Tractable Cre-*lox* system for stochastic alteration of genes in mice

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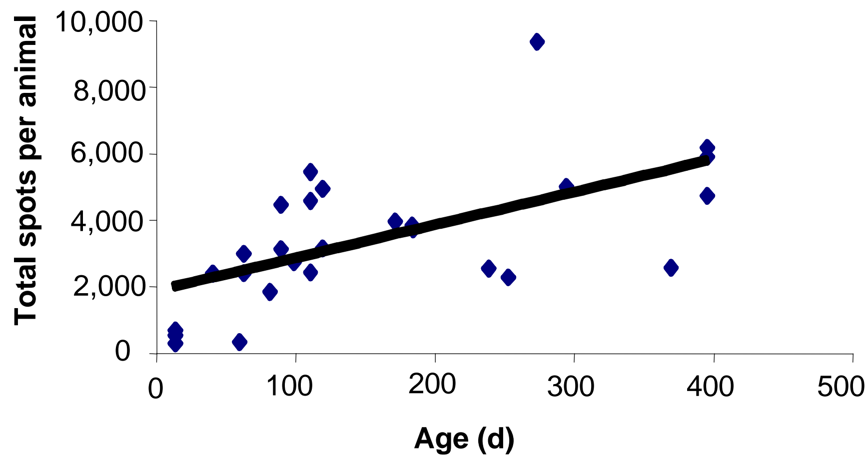
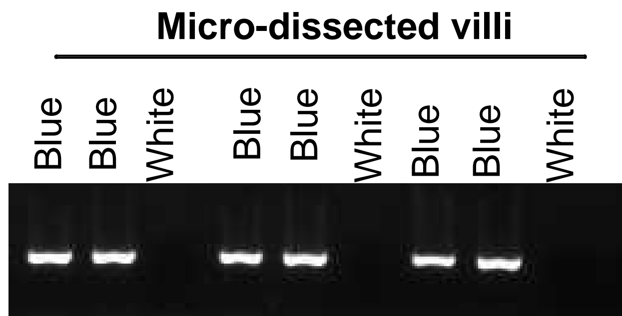
Supplementary figure and text:

Supplementary Figure 1 Characterization of Cre activation in *Pms2*^{cre} mice.

Supplementary Methods

Supplementary Figure 1. Characterization of Cre activation in *Pms2*^{cre} mice.





(**a**) Distribution of total number of "blue spots" per intestine as a function of age in *Pms2*$^{cre/cre}$, *Rosa26r* mice (n=27). (**b**) Post-Cre recombination PCR amplification products at the *Rosa26r* locus of individual micro-dissected villi.

Supplementary Methods

Generation of *Pms2*^{cre} carrying mice.

The SV40 NLS within Cre recombinase [4] was mutated from CCAAAGAAGAAGGAGAAG to CCAAAAAAAAAAAAGGAAG by site-directed mutagenesis. The changes preserved the NLS with the exception that a single additional adenosine nucleotide was added, inducing a +1bp frameshift. A splice acceptor (SA) and an internal ribosomal entry site (IRES) cloned 5' to the *cre* ATG and a *neo* cassette was cloned 3' to *cre*. The *IRES-cre-neo* was inserted between the 5' and 3' *Pms2* homology arms [5] using a 5' *Xho*1 adaptamer and a 3' blunt-end adaptamer, to put *Cre* under control of the *Pms2* promoter. To enrich for targeted clones by "negative selection", this construct was inserted into pTK1-TK2 using the *Xba*1 and *Bgl*II. The final targeting vector was linearized with *Sal*1 and electroporated into mouse ES cells G418-gancyclovir resistant ES cell colonies were screened by Southern for proper targeting at the *Pms2* locus as previously described [5]. Targeted ES cells were injected into blastocysts, implanted into pseudo-pregnant female mice, and chimeric animals were obtained. F1 offspring from chimera mice were genotyped with the following primers. The wild-type *Pms2* allele was detected with the primers PMS2A: TTCGGTGACAGATTTGTAAATG and PMS2W: TCACCATAAAAATAGTTTCCCG, giving a product of ~350bp. The *Pms2*^{cre} allele was detected with the primers CreF: AACATTCTCCCACCGTCAGT and CreR: CATTTGGGCCAGCTAAACAT, giving a product of ~300bp. Post-Cre recombination PCR amplification at the *Rosa26r* locus was detected with the primers PCbGAL1: GCAAGGCGATTAAGTTGGGTAACG and PCbGAL2: CAGTAGTCCAGGGTTTCCTTGATG, giving a product of ~320bp.

Pms2$^{cre/+}$ mice were mated with *Rosa26* conditional *LacZ* reporter mice (*Rosa26r*) [8] to generate *Pms2*$^{cre/+}$*,Rosa26r*, which were then bred to obtain *Pms2*$^{cre/cre}$*,Rosa26r*. *Pms2*$^{cre/+}$*,Rosa26r* mice were mated with *LSL-K-ras*$^{G12D\backslash+}$ [8] mice to generate *Pms2*$^{Cre/+}$,*LSL-K-ras*$^{G12D/+}$*,Rosa26r*. These were subsequently inbred to obtain *Pms2*$^{cre/cre}$,*LSL-K-ras*$^{G12D/+}$*,Rosa26r*. Animal protocols were approved by Oregon Health and Science University Department of Comparative Medicine and were in accordance with The Guide for Care and Use of Laboratory Animals as outlined by the Office of Laboratory Animal Welfare (OLAW) and the National Institutes of Health.

Fixation/staining/sectioning.

Whole mount β-galactosidase staining of intestines was performed as previously described [9]. Briefly, at appropriate time points mice were sacrificed by cervical dislocation. Intestines were dissected whole block, flushed with cold PLP (75mM Lysine buffer pH 7.4, 70mM NaPO4, 10mM NaIO4, 2% PFA), cut open along mesenteric line, pined onto dissection plates and PLP-fixed for 1 hour. Intestines were treated for 45 minutes with DTT solution (20mM DTT, 20% EtOH, 15mM Tris pH 8.0) followed by x-gal stain (2mM x-gal, 4mM each $K_3Fe(CN)_6$ $K_4Fe(CN)_6$-H2O, 2mM MgCl in PBS) 12h at 4°C. Blue villi emanating from a single crypt was designated as one "spot". Total number of spots were scored at 20x magnification. To determine the number of spots in each region, x-gal stained villi were counted from three 1-cm segments of each intestinal

region, averaged and extrapolated for the entire length of the region. "Spot size" was determined based on number of villi involved: 1 to 3, representing a single crypt, 4 to 10, representing 2-3 crypts, 11 to 50 and greater than 50, representing multiple crypts. Intestines were dehydrated to 70% ethanol, paraffin embedded and sectioned to 5 micron thickness. Sections were stained with hematoxylin and eosin.