

Figure S1

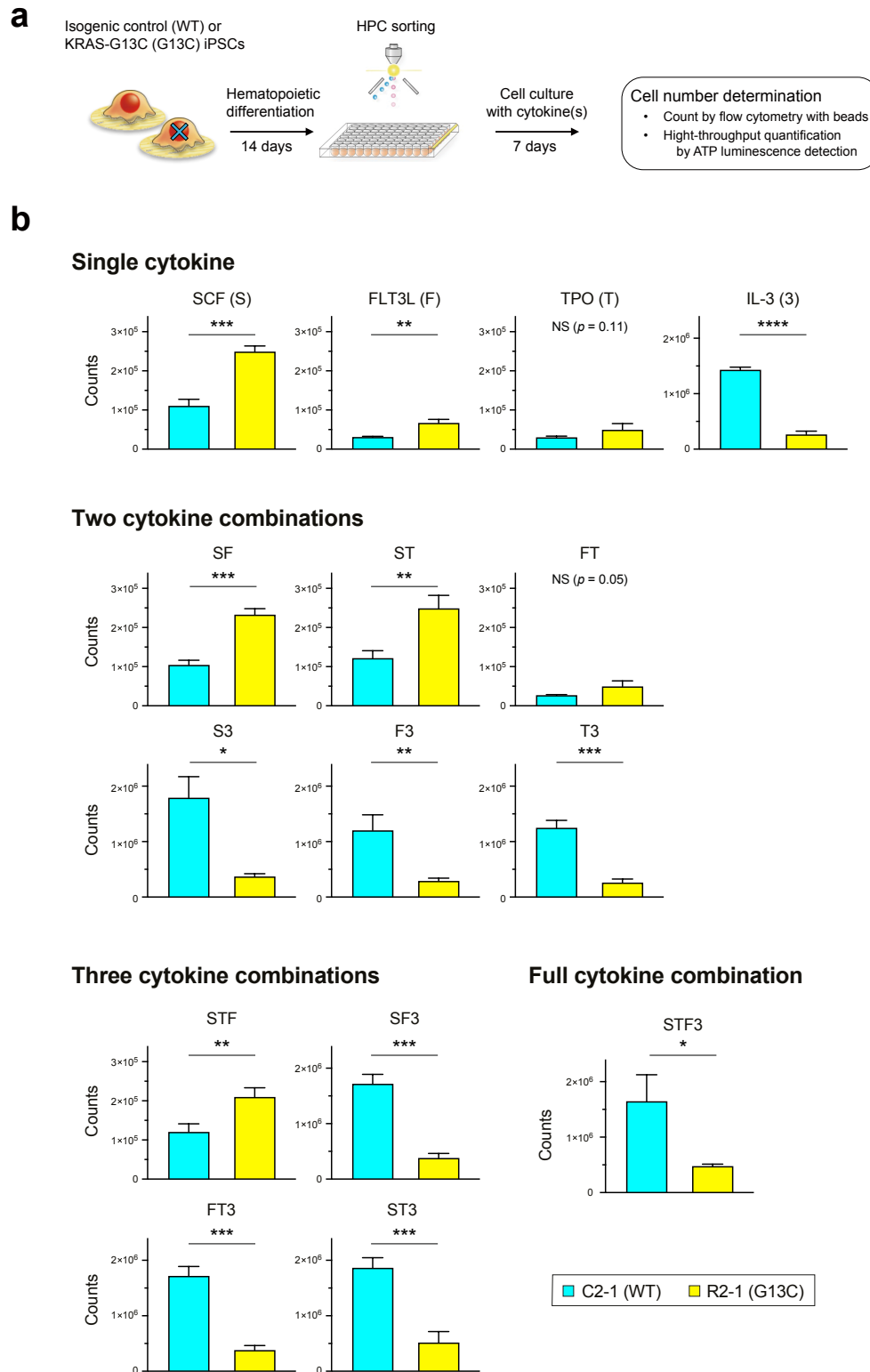


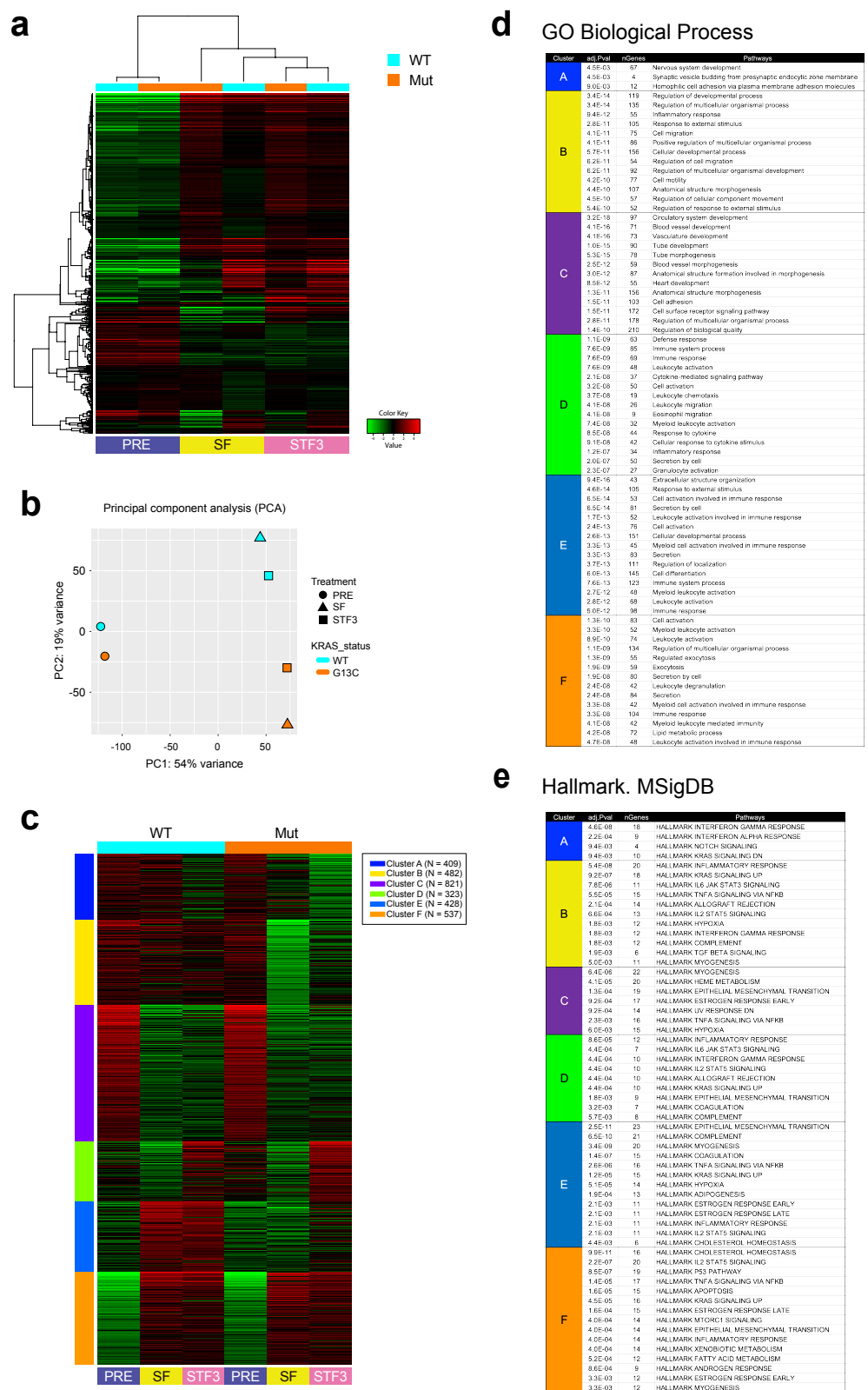
Fig. S1. Comprehensive screening of cytokine combinations to elucidate the effect of *KRAS* (G13C) mutation on iPSC-HPCs

(a) A pair of HPC samples obtained from an isogenic pair of patient 2-derived iPSCs (C2-1 and R2-1) were cultured for one week using every combination of the following cytokines as stated.

SCF (S), TPO (T), FLT3-ligand (F), and IL-3 (3).

(b) Shown are the representative cell count results determined by flow cytometry analysis using the fluorescence-beads. Mean $\pm$ SD values are shown (technical replicates: $n = 3$). The statistical analysis is based on an unpaired t test.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. NS, not significant.

Figure S2**Fig. S2. Characterization of altered transcriptome profiles brought by a single *KRAS* (G13C) mutation in iPSC-derived HPCs Patient 1 samples.**

Six samples from patient 1 were subjected to the iDEP program-based analysis.

(a) A heatmap demonstration with hierarchal clustering with the top 1,000 most variable genes. The remarkable similarity between genotypes (WT vs. Mut) is obvious for pre-expansion HPCs (PRE). Substantial changes upon stimulation in profiles are evident, with their difference between genotypes becoming greater.

(b) Principal component analysis (PCA) depicting a close relationship of PRE samples and distinct characteristics that become evident for control (WT) and mutant (G13C) samples after expansion cultures.

(c-e) A heatmap generated by K-means clustering with the use of 3,000 most variable genes (c). Enriched pathways were identified with reference to the pathway databases for the gene sets classified in each cluster by the functions provided with the iDEP. Shown are the result tables analyzed with the GO Biological Process (d) and the Hallmark. MSigDB pathway database (e). Note that pathways related to myeloid cell biology and immune/inflammatory responses are found enriched. The enrichment in pathways related to the *KRAS* signaling is also noted of hematopoietic progenitor cells differentiated from RALD patient-derived iPSCs.

Figure S3

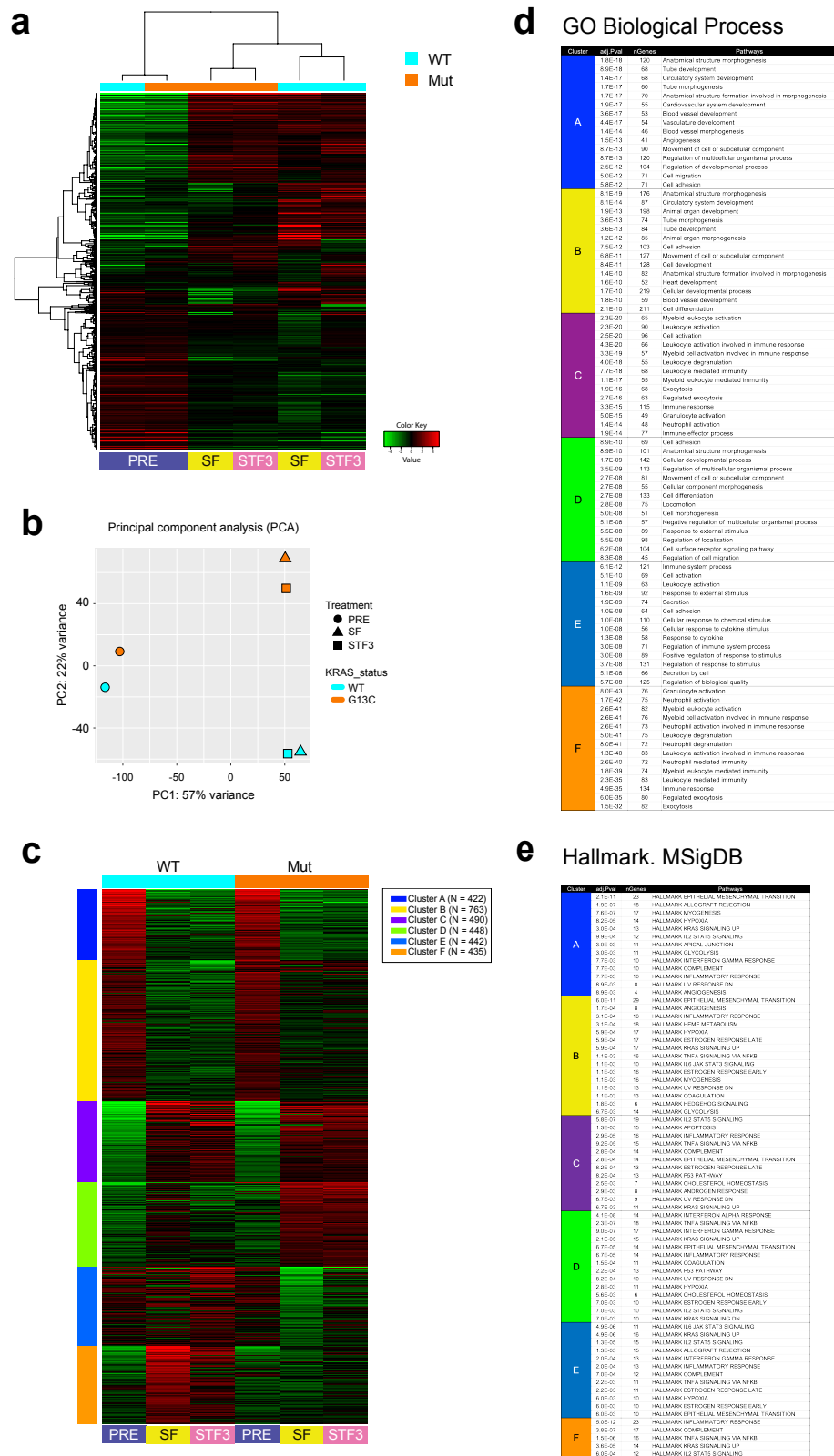


Fig. S3. Characterization of altered transcriptome profiles brought by a single *KRAS* (G13C) mutation in iPSC-derived HPCs (patient 2 samples), related to Figures 2 and 3.

Six samples from patient 2 were subjected to the iDEP program-based analysis.

(a) A heatmap demonstration with hierarchal clustering with the top 1,000 most variable genes. The remarkable similarity between genotypes (WT vs. Mut) is obvious for pre-expansion HPCs (PRE). Substantial changes upon stimulation in profiles are evident, with their difference between genotypes becoming greater.

(b) Principal component analysis (PCA) depicting a close relationship of PRE samples and distinct characteristics that become evident for control (WT) and mutant (G13C) samples after expansion cultures.

(c-e) A heatmap generated by K-means clustering with the use of 3,000 most variable genes (c). Enriched pathways were identified with reference to the pathway databases for the gene sets classified in each cluster by the functions provided with the iDEP. Shown are the result tables analyzed with the GO Biological Process (d) and the Hallmark. MSigDB pathway database (e). Note that pathways related to myeloid cell biology and immune/inflammatory responses are found enriched. The enrichment in pathways related to the *KRAS* signaling is also noted of hematopoietic progenitor cells differentiated from RALD patient-derived iPSCs.

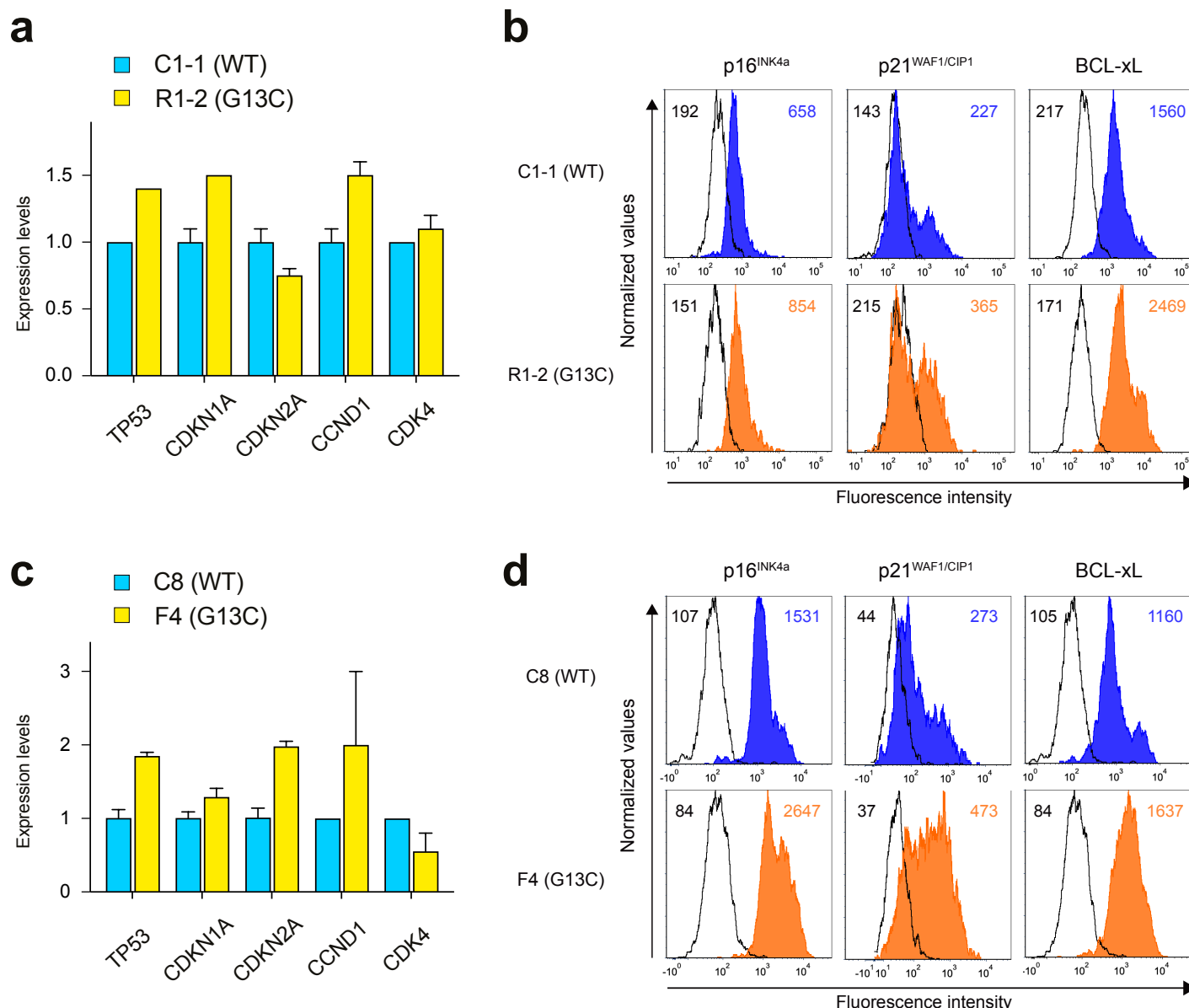
Figure S4

Fig. S4. Altered gene and protein expression of cell-cycle and apoptosis-related molecules in *KRAS*-mutant HPCs, related to Figure 4.

The analysis similar to that shown in Figure 4 was conducted with another isogenic pair of control (WT) and mutant (G13C) iPSCs, both derived from patient 1: C1-1 vs. R1-2 (primarily established clones) and C8 vs. F4 (gene-corrected vs. pre-correction mutant clones, originated from R1-2).

(a and c) Quantitative RT-PCR analysis of selected genes related to the regulation of cell cycle and apoptosis in expanded HPCs under standard conditions. The representative results obtained with each isogenic pair in two independent experiments are presented as mean $\pm$ SEM from duplicate samples.

(b and d) Intracellular flow cytometry analysis for the expression levels of indicated molecules in cultured HPCs. Shown are the representative overlay plots. Mean fluorescent values are shown for each histogram.

Figure S5

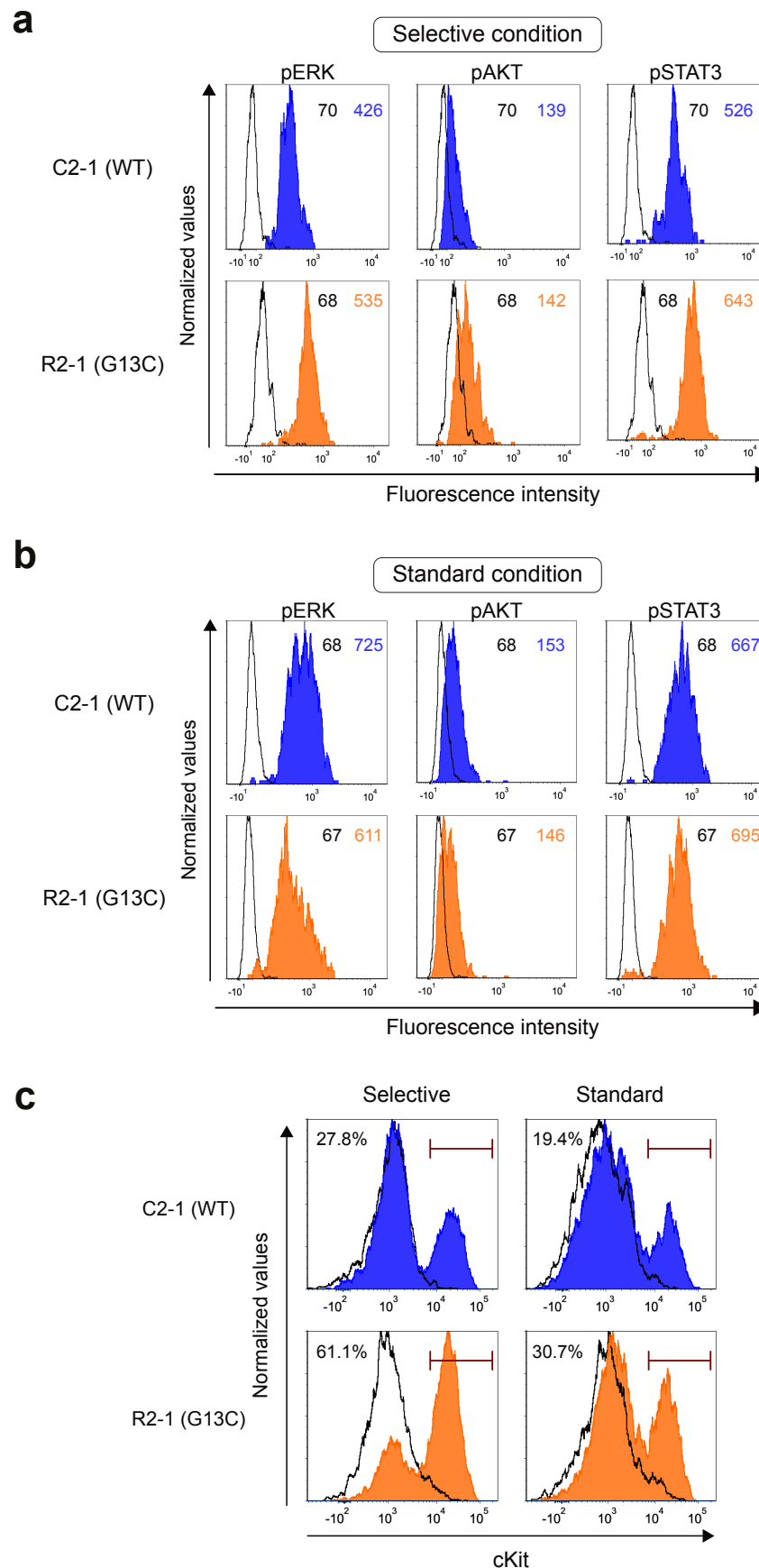


Fig. S5. Aberrancies in cell signaling pathways and cKit expression conferred on cultured iPSC-derived HPCs carrying the *KRAS* (G13C) mutation, related to Figure 5.

(a and b) Signaling events in expanded HPCs were determined by intracellular expression of pERK, pAKT, and pSTAT3, three well-known pathway molecules acting downstream of RAS, by flow cytometry analysis. Used are the isogenic pair derived from patient 2, C2-1 (WT) and R2-1 (G13C). HPCs were cultured before analysis either with Selective- (a) or Standard- (b) condition. Open histograms represent isotype antibody-control, while closed histograms show the specific staining for indicated molecules. Numbers indicate the mean fluorescence intensity.

(c) Cell surface expression levels of cKit determined by flow cytometry analysis. The isogenic pair of C2-1 and R2-1 was tested after expansion cultures. Open histograms, isotype-control; closed histograms, cKit-staining. The percent positive values are shown.

Figure S6

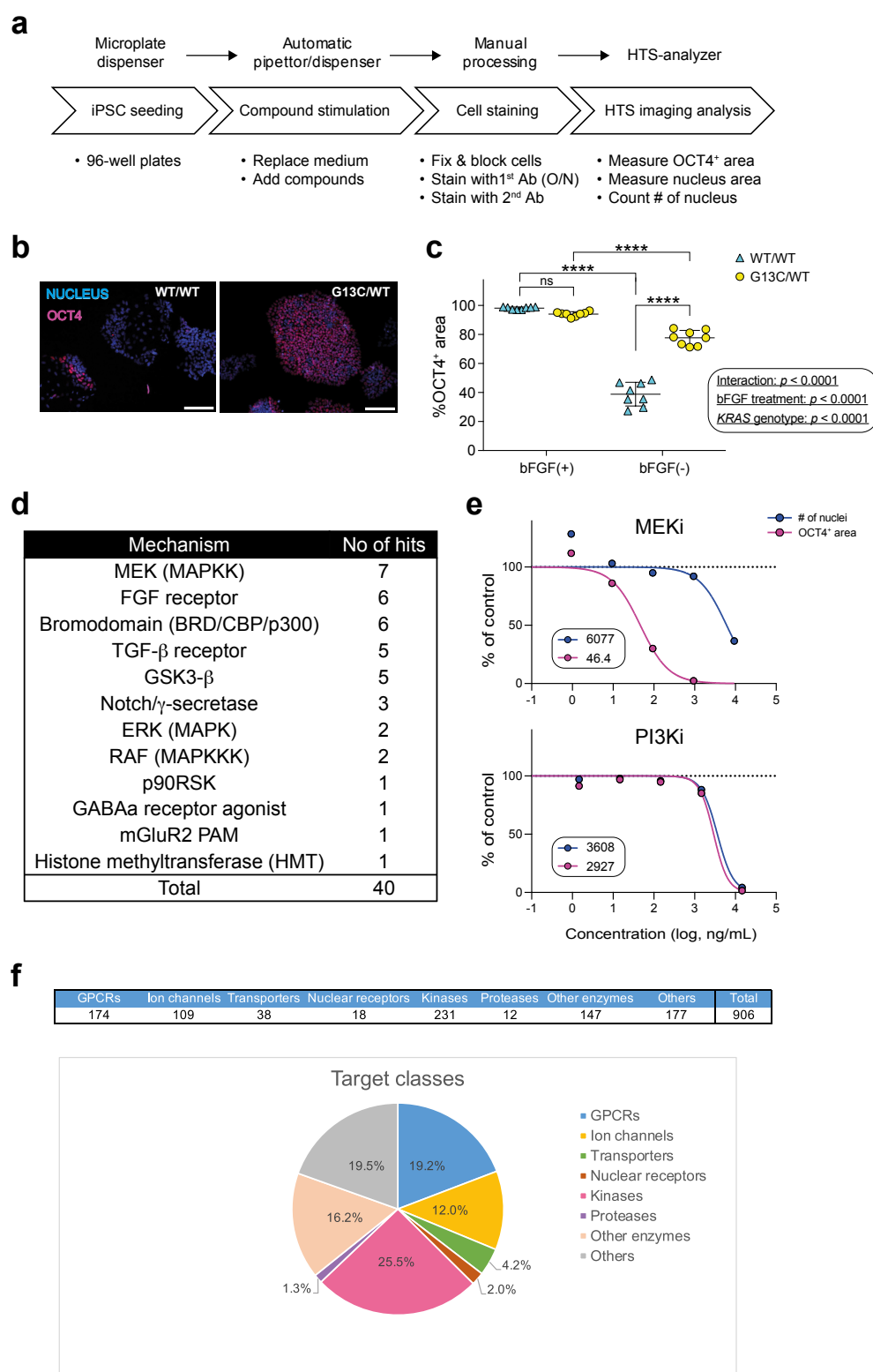


Fig. S6. Inhibitor library screening to identify effective modulators of aberrant KRAS signaling in iPSCs

(a) Schematic representation of an iPSC-based screening flow. iPSCs carrying the *KRAS*(G13C) mutation (R1-2) were seeded and maintained in 96-well plates using semi-automated processes. Test compounds were added on day 1 using an automatic pipettor/dispenser. Cells were cultured in the absence of bFGF, fixed and permeabilized on day 5, followed by overnight staining with the anti-OCT4 antibody. High-throughput screening (HTS) imaging analysis was performed on day 6 after being stained with the fluorescence-conjugated secondary antibody and Hoechst 33342.

(b) Immunohistochemistry of iPSCs cultured for 5 days in the absence of bFGF. The images show that while most control cells (C1-1, WT/WT) lose OCT4 expression (left), *KRAS*-mutant iPSCs (R1-2, G13C/WT) are still expressing the pluripotent marker (right). Scale bars: 100 μ m.

(c) Quantitative characterization for the ability specific to iPSCs carrying the *KRAS*(G13C) mutation, defined as “enforced retention of self-renewal.” The percentages of nucleated cells that expressed OCT4 were quantified using the isogenic pair of iPSCs (C1-1 and R1-2). Mean $\pm$ SD values are shown (experimental replicates: $n = 8$). The P values are shown based on the statistical analysis carried out with 2-way ANOVA, followed by Tukey’s multiple comparisons test. **** $p < 0.0001$; ns, not significant. Note that a majority of mutant iPSCs (77.7%) remain viable while expressing OCT4 in the absence of bFGF, reflecting the *KRAS* (G13C)-mediated abnormality that potentially serves as “druggable target.”

(d and e) A summary of screening results testing a 906 bioactive compound library. We sought to look for the ability to reverse the abnormality mentioned above (i.e., the effect to induce loss of OCT4 expression) without exhibiting significant cell toxicity. (d) Results shown in a table as 40 “hits” with their mode of action (MOA, Mechanism) and the number of compounds contained in each category. (e) Shown are representative examples of a “hit” (top, MEK inhibition) and a “non-hit” (bottom, PI3K inhibition). The IC_{50} values calculated from plotted reverse sigmoid curves are shown.

(f) MoA target classification of the compound library used in the screening experiments.

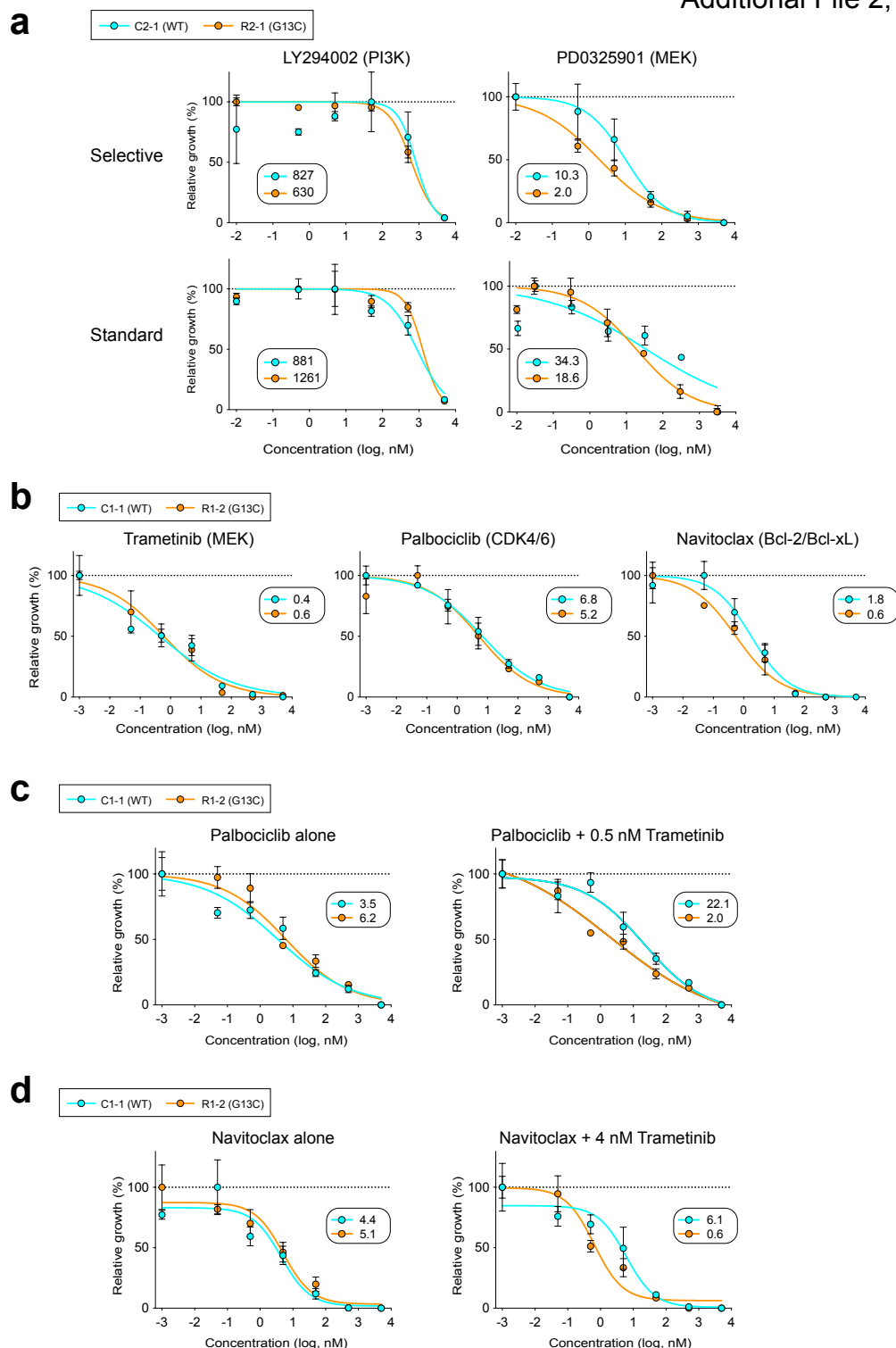


Fig. S7. Utilization of the established drug screening platform to explore inhibitory effects significantly selective on *KRAS*-mutant HPCs, related to Figures 6 and 7.

(a) Validation of the screening system with the well-characterized modulators of signaling pathways downstream of *KRAS*. Dose-response plots assessing growth inhibitory effects of a single reagent on either control (C2-1, blue) or *KRAS*-mutant (R2-1, orange) HPCs cultured in 2 distinct cytokine conditions (Selective or Standard). The percent growth was estimated relative to the control values (DMSO) with the ATP-measurement-based viability assay (technical replicates: $n = 3$). Calculated IC₅₀ values are indicated. Note the absence of selective inhibitory effects for the PI3K inhibitor LY294002 in both conditions. In contrast, the MEK inhibitor PD0325901 exhibits modest selectivity towards the mutant cells. Because the Selective condition (SCF and FLT3L only) often led to highly variable, non-informative outcomes due to overall poor cell viability, we decided to use only Standard culture for the subsequent experiments to ensure reliable consequences.

(b) Dose-response curves obtained with a patient 1-derived isogenic pair of iPSC-derived HPCs (C1-1 and R1-2) treated by a single reagent (technical replicates: $n = 3$). IC₅₀ values are shown. Note that the modest selective activity is observed for Navitoclax, whereas no evident selectivity is seen for Trametinib and Palbociclib.

(c and d) Potential enhancement of selective inhibitory effects on *KRAS*-mutant HPCs by combination treatment. Growth inhibitory effects were compared between single- and combined-treatments for the pair of C1-1 and R1-2 (technical replicates: $n = 3$). (c) Palbociclib dose-responses either alone (left) or in the presence of a low dose Trametinib (right). The IC₅₀ value for R1-2 samples gets lower (2.0 nM) in the presence of 0.5 nM Trametinib compared with that obtained with Palbociclib alone (6.2 nM), whereas it is not the case for C1-1 samples (3.5 nM with Palbociclib alone and 22.1 nM in combination).

(d) Navitoclax dose-responses either alone (left) or in the presence of a low dose Trametinib (right). The IC₅₀ value for R1-2 samples becomes lower (0.6 nM) in the presence of 4 nM Trametinib compared with that obtained with Navitoclax alone (5.1 nM), whereas the values remain unaltered for C1-1 samples (4.4 nM with Navitoclax alone and 6.1 nM in combination).