Analyzing differentially expressed genes and pathways of Bex2-deficient mouse lung via RNA-Seq

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Abstract: Bex2 is well known for its role in the nervous system, and is associated with neurological disorders, but its role in the lung’s physiology is still not reported. To elucidate the functional role of Bex2 in the lung, we generated a Bex2 knock-out (KO) mouse model using the CRISPR-Cas9 technology and performed transcriptomic analysis. A total of 652 genes were identified as differentially expressed between Bex2−/− and Bex2+/+ mice, out of which 500 were downregulated, while 152 were upregulated genes. Among these DEGs, Ucp1, Myh6, Coxa7a1, Myl3, Ryr2, Rnaset2b, Npy, Enob1, Krt5, Myl2, Hba-a2, and Nr0b2 are the most prominent genes. Myl2 was the most downregulated gene, followed by Npy, Hba-a2, Rnaset2b, nr0b2, KlrA8, and Ucp1. Tcte3, Enol1b, Zfp990, and Pcdha9 were the most upregulated DEGs. According to gene enrichment analysis, PPAR pathway, cardiac muscle contraction, and cytokine-cytokine receptor interaction were the most enriched pathways. Besides, the nuclear factor-kB signaling pathway and hematopoietic cell lineage pathways were also enriched. Chronic obstructive pulmonary disease (COPD) is enriched among KEGG disease pathways. RT-qPCR assays confirmed the RNA-Seq results. This study opens a new window toward the biological functions of Bex2 in different systems.

Key words: CRISPR Cas9, knock-out mouse model, transcriptomic study, differentially expressed genes, KEGG pathwayactor

1. Introduction
Brain expressed X-linked (BEX), a gene family related to X chromosome, consists of BEX1 to BEX5 in humans, while in rodents, Bex5 is missing, instead, Bex6 is found on chromosome 16 (Brown and Kay, 1999; Alvarez et al., 2005). All BEX genes consist of three exons, but only the third exon is coding for the protein (Fernandez et al., 2015).

hBEX2 gene was identified in 2002 in the embryonic cerebral cDNA library using high throughput sequencing technique (Yang et al., 2002), and mBex2 in 1999 (Brown and Kay, 1999). A robust expression of Bex2 has been observed in the mouse embryonic brain that plays a vital role in the development of the nervous system. BEX2 protein interacts with the hematopoietic transcription factor LIM domain only 2 (LMO2) and regulates transcriptional activity of an E-box sequence-binding complex that contains hBex2, LMO2, N5CL2 and LDB1 (Han et al., 2005). It has been reported that BEX2 is differentially expressed in breast tumors (Naderi et al., 2007). c-Jun and p65/RelA transcription factors targeting BEX2 are being phosphorylated in breast cancer cells. BEX2 activates the NF-kB pathway and thus inhibits ceramide 2 (C2) apoptosis of breast cancer cells (Naderi et al., 2007). An other group of researchers have identified that silencing of this gene promotes colorectal cancer metastasis through the Hedgehog signaling pathway (Tan et al., 2020).

Bex2 is detected to be an oncogene in multiple types of cancers (Kazi et al., 2015). The gene plays a critical role in malignancies (Naderi, 2019). A genome-wide association study (GWAS) revealed that Bex2 might act as a tumor inhibitor in glioma’s virulence (Foltz et al., 2006). BEX2 plays a role in promoting human glioblastoma cells’ propagation via NF-kB signaling pathway (Meng et al., 2014). A very recent study explains the involvement of Bex2 in the amelioration of allergic airway inflammation by adipose stem cell derived vesicles (Kim et al., 2020).

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These results show that Bex2 plays a critical role in multiple biological systems, especially in important oncogenic pathways. Gene expression analysis has found that Bex2 is highly enriched in the lungs, but its function in the lungs has never been studied.

The transcriptomic analysis utilizes the second-generation DNA sequencing technology to measure the genome-wide gene expression levels (Costa et al., 2010). RNA-seq has been extensively used as a comprehensive approach to investigate various cell types and transcriptional patterns in transgenic mouse models (Adlat et al., 2020; Sah, et al., 2020). It is helpful to disclose novel genes and alternative splicing events (Trappnell, et al., 2012). Gene enrichment analysis can give a broad picture of genes involvement in pathways that predict its function and regulatory network (Kumar et al., 2016).

To further elucidate Bex2 function in vivo, a mouse model was generated with Bex2 global deletion (Bex2−/−) using the CRISPR-Cas9 system. The lung transcriptome analysis was carried out by performing RNA sequencing (RNA-seq). Although a Bex2 knocked-out mouse has already been generated by other researchers (Ito et al., 2014), the transcriptomic analysis of lungs has not been studied to the best of our knowledge. The comparative analysis of differentially expressed genes (DEGs) between Bex2+/+ and Bex2−/− indicates that several pathways are being enriched. This study provides important information on the potential biological functions of Bex2.

2. Materials and methods

2.1. Experimental animals

This study was approved by the Institutional Animal Care Committee and Animal Experimental Ethics Committee of Northeast Normal University. The care has been taken to minimize the discomfort of the experimental animals. All the recommendations for the use of laboratory animals of NIH (USA) were followed strictly. The mice were kept at the 12/12 light-dark cycle rotation in the pathogen-free environment with free access to food and water. The temperature of 21 °C was maintained along with 30%–60% humidity.

2.2. Plasmid construction for microinjection

The plasmid used, pX330-U6-Chimeric_BB-CBh-hSpCas9, was gifted from Feng Zhang (Cong et al., 2013), through Addgene (https://www.addgene.org/42230/). The single guided RNAs (sgRNAs) were designed (Table), using the Benchling database website (https://www.benchling.com), authenticated using BLAST tool of the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and annealed according to the previously described methods (Ran et al., 2013). Briefly, the forward and reverse sgRNAs were annealed in a buffer containing 150 mM NaCl and heated for 2 min at 94 °C and then gradually cooled down to 25 °C at the rate of 5 degrees per minute using BioRad thermocycler. The annealed oligos were then ligated to BbsI-digested and gel-purified plasmid vector pX330-U6-Chimeric-B-CBhSpCas9, using a ligation kit from Takara (Takara, Dalian, China). The E. coli strain DH5a was used for the transformation of the ligation product. Large prep of plasmid was obtained using alkaline lysis plasmid prep method. The final plasmids, psgRNA-Bex2-1 and psgRNA-Bex2-2, were confirmed by Sanger sequencing (https://www.genewiz.com.cn) using U6 promoter primers (Table).

2.3. Microinjection

The microinjection procedures were followed by the manual “Manipulating the Mouse Embryo; A laboratory Manual, 4th Ed.” by Cold Spring Harbor Laboratory. Ten 4-6-week-old C57BL/6J F1 females were superovulated by intraperitoneal injection with hormone PMGS (5 units in 100 uL saline) followed by the same PCG dose 40 hr later. The mating plug was checked the next day, and oocytes were isolated in M2 and cultured in M16. The mixes of the two plasmids (5 ng/uL each) were microinjected to the pronucleus of the fertilized oocytes, following the previously described methods (Wang et al., 2013).

2.4. Genotyping

The primer-BLAST tool was used to design Bex2 specific primers located at the upstream and downstream of sgRNAs targeting sites (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). All primer sequences are provided in Table. Finger biopsies of two-week-old pups were digested, and PCR genotyped. Briefly, the finger biopsies were digested in GNTK buffer including Proteinase K at 60 °C overnight, boiled for 10 min next morning, centrifuged for 5 min at 12,000 rpm for 5 min, and 0.8 uL was used as the template. The PCR specific conditions include denaturation at 95°C for 4 min, amplification at 94 °C, 56 °C each for 30 s, and 72 °C for 45 s, consist of 32 cycles followed by 10 min extension at 72 °C and then cooled down to 4 °C. The PCR amplified gene fragment was analyzed using 0.8% agarose gel electrophoresis.

2.5. qRT-PCR

The total RNA was extracted from brain and lung tissues using RNAiso plus reagent (Takara, Dalian, China). The RNA concentration was measured using Nanodrop tools/primer-blast/). All primer sequences are provided in Table. Finger biopsies of two-week-old pups were digested, and PCR genotyped. Briefly, the finger biopsies were digested in GNTK buffer including Proteinase K at 60 °C overnight, boiled for 10 min next morning, centrifuged for 5 min at 12,000 rpm for 5 min, and 0.8 uL was used as the template. The PCR specific conditions include denaturation at 95°C for 4 min, amplification at 94 °C, 56 °C each for 30 s, and 72 °C for 45 s, consist of 32 cycles followed by 10 min extension at 72 °C and then cooled down to 4 °C. The PCR amplified gene fragment was analyzed using 0.8% agarose gel electrophoresis.

2.6. Extraction of RNA and library construction

Total RNA was extracted from the lung of Bex2+/+ and Bex2−/− mice, as mentioned above. The concentration, quality, and purity of RNA was confirmed on Nanodrop
ND-2000 spectrophotometer (Thermo Fisher Scientific, USA), Agilent 2100 Bioanalyzer (Santa Clara, CA, USA), and through gel electrophoresis. One μg RNA for each sample was used for the RNA-seq library preparation. RNA-seq was accomplished on the BGISEQ platform with paired-end reads.

2.7. RNA-Seq data analysis

Low value reads, adaptor-only, and reads with more than one undecoded base were distant using SOAPnuke (Cock et al., 2010) to acquire clean reads. Q20 (%), Q30 (%), and GC content (%) were analyzed. Reads were compiled into longer transcripts and aligned to reference genome (GCF_000001635.26_GRCm38.p6) using HISAT (Kim et al., 2015) and Bowtie2 tool (Langmead and Salzberg, 2012). The transcripts level was counted and presented as paired-end RNA-Seq FPKM (fragments per kilobase per million mapped reads) normalized reads. Differences in gene expression was recognized by annotation of two distinct libraries by applying Poisson distribution (Audic and Claverie, 1997) and expectation-maximization (RSEM) softwares (Li and Dewey, 2011). Following the normalization of the data, differentially expressed genes (DEGs) were analysed with [log2FC] ≥ +1 (upregulated), ≤ −1 (downregulated) and FDR <= 0.001 cut-off value. Kyoto Encyclopaedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment were executed for further functional analysis of distinctively expressed genes. According to DEGs, GO is functionally characterized as (i) biological process (BP), (ii) cellular component (CC), and (iii) molecular function (MF). According to their functional classes, GO functional enrichment was carried out using FDR ≤ 0.05 as the significant enrichment level. All the identified DEGs were aligned to the KEGG catalog for pathways analysis. Enrichment study of both GO and KEGG was executed using the phyper function in R software with a p-value < 0.05 (Kanehisa and Goto, 2000).

2.8. Statistical analysis

All the results are stated as means ± SEM (SEM). p-value < 0.05 (unpaired Student's t-test) was considered statistically significant. The graphs were executed with GraphPad Prism 8 for Mac (GraphPad Software).

3. Results

3.1. Bex2+/− mouse generation using CRISPR-Cas9 system

The Bex2 is a small gene and consists of only 1672 bps. Only the third exon contains the entire coding sequence (Figure 1A). To knock out the gene, the sgRNA1 was designed in front of the start codon, while the sgRNA2 (Table) was targeted downstream of the start codon but before stop codon (Figure 1A). To screen mutations, primer pair was designed flanking sgRNA targets with 752 bps of PCR product (Table).

Nine pups were obtained, numbered from 16 to 24 (Figure 1B). Fingers biopsies were used for genotyping, following the standard protocols, using forward and reverse primers (Table). The PCR product was subcloned into pMD18 plasmid (Takara, China) and sequenced. The sequencing results assured the correct deletion between two target sites (Figure 1C). Pups number 18 and 19 were found to be transgenic. These two mice were mated with wild-type C57BL/6 to confirm germline transmission. Downstream progenies were used for experimental analysis.

3.2. Confirmation of Bex2 expression level in lung and brain by qPCR

We performed quantitative real-time PCR (qPCR) to identify the distinctively expressed genes in the lung and brain between wild-type and knock-out mice. The qPCR primers are provided (Table). The mRNA levels in both lungs and brain were compared between Bex2+/+ and Bex2+/−. The relative mRNA expression results showed loss of the Bex2 (Figure 1D).

3.3. An overview of RNA-Seq libraries

The cDNA libraries were generated from lung mRNAs of Bex2+/+ and Bex2+/− mice (n = 3). BGISEQ platform was used for RNA-seq. The average yield was 6.77G for each sample. The average alignment ratio of each sample to reference sequence (Mus musculus genome reference GCF_000001635.26_GRCm38.p6) was 95.67%. The average alignment of gene set was 73.74% with a total of 17,244 genes detected. Number of genes in each FPKM level (FPKM <= 1, FPKM 1–10, FPKM >= 10) is listed in Table S1. In the first category, FPKM <=1, 4605 genes were expressed in Bex2+/+ while 4379 genes in Bex2+/−. In FPKM 1–10 category, 6281 genes were identified in Bex2+/+ and 6245 genes in Bex2+/−. In the category FPKM >=10, 6358 genes were found in Bex2+/+ and 6620 genes in Bex2+/−.

3.4. Differentially expressed genes identification in Bex2+/− lung

Hierarchical clustering was accomplished for the genes expressed only in Bex2+/− according to the difference in fold change values (log₂ FC). To see the gene expression and regulation (up/down) analysis, we performed a pairwise comparison between Bex2+/+ and Bex2+/−. A heatmap of the gene expression is provided (Figure 2A). We identified a total of 652 unigenes that were differentially expressed (DEG) (152 upregulated and 500 downregulated) in the lungs between Bex2+/+ and Bex2+/− (Figure 2B). According to each sample’s gene expression level, the significant DEGs detected were statistically plotted, and a volcano graph has been devised (Figure 2C).

3.5. DEG functional enrichment analysis

Gene ontologies offer a systematic language for genes and their products in three terms: molecular function (MF), biological process (BP) and cellular component...
Significant DEGs in the BP category contains regulation of the signaling receptor activity, inflammatory response and oxygen transport (Figure 3A). For the MF category, coreceptor activity, haptoglobin binding and protein binding bridging were prominent (Figure 3B). In the CC category, cardiac troponin complex, contractile fiber muscle myosin complex were the leading terms (Figure 3C).

Similarly, all the DEGs (652) were assigned for KEGG pathway analysis to see DEGs’ contribution in different pathways. Enrichment analysis confirmed a total of 217 KEGG pathways. The most enriched pathways are PPAR signaling pathway, cytokine-cytokine receptor interaction, adrenergic signaling in cardiomyocytes, adipocytokine signaling pathway, complement and coagulation cascades, calcium signaling pathway, hematopoietic cell lineage, carbon metabolism, NF-kB signaling pathway, and many more (Figure 4).

### 3.6. KEGG analysis of disease-associated pathways

All the DEGs were annotated to KEGG disease enrichment (Figure 5). The most significant pathways identified are dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), thalassemia, left ventricular noncompaction (LVNC), arterial septal defect, sickle cell anemia (SCA), alpha-1-antitrypsin (A1AT) deficiency, chronic obstructive pulmonary disease (COPD), etc. Among these, the COPD is related to lung physiology. Members of Serpina1 are involved in both A1AT and COPD.

### 3.7. Genes that encoding transcription factor proteins

Transcription factors (TFs) are the essential regulatory proteins that play roles in multiple biological processes. A certain number of TFs were identified in this study. The most enriched TFs are zf-C4 self-build, T-box, COE, TEA/ATTS domain, SRF transcription factor, cold shock DNA binding domain, and others (Figure 6).
3.8. DEGs validation by qPCR

To confirm the results of RNA-seq, ten genes (five upregulated and five downregulated) were measured for their relative expression level by qPCR. These genes were selected based on their important roles in lung development and function including *Tnfsf13*, *Csf3r,*
Figure 3. Gene Ontology (GO) analysis between Bex2⁻/⁻ and Bex2⁺/⁺ showing the different enriched ontologies of DEGs. (A) Biological process enrichment. (B) Molecular function enrichment. (C) Cellular component enrichment.

Figure 4. KEGG pathway enrichment of DEGs.
Retnlg, Dlg4, Mfap3, Myh6, Rnaset2b, Hba-a2, Acta1 and Car3. RNA-seq results are consistent with RT-qPCR results (Figure 7).

4. Discussion

BEX2 expression was favorably found in embryonic brain, which has a vital role in the nervous system development.
Bex2 synergistic effect with pathways under the control of Bex2−/− analysis from the lung tissue to investigate the genes and of the knocked-out mouse brain and lung indicated that relative mRNA expression level analysis by RT-qPCR shown that the is highly expressed in brain and lungs. Bex2 gene. The RT-qPCR analysis mouse model of the CRISPR-Cas9 technology to generate a global knock-out study has not been reported yet. In this study, we used using a gene targeting strategy, the lung’s transcriptomic KO mouse model has been generated (Ito et al., 2014), Bex2 embryonic liver, adult placenta, and lungs. Although the Bex2 KO mouse model has been generated (Ito et al., 2014), using a gene targeting strategy, the lung’s transcriptomic study has not been reported yet. In this study, we used CRISPR-Cas9 technology to generate a global knock-out mouse model of the Bex2 gene. The RT-qPCR analysis shown that the Bex2 is highly expressed in brain and lungs. The relative mRNA expression level analysis by RT-qPCR of the knocked-out mouse brain and lung indicated that the gene is successfully deleted. We performed RNA-seq analysis from the lung tissue to investigate the genes and pathways under the control of Bex2−/−. Surprisingly, most of the enrichment is related to the cardiac system. Similar results were obtained by other researchers also (Yee et al., 2018).

BEX2 is differentially expressed in breast tumors (Naderi et al., 2007), acute myeloid leukemia, and an increased expression was found in the MLL subtype (Rohrs et al., 2009). Lungs are not only the respiratory organs, but there are pieces of evidence that they also play a role in immunity (Lefrançais et al., 2017). We found that most of the KEGG pathways enriched in the immune system. PPAR signaling pathway, and cardiac muscle contraction, are the most significantly enriched pathways. Similarly, we found a prominent contribution of DEGs in complement and coagulation cascades and hematopoietic cell lineage pathways. We found that Tnfsf13, a member of TNFSF, is upregulated (DEG, \( \log_2 = 2.09 \)), indicates its role in synergistic effect with Bex2. Retnlg (DEG, \( \log_2 = 1.81 \)) is most likely expressed in nonadipose tissues (Lefrançais et al., 2017), indicates its possible role in the inflammation of lungs. CSF3, otherwise identified as G-CSF, exists within the primary 17q12-21 asthma predisposition and exacerbation locus (Bisgaard et al., 2009). Csf3r found in our upregulated DEGs (\( \log_2 = 1.96 \)) following the results of other researchers (Wang et al., 2019), may play a role in asthma. Similarly, the downregulated genes are comparable to the results of the other researchers. For instance, Myh6 among downregulated genes (\( \log_2 = -2.27 \)) is following the results of other researchers (Yee et al., 2018), where they found that neonatal hyperoxia reduced the gene expression involved in contractile function (like Myl4, Myl7, Myh6, Myh7) etc. A detailed study is needed to cross-check the synergism among these gene functions.

BEX2 has been shown that it activates the NF-kB signaling pathway in breast cancer cells (Naderi et al., 2007), promotes the human glioblastoma cells proliferation via the NF-kB signaling pathway (Meng et al., 2014). In brain tumors, BEX2 enhances cell moment and invasion in oligodendroglioma and glioblastoma cells. Besides, the expression of BEX2 protects glioma cells against apoptosis mediated via the JNK pathway and is essential for glioma cell proliferation through the NF-kB p65 (Naderi, 2019). In this study, NF-kB is one of the prominent enriched pathway. The most enriched gene among them is Tnf, which plays a central role in the development of the immune system. Several genes from mTOR Pathway were enriched including Prkat2 \( \log_2 = -1.08 \), Sox2 \( \log_2 = -1.10 \), Tnf 1.64 and Rragd \( \log_2 = -1.84 \). Similarly, Bcl2a1d, from NF-kB pathway is related to apoptotic process (Cartagena et al., 2013). Bdkrb2, \( \log_2 = 1.62 \), has a functional role related to reactive oxygen species (Perhal et al., 2019).

A more detailed study is needed to determine the synergism of Tnf and Bex2. A recent study explains the role of BEX2 gene in allergic airway inflammation (Kim et al., 2020).

In cell lines of leukemia, BEX2 was found to be expressed in MLLmu AML (Quentmeier et al., 2005). DEGs that enriched hematopoietic cell lineage pathway indicate that several genes e.g., Gm13305 \( \log_2 = 4.82 \), Cd19 \( \log_2 = 1.02 \), Cd22 \( \log_2 = 1.20 \), Csf3r \( \log_2 = 1.96 \), Il11a2 \( \log_2 = 3.92 \), Il1r2 \( \log_2 = 2.04 \), etc. were related to immune system. Besides, several DEGs identified were involved in the endocrine system and metabolic pathways. A more detailed study is required to use specific markers to validate the results of this bioinformatics analysis.

5. Conclusion

In this study, a mouse model of Bex2 global knock-out has been generated using the CRISPR Cas9 system. The research of genes and pathways under Bex2 regulation has identified many potentially important roles of this gene. We identified that several pathways are enriched related to the immune system. Besides the functions in respiration,
the lungs also play roles in the immune system and maybe a possible site for platelets' biogenesis. Moreover, several metabolic pathways are also identified. Several cardiac-related genes are found in DEGs. A detailed study is needed to determine the synergism between Bex2 and the most prominent DEGs.

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**Conflicts of interest**
The authors declare no conflict of interest.

**References**

Adlat S, Sah RK, Hayel F, Chen Y, Bah FB et al. (2020). Global transcriptome study of Dip2B-deficient mouse embryonic lung fibroblast reveals its important roles in cell proliferation and development. Computational and Structural Biotechnology Journal 18: 2381-2390.

Alvarez E, Zhou W, Witta SE, Freed CR (2005). Characterization of the Bex gene family in humans, mice, and rats. Gene 357: 18-28.

Ashburner M, Ball CA, Blake JA, Botstein D, Butler H et al. (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nature Genetics 25: 25-29.

Audic S, Claverie JM (1997). The significance of digital gene expression profiles. Genome Research 7: 986-995.

Bisgaard H, Bennelykke K, Sleiman PM, Brasholt M, Chawes B et al. (2009). Chromosome 17q21 gene variants are associated with asthma and exacerbations but not atopy in early childhood. American Journal Respiratory and Critical Care Medicine 179: 179-185.

Brown AL, Kay GF (1999). Bex1, a gene with increased expression in parthenogenetic embryos, is a member of a novel gene family on the mouse X chromosome. Human Molecular Genetics 8: 611-619.

Cartagena CM, Schmid KE, Phillips KL, Tortella FC, Dave JR (2013). Changes in apoptotic mechanisms following penetrating ballistic-like brain injury. Journal of Molecular Neuroscience 49: 301-311.

Cock PJ, Fields CJ, Goto N, Heuer ML, Rice PM (2010). The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. Nucleic Acids Research 38: 1767-1771.

Cong L, Ran FA, Cox D, Lin S, Barretto R et al. (2013). Multiplex genome engineering using CRISPR/Cas systems. Science 339: 819-823.

Costa V, Angelini C, De Feis I, Ciccodicola A (2010). Uncovering the complexity of transcriptomes with RNA-Seq. Journal of Biomedicine and Biotechnology 2010: 853916.

Fernandez EM, Diaz-Cespedes MD, Vilar M (2015). Brain expressed and X-linked (Bex) proteins are intrinsically disordered proteins (IDPs) and form new signaling hubs. PLoS One 10: e0117206.

Foltz G, Ryu GY, Yoon JG, Nelson T, Fahey J et al. (2006). Genome-wide analysis of epigenetic silencing identifies BEX1 and BEX2 as candidate tumor suppressor genes in malignant glioma. Cancer Research 66: 6665-6674.

Han C, Liu H, Liu J, Yin K, Xie Y et al. (2005). Human Bex2 interacts with LMO2 and regulates the transcriptional activity of a novel DNA-binding complex. Nucleic Acids Research 33: 6555-6565.

Ito K, Yamazaki S, Yamamoto R, Tajima Y, Yanagida A et al. (2014). Gene targeting study reveals unexpected expression of brain-expressed X-linked 2 in endocrine and tissue stem/progenitor cells in mice. Journal of Biological Chemistry 289: 29892-29911.

Kanehisa M, Goto S (2000). KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Research 28: 27-30.

Kazi JU, Kabir NN, Ronnstrand L (2015). Brain-Expressed X-linked (BEX) proteins in human cancers. Biochimica et Biophysica Acta 1856: 226-233.

Kim D, Langmead B, Salzberg SL (2015). HISAT: a fast spliced aligner with low memory requirements. Nature Methods 12: 357-360.

Kim SD, Kang SA, Kim YW, Yu HS, Cho KS et al. (2020). Screening and Functional Pathway Analysis of Pulmonary Genes Associated with Suppression of Allergic Airway Inflammation by Adipose Stem Cell-Derived Extracellular Vesicles. Stem Cells International 2020: 5684250.

Kumar D, Bansal G, Narang A, Basak T, Abbas T et al. (2016). Integrating transcriptome and proteome profiling: Strategies and applications. Proteomics 16: 2533-2544.

Langmead B, Salzberg SL (2012). Fast gapped-read alignment with Bowtie 2. Nature Methods 9:357-359

Lefrançois E, Ortiz-Muñoz G, Caudrillier A, Mallavia B, Liu F et al. (2017). The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. Nature 544: 105-109.

Li B, Dewey CN (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12: 323.
Meng Q, Zhi T, Chao Y, Nie E, Xu X et al. (2014). Bex2 controls proliferation of human glioblastoma cells through NF-κB signaling pathway. Journal of Molecular Neuroscience 53: 262-270.

Naderi A (2019). Molecular functions of brain expressed X-linked 2 (BEX2) in malignancies. Experimental Cell Research 376: 221-226.

Naderi A, Teschendorff AE, Beigel J, Cariati M, Ellis IO et al. (2007). BEX2 is overexpressed in a subset of primary breast cancers and mediates nerve growth factor/nuclear factor-kappaB inhibition of apoptosis in breast cancer cell lines. Cancer Research 67: 6725-6736.

Perhal A, Wolf S, Jamous YF, Langer A, Abd Alla J et al. (2019). Increased Reactive Oxygen Species Generation Contributes to the Atherogenic Activity of the B2 Bradykinin Receptor. Frontiers in Medicine (Lausanne) 6: 32.

Quentmeier H, Tonelli R, Geffers R, Pession A, Uphoff CC et al. (2005). Expression of BEX1 in acute myeloid leukemia with MLL rearrangements. Leukemia 19: 1488-1489.

Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA et al. (2013). Genome engineering using the CRISPR-Cas9 system. Nature Protocols 8: 2281-2308.

Rohrs S, Dirks WG, Meyer C, Marschalek R, Scherr M et al. (2009). Hypomethylation and expression of BEX2, IGSF4 and TIMP3 indicative of MLL translocations in acute myeloid leukemia. Molecular Cancer 8: 86.

Sah RK, Ma J, Bah FB, Xing Z, Adlat S et al. (2020). Targeted Disruption of Mouse Dip2B Leads to Abnormal Lung Development and Prenatal Lethality. International Journal Molecular Sciences 21: 8223.

Tan Y, Hu Y, Xiao Q, Tang Y, Chen H et al. (2020). Silencing of brain-expressed X-linked 2 (BEX2) promotes colorectal cancer metastasis through the Hedgehog signaling pathway. International Journal of Biological Sciences 16: 228-238.

Trapnell C, Roberts A, Goff L, Pertea G, Kim D et al. (2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature Protocols 7: 562-578.

Wang H, FitzPatrick M, Wilson NJ, Anthony D, Reading PC et al. (2019). CSF3R/CD114 mediates infection-dependent transition to severe asthma. Journal of Allergy and Clinical Immunology 143: 785-788 e786.

Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW et al. (2013). One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell 153: 910-918.

Yang QS, Xia F, Gu SH, Yuan HL, Chen JZ et al. (2002). Cloning and expression pattern of a spermatogenesis-related gene, BEX1, mapped to chromosome Xq22. Biochemical Genetics 40: 1-12.

Yee M, Cohen ED, Dommm W, Porter GA, Jr McDavid AN et al. (2018). Neonatal hyperoxia depletes pulmonary vein cardiomyocytes in adult mice via mitochondrial oxidation. American Journal Physiology-Lung Cellular and Molecular Physiol 314: L846-L859.
Supplementary Table.

Table S1. Statistics on the number of genes in three situations of FPKM (FPKM <= 1, FPKM 1–10, FPKM> = 10).

| Range     | Bex2−/− lung expression | Bex2+/+ lung expression |
|-----------|--------------------------|-------------------------|
| FPKM >=10 | 6358                     | 6620                    |
| FPKM 1–10 | 6281                     | 6245                    |
| FPKM <=1  | 4605                     | 4379                    |