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Identification of Compounds that Rescue *IKBKAP* Expression in Familial Dysautonomia-iPS Cells

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Abstract

Patient-specific induced pluripotent stem cells (iPSCs) represent a novel system for modeling human genetic disease and could develop into a key drug discovery platform. We recently reported disease-specific phenotypes in iPSCs from familial dysautonomia (FD) patients. FD is a rare but fatal genetic disorder affecting neural crest lineages. Here we demonstrate the feasibility of performing a primary screen in FD-iPSC derived neural crest precursors. Out of 6,912 compounds tested we characterized 8 hits that rescue expression of *IKBKAP*, the gene responsible for FD. One of those hits, SKF-86466, is shown to induce *IKBKAP* transcription via modulation of

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AUTHOR CONTRIBUTIONS

G.L.: conception and study design, maintenance/differentiation/isolation/expansion of FD-hiPSCs and FD-NCs, data analysis, cellular/molecular assays for validation/confirmation, data assembly, analysis and interpretation, and writing manuscript; C.N.R.: study design, development of assay, performing screening, data analysis and writing of manuscript; C.R.: performing screening; B.B.: HTS data analysis; H.K., N.Z., B.L., Y.J.K., I.Y.C. and B.M.C.: cellular/molecular assays for validation/confirmation; H.D.: study design, data analysis, interpretation of HTS data and writing manuscript; L.S.: conception and study design, data analysis and interpretation, and writing manuscript.

intracellular cAMP levels and PKA dependent CREB phosphorylation. SKF-86466 also rescues IKAP protein expression and the disease-specific loss of autonomic neuron marker expression. Our data implicate alpha-2 adrenergic receptor activity in regulating *IKBKAP* expression and demonstrate that small molecule discovery in an iPSC-based disease model can identify candidate drugs for potential therapeutic intervention.

Identification of Compounds that Rescue *IKBKAP* Expression in Familial Dysautonomia-iPS Cells

Familial dysautonomia (FD) is an autosomal recessive disease caused by a single point mutation in the I-κ-B kinase complex-associated protein (*IKBKAP*) gene leading to *IKBKAP* mis-splicing and a marked reduction in IKAP protein[1]. We previously reported the isolation of iPSC lines from FD-fibroblasts and identified multiple disease-specific phenotypes[2]. Among those phenotypes, we observed low levels of wild type (WT) *IKBKAP* expression, particularly in FD-iPSC derived neural crest (FD-NC), which may partly explain why the disease specifically affects the peripheral nervous system. FD is a progressive neurodegenerative disorder without effective treatment. The identification of compounds that increase *IKBKAP* expression in affected cell types could yield novel therapies for FD.

Developing screening conditions for FD-iPSC-derived NC

Several recent disease modeling studies have demonstrated the use of iPSC derived cell types for validating small sets of drug candidates[2–9]. However, to move from validating a few compounds to screening larger chemical libraries, it is critical to define disease-relevant conditions suitable for use in HTS (Fig. 1a). The first step in this process is the large-scale production of the pertinent cell type. We have previously reported the prospective isolation of FD-NC precursors by flow cytometry (Fig. 1b). Here we proliferated purified FD-NC precursors in the presence of FGF2 and EGF for 2 weeks (Fig. 1c) and cryo-preserved large batches (10^8–10^9 cells) for subsequent screening applications. NC precursors showed stable growth properties (population doubling time: 44.2 hrs; Ki-67+ cells: 51.7 ± 2.4%), high purity, maintenance of neurogenic differentiation potential, and excellent post-thawing recovery rates (91.6 ± 5.7 %; Fig. 1c, Supplementary Fig. 1). Optimized cell plating conditions for 384-well microtiter plates were obtained using a laminin/fibronectin-based coating method ("in house" coating; Supplementary Material & Methods, Supplementary Fig. 2a) that achieved reproducible cell attachment with high viability (Fig. 1d). Cellular growth was monitored using Alamar Blue[10] and Hoechst nuclear staining[11] which defined suitable plating densities at 2,500 – 7,500 cells/well (Fig. 1d, Supplementary Fig. 2b–c). DMSO had no major impact on growth of FD-NC precursors up to 1% DMSO (v/v). The final step in developing an FD-NC based HTS assay was the selection of a sensitive and disease-relevant readout. We hypothesized that promising compounds should increase levels of WT-*IKBKAP* in patient specific cells and thereby increase the levels of IKAP protein. Therefore, we developed a qRT-PCR assay for measuring levels of WT-*IKBKAP* against the *18S* internal control based on published primer sets[12] (Supplementary Fig. 2d). Levels of mutant (MU)-*IKBKAP* were also determined to address whether compounds increase both WT and MU-*IKBKAP* or act via *IKBKAP* splicing[12]. Transcript levels were measured

following cell lysis, RNA extraction, qRT-PCR reaction and data quantification (Supplementary Table 1). RT-PCR technology is not commonly used in HTS and is highly dependent on the quality and quantity of the isolated RNA. We found that a plating density of 7,500 cells/well yielded excellent reproducibility. To further validate our RT-PCR assay, we performed a control study mimicking the screening work flow (Suppl. Fig. 3) on three 384-well plates and demonstrating high consistency in Ct values (coefficient of variation (CV) ranging from 1 to 2%; average Z' values of 0.78 (Fig. 1e)). Differences in Ct values for WT-*IKBKAP* were minimal among replicate wells of the same plate. However, more marked differences were observed between wells of independent plates prompting us to perform the final screen in triplicates.

Screening of 6,912 compounds

We screened a library of 6,912 small molecules (20,736 treatment conditions in total). The library contained a broad set of biologically active and structurally diverse compounds from multiple commercial sources covering FDA approved drugs, known bioactives and experimental substances[13]. Compounds were screened at 10 μM in 1% DMSO (*v/v*) with each plate containing 1% DMSO (*v/v*) control wells. Z' values for the control plates in all three sets of the screen indicated excellent reproducibility (Suppl. Fig. 4). Using fold difference analysis of WT-*IKBKAP* to S18 ratios and a binning method taking into account robustness of replicate data, we nominated 28 compounds (0.4% hit rate) as potential rescuers of the WT-*IKBKAP* levels. In addition, we used an alternative rank-based selection algorithm that nominated an additional 15 candidate hits (Fig. 2a). For the rank-based method, we calculated the average WT-*IKBKAP* rank across the three replicate wells (ranks from 1–352), following elimination of compounds with low S18 values and correction for respective MU-*IKBKAP* ranks (see Material and Methods). This method was used as complementary strategy to the “fold difference” method to reduce the impact of variability in lowly expressed genes and to find potential compounds that selectively induce WT-*IKBKAP* (increase ratio of WT- to MU-*IKBKAP*). The list of 43 nominated compounds including structure information is provided in Supplementary Table 2). Out of 43 hits, only 35 were commercially available for further studies. After additional validations including dose-response and cytotoxicity assays (12 different concentration points), use of alternative housekeeping gene (GAPDH), and finally re-validation of WT-*IKBKAP* expression under standard 6-well culture conditions (Fig. 2a,b), we pursued 8 hits for further studies: Glucosaminic acid (**GA**); SKF-86466 Hydrochloride (**SKF**); Phenindione (**PD**); Dihydrojasmonic acid (**DHJ**); Cyclosporine (**CSP**); Cyproterone (**CPT**); Atropine (**AT**); 5-Thia-1-azabicyclo(4.2.0)oct-2-ene-2-carboxylic acid, 7-(((2Z)-(2-amino-4-thiazolyl)(methoxyimino)acetyl)amino)-3-(((5-(carboxymethyl)-4-methyl-2-thiazolyl)thio)methyl)-8-oxo-, disodium salt, (6R,7R) (**THIA**). Interestingly, the 8 compounds significantly increasing WT-*IKBKAP* levels in FD-NC precursors showed differential potency for *IKBKAP* induction in other cell types such as undifferentiated FD-iPSC, FD-fibroblasts and FD-lymphoblasts (Supplementary Fig. 5a–c). The tissue-specific bias in regulating WT-*IKBKAP* levels supports the argument for performing primary screens in symptom-relevant cells such as FD-NCs. Surprisingly, compounds did not trigger significant increases in WT-*IKBKAP* in control-NCs (derived from H9 hESCs; Supplementary Fig. 5d) suggesting a

possible feedback mechanism preventing supra-physiological WT-*IKBKAP* levels in control cells.

We next tested whether regulation of WT-*IKBKAP* expression by the 8 compounds is reproduced in FD-NCs from additional iPSC clones either from the same (clone 4) or a different (clone 101) patient (Fig. 2c). All compounds with the exception of THIA showed comparable potency across the various FD-NC precursor lines suggesting that the effects are generally not clone or patient dependent. THIA was shown to increase WT-*IKBKAP* in most clones when testing FD-iPSC NCs from yet additional patients (Supplementary Fig. 5e).

Hit validation and rescue studies

Only WT-*IKBKAP* but not MU-*IKBKAP* is translated into IKAP protein. We observed that several of the 8 compounds increased IKAP protein expression as measured by semi-quantitative Western blotting (Fig. 2d). Induction of IKAP protein levels was comparable to that observed following treatment with kinetin (**K**), the compound previously shown to partially rescue disease phenotypes in our FD-iPSC model[2], and comparable to the levels in NC precursors derived from hESCs or control iPSC lines (H9, C14; Fig. 2d). There was a significant ($p = 0.0343$) correlation between WT-*IKBKAP* transcript and IKAP protein levels, while no such correlation was seen between protein expression and MU *IKBKAP* (Supplementary Fig. 5f–h). None of the 8 compounds significantly affected cell proliferation or cell death, parameters that could indirectly affect *IKBKAP* levels (Supplementary Fig. 6).

While short-term (48 hours) compound treatment was sufficient to induce a robust increase in expression of WT-*IKBKAP* (Fig. 2b), it did not affect expression of autonomic markers such as *ASCL1* (*Mash1*) and *SCG10* (Supplementary Fig. 7a,b). Therefore we next tested the potency of each compound in long-term assays by treating cells for 28 days (from FD-iPSC through FD-NC stage). None of the compounds affected the percentage of HNK1+ FD-NC-precursors at day 28 (Supplementary Fig. 7c) suggesting that treatment does not interfere with cell differentiation. The levels of WT-*IKBKAP* in purified FD-NCs were increased for most compounds following long-term treatment compared with DMSO group (Supplementary Fig. 7d). Importantly, we observed significant increases in *ASCL1 (Mash1*) and *SCG10* expression, particularly following treatment with SKF (Fig. 2e,f). Remarkably, SKF rescued *ASCL1 (Mash1)* and *SCG10* expression to the levels found in NCs from wild-type lines. Despite a nearly complete rescue of autonomic marker expression, none of the 8 compounds rescued the reduced migratory propensity observed in FD-NC precursors (Fig. 2g), a result that mimicked our previous findings with kinetin[2]. It also remains to be determined whether long-term SKF treatment is required to capture the developmental stage most sensitive for rescuing autonomic neurogenesis or whether long-term treatment starting at late time points can be equally effective.

SKF implicates α2AR signaling in the regulation of *IKBKAP*

In a first attempt to address potential mechanisms by which compounds can increase *IKBKAP* expression, we focused on SKF (dose-response in Supplementary Fig. 7e). SKF is a known α_2-adrenergic receptor (α_2AR) antagonist without known inverse agonist activity[14]. SKF showed particularly promising results in rescuing expression of autonomic markers

raising the question whether α2AR activity could be involved in regulating *IKBKAP* expression. The family of α2ARs acts as G protein-coupled receptors (GPCRs) in association with the G_i heterotrimeric G-protein. There are three different subtypes of α2ARs: α_{2A}, α_{2B}, α_{2C}. The alpha subunit of the inhibitory G protein (dissociated Gi) associates with adenylcyclase and inhibits its activity resulting in decreased intracellular cAMP levels. We first confirmed expression for each of the three subunits (α_2A, α_2B and α_2C) of α_2AR in undifferentiated FD-iPSCs, differentiated FD-iPSC (Day 12 of neural induction) and purified/expanded FD-NCs by RT-PCR[15] (Fig. 3a, Supplementary Fig. 8a,b and Supplementary Table 1). Next we tested functional analogs of SKF that are either generic α_2AR antagonists (Yohimbine, YOH) or specific to each subunit of α_2AR (BRL-44408 (BRL) for subunit A, Imiloxan (IMI) for B, Spiroxatrine (Spi) and JP-1302 (JP) for C and RX821002 (RX) for D). YOH, BRL, IMI, Spi and JP (at 1 μM) significantly increased *IKBKAP* level immediately following exposure (Fig. 3b,c; 30 min. exposure), whereas no such effects were observed upon treatment with RX. RX exhibits selectivity for α2D-adrenoceptors[16], a receptor subtype expressed in murine but not in human tissues. IKAP protein levels were significantly increased compared to control (Fig. 3d) 48 hours after treatment with YOH or SKF. Despite the dramatic spike of *IKBKAP* mRNA induction 30 minutes after YOH treatment, the levels of mRNA and protein induction at 48 hours were comparable between YOH and SKF.

Functional analogs of SKF, such as YOH, were included in our HTS collection but were not identified as 'hit' in our initial analysis. This is likely due to the fact that the compound concentrations for the primary screen (10 μM) were suboptimal for YOH (Fig. 3b), and that a different time period was selected between compound exposure and *IKBKAP* measurement (30 minutes versus 48 hours). Exposure of FD-iPSC derived NCs to agonists of α_2AR (Xylazine or p-Iodoclonidine hydrochloride) resulted in time-dependent decreases in *IKBKAP* levels (Fig. 3e). However, the combination treatment of Xylazine and SKF or YOH did not significantly decrease *IKBKAP* levels (Fig. 3f) suggesting complex interactions of agonists and antagonists at α_2AR. To further address the specificity of α_2AR modulation on *IKBKAP* expression we tested for the impact of SKF and Xylazine following a sequential, time-dependent treatment paradigm. FD-NCs were exposed to DMSO, Xylazine or SKF (20uM, 10uM or 2uM) for 5 minutes and then switched to a medium containing DMSO, Xylazine or SKF (20uM, 10uM or 2uM) for an additional 30 minutes in various permutations (Fig. 3g). We observed that the effects of SKF pre-treatment on FD-NC were abolished by Xylazine. Conversely, the effects of Xylazine pre-treatment on FD-NC were overcome by SKF treatment in dose-dependent manner further arguing that SKF acts primarily through modulating A2AR.

Regulation of CREB phosphorylation is a well-known downstream mediator of α_2AR signaling mediated cAMP-induced regulation of pKA. Accordingly α2-adrenoceptor antagonists, treatment with either SKF or YOH led to an increase in intracellular cAMP levels that could be blocked by exposure to Xylazine (Fig. 3h, i). We observed that treatment with cell permeable dibutyryl-cAMP (cAMP) or Forskolin (FK) was able to mimic SKF effect (Fig. 3j). Furthermore, exposure to SKF, IMI and YOH increased the levels of phosphorylated CREB (p-CREB) in FD-NCs while exposure to H89 or KT5720

(selective Protein Kinase A inhibitors) blocked the effect of both SKF and YOH on *IKBKAP* expression (Fig. 3k,l). These data confirm that pKA activity is required to mediate SKF and YOH function. *IKBKAP* promoter analysis using the Transcription Element Search System (TESS, http://www.cbil.upenn.edu/cgi-bin/tess/tess) shows the presence of conserved CREB binding domains[17]. We developed a luciferase assay based on the corresponding human *IKBKAP* promoter region which demonstrated strong promoter activation following SKF treatment that was repressed by Xylazine or H89 (Fig. 3m). Our pharmacological studies strongly suggest that SKF induces *IKBKAP* expression, at least in part, via classical α_2AR signaling and cAMP-mediated activation of pKA and pCREB. The regulation of cAMP levels and its impact on *IKBKAP* expression levels appear to follow a complex temporal pattern and likely intersect with other signaling pathways. Interestingly, CSP, GA and AT also showed evidence of increased cAMP or p-CREB levels suggesting that this pathway could contribute to the action of other molecules from our screen (Supplementary Fig. 8c,d). Kinetin, a compound known to act on *IKBKAP* splicing, did not activate cAMP in FD-iPSC NC precursors supporting the specificity of this effect. The different mechanisms of action for SKF and Kinetin point to the possibility of combining modalities for future therapeutic applications.

DISCUSSION

Currently there are no animal models of FD that faithfully recapitulate the clinical symptoms of the disease beyond measuring tissue-specific *IKBKAP* splicing[18],[19]. Other unresolved issues include our lack of understanding as to why SKF or other compounds including Kinetin do not rescue the migratory defect of FD-NCs and whether any of the compounds will affect degenerative aspects of the disease such as the survival of postmitotic sensory or autonomic neurons. In either case, an important challenge for the future will be to make rational decisions in selecting the most promising compounds to pursue for clinical translation. While there are obvious criteria such as data on safe *in vivo* levels of a compound in relation to its *IKBKAP* activity or the ability to the cross blood-brain and blood-ganglia barriers, additional *in vivo* assays will be required for pre-clinical testing of our candidate compounds in a disease background. On the other hand, it is tempting to speculate whether an "*in vitro* clinical trial" that systematically tests compounds in iPSC-derived cells from FD-patients to be treated may contribute to a rationale clinical trial design. Since the first reports on establishing human iPSCs the use of disease-specific cells for drug validation and drug discovery has been an important goal. Our data demonstrate that it is feasible to perform primary screens in iPSC-derived cells for thousands of clinically relevant compounds. The development of similar assays for other diseases could herald a new drug discovery paradigm moving us closer towards the era of personalized medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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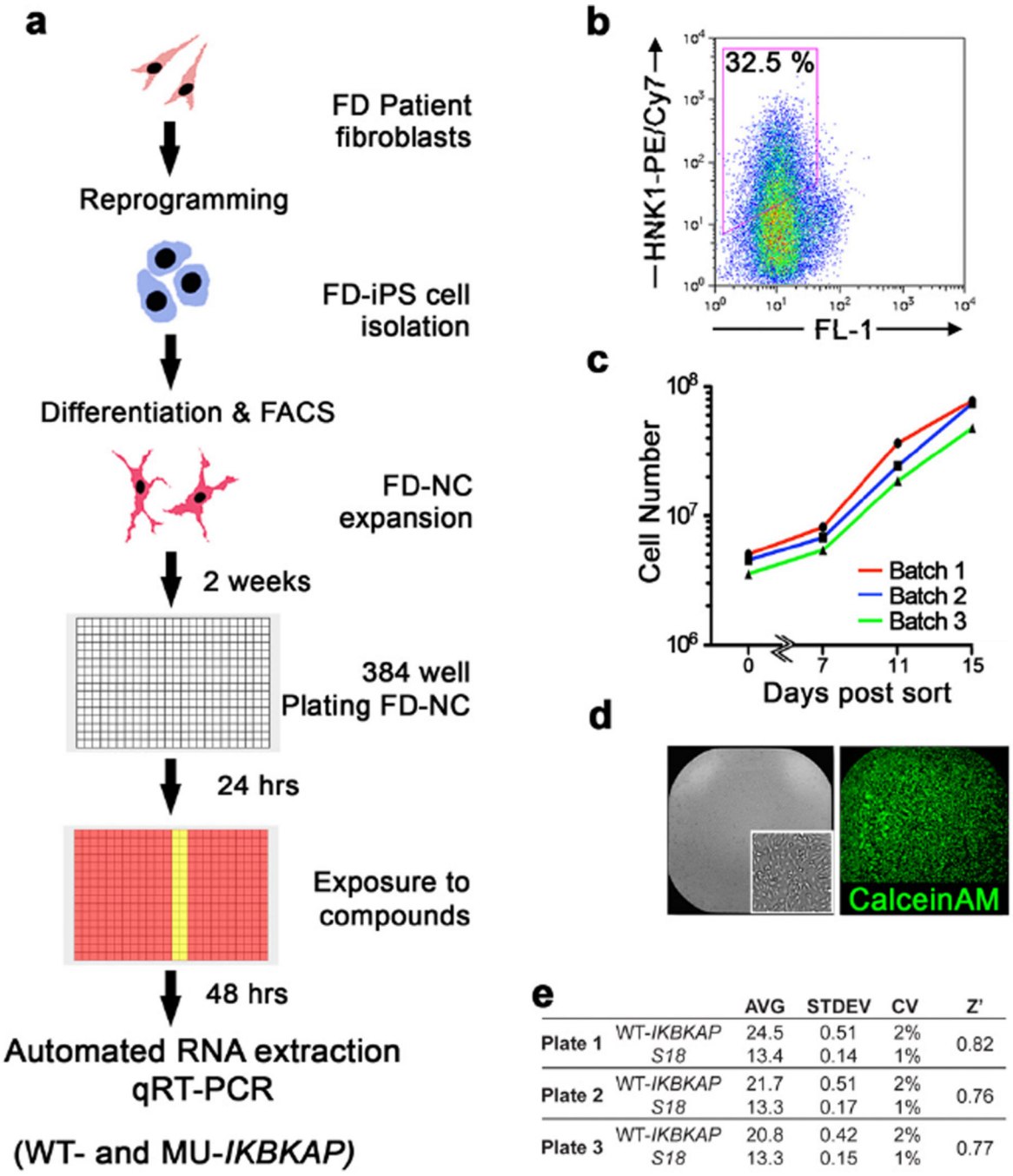
REFERENCES

1. Hims MM, et al. Therapeutic potential and mechanism of kinetin as a treatment for the human splicing disease familial dysautonomia. J Mol Med. 2007; 85:149–161. [PubMed: 17206408]
2. Lee G, et al. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature. 2009
3. Brennand KJ, et al. Modelling schizophrenia using human induced pluripotent stem cells. Nature. 2011; 473:221–225. [PubMed: 21490598]
4. Ebert AD, et al. Induced pluripotent stem cells from a spinal muscular atrophy patient. Nature. 2009; 457:277–280. [PubMed: 19098894]
5. Itzhaki I, et al. Modelling the long QT syndrome with induced pluripotent stem cells. Nature. 2011; 471:225–229. [PubMed: 21240260]
6. Marchetto MC, et al. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell. 2010; 143:527–539. [PubMed: 21074045]
7. Moretti A, et al. Patient-specific induced pluripotent stem-cell models for long-QT syndrome. N Engl J Med. 2010; 363:1397–1409. [PubMed: 20660394]
8. Yazawa M, et al. Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. Nature. 2011; 471:230–234. [PubMed: 21307850]
9. Yahata N, et al. Anti-Abeta drug screening platform using human iPS cell-derived neurons for the treatment of Alzheimer's disease. PLoS One. 2011; 6:e25788. [PubMed: 21984949]
10. O'Brien J, Wilson I, Orton T, Pognan F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. Eur J Biochem. 2000; 267:5421–5426. [PubMed: 10951200]
11. Desbordes SC, et al. High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. Cell Stem Cell. 2008; 2:602–612. [PubMed: 18522853]
12. Slaugenhaupt SA, et al. Rescue of a human mRNA splicing defect by the plant cytokinin kinetin. Hum Mol Genet. 2004; 13:429–436. [PubMed: 14709595]
13. Shum D, et al. High-content assay to identify inhibitors of dengue virus infection. Assay Drug Dev Technol. 2010; 8:553–570. [PubMed: 20973722]
14. Pauwels PJ, Tardif S, Wurch T, Colpaert FC. Facilitation of constitutive alpha(2A)-adrenoceptor activity by both single amino acid mutation (ThR373)Lys) and g(alphao) protein coexpression: evidence for inverse agonism. J Pharmacol Exp Ther. 2000; 292:654–663. [PubMed: 10640303]
15. Ooi YH, Oh DJ, Rhee DJ. Analysis of alpha2-adrenergic receptors and effect of brimonidine on matrix metalloproteinases and their inhibitors in human ciliary body. Invest Ophthalmol Vis Sci. 2009; 50:4237–4243. [PubMed: 19324857]
16. Trendelenburg AU, Wahl CA, Starke K. Antagonists that differentiate between alpha 2A-and alpha 2D-adrenoceptors. Naunyn Schmiedebergs Arch Pharmacol. 1996; 353:245–249. [PubMed: 8692278]
17. Pfenning AR, Schwartz R, Barth AL. A comparative genomics approach to identifying the plasticity transcriptome. BMC Neurosci. 2007; 8:20. [PubMed: 17355637]
18. Chen YT, et al. Loss of mouse Ikbkap, a subunit of elongator, leads to transcriptional deficits and embryonic lethality that can be rescued by human IKBKAP. Mol Cell Biol. 2009; 29:736–744. [PubMed: 19015235]
19. Hims MM, et al. A humanized IKBKAP transgenic mouse models a tissue-specific human splicing defect. Genomics. 2007; 90:389–396. [PubMed: 17644305]



e

		AVG	STDEV	CV	Z'
Plate 1	WT-*IKBKAP*	24.5	0.51	2%	0.82
	S18	13.4	0.14	1%	
Plate 2	WT-*IKBKAP*	21.7	0.51	2%	0.76
	S18	13.3	0.17	1%	
Plate 3	WT-*IKBKAP*	20.8	0.42	2%	0.77
	S18	13.3	0.15	1%	

Figure 1. HTS Assay Development

a, Schematic representation of HTS assay: FD patient specific fibroblasts were reprogrammed into FD-iPSC and FD-iPSCs were differentiated into neural crest cells. FD-NCs were purified and expanded for 2 weeks. After optimization, FD-NCs were plated in 384 well plate and further treated with compound library (24 hours after plating). Each plate contained 32 control wells (DMSO only, yellow columns). Treated FD-NCs were analyzed by quantitative RT-PCR (48 hours after treatment). **b,** FACS-purified HNK1+ FD-NCs. **c,** Number of different batch of purified FD-NCs during expansion. **d,** Representative images

of 384-well-plated FD-NC and CalceinAM staining. **e,** Robustness of primary HTS read-out: Control run statistics with average Ct values (AVG), their standard deviation (STDEV) and coefficient of variation (CV) and the Z' values are summarized for plates 1, 2, and 3.

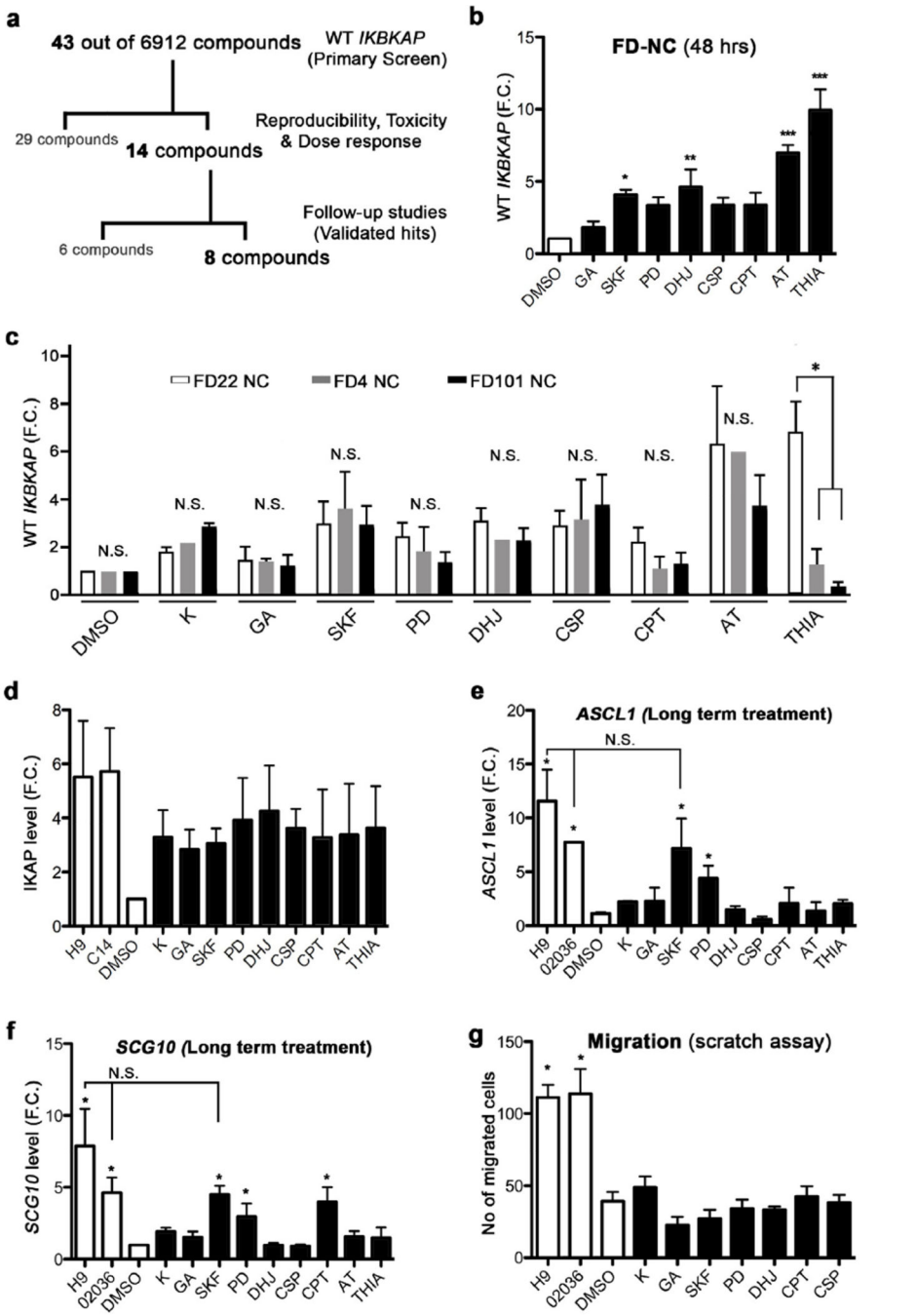


Figure 2. Results of 6912 compounds screen in FD-NCs

a, Out of 6,912 compounds 28 and 15 compounds were nominated as hits respectively using two distinct selection algorithms (see text for details). A total of 8 compounds were pursued for detailed follow up studies under non-HTS conditions. **b,** Validating impact of treatment with 8 compounds on WT-*IKBKAP* expression in FD-NC maintained under 6-well plate culture conditions. **c,** Reproducibility of HTS screening results in FD-NC derived from independent FD-iPSC clones from the same (FD4) and from a different (FD101) patient. **d**, IKAP protein levels after treatment with individual compounds. **e,f,** *ASCL1* (**e**) and *SCG10*

(**f**) expression in FD-NC following long-term treatment with 8 compounds from FD-iPSC to FD-NC stage. **g,** The wound-healing (scratch) assay in FD-NCs following long-term treatment. $n = 3–5$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. All values are mean and s.d. F.C., Fold Change based on DMSO. N.S., Not Significant.

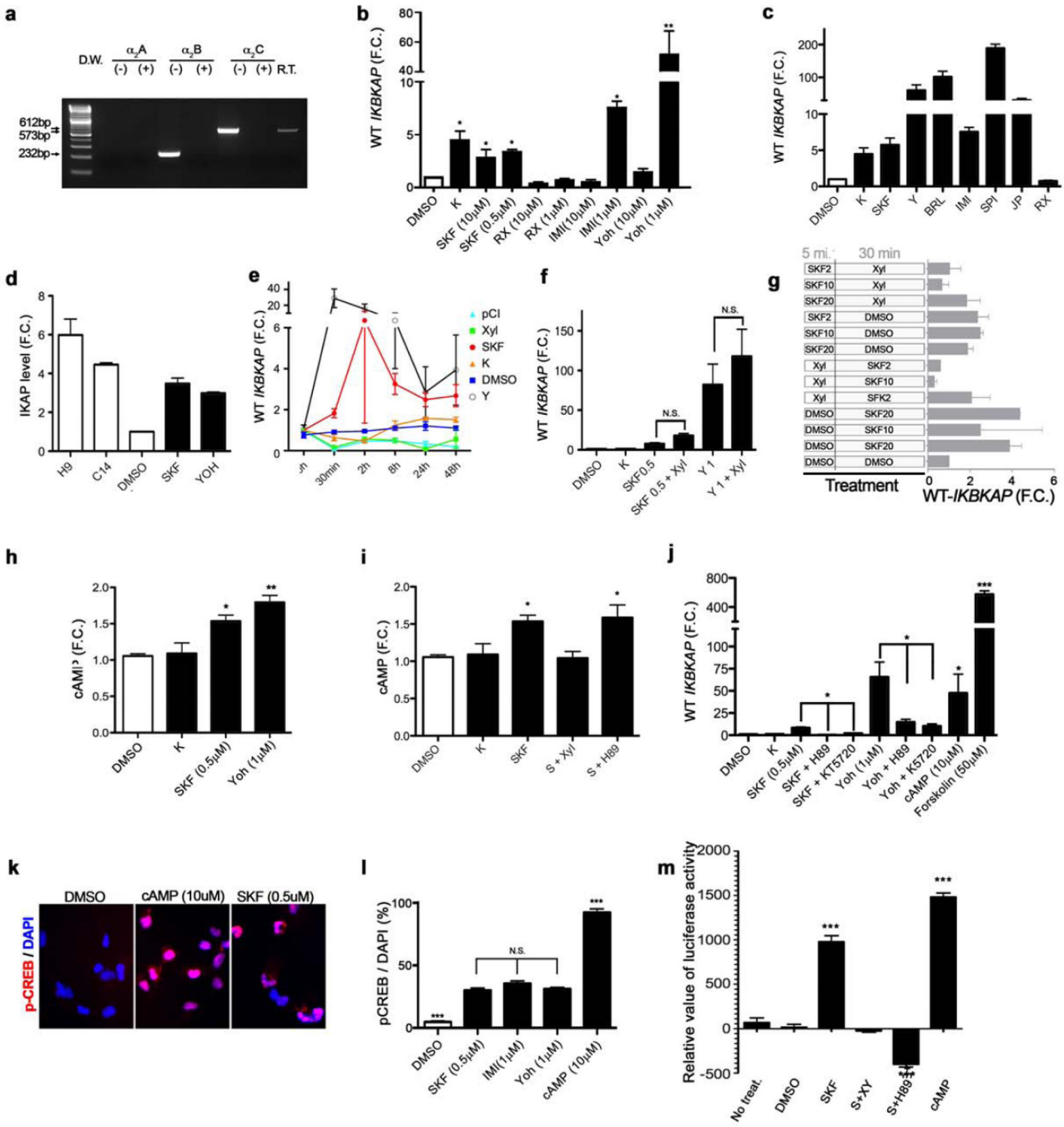


Figure 3. Functional studies on the mechanism of SKF action

a, Expression of α_2 adrenergic receptor subunits (A, B and C) in FD-NC. **b,c,** WT-*IKBKAP* expression in FD-NC following treatment with other α2-adrenergic receptor antagonists (RX, RX821002; IMI, Imiloxan; Yoh, Yohimbine; Spi, Spiroxatrine and JP, JP-1302). **d,** IKAP protein level after treatment of SKF and YOH (48 hrs). **e,** Time course of WT-*IKBKAP* expression following treatment with Xylazine (Xyl), p-Iodoclonidine hydrochloride (pCI), Kinetin (K), SKF (S) and Yohimbine (Y) or DMSO control (30 min). **f,g**, WT-*IKBKAP* expression in FD-NC after SKF or YOH (Y) treatment and co-treatment

with Xyl (30 min, **f**) or various pre-/post-treatments (**g**). **h,** cAMP levels following SKF or Yoh treatment **i,** cAMP levels after treatment of inhibitor and stimulators in α_2 adrenergic receptor pathway. **j,** WT-*IKBKAP* expression in FD-NC after treatment with SKF, Yoh, cAMP and Forskolin (FK) in the presence or absence of the specific PKA inhibitor compounds H89 and KT5720 (10 μM). **k,l**, Representative images (**k**) and quantification (**l**) of phosphorylated CREB levels in treated FD-NCs. **m,** Luciferase activity of *IKBKAP* promoter following each treatment. $n = 3–5$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. All values are mean and s.d. F.C., Fold Change based on DMSO. N.S., Not Significant. Scale bars, 20 μm.