ELF_Cytokine_Analysis Corr, Apr 23, 2024. Python 3.18

```
#------------------------------------------------------------------------
# ELF_Cytokine_Stats Analysis. Corr
# Apr 23, 2024 Python 3.18 by Teiji Sawa, MD, PhD
# Kyoto Prefectural University of Medicine, Japan
# For the analyses of the following publication:
# Authors: Sazuki Sudo, others & Teiji Sawa.
# Title: Case study observational research: inflammatory
# cytokines in the bronchial epithelial lining fluid of COVID-19
# patients with acute hypoxemic respiratory failure.
# Journal: Critical Care volume 28: 134, 2024.
# DOI: doi:10.1186/s13054-024-04921-3
#------------------------------------------------------------------------
```

```
#-[Python Code #1]--------------------------------------------------
import numpy as np
import pandas as pd
import seaborn as sns
import matplotlib.pyplot as plt
#-[END]---------------------------------------------------------------
```

```
#-[Python Code #2]--------------------------------------------------
data_all = pd.read_csv('Data1_ELF&Plasma_wNormal_20240423.csv')
data_all
#-[END]---------------------------------------------------------------
```

[Out]

	cytokine	group	source	conc
0	IL6	covid	ELF	91.28
1	IFNg	covid	ELF	4.39
2	MCP1	covid	ELF	1538.62
...	...	...	...	...
1599	IL13	normal	plasma	0.03

1600 rows × 4 columns

```
#-[Python Code #3]--------------------------------------------------
data_all_ELF = data_all[data_all.source=='ELF']
data_all_ELF
#-[END]---------------------------------------------------------------
```

[Out]

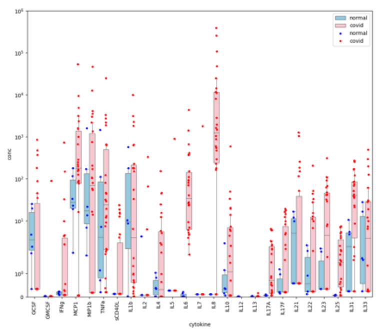
	cytokine	group	source	conc
0	IL6	covid	ELF	91.280000
1	IFNg	covid	ELF	4.390000
2	MCP1	covid	ELF	1538.620000
...	...	...	...	...
1574	IL13	normal	ELF	0.030000

800 rows × 4 columns

```
#-[Python Code #4]--------------------------------------------------
fig, ax = plt.subplots(figsize= (12, 10))
sns.boxplot(x='cytokine', y='conc', data=data_all_ELF, hue='group', \
                hue_order= ['normal', 'covid'],
                  order= ['GCSF','GMCSF','IFNg','MCP1','MIP1b','TNFa', \
```

```
                    'sCD40L','IL1b','IL2','IL4','IL5','IL6','IL7','IL8','IL10', \
                    'IL12p70','IL13','IL17A','IL17F','IL21','IL22','IL23', \
                    'IL25','IL31','IL33'],
                palette= ["skyblue", "pink"],
                dodge=True, color='black', ax=ax,
                linewidth=0.8,
                fliersize=2
            )

sns.stripplot(x='cytokine', y='conc', data=data_all_ELF, dodge=True, \
              jitter=True, hue='group', hue_order= ['normal', 'covid'],
             order= ['GCSF','GMCSF','IFNg','MCP1','MIP1b','TNFa', \
                    'sCD40L','IL1b','IL2','IL4','IL5','IL6','IL7','IL8','IL10', \
                    'IL12p70','IL13','IL17A','IL17F','IL21','IL22','IL23', \
                    'IL25','IL31','IL33'],
              palette= ["blue", "red"], ax=ax, size=4)

ax.set_yscale('symlog')
ax.set_ylim([0, 1000000])
ax.legend()

plt.xticks(rotation=90)
plt.savefig('Fig1_ELF_all.svg')
plt.savefig('Fig1_ELF_all.png')
plt.show()
#-[END]-----------------------------------------------------------------
```

[Out]



```
#-[Python Code #5]-----------------------------------------------
data_all_plasma = data_all[data_all.source=='plasma']
data_all_plasma
#-[END]-----------------------------------------------------------------
```

[Out]

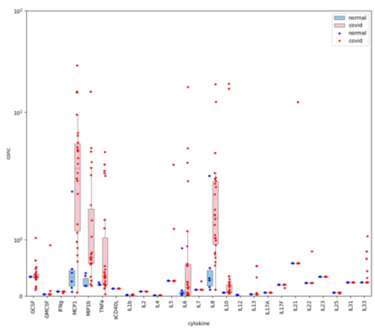
	cytokine	group	source	conc
25	IL6	covid	plasma	17.67
26	IFNg	covid	plasma	0.08
...	...	...	...	...
1599	IL13	normal	plasma	0.03

800 rows × 4 columns

```
#-[Python Code #6]-----------------------------------------------
fig, ax = plt.subplots(figsize=(12, 10))
sns.boxplot(x='cytokine', y='conc', data=data_all_plasma, \
```

```
                    hue='group', hue_order= ['normal', 'covid'],
                     order= ['GCSF','GMCSF','IFNg','MCP1','MIP1b','TNFa', \
                            'sCD40L','IL1b','IL2','IL4','IL5','IL6','IL7','IL8','IL10', \
                            'IL12p70','IL13','IL17A','IL17F','IL21','IL22','IL23', \
                            'IL25','IL31','IL33'],
                          palette= ["skyblue", "pink"],
                          dodge=True, color='black', ax=ax,
                          linewidth=0.8,
                          fliersize=2
                    )

sns.stripplot(x='cytokine', y='conc', data=data_all_plasma, dodge=True, \
                     jitter=True, hue='group', hue_order= ['normal', 'covid'],
                     order= ['GCSF','GMCSF','IFNg','MCP1','MIP1b','TNFa', \
                            'sCD40L','IL1b','IL2','IL4','IL5','IL6','IL7','IL8','IL10', \
                            'IL12p70','IL13','IL17A','IL17F','IL21','IL22','IL23', \
                            'IL25','IL31','IL33'],
                       palette= ["blue", "red"], ax=ax, size=4)

ax.set_yscale('symlog')
ax.set_ylim([0, 100])
ax.legend()

plt.xticks(rotation=90)
plt.savefig('Fig2_plasma_all.svg')
plt.savefig('Fig2_plasma_all.png')
plt.show()
#-[END]-----------------------------------------------------------------
```

[Out]



```
#-[Python Code #7]-------------------------------------------------
data_covid = pd.read_csv('Data2_ELFperPlasma_20240423.csv')
data_covid.head(3)
#-[END]-----------------------------------------------------------------
```

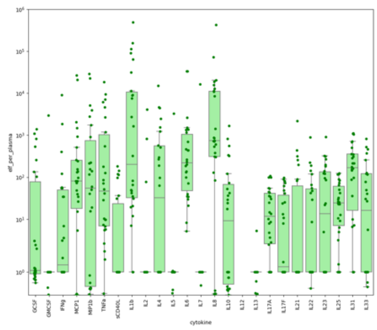
[Out]

	cytokine	group	source	elf_per_plasma
0	IL6	covid	ELF	17.67
1	IFNg	covid	ELF	0.08
2	MCP1	covid	ELF	15.93

```
#-[Python Code #8]-------------------------------------------------
fig, ax = plt.subplots(figsize= (12, 10))

sns.boxplot(x='cytokine', y='elf_per_plasma', data=data_covid,
           order= ['GCSF','GMCSF','IFNg','MCP1','MIP1b','TNFa', \
```

```
                    'sCD40L','IL1b','IL2','IL4','IL5','IL6','IL7','IL8','IL10', \
                    'IL12p70','IL13','IL17A','IL17F','IL21','IL22','IL23', \
                    'IL25','IL31','IL33'],
                        color='palegreen', ax=ax,
                        linewidth=1.2,
                        fliersize=0.1,
                        )

sns.stripplot(x='cytokine', y='elf_per_plasma', data=data_covid, \
                 jitter=True, color='green',
          order= ['GCSF','GMCSF','IFNg','MCP1','MIP1b','TNFa', \
                    'sCD40L','IL1b','IL2','IL4','IL5','IL6','IL7','IL8','IL10', \
                    'IL12p70','IL13','IL17A','IL17F','IL21','IL22','IL23', \
                    'IL25','IL31','IL33'],
                    ax=ax, size=5)

ax.set_yscale('symlog')
#ax.set_yscale('log')
ax.set_ylim([0.01, 1000000])

plt.xticks(rotation=90)
plt.savefig('Fig3_elf_per_plasma.svg')
plt.savefig('Fig3_elf_per_plasma.png')
plt.show()
#-[END]----------------------------------------------------------------
```

[Out]



Correlation Analysis¶

```
#-[Python Code #9]-----------------------------------------------
data = pd.read_csv('Data3_ELF_20240423.csv')
data.head(3)
#-[END]----------------------------------------------------------------
```

[Out]

	ch_no	psi_no	…	PSI	LIV	IL6
0	1	24		1	173	91.28
1	2	27		2	245	371.47
2	3	5		2	75	411.25

3 rows × 35 columns

```
#-[Python Code #10]-------------------------------------------------
data_corr = data[['PSI','LIV','TNFa','IL1b','IL8','GCF','MIP1b','IL2', \
                  'IL10','IL4','MCP1','IFNg','IL6','IL33','IL17F','IL22', \
                  'IL21','IL31','IL23','IL17A','IL25','sCD40L']]
data_all_r3 = data_corr.copy()
data_all_r3.head(3)
#-[END]-------------------------------------------------------------
```

[Out]

	PSI	....	IL10	IL4	...
0	173	65.79	49.74	5.11	
1	245	0.08	33.53	0.01	
2	75	0.08	6.20	0.01	

3 rows × 22 columns

```
#-[Python Code #11]-------------------------------------------------
###Logarithmic
# IL6: standardize
X1 = data_all_r3.loc[:, ['IL6']]
X1 = X1.apply(np.log)

# IL8: standardize
X2 = data_all_r3.loc[:, ['IL8']]
X2 = X2.apply(np.log)

# MCP1: standardize
X3 = data_all_r3.loc[:, ['MCP1']]
X3 = X3.apply(np.log)

# MIP1b: standardize
X4 = data_all_r3.loc[:, ['MIP1b']]
X4 = X4.apply(np.log)

# TNFa: standardize
X5 = data_all_r3.loc[:, ['TNFa']]
X5 = X5.apply(np.log)

# IL10: standardize
X6 = data_all_r3.loc[:, ['IL10']]
X6 = X6.apply(np.log)

# IL33: standardize
X7 = data_all_r3.loc[:, ['IL33']]
X7 = X7.apply(np.log)

# IFNg: standardize
X8 = data_all_r3.loc[:, ['IFNg']]
X8 = X8.apply(np.log)

# IL1b: standardize
X9 = data_all_r3.loc[:, ['IL1b']]
X9 = X9.apply(np.log)

# GCF: standardize
X10 = data_all_r3.loc[:, ['GCF']]
X10 = X10.apply(np.log)

# IL4: standardize
X11 = data_all_r3.loc[:, ['IL4']]
X11 = X11.apply(np.log)
```

```
# IL17F: standardize
X12 = data_all_r3.loc[:, ['IL17F']]
X12 = X12.apply(np.log)

# IL21: standardize
X13 = data_all_r3.loc[:, ['IL21']]
X13 = X13.apply(np.log)

# IL22: standardize
X14 = data_all_r3.loc[:, ['IL22']]
X14 = X14.apply(np.log)

# IL17A: standardize
X15 = data_all_r3.loc[:, ['IL17A']]
X15 = X15.apply(np.log)

# IL23: standardize
X16 = data_all_r3.loc[:, ['IL23']]
X16 = X16.apply(np.log)

# IL25: standardize
X17 = data_all_r3.loc[:, ['IL25']]
X17 = X17.apply(np.log)

# IL31: standardize
X18 = data_all_r3.loc[:, ['IL31']]
X18 = X18.apply(np.log)

# IL2: standardize
X19 = data_all_r3.loc[:, ['IL2']]
X19 = X19.apply(np.log)

# sCD40L: standardize
X24 = data_all_r3.loc[:, ['sCD40L']]
X24 = X24.apply(np.log)

# Output
data_all_r3.loc[:, ['IL6']] = X1
data_all_r3.loc[:, ['IL8']] = X2
data_all_r3.loc[:, ['MCP1']] = X3
data_all_r3.loc[:, ['MIP1b']] = X4
data_all_r3.loc[:, ['TNFa']] = X5
data_all_r3.loc[:, ['IL10']] = X6
data_all_r3.loc[:, ['IL33']] = X7
data_all_r3.loc[:, ['IFNg']] = X8
data_all_r3.loc[:, ['IL1b']] = X9
data_all_r3.loc[:, ['GCF']] = X10
data_all_r3.loc[:, ['IL4']] = X11
data_all_r3.loc[:, ['IL17F']] = X12
data_all_r3.loc[:, ['IL21']] = X13
data_all_r3.loc[:, ['IL22']] = X14
data_all_r3.loc[:, ['IL17A']] = X15
data_all_r3.loc[:, ['IL23']] = X16
data_all_r3.loc[:, ['IL25']] = X17
data_all_r3.loc[:, ['IL31']] = X18
data_all_r3.loc[:, ['IL2']] = X19
data_all_r3.loc[:, ['sCD40L']] = X24
#-[END]-----------------------------------------------------------------
```

```
#-[Python Code #12]-----------------------------------------------
data_all_r3.head(3)
#-[END]-----------------------------------------------------------------
```

[Out]

```
      PSI           ...         IL10

 0    173     4.186468    3.906809

 1    245    -2.525729    3.512441

 2     75    -2.525729    1.824549
```

3 rows × 22 columns

```
#-[Python Code #13]-----------------------------------------------
data_all_r3 = data_all_r3.iloc[:,:]
corr = data_all_r3.corr()
print(corr)
#-[END]-----------------------------------------------------------
```

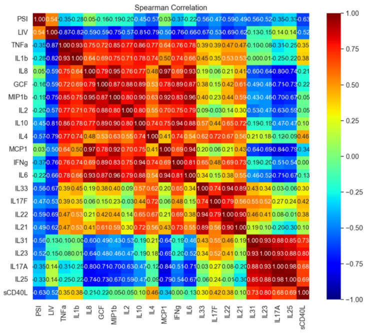
[Out]

```
              PSI        LIV       TNFa       IL1b        IL8        GCF      MIP1b   ¥
PSI      1.000000   0.346204   0.018715   0.166074   0.207175   0.030441  -0.011050
LIV      0.346204   1.000000  -0.484264  -0.325923  -0.278428  -0.175616  -0.453120
TNFa     0.018715  -0.484264   1.000000   0.830292   0.629645   0.570032   0.701376
.....
sCD40L  -0.229752  -0.408536   0.446174   0.444807   0.156168   0.027222   0.218299

              IL2       IL10        IL4   ...        IL6       IL33      IL17F   ¥
PSI     -0.108708  -0.217492  -0.162748   ...  -0.085738  -0.189133   0.049929
LIV     -0.196742  -0.392568  -0.259178   ...  -0.237532  -0.437492  -0.154480
TNFa     0.572178   0.731840   0.606350   ...   0.640329   0.263769   0.447039
.....
sCD40L   0.205723   0.223809   0.459109   ...   0.182824   0.196474   0.248289

             IL22       IL21       IL31       IL23      IL17A       IL25     sCD40L
PSI     -0.077068  -0.119359  -0.195992  -0.131076  -0.109583  -0.100648  -0.229752
LIV     -0.247969  -0.153879  -0.037292  -0.156624   0.062991   0.047808  -0.408536
TNFa     0.443901   0.335365   0.275557   0.270827   0.149964   0.250751   0.446174
.....
sCD40L   0.385023   0.160526   0.519911   0.646726   0.573544   0.518472   1.000000
[22 rows x 22 columns]
```

```
#-[Python Code #14]-----------------------------------------------
def display_correlation(df):
    r = df.corr(method="spearman")
    plt.figure(figsize= (12,10))
    heatmap = sns.heatmap(df.corr(), vmin=-1,
                          vmax=1, annot=True, cmap='jet', \
                          fmt='.2f', annot_kws={'fontsize': 12},)
    plt.title("Spearman Correlation")
    return(r)
#-[END]-----------------------------------------------------------
```

```
#-[Python Code #15]-----------------------------------------------
sns.set(font_scale=1.2)
display_correlation(corr)
plt.savefig('Fig4_spearman_elf.svg')
plt.savefig('Fig4_spearman_elf.png')
plt.show()
#-[END]-----------------------------------------------------------
```

[Out]




```
#-[Python Code #16]-----------------------------------------------
data2 = pd.read_csv('Data4_Plasma_20240423.csv')
data2.head(3)
#-[END]-----------------------------------------------------------
```

[Out]

	ch_no	.....	PSI
0	1	1	173
1	2	2	245
2	3	2	75

3 rows × 35 columns

```
#-[Python Code #17]-----------------------------------------------
data2_corr = data2[['PSI','LIV','IL8','MCP1','IL6','TNFa','IL13','IL33', \
                    'MIP1b','IL5','GMCF','GCF','IL10']]

data2_all_r3 = data2_corr.copy()
#-[END]-----------------------------------------------------------
```

```
#-[Python Code #18]-----------------------------------------------
###Logarithmic
# IL6: standardize
X1 = data2_all_r3.loc[:, ['IL6']]
X1 = X1.apply(np.log)

# IL8: standardize
X2 = data2_all_r3.loc[:, ['IL8']]
X2 = X2.apply(np.log)

# MCP1: standardize
X3 = data2_all_r3.loc[:, ['MCP1']]
X3 = X3.apply(np.log)

# MIP1b: standardize
X4 = data2_all_r3.loc[:, ['MIP1b']]
X4 = X4.apply(np.log)

# TNFa: standardize
X5 = data2_all_r3.loc[:, ['TNFa']]
X5 = X5.apply(np.log)

# IL10: standardize
X6 = data2_all_r3.loc[:, ['IL10']]
```

```
X6 = X6.apply(np.log)

# IL33: standardize
X7 = data2_all_r3.loc[:, ['IL33']]
X7 = X7.apply(np.log)

# GCF: standardize
X10 = data2_all_r3.loc[:, ['GCF']]
X10 = X10.apply(np.log)

## GMCF: standardize
X25 = data2_all_r3.loc[:, ['GMCF']]
X25 = X25.apply(np.log)

# Output
data2_all_r3.loc[:, ['IL6']] = X1
data2_all_r3.loc[:, ['IL8']] = X2
data2_all_r3.loc[:, ['MCP1']] = X3
data2_all_r3.loc[:, ['MIP1b']] = X4
data2_all_r3.loc[:, ['TNFa']] = X5
data2_all_r3.loc[:, ['IL10']] = X6
data2_all_r3.loc[:, ['IL33']] = X7
data2_all_r3.loc[:, ['GCF']] = X10
data2_all_r3.loc[:, ['GMCF']] = X25
#-[END]------------------------------------------------------------------
```

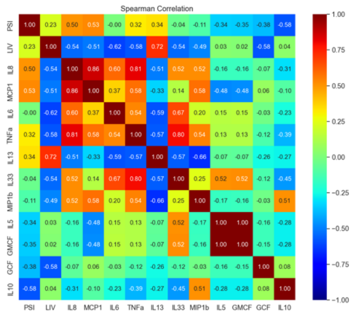
```
#-[Python Code #19]------------------------------------------------
corr2 = data2_all_r3.corr()
print(corr2)
#-[END]------------------------------------------------------------------
```

[Out]

```
        PSI          LIV          IL8        MCP1         IL6        TNFa         IL13   ¥
PSI     1.000000   0.346204   0.488543   0.494825   0.142918   0.367449   0.341706
LIV     0.346204   1.000000  -0.027189  -0.140894  -0.143893  -0.124760   0.481838
IL8     0.488543  -0.027189   1.000000   0.729544   0.468561   0.612345  -0.062737
....
IL10   -0.281407   0.186937  -0.022785   0.066774   0.050246  -0.135643  -0.095920

             IL33       MIP1b         IL5        GMCF         GCF        IL10
PSI     0.096084   0.140068   0.006554   0.002069  -0.078244  -0.281407
LIV    -0.144173  -0.033820   0.188067   0.183444  -0.264280   0.186937
IL8     0.426468   0.482982   0.175723   0.175037   0.098238  -0.022785
....
IL10   -0.182520   0.575821  -0.027637  -0.028236   0.104043   1.000000
```

```
#-[Python Code #20]------------------------------------------------
sns.set(font_scale =1.2)
display_correlation(corr2)
plt.savefig('Fig5_plasma_spearman.svg')
plt.savefig('Fig5_plasma_spearman.png')
plt.show()
#-[END]------------------------------------------------------------------
```

[Out]


Spearman Correlation
PSI
LIV
IL8
MCP1
IL6
TNFa
IL13
IL33
MIP1b
IL5
GMCF
GCF
IL10
1.00
0.75
0.50
0.25
0.00
−0.25
−0.50
−0.75
−1.00