

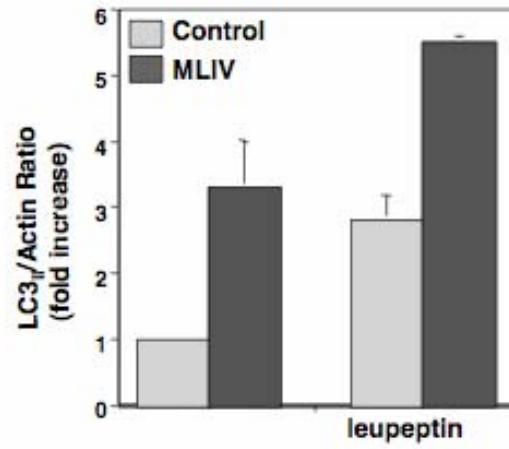
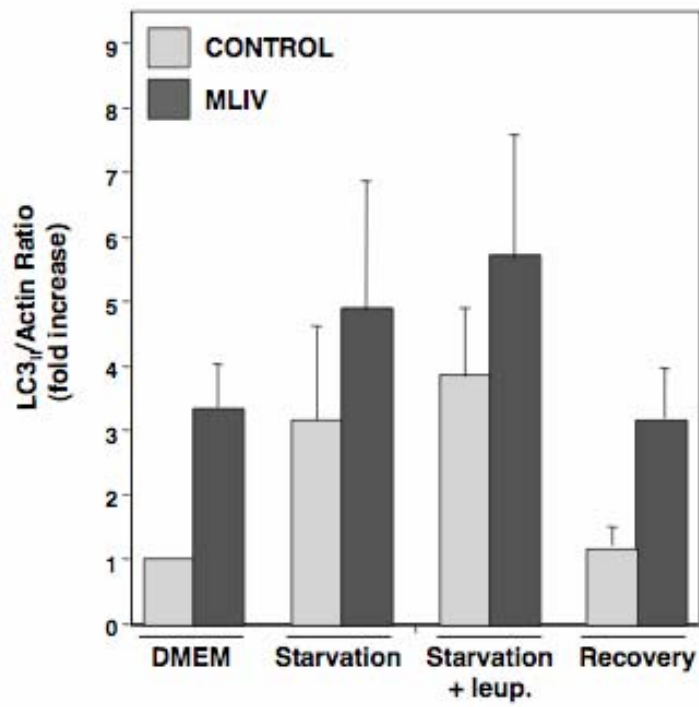
Supplementary Data

Supplementary Figure 1. Increased autophagy in MLIV fibroblasts. (A) Control and MLIV fibroblasts were treated without or with 50 $\mu\text{g/ml}$ leupeptin for 7 hours. Autophagy was measured as the ratio of LC3-II/Actin and is represented as a fold increase of the ratio from control cells without treatment. Results are means \pm S.D. (n=7). $P < 0.01$. (B) Lysates from control fibroblasts or fibroblasts from a MLIV patient were collected from untreated cells (DMEM), cells starved for 3 hours in the absence or presence of 50 $\mu\text{g/ml}$ leupeptin, or cells starved for 3 hours followed by 1 hour of recovery (recovery). Autophagy was measured as the ratio of LC3-II/Actin and is represented as a fold increase of the ratio from control cells in normal media (DMEM). Results are means \pm S.D. (n=4). $P < 0.01$.

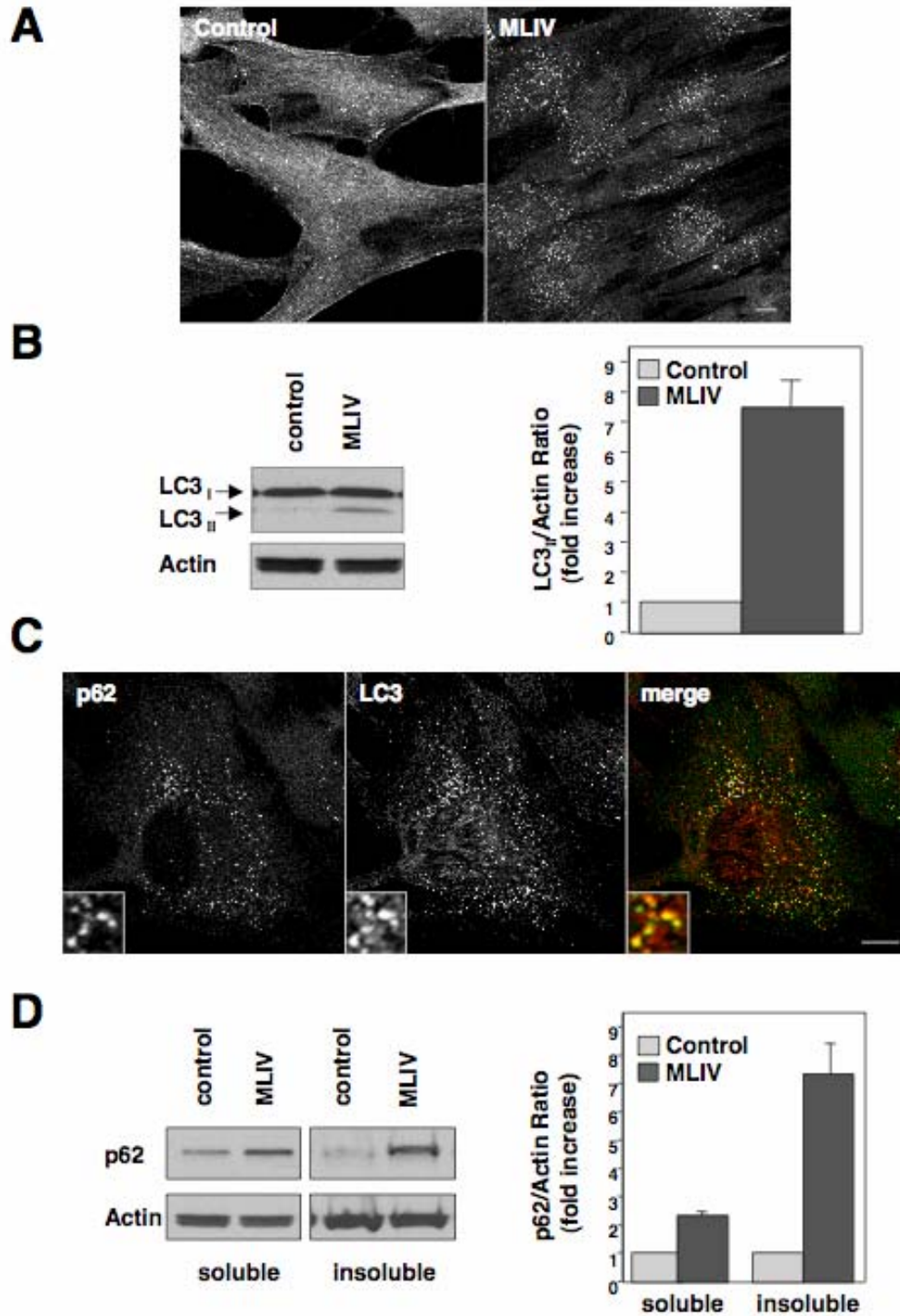
Supplementary Figure 2. Confirmation of increased autophagy in an independent MLIV cell line. (A) LC3 staining in untreated control and MLIV fibroblasts. (B) Control and MLIV fibroblasts cells were lysed and immunoblotted with anti-LC3 (top) or anti-actin (bottom). Autophagy was measured as the ratio of LC3-II/Actin and is represented as a fold increase of the ratio from control cells. Results are means \pm S.D. (n=3). $P < 0.01$ (C) MLIV fibroblasts grown in complete media were fixed, permeabilized and immunostained with a polyclonal antibody to LC3 (red) and a monoclonal antibody against p62 (green). Insets show a three-fold magnification of the indicated region. (D) Western blot showing the accumulation of p62 in the soluble and insoluble fractions of

MLIV fibroblasts. Quantification was performed by normalizing p62 with actin levels. Results are means \pm S.D. (n=3). Soluble $P < 0.001$; Insoluble $P < 0.05$.

Supplementary Figure 3. Degradation of autophagosomes is delayed in fibroblasts from a different MLIV patient. Control and MLIV fibroblasts were starved in EBSS media for 3 hours and then allowed to recover for 20 or 40 min in complete medium. Cells were then fixed and the number of LC3-positive structures was counted. Percentage of cells showing more than 20, between 10 and 20, or less than 10 LC3-positive structures after a 20 min recovery (left panel) or 40 min of recovery (right panel) is represented.

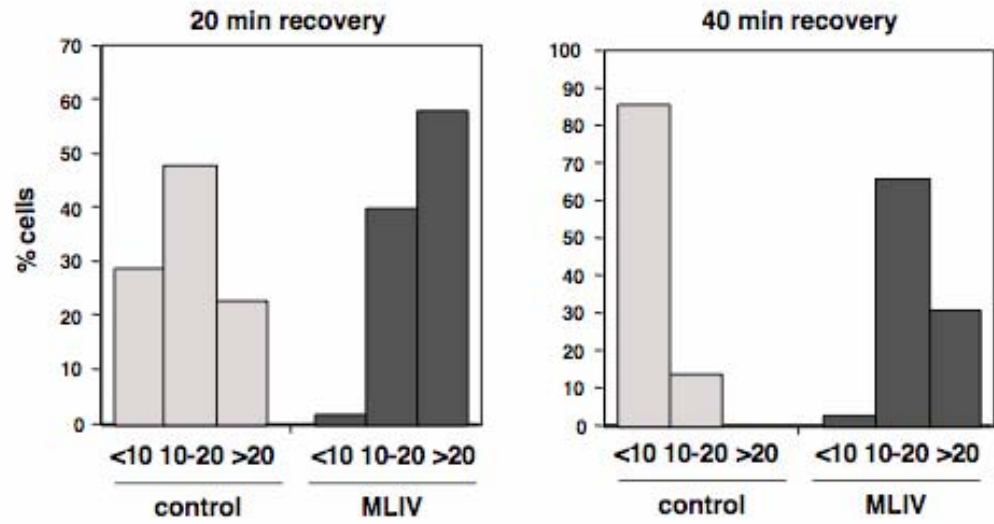
A**B**

Supplementary Figure 1



Supplementary Figure 2

Number of LC3-positive structures



Supplementary Figure 3