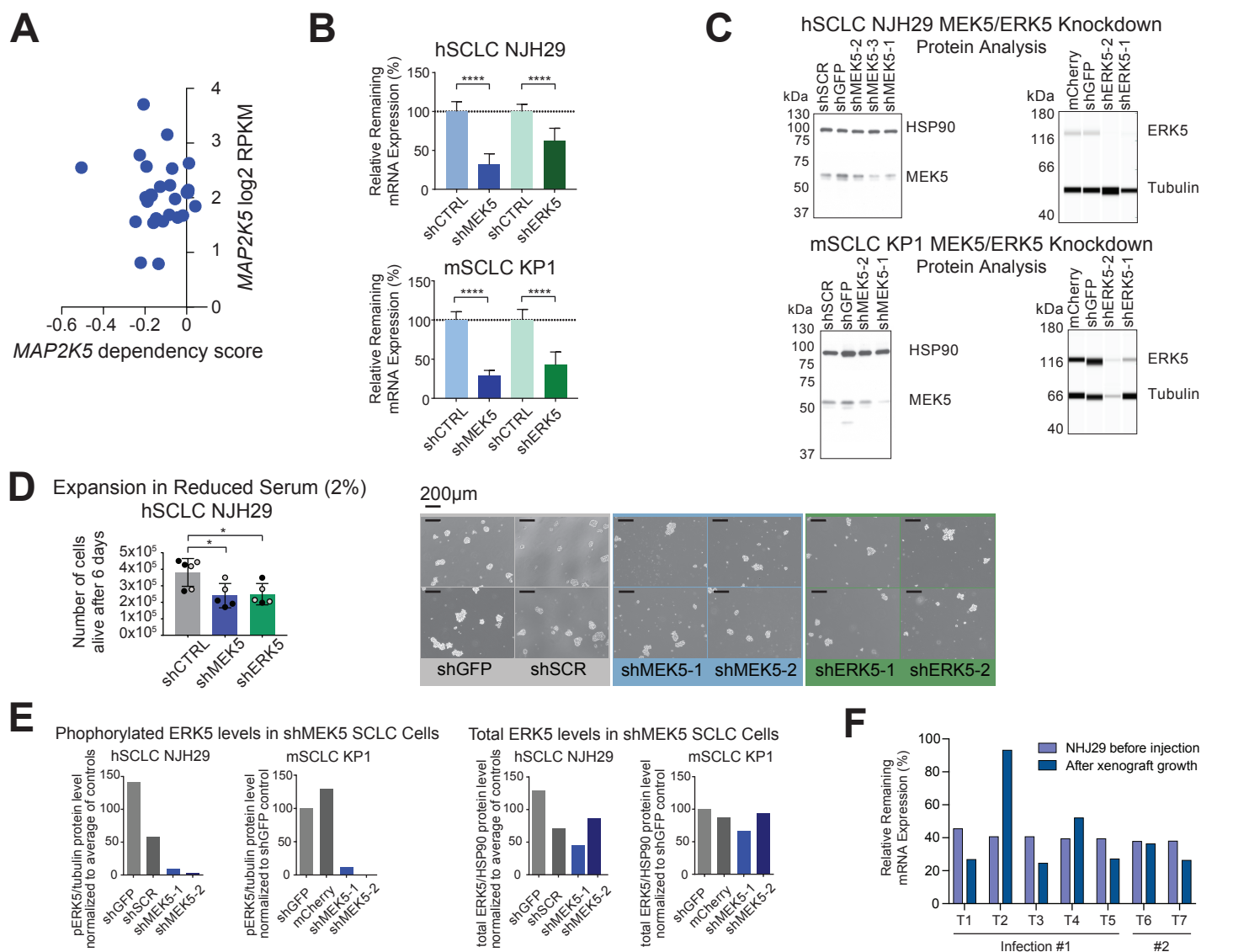


# Figure S1



**Figure S1: MEK5 and ERK5 mRNA and protein knock-down inhibits the expansion of SCLC cells in culture**

**(A)** Dependency on presence of MEK5 in 25 human SCLC (hSCLC) cell lines, as scored by DepMap; cells with lower scores have lower viability when MEK5 is knocked down, (a score of -1 is the median of all essential genes' dependency scores)..

**(B)** Relative mRNA expression after knockdown in hSCLC NJH29 (top) and mSCLC KP1 (bottom) cells, using two independent shRNAs per group; mRNA levels for each gene are normalized to the average expression level in the shCTRL (shGFP and shSCR samples); \*\*\*\* signifies p<0.0001 (t-test); for hSCLC NJH29, n=5-6, and for mSCLC KP1, n=3 independent experiments per individual hairpin.

**(C)** Protein analysis of hSCLC NJH29 (top) and mSCLC KP1 (bottom) cells with MEK5 (by immunoblot, on the left) and ERK5 (by Simple Western<sup>TM</sup> immunoassays, on the right) in control and knock-down cells. HSP90 was used as a loading control for shMEK5 samples, while tubulin was used as a loading control for shERK5 samples; n=1 per individual hairpin. Molecular weights (kDa) are indicated.

**(D)** Cell counts (left) of shMEK5/ERK5 and shCTRL hSCLC NJH29 cells after 6 days in reduced (2%) serum, with representative images (right); grey and black dots represent two independent shRNAs per group; \*, p<0.05 (t-test), n=2-3 per individual hairpin. Scale bar, 200 μm.

**(E)** Levels of phosphorylated ERK5 (pERK5) in shMEK5 samples compared to controls samples (left panel) of hSCLC NJH29 cells (far left) and mSCLC KP1 cells (near left); pERK5 protein levels were divided by levels of tubulin loading control in each sample, then normalized to the average value of shGFP and shSCR (control) samples for hSCLC cells, or simply to shGFP levels for mSCLC cells. Levels of total ERK5 in shMEK5 samples compared to controls samples (right panel) of hSCLC NJH29 cells (near right) and mSCLC KP1 cells (far right); total ERK5 protein levels were divided by levels of HSP90 loading control in each sample, then normalized to the average value of shGFP and shSCR (control) samples for hSCLC cells, or simply to shGFP levels for mSCLC cells; n=1 per individual hairpin. See Supplementary Table S16 for the raw data and representative images of the immunoassay.

**(F)** Changes in knock-down levels in NHJ29 cells before injection and after tumor growth and development of xenografts in NSG mice (27 days). Data shown for 7 tumors (T1-7) from two independent knock-down experiments. Note the variable changes in the knock-down (remaining expression is shown), with no consistent loss of knock-down.