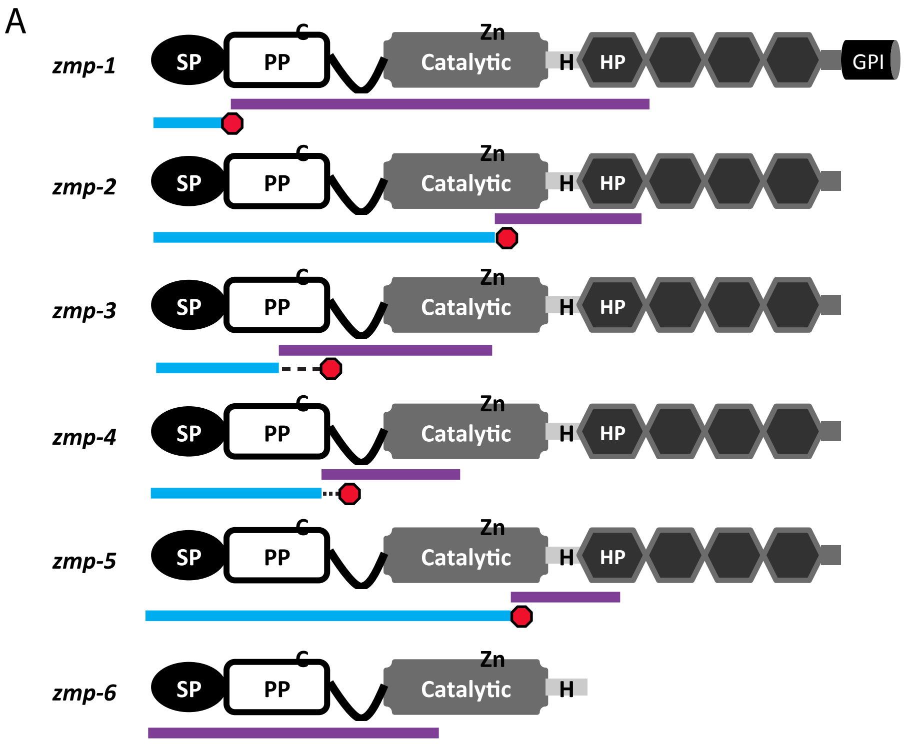
Kelley et al., Supplementary Figure 1



zmp-1 (cg115): 2306 bp (318 aa) deletion;
frameshift with premature stop codon

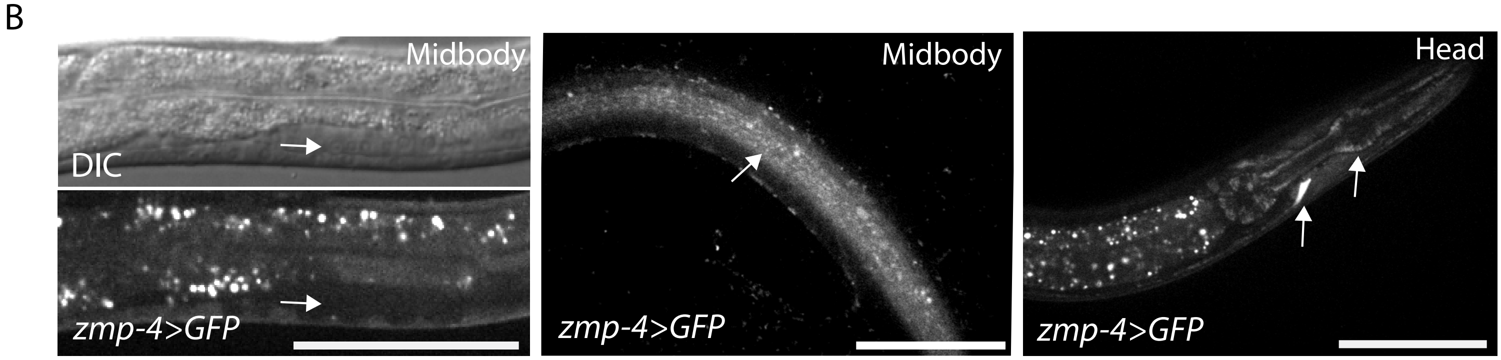
zmp-2 (tm3529): 290 bp (79 aa) deletion;
framshift with premature stop codon

zmp-3 (tm3482): 298 bp (100 aa) deletion;
frameshift with stop codon after 28 aa

zmp-4 (tm3484): 438 bp (77 aa) deletion;
frameshift with stop codon after 11 aa

zmp-5 (tm3209): 153 bp (50 aa) deletion;
frameshift with premature stop codon

zmp-6 (tm3073): 391 bp (82 aa) deletion;
start codon deleted; no predicted construct



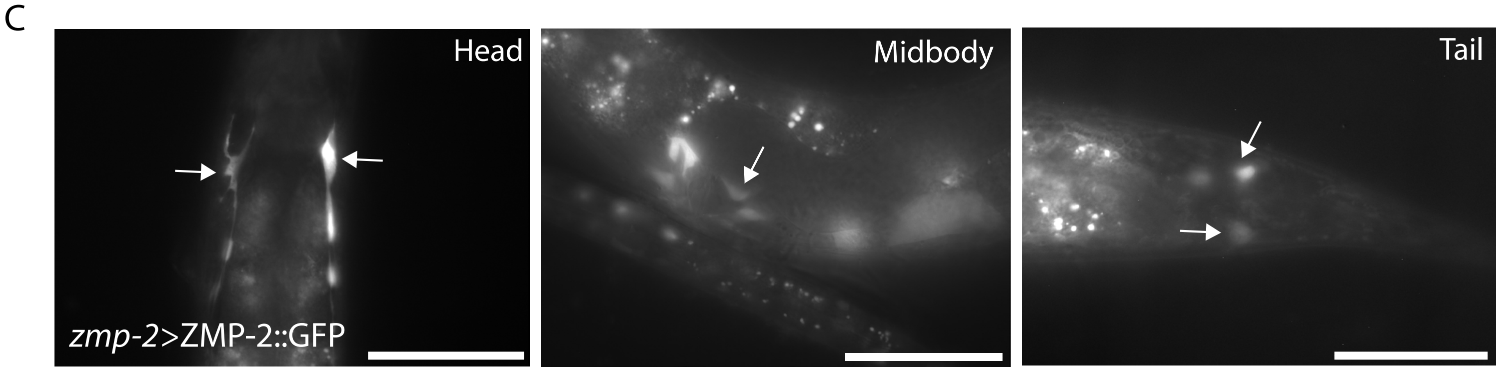


Figure S1. *C. elegans* MMP structure, mutants, and expression; Related to Figure 1 and Table 1. (**A**) Schematic representation of the protein domain structure of the six MMP (ZMP-1 through-6) orthologs are shown. All contain the catalytic metalloproteinase domain harboring the zinc (Zn) binding motif. Archetypal MMPs also include a signal peptide domain (SP), propeptide domain (PP) with a cysteine switch motif (C), a hinge region (H), and hemopexin-like domain (HP). The number of nucleotide base pairs (bp), the resulting number of amino acids (aa, referenced by span of the purple line) deleted in each *zmp* mutant allele ((*zmp-1 (cg115); zmp-2 (tm3529); zmp-3 (tm3482); zmp-4 (tm3484); zmp-5 (tm3209); zmp-6 (tm3073))*, and a brief description of the predicted protein product (referenced by blue lines, stop codon shown by red octagon) is written to the right of each structure. (**B**) Expression of the transcriptional reporter for *zmp-4 (zmp-4*>GFP*)* is not detectable in the uterine tissue (left, DIC and fluorescence, arrow points to the AC), but is visible in the body wall muscle (arrow in middle image) and in cells in the head (arrows) at the L3 larval developmental stage when AC invasion occurs. Scale bars, 100 μm. (**C**) The translational reporter for zmp-2 (*zmp-2*>ZMP-2::GFP) is expressed in the vulval muscles (middle image, see arrow) and in multiple cells in the head and tail (see arrows in left and right images) at the L4 developmental stage. Scale bars, 20 μm.

Kelley et al., Supplementary Figure 2

```
                                                                                                        
zmp-1 CCD65979  1     --MFTqLHDILIIILFLLVTLKIA----------------------------------QNVDHTKFLQKYGYL-------   36
zmp-2 CCD72394  1     mtKWSpNGNPLSTIYLILSLFTLAHTApt----TQHSRTTTQLRLEDED-ggggvdedSIHFVKGQMEKYGYL-------   68
zmp-3 CCD66298  1     ------MRLIYVIAILLVSTCQAGFFSslvsrfTGGGNSSPSSSSSSSSfsnsrkpslSDEKARSYLQTFGYVppsnslq   74
zmp-4 CAB01669  1     --MWP------LFLLGIIGVVSAGIPG------TNGDIRQPNFQKELMD------------EAKVYLFKFGYMip----e   50
zmp-5 CAB08559        --------------------------------------------------------------------------------
zmp-6 CCD61839        --------------------------------------------------------------------------------

                                                        cysteine switch
                                      v
zmp-1 CCD65979  37    ----TSGDNQLSSESLSDALKNMQRMAGLEETGELDERTIQMMERPRCGHPDVEDHQKSRGKRYAPPQF-KWKE-----K   106
zmp-2 CCD72394  69    -----KGIDHSSPQEFRQALMFFQEVLEVEQTGNVDEMTVEAASKPRCTQTDVRQEQTKRTKRFTLSKRaKWAHasgqsV   143
zmp-3 CCD66298  75    srngMAGDIQSAEQVFKSAIRKFQEFAGIAKTGFLDAATKAKMALSRCGVTDAPLALTSGSS-----QF-KWSK-----T   143
zmp-4 CAB01669  51    phqnVKTIQEPSEKQTKDALIRFQSAFLYYSSGDVDVPTQAKMNEWRCANNDMDKGQPIPPAS---KKE-LWTK-----K   121
zmp-5 CAB08559  1     ----------------MTVWRRWWYLYILSGFIINNVMLESKSRVKRYSIEGS ----------------YWGK-----S   42
zmp-6 CCD61839  1     ----------------MRSILLFILIFLIFAISEAQENIDKNLDFIKPIGFGSRE------KRYVIRAK-RWKK-----H   52
                                      ^

zmp-1 CCD65979  107   IITYGCKAVgTSTRISLDDLRRTMHQAASQWSELA--------DVEIVESSVK--NP---MMVISAGRENHYPcTVRFDT   173
zmp-2 CCD72394  144   TLKWYISDY-TS-DIDRLETRKVVEKAFKLWSSQSyiknekkvTLTFQEAS----SKDEADINILWAEGNHGD-EHDFDG   216
zmp-3 CCD66298  144   RLTYSIESW--SSDLSKDDVRRAISEAYGLWSKVT--------PLEFSEVPAG--ST--SDIKIRFGVRNHND-PWPFDG   208
zmp-4 CAB01669  122   SLTYKVVNT--PNTLSQAQIRSAAHEAFEQWTRAS--------GFKFVETTGA--T---PDITITFYDVPQSN--LRIAG   184
zmp-5 CAB08559  43    VITYRLKQP--SQRMSLSQQKAVFARAFATWEEHT--------RLWFVAVDDE--DEQKANIDIVFAAGDHED-GEPFDG   109
zmp-6 CCD61839  53    TLTWQLQTQ-NLLDPDVFIVRNTMHRAFNEWSTVS--------SVDFREIPPDlvTKQPPDIYIAFEKGEHSD-GFPFDG   122

                                                                  active site
zmp-1 CCD65979  174   -----------KTLAHAFFPT-----NGQIHINDRVQFAMTNY--TERMGANSLYSVVAHEMGHALGFSHSPDIDSVMFA   235
zmp-2 CCD72394  217   angkiegnkKENVLAHTFFPGyarplNGDIHFDDAEDWEIDVDQvGHGSNKRFFPYVLAHEIGHALGLDHSQKADALMHP   296
zmp-3 CCD66298  209   ---------EGGVLAHATMPE-----SGMFHFDDDENWTYKDARkIHNNEATDLLAVAIHEGGHTLGLEHSRDENAIMAP   274
zmp-4 CAB01669  185   ---------S------ASKPV-----NSHIILDKNQEWAYKSQA----PMGISLYHTLLHEIGHILGLPHTFYRGSIMHP   240
zmp-5 CAB08559  110   ---------KGNILAHAFFPRy----GGDVHFDEDELWS------ANKTKGVDLYAVAVHEIGHSLGLKHSSNHLSIMAP   170
zmp-6 CCD61839  123   ---------QDGVVAHAFYPR-----DGRLHFDAEEQWSLN------SVEGVNLFQTAVHEIGHLLGLEHSMDVRAAMFA   182

                                                   v                                              *
zmp-1 CCD65979  236   YDTPRKWK--------FTSMDKYNMRSYYGAKASKKENEEEErktENEDKR---RKTEKDR---grTREHESDDIRPNEC   301
zmp-2 CCD72394  297   YYKNVPINE-I----QLDIDDKCGVIWNYG--------GASD---FCLY-VWLMSQIVEAHN----SSAQNNHGVGSITS   355
zmp-3 CCD66298  275   FYQKTTDSSGNyvYPNLKSDDISAIQAIYGAGSGRSSSGSDFggsSGGGSRTTARPTTTTRSwfgrFFGDDDDDVRSRTT   354
                                                                              *
zmp-4 CAB01669  241   IFKPVLLPHGT--VDTVPNVDRLAIRKIYGLSSVDHSTPS----------------SDVDR-------------------   283
zmp-5 CAB08559  171   FYKQYTGA-----VMHLHQDDISAVKRLYGA------------------------------------------PVKIRKK   203
zmp-6 CCD61839  183   AKR--PYDPAF----TLGDDDVRAIRSLFPINETDANSGSEE---NSEDPVTTVKPISKEEG----IDEENND---PFDA   246
                                                   ^

zmp-1 CCD65979  302   RVENPI----------------------------------------VVQYR-GEYLIFKSQWVWRVSSDWKrLIIKAVPI   340
zmp-2 CCD72394  356   SRTNKK---SFKSEGFFLFQLKFPHSTL-------------THTDDVVMREKDKRSYRGDSKIPKCSSNNSsQRTLAEKK   419
                                                                *
zmp-3 CCD66298  355   TRRTTLwptTQSPFSGDDWGSGSGSSGRggsssgssgggcpSHIDAYTPSS-SFSYAFSGSQVYTISGT-K--VTKVQSI   430
zmp-4 CAB01669  284   ---------SKCP----------------------------KHLDSVVAINDQEWLFFRSNKVYKLNNR-K-FVDSGRPI   324
                                *
zmp-5 CAB08559  204   ASEHHIwrsELCT---------------------------kPYLDAVTTLKNGTILAFRGKMFFELKTTRK--WLLPRKI   254
zmp-6 CCD61839  247   SSTTTS---SSSPDSFFPFPLPSIEHFQ-------------RRNDWFVL-------------------------------   279

zmp-1 CCD65979  341   NQLFPGLPNPIDAAVTVG--HNLWVFVGEMIYVIYGNH-------MVHAPLRLSD-IGINEKYVDLAYEWHyfNPPAVYI   410
zmp-2 CCD72394  420   LTLGLHLSEADAKRYTEM---VCNFLAGLHMWRTNPNHHAs--ESLEKEYKGVSQeMGTFSGKSIAVRRLIr---HAEHQ   491
zmp-3 CCD66298  431   HDLFPSAPTPVNAALWNPisGSMLLFSSNRVYSYYFSNIRqifQMDSGFPKTLPS---DLGFSVSGALRWI--NGHQILM   505
zmp-4 CAB01669  325   QQVFPRGPQFVNATVSSG--PLTLLFVERTIYGYEYDGVQ--fTEAPNYPKELHD-RVLFYPQGAFPLNNG-----SVIL   394
zmp-5 CAB08559  255   NRIFP-FEGPLEAATTDR-hGNVYFFKKDTYWVMTKHG-----DMMNGYPKKISQgLTDTPDGINAALYYH--EDGKPYF   325
zmp-6 CCD61839        --------------------------------------------------------------------------------

zmp-1 CCD65979  411   WKGSRYWKLD-E----KMYHRRVDERYPKdtdlnwarVPKGVHSAFTYE----KEIHLLRGNQVFRMNSSRSvfDIaDGY   481
zmp-2 CCD72394  492   KERSEKGPLDpDYFDDDFFENFFMEYSK----------------------------------------------------   519
zmp-3 CCD66298  506   SSGDEFAVYD-EFWNQVTLKNRISSYFPN--------LPRGVKGVESPAG---SVITAFTSNQVFEYNSRTK--SI--GR   569
zmp-4 CAB01669  395   LSGNVFATYN-VQQNAPSFLNDKNRYFPN--------LPEDIKSGIEKTQgftDAYYMFDEATVSDYDMNSK-----QVL   460
zmp-5 CAB08559  326   FKKSYFWQYS-RYGKHKLWPRAIVSIFENqn------SPPEIDAAFQLNN---TSSFLFHQNKYWKVSGDPM--RIeKGF   393
zmp-6 CCD61839        --------------------------------------------------------------------------------

                                  transmembrane
                                   *
zmp-1 CCD65979  482   PQPLQSFFGFCPrneklvlnsssshfsliyatitililif   521
zmp-2 CCD72394        ----------------------------------------
                                 *
zmp-3 CCD66298  570   QSGFSS-YIAC-----------------------------   579
                                 *
zmp-4 CAB01669  461   QLQNIPDFLKCT----------------------------   472
                                 *
zmp-5 CAB08559  394   PRSLSRDWFNCL----------------------------   405
zmp-6 CCD61839        ----------------------------------------
```

signal sequence

v metallopeptidase domain boundary

*hemopexin-like domain bondary

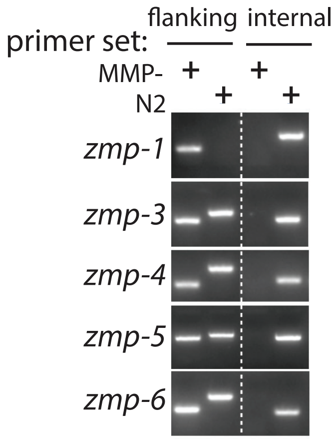
GPI anchor site

deleted sequence in MMP- animals

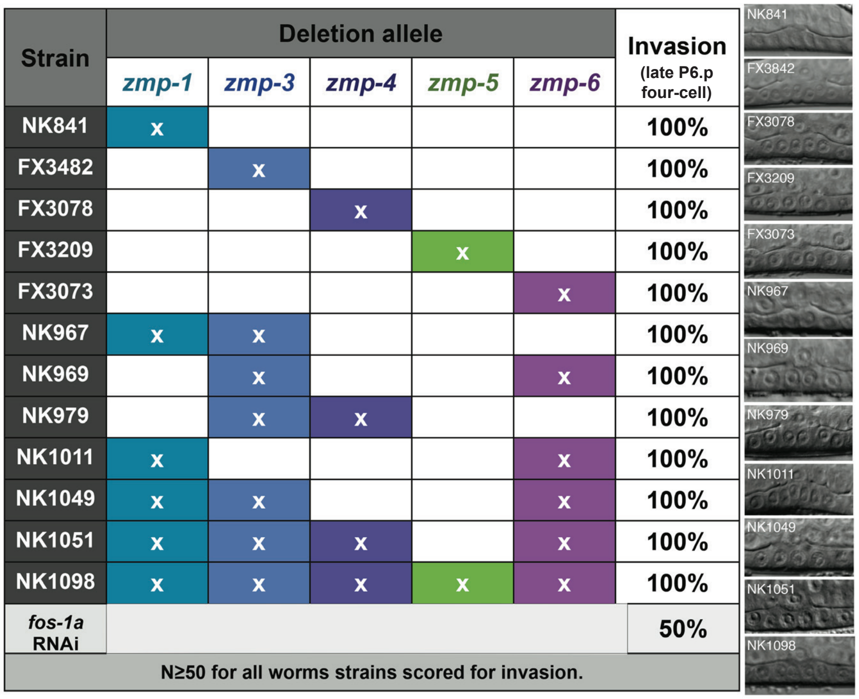
Figure S2. Amino acid sequences of the *C. elegans* MMPs; Related to Figure 1 and STAR Methods. The sequence alignment of the six *C. elegans* ZMP proteins are shown. All six ZMPs have a signal sequence (underlined), a highly conserved metallopeptidase and hemopexin-like domain (found within the arrowheads or asterisks, respectively), a cysteine switch, and an active site (both labeled and are bounded by black lines). In addition, ZMP-1 has a transmembrane domain and a GPI anchor site (yellow) at the end of the sequence. The span of amino acids included in the deletions used in the manuscript are highlighted in grey ((*zmp-1 (cg115); zmp-2 (tm3529); zmp-3 (tm3482); zmp-4 (tm3484); zmp-5 (tm3209); zmp-6 (tm3073))*.

Kelley et al., Supplementary Figure 3

A



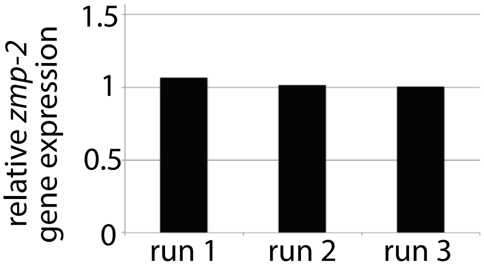
B



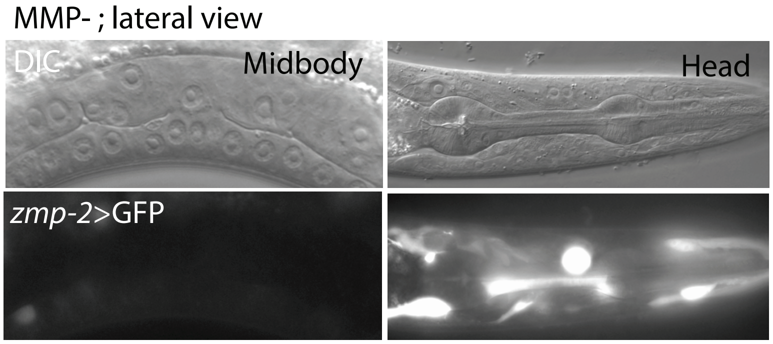
Strain	Deletion allele					Invasion (late P6.p four-cell)
	zmp-1	*zmp-3*	*zmp-4*	*zmp-5*	*zmp-6*	
NK841	x					100%
FX3482		x				100%
FX3078			x			100%
FX3209				x		100%
FX3073					x	100%
NK967	x	x				100%
NK969		x			x	100%
NK979		x	x			100%
NK1011	x				x	100%
NK1049	x	x			x	100%
NK1051	x	x	x		x	100%
NK1098	x	x	x	x	x	100%
fos-1a RNAi						50%

N≥50 for all worms strains scored for invasion.

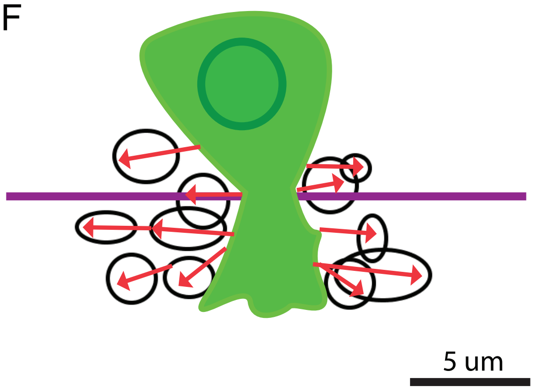
C



D



F



E

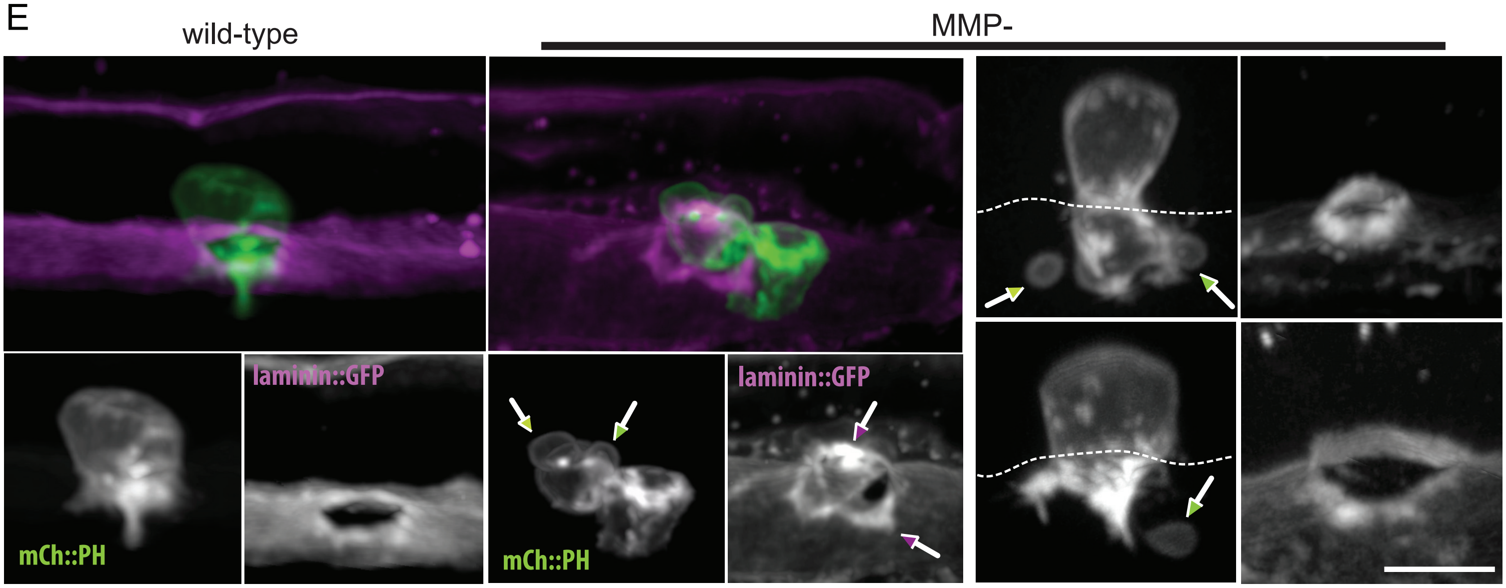
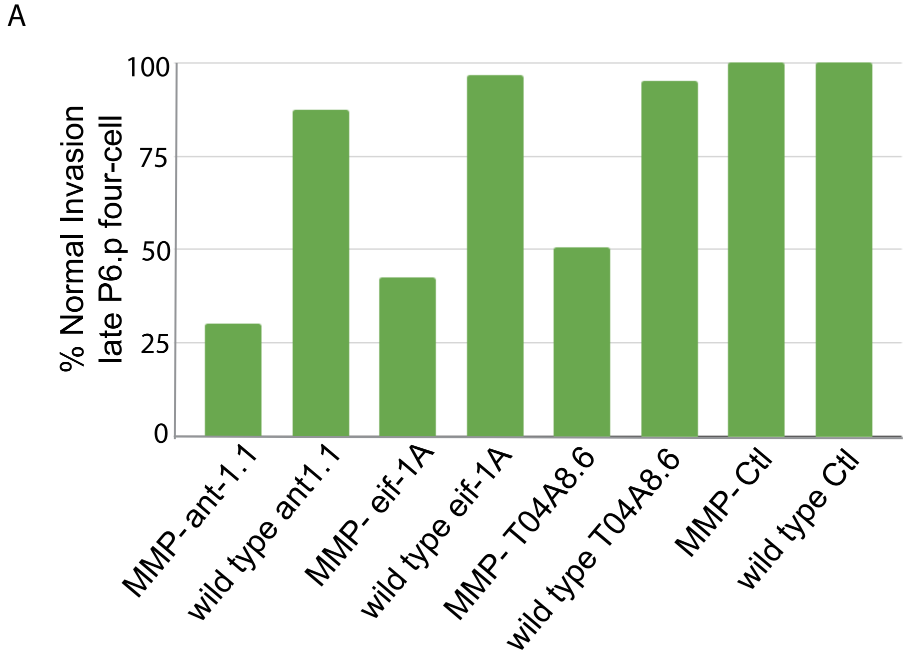
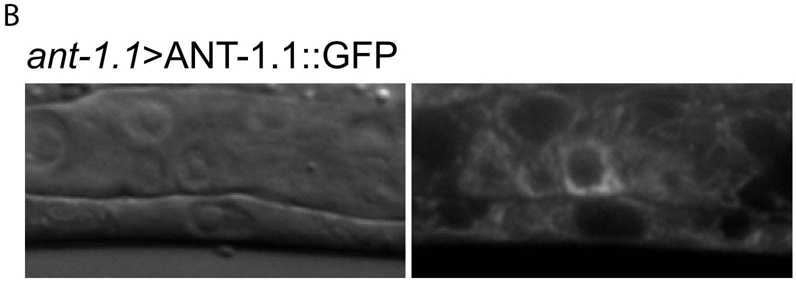


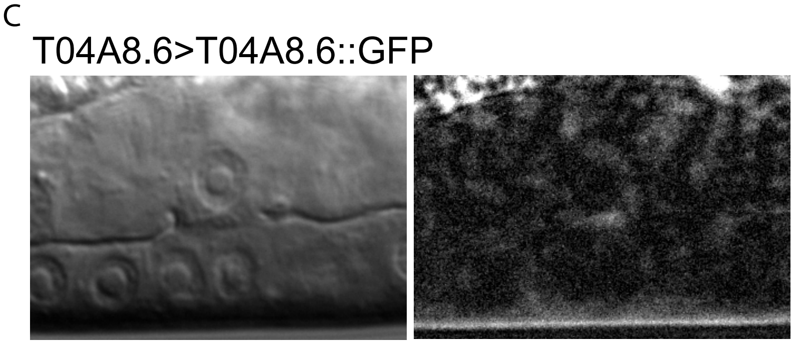
Figure S3. MMPs are not required for BM removal under the AC; Related to Table 1, Figure 3, and STAR Methods. (A) PCR genotyping analysis of wild-type (N2) and MMP- quintuple-mutant worms. Amplification products from primer sets flanking (left) or internal (right) to the region of genetic deletion are shown. **(B)** The table displays the putative *zmp*-deletion alleles analyzed alone, and in combination for invasion at the late P6.p four-cell VPC developmental stage (see Figure 1B). A representative DIC image for each strain is shown to the right. RNAi targeting *fos-1a* was used as a control for blocked invasion. **(C)** RT-qPCR analysis of whole worms shows no compensatory upregulation of *zmp-2* gene expression in MMP- animals. Values represent the relative gene expression of *zmp-2* in MMP- worms relative to wild type animals and normalized to the reference gene (Y45F10D.4). Three trials (runs) are shown (1.03 ± 0.03; Mean ± SD). **(D)** (Left panels) No expression of the transcriptional *zmp-2*> GFP reporter is detectable within or around the AC in animals in MMP- animals (images represent > 10 animals imaged for each strain). (Right panels) Similar exposure showing expression of *zmp-2*> GFP in the head region of the same animal. **(E)** Distinctive spherical membrane morphology displayed in MMP- animals. A representative wild type and MMP- animal shows an MMP-AC (visualized with membrane marker mCherry::PLCΔ^{PH}, grey, green in merged images) displaying membrane blebs and cell fragments (seen in ~30% of MMP- animals n > 100) that are not seen in wild-type worms. Laminin (laminin::GFP) is also shown alone (grey) or in merged images (magenta). **(F)** Illustration depicting the location (black circles) and attachment points (red arrows) on ACs (green) from 10 animals that harbored membrane spheres relative to the BM (magenta line). Scale bars, 5 μm.

Kelley et al., Supplementary Figure 4

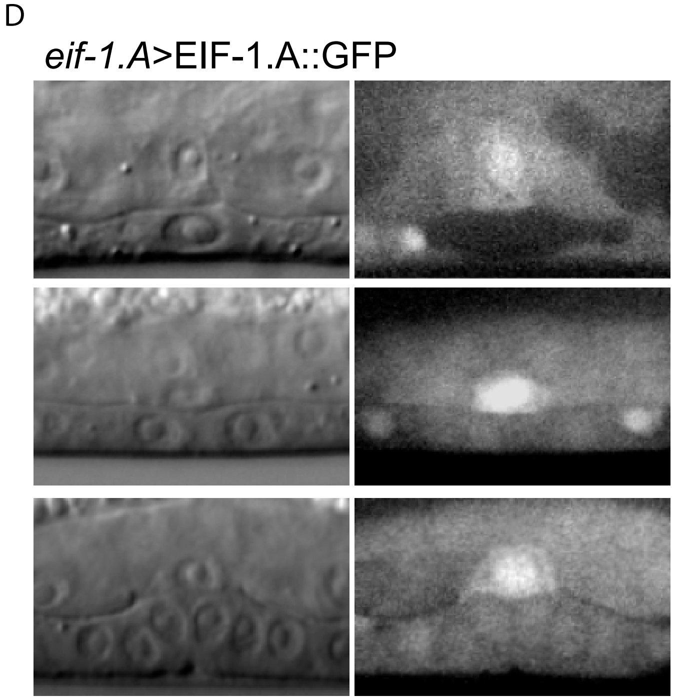




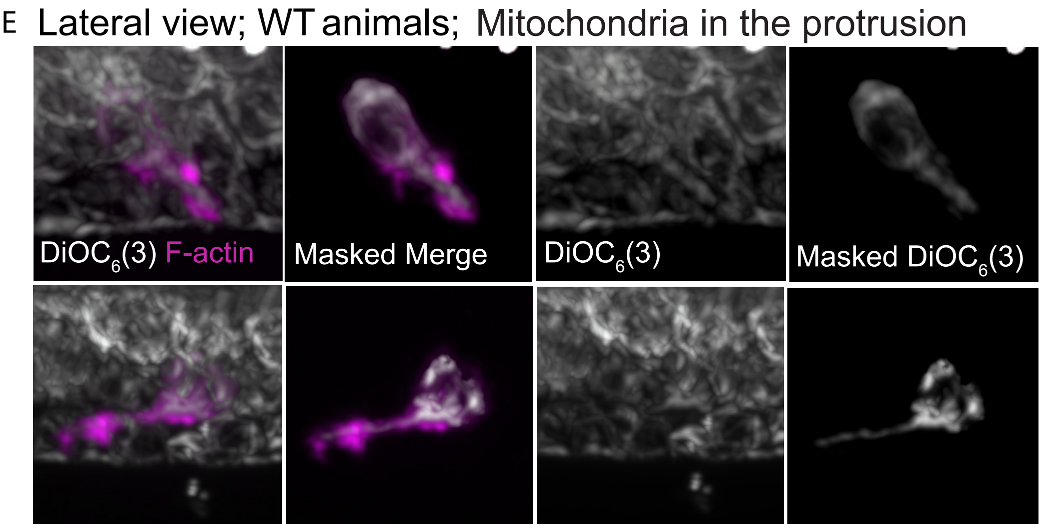
ant-1.1>ANT-1.1::GFP



T04A8.6>T04A8.6::GFP



eif-1.A>EIF-1.A::GFP



Lateral view; WT animals; Mitochondria in the protrusion

Figure S4. A whole-genome MMP- synergistic AC invasion interaction screen identifies three genes; Related to Figure 6. (A) The percent of animals showing normal invasion after RNAi targeting *ant-1.1*, *eif-1a*, *T04A8.6* or L4440 control at the early P6.p 4-cell VPC developmental stage are shown (data from Table S1). AC expression (arrows) of **(B)** ANT-1.1 protein (*ant-1.1*>ANT-1.1::GFP) at P6.p 2-cell stage **(C)** T04A8.6 protein (*T04A8.6*>T04A8.6::GFP) at P6.p 4-cell stage, and **(D)** EIF-1.A protein (*eif-1.A*>EIF-1.A::GFP) at P6.p 1- (top), 2- (middle), and 4-cell (bottom) VPC stages are shown to the right of corresponding DIC images. Scale bars, 5 µm. **(E)** Mitochondria and F-actin the invasive the invasive protrusion in wild type animals. Mitochondria staining is shown alone ($\text{DIOC}_6(3)$); white, or merged with F-actin (mCherry::moeABD; magenta).