



Supplementary material

Anti-Tumor Effects of Statins in Pancreatic Ductal Adenocarcinoma Cells

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The R programming language (ver. 4.4.2) was used together with libraries listed in the References section. Exploratory data analysis (EDA) summarized the percentage of cells using the median (lower–upper quartile) and visualized distributions across groups using boxplots. To address the study hypotheses on differences between statin-treated groups and the control (CTRL), robust linear regression models were fitted with Statin and Status/Phase as predictors, including their interaction where relevant. The hypotheses were then evaluated using planned post hoc contrasts of estimated marginal means (em-means), comparing each statin to CTRL within the corresponding Status/Phase strata, with p-values adjusted for multiple testing using Dunnett’s method [1–13].

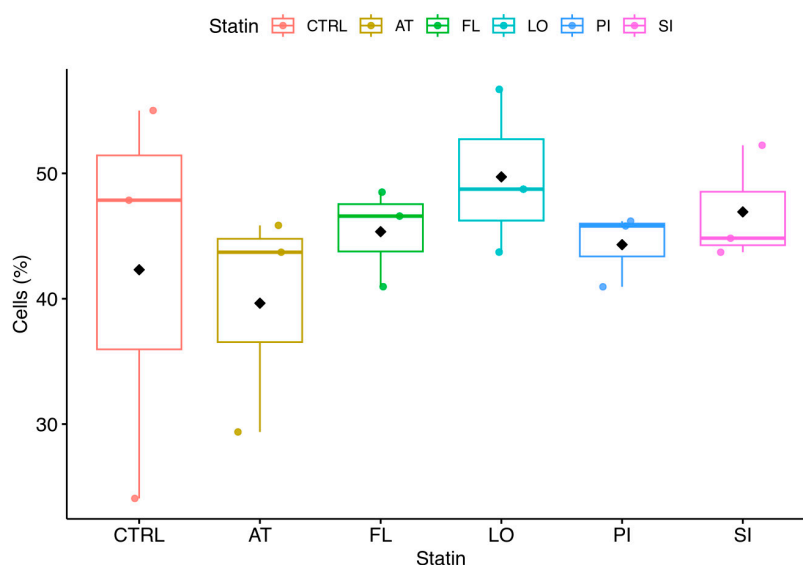


Figure S1. Percentage of ROS-positive cells by statin group. Boxplots display the percentage of ROS-positive cells for each statin group: line = median, box = lower/upper quartile, whiskers = most extreme values within 1.5×(upper–lower quartile), points beyond

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whiskers = potential outliers, black diamond = mean. Individual points represent values from the individual PDAC cell lines ($n = 3$).

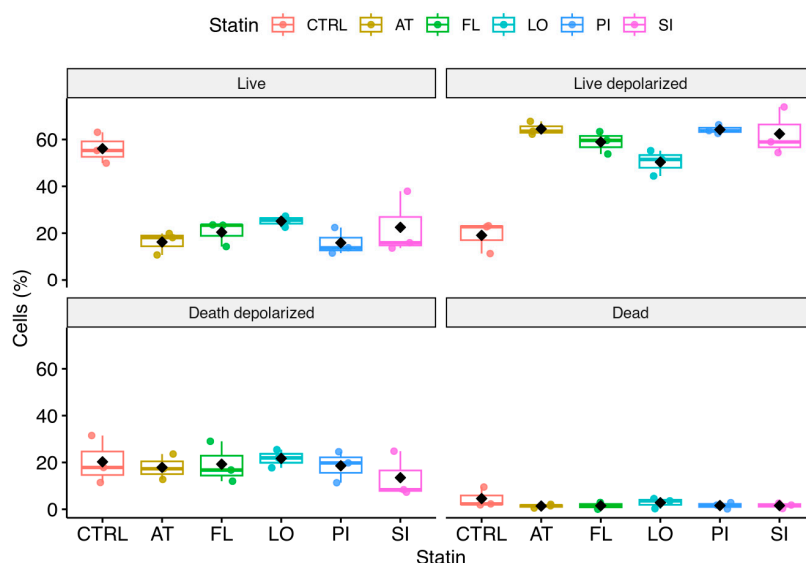


Figure S2. Mitochondrial membrane potential categories by statin group. Boxplots show the percentage of cells in each mitochondrial status category (Live, Live depolarized, Death depolarized, Dead) for each statin group. Boxplots display Cells (%) by statin group: line = median, box = lower/upper quartile, whiskers = most extreme values within 1.5×(upper–lower quartile), points beyond whiskers = potential outliers, black diamond = mean. Individual points represent values from the individual PDAC cell lines ($n = 3$).

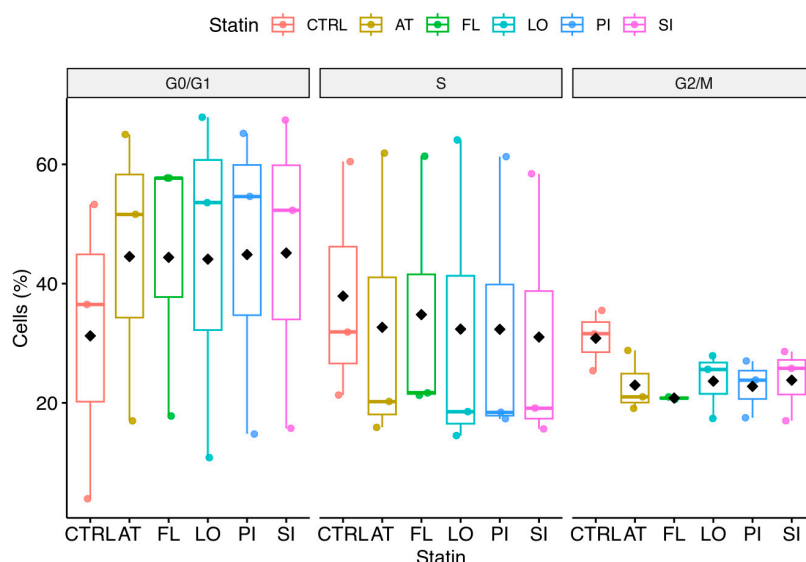


Figure S3. Cell-cycle phase distribution by statin group. Boxplots show the percentage of cells in each cell-cycle phase (G0/G1, S, G2/M) for each statin group. Boxplots display Cells (%) by statin group: line = median, box = lower/upper quartile, whiskers = most extreme values within 1.5×(upper–lower quartile), points beyond whiskers = potential outliers, black diamond = mean. Individual points represent values from the individual PDAC cell lines ($n = 3$).

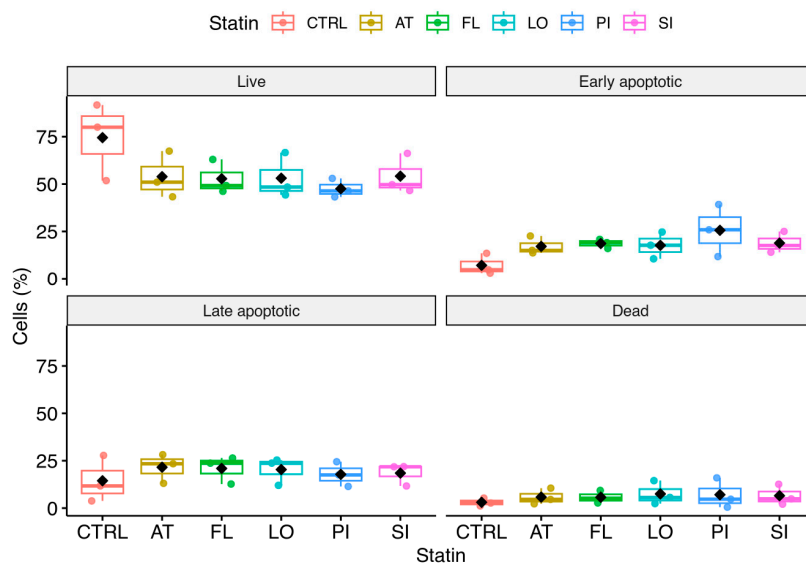


Figure S4. Annexin V/7-AAD apoptosis categories by statin group. Boxplots show the percentage of cells classified as Live, Early apoptotic, Late apoptotic, or Dead based on Annexin V/7-AAD staining for each statin group. Boxplots display Cells (%) by statin group: line = median, box = lower/upper quartile, whiskers = most extreme values within 1.5×(upper–lower quartile), points beyond whiskers = potential outliers, black diamond = mean. Individual points represent values from the individual PDAC cell lines ($n = 3$).

PDAC-1

significance

5 μ M	24h	48h	72h	96h	120h
AT	ns	***	***	***	***
FL	ns	ns	***	***	***
LO	ns	ns	***	***	***
PI	ns	*	***	***	***
PR	ns	ns	**	ns	ns
RO	ns	**	**	***	*
SI	ns	*	***	***	***

10 μ M	24h	48h	72h	96h	120h
AT	ns	***	***	***	***
FL	ns	***	***	***	***
LO	ns	***	***	***	***
PI	ns	***	***	***	***
PR	ns	ns	ns	***	ns
RO	ns	***	***	***	***
SI	ns	***	***	***	***

20 μ M	24h	48h	72h	96h	120h
AT	ns	***	***	***	***
FL	ns	***	***	***	***
LO	ns	***	***	***	***
PI	ns	***	***	***	***
PR	ns	**	ns	ns	ns
RO	ns	***	***	***	***
SI	ns	***	***	***	***

p-value

5 μ M	24h	48h	72h	96h	120h
AT	0.9923	3.9e-04	<1e-16	<1e-16	<1e-16
FL	0.5433	0.1022	<1e-16	<1e-16	<1e-16
LO	0.3159	0.4965	4.4e-16	<1e-16	<1e-16
PI	0.9874	0.0424	<1e-16	<1e-16	<1e-16
PR	0.2494	0.0527	0.0030	0.1004	0.7697
RO	9.53e-01	1.3e-03	1.7e-03	3.6e-05	1.06e-02
SI	0.7265	0.0171	<1e-16	<1e-16	<1e-16

10 μ M	24h	48h	72h	96h	120h
AT	0.9999	2.6e-06	<1e-16	<1e-16	<1e-16
FL	0.2950	4.0e-14	<1e-16	<1e-16	<1e-16
LO	0.3742	3.3e-13	<1e-16	<1e-16	<1e-16
PI	0.9041	9.2e-12	<1e-16	<1e-16	<1e-16
PR	0.6700	0.1167	0.4886	2.3e-04	0.4432
RO	0.5508	3.5e-06	9.5e-15	<1e-16	<1e-16
SI	0.1409	<1e-16	<1e-16	<1e-16	<1e-16

20 μ M	24h	48h	72h	96h	120h
AT	0.9919	4.6e-07	<1e-16	<1e-16	<1e-16
FL	0.9726	1.0e-06	<1e-16	<1e-16	<1e-16
LO	0.9999	4.3e-10	<1e-16	<1e-16	<1e-16
PI	0.9995	1.5e-07	<1e-16	<1e-16	<1e-16
PR	0.8330	0.0037	0.8988	0.6248	0.9990
RO	0.9683	5.4e-06	4.0e-11	<1e-16	<1e-16
SI	0.9950	4.8e-05	<1e-16	<1e-16	<1e-16

PDAC-2

significance

5 μ M	24h	48h	72h	96h	120h
AT	ns	ns	ns	ns	ns
FL	ns	*	ns	***	*
LO	ns	ns	ns	ns	ns
PI	ns	ns	ns	*	**
PR	ns	ns	ns	ns	ns
RO	ns	ns	ns	***	***
SI	ns	ns	ns	ns	ns

p-value

5 μ M	24h	48h	72h	96h	120h
AT	0.9994	0.9877	0.9936	0.4048	0.2462
FL	0.9605	0.0171	0.4299	6.4e-05	0.0311
LO	0.5604	0.8097	0.9448	0.0593	0.9672
PI	0.8993	0.9405	0.9999	0.0173	0.0020
PR	0.1453	0.7695	0.9976	0.8579	0.7576
RO	0.2812	0.9941	0.3220	1.0e-06	9.7e-08
SI	0.4833	0.4877	0.8960	0.9661	0.8275

10 μ M	24h	48h	72h	96h	120h
AT	ns	ns	ns	***	***
FL	ns	ns	ns	***	***
LO	ns	ns	ns	ns	***
PI	ns	ns	ns	***	***
PR	***	***	ns	ns	ns
RO	***	**	ns	***	***
SI	***	ns	ns	ns	*

10 μ M	24h	48h	72h	96h	120h
AT	0.9796	0.0865	0.8680	1.1e-10	< 1e-16
FL	0.7623	0.8683	0.9999	8.9e-08	< 1e-16
LO	0.9998	0.9910	0.0867	0.2651	7.8e-06
PI	0.7504	0.9996	0.9774	1.3e-11	< 1e-16
PR	6.5e-14	1.9e-07	0.9955	0.8314	0.1814
RO	2.7e-07	0.0029	0.5335	3.3e-05	2.7e-08
SI	3.3e-05	0.6654	0.7700	0.9957	0.0141

20 μ M	24h	48h	72h	96h	120h
AT	ns	ns	ns	***	***
FL	ns	ns	ns	**	***
LO	ns	ns	ns	ns	**
PI	ns	ns	ns	***	***
PR	**	ns	ns	ns	ns
RO	ns	***	**	***	***
SI	***	ns	ns	*	***

20 μ M	24h	48h	72h	96h	120h
AT	0.9961	0.1981	0.3063	3.1e-06	< 1e-16
FL	0.9984	0.9724	0.8013	0.0018	< 1e-16
LO	0.9837	0.9470	0.9197	0.9979	0.0087
PI	0.5783	0.9997	0.9901	4.3e-06	< 1e-16
PR	0.0055	0.4364	0.9261	0.8575	0.3505
RO	0.9421	6.3e-04	0.0047	5.1e-05	2.6e-09
SI	6.8e-05	0.9785	0.6627	0.0374	5.9e-08

PDAC-3

significance

5 μ M	24h	48h	72h	96h	120h
AT	ns	ns	***	***	ns
FL	ns	ns	***	***	***
LO	ns	ns	***	***	ns
PI	ns	ns	***	***	***
PR	ns	ns	ns	**	***
RO	ns	ns	ns	***	***
SI	ns	ns	**	ns	ns

p-value

5 μ M	24h	48h	72h	96h	120h
AT	0.7166	0.9847	1.2e-09	4.1e-08	0.9999
FL	0.6413	0.8973	9.5e-15	< 1e-16	1.3e-06
LO	0.8785	0.9999	1.2e-05	9.1e-04	0.0795
PI	0.9044	0.2784	< 1e-16	< 1e-16	< 1e-16
PR	0.9998	0.9717	0.2212	0.0017	2.8e-08
RO	0.9987	0.9460	0.9785	7.6e-04	< 1e-16
SI	0.6190	0.9339	0.0011	0.0782	0.2232

10 μ M	24h	48h	72h	96h	120h
AT	ns	ns	***	***	***
FL	ns	**	***	***	***
LO	ns	ns	***	***	***
PI	ns	**	***	***	***
PR	ns	ns	ns	ns	***
RO	ns	*	*	ns	***
SI	ns	ns	***	***	***

10 μ M	24h	48h	72h	96h	120h
AT	0.3972	0.6069	2.2e-16	< 1e-16	< 1e-16
FL	0.1819	0.0024	< 1e-16	< 1e-16	< 1e-16
LO	0.2671	0.8298	6.5e-07	1.6e-11	4.3e-05
PI	0.2676	0.0025	< 1e-16	< 1e-16	< 1e-16
PR	0.8443	0.8616	0.2989	0.1407	4.6e-05
RO	0.0544	0.0412	0.0116	0.9932	5.1e-11
SI	0.1374	0.8202	2.1e-07	1.1e-13	4.6e-11

20 μ M	24h	48h	72h	96h	120h
AT	ns	ns	***	***	***
FL	ns	*	***	***	***
LO	ns	***	***	***	***
PI	ns	***	***	***	***
PR	ns	*	ns	***	***
RO	ns	***	ns	ns	**
SI	ns	***	***	***	***

20 μ M	24h	48h	72h	96h	120h
AT	0.9925	0.1415	< 1e-16	< 1e-16	< 1e-16
FL	0.8846	0.0131	< 1e-16	< 1e-16	< 1e-16
LO	0.9114	8.2e-09	6.7e-14	< 1e-16	< 1e-16
PI	0.9999	8.1e-09	< 1e-16	< 1e-16	< 1e-16
PR	0.9994	0.0452	0.0518	7.2e-05	4.8e-12
RO	0.9999	3.9e-05	0.6829	0.2270	0.0055
SI	0.8653	2.0e-15	2.2e-16	< 1e-16	< 1e-16

Figure S5. Full statistical output for the MTT viability test. Tables summarize the statistical comparisons of each statin treatment versus the corresponding DMSO control within each PDAC cell line (PDAC-1, PDAC-2, PDAC-3) at each concentration (5, 10, 20 μ M) and time point (24–120 h) ($n = 3$). For each setting, the left table reports significance codes (*** $p < 0.001$,

** $p < 0.01$, * $p < 0.05$, ns – not significant) and the corresponding numerical p-values are provided in separate tables (right) in scientific notation. Abbreviations: atorvastatin (AT), fluvastatin (FL), lovastatin (LO), pitavastatin (PI), pravastatin (PR), rosuvastatin (RO), simvastatin (SI).

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