

Supplementary Figures

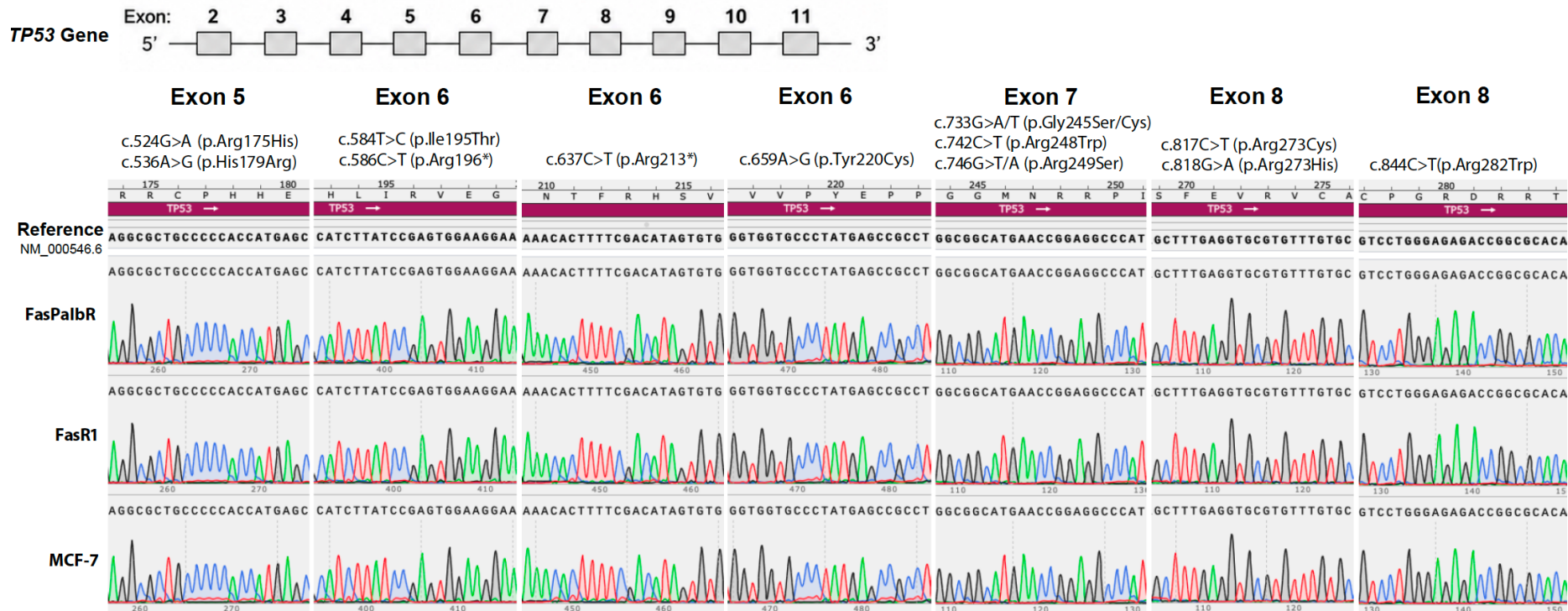
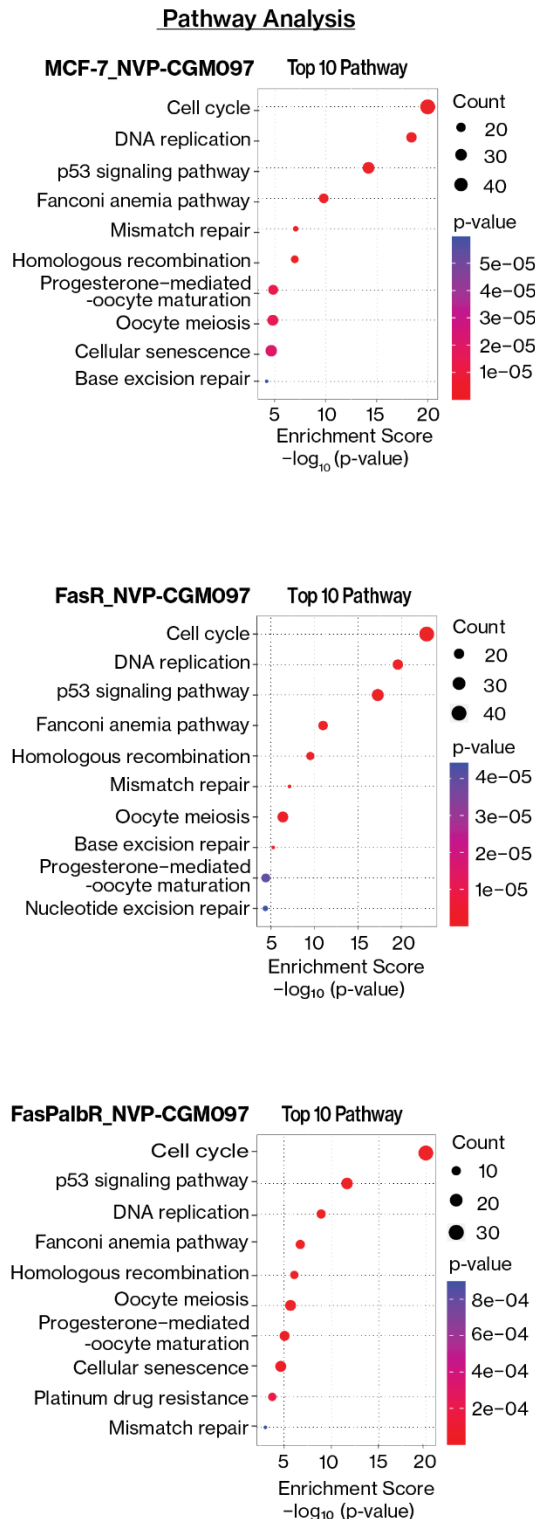


Figure S1. TP53 Sanger sequencing confirms the absence of coding mutations in parental and resistant cell lines. Representative Sanger sequencing chromatograms spanning selected TP53 hotspot regions in vehicle-treated MCF-7, fulvestrant-resistant (FasR), and fulvestrant/palboiclib-resistant (FasPalbR) cells. Sanger sequencing was performed across all TP53 coding exons (2-11). Sequence chromatograms were aligned to the TP53 reference transcript (NM_000546.6) and analysed using *JSI* software (v. 4.4.0). No coding pathogenic variants were identified in MCF-7, FasR, or FasPalbR cells.

A



B

Hallmark Gene Set Enrichment Analysis (GSEA)

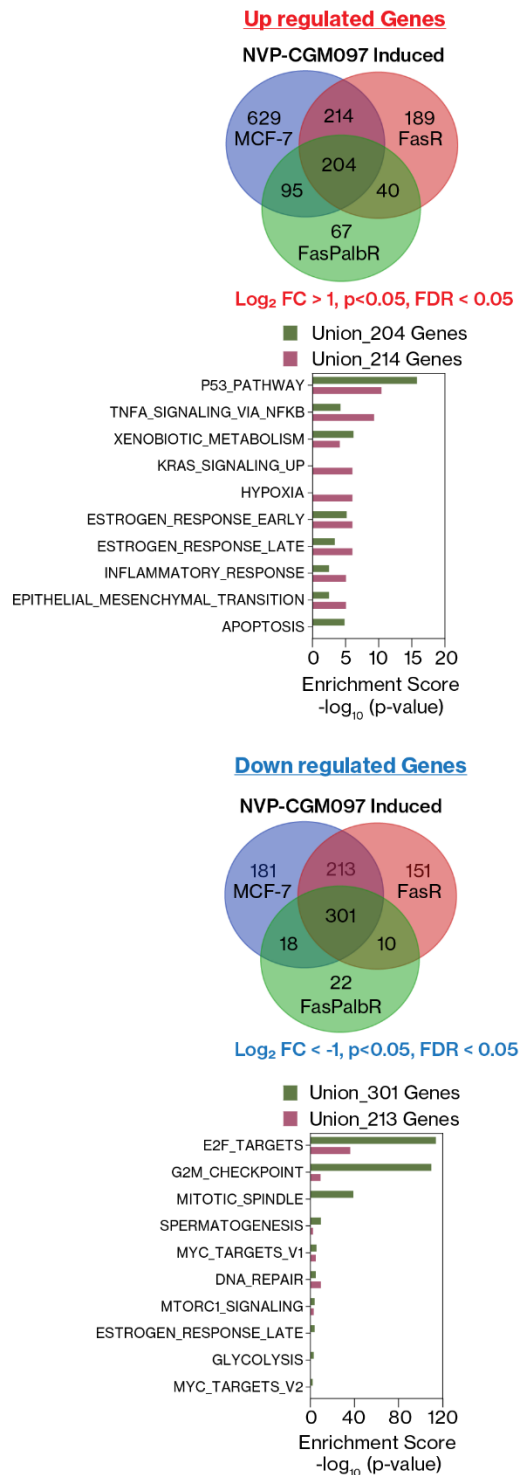
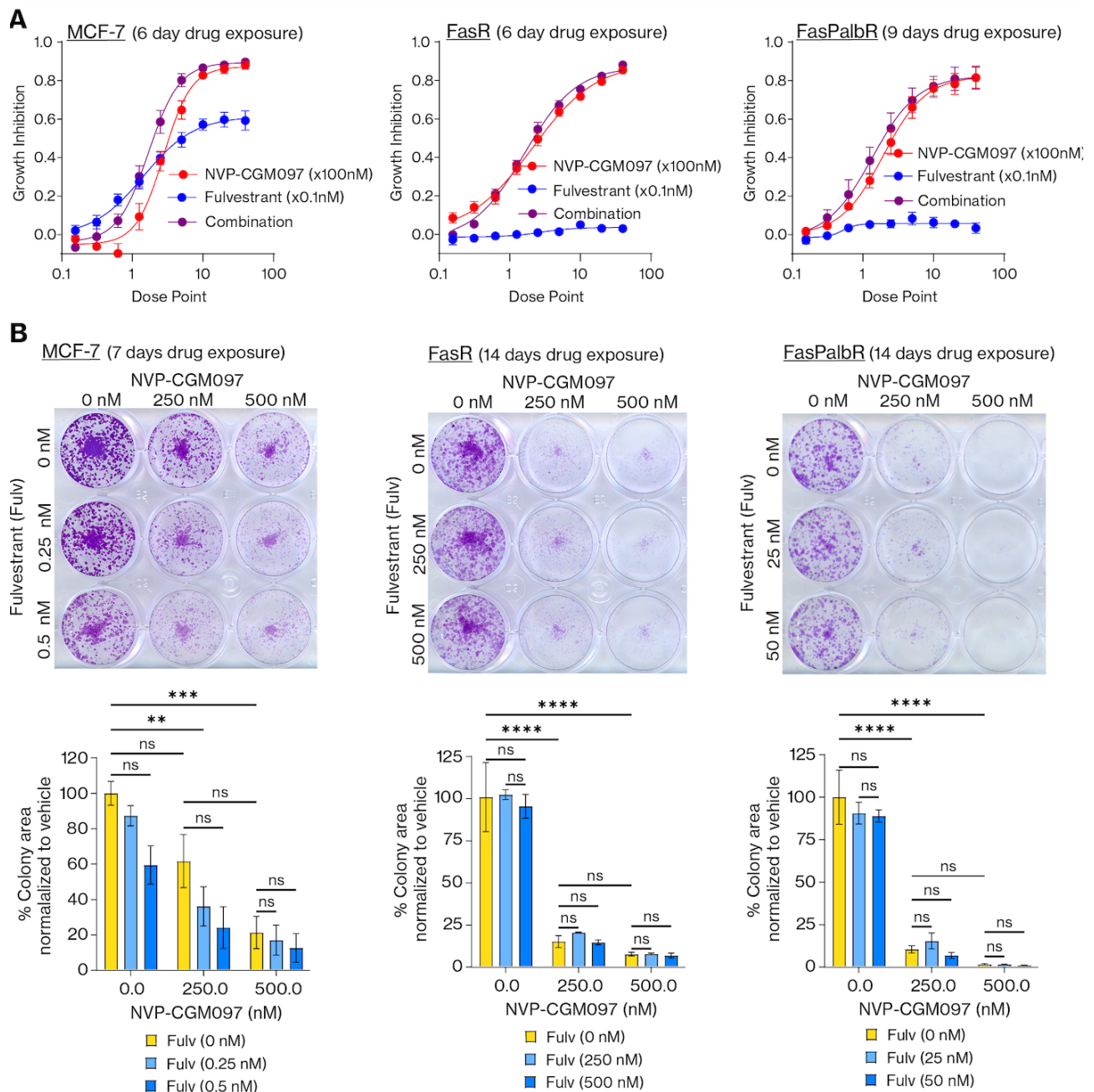


Figure S2. Differentially expressed genes and pathway enrichment in response to NVP-CGM097 in parental MCF-7, FasR, and FasPalbR cells. (A) Pathway enrichment analysis was performed using the ClusterProfiler *R* package on significantly regulated genes ($\text{FDR} < 0.05$) following 48 h treatment with NVP-CGM097 (500 nM). Top 10 enriched pathways are shown, ranked by p-value. **(B)** Gene Set Enrichment Analysis (GSEA) of differentially expressed genes with $\text{logFC} > 1$ (upregulated) or < -1 (downregulated) and $\text{FDR} q < 0.05$. Hallmark gene sets are ranked based on $\text{log}_{10}(\text{p-value})$ of enrichment. Venn diagrams highlight the overlap and uniqueness of DEGs across the three cell models.



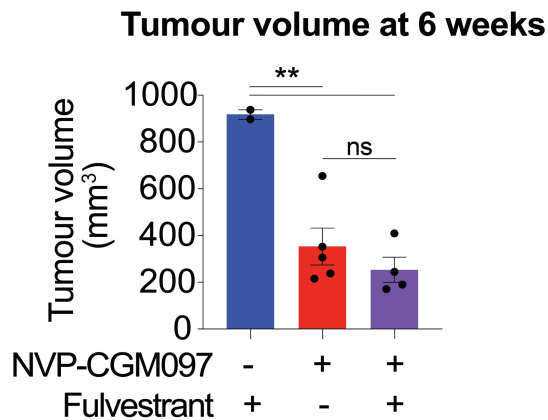


Figure S4. Tumour volume at the 6-week treatment endpoint in Gar15-13D-FPR PDX model. Tumour volumes were compared between the fulvestrant, NVP-CGM097, and fulvestrant plus NVP-CGM097 treatment groups using one-way ANOVA followed by Tukey's multiple-comparison test. Both NVP-CGM097 monotherapy and the combination of NVP-CGM097 plus fulvestrant significantly reduced tumour volume compared with fulvestrant alone (**P < 0.01), whereas no significant difference was observed between NVP-CGM097 monotherapy and the combination treatment (ns). Bars represent mean $\pm$ SEM, with individual tumours shown as dots.

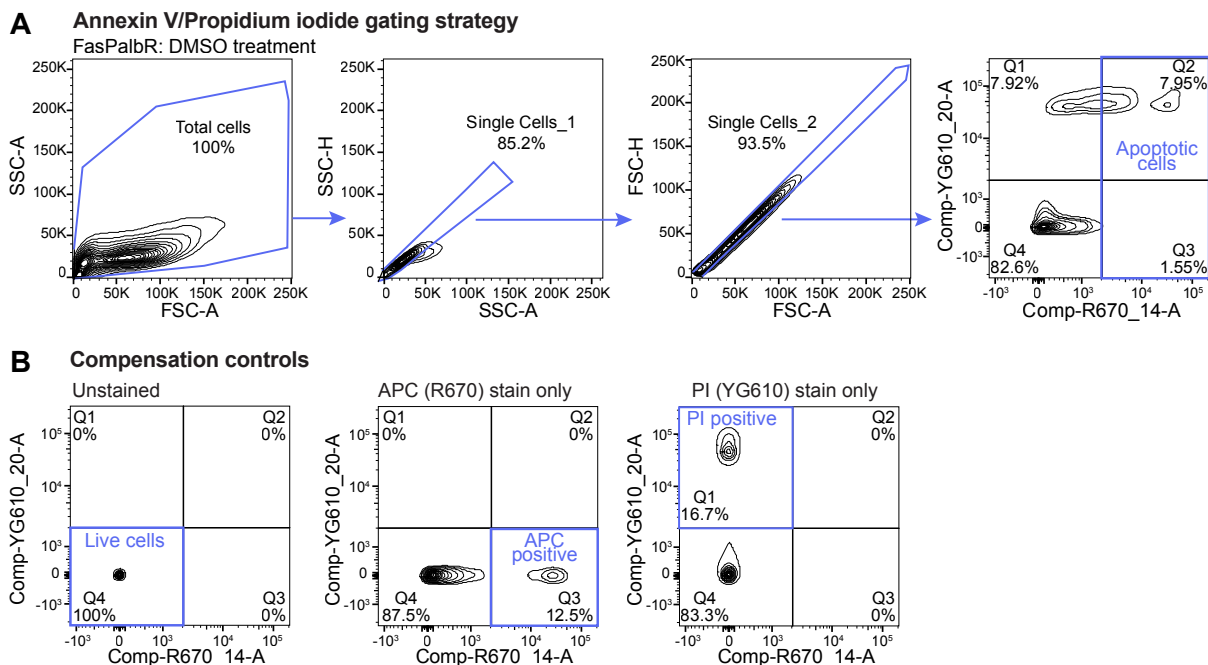


Figure S5. Gating strategy for Annexin V/propidium iodide (PI) flow cytometry to assess apoptosis in FasPalbR cells. **(A)** Gating strategy used to identify apoptotic FasPalbR cells. Initial gates selected total cell population, followed by gating on single cells. The final gate was applied to define Annexin V and PI staining profiles, with Q2 (Annexin V⁺/PI⁺) and Q3 (Annexin V⁺/PI⁻) representing the apoptotic population. **(B)** Compensation controls using unstained, Annexin V–APC only, and PI–YG610 only stained cells to define quadrant boundaries for apoptosis analysis. Gates were applied consistently across all experimental samples.

Supplementary Tables:

Table S1: KEGG pathway enrichment analysis of differentially expressed genes (DEGs) in parental MCF-7 cells following NVP-CGM097 treatment (48 h), performed using the *ClusterProfiler* R package.

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
hsa04110	Cell cycle	49/709	127/8223	1.02134E-20	3.23766E-18	2.93502E-18
hsa03030	DNA replication	25/709	36/8223	3.97739E-19	6.30416E-17	5.71488E-17
hsa04115	p53 signaling pathway	31/709	73/8223	6.33886E-15	6.69806E-13	6.07196E-13
hsa03460	Fanconi anemia pathway	22/709	54/8223	1.53326E-10	1.21511E-08	1.10153E-08
hsa03430	Mismatch repair	12/709	23/8223	8.55577E-08	5.42436E-06	4.91732E-06
hsa03440	Homologous recombination	16/709	41/8223	1.04366E-07	5.51402E-06	4.99860E-06
hsa04914	Progesterone-mediated oocyte maturation	23/709	102/8223	1.36575E-05	5.82721E-04	5.28251E-04
hsa04114	Oocyte meiosis	27/709	131/8223	1.47059E-05	5.82721E-04	5.28251E-04
hsa04218	Cellular senescence	30/709	156/8223	2.09462E-05	7.37770E-04	6.68807E-04
hsa03410	Base excision repair	11/709	33/8223	5.98447E-05	1.89708E-03	1.71975E-03
hsa00240	Pyrimidine metabolism	15/709	58/8223	8.23224E-05	2.25625E-03	2.04534E-03
hsa05222	Small cell lung cancer	20/709	92/8223	8.54099E-05	2.25625E-03	2.04534E-03
hsa04068	FoxO signaling pathway	25/709	131/8223	1.14553E-04	2.62686E-03	2.38131E-03
hsa03420	Nucleotide excision repair	13/709	47/8223	1.16013E-04	2.62686E-03	2.38131E-03
hsa01524	Platinum drug resistance	16/709	73/8223	3.87963E-04	8.19896E-03	7.43256E-03
hsa01232	Nucleotide metabolism	17/709	85/8223	7.95086E-04	1.57527E-02	1.42802E-02

hsa05166	Human T-cell leukemia virus 1 infection	32/709	222/8223	2.56174E-03	4.77689E-02	4.33037E-02
hsa05212	Pancreatic cancer	14/709	76/8223	4.93711E-03	8.69481E-02	7.88206E-02
hsa04210	Apoptosis	21/709	136/8223	6.06509E-03	1.01191E-01	9.17324E-02
hsa04512	ECM-receptor interaction	15/709	88/8223	7.75430E-03	1.19455E-01	1.08289E-01
hsa05169	Epstein-Barr virus infection	28/709	202/8223	7.91342E-03	1.19455E-01	1.08289E-01
hsa00380	Tryptophan metabolism	9/709	42/8223	8.35815E-03	1.20433E-01	1.09176E-01
hsa05161	Hepatitis B	23/709	162/8223	1.15666E-02	1.59418E-01	1.44516E-01
hsa05220	Chronic myeloid leukemia	13/709	76/8223	1.23437E-02	1.60399E-01	1.45406E-01
hsa00230	Purine metabolism	19/709	128/8223	1.32180E-02	1.60399E-01	1.45406E-01
hsa04380	Osteoclast differentiation	19/709	128/8223	1.32180E-02	1.60399E-01	1.45406E-01
hsa04915	Estrogen signaling pathway	20/709	138/8223	1.43913E-02	1.60399E-01	1.45406E-01
hsa04020	Calcium signaling pathway	31/709	240/8223	1.44859E-02	1.60399E-01	1.45406E-01
hsa04610	Complement and coagulation cascades	14/709	86/8223	1.46737E-02	1.60399E-01	1.45406E-01
hsa05162	Measles	20/709	139/8223	1.55006E-02	1.63789E-01	1.48479E-01
hsa00310	Lysine degradation	11/709	63/8223	1.77097E-02	1.81096E-01	1.64168E-01
hsa04750	Inflammatory mediator regulation of TRP channels	15/709	98/8223	2.00707E-02	1.98825E-01	1.80240E-01
hsa05030	Cocaine addiction	9/709	49/8223	2.24710E-02	2.15857E-01	1.95680E-01
hsa04923	Regulation of lipolysis in adipocytes	10/709	58/8223	2.51208E-02	2.34215E-01	2.12322E-01
hsa05214	Glioma	12/709	75/8223	2.58873E-02	2.34465E-01	2.12548E-01

hsa00830	Retinol metabolism	11/709	68/8223	2.98686E-02	2.59016E-01	2.34804E-01
hsa05226	Gastric cancer	20/709	149/8223	3.06464E-02	2.59016E-01	2.34804E-01
hsa05225	Hepatocellular carcinoma	22/709	168/8223	3.10492E-02	2.59016E-01	2.34804E-01
hsa05031	Amphetamine addiction	11/709	69/8223	3.28793E-02	2.67250E-01	2.42269E-01
hsa02010	ABC transporters	8/709	45/8223	3.64170E-02	2.88605E-01	2.61627E-01
hsa01522	Endocrine resistance	14/709	98/8223	4.07973E-02	3.15433E-01	2.85948E-01
hsa04933	AGE-RAGE signaling pathway in diabetic complications	14/709	100/8223	4.72053E-02	3.52101E-01	3.19189E-01
hsa05224	Breast cancer	19/709	147/8223	4.79789E-02	3.52101E-01	3.19189E-01
hsa04722	Neurotrophin signaling pathway	16/709	119/8223	4.88721E-02	3.52101E-01	3.19189E-01

Table S2: KEGG pathway enrichment analysis of differentially expressed genes (DEGs) in fulvestrant-resistant FasR cells following NVP-CGM097 treatment (48 h), performed using the *ClusterProfiler* R package.

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
hsa04110	Cell cycle	47/552	127/8223	1.20948E-23	3.85823E-21	3.69208E-21
hsa03030	DNA replication	24/552	36/8223	2.54098E-20	4.05287E-18	3.87835E-18
hsa04115	p53 signaling pathway	31/552	73/8223	5.15760E-18	5.48425E-16	5.24808E-16
hsa03460	Fanconi anemia pathway	21/552	54/8223	1.02657E-11	8.18687E-10	7.83433E-10
hsa03440	Homologous recombination	17/552	41/8223	2.98940E-10	1.90723E-08	1.82510E-08
hsa03430	Mismatch repair	11/552	23/8223	7.28414E-08	3.87273E-06	3.70596E-06
hsa04114	Oocyte meiosis	26/552	131/8223	4.31902E-07	1.96824E-05	1.88348E-05
hsa03410	Base excision repair	11/552	33/8223	5.61474E-06	2.23888E-04	2.14247E-04

hsa04914	Progesterone-mediated oocyte maturation	19/552	102/8223	3.95845E-05	1.40305E-03	1.34263E-03
hsa03420	Nucleotide excision repair	12/552	47/8223	4.42017E-05	1.41003E-03	1.34931E-03
hsa01524	Platinum drug resistance	15/552	73/8223	7.99173E-05	2.31760E-03	2.21780E-03
hsa04218	Cellular senescence	23/552	156/8223	2.76000E-04	7.33701E-03	7.02106E-03
hsa05222	Small cell lung cancer	16/552	92/8223	3.62529E-04	8.71655E-03	8.34120E-03
hsa00240	Pyrimidine metabolism	12/552	58/8223	3.82544E-04	8.71655E-03	8.34120E-03
hsa01232	Nucleotide metabolism	14/552	85/8223	1.44388E-03	3.07065E-02	2.93842E-02
hsa04068	FoxO signaling pathway	17/552	131/8223	6.45559E-03	1.28708E-01	1.23166E-01
hsa00100	Steroid biosynthesis	5/552	20/8223	8.91942E-03	1.65353E-01	1.58232E-01
hsa04210	Apoptosis	17/552	136/8223	9.33024E-03	1.65353E-01	1.58232E-01
hsa05216	Thyroid cancer	7/552	37/8223	1.04200E-02	1.74946E-01	1.67412E-01
hsa00480	Glutathione metabolism	9/552	57/8223	1.29059E-02	2.05849E-01	1.96984E-01
hsa05166	Human T-cell leukemia virus 1 infection	24/552	222/8223	1.36630E-02	2.07547E-01	1.98610E-01
hsa04215	Apoptosis - multiple species	6/552	32/8223	1.80445E-02	2.61645E-01	2.50378E-01
hsa05207	Chemical carcinogenesis - receptor activation	22/552	212/8223	2.69684E-02	3.74040E-01	3.57933E-01
hsa05210	Colorectal cancer	11/552	86/8223	2.82034E-02	3.74870E-01	3.58727E-01
hsa00830	Retinol metabolism	9/552	68/8223	3.69844E-02	4.71921E-01	4.51599E-01
hsa00983	Drug metabolism - other enzymes	10/552	80/8223	4.06299E-02	4.98497E-01	4.77031E-01
hsa04915	Estrogen signaling pathway	15/552	138/8223	4.32291E-02	4.98615E-01	4.77144E-01

hsa05161	Hepatitis B	17/552	162/8223	4.37656E-02	4.98615E-01	4.77144E-01
hsa00330	Arginine and proline metabolism	7/552	50/8223	4.79438E-02	5.27382E-01	5.04672E-01

Table S3: KEGG pathway enrichment analysis of differentially expressed genes (DEGs) in dual fulvestrant- and palbociclib-resistant FasPalbR cells following NVP-CGM097 treatment (48 h), performed using the *ClusterProfiler* R package.

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
hsa04110	Cell cycle	33/280	127/8223	1.05043E-20	2.79413E-18	2.63159E-18
hsa04115	p53 signaling pathway	19/280	73/8223	2.13664E-12	2.84173E-10	2.67642E-10
hsa03030	DNA replication	12/280	36/8223	1.15916E-09	1.02779E-07	9.68003E-08
hsa03460	Fanconi anemia pathway	12/280	54/8223	1.83437E-07	1.21986E-05	1.14890E-05
hsa03440	Homologous recombination	10/280	41/8223	7.85570E-07	4.17923E-05	3.93612E-05
hsa04114	Oocyte meiosis	17/280	131/8223	1.93826E-06	8.59295E-05	8.09308E-05
hsa04914	Progesterone-mediated oocyte maturation	14/280	102/8223	8.20111E-06	3.11642E-04	2.93513E-04
hsa04218	Cellular senescence	17/280	156/8223	2.09813E-05	6.97629E-04	6.57046E-04
hsa01524	Platinum drug resistance	10/280	73/8223	1.66233E-04	4.91310E-03	4.62729E-03
hsa03430	Mismatch repair	5/280	23/8223	8.97164E-04	2.38646E-02	2.24763E-02
hsa00480	Glutathione metabolism	7/280	57/8223	3.02901E-03	7.32470E-02	6.89861E-02
hsa00240	Pyrimidine metabolism	7/280	58/8223	3.34681E-03	7.41876E-02	6.98719E-02
hsa05222	Small cell lung cancer	9/280	92/8223	3.96393E-03	8.11080E-02	7.63898E-02
hsa03420	Nucleotide excision repair	6/280	47/8223	4.90383E-03	9.31728E-02	8.77528E-02
hsa01232	Nucleotide metabolism	8/280	85/8223	8.14039E-03	1.44356E-01	1.35959E-01
hsa05166	Human T-cell leukemia virus 1 infection	15/280	222/8223	8.73736E-03	1.45259E-01	1.36809E-01

hsa00230	Purine metabolism	10/280	128/8223	1.18163E-02	1.84890E-01	1.74135E-01
hsa05214	Glioma	7/280	75/8223	1.35630E-02	1.92530E-01	1.81330E-01
hsa04068	FoxO signaling pathway	10/280	131/8223	1.37521E-02	1.92530E-01	1.81330E-01
hsa04210	Apoptosis	10/280	136/8223	1.74942E-02	2.32673E-01	2.19138E-01
hsa03410	Base excision repair	4/280	33/8223	2.47673E-02	3.00077E-01	2.82621E-01
hsa05030	Cocaine addiction	5/280	49/8223	2.48184E-02	3.00077E-01	2.82621E-01
hsa05031	Amphetamine addiction	6/280	69/8223	2.95208E-02	3.40078E-01	3.20295E-01
hsa05226	Gastric cancer	10/280	149/8223	3.06837E-02	3.40078E-01	3.20295E-01
hsa04970	Salivary secretion	7/280	92/8223	3.69369E-02	3.93008E-01	3.70146E-01
hsa03320	PPAR signaling pathway	6/280	75/8223	4.19767E-02	4.29454E-01	4.04472E-01
hsa00534	Glycosaminoglycan biosynthesis - heparan sulfate / heparin	3/280	24/8223	4.66272E-02	4.59364E-01	4.32642E-01
hsa05219	Bladder cancer	4/280	41/8223	4.97113E-02	4.72257E-01	4.44785E-01