



PROJECT: ARMS\_NBL

Title: Differential methylation analysis

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# Study design

#### • 2 cancers:

- Alveolar Rhabdomiosarcoma (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
NtO	0	culture	primary	NBL	3
Nt1	1	tissue	tumor	NBL	3
Nt2	2	tissue	metastasi	NBL	3

# Research question

#### • 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
   →
- 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:

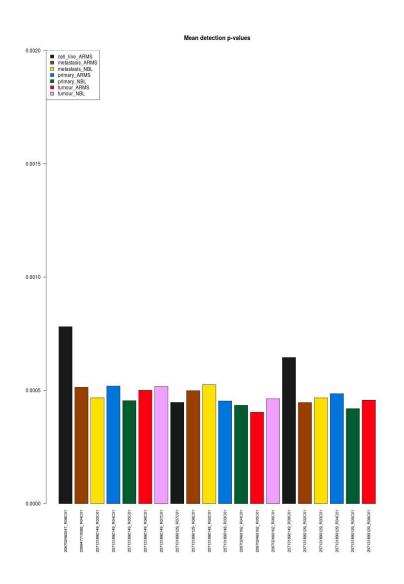
- Difference between Primary culture and mice tumor T0 vs T1
   →
- Difference between mice tumor and mice metastasis T1 vs T2
   →
   Tissue
- Difference between primary culture and mice samples T0 vs T1+T2 → culture/tissue

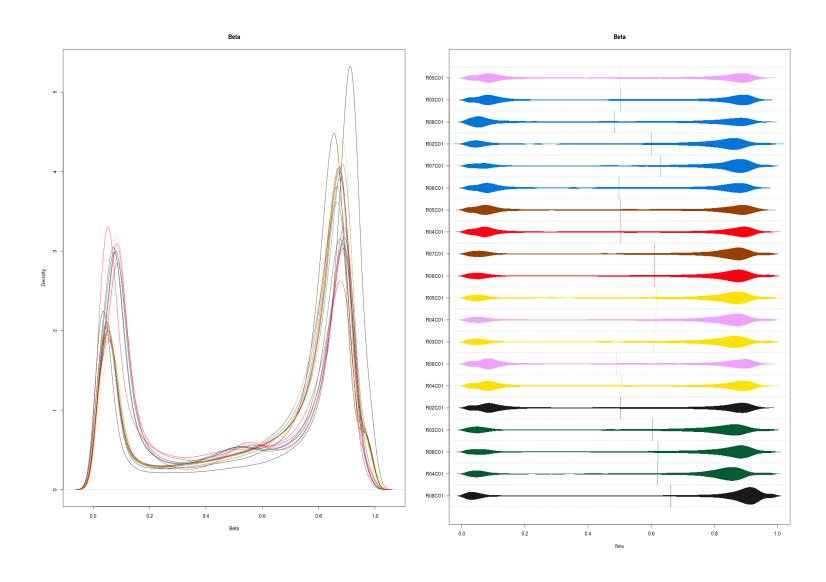
### Methods

- Signal pre-processing (Minfi):
  - Normalization: PreprocessNoob for background correction.
  - Quality filter: Signal detection Pvalue < 0.01
- Differentially Methylated Probes DMPs (limma):
  - Pvalue < 0.01</li>
  - 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

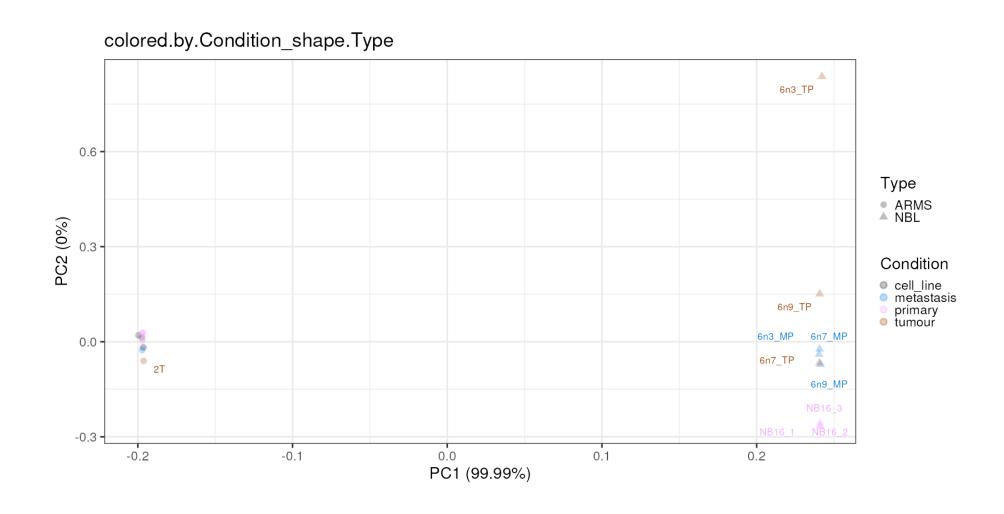
# Quality control





# Quality control: PCA - ALL

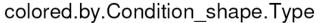
Very clear segregation depending on Cancer Type. Almost 100% of the variation.

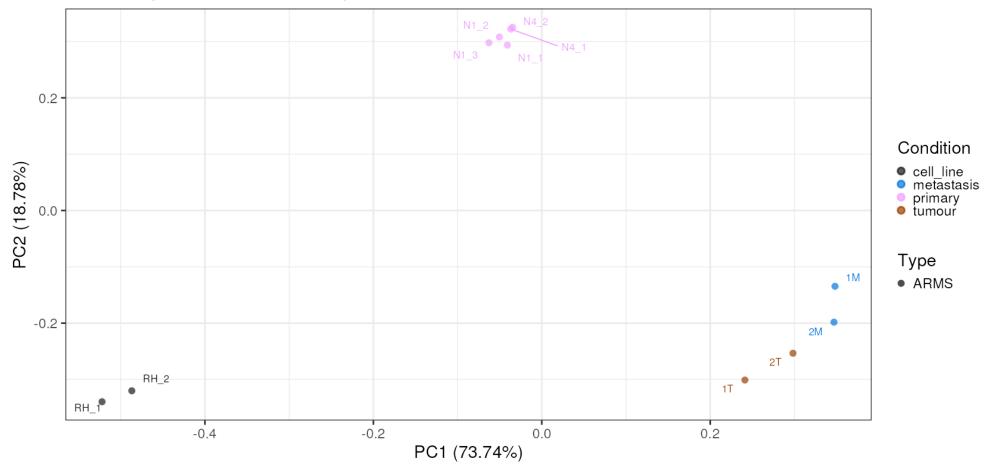


# **ARMS**

# Quality control: PCA - ARMS

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

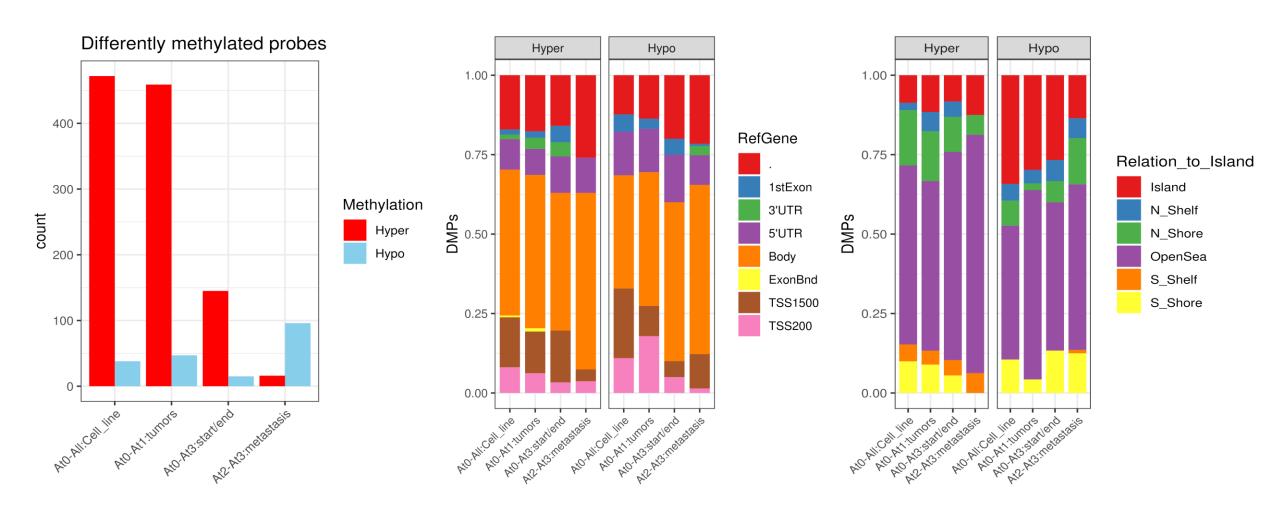




## Results: Summary - ARMS

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.gen es	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

MF         transmembrane signaling receptor act vity         32         2.32E-06 0.016908 CYSLTR1,MAS1L,CCR1,ORIN1,ADRA2C, At0-At3:start/end         GO_prom           MF         G protein-coupled receptor act vity         1426         35         2.59E-06 0.016908 CYSLTR1,MAS1L,CCR1,ORIN1,ADRA2C, At0-At3:start/end         GO_prom           MF         signaling receptor act vity         1426         35         2.95E-06 0.016908 CYSLTR1,MAS1L,CCR1,ORIN1,ADRA2C, At0-At3:start/end         GO_prom           MF         molecular transducer act vity         1426         35         2.95E-06 0.016908 CYSLTR1,MAS1L,CCR1,ORIN1,ADRA2C, At0-At3:start/end         GO_prom           BP         detect on of st mulus involved in sensory percept on         466         19         4.68E-06 0.026581 CN1N1,CST1,ORAC16,OR8B12,ORSA2,CAt0-At3:start/end         GO_prom           BP         sensory percept on of smell         382         17         7.46E-06 0.026581 CDH3,CYSLTR1,SCN11A,ORN1L,CST1,ORAC16,ORAt0-At3:start/end         GO_prom           BP         sensory percept on         924         26         1E-05 0.027642 CDH3,SCN11A,ORN1L,CST1,ORAC16,ORAt0-At3:start/end         GO_prom           MF         olfactory receptor act vity         334         16         1.38E-05 0.027642 OR1N1,CST1,ORAC16,OR8B12,ORSA2,ORADEAt0-At3:start/end         GO_prom           BP         detect bn of chemical st mulus involved in sensory percept on of smell	ONTOLO	GY TERM	N	DE	P.DE	FDR	SigGenesInSet	Contrast	method
MF         signaling receptor act vity         1426         35         2.95E-06         0.016908 CYSLTRI,MAS1L,CCRI,OR1N1,ADRA2C, At0-At3:start/end         GO_prom           MF         molecular transducer act vity         1426         35         2.95E-06         0.016908 CYSLTRI,MAS1L,CCRI,OR1N1,ADRA2C, At0-At3:start/end         GO_prom           BP         detect bit of st mulus involved in sensory percept bin         466         19         4.68E-06         0.021465 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         sensory percept on of smell         382         17         7.46E-06         0.026581 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end         GO_prom           BP         sensory percept on         924         26         1E-05         0.027642 CDR13,CYSLTR1,SCN11A,OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           MF         olfactory receptor act vity         354         16         1.38E-05         0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end         GO_prom           BP         detect bit of chemical st mulus involved in sensory percept bit of smell         356         16         1.47E-05         0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end         GO_prom           BP         response to blue light         6         3         1.57E-05         0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CA10-A	MF	transmembrane signaling receptor act vity	1207	32	2 2.32E-0	6 0.016908	CYSLTR1,MAS1L,CCR1,OR1N1,ADRA	2C, At0-At3:start/end	GO_prom
MF         molecular transducer act vity         1426         35         2.95E-06         0.016908 CYSLTR1,MAS1L,CCR1,OR1N1,ADRA2C, At0-At3:start/end         GG_prom           BP         detect bn of st mulus involved in sensory percept bn         466         19         4.68E-06         0.021465 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         sensory percept bn of smell         32         17         7.46E-06         0.026581 CDH3,CYSLTR1,SCN11A,OR1N1,CST2,OR5A2,OR4D6At0-At3:start/end         GO_prom           BP         sensory percept bn         924         26         1E-05         0.027642 CDH3,SCN11A,OR1N1,CST1,OR4C16,OR8D12,OR5A2,CAt0-At3:start/end         GO_prom           BP         detect bn of chemical st inulus involved in sensory percept bn         401         17         1.37E-05         0.027642 CDR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         detect bn of chemical st inulus involved in sensory percept bn of sensory percept bn of chemical st inulus involved in sensory percept bn of smell         356         16         1.47E-05         0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end         GO_prom           BP         sensory percept bn of chemical st inulus involved in sensory percept bn of smell         356         16         1.47E-05         0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end         GO_prom           BP <td>MF</td> <td>G protein-coupled receptor act vity</td> <td>781</td> <td>25</td> <td>5 2.59E-0</td> <td>06 0.016908</td> <td>CYSLTR1,MAS1L,CCR1,OR1N1,ADRA</td> <td>2C, At0-At3:start/end</td> <td>GO_prom</td>	MF	G protein-coupled receptor act vity	781	25	5 2.59E-0	06 0.016908	CYSLTR1,MAS1L,CCR1,OR1N1,ADRA	2C, At0-At3:start/end	GO_prom
BP         detect on of st mulus involved in sensory percept on of smell         466         19         4.68E-06         0.021465 OR1N1,CST1,OR4C16,OR8812,OR5A2,CAt0-At3:start/end         GO_prom           BP         sensory percept on of smell         382         17         7.46E-06         0.026581 OR1N1,OR4C16,OR8812,OR5A2,ORADeAt0-At3:start/end         GO_prom           BP         system process         2242         45         8.11E-06         0.026581 OR1N1,OR1A1,OR1N1,CST1,OR4C16,ORAB12,OR5A2,CAt0-At3:start/end         GO_prom           BP         sensory percept on         924         26         1E-05         0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         detect on of chemical st inulus involved in sensory percept on         354         16         1.38E-05         0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         detect on of chemical st inulus involved in sensory percept on of smell         356         16         1.47E-05         0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CR4D6At0-At3:start/end         GO_prom           BP         sensory percept on of chemical st inulus involved in sensory percept on of smell involved in sensory percept on of smell         356         16         1.47E-05         0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end         GO_prom           BP         response to blue light         6	MF	signaling receptor act vity	1426	3!	5 2.95E-0	6 0.016908	CYSLTR1,MAS1L,CCR1,OR1N1,ADRA	2C, At0-At3:start/end	GO_prom
BP         sensory percept bn of smell         382         17         7.46E-06 0.026581 OR1N1,OR4C16,OR8B12,OR5A2,OR4bEAt0-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 percept bn         924         26         1E-05 0.027642 CDH3,CYSLTR1,SCN11A,OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         detect bn of chemical st imulus involved in sensory percept bn         401         17         1.37E-05 0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         detect bn of chemical st imulus involved in sensory percept bn of smell         356         16         1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end         GO_prom           BP         sensory percept bn of chemical st imulus involved in sensory percept bn of smell         356         16         1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         sensory percept bn of chemical st imulus involved in sensory percept bn of smell         356         16         1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom           BP         sensory percept bn of chemical st imulus         457         18         1.52E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end         GO_prom </td <td>MF</td> <td>molecular transducer act vity</td> <td>1426</td> <td>3!</td> <td>5 2.95E-0</td> <td>06 0.016908</td> <td>CYSLTR1,MAS1L,CCR1,OR1N1,ADRA</td> <td>2C, At0-At3:start/end</td> <td>GO_prom</td>	MF	molecular transducer act vity	1426	3!	5 2.95E-0	06 0.016908	CYSLTR1,MAS1L,CCR1,OR1N1,ADRA	2C, At0-At3:start/end	GO_prom
System process 2242 45 8.11E-06 0.026581 CDH3,CYSLTR1,SCN11A,OR1N1,CRYAB, At0-At3:start/end GO_prom sensory percept on 924 26 1E-05 0.027642 CDH3,SCN11A,OR1N1,CST1,OR4C16,ORA610-At3:start/end GO_prom detect on of chemical st mulus involved in sensory percept on 17 1.37E-05 0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom detect on of chemical st mulus involved in sensory percept on 1354 16 1.38E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on of chemical st mulus involved in sensory percept on 1356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on of chemical st mulus involved in sensory percept on 1356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1356 18 1.52E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1356 18 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1356 18 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1354 18 1.52E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom detect on 0f chemical st mulus involved in sensory percept on 1354 18 1.52E-05 0.027642 OR1N1,OR4C16,OR8B12,OR	BP	detect on of st mulus involved in sensory percept on	466	19	9 4.68E-0	06 0.021465	OR1N1,CST1,OR4C16,OR8B12,OR5A	2,CAt0-At3:start/end	GO_prom
Sensory percept bn 924 26 1E-05 0.027642 CDH3,SCN11A,OR1N1,CST1,OR4C16,OFAt0-At3:start/end GO_prom detect bn of chemical st mulus involved in sensory percept bn 401 17 1.37E-05 0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom Olfactory receptor act vity 354 16 1.38E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom detect bn of chemical st mulus involved in sensory percept bn of smell 356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom Presponse to blue light 457 18 1.52E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end GO_prom Presponse to blue light 6 3 1.57E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom Presponse to blue light 6 3 1.57E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom Presponse to blue light 6 3 1.57E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom Presponse to blue light 6 3 1.57E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom Presponse to blue light 6 3 1.57E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom Presponse to blue light 6 3 1.57E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom Presponse to blue light 6 3 1.57E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom Proline:proton symporter act vity 6 2 2 2.3E-07 0.005276 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Proline transmembrane transporter act vity 7 2 4.05E-06 0.030999 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Proline transmembrane transporter act vity 10 2 1.01E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Proline transmembrane transporter act vity 10 2 1.32E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Proline transmembrane transport 11 2 1.31E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Proline transmembrane transport 11 2 1.32E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Proline transmembrane transporter act vity 15 1.32E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Proline transme	BP	sensory percept on of smell	382	17	7 7.46E-0	06 0.026581	OR1N1,OR4C16,OR8B12,OR5A2,OR	4D6At0-At3:start/end	GO_prom
detect bin of chemical st mulus involved in sensory percept bin  MF olfactory receptor act vity  354 16 1.37E-05 0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end  MF olfactory receptor act vity  354 16 1.38E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end  MF detect bin of chemical st mulus involved in sensory percept bin of smell  356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end  MF sensory percept bin of chemical st mulus  457 18 1.52E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end  MF proline:proton symporter act vity  6 3 1.57E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end  MF proline:proton symporter act vity  7 2 2.3E-07 0.005276 SLC36A2,SLC36A1  At2-At3:metastasis  MF L-alanine transmembrane transporter act vity  7 2 4.05E-06 0.03106 SLC36A2,SLC36A1  At2-At3:metastasis  MF L-alanine transmembrane transporter act vity  10 2 6.05E-06 0.03106 SLC36A2,SLC36A1  At2-At3:metastasis  MF glycine transmembrane transporter act vity  10 2 1.01E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis  MF glycine transmembrane transporter act vity  11 2 1.31E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis  MF proline transmembrane transport  MF proline transmembrane transporter act vity  10 2 1.01E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis  MF proline transmembrane transporter act vity  11 2 1.31E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis  MF proline transmembrane transporter act vity  11 2 1.32E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis  MF proline transmembrane transporter act vity  12 1.31E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis  MF proline transmembrane transporter act vity  13 2 1.31E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis  MF proline transmembrane transporter act vity  14 2 1.32E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis  MF proline transmembrane transporter act vity  15 2 1.91E-05 0.046693 SLC36A2,SLC36A1  At2-At3:metastasis  MF proline transmembrane transporter act vity  At2-At3:metastasis  MF prolin	BP	system process	2242	45	5 8.11E-0	06 0.026581	CDH3,CYSLTR1,SCN11A,OR1N1,CRYA	AB, At0-At3:start/end	GO_prom
MF olfactory receptor act vity 354 16 1.38E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end 60_prom detect bn of chemical st mulus involved in sensory percept bn of smell 356 16 1.47E-05 0.027642 OR1N1,OR4C16,OR8B12,OR5A2,OR4D6At0-At3:start/end 60_prom sensory percept bn of chemical st mulus 457 18 1.52E-05 0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end 60_prom response to blue light 6 3 1.57E-05 0.027642 CPT1B,DCT,TYR At0-At3:start/end 60_prom MF proline:proton symporter act vity 6 2 2 2.3E-07 0.005276 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF L-proline transmembrane transporter act vity 7 2 4.05E-06 0.03106 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF L-alanine transporter act vity 10 2 6.05E-06 0.03106 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF glycine transmembrane transporter act vity 10 2 1.01E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF glycine transmembrane transporter act vity 10 2 1.01E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF proline transport 11 2 1.31E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF proline transport 11 2 1.31E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis 60_prom MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2	BP	sensory percept on	924	20	6 1E-0	5 0.027642	CDH3,SCN11A,OR1N1,CST1,OR4C16	,ORAt0-At3:start/end	GO_prom
detect on of chemical st mulus involved in sensory percept on of smell sensory percept on of smell sensory percept on of chemical st mulus involved in sensory percept on of chemical st mulus sensory sensory percept on of chemical st mulus sensory percept on of chemical st mulus sensory sensor sensory sensor sensory sensor sensory sensor sen	BP	detect on of chemical st mulus involved in sensory percept on	401	17	7 1.37E-0	5 0.027642	OR1N1,CST1,OR4C16,OR8B12,OR5A	2,CAt0-At3:start/end	GO_prom
BP sensory percept bn of chemical st mulus 457 18 1.52E-05 0.027642 OR1N1,CST1,OR4C16,OR8B12,OR5A2,CAt0-At3:start/end GO_prom response to blue light 6 3 1.57E-05 0.027642 CPT1B,DCT,TYR At0-At3:start/end GO_prom MF proline:proton symporter act vity 2 2 2.3E-07 0.005276 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF amino acid:proton symporter act vity 6 2 1.72E-06 0.019766 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF L-proline transmembrane transporter act vity 7 2 4.05E-06 0.030999 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF L-alanine transmembrane transporter act vity 10 2 6.05E-06 0.03106 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF glycine transmembrane transporter act vity 10 2 6.77E-06 0.03106 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF glycine transmembrane transporter act vity 10 2 1.01E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF proline transport 11 2 1.31E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF proline transmembrane transport 11 2 1.32E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF alanine transmembrane transport 11 2 1.32E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Alanine transmembra	MF	olfactory receptor act vity	354	10	6 1.38E-0	5 0.027642	OR1N1,OR4C16,OR8B12,OR5A2,OR	4D6At0-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 act vity 2 2.3E-07 0.005276 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Amino acid:proton symporter act vity 6 2 1.72E-06 0.019766 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF L-proline transmembrane transporter act vity 7 2 4.05E-06 0.030999 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF L-alanine transmembrane transporter act vity 10 2 6.05E-06 0.03106 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF glycine transmembrane transporter act vity 11 2 6.77E-06 0.03106 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF glycine transmembrane transporter act vity 10 2 1.01E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF proline transport 11 2 1.31E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF proline transmembrane transport 11 2 1.31E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom MF alanine transmembrane transport 11 2 1.32E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom At2-At3:metastasis G	BP	detect on of chemical st mulus involved in sensory percept on of smell	356	10	6 1.47E-0	5 0.027642	OR1N1,OR4C16,OR8B12,OR5A2,OR	4D6At0-At3:start/end	GO_prom
MF proline:proton symporter act vity 2 2 2.3E-07 0.005276 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff amino acid:proton symporter act vity 6 2 1.72E-06 0.019766 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff L-proline transmembrane transporter act vity 7 2 4.05E-06 0.030999 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff L-alanine transmembrane transporter act vity 10 2 6.05E-06 0.03106 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff glycine transmembrane transporter act vity 10 2 6.77E-06 0.03106 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff glycine transmembrane transporter act vity 10 2 1.01E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff proline transport 11 2 1.31E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff proline transmembrane transport 11 2 1.32E-05 0.037723 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom Aff alanine transmembrane transporter act vity 15 2 1.91E-05 0.046	BP	sensory percept on of chemical st mulus	457	18	8 1.52E-0	5 0.027642	OR1N1,CST1,OR4C16,OR8B12,OR5A	2,CAt0-At3:start/end	GO_prom
MF amino acid:proton symporter act vity  MF L-proline transmembrane transporter act vity  MF L-alanine transmembrane transporter act vity  MF glycine transmembrane transport  MF glycine transmembrane	BP	response to blue light	6	3	3 1.57E-0	5 0.027642	CPT1B,DCT,TYR	At0-At3:start/end	GO_prom
MF L-proline transmembrane transporter act vity  MF L-alanine transmembrane transporter act vity  MF L-alanine transmembrane transporter act vity  BP L-alanine transmembrane transport  MF glycine transmembrane transport  BP proline transmembrane transport  BP proline transmembrane transport  MF glycine transmembrane transport  MF proline transport  BP proline transport  MF proline transmembrane transport  MF proline transmembrane transport  MF proline transmembrane transport  MF proline transmembrane transport  MF alanine transmembrane transport  MF alanine transmembrane transport  MF alanine transmembrane transporter act vity	MF	proline:proton symporter act vity	2	7	2 2.3E-C	7 0.005276	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
MF L-alanine transmembrane transporter act vity  BP L-alanine transport  I1 2 6.05E-06 0.03106 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  BP glycine transmembrane transporter act vity  I0 2 1.01E-05 0.037723 SLC36A2,SLC36A1  BP proline transport  BP proline transmembrane transport  I1 2 1.31E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  BP proline transmembrane transport  I1 2 1.31E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  BP proline transmembrane transport  I1 2 1.32E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  At2-At3:metastasis GO_prom  BP proline transmembrane transport  I1 2 1.32E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  At2-At3:metastasis GO_prom  At2-At3:metastasis GO_prom  At2-At3:metastasis GO_prom	MF	amino acid:proton symporter act vity	6	2	2 1.72E-0	06 0.019766	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
BP L-alanine transport  BP L-alanine transport  BP glycine transmembrane transporter act vity  BP proline transport  BP proline transmembrane transport  BP proline transmembr	MF	L-proline transmembrane transporter act vity	7	2	2 4.05E-0	06 0.030999	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
MF glycine transmembrane transporter act vity  10 2 1.01E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  Proline transmembrane transport  11 2 1.31E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  Proline transmembrane transport  12 1.32E-05 0.037723 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  At2-At3:metastasis GO_prom  15 2 1.91E-05 0.046693 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom  15 2 1.91E-05 0.046693 SLC36A2,SLC36A1  At2-At3:metastasis GO_prom	MF	L-alanine transmembrane transporter act vity	10	2	2 6.05E-0	0.03106	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 act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom	BP	L-alanine transport	11	2	2 6.77E-0	0.03106	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 A	MF	glycine transmembrane transporter act vity	10	2	2 1.01E-0	5 0.037723	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
MF alanine transmembrane transporter act vity 15 2 1.91E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom	BP	proline transport	11	2	2 1.31E-0	5 0.037723	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
, ,	BP	proline transmembrane transport	11	7	2 1.32E-0	5 0.037723	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
BP glycine transport 14 2 2.04E-05 0.046693 SLC36A2,SLC36A1 At2-At3:metastasis GO_prom	MF	alanine transmembrane transporter act vity	15	2	2 1.91E-0	5 0.046693	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom
	BP	glycine transport	14	2	2 2.04E-0	05 0.046693	SLC36A2,SLC36A1	At2-At3:metastasis	GO_prom

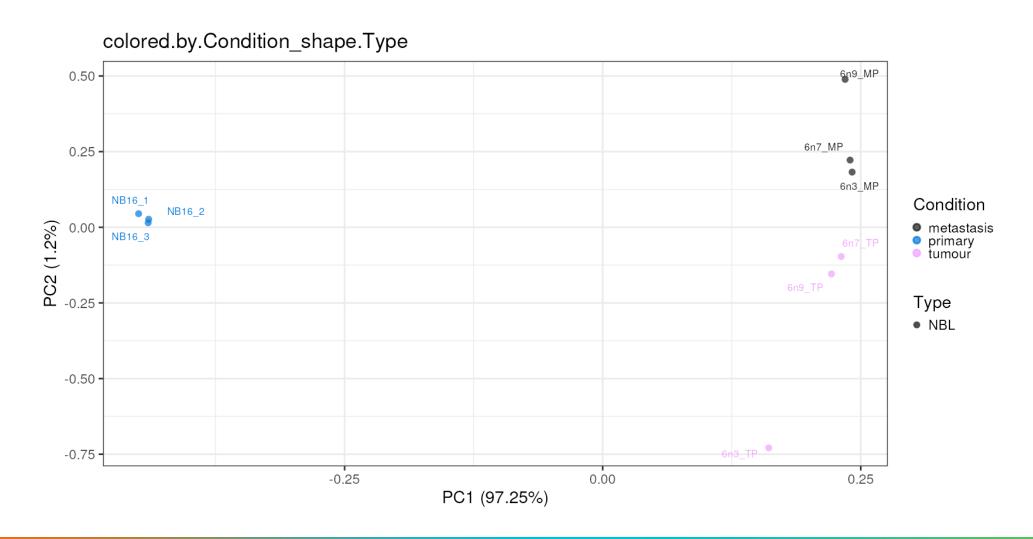
#### Pathways: Hypomethylated genes by contrast - ARMS

ONTOLOGY	TERM	N D	DE P.DE	FDR	SContrast	method
BP	striated muscle contraction	178	65.28922795772209e-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	63.74660559905911e-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	NAt2-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	NAt2-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	NAt2-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

# Quality control: PCA - NBL

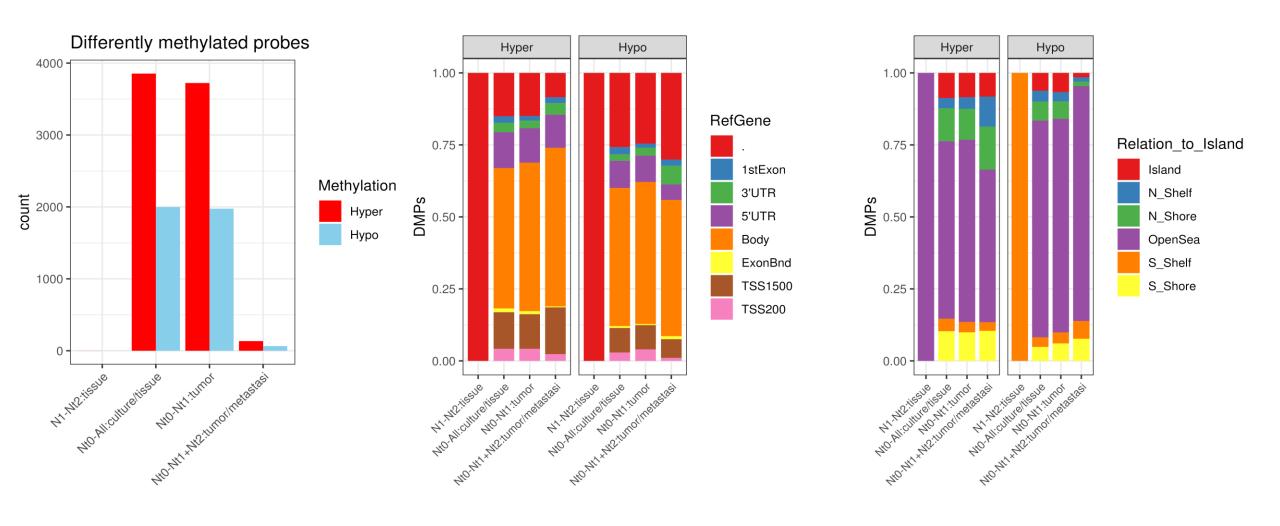
Very good seggregation where the origin of the sample (culture/tissue) accounts for most of the variation.



### 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

ITOLO	TERM	N	DE	FDR	SigGenesInSet	Contrast	method
CC	integral component of postsynapt c membrane	118		17 0.038798 0	CACNG2,GSG1L,ADRA1A,DCC,EPHB2,SORCS3,GABRA5,LRRTM2,GRIK	Nt0-Nt1:tumor	GO
CC	intrinsic component of postsynapt c membrane	123		17 0.038798 0	CACNG2,GSG1L,ADRA1A,DCC,EPHB2,SORCS3,GABRA5,LRRTM2,GRIK	Nt0-Nt1:tumor	GO
BP	inorganic ion transmembrane transport	914		56 0.038798 K	CNMB2,CACNG2,SLC26A1,PKD1L2,ADCYAP1R1,TMC1,ADRA1A,SLC	Nt0-Nt1:tumor	GO
CC	intrinsic component of synapt c membrane	166		20 0.038798 0	CACNG2,GSG1L,ADRA1A,DCC,EPHB2,NTNG1,SORCS3,GABRA5,LRRTI	Nt0-Nt1:tumor	GO
BP	vasoconstrict on	83		12 0.038798 K	CNMB2,ADRA1A,FGA,FGB,FGG,HRH1,HTR2B,SMTNL2,KEL,ASIC2,M	Nt0-Nt1:tumor	GO
BP	system process	2242	:	107 0.040922 K	CNMB2,CACNG2,BAIAP2,CELF2,WASF3,ADCY5,TMC1,LOXHD1,CRY	Nt0-Nt1:tumor	GO
BP	RNA processing	1323		39 9.11E-05 S	SNORD116-1,SNORD116-2,SNORD116-3,SNORD116-4,SNORD116-!	Nt0-Nt1:tumor	GO_prom
CC	nucleolus	1355		37 0.000777 S	SNORD116-1,SNORD116-2,SNORD116-3,SNORD116-4,SNORD116-!	Nt0-Nt1:tumor	GO_prom
BP	RNA processing	1323		43 8.56E-06 S	SNORD116-1,SNORD116-2,SNORD116-3,SNORD116-4,SNORD116-!	Nt0-All:culture/t issue	GO_prom
CC	nucleolus	1355		41 5.74E-05 S	SNORD116-1,SNORD116-2,SNORD116-3,SNORD116-4,SNORD116-!	Nt0-All:culture/t issue	GO_prom