Supplementary material

CD44^{+} cells determine fenofibrate-induced microevolution of drug-resistance in prostate cancer cell populations

Tomasz Wróbel[1], Marcin Luty[1], Jessica Catapano[1], Elżbieta Karnas[1], Małgorzata Szczygieł[2], Katarzyna Piwowarczyk[1], Damian Ryszawy[1], Grażyna Drabik[3], Ewa Zuba-Surma[1], Maciej Siedlar[4], Zbigniew Madeja[1], Martyna Elas[2] and Jarosław Czyż[1]

[1]Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Kraków, Poland;

[2]Department of Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, ul. Gronostajowa 7, 30-387 Kraków, Poland;

[3]Department of Transplantology, Institute of Paediatrics, Faculty of Medicine, Jagiellonian University Medical College, 265 Wielicka Str., 30-663 Kraków, Poland;

[4]Department of Clinical Immunology, Institute of Paediatrics, Faculty of Medicine, Jagiellonian University Medical College, 265 Wielicka Str., 30-663 Kraków, Poland.

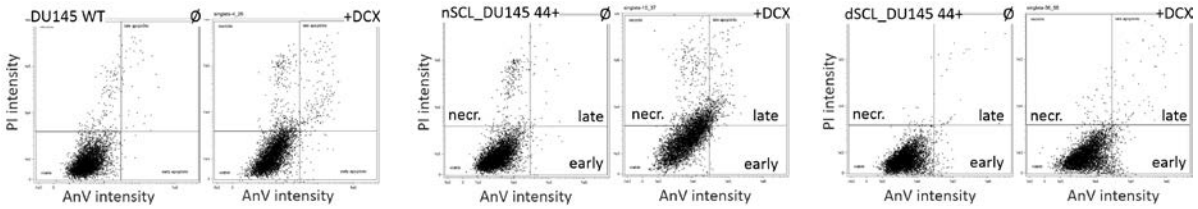

Figure S1. Pro-apoptotic activity of 10 nM DCX in the populations of WT, nSCL_ and dcxSCL_DU145 cells (cf. Figure 1F). Compensated plots comprise 50 000 annexinV/PI stained cells, classified based on their bright field ratios. Data representative of at least three independent experiments (N≥3). **Note the DCX-resistance of the "bulk" progenies of DCX-pretreated dSCL_DU145 cells.**

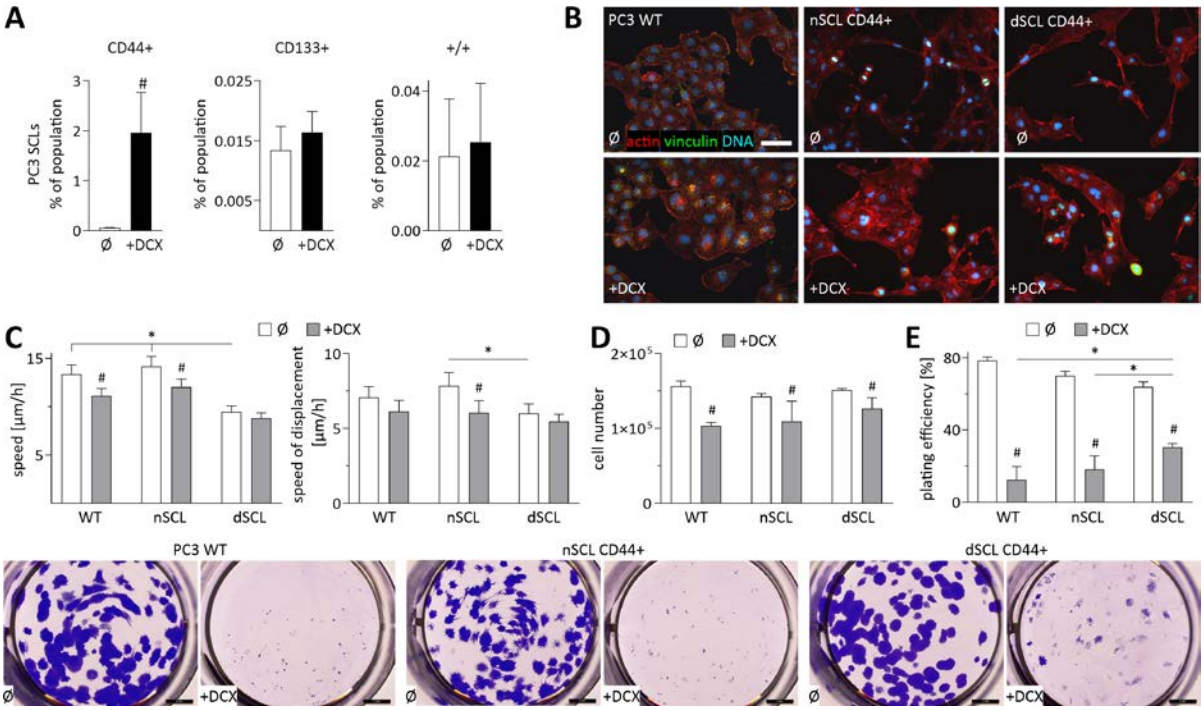

Figure S2. Potential of CD44$^+$ PC3 SCL cells. (A) PC3 cells, cultivated for 48 hours in control conditions or in the presence of DCX (10 nM), were classified based on their bright-field ratios and CD133/CD44 levels. The values in the plots represent relative SCL fractions calculated from the compensated dot-plots comprising 50 000 events. **(B)** Morphology of the progenies of CD44$^+$ PC3 cells isolated after pre-incubation in the absence (nSCL_PC3) or presence of DCX (10 nM, dSCL_PC3). Cells were cultivated for 2-6 passages and their morphology/actin cytoskeleton architecture was estimated in the absence or presence of DCX (10 nM) with fluorescence microscopy. Scale bar = 50 µm. **(C)** Motility of nSCL_PC3 and dSCL_PC3 cells cultivated in the absence/presence of DCX. Column charts show movement parameters at the population level, registered for 8 hours (N>50) with time-lapse videomicroscopy. **(D)** Proliferation of nSCL_PC3 and dSCL_PC3 cells cultivated in the absence/presence of DCX for 48 hours estimated with Coulter counter. **(E)** CD44$^+$ SCLs were isolated, propagated, and their direct progenies were seeded (500/cm^2), stained with CBB R250 and assessed towards clonogenic potential (72 hours). Scale bar = 2 mm. The statistical significance of the differences was tested with t-Student test (A, D, E); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars; or by one-way ANOVA followed by post-hoc Tukey's HSD (C); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars. **Note the DCX-induced drug-resistance of CD44$^+$ PC3 cell progenies accompanied by their lower plating efficiency in the presence of DCX.**

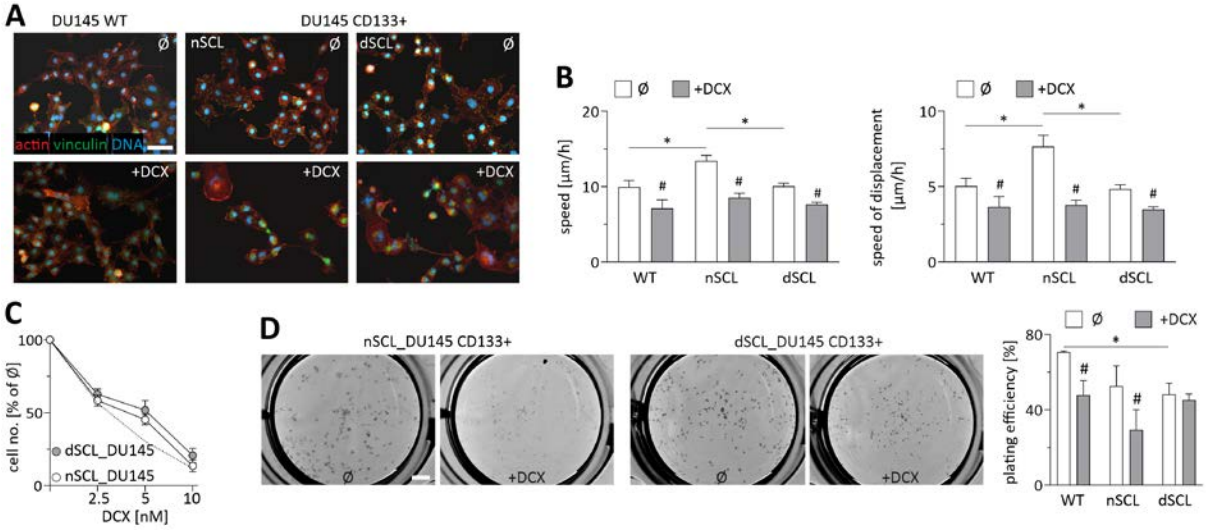

Figure S3. Potential of CD133$^+$ DU145 cells. (A) Morphology of the progenies of CD133$^+$ DU145 cells isolated isolated after pre-incubation in the absence (nSCL_DU145_CD133$^+$ cells) or presence of DCX (10 nM, dSCL_DU145_CD133$^+$ cells). Cells were cultivated for 2-6 passages and their morphology/actin cytoskeleton architecture was estimated in the absence or presence of DCX (10 nM) with fluorescence microscopy. Scale bar = 50 µm. **(B)** Motility of nSCL_DU145_CD133$^+$ and dSCL_DU145_CD133$^+$ cells cultivated in the absence/presence of DCX. Column charts show movement parameters at the population level, registered for 8 hours (N>50) with time-lapse videomicroscopy. **(C)** Proliferation of nSCL_DU145_CD133$^+$ and dSCL_DU145_CD133$^+$ cells cultivated in the absence/presence of DCX for 48 hours estimated with Coulter counter. Dotted line indicates proliferation of wtDU145 cells. **(D)** Clonogenic capacity of CD133$^+$ SCLs. Cells were isolated, propagated, and their direct progenies were seeded (500/cm^2), stained with CBB R250 and assessed towards clonogenic potential (72 hours). Scale bar = 2 mm. The statistical significance of the differences was tested with t-Student test (C, D); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars; or by one-way ANOVA followed by post-hoc Tukey's HSD (B); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars. **Note DCX-induced increase of the relative drug-resistance of CD133$^+$ DU145 cell progenies.**

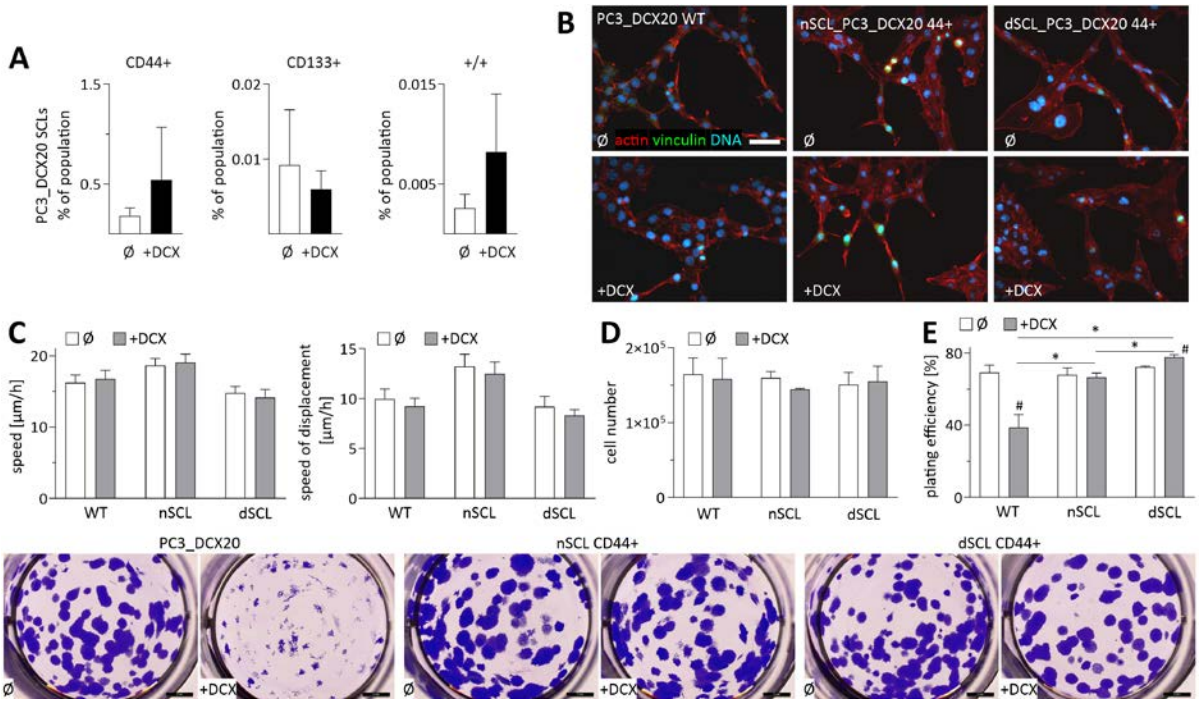

Figure S4. Potential of CD44$^+$ PC3_DCX20 SCL cells. (A) PC3_DCX20 cells, cultivated for 48 hours in control conditions or in the presence of DCX (10 nM), were classified based on their bright-field ratios and CD133/CD44 levels. The values in the plots represent relative SCL fractions calculated from the compensated dot-plots comprising 50 000 events. **(B)** Morphology of the progenies of CD44$^+$ PC3_DCX20 cells isolated after pre-incubation in the absence (nSCL_PC3 or presence of DCX (10 nM, dSCL_PC3). Cells were cultivated for 2-6 passages and their morphology/actin cytoskeleton architecture was estimated in the absence or presence of DCX (10 nM) with fluorescence microscopy. Scale bar = 50 μm. **(C)** Motility of nSCL_PC3_DCX20 and dSCL_PC3_DCX20 cells cultivated in the absence/presence of DCX. Column charts show movement parameters at the population level, registered for 8 hours (N>50) with time-lapse videomicroscopy. **(D)** Proliferation of nSCL_PC3 and dSCL_PC3_DCX20 cells cultivated in the absence/presence of DCX for 48 hours estimated with Coulter counter. **(E)** CD44$^+$ SCLs were isolated, propagated, and their direct progenies were seeded (500/cm^2), stained with CBB R250 and assessed towards clonogenic potential (72 hours). Scale bar = 2 mm. The statistical significance of the differences was tested with t-Student test (A, D, E); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars; or by one-way ANOVA followed by post-hoc Tukey's HSD (C); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars. **CD44$^+$ PC3_DCX20 cell progenies display similar drug-resistance to their maternal cells.**

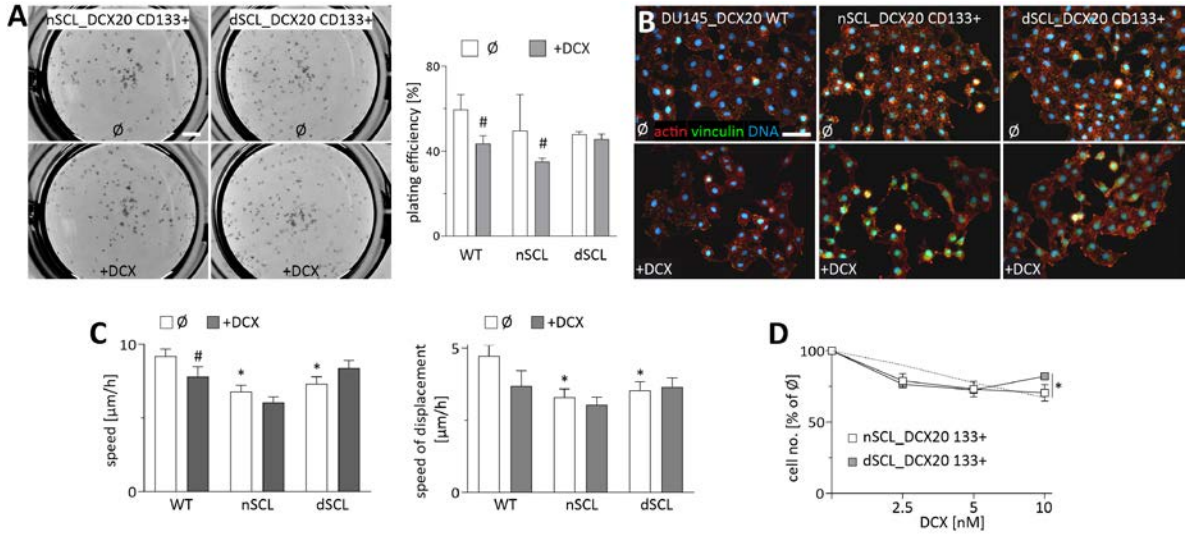

Figure S5. Potential of CD133$^+$ DU145_DCX20 cells. (A) Plating efficiency of CD133$^+$ DU145_DCX20 cells isolated after pre-incubation in the absence (nSCL_DCX20_CD133$^+$ cells) or presence of DCX (10 nM, dSCL_DCX20_CD133$^+$ cells). Cells were isolated, propagated, and their direct progenies were seeded (500/cm^2), stained with CBB R250 and assessed towards clonogenic potential (72 hours). Scale bar = 2 mm. **(B)** Morphology of nSCL_DCX20_CD133$^+$ and SCL_DCX20_CD133$^+$ cells. Cells were cultivated for 2-6 passages and their morphology/actin cytoskeleton architecture was estimated in the absence or presence of DCX (10 nM) with fluorescence microscopy. Scale bar = 50 µm. **(C)** Motility of nSCL_DCX20_CD133$^+$ and SCL_DCX20_CD133$^+$ cells cultivated in the absence/presence of DCX. Column charts show movement parameters at the population level, registered for 8 hours (N>50) with time-lapse videomicroscopy. **(D)** Proliferation of of nSCL_DCX20_CD133$^+$ and SCL_DCX20_CD133$^+$ cells cultivated in the absence/presence of DCX for 48 hours estimated with Coulter counter in relation to wt control (dotted line). The statistical significance of the differences was tested with t-Student test (A, D); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars; or by one-way ANOVA followed by post-hoc Tukey's HSD (C); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars. **CD133$^+$ DU145_DCX20 progenies display similar drug-resistance to their maternal cells.**

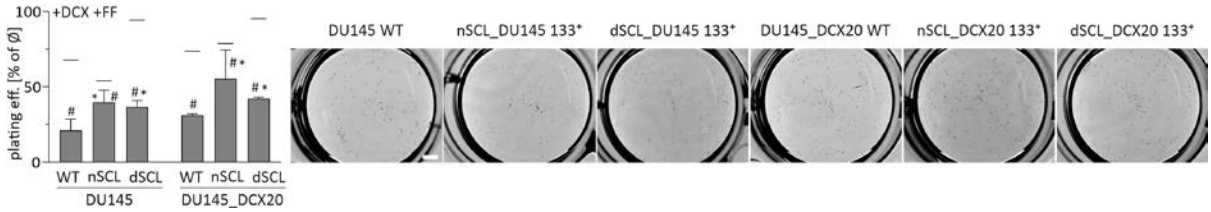

Figure S6. Effect of the combined DCX/FF treatment on the plating efficiency of CD133$^+$ DU145_DCX20 SCLs. Cells were isolated, propagated, and their progenies were seeded (500/cm^2), stained with CBB R250 and assessed towards clonogenic activity (72 hours) in comparison to DCX control (horizontal dotted lines). The statistical significance of the differences was tested with t-Student test; # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars. **Note the inhibition of DU145_DCX20 SCL clonogenic activity by the combined DCX/FF treatment.**

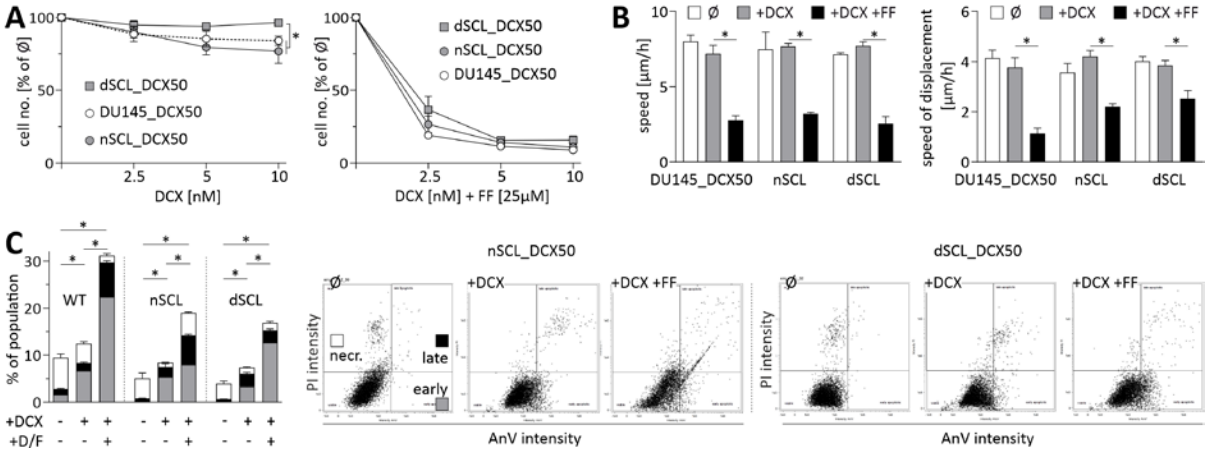

Figure S7. Effect of fenofibrate on the sensitivity of DU145_DCX50, nSCL_DCX50 and dSCL_DCX50 cells to DCX. (A) Progenies of SCLs derived from DU145_DCX50 cells were exposed to DCX or DCX/FF treatment for 48 hours. Their proliferation was then estimated with Coulter counter. (B-**C)** Progenies of SCLs derived from DU145_DCX50 cells were exposed to DCX or to the combined DCX/FF treatment. Their motility **(B)** and apoptosis **(C)** was estimated with time-lapse videomicroscopy and flow cytometry after 48 hours (B) or 72 hours (C). Column charts in (B) show movement parameters at the population level (registered for 8 h; N>50; plotted as % of control). Compensated dot-plots of annexinV/PI staining in C comprise 50 000 cells, classified based on their bright field ratios. Data representative of at least three independent experiments (N>3). The statistical significance of the differences was tested with t-Student test (A, C); *p ≤ 0.05 vs. selected bars; or by one-way ANOVA followed by post-hoc Tukey's HSD (B); *p ≤ 0.05 vs. selected bars.**Note relatively high sensitivity of DU145_DCX50, nSCL_DCX50 and dSCL_DCX50 cells to the combined DCX/FF treatment.**

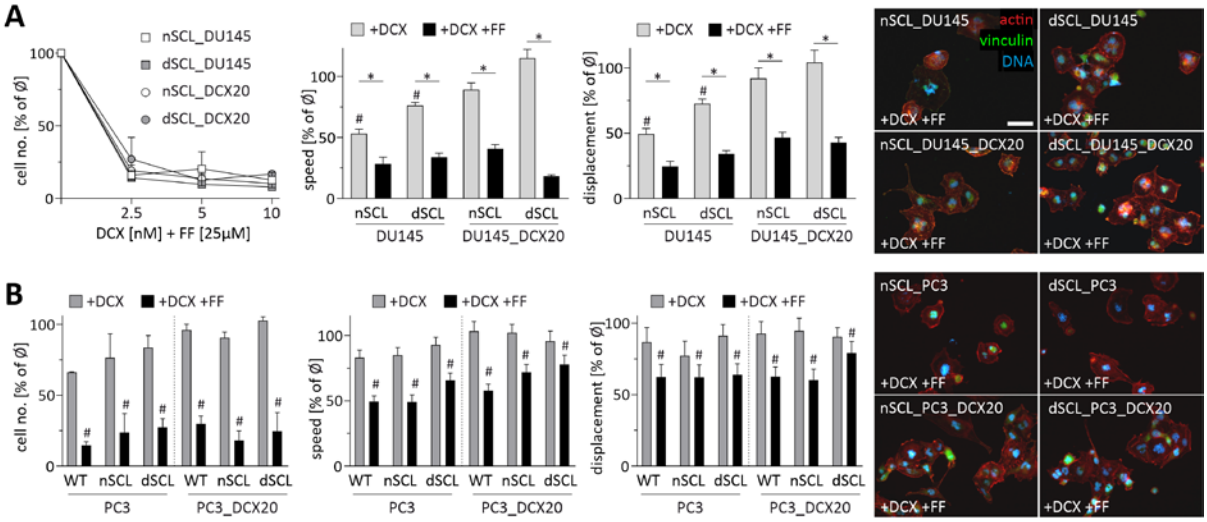

Figure S8. Effect of fenofibrate on the sensitivity of the progenies of CD133$^+$ DU145 and CD44$^+$ PC3 cells to DCX. Progenies of CD133$^+$ SCLs derived from DU145/DU145_DCX20 cells **(A)** or progenies of CD44$^+$ PC3/PC3_DCX20 cells **(B)** were exposed to DCX (10 nM) or to the combined DCX/FF treatment (10 nM/25 μM for 48 hours). Their proliferation (left) and motility (middle) was estimated with Coulter counter, and time-lapse videomicroscopy. Column charts show movement parameters (speed and displacement) at the population level (registered for 8 h; N>50; plotted as % of control). Cell morphology (right) was visualized by actin/vinculin staining and fluorescence microscopy. Scale bar = 50 μm. The statistical significance of the differences was tested with t-Student test (proliferation); # $p \leq 0.05$ vs. untreated control; or by one-way ANOVA followed by post-hoc Tukey's HSD (motility); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars. **Note the prominent cytostatic effects of DCX/FF and the differences in the reactivity of CD133$^+$ DU145 and CD44$^+$ PC3 progenies to the combined DCX/FF treatment.**

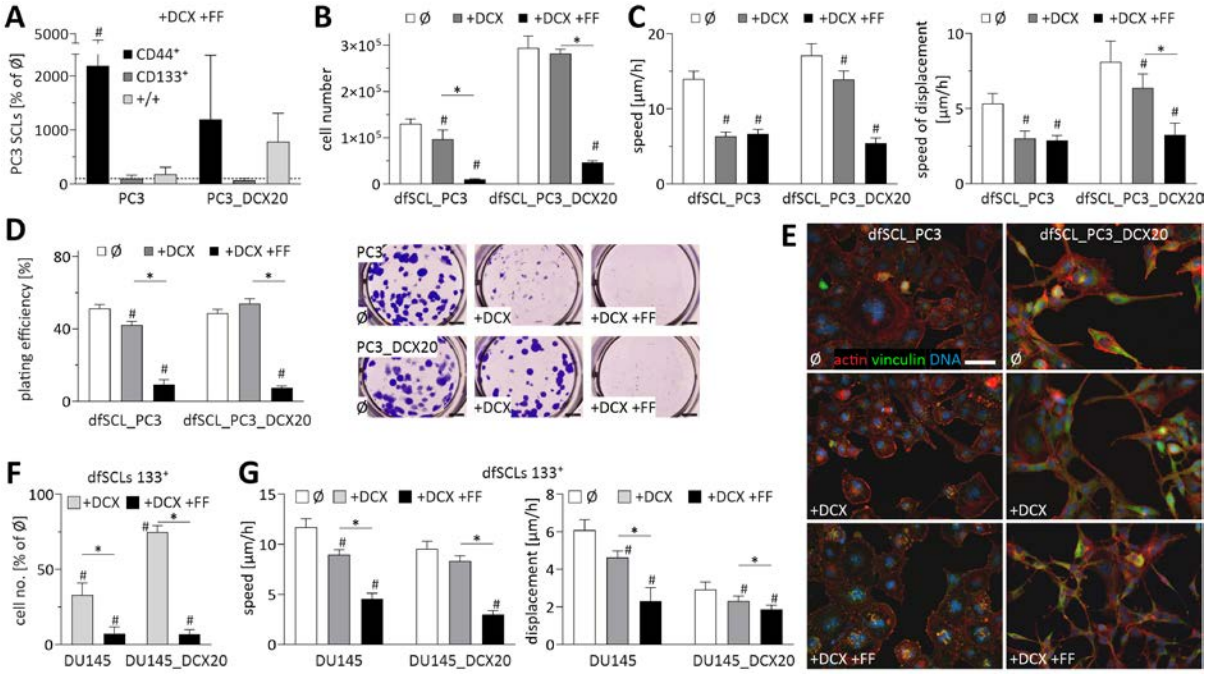

Figure S9. Effect of FF on the potential of CD44$^+$ PC3 and CD133$^+$ DU145 cells to generate DCX/FF-resistant offspring. (A) PC3 cells, cultivated for 48 hours in control conditions or in the presence of DCX (10 nM), were classified based on their bright-field ratios and CD133/CD44 levels. The values in the plots represent relative SCL fractions calculated from the compensated dot-plots comprising 50 000 events. **(B, C)** Progenies of DCX/FF-treated CD44$^+$ SCLs derived from PC3/PC3_DCX20 cells were exposed to DCX (10 nM) or to the combined DCX/FF (10 nM/25 µM) treatment. Their proliferation **(B)** and motility **(C)** was estimated after 48 hours with Coulter counter and time-lapse videomicroscopy. **(D)** Effect of the combined DCX/FF treatment on the plating efficiency of PC3/PC3_DCX20 SCLs. Cells were isolated, propagated, and their progenies were seeded (500/cm^2), stained with CBB R250 and assessed towards clonogenic activity (72 hours). Scale bar = 2 mm. **(E)** Morphology of dfSCL_PC3_DCX20 and dfSCL_PC3_DCX20 cells under DCX/FF stress. Cell morphology/actin cytoskeleton architecture was estimated in the absence or presence of DCX/FF with fluorescence microscopy. Scale bar = 50 µm. **(F, G)** Progenies of DCX/FF-treated CD133$^+$ SCLs derived from DU145/DU145_DCX20 cells were exposed to DCX (10 nM) or to the combined DCX/FF (10 nM/25 µM) treatment. Their proliferation **(F)** and motility **(G)** was estimated after 48 hours with Coulter counter and time-lapse videomicroscopy, respectively. Column charts **(in C and G)** show movement parameters at the population level (registered for 8 h; N>50; plotted as % of control). The statistical significance of the differences was tested with t-Student test (A, B, D, F)); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars or by one-way ANOVA followed by post-hoc Tukey's HSD (C, G); # $p \leq 0.05$ vs. untreated control; *$p \leq 0.05$ vs. selected bars. **Note that DCX/FF-induced preselection of CD133$^+$ DU145 and CD44$^+$ PC3 cells does not result in increased DCX/FF resistance of their offspring.**