

Mechanisms underlying the effect of environmental exposures on neurodevelopment



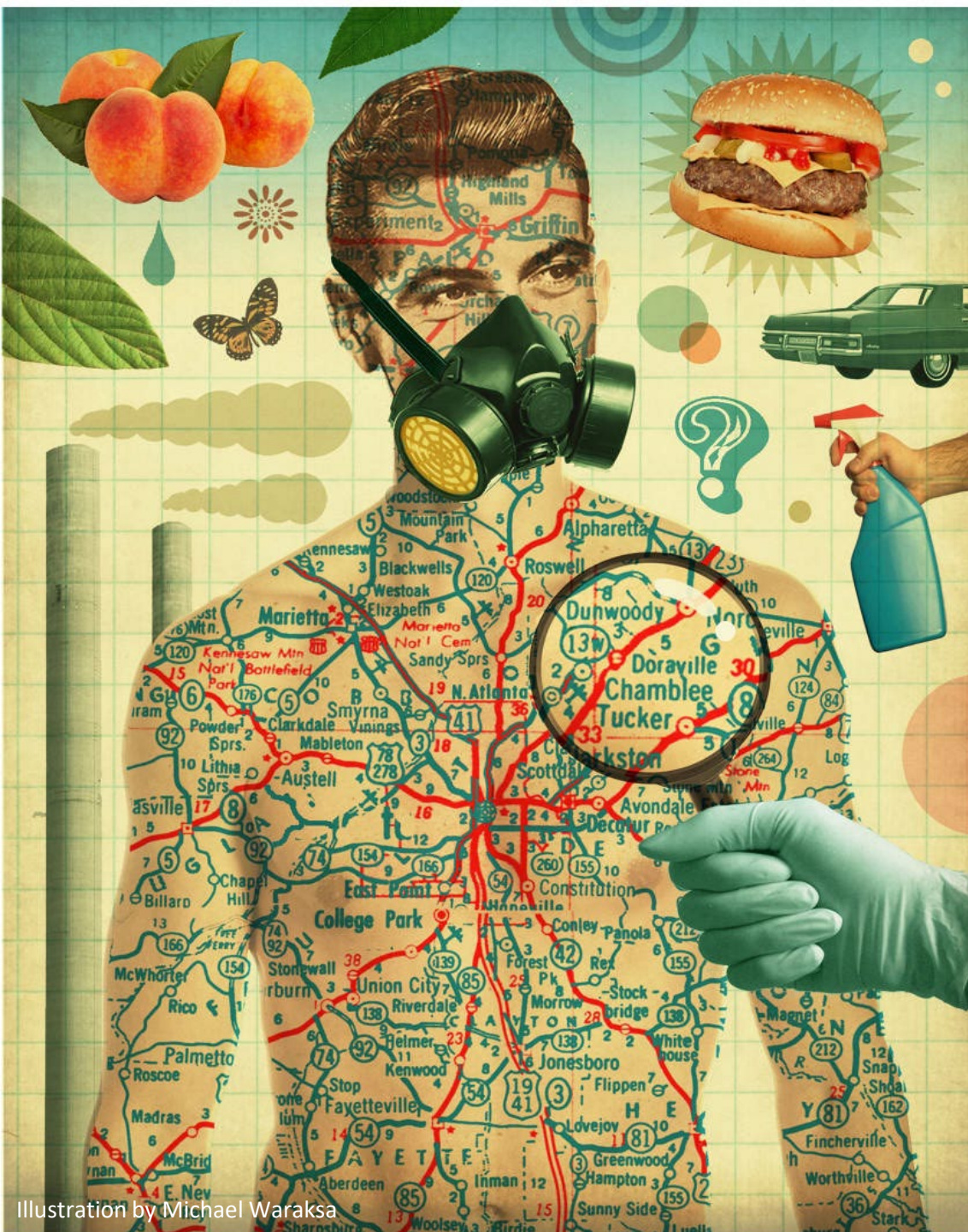
Hae-Ryung Park, PhD
Assistant Professor





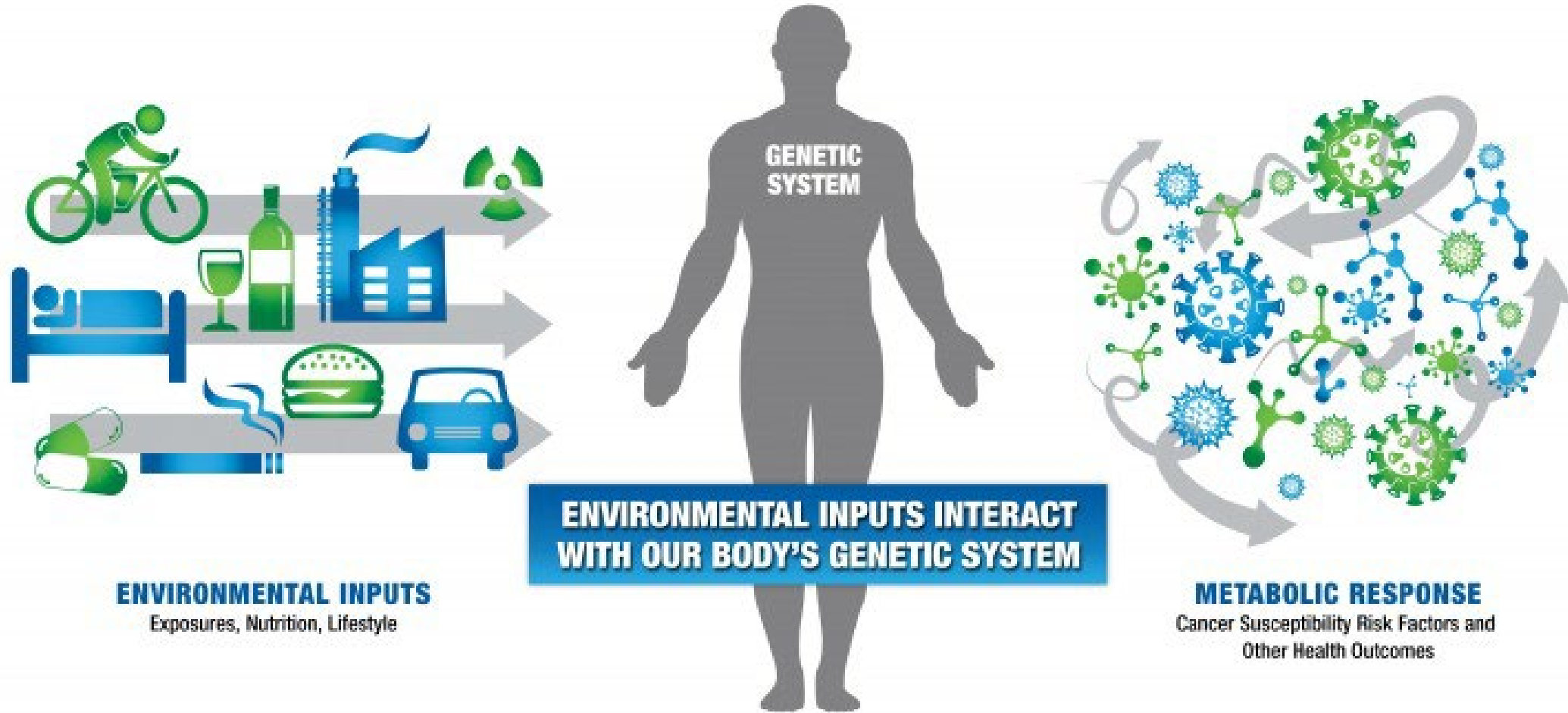
You are what you eat.

-Dr. Jean Anthelme Brillat-Savarin (1825, France)

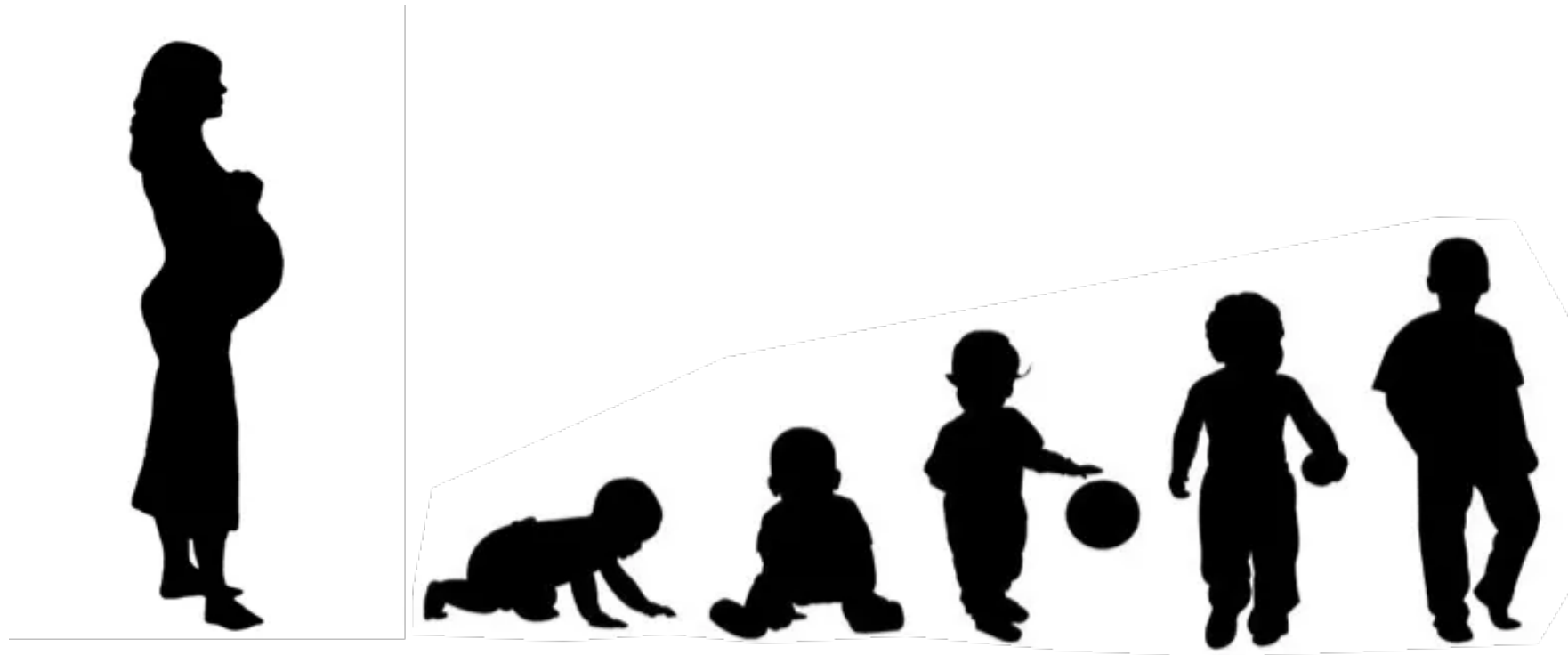


**You are what you get
exposed to.**

Gene-environment interaction determines health outcomes



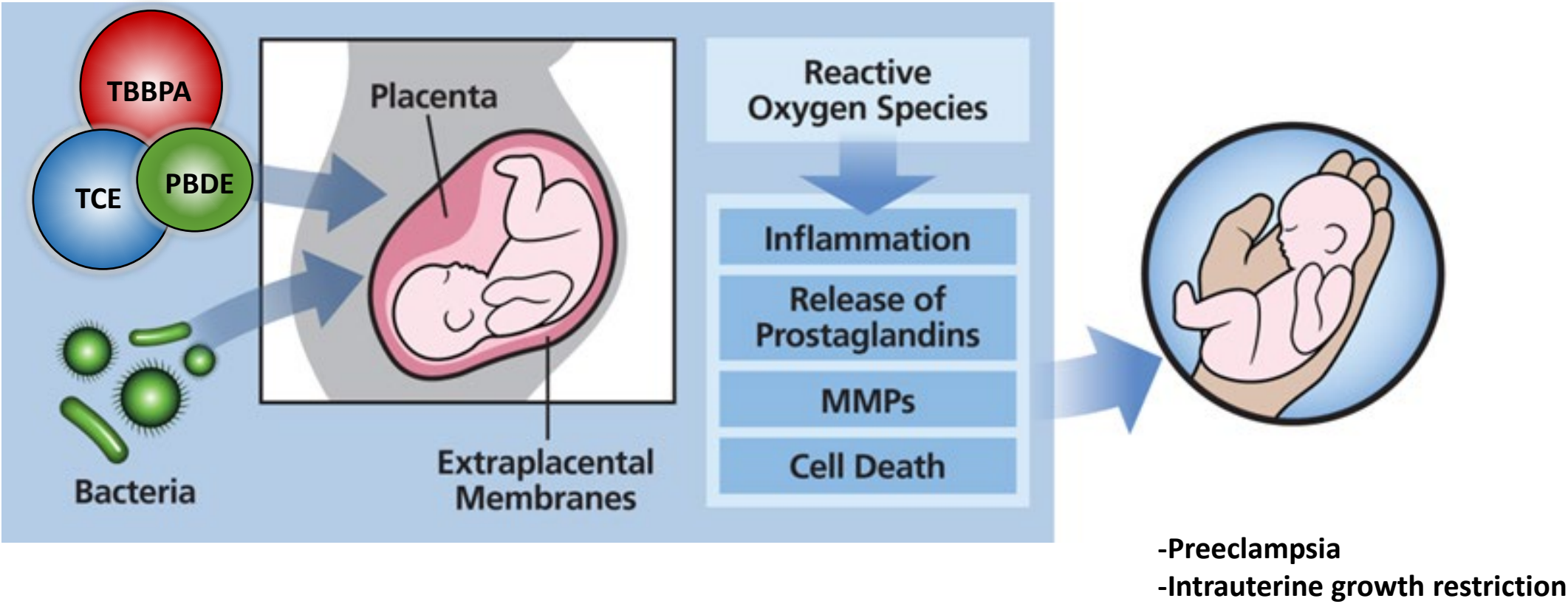
Vulnerable populations to environmental exposure



Pregnant mothers and
Developing fetuses

Infants and
developing children

Environmental Impacts on Pregnancy outcomes



-Preeclampsia
-Intrauterine growth restriction

Environmental impact on neurodevelopment in children



1. Identification of novel targets for metal exposure using genomics approach
2. *In vitro* mechanistic study
3. GWAS study to link identified targets with neurological outcomes in children

Environment Special:
The oceans—why 70%
of our planet is in danger

The Facebook Movie:
The secret history of
social networking

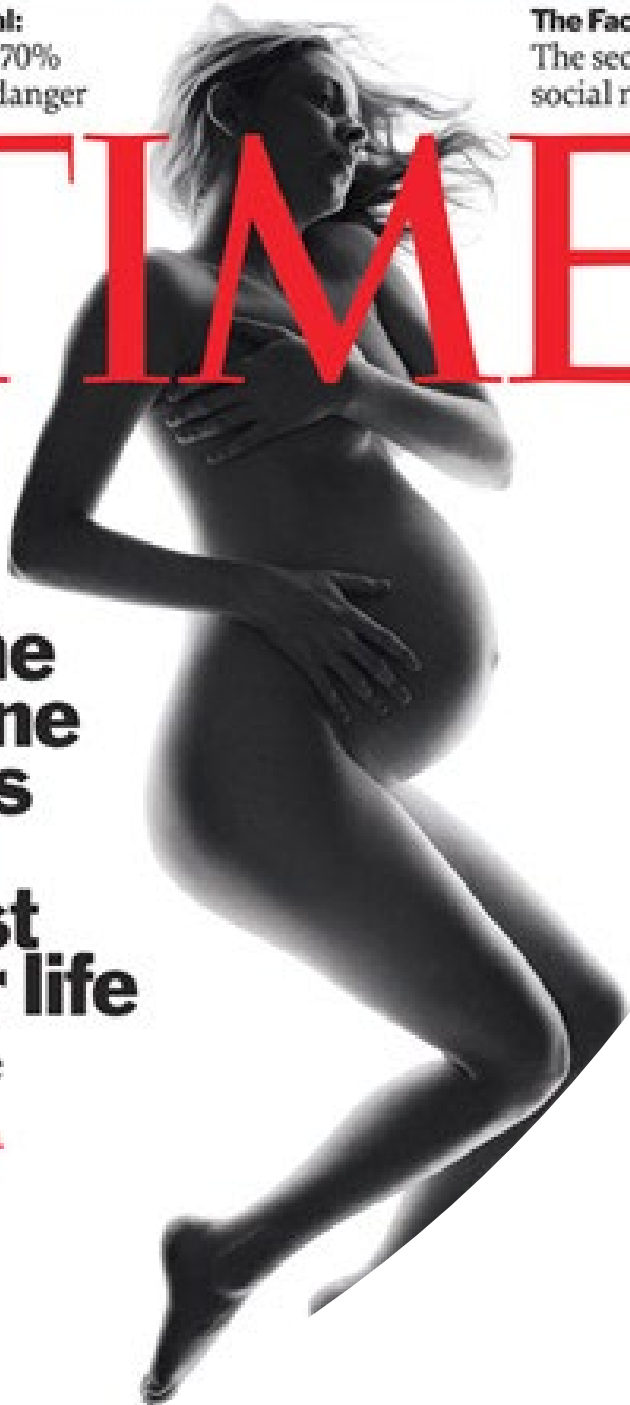
OCTOBER 4, 2010

TIME

**How the
first nine
months
shape
the rest
of your life**

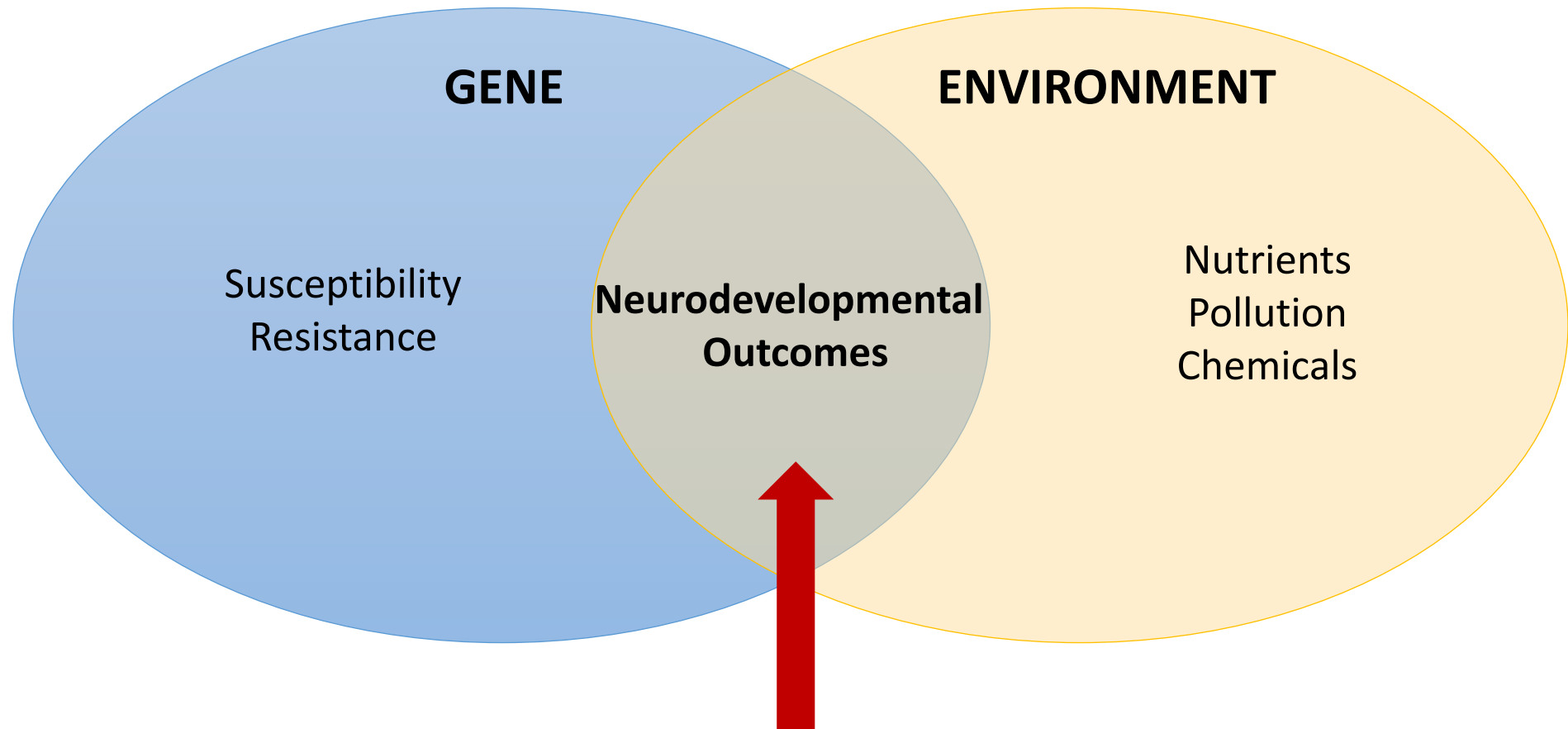
The new science
of fetal origins

BY ANNIE MURPHY PAUL



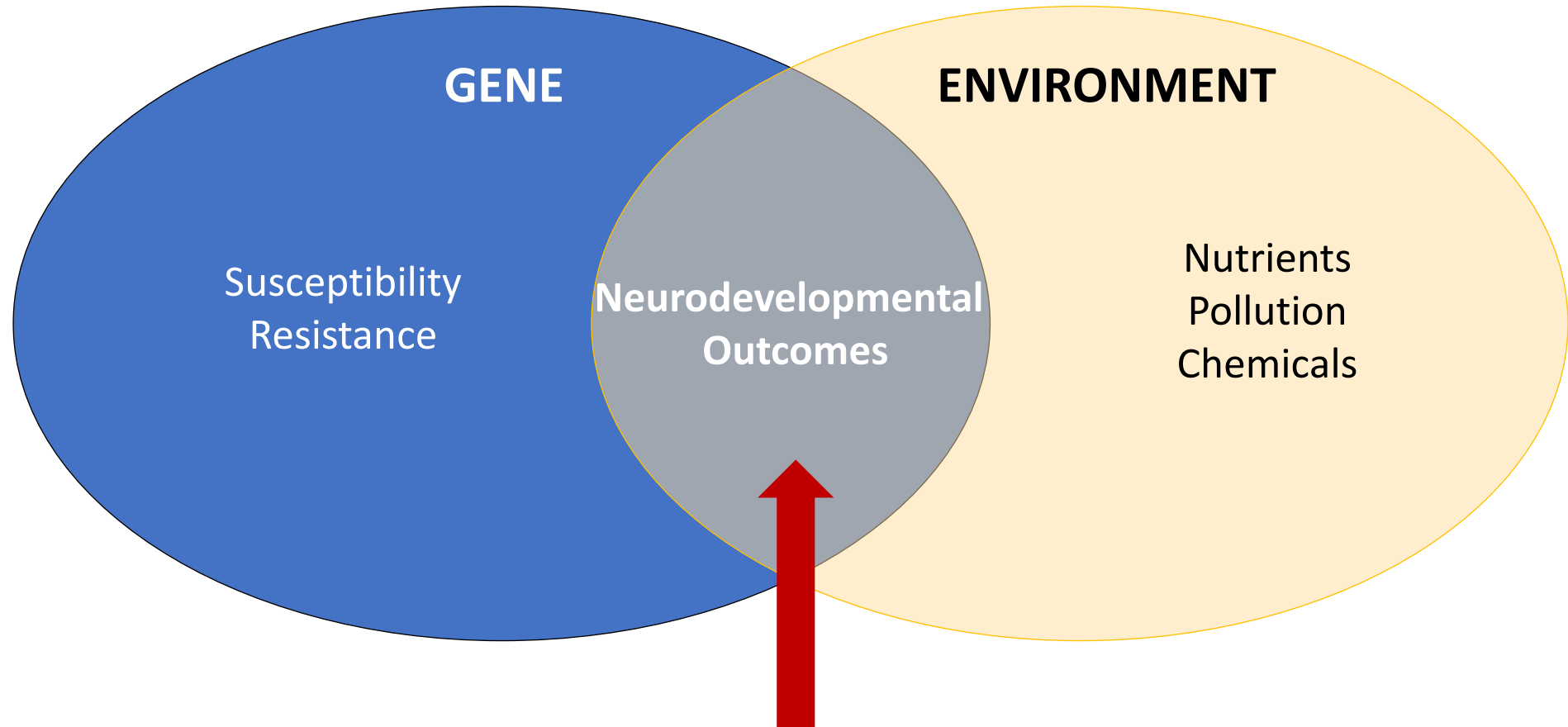
**Fetal
neuroprogramming
by interaction
between
neurotoxicants and
the placenta.**

Gene-Environment Interactions on Neurodevelopmental Outcomes



Roles of placenta/ placental extracellular vesicles

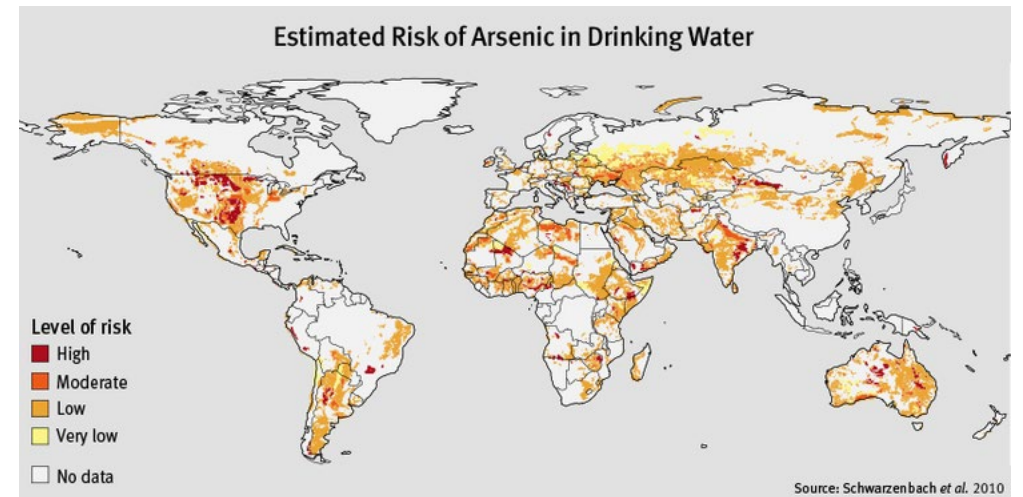
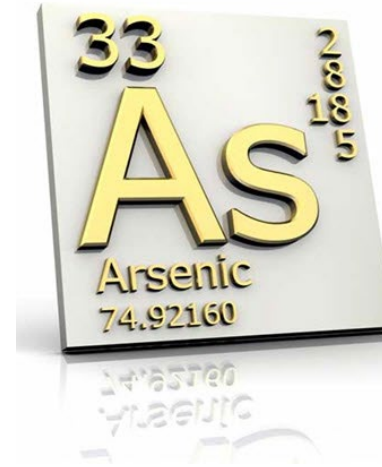
Gene-Environment Interactions on Neurodevelopmental Outcomes



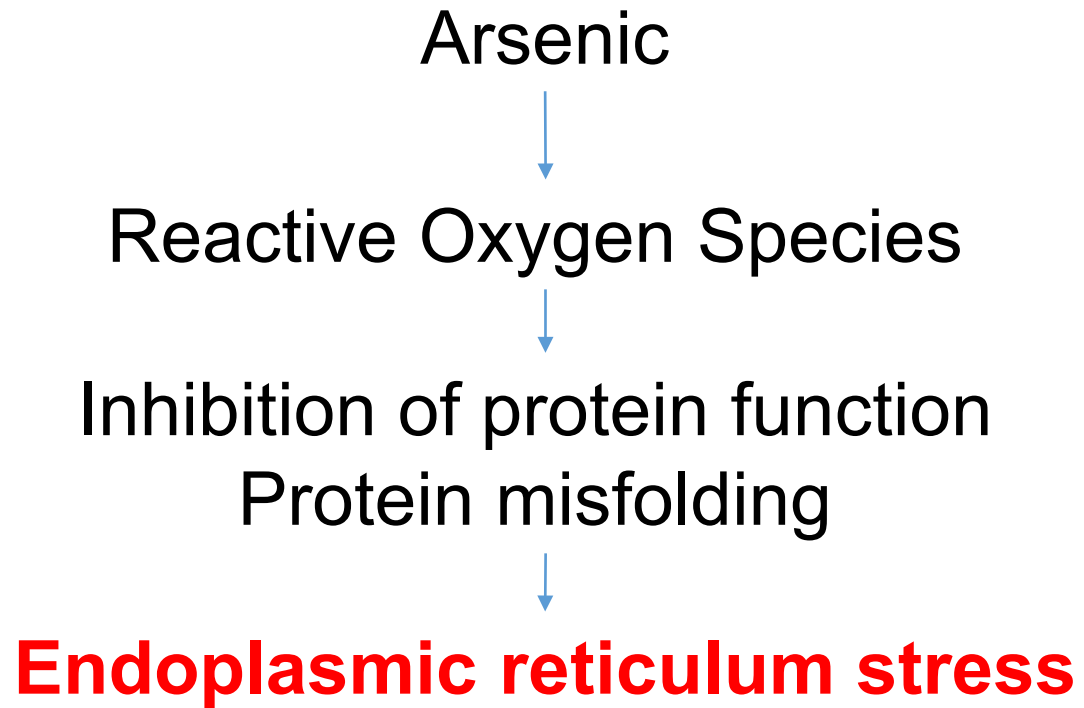
Roles of placenta/ placental extracellular vesicles

Arsenic (As) is a public health concern

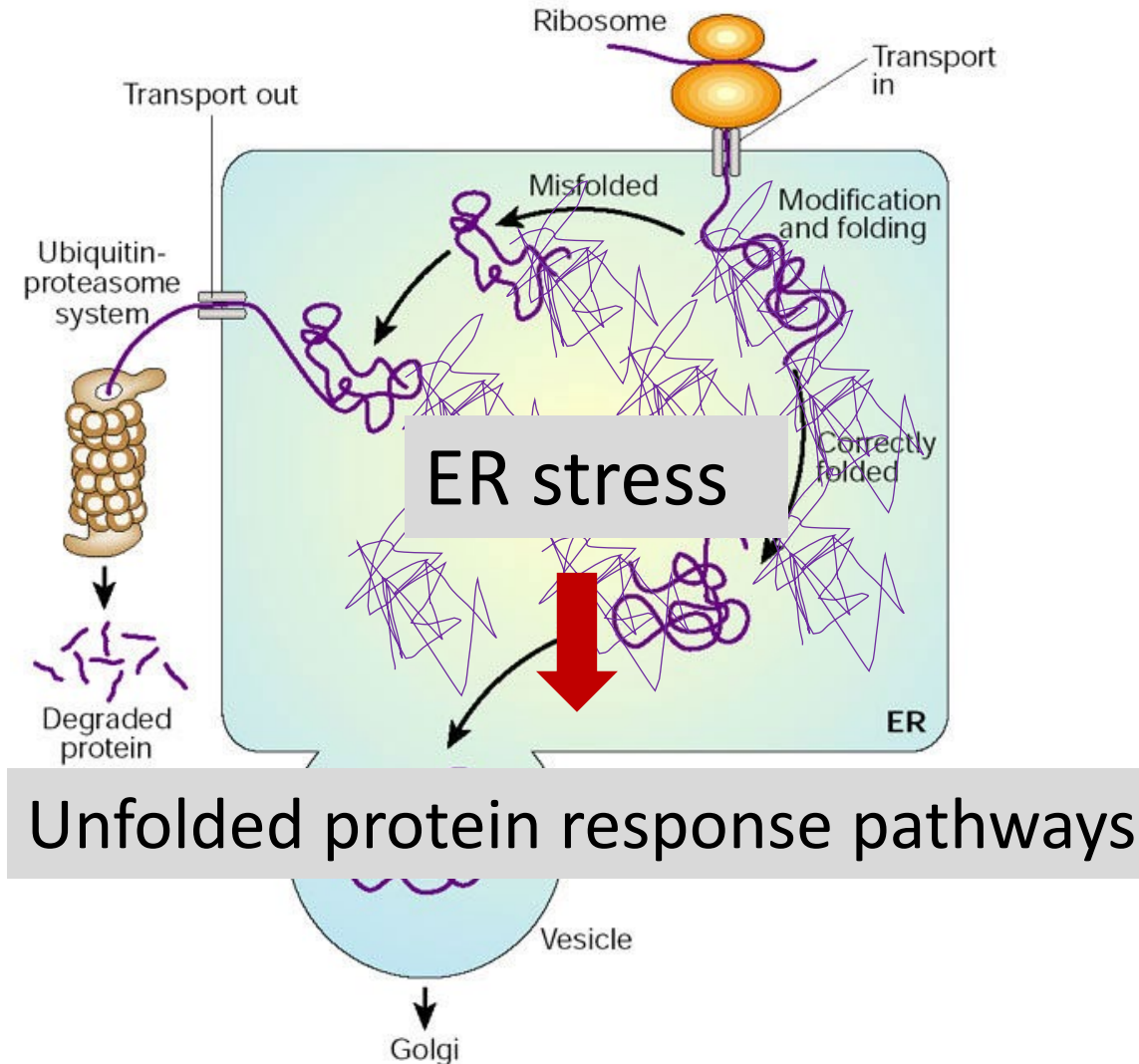
- A metalloid occurs naturally
- Uses
 - Wood preservatives
 - Pesticides
 - Alloying element
 - Semiconductor
- Affecting more than 150 million people globally
 - As levels above WHO guideline value of 10 ug/L
- Prenatal metal exposure to As has been associated with adverse neurodevelopmental outcomes in children.



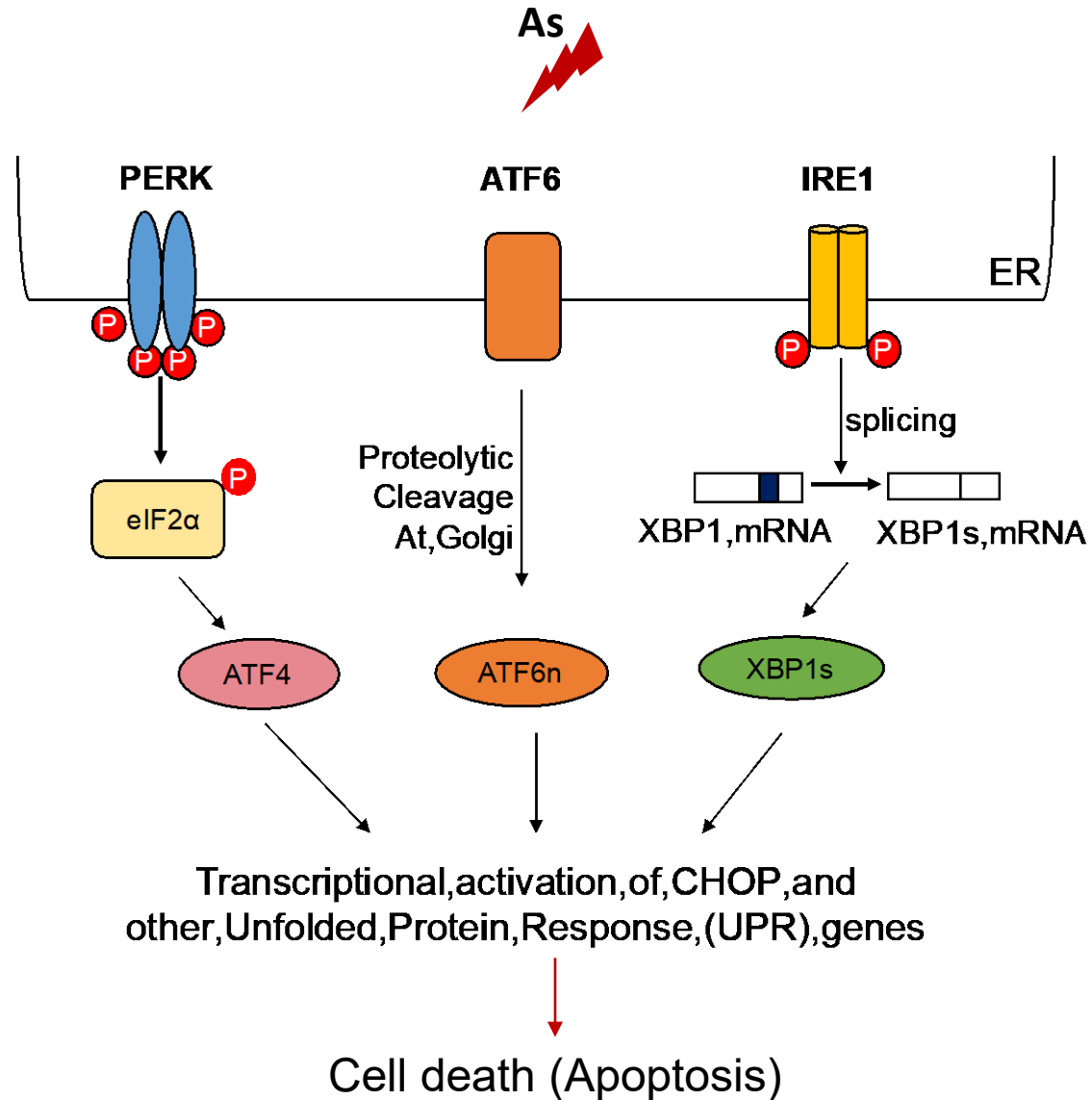
Mechanisms of As toxicity



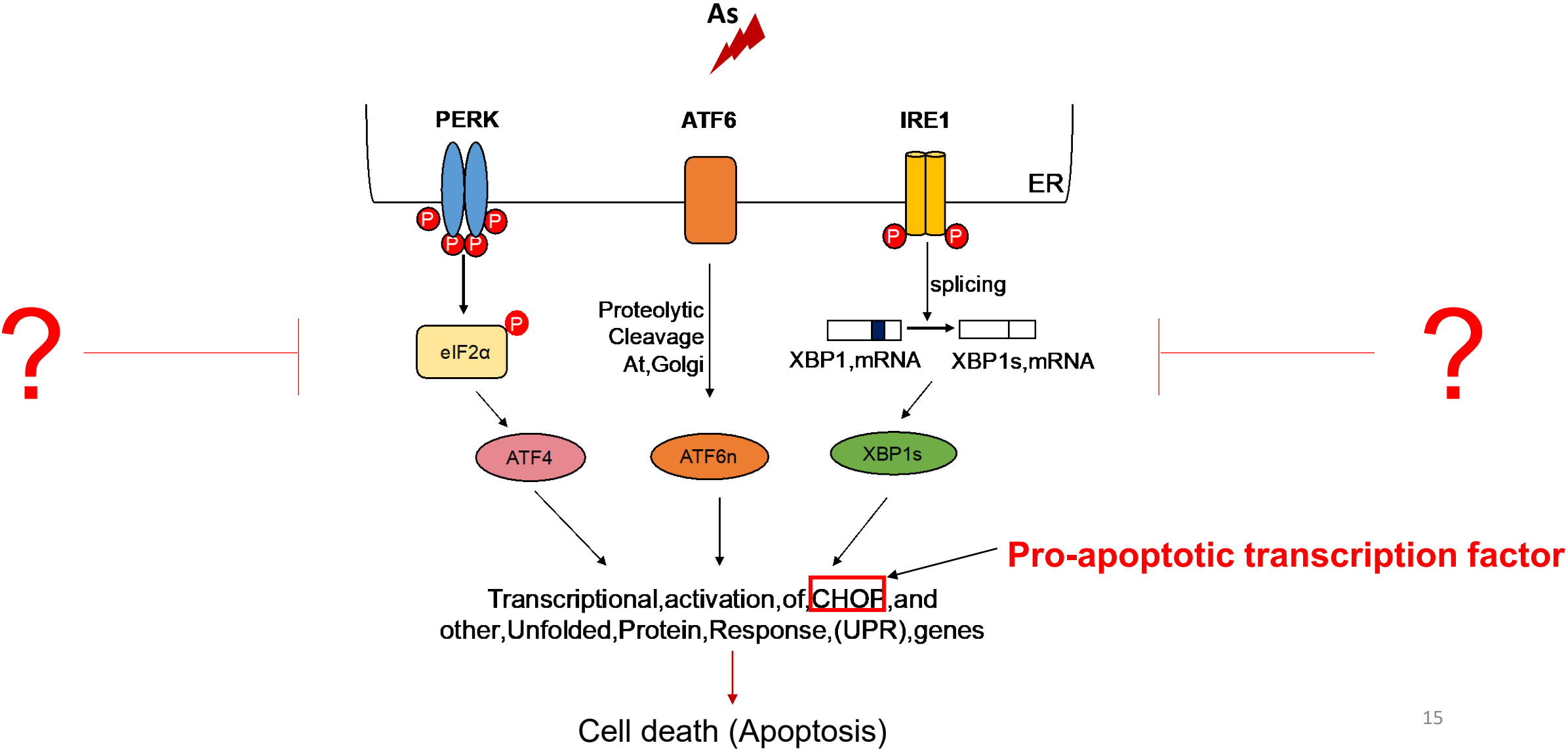
Accumulation of misfolded/unfolded proteins causes ER Stress



ER stress initiates unfolded protein response (UPR) pathways

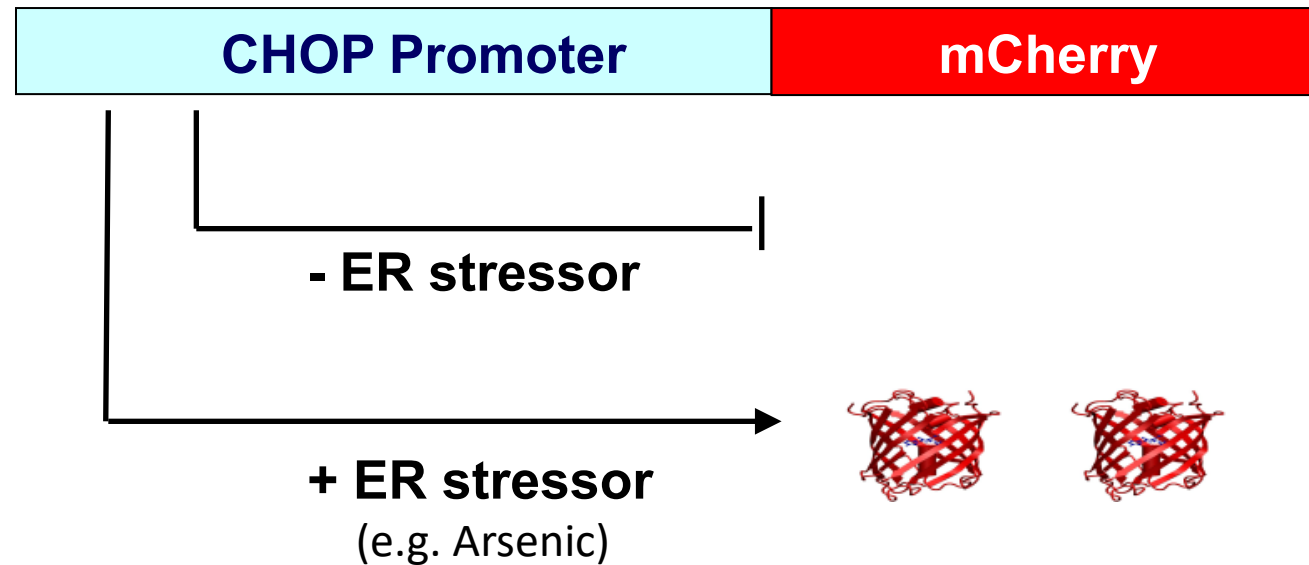


Research goals

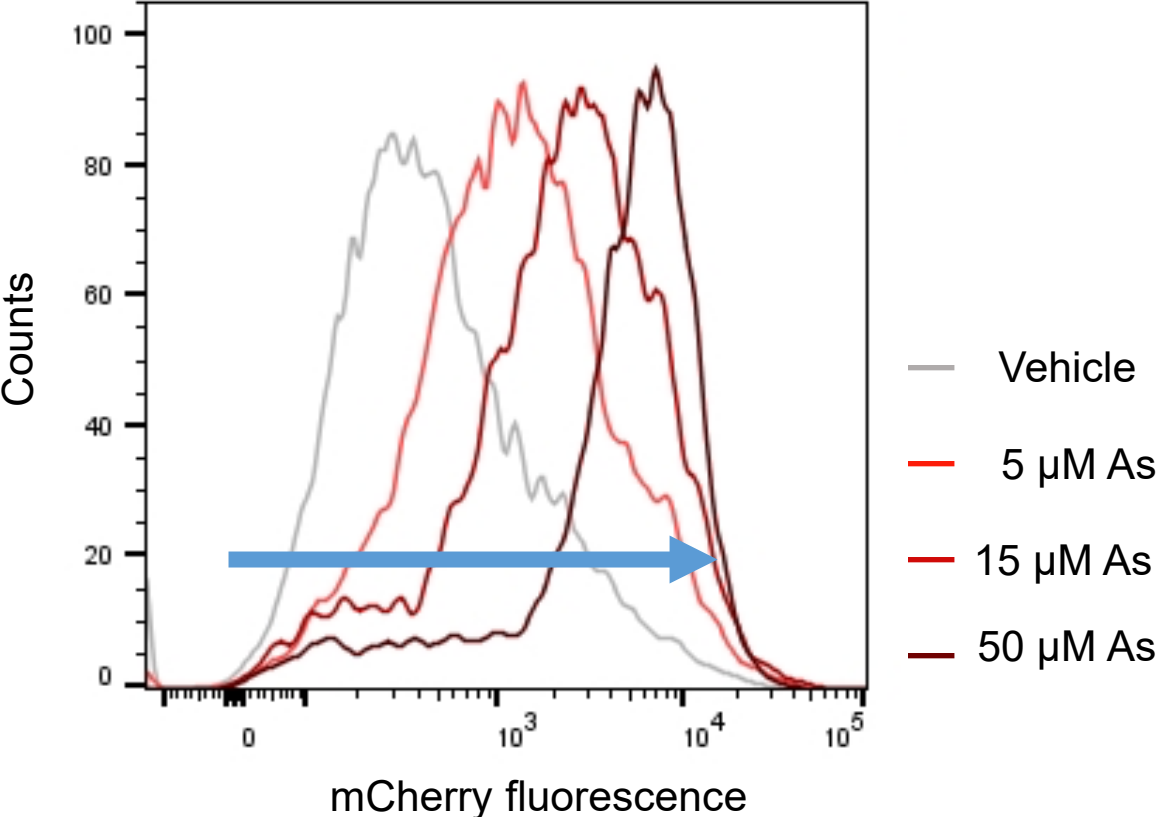
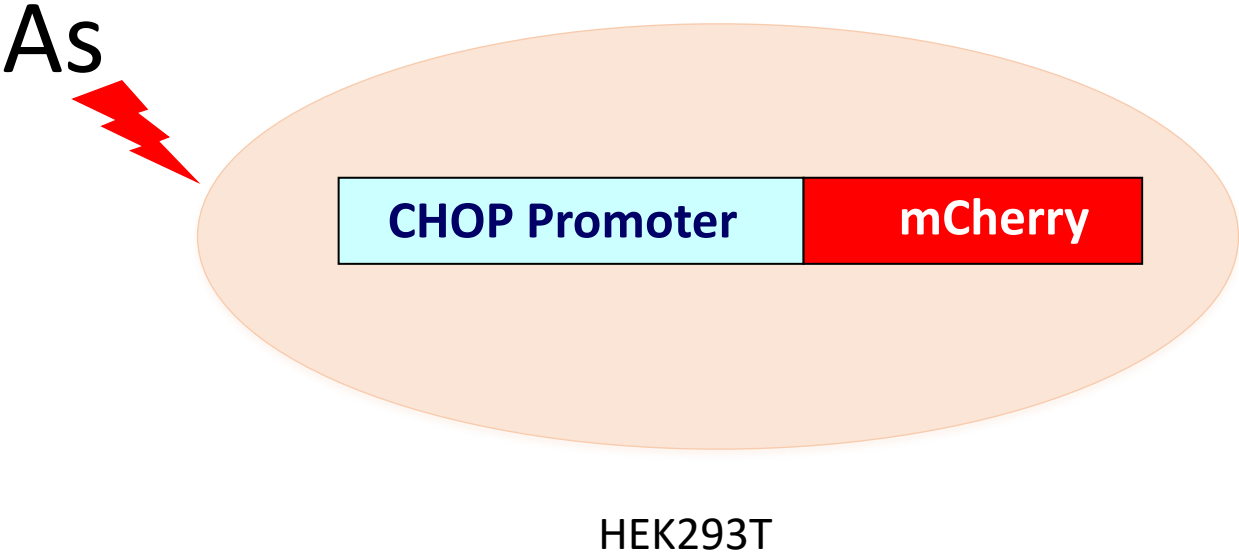


ER stress cell model: CHOP-mCherry reporter cells

- Human CHOP promoter (-649/+135) (*P. Farfounoux; Bruhat, 2000, MCB*)



ER stress cell model: CHOP-mCherry reporter cells

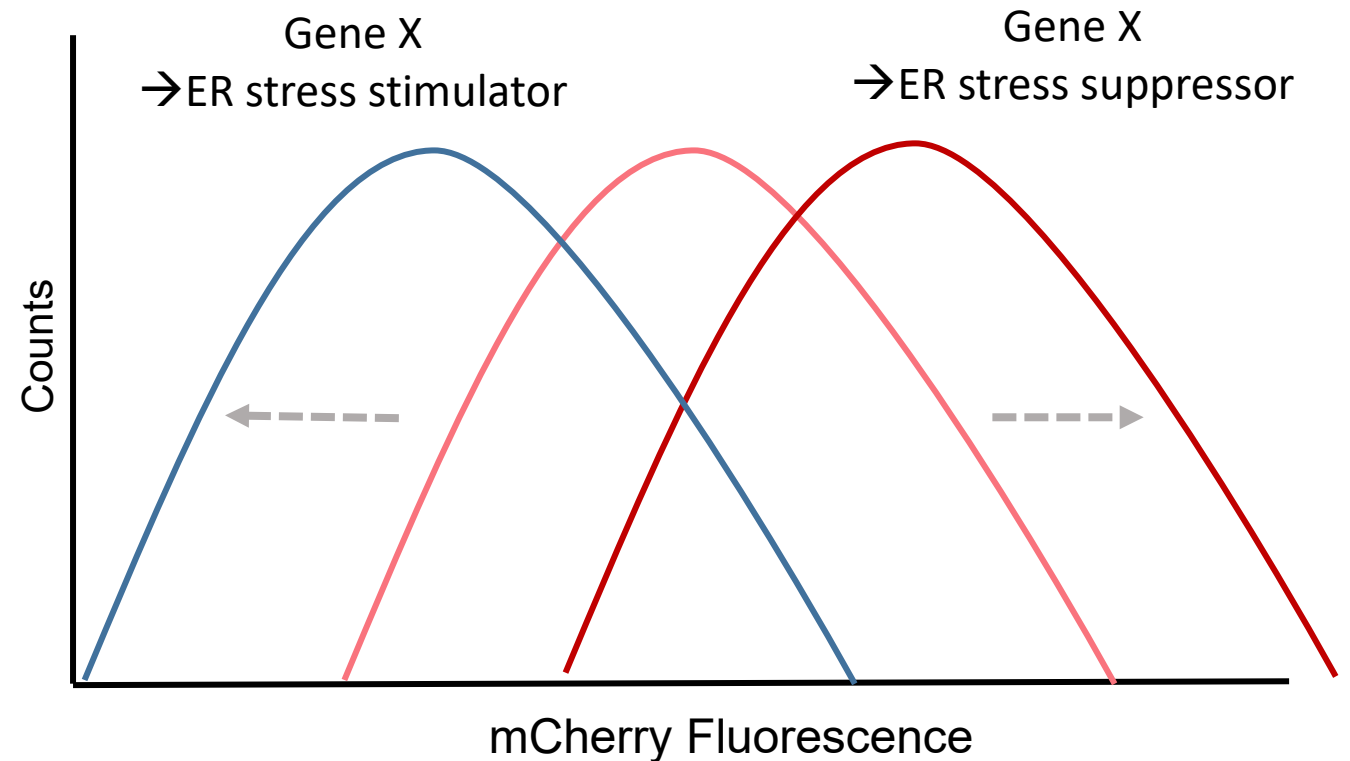
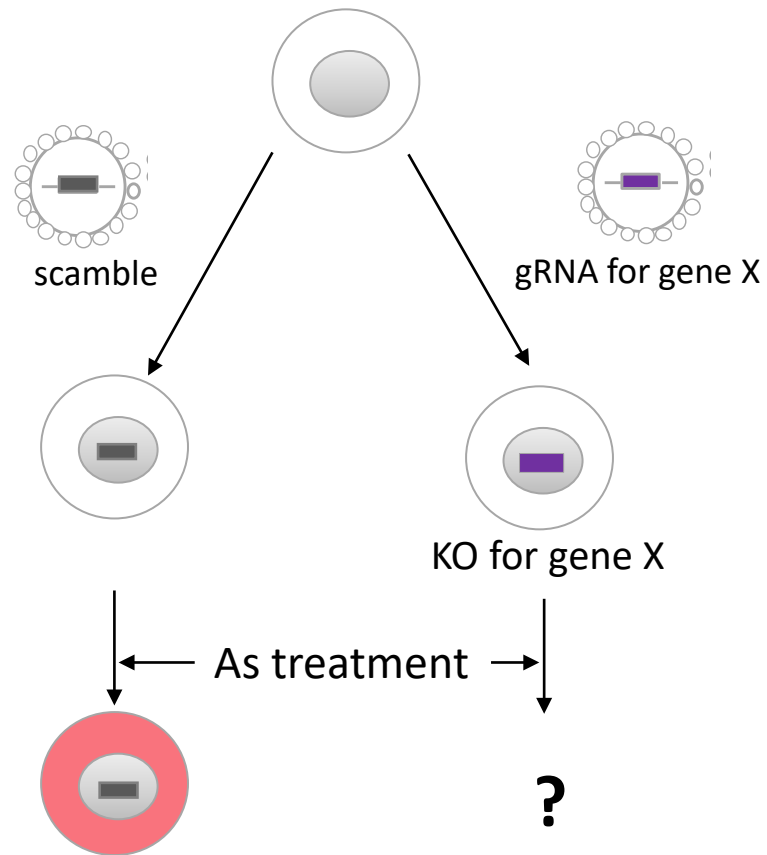


Genome-wide CRISPR Knockout Library

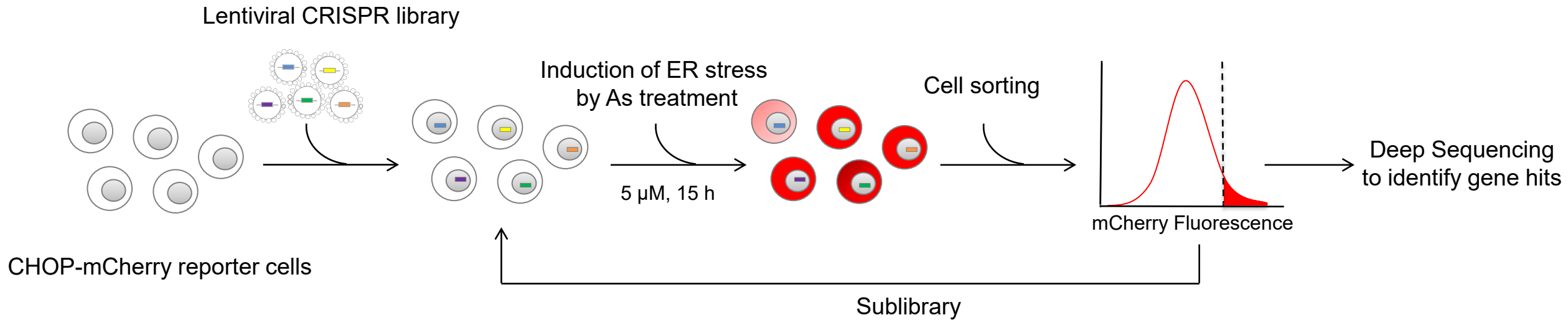
- Human CRISPR Knockout Pooled Library (GeCKO v2)
: Feng Zhang Lab (Broad Institute of MIT and Harvard)

Number of genes targeted	19,052
gRNAs per gene	6 (3 in library A and 3 in library B)
Number of miRNA targeted	1864
gRNAs per miRNA	4
Non-targeting gRNAs (scramble)	1000
Total gRNAs	122,417
Plasmid vector	Lentiviral

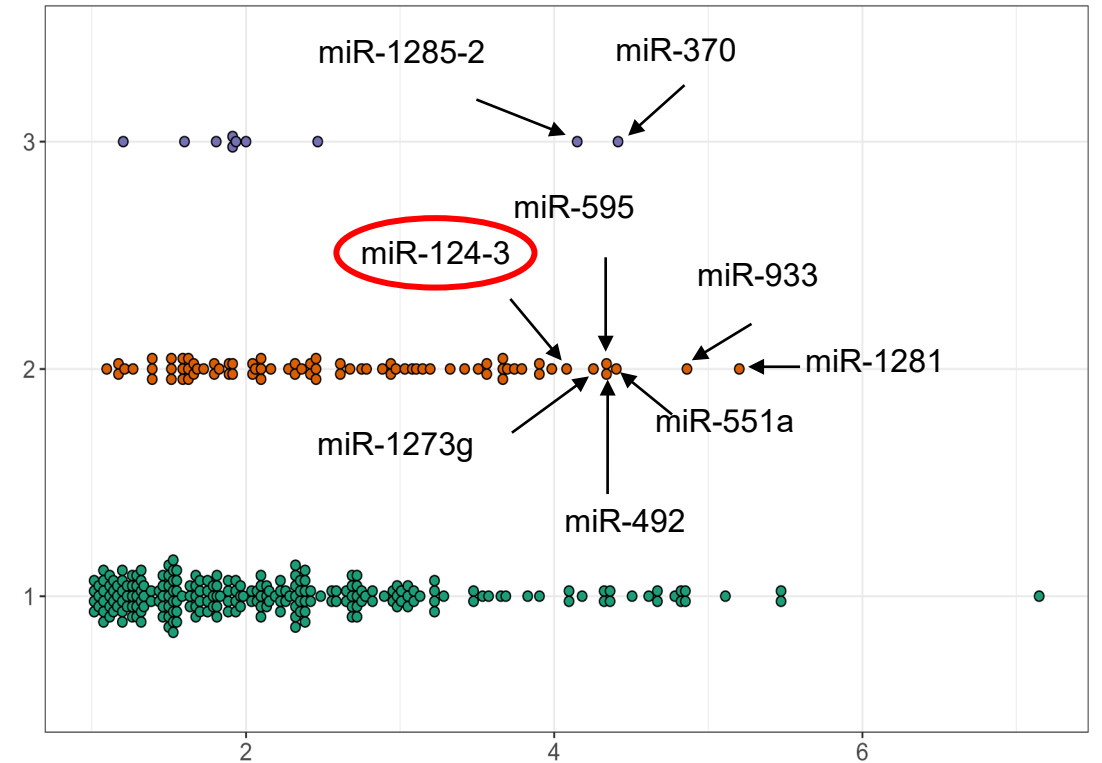
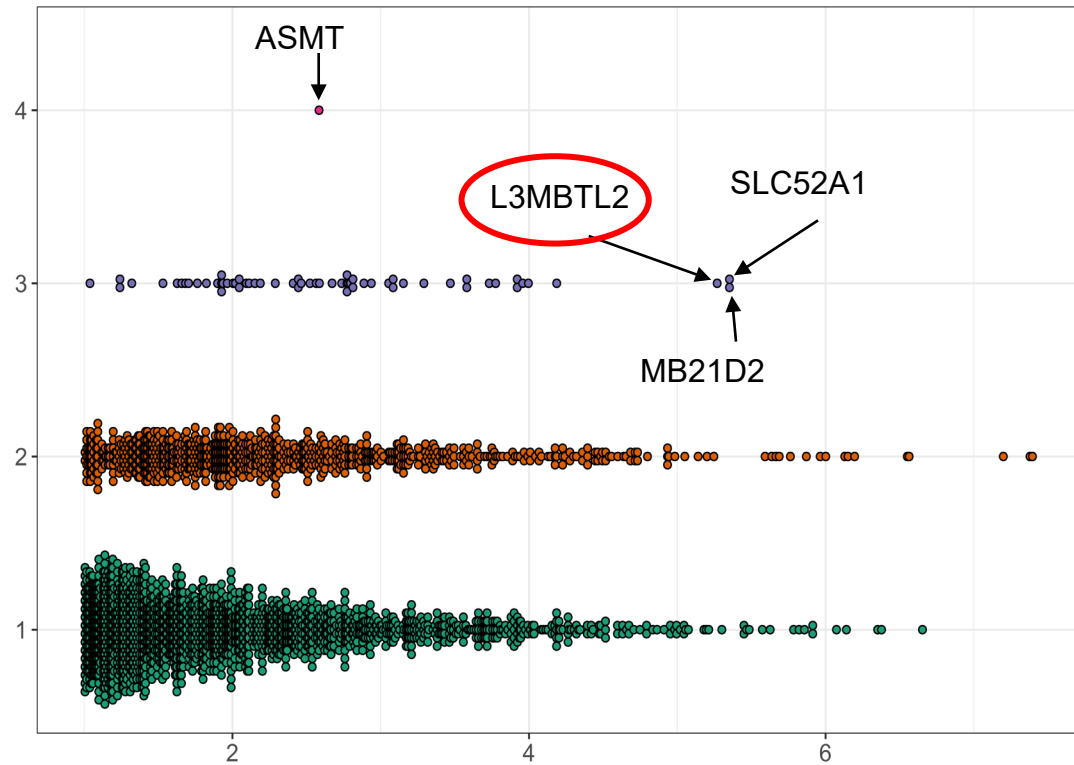
Principles of CRISPR screen in this study



CRISPR screen for Suppressors of As-induced ER Stress Response

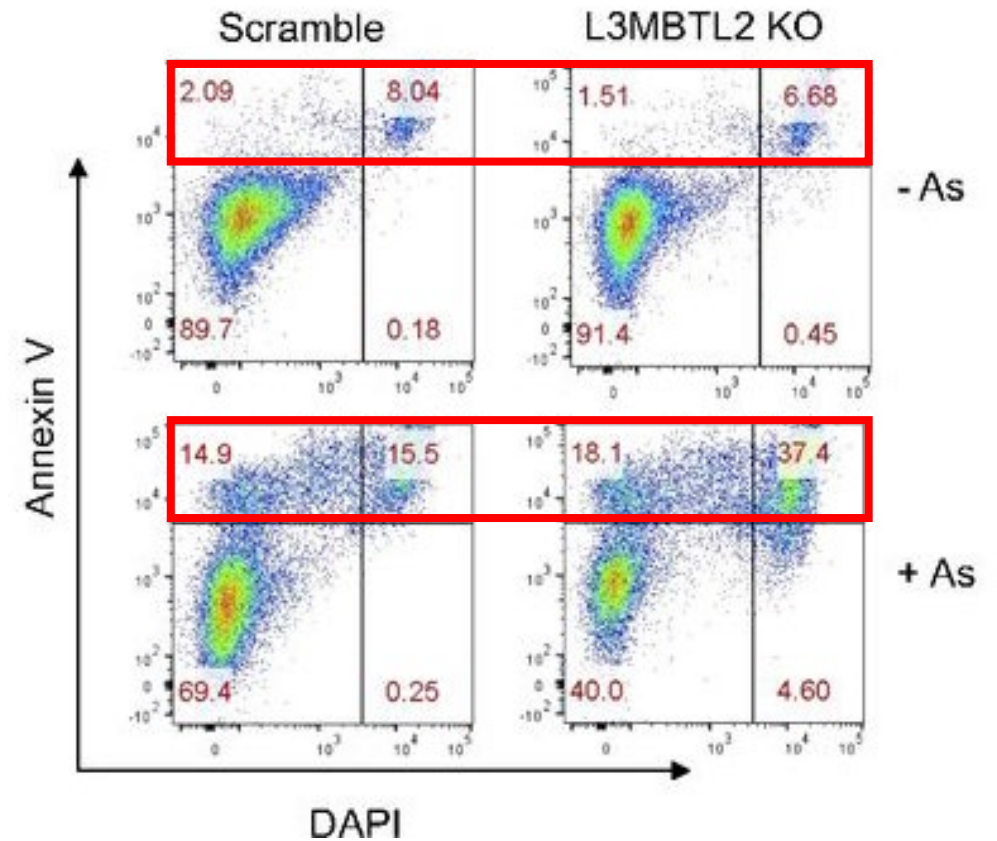
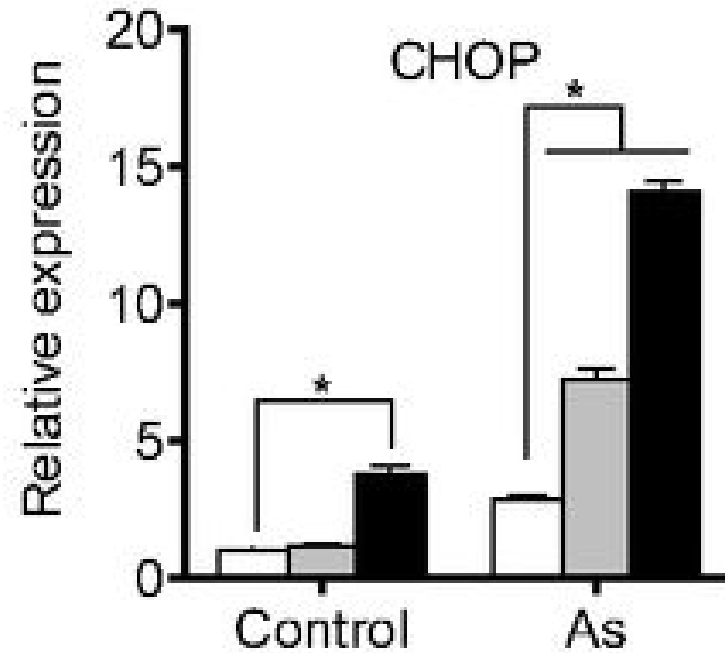


Hits from the screen



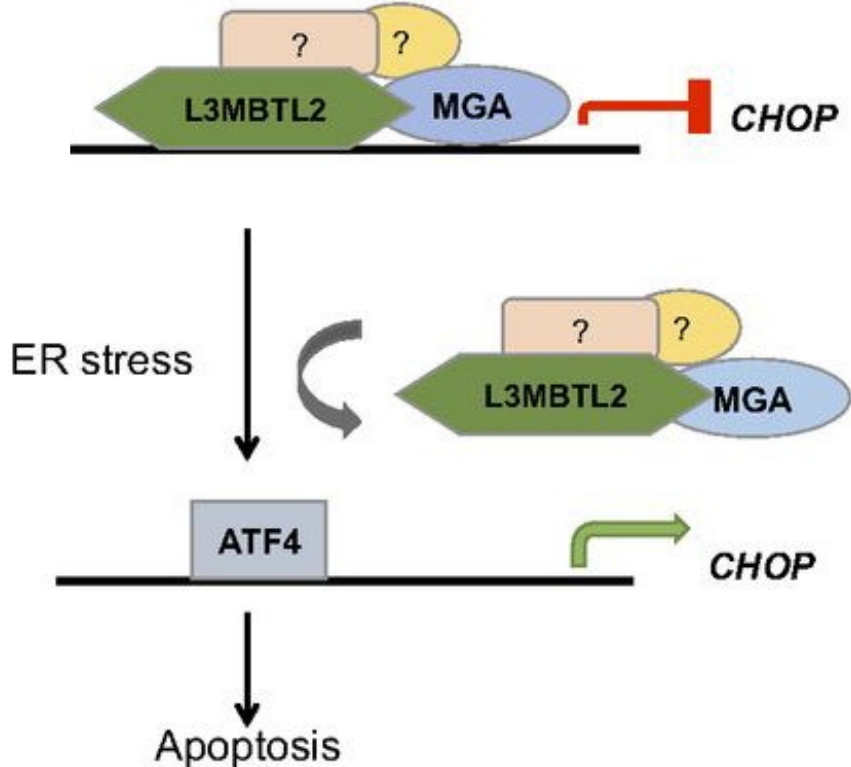
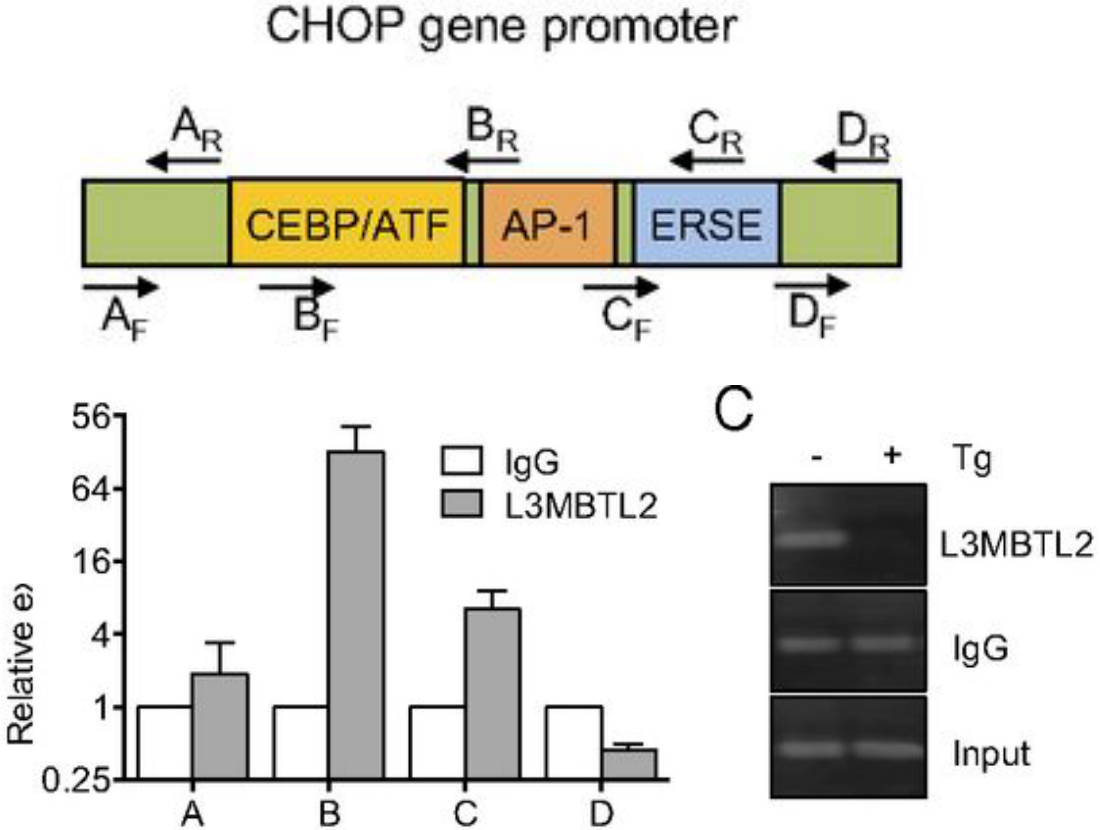
L3MBTL2 is a suppressor of As-induced ER stress response

□ Scramble □ L3MBTL2 gRNA1 ■ L3MBTL2 gRNA2



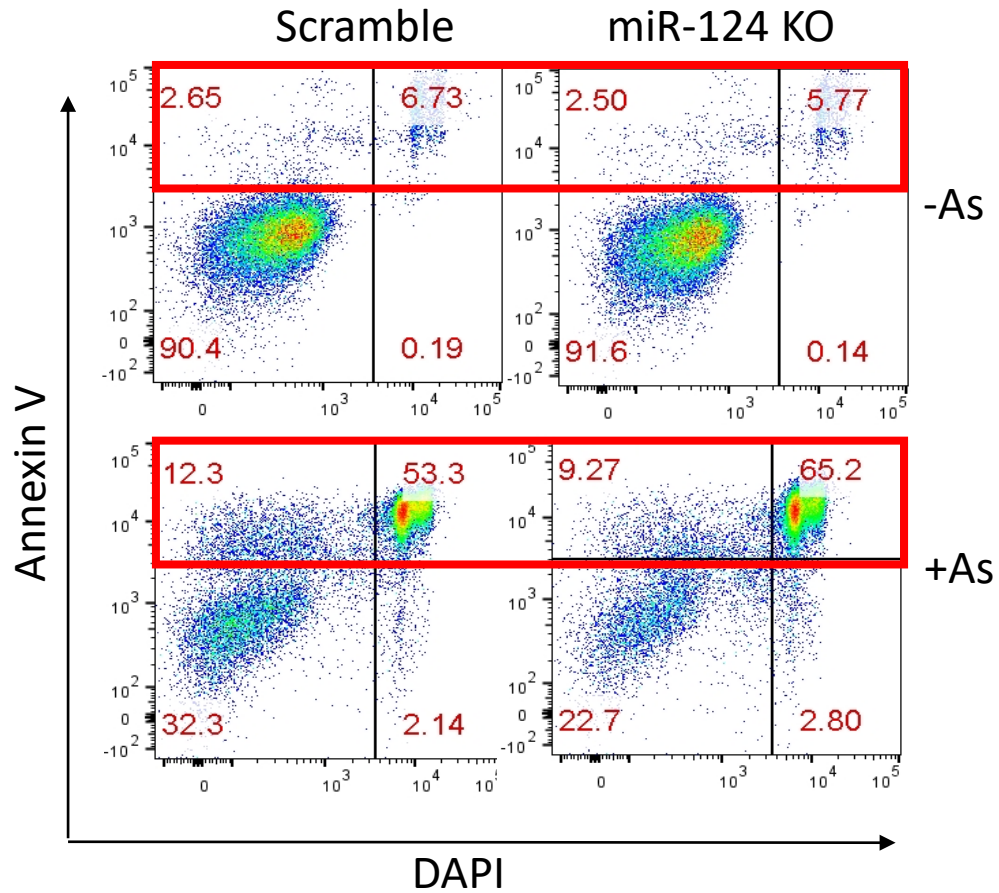
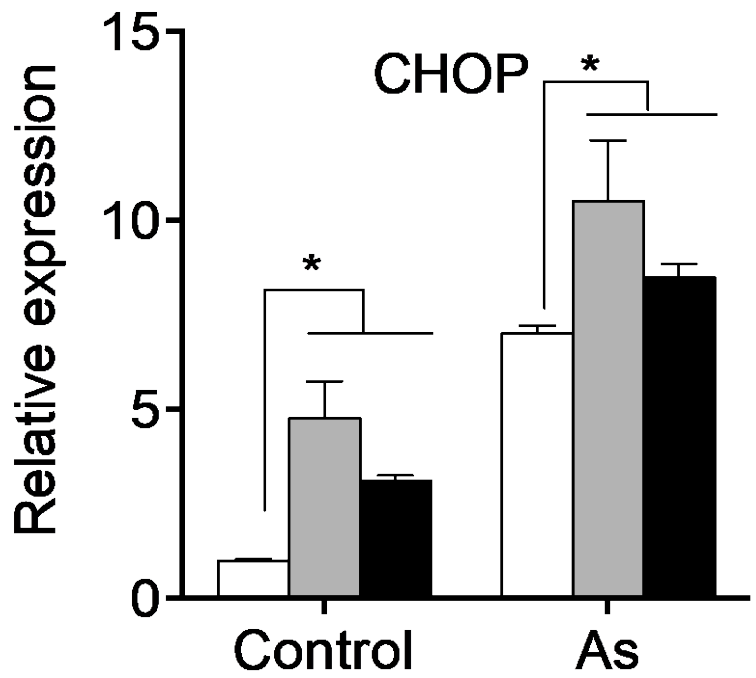
Apoptotic cells

L3MBTL2 associates with the CHOP promoter to repress its induction.



MiR-124 is a suppressor of As-induced ER stress response

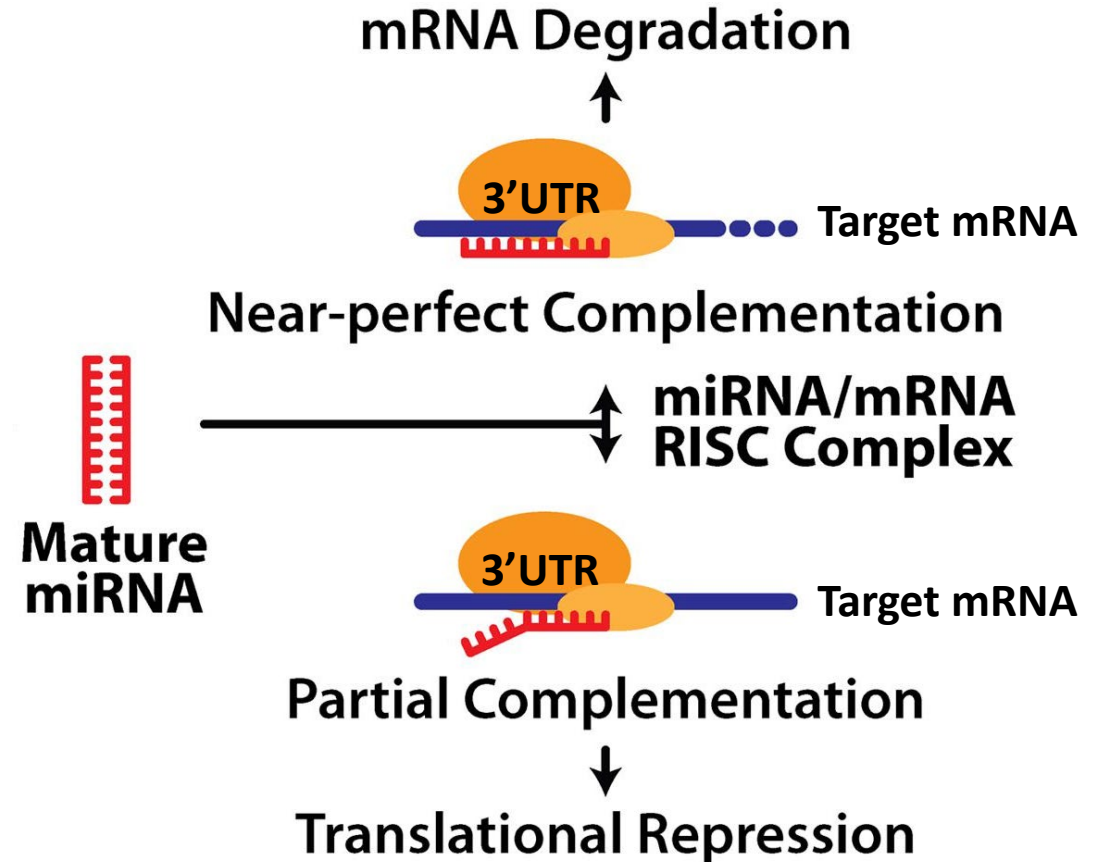
Scramble
 miR-124 gRNA1
 miR-124 gRNA2



Apoptotic cells

How miR-124 downregulates CHOP?

- MicroRNAs: small noncoding RNAs of 18–22 nucleotides
- Binding to 3'UTR(untranslated region) of target genes
 - Downregulation of target genes by mRNA degradation or by translational repression



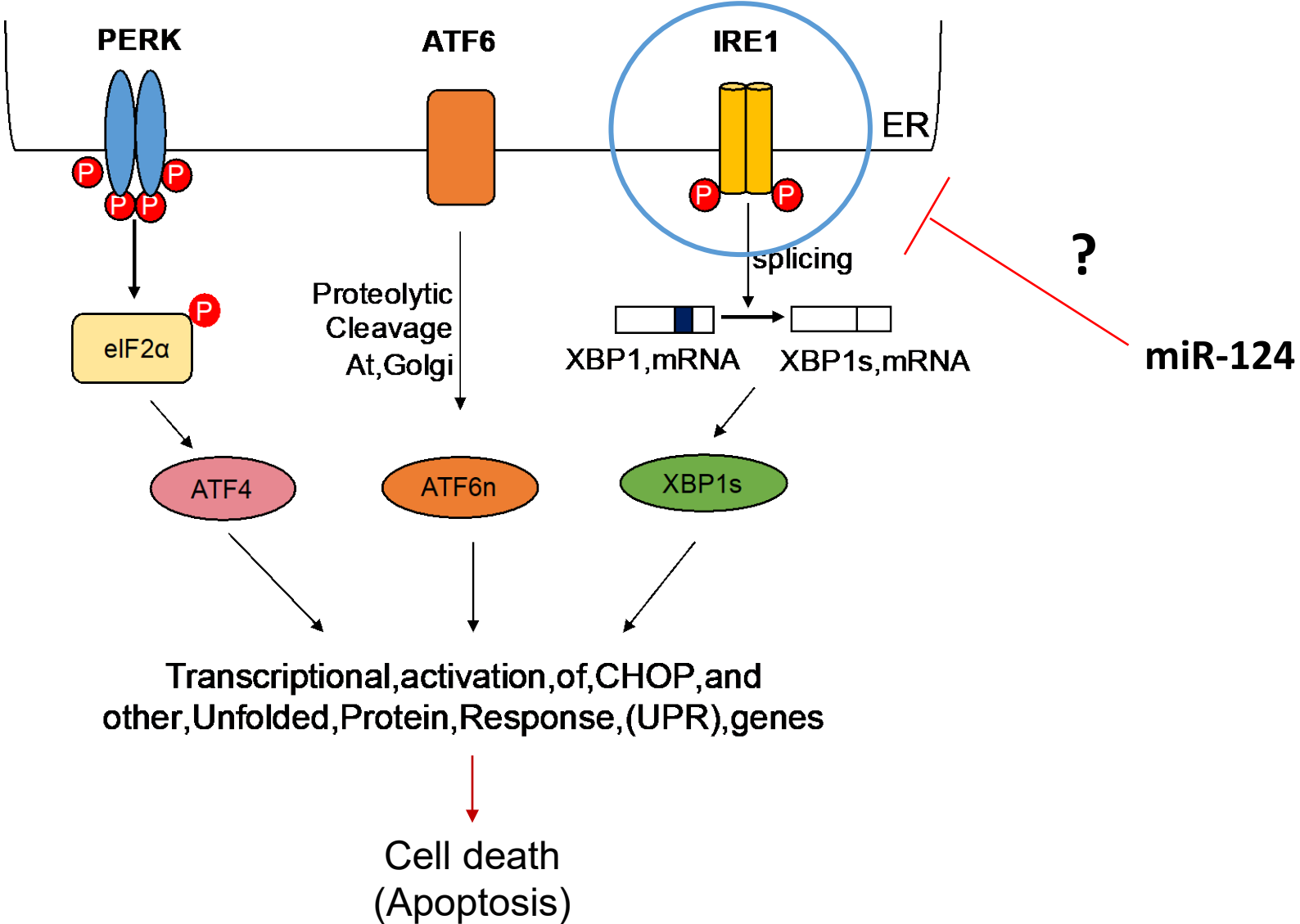
IRE1 is a predicted target of miRNA-124-3p

90.....	100.....	110.....	120.....	130.....	140.....	150.....
Human	-----AGGGAGA-----	CCAGGCUUCCCAAACCAA-	GUGCCUUGAGC---	UGCCUGC-----	UCUGCAG---	CCCACAGAGGAC----	AGU-----
Chimp	-----AGGGAGA-----	CCAGGCUUCCCAAACCAA-	GUGCCUUGAGC---	UGCCUGC-----	UCUGCAG---	CCCACAGAGGAC----	AGU-----
Rhesus	-----AGGGAGA-----	CCAGGCUUCCCAAACCAA-	GUGCCUUGAGC---	CGCCUGC-----	UCUGCAG---	CCCACAGAGGAC----	AGU-----
Squirrel	-----AGGGAGA-----	CCAGGCUUCCCAAACCAA-	GUGCCUUGAGC---	UGCCAC-----	UCUGCAG---	CCAGCAGACGAU----	AAU-----
Mouse	-----AGGAAGA-----	CUAAGCUUCGCAAUCA-	GUGCCUUGAGC---	UGCU-GA-----	UCUGCAG---	CCAGAAGAGGAU----	AAC-----
Rat	-----AGGAAGA-----	CUAAGCUUCUCAAACCAA-	GUGCCUUGAGC---	UGCU-GA-----	UCUGCAG---	CCAGAAGAGGAG----	UAU-----
Rabbit	-----AGGGAAC-----	CCAGGCUUCCCAAAC-AA-	GUGCCUUGAGC---	UGCCAC-----	UCCGCGG---	UCA-CAGAGGGU----	AAG-----
Pig	-----AGGGAGG-----	CCAGGCU-CUGGAGCCAA-	GUGCCUUGAGC---	UGCCGGC-----	UCUGCAG---	CCAGCAGGGGAG----	GAG-----
Cow	-----AGGGAGA-----	CCAGGCC-CGGAAGCCAA-	GUGCCUUGAGC---	UGCCAC-----	UCCGCAG---	CCAGCAGGGGAA----	GAU-----
Cat	-----AGGGAGA-----	CCAGGCC-CCCAAACCAA-	GUGCCUUGAGC-----	CUGC-----	UCUACAG---	CCAGCAGAAGAU----	GAU-----
Dog	-----AGGGAAA-----	CCAGGCU-CCCAAACCAA-	GUGCCUUGAGC---	UGCCCGC-----	CCUGCAG---	CCCGCAGGGGAAU----	GAU-----
Brown bat	-----AGAAGA-----	CCAGGCU-CCCAAACC-A-	GUGCCUUGAGC---	UGCCUGC-----	UCUGCAG---	CUGGCAGAGGAG----	GGU-----
Elephant	-----AGGGAGA-----	CCAGGGU-UCCCAAACCAA-	GUGCCUUGAGC---	UGCC-AU-----	UCUGCCG---	CCAGCAGAGGAAU----	AAU-----
Opossum	AGCCAUGAGAGAGAAG-----	CUGUGCUCUGAAAUUAA-	GUGCCUUGGAC---	UGCCUGCCGCGUUCUGCAG---	CCAGAGCAGGGG----	GAU-----	
Macaw	-----GAAGAAA-----	CCAAGCU-UCCAAA--AA-	GUGCCUUGUACCUCUGCCUGC---	GCUGGAC---	CAGGA----	GAU-----	GGU-----
Chicken	-----GAGGAAA-----	CCAAGCU-UCCAAA--AU-	GUGCCUUGUAU-UCUGCCUGC---	ACUGGAU---	CAGGA----	GAU-----	AGU-----
Lizard	-----GAAAGAU-----	CCAGCGU-CACCCA--AA-	GUGCCUUGAAC-UCUGCCUGU-----	CUGACU---	CAGGAAGUGGAUUCAGGU-----		
X. tropicalis	-----	-----	-----	-----	-----	-----	-----
				miR-124-3p.1			
ConAGGGAGA.....	CCAGGCU.CCCAAACCAA.	GUGCCUUGAGC...	UGCCuGC.....	UCUGCAG...	CCAGCAGAGGAu.....	ggU.....

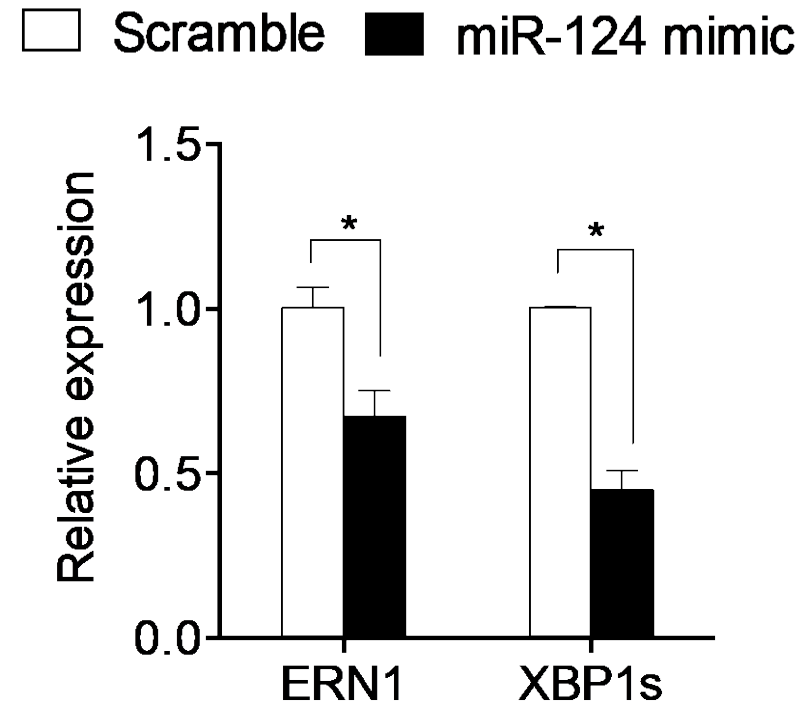
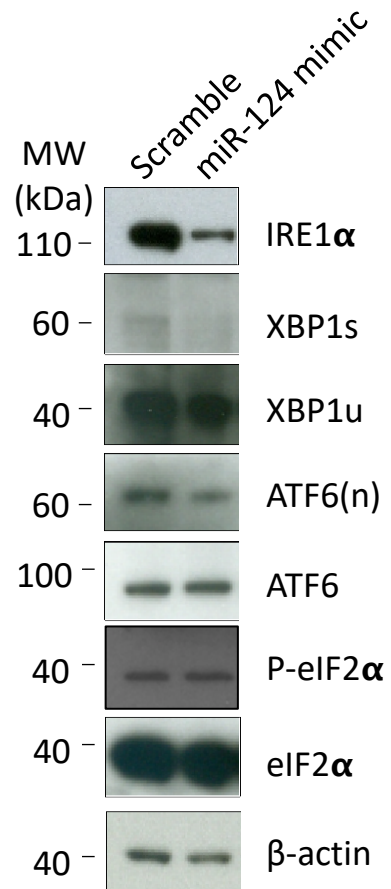
Human ERN1 ENST00000433197.3 3' UTR length: 4865

<http://www.targetscan.org/>

IRE1 is a predicted target of miR-124-3p



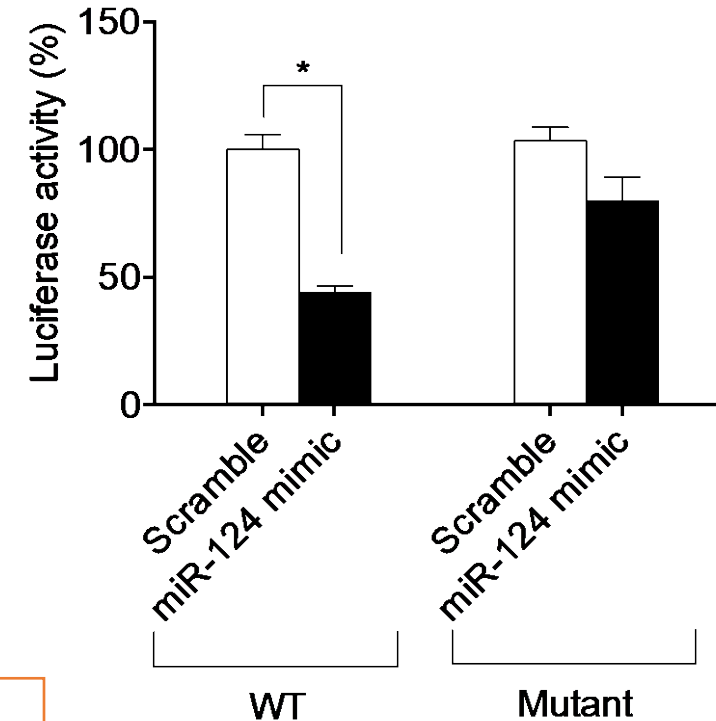
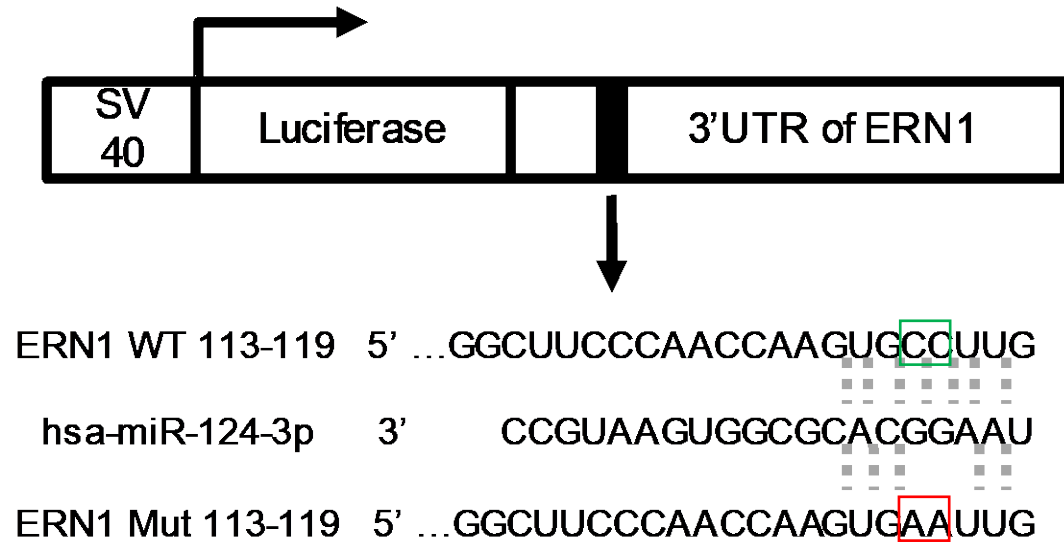
MiR-124 targets IRE1 pathway



ERN1: gene encoding IRE1

Panganiban* and Park* *et al*, PNAS (2019)

MiR-124 binds to 3'UTR of *ERN1* gene



Binding to 3'UTR of ERN1 → luciferase expression

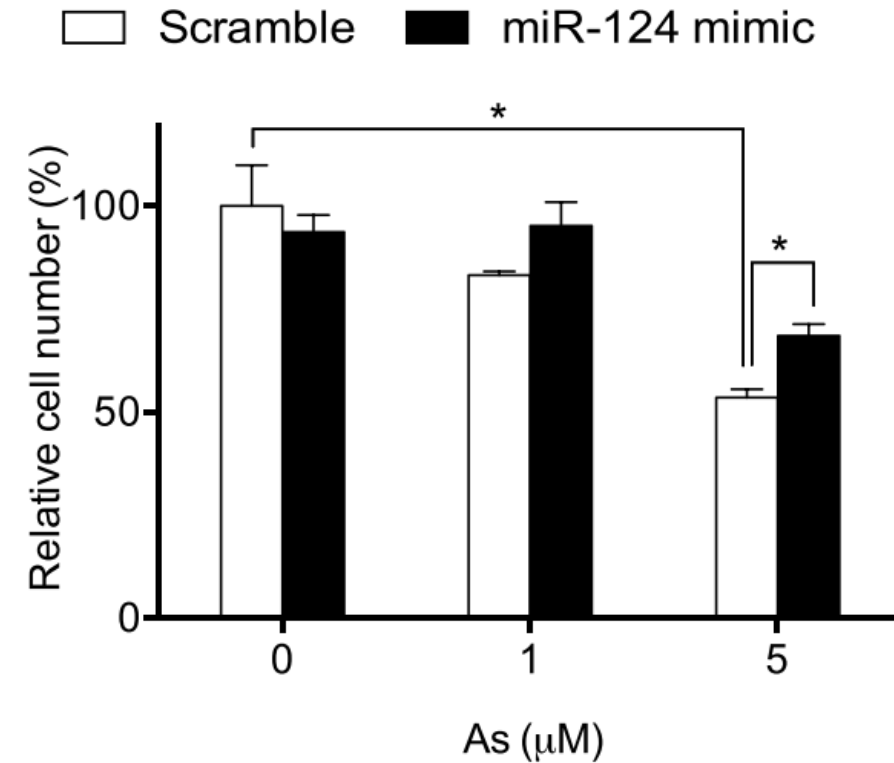
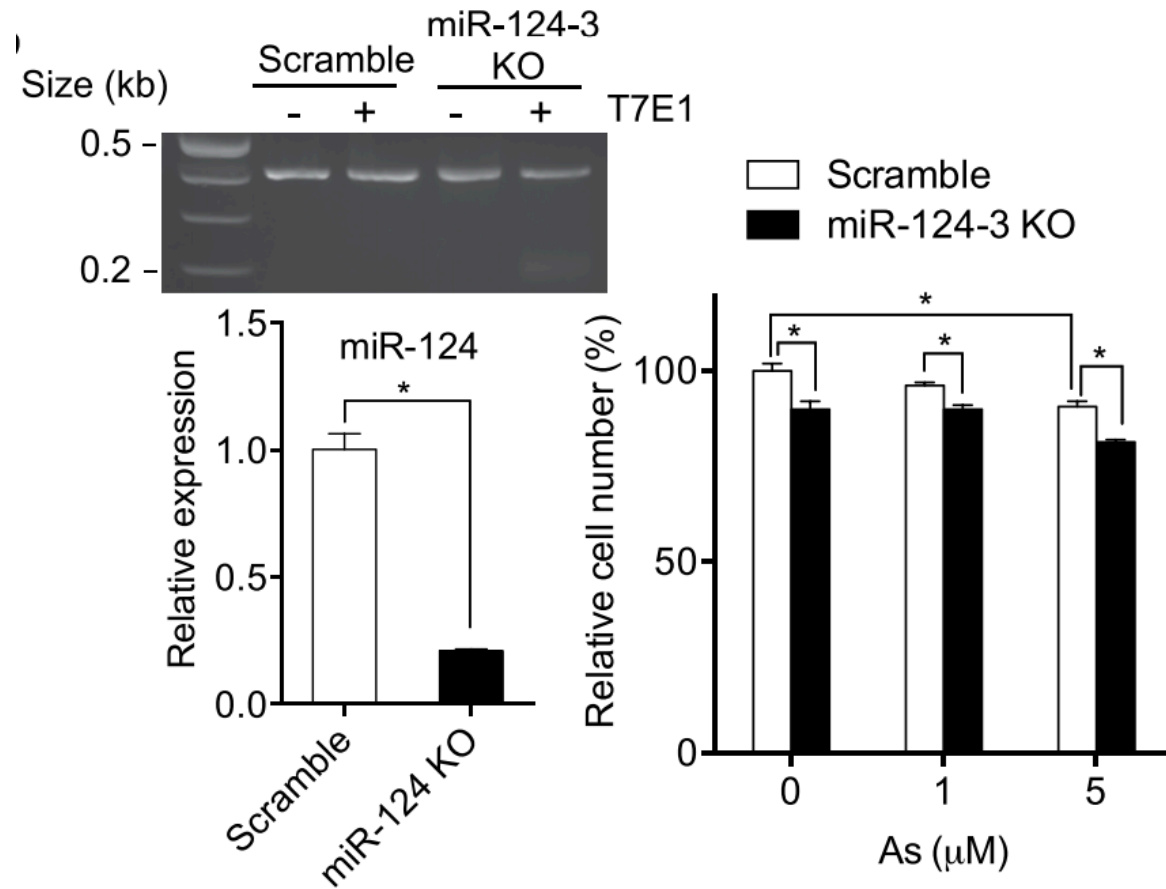


MiR-124 plays important roles in brain



- Highly and specifically expressed in all brain regions
- Play a crucial role in neurogenesis *in vitro* and *in vivo*.
- Dysregulation of miR-124 has been associated with pathological CNS conditions and Alzheimer's disease

MiR-124 protects against As-induced neurotoxicity



(Left) SH-SY5Y neuroblastoma cells (Right) Human neural stem cells

Study population-Bangladesh cohort

Characteristics		Mean (SD) or n (%)
		n=502
Sex, No. (%)	Male	256 (51)
	Female	246 (49)
Concentration of arsenic in umbilical cord blood ($\mu\text{g/dl}$) ^a		0.67 (0.61)
Gestational Age, weeks		38.2 (1.7)
Age at Exam, weeks		99.4 (18.5)
Mother's Education > Primary, n (%)		269 (54)
Smoking in Household Environment, n (%)		212 (42)
BSID Scores	Mental Composite	112.7 (10.5)
	Motor Composite	92.7 (5.0)

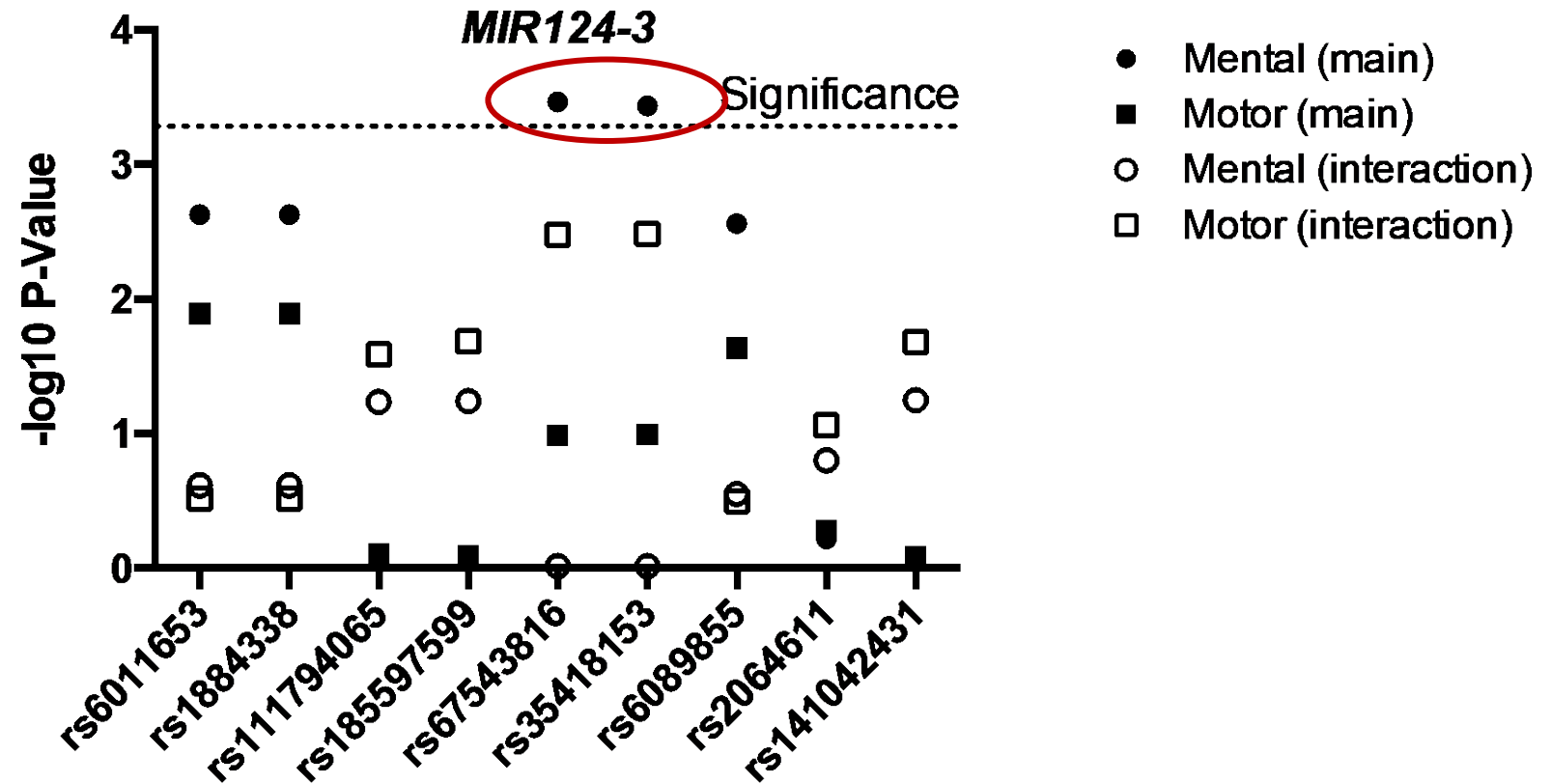
SD standard deviation

^aThere were 23 subjects in the Bangladesh cohort who did not have any recorded values for arsenic concentration.



AFP / Getty Images

MiR-124 genetic polymorphism is associated with neurocognitive score in children



Analysis by Dr. Ryan Sun (Christiani Lab)

Park *et al*, Scientific Reports (2020)

Summary

- A genome-wide CRISPR screen identifies suppressors of ER stress response.
- MiR-124 protects against As toxicity in human neural cells.
- Genetic polymorphisms of miRNA-124 are associated with neurodevelopmental outcomes in children.

Continuing study on miR-124 and As toxicity

- **K99/R00 a pathway to independence award from NIH/NIEHS**
- **Title:** microRNA, ER stress, and arsenic neurotoxicity
- **Specific Aims**
 - **Aim 1:** Investigate the role of As-induced ER stress on neural stem cell function
 - **Aim 2:** Elucidate the mechanisms through which miR-124 protects against As toxicity in neural stem cells
 - **Aim 3:** Investigate the functional association of miR-124 variants with neurodevelopment in children exposed to As

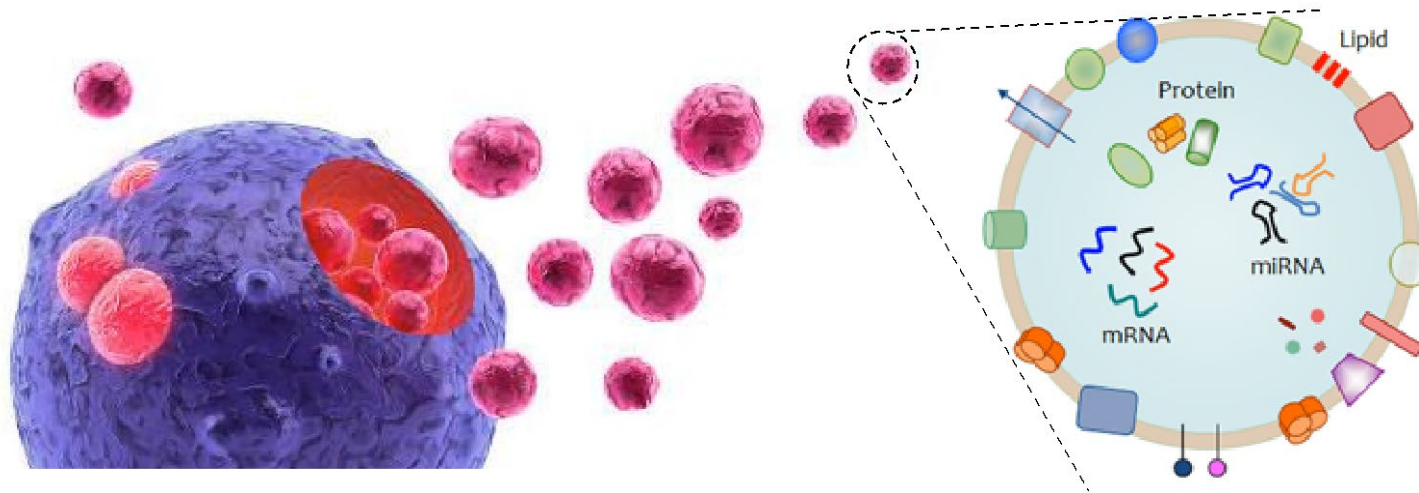
Placenta plays a critical role in fetal brain development



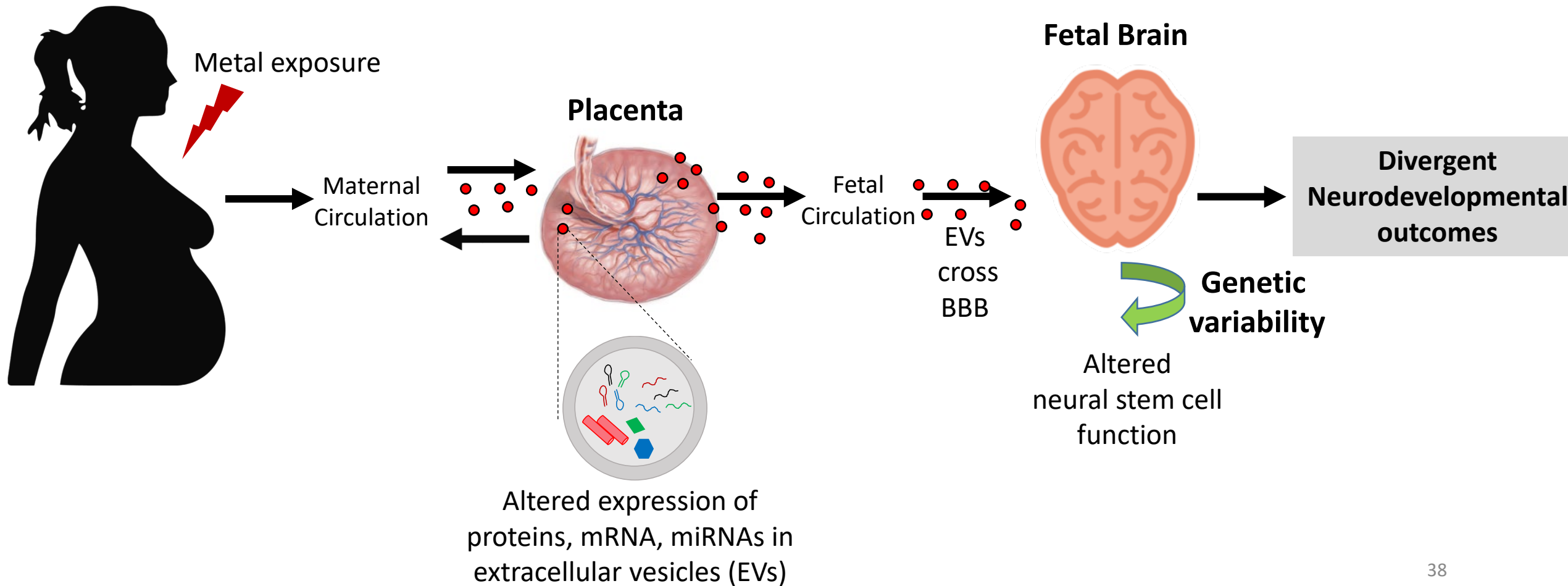
- Human placenta
 - Placental disk
 - Extraplacental membrane
 - Umbilical cord
- Preventing rejection by the maternal immune system
- Transport of nutrients and wastes between the mother and the embryo/fetus
- Placental cues can reach the fetal brain through the immature blood-brain barrier (BBB).

Extracellular vesicles are important mediators of communication

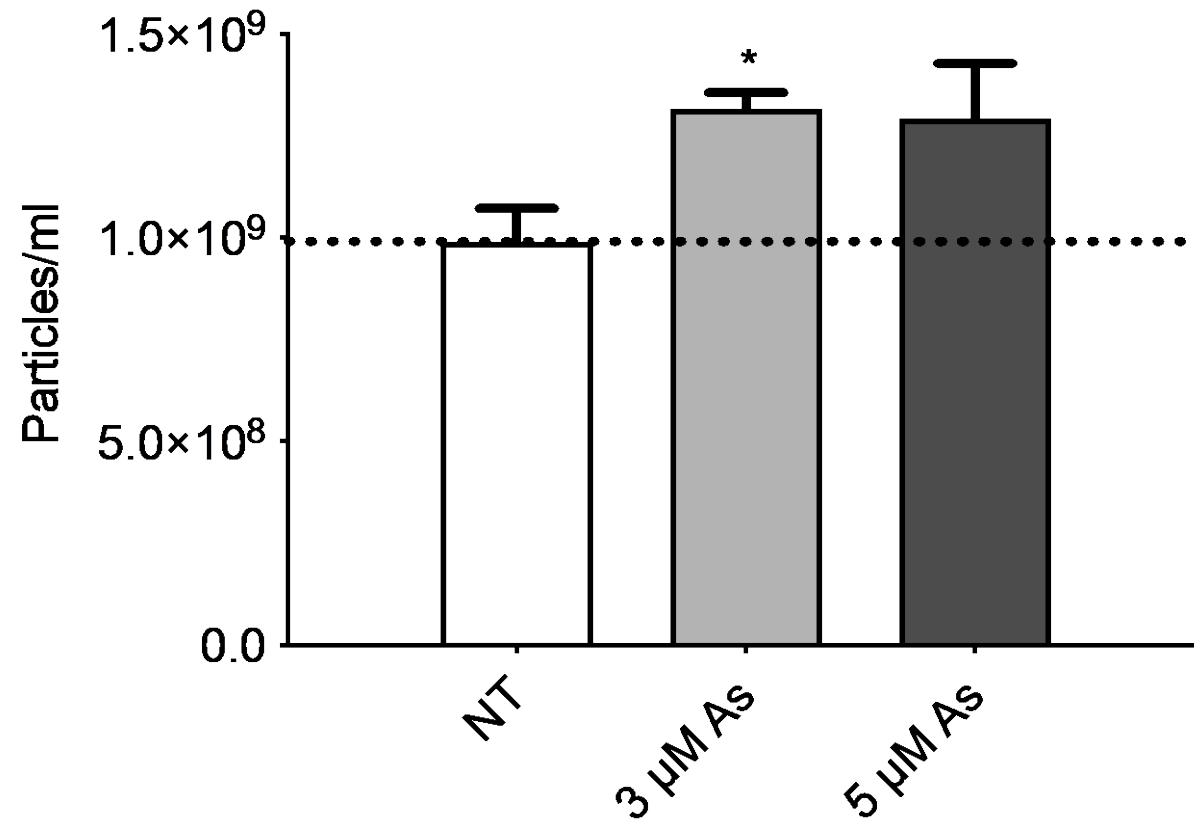
- Placental trophoblasts actively release EVs.
- Extracellular vesicles (EVs)
 - Nano-sized (.05-1 μm) membrane-bound vesicles.
 - Transfer proteins, lipids, and nucleic acids between cells
 - Involved in antigen presentation, tumor progression, immunosuppression
- EVs reach fetal neural cells after crossing the BBB.



Hypothesis

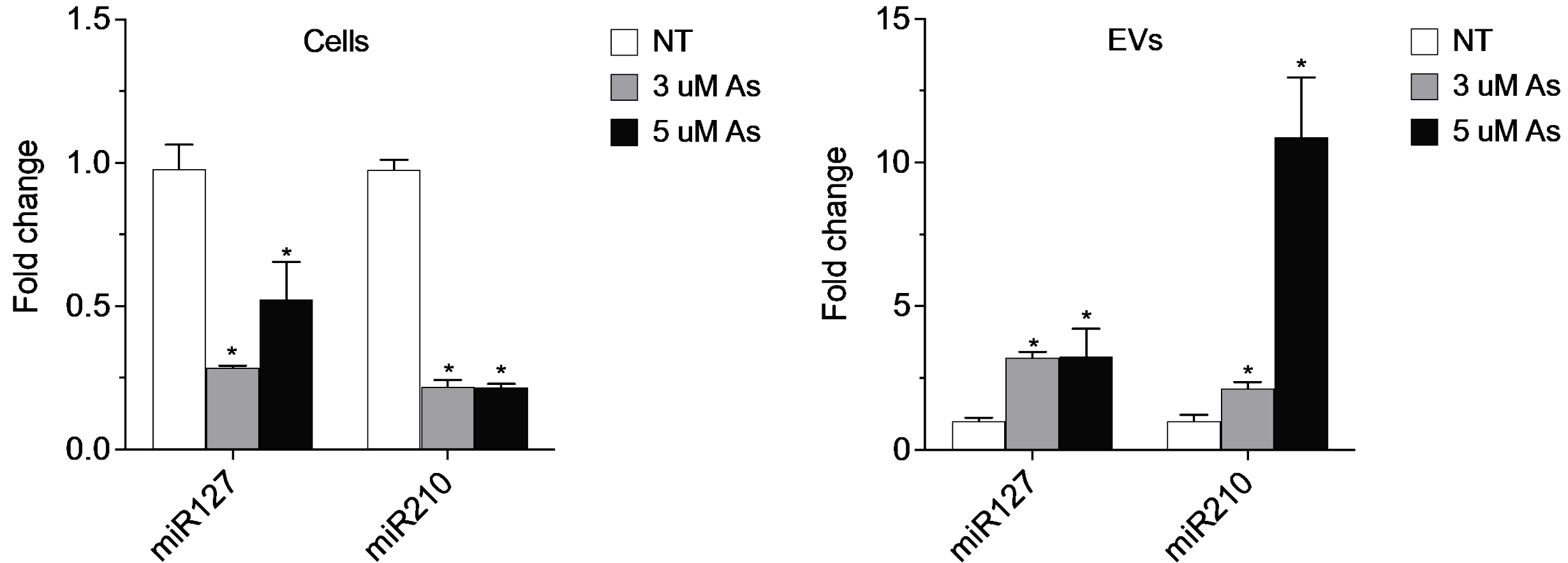


As treatment increases EV release from human placental cells



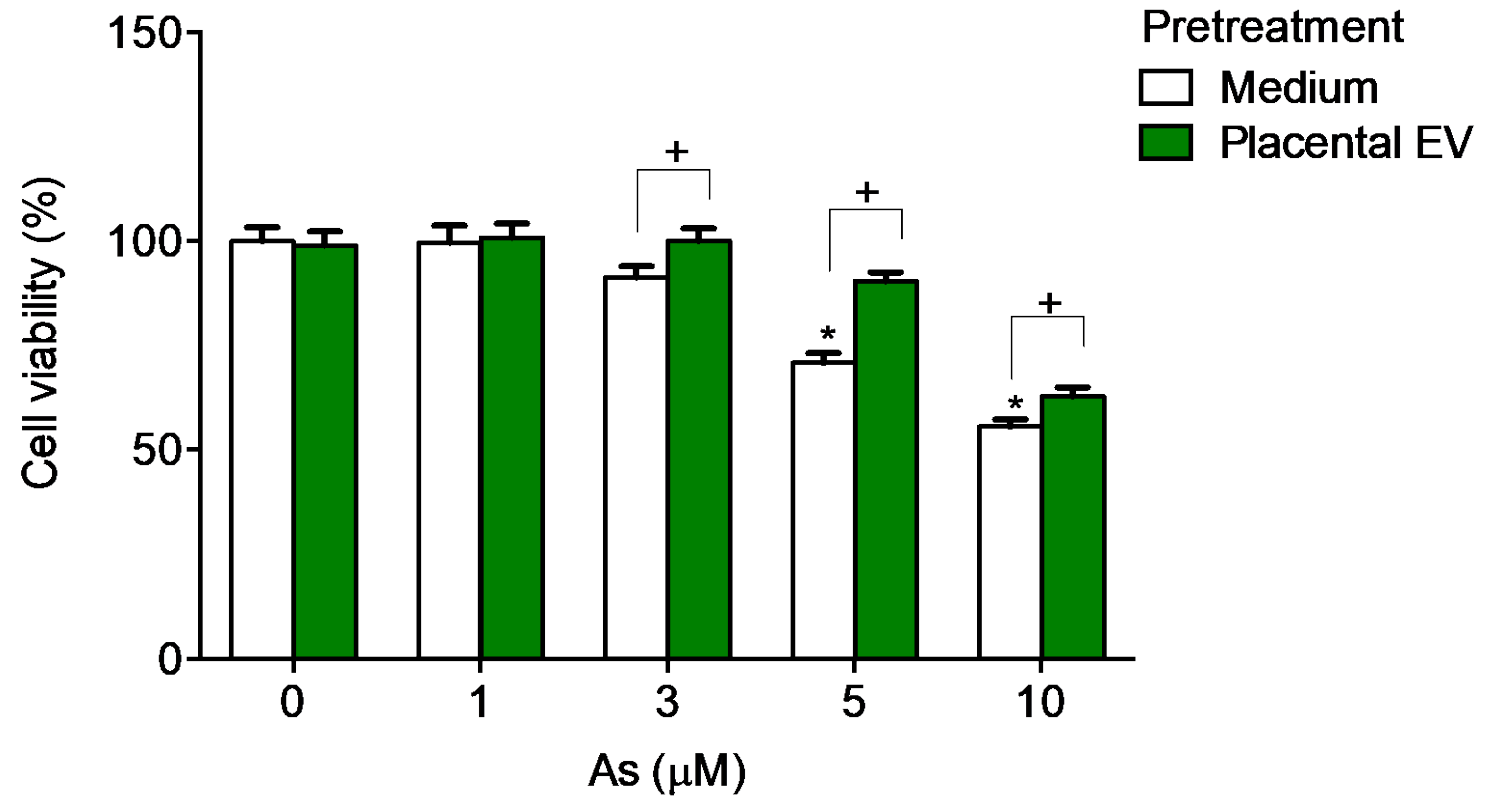
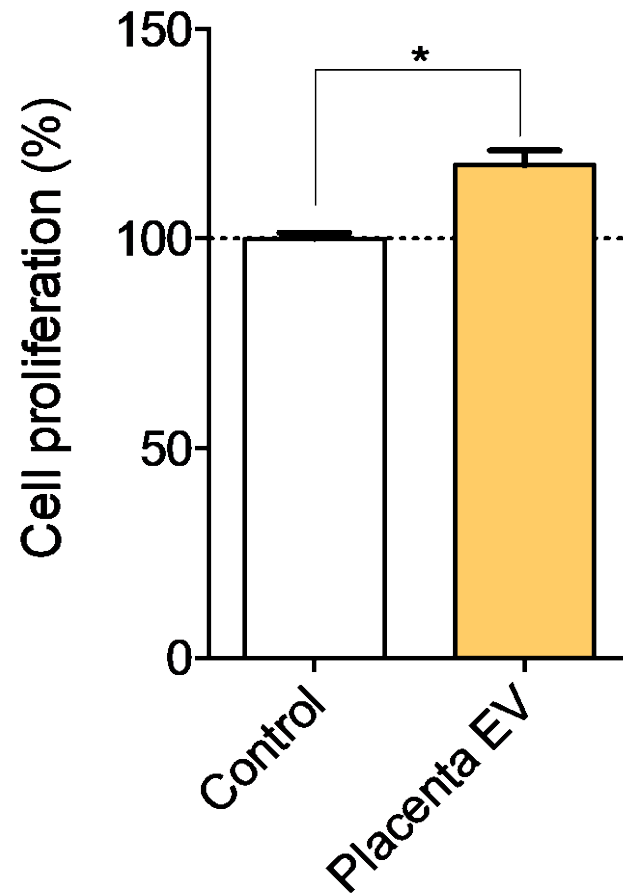
HTR-8/SVneo cells were treated with As for 24 h. The EV concentration in the medium was measured by Nanosight. N=3. *, P<0.05 compared to NT.

As treatment differentially regulates expression of placenta-specific miRNAs in human placental cells and EVs



HTR-8/SVneo cells were treated with As for 24 h. N=3. *, P<0.05 compared to NT.

Effect of placental EVs on neural cell proliferation

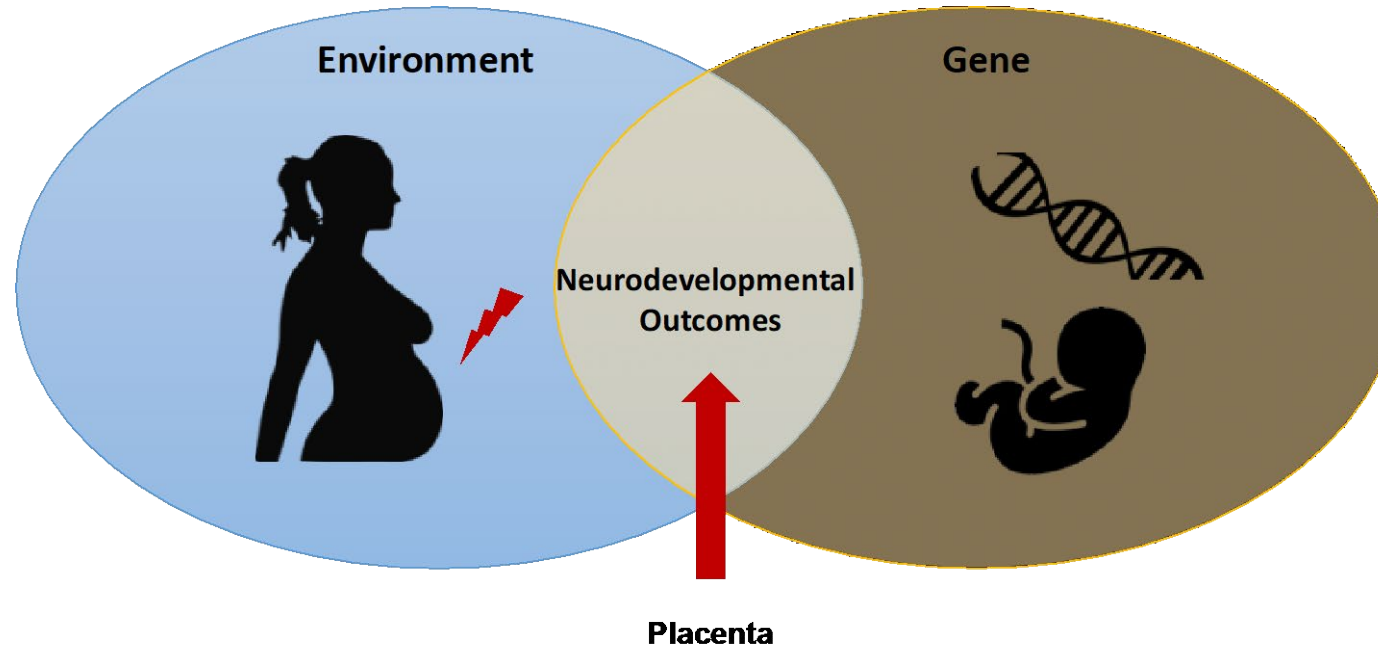


-ReNcell Cx neuroprogenitor cells
-EVs were collected from HTR-8 cell culture medium.

Proteins highly expressed in brain are detected in placental EVs

GENE	GENE DESCRIPTION	DISEASE INVOLVEMENT
TBR1	T-box, brain 1	Autism spectrum disorder, Disease mutation, Mental retardation
KIF5A	Kinesin family member 5A	Amyotrophic lateral sclerosis, Disease mutation, Epilepsy, Hereditary spastic paraplegia, Neurodegeneration
DNM1	Dynamin 1	Disease mutation, Epilepsy
DIRAS2	DIRAS family GTPase 2	
NAPB	NSF attachment protein beta	
YWHAH	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein eta	Cancer-related genes
LLGL1	LLGL1, scribble cell polarity complex component	
CNP	2',3'-cyclic nucleotide 3' phosphodiesterase	
ENO2	Enolase 2	Cancer-related genes
GDI1	GDP dissociation inhibitor 1	Disease mutation, Mental retardation
KLC1	Kinesin light chain 1	
TUBB2B	Tubulin beta 2B class IIb	Disease mutation, Mental retardation
TUBB3	Tubulin beta 3 class III	Disease mutation, FDA approved drug targets

Future work



- As effects on transcriptome of mouse placenta using scRNA-seq
- Effect of As/metal exposure on placental EVs cargoes (proteins, miRNA..) *in vivo* and *in vitro*
- Effect of placental EVs on NSC function

Acknowledgements

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