

SUPPLEMENTAL MATERIAL

Rodgers et al., <http://www.jem.org/cgi/content/full/jem.20132486/DC1>

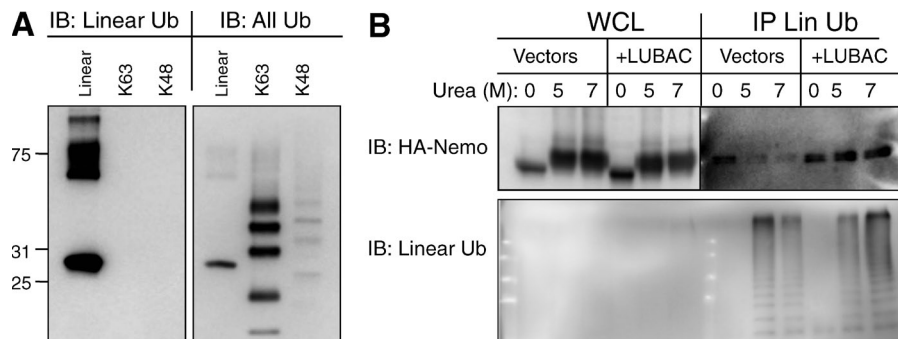
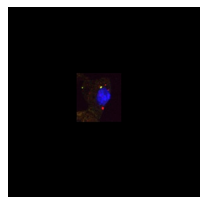


Figure S1. Controls for linear ubiquitin antibody (a) Purified K63, K48, and linear linkage ubiquitin chains were immunoblotted with the linear ubiquitin antibody or a linkage nonspecific ubiquitin antibody ("All Ub,"P4D1; Santa Cruz Biotechnology, Inc.). (b) HA-tagged Nemo was overexpressed in 293T with or without HOIL-1L and HOIP and immunoprecipitated. WCL (whole cell lysate) and IP were immunoblotted as labeled. Data are representative of three independent experiments.



Video 1. Linear ubiquitin is in the center of the ASC speck in WT BMDMs. The Z-stacks from the image in Fig. 4 c (WT Nigericin) were compiled into a 3-dimensional image and rotated in a movie.

Table S1. Transcripts with lower abundance in HOIL-1L^{-/-} BMDMs

WT versus HOIL-1L ^{-/-}	Untreated		LPS		TNF	
	Fold change	P-value	Fold change	P-value	Fold change	P-value
4930437M23Rik	-1.13	2.31 ⁻⁸	45.32	8.50 ⁻¹⁰	1.09	7.16 ⁻¹
Nxph2	1.02	1.05 ⁻⁵	33.15	2.47 ⁻⁷	-1.41	3.30 ⁻¹
chr2:176296720-176297353_F	1.67	2.02 ⁻⁹	32.38	4.99 ⁻¹¹	-1.44	4.20 ⁻²
A_55_P1964911	1.1	3.46 ⁻⁴	26.09	7.09 ⁻⁶	1.26	6.06 ⁻¹
Gm11541	-1.09	3.14 ⁻⁹	23.71	7.97 ⁻¹¹	1.04	8.13 ⁻¹
A_55_P2018054	-1.13	3.41 ⁻⁸	23.01	8.48 ⁻¹⁰	1.06	7.72 ⁻¹
chr5:77239012-77271614_F	1.07	1.23 ⁻⁷	21.3	6.67 ⁻⁹	-1.37	1.70 ⁻¹
9430018G01Rik	1.04	2.11 ⁻⁴	20.66	9.80 ⁻⁶	1.06	8.91 ⁻¹
ENSMUST00000108880	1.15	2.51 ⁻⁶	17.26	1.98 ⁻⁶	1.78	1.11 ⁻¹
Gm5072	1.35	6.45 ⁻⁵	15.39	1.59 ⁻⁶	-1.31	4.14 ⁻¹
ENSMUST00000112869	1.56	3.79 ⁻⁵	15.02	2.59 ⁻⁶	-2.3	2.55 ⁻²
ENSMUST00000101229	-1.6	8.64 ⁻⁵	13.81	4.85 ⁻⁶	-1.49	2.58 ⁻¹
ENSMUST00000120605	1.01	8.46 ⁻⁶	13.41	2.12 ⁻⁷	1.13	6.42 ⁻¹
Gm3853	1.19	3.53 ⁻⁶	11.66	8.84 ⁻⁸	-1.11	6.46 ⁻¹
ENSMUST00000046816	-1.02	1.58 ⁻⁵	11.63	2.01 ⁻⁶	-1.03	9.25 ⁻¹
chr9:46894029-46973977_R	1.44	4.52 ⁻⁷	10.46	1.59 ⁻⁸	-2.22	7.38 ⁻⁴
Mblac2	1.05	9.37 ⁻¹⁴	9.81	1.44 ⁻¹²	-1.23	2.03 ⁻²
Pkp1	3.34	3.93 ⁻⁶	9.79	7.99 ⁻⁶	-1.13	7.01 ⁻¹
ENSMUST00000108490	1.17	1.47 ⁻⁶	9.6	3.89 ⁻⁸	-1.06	7.50 ⁻¹
Zp2	1.11	5.31 ⁻⁸	9.58	1.63 ⁻⁹	-1.08	5.88 ⁻¹
ENSMUST00000063858	-1.04	7.56 ⁻⁵	9.49	4.11 ⁻⁶	-1.03	9.26 ⁻¹
A_55_P2135855	1.33	5.73 ⁻⁵	9.44	5.30 ⁻⁶	-1.31	3.72 ⁻¹
Slc17a7	-1.83	1.58 ⁻⁵	9.28	4.95 ⁻⁶	1.15	6.37 ⁻¹
chr6:140904129-140992655_F	1.07	3.60 ⁻⁵	9.22	8.15 ⁻⁷	1.1	7.01 ⁻¹
NAP059579-1	-1.12	1.61 ⁻⁷	8.34	7.37 ⁻⁸	1.03	8.66 ⁻¹
AK090094	1.94	3.66 ⁻⁵	8.02	6.31 ⁻⁶	-1.21	5.03 ⁻¹
Tubal3	2.01	7.52 ⁻⁸	7.88	1.38 ⁻⁶	-1.18	4.89 ⁻¹
Wfdc5	1.24	4.77 ⁻⁶	7.48	1.03 ⁻⁶	-1.14	5.59 ⁻¹
Cxadr	1	4.72 ⁻²⁰	6.4	2.14 ⁻¹²	-1.06	4.05 ⁻¹
Rslcan18	-1.01	3.87 ⁻¹⁰	5.98	6.66 ⁻⁸	-1.11	5.00 ⁻¹
A_55_P2025258	1.15	1.47 ⁻⁵	5.79	2.09 ⁻⁷	-1.13	4.82 ⁻¹
Lonrf2	1.09	1.14 ⁻⁸	5.75	3.07 ⁻¹⁰	-1.02	8.00 ⁻¹
TC1663651	1.33	2.14 ⁻⁵	5.74	5.60 ⁻⁷	1.4	8.86 ⁻²
A_55_P2126740	-1.25	6.07 ⁻⁵	5.49	1.48 ⁻⁶	1.34	1.58 ⁻¹
Nell2	-1.14	2.20 ⁻¹²	5.3	9.67 ⁻⁷	-1.01	9.78 ⁻¹
Tram111	-1.91	9.13 ⁻¹⁵	5.12	1.66 ⁻⁷	1.55	1.36 ⁻²
AK052049	-1.15	5.69 ⁻⁵	5.1	7.59 ⁻⁶	2.72	6.31 ⁻⁴
Vmn1r45	1.05	6.64 ⁻⁵	4.93	1.49 ⁻⁶	1.36	1.22 ⁻¹
Grem2	1.53	4.13 ⁻¹⁴	4.76	1.10 ⁻⁷	2.29	7.02 ⁻⁵
Prrx2	1.67	6.21 ⁻¹⁵	4.74	6.50 ⁻⁶	3.96	2.18 ⁻⁵
Tas2r139	1.46	3.46 ⁻⁶	4.53	1.47 ⁻⁷	-1.33	6.43 ⁻²
AW050000	-1.58	6.14 ⁻⁶	4.25	4.98 ⁻⁷	2.41	7.33 ⁻⁵
ENSMUST00000099198	1.99	1.49 ⁻⁶	4.03	1.47 ⁻⁶	1.87	2.02 ⁻³
Ahi1	1.32	2.89 ⁻¹¹	4.03	4.21 ⁻⁸	-1.66	8.65 ⁻⁴
1700088E04Rik	1.14	3.49 ⁻⁸	3.98	4.55 ⁻⁶	1.45	5.72 ⁻²
Dusp9	1.29	2.75 ⁻¹¹	3.97	1.21 ⁻⁶	1.31	1.08 ⁻¹
Fam171a1	1.55	6.82 ⁻¹⁴	3.84	4.02 ⁻⁶	1.63	1.37 ⁻²

The abundance of each transcript in WT BMDM samples was compared with the abundance in HOIL-1L^{-/-} BMDMs in untreated, LPS-treated, or TNF-treated conditions and the fold change was determined for each treatment condition. Transcripts are listed here that had >3-fold difference and a p-value < 1 × 10⁻⁵. Negative values indicate a lower abundance in WT samples and positive values indicate a higher abundance in WT samples.

Table S2. Transcripts with higher abundance in HOIL-1L^{-/-} BMDMs

WT versus HOIL-1L ^{-/-}	Untreated		LPS		TNF	
	Fold change	P-value	Fold change	P-value	Fold change	P-value
ENSMUST00000103305	-1.21	6.54 ⁻¹	-45.95	7.55 ⁻⁷	-1.02	9.62 ⁻¹
6030405A18Rik	1.1	8.31 ⁻¹	-43.17	1.62 ⁻⁶	1.27	5.97 ⁻¹
ENSMUST00000089097	1.08	8.38 ⁻¹	-27.87	1.46 ⁻⁶	-1.54	2.78 ⁻¹
Tmcc2	1.24	2.78 ⁻¹	-11.26	2.23 ⁻⁸	-1.14	5.00 ⁻¹
G530011006Rik	-1.47	2.10 ⁻¹	-10.7	3.19 ⁻⁶	-2.81	4.04 ⁻³
Kcns3	1.36	2.00 ⁻¹	-8.51	7.44 ⁻⁷	-1.16	5.31 ⁻¹
Wdr20b	-1.1	7.13 ⁻¹	-7.57	2.43 ⁻⁶	1.14	5.90 ⁻¹
ENSMUST00000147634	1.08	7.56 ⁻¹	-7.09	3.35 ⁻⁶	-1.07	7.72 ⁻¹
Gm10499	1.72	6.12 ⁻²	-6.71	9.71 ⁻⁶	1.02	9.48 ⁻¹
Olfr460	-1.83	1.25 ⁻²	-6.04	1.57 ⁻⁶	1.27	2.65 ⁻¹
Mid1	-2.01	2.46 ⁻³	-5.88	4.96 ⁻⁷	-5.23	1.02 ⁻⁶
Trim45	-1.03	8.47 ⁻¹	-4.38	4.87 ⁻⁷	1.07	6.74 ⁻¹
4933429K18Rik	1.25	3.40 ⁻²	-4.23	3.13 ⁻⁹	-1.14	2.01 ⁻¹
Pydc3	-1.61	8.63 ⁻⁶	-3.92	6.81 ⁻¹¹	-1.77	1.31 ⁻⁶
Rhox11	1.2	5.21 ⁻²	-3.63	3.05 ⁻⁹	-1.27	1.51 ⁻²
Ccdc64	1.16	1.51 ⁻¹	-3.4	3.10 ⁻⁸	-1.16	1.58 ⁻¹
Ccdc46	1.41	5.30 ⁻²	-3.4	5.39 ⁻⁶	1.12	4.90 ⁻¹
Galnt1	1.28	5.71 ⁻²	-3.17	4.23 ⁻⁷	-1.1	4.34 ⁻¹
2810432L12Rik	1.23	5.29 ⁻²	-3.16	6.39 ⁻⁸	1.01	9.02 ⁻¹
Nkain3	-1.06	5.88 ⁻¹	-3.14	2.28 ⁻⁷	1.14	2.41 ⁻¹
A_55_P2045380	-1.14	3.79 ⁻¹	-3.1	5.42 ⁻⁶	-1.28	1.19 ⁻¹
D330045A20Rik	-1.27	1.01 ⁻²	-3.06	6.97 ⁻⁹	-1.22	2.44 ⁻²

The abundance of each transcript in WT BMDM samples was compared to the abundance in HOIL-1L^{-/-} BMDMs in untreated, LPS-treated, or TNF-treated conditions and the fold change was determined for each treatment condition. Transcripts are listed here that had >3-fold difference and a P value <1 × 10⁻⁵. Negative values indicate a lower abundance in WT samples and positive values indicate a higher abundance in WT samples.

Table S3. qPCR primers in 5'→3' orientation

Gene	Forward primer	Reverse primer
18S	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG
IL-1β	AAGGGCTGCTTCCAAACCTTTGAC	ATACTGCCTGCCTGAAGCTCTGT
TNF	CTTCTGTCTACTGAACTTCGGG	TGATCTGAGTGTGAGGGTCTG
IL-6	CAAAGCCAGAGTCCTTCAGAG	GTCCCTAGCCACTCCTCTG
IκBα	ACGAGCAAATGTTGAAGGAG	TTCTGGAAGTTGAGGAAGGC
ICAM1	AAGGAGATCACATTCACGGTG	TTTGGGATGGTAGCTGGAAG
MyD88	GAGTCCGAGAAGCCTTTACAG	GTCTGTCTAGTTCGCCGATC
IL-1α	AGTTCTGCCATTGACCATCTC	TCTCAGAATCTTCCCCTTGC
IL-10	GCCAAGCCTTATCGGAAATG	AAATCACTCTTCACTGCTCC
IL-12b	CATTGAACTGGCGTTGGAAG	TGAGGGAGAAGTAGGAATGGG
COX2	GACTCTGCTCACGAAGGAAC	TGCTCATACTTCCCCACG