Supplementary Materials

Supplementary Figures

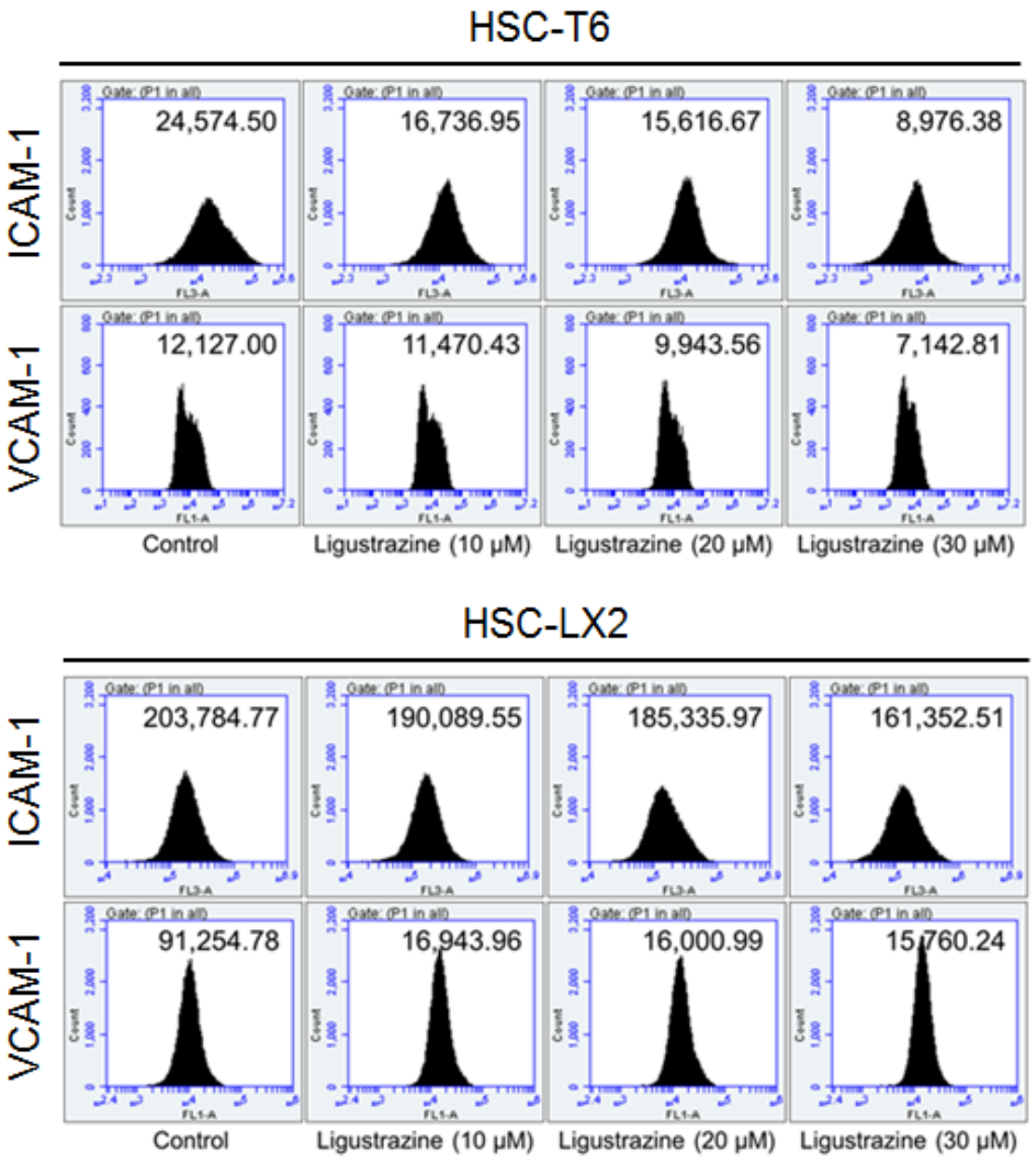

Figure S1. Ligustrazine reduces membrane abundance of adhesion molecules in HSCs.

Flow cytometry analyses of membrane distribution of ICAM-1 and VCAM-1 by determining their fluorescence intensity in HSCs treated with ligustrazine for 24 h. Representative histogram graphs were shown and corresponding fluorescence intensity was indicated.

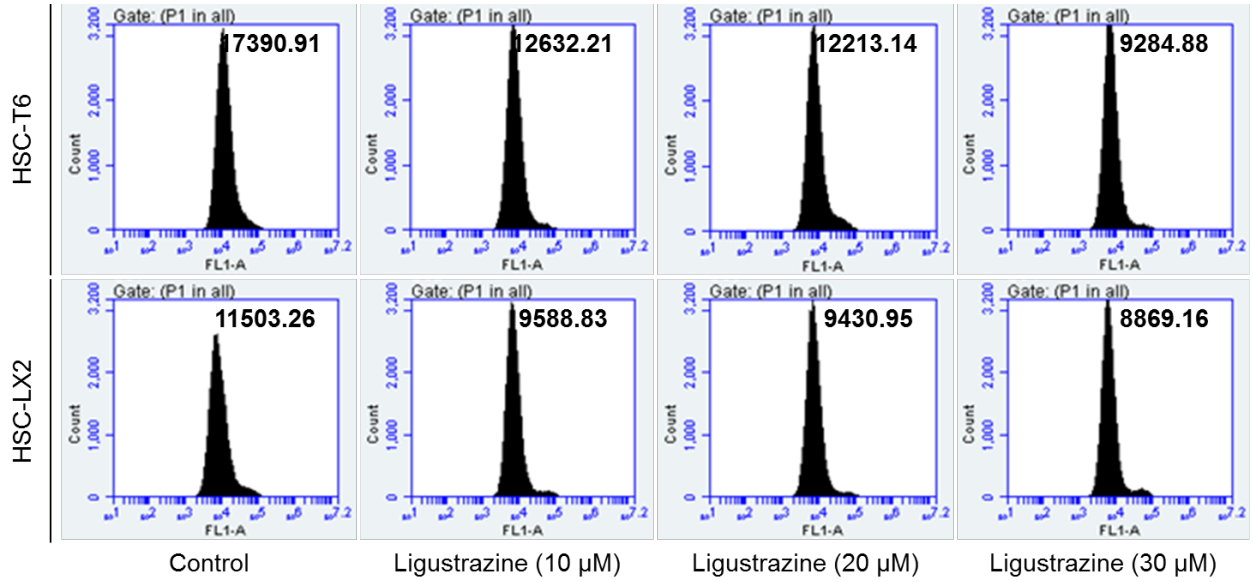

Figure S2. Ligustrazine decreases intracellular Ca^{2+} levels in HSCs. Flow cytometry analyses of intracellular Ca^{2+} levels by determining the fluorescence intensity of Fluo-3 in HSCs treated with ligustrazine for 24 h. Representative histogram graphs were shown and corresponding fluorescence intensity was indicated.

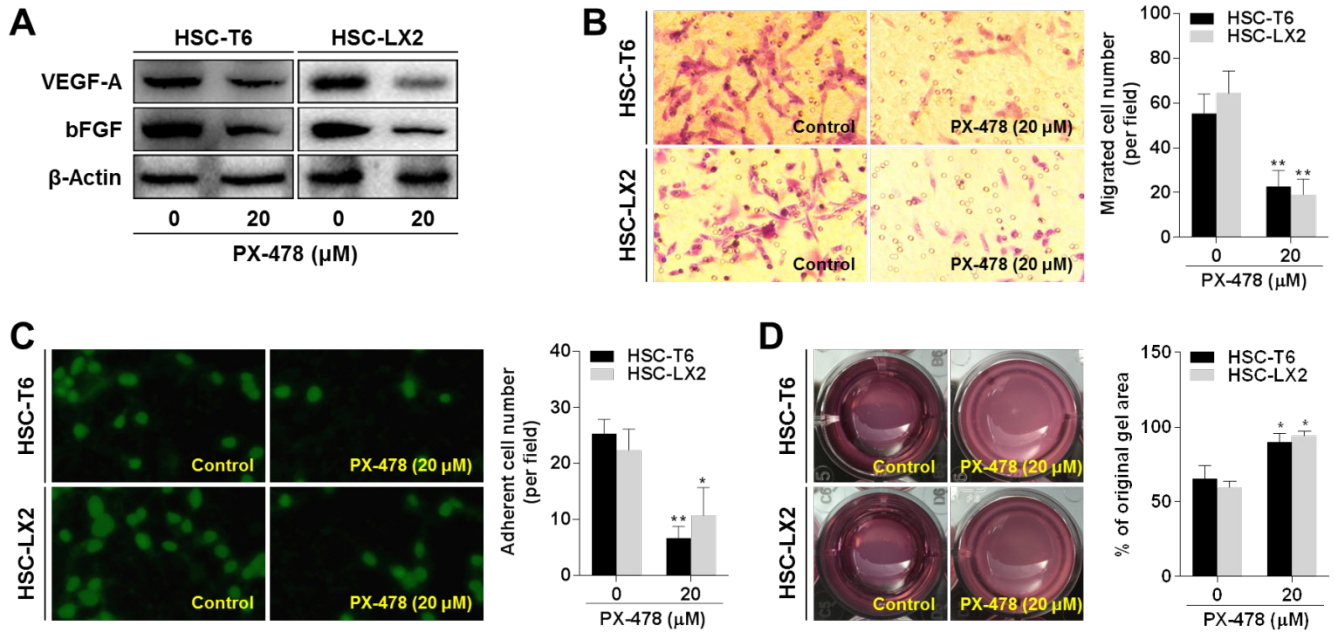

Figure S3. HIF-1α positively regulates HSC pericyte functions. (A) Western blot analyses of protein expression of pro-angiogenic cytokines in HSCs treated with PX-478 for 24 h. **(B)** Boyden chamber assay for evaluating migration of HSCs treated with PX-478 for 24 h (100× magnification). The number of migrated cells per field was counted. Significance: ***P*<0.01 versus control. **(C)** FDA staining for evaluating adhesion of HSCs treated with PX-478 for 24 h (100× magnification). The number of adherent cells per field was counted. Significance: **P*<0.05 versus control, ***P*<0.01 versus control. **(D)** Collagen gel assays for evaluating contraction of HSCs treated with PX-478 for 24 h. Percentages of original gel area were quantified. Significance: **P*<0.05 versus control.

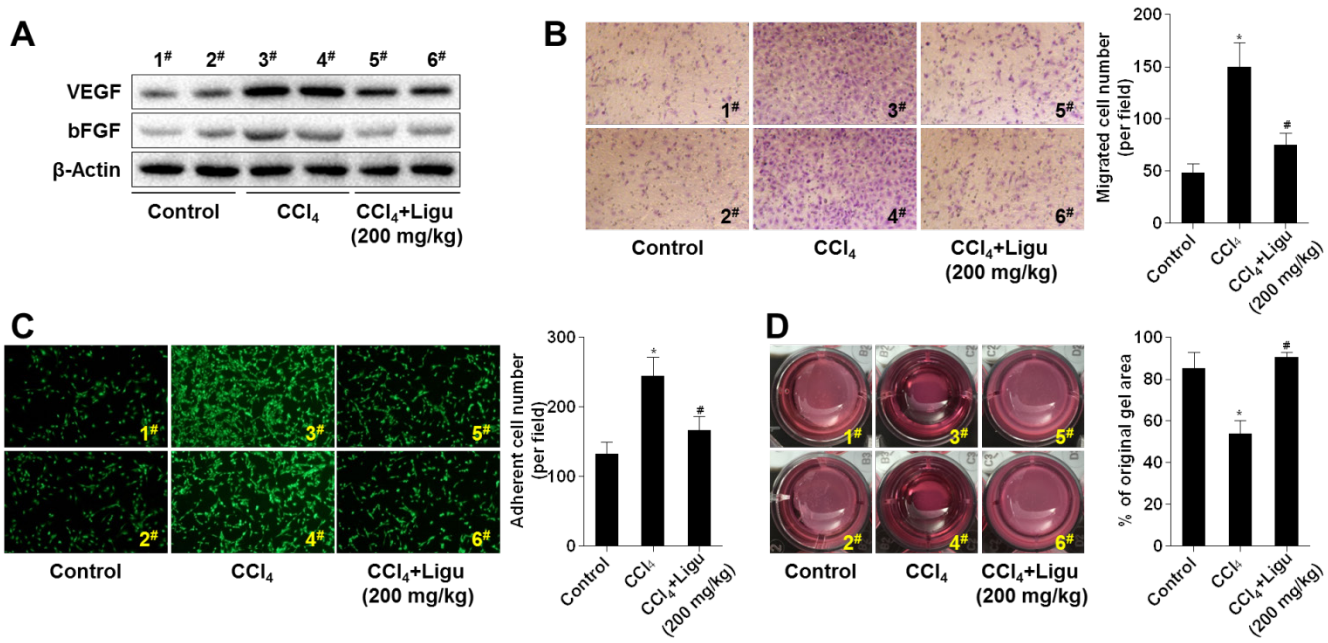

Figure S4. Ligustrazine represses pericyte functions of primary HSCs isolated from rats intoxicated with CCl_4. At the end of experiments, primary HSCs were isolated from two rats in each group of control (samples $1^{\#}$ and $2^{\#}$), CCl_4 (samples $3^{\#}$ and $4^{\#}$), and ligustrazine (200 mg/kg) treatment (samples $5^{\#}$ and $6^{\#}$), respectively. The freshly isolated HSCs were used for experiments immediately. **(A)** Western blot analyses of protein expression of pro-angiogenic cytokines in HSCs. **(B)** Boyden chamber assay for evaluating migration of HSCs (100× magnification). The number of migrated cells per field was counted. Significance: $^{*}P<0.05$ versus control, $^{\#}P<0.05$ versus CCl_4. **(C)** FDA staining for evaluating adhesion of HSCs (100× magnification). The number of adherent cells per field was counted. Significance: $^{*}P<0.05$ versus control, $^{\#}P<0.05$ versus CCl_4. **(D)** Collagen gel assays for evaluating contraction of HSCs. Percentages of original gel area were quantified. Significance: $^{*}P<0.05$ versus control, $^{\#}P<0.05$ versus CCl_4. In this figure, ligustrazine is abbreviated as ligu.

Supplementary Tables

Table S1. Primer sequences for site-directed mutagenesis

Recombinant plasmids	Sequences	
PPARγ (wild-type)	Forward	5’-TACCGGACTCAGATCTCGAGCGCCACCATGACC ATGGTTGACACAGAG-3’
	Reverse	5’-GATCCCGGGCCCGCGGTACCGTGTACAAGTCCT TGTAGATCTCCTGCAGGAGCGGGTG-3’
PPARγ (Ser289 mutant)	Forward	5’-TACCGGACTCAGATCTCGAGCGCCACCATGACC ATGGTTGACACAGAGA-3’
	Reverse	5’-GATCCCGGGCCCGCGGTACCGTGTACAAGTCCT TGTAGATCTCCTG-3’
PPARγ (Ser342 mutant)	Forward	5’-TACCGGACTCAGATCTCGAGCGCCACCATGACC ATGGTTGACACAGAGA-3’
	Reverse	5’-GATCCCGGGCCCGCGGTACCGTGTACAAGTCCT TGTAGATCTCCTG-3’
PPARγ (Ser289 and Ser342 mutant)	Forward	5’-TACCGGACTCAGATCTCGAGCGCCACCATGACC ATGGTTGACACAGAGA-3’
	Reverse	5’-GATCCCGGGCCCGCGGTACCGTGTACAAGTCCT TGTAGATCTCCTG-3’

Table S2. Primer sequences for real-time PCR.

Genes	Sequences	
VEGF-A (rat)	Forward	5’-GCACTGGACCCTGGCTTTACT-3’
	Reverse	5’-ATGGGACTTCTGCTCTCCTTCTG-3’
VEGF-A (human)	Forward	5’-CTGTCTAATGCCCTGGAGCC-3’
	Reverse	5’-ACGCGAGTCTGTGTTTTTGC-3’
bFGF (rat)	Forward	5’-CAACACTTACCGGTCACGGA-3’
	Reverse	5’- CCCCGTTTTGGATCCGAGTT-3’
bFGF (human)	Forward	5’-CCACCTATAATTGGTCAAAGTGGT-3’
	Reverse	5’-TCATCAGTTACCAGCTCCCC-3’
FATP (rat)	Forward	5'-GGTCTTCTGAGTCCCTGCTT-3'
	Reverse	5'-ACATCCAAGCTTTGCCAAGG-3'
FATP (human)	Forward	5'-TGCTCAGGTCTTGGAGAAGG-3'
	Reverse	5'-CAGCGGGTCTTCACAATAGC-3'
HIF-1α (rat)	Forward	5’-TAGACTTGGAAATGCTGGCTCCCT-3’
	Reverse	5’-TGGCAGTGACAGTGATGGTAGGTT-3’
HIF-1α (human)	Forward	5’-ACTTGGCAACCTTGGATTGGA-3’
	Reverse	5’-GCACCAAGCAGGTCATAGGT-3’
CD31 (rat)	Forward	5’-GACAGCCAAGGCAGATGCAC-3’
	Reverse	5’-ATTGGATGGCTTGGCCTGAA-3′
CD34 (rat)	Forward	5’-CCTGCCGTCTGTCAATGTTTC-3’
	Reverse	5’-GCACTCCTCGGATTCCTGAAC-3’
vWF (rat)	Forward	5’-GCGTGGCAGTGGTAGAGTA-3’
	Reverse	5’-GGAGATAGCGGGTGAAAT-3’
GAPDH (rat)	Forward	5’-GGCCCCTCTGGAAAGCTGTG-3’
	Reverse	5’-CCGCCTGCTTCACCACCTTCT-3’
GAPDH (human)	Forward	5’-TGACAACAGCCTCAAGAT-3’
	Reverse	5’-GAGTCCTTCCACGATACC-3’