SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Expression Vectors

pCDNA3-HA-FBXO3, pCAGGSnHC-MPHOSPH1, pECFP-Cdk2, pGL3-HDM2-luc and WWP-Luc (p21/WAF1), pEGFP-Myo5c, pCDNA3-DNMT2-Myc-GIluc, and pCI-SKP1-Myc were a gift from I. Kitabayashi, Y. Nakamura, R. Wolthuis, R. Agami, R. Bernards, R. Cheney, C.-L. Hsieh and J. Neefjes. pcDNA3.1-Lip5-Flag and pcDNA3.1-Chmp5-Flag were a gift from J. Kaplan.

Luciferase Activity Assay

Wild-type 293T cells (50.000 cells/well) were plated in 24-well plates. Conversely, 50.000 control knockdown and 70.000 FBXO3 knockdown cells were seeded in order to ensure a similar confluence at the time of transfection. The same was done for the FMN1 knockdown cells. One day after, cells were transfected with pGL3-HDM2-luc (100 ng) and either pcDNA3 Flag-mDia2 wt or MA (400 ng), or the empty pCDNA3 Flag vector (400 ng). U2OS cells (80.000 cells/well) were plated in 6-well plates and, 16 hours later, co-transfected with pGL3-HDM2-luc (500 ng) and either pcDNA3 Flag-mDia2 wt or MA (1000 ng) with or without pCDNA3-HA-FBXO3 (1000 ng). When pcDNA3-HA-FBXO3 was absent, empty vector (1000 ng) was added. In all experiments, independent triplicates were assayed and the Firefly Luciferase activity was measured 24 hours post-transfection with the Dual-Luciferase® Reporter Assay System (Promega) using an EnVision® Multilabel Reader (PerkinElmer). Initially, we added HDM2-Firefly luciferase-based reported and *Renilla* luciferase-based co-reported in a 1:10 ratio, which prevented *trans* effects between the two promoters (not shown). Incomplete quenching of the Firefly luciferase and low *Renilla* luciferase activity affected the normalization of the samples and dampened the stimulatory

effects of mDia2 (not shown). As total DNA amount and ratio between different plasmids were kept constant, we obtained very similar transfection efficiencies for different conditions and independent samples in both U2OS and 293T cells. Thus, the Firefly Luciferase activity was expressed as arbitrary units/μg of total proteins. Results are normalized against the lowest value of the control samples and represented as relative HDM2-promoter activities (mean ± SD), as obtained from at least three independent experiments.

Kinetics of Caspase-3/7 activity

U2OS cells were transfected in 6-well plates, trypsinized immediately after transfection and seeded in either triplicates or quadruplicates in a 384-well plate (1.200 cells/well) or 96-well plate (2000-3000 cells/well). After approximately one day, medium was replaced with fresh complete medium containing the Caspase-3/7 substrate (CellPlayer^{TM} 96-Well Caspase-3/7 Apoptosis Assay Kit, Essen Bioscience). IncuCyte FLR/ZOOM (Essen Bioscience) was used to track apoptosis over time. Image acquisition, processing and analyses were done with the Object Counting Software Module (Essen Bioscience). Apoptotic index was defined as the ratio between confluence of apoptotic objects and cell confluence and is expressed in arbitrary units.

Apoptosis and Cell Cycle Analysis by FACS

Cells were harvested by trypsinization, washed extensively with phosphate buffered saline and fixed in 70% Ethanol. Prior to FACS analysis, cells were stained overnight with Fluorochrome solution (20 μg/ml propidium iodide, 20 μg/ml RNAse A, 0.1% sodium citrate (w/v), 0.1% Triton-X100 (v/v) at 4°C in the dark. Samples were analysed for DNA content using a FACSCalibur.

qPCR Primers

p53 (TP53):

F_AGGCCTTGGAACTCAAGGAT, R_CCCTTTTTGGACTTCAGGTG

Cyclophillin (PPID):

F_CATCTGCACTGCCAAGACTGA, R_TTGCCAAACACCACATGCTT

Bax (BAX):

F_CAAGACCAGGGTGGTTGG, R_TGGAGGAAAAACACAGTCCA

p21 (CDKN1A):

F_TACCCTTGTGCCTCGCTCAG, R_ GAGAAGATCAGCCGGCGTTT

HDM2 (MDM2):

F_GAATCTACAGGGACGCATC, R_ TCCTTGATCCAACCAATCACC

mDia2 (DIAPH3):

F_GCGGGAAAAGGACTTCAGTAT, R_TCTGTCGGCTTCTCTTAAGACTTC

DAAM1 (DAAM1):

F_GGAGCTACAAGTTGGCCTGA, R_TCCTTCTCTAAAGCCAGCAGA

DAAM2 (DAAM2):

F_CAAAGCCCAAAGTGGAAGC, R_CATCTGTCTAAGACGCTTGCTG

FHOD1 (FHOD1):

F_AGTCTCGTGCCAAAGAGGTG, R_TCCAGCACTGTGGTCATTGT

FHOD3 (FHOD3):

F_AGGCCAGGTTGGAAAGGT, R_TCTGCTGCCAGTGACTCTTG

FMNL1 (FMNL1):

F_CTACGCGCCATCATGAACT, R_ACACAGGCTGGGTGGTTC

FMNL2 (FMNL2):

F_TGTGGAACTGGAAAAGCAACT, R_TGTGTGAACTTGAGTATTTGCATC

FMNL3 (FMNL3):

F_CCATCGAGGACATCATCACA, R_CCGAGAGGGTCTCAGTGG

INF1 (FHDC1):

F_GCATCATGTTCAGAAGACTGCTA, R_TGTCCTGACAAACAGCAAGTG

INF2 (INF2):

F_GAGGTCTTTGCCTCCCTGTT, R_GACAGGAGCTGGGCAGAC

FMN1 (FMN1):

F_GGCCCCTCTGATTCCAAA, R_GCTTGAAGTCTGCCAGGAGT

FMN2 (FMN2):

F_GCTTCCAGAACGTGTTCACAG, R_ATCCGGGAGCAAAACTTCTC

mDia1 (DIAPH1):

F_TTGGACATTCTTAAACGACTTCAT, R_GCTTGTTCCGGCTATCGTAA

mDia3 (DIAPH2):

F_TGCATTTTGAGAAGAACAAAGTG, R_CCAGCTTATCTTGATCTTTGCAG