SUPPLEMENTAL INFORMATION

Wild type N-Ras, overexpressed in basal-like breast cancer, promotes tumor formation by inducing IL8 secretion via JAK2 activation

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Supplemental figures

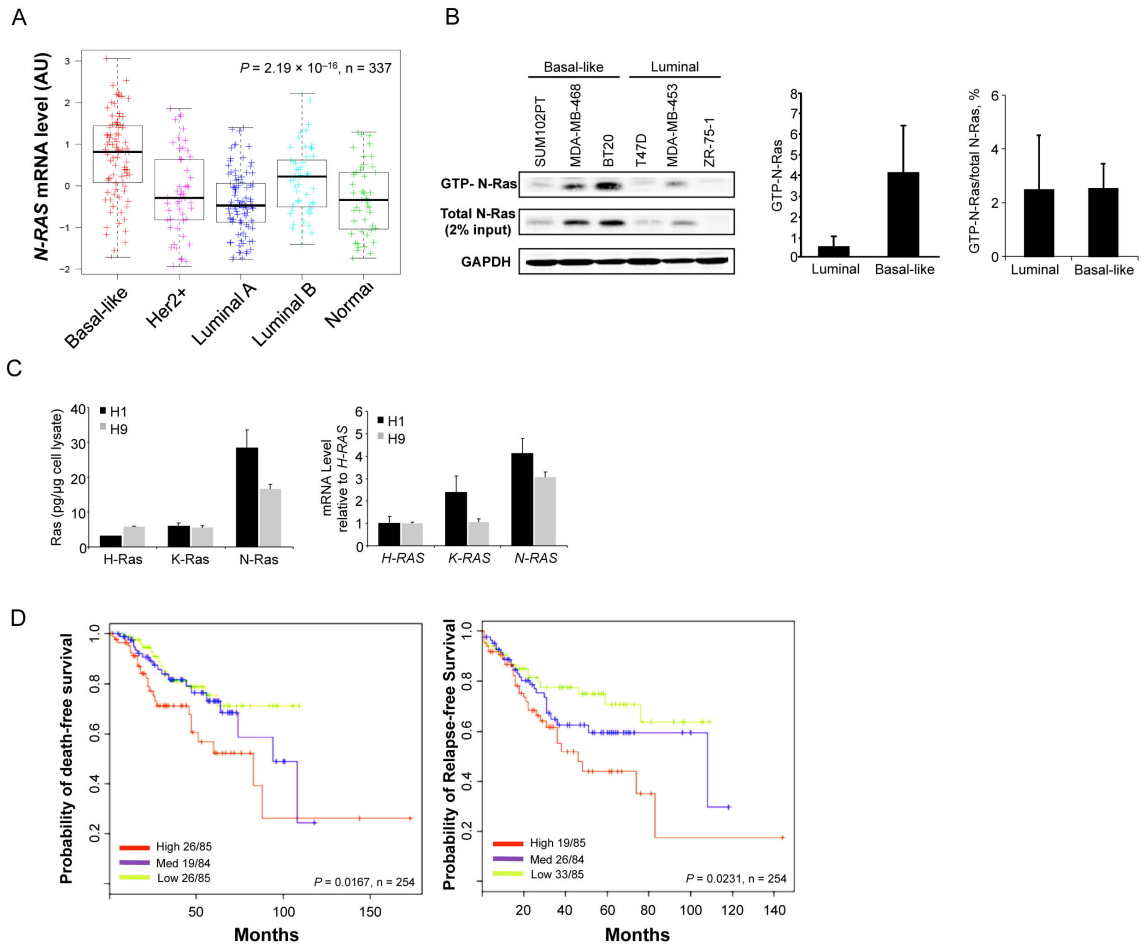

Figure S1, Related to Figure 1. (A) *N-RAS* mRNA levels in human tumors of different breast cancer subtypes were analyzed as in Figure 1A, except that a different database was used (Prat et al., 2010). AU, arbitrary unit. *P*-value by ANOVA. (B) The levels of GTP-bound N-Ras (as pull down by the Raf-bead), as well as 2% of total N-Ras, in the indicated cell lines were detected by Western blot (left). The absolute amounts of GTP-N-Ras (relative to those in luminal subtype cells) and GTP-N-Ras as percentages of total N-Ras were quantified on the right. Note the portions of GTP-N-Ras in the examined breast cancer cell lines are comparable to those seen in other cancer cell lines. Thus

BLBC cells have more GTP-N-Ras due to N-Ras overexpression, but not changes in how Ras activity is regulated. (C) Ras protein and mRNA levels in two human ES cell lines were analyzed as in Figure 1C. (D) The same database as in panel S1A was analyzed to decipher the impact of *N-RAS* expression levels on overall survival and relapse. n = 254 patients, *P*-value by long-rank test.

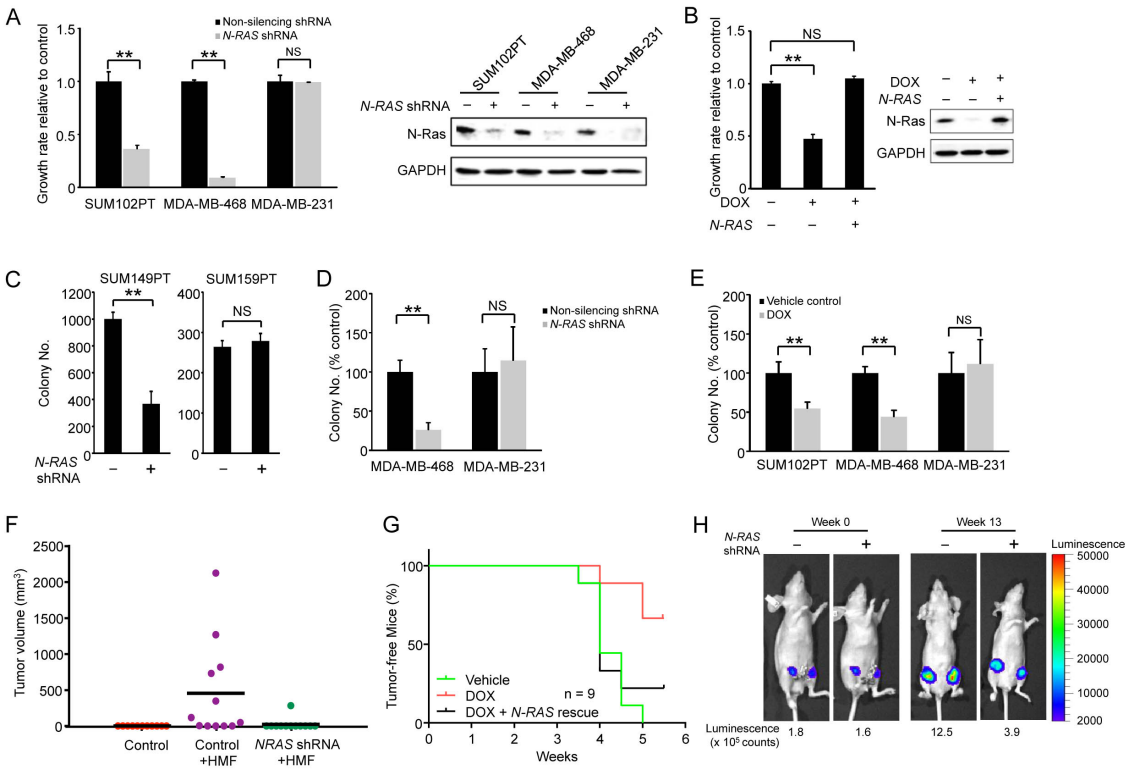

Figure S2, Related to Figure 2. (A) *N-RAS* was silenced by shRNA clone #2, confirmed by Western blot (right), and the resulting cells were seeded for growth rate measurement (left). (B) SUM102PT cells expressing inducible *N-RAS* shRNA (clone #3), with or without the rescue *N-RAS* cDNA, were treated with vehicle or DOX, and their growth rates were measured (left). The repression of N-Ras was confirmed by Western blot (right). (C) *N-RAS* was silenced as in Figure 2A and the resulting cells were seeded in soft agar for colony formation. (D) Cells were similarly examined as in panel S2D, except shRNA-2 was used. (E) Cells expressing the aforementioned inducible *N-RAS* shRNA were treated with vehicle or DOX and seeded in soft agar for colony formation. (F) Tumor volumes at week-10, as described in Figure 2F. (G) SUM102PT cells expressing inducible *N-RAS* shRNA with or without *N-RAS* rescue were co-injected with HMFs into nude mice (n = 9). The portions of tumor-free animals were plotted over time.

(H) A pair of mice in Figure 2G were imaged at Week-0 and Week-13 for the luciferase activity. The numbers below the picture show the total counts of luminescence, which were used to assess tumor size.

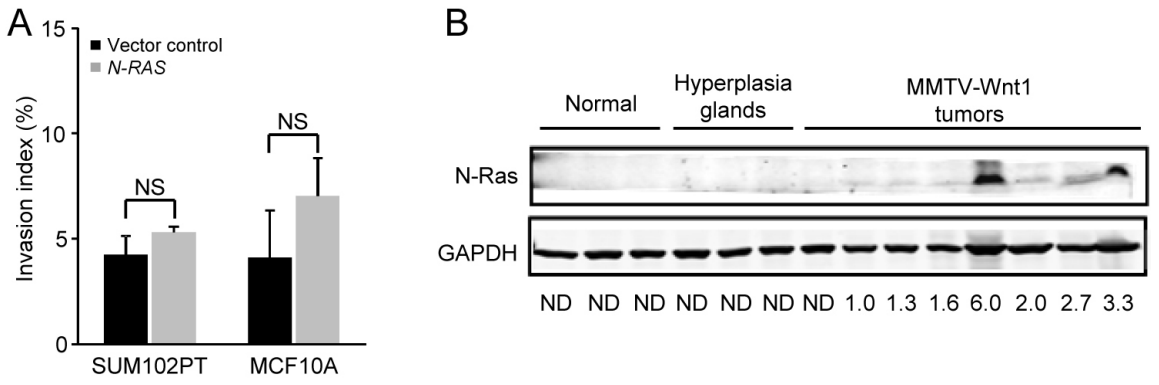

Figure S3, Related to Figure 3. (A) Control or N-Ras-overexpressed cells were measured for cell invasiveness. (B) Three normal and three hyperplasia glands, as well as 8 tumors from MMTV-Wnt1 mice were analyzed for N-Ras by Western blot (see Figure 1C). The numbers below show N-Ras levels relative to the one tumor sample with the lowest level of N-Ras. ND, not detectable.

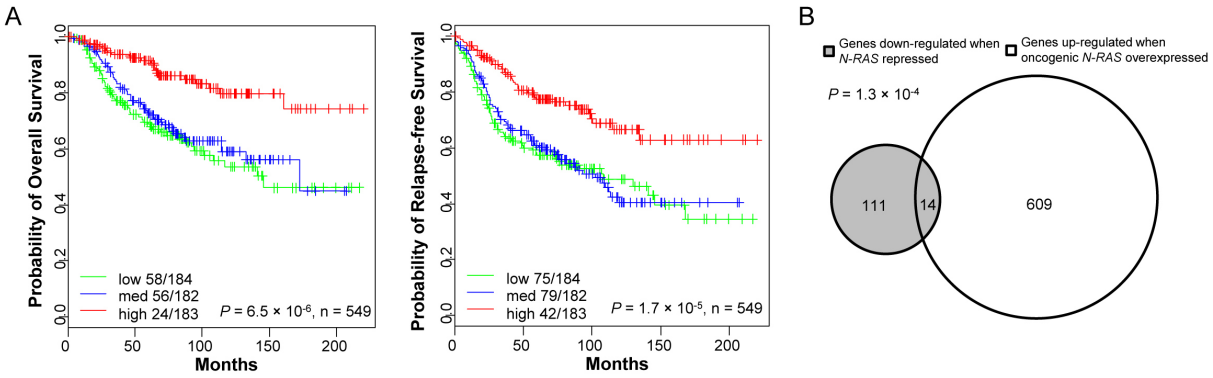

Figure S4, Related to Figure 4. (A) Principal Components Analysis (PCA) of N-Ras-controlled genes (Table S1) was used to compute a single composite score (first principal component). The results were shown here as Kaplan-Meier curves for survival in breast cancers (Prat et al., 2010) by tertiles of first principal component from this PCA analysis. We note that high scores do not necessarily imply high expression of genes, but the scores *ordinally* associate with outcome. (B) When we conducted the microarray analysis to detect gene expression changes after *N-RAS* repression, we also analyzed the same set of cells in parallel expressing an oncogenic form of N-Ras, N-Ras-(G12V). To assess whether N-Ras and N-Ras-(G12V) control the same set of genes, we compared those genes that are down-regulated upon *N-RAS* repression (Table S1) *vs.* those that are up-regulated by N-Ras-(G12V) (Table S3). By MSigDB analysis, while the N-Ras controlled genes are greatly enriched for those encoding cytokines and chemokines (Table S2), the genes up-regulated by N-Ras-(G12V) are not. Moreover, as depicted in the Venn diagram, only a very small set of genes from each set overlap, but most noticeably, IL8, is not among the overlapping genes. The *P* value of Venn diagram analysis was calculated by one-sided Fisher's exact test using software R.

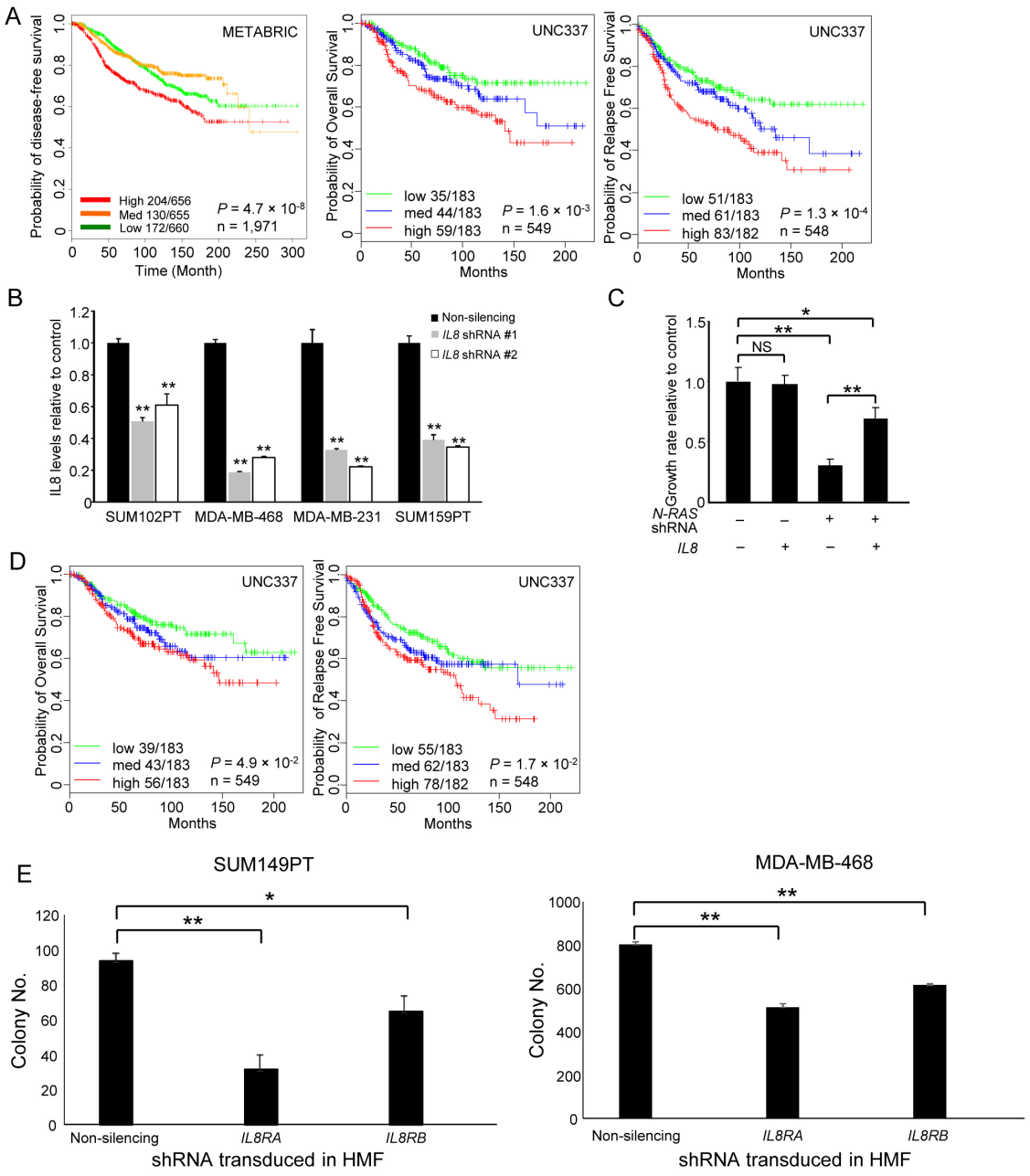

Figure S5, Related to Figure 5. (A) The indicated datasets were analyzed as in Figure 1E to decipher the impact of *IL8* expression levels on outcome. (B) IL8 secreted in various cell lines after silencing *IL8* expression was measured as in Figure 5B. (C) *N-RAS*

in SUM102PT cells was silenced as in Figure 2A, and the cells were also transfected to overexpress *IL8*, and seeded for growth rate measurement. (D) The UNC337 dataset was analyzed as in Figure 1E to determine the impact of *CCL3* expression levels on outcome. (E) Soft agar colony formation assay as performed in Figure 5I.

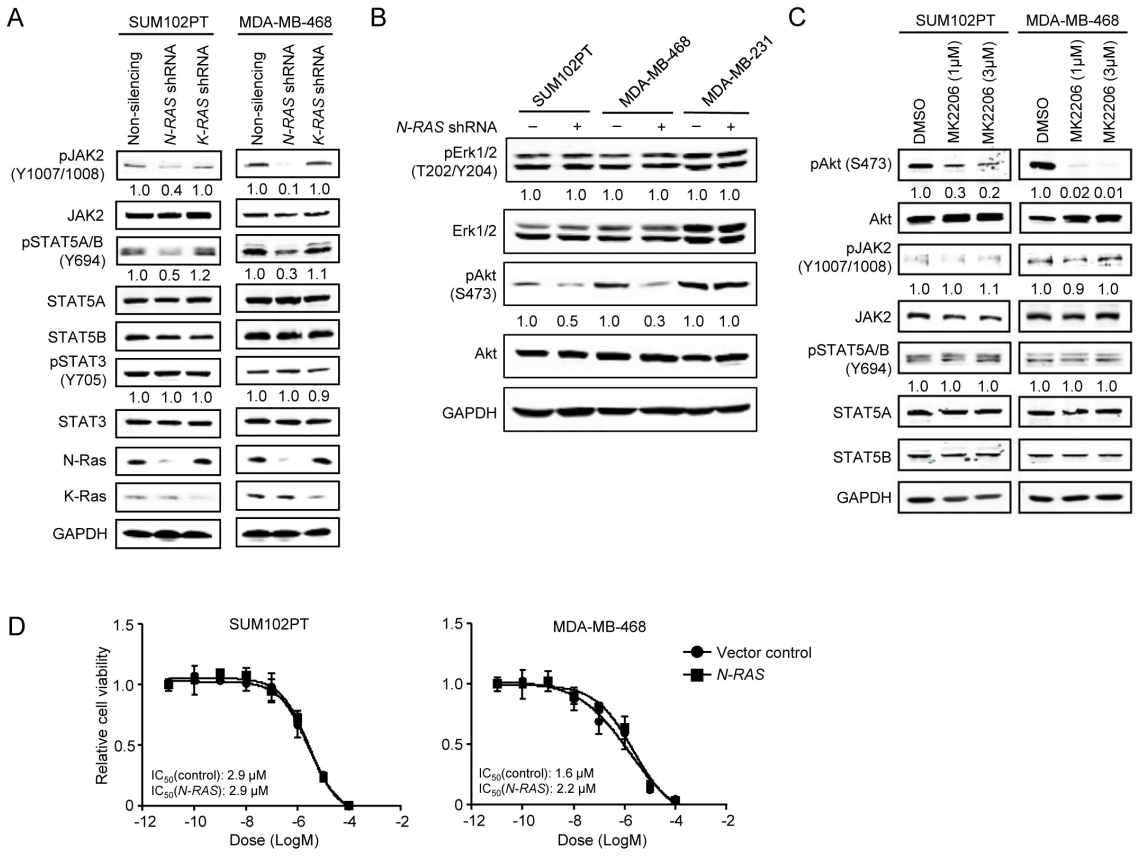

Figure S6, Related to Figure 6. (A) and (B) *N-RAS* was silenced as in Figure 2A, and the resulting cells were analyzed by Western blot for the indicated proteins. (C) Cells receiving MK2206 for 16 hours were analyzed for the indicated proteins by Western blot. (D) IC_{50} of MK2206 in the indicated cells.

Supplemental tables S1-S3 are submitted as separate Excel files

Table S1. Genes, up or down-regulated upon *N-RAS* repression, as identified by microarrays, Related to Figure 4 and Table 1

Table S2. Gene enrichment/MSigDB analysis of genes sensitive to *N-RAS* repression, Related to Figure 4

Table S3. Genes up-regulated by oncogenic N-Ras as identified by microarrays, Related to Figure S4 and Discussion

Supplemental experimental procedures

Cell culture

The cell lines and the culture methods used are described in a table at the end of this section.

Plasmid and plasmid construction

shRNAs against *N-RAS* (clone #1 and #2) and *K-RAS* in pLKO.1 were purchased from Sigma-Aldrich. shRNAs targeting IL8, IL8 receptors (IL8RA and IL8RB), and *N-RAS* (clone #3) in pGIPZ were obtained from Thermo Scientific. To build the inducible *N-RAS* shRNA, the #3 clone was released from pGIPZ by Xho I and Mlu I and cloned into the same sites in pINDUCER11 (Meerbrey et al., 2011). The targeting sequences in these shRNAs are shown in a table below. We note shRNA-1 was used in most of the gene silencing experiments unless otherwise indicated. Wild type *N-RAS* was amplified from the cDNA of human ES cells (H9) by PCR (see a table below for primer sequences). PCR was then performed to create the cDNA encoding oncogenic N-Ras-(G12V). The cDNA encoding palmitoylation-deficient N-Ras-(C181S) and dominant negative N-Ras-(S17N) were created by QuickChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies). The *N-RAS* cDNA that is refractory to shRNA-1 (*N-RAS**) or encode N-RasΔE, in which all amino acid residues in the effector-binding loop were mutated to alanine (Cheng et al., 2011), was constructed by mutating wild type *N-RAS* using the overlap extension PCR method. *K-RAS-4B* and *IL8* were amplified from pCGN-K-Ras-4B, pCGN-K-Ras-4B-(G12V) and pBMN-IL8-GFP (Schauer et al., 2009) by PCR. All PCR products were confirmed by sequencing, inserted into the pENTR shuttle vector

using the pENTR Directional TOPO Cloning system (Life Technologies), and finally transferred to destination vectors pLenti4, pINDUCER22 (Meerbrey et al., 2011), pBABE, or pBABE-nYn (Cheng et al., 2011) by the Gateway LR Clonase system (Life Technologies) to create pBABE-N-Ras, pBABE-N-Ras*, pBABE-N-Ras-(C181S), pINDUCER-N-Ras, pLenti4-N-Ras*, pBABE-nYn-N-Ras, pBABE-nYn-N-Ras-(G12V), pBABE-nYn-N-Ras-(S17N), pBABE-nYn-N-RasΔE, pLenti4-K-Ras-4B, pBABE-nYn-K-Ras-4B-(G12V), and pBABE-IL8. *JAK2-(V617F)* was created by QuikChange II XL Site-Directed Mutagenesis Kit using pDONR223-JAK2 (Johannessen et al., 2010) as the template. *JAK2* and *JAK2-(V617F)* were then transferred to pBABE-nYc and pBABE by the Gateway system to generate pBABE-nYc-JAK2 and pBABE-JAK2-(V617F). pWPT-FLuc-RFP, which expresses firefly luciferase and RFP separately, was constructed by inserting FLuc and RFP between the BamHI and SalI sites in pWPT (Addgene plasmid 12255, created by Didier Trono from École polytechnique fédérale de Lausanne, Switzerland). Plasmids expressing just Yc and Yc-RafRBD were constructed as described previously (Cheng et al., 2011).

Transfection, virus packaging, and viral infection

General transfection was performed by Lipofectamine 2000 (Life Technologies). In the BiFC assay, Lipofectamine 3000 (Life Technologies) was used due to lower toxicity. Viruses were produced in 293FT cells by cotransfecting an expression vector with the following packaging systems: (1) pAmpho for retroviral pBABE, (2) pMD2.G and psPAX2 for lentiviral pGIPZ and pWPT-FLuc-RFP, pINDUCER11, and pINDUCER22 (3) ViraPower Lentiviral Expression System (Life Technologies) for lentiviral pLKO.1 and pLenti4. Sixteen hours after transfection, the transfection medium was replaced with

fresh 293FT growth medium, and the supernatant containing the virus was harvested and filtered (0.45 μm, Nalgene) after another 48 hours. For transduction, the target cells were infected twice —16 hours after the first infection, they were infected again for another 24 hours. To enhance transduction efficiency, 4 μg/ml (retrovirus) or 6 μg/ml (lentivirus) polybrene (Sigma-Aldrich) was added to the media. The titer of pINDUCER22-N-Ras used for mouse work was determined by flow cytometry as described (Drayman and Oppenheim, 2011).

Measuring protein levels of each Ras protein in cell lines

To prepare Ras standards, we either purchased purified Ras protein, e.g., N-Ras from Santa Cruz Biotechnology, or purified our own. The cDNAs encoding *H-* or *K-RAS-4B* cDNA were cloned into pET-14b (Novagen) to express His-tagged Ras from *E. coli* (BL21) using Ni-HTA agarose beads (Qiagen). To determine the concentrations of purified Ras proteins, Ras proteins as well as the standard (bovine serum albumin) were separated by SDS-PAGE and stained by Coomassive blue. To detect Ras proteins in cell lines, cell lysates were prepared as described (Chiu et al., 2002). Purified Ras proteins were examined with the cell lysate simultaneously to determine the amount of a given Ras protein in the lysate. The cell lines used for Figure 1C were: BT20, HCC1187, HCC1569, HCC1806, HCC1937, HCC1954, HCC70, MDA-MB-468, SUM102PT, and SUM149PT for basal-like, CAMA1, HCC1419, HCC202, LY2, MDA-MB-134-VI, MDA-MB-175-VII, MDA-MB-361, MDA-MB-453, T47D, UACC-812, UACC-893, ZR-75-1, ZR-75-B, and ZR-75-30 for luminal, and BT549, HCC1395, HCC38, HS578T, MDA-MB-231, MDA-MB-436, and SUM159PT for claudin-low cells.

Protein measurement by ELISA and Western blots

Cell lysate preparation, and ELISA measurement of total and phosphorylated JAK2 levels were conducted following manufacturer's protocol (Life Technologies). IL8 levels in cell culture media were also measured by ELISA (PeproTech). For detecting all other proteins, cell or tumor lysates were prepared in RIPA buffer. Proteins were separated by SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad). The primary antibodies against N-Ras (F155, 1:500), K-Ras (F234, 1:500), H-Ras (F235, 1:500), IL8 receptor A/B (H-100, 1:500), STAT5A (L-20, 1:500), STAT5B (G-2, 1:500), STAT3 (F-2, 1:500) and GAPDH (6C5, 1:10,000) were from Santa Cruz Biotechnology. The antibodies for JAK2 (D2E12, 1:500), phospho-JAK2 (Tyr1007/1008, C80C3, 1:500), phospho-STAT5A/B (Tyr694, C11C5, 1:500), phospho-STAT3 (Tyr705, D3A7, 1:500), Erk1/2 (1:500), phospho-Erk1/2 (Thr202/Tyr204, 1:500), Akt (1:500), phospho-Akt (Ser473, 1:500) were from Cell Signaling. Antibodies against α-SMA (1A4, 1:1000) and GFP (1:1000) were from DAKO and Life Technologies, respectively. The fluorescein-conjugated secondary antibodies were from Li-COR Biosciences, and the protein levels were quantified by an Odyssey infrared imaging system (Li-COR Biosciences).

Measure GTP-Ras by the RAF-RBD pull-down assay

Cells were grown to 70-80% confluence, and the total and GTP-bound N-Ras in the lysates (800 μg) were measured by Active Ras Detection Kit (Cell Signaling) followed by Western blot using N-Ras specific antibody.

Cell growth and IC_{50} measurements

Cells were generally seeded into 96-well plates in triplicates (n = 3), except for Figure 5A (n = 4), and fed with fresh media (with or without drug) every two days. The number of

viable cells was measured by CellTiter 96 AQueous One Solution Cell Proliferation Assay kit (Promega) daily for 6 days. These numbers were normalized to the cell number on day-1 and plotted over time. The slope of the linear portion of the growth curve was defined as the growth rate. IL8 (72 amino acids, PeproTech), CCL3 (PeproTech), Repertaxin (raparixin-L-lysine, MedChem Express), and DOX (Sigma-Aldrich) were added at 2 ng/ml, 10 ng/ml, 200 μM, and 2 μg/ml respectively. To determine the IC_{50} of TG101348 and MK2206 (Selleck Chemicals), after seeding, the cells were treated with serially diluted drugs for 48 hours before the cell number was measured. The inhibition curves and the IC_{50} were generated by Prism 6 (GraphPad Software).

Soft agar colony formation assay

This was generally conducted as described previously (Cheng et al., 2011). Briefly, 1×10^4 cells were seeded into 6-well plates in triplicate (n = 3). For SUM102PT cells, twice as many cells were used. HMFs were typically mixed with cancer cells 1:1, except in Figure 3B in which only half as many HMFs were used. To treat the cells with the drug, 1 ml drug-containing medium was added, which was replaced twice weekly. The concentration used for repertaxin was 200 μM. For TG101348, 0.3 and 3 μM were used for SUM102PT and MDA-MB-468 cells respectively. The cells in soft agar were typically cultured for 21 days, unless otherwise indicated in the figure or the legends, before MTT staining.

Cell migration and invasion assays

For measuring migration, cells were first starved in serum-free media for 24 hours and then seeded to the cell culture inserts (Boyden chambers) with an 8 μm PET membrane

(BD Biosciences) in triplicate (n = 3). Growth medium containing 5% FBS was the chemoattractant. For measuring invasion, the same inserts were first coated with 1 mg/ml Matrigel (BD Biosciences) diluted in 0.01 M Tris-HCl (pH 8.0) and 0.7% NaCl. The similarly starved cells were then seeded in triplicate (n = 3) to the coated inserts, or to the uncoated inserts as control. HMF cells were seeded into the plate wells as the source of chemoattractants. In both assays, cells were incubated at 37 °C for 16 hours, and the inserts were then washed and stained with 0.5% crystal violet in 20% methanol. The stained colonies were quantified by Gelcount (Oxford Optronix), and the invasion index was defined as (Number of cells invading through Matrigel-coated insert/Number of cells migrating through uncoated insert) × 100%.

α-SMA induction in co-cultured HMFs

The cancer cells were loaded into the Millicell inserts (0.4 μm polycarbonate, Millipore), which were then placed into the 6-well plates preloaded with HMFs. These cells were co-cultured in cancer cells' growth media, which were replaced every 2 days for a week. HMF cell lysates were made to be analyzed by Western blot for α-SMA.

Gene expression measured by qPCR

RNA was isolated using the RNeasy kit (Qiagen), and cDNAs were synthesized from 5 μg of total RNA using the SuperScript III First-Strand Synthesis System (Life Technologies). Real-time PCR was performed with Power SYBR Green Master Mix on an ABI 7500 Fast Real-Time PCR System (Life Technologies). The PCR primers used are described in the table below. The relative amounts of PCR products generated from each primer set were determined on the basis of threshold cycle (Ct).

Identification of N-Ras responsive genes by microarrays

Cells were transduced with control or *N-RAS* shRNA and examined to confirm 70% of N-Ras knock down. The RNA was isolated from the cells by the RNeasy kit (Qiagen). All the microarray experiments were performed using UNC PerouLab 244K Custom Human Array (GEO:GPL19232, Agilent). After hybridization, the arrays were scanned by an Axon GenePix 4000B scanner, and the images were analyzed using Gene Pix Pro 5.0 software (Axon Instruments). Gene expression values were quantified by the base 2 log ratio of red channel (sample) intensity (mean) vs. green channel (universal human reference RNA) intensity (mean), followed by LOWESS normalization to remove the intensity dependent dye bias (Yang et al., 2002). We used the tool set available at UMD (UNC Microarray Database, https://genome.unc.edu/) to perform the filtering and preprocessing. If the intensity of one probe in one array was less than 10 in either channel, the results from that probe were treated as missing. If a probe had more than 30% of the expression values missing in all the arrays (due to low intensities or bad flags), the results from that probe were discarded. Data matrix was gene median centered and sample standardized, and missing data were imputed by the 10-nearest-neighbor imputation (Troyanskaya et al., 2001). The resulting primary data sets have been deposited to Gene Expression Omnibus (GEO, access number GSE61768).

Clinical database analysis

The METABRIC dataset was deposited in the European Genome-Phenome Archive under accession number EGAS00000000083 (Curtis et al., 2012), and its normalized data were obtained from "Cancer Research UK." The EMC-MSK dataset (Zhang et al., 2009) is a combined dataset of GSE2603, GSE5327, GSE2034, and GSE12276. The TCGA

data were from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/) (TCGA, 2012). The UNC337 dataset (Prat et al., 2010) and the dataset from Knudsen et al (Knudsen et al., 2012) were deposited in the GEO (GSE18229 and GSE33692, respectively). The breast cancer molecular subtypes in METABRIC, TCGA, and UNC337 datasets were determined by the PAM50 gene list and algorithm as described (Parker et al., 2009), while those in the EMC-MSK dataset were determined by Smid and colleagues (Smid et al., 2008). The subtypes in GSE33692 were determined as described (Knudsen et al., 2012). The expression level of a given gene in the analysis was generally defined by selecting the highest value of all the probes mapped to the same gene, unless otherwise indicated. The gene enrichment analysis was conducted at http://www.broadinstitute.org/gsea/msigdb/annotate.jsp using Molecular Signatures Database (MSigDB) v4.0 (Subramanian et al., 2005).

Cell lines and culture media used in this study

Cell Line	Culture Media[1]
BT20	MEM + 10% FBS + NEAA
HCC1187	RPMI + 10% FBS
HCC1569	RPMI + 10% FBS
HCC1806	RPMI + 10% FBS
HCC1937	RPMI + 10% FBS
HCC1954	RPMI + 10% FBS
HCC70	RPMI + 10% FBS
MDA-MB-468	DMEM + 10% FBS
SUM102PT	F12 + Serum Replacement[2] + In[3] + Hy + EGF
SUM149PT	F12 + 5% FBS + In + Hy
BT549	RPMI + 10% FBS + b-In
HCC1395	RPMI + 10% FBS
HCC38	RPMI + 10% FBS
HS578T	DMEM + 10% FBS + b-In
MDA-MB-231	DMEM + 10% FBS
MDA-MB-436	Leibovitz's L15 + 10% FBS
SUM159PT	F12 + 5% FBS + In + Hy
CAMA1	MEM + 10% FBS + NEAA
HCC1419	RPMI + 10% FBS
HCC202	RPMI + 10% FBS
LY2	DMEM + 10% FBS
MDA-MB-134-VI	DMEM + 10% FBS
MDA-MB-175-VII	DMEM + 10% FBS
MDA-MB-361	DMEM + 10% FBS
MDA-MB-453	DMEM + 10% FBS
T47D	RPMI + 10% FBS + b-In
UACC-812	DMEM + 10% FBS
UACC-893	DMEM + 10% FBS
ZR-75-1	RPMI + 10% FBS
ZR-75-B	DMEM + 10% FBS
ZR-75-30	RPMI + 10% FBS
MCF10A	DMEM/F12 + 5% HS + In + Hy + EGF + Ch
MCF12A	DMEM/F12 + 5% HS + In + Hy + EGF + Ch
hDCIS.01	DMEM/F12 + 5% HS + In + Hy + EGF + Ch
HEK293FT	DMEM + 10% FBS
HMF	DMEM + 10% FBS
HT1080	DMEM + 10% FBS
MEF	DMEM + 10% heat inactivated FBS + NEAA
H1, H9	MEF conditioned medium[4]

[1]Untransformed and breast cancer cells were cultured primarily according to ATCC or as described (Neve et al., 2006), but some changes were made to optimize cell growth. The

HMFs were obtained as described (Kuperwasser et al., 2004). Abbreviations: FBS, fetal bovine serum; HS, horse serum; NEAA, non-essential amino acids; In, human insulin; Hy, hydrocortisone; EGF, epidermal growth factor; b-In, bovine insulin; Ch, cholera toxin.

[2]Serum replacement: 1 g/L bovine serum albumin, 10 mM HEPES, 5mM ethanolamine, 5 μg/mL transferrin, 10 nM triiodo thyronine, 50 nM sodium selenite.

[3]Concentrations of supplements: In, 10 μg/ml for MCF10A, MCF12A and hDCIS.01, 5 μg/ml for other cell lines; Hy, 500 ng/ml for MCF10A, MCF12A and hDCIS.01, and 1 μg/ml for the rest; EGF, 20 ng/ml for MCF10A, MCF12A and hDCIS.01, and 10 ng/ml for the rest; Ch, 100 ng/ml; b-In, 10 ng/ml.

[4]These human ES cell lines were from WiCell Research Institute, and cultured in mouse embryonic fibroblast-conditioned medium as described previously (Xu et al., 2001).

DNA sequences used in this study

Gene		Sequence (5'-3')
shRNAs (antisense sequences):		
N-RAS-#1		CAAGAGTTACGGGATTCCATT
N-RAS-#2		CCATCAATAATAGCAAGTCAT
N-RAS-#3		TACAATATTTGAACATCTG
K-RAS		TAGTTGGAGCTGGTGGCGTAG
IL8-#1		CAGTGAAACTTCAAGCAAA
IL8-#2		CACAGTCAATATTAGTAAT
IL8RA		ACTGAGACACTCAACAAGT
IL8RB		TGTTAAATGATTTCATTCA
Cloning primers:		
N-RAS	Forward	CACCATGACTGAGTACAAACTG
	Reverse	TTACATCACCACACATGGCAA
N-RAS-(G12V)	Forward	CACCATGACTGAGTACAAACTGGTGGTGGTTGGAGCAGTTGGTGTT
	Reverse	TTACATCACCACACATGGCAA
*N-RAS**	Forward	GAAAGGTATTCCATAGCTTTTCGCCAGTTCGTGGGCTTGTTT
	Reverse	GCGAAAAGCTATGGAATACCTTTCATTGAAACCTCAGCCAAGAC
N-RASΔE	Forward	GCAGCAGCCGCTGCGGCGGCAGCAGCTTCATCTACAAAGTGGTTCT
	Reverse	GCTGCTGCCGCCGCAGCGGCTGCTGCCAGAAAACAAGTGGTTATAG
N-RAS-(C181S)	Forward	GGGACTCAGGGTTCTATGGGATTGCCATGTG
	Reverse	CACATGGCAATCCCATAGAACCCTGAGTCCC
K-RAS	Forward	CACCATGACTGAATATAAACTTGTG
	Reverse	TTACATAATTACACACTTTGTCTTTGA
IL-8	Forward	CACCATGACTTCCAAGCTGGCCG
	Reverse	TTATGAATTATCAGCCCTCTTCA
JAK2-(V617F)	Forward	GGTTTTAAATTATGGAGTATGTTTCTGTGGAGACGAGAATATTCTGG
	Reverse	CCAGAATATTCTCGTCTCCACAGAAACATACTCCATAATTTAAAACC
qPCR primers:		
CCL3	Forward	TGCAACCAGTTCTCTGCATC
	Reverse	TTTCTGGACCCACTCCTCAC
CCL4	Forward	CTTCCTCGCAACTTTGTGGT
	Reverse	CCAGGATTCACTGGGATCAG
CSF2	Forward	CAGCCACTACAAGCAGCACT
	Reverse	AAGGGGATGACAAGCAGAAA
GAPDH	Forward	ACCCACTCCTCCACCTTTG
	Reverse	CTCTTGTGCTCTTGCTGGG
HDAC9	Forward	CCAGCCACCCTCATGTTACT
	Reverse	AAGCTTTTGCTGTCGCATTT
H-RAS	Forward	GACGGAATATAAGCTGGTGG
	Reverse	AGGCACGTCTCCCCATCAAT
IL24	Forward	GCCTCTCAAATGCAGATGGT

	Reverse	GTCTTTCACAGCCCAGAAGG
IL8	Forward	GTGCAGTTTTGCCAAGGAGT
	Reverse	CTCTGCACCCAGTTTTCCTT
K-RAS	Forward	GTAGTTGGAGCTGGTGGCG
	Reverse	GGTCCCTCATTGCACTGTACTCC
N-RAS	Forward	TGGTGGTTGGAGCAGGTG
	Reverse	GCCTTCGCCTGTCCTCATGTA
Human *N-RAS*	Forward	TTATAGATGGTGAAACCTGTTTGTT
	Reverse	TAGGTACATCATCCGAGTCTTTTACTC

Supplemental references

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