Supplementary Figure Legends

Supplementary Figure S1. The BAC-Tg *RCAN1* gene is expressed in a pattern similar to though not identical to the endogenous mouse *Rcan1* gene.

Northern blotting showing the expression pattern of transgenic *RCAN1* mRNA in adult tissues from the middle copy number *RCAN1*$^{\text{BAC-Tg2}}$ mice. Duplicate blots were hybridized with human *RCAN1* and mouse *Rcan1* probes. Similar results for tissue-specific expression were obtained for the other two BAC-Tg lines (data not shown). EtBr, ethidium bromide–stained images of the northern gels showing 28S and 18S rRNA bands as a control for approximately equal loading of the lanes. Br: brain; Ht: heart; Lu :lung; Li: liver; Sp: spleen; Ki: kidney; Mu: muscle.

Supplementary Figure S2. Western blot analysis confirming epitope-tagged human RCAN1 protein expression in brain, heart and thymus tissues from the lowest copy number BAC-Tg line.

Total proteins were prepared from both transgenic and wild type tissues and blotted with HA antibody to recognize the tagged human RCAN1 protein. Both major isoforms RCAN1 are seen in brain, thymus and heart. The Coomasie-blue stain is shown as a control for equal loading.

Supplementary Figure S3. Luciferase reporter assays show a mild effect of LOC73419 on a CaN-responsive promoter.

Jurkat cell were transfected with 400 ng of pIL2-FF-luciferase plasmid and 200 ng pTK-RN-luciferase and increasing amounts of LOC73419 plasmid DNA. Twenty four hours later the

cells were stimulated with PMA (10 ng/ml) and ionomycin (1uM) for 8 hours followed by luciferase assays.

Supplementary Figure S4. Human RCAN1produced from the BAC-Tg enhances CaN activity in the RCAN1 protein complex *in vitro*.

Transgenic mouse brain lysate was first incubated with anti-CaN antibody, and then equal amounts of the lysate were incubated with protein A/G-agarose or anti-HA-agarose beads, respectively, to pull down total CaN or RCAN1-bound CaN. The beads were washed and applied to the CaN phosphatase assay. The CaN amount bound to the beads was determined by western blotting.

Supplementary Figure S5. RCAN1 enhances NFATc1 expression in BAC-Tg MEFs after thapsigargin treatment.

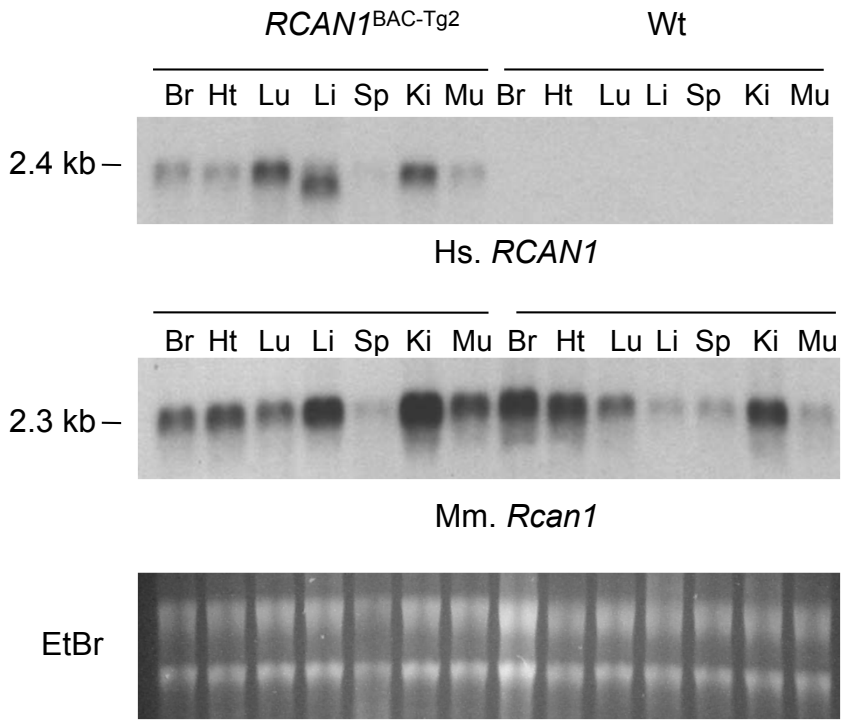
MEFs were treated with thapsigargin (100nM, 3 hours) to release Ca^{++} from intracellular stores. NFATc1 western blotting reveals increased expression of this protein in the BAC-Tg MEFs, providing further evidence that the tandem-tagged RCAN1 construct is biologically active. The anti-HA blots show that expression of the short isoform of the transgenic HA-tagged RCAN1 is induced by thapsigargin.

Supplementary Figure S6. Verification of deletion of the floxed Tp53 exon 7 in the mammary adenocarcinomas that form both in the presence and absence of the *RCAN1* BAC-Tg.

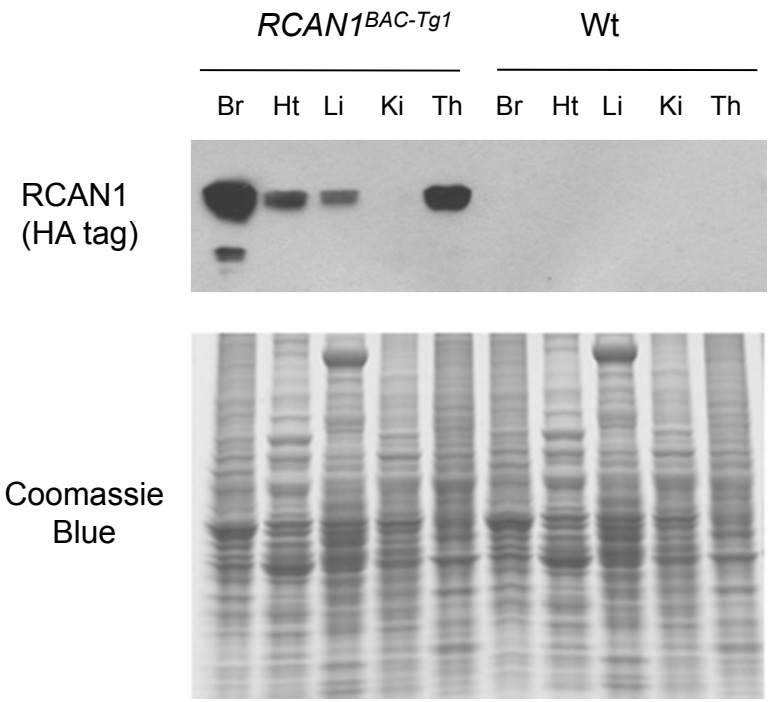
PCR of genomic DNA from the tumors, and from control mouse tail DNA, using primers flanking the *Tp53* CKO allele reveals the expected strong recombined band in all tumors, regardless of the presence or absence of the *RCAN1* Tg. There is a modest but reproducible

relative increase in intensity of the non-recombined *p53*^{flex7} band in the *p53*^{flex7}:*RCAN1*^{Tg} tumors, suggesting that these tumors may have slightly more stromal cells (*Tp53*-wild type) than the mammary tumors which formed in the absence of the *RCAN1* Tg.

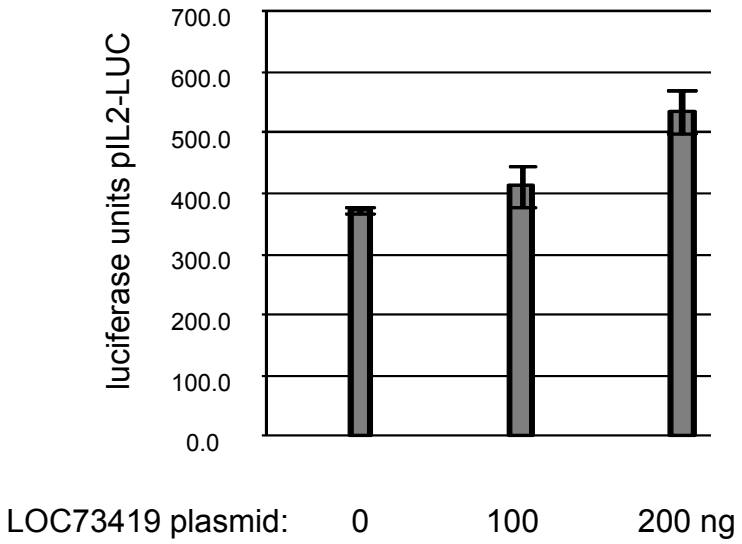
Suppl. Fig. S1


RCAN1BAC-Tg2
Wt
Br Ht Lu Li Sp Ki Mu Br Ht Lu Li Sp Ki Mu
2.4 kb
Hs. RCAN1
Br Ht Lu Li Sp Ki Mu Br Ht Lu Li Sp Ki Mu
2.3 kb
Mm. Rcan1
EtBr

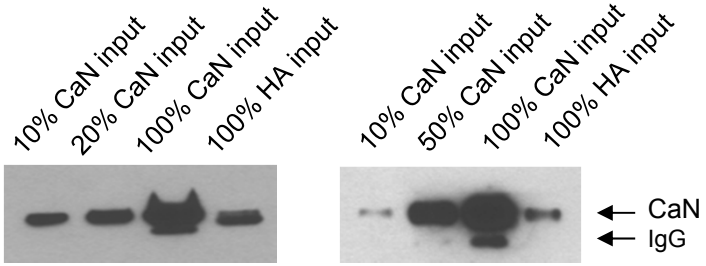
Suppl. Fig. S2

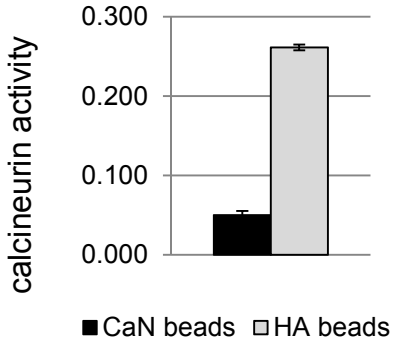

RCAN1BAC-Tg1
Wt
Br Ht Li Ki Th Br Ht Li Ki Th
RCAN1
(HA tag)
Coomassie
Blue

Suppl. Fig. S3


luciferase units pIL2-LUC
700.0
600.0
500.0
400.0
300.0
200.0
100.0
0.0
LOC73419 plasmid:
0
100
200 ng

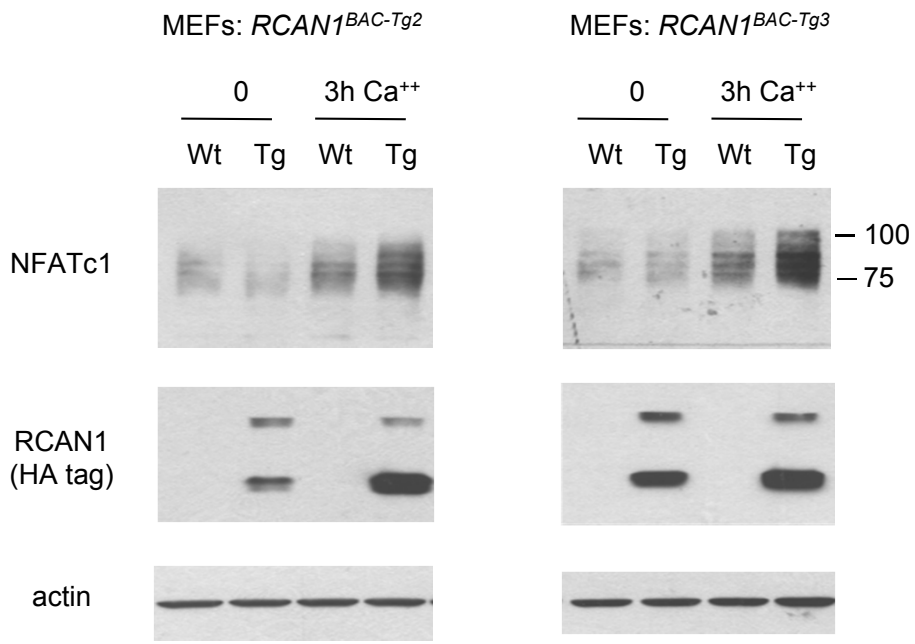
Suppl. Fig. S4



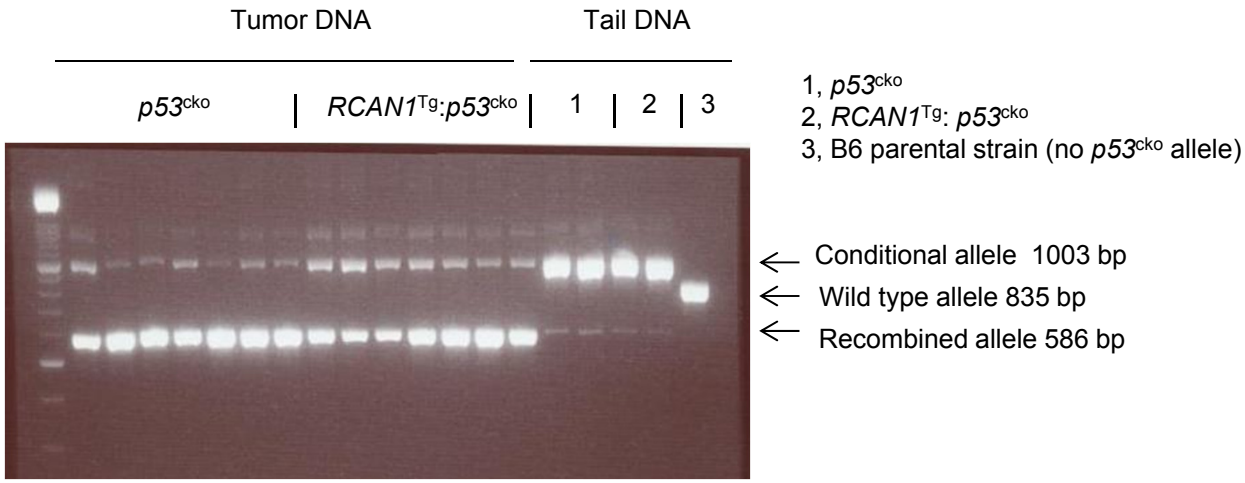


O.D. 620nm	CaN IP	HA IP
Total activity	0.159	0.380
Total activity	0.152	0.373
Total activity	0.162	0.377
Activity (with EGTA)	0.108	0.116
CaN activity	0.050 (“ 0.005)	0.261 (“ 0.004)

Suppl. Fig. S5


MEFs: RCAN1BAC-Tg2
MEFs: RCAN1BAC-Tg3
0
3h Ca++
0
3h Ca++
Wt
Tg
Wt
Tg
Wt
Tg
Wt
Tg
NFATc1
RCAN1
(HA tag)
actin
100
75

Suppl. Fig. S6



Genomic DNA PCR with primers flanking the *p53*$^{flex7/flex7}$conditional KO allele