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Supplemental Information

Latexin Inactivation Enhances Survival and Long-Term Engraftment of Hematopoietic Stem Cells and Expands the Entire Hematopoietic System in Mice

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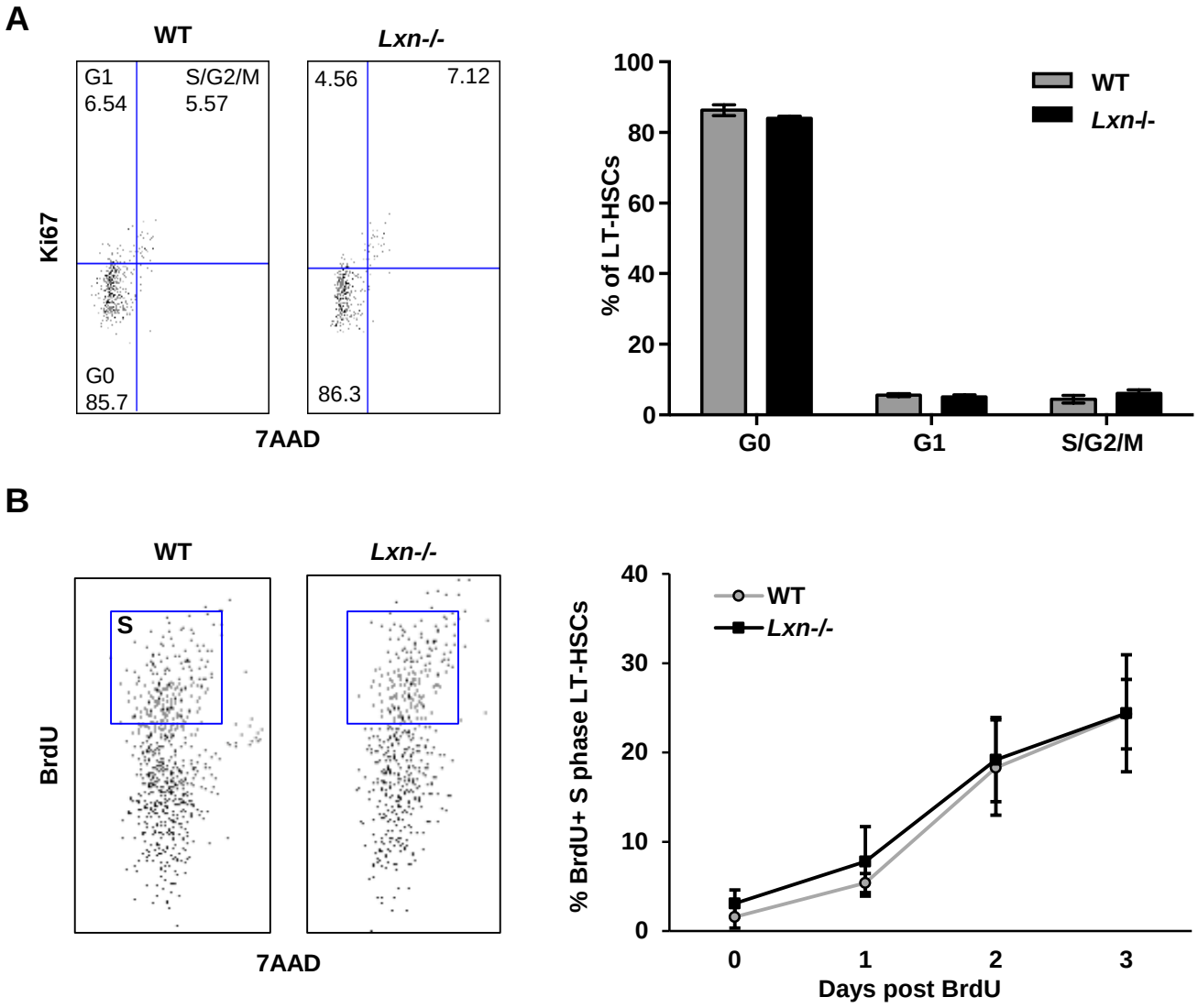


Supplementary Figure 1

Supplementary Figure 1.*Lxn* deletion does not alter HSC proliferation.
(**A**) Representative FACS plots showing the G0 (Ki67- and 7AAD-), G1 (Ki67+ and 7AAD-), and S/G2/M (Ki67+and 7AAD+) cell cycle phases in *Lxn-/-* and WT LT-HSCs (Lin-Sca1+c-Kit+CD34-Flt2- cells (left panel). The right panel shows the frequencies of each phase presented as the average ± SD of 6 measurements from 2 independent experiments. (**B**) Representative FACS plots showing proliferating S phase cells that are positive for BrdU incorporation. Right panel shows the accumulation of cycling *Lxn-/-* and WT LT-HSCs (BrdU+) over 3 days. Presented data are the average ± SD of 2 independent experiments, each performed with 3 mice (n=6).

A **Top functional categories significantly enriched in *Lxn-/-* HSCs**

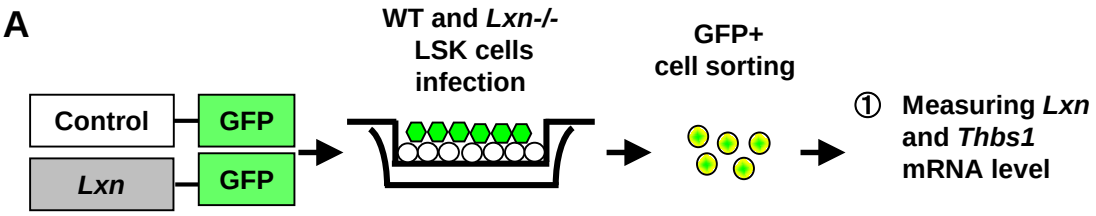
Functional Categories	FDR q-val	# of Genes in Leading Edge
Neuroactive Ligand Receptor Interaction	0.002	147
Cell Communication	0.003	88
Proteasome	0.004	14
Arachidonic Acid Metabolism	0.120	42
Systemic Lupus Erythematosus	0.143	66
ECM Receptor Interaction	0.188	44

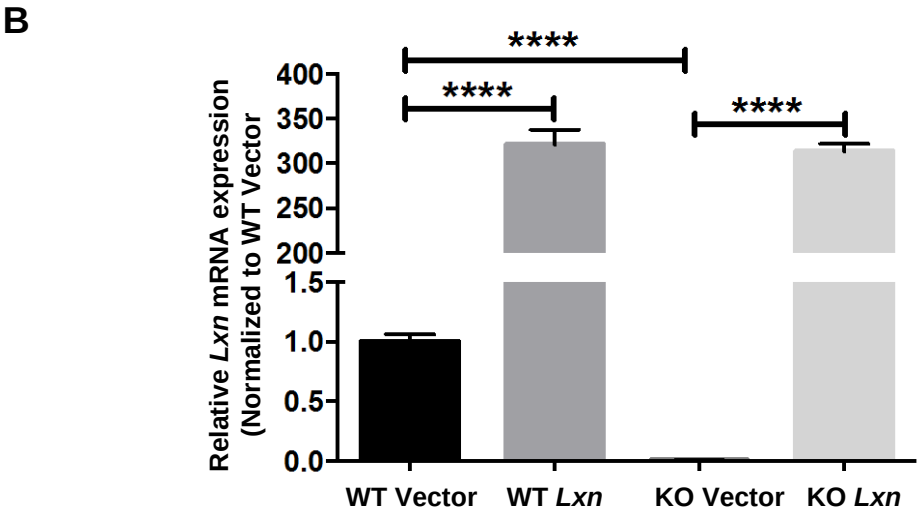
B **Top 10 genes significantly downregulated or upregulated in *Lxn-/-* HSCs**

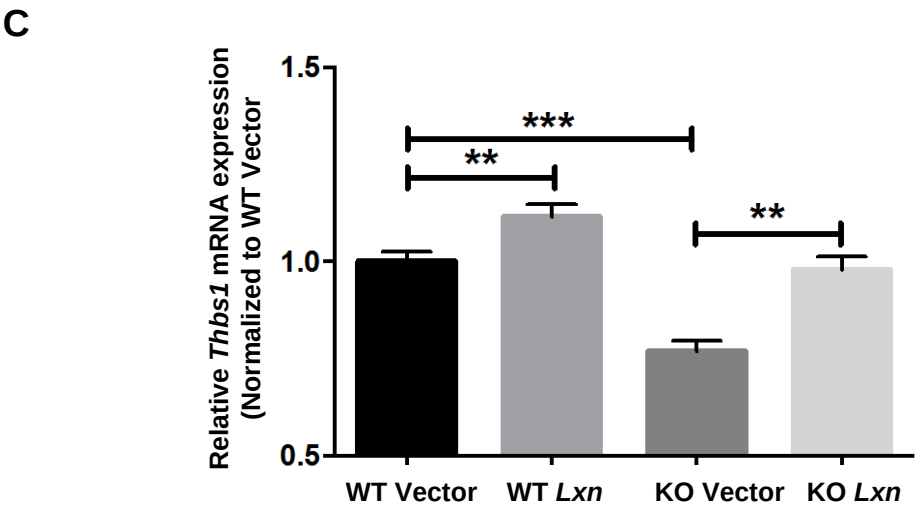
Downregulated	Upregulated
Xist	*Kdm5d*
Thbs1	*Hspa1a/Hspa1B*
Pgr	*Rnase3*
Trpc6	*Tsen15*
Slc18a2	*Gas5*
Gfi1b	*Hsph1*
Kir3dl2	*Mrpl18*
Slc35d3	*Trem1*
Prkaa2	*Ccr2*
Homx1	*Cspp1*

Supplementary Figure 2

Supplementary Figure 2. Genes and functional categories altered in *Lxn-/-* HSCs.
(A) Functional categories of genes dysregulated in *Lxn-/-* LT-HSCs and MPPs identified by GSEA based on microarray data with false discovery rate (FDR)<0.2. The FDR q value and the number of genes in the leading edge subset for each category are also presented.
(B) Top ten genes that are significantly up-regulated or down-regulated in *Lxn-/-* HSCs.

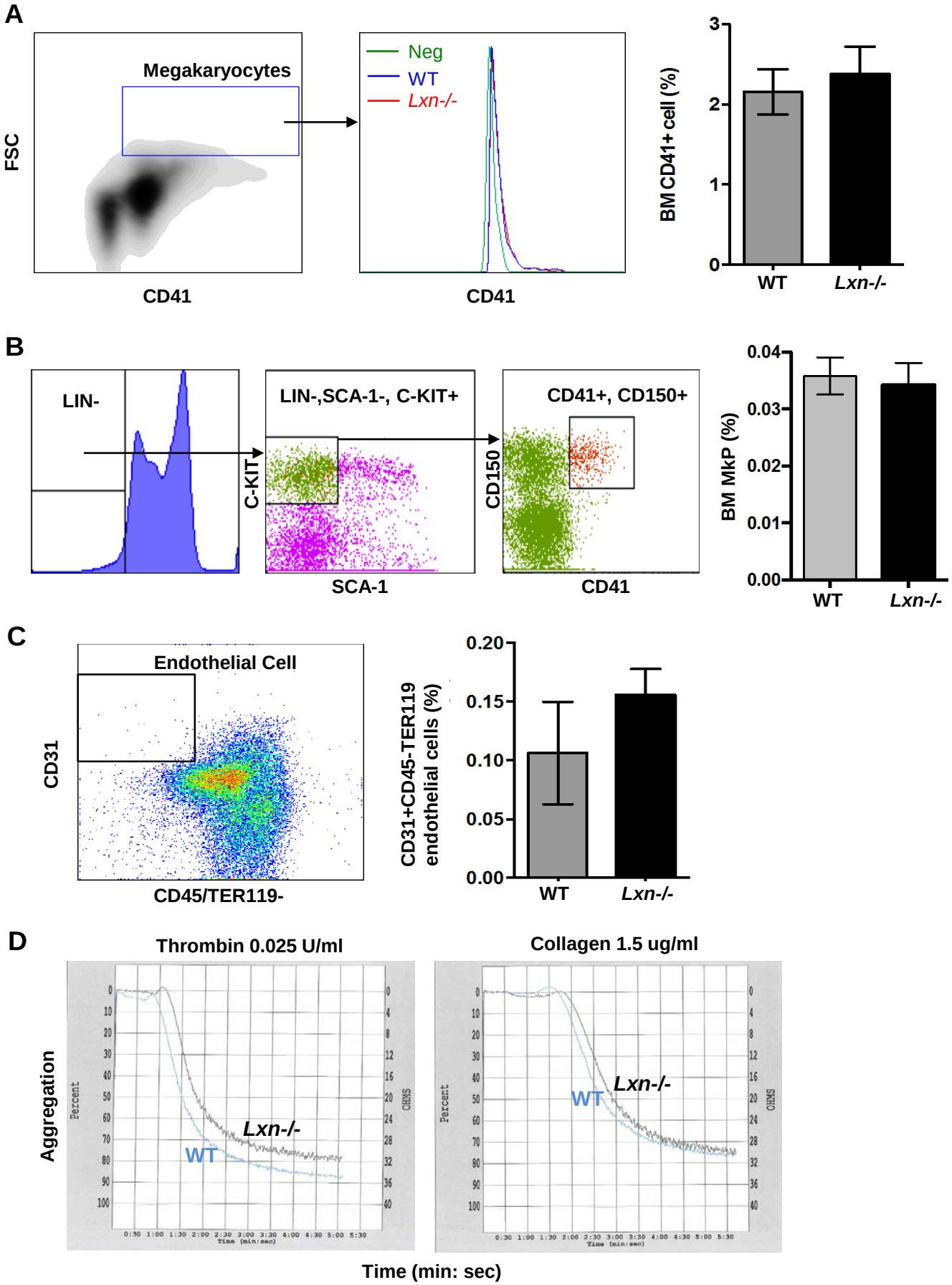






Supplementary Figure 3

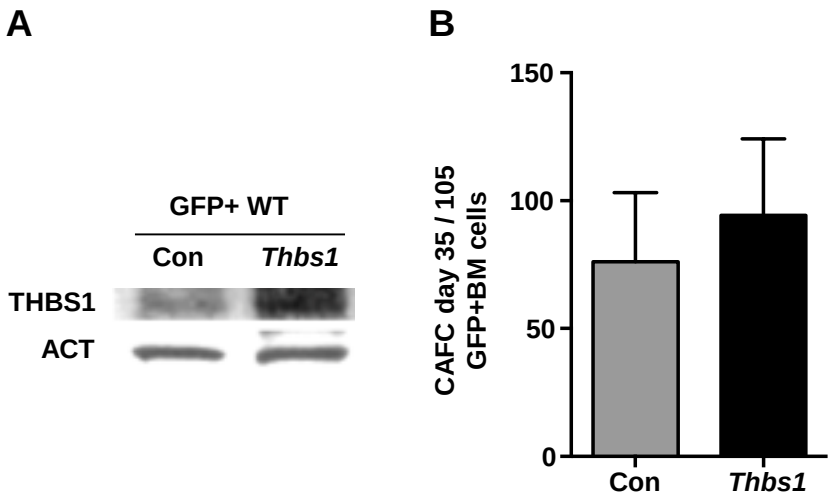
Supplementary Figure 3. Increased expression of *Thbs1* in *Lxn*-overexpressing LSK cells.
(A) Experimental scheme for lentivirus-mediated *Lxn* overexpression strategy in WT and *Lxn-/-* LSK cells. WT or *Lxn-/-* LSK cells were sorted and transduced with empty control or *Lxn*-containing lentiviral particles. Successfully transduced cells were sorted by GFP expression, and *Lxn* and *Thbs*1 mRNA levels were measured by Real-time quantitative PCR. **(B)** Confirmation of increased *Lxn* mRNA level in WT and *Lxn-/-* LSK cells overexpressing *Lxn*. **(C)** *Thbs1* expression was significantly increased in WT and *Lxn-/-* LSK cells overexpressing *Lxn.* Data shown are Mean ± SD of 3 replicates. **** indicates p value <0.0001, *** indicates p value <0.001, and ** indicate p value < 0.01.



Supplementary Figure 4

Supplementary Figure 4. *Lxn* deletion does not alter BM niche components and platelet function.

(A) Representative FACS plot showing the identification of CD41+ FSC^{high} megakaryocytes (MKs) (left panel). No difference in the CD41 expression in the histogram of MK cells (middle panel), or in the percentage of MK cells (right panel) between BM MKs from *Lxn-/-* and WT was observed. Neg represents unstained negative control. **(B)** Representative FACS plot showing megakaryocyte progenitor cells (MkPs) that are identified as LIN-, SCA-1-, C-KIT+, CD41+, CD150+ cells (left panel). No difference was shown in the percentage of MkP cells between *Lxn-/-* and WT BMs (right panel). **(C)** Representative FACS plot showing the identification of CD31+CD45-Ter119- endothelial cells in the BM (left panel). No difference was observed in the percentage of BM endothelial cells between *Lxn-/-* and WT mice. **(D)** Measurement of platelet aggregation and secretion in response to agonists, thrombin (left) and collagen (right). Whole blood was collected from the inferior vena cava using one-seventh volume of ACD (85mM trisodium citrate, 83mM dextrose, and 21mM citric acid) as anticoagulant. Platelets were washed twice with CGS (0.12M NaCl, 0.0129M trisodium citrate, and 0.03M D-glucose, pH 6.5), resuspended in modified Tyrode's buffer at $3x10^8$/ml, and incubated for 2h at 22°C before use. Luciferin/luciferase reagent (12ml) was added to 238ml of washed platelet suspension within 1min before stimulation. Platelet aggregation and secretion were elicited by adding platelet agonists and recorded in real time in a Chrono-log lumiaggregometer at 37°C with stirring (1000 rpm). No difference in platelet aggregation between $Lxn^{-/-}$ and WT platelets was observed. Data shown are the Mean ± SD of 3 independent replicates.



Supplementary Figure 5

Supplementary Figure 5. Overexpression *Thbs1* did not affect WT HSC number
(A) *Thbs1* was overexpressed in WT LSK cells using same strategy shown in **Figure 7A**. Western blot analysis of THBS1 protein in *Thbs1*-overexpressing (*Thbs1*) WT LSK cells. Actin (ACT) was the internal normalization control. Blots are representative of 2 independent experiments. **(B)** Absolute number of HSC clones, defined by cobblestone area forming cell (CAFC) assay, at d35 of culture in *Thbs1*-overexpressing (*Thbs1*) WT LSK cells.