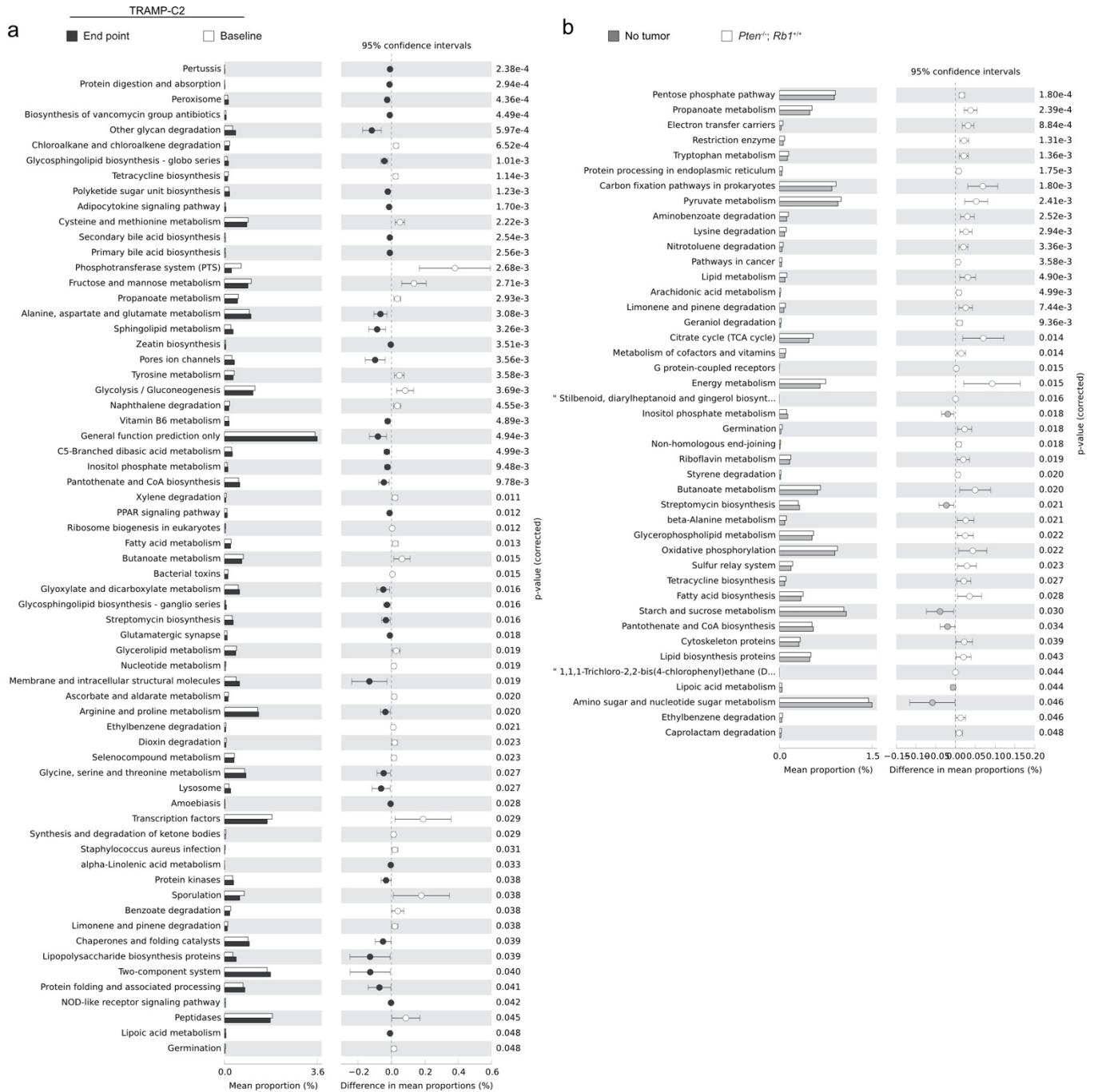
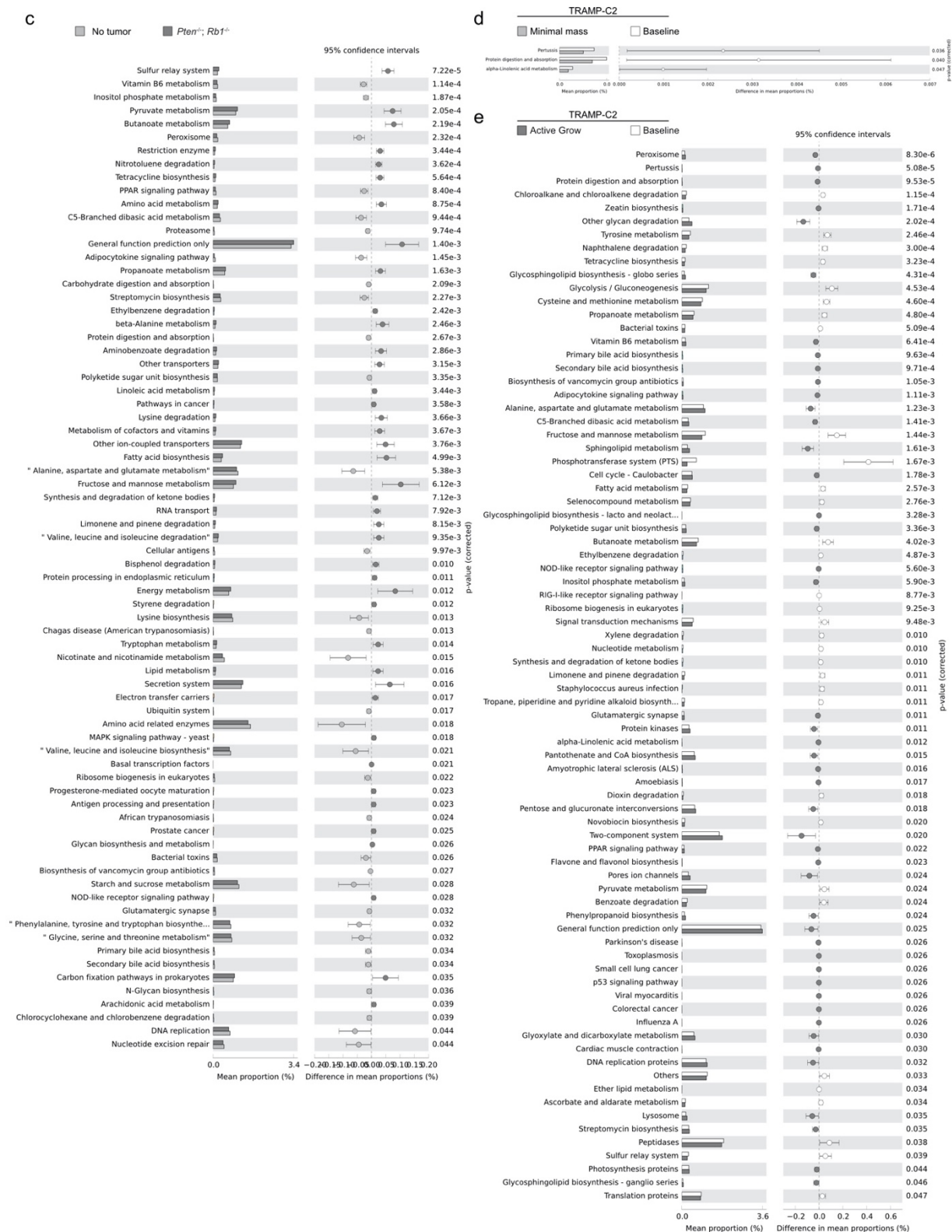


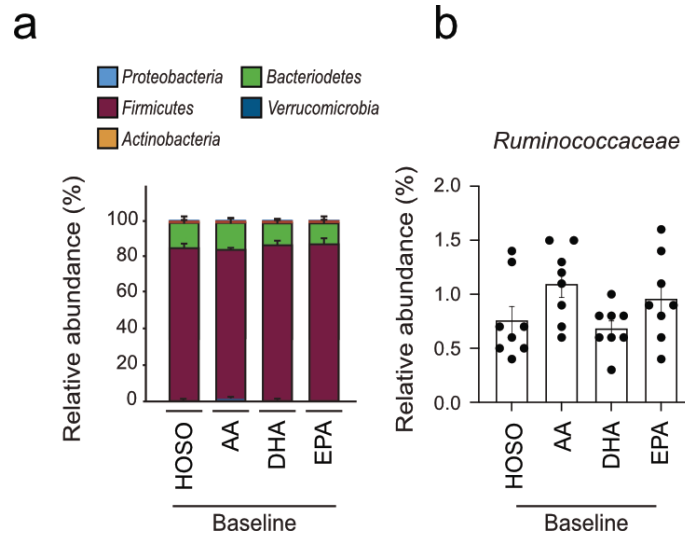
Supplementary Fig. 1. Differentially abundant bacteria at the genus level. **A** Fecal microbiota from mice with TRAMP-C2 tumours at the minimal mass growth stage were compared to the baseline before tumour cell injection (n=8 per group) using STAMP. **B** Fecal microbiota corresponding to mice with *Pten*^{-/-}; *Rb1*^{+/+} tumours compared to tumour-free mice (n=8 per group) using STAMP. **C** Relative levels (fold change) of total 16S rRNA sequences corresponding to *Parabacteroides distasonis* (left, n=8 per group) and *Lactobacillus murinus* (middle, n=8 per group) from fecal samples of mice with TRAMP-C2 tumour harvested at different stages of prostate cancer development. Relative levels (fold change) of total 16S rRNA sequences corresponding to *Parabacteroidetes distasonis* (right) from fecal samples of mice without tumour, with *Pten*^{-/-}; *Rb1*^{+/+} or *Pten*^{-/-}; *Rb1*^{-/-}, tumours (no tumour, n=8 and *Pten*^{-/-}; *Rb1*^{+/+} or *Pten*^{-/-}; *Rb1*^{-/-}, n=10). Graphs in A) and B) are mean ± SD. Graphs in C) are mean ± SEM, p-values in A) and B) are from two-sided Welch's T test and in C) p-values are from two-sided Student's T test.



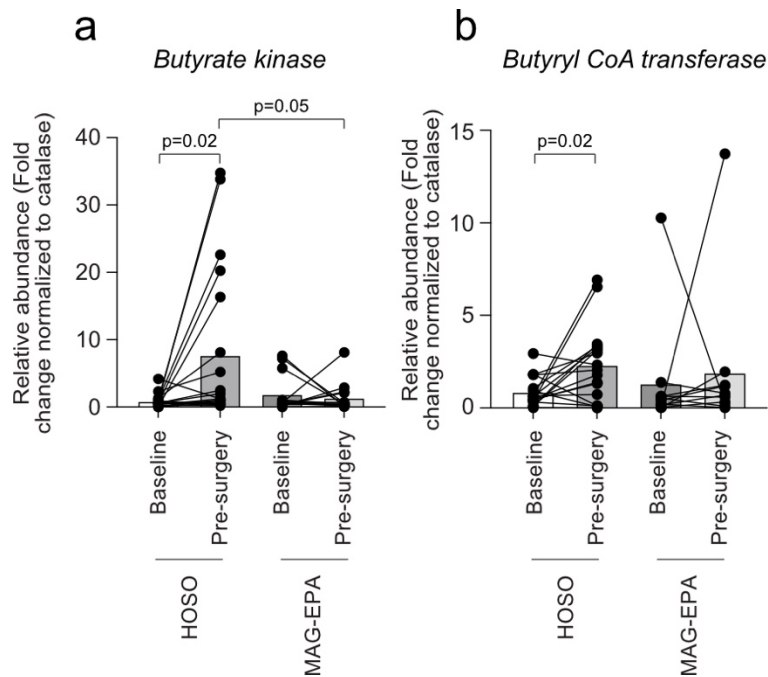
Supplementary Fig. 2 part 1. STAMP analysis of predicted functions. **A** Fecal microbiota comparison of mice with TRAMP-C2 tumours at the End point stage and baseline samples corresponding to main Fig. 2B. **B** Fecal microbiota comparison of mice with *Pten*^{-/-}; *Rb1*^{+/+} tumours and no tumour samples corresponding to main Fig. 2E. Graphs are mean ± SD, n=8 per group and p-values are from two-sided Welch's T test.



Supplementary Fig. 2 part 2. STAMP analysis of predicted functions. C Fecal microbiota comparison of mice with *Pten^{-/-};Rb1^{-/-}* tumours and no tumour samples corresponding to main Fig. 2E. **D** Fecal microbiota comparison of mice with TRAMP-C2 tumours at the Minimal mass stage and baseline corresponding to main Fig. 2B. **E** Fecal microbiota comparison of mice with TRAMP-C2 tumours at the Exponential growth stage and baseline samples corresponding to main Fig. 2B. Graphs are mean \pm SD, $n=8$ per group and p-values are from two-sided Welch's T test.



Supplementary Fig. 3. Baseline gut microbiota in a mouse model of prostate cancer development. **A** Relative abundance of baseline gut microbiota from mice before supplementation with high oleic sunflower oil (HOSO), monoglyceride arachidonic acid (MAG-AA), MAG-docosahexaenoic acid (DHA) or MAG-eicosapentaenoic acid (EPA) long-chain polyunsaturated fatty acids (PUFA) by oral gavage (n=8/group) at the phylum level. **B** Relative abundance of the 16S rRNA sequences corresponding to the Ruminococcaceae family (n=8/group). Data corresponds to the baseline conditions of Figure 4D. Graphs are mean \pm SEM.



Supplementary Fig. 4. Short-chain fatty acid-related enzymes are modulated by a dietary intervention in prostate cancer patients. A randomized clinical trial (NCT02333435) was performed at our clinical facility testing the effect of MAG-EPA PUFA on prostate cancer patients before radical prostatectomy. A subset of 41 patients donated fecal samples for research. Using DNA extracted from fecal samples, we used qPCR to compare the profiles corresponding to men before and after 4-10 weeks of MAG-EPA (n=21) supplementation or HOSO placebo (n=20). The relative abundance of sequences corresponding to rate-limiting enzymes implicated in the biosynthesis of butyric acid (A and B) were measured by qPCR and normalized with bacterial catalase DNA levels. **A** The levels of Butyrate kinase and **B** Butyryl CoA transferase were compared patients receiving MAG-EPA or HOSO 7.2 ± 0.43 weeks prior to radical prostatectomy (Pre-surgery) and their levels at baseline. Graphs are mean ± SEM, two-sided Welch's T test.

Supplementary table 1. Clinical and pathological characteristics of patients pre-radical prostatectomy (corresponding to Figure 1 A-D).

Characteristic	Tumor volume	Low ¹ (n=19)	Medium ² (n=20)	High ³ (n=23)	p-value
Age at radical prostatectomy (y.o)					0.75 ⁸
Mean (SD)		66.8 (6.9)	65.3 (5.4)	66.5 (7.6)	
Body mass index (kg/m ²)					0.54 ⁸
Mean (SD)		27.6 (4.6)	27.0 (3.3)	28.3 (3.8)	
PSA at prostate biopsy (ng/mL)					0.06 ⁸
Mean (SD)		7.4 (9.3)	5.8 (3.2) ⁴	16.0 (11.2)	
Min, max		1.1, 44.5	3.3, 9.8	4.5, 39.0	
Median (Q1, Q3)		5.7 (4.1, 6.5)	5.0 (4.4, 7.1)	12.4 (6.8, 21.0)	
PSA at radical prostatectomy (ng/mL)					0.16 ⁸
Mean (SD)		6.6 (8.7)	6.1 (3.8) ⁵	13.0 (9.4) ⁶	
Min, max		0.9, 41.4	1.4, 16.0	3.7, 32.9	
Median (Q1, Q3)		5.0 (3.6, 6.1)	5.0 (3.9, 7.3)	10.2 (6.4, 18.1)	
ISUP grade group at biopsy, n (%)					0.42 ⁹
1 (Gleason 6)		0 (0.0)	(0.0)	1 (4.3)	
2 (Gleason 3+4)		13 (68.4)	12 (60.0)	8 (34.8)	
3 (Gleason 4+3)		3 (15.8)	4 (20.0)	7 (30.4)	
4 (Gleason 8)		2 (10.5)	3 (15.0)	4 (17.4)	
5 (Gleason 9+)		1 (5.3)	1 (5.0)	3 (13.1)	
ISUP grade group at prostatectomy, n (%)					0.26 ¹⁰
2 (Gleason 3+4)		13 (68.4)	13 (65.0)	10 (43.5)	
3 (Gleason 4+3)		3 (15.8)	5 (25.0)	5 (21.8)	
4 (Gleason 8)		1 (5.3)	1 (5.0)	1 (4.3)	
5 (Gleason 9+)		2 (10.5)	1 (5.0)	7 (30.4)	
Clinical T stage at biopsy, n (%)					0.01 ¹¹
T2a or less		18 (94.7)	18 (90.0)	14 (90.0)	
T2b or T2c		0 (0.0)	2 (10.0)	7 (10.0)	
T3 or more		1 (5.3)	0 (0.0)	2 (0.0)	
Pathological T stage, n (%)					<0.001 ¹²
T2a or less		2 (10.5)	4 (20.0)	7 (30.4)	
T2b or T2c		15 (79.0)	9 (45.0)	3 (13.1)	
T3 or more		2 (10.5)	7 (35.0)	13 (56.5)	
Pathological N stage, n (%)					0.01 ¹³
N0		19 (100.0)	20 (100.0)	18 (78.3)	
N1		0 (0.0)	0 (0.0)	4 (17.4)	
N2		0 (0.0)	0 (0.0)	1 (4.3)	
Pathological M stage, n (%)					-----
M0		10 (52.6)	12 (60.0)	15 (65.2)	
M1		0 (0.0)	0 (0.0)	0 (0.0)	
Mx		9 (47.4)	5 (25.0)	8 (34.8)	
Estimated tumor mass at prostatectomy (g)					<0.001 ⁸
Mean (SD)		3.2 (1.4)	7.4 (1.4)	18.7 (12.7)	

¹ Estimated tumor mass <5.5g calculated as follow: prostate weight (g) * % of tumor at surgery. ² Estimated tumor mass ≥5.5g <10g. ³ Estimated tumor mass ≥10g. ⁴ One missing value. ⁵ 5 patients did not have a PSA measure at RP. ⁶ 6 patients did not have a PSA measure at RP. ⁷ 3 missing values. ⁸ Based on two-way ANOVA test. ⁹ Based on Exact Pearson Chi-Square Test, combining ISUP grade group 2 or less, as well as grade group 4 or more. ¹⁰ Based on Exact Pearson Chi-Square Test, combining ISUP grade group 4 or more. ¹¹ Based on Exact Pearson Chi-Square Test, combining clinical stage T2b or more. ¹² Based on Exact Pearson Chi-Square Test. ¹³ Based on Exact Pearson Chi-Square Test, combining clinical N stage N1/N2. Q: Quartile, PSA: Prostate-specific antigen, MAG-EPA: monoacylglyceride-conjugated eicosapentaenoic acid; ISUP: International society of urological pathology.

Supplementary table 2. Clinical and pathological characteristics of patients post-radical prostatectomy
(corresponding to Figure 1 E-H).

Characteristic	BCR status	None ¹ (n=20)	Low PSA ² (n=9)	High PSA ³ (n=18)	p-value
Age at RP (y.o)					0.10 ⁷
Mean (SD)		67.0 (5.6)	63.9 (6.2)	62.6 (7.2)	
Body mass index at RP (kg/m ²)					0.53 ⁷
Mean (SD)		27.9 (4.5)	26.5 (3.1)	26.9 (2.7)	
PSA at prostate biopsy (ng/mL)					0.54 ⁷
Mean (SD)		15.2 (9.1) ⁴	8.0 (6.7)	14.1 (24.0) ⁵	
Min, max		4.7, 39.0	3.0, 22.0	3.7, 105.9	
Median (Q1, Q3)		8.4 (7.1, 16.4)	5.2 (4.3, 8.6)	5.2 (4.3, 8.6)	
PSA at RP (ng/mL)		NA	NA	NA	NA
ISUP grade group at biopsy, n (%)					0.32 ⁸
1 (Gleason 6)		1 (5.0)	(0.0)	2 (11.1)	
2 (Gleason 3+4)		12 (60.0)	6 (66.7)	3 (16.7) ⁶	
3 (Gleason 4+3)		3 (15.0)	2 (22.2)	6 (33.3) ⁶	
4 (Gleason 8)		2 (10.0)	1 (11.1)	1 (5.6)	
5 (Gleason 9+)		2 (10.0)	0 (0.0)	3 (16.7)	
ISUP grade group at RP, n (%)					0.09 ⁸
1 (Gleason 6)		0 (0.0)	0 (0.0)	1 (5.6)	
2 (Gleason 3+4)		15 (75.0)	6 (66.7)	5 (27.8)	
3 (Gleason 4+3)		2 (10.0)	2 (22.2)	4 (22.2)	
4 (Gleason 8)		1 (5.0)	0 (0.0)	4 (22.2)	
5 (Gleason 9+)		2 (10.0)	1 (11.1)	4 (22.2)	
Clinical T stage, n (%)				⁵	0.46 ⁹
T2a or less		14 (70.0)	8 (88.9)	14 (77.8)	
T2b or T2c		5 (25.0)	1 (11.1)	2 (11.1)	
T3 or more		1 (5.0)	0 (0.0)	1 (5.6)	
Pathological T stage, n (%)					0.01 ¹⁰
T2a or less		9 (45.0)	1 (11.1)	1 (5.6)	
T2b or T2c		3 (15.0)	5 (55.6)	5 (27.8)	
T3 or more		8 (40.0)	3 (33.3)	12 (66.7)	
Pathological N stage, n (%)					0.06 ¹¹
N0		19 (95.0)	8(88.9)	12 (66.7)	
N1		1 (5.0)	1 (11.1)	4 (22.2)	
N2		0 (0.0)	0 (0.0)	2 (11.1)	
Pathological M stage, n(%)					-----
M0		10 (40.0)	4 (44.4)	16 (88.9)	
M1		0 (0.0)	0 (0.0)	0 (0.0)	
Mx		10 (45.0)	5 (55.6)	2 (11.1)	
Estimated tumor mass at RP ⁴ (g)					0.99 ⁷
Mean (SD)		12.9 (13.2)	13.2 (9.2)	17.5 (21.4) ⁵	
Min, Max		4.5, 63.7	1.6, 32.0	0.9, 88.0	
Median (Q1, Q3)		8.6 (6.3, 13.7)	14.0 (6.8, 17.6)	9.9 (4.8, 17.2)	

¹ Patients with no BCR following surgery, ² Patients who experienced ultrasensible BCR following a curative intent RP (PSA level <0.5ng/mL), ³ Patients who experienced a clinical BCR following a curative intent RP (PSA level >0.5ng/mL). ⁴ Estimated tumor mass calculated as follow: prostate weight (g) * % of tumor at surgery. ⁵ One missing value. ⁶ 3 patients had a Gleason 7 at BP, with no specification of primary and secondary pattern. ⁷ Based on two-way ANOVA test. ⁸ Based on Exact Pearson Chi-Square Test, combining ISUP grade group 2 or less, as well as grade group 4 or more. ⁹ Based on Exact Pearson Chi-Square Test, combining clinical stage T2b or more. ¹⁰ Based on Exact Pearson Chi-Square Test. ¹¹ Based on Exact Pearson Chi-Square Test, combining clinical N stage N1/N2. Q: Quartile, PSA: Prostate-specific antigen, MAG-EPA: monoacylglyceride-conjugated eicosapentaenoic acid, ISUP: International society of urological pathology, RP: Radical prostatectomy, BCR: Biochemical recurrence.

Supplementary table 3. Clinical characteristics of donor patients for fecal microbiota transfers
(Corresponding to Figure 3).

Characteristic	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
Age (y.o)	71.2	65.4	63.5	72.4	65.8	68.0
Body mass index (kg/m ²)	26.1	25.0	32.3	28.1	27.3	27.5
PSA (ng/mL)	44.48	5.91	4.82	20.27	6.69	11.63
ISUP grade group ¹	4	2	3	5	3	4
Cancer stage	T1c	T1c	T1c	T3	T1c	T2b
Estimated tumor mass at RP ² (g)	1.2	3.4	9.4	10.0	10.3	21.0

¹ International society of urological pathology grade group: 2 = Gleason 7 (3+4), 3 = Gleason 7 (4+3), 4= Gleason 8, 5= Gleason 9+.

² Estimated tumor mass calculated as follow: prostate weight (g) * % of tumor at surgery. RP: radical prostatectomy, PSA: Prostate-specific antigen.

Supplementary table 4. Baseline characteristics of patients enrolled in the clinical trial (corresponding to Figure 5).

Characteristic	Placebo (n=20)	MAG-EPA (n=21)	p-value
Age (y.o) Mean (SD)	65.8 (7.0)	65.9 (7.0)	0.97 ¹
Body mass index (kg/m ²) Mean (SD)	27.6 (4.5)	27.1 (2.9)	0.70 ¹
PSA (ng/mL) Mean (SD) Min, max Median (Q1, Q3)	6.3 (4.4) 1.1, 23.0 5.7 (4.2, 6.6)	10.8 (10.4) 2.3, 44.5 7.7 (4.7, 6.7)	0.08 ¹
ISUP grade group, n (%) 2 (Gleason 3+4) 3 (Gleason 4+3) 4 (Gleason 8) 5 (Gleason 9+)	12 (60.0) 5 (25.0) 3 (15.0) 0 (0.0)	9 (42.9) 6 (28.6) 4 (19.0) 2 (9.5)	0.47 ²
Cancer stage, n (%) T2a or less T2b or T2c T3 or more	19 (95.0) 1 (5.0) 0 (0.0)	17 (81.0) 2 (9.5) 2 (9.5)	0.17 ³

¹ Based on two-sided Student's t-test. ² Based on an Exact Pearson Chi-Square Test, combining ISUP grade group 4 or more. ³ Based on an Exact Pearson Chi-Square Test, combining cancer stage T2b or more. SD: Standard deviation, Q: Quartile, NCCN: National Comprehensive Cancer Network, PSA: Prostate-specific antigen, MAG-EPA: monoacylglyceride-conjugated eicosapentaenoic acid; ISUP: International society of urological pathology.

Supplementary table 5. Characteristics at prostatectomy of patients enrolled in the clinical trial (corresponding to Figure 5).

Characteristic	Placebo (n=20)	MAG-EPA (n=21)	p-value
PSA (ng/mL)			0.08 ²
Mean (SD)	5.6 (4.2) ¹	9.9 (10.0)	
Min, max	0.9, 20.6	2.0, 41.4	
Median (Q1, Q3)	4.9 (3.8, 6.2)	6.34 (3.8, 6.1)	
ISUP Grade group, n (%)			0.84 ³
2 (Gleason 3+4)	11 (55.0)	10 (47.6)	
3 (Gleason 4+3)	5 (25.0)	7 (33.3)	
4 (Gleason 8)	1 (5.0)	1 (4.8)	
5 (Gleason 9+)	3 (15.0)	3 (14.3)	
Pathological T stage, n (%)			0.81 ⁴
T2a or less	1 (5.0)	2 (9.5)	
T2b or T2c	12 (60.0)	13 (61.9)	
T3 or more	7 (35.0)	6 (28.6)	
Grade reclassification			0.05⁵
Upgrade	6 (30.0)	2 (9.5)	
Same	13 (65.0)	15 (71.4)	
Downgrade	1 (5.0)	4 (19.4)	

¹ One patient did not have a PSA measure at RP. ² Based on two-sided Student's t-test. ³ Based on an Exact Pearson Chi-Square Test, combining ISUP grade group 4 or more. ⁴ Based on an Exact Pearson Chi-Square Test. ⁵ Based on ordinal logistic regression comparing the change over the intervention time between groups. SD: Standard deviation, Q: Quartile, PSA: Prostate-specific antigen, MAG-EPA: monoacylglyceride-conjugated eicosapentaenoic acid; ISUP: International society of urological pathology; RP: radical prostatectomy.