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## Supplementary Materials for

## Identification of GPR83 as the receptor for the neuroendocrine peptide PEN

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Table S1. Description of statistical analysis for different figures.



fig. S1. Variable results with studies examining the effect of mPEN on adenylyl cyclase activity in hypothalamic membranes. The effect of mPEN on adenylyl cyclase (AC) activity in mouse hypothalamic membranes (2  $\mu$ g). (Left Panel) Data represent mean  $\pm$  SE of 6 individual experiments in triplicate. (Right Panel) Data represent mean  $\pm$  SE of 4 individual experiments in triplicate.



**fig. S2. The binding of mPEN to hypothalamic membranes from individual male mice.** The relative amount of [<sup>125</sup>I]Tyr-rPEN binding in 18 individual hypothalami was examined in triplicate. The number on the x-axis denotes the individual animal number.



fig. S3. Variable results with studies examining the effect of mPEN on PLC activity in hippocampal membranes. The effect of mPEN on PLC activity in mouse hippocampal membranes (10  $\mu$ g). (Left Panel) Data represent mean  $\pm$  SE of 3 individual experiments in triplicate. (Right Panel) Data represent mean  $\pm$  SE of 4 individual experiments in triplicate.



fig. S4. Evoked EPSC amplitude after sequential application of increasing concentrations of mPEN and washout. The time course of evoked EPSC amplitude following consecutive application of 10 nM and 100 nM of PEN in five PVN neurons from a single rat. Wash out of PEN effects following 5 minutes after removal of peptide from the perfusion bath. Data represent mean  $\pm$  SE (n=5 individual neurons).



**fig. S5. mPEN-stimulated neurite outgrowth in Neuro2A cells.** Cells treated without or with mPEN were imaged at 20X and scored for neurite outgrowth. Cells containing neurites that were longer than twice the diameter of the cell body were scored as positive. Data from multiple images were analyzed for the graphs shown in Figures 3A and 5E.



fig. S6. Expression of *GPR83* in heterologous cells confers PEN signaling and receptor endocytosis. (A) The effect of PEN (1  $\mu$  M) on intracellular Ca<sup>+2</sup> release in cells expressing *GPR83* along with a promiscuous chimeric hG $\alpha_{16/i3}$  protein. (Bar 1) scrambled peptide (1  $\mu$ M), (Bar 2) hPEN (1  $\mu$ M), (Bar 3) rlittleLEN (1  $\mu$ M), (Bar 4) mbigLEN (1  $\mu$ M) or (Bar 5) ATP (1  $\mu$ M). Similar experiments were performed with mGPR19, mGPR108, mGPR165, and mGPR171. (B) The effect of mPEN or hPEN (100 nM) on internalization of GPR83 in HEK293 cells expressing mGPR83 (2 x 10<sup>5</sup> cells). Data represent mean± SE (n=3-8 independent experiments). \*\*\*p<0.001(one-way Anova for A); details of the statistical analyses are in table S1.



fig. S7 Quantitative RT-PCR to confirm the presence of GPR83 mRNA in Neuro2A cells. n=6 different samples.



**fig. S8. Specificity of the GPR83 and GPR171 antibodies.** (**A**) Western blot for GPR83 in lysates (~20-35 μg protein) of CHO cells alone (CHO) or expressing either HA-tagged mGPR83 or myc-tagged mGPR171, of Neuro2A cells alone (N2A), Neuro2A cells overexpressing myc-tagged mGPR171 (N2A GPR171), Neuro2A cells stably expressing lentiviral shRNA to GPR171 (N2A-171V1 and N2A-171V5 generated as described in (13)), Neuro2A cells stably expressing lentiviral shRNA to GPR83 (N2A-83V1 for shRNA clone ID:TRCN0000026837, and N2A-83V2 for shRNA clone ID:TRCN0000026857) using GPR83 antibody raised in rabbit (green; 1:1000). Antibodies recognizing tubulin raised in mouse (red; 1:50,000) were used as loading control. (**B**) A parallel blot was probed with GPR171 antibody raised in rat (green; 1:5000) and antibodies recognizing tubulin (red; 1:50,000). (**C**) Western blot for GPR83 in lysates (~20-35 μg protein) of hypothalamic membranes from two individual wild type and four individual *GPR83*-knockout (KO) mice. GPR83 antibody raised in rabbit (green; 1:1000); antibodies recognizing tubulin raised in mouse (red; 1:50,000). (**D**) A parallel blot was probed with GPR171 antibody raised in rabbit (green; 1:1000); antibodies recognizing tubulin raised in mouse (red; 1:50,000). (**D**) A parallel blot was probed with GPR171 antibody raised in rat (green; 1:5000) and antibodies recognizing tubulin raised in mouse (red; 1:50,000). (**D**) A parallel blot was probed with GPR171 antibody raised in rat (green; 1:5000) and antibodies recognizing tubulin raised in mouse (red; 1:50,000).



**fig. S9**. **Confirmation of antibody specificity and accuracy of colocalization by analysis of the lateral septum**. Immunohistochemical localization using a GPR83 antibody raised in rabbit (green) and a GPR171 antibody raised in rat (red) in the lateral septum (white dashed line) to confirm specificity of colocalization.

**table S1. Description of statistical analysis for different figures.** ATP, adenosine triphosphate; Cont, control; k0, knockout; n.a., not applicable; Scr. pep., scrambled peptide; WT, wild type; Dfn, degrees of freedom (df) in the numerator; Dfd, degrees of freedom (df) in the denominator.

Fig.	t-test		One-way ANOVA	Two-way ANOVA			
	Group	p-value	Test	p-value	Test	p-	F (DFn, DEd)
						value	Dru)
2B	Baseline vs PEN	p<0.05; t=-5.41; df=7	n.a.	n.a.	n.a.	n.a.	n.a.
2D	Baseline vs PEN	p<0.05; -2.49; df=7	n.a.	n.a.	n.a.	n.a.	n.a.
ЗА ЗН	Cont v/s mPEN Cont v/s bigLEN Cont v/s SAAS mPEN v/s bigLEN mPEN v/s SAAS bigLEN v/s SAAS 5'Cont v/s 5'mPEN 30'Cont v/s 30'mPEN	p<0.0001; t=69.3; df=4 p<0.0001; t=36.3; df=8.9 p<0.0001; t=38; df=3.2 p<0.0001; t=11.8; df=8.9 p<0.0012; t=11; df=3.2 p<0.0001; t=16.3; df=9.9 p=0.005; t=4.77; df=5 p=0.73; t=0.365; df=5.03	One-Way ANOVA Post-hoc Dunnett's test Cont v/s mPEN Cont v/s bigLEN Cont v/s SAAS Tukey's Multiple Comparison Cont v/s mPEN Cont v/s bigLEN Cont v/s SAAS mPEN v/s bigLEN mPEN v/s SAAS bigLEN v/s SAAS n.a.	<pre>p&lt;0.0001; F=209.9 p&lt;0.0001; df=14 p&lt;0.0001; df=14 p&lt;0.0001; df=14 p&lt;0.0001; df=14 p&lt;0.0001; df=14 p&lt;0.0001; df=14 p&lt;0.0001; df=14 n.a.</pre>	n.a. n.a. Interaction Treatment Time Tukey's Multiple Comparison 5'Cont v/s 5'Cont v/s 30'Cont 5'Cont v/s 30'Cont 5'Cont v/s 30'Cont 5'mPEN v/s 30'Cont 5'mPEN v/s 30'mPEN 30'Cont v/s 30'mPEN 30'Cont v/s 30'mPEN	n.a. p=0.00 02 p<0.00 01 p=0.00 02 p<0.00 01 p=0.99 99 p=0.99 82 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 02 p<0.00 01 p<0.00 02 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 01 p<0.00 00 00 00 00 00 00 00 00 00	n.a. F $(1, 20) =$ 21.53 F $(1, 20) =$ 23.97 F $(1, 20) =$ 21.53 df=20 df=20 df=20 df=20 df=20 df=20 df=20 df=20 df=20
4A and S6A	n.a.	n.a.	GPR83 One-way ANOVA Dunnett's test Scr.pep v/s hPEN	p<0.0001; F=216.2	n.a.	p=0.99 82 n.a.	n.a.
			Scr.pep v/s littleLEN	p<0.0001; df=42			

		1	1	r		1	
			Scr.pep v/s bigLEN Scr.pep v/s ATP	p=0.9997; df=42 p=0.8748; df=42 p<0.0001; df=42			
			CDD10	p<0.0001, u1-42			
			One-way ANOVA				
				p<0.0001;			
			Dunnett's test	F=111.4			
			Scr.pep v/s hPEN				
			Scr.pep v/s littleLEN				
			Scr.pep v/s bigLEN	p=0.9755; df=18			
			Scr pep v/s ATP	n=0.9999. df=18			
			sen.pep visititi	p = 0.9997; df=18			
			CDD109	p=0.9997, u=10			
			GPRIUS	p<0.0001, d1−18			
			One-way ANOVA				
			Dunnett's test	p<0.0001;			
			Scr.pep v/s hPEN	F=2233			
			Scr.pep v/s littleLEN				
			Scr pep v/s bigLEN				
			Scr pep $v/s$ $\Delta TP$	$n=0.0832 \cdot df=17$			
			Serpep vis All	p = 0.0032, df = 17			
			CDD4/C	p=0.1219, d1=17			
			GPR165	p=0.9945; df=1/			
			One-way ANOVA	p<0.0001; df=17			
			Dunnett's test				
			Scr.pep v/s hPEN	p<0.0001;			
			Scr pep v/s littleLEN	F=2505			
			Scr pep v/s higt EN	1 2000			
			Ser pop v/s ATD				
			Sci.pep v/s ATF	0 5171 10 10			
				p=0.51/1; df=18			
			GPR171	p=0.9999; df=18			
			One-way ANOVA	p=0.9962; df=18			
			-	p<0.0001; df=18			
			Dunnett's test	1 /			
			Scr pen v/s hPFN				
			Ser pep v/s littleI EN	n<0.0001.			
			Server v/s httelEN	$\mu < 0.0001,$			
			Scr.pep V/s bigLEN	F=2019			
			Scr.pep v/s ATP				
				p>0.9999; df=18			
				p=0.9667; df=18			
				p<0.0001; df=18			
				p<0.0001: df=18			
4F	-PTX v/s +	$p=0.0005 \cdot t=4.307 \cdot$	na	na	na	na	na
	PTX	df=16					
5A	50 pmol cont	p<0.0001.t=30.82.	n.a.	n.a.	Interaction	p<0.00	F(2, 12) =
	v/s 50  nmol	df=4			SiRNA type	01	67.82
	CDD92				Doso	n<0.00	E(1, 12) =
	UPK83				Dose	p~0.00	$\Gamma(1, 12) =$
	100 pmol cont	p<0.0001;t=34.62;				01	5458
	v/s 100 pmol	dt=4				p<0.00	F(2,12) =
	GPR83				Tukey's	01	67.82
	200 pmol cont	p<0.0001;t=97.07;			Multiple		
	v/s 200 pmol	df=4			Comparison		
	GPR83				50 pmol cont $v/s$		
					50 nmol GPR 83		
					50 pmol cont w/a	n<0.00	df=12
					50 pinoi cont V/S	p~0.00	ui-12
					100 pmol cont	01	10.1-
					50 pmol cont v/s		df=12
					100 pmol GPR83	p>0.99	
					50 pmol cont v/s	99	df=12
					200 pmol cont		
					50 pmol cont v/s	p<0.00	df=12

					200 nmol CDD92	01	
					200 pillol GPR83	01	df=12
					$\frac{50}{v/s}$ 100 nmol	n>0.00	ui-12
					v/s 100 pilloi	p>0.99	df-12
					50 nmol CDD 93	77	ui-12
					50 pillol GPK85	<i>m</i> <0.00	46-10
					V/S 100 pmol	p<0.00	dI=12
					GPR83	01	10.10
					50 pmol GPR83		df=12
					v/s 200 pmol	p<0.00	
					cont	01	
					50 pmol GPR83		df=12
					v/s 200 pmol	p<0.00	
					GPR83	16	df=12
					100 pmol cont		
					v/s 100 pmol	p<0.00	df=12
					GPR83	01	
					100 pmol cont	-	df=12
					v/s 200 pmol	n<0.00	ui 12
					cont	01	df=12
					100 nmol cont	01	ui 12
					v/s 200 pmol	n < 0.00	df-12
					CDD 92	p<0.00	ui-12
						01	10, 10
					100 pmol GPR83		df=12
					v/s 200 pmol	p>0.99	
					cont	99	
					100 pmol GPR83		
					v/s 200 pmol	p<0.00	
					GPR83	01	
					200 pmol cont		
					v/s 200 pmol	p<0.00	
					GPR83	01	
						p<0.00	
						01	
						01	
						n<0.00	
						01	
5R	Cont v/s	$p < 0.0001 \cdot t = 8.84$	na	na	na	na	na
30	GPR83 siRNA	p < 0.0001, t = 0.04, df=6 941	11.d.	11.a.	11.a.	11.a.	11.a.
50	Cont ciDNA (	$u_1 = 0.941$ $u_2 = 0.0001.4 = 0.25.$		* 0	Interaction	m=0.00	E(1, 20) =
50	COILI SIKINA (-	p < 0.0001, t = 0.55,	11.a.	11. <b>a</b> .	SiDNA torna	p=0.00	$\Gamma(1, 20) = 0.42$
	PEIN) V/S	$u_{1-9.985}$			DEN sons	01	9.42
	(+PEN)	p=0.114; t=1.74;			PEN conc.	p=0.00	F(1, 20) =
	GPK85 SIKNA	ai=9.4/			<b></b>	61	9.42
	(-PEN) v/s				Tukey's	p<0.00	F(1, 20) =
	(+PEN)				Multiple	01	35.98
					Comparison		
					Cont siRNA (-		
					PEN) v/s (+PEN)		
					Cont siRNA (-	p<0.00	df=20
					PEN) v/s GPR83	01	
					siRNA(-PEN)		df=20
					Cont siRNA (-	p>0.99	
					PEN) v/s GPR83	99	
					siRNA(+PEN)		df=20
					Cont siRNA		-
					(+PEN) v/s	n=0.19	
					GPR83 eiRNA(	63	df=20
					DENI)	05	ui=20
					r EINJ Cont ciDNIA		
					( DEN)/~	m < 0.00	46-20
					(+PEN) v/s	p<0.00	df=20
					(+PEN) v/s GPR83	p<0.00 01	df=20

5D	Cont siRNA (- PEN) v/s (+PEN) GPR83 siRNA (-PEN) v/s (+PEN)	p<0.005; t=4.77; df=5 p<0.0001; t=31.07; df=5		GPR83 siRNA(- PEN) v/s GPR83 siRNA(+PEN) Interaction SiRNA type PEN conc. Tukey's Multiple Comparison Cont siRNA (- PEN) v/s (+PEN) Cont siRNA (- PEN) v/s GPR83 siRNA(-PEN) Cont siRNA (- PEN) v/s GPR83 siRNA(+PEN) Cont siRNA (+PEN) v/s GPR83 siRNA(- PEN) Cont siRNA (+PEN) v/s GPR83 siRNA(- PEN) v/s GPR83 siRNA(+PEN)	p<0.00 17 p=0.19 63 p<0.00 01 p<0.00 22 p<0.00 01 p>0.99 99 p=0.30 83 p<0.00 01	df=20 $F(1, 20) = 36.34$ $F(1, 20) = 36.34$ $F(1, 20) = 12.26$ $df=20$ $df=20$ $df=20$ $df=20$ $df=20$ $df=20$
				siRNA(+PEN) GPR83 siRNA(- PEN) v/s GPR83 siRNA(+PEN)	p<0.00 01 p=0.30 83	df=20
5E	Cont siRNA (- PEN) v/s (+PEN) GPR83 siRNA (-PEN) v/s (+PEN)	p<0.0001; t=69.3; df=10 p<0.3466; t=1; df=8		Interaction SiRNA type PEN conc. <b>Tukey's</b> <b>Multiple</b> <b>Comparison</b> Cont siRNA (- PEN) v/s (+PEN) Cont siRNA (- PEN) v/s GPR83 siRNA(-PEN) Cont siRNA (- PEN) v/s GPR83 siRNA(+PEN) Cont siRNA (+PEN) v/s GPR83 siRNA(- PEN) Cont siRNA	p<0.00 01 p<0.00 01 p<0.00 01 p>0.00 33 p>0.04 17	F (1, 20) = 1536 $F (1, 20) = 2017$ $F (1, 20) = 1667$ $df=20$ $df=20$ $df=20$ $df=20$
				GPR83 siRNA(+PEN)	01	ui 20

		1	1	r	1		
					GPR83 siRNA(- PEN) v/s GPR83 siRNA(+PEN)	p<0.00 01	df=20
						p<0.66 11	
5F	n.a.	n.a.	One-Way ANOVA	p<0.0001; F=116.3	n.a.	n.a.	n.a.
56	WT574(-PEN)	p<0.0001: t=6.699:	<b>Tukey's Multiple</b> <b>Comparison</b> WT 574 v/sWT 576 WT 574 v/s WT 578 WT 574 v/s GPR83 ko 572 WT 574 v/s GPR83 ko 582 WT 574 v/s GPR83 ko 585 WT 576 v/s GPR83 ko 572 WT 576 v/s GPR83 ko 582 WT 576 v/s GPR83 ko 582 WT 578 v/s GPR83 ko 582 WT 578 v/s GPR83 ko 582 WT 578 v/s GPR83 ko 582 WT 578 v/s GPR83 ko 582 GPR83 ko 572 v/s GPR83 ko 582 GPR83 ko 582 GPR83 ko 585 GPR83 ko 585 GPR83 ko 585 GPR83 ko 585	p<0.0022; df=12 p<0.0021; df=12 p<0.0001; df=12 p<0.0001; df=12 p<0.0001; df=12 p=0.0002; df=12 p=0.0002; df=12 p<0.0001; df=12 p<0.0001; df=12 p<0.0001; df=12 p=0.9998; df=12 p=0.9056; df=12 p=0.9694; df=12	Interaction	p<0.00	F(5, 60) =
	v/s WT574(+PEN ) WT576(-PEN) v/s WT576(+PEN )	df=9.89 p<0.0011; t=4.899; df=8.201 p<0.0011; t=8.357; df=9.983 p=0.0032; t=3.953; df=9.166			Mouse type PEN conc. Tukey's Multiple Comparison WT574(-PEN)	01 p<0.00 01 p<0.00 01	13.39 F (5, 60) = 13.51 F (1, 60) = 116.7
	WT578(-PEN) v/s WT578(+PEN	p=0.5360; t=0.6415: df=9 72			v/s WT574(+PEN) WT574(-PEN)	p<0.00	df=60

)			v/s WT576(-		df=60
GPR83 ko	p=0.8096;		PEN)	p>0.99	
572(-PEN) v/s	t=0.2530; df=5.402		WT574(-PEN)	99	df=60
GPR83 ko	,		v/s		
572(+PEN)			WT576(+PEN)	p<0.00	df=60
GPR83 ko			WT574(-PEN)	01	
582(-PEN) v/s			v/s WT578(-		df=60
GPR83 ko			PEN)	p>0.99	
582(+PEN)			WT574(-PEN)	99	df=60
GPR83 ko			v/s		
585(-PEN) v/s			WT578(+PEN)	p<0.00	
GPR83 ko			WT574(-PEN)	01	df=60
585(+PEN)			v/s GPR83 ko	01	
505(11210)			572(-PEN)	n>0.99	
			WT574(-PEN)	99	df=60
			v/s GPR83 ko		ur oo
			572 (+PEN)		
			WT574(-PFN)	n=0.16	df=60
			v/s GPR 83 ko	26	ui oo
			582(-PEN)	20	
			WT574(-PEN)		df=60
			v/s GPR 83 ko	n>0.00	ui oo
			$582 (\pm DEN)$	00	
			362 (TEN) WT574(DEN)	77	df-60
			$W_{13}/4(-TEN)$		ui-00
			585( DEN)	n>0.00	
			363(-1  EN) WT574( DEN)	p>0.99	df-60
			$W 13/4(-\Gamma EN)$	99	ui–00
			V/S  OPK65 KO		df-60
			$303 (\pm \Gamma EN)$ WT574(+DEN)	m>0.00	ui–00
			$WI3/4(\pm PEN)$	p>0.99	46-(0
			V/S W 15/0(-	99	d1=60
			PEN)		16-60
			W15/4(+PEN)		d1=60
			V/S	p>0.99	16-60
			WI5/0(+PEN)	99	d1=60
			W15/4(+PEN)		
			V/S W 15/8(-		16-60
			PEN)	p<0.00	d1=60
			WI3/4(+PEN)	01	
			V/S	> 0.00	10 (0
			W15/8(+PEN)	p>0.99	d1=60
			W15/4(+PEN)	1/	
			V/S GPR83 KO	-0.00	10 (0
			3/2(-PEN)	p<0.00	d1=00
			WI3/4(+PEN)	01	
			V/S GPK83 K0		10-00
			3/2(+PEN)	p>0.98	a1=60
			WI3/4(+PEN)	51	
			v/s GPR83 ko		10 (0
			582(-PEN)	p<0.00	df=60
			W15/4(+PEN)	01	
			v/s GPR83 ko		10 (0)
			582(+PEN)	0.00	dt=60
			WT574(+PEN)	p<0.00	10 60
			v/s GPR83 ko	13	dt=60
			585(-PEN)		10 65
			WT574(+PEN)		df=60
			v/s GPR83 ko	p<0.00	
			585(+PEN)	01	df=60
			WT576(-PEN)		
			v/s		
			WT576(+PEN)	p<0.00	df=60

vis W1578(-PEN) PEN) W1576(-PEN) Vis GPR3 ko Vis			WT576(-PEN)	01	
Image: second			v/s WT578(-		
Image: space of the space o			PEN)		df=60
vs         01         di-60           WT576(-PEN)         pc0.00         di-60           VS 576(-PEN)         pc0.09         di-60           VS 576(-PEN)         pc0.09         di-60           VS 576(-PEN)         pc0.09         di-60           VS 577(-PEN)         pc0.09         di-60           VS 577(-PEN)         pc0.09         di-60           VS 577(-PEN)         pc0.99         di-60           VS 577(-PEN)         pc0.99         di-60           VS 577(-PEN)         pc0.99         di-60           VS 577(-PEN)         pc0.99         di-60           VS 577(-PEN)         pc0.01         di-60           VS 577(-PEN)			WT576(-PEN)	p<0.00	
Image: State PEN, Sta			v/s	01	
Image: state in the state			WT578(+PEN)		df=60
vis         CFRR3 ko         p-0.00           S72(PEN)         p-0.00         dF=60           vis         S72(PEN)         p-0.00         dF=60           vis         GFR83 ko         p-0.99         dF=60           vis         GFR83 ko         p-0.14         dF=60           vis         GFR83 ko         p-0.99         dF=60           vis         GFR83 ko <td></td> <td></td> <td>WT576(-PEN)</td> <td></td> <td></td>			WT576(-PEN)		
572(-PEN)     01     d**60       WT376(-PEN)     9:00     d**60       WT376(-PEN)     9:00     d**60       WT376(-PEN)     01     d**60       WT376(-PEN)     9:00     d**60       VS     9:00     d**60			v/s GPR83 ko	n<0.00	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $			572(-PEN)	01	df=60
vis (1PR3)         p=0.00         df=60           vis (2PR3)         p=0.99         df=60           vis (2PR3)         p=0.90         df=60           vis (2PR3)         p=0.90         <			WT576(-PEN)	01	ui oo
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			v/s GPR83 ko		
$\begin{array}{c c c c c c c c c c c c c c c c c c c $			572(+PEN)	n<0.00	df=60
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			WT576(_PEN)	p \0.00	ui oo
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			v/s GPR83 ko	01	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $			582(-PEN)	n>0.00	df=60
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			362(-1  EN) WT576( DEN)	p>0.33	ui=00
$ \left  \begin{array}{ccccc} & & & & & & & & & & & & & & & & &$			W I 3 / 0 (-F E IN)	99	46-60
$ \begin{vmatrix} 332 (+PEX) & 0 & 0 & 0 & 0 \\ VX 576(+PEX) & 0 & 0 & 0 & 0 \\ VX 576(+PEX) & p & 0 & 0 & 0 \\ VX 576(+PEX) & p & 0 & 0 & 0 \\ VX 5776(+PEX) & 0 & 0 & 0 & 0 \\ VX 5778(+PEX) & 0 & 0 & 0 & 0 \\ VX 5778(+PEX) & 0 & 0 & 0 & 0 \\ VX 5778(+PEX) & 0 & 0 & 0 & 0 \\ VX 5778(+PEX) & 0 & 0 & 0 & 0 \\ VX 5778(+PEX) & 0 & 0 & 0 & 0 \\ VX 5778(+PEX) & 0 & 0 & 0 & 0 \\ VX 5778(+PEX) & 0 & 0 & 0 & 0 \\ VX 5778(+PEX) & 0 & 0 & 0 \\ VX $			V/S GPK85 K0 582( $\pm$ DEN)	m<0.00	d1-00
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			$362(\pm PEN)$	p<0.00	16-00
$ \begin{vmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0$			W I 3 / 0 (-PEIN)	01	d1-00
$ \left  \begin{array}{c c c c c c c c c c c c c c c c c c c $			V/S UPK83 KO	m> 0.00	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			363(-PEN)	p>0.99	16-00
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			WI3/6(-PEN)	99	ai=60
$ \left  \begin{array}{c c c c c c } & S35(+FEN) & p=0.14 \\ V:S WT578(+PEN) & p=0.14 \\ V:S WT578(+PEN) & p=0.99 \\ WT576(+PEN) & 99 \\ WT576(+PEN) & 99 \\ WT576(+PEN) & 99 \\ WT576(+PEN) & p>0.99 \\ WT576(+PEN) & p>0.09 \\ WT576(+PEN) & p>0.09 \\ WT576(+PEN) & p>0.00 \\ WT576(+PEN) & 01 \\ V:S GPR33 ko \\ S55(+PEN) & p=0.45 \\ WT578(+PEN) & 01 \\ V:S GPR33 ko \\ S55(+PEN) & p=0.45 \\ WT578(+PEN) & 01 \\ V:S GPR33 ko \\ S572(+PEN) & 01 \\ V:S GPR33 ko \\ S572(+PEN) & 01 \\ V:S GPR33 ko \\ S572(+PEN) \\ WT578(+PEN) \\ WT578(+PEN) \\ V:S GPR33 ko \\ S72(+PEN) \\ WT578(+PEN) \\ WT578(+$			V/S GPR83 KO		
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			585(+PEN)	0.1.4	10 60
$ \begin{vmatrix} v \otimes W 15/8(- PEN) & d^{1-60} \\ V \otimes W 15/8(+PEN) & d^{1-60} \\ V \otimes V$			W1576(+PEN)	p=0.14	dt=60
PEN)       PEN)       df=60         WT576(+PEN)       99         WT576(+PEN)       99         WT576(+PEN)       90         V/s GPR83 ko       572(PEN)         S82(-PEN)       90         WT576(+PEN)       90         V/s GPR83 ko       90.9         S82(-PEN)       99         V/s GPR83 ko       90.9         S85(-PEN)       90         V/s GPR83 ko       585(-PEN)         V/s GPR83 ko       585(-PEN)         V/s GPR83 ko       585(-PEN)         V/s GPR83 ko       10         V/s GPR83 ko       11			v/s W1578(-	42	
w1576(+PEN)       w1576(+PEN)       y20.9         w1576(+PEN)       99       w1576(+PEN)       y20.99         w1576(+PEN)       p20.99       w1576(+PEN)       y20.99         w1576(+PEN)       y20.99       w156(+PEN)       y46(+PEN)         w1576(+PEN)       y20.99       y362(+PEN)       y46(+PEN)         w1576(+PEN)       y20.00       w1576(+PEN)       y20.00         w1576(+PEN)       y20.00       w1576(+PEN)       y20.00         w1576(+PEN)       y20.00       w1576(+PEN)       y20.00         w1578(+PEN)       y20.00       w1578(+PEN)       y20.00         w1578(+PEN)       y20.00       w1578(+PEN)       y20.00         w1578(+PEN)       y20.00       w1578(+PEN)       y20.00         w1578(+PEN)       y20.00       w1578(+PEN)       y20.00         w1578(+PEN)       y20.00       y36(+PEN)       y20.00         v16 (PB38 ko       y72(+PEN)       y2			PEN)		
$ \left  \begin{array}{c c c c c c c c } & & & & & & & & & & & & & & & & & & &$			WT576(+PEN)		df=60
W1578(+PEN)       99         W1576(+PEN)       p>0.99         Vs GPR83 ko       99         S82(-PEN)       gf=60         W1576(+PEN)       p>0.99         df=60       W1576(+PEN)         W1576(+PEN)       p>0.99         S82(-PEN)       99         df=60       W1576(+PEN)         Vs GPR83 ko       p<0.00			v/s	p>0.99	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			WT578(+PEN)	99	
v/s GPR83 ko       572(PEN)       p>0.99         WT576(+PEN)       99       df=60         V/s GPR83 ko       572(+PEN)       p>0.99         WT576(+PEN)       p>0.99       df=60         V/s GPR83 ko       99       df=60         V/s GPR83 ko       p>0.99       gf=60         V/s GPR83 ko       p>0.99       df=60         V/s GPR83 ko       p>0.99       df=60         WT576(+PEN)       99       df=60         WT576(+PEN)       p<0.00			WT576(+PEN)		df=60
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			v/s GPR83 ko		
$ \begin{array}{ c c c c c } & WT576(+PEN) & 99 & df=60 \\ & V's GPR33 ko \\ 572(+PEN) & WT576(+PEN) & p>0.99 \\ & V's GPR33 ko & 99 & df=60 \\ & WT576(+PEN) & WT576(+PEN) & y>0.99 \\ & S82(-PEN) & 99 & df=60 \\ & WT576(+PEN) & y>0 & df=60 \\ & WT576(+PEN) & y>0 & df=60 \\ & WT576(+PEN) & y>0.00 & df=60 \\ & WT576(+PEN) & 01 & y's GPR33 ko & 585(-PEN) & 01 \\ & V's GPR33 ko & 585(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 572(-PEN) & 01 \\ & V's GPR33 ko & 77 \\ & 572(+PEN) & 01 \\ & V's GPR33 ko & 70 \\ & V's GPR3 ko & 70 \\ & V's GPR3 ko & 70 \\ & V's GPR3 ko $			572(-PEN)	p>0.99	
$ \left  \begin{array}{c c c c c c c c c c c c c c c c c c c $			WT576(+PEN)	99	df=60
$ \left  \begin{array}{c c c c c c c c c c c c c c c c c c c $			v/s GPR83 ko		
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			572(+PEN)		
v/s GPR83 ko       99         582(-PEN)       df=60         WT576(+PEN)       v/s GPR83 ko         v/s GPR83 ko       p>0.99         582(+PEN)       99         df=60         WT576(+PEN)         v/s GPR83 ko         585(-PEN)         v/s GPR83 ko         585(-PEN)         v/s GPR83 ko         585(+PEN)         v/s GPR83 ko         572(-PEN)         v/s GPR83 ko         572(-PEN)         v/s GPR83 ko         572(+PEN)         v/s GPR83 ko			WT576(+PEN)	p>0.99	df=60
582(-PEN)       df=60         WT576(+PEN)       v's GPR83 ko         y's GPR83 ko       p>0.99         585(-PEN)       p<0.00			v/s GPR83 ko	99	
$ \left  \begin{array}{c c c c c c c c c c c c c c c c c c c $			582(-PEN)		df=60
$ \begin{vmatrix} v'_{S} GPR3 k_{O} & p>0.99 \\ S82(+PEN) & 99 & df=60 \\ WT576(+PEN) & v'_{S} GPR3 k_{O} & 585(-PEN) & p<0.00 & df=60 \\ WT576(+PEN) & 01 & v'_{S} GPR3 k_{O} & 585(+PEN) & p=0.45 & df=60 \\ WT578(-PEN) & 01 & v'_{S} & WT578(-PEN) & 01 & v'_{S} & WT578(-PEN) & 01 \\ v'_{S} & WT578(-PEN) & 01 & v'_{S} & GPR3 k_{O} & 572(-PEN) & 01 \\ v'_{S} GPR3 k_{O} & 572(-PEN) & 01 & v'_{S} GPR3 k_{O} & 572(-PEN) & df=60 \\ WT578(-PEN) & 01 & v'_{S} GPR3 k_{O} & 47 & 572(-PEN) & df=60 \\ WT578(-PEN) & v'_{S} GPR3 k_{O} & 47 & df=60 \\ WT578(-PEN) & v'_{S} GPR3 k_{O} & 47 & df=60 \\ WT578(-PEN) & v'_{S} GPR3 k_{O} & 47 & df=60 \\ WT578(-PEN) & v'_{S} GPR3 k_{O} & 47 & df=60 \\ WT578(-PEN) & v'_{S} GPR3 k_{O} & 47 & df=60 \\ WT578(-PEN) & v'_{S} GPR3 k_{O} & 47 & df=60 \\ WT578(-PEN) & v'_{S} GPR3 k_{O} & df=60 \\ WT578(-PEN) & v'_{S} GPR3 k_{O}$			WT576(+PEN)		
$ \left  \begin{array}{c c c c c c c c c c c c c c c c c c c $			v/s GPR83 ko	p>0.99	
WT576(+PEN)       v/s GPR33 ko         585(-PEN)       p<0.00			582(+PEN)	99	df=60
v/s GPR83 ko       p<0.00			WT576(+PEN)		
$ \left  \begin{array}{c c c c c c c c c c c c c c c c c c c $			v/s GPR83 ko		
WT576(+PEN)       01         v/s GPR83 ko       p=0.45         S85(+PEN)       p=0.45         WT578(-PEN)       01         v/s       WT578(+PEN)         WT578(-PEN)       01         v/s       GPR83 ko         S72(-PEN)       01         v/s GPR83 ko       572(-PEN)         WT578(-PEN)       p=0.04         v/s GPR83 ko       47         S72(+PEN)       df=60         WT578(-PEN)       p<0.04			585(-PEN)	p<0.00	df=60
v/s GPR83 ko       p=0.45       df=60         WT578(-PEN)       01       v/s         WT578(+PEN)       p<0.00			WT576(+PEN)	01	
$ \left  \begin{array}{c c c c c c c c c c c c c c c c c c c $			v/s GPR83 ko		
WT578(-PEN)       01         v/s       WT578(+PEN)         WT578(-PEN)       01         WT578(-PEN)       01         v/s GPR83 ko       01         572(-PEN)       df=60         WT578(-PEN)       p=0.04         v/s GPR83 ko       47         572(+PEN)       df=60         WT578(-PEN)       p=0.04         v/s GPR83 ko       47         572(+PEN)       df=60         WT578(-PEN)       v/s GPR83 ko         v/s GPR83 ko       p<0.00			585(+PEN)	p=0.45	df=60
v/s       wT578(+PEN)       p<0.00			WT578(-PEN)	01	
WT578(+PEN)       p<0.00			v/s		
WT578(-PEN)       01         v/s GPR83 ko       01         v/s GPR83 ko       572(-PEN)         WT578(-PEN)       p=0.04         v/s GPR83 ko       47         572(+PEN)       df=60         WT578(-PEN)       v/s GPR83 ko         v/s GPR83 ko       p<0.00			WT578(+PEN)	p<0.00	df=60
v/s GPR83 ko       v/s GPR83 ko       df=60         572(-PEN)       p=0.04       v/s GPR83 ko       47         v/s GPR83 ko       47       df=60         WT578(-PEN)       wT578(-PEN)       df=60         WT578(-PEN)       v/s GPR83 ko       p<0.00			WT578(-PEN)	01	
572(-PEN)       p=0.04         WT578(-PEN)       p=0.04         v/s GPR83 ko       47         572(+PEN)       df=60         WT578(-PEN)       df=60         WT578(-PEN)       of=60         WT578(-PEN)       01         v/s GPR83 ko       p<0.00			v/s GPR83 ko		
WT578(-PEN)       p=0.04         v/s GPR83 ko       47         572(+PEN)       df=60         WT578(-PEN)       v/s GPR83 ko         v/s GPR83 ko       p<0.00			572(-PEN)		df=60
v/s GPR83 ko       47         572(+PEN)       df=60         WT578(-PEN)       v/s GPR83 ko         v/s GPR83 ko       p<0.00			WT578(-PEN)	p=0.04	
572(+PEN)       df=60         WT578(-PEN)       v/s GPR83 ko         v/s GPR83 ko       p<0.00			v/s GPR83 ko	47	
WT578(-PEN)       p<0.00			572(+PEN)		df=60
v/s GPR83 ko     p<0.00			WT578(-PEN)		
582(-PEN) 01 df=60 WT578(-PEN) v/s GPR83 ko			v/s GPR83 ko	p<0.00	
WT578(-PEN) v/s GPR83 ko			582(-PEN)	01	df=60
v/s GPR83 ko			WT578(-PEN)		
		 	 v/s GPR83 ko		

			0.00	10 60
		582(+PEN)	p<0.00	df=60
		WT578(-PEN)	01	
		v/s GPR83 ko		
		585(-PEN)		df=60
		WT578(-PEN)	n<0.00	
		v/c GDD 82 ko	01	
		$\sqrt{5}$ OF ROJ KU	01	16-00
		585(+PEN)		d1=60
		W15/8(+PEN)		
		v/s GPR83 ko	p<0.00	
		572(-PEN)	01	df=60
		WT578(+PEN)		
		v/s GPR83 ko		
		572(+PEN)	p < 0.00	df=60
		372(+12N) WT579(+DEN)	p <0.00	<b>u</b> 1 00
		$WIJ/\delta(\pm PEN)$	01	
		V/S GPR83 KO		
		582(-PEN)	p>0.99	df=60
		WT578(+PEN)	99	
		v/s GPR83 ko		
		582(+PEN)		df=60
		WT578(+PEN)	p=0.14	
		v/s GPR 83 ko	10	
		$\sqrt{5}$ OI ROJ KU	19	16-00
		585(-PEN)		d1=60
		W15/8(+PEN)		
		v/s GPR83 ko	p>0.99	
		585(+PEN)	99	df=60
		GPR83 ko 572(-		
		PEN) v/s GPR83		
		$k_0 572(+PEN)$	n>0.99	
		GDD83 ko 572(	00	df-60
		$\frac{\text{OF NO5 KO 572}}{\text{DEN}} = \sqrt{2} \frac{\text{CDD}}{2}$	<u>,,</u>	ui=00
		PEN) V/S GPK83		
		ko 582(-PEN)		
		GPR83 ko 572(-	p>0.99	
		PEN) v/s GPR83	99	df=60
		ko 582(+PEN)		
		GPR83 ko 572(-		
		PEN) v/s GPR83	n>0.99	
		$1 E(0) \sqrt{3} O(1003)$	p> 0.77	df-60
		K0.363(-PEN)	99	d1-00
		GPR83 ko 5/2(-		
		PEN) v/s GPR83		
		ko 585(+PEN)	p<0.00	
		GPR83 ko	01	df=60
		572(+PEN) v/s		
		GPR83 ko 582(-		
		PEN)	n<0.00	df=60
		GDR 82 kg	P .0.00	<b>a</b> 1 00
		577(_DEN)/~	01	
		$J/2(\top \Gamma E IN) V/S$		16-60
		GPK83 KO	0.65	d1=60
		582(+PEN)	p<0.00	
		GPR83 ko	01	
		572(+PEN) v/s		df=60
		GPR85 ko 582(-		
		PEN)	p<0.00	
		GPR83 kg	01	
		577(_DEN)/~	01	df-60
		$J/2(\pm PEIN) V/S$		ui-00
		GPK83 ko		
		585(+PEN)	p<0.00	
		GPR83 ko 582(-	01	df=60
		PEN) v/s GPR83		
		ko 582(+PEN)		
		GPR83 ko 582(-	n<0.00	
		PEN) v/s GDD 22	01	df=60
		$\frac{1}{100} \frac{505}{100} \frac{1}{100} \frac{505}{100} \frac{1}{100} \frac{505}{100} \frac{1}{100} \frac{505}{100} \frac{1}{100} \frac{1}{1$	01	ui=00
		KU 303(-PEN)		

- I			CDD021 502/		
			GPR83 ko 582(-		
			PEN) v/s GPR83	p=0.13	
			ko 585(+PEN)	96	
			GPR83 ko		
			582(+PEN) v/s		
			GPR83 ko 585(-	p>0.99	
			PEN)	99	
			GPR83 ko		
			582(+PEN) v/s		
			GPR83 ko	n>0 99	
			585(+PEN)	99	
			GPR83 ko 585(-	,,	
			PEN v/s GPR 83		
			$k_0 585(+\text{PEN})$	n>0.00	
			KU 303(+1 EIN)	p>0.99	
				77	
				> 0.00	
				p>0.99	
				99	
				p=0.14	
				40	
				p=0.47	
				28	
				-	
				n=0.14	
				24	
				54	
				<i>m</i> =0.10	
				p=0.19	
				80	
				p>0.99	
				99	
				_	
				p>0.99	
				99	
				p>0.99	
				99	
				p>0 99	
				99	
				,,	
				n > 0.00	
				p~0.99 00	
				77	

						p>0.99 99	
5H	WT (-PEN) v/s WT (+PEN) GPR83 ko (- PEN) v/s GPR83 ko(+PEN)	p<0.0372; t=1.969; df=11.06 p=0.3553; t=0.3781; df=15.18			Interaction Mouse type PEN conc. <b>Tukey's</b> Multiple Comparison WT (-PEN) v/s WT (-PEN) v/s GPR83 ko (- PEN) WT (-PEN) v/s GPR83 ko (- PEN) WT (+PEN) v/s GPR83 ko (- PEN) WT (+PEN) v/s GPR83 ko (- PEN) WT (+PEN) v/s GPR83 ko (- PEN) v/s GPR83 ko (+PEN)	p=0.09 85 $p<0.05$ 53 $p<0.24$ 21 $p=0.04$ 81 $p>0.99$ 99 $p=0.99$ 99 $p=0.04$ 81 $p=0.06$ 68 $p=0.99$ 99	F (1, 44) = 2.850 $F (1, 44) = 1.401$ $F (1, 44) = 3.877$ $df=44$ $df=44$ $df=44$ $df=44$ $df=44$ $df=44$
6B	hGPR83 v/s hGPR83+mGP R171 (t)	p=0.0092; t=4.049; df=5.156	n.a.	n.a.	n.a.	n.a.	n.a.
7B			One-way ANOVA Tukey's Multiple Comparison GPR171+GPR83+ v/s GPR171+GPR83- GPR171+GPR83+ v/s GPR171-GPR83+ GPR171+GPR83- v/s GPR171-GPR83+	<pre>p&lt;0.0001; F=157.3 p &lt; 0.0001; df= 87 p &lt; 0.0001; df = 87 p = 0.4180 ; df = 87</pre>			