

Experimental Design

1. Characterization of bone marrow and spleen hematopoietic stem and progenitor cell populations in myeloid Sulf deficient or overexpression (OE) mice.

Please note that this experiment will examine bone marrow hematopoiesis in mice with myeloid Sulf deletion or overexpression at two different ages. There is no experimental manipulation, thus there is no timeline of the experimental procedure. Diagram or flowchart is not necessary.

Cohort 1:

Groups	Age		# of mice
	8-12 weeks of age	8-12 months of age	
Sulf1 ^{flox/flox} Sulf2 ^{flox/flox} (control)	10 males 10 females	10 males 10 females	40
LysM-cre ^{+/-} (control)	10 males 10 females	10 males 10 females	40
LysM-Sulf KO mice (Sulf1 ^{flox/flox} Sulf2 ^{flox/flox} LysM-cre ^{+/-})	10 males 10 females	10 males 10 females	40
Total number of mice			120

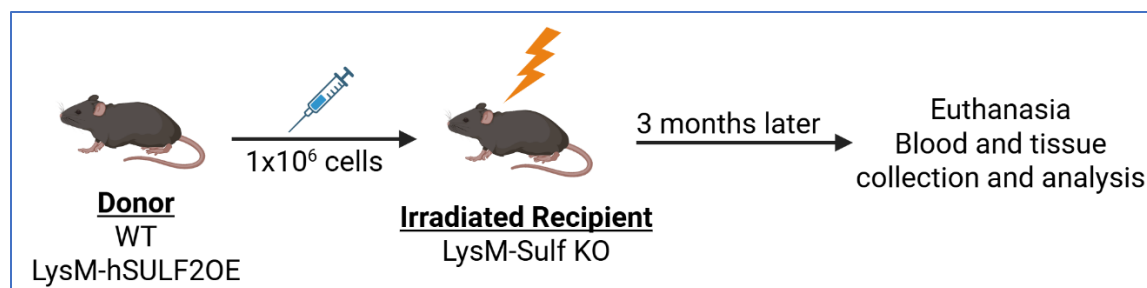
Cohort 2:

Groups	Age		# of mice
	8-12 weeks of age	8-12 months of age	
LoxP-STOP-LoxP (LSL)-hSULF2 (control)	10 males 10 females	10 males 10 females	40
LysM-cre ^{+/-} (control)	10 males 10 females	10 males 10 females	40
LysM-hSULF2 OE mice (LSL- hSULF2-LysM-cre ^{+/-})	10 males 10 females	10 males 10 females	40
Total number of mice			120

Brief description of experiment 1: Mice at two different ages (8-12 weeks of age and 8-12 months of age) will be euthanized, cells from the bone marrow and the spleen will be isolated and their hematopoietic stem and progenitor cell populations will be analyzed by flow cytometry and RNA/protein analyses. An updated panel of antibodies compared to our published findings in Matrix Biology (2024 Dec:134:107-118) will be used to further dissect how myeloid deletion or overexpression of the Sulf will alter bone marrow hematopoiesis.

2. Bone marrow transplantation.

Experimental diagram:

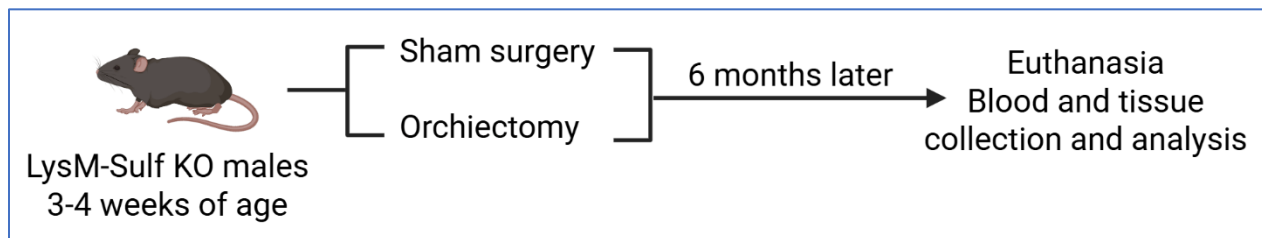


Donor		Recipient		# of mice
Genotype	# of mice	Genotype	# of mice	
WT	10 males	LysM-Sulf KO mice	10 males	20
LysM-hSULF2 OE mice	10 males	LysM-Sulf KO mice	10 males	20
Total number of mice				40

Brief description of experiment 2: Young male mice (8-12 weeks of age) will be used in this experiment. Bone marrow cells will be isolated from the donor mice and transplanted to irradiated recipient mice via tail vein injection. Blood cell production in the recipient mice will be examined at 3 months following the transplantation.

3. Role of male sex hormones in the development of bone marrow hematopoietic abnormalities in the LysM-Sulf KO mice.

Experimental diagram:



	Procedure	# of mice
LysM-Sulf KO male mice	Sham	10
LysM-Sulf KO male mice	orchiectomy	10
Total number of mice		20

Brief description of experiment 3: Only male mice will be used in this experiment. Male mice at 3-4 weeks of age will undergo bilateral orchiectomy and these mice will be examined 6 months later for changes in bone marrow hematopoiesis.

4. Examine how the Sulfs regulates macrophage phenotype in vitro.

Please note that in this experiment, alveolar and bone marrow derived macrophages (BMDMs) will be isolated from 8-12 weeks old mice, and then the rest of the experiment will be performed in vitro. There is no experimental manipulation in these mice, thus diagram or flowchart is not necessary.

Cohort 1: Macrophage-specific Sulf deletion Model

Group	Males	Females	# of mice
Sulf1 ^{flox/flox} Sulf2 ^{flox/flox} (control)	10	10	20
Cx3cr1-cre ^{+/-} (control)	10	10	20
Sulf1 ^{flox/flox} Sulf2 ^{flox/flox} Cx3cr1-cre ^{+/-}	10	10	20
Total number of mice			60

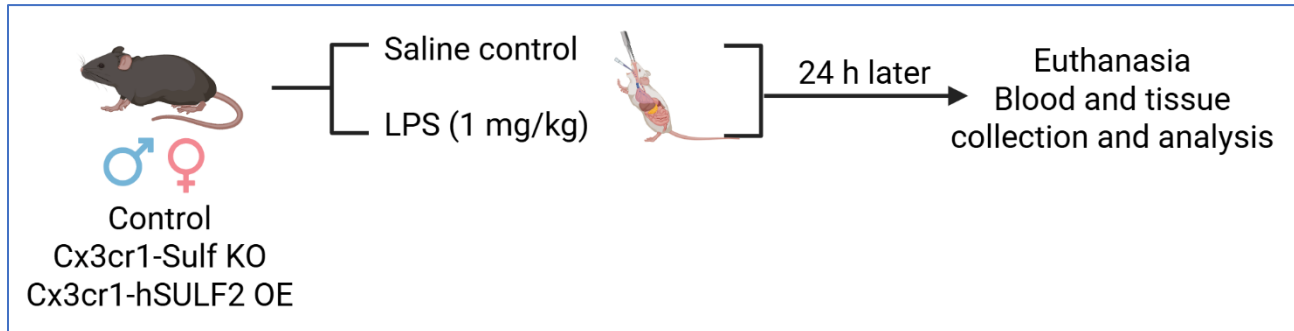
Cohort 2: Macrophage-specific hSULF2 OE Model

Group	Males	Females	# of mice
LoxP-STOP-LoxP (LSL)-hSULF2 (control)	10	10	20
Cx3cr1-cre ^{+/-} (control)	10	10	20
LSL-hSULF2-Cx3cr1-cre ^{+/-}	10	10	20
Total number of mice			60

Brief description of experiment 4: Because the LysM-Sulf KO mice have defects in bone marrow hematopoiesis, Cx3cr1-cre (more specific for macrophages) will be used to induce Sulf deletion or hSULF2 overexpression. Both alveolar macrophages and bone marrow derived macrophages (BMDMs) will be isolated from control and mice with macrophage-specific deletion of Sulfs or macrophage-specific OE of hSULF2 at 8-12 weeks of age, and these cells will be analyzed *in vitro*.

5. Examine how the Sulfs regulates macrophage phenotype in lung homeostasis and in LPS-induced lung injury and inflammation.

Experimental diagram:



Cohort 1: Macrophage-specific Sulf deletion Model

Group	Saline		LPS		# of mice
	Males	Females	Males	Females	
Sulf1 ^{lox/lox} Sulf2 ^{lox/lox} (control)	5	5	10	10	30
Cx3cr1-cre ^{+/-} (control)	5	5	10	10	30
Sulf1 ^{lox/lox} Sulf2 ^{lox/lox} Cx3cr1-cre ^{+/-}	5	5	10	10	30
Total number of mice					90

Cohort 2: Macrophage-specific hSULF2 OE Model

Group	Saline		LPS		# of mice
	Males	Females	Males	Females	
LSL-hSULF2 (control)	5	5	10	10	30
Cx3cr1-cre ^{+/-} (control)	5	5	10	10	30
LSL-hSULF2-Cx3cr1-cre ^{+/-}	5	5	10	10	30
Total number of mice					90

Brief description of experiment 4: Control, macrophage-specific Sulf KO, and macrophage-specific hSULF2 overexpression mice at 8-12 weeks of age will be treated with saline (control) or LPS (1 mg/kg) via oropharyngeal aspiration. These mice will be euthanized 24 hours later followed by blood and tissue collection.