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Supplemental Material to:

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Macroautophagy is deregulated in murine and human lupus T lymphocytes

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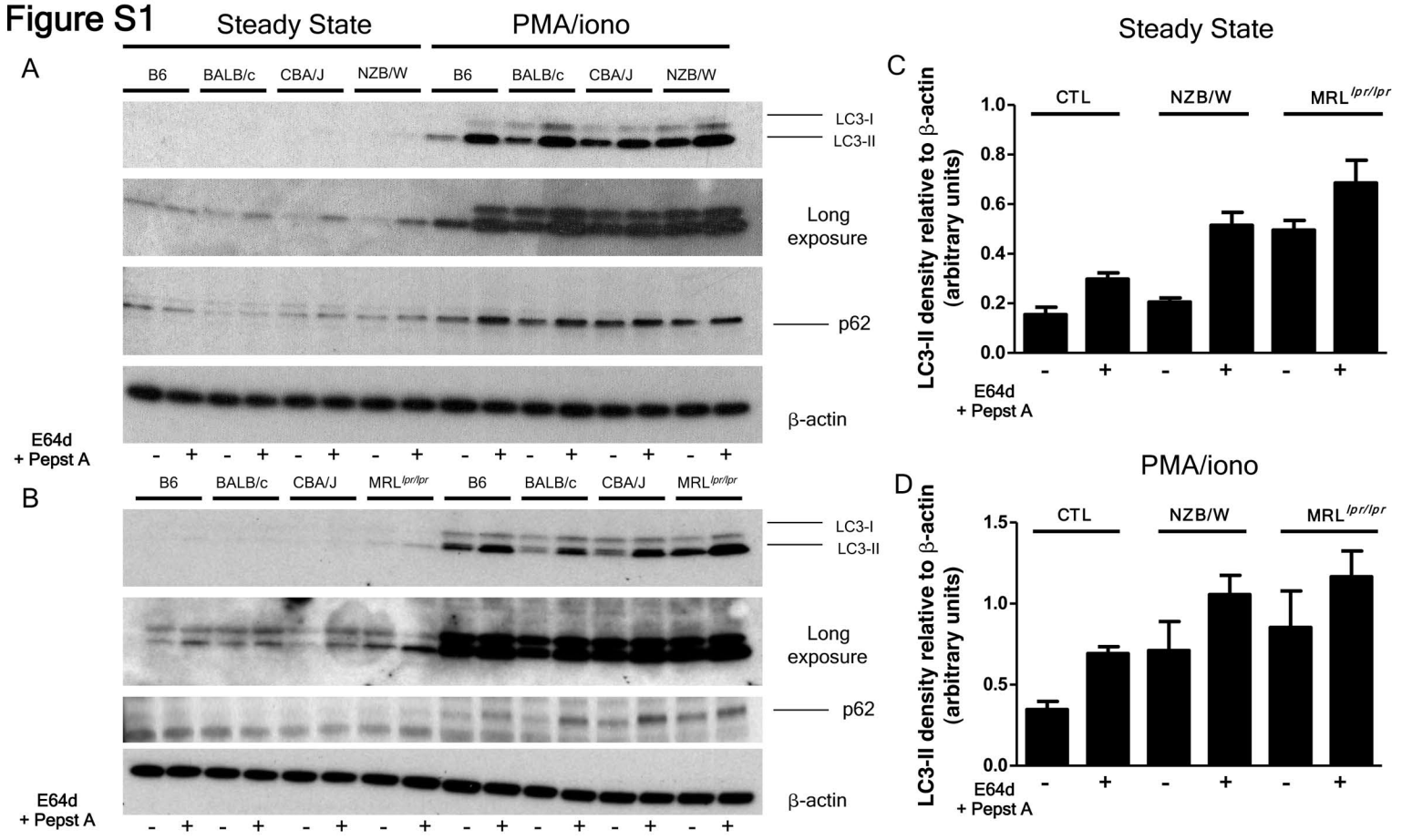

Figure S1. Autophagic activity is higher in lupus-prone mice compared to several control mouse strains. T cells were sorted from spleens of control CBA/J, C57BL/6 (B6), BALB/c and lupus-prone MRL$^{lpr/lpr}$ and NZB/W mice sacrificed at 12 weeks in (A) and 17 weeks in (B). Cells were left unstimulated at 37°C for 18 h (steady state) or stimulated for the same time with 50 ng/mL PMA and 1 µM ionomycin (PMA/iono). When indicated, cells were treated (+) or not (-) with 5 µg/mL pepstatin A and 5 µg/mL E64d to block lysosomal degradation. Cell lysates were resolved by SDS-PAGE, transferred onto PVDF membranes before staining with anti-LC3 and anti-p62 Abs. Loading controls were performed by staining actin b-chain. (C and D) Same experiment: means obtained from four independent experiments. CTL = merged data of C57BL/6, BALB/c and CBA/J (n = 4), NZB/W (n = 2) and MRL$^{lpr/lpr}$ (n = 2) lupus mice at steady state (C) and under PMA/Ionomycin stimulations (D).

Figure S2

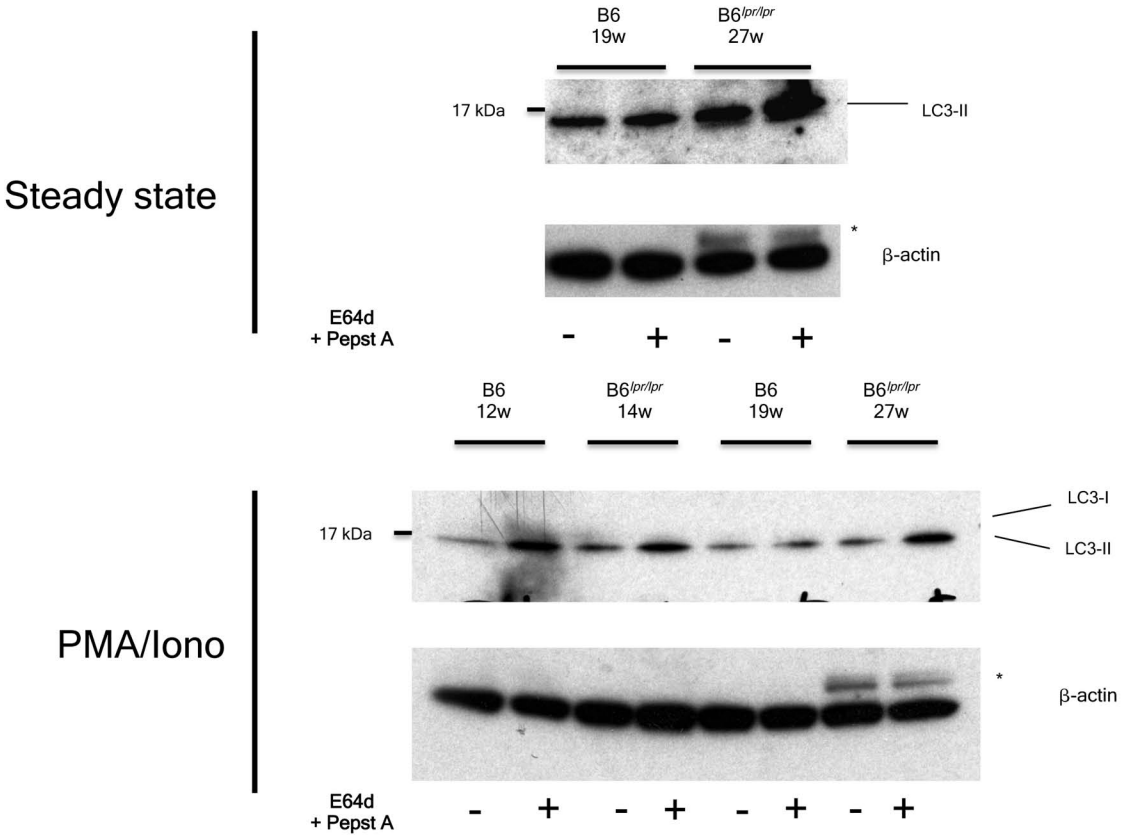

Figure S2. No difference in autophagic activity in T cells from lupus B6$^{lpr/lpr}$ mice compared to control B6 mice. T cells were sorted from spleens of C57BL/6 (B6) and C57BL/6$^{lpr/lpr}$ (B6$^{lpr/lpr}$) mice sacrificed at the indicated ages and left unstimulated at 37°C for 18 h (steady state) or stimulated for the same time with 50 ng/mL PMA and 1 µM ionomycin (PMA/Iono). When indicated, cells were treated (+) or not (-) with 5 µg/mL pepstatin A and 5 µg/mL E64d to block lysosomal degradation. Cell lysates were resolved by SDS-PAGE, transferred onto PVDF membranes before staining with anti-LC3 Ab. Loading controls were performed by staining actin b-chain. *Band corresponding to heavy and light chain of immunoglobulins retained in lysates obtained from oldest lupus mice.

Figure S3

Splenic B cells

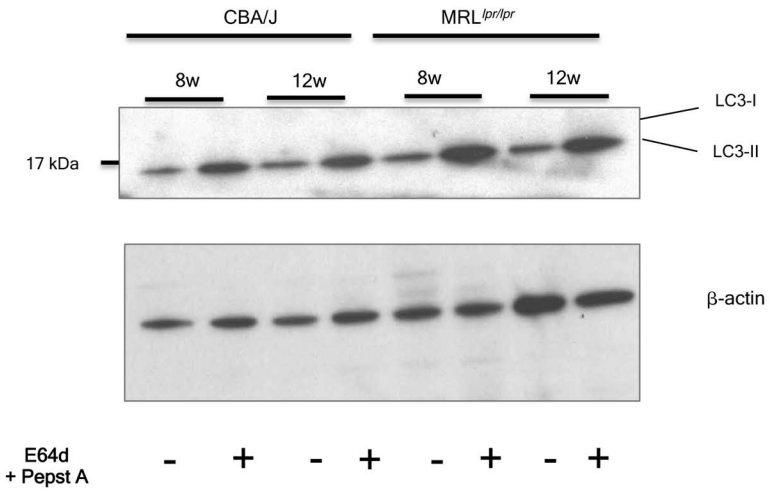

Figure S3. Autophagic activity is similar in splenic B cells from MRL$^{lpr/lpr}$ and CBA/J mice. B cells were sorted from spleens of control CBA/J and lupus-prone MRL$^{lpr/lpr}$ mice sacrificed at the indicated ages (8 and 12 weeks). Cells were left unstimulated at 37°C for 4 h. As indicated, cells were treated (+) or not (-) with 5 µg/mL pepstatin A and 5 µg/mL E64d to block lysosomal degradation. Cell lysates were resolved by SDS-PAGE, transferred onto PVDF membranes before staining with anti-LC3 Ab. Loading controls were performed by staining actin b-chain.

Figure S4

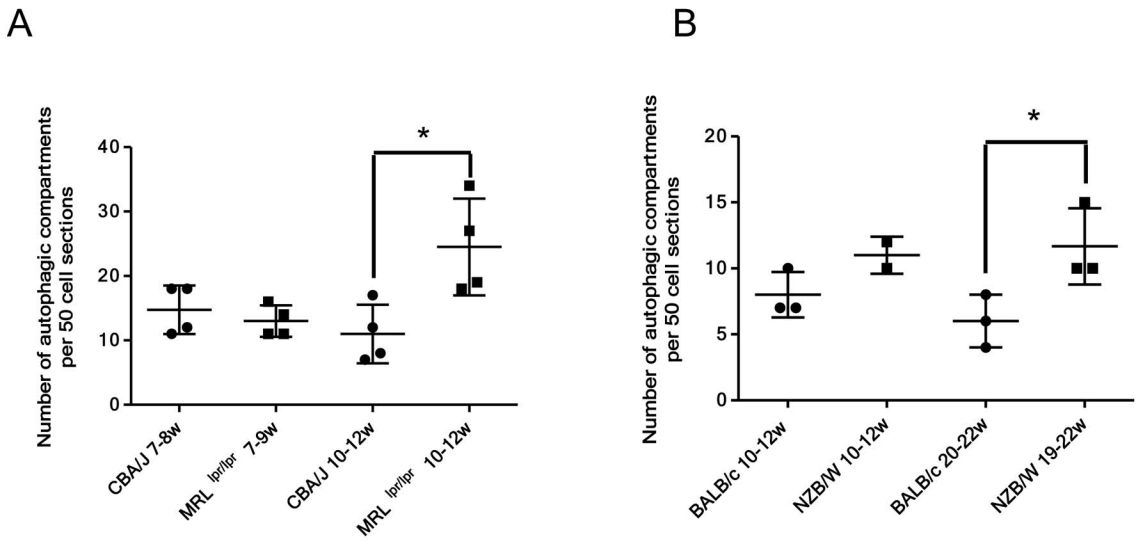

Figure S4. Autophagic activity is maintained or increased in lupus mice compared to control mice. Quantification by TEM of autophagosomes counted in 50 peripheral T lymphocyte sections sorted from spleens of control CBA/J and lupus MRL$^{lpr/lpr}$ mice (A) or from control BALB/c and lupus NZB/W (B). Mice were sacrificed at the indicated ages. Each point represents measurement of an individual mouse. Central bars refer to the mean and vertical bars stand for standard deviation.
*$p < 0.05$ using unpaired t test. w = week.

Figure S5

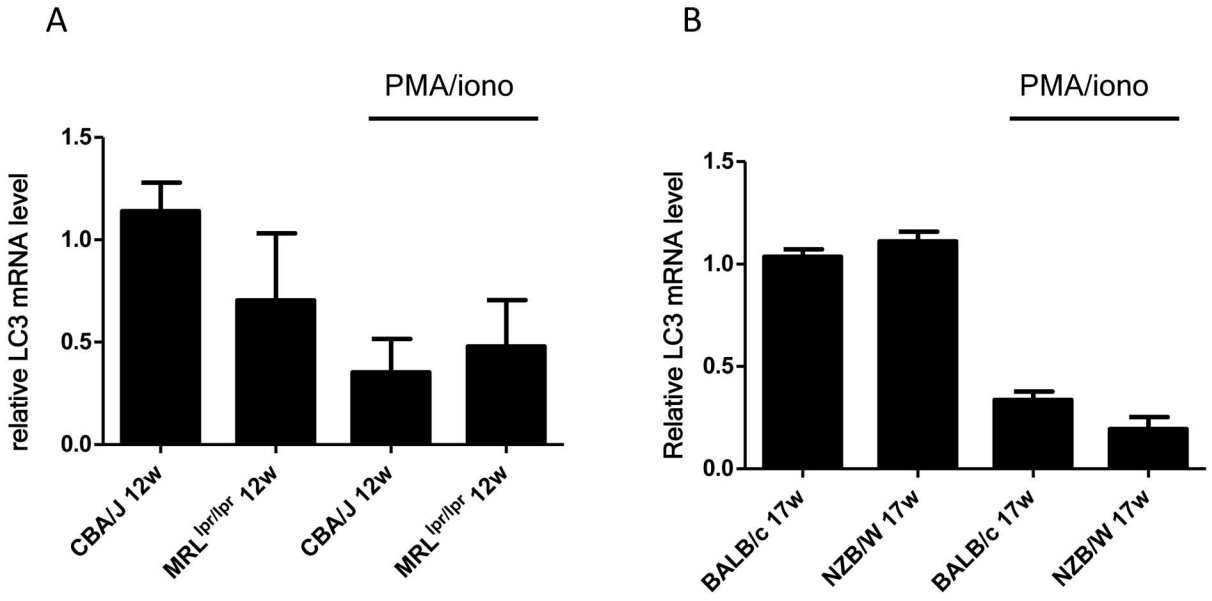

Figure S4. Higher LC3-II levels observed in lupus-prone mice are not a consequence of LC3 mRNA level increase. T cells were sorted from spleens of control CBA/J, BALB/c and lupus-prone MRL*lpr/lpr* and NZB/W mice sacrificed at 12 weeks (A) and 17 weeks (B). Cells were left unstimulated at 37°C for 18 h (steady state) or stimulated for the same time with 50 ng/mL PMA and 1 µM ionomycin (PMA/iono). RNA was isolated and retrotranscribed into cDNA. Quantitative RT-PCR was then performed for measurement of *Map1lc3a* transcripts and were normalized to *Actb* as described in material and methods section. mRNA levels are relative to one control mouse per PCR plate arbitrarily set to 1.