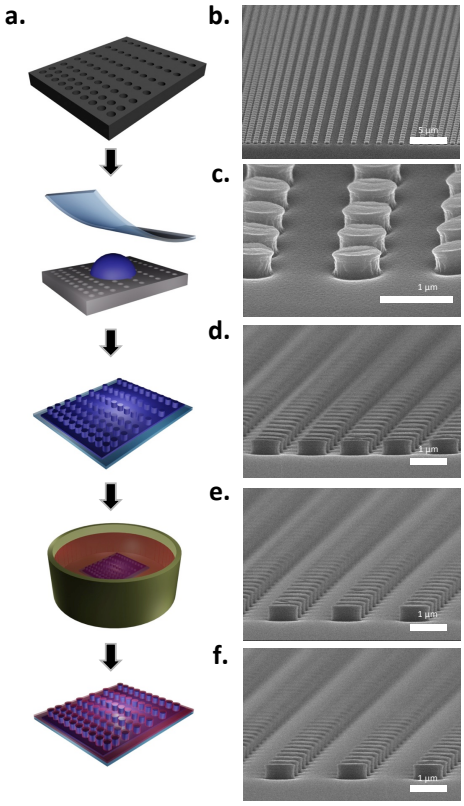
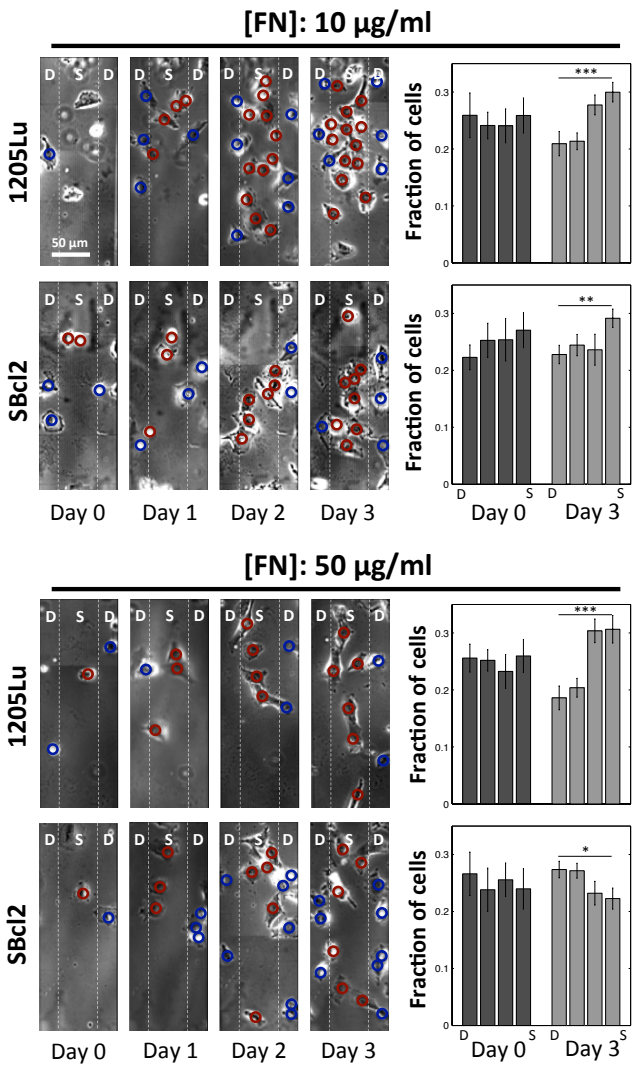
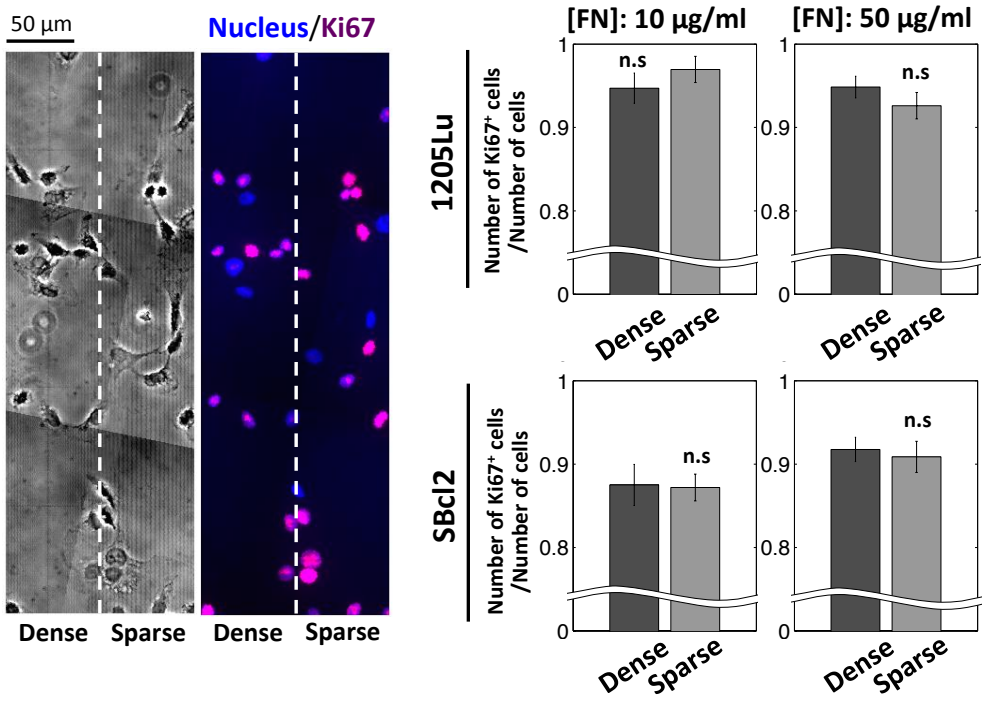


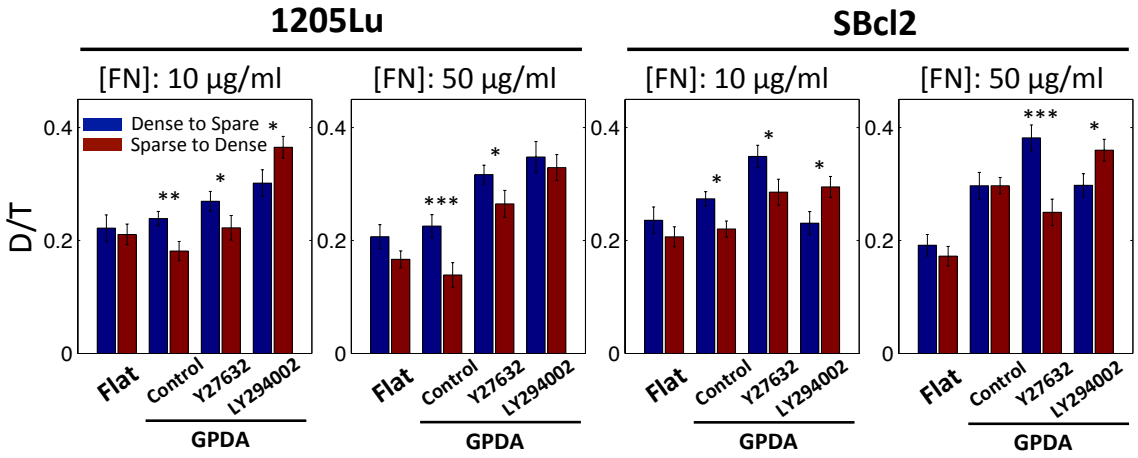
Supplementary figure 1. The schematic illustration of GDPA and its SEM images (a) Schematic sequence of steps of fabricating gradient post density array (GPDA). SEM images of PUA replications in the point of (b) large area (c) single pillar size (d) dense zone (e) intermediate zone (f) sparse zone.



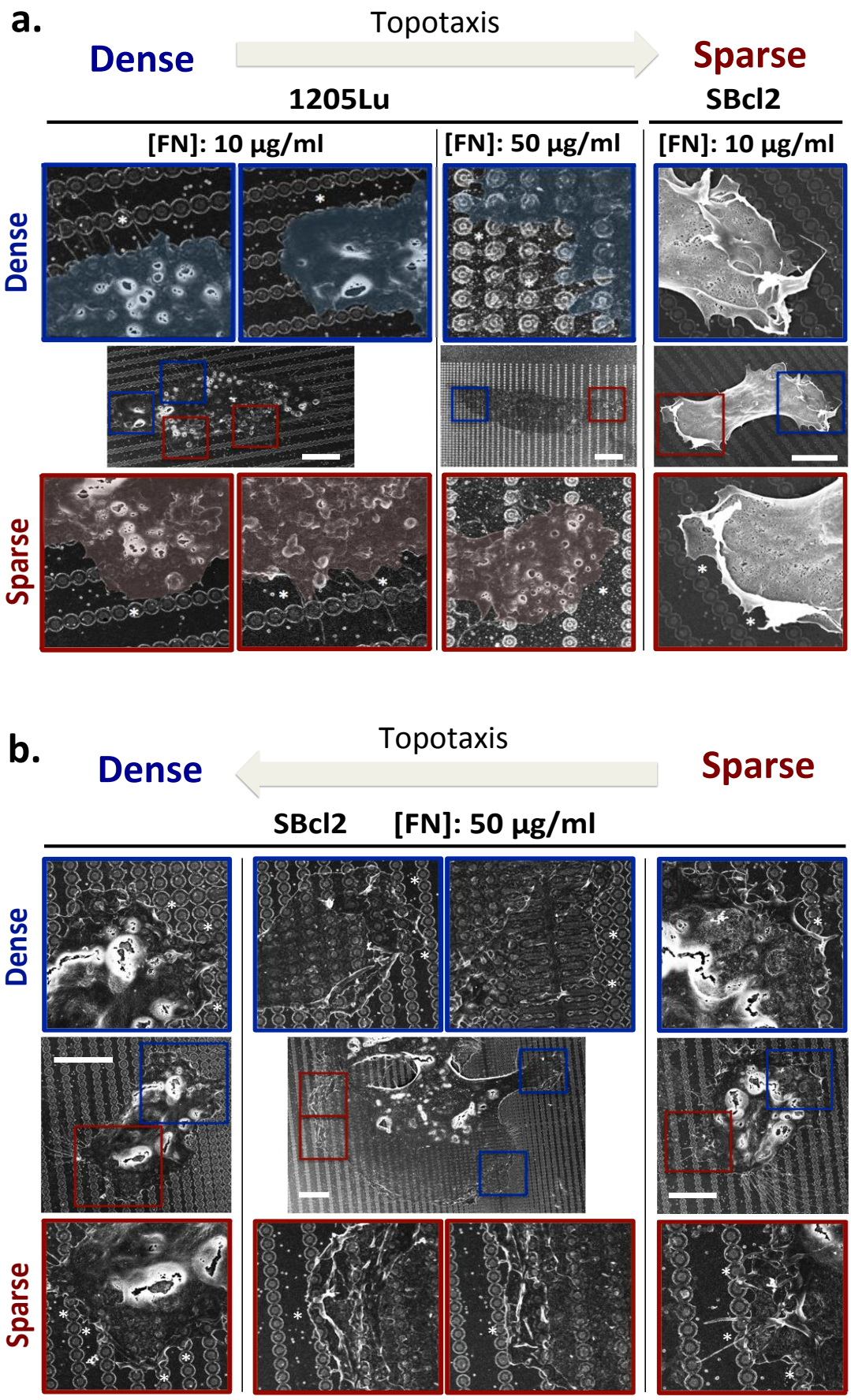
Supplementary figure 2. Directionally biased accumulation of 1205Lu and SBcl2 cells in zones of different post density and ECM density after 3 days of culture (D: dense regions and S: sparse regions). *= Significant difference of fraction of cells on dense and spare post density arrays on GPDA, *P<0.05, **P<0.01 and ***P<0.005. All paired two-sample Student's t-test. All error bars are S.E.M.



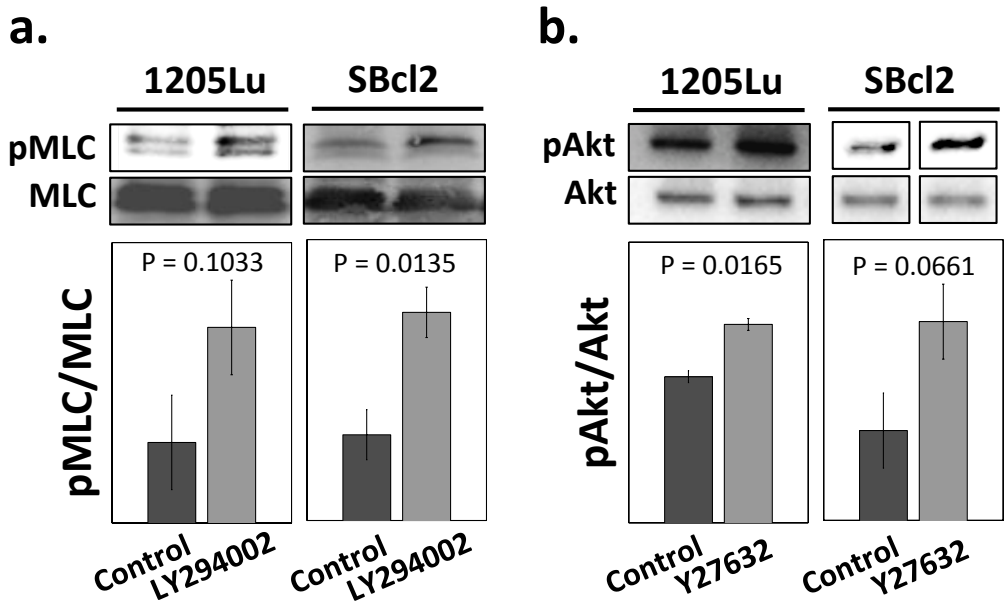
Supplementary figure 3. Analysis of proliferation of 1205Lu and SBcl2 cells in zones of different post density and ECM density. Ki67, a proliferation marker in the cells on GPDA, was stained for after 1 day of culture. The ratio of the number of Ki67 stained cells to total number of cells, representing proliferation rate, on either dense or sparse post arrays, was calculated. n.s= no significant difference between the ratios in dense and spare post array zones on GPDA. All paired two-sample Student's t-test. All error bars are S.E.M.



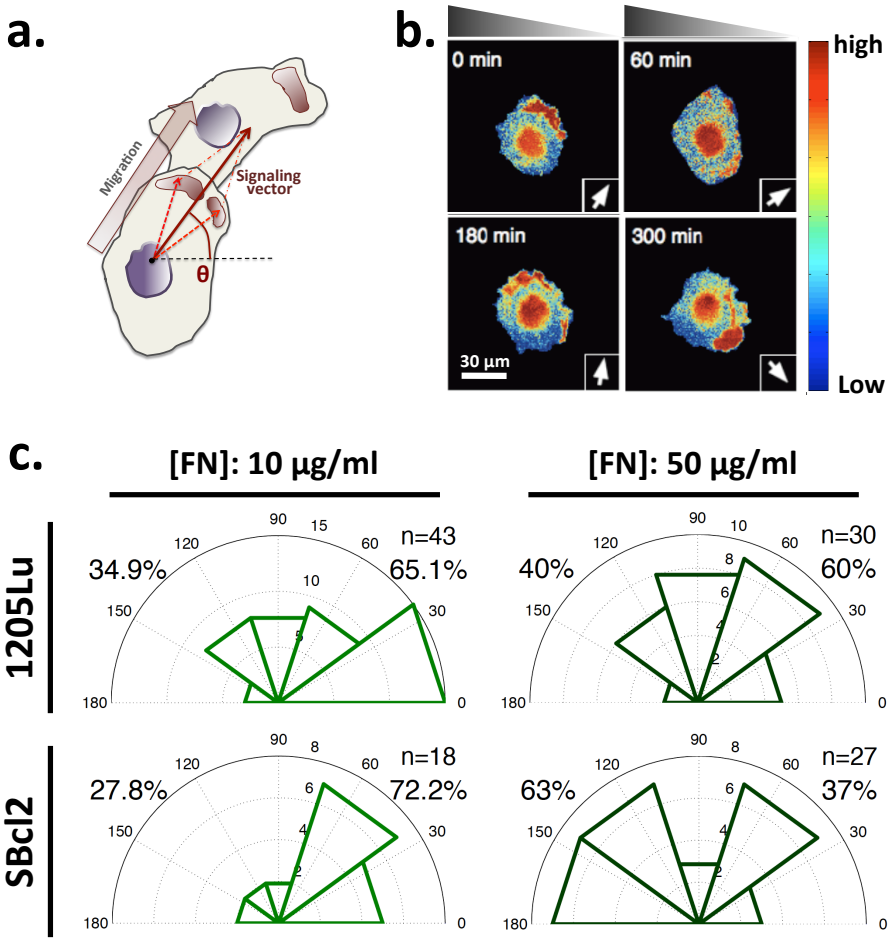
Supplementary figure 4. Analysis of persistence of topotactic cell migration. The D/T ratio described in the Methods is plotted for cells undergoing migration in two alternative directions under indicated perturbations. *= Significant difference between the values of cells moving toward dense and spare post arrays on GPDA, *P<0.05, **P<0.01 and ***P<0.005. All paired two-sample Student's t-test. All error bars are S.E.M.



Supplementary figure 5. SEM of 1205Lu and SBcl2 on pre-coated GPDA with 10 and 50 µg/ml of FN (a) Inhomogeneous vertical penetration of pseudopods depends on the post density. In the denser regions (top), pseudopods are localized on tops of the posts, whereas they are localized on the surface regions between posts in the sparser post array regions (marked by *) (bottom). (b) Non-invasive cells on GPDA coated with high FN surface density, migrating from sparser to denser regions, localize their pseudopods on the tops of the posts regardless of the post density (marked by *). Scale bars are 10 µm.



Supplementary figure 6. Crosstalk between PI3K and ROCK signaling. (a) ROCK activities of 1205Lu and SBcl2 cells were measured via immunoblotting, using MLC and phosphorylated MLC antibodies, following treatment with LY294002, a PI3K inhibitor. (b) PI3K activity in 1205Lu and SBcl2 cells was measured via immunoblotting with Akt and phosphorylated Akt antibodies, following treatment with Y27632, a ROCK inhibitor. All experiments were repeated three times. All P-values were calculated from paired two-sample Student's t-test. All error bars are S.E.M.



Supplementary figure 7. Spatially biased PI3K activity of melanoma cells depends on the post density and ECM density of GPDA (a) The schematic illustration of the signaling vector analysis, described in the Methods, representing spatial localization of PI3K activity over time. (b) Representative time-series images exhibiting spatial translocation of PI3K activity over time. Insets indicate the direction of signaling vectors (c) The distributions of the average PI3K signaling vectors of 1205Lu and SBcl2 cells with respect to different post density and ECM density, analyzed over 5 hours. The directions in the range from 0 to 90° point to sparser array zones, and the directions in the range from 90° to 180° point toward denser array zones.

Fig 4a-1

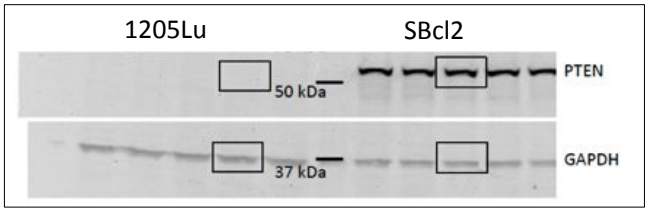


Fig 2d

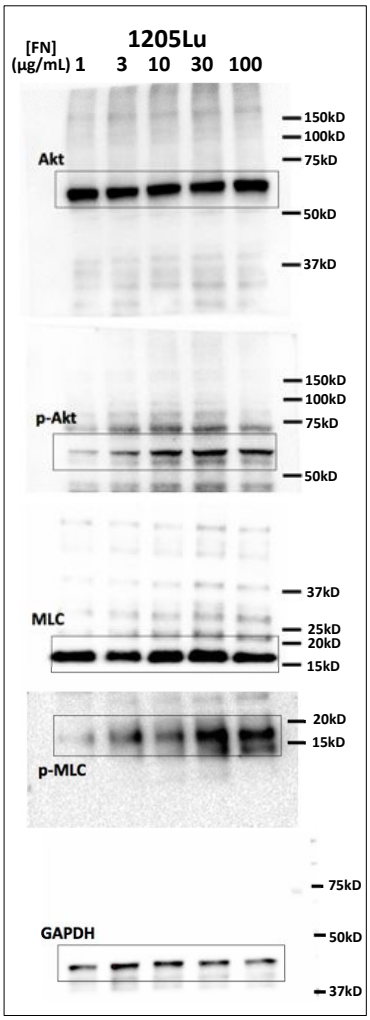


Fig 4a-2

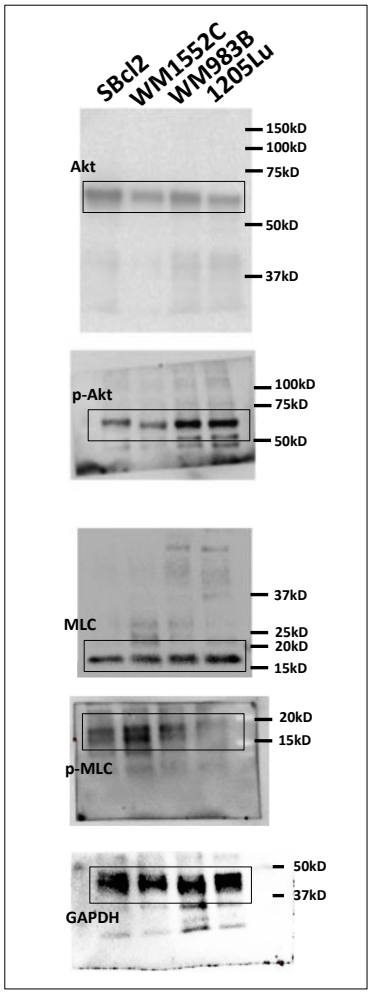
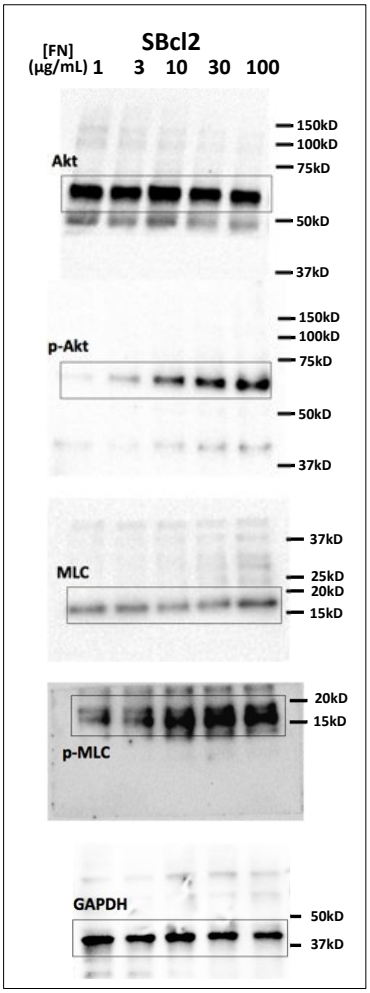
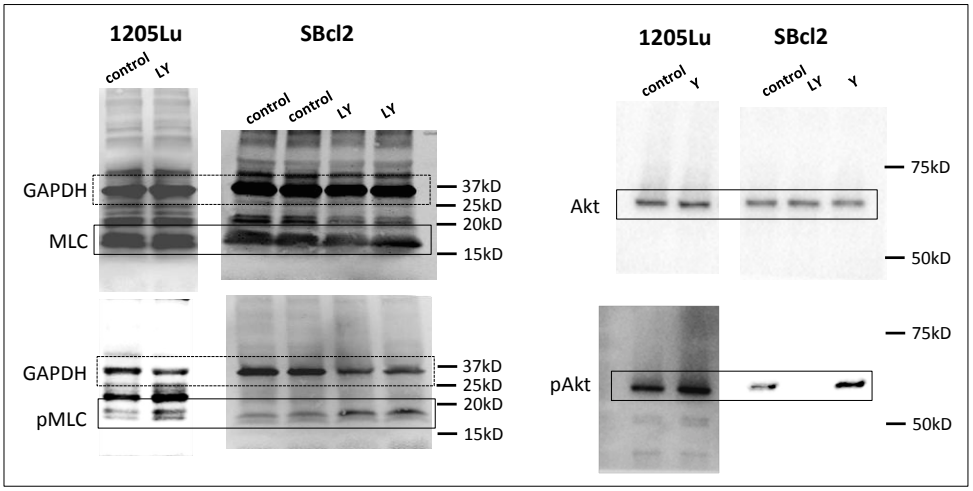


Fig 4b



Supplementary figure 6



Supplementary figure 8. Raw images of Western blots used for the figures in the paper.