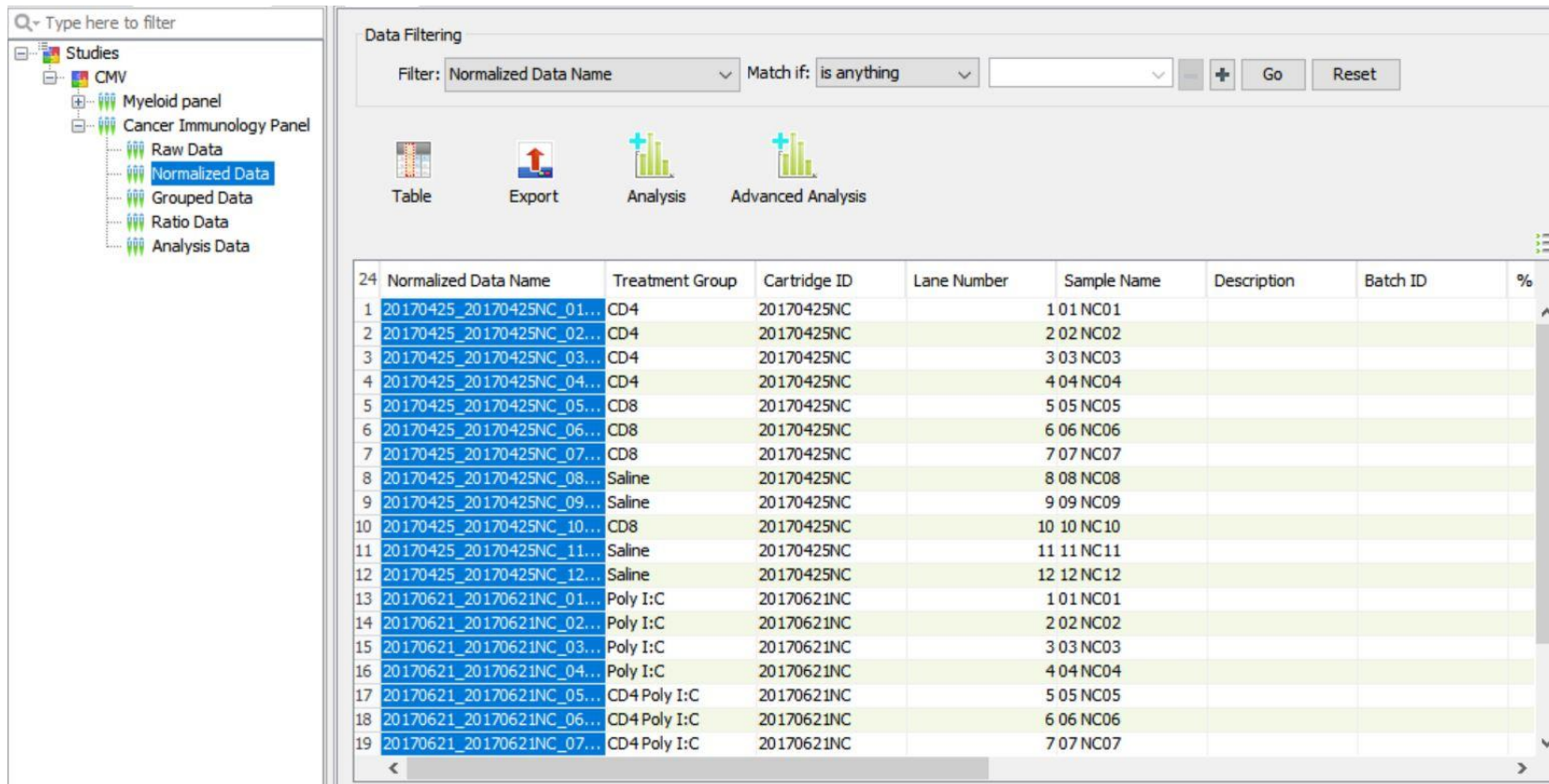


# Principle component analysis from Nanostring

Alex Bell

# 1. Import CSV to R from nSolver



The screenshot shows the nSolver software interface. On the left, a tree view under 'Studies' shows 'CMV' expanded to 'Cancer Immunology Panel', with 'Normalized Data' selected. The main area is titled 'Data Filtering' and contains a table with 24 rows. The table has columns: Normalized Data Name, Treatment Group, Cartridge ID, Lane Number, Sample Name, Description, Batch ID, and %. The data is as follows:

	Normalized Data Name	Treatment Group	Cartridge ID	Lane Number	Sample Name	Description	Batch ID	%
24								
1	20170425_20170425NC_01...	CD4	20170425NC		1 01 NC01			
2	20170425_20170425NC_02...	CD4	20170425NC		2 02 NC02			
3	20170425_20170425NC_03...	CD4	20170425NC		3 03 NC03			
4	20170425_20170425NC_04...	CD4	20170425NC		4 04 NC04			
5	20170425_20170425NC_05...	CD8	20170425NC		5 05 NC05			
6	20170425_20170425NC_06...	CD8	20170425NC		6 06 NC06			
7	20170425_20170425NC_07...	CD8	20170425NC		7 07 NC07			
8	20170425_20170425NC_08...	Saline	20170425NC		8 08 NC08			
9	20170425_20170425NC_09...	Saline	20170425NC		9 09 NC09			
10	20170425_20170425NC_10...	CD8	20170425NC		10 10 NC10			
11	20170425_20170425NC_11...	Saline	20170425NC		11 11 NC11			
12	20170425_20170425NC_12...	Saline	20170425NC		12 12 NC12			
13	20170621_20170621NC_01...	Poly I:C	20170621NC		1 01 NC01			
14	20170621_20170621NC_02...	Poly I:C	20170621NC		2 02 NC02			
15	20170621_20170621NC_03...	Poly I:C	20170621NC		3 03 NC03			
16	20170621_20170621NC_04...	Poly I:C	20170621NC		4 04 NC04			
17	20170621_20170621NC_05...	CD4 Poly I:C	20170621NC		5 05 NC05			
18	20170621_20170621NC_06...	CD4 Poly I:C	20170621NC		6 06 NC06			
19	20170621_20170621NC_07...	CD4 Poly I:C	20170621NC		7 07 NC07			

```
Cancerimmuno <- read.csv("~/Cancerimmuno.csv")  
View(Cancerimmuno)
```

	Probe.Name	Annotation	Accession..	NS.Probe.ID	Class.Name	Analyte.Type	X..Samples.above.Threshold	X20170425_20170425NC_01.NC01_01.RCC	X20170425_20170425NC_02.NC02_02.RCC	X20170425_20170425NC_03.NC03_03.RCC	X20170425_20170425NC_04.NC04_04.RCC
1		NA					NA	CD4	CD4	CD4	CD4
2		NA					NA				
3	A2m	NA	NM_175628.3	NM_175628.3:2128	Endogenous	mRNA	87.500	20.882	16.035	5.949	6.948
4	Abca1	NA	NM_013454.3	NM_013454.3:6865	Endogenous	mRNA	100.000	912.553	639.404	922.078	695.059
5	Abcb1a	NA	NM_011076.1	NM_011076.1:2600	Endogenous	mRNA	100.000	299.66	246.542	257.785	244.498
6	Abcg1	NA	NM_009593.1	NM_009593.1:295	Endogenous	mRNA	100.000	494.908	456.002	493.758	404.65
7	Abl1	NA	NM_009594.4	NM_009594.4:1378	Endogenous	mRNA	100.000	407.203	320.704	369.823	433.477
8	Ada	NA	NM_007398.3	NM_007398.3:795	Endogenous	mRNA	100.000	74.132	75.165	84.276	72.602
9	Adora2a	NA	NM_009630.2	NM_009630.2:2306	Endogenous	mRNA	100.000	25.059	11.024	12.889	19.218
10	Aicda	NA	NM_009645.2	NM_009645.2:552	Endogenous	mRNA	58.333	12.529	6.441	5.949	8.541
11	Aire	NA	NM_009646.1	NM_009646.1:656	Endogenous	mRNA	62.500	9.397	11.024	6.94	7.474
12	Akt3	NA	NM_011785.3	NM_011785.3:2494	Endogenous	mRNA	100.000	841.553	783.721	973.635	1021.768
13	Alcam	NA	NM_009655.1	NM_009655.1:2605	Endogenous	mRNA	100.000	1439.829	1234.712	1399.972	1686.932
14	Ambp	NA	NM_007443.3	NM_007443.3:410	Endogenous	mRNA	83.333	8.031	7.015	6.94	7.474
15	Amica1	NA	NM_001005421.4	NM_001005421.4:1002	Endogenous	mRNA	100.000	133.646	63.139	102.123	88.617
16	Angpt1	NA	NM_009640.3	NM_009640.3:1350	Endogenous	mRNA	100.000	41.764	27.059	41.642	48.046
17	Angpt2	NA	NM_007426.3	NM_007426.3:2020	Endogenous	mRNA	100.000	116.94	120.264	147.731	100.362
18	Anp32b	NA	NM_130889.2	NM_130889.2:480	Endogenous	mRNA	100.000	2974.672	2535.569	3147.953	2970.281
19	Anxa1	NA	NM_010730.2	NM_010730.2:400	Endogenous	mRNA	100.000	2063.163	2243.929	3037.899	2279.493
20	Apoe	NA	NM_009696.3	NM_009696.3:129	Endogenous	mRNA	100.000	21196.492	15694.472	15665.406	17496.043
21	App	NA	NM_007471.2	NM_007471.2:511	Endogenous	mRNA	100.000	2742.879	2208.852	2576.86	2285.899
22	Arg1	NA	NM_007482.3	NM_007482.3:626	Endogenous	mRNA	100.000	140.955	28.062	50.566	35.233
23	Arg2	NA	NM_009705.2	NM_009705.2:249	Endogenous	mRNA	100.000	24.015	24.053	25.779	22.421
24	Atf1	NA	NM_007497.3	NM_007497.3:1216	Endogenous	mRNA	100.000	145.131	142.313	152.688	170.829
25	Atf2	NA	NM_001284371.1	NM_001284371.1:1392	Endogenous	mRNA	100.000	570.084	462.015	532.425	526.365
26	Atg10	NA	NM_025770.3	NM_025770.3:46	Endogenous	mRNA	100.000	153.484	108.238	161.611	147.34
27	Atg12	NA	NM_026217.1	NM_026217.1:1800	Endogenous	mRNA	100.000	241.19	161.354	239.939	237.025
28	Atg16l1	NA	NM_001205391.1	NM_001205391.1:280	Endogenous	mRNA	100.000	353.954	273.601	401.55	384.364
29	Atg5	NA	NM_053069.5	NM_053069.5:2142	Endogenous	mRNA	100.000	107.543	113.249	117.986	135.595
30	Atg7	NA	NM_028835.1	NM_028835.1:855	Endogenous	mRNA	100.000	193.16	184.405	191.356	175.099
31	Atm	NA	NM_007499.2	NM_007499.2:5543	Endogenous	mRNA	100.000	441.659	431.949	407.499	475.117

# 2. Clean up data

Probe Name	Annotation	Accession	NS Probe ID	Class Name	Analytic Type	X_Samples above Threshold	X20170425_20170425NC.01.NC01_01.RCC	X20170425_20170425NC.02.NC02_02.RCC	X20170425_20170425NC.03.NC03_03.RCC	X20170425_2017
1	NA					NA	CD4	CD4	CD4	CD4
2	NA					NA				
3	Adm	NM_175038.3	NM_175038.3.01.08	Endogenous	miRNA	87,500	20,862	18,035	5,949	6,046
4	Abca1	NM_013454.3	NM_013454.3.06.05	Endogenous	miRNA	100,000	913,353	439,404	922,078	695,059
5	Abcb1a	NM_011076.1	NM_011076.1.02.00	Endogenous	miRNA	100,000	299,266	246,542	257,735	244,498
6	Abcg1	NM_005993.1	NM_005993.1.02.05	Endogenous	miRNA	100,000	454,908	456,002	493,758	404,465
7	Abi1	NM_005944.4	NM_005944.4.13.78	Endogenous	miRNA	100,000	407,203	320,704	369,823	433,477
8	Ada	NM_007398.3	NM_007398.3.7.95	Endogenous	miRNA	100,000	74,132	75,165	84,276	72,602
9	Adora2a	NM_006930.2	NM_006930.2.23.06	Endogenous	miRNA	100,000	25,059	11,024	12,889	19,218
10	Aldra	NM_009452	NM_009452.3.52	Endogenous	miRNA	58,333	12,229	6,441	5,949	6,541
11	Ahr	NM_009461	NM_009461.1.05	Endogenous	miRNA	62,500	9,397	11,224	6,94	7,474
12	Ahr1	NM_011705.3	NM_011705.3.04	Endogenous	miRNA	100,000	643,553	783,721	973,805	1021,700
13	Aicam	NM_009051.1	NM_009051.1.02.05	Endogenous	miRNA	100,000	1429,629	1224,712	1390,972	1096,532
14	Aimp	NM_007443.3	NM_007443.3.41.0	Endogenous	miRNA	83,333	8,631	7,015	6,94	7,474
15	Amic1	NM_001005421.4	NM_001005421.4.10.02	Endogenous	miRNA	100,000	133,646	63,139	102,123	88,617
16	Angpt1	NM_006460.3	NM_006460.3.13.50	Endogenous	miRNA	100,000	41,764	27,059	41,642	48,046
17	Angpt2	NM_007426.3	NM_007426.3.20.20	Endogenous	miRNA	100,000	114,94	120,264	147,731	100,362
18	Angpt2b	NM_130889.2	NM_130889.2.48.0	Endogenous	miRNA	100,000	2974,672	2335,569	3147,953	2970,281
19	Anxa1	NM_010730.2	NM_010730.2.40.0	Endogenous	miRNA	100,000	2063,163	2243,909	3037,890	2279,493
20	Apoa	NM_006966.3	NM_006966.3.13.9	Endogenous	miRNA	100,000	2196,482	1564,472	1565,498	1746,043
21	Apo	NM_007471.2	NM_007471.2.51.11	Endogenous	miRNA	100,000	2542,879	2208,852	2576,36	2285,809
22	Arg1	NM_007482.3	NM_007482.3.62.6	Endogenous	miRNA	100,000	140,955	28,262	30,566	35,233
23	Arg2	NM_009705.2	NM_009705.2.24.9	Endogenous	miRNA	100,000	24,015	24,053	25,779	22,421
24	Arg1	NM_007497.3	NM_007497.3.12.16	Endogenous	miRNA	100,000	143,131	142,313	152,688	170,829
25	Ahr2	NM_001284371.1	NM_001284371.1.13.02	Endogenous	miRNA	100,000	570,064	462,015	532,425	526,365
26	Atp10	NM_0023770.3	NM_0023770.3.46	Endogenous	miRNA	100,000	153,484	108,238	161,611	147,34
27	Atp12	NM_002171	NM_002171.11.00	Endogenous	miRNA	100,000	241,19	161,354	239,939	237,015
28	Atp10b	NM_001205913	NM_001205913.1.08	Endogenous	miRNA	100,000	353,564	273,601	401,35	384,344
29	Atp5	NM_002095	NM_002095.5.21.42	Endogenous	miRNA	100,000	107,543	112,249	117,866	125,595
30	Atp7	NM_008851	NM_008851.1.65	Endogenous	miRNA	100,000	193,16	184,405	191,356	175,099
31	Atm	NM_007499.2	NM_007499.2.55.43	Endogenous	miRNA	100,000	441,659	431,949	407,499	475,117



```
rownames(CIset) <- CIset[,1]
colnames(CIset) <- CIset[1,]
CIset1 <- CIset
CIset3 <- CIset1[-(1:7),-(1:2)]
CIset3 <- CIset3[,-(753:786)]
```

Need to change:

- Column names
- Row names
- Delete non-numerical data
- Remove all but genes of interest

# 2. Clean up data

	A2m	Abca1	Abcb1a	Abcg1	Abi1	Ada	Adora2a	Aicda	Aire	Akt3	Alcam	Ambp	Amica1	Angpt1	Angpt2	Anp32b	Anxa1	Apoe	App	Arg1	Arg2	Atf1	Atf2	Atg10	Atg12	Atg13
CD4	20.882	912.553	299.66	494.908	407.203	74.132	25.059	12.529	9.397	841.553	1439.829	8.031	133.646	41.764	116.94	2974.672	2063.163	21196.492	2742.879	140.955	24.015	145.131	570.084	153.484	241.19	301.19
CD4.1	16.035	639.404	246.542	456.002	320.704	75.165	11.024	6.441	11.024	783.721	1234.712	7.015	63.139	27.059	120.264	2535.569	2243.929	15694.472	2208.852	28.062	24.053	142.313	462.015	108.238	161.354	211.19
CD4.2	5.949	922.078	257.785	493.758	369.823	84.276	12.889	5.949	6.94	973.635	1399.972	6.94	102.123	41.642	147.731	3147.953	3037.899	15665.406	2576.86	50.566	25.779	152.688	532.425	161.611	239.939	401.19
CD4.3	6.948	695.059	244.498	404.65	433.477	72.602	19.218	8.541	7.474	1021.768	1686.932	7.474	88.617	48.046	100.362	2970.281	2279.493	17496.043	2285.899	35.233	22.421	170.829	526.365	147.34	237.025	301.19
CD8	42.457	2458.773	293.454	1689.548	509.487	101.148	63.686	13.736	11.239	528.218	469.527	12.487	404.592	42.457	275.972	2794.685	1843.144	42788.152	3301.674	319.678	66.183	157.342	514.482	218.53	380.866	301.19
CD8.1	34.101	2652.093	319.305	1537.625	390.606	105.402	51.151	6.318	6.318	522.358	545.609	15.5	337.905	49.601	246.454	2676.893	2028.983	36211.68	3506.156	533.209	83.701	124.002	489.808	187.553	296.055	401.19
CD8.2	47.917	2866.448	286.276	1669.74	399.312	110.579	72.491	9.829	22.116	504.977	491.461	13.515	346.48	36.86	399.312	2789.043	1664.825	38010.848	3384.94	837.942	82.32	153.582	420.199	200.27	314.535	401.19
Saline	5.695	537.912	290.453	437.591	417.526	85.034	20.064	7.644	5.733	989.834	1350.034	7.644	133.761	40.128	107.009	3301.994	2449.743	11709.849	2335.091	32.485	17.198	167.202	543.644	127.073	241.726	301.19
Saline.1	7.002	558.447	238.96	359.752	447.283	80.528	14.88	7.878	10.504	942.708	1656.086	8.753	85.78	46.391	98.91	3004.062	2516.515	14769.097	2322.196	30.636	25.384	140.05	526.061	135.673	241.585	401.19
CD8.3	47.223	3372.525	190.205	1669.866	347.615	114.123	61.653	11.806	11.806	425.009	299.08	13.118	339.745	26.235	342.368	2635.318	1635.76	49766.715	4092.679	3404.007	97.07	132.487	390.903	212.504	309.574	401.19
Saline.2	12.146	657.771	245.73	500.803	402.698	76.615	15.884	9.343	12.146	885.749	1337.966	11.212	116.792	34.57	163.508	3044.06	2517.096	15836.96	2431.137	41.111	25.227	163.508	554.06	122.398	229.846	301.19
Saline.3	18.09	762.786	306.019	547.216	431.14	78.389	18.09	12.06	9.045	1011.52	1444.167	13.567	223.107	51.254	143.211	3055.664	2553.673	17952.594	2808.438	37.687	25.627	161.3	476.364	150.748	218.585	401.19
Poly.I.C	15.616	863.572	327.939	792.519	463.018	66.369	23.424	8.448	8.448	1015.049	1255.537	8.448	139.764	33.575	129.614	3247.375	2162.835	16656.951	2551.677	22.643	24.205	164.75	588.728	158.504	228.776	301.19
Poly.I.C.1	10.006	808.162	304.793	755.824	511.066	73.119	13.854	8.421	8.421	1112.185	1299.986	8.421	122.379	43.872	147.778	3707.54	2803.168	14107.432	2527.623	18.472	30.787	191.65	643.451	140.081	291.708	401.19
Poly.I.C.2	8.766	710.778	289.278	661.104	477.748	71.589	18.263	8.036	9.497	937.963	1007.361	6	103.001	48.213	149.753	3613.789	2308.384	9795.29	2386.547	13.88	15.341	200.888	576.365	138.795	268.824	401.19
Poly.I.C.3	6.992	971.765	245.84	668.716	506.369	69.577	20.873	6.992	6.992	962.488	1258.579	9.277	87.358	37.881	130.651	3505.93	2169.27	16704.77	2347.079	24.739	32.469	183.221	565.896	151.524	272.125	301.19
CD4.Poly.I.C	16.005	885.315	356.316	712.633	481.827	62.334	37.064	7.433	7.433	769.913	1027.673	11.793	207.219	48.014	113.718	3389.637	2305.526	14654.454	2536.332	23.586	32.852	192.057	541.634	143.2	280.504	301.19
CD4.Poly.I.C.1	14.896	1054.078	326.825	747.405	529.229	66.592	28.039	7.01	7.886	865.693	1167.985	7.886	102.516	26.286	181.375	3526.736	2066.098	12157.381	2438.486	62.211	35.048	174.365	545.877	143.698	285.644	401.19
CD4.Poly.I.C.2	28.823	2330.725	280.368	2324.174	670.788	79.918	81.228	10.481	6.613	615.762	501.781	11.791	427.103	43.234	183.419	3714.225	1578.709	41812.863	2472.219	61.576	37.994	166.387	585.629	184.729	403.521	301.19
CD4.Poly.I.C.3	36.159	1778.788	368.588	1673.81	535.386	89.814	48.99	7.204	10.498	767.503	684.688	9.331	262.444	30.327	309.101	3367.45	1994.575	27500.646	2875.221	208.789	59.487	211.122	523.722	149.302	323.098	301.19
CD8.Poly.I.C	28.228	3500.235	169.366	1698.955	446.351	89.976	56.455	4.552	4.552	349.318	395.188	22.935	336.968	33.52	382.838	2732.794	2584.599	30916.393	3692.536	5086.279	95.268	162.309	451.643	165.838	352.846	301.19
CD8.Poly.I.C.1	47.131	2131.89	202.663	1157.85	446.173	131.967	67.554	4.072	9.426	364.479	375.477	18.852	465.025	18.852	394.329	3283.456	1729.706	17469.873	3696.638	2978.676	190.095	168.1	444.602	174.385	369.192	401.19
CD8.Poly.I.C.2	33.624	2408.807	231.332	1694.638	429.039	114.321	56.488	8.165	8.165	474.768	273.025	16.139	252.851	32.279	353.722	3049.004	1392.024	28238.592	3000.586	610.608	72.627	166.774	472.078	203.088	344.307	401.19
CD8.Poly.I.C.3	40.997	2425.672	265.116	1387.074	312.946	101.127	86.094	10.933	5.779	400.407	349.843	10.933	341.644	30.065	426.372	3070.696	2092.228	13870.744	3337.178	1238.118	61.496	168.089	426.372	168.089	323.878	401.19

### 3. Turn Characters into numbers

- Kept receiving this error report when trying to do PCA:

```
> prcomp(Cldata)
Error in colMeans(x, na.rm = TRUE) : 'x' must be numeric
```

- Realized my numbers were actually characters

```
> mode(CIset3)
[1] "character"
```

- Changed characters to numbers

```
CIset4 <- matrix(as.numeric(unlist(CIset3)),nrow=nrow(CIset3))
```

- Great success

```
> mode(CIset4)
[1] "numeric"
```

# 4. Perform PCA

```
my_pca <- prcomp(CIset4)
```



```
> my_pca
Standard deviations (1, ..., p=24):
 [1] 3.163669e+04 1.514445e+04 6.747373e+03 5.076125e+03 4.763752e+03 3.677696e+03 3.107976e+03
 [8] 2.179719e+03 1.828660e+03 1.718806e+03 1.516774e+03 1.272637e+03 1.004556e+03 8.791173e+02
[15] 8.098636e+02 7.525890e+02 5.718432e+02 5.053331e+02 4.767302e+02 3.837573e+02 3.517555e+02
[22] 2.902602e+02 2.541284e+02 9.108541e-12

Rotation (n x k) = (752 x 24):
      PC1      PC2      PC3      PC4      PC5      PC6
A2m    4.125191e-04 -2.779441e-04 4.365350e-04 -8.913626e-05 -1.844050e-04 -1.843134e-04
Abca1  2.850260e-02 -1.833015e-02 -1.781642e-02 -1.575116e-02 2.281739e-02 -3.463984e-02
Abcb1a -2.924434e-04 1.846023e-03 3.377987e-03 1.993240e-04 2.627546e-03 -1.707143e-03
Abcg1  1.640068e-02 -4.527342e-03 5.379973e-03 -1.166297e-02 4.015344e-02 2.262991e-02
Ab11   5.368374e-05 1.297021e-03 2.459613e-03 -6.939530e-03 6.745105e-03 1.058451e-02
Ada    4.216560e-04 -5.000221e-04 4.043788e-04 8.919508e-06 -1.420263e-03 -2.246141e-04
Adora2a 6.419432e-04 -6.078261e-04 4.491008e-04 1.685695e-04 9.512574e-04 9.061489e-04
Aicda  2.256848e-05 5.582497e-05 -5.109439e-05 1.724247e-04 6.228311e-05 1.541726e-04
Aire   3.083671e-05 6.333325e-05 1.211073e-04 3.366245e-05 -1.725326e-04 -1.049268e-04
Akt3   -6.444933e-03 7.836717e-03 -9.361617e-04 -7.727829e-04 4.036006e-04 3.136275e-03
Alcam  -1.286446e-02 1.166916e-02 -1.309005e-02 8.733677e-03 -2.123274e-02 4.829856e-03
Ambp   8.536563e-05 -1.253045e-04 -6.400581e-05 -1.324384e-04 -1.320902e-04 -1.631008e-04
Amical 3.417900e-03 -2.835743e-03 1.251176e-03 -3.578564e-03 -7.051968e-04 5.504371e-03
Angpt1 -5.345280e-05 3.057675e-04 -3.129832e-04 3.808833e-04 -4.852152e-06 5.964124e-05
Angpt2 2.608782e-03 -4.033528e-03 1.991380e-03 -7.405540e-04 1.870026e-03 -6.987922e-03
Anp32b -4.610727e-03 2.649847e-04 2.027838e-02 -2.370871e-02 3.019696e-02 2.741130e-02
Anxa1  -9.112292e-03 1.546517e-03 -2.767844e-02 -7.914400e-03 -2.752872e-03 -3.350442e-02
ApoE   3.370452e-01 1.749135e-01 -3.314441e-01 -2.040721e-01 2.459740e-02 4.187780e-01
App    1.389778e-02 -1.303021e-02 -7.874919e-03 -1.795893e-02 -2.044362e-02 -4.593727e-02
Arg1   2.063369e-02 -5.187353e-02 -6.678970e-02 -9.296309e-02 -1.512998e-02 -8.892657e-02
Arg2   7.899850e-04 -1.456829e-03 1.185899e-03 -2.524628e-03 -2.892905e-03 -8.118240e-04
Atf1   -2.411045e-04 -9.726245e-05 1.341327e-03 -1.502017e-03 2.474199e-03 7.888888e-05
Atf2   -1.131874e-03 1.804802e-03 1.336259e-03 -2.629906e-03 3.378465e-03 3.966624e-03
Ata10  8.092127e-04 8.502586e-06 -1.559208e-06 -1.985964e-04 -3.110500e-04 3.923812e-04
```

# 5. Graph PCA

- Install ggbiplot package

```
library(devtools)  
install_github("vqv/ggbiplot")
```

- Call ggbiplot

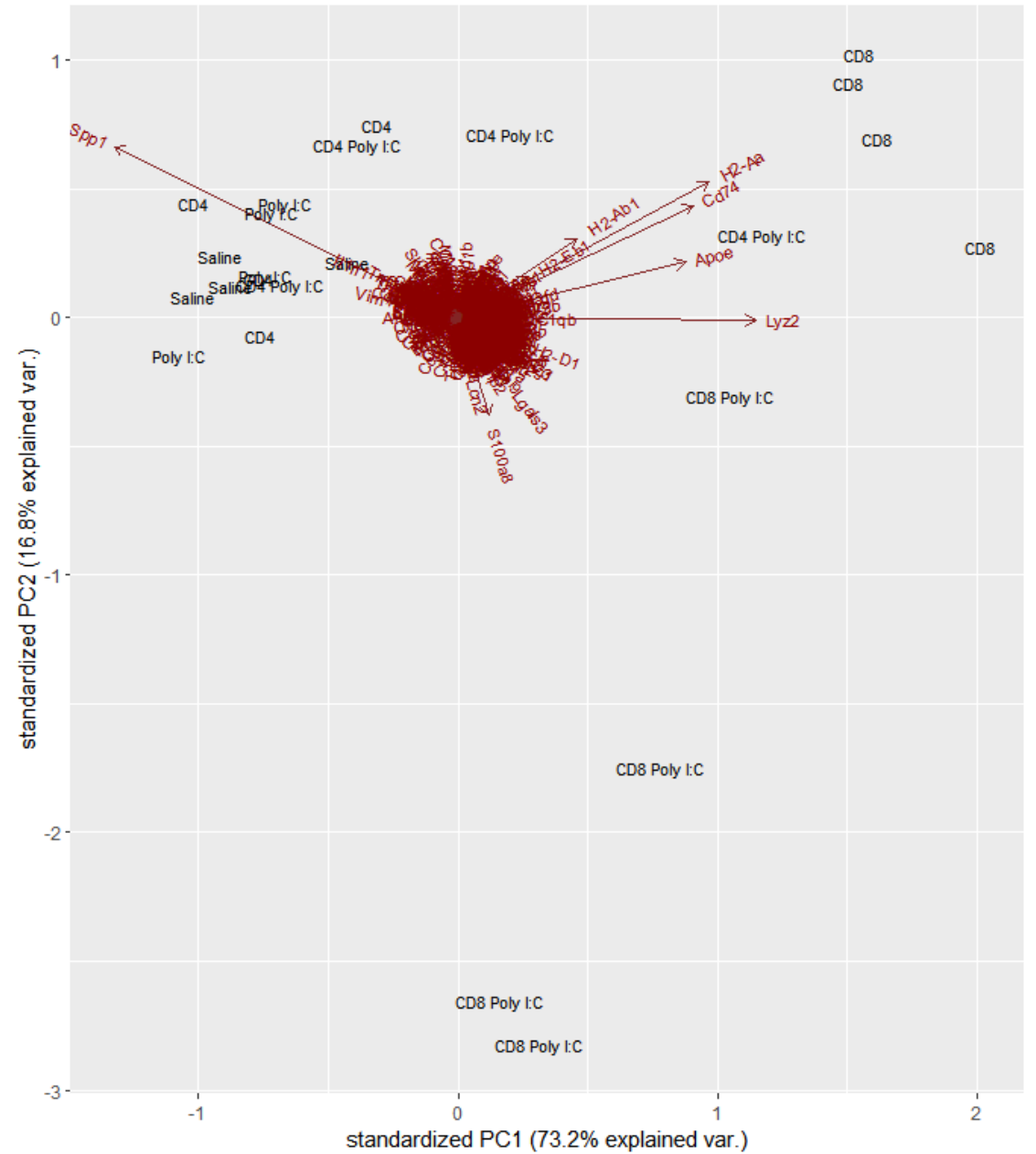
```
library(ggbiplot)
```

- Plot graph

```
ggbiplot(my_pca)
```

- Label points

```
ggbiplot(my_pca, labels=rownames(CIset4))
```





Thank you! 😊