Supplementary Information

GRIM-19 and p16^{INK4a} synergistically regulate cell cycle progression and E2F1-responsive gene expression

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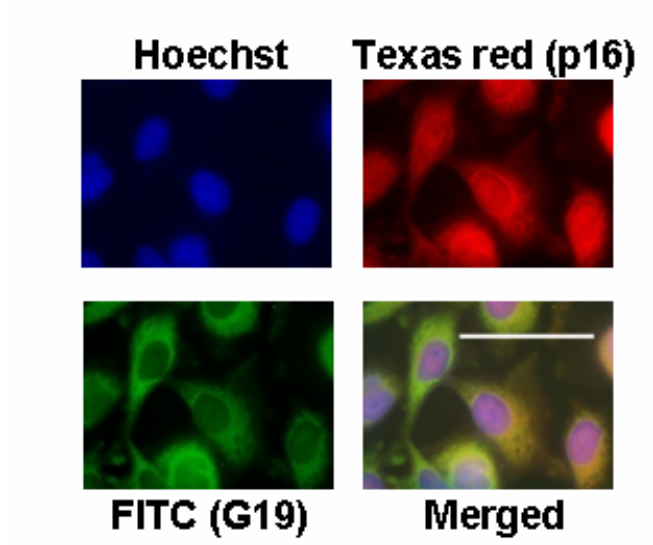
Fig.S1: Aminoacid sequence of human INK4A. Locations of INK4-related tryptic peptides detected by MALTI-TOF analysis.

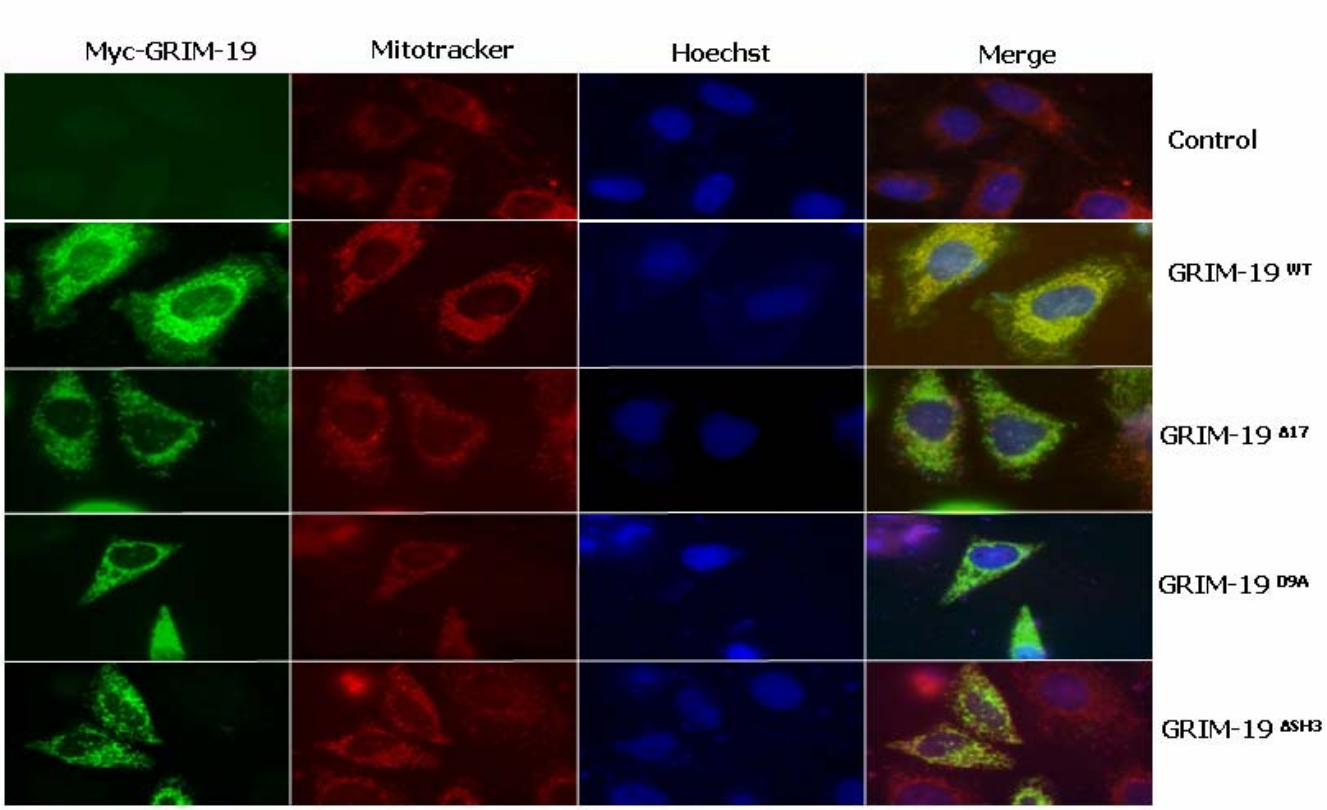
MEPAAGSSMEPSADWLATAAARGRVEEVRALLEAGALPNAPNSYGRRPIQVMMMGSARVAELLLLHGAEPNCADP
ATLTRPVHDAAREGFLDTLVVLHRAGARLDVRDAWGRLPVDLAEELGHRDVARYLRAAAGGTRGSNHARIDAAEG
PSDIPD

Fig.S2



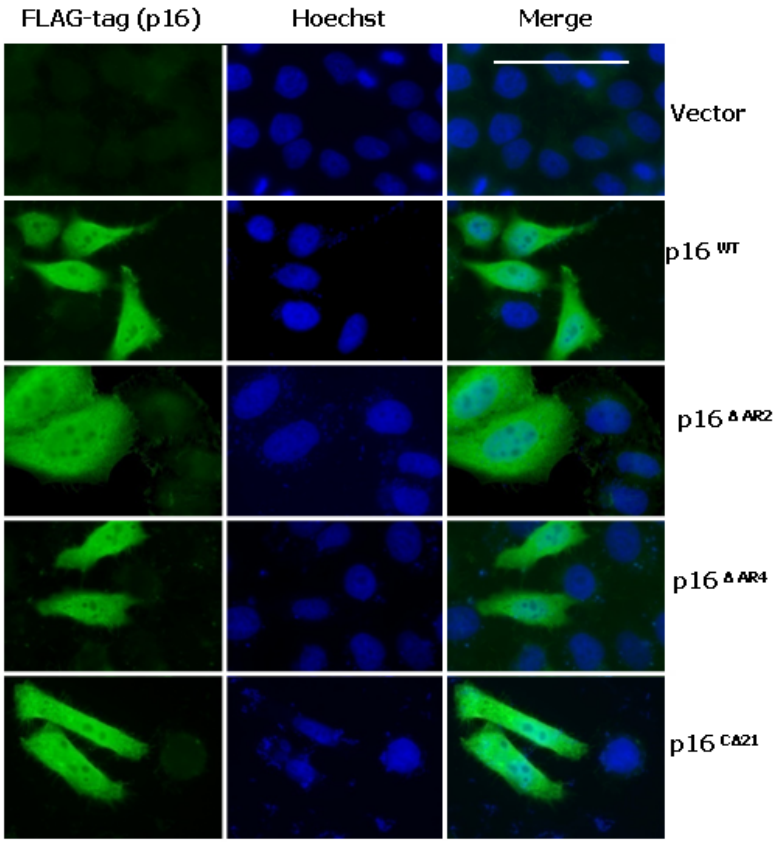
Immunofluorescence microscopy: Methanol-fixed HeLa cells were permeabilized and incubated with the indicated primary antibodies. For detecting GRIM-19 (Green channel) and p16 (Red channel), FITC-conjugated anti-mouse IgG and Texas red-conjugated anti-rabbit IgG were used, respectively. Hoechst was used to visualize nuclei (Blue channel). Images were captured using a fluorescence microscope (Olympus BX-FLA, Osaka) fitted with digital camera (QICAM) and processed by Q-capture pro 5.1 software (Q-Imaging corporation). Co-localization of p16 and GRIM-19 can be seen in the merged image. Scale Bar = 15 μm

Fig.S3



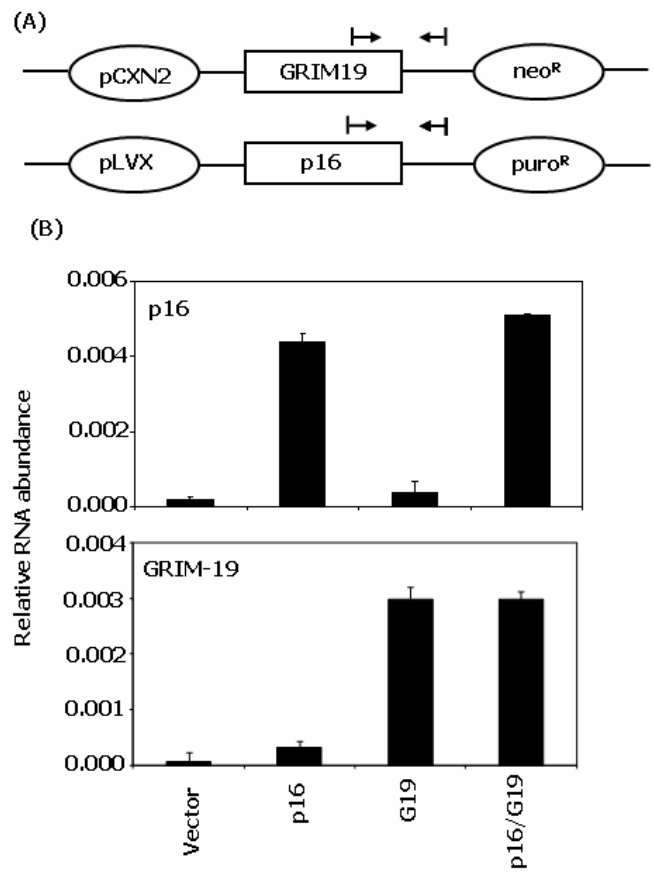
Immunofluorescence microscopy: Methanol-fixed cells were permeabilized and incubated with GRIM-19-specific monoclonal antibody. FITC-tagged secondary antibody was used to detect GRIM-19 (Green channel). For detecting mitochondria (Red channel), cells were incubated with Mito-tracker (Invitrogen) for 15 min followed by methanol fixation, permeabilization and incubation with the indicated antibodies. Hoechst was used to visualize nuclei (Blue channel). Images were captured using a fluorescence microscope (Olympus BX-FLA, Osaka) fitted with digital camera (QICAM) and processed by Q-capture pro 5.1 software (Q-Imaging corporation). For designations see Suppl Table 1A

Fig.S4



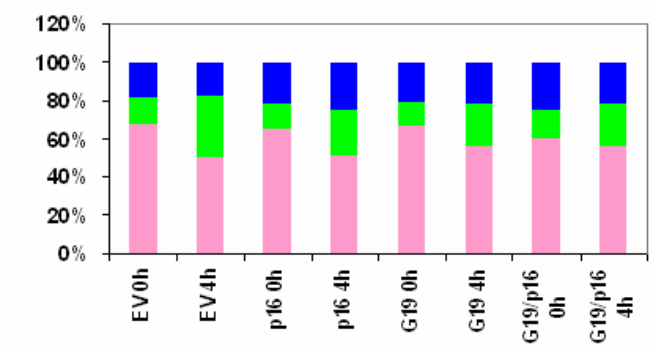
Immunofluorescence microscopy: Methanol-fixed cells were permeabilized and incubated with p16-specific monoclonal antibody. For detecting p16 (Green channel), FITC-conjugated anti-mouse IgG was used. Hoechst was used to visualize nuclei (Blue channel). Images were captured using a fluorescence microscope (Olympus BX-FLA, Osaka) fitted with digital camera (QICAM) and processed by Q-capture pro 5.1 software (Q-Imaging corporation). For designations see Suppl Table 1C

Fig.S5



Exogenous *GRIM19* and *CDKN2A* (p16) messages transcribe equivalently in MCF-7 cell line pairs. A) Schematic representation to detect transgene-derived mRNA. B) Relative abundance of transgene-derived *CDKN2A* and *GRIM19* mRNA as measured by real-time PCR.

Fig.S6



Co-expression of GRIM-19 and p16 exert synergistic inhibitory effect on G1/S progression. Double thymidine-blocked MCF-7 cell line pairs were released and cell population analyzed by FACS at 0h and 4h. Cells in G1 (Pink), S (Green) and G2-M (Blue) phase are shown as shown as a stack for each cell line. Raw data obtained at 0h and 4h for each sample was used to calculate cells exiting G1 and represented as % change compared to 0h.

Suupl Table 1A: Primers employed in PCR for generating point mutations and deletions in human *GRIM19* ORF

Construct ID	Sequence (5' → 3')	
	Sense oligo	**Anti-sense oligo**
WT	GCGGAATTCGCCACCATGGCGGCGTCAAAGGTG	CAGGGATCCCGTGTACCACATGAAGCCG
K5N	GCGGAATTCGCCACCATGGCGGCGTCAAACGTGAAG	
Q8A	GCGGAATTCGCCACCATGGCGGCGTCAAAGGTGAAGGCGGACATG	
D9A	GCGGAATTCGCCACCATGGCGGCGTCAAAGGTGAAGCAGGCGATGCC	
M10A	GCGGAATTCGCCACCATGGCGGCGTCAAAGGTGAAGCAGGACGCGCCTCC	
P11A	GCGGAATTCGCCACCATGGCGGCGTCAAAGGTGAAGCAGGACATGGCGCCGCC	
Δ1-17	GCGGAATTCGCCACCATGCCCATCGACTACAAGCGGAAC	
K99R*	GCGGAATTCGCCACCATGGCGGCGTCAAAGG	GGGCACGTCTCGCATGATGATGGC
	GCCATCATCATGCGAGACGTGCCC	GCGGGTACCCGTGTACCACATGAAGCCG
K105R*	GCGGAATTCGCCACCATGGCGGCGTCAAAGG	AGACTCCCCCACTCGCCAGTC
	GACTGGCGAGTGGGGGAGTCT	GCGGGTACCCGTGTACCACATGAAGCCG
ΔHLH*	GCGGAATTCGCCACCATGGCGGCGTCAAAGG	CACGGATCCCCACTTCATTATGCTCCA
	CTTGGATCCGCGCTGTTGCCACTGTTA	GCGGGTACCCGTGTACCACATGAAGCCG
ΔABD*	GCGGAATTCGCCACCATGGCGGCGTCAAAGG	TCTGGATCCCTTCATGATGATGGCCTC
	TTATTGGATCCGTGCCCCCCTTGATC	GCGGGTACCCGTGTACCACATGAAGCCG
ΔTPD*	GCGGAATTCGCCACCATGGCGGCGTCAAAGG	TTAGGATCCAGACTCCCCCACCTT
	TTATAGGATCCGAGCTGTACGGGCTG	GCGGGTACCCGTGTACCACATGAAGCCG
ΔSH3	GCGGAATTCGCCACCATGGCGGCGTCAAAGG	TTATTGGTACCGATCAAGGGGGGCAC

Restriction enzyme sites are underlined in the primer sequence. * cloned using overlap-extension PCR.

Suppl Table 1B: Primers employed in PCR for generating wild-type expression constructs of murine *Cdkn2* family and human CDKN2A.

Construct ID	Sequence (5' → 3')	
	Sense oligo	**Anti-sense oligo**
Cdkn2a	TACTGTGCTAGCGAGTCCGCTGCAGACAGA	CGTGAATTCGCTCTGCTCTTGGGATTG
Cdkn2c	TACTGTGCTAGCGCCGAGCCTTGGGGGAAC	CGTGAATTCCTGCAGGCTTGTGGCTCC
Cdkn2d	TACTGTGCTAGCCTTCTGGAAGAAGTCTGC	CGTGAATTCCATTGGGATCATCATGTG
CDKN2A	TACTGTGCTAGCGAGCCGGCGGCGGGGAGC	ATGGGATCCTCAATCGGGGATGTCTGAGGG

Restriction enzyme sites are underlined in the primer sequence.

Suppl. Table 1C: Primers employed in PCR for generating mutant *Cdkn2a/CDKN2A* constructs.

Construct ID	Sequence (5' → 3')	
	Sense oligo	**Anti-sense oligo**
Cdkn2a* ΔAR1	TACTGTGCTAGCGAGTCCGCTGCAGACAGA	CCGCCGAGCGCGCCAGTCTGTCTGC
	CGTGCGGGCGCGCCTGGAAGCCGG	GAAGGATCCGCTCTGCTCTTGGGATTG
Cdkn2a* ΔAR2	TACTGTGCTAGCGAGTCCGCTGCAGACAGA	AAAGAATTCGGGGCGTTGGGCG
	CCCGAATTCTCACGTAGCAGC	GAAGGATCCGCTCTGCTCTTGGGATTG
Cdkn2a* ΔAR3	TACTGTGCTAGCGAGTCCGCTGCAGACAGA	GCGTGTCTAGAAAGCCTTCCCGCGC
	GCTCGTCTAGATGTGCGCGATGCC	GAAGGATCCGCTCTGCTCTTGGGATTG
Cdkn2a* ΔAR4	TACTGTGCTAGCGAGTCCGCTGCAGACAGA	GCACCCCGCGGAACGCAAATATCGC
	GTGTACCGCGGCCAACGTCGCCC	GAAGGATCCGCTCTGCTCTTGGGATTG
***Cdkn2a* ΔC21**	TACTGTGCTAGCGAGTCCGCTGCAGACAGA	GAAGGATCCCCCGTCGGTCTGGG
CDKN2A* G139D	GGCACCAGAGACAGTAACCATGC	ATGGGATCCTCAATCGGGGATGTCTGAGGG
	TACTGTGCTAGCGAGCCGGCGGCGGGGAGC	GCATGGTTACTGTCTCTGGTGCC
CDKN2A* S140C	GCACCAGAGGCTGTAACCATGCC	ATGGGATCCTCAATCGGGGATGTCTGAGGG
	TACTGTGCTAGCGAGCCGGCGGCGGGGAGC	GGCATGGTTACAGCCTCTGGTGC
CDKN2A* H142R	GGCAGTAACCGTGCCCGCATAG	ATGGGATCCTCAATCGGGGATGTCTGAGGG
	TACTGTGCTAGCGAGCCGGCGGCGGGGAGC	CTATGCGGGCACGGTTACTGCC
CDKN2A* A147G	CGCATAGATGGCGCGGAAGG	ATGGGATCCTCAATCGGGGATGTCTGAGGG
	TACTGTGCTAGCGAGCCGGCGGCGGGGAGC	CCTTCCGCGCCATCTATGCG

Restriction enzyme sites are underlined in the primer sequence. * cloned using overlap-extension PCR.

Suppl. Table 2: Primer employed in real-time PCR for evaluating expression levels in human cell lines.

Transcript ID	Sequence (5' → 3')	
	Sense oligo	**Anti-sense oligo**
MCM4	GACGAAGCCTATGACAGGCG	TAGCATGGGCTTCTGCTAAGCG
DHFR	ACTGAACAACCAGAATTAGCA	TCAGAGAGAACACCTGGGTAT
MYBB	CGGAGCCCCATCAAGAAAGT	AGGTGTCGTGAAGTGGCTTC
TK1	AGACACTCGCTACAGCAGC	CTCTGGAAGGTCCCATCCAGT
GRIM19 **(native)**	TGCCACTGTTACAGGCAGAAACC	TGAAGCCGTGGCTGGCATGGAGAGC
GRIM19 **(transgene)**	TGCCACTGTTACAGGCAGAAACC	TTCTGAGATGAGTTTTTGTTCGGGCC*
CDKN2A **(transgene)**	GTCAGGGGCGCGCCTGGATGTGCGC	CCAGGTGAATATCAAATCCTCCTCG*

* Expression vector-borne sequence present in the transcript.