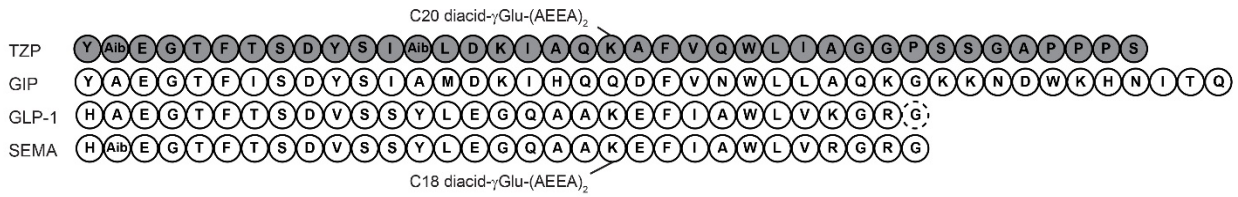
Supplemental Information – Table & Figures

Tirzepatide is an Imbalanced and Biased Dual GIP/GLP-1 Receptor Agonist

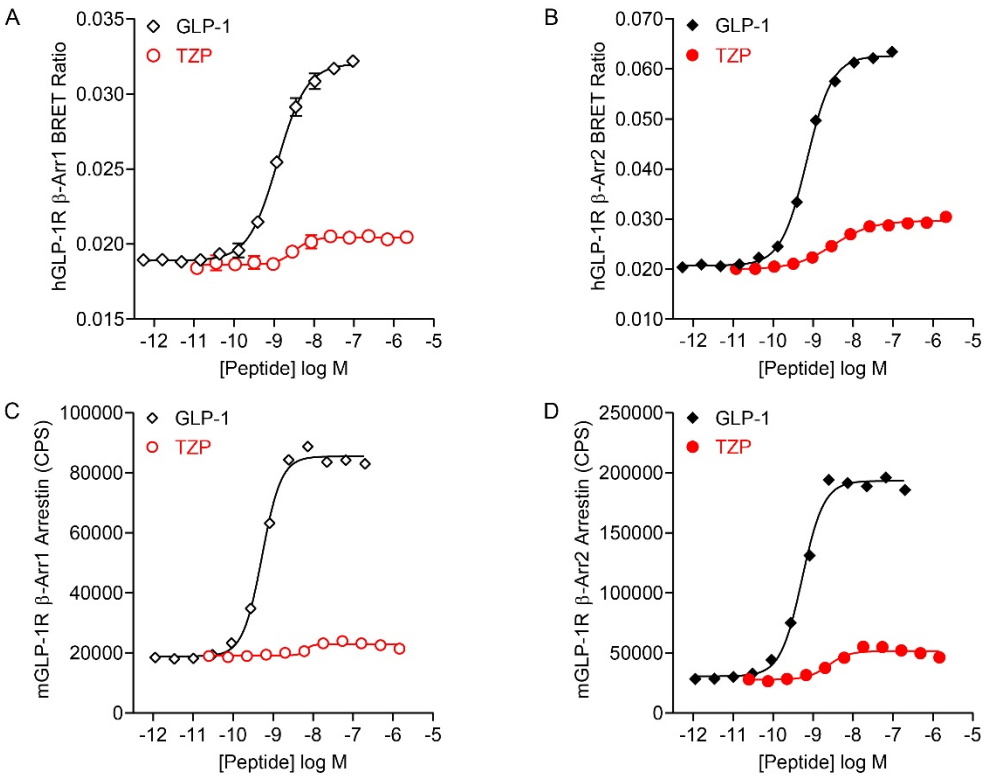
	[^{125}I]GIP Binding		GIPR Low Density cAMP			GIPR Medium Density cAMP			GIPR High Density cAMP		
Ligand	K_i nM [A] (SEM, n)	K_i [B] Ratio	Rec/Cell [C] (SEM, n)	EC_{50} nM (SEM, n)	EC_{50} [D] Ratio	Rec/Cell [C] (SEM, n)	EC_{50} nM (SEM, n)	EC_{50} [D] Ratio	Rec/Cell [C] (SEM, n)	EC_{50} nM (SEM, n)	EC_{50} [D] Ratio
GIP	0.203 (0.033, 12)	1.00		0.888 (0.114, 49)	1.00		0.0461 (0.0084, 12)	1.00		0.00224 (0.00040, 6)	1.00
Tzp	0.163 (0.029, 6)	1.25	1,700 (500, 5)	1.01 (0.15, 23)	0.88	6,400 (900, 6)	0.0536 (0.0157, 8)	0.86	86,000 (29,000, 6)	0.00196 (0.00019, 2)	1.14
Sema	>500 (ND, 2)	ND		>850 (ND, 10)	ND		>100 (ND, 2)	ND		80.3 (16.1, 2)	0.00

	[^{125}I]GLP-1 Binding		GLP-1R Low Density cAMP			GLP-1R Medium Density cAMP			GLP-1R High Density cAMP		
Ligand	K_i nM [A] (SEM, n)	K_i [B] Ratio	Rec/Cell [C] (SEM, n)	EC_{50} nM (SEM, n)	EC_{50} [D] Ratio	Rec/Cell [C] (SEM, n)	EC_{50} nM (SEM, n)	EC_{50} [D] Ratio	Rec/Cell [C] (SEM, n)	EC_{50} nM (SEM, n)	EC_{50} [D] Ratio
GLP-1	0.655 (0.055, 12)	1.00		0.366 (0.032, 57)	1.00		0.0721 (0.0061, 24)	1.00		0.00624 (0.00056, 14)	1.00
Tzp	2.94 (0.33, 6)	0.22	1,400 (200, 5)	6.54 (0.71, 22)	0.06	7,800 (1,300, 6)	1.38 (0.19, 11)	0.05	76,000 (10,000, 7)	0.132 (0.006, 9)	0.05
Sema	1.10 (0.28, 5)	0.60		0.364 (0.068, 9)	1.01		0.0571 (0.0117, 4)	1.26		0.00671 (0.00042, 3)	0.93

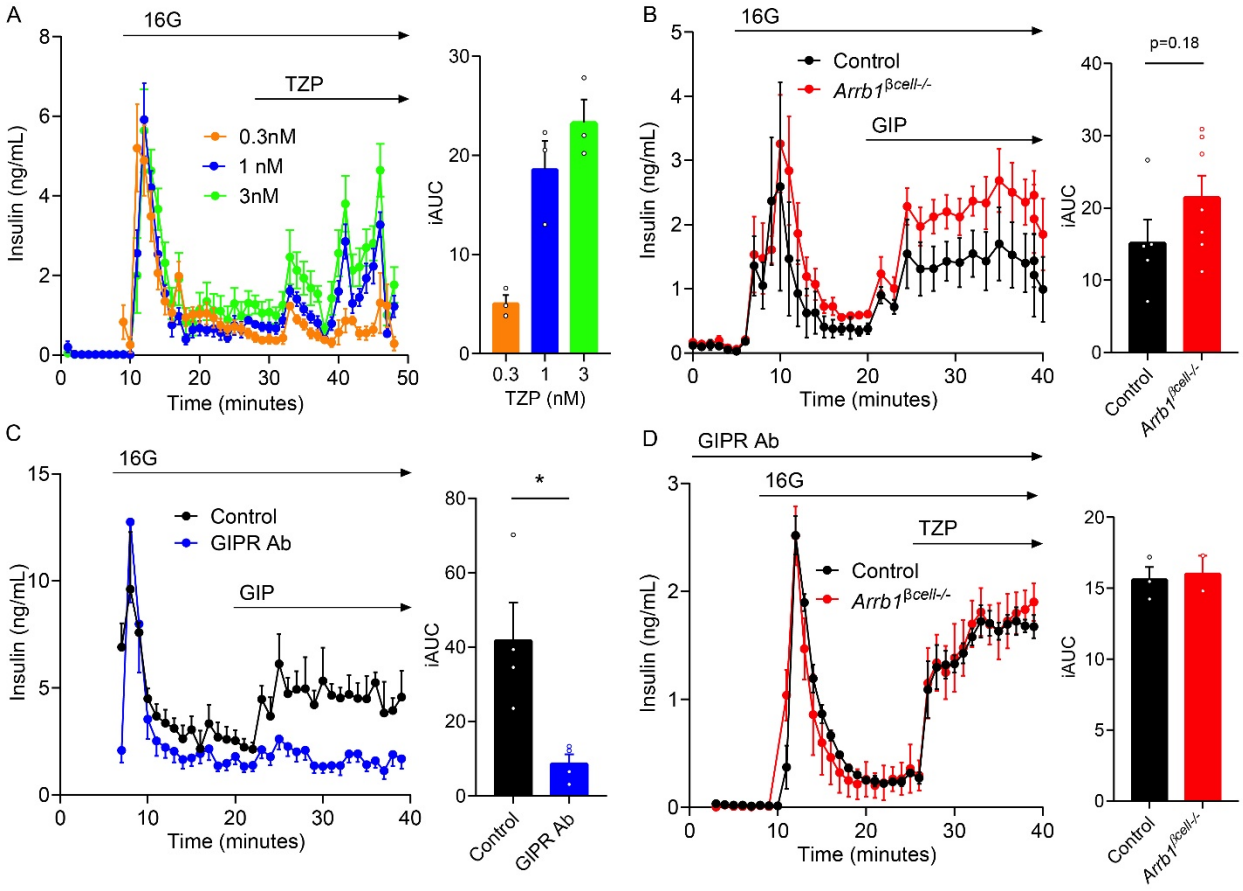
Supplemental Table 1. *In Vitro* Activity of Tirzepatide in Transgenic Cell Lines with Varying Receptor Densities. [A] Affinities were calculated from competition binding data using the Cheng-Prussoff correction (*Biochem Pharmacol.* 1973;22(23):3099-108). [B] Calculated by ratio of native ligand K_i to test ligand K_i. [C] Receptors per cell calculated by radioligand binding B_{MAX} determinations. [D] Calculated by ratio of native ligand EC_{50} to test ligand EC_{50}.



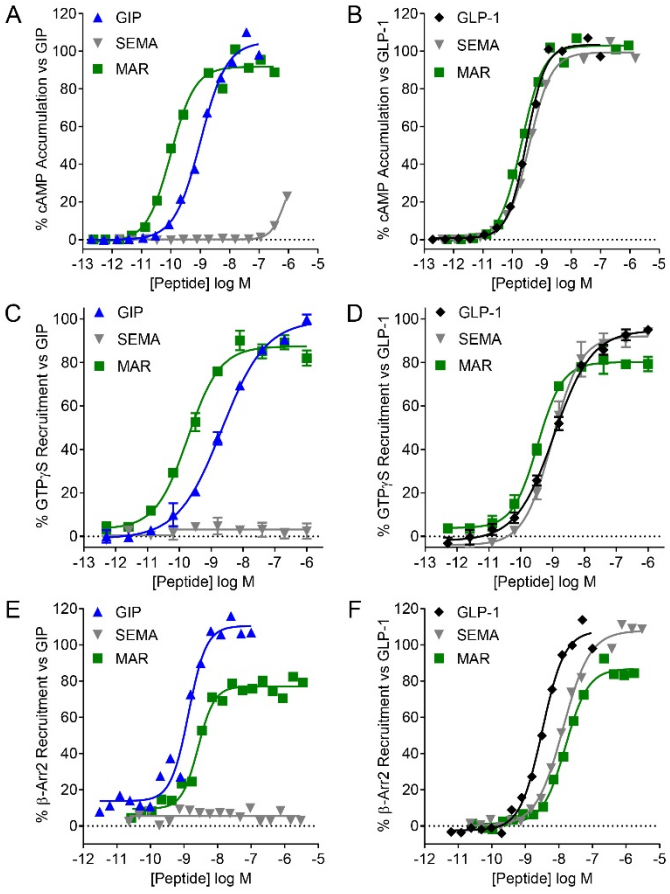
Supplemental Figure 1. Multiple Sequence Alignment of Ligands. Depicted is an amino acid sequence comparison of tirzepatide (TZP), human GIP(1-42), human GLP-1(7-36), and semaglutide (SEMA). For tirzepatide and semaglutide, the locations of the engineered acyl moieties are indicated. The dotted circle at the end of the GLP-1 sequence represents the terminal glycine residue that is present in the naturally occurring GLP-1(7-37) variant.



Supplemental Figure 2. β-Arrestin1 and β-Arrestin2 Recruitment to the GLP-1 Receptor. Arrestin recruitment was measured using bioluminescence resonance energy transfer (BRET) and NanoLuciferase complementation (NanoLuc). (**A**) Recruitment of β-Arr1 to human GLP-1R using BRET. EC_{50} values and efficacy relative to GLP-1: GLP-1 (1.3 nM, 100%) Tzp (3.1 nM, 12%) (**B**) Recruitment of β-Arr2 to human GLP-1R using BRET. EC_{50} values and efficacy relative to: GLP-1 (0.7 nM, 100%), Tzp (3.2 nM, 23%) (**C**) Recruitment of β-Arr1 to mouse GLP-1R using NanoLuc complementation. EC_{50} values and efficacy relative to GLP-1: (0.53 nM, 100%), Tzp (6.6 nM, 6%) (**D**) Recruitment of β-Arr2 to mouse GLP-1R using NanoLuc complementation. EC_{50} values and efficacy relative to GLP-1: GLP-1 (0.53 nM, 100%), Tzp (2.4 nM, 15%).



Supplemental Figure 3. Insulin Secretion in Perifused Islets. (**A**) Tirzepatide (TZP) enhances glucose stimulated insulin secretion from normal islets (wild-type male) in a concentration-dependent manner. Integrated area under the curve (iAUC) is determined from 25-39 minutes. (**B**) Insulin secretion in response to GIP is similar in control and *Arrb1*$^{\beta cell-/-}$ islets. iAUC is determined from 20-40 minutes. (**C**) GIP stimulated insulin secretion is prevented by a GIPR antagonist. iAUC is determined from 21-39 minutes. (**D**) Insulin secretion in response to TZP is similar in control and *Arrb1*$^{\beta cell-/-}$ islets in the presence of the GIPR antagonist. iAUC is determined from 25-39 minutes. Each panel depicts results of a representative experiment from at least 2 independent experiments. * - $P<0.05$, values are mean ± SEM. Statistical differences in iAUC values were determined by a 2-tailed student's *t* test.



Supplemental Figure 4. In Vitro Pharmacology of Comparator Ligands. The intrinsic activity of semaglutide (SEMA) and NNC00902746 (MAR/MAR709/RG7697) for intracellular cAMP accumulation (**A, B**), agonist stimulated GTPγS binding of $G\alpha_s$ (**C, D**), and recruitment of β-Arr2 (ARRB2) (**E, F**) in comparison to native peptide as previously shown in Figure 1 and Table 1. Graphs are representative curves for summarized data and number of independent experiments, n, mentioned below. (**A**) The potency (SEM, n) of GIP(1-42) for cAMP accumulation on GIPR is 0.888 nM (0.114, 49). SEMA: >1,000 nM at n=10. MAR: 0.130 nM (0.017, 7). (**B**) The potency of GLP-1(7-36) for cAMP accumulation on GLP-1R is 0.366 nM (0.032, 57). SEMA: 0.364 nM (0.068, 9). MAR: 0.248 nM (0.035, 5). (**C**) The potency of GIP(1-42) for GTP recruitment for GIPR is 1.43 nM (0.18, 27). SEMA: >1,000 nM at n=2. MAR: 0.182 nM (0.028, 3). (**D**) The potency of GLP-1(7-36) for GTP recruitment for GLP-1R is 1.63 nM (0.21, 26). SEMA: 1.01 nM (0.13, 3). MAR: 0.333 nM (0.045, 3). (**E**) The potency of GIP(1-42) for ARRB2 recruitment to GIPR is 1.58 nM (0.52, 6). SEMA: >10,000 nM at n=3. MAR: 2.01 nM (0.31, 3) and is trending toward partial agonism at 79.4% (2.9, 3) compared to native 118% (11, 6). (**F**) The potency of GLP-1(7-36) for ARRB2 recruitment to GLP-1R is 3.26 nM (0.71, 14). SEMA: 16.8 nM (11.9, n=4). MAR: 7.19 nM (3.23, 5).