Drug repositioning inferred from E2F1-coregulator interactions studies for the prevention and treatment of metastatic cancers

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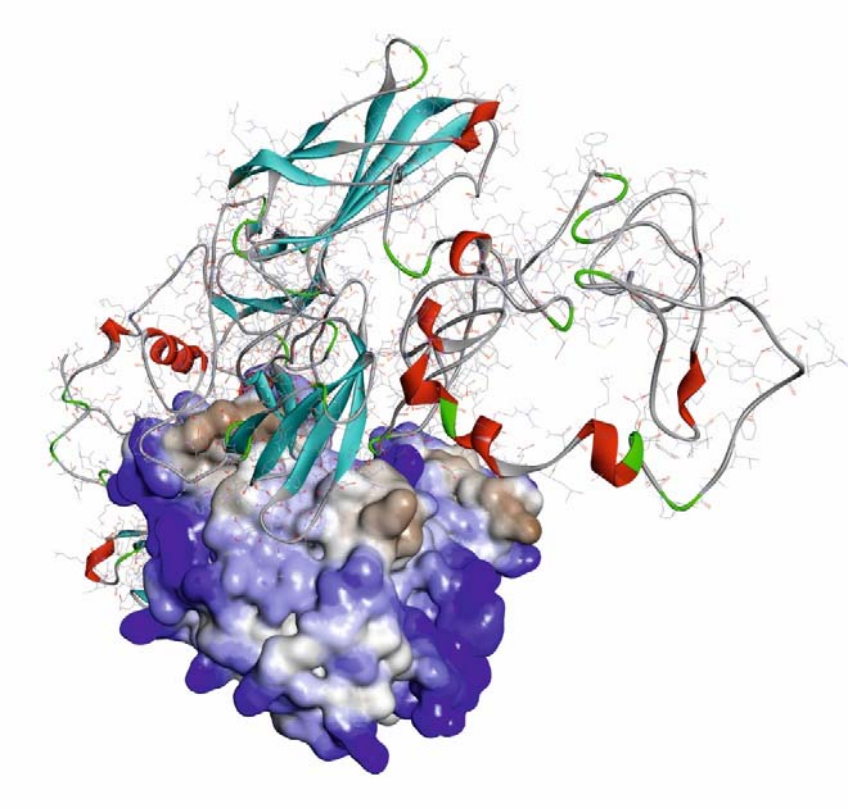


Figure S1. Prediction of E2F2 and MTA1 interaction. *In silico* prediction of E2F1 and MTA1 protein interaction indicates best complex formation of both proteins. E2F2 is shown as solid surface protein and colors are based on hydrophobicity index, whereas MTA1 is shown as solid ribbon with colors based on the secondary structure type. Atoms of MTA1 amino acid residues are shown as lines.

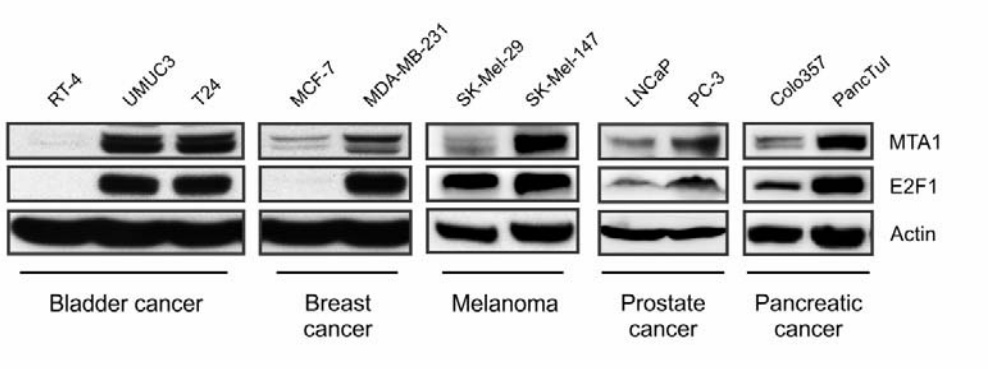


Figure S2. High protein levels of E2F1 and MTA1 correlate with aggressiveness of tumor cells in various cancer entities. Cells lines were seeded at the identical cell counts and allowed to grow to confluence. Protein expression levels were assessed by immunoblot using antibodies against depicted proteins. High protein levels of E2F1 and MTA1 correlate with aggressiveness of tumor cells. Actin was used for equal loading.

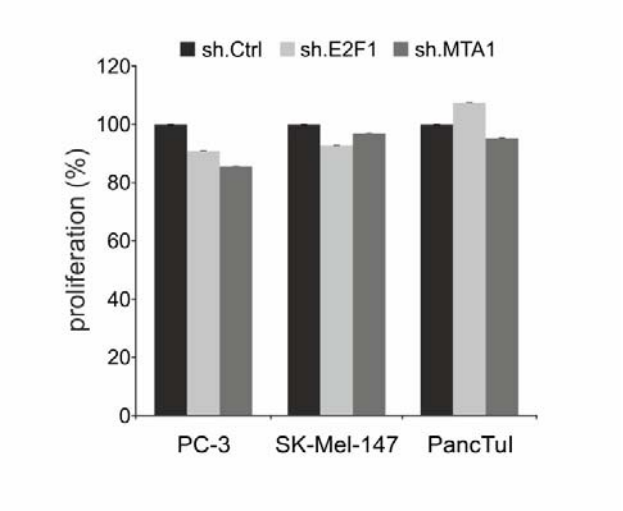


Figure S3. Effect of sh.E2F1 and sh.MTA1 on cell proliferation. XTT assays were performed 48 h after shRNA-mediated knockdown of E2F1 and MTA1 in three different cancer cell lines. The proliferation rate was calculated relative to the control (sh.ctrl). Bar graphs are represented as means ± SD.

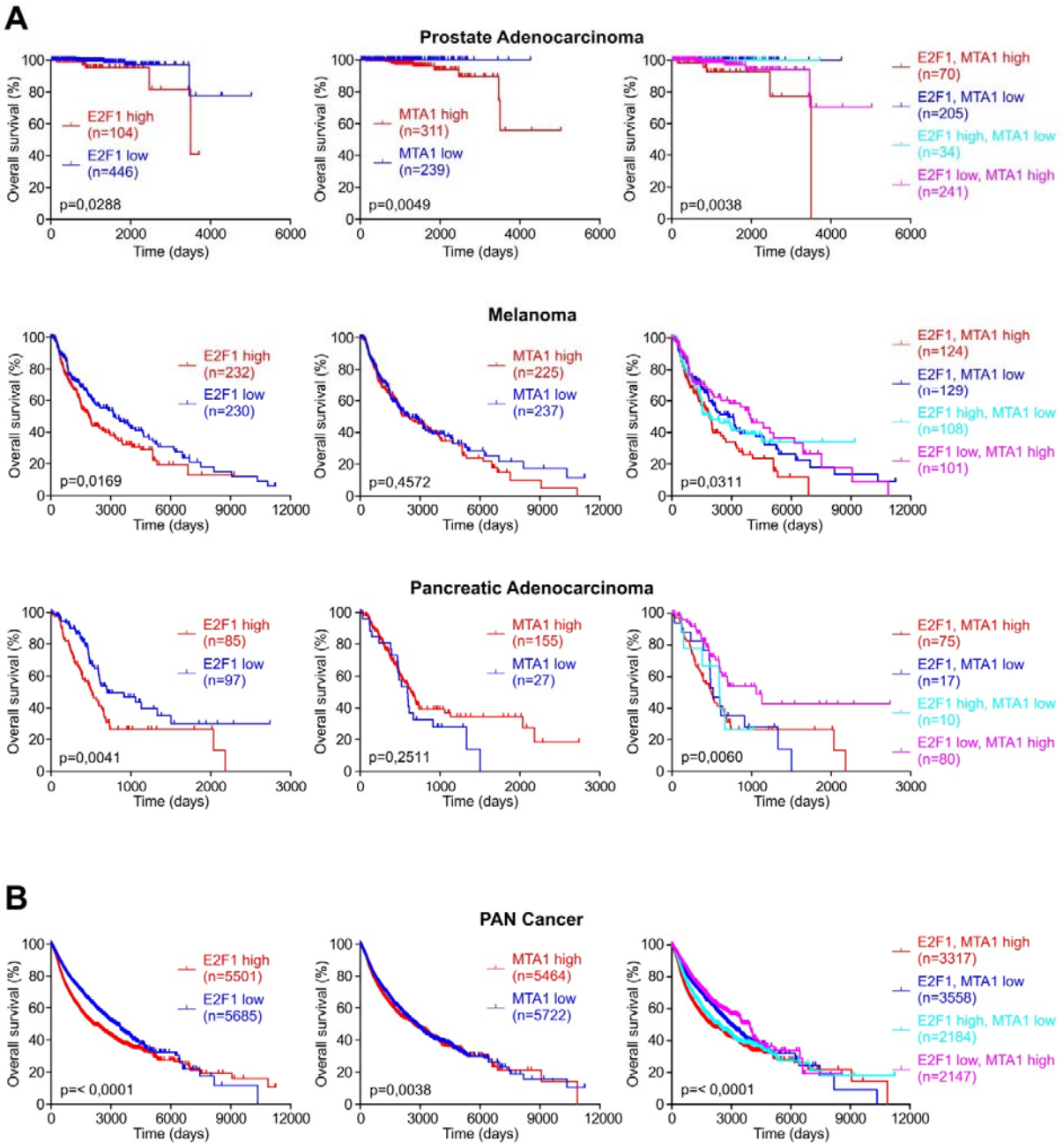


Figure S4. Correlation of E2F1 and MTA1 expression with patient survival. Using the UCSC Xena browser database Kaplan-Meier analyses were performed for overall survival of patients with **(A)** prostate adenocarcinoma (top), melanoma (center), and pancreatic adenocarcinoma (bottom) and **(B)** the Pan-Cancer cohort. Log-rank test p-values are depicted in the survival plots. High E2F1 expression is a prerequisite for poor survival (A and B left). While high MTA1 expression alone is a favourable parameter in some cancer entities (A and B middle and right), combined high MTA1 and E2F1 levels define bad prognoses (A and B right).

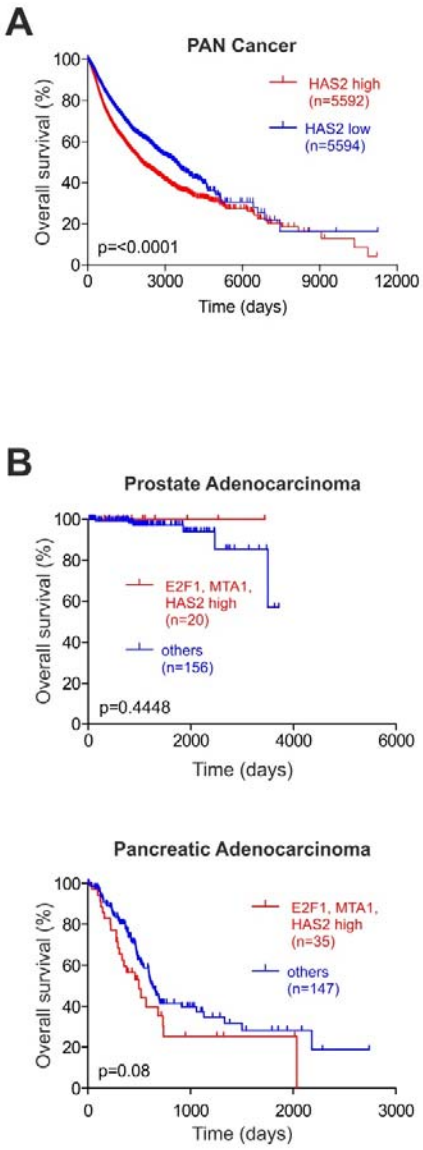


Figure S5. Survival data from patients with E2F1, MTA1 and HAS2 expression in different cancer cohorts. (A) Kaplan-Meier analyses in the Pan-Cancer cohort with high or low HAS2 levels. **(B)** Overall survival of patients with high E2F1, MTA1, and HAS2 expression in prostate (top) and pancreatic adenocarcinoma (bottom). Analyses were performed using the UCSC Xena browser database. Log-rank test p-values are depicted on the survival curves.

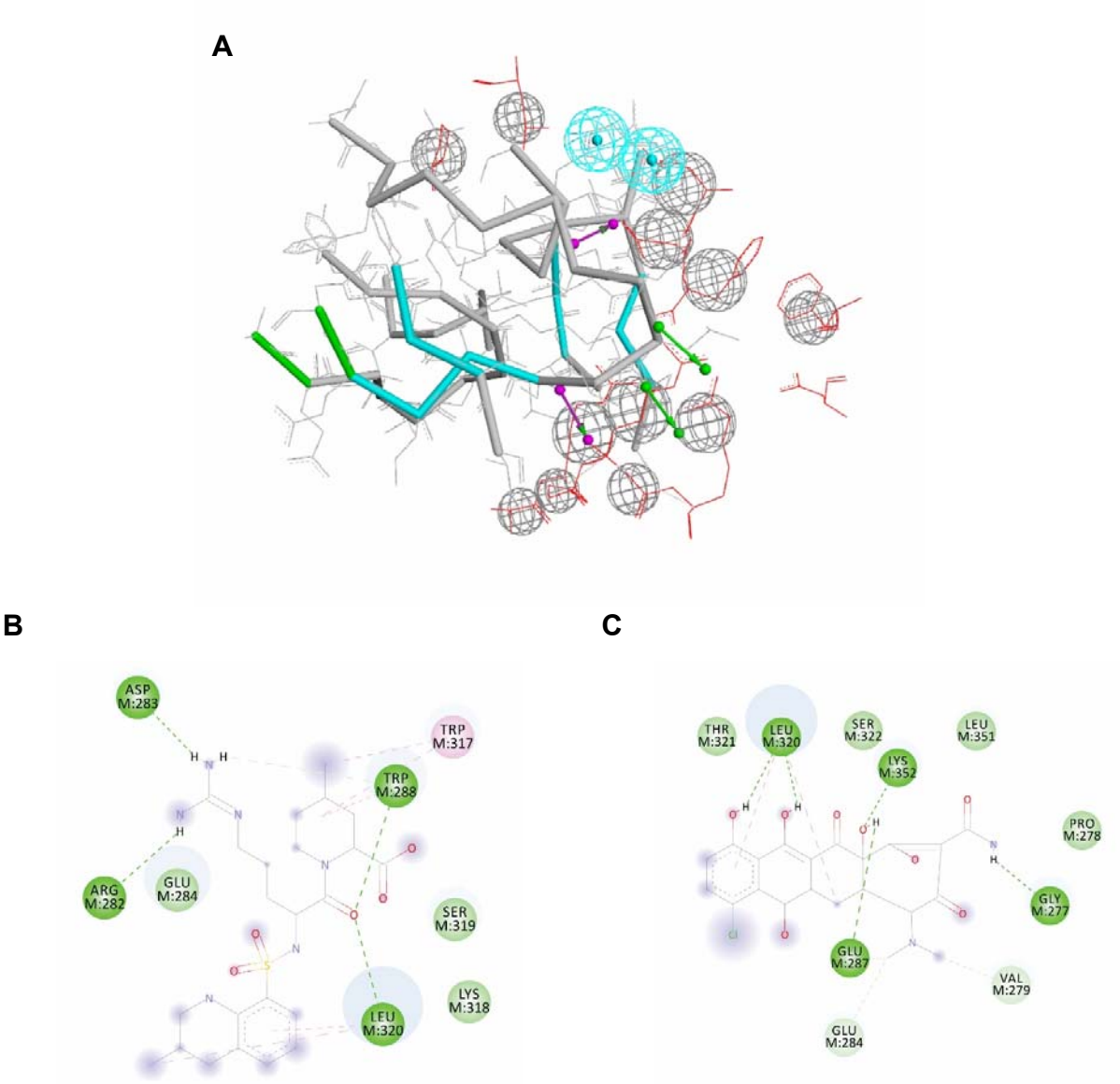


Figure S6. Structure based pharmacophore model generated from MTA1 residues interacting with E2F1 in the best pose. (A) Two hydrophobic groups are shown as *cyan spheres*, two hydrogen bond donors as *green arrows*, two hydrogen bond acceptors as *pink arrows*, and twelve excluded volumes as *gray spheres*. Amino acid residues of MTA1 and E2F1 involved in the binding interface are shown as *gray* and *red* lines. The MTA1 peptide backbone is highlighted as thick stick model. **(B, C)** Contact map of the best interaction poses of **(B)** argatroban and **(C)** demeclocycline in the MTA1 binding interface associated with E2F1. Interactions are shown as dotted lines. Green: conventional H-bond, light green: van der Waals interactions, orange: electrostatic interactions, pink: hydrophobic interactions. The best post identified for argatroban has a CDOCKER interaction energy of -45.68 kcal/mol. In case of demeclocycline the interaction energy for the best pose was found to be only -25.35 kcal/mol. Lower interaction energy of argatroban with MTA1 suggest more stable binding in comparison to demeclocycline.

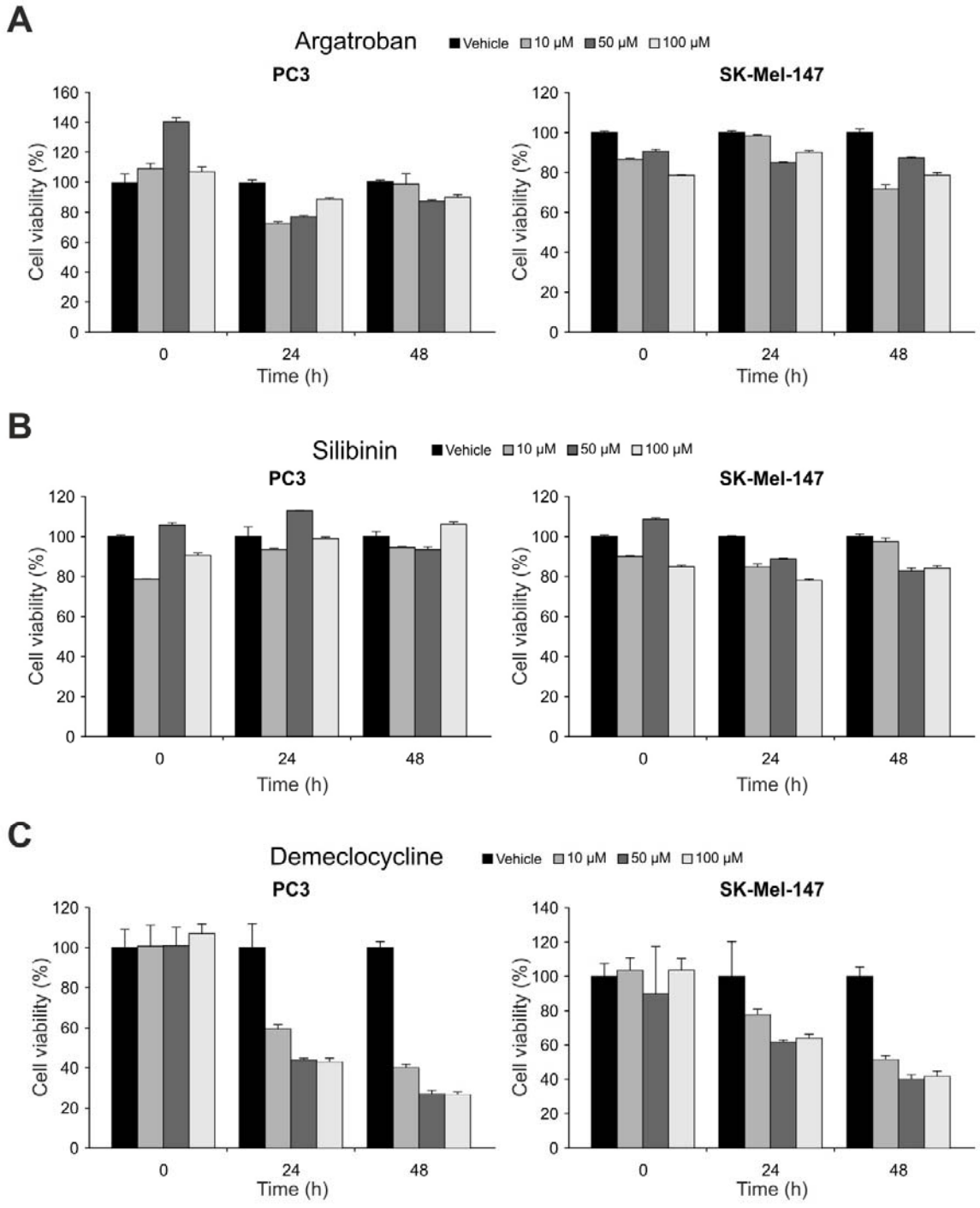


Figure S7. Cytotoxicity of demeclocycline, argatroban and silibinin in different cell lines. SK-Mel-147 and PC3 cells were treated with different concentrations of **(A)** argatroban, **(B)** silibinin or **(C)** demeclocycline. Cell viability was measured using XTT assay at indicated time points and calculated relative to the control (vehicle, set as 100%). Bar graphs are represented as means ± SD.

Amino acid residues	Distance (Å)	Bond Category	Bond Type
E2F1:ARG111:NH1 - MTA1:GLU287:OE2	2.95438	Electrostatic	Salt Bridge
MTA1:ARG225:NH2 - E2F1:ASP381:OD1	3.8743	Electrostatic	Salt Bridge
MTA1:ARG335:NH2 - E2F1:GLU320:OE1	1.85946	Electrostatic	Salt Bridge
MTA1:LYS352:NZ - E2F1:PHE437:OXT	2.535	Electrostatic	Salt Bridge
E2F1:ARG109:NH1 - MTA1:GLU287:OE2	5.00665	Electrostatic	Attractive Charge
E2F1:ARG111:NH1 - MTA1:GLU284:OE2	4.51285	Electrostatic	Attractive Charge
E2F1:ARG111:NH2 - MTA1:GLU287:OE1	5.41277	Electrostatic	Attractive Charge
E2F1:LYS125:NZ - MTA1:GLU133:OE1	1.65758	Electrostatic	Attractive Charge
E2F1:ARG127:NH2 - MTA1:GLU133:OE1	4.76786	Electrostatic	Attractive Charge
E2F1:ARG373:NH1 - MTA1:GLU292:OE1	5.53418	Electrostatic	Attractive Charge
E2F1:ARG422:NH2 - MTA1:ASP179:OD1	4.61986	Electrostatic	Attractive Charge
MTA1:ARG189:NH1 - E2F1:GLU407:OE2	5.23428	Electrostatic	Attractive Charge
MTA1:ARG225:NH1 - E2F1:ASP381:OD2	4.62703	Electrostatic	Attractive Charge
MTA1:LYS331:NZ - E2F1:ASP277:OD2	4.44111	Electrostatic	Attractive Charge
MTA1:ARG335:NH1 - E2F1:GLU319:OE2	5.04002	Electrostatic	Attractive Charge
MTA1:ARG335:NH1 - E2F1:GLU320:OE2	4.97341	Electrostatic	Attractive Charge
MTA1:LYS340:NZ - E2F1:ASP277:OD2	5.05875	Electrostatic	Attractive Charge
E2F1:ARG113:NH1 - MTA1:LEU320:O	2.66335	Hydrogen Bond	Conventional H-bond
E2F1:LYS120:NZ - MTA1:THR134:O	1.69271	Hydrogen Bond	Conventional H-bond
E2F1:ARG127:NH2 - MTA1:LEU137:O	3.24795	Hydrogen Bond	Conventional H-bond
E2F1:THR228:OG1 - MTA1:LYS138:O	2.76769	Hydrogen Bond	Conventional H-bond
E2F1:PHE437:N - MTA1:GLU287:OE2	2.99446	Hydrogen Bond	Conventional H-bond
MTA1:ASN132:N - E2F1:LYS125:O	2.43103	Hydrogen Bond	Conventional H-bond

MTA1:THR134:OG1 - E2F1:THR130:O	2.04248	Hydrogen Bond	Conventional H-bond
MTA1:ARG189:NH1 - E2F1:PRO405:O	2.24207	Hydrogen Bond	Conventional H-bond
MTA1:ARG225:NH1 - E2F1:VAL378:O	2.15437	Hydrogen Bond	Conventional H-bond
MTA1:ARG225:NH2 - E2F1:LEU377:O	1.97973	Hydrogen Bond	Conventional H-bond
MTA1:HIS253:N - E2F1:ASP381:OD1	2.81002	Hydrogen Bond	Conventional H-bond
MTA1:ALA254:N - E2F1:ASP381:OD1	2.89143	Hydrogen Bond	Conventional H-bond
MTA1:SER322:N - E2F1:ARG111:O	3.19967	Hydrogen Bond	Conventional H-bond
MTA1:ASN371:ND2 - E2F1:ILE293:O	2.94273	Hydrogen Bond	Conventional H-bond
E2F1:ARG109:CD - MTA1:THR257:OG1	3.71617	Hydrogen Bond	Carbon H-bond
E2F1:HIS114:CE1 - MTA1:ASN378:O	1.89129	Hydrogen Bond	Carbon H-bond
MTA1:LEU137:CA - E2F1:SER131:OG	2.61621	Hydrogen Bond	Carbon H-bond
MTA1:SER322:CB - E2F1:ARG111:O	3.62722	Hydrogen Bond	Carbon H-bond
MTA1:ARG335:CA - E2F1:LYS289:O	3.4143	Hydrogen Bond	Carbon H-bond
MTA1:GLY379:CA - E2F1:TYR128:OH	3.24445	Hydrogen Bond	Carbon H-bond
E2F1:ASP436:OD2 - MTA1:TRP317	4.58869	Electrostatic	Pi-Anion
E2F1:ASP436:OD2 - MTA1:TRP317	4.48457	Electrostatic	Pi-Anion
E2F1:LEU191:CD2 - MTA1:TYR140	3.09206	Hydrophobic	Pi-Sigma
E2F1:LEU377:CD1 - MTA1:TYR355	3.59324	Hydrophobic	Pi-Sigma
E2F1:LEU435:CD2 - MTA1:TRP317	3.34605	Hydrophobic	Pi-Sigma
E2F1:PRO122 - MTA1:LEU137	4.65475	Hydrophobic	Alkyl
E2F1:LYS125 - MTA1:LEU160	5.0351	Hydrophobic	Alkyl
E2F1:ALA275 - MTA1:MET329	4.98148	Hydrophobic	Alkyl
E2F1:LYS289 - MTA1:VAL337	5.43881	Hydrophobic	Alkyl
E2F1:LYS289 - MTA1:LYS340	5.27173	Hydrophobic	Alkyl

E2F1:PRO292 - MTA1:MET329	4.86215	Hydrophobic	Alkyl
E2F1:PRO292 - MTA1:VAL369	3.91604	Hydrophobic	Alkyl
E2F1:LEU374 - MTA1:MET285	5.47113	Hydrophobic	Alkyl
E2F1:ARG422 - MTA1:LEU181	3.4059	Hydrophobic	Alkyl
MTA1:ARG189 - E2F1:PRO404	4.42438	Hydrophobic	Alkyl
MTA1:ALA224 - E2F1:VAL378	4.16551	Hydrophobic	Alkyl
MTA1:ARG282 - E2F1:ILE400	5.33682	Hydrophobic	Alkyl
MTA1:LYS373 - E2F1:ILE293	4.87702	Hydrophobic	Alkyl
MTA1:ALA382 - E2F1:MET362	5.44388	Hydrophobic	Alkyl
E2F1:HIS406 - MTA1:ARG189	4.86253	Hydrophobic	Pi-Alkyl

Table S1. Intermolecular hydrogen interactions between E2F1 and MTA1 in the top interacting pose.

S.No.	Mutation	Single amino acid mutation	
		Mutation energy (kcal/mol)	Effect of mutation
1	M:ASN132>ALA	-0.63	Stabilizing
2	M:GLU133>ALA	3	Destabilizing
3	M:THR134>ALA	2.23	Destabilizing
4	M:LEU137>ALA	-4.28	Stabilizing
5	M:LYS138>ALA	-3.51	Stabilizing
6	M:TYR140>ALA	1.33	Destabilizing
7	M:LEU160>ALA	1.15	Destabilizing
8	M:ASP179>ALA	-1.3	Stabilizing
9	M:LEU181>ALA	0.99	Destabilizing
10	M:ARG189>ALA	2.41	Destabilizing
11	M:ARG225>ALA	4.91	Destabilizing
12	M:HIS253>ALA	0.99	Destabilizing
13	M:ALA254>ALA	0	Neutral
14	M:ARG282>ALA	1.6	Destabilizing
15	M:MET285>ALA	3.52	Destabilizing
16	M:GLU287>ALA	-13.81	Stabilizing
17	M:GLU292>ALA	-2.1	Stabilizing
18	M:TRP317>ALA	-5.33	Stabilizing
19	M:LEU320>ALA	2.24	Destabilizing
20	M:SER322>ALA	0.07	Neutral
21	M:MET329>ALA	-1.47	Stabilizing
22	M:LYS331>ALA	-43.19	Stabilizing
23	M:ARG335>ALA	1.62	Destabilizing
24	M:VAL337>ALA	0.71	Destabilizing
25	M:LYS340>ALA	1.08	Destabilizing
26	M:LYS352>ALA	-3.17	Stabilizing
27	M:TYR355>ALA	-0.68	Stabilizing
28	M:VAL369>ALA	-0.07	Neutral
29	M:GLY379>ALA	-0.37	Neutral

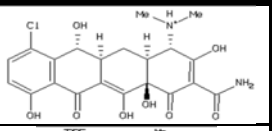
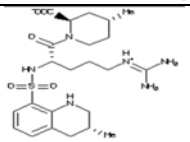
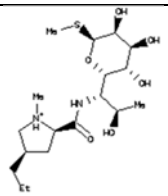
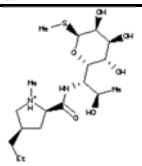
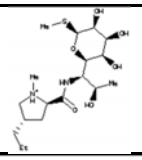
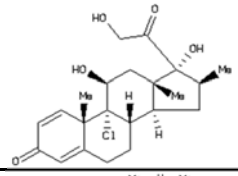
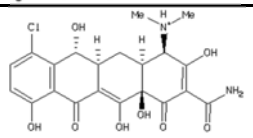
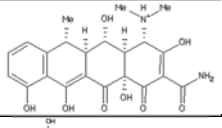
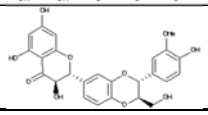
Table S2: Site directed mutagenesis for MTA1 amino acid residues interacting with E2F1.

S.No.	Mutation	Single amino acid mutation	
		Mutation energy (kcal/mol)	Effect of mutation
1	E:ARG109>ALA	3.1	Destabilizing
2	E:ARG111>ALA	3.56	Destabilizing
3	E:ARG113>ALA	1.69	Destabilizing
4	E:HIS114>ALA	2.09	Destabilizing
5	E:LYS120>ALA	3.78	Destabilizing
6	E:PRO122>ALA	0.12	Neutral
7	E:LYS125>ALA	0.87	Destabilizing
8	E:ARG127>ALA	-26.72	Stabilizing
9	E:TYR128>ALA	3.41	Destabilizing
10	E:THR130>ALA	0.67	Destabilizing
11	E:SER131>ALA	0.31	Neutral
12	E:GLN189>ALA	-0.51	Stabilizing
13	E:LEU191>ALA	1.05	Destabilizing
14	E:THR228>ALA	-2.38	Stabilizing
15	E:VAL262>ALA	0.14	Neutral
16	E:ASP277>ALA	2.15	Destabilizing
17	E:LYS289>ALA	0	Neutral
18	E:PRO292>ALA	-0.1	Neutral
19	E:ILE293>ALA	1.48	Destabilizing
20	E:GLU319>ALA	0.13	Neutral
21	E:GLU320>ALA	0.62	Destabilizing
22	E:LEU374>ALA	2.45	Destabilizing
23	E:LEU377>ALA	1.27	Destabilizing
24	E:VAL378>ALA	0.71	Destabilizing
25	E:ASP381>ALA	0.61	Destabilizing
26	E:ILE400>ALA	-1.22	Stabilizing
27	E:PRO404>ALA	0.81	Destabilizing
28	E:PRO405>ALA	0.53	Destabilizing
29	E:HIS406>ALA	0.23	Neutral
30	E:GLU407>ALA	-0.39	Neutral
31	E:ARG422>ALA	2.47	Destabilizing
32	E:LEU435>ALA	-0.71	Stabilizing
33	E:ASP436>ALA	-7.77	Stabilizing
34	E:PHE437>ALA	1.06	Destabilizing

Table S3: Site directed mutagenesis for E2F1 amino acid residues interacting with MTA1.

Rank	Gene	LOG2_FC	LOG2_FC	GO grouping
1	NTSR1	-6,47	-1,88	regulation of apoptotic process
2	HAS2	-7,06	-1,51	extracellular matrix adhesion/cell adhesion/cell migration
3	NOG	-6,41	-1,76	extracellular matrix adhesion/cell adhesion/cell migration
4	RIN2	-7,08	-1,14	intracellular transport
5	ITGB4	-5,6	-1,6	extracellular matrix adhesion/cell adhesion/cell migration
6	NPY1R	-4,83	-2,54	biosynthetic process
7	PYGB	-5,11	-1,56	immune system process
8	LXN	-5,8	-1,02	immune system process
9	AHNAK2	-5,09	-1,09	regulation of gene expression
10	FZD4	-4,13	-1,13	vasculogenesis
11	FN1	-3,62	-1,36	extracellular matrix adhesion/cell adhesion/cell migration
12	MUC5B	-3,02	-2,05	immune system process
13	SPRY4	-4,35	-1,07	regulation of MAPK cascade
14	TRIB2	-3,43	-1,28	regulation of MAPK cascade
15	SULF2	-4,02	-1,04	extracellular matrix adhesion/cell adhesion/cell migration
16	SPTSSB	-3,08	-1,1	biosynthetic process
17	HSPG2	-2,34	-1,17	vasculogenesis
18	TMEM200C	-2,2	-1,67	-
19	NFIC	-2,8	-1,11	regulation of gene expression
20	TBXAS1	-1,59	-1,69	vasculogenesis
21	PCOLCE2	-1,28	-1,79	immune system process
22	MYO1D	-2,22	-1,1	intracellular transport
23	FAM131B	-1,46	-1,15	extracellular matrix adhesion/cell adhesion/cell migration
24	CADM2	-1,03	-1,05	extracellular matrix adhesion/cell adhesion/cell migration

Table S4: List of downregulated genes after knockdown of E2F1 and MTA1. The ranking is based on the weighted sum of fold changes using the ratio of median $\log_2$ fold change of the downregulated targets in shE2F1 versus shMTA1 microarrays.

No.	Zink ID	Generic name	FIT SCORE	Biological properties	structure
1	ZINC08034234	Demeclocycline	3.2139	Antibacterial drug	
2	ZINC12466745	Argatroban	2.22492	Anticoagulant drug	
3	ZINC11592628	Lincomycin	2.11888	Antibacterial drug	
4	ZINC11592629	(2R,4S)-N-[(1S,2R)-2-hydroxy-1-[(2S,3S,4S,5S,6S)-3,4,5-trihydroxy-6-methylsulfanyl-tetrahydropyran-2	1.26729	Antibacterial drug	
5	ZINC03830996	Lincomycin hydrochloride	1.09165	Antibacterial drug	
6	ZINC04097285	Beclomethasone	0.970538	Antibacterial drug	
7	ZINC11592647	(4R,4aS,5aS,6R,12aR)-7-chloro-4-dimethylamino-3,6,10,12,12a-pentahydroxy-1,11-dioxo-4a,5,5a,6-tetrah	0.762399	Antibacterial drug	
8	ZINC16052277	DOXYCYCLINE HYDROCHLORIDE	0.718734	Antibacterial drug	
9	ZINC02033589	Silibinin	0.686234	Antioxidant	

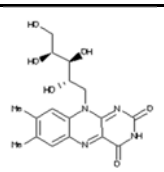
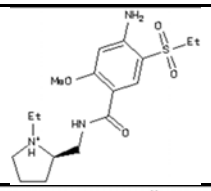
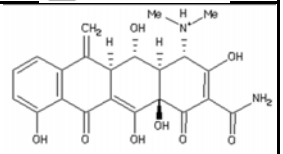
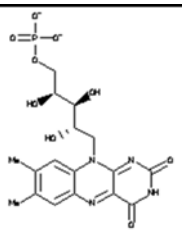
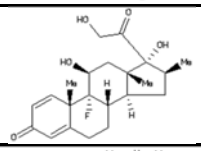
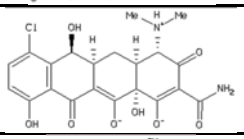
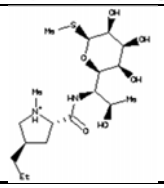
10	ZINC01769096	Vitamin B2 / Lyxoflavine	0.56853	Growth promoter for agricultural products	
11	ZINC00601255	Amisulpride	0.536854	Antipsychotic drug	
12	ZINC08034263	4-dimethylamino-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-4,4a,5,5a-tetrahydrotetracene-2-ca /Metacycline	0.484193	Antibacterial drug	
13	ZINC08551108	Flavin mononucleotide	0.452035	Vitamin	
14	ZINC03876136	Betamethasone	0.345639	Anti-inflammatory drug	
15	ZINC19795961	Demeclocycline hydrochloride	0.079402 8	Antibacterial drug	
16	ZINC03830997	Lincomycin hydrochloride	0.072037 7	Antibacterial drug	

Table S5. List of approved drugs/nutraceuticals mapped with the generated pharmacophore model.