Supplementary Figures

Figure S1

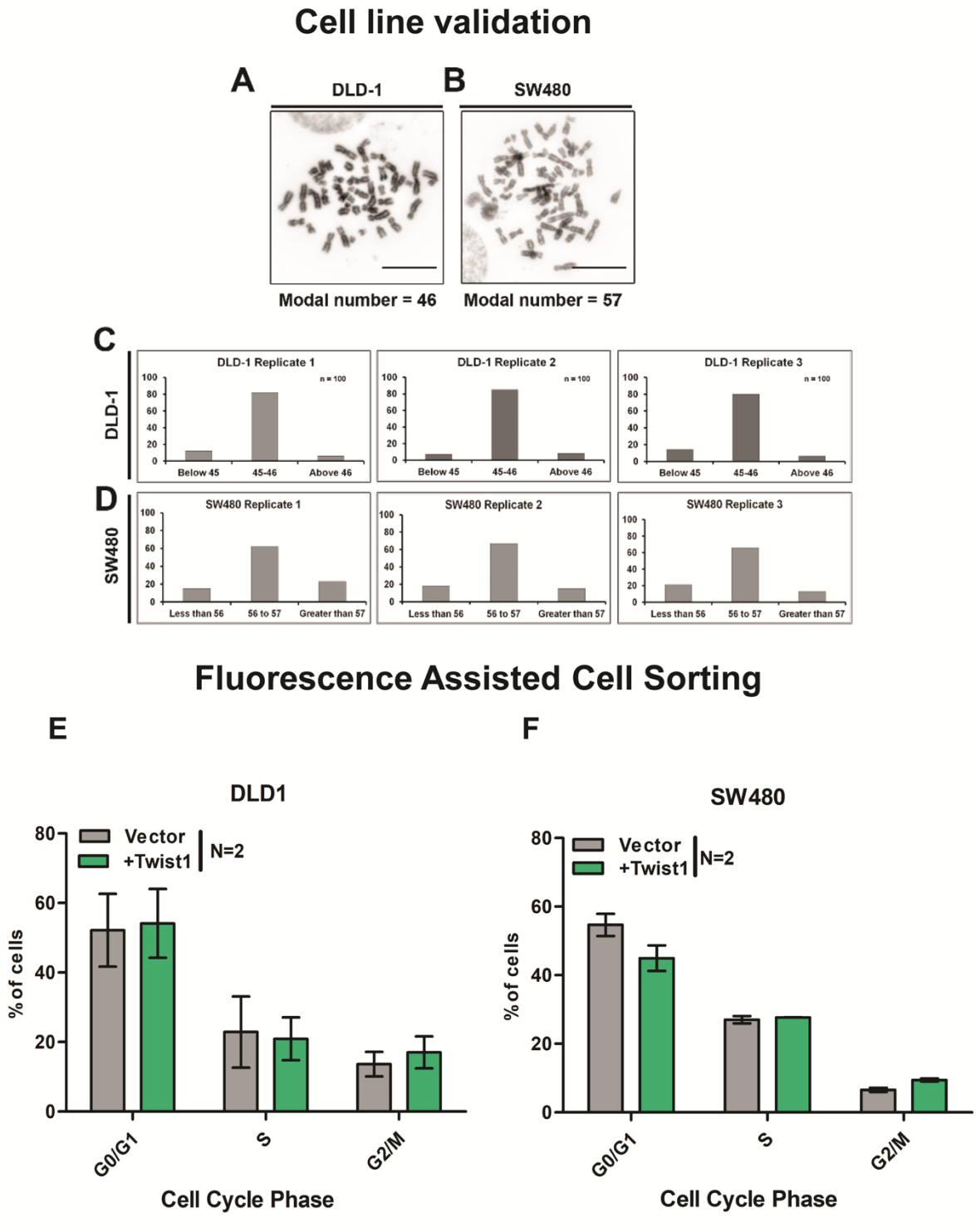
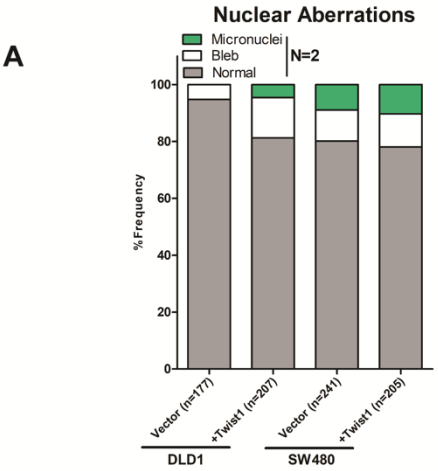


Figure S1: Cell line validation by chromosome counting and FACS

(A-B) Representative metaphase spreads of colorectal cancer cell lines DLD1 and SW480. **(C)** Quantification of chromosome numbers from metaphase spreads showing modal chromosome numbers of 44-45 for DLD1 cells. Data from N=3 independent biological replicates. Scale bar ~10 μm. **(D)** Quantification of chromosome numbers from metaphase spreads showing modal chromosome number of 56-57 for SW480 cells. Data from N=3 independent biological replicates. **(E-F)** Quantification of distribution of cells across cell cycle phases from Fluorescence Assisted Cell Sorting shows no significant effect of Twist1 overexpression on cell cycle. Data quantified from N=2 independent biological replicates (N=2, Mean with Range).

Figure S2 Nuclear and Mitotic Aberrations



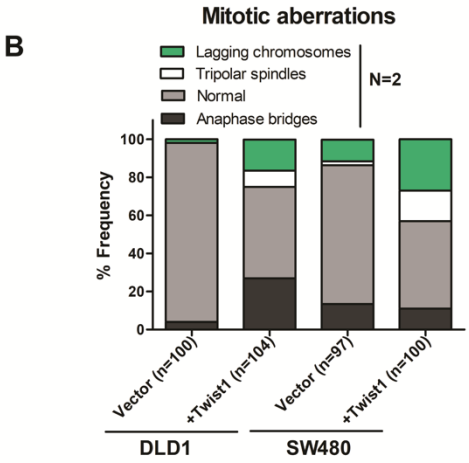
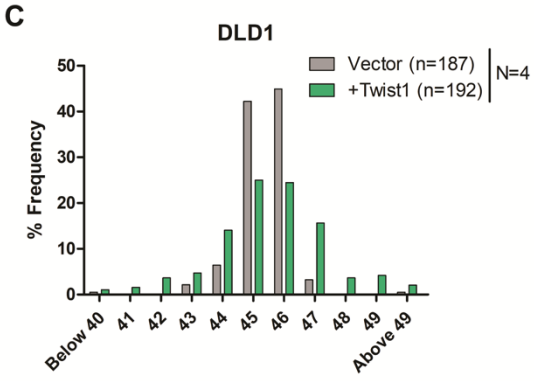


Table S1

	DLD1		SW480	
	Vector	+Twist1	Vector	+Twist1
Normal	95%	81%	80%	78%
Blebs	5%	14%	11%	12%
Micronuclei	0%	5%	9%	10%

Table S2

	DLD1		SW480	
	Vector	+Twist1	Vector	+Twist1
Lagging Chromosomes	2%	16%	11%	27%
Anaphase Bridges	4%	27%	14%	11%
Tri-polar Spindles	0%	9%	2%	16%
Normal Anaphases	94%	48%	73%	46%



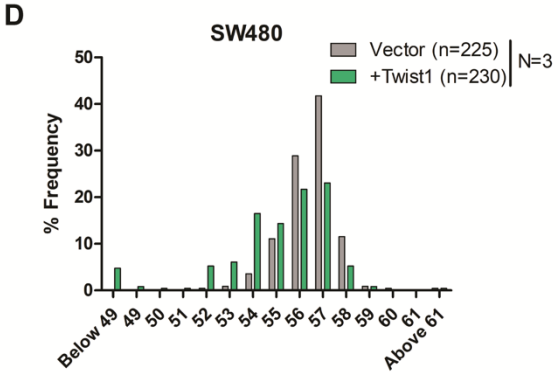
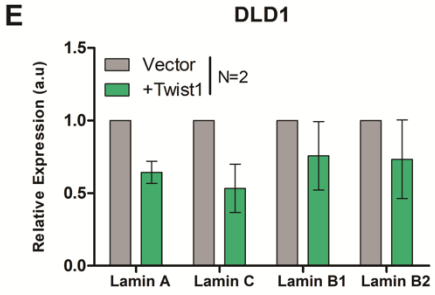


Table S3

	DLD1			SW480		
	<45	45-46	>46	<56	56-57	>57
Vector	9%	87%	4%	16%	71%	13%
+Twist1	25%	49%	26%	49%	45%	6%



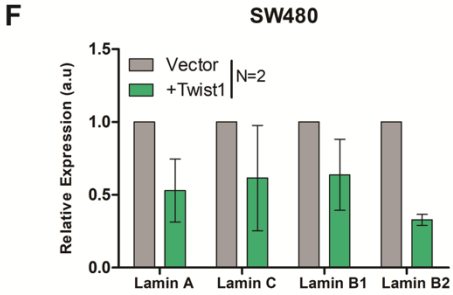


Figure S2: Nuclear and Mitotic Aberrations

(A) Quantification of nuclear aberrations plotted with frequency of normal cells observed across both cell lines (N=2, Mean). **(B)** Quantification of mitotic aberrations plotted with frequency of normal cells observed across both cell lines (N=2, Mean). **(Table S1)** Table representing the average % frequency changes in nuclear aberrations observed upon Twist1 overexpression across cell lines. Upon Twist1 overexpression, the % frequency of normal cells decreases, while the % frequency of blebs and micronuclei increases across cell lines. Data quantified from two independent biological replicates (N=2, Mean). **(Table S2)** Table representing the average % frequency changes in mitotic aberrations observed upon Twist1 overexpression across cell lines. Upon Twist1 overexpression, the % frequency of normal cells decreases, while the % frequency of anaphase bridges, lagging chromosomes and tripolar spindles increases in DLD1 cells. The % frequency of anaphase bridges decreases, while that of lagging chromosomes and tripolar spindles increases in SW480 cells. Data quantified from two independent biological replicates (N=2, Mean). **(C)** Representative histograms of chromosome count upon Twist1 overexpression across DLD1 cell line. Data quantified from n>180 independent metaphase spreads collected from four independent biological replicates (N=4). **(D)** Representative histograms of chromosome count upon Twist1 overexpression across SW480 cell line. Data quantified from n> 200 independent metaphase spreads collected from three independent biological replicates (N=3). **(Table S3)** Table representing the % frequency of gains and losses as compared to normal cells upon Twist1 overexpression. **(E-F)** Quantification of lamin levels upon Twist1 overexpression in **(E)** DLD1 **(F)** SW480**, (Fig.2I).** Quantification of band intensities from two independent biological replicates, normalized to GAPDH (N=2, Mean with Range).

Figure S3

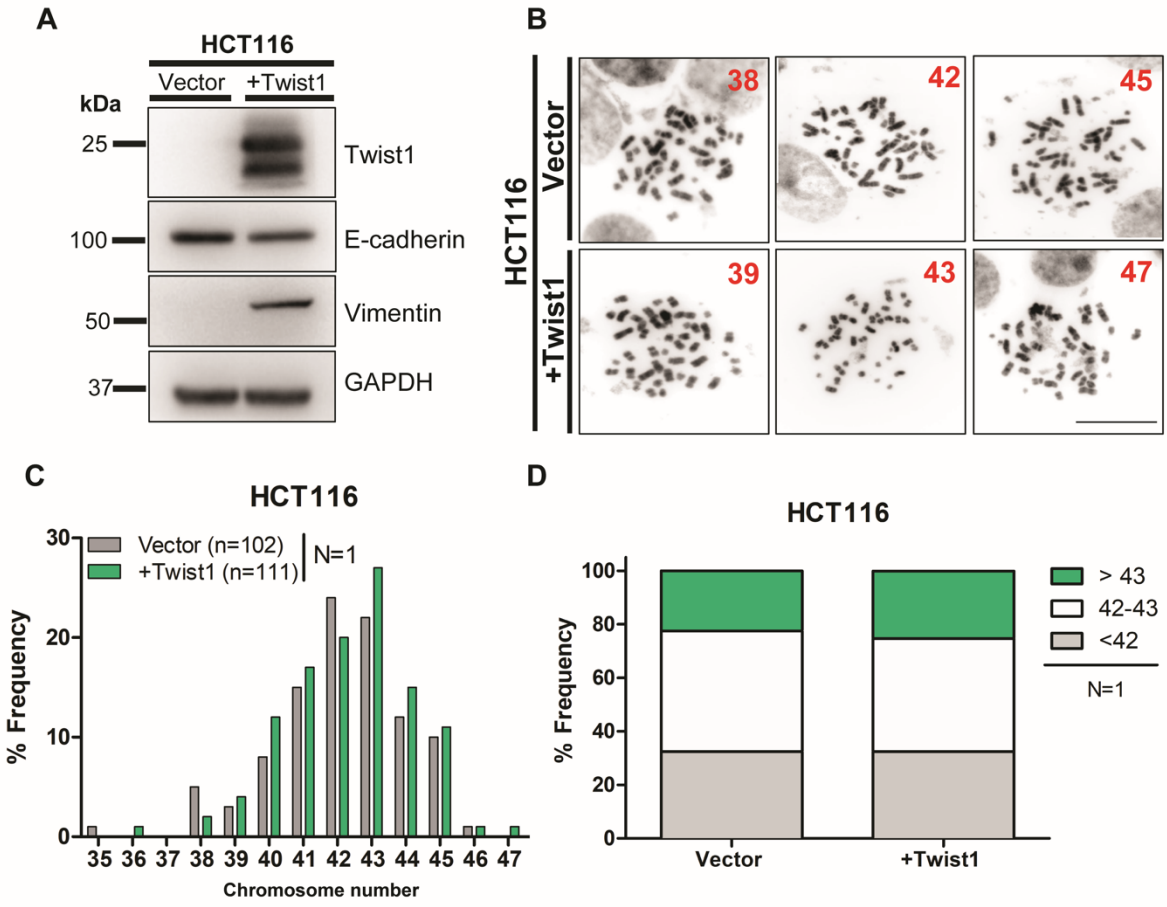


Table S4

	<42	42-43	44-45	<45
Vector	32%	45%	22%	1%
+Twist1	32%	42%	23%	2%

Figure S3: Twist1 overexpression in HCT116 colorectal cancer cells

(A) Representative immunoblot showing EMT induction upon Twist1 overexpression. E-cadherin shows a marginal decrease, while Vimentin shows a concomitant increase in levels in HCT116 cells. **(B)** Representative images of metaphase spreads derived from HCT116 upon Twist1 overexpression. Scale bar ~20 μm. **(C)** Histogram showing quantification of metaphase spreads derived from cells treated with vector control (N=1, n=102) and Twist1 overexpression respectively (N=1, n=111). **(D)** Quantification of whole chromosomal gains and losses in HCT116 cells. (Data quantified from n=100 independent metaphase spreads collected from N=1 single experiment). **(Table S4)** Table representing percentage frequency of whole chromosomal gains and losses in HCT116 cells upon Twist1 overexpression.

Figure S4

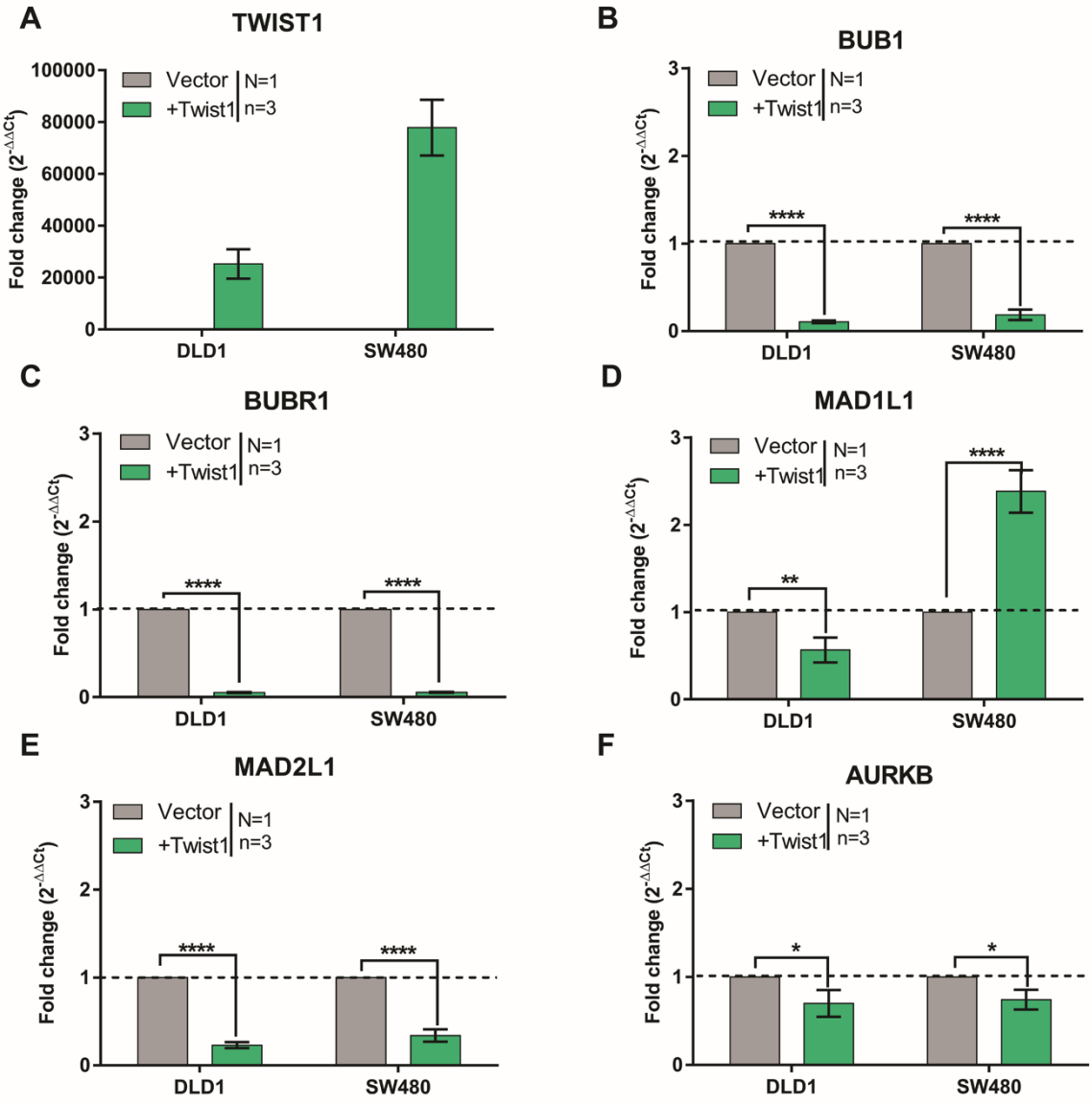
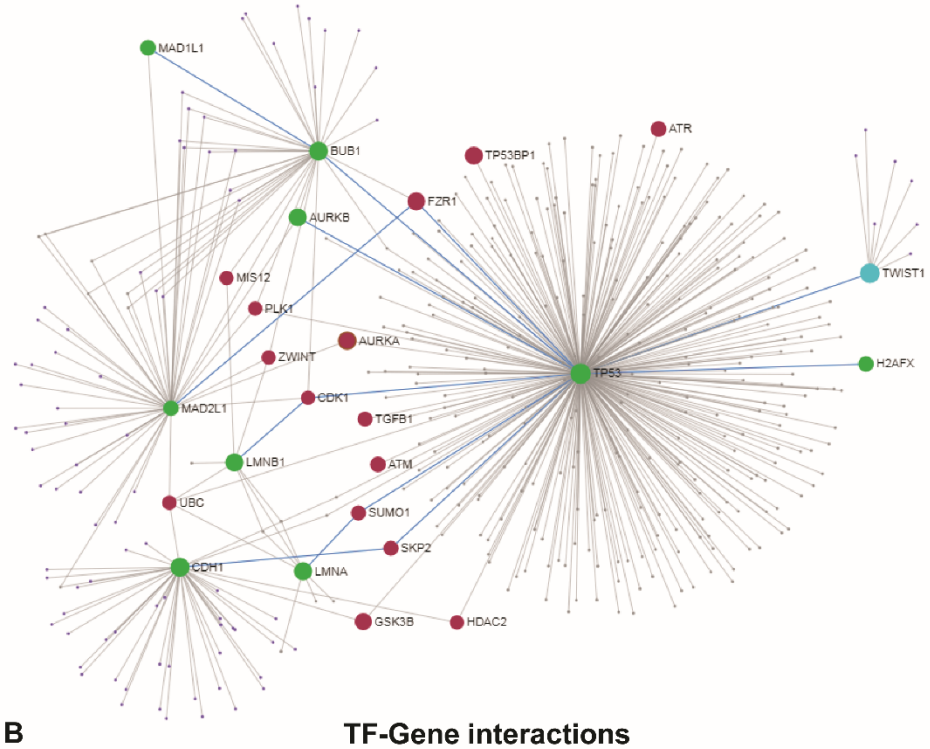

A
TWIST1
Vector
+Twist1
N=1
n=3
Fold change (2^-ΔΔCt)
100000
80000
60000
40000
20000
0
DLD1
SW480
B
BUB1
****
C
BUBR1
D
MAD1L1
**
E
MAD2L1
F
AURKB
*

Figure S4: Effect of Twist1 overexpression on transcript levels of checkpoint genes

(A) Gene expression levels determined by RT-PCR of Twist1 upon Twist1 overexpression in DLD1 and SW480 cells, normalized to vector control. **(B-F)** Gene expression profiling of checkpoint genes determined by RT-PCR for checkpoint genes upon Twist1 overexpression in DLD1 & SW480. Data normalised to its respective vector control. (Data quantified from N=1 single experiment that include n=3 independent technical replicates, unpaired t-test, Mean±SD, *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

Figure S5

A **Protein- Protein interactions**



B **TF-Gene interactions**

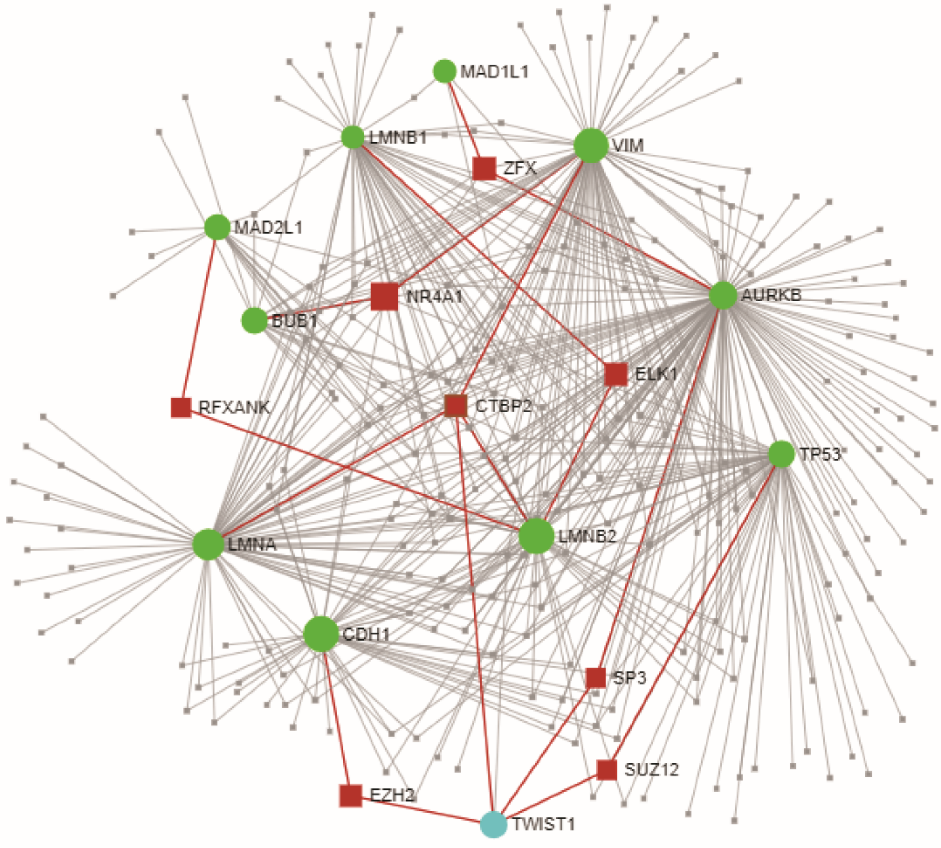


Figure S5: Correlation between levels of Twist1, EMT and CIN factors

(A) Generic Protein-Protein interactions of Twist1 & other proteins examined in this study (E-cadherin, Vimentin, Bub1, BubR1, Mad1, Mad2, Aurora B kinase, p53, LaminA/C, LaminB1, LaminB2) generated using NetworkAnalyst. Blue line: Candidate and key protein-protein interactions in the context of Twist1, EMT and CIN factors. Gray line: All other protein-protein interactions. Candidate EMT and CIN factors reported in this study

Potential protein-protein interactors connecting Twist1 with EMT, CIN and DNA damage.

(B) Transcription factor-Gene interactions between TWIST1 & other target genes (CDH1, VIM, BUB1, MAD1L1, MAD2L1, AURKB, TP53, LMNA, LMNB1 and LMNB2) examined in this study generated using NetworkAnalyst. TWIST1 Input candidate genes transcription factor enrichment on genes derived from ENCODE ChIP-seq data.

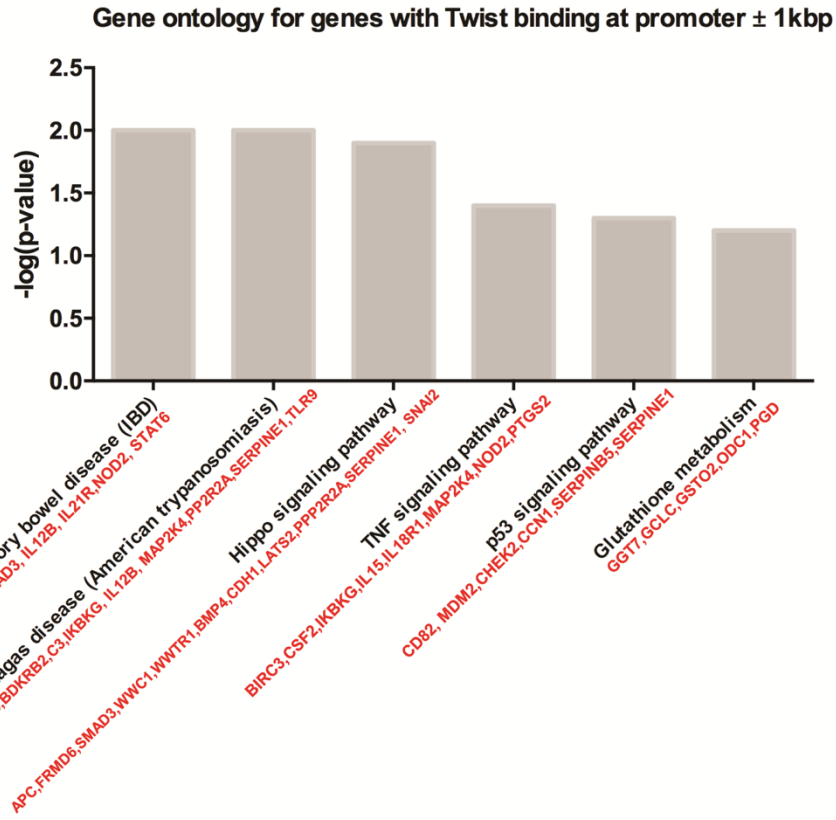
Table S5 Analysis of Twist1 occupancy from ChIP seq data of HMLE cells

Gene Category	Gene Name	Occupancy	Chromosome	Start	End	Annotation	Distance to TSS (bp)
EMT	CDH1	+	Chr.16	67298818	67299408	Intergenic	-29583
	CDH1	+	Chr.16	67328548	67328884	promoter-TSS (NM_004360)	+20
	CDH1	+	Chr.16	67377982	67378379	intron (NM_004360, intron 2 of 15)	+49484
	VIM	-	Chr.10	N.A.	N.A.	N.A.	N.A.
Checkpoint	BUB1	-	Chr.2	N.A.	N.A.	N.A.	N.A.
	BUBR1	-	Chr.15	N.A.	N.A.	N.A.	N.A.
	MAD1L1	+	Chr.7	2201955	2202527	intron (NM_001013836, intron 10 of 18)	+36868
	MAD1L1	+	Chr.7	2122201	2122623	intron (NM_001013836, intron 11 of 18)	+116697
	MAD2L1	+	Chr.4	121632168	121632386	Intergenic	-424816
	AURKB	-	Chr.17	N.A.	N.A.	N.A.	N.A.
	TP53	-	Chr.17	N.A.	N.A.	N.A.	N.A.
Nuclear Envelope	LMNA	+	Chr.1	154339840	154340272	intron (NM_001282625, intron 3 of 12)	-11029
	LMNA	+	Chr.1	154360004	154360693	intron (NM_005572, intron 1 of 9)	-2184
	LMNA	+	Chr.1	154365724	154366780	intron (NM_001282624, intron 2 of 10)	+3720
	LMNA	+	Chr.1	154355937	154356303	intron (NM_005572, intron 1 of 9)	+5035
	LMNB1	-	Chr.5	N.A.	N.A.	N.A.	N.A.
	LMNB2	+	Chr.19	2404030	2404390	intron (NM_032737, intron 1 of 11)	+3756

Table S5 ChIP-Sequencing analyses of Twist1 occupancy from HMLE (human mammary epithelial) cells upon Twist1 overexpression.

Figure S6

A


Gene ontology for genes with Twist binding at promoter ± 1kbp
-log(p-value)
2.5
2.0
1.5
1.0
0.5
0.0
Inflammatory bowel disease (IBD)
RORC,SMAD3, IL12B, IL21R,NOD2, STAT6
Chagas disease (American trypanosomiasis)
SMAD3,BDKRB2,C3,IKBKG, IL12B, MAP2K4,PP2R2A,SERPINE1,TLR9
Hippo signaling pathway
APC,FRMD6,SMAD3,WWC1,WWTR1,BMP4,CDH1,LATS2,PPP2R2A,SERPINE1, SNAI2
TNF signaling pathway
BIRC3,CSF2,IKBKG,IL15,IL18R1,MAP2K4,NOD2,PTGS2
p53 signaling pathway
CD82, MDM2,CHEK2,CCN1,SERPINB5,SERPINE1
Glutathione metabolism
GGT7,GCLC,GSTO2,ODC1,PGD

B

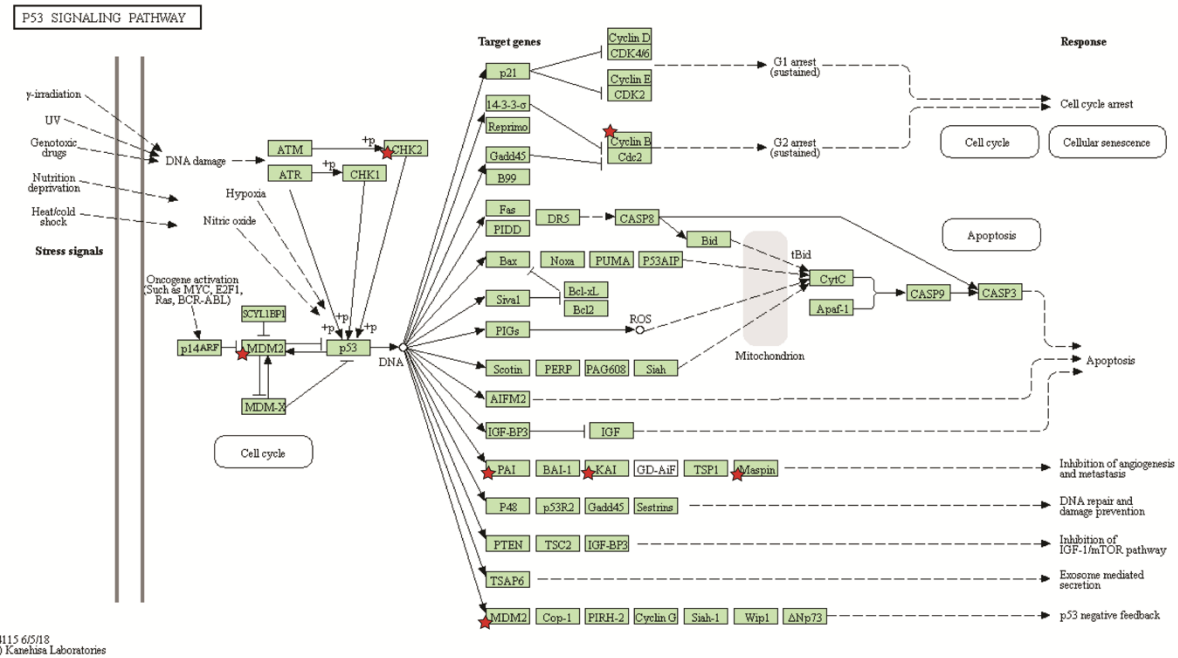

P53 SIGNALING PATHWAY
γ-irradiation
UV
Genotoxic drugs
Nutrition deprivation
Heat/cold shock
Stress signals
DNA damage
ATM
ATR
CHK2
CHK1
Hypoxia
Nitric oxide
Oncogene activation (Such as MYC, E2F1, Ras, BCR-ABL)
3CYL1BP1
p14ARF
MDM2
p53
MDM-X
DNA
Cell cycle
Target genes
p21
14-3-3-σ
Reprimo
Gadd45
B99
Cyclin D
CDK4/6
Cyclin E
CDK2
Cyclin B
Cdc2
G1 arrest (sustained)
G2 arrest (sustained)
Response
Cell cycle arrest
Cell cycle
Cellular senescence
Fas
PIDD
DR5
CASP8
Bid
Apoptosis
Bax
Noxa
PUMA
P53AIP
tBid
CytC
Apaf-1
CASP9
CASP3
Siva1
Bcl-xL
Bcl2
PIGs
ROS
Mitochondrion
Scotin
PERP
PAG608
Siah
AIFM2
IGF-BP3
IGF
PAI
BAI-1
KAI
GD-AiF
TSP1
Maspin
Inhibition of angiogenesis and metastasis
P48
p53R2
Gadd45
Sestrins
DNA repair and damage prevention
PTEN
TSC2
IGF-BP3
Inhibition of IGF-1/mTOR pathway
TSAP6
Exosome mediated secretion
MDM2
Cop-1
PIRH-2
Cyclin G
Siah-1
Wip1
ΔNp73
p53 negative feedback
04115 6/5/18
(c) Kanehisa Laboratories

Figure S6: Twist1 is enriched on promoters of genes in the p53 signaling pathway

(A) Gene Ontology analysis of genes that show Twist1 occupancy enriched within (-1kbp to +1kbp) of the promoter region using DAVID Bioinformatics Resources 6.8. **(B)** p53 pathway from KEGG. Red stars indicate genes of the p53 pathway showing Twist1 occupancy in their promoter region.