

The systemic deletion of interleukin-1 α reduces myocardial inflammation and attenuates ventricular remodeling in murine myocardial infarction

Short title: IL-1 α deficiency reduces post-MI inflammation and remodeling

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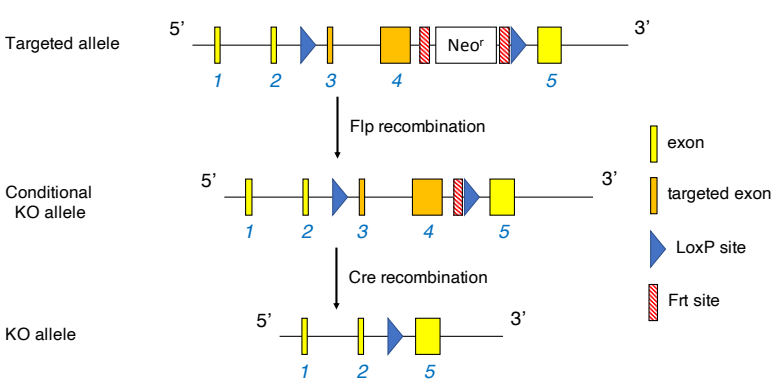
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Supplementary Figure 1. Generation of mice with *Il1a*-floxed allele spanning exons 3 and 4 of *Il1a* gene. *Il1a*-floxed mice were generated by Cyagen Biosciences Inc. (Santa Clara, CA, USA) using the following targeting strategy. LoxP sites spanning exons 3 and 4 was done by homologous recombination with a targeting vector. Neomycin resistance cassette (Neo^r) was removed by Flp recombination producing the *Il1a*^{fl} allele. Upon Cre recombinase expression and Cre recombination, exons 3 and 4 are deleted producing the KO allele.

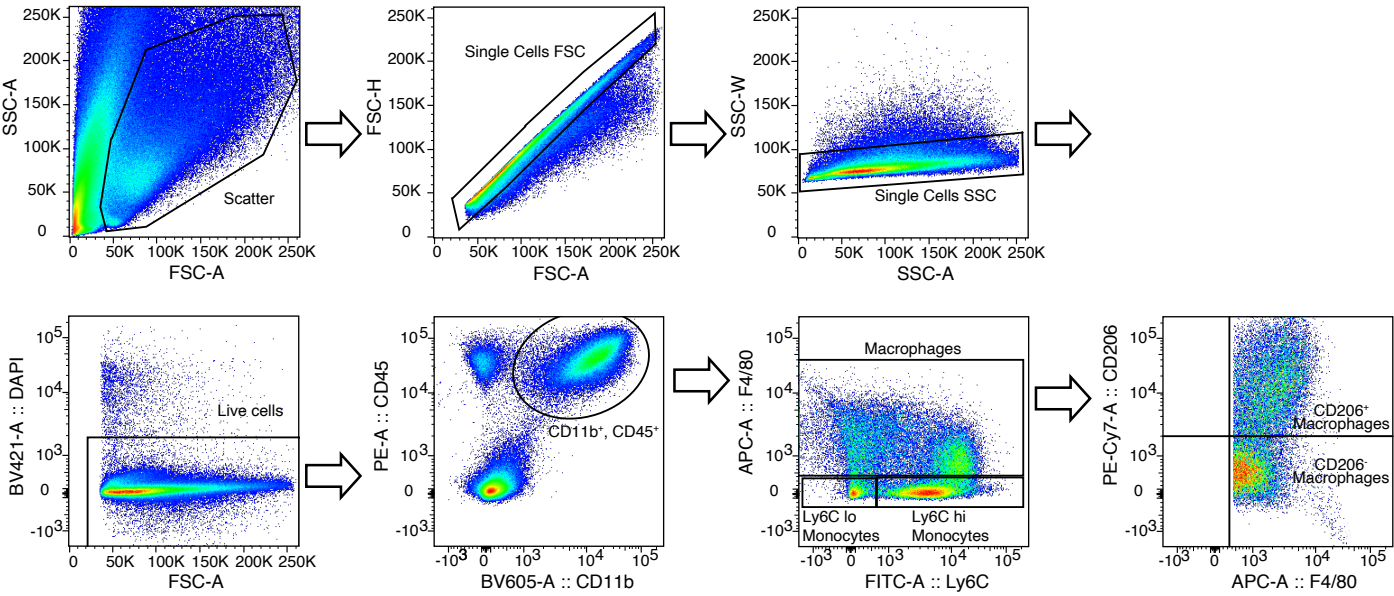
Supplementary Figure 2. Gating strategy for FACS analysis. Cell debris, cell doublets and dead cells (according to DAPI staining) were excluded from analysis. Analysis was done on single live cells. Monocytes/macrophages were identified as CD11b⁺/CD45⁺ positive cells. Macrophages were characterized by expression of F4/80 and monocytes as F4/80⁻ population. Pro-inflammatory monocytes were Ly6C⁺ and anti-inflammatory monocytes Ly6C⁻. Macrophages were further characterized as M1 pro-inflammatory (CD206⁻) and M2 anti-inflammatory (CD206⁺) macrophages.

Supplementary Figure 3. Time course expression of pro-inflammatory genes mRNA and pro-inflammatory cytokines during the first week after MI. Myocardial mRNA expressions of (a) *Il1a*, (b) *Il6*, (c) *Ccl2/Mcp1*, (d) *Tnf* and (e) *Mpo* pro-inflammatory genes at days 1, 3 and 7 post sham and MI surgery. mRNA expressions of target genes were detected by RT-PCR, normalized to *Rps18* housekeeping gene expression. (f) Myocardial IL-6 and (g) MCP-1 proteins levels at days 1, 3 and 7 post sham and MI surgery. Proteins from tissue lysates were analyzed by ELISA and expressed as pg/mg of total proteins. N=5/group, * p < 0.05. N.D., not detected.

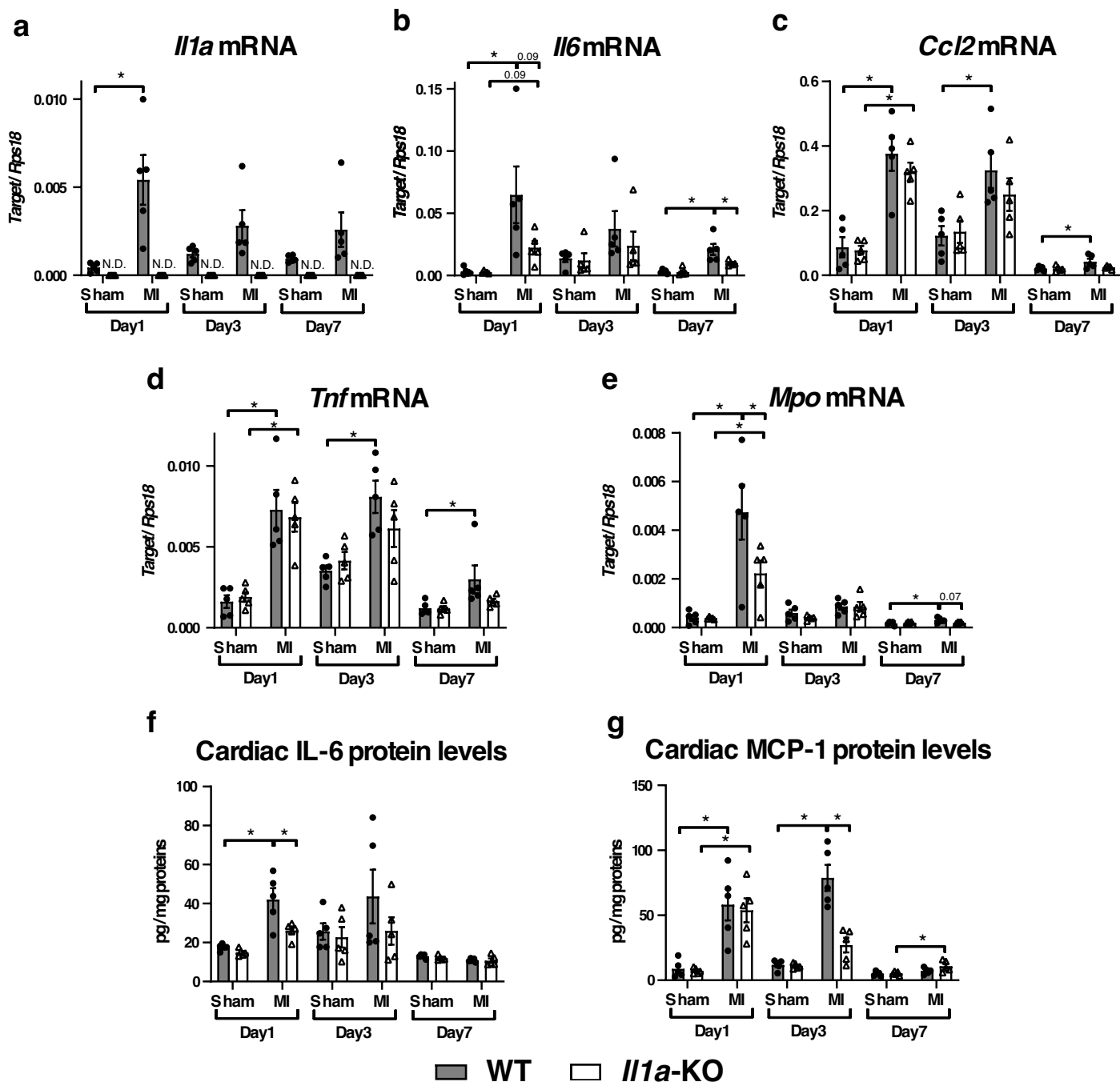
SUPPLEMENTARY FIGURE 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Table 1 : Oligonucleotides

Gene name	Protein	Forward sequence (5'-3')	Reverse Sequence (5'-3')	Use
<i>Acta2</i>	α -SMA	TTCATTGGGATGGAGTCAGCG	AATGCCTGGGTACATGGTGG	RT-PCR
<i>Bambi</i>	BMP and activin membrane-bound inhibitor homolog	ACAGCTTTCACGGACACCAT	TTGCCGCATTTTGTACAGG	RT-PCR
<i>Ccl2</i>	CCL2/MCP-1	GCACCAGCACCAGCCAACTCT	GCAGGCCCAAGCATGACAGG	RT-PCR
<i>Col1a1</i>	Collagen alpha-1(I) chain	GAGAGGTGAACAAGGTCCCG	AAACCTCTCTCGCTCTTGC	RT-PCR
<i>Ccn2/Ctgf</i>	CCN family member 2/Connective tissue growth factor	CACTCTGCCAGTGGAGTTCA	AGATGTCATTGTCCCAGGA	RT-PCR
<i>Il1a</i>	Interleukin-1 α	GCAACGGGAAGATTCTGAAG	TGACAACTTCTGCCTGACG	RT-PCR
<i>Il6</i>	Interleukin-6	GTGGCTAAGGACCAAGACCA	ACCACAGTGAGGAATGTCCA	RT-PCR
<i>Mpo</i>	Myeloperoxidase	CACCTCTTTGTTTCGAGAGC	AGAGCTTCTCCCATTCCAT	RT-PCR
<i>Myh7</i>	Myosin-7/Myosin heavy chain 7	GGCCCTTTGACCTCAAGAAAGA	TCACCGTCTTGCCATTCTCC	RT-PCR
<i>Nppb</i>	Natriuretic peptides B / Brain natriuretic peptide B	CTGAAGGTGCTGTCCCAGAT	CCTTGGTCTTCAAGAGCTG	RT-PCR
<i>Postn</i>	Periostin	GCAAACCACTTTCACCGACC	CGTTGGTCCATGCTCAGAGT	RT-PCR
<i>Rps18</i>	40S ribosomal protein S18	ACTTTGGGGCCTTCGTGTC	GCCCAGAGACTATTCTTCTTG	RT-PCR
<i>Tgfb1</i>	Transforming growth factor beta-1 proprotein	CACCGGAGAGCCCTGGATA	TGTACAGCTGCCGCACACA	RT-PCR
<i>Tgfb1</i>	TGF-beta receptor type-1	AGCGTTCATGGTTCCGAGAG	TCTGACACCAACCACAGCTG	RT-PCR
<i>Tnf</i>	Tumor necrosis factor	TGTTGCCTCTCTTTTGCTT	TGGTCACCAATCAGCGTTA	RT-PCR
Gene name	Target	Forward sequence (5'-3')	Reverse Sequence (5'-3')	Use
<i>Myh6-Cre</i>	Wild-type allele	TCTATTGCACACAGCAATCCA	CCAACCTTGTGAGAGGAGCA	genotyping
<i>Myh6-Cre</i>	Mutant allele (Cre expressing)	TCTATTGCACACAGCAATCCA	CCAGCATTGTGAGACAAGG	genotyping
<i>Il1a-floxed</i>	Region spanning LoxP site located in 3' of exon 4	TAACATAAGGGAAAGTCTAGTAGG	GTTTTGGAGCTTAGAGAGAGTGA	genotyping
<i>Il1a-del</i>	Amplification of a 750bp fragment upon deletion of exons 3 and 4	ACAGCCACTGGTGTCTCTT	GTTTTGGAGCTTAGAGAGAGTGA	genotyping
Gene name	Target	Sequence (5'-3')	Comment	Use
<i>Il1a</i>	Murine <i>Il1a</i>	AAAgattcATGGCCAAAGTTCCTGACTTGT	BamHI restriction site	cloning
<i>Il1a</i>	Murine <i>Il1a</i>	AAAgcgccgcTTATGATATCTGGAAGTCTGCATAGAGG	NotI restriction site	cloning

Supplementary Table 2 : Antibodies used for western blotting and flow cytometry.

Western Blot Antibodies	Brand	Catalog n°	Dilution
α-Tubulin	Adipogen	AG-27B-0005	1:2000
Flag-tag	Sigma	F1804-50ug	1:1000
IL-1α	eBioscience	14-7011-85	1:1000
SMAD2/3	Cell Signaling	#3102	1:1000
P-SMAD2	Cell Signaling	#3108	1:1000
VCAM-1	R&D Systems	AF643	1 :1000
GAPDH	Origene	TA302944	1 :10000

Flow Cytometry Antibodies			
CD16/32 (Fc Block)	Biolegend	101302	1 :50
CD45-PE	Biolegend	101306	1:2000
CD11b-BV605	Biolegend	101257	1:2000
Ly-6C-FITC	Biolegend	128006	1:200
F4/80-AF647	Biolegend	123122	1:200
CD206-PE/Cy7	Biolegend	141720	1:100