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Normal Viability of Kai1/Cd82 Deficient Mice

John I. Risinger[1,2,*], **Mary Custer**[2], **Lionel Feigenbaum**[2], **R. Mark Simpson**[2], **Shelley B. Hoover**[2], **Joshua D. Webster**[2], **Gadisetti V. R. Chandramouli**[1], **Lino Tessarollo**[2], **J. Carl Barrett**[2,3]

[1]Department of Obstetrics, Gynecology and Reproductive Biology, Michigan State University College of Human Medicine, Grand Rapids, Michigan [2]Center for Cancer Research, National Cancer Institute, Bethesda, Maryland [3]Translational Science Oncology Innovative Medicine AstraZeneca Pharmaceuticals LP, Waltham, Massachusetts

Abstract

The KAI1/CD82 tetraspanin is a widely expressed cell surface molecule thought to organize diverse cellular signaling processes. KAI1/CD82 suppresses metastasis but not tumorigenicity, establishing it as one of a class of metastasis suppressor genes. In order to further assess its functions, we have characterized the phenotypic properties of Kai1/Cd82 deleted mice, including viability, fertility, lymphocyte composition, blood chemistry and tissue histopathology, and of their wild-type and heterozygote littermates. Interestingly, Kai1/Cd82$^{-/-}$ showed no obvious genotype associated defects in any of these processes and displayed no genotype associated histopathologic abnormalities after 12 or 18 months of life. Expression profiles of non-immortal, wild-type and Kai1/Cd82$^{-/-}$ mouse embryo fibroblast (MEFs) indicated distinct sex-specific and genotype-specific profiles. These data identify 191 and 1,271 differentially expressed transcripts (by twofold at $P < 0.01$) based on Kai1/CD82 genotype status in female and male MEFs, respectively. Differentially expressed genes in male MEFs were surprisingly enriched for cell division related processes, suggesting that Kai1/Cd82 may functionally affect these processes. This suggests that Kai/Cd82 has an unappreciated role in the early establishment of proliferation and division when challenged with a new environment that might play a role in adaptability to new metastatic sites.

Keywords

metastasis suppressor; gene knockout; CD82

INTRODUCTION

Cancer progression, specifically the process of metastasis, is the critical step in determining whether many cancers become fatal. Many gene products have been implicated in growth, maintenance, and suppression of cancer, yet few function specifically to suppress metastasis

*Correspondence to: Department of Obstectrics, Gynecology and Reproductive Biology, Michigan State University College of Human Medicine, Grand Rapids, MI 49503.

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without affecting the overall growth of the cancer. These genes collectively called metastasis suppressors are defined by their ability to functionally impede metastatic spread but have no effect on the growth or maintenance of the primary lesion in orthotopic tumor models. One of the first genes identified that met this definition was the *KAI1* gene. KAI1 was originally identified for its ability to suppress prostate cancer cell metastasis. Specifically, KAI1 impeded lung metastasis formation when introduced into the highly metastatic AT6.1 Dunning rat prostate cancer cell line, whereas no effect was noted in primary tumor formation [1]. Since this seminal discovery, KAI1 has been implicated in the metastatic spread of numerous cancer types. Reduced or lack of KAI1 expression in cancers is often linked with poor prognosis and its elevated expression is associated with a less severe clinical course [2–13].

KAI1, also known as CD82, is a member of the tetraspanin family of membrane spanning cell surface molecules. Tetraspanins play a wide variety of roles in cell adhesion, migration, and in the organization of effective cell signaling. Much research has been undertaken to understand the role of tetraspanins and specifically KAI1 in these processes. Thus, Kai1/Cd82 is hypothesized to function as an adaptor molecule involved in a wide variety of processes. KAI1/CD82 is known to interact with a wide variety of molecules including integrins, other tetraspanins as well as cell surface receptor tyrosine kinases such as c-met and epidermal growth factor receptor [14,15]. Deregulation of tetraspanins and integrins are common findings in cancer cells further implicating Kai1 in these processes. Kai1 has also been shown to functionally interact with DARC, the Duffy antigen receptor for chemokines, and this interaction has also been suggested as key to its function [16,17]. The role of Kai1 as a metastasis suppressor has been expertly reviewed [14,18,19].

KAI1/CD82 is highly expressed in immune cells of the blood where its expression is integral to viral infection of T cells [20,21]. The C33 monoclonal antibody to KAI1/CD82 was able to block virus-induced syncytium formation in T cells. These studies pointed to the necessity of the molecule to allow syncytium formation and selective viral uptake [20,21]. The role of KAI1/CD82 in epithelial cells is much less clear. KAI1/CD82 mRNA is expressed in most tissues, unlike some other tetraspanins which exhibit more restricted tissue expression [22,23]. In our previous study, we developed the first mouse specific Cd82 antibody and confirmed Kai1/Cd82 protein expression in specific cell types in most mouse organs. We found varying levels of Kai/Cd82 expression in all tissues with the exception of smooth muscle [24]. Murine and human KAI1/CD82 are fairly conserved at the amino acid level principally differing only in the number of *n*-linked glycosylation sites, further suggesting that the murine model is appropriate for study of the normal functions of KAI1/CD82.

The effect of germline deletion has been examined for several tetraspanins but has not been reported for Kai1/Cd82. These murine germline deletion experiments have uncovered several unexpected functions of some tetraspanins. Most notably germline deletion of CD9 results in the inability of sperm egg fusion with CD9 required on egg plasma membrane [25–27]. However, more commonly germline deletion of tetraspanins in mice results in few grossly detectable defects noted in vivo. Deletion of CD37, CD81, CD151, CD63, and TSSC6 all result in viable mice having only subtle or no altered phenotypes [28–32]. Notable phenotypes were not seen for CD37 or Tssc6 mice, while increased brain size was

noted as the only major phenotype alteration in CD81 null animals [31]. CD151 null mice, although outwardly phenotypically normal, exhibit an increased propensity to bleed and demonstrate a failure in wound healing [33]. CD151 mice also exhibit an increased resistance to metastasis [34]. CD63 mice exhibit abnormal water balance due to kidney dysfunction [28]. Several of these models exhibit increased proliferation of lymphocytes upon stimulation in vitro [35,36]. The lack of appreciable phenotypes in tetraspanin knockouts is perhaps unsurprising given the high level of protein conservation among the tetraspanins. The redundancy of tetraspanin function was further noted by the ability of CD81 to rescue the CD9 null sperm egg fusion phenotype [37]. In order to further examine the function of Kai1 we deleted it in the mouse and performed an initial characterization of its gross phenotypes and effect on viability.

METHODS

Targeting Vector

A mouse BAC (Life Technologies, Foster City, CA) containing murine Kai1 was recombined into PLMJ235. *Sal*1 and *Eco*RV flanked 5′ homology and *Eco*RV and *Not*1 flanked 3′ homology arms were generated by PCR ligation to PBSK and used to create a loxP-neo cassette via ligation to PLMJ237. The cassette was used to recombine with PLMJ235 genomic DNA plasmid. The final recombined plasmid was linearized and used to target ES cells. The recombination strategy for building the targeting vector utilized has been expertly detailed [38].

Mice

ES cells were screened by Southern blotting. Targeted cells were introduced to pseudo-pregnant mice. Resultant chimeric offspring were bred and Kai1/Cd82-targeted strains were identified. Two independent strains of mice were backcrossed to the C57BL/6 strain using the NCI speed congenic service, SAIC Frederick MD. Southern blotting, Northern blotting and immunoblotting and immunohistochemistry with mouse anti-Kai1 all confirmed absence of Kai1/Cd82 in knockout mice.

Genotyping and Sex Determination

Probes were constructed by polymerase chain reaction at both the 5′ and 3′ end; primers for probes were 32623-F-ctcttgggctgtttatcctgcaa and 33040-R-tcaagttgtgcccaaagttgcc for 5′ and 43367-F-ggtcatgtgagaggcaggatca and 44134-R-cagtccccagaaaagtcaccag for 3′. These were cloned in vector PCR-4-TOPO (Life Technology). Plasmids were isolated, confirmed by sequence and inserts purified following *Eco*R1 digestion. Five microgram genomic DNA was digested with *Xho*1 and *Kpn*1 and separated on 1% gels. Radio-labeled random primed probes were prepared and hybridized as previously described [39]. Sex of mouse embryo fibroblasts (MEFs) were determined using PCR and primers targeting Sry and Tsh essentially as described [40].

Pathology

Mice at 6–8 wk, 12 and 18 months of age were evaluated for hematology, serum biochemistry, and histologic abnormalities of all major organs without prior knowledge of

genotypes by board certified veterinary pathologists (R.M.S. and J.D.W.). Gross necropsies and blood analyses were performed at the NCI-Frederick (Frederick, MD) contract facilities. Blood collection (<0.2 ml) was performed by mandibular bleeding. For the complete blood count (CBC) analysis, whole blood was collected in Microvette EDTA collection tubes (Sarstedt, Inc., Newton, NC) and 20 μl was analyzed using a Hemavet 950 Multispecies Hematology Analyzer (Drew Scientific, Waterbury, CT). For blood chemistry analysis, blood was collected in serum separator tubes (Becton Dickinson, Palo Alto, CA) and allowed to sit at room temperature for 30 min. Tubes were centrifuged at 2,000 rpm for 10 min. The chemistry analysis was done using 100 μl of serum on the VetScan (Abaxis, Union City, CA) and/or 40–50 μl of serum on the Vitros analyzer (Ortho Clinical Diagnostics Raritan, NJ). Immunohistochemistry and immunoblotting were performed on murine tissues using rabbit anti-Kai1 antibodies as previously described by our group [24].

Gene Expression

Embryonic day 12.5 embryos were collected, extensively rinsed in PBS, and cells were harvested in 100 mm dishes for in vitro culture. When wells became confluent, cells were harvested, counted, and replated 1:4 into 60 mm dishes. Three dishes were used for RNA isolation and the remaining dish was used for future growth studies, as well as genotyping and sex determination. Selected MEFs were serially passaged until immortal and are available for future study. Total RNAs were used to evaluate gene expression on Affymetrix GeneChip® Mouse Genome 430 2.0 Array (Affymetrix, Inc., Santa Clara, CA).

Expression Analysis

Expression Console software Ver 1.1 (Affymetrix, Inc.) was used to determine the signal values according to the MAS5 algorithm. Total intensity normalization was applied by normalizing each of the arrays to trimmed mean signal value of 500. All the statistical calculations were done on logarithmic values of signals to the base 2. Global expression profiles were examined by multidimensional scaling (MDS) using 1-correlation as distance metric. About 25,000 transcripts that were detected at least in half of the arrays were included for this analysis. The 3D graph of arrays from this analysis indicated that male Kai1/Cd82$^{-/-}$ MEFs (16 cases) inappropriately clustered together with male wild type, though male to female MEFs were well segregated as expected. Examination of Cd82 expression levels of MEF (16 cases) indicated similar values to those of wild-type MEFs. The Xist gene expressions of all male MEFs were much lower than female MEFs, rationalizing the dissimilarities observed in MDS. Global expression profiles between WT and Kai1/Cd82$^{-/-}$ male MEFs were found to be significantly different at global test $P = 0.001$ after excluding MEF (16 cases; BRB ArrayTools software ver. 4). Differentially expressed genes between WT and Kai1/Cd82$^{-/-}$ MEFs were determined by two-sample *t*-tests at two-tailed *P*-value < 0.01. There were 1271 transcripts up- or down-regulated by twofold at this *P*-value threshold and having a geometric average signal value >100. Similar comparison of female MEFs identified 191 transcripts up- or down-regulated by twofold at $P < 0.01$ where the global test *P*-value = 0.01. Gene ontology analysis of 1,271 transcripts by Expression Analysis Systematic Explorer (EASE) software identified enrichment for down-regulated *immune response* and *cell proliferation* pathway genes. Similar analysis of 191

transcripts altered by twofold in female MEFs indicated enrichment for up-regulated *morphogenesis* and *cell migration*-related genes.

RESULTS

We isolated the murine Kai1 locus in a bacterial artificial chromosome (BAC) following a PCR screen. This BAC was used to create a gene targeting vector with the goal to remove the second exon of the Kai1/Cd82 gene. In the mouse, this exon contains the translation initiation codon and we predicted that its elimination would result in a null allele. We followed an established recombination vector creation strategy in use at our institution [38]. Our vector was designed to be multifunctional and could result in subsequent Cre recombinase driven deletion (Figure 1A and 1B). We introduced linearized vector to ES cells and screened for recombinants. Of the initial 37 clones analyzed, 12 contained the expected bands following Southern blot analysis using both 5′ and 3′ specific probes. In addition, we amplified across the entire locus and sequenced the recombined locus to confirm the integration. Upon this sequencing we noted that an internal recombination within the targeting vector had removed an internal loxP site (and exon 2). This unintended loss will affect the utility of the resultant mouse strain for use in promoter specific Cre driven recombination of this locus. The resultant recombinant mice described in this article therefore represent a conventional gene deletion for exon 2 of the Kai1/Cd82 locus.

We crossed resultant chimeric mice in order to generate Kai1-targeted mice and created two independent lines of Kai1-targeted mice. These initial crosses resulted in normal litter sizes and offspring with apparent normal Mendelian ratios of targeted and non-targeted alleles and a roughly equal distribution of female and male offspring (data not shown). We examined these offspring for Kai1/Cd82 mRNA message and protein. First, we utilized RT-PCR assays to examine the expression of Kai1 message in mice that had been genotyped as +/+ and −/− by Southern blotting. Assays utilizing RNA isolated from the spleen, an organ with abundant Kai1 expression, confirmed a lack of message in −/− mice (data not shown). To confirm that the lack of message results in mice null for Kai1 protein, we examined the expression of Kai1 in the spleens from wild-type (+/+) and suspected null (−/−) mice. Wild-type mice expressed abundant Kai1 protein, whereas −/− mice exhibited no detectable protein (Figure 1C). We further examined Kai1 expression using immunohistochemistry and confirmed the lack of Kai1 in null mice (Figure 1D). These data confirmed that our targeted strategy resulted in efficient removal of Kai1/Cd82 exon 2 and that this removal results in a locus incapable of producing normal Kai1/Cd82 protein.

As a first pass to determine if Kai1/Cd82 loss contributed to any noticeable phenoptyic changes in mice, we examined a limited number of these mixed background mice at approximately 6 months of age. Specifically, we performed a detailed histopathologic analysis of three mice for each sex and genotype at this age. In general, there were no obvious genotype-specific differences. However, histopathologic examination identified several pancreatic abnormalities in two male −/− and in one male heterozygous mouse. The pancreases from these mice contained cystic, hyperplastic foci, suggestive of early islet cell adenoma (data not shown).

To follow up these observations, we designed a phenotypic study with greater statistical and longitudinal power. Furthermore, we performed extensive background crossing to C57BL/6 mice in order to produce near isogenic animals. These backcrosses were supported by genotypic speed congenics, which selected animals with the highest percentage of C57BL/6 background for successive breeding. Following these speed congenic background crosses, we obtained mice with the Kai1/Cd82 null allele that were confirmed to be comprised of >99% C57BL/6 background (data not shown). Using these mice we performed more extensive histopathologic analyses at 12 and 18 months of age, as well as performed CBC and blood chemistry analyses.

The results of breeding on the C57BL/6 background supported our initial breeding results in mixed background mice. These breedings resulted in 31 broods with 260 males and 206 females born. These included 100 wild-type, 240 heterozygote, and 122 homozygous knock-out mice. Near Mendelian ratios of offspring indicated that Kai1/Cd82 did not affect viability (Table 1). Furthermore, we examined whether Kai1 null animals were fertile. Eighteen matings of −/− animals from two independent knockout strains produced 112 offspring resulting in an average litter size of 6.22. These data were entirely consistent with data obtained from with 16 matings of +/+ animals which produced 98 offspring with an average litter of 6.12. These data are also in agreement with average litter size of 6.2 reported for this strain of mice [41]. Taken together these data are consistent with there being no effect of Kai1 on viability or fertility.

No genotypic-related histopathologic abnormalities were observed in the pancreas of these mice, in contrast to proliferative changes noted in small numbers of mixed background mice. As expected from older animals, we detected numerous lesions in 12 and 18-month-old mice including benign neoplasms and some malignancies (Table 2). Specifically, we noted 37 neoplasms among all aged mice. However, none of these occurred with unexpected frequency compared to conventional C57BL/6 mice, and therefore were not associated with any specific genotype.

We performed analysis of blood chemistry (Table 3) and blood composition (Table 4) in littermates from Kai1/Cd82+/− matings. While some of the values were statistically different none of these apparent abnormalities in blood composition relating to genotype fell outside the normal range for this strain of mouse.

Because we noted no obvious Kai1/Cd82 genotype knockout effects on mouse physiology and longevity, we performed a gene expression array analysis designed to test whether Kai1/Cd82 had any effect on gene expression. Rather than examine an organ or tissue from adult mice we instead chose to examine non-immortalized cells grown from Day 12.5 mouse embryos. Specifically, we chose to examine these cells at the point where they first begin to grow in culture to simulate an adaptive challenge. We harvested Day 12.5 embryos and established them in culture. At the first passage these cells were harvested in triplicate for gene expression array, genotype, and sex determination. We performed Affymetrix gene expression analysis on the resultant samples.

Unsupervised MDS analysis showed that gene expression from MEFs was tightly linked to the sex of the derived cells as well as to the Kai1/Cd82 genotype. Specifically, total gene expression in MDS showed four distinct groupings of samples based on sex and genotype (Figure 2A). These initial findings suggested that there will be many significant sex-associated transcripts as well as those whose differential expression is attributable to Kai1/Cd82. As expected we identified several well known and established sex-associated transcripts in comparison of male to female mice, including Xist, which likely drive the unsupervised clustering of these samples (Supplementary Tables S1 and S2). To determine the effect of Kai1/Cd82, we also performed differential gene expression analysis of males and females separately and according to genotype. Many genes were identified that were associated with Kai1/Cd82 genotype (presence or absence) in both male and female mice and some were only sex dependent (Supplementary Tables S3 and S4). The top differentially expressed genes in male and female knockout mice are provided in Tables 5 and 6.

Of these differentially expressed transcripts we noted a marked up-regulation of the Tmem87A transcript in both male and female Kai1/Cd82 null cells. This uncharacterized transcript putatively encodes a transmembrane protein of unknown function (Figure 2B). Interestingly, we also noted the loss of the DPCA2 transcript in Kai1/Cd82 null cells. This transcript encodes the Dresden Prostate Cancer Associated 2 transcript. This transcript is normally considered a prostate-specific transcript, but was detected in both male and female MEFs in our experiment. Loss of DPCA2 was noted in both male and female Kai1/Cd82−/− MEFS (Figure 2C). Real-time quantitative PCR confirmed the differential expressions of these transcripts (Figure 2).

Because we examined these MEF cultures at a point where they were still establishing growth in culture and prior to immortalization we were interested in seeing if Kai1/Cd82 genotype affected any global processes. Therefore, we examined the differentially expressed genes related to genotype using Gene Ontology analysis. As might be expected based on known Kai1/Cd82 function, we found that gene ontologies related to immune response and cell adhesion were among the four most highly over-represented gene ontologies (Table 7). Surprisingly, we also noted that genes associated with cell proliferation and mitosis were also among the top four ontologies (Table 7). Finally, we examined the expression of known metastasis associated genes in Kai1/Cd82 wild-type and null cell (Figure 3 and Table 8) and noted differentially expressed genes in this list.

DISCUSSION

We successfully deleted the Kai1/Cd82 gene in the germline of mice. These mice were viable and exhibited no genotype-associated lesions or physiological abnormalities affecting health or growth at advanced age (18 months). The Kai1/Cd82 null mice, unlike other tetraspanin-deleted models, did not have any observed effect on reproductive viability. These mice showed no increased or decreased propensity to neoplasia and exhibited blood chemistries and hematologic parameters in the normal range for mice on the C57BL/6 background.

Although these mice were outwardly phenotypically normal, subtle defects may exist for which we did not test. Several tetraspanins exhibit alterations in lymphocyte proliferation following stimulation. We did not test this in Kai1/Cd82 mice but it is possible that this process is also affected in Kai1/Cd82 mice. Given the well-known role of CD82 in immune functions, several detailed examinations of altered immune activities could be examined in future studies utilizing these mice.

The Cd151 tetraspanin is known to affect wound healing in mice. Specifically, mice with deleted Cd151 have decreased wound healing function [33]. Furthermore, this defect may be related to angiogenesis [42]. Although we do not present the data in this article, preliminary experiments did not reveal an overtly obvious defect in wound healing in Kai1/Cd82 null mice (Riss, Risinger, and Barrett, unpublished data). Some emerging evidence suggests a reciprocal function of CD151 and CD82. Data on cancers suggest loss of Kai1/Cd82 contributes to metastasis but similar data suggest gain or retention of CD151 is associated with advanced and metastatic disease and that Cd151 actively promotes invasion and motility [43–45]. Cd151 null mice are also less prone to metastasis than wild-type mice [34]. Recently, interdependence of Cd151 and Kai1/Cd82 were also demonstrated on the vitro motility and invasion of cancer cells [46]. Interestingly, our lab has also determined that CD151 and CD82 physically interact in yeast 2-hybrid studies (Risinger and Barrett, unpublished). The reciprocal roles of Cd151 and Kai1/Cd82 will require future studies to unravel their specific functions in cells when both are expressed or when one member is preferentially expressed.

We examined the global mRNA expression of Kai1/Cd82 mice and their wild-type littermate MEFs in cell culture. We performed an unbiased assessment of gene expression in passage 1 MEFs at the initial phases of cell growth in culture when these cells are challenged with a new environment. We hoped to uncover novel functions of Kai1 which could be related through downstream affects on gene expression. Data from these studies clearly showed sex and genotype effects on global expression. In fact both these were globally significant suggesting a profound effect of Kai1/Cd82 on the transcriptome of mice. The lists of differentially expressed genes identified several interesting genes. These included a dramatic up-regulation of TMEM87a, Desmoplakin and Palladin in Kai1/Cd82 null cells. TMEM87a is an unstudied gene whose protein sequence suggests a protein in a transmembrane location. This gene was the most highly up-regulated transcript in response to Kai1 loss in MEFs. Message levels of desmoplakin an integral component of desmosome mediated cell attachments was also significantly increased in Kai1 null cells. The palladin gene suspected of association with actin cytoskelelon was also up-regulated. These findings suggest that Kai1 influences cell attachments and cell shape.

CONCLUSION

Early work defining metastasis suppressors noted the ability of certain human chromosomes to suppress metastasis in orthotopic cancer models without affecting tumor formation [47–50]. Several different human chromosomes including 7, 10, 11, and 17 were shown to possess this property. Kai1/Cd82 was originally identified as the metastasis suppressor from chromosome 11. The human KAI1 gene was able to effectively suppress the formation of

lung metastasis in the Rat AT6 cell line without affecting tumorigenicity. These studies have resulted in focusing future metastasis suppressor research on metastatic processes such as motility, invasion, etc. rather than those related to central tumor processes which might include apoptosis or proliferation. However, the results of our ontology analysis on the differentially expressed genes identified in the comparison of wild-type to Kai1/Cd82 null mice showed both expected and unexpected results. Unsurprisingly, based on known Kai1/Cd82 functions, we noted cell adhesion and immune function as ontologies over-represented. However, we were surprised to see that mitosis and cell proliferation were the other two top ontologies. This suggests that Kai/Cd82 has an unappreciated role in the early establishment of proliferation and division processes when cells are challenged with a new environment, in our case cell culture; in vivo this might be adaptability to new metastatic sites.

In summary, we report the generation of Kai1/Cd82 null mice. Although these mice were outwardly normal they are likely to yield further valuable information in other studies related to metastasis and normal biology regarding immune function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations:

MEF	mouse embryo fibroblast
CBC	complete blood count
MDS	multidimensional scaling
EASE	Expression Analysis Systematic Explorer
BAC	bacterial artificial chromosome

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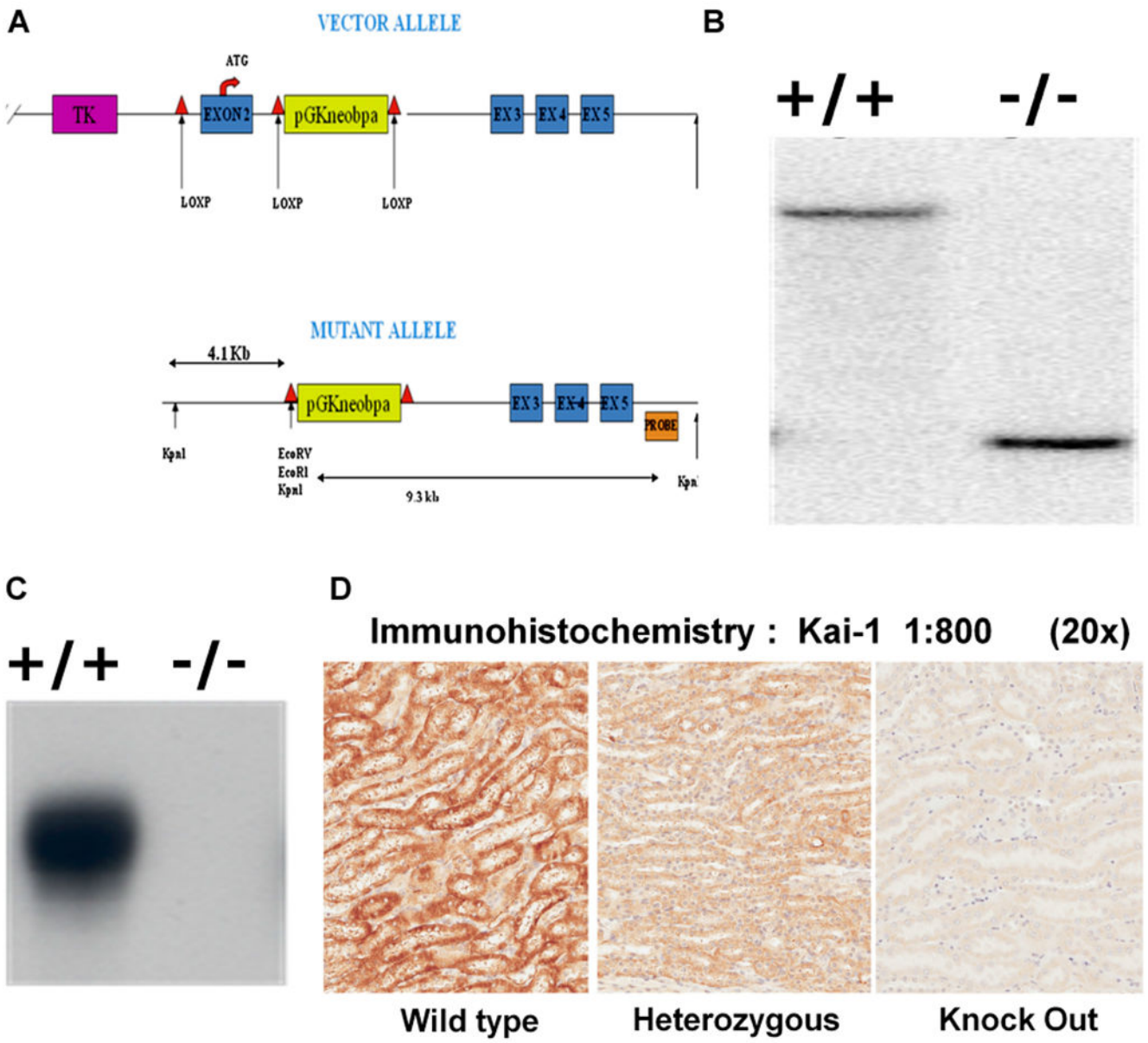

Figure 1.

(A) Knock-out recombination strategy. (B) Southern blotting of wild-type (+/+) and homozygous deleted (−/−) Kai1/Cd82 mice. (C) Kai1/Cd82 immunoblot in proteins isolated from wild-type (+/+) and homozygous deleted (−/−) Kai1/Cd82 mouse spleens. (D) Immunohistochemical detection of Kai1/Cd82 in kidney sections from wild-type (+/+), heterozygous (+/−), and knockout (−/−) mice.

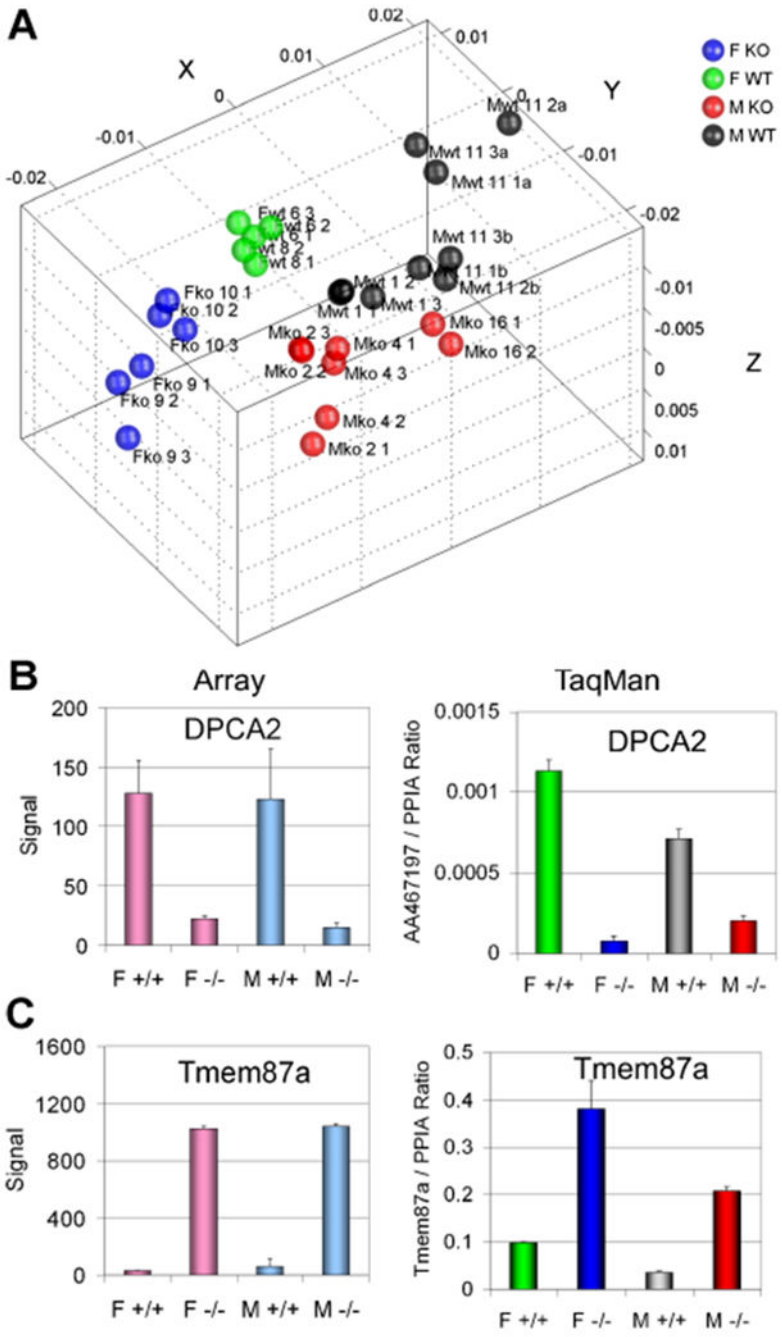

Figure 2.

(A) Gene expression of passage 1 cultures of Day 12.5. WT and null mouse embryos identify genes systemically regulated by Kai1/Cd82. Global effect of gene expression demonstrated using unsupervised multidimensional scaling analysis. F KO, female knockout (−/−); F WT, female wild type; M KO, male knockout (−/−); M WT, male wild-type mice. (B,C) Tmem87a and DPCA2 gene expressions that are affected by Cd82/Kai1 genotype. F = female, M = male, +/+ = WT, −/− = KO. Left: Microarray. Right: TaqMan validation.

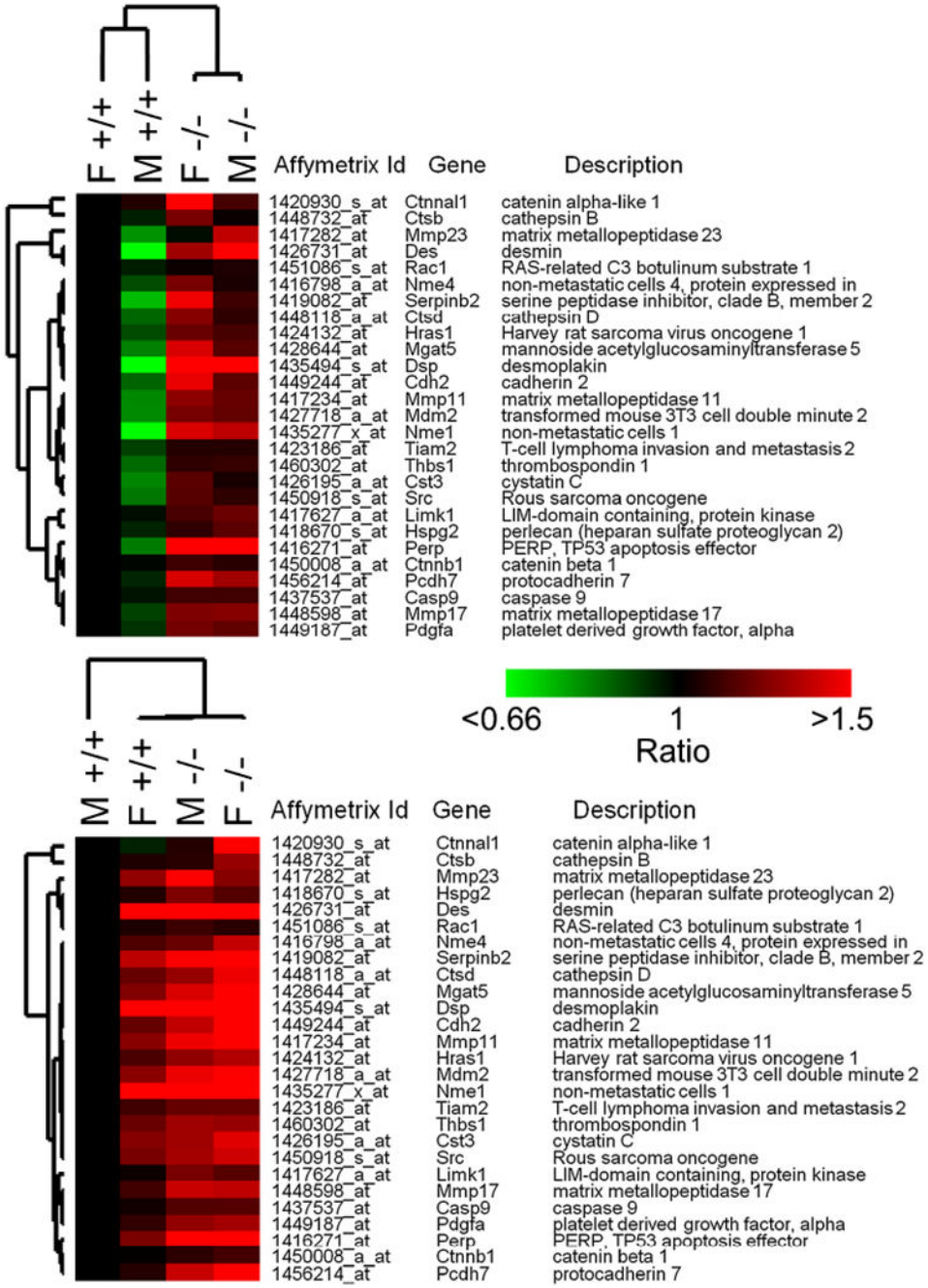

Figure 3.

Metastasis-related genes differentially expressed in MEFs derived from wild-type and knock-out Kai1/Cd82 mice. Red is up-regulated, Green is down-regulated. Top: reference is male $Cd82^{+/+}$. Bottom: reference is female $Cd82^{+/+}$.

Table 1.

Breedings Resulted in Near Mendelian Ratios of Offspring Indicating That Kai1/Cd82 Did Not Affect Viability

	Ratio
Female hets	0.507
Female KO	0.275
Female WT	0.217
Male hets	0.525
Male KO	0.259
Male WT	0.216

Table 2.

Neoplasms in 12- and 18-Month-Old Kai1/Cd82 Null Mice and Littermates

Tumor	12 Months	Genotype (WT/het/KO)	18 Months	Genotype WT/het/KO
Lymphoma	2	0/1/1	16	5/7/4
Pulmonary adenoma/adenocarcinoma	1	0/1/0	4	1/3/0
Harderian gland adenoma	0		2	0/1/1
Histiocytic sarcoma	1	0/0/1	4	0/1/3
Anaplastic round cell tumor (sarcoma)	0		1	1/0/0
Pheochromocytoma	0		1	0/0/1
Adrenocortical adenoma	0		1	1/0/0
Hepatocellular carcinoma	0		2	0/0/2
Intestinal polyp	0		2	1/1/0

Table 3.

Blood Chemistry Analysis of Male and Female Wild-Type (WT) and Cd82$^{-/-}$ (KO) Mice

		Male					Female							
				Normal range					Normal range					
Assay	**Units**	**WTO**	**KO**	**Low**	**High**	***P*-value**	**WT**	**KO**	**Low**	**High**	***P*-value**	**WT M vs. F (*P*-value)**	**KO M vs. F (*P*-value)**	**WT vs. KO M & F (*P*-value)**
ALB	g/dl	3.4	3.5	2.1	2.46	0.572	4.1	3.6	2.72	2.32	0.082	**0.013**	0.151	0.836
ALP	U/L	82	83	125	176	0.943	155	129	153	210	0.276	**0.037**	**0.022**	0.880
ALT	U/L	57	51	45	93	0.430	37.3	40.6	41	76	0.728	0.091	0.166	0.383
AMY	U/L	1,034	1,050			0.818	1,116	974			0.495	0.679	0.472	0.609
TBIL	mg/dl	0.3	0.3	0.1	0.29	1.000	0.3	0.3	0.1	0.32	0.688	0.511	0.710	0.925
BUN	mg/dl	23.5	21.6	17.5	23.2	0.067	23.0	19.4	13.7	20.4	0.251	0.575	0.106	**0.027**
Ca++	mg/dl	9.9	9.8	9.85	10.54	0.743	10.0	9.8	9.94	10.55	0.560	0.444	0.985	0.531
PHOS	mg/dl	8.3	8.3	10	12.37	0.935	8.7	8.3	8.51	12.23	0.714	0.524	0.973	0.779
CRE	mg/dl	0.4	0.2	0.24	0.32	0.020	0.3	0.4	0.22	0.31	**0.049**	0.604	**0.003**	0.634
GLU	mg/dl	148	146	139.3	184.3	0.911	152	132	139.2	194.1	0.465	0.859	0.519	0.520
Na+	mmol/L	159	157	149.4	153.4	0.247	162	154	147.7	151.2	**0.025**	0.125	**0.002**	**0.010**
K+	mmol/L	6.6	6.7	5.54	7	0.490	6.8	6.2	5.78	6.61	0.173	0.802	**0.014**	0.410
TP	g/dl	6.0	6.1	4.92	5.5	0.688	6.4	6.1	5.1	5.57	0.241	0.171	0.890	0.678
GLOB	g/dl	2.7	2.6			0.802	2.3	2.4			0.581	0.058	0.081	0.736
CLORIDE	mmol/L	116	112	110.8	116.9	0.149	117	114	112.1	116.3	0.532	0.286	0.611	0.103
LIPASE	U/L	904	948			0.301	1,385	1,145			0.143	**0.003**	**0.004**	0.974
CHOL.	mg/dl	106	103	100	116	0.673	106	87	88	110	0.340	0.865	0.124	0.214
TRIGLY	mg/dl	130	128			0.884	80	109			0.137	**0.009**	0.171	0.752
AST	U/L	107	101	65	142	0.738	73	102	57	199	0.387	0.221	0.941	0.884
IRON	mg/dl	148	167	145.1	215.7	0.286	156	164	146.2	223.3	0.693	0.686	0.890	0.230

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMY, amylase; TBIL, total bilirubin; BUN, blood urea nitrogen; PHOS, phosphorus; CRE, creatinine; GLU, glucose; TP, total protein; GLOB, globulin; CHOL., cholesterol; TRYGLY, tryglycerides; AST, aspartate aminotransferase.

Values of pooled samples from eight mice each of male WT, KO and female KO and of four female WT mice.

P-values < 0.05 are indicated in bold face.

Table 4.

Blood Composition (CBC) Analysis of Male and Female Wild-Type (WT) and Cd82$^{-/-}$ (KO) Mice

	Male			Female			Normal range				
Assay	**WT**	**KO**	***P*-value**	**WT**	**KO**	***P*-value**	**Low**	**High**	**WT M vs. F (*P*-value)**	**KO M vs. F (*P*-value)**	**WT vs. KO M & F (*P*-value)**
Leukocytes											
WBC	9.00	8.47	0.506	5.48	5.74	0.755	1.8	10.7	**0.003**	**0.0004**	0.141
NE	2.46	2.49	0.931	1.07	1.48	0.123	0.1	2.4	**0.001**	**0.004**	0.646
LY	6.21	5.60	0.261	4.20	4.02	0.802	0.9	9.3	**0.019**	**0.003**	0.059
MO	0.274	0.309	0.471	0.175	0.218	0.376	0	0.4	**0.037**	0.074	0.704
EO	0.044	0.054	0.567	0.027	0.031	0.748	0	0.2	0.335	0.186	0.810
BA	0.008	0.014	0.260	0.003	0.008	0.326	0	0.2	0.193	0.353	0.234
Leukocytes (%)											
NE	27.43	28.58	0.708	20.69	26.60	0.150	6.6	38.9	0.055	0.557	0.415
LY	68.84	67.19	0.619	75.31	68.53	0.163	55.8	91.6	0.103	0.723	0.308
MO	3.13	3.51	0.362	3.28	4.03	0.418	0	7.5	0.789	0.411	0.143
EO	0.50	0.57	0.746	0.62	0.65	0.927	0	3.9	0.642	0.738	0.653
BA	0.09	0.15	0.290	0.11	0.19	0.524	0	2	0.835	0.684	0.196
Erythrocytes											
RBC	10.11	10.48	0.148	9.83	9.94	0.712	6.36	9.42	0.377	**0.039**	0.412
Hb	14.33	14.55	0.571	14.30	14.43	0.736	11	15.1	0.961	0.720	0.530
HCT	41.68	42.74	0.535	42.17	41.71	0.796	35.1	45.4	0.815	0.507	0.746
MCV	41.14	40.69	0.603	42.87	41.94	0.269	45.4	60.3	0.108	0.105	0.678
MCH	14.16	13.89	0.085	14.55	14.54	0.961	14.1	19.3	0.126	**0.0004**	0.818
MCHC	34.48	34.22	0.657	34.02	34.71	0.310	30.2	34.2	0.489	0.410	0.765
RDW	18.30	18.36	0.803	18.10	17.88	0.511	12.4	27	0.595	**0.046**	0.481
Thrombocytes											
PLT	980	969	0.840	771	879	0.148	592	2,972	**0.008**	0.140	0.961
MPV	4.17	4.24	0.635	4.17	4.26	0.615	5	20	0.991	0.852	0.444

WBC, white blood cells; NE, neutrophils; LY, lymphocytes; MO, monocytes; EO, eosinophils; BA, basophils; RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; PLT, platelets; MPV, mean platelet volume.

Average values of eight mice each of male WT, KO and female KO and of four female WT mice.

P-values < 0.05 are indicated in bold face.

Table 5.

Differentially Expressed Genes Up- and Down-Regulated by Kai1/Cd82$^{-/-}$ in Male MEFs

Affymetrix Id	Gene	Description	M KO signal	M WT signal	F KO signal	F WT signal	Male KO/WT	*P*-value	Female KO/WT	*P*-value
Male										
1421058_at	Adh7	Alcohol dehydrogenase 7, mu or sigma	11	184	13	22	0.0609	6E–06	0.5882	0.2169
1460248_at	Cpxm2	Carboxypeptidase X 2 (M14 family)	51	488	39	39	0.1045	5E–10	1.0055	0.9806
1419728_at	Cxcl5	Chemokine (C-X-C motif) ligand 5	7	231	5	5	0.0302	3E–06	1.1236	0.8662
1418283_at	Cldn4	Claudin 4	170	20	283	144	8.5366	0.0023	1.9665	0.0001
1453092_at	Crct1	Cysteine-rich C-terminal 1	1,711	196	2,332	1,247	8.7516	1E–09	1.8697	2E–08
1435494_s_at	**Dsp**	Desmoplakin	109	16	247	47	6.7231	4E–05	5.2668	8E–06
1439746_at	Dusp27	Dual specificity phosphatase 27 (putative)	233	24	246	184	9.6682	0.0015	1.3407	0.021
1422947_at	Hist1h3a	Histone cluster 1, H3a	233	15	314	118	16.013	5E–06	2.6684	1E–06
1428014_at	Hist1h4h	Histone cluster 1, H4h	241	27	309	135	8.9152	1E–05	2.3	3E–05
1449499_at	Hoxa7	Homeo box A7	270	16	245	232	16.514	2E–09	1.0549	0.5373
1457666_s_at	Ifi202b	Interferon-activated gene 202B	1	3,269	3	1	0.0004	7E–11	2.2985	0.1337
1451567_a_at	Ifi203	Interferon-activated gene 203	82	1,184	116	182	0.0689	3E–10	0.6393	0.0131
1426278_at	Ifi27	Interferon, alpha-inducible protein 27	115	1,337	191	200	0.0864	2E–08	0.9537	0.5953
1427300_at	Lhx8	LIM homeobox protein 8	37	514	68	38	0.0729	4E–08	1.7899	0.0573
1425828_at	Nkx6-1	NK6 homeobox 1	125	19	157	108	6.4826	1E–05	1.4504	0.0832
1434046_at	**AA467197**	Dresden Prostate Cancer Associated 2	14	220	22	128	0.0644	2E–06	0.1748	2E–05
1457088_at	**Pldn**	Pallidin	324	35	342	42	9.3682	1E–09	8.0977	7E–09
1421917_at	Pdgfra	Platelet-derived growth factor receptor, alpha	121	1,293	69	112	0.0936	6E–10	0.6186	0.0164
1417466_at	Rgs5	Regulator of G-protein signaling 5	882	118	1,277	702	7.4665	4E–09	1.8201	0.0007
1449340_at	Sostdc1	Sclerostin domain containing 1	31	334	47	36	0.0919	1E–05	1.3274	0.1462
1418105_at	Stmn4	Stathmin-like 4	278	22	344	251	12.612	0.0003	1.3722	0.0015
1442140_at	Tnn	Tenascin N	92	1,855	46	43	0.0495	2E–10	1.0673	0.9125
1424454_at	**Tmem87a**	Transmembrane protein 87A	1,041	19	1,025	29	54.054	4E–06	35.777	2E–09
1429947_a_at	Zbp1	Z-DNA binding protein 1	29	390	81	70	0.0736	7E–05	1.163	0.5703

M, male; F, female; WT, Cd82$^{+/+}$; KO, Cd82$^{-/-}$; KO/WT, Cd82$^{-/-}$/Cd82$^{+/+}$ signal ratio.

Signal values are geometric mean values.

Table 6.

Differentially Expressed Genes Up- and Down-Regulated by Kai1/Cd82$^{-/-}$ in Female MEFs

Affymetrix Id	Gene	Description	M KO signal	M WT signal	F KO signal	F WT signal	Male KO/WT	*P*-value	Female KO/WT	*P*-value
1458560_at	Aspm	Abnormal spindle-like, microcephaly associated	85	223	63	150	0.3821	0.0038	0.4178	0.0003
1448595_a_at	Bex1	Brain expressed gene 1	982	305	3,461	850	3.2185	3E–09	4.0696	4E–06
1449434_at	Car3	Carbonic anhydrase 3	61	10	166	37	6.0038	0.0019	4.5467	2E–05
1418710_at	Cd59a	CD59a antigen	295	690	390	1,305	0.4274	5E–06	0.2987	2E–10
1454112_a_at	Cep27	centrosomal protein 27	121	325	114	303	0.3716	7E–08	0.3765	7E–06
1448734_at	Cp	Ceruloplasmin	76	38	347	64	2.0054	0.0348	5.3877	2E–06
1424131_at	Col6a3	Collagen, type VI, alpha 3	3,119	10,666	1,471	3,923	0.2924	1E–07	0.3749	1E–09
1435494_s_at	**Dsp**	Desmoplakin	109	16	247	47	6.7231	4E–05	5.2668	8E–06
1452406_x_at	Erdr1	Erythroid differentiation regulator 1	411	1,191	237	1,270	0.3448	0.0432	0.1868	0.0004
1445191_at	Exdl1	Exonuclease 3′–5′ domain-like 1	131	34	176	27	3.8183	3E–05	6.5177	0.0004
1456655_at	Ext1	Exostosin-1	1,510	2,182	59	1190	0.6917	0.0552	0.0495	3E–08
1416200_at	Il33	Interleukin 33	130	45	363	83	2.9143	0.0002	4.3525	3E–06
1425045_at	Jmjd7	jmjC domain-containing protein 7	62	206	79	252	0.3013	1E–07	0.3119	1E–06
1417595_at	Meox1	Mesenchyme homeobox 1	92	366	56	143	0.2522	0.0001	0.3911	0.0005
1434046_at	**AA467197**	Dresden prostate cancer associated 2	14	220	22	128	0.0644	2E–06	0.1748	2E–05
1419271_at	Pax6	Paired box gene 6	34	21	122	26	1.5808	0.361	4.7538	0.0002
1457088_at	**Pldn**	Pallidin	324	35	342	42	9.3682	1E–09	8.0977	7E–09
1449586_at	Pkp1	Plakophilin 1	141	264	77	258	0.5355	0.0015	0.2994	0.0081
1434365_a_at	Scyl3	Protein-associating with the carboxyl-terminal	93	217	52	142	0.4284	0.0002	0.3639	0.0002
1444061_at	A030004J04Rik	RIKEN cDNA A030004J04 gene	98	45	328	77	2.1624	0.0101	4.2384	4E–07
1422198_a_at	Shmt1	Serine hydroxymethyltransferase 1 (soluble)	59	234	32	101	0.2538	0.0027	0.318	0.0029
1454211_a_at	Shroom3	Shroom family member 3	93	30	203	25	3.067	0.0224	8.2233	0.0027
1424454_at	**Tmem87a**	Transmembrane protein 87A	1,041	19	1,025	29	54.054	4E–06	35.777	2E–09

M, male; F, female; WT, Cd82$^{+/+}$; KO, Cd82$^{-/-}$; KO/WT, Cd82$^{-/-}$/Cd82$^{+/+}$ signal ratio.

Signal values are geometric mean values.

Table 7.

Gene Ontology Analysis Selected Ontology Terms at Top EASE Scores

Gene category	No. of genes	EASE score	List of genes
GO Biological Process: immune response	71	5.56	Up: Cxcl12, Rnf128, Cd55, Ccl17, Selp, Cd40
		E–14	Down: Psmb9, C2, Serping1, Irf8, C1qb, Ccl6, C1qa, Hells, Rgs1, Rac2, Ccl9, Gbp2, Fcer1g, Gbp3, Ptx3, Cxcl16, Sfpi1, Tnfsf11, Tlr2, Cxcl1, C3ar1, Cd93, Ccr1, Was, Mx2, Cxcl5, Slc11a1, Ccl2, Ifi202b, Ccl4, Trem2, Tlr7, Ptprc, C5ar1, Hfe, Tlr3, Dock2, Ly86, Psmb8, Cybb, Oasl1, Oas1g, Oas2, Gbp6, H2-K1, Oas3, H2-L, Lst1, Ncf1, Ifi205, Fcgr2b, S1pr3, Lyz2, Ncf2, Cxcr4, Ifi203, Ifit3, C1qc, Itgb2, Psmb9, Ifit1, Tyrobp, H2-D1, Adam33, Mx1, Cfp, Oasl2
GO Biological Process: cell proliferation	91	1.35	Up: Fabp3, Prkar1b, Crip2, Cxcl12, Cdkn1c, Foxg1, Pgf, Tgfb2, 2810003C17Rik, Pitx2, 1434378_a_at, 1443620_at, Ccng2, Cdkn2a, Grpr, Hoxb4
		E–10	Down: Uhrf1, Mcm5, Mcm7, Ccnb1-rs1, Rad21, Coro1a, Cdc45l, Gas1, Cdc6, Psip1, Nek2, Pycard, Tacc3, Gmnn, Rac2, Mybl2, Ccna2, Rad51, Prim1, Clec11a, Cxcl1, Egfl6, Pola1, Ect2, Plk4, Csf1r, Ccnb1, Jag1, Pdgfra, Cdc25b, Igfbp4, Cenph, Mad2l1, Cables1, Ccnf, Ccne2, Dock2, Incenp, Hdgfrp3, Prc1, Ncapd2, Bub1, Aurkb, Cdt1, Rbl1, Rfc4, Brca1, Ncf1, Cdc7, Mki67, Pold3, Pole2, Smc4, Smc2, Bcl11b, Mcm4, S1pr3, Mcm6, Figf, Top2a, Hgf, Bub1b, Plk1, Cxcr4, Cd68, Evi2a, Rad54l, Gsg2, Ifrd2, Adam33, Chtf18, Rfc5, Rpa2, Cdc25c, Smc6
GO Biological Process: mitotic cell cycle	39	2.72	Up: Foxg1, Ccng2, Cdkn2a
		E–08	Down: Mcm5, Mcm7, Ccnb1-rs1, Rad21, Coro1a, Cdc45l, Gas1, Nek2, Ccna2, Prim1, Pola1, Ccnb1, Cdc25b, Cenph, Mad2l1, Cables1, Ccnf, Ccne2, Incenp, Ncapd2, Bub1, Cdt1, Rfc4, Pold3, Pole2, Smc4, Smc2, Mcm4, Mcm5, Mcm6, Top2a, Bub1b, Plk1, Chtf18, Rfc5, Rpa2, Cdc25c
GO Biological Process: cell adhesion	51	5.80	Up: Mcam, Cxcl12, Itga7, Selp, Col19a1, Itga3, Col10a1, Nell2, Itgbl1, Jup, Lama5, Aplp1, Cldn1, Cpxm1, Col2a1, Arvcf, Chodl
		E–05	Down: Cd34, Cd82, Adam8, Sirpa, Rac2, Omd, Clec11a, Cpxm2, Wisp2, Cd93, Clec4n, Col5a3, Pcdha1, Clec4d, Cd36, Plxnc1, Tpbg, Mfap4, Icam1, Col6a3, Pcdha6, Lama4, Lpxn, Cd72, Col6a2, Tspan11, Col8a2, Emilin2, Gpnmb, Clstn2, Col6a1, Col15a1, Acan, Itgb2, Emr1

Table 8.

Metastasis-Related Genes Differentially Regulated Between Wild-Type and Kai1/Cd82$^{-/-}$ MEFs by Twofold at $P < 0.01$

Affymetrix Id	Gene	Description	M KO signal	M WT signal	F KO signal	F WT signal	KO/WT Male	*P*-value	KO/WT Female	*P*-value
1419872_at	Csf1r	Colony-stimulating factor 1 receptor	524	2,160	1,353	919	0.243	2E–08	1.472	4E–06
1416298_at	Mmp9	Matrix metallopeptidase 9	147	604	110	144	0.243	4E–06	0.760	0.1135
1416401_at	Cd82	CD82 antigen	192	640	234	466	0.299	2E–08	0.502	1E–06
1451866_a_at	Hgf	Hepatocyte growth factor	44	136	37	56	0.324	3E–05	0.664	0.0535
1420798_s_at	Pcdha1	Protocadherin alpha 1	55	145	88	55	0.376	0.0001	1.587	0.016
1424341_s_at	Pcdha6	Protocadherin alpha 6	42	103	57	42	0.405	9E–05	1.343	0.0479
1433930_at	Hpse	Heparanase	56	115	45	53	0.484	0.0006	0.843	0.5041
1450040_at	Timp2	Tissue inhibitor of metalloproteinase 2	5,720	2,757	6,422	4,914	2.074	8E–07	1.307	0.0009
1435277_x_at	Nme1	Non-metastatic cells 1	9,273	4,221	9,701	6,782	2.197	0.0022	1.430	0.0506
1425092_at	Cdh10	Cadherin 10	1,178	510	881	1,085	2.310	2E–07	0.812	0.013
1449153_at	Mmp12	Matrix metallopeptidase 12	566	213	1,001	602	2.654	3E–06	1.663	8E–05
1420558_at	Selp	Selectin, platelet	142	48	179	118	2.987	7E–07	1.522	0.0162
1419675_at	Ngf	Nerve growth factor	4,689	1,450	4,026	4,006	3.234	6E–10	1.005	0.9273
1426731_at	Des	Desmin	776	239	513	394	3.247	2E–07	1.301	0.0391
1421997_s_at	Itga3	Integrin alpha 3	105	28	217	180	3.701	0.0021	1.202	0.1766
1435494_s_at	Dsp	Desmoplakin	109	16	247	47	6.723	4E–05	5.267	8E–06
1416271_at	Perp	PERP, TP53 apoptosis effector	849	455	1,436	557	1.864	6E–06	2.579	0.0002

M KO, male Cd82$^{-/-}$; M WT, male Cd82$^{+/+}$; F KO, female Cd82$^{-/-}$; F WT, female Cd82$^{+/+}$; KO/WT, ratio of Cd82$^{-/-}$ to Cd82$^{+/+}$ signals.

All of them are altered by twofold in male MEFs except for Perp that is 1.86-fold. In female MEFs, Dsp, Perp are altered by twofold other than Kai1 down-regulation.

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