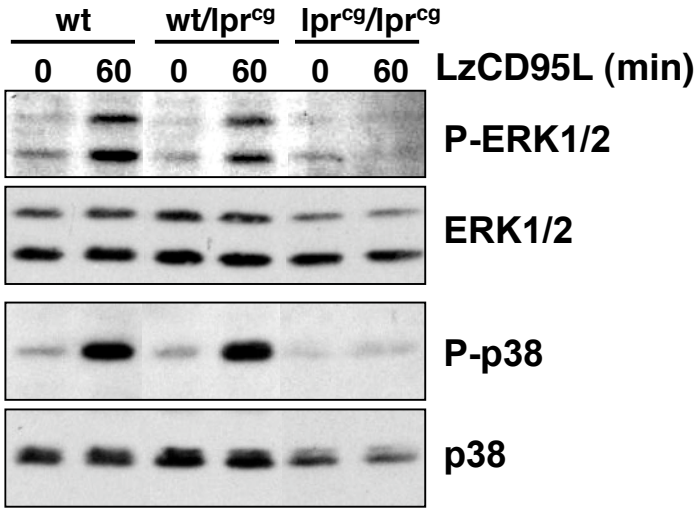
A



B

Phenotype	CD95 expression (MFI)	% positive cells
wt	**43.4**	**93.9**
wt/lpr	**25.4**	**83.9**
lpr/lpr	**4.7**	**1.11**
Isotype control	**4.8**	**2.8**

Supplementary Figure 2: (**A**) Activated splenocytes from the indicated mouse strains were left untreated (0) or stimulated with 1 μg/ml LzCD95L for 60 min (60) and lysates of the cells subjected to Western blot analysis for phosphorylation of indicated MAP kinases. (**B**) Surface expression of CD95 on splenocytes from C3H lpr/wt mice. Surface staining for CD95 was done in duplicate and the mean is shown. Data represent CD95 expression and % positive cells in activated (anti-CD3ε/IL2) splenocytes. MFI: mean fluorescence intensity. Surface staining was performed as previously described (Algeciras-Schimnich et al., 2003).

Western Blot Analysis. Cells were lysed in denaturating buffer (10% glycerol, 2% SDS (w/v), 5% 2-mercaptoethanol (v/v), 250 μg/l bromophenol blue), boiled 5 min and then separated in a 12% SDS-PAGE. Western blot analysis was performed as previously described (Barnhart et al., 2004) using the following antibodies: Phospho-specific and whole protein anti-p38 and anti-ERK1/2 antibodies (Cell signaling), anti-mouse caspase-8 (clone 3B10) (see Fig 3F) provided by A. Strasser (The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia) and appropriate secondary antibodies.

References

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Barnhart B.C., Legembre, P., Pietras, E.M., Bubici, C., Franzoso, G. and Peter, M.E. (2004) CD95 ligand induces motility and invasiveness of apoptosis resistant tumor cells. *EMBO J*, **23**, 3175-3185.