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SUMO enriched proteome for *Drosophila* innate immune response

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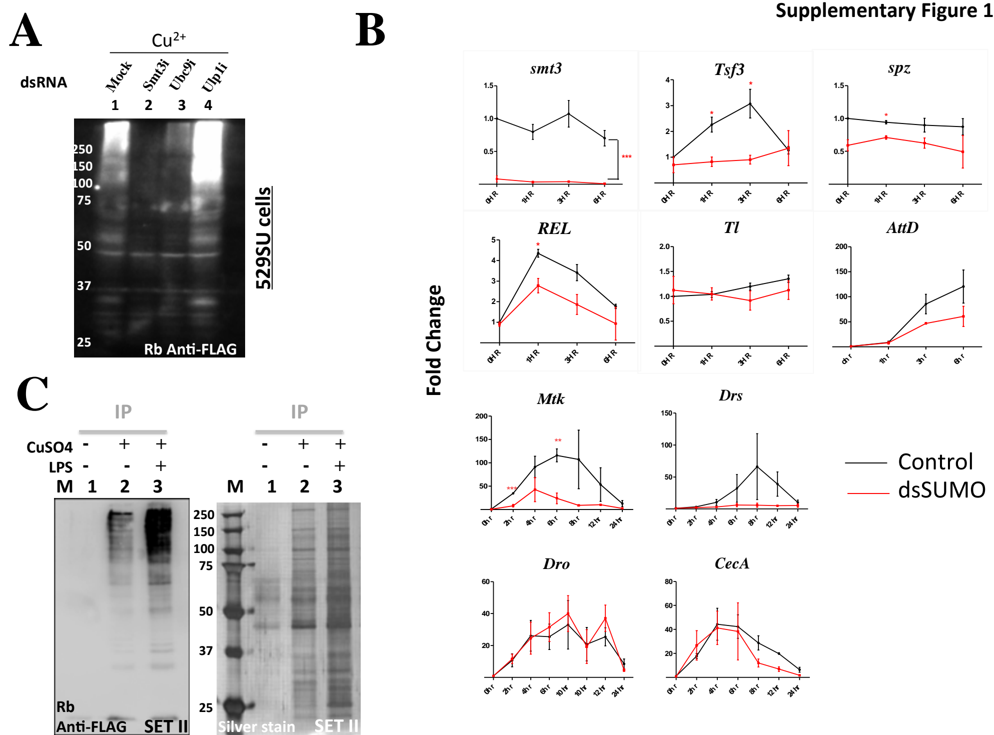

Figure S1

A. Global changes to the SUMO proteome on knockdown of *SUMO*, *Ubc9* and *Ulp1*. A stable cell line (529SU), expressing FLAG-SUMO and HA-Ubc9 under a Copper inducible metallothionein promoter was used to express tagged SUMO cycle components. FLAG-SUMO conjugates to a large number of proteins, generating a 'SUMO' ladder in the Western blot (lane 1) of an SDS-PAGE gel where equal numbers of lysed cells are loaded in each lane. On dsRNA interference of *SUMO* (*Smt3i*; lane 2) or *Ubc9* (*Ubc9i*; lane 3) transcripts in cells, there is a global decrease in SUMOylation, while reduction of *Ulp1* (*Ulp1i*, Lane 4) leads to a global increase of SUMOylation.

B. Regulation of defense genes. Real Time PCR is used to measure the up-regulation of defense genes, in response to a mock infection (LPS; 10 ug/ml), over a time period of 6 days in 529SU cells. Graph shows relative abundance of transcript levels of wild type (**black line**) and after SUMO knockdown (~90%; red line). In each panel, plots for average and standard deviation (whiskers) for three biological replicates are displayed. Data for the top two rows are collected using TaqMan probes while those in the bottom two rows are using SYBR green to measure transcript levels. The data indicates differential changes to the activation of downstream NF-kappaB genes in the absence of *SUMO*. Distinct temporal changes were seen in the expression of *Tsf3, REL, Mtk, Drs* and *AttD* but not for *CecA, Dro* and *Tl*. Data for *Dipt, Def, AttA,B, SAE1 and SAE2* also did not show significant changes in their transcriptional profiles. A red asterisk denotes a $p<0.01$, while two (**) denote a $p<0.005$ and '***' a $p<0.001$. The *p* values were calculated using an unpaired students t-test. The mechanistic reasons for effects on the transcriptional profile of some target genes and not on others is unclear.

C. Affinity purification of SUMOylated proteins (Set II): 529SU cells lysates, pre and post LPS treatment, that were affinity purified using Mouse anti-FLAG agarose (Sigma). 5% of the total, affinity purified FLAG eluate was loaded on the gels. Lane 1 is the affinity purified, un-induced master control, showing non-specific pull down that is ≥5% of the total protein, while Lane 2 & 3 are affinity purification of induced cells with (lane 3) and without (lane 2) LPS. The panel on the LHS side shows a Western blot probed with Rb anti-FLAG while that on the RHS shows a silver stained gel.

Tables S1-S2

Available for download at www.g3journal.org/lookup/suppl/doi:10.1534/g3.115.020958/-/DC1

Table S1. **An xls database file with processed ITRAQ data.**

Sheet A) (Row 2-1620) List of 1619 unique proteins that reflect the SUMO enriched immune proteome, ITRAQ ratio for each Set (1,2 &3), arranged in descending order of average ITRAQ ratio. Rows 1623-1818 (Cells painted RED) lists the proteins identified in the Master Control; these have not been included in the 1629 list. Rows 1821-1840 (cells painted YELLOW) represent proteins found in the Master Control dataset, but not common to any of the biological replicates (1, 2 or 3).

Sheet B) List of 923 proteins that are represented in two of the three biological replicates.

Sheet C) List of 710 proteins that are represented in two of the three biological replicates and have a ITRAQ ration $\geq$ 2.00. This represents the confident set used for global analysis or protein domains, pathways and complexes.

Table S2. **Raw data from all three ITRAQ data sets**. Three xls files (Set1, Set 2, Set3) that are zipped into one file. These tables contain data on peptides identified (Gene Symbol, Description, Score, Coverage, Peptides, ITRAQ fold change).