

Figures

```
In [1]: import numpy as np
import pandas as pd
import scanpy as sc
import scvelo as sv
import matplotlib.pyplot as plt
from matplotlib_venn import venn3, venn3_circles
from scipy import stats
import seaborn as sns
import sciripy as ir
import itertools
import gseapy

sc.settings.verbosity = 0 # scientific computing toolkit
(3) sc.logging.print_header() # data analysis toolkit
# scanpy is referred to with sc.*** # RNA velocity toolkit
# Matplotlib is referred to with plt.*** # Venn diagrams
# for linear regressions
# for easy heatmaps
# TCR analysis

sc.set_versions(scipy=1.5.1, pandas=1.1.5, scikit-learn=0.21.3,
statsmodels=0.11.1, python-igraph=0.7.1+4.bed07760, louvain=0.6.1, leidenalg=0.7.0)
```

```
In [2]: sc.settings.set_figure_params(dpi=80, dpi_save=200, color_map='viridis')
clonotypes_re_expression=['2','4','5','13','14','23','49','74','184','471']
order=['Blood_before', 'Infusion_product', 'Blood_after']
adata = sc.read('adata_figures.h5ad')
clonotypes_greater_2=adata.obs.clonotype[adata.obs.clonotype_size>2].unique().tolist()
adata=adata[adata.obs.leiden!='6'] #exclude cluster containing non T cells (Monocyte impurities)
```

```
In [3]: chain_pairing_all=np.round((adata.obs.chain_pairing.value_counts(normalize=True)*100),1).astype('str')+' %'
chain_pairing_T=np.round((adata[adata.raw[:, 'CD3D'].X>0, : ].obs.chain_pairing.value_counts(normalize=True)*100),1).astype('str')+' %'
```

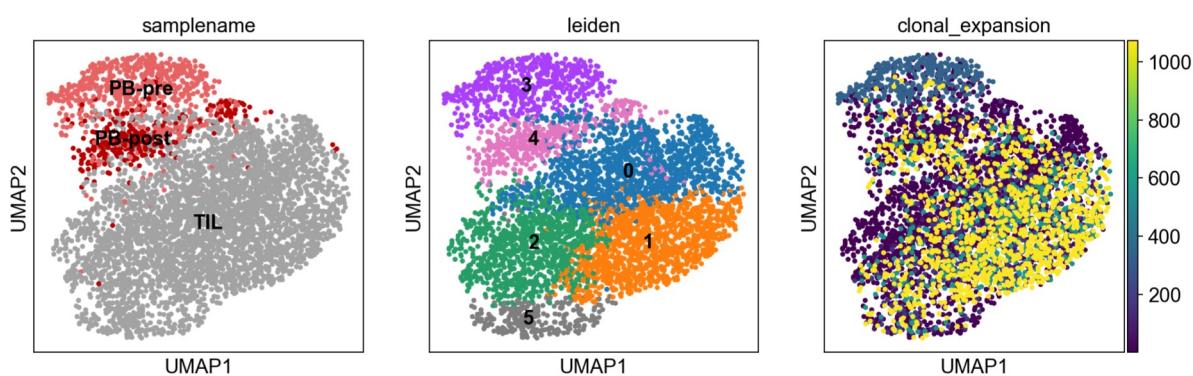
```
In [4]: print('%.2f' % ((adata[adata.obs.chain_pairing.isin(['Single pair', 'Extra beta', 'Extra alpha', 'Two full chains'])].shape[0]/adata.shape[0])*100))+' % of the cells have at least one alpha/beta pair'
print('%.2f' % ((adata[adata.obs.chain_pairing.isin(['Single pair', 'Extra beta', 'Extra alpha', 'Two full chains'])].shape[0]/adata[adata.raw[:, 'CD3D'].X>0, : ].shape[0])*100))+' % of the T cells have at least one alpha/beta pair')
```

59.92 % of the cells have at least one alpha/beta pair
78.90 % of the T cells have at least one alpha/beta pair

```
In [5]: adata.obs.samplename.cat.rename_categories({'Blood_before':'PB-pre', 'Blood_after':'PB-post', 'Infusion_product':'TIL'}, inplace=True)
adata.uns['samplename_colors']=[ '#b80000', '#e86464', '#a3a3a3'] #[ '#1f77b4', '#ff7f0e', '#279e68']
adata.uns['leiden_colors']=[ '#1f77b4', '#ff7f0e', '#279e68', '#aa40fc', '#e377c2', '#7f7f7f'] #[ '#1f77b4', '#ff7f0e', '#279e68', '#d62728', '#aa40fc', '#8c564b']
```

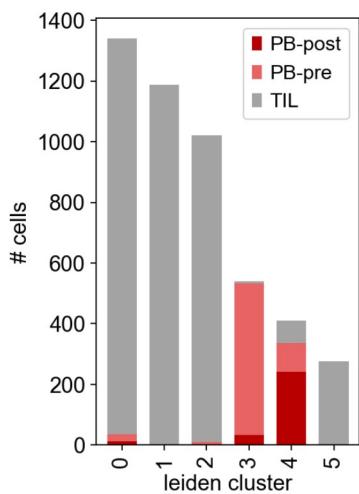
Trying to set attribute `uns` of view, copying.

```
In [6]: fig,ax = plt.subplots(ncols=3, figsize=(14,4))
sc.pl.umap(adata, color='samplename', show=False, legend_loc='on data', s=50, ax=ax[0])
sc.pl.umap(adata, color='leiden', show=False, legend_loc='on data', s=50, ax=ax[1])
sc.pl.umap(adata, color='clonal_expansion', s=50, show=False, ax=ax[2])
fig.savefig('./figures_paper/umaps.svg', dpi=300, bbox_inches='tight')
```

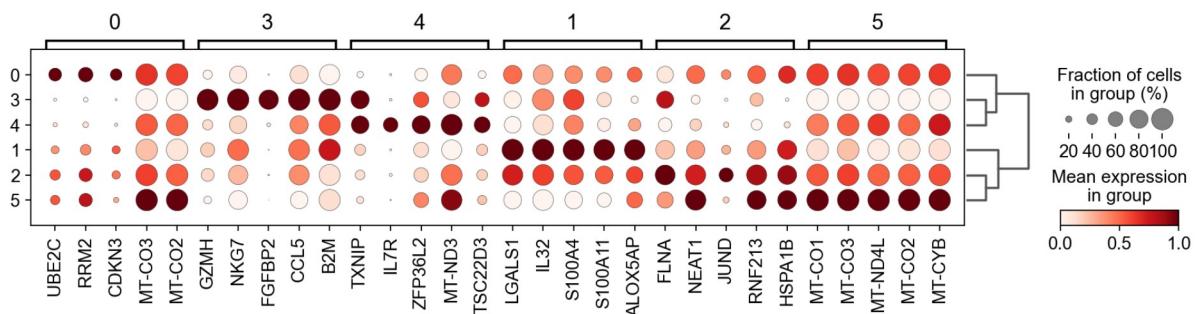


```
In [7]: data=adata.obs.groupby(['samplename']).count()
data=pd.DataFrame(index=['PB-post', 'PB-pre', 'TIL'], columns=['0','1','2','3','4','5'])
for column in data.columns.tolist():
    for row in data.index.tolist():
        data.loc[row,column]=adata[(adata.obs.samplename==row) & (adata.obs.leiden==column)].shape[0]
```

```
In [8]: fig, ax=plt.subplots(figsize=(3,5))
data[['0','1','2','3','4','5']].T.plot(kind='bar', stacked=True, color=['#b80000','#e86464','#a3a3a3'],
ax=ax, grid=False, width=0.7)
ax.set_xlabel('leiden cluster')
ax.set_ylabel('# cells')
plt.show()
fig.savefig('./figures_paper/n_cells_clusters.svg', dpi=300, bbox_inches='tight')
```

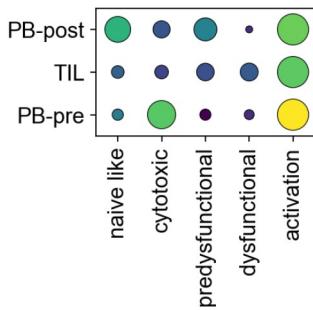


```
In [9]: sc.tl.dendrogram(adata, groupby='leiden')
sc.tl.rank_genes_groups(adata, 'leiden', use_raw=True, method='t-test')
sc.pl.rank_genes_groups_dotplot(adata, n_genes=5, show=False, standard_scale='var')
plt.savefig('./figures_paper/dotplot_leiden.svg', dpi=300, bbox_inches='tight')
```

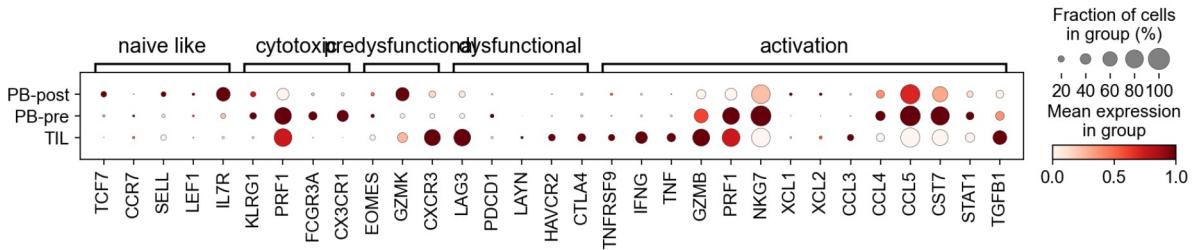


```
In [10]: fig,ax=plt.subplots(figsize=(2.5,1.5))
df_dotplot=adata.obs.groupby('samplename').agg({'naive_like_state':'mean',
                                                'cytotoxic_state':'mean',
                                                'predysfunctional_state':'mean',
                                                'dysfunctional_state':'mean',
                                                'activation_score':'mean'})
df_dotplot=df_dotplot.loc[['PB-pre','TIL','PB-post']]
df_size=df_dotplot.copy()
for column in df_size.columns:
    df_size[column]=adata.obs[adata.obs[column] > 0].groupby('samplename')['cytotoxic_activation'].count() / adata.obs.groupby('samplename')['cytotoxic_activation'].count()

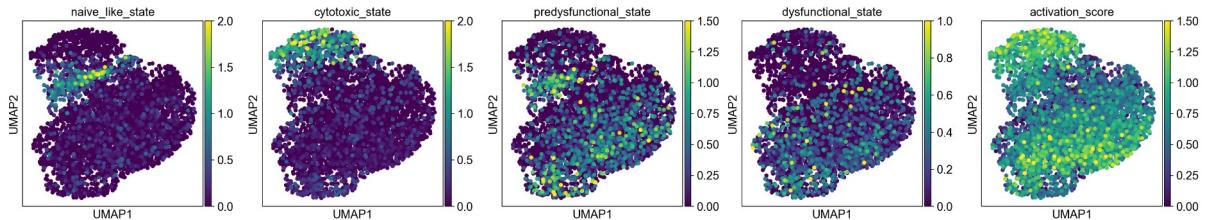
sns.scatterplot(x=df_dotplot.columns.tolist()*df_dotplot.index.shape[0],
                 y=list(itertools.chain(*[df_dotplot.index.tolist()[i]*df_dotplot.columns.shape[0] for i in range(df_dotplot.shape[0])])), size=df_size.values.flatten().tolist(),
                 hue=df_dotplot.values.flatten().tolist(), edgecolor='black', linewidth=.5, ax=ax,
                 sizes=(20,400), palette='viridis', vmin=0)
ax.set_xticklabels(['naive like', 'cytotoxic', 'predysfunctional', 'dysfunctional', 'activation'], rotation=90)
ax.get_legend().remove()
ax.set_xlim([-0.5,4.5])
ax.set_ylim([-0.5,2.5])
ax.grid(None)
plt.savefig('./figures_paper/dotplot_scores_absolute.svg', dpi=300, bbox_inches='tight')
plt.show()
```



```
In [11]: t=sc.pl.dotplot(adata, var_names=['TCF7', 'CCR7', 'SELL', 'LEF1', 'IL7R',
                                         'KLRG1', 'PRF1', 'FCGR3A', 'CX3CR1',
                                         'EOMES', 'GZMK', 'CXCR3',
                                         'LAG3', 'PDCD1', 'LAYN', 'HAVCR2', 'CTLA4',
                                         'TNFRSF9', 'IFNG', 'TNF', 'GZMB', 'PRF1', 'NKG7', 'XCL1', 'XCL2', 'CCL3', 'CCL4',
                                         'CCL5', 'CST7', 'STAT1', 'TGFB1'],
                     var_group_positions=[(0,4), (5,8), (9,11), (12,16), (17,30)],
                     var_group_labels=['naive like', 'cytotoxic', 'predysfunctional', 'dysfunctional', 'activation'],
                     groupby='samplename',
                     standard_scale='var', var_group_rotation=0, show=False)
plt.savefig('./figures_paper/dotplot_score_genes.svg', dpi=300, bbox_inches='tight')
```

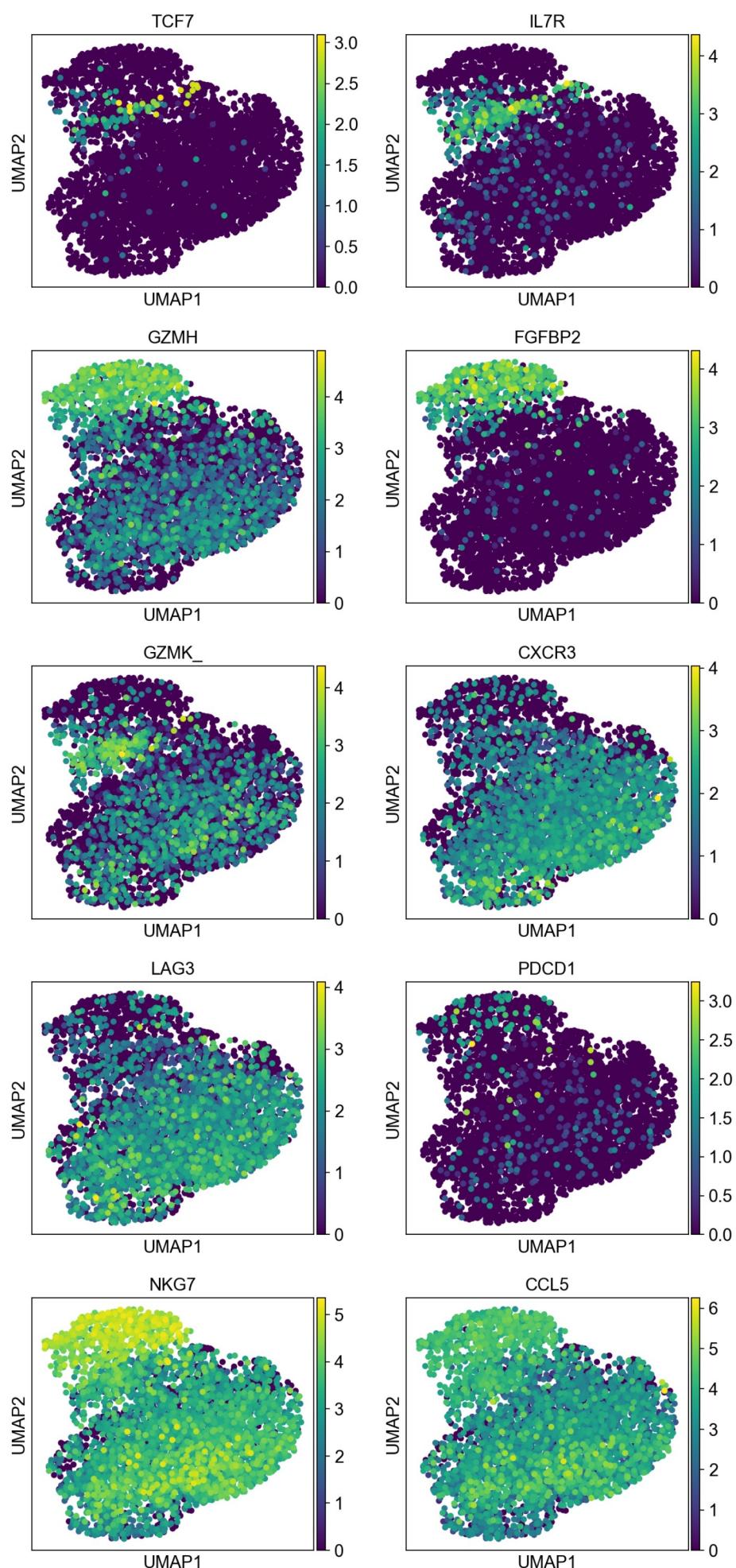


```
In [12]: sc.pl.umap(adata, color=['naive_like_state', 'cytotoxic_state', 'predysfunctional_state', 'dysfunctional_state', 'activation_score'], ncols=5, s=100, vmax=[2,2,1.5,1,1.5], vmin=0, show=False, color_map='viridis')
plt.savefig('./figures_paper/feature_plot_scores.svg', dpi=300, bbox_inches='tight')
```

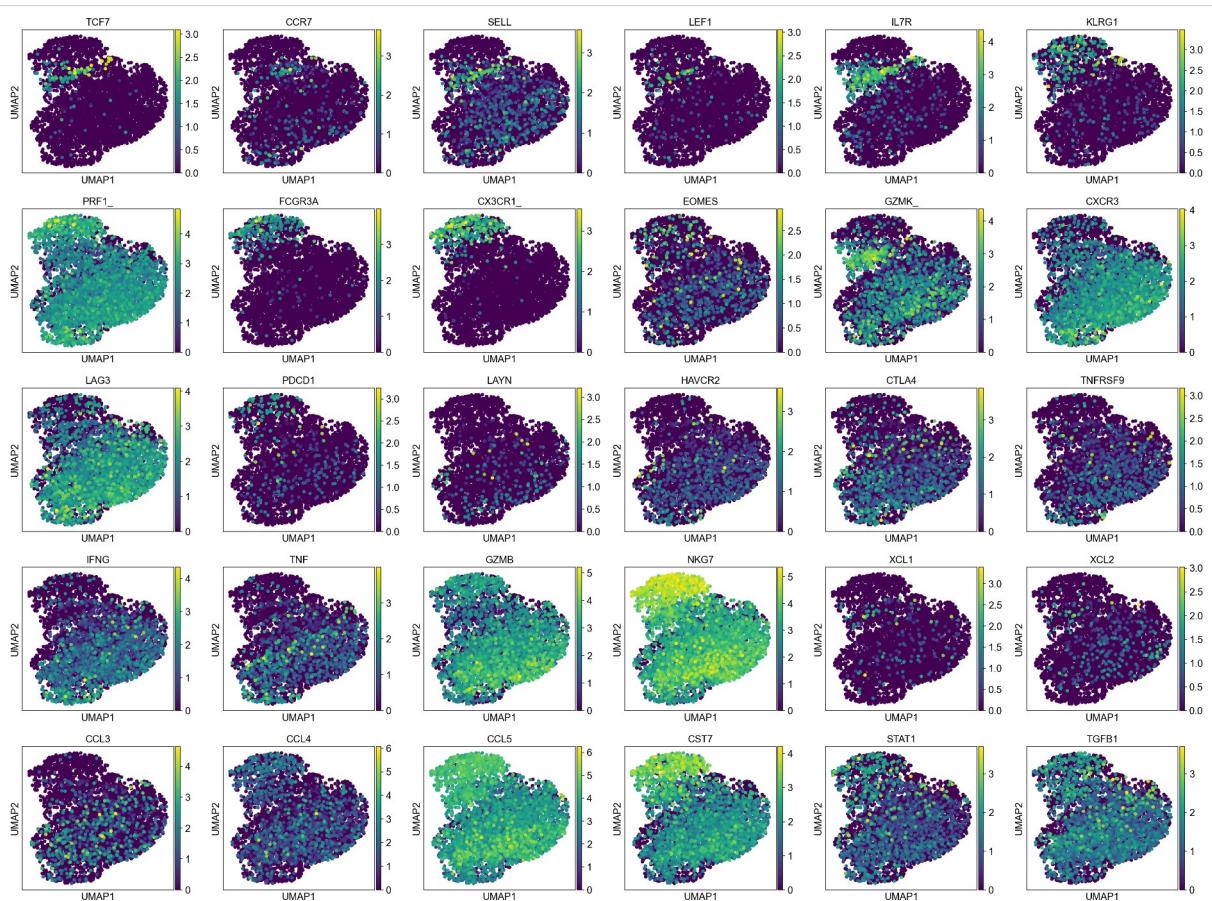


```
In [13]: test=adata.raw  
adata.obs['CX3CR1_']=[x[0] for x in test[:,test.var_names=='CX3CR1'].X.todense().tolist()]  
adata.obs['GZMK_']=[x[0] for x in test[:,test.var_names=='GZMK'].X.todense().tolist()]  
adata.obs['PRF1_']=[x[0] for x in test[:,test.var_names=='PRF1'].X.todense().tolist()]
```

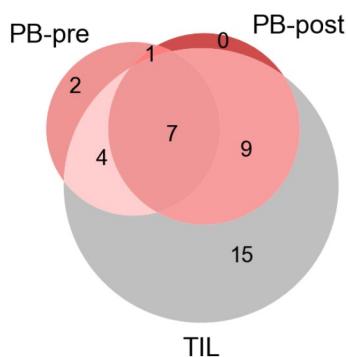
```
In [14]: sc.pl.umap(adata, color=['TCF7', 'IL7R', 'GZMH', 'FGFBP2', 'GZMK_', 'CXCR3', 'LAG3', 'PDCD1', 'NKG7', 'CCL5'], n  
ols=2, s=100, show=False, color_map='viridis')  
plt.savefig('./figures_paper/feature_plot_score_genes.svg', dpi=300, bbox_inches='tight')
```



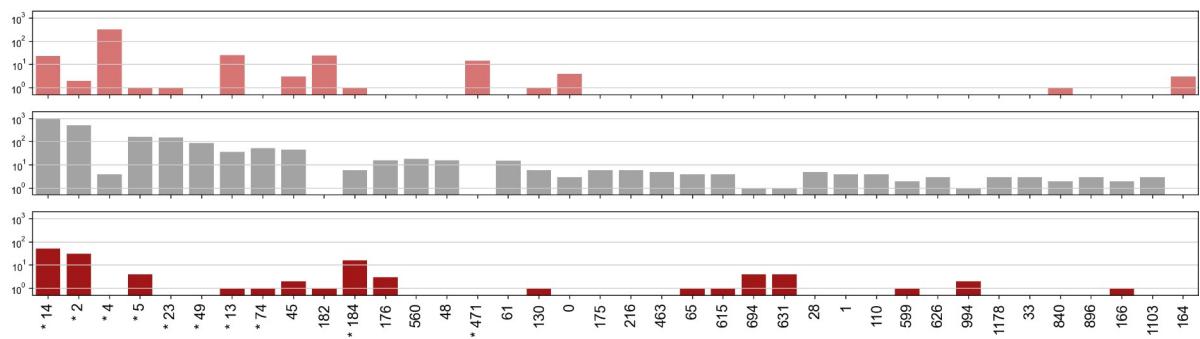
```
In [15]: sc.pl.umap(adata, color=['TCF7', 'CCR7', 'SELL', 'LEF1', 'IL7R',
                                'KLRG1', 'PRF1', 'FCGR3A', 'CX3CR1',
                                'EOMES', 'GZMK', 'CXCR3',
                                'LAG3', 'PDCD1', 'LAYN', 'HAVCR2', 'CTLA4',
                                'TNFRSF9', 'IFNG', 'TNF', 'GZMB', 'NKG7', 'XCL1', 'XCL2', 'CCL3', 'CCL4', 'CCL5',
                                'CST7', 'STAT1', 'TGFB1'], ncols=6, s=100, show=False)
plt.savefig('./figures_paper/feature_plot_score_genes.svg', dpi=300, bbox_inches='tight')
```



```
In [16]: list_pb_pre=adata.obs[(adata.obs.samplename=='PB-pre') & (adata.obs.clonotype_size>2)].clonotype.unique().tolist()
list_pb_post=adata.obs[(adata.obs.samplename=='PB-post') & (adata.obs.clonotype_size>2)].clonotype.unique().tolist()
list_TIL=adata.obs[(adata.obs.samplename=='TIL') & (adata.obs.clonotype_size>2)].clonotype.unique().tolist()
venn3([set(list_pb_pre), set(list_pb_post), set(list_TIL)], set_labels=('PB-pre', 'PB-post', 'TIL'), set_colors=[ '#e86464', '#b80000', '#a3a3a3'], alpha=0.7)
plt.savefig('./figures_paper/cloneotype_venn.svg', dpi=300, bbox_inches='tight')
```



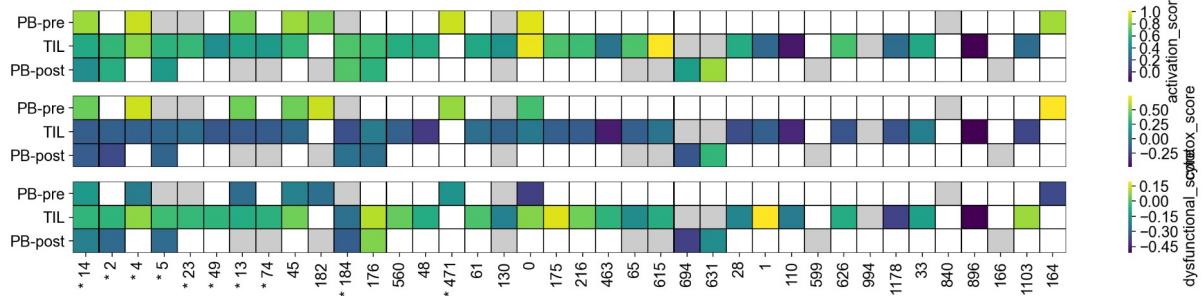
```
In [17]: fig, ax=plt.subplots(nrows=3, figsize=(20,5), sharex=True)
data=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby(['clonotype','samplename'], sort=False).agg({'clonotype_size':'count', 'clonotype':'first', 'samplename':'first'})
sns.barplot(data=data, x='clonotype', y='clonotype_size', ax=ax[0], hue='samplename', hue_order=['PB-pre'], palette=['#e86464'])
sns.barplot(data=data, x='clonotype', y='clonotype_size', ax=ax[1], hue='samplename', hue_order=['TIL'], palette=['#a3a3a3'])
sns.barplot(data=data, x='clonotype', y='clonotype_size', ax=ax[2], hue='samplename', hue_order=['PB-post'], palette=['#b80000'])
for ax in ax:
    ax.set_yscale('log')
    ax.set_xlabel('')
    ax.set_ylabel('')
    ax.get_legend().remove()
    ax.set_ylim([0.5,2000])
    ax.set_yticks([1,10,100,1000])
    ax.tick_params(axis='y', which='major', labelsize=9)
ax.set_xticklabels(['*' + x if x in clonotypes_re_expression else x for x in data.index.get_level_values(0).categories.tolist()], rotation=90)
fig.savefig('./figures_paper/clonotype_size_samplename.svg', dpi=300, bbox_inches='tight')
plt.show()
```



```
In [18]: mask=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby('clonotype', sort=False).agg({'size_Infusion_product':'mean','size_Blood_before':'mean', 'size_Blood_after':'mean'}).T==0
mask=mask.loc[['size_Blood_before','size_Infusion_product','size_Blood_after'],mask.columns.isin(clonotypes_greater_2)].values
fig,ax=plt.subplots(nrows=3, figsize=(20,4))
for i, measure in enumerate(['activation_score','cytotox_score', 'dysfunctional_score']):
    sizetable=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby('clonotype', sort=False).agg({'size_Infusion_product':'mean','size_Blood_before':'mean', 'size_Blood_after':'mean'}).T
    mask2=~((sizetable<3)&(sizetable!=0))
    mask2=mask2.loc[['size_Blood_before','size_Infusion_product','size_Blood_after'],mask2.columns.isin(clonotypes_greater_2)].values
    df=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby('clonotype', sort=False).agg({measure+'_Blood_before':'mean',measure+'_Infusion_product':'mean', measure+'_Blood_after':'mean'})
    df=df.T
    df=df.loc[:,df.columns.isin(clonotypes_greater_2)]
    max_value=df.values.flatten()[(sizetable.loc[['size_Blood_before','size_Infusion_product','size_Blood_after']],sizetable.columns.isin(clonotypes_greater_2))>2].values.flatten().max()
    min_value=df.values.flatten()[(sizetable.loc[['size_Blood_before','size_Infusion_product','size_Blood_after']],sizetable.columns.isin(clonotypes_greater_2))>2].values.flatten().min()
    sns.heatmap(df.values.tolist(), ax=ax[i], cmap='viridis', cbar_kws={'label': measure}, mask=mask,
                linewidths=.5, linecolor='black', vmin=min_value, vmax=max_value)
    #mark sizes<3 in gray
    df2=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby('clonotype', sort=False).agg({'size_Blood_before':'mean','size_Infusion_product':'mean', 'size_Blood_after':'mean'})
    df2=df2.T
    df2=df2.loc[:,df2.columns.isin(clonotypes_greater_2)]
    sns.heatmap(df2.values.tolist(), ax=ax[i], cmap='gray', mask=mask2,
                linewidths=.5, linecolor='black', vmin=-3, vmax=2, cbar=False)

    ax[i].set_yticklabels(['PB-pre', 'TIL', 'PB-post'], rotation=0)
    ax[i].set_xticklabels('')
    ax[i-1].set_xticks([])
    ax[i].set_xticklabels(['*' +x if x in clonotypes_re_expression else x for x in df.columns], rotation=90)
plt.show()

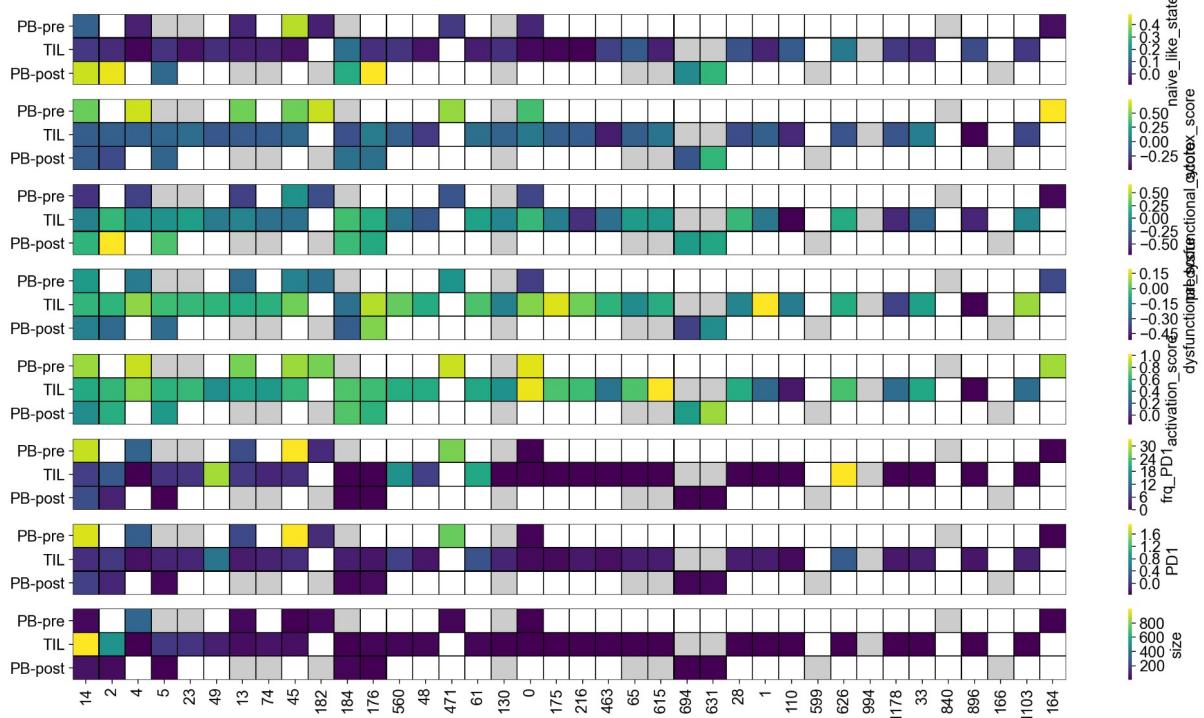
fig.savefig('./figures_paper/clonotypes_scores.svg', bbox_inches='tight')
plt.show()
```



```
In [19]: mask=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby('clonotype', sort=False).agg({'size_Infusion_product':'mean','size_Blood_before':'mean', 'size_Blood_after':'mean'}).T==0
mask=mask.loc[['size_Blood_before','size_Infusion_product','size_Blood_after'],mask.columns.isin(clonotypes_greater_2)].values
fig,ax=plt.subplots(nrows=8, figsize=(20,11))
for i, measure in enumerate(['naive_like_state','cytotox_score','predysfunctional_score', 'dysfunctional_score','activation_score','frq_PD1','PD1','size']):
    sizetable=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby('clonotype', sort=False).agg({'size_Infusion_product':'mean','size_Blood_before':'mean', 'size_Blood_after':'mean'}).T
    mask2=~((sizetable<3)&(sizetable!=0))
    mask2=mask2.loc[['size_Blood_before','size_Infusion_product','size_Blood_after'],mask2.columns.isin(clonotypes_greater_2)].values
    df=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby('clonotype', sort=False).agg({measure+'_Blood_before':'mean',measure+'_Infusion_product':'mean', measure+'_Blood_after':'mean'})
    df=df.T
    df=df.loc[:,df.columns.isin(clonotypes_greater_2)]
    max_value=df.values.flatten()[(sizetable.loc[['size_Blood_before','size_Infusion_product','size_Blood_after']],sizetable.columns.isin(clonotypes_greater_2))>2].values.flatten().max()
    min_value=df.values.flatten()[(sizetable.loc[['size_Blood_before','size_Infusion_product','size_Blood_after']],sizetable.columns.isin(clonotypes_greater_2))>2].values.flatten().min()
    sns.heatmap(df.values.tolist(), ax=ax[i], cmap='viridis', cbar_kws={'label': measure}, mask=mask,
                linewidths=.5, linecolor='black', vmin=min_value, vmax=max_value)
    #mark sizes<3 in gray
    df2=adata.obs[adata.obs.clonotype_size>2].sort_values('clonotype_size', ascending=False).groupby('clonotype', sort=False).agg({'size_Blood_before':'mean','size_Infusion_product':'mean', 'size_Blood_after':'mean'})
    df2=df2.T
    df2=df2.loc[:,df2.columns.isin(clonotypes_greater_2)]
    sns.heatmap(df2.values.tolist(), ax=ax[i], cmap='gray', mask=mask2,
                linewidths=.5, linecolor='black', vmin=-3, vmax=2, cbar=False)

    ax[i].set_yticklabels(['PB-pre', 'TIL', 'PB-post'], rotation=0)
    ax[i].set_xticklabels([''], '')
    ax[i-1].set_xticks([])
    ax[i].set_xticklabels([x for x in df.columns], rotation=90)
plt.show()

fig.savefig('./figures_paper/clonotypes_scores_supp.svg', bbox_inches='tight')
plt.show()
```



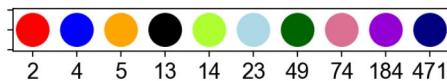
Correlations with functional data

```
In [20]: df_correlation=adata.obs[adata.obs.clonotype.isin(clonotypes_re_expression)]
df_correlation=df_correlation.groupby('clonotype').agg('mean')
df_correlation=df_correlation[df_correlation.index.isin(clonotypes_re_expression)]

FACS_values=pd.read_csv('FACS_mean_values_new.csv', sep=';', decimal=',', index_col=0)
interesting_clonotypes_colors=['red','blue','orange','black','greenyellow','lightblue', 'darkgreen', 'purple', 'darkvioletred', 'darkviolet','navy']

for valuename in FACS_values.columns.tolist():
    df_correlation[valuename]=FACS_values[valuename].tolist()
df_correlation['clonotype']=df_correlation.reset_index().clonotype.values.tolist()
df_correlation['clonotype']="TCR_"+df_correlation.clonotype
df_correlation.rename(columns={'cytotoxic_state':'cytotox_score',
                               'predysfunctional_state':'predysfunctional_score',
                               'dysfunctional_state':'dysfunctional_score'}, inplace=True)
```

```
In [21]: fig,ax=plt.subplots(figsize=(5,.5))
sns.scatterplot(x=df_correlation.index.tolist(), y=[0]*df_correlation.index.shape[0], hue=df_correlation.clonotype.tolist(),
                 legend=None, palette=interesting_clonotypes_colors, s=500)
ax.grid(None)
ax.set_yticklabels('')
plt.show()
```



```
In [22]: #remove values smaller than 3 from sizes
for size in ['size_Infusion_product', 'size_Blood_before','size_Blood_after']:
    df_correlation[size][df_correlation[size]<2]=0
```

```
In [23]: samples=['Blood_before', 'Infusion_product', 'Blood_after']
```

```
In [24]: def convert_significance(p):
    sig=', n.s.'
    if p < 0.05:
        sig='*'
    if p < 0.01:
        sig='**'
    if p < 0.001:
        sig='***'
    if p < 0.0001:
        sig='****'
    return sig
```

```
In [25]: measures=['naive_like_state','cytotox_score','predysfunctional_score', 'dysfunctional_score', 'activation_score',
               'frq_PD1','PD1', 'size', 'TRA_expr', 'TRB_expr', 'TCR_expr']
correlations_PB=pd.DataFrame(index=measures, columns=['EC50_IFNg','EC50_IL2','EC50_TNFa'])
correlations_PB_p=correlations_PB.copy()
correlations_TIL=pd.DataFrame(index=measures, columns=['EC50_IFNg','EC50_IL2','EC50_TNFa'])
correlations_TIL_p=correlations_TIL.copy()
```

```
In [26]: old_samplenames=['Infusion_product', 'Blood_before', 'Blood_after']
for samplename in old_samplenames:
    df_correlation['TRA_expr_'+samplename]=0
    df_correlation['TRB_expr_'+samplename]=0
    df_correlation['TCR_expr_'+samplename]=0

for i, clonotype in enumerate([x[4:] for x in df_correlation.clonotype.unique()]):
    for j, samplename in enumerate(adata.obs.samplename.unique().tolist()):
        df_temp1=pd.DataFrame(adata[np.logical_and(adata.obs.clonotype==clonotype, adata.obs.samplename==samplename)].obs.TRA_1_expr, columns=['TRA_1_expr'])
        if df_temp1.shape[0]!=0:
            df_correlation['TRA_expr_'+old_samplenames[j]][df_correlation.clonotype=='TCR_'+clonotype]=df_temp1.TRA_1_expr.mean()

        df_temp2=pd.DataFrame(adata[np.logical_and(adata.obs.clonotype==clonotype, adata.obs.samplename==samplename)].obs.TRB_1_expr, columns=['TRB_1_expr'])
        if df_temp2.shape[0]!=0:
            df_correlation['TRB_expr_'+old_samplenames[j]][df_correlation.clonotype=='TCR_'+clonotype]=df_temp2.TRB_1_expr.mean()

        df_temp=pd.DataFrame(df_temp1['TRA_1_expr']+df_temp2['TRB_1_expr'], columns=['TCR_expr'])
        if df_temp.shape[0]!=0:
            df_correlation['TCR_expr_'+old_samplenames[j]][df_correlation.clonotype=='TCR_'+clonotype]=df_temp.TCR_expr.mean()

print(str(np.round(((i+1)/len(df_correlation.clonotype.unique()))*100,1))+ "%", end="\r", flush=True)
```

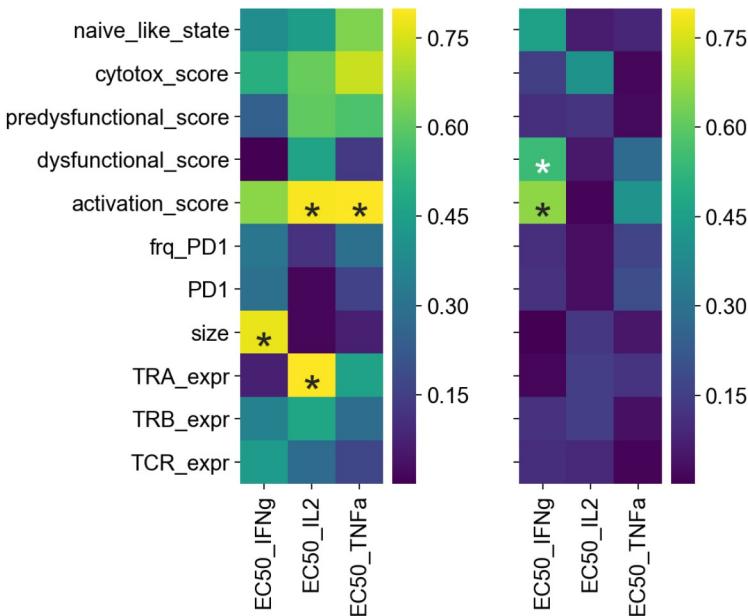
100.0%

```
In [27]: for i, cytokine in enumerate(['EC50_IFNg_pool', 'EC50_IL2_pool', 'EC50_TNFa_pool']):
    for j, measure in enumerate(measures):
        mask = ~np.isnan(df_correlation[cytokine]) & ~ (df_correlation['size_Blood_before']==0)
        slope, intercept, r_value, p_value, std_err = stats.linregress(df_correlation[cytokine][mask], df_correlation[measure+'_Blood_before'][mask])
        correlations_PB.loc[measure, cytokine[:-5]]=r_value**2
        correlations_PB_p.loc[measure, cytokine[:-5]]=convert_significance(p_value)

        mask = ~np.isnan(df_correlation[cytokine]) & ~ (df_correlation['size_Infusion_product']==0)
        slope, intercept, r_value, p_value, std_err = stats.linregress(df_correlation[cytokine][mask], df_correlation[measure+'_Infusion_product'][mask])
        correlations_TIL.loc[measure, cytokine[:-5]]=r_value**2
        correlations_TIL_p.loc[measure, cytokine[:-5]]=convert_significance(p_value)
```

```
In [28]: correlations_TIL_p[correlations_TIL_p=='', n.s.]=''
correlations_PB_p[correlations_PB_p=='', n.s.]=''
```

```
In [29]: fig,ax=plt.subplots(ncols=2, figsize=(5,5))
fig.tight_layout(w_pad=2)
sns.heatmap(correlations_PB.astype(float), cmap='viridis', ax=ax[0], vmax=.8, annot=correlations_PB_p, fmt = '', annot_kws={'size':30, 'va':'top'})
sns.heatmap(correlations_TIL.astype(float), cmap='viridis', ax=ax[1], vmax=.8, annot=correlations_TIL_p, fmt = '', annot_kws={'size':30, 'va':'top'})
ax[1].set_yticklabels([''])
fig.savefig('./figures_paper/heatmap_correlation.svg', bbox_inches='tight')
```

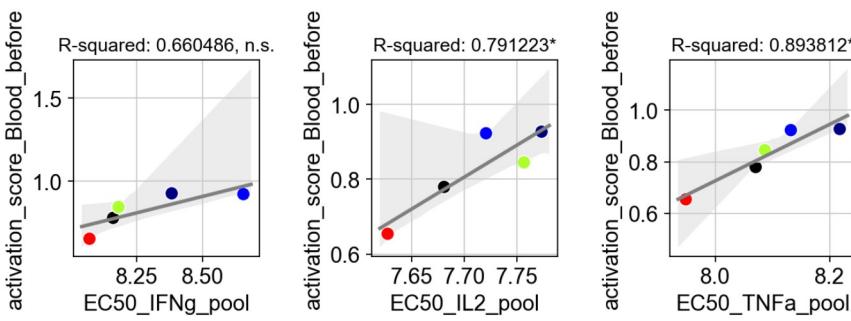


```
In [30]: value_pairs_PB=[['EC50_IFNg','activation_score_Blood_before'],
                     ['EC50_IL2','activation_score_Blood_before'],
                     ['EC50_TNFa','activation_score_Blood_before']]

value_pairs_TIL=[['EC50_IFNg','activation_score_Infusion_product'],
                 ['EC50_IFNg','dysfunctional_score_Infusion_product']]

In [31]: df_correlation['colors']=interesting_clonotypes_colors

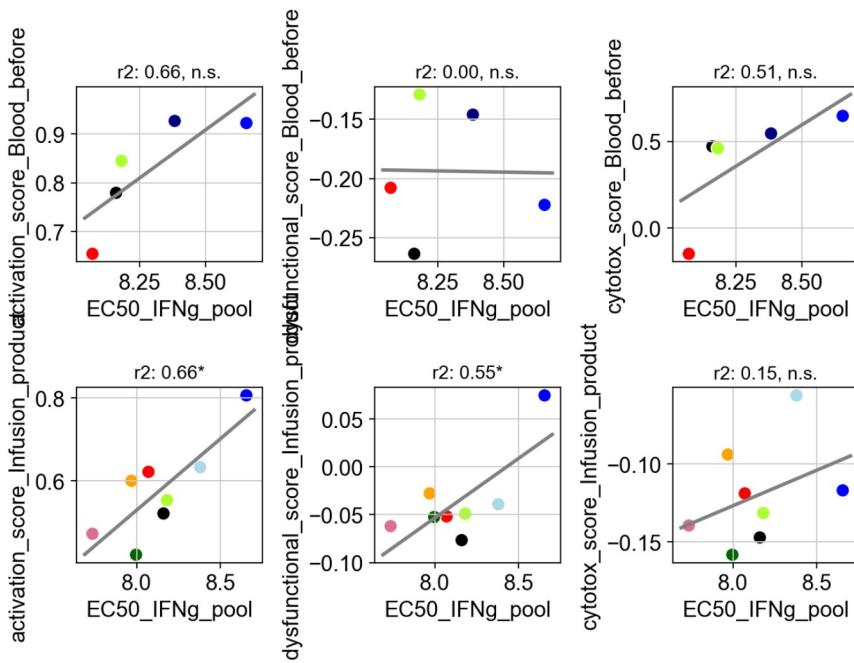
In [32]: fig,ax=plt.subplots(ncols=3, figsize=(8,2.5))
fig.tight_layout(w_pad=2)
colors=interesting_clonotypes_colors
samplename='PB-pre'
for i, pair in enumerate(value_pairs_PB):
    mask = ~np.isnan(df_correlation[pair[0]+'_pool']) & ~df_correlation['size_Blood_before']==0
    sns.regplot(df_correlation[mask][pair[0]+'_pool'], df_correlation[mask][pair[1]], ax=ax[i], color='gray', ci=95)
    sns.scatterplot(df_correlation[mask][pair[0]+'_pool'], df_correlation[mask][pair[1]], hue=df_correlation[mask].clonotype, palette=df_correlation[mask].colors, legend=None, ax=ax[i], s=75)
    slope, intercept, r_value, p_value, std_err = stats.linregress(df_correlation[pair[0]+'_pool'][mask], df_correlation[pair[1]][mask])
    ax[i].set_title(label=("R-squared: %f" % r_value**2)+convert_significance(p_value), size=12)
fig.savefig('./figures_paper/correlation_examples.svg', bbox_inches='tight')
```



```
In [33]: value_pairs_PB=[['EC50_IFNg','activation_score_Blood_before'],
                     ['EC50_IFNg','dysfunctional_score_Blood_before'],
                     ['EC50_IFNg','cytotox_score_Blood_before']]

value_pairs_TIL=[['EC50_IFNg','activation_score_Infusion_product'],
                 ['EC50_IFNg','dysfunctional_score_Infusion_product'],
                 ['EC50_IFNg','cytotox_score_Infusion_product']]
```

```
In [34]: fig,ax=plt.subplots(nrows=2, ncols=3, figsize=(8,5))
fig.tight_layout(w_pad=2, h_pad=4)
colors=interesting_clonotypes_colors
samplename='PB-pre'
for i, pair in enumerate(value_pairs_PB):
    mask = ~np.isnan(df_correlation[pair[0]+'_pool']) & (df_correlation['size_Blood_before']>=2)
    sns.regplot(df_correlation[mask][pair[0]+'_pool'], df_correlation[mask][pair[1]], ax=ax[0,i], color='gray', ci=0, line_kws={'linestyle': '-'})
    sns.scatterplot(df_correlation[mask][pair[0]+'_pool'], df_correlation[mask][pair[1]], hue=df_correlation[mask].clonotype, palette=df_correlation[mask].colors, legend=None, ax=ax[0,i], s=75)
    slope, intercept, r_value, p_value, std_err = stats.linregress(df_correlation[pair[0]+'_pool'][mask], df_correlation[pair[1]][mask])
    ax[0,i].set_title(label=("r2: %.2f" % r_value**2)+convert_significance(p_value), size=12)
for i, pair in enumerate(value_pairs_TIL):
    mask = ~np.isnan(df_correlation[pair[0]+'_pool']) & (df_correlation['size_Infusion_product']>=2)
    sns.regplot(df_correlation[mask][pair[0]+'_pool'], df_correlation[mask][pair[1]], ax=ax[1,i], color='gray', ci=0, line_kws={'linestyle': '-'})
    sns.scatterplot(df_correlation[mask][pair[0]+'_pool'], df_correlation[mask][pair[1]], hue=df_correlation[mask].clonotype, palette=df_correlation[mask].colors, legend=None, ax=ax[1,i], s=75)
    slope, intercept, r_value, p_value, std_err = stats.linregress(df_correlation[pair[0]+'_pool'][mask], df_correlation[pair[1]][mask])
    ax[1,i].set_title(label=("r2: %.2f" % r_value**2)+convert_significance(p_value), size=12)
fig.savefig('./figures_paper/correlation_examples.svg', bbox_inches='tight')
```



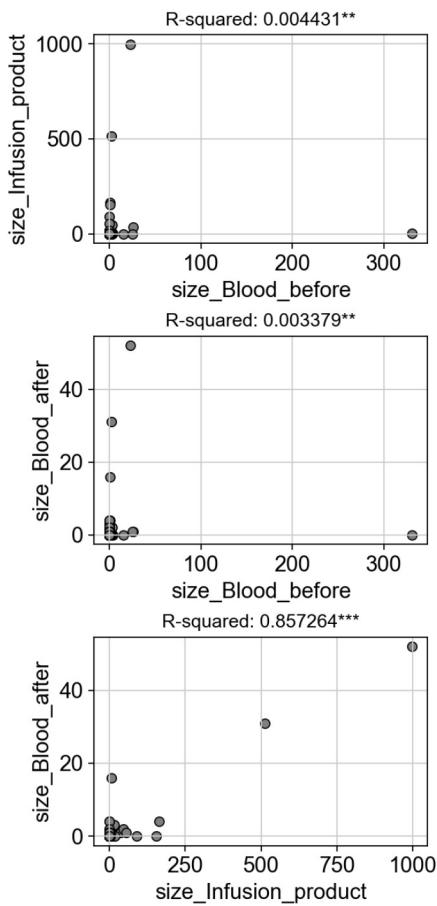
```
In [35]: data=adata.obs.groupby('clonotype').agg({'size_Blood_before':'mean',
                                              'size_Infusion_product':'mean',
                                              'size_Blood_after':'mean'})
```

```
In [36]: fig, ax = plt.subplots(nrows=3, figsize=(4,8))
fig.tight_layout(h_pad=2.5)
sns.scatterplot(data=data, x='size_Blood_before', y='size_Infusion_product', edgecolor='black', color='gray', ax=ax[0])
slope, intercept, r_value, p_value, std_err = stats.linregress(data['size_Blood_before'], data['size_Infusion_product'])
ax[0].set_title(label=("R-squared: %f" % r_value**2)+convert_significance(p_value), size=12)

sns.scatterplot(data=data, x='size_Blood_before', y='size_Blood_after', edgecolor='black', color='gray', ax=ax[1])
slope, intercept, r_value, p_value, std_err = stats.linregress(data['size_Blood_before'], data['size_Blood_after'])
ax[1].set_title(label=("R-squared: %f" % r_value**2)+convert_significance(p_value), size=12)

sns.scatterplot(data=data, x='size_Infusion_product', y='size_Blood_after', edgecolor='black', color='gray', ax=ax[2])
slope, intercept, r_value, p_value, std_err = stats.linregress(data['size_Infusion_product'], data['size_Blood_after'])
ax[2].set_title(label=("R-squared: %f" % r_value**2)+convert_significance(p_value), size=12)

fig.savefig('./figures_paper/size_correlations.svg', bbox_inches='tight')
```

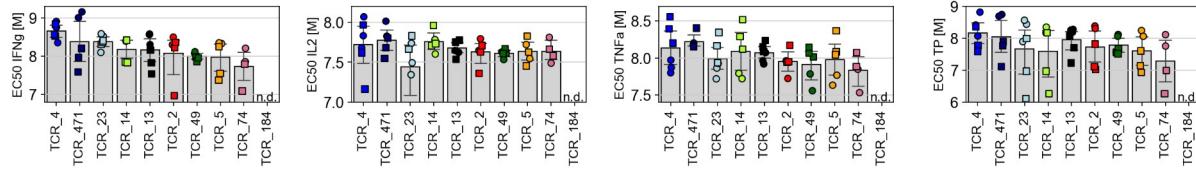


```
In [37]: ICCS_data=pd.read_csv('values_ICCS_pooled_TP.csv', sep=';', decimal=',')
```

```
In [38]: ICCS_data['experiment']='none'
ICCS_data['experiment'][ICCS_data.Replicate.isin(['Rep1','Rep2','Rep3'])]='Exp 1'
ICCS_data['experiment'][ICCS_data.Replicate.isin(['Rep4','Rep5','Rep6'])]='Exp 2'
```

```
In [39]: ICCS_data.TCR=ICCS_data.TCR.str.replace(' ', '_')
```

```
In [40]: fig,axes=plt.subplots(ncols=4, figsize=(16,2))
fig.tight_layout(w_pad=2)
for i, cytokine in enumerate(['IFNg','IL2','TNFa','TP']):
    ax=axes[i]
    sns.barplot(data=ICCS_data[ICCS_data.Cytokine==cytokine], x='TCR',y='EC50',ax=ax, palette=['lightgray'],
                 order=df_correlation.sort_values('EC50_IFNg_pool', ascending=False) ['clonotype'].tolist(),
                 edgecolor='black',
                 linewidth=1,errwidth=1, capsize=.5)
    sns.stripplot(data=ICCS_data[(ICCS_data.Cytokine==cytokine) & (ICCS_data.experiment=='Exp 1')], x='TCR',
                  y='EC50',ax=ax,
                  edgecolor='black', linewidth=1, marker='o',
                  palette=df_correlation.sort_values('EC50_IFNg_pool', ascending=False) ['colors'], s=6,
                  order=df_correlation.sort_values('EC50_IFNg_pool', ascending=False) ['clonotype'].tolist())
    sns.stripplot(data=ICCS_data[(ICCS_data.Cytokine==cytokine) & (ICCS_data.experiment=='Exp 2')], x='TCR',
                  y='EC50',ax=ax,
                  edgecolor='black', linewidth=1, marker='s',
                  palette=df_correlation.sort_values('EC50_IFNg_pool', ascending=False) ['colors'], s=6,
                  order=df_correlation.sort_values('EC50_IFNg_pool', ascending=False) ['clonotype'].tolist())
    ax.set_xticklabels(ax.get_xticklabels(), size=12, rotation=90)
    ax.set_xlabel('')
    ax.text(0.9,0.015,'n.d.', transform=ax.transAxes, size=12)
    axes[0].set_ylim(6.8,9.3)
    axes[0].set_ylabel('EC50 IFNg [M]', size=12)
    axes[1].set_ylim(7,8.2)
    axes[1].set_ylabel('EC50 IL2 [M]', size=12)
    axes[2].set_ylim(7.4,8.7)
    axes[2].set_ylabel('EC50 TNFa [M]', size=12)
    axes[3].set_ylim(6,9)
    axes[3].set_ylabel('EC50 TP [M]', size=12)
    plt.savefig('./figures_paper/EC50_IFNg.svg', bbox_inches='tight')
    plt.show()
```



Genes correlating with TCR functionality

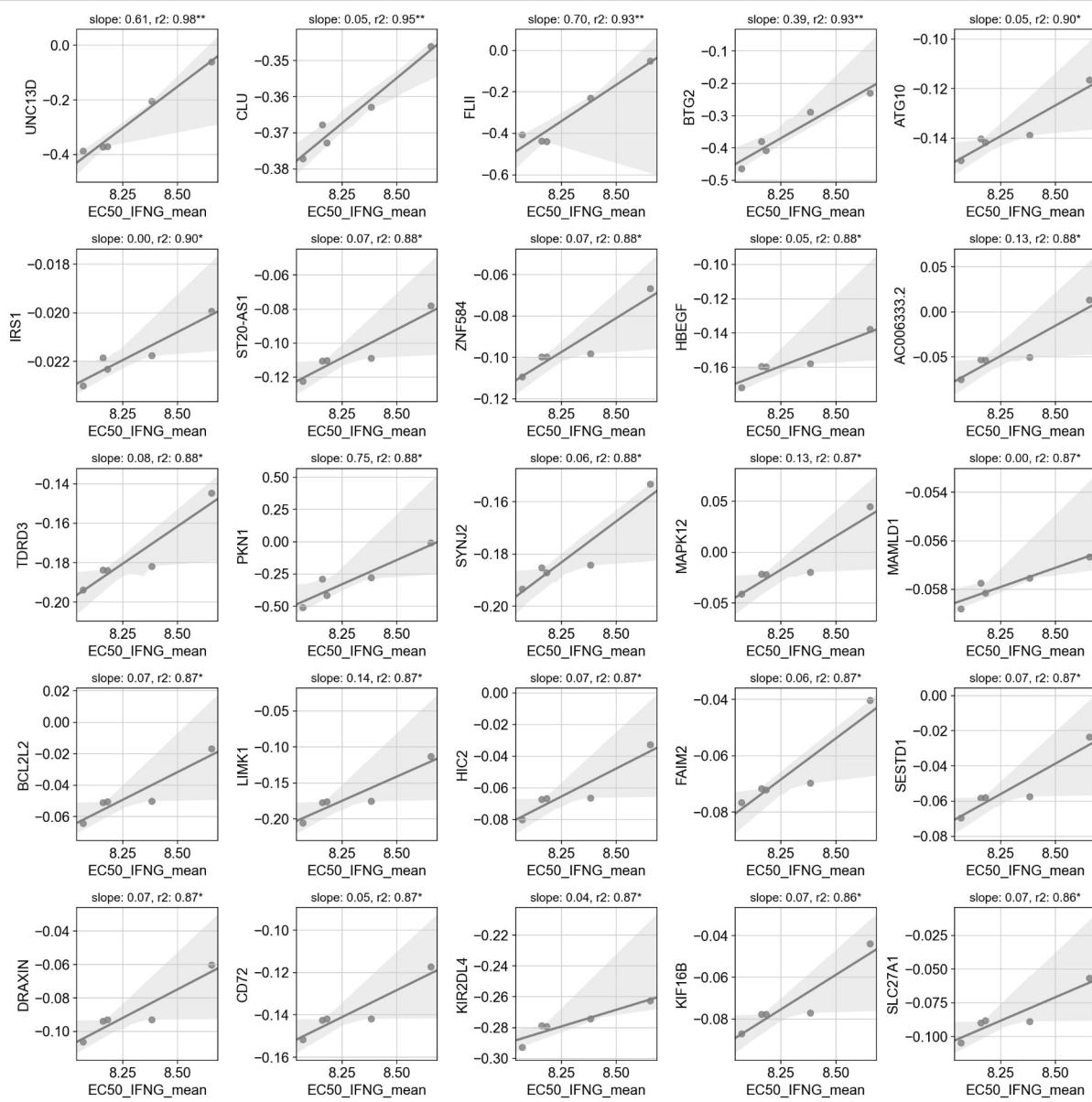
```
In [41]: ICCS_data['clonotype']=[x[4:] for x in ICCS_data.TCR]
adata_selected=adata[np.logical_and(adata.obs.clonotype.isin(['2','4','13','14','471']),adata.obs.sample.name=='PB-pre')]
adata_selected.obs['functionalities']=0
adata_selected.obs['functionalities'][adata_selected.obs.clonotype.isin(['4','23','471'])]='high'
adata_selected.obs['functionalities'][adata_selected.obs.clonotype.isin(['74'])]='low'
adata_selected.obs['functionalities'][adata_selected.obs.clonotype.isin(['5','2','49','13','14'])]='intermediate'
adata_selected.obs['functionalities'][adata_selected.obs.clonotype.isin(['184'])]='non-functional'
adata_selected.obs['EC50_IFNG_mean']=0
for clonotype in adata_selected.obs.clonotype.unique():
    adata_selected.obs['EC50_IFNG_mean'][adata_selected.obs.clonotype==clonotype]=df_correlation.loc[clonotype,'EC50_IFNG_pool']
sc.pp.highly_variable_genes(adata_selected)
```

Trying to set attribute `obs` of view, copying.

```
In [42]: generate_corr_data=True
if generate_corr_data==True:
    correlation_data=pd.DataFrame(index=adata_selected.var_names.tolist(), columns=['slope','intercept',
    'r_value','p_value','std_err'])
    for i, gene in enumerate(adata_selected.var_names.tolist()):
        adata_selected.obs[gene]=[x[0] for x in adata_selected[:,adata_selected.var_names==gene].X]
        data=adata_selected.obs.groupby('clonotype').agg({gene:'mean','EC50_IFNG_mean':'mean'})
        correlation_data.loc[gene] = stats.linregress(data['EC50_IFNG_mean'], data[gene])
        print(str(np.round(((i+1)/len(adata_selected.var_names.tolist()))*100,1))+' %',end="\r", flush=True)
    correlation_data['r_squared']=correlation_data['r_value']**2
    correlation_data.sort_values('slope', ascending=False).to_excel('correlating_genes_IFNG.xls')
    correlation_data[(correlation_data.p_value<0.05)].sort_values('slope', ascending=False).to_excel('correlating_genes_IFNG_significant.xls')
if generate_corr_data==False:
    correlation_data=pd.read_excel('correlating_genes_IFNG.xls', index_col=0)
```

100.0 %

```
In [43]: fig, ax=plt.subplots(ncols=5, nrows=5, figsize=(15,15))
fig.tight_layout(w_pad=3, h_pad=3)
for i, gene in enumerate(correlation_data.sort_values('r_value', ascending=False).index.tolist()[:25]):
    data=adata_selected.obs.groupby('clonotype').agg({gene:'mean','EC50_IFNG_mean':'mean'})
    sns.regplot(data=data, x='EC50_IFNG_mean',y=gene, color='gray', marker='o', ax=ax.reshape(-1)[i])
    ax.reshape(-1)[i].set_title('slope: %.2f' % correlation_data.loc[gene,'slope']+r2: %.2f' % correlation_data.loc[gene,'r_squared']+convert_significance(correlation_data.loc[gene,'p_value']), size=12)
```



```
In [44]: cytotox_genes=['KLRLG1', 'PRF1', 'FCGR3A', 'CX3CR1']
dysfunctional_genes=['LAG3', 'PDCD1', 'LAYN', 'HAVCR2', 'CTLA4']
activation_genes=['TNFRSF9', 'IFNG', 'TNF', 'GZMB', 'PRF1', 'NKG7', 'XCL1', 'XCL2', 'CCL3', 'CCL4', 'CCL5', 'CST7',
'STAT1', 'TGFB1']
```

```
In [45]: plot_data=correlation_data.sort_values('r_value', ascending=False)
plot_data=plot_data[plot_data.p_value<0.05]
plot_data['gene']=plot_data.index.tolist()
plot_data['color']='gray'
plot_data['color'][plot_data.gene.isin(cytotox_genes)]= 'red'
plot_data['color'][plot_data.gene.isin(dysfunctional_genes)]= 'black'
plot_data['color'][plot_data.gene.isin(['TCF7', 'CCR7', 'SELL', 'LEF1', 'IL7R',
'KLRLG1', 'PRF1', 'FCGR3A', 'CX3CR1',
'EOMES', 'GZMK', 'CXCR3',
'LAG3', 'PDCD1', 'LAYN', 'HAVCR2', 'CTLA4',
'TNFRSF9', 'IFNG', 'TNF', 'GZMB', 'PRF1', 'NKG7', 'XCL1', 'XCL2', 'CCL3', 'CCL4', 'CCL5', 'CST7', 'STAT1', 'TGFB1'])]= 'orange'
```

```
In [46]: from matplotlib.patches import Rectangle
fig, ax = plt.subplots(1, 2, gridspec_kw={'width_ratios': [2, 1]}, figsize=(15,3))
s=pd.Series(plot_data['r_value'], index=plot_data['gene'])
s.plot(kind='bar', ax=ax[1], color=plot_data.color.tolist(), width=1, grid=False)
s[:50].plot(kind='bar', ax=ax[0], color=plot_data.color.tolist(), width=.8, grid=False)

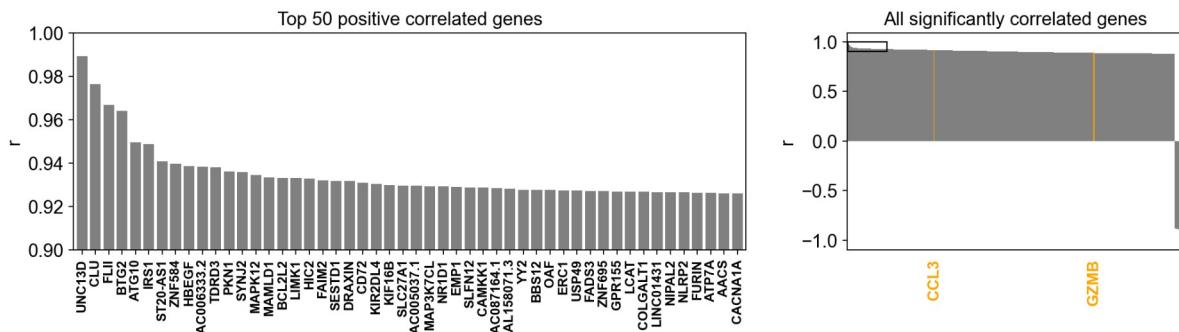
ax[1].set_xticklabels([plot_data.iloc[i].gene if plot_data.iloc[i].color != 'gray' else '' for i in range(plot_data.shape[0])], fontdict={'fontsize': '12', 'fontweight': 'bold'})
ax[0].set_xticklabels(ax[0].get_xticklabels(), fontdict={'fontsize': '10', 'fontweight': 'semibold'})

ax[1].add_patch(Rectangle(xy=(0, 0.9), width=50, height=0.1, fill=False, edgecolor='black'))

ax[0].set_title('Top 50 positive correlated genes')
ax[1].set_title('All significantly correlated genes')
ax[0].set_ylabel('r')
ax[1].set_ylabel('r')
ax[0].set_ylim([0,9,1])
ax[1].xaxis.set_ticks_position('none')

for ticklabel, tickcolor in zip(ax[1].get_xticklabels(), plot_data.color.tolist()):
    ticklabel.set_color(tickcolor)

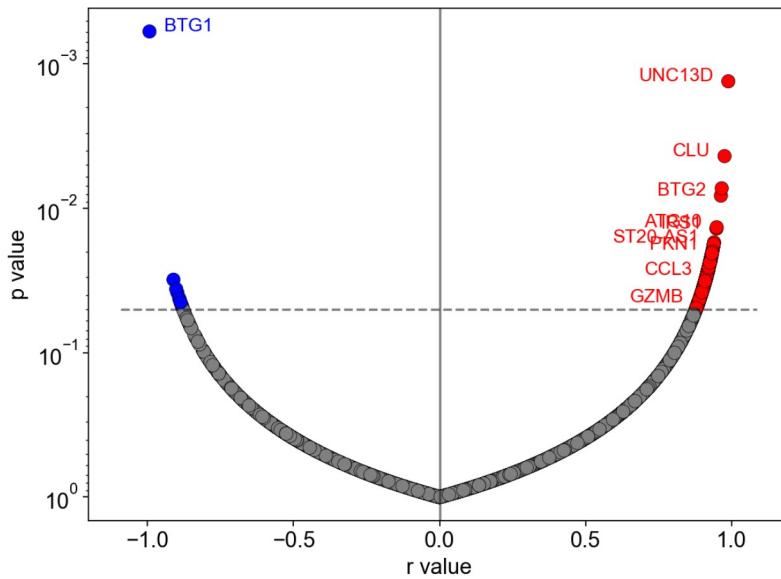
for ax in ax:
    ax.set_xlabel('')
fig.savefig('./figures_paper/waterfall_new.pdf', bbox_inches='tight')
plt.show()
```



```
In [47]: correlation_data['correlation']='not significant'
correlation_data['correlation'][ (correlation_data.p_value<0.05) & (correlation_data.r_value>0) ]='significantly pos'
correlation_data['correlation'][ (correlation_data.p_value<0.05) & (correlation_data.r_value<0) ]='significantly neg'
```

```
In [48]: annotation=['GZMB', 'CCL3', 'BTG1', 'UNC13D', 'CLU', 'BTG2', 'ATG10', 'IRS1', 'ST20-AS1', 'PKN1']
correlation_data['annotation']=False
correlation_data['annotation'][correlation_data.index.isin(annotation)]=True
```

```
In [49]: fig,ax=plt.subplots(figsize=(8,6))
sns.scatterplot(data=correlation_data, x='r_value', y='p_value', hue='correlation', palette=['gray', 'red', 'blue'], ax=ax,
                 edgecolor='black', linewidth=.3, s=75)
ax.set_yscale('log')
ax.set_ylim(ax.get_ylim()[::-1])
for line in range(0,correlation_data.shape[0]):
    if (correlation_data.iloc[line].correlation=='significantly pos') & (correlation_data.index[line] in annotations):
        ax.text(correlation_data.r_value[line]-0.05, correlation_data.p_value[line], correlation_data.index[line],
                horizontalalignment='right', size=12, color='red')
    if (correlation_data.iloc[line].correlation=='significantly neg') & (correlation_data.index[line] in annotations):
        ax.text(correlation_data.r_value[line]+0.05, correlation_data.p_value[line], correlation_data.index[line],
                horizontalalignment='left', size=12, color='blue')
    if (correlation_data.iloc[line].correlation=='not significant') & (correlation_data.index[line] in annotations):
        ax.text(correlation_data.r_value[line]+0.05, correlation_data.p_value[line], correlation_data.index[line],
                horizontalalignment='left', size=12, color='black')
ax.vlines(x=0, ymin=ax.get_ylim()[0], ymax=ax.get_ylim()[1], alpha=.5)
ax.hlines(y=0.05, xmin=ax.get_xlim()[0], xmax=ax.get_xlim()[1], linestyles='--', alpha=.5)
ax.grid(False)
ax.get_legend().remove()
ax.set_xlabel('r value')
ax.set_ylabel('p value')
fig.savefig('./figures_paper/volcano.svg', bbox_inches='tight')
```



```
In [50]: fig, ax=plt.subplots(ncols=5, nrows=2, figsize=(15, 6))
fig.tight_layout(w_pad=3, h_pad=3)
for i, gene in enumerate(annotation):
    data=adata_selected.obs.groupby('clonotype').agg({gene:'mean', 'EC50_IFNG_mean':'mean'})
    sns.regplot(data=data, x='EC50_IFNG_mean',y=gene, color='gray', marker='o', ax=ax.reshape(-1)[i])
    ax.reshape(-1)[i].set_title('slope: %.2f' % correlation_data.loc[gene,'slope']+', r2: %.2f' % correlation_data.loc[gene,'r_squared']+convert_significance(correlation_data.loc[gene,'p_value']), size=12)
fig.savefig('./figures_paper/single_correlations.pdf', bbox_inches='tight')
```

