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Glucocorticoids reprogram beta cell signaling to preserve insulin secretion

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ABSTRACT

Excessive glucocorticoid exposure has been shown to be deleterious for pancreatic beta cell function and insulin release. However, glucocorticoids at physiological levels are essential for many homeostatic processes, including glycemic control. Here, we show that corticosterone and cortisol and their less active precursors, 11-dehydrocorticosterone (11-DHC) and cortisone, suppress voltage-dependent Ca^{2+} channel function and Ca^{2+} fluxes in rodent as well as human beta cells. However, insulin secretion, maximal ATP/ADP responses to glucose and beta cell identity were all unaffected. Further examination revealed the upregulation of parallel amplifying cAMP signals, and an increase in the number of membrane-docked insulin secretory granules. Effects of 11-DHC could be prevented by lipotoxicity and were associated with paracrine regulation of glucocorticoid activity, since global deletion of 11β-hydroxysteroid dehydrogenase type 1 normalized Ca^{2+} and cAMP responses. Thus, we have identified an enzymatically-amplified feedback loop whereby glucocorticoids boost cAMP to maintain insulin secretion in the face of perturbed ionic signals. Failure of this protective mechanism may contribute to diabetes in states of glucocorticoid excess such as Cushing's syndrome, which are associated with frank dyslipidemia.

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INTRODUCTION

Circulating glucocorticoids exert potent metabolic effects including lipolysis, hepatic gluconeogenesis, amino acid mobilization and reduced skeletal muscle glucose uptake (1). This is facilitated by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1), which (re)activates glucocorticoid in a tissue-specific manner to determine bioavailability (2). As such, states of excess glucocorticoids (*e.g.* Cushing's syndrome) are pro-diabetic, since they cause profound glucose intolerance and insulin resistance.

While systemic administration of glucocorticoids induces a compensatory increase in beta cell mass, and eventually insulin secretory failure due to insulin resistance (3), effects directly on beta cell function are less well understood. Suggesting an important link between glucocorticoids and insulin release, beta cell-specific glucocorticoid receptor (GR) overexpression reduces glucose tolerance (4). However, *in vitro* studies using isolated islets have shown inhibitory or no effect of glucocorticoids on glucose-stimulated insulin secretion depending on the steroid potency, concentration and treatment duration (5-9). By contrast, HSD11B1 increases ligand availability at the GR by converting less active to more active glucocorticoid (11-dehydrocorticosterone (11-DHC) -> corticosterone in rodents; cortisone -> cortisol in man), impairing beta cell function in islets both *in vitro* and *in vivo* (6; 10; 11). Whereas 11-DHC has consistently been shown to impair beta cell function in islets from obese animals, conflicting reports exist regarding its effects on normal islets (7; 10).

More generally, the signaling components targeted by glucocorticoids are not well defined. While exogenous application of glucocorticoid subtly decreases insulin release, and nicotinamide adenine dinucleotide phosphate, 3',5'-cyclic adenosine monophosphate (cAMP) and inositol phosphate production (5), these studies were performed using high dose dexamethasone (25x relative potency compared to cortisol). Conversely, administration of the same glucocorticoid in drinking water augments insulin release by increasing the number of

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docked exocytotic vesicles, as well as beta cell mitochondrial potential/metabolism (12). However, indirect effects of insulin resistance cannot be excluded, as studies in high fat diet-fed mice have shown that compensatory beta cell responses, including proliferation, occur within a few days (13). Lastly, glucocorticoid administration or GR deletion in the early neonatal period alters beta cell development, leading to reductions in the expression of key maturity markers including Pdx1, Nkx6.1 and Pax6 (14; 15). Whether this is also seen in adult islets, as may occur during diabetes (16), is unknown.

In the present study, we therefore sought to investigate the mechanisms by which the endogenous glucocorticoids corticosterone and cortisol affect beta cell function. Using *in situ* imaging approaches together with biosensors, we reveal that glucocorticoids perturb cytosolic Ca^{2+} concentration through effects on voltage-dependent Ca^{2+} channel (VDCC) function, without altering beta cell maturity, glucose-induced changes in ATP/ADP ratio or incretin responsiveness. This however does reduce insulin secretion, since glucocorticoids upregulate parallel cAMP signaling pathways. The less active glucocorticoids 11-DHC and cortisone showed identical effects, which could be reversed in mouse following global deletion of *Hsd11b1*. Thus, a steroid-regulated feedback loop, encompassing an enzymatic amplification step, maintains normal insulin secretory output in the face of impaired beta cell ionic fluxes.

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MATERIALS AND METHODS

Animals

CD1 mice (8-12 weeks, males) were used as wild-type tissue donors. *Hsd11b1*$^{-/-}$ mice were generated as described (17). Studies were regulated by the Animals (Scientific Procedures) Act 1986 of the United Kingdom, and approval granted by the University of Birmingham's Animal Welfare and Ethical Review Body.

Islet isolation

Islets were isolated using collagenase digestion and cultured in RPMI supplemented with 10% fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin. Vehicle (ethanol 0.2%), 11-DHC (20/200 nM) or corticosterone (20 nM) (*i.e.* within the circulating free glucocorticoid range) were applied for 48 hrs. BSA-conjugated palmitate was applied at 0.5 mM.

Human islet culture

Islets were obtained from isolation centers at Alberta (IsletCore) (18), Pisa and Milan, with local and national ethical permissions. Islets were cultured in RPMI containing 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone, supplemented with 5.5 mM D-glucose, and treated with either vehicle (ethanol 0.2%), cortisone (200 nM) or cortisol (20 nM) for 48 hrs. See Supplemental Table 1 for donor characteristics. Studies were approved by the National Research Ethics Committee (NRES), REC reference 16/NE/0107.

Calcium, ATP/ADP and cAMP imaging

Islets were loaded with 10 μM Fluo8 AM for 45 mins at 37 °C before washing and incubation in buffer for a further 30 min to allow cleavage by intracellular esterase. Imaging was conducted using either: 1) a Crest X-Light spinning disk and 10 x/0.4 NA objective; or 2) a Zeiss LSM780 and 10 x/0.45 NA objective. For the Crest system, excitation was

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delivered at λ = 458–482 nm (400 ms exposure; 0.33 Hz) and emitted signals detected at λ = 500–550 nm using an EMCCD. For the Zeiss system, excitation was delivered at λ = 488 nm, and emitted signals detected at λ = 499–578 nm using a PMT. Fura2 was loaded as for Fluo8 and imaging performed using LEDs (excitation λ = 340/385 nm; emission λ = 470-550 nm).

ATP/ADP ratios and cAMP responses were measured using adenovirus harboring either Perceval (excitation/emission as for Fluo8) or the FRET probe Epac2-camps (excitation λ = 430-450 nm; emission λ = 460-500 nm and 520-550 nm) (19; 20). For Perceval, glucose was increased from 3-11 mM, which leads to plateau responses (21). An effect of glucocorticoid on Epac2-camps expression was unlikely, since single and dual channel fluorescence under maximal stimulation was similar for all treatments (Supplemental Table 2). In all cases, HEPES-bicarbonate buffer was used, containing (in mM): 120 NaCl, 4.8 KCl, 24 $NaHCO_3$, 0.5 Na_2HPO_4, 5 HEPES, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 3-17 *D*-glucose. Ca^{2+}, cAMP and ATP/ADP traces were normalized as F/F_{min}, where F = fluorescence at any given time point and F_{min} = minimum fluorescence during the recording (*i.e.* under basal conditions).

Electrophysiology

VDCC currents were recorded from dispersed mouse beta cells, as previously described (22). Patch electrodes were pulled to a resistance of 3-4 MΩ then filled with an intracellular solution containing (in mM): 125 CsCl, 10 tetraethylammonium Cl (TEA), 1 MgCl2, 5 EGTA, 10 HEPES, 3 MgATP, pH 7.22 with CsOH. Cells were patched in HEPES-buffered solution + 17 mM glucose. Upon obtaining the whole-cell configuration with a seal resistance > 1 GΩ, the bath solution was exchanged for a modified HEPES-buffered solution containing (in mM): 62 NaCl, 20 TEA, 30 $CaCl_2$, 1 $MgCl_2$, 5 CsCl, 10 HEPES, 17 glucose, 0.1 tolbutamide, pH 7.35 with NaOH. Beta cells were perfused for 3 mins with this solution prior to initiating the VDCC recording protocol. Voltage steps of 10 mV were applied from a

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holding potential of -80 mV; linear leak currents were subtracted online using a P/4 protocol. Data were analyzed using Clampfit (Molecular Devices).

Immunohistochemistry and super-resolution imaging

Islets were fixed overnight at 4ºC in 4% parafolmadehyde before immunostaining using rabbit monoclonal anti-insulin (Cell Signaling Technology; 1:400) and goat anti-rabbit Alexa568 (1:1000). Super-resolution imaging was performed using a VisiTech iSIM and a 100x/1.49 NA objective. Excitation was delivered using a λ = 561 nm and emitted signals captured at λ = 633-647 nm using a sCMOS. Image stacks were cropped to include only the near-membrane regions and exclude out-of-focus signal, converted to 8-bit grayscale, before obtaining the maximum intensity projection. Auto-thresholding was performed in Fiji (NIH) to produce a binary snapshot from which the area occupied by insulin granules could be quantified as a unitary ratio (V/v) *versus* the total membrane area using the analyze particle plugin, as previously described (20).

Real-time PCR

Relative mRNA abundance was determined using SYBR Green chemistry and fold-change in mRNA expression calculated compared to *Actb* using the $2^{-\Delta\Delta Ct}$ method (see Supplemental Table 3 for primer sequences). *Hsd11b1* mRNA abundance was determined using TaqMan assays for mouse (Cat. # 4331182) and human (Cat. # 4331182) tissue, *Hsd11b1* expression calculated using $2^{-\Delta Ct}$ x 1000, and transformed values presented as arbitrary units.

Measurements of insulin secretion and ATP in isolated islets

Batches of eight islets were placed in low-bind Eppendorf tubes, incubated for 30 mins at 37 °C in HEPES-bicarbonate buffer containing 3 mM glucose, before addition of either 3 mM glucose, 17 mM glucose or 17 mM glucose + 10 mM KCl for a further 30 mins and collection of supernatant. Total insulin was extracted into acid-ethanol. Insulin concentration was determined using a Homogeneous Time Resolved Fluorescence (HTRF)-based assay

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(Cisbio) according to the manufacturer's instructions. Total ATP at 3 and 17 mM glucose was measured in batches of 25 islets using a luciferase-based assay (Invitrogen), and values normalized to total protein.

Statistical analyses

Pairwise comparisons were performed using paired or unpaired Student's t-test. Interactions between multiple treatments were determined using one-way ANOVA (adjusted for repeated measures as necessary), followed by Bonferonni's or Tukey's post hoc test. Analyses were conducted using Graphpad Prism and IgorPro.

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RESULTS

Glucocorticoids alter ionic but not metabolic fluxes

Fluo8-loaded beta cells residing within intact islets of Langerhans were subjected to multicellular Ca^{2+}-imaging approaches (23). Individual beta cells responded to elevated glucose (3 mM -> 17 mM) with large increases in cytosolic Ca^{2+} levels (Fig. 1A and B). Whereas 11-DHC 20 nM was without effect, higher (200 nM) concentrations suppressed the amplitude and area-under-the-curve (AUC) of Ca^{2+} rises in response to glucose and glucose + 10 mM KCl by ~30% (Fig. 1A-E) (Supplemental Fig. 1A and B) (Supplemental Fig. 2A-C), and this reached ~50% in the presence of corticosterone 20 nM. Results were confirmed using the ratiometric Ca^{2+} indicator Fura2, excluding a major contribution of basal Ca^{2+} levels to the magnitude changes detected here (Supplemental Fig. 2A-C). No effect of glucocorticoid on the time to onset of Ca^{2+} rises was detected (lag period ± SD = 22.5 ± 7.7 *versus* 26.3 ± 9.7 *versus* 24.0 ± 6.2 s for control, 11-DHC and corticosterone, respectively; non-significant, one-way ANOVA). The peak Ca^{2+} response to KCl depolarization in low ~~(~~(3 mM) glucose was unaffected by 11-DHC and significantly increased by corticosterone (Supplemental Fig. 2D and E), although both glucocorticoids reduced Ca^{2+} amplitude when KCl concentration was increased from 10 to 30 mM (Supplemental Fig. 2F and G) (24). While both 11-DHC and corticosterone led to more sustained Ca^{2+} influx in response to 3 mM glucose + 10 mM KCl (Supplemental Fig. 2E), this was not the case with 30 mM KCl (Supplemental Fig. 2G). An effect of treatment on basal Ca^{2+} levels at 3 mM glucose was unlikely, since the Fura2 340/385 ratio was not significantly affected by 11-DHC or corticosterone (Supplemental Fig. 2H).

Supporting an action on later steps in ionic flux generation, 11-DHC and corticosterone reduced Ca^{2+} oscillation frequency at moderately (11 mM) elevated glucose concentration (Fig. 1F and G). Glucocorticoids (cortisone and cortisol) also suppressed Ca^{2+}

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responses to glucose and glucose + 10 mM KCl in human islets (Fig. 1H-J), without significantly altering basal Ca^{2+} concentration (Supplemental Table 4). The reported glucocorticoid actions were specific to glucose, as both 11-DHC and corticosterone were unable to influence Ca^{2+} responses to exendin-4 in mouse islets in terms of oscillation frequency and AUC (Fig. 1K-M), these parameters being the primary drivers of incretin-stimulated Ca^{2+} fluxes in this species (23).

Beta cells remain differentiated in the presence of glucocorticoids

Immature or de-differentiated beta cells fail to respond properly to glucose, a defect that can partly be explained by lowered transcription factor expression, and impairments in metabolism and Ca^{2+} flux generation (25). This was unlikely to be the case here, however, as 11-DHC and corticosterone did not significantly affect mRNA abundance of the key beta cell maturity markers *Pdx1* (Fig. 2A-C) and *Nkx6.1* (Fig. 2D-F). Moreover, maximal ATP/ADP increases in response to glucose, measured using the biosensor Perceval (26), were not significantly different (Fig. 2G and H). 11-DHC and corticosterone did not affect the time to onset (Supplemental Fig. 3A) or the amplitude (Supplemental Fig. 3B) of the initial, transient decrease in ATP/ADP. No significant effects of glucocorticoid on basal or glucose-stimulated ATP levels were detected using luciferase-based assays (Supplemental Fig. 4). Patch-clamp electrophysiology revealed abnormal VDCC function in the presence of glucocorticoids, with voltage-current (I-V) curves showing a marked reduction in Ca^{2+} conductance (Fig. 2I and J). Suggestive of changes in VDCC function rather than expression, transcript levels of the major alpha and beta subunits *Cacna1c (*Fig. 2K and L), *Cacnb2* (Fig. 2M and N) and *Cacna1d* (Fig. 2O and P) were not significantly altered.

Glucocorticoids do not affect insulin secretory responses

In response to glucose, increases in ATP/ADP ratios lead to closure of K_{ATP}-channels, opening of VDCCs and Ca^{2+}-dependent insulin secretion (27). Thus, perturbed cytosolic Ca^{2+}

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fluxes/levels generally translate to reductions in insulin secretory output (27). However, glucose and glucose + KCl-stimulated insulin release were not significantly different following 48 hr exposure of islets to 11-DHC or corticosterone (Fig. 3A). This was not due to an increase in insulin expression, since *Ins1* mRNA levels were similar in the presence of both glucocorticoids (Fig. 3B-D). Likewise, total insulin content was not significantly different between treatments under all stimulation conditions examined (Fig. 3E). Insulin secretion was also unaffected by cortisone and cortisol treatment in primary human islets (Fig. 3F and G) (Supplemental Table 1).

cAMP signals are upregulated by glucocorticoids

Granule release competency can be increased by signals including cAMP, which acts directly upon protein kinase A (PKA) and exchange protein directly activated by cAMP 2 (Epac2) (28). Using the FRET probe Epac2-camps to dynamically report cytosolic cAMP (20), glucose induced a robust increase in levels of the nucleotide (Fig. 4A). Both 11-DHC and corticosterone upregulated cAMP responses to glucose by ~1.5-fold (Fig. 4A-C). This appeared necessary for maintenance of secretory output, since chemical inhibition of PKA significantly reduced glucose-stimulated insulin release in 11-DHC-treated islets (Fig. 4D). Indeed, more granules were present at the membrane in glucocorticoid-treated islets, revealed using super-resolution structured illumination microscopy (SIM) (Fig. 4E and F). Similar results were seen in human islets, with cortisone and cortisol both augmenting cAMP responses to glucose (Fig. 4G and H). As for Ca^{2+}, the actions of glucocorticoid were glucose-specific, as neither 11-DHC nor corticosterone altered cAMP responses to exendin-4 (Fig. 4I and J). Supporting a central role for adenylate cyclase (Adcy) in this effect, expression of *Adcy1* was increased by both glucocorticoids (Fig. 4K and L), and induction of lipotoxicity with palmitate- shown previously to lower *Adcy9* mRNA (29)- prevented glucocorticoid from augmenting cAMP responses to glucose (Fig. 4M and N).

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Hsd11b1 is expressed in islets of Langerhans

HSD11B1 is responsible for catalyzing the conversion of 11-DHC to corticosterone and is an important mechanism that determines local glucocorticoid activity (30). Expression of *Hsd11b1* in islets has previously been shown to be sufficient for 11-DHC → corticosterone conversion (7). We therefore repeated studies in islets obtained from mice globally lacking one (*Hsd11b1*$^{+/-}$) or both (*Hsd11b1*$^{-/-}$) alleles of Hsd11b1. While *Hsd11b1* mRNA levels were low in mouse islets compared to liver and muscle, it was still detectable (ΔCt = 7.33. ± 1.80) (Supplemental Fig. 5A). Moreover, *Hsd11b1* mRNA abundance was 55-75% lower in islets from animals expressing a single copy of *Hsd11b1* and undetectable in those deleted for both alleles (Supplemental Fig. 5B), as assessed using specific TaqMan assays. Quantification of *HSD11B1* mRNA revealed similar levels in human and mouse islets, with expression an order of magnitude lower than in human subcutaneous and omental adipose tissue (Supplemental Fig. 5C), a major site of enzyme activity and steroid reactivation (31).

Hsd11b1 deletion reverses the effects of glucocorticoids on beta cell Ca^{2+} and cAMP signaling

As expected, both 11-DHC and corticosterone impaired cytosolic Ca^{2+} fluxes in beta cells residing within islets from *Hsd11b1*$^{+/-}$ animals (Fig. 5A-D) (Supplemental Fig. 6A and B). However, deletion of *Hsd11b1*$^{-/-}$ throughout the islet reversed these effects, with 11-DHC and corticosterone no longer able to suppress Ca^{2+} rises in response to glucose- or glucose + KCl (Fig. 5E-H) (Supplemental Fig. 6C and D). This suggests that local regulation of glucocorticoid activity in the islet may mediate the effects of 11-DHC and corticosterone on beta cell Ca^{2+} fluxes. 11-DHC was able to significantly elevate cAMP responses to glucose in *Hsd11b1*$^{+/-}$ (Fig. 6A-D) (Supplemental Fig. 7A), but not *Hsd11b1*$^{-/-}$ islets (Fig. 6E-H) (Supplemental Fig. 7B). However, corticosterone still improved cAMP responses to glucose, even following deletion of *Hsd11b1* (Fig. 6A-H) (Supplemental Fig. 7A and B). Glucose-

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stimulated insulin secretion was significantly higher in corticosterone- *versus* control- or 11-DHC-treated *Hsd11b1*$^{-/-}$ islets (Fig. 6I), consistent with the Ca^{2+} and cAMP results. Similarly, QRT-PCR analyses revealed upregulation of *Adcy1* expression by corticosterone, but not by 11-DHC, in *Hsd11b1*$^{-/-}$ islets (Fig. 6J and K). Ca^{2+} responses to glucose, glucose + KCl and KCl were not significantly decreased by 11-DHC (Fig. 7A-D) (Fig. 7E and F) (Supplemental Fig. 8A and B) in islets pre-treated with RU486. Similarly, corticosterone was unable to impair Ca^{2+} responses to glucose and KCl in RU486-treated islets (Fig. 7E) (Supplemental Fig. 8C and D), although Ca^{2+} responses to glucose + KCl were unaffected (Fig. 7F). Thus, the inhibitory actions of the glucocorticoids are partly mediated by the GR.

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DISCUSSION

We show here that corticosterone and cortisol and their less active precursors, 11-DHC and cortisone, impair glucose-, glucose + KCl- and KCl-stimulated ionic fluxes in rodent and human beta cells. However, insulin secretory output is likely preserved because both glucocorticoids upregulate cAMP signals to increase insulin granule number at the membrane. Invoking a critical role for glucocorticoid interconversion, the effects of 11-DHC could be prevented following islet-wide deletion of HSD11B1. Thus, an enzyme-assisted steroid-regulated feedback loop maintains insulin secretion in the face of altered beta cell ionic signaling (Fig. 8).

Both corticosterone and 11-DHC have previously been shown to exert inhibitory effects on insulin release (6; 7; 10; 11). However, these studies either used islets from *ob/ob* mice that display highly upregulated *Hsd11b1* expression (6; 10), or incubated wild-type islets with glucocorticoid for only two hours (7; 11), which is unlikely to fully compensate the loss of adrenal input that occurs following islet isolation. Likewise, studies in which glucocorticoids are administered in the drinking water are confounded by insulin resistance and compensatory islet expansion (12). Thus, the effects observed in the present study more likely reflect the cellular/molecular actions of circulating glucocorticoids under normal conditions.

Cytosolic Ca^{2+} responses to glucose were impaired in the presence of either 11-DHC or corticosterone. This was unlikely due to defects in metabolism and K_{ATP}-channel function, since glucose-induced ATP/ADP maximal rises were unaffected. However, KCl- and KCl + glucose-induced Ca^{2+} influx, as well as VDCC conductance, were markedly suppressed, although QRT-PCR analyses of expression levels of the key L-type VDCC subunits showed no differences. Paradoxically, glucocorticoid improved the sustained Ca^{2+} responses to 3 mM glucose + 10 mM KCl. While this may reflect basal cAMP generation due to upregulated

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Adcy1, it should be noted that VDCCs do not open fully under these conditions (Supplemental Table 5), meaning that true defects in their activity are likely to be missed. Indeed, glucocorticoids may induce changes that only restrict Ca^{2+} entry when VDCC open probability increases to support insulin secretion (*i.e.* 17 mM glucose and/or 30 mM KCl). Ca^{2+} oscillation frequency was also affected, suggesting that glucocorticoids may conceivably target more distal steps in Ca^{2+} flux generation, such as intracellular stores (*e.g.* by depleting them through cAMP-sensitization of IP_3 receptors (32)), upregulate ion channels involved in voltage-inactivation (*i.e.* large-conductance Ca^{2+}-activated K^+ channels (33)), or alter glucose-regulated inputs other than cAMP (34). These effects are presumably specific to glucose-stimulated Ca^{2+} rises, as responses to the incretin-mimetic exendin 4 remained unchanged by glucocorticoid exposure, possibly due to PKA-mediated rescue of VDCC function or organellar Ca^{2+} release (35).

Recent RNASeq analyses of purified mouse beta cells have shown that *Hsd11b1* mRNA levels are unusually low in these and other islet neuroendocrine cells (*i.e.* it is an islet "disallowed" gene) (36). Likewise, *HSD11B1* mRNA levels were low in human beta and alpha cells (37). These findings contrast with reports that protein expression co-localizes with glucagon or insulin in rodent islets depending on the antibody used (7; 38). The reasons for these discrepancies are unclear, but in the present study specific TaqMan assays showed consistently detectable mRNA levels in both rodent and human islets. Moreover, 11-DHC effects could be prevented in global *Hsd11b1*$^{-/-}$ islets in which mRNA was largely absent, and *HSD11B1* expression in human islets was an order of magnitude lower than in adipose tissue, a major site for steroid reactivation after the liver (31). Thus, 11-DHC likely affects beta cell function in a paracrine manner, possibly through the actions of HSD11B1 in non-endocrine islet cell types (*e.g.* endothelial cells where expression levels are higher (37)). This may form the basis of an adaptive mechanism to prevent the build-up of high local

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corticosterone/cortisol concentrations. Together, these data highlight the importance of the islet context for the regulation of insulin secretion, and underline the requirement to consider cell-cell cross-talk when assessing the functional consequences of beta cell gene disallowance.

Global deletion of *Hsd11b1* prevented the effects of 11-DHC on ionic and cAMP fluxes, as expected, suggesting that local regulation of glucocorticoid activity is important for beta cell function. However, corticosterone was unable to impair Ca^{2+} responses in *Hsd11b1*$^{-/-}$ islets, whereas potentiation of cAMP remained intact. Together, these observations raise the possibility that corticosterone may undergo substantial oxidation to 11-DHC *via* HSD11B2 (37), with local concentrations dropping below the threshold for suppression of Ca^{2+} but not cAMP following *Hsd11b1* knockout. While previous studies have shown that a single *Hsd11b1* allele is sufficient for full enzymatic activity (39), further studies are required to determine whether this is also the case in islets.

Consistent with upregulated cAMP signaling, an increase in the number of sub-membrane insulin granules was observed in glucocorticoid-treated islets. cAMP has been shown to recruit non-docked insulin granules to the membrane, as well as increase the size of the readily-releasable granule pool *via* Epac2 and PKA (40; 41), and this may account for the intact secretory responses to glucose and KCl. The exact mechanisms by which 11-DHC and corticosterone boost cAMP signaling are unknown, but likely involve specific adenylate cyclases, since *Adcy1* gene expression was increased in 11-DHC- and corticosterone-treated islets compared to controls. Moreover, palmitate, which downregulates *Adcy9* and impairs cAMP responses to glucose (29), prevented 11-DHC from increasing cAMP levels. While *Adcy9* mRNA expression was not significantly affected by glucocorticoid, other mechanisms can account for cAMP generation, including organization of the enzyme into microdomains (42). Pertinently, *Adcy1* and *Adcy9* knockdown have been shown to reduce glucose-

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stimulated cAMP rises and insulin secretion in beta cells (29; 43). Further studies are thus warranted in glucocorticoid-treated *Adcy1*- and *Adcy9*-null islets. Upregulated cAMP signaling may represent a protective mechanism that is disrupted by free fatty acids to induce beta cell failure/decompensation in the face of excess glucocorticoid. Of note, endogenous elevation of glucocorticoids leads to dyslipidemia due to lipolysis, *de novo* fatty acid production/turnover and hepatic fat accumulation (44).

It has previously been shown in mouse islets that cAMP responses to glucose are oscillatory (29), albeit noisier than those in MIN6/INS-1E cells (45). However, the latter studies used TIRF microscopy to study sub-membraned cAMP responses, whose changes may be larger and more dynamic than those recorded throughout the cytosol (46). Similar studies using epifluorescence techniques show non-oscillatory cAMP increases in response to high glucose concentrations (47). Thus, further studies are required to investigate the impact of glucocorticoids on cAMP oscillations, which were not detectable at the axial resolutions employed here. While ATP/ADP responses were oscillatory in single islets, a transient dip was present following introduction of high glucose. This has also been seen in previous studies (19) and may reflect net ATP consumption secondary to Ca^{2+} transporter activity (48), glucokinase activity (49) and the initial steps of exocytosis (50), or an uncoupling effect of highly elevated Ca^{2+} levels on mitochondrial function (21). While similar results were seen using luciferase-based ATP measures, a change in intracellular pH and Perceval intensity cannot be excluded.

In summary, we have identified a novel mechanism by which glucocorticoids maintain beta cell function in rodent and human beta cells through engagement of parallel cAMP pathways. Failure of this protective feedback loop may contribute to impaired insulin release during states of glucocorticoid excess (e.g. Cushing's syndrome).

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AUTHOR CONTRIBUTIONS

N.H.F.F. conceived and devised the study, performed the experiments and analysed data. C.L.D., Y.S.E., N.V. and D.A.J. performed experiments and analyzed data. G.A.R. provided reagents. G.G.L. provided reagents and analyzed data. M.P., M.B., L.P. and R.N. isolated and provided human islets. D.J.H. supervised the research, conceived and devised the study, performed analysis and wrote the manuscript with input from all authors. D.J.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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FIG.S AND LEGENDS

Figure 1 - Glucocorticoids suppress cytosolic Ca^{2+} fluxes in response to glucose and glucose + KCl. *A*: Mean ± S.E.M intensity-over-time traces showing glucose- and glucose + KCl-stimulated Ca^{2+} rises in mouse islets treated for 48 hrs with 11-DHC or corticosterone (*n* = 14-28 islets from 6 animals). *B*: Representative max intensity projection images showing impaired Ca^{2+} signaling in glucose-stimulated control (Con)-, 11-DHC 200 nM-, and corticosterone (Cort)-treated islets (scale bar, 20 μm) (images cropped to show a single islet). *C*: Summary bar graph showing a significant reduction in the amplitude of glucose-stimulated Ca^{2+} rises following treatment with either glucocorticoid (*n* = 14-28 islets from 6 animals). *D*: As for *C*, but area-under-the-curve (AUC). *E*: As for *C* but glucose + KCl. *F*: Corticosterone and 11-DHC significantly decrease Ca^{2+} spiking frequency at high glucose (representative traces shown) (*n* = 14 islets from 3 animals). G: As for *F*, but summary bar graph showing Ca^{2+} oscillations per min. *H.* Cortisone and cortisol blunt glucose- and glucose + KCl-stimulated Ca^{2+} rises in human islets (representative traces shown) (*n* = 15-18 islets from 3 donors, 48 hrs). *I* and *J*: As for *H*, but summary bar graphs showing amplitude of Ca^{2+} responses to glucose (*I*) and glucose + KCl (*J*). *K*: 11-DHC and corticosterone do not affect Ca^{2+} responses to the incretin-mimetic, exendin 4 (Ex4) 10 nM (representative traces shown) (*n* = 14-17 islets from 3 animals). *L* and *M*: As for *K*, but summary bar graphs showing oscillation frequency (*L*) and AUC (*M*). G3 = 3 mM glucose; G11 = 11 mM glucose; G17 = 17 mM glucose. KCl was applied at 10 mM. *P<0.05, **P<0.01 and NS, non-significant; one-way ANOVA (Bonferroni's post hoc test). Corticosterone was applied at 20 nM for 48 hr. Traces in *F*, *H* and *K* share the same F/F_{min} scale but are offset in the y-axis. Unless otherwise stated, data represent the mean ± S.D.

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Figure 2 – Glucocorticoids impair VDCC function despite preserved beta cell identity and metabolism. *A-F*: Expression of mRNA for the beta cell maturity markers *Pdx-1* (*A-C*) and *Nkx6.1* (*D-F*) are similar in control and 11-DHC/corticosterone-treated islets (*n* = 4-7 animals, 48 hrs). *G*: Mean ± S.E.M traces showing no effect of glucocorticoids on maximal ATP/ADP responses to glucose, measured using the biosensor Perceval. *H:* As for G, but summary bar graph showing the amplitude of ATP/ADP rises (*n* = 7 islets from 4 animals). *I:* 11-DHC and corticosterone reduce VDCC conductance, as shown by the voltage-current (I-V) relationship (*n* = 4 animals) (* 11-DHC *versus* control; # corticosterone *versus* control). *J:* As for *I*, but representative Ca^{2+} current traces. *K-P*: Expression levels of the VDCC α/β subunits Cacna1c (*K* and *L*), Cacnb2 (*M* and *N*) and Cacna1d (*O* and *P*) are not significantly altered by 11-DHC or corticosterone (*n* = 4-6 animals, 48 hrs). G3 = 3 mM glucose; G17 = 17 mM glucose. $^{\#}$/*P<0.05, $^{\#\#}$/**P<0.01 and NS, non-significant; Student's t-test, Student's paired t-test or one-way ANOVA (Bonferroni's post hoc test). Corticosterone was applied at 20 nM for 48 hr. Unless otherwise stated, data represent the mean ± S.D.

Figure 3 - Insulin secretion from islets is maintained in the face of excess glucocorticoid. *A*: Basal, glucose-stimulated and glucose + KCl-stimulated insulin secretion is unaffected following 48 hr treatment of mouse islets with either 11-DHC or corticosterone (*n* = 5 animals). *B-D*: QRT-PCR analyses of *Ins1* mRNA expression shows no significant changes in response to 11-DHC 20 nM (B), 11-DHC 200 nM (C) or corticosterone (D) exposure (*n* = 4-7 animals). *E*: Total insulin content is unaffected by exposure to 11-DHC or corticosterone (*n* = 3 animals). *F*: Basal, glucose-stimulated and glucose + KCl-stimulated insulin secretion is unaffected following 48 hr treatment of human islets with either cortisone 200 nM or cortisol 20 nM (*n* = 3 donors). *G*: As for *F*, but stimulation index to better account for differences in basal secretion between islet batches from the different isolation centres.

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*P<0.05, **P<0.01 and NS, non-significant; Student's t-test, one-way ANOVA (Bonferroni's post hoc test) or two-way ANOVA. G3 = 3 mM glucose; G17 = 17 mM glucose. Corticosterone was applied at 20 nM for 48 hr. KCl was applied at 10 mM. Unless otherwise stated, data represent the mean ± S.D or range.

Figure 4 - Glucocorticoids potentiate cAMP signaling. *A*: Both 11-DHC and corticosterone (Cort) amplify glucose-stimulated cAMP generation, as measured online using the biosensor Epac2-camps (FSK, forskolin; positive control) (mean ± S.E.M traces shown) (*n* = 20-24 islets from 5 animals). *B*: Summary bar graph showing significant effects of either glucocorticoid on the AUC of cAMP responses to glucose. *C*: Representative images of FRET responses in control-, 11-DHC- and corticosterone (Cort)-treated beta cells expressing Epac2-camps (scale bar, 10 μm). *D*: Inhibition of PKA decreases glucose-stimulated insulin in the presence of 11-DHC but not control (*n* = 3 animals) (mean and range shown). *E*: 11-DHC and corticosterone (Cort) increase the fraction of the cell membrane occupied by insulin granules (V/v). *F*: Representative Structured Illumination Microscopy (SIM) images showing insulin granules in control-, 11-DHC- and corticosterone (Cort)-treated islets (*n* = 8 cells from 3 animals) (scale bar = 5 μm) (lower panel shows zoom-in). *G:* Cortisone and cortisol augment glucose-stimulated cAMP generation in human islets (mean ± S.E.M traces shown). *H:* Summary bar graph showing significant effects of cortisone and cortisol on the AUC of cAMP responses to glucose (*n* = 10-11 islets from 3 donors). *I:* Glucocorticoid does not affect cAMP responses to exendin-4 (Ex4) 10 nM (*n* = 24-46 islets from 4 animals). *J:* As for *I*, but summary bar graph showing AUC of cAMP responses. *K* and *L:* Relative (fold-change) expression levels of *Adcy1*, *5*, *6*, *8* and *9* in 11-DHC (*K*) and corticosterone (*L*)-treated islets (*n* = 4-5 animals). *M:* Palmitate but not BSA control prevents 11-DHC from augmenting cAMP responses to glucose (Palm, palmitate) (*n* = 23-27 islets from 4 animals) (traces

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represent mean ± S.E.M). *N.* As for *M*, but summary bar graph showing AUC of cAMP responses. G3 = 3 mM glucose; G11 = 11 mM glucose; G17 = 17 mM glucose. *P<0.05, **P<0.01 and NS, non-significant; Student's t-test or one-way ANOVA (with Bonferroni's or Tukey's post hoc test). 11-DHC and corticosterone were applied for 48 hr at 200 nM and 20 nM, respectively. Unless otherwise stated, data represent the mean ± S.D.

Figure 5 - Deletion of *Hsd11b1* reverses the effects of glucocorticoids on Ca^{2+} signaling. *A*: Mean intensity-over-time traces showing a reduction in glucose- and glucose + KCl-stimulated Ca^{2+} rises in *Hsd11b1*$^{+/-}$ islets treated for 48 hrs with 11-DHC or corticosterone (Cort) (*n* = 15-19 islets from 3 animals). *B* and *C*: As for *A*, but summary bar graphs showing the amplitude of Ca^{2+} responses to glucose (*B*) and glucose + KCl (*C*). *D*: Representative max intensity projection images showing impaired glucose-stimulated Ca^{2+} rises in 11-DHC- and corticosterone- compared to control (Con)-treated *Hsd11b1*$^{+/-}$ islets (scale bar, 20 μm) (images cropped to show a single islet). *E*: Mean ± S.E.M intensity-over-time traces showing intact glucose- and glucose + KCl-stimulated Ca^{2+} rises in *Hsd11b1*$^{-/-}$ islets treated for 48 hrs with 11-DHC or corticosterone (*n* = 19-28 islets from 3 animals). *F* and *G*: As for *E*, but summary bar graphs showing the amplitude of Ca^{2+} responses to glucose (*F*) and glucose + KCl (*G*). *H*: Representative max intensity projection images showing similar glucose-stimulated Ca^{2+} rises in 11-DHC- and corticosterone- compared to control (Con)-treated *Hsd11b1*$^{-/-}$ islets (scale bar, 20 μm) (images cropped to show a single islet). G3 = 3 mM glucose; G17 = 17 mM glucose. *P<0.05, **P<0.01 and NS, non-significant, one-way ANOVA (Bonferroni's post hoc test). 11-DHC and corticosterone were applied for 48 hr at 200 nM and 20 nM, respectively. KCl was applied at 10 mM. Unless otherwise stated, data represent the mean ± S.D.

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Figure 6 - Deletion of *Hsd11b1* reverses the effects of 11-DHC on cAMP signaling. *A*: Mean ± S.E.M intensity-over-time traces showing cAMP responses to glucose in 11-DHC- and corticosterone (Cort)-treated *Hsd11b1*$^{+/-}$ islets (FSK, forskolin; positive control) (*n* = 15-19 islets from 3 animals). *B* and *C*: As for *A*, but summary bar graphs showing the amplitude (*B*) and AUC (*C*) of cAMP responses. *D*: Representative images of cAMP responses to glucose in control (Con)-, 11-DHC- or corticosterone-treated *Hsd11b1*$^{+/-}$ islets expressing Epac2-camps (scale bar, 10 μm). *E*: Mean ± S.E.M intensity-over-time traces showing that cAMP responses to glucose are potentiated by 11-DHC, but not corticosterone, in *Hsd11b1*$^{-/-}$ islets (*n* = 22-23 islets from 3 animals). *F* and *G*: As for *E*, but summary bar graphs showing the amplitude (*F*) and AUC (*G*) of cAMP responses. *H*: Representative images of cAMP responses to glucose in control (Con)-, 11-DHC- and corticosterone-treated *Hsd11b1*$^{-/-}$ islets expressing Epac2-camps (scale bar, 10 μm). *I*: Insulin secretion in response to glucose is significantly improved in corticosterone- compared to control and 11-DHC-treated *Hsd11b1*$^{-/-}$ islets (*n* = 4 animals). *J* and *K*: Relative (fold-change) expression levels of *Adcy1*, *5*, *6*, *8* and *9* in 11-DHC (*J*) and corticosterone (*K*)-treated *Hsd11b1*$^{-/-}$ islets (*n* = 5 animals). G3 = 3 mM glucose; G17 = 17 mM glucose. *P<0.05, **P<0.01 and NS, non-significant, Student's t-test or one-way ANOVA (Bonferroni's post hoc test). 11-DHC and corticosterone were applied for 48 hr at 200 nM and 20 nM, respectively. KCl was applied at 10 mM. Unless otherwise stated, data represent the mean ± S.D.

Figure 7 - 11-DHC effects are mediated through the glucocorticoid receptor. *A*: The glucocorticoid receptor antagonist RU486 prevents the suppressive effects of 11-DHC on glucose- and glucose + KCl-stimulated Ca^{2+} signals (mean ± S.E.M traces shown) (*n* = 12-13 islets from 4 animals). *B* and *C*: As for, *A* but summary bar graphs showing that 11-DHC does not affect Ca^{2+} responses to glucose (*B*) or glucose + KCl (*C*) in RU486-treated islets.

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D: Representative max intensity projection images showing impaired Ca^{2+} rises in 11-DHC-treated islets, which can be reversed using the glucocorticoid receptor antagonist RU486 (scale bar, 20 μm) (images cropped to show a single islet). *E*: RU486 blocks the effects of corticosterone (Cort) on Ca^{2+} responses to glucose (*n* = 14-17 islets from 6 animals). *F:* As for *E*, but RU486 is unable to significantly affect Ca^{2+} responses to glucose + KCl in corticosterone-treated islets (*n* = 14-17 islets from 6 animals). *P<0.05, **P<0.01 and NS, non-significant, one-way ANOVA (Bonferroni's post hoc test). 11-DHC and corticosterone were applied for 48 hr at 200 nM and 20 nM, respectively. KCl was applied at 10 mM. Unless otherwise stated, data represent the mean ± S.D.

Figure 8 - Glucocorticoids impair K_{ATP}-independent signals to reduce ionic fluxes in glucose-stimulated beta cells. This is further exacerbated by Hsd11b1, which increases availability of more active glucocorticoid (11-DHC/cortisone -> corticosterone/cortisol) in a paracrine manner. However, insulin secretion is preserved, since glucocorticoids are able to reprogram the beta cell signaling cassette towards a cAMP phenotype, most likely through upregulation of specific Adcy isoforms.

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Figure 1

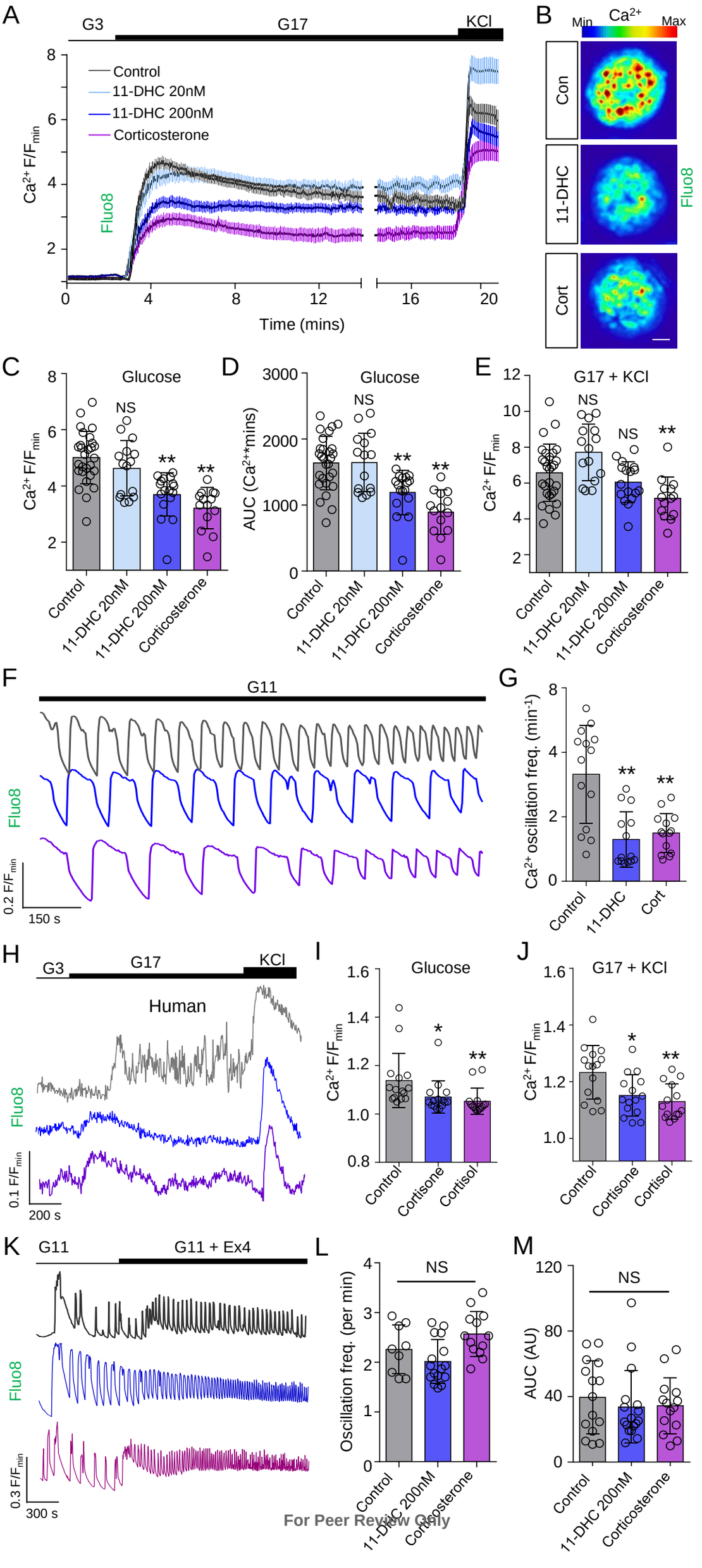

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Figure 2

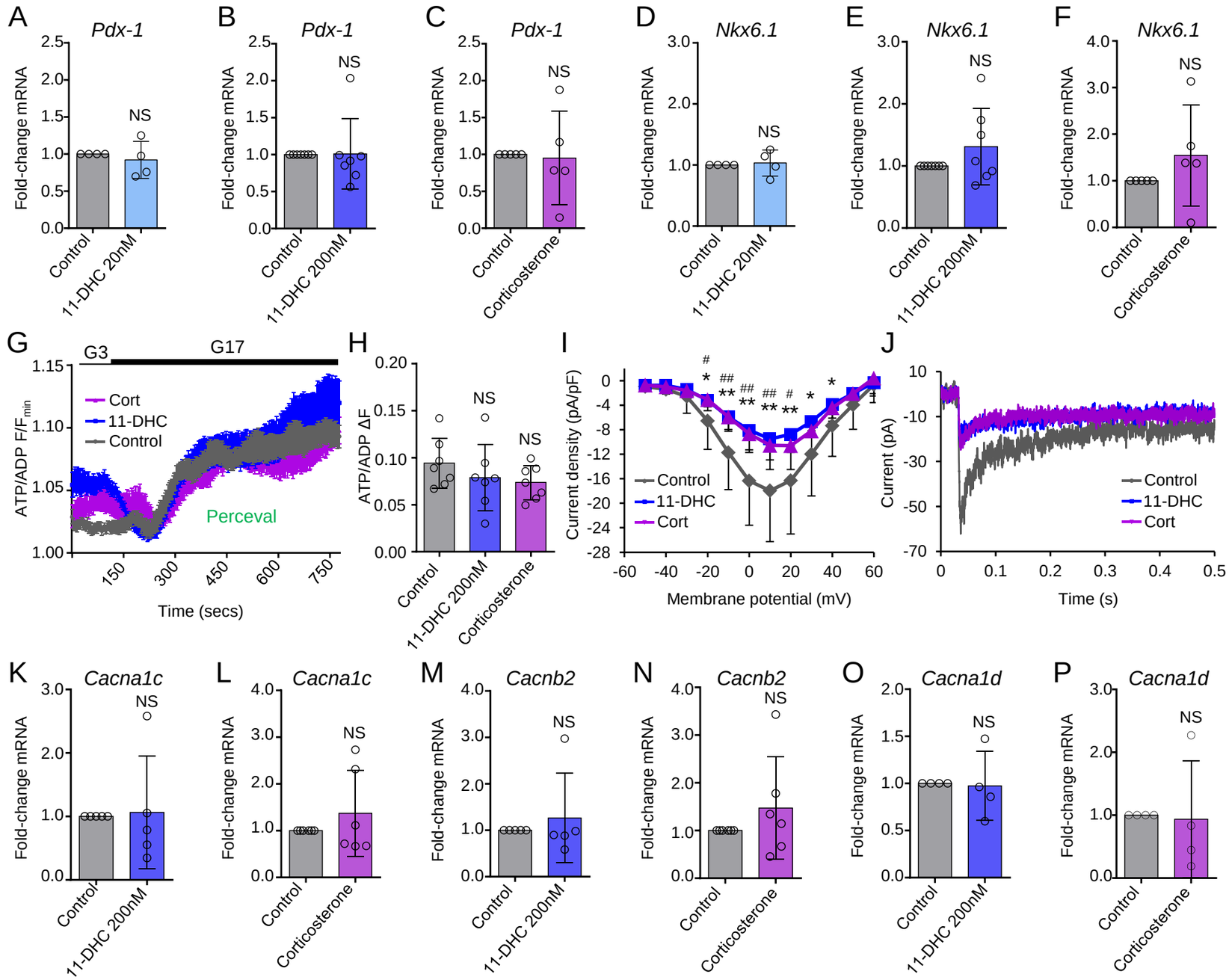
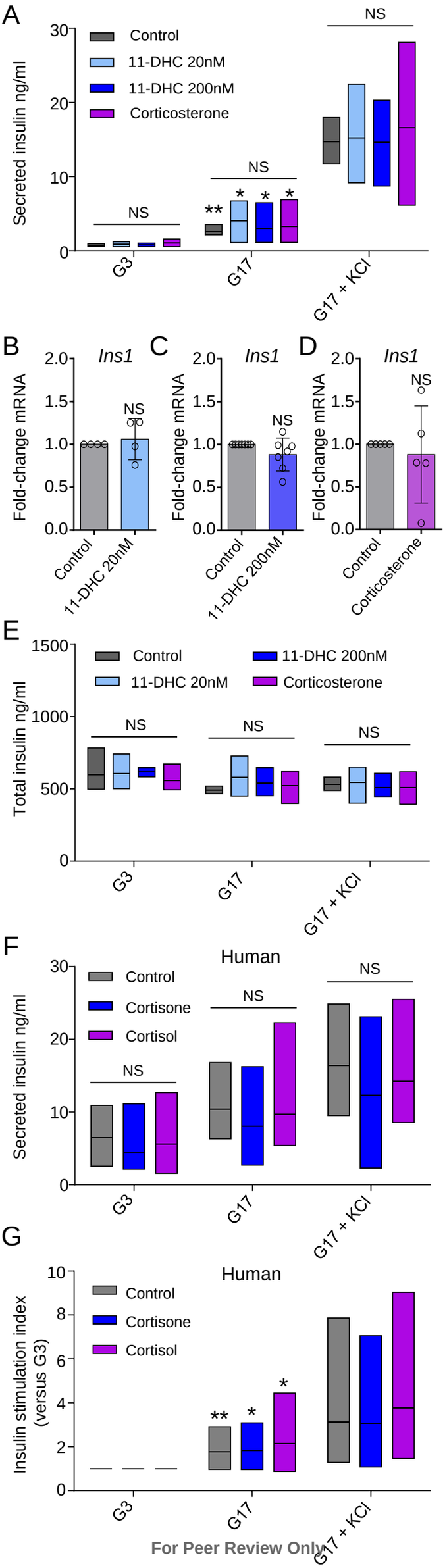
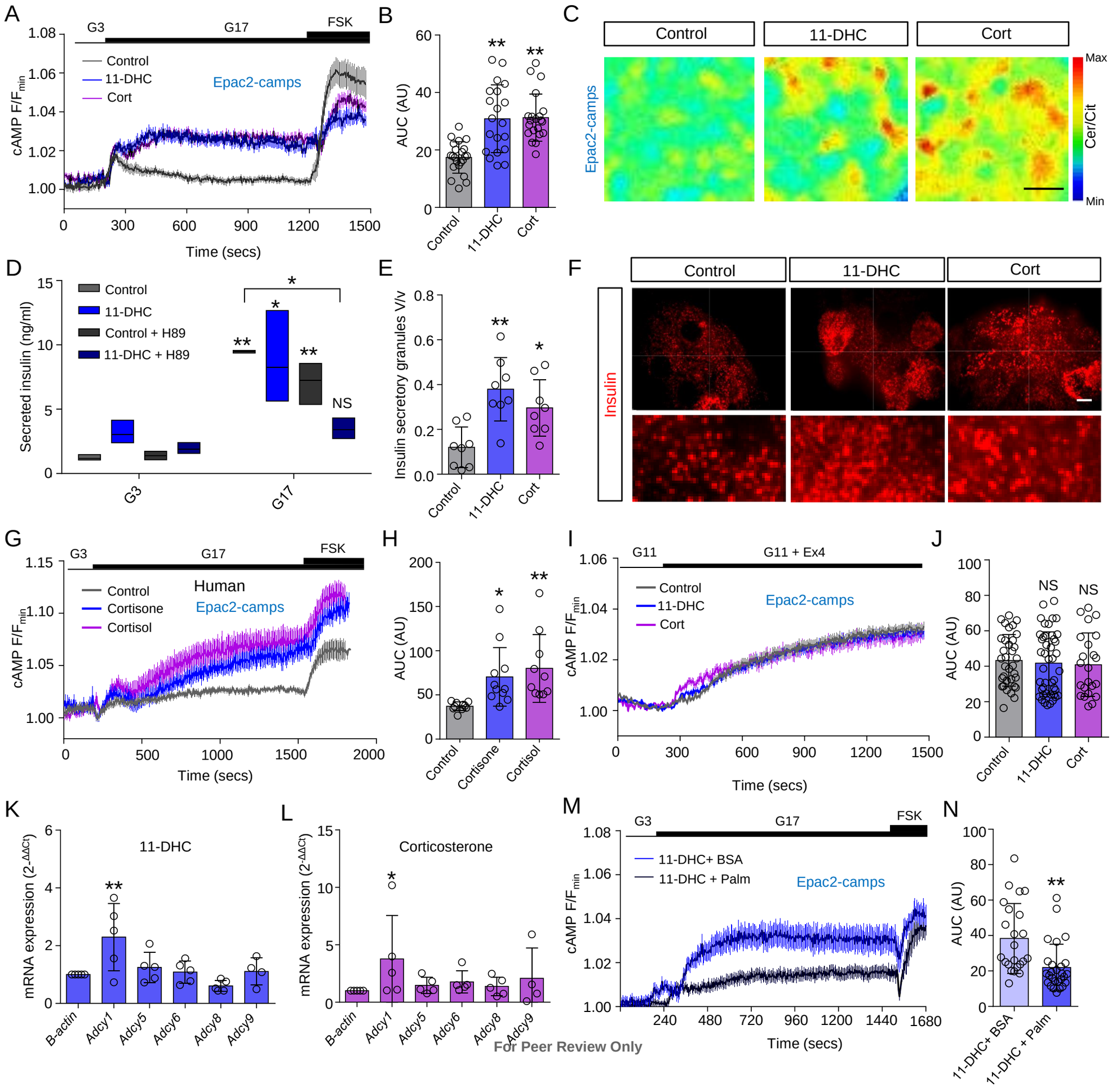

A
Pdx-1
Fold-change mRNA
NS
Control
11-DHC 20nM
B
Pdx-1
Fold-change mRNA
NS
Control
11-DHC 200nM
C
Pdx-1
Fold-change mRNA
NS
Control
Corticosterone
D
Nkx6.1
Fold-change mRNA
NS
Control
11-DHC 20nM
E
Nkx6.1
Fold-change mRNA
NS
Control
11-DHC 200nM
F
Nkx6.1
Fold-change mRNA
NS
Control
Corticosterone
G
G3
G17
Cort
11-DHC
Control
Perceval
ATP/ADP F/Fmin
Time (secs)
H
ATP/ADP ΔF
NS
NS
Control
11-DHC 200nM
Corticosterone
I
Current density (pA/pF)
Control
11-DHC
Cort
Membrane potential (mV)
J
Current (pA)
Control
11-DHC
Cort
Time (s)
K
Cacna1c
Fold-change mRNA
NS
Control
11-DHC 200nM
L
Cacna1c
Fold-change mRNA
NS
Control
Corticosterone
M
Cacnb2
Fold-change mRNA
NS
Control
11-DHC 200nM
N
Cacnb2
Fold-change mRNA
NS
Control
Corticosterone
O
Cacna1d
Fold-change mRNA
NS
Control
11-DHC 200nM
P
Cacna1d
Fold-change mRNA
NS
Control
Corticosterone

Figure 3



For Peer Review Only

Figure 4



For Peer Review Only

Figure 5

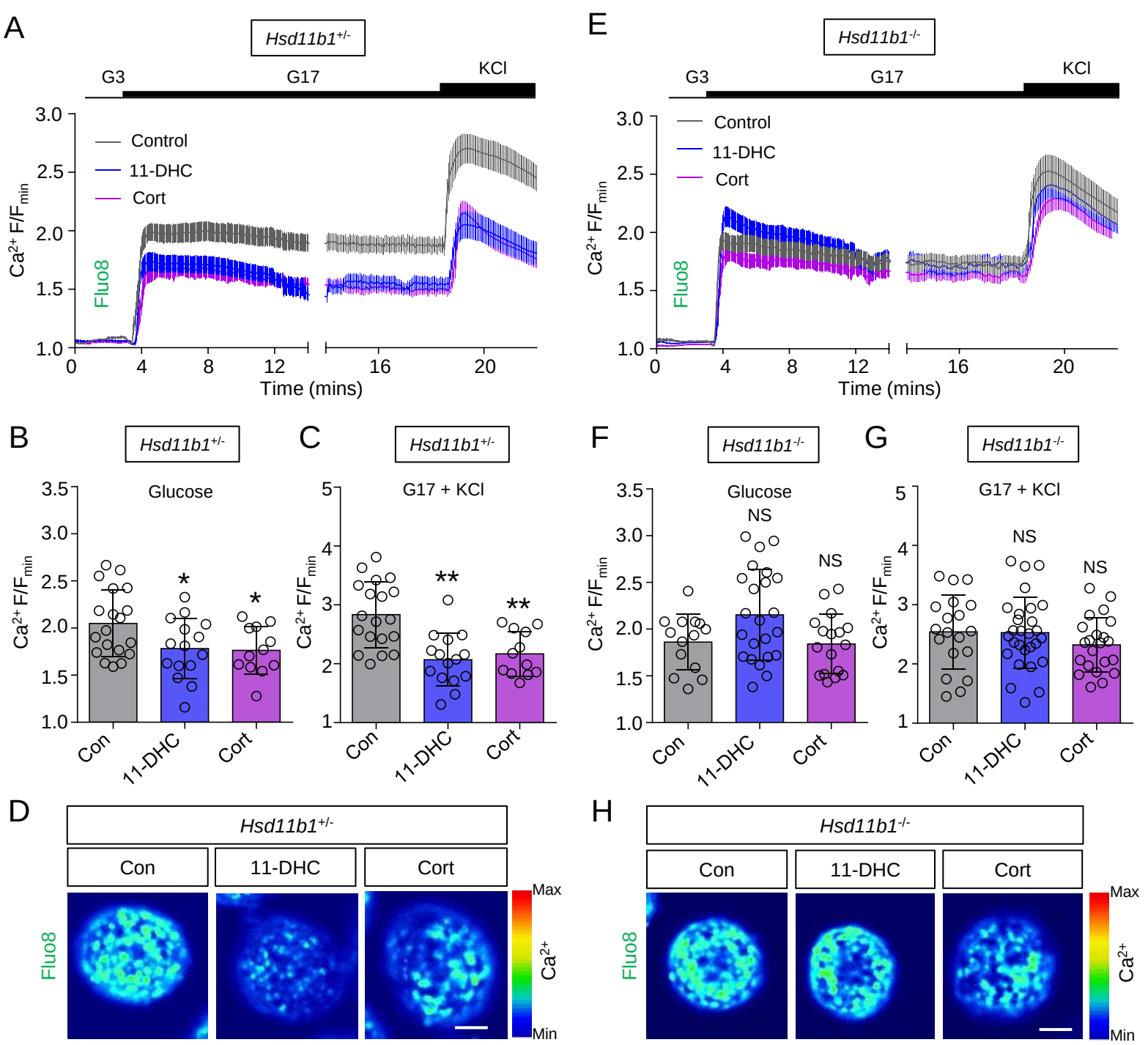

A
Hsd11b1+/-
G3
G17
KCl
Control
11-DHC
Cort
Ca2+ F/Fmin
Fluo8
Time (mins)
E
Hsd11b1-/-
B
C
F
G
Glucose
G17 + KCl
NS
Con
11-DHC
Cort
D
H
Max
Min
Ca2+

Figure 6

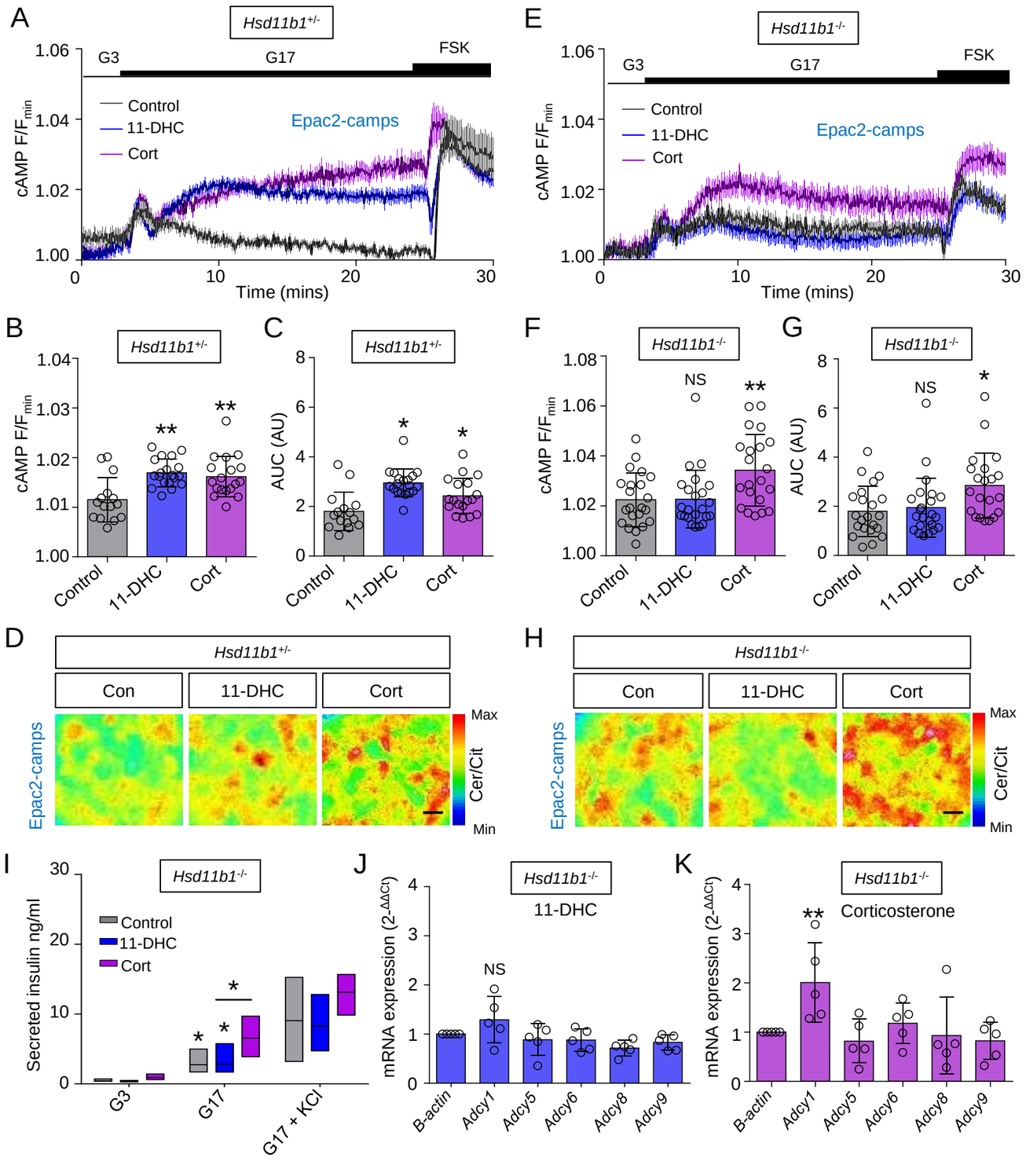
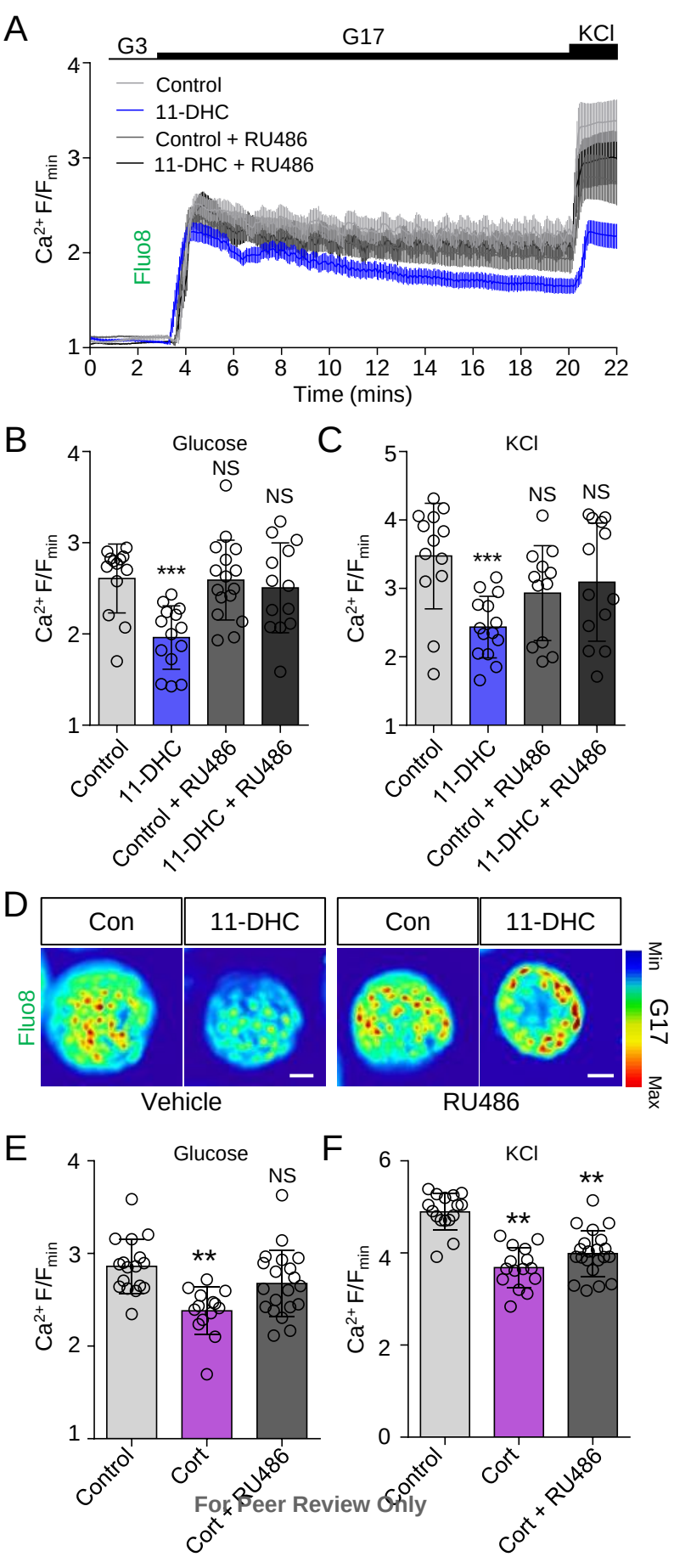
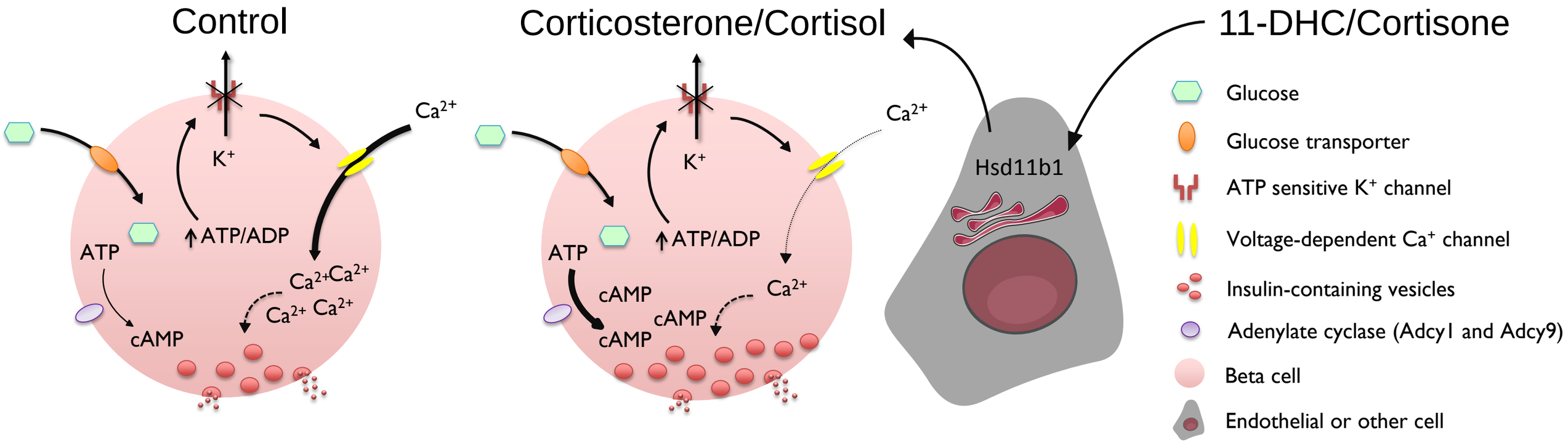

A
Hsd11b1+/-
G3
G17
FSK
Control
11-DHC
Cort
Epac2-camps
cAMP F/Fmin
Time (mins)
E
Hsd11b1-/-
B
C
F
G
AUC (AU)
NS
D
H
Con
Max
Min
Cer/Cit
I
Secreted insulin ng/ml
G3
G17
G17 + KCl
J
11-DHC
mRNA expression (2-ΔΔCt)
B-actin
Adcy1
Adcy5
Adcy6
Adcy8
Adcy9
K
Corticosterone

Figure 7



For Peer Review Only


Control
Corticosterone/Cortisol
11-DHC/Cortisone
K+
ATP/ADP
ATP
cAMP
Ca2+
Ca2+Ca2+
Ca2+ Ca2+
Hsd11b1
Glucose
Glucose transporter
ATP sensitive K+ channel
Voltage-dependent Ca+ channel
Insulin-containing vesicles
Adenylate cyclase (Adcy1 and Adcy9)
Beta cell
Endothelial or other cell

SUPPLEMENTAL INFORMATION

Supplemental Figure 1 - Delta Ca^{2+} rises in response to glucose and KCl at high glucose in glucocorticoid-treated islets.

Supplemental Figure 2 - Glucocorticoids impair Ca^{2+} responses to glucose and KCl at high glucose as measured using Fura2.

Supplemental Figure 3 - Glucocorticoids do not influence the time to onset or amplitude of ATP/ADP responses to glucose.

Supplemental Figure 4 - Glucocorticoids do not affect glucose-stimulated ATP production

Supplemental Figure 5 - *Hsd11b1* and *HSD11B1* mRNA expression in mouse and human tissue.

Supplemental Figure 6 -11-DHC suppresses delta Ca^{2+} rises in *Hsd11b1*$^{+/-}$ but not *Hsd11b1*$^{-/-}$ islets.

Supplemental Figure 7 -11-DHC augments delta cAMP rises in *Hsd11b1*$^{+/-}$ but not *Hsd11b1*$^{-/-}$ islets.

Supplemental Figure 8 - R486 blocks the effects of glucocorticoids on Ca^{2+} rises.

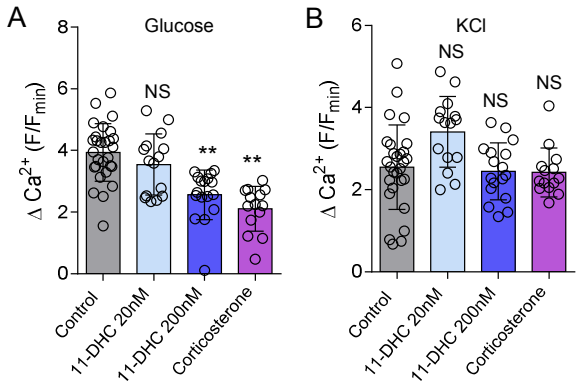
Supplemental Table 1 - Human islet donor characteristics.

Supplemental Table 2 – Epac2-camps single and dual channel fluorescence under maximal stimulation in mouse islets

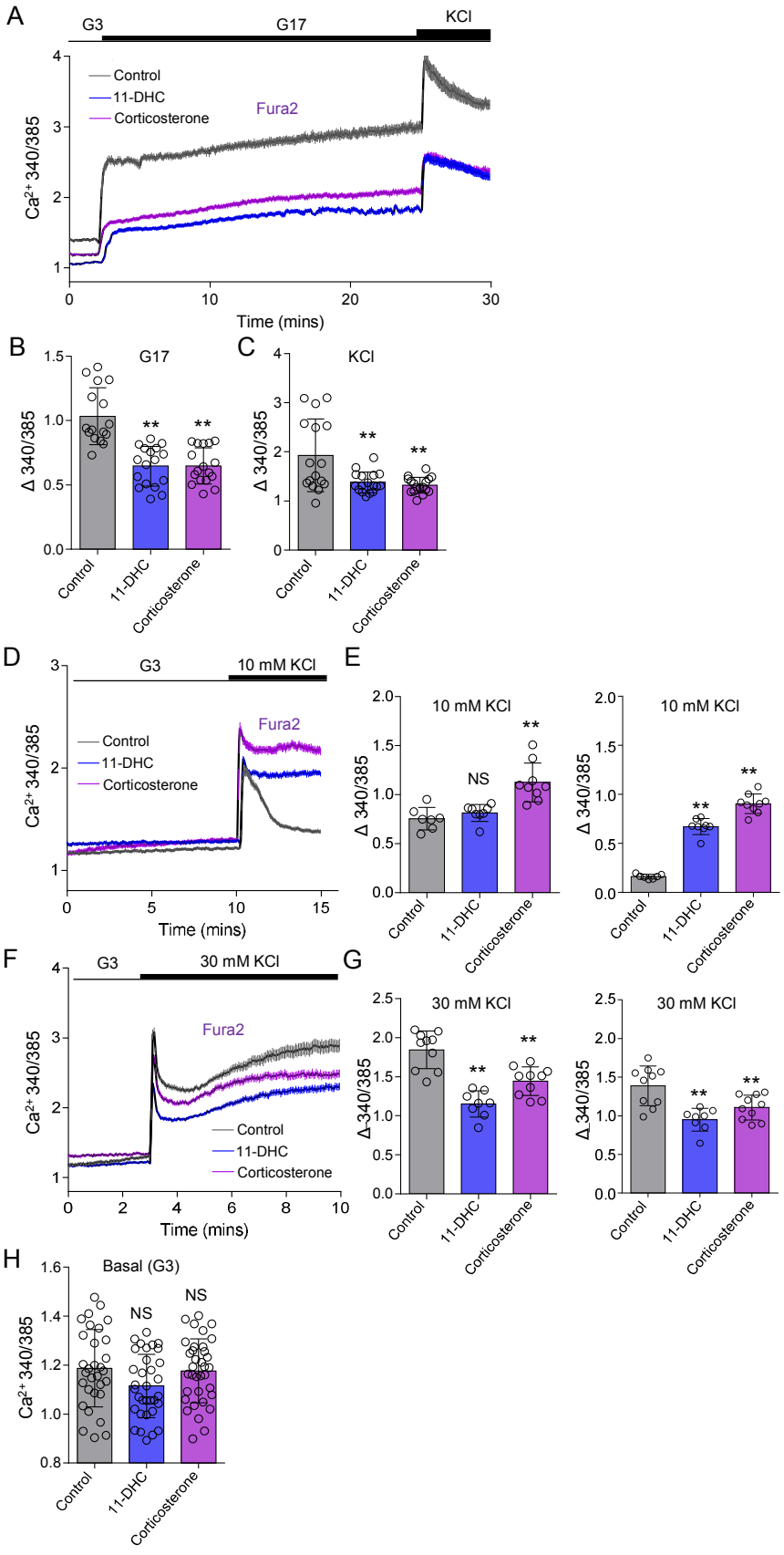
Supplemental Table 3 - Primer sequences.

Supplemental Table 4 – Basal intracellular Ca^{2+} concentration in human islets.

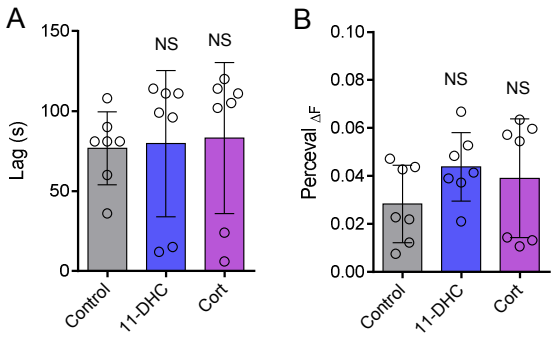
Supplemental Table 5 – Effect of KCl concentration on amplitude Ca^{2+} responses at 3 mM glucose.



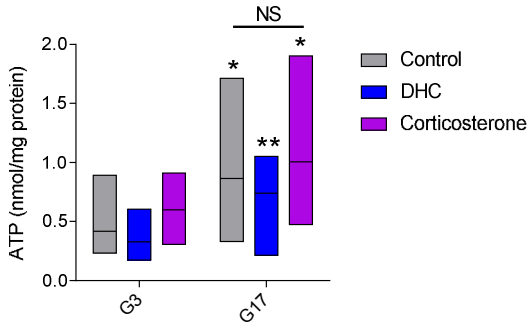
Supplemental Figure 1 - Delta Ca^{2+} rises in response to glucose and KCl at high glucose in glucocorticoid-treated islets. *A*: Gluococorticoids significantly impair the amplitude of Ca^{2+} responses to glucose. *B*: As for *A*, but Ca^{2+} responses to 10 mM KCl (amplitude measured *versus* 17 mM glucose; G17). *P<0.05, **P<0.01 and NS, non-significant; one-way ANOVA (Bonferroni's post hoc test). 11-DHC and corticosterone were applied at 200 nM or 20 nM, respectively. Data represent the mean ± S.D. N numbers as for Figure 1.


A
G3
G17
KCl
Control
11-DHC
Corticosterone
Fura2
Ca2+ 340/385
Time (mins)
B
G17
Δ 340/385
C
KCl
Δ 340/385
Control
11-DHC
Corticosterone
D
G3
10 mM KCl
Fura2
Control
11-DHC
Corticosterone
Ca2+ 340/385
Time (mins)
E
10 mM KCl
Δ 340/385
NS
F
G3
30 mM KCl
Fura2
Control
11-DHC
Corticosterone
Ca2+ 340/385
Time (mins)
G
30 mM KCl
Δ-340/385
H
Basal (G3)
NS
Ca2+ 340/385

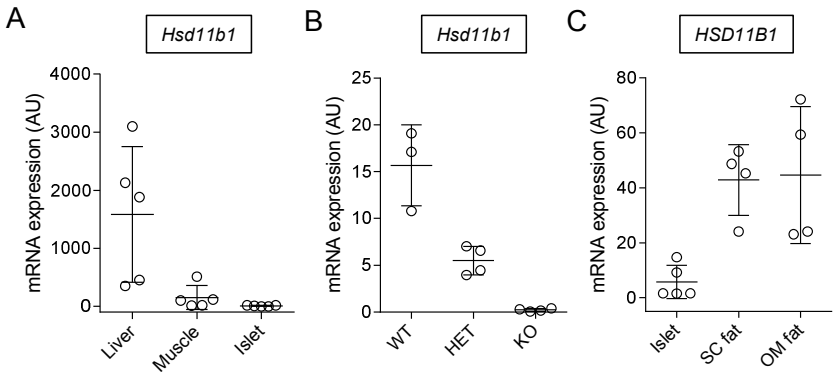
Supplemental Figure 2 - Glucocorticoids impair Ca^{2+} responses to glucose and KCl at high glucose as measured using Fura2. *A:* Ratiometric Fura2 recordings showing glucose- and glucose + 10 mM KCl-stimulated Ca^{2+} rises in mouse islets treated for 48 hrs with 11-DHC or corticosterone (mean ± S.E.M intensity-over-time traces shown) (*n* = 16-17 islets from 4 animals). *B*-*C*: Summary bar graphs showing a significant reduction in the amplitude of glucose- (*B*) and 10 mM KCl- (*C*) stimulated Ca^{2+} rises following treatment with either glucocorticoid (KCl amplitude measured *versus* 17 mM glucose; G17). *D*: Peak Ca^{2+} responses to 10 mM KCl at low (3 mM) glucose are not affected or significantly increased by 11-DHC or corticosterone exposure, respectively. Sustained Ca^{2+} responses to 10 mM KCl at low (3 mM) glucose are significantly increased by both glucocorticoids. *E:* As for *D*, but summary bar graph (peak Ca^{2+} responses, left panel; sustained Ca^{2+} responses, right panel) (*n* = 7-9 islets from 2 animals). *F*: Peak and sustained Ca^{2+} responses to 30 mM KCl at low (3 mM) glucose are significantly reduced by treatment with 11-DHC or corticosterone (*n* = 31-35 islets from 9 animals). *G:* As for *F*, but summary bar graph (peak Ca^{2+} responses, left panel; sustained Ca^{2+} responses, right panel) (*n* = 31-35 islets from 9 animals). *H:* Glucocorticoid does not significantly alter the Fura2 340/385 ratio (*n* = 8-10 islets from 3 animals). G3 = 3 mM glucose; G17 = 17 mM glucose. **P<0.01 and NS, non-significant; one-way ANOVA (Bonferroni's posthoc test). 11-DHC and corticosterone were applied for 48 hrs at 200 nM or 20 nM, respectively. Unless otherwise stated, data represent the mean ± S.D.



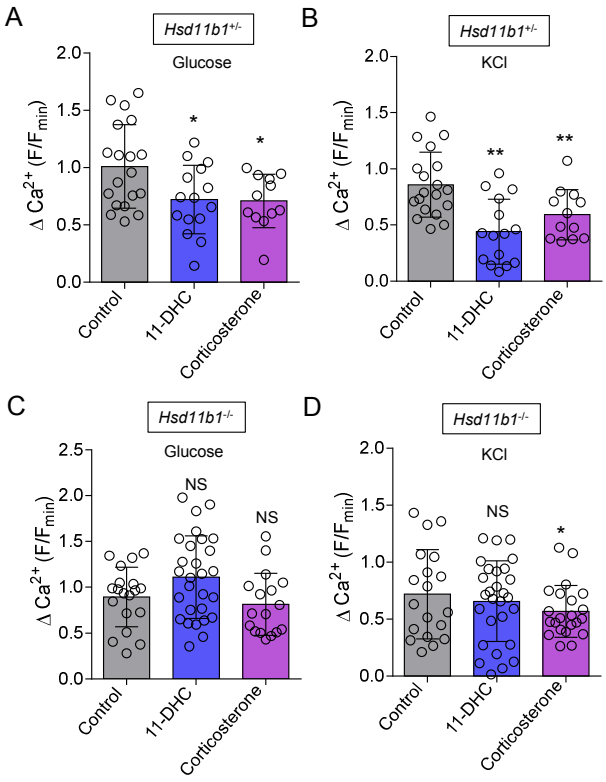
Supplemental Figure 3 - Glucocorticoids do not influence the time to onset or amplitude of ATP/ADP responses to glucose. *A:* Bar graph showing no effect of 11-DHC or corticosterone (Cort) on the time to the initial decrease in ATP/ADP. *B*: As for, A but amplitude of the decrease. 11-DHC and corticosterone were applied for 48 hrs at 200 nM or 20 nM, respectively. NS, non-significant; one-way ANOVA (Bonferroni's posthoc test). Data represent the mean ± S.D.



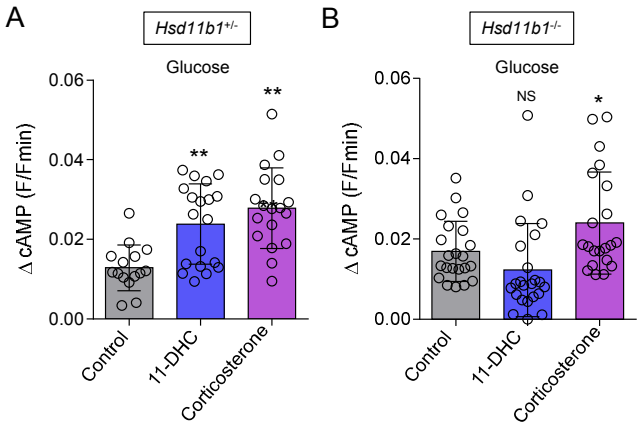
Supplemental Figure 4 - Glucocorticoids do not affect glucose-stimulated ATP production. High (17 mM) glucose concentration significantly increases ATP levels under all conditions examined. No differences were detected between control-, 11-DHC- and corticosterone-treated islets (n = 12 animals). 11-DHC and corticosterone were applied for 48 hrs at 200 nM or 20 nM, respectively. *P<0.05, **P<0.01; Student's t-test. NS, non-significant; one-way ANOVA. Data represent the mean and range.



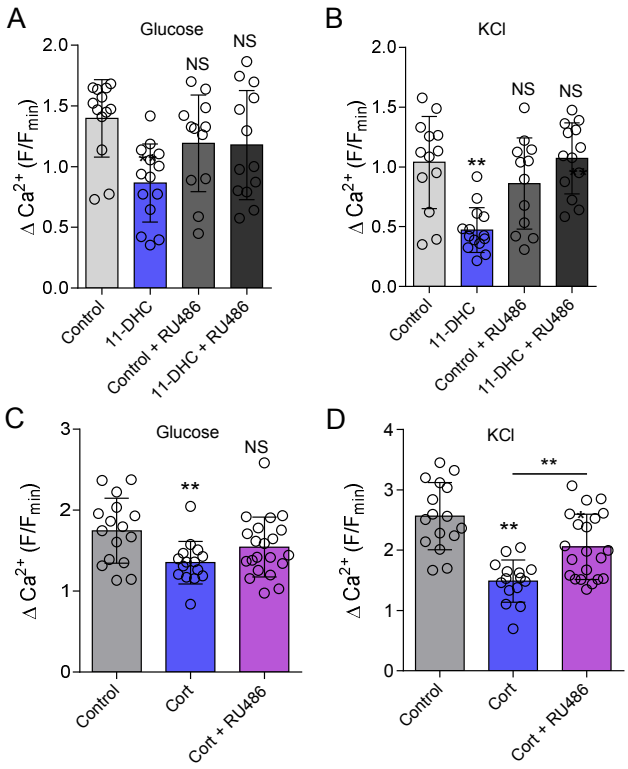
Supplemental Figure 5 - *Hsd11b1* and *HSD11B1* mRNA expression in mouse and human tissue. *A*: Relative *Hsd11b1* gene expression in muscle, liver and islets in mice (n = 5 animals). *B*: *Hsd11b1* is expressed in islets from *Hsd11b1*$^{+/+}$ and *Hsd11b1*$^{+/-}$ mice, but not *Hsd11b1*$^{-/-}$ animals (n = 3-4 animals). *C*: *HSD11B1* levels in human islets are only an order of magnitude lower than in subcutaneous (SC) and omental (OM) fat (n = 4-5 donors). Data represent the mean ± S.D.



Supplemental Figure 6 -11-DHC suppresses delta Ca^{2+} rises in *Hsd11b1*$^{+/-}$ but not *Hsd11b1*$^{-/-}$ islets. *A*: Both 11-DHC and corticosterone significantly impair the amplitude of Ca^{2+} responses to glucose in *Hsd11b1*$^{+/-}$ islets. *B*: As for *A*, but 10 mM KCl (amplitude measured *versus* 17 mM glucose; G17). *C*: Deletion of *Hsd11b1* (*Hsd11b1*$^{-/-}$) restores Ca^{2+} responses to glucose. *D*: As for *A*, but 10 mM KCl (amplitude measured *versus* 17 mM glucose; G17). Data represent the mean ± S.D. *P<0.05, **P<0.01 and NS, non-significant; one-way ANOVA (Bonferroni's post hoc test). 11-DHC and corticosterone were applied for 48 hrs at 200 nM or 20 nM, respectively. Data represent the mean ± S.D. N numbers as for Figure 5.



Supplemental Figure 7 - 11-DHC augments delta cAMP rises in *Hsd11b1*$^{+/-}$ but not *Hsd11b1*$^{-/-}$ islets. *A*: Both 11-DHC and corticosterone potentiate cAMP responses to glucose in *Hsd11b1*$^{+/-}$ islets. *B*: Only corticosterone potentiates cAMP responses to glucose in *Hsd11b*$^{-/-}$ islets. *P<0.05, **P<0.01 and NS, non-significant; one-way ANOVA (Bonferroni's post hoc test). 11-DHC and corticosterone were applied for 48 hrs at 200 nM or 20 nM, respectively. Data represent the mean ± S.D. N numbers as for Figure 6.



Supplemental Figure 8 - R486 blocks the effects of glucocorticoids on Ca^{2+} rises. *A*: RU486 prevents 11-DHC from impairing Ca^{2+} responses to glucose. *B*: As for *A*, but 10 mM KCl (amplitude measured *versus* 17 mM glucose; G17). *C*: RU486 prevents corticosterone (Cort) from impairing Ca^{2+} responses to glucose. *D*: As for *C*, but 10 mM KCl (amplitude measured *versus* G17). *P<0.05, **P<0.01 and NS, non-significant; one-way ANOVA (Bonferroni's post hoc test). 11-DHC and corticosterone were applied for 48 hrs at 200 nM or 20 nM, respectively. Data represent the mean ± S.D. N numbers as for Figure 7.

Age	Gender	BMI	Source
55	F	26	Milan
49	F	23.9	Milan
73	F	28.4	Alberta
71	F	35.5	Alberta
54	M	26.5	Milan
57	F	26	Milan
64	M	24.5	Pisa
44	M	34.4	Alberta

Supplemental Table 1 - Human islet donor characteristics.

Treatment	YFP intensity ± SD (AU)	CFP/YFP ± SD
Control	$2.3 \times 10^4 \pm 6.7 \times 10^3$	1.08 ± 0.03
11-DHC	$2.4 \times 10^4 \pm 8.8 \times 10^3$ NS	1.08 ± 0.05 NS
Corticosterone	$2.7 \times 10^4 \pm 5.0 \times 10^3$ NS	1.07 ± 0.04 NS

Supplemental Table 2 – Epac2-camps single and dual channel fluorescence in mouse islets during maximal stimulation with forskolin. NS, non-significant *versus* control, one-way ANOVA (Bonferroni's post hoc test).

Gene	Forward	Reverse
Ins1	GCTGGTGGGCATCCAGTAA	AATGACCTGCTTGCTGATGGT
Pdx-1	CCAAAGCTCACGCGTGGA	TGTTTTCCTCGGGTTCCG
Nkx6.1	GCCTGTACCCCCCATCAAG	GTGGGTCTGGTGTGTTTTCTCTT
Cacna1d	GAAGCTGCTTGACCAAGTTGT	AACTTCCCCACGGTTACCTC
Cacna1c	CCAACCTCATCCTCTTCTTCA	ACATAGTCTGCATTGCCTAGGAT
Cacnb2	GCAGGAGAGCCAGATGGA	TCCTGGCTCCTTTTCCATAG
Adcy1	CGGAATTGCATGCCTTGAA	TCCATTCTTTTGTGCATGCTACAT
Adcy5	CTTCACCAGCCCCAAGAAAC	GAAGCGGCAGAGCACAGAAC
Adcy6	AGCCTTGGATAGGAAGGGACTACT	CTCCCTGCTTTGGCTTATATACCT
Adcy8	TTGGGCTTCCTACACCTTGACT	CGGTAGCTGTATCCTCCATTGAG
Adcy9	CATACAGAAGGCACCGATAG	CCGAACAGGTCATTGAGTAG
β-actin	CGAGTCGCGTCCACCC	CATCCATGGCGAACTGGTG

Supplemental Table 3 - Primer sequences

Treatment	Ca^{2+} concentration ± SD (nM)
Control	61.1 ± 16.2
Cortisone	60.9 ± 18.2 ^{NS}
Cortisol	52.7 ± 19.8 ^{NS}

Supplemental Table 4 - Basal intracellular Ca^{2+} concentration in human islets. Free Ca^{2+} concentrations were calculated using $K_d*(F-F_{min})/(F_{max}-F)$ where F_{max} and F_{min} represent fluorescence in the presence of 10 μM ionomycin or 0.1% Triton + 5 mM EGTA, respectively, and K_d = 389 nM. NS, non-significant *versus* control, one-way ANOVA (Bonferroni's post hoc test).

Treatment	ΔCa^{2+} ± SD (340/385)
3 mM glucose + 10 mM KCl	0.76 ± 0.12
3 mM glucose + 30 mM KCl	1.85 ± 0.24**

Supplemental Table 5 – Effect of KCl concentration on amplitude Ca^{2+} responses at 3 mM glucose. **P<0.01 *versus* 3 mM glucose + 10 mM KCl, Student's t-test.