Expanded View Figures

Figure EV1. (related to Fig 1) Co-Precipitation of GR and LRH-1 and validation of BiFC system.

A HEK 293T cells transiently transfected with expression vectors coding for myc/His tagged human GR (mh-hGR) and/or eGFP-tagged human LRH-1 (eGFP-hLRH 1) were treated for 90 min with dexamethasone (Dexa). Cytoplasmic (CP) and nucleoplasmic (NP) lysates were prepared and subjected to his-tag pulldown with Ni^{2+} beads. Input samples and eluates were analyzed by SDS-Page and subsequent Western blotting using anti-GFP and anti-Myc antibodies. Lanes confirming co-precipitation of eGFP-hLRH-1 with mh-hGR are highlighted in blue. Representative results from two are shown.

B, C Absence of eYFP fluorescence in HEK 293H cells transiently transfected with YFP1-LRH-1 or GR-YFP2 after 2 h Dexa treatment (B) and BiFC of YFP1-GR and GR-YFP2 in presence and absence (dimethyl sulfoxide (DMSO)) of Dexa (C). Cells were analyzed by fluorescence microscopy using a 10x objective (B, Scale Bar: 100 μM) and 40x objective (C; Scale bar: 20 μm), respectively. Boxed areas in (C) are 3x magnified and shown at the right. Representative images of $n > 10$ biological replicates are shown.

D Flow cytometry-based quantification of YFP+ HEK 293H cells as a percentage (%) of viable cells after transient transfection with YFP1-LRH-1, YFP1-GR or/and GR-YFP2 in presence and absence of 500 nM Dexa (2 h). Data are represented as mean ± SD of nine. Two-way ANOVA; ns, not significant.

E Corresponding to Fig 1D: Representative fluorescence microscopy images of HEK 293H cells transiently transfected with YFP1-LRH-1 and GR-YFP2 after 6 h pre-treatment with 20 μM 3d2 or 5 μM SR1848 and following 2 h stimulation with 500 nM Dexa. eYFP fluorescence indicates BiFC and Hoechst33342 was used for nuclear counterstain. Pictures were merged with the corresponding brightfield (BF) image. Cells were analyzed by fluorescence microscopy using a 40x objective (Scale bar: 20 μm). Boxed areas are 3x magnified and shown below.

Source data are available online for this figure.

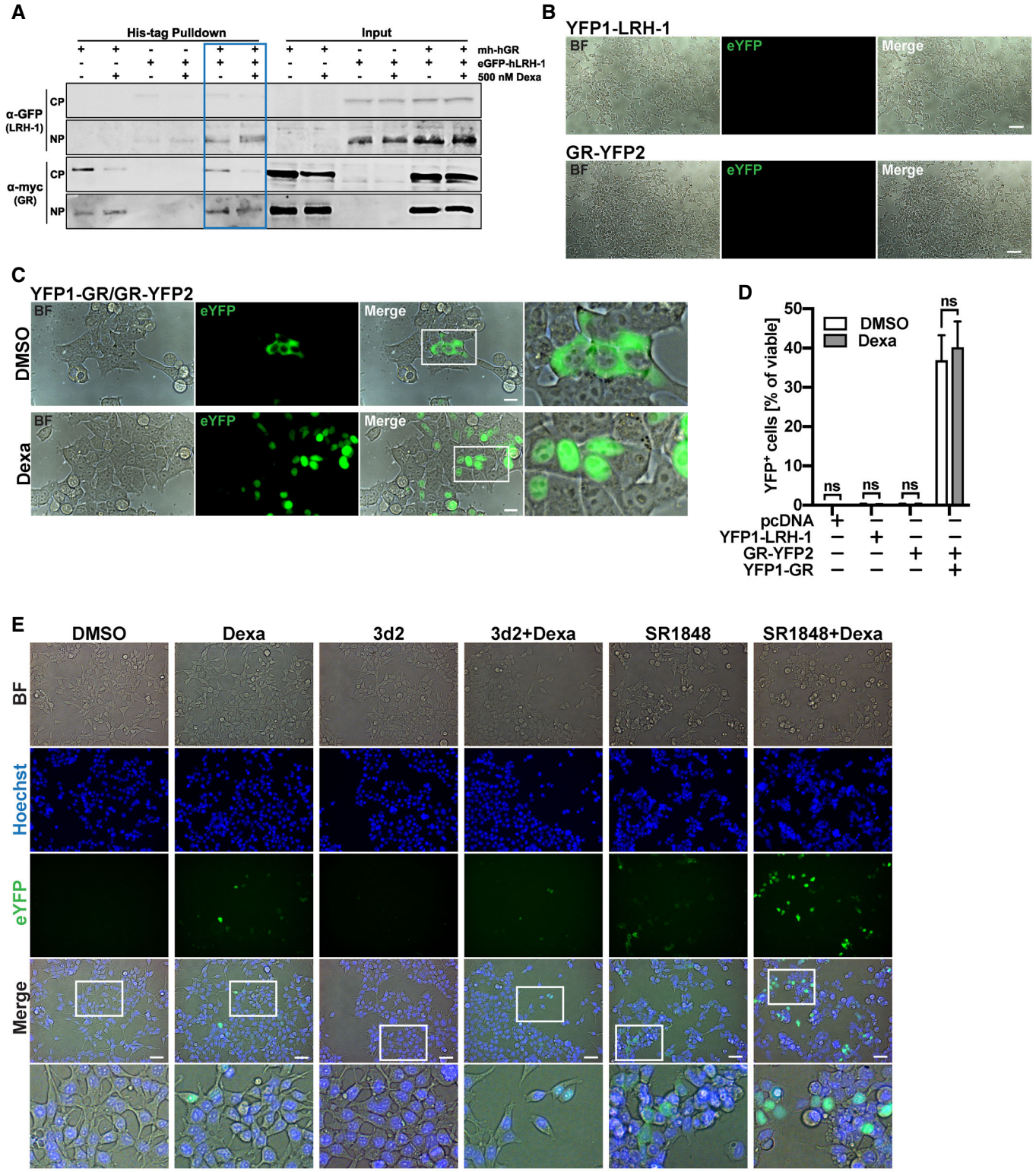

Figure EV1.

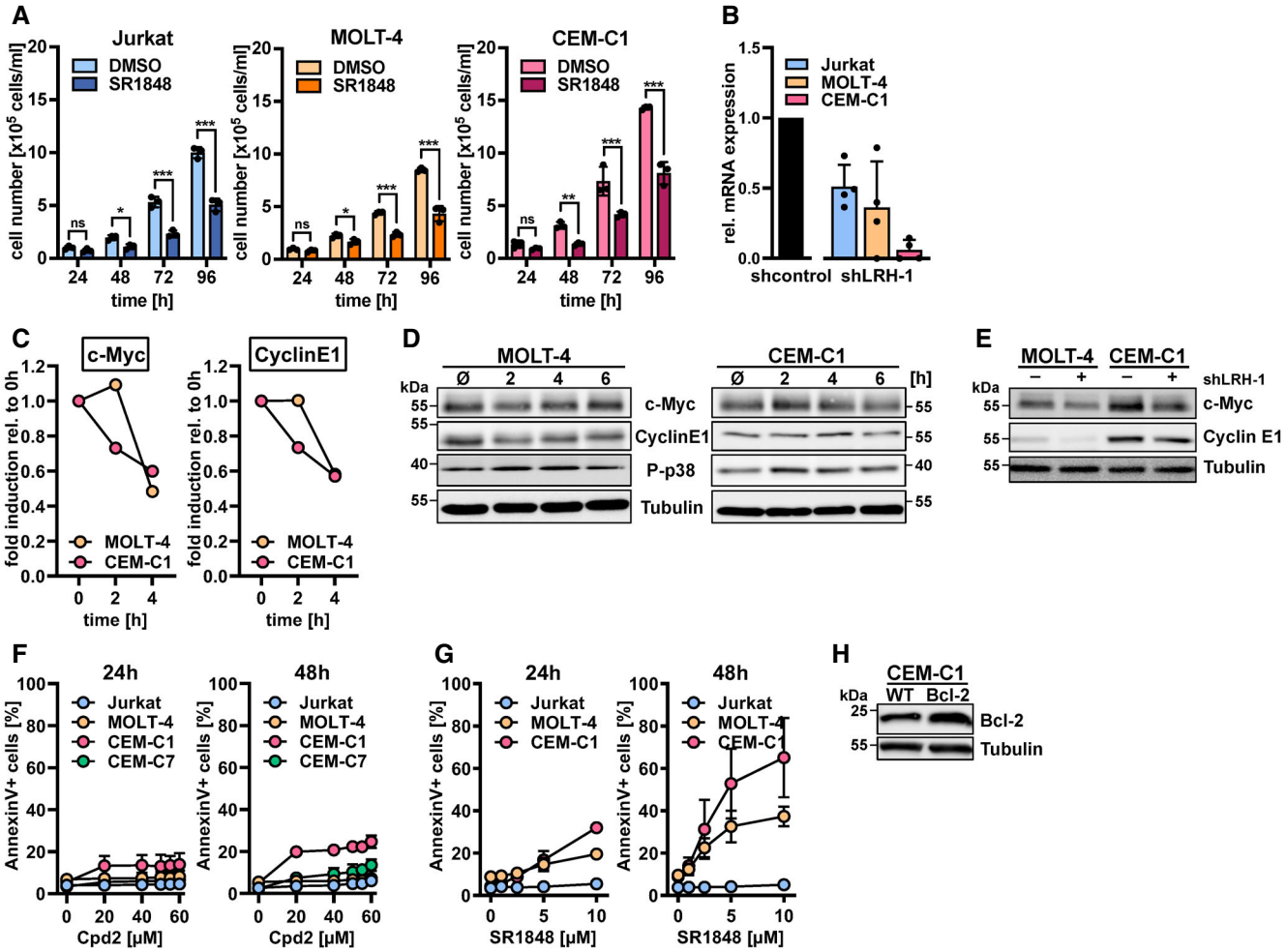

Figure EV2. (related to Fig 4) SR1848 impairs viability of leukemic T cells; verification and characterization of LRH-1 knockdown T-ALL cell lines.

A Daily cell counting-based comparison of cellular proliferation of T-ALL cells after application of 4 µM SR1848 in Jurkat and 1 µM SR1848 in CEM-C1 and MOLT-4 or corresponding amounts of dimethyl sulfoxide (DMSO, solvent control). Individual and mean values of technical triplicates of one representative experiment ($n = 3$) ± SD are shown. Two-way ANOVA; ns, not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

B Verification of LRH-1 knockdown in T-ALL cell lines stably expressing a small hairpin RNA (shRNA) construct targeting LRH-1 (shLRH-1) by quantification of LRH-1 mRNA levels with probe-based real-time quantitative PCR. Fold induction relative to the control transduced with a non-targeting shRNA construct (shcontrol) was calculated after normalization to beta-actin. Individual and mean values of $n = 4$ (generated cell lines) ± SD are shown.

C mRNA expression levels of human c-Myc and Cyclin E1 in Jurkat and MOLT-4 were determined by real-time quantitative PCR after treatment with 20 µM 3d2 for 2/4/6 h. Fold induction relative to the DMSO-treated control (0 h) was calculated after normalization to beta-actin (one biological replicate).

D Immunoblots of c-Myc, Cyclin E1, phospho-p38 (P-p38) and Tubulin from T-ALL cells treated as described in (C).

E Immunoblots of c-Myc, Cyclin E1, and Tubulin from untreated CEM-C1 and MOLT-4 cells expressing shLRH-1 (+) or shcontrol (–). Representative results from $n > 3$ are shown.

F, G Flow cytometric analysis of cell death by AnnexinV staining in T-ALL cell lines upon treatment with indicated Cpd2 (F) and SR1848 (G) concentrations for 24 h and 48 h. Mean values of $n = 3$ ± SD are shown.

H Immunoblots of Bcl-2 and Tubulin from untreated CEM-C1 wild-type (WT) or Bcl-2-overexpressing (Bcl-2) cells. Representative results from three are shown.

Source data are available online for this figure.

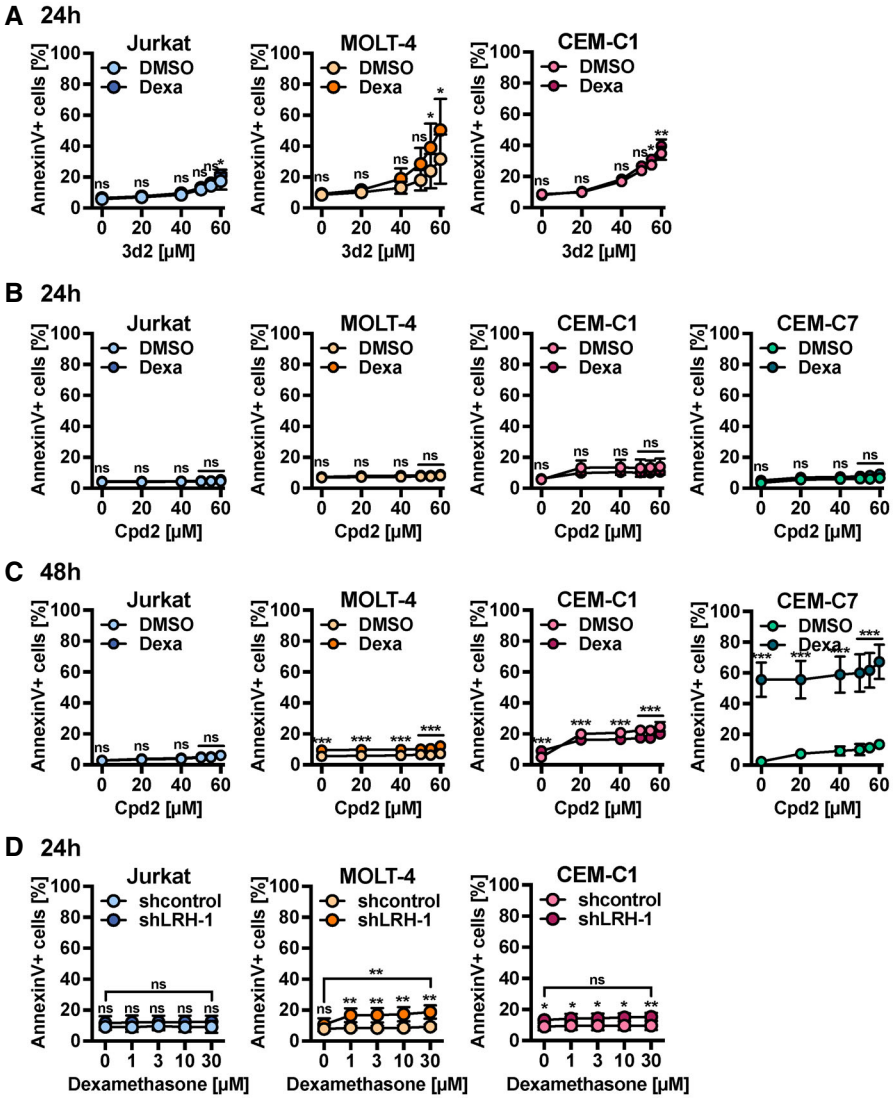

Figure EV3. (related to Fig 5) Specificity of 3d2-mediated sensitization towards GC-induced apoptosis.

A–D Flow cytometric analysis of cell death by AnnexinV staining in T-ALL cell lines after 24 h or 48 h treatment with dexamethasone and 3d2 (A), Cpd2 (B, C) or (D) small-hairpin RNA-mediated LRH-1 (shLRH-1) respectively control (shcontrol) knockdown. Mean values of (A) $n = 4$ and (B–D) $n = 3 \pm$ SD are shown.

Data information: For all displayed experiments dimethyl sulfoxide (DMSO; represented by 0) was used as a solvent control. Two-way ANOVA; ns, not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

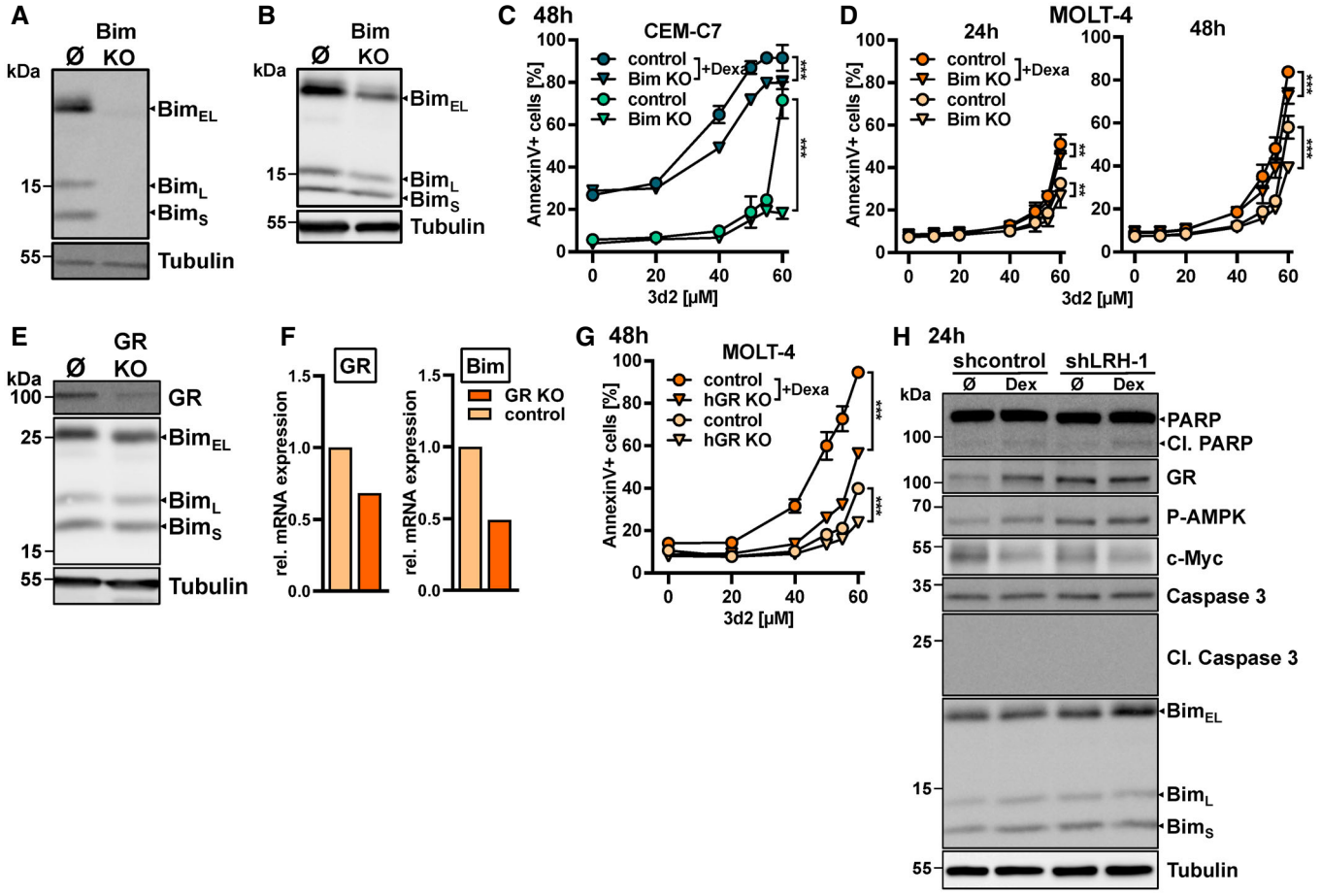

Figure EV4. (related to Fig 6) Verification and characterization of Bim/GR knockout and LRH-1 knockdown T-ALL cell lines.

A, B Immunoblots of Bim_{EL}, Bim_L and Bim_S and Tubulin from control (Ø) and Bim knockout (Bim KO) CEM-C7 (A) and MOLT-4 (B) cells (one biological replicate).

C, D Flow cytometric analysis of cell death by AnnexinV staining in control and Bim KO CEM-C7 (C) and MOLT-4 (D) treated with 3d2 and 30 µM Dexa, for 24 h and/or 48 h. Mean values of (C) technical triplicates of one representative out of four and (D) $n = 3 \pm$ SD are shown.

E Immunoblots of the glucocorticoid receptor (GR), Bim_{EL}, Bim_L and Bim_S and Tubulin from control (Ø) and GR knockout (GR KO) MOLT-4 cells (one biological replicate).

F Quantification of mRNA expression levels of human GR and Bim in control and GR KO MOLT-4 by real-time quantitative PCR. Fold induction relative to the control was calculated after normalization to beta-actin (one biological replicate).

G Flow cytometric analysis of cell death by AnnexinV staining in control and GR KO MOLT-4 treated with 3d2 and 30 µM Dexa, for 48 h. Mean values of $n = 3 \pm$ SD are shown.

H Immunoblots of full-length and cleaved (cl.) PARP, GR and phospho-AMPK (P-AMPK), c-Myc, full-length and cleaved (Cl.) Caspase 3, Bim_{EL}, Bim_L and Bim_S and Tubulin from CEM-C1 cells treated for 24 h with 10 µM Dexa. Representative results from two are shown.

Data information: For all displayed experiments dimethyl sulfoxide (represented by 0 or Ø) was used as a solvent control. Two-way ANOVA, $^{**}P < 0.01$, $^{***}P < 0.001$.
Source data are available online for this figure.