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Membrane-bound but not Secreted Fas Ligand Is Essential for Fas-Induced Apoptosis and Prevention of Autoimmunity and Cancer

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Abstract

Fas ligand (FasL), an apoptosis-inducing member of the TNF cytokine family and its receptor, Fas, are critical for shutdown of chronic immune responses1-3 and prevention of autoimmunity4,5. Accordingly, mutations in their genes cause severe lymphadenopathy and autoimmune disease in mice6,7 and humans8,9. FasL function is regulated by deposition in the plasma membrane and metalloprotease-mediated shedding10,11. We generated gene-targeted mice that selectively lack either secreted FasL (ΔsFasL) or membrane-bound FasL (ΔmFasL) to resolve which of these forms is required for cell killing and to explore their hypothetical non-apoptotic activities. Mice lacking sFasL ($FasL^{\Delta s/\Delta s}$) appeared normal and their T cells readily killed target cells, whereas T cells lacking mFasL ($FasL^{\Delta m/\Delta m}$) could not kill cells through Fas activation. $FasL^{\Delta m/\Delta m}$ mice developed lymphadenopathy and hyper-gammaglobulinaemia, similar to $FasL^{gld/gld}$ mice, which express a mutant form of FasL that cannot bind Fas, but surprisingly, (on a C57BL/6 background) $FasL^{\Delta m/\Delta m}$ mice succumbed to SLE-like autoimmune kidney destruction and histiocytic sarcoma, diseases that occur only rarely and considerably later in $FasL^{gld/gld}$ mice. These results demonstrate that mFasL is essential for cytotoxic activity and constitutes the guardian against lymphadenopathy, autoimmunity and cancer whereas excess sFasL appears to promote autoimmunity and tumorigenesis through non-apoptotic activities.

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Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Author Contributions L.A.O'R. planned and performed most experiments and wrote manuscript. L.T., L.L., E.A.K., S.G., W.D.F., N.M.H., D.M.T., J-G.Z., G.T.B., M.J.S., P.B. and L.R. contributed to planning and execution of experiments and writing of the manuscript. A.S. conceived study, planned experiments and wrote manuscript.

Author Information The authors make the newly generated gene-targeted mice described in this paper freely available. The authors have no conflicts of interest to declare.

Keywords

apoptosis; Fas ligand; autoimmunity; cancer; allergy

Although Fas-induced apoptosis is thought to require extensive aggregation of pre-assembled Fas trimers12-15, it has not been resolved whether mFasL, sFasL or both cause cell killing when expressed physiologically 4,5. It is also debated whether either or both of these forms of FasL may have non-apoptotic activities, such as induction of inflammatory responses5,16,17. Indeed, sFasL is capable of activating the NF-κB pathway (Supplementary Fig. 1 and 18,19). We sought to determine the physiological functions of mFasL and sFasL by generating gene-targeted mice that cannot shed FasL but do express membrane-bound FasL ($FasL^{\Delta s/\Delta s}$) or conversely mice that lack membrane-bound FasL but are capable of producing sFasL ($FasL^{\Delta m/\Delta m}$). Studies using FasL over-expression in transfected cell lines showed that the former can be accomplished, by mutating the sequences in the *fasl* gene encoding the amino acids required for metalloprotease-mediated cleavage12-14 (Fig. 1a, Supplementary Fig. 2a,b,d). Conversely, the latter can be achieved by replacing the sequences in the *fasl* gene encoding the trans-membrane and intra-cellular regions of FasL with those encoding the signal peptide of the cytokine G-CSF12-14 (Fig. 1a, Supplementary Fig. 2a,c,e).

To verify that the mutations had the intended consequences, we compared the expression and subcellular localisation of FasL between mitogenically activated T lymphocytes from $FasL^{\Delta s/\Delta s}$, $FasL^{\Delta m/\Delta m}$ and wt mice. Immunofluorescent staining and confocal microscopic analysis of fixed cells showed that intracellular localisation and levels of the $FasL^{\Delta s}$ and $FasL^{\Delta m}$ mutant proteins were comparable to those of wt FasL (Fig. 1b). ELISA demonstrated that mitogen-activated T cells from $FasL^{\Delta m/\Delta m}$ and wt mice contained substantial levels of FasL in their supernatants whereas $FasL^{\Delta s/\Delta s}$ T cells had significantly less (Fig. 1c). FasL in cellular supernatants can be found in two forms: secreted sFasL derived by metalloprotease-mediated cleavage or mFasL present on vesicles that had been shed by cells12. The latter can efficiently trigger Fas-mediated apoptosis in cultured cells12, although the physiological relevance of this remains unclear. Regardless, FPLC and ultra-centrifugation revealed that in contrast to FasL from supernatants of wt or $FasL^{\Delta m/\Delta m}$ T cells, a substantial fraction of FasL in supernatants of $FasL^{\Delta s/\Delta s}$ T cells resided in membranous fractions (Supplementary Fig. 3). Finally, immunofluorescent cell surface staining and FACS analysis identified significantly higher levels of membrane-bound FasL on activated T cells from $FasL^{\Delta s/\Delta s}$ mice compared to wt T cells (Fig. 1d), consistent with the notion that metalloprotease-mediated cleavage reduces the levels of mFasL11-14. As expected, no FasL was detected on the surface of $FasL^{\Delta m/\Delta m}$ T cells (Fig. 1e). These results verify that $FasL^{\Delta s/\Delta s}$ mice produce mFasL that cannot be shed by metalloproteases, whereas $FasL^{\Delta m/\Delta m}$ mice lack mFasL but produce sFasL.

Since FasL contributes to the killing of virus-infected and other target cells4, we examined which form is critical. We used mitogen-activated T cells from wt or the mutant *fasl* knock-in mice as killers and the FasL sensitive CH1 mouse B lymphoma cells (Supplementary Fig. 4a,b) as targets. $FasL^{\Delta s/\Delta s}$ T cells killed CH1 cells with significantly higher efficiency than

wt T cells (Fig. 2a). In contrast, *FasL*$^{\Delta m/\Delta m}$ T cells possessed only poor cytotoxic activity, comparable to those from FasL-deficient *FasL*$^{gld/gld}$ mice (Fig. 2b). FasL neutralisation inhibited the cytotoxicity of wt and *FasL*$^{\Delta s/\Delta s}$ T cells but did not further reduce the poor killing by *FasL*$^{\Delta m/\Delta m}$ or *FasL*$^{gld/gld}$ T cells (Supplementary Fig. 4c-e), demonstrating that only the former triggered a FasL/Fas-dependent apoptotic process. Restimulation of activated T cells *in vitro* causes activation induced cell death (AICD), which is largely dependent on FasL-mediated (paracrine and/or autocrine) Fas activation4. Stimulation with mitogenic antibodies to CD3 triggered AICD in *FasL*$^{\Delta s/\Delta s}$ T cell blasts as efficiently as in wt T cell blasts (Fig. 2c). In contrast, AICD was abnormally reduced in *FasL*$^{\Delta m/\Delta m}$ T cells, indeed to a similar extent as seen with their *FasL*$^{gld/gld}$ counterparts (Fig. 2d). FasL neutralisation significantly reduced AICD in wt and *FasL*$^{\Delta s/\Delta s}$ T cells but did not further diminish the already reduced killing of *FasL*$^{\Delta m/\Delta m}$ or *FasL*$^{gld/gld}$ T cells (Supplementary Fig. 5). This is consistent with the notion that AICD involves FasL/Fas-dependent as well as -independent mechanisms4,20. Collectively, these results demonstrate that mFasL but not sFasL is essential for Fas-induced killing of target cells and AICD and they indicate that metalloprotease-mediated cleavage of mFasL reduces the cytotoxic activity of activated T cells. This is consistent with certain findings with transfected cell lines over-expressing FasL12-14 and the notion that apoptosis induction requires not only binding of FasL trimers to pre-assembled Fas trimers but more extensive aggregation of Fas trimers12-15. This contrasts with TNFα, which can kill TNF-R1^{+} target cells in both membrane-bound as well as secreted form4,5.

Defects in AICD of mature T cells are thought to be the cause of the lymphadenopathy, hyper-gammaglobulinaemia and autoimmunity in mice and humans deficient in FasL (e.g. *FasL*$^{gld/gld}$) or Fas (e.g. *Fas*$^{lpr/lpr}$)4. As reported21, by ∼100 days of age *FasL*$^{gld/gld}$ mice (on an inbred C57BL/6 background) started to show signs of lymphadenopathy and splenomegaly (Fig. 3a,b), including accumulation of large numbers of ‘unusual’ TCRα/β^{+}CD4^{-}CD8^{-}B220^{+} T cells (Fig. 3c,d). This was accompanied by hyper-gammaglobulinaemia (Fig. 3e) with high titers of anti-nuclear auto-antibodies (ANA, Fig. 3f,g). *FasL*$^{\Delta m/\Delta m}$ mice developed lymphadenopathy, splenomegaly, hyper-gammaglobulinaemia and ANA at a similar rate and extent as *FasL*$^{gld/gld}$ mice (Fig. 3). Remarkably, however, the titres of anti-DNA auto-antibodies were significantly higher in *FasL*$^{\Delta m/\Delta m}$ mice compared to *FasL*$^{gld/gld}$ mice (67% vs 14% symptomatic of SLE-like disease (>12 IU/mL), $p<0.05$; Fig. 3h). In contrast, *FasL*$^{\Delta s/\Delta s}$ mice exhibited none of these abnormalities and had a normal lifespan (Supplementary Fig. 6). These results show that mFasL but not sFasL is essential for the killing of unwanted lymphocytes that is required to prevent lymphadenopathy, hyper-gammaglobulinaemia and accumulation of auto-antibodies.

FasL$^{gld/gld}$ mice express a mutated FasL^{gld} protein (in membrane-bound and secreted form) that is unable to bind to its receptor Fas7, whereas the secreted FasL$^{\Delta m}$ protein (like wt sFasL) can bind to Fas (Supplementary Fig. 1 and12-14). If sFasL plays a role in inflammation, for example through NF-κB activation (Supplementary Fig. 1 and 18,19), one would expect significant differences in morbidity and mortality between *FasL*$^{\Delta m/\Delta m}$ and *FasL*$^{gld/gld}$ mice. It was therefore remarkable that *FasL*$^{\Delta m/\Delta m}$ mice became sick significantly

earlier than *FasL*$^{gld/gld}$ mice (Fig. 4a, median latency 406 vs 658 days; p<0.0001). At autopsy 62% of terminally ill *FasL*$^{\Delta m/\Delta m}$ mice showed signs of SLE-like autoimmune disease, including cellular crescents, protein casts (Fig. 4b,c), deposition of IgM as well as IgG antibodies and complement in renal glomeruli (Supplementary Fig. 7). In contrast, such pathologies were only rarely observed in *FasL*$^{gld/gld}$ mice, and then only at a considerably older age and with less severity. Remarkably, by 400 days ∼50% of *FasL*$^{\Delta m/\Delta m}$ but only ∼15% of the *FasL*$^{gld/gld}$ mice had developed fatal SLE-like autoimmune kidney disease (Fig. 4b).

By 5 months 46% of *FasL*$^{\Delta m/\Delta m}$ mice presented with very high (>3000 ng/mL) serum IgE levels and ∼30% had developed severe dermatitis with lesions appearing on their ears and necks (Supplementary Fig. 8a,b). Although not previously reported, we observed this autoimmune pathology also in some *FasL*$^{gld/gld}$ mice, albeit at decidedly lower incidence (∼10%) and later in life compared to *FasL*$^{\Delta m/\Delta m}$ mice. In *Fas*$^{lpr/lpr}$ mice lymphadenopathy and accumulation of TCRα/β^{+}CD4^{-}CD8^{-}B220^{+} T cells are accompanied by abnormally increased serum levels of pro-inflammatory cytokines, including TNFα, IL-6, Ifnγ and FasL itself22. FasL was shown to activate NF-κB transcription factors and expression of pro-inflammatory cytokines and chemokines (Supplementary Fig. 1 5,18,19). We therefore hypothesised that *FasL*$^{\Delta m/\Delta m}$ mice may develop autoimmune disease more rapidly and at higher incidence than *FasL*$^{gld/gld}$ animals because only the former produce excess sFasL that can bind to its receptor Fas, which may then activate NF-κB and thereby drive production of pro-inflammatory cytokines (Supplementary Figs. 8 and 9). Consistent with this idea, at 3-5 months *FasL*$^{\Delta m/\Delta m}$ mice contained substantial numbers of cells with high levels of nuclear (i.e. active) p65/NF-kB in the spleen, liver (Supplementary Fig. 10) and kidneys (not shown) and high serum levels of TNFα (Supplementary Fig. 8; p<0.0001 for TNFα). These abnormalities were significantly less prevalent in *FasL*$^{gld/gld}$ mice and were not detected in *FasL*$^{\Delta s/\Delta s}$ or wt animals (Supplementary Figs. 8, 9 and 10).

Fas is expressed on hepatocytes and its activation causes apoptosis23,24. FasL can also be found in the liver, produced by infiltrating T lymphocytes or resident myeloid cells, and it has therefore been hypothesised that FasL-Fas induced apoptosis prevents tumorigenesis in this organ25. Interestingly, a significant fraction (27% by 18 months) of both strains of *FasL*$^{\Delta m/\Delta m}$ mice developed hepatic tumours with deposits in the spleen and lungs (Fig. 4d,e). Microscopically and by immuno-phenotype (Mac-1^{+}Mac-2^{+}F4/80^{+}B220^{-}Thy-1^{-}CD3^{-}) these tumours were characteristic of histiocytic sarcoma (Fig. 4e,f and Supplementary Fig. 11), being composed of oval cells with eosinophilic cytoplasm and elongated or folded nuclei26. These tumours were transplantable in C57BL/6 mice (Supplementary Fig. 11), confirming their malignant status. Histiocytic sarcoma was rarely seen in *FasL*$^{\Delta s/\Delta s}$ or *FasL*$^{gld/gld}$ animals and in C57BL/6 (wt) mice such tumours are observed only at very low frequency late in life (∼5% >18 months26,27; Fig. 4d and Supplementary Fig. 6).

Our findings that mFasL but not sFasL is critical for AICD of T cells *in vitro* and for prevention of lymphadenopathy, hyper-gammaglobulinemia and accumulation of auto-antibodies within the whole animal are consistent with the notion that repeated TCR stimulation kills chronically activated T cells that are specific for auto-antigens or persistent pathogens through FasL-Fas signalling, thereby preventing lymphadenopathy1-4. The

observation that *FasL*$^{\Delta m/\Delta m}$ mice develop SLE-like glomerulonephritis and histiocytic sarcoma considerably earlier and with higher incidence than *FasL*$^{gld/gld}$ mice indicates that the high levels of sFasL produced in the *FasL*$^{\Delta m/\Delta m}$ mice, which in contrast to FasL^{gld} can engage its receptor Fas12,13, may promote autoimmunity and tumorigenesis. sFasL may achieve this by triggering non-apoptotic signalling pathways, such as NF-κB-dependent inflammatory processes. Alternatively, differences between *FasL*$^{gld/gld}$ and *FasL*$^{\Delta m/\Delta m}$ mice may be due to the fact that retrograde signalling through FasL28,29 can only occur in the former but not the latter, although upon challenge with influenza virus *in vivo* or stimulation with suboptimal doses of anti-CD3 antibodies *in vitro*, CD8^{+} T cell responses were indistinguishable between wt, *FasL*$^{gld/gld}$ and *FasL*$^{\Delta m/\Delta m}$ mice (Supplementary Figures 12 and 13). It is theoretically also possible that *FasL*$^{\Delta m/\Delta m}$ mice die earlier than *FasL*$^{gld/gld}$ mice because the *FasL*$^{\Delta m}$ mutation causes complete loss of function whereas *FasL*^{gld} represents a partial loss of function mutation. For two reasons this appears unlikely: (1) lymphadenopathy and hyper-gammaglobulinemia occur in the two mutant strains (*FasL*$^{\Delta m/\Delta m}$ and *FasL*$^{gld/gld}$) with comparable kinetics and magnitude (Fig. 3), indicating that the two mutations do not differ markedly in their potency, and (2) histiocytic sarcoma has not been reported in FasL knock-out mice30. We therefore hypothesise that tumorigenesis may be driven by a combination of loss of mFasL-mediated apoptosis of cells undergoing transformation and sFasL-Fas induced non-apoptotic signals, perhaps NF-κB-mediated stimulation of cell proliferation, survival and/or inflammation within an elevated cytokine milieu.

Methods Summary

Generation of FasL mutant mice

The mouse *fasl* locus and known restriction sites were used to construct the targeting vector and diagnose homologous recombination in embryonic stem (ES) cells and gene-targeted (*FasL*$^{\Delta m/\Delta m}$, *FasL*$^{\Delta s/\Delta s}$) mice. Targeting knock-in vectors were made with the *loxP/pGKNeo/loxP* cassette cloned into the Pac1 site. Targeting constructs for the mutant FasL mice (Supplementary Fig. 2) were linearised and electroporated into C57BL/6-derived Bruce-4 ES cells.

Analysis of FasL mutant mice

All experiments with mice were performed according to the guidelines of the Animal Ethics committees of our institutions. Mice were killed at 6, 12 or 20 weeks for analysis and further cohorts were monitored daily for morbidity and killed when showing signs of illness. Tissues were fixed for microscopic analysis in 80% Histochoice (Amresco)/20% ethanol or 10% buffered formalin and embedded in paraffin and conventional histopathology was performed on hematoxylin plus eosin stained sections. For detailed methods for immunohistochemical staining, immunofluorescent staining, confocal microscopy, cell preparation, flow cytometric analysis, ELISA, AICD, chromatography, viral infection and target cell killing, T cell proliferation assays and Western blotting refer to the online methods version.

Statistical analysis was performed using the student's T-test, log rank (Mantel-Cox) test for survival curves or one-way analysis of variance using Turkey's comparison test to compare multiple groups where appropriate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

FasL$^{gld/gld}$	generalized lymphoproliferative disorder = spontaneous mutation in the Fas ligand gene
FasL	Fas ligand
sFasL	secreted Fas ligand
mFasL	membrane-bound FasL
Fas$^{lpr/lpr}$	lymphoproliferation = spontaneous mutation in the Fas gene
FasL$^{\Delta m/\Delta m}$	mice lacking membrane bound FasL

***FasL*$^{\Delta s/\Delta s}$**	mice lacking secreted FasL
TNFα	tumour necrosis factor α
Ifnγ	interferon-γ
SLE	systemic lupus erythematosus
GN	glomerulonephritis
ANA	anti-nuclear auto-antibodies
G-CSF	granulocyte colony stimulating factor

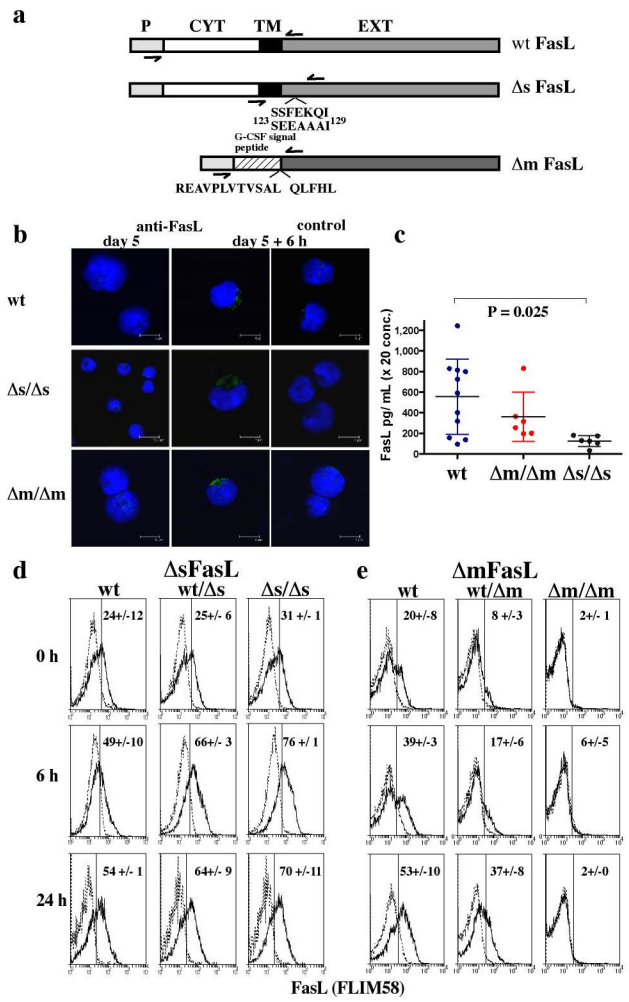

Figure 1. Generation of mutant mice that specifically lack either secreted FasL or membrane-bound FasL

a, Schematic diagram of the wt mouse *fasl* gene, the mutation for creating the $FasL^{\Delta s}$ allele, in which the metalloproteinase recognition site (middle sequence) was altered to prevent FasL shedding and the mutation for creating the $FasL^{\Delta m}$ allele, in which sequences encoding the human G-CSF signal sequence were fused in frame with those for the extracellular region of mouse FasL (lower sequence) to preclude insertion of FasL into the plasma membrane but allowing secretion of FasL (sFasL). P, CYTO, TM and EXT represent the promoter, cytoplasmic, transmembrane and extracellular regions, respectively. The positions of PCR primers used for genotyping of the gene-targeted mice are indicated. **b**, Immunofluorescent staining and confocal microscopy to demonstrate intracellular localisation of FasL (green) in activated T cells from wt, $FasL^{\Delta s/\Delta s}$ and $FasL^{\Delta m/\Delta m}$ mice. DAPI (blue) was used to label nuclei. **c**, ELISA to quantify the levels of FasL in the supernatants of activated T cells from wt, $FasL^{\Delta s/\Delta s}$ and $FasL^{\Delta m/\Delta m}$ mice. Each dot represents a single mouse and the bar indicates the mean +/- SD. $FasL^{\Delta s/\Delta s}$ T cells had significantly ($p<0.03$) less FasL in their supernatants compared to wt T cells. **d**,**e**, Cell surface immunofluorescent staining and FACS analysis to measure expression of membrane-bound FasL on activated T cells from wt, $FasL^{\Delta s/\Delta s}$ (**d**) and $FasL^{\Delta m/\Delta m}$ (**e**) mice.

Solid lines show staining with an anti-FasL antibody and dotted lines show staining with an isotype-matched control antibody. Values represent mean +/- SD of $mFasL^+$ T cells from 3 independent experiments ($p<0.05$ wt vs $FasL^{\Delta m/\Delta m}$ mice at 6 and 24 h; $p<0.05$ wt vs $FasL^{\Delta s/\Delta s}$ mice at 6 h). For all experiments (**b**,**c**,**d**,**e**) T cells were stimulated with ConA for 3 days, rested for 2 days in IL-2 (0 h) and then restimulated with PMA plus ionomycin for 6 or 24 h.

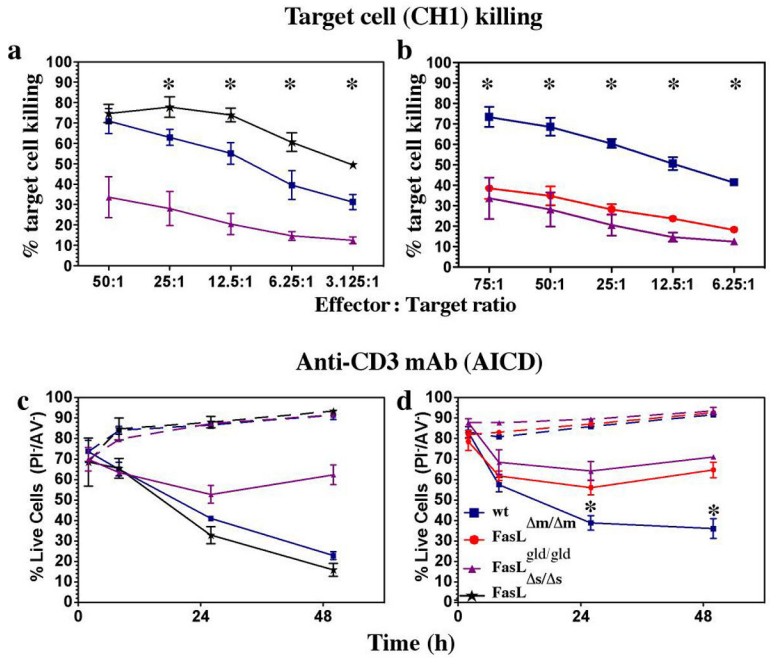

Figure 2. Membrane-bound but not secreted FasL is essential for target cell killing and AICD

a,**b**, CH1 target cells (FasL sensitive) were co-cultured with activated T cells from wt (blue squares), *FasL*$^{\Delta s/\Delta s}$ (black stars) or *FasL*$^{gld/gld}$ (purple triangles**)** mice (**a**) or from wt (blue squares), *FasL*$^{\Delta m/\Delta m}$ (red circles**)** or *FasL*$^{gld/gld}$ (purple triangles**)** mice (**b**) at the indicated effector:target ratio. The percentage of CH1 target cell killing was measured by FACS analysis after 24 h. **c**,**d**, Activated T cells from wt (blue squares), *FasL*$^{\Delta s/\Delta s}$ (black stars) or *FasL*$^{gld/gld}$ (purple triangles**)** mice (**c**) or from wt (blue squares) *FasL*$^{\Delta m/\Delta m}$ (red circles) or *FasL*$^{gld/gld}$ (purple triangles) mice (**d**) were either cultured in medium plus IL-2 (broken lines) or restimulated for 6, 24 or 48 h with antibodies to CD3 (solid lines). Cell survival was measured by staining with PI plus FITC-coupled annexin V and FACS analysis. Values in graphs in **a**,**b**,**c**,**d** represent mean +/- SEM from 3 independent experiments. * $p<0.05$ **a**,**c** *FasL*$^{\Delta s/\Delta s}$ vs wt, **b**,**d** *FasL*$^{\Delta m/\Delta m}$ vs wt.

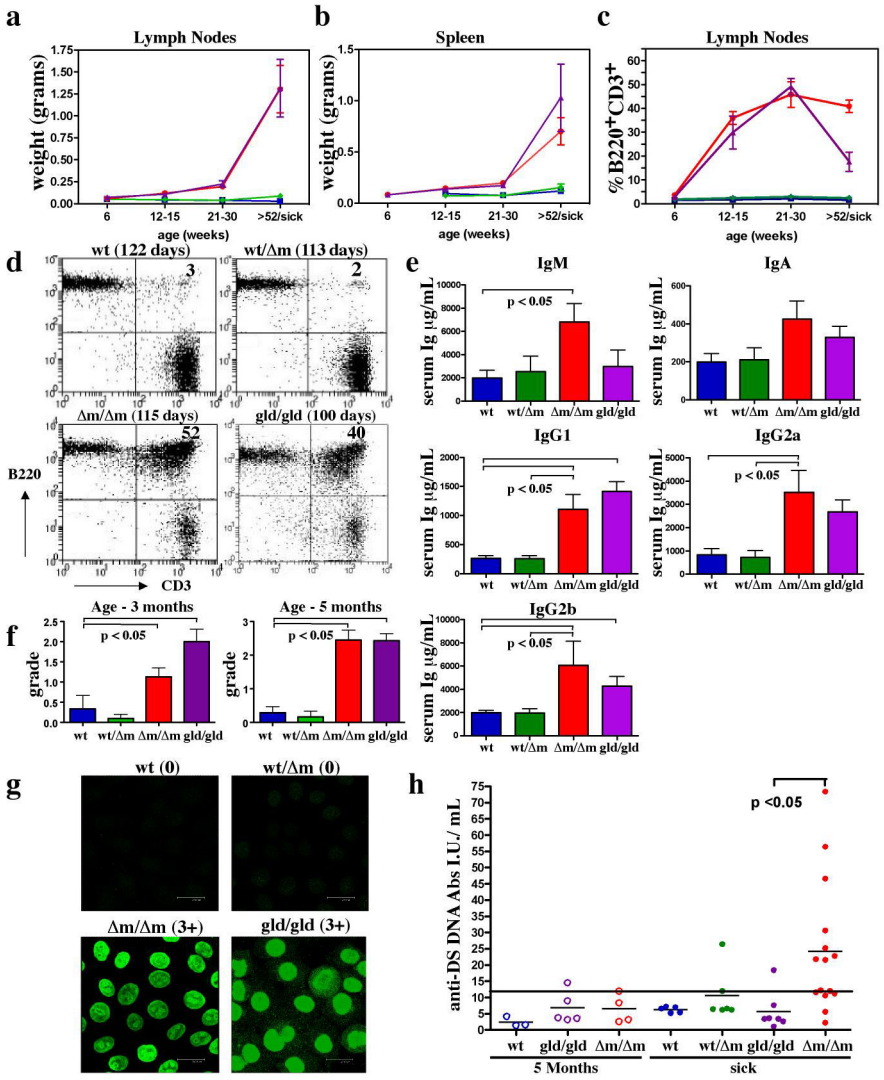

Figure 3. Membrane-bound FasL but not secreted FasL is essential to prevent lymphadenopathy, splenomegaly and hyper-gammaglobulinemia with anti-nuclear autoantibodies

a, Lymph nodes (axillary, brachial, inguinal and mesenteric) and **b**, spleens from wt (blue), *FasL*$^{gld/gld}$ (purple), *FasL*$^{\Delta m/wt}$ (green) or *FasL*$^{\Delta m/\Delta m}$ (red) mice of the ages indicated were weighed. **c**,**d**, The percentages of the 'unusual' CD3^{+}B220^{+} T cells in the lymph nodes of these mice were determined by FACS analysis (numbers indicate % of cells in each quadrant). **e**, The levels of the indicated immunoglobulin isotypes in the sera of these mice (age 5 months) were determined by ELISA, mean +/- SD. **f**, The levels of anti-nuclear autoantibodies (ANA) in the sera of these mice, aged 3 (left panel) or 5 months (right panel) were quantified by indirect immunofluorescence staining (1/100 serum dilution) of slides covered with human HEp-2 epithelial cells and scoring of brightness of fluorescence intensity on a scale of 0: no fluorescence to 3+: maximal fluorescence. **g**, Examples of ANA quantification in sera of mice of the indicated genotypes. Immunofluorescence intensity score is indicated in brackets. Values in graphs in **a,b** represent mean +/-SEM and in **c,e,f** they represent mean +/-SD from a minimum of 10 to 52 *FasL*$^{\Delta m/\Delta m}$ mice. **h**, The levels of

anti-DNA auto-antibodies in sera of mice of the indicated genotypes and ages were measured by ELISA (each dot represents the value for a single mouse). Bar at 12.5 IU/ml indicates the level diagnostic for SLE.

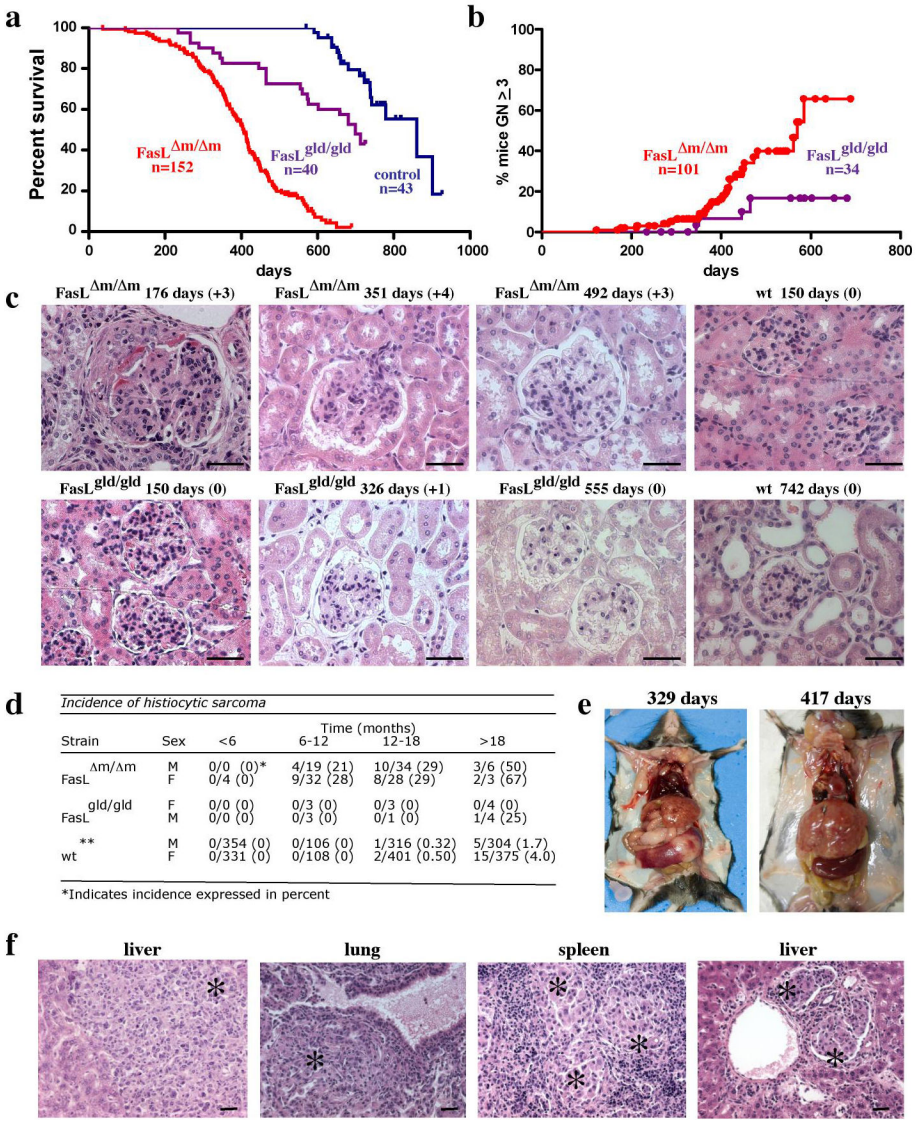

Figure 4. *FasL*$^{\Delta m/\Delta m}$ mice die considerably earlier than *FasL*$^{gld/gld}$ mice due to SLE-like fatal glomerulonephritis and histiocytic sarcoma

a, Kaplan-Meyer survival curves for control (blue line, wt and *FasL*$^{\Delta m/wt}$ combined), *FasL*$^{\Delta m/\Delta m}$ (red line) and *FasL*$^{gld/gld}$ mice (purple line) (control vs *FasL*$^{gld/gld}$: p<0.0001; control vs *FasL*$^{\Delta m/\Delta m}$: p<0.0001; *FasL*$^{\Delta m/\Delta m}$ vs *FasL*$^{gld/gld}$: p<0.0001). **b**, Incidence of severe autoimmune kidney disease (glomerulonephritis (GN) score ≥3 in *FasL*$^{\Delta m/\Delta m}$ (red line) and *FasL*$^{gld/gld}$ (purple line) mice. (*FasL*$^{\Delta m/\Delta m}$ vs *FasL*$^{gld/gld}$: p<0.0175). **c**, H&E stained sections of kidneys from mice of the indicated genotypes and ages were examined for pathological changes, such as hypercellularity, cellular crescents, dilated tubules or sclerotic glomeruli and the % of total glomeruli affected scored on a scale of 0-4 (0=normal, 1=0-25%, 2=25-50%, 3=50-75%, 4=>75% affected; scores indicated in brackets). **d**, Incidence of histiocytic sarcoma in *FasL*$^{\Delta m/\Delta m}$, *FasL*$^{gld/gld}$ and control wt mice (*FasL*$^{\Delta m/\Delta m}$ vs *FasL*$^{gld/gld}$: p=0.03), ** 27. **e**. Pictures of two *FasL*$^{\Delta m/\Delta m}$ mice with histiocytic sarcoma (aged 329 and 417 days). Macroscopic lesions are readily seen in the livers and spleens. **f**,

H&E stained sections of livers, spleens and lungs of histiocytic sarcoma-burdened *FasL*$^{\Delta m/\Delta m}$ mice (aged 329 and 417 days; * indicates histiocytic lesions). Scale bar represents 155 μm.