

Fig. S1. *alk* mutant VM cells are Org-1 negative and Vrp1 positive. (Upper panel) In wild-type embryos (stage 13), Org-1 protein (red) is observed in the fusing cells of the VM. All VM cells express Alk (green). (Lower panel) Org-1 protein (red) expression is absent in VM cells of *alk*^{-/-} embryos. Vrp1-positive cells denoted in white or blue (merged panel).

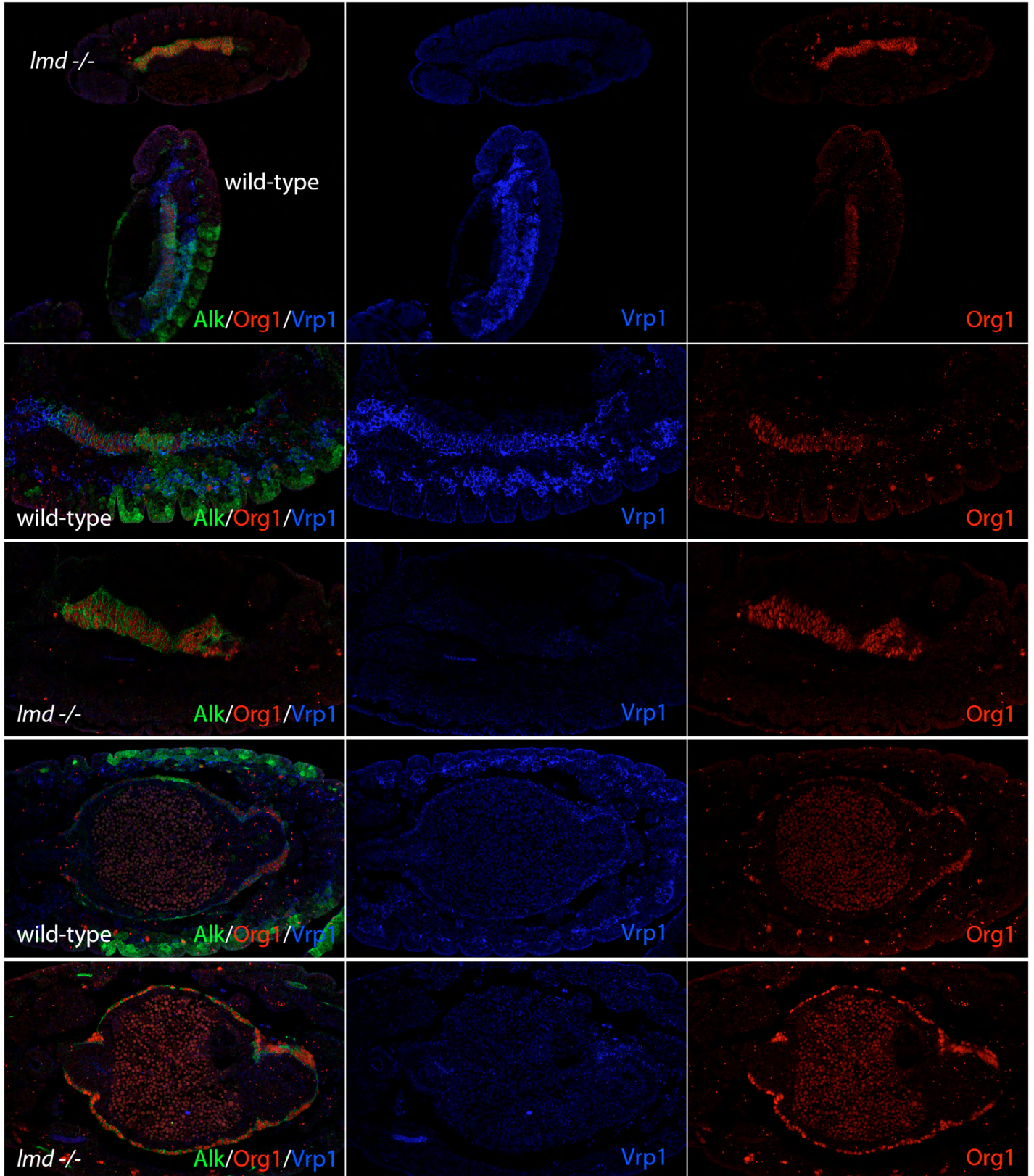


Fig. S2. VM in *lmd* mutants fail to express Vrp1 protein at later stages of embryonic development. In wild-type embryos, Org-1 protein (red) is observed in the fusing cells of the VM, together with Vrp1 protein (blue). All VM cells express Alk (green). VM cells in *lmd*^{-/-} embryos (marked with Alk in green) robustly express Org-1 (red) but lack Vrp1 protein (blue).

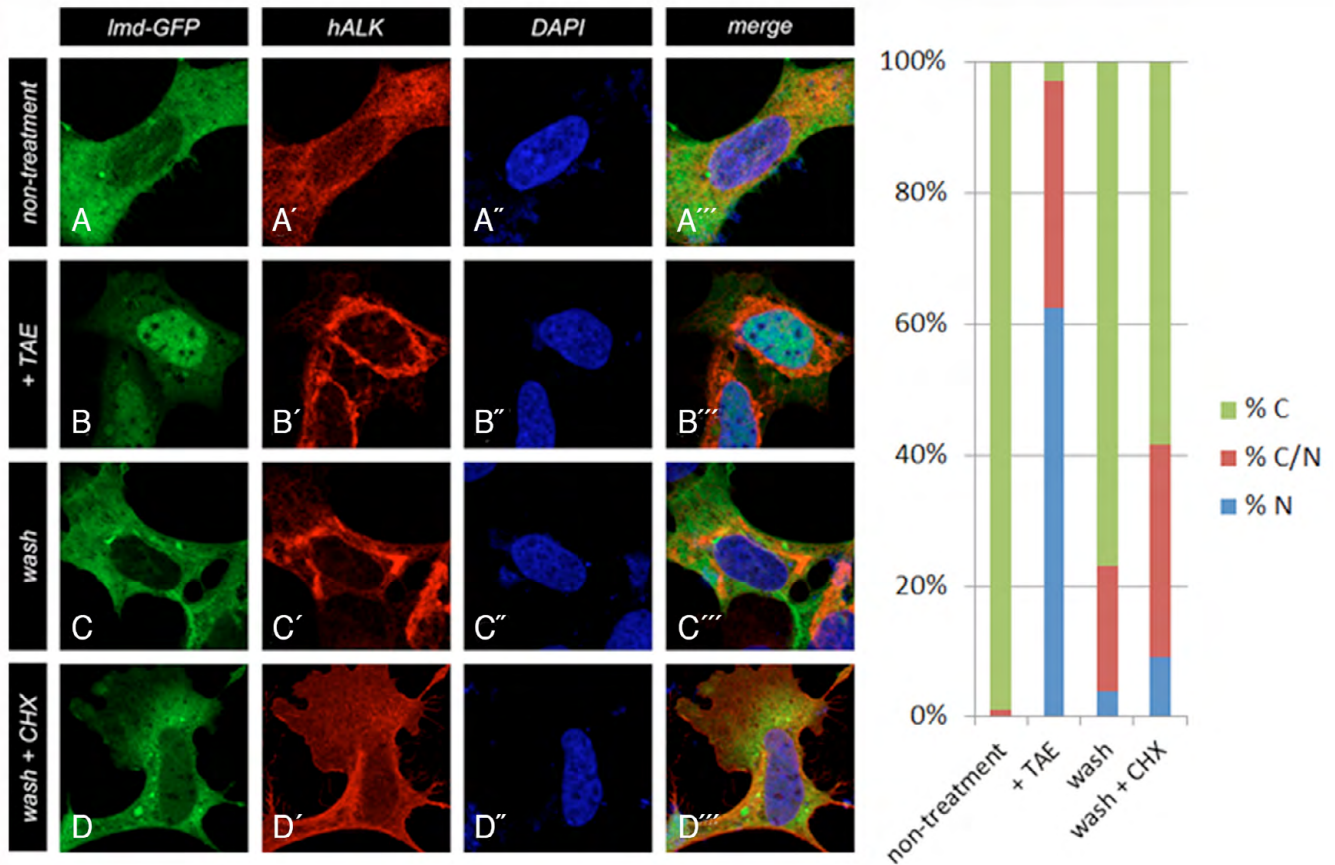


Fig. S3. ALK activity-dependent Lmd translocation does not require *de novo* protein synthesis. HEK293 cells transfected with Lmd-GFP and gain-of-function human ALK^{F1174S} were pretreated with 0.2 μ M NVP-TAE684, a specific ALK inhibitor, for 18 hours. Following a 2-hour pretreatment with 50 μ g/ml cycloheximide (CHX) to block *de novo* protein synthesis, NVP-TAE684 was washed away and cells were further incubated for 30 minutes in the presence of CHX. ALK was visualized by immunostaining (red) and nuclei marked by DAPI (blue). (A,B) Translocation of Lmd protein (green) to cytosol in non-treated cells (A) is blocked in the presence of NVP-TAE684 (B). (C,D) After removal of ALK inhibitor, Lmd protein re-distributes to cytosol (C), even in the presence of CHX (D). Subcellular localisation of Lmd protein is quantified on the right. C, cytosol; N, nucleolus; C/N, both.

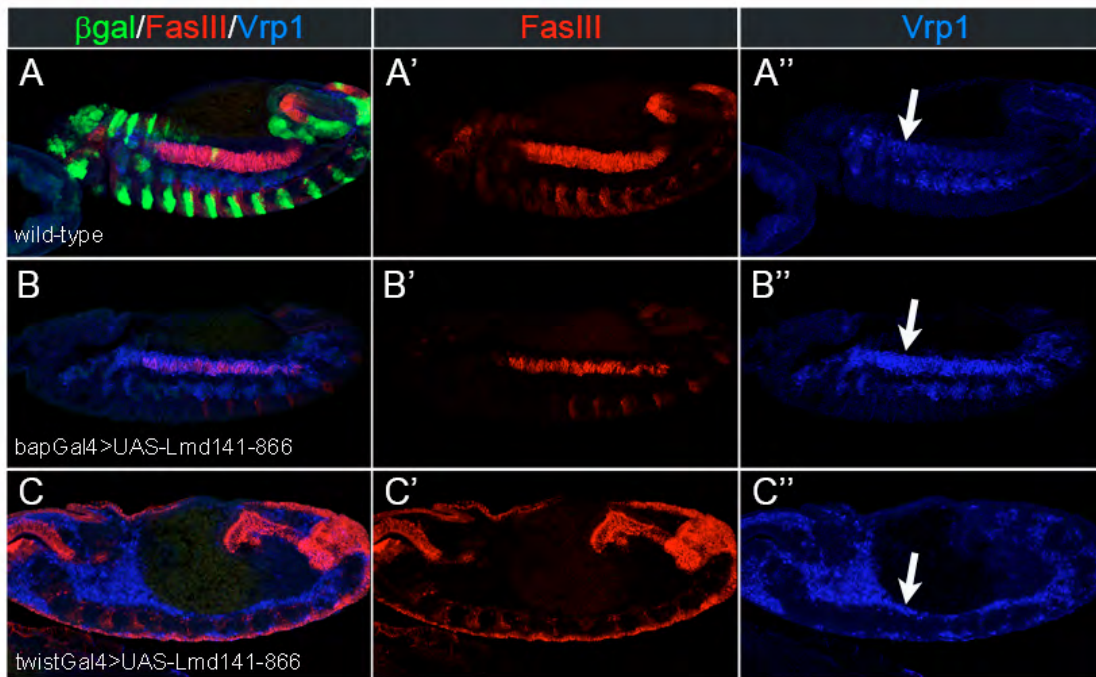
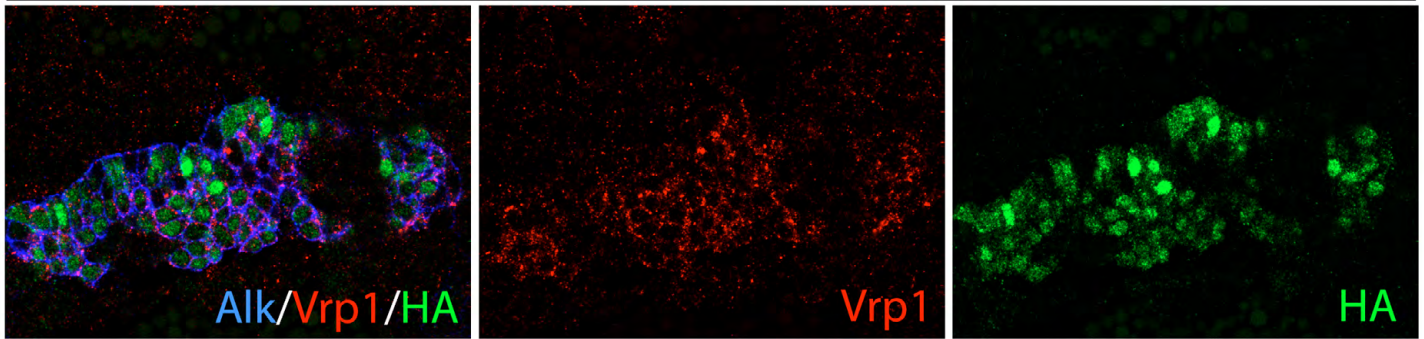


Fig. S4. Expression of the *Lmd*¹⁴¹⁻⁸⁶⁶ mutant protein in the mesoderm drives robust expression of *Vrp1*. (A-A'') In wild-type embryos (stage 13), *Vrp1* protein (blue) is observed in the FCM of the VM and is downregulated after fusion (arrow). (B-B'') All VM cells in *bapGal4>UAS-lmd*¹⁴¹⁻⁸⁶⁶-expressing embryos (marked with *Fas3* in red) express high levels of *Vrp1* (blue, arrow), which is not downregulated. (C-C'') In *twistGal4>UAS-lmd*¹⁴¹⁻⁸⁶⁶, both VM and SM express high levels of the *Lmd* target *Vrp1* (blue, arrow). No *Fas3*-positive VM can be observed (red).

A *bapGal4>UAS-Lmd141-866*



B *twistGal4>UAS-Lmd141-866*

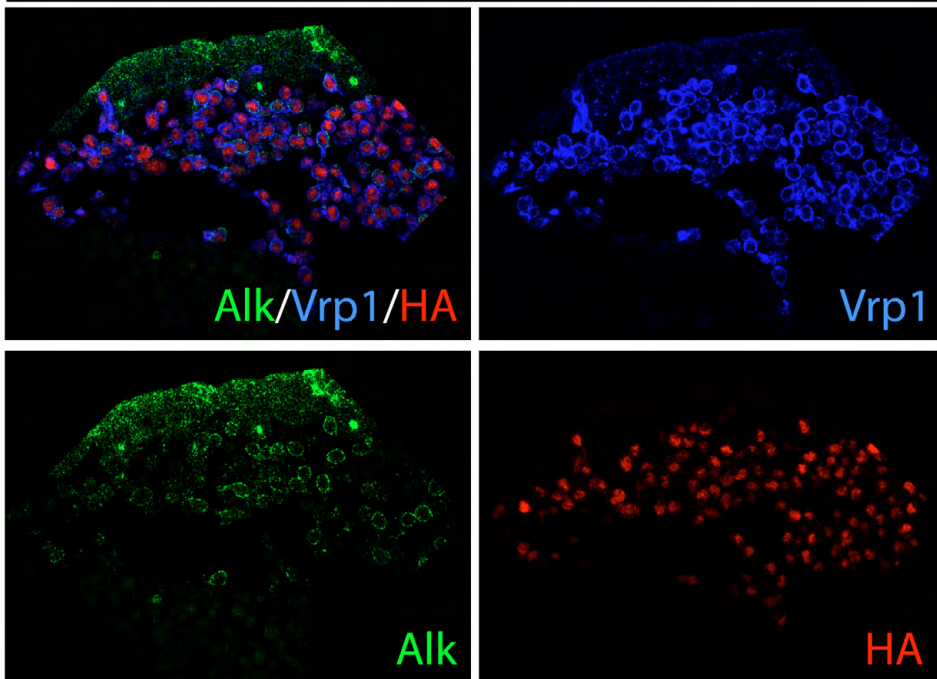


Fig. S5. The $Lmd^{141-866}$ mutant protein persists in Alk positive VM cells. (A) VM FCs in *bapGal4>UAS-lmd¹⁴¹⁻⁸⁶⁶*-expressing embryos (marked with Alk in blue) express Vrp1 (red). Both FCs and FCMs display nuclear $Lmd^{141-866}$ mutant protein (HA in green). (B) In *twistGal4>UAS-lmd¹⁴¹⁻⁸⁶⁶*, both visceral and somatic mesoderm persistently express nuclear $Lmd^{141-866}$ mutant protein (HA in green). This is associated with high levels of expression of the Lmd target Vrp1 (blue) in these cells. In this case, a subset of the Vrp1, Lmd-positive cells are Alk positive (green).