

# PROJECT: ARMS\_NBL

## Title: Differential methylation analysis

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# Study design

- 2 cancers:
  - Alveolar Rhabdomyosarcoma (ARMS):
    - 4 timepoints
    - 11 samples
  - Neuro Blastoma (NBL):
    - 3 timepoints
    - 9 samples

Name	timepoint	Origin	type	cancer	N
At0	0	culture	cell line	ARMS	2
At1	1	tissue	tumor	ARMS	2
At2	2	tissue	metastasi	ARMS	2
At3	3	culture	primary	ARMS	5
Nt0	0	culture	primary	NBL	3
Nt1	1	tissue	tumor	NBL	3
Nt2	2	tissue	metastasi	NBL	3

# Research question

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- ARMS:

- Difference in methylation between cell line (culture) and mice tumor T0 vs T1 → Tumors
- Difference between cell line and primary culture T0 vs T3 start vs end →
- Difference between tumor and metastasis tissue samples T1 vs T2 → Tissue
- Difference between metastasis samples in vivo and in vitro T2 vs T3 Metastasis →
- Difference between cell line and the rest T0 vs T1+T2+T3 Cell line. →

- NBL:

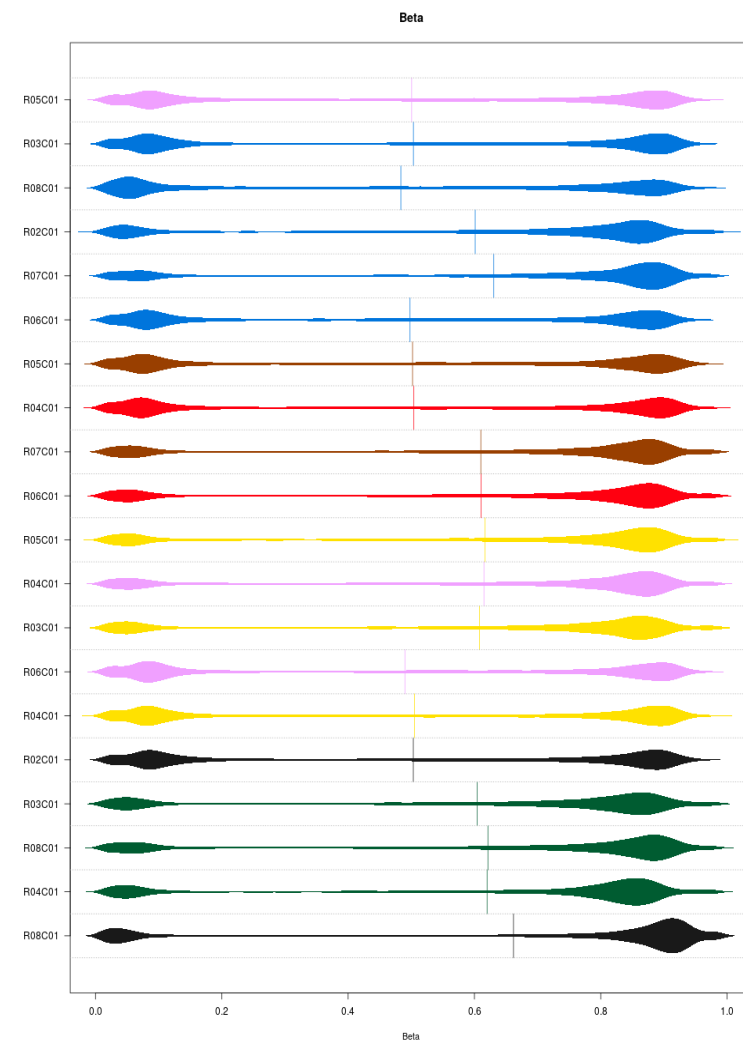
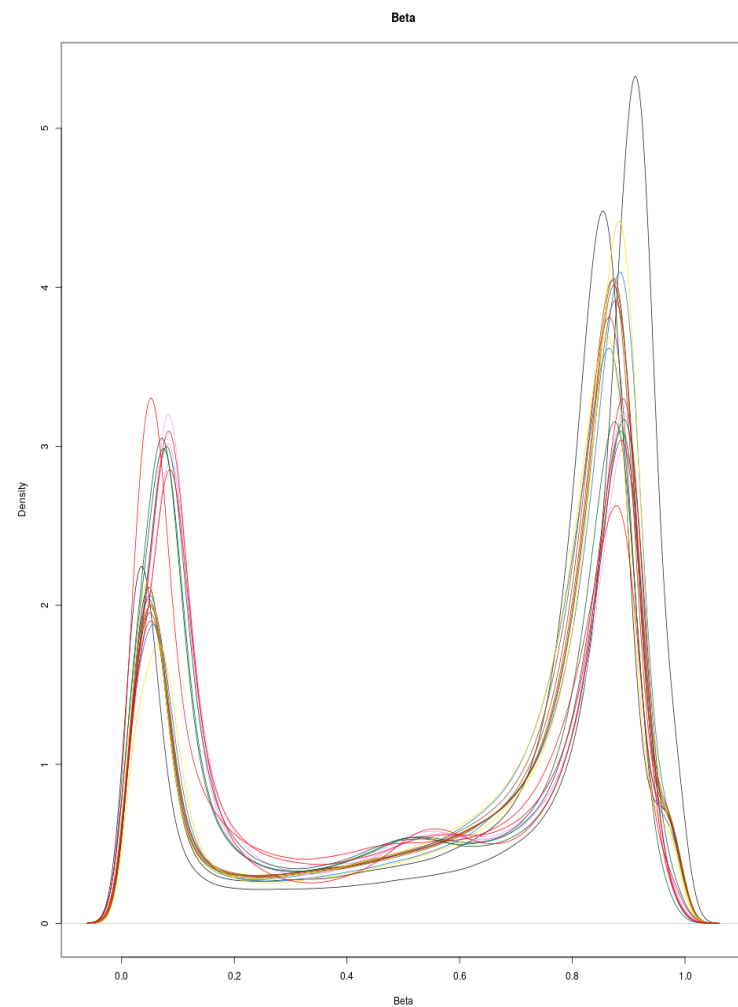
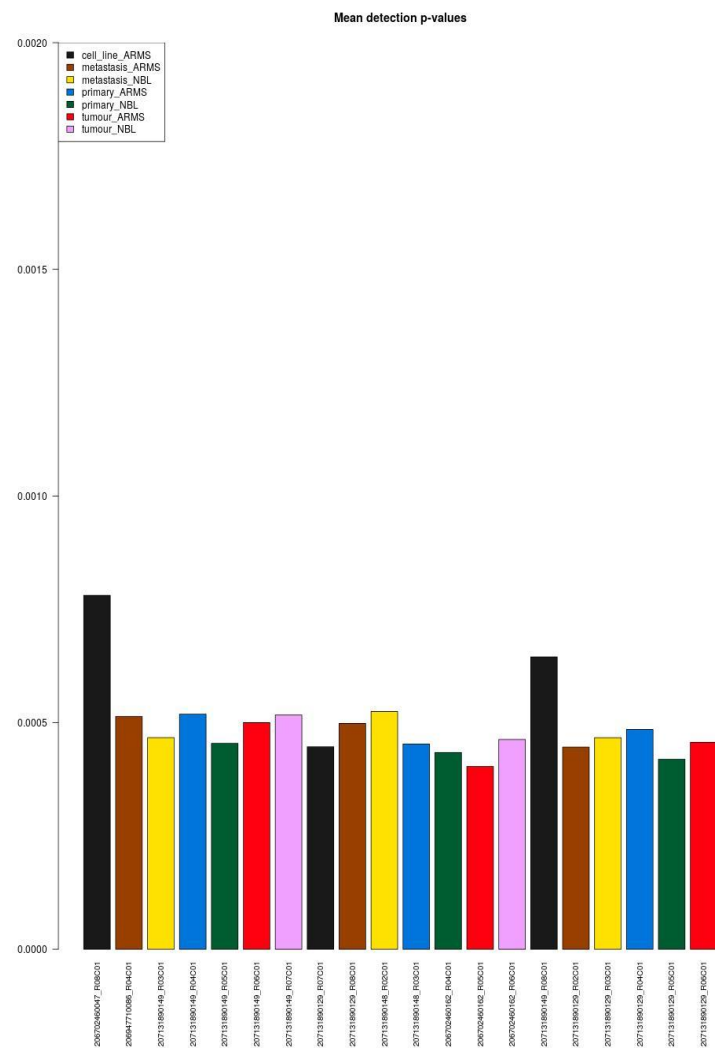
- Difference between Primary culture and mice tumor T0 vs T1 → Tumors
- Difference between mice tumor and mice metastasis T1 vs T2 → Tissue
- Difference between primary culture and mice samples T0 vs T1+T2 → culture/tissue

# Methods

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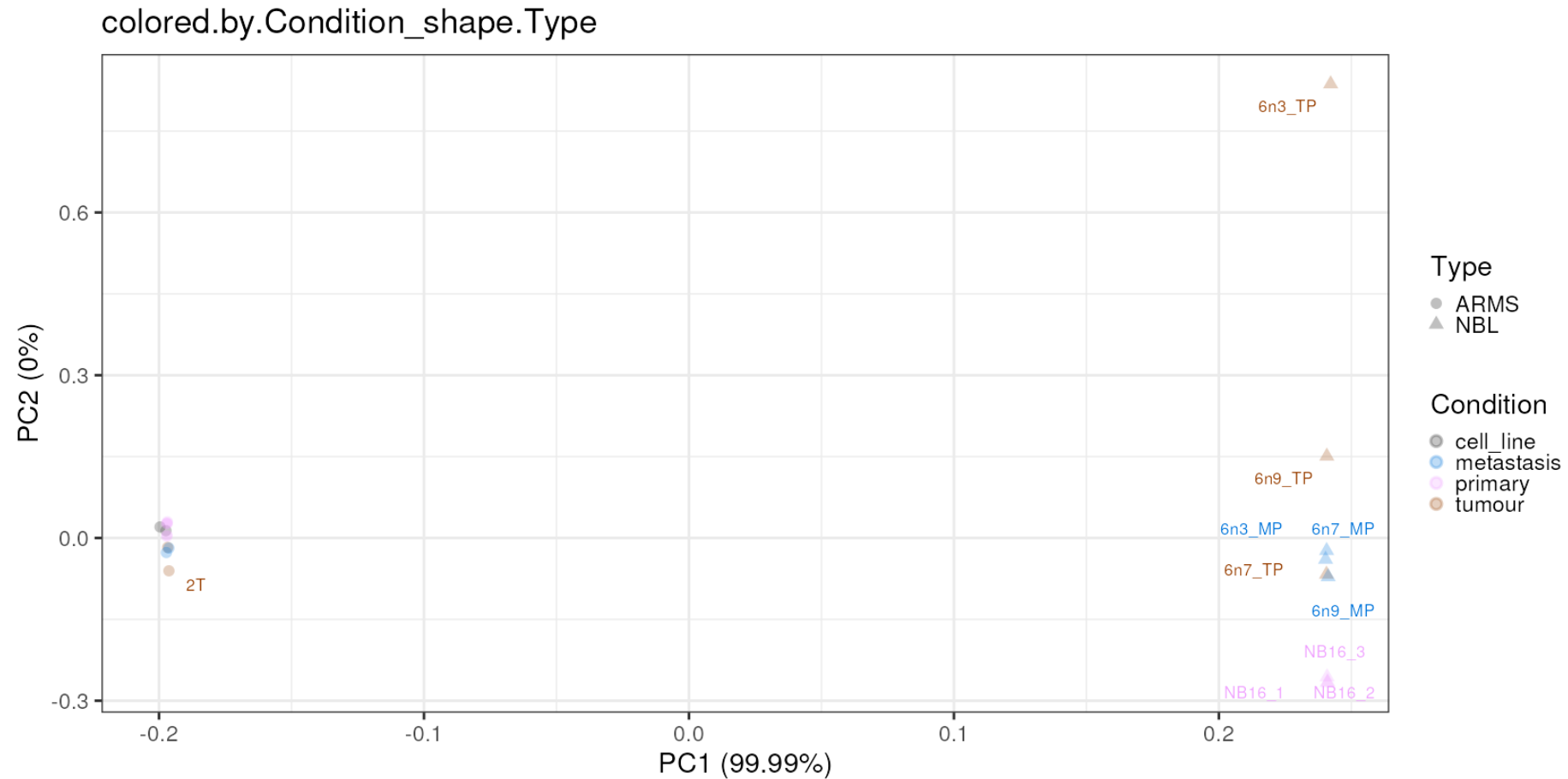
- Signal pre-processing (*Minfi*):
  - Normalization: PreprocessNoob for background correction.
  - Quality filter: Signal detection Pvalue < 0.01
- Differentially Methylated Probes - DMPs (*limma*):
  - Pvalue < 0.01
  - Mean methylation difference > 0.5
- Differentially Methylated Regions - DMRs (*DMRcate*):
  - Pvalue < 0.01; FDR < 0.05
  - Mean methylation difference > 0.2
  - Minimum number of CpG per region: 3
- Functional enrichment DM genes (*missMethyl*):
  - FDR < 0.05
  - Categories
    - Pathways: KEGG, Reactome
    - Gene Ontology (GO):
      - Cellular Component (CC)
      - Molecular Function (MF)
      - Biological Process (BP)
    - GO promoters

# Quality control



# Quality control: PCA - ALL

Very clear segregation depending on Cancer Type. Almost 100% of the variation.

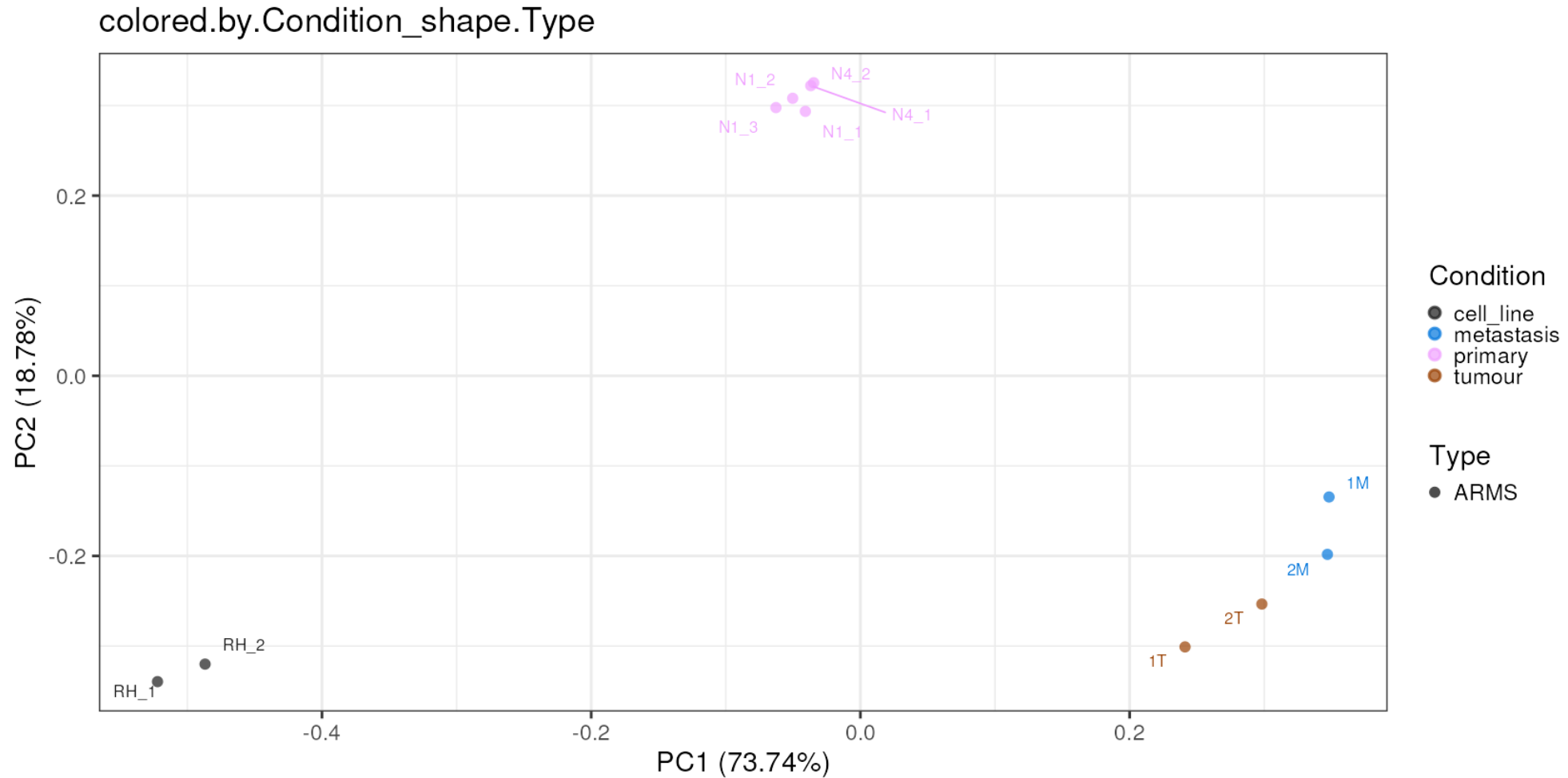


ARMS

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# Quality control: PCA - ARMS

Very good segregation where the origin of the sample (culture/tissue) accounts for most of the variation.



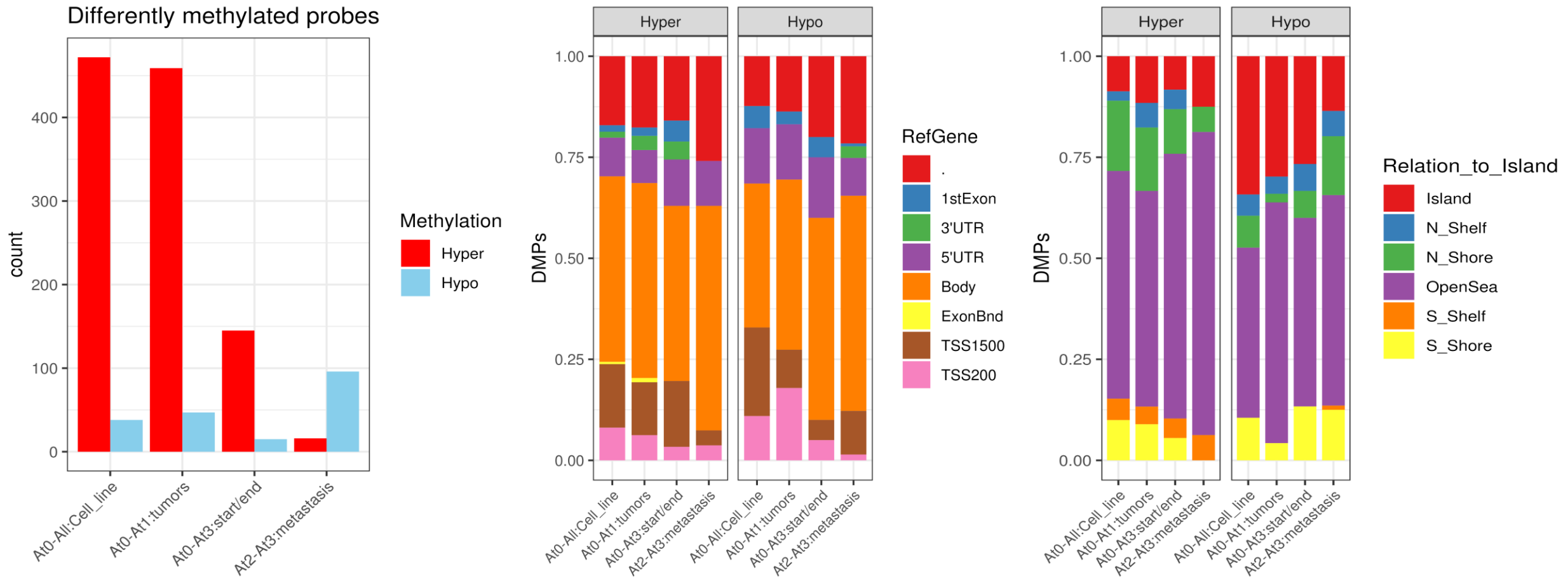


## Results: Summary - ARMS

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Contrast	Hyper. DMPs	Hypo. DMPs	Hyper.DMRS	Hypo.DMRS	Hyper.Genes	Hypo.Genes
At0-All:Cell_line	472	38	1365	68	1083	55
At1-At2:tissue	-	-	-	-	-	-
At0-At1:tumors	459	47	1436	86	1118	67
At0-At3:start/end	145	15	1050	29	825	26
At2-At3:metastasis	16	96	92	415	71	335

# Results: DMPs -ARMs



Metastasis T2 vs T3 methylation changes are located in different regions compared to the rest of the contrasts.

## Differentially methylated regions (DMRs) by Contrast - ARMS top20

seqnames	start	end	width	no.cpgs	HMFDR	overlapping.genes	Contrast
chr7	157381078	157381240	163	3	0.038462567457967	PTPRN2	At1-At2:tissue
chrX	9982230	9982520	291	4	0.062194871799378		At1-At2:tissue
chr7	2069790	2071166	1377	7	0.081505655803248	MAD1L1	At1-At2:tissue
chr13	29933441	29933565	125	3	0.083198649998193	MTUS2	At1-At2:tissue
chr12	115255983	115256052	70	3	0.104783347289206		At1-At2:tissue
chrX	3629716	3630619	904	3	0.110330124034833	PRKX	At1-At2:tissue
chr2	189837928	189839474	1547	6	0.110674969102852	COL3A1	At1-At2:tissue
chr6	168748440	168748530	91	3	0.140434781321334		At1-At2:tissue
chr18	10588980	10589360	381	5	0.143647990360319		At1-At2:tissue
chr13	112815083	112815961	879	5	0.153448439964894		At1-At2:tissue
chr17	1366091	1366970	880	4	0.222729888696244	CRK	At1-At2:tissue
chr17	76556745	76558006	1262	6	0.249118423028609	DNAH17	At1-At2:tissue
chr17	9335613	9336502	890	3	0.286428737523276	STX8	At1-At2:tissue
chr15	31213336	31215822	2487	7	0.422226746106167	FAN1	At1-At2:tissue
chr4	111539291	111543114	3824	17	0.424757089579034	PITX2	At1-At2:tissue
chr2	115177000	115177805	806	4	0.467875617500192	RNU2-41P	At1-At2:tissue
chr10	459697	459968	272	3	0.474537302710113	DIP2C	At1-At2:tissue

## Pathways: Hypermethylated genes by contrast - ARMS

ONTOLOGY	TERM	N	DE	P.DE	FDR	SigGenesInSet	Contrast	method
MF	transmembrane signaling receptor activity	1207	32	2.32E-06	0.016908	CYSLTR1,MAS1L,CCR1,OR1N1,ADRA2C,	At0-At3:start/end	GO_prom
MF	G protein-coupled receptor activity	781	25	2.59E-06	0.016908	CYSLTR1,MAS1L,CCR1,OR1N1,ADRA2C,	At0-At3:start/end	GO_prom
MF	signaling receptor activity	1426	35	2.95E-06	0.016908	CYSLTR1,MAS1L,CCR1,OR1N1,ADRA2C,	At0-At3:start/end	GO_prom
MF	molecular transducer activity	1426	35	2.95E-06	0.016908	CYSLTR1,MAS1L,CCR1,OR1N1,ADRA2C,	At0-At3:start/end	GO_prom
BP	detect ion of stimulus involved in sensory perception	466	19	4.68E-06	0.021465	OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAT0-At3:start/end	At0-At3:start/end	GO_prom
BP	sensory perception of smell	382	17	7.46E-06	0.026581	OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end	At0-At3:start/end	GO_prom
BP	system process	2242	45	8.11E-06	0.026581	CDH3,CYSLTR1,SCN11A,OR1N1,CRYAB,	At0-At3:start/end	GO_prom
BP	sensory perception	924	26	1E-05	0.027642	CDH3,SCN11A,OR1N1,CST1,OR4C16,OR4At0-At3:start/end	At0-At3:start/end	GO_prom
BP	detect ion of chemical stimulus involved in sensory perception	401	17	1.37E-05	0.027642	OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAT0-At3:start/end	At0-At3:start/end	GO_prom
MF	olfactory receptor activity	354	16	1.38E-05	0.027642	OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end	At0-At3:start/end	GO_prom
BP	detect ion of chemical stimulus involved in sensory perception of smell	356	16	1.47E-05	0.027642	OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end	At0-At3:start/end	GO_prom
BP	sensory perception of chemical stimulus	457	18	1.52E-05	0.027642	OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAT0-At3:start/end	At0-At3:start/end	GO_prom
BP	response to blue light	6	3	1.57E-05	0.027642	CPT1B,DCT,TYR	At0-At3:start/end	GO_prom
MF	proline:proton symporter activity	2	2	2.3E-07	0.005276	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
MF	amino acid:proton symporter activity	6	2	1.72E-06	0.019766	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
MF	L-proline transmembrane transporter activity	7	2	4.05E-06	0.030999	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
MF	L-alanine transmembrane transporter activity	10	2	6.05E-06	0.03106	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
BP	L-alanine transport	11	2	6.77E-06	0.03106	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
MF	glycine transmembrane transporter activity	10	2	1.01E-05	0.037723	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
BP	proline transport	11	2	1.31E-05	0.037723	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
BP	proline transmembrane transport	11	2	1.32E-05	0.037723	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
MF	alanine transmembrane transporter activity	15	2	1.91E-05	0.046693	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
BP	glycine transport	14	2	2.04E-05	0.046693	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom

## Pathways: Hypomethylated genes by contrast - ARMS

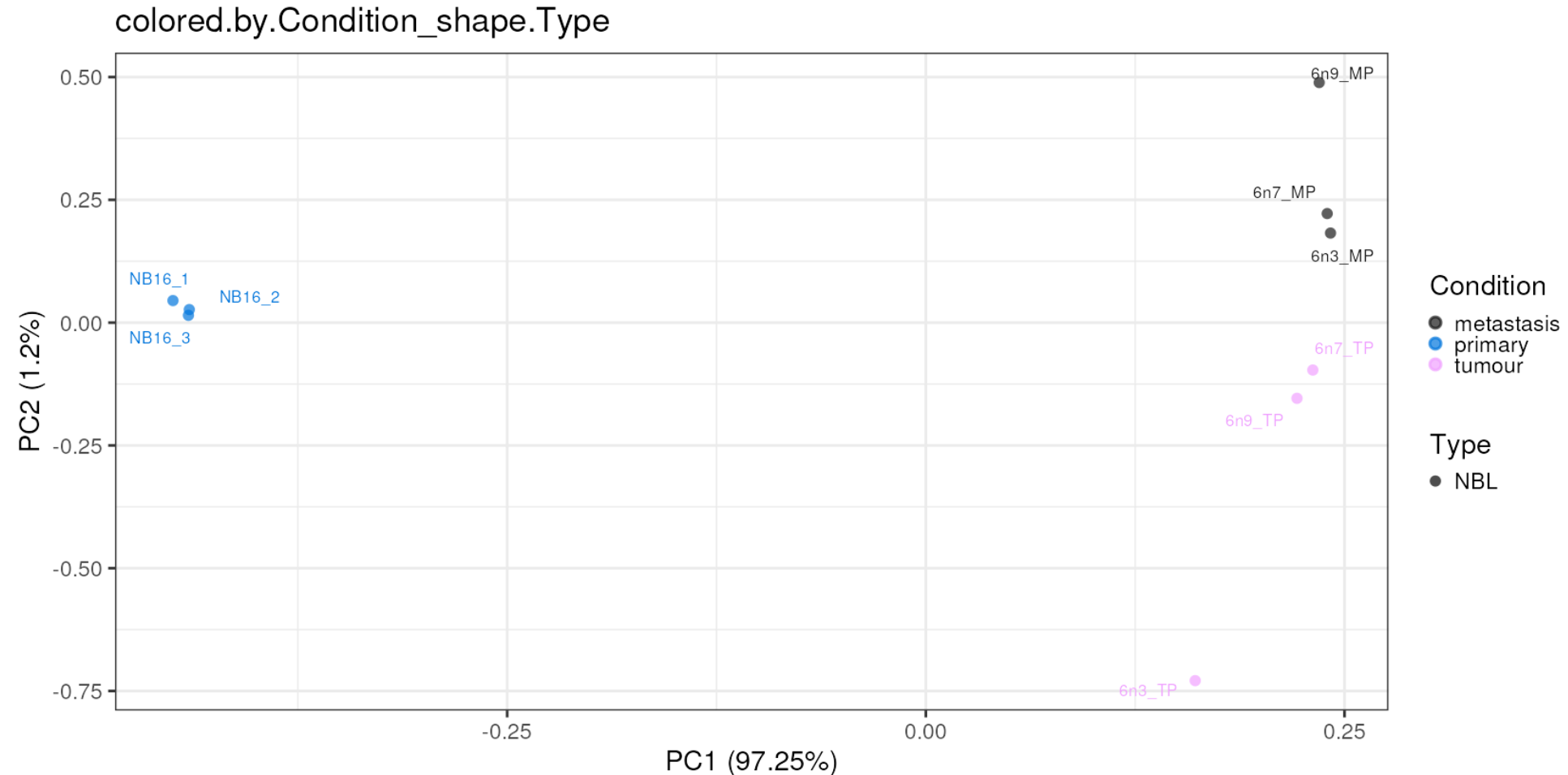
ONTOLOGY	TERM	N	DE	P.DE	FDR	SContrast	method
BP	striated muscle contraction	178	6	5.28922795772209e-06	0.121340178578102	SAt2-At3:metastasis	GO_prom
BP	muscle system process	452	8	1.63024867238678e-05	0.144356404248889	SAt2-At3:metastasis	GO_prom
BP	muscle contraction	348	7	2.49124833372515e-05	0.144356404248889	SAt2-At3:metastasis	GO_prom
BP	sarcomerogenesis	3	2	2.51700282025873e-05	0.144356404248889	TAt2-At3:metastasis	GO_prom
CC	contractile fiber	242	6	3.74660559905911e-05	0.168034820160168	SAt2-At3:metastasis	GO_prom
BP	blood circulation	520	8	4.39479064104008e-05	0.168034820160168	AAt2-At3:metastasis	GO_prom
BP	system process	2242	16	5.17574395403375e-05	0.169623917213555	MAAt2-At3:metastasis	GO_prom
BP	circulatory system process	605	8	0.000142919602992051	0.409839826530081	AAt2-At3:metastasis	GO_prom
BP	sarcomere organization	44	3	0.000201193815742095	0.512843036326599	MAAt2-At3:metastasis	GO_prom
CC	sarcomere	214	5	0.000239334983185797	0.549058384926537	SAt2-At3:metastasis	GO_prom
CC	myofibril	233	5	0.000348152605168136	0.670194031098178	SAt2-At3:metastasis	GO_prom
BP	heart contraction	249	5	0.000403555906608205	0.670194031098178	AAt2-At3:metastasis	GO_prom
BP	cardiac muscle contraction	139	4	0.00040716432009912	0.670194031098178	FAt2-At3:metastasis	GO_prom
MF	molecular sequestering activity	14	2	0.000408993349695937	0.670194031098178	CAt2-At3:metastasis	GO_prom
CC	muscle myosin complex	15	2	0.000438225776205094	0.670222502128071	MAAt2-At3:metastasis	GO_prom
BP	heart process	260	5	0.000498819053395672	0.715212993996882	AAt2-At3:metastasis	GO_prom
MF	platelet-derived growth factor binding	11	1	0.000145454022240033	0.737963899516187	CAt1-At2:tissue	GO
CC	collagen type III trimer	1	1	0.000145454022240033	0.737963899516187	CAt1-At2:tissue	GO
BP	limb joint morphogenesis	5	1	0.000145454022240033	0.737963899516187	CAt1-At2:tissue	GO
BP	layer formation in cerebral cortex	14	1	0.0002431621788919	0.737963899516187	CAt1-At2:tissue	GO
BP	aorta smooth muscle tissue morphogenesis	6	1	0.000245347325565539	0.737963899516187	CAt1-At2:tissue	GO
CC	fibrillar collagen trimer	12	1	0.000313228368642091	0.737963899516187	CAt1-At2:tissue	GO
CC	banded collagen fibril	12	1	0.000313228368642091	0.737963899516187	CAt1-At2:tissue	GO
BP	elastic fiber assembly	11	1	0.000341645055802608	0.737963899516187	CAt1-At2:tissue	GO
BP	basement membrane organization	36	1	0.000362315592437518	0.737963899516187	CAt1-At2:tissue	GO
BP	smooth muscle tissue development	25	1	0.000385065399154555	0.737963899516187	CAt1-At2:tissue	GO
BP	negative regulation of neuron migration	12	1	0.000448411653500659	0.737963899516187	CAt1-At2:tissue	GO
CC	complex of collagen trimers	22	1	0.000450018750330211	0.737963899516187	CAt1-At2:tissue	GO
BP	cerebral cortex radial glia-guided migration	25	1	0.000450350664453451	0.737963899516187	CAt1-At2:tissue	GO
BP	telencephalon glial cell migration	25	1	0.000450350664453451	0.737963899516187	CAt1-At2:tissue	GO

NBL

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# Quality control: PCA - NBL

Very good seggregation where the origin of the sample (culture/tissue) accounts for most of the variation.

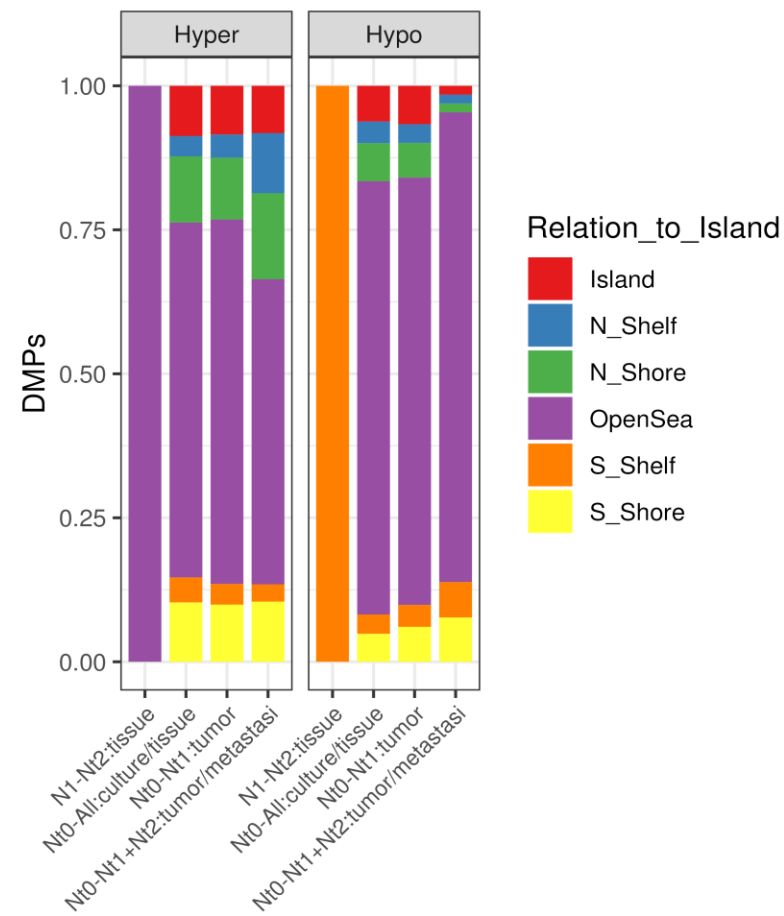
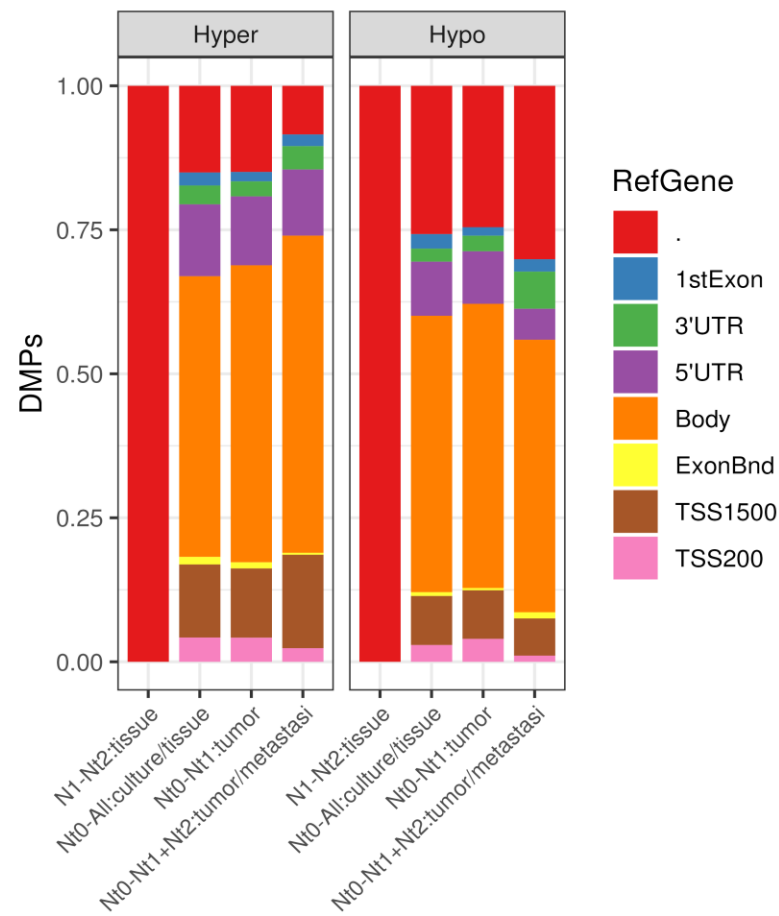
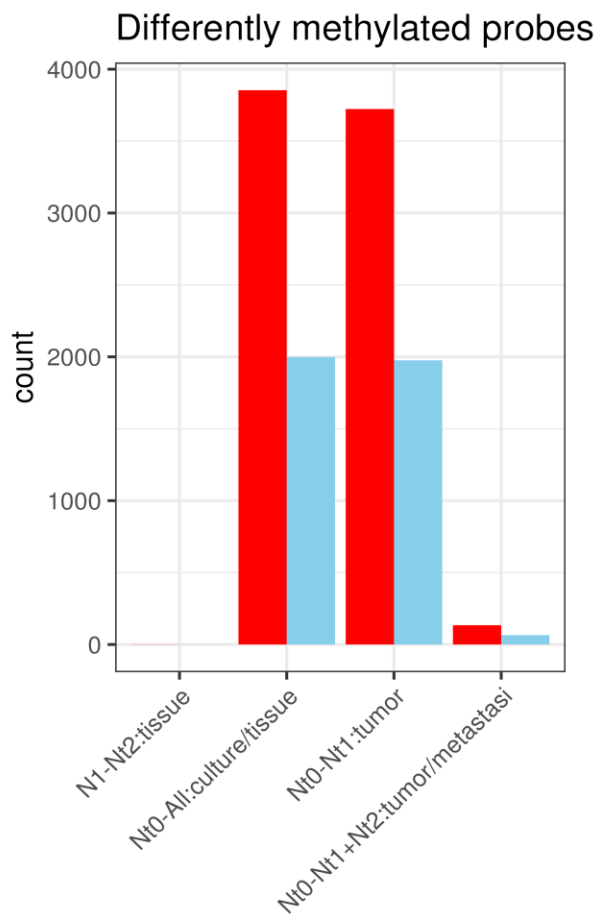


# Results: Summary - NBL

Contrast	Hyper.DMPs	Hypo.DMPs	Hyper.DMRs	Hypo.DMRs	Hyper.Genes	Hypo.Genes
N1-Nt2:tissue	1	1	57	65	47	54
Nt0-All:culture/tissue	3854	1998	2511	1284	1897	864
Nt0-Nt1+Nt2:tumor/metastasi	134	65	549	317	455	229
Nt0-Nt1:tumor	3723	1976	2463	1222	1884	817



# Results: DMPs -NBL



## Differentially methylated genes (DMRs) by Contrast -NBL

seqnames	start	end	width	no.cpgs	HMFDR	overlapping.genes	Contrast
chr5	38025467	38026056	590	4	0.022	CTD-2116N24.1	N1-Nt2:tissue
chr21	31859610	31860473	864	3	0.029	KRTAP19-2	N1-Nt2:tissue
chr6	35025887	35027324	1438	5	0.034	ANKS1A	N1-Nt2:tissue
chr21	32715908	32716820	913	6	0.041	TIAM1	N1-Nt2:tissue
chr17	14641538	14642137	600	3	0.042		N1-Nt2:tissue
chr11	124514419	124514971	553	3	0.042	SIAE	N1-Nt2:tissue
chr3	133191078	133191407	330	4	0.049	BFSP2	N1-Nt2:tissue
chr20	868102	868404	303	3	0.052	ANGPT4	N1-Nt2:tissue
chr4	37435982	37436666	685	3	0.054	KIAA1239	N1-Nt2:tissue
chr8	41732895	41733931	1037	4	0.055	ANK1	N1-Nt2:tissue
chr2	25861882	25862770	889	4	0.056	DTNB	N1-Nt2:tissue
chr5	110344874	110346052	1179	3	0.057		N1-Nt2:tissue
chr10	130833836	130834003	168	4	0.057		N1-Nt2:tissue
chr12	103357707	103359836	2130	10	0.058		N1-Nt2:tissue
chr11	122713816	122714824	1009	3	0.061	CRTAM	N1-Nt2:tissue
chrX	11284010	11284830	821	3	0.063	ARHGAP6	N1-Nt2:tissue
chr22	17586723	17586850	128	3	0.064	IL17RA	N1-Nt2:tissue
chr14	105438681	105440509	1829	4	0.066	AHNAK2	N1-Nt2:tissue

## Pathways: Hypermethylated genes by contrast - ARMS

ITOL	TERM	N	DE	FDR	SigGenesInSet	Contrast	method
CC	integral component of postsynaptic membrane	118	17	0.038798	CACNG2,GSG1L,ADRA1A,DCC,EPHB2,SORCS3,GABRA5,LRRTM2,GRIKNT0-Nt1:tumor		GO
CC	intrinsic component of postsynaptic membrane	123	17	0.038798	CACNG2,GSG1L,ADRA1A,DCC,EPHB2,SORCS3,GABRA5,LRRTM2,GRIKNT0-Nt1:tumor		GO
BP	inorganic ion transmembrane transport	914	56	0.038798	KCNMB2,CACNG2,SLC26A1,PKD1L2,ADCYAP1R1,TMC1,ADRA1A,SLCNT0-Nt1:tumor		GO
CC	intrinsic component of synaptic membrane	166	20	0.038798	CACNG2,GSG1L,ADRA1A,DCC,EPHB2,NTNG1,SORCS3,GABRA5,LRRTINT0-Nt1:tumor		GO
BP	vasoconstriction	83	12	0.038798	KCNMB2,ADRA1A,FGA,FGB,FGG,HRH1,HTR2B,SMTNL2,KEL,ASIC2,MNT0-Nt1:tumor		GO
BP	system process	2242	107	0.040922	KCNMB2,CACNG2,BAIAP2,CELF2,WASF3,ADCY5,TMC1,LOXHD1,CRYNT0-Nt1:tumor		GO
BP	RNA processing	1323	39	9.11E-05	SNORD116-1,SNORD116-2,SNORD116-3,SNORD116-4,SNORD116-!Nt0-Nt1:tumor		GO_prom
CC	nucleolus	1355	37	0.000777	SNORD116-1,SNORD116-2,SNORD116-3,SNORD116-4,SNORD116-!Nt0-Nt1:tumor		GO_prom
BP	RNA processing	1323	43	8.56E-06	SNORD116-1,SNORD116-2,SNORD116-3,SNORD116-4,SNORD116-!Nt0-All:culture/tissue		GO_prom
CC	nucleolus	1355	41	5.74E-05	SNORD116-1,SNORD116-2,SNORD116-3,SNORD116-4,SNORD116-!Nt0-All:culture/tissue		GO_prom