# **Expanded View Figures**

### Figure EV1. DUB cDNA screening identified USP4 as the most potent activator of activin signaling.

- A This graph depicts the activin (5 ng/ml)-induced ARE-Luc transcriptional reporter activity compared to control cells and the effects of a representative set of DUB cDNAs, n = 3. The top activator USP4 is indicated with an arrow. The data are presented as means  $\pm$  SD.
- B List of the results of screen performed in Appendix Fig S1A.

C Left panel: effect of USP4 and USP9x on the ARE-Luc transcriptional response induced by activin A (50 ng/ml) in HepG2 cells. Right panel (mentioned in the discussion): USP4, but not the USP9x rescued the defective ARE-Luc response caused by sh-USP4 that target USP4 3'UTR. The data are presented as means ± SD. Covec, empty vector; Co.sh, non-targeting shRNA. \* indicates statistical significance (*P* < 0.05). Two-tailed, unpaired *t*-test. Experiments were performed in triplicate.

D Q-PCR analysis of USP4 mRNA level in HepG2 cells infected with lentivirus encoding control (Co.sh) or USP4 shRNA (#1&#2). Experiments were performed in triplicate. Two-tailed, unpaired *t*-test. \*\**P* < 0.01 and \*\*\**P* < 0.001. The data are presented as means ± SD.

Α



Figure EV1.

Relative Fold change	Relative Fold change (+activin A)	
1.00	5.98	
0.51	0.97	
1.43	2.08	
0.37	2.08	
0.45	2.38	
1.13	2.60	
1.22	3.25	
1.68	3.50	
0.89	4.10	
2.37	4.21	
1.19	4.60	

В

Sequence

DUB cDNA

1 Flag GFP

2 OTUB1

3 UCHL3 4 USP42 5 COPS6 6 USP3

8         OTUD1         1.68         3.60           9         OTUD7B         0.89         4.11           10         YOD1         2.37         4.22           11         USP25         0.56         4.82           13         USP33         1.06         5.33           14         EIF3S3         0.86         5.44           16         USP43         0.54         5.61           17         USP46         1.41         5.86           18         PSMD7         1.16         5.90           19         USP20         1.21         6.00           20         CYLD         1.78         6.16           21         USP50         1.36         6.22           22         USP15         1.03         6.22           21         USP50         1.38         6.66           27         USP30         1.03         6.62           28         OTUD5         2.14         6.66           29         OTUD5         2.14         6.66           29         OTUD5         2.14         6.66           29         OTUD5         2.14         6.66           29		7	USP22	1.22	3.25
9         OTUD7B         0.89         4.10           10         YOD1         2.37         4.21           11         USP25         0.56         4.92           13         USP33         1.06         5.37           14         EIF3S3         0.86         5.44           15         DUB3         0.81         5.44           16         USP46         1.41         5.88           18         PSMD7         1.16         5.99           19         USP20         1.21         0.67           20         CYLD         1.78         6.16           21         USP50         1.38         6.22           22         USP15         1.03         6.26           26         USP5         0.68         6.66           27         USP30         1.03         6.64           28         OTUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.97           33         BRCC3         1.18         9.96           34		8	OTUD1	1.68	3.50
10         YOD1         2.37         4.21           11         USP25         0.56         4.66           12         USP33         1.06         5.37           14         EIF3S3         0.86         5.47           15         DUB3         0.81         5.44           16         USP43         0.54         5.66           17         USP46         1.41         5.88           18         PSMD7         1.16         5.99           19         USP20         1.21         6.07           20         CYLD         1.78         6.12           21         USP50         1.33         6.22           22         USP15         1.03         6.22           21         USP50         1.38         6.22           22         USP15         1.03         6.62           26         USP30         1.03         6.86           27         USP30         1.03         6.86           28         OTUD5         2.14         6.66           29         OTUD6         2.14         6.66           29         OTUD6         2.13         7.02           33		9	OTUD7B	0.89	4 10
10         USP21         1.19         4.66           12         USP25         0.56         4.83           13         USP33         1.06         5.33           14         EIF333         0.86         5.44           15         DUB3         0.81         5.44           16         USP43         0.54         5.66           17         USP46         1.41         5.83           18         PSMD7         1.16         5.96           19         USP20         1.21         6.07           20         CYLD         1.78         6.16           21         USP50         1.38         6.22           22         USP15         1.03         6.26           26         USP5         0.68         6.66           27         USP30         1.03         6.64           28         OTUD5         2.14         6.64           29         OTUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP38         0.61         6.99           33		10	YOD1	2.37	4.21
11         USP25         0.66         4.82           13         USP33         1.06         5.33           14         EIF3S3         0.86         5.47           16         USP43         0.54         5.66           17         USP46         1.41         5.86           18         PSMD7         1.16         5.96           19         USP20         1.21         6.07           20         CYLD         1.78         6.14           21         USP50         1.38         6.22           22         USP50         1.38         6.26           22         USP50         1.38         6.26           23         USP29         1.32         6.44           25         PSMD14         1.43         6.66           26         USP5         0.168         6.66           27         USP30         1.03         6.64           29         OTUD4         0.87         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           34         JOSD2         2.13         7.02           35	-	11	LISP21	1 10	4.60
12         USP23         0.36         4.66           14         EIF3S3         0.86         5.47           15         DUB3         0.81         5.44           16         USP46         1.41         5.86           17         USP46         1.41         5.86           18         PSMD7         1.16         5.99           19         USP20         1.21         6.00           20         CYLD         1.78         6.16           21         USP50         1.33         6.22           22         USP15         1.03         6.22           23         USP29         1.32         6.44           24         MYMS1         1.76         6.44           25         PSMD14         1.43         6.65           20         GTUD4         0.87         6.77           30         USP44         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           33         BRC3         1.18         9.96           34         JOSD2         2.13         7.02           38	-	10	03F21	1.10	4.00
13         USP33         1.06         5.34           14         EF3533         0.86         5.44           16         USP43         0.54         5.61           17         USP46         1.41         5.86           17         USP46         1.41         5.86           19         USP20         1.21         6.07           20         CYLD         1.76         6.16           21         USP50         1.33         6.62           22         USP15         1.03         6.22           23         USP29         1.32         6.44           26         PSMD14         1.43         6.56           26         USP5         0.68         66           27         USP30         1.03         0.64           28         OTUD5         2.14         0.66           29         OTUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.48           32         USP36         0.61         6.99           33         BRC3         1.18         9.96           34	-	12	USP25	0.50	4.82
14         EIF3S3         0.86         5.44           16         USP43         0.54         5.66           17         USP46         1.41         5.86           18         PSMD7         1.16         5.96           19         USP20         1.21         6.07           20         CYLD         1.78         6.16           21         USP50         1.36         6.22           22         USP15         1.03         6.27           23         USP5         0.68         6.66           26         USP5         0.68         6.66           27         USP30         1.03         6.67           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           34         JOSD2         2.13         7.02           38	_	13	USP33	1.06	5.37
15         DUB3         0.81         5.44           16         USP46         1.41         5.86           18         PSMD7         1.16         5.96           19         USP20         1.21         6.07           20         CYLD         1.78         6.16           21         USP50         1.36         6.22           22         USP15         1.03         6.27           23         USP29         1.32         6.44           24         MYMS1         1.76         6.42           25         PSMD14         1.43         6.66           26         USP30         1.03         6.64           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.77           31         USP38         1.23         6.84           32         USP38         1.23         6.84           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           38         USP47         1.21         7.16           37         UCHL1         1.57         7.22           38		14	EIF3S3	0.86	5.47
16         USP46         1.41         5.88           17         USP46         1.41         5.88           18         PSMD7         1.16         5.99           19         USP20         1.21         6.07           20         CYLD         1.78         6.16           21         USP50         1.36         6.22           23         USP15         1.03         6.62           24         MYMS1         1.76         6.44           25         PSMD14         1.43         6.65           26         USP5         0.68         6.66           27         USP30         1.03         8.64           28         OTUD5         2.14         6.66           20         OTUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           33         BRC3         1.18         9.96           34         JOSD2         2.13         7.02           38         BAP1         1.10         7.33           40		15	DUB3	0.81	5.48
17         USP46         1.41         5.88           18         PSMD7         1.16         5.99           19         USP20         1.21         6.07           20         CYLD         1.78         6.16           21         USP50         1.36         6.22           23         USP29         1.32         6.41           24         MYMS1         1.76         6.42           25         PSMD14         1.43         6.56           26         USP5         0.68         6.66           27         USP30         1.03         6.64           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.77           30         USP44         0.90         6.77           31         USP28         1.23         6.84           32         USP30         2.13         7.00           33         BRC3         1.18         9.96           34         JOSD2         2.13         7.02           33         BR241         1.07         7.33           39         BAP1         1.10         7.33           34		16	USP43	0.54	5.61
18         PSMD7         1.16         5.96           19         USP20         1.21         6.07           20         CYLD         1.78         6.16           21         USP50         1.36         6.22           22         USP15         1.03         6.21           23         USP50         1.32         6.44           26         PSMD14         1.43         6.66           27         USP50         0.68         6.66           27         USP50         1.03         6.64           28         OTUD5         2.14         6.66           29         OTUD5         2.14         6.66           29         OTUD5         2.14         6.66           30         USP14         0.90         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.64           32         USP36         0.61         6.99           33         BRC3         1.18         9.99           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           37		17	USP46	1.41	5.88
10         USP20         1.21         6.00           20         CYLD         1.78         6.14           21         USP50         1.36         6.22           22         USP15         1.03         6.21           23         USP29         1.32         6.41           24         MYMS1         1.76         6.44           26         PSMD14         1.43         6.56           20         USP5         0.68         6.66           27         USP30         1.03         6.64           29         OTUD5         2.14         6.66           20         USP14         0.90         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           36         USP42F         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.33           30	_	18	PSMD7	1.16	5.96
13         USF20         1.21         0.01           20         CYLD         1.78         6.16           21         USP50         1.36         6.22           22         USP15         1.03         6.21           23         USP29         1.32         6.44           25         PSMD14         1.43         6.66           20         USP5         0.68         6.66           21         USP30         1.03         6.64           20         OTUD5         2.14         6.66           29         OTUD5         2.14         6.66           29         OTUD5         2.14         6.66           29         OTUD5         2.14         6.66           30         USP14         0.90         6.77           30         USP4         0.90         6.77           31         USP28         1.23         6.68           32         USP36         0.61         6.99           33         BRC3         1.18         9.90           34         JOSD2         2.13         7.02           38         BAP1         1.10         7.33           9	-	10	119020	1.10	6.07
20         CTLD         1.76         8.62           22         USP50         1.36         6.22           23         USP29         1.32         6.41           24         MYMS1         1.76         6.44           25         PSMD14         1.43         6.65           26         USP5         0.68         6.60           27         USP30         1.03         6.64           28         OTUD5         2.14         6.66           20         OTUD4         0.87         6.74           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           36         OTUD6B         1.67         7.11           37         UCHL1         1.57         7.22           38         BAP1         1.10         7.33           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41	-	19	CVID	1.21	0.07
21         USF50         1.36         6.22           22         USP15         1.03         6.21           23         USF29         1.32         6.44           24         MYMS1         1.76         6.44           25         PSMD14         1.43         6.66           26         USP5         0.68         6.60           27         USP30         1.03         6.64           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.00           36         OTUD6B         1.67         7.10           36         OTUD6A         2.03         7.46           41         USP44         1.39         7.32           39         BAP1         1.10         7.37           40         OTUD6A         2.03         7.46           41	-	20	USDES	1.78	0.10
22         USP15         1.03         6.21           23         USP29         1.32         6.41           24         MYMS1         1.76         6.44           25         PSMD14         1.43         6.56           26         USP5         0.68         6.60           27         USP30         1.03         6.64           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.74           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.00           35         OTUD6B         1.67         7.10           36         USP42F         1.21         7.10           37         UCH1         1.57         7.22           38         USP48         1.39         7.33           39         BAP1         1.10         7.31           40         OTUD6A         2.03         7.44           41	_	21	USP50	1.36	6.20
23         USP29         1.32         6.44           24         MYMS1         1.76         6.42           25         PSMD14         1.43         6.65           26         USP5         0.68         6.60           27         USP30         1.03         6.64           28         OTUD5         2.14         6.65           29         OTUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.91           33         BRCC3         1.18         9.92           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           36         USP4 ZF         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.35           39         BAP1         1.10         7.37           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           43 <td></td> <td>22</td> <td>USP15</td> <td>1.03</td> <td>6.21</td>		22	USP15	1.03	6.21
24         MYMS1         1.76         6.42           25         PSMD14         1.43         6.66           26         USP5         0.68         6.66           27         USP30         1.03         6.64           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.74           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.91           33         BRCC3         1.18         9.92           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           36         USP48         1.39         7.32           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.51           42         USP11         1.56         7.63           43         MYPND         1.56         7.66           45         USP1         1.52         7.77           46		23	USP29	1.32	6.41
25         PSMD14         1.43         6.56           26         USP5         0.68         6.60           27         USP30         1.03         6.64           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.74           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.97           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.10           36         USP4 ZF         1.21         7.16           37         UCH1         1.57         7.22           38         USP48         1.39         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           42         USP11         1.26         7.70           44         COPS5         1.25         7.70           44         USP37         1.28         7.86           45 <td></td> <td>24</td> <td>MYMS1</td> <td>1.76</td> <td>6.42</td>		24	MYMS1	1.76	6.42
26         USP5         0.68         6.60           27         USP30         1.03         6.64           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.77           31         USP36         0.61         6.91           33         BRCC3         1.18         9.90           34         JOSD2         2.13         7.00           35         OTUD6B         1.67         7.10           36         USP4 ZF         1.21         7.16           37         UCH1         1.57         7.22           38         USP48         1.39         7.33           39         BAP1         1.10         7.31           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           42         USP11         1.26         7.55           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           45         USP13         1.65         8.14           50		25	PSMD14	1.43	6.56
Los         0.03         0.64           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.91           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           35         OTUD6A         2.03         7.44           40         OTUD6A         2.03         7.44           41         USP49         1.40         7.51           42         USP11         1.26         7.52           43         MYPND         1.56         7.66           44         COP55         1.25         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.99           48         USP10         1.19         7.97           49         USP7         2.18         8.04           50         USP13 <td></td> <td>26</td> <td>USP5</td> <td>0.68</td> <td>6.60</td>		26	USP5	0.68	6.60
27         361 30         1.03         0.04           28         OTUD5         2.14         6.66           29         OTUD4         0.87         6.74           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.91           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           36         USP4 ZF         1.21         7.16           37         UCH1         1.57         7.22           38         USP48         1.39         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           42         USP11         1.26         7.70           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           44         USP37         1.28         7.66           45         USP13         1.65         8.14           51 <td>-</td> <td>20</td> <td>LISP30</td> <td>1.02</td> <td>6.64</td>	-	20	LISP30	1.02	6.64
20         010D3         2.14         0.05           29         0TUD4         0.87         6.77           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.91           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           36         USP4ZF         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.35           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           42         USP11         1.52         7.70           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         2.18         8.01           50         USP1         1.52         7.70           46	-	21	OTUDE	1.03	0.04
29         010D4         0.87         6.74           30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.91           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.10           35         OTUD6B         1.67         7.10           37         UCH1         1.57         7.22           38         USP48         1.39         7.33           39         BAP1         1.10         7.31           40         OTUD6A         2.03         7.44           41         USP49         1.40         7.61           42         USP11         1.26         7.52           43         MYPND         1.56         7.71           44         COPS5         1.25         7.66           44         COPS5         1.25         7.66           45         USP10         1.19         7.97           48         USP7         2.18         8.01           50	-	28	01005	2.14	0.65
30         USP14         0.90         6.77           31         USP28         1.23         6.84           32         USP36         0.61         6.99           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           36         USP4 ZF         1.21         7.12           37         UCHL1         1.57         7.22           38         USP48         1.39         7.35           39         BAP1         1.10         7.37           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           43         MYPND         1.56         7.66           44         COPS5         1.25         7.76           44         USP10         1.19         7.97           44         USP17         2.18         8.01           50         USP77         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52 <td>-</td> <td>29</td> <td>01004</td> <td>0.87</td> <td>6.74</td>	-	29	01004	0.87	6.74
31         USP28         1.23         6.84           32         USP36         0.61         6.91           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.00           35         OTUD6B         1.67         7.10           36         USP4 ZF         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.35           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           42         USP11         1.26         7.63           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.95           48         USP10         1.19         7.99           49         USP7         2.18         8.01           50	_	30	USP14	0.90	6.77
32         USP36         0.61         6.91           33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.02           35         OTUD6B         1.67         7.11           36         USP4 ZF         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.33           39         BAP1         1.10         7.37           40         OTUD6A         2.03         7.44           41         USP49         1.40         7.51           42         USP11         1.26         7.55           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           44         COPS5         1.25         7.66           45         USP11         1.52         7.77           46         USP37         1.28         7.86           47         ATXN3         2.66         7.99           48         USP7         2.18         8.00           50         USP13         1.65         8.14           51		31	USP28	1.23	6.84
33         BRCC3         1.18         9.96           34         JOSD2         2.13         7.00           35         OTUD6B         1.67         7.10           36         USP4 ZF         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.33           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           43         MYPND         1.56         7.66           44         COPS5         1.25         7.76           44         COPS5         1.28         7.86           47         ATXN3         2.66         7.99           48         USP1         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.25           54		32	USP36	0.61	6.91
34         JOSD2         2.13         7.00           35         OTUD6B         1.67         7.11           36         USP4 ZF         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.33           39         BAP1         1.10         7.37           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.51           42         USP11         1.26         7.55           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           45         USP11         1.52         7.77           46         USP37         1.28         7.86           47         ATXN3         2.66         7.95           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP52         1.59         8.20           53         UCHL5         2.43         8.22           54         USP45         0.37         8.43           55		33	BRCC3	1.18	9.96
61         07UD6B         1.67         7.10           36         USP4 ZF         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.35           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.51           42         USP11         1.26         7.55           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.95           48         USP10         1.19         7.97           48         USP7         2.18         8.00           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53		34	JOSD2	2.13	7.02
35         01000         1.01         1.10           36         USP4 ZF         1.21         7.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.33           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.53           43         MYPND         1.56         7.63           44         COPS5         1.25         7.66           44         COPS5         1.25         7.70           46         USP1         1.52         7.70           46         USP1         1.52         7.70           46         USP1         1.52         7.70           48         USP1         1.19         7.97           49         USP1         1.65         8.14           51         WPIP1         0.77         8.16           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP45         0.37         8.43           56	_	25	OTUDER	1.67	7.10
36         USP42F         1.21         1.16           37         UCHL1         1.57         7.22           38         USP48         1.39         7.33           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.51           42         USP11         1.26         7.55           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.96           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.22           54         USP53         0.37         8.43           55	-	30		1.07	7.10
37         UCHL1         1.57         7.22           38         USP48         1.39         7.35           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.55           42         USP11         1.26         7.53           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.96           47         ATXN3         2.66         7.96           48         USP10         1.19         7.97           48         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.20           53         UCHL5         2.43         8.22           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56	-	30	USP4 ZF	1.21	7.10
38         USP48         1.39         7.33           39         BAP1         1.10         7.33           40         OTUD6A         2.03         7.44           41         USP49         1.40         7.51           42         USP11         1.26         7.53           43         MYPND         1.56         7.61           44         COPS5         1.25         7.66           44         COPS5         1.25         7.66           44         COPS5         1.25         7.66           44         COPS5         1.25         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.99           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.25           54         USP45         0.37         8.43           56	_	37	UCHL1	1.57	1.22
39         BAP1         1.10         7.37           40         OTUD6A         2.03         7.46           41         USP49         1.40         7.51           42         USP11         1.26         7.55           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.99           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.22           54         USP52         1.59         8.42           55         USP39         1.78         8.44           56         USP16         2.16         8.42           57         OTUD3         1.44         8.52           59		38	USP48	1.39	7.35
40         OTUD6A         2.03         7.46           41         USP49         1.40         7.51           42         USP11         1.26         7.53           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.95           48         USP10         1.19         7.97           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.20           53         UCHL5         2.43         8.22           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56         USP16         2.16         8.44           57         OTUD3         1.44         8.50           58		39	BAP1	1.10	7.37
41         USP49         1.40         7.51           42         USP11         1.26         7.55           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           44         COPS5         1.25         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.77           46         USP37         1.28         7.86           47         ATXN3         2.66         7.99           48         USP10         1.19         7.97           49         USP7         2.18         8.00           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.22           54         USP45         0.37         8.42           56         USP45         0.37         8.42           56         USP45         0.37         8.42           56         USP45         0.37         8.42           57		40	OTUD6A	2.03	7.46
11         126         7.55           42         USP11         1.26         7.65           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.99           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.25           54         USP39         1.78         8.44           56         USP39         1.78         8.44           56         USP33         0.69         8.52           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.66           61         USP19         2.17         8.92           62         PARP11		41	USP49	1 40	7.51
42         007 H         1.20         1.30           43         MYPND         1.56         7.66           44         COPS5         1.25         7.66           45         USP1         1.52         7.77           46         USP37         1.28         7.86           47         ATXN3         2.66         7.95           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.22           54         USP53         0.37         8.43           55         USP39         1.78         8.44           56         USP53         0.69         8.52           59         STAMBPL1         2.16         8.63           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63		42	USP11	1.26	7.53
43         MITPNO         1.30         7.01           44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.95           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.22           54         USP45         0.37         8.43           56         USP39         1.78         8.44           56         USP45         0.69         8.52           59         STAMBPL1         2.16         8.63           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64 <td>-</td> <td>42</td> <td></td> <td>1.20</td> <td>7.61</td>	-	42		1.20	7.61
44         COPS5         1.25         7.66           45         USP1         1.52         7.70           46         USP37         1.28         7.86           47         ATXN3         2.66         7.99           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.25           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56         USP39         1.78         8.44           56         USP39         1.78         8.45           56         USP39         1.78         8.45           57         OTUD3         1.44         8.50           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.66           61         USP19         2.17         8.92           62	-	43	MITFIND	1.00	7.01
45         USP1         1.52         7.77           46         USP37         1.28         7.86           47         ATXN3         2.66         7.99           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.25           54         USP52         0.37         8.43           55         USP39         1.78         8.44           56         USP53         0.69         8.52           59         STAMBPL1         2.16         8.66           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.35           64         USP26         2.02         9.55           65         USP2         2.20         9.55           65	_	44	COPSS	1.25	7.08
46         USP37         1.28         7.86           47         ATXN3         2.66         7.95           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.20           53         UCHL5         2.43         8.22           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56         USP16         2.16         8.44           57         OTUD3         1.44         8.50           59         STAMBPL1         2.16         8.63           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.55           66         USP38         1.92         9.93           69 <td>_</td> <td>45</td> <td>USP1</td> <td>1.52</td> <td>1.70</td>	_	45	USP1	1.52	1.70
47         ATXN3         2.66         7.99           48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.25           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56         USP16         2.16         8.44           57         OTUD3         1.44         8.50           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.52           66         USP18         1.68         9.62           67         STAMBP         1.91         9.82           68 <td>_</td> <td>46</td> <td>USP37</td> <td>1.28</td> <td>7.86</td>	_	46	USP37	1.28	7.86
48         USP10         1.19         7.97           49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.20           53         UCHL5         2.43         8.22           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56         USP16         2.16         8.44           57         OTUD3         1.44         8.56           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.66           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.55           66         USP18         1.68         9.66           67         STAMBP         1.91         9.82           68 <td></td> <td>47</td> <td>ATXN3</td> <td>2.66</td> <td>7.95</td>		47	ATXN3	2.66	7.95
49         USP7         2.18         8.01           50         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.20           53         UCHL5         2.43         8.22           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56         USP16         2.16         8.44           57         OTUD3         1.44         8.55           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.63           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.55           66         USP18         1.68         9.66           67         STAMBP         1.91         9.92           69         USP44         2.39         10.25           70 </td <td></td> <td>48</td> <td>USP10</td> <td>1.19</td> <td>7.97</td>		48	USP10	1.19	7.97
10         USP13         1.65         8.14           51         WPIP1         0.77         8.16           52         USP52         1.59         8.22           53         UCHL5         2.43         8.24           54         USP45         0.37         8.44           55         USP45         0.37         8.44           56         USP16         2.16         8.44           56         USP16         2.16         8.44           57         OTUD3         1.44         8.50           58         USP53         0.69         8.55           59         STAMBPL1         2.16         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.55           66         USP18         1.68         9.66           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70<		49	USP7	2.18	8.01
50         100         100         0.19           51         WPP1         0.77         8.16           52         USP52         1.59         8.20           53         UCHL5         2.43         8.25           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56         USP16         2.16         8.44           57         OTUD3         1.44         8.50           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.46           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.35           64         USP26         2.02         9.53           65         USP2         2.20         9.63           66         USP18         1.68         9.63           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69		50	USP13	1.65	8 14
S1 Win 1         0.17         8.16           52 USP52         1.59         8.22           53 UCHL5         2.43         8.25           54 USP45         0.37         8.43           55 USP39         1.78         8.44           56 USP16         2.16         8.44           57 OTUD3         1.44         8.55           58 USP53         0.69         8.52           59 STAMBPL1         2.16         8.63           60 JOSD1         1.51         8.66           61 USP19         2.17         8.92           62 PARP11         1.68         9.12           63 JOSD3         1.23         9.33           64 USP26         2.02         9.51           65 USP2         2.20         9.52           66 USP18         1.68         9.69           67 STAMBP         1.91         9.92           68 USP38         1.92         9.03           69 USP44         2.39         10.25           70 EIF3S5         2.30         10.77           71 OTUB2         1.89         11.00           72 USPL1         2.30         11.46           73 USP8         1.03         14.02 <td>-</td> <td>51</td> <td></td> <td>0.77</td> <td>9.16</td>	-	51		0.77	9.16
32         USP32         1.39         8.24           53         UCHL5         2.43         8.24           54         USP45         0.37         8.44           55         USP39         1.78         8.44           56         USP16         2.16         8.44           57         OTUD3         1.44         8.50           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.55           66         USP18         1.68         9.66           67         STAMBP         1.91         9.82           68         USP28         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USP41         2.30         11.44 <td< td=""><td>-</td><td>50</td><td>LICDEO</td><td>0.77</td><td>0.10</td></td<>	-	50	LICDEO	0.77	0.10
53         UCHL5         2.43         8.22           54         USP45         0.37         8.43           55         USP39         1.78         8.44           56         USP16         2.16         8.44           56         USP39         1.78         8.44           57         OTUD3         1.44         8.56           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.66           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.35           64         USP26         2.02         9.55           66         USP28         1.08         9.66           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.77           71         OTUB2         1.89         11.00	-	52	05P52	1.59	8.20
54         USP45         0.37         8.43           55         USP39         1.78         8.45           56         USP16         2.16         8.45           57         OTUD3         1.44         8.55           59         STAMBPL1         2.16         8.66           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.35           64         USP26         2.02         9.55           65         USP18         1.68         9.66           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.77           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.44           73         USP8         1.03         14.40           74         USP4         1.00         15.86		53	UCHL5	2.43	8.25
55         USP39         1.78         8.45           56         USP16         2.16         8.45           57         OTUD3         1.44         8.50           58         USP53         0.69         8.55           59         STAMBPL1         2.16         8.63           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.55           66         USP18         1.68         9.66           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.76           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86	_	54	USP45	0.37	8.43
56         USP16         2.16         8.48           57         OTUD3         1.44         8.50           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.66           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.53           65         USP2         2.20         9.53           66         USP18         1.68         9.06           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.77           71         OTUB2         1.89         11.00           72         USP1         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86		55	USP39	1.78	8.45
57         OTUD3         1.44         8.50           58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.63           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.35           64         USP26         2.02         9.55           66         USP18         1.68         9.65           66         USP18         1.68         9.65           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.22           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.42           73         USP8         1.03         14.02           74         USP4         1.00         15.86		56	USP16	2.16	8.48
58         USP53         0.69         8.52           59         STAMBPL1         2.16         8.63           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.11           63         JOSD3         1.23         9.33           64         USP26         2.02         9.51           65         USP2         2.20         9.52           66         USP18         1.68         9.69           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.77           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86		57	OTUD3	1.44	8.50
00         01.00         01.00           59         STAMBPL1         2.16         8.63           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.53           66         USP18         1.68         9.66           67         STAMBP         1.91         9.82           69         USP38         1.92         9.93           69         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.77           71         OTUB2         1.89         11.00           72         USP1         2.30         11.44           73         USP8         1.03         14.40           74         USP4         1.00         15.86		58	USP53	0.69	8.52
Struct 1         2.10         8.05           60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.51           65         USP2         2.20         9.53           66         USP18         1.68         9.06           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.42           73         USP8         1.03         14.02           74         USP4         1.00         15.86	-	50	STAMBPL 1	2.16	8.62
60         JOSD1         1.51         8.66           61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.35           64         USP26         2.02         9.55           65         USP2         2.20         9.55           66         USP18         1.68         9.65           66         USP18         1.68         9.65           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86	-	39		2.10	0.03
61         USP19         2.17         8.92           62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.51           65         USP2         2.20         9.55           66         USP18         1.68         9.66           67         STAMBP         1.91         9.82           69         USP38         1.92         9.93           69         USP38         1.92         9.93           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USP81         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86	_	00	10501	1.51	8.68
62         PARP11         1.68         9.12           63         JOSD3         1.23         9.33           64         USP26         2.02         9.51           65         USP2         2.20         9.55           66         USP18         1.68         9.66           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.42           73         USP8         1.03         14.02           74         USP4         1.00         15.86	_	61	05P19	2.17	8.92
63 JOSD3         1.23         9.33           64 USP26         2.02         9.51           65 USP2         2.20         9.53           66 USP18         1.68         9.66           67 STAMBP         1.91         9.82           68 USP38         1.92         9.93           69 USP44         2.39         10.22           70 EIF3S5         2.30         10.75           71 OTUB2         1.89         11.00           72 USPL1         2.30         11.42           73 USP8         1.03         14.02           74 USP4         1.00         15.86	_	62	PARP11	1.68	9.12
64         USP26         2.02         9.51           65         USP2         2.20         9.52           66         USP18         1.68         9.65           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.76           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86		63	JOSD3	1.23	9.35
65         USP2         2.20         9.53           66         USP18         1.68         9.66           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.45           73         USP8         1.03         14.00           74         USP4         1.00         15.86		64	USP26	2.02	9.51
66         USP18         1.68         9.65           67         STAMBP         1.91         9.82           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.42           73         USP8         1.03         14.02           74         USP4         1.00         15.86		65	USP2	2.20	9.53
Image: Non-Stramb         Image: Non-Stramb		66	USP18	1.68	9.69
61         010001         1.01         0.02           68         USP38         1.92         9.93           69         USP44         2.39         10.25           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86	-	67	STAMBP	1.00	0.00
00         USP 30         1.92         9.93           69         USP44         2.39         10.29           70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.45           73         USP8         1.03         14.02           74         USP4         1.00         15.86	-	07	LICE20	1.01	9.02
b9         USP44         2.39         10.28           70         EIF3S5         2.30         10.79           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86	-	08	03530	1.92	9.93
70         EIF3S5         2.30         10.75           71         OTUB2         1.89         11.00           72         USPL1         2.30         11.44           73         USP8         1.03         14.02           74         USP4         1.00         15.86	_	69	05P44	2.39	10.29
71         OTUB2         1.89         11.00           72         USPL1         2.30         11.45           73         USP8         1.03         14.02           74         USP4         1.00         15.86	_	70	EIF3S5	2.30	10.79
72         USPL1         2.30         11.49           73         USP8         1.03         14.02           74         USP4         1.00         15.86		71	OTUB2	1.89	11.00
73         USP8         1.03         14.02           74         USP4         1.00         15.86		72	USPL1	2.30	11.49
74 USP4 1.00 15.86		73	USP8	1.03	14.02
14 0014 1.00 10.00	-	74	USP4	1.00	15.86
	_	74	0014	1.00	10.00

## Figure EV2. USP4 is required for BMP-SMAD signaling.

- A qRT\_PCR analysis of activin target genes SMAD7, PAI-1, P21, and P15 in USP4<sup>+/+</sup> and USP4<sup>-/-</sup> MEFs in the absence or presence of ectopically expressed USP4-WT or USP4-CS and treated with activin A (50 ng/ml) for 1 h. The values and error bars represent the mean ± SD of triplicate assays and at least two independent experiments.
- B, C The effect of USP4 wild-type (USP4-WT) and the USP4 mutant in which the de-ubiquitinating enzyme activity is inactive (USP4-CS) (B) or USP4 knockdown (shUSP4 #1 and #2) (C) on the BRE-Luc transcriptional response induced by BMP6 (25 ng/ml) in HepG2 cells. These data are presented as the means ± SD (*n* = 3). Co.vec, empty vector; Co.sh, non-targeting shRNA.
- D, E Immunoprecipitation (IP) and immunoblotting (IB) analyses of SMAD1/5-SMAD4 complex formation and SMAD1/5 phosphorylation in HepG2 cells that stably overexpressed USP4-WT or USP4-CS (D) or in which stable knockdown of USP4 by shRNA (shUSP4 #1 and #2) (E) was achieved and after stimulation with BMP6 (25 ng/ml) for the indicated times. IB for actin on TCL is included as loading control.
- F, G Effect of morpholino-mediated depletion of usp4 on activin- and bmp4-induced phenotype as measured by expression of gata5 and sox32 (F) and eve1 and bmp2 (G). These figures relate to Fig 11 and J.
- H The relative expression levels of zebrafish *vox* and *vent* as determined by qRT–PCR. The quantified mRNA levels were normalized to  $\beta$ -actin mRNA level and are presented relative to control embryos. \* indicates statistical significance (P < 0.05), and \*\* indicates statistical significance (P < 0.005). Two-tailed, unpaired *t*-test. These data are presented as the means  $\pm$  SD (n = 3).

Source data are available online for this figure.



Figure EV2.



#### Figure EV3. Genetic interaction between zebrafish usp4 and sqt.

- A The depletion of *usp4* partially inhibited the *sqt*-induced phenotype. Single-cell embryos were injected with control MO, *usp4* MO, *sqt* mRNA, or their combination. Embryos at the shield stage or 70% epiboly stage were fixed and hybridized with probes for markers of the endoderm and mesoderm including *sox32*, *gata5*, and *goosecoid* (*gsc*). Each group of embryos is oriented differently: animal pole view for *sox32*, and *gata5* panels; organizer view for the *gsc* panel.
- B The relative expression levels of zebrafish *goosecoid* (*gsc*), *sox32*, and *gata5* were monitored by quantitative real-time PCR (qRT–PCR). The quantified mRNA levels were normalized to  $\beta$ -actin and are presented relative to the control embryos. \* indicates statistical significance (P < 0.05). Two-tailed, unpaired *t*-test. Experiments were performed on at least three independent injected embryos. These data are presented as the means  $\pm$  SD.





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# Figure EV4. Regulation of SMAD4 monoubiquitination.

- A R-SMAD preferred to associate with free SMAD4 rather than monoubiquitinated SMAD4. IB analysis of the input and immunoprecipitates derived from the incubation of purified SMAD1 with SMAD4 or SMAD4 -1xUb.
- B USP4 prevented the monoubiquitination of SMAD4 upon stimulation of cells with TGF-β. IB analysis of total cell lysates (TCL) and immunoprecipitates derived from HA-Ub expressing HEK293T cells that were transfected with Myc-USP4 wild-type (WT) or Myc-USP4 C311S mutant (CS), with or without TGF-β treatment (5 ng/ml) for 1 h.
- C USP4 bound to the SMAD4 MH2 domain. HEK293T cells were transfected with Myc-tagged SMAD4 deletions and Flag-tagged USP4 CS. The cells were harvested for immunoprecipitation (IP) with anti-Flag affinity resin and subjected to IB analysis with indicated antibodies (left panel). TCL were analyzed by IB using myc antibodies (right panel).
- D, E The SMAD4 bound to the MH2 domain of SMAD3 or SMAD5. HEK293T cells were transfected with Myc-tagged SMAD3 deletions (D) or SMAD5 deletion (E) and Flagtagged SMAD4. The cells were harvested for IP with anti-Flag affinity resin and IB with the indicated antibodies (upper two panels). TCL were analyzed by IB using myc antibodies (bottom panel).
- F, G The MH2 domain of SMAD3 or SMAD5 displaced USP4 from SMAD4. IB analysis of total cell lysate (TCL) and immunoprecipitates derived from HEK293T cells that were transected with Flag-USP4 CS and Myc-SMAD3 (F) or Myc-SMAD5 (G) deletions as indicated.
- H IB analysis of immunoprecipitates derived from Flag-SMURF2-transfected HEK293T cells treated with or without activin A (50 ng/ml), BMP2 (50 ng/ml), or TGF-β (5 ng/ml) for 1 h.
- I BMP6 promoted the formation of a trimeric complex of SMURF2, SMAD1, and SMAD4. Flag-SMURF2 CA and Myc-SMAD1 were co-expressed in HEK293T cells. The cells were treated with BMP6 (50 ng/ml) for 1 h and then harvested for immunoprecipitation with anti-Flag beads and elution with Flag-peptide. The second IP with anti-SMAD4 antibody was performed, and the resulting samples were subjected to IB with anti-Flag, anti-SMAD4, or anti-Myc antibodies.

Source data are available online for this figure.





# Figure EV5. Effect of usp4 morphants on embryonic cell movements.

A Related to Fig 7A, overview of zebrafish embryos. The usp4 depletion partially inhibits smad4-induced phenotype. Single-cell embryos were injected with control morpholino (MO), usp4 MO, smad4 mRNA, or their combination (as indicated).

B Injection of *usp4* morpholino led to impaired epiboly and convergent extension (CE) movement. Single-cell embryos were injected with *usp4* MO or control morpholino (control MO). The stages of the embryos are shown, and the number of described embryos and total number are listed in the bottom.