

## Supplementary Information

***Sargassum fusiforme* together with turmeric extract and pomegranate peel extract alleviates obesity in high fat-fed C57BL/6J mice**

### Authors

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**This Supplementary Information Includes:**

**Supplementary Materials and Methods**

**Supplementary Tables**

**Supplementary Figures**

## Supplementary Materials and Methods

### Preparation of water extract of *Sargassum fusiforme*

Dried *Sargassum fusiforme* were soaked in water at room temperature for 10 h, and then milled into powder. Milled seaweed powder and distilled water were evenly mixed at the ratio of material to solution of 1 : 50, and stirred in a water bath at 85 °C for 6 h. The extract was centrifuged at 4500 rpm for 15 min at room temperature, and then the supernatant was collected and concentrated by rotary evaporation at 45 °C. The supernatant was transferred to an air-drying cabinet for drying at 35 °C when the condensation tube was free of water droplets. The dry powder obtained was water extract of *Sargassum fusiforme*. The extraction rate was calculated according to the following formula.

$$\text{Extraction rate}(\%) = \frac{x}{S} \times 100\%$$

Where x is the mass of dried water extract of *Sargassum fusiforme*, S is the mass of dried *Sargassum fusiforme*.

## Supplementary Tables

### Supplementary Table S1

**Table S1** Formulation information

Formulation	Ingredient	Content	Compound proportion
Water extract of <i>Sargassum fusiforme</i>	water extract of <i>Sargassum fusiforme</i>	100 %	
together with curcumin	curcumin	95 %	150 : 6 : 3
and punicalagin	punicalagin	98 %	
<i>Sargassum fusiforme</i>		15 % water extract of	
together with turmeric	<i>Sargassum fusiforme</i>	<i>Sargassum fusiforme</i> (by	
extract and pomegranate		calculating the extraction	1000 : 28 : 15
peel extract (C)	turmeric extract	20 % curcumin	
	pomegranate peel extract	20 % punicalagin	

## Supplementary Table S2

**Table S2** Formula and caloric information for D14060603 (LFD)

Ingredient	Grams
Casein	200
L-Cystine	3
Corn Starch	452.2
Maltodextrin 10	75
Sucrose	172.8
Cellulose, BW200	50
Corn Oil	25
Lard	20
Mineral Mix S10026	10
DiCalcium Phosphate	13
Calcium Carbonate	5.5
Potassium Citrate, 1 H <sub>2</sub> O	16.5
Vitamin Mix V10001	10
Choline Bitartrate	2
Total	1055

Caloric Information	
Protein	20% kcal
Fat	10% kcal
Carbohydrate	70% kcal
Energy Density	3.8 kcal/g

### Supplementary Table S3

**Table S3** Formula and caloric information for D14060606 (HFD)

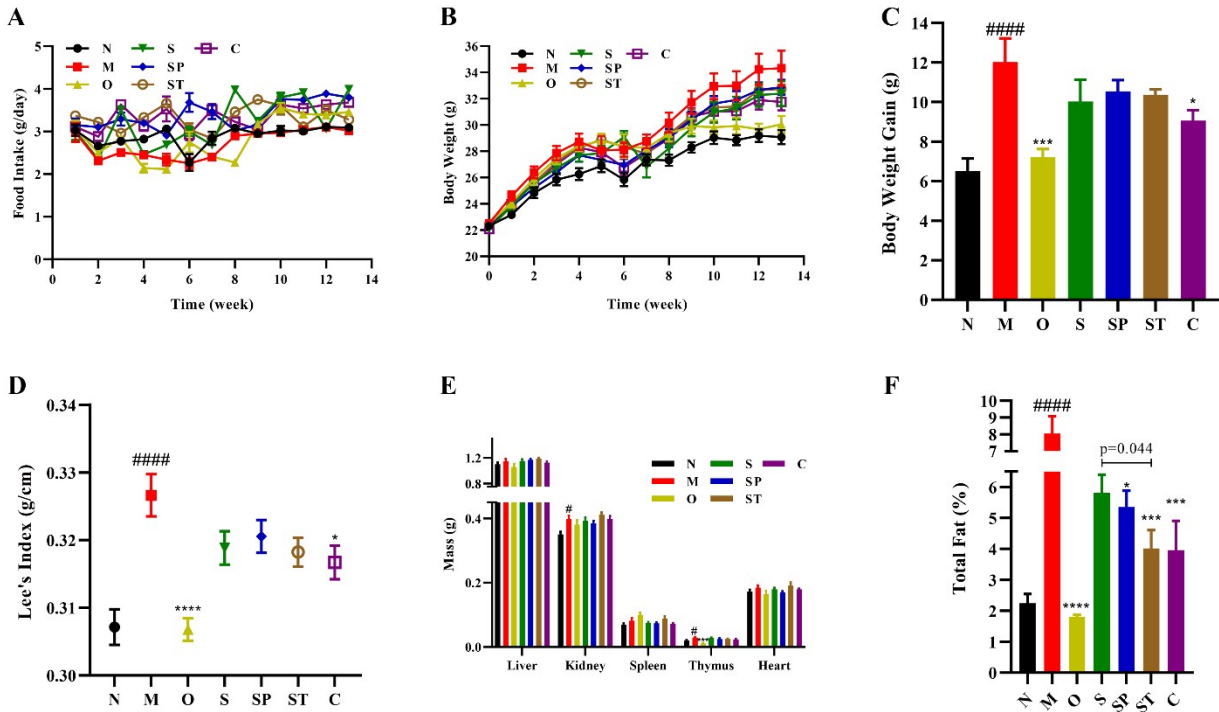
Ingredient	Grams
Casein	200
L-Cystine	3
Corn Starch	72.8
Maltodextrin 10	100
Sucrose	172.8
Cellulose, BW200	50
Corn Oil	25
Lard	177.5
Mineral Mix S10026	10
DiCalcium Phosphate	13
Calcium Carbonate	5.5
Potassium Citrate, 1 H <sub>2</sub> O	16.5
Vitamin Mix V10001	10
Choline Bitartrate	2
Total	858.1

Caloric Information	
Protein	20% kcal
Fat	45% kcal
Carbohydrate	35% kcal
Energy Density	4.7 kcal/g

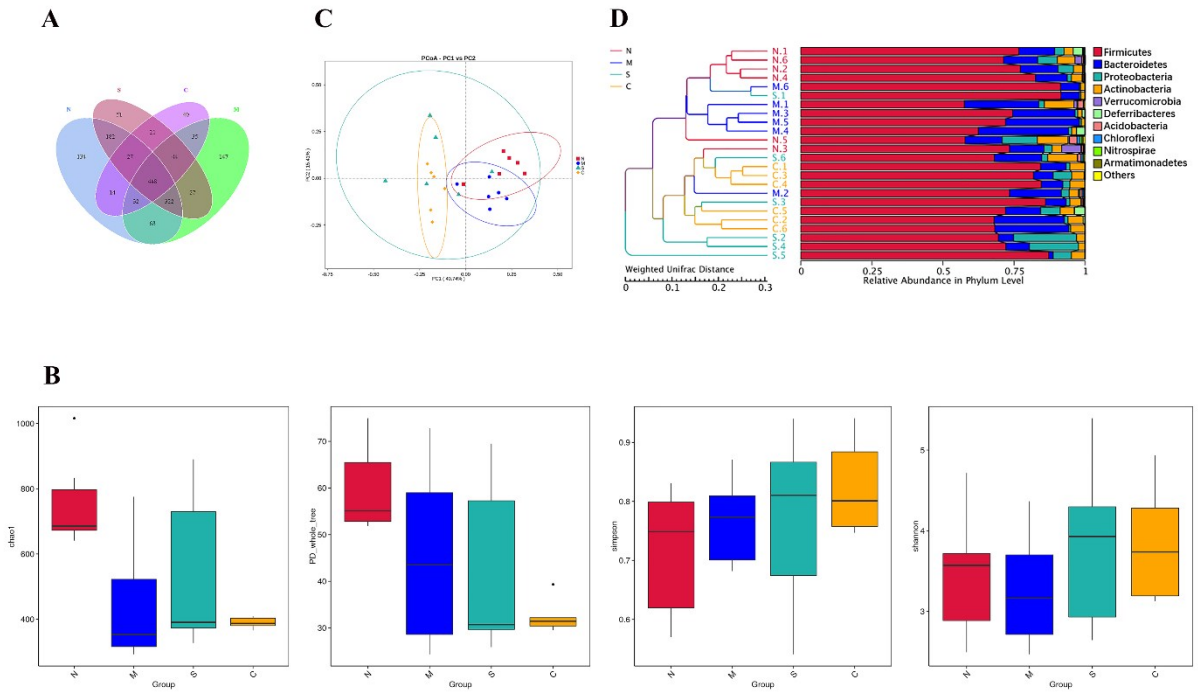
## Supplementary Figures

### Supplementary Figure S1



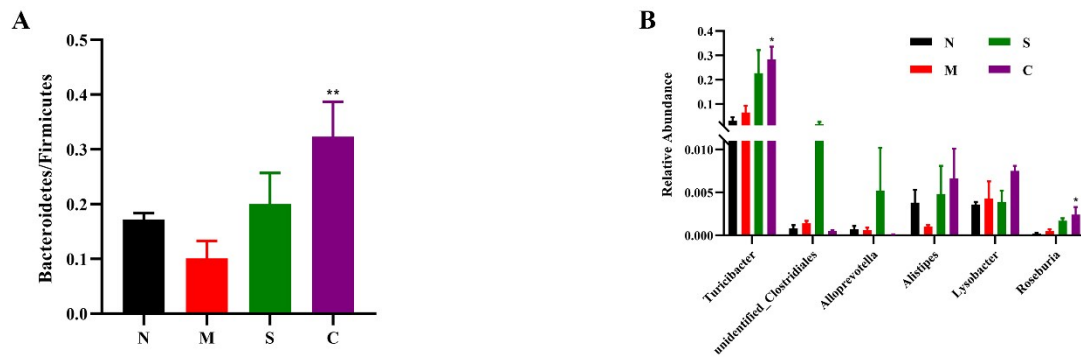
**Fig. S1** The effects of diet on phenotype of overweight C57BL/6J mice. (A) Food intake throughout the experiment, (B) body weight fluctuations, (C) weight gain, (D) Lee's index, (E) weight of organs, (F) total fat percentage. N = Normal, M = Model, O = Orlistat, S = *Sargassum fusiforme*, SP = *Sargassum fusiforme* together with pomegranate peel extract, ST = *Sargassum fusiforme* together with turmeric extract, C = *Sargassum fusiforme* together with turmeric extract and pomegranate peel extract. Values are given as mean  $\pm$  SEM, n = 10. Statistical significances were calculated using one-way ANOVA (# and \*  $p < 0.05$ , \*\*\*  $p < 0.001$ , #### and \*\*\*\*  $p < 0.0001$ ), # denotes versus N, \* denotes versus M. The statistical analysis between C (or SP, ST) and S was assessed by t test.

## Supplementary Figure S2



**Fig. S2** (A) Venn diagram of microbiota differences, (B)  $\alpha$  diversity indices (from left: Chao1, PD whole tree, Simpson, and Shannon), (C) PCoA based on Weighted UniFrac, (D) UPGMA cluster tree based on Weighted UniFrac. N = Normal, M = Model, S = *Sargassum fusiforme*, C = *Sargassum fusiforme* together with turmeric extract and pomegranate peel extract; n = 6.

### Supplementary Figure S3



**Fig. S3** (A) The ratio of Bacteroidetes to Firmicutes, (B) the relative abundance of selected bacteria at genus level. N = Normal, M = Model, S = *Sargassum fusiforme*, C = *Sargassum fusiforme* together with turmeric extract and pomegranate peel extract. Values are given as mean ± SEM, n = 6. Statistical significances were calculated using one-way ANOVA (\*  $p < 0.05$ , \*\*  $p < 0.01$ ), \* denotes versus M.