## **Supplementary Material**

## LncRNA-SLC16A1-AS1 induces metabolic reprogramming during bladder

## cancer progression as target and co-activator of E2F1

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## <u>Tables</u>

Table S1: Primers used for PCR and mutagenesis

primer name	sequence (5´→3´)
Actin_FWD	CGGGAAATCGTGCGTGACATTA
Actin_RVS	ACCGCTCATTGCCAATGGTGAT
ASNS_FWD	CGACCAAAAGAAGCCTTCAG
ASNS_RVS	CCACTTGGGCATCCAGTAAT
GAPDH_FWD	ATCGTGGAAGGACTCATGACCACA
GAPDH_RVS	AAGGCCATGCCAGTGAGCTTC
GFPT1_FWD	CAAAGGCCTTCAGAGACTGG
GFPT1_RVS	AGGACTGGGTTCTCCATGTG
E2F1_FWD	GCTGGACCACCTGATGAA
E2F1_REV	GGAGGGGCTTTGATCACC
KYNU_FWD	CAAGAGAGGGGGAAGAAACC
KYNU_RVS	TAACAACCCTTCGCTTGTCC
MTHFD2_FWD	TTGACCTCCTGACCTCTGCT
MTHFD2_RVS	TTCCAAGGGATACTGCCAAG
ODC1_FWD	CAAAGTTGGTTTTGCGGATT
ODC1_RVS	CGAAGGTCTCAGGATCGGTA
PHKB_FWD	ACGTGAGCATGAGGTTTTCC
PHKB_RVS	TGTCTGGCCTTCCAGAGAGT
PPARA_RVS	GATCTTGGCATTCGTCCAA
PPARA-FWD	GAGCATTGAACATCGAATGTAG
SH3BGRL_FWD	TTGCATCTTCCTCTGGCTCT
SH3BGRL_RVS	CTGGCTTCAAAGAAGGCATC
SLC16A1_FWD	TCAGGCTGTGGCTTGATTGC
SLC16A1_RVS	GCCAATGGTCGCCTCTTGTAGAA
SLC16A1-AS1 KO1_FWD	AGAGGTCCCATGTGCTTTCCAA
SLC16A1-AS1 KO1_RVS	CAATGACCCGAAATGCCTTCCCTT
SLC16A1-AS1 KO2_FWD	AGCTACCACGCCCTGCCAGACT
SLC16A1-AS1 KO2_RVS	TTGCCCAGGCTGGAGTGCAATGAT
SLC16A1-AS1_FWD	GGGAGACTTAGGCACAAATTAACC
SLC16A1-AS1_RVS	ATGTTGGTGTGCTTGAAATCTTCC
SNAI2_FWD	ATGAGGAATCTGGCTGCTGT
SNAI2_RVS	TGAATTCCATGCTCTTGCAG
MCT1 mut-Prom FWD	ACATCCGAACTGCCCTCCCCGCCACG
MCT1 mut-Prom REV	CGTGGCGGGGGGGGGGGCAGTTCGGATGT
SLC16A1-AS1_del1-FWD	CCGAGAGGCCTGACCCAGCATTTGAATGGATG
SLC16A1-AS1_del1-REV	CATCCATTCAAATGCTGGGTCAGGCCTCTCGG
SLC16A1-AS1_del2-FWD	CGGGGAATGGGCAGCATTTCATGGGCTGGG
SLC16A1-AS1_del2-REV	CCCAGCCCATGAAATGCTGCCCATTCCCCG
SLC16A1-AS1_del4-FWD	GGGGGAGATGCGCGCGACTGAGGAGC
SLC16A1-AS1_del4-REV	GCTCCTCAGTCGCGCGCATCTCCCCC
SLC16A1-AS1_del core-FWD	GATGCGCGTGCGCGGGAAGCGACT
SLC16A1-AS1_del core-REV	AGTCGCTTCCCGCGCACGCGCATC

U6-FWD	CTCGCTTCGGCAGCACATATAC
U6-REV	AACGCTTCACGAATTTGCGTGTC

**Table S2**: GSEA of genes from TANRIC atlas positively (n = 1245) and negatively (n = 991) correlated ( $|k| \ge 0.4$ ) with SLC16A1-AS1 expression in TCGA-BC.

TANRIC - positively correlated						
Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q- value	-log₁₀ (p- value)
GO_CELL_CYCLE	1847	217	0.1175	3.56E-63	2.64E-59	62.45
GO_MITOTIC_CELL_CYCLE	1009	155	0.1536	5.37E-60	1.99E-56	59.27
GO_CELL_CYCLE_PROCESS	1383	181	0.1309	2.44E-59	6.01E-56	58.61
HALLMARK_E2F_TARGETS	200	65	0.325	1.79E-46	3.32E-43	45.75
GO_PROTEIN_PHOSPHORYLATION	1967	185	0.0941	1.5E-39	2.22E-36	38.82
GO_REGULATION_OF_RESPONSE_TO_STRESS	1497	154	0.1029	2.75E-37	3.4E-34	36.56
GO_CELL_CYCLE_PHASE_TRANSITION	621	95	0.153	1.29E-36	1.36E-33	35.89
GO_RESPONSE_TO_CYTOKINE	1192	134	0.1124	1.95E-36	1.8E-33	35.71
GO_REGULATION_OF_CELL_CYCLE	1212	135	0.1114	2.78E-36	1.97E-33	35.56
HALLMARK_G2M_CHECKPOINT	200	56	0.28	2.89E-36	1.97E-33	35.54
GO_REGULATION_OF_IMMUNE_SYSTEM_PROCESS	1631	160	0.0981	2.94E-36	1.97E-33	35.53
GO_CYTOSKELETON_ORGANIZATION	1298	140	0.1079	4.3E-36	2.65E-33	35.37
GO_DEFENSE_RESPONSE	1709	164	0.096	5.19E-36	2.96E-33	35.28
GO_APOPTOTIC_PROCESS	1980	176	0.0889	1.94E-34	1.03E-31	33.71
GO_DNA_METABOLIC_PROCESS	850	107	0.1259	2.08E-33	1.02E-30	32.68
GO_IMMUNE_EFFECTOR_PROCESS	1253	130	0.1038	8.01E-32	3.7E-29	31.10
GO_CHROMOSOME_ORGANIZATION	1207	127	0.1052	1.09E-31	4.76E-29	30.96
GO_REGULATION_OF_PROTEIN_MODIFICATION_PROCESS	1843	162	0.0879	4.34E-31	1.78E-28	30.36
GO_POSITIVE_REGULATION_OF_IMMUNE_SYSTEM_PROCESS	1166	123	0.1055	8.38E-31	3.26E-28	30.08
GO_REGULATION_OF_CELL_DIFFERENTIATION	1863	162	0.087	1.49E-30	5.51E-28	29.83

TANRIC- negatively correlated						
Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q-value	-log <sub>10</sub> (p-value)
GO_LIPID_METABOLIC_PROCESS	1241	139	0.112	4.59E-49	3.39E-45	48.34
GO_CELLULAR_LIPID_METABOLIC_PROCESS	885	113	0.1277	2.31E-45	8.55E-42	44.64
GO_SMALL_MOLECULE_METABOLIC_PROCESS	1688	146	0.0865	3.5E-38	8.63E-35	37.46
GO_ORGANIC_ACID_METABOLIC_PROCESS	903	94	0.1041	1.26E-30	2.33E-27	29.90
GO_MONOCARBOXYLIC_ACID_METABOLIC_PROCESS	475	65	0.1368	4.38E-28	6.48E-25	27.36
GO_LIPID_CATABOLIC_PROCESS	325	51	0.1569	4.71E-25	5.81E-22	24.33
GO_FATTY_ACID_METABOLIC_PROCESS	257	45	0.1751	2.82E-24	2.98E-21	23.55
GO_LIPID_BIOSYNTHETIC_PROCESS	669	71	0.1061	8.05E-24	7.45E-21	23.09
GO_CELLULAR_LIPID_CATABOLIC_PROCESS	207	40	0.1932	2.2E-23	1.81E-20	22.66
GO_OXIDATION_REDUCTION_PROCESS	970	86	0.0887	2.82E-23	2.09E-20	22.55
GO_ORGANOPHOSPHATE_METABOLIC_PROCESS	532	61	0.1147	2.47E-22	1.66E-19	21.61
GO_ORGANIC_ACID_CATABOLIC_PROCESS	250	42	0.168	5.01E-22	3.09E-19	21.30
GO_SMALL_MOLECULE_CATABOLIC_PROCESS	396	50	0.1263	2.5E-20	1.42E-17	19.60
HALLMARK_ESTROGEN_RESPONSE_EARLY	200	35	0.175	3.59E-19	1.90E-16	18.44
GO_INTRACELLULAR_TRANSPORT	1825	111	0.0608	5.94E-17	2.93E-14	16.23
GO_MONOCARBOXYLIC_ACID_CATABOLIC_PROCESS	122	26	0.2131	8.81E-17	4.07E-14	16.06
GO_FATTY_ACID_CATABOLIC_PROCESS	103	24	0.233	1.52E-16	6.63E-14	15.82
GO_CARBOHYDRATE_DERIVATIVE_METABOLIC_PROCESS	940	72	0.0766	3.21E-16	1.32E-13	15.49
GO_LIPID_MODIFICATION	235	34	0.1447	4.80E-16	1.87E-13	15.32
GO_LIPID_OXIDATION	101	23	0.2277	1.12E-15	4.14E-13	14.95

**Table S3 (related to Figure 4B)**: Top 20 potential interaction sites between E2F1 and SLC16A1-AS1 predicted by catRAPID. Discriminative Power (DP) ranges from 0% (unpredictability) to 100% (predictability). DP values above 50% indicate that the interaction is likely to take place, whereas DPs above 75% represent high-confidence predictions.

E2F1 region (amino acides)	SLC16A1-AS1 region (base pairs)	discriminative power (%)
312-363	241-302	63
51-102	241-302	59
87-138	241-302	59
162-213	241-302	59
276-327	241-302	59
312-363	262-323	57
87-138	262-323	54
212-263	241-302	54
251-302	241-302	54
301-352	241-302	54
51-102	262-323	52
276-327	262-323	52
51-102	232-293	50
162-213	232-293	50
162-213	262-323	50
201-252	241-302	50
276-327	232-293	50
312-363	151-212	50
312-363	172-233	50
312-363	211-272	50

Table S4 (related to Figure 4B): Secondary structure level analysis of top 20 potential interaction sites between E2F1 and SLC16A1-AS1. Surface accessibility is calculated using NetSurfP-2.0. RSA, Relative Surface Accessibility; ASA, Absolute Surface Accessibility. RSA reflects the degree to which a residue interacts with the solvent molecule. Accessible surface area or Absolute Surface Accessibility is the surface area of a biomolecule that is accessible to a solvent. RSA=ASA/max ASA. The asterisk marks the best combination of the E2F1:SLC16A1-AS1 complex based on the secondary structure parameters.

complex binding site (no.)	protein region (amino acids)	average RSA	average ASA	IncRNA region (base pairs)	number of stem forming bases (SLC16-A1-AS1)	number of loop forming bases (SLC16-A1-AS1)
1	312-363	0.623423077	92.69209615	241-302 (62)	45 (72.6%)	17 (27.4%)
2	51-102	0.574923077	86.88392308	241-302 (62)	45 (72.6%)	17 (27.4%)
3	87-138	0.539538462	86.16046154	241-302 (62)	45 (72.6%)	17 (27.4%)
4	162-213	0.372019231	60.44236538	241-302 (62)	45 (72.6%)	17 (27.4%)
5	276-327	0.484346154	72.46588462	241-302 (62)	45 (72.6%)	17 (27.4%)
6	312-363	0.623423077	92.69209615	262-323 (62)	48 (77.4%)	14 (22.6%)
7	87-138	0.539538462	86.16046154	262-323 (62)	48 (77.4%)	14 (22.6%)
8	212-263	0.377961538	62.41503846	241-302 (62)	45 (72.6%)	17 (27.4%)
9	251-302	0.299269231	47.28023077	241-302 (62)	45 (72.6%)	17 (27.4%)
10	301-352	0.634346154	90.76119231	241-302 (62)	45 (72.6%)	17 (27.4%)
11	51-102	0.574923077	86.88392308	262-323 (62)	48 (77.4%)	14 (22.6%)
12	276-327	0.484346154	72.46588462	262-323 (62)	48 (77.4%)	14 (22.6%)
13	51-102	0.574923077	86.88392308	232-293 (62)	47 (75.8%)	15 (24.2%)
14	162-213	0.372019231	60.44236538	232-293 (62)	47 (75.8%)	15 (24.2%)
15	162-213	0.372019231	60.44236538	262-323 (62)	48 (77.4%)	14 (22.6%)
16	201-252	0.407384615	68.73494231	241-302 (62)	45 (72.6%)	17 (27.4%)
17	276-327	0.484346154	72.46588462	232-293 (62)	47 (75.8%)	15 (24.2%)
18	312-363	0.623423077	92.69209615	151-212 (62)	39 (62.9%)	23 (28.1%)
19*	312-363	0.623423077	92.69209615	172-233 (62)	36 (58.1%)	26 (41.9%)
20	312-363	0.623423077	92.69209615	211-272 (62)	48 (77.4%)	14 (22.6%)

Table S5 (related to Figure 4F and G): Overview of the minimum free energies (MFE) of the mutants of SLC16A1-AS1 and binding energies of the corresponding complex structures with E2F1.

In silico mutagenesis	MFE (Kcal/mol) of the IncRNA (RNAfold)	Binding energy (Kcal/mol) of the E2F1:IncRNA complex (FireDock)
wild-type	-525.6	-35.68
del1	-492.3	-43.67
del2	-480.7	-28.51
del3	-449.7	-49.61
del4	-518.5	-33.47
del5	-524.8	-57.29
del6	-517.3	-24.66

 Table S6 (related to Figure 4H):
 Sequence based identification of IncRNA-DNA triplex formation sites on the MCT1 promoter using the LongTarget tool (http://lncrna.smu.edu.cn/show/DNATriplex)

	QueryStart (SLC16A1- AS1)	QueryEnd (SLC16A1- AS1)	StartInSeq (MCT1 promoter)	EndInSeq (MCT1 promoter)	Direction	Mean Stability	Mean Identidy (%)	Strand	Rule	Score	base pairs	Class	Mid Point	Center
1	1077	1130	-262	-315	L	1.49	61.11	AntiPlus	17	1.5	54	1	1103	1106
2	1059	1125	-253	-315	L	1.71	61.19	AntiPlus	14	1.15	67	1	1092	1106
3	1065	1129	-315	-253	R	2.73	60.61	ParaPlus	2	1.09	66	1	1097	1106
4	1075	1133	-252	-313	L	1.83	65.08	AntiPlus	14	1.29	63	1	1104	1106
5	1079	1142	-252	-315	L	1.91	63.08	AntiPlus	14	1.31	65	1	1110	1106
6	1091	1142	-255	-307	L	1.88	66.04	AntiPlus	14	1.72	53	1	1116	1106
7	1064	1130	-314	-252	R	2.67	61.19	ParaPlus	5	1.15	67	1	1097	1106
8	1080	1130	-314	-262	R	2.74	64.15	ParaPlus	5	1.55	53	1	1105	1106
9	1091	1142	-313	-265	R	2.8	61.54	ParaPlus	2	1.31	52	1	1116	1106
10	1079	1142	-252	-315	L	1.9	62.69	AntiPlus	8	0.93	67	1	1110	1106
11	1078	1129	-265	-315	L	1.95	63.46	AntiPlus	5	1.48	52	1	1103	1106
12	1077	1133	-255	-311	L	1.84	61.4	AntiPlus	5	1.53	57	1	1105	1106
13	1073	1132	-312	-252	R	2.58	62.3	ParaPlus	2	1.41	61	1	1102	1106
14	1075	1142	-252	-315	L	1.88	62.32	AntiPlus	2	1.09	69	1	1108	1106
15	1054	1115	-260	-315	L	1.81	66.67	AntiPlus	14	1.24	63	2	1084	1089
16	1052	1115	-251	-315	L	1.99	61.54	AntiPlus	2	1.35	65	2	1083	1089
17	1059	1120	-313	-252	R	2.57	60.0	ParaPlus	2	1.03	65	2	1089	1089
18	1059	1110	-303	-252	R	2.87	64.15	ParaPlus	2	1.32	53	2	1084	1089
19	1055	1113	-259	-315	L	1.87	60.0	AntiPlus	2	1	60	2	1084	1089
20	1470	1521	-253	-302	L	1.1	63.46	AntiPlus	9	1.48	52	3	1495	1495
21	617	679	-311	-251	R	1.85	60.32	ParaPlus	4	1.05	63	4	648	648
22	575	625	-262	-312	L	1.77	63.46	AntiPlus	10	1.25	52	5	600	600
23	251	313	-315	-253	R	1.96	60.0	ParaPlus	4	0.85	65	6	282	282
24	157	207	-268	-315	L	1.77	62.75	AntiPlus	10	1.18	51	7	182	182

### Table S6 (related to Figure 4H): continued

	TFO sequence	TTS sequence
1	CCUCCCUUCCAGGACUCACCUCACUUACCCUCCCCUCACUUACCCUCCCC	TCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
2	CCCUUCCCCUCGUGUCUUCCUCCCCUCAGGACUCACCCCCCCC	GCCCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCTCTAGCTCGCCCGCGCGCG
3	CCCUCGUGUCUUCCUCCCUCAGGACUCACCCCCCCCCCC	GGCGCCGGCGCGCGGGGGGGGGGGGGGGGGGGGGGGGGG
4	UUCCUCCCUUCCAGGACUCACCUCACUUACCCUCCCCCUCACUUACCCUCCCCCUC	AGCCCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
5	UCCCUUCCAGGACUCACCCCACUUACCCUCCCCUCACUUACCCUCCCCCUCAAUUCUCAC	AGCCCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
6	CUCACCCCUCACUUACCCUCCCCUCACUUACCCUCCCCCUCAAUUCUCAC	CCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
7	CCCCUCGUGUCUUCCUCCCUUCCAGGACUCACCCCCCUCACUUACCCUCCCCCUCACUUACCCUCCCC	GCGCCGGCGCGCGGGGGGGGGGGGGGGGGGGGGGGGGGG
8	CCCUUCCAGGACUCACCUCACUUACCCUCCCCUCACUUACCCUCCCC	GCGCCGGCGCGCGGGGGGGGGGGGGGGGGGGGGGGGGGG
9	CUCACCCCUCACUUACCCUCCCCUCACUUACCCUCCCCCUCAAUUCUCAC	CGCCGGCGCGCGGGGGGGGGGGGGGGGGGGGGGGGGGGG
10	UCCCUUCCAGGACUCACCCCACUUACCCUCCCCUCACUUACCCUCCCCCUCAAUUCUCAC	AGCCCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
11	CUCCCUUCCAGGACUCACCUCACUUACCCUCCCCUCACUUACCCUCCC	ACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
12	CCUCCCUUCCAGGACUCACCCCACUUACCCUCCCCUCACUUACCCUCCCCCUC	CCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
13	UCUUCCUCCCUUCCAGGACUCACCUCACUUACCCUCCCCUCACUUACCCUCCCCCU	GCCGGCGCGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
14	UUCCUCCCUUCCAGGACUCACCUCACUUACCCUCCCCUC	AGCCCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
15	CCCUGCCCUCCCUCGUGUCUUCCUCCCUUCCAGGACUCACCCCCCCACUUACCCUCCCCC	CCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
16	GACCCUGCCCUCCCUCGUGUCUUCCUCCCUUCCAGGACUCACCCCCCCACUUACCCUCCCCC	AGCCCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
17	CCCUUCCCCUCGUGUCUUCCUCCCCUCAGGACUCACCCCCCACUUACCCUCCCCCUCACU	CGCCGGCGCGCGGGGGGGGGGGGGGGGGGGGGGGGGGGG
18	CCCUUCCCCUCGUGUCUUCCUCCCGGGACUCACCCCCCCACUUACCCU	CGCGGGCGAGCTAGAGGGCGCGCGCGCGGGCTGAAAGCGTGTGGAGGCGCGGGCT
19	CCUGCCCUUCCCCUGUGUCUUCCUCCCGGACUCACCCCCUCACUUACCCUCCC	GCCTCCACACGCTTTCAGCCGCGCGCGCGCCCTCTAGCTCGCCCGCGCGCG
20	AAACUUAAAGUGUAACAAUAAUAAAAUUUUUUUAAAAAAAA	GCCCGCGCCTCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCG
21	GCCGGACGCUUAUGGACGAUGCAUAUGUGGGGCCGGAUAGAAUUGUGGCAGGCGGCGCUGGGG	CCGGCGCGCGGGCGAGCTAGAGGGCGCGCGCGCGCGGCTGAAAGCGTGTGGAGGCGCGGGCT
22	GGGGUGUGCACCUCGGCCACGUGGAAAAGCGAGAAUGCAGAGGCCGGACGC	TCCACACGCTTTCAGCCGCGCGCGCCCCTCTAGCTCGCCCGCGCGCG
23	GGGUGCCGCUGGCACCCGGGAAGACGCUGGGGGCCGGCGCUGUAGAGCCGGGCAUGGGCUGGG	GGCGCCGGCGCGCGGGGGGGGGGGGGGGGGGGGGGGGGG
24	CGAGAGGCCUGACCCUGACGGGUGGGGGGGGGGGGGGGG	CGCTTTCAGCCGCGCGCGCCCTCTAGCTCGCCCGCGCGCG

**Table S7 (related to Figure 6A):** GSEA of genes responsive (up- or downregulated, |fold change|  $\geq$  1.5) to MCT1 knockdown in SUM149 cell line (from GSE76675) and to SLC16A1-AS1 knockout in UMUC-3.

GSEA of SUM149.shMCT1(up, down) vs. UMUC-3-KO (up, down)						
Gene Set Name	# Genes in Gene Set (K)	# Genes in Overlap (k)	k/K	p-value	FDR q- value	-log₁₀ (p-value)
GO_RESPONSE_TO_ENDOGENOUS_STIMULUS	1648	84	0.051	3.67E-26	2.72E-22	25.44
GO_NEGATIVE_REGULATION_OF_RESPONSE_TO_STIMULUS	1655	82	0.0495	9.87E-25	3.65E-21	24.01
GO_RESPONSE_TO_OXYGEN_CONTAINING_COMPOUND	1616	80	0.0495	4.19E-24	1.03E-20	23.38
GO_APOPTOTIC_PROCESS	1980	89	0.0449	6.26E-24	1.16E-20	23.20
GO_CELLULAR_RESPONSE_TO_ENDOGENOUS_STIMULUS	1384	72	0.052	6.11E-23	9.04E-20	22.21
GO_LOCOMOTION	1943	86	0.0443	1.12E-22	1.39E-19	21.95
HALLMARK_MTORC1_SIGNALING	200	29	0.145	1.81E-21	1.92E-18	20.74
GO_REGULATION_OF_CELL_DEATH	1723	78	0.0453	3.78E-21	3.5E-18	20.42
GO_CELL_CYCLE	1847	80	0.0433	1.59E-20	1.31E-17	19.80
GO_CELL_MOTILITY	1719	76	0.0442	5.11E-20	3.78E-17	19.29
GO_CELLULAR_RESPONSE_TO_OXYGEN_CONTAINING_COMP OUND	1126	59	0.0524	4.85E-19	3.26E-16	18.31
GO_RESPONSE_TO_NITROGEN_COMPOUND	975	54	0.0554	1.54E-18	9.49E-16	17.81
GO_NEGATIVE_REGULATION_OF_SIGNALING	1394	65	0.0466	2.49E-18	1.35E-15	17.60
GO_RESPONSE_TO_ORGANIC_CYCLIC_COMPOUND	917	52	0.0567	2.56E-18	1.35E-15	17.59
GO_REGULATION_OF_CELL_POPULATION_PROLIFERATION	1708	72	0.0422	7.20E-18	3.55E-15	17.14
GO_RESPONSE_TO_HORMONE	977	53	0.0542	7.95E-18	3.67E-15	17.10
GO_POSITIVE_REGULATION_OF_MOLECULAR_FUNCTION	1756	73	0.0416	8.73E-18	3.80E-15	17.06
GO_RESPONSE_TO_DRUG	1018	54	0.053	1.00E-17	4.13E-15	17.00
GO_PROTEIN_PHOSPHORYLATION	1967	77	0.0391	2.82E-17	1.10E-14	16.55
GO_RESPONSE_TO_EXTRACELLULAR_STIMULUS	523	38	0.0727	4.08E-17	1.51E-14	16.39

**Table S8 (related to Figure 6B):** Common differentially regulated genes (|fold change|  $\geq$  2) upon SLC16A1-AS1 overexpression in RT-4 cell line or knockout in UMUC-3 cell line.

RT-4 up & UMUC-3 down	RT-4 down & UMUC-3 up
ARRDC3	ACTL6A
ATL1	ADGRL2
C5orf15	ARCN1
CARF	ASNS
COMMD7	AURKA
CYBRD1	BAZ1A
DPY19L4	CDC20P1
DRAM1	COPG1
ERAP2	CSE1L
GBP3	FGD6
KATNAL1	GFPT1
LPCAT2	GNL2
NRIP1	HMGB3
РНКВ	HSPA4L
PPARA	INTS4
RAB23	KIF2C
RBBP9	KYNU
RP11-472B18.2	LINC00958
SAMD9L	MCM2
SCAMP1	MICB
SH3BGRL	MTHFD2
SNAI2	МҮВ
SNORD116-14	NAV2
SNORD116-15	NCAPD3
SNORD116-16	ODC1
SNORD116-17	ORC1
SNORD116-18	RNF168
SNORD116-19	RRM1
SNORD116-20	SBNO1
SNORD116-21	SLC3A2
SNORD116-24	SLC7A11
SNORD116-3	TFRC
SNORD116-9	TM4SF1
SYTL4	TNFAIP3
TCP11L2	UBE2C
TGFBI	UTP18
	WDHD1
	ХРОТ

**Table S9 (related to Figure 6B):** Pathways and corresponding target genes differentially regulated by SLC16A1-AS1 identified using KEGG Mapper tool.

KEGG pathway	SLC16A1-AS1 targets
Metabolic pathways	ASNS, GFPT1, KYNU, LPCAT2, MTHFD2,
	ODC1, RRM1
Ferroptosis	SLC3A2, SLC7A11, TFRC
NOD-like receptor signaling pathway	GBP3, TNFAIP3
Ribosome biogenesis in eukaryotes	GNL2, UTP18
Glucagon signaling pathway	PHKB, PPARA
Insulin resistance	GFPT1, PPARA
Alanine, aspartate and glutamate metabolism	ASNS, GFPT1
Cell cycle	MCM2, ORC1
Glutathione metabolism	ODC1, RRM1

**Table S10 (related to Figure 6F):** Construction of the consensus position weight matrix (PWM) of E2F1 from the three known PWMs of E2F1 (MA.00024.1, -2, -3; JASPAR).

matrix position	1	2	3	4	5	6	7	8	9	10	11	12
MA.00024.1	Ŧ	т	т	C	C	c	C	c				
	0	0	0	0	0	0	0	0				
	0	0	0	4	2	10	0	q				
	0	0	0	- 6	2	0	10	1				
	10	10	10	0	0	0	0	0				
	10	10	10	0	0	0	Ū	0				
			C	G	G	C	G	GIC	GIC	^		C/A
		259	218	144	0	0	0	0	0	<b>A</b> 1059	G/A 508	305
		317	0	274	0	1059	0	337	286	0	0	269
MA.00024.2		280	628	641	1059	0	1059	722	773	0	551	485
		203	213	0	0	0	0	0	0	0	0	0
		200	210	0	0	0	Ū	0	Ū	0	0	Ū
	F	т	т	6	6	6	6	<u> </u>	6	•	٨	Δ
	1 254	ı 241	1 208	54	0		0	2	0	<b>A</b> 524	A 510	A 493
	115	46	200	71	32	599	1	588	577	171	80	59
MA.00024.3	80	100	145	888	950	0	1009	17	46	20	58	112
	565	638	627	1	0	2	0	0	50	110	182	210
	000	000	021		0	2	U	0	00	115	102	215
	т	т	т	G	G	C	G	C/G	C/G	Δ	Δ	Δ
	254	500	426	198	0	0	0	2	0	1583	1018	798
CONSENSUS	115	363	36	349	34	1668	1	934	863	171	80	328
(Sum)	89	380	773	1535	2017	0	2078	740	819	20	609	597
	575	851	850	1	0	2	0	0	50	119	182	219
	0.0		000	•	Ũ	_	Ŭ	Ũ	00		102	2.0
CONSENSUS (%)	т	т	т	G	G	С	G	C/G	C/G	А	A/G	A/G
	24.6	23.9	20.4	9.5	0.0	0.0	0.0	0.1	0.0	83.6	53.9	41.1
	11.1	17.3	1.7	16.8	1.7	99.9	0.0	55.7	49.8	9.0	4.2	16.9
	8.6	18.1	37.1	73.7	98.3	0.0	100	44.2	47.3	1.1	32.2	30.7
	55.7	40.6	40.8	0.0	0.0	0.1	0.0	0.0	2.9	6.3	9.6	11.3

## **Figures**

Figure S1 (related to Figure 2)



**Figure S1.** Representative images of migrated cells from Boyden chamber assays of indicated cell lines and conditions.

#### Figure S2 (related to Figure 3A)

# HUMAN>NR\_103743.1 Homo sapiens SLC16A1 antisense RNA 1 (SLC16A1-AS1), transcript variant 1, long non-coding RNA

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#### GORILLA>1 dna:chromosome chromosome:gorGor3.1:1:115899284:115901331:1

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#### ORANGUTAN>1 dna:chromosome chromosome:PPYG2:1:115286199:115288261:-1

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#### MACAQUE>chromosome:Mmul\_8.0.1:1:113937657:113939708:1

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#### MARMOSET>chromosome:C\_jacchus3.2.1:7:148928200:148930498:1

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#### RAT>2 dna:chromosome chromosome:Rnor\_6.0:2:207107357:207108366:-1

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#### COW>3 dna:chromosome chromosome:UMD3.1:3:30531596:30533610:-1

GCGCGCGCGCCCCCCGCTATGCGGCAGGGACCTTGTAGCCGCGCCCCTCTGCGGAGGTCTCCCCTTCGGCCCGCCGTCGGAGGA GAATGGACGCGGGTGCATCTCGGACCGGGGAAGAGGCCGGAAAGCCGGCAGTTTAGAGCGCGGGCTGCGGCCTCGCTTCTTGAG ATCAGTGACCTCGGGGGAAGTCACTCAAAATCCTCAGGGCCTGCTTCCTGCTCTCTGAAATGAAAACAGTAATACAGGCGACCTC ATAAGGGCTTTTGGTGATTTTGATGAGAGAATTTATGGAACTTTTTTACATTTGATGAATGGTGAGTATGAACACAGAAGACG GGAGAAAAGAGCTGGTCTGACGGTGGTGACACTGGCCCCCACGAGAGGATCTGTGTGTCGGAAATATGAAGAAATGAGACTGCA GAGAGGCCAAATTTTTATGGACGCTGAGGGTTTGGAACAAAATATAAATTATAATTAAAGGTTCTGAAGGGCCGATTATATACT TAGCAGTTCACCTGAACTATCCTCAGGACCCCTATGATGTTGAAGTGATCAAACTGAATAAACATGGAGTGCCTCTGATGTCCG TTCAGCCTTTTGTTTTGCAAATGAAGAATCTAGATTGTAATGGAATGGGCCCTGAGGGATACGATTCTGAAAGTTTCCAAATA CTTTCTCCCTCTCACCCACTCCGCCTCTGTTTCAGGCTAGAAAAAGTTTTCAGTAACAGTTAAACATGTTAGAAAAACAGCATG ACAAACTAGGACTGGTTCAGATATGAACTGTCAGGACCTGAACGGTGGAATGTAGGAGGAGCATAGTATGGTAGATTCACAAT  ${\tt ATGGACCCAGGATCAGATCTGAATTCGAGCTTGAATACTTGGCACTTACCACGTCTTTGTCCTTAGGTAAATCACCCCCGCTC}$  ${\tt CCACGCCCCCGCCGCTGCTCTGAGCCTCATTCATTCTCTGCTTTGTAAAACAAGGACAGTACCTTCCTCATAAGGTTGTTATA}$ ATGCTAAAGATTAACCCTAAAGTTCCCTCACTTGGTCCTAACAGTGTAGTCACAGGATCATACTTAGTGATGTATTGTACTTT GACCTATTCTGGGCCTGTTTTTTTTAACCAGGTAATTTGAAGATAGTATCCATCAATCTGAAATTGTTGTGACAGTCAGGTAAG ATAGTATTTGCAAAAGTGATTGGTGGAACTTGATAAGTGCCCTATAAATGTTTATGAATTAATATTATAATTTTTCCACAAAG TTTATAAAAGGCAAAGATAAAAA

#### OPOSSUM>chromosome:BROADO5:2:483438127:483440716:1

AGCCTCAGGCACTTACTAGCTGCATGACCCTGGGCAAGTCACTTAACCTCGTTTGCCTCAGTTTCCTCCTCTGTAAAATGAGC TAGAGAAGTAAATGGCAAACCACTCTAATCTCTGCCTAGAAAACCCCCAAATGGAGTCACAAGTTGAACATGAACTCAACAATG AGAATGGTAAAAGGTTTCCAGTGCAAAACTGCAAAAATGCAACAGCTTTGAAGACACCTTATTGTTCCCATTAATCGGGACCTGG CTCCAATTCTTTGTAGAGGCTGTAAATTCCATTAGATCACCACCGTTCTGTACAGTCTAGGCAGTAGCCATTAGGATCAATTT ATCTAGCCTTTGGCCGTTCGTTACCCGAGTGATCTTGGGCTCGAGCTACGGGCCTCAGTTTCCTCCAGAGTAAAAAAGGGAGG AGCCTGACCACCATGCCTCCCCAGTCTCTTCCCGCGCTCTCGAAGTGGAGTGACAAAAAGGCCTTTCTGGCAGTCCCAGGCAG AAGGTTAGGCTCATCCATCCTCCGCCTCGGCCCCACGCTAAGCCCCACTGCTAACGTCGGGGCAGGGAATGCCTGGAAGCCG GACGGGGCCACGTGGAGGAGGCCTCTCCAATCGCGGCCAGAAGAAACTAGGAGGGCTAGTACCATTCCGCGTGCCAGTGTG GTGCTCTCCCCGTCGGAAGCGCCCGCTCAGCTCTGACCACCGCTGCCTCCTGCCACTGGCTTATAATGCACCCGGGGCGGGG GAACATGCGGCTCCCCACGACCCGCCACGTGATCCCTACTGGGAAGAGCTGCTCATCAGGGCAACCCCCACACGGGTGTCGGA  ${\tt CCTCCTCCACGGAGGACACCCATGAGATGGTTACCCGCCCCTGGGGTGGGAGGTGTGCTTAACCTCCCAGTGCCTCCTGTTTC}$ CCACTCCAAGCTTGAAGTCACTTAATGTCTACACTTGCCAGAGCAGGAAATTTCCCCCCACTCCGCTGCTCATGACATGGAAAG TTTACACAGATGGATGCTCAGGAATCGTCGAAGAGACACGCTGCGTGGAACACAGGGGTTTGGAAGGATTCTAGGGAAGGCAG TGCACAGCCGTGGAGTGCTTAAGCTAAACGCTAGGGAAGGCAAAGGAGCTCCCTTTTTAAGCAGGAAACTATGTAAATAGGTA  ${\tt CTTGCAAGACAGACAGAGCAGCGCTTGGTTGGGTCGTTGCAGTCGGGTCTGATTCCTCAAGACGCCATCTGGTTTTCTTGGC$ AGAGATACTGGAGTGGTCTGCCCCATCCCAGCTCATTTTACAGATGAGGAAACTGAGGCAAACAGGCTTAAATAACTTGTCCA GTGTCACACAGCTAGTATTTTCCATTTCCTTCTCCAACCTATTTTACAGATGAGGAAACTAAAGCAAACAGGATCAAATGACA TGCCCAGGGTCACACAGTAAGCATCTGAGATCAGATTTGAATGCAGGTTTTCCTGATTCCAGGTTGAGCACTGGTATCTACTG TGCCACCTAGCTGCCCCCAGATAGAGTAATCTCATGGGAGAAAACCTGATATAGAATTTGGGGCTTGGACTGAGTCCAGAAGCA ATGGAATAACCTCCGTAAAGAGCAGCAGGAATGATTGTATCTCTGGATCACAGTGTCTGAAAGGGGAGAAGGCGAGAAAAGACA AGGATTCAAGTTACAAAGAACTTCCTTACATTGCCAAATACAGCATTTTATATAGTTGATCCTGGAAAACATTTAAAAAATAAA TATATATGTATATATTTAATCCTGGAGCTAATGGAGTTATTGAAAGGGATGTGGAGGTGACACATGGCCAGATCTACACTTGA GGAAAGTGACTTTGGCACCTAAATTTGGGGAGTGATTTGAGGTAATCCGCAGGCCAATTAGAAGGCTATTTCATTTGTCCAAA AGGGCTATCATGAGGAA

**Figure S2.** Sequences of SLC16A1-AS1 homologues in representative eutheria and metatheria species.

## Figure S3 (related to Figure 3A)

HUMAN	ccctcc <mark>ccgccaca</mark> cagacatccgaact-gcagcccgcgcctccacac
GORILLA	ccctcc <mark>ccgccaca</mark> cagacatccgaact-gcagcccgcgcctccacac
ORANGUTAN	ccctcc <mark>ccgccaca</mark> cagacatccgaact-gcagcccgcgcctccacac
MACACA	ccctcc <mark>ccgccaca</mark> cagacatccgaact-gcagcccgcatctccacac
MARMOSET	attacactccagcctgggtaacaaga <mark>gc-gaaact</mark> c-cgtctcaaaaaaaaaaaaaaaaaaaaaaaaaa
COW	ccctccccctacacttacacccgaacc-gc <mark>gactcgcgcc</mark> tccaca
DOG	cccccccccccccg <mark>gacgccaaa</mark> tc-gcg <mark>actcgcg-c</mark> tccaca
MOUSE	ccctccctgccatacatactcccagctcggc <mark>atctcgcgcc</mark> tccacac
RAT	ccctccctgccatacatacaccctgctcggcatctcgcgcctccacaa

position in block: -131 to -85

HUMAN	gcctttcagccgcgcgcgccctctagctcgccgcgcgcgcgcgcgcgcgcgcgcgcg
GORILLA	gccctctagctcgccgcgcgcgcgcgcgcgcgcgcgcgcg
ORANGUTAN	gcctttagctgcgcgcgcgcgccttagctcgccgcgcgcg
MACACA	gctctcagccgcgcgcgccctctagctcgccgcgcgcgcg
MARMOSET	aaaagaagaagaagaaggacctctccgctgcgtgataacgcgccccgggccgagcctgcgctccg
COW	gctctcaggcgcgcgcccgcagagtc <mark>gctttctc</mark> tc <mark>gctcgcgc</mark> gcgctg
DOG	cgcccgcgc <mark>tt</mark> <mark>tgcgcgcg</mark> ccg
MOUSE	gctgcgggctgcgcgtgcccgctcgctcgc
RAT	gctgcggtctgcgcgtgcccgctcgctcgc

position in block: -84 to -43

HUMAN	gcg-cccctcc <mark>ccgccacg</mark> tgaccggcgtcccagtccg-accc				
GORILLA	gcg-cccctcc <mark>ccgccacg</mark> tgaccggcgtcccagtccg-accg				
ORANGUTAN	gcg-cccctcc <mark>ccgccacg</mark> tgaccggcgtcccagtccg-accc				
MACACA	gcgccccctcc <mark>ccgccacg</mark> tgaccggcgtcccagtccg-accc				
MARMOSET	ggg-gcggagtcggc <mark>cctcgcgc</mark> tcgctgctctggcagacaggc				
COW	gcgccccctcc <mark>ccgccacg</mark> tgacccgcggcccggcaag-accc				
DOG	ccg-cccctcccagccacgtgacccgcgg				
MOUSE	c				
RAT	c				
position in block: -42 to -1					

**Figure S3.** SLC16A1-AS1 promoters from depicted species bear putative E2F1 binding sites (highlighted in yellow).

## Figure S4 (related to Figure 3E)



**Figure S4.** Immunofluorescence staining of MCT1 in bladder cancer cells. MCT1 (green) localizes in the cytoplasm and the cell membrane. DAPI (blue) represents nuclei. Bar graph: 20µm



**Figure S5.** 3D model of SLC16A1-AS1. Based on secondary structure folding a 3D model was generated using 3DRNA v2.0 software tool which is based on a fragment assembly approach to build RNA 3D structures. After manually cross-checking for bond length and missing interactions the model of SLC16A1-AS1 was optimized using 'Smart Minimizer protocol' available in DS2017 using CHARMM force field.

Α

В



del4: bases 193-209 MFE: -518.50 kcal/mol

del6: bases 172-175 &193-209 MFE: -517.30 kcal/mol

**Figure S6.** (A) Positions of the deletions in SLC16A1-AS1. (B) Minimum free energy (MFE) fold structures from wild type and *in silico* mutated SLC16A1-AS1. In all deletions MFE per mol of the IncRNA is reduced in comparison to wild-type. We calculated number of hairpin, interior and multiloops (junction) with at least three bases. For all mutants, the numbers of hairpin loops are reduced. In *del1*, *del2*, *del4* and *del6* the amount of interior loops is slightly increased in comparison to wild-type.

del5: bases 172-175 MFE: -524.80 kcal/mol

E2F1 residues	del1	del2	del3	del4	del5	del6
ALA67						
ARG80						
PRO87						
VAL88						
LYS89						
ARG90						
ARG91						
LEU92						
GLN99						
ALA102						
SER105						
GLY106						
PRO107						
ARG109						
ARG111						
SER121						
PRO122						
VAL246						
GLN249						
ARG252						
SER337						
PRO339						
SER340						
ASP345						
SER347						
GLN348						
SER349						
LEU350						
LEU351						
LEU374						
SER375						
VAL378						
ALA380						
ASP381						
SER382						
LEU384						
ILE400						
LEU402						

### Figure S7 (related to Figure 4F, G)

**Figure S7.** Pattern of E2F1 interacting residues with SLC16A1-AS1 wild-type compared to the mutants. E2F1 residues that are interacting with wild-type SLC16A1-AS1 appear in the first column. Orange indicates that the residue is involved in the same wild-type specific lncRNA interaction with E2F1whereas red shows non-interacting residues. All deletions lead to a strong reduction of protein:RNA associated groups. Especially, for *del1* no binding was predicted between E2F1 residues and the mutated lncRNA model, supporting a relevant role of this region for a functional E2F1 binding.

Α







**Figure S8.** GSEA of genes from TANRIC atlas (A) positively (n = 1245) and (B) negatively (n = 991) correlated ( $|k| \ge 0.4$ ) with SLC16A1-AS1.

#### Figure S9 (related to Figure 5E, F)



**Figure S9.** Quantification of SLC16A1 and SLC16A1-AS1 in UMUC-3 cells. Cells were treated with MCT1 inhibitor (AR-C155858) at indicated concentration and mRNA/IncRNA level were measured by qPCR 24 h later. The expression was normalized (GAPDH) and compared to the DMSO control (set as1). Bar graphs are represented as means  $\pm$  SD (n=3).