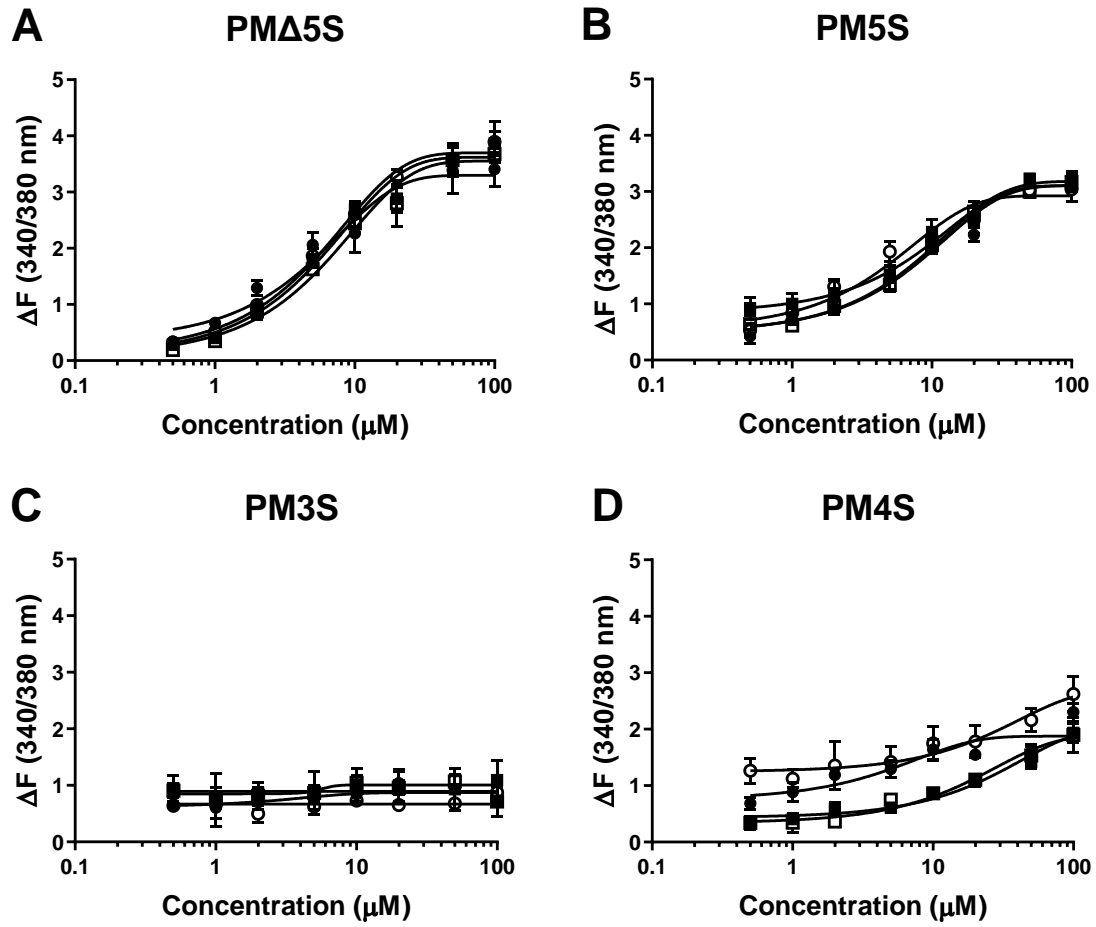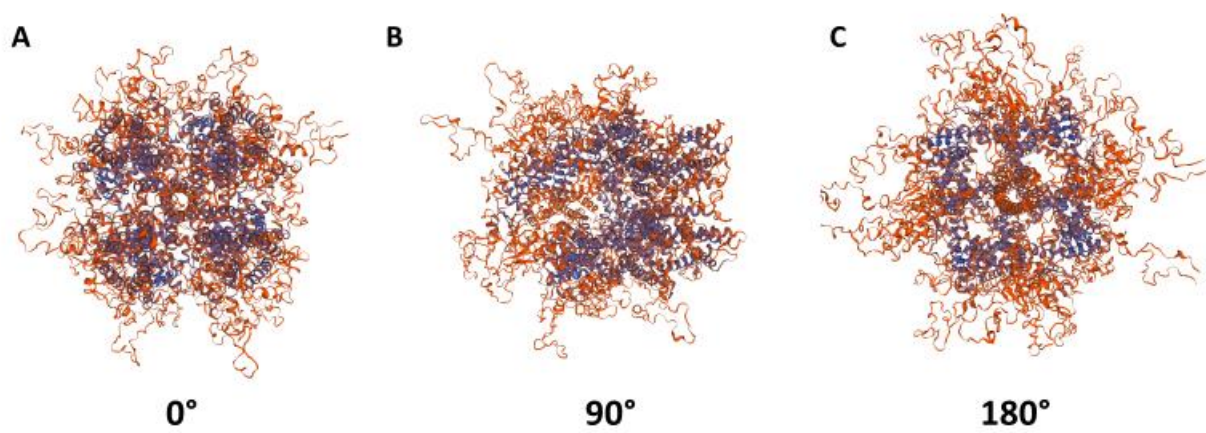Supplementary Figure 1: PM5S increases glucose-stimulated insulin secretion in murine islets and is not altered in mice deficient of TGR5 or FXR

Islets from mice were isolated and incubated with PM5S at low (2 or 3 mmol/L) and high (20 mmol/L) concentrations of glucose. Insulin secretion in response to incubation with PM5S was assessed from islets that were isolated from (A) WT and Tgr5^{-/-} mice or (B) WT and Fxr^{-/-} mice. Unless indicated differences between groups were not significant; significance differences are indicated by: * P < 0.05, *** P < 0.001 as determined by one-way ANOVA followed by Tukey's multiple comparisons test or Kruskal-Wallis test followed by Dunn's multiple comparisons test. Data expressed as mean ± SEM, for each graph n= 3 independent experiments, each group contained 5 sized matched islets.
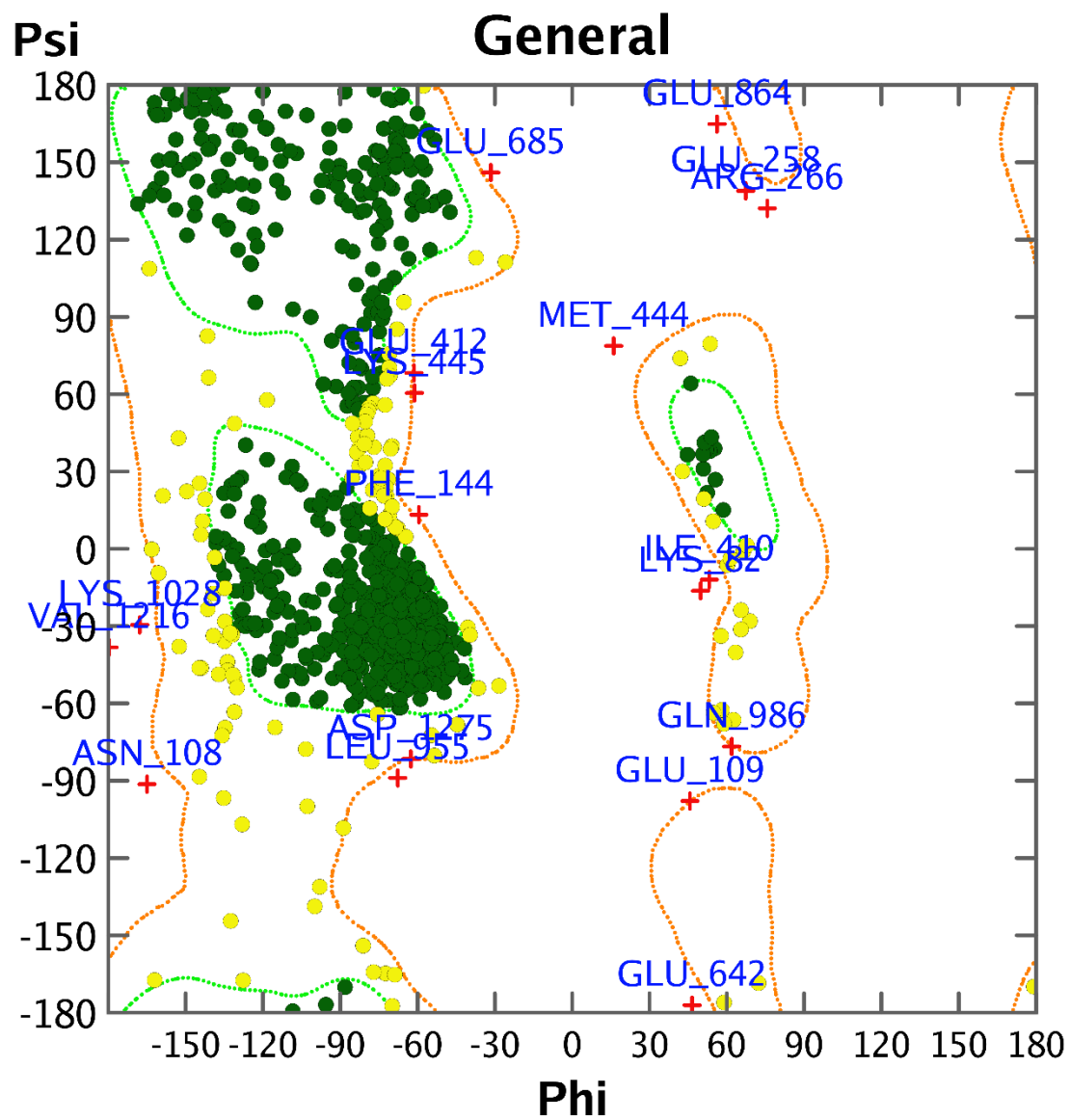
Supplementary Figure 2: Ca^{2+} concentration is altered by PMΔ5S, PM5S and PM4S

HEK cells transfected with TRPM3 were dyed with Fura-2 to image calcium fluorescence. Increasing concentrations of progesterone sulfates were given. Each graph displays 4 replicate experiments for each progesterone sulfate investigated. (A) PMΔ5S, (B) PM5S, (C) PM3S, (D) PM4S. n= 4 independent experiments for each progesterone sulfate.
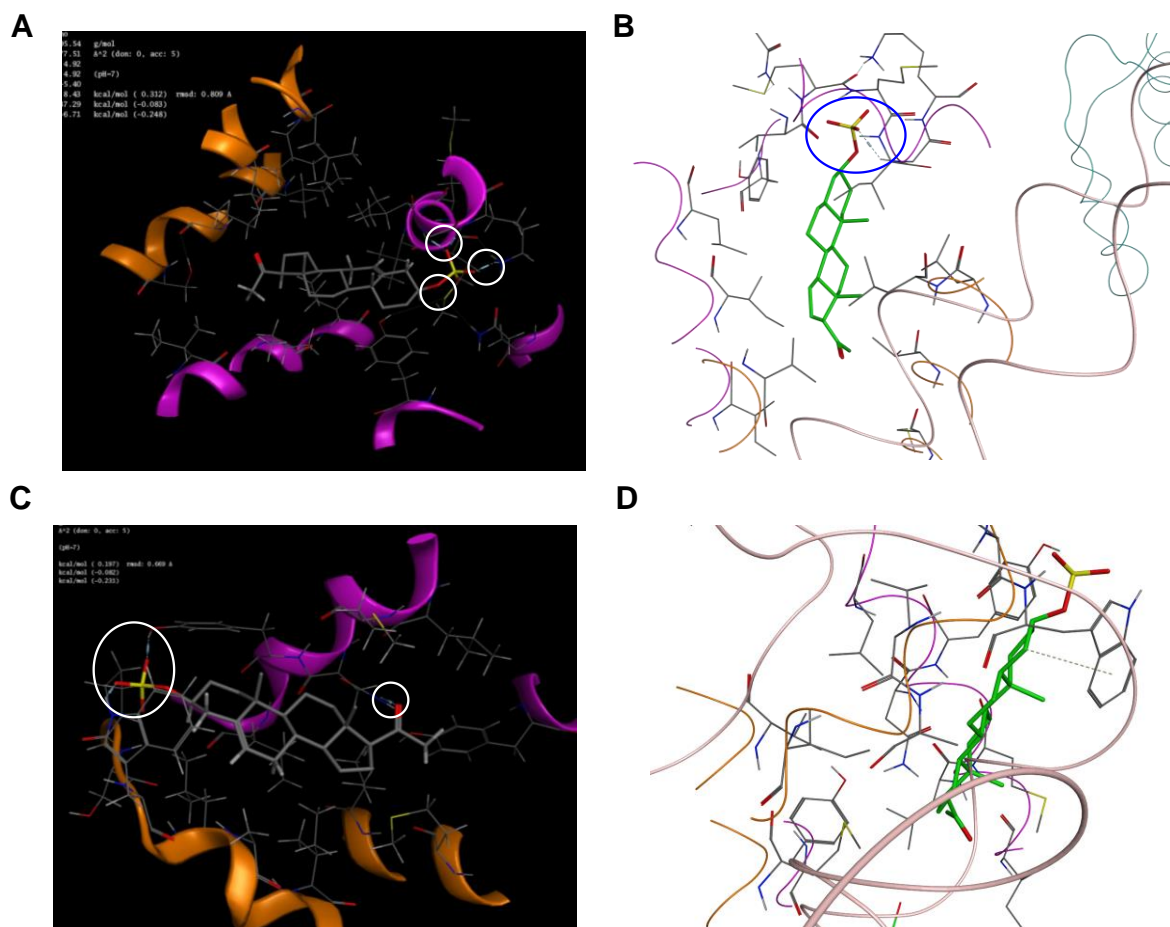
Supplementary Figure 3: Computational structure of TRPM3

Homology model of TRPM3 generated using TRPM7 (PDB code 5ZX5) as a template. TRPM3 is represented at different horizontal rotations: (A) 0° (B) 90° (C) 180°.

Supplementary Figure 4: Ramachandran plot of human TRPM3.

Supplementary Figure 5: 3D images of PMΔ5S and PM5S binding to first 2 sites

Progesterone sulfate binding at both sites. (A) and (B) shows the 3D structure of pregnenolone sulfate (PMΔ5S, carbon backbone in grey) and epiallopregnanolone sulfate (PM5S, carbon backbone in green), respectively, binding at site 1. The 3D structures of pregnenolone sulfate and epiallopregnanolone sulfate binding at site 2 is shown in (C) and (D) respectively. The white and blue circles highlight key residue interaction with ligand according to Supplementary Figure 6.

Supplementary Figure 6: Ligand interactions with PMΔ5S and PM5S in sites 1 and 2

Ligand interaction at site 1 (A and B) and site 2 (C and D) for pregnenolone sulfate (PMΔ5S) (A+C) and

epiallopregnanolone sulfate (PM5S) (B+D).

Age (years)	Gender	BMI kg/m^2
46	Female	29.03
38	Female	27
40	Female	27.01
43	Female	30

Supplementary Table 1: Details of each donor human islets

Cohort 2 – BMI correlation		
Progesterone Sulfate	P	Rho
PM5S	0.0166	0.255
PM3S	0.0020	0.424
PM3DiS	0.4682	-0.078
PM2DiS	0.5730	-0.061
PM4S	0.0112	-0.269
PMΔ5S	0.0410	-0.218

Supplementary Table 2: BMI correlations with progesterone sulfates in women with GDM (Cohort 2).

Spearman's rank correlation coefficient (Rho) was used to assess correlations.

Cohort 2	PM3S	PM2DiS	PM3DiS	PM5S	PM4S	PMΔ5S
All patients (Non-GDM and GDM)	-0.145	0.029	-0.065	0.025	-0.029	-0.119
GDM Patients only	-0.215	0.229	0.088	-0.022	-0.182	-0.342
Cohort 3						
All patients (Non-GDM and GDM)	-0.254*	-0.067	0.076	-0.333*	-0.239*	-0.060
GDM Patients only	-0.121	0.018	0.136	-0.082	-0.036	-0.034

Supplementary Table 3: Fasting glucose correlations with progesterone sulfates in women with GDM (Cohort 2+3).

Spearman's rank correlation coefficient was used to assess correlations, results with * signifies $P < 0.05$. Cohort 2, all patients n= 89, GDM patients only n= 25. Cohort 3 each group were collated irrespective of BMI, all patients n= 266, GDM patients only n= 114.

A

Cohort 4: BMI≤25 kg/m²			
Biochemical marker	OR (95% CI)	P	Area under ROC curve
PM5S	0.82 (0.46-1.45)	0.49	0.46 (0.34-0.57)
PM3S	0.98 (0.51-1.86)	0.94	0.50 (0.38-0.61)

B

Cohort 4: BMI≥35 kg/m²			
Biochemical marker	OR (95% CI)	P	Area under ROC curve
PM5S	1.66 (0.99-2.77)	0.05	0.60 (0.49-0.72)
PM3S	0.76 (0.38-1.53)	0.44	0.46 (0.35-0.58)

Supplementary Table 4: Logistic regression analysis of PM5S and PM3S as predictors of GDM at 11-13 weeks gestation.

A

Energy (kcal/mol)	RMSD	Ligand
-6.8199997	1.1125467	PM5S
-6.6702867	1.4927102	PMΔ5S
-6.5948405	2.1555789	PMΔ5S
-6.5424743	1.2312251	PM4S
-6.439683	1.8100771	PM5S
-6.4153433	2.1325195	PMΔ5S
-6.4101419	1.6385957	PM3S
-6.4068756	1.9603847	PM5S
-6.3827085	1.5345517	PMΔ5S
-6.3573208	1.8047764	PMΔ5S
-6.3540144	2.3486347	PM3S
-6.3538809	2.0648024	PM5S
-6.3411345	2.4843247	PM5S
-6.0677705	3.4442627	PM3S
-6.063601	1.3541986	PM4S
-5.8733807	1.5544147	PM4S
-5.8704696	2.3242292	PM3S
-5.8615518	1.7133332	PM4S
-5.8240767	2.1893353	PM3S
-5.7720551	1.5481328	PM4S

B

Energy (kcal/mol)	RMSD	Ligand
-6.8524556	1.4652321	PMΔ5S
-6.7510605	1.3558685	PM5S
-6.6705761	0.96520036	PMΔ5S
-6.6407328	3.300529	PM5S
-6.5805449	1.3545821	PMΔ5S
-6.5199375	2.2168357	PMΔ5S
-6.4493017	1.5938253	PMΔ5S
-6.3897424	3.51401	PM3S
-6.3854003	2.3206003	PM5S
-6.3802776	2.2265522	PM3S
-6.322556	1.9600726	PM3S
-6.3022604	2.1742473	PM5S
-6.2860308	1.9575782	PM5S
-6.2518678	2.4348776	PM3S
-6.2481518	0.88207519	PM4S
-6.1969061	4.4030252	PM3S
-6.0141978	1.0526633	PM4S
-6.0022035	2.6718736	PM4S
-5.987834	0.97104383	PM4S
-5.9865279	2.0926847	PM4S

	Energy (kcal/mol)	RMSD	Ligand
C	-6.0231051	2.7763674	PM5S
	-5.9996939	1.6458317	PM5S
	-5.9769263	1.0221686	PM5S
	-5.9701791	2.7118475	PMΔ5S
	-5.9436126	3.4987359	PMΔ5S
	-5.9239669	1.8993984	PMΔ5S
	-5.9137206	3.8773561	PM3S
	-5.9122968	2.45784	PM5S
	-5.8920221	1.3614609	PM4S
	-5.8661146	2.0471373	PM5S
	-5.8643146	1.8455839	PMΔ5S
	-5.8574042	1.4660836	PM4S
	-5.8362899	4.5342412	PM3S
	-5.754652	1.5124441	PMΔ5S
	-5.6872506	2.2099655	PM4S
	-5.6868806	3.8968232	PM3S
	-5.5618906	1.6737609	PM4S
	-5.5287905	3.2175353	PM3S
	-5.5134492	3.1202154	PM3S
	-5.4297891	1.4013846	PM4S

Supplementary Table 5: Table detailing the affinity of the poses the progesterone sulfates form at each docking site.

(A) and (B) shows the positions tested in sites 1 and 2 respectively, the highlighted row indicates the best position PM5S binds in both sites. (C) shows the positions tested in site 3. PMΔ5S, PM3S, PM4S and PM5S were all tested at these docking sites. The energy represents the affinity for that dock pose in the active site. RMSD, root-mean-square deviation of atomic position.