A.

In vivo model of acquired resistance to fluvastatin

Study schema

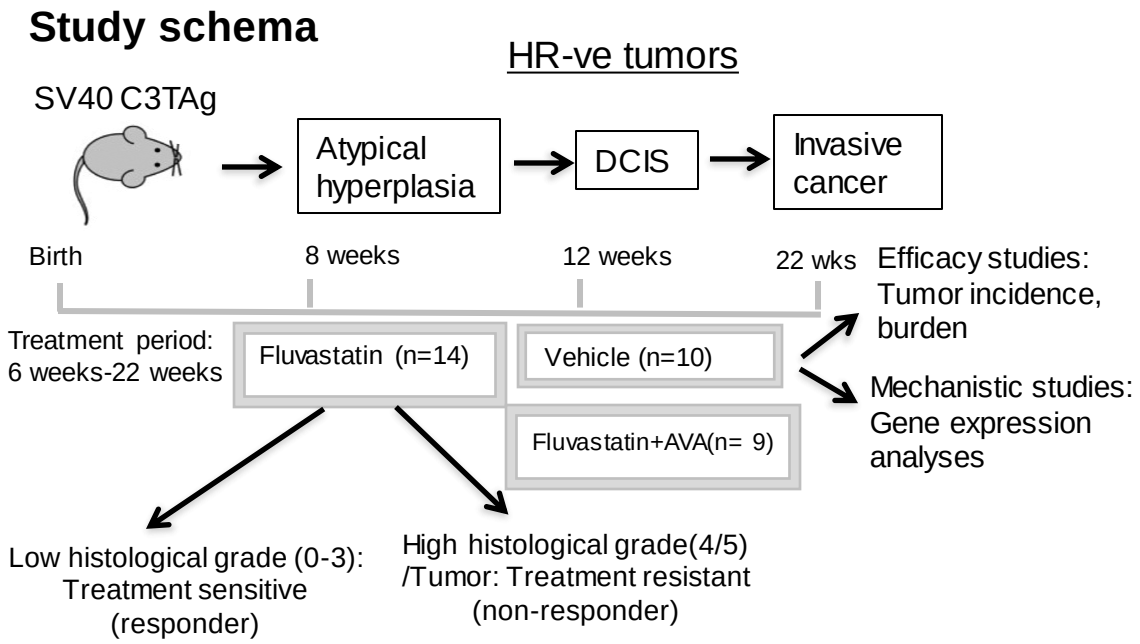
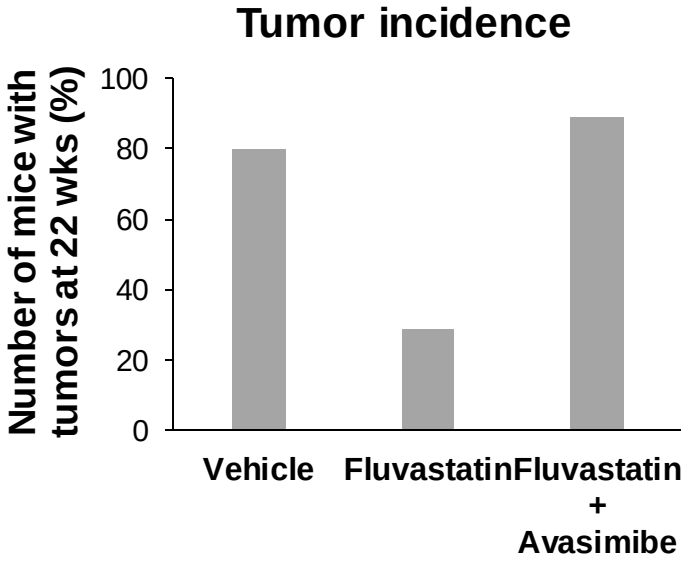
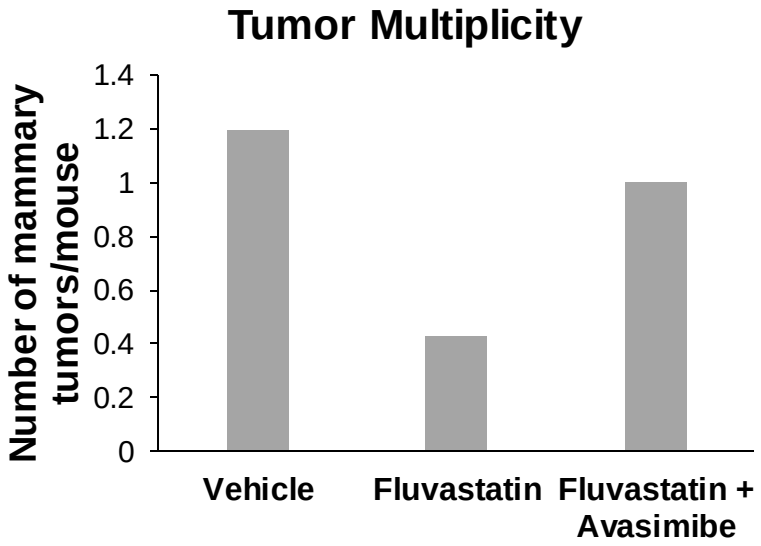


Fig. 1: (A). Schematic showing the schedule of drug treatment in SV40C3 TAg mouse model of breast cancer progression and the end point analyses. *HR* hormone receptor. Fluvastatin treatment reduces tumor incidence, delays onset of tumor, and inhibits tumor growth in the SV40C3 TAg mouse model of breast cancer progression. **(B).** Fluvastatin treatment (10 mg/kg/day) for 16 weeks inhibited the percentage of mice that developed mammary tumors as determined by the macroscopic lesions at the time of necropsy at 22 weeks of age **(C).** and the average number of tumors per mouse relative to the vehicle control group after 16 weeks of treatment. **(D).** and delayed the average age at which tumors appear, as shown here as the % incidence of palpable tumor bearing mice during study (12 week of age to 21-week age), relative to the vehicle control group. $*p < 0.05$

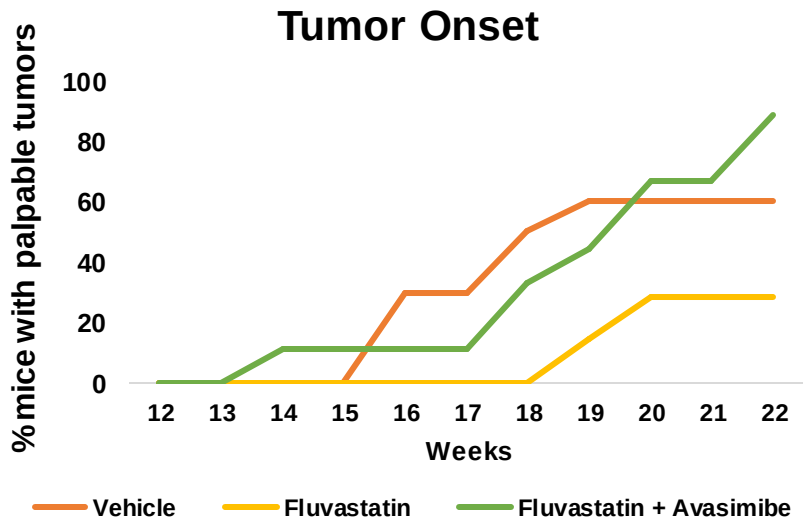
B.



C.



D.



Gene signature of acquired resistance to statins

Statin resistance signature derived from in vitro model

In vivo validation

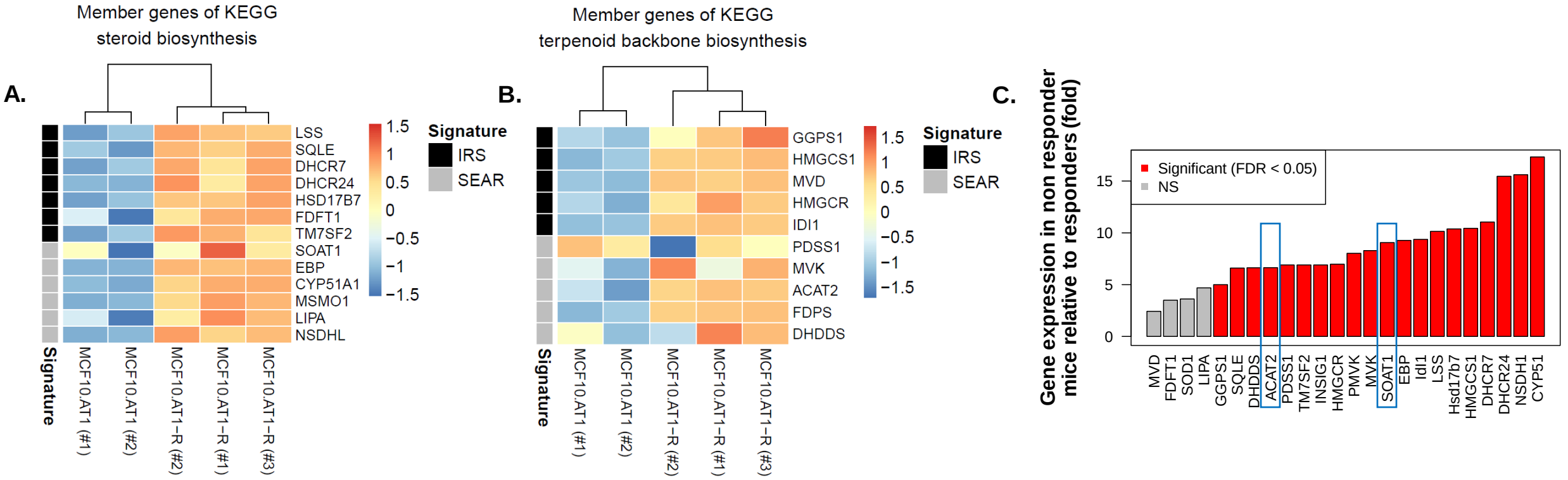
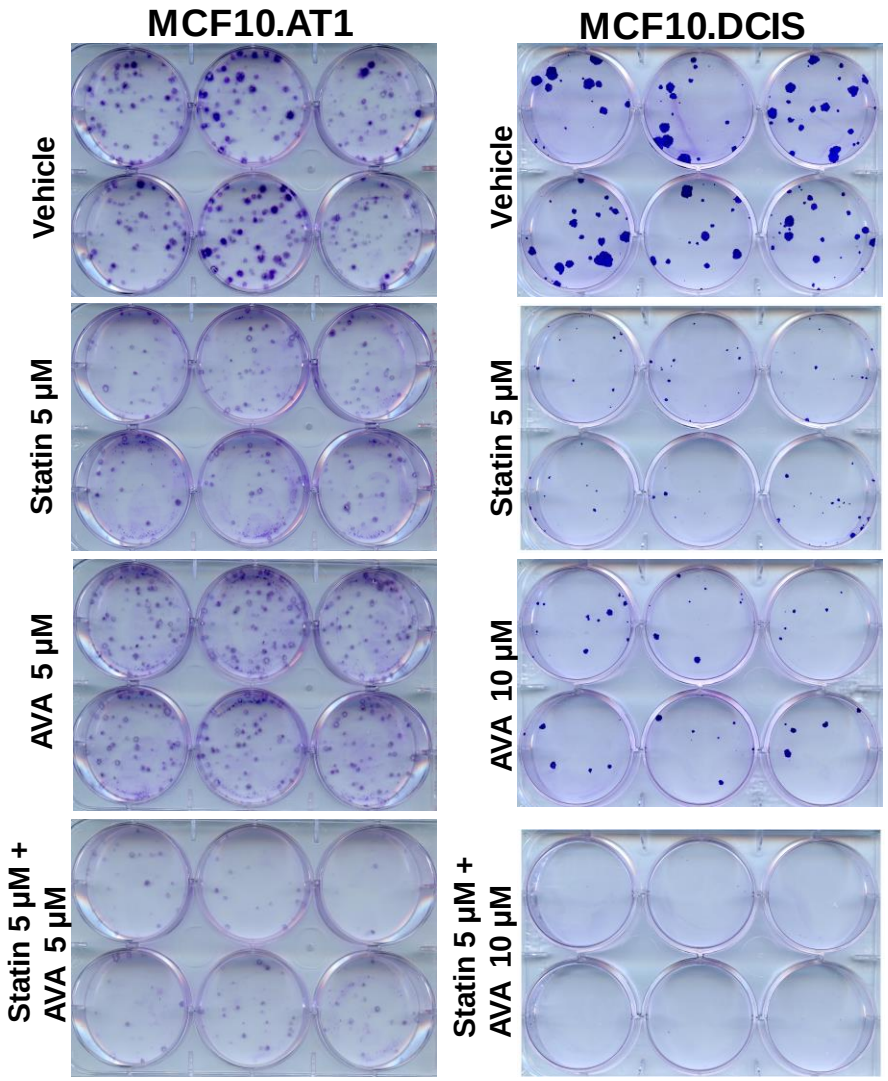


Fig. 2: Acquired resistance to fluvastatin causes restorative metabolic reprogramming. (**A & B).** Heatmap showing the genes that map to steroid biosynthesis and terpenoid backbone biosynthesis in the fluvastatin resistant MCF10.AT-R cells, including ACAT1 (SOAT1) and ACAT2. (**C**). Bar diagrams show qPCR validation where over expression of genes (including ACAT1/ SOAT1 and ACAT2 statin resistance gene panel) correlates tumor outcome in fluvastatin treated SV40C3TAg mice. The Y axis depicts the fold changes of average gene expression that was calculated by using the ΔΔCt method after normalizing with ribosomal protein L19. Red color in bar diagram represents the fold changes were significant and gray represents non-significant (NS) changes at an FDR of < 5%

A.



B.

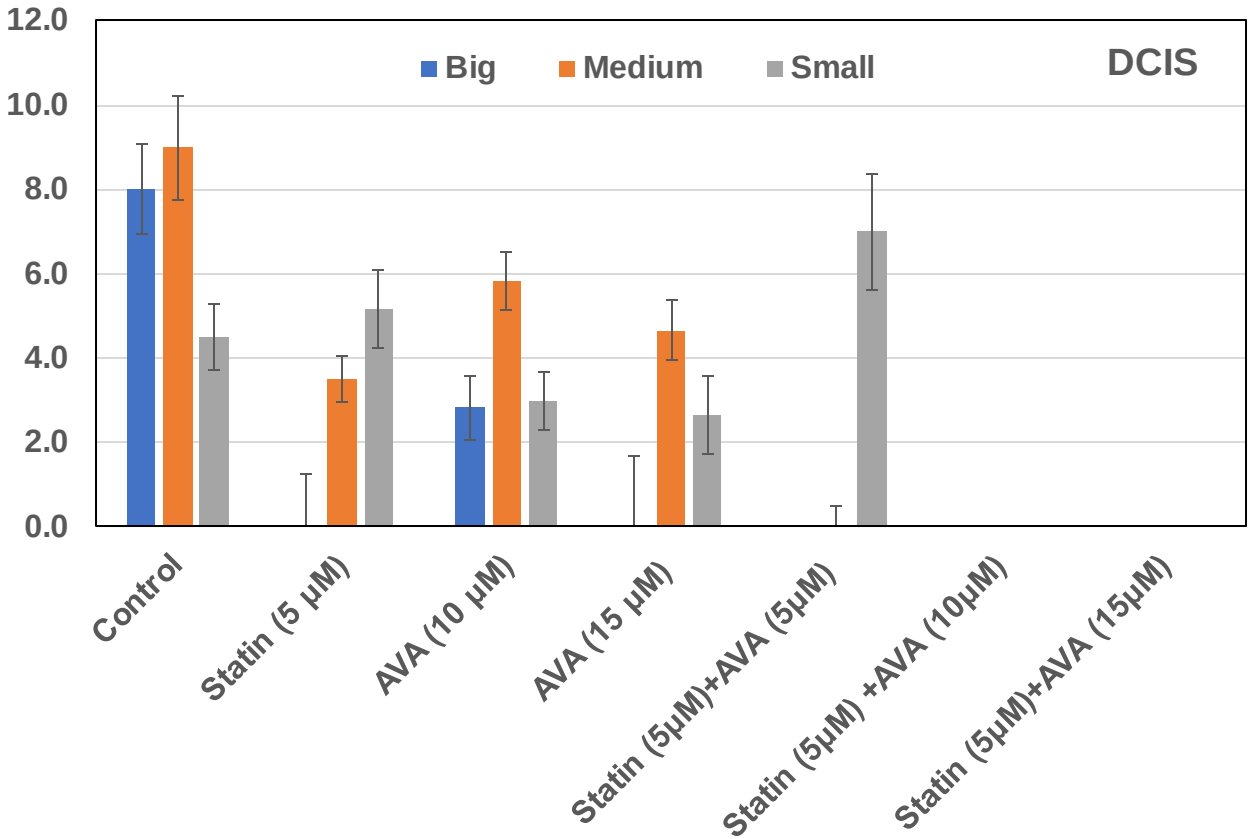


Fig. 3: Fluvastatin inhibits colonization ability and proliferation of breast preneoplastic cells. (**A).** Clonogenic survival assay showing crystal violet-stained colonies formed by preneoplastic MCF10.AT1 and MCF10.DCIS cells after 12 days of treatment with fluvastatin or vehicle control. **(B).** Quantification of colonies formed by MCF10.DCIS cells treated with fluvastatin or vehicle control. Values represent number of colonies (%) ± SEM. *$p < 0.001$.