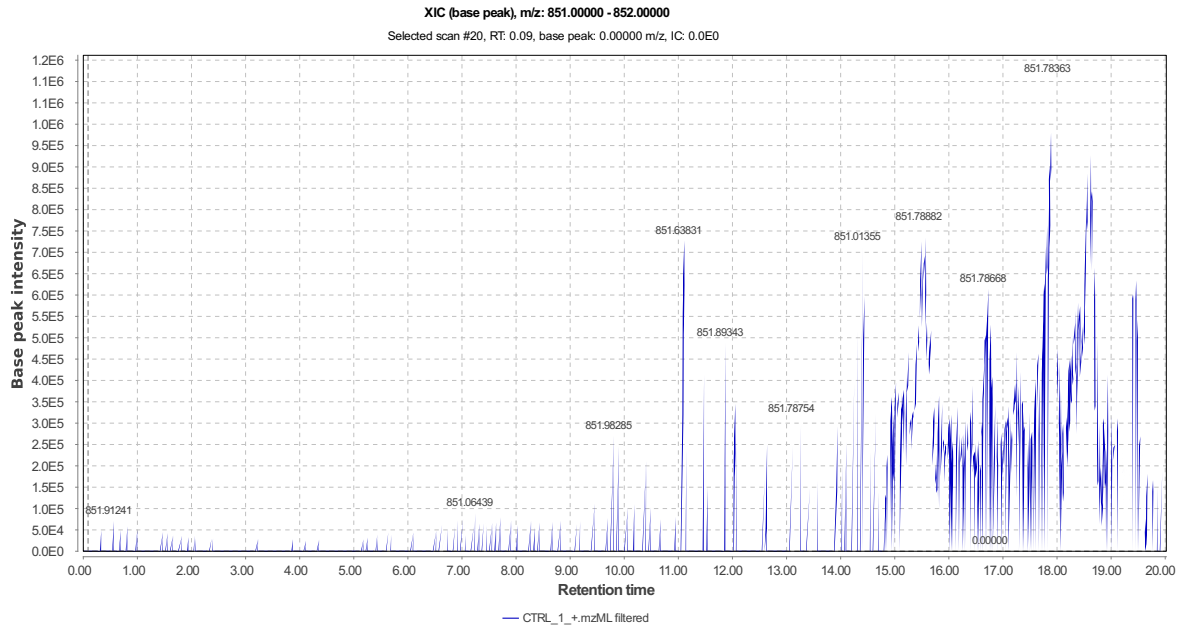
Start	Stop	Molecular Weight	Peptide Sequence
1	35	3436.5446	--.MYNMMETELKPPGPQQTSGGGGGNSTAAAAGGNQK.NS
36	40	587.2663	QK.NSPDR.VK
41	42	245.1739	DR.VK.RP
43	53	1393.6747	VK.RPMNAFMVWSR.GQ
54	56	359.1917	SR.GQR.RK
57	57	174.1117	QR.R.KM
58	58	146.1055	RR.K.MA
59	65	816.38	RK.MAQENPK.MH
66	73	944.4386	PK.MHNSEISK.RL
74	74	174.1117	SK.R.LG
75	80	702.3701	KR.LGAEWK.LL
81	87	818.4385	WK.LLSETEK.RP
88	95	974.5185	EK.RPFIDEAK.RL
96	96	174.1117	AK.R.LR
97	98	287.1957	KR.LR.AL
99	103	598.3261	LR.ALHMK.EH
104	109	787.3501	MK.EHPDYK.YR
110	113	590.3289	YK.YRPR.RK
114	114	174.1117	PR.R.KT
115	115	146.1055	RR.K.TK
116	117	247.1532	RK.TK.TL
118	121	491.2778	TK.TLMK.KD
122	122	146.1055	MK.K.DK
123	124	261.1325	KK.DK.YT
125	156	2897.4817	DK.YTLPGGLLAPGGNSMASGVGVGAGLGAGVNQR.MD
157	199	4831.0454	QR.MDSYAHMNGWSNGSYSMMQDQLGYPQHPLNAHGAAQMOPMHR.YD
200	245	4891.1432	HR.YDVSALQYNSMTSSQTYMNGSPTYSMSYSQQGTPGMALGSMGSSVK.SE
246	262	1700.7966	VK.SEASSSPPVVTSSSHSR.AP
263	271	929.4389	SR.APCQAGDLR.DM
272	291	2130.0125	LR.DMISMYLPGAEVPEPAAPSR.LH
292	317	2772.3476	SR.LHMSQHYQSGPVPGTAINGTLPISHM.--

Using Human Proteome Map (<http://www.humanproteomemap.org/>), we next determined that the unmodified peptide would carry a charge of 2 and a predicted m/z of 851.41.

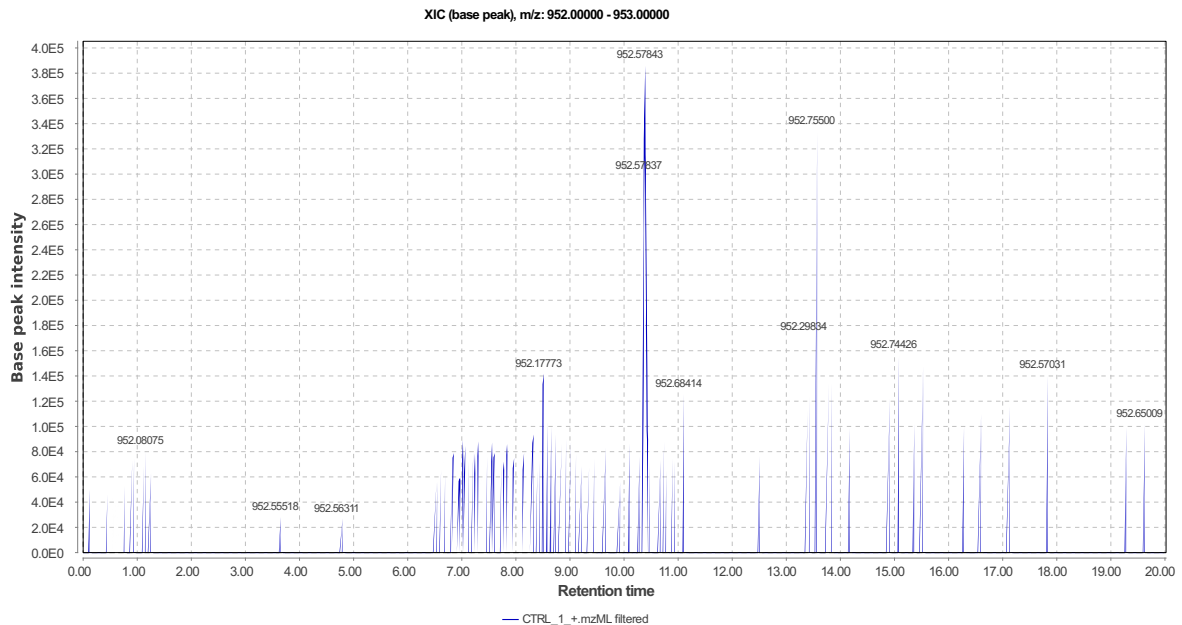
Peptide sequence	Modifications	m/z	Charge
SEASSSPPVVTSSSHSR		851.41	2
sSSHSR	N-Term(Acetyl)	351.66	2

Next, we used the NIST glycol mass calculator (<https://www.nist.gov/mml/biomolecular-measurement/mass-spectrometry-data-center/nist-glyco-mass-calculator>) to determine the

predicted m/z of the peptide, if it is indeed O-GlcNAc modified at Ser246. The predicted m/z value was **952.9**



Finally, we immunoprecipitated Sox2 as described in the manuscript, SDS-PAGE purified the band and analyzed it with LC-MS (MS1) after tryptic digestion. As seen in the traces below, we see representative peptide(s) at m/z between 851-852, as well as between 952-953. Please note that the traces presented here are only 1m/z wide and based upon the predicted m/z value of the unmodified or modified peptide



respectively. Based on this, we can conclude that both Ser246 glycosylated and deglycosylated Sox2 is present in the cells.

Gene ID	Transcript ID	Total counts	Q-value (OGTi vs. Sc-OGT)	P-value (OGTi vs. Sc-OGT)
AKAP13	AKAP13-001	2.57E+01	9.19E-01	3.96E-02
ARPC2	ARPC2-016	3.68E+01	9.19E-01	4.82E-02
CARS	CARS-004	1.58E+01	9.19E-01	4.11E-02
CDC20P1	CDC20P1-201	4.43E+01	9.19E-01	3.53E-02
CFL2	CFL2-004	4.22E+01	9.19E-01	1.67E-02
CREB1	CREB1-011	3.09E+01	9.19E-01	4.74E-02
CTC-575D19.1	CTC-575D19.1-001	1.02E+01	9.19E-01	3.86E-02
DHX36	DHX36-201	8.18E+01	9.19E-01	3.20E-02
EZH2	EZH2-010	5.85E+01	9.19E-01	2.88E-02
FAM179B	FAM179B-002	7.54E+01	9.19E-01	2.25E-02
FAM49B	FAM49B-010	9.99E+01	9.19E-01	3.22E-02
HNRNPL	HNRNPL-009	1.38E+02	9.19E-01	4.19E-02
IGF2BP2	IGF2BP2-008	6.82E+01	9.19E-01	2.64E-02
ITSN2	ITSN2-003	1.67E+01	9.19E-01	1.23E-02
KDM3A	KDM3A-202	1.04E+02	9.19E-01	3.20E-02
KDM7A	KDM7A-002	3.23E+01	9.19E-01	4.41E-02
LAMC2	LAMC2-003	2.53E+02	9.19E-01	4.70E-02
LMO4	LMO4-002	3.83E+02	9.19E-01	2.46E-02
LMO4	LMO4-004	4.66E+01	9.19E-01	4.47E-02
MMS19	MMS19-007	2.07E+01	9.19E-01	2.09E-02
MTDH	MTDH-003	3.19E+01	9.19E-01	3.06E-02
MYOF	MYOF-004	6.49E+02	9.19E-01	3.95E-02
NMT2	NMT2-002	7.64E+01	9.19E-01	2.55E-02
NRP2	NRP2-005	3.33E+01	9.19E-01	4.29E-02
NUP98	NUP98-002	8.96E+00	9.19E-01	2.42E-02
NVL	NVL-018	3.01E+01	9.19E-01	2.58E-02
OTUB1	OTUB1-003	3.39E+01	9.19E-01	4.59E-02
PBRM1	PBRM1-008	1.33E+02	9.19E-01	2.89E-02
PCBP2	PCBP2-011	3.45E+01	9.19E-01	7.40E-03
PLK2	PLK2-009	3.44E+01	9.19E-01	4.30E-02
PTMAP5	PTMAP5-001	2.54E+02	9.19E-01	4.26E-02
PTP4A2	PTP4A2-009	4.66E+01	9.19E-01	3.56E-03
RDX	RDX-201	5.11E+01	9.19E-01	2.60E-02
RPGRIP1L	RPGRIP1L-005	1.29E+01	9.19E-01	4.15E-02
SGK1	SGK1-019	4.94E+01	9.19E-01	2.25E-02
SMOX	SMOX-001	1.48E+01	9.19E-01	3.18E-02
SPRY2	SPRY2-001	2.54E+02	9.19E-01	2.25E-02
ST7	ST7-007	1.61E+01	9.19E-01	1.68E-02
TMEM123	TMEM123-006	3.87E+01	9.19E-01	4.76E-02
WDR20	WDR20-204	2.40E+01	9.19E-01	2.99E-02
WNK1	WNK1-003	2.95E+01	9.19E-01	4.67E-02
ZEB1	ZEB1-018	9.30E+01	9.19E-01	4.85E-02
ZFAND6	ZFAND6-004	4.46E+01	9.19E-01	2.14E-02

*target genes of the **SOX2** transcription factor in low- or high-throughput transcr*
http://amp.pharm.mssm.edu/Harmonizome/gene_set/SOX2/CHEA+Transcription+Factor+Targets

FDR step up (OGTi vs. Sc-OGT)

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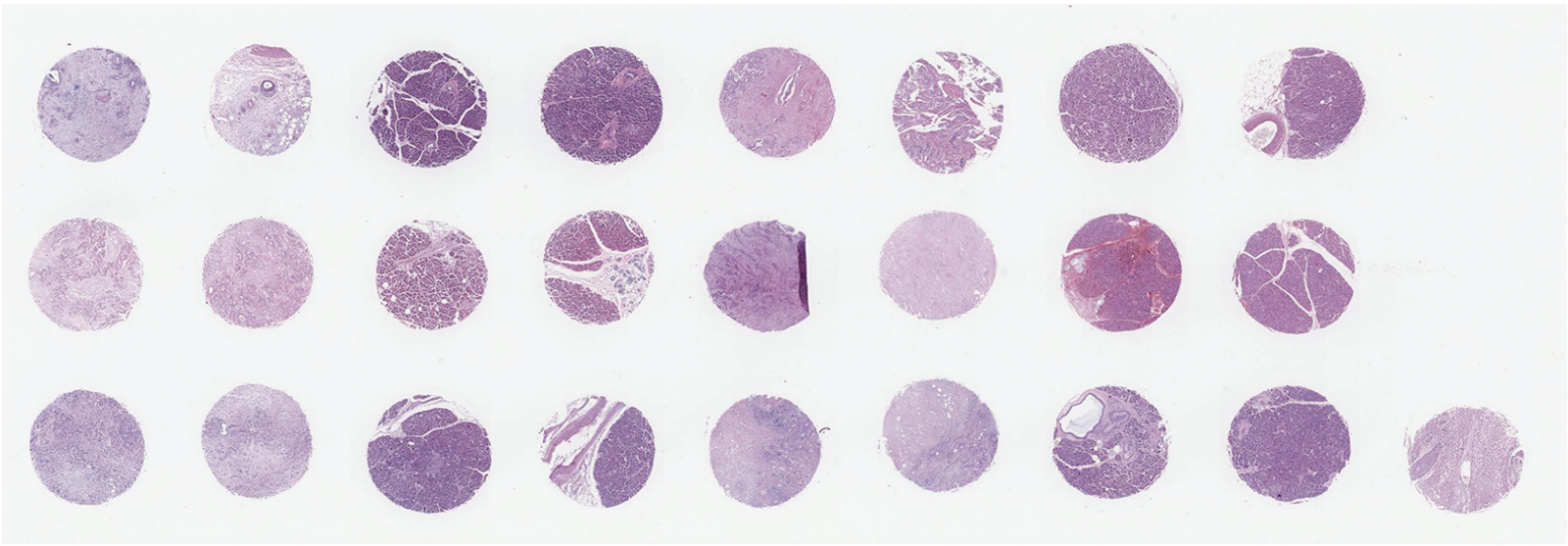
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ption factor functional studies from the CHEA Transcription Factor 1

targets dataset.

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PTP4A2	-2.75E-02
PCBP2	-7.53E-03
ITSN2	-3.37E-02
CFL2	-5.44E-03
ST7	-2.44E+01
MMS19	-1.12E+01
ZFAND6	-5.96E+00
FAM179B	-2.11E-01
SPRY2	-1.57E-01
SGK1	-8.29E-02
NUP98	-5.18E+01
LMO4	-3.40E-01
NMT2	-2.43E-01
NVL	-3.38E-02
RDX	-1.14E-01
IGF2BP2	-8.03E-02
EZH2	-1.18E-01
PBRM1	-1.01E-01
WDR20	-3.48E+01
MTDH	-5.19E-02
SMOX	-4.14E-02
DHX36	-3.99E+00
KDM3A	-3.91E-02
FAM49B	-5.81E+00
CDC20P1	-4.64E-02
CTC-575D19.1	-4.22E-02
MYOF	-2.62E+00
AKAP13	-7.09E+00
CARS	-1.16E-01
RPGRIP1L	-7.47E-02
HNRNPL	-3.71E+00
PTMAP5	-1.29E-01
NRP2	-5.63E+00
PLK2	-1.41E-01
KDM7A	-7.45E+00
LMO4	-2.28E-01
OTUB1	-6.11E-02
WNK1	-4.95E-02
LAMC2	-1.78E+01
CREB1	-2.56E-02
TMEM123	-1.19E-01
ARPC2	-2.49E+01
ZEB1	-2.63E-01

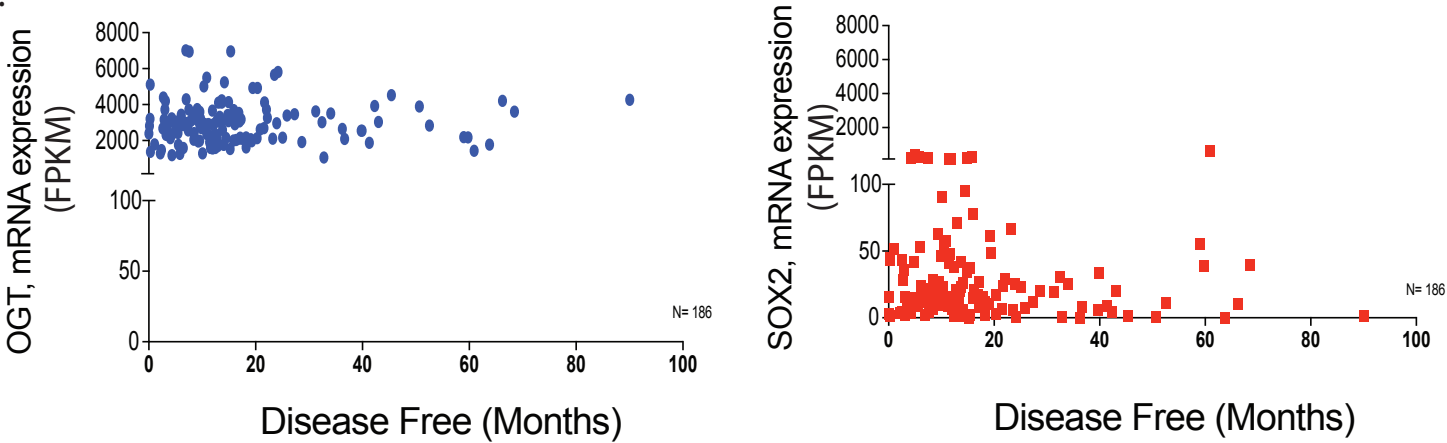
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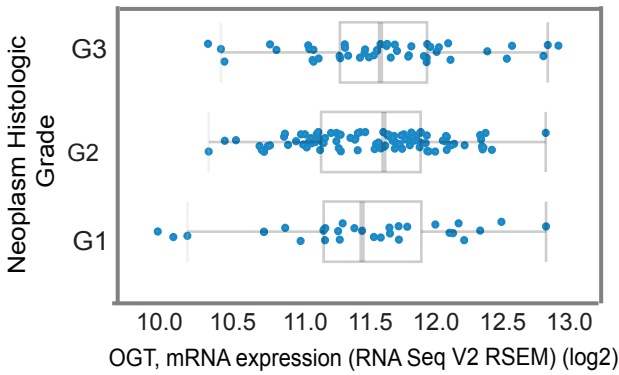
B.

A1	1	62	F	Pancreas	Duct adenocarcinoma	T2N1M0	2	IIB	Malignant		
A2	2	62	F	Pancreas	Duct adenocarcinoma	T2N1M0	2	IIB	Malignant		
A3	3	63	M	Pancreas	Duct adenocarcinoma	T2N0M0	2	IB	Malignant		
A4	4	63	M	Pancreas	Duct adenocarcinoma	T2N0M0	2	IB	Malignant		
A5	5	68	F	Pancreas	Duct adenocarcinoma	T2N0M0	2	IB	Malignant		
A6	6	68	F	Pancreas	Duct adenocarcinoma	T2N0M0	2	IB	Malignant		
B1	7	70	M	Pancreas	Duct adenocarcinoma	T2N0M0	2	IB	Malignant		
B2	8	70	M	Pancreas	Duct adenocarcinoma	T2N0M0	2	IB	Malignant		
B3	9	67	M	Pancreas	Duct adenocarcinoma	T2N0M0	2	IB	Malignant		
B4	10	67	M	Pancreas	Duct adenocarcinoma	T2N0M0	2	IB	Malignant		
B5	11	64	M	Pancreas	Adenocarcinoma	T3N0M0	3	IIA	Malignant		
B6	12	64	M	Pancreas	Adenocarcinoma	T3N0M0	3	IIA	Malignant		
C1	13	45	M	Pancreas	Adenocarcinoma	T2N1M0	3	IIB	Malignant		
C2	14	45	M	Pancreas	Adenocarcinoma	T2N1M0	3	IIB	Malignant		
C3	15	55	M	Pancreas	Adenocarcinoma	T2N0M0	3	IB	Malignant		
C4	16	55	M	Pancreas	Adenocarcinoma	T2N0M0	3	IB	Malignant		
C5	17	62	F	Pancreas	Adenocarcinoma	T3N0M0	3	IIA	Malignant		
C6	18	62	F	Pancreas	Adenocarcinoma	T3N0M0	3	IIA	Malignant		
D1	19	51	F	Pancreas	Mucinous adenocarcinoma	T2N0M0	3	IB	Malignant		
D2	20	51	F	Pancreas	Mucinous adenocarcinoma	T2N0M0	3	IB	Malignant		
D3	21	60	F	Pancreas	Adjacent normal pancreas tissue	-	-	-	NAT		
D4	22	60	F	Pancreas	Adjacent normal pancreas tissue	-	-	-	NAT		
D5	23	69	F	Pancreas	Adjacent normal pancreas tissue	-	-	-	NAT		
D6	24	69	F	Pancreas	Adjacent normal pancreas tissue	-	-	-	NAT		

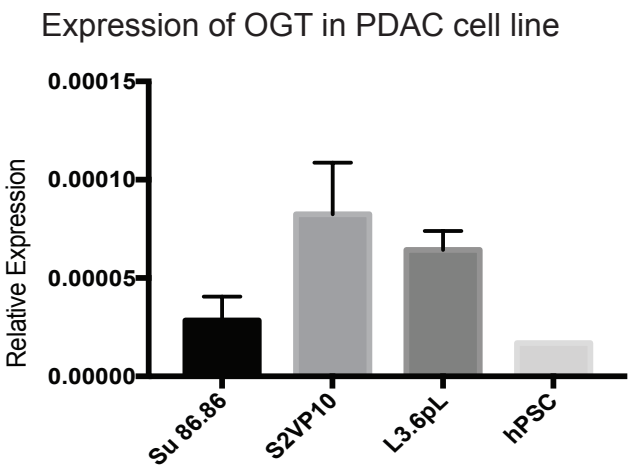
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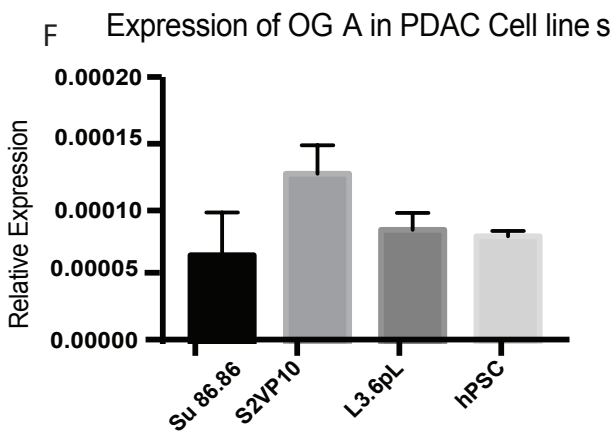
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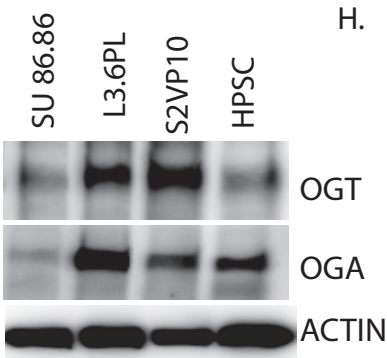
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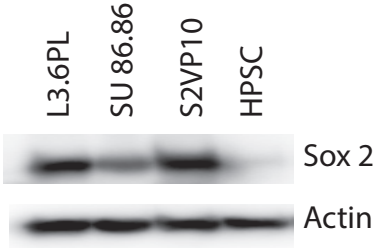
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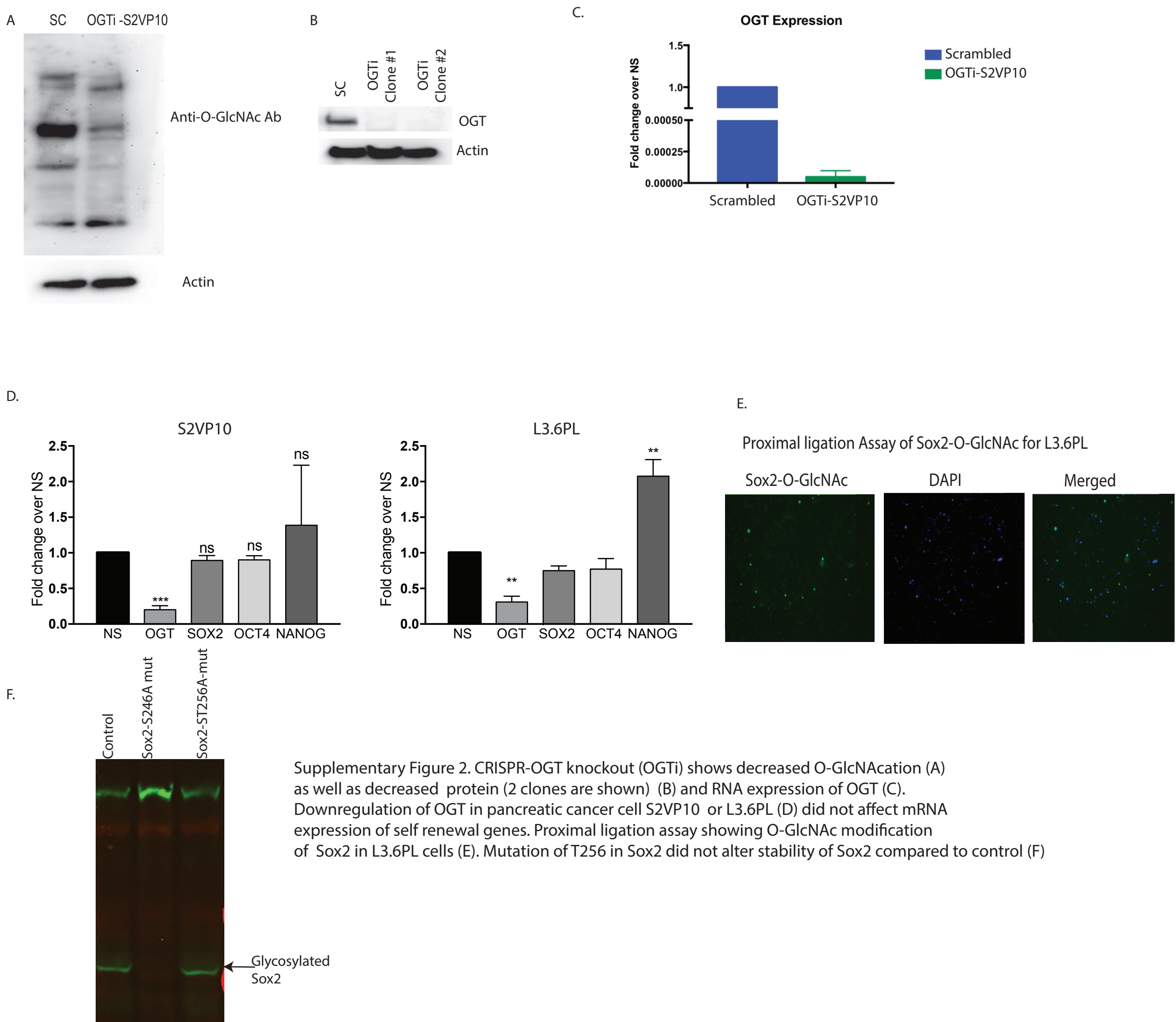
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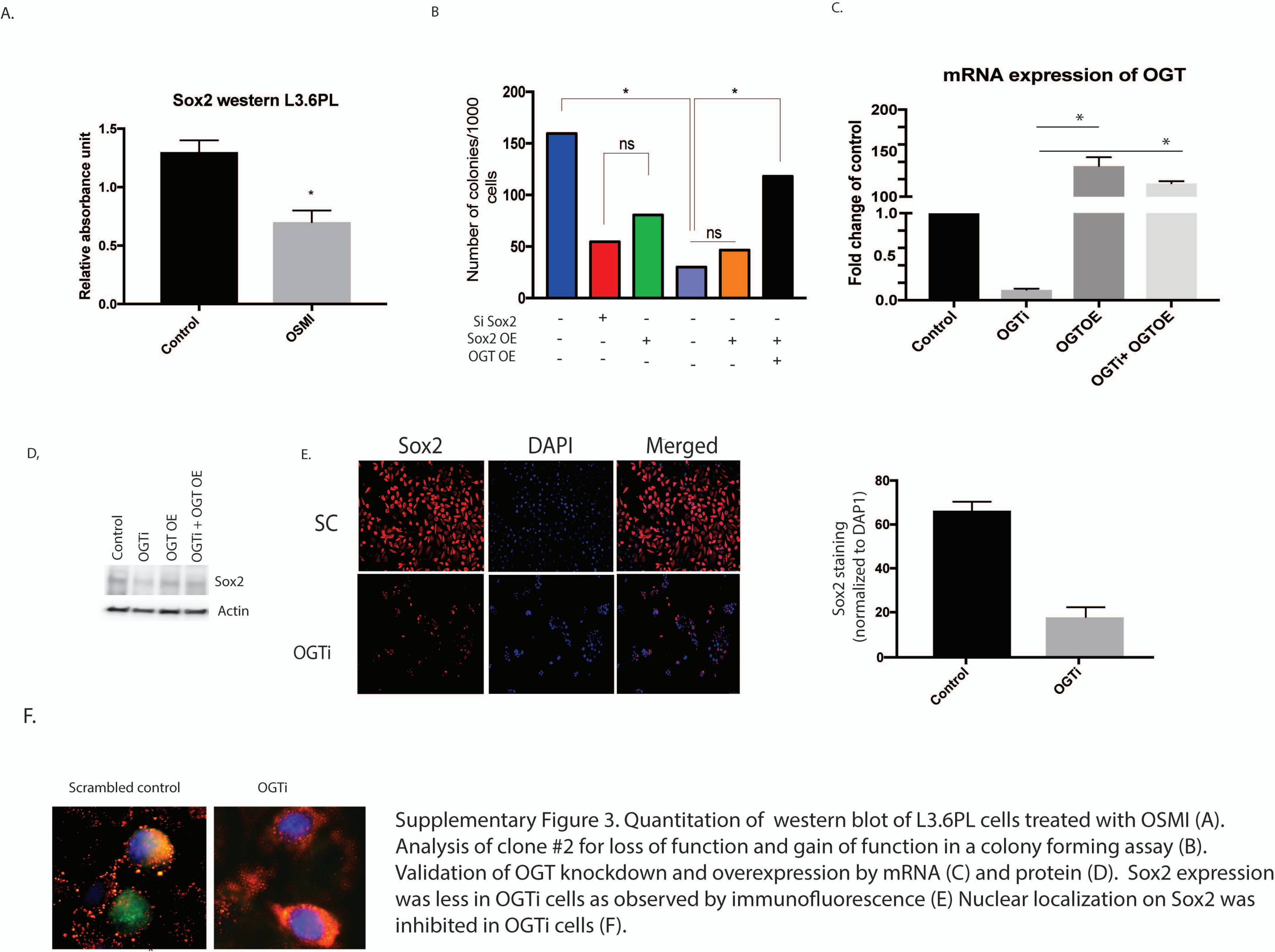


H.

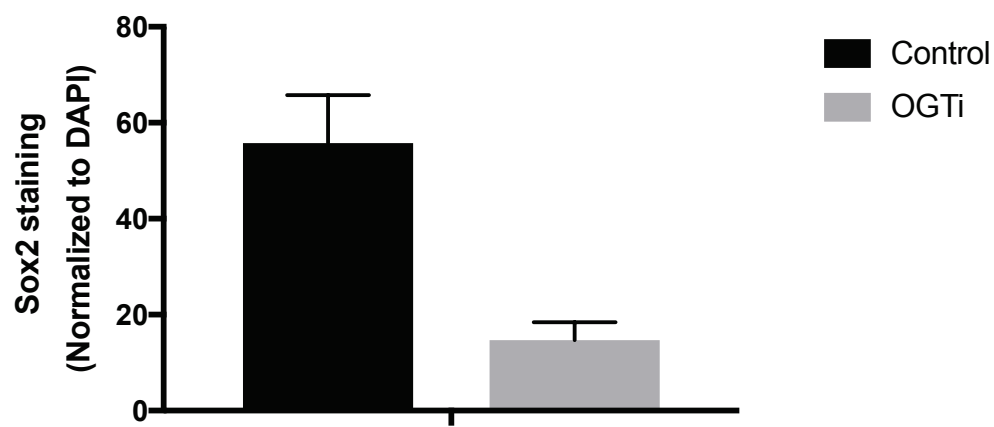


Supplementary Figure 1. Images of tumor tissue microarray (A) with TNM staging (B). Analysis of samples in TCGA at www.cbioportal.org showed that high OGT and Sox2 expression correlated with less disease free survival (C). Further, histologic grade showed increased OGT expression correlated with advanced histologic grade (D) RNA expression of OGT (E), OGA(F) in pancreatic cancer cell lines; Protein expression of OGT and OGA in pancreatic cancer cell lines (G). Sox 2 expression in the different cell lines correlated with the OGT expression in the cell lines (H).

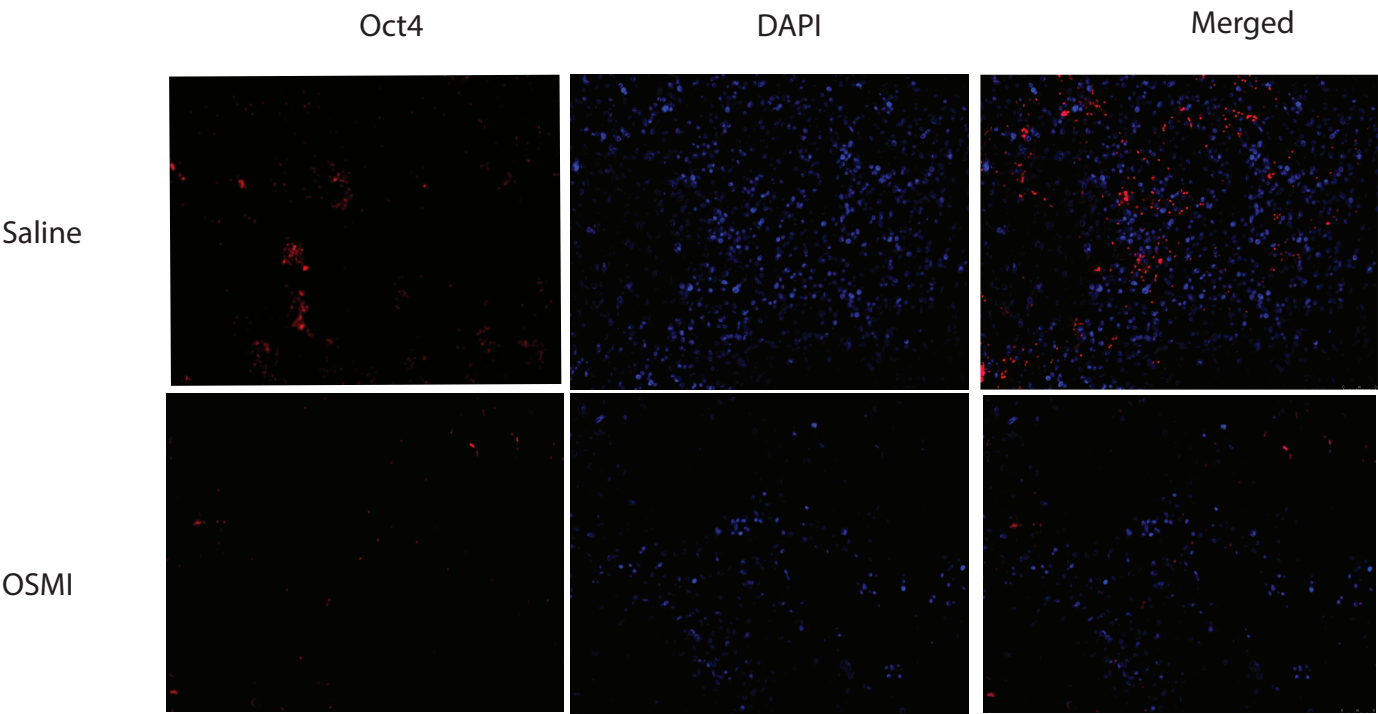




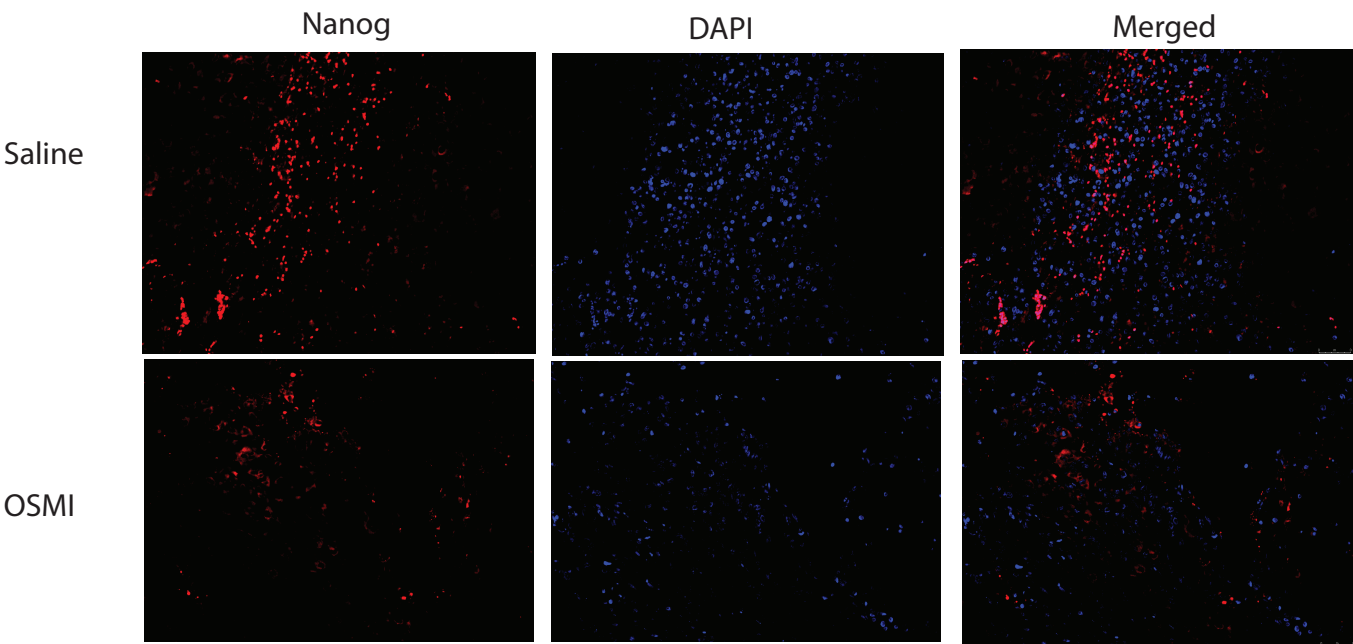
A.



B.



C.



Supplementary Figure 4. Staining of Sox2 in tumors quantitated and normalized to DAPI (A) Treatment with OSMI decreases OCT4 expression (B) and NANOG expression (C) in the tumors compared to control.