SUPPLEMENTAL

Primer Name	Sequence
R ntrk1 center	GCACTCAGCAAGGAAGACCT
F ntrk1 center	GGCAGAGGTCTCTGTTCAGG
R ntrk1 RC213091	TTGCTGCCAGATCCTCTTCT
F ntrk1 RC213091	GATCCGGTACCGAGGAGAT
NotI-ntrk1 R Long	CATTAGGCGGCCGCACCTAGGCCCAGGACATCCAGGTAGACAGGAGGTG
NotI-ntrk1 Reverse	CATTAGGCGGCCGCACCTAGGCCCAGGACATCCAGGTAGA
NotI-ntrk1 Forward	GATTACAGCGGCCGCACCATGCTGCGAGGCGGACGGCG
NTRK1cDNA R2	TTGTCCATGAAGGCAGCCAT
NTRK1 cDNA F2	TGGTCTCATTGAGCACGGAG
NTRK1 cDNA R1	AATGGCTCCGTGCTCAATGA
NTRK1 cDNA F1	AGGGTTGTCCATGAAGGCAG
pIRES-MCS-ntrk1 R2	AACGCCACAGCATCAAGGAT
pIRES-MCS-ntrk1 F2	GTACTCACCCCAACAGCTGG
pIRES-MCS-ntrk1 R1	CAGCATCAAGGATGTGCACG
pIRES-MCS-ntrk1 F1	GGAGTACTCACCCCAACAGC

Supplemental Table S1: NTRK primers for generation and confirmation of TrkA cell lines

Altered Genes > logFC 1

Upregulated genes				Downregulated genes			
Gene Name	logFC	Gene Name	logFC	Gene Name	logFC	Gene Name	logFC
HIST1H2BM	5.300685	TMEM126B	1.479312	C1orf210	-1.01315	MYCL	-1.49604
HIST1H3B	4.232445	ZNF165	1.421289	PYGO2	-1.02806	OTUD1	-1.53782
FAM72D	3.916438	IL1A	1.415549	SCD5	-1.063	DSEL	-1.57764
GJB2	3.547973	FDX1	1.363108	ZNF57	-1.06517	SENP8	-1.58834
SLITRK6	3.305954	B3GNT2	1.355046	PERP	-1.07004	PRODH	-1.62498
FEN1	3.117373	EEF1E1	1.339461	ACOT1	-1.1152	SCNN1G	-1.6784
SPINK6	2.939614	GPR3	1.325148	SYT15	-1.12024	RAB7B	-1.69222
CRABP2	2.870189	HS3ST3A1	1.310651	PCDHB10	-1.12618	WBP5	-1.71479
HIST1H3I	2.862593	RPL39L	1.306733	UCN	-1.12681	NFIL3	-1.80119
GSG2	2.574647	HIST1H2BF	1.300464	SPDY5	-1.13705	MAF	-1.96775
HIST1H2AG	2.236954	C2orf44	1.286178	PARK2	-1.14638	GCSAM	-2.24386
AMTN	2.226482	SLC35C1	1.266293	TSHZ2	-1.15562	KCNB1	-2.27449
CHAC2	2.193323	PGP	1.258337	CFAP53	-1.20364	TSC22D3	-2.37186
HIST1H2AE	2.070118	MZT1	1.234009	ADRB2	-1.21347	MAFB	-2.77564
HYLS1	2.065507	HIST2H2AB	1.21936	ARL4A	-1.21535	KLHL38	-2.79431
TMEM171	2.029894	SOWAHC	1.196201	HCAR2	-1.2216	GNG7	-2.93085
PMCH	2.020629	LLPH	1.1825	TSSK3	-1.22651	CRYAB	-3.10802
AMIGO2	2.01445	RBPMS2	1.177211	NAP1L5	-1.2376	CITED2	-4.15083
H2AFX	1.992244	HIST1H3G	1.167181	DGCR6	-1.24567	METTL7A	-4.38533
MT1A	1.970384	TRMT10C	1.164855	SPDYE2	-1.25754		
HSD17B2	1.877982	DNAJB5	1.159799	SPDYE2B	-1.25754		
HIST1H4D	1.835552	AK1	1.153043	IGIP	-1.26463		
PIGW	1.807824	RPE65	1.137656	PCDHB14	-1.26715		
TMEM176B	1.799355	PDE12	1.105626	ZBTB22	-1.27625		
RTKN2	1.791073	HIST1H2BC	1.09568	HIST2H4B	-1.28258		
HIST1H2BL	1.664501	TRIM59	1.083583	HIST3H2A	-1.33157		
MT1G	1.550813	FBXO45	1.068118	CHAD	-1.3747		
RMI1	1.509626	NXT2	1.062884	BBS10	-1.37627		
HIST1H2BI	1.505982	HIST1H4L	1.047807	DNAJC28	-1.48722		

Supplemental Table S2: Genes altered in nontumorigenic breast cells with TrkA overexpression. Genes with > 1 log fold change (FC) in TrkA overexpressing cells when compared to wildtype parental control.

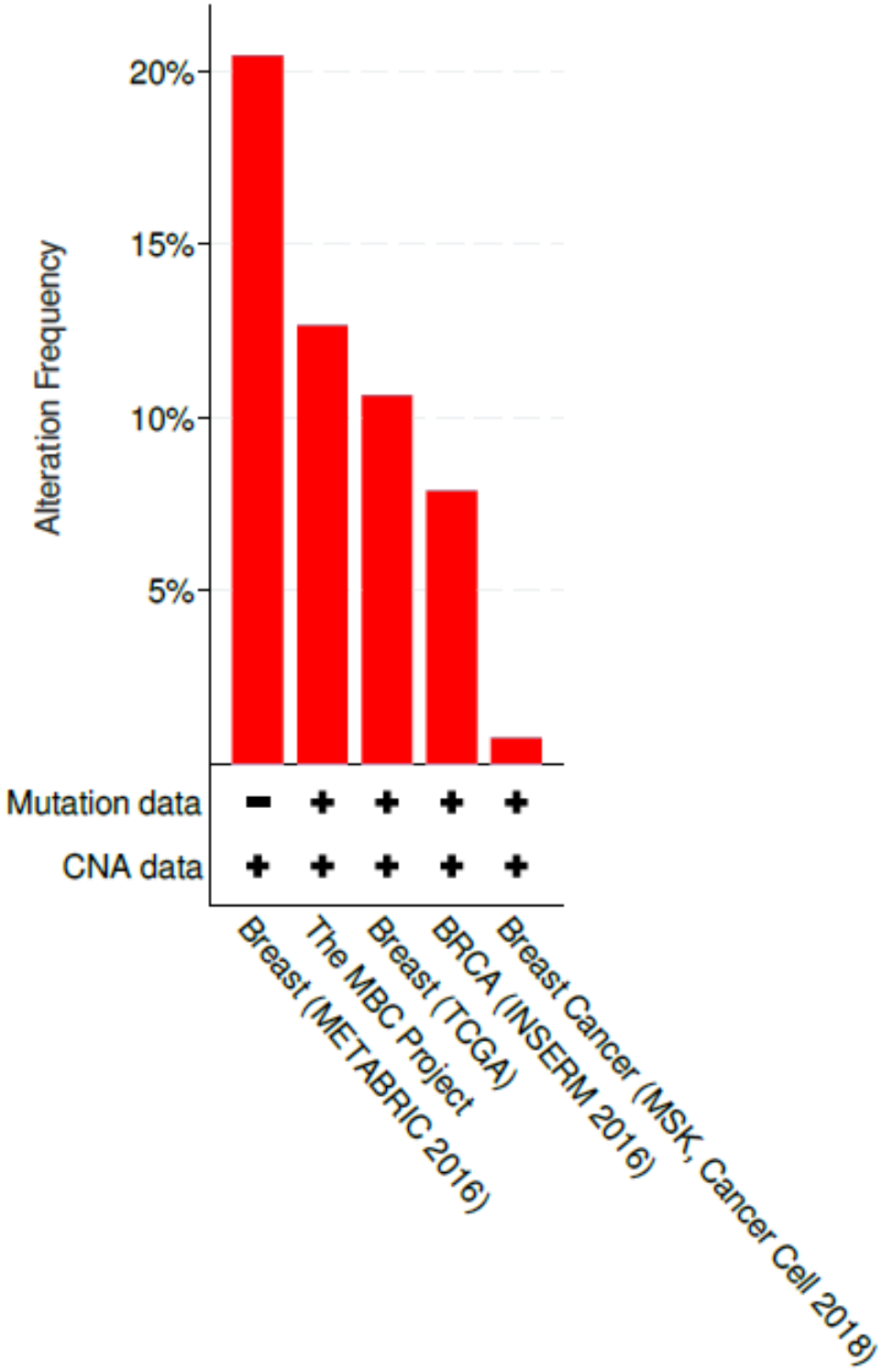


Figure S1: NTRK1 amplification in patients with breast cancer. Interrogation of cBioPortal revealed NTRK1 amplification in patients with breast cancer. Data represents 5762 patients / 5988 samples with amplification across 5 studies (METABRIC: 2173, The MBC Project: 237, TCGA: 117, INSERM: 17, MSK: 14). Percentages are based on total patients within study. cBioPortal was accessed in November 2019.

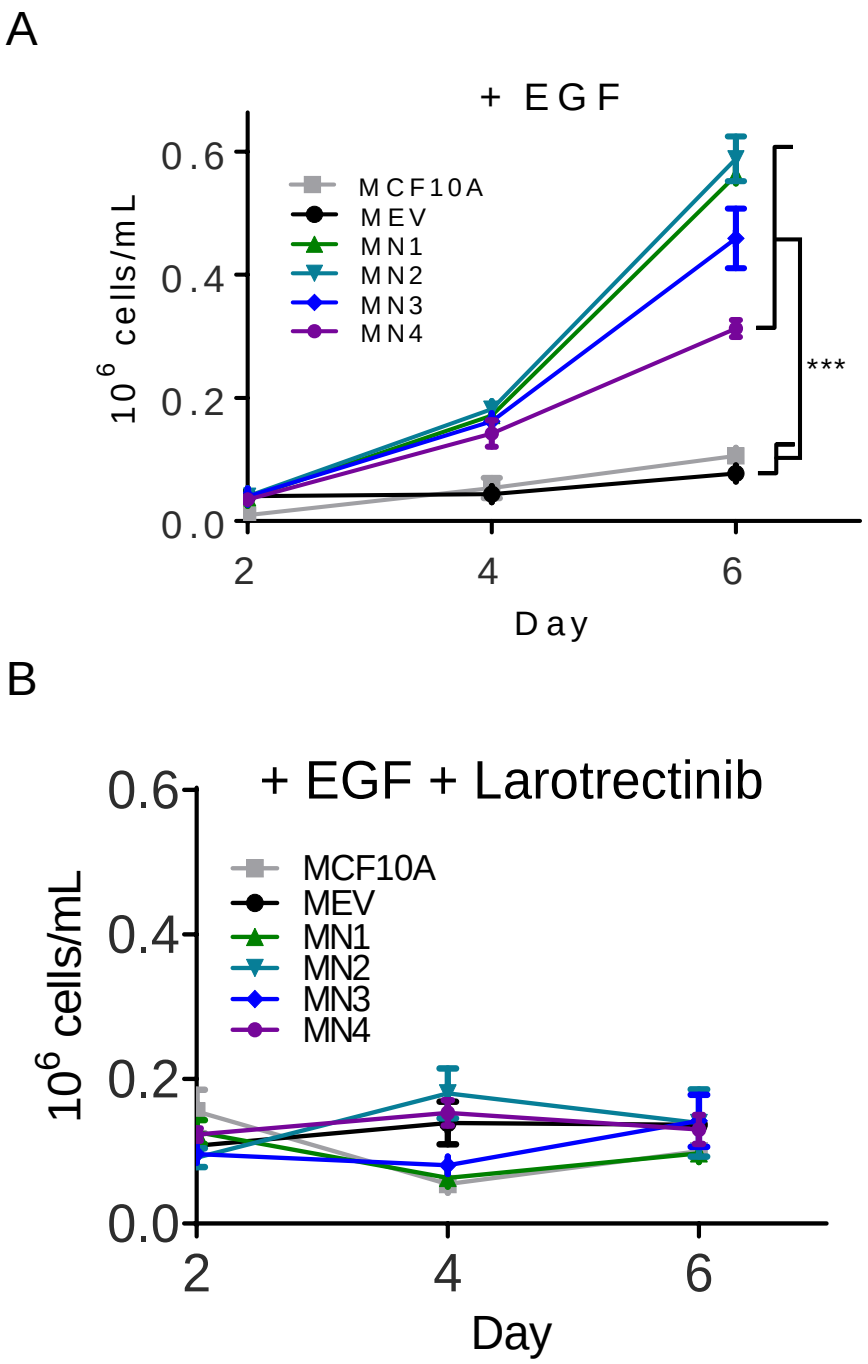


Figure S2: TrkA overexpression in MCF10A confers growth advantage in the presence of EGF. (A) Proliferation analysis of the MCF10A TrkA overexpression panel in the presence of 0.2 ng/mL epidermal growth factor (+EGF) and (B) + 1.5 µM Larotrectinib. Cells were plated at a density of 30,000 cells/well in 24-well plates and cell counted on 2, 4, and 6 days. Mean ± SEM shown, ***P ≤ 0.001, by ANOVA at 6 day time point.

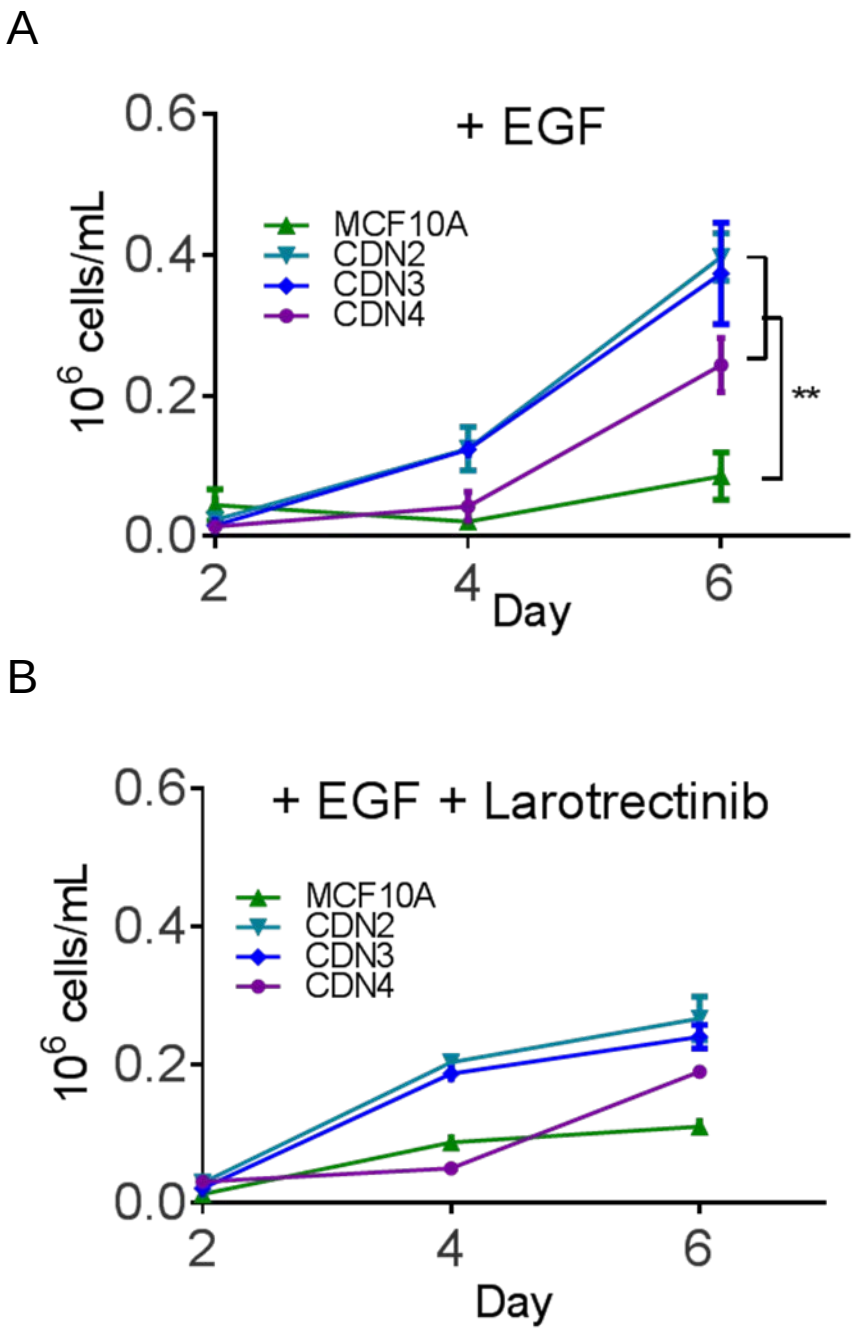


Figure S3: Proliferation of CD74-NTRK1 fusions in MCF10A. (A) Proliferation analysis of the MCF10A CD74-NTRK1 fusion panel in the presence of 0.2 ng/mL EGF and (B) + 2 µM Larotrectinib. Cells were plated at a density of 30,000 cells/well in 24-well plates and cell counted on 2, 4, and 6 days. Mean ± SEM shown, **P ≤ 0.01, by ANOVA at 6 day time point.

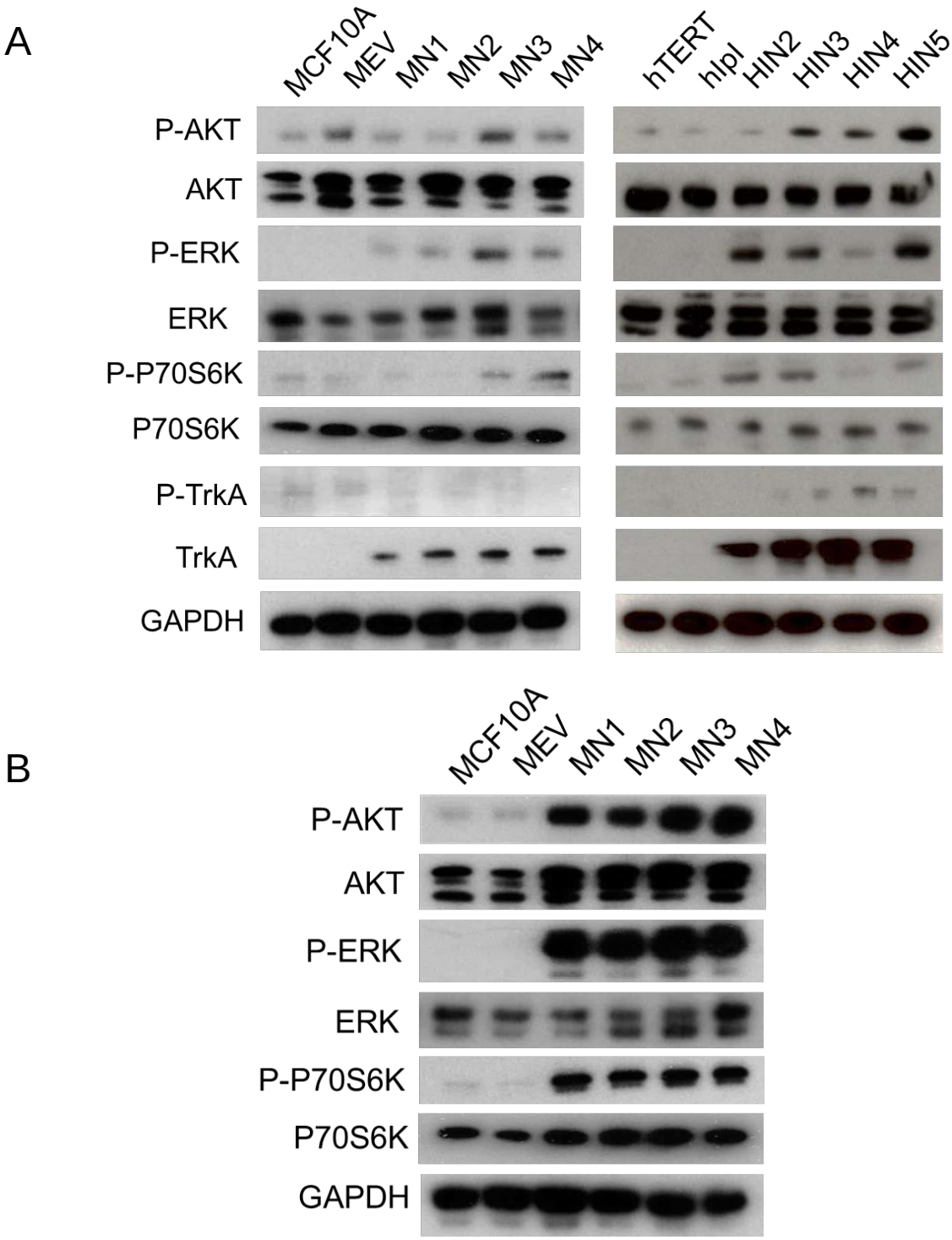
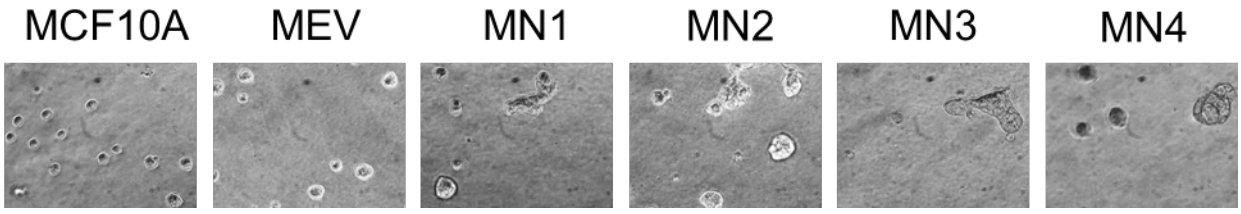


Figure S4: TrkA overexpression leads to increased MAPK/PI3K signaling and dysregulation of genes in oncogenic pathways. (A) Immunoblot analysis of the MCF10A and hTERT-IMEC TrkA overexpression panels in the absence of growth factors (B) Immunoblot analysis of the MCF10A TrkA overexpression panel in the presence of 0.2 ng/mL neuronal growth factor (NGF) and no epidermal growth factor (EGF).

A



B

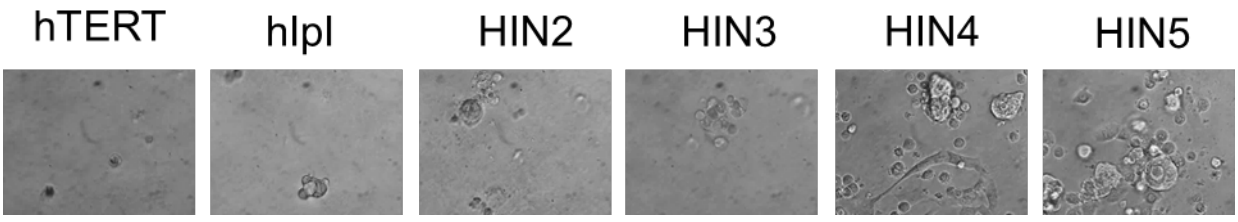


Figure S5: TrkA overexpression leads to acini formation in growth-factor reduced media (A) MCF10A TrkA overexpressing panel and (B) hTERT-IMEC overexpressing panel were cultured at low density in matrigel in the abesence of EGF and NGF.

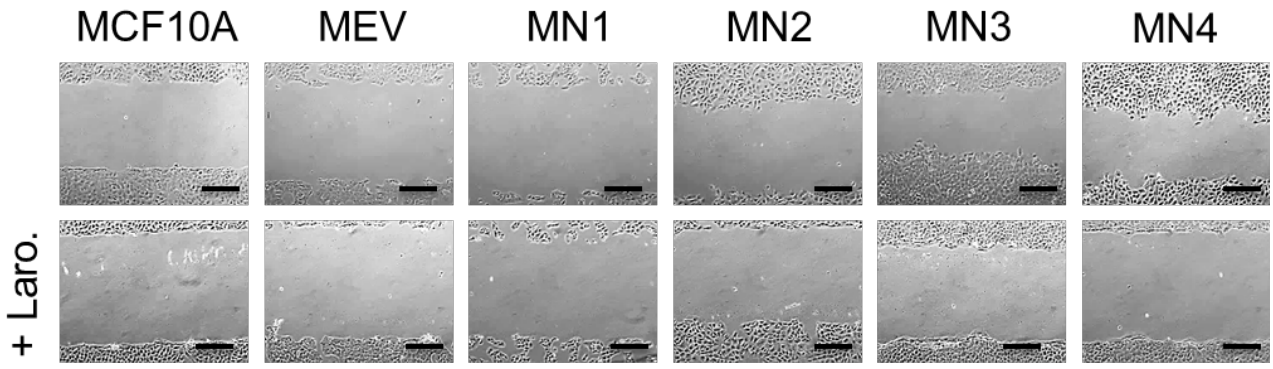


Figure S6: TrkA overexpression leads to increased wound healing in MCF10A panel. Representative images of MCF10A scratch assays at 16 hours. Quantified data presented in main figure 5A (scale bar, 50 µm).

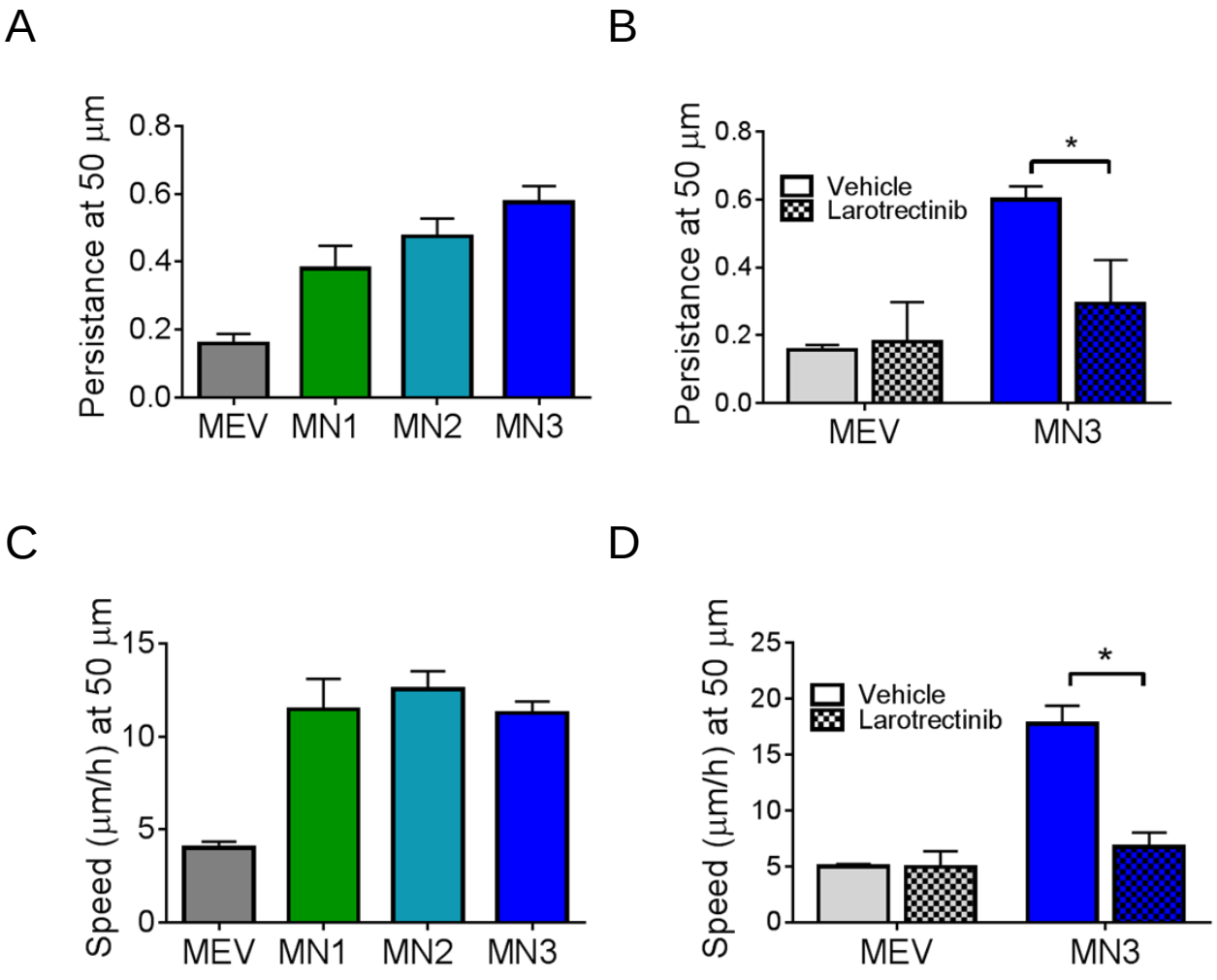


Figure S7: TrkA overexpression leads to increased migration in MCF10A panel. Microchannel migration assays were performed in 50 µm channels. Individual cells from the MCF10A TrkA overexpression panel were tracked along a growth factor gradient. (A) Persistence of migrating cells as a measure of net cell displacement to total distance traveled (B) + 1.5 µM larotrectinib. (C) Instantaneous speed of migrating cells in a linear direction (D) + 1.5 µM larotrectinib.

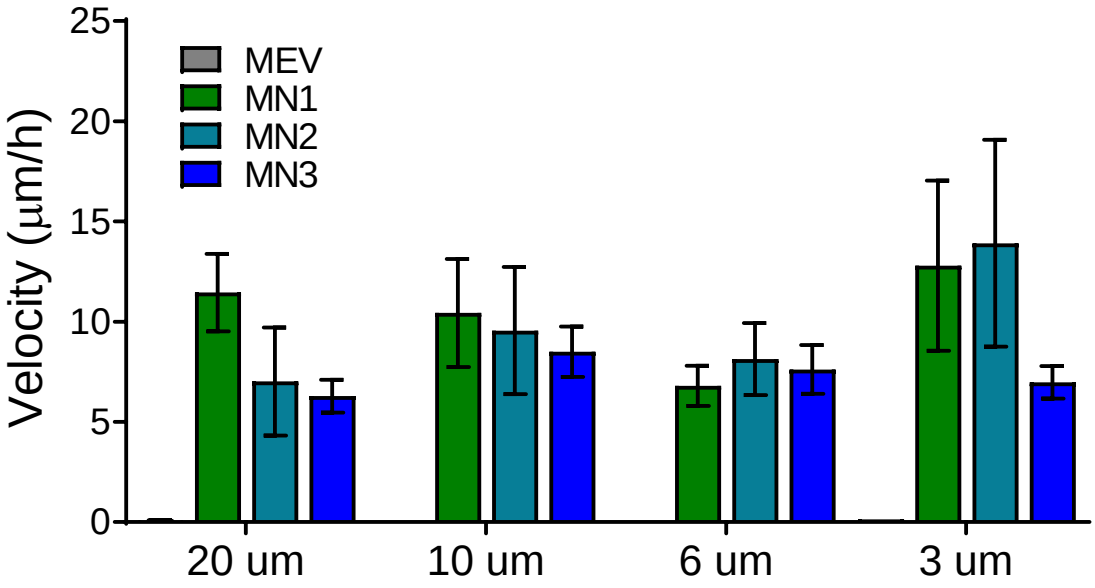


Figure S8: TrkA overexpression leads to increased migration in MCF10A panel in varying channel sizes. Additional microchannel migration assays were performed in 20, 10, 6, and 3 µm channels. Individual cells from the MCF10A TrkA overexpression panel were tracked along a growth factor gradient.