Supporting Information

Yun *et al*. 10.1073/pnas.0707025105

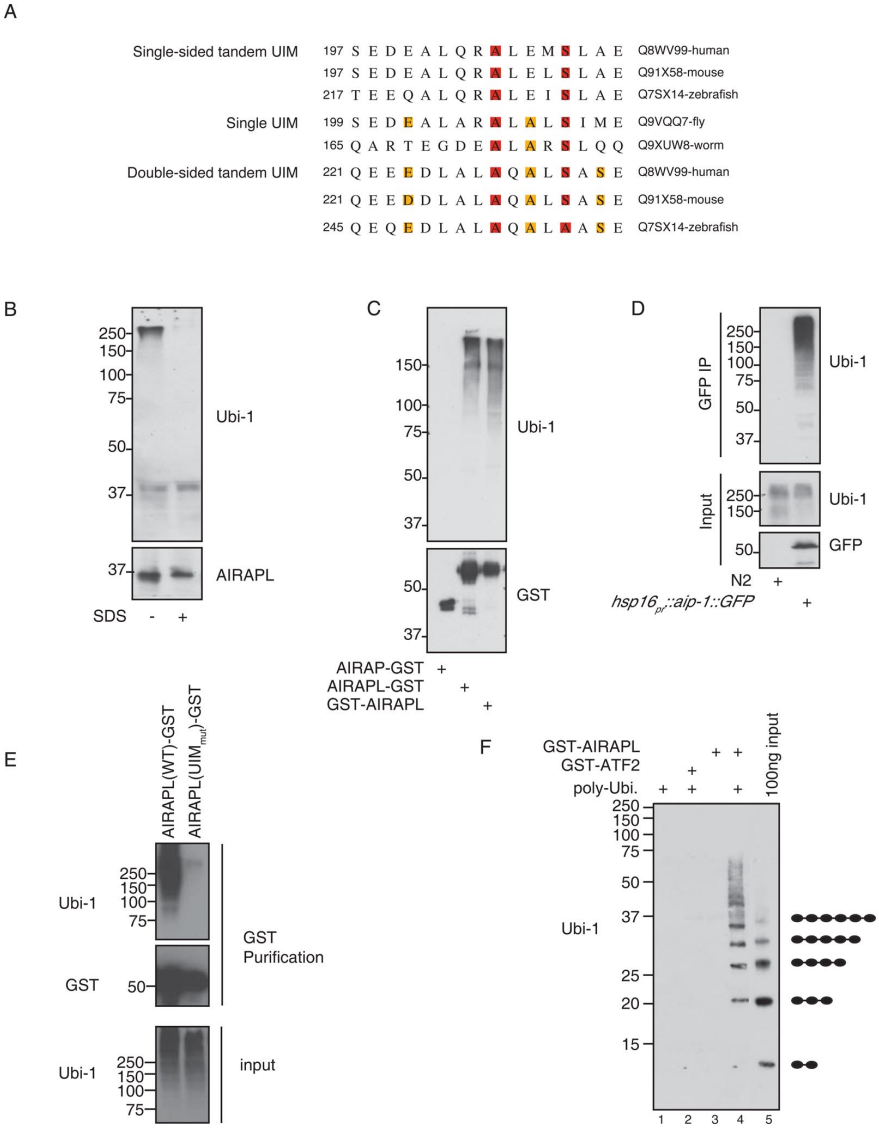

Fig. S1. AIRAPL binds polyubiquitin via its ubiquitin-interacting motifs (UIMs). (*A*) UIM alignment of AIRAPL from the indicated species. The SwissProt/TrEMBL accession numbers and species are given in the rightmost column. Residues in positions important for conventional (one-sided) UIMs are in red; residues in positions corresponding to site 2 in the double-sided UIMs are in orange (1). (*B*) Immunoblot of endogenous polyubiquitinated proteins in complex with endogenous AIRAPL immunoprecipitated from lysates of 293T cells exposed to arsenite. Where indicated, the complex was disrupted by treating the lysate with 2% SDS before dilution to 0.1% SDS and immunopurification. The AIRAPL immunoblot (*Lower*) reports on the recovery of endogenous AIRAPL (''the bait'') in the immune complex. (*C*) Immunoblot of endogenous polyubiquitinated proteins in complex with the indicated GST fusion proteins purified from lysates of transfected 293T cells by glutathione affinity chromatography. (*D*) Immunoblot of endogenous polyubiquitinated (poly-Ubi.) proteins in complex with AIP-1::GFP immunopurified (IP) with anti-GFP serum from lysates of *hsp-16*$_{pr}$::*aip-1*::*gfp* transgenic worms after heat shock induction of the transgene. Identically treated parental (nontransgenic N2) worms serve as a negative control. (*E*) Immunoblot of endogenous polyubiquitinated proteins in complex with wild-type AIRAPL-GST (WT) and triple mutant AIRAPL-GST (Ala205→Gln, Ala228→Gln, Ala230→Gln) with inactivating mutations in both UIMs (UIM$_{mut}$) purified from lysates of transfected 293T cells by glutathione affinity chromatography. (*F*) Immunoblot of ubiquitin multimers recovered in complex with bacterially expressed GST-AIRAPL and GST-ATF2 (a control) immobilized on glutathione Sepharose following incubation with pure, *in vitro* assembled polyubiquitin (BostonBiochem) and extensive washing of the resin as described (1). The input material is presented in lane 5. The length of the ubiquitin chain is cartooned to the right of the immunoblot. Note that high molecular weight ubiquitin polymers bind preferentially to GST-AIRAPL in this assay.

1. Hirano S, *et al.* (2006) Double-sided ubiquitin binding of Hrs-UIM in endosomal protein sorting. *Nat Struct Mol Bio* 13:272–277.

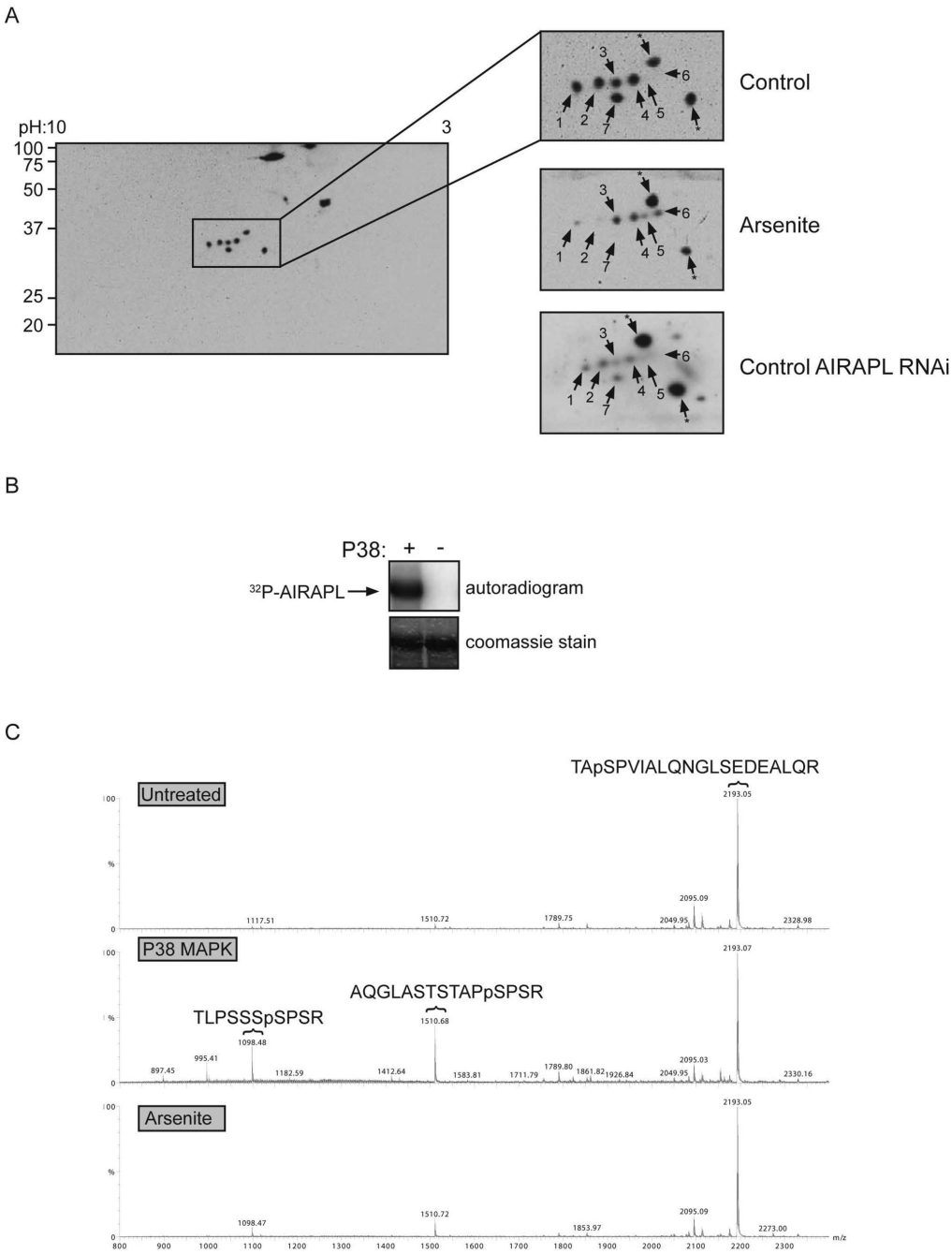

Fig. S2. Characterization of AIRAPL phosphorylation. (*A*) AIRAPL immunoblot of proteins in lysates from untreated, wild-type, and AIRAPL RNAi knockdown cells and arsenite-treated wild-type 293T cells resolved on a two-dimensional PAGE (pI 3–10) and transferred to a nitrocellulose filter. Note the arrangement of spots (1–6) on a diagonal line characteristic of phosphoisomers and the shift to a preponderance of more acidic, slower-migrating spots following exposure to arsenite. Asterisks indicate irrelevant proteins reactive with the AIRAPL-antiserum that are not reduced in intensity by AIRAPL RNAi in 293T cells. The significance of spot 7 remains unknown. (*B*) Autoradiogram and Coomassie stain of an SDS/PAGE of bacterially expressed GST-AIRAPL phosphorylated with purified constitutively active P38α MAP kinase in the presence of γ^{32}P-labeled ATP *in vitro*. (*C*) Mass spectra and MS-MS sequencing of tryptic phosphopeptides derived from purified AIRAPL-GST expressed in 293T cells. Where indicated, the cells were cotransfected with active P38α MAP kinase or exposed to arsenite. Tryptic phosphopeptides were enriched by using titanium dioxide chromatography. The sequence of phosphopeptides whose level increased after arsenite exposure or P38α MAP kinase coexpression was derived by MS-MS fragmentation and is indicated above the corresponding peaks. (*D*) Sequence of the mouse AIRAPL (accession number Q91X58). The tryptic phosphopeptides identified in *C* are highlighted, and phosphorylated residues are bolded. The two UIMs are underlined, and the residues mutated in the constructs used in Figs. S1*E* and S3 are boxed. (*E*) Immunoblot of wild-type and mutant AIRAPL proteins and endogenous PSMA1 recovered in association with proteasome immunopurified (IP) with a monoclonal antibody to PSMA1 from transfected AIRAPL (RNAi) 293T cells. Where indicated, the cells were exposed to arsenite (ARS). Residues Ser-163, Ser-173, and Ser-187 were mutated to alanine in AIRAPL S $\rightarrow$ A or glutamic acid in AIRAPL S $\rightarrow$ E. Note that these mutations do not affect proteasome association of AIRAPL.

D

Q91X58

MEFPDLGAHC SEPSCQRLDF LPLKCDACSG IFCADHVAYA QHHCGSAYQK DIQVPVCPLC
NVPVPVARGE PPDRAVGEHI DRDCRSDPAQ QKRKIFTNKC ERSGCRQREM MKLTCDRCGR
NFCIKHRHPL DHECSGEGHQ TSRAGLAAIS RAQGLASTST APSPSRTLPS SSSPSRATPQ
LPTRTASPVI ALQNGLSEDE ALQRALELSL AEAKPQVLSS QEEDDLALAQ ALSASEAEYQ
QQQAQSRSLK PSNCSLC

E

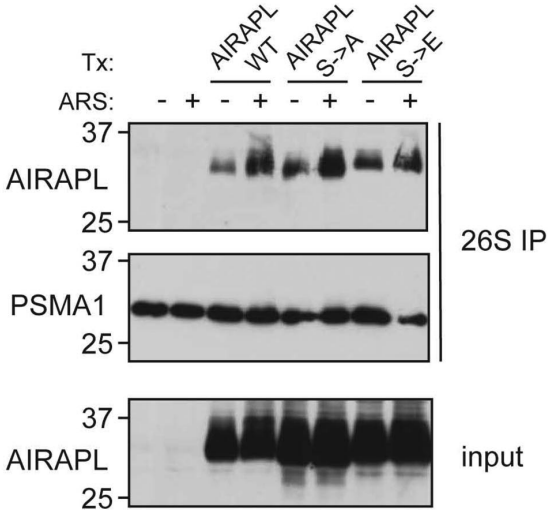

Fig. S2. *Continued*

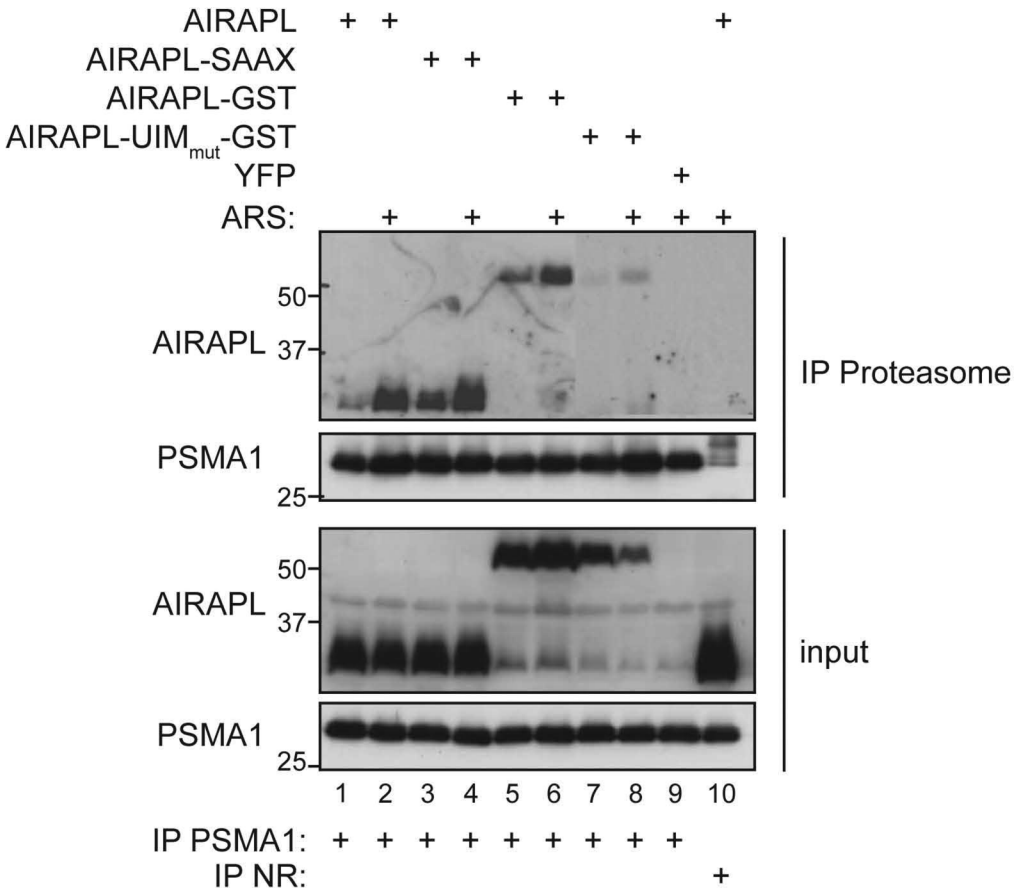

Fig. S3. Neither the CAAX nor the UIM motives are required for regulated association of AIRAPL with proteasomes. An immunoblot of wild-type and mutant AIRAPL proteins and endogenous PSMA1 recovered in association with proteasomes immunopurified with a monoclonal antibody to PSMA1 from transfected AIRAPL(RNAi) 293T cells is shown. Where indicated, the cells were exposed to arsenite (ARS). The Cys254→Ser mutation converts the CAAX to SAAX, and the Ala205→Gln, Ala228→Gln, and Ala230→Gln mutations inactivate the UIMs. Note that these mutations do not affect proteasome association.

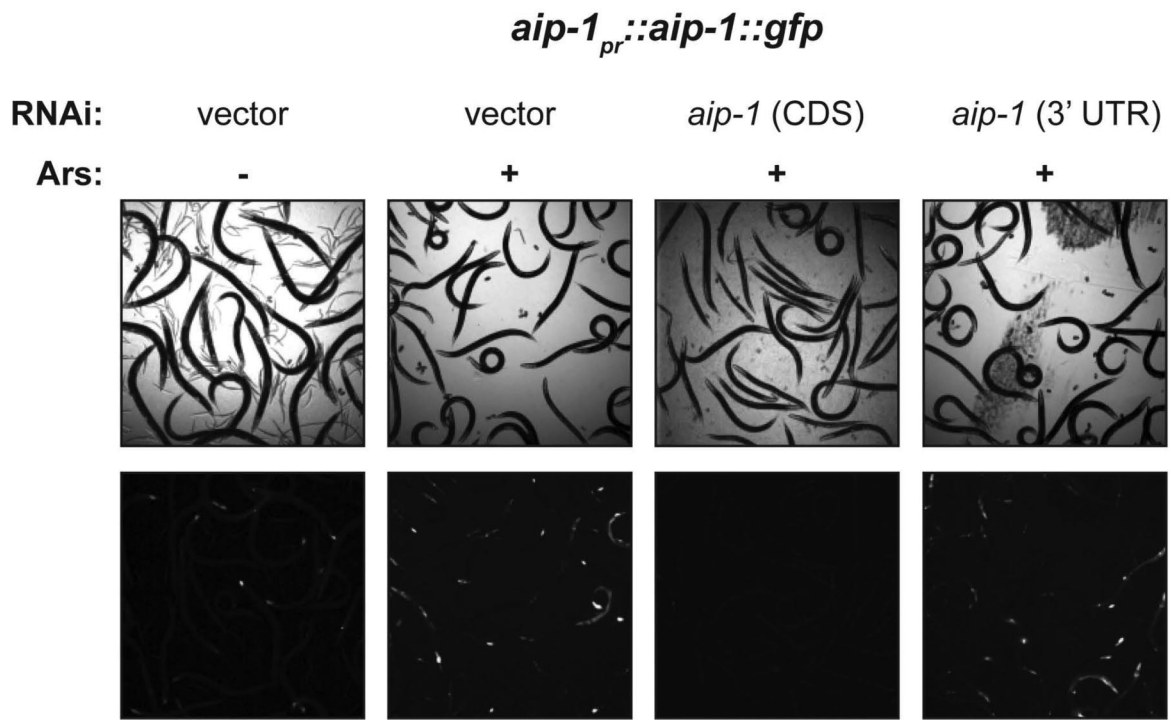

Fig. S4. Selective inactivation of the *aip-1*$_{pr}$::*aip-1:gfp*-rescuing allele with coding-sequence directed *aip-1*(RNAi). Bright field (*Upper*) and fluorescent photomicrographs (*Lower*) of *aip-1*$_{pr}$::*aip-1:gfp* transgenic worms that had been raised on bacteria expressing mock double-stranded RNA (vector) or RNAi constructs targeted to the *aip-1* coding sequence (CDS) or *aip-1* 3′-UTR are shown. Where indicated, the animals were exposed to arsenite (Ars) as described [Sok J, *et al.* (2001) Arsenite-inducible RNA-associated protein (AIRAP) protects cells from arsenite toxicity. *Cell Stress Chaperones* 6:6–15]. Note that the transgene, which has a heterologous 3′-UTR, is selectively silenced only by the coding sequence-directed RNAi construct.

Table S1. *aip-1* is dispensable for dauer formation

Strain	Gene inactivation by RNAi	dauer, %	*n*
daf-2 (e1370)	vector	100	713
daf-2 (e1370)	*daf-16*	45	777
daf-2 (e1370)	*aip-1*	100	695
N2	*vector*	0	850
N2	*daf-2*	17	588
N2	*daf-16*	0	815
N2	*aip-1*	0	760

Dauer formation of N2 and *daf-2(e1370)* animals at 25°C under the indicated RNAi conditions. Dauer formation was calculated as the percentage of animals resisting 1% SDS treatment. Wild-type (N2) and *daf-2(e1370)* strains were grown at 16°C. Once gravid, animals were treated with bleach to release fertilized eggs and placed at 25°C. After 72 h, plates were flooded with 1% SDS, and the percentage of surviving SDS-resistant dauer animals was calculated. Dauer assays were repeated three times.