UAB RESEARCH CENTER OF EXCELLENCE IN ARSENICALS

CounterACT

Molecular Underpinning of Vesicant Chemical Injury and MCM Development

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Introduction

- **A large number of highly toxic chemicals which are used in chemical warfare or for industrial purposes pose significant threat to the environment and human health if exposed accidently or during the terrorist attack (**Homeland Security identified 200 chemicals segregated as Toxidrome**)**
- **Arsenicals are one group of such chemicals in the category called vesicants, which were developed and weaponized during world wars I and II**
- **Of these, Lewisite (2-chlorovinyl-dichloroarsine) is the best-known rapid action chemical weapon given the nick name 'Dew of Death'**

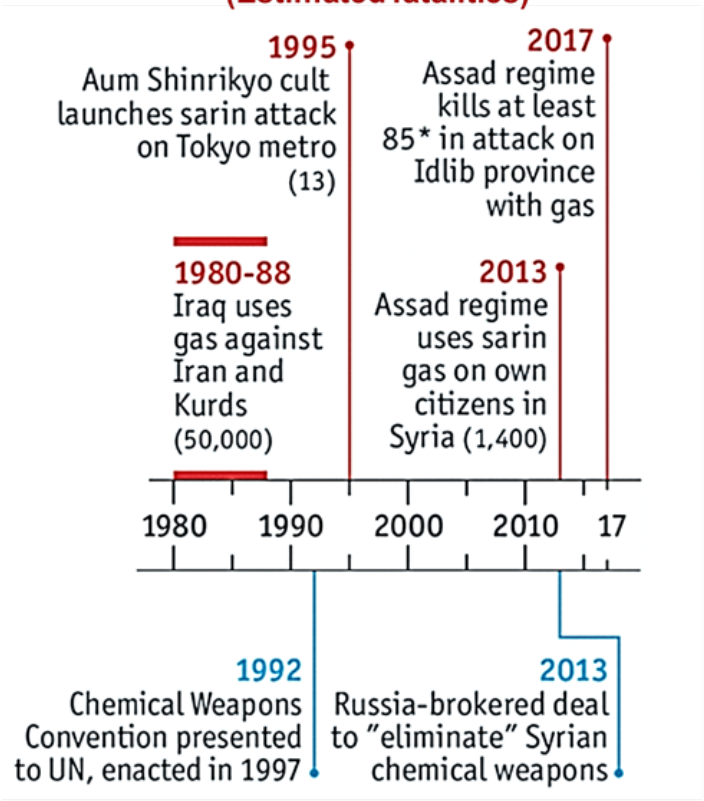
Li et al. Ann N Y Acad Sci. 2016, 1378(1):143-157.

- Arsenicals cause severe painful inflammation in the skin, eye and lungs
- Their cutaneous exposure also causes severe systemic injury often leading to lethality in humans. These include acute kidney damage, lung, liver, immune and neural injury
- The molecular pathogenesis of lesions caused by these chemicals remains largely undefined. Therefore, our focus is on unraveling the molecular pathogenesis of arsenicals in the skin, lung and kidney following their cutaneous exposure
- To develop mechanism-based effective counter-measures which can effectively block tissue damage, morbidity and lethality in animal models

Organization for the Prohibition of Chemical Weapons (OPCW), Hague

Sezigen, S et al Toxicol. Lett., 2019

USE OF CHEMICAL WEAPONS (Estimated fatalities)

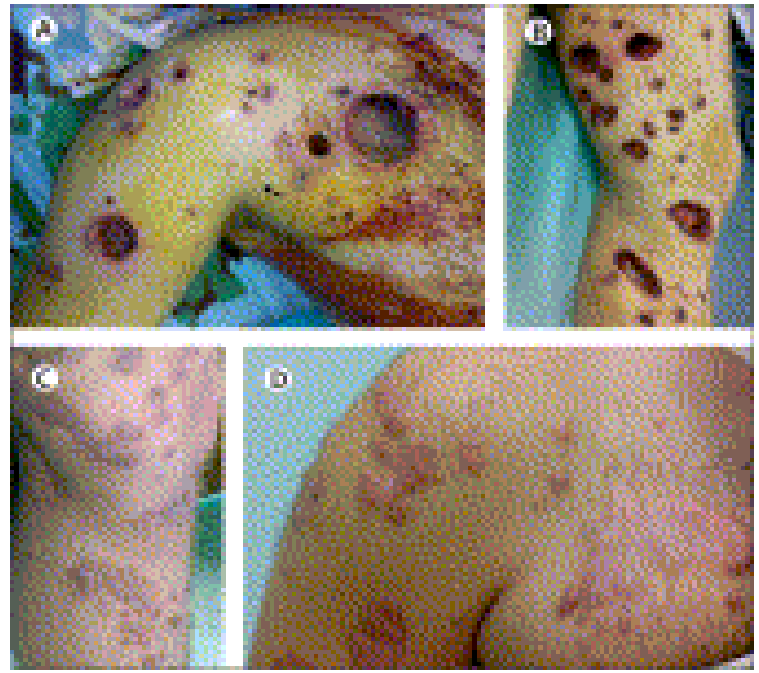


Source: *The Economist* 04/05/2017

* As of 5th April 2017

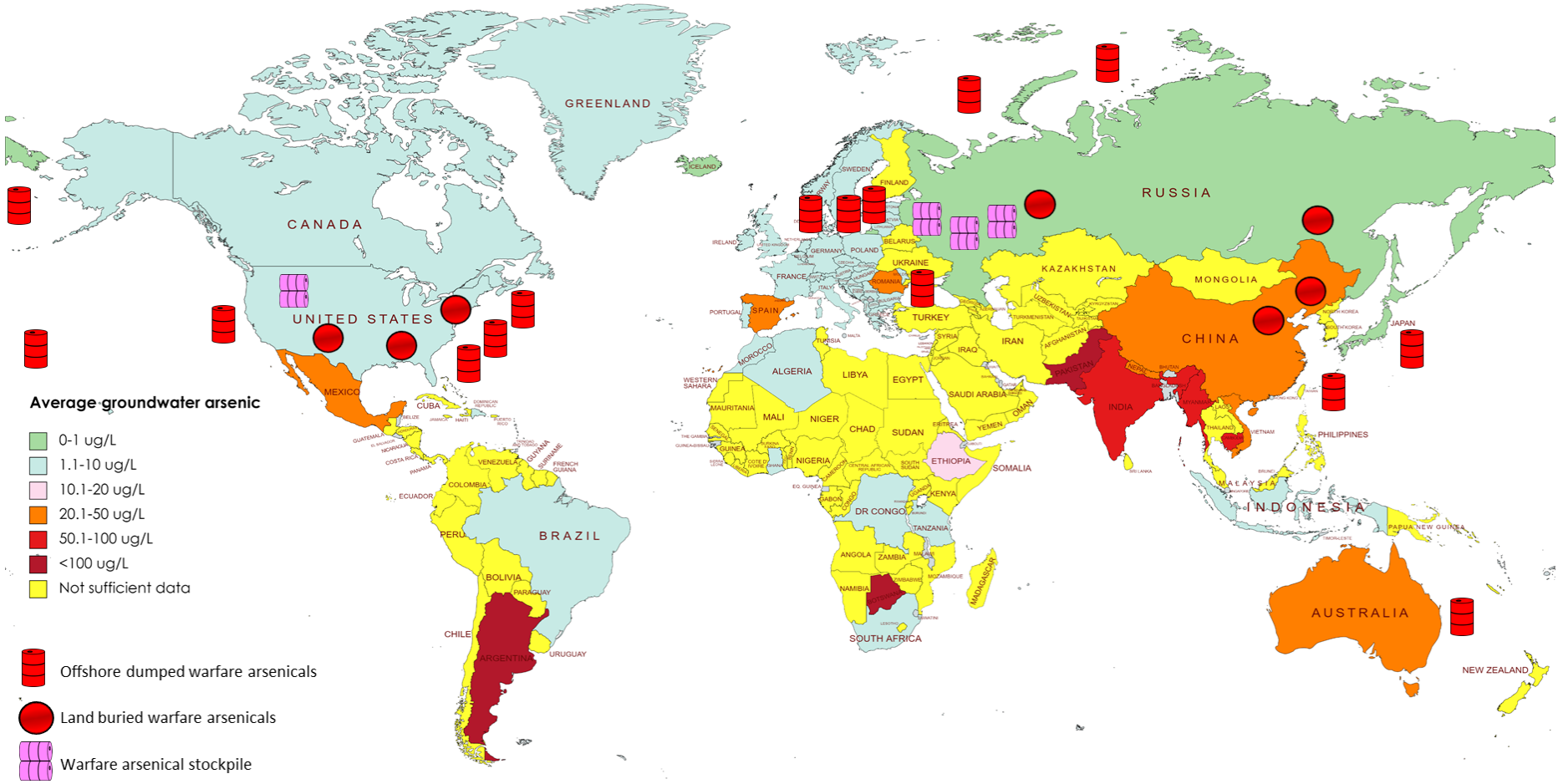
U.S. Accuses Russia of Using Chemical Weapons in Ukraine

The State Department said Russia had used chloropicrin, a poison gas widely used during World War I, against Ukrainian forces, an act that would violate a global ban signed by Moscow. Nicole Tung for The New York Times, May 2, 2024



Al Berquoni, NL et al, Lancet, 2010

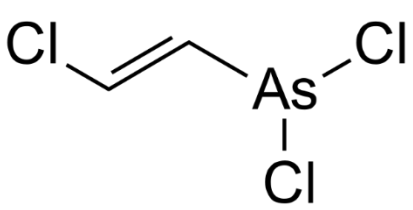
Global distribution of groundwater warfare arsenicals and arsenic



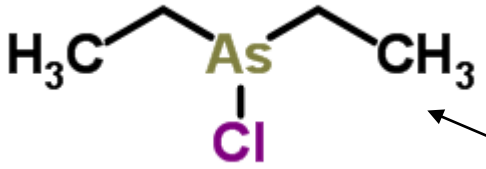
This map overlays the distribution of warfare arsenicals (existing stockpiles, land buried and offshore dumped arsenicals) with the average groundwater arsenic concentrations in different countries around the world (Podgorski et al; 2020) .

Muzaffar et al., 2023

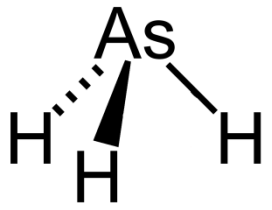
Structures of Organic & Inorganic Arsenicals



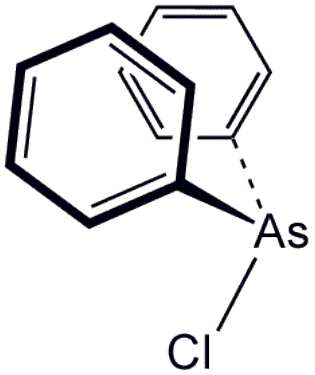
Lewisite



Diethylchloroarsine

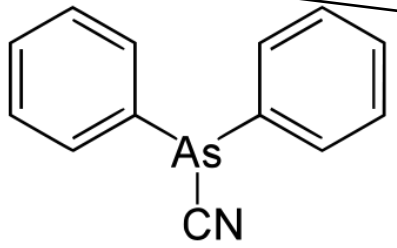


Arsine



Diphenylchlorarsine (Clark1)

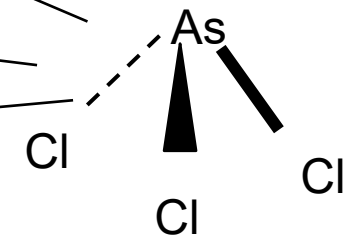
(Sneezing agent)



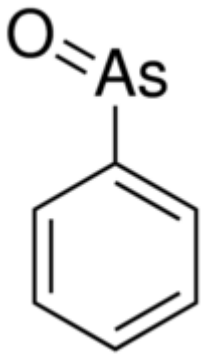
Diphenylcyanoarsine

Diphenylcyanoarsine (Clark 2)

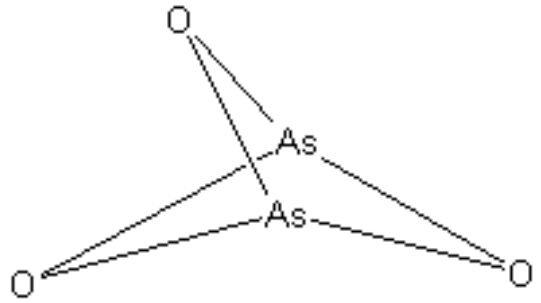
(Vomiting agent)



Arsenic Trichloride



Phenylarsine Oxide



Arsenic Trioxide

Needs for Developing Medical Countermeasures (MCMs) for Toxic Agents

- Suitable animal models which can faithfully recapitulate the human pathogenesis of toxic chemical (more than one model) & identifying a surrogate chemical that can be used in the laboratory safety conditions
- Defined molecular pathogenesis of lesions caused by exposure to these chemicals with druggable molecular target(s)
- Defined molecular toxidrome (identifying common targets)
- Availability drugs that can be repurposed or novel small molecules with IP rights

Warfare Vesicants Induce Severe Tissue Damage by Disrupting Multiple Innate Immune Regulatory Control

Translational control

Lewisite, Diphenylchlorarsine, Diphenylcyanoarsine, Diethylchlorarsine and Phenylarsine oxide

Vesicants

Epigenetic targets

Post Transcriptional regulation (RNA binding proteins)

Percutaneous Exposure

Models

Murine & Porcine

Damage

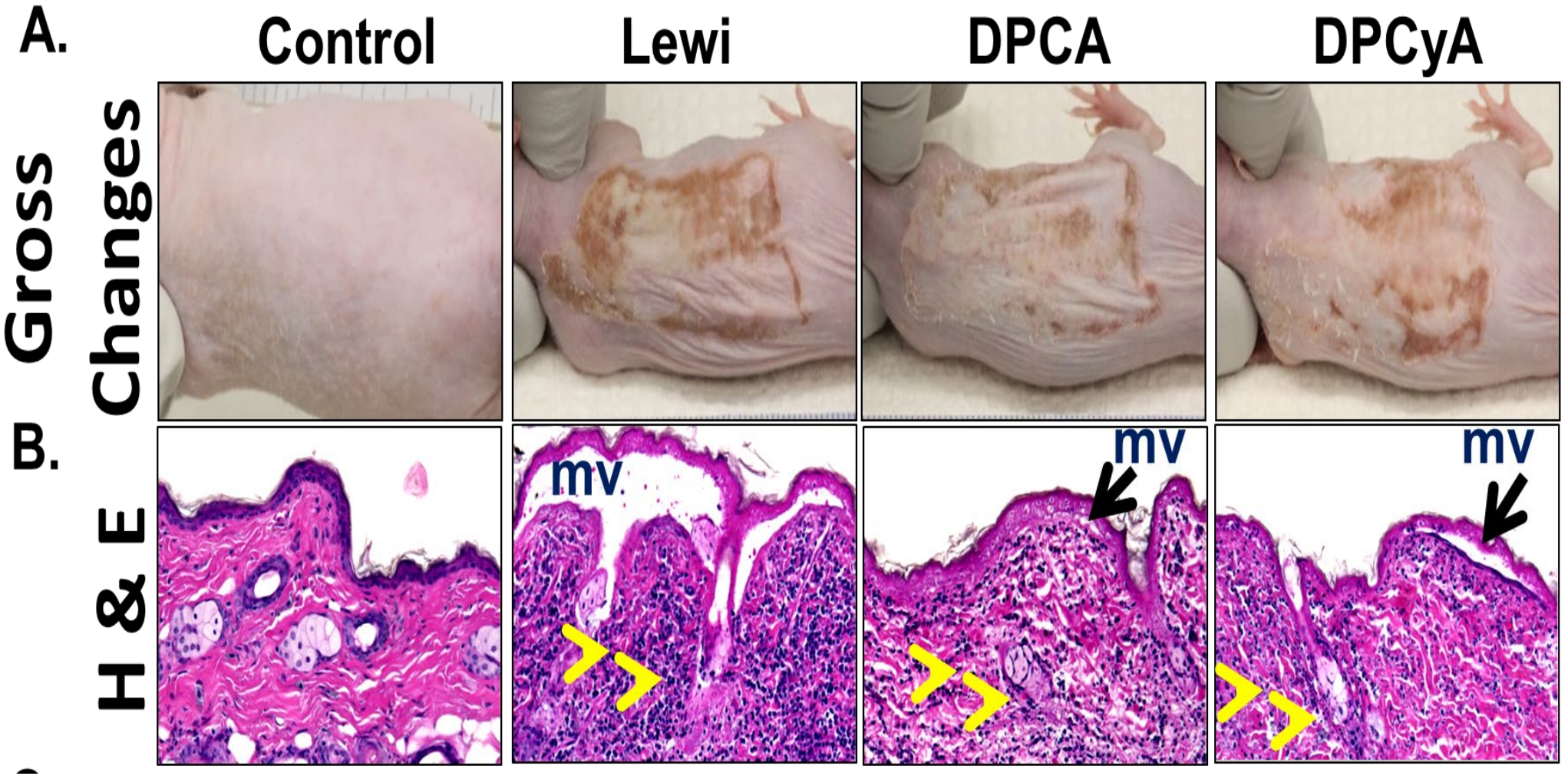
Skin Lung Kidney

Inflammation

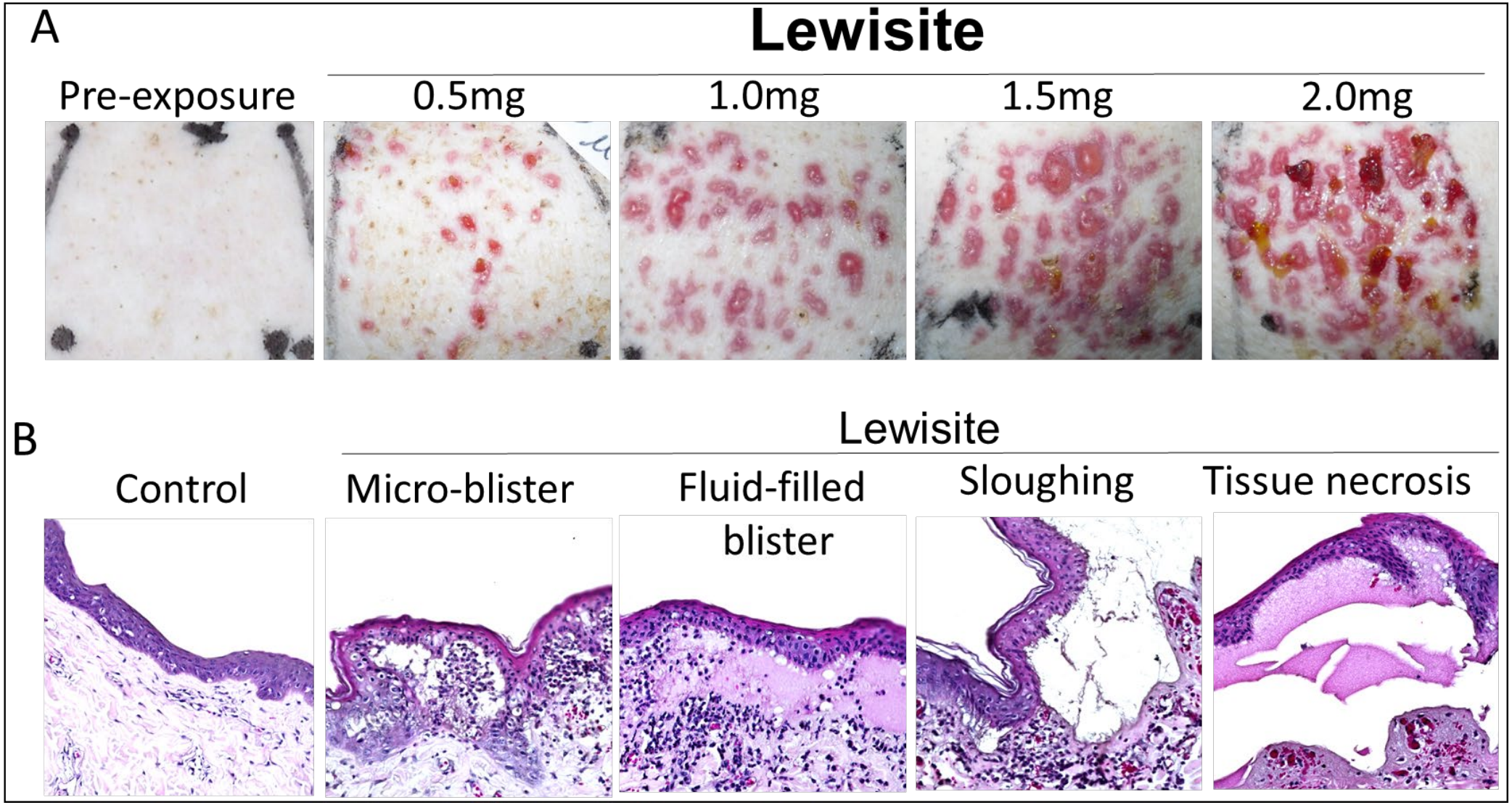
Tissue Disruption/Remodeling

Pain

Lewisite, Diphenylchlorarsine, and Diphenylcyanoarsine Induce Similar Micro-blisters and Cutaneous Injury in Mice

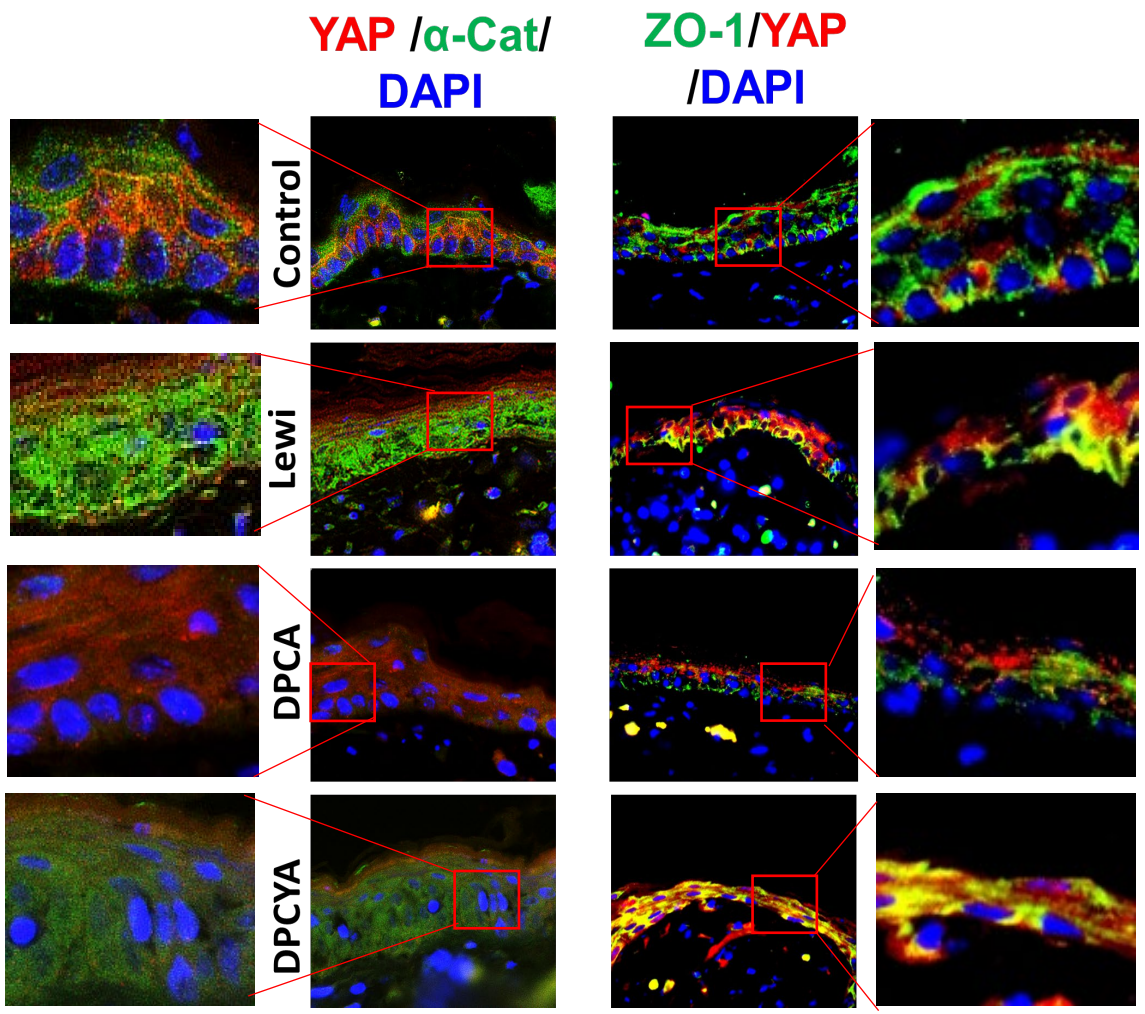


Lewisite-induced Cutaneous Blisters in Gottingen Minipig Skin

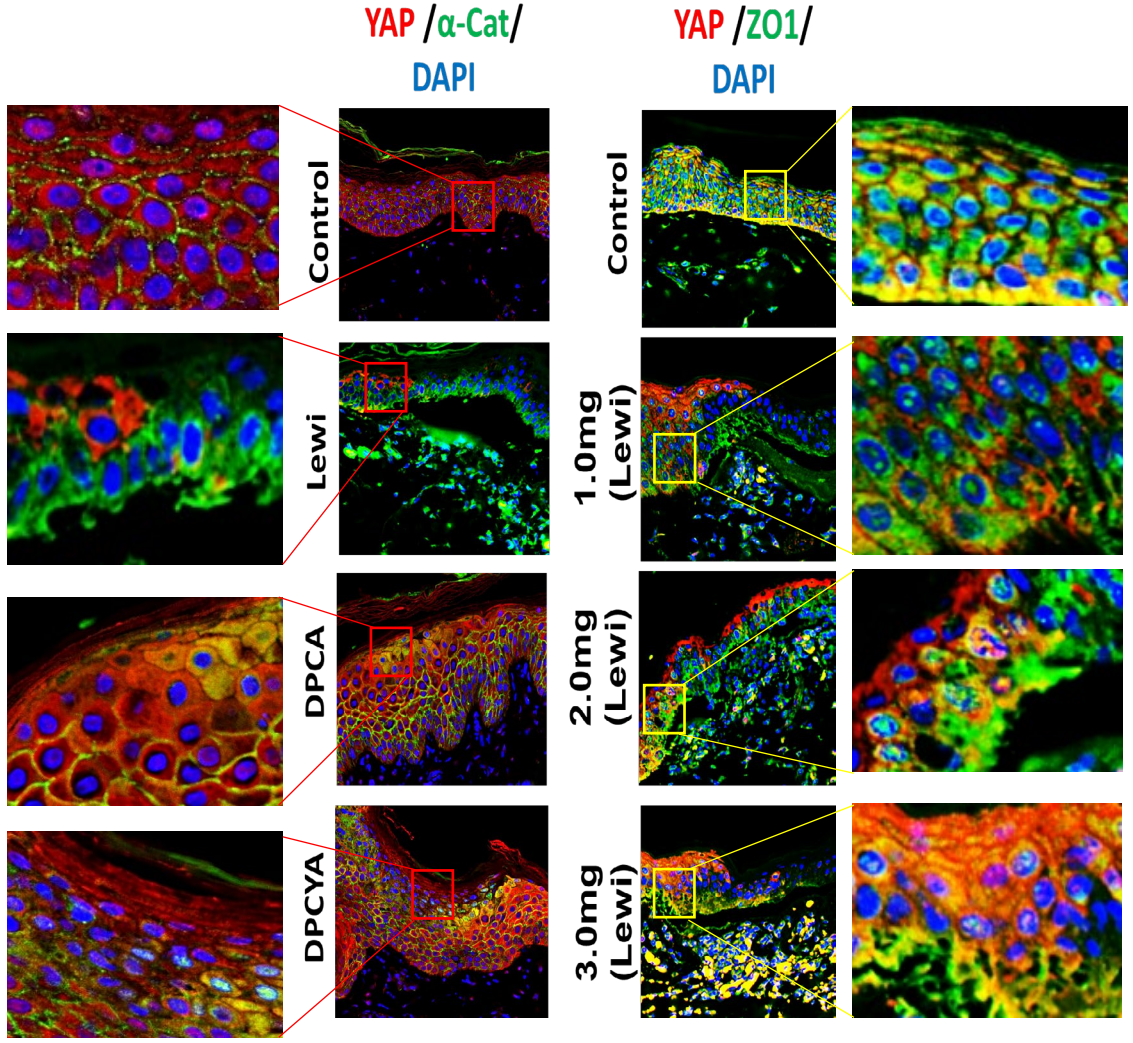


Effects of Arsenicals on Disruption of Tight & Adherens Junctions

Murine Model

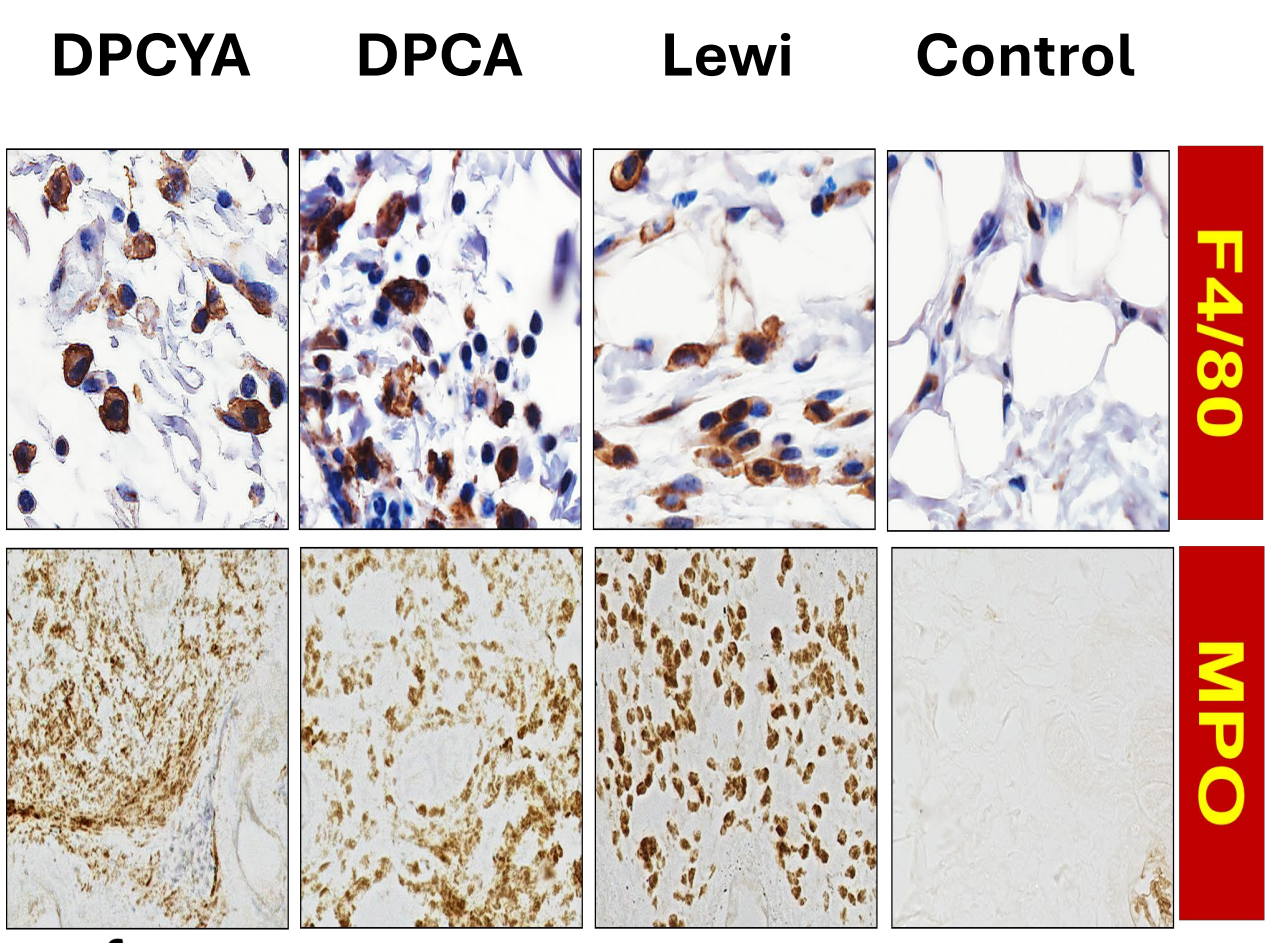


Porcine Model

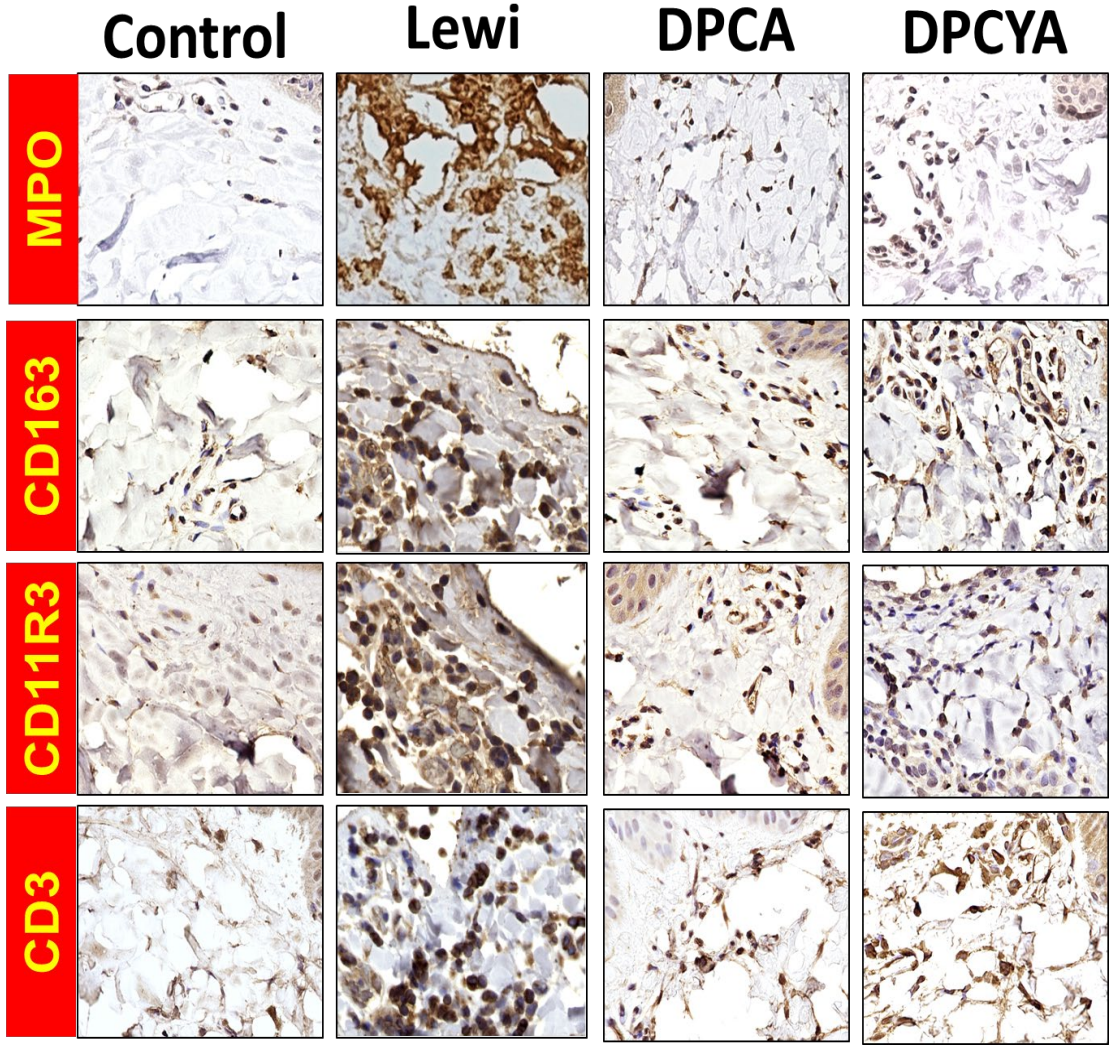


Lewisite, DPCA, DPCYA Induce Severe Cutaneous Inflammation

Mice



Minipig



Development of Arsenical Surrogate

SCIENTIFIC REPORTS

OPEN

Defining cutaneous molecular pathobiology of arsenicals using phenylarsine oxide as a prototype

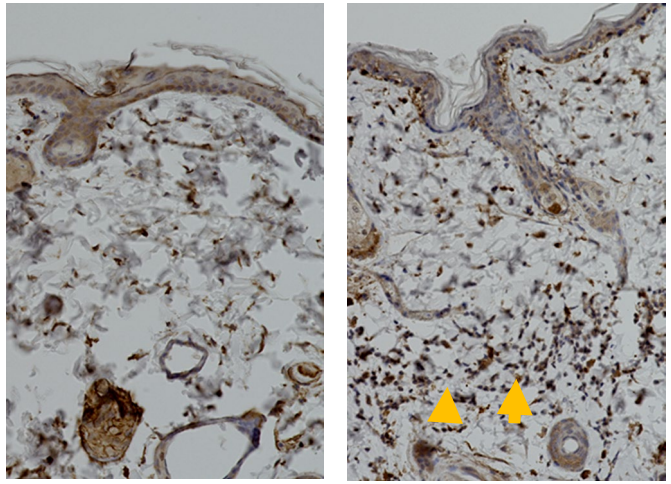
Received: 09 June 2016
Accepted: 16 September 2016
Published: 11 October 2016

Ritesh K. Srivastava[1,*], Changzhao Li[1,*], Zhiping Weng[1], Anupam Agarwal[2], Craig A. Elmets[1], Farrukh Afaq[1] & Mohammad Athar[1]

Arsenicals are painful, inflammatory and blistering causing agents developed as chemical weapons in

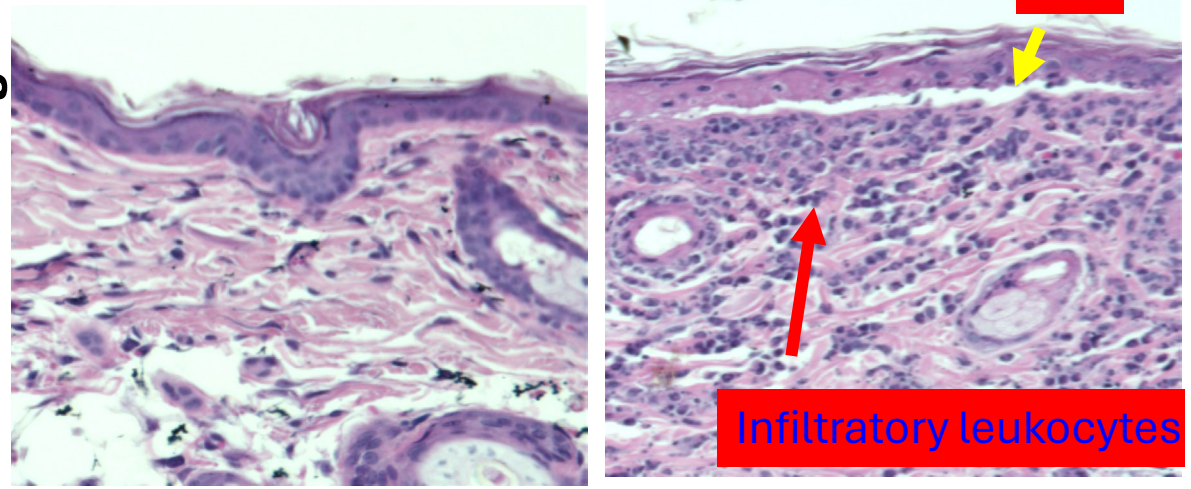
Control PAO

F4/80



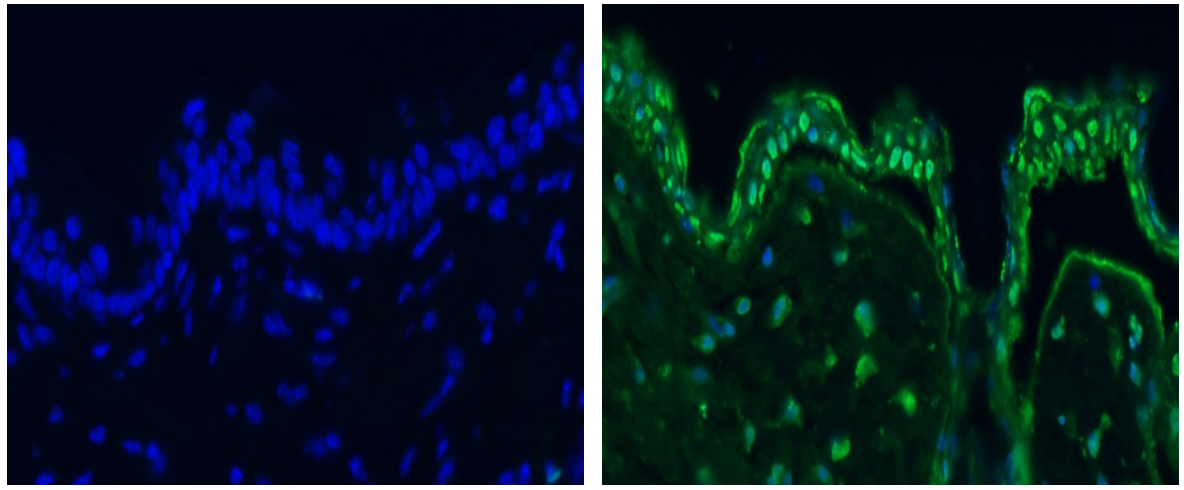
Control PAO

H & E Staining

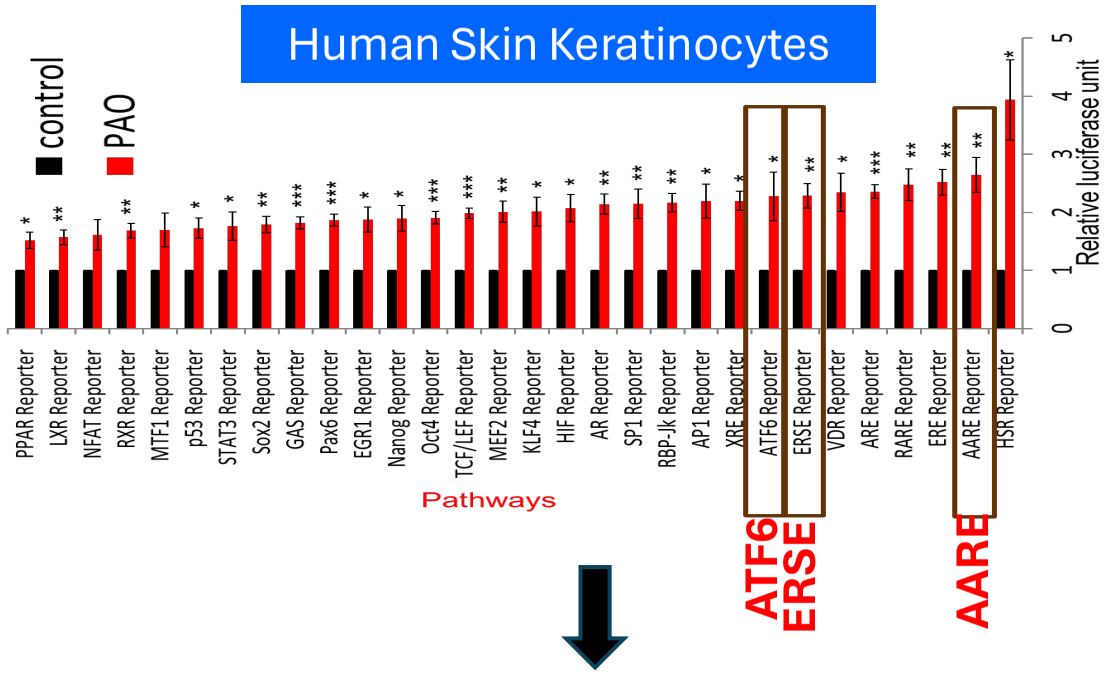


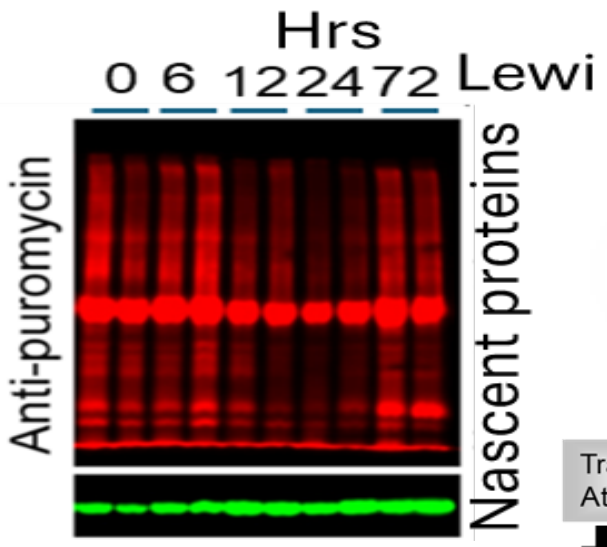
Control PAO

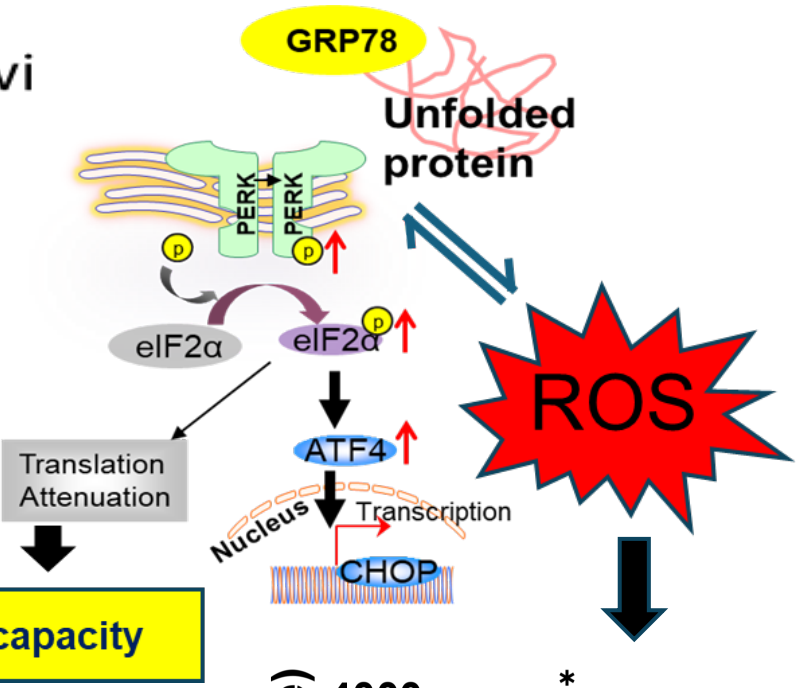
TUNEL/DAPI



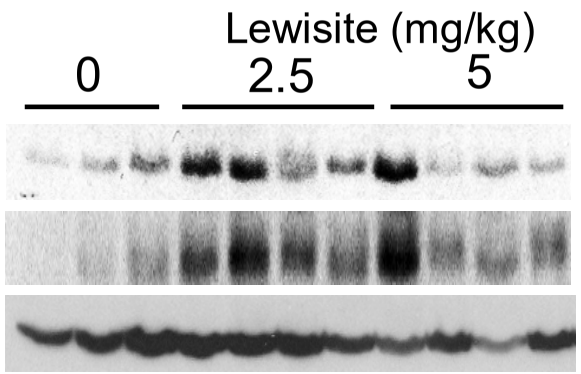
Unfolded Protein Responses is Associated with Arsenicals-Induced Cutaneous Injury



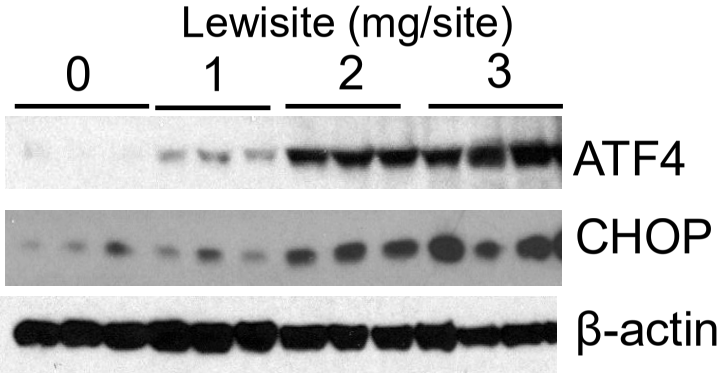


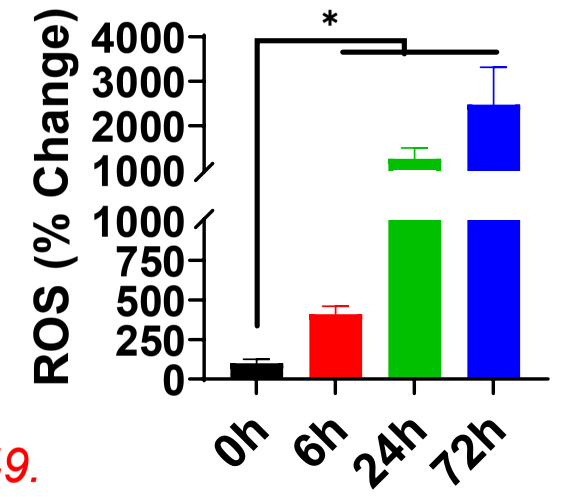


Validation in Mice Skin



Validation in Pig Skin





Li C. et. al. Am J Pathol. 2016 Oct;186(10):2637-49.

Chaperone/Antioxidant Therapy

4-Phenyl Butyric Acid (4-PBA)

(FDA approved for Urea Cycle Disorders on May13, 1996)

Marketed by:

i. Ucyclyd Pharma

ii. Swedish Orphan International

iii. Fyrlklovern Scandinavia

4-PBA+taurursodiol

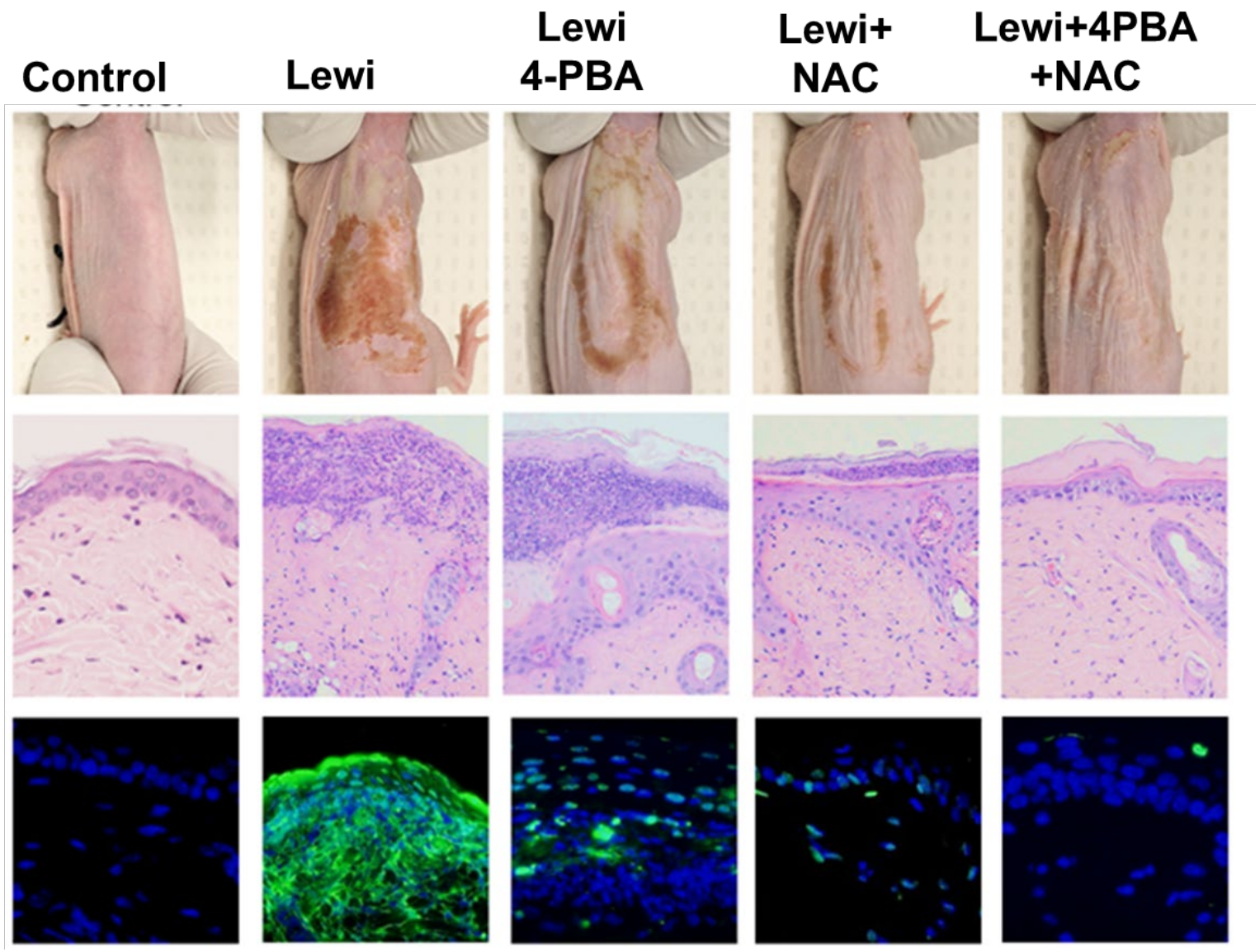
(FDA approved for Amyotrophic Lateral Sclerosis 09/2022)

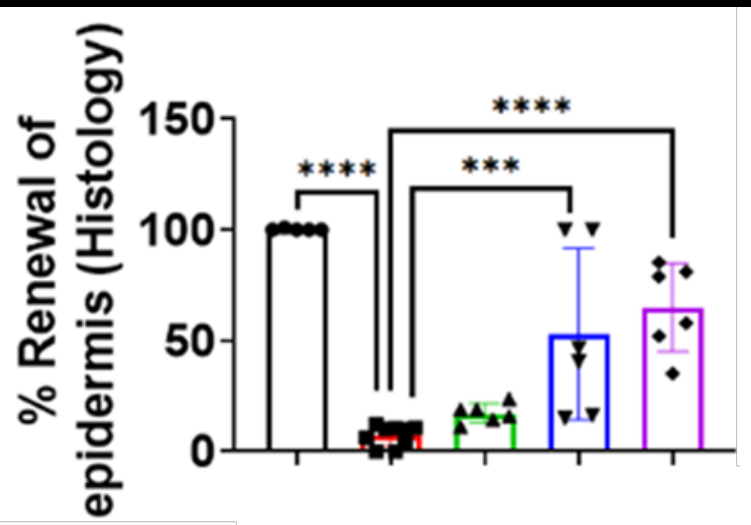
N-acetyl Cysteine (NAC)

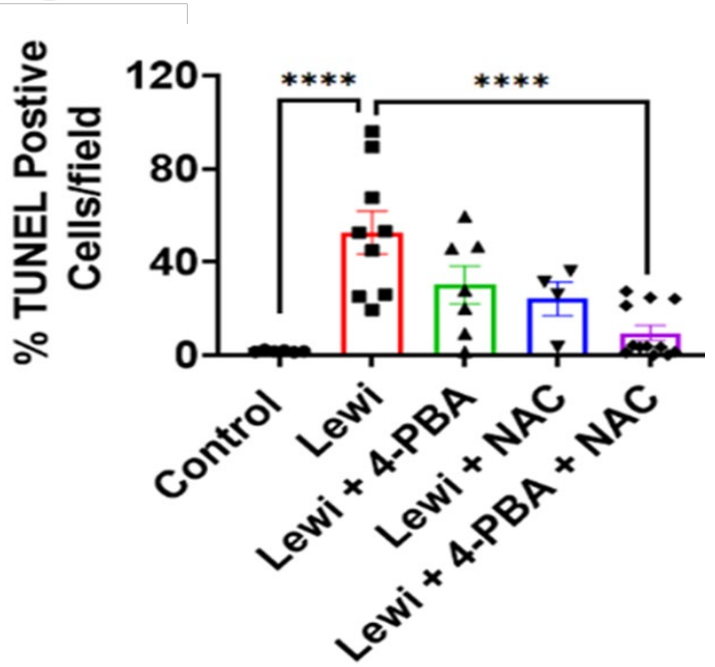
(FDA approved for overdose toxicity of Acetaminophen on January 23, 2004)

Marketed by: Cumberland Pharma

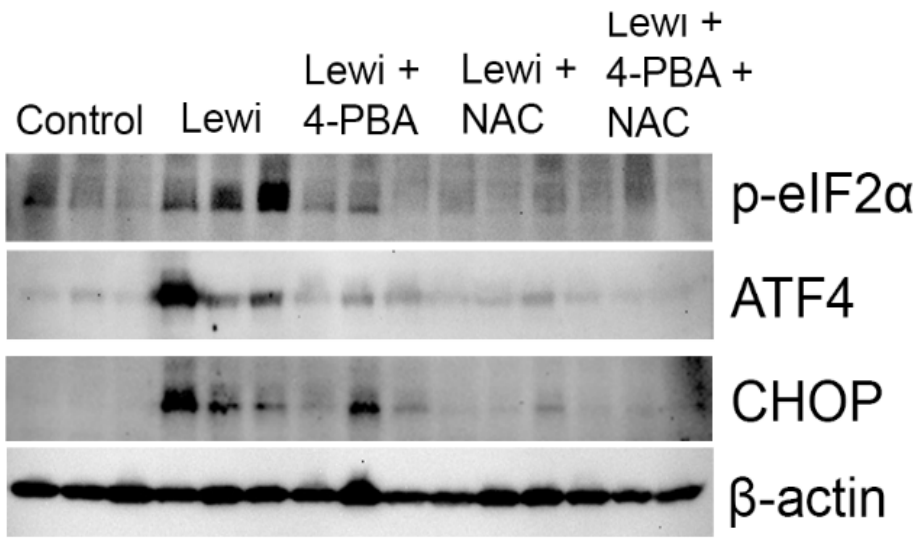
Combination of 4-PBA+NAC is Highly Effective in Attenuating Arsenical-induced Cutaneous Injury in Mice

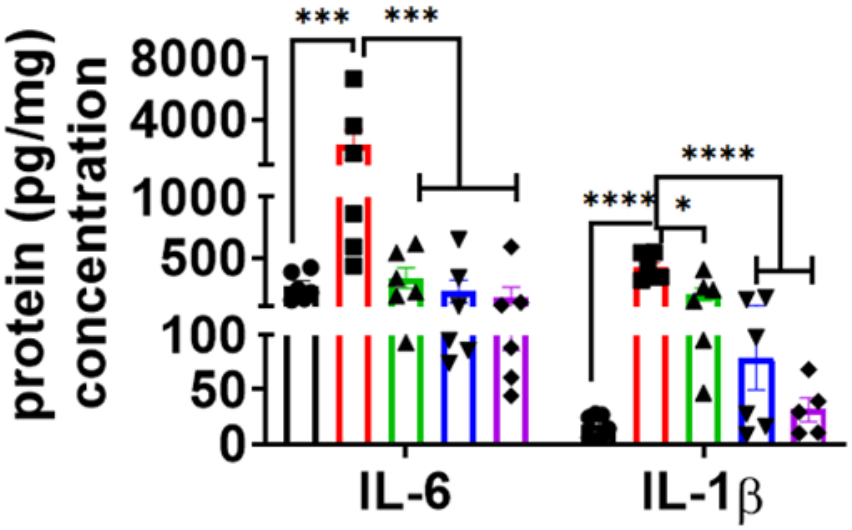


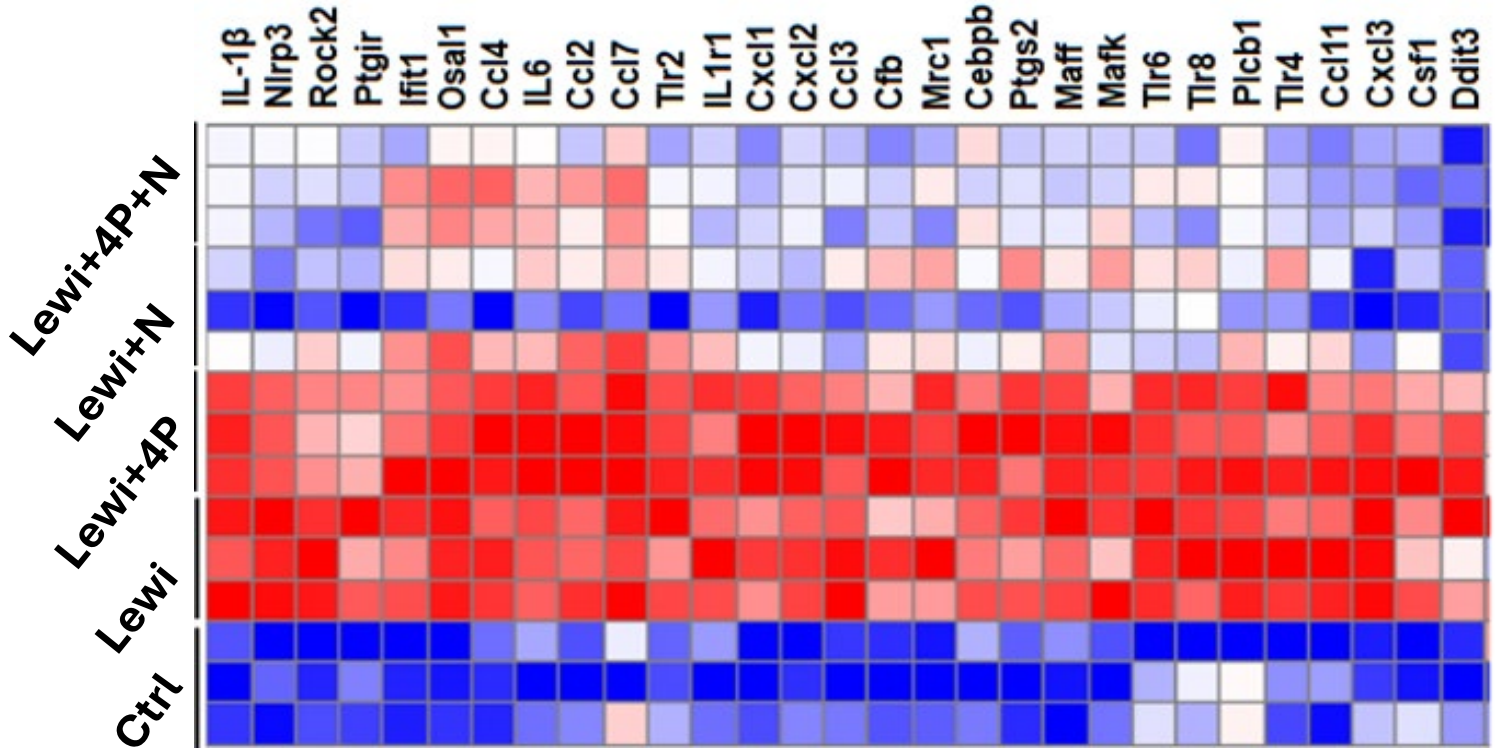




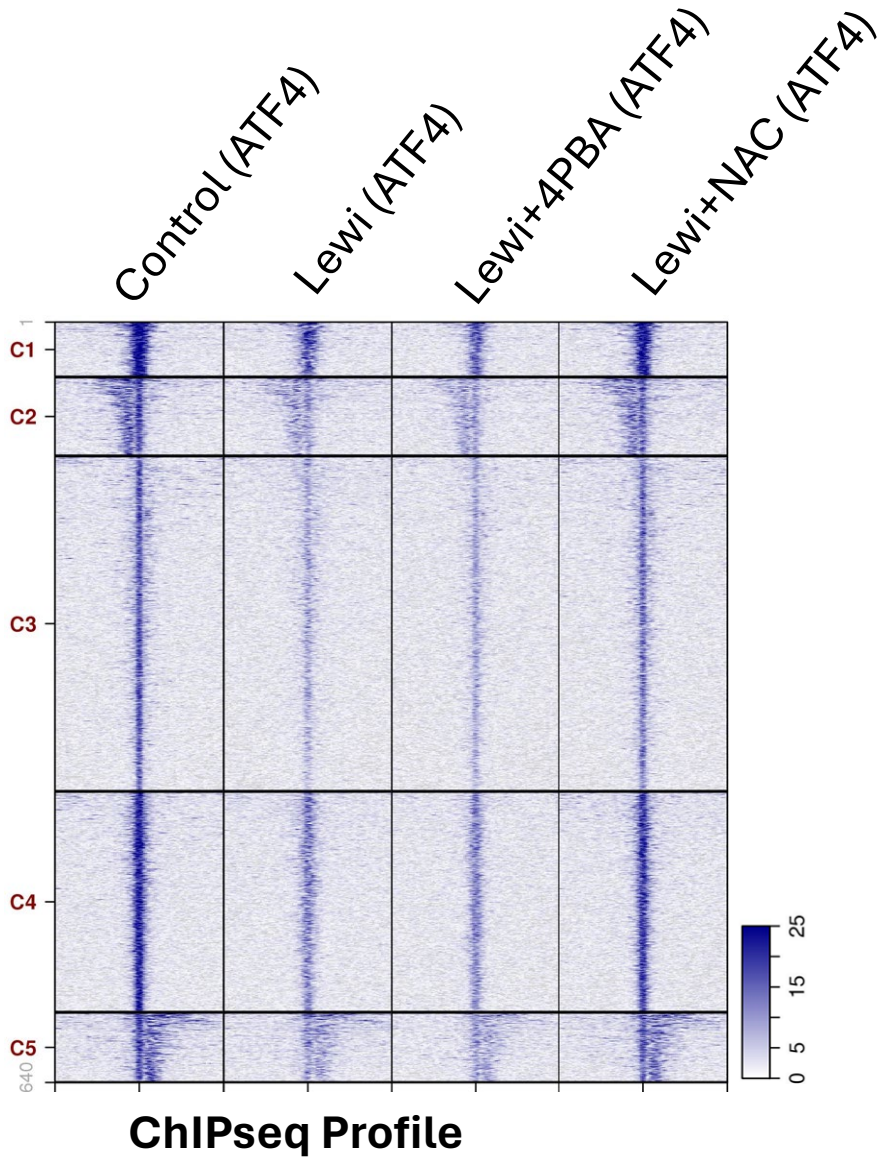
Combination of 4-PBA+NAC Reverses Lewisite-induced Inflammatory Phenotype in Mouse Skin

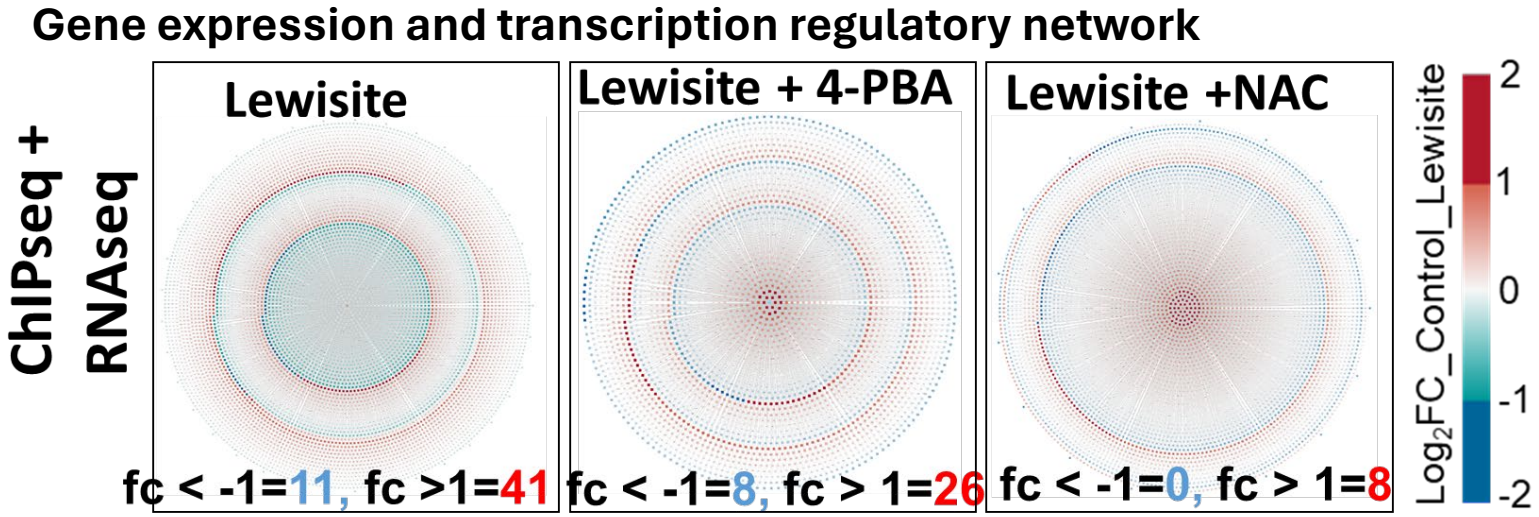


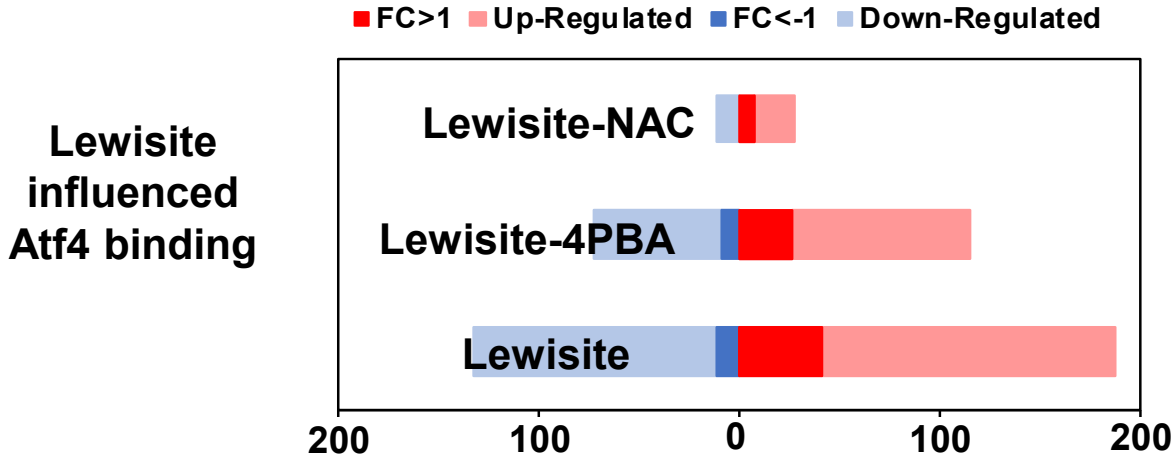




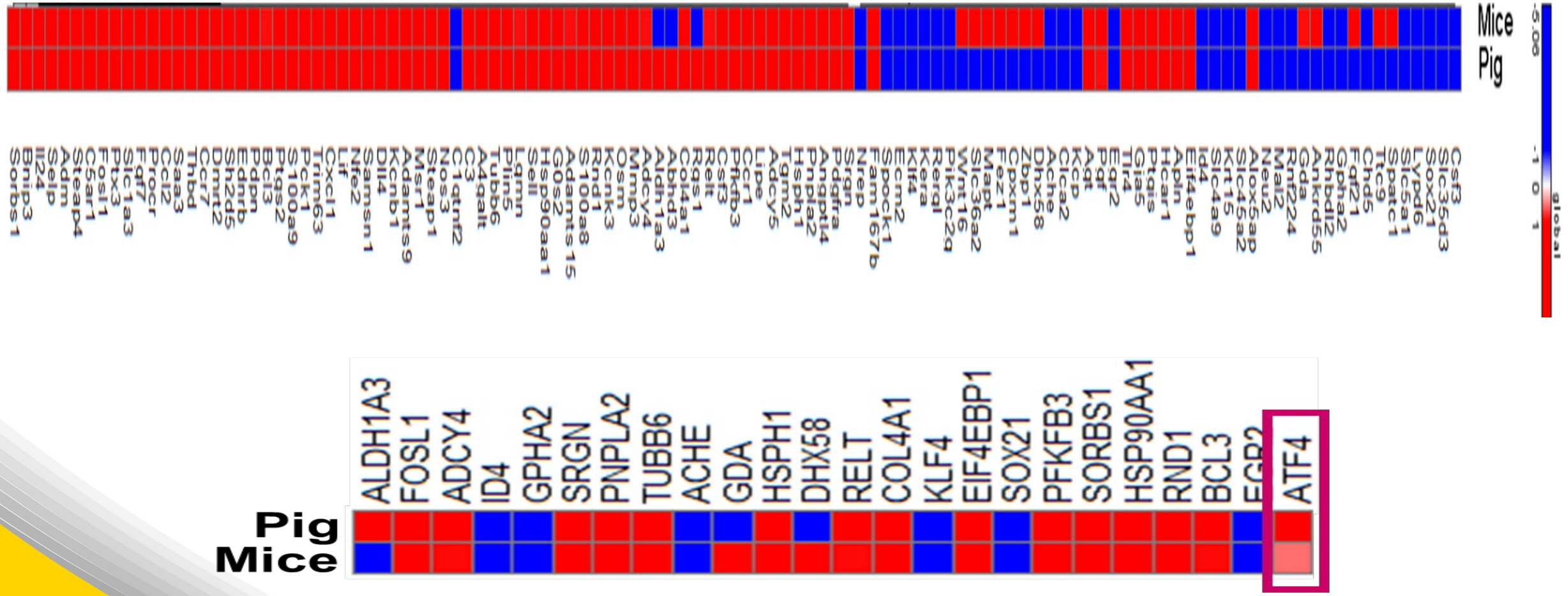
Combination of 4-PBA+NAC Reverses Lewisite-induced Alterations in Genomic Occupancy of ATF4 in Mouse Skin



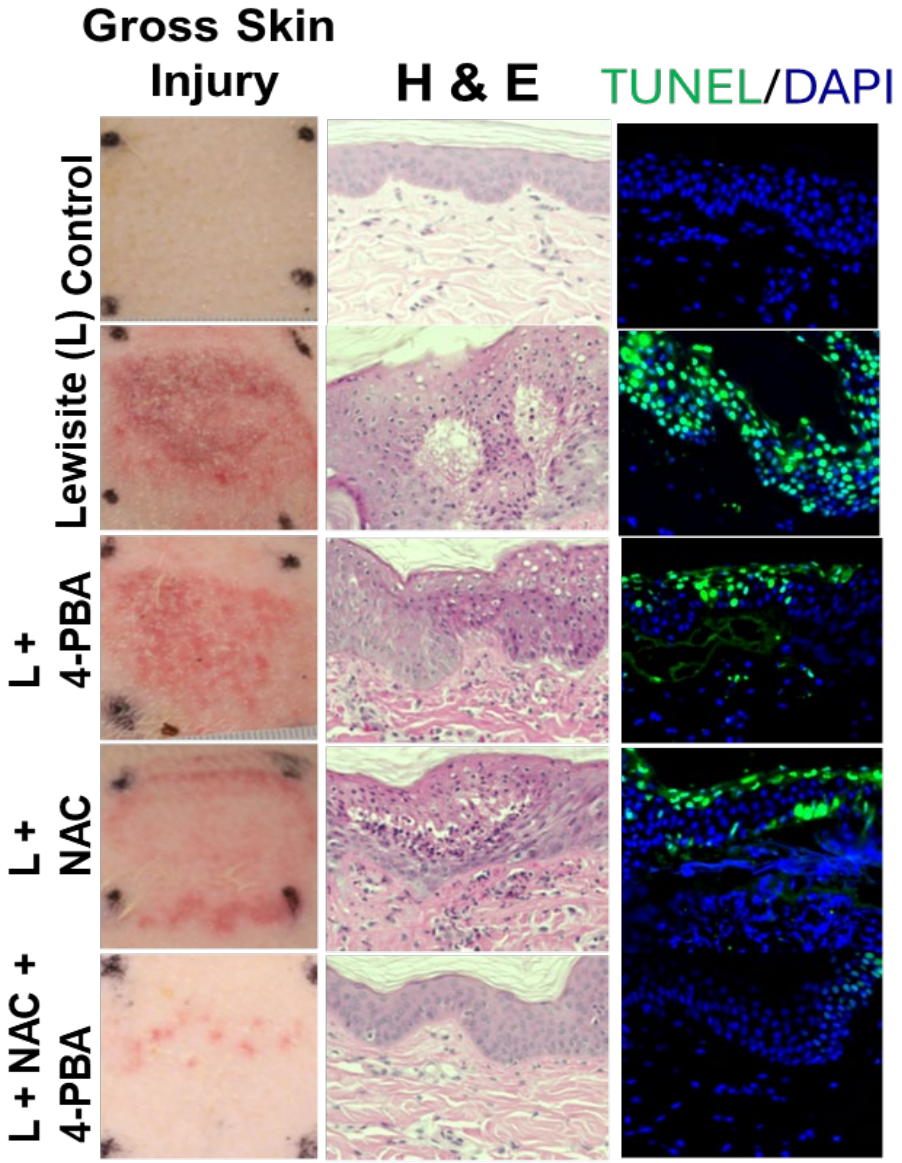


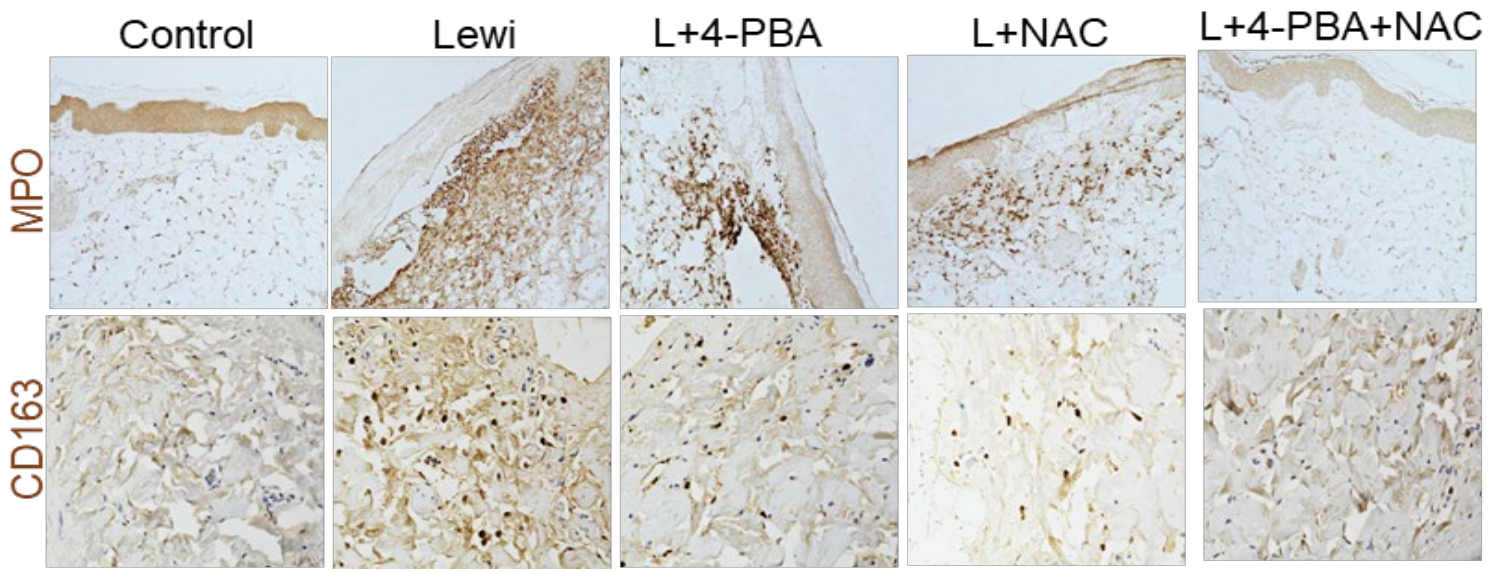


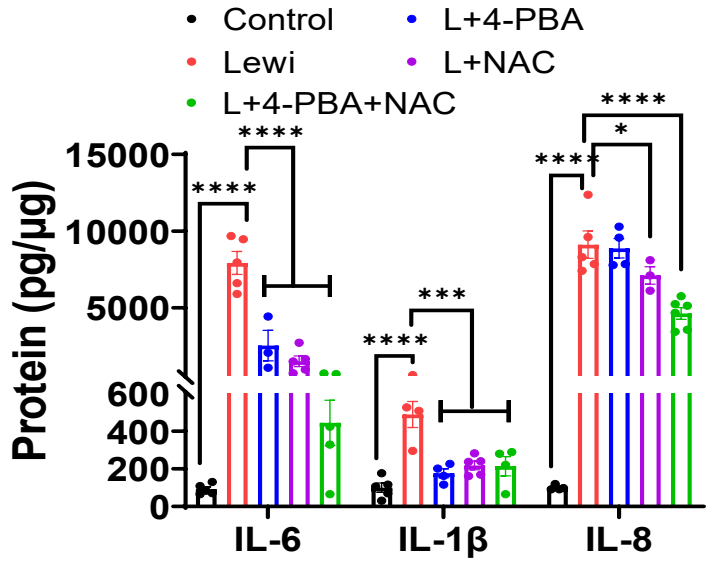
Expression of Common Genes (RNASeq) in Mouse and Porcine Models

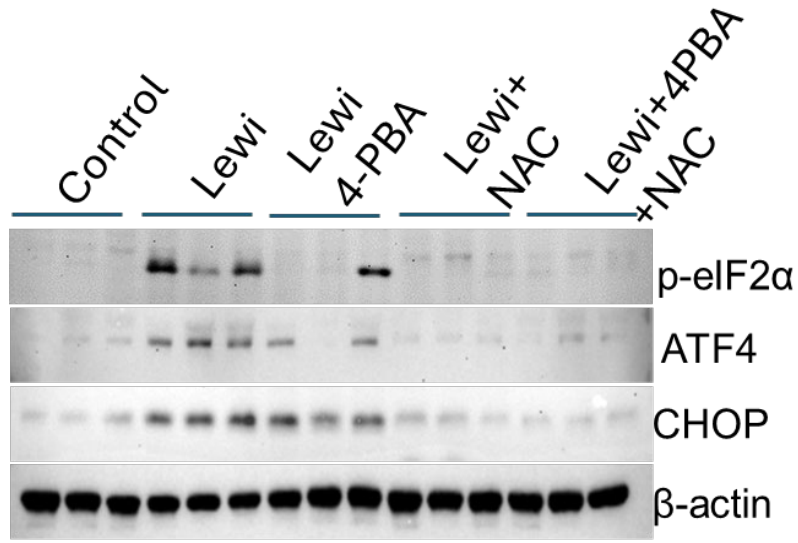


Combination of 4-PBA+NAC is Highly Effective in Attenuating Arsenical-induced Cutaneous Injury in Minipig









Conclusions

- 4-PBA in combination with NAC provides a highly effective MCM against Lewisite and acts by attenuating ER stress and protein translation.

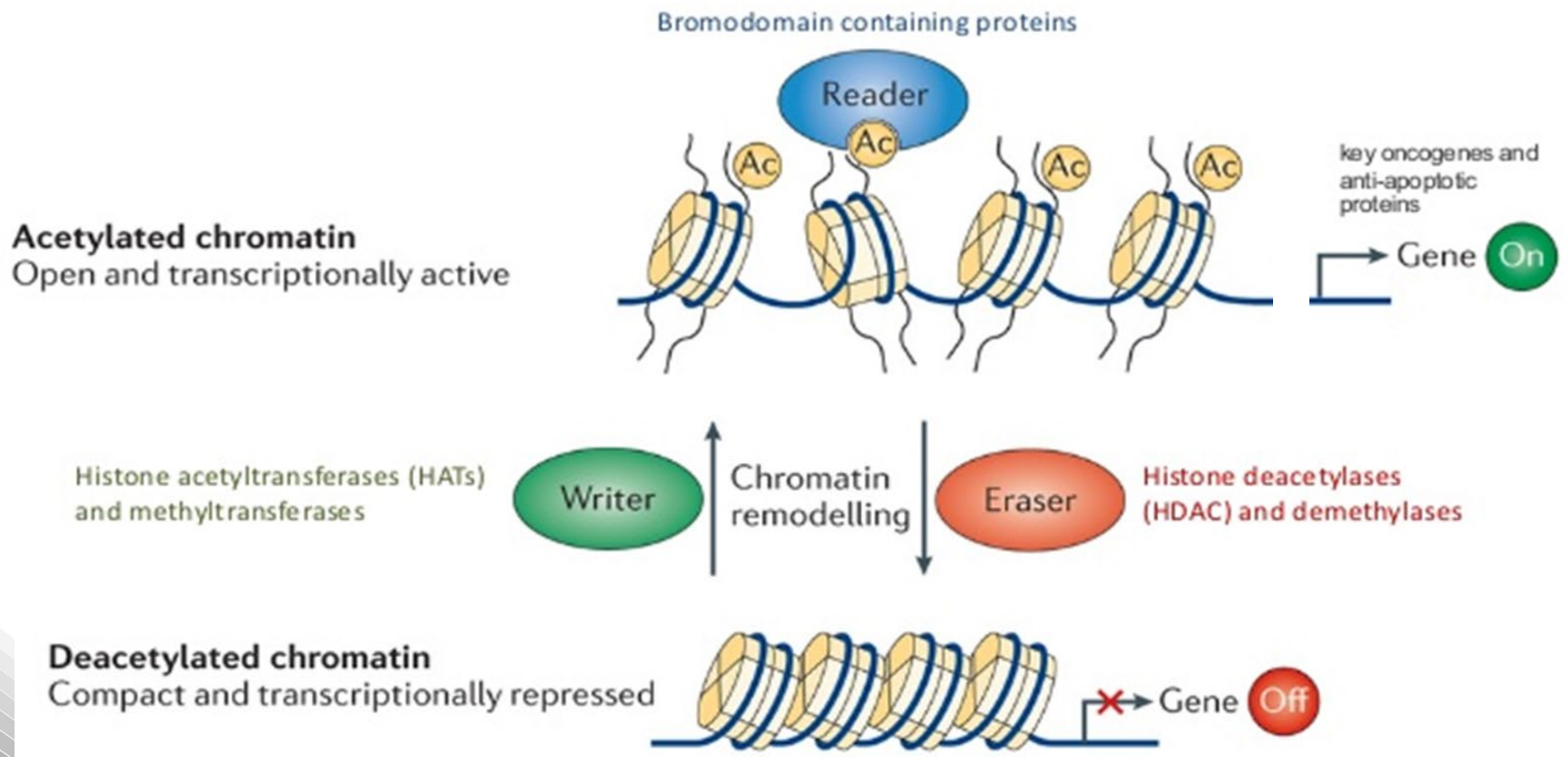
- This treatment is equally effective against other arsenicals induced vesicant injury

Epigenetic Mechanisms Regulating Blistering, Nociception and Tissue Disruption

Hypothesis

The unifying hypothesis is to uncover the role histone hyper-acetylation-dependent epigenetic regulation of multiple signaling pathways, which orchestrate the robust inflammatory and tissue damaging responses induced by arsenicals. The epigenetic reader bromodomain 4 (BRD4) as a novel, potent therapeutic target for mitigating arsenicals-induced tissue injury will be investigated.

Targeting Bromodomains: Epigenetic Readers of Lysine Acetylation



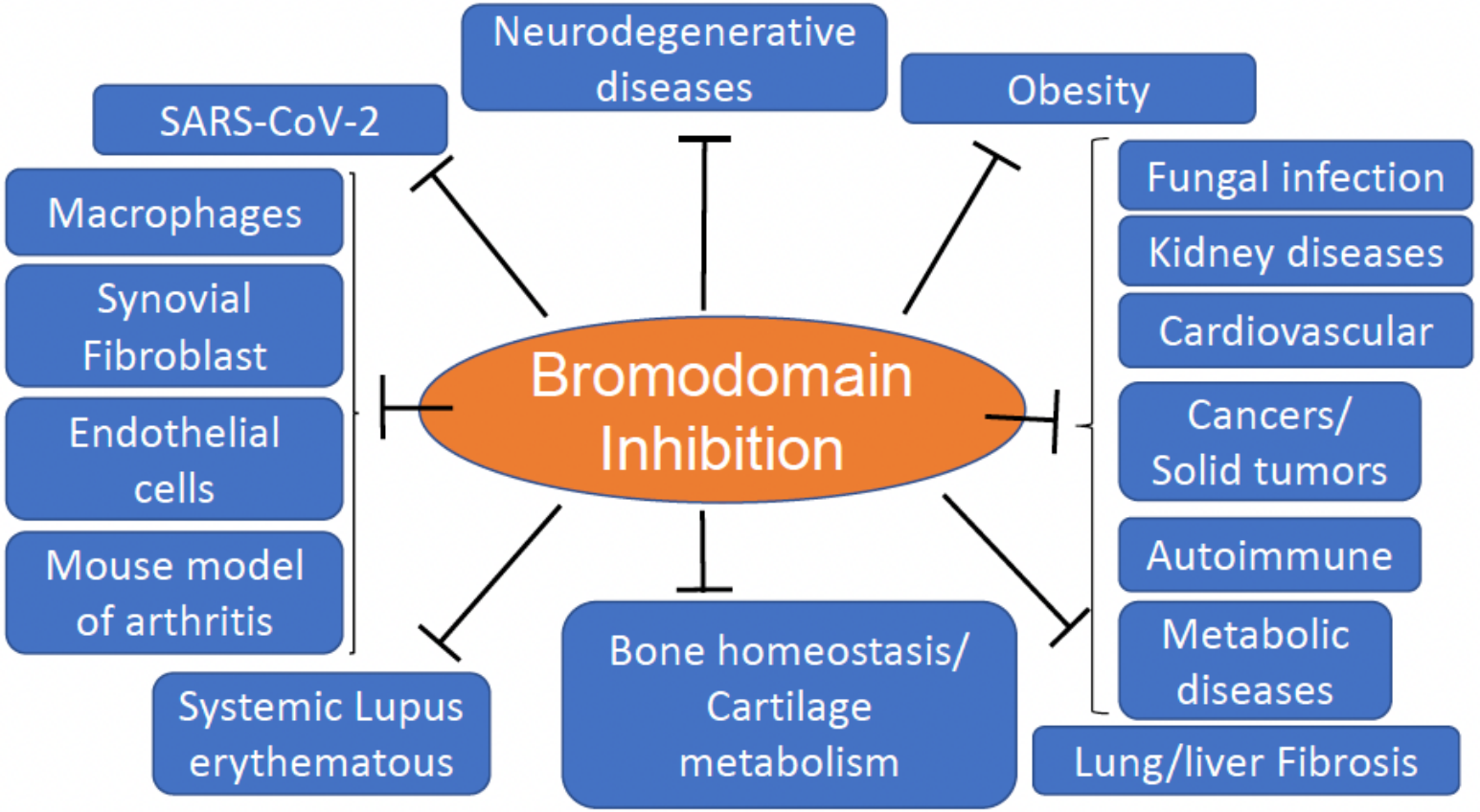
Transcription of Genes in Inflammation, Tissue Damage/ Remodeling

Nature Reviews Molecular Cell Biology 16, 258–264 (2015)

BET Bromodomain Therapeutic Activity

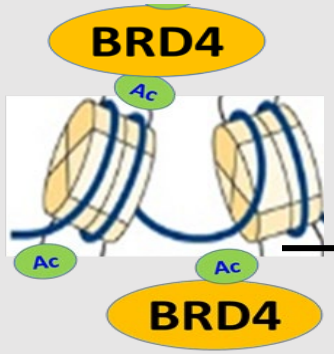
- Bromodomain family of proteins are involved in reading of acetylated lysine on histones DNA and facilitate the transcriptional regulation of various inflammatory and oncogenes.

- Bromodomain inhibition leads to gene expression changes that may affect pathological conditions of various inflammatory diseases.



Arsenicals-induced BRD4 targets

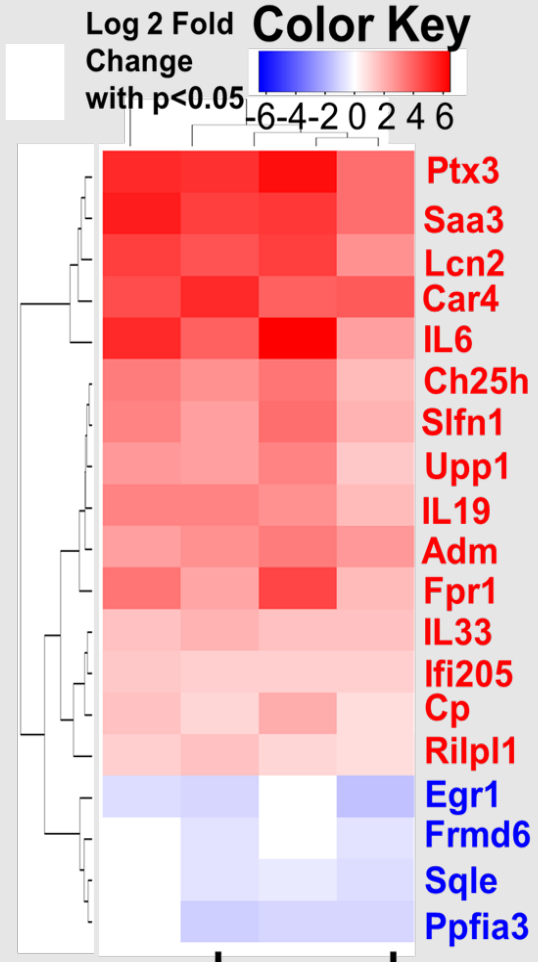
Arsenicals



target genes

RNA seq

BRD4 regulated targets

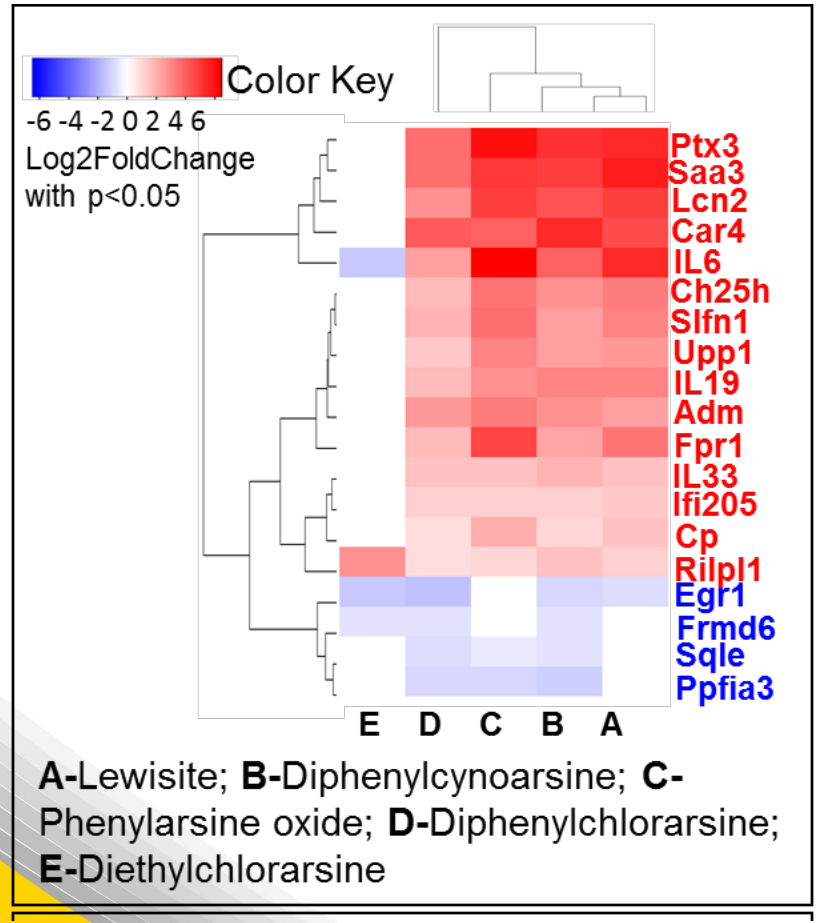


a-Lewi, b-DPCA
c-PAO, d-DPCyA

Confirmed BRD4 targets by RT-PCR in Skin

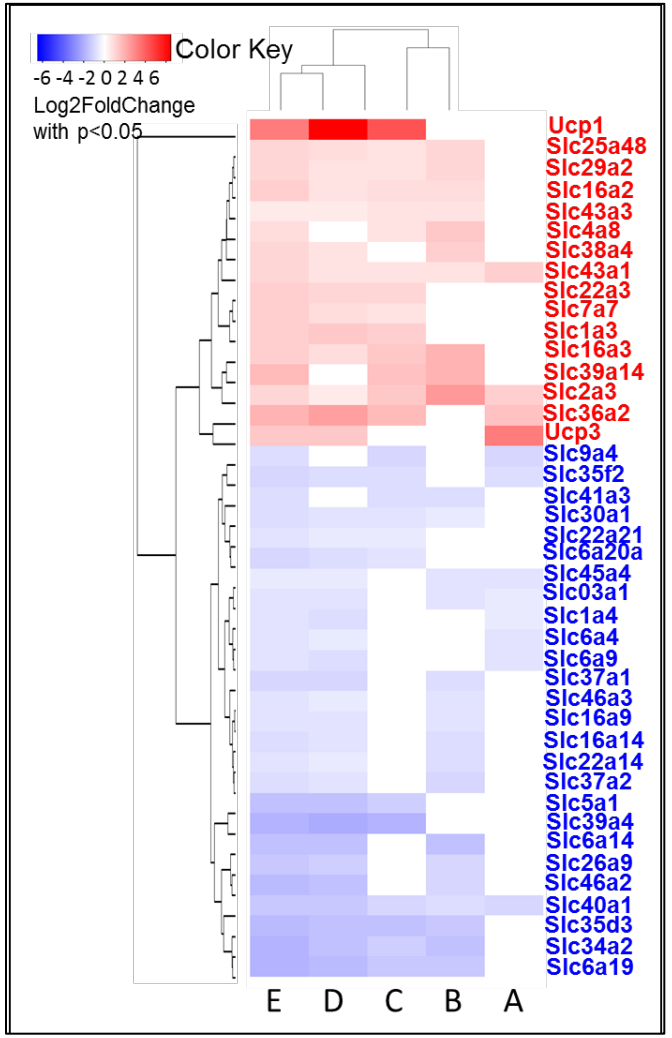
BRD4-regulated Transcription of Genes by Various Arsenicals in Multiple Organs

Skin

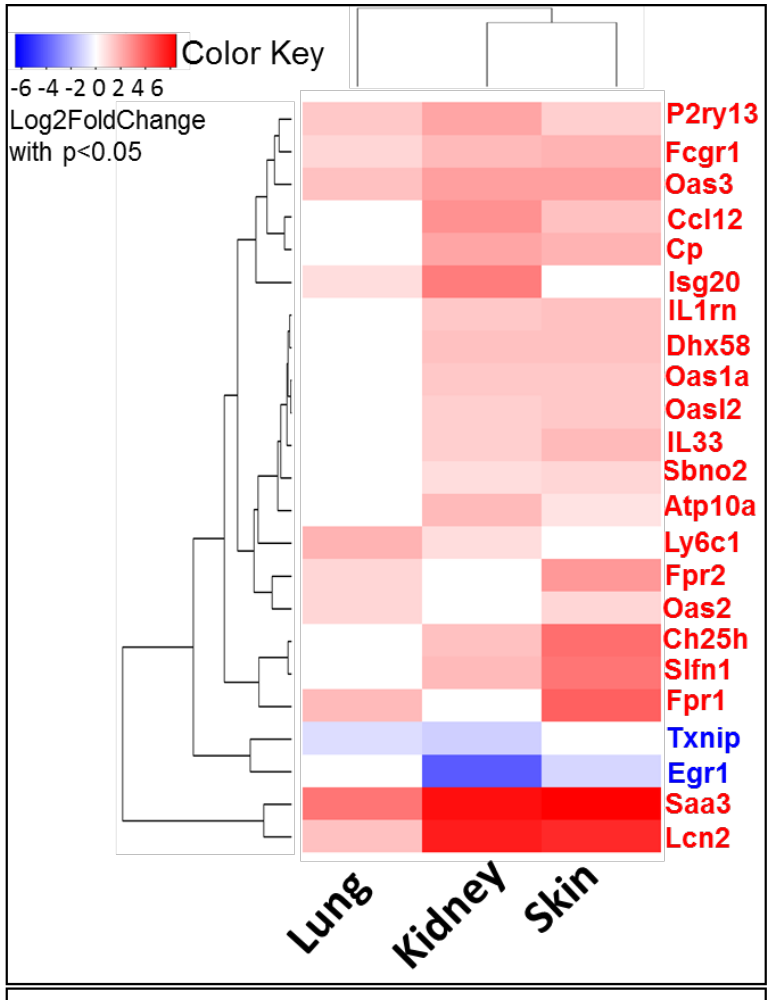


A-Lewisite; **B-**Diphenylcynoarsine; **C-**Phenylarsine oxide; **D-**Diphenylchlorarsine; **E-**Diethylchlorarsine

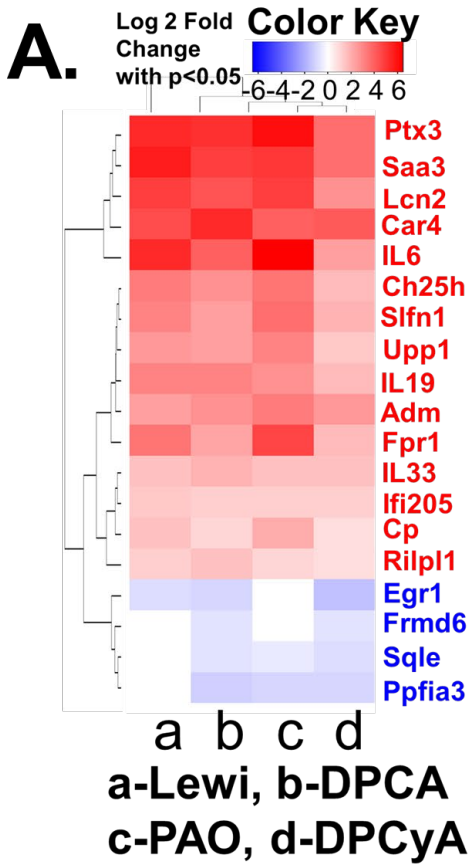
Solute Carrier

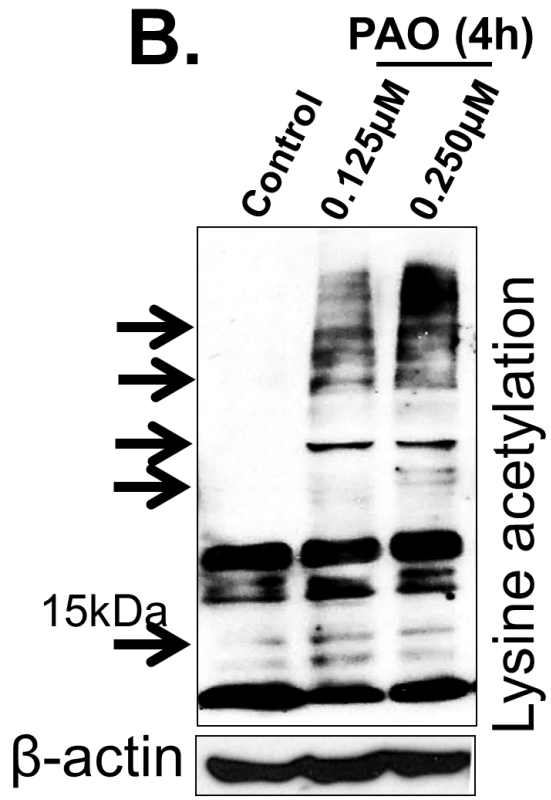


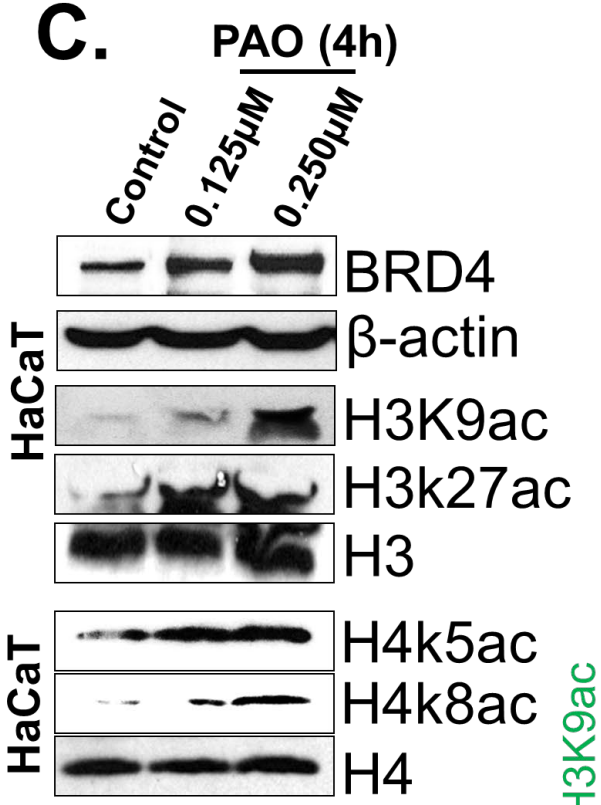
Lewisite

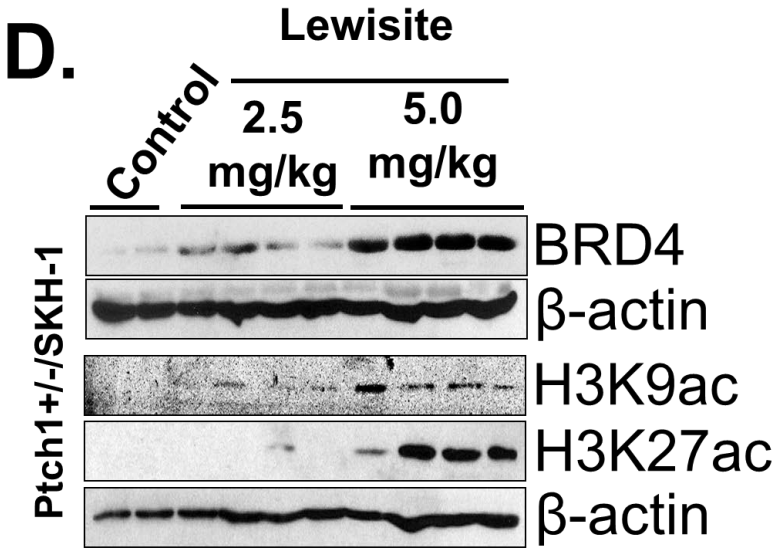


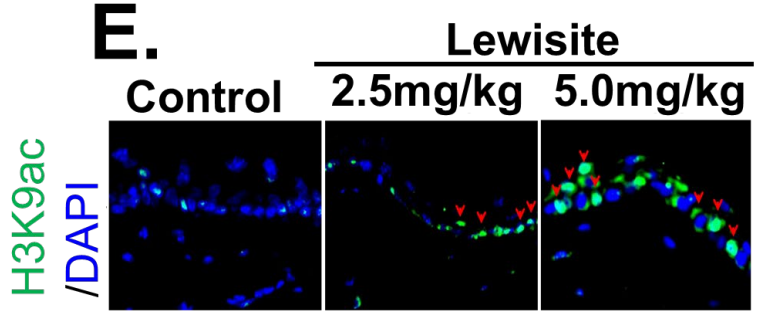
Arsenicals Induce BRD4 Expression and Lysine Hyperacetylation of Histones



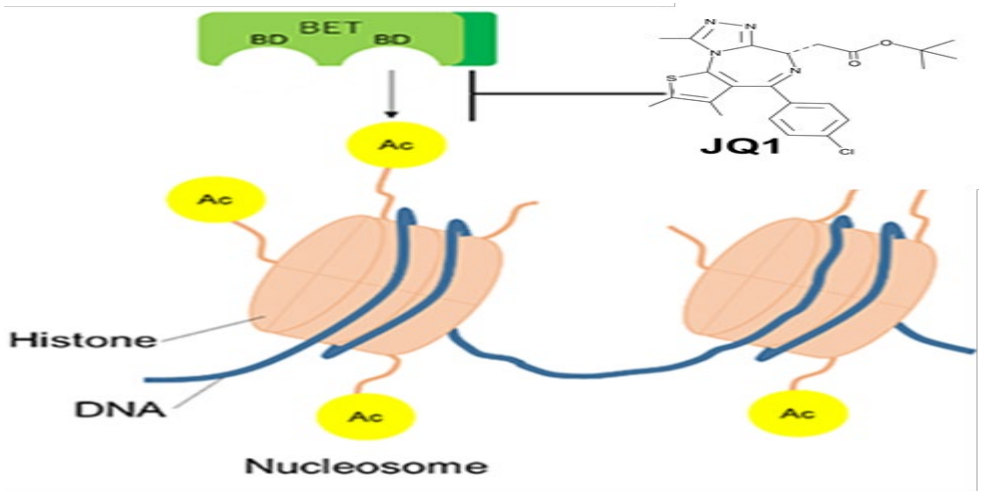


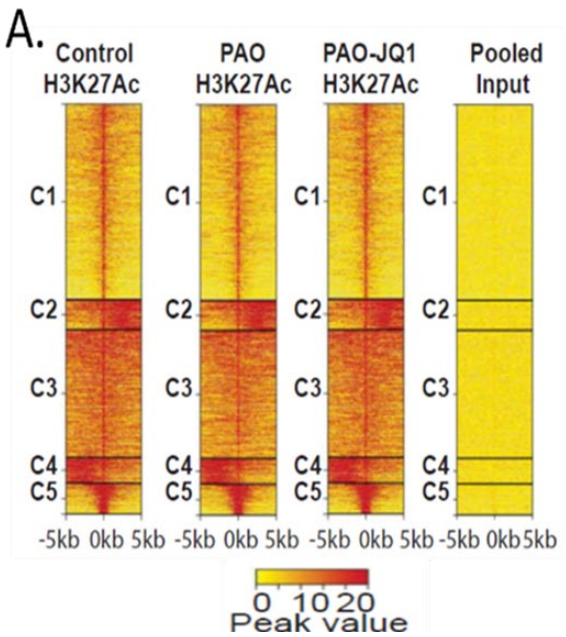






BRD4 Inhibitor, JQ1 Block PAO-induced Histone Hyperacetylation and Inflammation in Mouse Skin





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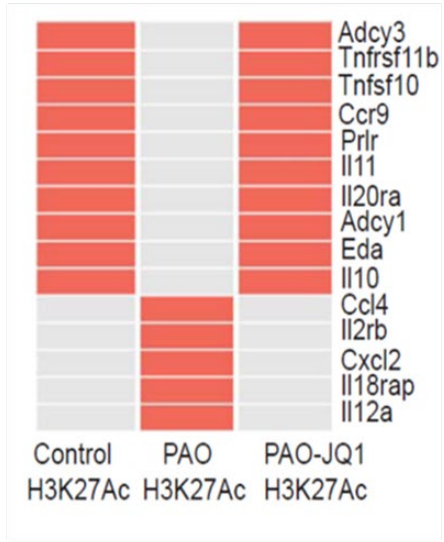
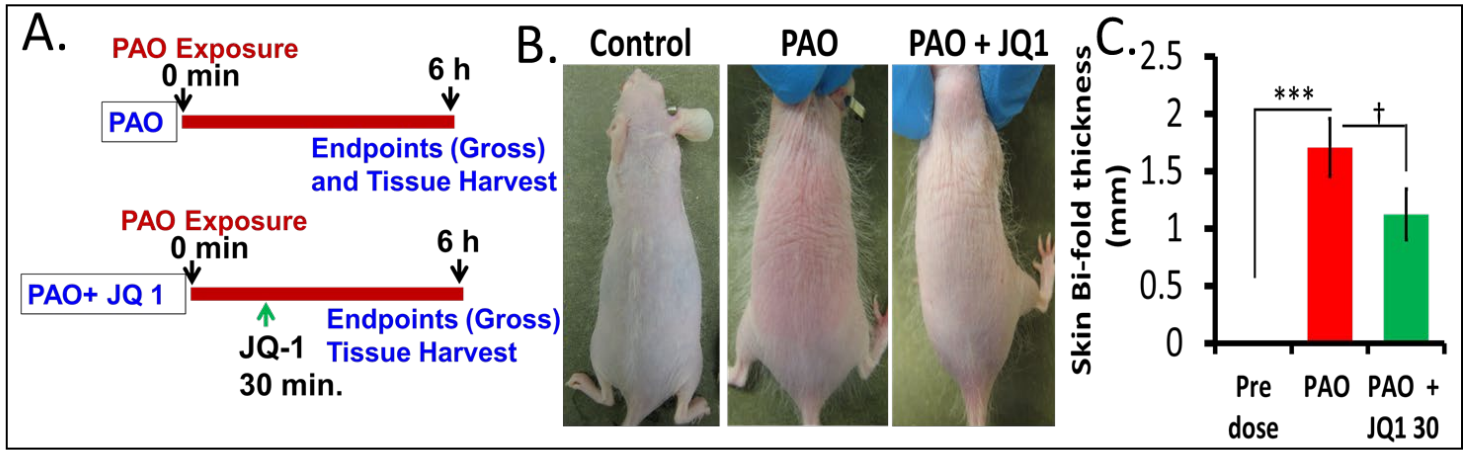
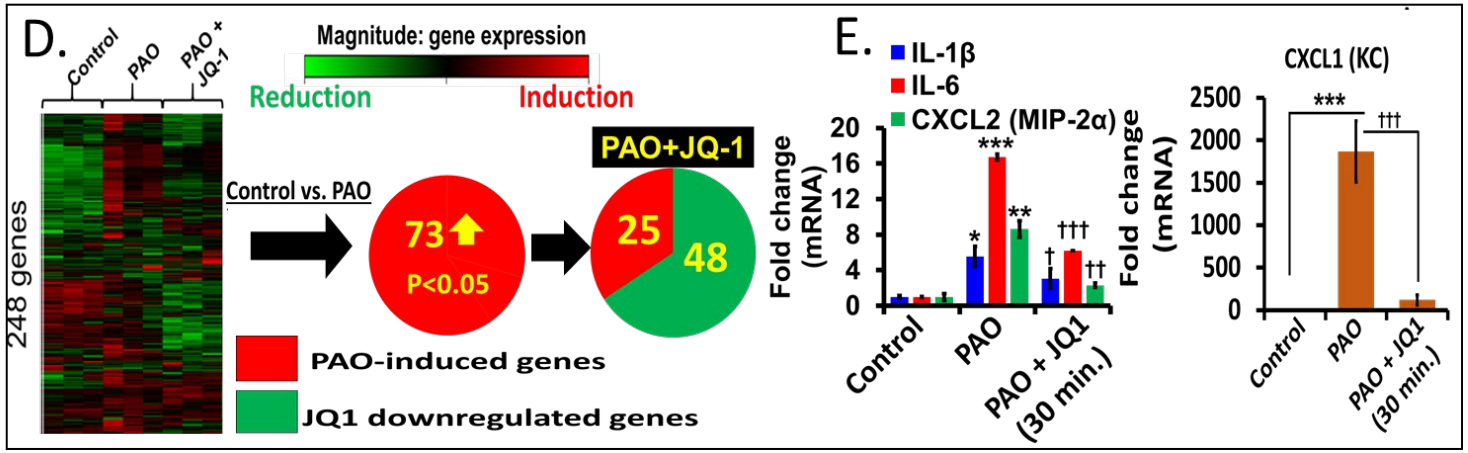
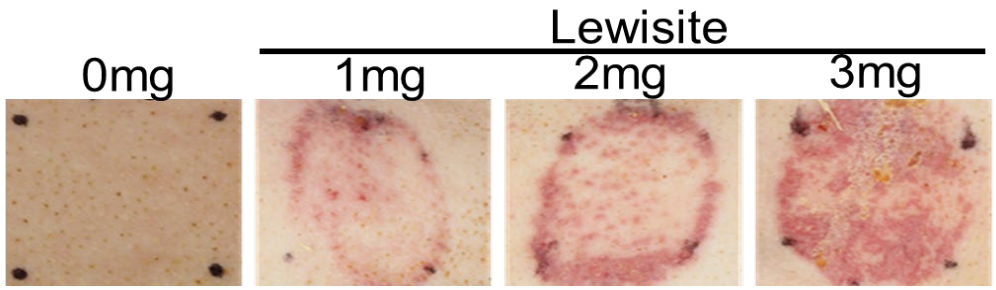
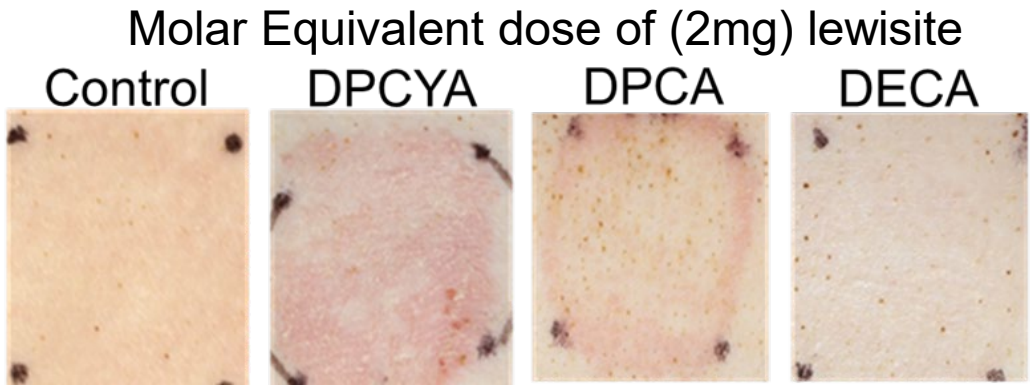


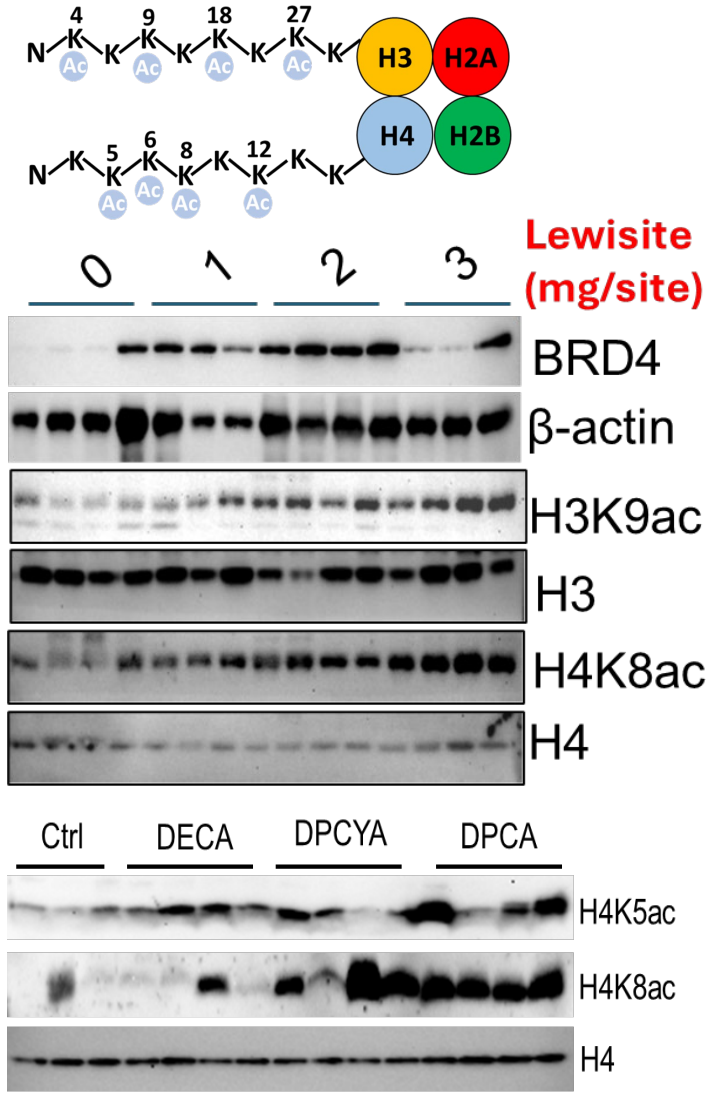


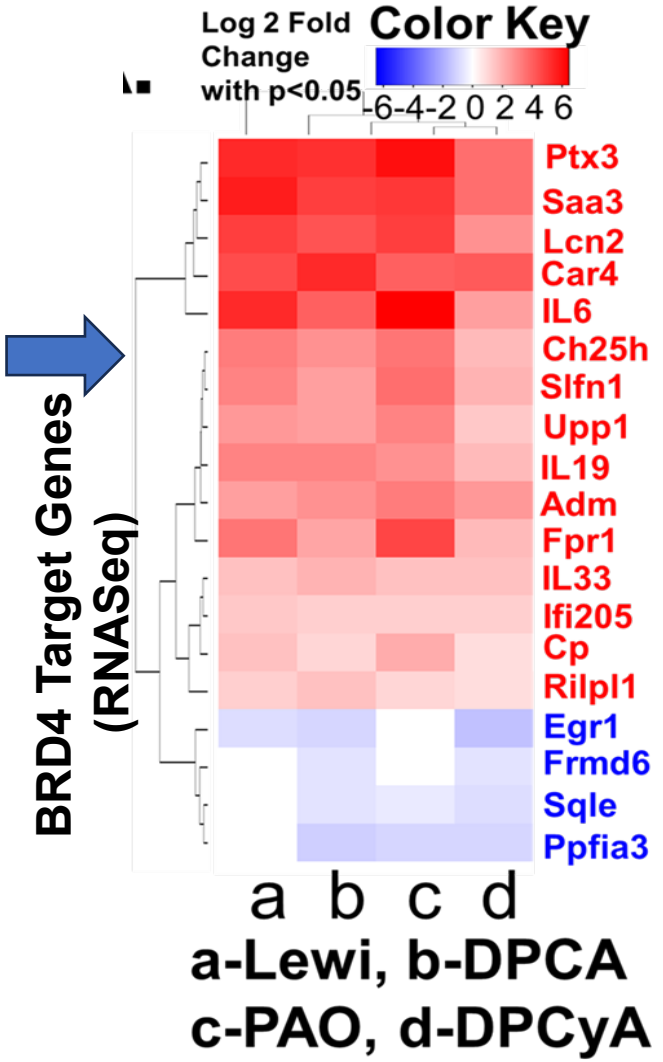


Lewisite and other Arsenicals Induce Hyperacetylation of H3 & H4 Histones in Porcine Skin



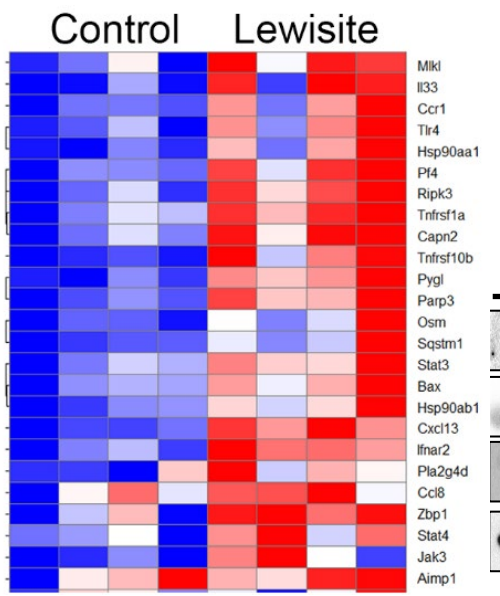


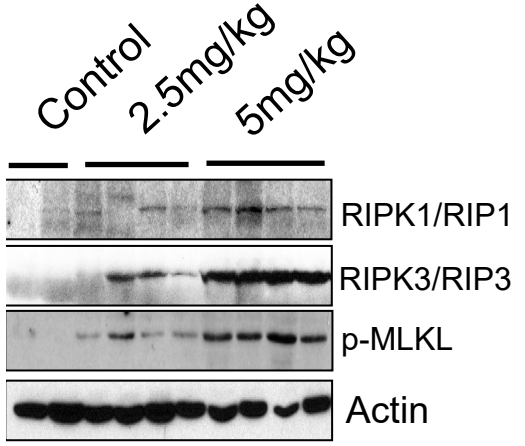




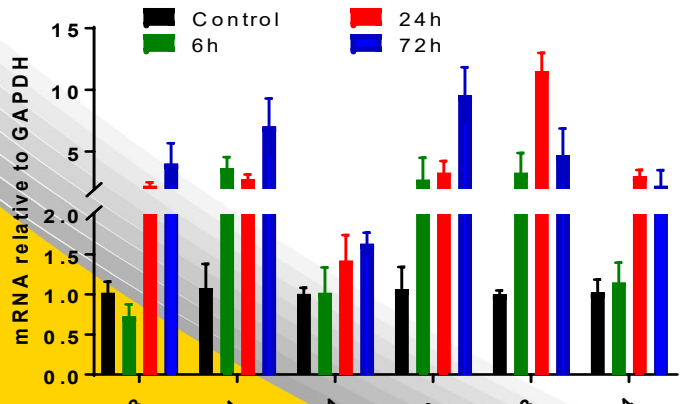
Necroptosis Regulating Signaling in Tissue Inflammation and Damage

Lewisite

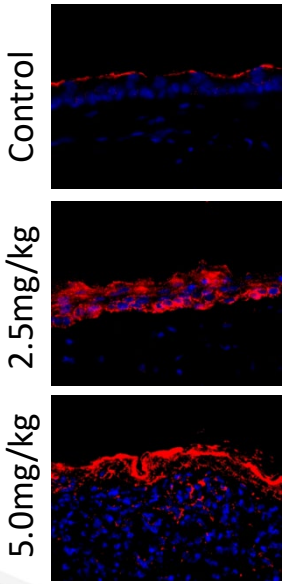




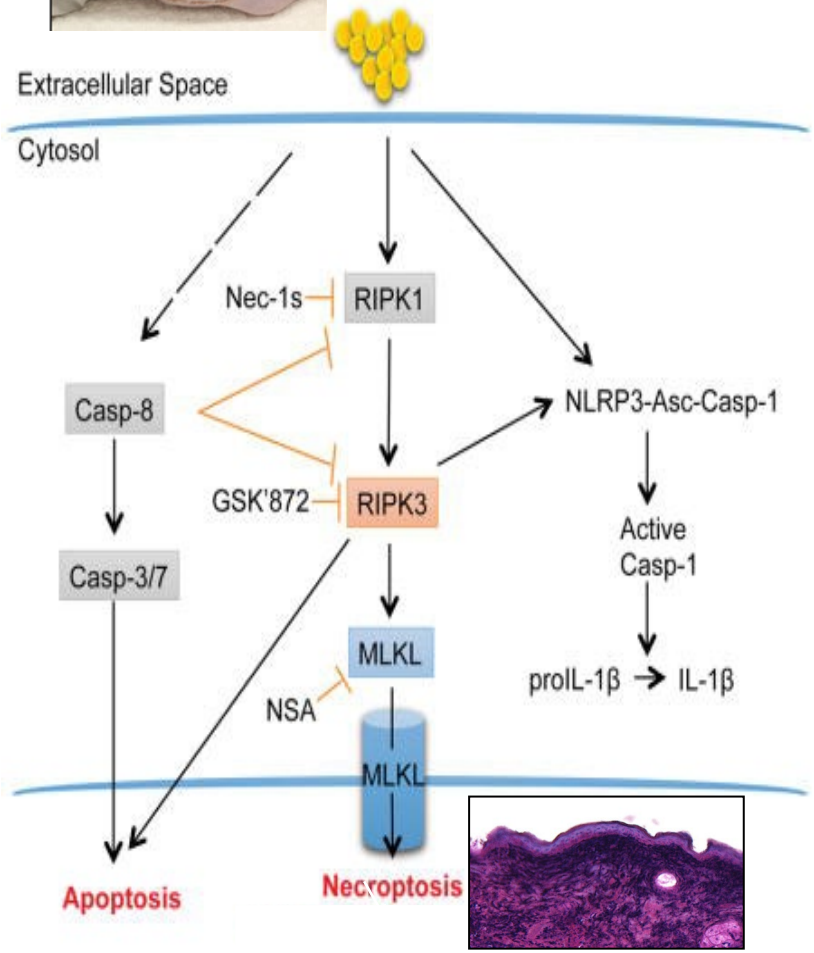
RT-PCR confirmation



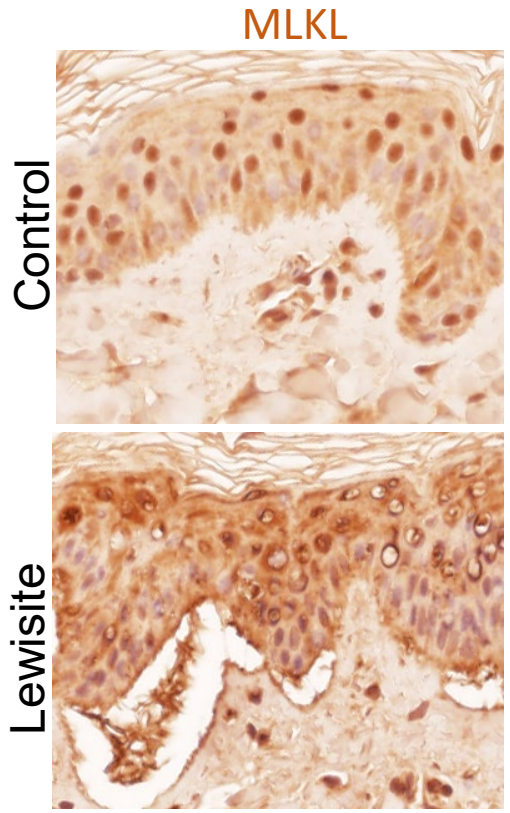
RIPK3/DAPI

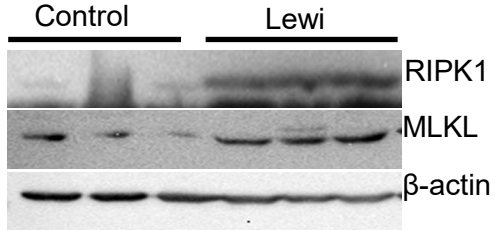


Murine Skin



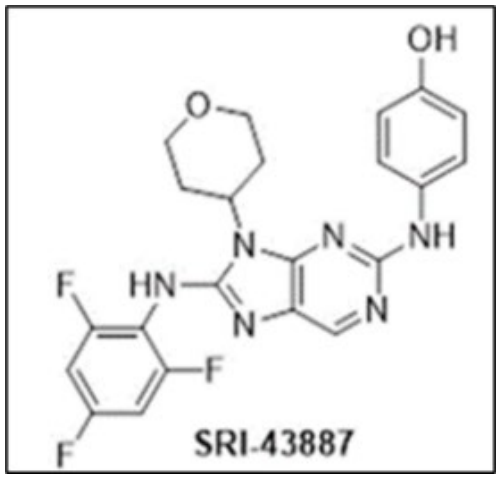
Porcine Skin





Necrosis in Arsenicals- treated Skin

Pharmacology and Tox Data for Dual BRD4-RIPK3 Inhibitor



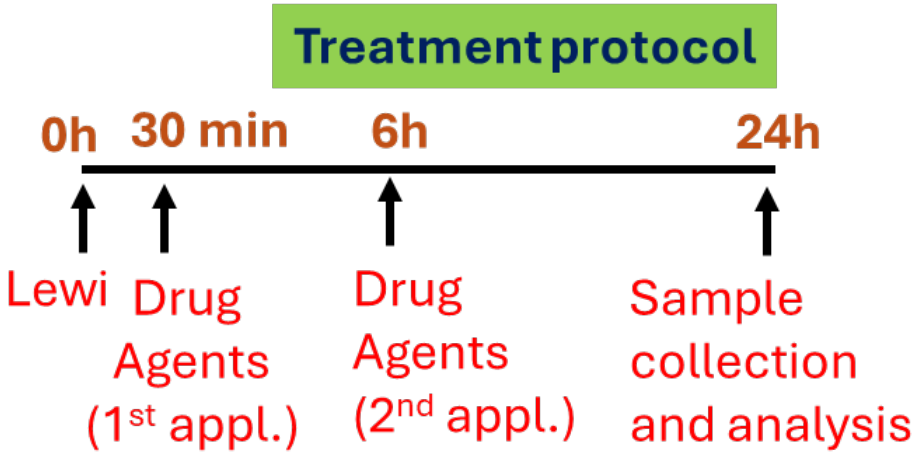
SRI-43887

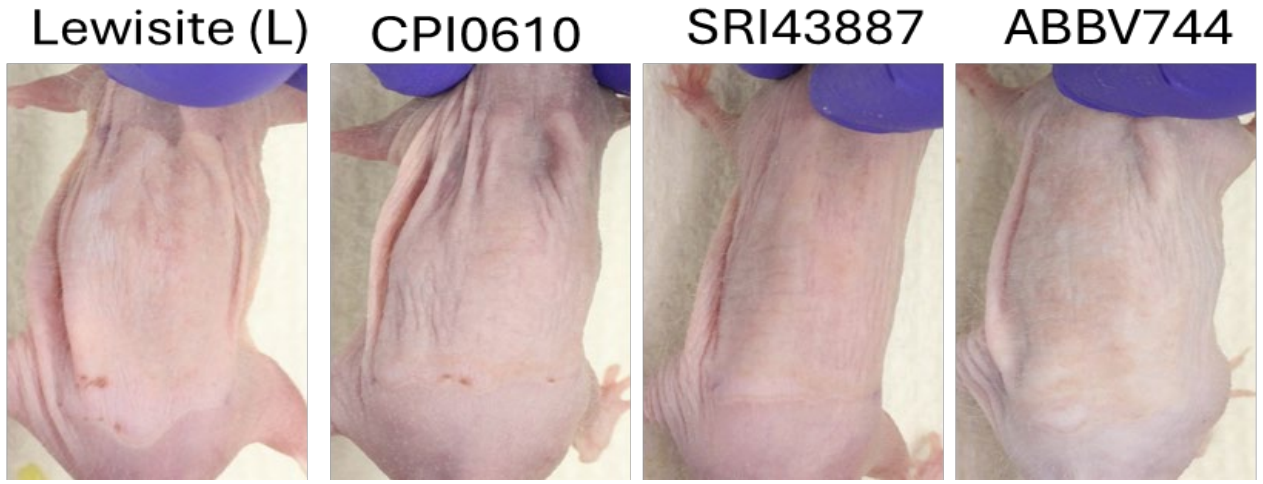
Table 1: PK parameters of **SRI-43887** in mice plasma

Parameters	SRI-43887		
Route	IP	IV	PO
Dose (mg/kg)	10	1	5
$t_{1/2}$ (h)	3.63	1.70	2.85
T_{max} (h)	0.25	----	0.50
C_{max} (ng/mL)	2937	----	225
AUC_{last} (h*ng/mL)	2010	284	361
%F	-----	----	28.5

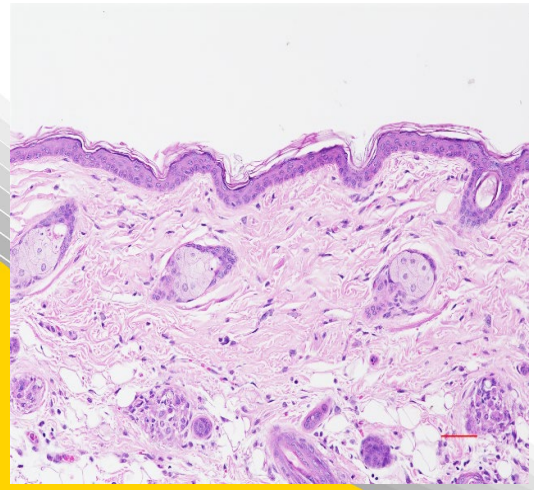
Assay	SRI-43887 (HCl salt)
Mini-Ames	Negative
hERG	IC_{50} = 13.35 µM (low inhibition)
Cyp Inhibition	CYP1A2, CYP2A6, CYP2C19, CYP2D6, CYP2E1 >50 µM; CYP2B6 = 40.92 µM; CYP2C8 = 14.93 µM; CYP2C9 = 42.27 µM; CYP3A4/5_MIDAZOLAM = 11.39 µM; CYP3A4/5_TESTOSTERONE = 36.52 µM
Maximum Tolerated Dose	IP Dosing: 1, 10, 30 mg/kg Vehicle: DMSO/PEG400/Water (5/30/65) No abnormality was observed at 1, 10, 30 mg/kg
Kinase Panel (39 kinases)	10 µM: ≥80% inhibition against 14 kinases
Protein binding	94.89%
Drug SafetyScreen44	10 µM: ≥50% inhibition (inh.) in 6 primary assays: Phosphodiesterase PDE4D2 (58% inh.); Cyclooxygenase COX-1 (71% inh.); Cholecystokinin CCK_1 (CCK_A) (84% inh.);

Effects of BRD4 inhibitors on Lewisite-induced Skin Injury in Murine Model

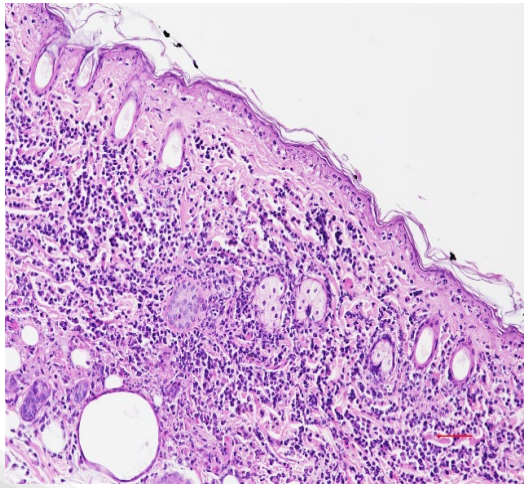




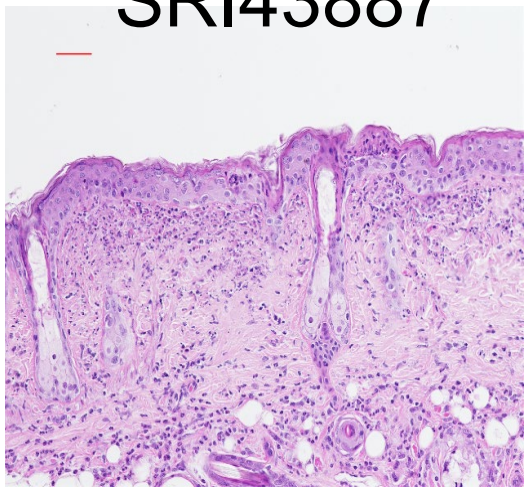
Control

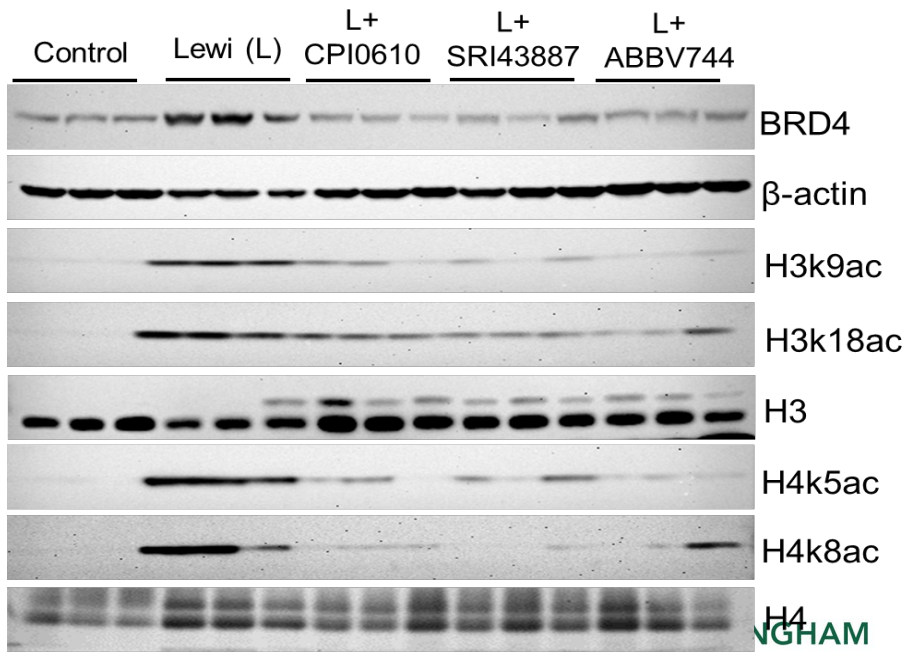


Lewisite

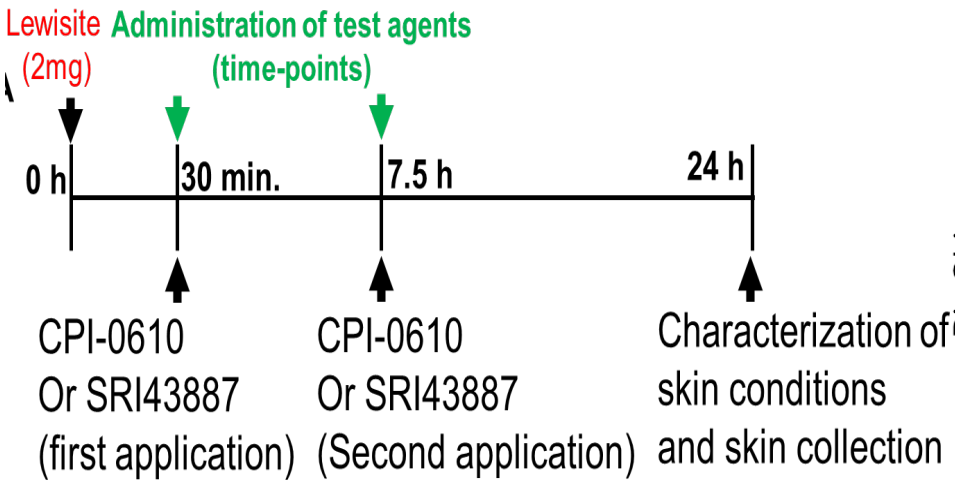


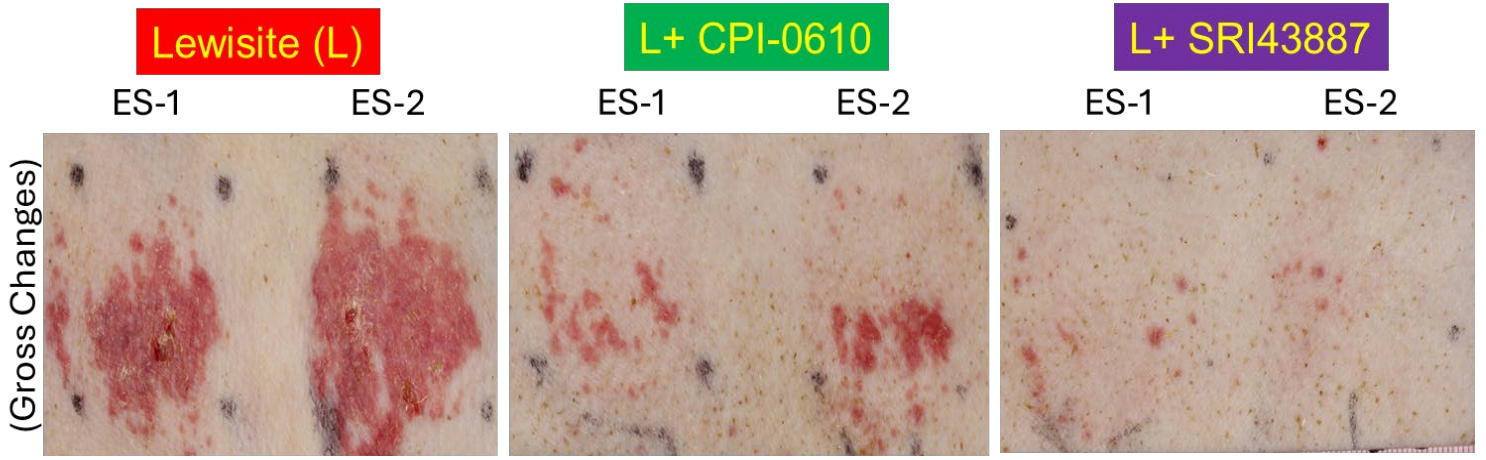
Lewisite+ SRI43887





Effects of BRD4 inhibitors on Lewisite-induced Skin Injury in Porcine Model





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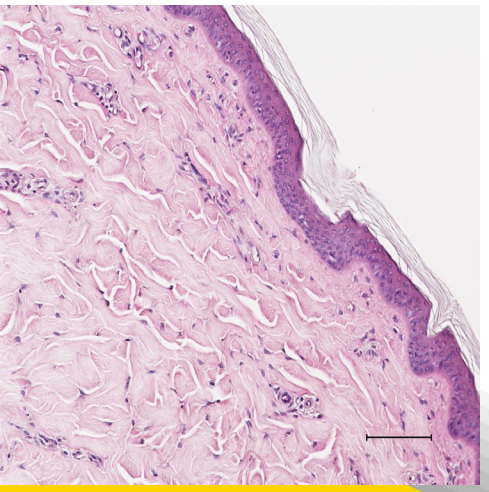
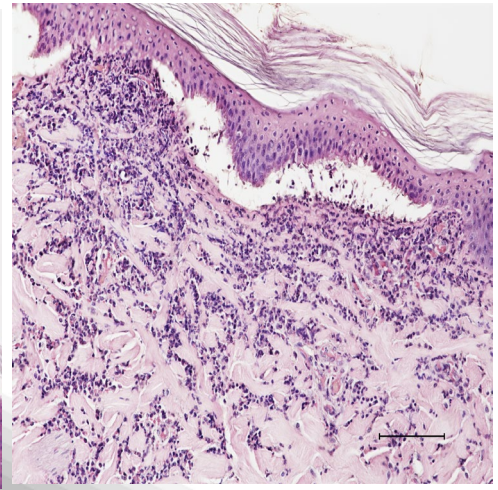
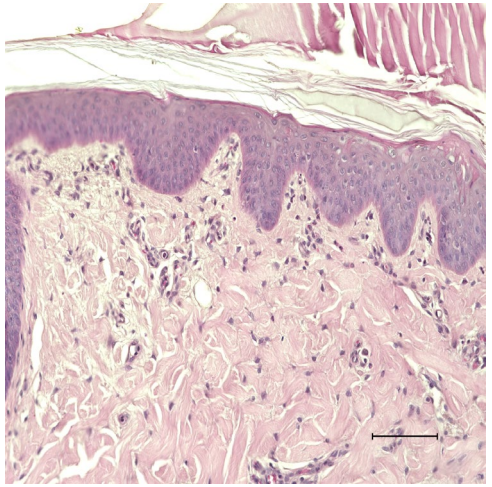
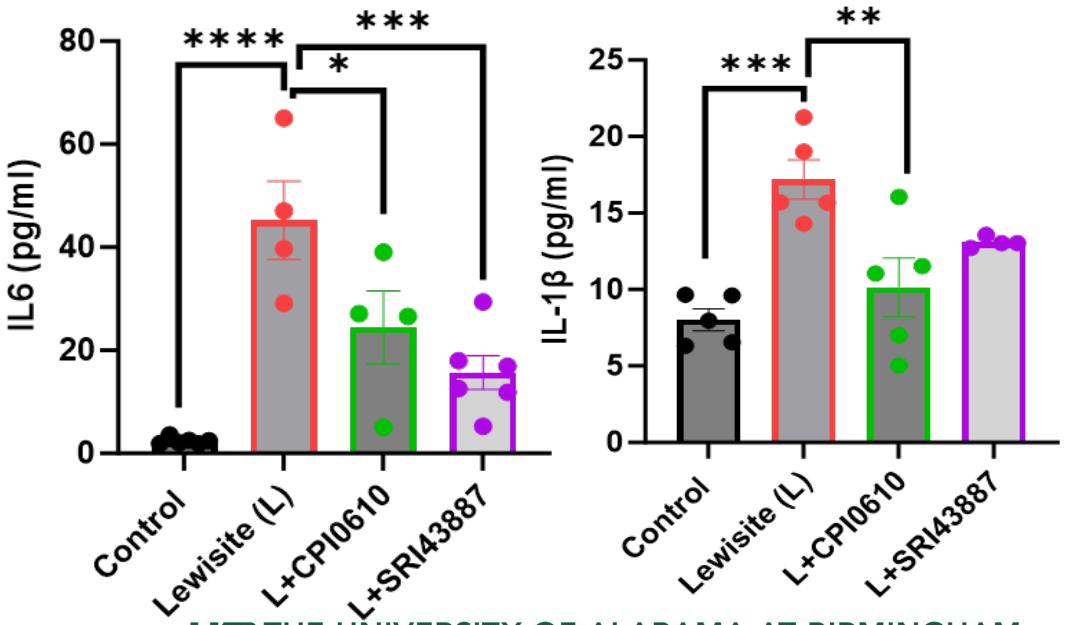


Lewisite



Lewisite+ SRI43887



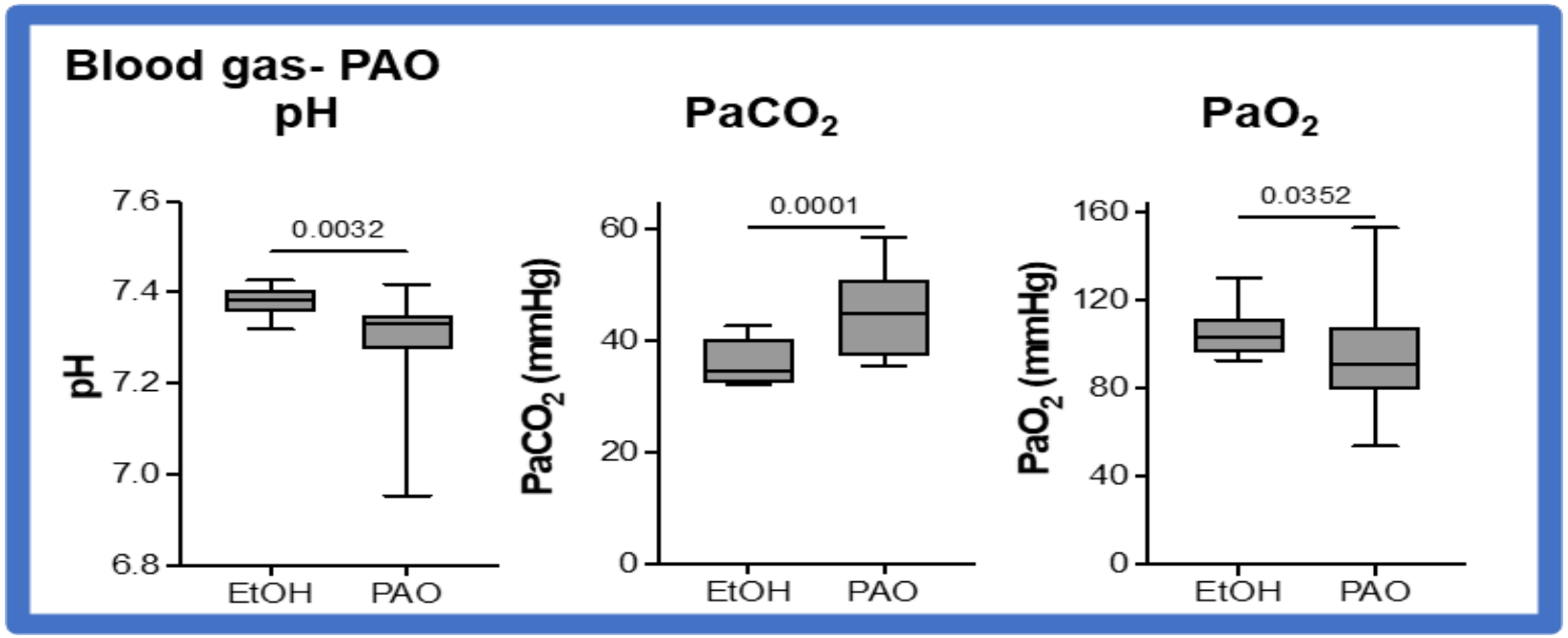


Model of cutaneous arsenical-induced Acute Lung Injury: Utilizing PAO as a surrogate for Lewisite

C57BL/6J mice

Skin

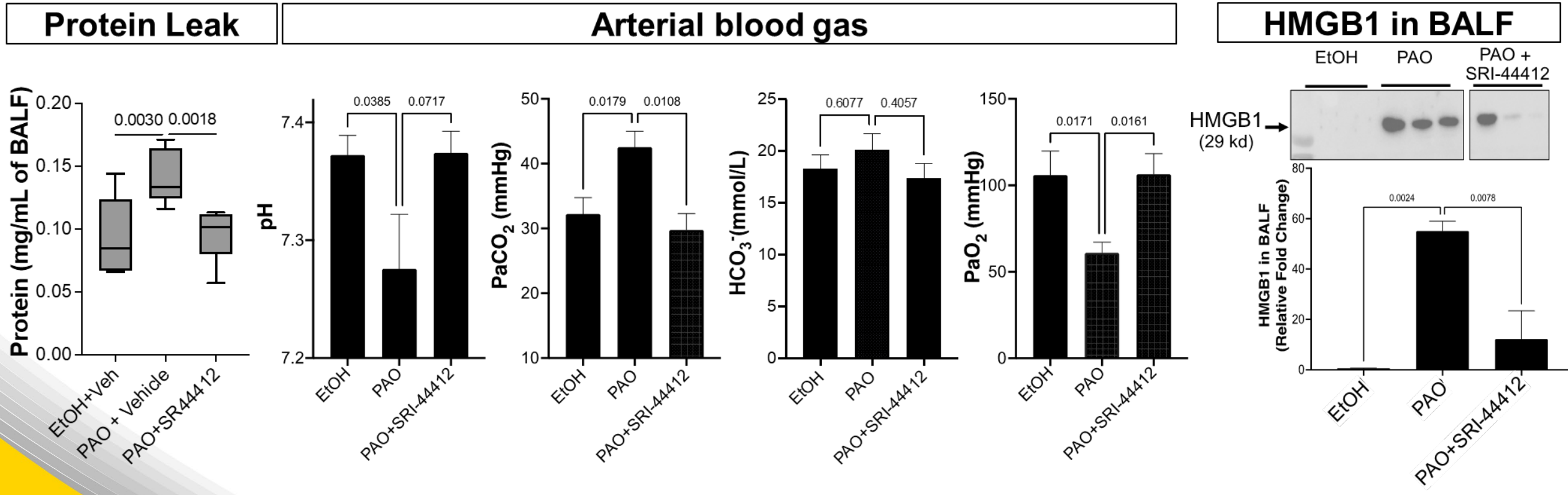
EtOH PAO



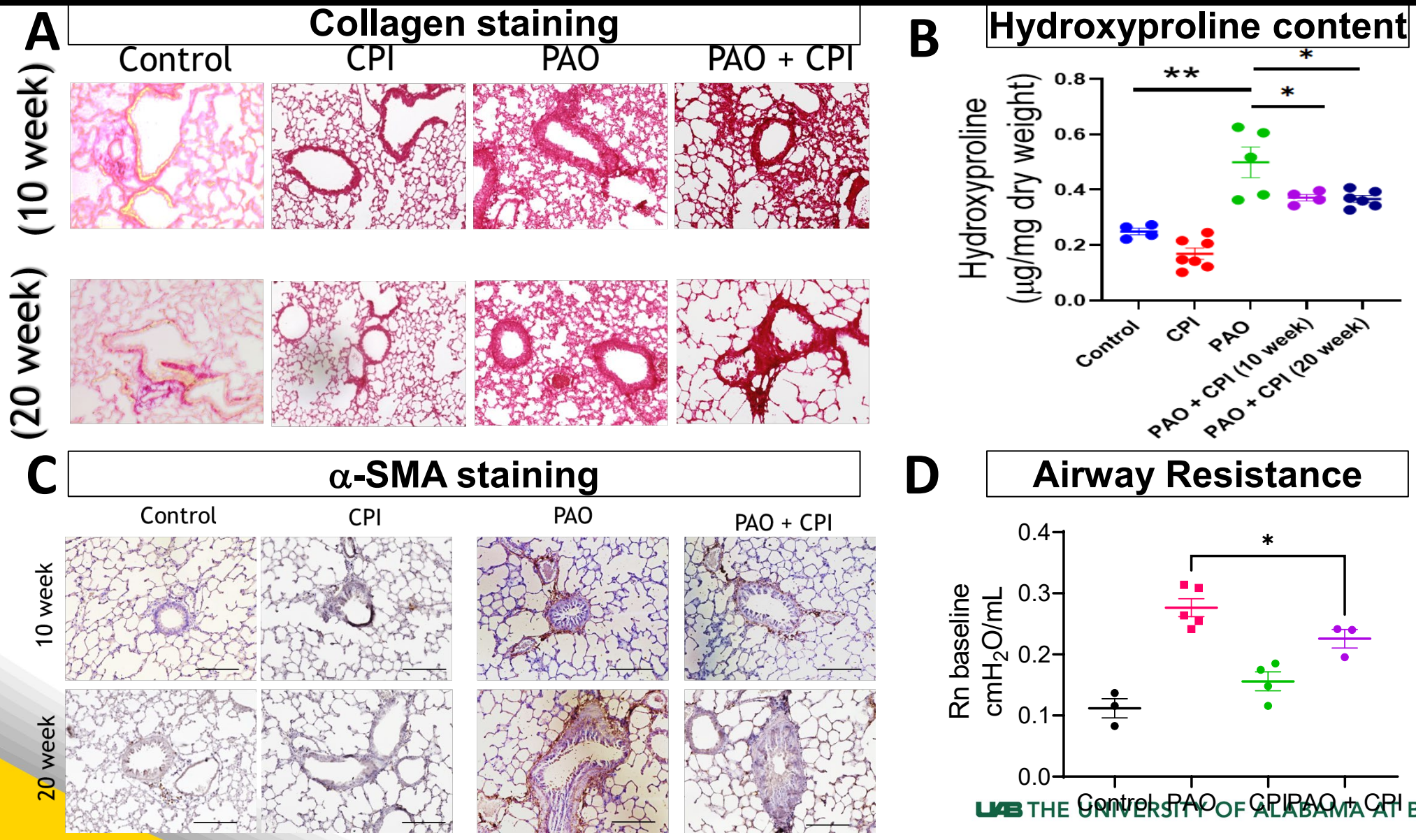
Gas Exchange

PAO:
Phenylarsine Oxide

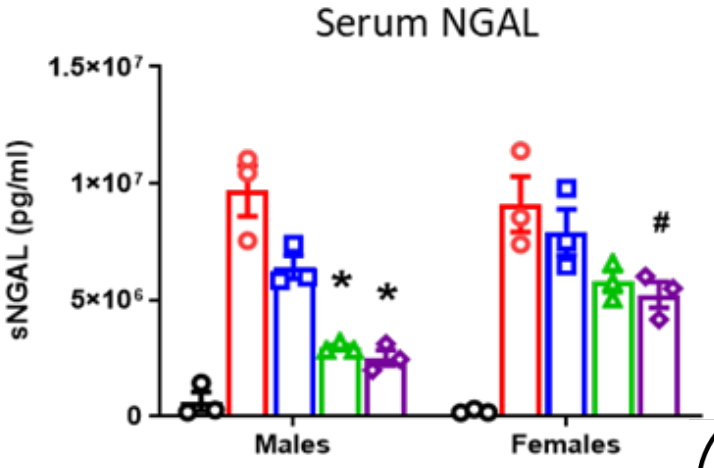
BRD4i Mitigates Cellular Injury & Reverses ALI in Mice Challenged with Cutaneous PAO

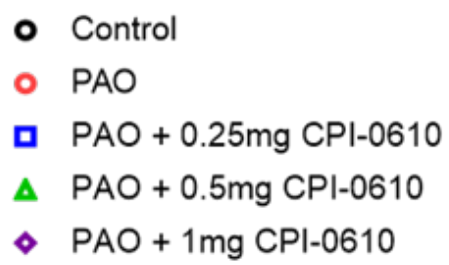


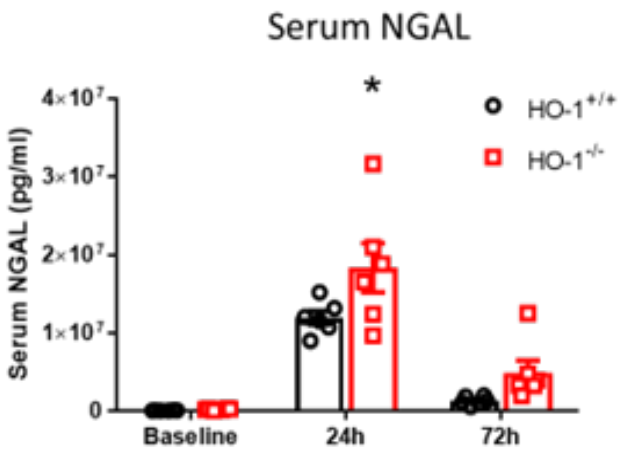
BRD4i CPI-0610 Reverses DLI in Mice Challenged with Cutaneous PAO

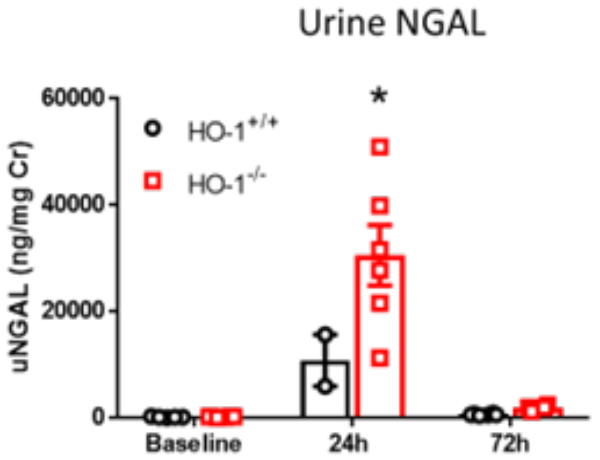


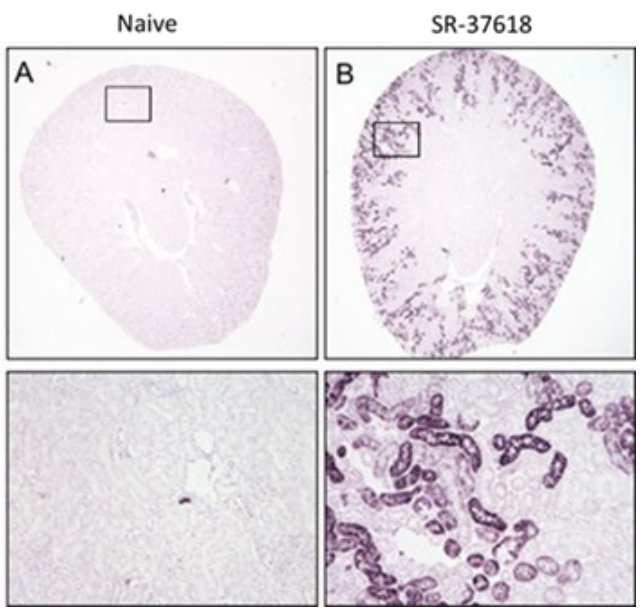
BRD4i CPI-0610 Reduces Arsenical-induced AKI

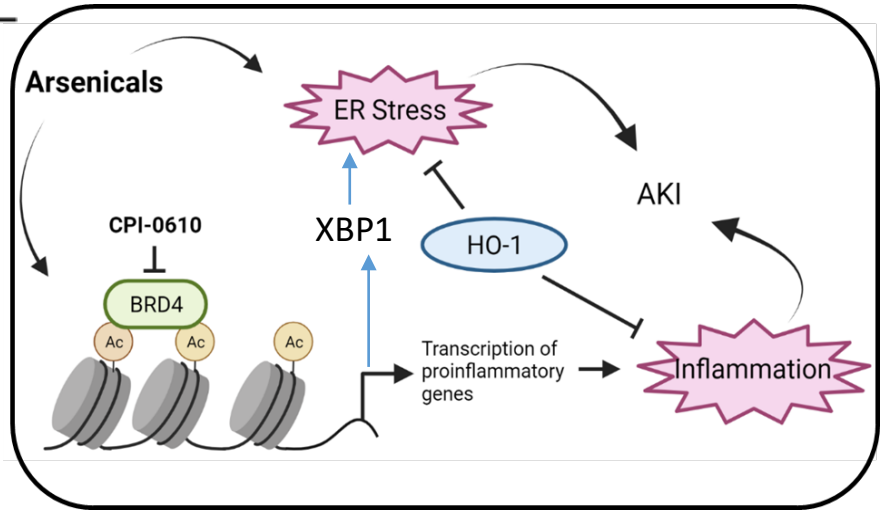


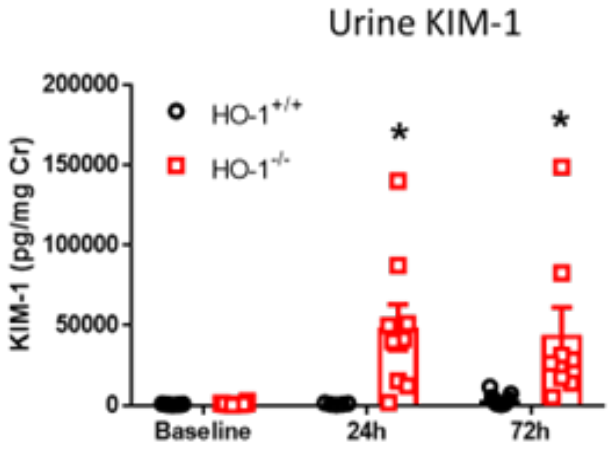


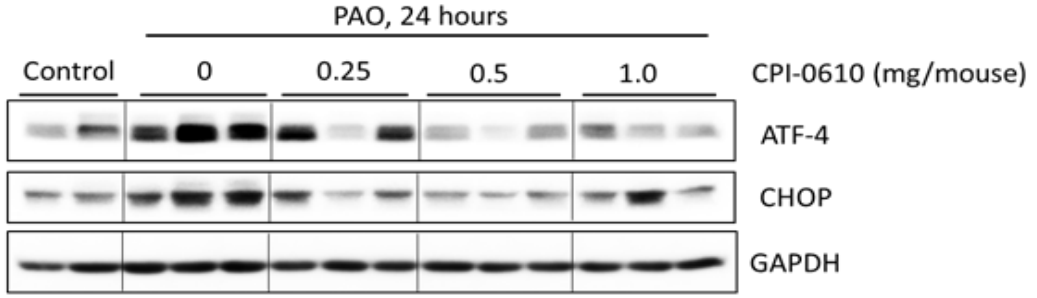












PIPELINE of SMALL MOLECULES under DEVELOPMENT as MCMs AGAINST ARSENICALS

	AGENTS	TARGET	STAGE OF DEVELOPMENT
1.	N-acetyl cysteine (NAC)	Reactive oxygen species (ROS)	Efficacy completed & formulation in progress
2.	4-phenyl butyric acid (4-PBA)	Unfolded Proteins	Efficacy completed & formulation in progress
3.	NAC + 4-PBA	ROS + Unfolded proteins	Efficacy completed & formulation in progress
4.	SRI-44412	BET Bromodomain	Efficacy studies completed
5.	SRI-43887 dual inhibitor	Dual -BRD4 + RIPK3	Efficacy studies completed
	SRI-44078	RIPK3	Efficacy studies completed
	CPI-0610/ABBV-744/ JQ1/ OTX0515/ ZL0420	BET Bromodomain	Efficacy studies completed
	ISRIB/Salubrinal	eIF2αand stress Granules	Efficacy studies completed
	Necrosulfonamide	MLKL	Efficacy studies completed
	Nordihydroguaiaretic acid (NDGA)	Lipoxygenase (LOX), p300	Efficacy studies completed
11.	H-151/C-176	Stimulator of interferon genes (STING)	In progress
12.	G140/RU.521	dsDNA sensor, cGAS	In progress
13.	8-Azaadenosine	dsRNA sensor-ADAR1 inhibitor	In progress

Summary

- Established murine and porcine models of skin exposures to single high dose of vesicants that recapitulate human pathobiology of cutaneous and systemic injury
- Developed molecular toxidrome of blistering agents with an objective to develop single MCM for multiple toxic chemicals
- Identified additional multiple druggable molecular targets and assessed their potential utility in MCM development

Summary (continued.)

- Two distinct MCMs have advanced with remarkable efficacy in two animal models against lewisite and other vesicants with defined molecular targets and mechanisms of action
- Developed impressive pipeline of MCMs which are at different stages of preclinical development and include novel (with IP rights) and repurposed drugs

Conclusion

Our translational approach is “Mechanism to Clinic”. In this regard, we are also identifying surrogate disease with similar molecular pathogenesis for FDA approval and stock piling.

Thank You

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U01 AR078544

Acknowledgements

Collaborators

Project -01 Team

Education Core Team

Project-03 Team

Project-02 Team

Dr. Antony's Lab

Drug Discovery Team

Acknowledgment

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