**A**

tk neo

Insertion vector

1 2* 3

Mouse genome

1 2 3

BstEII

Select with G418

A B C

1 2* 3 neo tk 1 2 3

Intrachromosomal recombination

tk neo

Select with gancidovir

A B C

desired product

*

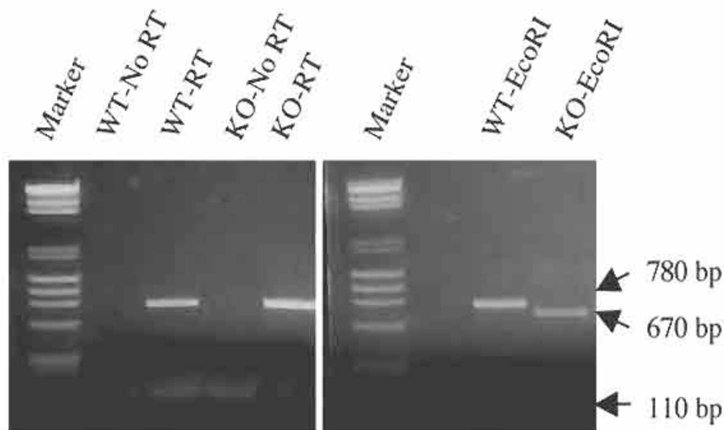
gancidovir resistant

excised from chromosome

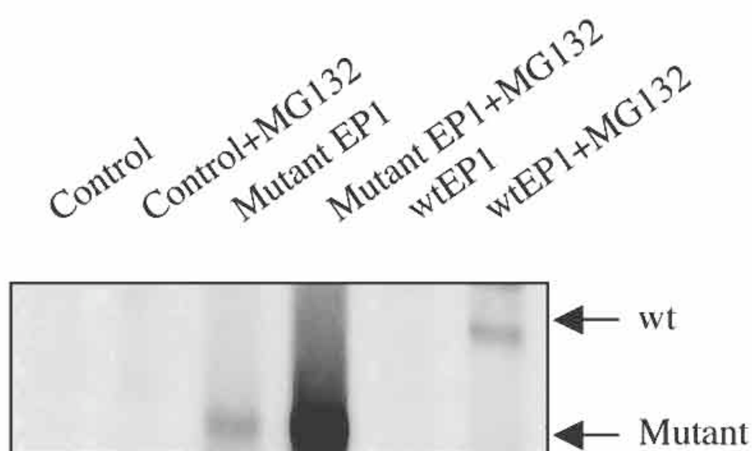
tk neo

TK Neo

**B**



A




B

anti-HA Ab

anti-EP1 Ab

control

wild type

mutant

**Supplemental materials:**

**Figure 1:**

A) Schematic of the "Hit & Run" strategy used to disrupt mouse EP1 gene function. The targeting plasmid was linearized with BstEII and electroporated into TL1 embryonic stem cells. A single cross-over event occurring via homologous recombination was selected for using G418, resulting in the tandem insertion of the mutant EP1 gene as a direct repeat. Spontaneous intra-chromosomal recombination between the direct repeats was then selected for using ganciclovir to delete the tandem selection cassettes (Neo & TK), the plasmid backbone and one repeat of the targeted locus. In half of these recombination events the native EP1 gene is deleted leaving the mutant EP1 fragment containing a premature stop codon and a newly-created EcoRI site. The dashed line indicates the premature stop codon and * represents the new EcoRI restriction site. Correctly targeted ES cells were then injected into 3.5 d blastocysts obtained from naturally mated C57Bl/6 females and transferred to pseudo-pregnant ICR recipients.

B) Sequential selection for the desired targeting events were examined by Southern Blot analysis using probe A, B, and C (Figure 1b). Mice with targeted EP1 gene allele were backcrossed to either C57Bl/6 or 129S6/Sv/EvTac background until congenic background was reached (N10). Wild-type and targeted EP1 alleles were genotyped using both Southern blot and PCR-based methods (figure 1c). Intercrossing mice heterozygous for the EP1 receptor yielded a near-perfect Mendelian pattern of inheritance (27%/52.4%/20.6% for wild-type/heterozygous/homozygous ratio) with male to female ratio of 1:1.08.

**Supplemental Figure 2: Truncation of the mutant EP1 receptor.** To confirm that the engineered EP1 mutation resulted in the expected truncation of the EP1 receptor protein, HEK293 cells transfected with pcDNA3.1 inserts of hemagglutinin (HA) tagged EP1 cDNAs in which hemagglutinin (HA) nucleotide fragment was fused in-frame to the 5' end of the $EP1^{+/+}$ and $EP1^{-/-}$ coding sequences.

A) HEK293 cells were metabolically labeled with $^{35}S$-methionine, and the protein lysate immuno-precipitated with anti-hemagglutinin antibody (HA-ab). To increase receptor protein expression levels, degradation was inhibited with the proteosomal inhibitor MG132. Anti-HA-Ab pulled down a radiolabeled protein fragment of the expected size of 42 kDa from lysates of HEK293 cells transfected with wild type EP1 expression vector. In contrast, transfection with the mutant EP1 receptor precipitated a smaller protein of ~31 kDa.

B) Immuno-fluorescent labeling of transiently transfected COS-1 cells revealed that N-terminal HA-tag was detected in COS-1 cells transfected with either the wild-type or mutant EP1 construct, In contrast a C-terminal anti-EP1 antibody failed to detect EP1 protein expression in the mutant EP1-transfected cells, consistent with truncation EP1 protein was made by mutant EP1 cDNA. Immunostains of anti-HA and anti-EP1 C-terminal antibody and visualized using FITC- or Cy3-conjugated $2^0$ Ab respectively.