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Supplemental Information

A Conditional System to Specifically Link Disruption of Protein-Coding Function with Reporter Expression in Mice

Shin-Heng Chiou, Caroline Kim-Kiselak, Viviana I. Risca, Megan K. Heimann, Chen-Hua Chuang, Aurora A. Burds, William J. Greenleaf, Tyler E. Jacks, David M. Feldser, and Monte M. Winslow

Supplemental Information

Targeting vector and animal availability.

To obtain the targeting vectors in any of the three reading frames, contact the corresponding author. Complete sequence files are available upon request. *Hmga2*^{CK} and *Hmga2*^{GFP} mice can also be obtained from the corresponding author. *Rosa26*^{FLPe} (JAX Stock number 003946)[7] and β-*actin*-*Cre* (JAX Stock number 003376)[8] are available from The Jackson Laboratory.

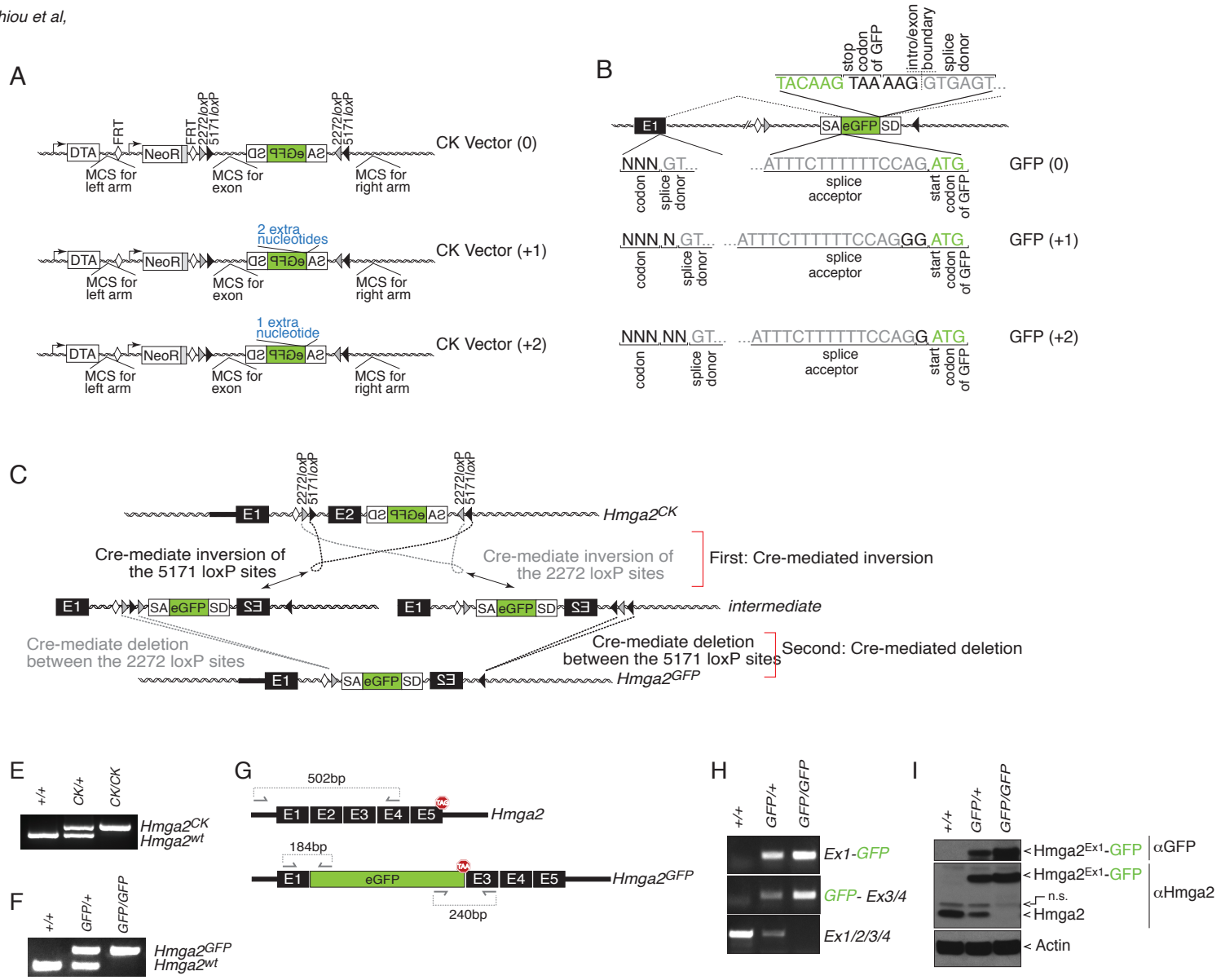

Figure S1, related to Figure 1. Targeting vectors for the creation of conditional-null/conditional-reporter alleles.
(A) Base vectors for the generation of conditional-null/conditional-reporter CK alleles were generated with GFP in each of the three reading frames. Depending on the frame of the first exon the correct CK targeting backbone must be chosen. These plasmids contain diptheria toxin fragment A (DTA) 5' of the multiple cloning site (MCS) for the "left" arm. A FRT flanked neomycin resistance gene allows positive selection followed by removal *in vitro* or *in vivo* using Flp-recombinase. Pairs of incompatible *lox*P (2272 loxP and 5171 loxP) sites flank the splice acceptor(SA)-GFP-splice donor(SD) cassette. Details for the generation of CK alleles are in the methods sections. **(B)** Nucleotide sequence within the splice acceptor (SA)-eGFP. Depending on the reading frame of exon 1 (E1) of the targeted gene, CK vector (0), CK vector (+1), or CK vector (+2) should be used. After inversion these vectors create GFP(0), GFP (+1), and GFP(+2) respectively. **(C)** Two Cre-mediated recombination events stably invert the SA-GFP-SD cassette. The first Cre-mediated event inverts the cassette by recombination of either the head to head 5171*lox*P(left) or 2272*lox*P(right) sites. This creates one of two intermediates followed by a second Cre-mediated event that deletes the region between the 2272*lox*P(left) or 5171*lox*P(right) sites.The first Cre-mediated inversion event is reversible but the second Cre-mediated event would likely be favored by virtue of the small distance between the head to head *lox*P sites. The final conformation cannot undergo subsequent recombination events due to the incompatibility between the remaining 2272*lox*P and 5171*lox*P sites. **(E,F)** Representative genotyping of mice with the $Hmga2^{+}$,$Hmga2^{CK}$, and $Hmga2^{GFP}$ alleles. **(E)** Schematic of the $Hmga2^{+}$ and $Hmga2^{GFP}$ mRNA. RT-PCR primer locations are indicated. **(F)** RT-PCR analysis of E15.5 embryos documents the expression of the $Hmga2^{GFP}$ transcript and the absence of the $Hmga2^{+}$ transcript in $Hmga2^{GFP/GFP}$ cells. **(G)** The Hmga2-GFP fusion in $Hmga2^{GFP/+}$ and $Hmga2^{GFP/GFP}$ E15.5 embryos is detected by both the anti-Hmga2 antibody (which recognizes an epitope in the N-terminal portion of Hmga2) and the anti-GFP antibody. No endogenous Hmga2 is detected in the $Hmga2^{GFP/GFP}$ cells. Actin shows equal loading. A non-specific band (n.s.) is indicated. **(H)** RT-PCR analysis of E15.5 embryos documents the expression of the $Hmga2^{GFP}$ transcript and the absence of the $Hmga2^{+}$ transcript in $Hmga2^{GFP/GFP}$ cells. **(I)** The Hmga2-GFP fusion in $Hmga2^{GFP/+}$ and $Hmga2^{GFP/GFP}$ E15.5 embryos is detected by both the anti-Hmga2 antibody (which recognizes an epitope in the N-terminal portion of Hmga2) and the anti-GFP antibody. No endogenous Hmga2 is detected in the $Hmga2^{GFP/GFP}$ cells. Actin shows equal loading. A non-specific band (n.s.) is indicated.

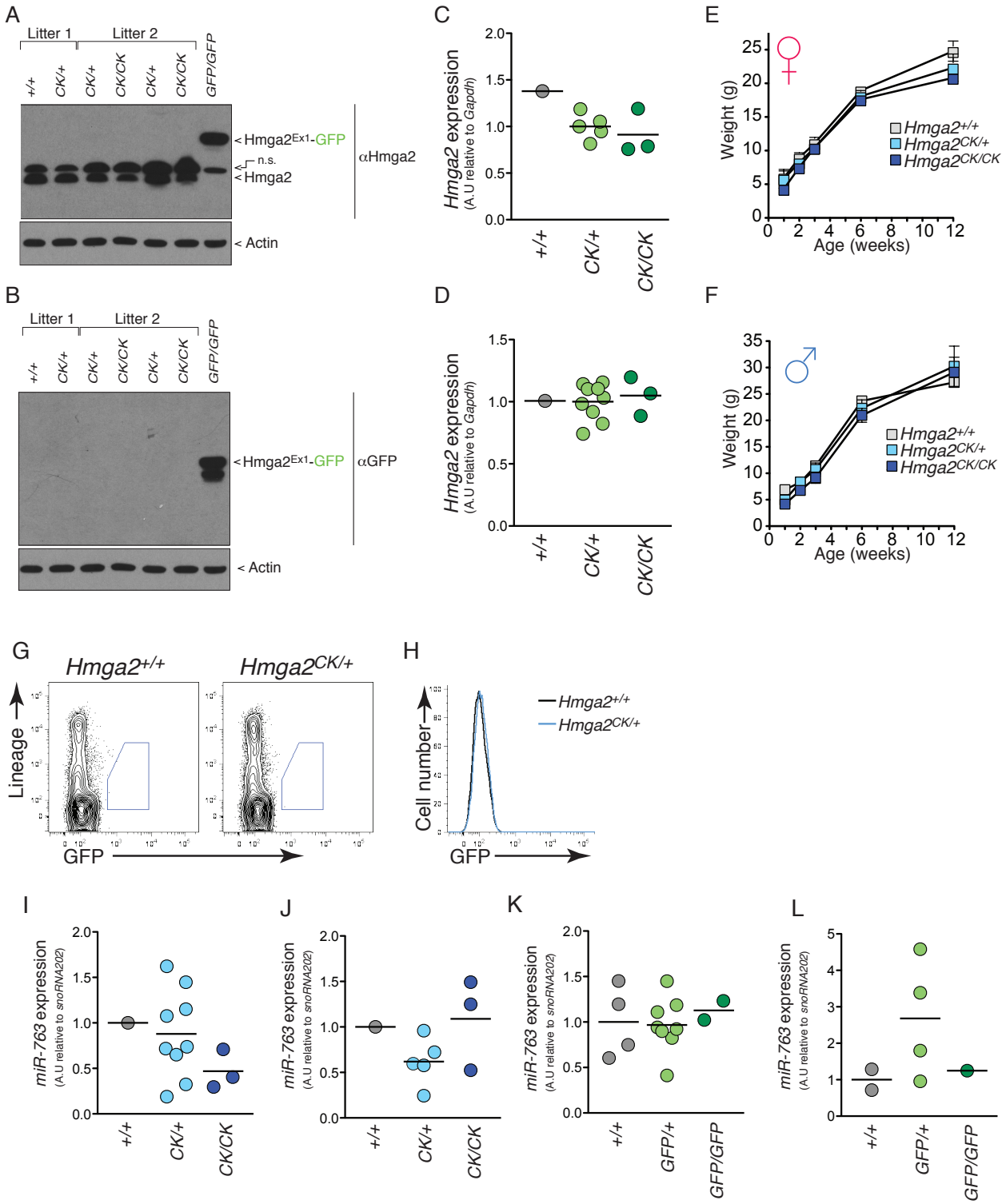

Figure S2, related to Figures 1 and 2. No phenotypic consequences of the *Hmga2^{CK}* allele.
(A) *Hmga2* targeting prior to Cre expression does not alter Hmga2 protein expression. Western blot analysis of MEFs is shown. Actin shows equal loading. A non-specific band (n.s.) is indicated. **(B)** No GFP expression in MEFs with the *Hmga2^{CK}* allele. *Hmga2$^{GFP/GFP}$* MEFs are shown as a positive control. Actin shows equal loading. **(C, D)** *Hmga2* targeting prior to Cre expression does not significantly alter *Hmga2* mRNA expression. qRT-PCR analysis of MEFs **(C)** and embryos **(D)** is shown. Expression is shown relative to *Gapdh* control. To compile data from multiple litters expression was normalized so that the average *Hmga2* expression in *Hmga2$^{CK/+}$* samples in each litter was 1. **(E, F)** The *Hmga2^{CK}* allele does not reduce body weight. Growth curves for female **(E)** and male **(F)** mice are shown. **(G,H)** The *Hmga2^{CK}* allele does not express GFP. *Hmga2$^{CK/+}$* and *Hmga2$^{CK/CK}$* cell do not have GFP expression above background green fluorescence of *Hmga2$^{+/+}$* cells. Overlays of the GFP intensities of the fetal liver cells of the indicated genotypes further illustrate the absence of GFP expression. **(I,J)** *Hmga2* targeting prior to Cre expression (*Hmga2^{CK}*) does not alter the expression of the intronic miRNA *miR-763*. qRT-PCR analysis of embryos **(I)** and MEFS **(J)** is shown. The difference between *Hmga2$^{CK/+}$* and *Hmga2$^{CK/CK}$* in embryos is not significant. *p*-value < 0.2 Student's t-test. **(K, L)** The *Hmga2^{GFP}* does not alter the expression of the intronic miRNA *miR-763*. qRT-PCR analysis of embryos **(K)** and MEFS **(L)** is shown. Expression is shown relative to *snoRNA202* control. "(**C,D,** and **I-L**) Each dot represents MEFs derived from an embryo (**C, J,** and **L**) or an embryo (**D, I,** and **K**) and the bar represents the mean. (**E, F**) Mean +/- SD of each time point is shown.

A

	Hmga2	Total Radiant Efficiency [p/s]/[μW/cm^2]x10^8
Litter 1 (E13.5)	*+/+*	3.1
	GFP/+	15.4
	GFP/+	14.3
	GFP/+	15.0
	GFP/GFP	27.8
	GFP/GFP	27.4
	GFP/GFP	25.6
Litter 2 (E15.5)	*+/+*	5.6
	GFP/+	16.0
	GFP/+	12.8
	GFP/+	12.7
	GFP/+	17.3
Litter 1 (E14.5)	*+/+*	4.1
	CK/+	3.0
	CK/+	2.6
	CK/CK	3.1
	CK/CK	2.6
Litter 2 (E15.5)	*CK/+*	4.8
	CK/+	4.4
	CK/CK	5.3

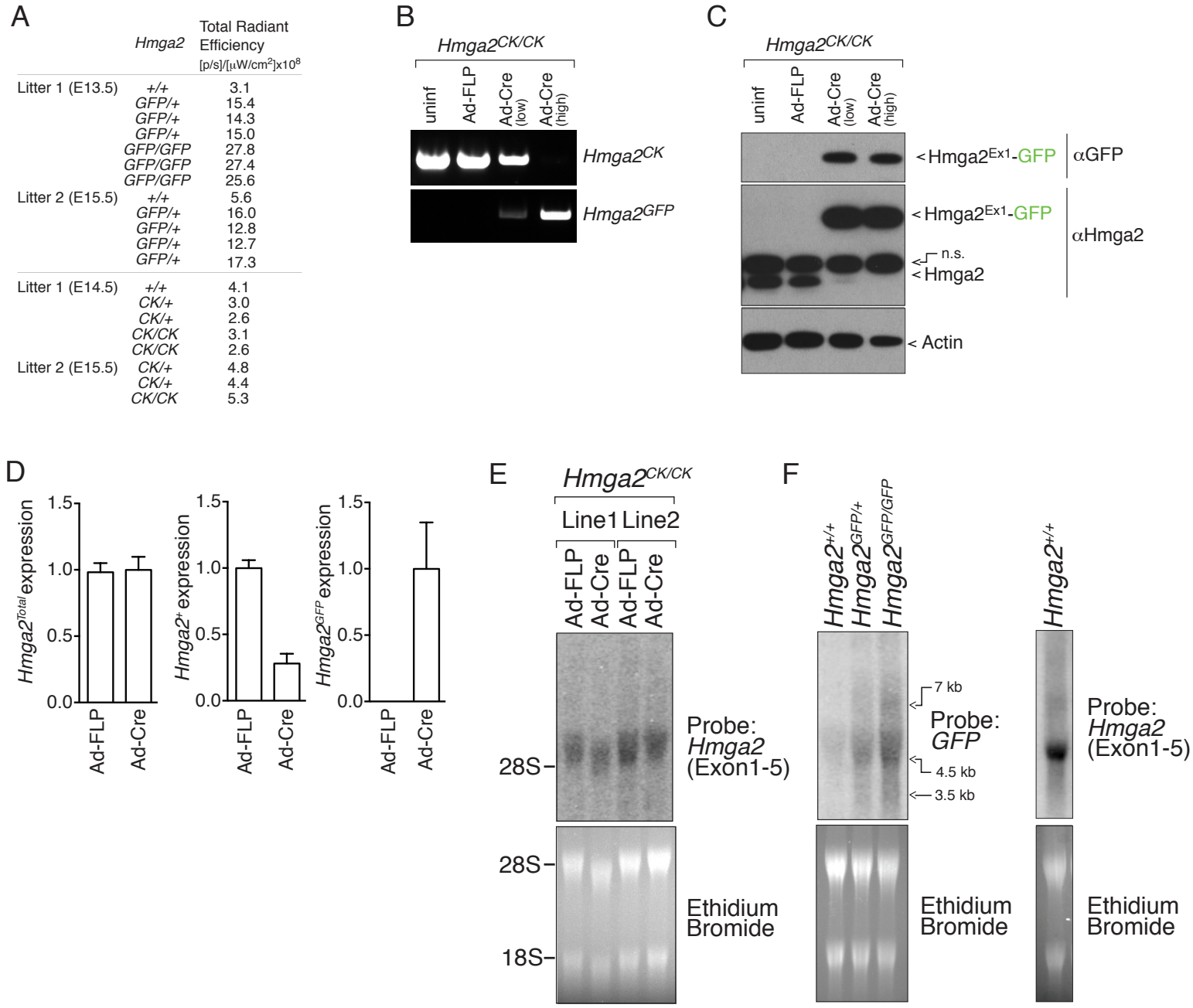

Figure S3, related to Figures 1, 2, and 3. Visualization of GFP expression from the *Hmga2^{GFP}* allele.
(A) Quantification of the GFP intensity of embryos with different combinations of *Hmga2^{+}*,*Hmga2^{GFP}*, and *Hmga2^{CK}* alleles. **(B)** Genotyping of *Hmga2$^{CK/CK}$* MEFs after Cre expression. Uninfected (uninf) and Adenoviral-FLP (Ad-FLP) infected are controls. Low-dose and high-dose Adenoviral-Cre (Ad-Cre) are shown. High-dose Ad-Cre entirely converts the CK alleles to their GFP conformation. **(C)** Western blot analysis of *Hmga2$^{CK/CK}$* MEFs after Cre expression. High-dose Ad-Cre infected *Hmga2$^{CK/CK}$* MEFs lack endogenous Hmga2 and express Hmga2^{Ex1}-GFP. Actin shows loading. **(D)** qRT-PCR analysis of *Hmga2$^{CK/CK}$* MEFs 3 days after control Ad-FLP or Ad-Cre infection documents unchanged total *Hmga2^{wt}*, reduced *Hmga2^{wt}*, and the presence of *Hmga2^{GFP}*. Mean +/- SD of triplicate wells is shown. **(E)** Northern blot on two independant *Hmga2$^{CK/CK}$* MEF line shows comparable total *Hmga2*. **(F)** Nothern blot with a GFP specific probes (left panel) shown the quantitative increase in intensities that is proportional to the copy number of *Hmga2^{GFP}* alleles. Right panel, northern blot on *Hmga2$^{+/+}$* MEFs with the *Hmga2-Ex1-5* probes as in **(E)** revealed a predominant band that is equivalent to the predicted size of *Hmga2* mRNA (~4.2 kb).

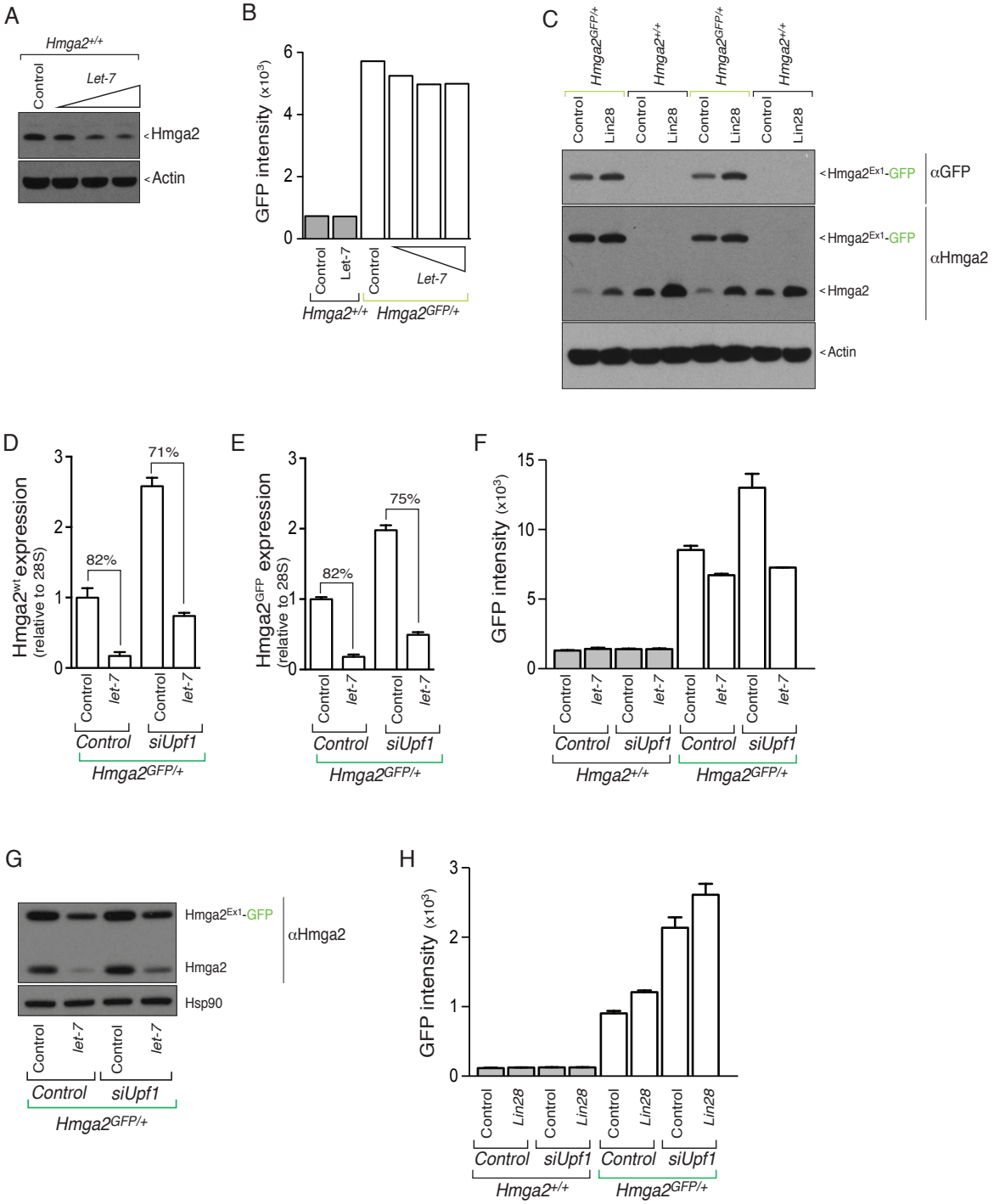

Figure S4, related to Figures 3 and 4. Hmga2^{Ex1}-GFP remains under *let-7* regulation.
(A) Transfection of wild-type *Hmga2$^{+/+}$* MEFs with *let-7* reduces Hmga2. Cells were transfected with a control miRNA mimic (36 pmol) or increasing amounts of *let-7* mimic (2, 10, and 36 pmol). Hmga2 expression decreases in a dose-dependent manner. Actin shows equal loading. **(B)** Quantification of GFP mean fluorescence intensity after transfection with a control miRNA mimic or increasing amounts of *let-7* mimic (2, 10, and 36 pmol - only 36pmol *let-7* mimic is shown for *Hmga2$^{+/+}$* cells). This analysis corresponds to the cells used for the western blot analysis shown in Figure 3c. **(C)** Lin28 expression increases Hmga2 and Hmga2^{Ex1}-GFP levels in MEFs. Two sets of *Hmga2$^{+/+}$* and *Hmga2$^{GFP/+}$* MEFs infected with a control retroviral vectors or a vector expressing Lin28 are shown. The levels of both Hmga2 and Hmga2^{Ex1}-GFP are increased in the presence of Lin28. The pair of *Hmga2$^{GFP/+}$* and *Hmga2$^{+/+}$* MEFs on the right are the samples analyzed by FACS in Figure 3d. **(D, E)** Quantitative PCR of *Hmga2^{wt}* **(D)** and *Hmga2^{GFP}* **(E)** transcripts 3 days after transfection of *Hmga2$^{GFP/+}$* MEFs with *let-7* and/or *Upf1* siRNA (**D**, N =2; **E**, N = 3). **(F, G)** As in **(D, E)**, transfected MEFs (**F**, both *Hmga2$^{+/+}$* and *Hmga2$^{GFP/+}$* MEFs; **G**, *Hmga2$^{GFP/+}$* MEFs) were prepared for quantification of GFP expression by FACS (**F**, mean fluorescent intensities or MFI, N = 2) and both Hmga2^{Ex1}-GFP and Hmga2^{wt} protein by western blot analyses (**G**, N =2). **(H)** *Hmga2$^{+/+}$* and *Hmga2$^{GFP/+}$* MEFs retrovirally transduced with control or virus expressing Lin28 were subsequently transfected with control or *Upf1* siRNA as indicated. MFI of GFP was quantified by FACS as in **(B)** and **(F)** (N = 2 for *Hmga2$^{GFP/+}$* MEFs). (**D, E, F,** and **H**) Mean +/- SD of triplicate wells is shown.