Cesarini et al., http://www.jcb.org/cgi/content/full/jcb.201504035/DC1

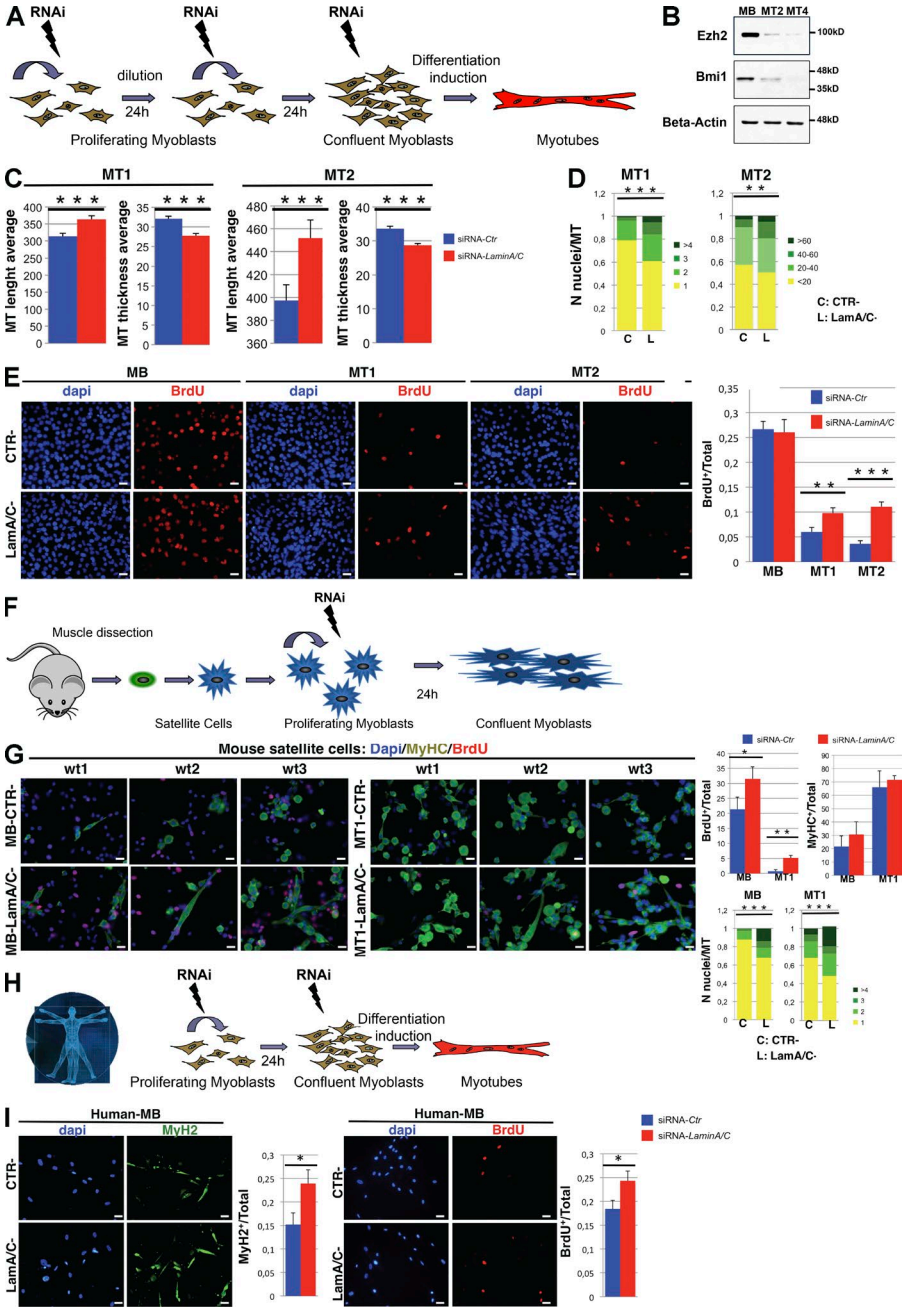

Figure S1. ***Lamin A/C* depletion generates two distinct phenotypes in cultured cells.** (A) Schematic representation of the experimental design using siRNA for knock-down experiments in C2C12 cells. (B) Western blot of total protein extracts of MBs or MTs at MT2 or MT4 hybridized with indicated antibodies. (C–D) Differentiation parameter measurements of C2C12 cells transfected with indicated siRNAs. (C) Mean MT length and thickness (measured in pixels) at MT1 and MT2. $n > 222$ from three independent experiments. (D) Nuclei per MT distribution at MT1 and MT2. $n > 191$ from three independent experiments. (E, left) Representative images of immunostaining for BrdU incorporation of cells transfected as indicated. Red, Alexa Fluor 594. Bar, 20 µm. (right) Proliferative rate calculated as a percentage of cells positive for BrdU with respect to the total number of nuclei. $n > 2{,}955$ from three independent experiments. (F) Schematic representation of the experimental design using siRNAs for KD experiments in satellite cells. (G, left) Representative images of immunostaining for MyHC and BrdU of satellite cells extracted from three different wild-type mice transfected with indicated siRNAs. Cells were analyzed as MB or MT1. Green, Alexa Fluor 488; red, Alexa Fluor 594. Bar, 20 µm. (right) Fusion index and proliferative rate (top); nuclei per fiber distribution (bottom). $n > 1{,}082$ from three independent experiments. (H) Schematic representation of the experimental design using siRNAs for KD experiments in human primary MBs. (I) Representative images of MyHC and BrdU immunostaining in human primary MBs transfected with indicated siRNAs. Green, Alexa Fluor 488; red, Alexa Fluor 594. Bar, 20 µm. Fusion index and proliferative rate are indicated. Data points were generated from the mean of three independent populations. Two-tailed *t* test was applied for statistical analysis in C, E, G, and I. SEM is indicated. Mann–Whitney two-tailed test was applied for statistical analysis in D and G. Statistically relevant differences ($\alpha = 0.05$): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

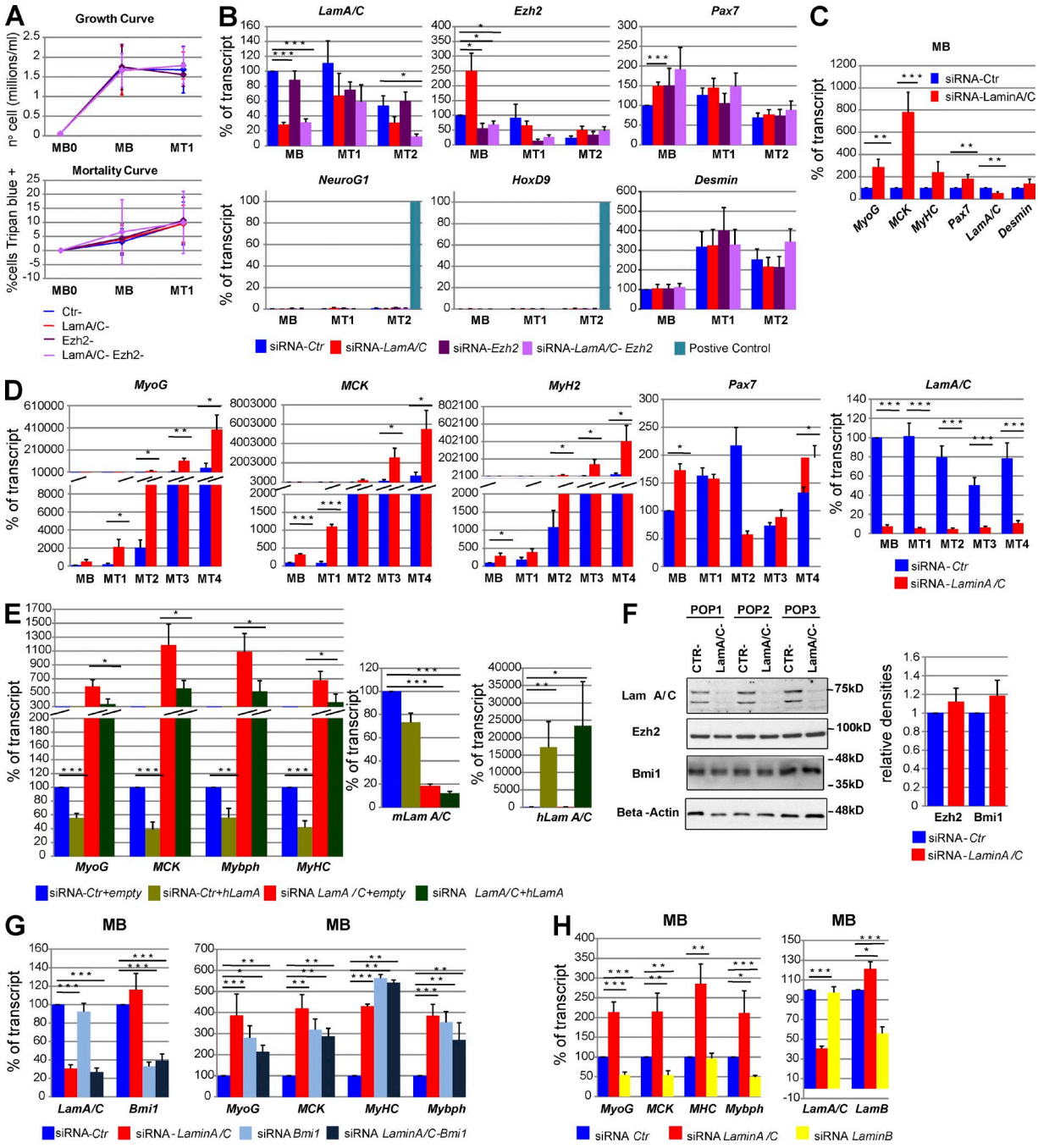

Figure S2. ***Lamin A/C* depletion is accompanied by de-repression of PcG-regulated muscle genes.** (A) Growth and mortality curves of C2C12 cells transfected with indicated siRNAs. The growth curve represents the number of cells/ml 24 or 48 h after transfection. The mortality curve is calculated at the same time points, as a percentage of nuclei stained with Trypan blue with respect to the total number of nuclei. (B) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in C2C12 cells transfected as indicated in A. Positive controls, represented in green, were *NeuroG* expression in the cortex of a 17-d embryo and *HOXD9* expression in 18-d embryoid bodies. Measurements were done on C2C12 cells as confluent MBs at MT1 and MT2. (C) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in satellite-derived MBs transfected with indicated siRNAs. (D) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in primary human MBs transfected with indicated siRNAs. Measurements were done on confluent MBs at MT1, MT2, MT3, and MT4. (E) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in C2C12 MBs transfected with a plasmid-overexpressing human lamin A or an empty vector as control. C2C12 cells were cotransfected with indicated siRNAs. (F) Western blot of total protein extracts hybridized with indicated antibodies in cells transfected as indicated in A. β-Actin was used as loading control. The graph indicates quantification of protein bands normalized to β-actin and relative to MB control. (G) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in C2C12 MBs transfected with indicated siRNAs. (H) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in C2C12 MBs transfected with indicated siRNAs. All data points in Fig. S2 were generated from the mean of at least four independent experiments. Two-tailed *t* test was applied for statistical analysis. SEM is indicated. Statistically relevant differences ($\alpha = 0.05$): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

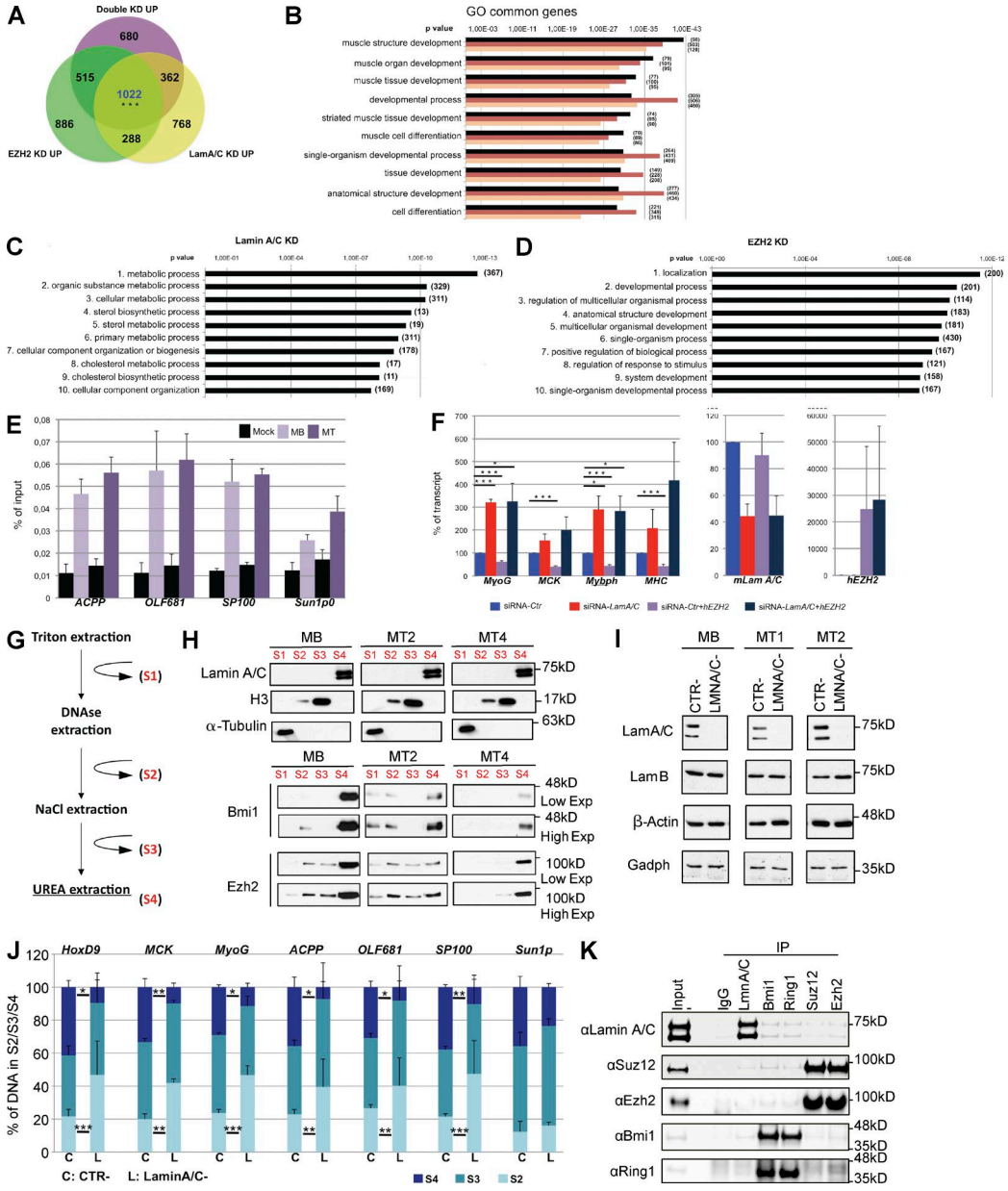

Figure S3. **PgC proteins and lamin A/C coregulate muscle differentiation.** (A) Venn diagram showing intersection between genes up-regulated in *Lamin A/C*, *Ezh2*, or double *Lamin A/C–Ezh2* KD. (B) Comparison in terms of P values of GO analysis (Biological Process) performed on common up-regulated genes in both *Lamin A/C* and *Ezh2* depletions (black) and up-regulated genes after single *Lamin A/C* (red) or *Ezh2* (orange) KD. The number of genes in each category is reported in brackets. (C) GO analysis (biological process) of the genes up-regulated in *Lamin A/C* KD but not in *Ezh2* KD, showing the top represented GO terms according to P values. The number of genes in each category is reported in brackets. (D) GO analysis (biological process) of the genes up-regulated in *Ezh2* KD but not in *Lamin A/C* KD, showing the top represented GO terms according to P values. The number of genes in each category is reported in brackets. (E) ChIP analyses with antibodies against lamin A/C are presented as a percentage of input chromatin precipitated for indicated regions. Each graph shows the mean result from at least three independent IP reactions on different chromatin preparations. *ACPP*, *OLF681*, and *SP100* were used as positive controls (Kubben et al., 2012), and *Sun1p0* was used as negative control. (F) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in C2C12 MBs infected with a virus overexpressing human Ezh2 or an empty vector as control. C2C12 cells were cotransfected with indicated siRNAs. All data points were generated from the mean of at least five independent experiments. (G) Scheme for chromatin fractionation experiments. Sequential protein extractions were performed to isolate soluble proteins (S1 fraction), DNase-sensitive proteins (S2 fraction), DNase-resistant proteins (S3 fraction), and nuclear matrix–associated proteins (S4 fraction). (H) Chromatin fractionation experiments of C2C12 cells during muscle differentiation. Cells were collected as MBs and MTs at MT2 and MT4. Equal amounts of each fraction were immunoblotted and hybridized with indicated antibodies. Loading controls: α-tubulin (S1), histone H3 (S2 and S3), and lamin A/C (S4). Data shown are from a single representative experiment of two repeats. (I) Western blot of total protein extracts hybridized with indicated antibodies in cells transfected with siRNA control or *lamin A/C*. β-Actin was used as loading control. Data shown are from a single representative experiment of two repeats. (J) Relative enrichment of the indicated genomic regions in S2, S3, and S4 chromatin fractions in cells transfected with siRNA control or *lamin A/C*. ACPP, OLF681, and SP100 were used as positive controls (Kubben et al., 2012), and Sun1p0 was used as negative control. Data points represent the mean of four independent experiments. (K) Western blot analysis of coIP performed in C2C12 cells. Nuclear extracts immunoprecipitated with lamin A/C, Bmi1, Ring1, Suz12, or Ezh2 antibodies, together with inputs, were immunoblotted and hybridized with indicated antibodies. An unrelated antibody (mouse IgG) was used as negative control. The data shown are from a single representative experiment of two repeats. Hypergeometric test was applied for statistical analysis in A. Two-tailed *t* test was applied for statistical analysis in F. SEM is indicated. Statistically relevant differences ($\alpha = 0.05$): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

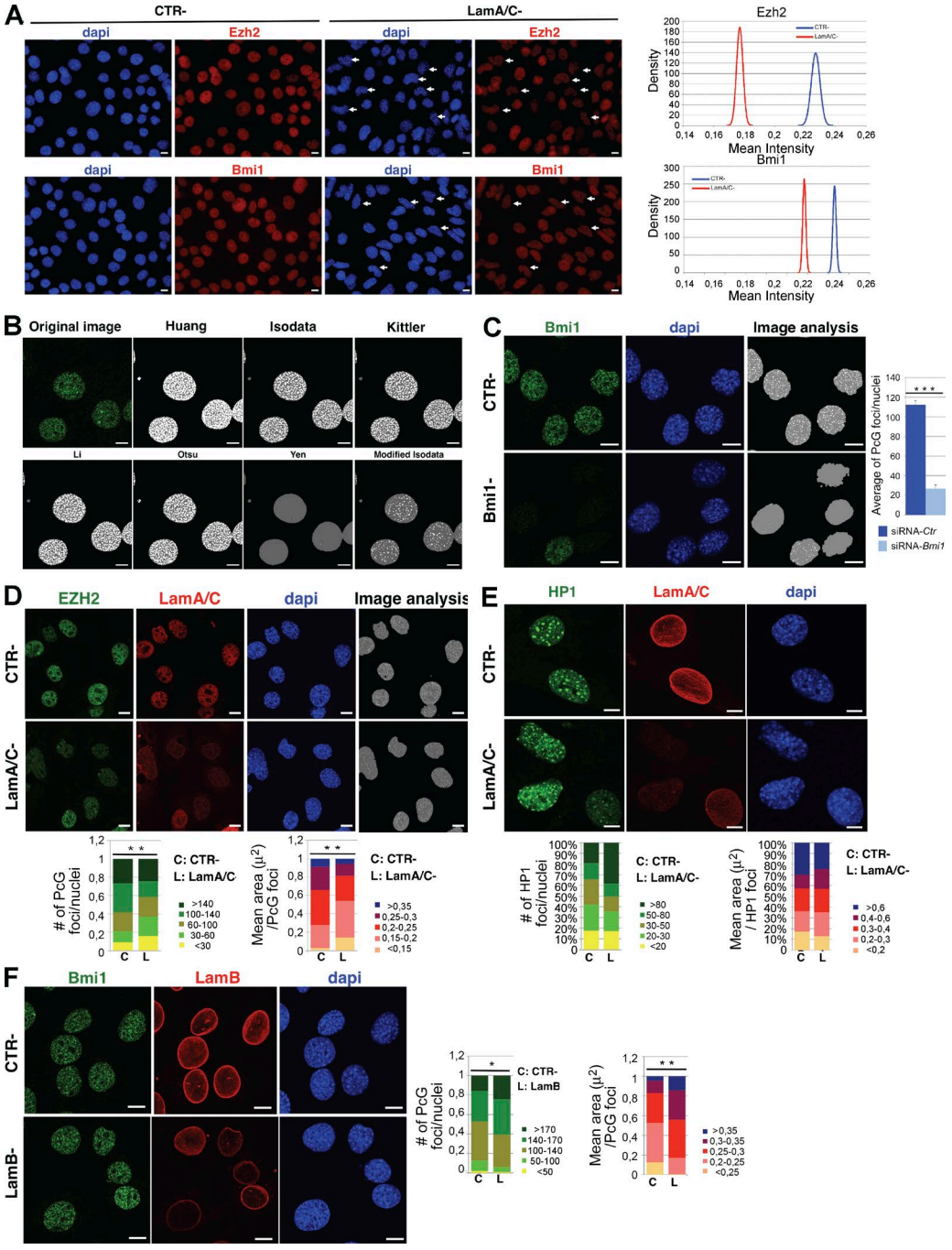

Figure S4. **Intranuclear localization of PcG proteins depends on lamin A/C.** (A, left) Representative examples of confluent C2C12 MBs transfected with indicated siRNAs, immunostained using Ezh2 and Bmi1 antibodies, as indicated. Bar, 10 µm. Arrows indicate misshapen nuclei indicative of lamin A/C depletion. (right) Intranuclear mean fluorescence intensities were determined using an automated pipeline (see Image analysis). Density indicates the number of cells. Images from four independent experiments were recorded using constant image acquisition parameters. Two-tailed *t* test was applied for statistical analysis ($\alpha = 0.05$). P values are <0.001. $n > 1{,}492$ from four independent experiments. (B) Qualitative segmentation comparison of the MID algorithm with some of the more well-known thresholding algorithms (Sezgin and Sankur, 2004). Bar, 10 µm. (C, left) Representative examples of C2C12 MBs transfected with indicated siRNAs and immunostained using Bmi1 antibodies and DAPI, as indicated. Bar, 10 µm. (right) Mean of number of PcG protein foci per nucleus among the cellular population. $n > 87$ from two independent experiments. (D, top) Representative examples of C2C12 MBs transfected with indicated siRNAs and immunostained using Ezh2, lamin A/C antibodies, and DAPI, as indicated. Bar, 10 µm. right: Contour of nuclei and PcG foci in segmentation analysis. (bottom) Distribution of number of PcG foci per nucleus and PcG foci area (measured in µm²) among the cellular population. $n > 157$ from two independent experiments. (E, top) Representative examples of C2C12 MBs transfected with indicated siRNAs and immunostained using HP1, lamin A/C antibodies, and DAPI, as indicated. Bar, 10 µm. (bottom) Distribution of number of HP1 foci per nucleus and HP1 foci area (measured in µm²) among the cellular population. $n > 251$ from four independent experiments. (F, left) Representative examples of C2C12 MBs transfected with indicated siRNAs and immunostained using Bmi1, lamin B antibodies, and DAPI, as indicated. Bar, 10 µm. (right) Distribution of number of PcG foci per nucleus and PcG foci area (measured in µm2) among the cellular population. $n > 196$ from two independent experiments. All data points were generated from the mean of at least two independent experiments. Two-tailed *t* test was applied for statistical analysis in A and C. SEM is indicated. Mann–Whitney two-tailed test was applied for statistical analysis in D, E, and F. Statistically relevant differences ($\alpha = 0.05$): *, $P < 0.05$ **, $P < 0.01$; ***, $P < 0.001$. Green, Alexa Fluor 488; red, Alexa Fluor 594.

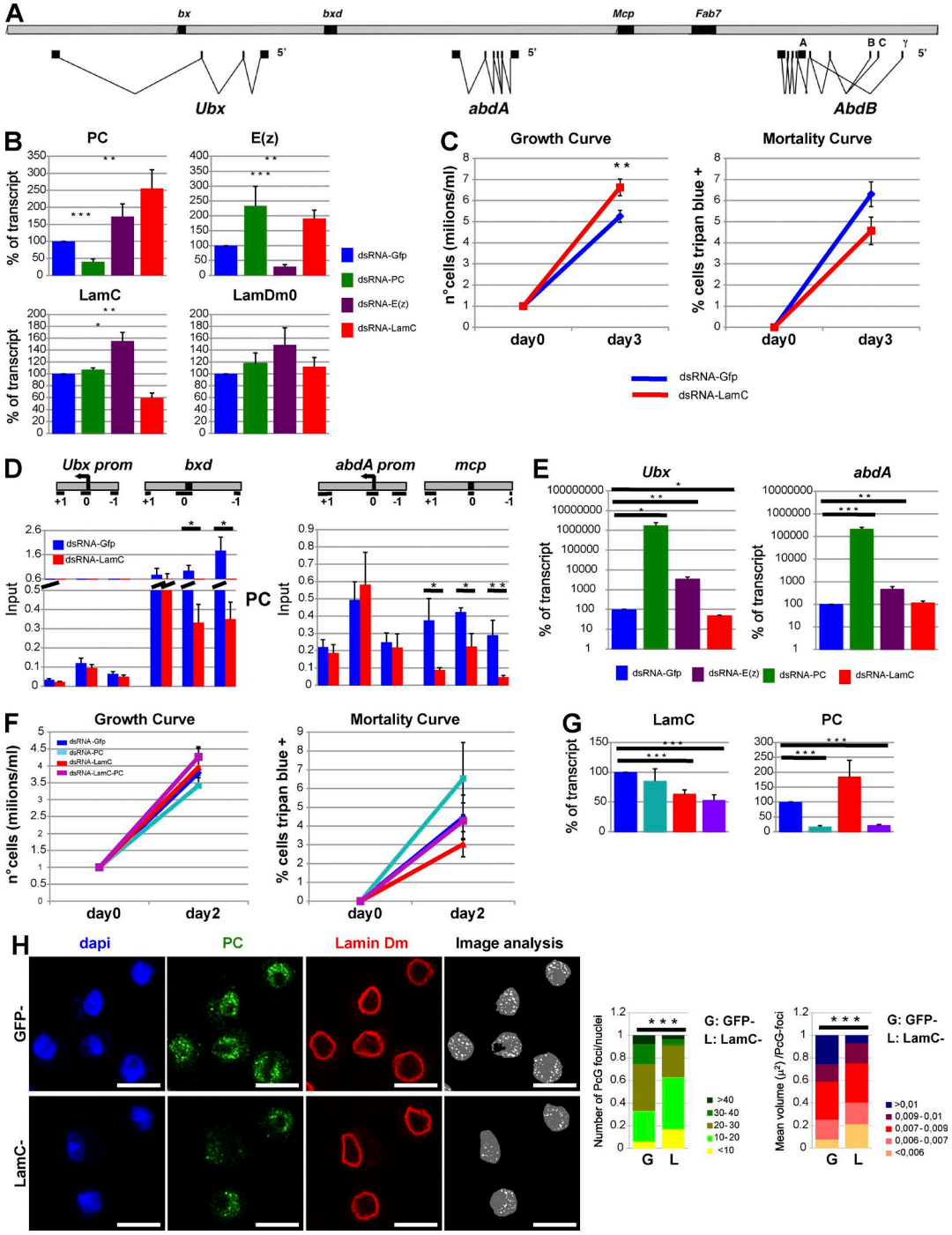

Figure S5. ***Drosophila* lamin C is necessary for correct PcG protein subcompartmentalization and function.** (A) Schematic representation of BX-C including homeotic genes and characterized PREs (black). (B) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in cells transfected with indicated dsRNAs. Data points represent the mean of at least 10 independent experiments. (C) Growth and mortality curves of dsRNA-transfected cells. The growth curve represents the number of cells/ml on the day of transfection (d0) and 3 d after transfection. The mortality curve is calculated at the same time points as the percentage of nuclei stained with Trypan blue with respect to total number of nuclei. (D) ChIP analysis with antibodies against PC, presented as the percentage of input chromatin precipitated for each region. The position of the amplicons, overlapping PcG protein binding sites, and their flanking regions at +1 or −1 Kb, is indicated on the map at top. Mock enrichment is <0.003% of the input. Data points represent the mean result from at least six independent IP reactions on different chromatin preparations. (E) Quantification by real-time PCR of transcript levels, relative to GAP DH, of homeotic genes in cells transfected as indicated in B. Data points represent the mean of at least 10 independent experiments. (F) Growth and mortality curves measured as indicated in C in cells transfected with indicated dsRNAs. (G) Quantification by real-time PCR of transcript levels, relative to GAPDH, of indicated genes in cells transfected with indicated dsRNAs. Data points represent the mean of six independent experiments. (H, top) left: Representative examples of S2 cells transfected with indicated dsRNAs and immunostained using PC and DAPI, as indicated. Green, Alexa Fluor 488; red, Alexa Fluor 594. Magnification 4. top right: Contour of nuclei and PcG protein foci in segmentation analysis. bottom: Distribution of number of PcG foci per nucleus (left) and PcG foci area (right; measured in µm^2) among the cellular population. $n > 89$ from three independent experiments. Two-tailed *t* test was applied for statistical analysis in B–G. SEM is indicated. Mann–Whitney two-tailed test was applied for statistical analysis in H. Statistically relevant differences ($\alpha = 0.05$): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table S1. **List of antibodies used in this study**

Protein	Company or laboratory	Reference number	Source	Immunofluorescence	Western blot	CoIP	ChIP	PLA	EM	3D-SIM
MyH	SantaCruz	sc-20641	Rabbit	X	X					
BrdU	Becton Dickinson	347580	Mouse	X						
Lamin A/C	SantaCruz	sc-6215	Goat	X	X	X	X			
Lamin A	SantaCruz	sc-20680	Rabbit	X				X		
Lamin A/C	SantaCruz	sc-7292	Mouse	X				X		
Lamin A/C	SantaCruz	sc-20681	Rabbit							X
Bmi1	Millipore	05-637	Mouse	X	X			X	X	
Bmi1	Abcam	ab14389	Mouse	X				X	X	X
Bmi1	Abcam	ab85688	Rabbit		X	X				
Ezh2	Cell Signaling	3147S	Mouse	X	X			X		
Ezh2	Cell Signaling	5246S	Rabbit			X		X		
Ezh2	Diagenode	pAb039-050	Rabbit				X			
Myogenin	SantaCruz	sc-12732	Mouse		X					
β-Actin	SantaCruz	sc-1616-R	Rabbit		X					
α-Tubulin	Sigma	T5168	Mouse		X					
Histone H3	Abcam	ab1791	Rabbit		X					
Lamin B	SantaCruz	sc6216	Goat		X					
Lamin B	SantaCruz	sc374015	Mouse							X
Lamin B	Abcam	ab16048	Rabbit					X		
Ring1b	Cell Signaling	D22F2	Rabbit	X	X	X		X		X
RNAPolII	Abcam	Ab5408	Mouse							X
HP1	Millipore	MAB3448	Mouse	X						
Suz12	Cell Signaling	D39F6 XP	Rabbit		X	X				
Gapdh	SantaCruz	sc-137179	Mouse		X					
IgG	Genetex	GTX26708	Mouse			X				
E(z)	J. Muller (Max Planck Institute of Biochemistry, Martinsried, Germany	Homemade	Rabbit		X					
PC	R. Paro (ETH Zurich, Zurich, Switzerland)	Homemade	Rabbit	X	X		X			
LaminDm0	P.A. Fisher (SUNY Stony Brook, Stony Brook, NY)	Homemade	Mouse	X	X					
Lamin C	P.A. Fisher	Homemade	Mouse		X					
β-Tubulin (E7)	University of Iowa	Hybridoma Bank	Mouse		X					
Type II topoisomerase	D. Arndt-Jovin (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany)	Homemade	Rabbit		X					

Included online are Tables S2, S3, and S4, showing genes up-regulated in *Lamin A/C* KD, common genes up-regulated in *Lamin A/C* and *Ezh2* KD, and genes up-regulated in *Ezh2* KD and not *Lamin A/C* KD, respectively.

The zip archive file provided online contains the following source code files: main.c, main MCV-MID program file; MCV-MID.h, prototypes and structures for MCV-MID; parse_option.c, function for the program command line arguments parsing; ReadMicroscopyImage.c, function for reading TIFF microscopy image files; utils.c, utility functions for image filename manipulation; utils.h, prototypes for functions in utils.c; filtering.c, functions for image mean filtering; MCV.c, implementation of MCV algorithm; and MID.c, implementation of MID algorithm.

References

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