Figure S1. Hsf1 loss significantly reduced human T-ALL cell survival.

(A-B) Representative Western blots showing HSF1 protein expression in human T-ALL cell lines (CCL119 and CUTLL1) following silencing of Hsf1 by doxycycline-inducible shRNA expression for the indicated times. Cells expressing scramble shRNA were used as control (n=3 experiments). α-tubulin was used as loading control.

(C-D) Cell-growth kinetics of CCL119 and CUTLL1 cell lines following silencing of Hsf1 as described in panels A and B. Cell growth assays were performed by plating $2x10^5$ cells in triplicate in 6-well plates in media containing doxycycline for the indicated times (n=4 independent analyses).

(E-F) Apoptosis or necrosis monitored by flow cytometric analysis (FACS) of T-ALL cells (CCL119 and CUTLL1) treated as described in panels A and B, and stained for annexin V and 7-AAD. The percent of apoptotic (annexinV^{pos}-7$AAD^{neg/pos}$) and necrotic (annexinV^{neg} -7-AAD^{pos}) cells is indicated in the quadrants. Quantification of apoptotic and necrotic cells is presented in the right panels (n=4 independent analyses).

For all panels, scale bars represent mean ± SD. Statistical significance is indicated (* p<0.05, ** p< 0.01, *** p< 0.001).

Figure S2. Pharmacological inhibitors of the HSF1 pathway exhibit potent anti-tumor activity in human T-ALL cell lines.

Human T-ALL (CCL119, CUTLL1, MOLT4, and CCRF-HSB2) cells were treated with increasing concentrations of the indicated HSF1 inhibitors (KRIB11 or Recoglamide), and cell viability/growth was recorded at 1 to 4 days. Drugs were replenished every 48 hours. Bars are means ± SD (n=4 assays per group).

Figure S3. HSF1 knockdown in human T-ALL cell lines reduces the mitochondrial membrane potential and increases cellular ROS levels. T-ALL cell lines (CCL119 and CUTLL1) following silencing of Hsf1 by doxycycline-inducible shRNA expression for 3 days were analyzed. T-ALL cells (CCL119 and CUTLL1) expressing scramble shRNA were used as control. Cells were assayed for **(A)** Mitochondrial membrane potential and **(B)** cellular ROS activity by FACS analysis. For all panels, FACS profile or bars represent cells expressing Hsf1 shRNA (open) or scramble shRNA (filled). Scale bars represent mean ± SD. (n=4 samples). Statistical significance is indicated (* $p<0.05$, ** $p< 0.01$).

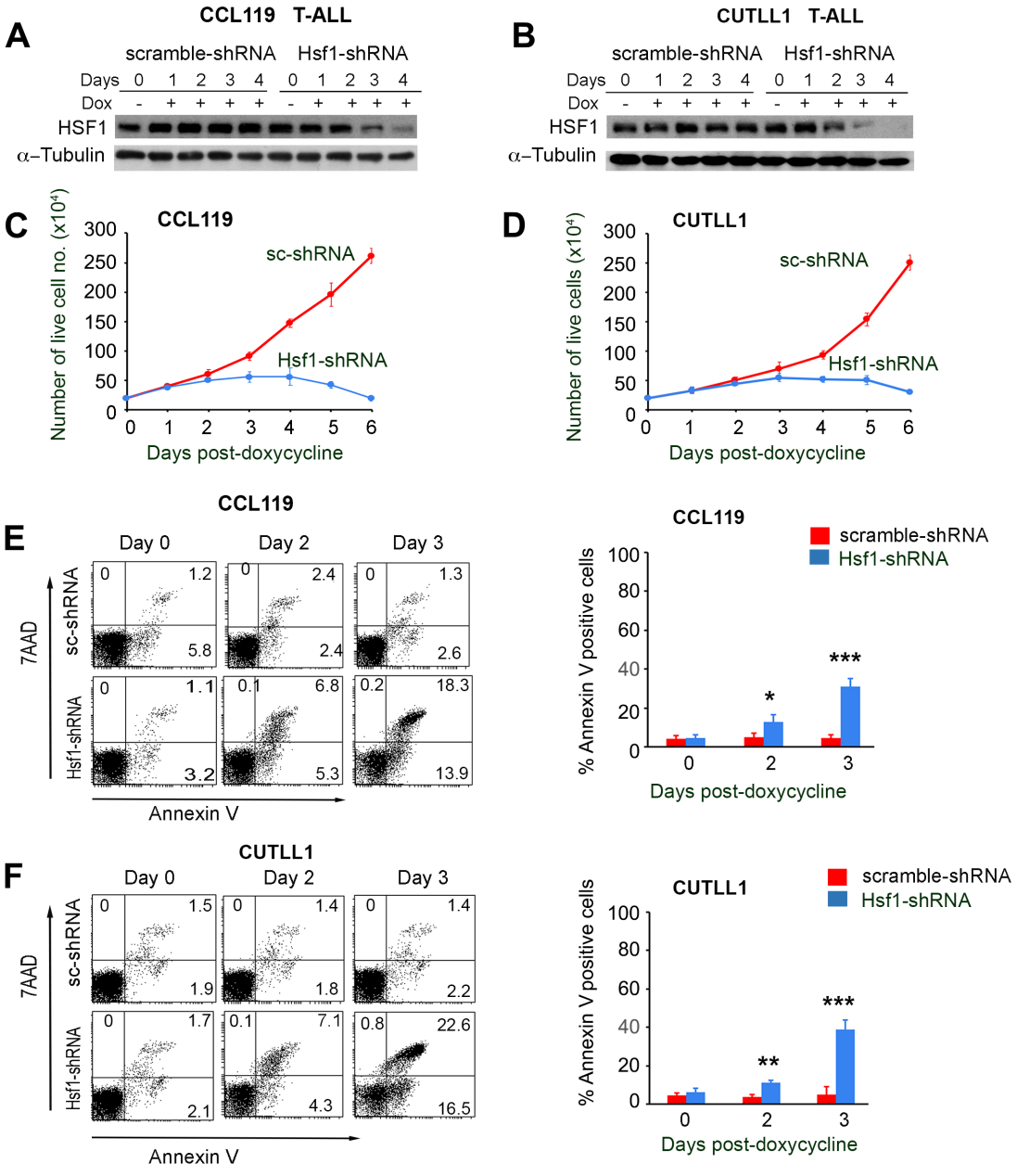
A
CCL119 T-ALL
scramble-shRNA
Hsf1-shRNA
Days 0 1 2 3 4 0 1 2 3 4
Dox - + + + + - + + + +
HSF1
α–Tubulin
B
CUTLL1 T-ALL
scramble-shRNA
Hsf1-shRNA
Days 0 1 2 3 4 0 1 2 3 4
Dox - + + + + - + + + +
HSF1
α–Tubulin
C
CCL119
Number of live cell no. (x10⁴)
sc-shRNA
Hsf1-shRNA
Days post-doxycycline
D
CUTLL1
Number of live cells (x10⁴)
sc-shRNA
Hsf1-shRNA
Days post-doxycycline
E
CCL119
Day 0
Day 2
Day 3
7AAD
sc-shRNA
Hsf1-shRNA
Annexin V
CCL119
scramble-shRNA
Hsf1-shRNA
% Annexin V positive cells
Days post-doxycycline
F
CUTLL1
Day 0
Day 2
Day 3
7AAD
sc-shRNA
Hsf1-shRNA
Annexin V
CUTLL1
scramble-shRNA
Hsf1-shRNA
% Annexin V positive cells
Days post-doxycycline

Fig. S1 A-F

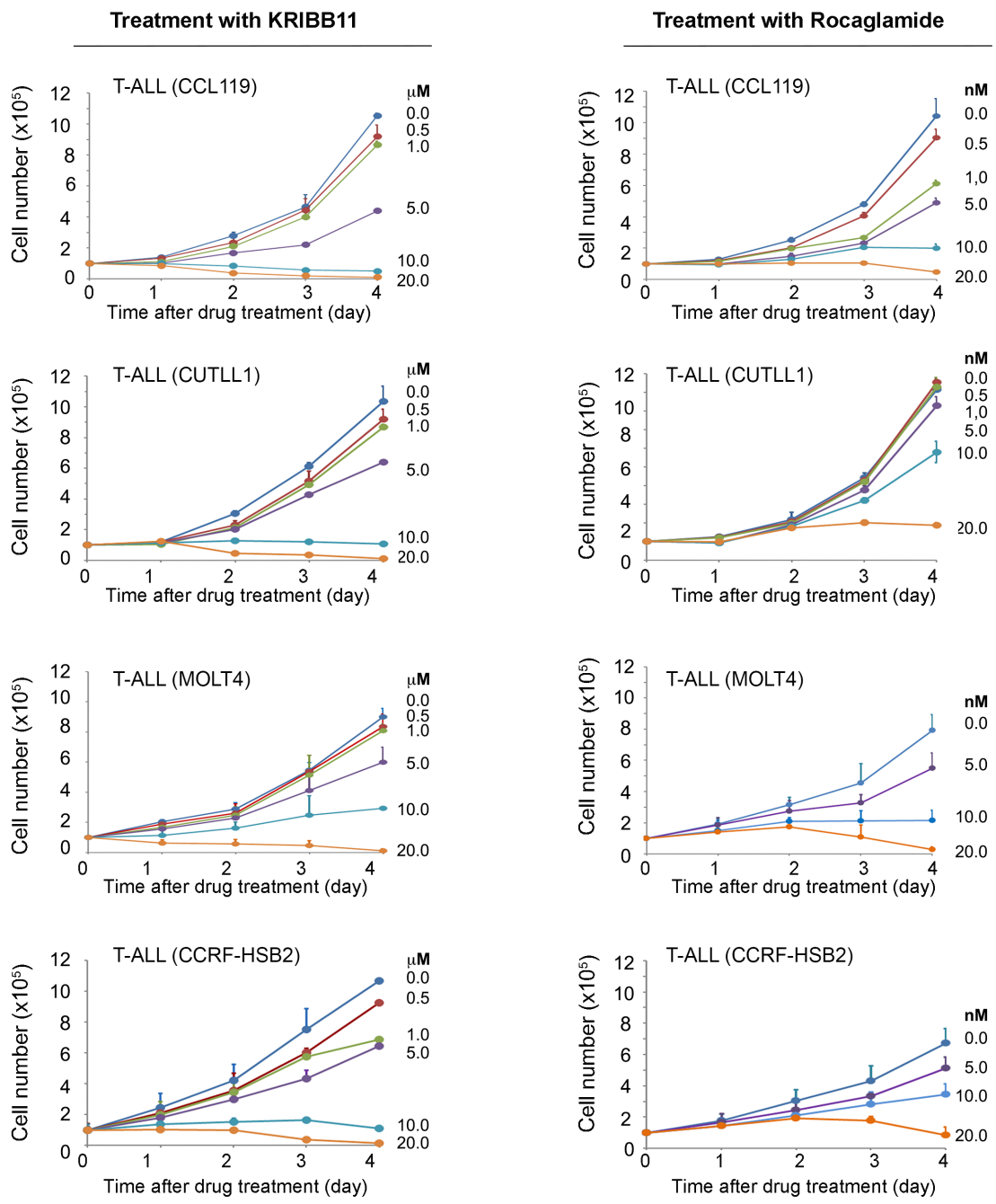
Treatment with KRIBB11
Treatment with Rocaglamide
T-ALL (CCL119)
T-ALL (CUTLL1)
T-ALL (MOLT4)
T-ALL (CCRF-HSB2)
Cell number (x10⁵)
Time after drug treatment (day)
μM
nM
0.0
0.5
1.0
1,0
5.0
10.0
20.0

Fig. S2

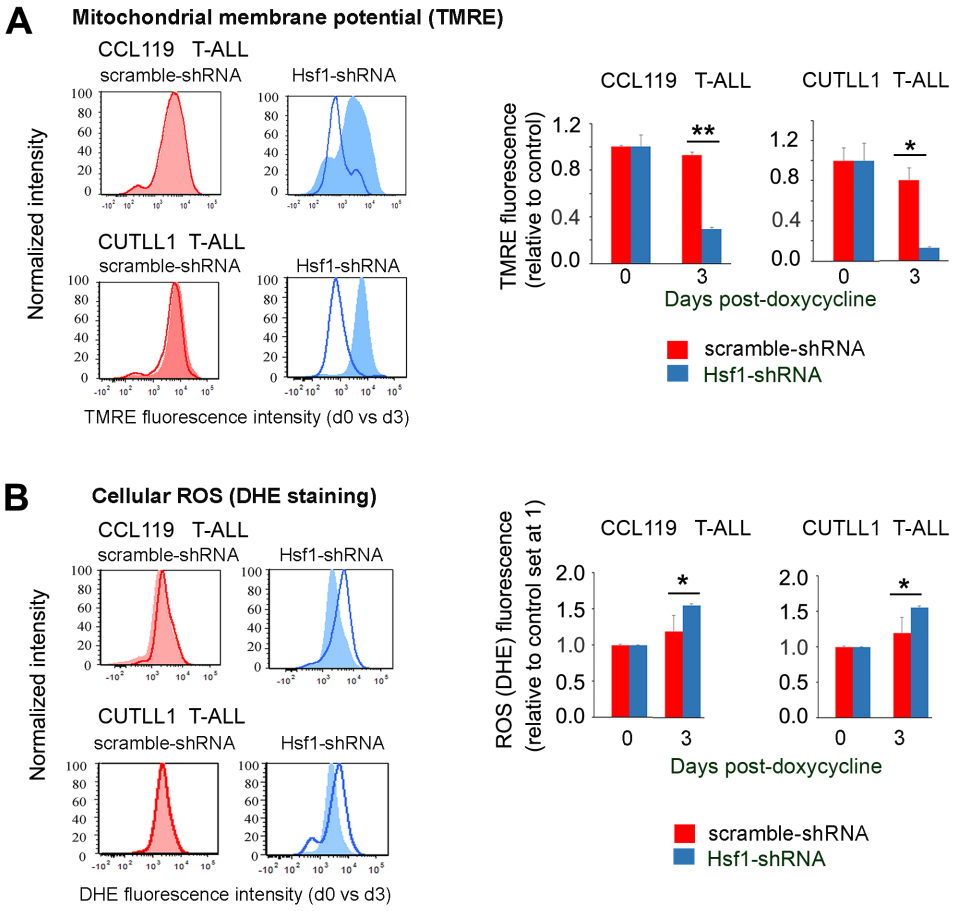
A
Mitochondrial membrane potential (TMRE)
CCL119 T-ALL
scramble-shRNA
Hsf1-shRNA
CUTLL1 T-ALL
scramble-shRNA
Hsf1-shRNA
Normalized intensity
TMRE fluorescence intensity (d0 vs d3)
CCL119 T-ALL
CUTLL1 T-ALL
TMRE fluorescence (relative to control)
**
*
Days post-doxycycline
scramble-shRNA
Hsf1-shRNA
B
Cellular ROS (DHE staining)
CCL119 T-ALL
scramble-shRNA
Hsf1-shRNA
CUTLL1 T-ALL
scramble-shRNA
Hsf1-shRNA
Normalized intensity
DHE fluorescence intensity (d0 vs d3)
CCL119 T-ALL
CUTLL1 T-ALL
ROS (DHE) fluorescence (relative to control set at 1)
*
*
Days post-doxycycline
scramble-shRNA
Hsf1-shRNA

Fig. S3 A-B