RNA damage induced DHX9 granules

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Stress
heat shock, arsenite, infection
starvation, UVC…

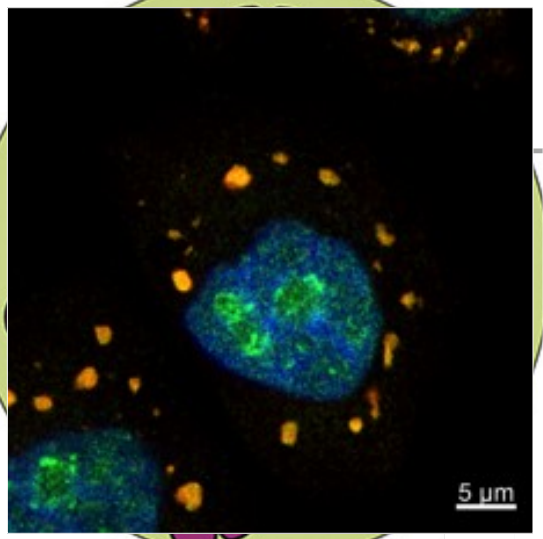
DNA

mRNA AAAAA

Translation AAAAA

AA

Membraneless liquid granules



LLPS

RNA

RBP…
ATPase
Helicase
Ub ligase
etc…

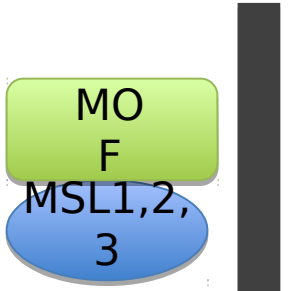
P body

autophagy **antiviral** **survival**

DHX9: a nuclear RNA helicase binding dsRNA

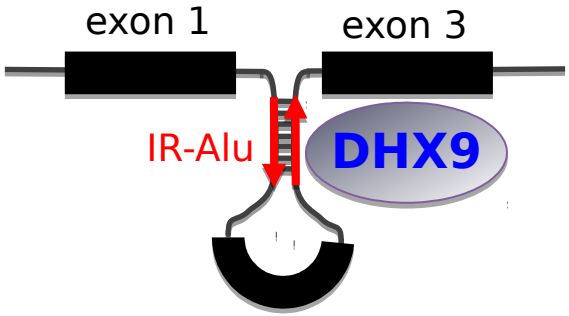
Drosophila

roX1/2 RNA

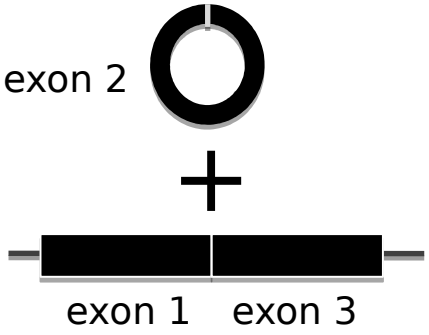


X chr Dosage Compensation

Mammalian



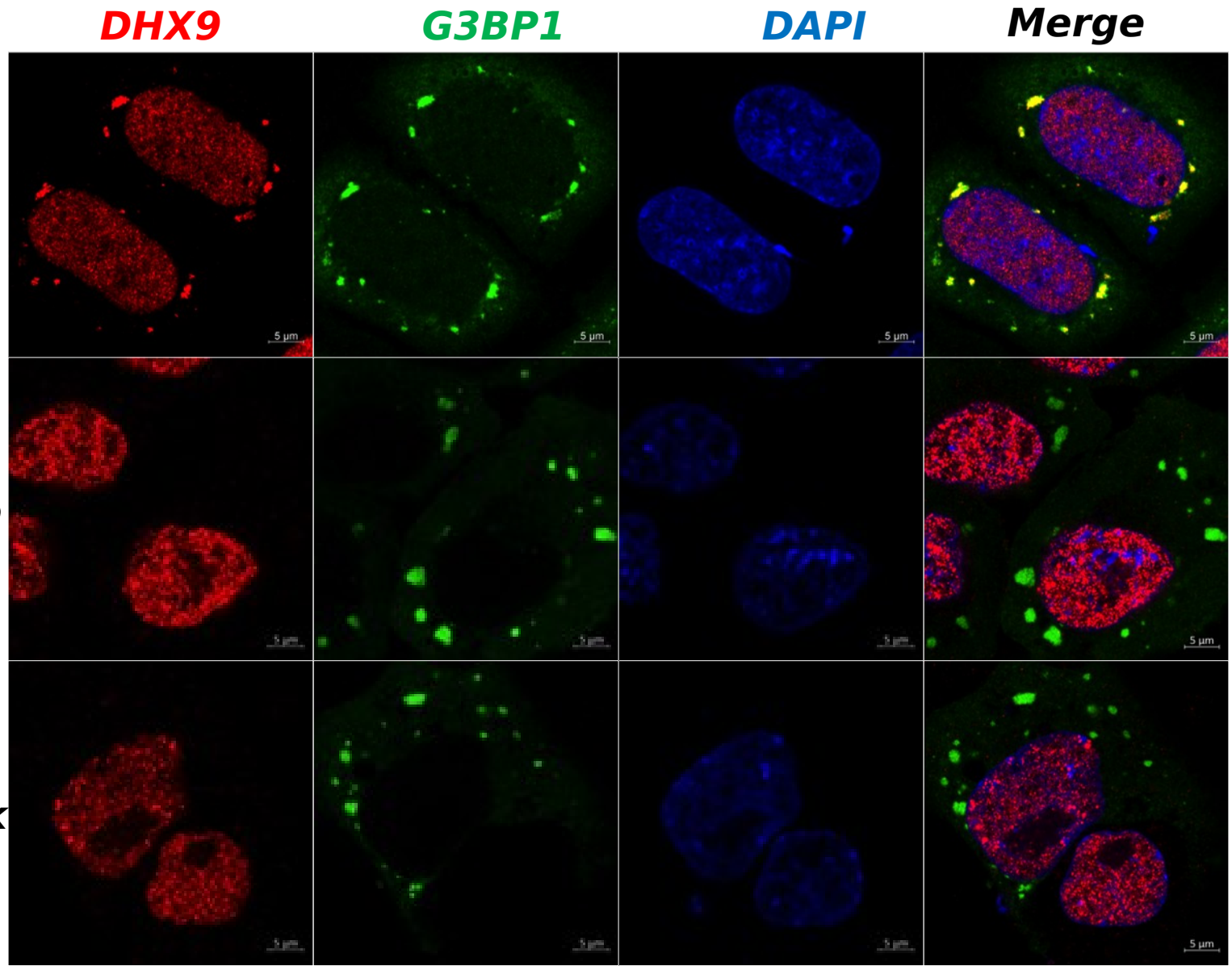
Circular RNA



self dsRNA

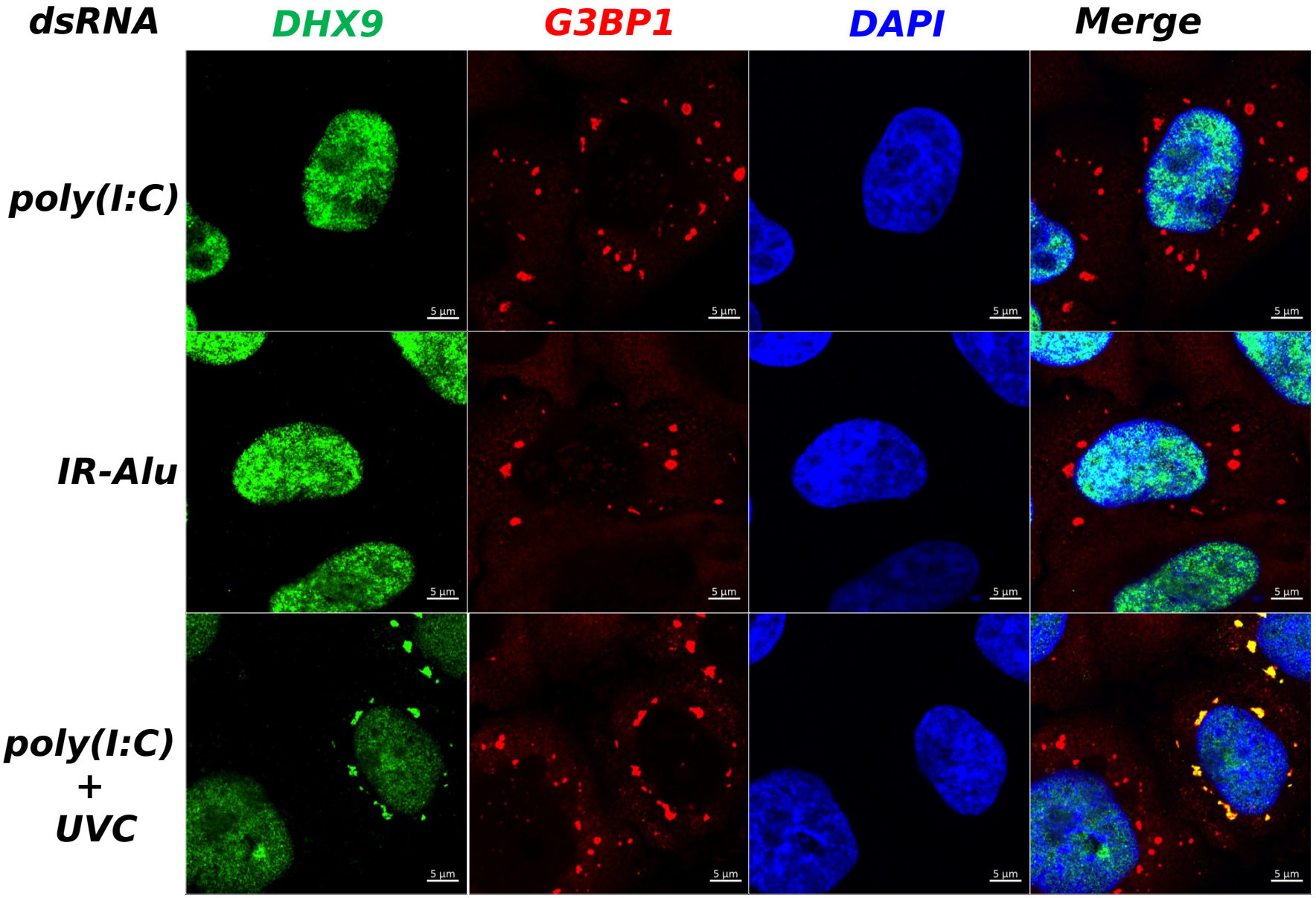
Hallacli et al. Mol cell. 2012
Ilik et al. Mol cell. 2013
Valsecchi et al.Nature.2020
Aktas et al.Nature.2017

UVC induces cytoplasmic DHX9 granules



Hela

dsRNA did not induce cytoplasmic DHX9 granules

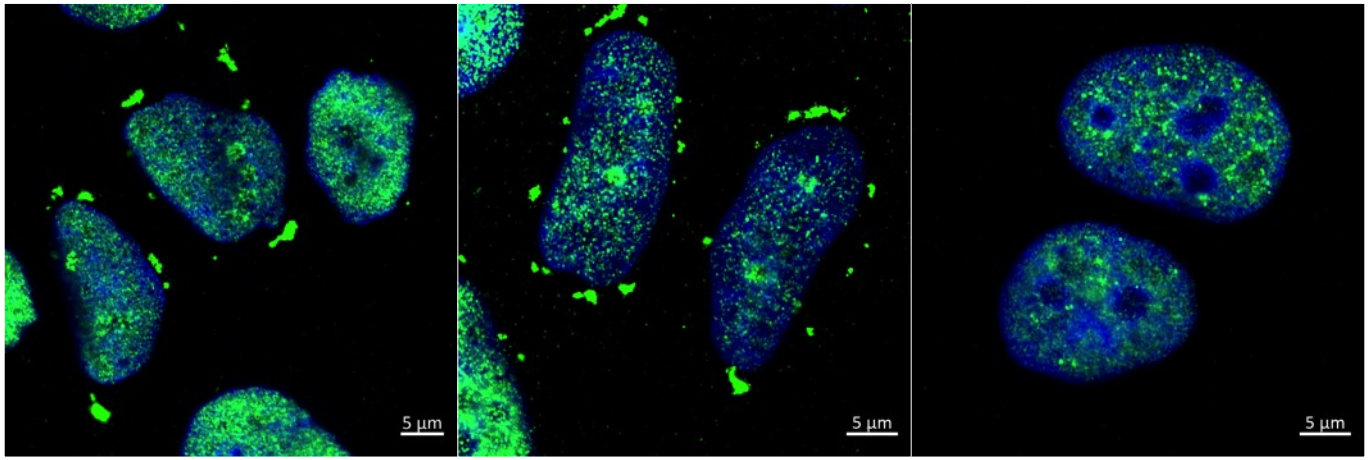


UVC and UVB induce DHX9 granules

VC(254nm) and UVB(312nm): high energy, directly damage DNA

VA(365nm): low energy, weakly damage DNA indirectly by ROS in high dose

DHX9, DAPI



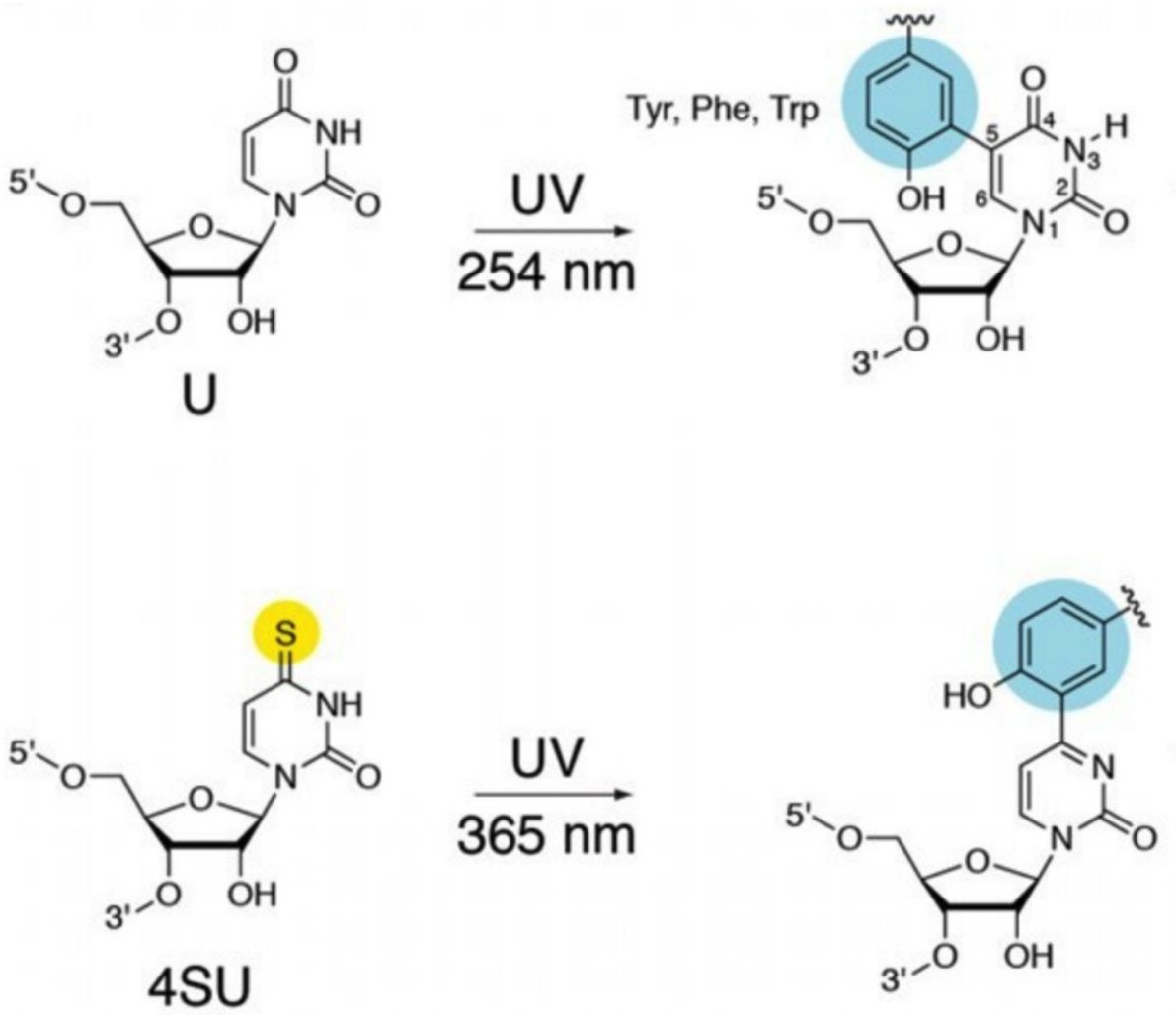
UVC UVB UVA

Hela

UVC and UVB induce DHX9 granules

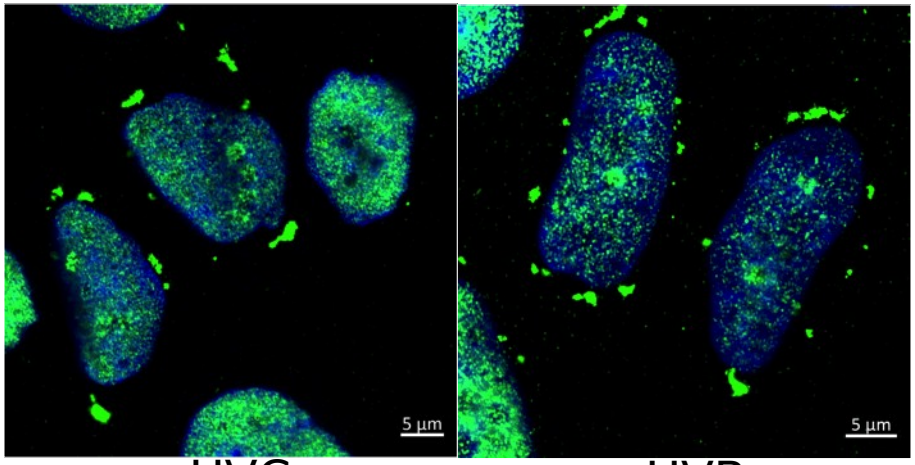
Stress	Cytosolic DHX9 granules	
1. UVC, UVB	+	
2. UVA	-	
3. Oxidative stress (Arsenite)	-	
4. Heat shock stress (42°C)	-	
5. Hypersomic stress (NaCl)	-	
6. dsRNA, starvation….	-	DNA damage?
7. Etoposide/Doxorubicin/Gamma ray/ Hydroxyurea/DMBA/Camptothecin/ H2O2/ MMS/4NQO…	-	RNA damage?
8. Etoposide + poly(I:C)/arsenite	-	

4sU+UVA to mimic UVC- induced RNA damage but not DNA damage



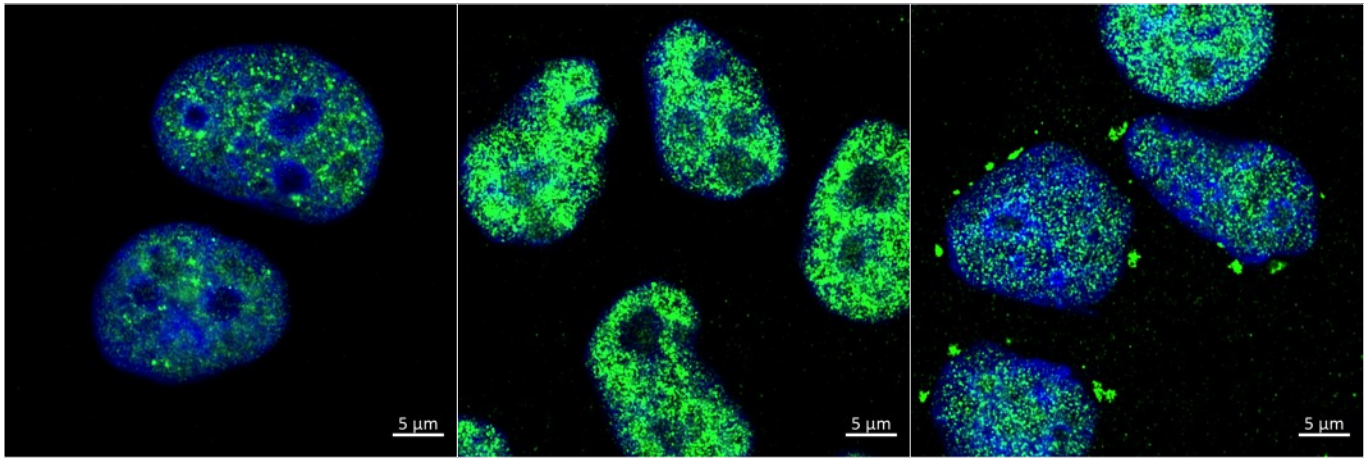
4-thiouridine (4SU): analog of Uridine, activated specifically by **UVA** and crosslink proximal proteins

RNA cross-linking induces DHX9 granules



UVC UVB

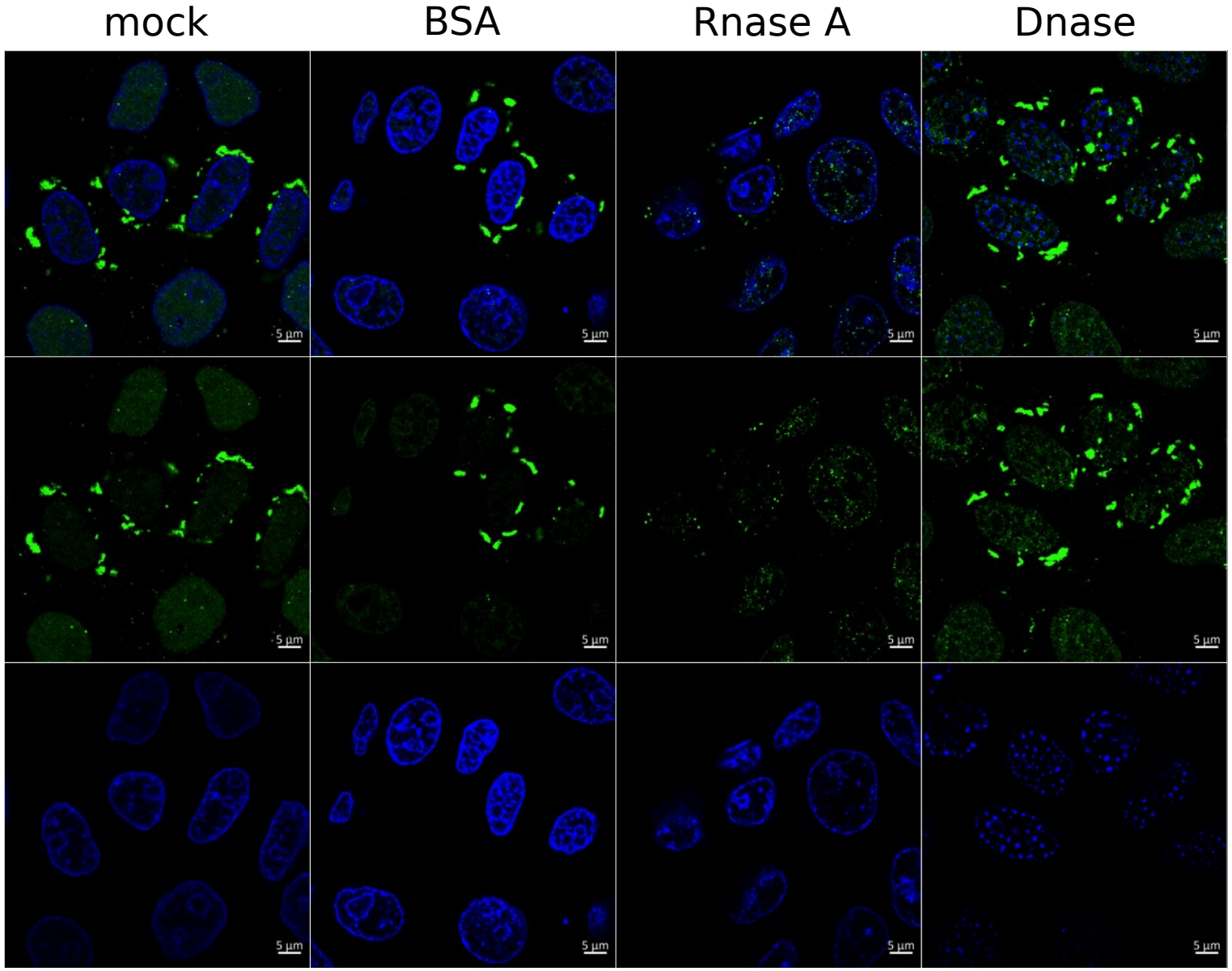
DHX9, DAPI



UVA 4-s-U 4-s-U + UVA(0.05J/cm²)

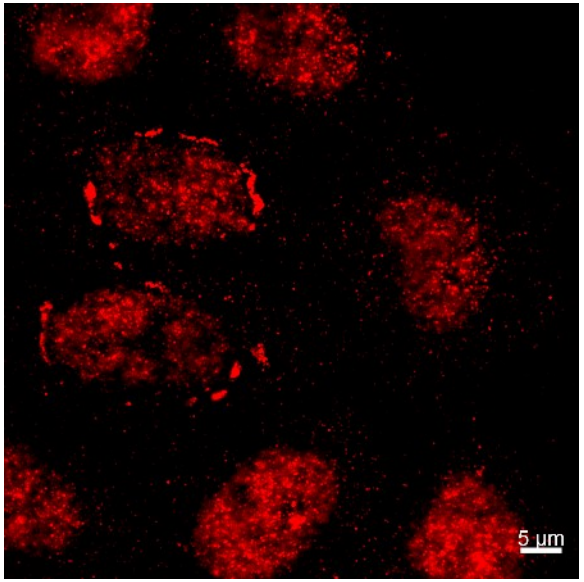
aily UVA dose in Berlin in April: 70J/cm² ----------Christiaens et al.2

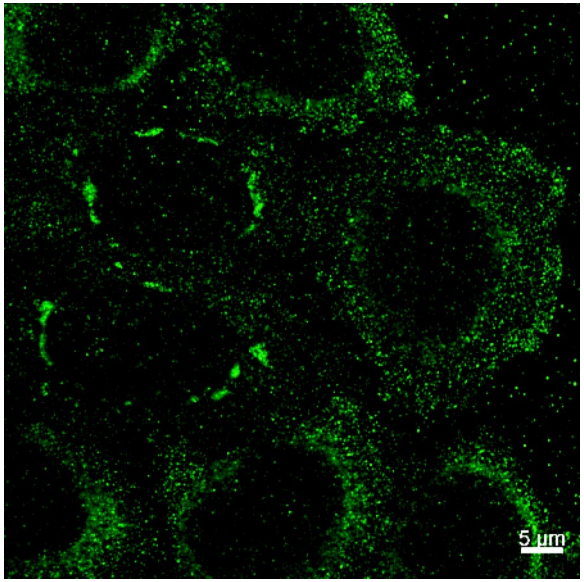
RNA is essential for the DHX9 granule



DHX9-endo-GFP-Hela

DHX9 granules contains dsRNA

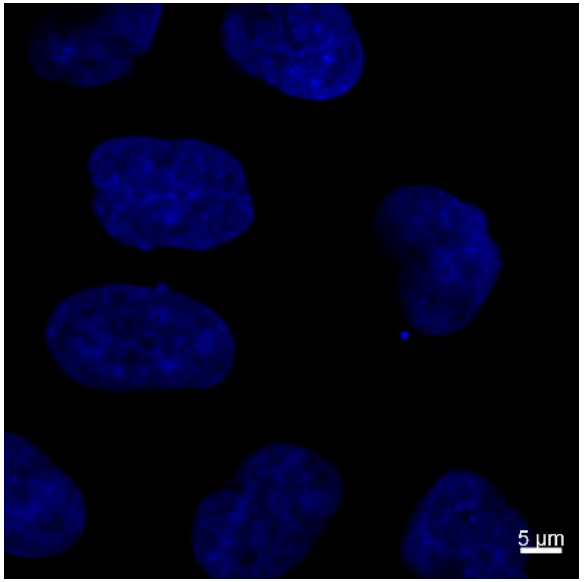


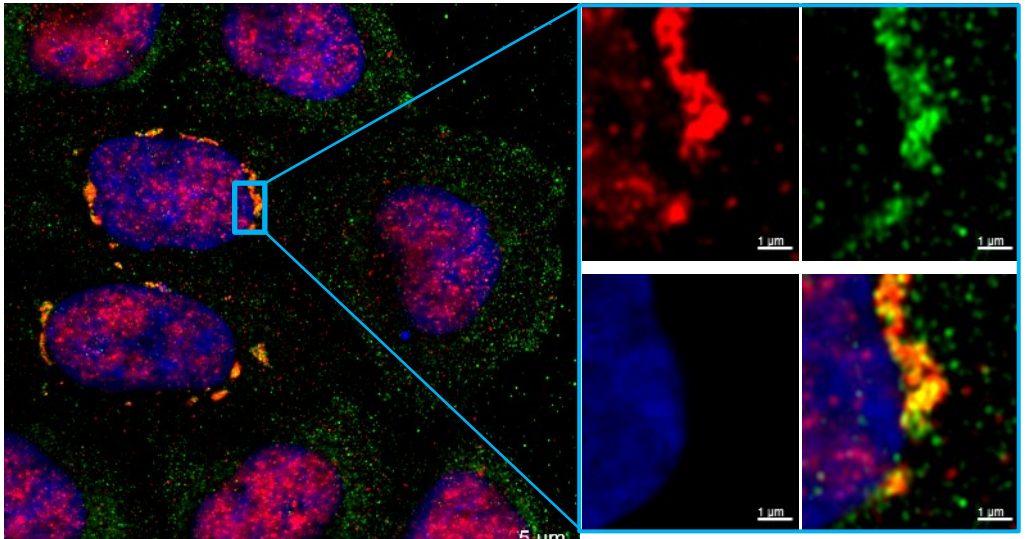


DHX9

J2: dsRNA antibody

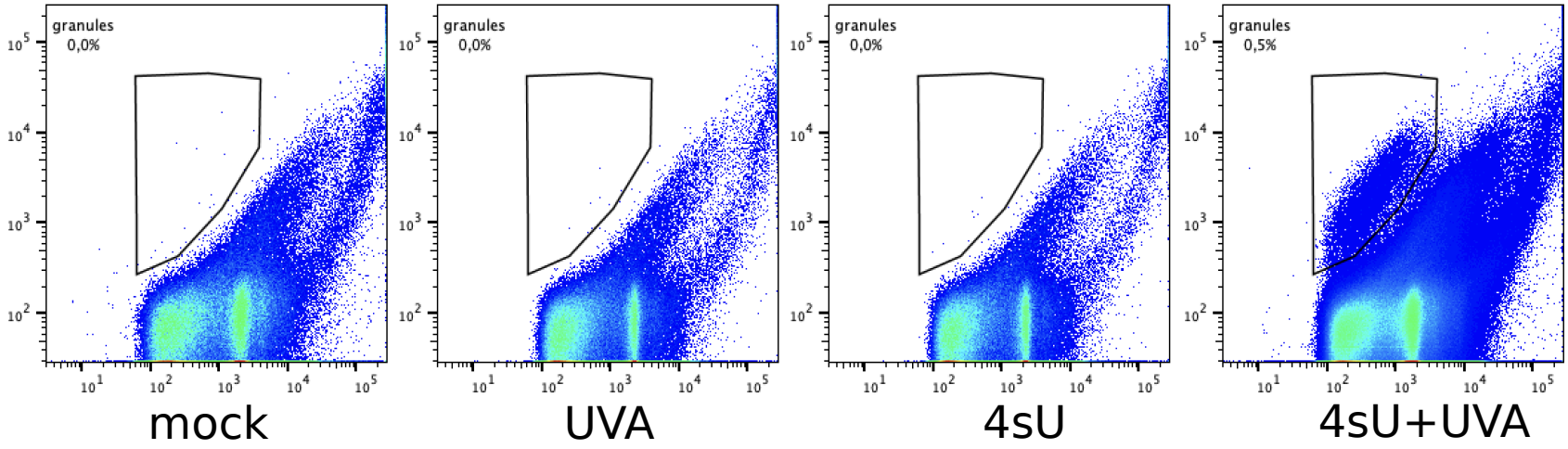
DAPI





FANCI: Fluorescence Activated Non-membrane Condensates Isolation

a novel methods to isolate stress granules

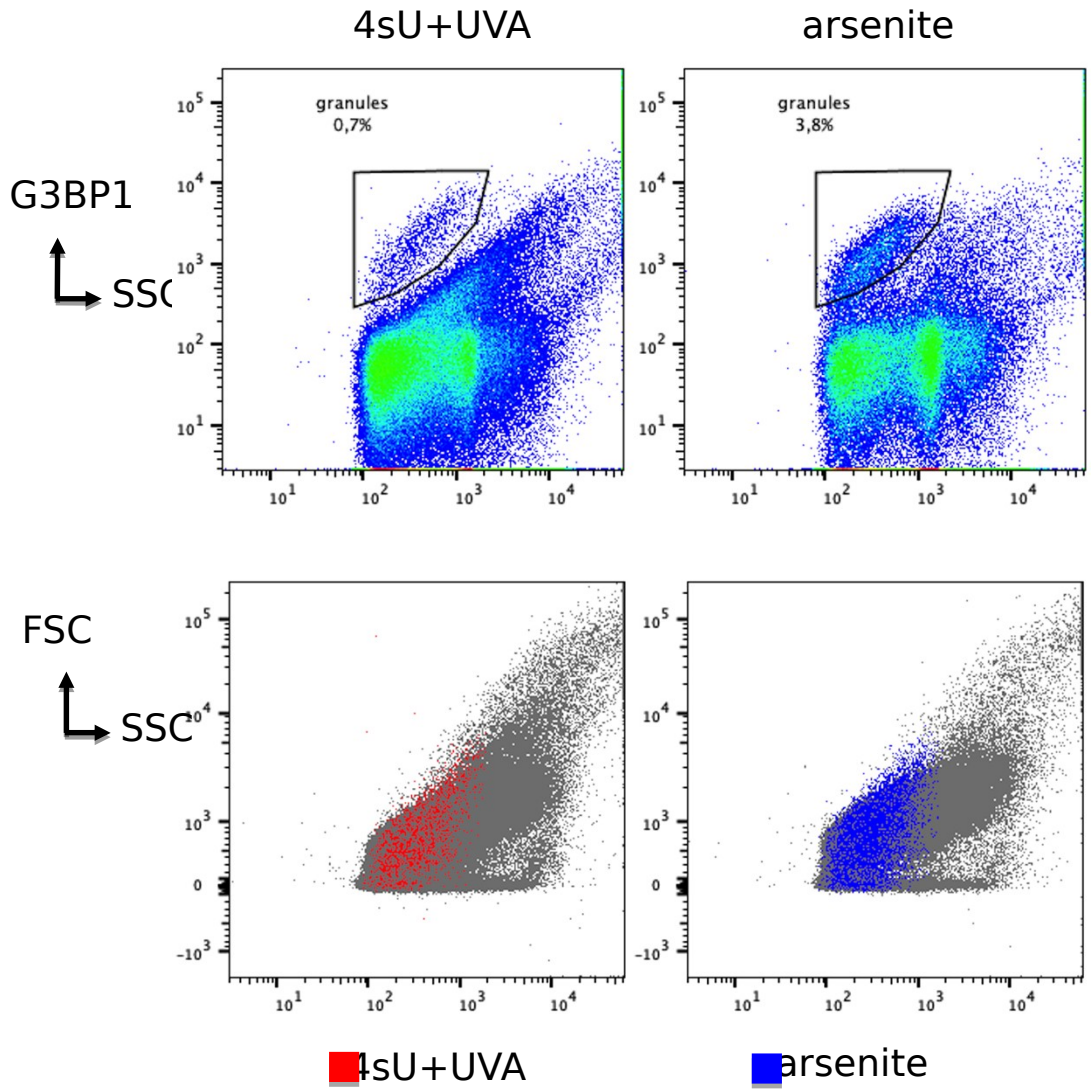


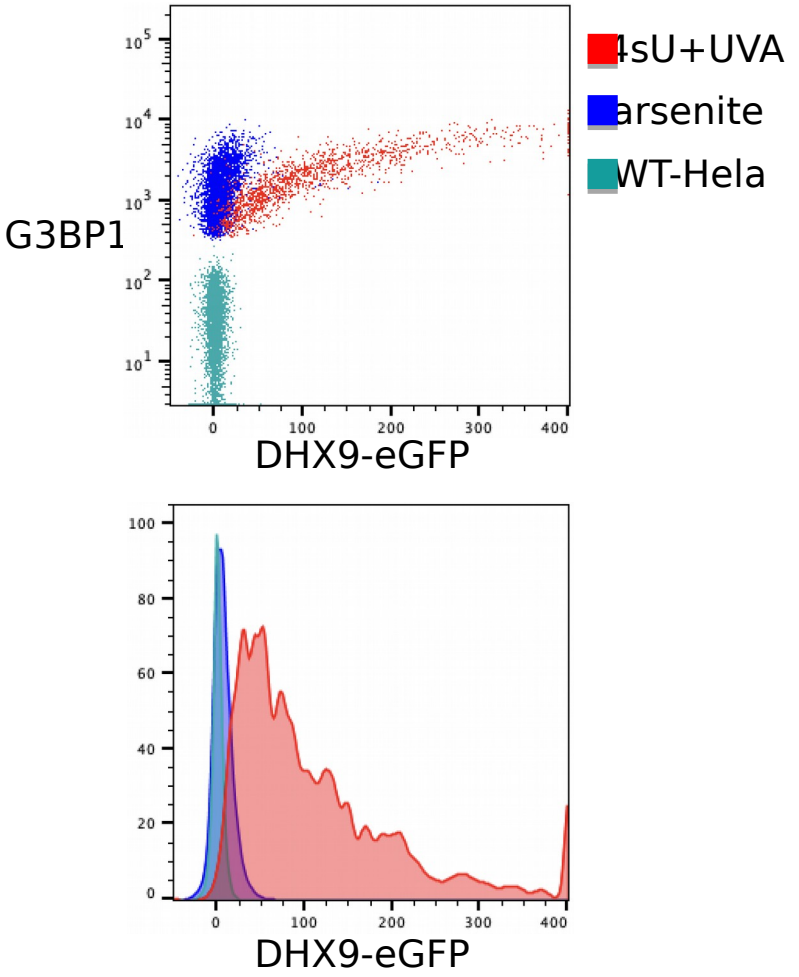
G3BP1-mCherry

SSC

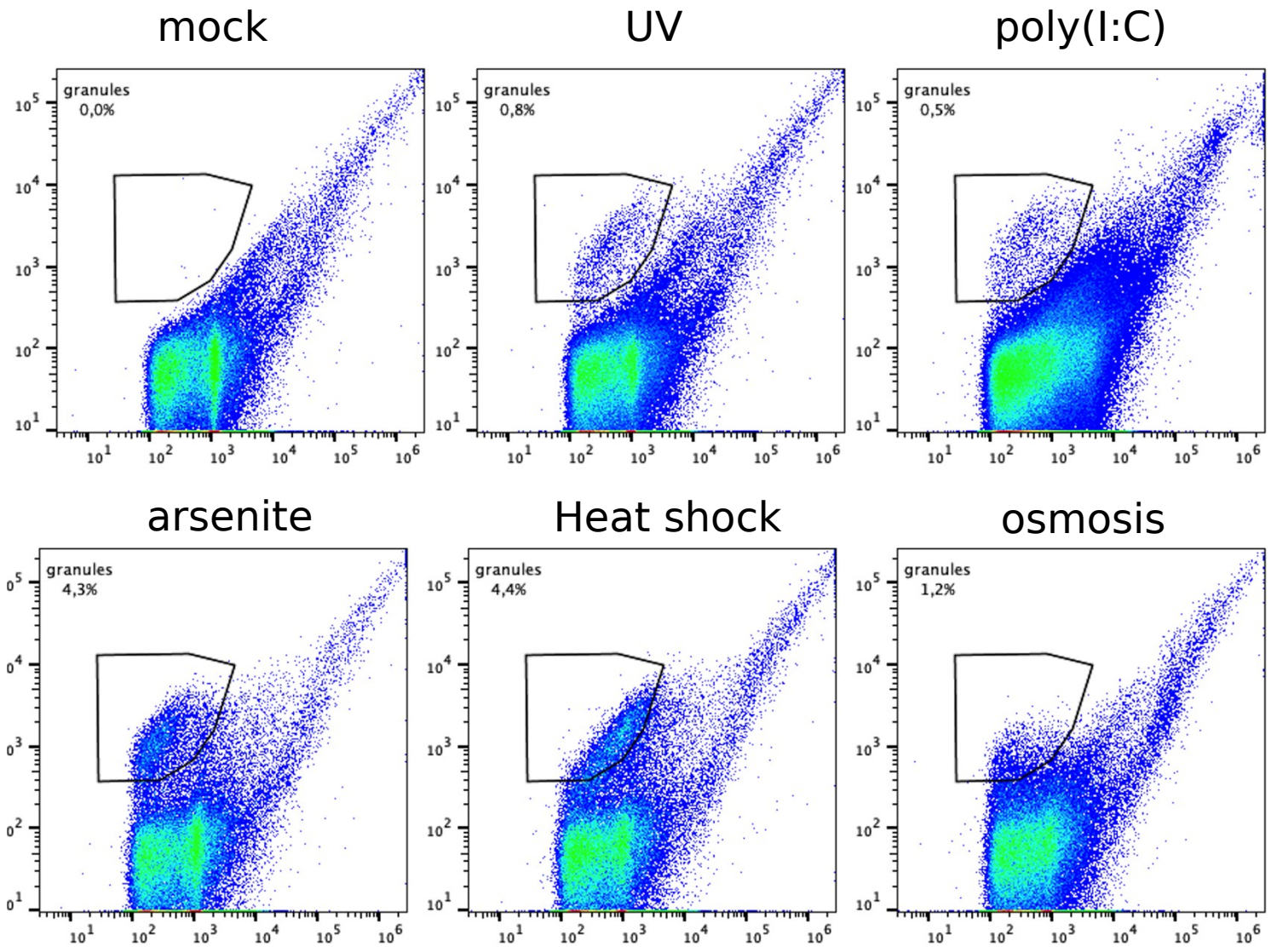
FANCI of UV stressed cell

DHX9 is only expressed in UV- but not as- granules

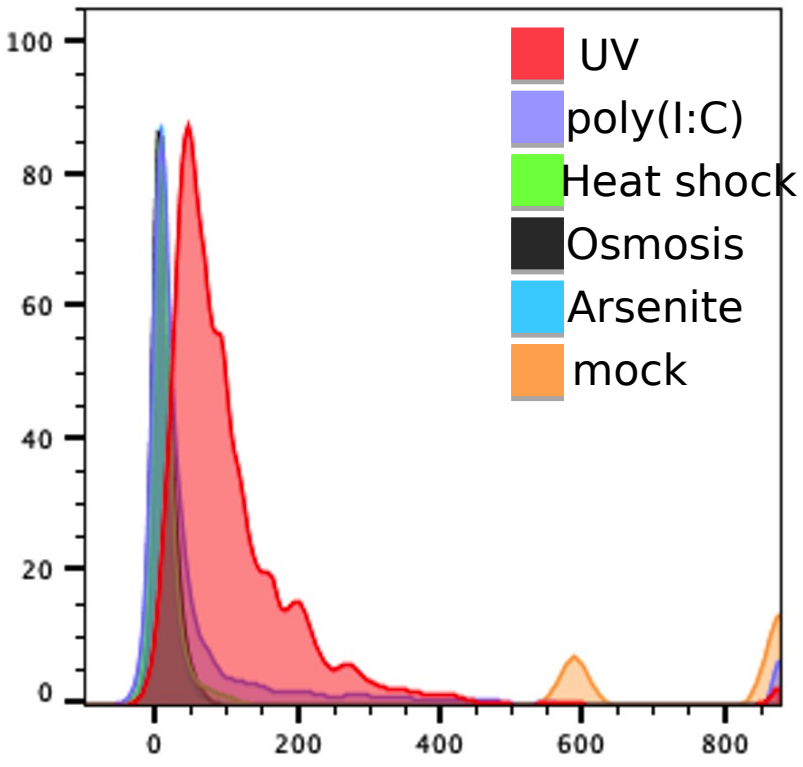




FANCI other stress granules



FANCI can be applied to all other stress induced granules



DHX9-eGFP

Stress	DHX9
1. Oxidative stress (Arsenite)	-
2. Heat shock stress (42°C)	-
3. Hypersomic stress (NaCl)	-
4. Virus dsRNA (poly(I:C))	-
5. mTOR (starvation)	-
6. Rocaglamide	?
7. eIF4A inhibition(Pateamine)	-
8. ER stress (Thaps)	-
9. Protease inhibition(MG132)	-
10. Etoposide/Doxorubicin/Gamma ray	-
11. Etoposide + arsenite	-
12. Etoposide + poly(I:C)	-
13. UV	+

IA-seq sample annotation_project 2201

sample name	note
1	total RNA of mock-1
2	total RNA of mock-2
3	total RNA of mock-3
4	total RNA of arsenite-1
5	total RNA of arsenite-2
6	total RNA of arsenite-3
7	total RNA of UV-1
8	total RNA of UV-2
9	total RNA of UV-3
10	SG RNA of arsenite-1
11	SG RNA of arsenite-2
12	SG RNA of arsenite-3
13	SG RNA of UV-1
14	SG RNA of UV-2
15	SG RNA of UV-3

400000 stress granule particles are collected, generated 10ng RNA and sequenced

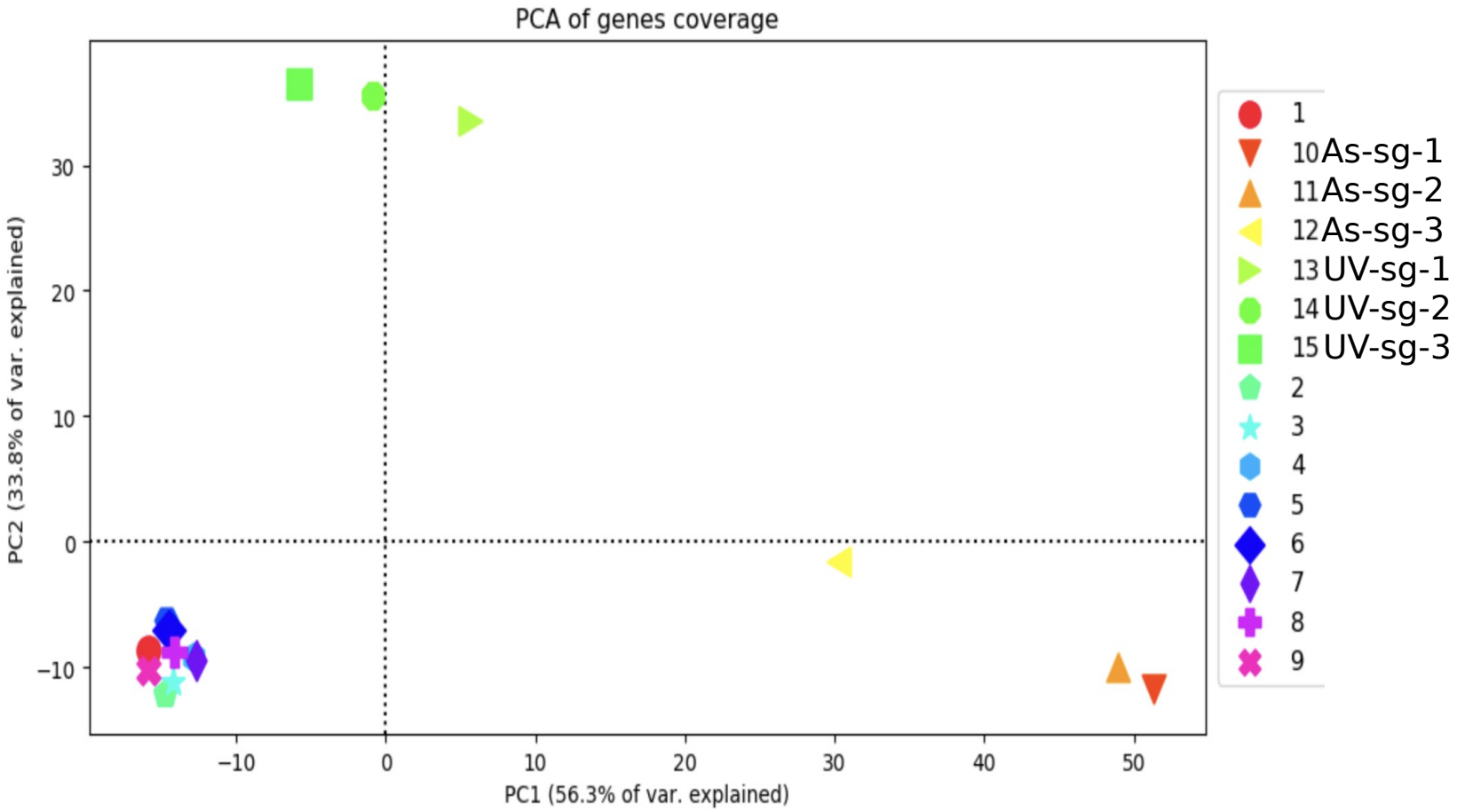
One 10 cm dish of cell is enough for UV SG

One well of Hela from a 6 well plate is enough for As

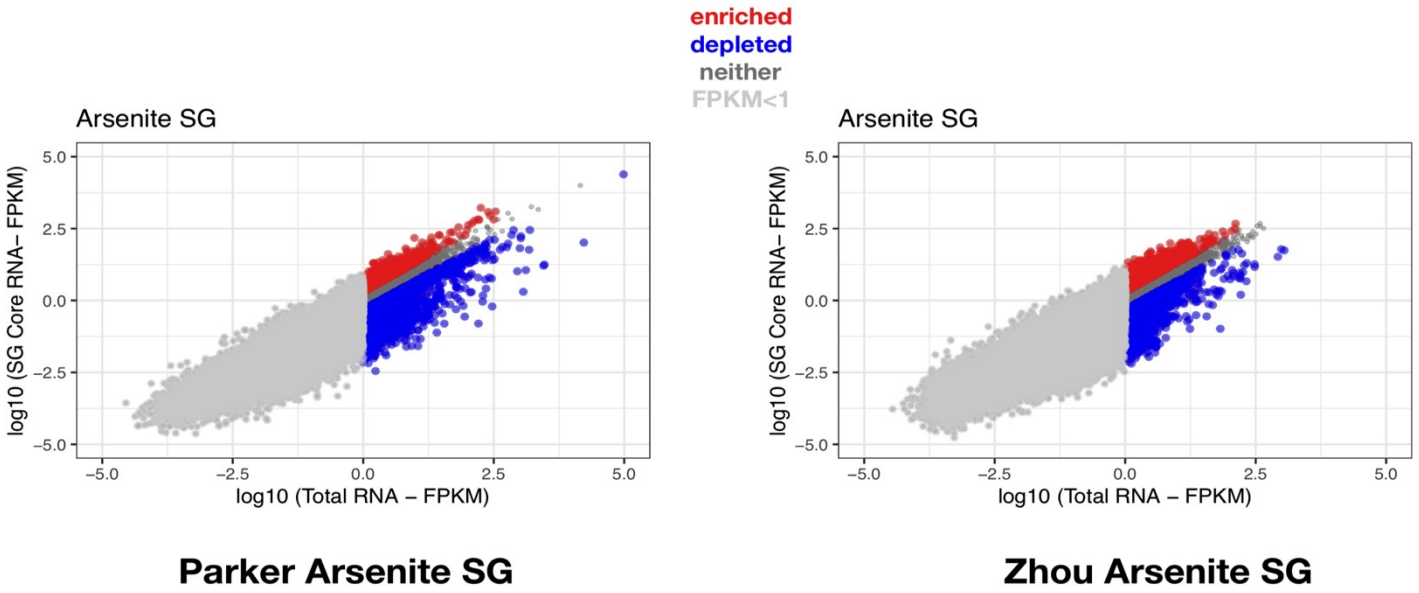
stored at −80 °C. Of note, for mammalian cells, we routinely combine and snap freeze 5 × 15 cm plates for a single pellet.

Wheeler et al.Methods.2018

PCA analysis



Comparison with Parker's arsenite SG RNA profile

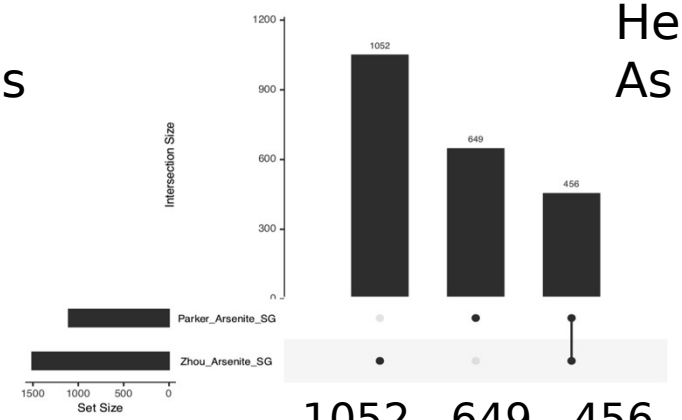


Parker Arsenite SG

Zhou Arsenite SG

U2OS
As 60mins

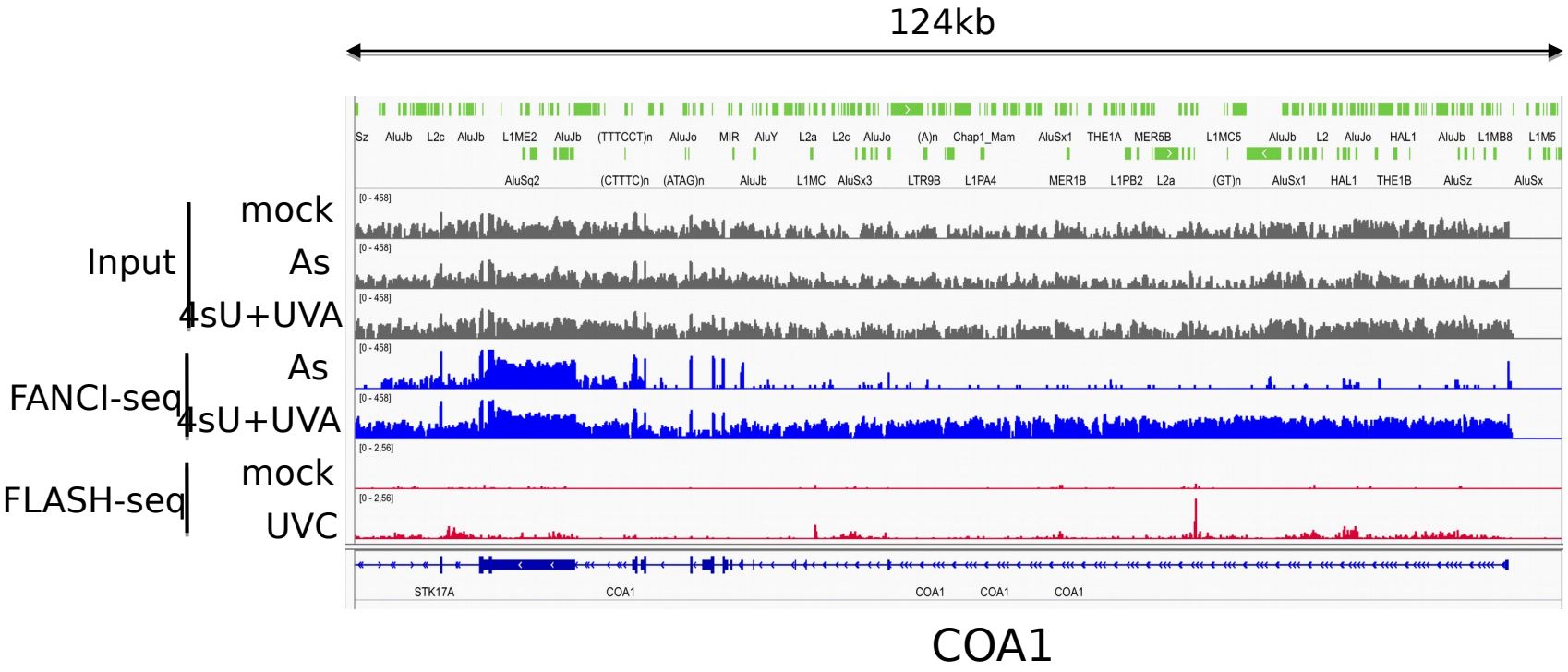
Hela
As 30mins



1052 649 456

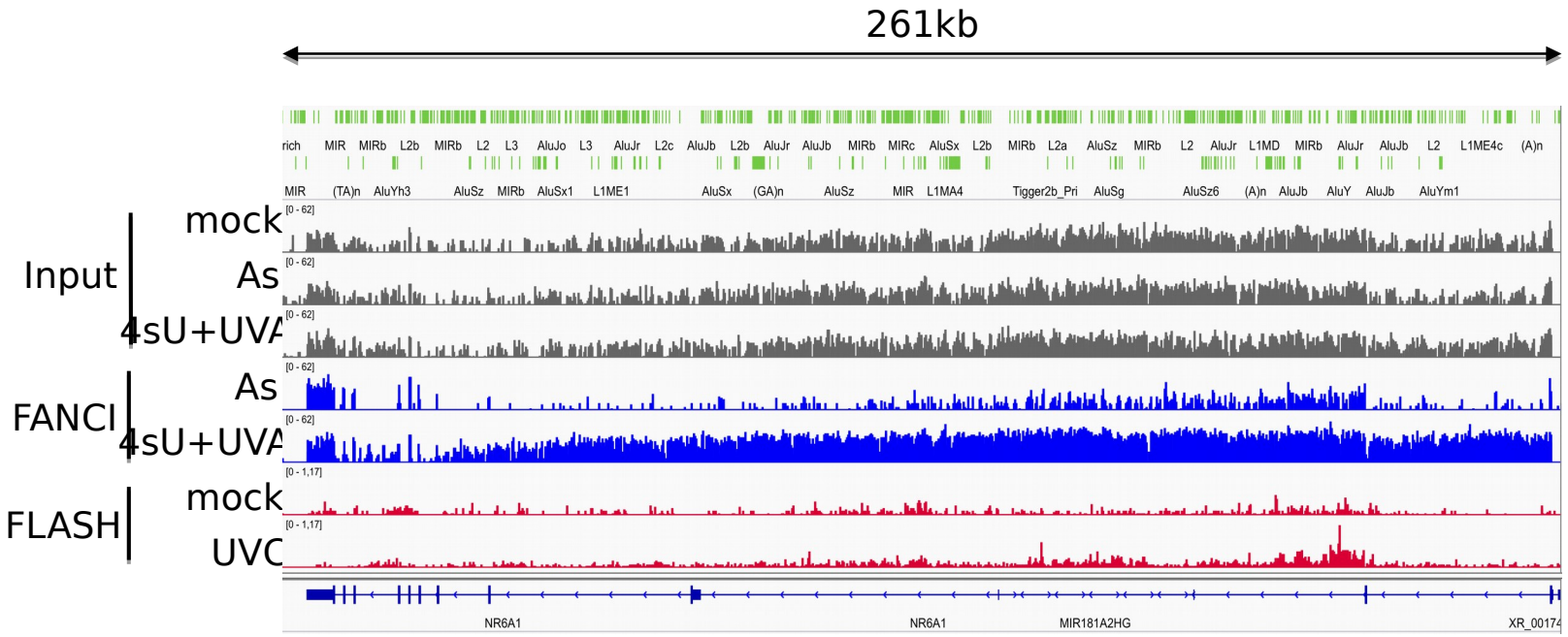
Analysis by Umut

Genes of long introns with high transposon occupation is enriched in UV SG



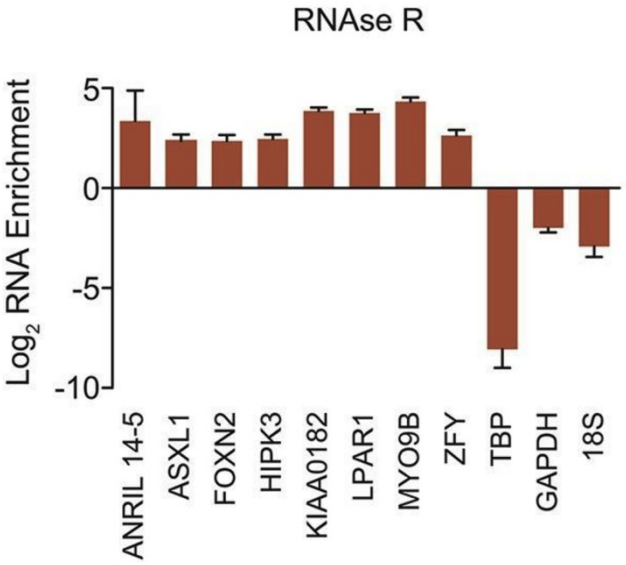
COA1

Genes of extreme long introns with high transposon occupation is enriched in UV SG

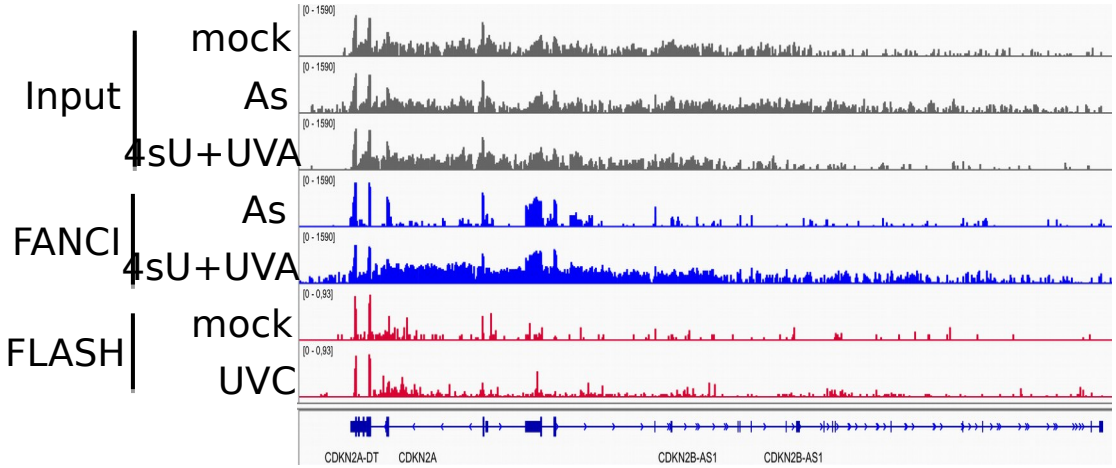


NR6A1

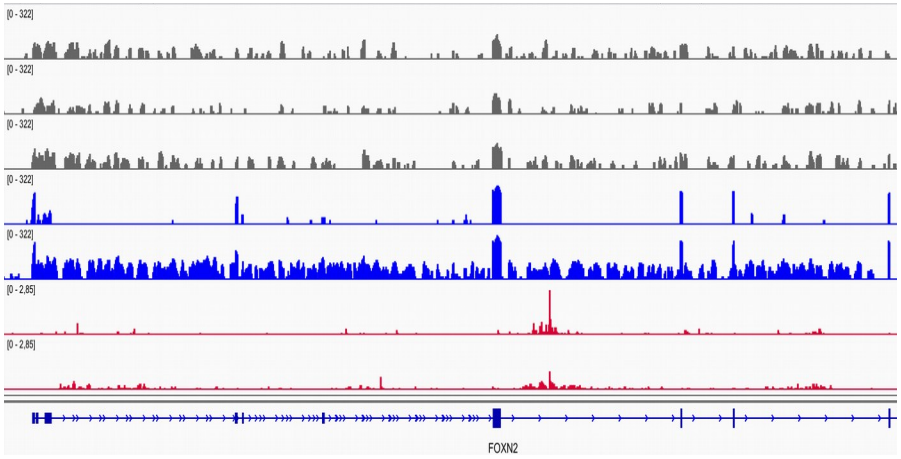
Circular RNA?



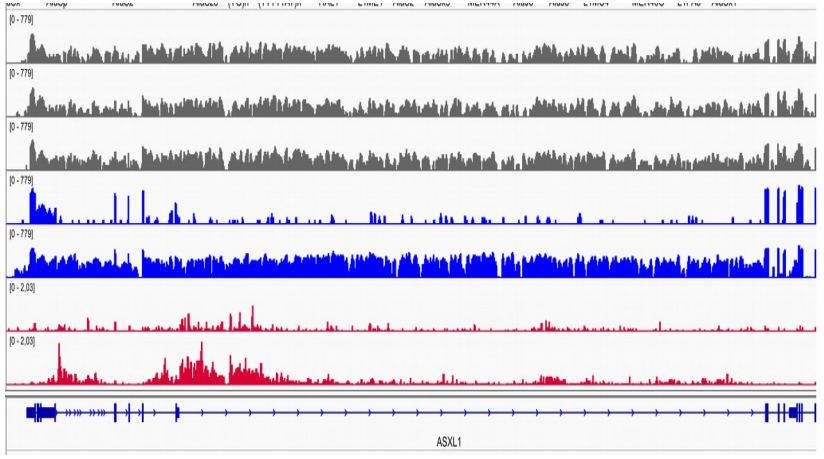
Jeck et al.RNA.2013



ANRIL



FOXN2



ASXL1

Hypothesis

1, RNA damage disrupt splicing process, cause intron-retention or generation of circular RNA. These abnormal mRNA are sequestered into stress granules

1, A general RNA seq analysis of total RNA sample: (1,2,3) vs (4,5,6) vs(7,8,9)
Do we need a different tool as our RNA is total RNA but not polyA RNA?
Splicing might be very important for our project, what kind of splicing analysis we should do?

2, A RNA enrich analysis similar as the Mol cellular paper.
They take high expression genes and calculated the ratio of these genes in total and granule samples, then calculated whether there is an enrichment or not.
This might not work so good to us as for many genes, you can see mature mRNA in as granules(10,11,12) and immature mRNA in UV granules(13,14,15). They will be considered as genes in both granules but in fact their case is different.

3, maybe we can first calculate the ratio of extron/intron/UTR in each sample, and find that there are a lot of intron in UV granules but not as granules, then we check what these intron are, what genes they are and feature of these enriched introns.

4, we can overlap these introns or genes with published circular RNA dataset or m6A datasets, or calculate the tendency to form circular RNA
From literature: longer exons than average, surrounded by small introns having reversed Alu repeats, appear to be main features present in the RNA circularisation mechanism.

5, is it possible to distinguish mRNA and immature RNA from the reads? If yes, maybe we can check the correlation between mRNA abundance and intron-retention(or circular RNA) possiblity.
There seems to be a negative correlation between intron reads and 3UTR reads in granules.