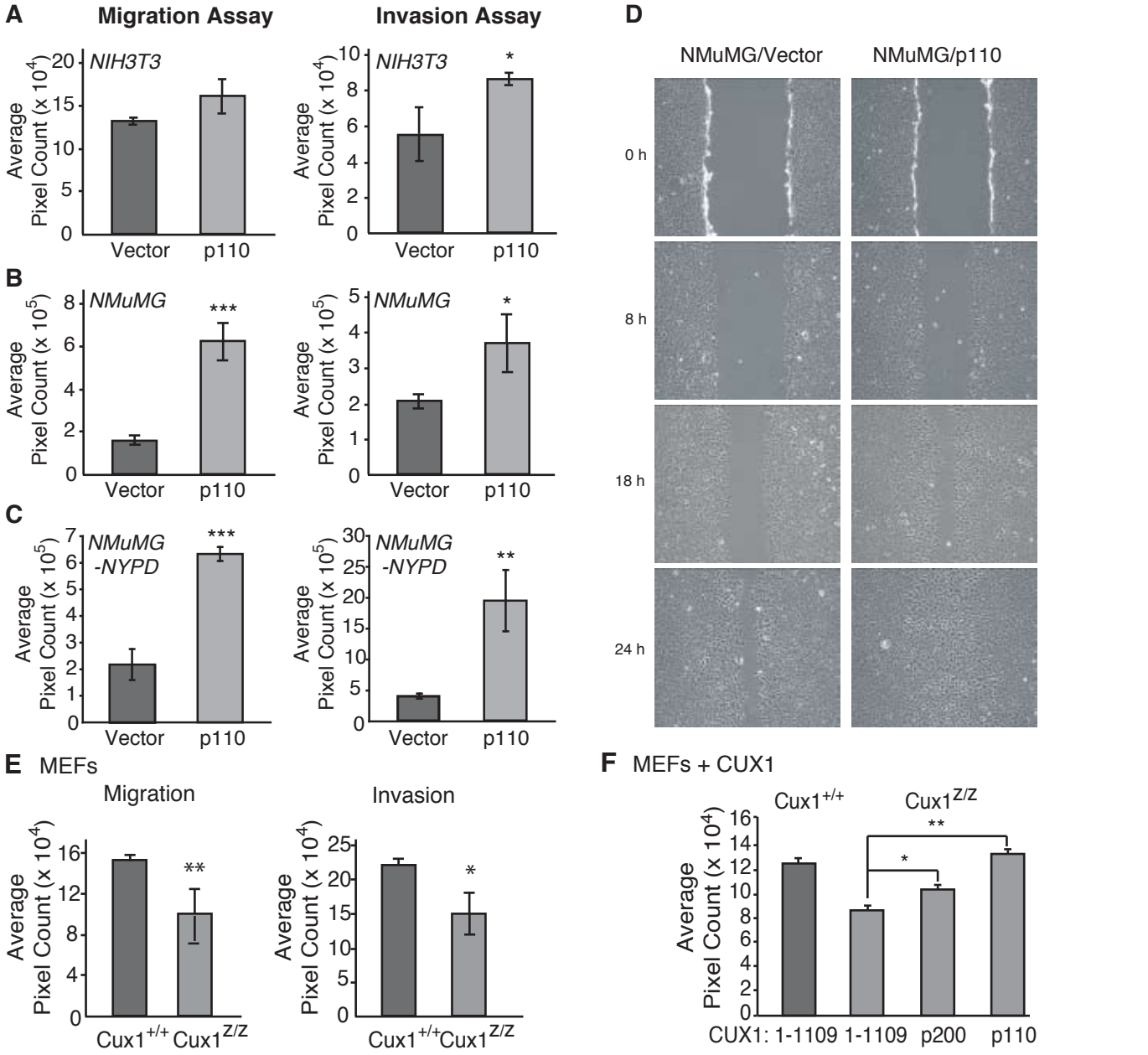
Supplemental Figure 1



Supplemental Figure 1

p110 CUX1 stimulates migration and invasion in various cell lines

(A), (B) and (C) Populations of cells stably carrying a retroviral vector, either empty or expressing p110 CUX1, were submitted to two-chamber migration and invasion assays. **(A)** Murine fibroblastic NIH3T3 cells. **(B)** Murine mammary epithelial NMuMG cells. **(C)** NMuMG-NYPD cells that express the NYPD erbB2 mutant. Each experiment was done in triplicate, and the graphs represent an average of the 3 wells.

(D) Wound healing assay was performed using NMuMG/Vector and NMuMG/p110 cell populations. Scratches were done on highly confluent cells and closure was monitored by taking pictures at different time points. Representative results from at least three independent experiments are presented. *, 0.05> pValue >0.01; **, 0.01> pValue> 0.001; *** , pValue < 0.001.

(E) Wild type (Cux1+/+) and CUX1 deficient (Cux1Z/Z) MEFs were submitted to two-chamber assays either without or with a matrigel coating to evaluate their migration and invasion ability, respectively. 80 000 cells were plated at the top of the chamber in medium without serum, and 10% FBS medium was placed at the bottom of the chamber. After 16 h incubation at 37 °C, cells were fixed in 10% formalin and stained with 0.1% crystal violet. Top cells were removed and the average pixel count was measured to evaluate the number of migrating cells.

(F) CUX1Z/Z MEFs were infected with adenoviral vectors expressing p200 CUX1, p110 CUX1 or, as a control, a recombinant CUX1 protein that does not localize to the nucleus, 1-1109. In parallel, CUX1+/+ MEFs were infected with the CUX1(1-1109) adenoviral vector. A two-chamber migration assay with a matrigel coating was performed as described in (D).

Supplementary Table 2: Putative targets of p110 CUX1 that are involved in cell motility. A list of putative targets of CUX1 was established from genome-wide location arrays performed in cell lines of various cell types (Supplemental data, Table 1). Genes were classified into functional categories using programs from DAVID (http://niaid.abcc.ncifcrf.gov/content.jsp?file=functional_annotation.html#intro) (left column). Genes with a function related to migration and invasion are shown. Their full name and symbol are indicated. P-values from the location arrays are indicated in the right column.

Function	SYMBOL	Gene Name	p Value
GTPases Related Proteins	ARHGEF7	Rho guanine nucleotide exchange factor (GEF) 7	9,7E-5
	ARHGAP6	Rho GTPase activating protein 6	2,0E-3
	ARHGAP11A	similar to human GTPase-activating protein	6,6E-4
	ARHGAP21	Rho GTPase activating protein 21	4,5E-3
	ARHGAP18	Rho GTPase activating protein 18	1,9E-3
	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	4,7E-12
	ARHGEF2	rho/rac guanine nucleotide exchange factor (GEF) 2	1,5E-3
	ARHGEF10	Rho guanine nucleotide exchange factor (GEF) 10	1,1E-3
	ACK1	activated Cdc42-associated kinase 1	1,5E-3
	CDC42	cell division cycle 42 (GTP binding protein, 25kDa)	6,7E-4
	GRAF	GTPase regulator associated with focal adhesion kinase pp125(FAK)	1,4E-5
	MARCKS	myristoylated alanine-rich protein kinase C substrate	1,2E-4
	RAB36	RAB36, member RAS oncogene family	5,5E-5
	RAIN	Ras-interacting protein	1,4E-3
	RAP1A	RAP1A, member of RAS oncogene family	8,7E-5
	RHOBTB3	Rho-related BTB domain containing 3	4,0E-3
	RAC2	ras-related C3 botulinum toxin substrate 2	7,4E-4
	RACGAP1	Rac GTPase activating protein 1	8,4E-4
	RICS	Rho GTPase-activating protein	5,9E-4
Cell Adhesion Proteins	CLDN12	claudin 12	4,0E-3
	CLDN17	claudin 17	1,7E-3
	CLDN4	claudin 4	1,3E-3
	CTNNA3	catenin (cadherin-associated protein), alpha 3	7,1E-6
	GJA10	gap junction protein, alpha 10, 59kDa	2,0E-3
	ITGBL1	integrin, beta-like 1 (with EGF-like repeat domains)	3,2E-4
	ITGB1BP2	integrin beta 1 binding protein (melusin) 2	6,0E-4
	ITGB1	integrin, beta 1	4,8E-3
	ITGB3BP	integrin beta 3 binding protein (beta3-endonexin)	3,6E-4
	ITGB2	integrin, beta 2 (antigen CD18 (p95)	7,7E-4
	ITGB7	integrin, beta 7	1,3E-3
	OCLN	occludin	2,9E-4
	PCDH10	protocadherin 10	4,5E-3
	PNN	pinin, desmosome associated protein	2,2E-6
	PTK2	PTK2 protein tyrosine kinase 2	1,2E-3
	TJP4	tight junction protein 4 (peripheral)	9,4E-4
	ALCAM	activated leukocyte cell adhesion molecule	2,4E-3
	TM4SF8	transmembrane 4 superfamily member 8	1,5E-3
EMT	CDH2	cadherin 2, type 1, N-cadherin (neuronal)	3,2E-3
	VIM	vimentin	7,1E-4
Matrix Metallo-proteases	MMP10	matrix metalloproteinase 10 (stromelysin 2)	3,6E-3
	MMP11	matrix metalloproteinase 11 (stromelysin 3)	1,1E-4
	MMP12	matrix metalloproteinase 12 (macrophage elastase)	2,2E-3
	MMP23B	matrix metalloproteinase 23B	2,0E-3

Cytosketal Related Proteins	DSC2	desmocollin 2	1,6E-3
	ELMO1	engulfment and cell motility 1 (ced-12 homolog, C. elegans)	4,7E-3
	EMP1	epithelial membrane protein 1	4,8E-6
	ENG	endoglin (Osler-Rendu-Weber syndrome 1)	5,0E-4
	EPLIN	epithelial protein lost in neoplasm beta	9,7E-4
	EPN1	epsin 1	2,2E-3
	FLNA	filamin A, alpha (actin binding protein 280)	9,6E-4
	GSN	gelsolin (amyloidosis, Finnish type)	2,2E-3
	PACE-1	ezrin-binding partner PACE-1	1,6E-3
	PXN	paxillin	1,2E-3
	SVIL	supervillin	1,9E-4
	THBS1	thrombospondin 1	3,8E-3
	PKP2	plakophilin 2	3,5E-7
	CFL1	cofilin 1 (non-muscle)	1,0E-8
Other Functions	CD2AP	CD2-associated protein	1,5E-3
	CD151	CD151 antigen	4,0E-3
	CTGF	connective tissue growth factor	6,9E-4
	GAS8	growth arrest-specific 8	7,7E-4
	DACT1	dapper homolog 1, antagonist of beta-catenin (xenopus)	4,2E-3
	LIMS1	LIM and senescent cell antigen-like domains 1	1,2E-3
	PAK2	p21 (CDKN1A)-activated kinase 2	9,1E-5
	WISP2	WNT1 inducible signaling pathway protein 2	2,6E-4
	WASPIP	Wiskott-Aldrich syndrome protein interacting protein	2,5E-3

Supplemental Table 3: Primer sequences in the coding sequences of genes

Gene	length(bp)	primer 1	primer 2
ARHGDIB	372	TTCCCAATGTGACTGTTACCCG	TGAGGTGGTCCTGTTTGTCGTC
Rab36	379	ACAGCCTTTGACCTCACTGACG	GCCTCTTGTTCTCCTGGGTTTTG
RAIN	290	ATTGGAGACCGCCAGCCAGAGAAC	TTGGTGAGGTAGTAGACCGACTGC
RHOBTB3	331	GCACGATGTGAAGTGATGGCTG	TGGAAAGGCAGTCTGAGTGGTG
RICS	382	CGCACGCACAGAACTAATCGG	CAGCCATCTTGCTCTCCATTGTC
ITGB2	184	ATCCAGGAGCAGTCGTTTGTCA	CACAGTTTTTCCCAATGTAGCCAG
PNN	420	GAATCTACTGTTGCTACTGAACGGC	CACCACCTGATGCTCTTTTTCC
PCDH10	303	CGCAAGAAGAAACTCAGCAAGTC	CCTTACCTCGTTGGACAAAATGC
CDH2	375	GACAATCCTCCAGAGTTTACTGCC	AGGATTGGGGGCAAAATAAGG
CDH1	266	GGAGAACGAGGAACCCTTTGAG	GCGTGAAAATGGCACCAGTC
OCLN	126	GCCTCTTGAAAGTCCACCTCCTTAC	AAAGAGTAGGCTGGCTGAGAGAGAGC
VIM	351	AAGAACACCCGCACCAACGAGAAG	CGCATTGTCAACATCCTGTCTG
EPLIN	326	GGAGCAAAAGGAGAATGTGCC	TGTGAAACACCTGCTGGTTGG
FLNA	259	GGTCCTCGTCTTGTAAGCAACCAC	TCTCTTCACAGGGTGTCCTTGG
MARCKS	240	GTCGCCTTCCAAAGCAAATGGG	GCCTCCGCAGCCTCCTTGTC
PTK2	116	GCACCATCCCTAACCATTGC	GCCCGTTCACCTTCTTTCTG
THBS1	281	ACACAGACAAAAACGGGGAGG	GGCATTAGGCACATAGGGACAG
WISP2	334	AGGAGAATACAGGTGCCAGGAAG	TATCCACATCCCCGCAATGG
SNAIL	137	CAACCGTGCTTTTGCTGACC	AGCCAGACTCTTGGTGCTTGTG
SLUG	237	CATTTCAACGCCTCCAAGAAGC	ACTTACACGCCCCAAGGATGAG

Supplemental Table 4: Primer sequences in gene promoters for ChIP experiments

Gene	length (bp)	primer 1	primer 2
ARHGDIB	176	AGCCCGTGTGACGTG	ACAAAGTAAAGCCGATGTGA
Rab36	207	AGAACCTGCGCGAATCTCTCCACGGTCA	ACCGGCGATCACCATAGCAACGAGAACCTG
RAIN	147	TTCACTTCCCTTTCAGAACGACAG	TCAAGCCCACCATAAGCCAG
RHOBTB3	226	AGTTGGTCTGCGGAGCTA	AATTTGCTAATGTCGCCTTG
RICS	201	TGCCCAAATATAATCTAAAC	TACACTCTTGTACGGAATG
ITGB2	294	GGGCCTGAGACCGTCACCAAGACCCCTTC	GGCCCTCGGGAAACAGCACAGCTACAGCCT
PNN	251	CCGGAAGACAACGCTCATC	GTTCCCGTTAGGCCCTTACC
PCDH10	191	CTCACGGCTCGAAGCTAGGT	GCGAAGCATACGGAGTAGGG
CDH2	191	TGTGAGCGTCTGCGAGTGTG	AAACCACGAGCCCGAAACCT
CDH1	138	CCTGTGAGCTTGCGGAAGTC	GCAGCAGCGCCGAGAG
OCLN	233	GCTGCCATCATCTGAAATACCTC	TAACTATCTGCATTGAAAGCGAACT
VIM	278	CGGTGCAATCGTGATCTG	GAGGGCTCCTAGCGGTTTAG
EPLIN	103	AGCAGCACTGAGATTAGGCACAG	TGGGGTGGATTTGTTCTTCTTG
FLNA	197	ATAGTGTCTTGCTGTGTTGCCCAGGTTGGT	GGCAGCAGAAACAGGATCGCTCAGG
MARCKS	141	GCAAACACACAAATCGCAACC	TTGACTCGCATTGACTCTCGTCGG
PTK2	170	ACAGCATAATCTGAACGGAACCTT	TTTGTAGATCTGCCCTTTTGACATT
THBS1	163	GTATCCACCTCTCGCCATCA	TCGGCGCTCGTACTCTTG
WISP2	190	GCAAAGGGGTTGTTTACTGAATGC	TGTGTGTGTGTGAAAGCCTGAGC
CDH1-pGL3	156	CCTGTGAGCTTGCGGAAAGTC	AGCGGTCCATCTTCCAGCGGATAGA

Supplemental Table 5: Primer sequences for scanning ChAP on Snail and Slug promoters					
Gene	**Primers**	**distance from transcription start site**	**length(bp)**	**primer 1**	**primer 2**
Snail	**A**	-2256 to -2027	229	AATCAAGATGTGGGTGGGGG	GTCGGTGGGCATAGTCACTTTG
	B	-1863 to -1630	233	GAATGAAAGGAAGCCAGCGTG	CTGTCTCAGAGTAAAAGCCAAAGTCC
	C	-1431 to -1101	330	CGGCACCAAGTGACTAAACAGAC	AGGCTGGCGGGAGAAAGAAG
	D	-913 to -606	307	CAGTTGCCACTTCTTCCCTCG	CACCCGTTCCTTCCCTTATCC
Slug	**A**	-3257 to -3000	258	TGGGATTTTTCAGCCTATGCC	CTGGATGGGTGGGGTTTCTC
	B	-2682 to -2389	293	CCTTCCTTTTGTCTTACCCGTGC	GAAATCTTTGTGGAGGCAACTGC
	C	-1987 to -1686	301	ACCCTCGGATACCTGCTGATG	CACTCCATTTCCCACAAAACACAC
	D	-1578 to -1378	200	CCAGTTTGTGTGTGGAGAAATCG	ATGTGTGTTTGGAGGGTGAGGTGGGGCAC
	E	-789 to -478	311	TCCAAATGACAGTTACCTCTTGCC	GAAAAAGGAAGGGGGAAGCG