Supporting Information

Osborne et al. 10.1073/pnas.1303932110

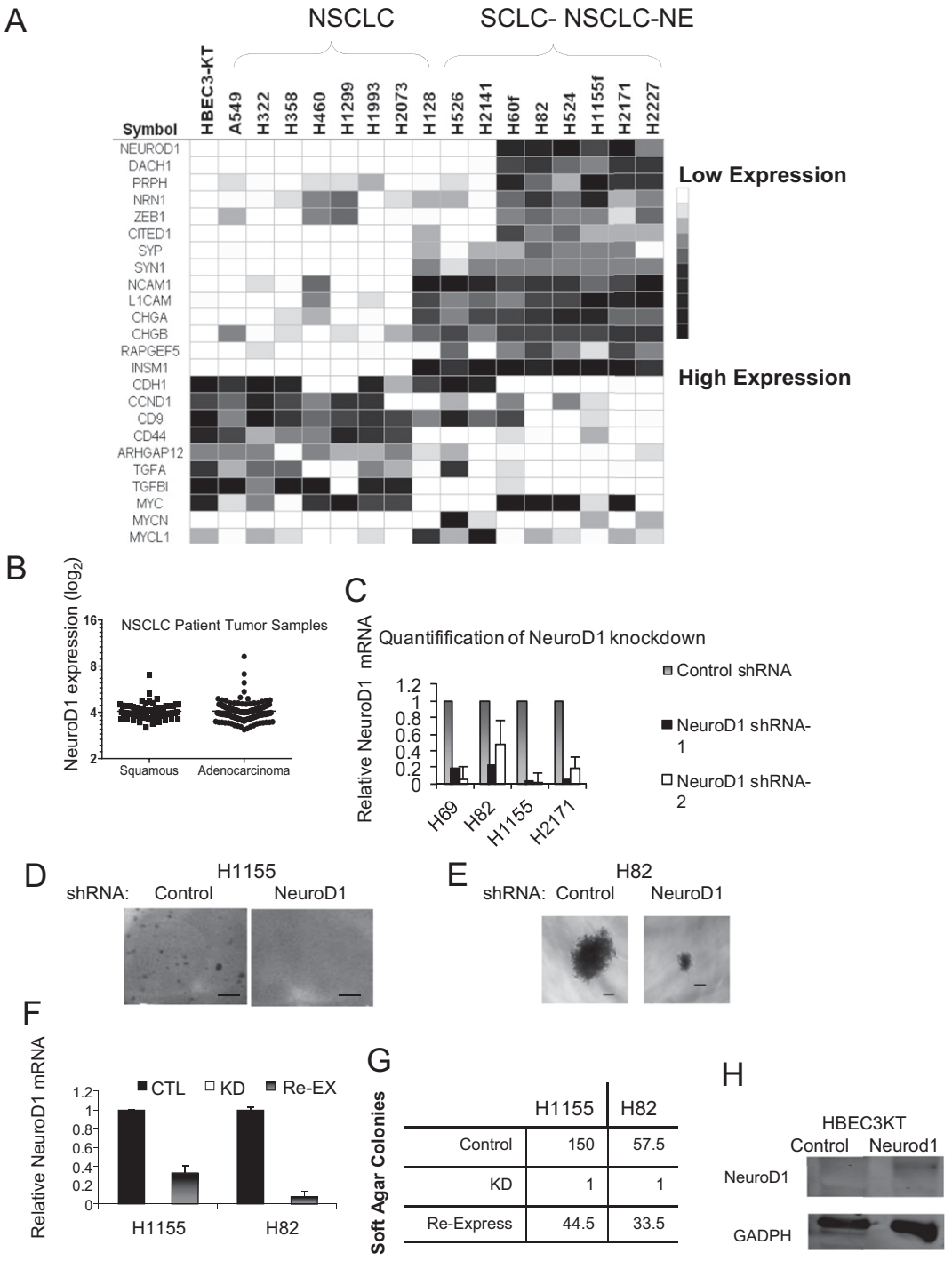

Fig. S1. Neurogenic differentiation 1 (NeuroD1) microarray and functional assay (associated with Fig. 1). (*A*) mRNA expression was analyzed in human bronchial epithelial cell (HBEC), non–small-cell lung cancer (NSCLC), and small-cell lung cancer (SCLC) using Affymetrix HG-U133A and B GeneChips. (*A* and B) mRNA expression in 275 NSCLC lung cancer patient samples assessed using Illumina BeadChip HumanWG-6 V3. (*C*) The stable cell lines created and tested in this figure were used throughout the paper in experiments using NeuroD1 knockdown. RNA was extracted from cells, reverse transcribed to cDNA, and used for quantitative RT-PCR (qRT-PCR) to quantitate knockdown efficiency. Subsequent studies used shRNA-2 unless otherwise stated. (*D* and *E*) Formation of colonies in soft agar by H1155 and H82 cells in which NeuroD1 was stably knocked down was measured. Pictures are at 4× (H1155) and 10× (H82) magnification. (*F*) Cells as in *D* and *E* were transiently transfected with a plasmid encoding mouse Neurod1. Expression was confirmed by mRNA analysis (*Left*). (*G*) Quantitation of panel *F*. (*H*) Overexpression of NeuroD1 in HBEC3KT (See Fig. 1 *F* and *G*).

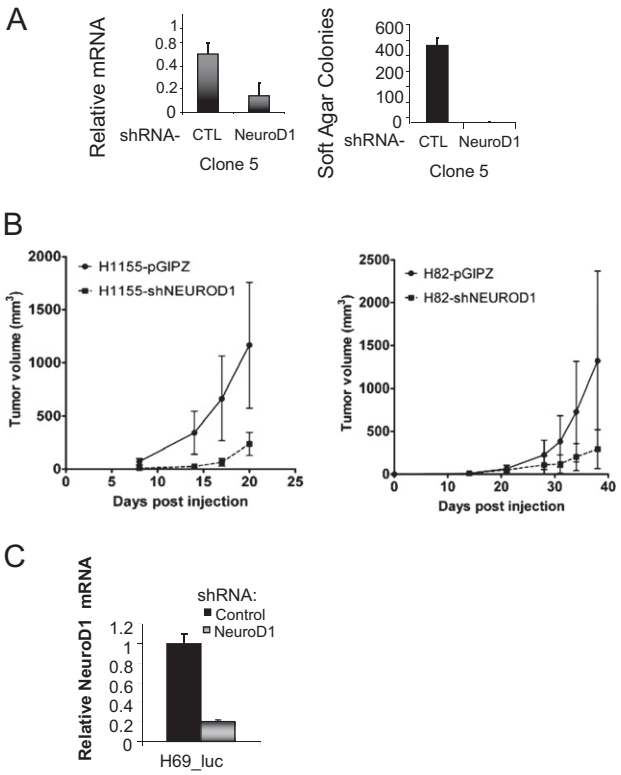

Fig. S2. Analysis of aspects of NeuroD1 function (associated with Fig. 2). (*A*) HBEC3KTRL53-clone 5 cells were infected with viruses expressing NeuroD1-1 or control shRNAs. NeuroD1 mRNA was quantitated by qRT-PCR (*Left*). Formation of colonies in soft agar by clone 5 cells was measured (*Right*). (*B*) Nonobese diabetic (NOD)/SCID female mice were injected with 10^6 H1155 or H82 cells infected with either control shRNA or NeuroD1 shRNA. Tumors were measured every 3–5 d until maximum tumor burden was reached ($n = 5$ mice per group). *P* values were computed by linear regression (of slopes). Data means are ±SEM. (*C*) The H69-luc cell line was infected with control or NeuroD1 shRNA and knockdown was quantified via qRT-PCR for Fig. 2 *A* and *B*.

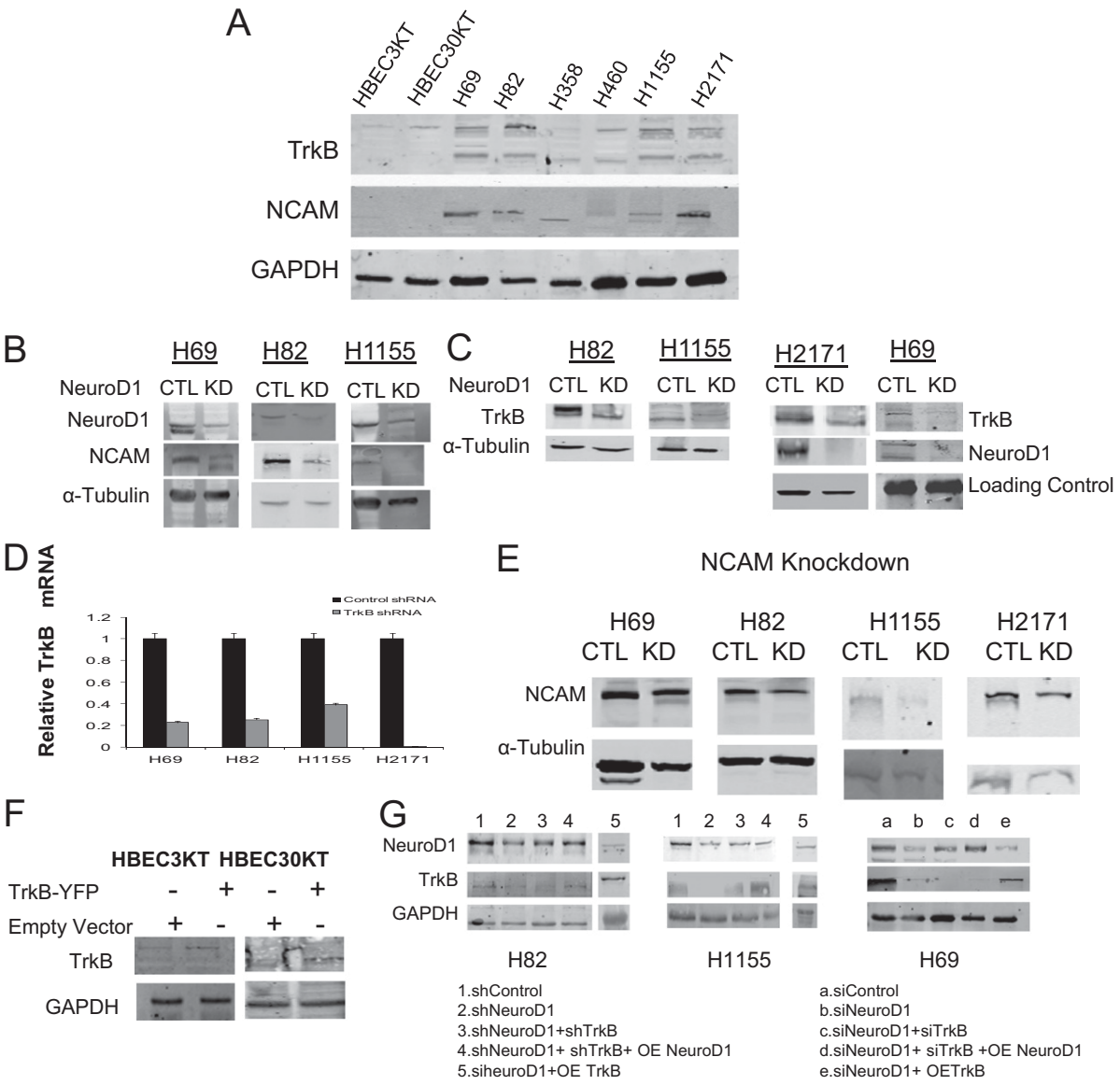

Fig. S3. Studies of neural cell adhesion molecule (NCAM) and tropomyosin-related kinase B (TrkB) (associated with Fig. 3). (*A*) Lysates of HBEC cell lines, SCLC and NSCLC immunoblotted for NCAM and TrkB. (*B* and *C*) Lysates of cells in which NeuroD1 was stably knocked down were blotted for NCAM (*B*) and TrkB (*C*). Loading control for H69 is GAPDH, and loading control for H2171 is α-tubulin. (*D*) Knockdown of TrkB was quantified by qRT-PCR. (*E*) Knockdown of NCAM was quantified by immunoblotting. (*F*) HBEC3KT and HBEC30KT were transfected with a plasmid encoding TrkB or control vector. Cell lysates were immunoblotted for TrkB and for GAPDH as loading control. (*G*) H69, H82, and H1155 cell lines were transfected with siRNA oligonucleotides or shRNA vectors against NeuroD1 and NeuroD1/TrkB. Knockdown cells were subjected to overexpression of either NeuroD1 or TrkB mammalian expression vectors. Cells were subjected to migration experiments and lysed and immunoblotted for NeuroD1, TrkB, and GAPDH as loading control.

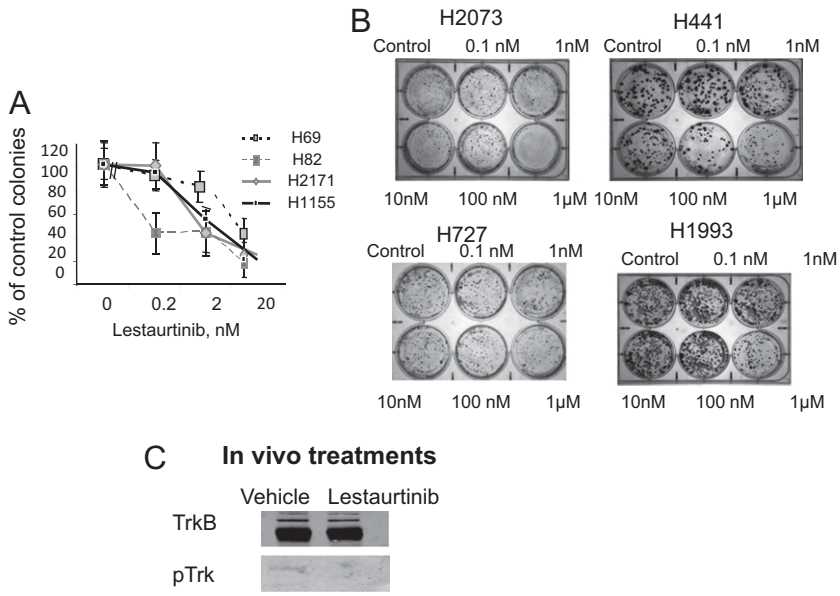

Fig. S4. Colony formation with lestaurtinib (associated with Fig. 4). (*A*) Formation of colonies in soft agar was assayed in three SCLC and one NSCLC-NE cell lines, and then exposed to 0.2, 2, and 20 nM lestaurtinib or control. (*B*) Liquid colony assays was examined in four NSCLC cell lines (H441, H2073, H1993, adenocarcinoma; and H727, a neuroendocrine carcinoid) treated once with increasing concentrations of lestaurtinib. Colonies were stained after 2 wk. (*C*) Xenograft tumors were homogenized, and TrkB was immunoprecipitated and immunoblotted with pTrk antibody.

Table S1. Human bronchial epithelial cell (HBEC) manipulations

Cell lines	Genetic manipulations
HBEC3KT	K = CDK4
HBEC30KT	T = hTERT
	Immortalized—not tumorigenic
HBEC3KTRL53	K = CDK4
	T = hTERT
	RL = oncogenic KRAS V12
	53 = knockdown of p53
	Tumorigenic
HBEC3KTRL53	K = CDK4
Clone 5	T = hTERT
	RL = oncogenic KRAS V12
	53 = knockdown of p53
	Tumorigenic

HBEC lines derived from different individuals were randomly assigned a number, e.g., 3, 30, etc., and immortalized with cyclin-dependent kinase 4 (CDK4) and human telomerase reverse transcriptase (hTERT). The tumor suppressor p53 was knocked down stably and the K-Ras mutant V12 was stably expressed. The resulting HBEC3KTRL53 was subcultured and a number of clonal populations, including clone 5, were isolated.