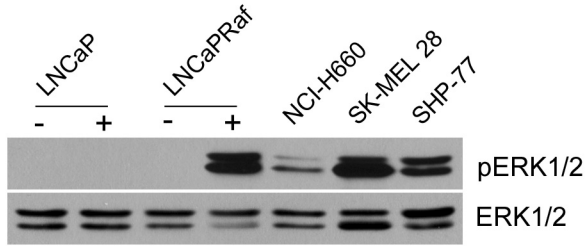
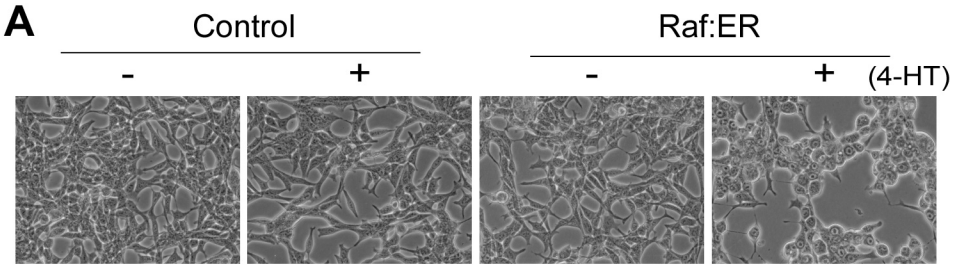
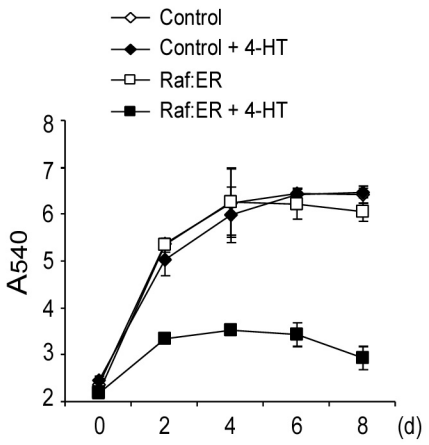
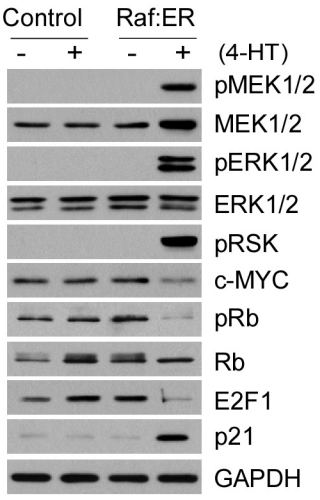


Supplementary data 1. Raf:ER induces similar levels of ERK1/2 activity to oncogenically altered ERK1/2 activity in different cancer cell lines. LNCaP and LNCaPRaf cells treated with 1 μM 4-hydroxytamoxifen (+) were examined for the levels of ERK1/2 phosphorylation (Thr 202/Tyr 204 of ERK1 and Thr 183/Tyr 185 of ERK2) in comparison with the tumor cell lines exhibiting aberrant activation of the Raf/MEK/ERK pathway. NCI-H660 is the neuroendocrine tumor of prostate. SK-MEL 28 is a melanoma line containing the oncogenic B-Raf (V600E) mutation. SHP-77 is a lung cancer line with K-Ras (G12V) mutation. This data indicates that the intensity of Raf:ER-mediated ERK1/2 activation is not significantly different from oncogenically altered ERK1/2 activation levels.



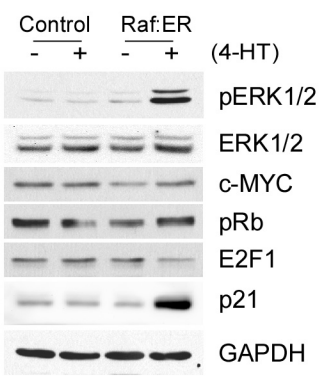




C

% of Raf:ER-infected cells in phase

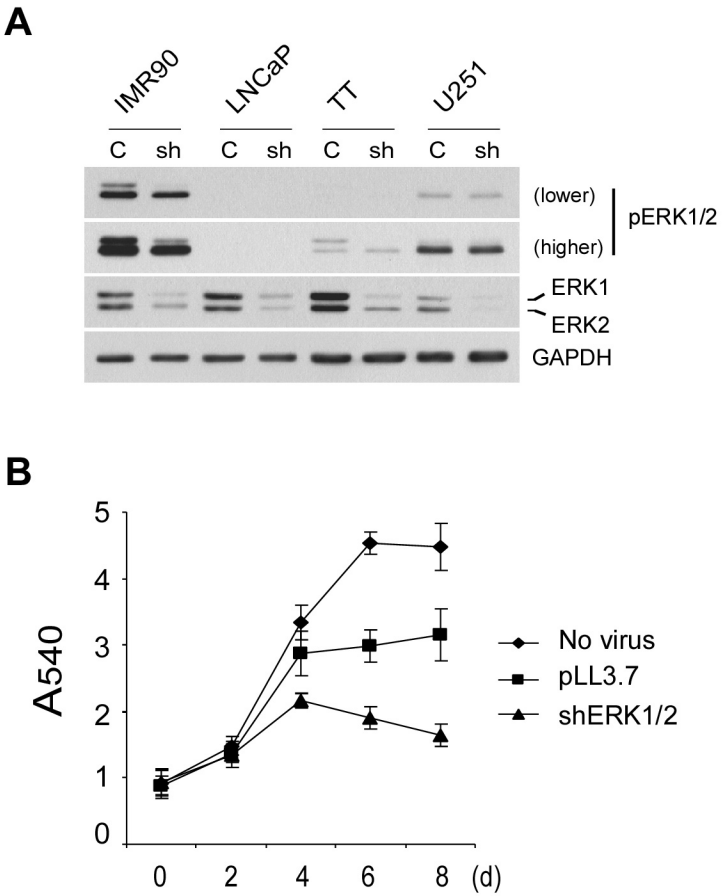
		-	+ (4-HT)
Day 2	G0/G1	63.2 +/- 0.31	65.9 +/- 0.88
	S	25.5 +/- 0.19	14.2 +/- 1.27
	G2/M	11.3 +/- 0.49	19.9 +/- 0.39
Day 4	G0/G1	80.6 +/- 0.10	70.8 +/- 0.85
	S	14.3 +/- 2.43	6.52 +/- 1.58
	G2/M	5.09 +/- 2.42	22.6 +/- 0.73



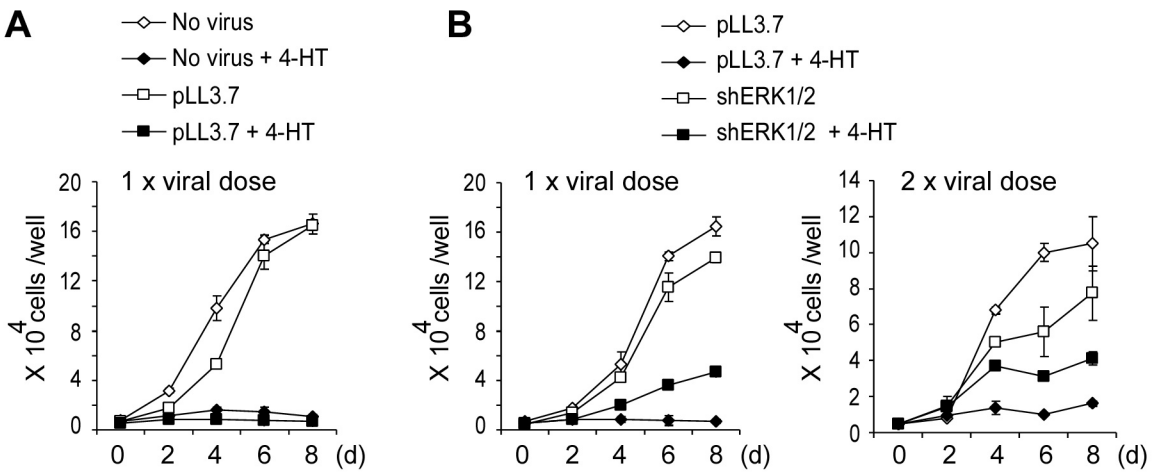
Supplementary data 2. Raf:ER-mediated growth arrest in cancer cells. (A to D) LNCaP cells stably infected with pHAGE (control) or pHAGE-Raf:ER lentivirus were treated with 1 μM 4-hydroxytamoxifen (4-HT) and were examined for morphological changes at day 2 (A), cell proliferation for 8 days by MTT assay (B), cell cycle analysis using propidium iodide at day 2 and 4 (C), and expression of phosphorylated MEK1/2 (pMEK1/2), phosphorylated ERK1/2 (pERK1/2), phosphorylated p90RSK (pRSK), c-MYC, phosphorylated Rb (pRb), E2F1, and p21CIP1 by Western blot analysis (D). The downshift of Rb band in (D) indicates downregulation of its phosphorylation. Data (mean +/- standard error) are from a representative experiment performed in triplicate. These data indicate that activation of the Raf:ER construct induces cell cycle arrest mainly at G2/M phase to suppress LNCaP cell proliferation. This growth arrest is accompanied by activation of the MEK/ERK pathway, phosphorylation of the ERK substrate p90RSK, and changes in protein levels of c-MYC and the cell cycle regulators. E) U251 cells stably infected with pHAGE-Raf:ER lentivirus were treated with 1 μM 4-hydroxytamoxifen and were examined at day 2 for expression of the indicated proteins by Western blot analysis. Raf activation also controlled E2F1 and p21CIP1, but not c-MYC and Rb phosphorylation, in U251 cells. These data also indicate that 1 μM 4-hydroxytamoxifen has no effects by itself and, therefore, does not interfere with the use of Raf:ER in studying the Raf/MEK/ERK pathway-mediated growth arrest signaling.

	% of cells in phase	
	Control	MEK1CA
G0/G1	75.2 +/- 0.08	79.4 +/- 0.64
S	17.8 +/- 0.68	4.85 +/- 4.85
G2/M	6.97 +/- 0.76	15.7 +/- 4.22

Supplementary data 3. Constitutively active MEK1 induces cell cycle arrest in LNCaP cells. LNCaP cells were infected with pHAGE (control) or pHAGE-MEK1CA lentivirus for 4 days. Data (mean +/- standard error) are from a representative experiment performed in triplicate. These data indicate that constitutively active MEK1 induces cell cycle arrest mainly at G2/M phase similar to the Raf activation shown in Supplementary data 2C.



Supplementary data 4. The primary normal fibroblast IMR90 is more sensitive to ERK1/2 depletion than the Ras/Raf-responsive tumor lines, LNCaP, TT and U251. A) ERK1 and ERK2 were doubly knocked down in IMR90, LNCaP, TT and U251 cells using the same dose of shERK1 and shERK2 viruses that specifically target ERK1 and ERK2 (sh). pLL3.7 is the control virus. The data shows that the efficacy of ERK1/2 knockdown in these cells was similar. B) The effect of ERK1/2 knockdown on IMR90 cell proliferation was determined by MTT assay. Data (mean +/- standard error) are from a representative experiment performed in triplicate. The data shows that ERK1/2-depleted IMR90 cells could not proliferate as well as the cells infected with the control pLL3.7 virus. Given that IMR90 cells maintain higher basal ERK1/2 activity than LNCaP, TT and U251 and that the tumor lines could still proliferate when ERK1/2 was knockdown (Supplementary data 5 and Fig. 7), IMR90 cells appear to be more dependent on ERK1/2 than the tumor cell lines. This supports that the Ras/Raf-responsive tumor lines have advantage for the characterization of ERK1/2 signaling required for the Raf/MEK/ERK pathway-induced growth arrest.



Supplementary data 5. ERK1/2 double-knockdown by transient infection blocks Raf-mediated growth arrest in LNCaP cells. A) The effect of transient lentiviral infection on LNCaP proliferation and its response to Raf activation was determined by counting cells of LNCaPRaf, infected with the pLL3.7 lentivirus and treated with 1 μM 4-hydroxytamoxifen (4-HT). The data indicates that virus used at the dose (1 x) did not significantly affect basal cell growth or Raf-induced growth arrest. B) The effect of transient ERK1/2 knockdown on LNCaP proliferation and its response to Raf activation was determined by counting cells of LNCaPRaf, coinfected with two different doses (1 x and 2 x) of shERK1 and shERK2 lentiviruses and treated with 4-HT. The efficiency of infection and ERK1/2 knockdown (at 1 x viral dose) is shown in Fig. 2A and 2B, and Supplementary data 3A. Data (mean +/- standard error) are from a representative experiment performed in triplicate. The data indicates that ERK1/2 depletion by transient infection can rescue cells from Raf-induced growth arrest. The "rescue" effect was proportonal to the dose of shERK1 and shERK2 viruses used, although higher viral dose slightly decreased cell growth rates. The inhibitory effect of ERK1/2 depletion on Raf-induced growth arrest was more apparent when ERK1 and ERK2 were stably knocked down (Fig. 2D).