HDL-MEDIATED REDUCTION OF CHOLESTEROL CONTENT INHIBITS THE PROLIFERATION OF PROSTATE CANCER CELLS INDUCED BY LDL: ROLE OF ABCA1 AND PROTEASOME INHIBITION

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SUPPLEMENTAL FILE

Supplementary table 1. Primers for real time PCR

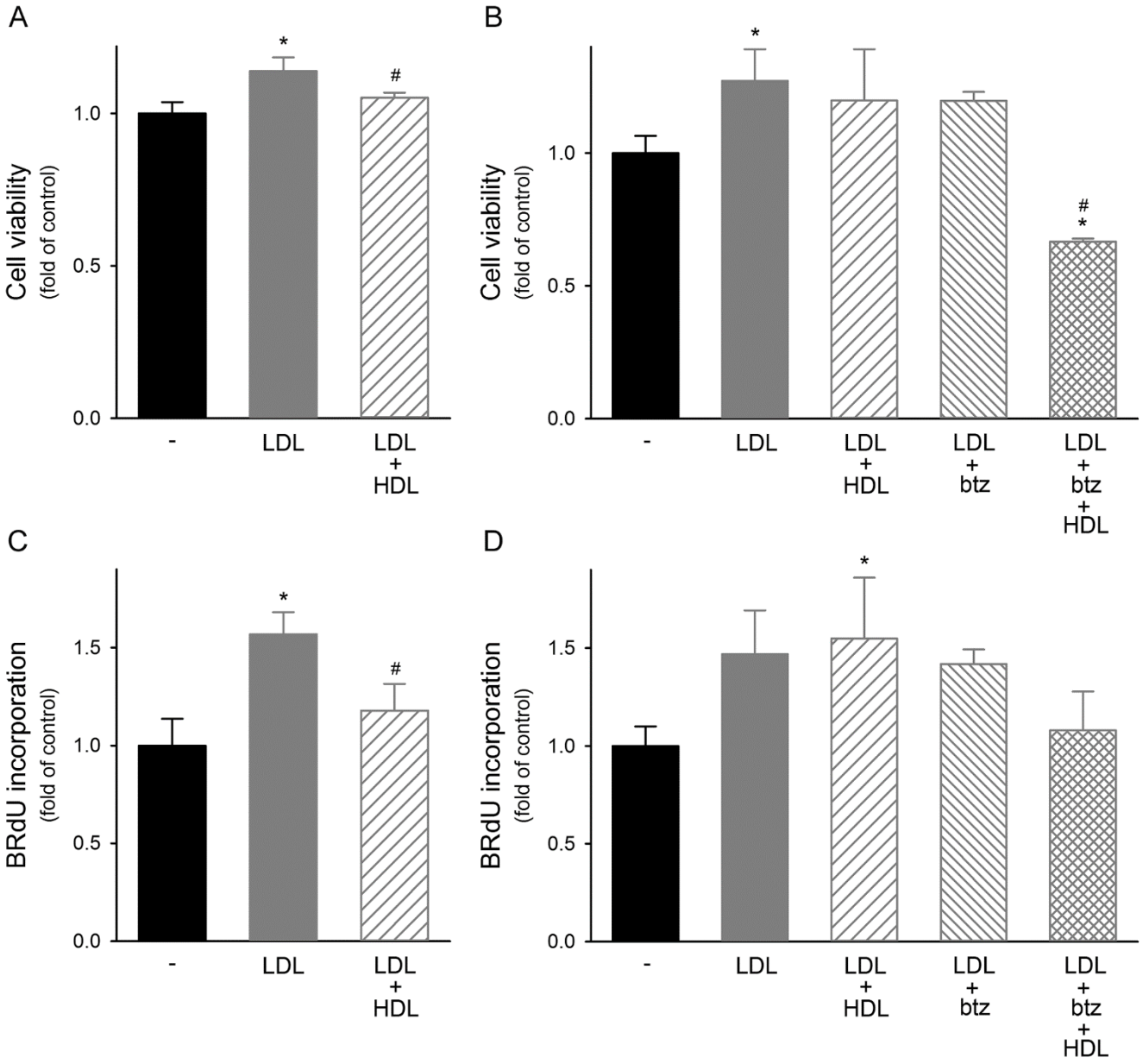
Target	forward	reverse
LDLR	5'-CAATGTCTCACCAAGCTCTG-3'	5'-TCTGTCTCGAGGGGTAGCTG-3'
LRP1	5'-GACCAGGTGTTGGACGCAGACG-3'	5'-AATCGTTGTCTCCGTCACACTTC-3'
SRD5A	5'-CCTGTTGAATGCTTCATGACTTG-3'	5'-TAAGGCAAAGCAATGCCAGATG-3'
HMGCR	5'-GGCCCAGTTGTGCGTCTT-3'	5'-TTTCGAGCCAGGCTTTCACT-3'
SREBF2	5'-CCGCCTGTTCCGATGTACAC-3'	5'-TGCACATTCAGCCAGGTTCA-3'
ABCA1	5'-GCACTGAGGAAGATGCTGAAA-3'	5'-AGTTCCTGGAAGGCTTTGTTCAC-3'
GAPDH	5'-CCCTTCATTGACCTCAACTACATG-3'	5'-TGGGATTTCCATTGATGACAAGC-3'

Supplementary table 2. Antibodies for western blotting and immunofluorescence

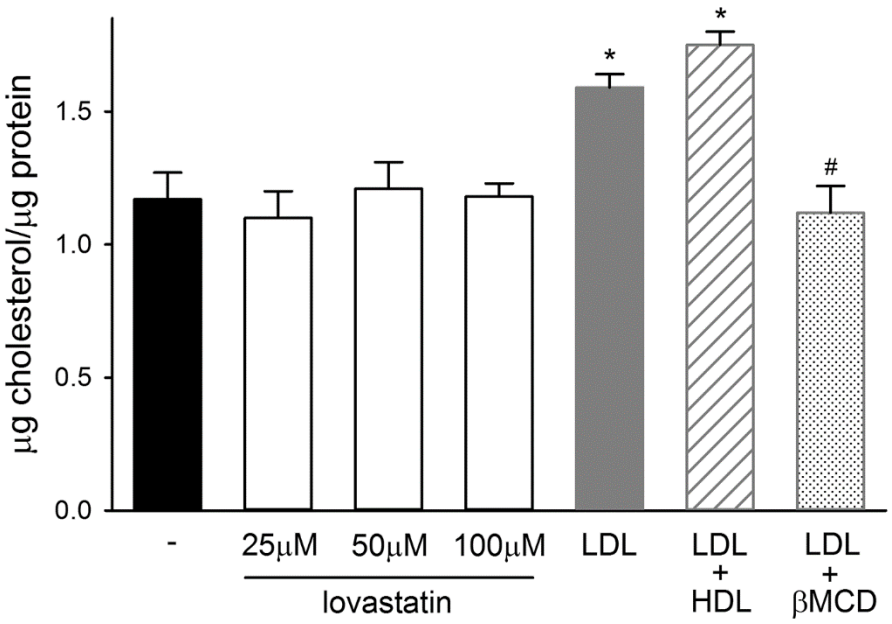
Target	Source	Catalogue number
Human LDL-receptor	ThermoFisher Scientific, IL, USA	PA5-22976
Human LRP1	Abcam, UK	ab92544
Human HMGCoA-reductase	Abcam, UK	ab174830
Human SR-BI	Novus Biologicals, CO, USA	NB400-104
Human p21	Santa Cruz biotechnology, TX, USA	Sc-817
Human ABCA1	ThermoFisher Scientific, IL, USA	PA1-16789
Human α-tubulin	Sigma-Aldrich, MO, USA	T9026
Human phosphorylated NF-kB p65 (Ser536)	Cell Signaling Technology, MA, USA	3033
Human NF-kB p65	Cell Signaling Technology, MA, USA	8242
Polyclonal goat anti-rabbit immunoglobulins/HRP	Dako Cytomation, Denmark	P0448
Polyclonal rabbit anti-mouse immunoglobulins/HRP	Dako Cytomation, Denmark	P0260
anti-mouse AlexaFluor-488	ThermoFisher Scientific, IL, USA	A32723
anti-rabbit Rhodamine conjugated	ThermoFisher Scientific, IL, USA	31670

Supplementary table 3. Polymorphisms in the *ABCA1* gene of PC3 cells

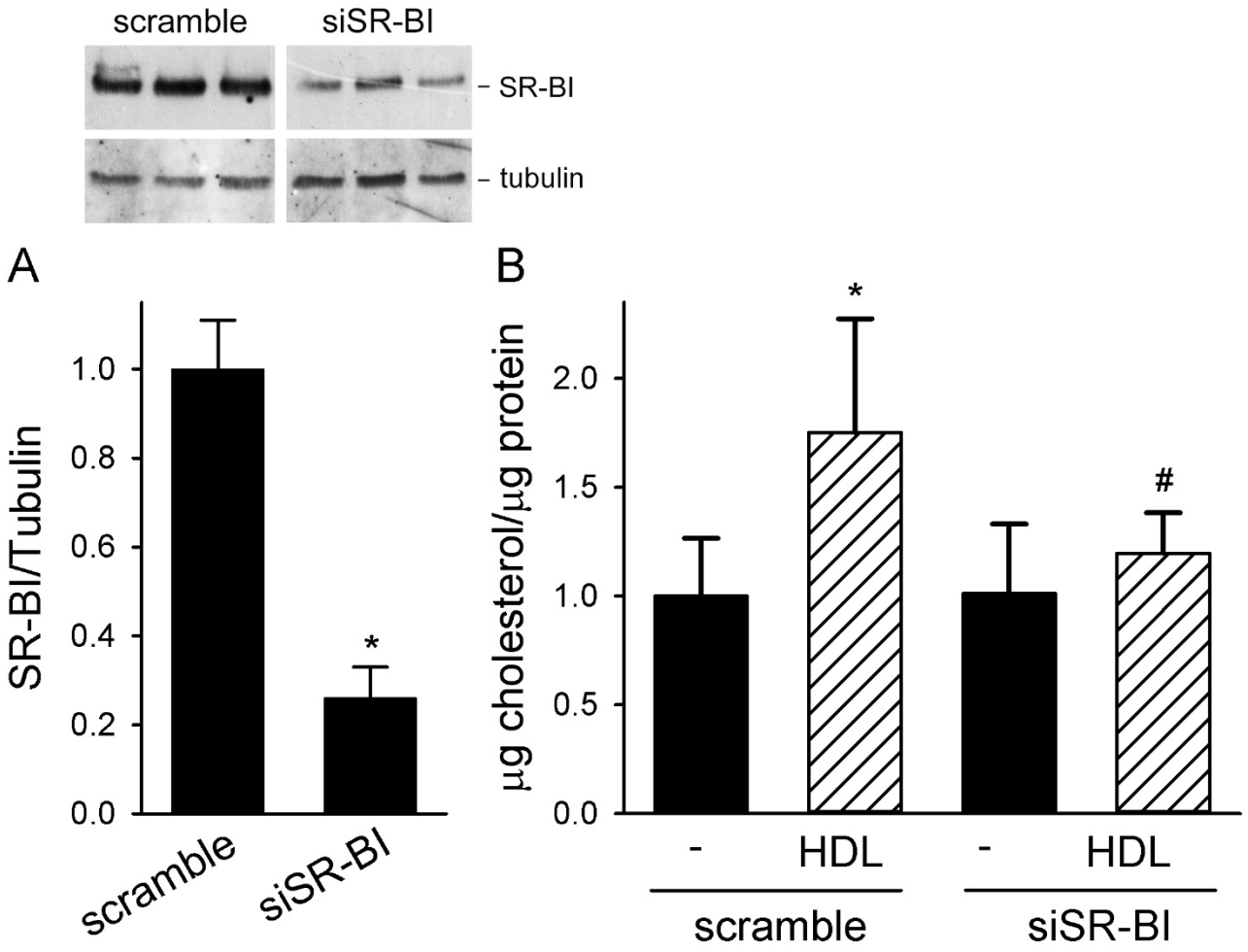
Region	Variant	N. of mutated alleles
Exon 2	c.-76delG	2
Exon 2	c.-18 C>G	2
Exon 8	c.765 C>T (p.Ala255Ala)	2
Intron 32	c.4559+30 G>T	2
Exon 35	c.4760 A>G (p.Lys1587Arg)	2
Exon 44	c.5921 G>A (p.Arg1974Lys)	2
Intron 48	c.6401+86 C>A	2
Exon 50	c.*39 G>A	2



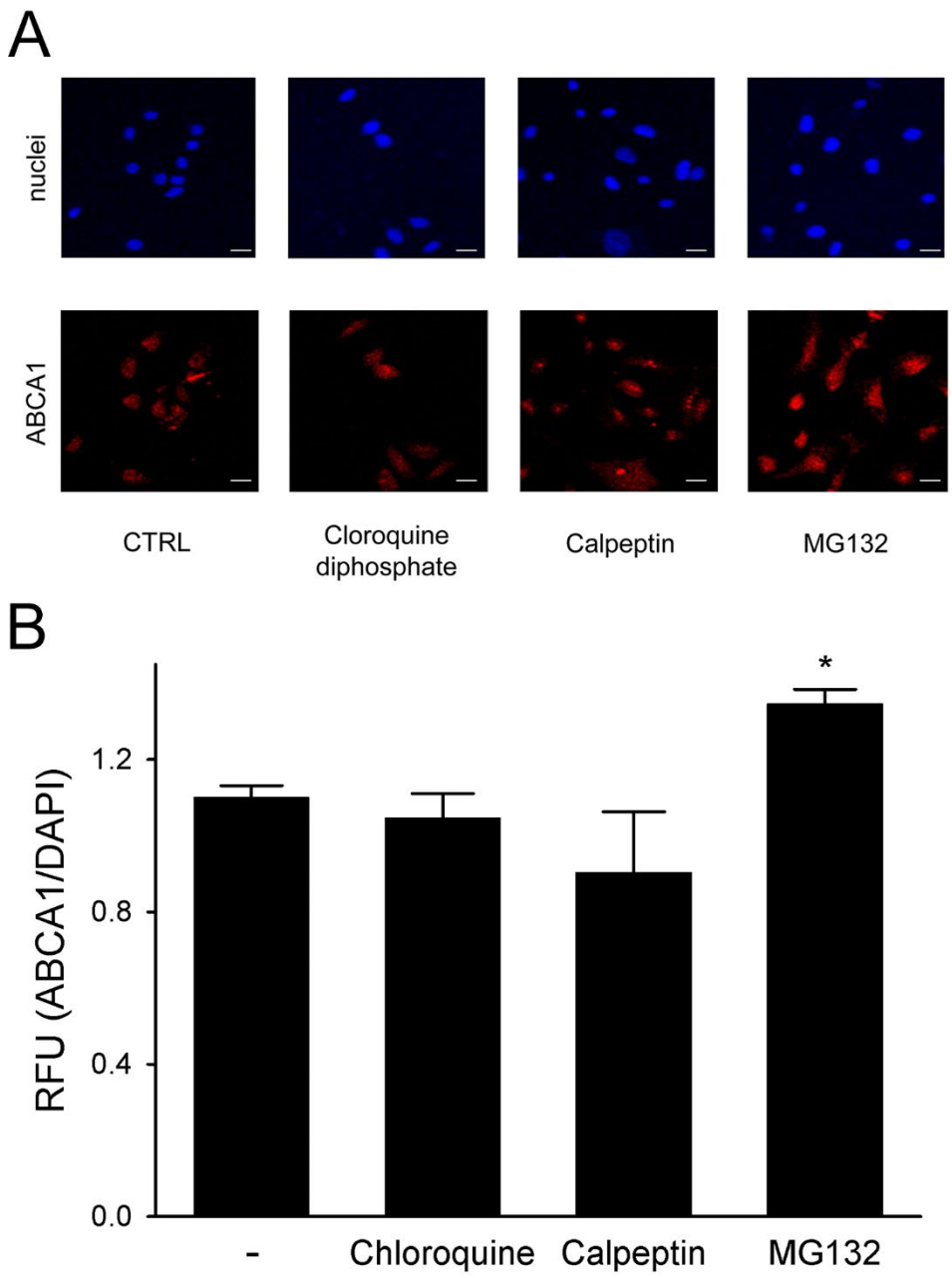
Supplementary figure 1. Proliferation assays in LNCaP and PC3 cells. LNCaP proliferation was evaluated by measuring ATP levels (Panel A) or BrdU incorporation (Panel C) at 72h in untreated cells (black bars) or in cells incubated with LDL at 20 ug/ml for the first 24h followed (grey dashed bars) or not (grey bars) by HDL at 0.5 mg/ml for 48h. Data are expressed as fold of untreated cells, mean±SD, n=3. *P<0.05 vs untreated cells, #P<0.05 vs LDL. Proliferation of PC3 cells was evaluated by measuring ATP levels (Panel B) or BrdU incorporation (Panel D) at 72h in untreated cells (black bars) or in cells incubated with (i) LDL at 20 ug/ml for 24h (grey bars), (ii) LDL at 20 ug/ml for the first 24h followed by HDL at 0.5 mg/ml for 48h (grey dashed bars), (iii) LDL at 20 ug/ml plus bortezomib 5 nM for 24h followed (grey crossed bars) or not (grey dashed bars) by HDL at 0.5 mg/ml for 48h. Data are expressed as fold of untreated cells, mean±SD, n=3. *P<0.05 vs untreated cells, #P<0.05 vs LDL.



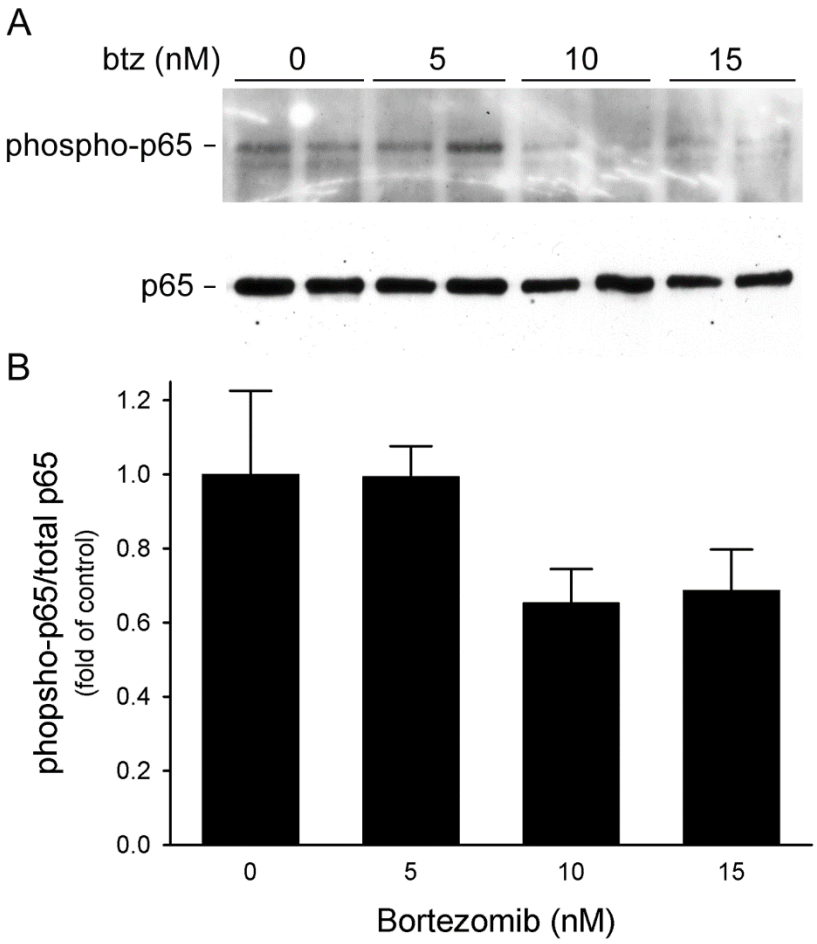
Supplementary figure 2. Effect of lovastatin and βMCD on PC3 cholesterol content. Cell cholesterol content was measured in untreated cells (black bar) and in cells incubated with: (i) lovastatin at the indicated concentrations for 24h (open bars), (ii) LDL at 50 ug/ml for 24h (grey bar), (iii) LDL at 50 ug/ml for 24h followed by HDL at 0.5 mg/ml (dashed bar) or β-methylcyclodextrin at 2.5mM (βMCD, dotted bar) for 1h. Data are expressed as mean±SD, n=3. *P<0.05 vs untreated cells, #P<0.05 vs LDL. Before use, lovastatin was converted to its active form by incubation with NaOH at 50°C followed by neutralization with HCl.



Supplementary figure 3. Silencing of SR-BI in PC3 cells. Panel A, SR-BI protein levels by Western blotting in PC3 treated with noncoding (scramble) or SR-BI specific siRNA for 48h. Results are expressed as fold of scramble-treated cells, mean±SD, n=3. The blot is shown at the top. Panel B, Cell cholesterol content in PC3 cells pre-treated with noncoding (scramble) or SR-BI specific siRNA. Cell were exposed (dashed bars) or not (black bars) to HDL 0.5 mg/ml for 1h. Data are expressed as mean±SD, n=5. **P*<0.05 vs scramble control cells, #*P*<0.05 vs scramble HDL-treated cells.



Supplementary figure 4. Effect of the inhibition of protein degradation systems on ABCA1 expression in PC3 cells. PC3 cells were treated for 4h with MG132 50 μM, chloroquine diphosphate 100 μM or calpeptin 30 ug/ml. Panel A, Representative confocal immunofluorescence images for ABCA1 (red), and DAPI (blue). Scale bar, 50 μm. Panel B, Quantitative assessment of ABCA1 fluorescence intensities in untreated cells (-) and in cells incubated with the indicated inhibitors. Data are expressed as ABCA1/DAPI signal ratio, mean±SD, n=3. *P<0.05 vs untreated cells.



Supplementary figure 5. NF-kB activation after bortezomib. Representative blot (Panel A) and phosphorylated/total NF-kB p65 ratio (Panel B) by western blotting in PC3 cells incubated with increasing concentrations of bortezomib. Data are expressed as fold increase of untreated cells, mean±SD, n=3.