Supplementary information for

Bacterial–host adhesion dominated by collagen subtypes remodelled by osmotic pressure

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Supplementary Figure 1 to 15

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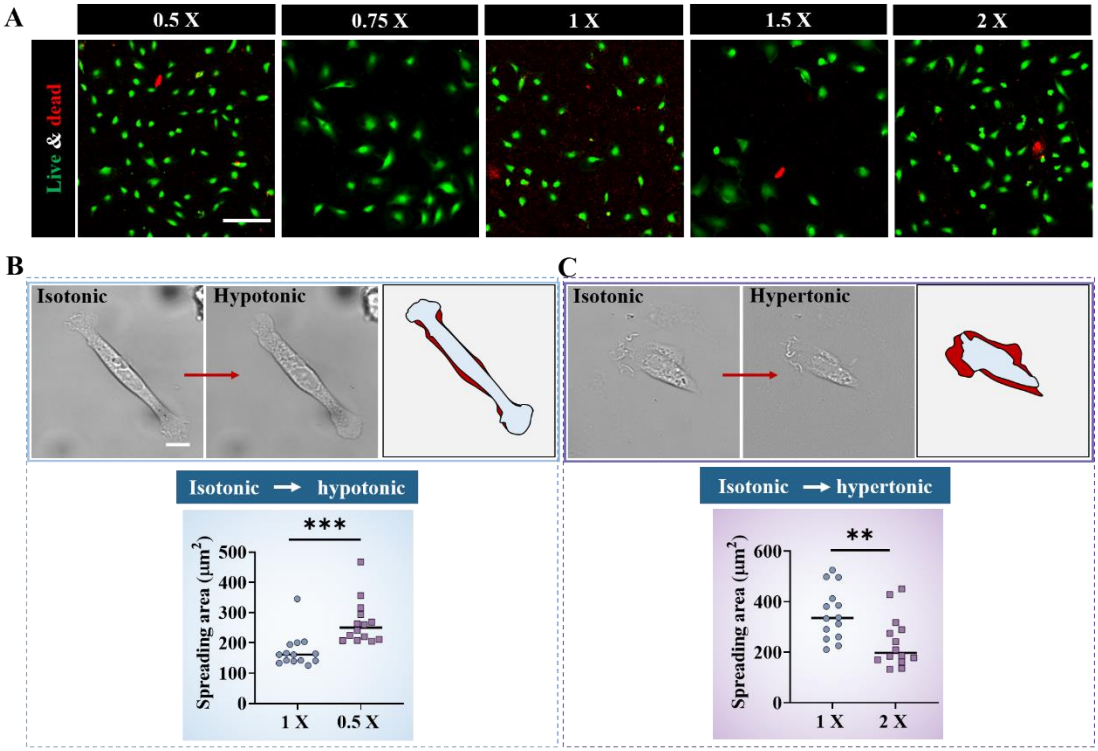
Other Supplementary Materials for this manuscript include the following:

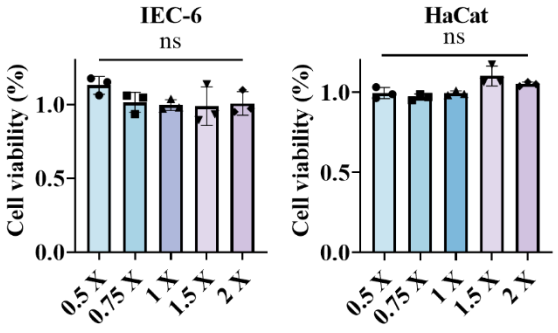
Supplementary Movie 1

Supplementary Data 1

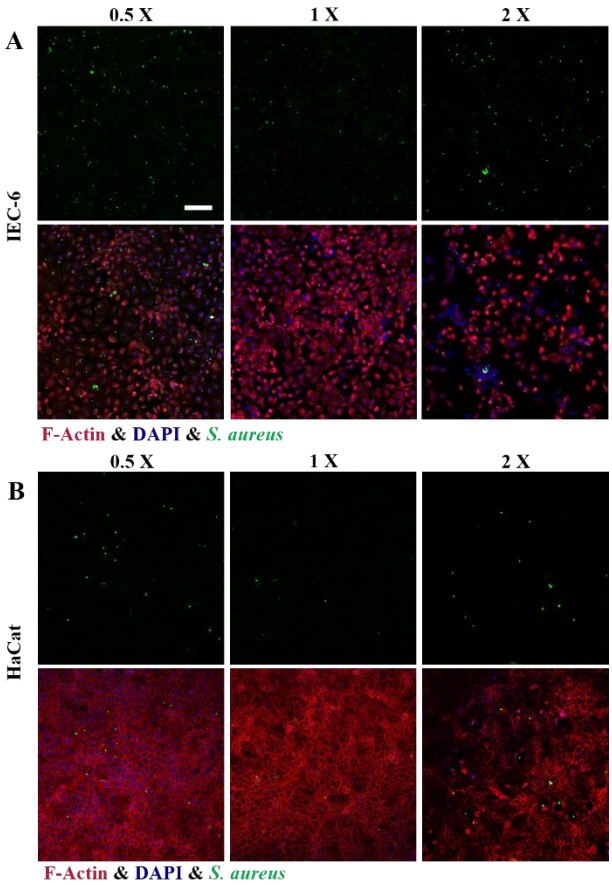
Supplementary Text



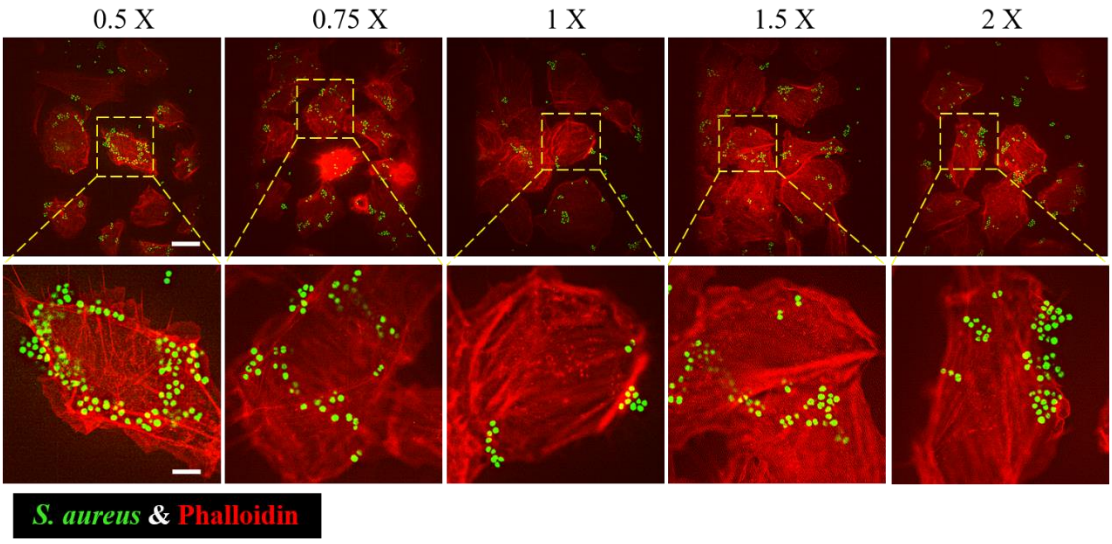
Supplementary Figure 1. Cytotoxicity assay and morphological changes under osmotic stresses. (A) Results of live/dead staining after IEC-6 cells were exposed to hypotonic (0.5X and 0.75X), isotonic (1X), and hypertonic (1.5X and 2X) solutions for 3 h, where green denoted the live cells whereas red indicated the dead ones. Scale bar: 100 μm. (B) and (C) changes in cell morphology after 3 min of hypotonic and hypertonic stimulation. The spreading areas of the cells were quantified via the software of ImageJ. Scale bar: 10 μm. At least three independent experiments were carried out for each condition. Two-sided unpaired t test was used for statistical analysis. **and*** indicated $P < 0.01$ and $P < 0.001$, respectively.



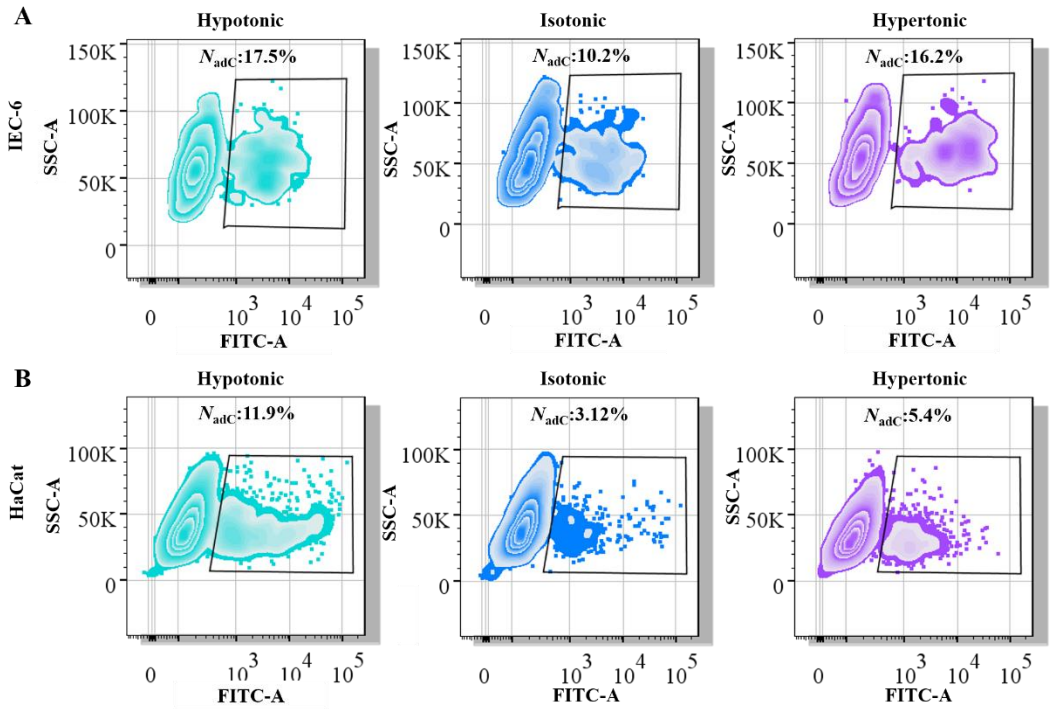
Supplementary Figure 2. Cytotoxicity tests of IEC-6 cells (A) and HaCat cells (B) under different osmotic pressure conditions quantified through the CCK8 assay (n=3). The CCK8 assay was performed after exposing cells to the hypotonic (0.5X and 0.75X), isotonic (1X), and hypertonic (1.5X and 2X) solutions for 3 h, respectively. All statistical data were presented as Mean±SD, and one-way analysis of variance (ANOVA) was used for data analysis, with ns indicating no statistically significant difference (P>0.5).



Supplementary Figure 3. Interactions between Staphylococcus aureus (S. aureus) expressing green fluorescent protein (GFP) and host cell monolayers of IEC-6 (A) or HaCat cell (B). Scale bar: 50 μm.

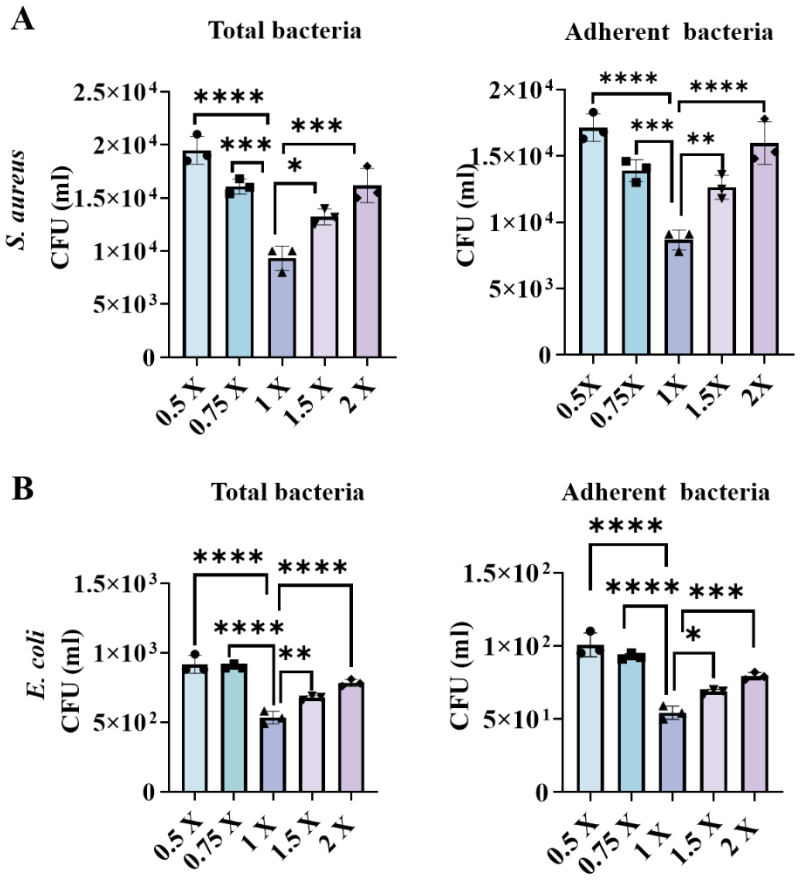


Supplementary Figure 4. Super-resolution imaging results of interactions between *S. aureus* and host cell (IEC-6) under the hypotonic (0.5X and 0.75X), isotonic (1X), and hypertonic (1.5X and 2X) conditions. The scale bars in the upper and lower panels were 20 and 5 μm, respectively.



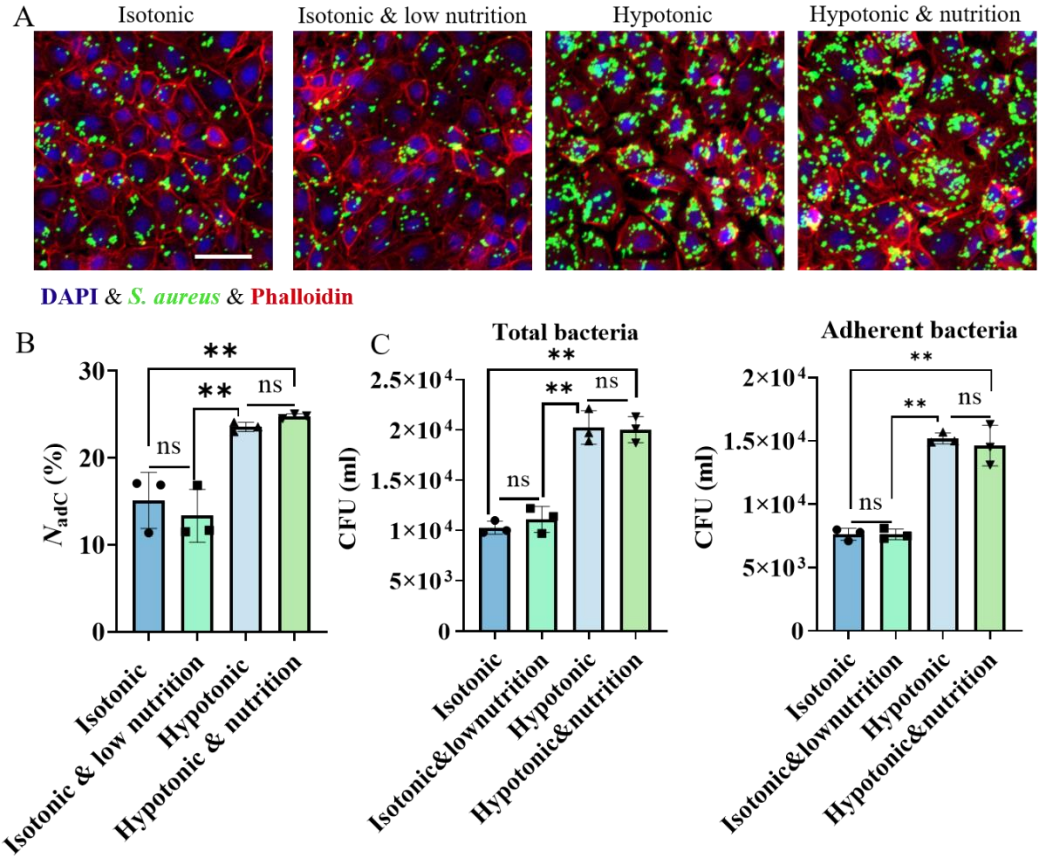
Supplementary Figure 5. Interactions between bacteria (*S. aureus*) and host cells including (A) IEC-6 cells and (B) HaCat cells, regulated with environmental osmotic pressures, where the host cells carrying bacteria, *i.e.*, the host cells to which bacteria

adhered and internalized, were quantified through the flow cytometry. The percentages of the host cells carrying bacteria were also presented in the images.



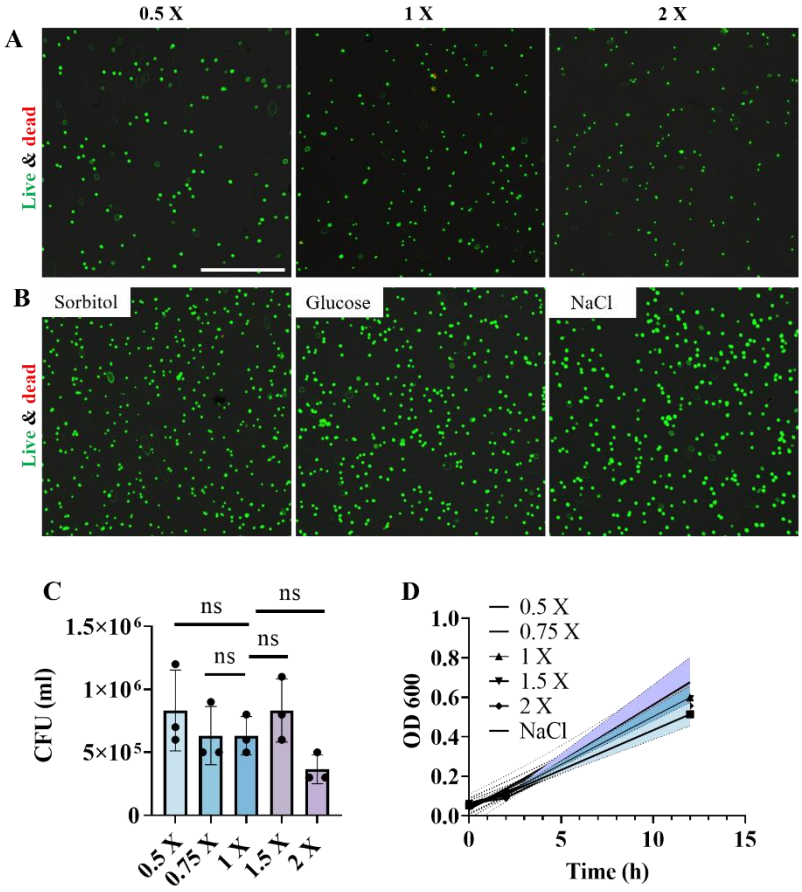
Supplementary Figure 6. Colony counts of bacteria that directly interacted with the host cells, including adherent bacteria and internalized bacteria (hereafter referred to as total bacteria) and purely adherent bacteria. (A) and (B) were the corresponding experimental results, in which the adopted bacteria were gram-positive bacterium *S. aureus* and gram-negative bacterium *E. coli*, respectively. In these experiments, the host cells were allowed to interact with GFP-expressing *S. aureus*. Then, they were rinsed three times with Dulbecco's phosphate buffered saline (DPBS) solution. To quantify the number of total bacteria that included adherent bacteria and internalized bacteria, the host cells harboring adherent and internalized bacteria were lysed with 1% Triton X-100 and subsequently serially diluted for analysis based on the flat colony counting method. On the other hand, the host cells were treated with 200 μg/mL gentamicin to kill extracellularly adherent bacteria. In this way, one could determine the number of internalized bacteria. Further, the cells were lysed with 1% Triton X-100

and serially diluted for analysis using the same flat colony counting method. Finally, the purely adherent bacteria might be quantitatively estimated. At least three independent experiments were carried out for each specific experimental condition. All the statistical data were denoted as Mean±SD, and one-way analysis of variance (ANOVA) was employed in the data analyses with ** and *** indicating $P < 0.01$ and $P < 0.001$, respectively.



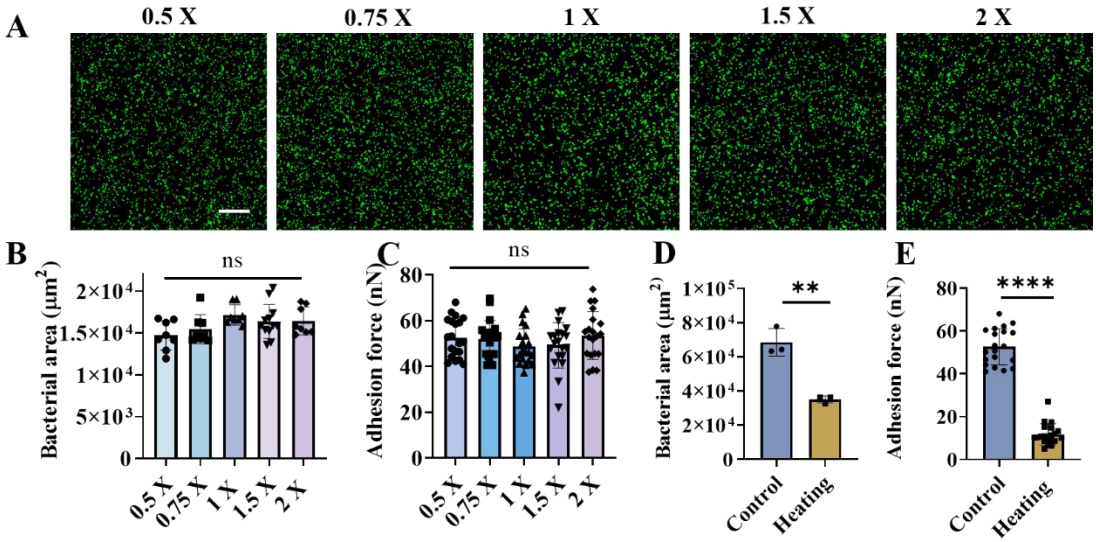
Supplementary Figure 7. Effect of nutrient levels on bacterial-host cell interactions under different nutrition and osmotic pressure (Isotonic, isotonic & low nutrition, hypotonic and hypotonic & normal nutrition). (A) Confocal imaging, (B) flow cytometry analysis and (C) bacterial colony counts, where the left image showed the statistical results of total bacteria whereas the right image presented those of adherent bacteria. All the statistical data were denoted as Mean±SD, and one-way analysis of

variance (ANOVA) was employed in the data analyses with ** and *** indicating P < 0.01 and P < 0.001, respectively. Scale bar: 50 μm.

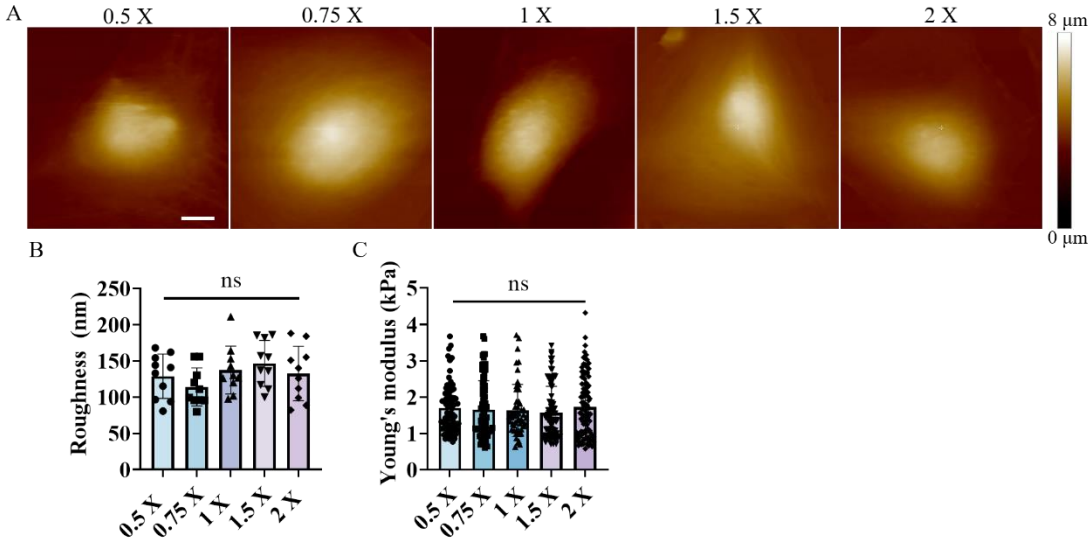


Supplementary Figure 8. Bacterial viability tests under different osmotic pressures. (A) Fluorescence images presenting the viability of *S. aureus*, where the live and dead bacteria were labelled as green and red by SYTO/PI dead-live double staining, respectively, after they were treated with hypotonic (0.5X, left), isotonic (1X, middle) and hypertonic (2X, right) solutions for 6 h. (B) Typical fluorescence images presenting the viability of *S. aureus* after they were treated with the hypertonic solutions prepared with sorbitol (left), glucose (middle) and NaCl (right), respectively. (C) Colony count-based statistical results of the bacteria treated with hypotonic (0.5X, 0.75X), isotonic (1X) and hypertonic (1.5X, 2X) solutions for 6 h, respectively. (D) Growth curves (OD 600) of the bacteria in the hypotonic (0.5X and 0.75X), isotonic (1X) and hypertonic (1.5X and 2X) solutions. All the statistical data were presented as Mean±SD from at least three independent experiments for each specific condition. Statistical analyses

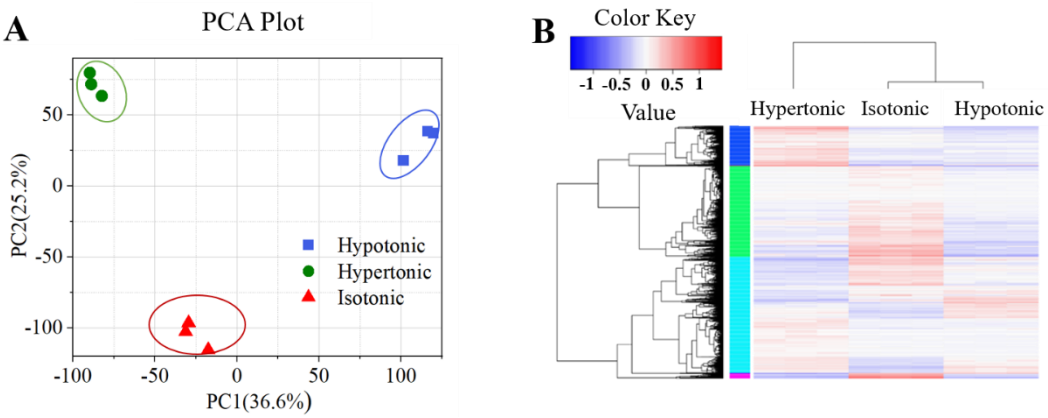
based on one-way ANOVA were used in the experiments and *, **, *** and **** denoted $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively. Scale bar: 100 μm.



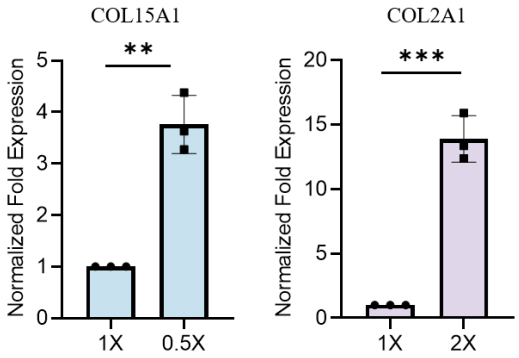
Supplementary Figure 9. Experimental results of bacterial adhesion to collagen-modified substrates under different osmotic pressures. In these experiments, bacteria (*S. aureus*) were added to collagen-modified substrates and then treated with the hypotonic (0.5X and 0.75X), isotonic (1X) and hypertonic (1.5X and 2X) solutions for 6 h, respectively. Subsequently, they were rinsed three times to remove the nonadherent bacteria and imaged with an inverted confocal laser scanning microscope (Nikon A1, Japan). (A) Typical Fluorescence images of bacterial adhesion to collagen-modified substrates under different osmotic pressures. (B) Bacterial adhesion areas under different osmotic pressures. (C) Adhesion forces between the bacteria and the underlying collagen-modified substrates under different osmotic pressures. (D) Comparison of bacterial adhesion area between unheated and heated treatments. (E) Comparison of adhesion force between unheated and heated treatments. All these statistical results were presented as Mean±SD from at least three independent experiments for each specific condition. Statistical analyses based on one-way ANOVA were utilized in the experiments and *, **, ***and **** denoted $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively. Scale bar 100 μm.



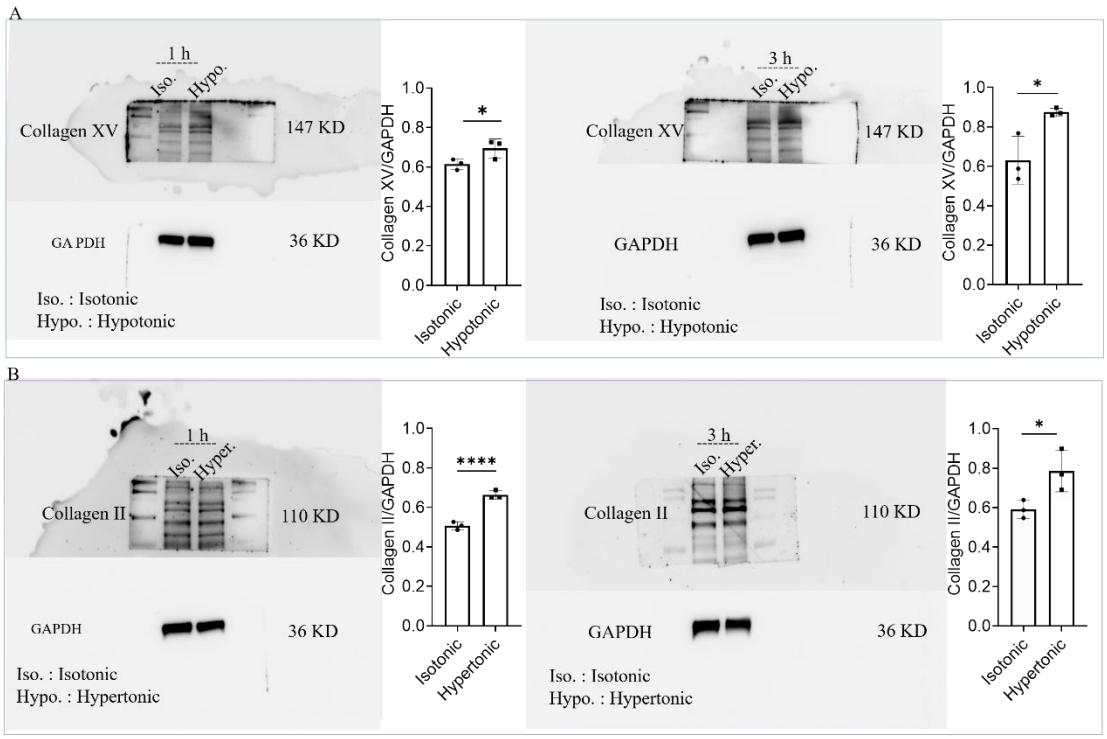
Supplementary Figure 10. Morphology, roughness and stiffness (Young's moduli) of the host cell (IEC-6) characterized by atomic force microscope (AFM), under different osmotic pressure (Hypotonic: 0.5X; 0.75X; Isotonic: 1X; Hypertonic: 1.5 X, 2X). Scale bar: 5µm.



Supplementary Figure 11. RNA sequencing analysis were performed on host cells (IEC-6) treated with the hypotonic (0.5X), isotonic (1X) and hypertonic (2X) solutions, respectively. (A) Principal component analysis (PCA) results of the RNA sequencing data. (B) Cluster gram heat map. These data demonstrated that there were significantly up-regulated/down-regulated genes in the host cells under different osmotic pressures.

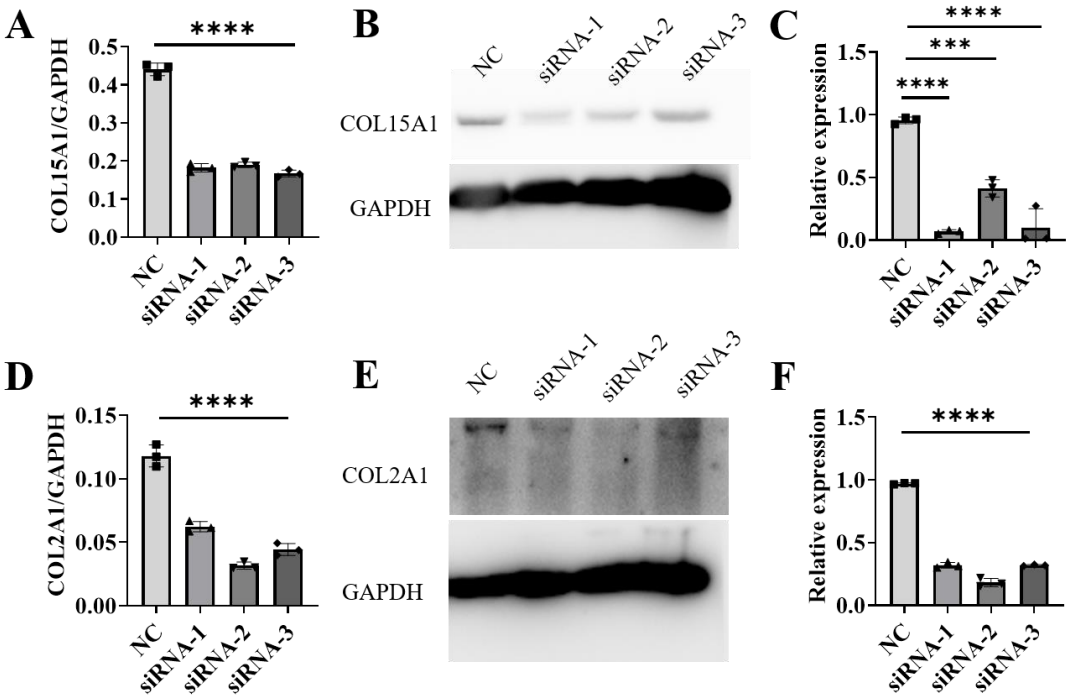


Supplementary Figure 12. The IEC-6 cells were treated with isotonic (1X), hypotonic (0.5X) and hypertonic (2X) solution, and the relative expression levels of the COL15A1 and COL2A1 genes were quantified by qPCR and normalized to that of GAPDH. Relative expression was calculated using the 2−Δ Δ CT method. Two-sided unpaired t test was used for statistical analysis. **and*** indicated P < 0.01 and P < 0.001, respectively.

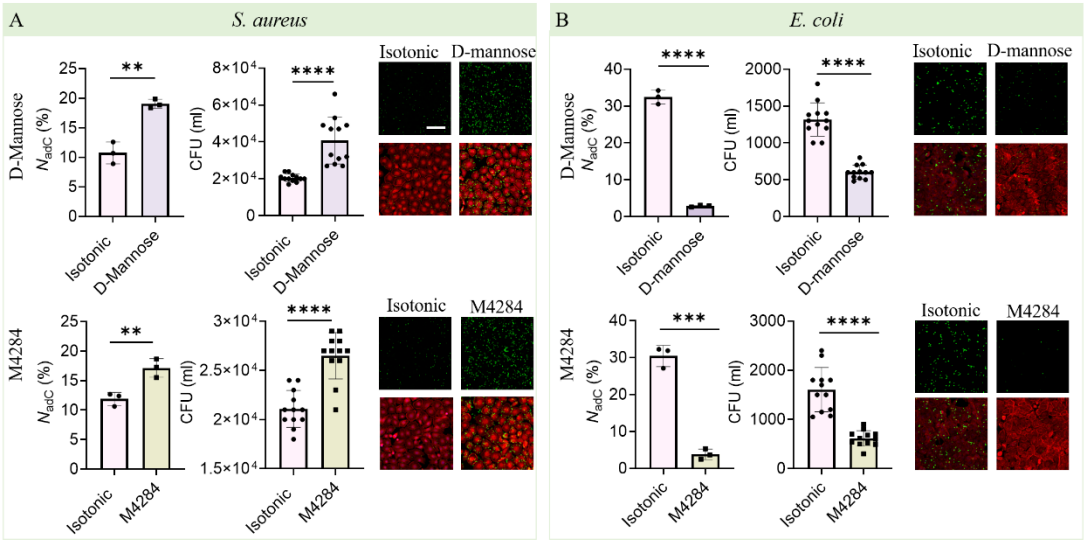


Supplementary Figure 13. Western blotting (WB) results for IEC-6 cells that were treated within hypotonic (A) and hypertonic (B) solution for 1 h and 3 h. Two-sided

unpaired t test was used for statistical analysis. *and**** indicated $P < 0.05$ and $P < 0.0001$, respectively.



Supplementary Figure 14. Experiments on siRNA-mediated Knockdown of type XV and type II collagen in IEC-6 cells. (A) Relative expression of COL15A1 in the mRNA level. (B) Western blot (WB) and (C) the corresponding quantitative results for IEC-6 cells that were transfected with the designed siRNA sequences targeting COL15A1 or negative control (NC) siRNA. (D) Relative expression of COL2A1 in the mRNA level. (B) Western blot (WB) and (C) the corresponding quantitative results for IEC-6 cells that were transfected with the designed siRNA sequences targeting COL2A1 or negative control (NC) siRNA. All the statistical data were presented as Mean±SD from at least three independent experiments for each specific condition. Statistical analyses based upon one-way ANOVA were used in the experiments, and *, **, *** and **** indicated $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively.



Supplementary Figure 15. (A) Infection of host cells by S. aureus in the presence of M4284 (10μM) or D-mannose (200 mM). (B) Infection of host cells by *E. coli* in the presence of M4284 (10uM) or D-mannose (200 mM). All statistical data are presented as Mean ± SD, and one-way analysis of variance (ANOVA) was utilized for data analysis, with **** indicating $P < 0.0001$, respectively. Scale bar: 100 μm.

Supplementary Table 1. Preparation of osmotic stimulation solutions with different osmotic pressures.

Experimental group	Reagent formula	Measured osmotic pressure (mOsm kg^{-1})
0.5 X	Regular DMEM Medium[a] + ddH_2O (v/v=1:1)	168
0.75 X	Regular DMEM Medium + ddH_2O (v/v=2:1)	227
1 X	Regular DMEM Medium	344
1.5 X	Regular DMEM Medium + 30mg/ml mannitol	499
2 X	Regular DMEM Medium + 50mg/ml mannitol	612

[a] DMEM/High glucose produced by Hyclone (Cat No.: SH30243.01) was referred to as isotonic solution (1 X) in the experiments.

Supplementary Table 2. Preparation of culture media containing various nutrients in the hypotonic or isotonic solution.

Experimental group	**Isotonic**	**Isotonic & low nutrition**	**hypotonic**	**Isotonic & normal nutrition**
Component description	mg/L			
Calcium chloride	200	100	100	200
Ferric nitrate $9H_2O$	0.1	0.05	0.05	0.1
Potassium chloride	400	200	200	400
Magnesium sulfate	97.6	48.8	48.8	97.6
Sodium chloride	6400	3200	3200	6400
Sodium phosphate monobasic H_2O	125	62.5	62.5	125
L-Arginine-HCl	84	42	42	84
L-Cystine-2HCl	62.5	31.25	31.25	62.5
L-Glutamine	584	297	297	584
Glycine	30	15	15	30
L-Histidine-HCl H_2O	42	21	21	42
L-Isoleucine	104.8	52.4	52.4	104.8
L-Leucine	104.8	52.4	52.4	104.8
L-Lysine-HCL	146.3	73.1	73.1	146.3
L-Methionine	30	15	15	30
L-Phenylalanine	66	33	33	66
L-Threonine	95.2	47.6	47.6	95.2
L-Tryptophan	16	8	8	16
L-Tyrosine-2Na-$2H_2O$	103.7	51.85	51.85	103.7
L-Valine	93.6	46.8	46.8	93.6
FBS	volume ratio (v/v)			
	1%			

Supplementary Table 3. The number of significantly up-regulated and down-regulated genes in IEC-6 cells under the hypertonic and hypotonic conditions

Sample comparison	Up-regulated	Down-regulated
Hypotonic vs. Isotonic	2399	667
Hypertonic vs. Isotonic	2113	566

Supplementary Table 4. Major genes significantly differentially expressed in the hypertonic condition.

GeneID	log2Foldchange	pvalue	padj	Regulation	Genesymbol
ENSRNOG00000035596	9.691420003	4.45E-16	9.75E-15	Ups	Mir207
ENSRNOG00000036703	9.623171199	7.30E-16	1.56E-14	Ups	Itgax
ENSRNOG00000050714	9.546842231	1.25E-15	2.59E-14	Ups	Islr2
ENSRNOG00000012034	9.506498986	1.65E-15	3.39E-14	Ups	Ces2i
ENSRNOG00000019404	9.41626847	3.00E-15	15.97E-14	Ups	Hhatl
ENSRNOG00000012719	9.413728394	3.91E-15	7.65E-14	Ups	Tdrd12
ENSRNOG00000027940	9.354837022	1.41E-116	5.74E-14	Ups	Plppr3
ENSRNOG00000033173	9.215361257	1.13E-09	1.17E-08	Ups	Fam71e2
ENSRNOG00000019390	9.189606725	1.62E-14	2.97E-13	Ups	Klhl40
ENSRNOG00000021199	9.103956049	3.44E-14	6.10E-13	Ups	Fcgr1a
ENSRNOG00000007044	8.987641701	7.11E-14	1.23E-12	Ups	L3mbtl1
ENSRNOG00000058560	8.686272396	5.64E-13	8.96E-12	Ups	Col2a1
ENSRNOG00000017209	8.662582869	6.90E-13	1.08E-11	Ups	Tubb3
ENSRNOG00000018434	8.634353176	7.76E-13	1.21E-11	Ups	Stab1
ENSRNOG00000042847	8.540575704	2.21E-12	3.22E-11	Ups	LOC687707
ENSRNOG00000000335	8.488855347	2.52E-12	3.66E-11	Ups	Ermap
ENSRNOG00000019728	8.458008602	3.83E-12	5.42E-11	Ups	Itgam
ENSRNOG00000004630	8.426234866	3.30E-12	4.72E-11	Ups	Rag1
ENSRNOG00000016703	8.286082695	1.12E-11	1.51E-10	Ups	Gtf2a1l
ENSRNOG00000019486	8.285997838	8.83E-12	1.21E-10	Ups	Trpv1

Supplementary Table 5. Major genes significantly differentially expressed in the hypotonic condition.

GeneID	log2Foldchange	pvalue	padj	Regulation	Genesymbol
ENSRNOG00000010278	9.321691	8.14E-14	8.14E-14	Ups	Il6
ENSRNOG00000016073	8.961566	1.02E-12	1.02E-12	Ups	Taar1
ENSRNOG00000049191	8.555186	1.42E-11	1.42E-11	Ups	Olr1159
ENSRNOG00000007640	8.189205	1.50E-10	1.50E-10	Ups	Npbwr1
ENSRNOG00000046763	7.711738	1.60E-06	1.60E-06	Ups	Adssl1
ENSRNOG00000017209	7.687042	4.35E-09	4.35E-09	Ups	Tubb3
ENSRNOG00000027030	7.506298	3.68E-56	3.68E-56	Ups	Adm
ENSRNOG00000003300	7.328737	2.52E-170	5.21E-168	Ups	Btg2
ENSRNOG00000009919	7.191162	6.46E-08	6.46E-08	Ups	Acod1
ENSRNOG00000059956	6.956704	3.41E-07	3.41E-07	Ups	Bcl6b
ENSRNOG00000003104	6.945858	3.91E-07	3.91E-07	Ups	Trpv2
ENSRNOG00000008015	6.741163	0	0	Ups	Fos
ENSRNOG00000060381	6.003268	5.81E-05	5.81E-05	Ups	Col15a1
ENSRNOG00000005082	5.992952	1.89E-07	1.89E-07	Ups	Irf6
ENSRNOG00000009822	5.975929	1.13E-05	1.13E-05	Ups	Tlr2
ENSRNOG00000022884	5.960116	7.52E-05	7.52E-05	Ups	Cd84
ENSRNOG00000012509	5.954869	1.54E-05	1.54E-05	Ups	Il17f
ENSRNOG00000006579	5.941042	3.75E-07	3.75E-07	Ups	Reg3g
ENSRNOG00000021318	5.933944	6.26E-05	6.26E-05	Ups	Epas1
ENSRNOG00000047511	5.929741	7.71E-05	7.71E-05	Ups	Olr1381

Supplementary Table 6. List of primer sequences designed for qPCR.

Target Gene		Sequence(5'—3')
COL15A1	Forward primer	GCCCCCTACTTCATCCTCTC
	Reverse primer	CAGTACGGACCTCCAGGGTA
COL2A1	Forward primer	ACGCTCAAGTCGCTGAACAA
	Reverse primer	TCAATCCAGTAGTCTCCGCTCT
GAPDH	Forward primer	CCGCATCTTCTTGTGCAGTG
	Reverse primer	CGATACGGCCAAATCCGTTC

Supplementary Table 7. List of siRNA sequences designed in the experiments.

Species	Target Gene	Name		Sequence(5'—3')
Rattus norvegicus	COL15A1	Si-1	sense	GCU CAU UGG UGU CCC AUU ATT
			antisense	UAA UGG GAC ACC AAU GAG CTT
		Si-2	sense	GGA AGU AGA CAU GCU GGA UTT
			antisense	AUC CAG CAU GUC UAC UUC CTT
		Si-3	sense	GCC UAA AGA AGC ACA CGU UTT
			antisense	AAC GUG UGC UUC UUU AGG CTT
	COL2A1	Si-1	sense	GCU GGU GCA CAA GGU CCU ATT
			antisense	GCU GGU GCA CAA GGU CCU ATT
		Si-2	sense	UAG GAC CUU GUG CAC CAG CTT
			antisense	GCU CAU CCA GGG CUC CAA UTT
		Si-3	sense	GGG UGA AGG UGG AAA GCA ATT
			antisense	UUG CUU UCC ACC UUC ACC CTT
	Negative control		sense	UUC UCC GAA CGU GUC ACG UTT
			antisense	ACG UGA CAC GUU CGG AGA ATT

Supplementary Table 8. Preparation of osmotic stimulation solution based on the FimH antagonist (M4284 and D-mannose).

Component	Concentration	Osmotic pressure (mOsm/kg)
DMEM/High glucose		344
M4284	10uM[1-3]	425
D-mannose	1mM[2]	355
D-mannose	200mM[4]	508

Supplementary References

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2. Tomasek, K. et al. Type 1 piliated uropathogenic Escherichia coli hijack the host immune response by binding to CD14. eLife 11, e78995 (2022).
3. Han, Z. et al. Lead Optimization Studies on FimH Antagonists: Discovery of Potent and Orally Bioavailable Ortho-Substituted Biphenyl Mannosides. Journal of Medicinal Chemistry 55, 3945-3959 (2012).
4. Plescher, M., Teleman, A. A. & Demetriades, C. TSC2 mediates hyperosmotic stress-induced inactivation of mTORC1. Scientific Reports 5, 1-12(2015).