

A proteomic analysis of an in vitro knock-out of miR-200c

Bojan Ljepoja^{1#¶}, Jonathan García-Roman^{1#¶}, Ann-Katrin Sommer¹, Thomas Fröhlich², Georg J. Arnold², Ernst Wagner¹, Andreas Roidl^{1*}

¹Pharmaceutical Biotechnology, Department of Pharmacy, Ludwig-Maximilians-Universität München, Munich, Germany

²Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, Ludwig-Maximilians-Universität München, Munich, Germany

* E-mail: andreas.roidl@cup.uni-muenchen.de

These authors contributed equally to this work.

S1

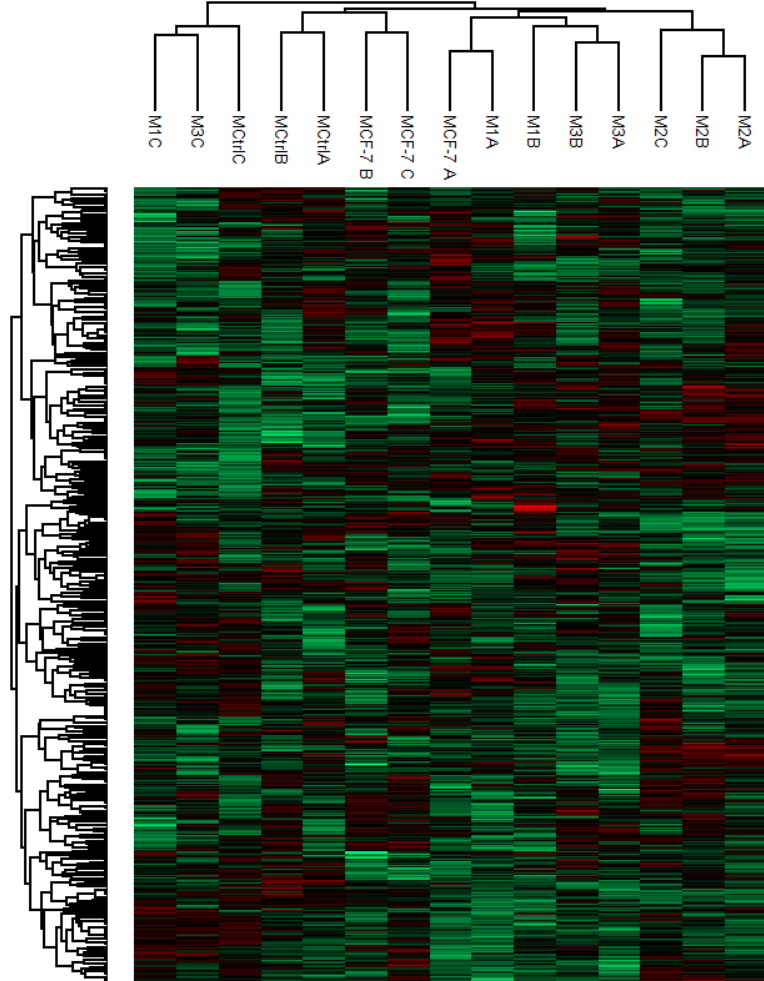


Figure S1 Clustering analysis of the measurements after vertical and horizontal z-score normalization

S2

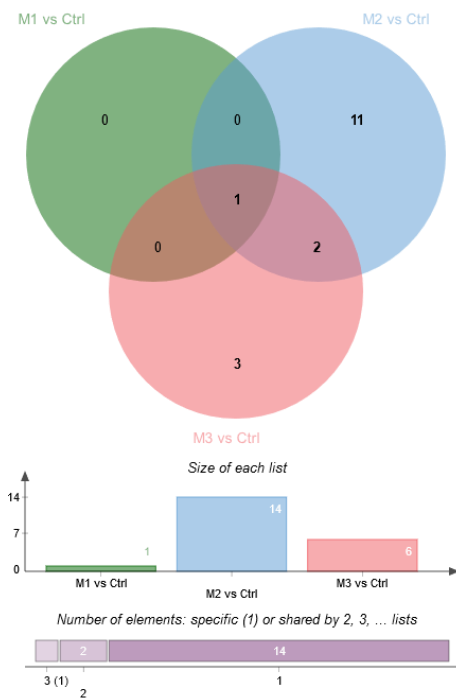


Figure S2 Venn-diagramm with results of the volcano blot analysis of M1 or M2 or M3 vs (MCF7 and MCtrl) each N=675 with 250 randomizations, FDR 0.05 and S0 of 0.1 as shown in Table 2

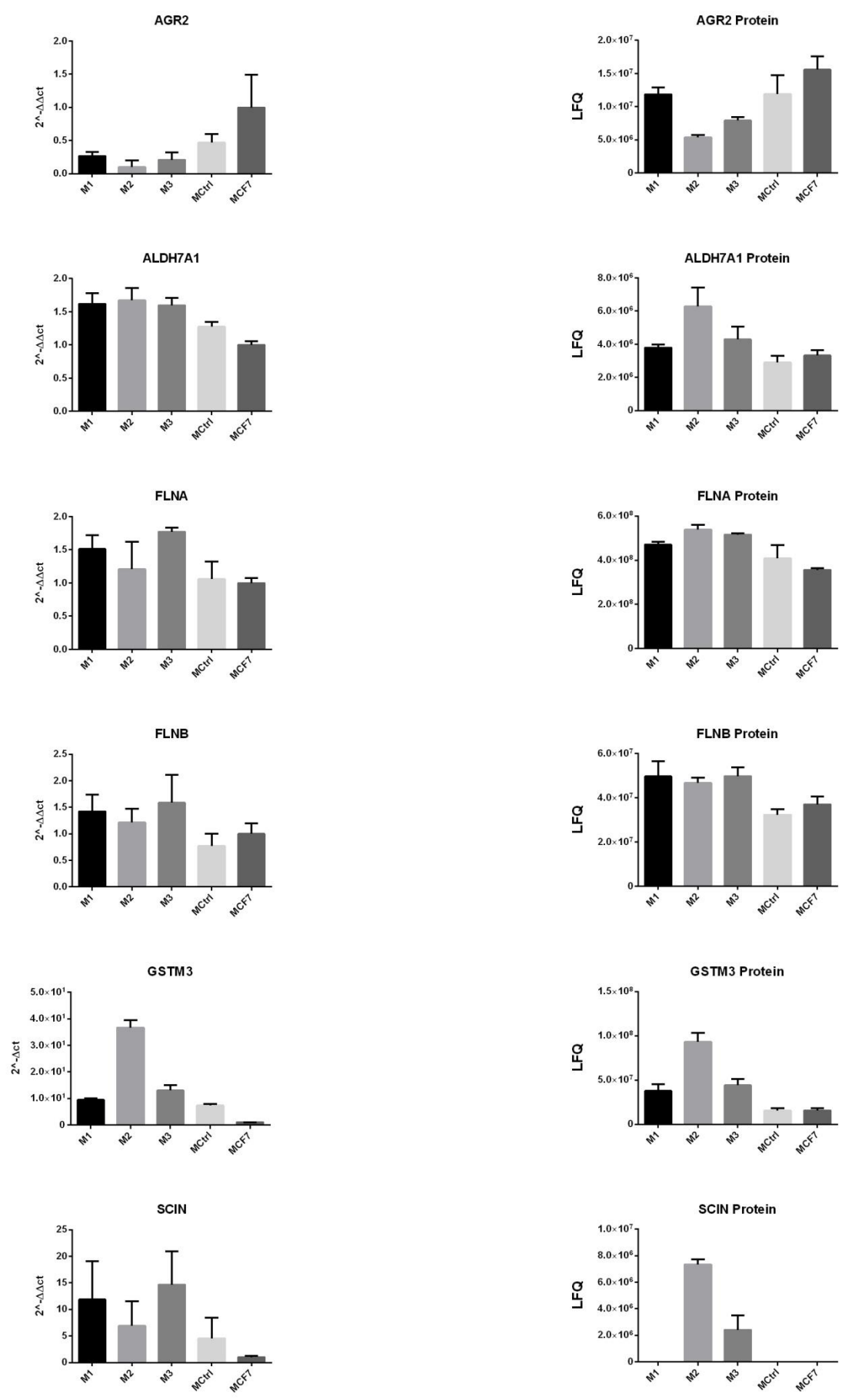
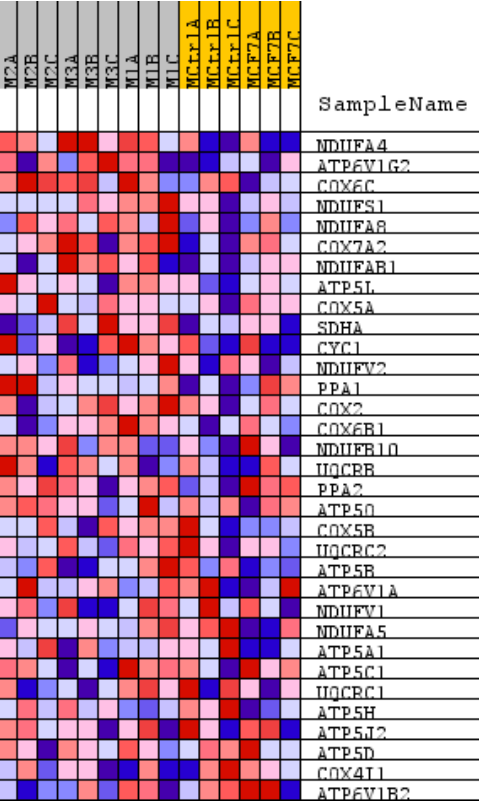


Figure S3 Detailed results of Figure 3F, with mRNA measurements compared to the protein expression data for each clone

KEGG OXIDATIVE
PHOSPHORYLATION



KEGG FOCAL ADHESION

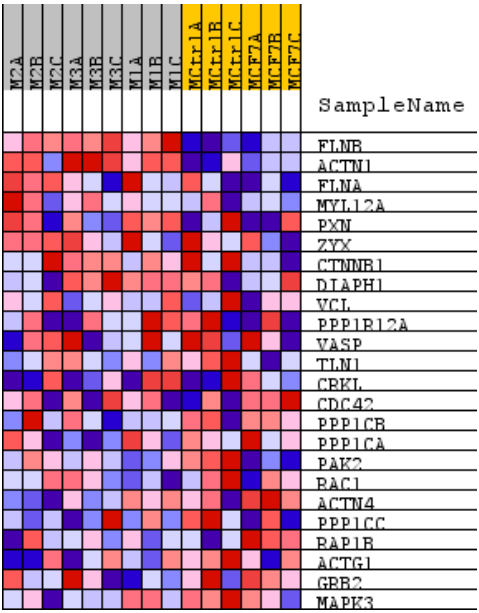


Figure S4 Heatmaps corresponding to the Enrichment blots in Figure 4C

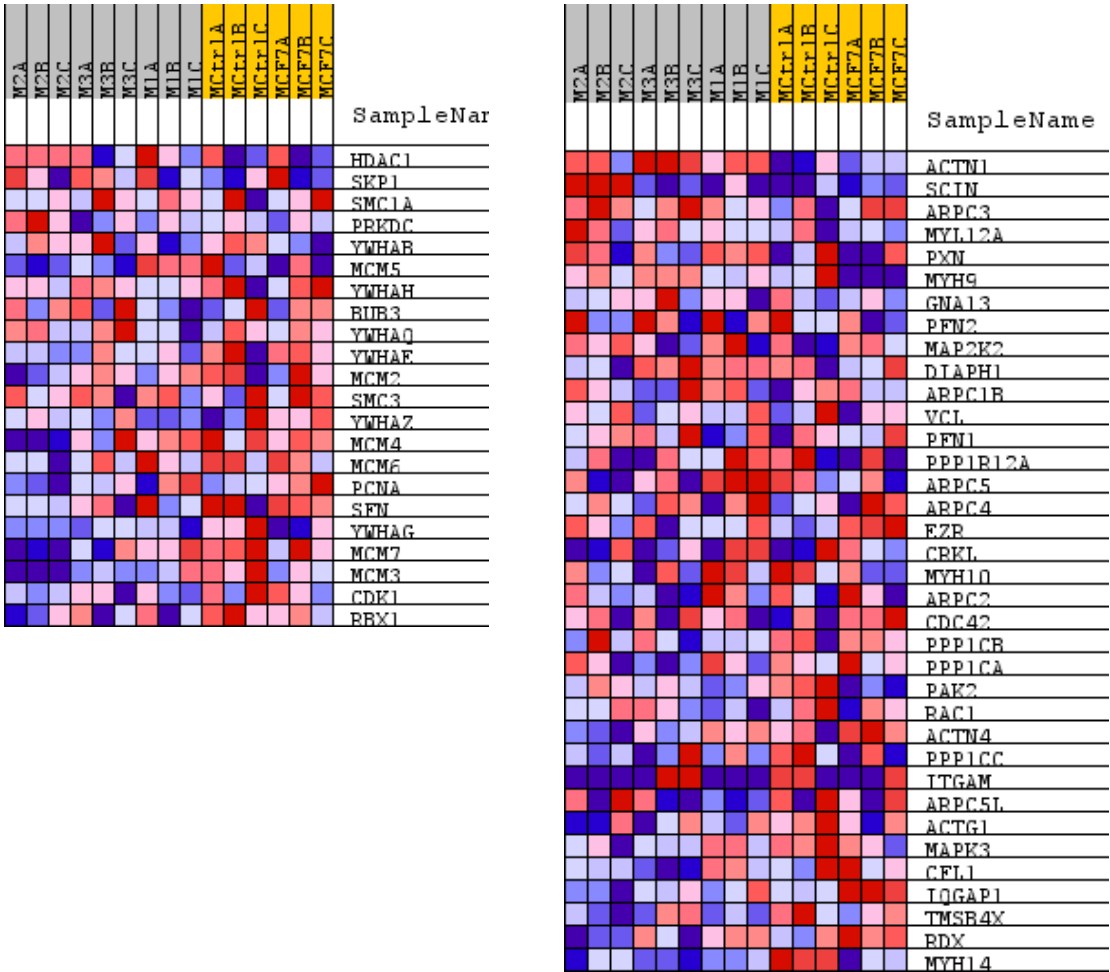


Figure S5 Heatmaps corresponding to the Enrichment blots in Figure 4D

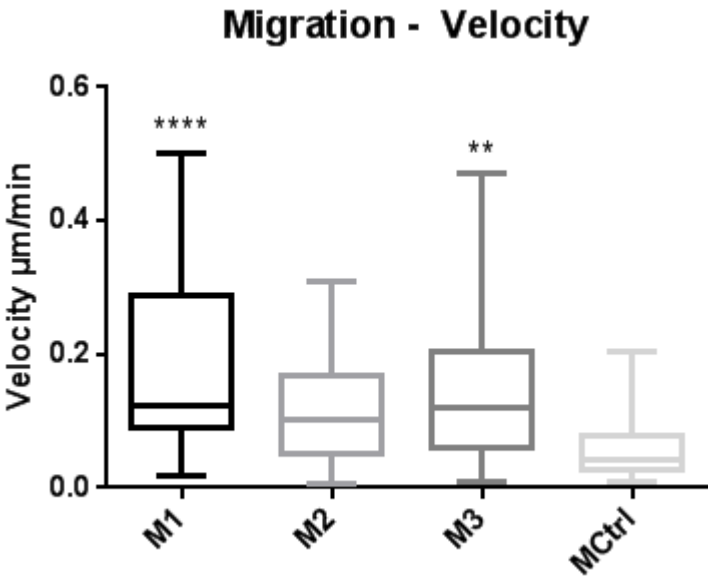


Figure S6 Evaluation of migration data in Figure 5B (N=30, * $p \leq 0.05$, one-way ANOVA with post-hoc Bonferroni's multiple comparison)

S7

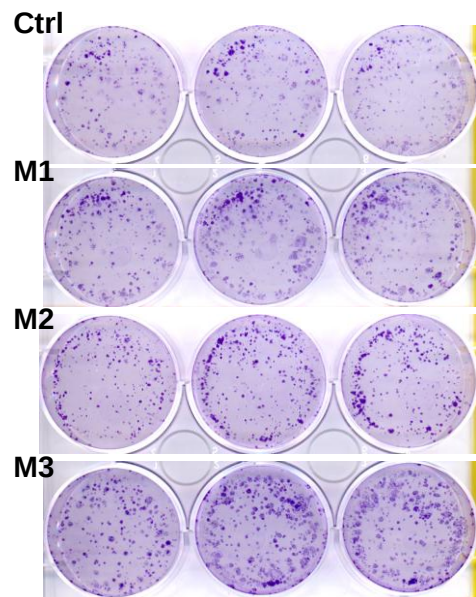


Figure S7 Clonogenic assay – imaging of colonies, as evaluated in the pooled analysis in figure 5d

S8

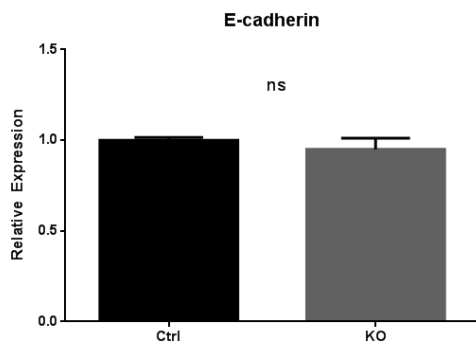


Figure S8 qPCR measurements of E-Cadherin mRNA levels in Ctrl vs KOs shows no significant difference

Table S1 15 / 32 gene sets are enriched in phenotype KO

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
KEGG PARKINSONS DISEASE	34	0.46907002	15.940.783	0.009861933	0.24380365	0.214	345	tags=50%, list=28%, signal=67%
KEGG OXIDATIVE PHOSPHORYLATION	33	0.48710477	15.471.032	0.018480493	0.17806831	0.293	345	tags=52%, list=28%, signal=70%
KEGG ALZHEIMERS DISEASE	35	0.420356	14.566.842	0.046	0.24040706	0.479	292	tags=40%, list=24%, signal=51%
KEGG CITRATE CYCLE TCA CYCLE	20	0.4615872	13.821.458	0.12048193	0.29699662	0.648	132	tags=30%, list=11%, signal=33%
KEGG HUNTINGTONS DISEASE	43	0.36943752	13.720.317	0.03508772	0.2523027	0.664	292	tags=42%, list=24%, signal=53%
KEGG GLUTATHIONE METABOLISM	15	0.44105875	13.544.401	0.08317215	0.23395455	0.703	78	tags=27%, list=6%, signal=28%
KEGG MAPK SIGNALING PATHWAY	16	0.4151188	13.413.316	0.10453649	0.22068681	0.734	136	tags=25%, list=11%, signal=28%
KEGG AMINOACYL TRNA BIOSYNTHESIS	20	0.44356683	13.299.485	0.15369262	0.20533033	0.744	263	tags=40%, list=21%, signal=50%
KEGG CARDIAC MUSCLE CONTRACTION	17	0.46564567	12.798.785	0.18257262	0.23733874	0.82	292	tags=53%, list=24%, signal=68%
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	15	0.42715377	12.191.079	0.24395162	0.285524	0.892	308	tags=47%, list=25%, signal=61%
KEGG PYRUVATE METABOLISM	15	0.49441242	11.757.169	0.28846154	0.31522772	0.919	122	tags=33%, list=10%, signal=37%
KEGG GLYCOLYSIS GLUCONEOGENESIS	23	0.39739954	11.214.875	0.33840305	0.35995352	0.95	200	tags=30%, list=16%, signal=36%
KEGG FOCAL ADHESION	24	0.30988422	10.625.255	0.36055776	0.41783723	0.972	277	tags=29%, list=22%, signal=37%
KEGG LYSOSOME	17	0.24309203	0.69935	0.9089184	0.94843	1.0	162	tags=18%, list=13%, signal=20%
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	17	0.19984435	0.660395	0.934236	0.92140806	1.0	195	tags=18%, list=16%, signal=21%

Table S2 17 / 32 gene sets are upregulated in phenotype Ctrl

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val	RANK AT MAX	LEADING EDGE
KEGG CELL CYCLE	22	-0.51119137	-16.219.078	0.035643563	0.19916053	0.172	348	tags=55%, list=28%, signal=75%
KEGG RIBOSOME	68	-0.31785846	-14.187.368	0.016746411	0.49701187	0.529	392	tags=41%, list=32%, signal=57%
KEGG ENDOCYTOSIS	20	-0.40655762	-13.007.351	0.12331407	0.70801485	0.78	193	tags=30%, list=16%, signal=35%
KEGG PURINE METABOLISM	23	-0.36597934	-11.775.029	0.24390244	0.9449636	0.925	14	tags=13%, list=1%, signal=13%
KEGG PROTEASOME	34	-0.30099234	-11.316.409	0.29045644	0.9364207	0.95	457	tags=53%, list=37%, signal=82%
KEGG ADHERENS JUNCTION	15	-0.35883263	-11.289.837	0.28879312	0.78668696	0.95	338	tags=53%, list=27%, signal=73%
KEGG TIGHT JUNCTION	20	-0.3472254	-10.904.311	0.3391473	0.7903667	0.964	294	tags=40%, list=24%, signal=52%
KEGG OOCYTE MEIOSIS	19	-0.3068673	-0.99786586	0.49278352	0.9669546	0.988	440	tags=58%, list=36%, signal=89%
KEGG UBIQUITIN MEDIATED PROTEOLYSIS	16	-0.319742	-0.979242	0.4989059	0.909404	0.991	251	tags=31%, list=20%, signal=39%
KEGG INSULIN SIGNALING PATHWAY	17	-0.29883376	-0.95862424	0.51827955	0.8756116	0.994	256	tags=35%, list=21%, signal=44%
KEGG REGULATION OF ACTIN CYTOSKELETON	36	-0.24238425	-0.9287425	0.6079295	0.8686062	0.998	275	tags=31%, list=22%, signal=38%
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	22	-0.28710213	-0.9233835	0.595092	0.8081302	0.998	514	tags=55%, list=42%, signal=92%
KEGG NEUROTROPHIN SIGNALING PATHWAY	17	-0.29578927	-0.90542006	0.5875831	0.7824528	0.999	440	tags=59%, list=36%, signal=90%
KEGG SPLICEOSOME	58	-0.22438549	-0.8703807	0.6956522	0.7967216	0.999	364	tags=38%, list=29%, signal=51%
KEGG PATHWAYS IN CANCER	27	-0.22919808	-0.7894147	0.8729839	0.8798361	0.999	338	tags=41%, list=27%, signal=55%
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	16	-0.24920684	-0.7617598	0.872	0.8646141	1.0	218	tags=25%, list=18%, signal=30%
KEGG SYSTEMIC LUPUS ERYTHEMATOSUS	16	-0.28722718	-0.7255098	0.8017058	0.8583328	1.0	362	tags=44%, list=29%, signal=61%

Table S3 – Overview of predicted transcription-factor binding sites

Gene	Quality* of predicted miR-200c binding-site
ALDH7A1	7mer-m8
CA2	7mer-A1
GSTM3	7mer-m8
KYNU	8mer / 7mer-m8 / 7mer-A1
SCIN	8mer / 7mer-A1

* as described on <https://www.targetscan.org>

[illegible]

TF: Transcription Factor, NA: Algorithm was not providing any binding site or the intergenic region was too short for valid prediction