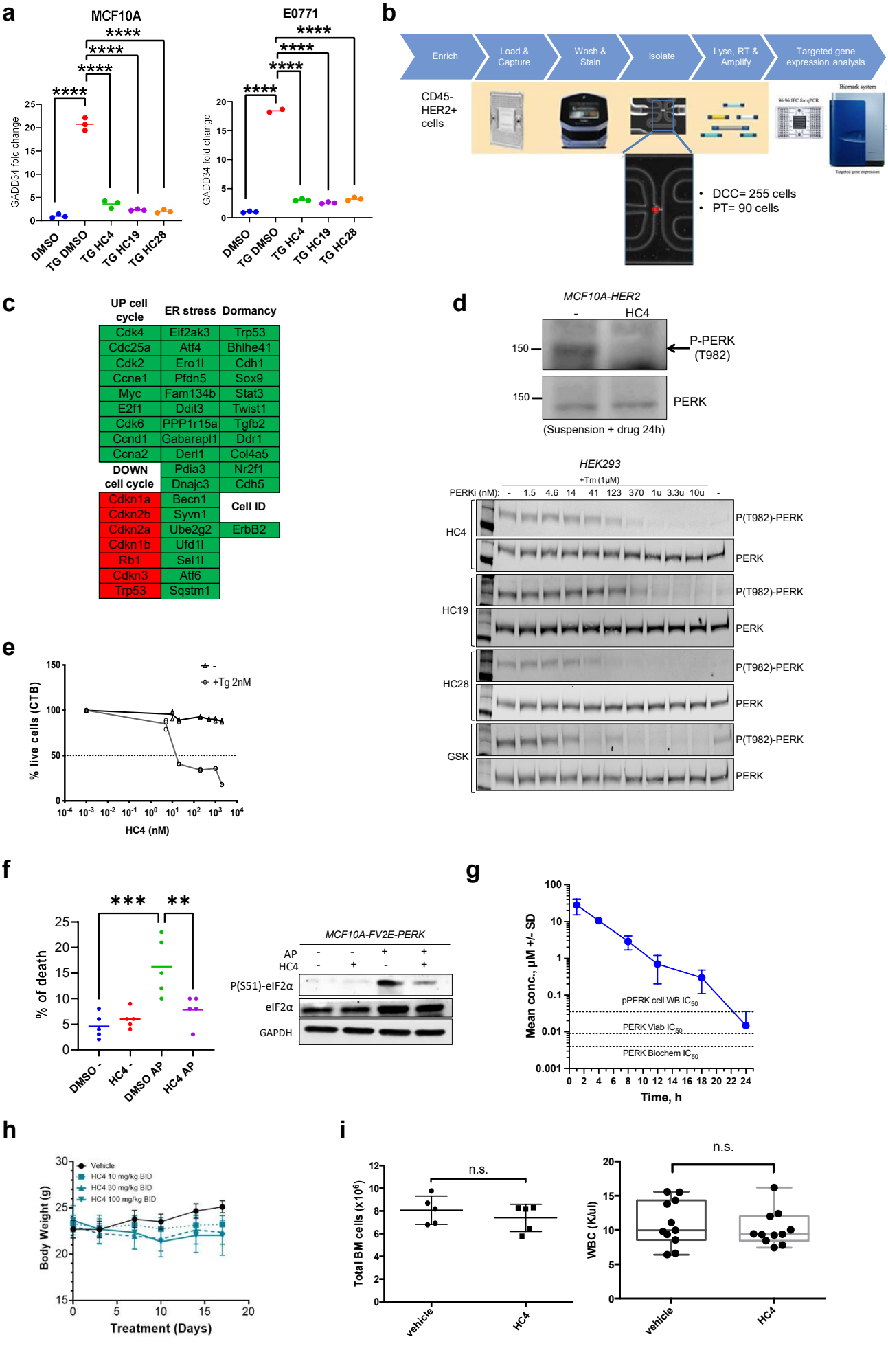
a
MCF10A
E0771
GADD34 fold change
DMSO
TG DMSO
TG HC4
TG HC19
TG HC28
b
Enrich
Load & Capture
Wash & Stain
Isolate
Lyse, RT & Amplify
Targeted gene expression analysis
CD45- HER2+ cells
• DCC= 255 cells
• PT= 90 cells
c
UP cell cycle
ER stress
Dormancy
Cdk4
Eif2ak3
Trp53
Cdc25a
Atf4
Bhlhe41
Cdk2
Ero1l
Cdh1
Ccne1
Pfdn5
Sox9
Myc
Fam134b
Stat3
E2f1
Ddit3
Twist1
Cdk6
PPP1r15a
Tgfb2
Ccnd1
Gabarapl1
Ddr1
Ccna2
Derl1
Col4a5
DOWN cell cycle
Pdia3
Nr2f1
Dnajc3
Cdh5
Cdkn1a
Becn1
Cell ID
Cdkn2b
Syvn1
Cdkn2a
Ube2g2
ErbB2
Cdkn1b
Ufd1l
Rb1
Sel1l
Cdkn3
Atf6
Trp53
Sqstm1
d
MCF10A-HER2
HC4
P-PERK (T982)
PERK
(Suspension + drug 24h)
HEK293
+Tm (1μM)
PERKi (nM):
HC4
HC19
HC28
GSK
P(T982)-PERK
PERK
e
% live cells (CTB)
+Tg 2nM
HC4 (nM)
f
% of death
DMSO -
HC4 -
DMSO AP
HC4 AP
MCF10A-FV2E-PERK
AP
HC4
P(S51)-eIF2α
eIF2α
GAPDH
g
Mean conc., μM +/- SD
pPERK cell WB IC50
PERK Viab IC50
PERK Biochem IC50
Time, h
h
Body Weight (g)
Vehicle
HC4 10 mg/kg BID
HC4 30 mg/kg BID
HC4 100 mg/kg BID
Treatment (Days)
i
Total BM cells (x10^6)
n.s.
WBC (K/ul)
vehicle
HC4

Supplementary Figure S1. (a) MCF10A cells were treated with DMSO, thapsigargin (0.2 μM) alone or in combination with PERK inhibitors (2 μM) for 24 h and assayed for GADD34 expression by RT-qPCR. N = 3 wells per group (left graph). E0771 cells were treated with DMSO, thapsigargin (0.1 μM) alone or in combination with PERK inhibitors (2 μM) for 16 h and assayed for GADD34 expression by RT-qPCR. N = 3 wells per group (right graph) ****, p <0.0001 by t-test. (b) Flow diagram of the steps followed for single cell gene expression analysis with C1 and Biomark HD Fluidigm. A total of 255 DCCs and 90 primary tumor (PT) cells were analyzed. (c) List of the genes analyzed by high-throughput qPCR. (d) (upper) Immunoblot showing the inhibition of PERK phosphorylation (T982) by the PERK inhibitor HC4 (2 μM) in MCF10A-HER2 cells plated on low attachment plates and incubated with inhibitor for 24 h. (lower) Immunoblot showing inhibition of PERK phosphorylation by treatment with increasing doses of HC4, HC19, HC28 and GSK2656157 for 4 h upon incubation with ER stress inducer tunicamycin in HEK293 cells. (e) The PERK inhibitor HC4 sensitizes to low dose ER stress-induced cell death in vitro. HC4 dose-response viability curve (Cell Titer Blue, CTB) in MCF10A-HER2 cells, in the absence (-) or in the presence of stress (low dose thapsigargin, Tg 2 nM) after 48 h. Dashed line indicates IC50 (≈ 9 nM). (f) MCF10A-Fv2E-PERK cells in 2D culture were treated daily with 2 nM AP20187 (AP), HC4 2 μM and combination and assayed for cell death (left) or protein expression (right) after 24 h. (g) In vivo pharmacokinetic profile of HC4 in mouse plasma. Dashed lines indicate the different biochemical, cellular and in vitro viability IC50s. (h) Effect of HC4 on body weight in female BALB/c mice (7–8-week-old) females treated twice per day (BID) with HC4 at indicated concentrations. (i) Effect of HC4 on total bone marrow cells (in two lower limbs) in MMTV-HER2 females treated for 2 weeks (N=5 per group) (left graph); effect of HC4 on total white blood cells in MMTV-HER2 females treated for 2 weeks (N=11 per group) (middle graph).