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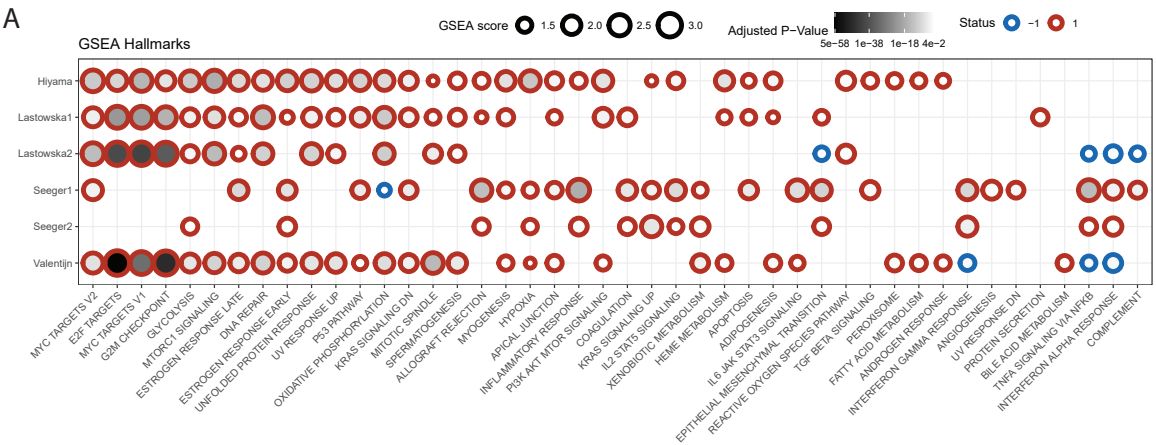
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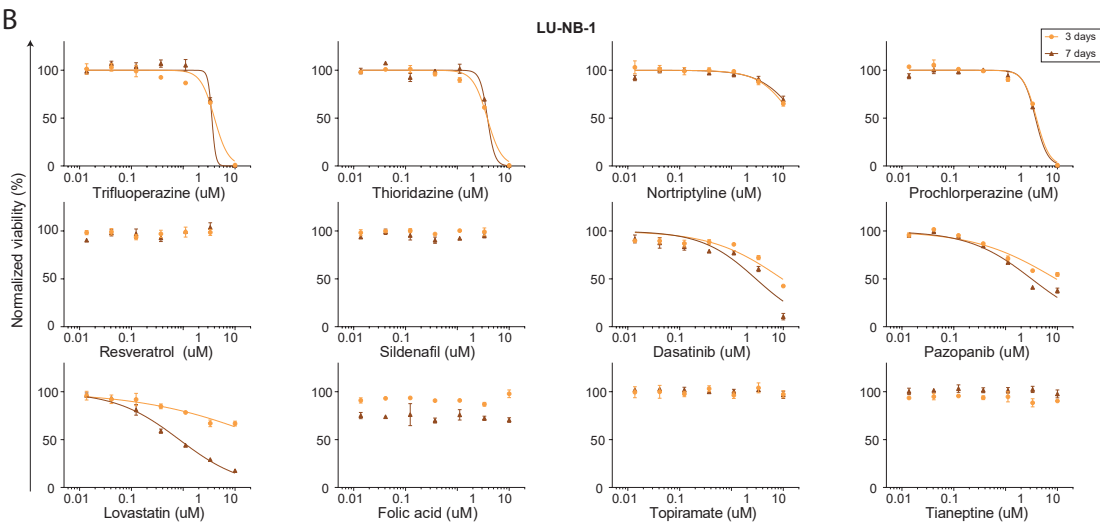
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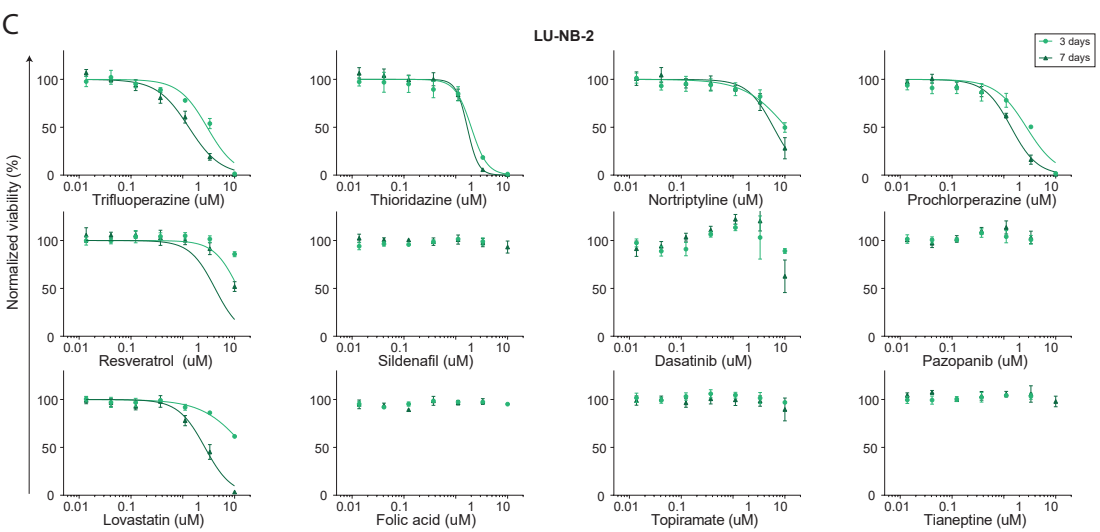
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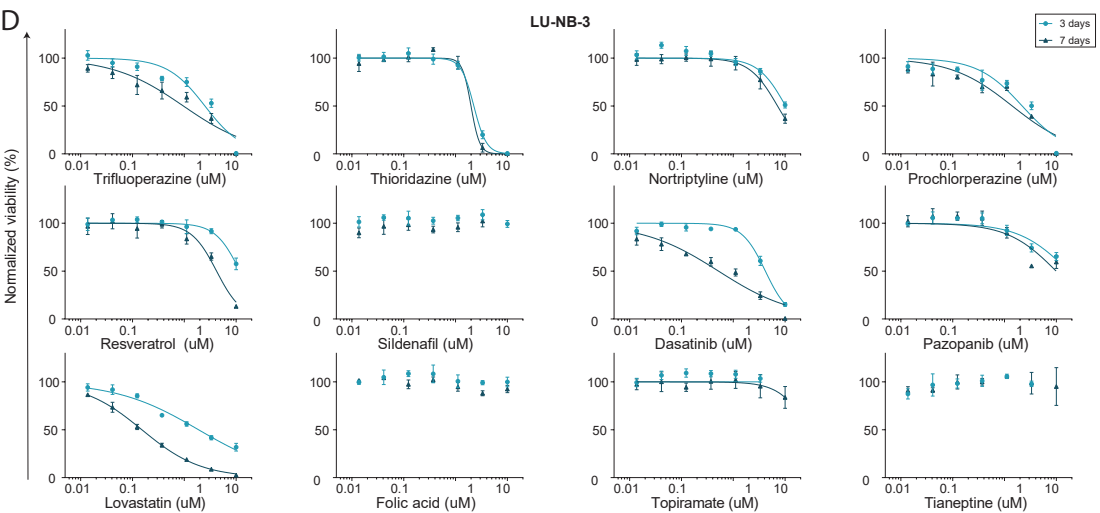
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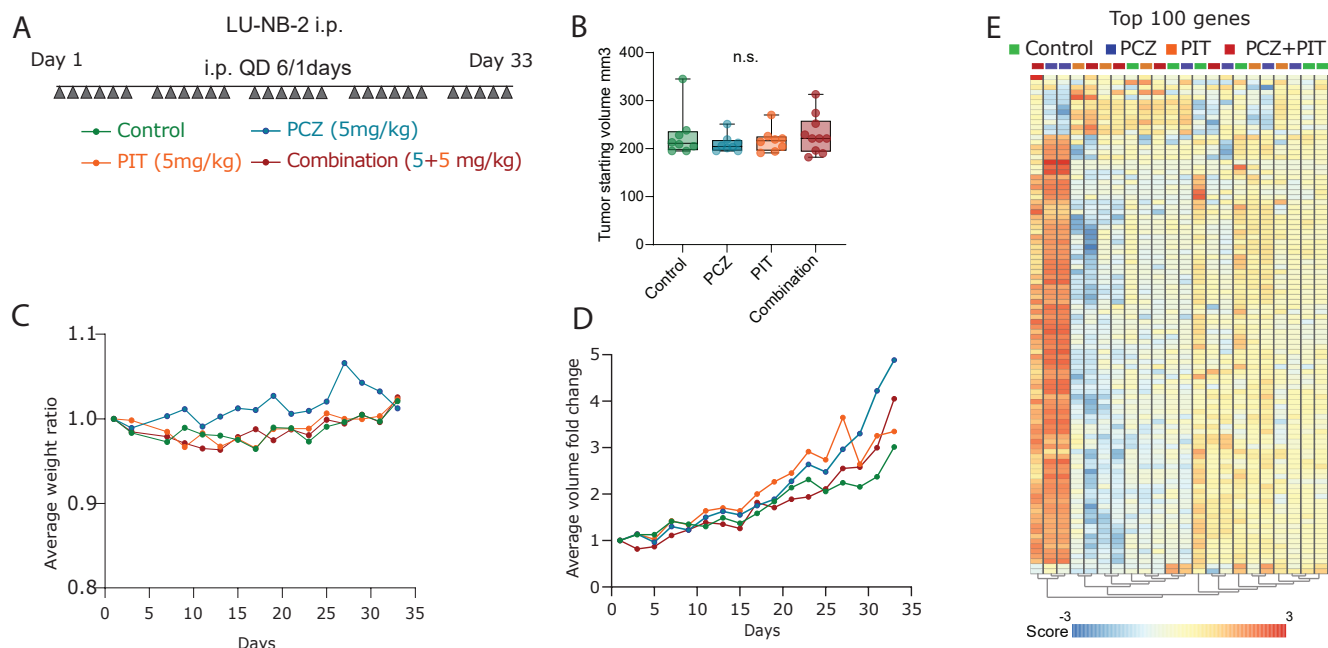
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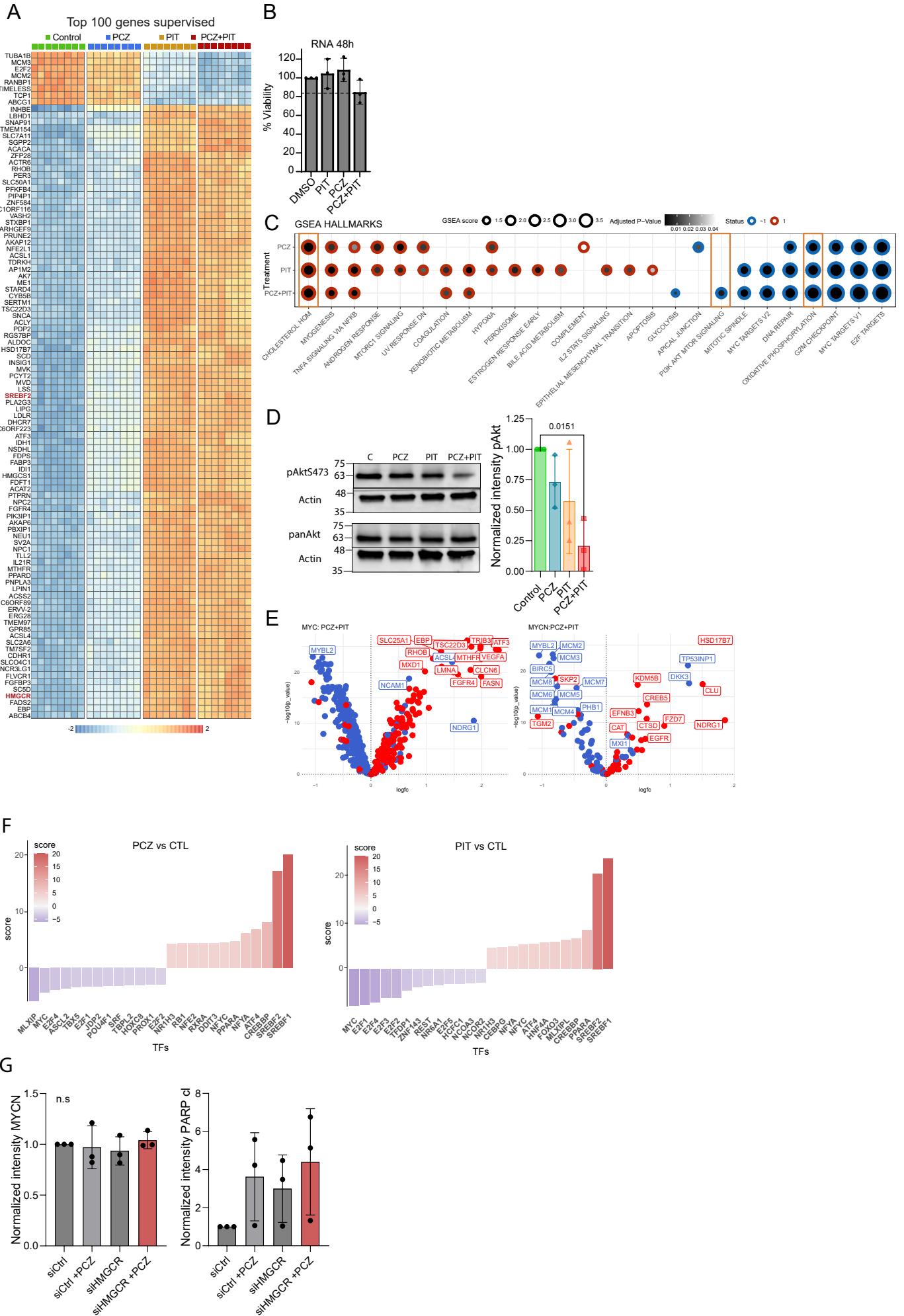
D



Appendix Figure S1. Gene expression profile (GEP) analysis and *in vitro* response of predicted drugs. **A** Gene set enrichment analysis (Hallmarks database) of GEPs across datasets included in the original drug predictions (Table S1). **B-D** Single dose-response curves for the predicted 12 drugs (Table 1). NB PDX-derived organoids (n=3) treated for 3 or 7 days: LU-NB-1, LU-NB-2, and LU-NB-3; data represent mean $\pm$ SD.

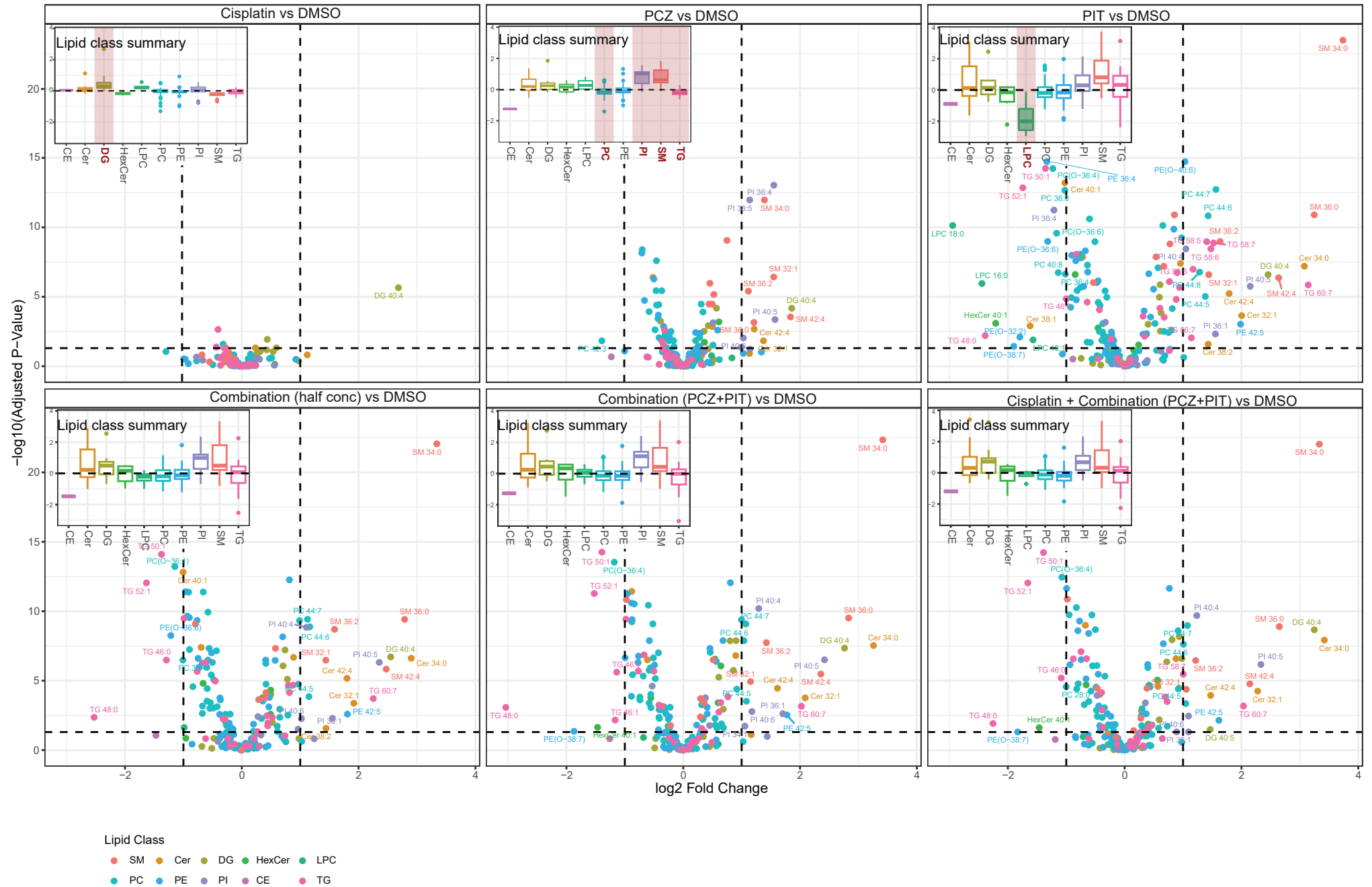


Appendix Figure S2. Effects of intraperitoneal treatment in LU-NB-2. **A** Schematic overview of the LU-NB-2 treatment study *in vivo*. Drugs administered intraperitoneally (i.p.) six times a week for 33 days. Control n=8; PCZ n=8; PIT n=8; Combination n=10 **B** Tumor starting volume (control n=8; PCZ n=8; PIT n=8; Combination n=10, one-way ANOVA followed by Tukey's multiple comparisons test, n.s. boxes represent the interquartile range and whiskers indicate minimum and maximum values). **C** Average weight ratio. **D** Average tumor size in each treatment group throughout the study duration. **E** Unsupervised gene expression analysis of the top 100 most variable genes from tumor samples collected on day 33. PCZ-prochlorperazine; PIT-pitavastatin.

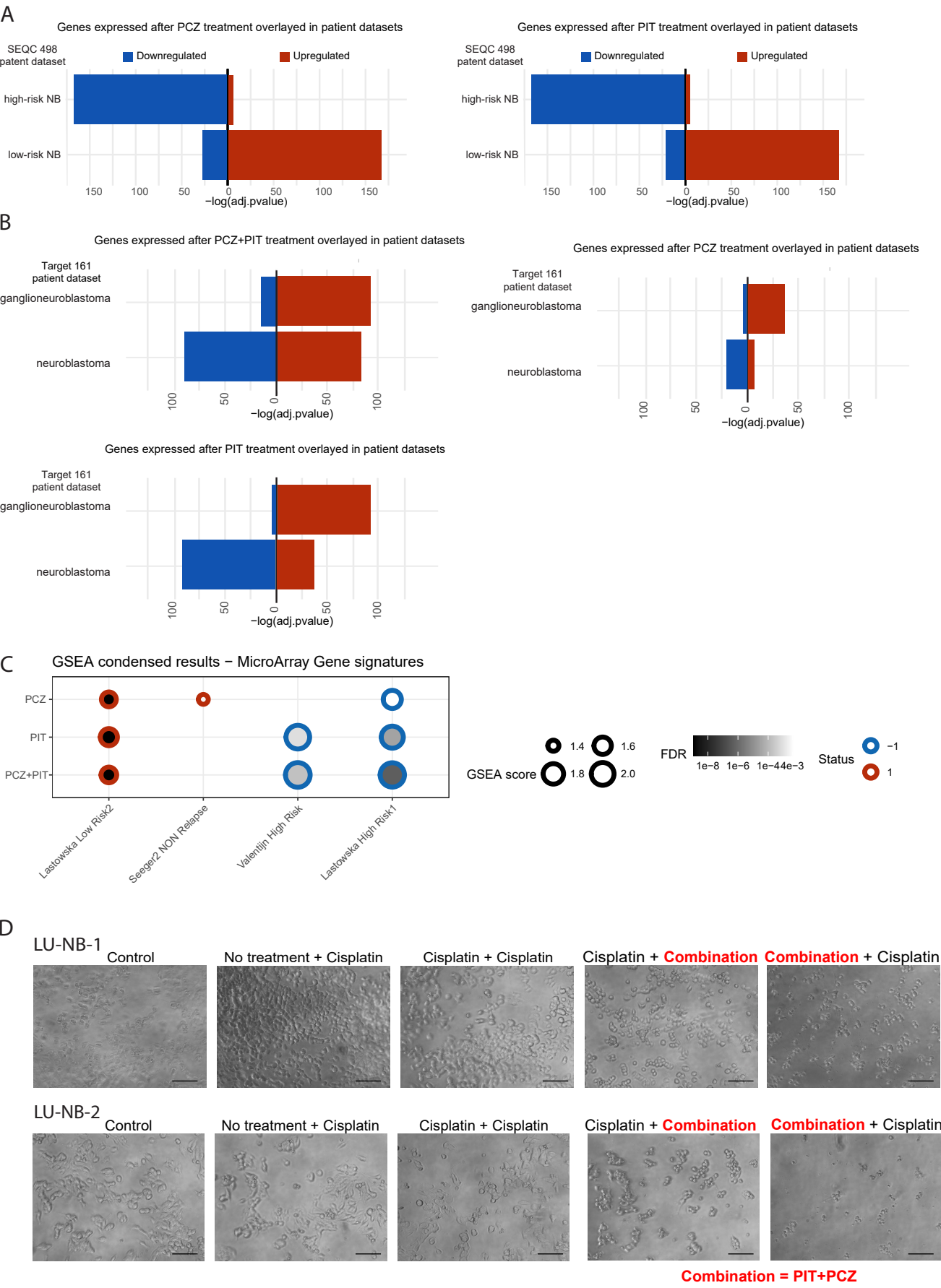


Appendix Figure S3. Mechanistic consequences of treatment with single drugs and PCZ + PIT combination. **A** Heatmap showing differential gene expression between all treatment groups (top 100 most differentially expressed genes) of LU-NB-2 organoids after 48 h treatment with single drugs or PCZ+PIT combination. Corresponding cell viability of the RNAseq experiment shown in **B** (RNAseq: n=8 in each group, viability test: n=3; data represent mean $\pm$ SD). **C** GSEA (Hallmarks database) of the respective treatment groups vs. control. **D** Western blot analysis of pAkt-S473 and panAkt protein expression (n=3, one-way ANOVA followed by Tukey's multiple comparison test; data represent mean $\pm$ SD). **E** Volcano plots displaying expression of the downstream targets of *MYC*, and *MYCN* after PCZ+PIT combination treatment. **F** Transcription factor (TF) activity after treatment with single drugs (CollecTri) compared to control. **G** Western blot quantification of *MYCN* and PARP after siRNA treatment (n=3), one-way ANOVA; data represent mean $\pm$ SD). PCZ-prochlorperazine; PIT-pitavastatin.

S4



Appendix Figure S4. Lipidomics changes after single drugs, combination, and combination + cisplatin treatment *in vitro*. Volcano plot presenting upregulated and downregulated lipids (species level nomenclature) after treatment with cisplatin, PCZ, PIT, PCZ+PIT at halved and full concentrations and cisplatin+PCZ+PIT. Upper left corner graphs summarize logFC of each lipid class change after treatment with significant changes indicated with the red background (boxes represent the interquartile range (IQR), center lines indicate medians, whiskers extend to 1.5×IQR, and points denote outliers). DMSO treated cells were used as a baseline. (n=6, p-values calculated by permutation test, corrected with Benjamini-Hochberg test. PCZ-prochlorperazine; PIT-pitavastatin.



1 **Appendix Figure S5. Transcriptional phenotype correlates with risk in patient datasets.**
2 **A** Association of DEGs (after single 1131 PCZ and PIT treatment) with risk stratification in the
3 SEQC498 patient dataset. **B** Genes expressed after PCZ and/or PIT treatment overlayed in the
4 Target161 dataset including patients with ganglioneuroblastoma and patients with aggressive
5 neuroblastoma. **C** GSEA of RNA expression after treatment with single drugs and combination
6 (LU-NB-2, 48 h) compared with risk in original datasets (Table S1). Only significant (FDR p-
7 value < 0.05) results are displayed. **D** Brightfield images of cells seeded on laminin at different
8 treatment conditions. Corresponding pictures of normalized viability graphs in Fig 4E. Picture
9 magnification: 10x, scale bar 100 μ m. PCZ-prochlorperazine; PIT-pitavastatin.

Appendix Table S1. Neuroblastoma expression datasets used for drug predictions.

Author	NCBI GEO	Reference name	Benign group	Progression/higher severity group	Patient group	Number of patients
Valentijn et al. (2015) (87)	GSE73537	Valentijn	Alive	Dead	All stages	34
Ohtaki et al. (2010)(88)	GSE16237	Hiyama	Alive + stages 1/2/4s	Dead of disease + stages 3/4	All stages	51
Asgarzadeh et al.(2006) (89)	GSE3446 HG-U133A	Seeger 1	No relapse	Relapse/metastasis	<i>MYCN</i> non-amplified	117
Asgarzadeh et al.(2006) (89)	GSE3446 HG-U133B	Seeger 2	No relapse	Relapse/metastasis	<i>MYCN</i> non-amplified	117
Lastowska et al.(2007)(90)	GSE13136	Lastowska 1	Low stage, <i>MYCN</i> non-amplified	<i>MYCN</i> amplified, high stage, 1p del	All stages, 17q gain	30
Lastowska et al. (2007) (90)	GSE13136	Lastowska 2	Low stage, <i>MYCN</i> non-amplified	<i>MYCN</i> non-amplified, high stage, 11q del	All stages, 17q gain	30

Appendix Table S2. Neuroblastoma PDX models included in the study.

PDX	Sample type	SNP profile	Stage	Chemotherapy
LU-NB-1	Primary (adrenal gland)	<i>MYCN</i> amp, 1p del, 17q gain	IV	No
LU-NB-2	Metastasis (after relapse)	<i>MYCN</i> amp, 1p del, 17q gain	IV	Yes
LU-NB-3	Primary (adrenal gland)	<i>MYCN</i> amp, 1p del, 17q gain	III	No

Appendix Table S3. Effect of four selected drugs in three different high-risk neuroblastoma organoid models. Single dose curves for statistical comparison between 3 and 7 days. NA- not enough data points around IC₅₀ to compute the CI.

	LU-NB-1		LU-NB-2		LU-NB-3	
	3 days	7 days	3 days	7 days	3 days	7 days
Trifluoperazine						
IC ₅₀ (μM)	4.01	3.57	2.95	1.31	2.50	0.96
95% CI IC ₅₀	NA	NA	2.4 – 3.6	1.1 – 1.5	1.8 – 3.4	0.62 – 1.49
Sig 3 vs 7 days	no	no	yes	yes	yes	yes
AUC	495	542	427	240	408	304
95% CI AUC	486 – 504	521 – 563	390 – 63.4	211 – 268	376 – 441	265 – 342
Sig 3 vs 7 days	yes	yes	yes	yes	yes	yes
Thioridazine						
IC ₅₀ (μM)	3.78	3.81	1.99	1.67	2.25	1.99
95% CI IC ₅₀	3.53 – 4.06	NA	1.70 – 2.33	NA	2.06 – 2.50	NA
Sig 3 vs 7 days	no	no	no	no	no	no
AUC	478	533	278	224	300	247

95% CI AUC	468 – 487	521 – 545	258 – 298	205 – 244	270 – 331	212 – 283
Sig 3 vs 7 days	yes	yes	yes	yes	no	no
Prochlorperazine						
IC ₅₀ (μ M)	3.93	3.77	2.73	1.38	2.20	1.41
95% CI IC ₅₀	NA	NA	2.08 – 3.50	1.24 – 1.53	1.48 – 3.16	0.87 – 2.23
Sig 3 and 7 days	no	no	yes	yes	no	no
AUC	498.2	488.2	409.6	232.4	393.0	333.4
95% CI AUC	486 – 510	477 – 499	388 – 432	199 – 266	363 – 423	318 – 349
Sig 3 vs 7 days	no	no	no	no	no	no
Lovastatin						
IC ₅₀ (μ M)	45.9	0.84	15.9	2.67	1.89	0.15
95% CI IC ₅₀	24.0 – 119	0.73 – 0.97	13.0 – 21.4	2.30 – 3.09	1.54 – 2.33	0.14 – 0.17
Sig 3 vs 7 days	yes	yes	yes	yes	yes	yes
AUC	700	303	796	398	427	106
95% CI AUC	670 – 730	290 – 315	780 – 811	343 – 453	396 – 458	97.2 – 115
Sig 3 vs 7 days	yes	yes	yes	yes	yes	yes

Appendix Table S4. Neuroblastoma gene signatures included in the analysis.

Author	Material	States	Omics method
Van Groningen <i>et al.</i> 2017(12)	Cell lines (N=30) Adr-mes cell line pairs (N=4)	Adrenergic Mesenchymal	Chip-Seq, RNA-seq,
Boeva <i>et al.</i> 2017(13)	Patient tumors (N=10) NB cell lines (N=25) PDXs (N=6)	Noradrenergic NCC-like	Chip-Seq, RNA-seq,
Gartlgruber <i>et al.</i> 2021(16)	Patient tumors (N=60) Cell lines (N=25)	<i>MYCN</i> amp <i>MYCN</i> non-amp, high risk <i>MYCN</i> non-amp, low risk MES/Schwann cell precursor	Chip-Seq (H3K27ac), RNAseq, ATAC-seq
Olsen <i>et al.</i> 2024(18)	Patient tumors (N=17)	Adrenergic Mesenchymal SCP-like bridge cells	scRNA-seq
Manas <i>et al.</i> 2022(19)	Integration of signatures from PDX models (N=3) and multiple published signatures	Adrenergic Mesenchymal-like	RNA-seq
Bedoya-Reina <i>et al.</i> 2021(15)	Patient tumors (N=11) Normal adrenal gland (human+mouse) (N=3+5)	Undiff (nC2,3) NOR (nC5,7,8,9) Stromal clusters	sc/snRNA-seq
Yuan <i>et al.</i> 2022(17)	Patient tumors (N=10 peripheral neuroblastic tumors)	Adrenergic, Transitional Mesenchymal	scRNA-seq
Patel <i>et al.</i> bioRxiv(22)	Patient tumors (N=51) PDX1(N=1)	Adrenergic Mesenchymal Sympathoblast	sc/snRNA-seq Spatial transcriptomics

Appendix Table S5. Multispectral staining antibody-opal pairing.

Antibody name	Company and number	Dilution	Opal name/product number	Dilution
NCAM	DAKO NCL-L-CD56-504	1:50	Opal 780	1:100
SOX9	Abcam Ab76997	1:500	Opal 480	1:100
TH	Abcam Ab112	1:1000	Opal 620	1:100
CD44	Abcam Ab157107	1:500	Opal 520	1:100
PHOX2B	Abcam Ab183741	1:1000	Opal 570	1:100