Supplemental Figures Legends SC-06-0745

Supplement Fig. S1. FACS for FasL and GFP. The anti-FasL antibodies MFL-3, MFL-4, N20, Kay-10, AB1665, and FLIM58 were tested on IL-2 activated rat lymphocytes cultured for 7 days in 20 IU IL-2. The antibodies Flim58 and AB1665 revealed a shift in the FACS and were used for subsequent analyses (**a**). (**b**) FACS on naïve HEK (black) and rFasL-transfected HEK (HEK.XF1, gray) cells using Flim58 and AB1665. There was high background staining and no evidence for recombinant rFasL expression. HEK.XF1 cells were also positive for GFP demonstrating the functionality of the IRES in the XF plasmid. (**c**) FACS analyses on naïve D3 ES (black) and rFasL-recombinant mES cells (ES.XF1, gray) using Flim58 and AB1665. Like seen with the HEK cells, there was no indication of rFasL expression. Similar results were observed with the N-20 antibody (data not shown). FACS for GFP fluorescence demonstrated that the ES.XF1 cells did not express the GFP gene, while pCX-EGFP-transfected control cells were positive for GFP expression. (**d**) FACS using the AB1665 and N-20 antibodies on differentiated D3 ES (black) and ES.XF1 (gray) mES cells at ND stage 5:8. As seen with the immature ES cells, there was no indication for expression of recombinant rFasL. In addition, differentiated cells from ES.X_{gfp} expressed GFP (gray), while ES.XF1 cells were GFP negative (black). These data show that in immature and differentiated mES cells the CBA promoter is active and that the IRES in pCX-rFasL-IRES-EGFP (XF) is not functional.

Supplement Fig. S2. FACS analysis of co-culture experiments using Jurkat target cells with naïve or rFasL-transfected HEK effector cells. After incubation for 24 to 36 hrs, the cultured cells were analyzed by PI staining and FACS measuring the cell amounts in two gates, the target (Jurkat) and the effector cell (HEK and HEK.XF) gate. (**a**) FACS of control experiment using Jurkat cells treated with sFasL. Untreated Jurkat cells (left panel, -FasL) were gated and the gate applied to Jurkat cells treated with 50 ng/ml sFasL for 36 hrs (right panel , +FasL). The cell amounts decreased from 87.7% in the untreated to 3.8% in the treated cell population demonstrating sFasL-induced apoptosis. (**b**) The same strategy as described in (**a**) was applied to co-culture experiments using HEK or HEK.XF as effector cells. Left upper panel: gated HEK cells alone and the target gate for Jurkat cells. Right upper panel: gated HEK cells from co-

culture with Jurkat cells present in the Jurkat cell gate (66%). Left lower panel: gated HEK.XF cells alone and the gate for Jurkat cells. Right lower panel: rFasL-transfected HEK.XF cells from co-culture with Jurkat cells. There were almost no Jurkat cells in the target cell gate (8%) demonstrating FasL-mediated apoptosis. To compensate for variations within the experiments, the data were plotted as ratios between Jurkat and effector cell population (see Figs. 1, 2, and 3).

Supplement Fig. S3. Testing rFasL expression in the rat neuroblastoma cell line B104. B104 cells were co-transfected with pCX-rFasL-IRES-EGFP and the puromycin-resistance gene expressing plasmid PGK-puro. Transfected cells were selected in 2 µg/ml puromycin (Sigma) and named B104.XF. (**a**) RT-PCR for GAPDH and FasL (lower panels), and Western blots using the rabbit anti-mouse FasL antibody N-20 (upper panel) on naïve B104 and B104.XF cells. In both naïve and rFasL-transfected B104, there was a rFasL transcript and a 32 kDa product that was also present in thymus lysates. (**b**) FACS analyses of naïve B104 and B104.XF cells using the anti-FasL antibodies AB1665, N-20, MFL-3, and Flim58. There was no evidence for surface FasL expression. (**c**) FACS of Jurkat cell co-culture experiments with B104 and B104.XF cells. There was no apoptosis of Jurkat cells in the presence of the naïve or rFasL-tansfected B104 cells. (**d**) FACS of Jurkat cell assays using sFasL or supernatants from B104 or B104.XF cells. The conditioned media from both naïve and rFasL-transfected B104 cells did not kill the Jurkat cells indicating that no sFasL was released.

Antibody name	Company	Antigen	Species-specificity	Isotype	Concentration	Results for rat thymus lysates	Results for HEK cells	Results for ES cells
N-20 (sc-834)	Santa Cruz Biotechnologies	amino terminus rat FasL	mouse, rat, human	rabbit IgG	1 µg/ml	expected band at 32 kDa	multiple signals plus strong 32 kDa in rat FasL transfectants	multiple signals plus 32 kDa in naïve and rat FasL transfectant cells
N-20 (sc-834)	Santa Cruz Biotechnologies	amino terminus rat FasL	mouse, rat, human	goat IgG	1 µg/ml	expected band at 32 kDa	multiple weak signals, but not conclusive for 32 kDa in rat FasL transfectants	multiple weak signals, but not conclusive for 32 kDa in naïve and rat FasL transfectant cells
H11	Alexis Laboratories	synthetic peptide (aa 196-220 mouse FasL)	mouse	rat IgG	2 µg/ml	expected band at 32 kDa	no signal in naïve and rat FasL transfectants	no signal in naïve and rat FasL transfectants
F2928	Sigma-Aldrich	synthetic peptide (aa 132-279 mouse FasL)	mouse	rat IgG	0.2 µg/ml	expected band at 32 kDa	no signal in naïve and rat FasL transfectants	multiple signals plus 32 kDa in naïve and rat FasL transfectant cells

Table S1: Summary of Western blot experiments using four different anti-FasL antibodies. Listed are the antibody information and the results for rat thymus lysates, and for naïve and rat FasL-recombinant HEK and mouse ES cells. Note that there are 2 amino acid mismatches and 4 mismatches in the peptide sequences between mouse and rat FasL for H11 and F2928, respectively. The expected size for the FasL protein is 32 kDa.

Fig. S1

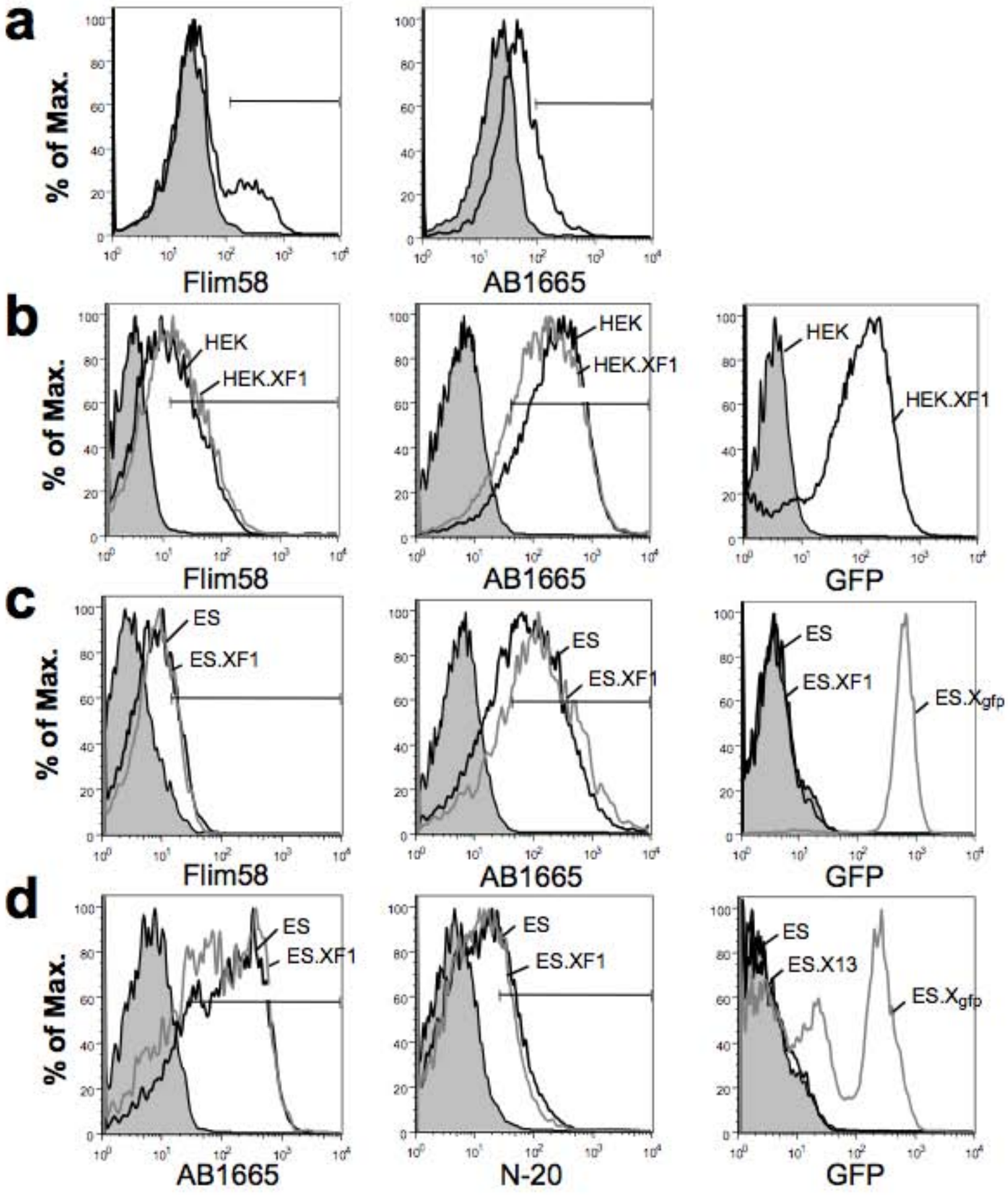

a
% of Max.
Flim58
AB1665
b
% of Max.
HEK
HEK.XF1
Flim58
AB1665
GFP
c
% of Max.
ES
ES.XF1
ES.X$_{gfp}$
Flim58
AB1665
GFP
d
% of Max.
ES
ES.XF1
ES.X13
ES.X$_{gfp}$
AB1665
N-20
GFP

Fig. S2

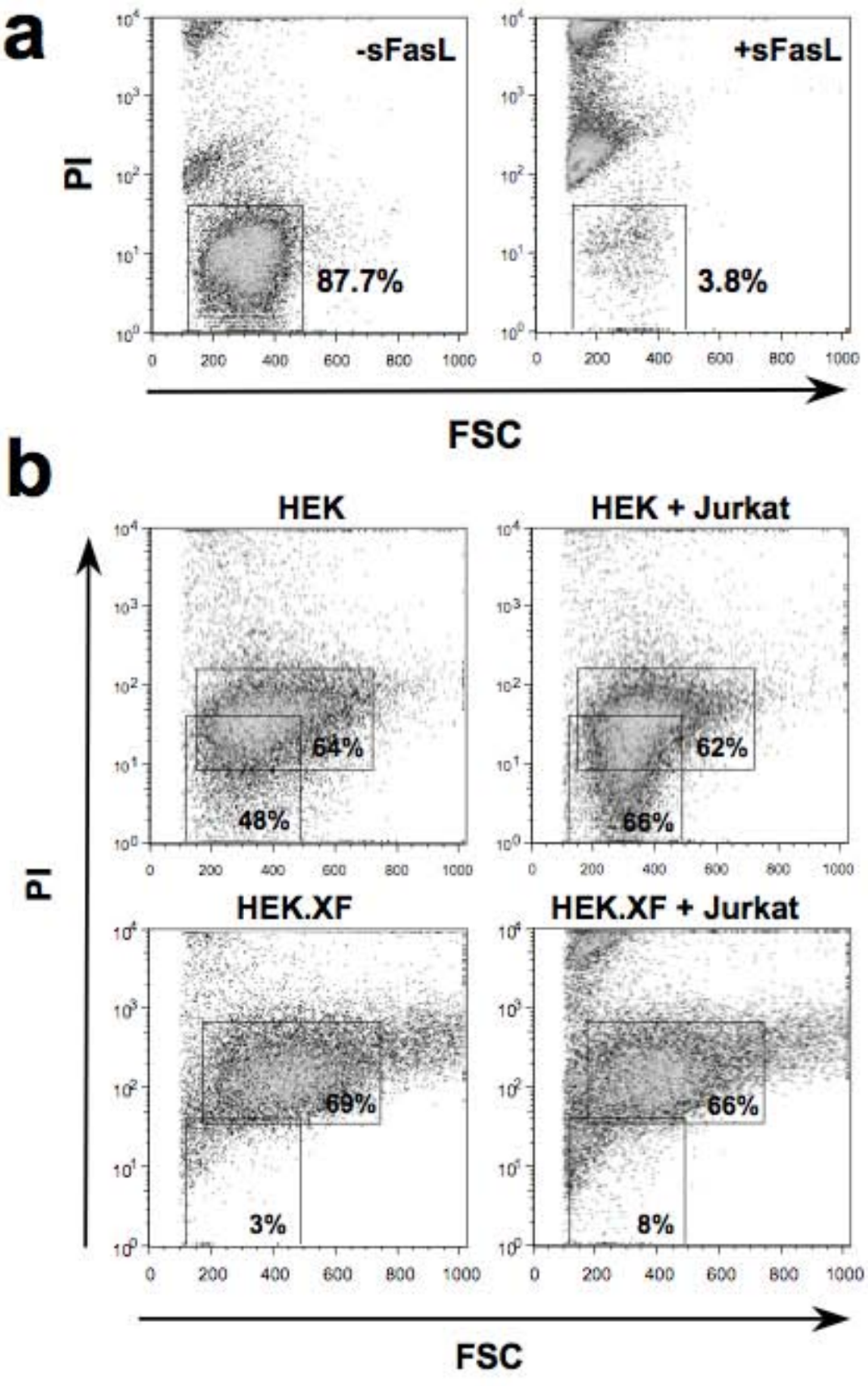
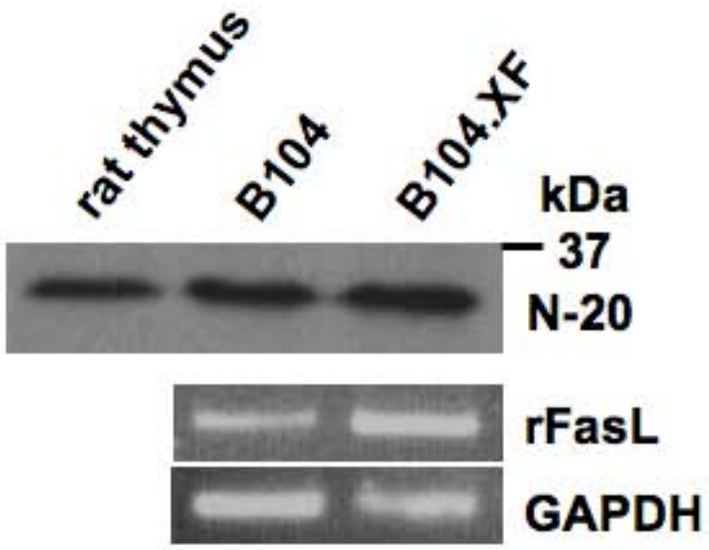
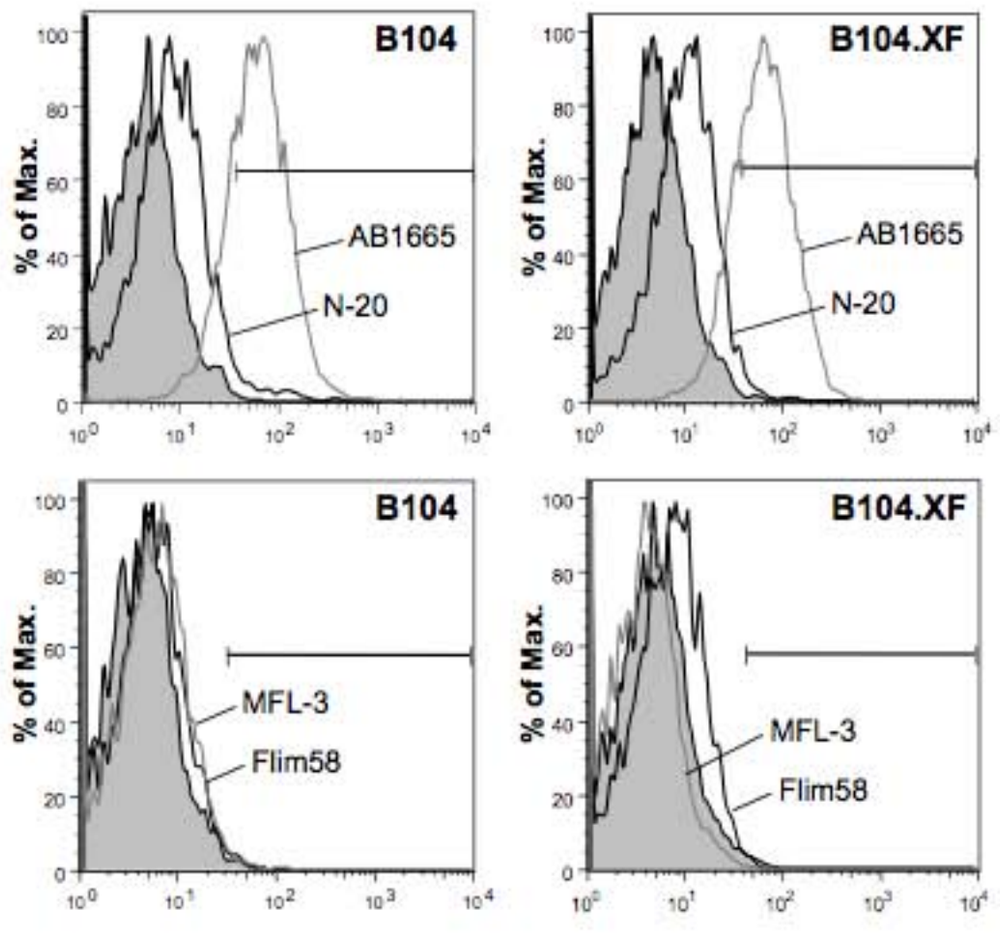
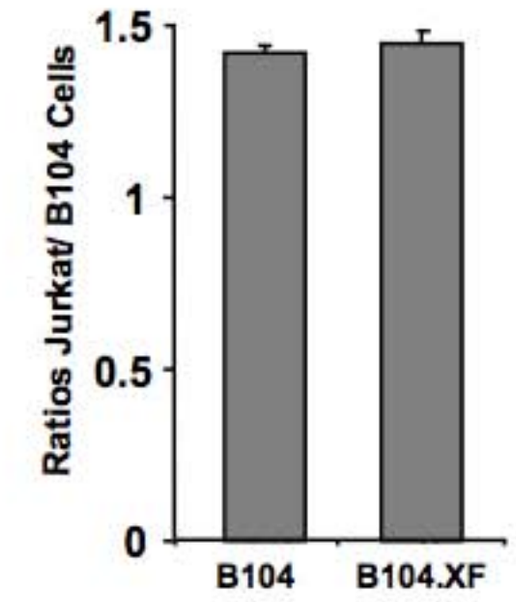
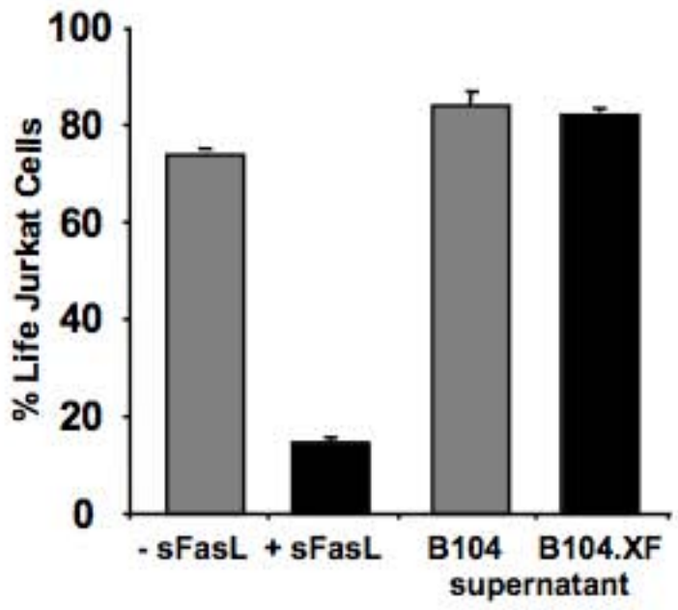

a
-sFasL
+sFasL
PI
87.7%
3.8%
FSC
b
HEK
HEK + Jurkat
64%
48%
62%
66%
HEK.XF
HEK.XF + Jurkat
69%
3%
66%
8%
PI
FSC

Fig. S3


a
rat thymus
B104
B104.XF
kDa
37
N-20
rFasL
GAPDH


b
B104
B104.XF
% of Max.
AB1665
N-20
MFL-3
Flim58


c
Ratios Jurkat/ B104 Cells
1.5
1
0.5
0
B104
B104.XF


d
% Life Jurkat Cells
100
80
60
40
20
0
- sFasL
+ sFasL
B104
B104.XF
supernatant