**Table S1**

|  |  |
| --- | --- |
| **KPNA2 genotype of parents** | **LacZ-positive embryos** |
| HET mother  WT father | 56/57 zygotes (98%)  25/51 2cell embryos (49%)  43/78 4cell embryos (55%) |

Breeding of females with one allel of β galactosidase gene under the KPNA2 promoter and WT males results in embryos that either express β galactosidase or not. According to Mendelian rules, around 50% of embryos should be positive. Number of females n=8.

**Table S2**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **gene** | **primer sequence** | **fragment length (bp)** | **annealing temp.** | **number of cycles** |
| Kpna2  (genotyping) | 5’-AGT TCT GAT GGG CAA CCA AG-3’  5’-CTT AAT GTG GGC AGC ACC ATC-3’  5’-CCT CCG CAA ACT CCT ATT TC-3’ | WT: 794  KO: 597 | **59°C** | **35** |
| Kpna2 (RT-PCR) | 5‘-GAA GGG TAG CAG ACG TTT CC-3‘  5’-AAC AAT GTC CTC AAC AGA CC-3‘ | 356 | **62°C** | **30** |
| Kpna1 (RT-PCR) | 5‘-CGT CTC CCT CTT CGT AGT G-3‘  5‘-CTT CTT CCT CCC TCC TCC TG-3‘ | 144 | **62°C** | **30** |
| Kpna3 (RT-PCR) | 5’-TGA CAG TTG AAC TCC GGA AG-3’  5’-AGC ACT CAA CTG GAC TAC AG-3’ | 179 | **62°C** | **30** |
| Nme2(RT-PCR) | 5’-CGA CTA CAC TTC TTG CCT CC-3’  5’-GCT TCA GGT GTT CTT CAG AG-3’ | 176 | **62°C** | **30** |
| Eif1a (RT-PCR) | 5'-ATG CTG GGA AAT GGA CGG TT-3'  5‘-CAG AGA ACT TGG AAG GTA GC-3’ | 350 | **62°C** | **30** |

Primer sequences and PCR conditions

**Table S3**

|  |  |  |
| --- | --- | --- |
| **name** | **company** | **conditions** |
| anti-KPNA1 (rabbit) | selfmade (peptide MTTPGKENFCL) | 1:2,000 |
| anti-KPNA2 (goat) | Origene (TA302917) | 1:8,000 (Western Blot);  1:250 (Immunofluorescence) |
| anti-KPNA3 (rabbit) | selfmade (peptide MAENPGLENHRIC) | 1:5,000 |
| anti-KPNA4 (rabbit) | selfmade (peptide NSSANVPTEGFQF) | 1:2,000 |
| anti-KPNA6 (rabbit) | selfmade (peptide QPEAPMEGFQL) | 1:10,000 |
| anti-PCNA (mouse) | Cell Signaling (2586) | 1:1,000 |
| anti-α-tubulin-FITC | Sigma Aldrich(F2168) | 1:2,000 |
| anti-Oct4 (mouse) | Santa Cruz (sc-5279) | 1:100 |
| anti-GST (mouse) | Santa Cruz (sc-138) | 1:1,000 |
| anti-Sall4 (mouse) | Santa Cruz (sc-101147) | 1:100 |
| anti-Brg-1 (rabbit) | Santa Cruz (sc-10768) | 1:100 |
| anti-Stella (mouse) | Millipore (MAB 4388) | 1:100 |
| anti-H3K9me2 (rabbit) | Millipore (07-441) | 1:100 |
| anti-HDAC1 (mouse) | Abnova (11238-3E1) | 1:100 |
| anti-Npm2 (goat) | Santa Cruz (sc-98049) | 1:100 |
| anti-RSL1D1 (rabbit) | Biorbyt (orb704476) | 1:150 |
| anti GAPDH (rabbit) | Cell Signaling (2118) | 1:1,000 |
| IRDye 800 donkey anti-mouse | LiCor (926-32212) | 1:10,000 |
| IRDye 800 donkey anti-rabbit | LiCor (926-32213) | 1:10,000 |
| IRDye 800 donkey anti-goat | LiCor (926-32214) | 1:10,000 |
| goat anti mouse HRP | DAKO (P0447) | 1:5,000 |
| goat anti-mouse Alexa 488 | Invitrogen (A110019) | 1:500 |
| donkey anti-rabbit Cy3 | Jackson ImmunoResearch (711-165-152) | 1:500 |
| donkey anti-rabbit Alexa 488 | Invitrogen (A21206) | 1:500 |
| donkey anti-goat Alexa 488 | Invitrogen (A11055) | 1:500 |

**Supporting Figure Legend**

**Fig. S1 Generation and Characterization of KPNA2 KO mice.** (A) A genetrap cassette containing β-galactosidase and neomycin resistency was introduced into intron 3/4 of the KPNA2 gene. This results in aborted transcription of KPNA2 with formation of a fusion protein containing the importin beta binding domain of KPNA2 and β-galactosidase-neomycin fusion protein. (B) RT-PCR of KPNA2 in different tissues. M: 100bp marker. (C) Western Blot for KPNA2 in different tissues. KPNA2 is expressed mainly in spleen, thymus, testis, and, to a lesser extend, ovary of wildtype mice. *C*: Mouse testis extract served as positive control. GAPDH served as housekeeping control. (D) Body weight of female KPNA2 KO, HET and WT mice at various ages.

**Fig. S2 KPNA2 KO embryos have a tendency for embryonic developmental delay and are born at lower numbers.** (A) Intrauterine implantation sites reveal a slight reduction in embryo number in KPNA2 HET mothers compared to WT. (B) Higher tendency for empty implantation sites in KPNA2 HET mothers. (C) No difference could be observed in the ratio of avital or developmentally delayed embryos in KPNA2 HET mothers compared to KO. (C) Genotyping of offspring at different time points. Note that KPNA2 KO embryos display a tendency to be smaller. 5 WT females and 12 HET females ware analyzed. *N:* Number of embryos analyzed.

**Fig. S3 Normal nuclear expression of the transcription factor Oct4 in KPNA2 KO embryos.** (A) Immunofluorescence staining of Oct4 reveales a regular localization in germinal vesicles, pronuclei and nuclei (green); DNA is visualized with DAPI (blue). Scale bar 25μm. (B) Oct4 binding assay using KPNA-GST and 35S-methionine labelled in vitro transcribed and translated (IVTT) Oct4. The binding assay confirms a specific Importin β-dependent binding of all tested KPNA paralogues to Oct4. *M* marker; *C* positive control (IVTT Oct4 10% input).

**Fig. S4 Nuclear localization of various maternal effect genes in early embryos from KPNA2 KO mothers.** Immunofluorescence analysis reveals a regular nuclear localization of the transcription factor Sall4, the transcriptional activator Brg-1, the germ cell specific protein Stella and the histone deacetylase HDAC1 in embryos of KPNA2 KO females. The methylation of histone H3 at lysin9 did not reveal any differences between WT and KO zygotes. Only embryonic stages with a relevant expression in WT embryos are displayed. Scale bar 25 μm.