

# Supplementary Materials

## **Loss of HtrA2/Omi activity in non-neuronal tissues of adult mice causes premature aging**

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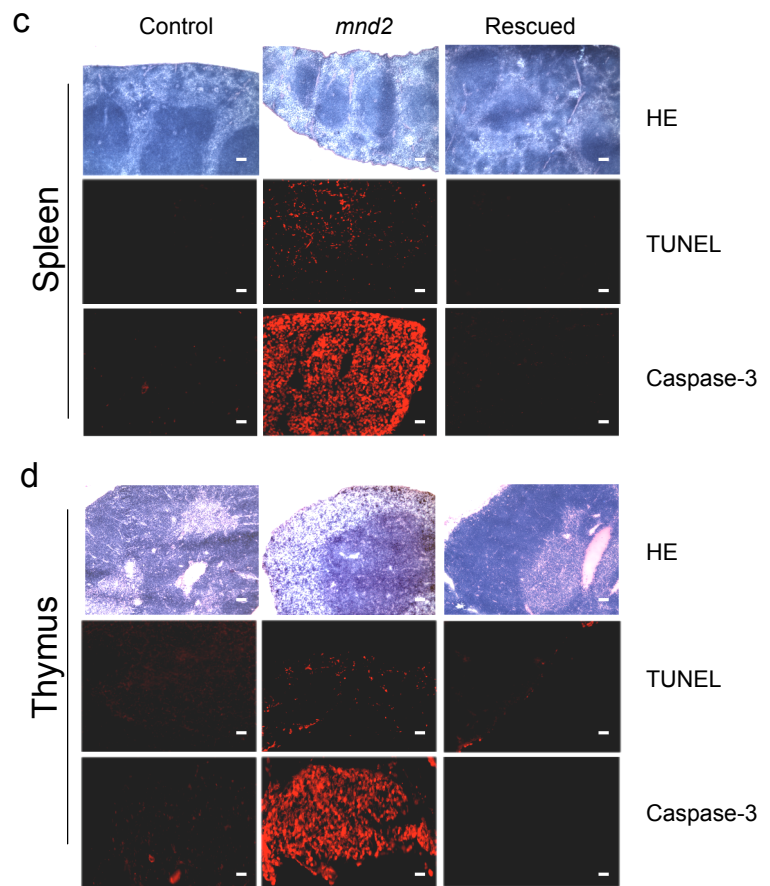
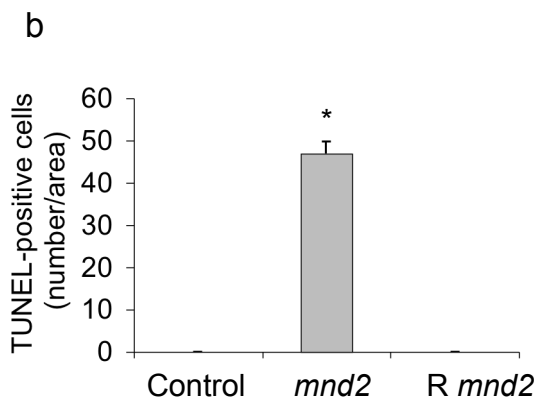
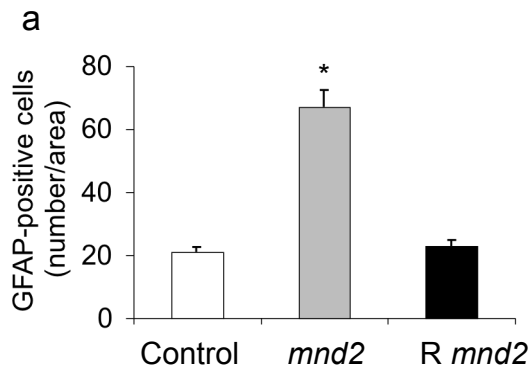
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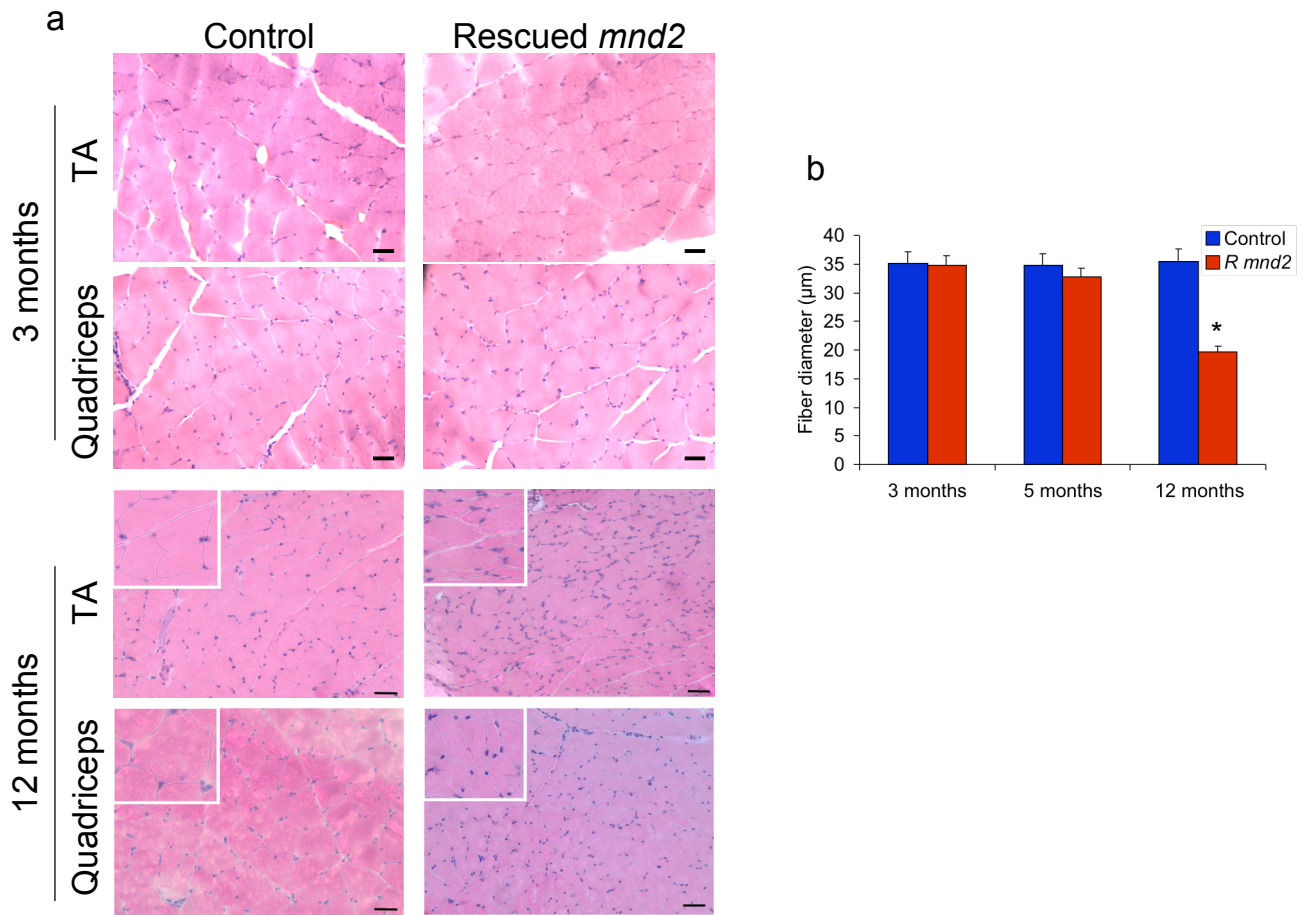
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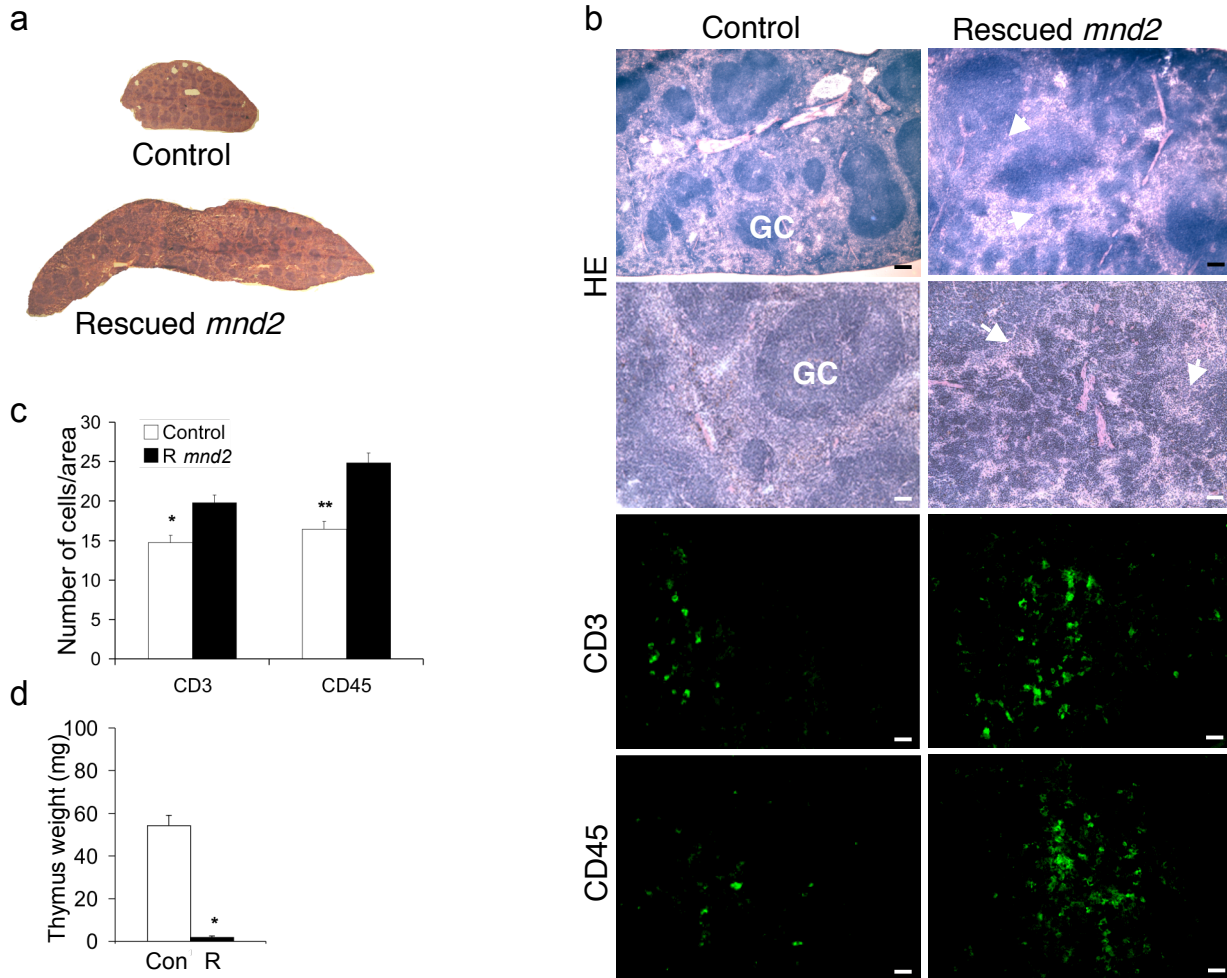
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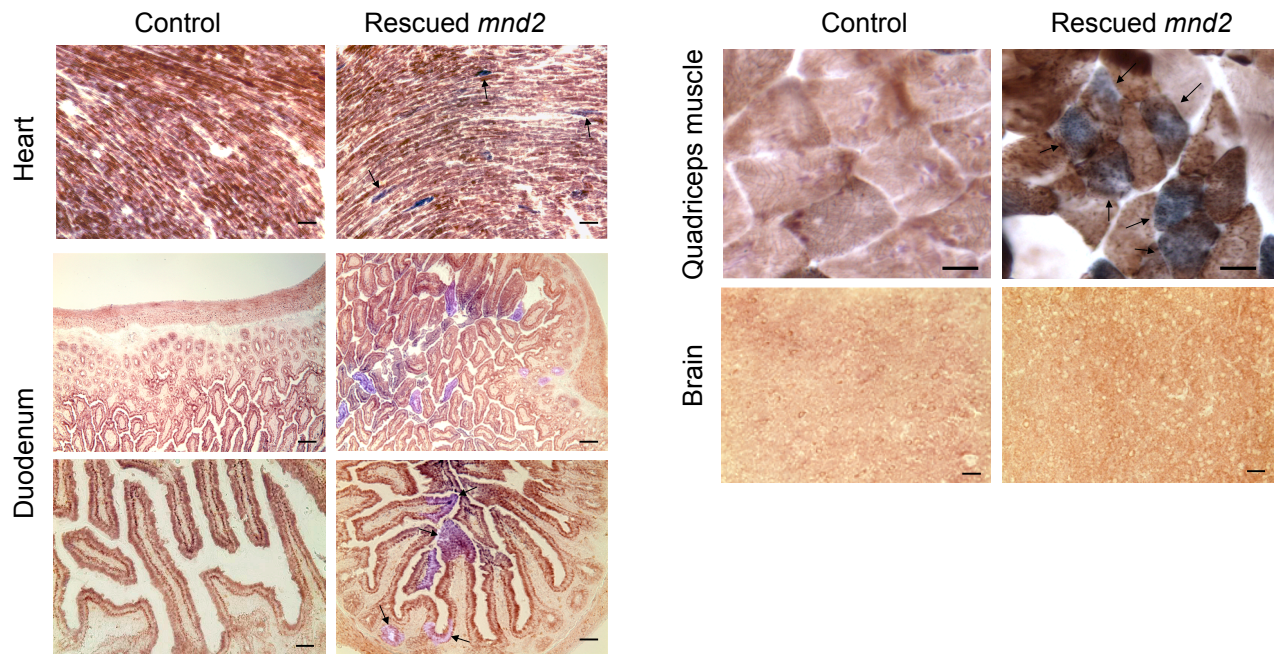
**Supplementary Figure 1 (a, b)** Astroglial infiltration and apoptosis in the striatum of WT, *mnd2* and rescued *mnd2* mice. Graphs show GFAP- (a) and TUNEL- (b) positive cells in cryostat sections of the striatum of 4-week-old control WT, *mnd2* and rescued *mnd2* mice (n = 4 in each group with at least 5 sections randomly examined per striatum). Asterisk,  $P < 0.01$ . (c, d) Histological analysis of apoptosis in spleen of WT, *mnd2* and rescued *mnd2* mice. Cryostat sections of the spleen (c) or thymus (d) of 4 week old control (WT), *mnd2*, and rescued *mnd2* mice (R) stained with hematoxylin-eosin (HE, 100x magnification) (upper panels), or TUNEL (middle panels), or immunostained for active caspase 3. Note the large presence of TUNEL- and active caspase-3-positive cells in spleen and thymus of *mnd2* mice. Bar: Top row: 150  $\mu\text{m}$ ; middle and lower row: 90  $\mu\text{m}$ . All error bars indicate s.e.m.



**Supplementary Figure 2** Increased muscle atrophy in aged rescued *mnd2* mice. **(a)** Cryostat sections of Tibialis Anterior (TA) and Quadriceps muscles from 3- and 12-month-old rescued *mnd2* and control *mnd2*<sup>+/+</sup>;Tg mice stained with hematoxylin-eosin. Skeletal muscle fibers from 12-month-old rescued *mnd2* mice are smaller and angulated. **(b)** Graph shows a significant reduction in the diameter of skeletal muscle fibers of 12-month-old rescued *mnd2* (R *mnd2*) mice compared to control *mnd2*<sup>+/+</sup>;Tg mice. No significant difference is seen in muscle fibers diameter between young (3- and 5-month-old) rescued *mnd2* mice and control mice. Bar: 35 µm, insert: 20 µm. Asterisk,  $P < 0.01$ , All error bars indicate s.e.m.



**Supplementary Figure 3** Splenomegaly, increased extramedullary hematopoiesis and thymus atrophy in aged rescued *mnd2* mice. **(a)** Haematoxylin and eosin (HE)-stained sections of spleen from 15-month rescued *mnd2* mouse and control *mnd2*<sup>+/+</sup>;Tg littermate. **(b)** Cryostat sections of spleen from 15-month rescued *mnd2* and control *mnd2*<sup>+/+</sup>;Tg mice stained with HE and immunostained for CD3 and CD45. HE-stained sections show that germinal centers are well defined in control mice but are small and disorganized in rescued *mnd2* mice. Note extramedullary hematopoiesis in the red pulp of rescued *mnd2* mice (arrows). Numerous CD3- and CD45-positive cells were observed in the spleen of aged R-*mnd2* mice. **(c)** Graphs shows an increase in the number of CD3- and CD45-positive cells in the spleen of aged rescued *mnd2* mice. CD3- and CD45-positive cells were enumerated on at least 5 random sections of 15-month-old rescued *mnd2* (R *mnd2*) mice and control littermates. Asterisk,  $P < 0.05$ , double asterisk,  $P < 0.01$ . Bar: 1<sup>st</sup> row from top 100  $\mu$ m; 2<sup>nd</sup> row from top 50  $\mu$ m; 3<sup>rd</sup> and 4<sup>th</sup> row from top 40  $\mu$ m. **(d)** Graphs shows average weight of thymi from 15-month-old rescued *mnd2* (R) mice and control (con) heterozygous *mnd2*<sup>+/+</sup>;Tg littermates. Asterisk,  $P < 0.001$ . All error bars indicate s.e.m.



**Supplementary Figure 4** COX deficiency in tissues of rescued *mnd2* mice. Cryostat sections of heart, duodenum, skeletal muscle and brain from 15-month-old rescued *mnd2* and control *mnd2*<sup>+/+</sup>;Tg mice sequentially stained for COX and SDH. arrows indicate COX-negative areas. No COX-negative cells could be detected in the brain. Bar: 1<sup>st</sup> and 2<sup>nd</sup> row, 50  $\mu$ m; 3<sup>rd</sup> and 5<sup>th</sup> row, 30  $\mu$ m; 4<sup>th</sup> row, 15  $\mu$ m.