Anatomical profiling of G protein-coupled receptor expression

Jean B. Regard, **Isaac T. Sato**, and **Shaun R. Coughlin**
*Cardiovascular Research Institute University of California, San Francisco San Francisco, CA 94158
USA*

Summary

G protein-coupled receptors (GPCRs) comprise the largest family of transmembrane signaling
molecules and regulate a host of physiological and disease processes. To better understand the
functions of GPCRs in vivo, we quantified transcript levels of 353 non-odorant GPCRs in 41 adult
mouse tissues. Cluster analysis placed many GPCRs into anticipated anatomical and functional
groups and predicted novel roles for less studied receptors. From one such prediction, we showed
that the Gpr91 ligand succinate can regulate lipolysis in white adipose tissue suggesting that signaling
by this citric acid cycle intermediate may regulate energy homeostasis. We also showed that pairwise
analysis of GPCR expression across tissues may help predict drug side effects. This resource will
aid studies to understand GPCR function in vivo and may assist in the identification of therapeutic
targets.

Introduction

Mammalian cells sense myriad signals in their environment via G protein-coupled receptors
(GPCRs), the largest family of transmembrane signaling molecules. GPCRs can be partitioned
into two groups: odorant/sensory and non-odorant. Odorant/sensory receptors are restricted to
specialized cells that detect external cues — odors, tastes and pheromones — and regulate
organismal behaviors such as feeding and mating. Non-odorant GPCRs are differentially
expressed throughout the organism, respond to diverse endogenous ligands, and regulate a host
of physiological processes including hematopoiesis, hemostasis, immune function,
metabolism, neurotransmission, reproduction, cardiac function and vascular tone.
Accordingly, such receptors are the targets for about one third of all approved drugs (Hopkins
and Groom, 2002; Muller, 2000). Although in vivo roles have been defined for many of the
approximately 370 non-odorant GPCRs in mice and humans, the expression and function of
many such receptors are incompletely characterized, and a significant fraction remain orphans
(Fredriksson et al., 2003; Fredriksson and Schioth, 2005; Hill et al., 2002; Joost and Methner,
2002; Vassilatis et al., 2003).

To support studies of non-odorant GPCR function, we analyzed the pattern of GPCR mRNA
expression across tissues and the relative abundance for each of 353 non-odorant GPCRs in
41 tissues from adult mouse. Hierarchical clustering analysis revealed groupings of tissues and
receptors that predicted physiological functions for individual receptors and receptor clusters.

We tested one such prediction by examining Gpr91, a receptor for the citric acid cycle intermediate succinate (He et al., 2004). Gpr91 was grouped in the "adipose cluster", but neither Gpr91 nor succinate was known to regulate adipocyte functions. We demonstrated that extracellular succinate can inhibit lipolysis in white adipose tissue in a manner consistent with its acting via adipocyte Gpr91. Overall, this data set provides a resource for those interested in finding new roles for GPCRs with known ligands and hints regarding the functions of orphan GPCRs and the sources of their ligands. When compared with human expression data (SymAtlas, SAGEmap), these mouse data will aid the rational use of mice to model GPCR function in human physiology and disease and may help point up new therapeutic targets and predict on-target side effects. In addition, these quantitative data describing expression of a large number of related and relatively small genes across many tissues may support studies aimed at identifying cis-acting elements and transcription factors that dictate expression in particular tissues.

Results

Tissue profiling of GPCRs by qPCR

GPCRs are usually expressed at low levels. Indeed, non-odorant GPCRs comprise about 1% of genes in the genome, but only 0.001−0.01% of expressed sequence tags (ESTs) correspond to GPCRs (Fredriksson and Schioth, 2005). Accordingly, we chose TaqMan-type quantitative real-time polymerase chain reaction (qPCR) for its high sensitivity, specificity and broad dynamic range to measure GPCR mRNA expression. Primer/probe sets were validated as described in Experimental Procedures; their sequences are provided in Supplement S1.

Transcript levels for 353 GPCRs were profiled in 41 adult tissues isolated from C57BL/6 mice. Bar graphs indicating transcript levels for each receptor in each tissue relative to internal controls (β-actin, cyclophilin, GAPDH and ribosomal protein S9) are found in Supplement S2 and at http://pdsp.med.unc.edu/apGPCRe/.

GPCR expression levels varied dramatically by tissue. Predictably, rhodopsin was the most abundantly expressed GPCR and among the most tissue-specific, present at ~350,000 arbitrary units (a.u.) in eye but below 25 a.u. in other tissues (Fig. 2A). Because rhodopsin presumably serves no function in extraocular tissues, we adopted the convention that receptors with expression values below 25 a.u. in a given tissue were "absent". Using this criterion, only 25 GPCRs were expressed above background in all 41 tissues assayed (Table 1); 90 were expressed in greater than half of tissues and 238 in less than half (Fig. 1A). Some ubiquitously expressed GPCRs were highly expressed in blood vessels (i.e. *Edg1*(*S1p1*), *F2r(Par1)*, *Ednra*, *Ptger1*), perhaps accounting for their presence in all tissues (Table 1). Other ubiquitously expressed receptors, including Gpr56, Lec1(Lphn2), Lec2(Lphn1), Gpr107, Gpr108, Tm7sf1(Gpr137b), Tm7sf1l1(Gpr137), Tm7sf3 and Tpra40(Gpr175), were expressed in 5 of 5 different nonvascular cell lines tested and may indeed be expressed by most cell types in vivo (Table 1 and data not shown).

Save rhodopsin, nearly all GPCRs were expressed at levels below 10,000 a.u. To provide an overview of GPCR distribution, receptors expressed at 25−250, 250−2500, and >2500 a.u. were designated as low, medium and high expressors, respectively, and tissues were grouped by system (e.g. CNS, endocrine, cardiovascular, pulmonary, metabolic, gastrointestinal, immune, reproductive and cutaneous/barrier) (Fig. 1B). By these criteria, 15 or fewer receptors were expressed at high level in any tissue group but CNS.

As expected, receptors that were highly expressed in a given tissue included receptors established to play an important role in that tissue (Table 1). For example, light-detecting opsins were highly expressed in eye, and dopamine, gamma-aminobutyric acid (GABA) and

glutamate receptors were highly expressed in CNS (Table 1 and Fig. 2A). In endocrine tissues, the extracellular calcium-sensing receptor (Casr), which regulates parathyroid hormone secretion (Ho et al., 1995), was highly expressed in parathyroid/thyroid; the growth hormone releasing hormone receptor (Ghrhr), which regulates growth hormone secretion (Lin et al., 1993), was high in pituitary; and the glucagon-like peptide receptor 1 (Glp1r), which regulates insulin secretion (Scrocchi et al., 1996), was high in islets (Table 1,Fig. 2B and Supplement S2). The β3 adrenergic receptor (Adrb3) and the niacin/ketone body receptor Hm74(Gpr109A) (Susulic et al., 1995;Tunaru et al., 2003), which regulate lipolysis, were among the most highly expressed receptors in adipose tissue (Table 1 and Fig. 4B). Glucagon receptor (Gcgr), an important regulator of glucose homeostasis (Gelling et al., 2003), was highly expressed in liver, and the parathyroid hormone receptor (Pthr1), which regulates calcium and phosphate levels and modulates the activity of 25-hydroxyvitamin D 1-hydroxylase (Amizuka et al., 1997), was highly expressed in kidney (Table 1 and Fig. 2C). In heart and blood vessels, angiotensin type 1a receptor (Agtr1), which regulates vascular tone (Ito et al., 1995;Sugaya et al., 1995), thrombin receptor (F2r; Par1) and sphingosine-1-phosphate receptor (Edg1; S1p1), which play important roles in vascular development (Connolly et al., 1996;Liu et al., 2000), and the M2 muscarinic acetylcholine receptor (Chrm2), which regulates heart rate (Gomeza et al., 1999) (Table 1 and Fig. 2D), were all highly expressed. Ccr9, Cxcr4 and Il8rb(Cxcr2), which regulate leukocyte formation and function (Nagasawa et al., 1996;Shuster et al., 1995;Wurbel et al., 2001), were abundant in immune tissues (Table 1). Taken together, these results provided confidence that high-level expression in specific tissues and tissue clusters correlates with physiological function and might predict roles for less well-characterized receptors.

Hierarchical clustering of GPCR expression

GPCR qPCR results were analyzed by unsupervised, hierarchical clustering to further explore potential relationships between receptor expression and tissue function. The resulting dendograms for both the tissue and receptor axes showed functional clusters (Fig. 3A for a thumbnail image and supplemental S3 for a full size form). CNS tissues cerebellum, brainstem, hypothalamus, cerebral cortex, hippocampus, striatum, olfactory bulb, retina and whole eye clustered together as did the immune/hematopoietic tissues spleen, thymus and bone marrow. The steroidogenic organs adrenal gland and ovary clustered, but testes showed a very distinct pattern of GPCR expression. Liver, kidney and gall bladder formed a group, as did large intestine, small intestine, pancreas and stomach. Skin, esophagus and tongue also formed a cluster, perhaps related to their common barrier function. Cardiac atrium and ventricle, skeletal muscle, aorta and urinary bladder formed a cluster, perhaps in part due to their sharing a relative abundance of muscle cells. Brown adipose tissue (BAT), white adipose tissue (WAT), isolated adipocytes and vena cava formed an "adipose" cluster. The presence of vena cava in this cluster likely represents the incomplete removal of surrounding fat from the samples.

A number of receptor axis clusters were easily recognized (Fig. 3). A portion of the "immune/hematopoietic" cluster is shown in Fig. 3B. Included are Ccr9, Cxcr6, Cxcr4, Ccr3, Ccxcr1 (Xcr1), Blr1, Pgr16(Emr4), Gpr33, Ccr6, Gpr65(Tdag8), Cnr2, Edg6(S1p4), Gpr9(Cxcr3), Ccr7, Fksg79(Gpr174), G2a(Gpr132), P2Y10, Pgr27(Gpr114), Ebi2, Gpr18 and H963 (Gpr171). Most of these receptors are known to be expressed in and/or to play a role in immune cells(Birkenbach et al., 1993;Forster et al., 1996;Forster et al., 1999;Humbles et al., 2004;Karsak et al., 2007;Kim et al., 2001;Le et al., 2001;Malone et al., 2004;Nagasawa et al., 1996;Rao et al., 1999;Soto et al., 1998;Stacey et al., 2002;Varona et al., 2001;Wurbel et al., 2001), but no role for Fksg79(Gpr174), Pgr27(Gpr114) and H963(Gpr171) in this context has been described.

The pituitary cluster (Fig. 3C) includes Bdkrb2, Gpr30(Gper), Ghrhr, Gnrhr, Drd3, Mc3r, Sstr5, Gpr2(Ccr10) and Hcrtr1(Ox1r); among these, Gpr2(Ccr10) has not been previously implicated

in pituitary gland function (Brailoiu et al., 2007;Date et al., 2000;Kumar et al., 1997;Lin et al., 1993;Lorsignol et al., 1999;Tsutsumi et al., 1992).

The CNS cluster was by far the largest. Our analysis (Fig. 1B and Fig. 3), as well as others (Vassilatis et al., 2003), suggests that >80% of all non-odorant GPCRs are expressed in CNS. In Fig. 3D we show a small portion of the CNS cluster that includes Gpr101, Hcrtr2(Ox2r), Oprk1, Gpr83, Ntsr1, Gpr45, Htr1a, Htr7, Oprm1 and Npy5r, all of which have been implicated in regulation of neuronal function (Bates et al., 2006;Filliol et al., 2000;Lovenberg et al., 1993;Marsh et al., 1998;Mazella et al., 1996;Popova et al., 2007;Simonin et al., 1995;Willie et al., 2003).

Finally Fig. 2E shows the "eye/retina" cluster which contains Glp2r, Drd4, Grm6(Mglur6), Opn1mw, Opn1sw, Pgr5(Gpr152), Rho, Rrh, Vlgr1(Gpr98), Oa1(Gpr143) and Rgr. Save Glp2r and Pgr5(Gpr152), all are known to function in the eye (Chen et al., 2001;Chiu et al., 1994;Cohen et al., 1992;Dryja et al., 2005;Incerti et al., 2000;McGee et al., 2006;Nathans and Hogness, 1983;Sun et al., 1997a;Sun et al., 1997b).

Taken together, the results outlined above reveal that tissues cluster into largely expected functional groups based purely on their GPCR repertoires, and both expected and unique groups of receptors cluster by tissue function. These data identify sets of receptors involved in specific aspects of physiology and should prove useful in providing clues regarding in vivo roles for orphan GPCRs and new roles for receptors with known ligands. To test the latter prediction, we sought a possible role for a recently de-orphanized receptor, Gpr91, found in the adipose cluster.

Extracellular succinate inhibits lipolysis, likely via Gpr91

Thirteen GPCRs defined an adipose cluster (Fig. 4A and circled area in Fig. 3A): Gpr64(He6), Hm74(Gpr109a), Adrb3, Gpr81, Pgr4(Gpr120), Gpr23(Lpa4), Tshr, Opn3, Oxtr, Sctr, Gpr91 (Suncr1), Pthr1 and Ptger3(Ep3). Ligands for most of these receptors are known to affect adipocyte function (Butcher and Carlson, 1970;Gotoh et al., 2007;Moskowitz and Fain, 1969;Rodbell, 1964;Sinha et al., 1976;Susulic et al., 1995;Tunaru et al., 2003;Valet et al., 1998), but to our knowledge, no role for Gpr64(He6), Gpr81, Opn3 or Gpr91(Suncr1) in adipose tissue has been reported. He et al. demonstrated that the citric acid cycle intermediate succinate can activate Gpr91 and that Gpr91, presumably in kidney, mediates elevation of plasma renin levels and blood pressure in response to exogenous succinate (He et al., 2004). Like *Adrb3* and *Hm74(Gpr109a)*, *Gpr91(Suncr1)* mRNA was most highly expressed in white adipose tissue (WAT) and was abundant in purified adipocytes (Fig. 4B). Gpr91 is at least partially G_i-coupled (He et al., 2004), and G_i-coupled GPCRs are known to inhibit lipolysis in adipose tissues (Moreno et al., 1983). Accordingly, we examined the effect of succinate on isoproterenol-induced lipolysis in isolated WAT (Isoproterenol has relatively low affinity for Adrb3 and most likely acts via Adrb2 in this context).

Succinate inhibited lipolysis in a dose-dependent manner with an apparent IC_{50} of 44μM (Fig. 4C), a concentration similar to the EC_{50} for succinate activation of Gpr91 heterologously expressed in 293 cells (He et al., 2004). Inhibition of lipolysis by succinate was ablated by pertussis toxin pretreatment. 3T3-L1 adipocyte-like cells, which do not express *Gpr91* mRNA (unpublished observation) and were not sensitive to the anti-lipolytic effects of succinate, became succinate-sensitive when transfected with a mammalian expression vector for GPR91 (Fig. 4E). These data strongly suggest that succinate inhibits lipolysis in WAT via a G_i-coupled GPCR, presumably Gpr91.

Pair-wise analysis of GPCR co-expression may help identify multiple roles for individual GPCRs

To explore whether co-expression of GPCRs might provide additional clues to function, we compared the expression pattern of each GPCR to that of every other to generate the map shown in Fig. 5. Positive and negative correlation of expression patterns are indicated by yellow and blue colors, respectively (Fig. 5A and Supplemental S4). Such correlation maps are a powerful tool for organizing and analyzing gene-gene and protein-protein interactions on a global scale (Collins et al., 2007a;Collins et al., 2007b;Krogan et al., 2006;Schuldiner et al., 2005;Segre et al., 2005).

Not surprisingly, tissue-specific receptor clusters similar to those seen in Fig. 3 were, for the most part, recapitulated as distinct blocks along the diagonal. Examples are shown in Fig. 5A, B and C, which largely reproduce the immune/hematopoietic, eye/retina, and adipose clusters of Fig. 3B and E and 4A, respectively. Off-diagonal blocks drew attention to possible roles for receptors in other contexts. By pointing up possible roles for receptors outside of their main physiological cluster, such analysis may be useful in understanding and predicting on-target drug side effects (See Discussion).

Discussion

We have quantitated mRNA levels for the non-odorant G protein-coupled receptors encoded in the mouse genome in 41 tissues and provide this data set as a resource for predicting roles for incompletely characterized GPCRs, exploring tissue-specific gene expression, and other purposes. The fact that tissues that comprise classical physiological systems (cardiovascular, gastrointestinal, etc.) were clustered together simply on the basis of their GPCR repertoires speaks to the key roles that GPCRs play in homeostatic regulation.

Our anatomic expression profiling yielded a large amount of information consistent with known physiology, and high-level expression of a GPCR in a particular tissue cluster or specific tissue correlated well with its physiological role. While this result is not surprising, it does provide confidence that roles for orphan receptors or GPCRs not known to play a role in a particular physiological process might be predicted by presence in a given cluster. Our demonstration that Gpr91 expression pointed to a role for extracellular succinate in regulating lipolysis in adipocytes validates this notion and is also of intrinsic interest.

The concentration of succinate in plasma has been reported at $5-125$ μM, a range that surrounds the EC_{50} for Gpr91 activation (He et al., 2004) and the IC_{50} for inhibition of lipolysis in WAT (Fig. 4). Succinate concentrations increase during exercise and metabolic acidosis and, in rodents, in hyperglycemic metabolic states (Forni et al., 2005; Hochachka and Dressendorfer, 1976; Krebs, 1950; Kushnir et al., 2001; Nordmann and Nordmann, 1961; Sadagopan et al., 2007). Thus, excursions in the levels of extracellular succinate do occur and might regulate adipocyte function in vivo. Adipocyte function was not investigated in mice lacking Gpr91, which are grossly healthy (He et al., 2004). Overall, a physiological role for succinate in regulating adipocyte metabolism is plausible, but when and how such a system might be important and/or redundant with other systems that govern adipocyte function remains unknown.

49 of the 353 GPCRs profiled were expressed in only one or two of the 41 tissues examined (see Table 2 in the Supplement). Such confined expression might point up targets of pharmaceutical interest. For example, testes showed a GPCR expression pattern very distinct from that of other tissues. Gpr150, Gpr66 and Gpr15, Mtnr1a, Pgr23 were almost perfectly specific to testes. Whether such receptors play a role in spermatogenesis or other testicular functions and their potential utility as targets for drugs aimed at controlling fertility is unknown.

A comparison of each receptor's expression pattern with that of every other (Fig. 5) provided a means of pointing up possible roles for a given receptor outside its main physiological cluster. Hm74(Gpr109a), the ketone body receptor that is activated therapeutically by niacin to treat dyslipidemias (Soga et al., 2003;Tunaru et al., 2003;Wise et al., 2003) provides an interesting example. By traditional clustering analysis Hm74(Gpr109a) is placed in the "adipose" cluster (Fig. 4A), and activation of adipocyte Hm74 likely mediates the anti-lipolytic actions of niacin (Tunaru et al., 2003). It was recently shown that the skin flushing side effect of niacin (Carlson, 2005) is mediated by Hm74 expression by bone-marrow derived epidermal Langerhans cells that release of vasodilatory prostanoids (Benyo et al., 2006;Benyo et al., 2005). By quantitative profiling across tissues, Hm74 was noted to be expressed relatively highly in skin and other "barrier-cluster" tissues as well as adipose (Fig. 4B). By expression correlation analysis, Hm74 was not found with the adipose cluster on the diagonal but instead clustered with receptors with more widespread expression (Supplemental Figures S4 & S5). A search of off-diagonal interactions revealed that Hm74 interacts not only with the adipose cluster, but also with receptors in both immune and barrier clusters (Supplemental Figure S4 & S5). Thus, analysis of GPCR expression data from these different perspectives may generate hypotheses regarding on-target side effects of drugs.

GPCR genes are usually relatively small, often intronless, and range from closely to distantly related. These features plus the availability of quantitative expression data across multiple tissues for hundreds of related genes that show clusters based on shared tissue-specific patterns may provide a resource for those interested in identifying the combinations of cis-acting elements that specify gene expression in a given cell type.

Some GPCRs are thought to function as heterodimers, and cluster analysis for co-expression might point to potential receptor pairs. For example, Gabbr1 is currently thought to function as an obligate heterodimer with Gpr51(Gabbr2) (Jones et al., 1998; Kaupmann et al., 1998; Kuner et al., 1999; White et al., 1998). Our data (Supplement S2) and that of others (Calver et al., 2000) demonstrate similar expression patterns for these receptors in CNS and divergent expression in the periphery. These results are consistent with Gabbr1 and Gabbr2 heterodimer formation in the CNS but raise the possibility that in the periphery, expression of one or the other partner is regulated, that these receptors may use other partners, and/or that heterodimerization may not be required in all settings (Cheng et al., 2007).

Several caveats should be stated regarding interpreting our expression data. 1) Relative mRNA levels, of course, will not always reflect relative protein expression levels nor the relative importance of a particular receptor in a particular tissue. Indeed, some important receptors such as adrenergic receptors were expressed at relatively low levels. 2) Tissues are comprised of multiple cell types, and receptor expression can be restricted to a minority cell type. In the extreme, receptor expression in a minority population can be missed by whole tissue analysis. For example, neither our qPCR data (supplemental S2) nor those of others (Liberles and Buck, 2006) detected trace amine associated receptor 1 (Taar1) expression in brain, but a Taar 1 Lac-Z knock-in mouse revealed Taar1 expression in discrete neuronal populations and Taar1-dependent regulation of dopaminergic activity (Lindemann et al., 2008). 3) The presence of a receptor within a particular anatomical cluster does not exclude important functions in tissues outside that cluster. For example, the D2 dopamine receptor (Drd2) is recognized as the major dopamine receptor subtype in pituitary (Kelly et al., 1997; Saiardi et al., 1997), but Drd2 was in the CNS cluster while the D3 dopamine receptor (Drd3) was in the pituitary cluster (Figure 3C). The cluster analysis presented here used the Pearson correlation, which normalizes expression levels (see Methods) to focus on gaining information from the pattern of gene expression at the cost of ignoring absolute levels of receptor expression, and the Drd cluster results are presumably a result of the fact that Drd2 is expressed at moderate levels in numerous CNS structures while Drd3 is expressed at very low levels in only a few structures

(supplemental S2). However, our quantitative expression data (supplemental S2) reveals that Drd2 is expressed approximately 100-fold higher than Drd3 in pituitary, consistent with the dominant role of Drd2 in pituitary function. Thus, this resource is best utilized when the data are analyzed from several perspectives and should be viewed as a means of generating hypotheses to be tested experimentally. Raw expression data for the 353 GPCRs in the 41 organ samples are available online at http://pdsp.med.unc.edu/apGPCRe/. for those who wish to perform their own analyses.

Lastly, expression of individual GPCRs in specific tissues can be different in human and mouse. When used together with GPCR expression patterns in human (SymAtlas, SAGEmap), our dataset should facilitate rational use of mouse to model the roles of GPCRs in human physiology and disease.

Materials and Methods

qRT-PCR

10 week old C56BL/6 mice (Jackson Labs) were housed in UCSF animals facilities for 2 weeks prior to organ harvest. Mice were anesthetized with ketamine/xylazine and transcardially-perfused with saline to remove blood. Organs were dissected, rapidly frozen in liquid nitrogen and stored at −80°C until time of RNA isolation. Corresponding organs from 2 male and 2 female mice were pooled to yield a single tissue RNA sample (i.e., for each separate replicate, organs of 4 mice were pooled), with the exception of sex-specific organs, which were pooled from 2 mice. Pancreas was removed and stored for 24 hours in RNAlater at 4°C prior to storage at −80°C. Islets of Langerhans were isolated from 20 male C57BL/6 males by the UCSF islet isolation core. Primary adipocytes were isolated from sex organ fats pads as described (Rodbell, 1964). Tissues were first homogenized and crude RNA extracted with Trizol (Invitrogen). RNA was further purified using RNeasy columns (Qiagen) with on-column DNAse I digestion. RNA samples were DNAse I-treated a second time and concentrated using the Zymo Research DNA-free RNA kit. First strand synthesis was performed using the iScript kit (Biorad). qPCR assays were performed in a 384-well format using an ABI7900HT, Platinum qPCR mix (Invitrogen) and 10μL reactions. Cycle threshold (Ct) values were collected at 0.2 for all samples; Ct values for individual GPCRs were compared to Ct values for 4 internal controls (β-actin, cyclophilin, GAPDH and ribosomal protein S9) for all tissues. Taqman primer/probes have been described (Regard et al., 2007) and sequences are available in supplemental data (S1). There were 22 mouse GPCRs for which we were unable to generate primer/probe sets that produced a signal above background in any tissue. A lack of data should be viewed as "no data" for these receptors, which are listed in Supplemental Table S3. PCR efficiencies were calculated as described (Peirson et al., 2003). $2^{(Ct_{GPCR} - Ct_{control})}$ multiplied by 2^{15} was used for graphical representation of qPCR data. Repeated qPCR analysis of a given tissue RNA sample, i.e., technical replicates, yielded variations of less than 10%. 2 to 5 independently prepared RNA samples from separate tissue isolations were analyzed to assess in vivo expression variability, which was also generally less than 10%. Data presented in Supplement 2 and elsewhere represent the mean ± SEM (n=2−5).

Lipolysis

C57BL6 mice were euthanized by cervical dislocation. Sex organ fat pads were dissected and finely minced and washed 4 times with Krebs-Ringer Hepes buffer containing 4% fatty acid free BSA, 5mM glucose, 0.1mM ascorbic acid (KRH-BSA). 25−30 mg of WAT mincate was placed in the upper well of transwells in a 24-well plate and placed in a tissue culture incubator at 37°C with the specified cocktail for the indicated amount of time. KRH-BSA samples were taken from the wells at the specified time to quantify glycerol release and divided by the weight of the mincate. Pertussis toxin (Calbiochem) pretreatment was performed for 4 hours in a tissue

culture incubator with mild shaking. 3T3-L1 cells (ATCC) were cultured and differentiated using standard protocols. Fully differentiated 3T3-L1 cells were transfected (Amaxa, Nucleofection) with mammalian expression vectors containing β2-adrenergic receptor (Adrb2 – generously provided by Dr. J. Silvio Gutkind) and GPR91 (Missouri S&T) and allowed to recover 48−72 hours prior to lipolysis assays. Free glycerol was quantified using the Free Glycerol reagent (Sigma). IC_{50} for succinate inhibition of isoproterenol-induced lipolysis was calculated using Prism4.

Hierarchical Clustering

All analysis of the data was performed using Python, Scipy and Excel. For pair-wise correlation in Figure 5, Pearson correlation r values were computed between the expression vectors of every pair of genes in the data set. Transformed data was hierarchically clustered using Cluster 3.0 (Eisen et al., 1998) and visualized using Java TreeView (Saldanha, 2004).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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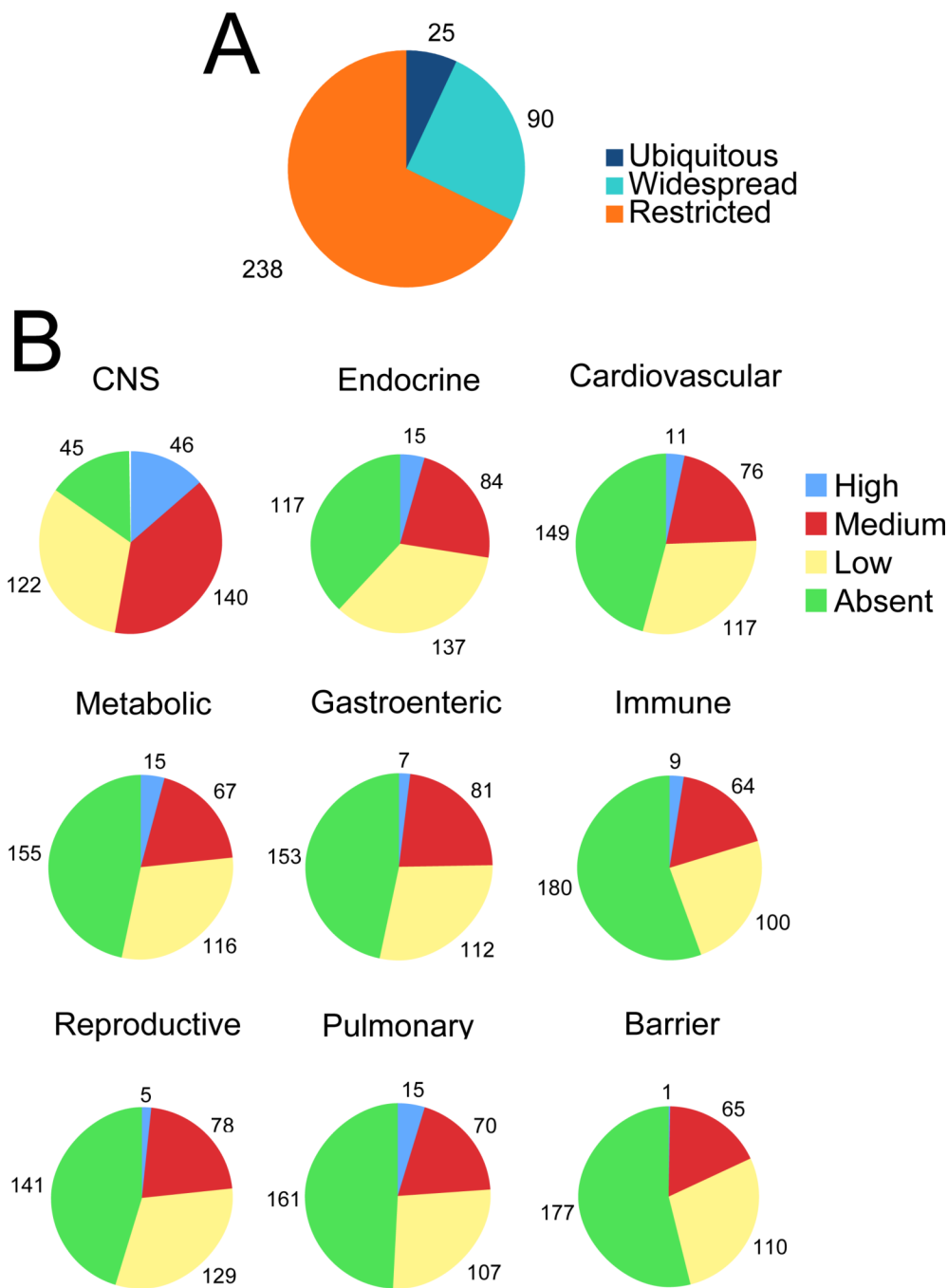
Fig. 1. Distribution of mouse GPCR mRNA expression in vivo
A) The number of GPCRs expressed in tissues presented in pie chart form. Receptors expressed in all 41 tissues assayed are "ubiquitous"; receptors expressed in more than or less than half are "widespread" or "restricted", respectively. A list of the "ubiquitous" receptors is included in Table 1. B) GPCR expression by tissue systems. High, medium, low and absent are defined in Results. Tissue systems are defined as: Central Nervous System (CNS: cerebellum, brainstem, hypothalamus, cerebral cortex, hippocampus, striatum, olfactory bulb, olfactory epithelium, retina, whole eye), Endocrine (pituitary gland, islets of Langerhans, adrenal gland, thyroid/parathyroid), Cardiovascular (aorta, vena cava, heart atrium, heart ventricle), Pulmonary (lung, trachea), Metabolic (brown adipose tissue, white adipose tissue, isolated

adipocytes, liver, skeletal muscle, kidney), Gastroenteric (pancreas, gall bladder, large intestine, small intestine, stomach, urinary bladder), Reproductive (ovary, testes, uterus), Barrier (tongue, esophagus, skin) and Immune (spleen, thymus, bone marrow). A list of the highly expressed GPCRs in individual tissue systems is included in Table 1.
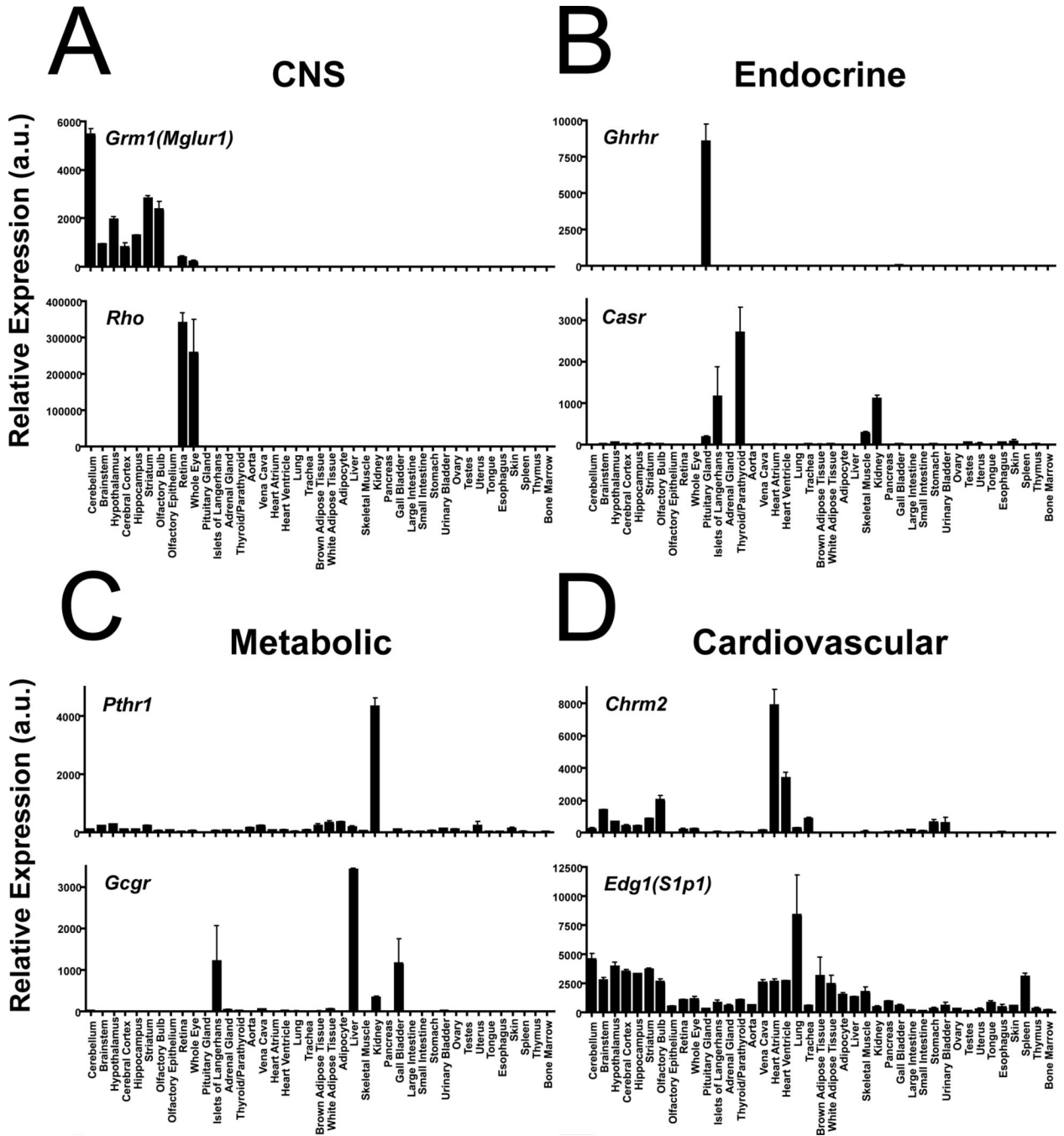
Fig. 2. qRT-PCR tissue distribution yields predicted patterns of expression
Representative GPCRs that were highly expressed in distinct tissue systems were selected from
Fig. 1B and Table 1 to illustrate tissue specificity, range of expression levels, and
reproducibility. Examples are shown for A) CNS: metabotropic glutamate receptor 1 [Grm1
(Mglur1)] and rhodopsin [Rho]. B) Endocrine system: growth hormone releasing hormone
receptor [Ghrhr] and the extracellular calcium-sensing receptor [Casr]. C) Metabolic tissues:
parathyroid hormone receptor 1 [Pthr1] and glucagon receptor [Gcgr]. D) Cardiovascular
system: M2 muscarinic receptor [Chrm2] and the sphingosine-1-phosphate receptor 1 [Edg1
(S1p1)]. Values are plotted as the mean ± SEM; n=2−5.
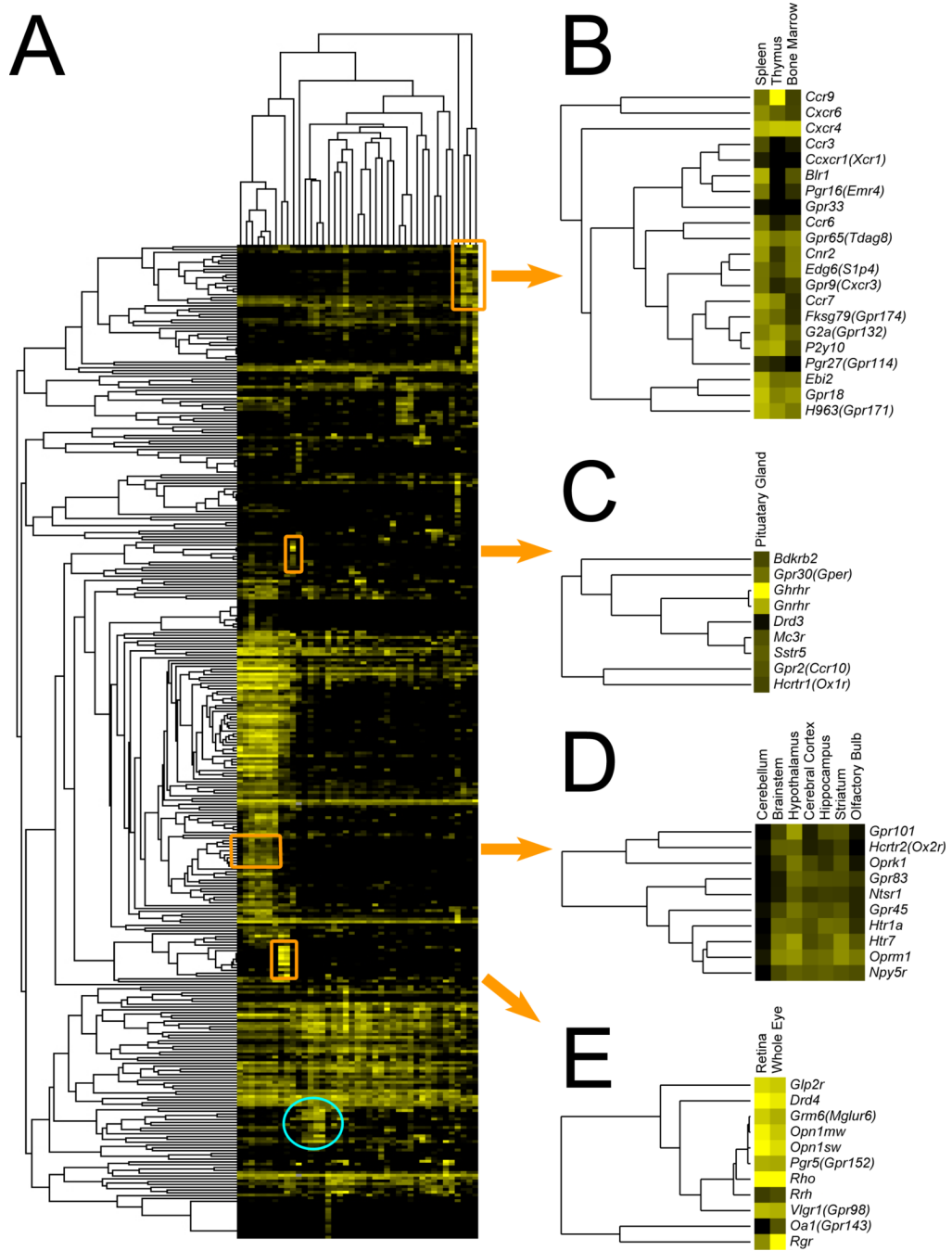
Fig. 3. Unsupervised hierarchical clustering of GPCR expression across tissues
A) Transformed qRT-PCR data for the 353 GPCRs assayed in the 41 tissues was evaluated by
unsupervised hierarchical clustering with average linkage using Cluster 3.0 and visualized
using Java TreeView (see Experimental Procedures). A thumbnail image is shown here, a full
size version is available in Supplement S3. Multiple clusters and subclusters were seen, 5 were
chosen for further analysis. The fifth cluster (blue circle towards the bottom) is discussed in
Fig. 4. B) A portion of the "immune/hematopoietic" cluster is shown; note the abundance of
chemokine receptors. C) The "pituitary" cluster contains many well-documented regulators of
pituitary function, including Ghrhr and Gnrhr. D) A small portion of the "CNS" cluster, by far
the largest. This portion contains receptors for important neurotransmitters including serotonin,

neuropeptide Y, orexin and opiates. E) The "eye/retinal" cluster contains light-sensing opsins as well as other receptors known to regulate vision.
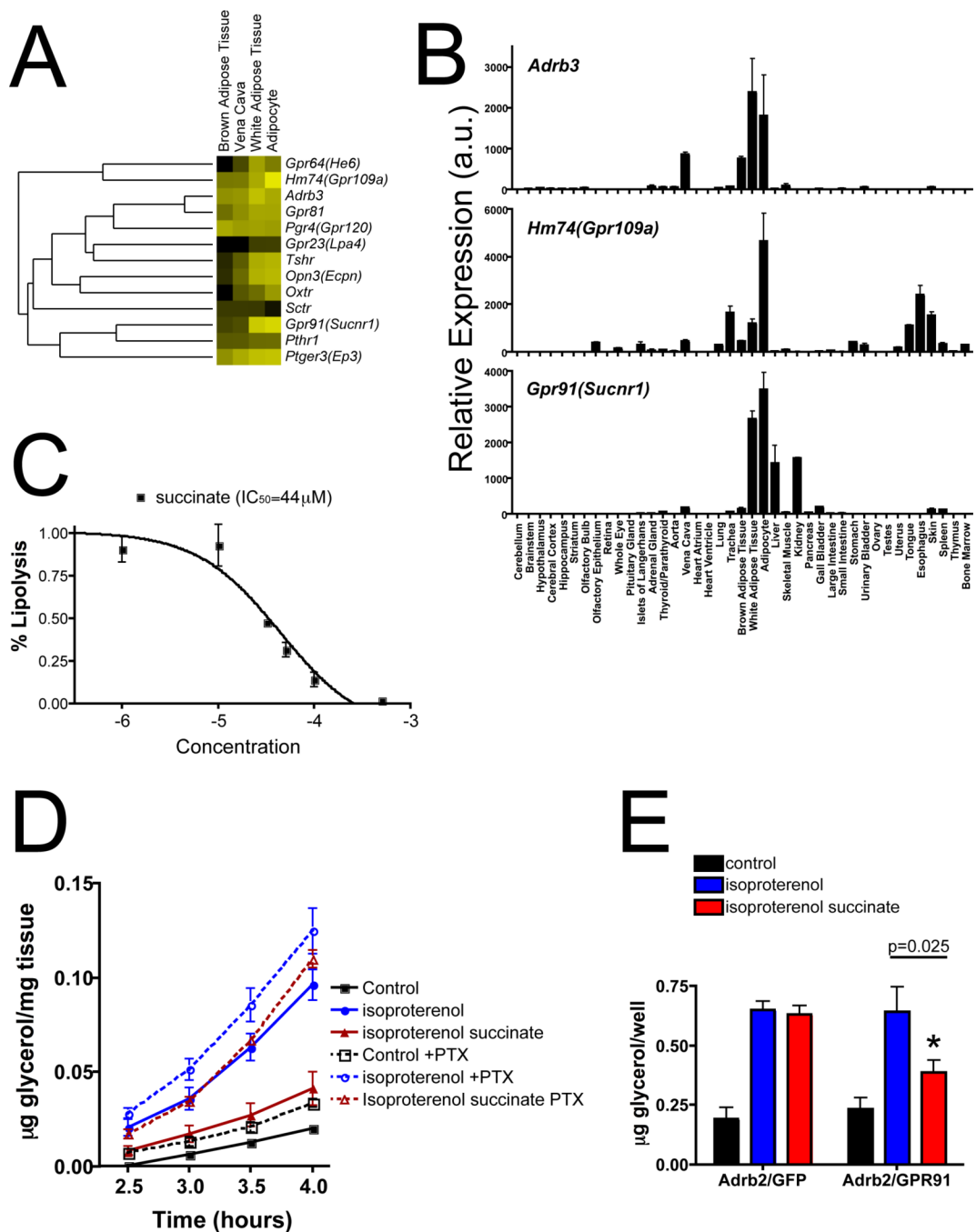
Fig. 4. *Gpr91(Sucnr1)* and its ligand, succinate, can inhibit lipolysis in adipocytes
A) The "adipose" cluster (blue circle, lower part Fig. 3A) contained numerous regulators of adipocyte function. B) *Adrb3*, *Hm74(Gpr109a)* and *Gpr91(Sucnr1)* were expressed at similarly high levels in white adipose tissues (WAT) and isolated adipocytes. C) Isolated WAT was treated with isoproterenol (20nM) to stimulate lipolysis, as measured by glycerol release at 3 hours. Succinate added concurrently with isoproterenol inhibited lipolysis in a concentration-dependent manner with an apparent IC_{50} of approximately 44μM. D) Isolated WAT was pretreated either with KRH/BSA or KRH/BSA containing pertussis toxin (PTX; 100 ng/mL) for 3 hours prior to exposure to the indicated conditions. Isoproterenol (20nM)-stimulated glycerol release was inhibited by the addition of 80μM succinate. Pretreatment of WAT with

PTX abrogated this effect, suggesting succinate's inhibition of lipolysis occurred in a $G_{i/o}$-dependent manner, consistent with Gpr91 activation. E) Differentiated 3T3-L1 cells, which do not express *Gpr91* mRNA, were transfected with mammalian expression vectors containing β2 adrenergic receptor (Adrb2) and control (green fluorescent protein; GFP) or Adrb2 and GPR91. Succinate (100μM) inhibited isoproterenol (10nM)-induced lipolysis in cells co-transfected with GPR91, but not GFP. The data shown are mean ± SD, n=3; p=0.025, unpaired student's t-test. This experiment was done three times with similar results.
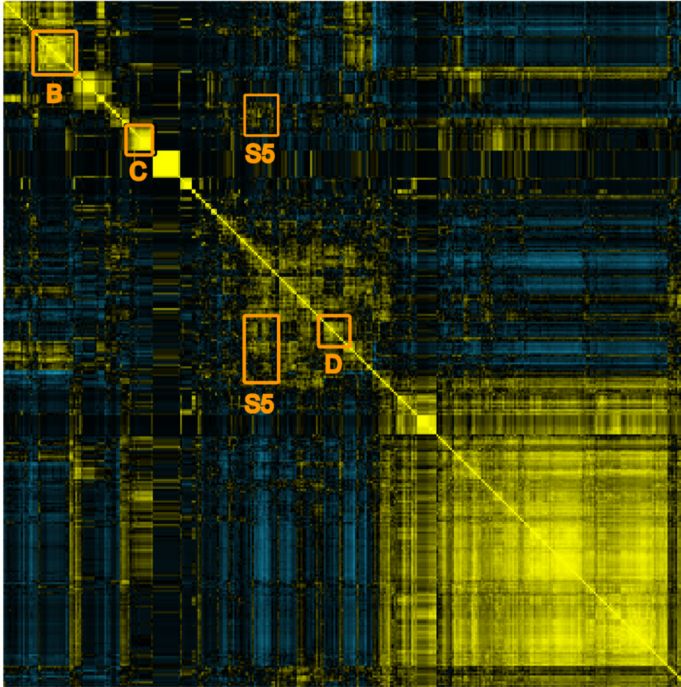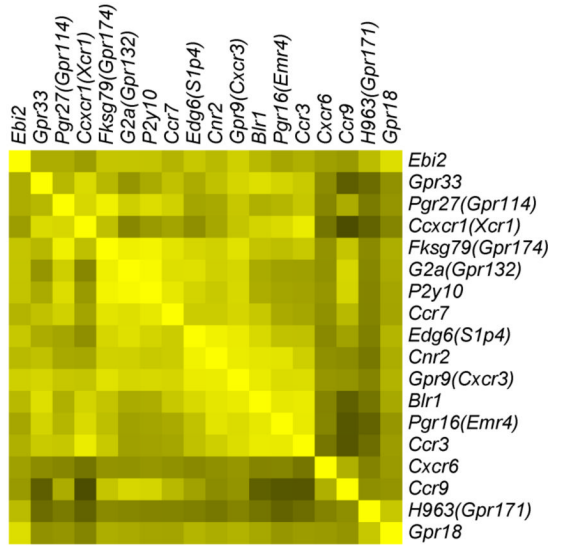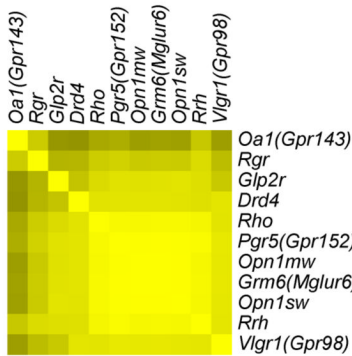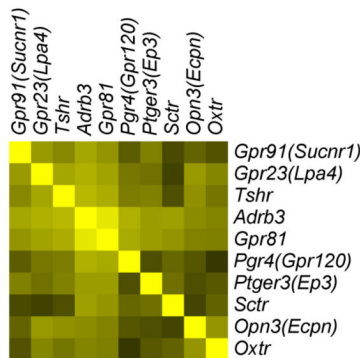
Fig. 5. Hierarchical-Cluster analysis of pairwise GPCR expression reveals additional levels of interaction

A) Pearson correlation r coefficients were calculated for interactions between each GPCR with all others based on tissue expression patterns. The resulting data set was further analyzed using Cluster 3.0 with complete linkage and visualized using TreeView. A thumbnail image is shown here, a full size image is available in Supplemental S4. Receptors with similar distributions are shown in yellow; distinct distributions are shown in blue; X- and Y-axis are mirror images of one another. The diagonal represents each receptor interacting with itself (perfect similarity in distribution). B) Clustering of receptors by similarity of expression reveals a immune/hematopoietic grouping very similar to that in Fig. 3B; C) an eye/retinal cluster similar to that

in Fig. 3E; and D) an adipose cluster similar to that in Fig. 4A. However, analysis of GPCR interaction clusters off the diagonal suggested receptor functions outside of their most obvious physiological roles. This might aid in understanding and predicting on-target drug side effects (see Discussion and supplementary figure S5).

Table 1

GPCRs ubiquitously and abundantly expressed in vivo.

Ubiquitous	CNS	Endocrine	Cardiovascular
Cd97	Adcyap1r1	Adora1	Agtr1(Agtr1a)
Ebi2	Adora1	Agtr1(Agtr1a)	Chrm2
Edg1(S1p1)	Bai1	Casr	Edg1(S1p1)
Edg3(S1p3)	Bai2	Drd2	Ednra
Ednra	Celsr2	F2r(Par1)	Etl(Eltd1)
Etl(Eltd1)	Chrm1	Ghrhr	F2r(Par1)
F2r(Par1)	Cnr1	Glp1r	Fzd1
Fzd1	Drd1	Gpr108	Fzd2
Fzd7	Drd4	Gpr48(Lgr4)	Fzd4
Gabbr1	Edg1(S1p1)	Gpr56	Kiaa0758(Gpr116)
Gpr10(Prlhr)	Ednrb	Lec2(Lphn1)	Rdc1(Cxcr7)
Gpr105(P2ry14)	Fy(Darc)	Rai3(Gprc5a)	
Gpr107	Fzd1	Rdc1(Cxcr7)	
Gpr108	Fzd3	Tm7sf1(Gpr137b)	**Barrier**
Gpr19	Gabbr1	Tm7sf1l1(Gpr137)	Hm74(Gpr109a)
Gpr56	Glp2r		
Il8rb(Cxcr2)	Gpr108	**Metabolic**	
Lec1(Lphn2)	Gpr22	Adrb3	**Pulmonary**
Lec2(Lphn1)	Gpr26	Agtr1(Agtr1a)	Adrb2
P2y5	Gpr37(Paelr)	Cmkbr1l1(Ccr1l1)	Calcrl
Ptger1(Ep1)	Gpr37l1	Edg1(S1p1)	Cckar
Tm7sf1(Gpr137b)	Gpr49(Lgr5)	Etl(Eltd1)	Cd97
Tm7sf1l1(Gpr137)	Gpr51(Gabbr2)	Fzd4	Edg1(S1p1)
Tm7sf3	Gpr56	Gcgr	Edg3(S1p3)
Tpra40(Gpr175)	Gprc5b(Raig2)	Gpr10(Prlhr)	Etl(Eltd1)
	Grca(Gpr162)	Gpr48(Lgr4)	F2r(Par1)
	Grm1(Mglur1)	Gpr56	Glp1r
	Grm3(Mglur3)	Gpr91(Sucnr1)	Gpr108
	Grm4(Mglur4)	Hm74(Gpr109a)	Gpr56
	Grm5(Mglur5)	Ptger3(Ep3)	Kiaa0758(Gpr116)
	Grm6(Mglur6)	Pthr1	Rai3(Gprc5a)
	Grm7(Mglur7)	Tm7sf1(Gpr137b)	Tm7sf1(Gpr137b)
	Hrh3		Tm7sf1l1(Gpr137)
	Il8rb(Cxcr2)	**Gastroenteric**	
	Kiaa1828(Gpr123)	Cckar	
	Lec2(Lphn1)	Flj14454(Gpr128)	**Immune**
	Lec3(Lphn3)	Fzd1	Adrb2
	Ntsr2	Glp1r	Ccr9
	Opn1mw	Gpr108	Cxcr4
	Opn1sw	Ptger3(Ep3)	Edg1(S1p1)
	Oprl1	Rai3(Gprc5a)	Fpr-rs2
	Pgr15(Gpr165)		
	Pgr22(Gpr155)		Gpr18
	Rgr	**Reproductive**	Il8rb(Cxcr2)
	Rho	Agtr1(Agtr1a)	Mrga3
	Tm7sf1l1(Gpr137)	Gpcr150	P2y5
		Gpr108	
		Gpr19	
		Tm7sf1l1(Gpr137)	