Supporting Information

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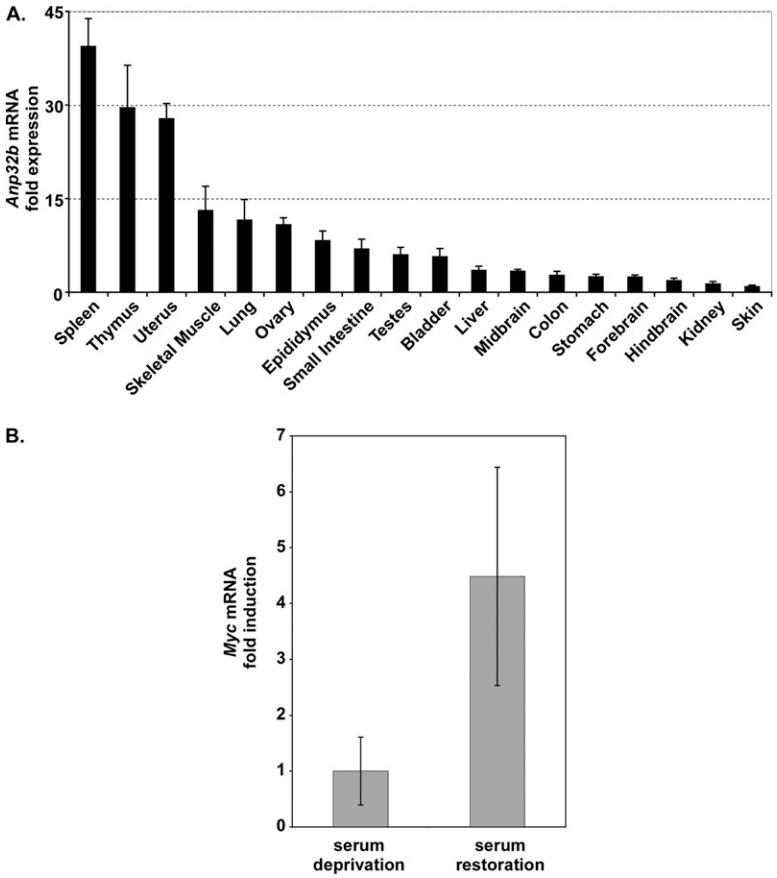

Fig. S1. Correlation of elevated *Anp32b* mRNA expression with cell proliferation. (*A*) Quantitation of *Anp32b* mRNA levels in normal tissues. Total RNA from the indicated tissues of 8-wk-old wild-type (WT) C57BL6 mice was harvested and subjected to quantitative RT-PCR to detect expression of *Anp32b* mRNA. Values were normalized to TATA-box–binding protein (*Tbp*) expression and compared with the expression level in the skin. Data shown are the mean fold induction in relative *Anp32b* mRNA expression ± SE. Results shown are representative of independent RNA isolations from three pairs of different WT mice. (*B*) Up-regulation of *Myc* mRNA expression. WT mouse embryo fibroblasts (MEFs) were deprived of serum for 72 h and then resupplied with medium containing 10% FBS for 24 h. *Myc* mRNA levels were determined by quantitative RT-PCR relative to *Actb* (β-actin). Data shown are the mean ± SD of two independent WT MEF cultures. No statistically significant differences were detected.

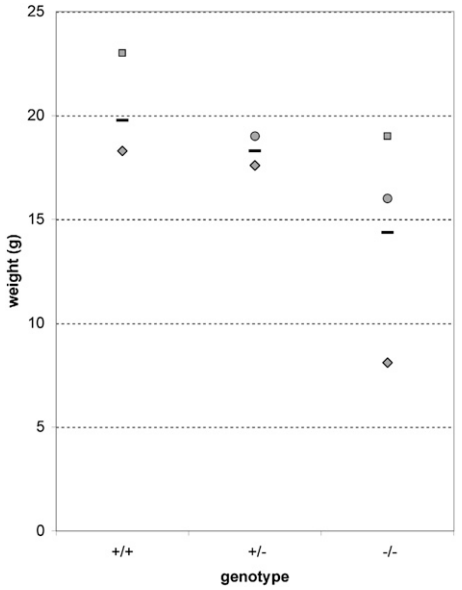

Fig. S2. Weights of Anp32b-deficient male mice. *Anp32b*$^{-/-}$ male mice were weighed alongside their *Anp32b*$^{+/+}$ or *Anp32b*$^{+/-}$ littermates at 3 wk of age. Results shown are values per individual mouse. Horizontal bars are mean values. Although no statistically significant difference was apparent for male mice in this analysis, when these data were combined with those for female *Anp32b*$^{-/-}$ mice, a statistically significant relationship between loss of *Anp32b* and reduced body weight became apparent.

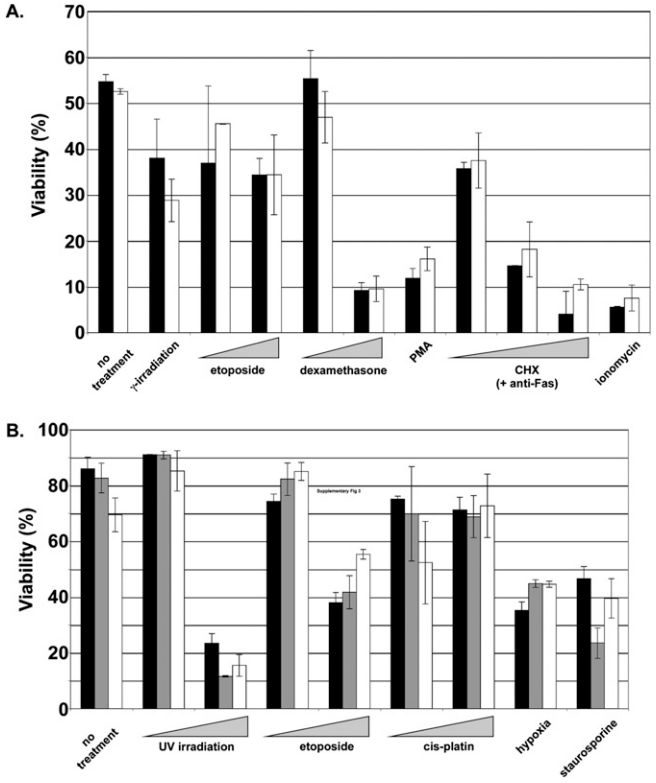

Fig. S3. No aberrant apoptotic response in Anp32b-deficient cells. Viability of (*A*) thymocytes and (*B*) transformed mouse embryo fibroblasts from *Anp32b*$^{+/+}$ (black bars), *Anp32b*$^{+/-}$ (gray bars), or *Anp32b*$^{-/-}$ (open bars) mice as determined by propidium iodide exclusion. Cells were exposed to the following apoptotic stimuli for 18 h: 1.0 Gy γ-irradiation; 0.2 or 1.0 μM etoposide; 0.1 or 1.0 μM dexamethasone; 50 ng/mL phorbol myristate acetate (PMA); 1 μg/mL anti-Fas antibody (clone Jo2; BD Biosciences) plus 0.01, 0.1, or 1.0 μg/mL cycloheximide (CHX); 1 μM ionomycin; 20 or 60 mJ/cm^2 UV light; 1.0 or 5.0 μM cis-platin; culture for 20 h in 0.2% oxygen (hypoxia) or 10 μM staurosporine. Results shown are the mean ± SD (n = 3/stimulus) and represent two independent trials. No significant differences were detected.

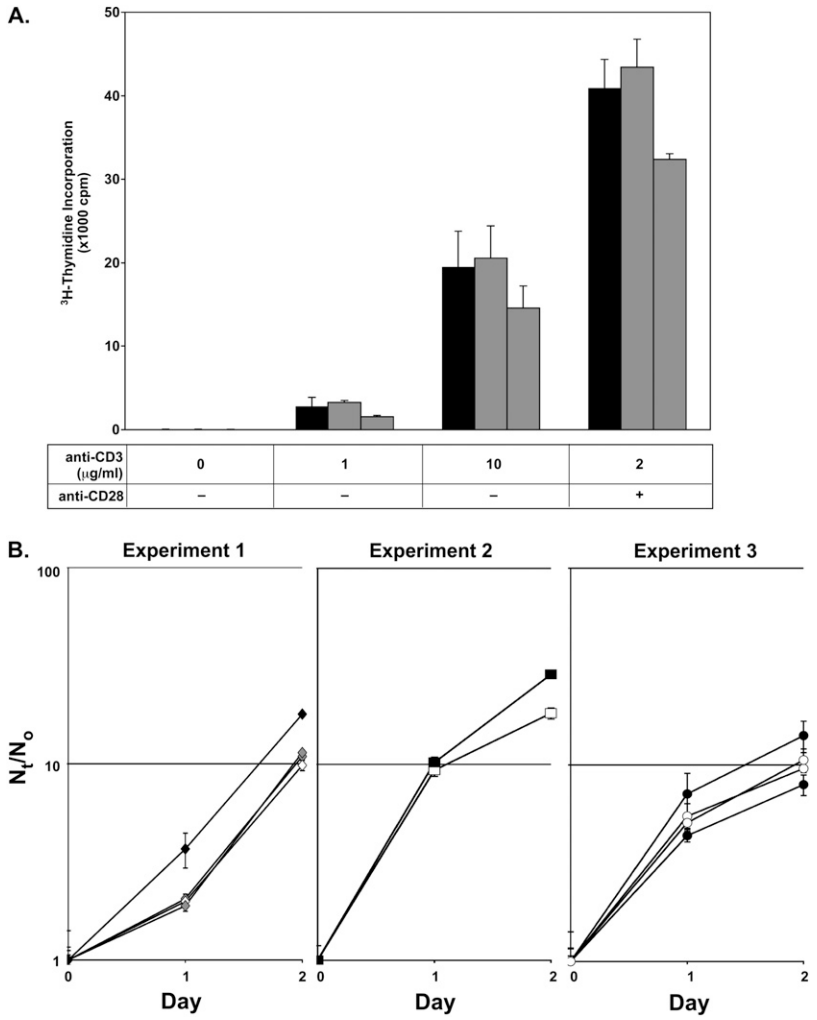

Fig. S4. No proliferation defects in Anp32b-deficient cells. (*A*) Normal T-cell proliferation. Peripheral T cells purified from *Anp32b*$^{+/+}$ (black bars) or *Anp32b*$^{-/-}$ (gray bars) mice were stimulated with plate-bound anti-CD3 antibody with or without 0.1μg/mL anti-CD28 antibody. Proliferation was determined by [^{3}H]-thymidine incorporation assays. Results shown are the mean ± SD (n = 3). (*B*) Normal primary mouse embryo fibroblast (MEF) proliferation. Primary MEFs were established from *Anp32b*$^{+/+}$ (black symbols), *Anp32b*$^{+/-}$ (gray symbols), or *Anp32b*$^{-/-}$ (open symbols) mice, and proliferation in culture was monitored for 2 d. N_t/N_o represents the cell number at a given time normalized to the cell number at day 0. Three experiments involving three independent MEF isolations are shown. Results shown for each data point are the mean ± SD of technical duplicates. For *A* and *B*, no significant differences were detected.

Table S1. Single functional *Anp32b* allele is sufficient for mouse survival

Anp32b status:	+/+									+/–									–/–								
Anp32a status:	+/+			+/–			–/–			+/+			+/–			–/–			+/+			+/–			–/–		
Anp32e status:	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	–/–	+/+	+/–	–/–
Mendelian	3	5	3	5	11	5	3	5	3	5	11	5	11	21	11	5	11	5	3	5	3	5	11	5	3	5	3
Expected	4	8	4	8	16	8	4	8	4	6	12	6	12	24	12	6	12	6	1	2	1	2	3	2	1	2	1
Observed	6	11	4	6	19	15	1	9	2	6	13	8	9	27	17	6	4	6	0	0	1	0	0	0	0	0	0

Numbers of triple-mutant mice of the indicated genotypes arising from the intercrossing of triply heterozygous *Anp32a*$^{+/-}$;*Anp32b*$^{+/-}$;*Anp32e*$^{+/-}$ mice. "Mendelian" numbers are the distribution for the indicated total number of mice under conditions of normal Mendelian segregation. "Expected" numbers are the distribution for the Mendelian distributed numbers with integration of the observed rates of *Anp32b* lethality (Table 1). "Observed" indicates the actual numbers of mice of the indicated genotypes obtained.

Table S2. Primer sequences used in this analysis

Name	Use	Sequences
32b 1	*Anp32b* qPCR	5′-AGCCGTTCGAGAACTTGTCTT-3′
32b 2	*Anp32b* qPCR	5′-CAGGTTATTGCCACTTAGGTTCA-3′
Tbp 1	*Tbp* qPCR	5′-GCTCTGGAATTGTACCGCAG-3′
Tbp 2	*Tbp* qPCR	5′-CTGGCTCATAGCTCTTGGCTC-3′
Myc 1	*Myc* qPCR	5′-CTGGATTTCCTTTGGGCGTT-3′
Myc 2	*Myc* qPCR	5′-TGGTGAAGTTCACGTTGAGGG-3′
Actin 1	*Actb* (β-actin) qPCR	5′-TAGCCATCCAGGCTGTGC-3′
Actin 2	*Actb* (β-actin) qPCR	5′-TCAGGATCTTCATGAGGTAG-3′
18S 1	*Rn18S* (18S rRNA) qPCR	5′-AGTTCCAGCACATTTTGCGAG-3′
18S 2	*Rn18S* (18S rRNA) qPCR	5′-TCATCCTCCGTGAGTTCTCCA-3′
uAOH 1	Cloning upstream arm of homology	5′-CCCCTCGAGTCTTTGGACCATGTTATAAATGTGTACTAGCTGGC-3′
uAOH 2	Cloning upstream arm of homology	5′-GGGGTCGACTCACTACCATCACTCAGAGTTCCAATAGTCTTCTG-3′
dAOH 1	Cloning downstream arm of homology	5′-GGGTCTAGAAACTCAATAGTAGATCAGGCTGGC-3′
dAOH 2	Cloning downstream arm of homology	5′-GGGTCTAGACCACACAACTCAGCAGTTCTCAG-3′
FP fwd	Flanking Southern probe synthesis	5′-CACCTGGAGGGTTCACTGAGAATAAATTG-3′
FP rev	Flanking Southern probe synthesis	5′-CACTACCAAAATGCACAGACGTAAGGTTAAG-3′
32b wt-rev	*Anp32b* wt genotyping	5′-GGCACACTTACAGAGTTCGGTTCACAAGTTGAGC-3′
32b-fwd	*Anp32b* wt and mutant genotyping	5′-GGTCACAGTGTCTCTTCACATCAGTAAAACCCTAAGTAAG-3′
32a wt-rev	*Anp32a* wt genotyping	5′-GAATGAGGTGAGAGGTCAAGATTCAGCTGC-3′
32a-fwd	*Anp32a* wt and mutant genotyping	5′-CTAATCCCTCTTCAGAGAACTGCCCTGTTCAAG-3′
32e wt-rev	*Anp32e* wt genotyping	5′-GGAGTCACGAGCTAACTGCACTGTCACCAAC-3′
32e-fwd	*Anp32e* wt and mutant genotyping	5′-GGAGGAAGATGACGATGAGGATGAAGCTGG-3′
neo-rev1	*Anp32b*, *Anp32a*, and *Anp32e* mutant genotyping	5′-CTACCGGTGGATGTGGAATGTGTGCG-3′

qPCR, quantitative real-time PCR.