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SUPPLEMENTAL DATA

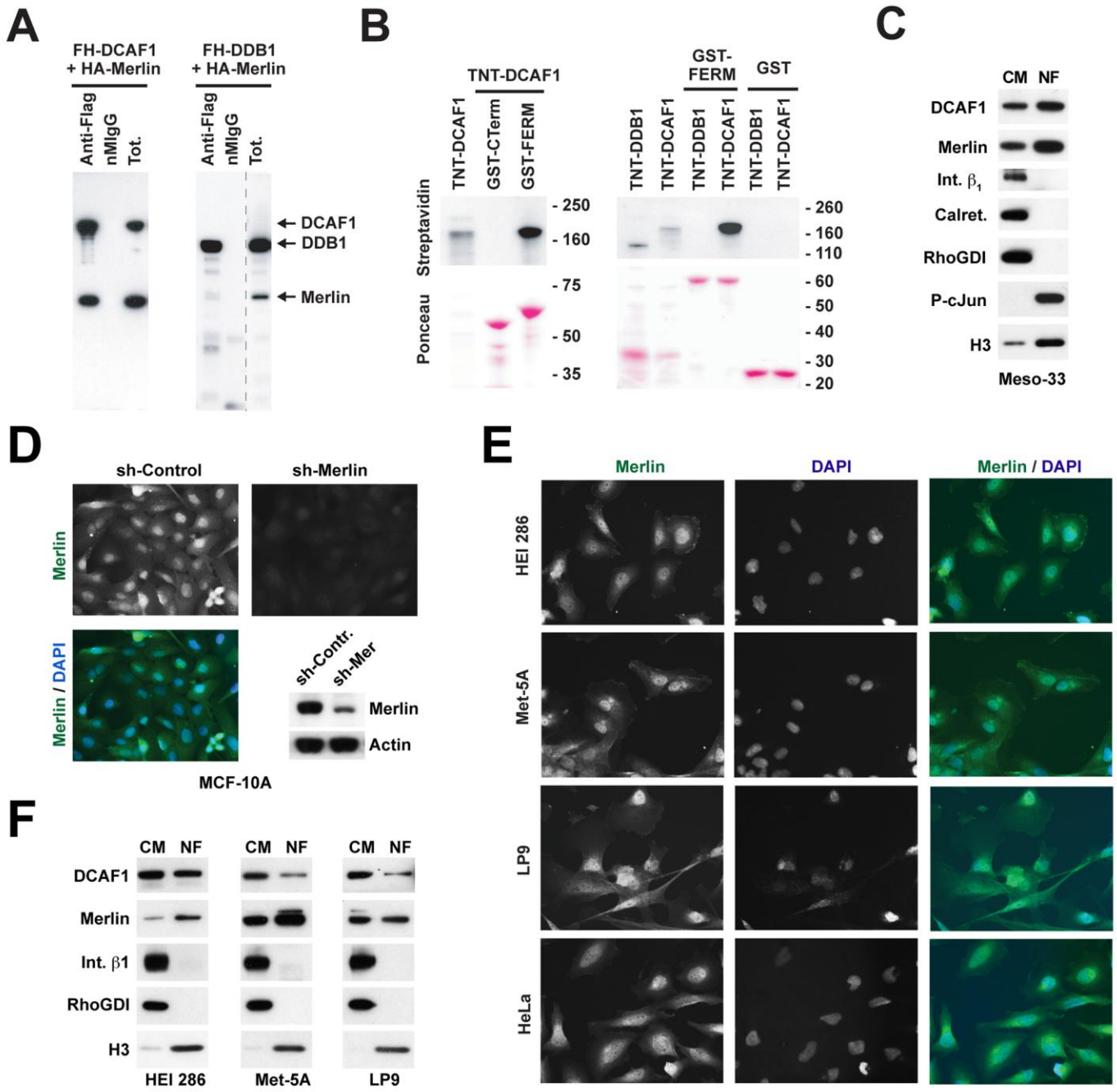

Figure S1, related to Figure 1.

(A) Cos7 cells were transfected with HA-Merlin in combination with FH-DCAF1 or FH-DDB1, immunoprecipitated with anti-Flag (M2) or control (nMIgG) antibodies, and immunoblotted with anti-HA. Total lysates were also subjected to immunoblotting.

(B) TNT-translated and biotinylated DCAF1 was subjected to pull down with GST fusion proteins comprising the α-helical and C-terminal domain (GST-CTerm) or the FERM domain of Merlin (GST-FERM). An aliquot of TNT-DCAF1 was run as a control (left). TNT-translated and biotinylated DDB1 and DCAF1 were subjected to pull down with a GST fusion protein comprising the FERM domain of Merlin (GST-FERM) or with GST alone. Aliquots of TNT-DDB1 and TNT-DCAF1 were run as controls (right). Blots were stained with Ponceau and reacted with peroxidase-conjugated Streptavidin.

(C) Meso-33 cells were transiently transfected with moderate amounts of HA-Merlin and subjected to subcellular fractionation. Equal amounts of proteins from the nuclear (NF) and non-nuclear (CM; cytosol + crude membranes) fractions were subjected to immunoblotting with antibodies to the indicated proteins.

(D) MCF-10A cells were infected with lentiviral vectors encoding a sh-RNA targeting Merlin or a control sh-RNA and stained with affinity-purified antibodies to the C-terminus of Merlin. Nuclei were labeled by DAPI. The efficiency of silencing was verified by immunoblotting (lower right panel).

(E) The indicated cells were subjected to staining with anti-Merlin as above and counterstained with DAPI.

(F) The indicated cells were subjected to subcellular fractionation as described above.

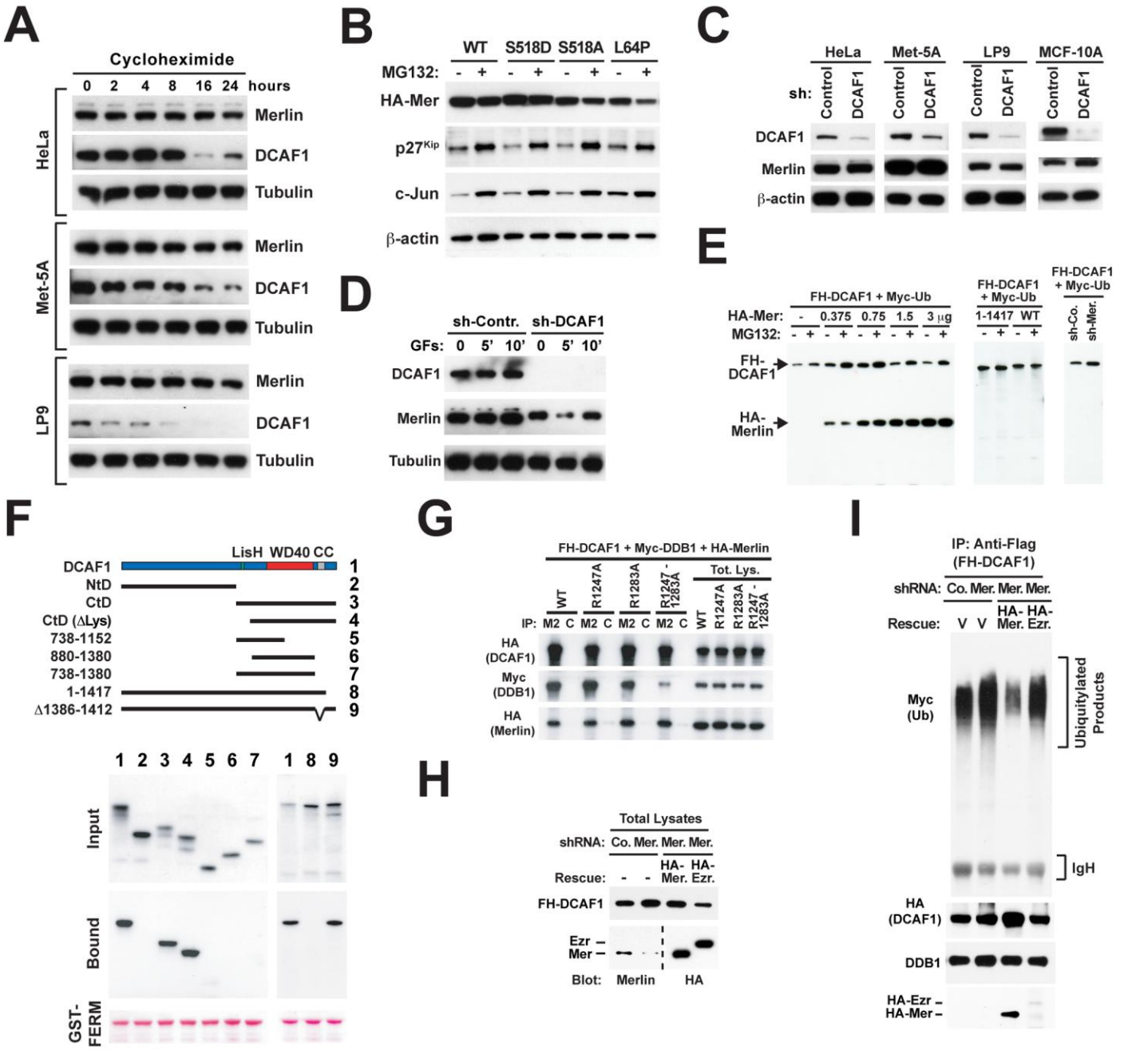

Figure S2, related to Figure 2.

(A) Cells were treated with 75 μg/ml Cycloheximide for the indicated times and subjected to immunoblotting as indicated.

(B) Cos7 cells transiently transfected with HA-Merlin or the indicated mutants were treated for 6 hours with 25 μM MG132 and subjected to immunoblotting with the indicated antibodies.

(C) Cells were infected with a shRNA targeting DCAF1 or a control shRNA and subjected to immunoblotting.

(D) Hela cells infected with lentiviruses encoding a control shRNA (sh-Contr.) or a shRNA targeting DCAF1 (sh-DCAF1) were starved for 48 hours, restimulated with 10% serum for the indicated times, and subjected to immunoblotting.

(E) Total lysates from the samples of Figure 2A (left), 2C (middle), and 2D (right) were blotted to verify expression levels.

(F) TNT-translated and biotinylated DCAF1 and the indicated mutants were subjected to pull down with GST-FERM. Aliquots of input TNT-proteins and the GST-FERM bound fractions were blotted with peroxidase-conjugated Streptavidin. Staining with Ponceau was used to verify levels of GST-FERM in each sample.

(G) Cos7 cells were transfected with FH-DCAF1 or the indicated mutants in combination with Myc-DDB1 and HA-Merlin. Anti-Flag (M2) or control Mab (C) precipitates and total lysates were immunoblotted as indicated.

(H) Cos7 cells were infected with lentiviruses encoding a control sh-RNA (Co.) or a sh-RNA targeting Merlin (Mer.) and then transfected with FH-DCAF1 and Myc-Ubiquitin plus HA-Merlin (HA-Mer.), HA-Ezrin (HA-Ezr.), or empty vector (V). Lysates were immunoblotted to verify knock down and expression levels.

(I) Samples from H were immunoprecipitated with anti-Flag and immunoblotted as indicated.

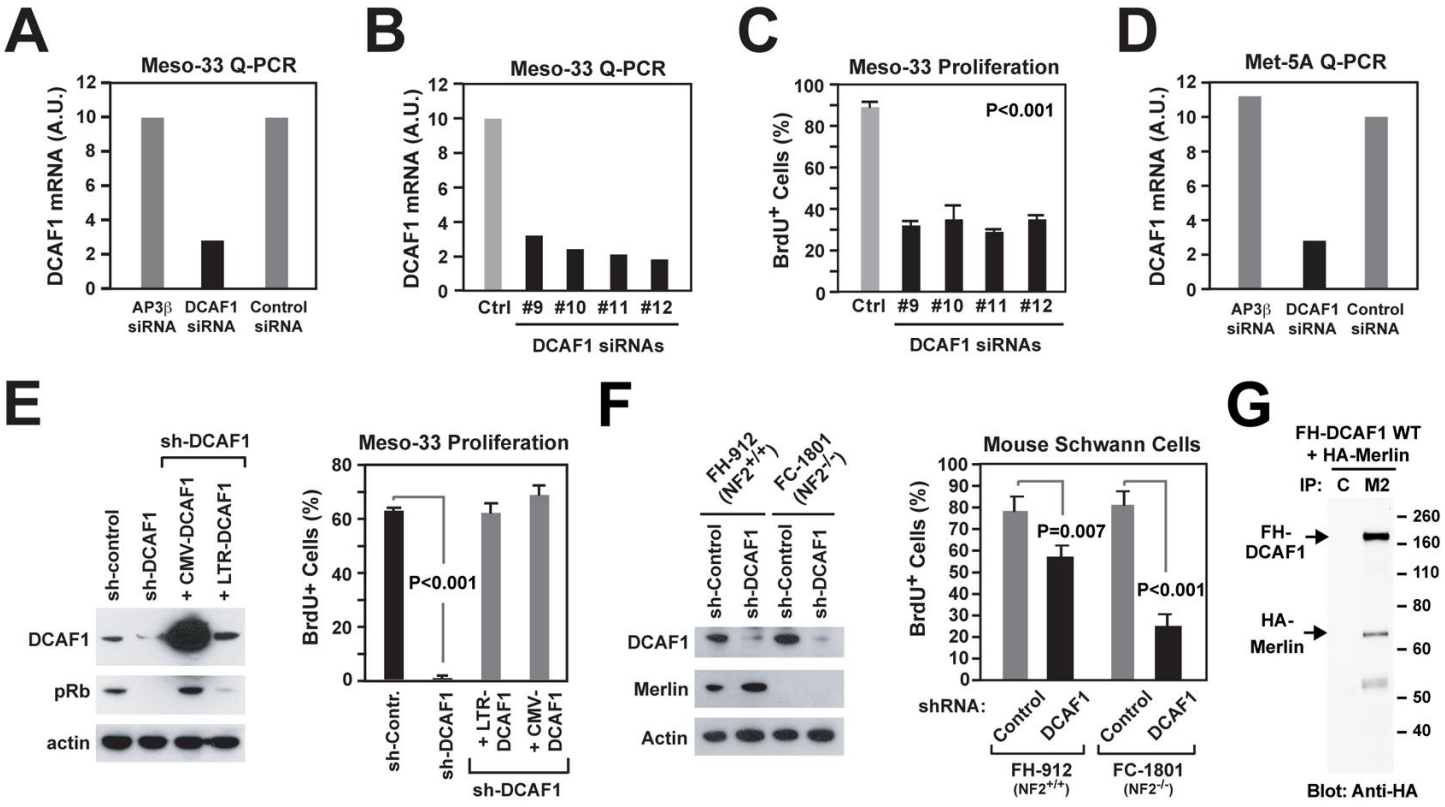

Figure S3, related to Figure 3.

(A) Meso-33 were transfected with a SMARTpool of siRNAs targeting the adaptor protein AP3β, DCAF1, or a control non-targeting pool and subjected to Q-PCR to verify knock down of DCAF1.

(B) Meso-33 cells were transfected with 75 pmol of a control siRNA or each of four siRNAs targeting DCAF1 and subjected to Q-PCR as above.

(C) Meso-33 cells treated as above were deprived of growth factors, incubated with BrdU in the presence of mitogens for 24 hours, and subjected to anti-BrdU staining. The graph indicates the percentage (+ SEM) of BrdU-positive cells.

(D) Met-5A cells were transfected with a SMARTpool of siRNAs targeting the adaptor protein AP3β, DCAF1, or a control non-targeting pool and subjected to Q-PCR to verify knock down of DCAF1.

(E) Meso-33 cells were infected with lentiviral constructs encoding a control shRNA (sh-control) or a sh-RNA targeting DCAF1 (sh-DCAF1). Meso-33 cells expressing sh-DCAF1 were also infected with a CMV-based lentiviral vector encoding a sh-RNA-resistant form of DCAF1 (CMV-DCAF1) or a LTR-based retroviral vector encoding a sh-RNA-resistant form of DCAF1 (LTR-DCAF1). Cells were lysed and equal amounts of total proteins were subjected to immunoblotting with antibodies reacting with DCAF1, phospho-RB and β-actin (left) or they were deprived of growth factors, detached, plated on fibronectin-coated coverslips in complete medium with BrdU for 24 hours, and stained with anti-BrdU. The graph indicates the percentage (+ SEM) of BrdU-positive cells.

(F) FH-912 and FC-1801 cells were infected with lentiviruses encoding a control shRNA (sh-control) or a sh-RNA targeting DCAF1 (sh-DCAF1). Cell were lysed and subjected to immunoblotting with antibodies reacting with DCAF1, Merlin and β-actin (left) or they were starved, plated on fibronectin-coated coverslips in complete medium with BrdU for 24 hours, and stained with anti-BrdU. The graph indicates the percentage (+ SEM) of BrdU-positive cells.

(G) Lysates containing 0.3 mg of total proteins from Meso-33 cells transfected with FH-DCAF1 WT and HA-Merlin(as in Figure 3E) were immunoprecipitated with anti-Flag Mab (M2) or a control Mab (C) followed by immunoblotting with anti-HA (right). Note that DCAF1 is not saturated by Merlin under these experimental conditions.

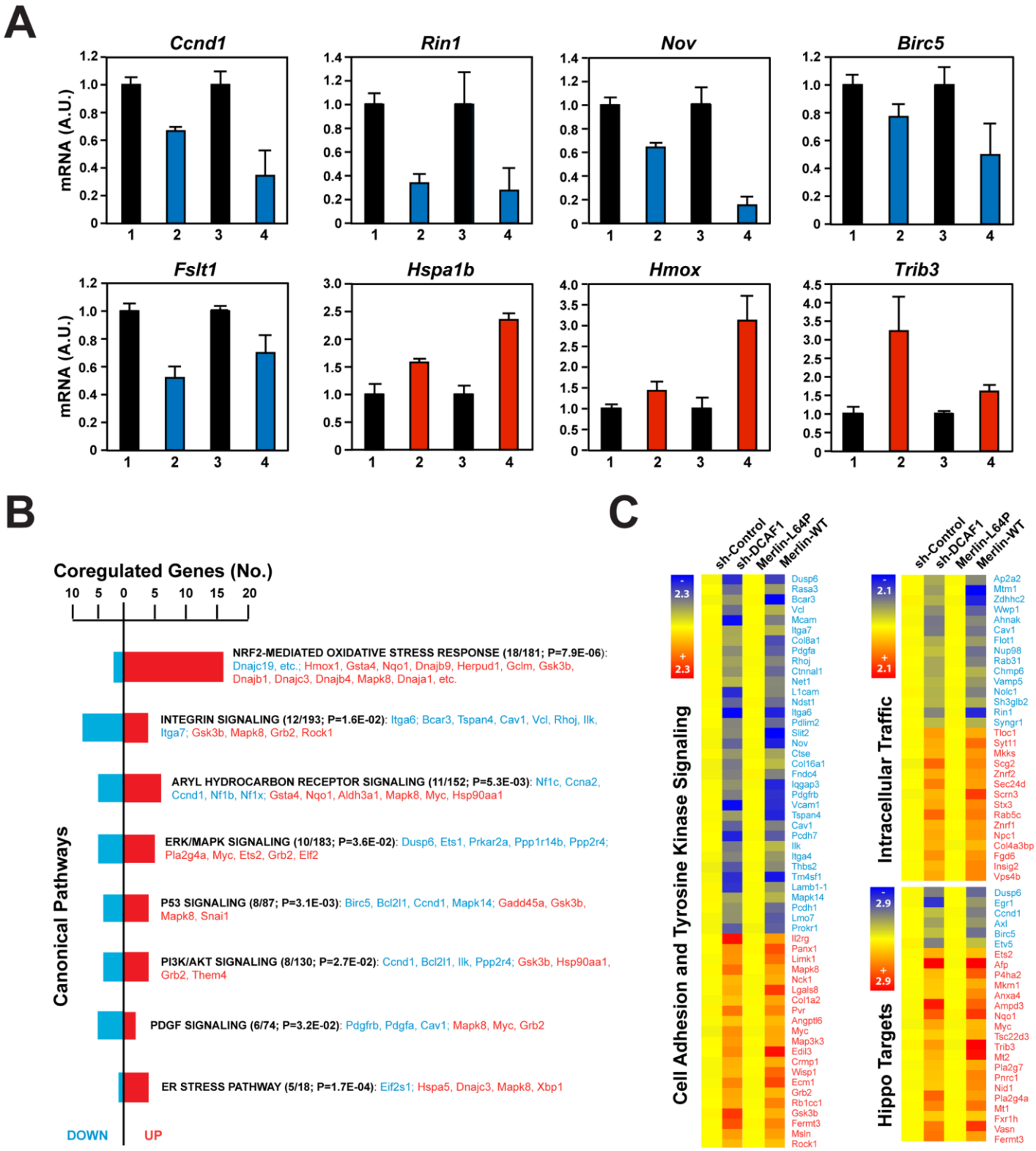

Figure S4, related to Figure 4.
(A) FC-1801 cells expressing a control sh-RNA (1), a sh-RNA targeting DCAF1 (2), no insert (3), or Merlin (4) were subjected to Q-PCR analysis to confirm that the indicated genes are coregulated by knock down of DCAF1 and expression of Merlin.
(B) Ingenuity Pathway analysis of 667 coregulated genes. Down-regulated and upregulated genes are visualized separately in the category of Canonical Pathways. Representative downregulated genes are shown in blue and upregulated genes in red in order of decreasing average fold change.
(C) Coregulated genes implicated in cell adhesion and tyrosine kinase signaling (56), intracellular traffic (30) and Hippo targets (25) were subjected to hierarchical clustering. Downregulated genes are shown in blue and upregulated genes in red.

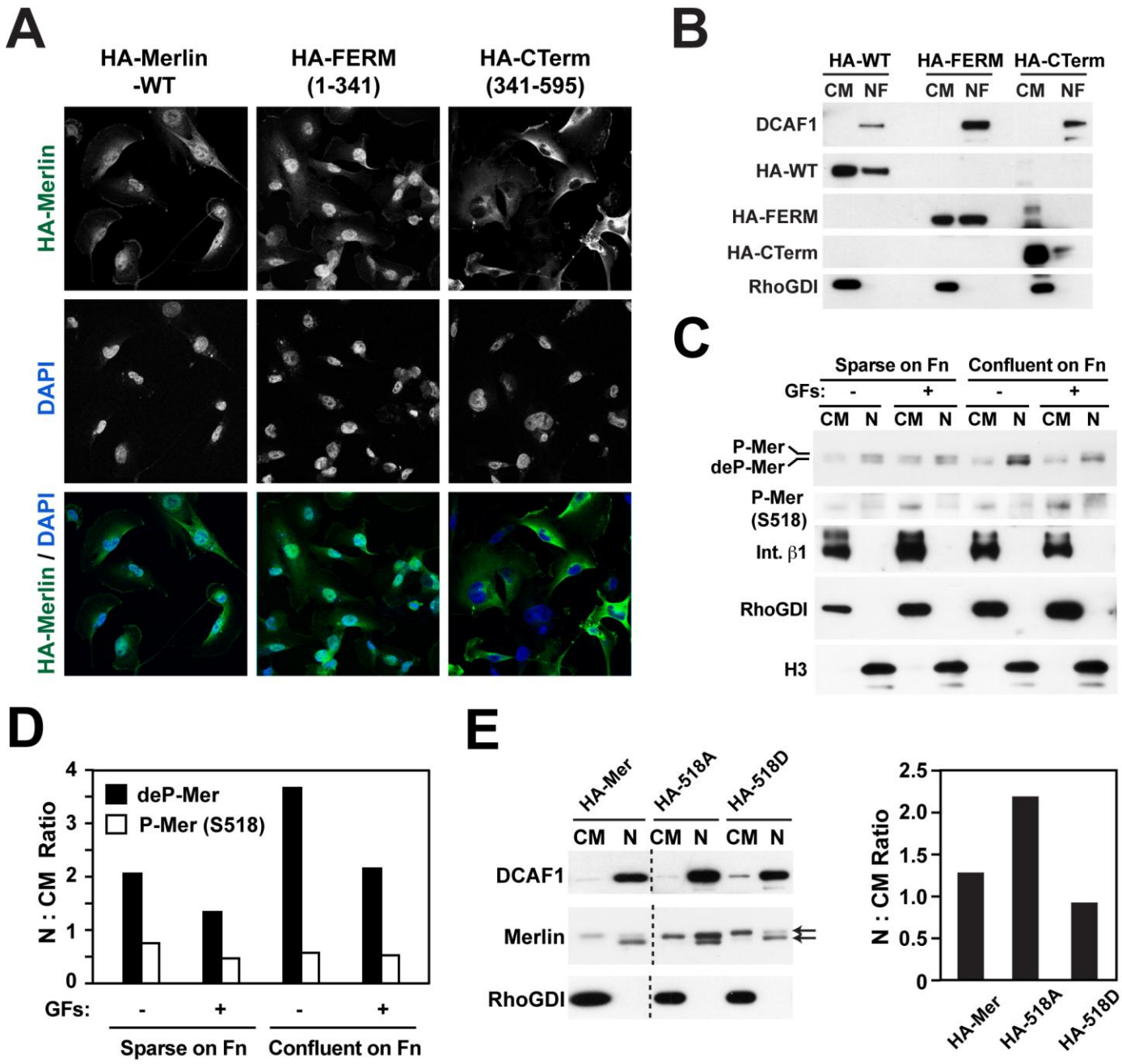

Figure S5, related to Figure 5.

(A) Meso-33 cells were transiently transfected with moderate amounts of constructs encoding HA-Merlin or the indicated truncation mutants and subjected to immunofluorescent staining with anti-HA and DAPI.

(B) Meso-33 cells transfected as indicated above were subjected to subcellular fractionation. Equal amounts of total proteins from the non-nuclear (CM; cytosol + crude membranes) and nuclear (NF) fraction were subjected to immunoblotting with antibodies against the indicated proteins.

(C) HUVECs were deprived of growth factors, detached, replated on fibronectin (Fn)-coated plates under sparse or confluent conditions, and then treated or not with mitogens (bFGF + Hep, EGF,and ITS) for 2 hours. Equal amounts of total proteins from the non-nuclear (CM) and nuclear (NF) fraction were subjected to SDS-PAGE on a gel containing a decreased Acrylamide/Bis-Acrylamide ratio (16:1) to separate the phosphorylated and unphosphorylated form of Merlin (top 2 blots) or on a regular gel (other blots). Samples were subjected to immunoblotting with antibodies against the indicated proteins. The slower migrating form of merlin present in the nucleus may be phosphorylated at a site distinct from S518, as it is not recognized by anti-P-Merlin (S518) antibodies.

(D) Data were quantified by densitometry. The graph illustrates the ratio of unphosphorylated Merlin or Merlin phosphorylated at S518 present in the nuclear over the non-nuclear fraction.

(E) Meso-33 cells were transiently transfected with moderate amounts of HA-Merlin, HA-Merlin-S518A, or HA-Merlin-S518D and subjected to subcellular fractionation. Equal amounts of total proteins from the non-nuclear (CM) and nuclear (NF) fraction were subjected to immunoblotting as indicated. Lower arrow points to a band, which we have detected only after high-resolution SDS-PAGE. This band is recognized by anti-Merlin but not anti-HA antibodies and thus may represent an N-terminally cleaved form of Merlin. The amount of Merlin in each fraction was estimated by densitometry. The graph illustrates the ratio between the nuclear and non-nuclear fraction for the wild type and each of the two mutant forms of Merlin.

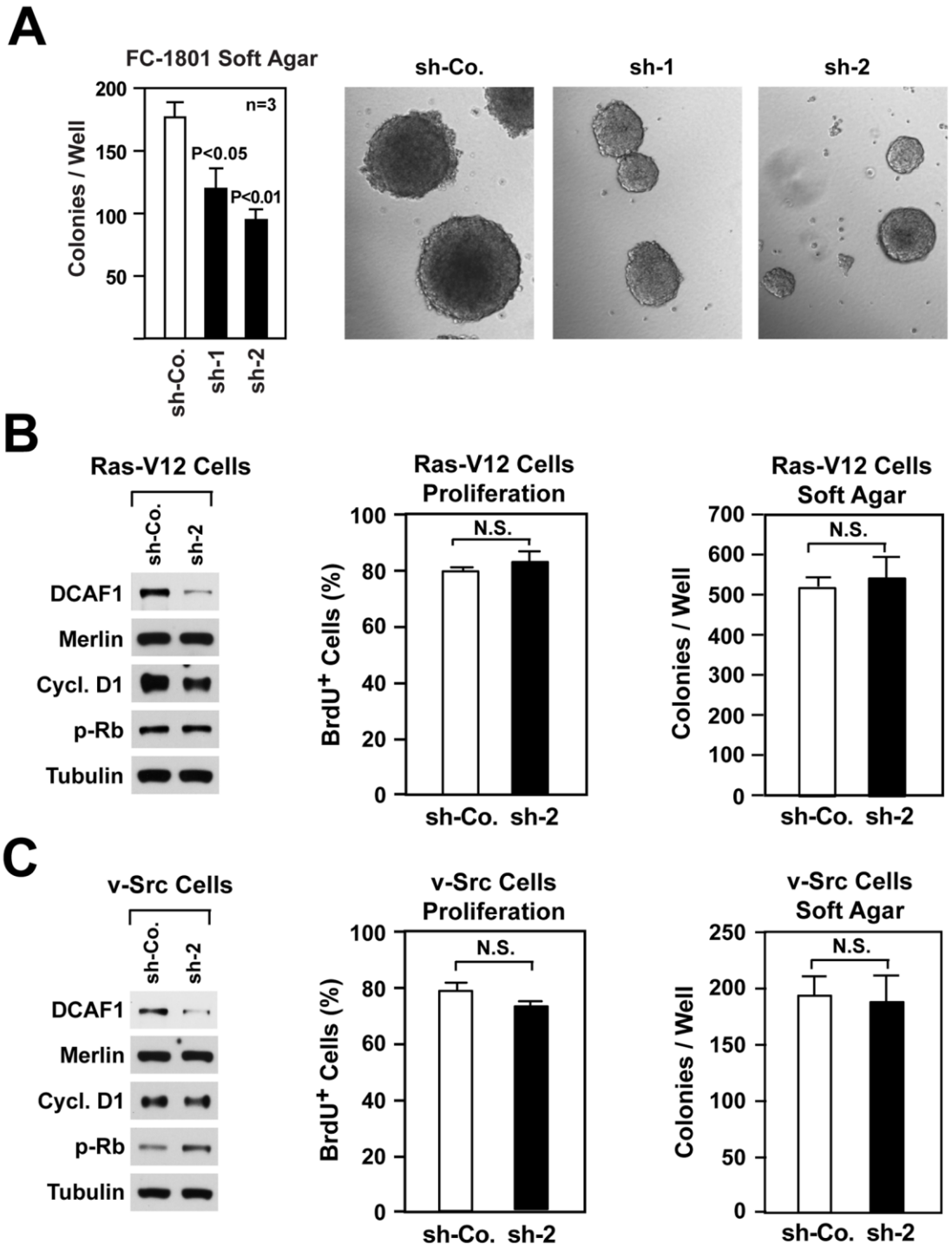

Figure S6, related to Figure 7.
(A) FC-1801 cells infected with lentiviral vectors encoding two distinct sh-RNAs targeting DCAF1 (sh-1 and sh-2) or a control sh-RNA (sh-Co.) were subjected to soft agar assay. The graph shows the total number (+ SEM) of colonies present in each well after 2 weeks of culture. The pictures show representative images.
(B) Ras-V12-transformed 3T3 cells were infected with lentiviral vectors encoding a control sh-RNA (sh-Co.) or a sh-RNA targeting DCAF1 (sh-2) and subjected to immunoblotting with antibodies against the indicated proteins (left), BrdU incorporation assay (middle), and soft agar assay (right). The graph on the right shows the total number (+ SEM) of colonies present in each well after 2 weeks of culture.
(C) v-Src-transformed 3T3 cells were infected with lentiviral vectors encoding a control sh-RNA (sh-Co.) or a sh-RNA targeting DCAF1 (sh-2) and subjected to immunoblotting with antibodies against the indicated proteins (left), BrdU incorporation assay (middle), and soft agar assay. The graph on the right shows the total number (+ SEM) of colonies present in each well after 3 weeks of culture.

Table S1. nano-LC-MS/MS analysis results on protein gel stacks annotated wild-type(Wt) Merlin and Merlin-L64P mutant. Related to Figure 1.

NCBI Accession #	kDa	Unique Peptide count		Mascot Score		Sequence Coverage		Protein Name	
		Wt	L64P	Wt	L64P	Wt	L64P		
3980301	71.9	29	21	1316	658	36.4	31.5	NF2	Neurofibromin 2 (merlin)
7662316	168.9	23	0	481	0	16.4	0	DCAF1	DDB1-and Cul4-Associated Factor 1
2632123	126.8	8	0	195	0	8.3	0	DDB1	Damage-specific DNA Binding protein 1
13259127	90.6	1	0	51	0	1	0	CUL4B	Cullin 4B

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell Lines

LP9 and Met5-A immortalized mesothelial cells and Merlin-deficient mesothelioma Meso-33 cells were cultured in MCDB 110 supplemented with EGF (10 ng/ml, Invitrogen #PHG0311), Hydrocortisone (50 μg/ml, CalBioChem #3867), ITS (1%, Invitrogen #I2521), Antibiotics (1%, GEMINI BIO # 400-101), Fetal Bovine Serum (15%, Invitrogen #10437-028), and L-Glutamine (2 mM, Invitrogen # 25030-081). HEI-286 immortalized human Schwann cells, HeLa squamous carcinoma cells, Cos7, 293FT, and Phoenix cells were cultured in DMEM-HG supplemented with Antibiotics (1%, GEMINI BIO # 400-101), Fetal Bovine Serum (10%, Invitrogen #10437-028), L-Glutamine (2 mM, Invitrogen # 25030-081). Primary Human Umbilical Vein Endothelial Cells (HUVECs) and MCF-10A mammary epithelial cells were cultured as described previously (Debnath et al.; Okada et al., 2005). To generate the FC-1801 *Nf2*$^{-/-}$ mouse Schwannoma cells, primary mouse Schwann cell cultures from E12.5 *Nf2*$^{flox/flox}$ embryos (Giovannini et al., 2000) were infected with adenoviral Cre (Ad5CMVCre, University of Iowa Gene Transfer Vector Core, Iowa City, IA, USA) and immortalized in vitro (Lallemand et al., 2009). To obtain the FH-912 *Nf2*$^{+/+}$ mouse Schwann cells, primary Schwann cell cultures from adult *Nf2*$^{flox/flox}$ mouse sciatic nerve were spontaneously immortalized in vitro. Schwann cells were purified by anti-p75 cell sorting (Manent et al., 2003) and routinely cultured in N2 media (DMEM-F12 with 2 mM forskolin, 10 ng/ml heregulin, 50 μg/ml gentamicin, 2.5 μg/ml fungizone and N2 supplement (GIBCO) (Lallemand et al., 2009). Unless otherwise indicated, experiments were performed on cells grown to 70-90% confluency in complete medium.

Antibodies

To generate monospecific antibodies to DCAF1, we constructed a bacterial expression vector encoding a 6xHis-tagged N-terminal fragment of DCAF1 (N1-201) by subcloning residues 1-201 of a cDNA encoding human DCAF1 (ATCC) into pET21d (Novogene). Recombinant N1-201 was produced in DE3 cells (Novogene) and purified by using Ni-NTA agarose (Qiagen) according to manufacturer's instructions. Rabbit antibodies were generated by Washington Biotechnology and affinity purified by us on recombinant N1-201 conjugated to CNBr-activated Sepharose 4B (Amersham) following manufacturer's instructions. Rabbit antibodies to a peptide modeled after the cytoplasmic domain of the integrin β1 subunit and anti-MHC control Mab W6.32 were previously described (Giancotti and Ruoslahti, 1990; Wary et al., 1998). Antigen affinity-purified rabbit antibodies to Merlin (#9168, to a N-terminal peptide), to Ezrin (#3142, to a C-terminal peptide), and to Histone H3 (#9715, to a C-terminal peptide) were purchased from Cell Signaling Technology. Antigen affinity-

purified rabbit antibodies to Merlin (A-19, # Sc-331, to a N-terminal peptide, and C-18, # Sc-332, to a C-terminal peptide) and to c-Jun (#sc-44, to a conserved DNA binding domain peptide) and antigen affinity-purified goat antibodies to Cul4 (C-19, #sc-8557, C-terminal peptide) were obtained from Santa Cruz Biotechnology. Antigen affinity-purified rabbit antibodies to DDB1 (BL1999) were purchased from Bethyl. Purified mouse monoclonal antibodies to the HA tag (MMS-101) were obtained from Covance, to Myc (9B11) from Cell Signaling Technology, to β-actin (A3376) from Sigma, to calreticulin (# 612136) and to RhoGDI (# 610255) from BD Biosciences, to P-c-Jun (KM-1, #sc-822) from Santa Cruz Biotechnology, and to p27 (Ab-1, #MS-256) from Thermo Fisher Scientific. EZview Red Anti-FLAG M2 affinity gel, agarose-conjugated anti-HA monoclonal antibody, and agarose-conjugated normal mouse IgGs were purchased from Sigma, A0919.

Vectors

To generate a vector encoding Merlin bearing a N-terminal Flag-HA tag, a cDNA encoding the Flag peptide was subcloned upstream of the sequences encoding the HA tag in pXJ40-HA-Merlin and the resulting cDNA encoding Flag-HA-Merlin was subcloned in pRK5. To generate point mutations in Merlin, the resulting expression vector, pRK5-FH-Merlin, was subjected to site-directed mutagenesis using the Quickchange mutagenesis Kit (Stratagene). To generate pRK5-Merlin-FH, which encodes Merlin bearing a C-terminal Flag-HA tag, PCR products encoding Merlin and the Flag-HA tag were subcloned in pRK5. pRK5-Myc-DCAF1 was constructed by subcloning a cDNA encoding human DCAF1 (ATCC) in pRK5-Myc. To generate pRK5-FH-DCAF1, we replaced the sequences encoding the Myc epitope in pRK5-Myc-DCAF1 with sequences encoding the Flag-HA tag. pRK5-Myc-DDB1 was constructed by amplifying a cDNA encoding human DDB1 (ATCC) and subcloning it in pRK5-Myc. pHis-Myc-Ub was generously provided by Michele Pagano (Department of Pathology, NYU School of Medicine, New York, NY) and pHA-Ub was described previously (Treier et al., 1994). Constructs encoding truncation mutants of DCAF1 were generated by subcloning PCR products of a cDNA encoding human DCAF1 in pRK5-Myc and pRK5-FH. Retroviral vectors encoding FH-DCAF1 and mutants thereof were generated by subcloning the corresponding cDNAs in pBabe-hygro. Lentiviral vectors encoding Merlin-WT and Merlin-L64P were generated by subcloning the corresponding cDNAs in pLenti6/V5-D-TOPO (Invitrogen). The vector pHR-Vpr, encoding the HIV Vpr protein, was provided by Junjie Chen (Department of Therapeutic Radiology, Yale University School of Medicine, New haven, CT).

Tandem Affinity Purification and Mass Spectrometry

Ten cm diameter plates of Cos7 cells were transiently transfected with 8 μg of pRK5 encoding Flag-HA-Merlin by using Lipofectamine 2000 (Invitrogen) and lysed on ice with 1 ml of RIPA buffer (50 mM Tris pH 7.4, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 0.5% sodium deoxycholate, 10% glycerol, 1 mM EGTA, 4 mM EDTA,1 mM sodium orthovanadate, 1 mM NaF,1 mM AEBSF, 1 μg/ml Aprotinin, 1 μg/ml Leupeptin, 1 μg/ml pepstatin). The lysate obtained from 15 plates consisting of approximately 45 mg of total proteins was sonicated, clarified, and incubated with 0.8 ml of mouse IgG agarose for 1 hour at 4°C. Merlin and associated proteins were purified from the supernatant following the instructions of the FLAG HA Tandem Affinity Purification Kit (Sigma TP0010). Proteins were separated on a 4-12% Bis-Tris SDS-PAGE (Invitrogen) and visualized using Gelcode (Pierce). Visible bands were excised and subjected to protein identification using MALDI-reTOF-MS/MS analysis. For nanoLC-MS/MS analysis of the affinity protein complex, samples were run on SDS-PAGE gels consisting of 4% stacking and 10% separating gel and were allowed to enter (stacked) into the separating portion by 1-2 mm before processing for mass spectrometry.

Protein Identification

For MALDI-reTOF-MS/MS, gel-resolved proteins were digested with trypsin, batch fractionated on a Poros 50 R2 RP micro-tip, and resulting peptide pools analyzed by matrix-assisted laser-desorption / ionization reflectron time-of-flight (MALDI-reTOF) MS using a BRUKER UltraFlex TOF/TOF instrument (Bruker Daltonics; Bremen, Germany), as described previously (Erdjument-Bromage et al., 1998; Sebastiaan Winkler et al., 2002). Selected experimental masses (m/z) were taken to search the human segment of a non-redundant protein database ('NR'; ~ 217,656 entries; National Center for Biotechnology Information; Bethesda, MD), utilizing the Mascot Peptide Mass Fingerprint (PMF) program, version 2.2.04 for Windows (www.matrixscience.com), with a mass accuracy restriction better than 35 ppm, and maximum one missed cleavage site allowed per peptide. To confirm PMF results with scores $\leq$ 40, mass spectrometric sequencing of selected peptides was done by MALDI-TOF/TOF (MS/MS) analysis on the same prepared samples, using the UltraFlex instrument in 'LIFT' mode. Fragment ion spectra were taken to search NR using the MASCOT MS/MS Ion Search program (Matrix Science). For nano-LC-MS/MS, protein mixtures were denatured, and concentrated into a single, 1-mm wide "stack" by electrophoresing through an SDS 'stacking gel' until entering the 'separation gel', followed by brief staining with Gelcode and excision of the band. In-gel tryptic digests were subjected to a micro-clean-up procedure on 2 μl bed-volume of Poros 50 R2 (Applied Biosystems – 'AB') reversed-phase beads, packed in an Eppendorf gel-loading tip, and the eluant diluted with 0.1% formic acid (FA) to yield a final concentration of 7% acetonitrile (MeCN).

Analysis of the resulting peptides was performed using a QSTAR-Elite hybrid quadrupole time-of-

flight mass spectrometer (AB/MDS Sciex) equipped with a NanoSpray ion source (AB/MDS Sciex). Peptide mixtures (in 20 μl) were loaded onto a trapping guard column (0.3 x 5-mm PepMap C18 100 cartridge from LC Packings) using a Tempo nano MDLC system (Applied Biosystems) at a flow rate of 20 μl/min. After washing, the flow was reversed through the guard column and the peptides eluted with a 5-45% MeCN gradient (in 0.1% FA) over 85 min at a flow rate of 200 nl/min, onto and over a 75-μm x 15-cm fused silica capillary PepMap C18 column (LC Packings); the eluant was directed to a 75-μm (with 8-μm orifice) fused silica nano-electrospray needle (New Objective). Electrospray ionization (ESI) needle voltage was set at about 1800 V. The mass analyzer was operated in automatic, data-dependent MS/MS acquisition mode, with the threshold set to 10 counts per second of doubly or triply charged precursor ions selected for fragmentation scans. Survey scans of 0.25 sec were recorded from 400 to 1800 amu; up to 3 MS/MS scans were then collected sequentially for the selected precursor ions, recording from 100 to 1800 amu. The collision energy was automatically adjusted in accordance with the *m/z* value of the precursor ions selected for MS/MS. Selected precursor ions were excluded from repeated selection for 60 sec after the end of the corresponding fragmentation duty cycle.

Initial protein identifications from LC-MS/MS data was performed using the Mascot search engine (Matrix Science, version 2.2.04; www.matrixscience.com) and the NCBI (National Library of Medicine, NIH) and IPI (International Protein Index, EBI, Hinxton, UK) databases. One missed tryptic cleavage site was allowed, precursor ion mass tolerance = 0.4 Da, fragment ion mass tolerance = 0.4 Da, protein modifications were allowed for Met-oxide, Cys-acrylamide and N-terminal acetylation. MudPit scoring was typically applied with 'require bold red' activated, and using significance threshold score $p < 0.01$. Unique peptide counts (or 'spectral counts') and percent sequence coverages for all identified proteins were exported to Excel for further analysis.

Immunoprecipitation and Immunoblotting

Ten cm diameter dishes of Cos7 cells were transiently transfected with 8 μg of pRK5 plasmids expressing tagged wild type Merlin or mutants thereof by using Lipofectamin 2000 (Invitrogen) and were lysed 24 hours later with RIPA buffer. To isolate Merlin and associated proteins, extracts were precleared with agarose-mouse IgGs and incubated with anti-FLAG M2 Affinity Gel (Sigma) at 4^0 C for 2 hours. Unless otherwise indicated, agarose-mouse IgGs were used as a control. The immunoprecipitates were washed four times with RIPA buffer, and bound proteins were dissociated in 20 μL 1 x SDS sample buffer (25 mM Tris pH 6.8, 4% SDS, 5% Glycerol, bromophenol blue). Eluted proteins were separated on 4-12% Bis-Tris SDS-PAGE gels (Invitrogen) and transferred to Immobilon-P membranes (Millipore). Membranes were incubated in blocking buffer (5% skim milk,

0.1% Tween, 10 mM Tris at pH 7.6, 100 mM NaCl) for 1 hour at room temperature and then with primary antibodies diluted in blocking buffer for another hour at the same temperature. After three washes, the membranes were incubated with goat anti Rabbit HRP-conjugated antibody (1:5,000; Santa Cruz sc-2054) or goat anti-mouse HRP-conjugated antibody (1:10,000; Santa Cruz sc-2005) at room temperature for 1 hour and subjected to chemiluminescence using ECL (Pierce #1856136).

Subcellular Fractionation

Nuclear and non-nuclear (crude membranes and cytosol) fractions were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Reagents following manufacturer's instructions (Thermo Fisher Scientific). The chromatin fraction was prepared from nuclear pellets extracted with micrococcal nuclease as described previously (Groisman et al., 2003). When indicated, cells were washed with PBS, irradiated with UV light at 25 J/m^2, and incubated in fresh medium for 30 minutes before subcellular fractionation.

Ubiquitylation Assay

Ten cm diameter plates of Cos7 cells were transfected using Lipofectamine 2000 (Invitrogen) with 3 μg of pRK5-Flag-HA-DCAF1 and 2 μg of pHis-Myc-Ub in combination with indicated quantities of pRK5-HA-Merlin, with 6 μg of pRK5-Flag-HA-DCAF1 WT or pRK5-Flag-HA-DCAF1 (1-1417) in combination with 2 μg of pHis-Myc-Ub, or with 3 μg of pRK5-Flag-HA-DCAF1 and 2 μg of pHA-Ub in combination with 3 μg of pRK5-Myc-Merlin-WT, pRK5-Myc-Merlin1-339, pRK5-Myc-Ezrin or empty vector. When indicated, cells were treated with 25 μM MG132 for 6 hrs. Lysates containing 0.5 mg of proteins were incubated with either anti-Flag beads or control mouse IgG beads overnight at 4^0 C and subjected to immunoblotting as indicated.

GST Pull Down Assay

GST fusion proteins containing the FERM domain of Merlin and mutants thereof (WT, L46R, F62S, L64P, L141P, G197C, A211D, E270G) were expressed in BL21 (DE3) competent cells (Invitrogen C6000-03) and purified from lysates using glutathione-Sepharose 4B beads (GE Healthcare 17-0756-01). A TnT SP6 High-Yield Expression System (Promega L3261) was used to transcribe and translate DCAF1 in the presence of biotinylated lysine tRNA (Promega L5061). Ten μl of the TnT reaction, containing less than or equal to 1 μg of DCAF1, was diluted in 0.5 ml of Binding Buffer (20 mM Tris pH7.5, 100 mM NaCl, 2 mM EDTA, 10% Glycerol, 0.05% NP-40, 0.5 mM DTT, and protease inhibitors) and incubated with 0.5 μg, 1 μg, or 2 μg of each GST-FERM fusion protein immobilized on

Sepharose-Glutathione for 2 hours at 4°C. After washing with Binding Buffer, the samples were analyzed by SDS-PAGE and immunoblotting.

Gene Expression and Silencing

Transient transfections were performed using Lipofectamine 2000 or Oligofectamine (Invitrogen). Retroviral and lentiviral vectors encoding recombinant proteins or shRNAs targeting Merlin (#1: RHS3979-97079645 and #2: RHS3979-97079647 from Open Biosystems) or DCAF1 (#1: TRCN0000129909; #2: TRCN0000129831 from Open Biosystems) were generated as previously described (Pylayeva et al., 2007). Cells were incubated with infectious particles in the presence of 10 μg/ml hexadimethrine bromide (Sigma) overnight. After recovery in complete medium for 24 hours, Meso-33 cells and FC-1801 cells were placed under selection in 2 μg/ml puromycin, 200 μg/ml hygromycin, 500 μg/ml G418, or 10 μg/ml blasticidin. Meso-33 and Met-5A cells were transfected with 75 pmol of ON-TARGETplus SMARTpool against DCAF1 or ON-TARGETplus siCONTROL Non-targeting pool (Dharmacon). HEI-286 cells and HUVECs were transfected with a previously validated si-RNA targeting Merlin, si-Merlin (Okada et al., 2005), with si-DCAF1 #9 or #11 (J-021119-09 and J-021119-11, Dharmacon), or with both. Efficiency of knock down was verified by Q-PCR or immunoblotting. For Q-PCR, total RNA was extracted from half of the transfected cells with Rneasy Mini Kit (Qiagen) and subjected to reverse transcription with SuperScript III First-Strand Synthesis SuperMix (Invitrogen). Q-PCR was performed with Taqman Gene Expression Assay (Applied Biosystems).

BrdU Incorporation Assay

Unless indicated otherwise, Meso-33, Met-5A cells, and HEI-286 cells were plated at 2.5 x 10^4 per cm^2 and HUVECs at 1.5 x 10^5 per cm^2 on fibronectin-coated coverslips and were deprived of growth factors for 24 hours. They were then incubated with complete medium supplemented with BrdU for 24 hours. After fixation with 100% cold methanol, cells were stained with BrdU Labeling and Detection Kit I (Roche). When indicated, cells were lysed in SDS-boiling buffer (10 mM Tris ph 7.5, 1% SDS, 50 mM NaF, 1 mM $NaVO_4$) and subjected to SDS-PAGE and immunoblotting.

DNA Microarray Analysis

FC-1801 cells were left untreated or transduced with lentiviruses encoding sh-control, sh-DCAF1, Merlin-WT, Merlin-L64P, or a combination of viruses encoding sh-DCAF1 and Merlin-WT and were plated in triplicate at subconfluent density in complete medium. cDNAs were hybridized to GeneChip

Mouse Genome 430a 2.0 Array chips (Affymetrix). Raw expression data were generated using GeneSpring software version 9.0 (Silicon Genetics). Robust Multichip Averaging (RMA) was used to summarize the data and normalize them to the median value for parental cells. Principal Component Analysis (PCA) was used to verify the quality of data. Various filtering and statistical analysis constraints were applied to the expression data to exclude those genes that did not vary significantly between comparison groups. First, statistical group comparison was carried out to find genes showing statistically significant differences in mean expression level between selected groups (sh-control versus sh-DCAF1 cells and Merlin-L64P mutant versus Merlin-WT). Log of ratio normalized expression data were analyzed by using the 1-Way ANOVA test, with a P-value cut-off of 0.01. Benjamini and Hochberg false discovery rate was used for multiple test correction. Of the 22,690 probesets in the Affymetrix Mouse *Genome* 430a 2.0 chip, 14,166 genes passed this restriction: 7,708 entities differentially expressed between cells expressing sh-control and sh-DCAF1 and 9,217 entities between those expressing Merlin-L64P and Merlin WT. To identify genes differentially expressed upon knock down of DCAF1 or expression of Merlin, we used a threshold fold change of 2. By using this criterion, 417 entities were identified as differentially expressed in cells expressing sh-DCAF1 as compared to cells expressing sh-Control and 1,288 between cells expressing Merlin-WT as compared to those expressing Merlin-L64P. Co-regulated genes (885) were identified as differentially expressed ≥ 2 fold in one comparison and showing a concordant and statistically significant difference in expression ($p \leq 0.01$ by 1-way ANOVA test) in the other. Centroid linkage clustering and a Pearson Centered distance metric (Genespring 9.0, Silicon Genetics) were used for hierarchical clustering. For functional profiling, we used more stringent criteria. We considered as coregulated only those genes showing a consensual differential expression of ≥ 1.5 fold within both comparisons and in comparison with parental cells. A total of 667 entities (353 downregulated and 314 upregulated consensually under both conditions) were grouped in Biological Functions and Canonical Pathways by Ingenuity Pathways Analysis (IPA) Software (2008 Ingenuity Systems Inc). Literature searches were used to allocate genes to three additional categories: Cell Adhesions and Tyrosine Kinase Signaling, Intracellular Traffic, and Hippo Targets. Hippo pathway target genes were identified in previous studies (Dong et al., 2007; Zhao et al., 2008).

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Supplemental Figure 1

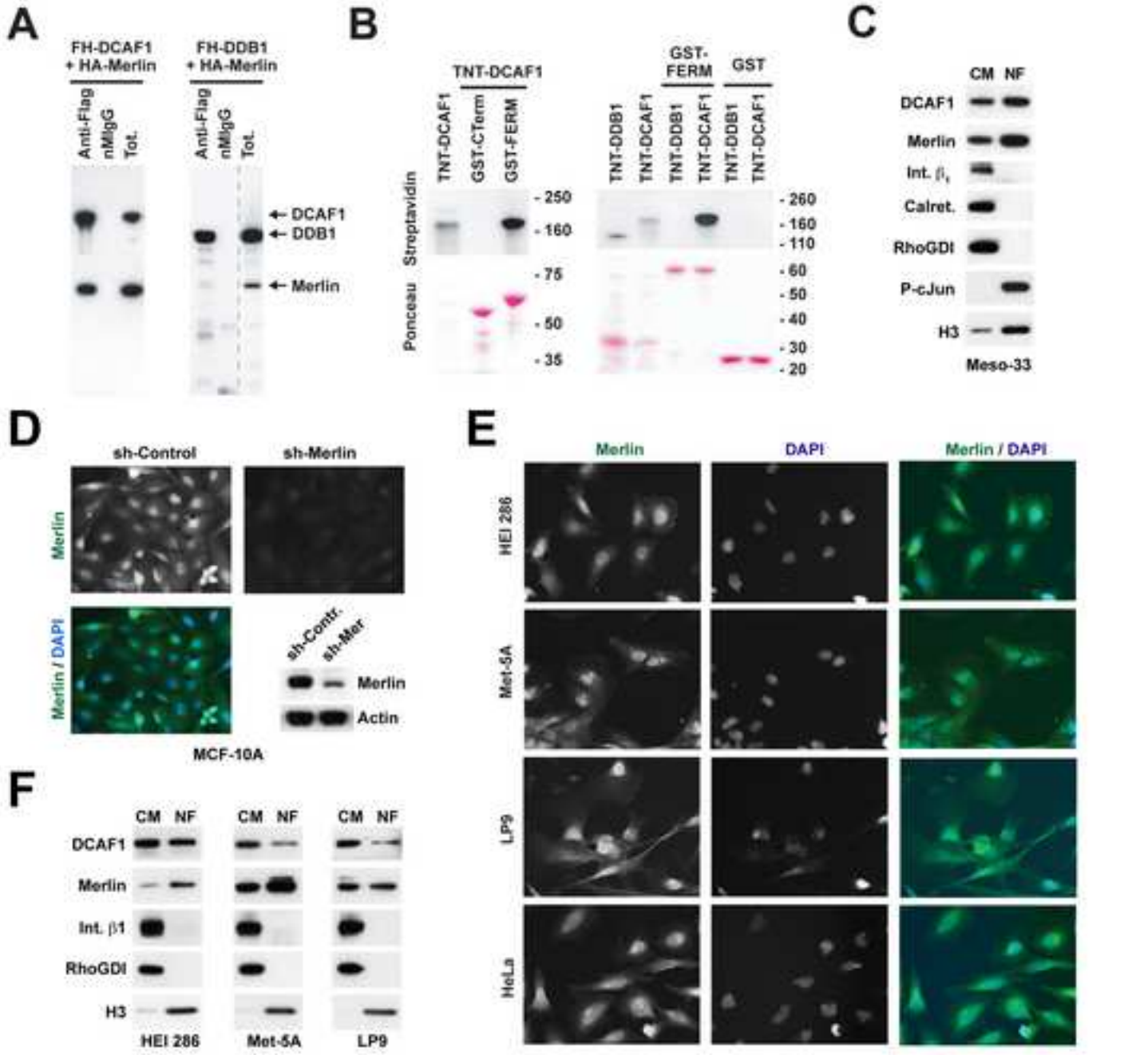

Figure S1, related to Figure 1.

(**A**) Cos7 cells were transfected with HA-Merlin in combination with FH-DCAF1 or FH-DDB1, immunoprecipitated with anti-Flag (M2) or control (nMIgG) antibodies, and immunoblotted with anti-HA. Total lysates were also subjected to immunoblotting.

(**B**) TNT-translated and biotinylated DCAF1 was subjected to pull down with GST fusion proteins comprising the α-helical and C-terminal domain (GST-CTerm) or the FERM domain of Merlin (GST-FERM). An aliquot of TNT-DCAF1 was run as a control (left). TNT-translated and biotinylated DDB1 and DCAF1 were subjected to pull down with a GST fusion protein comprising the FERM domain of Merlin (GST-FERM) or with GST alone. Aliquots of TNT-DDB1 and TNT-DCAF1 were run as controls (right). Blots were stained with Ponceau and reacted with peroxidase-conjugated Streptavidin.

(**C**) Meso-33 cells were transiently transfected with moderate amounts of HA-Merlin and subjected to subcellular fractionation. Equal amounts of proteins from the nuclear (NF) and non-nuclear (CM; cytosol + crude membranes) fractions were subjected to immunoblotting with antibodies to the indicated proteins.

(**D**) MCF-10A cells were infected with lentiviral vectors encoding a sh-RNA targeting Merlin or a control sh-RNA and stained with affinity-purified antibodies to the C-terminus of Merlin. Nuclei were labeled by DAPI. The efficiency of silencing was verified by immunoblotting (lower right panel).

(**E**) The indicated cells were subjected to staining with anti-Merlin as above and counterstained with DAPI.

(**F**) The indicated cells were subjected to subcellular fractionation as described above.

Supplemental Figure 2

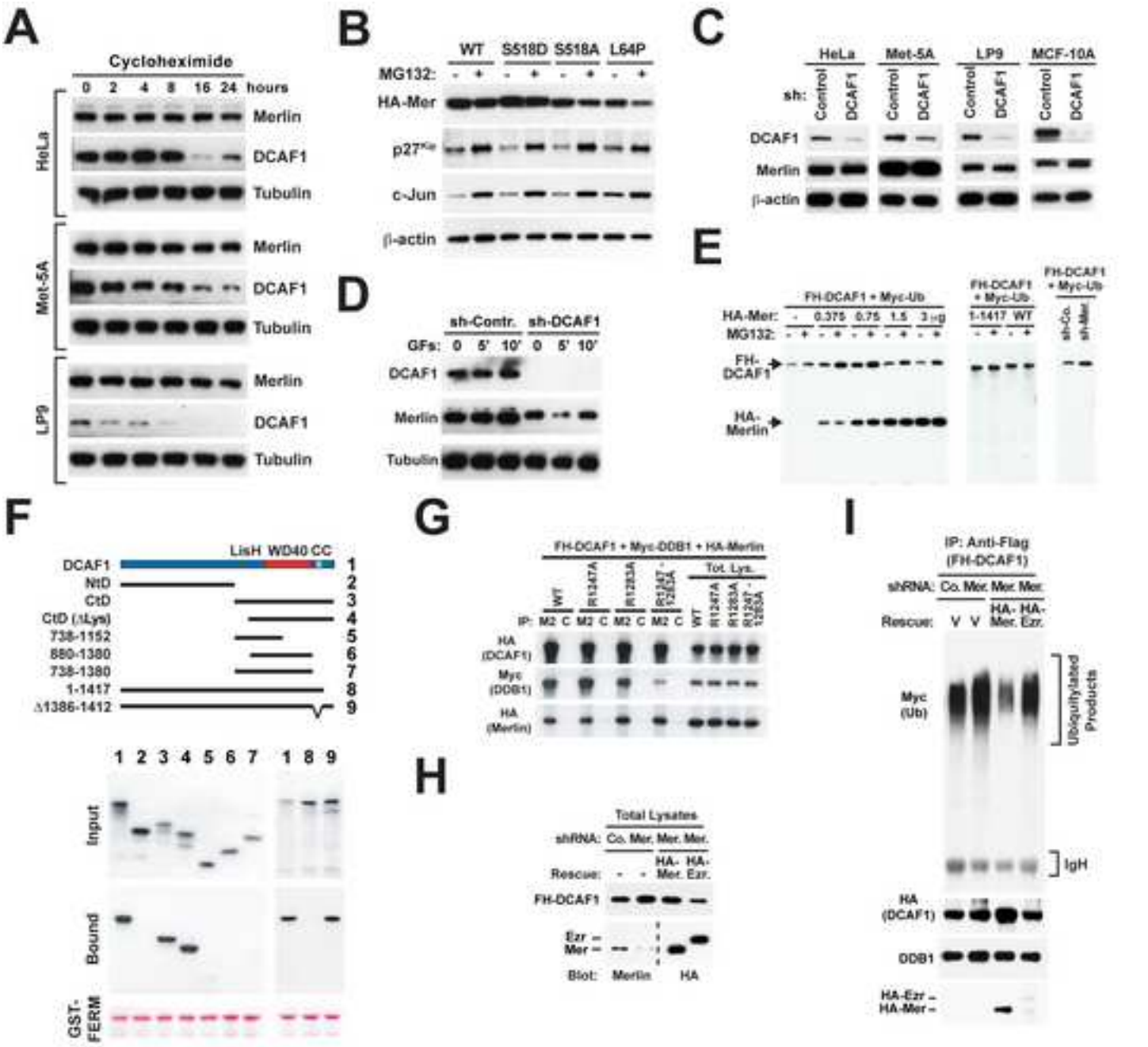

Figure S2, related to Figure 2.

(**A**) Cells were treated with 75 μg/ml Cycloheximide for the indicated times and subjected to immunoblotting as indicated.

(**B**) Cos7 cells transiently transfected with HA-Merlin or the indicated mutants were treated for 6 hours with 25 μM MG132 and subjected to immunoblotting with the indicated antibodies.

(**C**) Cells were infected with a shRNA targeting DCAF1 or a control shRNA and subjected to immunoblotting.

(**D**) Hela cells infected with lentiviruses encoding a control shRNA (sh-Contr.) or a shRNA targeting DCAF1 (sh-DCAF1) were starved for 48 hours, restimulated with 10% serum for the indicated times, and subjected to immunoblotting.

(**E**) Total lysates from the samples of Figure 2A (left), 2C (middle), and 2D (right) were blotted to verify expression levels.

(**F**) TNT-translated and biotinylated DCAF1 and the indicated mutants were subjected to pull down with GST-FERM. Aliquots of input TNT-proteins and the GST-FERM bound fractions were blotted with peroxidase-conjugated Streptavidin. Staining with Ponceau was used to verify levels of GST-FERM in each sample.

(**G**) Cos7 cells were transfected with FH-DCAF1 or the indicated mutants in combination with Myc-DDB1 and HA-Merlin. Anti-Flag (M2) or control Mab (C) precipitates and total lysates were immunoblotted as indicated.

(**H**) Cos7 cells were infected with lentiviruses encoding a control sh-RNA (Co.) or a sh-RNA targeting Merlin (Mer.) and then transfected with FH-DCAF1 and Myc-Ubiquitin plus HA-Merlin (HA-Mer.), HA-Ezrin (HA-Ezr.), or empty vector (V). Lysates were immunoblotted to verify knock down and expression levels.

(I) Samples from H were immunoprecipitated with anti-Flag and immunoblotted as indicated.

Supplemental Figure 3

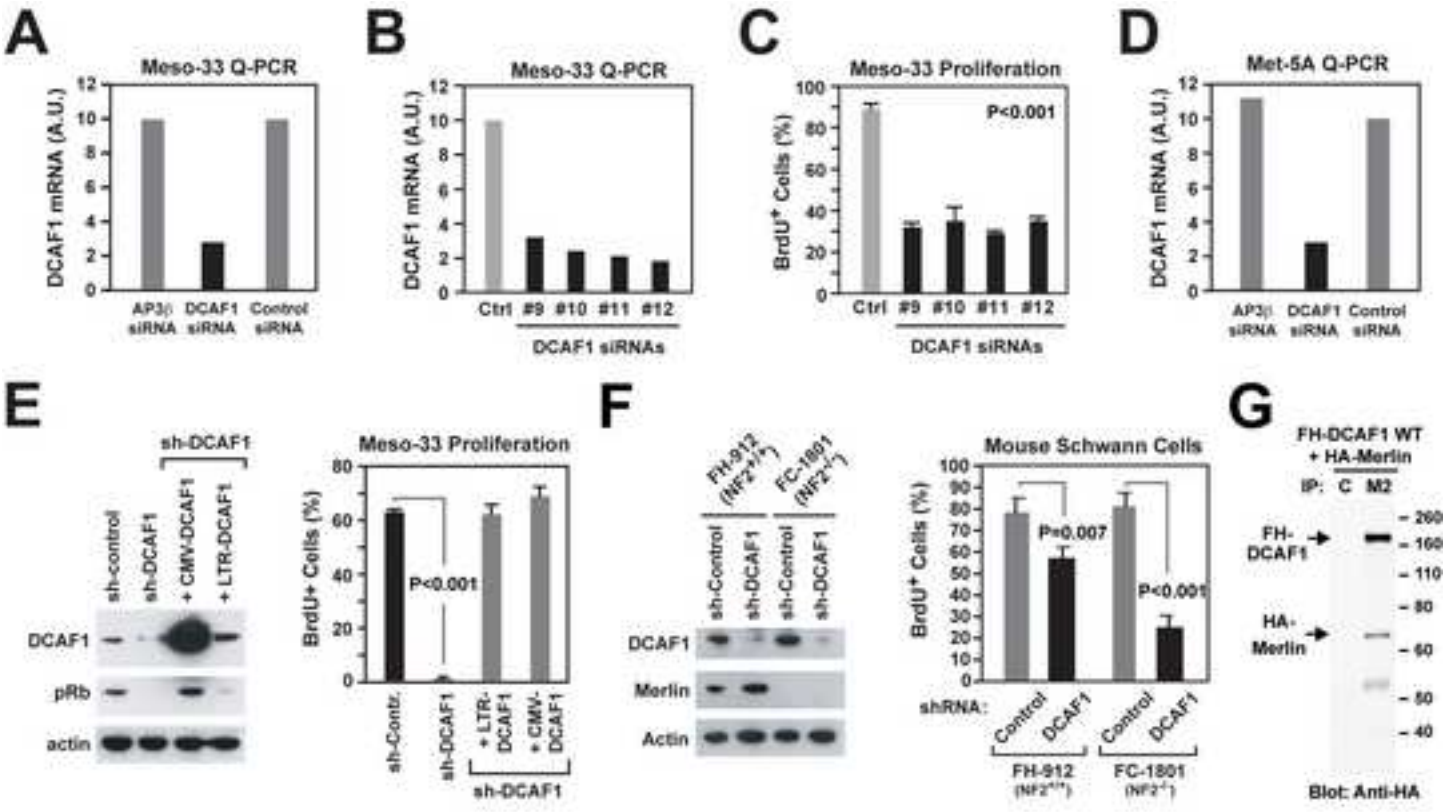

Figure S3, related to Figure 3

(**A**) Meso-33 were transfected with a SMARTpool of siRNAs targeting the adaptor protein AP3β, DCAF1, or a control non-targeting pool and subjected to Q-PCR to verify knock down of DCAF1.

(**B**) Meso-33 cells were transfected with 75 pmol of a control siRNA or each of four siRNAs targeting DCAF1 and subjected to Q-PCR as above.

(**C**) Meso-33 cells treated as above were deprived of growth factors, incubated with BrdU in the presence of mitogens for 24 hours, and subjected to anti-BrdU staining. The graph indicates the percentage (+ SEM) of BrdU-positive cells.

(**D**) Met-5A cells were transfected with a SMARTpool of siRNAs targeting the adaptor protein AP3β, DCAF1, or a control non-targeting pool and subjected to Q-PCR to verify knock down of DCAF1.

(**E**) Meso-33 cells were infected with lentiviral constructs encoding a control shRNA (sh-control) or a sh-RNA targeting DCAF1 (sh-DCAF1). Meso-33 cells expressing sh-DCAF1 were also infected with a CMV-based lentiviral vector encoding a sh-RNA-resistant form of DCAF1 (CMV-DCAF1) or a LTR-based retroviral vector encoding a sh-RNA-resistant form of DCAF1 (LTR-DCAF1). Cells were lysed and equal amounts of total proteins were subjected to immunoblotting with antibodies reacting with DCAF1, phospho-RB and β-actin (left) or they were deprived of growth factors, detached, plated on fibronectin-coated coverslips in complete medium with BrdU for 24 hours, and stained with anti-BrdU. The graph indicates the percentage (+ SEM) of BrdU-positive cells.

(**F**) FH-912 and FC-1801 cells were infected with lentiviruses encoding a control shRNA (sh-control) or a sh-RNA targeting DCAF1 (sh-DCAF1). Cell were lysed and subjected to immunoblotting with antibodies reacting with DCAF1, Merlin and β-actin (left) or they were starved, plated on fibronectin-coated coverslips in complete medium with BrdU for 24 hours, and stained with anti-BrdU. The graph indicates the percentage (+ SEM) of BrdU-positive cells.

(**G**) Lysates containing 0.3 mg of total proteins from Meso-33 cells transfected with FH-DCAF1 WT and HA-Merlin (as in Figure 3E) were immunoprecipitated with anti-Flag Mab (M2) or a control Mab (C) followed by immunoblotting with anti-HA (right). Note that DCAF1 is not saturated by Merlin under these experimental conditions.

Supplemental Figure 4

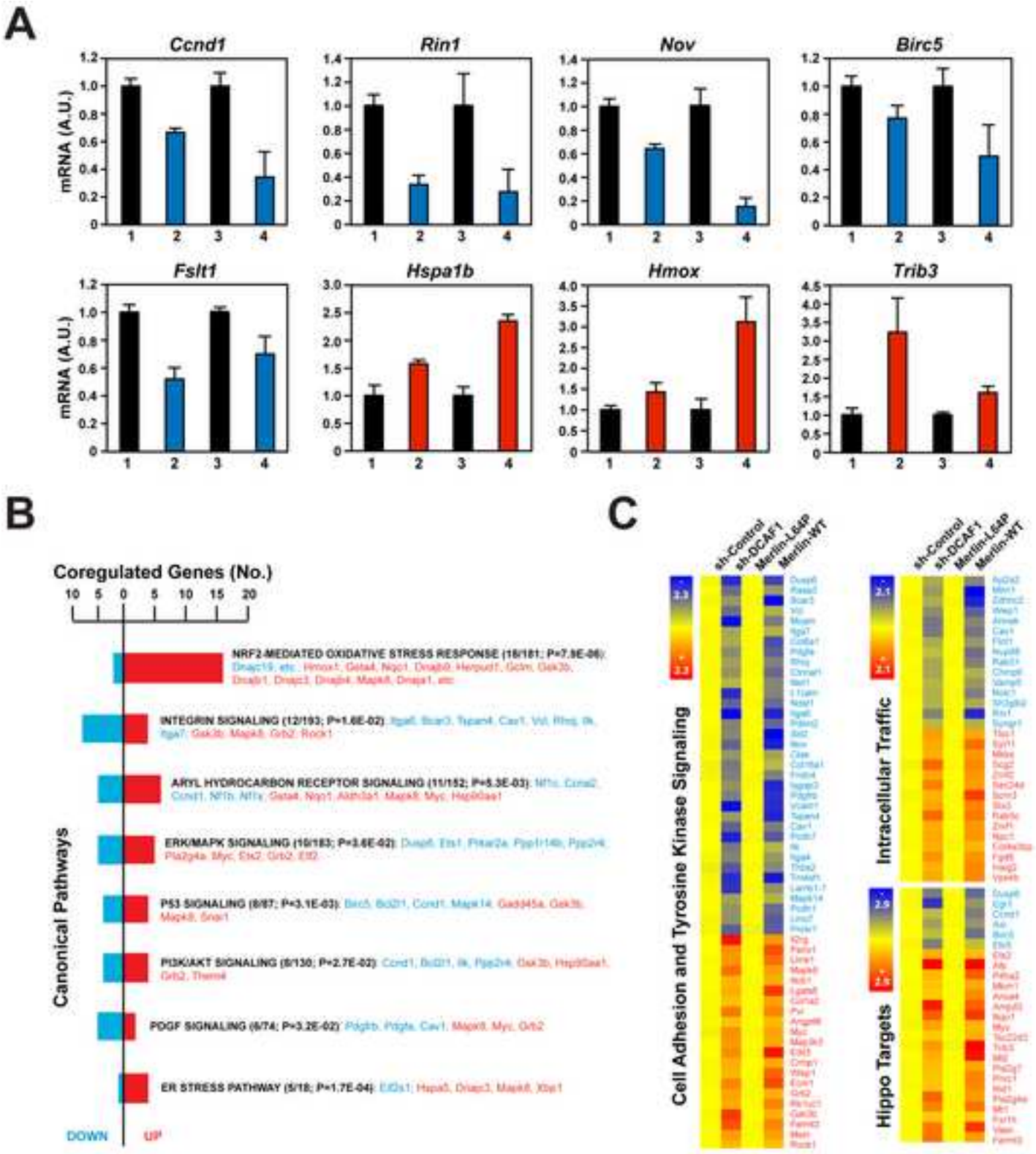

Figure S4, related to Figure 4.

(**A**) FC-1801 cells expressing a control sh-RNA (1), a sh-RNA targeting DCAF1 (2), no insert (3), or Merlin (4) were subjected to Q-PCR analysis to confirm that the indicated genes are coregulated by knock down of DCAF1 and expression of Merlin.

(**B**) Ingenuity Pathway analysis of 667 coregulated genes. Down-regulated and upregulated genes are visualized separately in the category of Canonical Pathways. Representative downregulated genes are shown in blue and upregulated genes in red in order of decreasing average fold change.

(**C**) Coregulated genes implicated in cell adhesion and tyrosine kinase signaling (56), intracellular traffic (30) and Hippo targets (25) were subjected to hierarchical clustering. Downregulated genes are shown in blue and upregulated genes in red.

Supplemental Figure 5

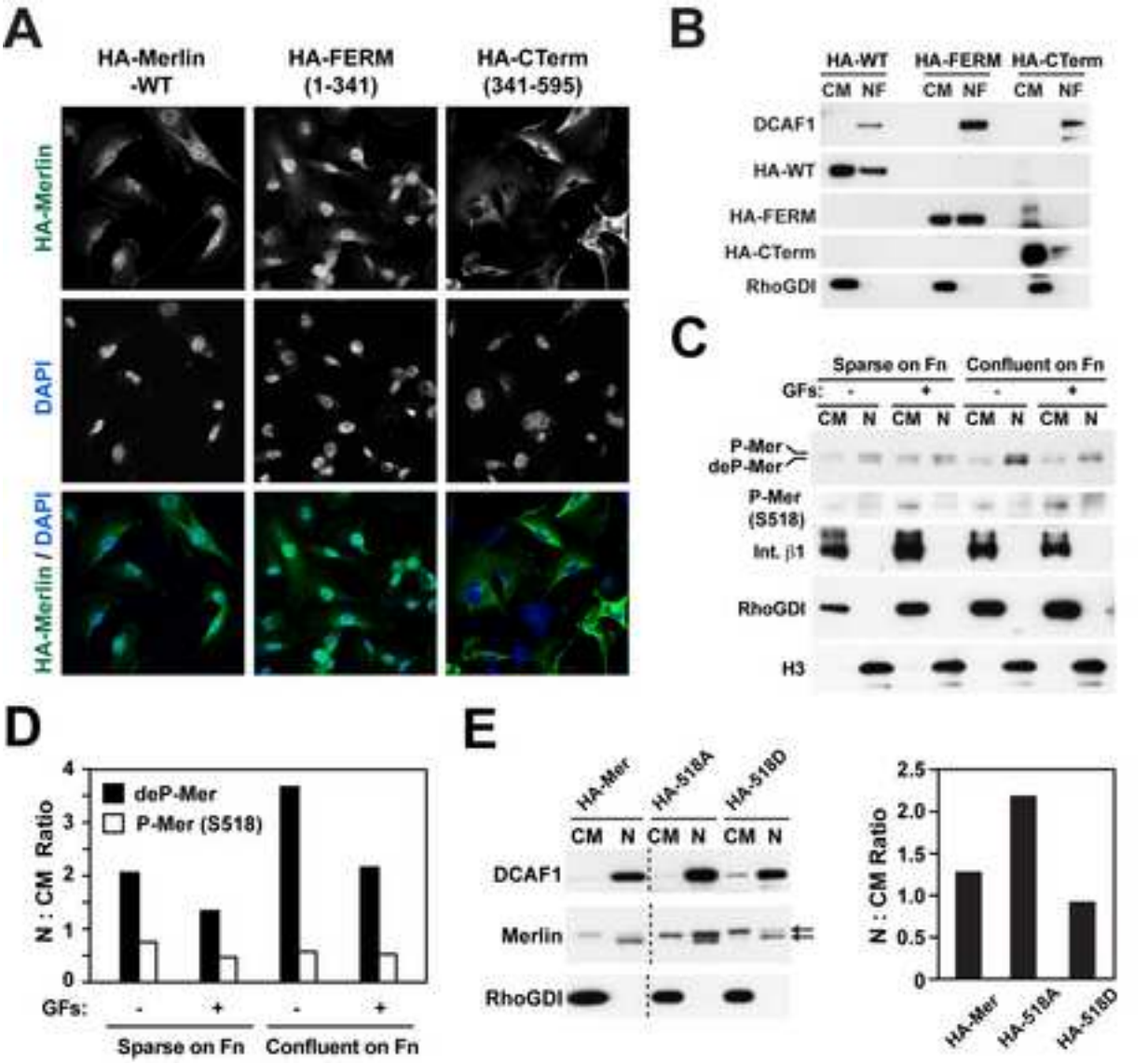

Figure S5, related to Figure 5.

(**A**) Meso-33 cells were transiently transfected with moderate amounts of constructs encoding HA-Merlin or the indicated truncation mutants and subjected to immunofluorescent staining with anti-HA and DAPI.

(**B**) Meso-33 cells transfected as indicated above were subjected to subcellular fractionation. Equal amounts of total proteins from the non-nuclear (CM; cytosol + crude membranes) and nuclear (NF) fraction were subjected to immunoblotting with antibodies against the indicated proteins.

(**C**) HUVECs were deprived of growth factors, detached, replated on fibronectin (Fn)-coated plates under sparse or confluent conditions, and then treated or not with mitogens (bFGF + Hep, EGF, and ITS) for 2 hours. Equal amounts of total proteins from the non-nuclear (CM) and nuclear (NF) fraction were subjected to SDS-PAGE on a gel containing a decreased Acrylamide/Bis-Acrylamide ratio (16:1) to separate the phosphorylated and unphosphorylated form of Merlin (top 2 blots) or on a regular gel (other blots). Samples were subjected to immunoblotting with antibodies against the indicated proteins. The slower migrating form of Merlin present in the nucleus may be phosphorylated at a site distinct from S518, as it is not recognized by anti-P-Merlin (S5518) antibodies.

(**D**) Data were quantified by densitometry. The graph illustrates the ratio of unphosphorylated Merlin or Merlin phosphorylated at S518 present in the nuclear over the non-nuclear fraction.

(**E**) Meso-33 cells were transiently transfected with moderate amounts of HA-Merlin, HA-Merlin-S518A, or HA-Merlin-S518D and subjected to subcellular fractionation. Equal amounts of total proteins from the non-nuclear (CM) and nuclear (NF) fraction were subjected to immunoblotting as indicated. Lower arrow points to a band, which we have detected only after high-resolution SDS-PAGE. This band is recognized by anti-Merlin but not anti-HA antibodies and thus may represent an N-terminally cleaved form of Merlin.The amount of Merlin in each fraction was estimated by densitometry. The graph illustrates the ratio between the nuclear and non-nuclear fraction for the wild type and each of the two mutant forms of Merlin.

Supplemental Figure 6

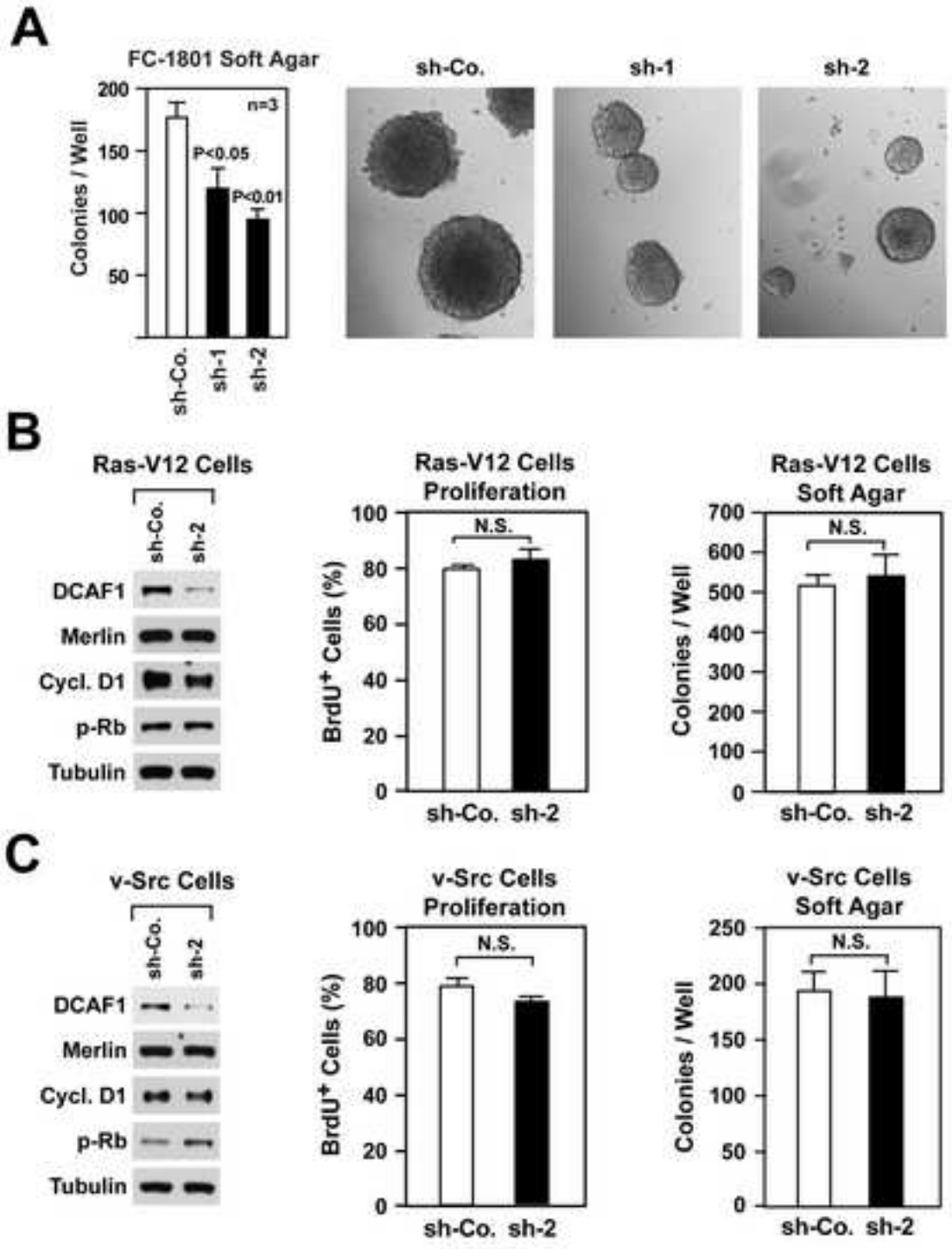

Figure S6, related to Figure 7.

(**A**) FC-1801 cells were infected with lentiviral vectors encoding two distinct sh-RNAs targeting DCAF1 (sh-1 and sh-2) or a control sh-RNA (sh-Co.) and subjected to soft agar assay. The graph shows the total number (+ SEM) of colonies present in each well after 2 weeks of culture. The pictures show that knock down of DCAF1 reduces also average colony size.

(**B**) Ras-V12-transformed 3T3 cells were infected with lentiviral vectors encoding a control sh-RNA (sh-Co.) or a sh-RNA targeting DCAF1 (sh-2) and subjected to immunoblotting with antibodies against the indicated proteins (left), BrdU incorporation assay (middle), and soft agar assay (right). The graph on the right shows the total number (+ SEM) of colonies present in each well after 2 weeks of culture.

(**C**) v-Src-transformed 3T3 cells were infected with lentiviral vectors encoding a control sh-RNA (sh-Co.) or a sh-RNA targeting DCAF1 (sh-2) and subjected to immunoblotting with antibodies against the indicated proteins (left), BrdU incorporation assay (middle), and soft agar assay. The graph on the right shows the total number (+ SEM) of colonies present in each well after 3 weeks of culture.