Supplemental Figures

Figure S1: Isolation of WDR62 by the Ras Recruitment System and WDR62 expression. A. CDC25-2 temperature sensitive yeast strain transformed with the indicated Met425 Ras-bait fusion protein expression plasmid and pMyr expression plasmid encoding the myristoylated prey fusion protein. The bait plasmid confers yeast growth on medium lacking leucine and expresses the bait when grown on medium lacking methionine. The prey plasmid confers yeast growth on medium lacking uracil and expresses the myristoylated prey fusion when grown on galactose containing medium. Single colonies were selected and grown on minimal Ynb glucose containing agar plates lacking leucine and uracil for 2 days at 24^{o}C (left panel). Subsequently, plate was replica plated onto minimal Ynb galactose containing agar plate lacking leucine, uracil and methionine incubated at 37^{o}C for 4 days (right panel). Baits used: JNK1KM-Ras - JNK1 ATP binding mutant K149M, and Ras-Pak - p21 activating kinase regulatory domain (Hubsman, 2001). Prey used: JBP5 - clone #5 encoding 505 a.a. derived from WDR62 C-terminal, WDR62-C - encoding 309 a.a. derived from WDR62 C-terminal, and Chp - CDC42 homologous protein (Aronheim *et al.*, 1998). **B.** PCR of cDNA expression panel derived from the indicated human tissue mRNA. Expected PCR fragment size is 366 bp. DNA 1 KB ladder (Mr) is indicated. **C.** cDNA prepared from HEK-293 (HEK), H1299 and HeLa cells with the indicated oligonucleotides. PCR was performed in the absence (-) or presence (+) of cDNA template. Samples were separated by a 15% non-denaturing polyacrylamide gel. DNA size markers are indicated.

Figure S2: WDR62 expression in different cell lines analyzed by different WDR62 antibodies. A. Western blot analysis with cell lysate derived from either transfected with Myc-WDR62 encoding plasmid (lanes 1-2) or un-transfected HEK-293T (lanes 3-4) probed with the indicated antibodies. Cell lysate was either boiled for 5 minutes (lanes 1 and 3) or non-heated prior to loading (lanes 2 and 4). **B.** Western blot analysis of lysate derived from different human cell lines including HeLa, H1299, HEK-293 (HEK) and HEK-293T (293T) or mouse embryo fibroblast (MEF). Samples were either boiled for 5 minutes prior to gel loading (+) or non-heated (-). Expression level of WDR62 and α-tubulin were examined with the indicated corresponding antibodies. **C.** Western blot analysis of HEK-293T cells un-transfected (lane 4) or transfected with plasmids encoding either the human WDR62

(hWDR62) or the mouse WDR62 homologue (mWDR62). Lysate was loaded directly without heating or was boiled for 5 minutes where indicated (+). Due to high level of expression of the transfected mouse and human WDR62 proteins, endogenous WDR62 proteins are not observed in un-transfected cells due to the short exposure of the membrane.

Figure S3: Myc-WDR62 associates with HA-JNK1/2 and HA-MKK7. A. HEK-293T cells were co-transfected as indicated. Cell lysate was immuno-precipitated with anti-HA antibody and eluted proteins were separated by 10% SDS-PAGE. The purified proteins were detected with anti-JBP5 antibodies (top panel). Total cell lysate of transfected HEK-293T was used for staining with anti-JBP5 (middle panel) and various HA-JNK1/2 and MKK7 antibodies (bottom panel). The migration of transfected proteins is indicated by arrows. **B.** Western blot analysis of cell lysate derived from rat hippocampus (lane 1) and HEK-293T cells. Membrane was probed with anti-JIP antibody and anti-GAPDH as loading control. The migration of JIP3 is indicated.

Figure S4: Lack of co-localization between Myc-WDR62 and different fluorescent cellular markers. Immuno-fluorescence of HEK-293T cells co-transfected with the indicated fluorescently labeled protein markers together with either vehicle pCAN expression vector (-) or Myc-WDR62 (+). Cells were stained with anti-3G8 followed by the fluorescently labeled secondary antibody (red fluorescence). Fluorescently-fused protein markers used: early endosomes (EEA1-GFP), ER (M1-CFP) and golgi (GALT-CFP). Cells were visualized by confocal microscope. The CFP and GFP fluorescence of the protein markers are shown in green for convenience purpose only.

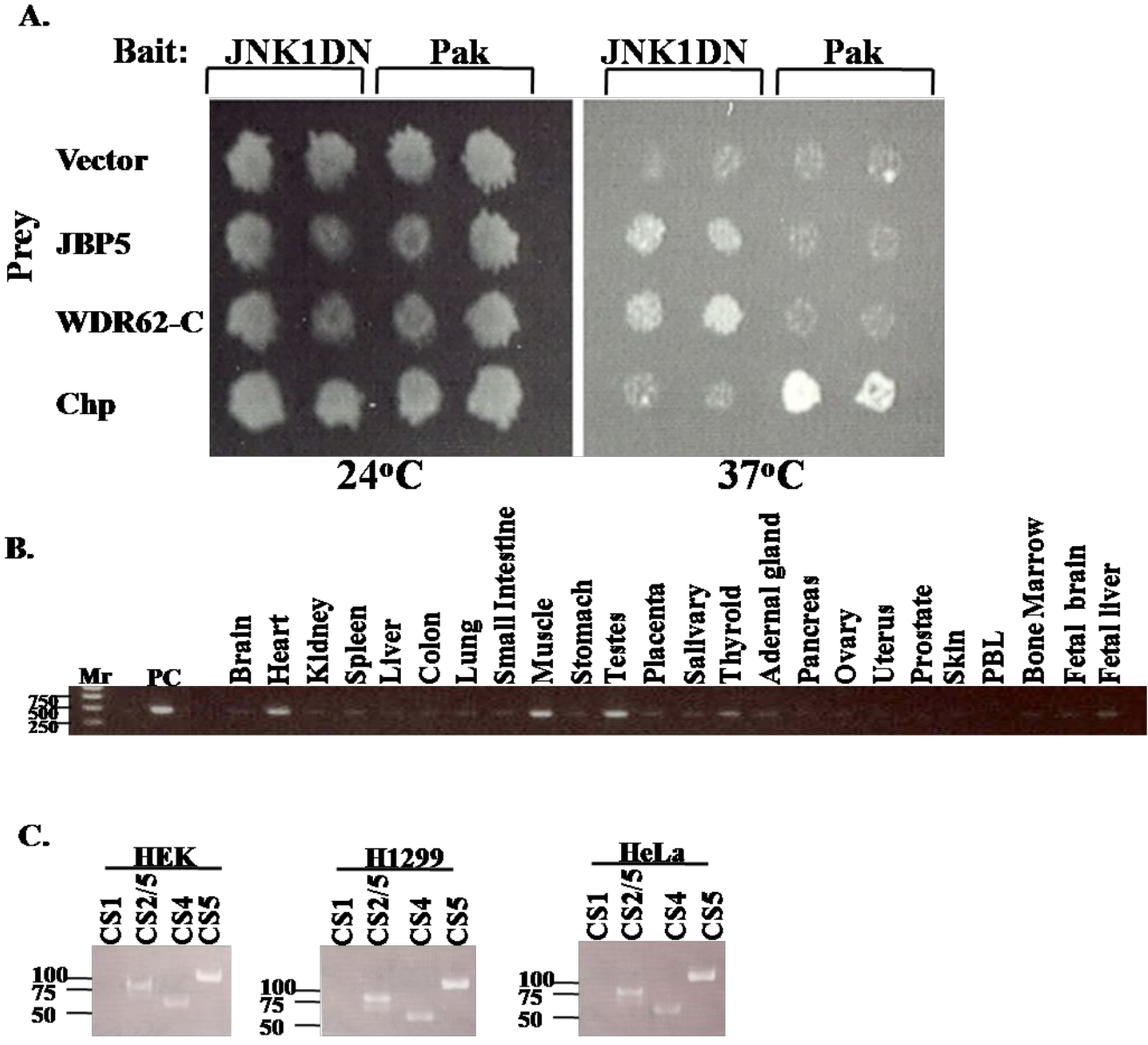
Figure S5: Statistical analysis for WDR62-TIA and pJNK-DCP1α co-localization. Co-localization coefficient was calculated as described in methods section. Co-localization between WDR62 and TIA, and phospho-JNK and DCP1α is shown. The number of cells analyzed is indicated (n). Coefficient value ranges from zero to one corresponds to either no localization or 100% localization respectively.

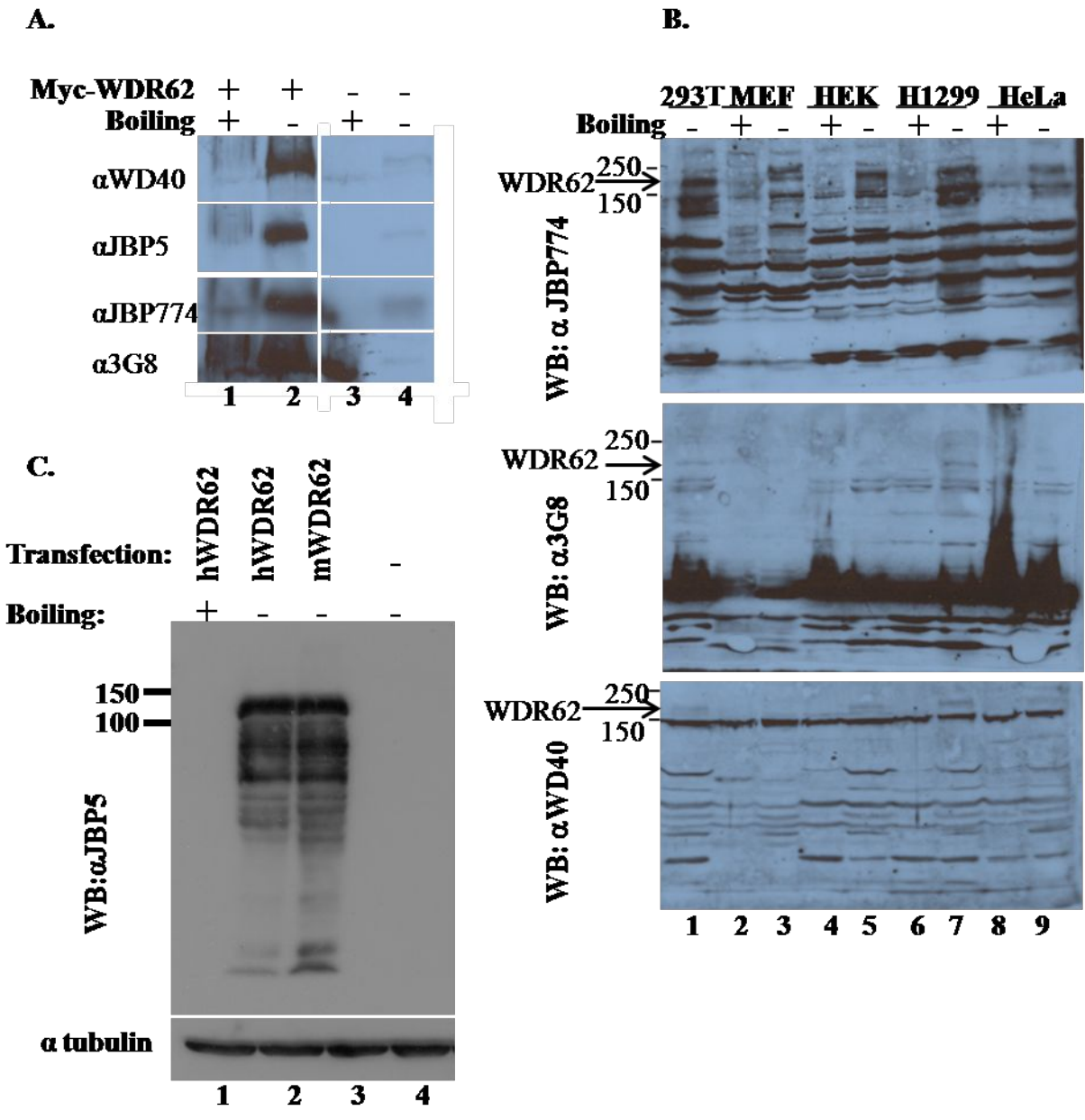
Figure S6: G3BP co-localize with the SG marker TIA following arsenite treatment. Immuno-fluorescence of fixed HEK-293T cells either untreated or treated for 1h with arsenite. Cells were co-stained with: anti-G3BP (αG3BP, purple), anti-phospho-JNK (αpJNK, red), anti-TIA (αTIA, green) and DAPI nuclear stain (blue).

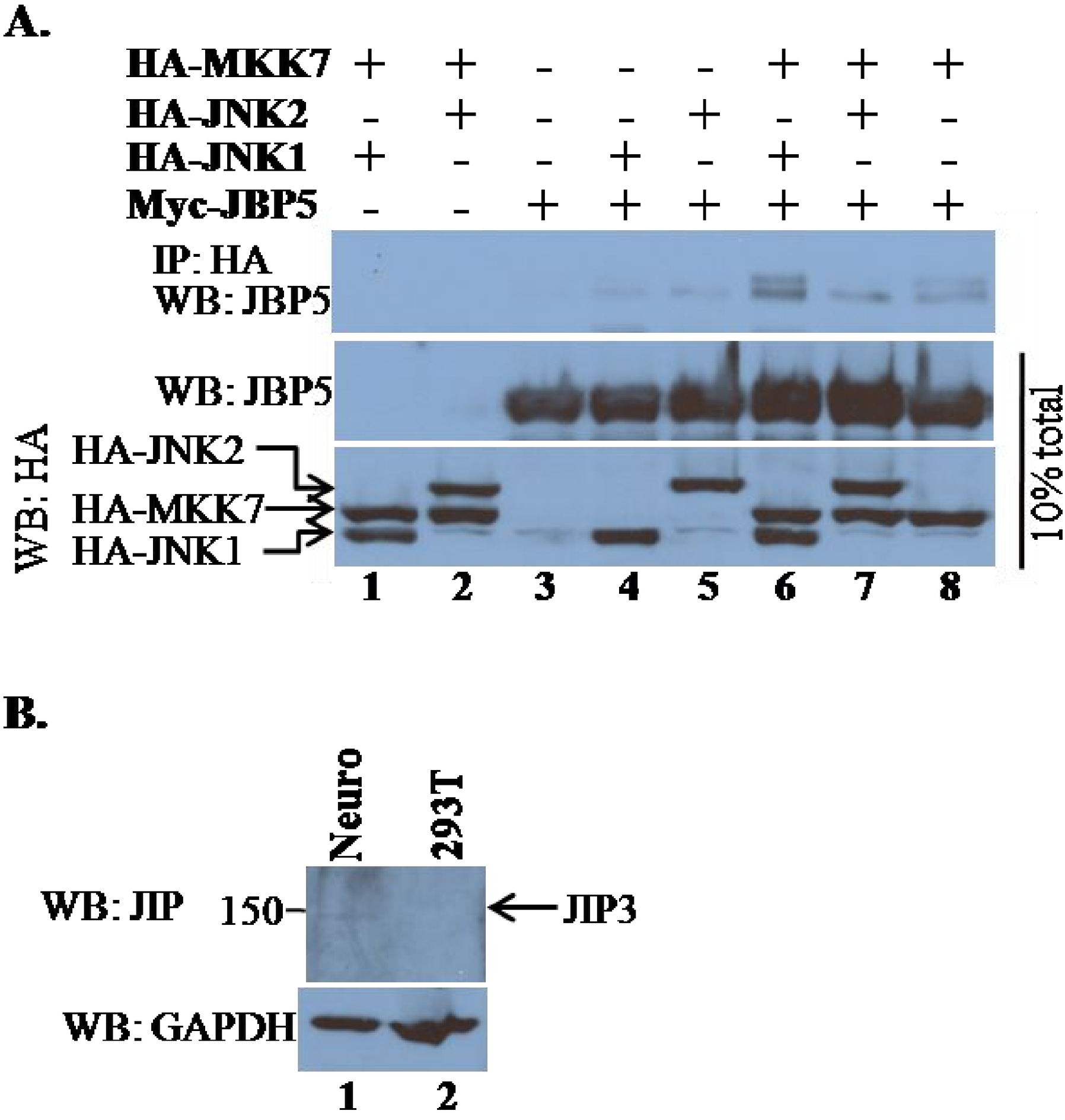
Figure S7: WDR62 C-terminal domain serves as JNK substrate. *In vitro* kinase assay with UV irradiated immune-precipitated HA-JNK1 in the presence of the indicated bacterially purified GST-fusion proteins (10 μg) and [γ^{32}P]-ATP. Following kinase reaction, samples were separated by 10% SDS-PAGE. Gel was coomassie blue stained (CBB, bottom panel), dried and exposed to autoradiography (^{32}P, top panel).

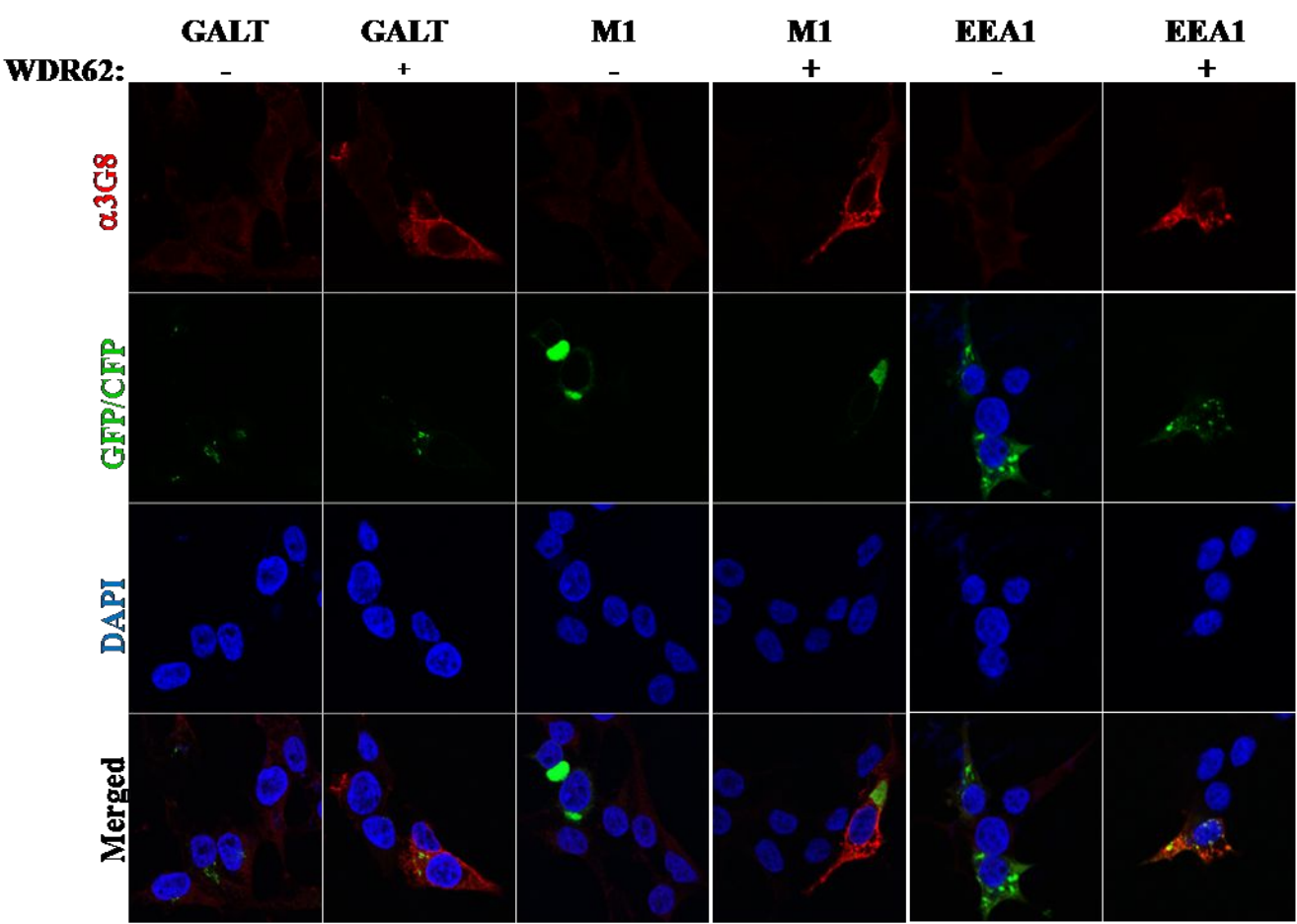
Figure S8: WDR62 over-expression inhibits JNK translocation to the nucleus. Immuno-fluorescence of HEK-293T cells either untreated or treated with arsenite for 1h. Cells were co-stained with: anti-3G8 (green), anti-phospho-JNK (red) and DAPI nuclear stain (blue). White arrow indicates WDR62 over-expressing cell nuclei.

Figure S9: Statistical analysis of number and size of SG and PB following arsenite treatment in the presence or absence of SP600125. The number and size of stained SG and PB was analyzed using the 510 LSM software is described in methods section. * and ** represents P values < 0.05 and < 0.01 respectively.

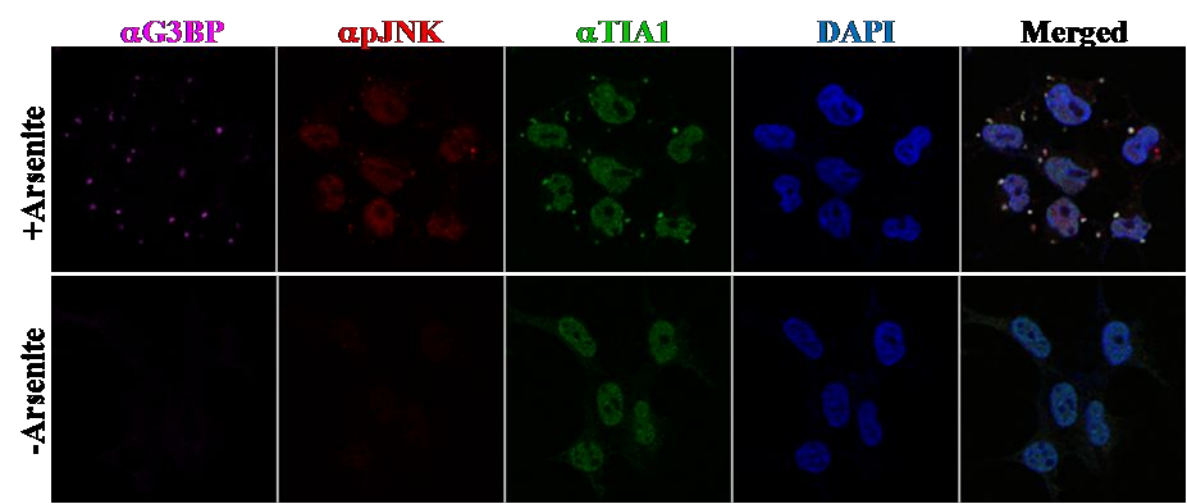

A.
Bait:
JNK1DN
Pak
JNK1DN
Pak
Prey
Vector
JBP5
WDR62-C
Chp
24°C
37°C
B.
Mr
PC
Brain
Heart
Kidney
Spleen
Liver
Colon
Lung
Small Intestine
Muscle
Stomach
Testes
Placenta
Sallvary
Thyrold
Adernal gland
Pancreas
Ovary
Uterus
Prostate
Skin
PBL
Bone Marrow
Fetal brain
Fetal liver
750
500
250
C.
HEK
H1299
HeLa
CS1
CS2/5
CS4
CS5
100
75
50

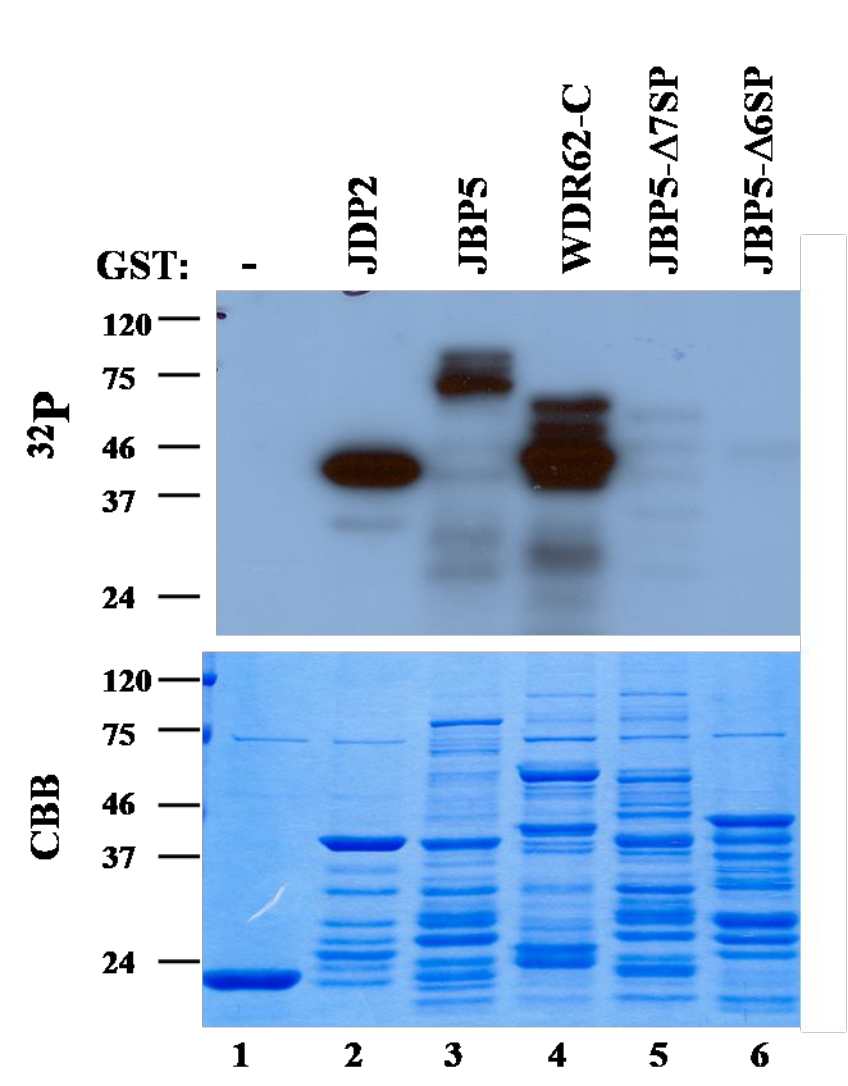

A.
Myc-WDR62 + + - -
Boiling + - + -
αWD40
αJBP5
αJBP774
α3G8
1 2 3 4
B.
293T MEF HEK H1299 HeLa
Boiling - + - + - + - + -
WDR62
250
150
WB: α JBP774
WDR62
250
150
WB: α3G8
WDR62
250
150
WB: αWD40
1 2 3 4 5 6 7 8 9
C.
Transfection: hWDR62 hWDR62 mWDR62 -
Boiling: + - - -
150
100
WB:αJBP5
α tubulin
1 2 3 4

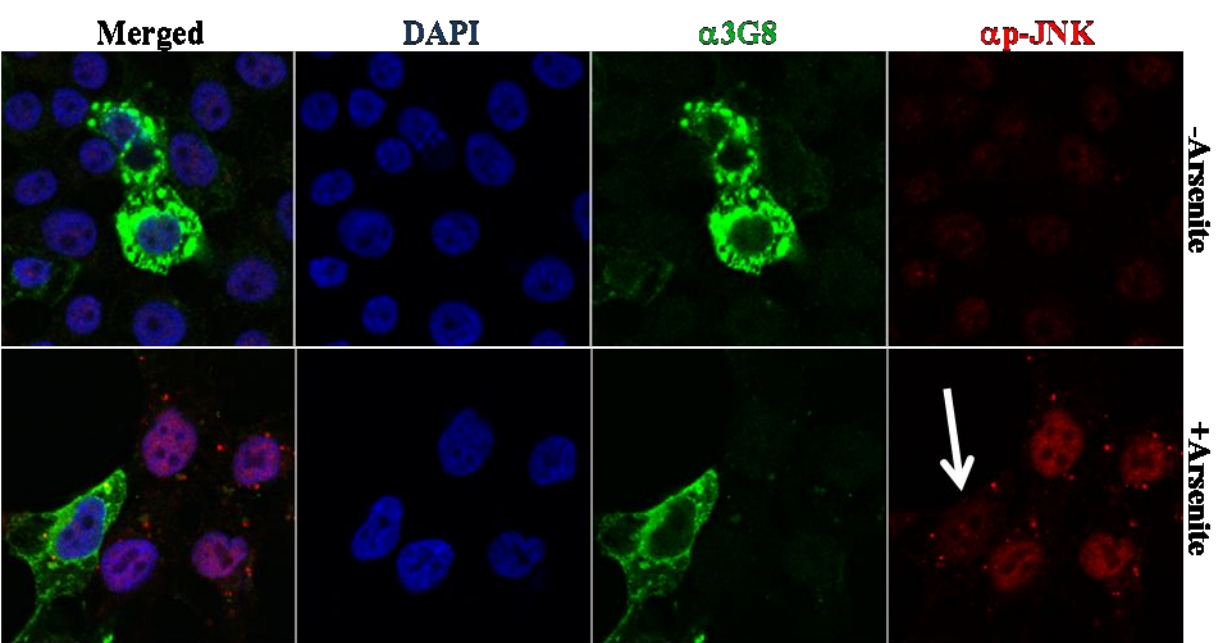

A.
HA-MKK7 + + - - - + + +
HA-JNK2 - + - - + - + -
HA-JNK1 + - - + - + - -
Myc-JBP5 - - + + + + + +
IP: HA
WB: JBP5
WB: JBP5
WB: HA
HA-JNK2
HA-MKK7
HA-JNK1
10% total
1 2 3 4 5 6 7 8
B.
Neuro
293T
WB: JIP
150
JIP3
WB: GAPDH
1 2


GALT
GALT
M1
M1
EEA1
EEA1
WDR62:
-
+
-
+
-
+
α3G8
GFP/CFP
DAPI
Merged

	Channel	Weighted Co -localization coefficient	n
WDR62 and TIA1 co-localization	WDR62	0.94±0.1	55
	TIA1	1±0.01	
phospho-JNK and DCP1α co-localization	pJNK	0.9±0.08	44
	DCP1a	0.94±0.07	


αG3BP
αpJNK
αTIA1
DAPI
Merged
+Arsenite
-Arsenite


GST:
-
JDP2
JBP5
WDR62-C
JBP5-Δ7SP
JBP5-Δ6SP
32P
120
75
46
37
24
CBB
120
75
46
37
24
1
2
3
4
5
6


Merged
DAPI
α3G8
αp-JNK
-Arsenite
+Arsenite

	Stress Granules			Processing Bodies		
Treatment	**No. per Cell**	**Average Size (pxl^2)**	**n**	**No. per Cell**	**Average Size (pxl^2)**	**n**
Arsenite	11.3±1.8*	58.6±7.7**	32	13.5±1.8*	26.2±5.3*	30
SP600125 Arsenite	13.6±1.4	42.5±5.2	25	15.1±2.0	20±2.0	31