Supplementary information

Functional parsing of driver mutations in the colorectal cancer genome reveals numerous suppressors of anchorage-independent growth

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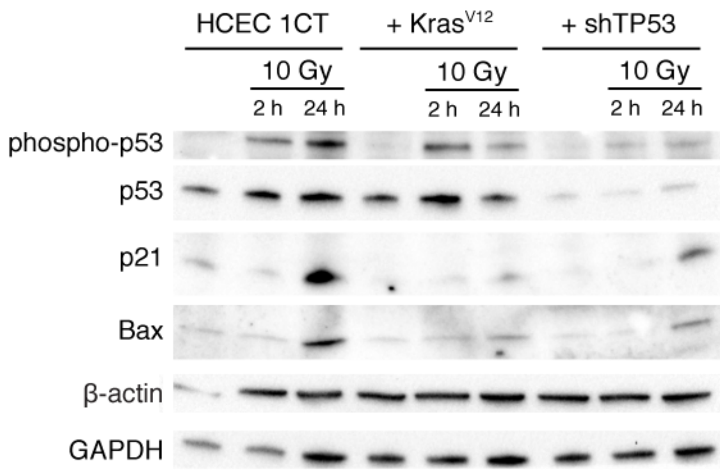
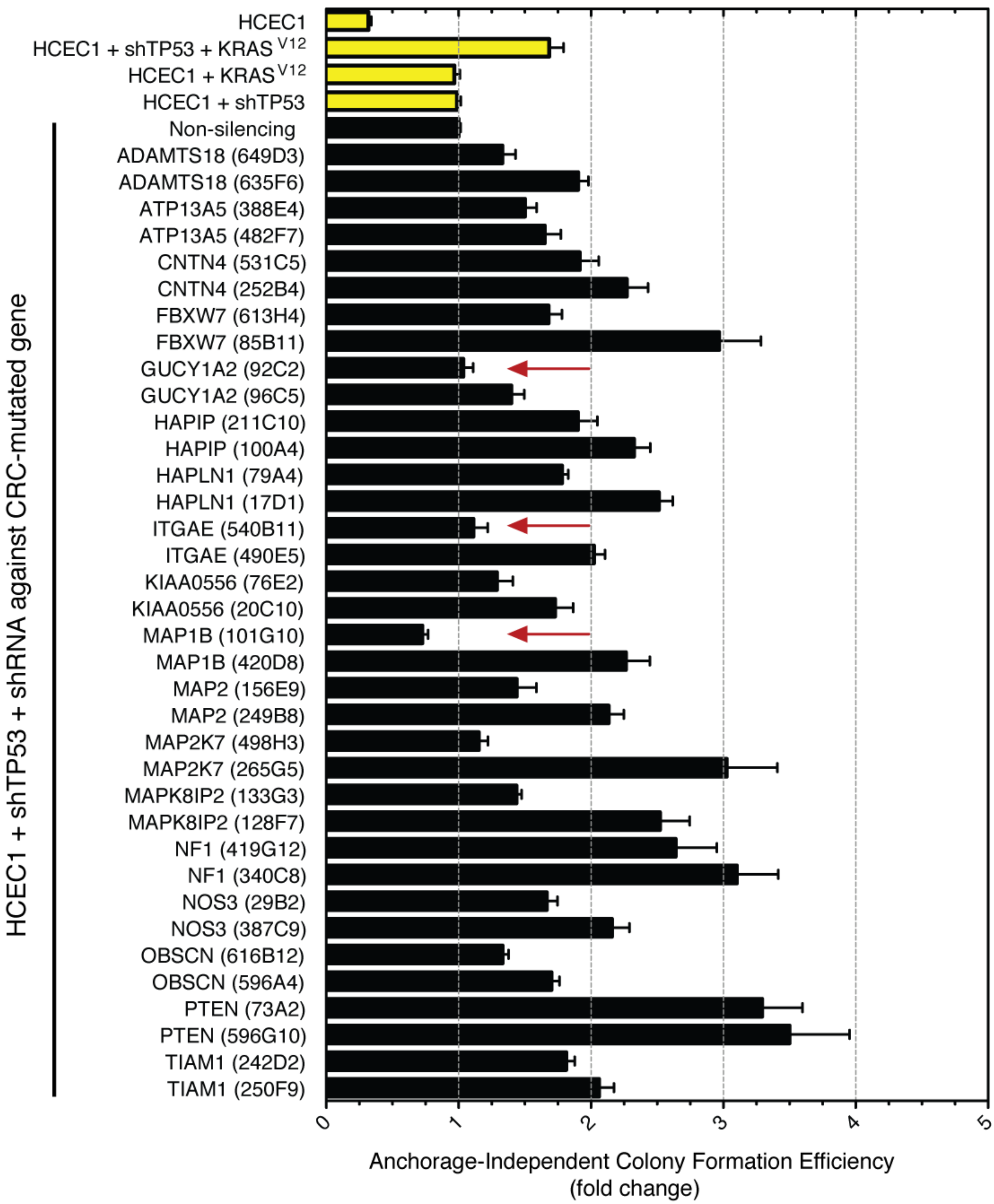
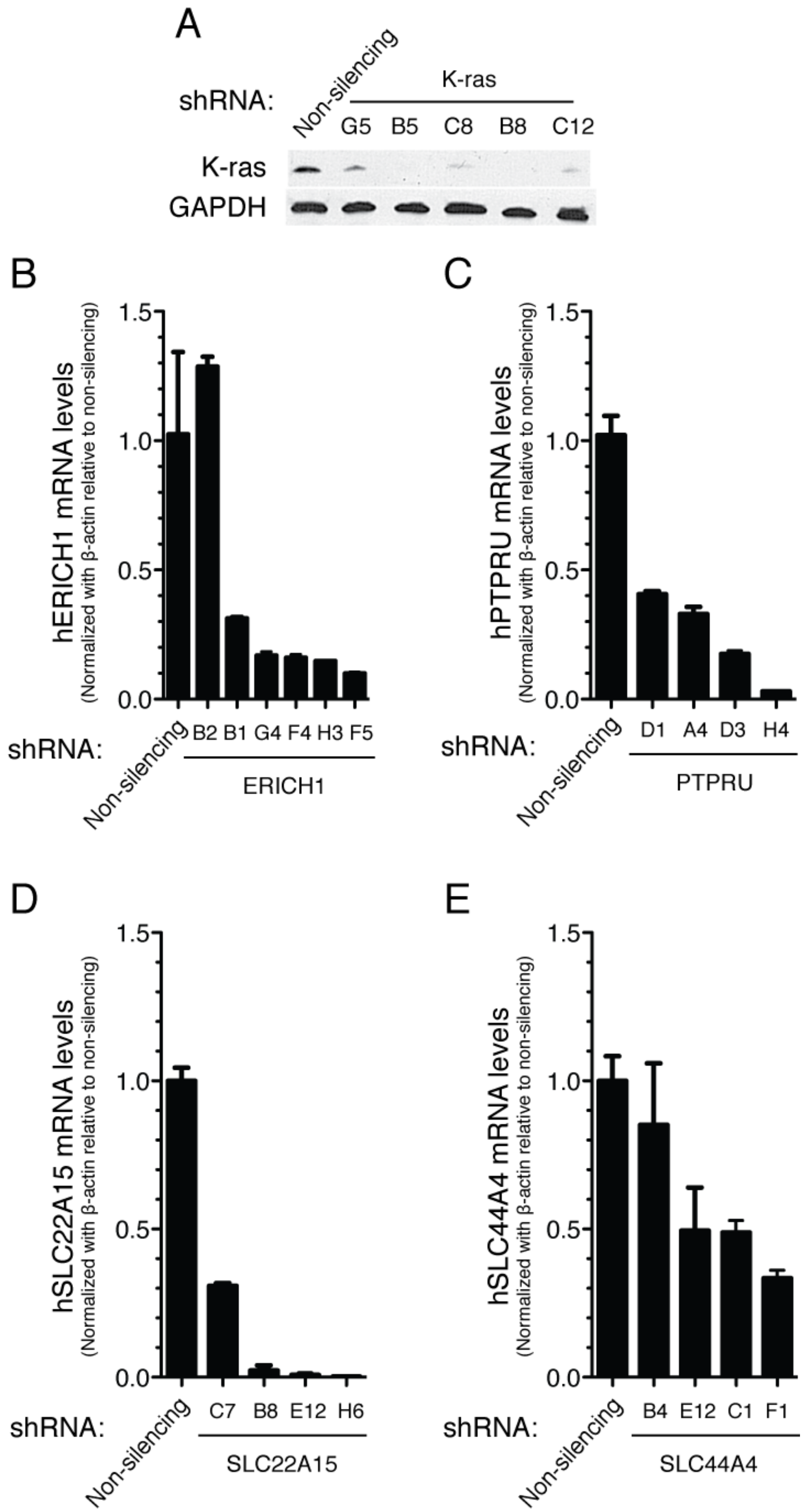


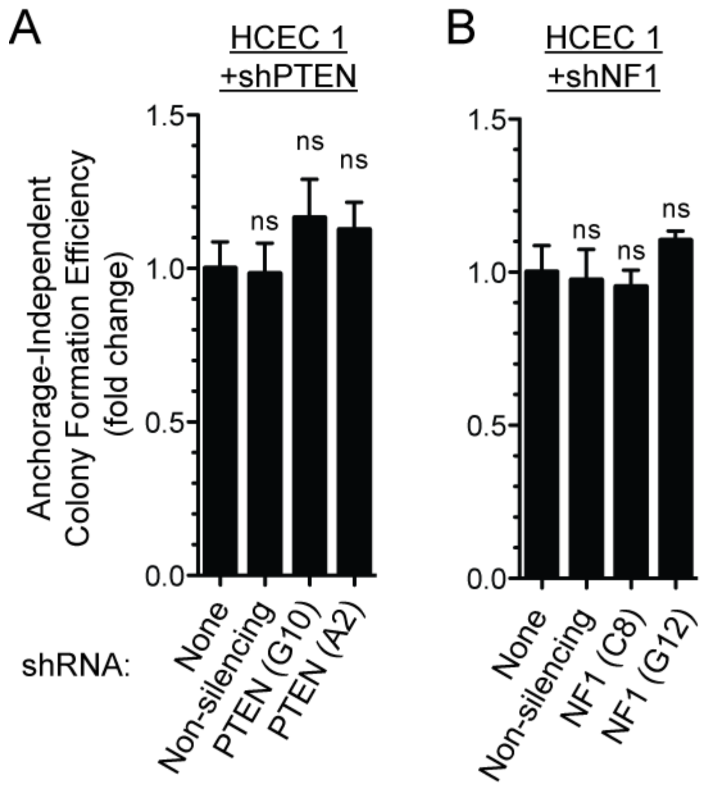
Supplementary Figure S1. K-ras^{V12} expressing cells are resistant to p53 induced apoptosis. Whole-cell extracts from immortalized K-ras^{V12} or p53 down regulated HCECs were immunoblotted with p53 and its down-stream effectors after 10 Gy gamma-radiation.



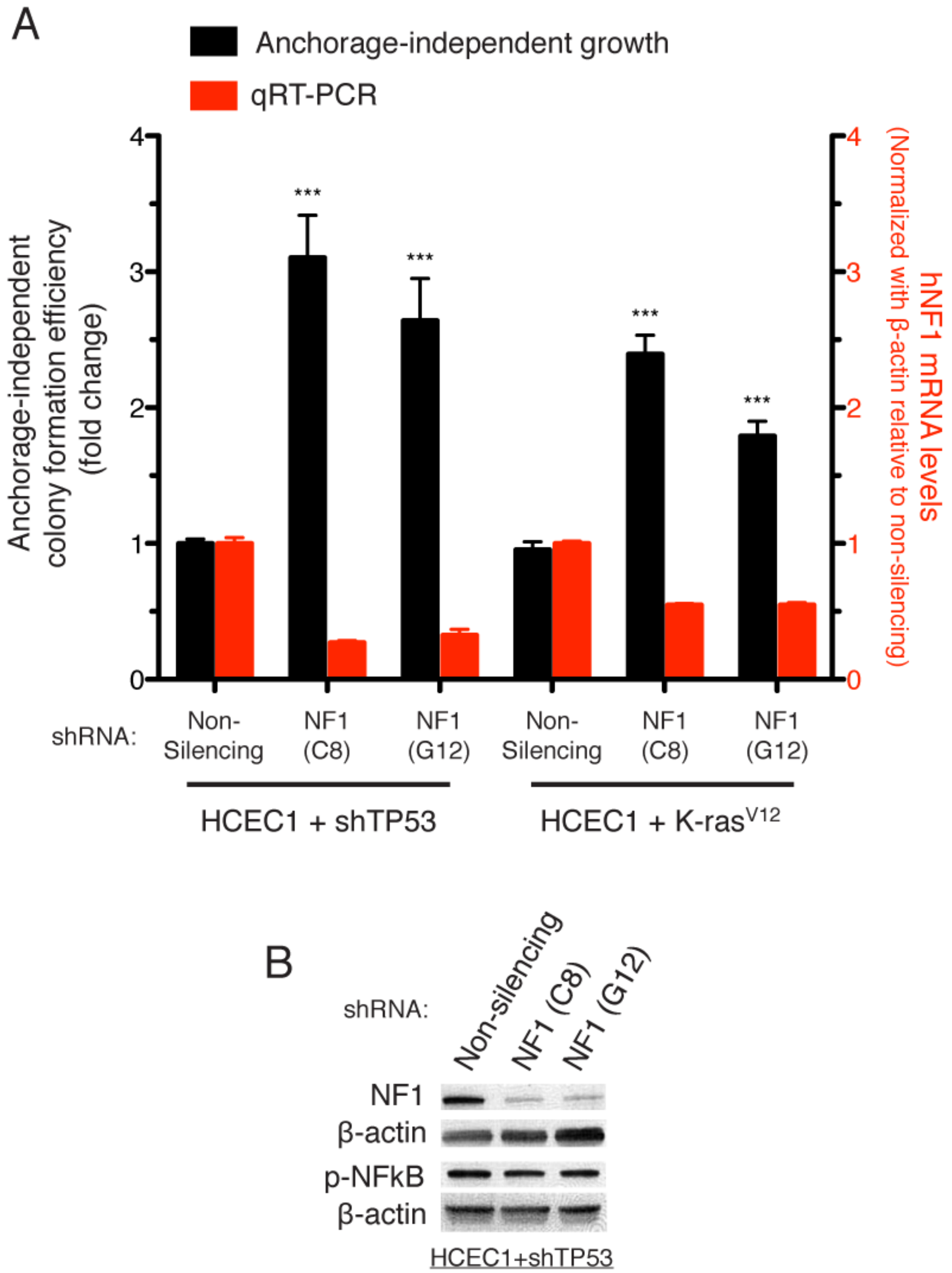
Supplementary Figure S2. Quantitative validation of selected shRNAs for their ability to enhance soft-agar growth of immortalized shTP53 expressing HCECs. Each bar represents 8 data points (quadruplicates from two separate experiments). Arrows denote shRNAs that failed to enhance anchorage-independent growth in a statistically significant manner. Enhancement for all other shRNAs were significant (two tailed Student's t-test, compared to none, mean ± s.e.m., $P<0.05$).



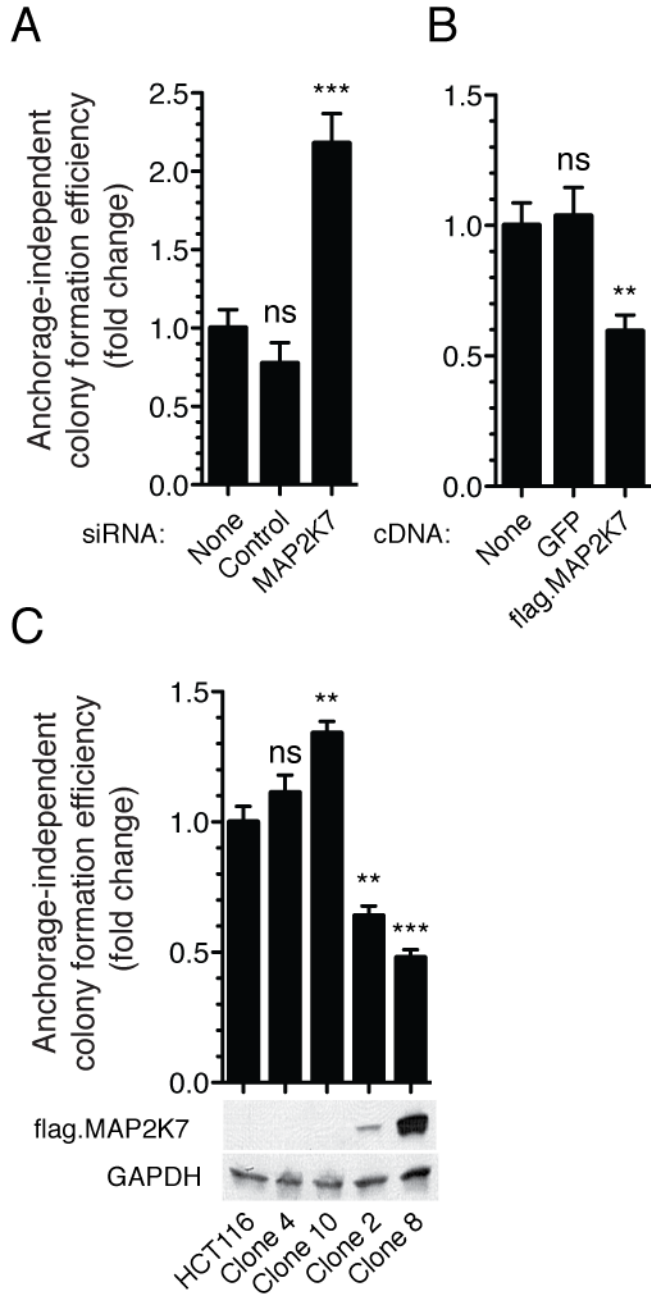
Supplementary Figure S3. Ability of shRNAs to knockdown expression was demonstrated by A, immunoblotting for K-ras or B-E, Quantitative RT-PCR for ERICH1, PTPRU, SLC22A15 and SLC44A4 48 hours after transfection into 293FT cells. Two out of 23 tested shRNAs did not provide any knockdown.



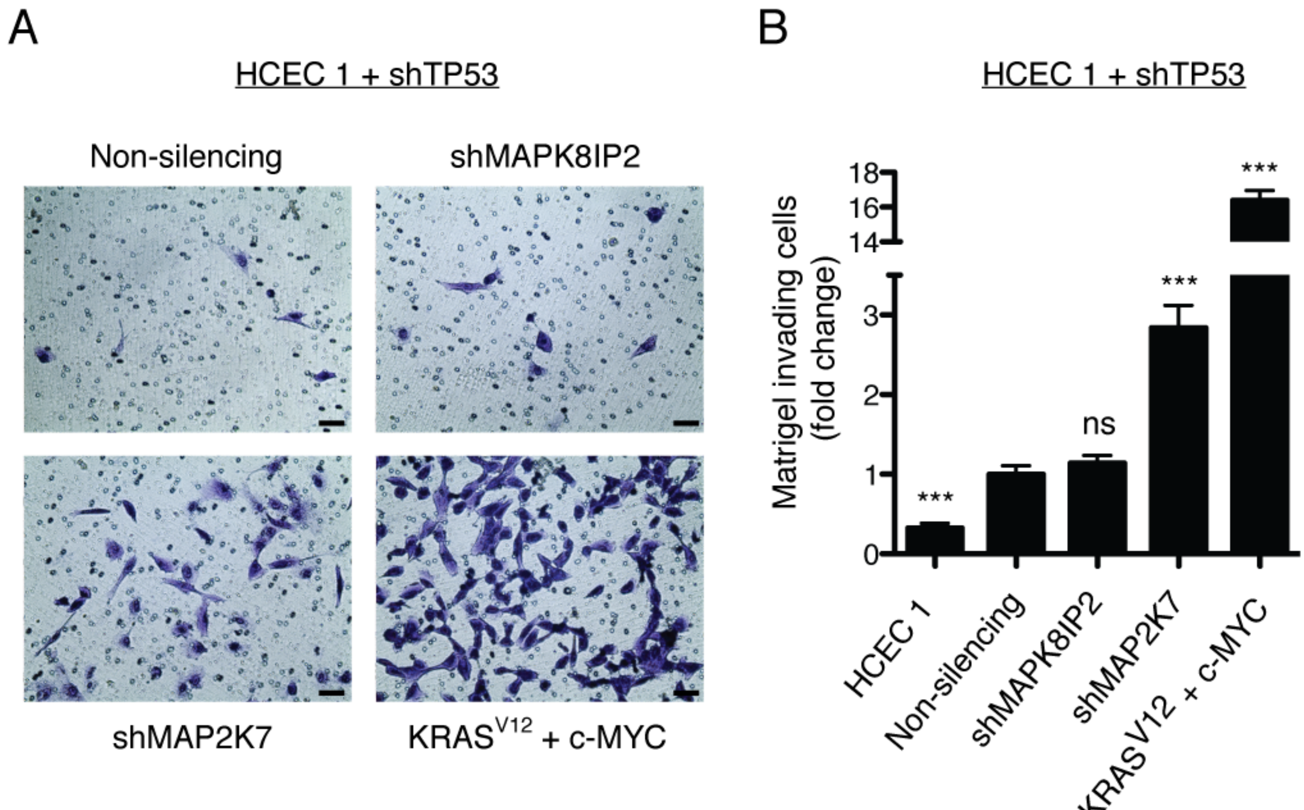
Supplementary Figure S4. shRNAs against A, PTEN and B, NF1 do not enhance soft agar growth in HCECs without oncogenic manipulations (Student's t-test, compared to none, mean ± s.e.m., ns= non-significant).



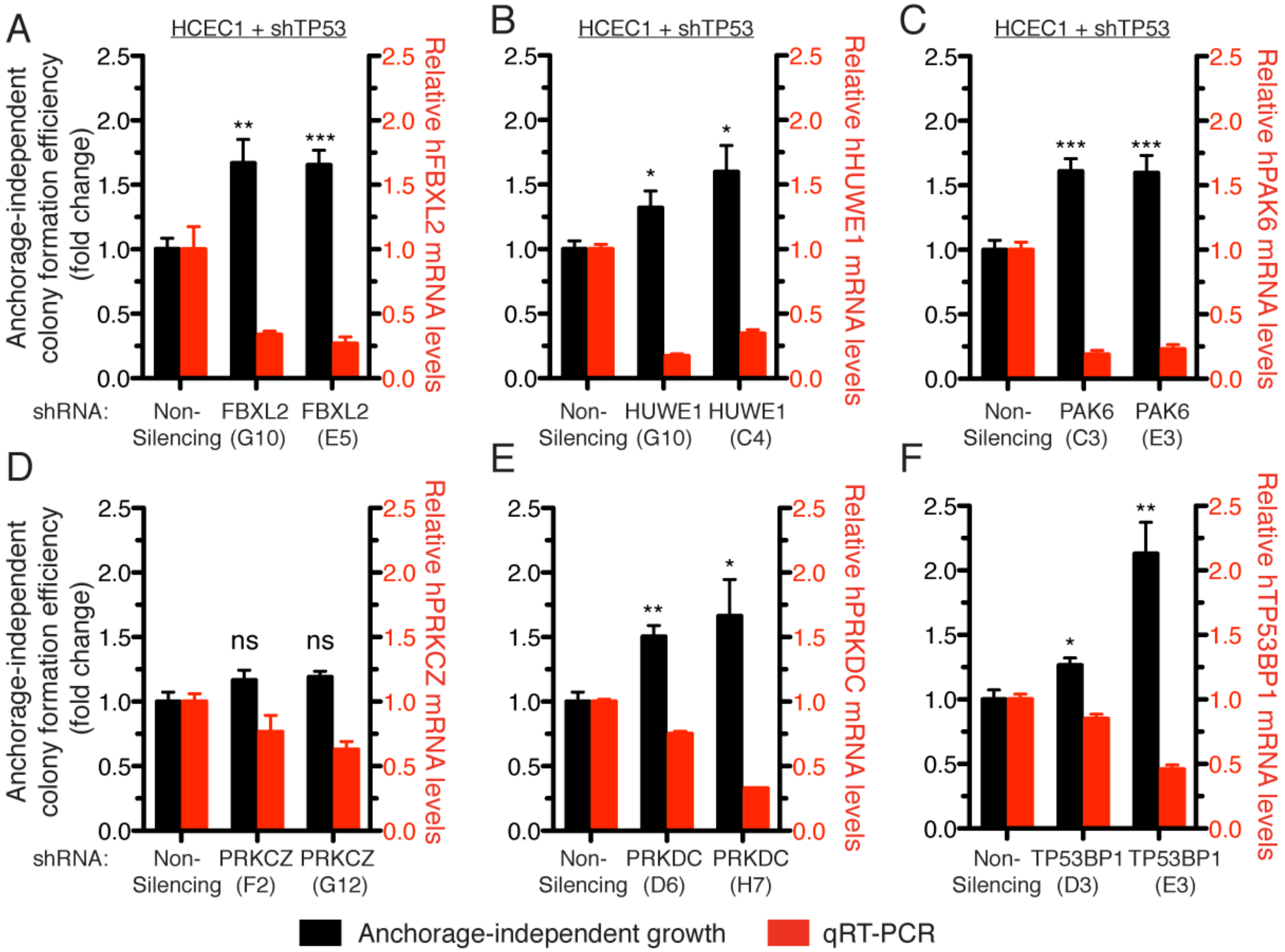
Supplementary Figure S5. A, shRNAs against NF1 enhance soft agar growth HCECs both in shTP53 and oncogenic K-ras background (left axis, black bars, two tailed Student's t-test, compared to non-silencing, mean ± s.e.m., ****P*<0.0001) and provide knock down measured by quantitative RT-PCR analysis for human NF1 mRNA (right axis, red bars). B, Whole-cell extracts from shTP53 expressing cells were immunoblotted for the indicated antibodies.



Supplementary Figure S6. A, HCT116 cells were transfected with control or MAP2K7 siRNAs, cultured in soft agar for two weeks and colonies larger than 0.2mm were counted. Each bar represents 8 data points (quadruplicates from two separate experiments). B, HCT116 cells were transfected with control or flag tagged MAP2K7 cDNAs, cultured in soft agar for two weeks and colonies larger than 0.2mm were counted. Each bar represents 8 data points (quadruplicates from two separate experiments). C, Cells in B selected for G418 resistance that is co-expressed from flag.MAP2K7 cDNA vector. Parental cells, two clones without detectable flag expression (as a control for cloning protocol and clonal variation) and two with varying flag expression were cultured in soft agar for a week and colonies larger than 0.2mm were counted. Each bar represents 6 data points (triplicates from two separate experiments, two tailed Student's t-test, compared to none, mean ± s.e.m., ns= non-significant, $^{**}P<0.001$, $^{***}P=0.0001$).



Supplementary Figure S7. shRNAs against *MAP2K7*, but not *MAPK8IP2*, enhance matrigel invasion of HCECs**.** A, Representative images (bars: 50 μm) B, quantification of A (two tailed Student's t-test, compared to non-silencing, mean ± s.e.m., ***P<0.0001).



Supplementary Figure S8. shRNAs against A, *FBXL2*; B, *HUWE1*; C, *PAK6*; D, *PRKCZ;* E, *PRKDC* and F, *TP53BP1* were tested for their ability to enhance anchorage-independent growth in p53 down-regulated cells (left axis, black bars, two tailed Student's t-test, compared to non-silencing, mean ± s.e.m., *P<0.05, **P<0.005, ***P<0.0001)) and provide knockdown measured by quantitative RT-PCR analysis for human mRNA levels (right axis, red bars).

Supplementary Table S1. List of all screened shRNAs targeting CRC-mutated genes

Gene Symbol	Clone ID	1CTR	1CTP
ABCA1	V2LHS_64364		
ABCA1	V2LHS_64362		
ABCA1	V2LHS_64365		
ABCB11	V2LHS_23959		
ABCB11	V2LHS_23961		
ABCB11	TRCN0000059362		
ACAN	V2LHS_193758		
ACAN	TRCN0000071732		
ACAN	V2LHS_193758		
ACSL5	V2LHS_115033		
ACSL5	V2LHS_115032		
ADAM29	V2LHS_252416		
ADAM29	V3LHS_397683		
ADAM29	V3LHS_397686		
ADAMTS15	V2LHS_70921	+	+
ADAMTS15	V3LHS_364310	+	
ADAMTS15	V3LHS_364309	+	
ADAMTS18	V2LHS_123010	+	+
ADAMTS18	V2LHS_70909		
ADAMTS18	V2LHS_123009		
ADAMTS18	V2LHS_70905		+
ADAMTS18	V2LHS_70907	+	
ADAMTS18	V2LHS_70906		+
ADAMTS20	V2LHS_272489		
ADAMTS20	V2LHS_136558	+	+
ADAMTS20	V3LHS_302842	+	+
ADAMTSL3	V2LHS_54992		
ADAMTSL3	V2LHS_54991	+	
ADAMTSL3	V2LHS_54989		
ADARB2	V2LHS_26757		
ADARB2	V2LHS_26756		+
ADARB2	V2LHS_26755		
AKAP12	V2LHS_220134		
AKAP12	V2LHS_221508	+	+
AKAP12	V2LHS_50557	+	+
AKAP12	V2LHS_50556		+
AKAP6	V2LHS_27894		+
AKAP6	V2LHS_27895		
ALK	V2LHS_70308		
ALK	V2LHS_15557		
APC	V2LHS_153492		
APC	V2LHS_240271		+
APC	V2LHS_153494		
APC	V2LHS_89656		
ARHGEF10	V2LHS_80181		
ARHGEF10	V2LHS_80180		
ATP11A	V2LHS_249221	+	
ATP11A	V2LHS_61340		
ATP11A	V2LHS_61338		
ATP13A1	V2LHS_34066		
ATP13A1	TRCN0000051608	+	
ATP13A1	TRCN0000051609	+	+
ATP13A5	V2LHS_179229		
ATP13A5	V2LHS_23942	+	+
ATP13A5	V2LHS_164725		+
ATP13A5	V2LHS_179230	+	+
ATP13A5	V2LHS_164724	+	
ATP13A5	V2LHS_23938	+	+
BCL9	V2LHS_15242	+	
BCL9	V2LHS_287024		
BCL9	V2LHS_287023		
C10orf137	TRCN0000116737		
C10orf137	TRCN0000144797		
C14orf115	V2LHS_156410		
C15orf2	V2LHS_51792	+	
C15orf2	V2LHS_51795		+
C15orf2	V2LHS_245404	+	
CACNA2D3	V2LHS_175409		
CACNA2D3	V2LHS_175404		
CD109	V2LHS_99968	+	+
CD109	TRCN0000073648		
CD109	TRCN0000073649		
CD248	V2LHS_34217		+
CD248	TRCN0000053453	+	+
CD248	TRCN0000053454		
CD46	V2LHS_151639		
CD46	V2LHS_151637	+	
CD46	V2LHS_151638	+	
CD46	V2LHS_151636	+	
CD93	V2LHS_28878		
CD93	TRCN0000029084		
CD93	TRCN0000029085		

Gene Symbol	Clone ID	1CTR	1CTP
CHL1	V2LHS_80198	+	+
CHL1	V2LHS_258073	+	+
CHL1	V2LHS_260545		
CHL1	V2LHS_91574		
CHL1	V2LHS_91578	+	+
CLSTN2	V2LHS_201036		+
CLSTN2	V2LHS_200976	+	
CLSTN2	V2LHS_11827		
CNTN4	V2LHS_38255	+	+
CNTN4	V2LHS_245484		
CNTN4	V2LHS_38256	+	
COL3A1	V2LHS_88556		
COL3A1	V2LHS_259244		+
COL3A1	TRCN0000003295	+	+
COL3A1	TRCN0000003293	+	
COL3A1	TRCN0000003294	+	+
COL3A1	TRCN0000091487	+	+
CPAMD8	V2LHS_225403		
CPAMD8	V2LHS_114636		+
CSMD3	V2LHS_166348		
CSMD3	V2LHS_119271		
CSMD3	V2LHS_119274	+	
CUX1	V2LHS_151077		
CUX1	V2LHS_151079		
DPP10	V2LHS_213795		+
DPP10	TRCN0000046663		
DPP10	TRCN0000046664		
DSCAML1	V2LHS_57527	+	
DSCAML1	V2LHS_57526	+	
DTNB	V2LHS_74472	+	
DTNB	V2LHS_74470		
DTNB	V2LHS_74471	+	
EPHA3	V2LHS_43360		
EPHA3	V3LHS_639472		
EPHA3	V3LHS_639471		
EPHB6	V2LHS_17714		
EPHB6	V2LHS_17715	+	+
EPHB6	V2LHS_17717		
EPHB6	V2LHS_17717		
ERCC6	V2LHS_182155		
ERCC6	V2LHS_182151		+
ERCC6	V2LHS_182153	+	
ERCC6	V2LHS_83786		+
ERCC6	V2LHS_100066	+	
ERCC6	V2LHS_83785	+	
ERCC6	V2LHS_182154		+
ERCC6	V2LHS_83784	+	+
ERGIC3	V2LHS_262029		
ERGIC3	V2LHS_134570		
ERICH1	V2LHS_163215		
ERICH1	V2LHS_163219		
ERICH1	V2LHS_91292		
ERICH1	V2LHS_91293		
ERICH1	V2LHS_163216		
ERICH1	V2LHS_91291		
EVL	V2LHS_266512		
EVL	V2LHS_115473		
EXOC4	V2LHS_81246		
EXOC4	V2LHS_81245		
EYA4	V2LHS_172982		
EYA4	V2LHS_172983		
EYA4	V2LHS_172985		
F8	V2LHS_255926	+	
F8	V2LHS_83667		+
F8	V2LHS_67564	+	+
F8	V2LHS_259026		
F8	V2LHS_256236		
FAM161A	V2LHS_216416	+	
FAM161A	V2LHS_254445	+	+
FAM161A	V3LHS_330732		
FAM193B	V2LHS_176467		
FAM193B	V2LHS_176466		
FBN2	V2LHS_151526		
FBN2	V2LHS_151530		
FBXW7	V2LHS_14559	+	+
FBXW7	V2LHS_254965	+	+
FBXW7	V2LHS_202810		+
FBXW7	V2LHS_203045		+
FBXW7	V2LHS_89326		+
FBXW7	V2LHS_89328		+
FBXW7	V2LHS_203036	+	+
FBXW7	V2LHS_202932	+	+

Gene Symbol	Clone ID	1CTR	1CTP
FBXW7	V2LHS_254965	+	+
FLNC	V2LHS_238475		+
FLNC	V3LHS_327303		
FLNC	V3LHS_327300		
FN1	V2LHS_113935		
FN1	V2LHS_113940		
FN1	V3LMM_433067		
GALNS	V2LHS_93416	+	
GALNS	V2LHS_93414	+	
GALNS	V3LHS_358773		
GJD4	V2LHS_39029	+	+
GJD4	TRCN0000074124		
GLI3	V2LHS_82965	+	+
GLI3	V2LHS_82970		
GLI3	V3LHS_372606		
GNAS	V2LHS_93436		+
GNAS	V2LHS_268469		+
GNAS	V2LHS_202713		
GPR112	V2LHS_215633		
GPR112	V2LHS_211038		
GPR158	V2LHS_90354		
GPR158	TRCN0000062923		
GPR158	TRCN0000062924		
GRID1	V2LHS_222616		
GRID1	TRCN0000063034		
GRID1	TRCN0000063033		
GRM1	V2LHS_130872	+	+
GRM1	V2LHS_130874		
GRM1	V2LHS_130870		+
GUCY1A2	V2LHS_130956		+
GUCY1A2	V2LHS_130958		+
GUCY1A2	V2LHS_130955		+
GUCY1A2	V2LHS_237217		
HAPLN1	V2LHS_150924	+	
HAPLN1	V2LHS_150925		
HAPLN1	V2LHS_191024	+	+
HAPLN1	TRCN0000150533	+	+
HIST1H1B	V2LHS_37955	+	+
HIST1H1B	V2LHS_37954	+	+
IGFBP3	V2LHS_111629	+	
IGFBP3	V2LHS_111628		+
IGFBP3	V2LHS_225584		
IGSF22	V2LHS_178658		
IGSF22	V2LHS_178659		+
IGSF22	V2LHS_178660	+	+
IRS4	V2LHS_28086	+	+
IRS4	V2LHS_28015	+	+
IRS4	V2LHS_28085		+
ITGAE	V2LHS_275031	+	+
ITGAE	V2LHS_133454	+	+
ITGAE	V2LHS_133451	+	+
KALRN	V2LHS_199006		
KALRN	V2LHS_263240		+
KALRN	V2LHS_262322	+	+
KALRN	V2LHS_46992	+	+
KALRN	V2LHS_46991		+
KCNQ5	V2LHS_34566		
KCNQ5	V2LHS_34562	+	
KCNQ5	V2LHS_34563	+	
KIAA0182	V2LHS_274950	+	+
KIAA0182	V2LHS_139767		+
KIAA0182	V2LHS_139766	+	+
KIAA0556	V2LHS_15592	+	+
KIAA0556	V2LHS_15593	+	
KIAA0556	V2LHS_240500	+	+
KIAA0556	V2LHS_241707		
KIAA1409	V2LHS_161077		
KIAA1409	V2LHS_161079		
KIAA2022	V2LHS_86535		+
KIAA2022	V2LHS_86537	+	+
KRAS	V2LHS_178283		
KRAS	V2LHS_202204		
KRAS	V2LHS_169384		
KRAS	V2LHS_203252		
KRAS	V2LHS_275818		
KRT73	V2LHS_286579		+
KRT73	V2LHS_193029		+
LAMA1	V2LHS_76891		
LAMA1	V2LHS_76887		
LCN9	V2LHS_284827		
LCN9	V2LHS_284623		
LGR6	V2LHS_28493		

Gene Symbol	Clone ID	1CTR	1CTP
LMO7	V2LHS_114789		
LMO7	V2LHS_114791	+	+
LMO7	V2LHS_114786	+	
LMO7	V2LHS_114787		
LMO7	V2LHS_114790		+
LRP2	V2LHS_48526		
LRP2	V2LHS_48528	+	+
MAP1B	V2LHS_209336	+	+
MAP1B	V2LHS_197483	+	+
MAP2	V2LHS_151558	+	
MAP2	V2LHS_151557	+	+
MAP2	V2LHS_151560	+	
MAP2K7	V2LHS_19417	+	+
MAP2K7	V2LHS_262276	+	+
MAPK8IP2	V2LHS_50504		+
MAPK8IP2	V2LHS_50507	+	+
MCM3AP	V2LHS_47913		
MCM3AP	V2LHS_47912		
MKRN3	V2LHS_56062		
MKRN3	V2LHS_56060		
MKRN3	V2LHS_56057		
MLL3	V2LHS_60368		+
MLL3	V2LHS_282577		+
MLL3	V2LHS_248916		+
MMP2	V2LHS_48434		
MMP2	V2LHS_48430		
MMP2	V2LHS_48431		
MYO18B	V2LHS_159519		
MYO18B	TRCN0000117367		
MYO18B	TRCN0000117368		
MYO19	V2LHS_137025		
MYO19	V2LHS_137024		+
MYO19	V2LHS_137020		+
MYO5C	V2LHS_26316		
NAV3	V2LHS_95965	+	
NAV3	V2LHS_95967	+	
NF1	V2LHS_189526	+	
NF1	V2LHS_193129	+	
NF1	V2LHS_76028		+
NF1	V2LHS_76027	+	+
NF1	V2LHS_260806	+	+
NF1	V2LHS_76029		+
NF1	V2LHS_190255		+
NF1	V2LHS_76032	+	
NOS3	V2LHS_111653		+
NOS3	V2LHS_111652		+
NOS3	V2LHS_264475		+
NTNG1	V2LHS_96052		+
NTNG1	V2LHS_96051		+
NTNG1	V2LHS_96054		
NUP210	V2LHS_176870		
NUP210	V2LHS_219299		
NUP210	V2LHS_176868		
OBSCN	V2LHS_28312	+	
OBSCN	V2LHS_238841		+
OBSCN	V2LHS_202462		+
OBSCN	V2LHS_202481	+	+
OR51E1	V2LHS_20776		
OR51E1	V2LHS_20775		
P2RX7	V2LHS_152543	+	+
P2RX7	V3LHS_379761		
P2RX7	V3LHS_379765		
P2RY14	V2LHS_95836		
P2RY14	V2LHS_95837		
P2RY14	V2LHS_95834	+	+
PCDH11X	V2LHS_86840	+	+
PCDH11X	V2LHS_86845		
PCDH11X	V3LHS_337355		
PCDHA9	V3LHS_336241		
PCDHA9	V3LHS_306867		+
PCDHA9	V3LHS_306865		
PHIP	V2LHS_55558	+	+
PHIP	V2LHS_174594	+	
PHIP	V2LHS_174592		+
PHIP	V2LHS_174591		
PHIP	V2LHS_174593		
PHIP	V2LHS_55555		
PIK3CA	V2LHS_1227		
PIK3CA	V2LHS_58550		
PKNOX1	V2LHS_47744		
PKNOX1	V2LHS_47746		
PLB1	V2LHS_22403		

Gene Symbol	Clone ID	1CTR	1CTP
PLCG2	V2LHS_262144		
PLCG2	V2LHS_170087		
PRDM9	V2LHS_189753		+
PRDM9	V2LHS_191050	+	+
PRKD1	V2LHS_170466		+
PRKD1	V2LHS_170467	+	+
PRKD1	V2LHS_170464	+	+
PRKD1	V2LHS_170465	+	+
PRKD1	V2LHS_170466		+
PRKD1	V2LHS_170467	+	+
PRKD1	V2LHS_170464	+	+
PRKD1	V2LHS_170465	+	+
PRUNE2	V2LHS_212836		
PRUNE2	V2LHS_204508	+	
PRUNE2	V2LHS_253448		
PRUNE2	V2LHS_207055		+
PTEN	V2LHS_119551	+	+
PTEN	V2LHS_265808		+
PTEN	V2LHS_231477	+	+
PTEN	V2LHS_92317	+	+
PTEN	V2LHS_92314		+
PTEN	V2LHS_92319		
PTEN	V2LHS_228508	+	+
PTEN	V2LHS_192536		+
PTPRD	V2LHS_221647	+	+
PTPRD	V2LHS_170974	+	+
PTPRD	V2LHS_211074	+	+
PTPRD	V2LHS_170972	+	+
PTPRD	V2LHS_170976	+	+
PTPRS	V2LHS_171032		
PTPRS	V3LHS_634063		
PTPRS	V3LHS_634062		
PTPRU	V2LHS_197528		
PTPRU	V2LHS_239079	+	
PTPRU	V2LHS_197656		
PTPRU	V2LHS_196429		
RASGRF2	V2LHS_201735		
RASGRF2	V2LHS_203362		
RASGRF2	V2LHS_202571		
RET	V2LHS_203395		
RET	TRCN0000040024		
RNF219	V2LHS_136271	+	+
RNF219	V2LHS_136273		+
RNF219	V3LHS_644374		
RNF219	V3LHS_644375		
ROBO1	V2LHS_32195		
ROBO1	V2LHS_32196		
ROBO1	V2LHS_32197		
RUNX1T1	V2LHS_15002		
RUNX1T1	TRCN0000096598		
SCN3B	V2LHS_175419		
SCN3B	V2LHS_175417		
SCN3B	V3LHS_358981		
SEMA3D	V2LHS_38000		
SEMA3D	TRCN0000063214		
SEMA3D	TRCN0000063213		
SFRS6	V2LHS_203007		
SFRS6	V2LHS_263096	+	+
SFRS6	V2LHS_263152	+	+
SFRS6	V2LHS_201643	+	
SFRS6	V2LHS_20637	+	+
SFRS6	V2LHS_20638		+
SFRS6	V2LHS_263096	+	+
SH3TC1	V2LHS_51198		
SH3TC1	TRCN0000062608	+	+
SH3TC1	TRCN0000062609		+
SHANK1	V2LHS_97792	+	+
SHANK1	TRCN0000136791		
SHANK1	TRCN0000138405		
SLC22A15	V2LHS_276310		
SLC22A15	V2LHS_275692		
SLC22A15	V2LHS_175529		
SLC22A15	V2LHS_175527		
SLC29A1	V2LHS_249880	+	
SLC29A1	V2LHS_61922	+	+
SLC29A1	TRCN0000043643	+	+
SLC44A4	V2LHS_158453		
SLC44A4	V2LHS_158451		
SLC44A4	V2LHS_158455		
SLC44A4	V2LHS_158456		
SMAD2	V2LHS_213986		
SMAD2	V2LHS_197144		+

Gene Symbol	Clone ID	1CTR	1CTP
SMAD2	V2LHS_206703		
SMAD2	V2LHS_285936		
SMAD2	V2LHS_216709		+
SMAD3	V2LHS_215032	+	
SMAD4	V2LHS_37199		
SMAD4	V2LHS_37196		
SMAD4	V2LHS_37198		
SMAD4	V2LHS_37195		
SMTN	V2LHS_94901		
SMTN	TRCN0000123229		
SMTN	TRCN0000123230		
SORL1	V2LHS_153328		
SORL1	TRCN0000062948		
SORL1	TRCN0000062949		
STAB1	V3LHS_386813		
STAB1	V3LHS_386815		
SYNE1	V2LHS_79914	+	
SYNE1	V2LHS_79910		
SYNE1	V2LHS_79911	+	
SYNE1	V3LHS_334366		
SYT14L	V2LHS_165008		
SYT14L	V3LHS_353747		
SYT14L	V3LHS_353743	+	+
TAF2	V2LHS_153737	+	+
TAF2	V2LHS_153736	+	
TAF2	V2LHS_153738		
TBX22	V2LHS_154236	+	
TBX22	V2LHS_154237		
TCERG1L	V2LHS_41904		+
TCERG1L	V2LHS_41900	+	+
TCERG1L	V2LHS_244266		+
TCF7L2	V2LHS_116684		+
TCF7L2	V2LHS_116688	+	+
TCF7L2	V2LHS_116683		+
TGFBR2	V2LHS_153994	+	+
TGFBR2	V3LHS_645438	+	+
TGM3	V2LHS_154012	+	+
TGM3	V2LHS_154017		
TGM3	TRCN0000036129		
TIAM1	V2LHS_154064	+	+
TIAM1	V2LHS_154060	+	+
TLR9	V2LHS_154388		
TLR9	V3LHS_635783		
TLR9	V3LHS_636051		
TNN	V2LHS_77556		
TNN	V2LHS_77554		+
TP53	V2LHS_93615	+	+
TP53	V2LHS_93613	+	+
TP53	TRCN0000003753		
TTLL3	V2LHS_223927		
TTLL3	TRCN0000048529		
TTLL3	TRCN0000048528		
UHRF2	V2LHS_149460	+	+
UHRF2	V2LHS_149462	+	+
UHRF2	V2LHS_149463		+
UQCRC2	V2LHS_220312	+	+
UQCRC2	V2LHS_276849		+
UQCRC2	V2LHS_171876		
ZMYM4	V2LHS_17818		
ZMYM4	V2LHS_17815		+
ZMYM4	V2LHS_241770		+
ZNF442	V2LHS_117028	+	
ZNF442	V2LHS_225430		
ZNF442	V2LHS_226701		+
ZNF442	V2LHS_190261	+	
ZNF442	V2LHS_30713		+
ZNF442	V2LHS_117027		
ZNF442	V2LHS_117029	+	
ZNF521	V2LHS_233336	+	
ZNF521	V2LHS_96617	+	
ZNF521	V2LHS_96615		

+ denotes enhanced growth in soft agar (in at least 6 out of 8 replicates).
Clone ID could be used to retrive shRNA sequences from Open Biosystem's website.

Supplementary Table S2. List of all screened shRNAs targeting random genes

Clone ID	Gene Symbols	1CTP
V2LHS_101114	HSF5	
V2LHS_101618	-	
V2LHS_101768	-	
V2LHS_103872	SLC35E2, RP11-345P4.4	
V2LHS_104531	-	
V2LHS_105645	-	
V2LHS_108415	-	
V2LHS_109255	-	
V2LHS_110154	-	
V2LHS_110452	-	
V2LHS_110633	-	
V2LHS_110767	-	
V2LHS_111327	RP11-151A6.2	
V2LHS_111896	ACACA	
V2LHS_112050	ALDH3A1	
V2LHS_112366	CHRNB1	
V2LHS_113117	CCR1	
V2LHS_113258	CSTF2	
V2LHS_113545	DPYSL2	
V2LHS_113750	EPAS1	
V2LHS_113857	FHIT	
V2LHS_114304	GTF2E2	
V2LHS_116102	MRPL51, RAB3IP	
V2LHS_116821	GPR63	
V2LHS_118342	MAS1L	
V2LHS_119091	ELOVL7	
V2LHS_119132	MORC3	
V2LHS_120978	LOC100130673, LOC100134684	
V2LHS_123189	ZNF624	
V2LHS_123448	-	
V2LHS_123619	-	
V2LHS_124058	-	
V2LHS_124550	-	
V2LHS_125451	-	
V2LHS_126622	-	
V2LHS_128925	-	
V2LHS_129834	-	
V2LHS_132025	GPR39	
V2LHS_133095	HMGB2	
V2LHS_134135	LY75	
V2LHS_134397	NOP5/NOP58	
V2LHS_135471	FOLR1	
V2LHS_135551	SOBP	
V2LHS_137268	DEPDC2	
V2LHS_137913	ARPC5L	
V2LHS_138593	C8orf53	
V2LHS_138639	CHCHD6	
V2LHS_138988	PLAT	
V2LHS_139519	EBF2	
V2LHS_140914	-	
V2LHS_141304	RSBN1L	
V2LHS_141373	LOC285501	
V2LHS_142260	-	
V2LHS_144752	RSHL3	
V2LHS_144815	-	
V2LHS_145056	-	
V2LHS_145188	LOC100131353	
V2LHS_145271	-	
V2LHS_146481	-	
V2LHS_147158	-	
V2LHS_148300	-	
V2LHS_150657	CLTCL1	
V2LHS_150823	COX7A2	
V2LHS_150860	CPM	
V2LHS_152058	MYD88	
V2LHS_152085	MYH11	
V2LHS_152804	SEPW1	
V2LHS_153498	SRP54, LOC650638	
V2LHS_15365	DNLZ	
V2LHS_153812	TCEB3	
V2LHS_154118	ADD3	
V2LHS_156230	HHAT	
V2LHS_156875	ZCCHC6	
V2LHS_157244	BBS10	
V2LHS_157296	C6orf103	
V2LHS_157493	MYH14	
V2LHS_158349	C10orf81	

Clone ID	Gene Symbols	1CTP
V2LHS_162266	KIAA2026	
V2LHS_163209	FBXO43	
V2LHS_165401	-	
V2LHS_165590	-	
V2LHS_165596	-	
V2LHS_16782	FBP2	
V2LHS_168539	-	
V2LHS_169173	-	
V2LHS_169387	-	
V2LHS_169577	-	
V2LHS_16967	GOLGB1	
V2LHS_169974	SERPINB8	
V2LHS_171239	RAP1A	
V2LHS_171350	TLR4	
V2LHS_171493	TPM4	
V2LHS_17212	-	
V2LHS_172145	ZNF264	
V2LHS_17240	TMC4	
V2LHS_172493	TAF15	
V2LHS_174041	C21orf55	
V2LHS_174072	LPCAT2	
V2LHS_174609	FLJ20712	
V2LHS_175228	RSBN1	
V2LHS_175239	CNO	
V2LHS_176261	RNF216	
V2LHS_176549	ING3	
V2LHS_176630	CRLS1	
V2LHS_177218	C19orf48	
V2LHS_179779	MOXD1	
V2LHS_18068	LOC149684	
V2LHS_180778	-	
V2LHS_181420	PGAP1	
V2LHS_181966	TMEM17	
V2LHS_182233	-	
V2LHS_182438	-	
V2LHS_182486	-	
V2LHS_183592	LOC284865	
V2LHS_184128	ORAI2	
V2LHS_184307	LOC286114	
V2LHS_184442	-	
V2LHS_184523	LOC286254	
V2LHS_185846	-	
V2LHS_18592	DYNC1I1	
V2LHS_186617	-	
V2LHS_18664	TBC1D20	
V2LHS_188068	LOC643166	
V2LHS_190449	LOC344328, LOC728162	
V2LHS_19083	LOC146325	
V2LHS_191378	-	
V2LHS_192413	PRO2012	
V2LHS_193874	-	
V2LHS_19417	MAP2K7	+
V2LHS_195354	-	
V2LHS_196157	USP26	
V2LHS_196370	LHFP	
V2LHS_196442	WWP2	
V2LHS_197782	LHX6	
V2LHS_199955	HYOU1	
V2LHS_20081	ZNF597	
V2LHS_202139	STK33	
V2LHS_204157	-	
V2LHS_204426	RNF151	
V2LHS_20445	RAD9B	
V2LHS_20531	MYO1B	
V2LHS_20685	LOC441086	
V2LHS_207008	-	
V2LHS_207961	-	
V2LHS_208532	-	
V2LHS_208575	-	
V2LHS_209406	-	
V2LHS_21029	HMGB4	
V2LHS_211123	-	
V2LHS_214308	INTS1	
V2LHS_214638	GPRIN1	
V2LHS_21650	-	
V2LHS_216983	LYRM7	
V2LHS_21728	-	

Clone ID	Gene Symbols	1CTP
V2LHS_217322	CDGAP	
V2LHS_218229	PLN	
V2LHS_218270	SMPD4, FLJ41352	
V2LHS_218527	TSPAN4	
V2LHS_218834	SLC16A10	+
V2LHS_219156	OR5K4	
V2LHS_219922	LOC130355	
V2LHS_220477	-	
V2LHS_220779	ORC1L	+
V2LHS_221197	ZNF85	
V2LHS_221407	ACTBL2	
V2LHS_221662	SERPINF1	
V2LHS_221966	PINX1	
V2LHS_222117	WDR44	
V2LHS_222727	LOC284262	
V2LHS_222883	UBE2V2	
V2LHS_222945	DEPDC1	
V2LHS_223321	PLS1	
V2LHS_223430	PIGA	
V2LHS_22475	-	
V2LHS_225011	NAP1L6	
V2LHS_225587	RCBTB2	
V2LHS_227034	C6orf32	
V2LHS_227310	CXCL12	
V2LHS_228842	ADSS	
V2LHS_229142	LETMD1	
V2LHS_230644	KRT10	
V2LHS_230654	NAT5	
V2LHS_23124	-	
V2LHS_232882	PKD2L1	
V2LHS_235586	HEATR5B	
V2LHS_235702	LY75	
V2LHS_236794	LOC283435	
V2LHS_237284	-	
V2LHS_23774	SRD5A2L2	
V2LHS_238193	POLR2B	
V2LHS_238546	TRIM46	
V2LHS_238690	VPS54	
V2LHS_238825	RAD54B	
V2LHS_239255	NR0B1	
V2LHS_239291	HNF4G	
V2LHS_240019	GTF3C5	
V2LHS_240036	ZFP62, LOC643836	
V2LHS_241222	-	
V2LHS_241276	GTF2A2	
V2LHS_242130	OR51E2	
V2LHS_242428	-	
V2LHS_242962	GPR39, LYPD1	
V2LHS_243155	ZFP62, LOC643836	
V2LHS_24350	C10orf6	
V2LHS_243686	-	
V2LHS_244007	RAB3GAP2	
V2LHS_245782	ZNF648	
V2LHS_246137	-	
V2LHS_246972	UBIAD1	
V2LHS_247631	-	
V2LHS_247865	-	
V2LHS_247951	EXOD1	
V2LHS_248572	-	
V2LHS_249542	UCHL3	
V2LHS_24967	LOC285401	
V2LHS_250042	-	
V2LHS_250215	PDZK1IP1	
V2LHS_251838	-	
V2LHS_25191	GLRX	+
V2LHS_251919	C3orf43	
V2LHS_252337	ZMYM6	
V2LHS_253357	LOC114227	
V2LHS_25459	-	
V2LHS_254594	USP8	
V2LHS_254879	HECTD3	
V2LHS_254965	FBXW7	+
V2LHS_255392	ALAD	
V2LHS_25671	-	
V2LHS_257412	LOC440131	
V2LHS_257476	STARD3	
V2LHS_257994	SPIN1	

Clone ID	Gene Symbols	1CTP
V2LHS_258949	-	
V2LHS_25956	-	
V2LHS_259783	NF2	
V2LHS_260651	-	
V2LHS_261232	-	
V2LHS_262372	ST5	
V2LHS_262537	VCAM1	
V2LHS_262629	ACYP2	
V2LHS_263096	SFRS6	+
V2LHS_264104	CCDC80	
V2LHS_264773	-	
V2LHS_265855	-	
V2LHS_268217	ARF4	
V2LHS_26908	-	
V2LHS_269098	-	
V2LHS_276463	MFSD5	
V2LHS_277826	POLR1B	
V2LHS_27935	LY86	
V2LHS_27966	RAE1	
V2LHS_28203	-	
V2LHS_285279	HSN2	
V2LHS_285980	C1orf49, C1orf220	
V2LHS_286436	RBM11	
V2LHS_29175	RNF183	
V2LHS_29248	C19orf40	
V2LHS_29339	RPUSD2	
V2LHS_29824	FLJ16124	
V2LHS_30865	MXD1	
V2LHS_31264	-	
V2LHS_31648	-	
V2LHS_31708	RAVER1	
V2LHS_32326	TTF2	
V2LHS_33383	SLC30A8	
V2LHS_33437	HIST1H4C	
V2LHS_33452	-	
V2LHS_35058	DEFA5	
V2LHS_3553	DUSP8	
V2LHS_36444	SLC16A7	
V2LHS_36857	SSPO	
V2LHS_37991	-	
V2LHS_38134	GRB10	
V2LHS_40790	-	
V2LHS_40923	-	
V2LHS_44557	-	+
V2LHS_45778	-	
V2LHS_45803	-	
V2LHS_47453	MBD3	
V2LHS_47505	MBD4	
V2LHS_47557	-	
V2LHS_47605	-	
V2LHS_49921	NR1D2	
V2LHS_49989	CCS	
V2LHS_50715	C11orf30	
V2LHS_51042	PMEPA1	
V2LHS_51716	SMARCAD1	
V2LHS_51754	C11orf60	
V2LHS_51796	DAZAP1	
V2LHS_52116	-	
V2LHS_52452	-	
V2LHS_52876	LOC127295	
V2LHS_53720	CNTNAP2	
V2LHS_54334	-	
V2LHS_54537	-	
V2LHS_55636	-	
V2LHS_56193	TP53BP1	+
V2LHS_56749	STK3	
V2LHS_58426	-	
V2LHS_59014	PDE4D	
V2LHS_59352	C9orf32	
V2LHS_60022	METTL7A	
V2LHS_60927	-	
V2LHS_61314	-	
V2LHS_61719	IDE	
V2LHS_61795	FUS	
V2LHS_62078	DEFB4, DEFB4P	
V2LHS_65159	LOH3CR2A	
V2LHS_66511	ANGPTL7	

Clone ID	Gene Symbols	1CTP
V2LHS_66661	MRPL23	
V2LHS_68122	PRELID2	
V2LHS_68738	LOC157381	
V2LHS_69357	EOMES	
V2LHS_69401	-	
V2LHS_69715	LYG2	
V2LHS_70215	-	
V2LHS_70516	UHRF1, LOC100133565	
V2LHS_70718	-	
V2LHS_70802	CCDC104	
V2LHS_71965	B4GALNT2	
V2LHS_72116	-	
V2LHS_72154	-	
V2LHS_72199	-	
V2LHS_72249	CAMK1G	
V2LHS_72587	-	
V2LHS_74033	HDAC9	
V2LHS_74041	C2orf43	
V2LHS_74615	ASXL1	
V2LHS_75292	LOC121906	
V2LHS_75840	-	
V2LHS_76027	NF1	+
V2LHS_78198	-	
V2LHS_80182	DMN	
V2LHS_81798	-	
V2LHS_82128	-	
V2LHS_82529	HK2	
V2LHS_82896	IRX6	
V2LHS_83245	FLJ32682	
V2LHS_83376	ANKRD57	
V2LHS_83817	ERCC3	
V2LHS_83945	EPB42	
V2LHS_83962	-	
V2LHS_84218	-	
V2LHS_84707	SYPL1	
V2LHS_86605	KIAA0922	
V2LHS_86870	-	
V2LHS_88470	CP	
V2LHS_88888	-	
V2LHS_89932	PSEN1	
V2LHS_89945	-	
V2LHS_90115	-	
V2LHS_90366	NMU	
V2LHS_91329	EML5	+
V2LHS_91553	-	
V2LHS_91573	-	
V2LHS_91751	KLHL18	
V2LHS_92232	PLA2G2A	
V2LHS_93320	COL17A1	
V2LHS_93663	DCX	
V2LHS_94561	APBB1	
V2LHS_95305	KIAA0141	
V2LHS_96867	LOC26010	
V2LHS_96902	PTCD1	
V2LHS_97427	HDGFRP3	
V2LHS_97516	EIF3EIP	
V2LHS_98768	NUDT9	
V2LHS_99249	SEH1L	
V2LHS_99336	TEX11	
Negative Control		
Negative Control		
Negative Control		
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Negative Control		
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Negative Control		
Negative Control		
Negative Control		
Negative Control		
Negative Control		
Negative Control		
Negative Control		
Negative Control		

+ denotes enhanced growth in soft agar (in at least 6 out of 8 replicates). Blue highlight denotes positive controls that were idenitified in our original screen. Green highlight denotes soft-agar enhancing random shRNAs
Clone ID could be used to retrive shRNA sequences from Open Biosystem's website.

Supplementary Table S3. Categorization of candidate tumor suppressor genes

Common Function or Process	Candidate tumor suppressors
Apoptosis	CHL1, CNTN4, DSCAML1#, MAP2, OBSCN, PRKD1, PTEN, TP53
ATPases	ATP13A1, ATP13A5, ERCC6, MYO19*
Cell adhesion	ADAMTS15, ADAMTS18, ADAMTS 20, CD248, CHL1, CNTN4, COL3A1, DSCAML1#, F8, HAPLN1, IGSF22, ITGAE, OBSCN, PTEN, PTPRD, TCERG1L
Chromatin remodeling/ transcription	ERCC6, HIST1H1B, KRT73*, MLL3*, PRDM9, SFRS6, SMAD2*, TAF2, TCERG1L, TCF7L2, TP53, UHRF2, ZMYM4*
Cytoskeleton remodeling	AKAP12, DTNB#, IGSF22, LMO7, MAP1B, MAP2, MYO19*, SYNE1#
ECM remodeling	CD248, COL3A1, ADAMTS15, ADAMTS18, ADAMTS20, F8, HAPLN1, PTPRD
JNK signaling	MAP1B, MAP2, MAP2K7, MAPK8IP2
Nitrous oxide signaling	GUCY1A2*, NOS3*
Small GTPase regulators	IGSF22, KALRN1, NF1, MYO19*, OBSCN, TIAM1
TGF-beta signaling	SMAD2*, TGFBR2
Ubiquitin-protein ligase	FBXW7, UHRF2
Unknown	C15orf2, FAM161A, KIAA0182, KIAA0556, KIAA2022, PHIP, RNF219, SH3TC1
Uncategorized	CD46#, GALNS#, GNAS*, GRM1, IRS4, KCNQ5#, NAV3#, NTNG1*, SLC29A1, UQCRC2

This table categorize candidate tumor suppressor genes into a common function or process based on gene ontology molecular function, biological process and/or literature curation. ECM: extracellular matrix. * enhanced soft agar growth only in p53 down-regulated cells or # only in K-ras^{V12} expressing cells.

Supplementary Table S4. Distribution of anchorage independent genes for each sequenced sample

Tumor*	All mutated genes*	CAN genes*	AI genes~	AI gene symbols~
co108	82	15	**4**	OBSCN, NTNG1, NOS3, IRS4
co74	123	27	**9**	TP53, TGFBR2, SYNE1, SFRS6, PRKD1, MLL3, GALNS, FBXW7, CNTN4
co92	90	15	**4**	TP53, MAP2, KRT73 HAPLN1, AKAP12
mx22	69	14	**5**	ZMYM4, TCERG1L, MAP1B, KIAA0182, GNAS
mx27	86	16	**6**	TIAM1, MYO19, KALRN, IGSF22, F8, DTNB
mx30	57	9	**3**	SMAD2, PRDM9, MAP2K7
mx32	76	19	**6**	UHRF2, TP53, SYNE1, NAV3, KCNQ5, FAM161A, ATP13A5, ADAMTS18
mx38	77	14	**8**	TP53, SH3TC1, RNF219, NF1, NAV3, KIAA2022, ITGAE, ERCC6
mx41	99	26	**14**	UQCRC2, TCF7L2, TAF2, SYNE1, PHIP, OBSCN, LMO7, GUCY1A2, GRM1, COL3A1, CD248, ATP13A1, ADAMTS20, ADAMTS15
mx42	81	14	**6**	TP53, PTPRD, MAPK8IP2, KIAA0556, DSCAML1, C15orf2
mx43	102	18	**5**	SYNE1, SLC29A1, PTEN, HISTH1B, CHL1

* Data from Wood et. al. ~ Data from this study. AI: Anchorage independent growth

This table lists 11 completely sequenced colon cancer samples with the number of all genes (Column 2) and *CAN*-genes (Column 3) mutated in each sample. Genes involved in anchorage independent growth that were identified through a loss of function screen in immortalized human colonic epithelial cells expressing either mutant K-*ras* or shRNA against *TP53* are listed in column 4. Approximately 37% of all *CAN*-genes in an individual tumor enhance anchorage independent growth when downregulated in these sensitized non cancer cells.

Supplementary Table S5. Anchorage independent colony formation efficiencies

Cell Type	No. of seeded cells per well	Colony Formation Efficiency (%)
HCEC 1CT	3000 - 6000	4.8
HCEC 1CT + shTP53	1000 - 2000	14.8
HCEC 1CT + KrasV12	1000 - 2000	14.5
HCEC 1CT + shTP53 + KrasV12	500 - 1000	25.3
HCEC 1CT + shTP53 + shPTEN	500 - 1000	52.5
VaCo576	500 - 1000	43.4
HCT116	250 - 500	60.1
DLD1	250 - 500	48.8
RKO	250 - 500	53.2
HCEC 2CT	3000 - 6000	4.6
HCEC 2CT + shTP53	1000 - 2000	14.4
HCEC 2CT + KRASV12	1000 - 2000	14.6

Supplemental Materials and Methods

Anchorage-independent colony formation assay. Generated cells were seeded in soft-agar as described (1) with following modifications: cells were seeded in two different densities (adjusted depending on the cell type) in 24-well plates, each density was seeded at least in triplicates and each assay was performed at least from two different cell suspensions at different times. Colony formation efficiency was calculated by average number of colonies counted per well divided by number of seeded cells. Non-silencing shRNA expressing cells were seeded with each assay to be used as normalization control to correct for plate-to-plate variations. Data is plotted as fold change compared to non-silencing or vector only infected cells. Absolute colony formation efficiencies of the parental lines as well as some of the HCEC derivatives are shown in Supplementary Table S5. GraphPad Prism 5 (GraphPad Software, Inc.) was used to plot data and perform two tailed Student's t-tests.

Transient transfections. For cDNA expression experiments 2 million HCT116 cells were transfected with 1 μg of flag.MAP2K7 or pMAX.GFP construct using Effectene as described above. Cells were collected and seeded in soft agar 24 hours after transfection, cultivated for 10 days and analysed as described above. Remaining cells were selected with 800 $\mu g\ ml^{-1}$ G418 to isolate clones with stable integrations. For siRNA experiments, 100nM pooled siMAP2K7 (M004016, Dharmacon) or siControl (D001206, Dharmacon) was transfected to HCT116 cells using RNAiMAX (InVitrogen) according to manufacturer's instructions. Cells were collected and seeded in soft agar 24 hours after transfection, cultivated for 10 days and analysed as described above.

Immunofluorescence. Cells were either seeded in monolayer or in soft-agar in chamber slides. Twenty four hours after seeding, cells were fixed and stained as previously described (1) with phospho-JNK antibody (Cell Signalling, 9251).

Immunobloting. Immunoblotting studies were performed as described previously (1) with following antibodies: p53 (Biomeda), phospho-JNK (Cell Signalling, 9251), JNK1/3 (Santa Cruz, SC-474), MAP2K7 (Santa Cruz, SC-25288), MAP2 (BD, 5190021018), Cleaved PARP (Cell Signalling, 9541), b-actin (Sigma, A1978), MAPK8IP2 (Abcam, 65211), GAPDH (Cell Signalling, 2118), APC (Calbiochem, OP44), KRAS (Santa Cruz, SC-30), PTEN (Cell Signalling, 9552), Flag (Sigma, F3165), NF1 (Santa Cruz, SC-67), phospho-NF-kB (Cell Signalling, 3031).

qRT-PCR. qRT-PCR assays were performed as described previously(1) for the following target genes: ERICH1 (5p primer, tgagccagaaacatgctgag; 3p primer, ccgctggcagtgtagagc), PTPRU (5p primer, ggagcaagtgcgaatcca; 3p primer, gaagtgttgaccatcaagtaggag), SLC22A15 (5p primer, ttgtcttattgtaatgtttcttccaga; 3p primer, gttaaaggcagcactgatggt), SLC44A4 (5p primer, cctggtattggattcttgttgc; 3p primer, ggcgcagaagcaagataaac), NF1 (5p primer, tgtacccatctattcaagcaaaaa; 3p primer, agtacaacatcaagcagatctgtaatc).

Network analysis. The comprehensive network of human genes was previously described (2). Briefly, the network was constructed by compiling protein-protein interactions in HPRD (3), Gene (4), BIND (5) and IntAct (6). Signaling interactions were compiled from Biocarta and KEGG (7) as well as through manual curation. Transcription factor-target interactions were obtained from ORegAnno (8) and TRANSFAC (9).

Functional interactions between genes were constructed based on the significance of overlap of their Gene Ontology (10). These interactions were supplemented by neighbouring interactions from the Reactome (11) and KEGG metabolic interactions. For the latter, an interaction to a pair of genes was assigned if the reactions performed by their respective enzymes shared a metabolite (e.g. Hexokinase II and glucokinase, shared metabolite: glucose phosphate).

Supplementary References

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