# **Raw Data for Western Blot Experiments**

The total proteins from tissues of 9 mice in each group were extracted. When loading the samples for electrophoresis, a mixed sample of protein extracts from 3 mice were used. Therefore, there were 3 replicates of western blot experiments for each group. All raw pictures and analyzed data were shown in the following.

1. Raw pictures for Fig.2I (The representative pictures in Fig.2I were from the Western

blot pictures in Replicate 3) **Replicate 1** 



Note: 1, NC group; 2, HFD group; 3, GDM group; 4, GDM+XOS group; M, Marker

**Replicate 2 and 3** (These two replicates were ran in one Western blot gel. We did not use markers in order to save costs. It is easy to identify the bands of p-Akt and Akt in the gel based on the first time running of replicate 1 with marker.)



Image J plots for western blot pictures Replicate 1





		Grey value produced by Image J software			Corre	cted value by <b>f</b>	Relative ratio to the NC group	
		p-Akt Akt β-actin		β-actin	p-Akt/β-actin	tin Akt/β-actin p-Ak		Relative faile to the ree group
	NC	7938.569	7287.326	11038.518	0.71916982	0.660172498	1.089366525	1.00
D. P. A.I	HFD	4619.983	6684.69	50668.522	0.091180536	0.13192984	0.691128983	0.63
Replicate 1	GDM	4080.276	8291.933	10346.811	0.394351071	0.801399871	0.492077782	0.45
	GDM+XOS	5403.832	5813.589	12129.347	0.445517141	0.479299421	0.929517377	0.85
	NC	4949.69	8315.095	7187.761	0.688627516	1.156840774	0.595265598	1.00
Deplicate 2	HFD	2690.397	7814.024	7279.054	0.369608056	1.07349444	0.344303652	0.58
Replicate 2	GDM	2058.326	8378.418	7365.225	0.279465461	1.137564433	0.245670006	0.41
	GDM+XOS	4033.518	8233.125	7296.64	0.552791148	1.12834469	0.489913368	0.82
	NC	6235.154	5558.347	7995.225	0.779859729	0.695208327	1.121764078	1.00
Replicate 3	HFD	4197.033	6155.74	7431.225	0.564783464	0.828361407	0.681808036	0.61
	GDM	2651.376	5981.79	7676.225	0.345401027	0.779261942	0.443241237	0.40
	GDM+XOS	5607.619	6208.619	8258.811	0.678986237	0.751757002	0.903199085	0.81

# 2. Raw pictures for Fig.5

The primary antibodies for occludin and ZO-1 are expensive. Therefore, the western blot gel containing the target protein were first cut into small pieces based on the position of markers before incubation with the primary antibody in order to save the costs.

# 2.1 Occludin (Fig.5 C)

## **Replicate 1**

Original western blot gel picture for occludin (1)



#### Cut blot picture for occludin (1)



Plot of occludin picture (1) by using Image J software



Original western blot gel picture for  $\beta$ -actin (1)



Cut blot picture for  $\beta$ -actin (1)



Plot of  $\beta$ -actin picture (1) by using Image J software



## Replicate 2

Original western blot gel picture for occludin (2)





Plot of occludin picture (2) by using Image J software



Original western blot gel picture for  $\beta$ -actin (2)





Plot of  $\beta$ -actin picture (2) by using Image J software



# Replicate 3

Original western blot gel picture for occludin (3)



#### Cut blot picture for occludin (3)



#### Plot of occludin picture (3) by using Image J software



Original western blot gel picture for  $\beta$ -actin (3)



# Cut blot picture for β-actin (3) NC HFD GDM GDM+XOS



Plot of  $\beta$ -actin picture (3) by using Image J software

The original grey value produced by Image J software and their relative ratio to $m eta$ -actin or to the NC group												
	Replicate 1				Replicate 2				Replicate 3			
Groups	occludin	β-actin	occludin/β- actin	Relative ratio to the NC group	occludin	β-actin	occludin/β- actin	Relative ratio to the NC group	occludin	β-actin	occludin/β- actin	Relative ratio to the NC group
NC	29174.953	32033.459	0.910764991	1.00	28798.51	31235.581	0.921977728	1.00	22841.146	20456.388	1.116577668	1.00
HFD	9794.075	14740.752	0.664421666	0.73	9778.447	16755.246	0.583605099	0.63	21831.217	28321.024	0.770848434	0.69
GDM	2440.154	10137.125	0.240714601	0.26	8391.054	30267.539	0.277229477	0.30	10046.246	29679.711	0.338488673	0.30
GDM+XOS	19825.024	39010.551	0.508196462	0.56	15510.933	30499.024	0.508571455	0.55	17456.51	27565.217	0.63328034	0.57

# 2.2 ZO-1 (Fig.5 D)

#### **Replicate 1**

Original western blot gel picture for ZO-1 (1)



# Cut blot picture for ZO-1 (1)







Original western blot gel picture for  $\beta$ -actin (1)





Plot of  $\beta$ -actin picture (1) by using Image J software



#### **Replicate 2**

Original western blot gel picture for ZO-1 (2)

NC	HFD	GDM	GDM+XOS
-	-	-	
and a	Jones	far. 1	



Plot of ZO-1 picture (2) by using Image J software



Original western blot gel picture for  $\beta$ -actin (2)



Cut blot picture for  $\beta$ -actin (2)



Plot of  $\beta$ -actin picture (2) by using Image J software



# **Replicate 3**

Original western blot gel picture for ZO-1 (3)





#### Plot of ZO-1 picture (3) by using Image J software



Original western blot gel picture for  $\beta$ -actin (3)



Plot of  $\beta$ -actin picture (3) by using Image J software



The original grey value produced by Image J software and their relative ratio to β-actin or to the NC group												
	Replicate 1				Replicate 2				Replicate 3			
Groups	ZO-1	β-actin	ZO-1/β-actin	Relative ratio to the NC group	ZO-1	β-actin	ZO-1/β-actin	Relative ratio to the NC group	ZO-1	β-actin	ZO-1/β-actin	Relative ratio to the NC group
NC	24161.175	27802.903	0.869016268	1.00	14235.347	16755.752	0.849579714	1.00	18922.66	16077.803	1.176943143	1.00
HFD	23545.095	34237.024	0.687708575	0.79	15522.853	23027.388	0.674103941	0.79	17617.246	20052.853	0.878540625	0.75
GDM	11776.418	28417.146	0.414412411	0.48	7320.66	18911.974	0.387091268	0.46	12592.317	24578.418	0.512332283	0.44
GDM+XOS	18492.803	31790.681	0.581705154	0.67	20531.66	34866.823	0.588859501	0.69	15357.61	20136.752	0.762665697	0.65