Supplemental Figure Legends

Figure S1. RIN1 expression in mammary cell lines. **A.** Immunohistochemical staining for RIN1 in cell lines. Paraffin-embedded MCF10A and KPL-1 cells were sectioned and stained with anti-RIN1 using the same technique applied to tissue samples (Figs. 1B & 1C). **B.** Expression of RIN1 in normal mammary epithelial cells. RIN1 was immunoprecipitated from HMLE (immortalized primary human mammary epithelial cells) and from MCF10A cells, then analyzed by immunoblot with anti-RIN1. Three percent of each cell extract used in the immunoprecipitations was immunoblotted directly with anti-β-tubulin (TUBB) for normalization.

Figure S2. Alignment of RIN1 promoter sequences from human (*H. sapiens*), monkey (*M. mulatta*), cow (*B. taurus*), dog (*C. familiarus*), rat (*R. novegicus*) and mouse (*M. musculus*). Nucleotide -809 is the first nucleotide following the transcription stop of the upstream gene *BRMS1*. Nucleotide +1 is the transcription start of RIN1 and nucleotide +128 is the translation start of RIN1. Nucleotide sequences were obtained from ENSEMBL (www.ensembl.org) and SNAI1 sites were identified using CONSITE (mordor.cgb.ki.se/cgi-bin/CONSITE/consite). Human SNAI1 sites are underlined. Conserved SNAI1 sites (no more than one nucleotide variation in one species) are in bold.

Figure S3. Ectopic expression of RIN1 in breast tumor cells. **A**. Comparison of endogenous and ectopic RIN1 expression in KPL-1 (left) and MDA-MB-231 cells (right). MCF10A cells show normal mammary epithelial cell expression of RIN1 (third lane on left, second lane on right), and a β-tubulin (TUBB) control blot is shown below. **B.** RIN1 expression in tumors derived from transduced KPL-1 cells. RIN1 immunohistochemical staining of tumors obtained from KPL-1 blast transduced cells or KPL-1 RIN1 transduced cells injected into nude mouse mammary fat pads (Fig. 5).

Figure S4. A. Expression of wild type RIN1 and RIN1^{QM} constructs in transduced MDA-MB-231 cells. **B.** Silencing of endogenous RIN1 expression in MDA-MB-231 cells by shRNA. Note that the amount of extract and exposure used for vector control MDA-MB-231 cells was increased compared with "A" to highlight the silencing of RIN1. **C.** Analysis of RIN1^{QM}, a signaling defective mutant of RIN1. RIN1^{QM} mutant is defective for ABL2 (Arg) activation. HEK293T cells were transfected with Flag-ABL2, RIN1 and RIN1^{QM} constructs as indicated. Whole cell lysates (50 µg) were immunoblotted with anti-pTyr (left). Endogenous CRKL, a known ABL substrate, was immumo-precipitated with anti-CRKL and immunoblotted with anti-p-Tyr (right, top). Whole cell lysates were immunoblotted with anti-CRKL, anti-Flag (ABL2) and anti-RIN1 to validate expression.

Fig. S1
Milstein et al.

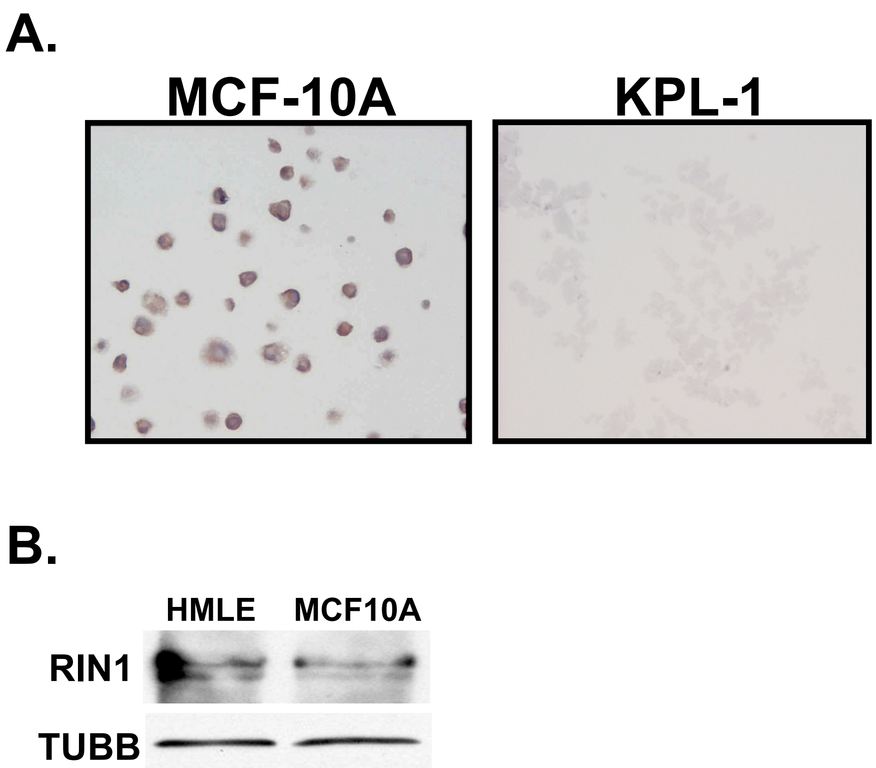


Fig. S2

Alignment of mammalian RIN1 promoters

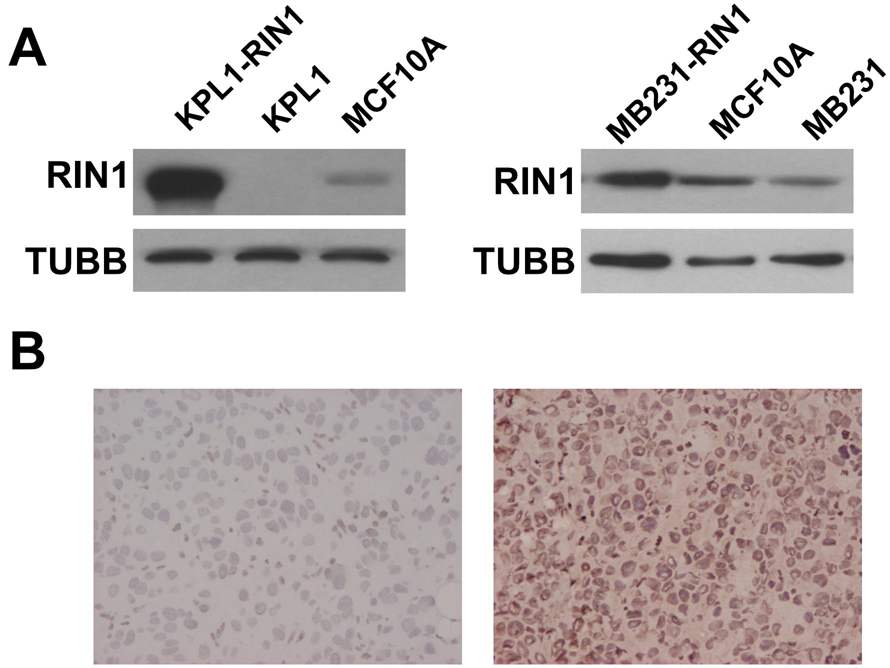
```
               -809
H. sapiens     CTCTGGCGTGCCCTGTGTGTGTGTGTGATTTACCTTGCAAGAGAATCAGGGCTCCCGTGGGGA
M. mulatta     TTCTGGTGTGCCCCGTGTATGTGTG--ATTTACCTTGCAAGAGAATCAGGGCTTCTGTGGGGA
B. taurus      CTCTGGCATGACCCGCCTGTGTCTA--ATTTACCGTGCAAGGGAATCAGGACTCTGGTGGAAA
C. familiaris  CCCTAGCATGACCTGTGTGTGTCTG--ATTTACTTTGCGAGGGAATCAGGGCTCCCCTGGGGA
R. novegicus   CCTGGATATGACTGCTGTGTGGTTG--TTGTATCTTACAAGGGAATCAAGGCTTTTGTGGGGA
M. musculus    CCTTGGTGTGGCTGATGTGTGGCTG--CTGTATCTTAAAAGGGAATCAAGGCTTT-GTGGG-A
                       ** *     * **  *    * **   *   ** ****** * **    ***  *

Hs    GCACCTGAACCAGGCCGACTTCTAGGGTGCCTGCAG---ATTGCCACTCCTCACACTACCCAGCAGAGGCTAGAGCACTG
Mmul  GCACCTGAACCAGGCTGACTTCTGAGGTGCCTGCAG---ATTGCCACTCCTTGCACTGCCCAGCAGAGGCTGGAGCACTG
Bt    GTGCTTGAGACAGGCAGTCTCTTCTGGGGGCCCTGG-------TATCTCCTCCAG-----TGAGTGGAGCCTACCCCTTA
Cf    GTGCCTGAGTCAGGTGGGCTCTCCTGGAGTCCCTGG-------TACCTCCTCTCTCTACCTGGCAGGAGCCCACCTACTC
Rn    GCACCTAAGACA----GACTT--CTGGGGTCCCTA--------CAGTTTGAGACA----------------------TTA
Mmus  GCACCTAAGACA----GACTTTGCTGGGGTCCCTG--------TAGTTTGTGAC--------------------------
      *  * * *  **    * **     ** * *                *

Hs    TG-CGTGGGGC-CAGGCTGGG---GAGGG----------------------GGAGGTGGGCGCTGGGCTGC-------CT
Mmul  TG-CGTGGGGC-CACACTGGG---GAGGG----------------------GGAGGTGGGCGCTGGGCTGC-------CT
Bt    TGACTTGGGGTATAGGCTGGGGAAGGAGGCATTCCCCCACACATCTGCTCCAGATGTGGGGGGCTGGG--------TGCT
Cf    TGAGTTGGGGTATGGG--GGGGGC-------CCTCCCAGCACACCT--CCCAGAGGTGGGGGA-----------------
Rn    GTTCTTAGAGAAAAGCCTGAGGAAGGAAGT--------------TGGGACCAGGGCAGG---------------------
Mmus  ----TTAGAGAAAAGCCTGGGAGAGGAAGT--------------TGGGACCAGAGCAGGAAGCGCCGC------------
           * * *        * *                               *    **

Hs    GGCGC------TGGGCAGAGTCAACATAT-GGCACCGGGAGAGTGCCTCGG-C-----------GGGGAGGGTGGATGCG
Mmul  GGCAC------TGGGCAGAG--AACATAT-GGCGCCAGCGGAGTGCCTCGGGT-----------GGGGAGGATGGGTGCG
Bt    GGTGA-GC--AAAGTCTGAGGCAACTT---GGGGCACAGGCCGAGCACGTGGC------------------CTGGGTGCT
Cf    -GTGA-GC--AAAGTCTGAAGCAACAT---GTGGCACAGGCCGATCATGTGTCG----GGGGTGGGGAGGGTTGTGTGCT
Rn    -----------CAAACAGTCGAAGCAC---GTGGCCTCGGGTGGGGAAGGGGC------------------TTCTGCAC-
Mmus  -ATGGCACAGGCAAGCCAGAGAAGCAC---ATGGCCTTGTGTGGTGAAGGGAC------------------TTTTGGAC-
                     *      * *         *       *       *                     *     *

Hs    -TGTGCTTGGAGCCTGGGTCATGTCTGCACCTGTCCCAGTTGCCTGGCAGATTGGCAAGGCAGGCTGGCAGA--GCCTGC
Mmul  -TGTGCTTGAAGCCTGGGTCATGTCTGCACCTGTCCCAGTTGCCTGGCAGATTGGCAAGGCAGGCTGGCAGA--GCCTGC
Bt    CCGTGCCTAGAGACTGGATCGTGTCTGCACCTGCCCCAGTCTCTTGGCAGATTGGCAAGGCAGGCTGT------------
Cf    CTGTGCTTGGAGCCTGGGTCATGTCTGCACCTGCCCCAGTCACTTGGCAGATTGGTAAGGCAGGCTGGCGGG--GCCTGG
Rn    --ATTTCTGGAGGTCAGGTCATGTCTGCACCTGCCCCAAACACTTGGCAAATTGGCAAGGCGGGCTAGCTAGCAGGGTCT
Mmus  --ATGCCTGGAGGCCAGGTCATGTCTGCACCTGCCCCAGACACTCGGCAGATTGGCAAGGCAGGCTAGCTAGCAGGGCCT
         *   *  **    * ** ************ ****       **** ***** ***** ****

Hs    GTGCGTGGGAGGCAGCCGGCAGCACCGCCATGTGCTGACGACCTCACAGCCAGACTCCCCC----------TGGTTA-GC
Mmul  ATGCGTAGGAGGCAGCCGGCAGCAGCACCATGTGCTAACGACCTCACAGCCAGACTCCCCC----------TGGTTA-GT
Bt    GTGCGTGGGAGGCAGCCAGCAGCAGCGCCGTGTGCCGATGACCTCACAGCCAGACTCCCCC---------CTGTTTA-GT
Cf    GAGTGTAGGAGGCAGCTGGCAGCAGCACCATGTGCTGACGACCTCACAACCAAACTCCCCCACCCCCATCCCATTTA-GC
Rn    GTGTGTGGGAGACAGCTAGCAGCAGCACCATGTGCTGTCGACCTCACAGCCACACGCCCCC--------TCTGCTTA-GC
Mmus  GTGTGTGGGAGGCAGCTAGCAGCAGCACCAAGTGCTGTCGACCTCACAGCCACACTCCCCC--------TCTGGTTA-AC
        * ** **** ****  ****** * **  **** *  ********* *** ** *****             ***

Hs    ACTGAGTGTCAGAACCACCCCCAGAACCTCAGTTAAGCAAATAGGTTGGTTTCCATGGAGACATGGCATATTTTCTCCAT
Mmul  GCTGAGTGTCAGAACCACCCCCAGAACCTCAGTTAAGCAAATAGGTTGGTTTCCATGGAGACGTGGCATATTTTCTCCAT
Bt    GCTGAGTGTCAGAACCACCCCCAGAACCTCCACTAAGCAAAGGGGTTAGTTTCCATGGAGACACAGCATCCTTTCTCCAT
Cf    GCCAAGTGTTAGAACCACCCCCAGAACCTCAGTTAAGCAAAGGGGTTAGTTTCCAAGGAGACACAGCATCCTTTCTCCAT
Rn    AGTGAGTGTCAGAACCACCCCCAGAACCTCAGTTAAGCAAAGAGCTTAGTTTCCATGGAGACACAGCATCCTTTCTCCAT
Mmus  AGTGAGTGTCAGAACCACCCCCAGAACCTCAGTTAAGCAAAGAGCTTAGTTTCCATGGAGACACAGCATCCTTTCTCCAT
          ***** ********************   ********  * ** *** *** *******  ****  *********

Hs    GGCAACCTAAAATCCTAAAACCCAGGTT-----CAGGAATGAGTCATCTATGGACAGTAGAGGGTGCTCTCCCACAAGGC
Mmul  GGCAACCTAAAATCCTAAAACCCATGTT-----CAGGAATGAGTCATCTATGGACAGTAGAGGGTGCTCTCCCACAAGGC
Bt    GGCAACCTGAAATCCCCAAAACCACGTTAAGCTCAGGCATGAGTCATCTGTGGCCAGTAGGGGGCACTCACCCAAAAGGC
Cf    GGCAACCTGAAAATCCAAAACCCAAGTTAGGCTCGAGAATGAGTCATCTGTGACCAGTAGGGGGTGCCCTCCCACTGGGC
Rn    AGCAACCTGAAATCACAAAACCCAAGTTAGATTCAGGAATGAGTCATCTGCAACCAGTAGGGGGTGCTCTCCCACAAGGC
Mmus  AGCAACCTGAAATCACAAAACCCAAGTTAGGTTCAGGAATGAGTCATCTGCAACCAGTAGGGGGTGCTCTCCCACAAGGC
       ******* ***     *** *** ***     *  * ***********     ****** ***  * * ****   ***
```

Fig. S2

```
Hs    TGACCTGAGG---------------------CCAGGGG---CAGATGAGCAACTGACCACAGAGCC----AAAGGTGCCC
Mmul  TGACCTGAGG---------------------CCAGGGG---CAGATGAGCAACTGACCACAGACCC----AGAGGTGCCC
Bt    TGACCTTAGG--------------------GCCAGGGA---CAGATGAGCAGTTGACCACAGACTT----GAAGGTGCCC
Cf    TGACGGGGGCG-------------------GGGAGGGCACACAAATGAGCAGTTGACCACAAACTTAATTAAAGGTGCCC
Rn    TGACC-----------CCGCCTTC-------AAGGCTGGAACAGATGAGCCAGTGACCACAGACTT-CTT-AAGGTGACC
Mmus  TGACCTTCCCCCCCCCCCGCCCCCCCTTTGCAAGGCTGGAGCCGATGAGCCAGTGACCACAGACTT-CTT-AAGGTGACC
      ****                              *      *  ******   ******** **        ***** **

Hs    CCAGAGCATGGCTGGGGCCTGTGGGGACTGAGGCCG------CTTAACCACTTCCCGCCCACGTGTGCAATGTGAGTAG-
Mmul  CCAGAGTGTGGCTGGGGCCTGCGGGGACTGAGTCGG---CCGTTTAACCACTTCCCGCCCACCTGTGCAATTTGAGGAG-
Bt    CAAGGGCG-A-----GGCCCGTGGAGACCTTGGCGGGCACAGCTTAACCCCTTCCTGTCCACCTGTGCGTTTTGAGGAGT
Cf    CATGGGCA-AACTTGGGCCTGTGG-GACCCAGATGGGCCCAGCTTAACCCCTTCCCGCCTGCTCGTGTGCCTCGAGGAGG
Rn    CAGGGGTGCGGCTTGGGCCTGTGGGAACTT-GGTTGGCCGTGTTTAACCCCTCCCTATCCCACTGCATAGTGTGAGG---
Mmus  CAGGGGTGTGGCTTGGGTCTATGGGAACTT-GGCTGATCCAGCCTAACCCCTCCCTCCCCCACTGCCTAGTGTGAGG---
      *  * *         ** *   **  **   *   *        ***** ** **   *     *        ***

Hs    CTGGTGAGGCTGCAGGAGGCCGTGTGTGGAGGTGGCATCCCC-CAGCTGGGGCCCTCAGAGCAGGGCTAGCATGGAGGGG
Mmul  CTGGTGAGGCTGCAGGAGGCCCTGTGTGGAGGTGGCACCCCC-CAGCTGGGGCCCTCAGAGCAGGGCTAGCACGGAGGGG
Bt    CTGGCAAGGCTGCAGGAAGCGCCGTGTGGAGGTGGCAGCCCCACAGCTAGAACTCTGGGCAGGCCACCTGCATGGAATGG
Cf    CTGGCGAGGCTTCAGGATGCGCTGTGTGGAGGTGGCAGCCCCACAGCCGGGGCTCGTGGAGTGGCAC-TGCACGGAGAGG
Rn    ------AGGCTACAGGAAGTGCTGTGTGGAGGTGCTCCTCCCACAGCGGGGGCCCTGATA--GACTCTAGTGTGGAAG--
Mmus  ------AGGCTGCAGGAGGTGCTGTGTGGGGGTGGTCCTCCCTTAGCCGGGGC-CTGATGATGACACTGGCATGGAGGGC
            ***** ***** *    ****** ****     ***  ***  *  * *           *  *   ***

                                                            +1
Hs    ACCCCTGACCCCCAAAGCAGGGTCCAGA-ACAGAGGAGGCGGGCACTGGCCAGGCAGGGTGGACACACAAGAGTTAACTG
Mmul  ATCCCTGACCCCCGAAGCAGGGTCCAGA-ACAGAGGAGGCAGGCACTGGCCAGGCGGGGTGGACACACAAGAGTTAACAG
Bt    ATCCCTGCCCCCAGAGGCTGGGCCCAGA-GCAGAGGAAGCAGGCACTGCCCGGGCTGAGTGGACACAAAAGAGTTAACTG
Cf    ACCCTTGCTCCCTGAGGCTGGGCCTGAACACGGAGGAAGCGGGCACTGCCCGGGCTAAGGGGACACACAAGAGTTAACTG
Rn    -CCTCTGCCATTCAAGACTGGGCCAAGA-GCAAAAGAAGTGGACACTGGCTACATGGAGTGGACATACAAGAGTTAACTG
Mmus  TTCTCTGCCATTCAAGACTGT-CTAAGA-GTAGAAGAAGTGGACACTGGCTACATGGAGTGGACATACAAGAGTTAACTG
        *  **       *  * *       *     * ** *  * ***** *        * ***** * ************

Hs    GCGGG-TGTGACAGG-CGGAC------CGCCCTCAGGAAGTGTTACTCAC---TGGGGATGTGC-GTGC-CTTGCCTTGG
Mmul  GCGGG-TGTGACAGG-CGGAC------CGCCCTCAGGAAGTGTTACTCAC---TGGGGATGTGC-GTGC-CTTGCCTTGG
Bt    GCGGG-TGTGACCGGGCGAAC------CGCCCTCAGGAAGTGTTACTCACCACTGGGAATGTGA-GTGCTCTCGCCTTGG
Cf    GCGGGGCGTGACAGGGCGAAC------CGCCCTCAGGAAGTGTTACTCACTGCTGGGAATGTGA-GCGCTCTTGCCTTGG
Rn    GCGGGGTGTGACCGGGCGAAC------CGCCTTCAGGAAGTGTTACTCACCGCCAGGAATGTGC-ACGCCTGTGCCTTGG
Mmus  GCGGGGTGTGACAGGGCGAAC------CGC-TTCAGGAAGTGTTACTCACCGCCAGGAATGTGC-ACGCCTGTGCCTTGG
      *****  ***** ** ** **      * *  *****************      ** *****    **    *******

                                                             +128
Hs    GA-CTGGATTCTCT-------TCCTGAAGC-GAA--------------GGAGCTCCCAGCCATGGAAAGCCCTGGAGAGT
Mmul  GG-TTGGATTCTCT-------TCCTGAAGCCGAA--------------GGAGCTCCCAGCCATGGAAAGCCCTGGAGAGT
Bt    GGGCTGGCTTCTCT----GAGTCCCGGGAGAGACTGG----------AGGAGCTCCCAGCCATGGATACCCCCCGGGAGC
Cf    GGGCTGGATTCTCTTCCTGAGTCCTGGGAGAAACCGGGAGAAACCAAAGGAGCTCCCAGCCATGGAAACCCCTGGGGAGC
Rn    GGGTTGGATTCTCTTCCTGAGTCCCAGGAGAAGCTGA----------AGGAGCTCCCAGCCATGGAAGACCCTGGTGAGA
Mmus  GGGTTGGATTGTCTTCCTGAGTCCCAGGAGAAGCCGA----------AGGAGCTCCCAGCCATGGAAGACCCTGGTGAGA
      *   *** ** ***       ***                        *****************    ***  * ***
```

Fig. S3

Milstein et al.



KPL-1-blast tumor
IHC: anti-RIN1

KPL-1-RIN1 tumor
IHC: anti-RIN1

Fig. S4

Milstein et al.