Appendix A: Supplementary data

An actionable sterol-regulated feedback loop modulates statin sensitivity in prostate cancer

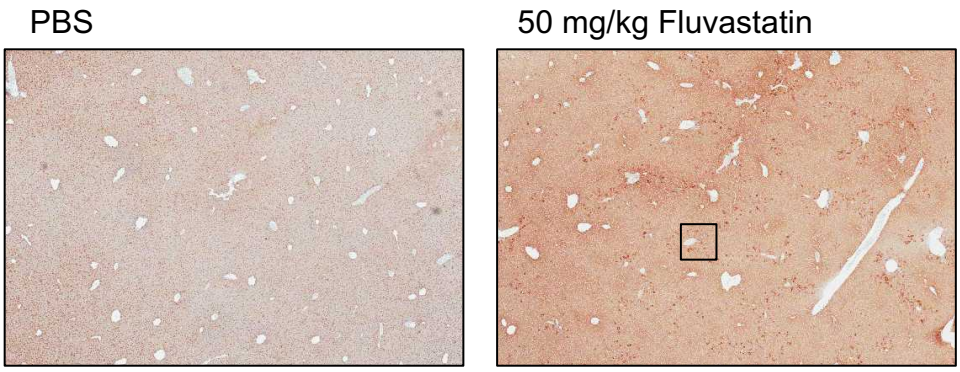
Joseph Longo, Peter J. Mullen, Rosemary Yu, Jenna E. van Leeuwen, Mehdi Masoomian, Dixon T. S. Woon, Yuzhuo Wang, Eric X. Chen, Robert J. Hamilton, Joan M. Sweet, Theodorus H. van der Kwast, Neil E. Fleshner, Linda Z. Penn

This PDF file includes:

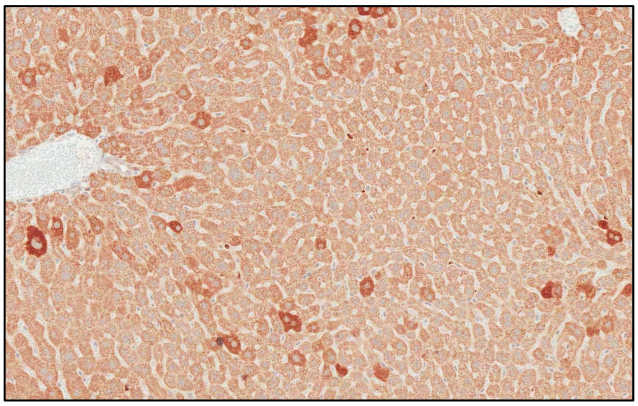
- Supplementary Figures 1-6
- Supplementary Table 1

Supplementary Figure 1

A



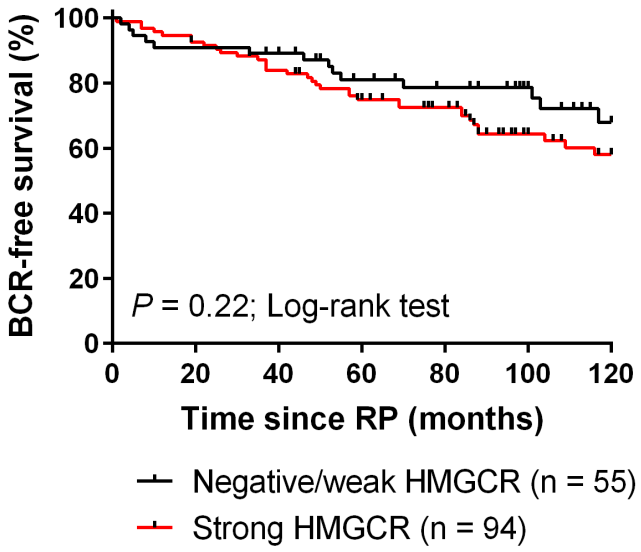
B



Supplementary Figure 1: Immunohistochemistry (IHC) validation of a monoclonal antibody against HMGCR in liver tissues of control and fluvastatin-treated mice. (A) HMGCR staining in liver tissues of male NOD/SCID mice treated with either PBS or 50 mg/kg/day fluvastatin by oral gavage for 4 consecutive days. Liver tissues were harvested 2 hours after the last treatment, and the formalin-fixed and paraffin-embedded tissues were subsequently subjected to IHC using the A9 mouse monoclonal antibody against HMGCR. As expected, fluvastatin treatment induced the expression of HMGCR in the liver. (B) Higher magnification image (20x) of the liver tissue from the fluvastatin-treated mouse in (A).

Supplementary Figure 2

A

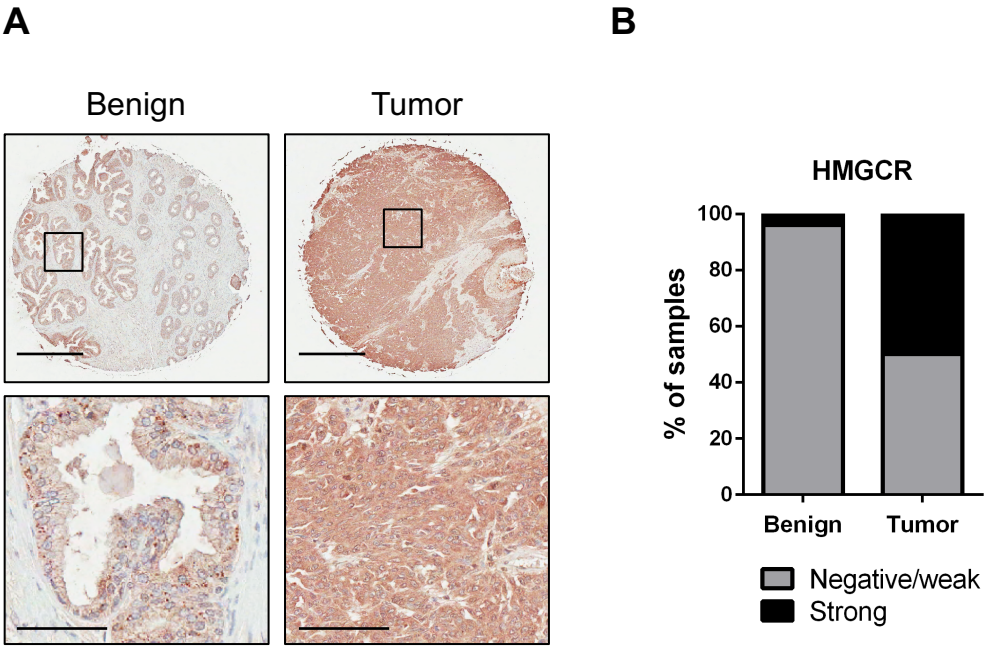


B

	HMGCR staining	
	Negative/weak	**Strong**
Patients	55	94
Statin users	17 (31%)	19 (20%)
Pre-treatment PSA Median (IQR), ng/mL	5.6 (3.5-11.3)	6.5 (5.1-8.6)
Gleason score		
< 7	23 (41.8%)	38 (40.4%)
7	29 (52.7%)	50 (53.2%)
> 7	3 (5.5%)	6 (6.4%)
Positive margins	19 (35%)	19 (20%)
Extracapsular extension	20 (36%)	33 (35%)

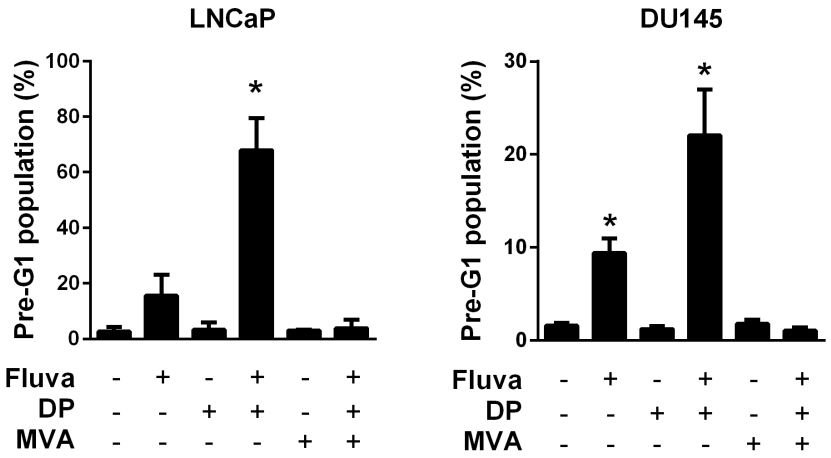
Supplementary Figure 2: HMGCR expression in prostate tumors is not associated with biochemical relapse-free survival when considering both statin users and non-users. (A) HMGCR expression in prostate tumors is not associated with early biochemical relapse (BCR) when both statin users and non-users are considered. Hazard Ratio (95% confidence interval) = 0.68 (0.39-1.25); *P* = 0.22 (Log-rank test). (B) HMGCR expression among all patients (statin users and non-users) by clinical and pathological features. IQR = interquartile range.

Supplementary Figure 3



Supplementary Figure 3: HMGCR expression is elevated in primary PCa tissues in a validation cohort. (A) Representative images of a benign prostate tissue and malignant prostate tissue sample stained for HMGCR expression. Scale bars = 500 μm (top row) and 100 μm (bottom row). (B) Prostate tumor tissues stained more intensely for HMGCR expression compared to benign prostate tissue controls. N = 30 benign (normal and hyperplasia) prostate and 45 PCa tissue samples. $P < 0.0001$ (Fisher's exact test).

Supplementary Figure 4



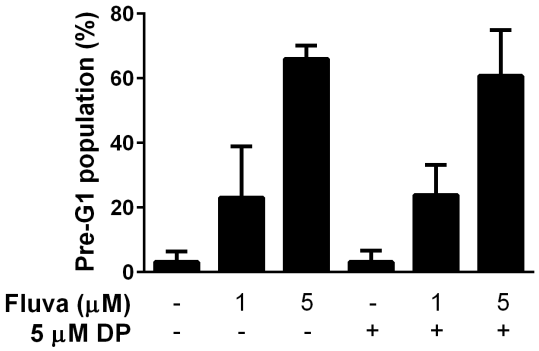
Supplementary Figure 4: Fluvastatin and dipyridamole-induced cell death is abrogated by mevalonate. LNCaP and DU145 cells were treated as indicated with 10 μM fluvastatin, 5 μM dipyridamole (DP) and/or 200 μM mevalonate (MVA) for 72 hours, fixed in ethanol and assayed for DNA fragmentation (% pre-G1 population) as a marker of cell death by propidium iodide staining. Error bars represent the mean + SD, n = 3, *p < 0.05 (one-way ANOVA with Tukey's multiple comparisons test).

Supplementary Table 1: shRNA sequences (from 5' to 3').

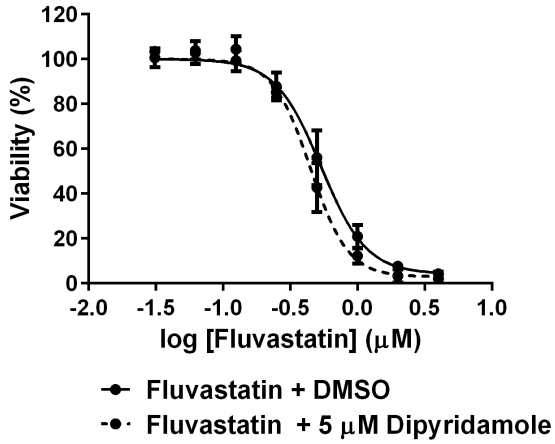
shRNA	TRC ID	Targeting sequence	Forward sequence	Reverse sequence
Scramble	N/A	GCAAGCTGACCCT GAAGTTCA	CCGGGCAAGCTGACCCTGAA GTTCACTCGAGTGAACTTCAG GGTCAGCTTGCTTTTTG	AATTCAAAAAGCAAGCTGACC CTGAAGTTCACTCGAGTGAAC TTCAGGGTCAGCTTGC
SREBF2 #1	TRCN0000215753	GCCATTGATTACAT CAAATAT	CCGGGCCATTGATTACATCAA ATATCTCGAGATATTTGATGTA ATCAATGGCTTTTTG	AATTCAAAAAGCCATTGATTAC ATCAAATATCTCGAGATATTTG ATGTAATCAATGGC
SREBF2 #2	TRCN0000431900	CCTTCAGTGCAAC GGTCATTC	CCGGCCTTCAGTGCAACGGTC ATTCCTCGAGGAATGACCGTT GCACTGAAGGTTTTTG	AATTCAAAAACCTTCAGTGCAA CGGTCATTCCTCGAGGAATGA CCGTTGCACTGAAGG

Supplementary Figure 5

A

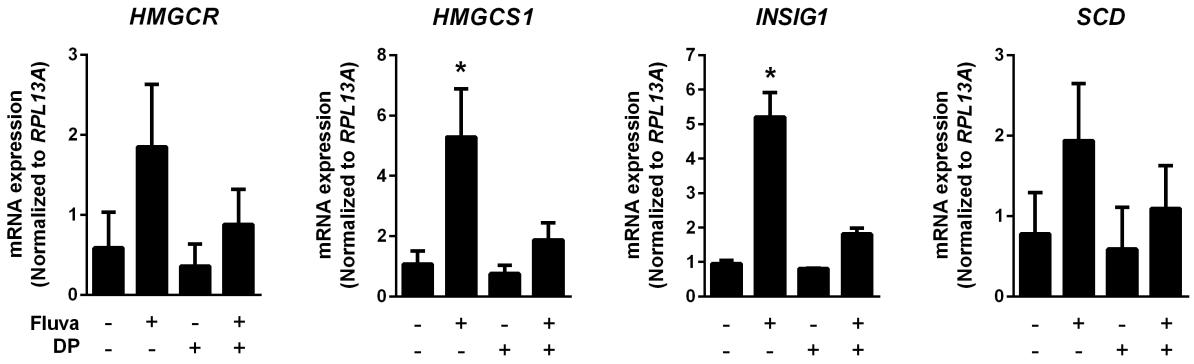


B



Supplemental Figure 5: Dipyridamole does not potentiate fluvastatin-induced cell death in PC-3 cells. (A) Cells were treated with 1 or 5 μM fluvastatin ± 5 μM dipyridamole (DP) for 72 hours, fixed in ethanol and assayed for DNA fragmentation (% pre-G1 population) as a marker of cell death by propidium iodide staining. Error bars represent the mean + SD, n = 3. (B) Cells were treated with a range of fluvastatin doses ± 5 μM DP for 72 hours, and cell viability was determined using an MTT assay. Error bars represent the mean ± SD, n = 3.

Supplementary Figure 6



Supplementary Figure 6: Dipyridamole inhibits fluvastatin-induced upregulation of SREBP target genes in DU145 cells. Cells were treated with 10 μM fluvastatin ± 5 μM dipyridamole (DP) for 16 hours, and RNA was isolated to assay for *HMGCR*, *HMGCS1*, *INSIG1* and *SCD* expression by qRT-PCR. mRNA expression data are normalized to *RPL13A* expression. Error bars represent the mean + SD, n = 3, *p < 0.05 (one-way ANOVA with Bonferroni's multiple comparisons test, where each group was compared to the solvent controls group).