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Supplemental Information

TIGAR Is Required for Efficient

Intestinal Regeneration and Tumorigenesis

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Inventory of Supplemental Information:

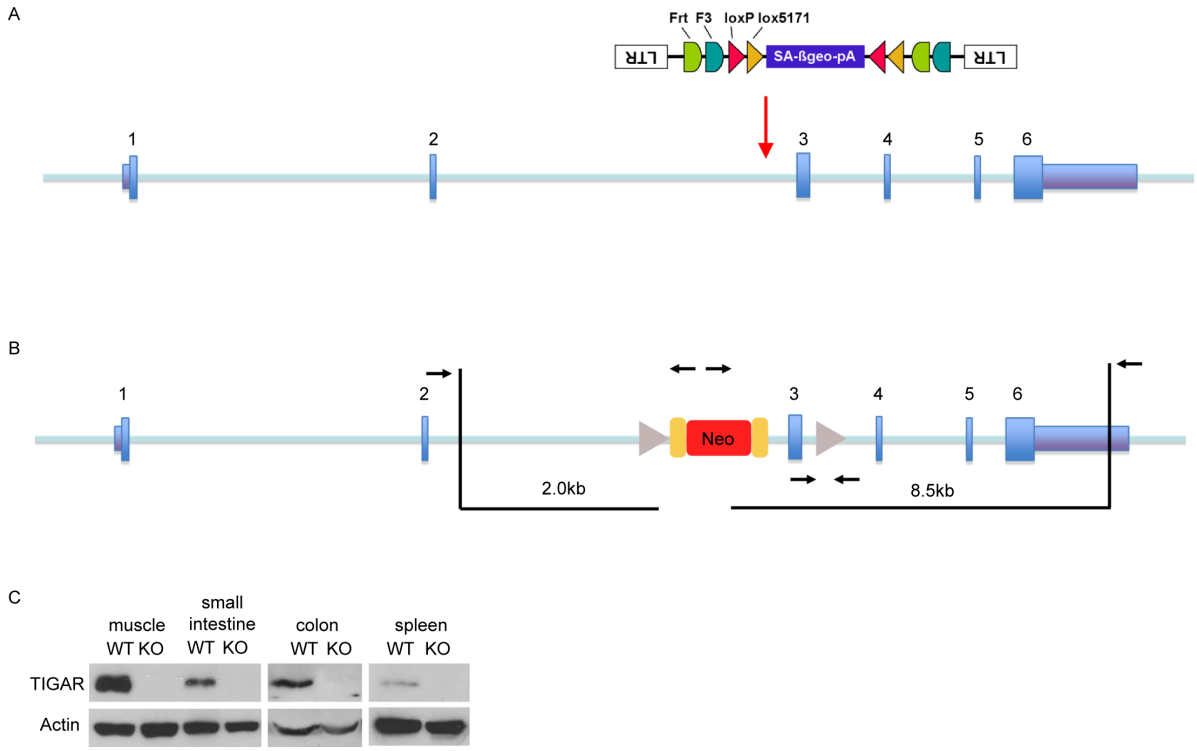

Figure S1: Generation of *TIGAR*$^{-/-}$ and *TIGAR*$^{fl/fl}$ mice, related to Figure 1. (A) Diagram of the gene trap TIGAR allele in ES cell clone 9630033F20RikGt(EUCE0047g05) Hmgu (Helmholtz Zentrum, Munich) used in generating *TIGAR*$^{-/-}$ mice, showing the splice acceptor (SA) site, β-Geo, and polyadenylation site (PA) integrated into intron 2. (B) Diagram of the TIGAR conditional allele (*TIGAR*$^{fl/fl}$). Conditional KO is designed to remove exon 3 of the transcript following recombination at the loxP sites (grey) causing a frame-shift in the mRNA. The Neo selectable marker is flanked by FRT sites (orange) to allow removal. Primer binding sites are indicated by arrows. (C) Western blot analysis of the indicated tissues from wild type (*TIGAR*$^{fl/fl}$) and TIGAR deficient (deleter cre+/ *TIGAR*$^{fl/fl}$) animals.

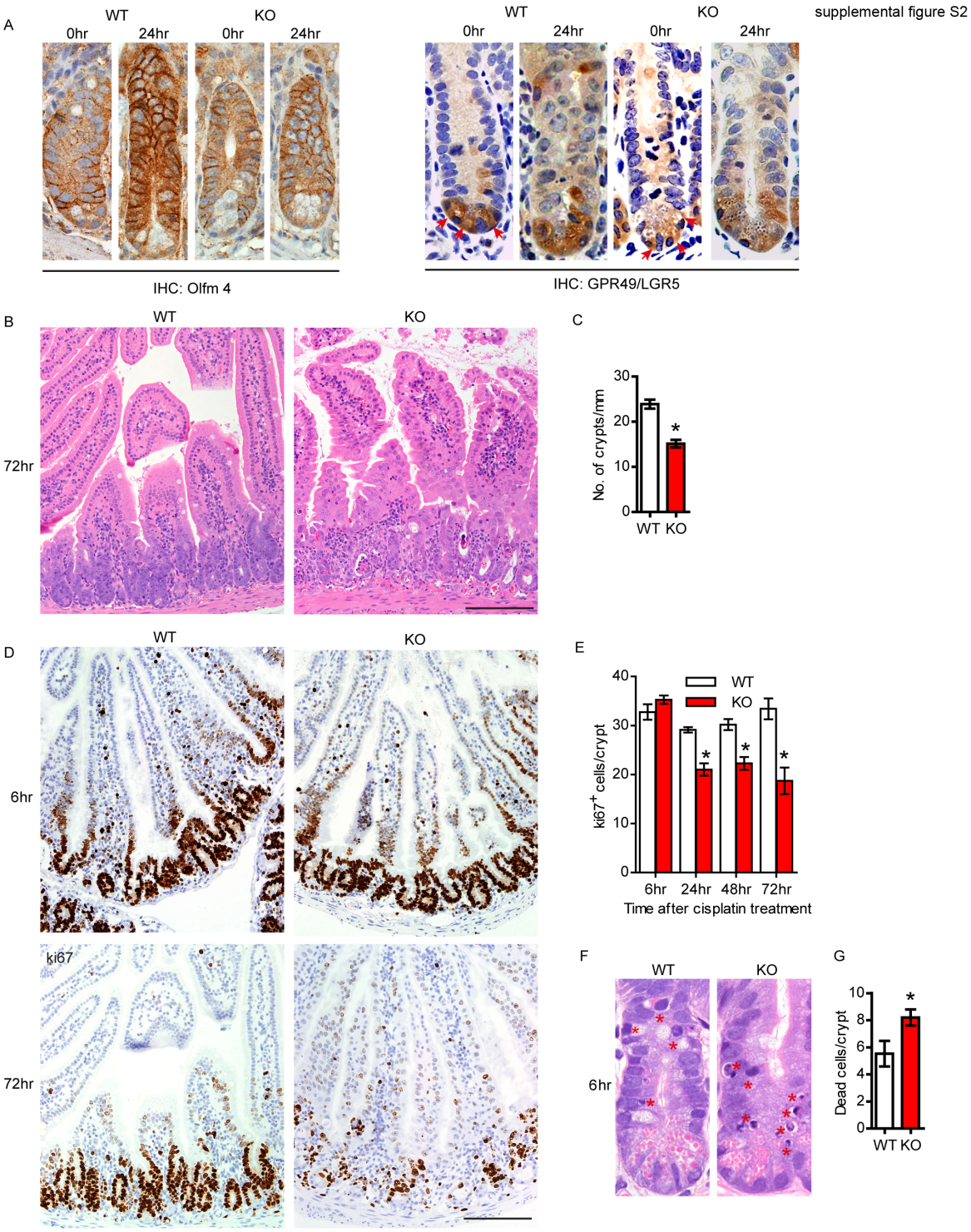

Figure S2: TIGAR deficient mice have reduced regenerative capacity in the intestinal crypt, related to Figure 2. (A) Olfm 4 and LGR5 staining of wildtype (WT) and *TIGAR*$^{-/-}$ (KO) intestines before and 24 hours after IR treatment. (B) Small intestine from WT and KO animals 72 hours after cisplatin treatment. Bar = 200µm (C) Number of crypts per mm 72 hours after cisplatin treatment. * $p<0.05$ compared to WT (n=3). (D) Ki67 staining of WT and KO intestines at 6 and 72 hours after cisplatin treatment. Bar = 200µm (E) Quantification of Ki67^{+} cells at the indicated times after cisplatin treatment. * $p<0.05$ compared to WT (n=3). (F) Apoptosis in the small intestine 6 hours after cisplatin treatment. Asterisks denote cells with apoptotic nuclear morphology. (G) Number of apoptotic cells in the crypts 6 hours after cisplatin treatment. * $p<0.05$ compared to WT (n=3). Data are represented as mean ± SEM.

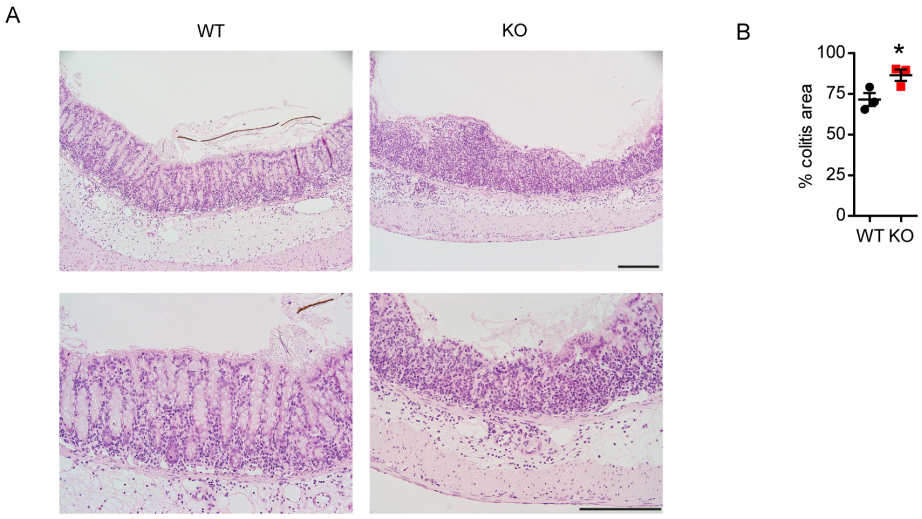

Figure S3: TIGAR deficient mice are more sensitive to DSS-induced colitis, related to Figure 3. (A) Colon from WT (*TIGAR*$^{fl/fl;+/+}$) and KO (*TIGAR*$^{fl/fl;cre/+}$) animals 2 days after 2% DSS treatment. Bar = 200µm (B) Percentage of colitis area 1 day after 3.5% DSS in WT and KO animals. * $p<0.05$ compared to WT (n=5). Data are represented as mean ± SEM.

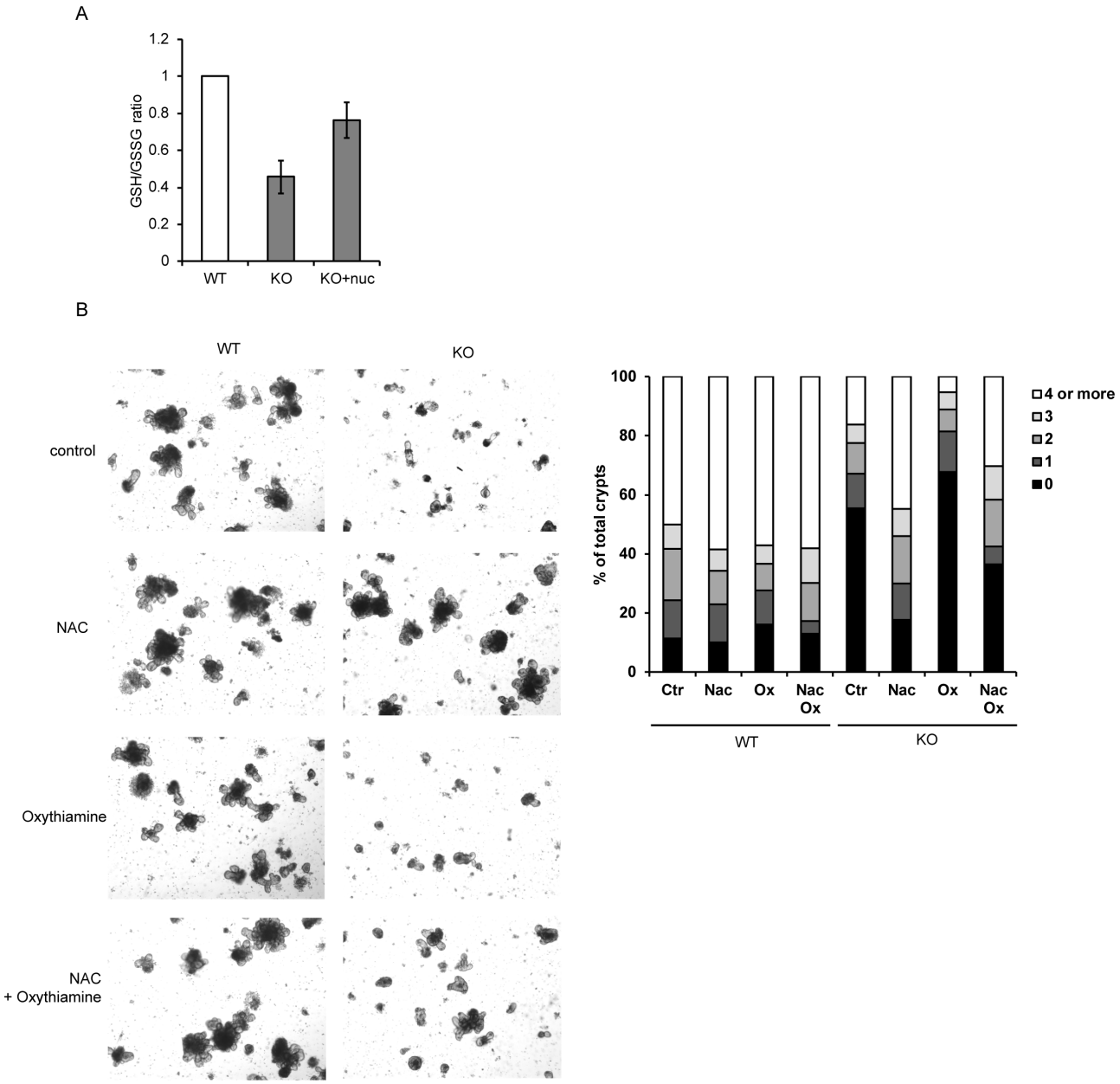

Figure S4: Effect of nucleosides, NAC and oxythiamine on *TIGAR*$^{-/-}$ intestinal crypt cultures, related to Figure 4. (A) Glutathione level (GSH/GSSG ratio) in WT, *TIGAR*$^{-/-}$ (KO), and KO cultures in the presence of nucleosides. (B) Crypt growth of WT and KO crypts in the presence of NAC and/or oxythiamine (Ox) (10mm). Data are represented as mean ± SEM (n=3).

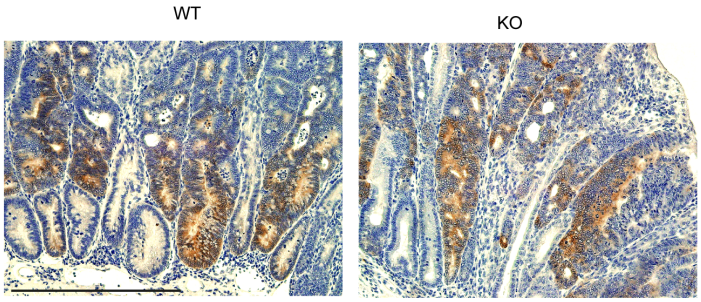

Figure S5: GFP staining in a mouse model of intestinal adenoma in the presence (WT) or absence (KO) of TIGAR, related to Figure 5. WT = *TIGAR*$^{+/+}$ *Lgr5-EGFP-IRES-creER*^{T2}*/APC*$^{fl/fl}$; KO = *TIGAR*$^{-/-}$ *Lgr5-EGFP-IRES-creER*^{T2}*/APC*$^{fl/fl}$. Bar = 200mm.

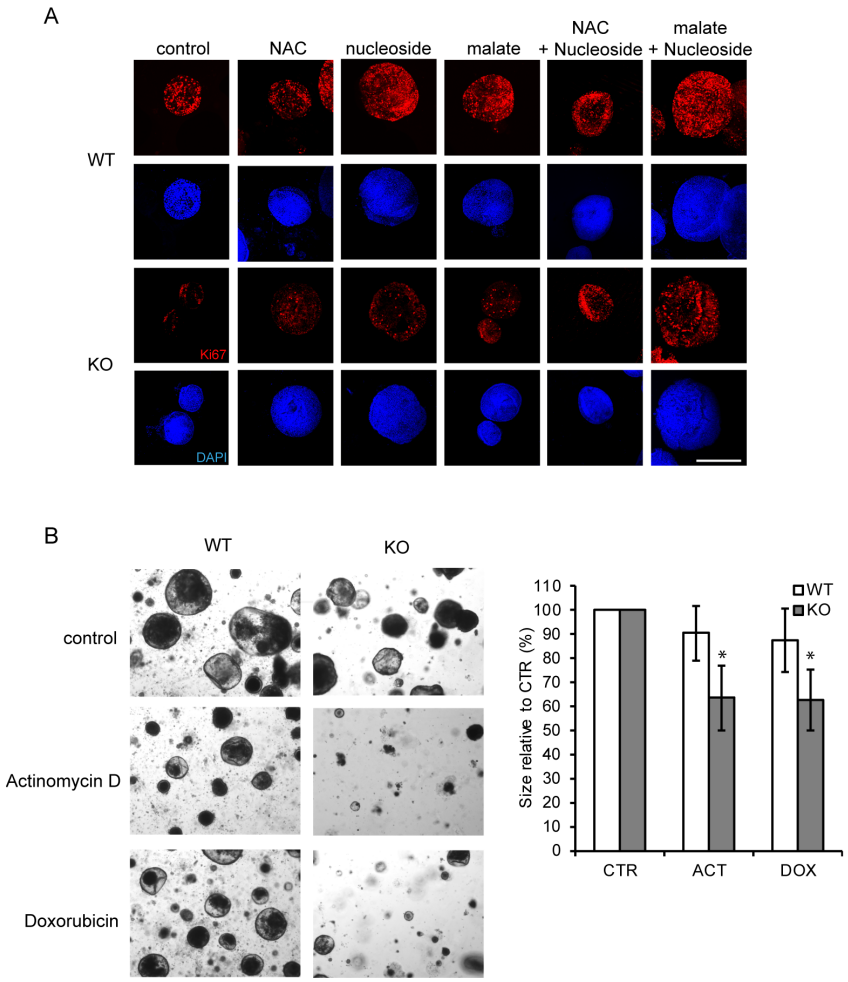

Figure S6: TIGAR deficient tumor organoid cultures, related to Figure 6. (A) Ki67 staining of the tumor organoid cultures from WT (*TIGAR*$^{+/+}$ *Lgr5-EGFP-IRES-creER*^{T2}*/APC*$^{fl/fl}$) and KO (*TIGAR*$^{-/-}$ *Lgr5-EGFP-IRES-creER*^{T2}*/APC*$^{fl/fl}$) animals with the indicated treatments for 5 days. Bar = 100µm. (B) WT and KO tumor organoid cultures were treated with the indicated drugs day two after plating, then three days later crypt size was measured. * $p<0.05$ compared to WT. Data are represented as mean ± SEM (n=3).

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIAL AND METHODS

Creation of *TIGAR*$^{-/-}$ and *TIGAR*$^{fl/fl}$ mice

TIGAR$^{-/-}$ mice

The gene trap embryonic stem (ES) cell line 9630033F20RikGt$^{(EUCE0047g05)Hmgu}$ was obtained from Helmholtz Zentrum, Munich. Location of the gene trap cassette was verified by screening on both the 5' and 3' sides of the gene trap using the primers GTTTTGCTTTGAGTCTCATGCTC and AAGGCGATTAAGTTGGGTAACGCC (on the 5' side) and GACGAGTTCTTCTGAGGGGATCAAT and GTGTAGGAGGACTGAGGGTACAG (on the 3' side). Mouse lines were derived by injection of ES cells into C57BL/6J blastocysts according to standard protocols (Nagy et al., 2003). Germline transmission was identified by coat colour and correct transmission of the transgene was confirmed by PCR to the Neo sequences in the gene trap cassette according to standard protocols using the primers GTTGTCACTGAAGCGGGAAG and GCGATACCGTAAAGCACGAG. Mice could subsequently be genotyped using the primers: CATTCCAAGTCCCATAGTGAGG and CAGTGCTACAGAGACAGCTTGC for the wild-type allele; GACACTAGACAATCGGACAGACAC and CAGTGCTACAGAGACAGCTTGC to detect the gene trap allele.
All experiments were performed under the UK Home Office guidelines.

TIGAR conditional (*TIGAR*$^{fl/fl}$) mice

To generate the conditional gene targeting vector, a 129 bacterial artificial chromosome for the *TIGAR* genomic locus was initially identified on Ensembl

(Adams et al., 2005). Three homology arms were subcloned from the BAC by recombineering in DY380 E. coli according to standard protocols (Liu et al., 2003). The three genomic homology arms were subsequently subcloned into pFlexible to generate the final targeting vector (van der Weyden et al., 2005). Conditional KO was designed to remove exon 3 (OTTMUSE00000313695) of the transcript and cause a frameshift in the message (Havana transcript RP23-8L20.1-001; OTTMUST00000058971).

$8x10^6$ mid-log phase HM1 ES cells were resuspended in 800µl Embryomax electroporation solution (Millipore) and mixed with 40µg of AscI-linearised targeting vector. Electroporation was performed under standard conditions (250V; 500µF; infinite resistance; cuvette width: 4mm) in a Biorad GenePulser XCell with capacitance extender. After plating onto DR4 irradiated MEF monolayers (Tucker et al., 1997) cells were maintained under regular ES medium for 24 hours before being placed under G418 selection for 6 days. G418 -resistant colonies were picked onto DR4 monolayers in 96-well plates and grown under G418 for 3 days. Colonies were subsequently harvested and saved as stock plates [under N2(l)] or plated onto gelatin-coated plates for growth and isolation of gDNA for screening.

Correct targeting of the vector to the *TIGAR* locus on both the 5' and 3' sides was confirmed using PCR on genomic DNA prepared from G418 -resistant colonies. PCR genotyping was done using Expand Long Template (Roche) according to the manufacturer's recommendations. Following confirmation of targeting by PCR, clones were recovered and expanded under G418 -ESC medium for 2-3 days then regular ES medium prior to storage. Primers used for genotyping targeted ES cells

were 5′ GGAGCCTGTTTTGCTTTGAG and TAGTTGCCAGCCATCTGTTG and 3' TGGCTGGACGTAAACTCCTC and TCTGCGCCTACAGAACACTG. The presence of the isolated 3' loxP was confirmed by PCR with the primers CCAGGAACACATTCCAGGTC and ACCACAGAGACGGACAAACC.

Following identification of correctly targeted clones, mouse lines were derived by C57BL/6J blastocysts as described above. Germline offspring were identified by coat colour and the presence of the modified allele was confirmed with the 3' loxP primers described above. Mice were subsequently crossed with a mouse line expressing Flpe (Tg(ACTFLPe)9205Dym) to delete the selectable marker by recombination at the FRT sites (Rodriguez et al., 2000). To generate null alleles of *TIGAR* from these floxed mice, we crossed the *TIGAR*$^{fl/fl}$ mice to a Cre deleter strain expressing Cre in the germline.

REFERENCES

Adams, D.J., M.A. Quail, T. Cox, L. van der Weyden, B.D. Gorick, Q. Su, W.I. Chan, R. Davies, J.K. Bonfield, F. Law, S. Humphray, B. Plumb, P. Liu, J. Rogers, and A. Bradley. 2005. A genome-wide, end-sequenced 129Sv BAC library resource for targeting vector construction. *Genomics*. 86:753-758.

Liu, P., N.A. Jenkins, and N.G. Copeland. 2003. A highly efficient recombineering-based method for generating conditional knockout mutations. *Genome Res*. 13:476-484.

Nagy, A., M. Gertsenstein, K. Vintersten, and R. Behringer. 2003. Manipulating the mouse embryo: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Rodriguez, C.I., F. Buchholz, J. Galloway, R. Sequerra, J. Kasper, R. Ayala, A.F. Stewart, and S.M. Dymecki. 2000. High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. *Nat Genet*. 25:139-140.

Tucker, K.L., Y. Wang, J. Dausman, and R. Jaenisch. 1997. A transgenic mouse strain expressing four drug-selectable marker genes. *Nucleic Acids Res*. 25:3745-3746.

van der Weyden, L., D.J. Adams, L.W. Harris, D. Tannahill, M.J. Arends, and A. Bradley. 2005. Null and conditional semaphorin 3B alleles using a flexible puroDeltatk loxP/FRT vector. *Genesis*. 41:171-178.