Supplementary material

Microsomal triacylglycerol transfer protein (MTP) is required cell autonomously to expand tracheal lumen in Drosophila

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Supplementary Figure Legends

Figure S1. *MTP* gene and protein in wild type and in *MTP*^{2L3637} and *MTP*^{2L4501}

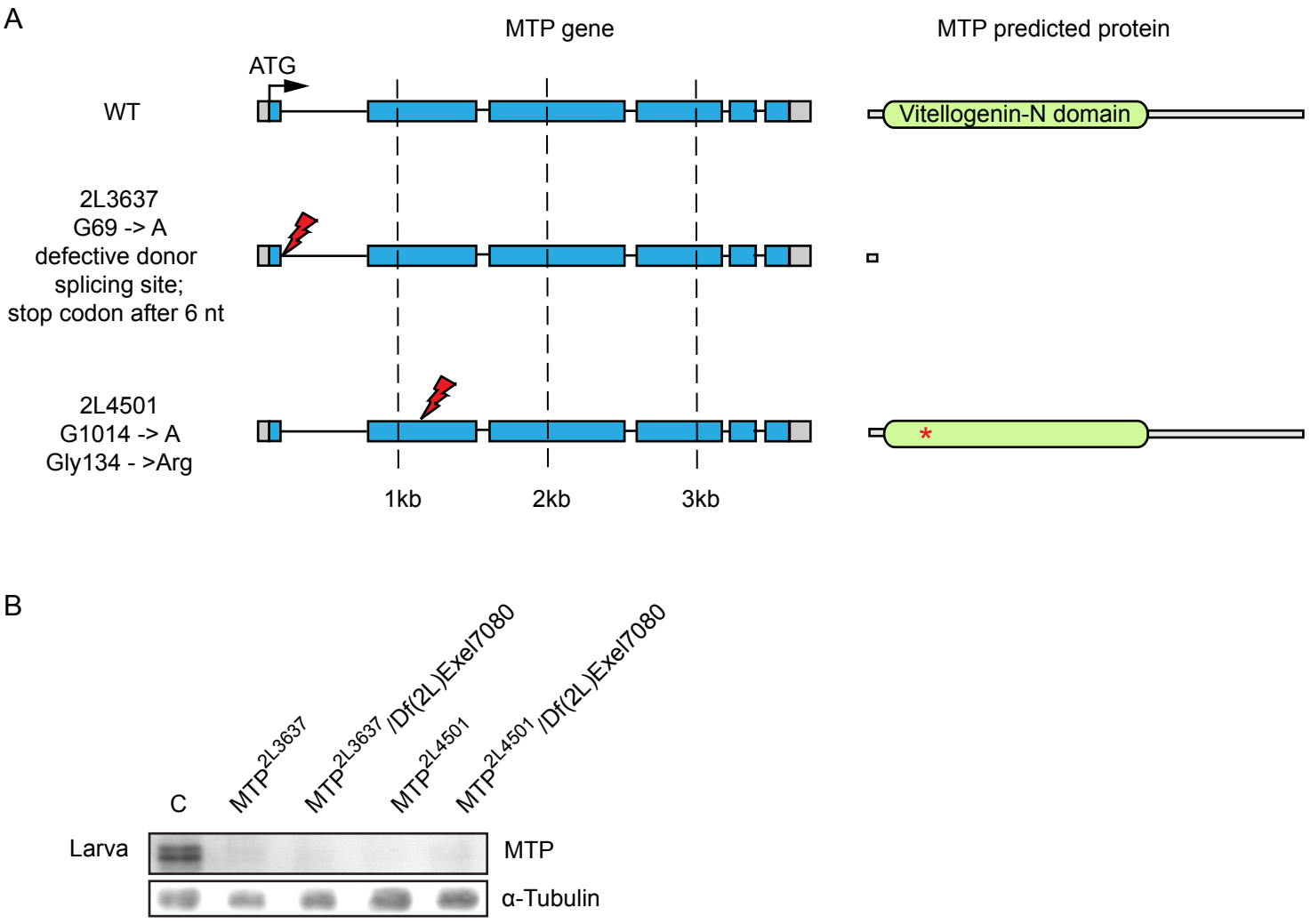
MTP gene consists of 6 exons (blue) and encodes a protein of 886 amino acids with N-terminal Vitellogenin-N domain (green). There is only one transcript and one polypeptide reported. In allele 2L3637 G nucleotide at position 69, counting from the ATG in the annotated transcript, is exchanged for A nucleotide – this leads to a defect in the donor splicing site of the first exon. As the result, a stop codon follows after 6 nucleotides. Translation of this transcript results in a truncated protein of 24 amino acids in length, missing the Vitellogenin-N domain. Allele 2L4501 carries a nucleotide substitution of G to A at position 1014, leading to an amino acid residue change at position 134 from Glycine to Arginine. This mutation falls within the Vitellogenin-N domain (asterisk) (A). In larvae homozygous for either of the lines or in combination with a deficiency removing the MTP coding region and neighbouring genes, only residual full length protein can be detected on Western blot (B). As there is no difference between the homozygous and hemizygous larvae, we assume that the observed band is due to the maternal contribution. Tubulin was used as a loading control.

Figure S2. Lipoproteins in the fat bodies of wild type and MTP mutant larvae.

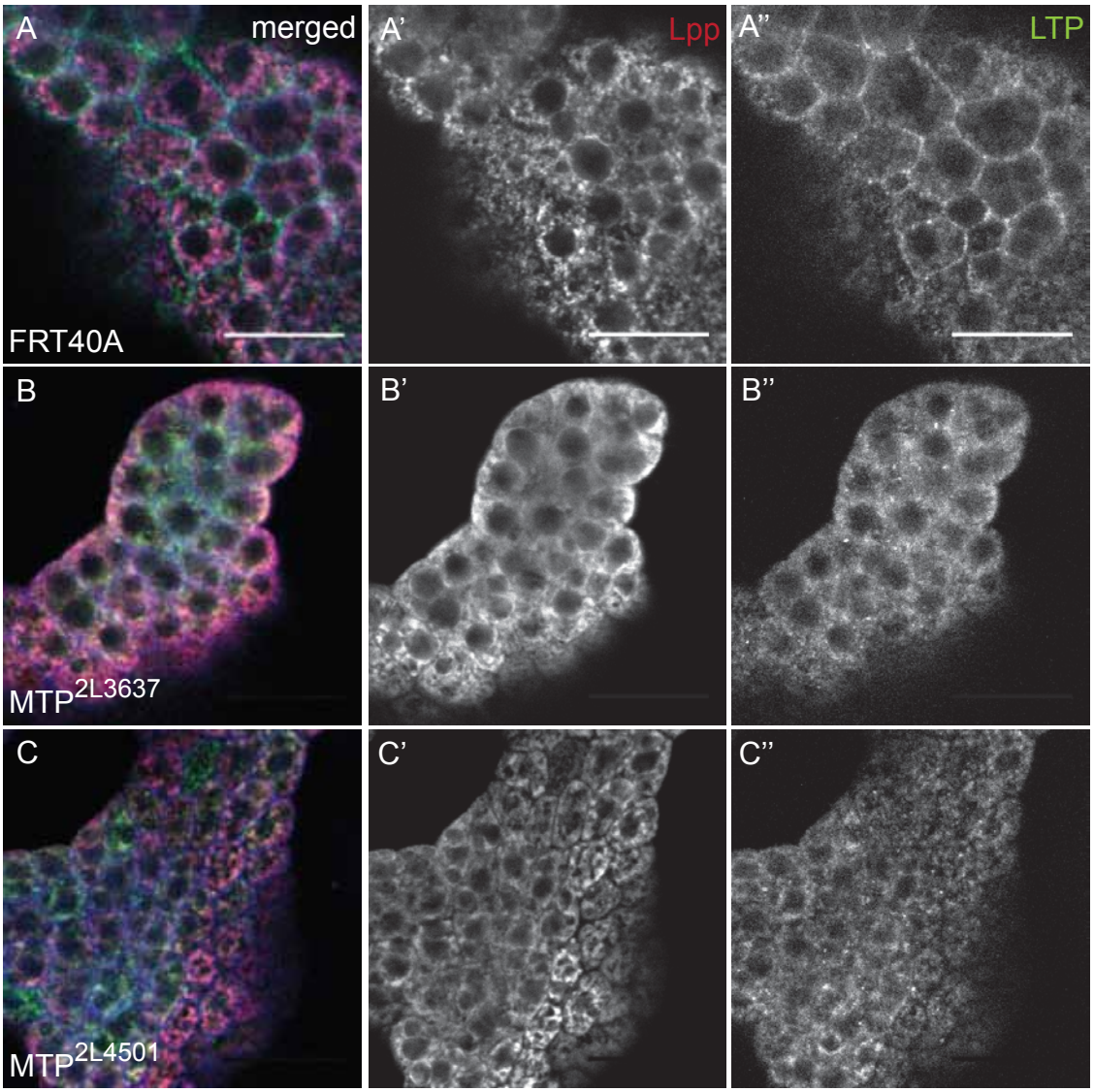
Lipoproteins assemble in the cells of the fat body and show distinct cellular localization. In wild type (A'') LTP localizes at the cell membrane, whereas Lpp is distributed broadly in the cell body (A'). *In absence of MTP levels of both lipoproteins in fat body cells are not reduced (B-B'', C-C''). However, membrane localization of LTP is lost (B'' and C'') whereas Lpp preserves its broad cytoplasmic localization.* Scale bars: 20µm.

Figure S3. Phenotype analysis over time.

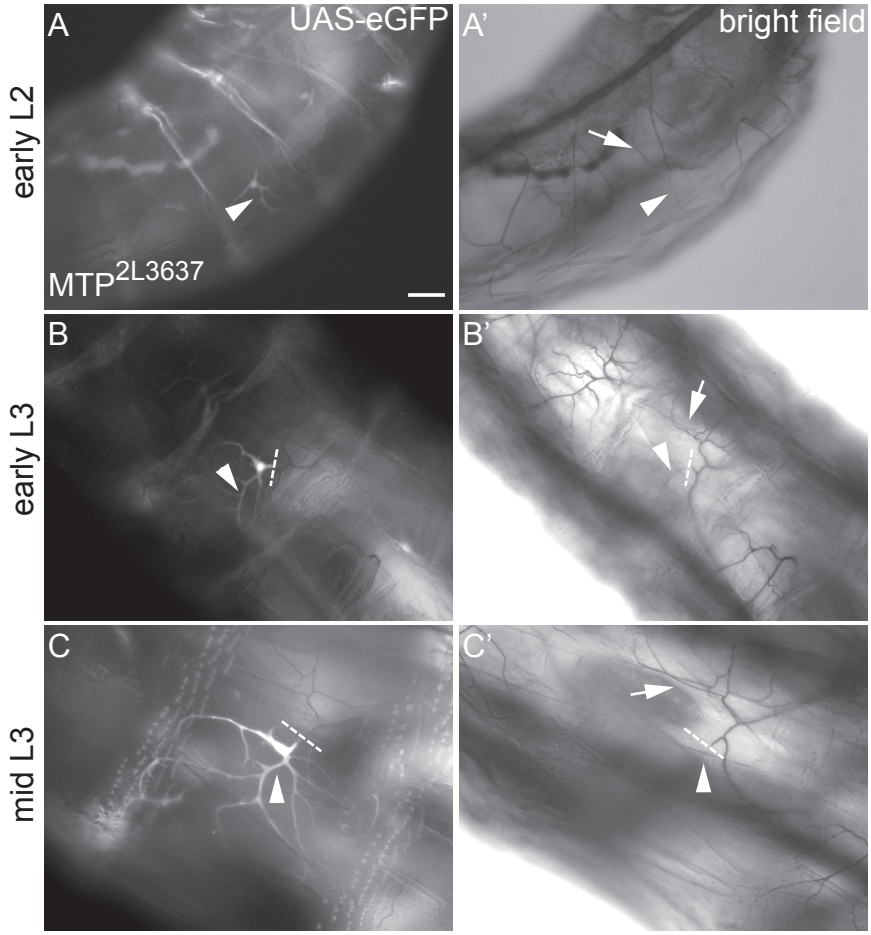
Shortly after the L1 to L2 molt (A, A') there is no obvious difference in gas-filling of the lumen between the mutant (arrowhead) and the wild type (arrow) terminal cells. During next 24 hours and after the L2 to L3 molt (B, B') the wild type cell formed new gas-filled branches (arrow). The mutant cell also expanded its branches, but failed to fill with gas (arrowhead). Within the following 24 hours (C, C'), both wild type and mutant cells made new branches and elongated the existing ones, but the gas-filled lumen in the mutant cell did not expand. The images show the same larva with GFP-marked mutant cells at different developmental stages. The non-marked collateral cell was used as an internal control of lumen growth and gas filling. Dashed lines indicate the proximal end of the mutant cell. Scale bar: 50µm.



Baer et al., Figure S1



Baer et al., Figure S2



Baer et al., Figure S3