multi_shrna_screening_ap009

March 19, 2025

1 Multiplexed shRNA screening AP009 analytics

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This notebook includes analysis scripts of processing count table of multiplexed shRNA screening experiments on AP009 cell line received from Cellecta, and evaluating * whether multi-shRNA system works as expected * prognostic effect of genotype X, and * predictive effect of genetype X on response to treatment

Note - bash commands to convert notebook to pdf:

> `jupyter nbconvert --to latex multi_shrna_screening_ap009.ipynb`

> `xelatex multi_shrna_screening_ap009.tex`

1.1 (ToDos)

- 0 - separate D18-B (technical replicate of D18-A) from the analysis
- 1 - polish up ordering of conditions in plots
- 2 - for each target gene, aggregate across multiple shRNAs
  - currently evaluating each shRNA of each target gene separately
- 3 - spot check QC'ing fastq processing to validate Cellecta's count table
  - also inquiring about counts of clonal barcodes
- 4 - check correlation between cell counts and plasmid counts of all shRNAs for each of pre-Tx NoDox samples

[3]:
```
!pip install matplotlib_venn --trusted-host pypi.python.org --trusted-host pypi.
↪org --trusted-host files.pythonhosted.org
```

```
Requirement already satisfied: matplotlib_venn in
/opt/anaconda3/lib/python3.11/site-packages (1.1.2)
Requirement already satisfied: matplotlib in /opt/anaconda3/lib/python3.11/site-
packages (from matplotlib_venn) (3.8.0)
Requirement already satisfied: numpy in /opt/anaconda3/lib/python3.11/site-
packages (from matplotlib_venn) (1.23.4)
Requirement already satisfied: scipy in /opt/anaconda3/lib/python3.11/site-
packages (from matplotlib_venn) (1.9.3)
Requirement already satisfied: contourpy>=1.0.1 in
/opt/anaconda3/lib/python3.11/site-packages (from matplotlib->matplotlib_venn)
(1.2.0)
Requirement already satisfied: cycler>=0.10 in
```

```
/opt/anaconda3/lib/python3.11/site-packages (from matplotlib->matplotlib_venn)
(0.11.0)
Requirement already satisfied: fonttools>=4.22.0 in
/opt/anaconda3/lib/python3.11/site-packages (from matplotlib->matplotlib_venn)
(4.25.0)
Requirement already satisfied: kiwisolver>=1.0.1 in
/opt/anaconda3/lib/python3.11/site-packages (from matplotlib->matplotlib_venn)
(1.4.4)
Requirement already satisfied: packaging>=20.0 in
/opt/anaconda3/lib/python3.11/site-packages (from matplotlib->matplotlib_venn)
(23.1)
Requirement already satisfied: pillow>=6.2.0 in
/opt/anaconda3/lib/python3.11/site-packages (from matplotlib->matplotlib_venn)
(10.2.0)
Requirement already satisfied: pyparsing>=2.3.1 in
/opt/anaconda3/lib/python3.11/site-packages (from matplotlib->matplotlib_venn)
(3.0.9)
Requirement already satisfied: python-dateutil>=2.7 in
/opt/anaconda3/lib/python3.11/site-packages (from matplotlib->matplotlib_venn)
(2.8.2)
Requirement already satisfied: six>=1.5 in /opt/anaconda3/lib/python3.11/site-
packages (from python-dateutil>=2.7->matplotlib->matplotlib_venn) (1.16.0)
```

1.2 Dependency pkgs

[4]:
```
import pandas as pd
import numpy as np
from prettytable import PrettyTable
from IPython.display import display
import matplotlib.pyplot as plt
import seaborn as sns
import numpy as np

from matplotlib_venn import venn3

# seed of random number generator
rng_seed = 1234
```

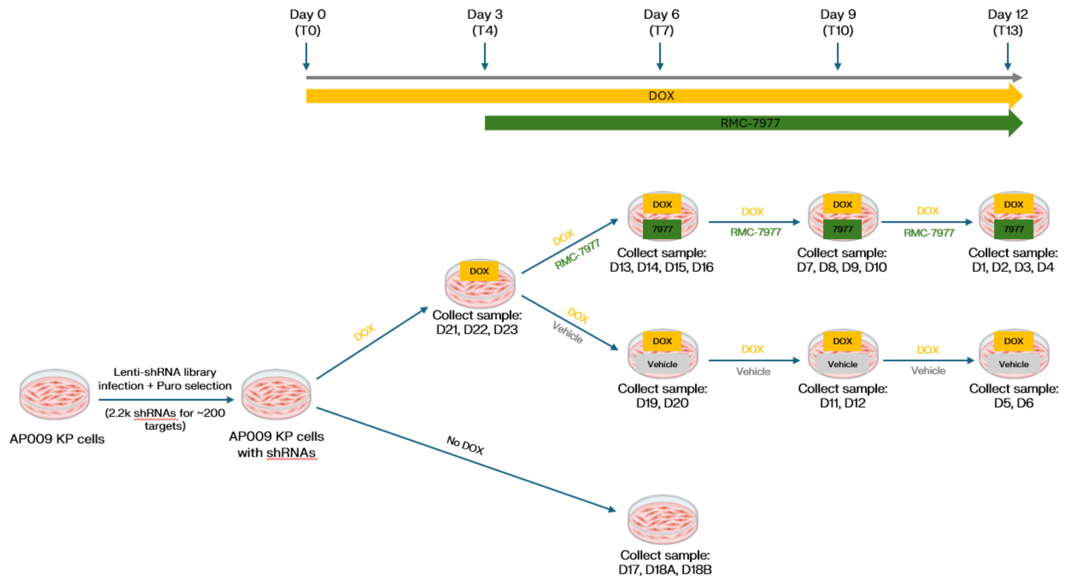
1.3 Experimental design and parameters

Load experimental design xlsx file (from Ian) * Master list tab of target genes, vector types, and annotations * Experimental design tab of groups and samples

Load sample description xlsx file (from Cellecta) * Actual Sample ID and description on flowcell, and inferred information of Group, Day_Tx, Replicate, Dox, etc.

Also check out schematic workflow of experimental design (from Zheng)

Study Design



[5]:
```
# #### experimental design xlsx file
# file_path = "~/Documents/Projects/Multi_shRNA_screening_AP009/data/
↪Multiplexed shRNA screen in 2D AP009 - Cellecta.xlsx"

# xls = pd.ExcelFile(file_path)

# ## Gene targets
# gene_targets_df = pd.read_excel(xls, sheet_name="Master list")
# # Filter out "Individual dual shRNA vector" from gene targets
# filtered_gene_targets_df = gene_targets_df[gene_targets_df["Vector type"] !=␣
↪"Individual dual shRNA vector"]
# filtered_gene_targets = filtered_gene_targets_df["Mouse gene symbol"].
↪dropna().unique()

# # ## Define experimental parameters
# # # Num clonal barcodes per gene
# # num_clonal_barcodes = 12000
# # # Num shRNAs per gene
# # num_shRNAs_per_gene = 10
# # # N_reps per condition per timepoint
# # num_replicates = 2

# ## Define experimental conditions and timepoints
# # available timepoints
# time_points = ["0d", "3d", "6d", "9d"]
# # experimental conditions based on design
# conditions = [
#     "Baseline_NoDox_Vehicle",
#     "Baseline_Dox_PreTx",  # Only at 0d
#     "Prognostic_Dox_Vehicle",
#     "Predictive_Dox_7977_LowDose",  # IC30 early, IC50 later
#     "Predictive_Dox_7977_HighDose"  # IC90
# ]
```

[6]:
```
#### sample description xlsx file
file_path_cellecta = "~/Documents/Projects/Multi_shRNA_screening_AP009/data/
  ↪sample_description_rectified.xlsx"

df_sd = pd.read_excel(file_path_cellecta, sheet_name='Sheet1')

# Set the first row as column headers and remove it from the data
df_sd.columns = df_sd.iloc[0]
df_sd = df_sd[1:].reset_index(drop=True)

# Rename columns to remove any unintended whitespace
df_sd.columns = df_sd.columns.str.strip()
```

Utility function of table viewing

[7]:
```
## utility function of printing table
def ViewTable(df, top_n_rows = None):
    table = PrettyTable(df.columns.tolist())
    if top_n_rows:
        df_tmp = df.head(top_n_rows)
    else:
        df_tmp = df
    for row in df_tmp.itertuples(index=False, name=None):
        table.add_row(row)
    print(table)

ViewTable(df_sd, 5)
```

```
+-----------+--------------------+----------------+----------------------------
-------------------+-------------------+---------+-------+--------+-----------+-
----+------+
| Sample_ID | Sample_Description |    Library     |                     Vector
|      Flowcell      |     Tx    | Group | Day_Tx | Replicate | Dox | Note |
+-----------+--------------------+----------------+----------------------------
-------------------+-------------------+---------+-------+--------+-----------+-
----+------+
|     D1    |    T13_Dox_0.6nM    | 2.2K-REVMED-ZZ |  pRSIT16cb-U6tet-sh-CMV-
tetR-2A-TagRFP-2A-Puro | 25-03-11  102190  |   Low    |    5    |    9     |     2
|  Y  | nan  |
|     D2    |    T13_Dox_0.6nM    | 2.2K-REVMED-ZZ |  pRSIT16cb-U6tet-sh-CMV-
tetR-2A-TagRFP-2A-Puro | 25-03-11  102190  |   Low    |    5    |    9     |     1
|  Y  | nan  |
|     D3    |    T13_Dox_3.5nM    | 2.2K-REVMED-ZZ |  pRSIT16cb-U6tet-sh-CMV-
tetR-2A-TagRFP-2A-Puro | 25-03-11  102190  |   High  |   4   |   9    |     2
|  Y   | nan  |
|     D4    |    T13_Dox_3.5nM    | 2.2K-REVMED-ZZ |  pRSIT16cb-U6tet-sh-CMV-
tetR-2A-TagRFP-2A-Puro | 25-03-11  102190  |   High  |   4   |   9    |     1
|  Y   | nan  |
|     D5    |  T13_Dox_Vehicle    | 2.2K-REVMED-ZZ |  pRSIT16cb-U6tet-sh-CMV-
tetR-2A-TagRFP-2A-Puro | 25-03-11  102190  | Vehicle |   2   |   9    |     2
|  Y   | nan  |
+-----------+-------------------+----------------+------------------------------
-------------------+------------------+---------+-------+--------+-----------+-
----+------+
```

1.4 Barcodes (shRNA) count table

Load count table (from Cellecta) and extract fields of sample IDs and target gene

[8]:
```python
file_path_count_table = "~/Documents/Projects/Multi_shRNA_screening_AP009/data/
↪Count_Table.csv"
df_counts = pd.read_csv(file_path_count_table)

# Extract columns that start with 'D' plus the 'Gene Symbol / Target Name'␣
↪column
selected_columns = [col for col in df_counts.columns if col.startswith("D")] +␣
↪["Gene Symbol / Target Name"]

# Create a new dataframe with selected columns
df_counts = df_counts[selected_columns].copy()

# Rename 'Gene Symbol / Target Name' to 'target_gene'
df_counts = df_counts.rename(columns={"Gene Symbol / Target Name":␣
↪"Target_Gene"})

# Rename 'Non-Targeting-Mouse' to 'NT' in the target_gene column
df_counts["Target_Gene"] = df_counts["Target_Gene"].
↪replace("Non-Targeting-Mouse", "NT")

# Ensure there are no NaN values in the target_gene column before counting␣
↪repeats
df_counts["Target_Gene"] = df_counts["Target_Gene"].fillna("Unknown")

# Generate a sequential ID for each occurrence of target_gene
df_counts["target_gene_repeat_ID"] = df_counts.groupby("Target_Gene").
↪cumcount() + 1

# Format as "01", "02", "03", etc.
```

```
df_counts["target_gene_repeat_ID"] = df_counts["target_gene_repeat_ID"].
 ↪astype(int).apply(lambda x: f"{x:02d}")

# Combine with target_gene to create a unique ID
df_counts["shRNA_ID"] = df_counts["Target_Gene"] + "_" +␣
 ↪df_counts["target_gene_repeat_ID"]

# Drop the temporary repeat ID column
df_counts = df_counts.drop(columns=["target_gene_repeat_ID"])

# Update the target_gene_ID column accordingly
df_counts["shRNA_ID"] = df_counts["Target_Gene"] + "_" + df_counts["shRNA_ID"].
 ↪str.split("_").str[-1]

# melt the dataframe to long format
df_long = df_counts.melt(id_vars=["Target_Gene", "shRNA_ID"],␣
 ↪var_name="Sample_ID", value_name="Read_Counts")
```

[9]:
```
display(df_counts)
```

```
        D1    D2    D3    D4    D5    D6    D7    D8    D9   D10  …   D17  \
0     1399  2777  1312  2281  2056  1579  1072  3116  2532  3019  …  1683
1     1785  3277  1776  3414  3139  2482  1289  3877  3373  3953  …  2239
2      569  1270   539  1025  1075   892   484  1728  1201  1301  …   727
3      955  1703   912  1607  1714  1235   817  2199  1727  2160  …  1234
4     1150  2403  1177  2281  1981  1467  1055  2641  2071  2504  …  1462
…      …     …     …     …     …     …     …     …     …     …   …   …
2182   460   889   548   870   810   571   332  1141   935  1101  …   723
2183   725  1451   709  1416  1151  1017   483  1693  1601  1726  …   936
2184   547  1133   575  1052  1093   843   477  1365  1246  1497  …   777
2185  1354  2584  1371  2570  2230  1773  1047  3223  2530  3248  …  1740
2186   479   960   497   907   832   562   368  1276   813  1025  …   593

      D18-A  D18-B   D19   D20   D21   D22   D23  Target_Gene  shRNA_ID
0      1411   1717  1682  2105  1482  1257  1965           NT     NT_01
1      2000   2510  2559  2959  2321  1782  2763           NT     NT_02
2       726    804   774  1094   768   601   945           NT     NT_03
3      1153   1372  1268  1481  1010   855  1385           NT     NT_04
4      1415   1599  1556  2025  1541  1126  1868           NT     NT_05
…       …      …     …     …     …     …     …           …       …
2182    679    758   709   892   658   475   859         Zeb1   Zeb1_06
2183    782    970   933  1242   931   757  1071         Zeb1   Zeb1_07
2184    771    868   820  1157   698   637   846         Zeb1   Zeb1_08
2185   1610   1877  1745  2160  1681  1329  1973         Zeb1   Zeb1_09
2186    534    597   583   787   609   474   691         Zeb1   Zeb1_10

[2187 rows x 26 columns]
```

[10]:
```
display(df_long)
```

```
      Target_Gene shrNA_ID Sample_ID  Read_Counts
0              NT    NT_01        D1         1399
1              NT    NT_02        D1         1785
2              NT    NT_03        D1          569
3              NT    NT_04        D1          955
4              NT    NT_05        D1         1150
…             …        …          …           …
52483        Zeb1  Zeb1_06       D23          859
52484        Zeb1  Zeb1_07       D23         1071
52485        Zeb1  Zeb1_08       D23          846
52486        Zeb1  Zeb1_09       D23         1973
52487        Zeb1  Zeb1_10       D23          691

[52488 rows x 4 columns]
```

[11]:
```
### total number of barcodes per sample / condition
```

[12]:
```
df_full = df_long.merge(df_sd[["Sample_ID", "Sample_Description", "Day_Tx",␣
 ↪"Tx", "Dox", "Replicate"]],
                        left_on = "Sample_ID", right_on = "Sample_ID", how =␣
 ↪"left")
df_full.to_csv("~/Documents/Projects/Multi_shRNA_screening_AP009/data/
 ↪long_format_joint_count_data.csv", index = False)
```

[13]:
```
display(df_full)
print(df_full["Target_Gene"].value_counts())
```

```
      Target_Gene shRNA_ID Sample_ID  Read_Counts Sample_Description Day_Tx  \
0              NT    NT_01        D1         1399      T13_Dox_0.6nM      9
1              NT    NT_02        D1         1785      T13_Dox_0.6nM      9
2              NT    NT_03        D1          569      T13_Dox_0.6nM      9
3              NT    NT_04        D1          955      T13_Dox_0.6nM      9
4              NT    NT_05        D1         1150      T13_Dox_0.6nM      9
…             …        …          …           …                 …     …
52483        Zeb1  Zeb1_06       D23          859        T4_Dox_NoTx      0
52484        Zeb1  Zeb1_07       D23         1071        T4_Dox_NoTx      0
52485        Zeb1  Zeb1_08       D23          846        T4_Dox_NoTx      0
52486        Zeb1  Zeb1_09       D23         1973        T4_Dox_NoTx      0
52487        Zeb1  Zeb1_10       D23          691        T4_Dox_NoTx      0

         Tx Dox Replicate
0       Low   Y         2
1       Low   Y         2
2       Low   Y         2
3       Low   Y         2
4       Low   Y         2
```

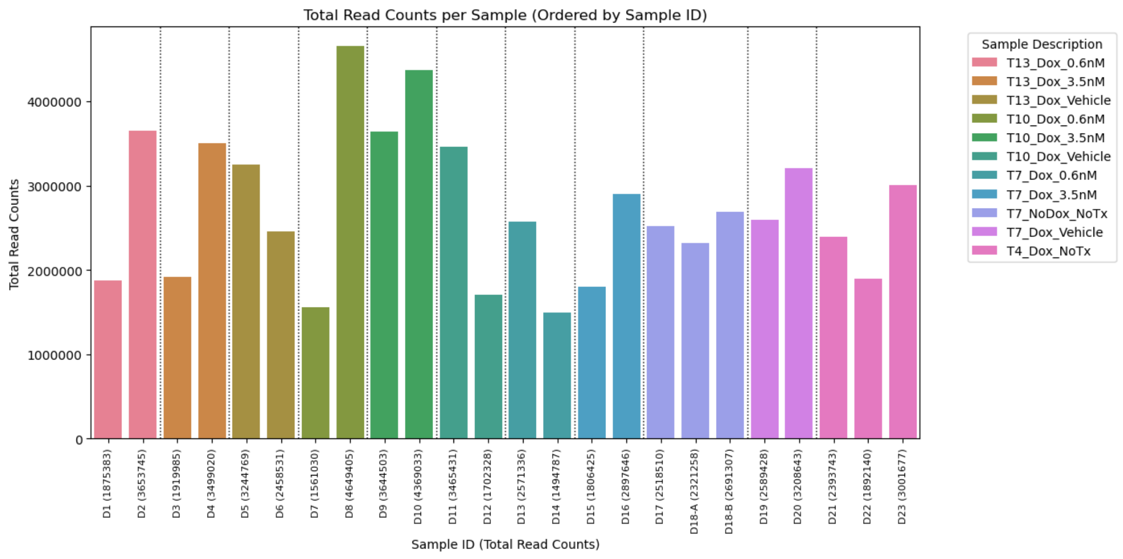
```
…      …   ..        …
52483  None   Y          1
52484  None   Y          1
52485  None   Y          1
52486  None   Y          1
52487  None   Y          1

[52488 rows x 9 columns]
```

```
NT                 4800
Pdgfra              240
Nf1                 240
Nf2                 240
Nfe2l2              240
                   …
Erbb2               240
Erbb3               240
Ern1                240
Zeb1                240
Cdkn2a(Ink4a)       168
Name: Target_Gene, Length: 200, dtype: int64
```

Plot total read counts per sample

[14]:

```
# Aggregate total read counts per sample
df_sample_counts_simple = df_full.groupby(["Sample_ID",␣
↪"Sample_Description"])["Read_Counts"].sum().reset_index()

# Ensure Sample_ID is sorted numerically rather than lexicographically
df_sample_counts_simple["Sample_ID_Sort"] =␣
↪df_sample_counts_simple["Sample_ID"].str.extract('(\d+)').astype(int)
df_sample_counts_simple = df_sample_counts_simple.
↪sort_values(by="Sample_ID_Sort").reset_index()

# Modify Sample_ID labels to include total counts in parentheses
df_sample_counts_simple["Sample_ID_Label"] = df_sample_counts_simple.apply(
    lambda row: f"{row['Sample_ID']} ({int(row['Read_Counts'])})", axis=1
)

# Plot the bar chart with modified x-axis labels
plt.figure(figsize=(12, 6))
ax = sns.barplot(data=df_sample_counts_simple, x="Sample_ID_Label",␣
↪y="Read_Counts", hue="Sample_Description", dodge=False)

# Identify group transitions for adding vertical dotted lines
prev_desc = None
for index, row in df_sample_counts_simple.iterrows():
    current_desc = row["Sample_Description"]
    if prev_desc is not None and prev_desc != current_desc:
        plt.axvline(x=index - 0.5, color="black", linestyle="dotted",␣
 ↪linewidth=1)  # Add vertical separator
    prev_desc = current_desc

# Set y-axis to exact number format
plt.ticklabel_format(style='plain', axis='y')  # Disable scientific notation

plt.xticks(rotation=90, fontsize=8)
plt.xlabel("Sample ID (Total Read Counts)")
plt.ylabel("Total Read Counts")
plt.title("Total Read Counts per Sample (Ordered by Sample ID)")
plt.legend(title="Sample Description", bbox_to_anchor=(1.05, 1), loc='upper␣
 ↪left')  # Move legend outside
plt.show()
```



1.5 Non-targeting shRNAs quantification and selection

Assuming that for each non-targeting shRNA, its reads proportion should be minimally variable among replicates of each experimental condition (as denoted by `Sample_Description` field of `df_full`

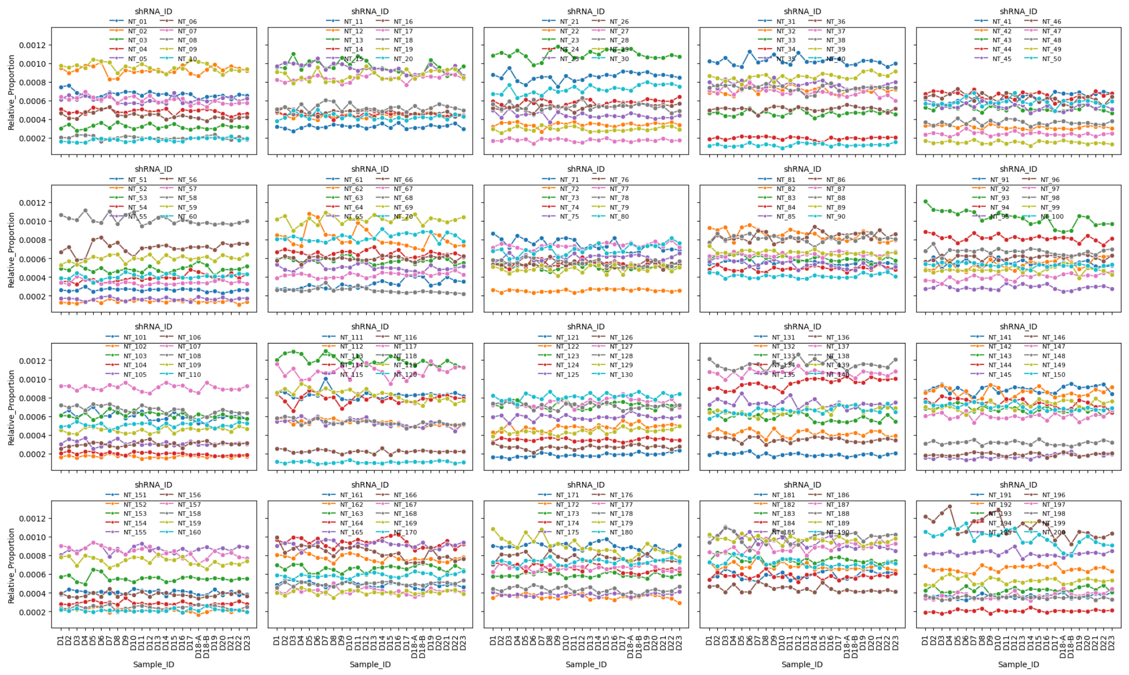
[15]:
```
# Reload full count table
df_full_path = "~/Documents/Projects/Multi_shRNA_screening_AP009/data/
 ↪long_format_joint_count_data.csv"
df_full = pd.read_csv(df_full_path)

# Filter for only NT (Non-Targeting) shRNAs
```

```
df_nt = df_full[df_full["Target_Gene"] == "NT"]

# Compute total counts per sample
df_total_counts = df_full.groupby("Sample_ID")["Read_Counts"].sum().
  ↪reset_index()
df_total_counts = df_total_counts.rename(columns={"Read_Counts":␣
  ↪"Total_Read_Counts"})

# Merge total counts back to the NT dataset
df_nt = df_nt.merge(df_total_counts, on="Sample_ID", how="left")

# Compute relative proportion for each shRNA within each sample
df_nt["Relative_Proportion"] = df_nt["Read_Counts"] / df_nt["Total_Read_Counts"]
```

```
[16]: display(df_nt)
      print(df_nt["Target_Gene"].value_counts())
```

```
     Target_Gene shRNA_ID Sample_ID  Read_Counts Sample_Description  Day_Tx  \
0             NT    NT_01        D1         1399      T13_Dox_0.6nM       9
1             NT    NT_02        D1         1785      T13_Dox_0.6nM       9
2             NT    NT_03        D1          569      T13_Dox_0.6nM       9
3             NT    NT_04        D1          955      T13_Dox_0.6nM       9
4             NT    NT_05        D1         1150      T13_Dox_0.6nM       9
…            …       …        …           …                  …     …
4795          NT   NT_196       D23         3103        T4_Dox_NoTx       0
4796          NT   NT_197       D23         1325        T4_Dox_NoTx       0
4797          NT   NT_198       D23          991        T4_Dox_NoTx       0
4798          NT   NT_199       D23         1613        T4_Dox_NoTx       0
4799          NT   NT_200       D23         2721        T4_Dox_NoTx       0

        Tx Dox  Replicate  Total_Read_Counts  Relative_Proportion
0      Low   Y          2            1875383             0.000746
1      Low   Y          2            1875383             0.000952
2      Low   Y          2            1875383             0.000303
3      Low   Y          2            1875383             0.000509
4      Low   Y          2            1875383             0.000613
…     …  ..        …                …                  …
4795  None   Y          1            3001677             0.001034
4796  None   Y          1            3001677             0.000441
4797  None   Y          1            3001677             0.000330
4798  None   Y          1            3001677             0.000537
4799  None   Y          1            3001677             0.000906

[4800 rows x 11 columns]

NT    4800
Name: Target_Gene, dtype: int64
```

[17]:
```
# Get unique shRNA_IDs
unique_shRNAs = df_nt["shRNA_ID"].unique()

# Create a 4x5 subplot grid
fig, axes = plt.subplots(4, 5, figsize=(20, 12), sharex=True, sharey=True)
axes = axes.flatten()  # Flatten 2D array of subplots

# Plot in batches of 10 shRNAs per subplot
batch_size = 10
for i in range(0, len(unique_shRNAs), batch_size):
    shRNA_subset = unique_shRNAs[i:i + batch_size]
    ax = axes[i // batch_size]

    sns.lineplot(data=df_nt[df_nt["shRNA_ID"].isin(shRNA_subset)],
                 x="Sample_ID", y="Relative_Proportion", hue="shRNA_ID",␣
 ↪marker="o", ax=ax)

    ax.tick_params(axis='x', rotation=90)

    # Move legend on top of each subplot and shrink marker size to avoid clutter
    legend = ax.legend(title="shRNA_ID", bbox_to_anchor=(0.5, 1.2), loc="upper␣
 ↪center", fontsize=8, ncol=2, frameon=False)
    for line in legend.get_lines():
        line.set_markersize(4)  # Reduce marker size

# Adjust layout
plt.tight_layout()
plt.show()
```



```
[ ]:
```

Quantifying CV of reads proportion for each NT shRNA among samples within each experimental condition of `Sample_Description`

```
[18]: # Group by shRNA within each Sample_Description
      df_nt_variation = df_nt.groupby(["shRNA_ID",␣
       ↪"Sample_Description"])["Relative_Proportion"].agg(
          Mean_Proportion="mean",
          Std_Proportion="std"
      ).reset_index()

      # Compute Coefficient of Variation (CV)
      df_nt_variation["CV"] = df_nt_variation["Std_Proportion"] /␣
       ↪df_nt_variation["Mean_Proportion"]
```

```
[19]: display(df_nt_variation)
      df_nt_variation.to_csv("~/Documents/Projects/Multi_shRNA_screening_AP009/data/
       ↪non_targeting_shrna_variation_table.csv", index = False)
```

```
     shRNA_ID Sample_Description  Mean_Proportion  Std_Proportion        CV
0       NT_01       T10_Dox_0.6nM         0.000678        0.000012  0.017231
1       NT_01       T10_Dox_3.5nM         0.000693        0.000003  0.003822
2       NT_01     T10_Dox_Vehicle         0.000673        0.000028  0.041265
3       NT_01       T13_Dox_0.6nM         0.000753        0.000010  0.013204
4       NT_01       T13_Dox_3.5nM         0.000668        0.000022  0.033302
```

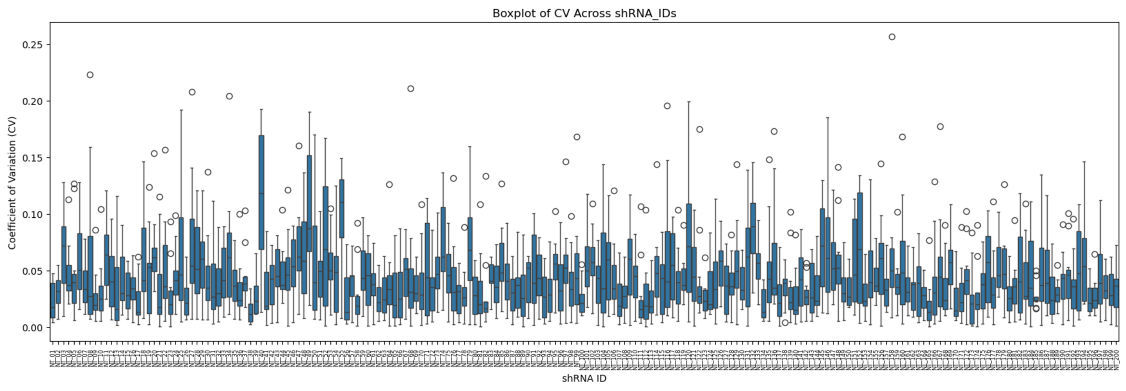
```
…          …                 …                 …             …          …
2195     NT_99        T4_Dox_NoTx        0.000457        0.000028  0.061696
2196     NT_99       T7_Dox_0.6nM        0.000470        0.000032  0.068284
2197     NT_99       T7_Dox_3.5nM        0.000461        0.000037  0.080397
2198     NT_99     T7_Dox_Vehicle        0.000444        0.000075  0.167892
2199     NT_99       T7_NoDox_NoTx       0.000531        0.000020  0.037855

[2200 rows x 5 columns]
```

[20]:
```
display(df_nt_variation[df_nt_variation["Sample_Description"] == "T10_Dox_0.
↪6nM"])
```

```
     shRNA_ID Sample_Description  Mean_Proportion  Std_Proportion        CV
0       NT_01      T10_Dox_0.6nM         0.000678        0.000012  0.017231
11      NT_02      T10_Dox_0.6nM         0.000830        0.000006  0.006931
22      NT_03      T10_Dox_0.6nM         0.000341        0.000044  0.127808
33      NT_04      T10_Dox_0.6nM         0.000498        0.000036  0.071551
44      NT_05      T10_Dox_0.6nM         0.000622        0.000076  0.122570
…          …                 …                 …             …          …
2145    NT_95      T10_Dox_0.6nM         0.000277        0.000010  0.035703
2156    NT_96      T10_Dox_0.6nM         0.000595        0.000042  0.070011
2167    NT_97      T10_Dox_0.6nM         0.000403        0.000026  0.063755
2178    NT_98      T10_Dox_0.6nM         0.000689        0.000017  0.025127
2189    NT_99      T10_Dox_0.6nM         0.000474        0.000003  0.007054

[200 rows x 5 columns]
```

Boxplot of CV of `df_nt_variation` across NT shRNAs

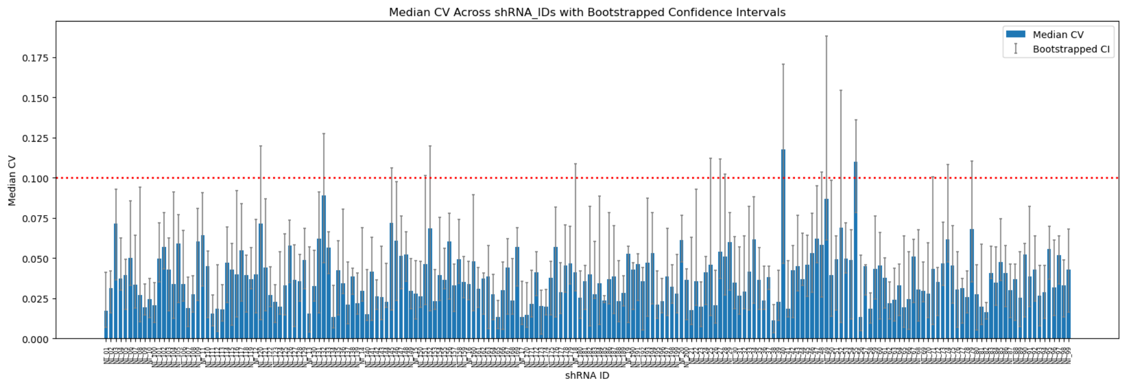
[21]:
```
# Extract numeric part of shRNA_ID and sort sequentially from 1 to 200
df_nt_variation["shRNA_Seq"] = df_nt_variation["shRNA_ID"].str.
↪extract(r'(\d+)').astype(int)
df_nt_variation = df_nt_variation.sort_values(by="shRNA_Seq")

# Create a wider boxplot with ordered shRNA_IDs
plt.figure(figsize=(20, 6))
sns.boxplot(data=df_nt_variation, x="shRNA_ID", y="CV")

# Customize the plot
plt.xticks(rotation=90, fontsize=6)  # Smaller font size for x-axis labels
plt.xlabel("shRNA ID")
plt.ylabel("Coefficient of Variation (CV)")
plt.title("Boxplot of CV Across shRNA_IDs")

# Show the plot
plt.show()
```



Barchart of Median CV of `df_nt_variation` across NT shRNAs with bootstrapped confience internal

[22]:
```
# Define function for bootstrapped confidence interval estimation
def bootstrap_median_ci(data, num_resamples=1000, ci=95):
    boot_medians = [np.median(np.random.choice(data, size=len(data),␣
 ↪replace=True)) for _ in range(num_resamples)]
    lower_bound = np.percentile(boot_medians, (100 - ci) / 2)
    upper_bound = np.percentile(boot_medians, 100 - (100 - ci) / 2)
    return lower_bound, upper_bound

# Compute median and bootstrapped confidence intervals for each shRNA_ID
df_cv_stats = df_nt_variation.groupby("shRNA_ID")["CV"].agg(
    Median_CV="median"
).reset_index()

# Apply bootstrapping for confidence intervals
df_cv_stats["Lower_CI"], df_cv_stats["Upper_CI"] = zip(*df_nt_variation.
 ↪groupby("shRNA_ID")["CV"].apply(lambda x: bootstrap_median_ci(x)))

# Calculate error bars (difference between median and lower/upper bounds)
df_cv_stats["Error_Lower"] = df_cv_stats["Median_CV"] - df_cv_stats["Lower_CI"]
df_cv_stats["Error_Upper"] = df_cv_stats["Upper_CI"] - df_cv_stats["Median_CV"]
```

[23]:
```
# Create a bar chart with bootstrapped confidence intervals
plt.figure(figsize=(20, 6))

# Plot bars for Median_CV
plt.bar(df_cv_stats["shRNA_ID"], df_cv_stats["Median_CV"], label="Median CV")

# Add error bars with small caps at both ends
plt.errorbar(df_cv_stats["shRNA_ID"], df_cv_stats["Median_CV"],
             yerr=[df_cv_stats["Error_Lower"], df_cv_stats["Error_Upper"]],
             fmt='none', ecolor="gray", elinewidth=1.2, capsize=1.5, capthick=1.
↪2, label="Bootstrapped CI")
plt.axhline(y=0.1, color='red', linestyle='dotted', linewidth=2)
# Customize the plot
plt.xticks(rotation=90, fontsize=6)
plt.xlabel("shRNA ID")
plt.ylabel("Median CV")
plt.title("Median CV Across shRNA_IDs with Bootstrapped Confidence Intervals")
plt.legend()

# Show the plot
plt.show()
```



There are 183 NT shRNA whose bootstrapped confidence internals of median CVs < 0.1

```
[24]: df_selected_nt_boot_median = df_cv_stats[(df_cv_stats["Upper_CI"] < 0.1)]
display(df_selected_nt_boot_median)
df_selected_nt_boot_median.to_csv("~/Documents/Projects/
↪Multi_shRNA_screening_AP009/data/
↪selected_non_targeting_shRNA_table_median_cv_bootstrapped.csv", index =␣
↪False)
```

```
    shRNA_ID  Median_CV  Lower_CI  Upper_CI  Error_Lower  Error_Upper
0      NT_01   0.017231  0.007015  0.041265     0.010216     0.024034
1      NT_02   0.031408  0.016015  0.042011     0.015393     0.010603
2      NT_03   0.071599  0.036633  0.092930     0.034966     0.021331
3      NT_04   0.037188  0.029977  0.062541     0.007211     0.025353
4      NT_05   0.039316  0.017949  0.049489     0.021367     0.010173
..       …         …         …         …           …            …
195    NT_95   0.055613  0.036926  0.069820     0.018688     0.014206
196    NT_96   0.031795  0.014230  0.061475     0.017565     0.029680
197    NT_97   0.051950  0.033848  0.063755     0.018101     0.011805
198    NT_98   0.033246  0.013237  0.048943     0.020009     0.015697
199    NT_99   0.043054  0.016347  0.068284     0.026707     0.025230
```

```
[183 rows x 6 columns]
```

Quantifying overall variability for each NT shRNA by summarizing mean, median, inter-quartile-range, and max CVs, where * mean is the average across all conditions * median is more robust to outliers * IQR measures spread * max captures extreme values

[25]:
```
df_nt_cv_summary = df_nt_variation.groupby("shRNA_ID")["CV"].agg(
    Mean_CV="mean",
    Median_CV="median",
    IQR_CV=lambda x: x.quantile(0.75) - x.quantile(0.25),  # Interquartile Range
    Max_CV="max"
).reset_index()
```

[26]:
```
display(df_nt_cv_summary)
```

```
    shRNA_ID   Mean_CV  Median_CV    IQR_CV    Max_CV
0      NT_01  0.023505   0.017231  0.030772  0.047327
1      NT_02  0.031011   0.031408  0.019975  0.054954
2      NT_03  0.066902   0.071599  0.050576  0.127808
3      NT_04  0.046917   0.037188  0.022093  0.112538
4      NT_05  0.047722   0.039316  0.025738  0.126396
..       …         …          …         …         …
195    NT_95  0.054365   0.055613  0.021983  0.102406
196    NT_96  0.037031   0.031795  0.041091  0.092345
197    NT_97  0.056563   0.051950  0.024747  0.146140
198    NT_98  0.037734   0.033246  0.029870  0.097928
199    NT_99  0.051712   0.043054  0.045255  0.167892

[200 rows x 5 columns]
```

From a total of 200 non-targeting shRNAs Selecting 49 that have * low Mean_CV (ensuring overall low variability) * low IQR_CV (ensuring tight distribution of variability) * filtered out cases of extreme outliers (in case Max_CV >= 3 x Median_CV)

[27]:
```
mean_cv_threshold = df_nt_cv_summary["Mean_CV"].quantile(0.5)
iqr_cv_threshold = df_nt_cv_summary["IQR_CV"].quantile(0.5)
max_cv_threshold = df_nt_cv_summary["Max_CV"].quantile(0.8)

df_selected_nt = df_nt_cv_summary[
    (df_nt_cv_summary["Mean_CV"] < mean_cv_threshold) &
    (df_nt_cv_summary["IQR_CV"] < iqr_cv_threshold) &
    (df_nt_cv_summary["Max_CV"] < 3 * df_nt_cv_summary["Median_CV"])
]

print(df_selected_nt.shape)
```

```
(49, 5)
```

[28]:
```
display(df_nt_variation)
```

```
     shRNA_ID Sample_Description  Mean_Proportion  Std_Proportion        CV  \
0       NT_01       T10_Dox_0.6nM         0.000678        0.000012  0.017231
1       NT_01       T10_Dox_3.5nM         0.000693        0.000003  0.003822
2       NT_01     T10_Dox_Vehicle         0.000673        0.000028  0.041265
3       NT_01       T13_Dox_0.6nM         0.000753        0.000010  0.013204
4       NT_01       T13_Dox_3.5nM         0.000668        0.000022  0.033302
…         …                 …               …             …        …
1322   NT_200     T10_Dox_Vehicle         0.001081        0.000013  0.011939
1321   NT_200       T10_Dox_3.5nM         0.001017        0.000002  0.001932
1320   NT_200       T10_Dox_0.6nM         0.000998        0.000055  0.054743
1324   NT_200       T13_Dox_3.5nM         0.001059        0.000046  0.043319
1329   NT_200      T7_Dox_Vehicle         0.000975        0.000041  0.041774

      shRNA_Seq
0             1
1             1
2             1
3             1
4             1
…           …
1322        200
1321        200
1320        200
1324        200
1329        200

[2200 rows x 6 columns]
```

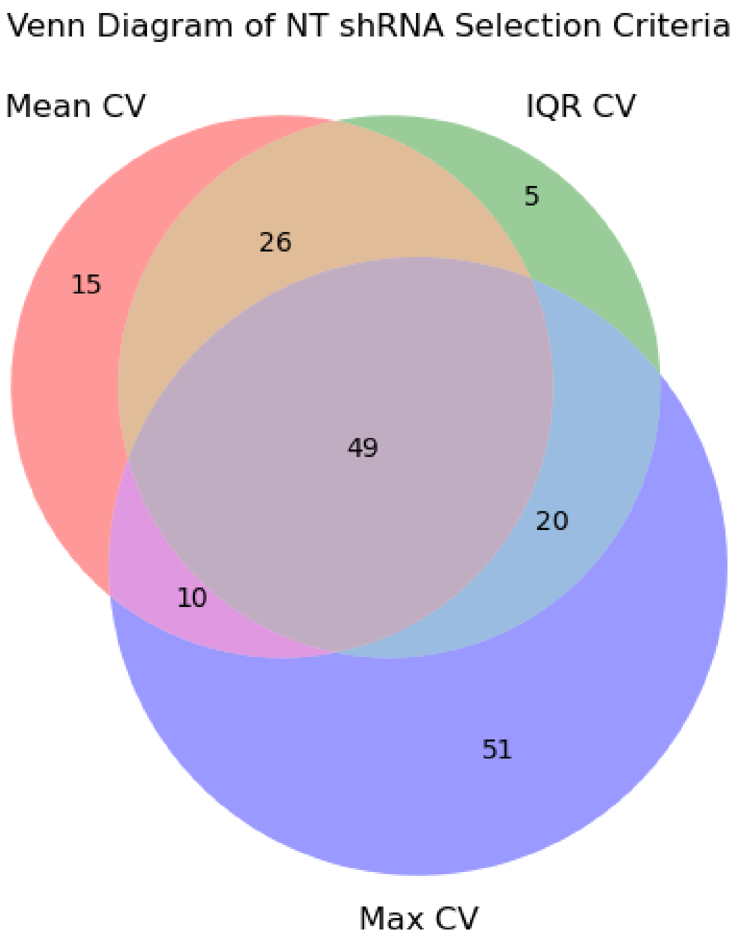
[29]:
```
display(df_selected_nt)
df_selected_nt.to_csv("~/Documents/Projects/Multi_shRNA_screening_AP009/data/
↪selected_non_targeting_shRNA_table.csv", index = False)
```

```
    shRNA_ID   Mean_CV  Median_CV    IQR_CV    Max_CV
0      NT_01  0.023505   0.017231  0.030772  0.047327
1      NT_02  0.031011   0.031408  0.019975  0.054954
6      NT_07  0.036874   0.033410  0.028563  0.077838
10    NT_100  0.024578   0.020823  0.016112  0.055441
18    NT_108  0.028896   0.027380  0.019566  0.062194
29    NT_118  0.039625   0.039568  0.028879  0.103221
35    NT_123  0.025248   0.022711  0.017982  0.061534
40    NT_128  0.038933   0.035748  0.019580  0.081445
43    NT_130  0.037138   0.032867  0.028425  0.067972
51    NT_138  0.034664   0.038686  0.014556  0.059808
56    NT_142  0.027838   0.026430  0.012434  0.056190
63    NT_149  0.033895   0.029703  0.021475  0.074647
64     NT_15  0.030534   0.027832  0.031760  0.058035
65    NT_150  0.032096   0.026365  0.024393  0.060276
68    NT_153  0.028462   0.023164  0.019306  0.064539
```

```
72     NT_157  0.035296   0.032896  0.023889  0.072380
74     NT_159  0.040324   0.035262  0.017661  0.101391
75      NT_16  0.033799   0.033751  0.019478  0.063102
77     NT_161  0.035307   0.030762  0.021791  0.071762
78     NT_162  0.033030   0.037519  0.024529  0.070705
80     NT_164  0.027160   0.027567  0.018410  0.050325
92     NT_175  0.033889   0.037758  0.030912  0.075183
98     NT_180  0.026386   0.025270  0.021713  0.062622
99     NT_181  0.035716   0.035482  0.022273  0.094494
103    NT_185  0.027230   0.023812  0.003720  0.049740
107    NT_189  0.029907   0.028455  0.012479  0.054757
109    NT_190  0.039326   0.042766  0.022109  0.090807
111    NT_192  0.035765   0.035747  0.025694  0.095609
114    NT_195  0.025289   0.021189  0.020744  0.051631
115    NT_196  0.026629   0.023246  0.017673  0.064515
117    NT_198  0.033319   0.032052  0.020271  0.060411
118    NT_199  0.030918   0.027801  0.027543  0.062237
120    NT_200  0.032716   0.036554  0.025351  0.072641
126     NT_26  0.024747   0.020779  0.024054  0.062149
135     NT_35  0.038134   0.036376  0.027838  0.076903
138     NT_38  0.013404   0.011438  0.015817  0.034109
139     NT_39  0.027851   0.022704  0.024164  0.064824
157     NT_57  0.038328   0.045156  0.018738  0.071960
161     NT_61  0.037073   0.037731  0.019360  0.073953
162     NT_62  0.024643   0.021960  0.021308  0.054732
163     NT_63  0.029498   0.023928  0.030850  0.062410
169     NT_69  0.034701   0.029850  0.022383  0.062249
172     NT_72  0.033047   0.035258  0.029135  0.085999
177     NT_77  0.028418   0.031385  0.024956  0.056686
184     NT_84  0.038012   0.034941  0.031921  0.073133
187     NT_87  0.029659   0.030047  0.028226  0.062630
188     NT_88  0.034934   0.036721  0.026990  0.083816
194     NT_94  0.030844   0.028636  0.029478  0.075226
198     NT_98  0.037734   0.033246  0.029870  0.097928
```

[30]:
```
# Define sets for each selection criterion
set_mean_cv = set(df_nt_cv_summary[df_nt_cv_summary["Mean_CV"] <␣
 ↪mean_cv_threshold]["shRNA_ID"])
set_iqr_cv = set(df_nt_cv_summary[df_nt_cv_summary["IQR_CV"] <␣
 ↪iqr_cv_threshold]["shRNA_ID"])
set_max_cv = set(df_nt_cv_summary[df_nt_cv_summary["Max_CV"] < 3 *␣
 ↪df_nt_cv_summary["Median_CV"]]["shRNA_ID"])

# Create Venn diagram
plt.figure(figsize=(6, 6))
venn = venn3([set_mean_cv, set_iqr_cv, set_max_cv], ('Mean CV', 'IQR CV', 'Max␣
 ↪CV'))
```

```
# Customize colors and labels
plt.title("Venn Diagram of NT shRNA Selection Criteria")
plt.show()
```



1.6 RCC (relative cell count)

For each shRNA (`shRNA_ID`) of each target gene (`Target_Gene`) of each condition (`Sample_Description`), RCC is defined and calculated as ratio of total read counts of the shRNA of the target gene divided by total read counts of (selected) non-target genes

[31]:
```
## Create df_full_nt_sel by filtering NT shRNAs based on df_selected_nt (or␣
↪df_selected_nt_boot_median) and keeping all other target genes
df_full_nt_sel = df_full.copy()

# Use df_selected_nt_boot_median to filter on NT shRNAs
df_nt_filtering = df_selected_nt_boot_median
```

```
# df_nt_filtering = df_selected_nt

df_full_nt_sel = df_full_nt_sel[
    (df_full_nt_sel["Target_Gene"] != "NT") |  # Keep all non-NT genes
    ((df_full_nt_sel["Target_Gene"] == "NT") & df_full_nt_sel["shRNA_ID"].
  ↪isin(df_nt_filtering["shRNA_ID"]))  # Keep only selected NT shRNAs
]
print(df_full_nt_sel.shape)
```

```
(52080, 9)
```

[32]:
```
display(df_full_nt_sel)
```

```
      Target_Gene shRNA_ID Sample_ID  Read_Counts Sample_Description  Day_Tx  \
0              NT    NT_01        D1         1399      T13_Dox_0.6nM       9
1              NT    NT_02        D1         1785      T13_Dox_0.6nM       9
2              NT    NT_03        D1          569      T13_Dox_0.6nM       9
3              NT    NT_04        D1          955      T13_Dox_0.6nM       9
4              NT    NT_05        D1         1150      T13_Dox_0.6nM       9
…             …       …        …          …                 …     …
52483        Zeb1  Zeb1_06       D23          859        T4_Dox_NoTx       0
52484        Zeb1  Zeb1_07       D23         1071        T4_Dox_NoTx       0
52485        Zeb1  Zeb1_08       D23          846        T4_Dox_NoTx       0
52486        Zeb1  Zeb1_09       D23         1973        T4_Dox_NoTx       0
52487        Zeb1  Zeb1_10       D23          691        T4_Dox_NoTx       0

         Tx Dox  Replicate
0       Low   Y          2
1       Low   Y          2
2       Low   Y          2
3       Low   Y          2
4       Low   Y          2
…      …  ..        …
52483  None   Y          1
52484  None   Y          1
52485  None   Y          1
52486  None   Y          1
52487  None   Y          1

[52080 rows x 9 columns]
```

[33]:
```
## Compute sum of Read_Counts for each shRNA_ID of each Target_Gene within each␣
  ↪Sample_Description
df_summed_counts = df_full_nt_sel.groupby(["Sample_Description", "Target_Gene",␣
  ↪"shRNA_ID", "Day_Tx", "Tx", "Dox"])\
    ["Read_Counts"].sum().reset_index()
```

[34]:
```
display(df_summed_counts)
```

```
      Sample_Description Target_Gene  shRNA_ID  Day_Tx    Tx Dox  Read_Counts
0            T10_Dox_0.6nM       Abcb1  Abcb1_01       6   Low   Y         1952
1            T10_Dox_0.6nM       Abcb1  Abcb1_02       6   Low   Y         2035
2            T10_Dox_0.6nM       Abcb1  Abcb1_03       6   Low   Y         1532
3            T10_Dox_0.6nM       Abcb1  Abcb1_04       6   Low   Y         4146
4            T10_Dox_0.6nM       Abcb1  Abcb1_05       6   Low   Y         4395
…                      …           …         …       …   …  ..          …
23865        T7_NoDox_NoTx        Zeb1   Zeb1_06       3  None   N         2160
23866        T7_NoDox_NoTx        Zeb1   Zeb1_07       3  None   N         2688
23867        T7_NoDox_NoTx        Zeb1   Zeb1_08       3  None   N         2416
23868        T7_NoDox_NoTx        Zeb1   Zeb1_09       3  None   N         5227
23869        T7_NoDox_NoTx        Zeb1   Zeb1_10       3  None   N         1724

[23870 rows x 7 columns]
```

[35]:
```
## Calculate RCCs

# Compute the summed Read_Counts across all NT shRNA_IDs for each␣
↪Sample_Description
df_nt_summed = df_summed_counts[df_summed_counts["Target_Gene"] == "NT"]\
    .groupby("Sample_Description")["Read_Counts"].sum().reset_index()

df_rtn = df_summed_counts.merge(df_nt_summed, on="Sample_Description",␣
↪suffixes=("", "_NT"))

# df_rtn = df_summed_counts.merge(
#     df_summed_counts[df_summed_counts["Target_Gene"] ==␣
↪"NT"][["Sample_Description", "Read_Counts"]],
#     on="Sample_Description",
#     suffixes=("", "_NT")
# )

# Compute RTN (Read_Counts / Read_Counts of NT)
df_rtn["RTN"] = df_rtn["Read_Counts"] / df_rtn["Read_Counts_NT"]

# Drop the redundant NT read count column
df_rtn = df_rtn.drop(columns=["Read_Counts_NT"])

df_rtn.to_csv("~/Documents/Projects/Multi_shRNA_screening_AP009/data/
↪shrna_target_gene_relative_tumor_number_table.csv", index = False)
```

[36]:
```
display(df_rtn)
```

```
      Sample_Description Target_Gene  shRNA_ID  Day_Tx    Tx Dox  Read_Counts  \
0            T10_Dox_0.6nM       Abcb1  Abcb1_01       6   Low   Y         1952
1            T10_Dox_0.6nM       Abcb1  Abcb1_02       6   Low   Y         2035
2            T10_Dox_0.6nM       Abcb1  Abcb1_03       6   Low   Y         1532
3            T10_Dox_0.6nM       Abcb1  Abcb1_04       6   Low   Y         4146
```

```
4          T10_Dox_0.6nM       Abcb1  Abcb1_05       6   Low    Y          4395
…                   …          …        …       …    …   ..          …
23865      T7_NoDox_NoTx        Zeb1   Zeb1_06       3  None    N          2160
23866      T7_NoDox_NoTx        Zeb1   Zeb1_07       3  None    N          2688
23867      T7_NoDox_NoTx        Zeb1   Zeb1_08       3  None    N          2416
23868      T7_NoDox_NoTx        Zeb1   Zeb1_09       3  None    N          5227
23869      T7_NoDox_NoTx        Zeb1   Zeb1_10       3  None    N          1724

            RTN
0      0.002824
1      0.002944
2      0.002216
3      0.005998
4      0.006359
…         …
23865  0.002631
23866  0.003274
23867  0.002943
23868  0.006366
23869  0.002100

[23870 rows x 8 columns]
```

[37]:
```
print(df_rtn["Sample_Description"].value_counts())
```

```
T10_Dox_0.6nM      2170
T10_Dox_3.5nM      2170
T10_Dox_Vehicle    2170
T13_Dox_0.6nM      2170
T13_Dox_3.5nM      2170
T13_Dox_Vehicle    2170
T4_Dox_NoTx        2170
T7_Dox_0.6nM       2170
T7_Dox_3.5nM       2170
T7_Dox_Vehicle     2170
T7_NoDox_NoTx      2170
Name: Sample_Description, dtype: int64
```

[38]:
```
# Split shRNA_ID into two parts: the main ID and the repetition number
df_rtn["shRNA_Rep"] = df_rtn["shRNA_ID"].str.split("_").str[1]
```

1.7 Prognostic effects

A vs. B <-> Dox (target gene vs NT) vs. NoDox (target gene vs NT) * ratio of ratios - `T13_Dox_Vehicle` vs. `T7_NoDox_NoTx`

[39]:
```
# Define conditions for A and B
# condition_A = "T7_Dox_Vehicle"
```

```
condition_A = "T13_Dox_Vehicle"
condition_B = "T7_NoDox_NoTx"

# Include the Target_Gene field in both condition datasets before merging
df_A = df_rtn[df_rtn["Sample_Description"] == condition_A][["shRNA_ID",␣
 ↪"Target_Gene", "RTN"]].rename(columns={"RTN": "RTN_A"})
df_B = df_rtn[df_rtn["Sample_Description"] == condition_B][["shRNA_ID",␣
 ↪"Target_Gene", "RTN"]].rename(columns={"RTN": "RTN_B"})

# Merge the two datasets on shRNA_ID and Target_Gene
df_prognostic = df_A.merge(df_B, on=["shRNA_ID", "Target_Gene"], how="inner")

# Compute the prognostic effect (A / B)
df_prognostic["Prognostic_Effect"] = df_prognostic["RTN_A"] /␣
 ↪df_prognostic["RTN_B"]
```

[40]:
```
display(df_prognostic)
```

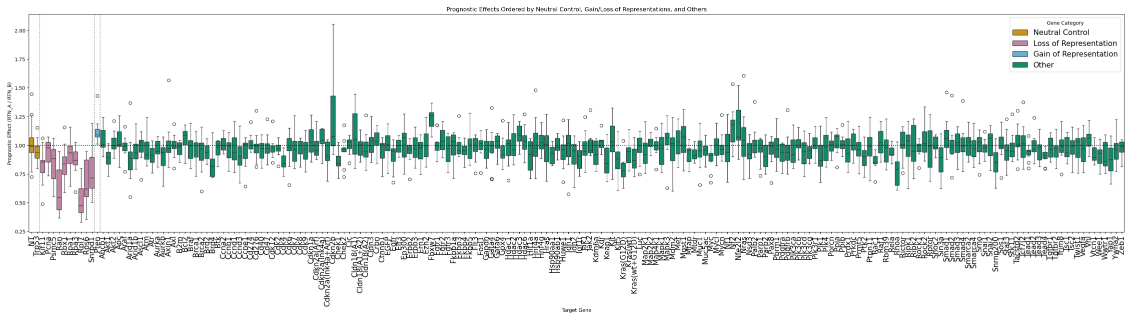
```
      shRNA_ID Target_Gene     RTN_A     RTN_B  Prognostic_Effect
0     Abcb1_01       Abcb1  0.002883  0.003196           0.901969
1     Abcb1_02       Abcb1  0.002869  0.003036           0.944746
2     Abcb1_03       Abcb1  0.002546  0.002592           0.982345
3     Abcb1_04       Abcb1  0.006731  0.005934           1.134384
4     Abcb1_05       Abcb1  0.007275  0.005840           1.245715
…          …         …        …         …                 …
2165   Zeb1_06        Zeb1  0.002152  0.002631           0.817944
2166   Zeb1_07        Zeb1  0.003378  0.003274           1.031843
2167   Zeb1_08        Zeb1  0.003017  0.002943           1.025161
2168   Zeb1_09        Zeb1  0.006237  0.006366           0.979753
2169   Zeb1_10        Zeb1  0.002172  0.002100           1.034449

[2170 rows x 5 columns]
```

[41]:
```
# Define control gene lists
loss_of_representation_target_genes = ["Rpa1", "Rpa3", "Rps6", "Pcna", "Psmc5",␣
 ↪"Rbx1", "Ran", "Snrpd1", "Rpl7", "Kif11"]
neutral_control_target_genes = ["NT", "Trp53"]
gain_of_representation_target_genes = ["Pten"]

# Assign categories for sorting
df_prognostic["Gene_Category"] = "Other"  # Default category
df_prognostic.loc[df_prognostic["Target_Gene"].
 ↪isin(neutral_control_target_genes), "Gene_Category"] = "Neutral Control"
df_prognostic.loc[df_prognostic["Target_Gene"].
 ↪isin(loss_of_representation_target_genes), "Gene_Category"] = "Loss of␣
 ↪Representation"
```

```
df_prognostic.loc[df_prognostic["Target_Gene"].
 ↪isin(gain_of_representation_target_genes), "Gene_Category"] = "Gain of␣
 ↪Representation"

# Sort Target_Gene first by category, then alphabetically within each category
df_prognostic["Sort_Order"] = df_prognostic["Gene_Category"].map({"Neutral␣
 ↪Control": 1,
                                                                   "Loss of␣
 ↪Representation": 2,
                                                                   "Gain of␣
 ↪Representation": 3,
                                                                   "Other": 4})
df_prognostic = df_prognostic.sort_values(by=["Sort_Order", "Target_Gene"])
```

[42]:
```
plt.figure(figsize=(40, 8))

# Use a distinct color palette for better differentiation
palette = {"Neutral Control": "#E69F00",
           "Gain of Representation": "#56B4E9",
           "Loss of Representation": "#CC79A7",
           "Other": "#009E73"}

# Create the boxplot
ax = sns.boxplot(data=df_prognostic, x="Target_Gene", y="Prognostic_Effect",␣
 ↪hue="Gene_Category", dodge=False, palette=palette)

# Add a horizontal reference line at 1.0
plt.axhline(y=1.0, color="black", linestyle="dotted")

# Compute correct category boundaries
neutral_control_count = df_prognostic[df_prognostic["Gene_Category"] ==␣
 ↪"Neutral Control"]["Target_Gene"].nunique()
loss_of_representation_count = df_prognostic[df_prognostic["Gene_Category"] ==␣
 ↪"Loss of Representation"]["Target_Gene"].nunique()
gain_of_representation_count = df_prognostic[df_prognostic["Gene_Category"] ==␣
 ↪"Gain of Representation"]["Target_Gene"].nunique()

# Add vertical dotted lines at the correct positions
plt.axvline(x=neutral_control_count - 0.5, color="gray", linestyle="dotted")  #␣
 ↪End of neutral controls
plt.axvline(x=neutral_control_count + loss_of_representation_count - 0.5,␣
 ↪color="gray", linestyle="dotted")  # End of loss of representation
plt.axvline(x=neutral_control_count + loss_of_representation_count +␣
 ↪gain_of_representation_count - 0.5, color="gray", linestyle="dotted")

# Customize the plot
```

```
plt.xticks(rotation=90, fontsize=10)  # Smaller font size for x-axis labels
plt.xlabel("Target Gene")
plt.ylabel("Prognostic Effect (RTN_A / RTN_B)")
plt.title("Prognostic Effects Ordered by Neutral Control, Gain/Loss of␣
 ↪Representations, and Others")
plt.xticks(rotation = 90, fontsize = 15)

# Move legend inside the plot at the top-right corner
plt.legend(title="Gene Category", loc="upper right", fontsize=15, frameon=True)

# Show the plot
plt.show()
```



[43]:
```
display(df_prognostic)
```

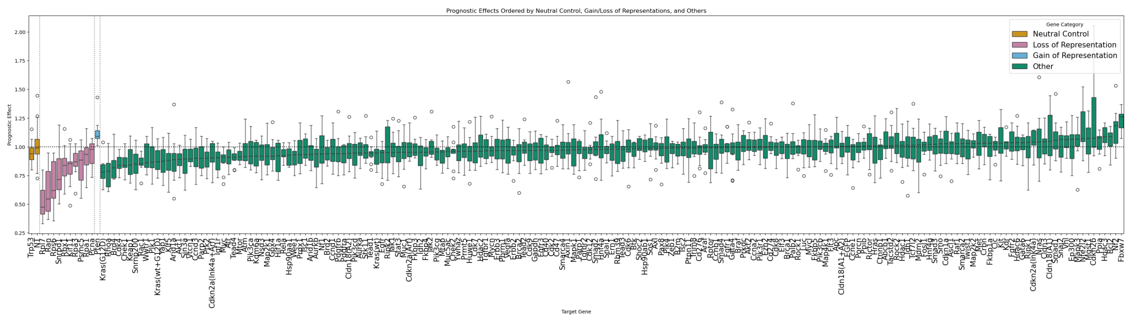
```
     shRNA_ID Target_Gene     RTN_A     RTN_B  Prognostic_Effect  \
1147    NT_01          NT  0.005664  0.005860           0.966613
1148    NT_02          NT  0.008759  0.008220           1.065510
1149    NT_03          NT  0.003065  0.002749           1.114952
1150    NT_04          NT  0.004595  0.004578           1.003659
1151    NT_05          NT  0.005373  0.005452           0.985509
…         …         …         …         …                 …
2165  Zeb1_06        Zeb1  0.002152  0.002631           0.817944
2166  Zeb1_07        Zeb1  0.003378  0.003274           1.031843
2167  Zeb1_08        Zeb1  0.003017  0.002943           1.025161
2168  Zeb1_09        Zeb1  0.006237  0.006366           0.979753
2169  Zeb1_10        Zeb1  0.002172  0.002100           1.034449

        Gene_Category  Sort_Order
1147  Neutral Control           1
1148  Neutral Control           1
1149  Neutral Control           1
1150  Neutral Control           1
1151  Neutral Control           1
…                 …         …
2165            Other           4
```

```
2166              Other           4
2167              Other           4
2168              Other           4
2169              Other           4

[2170 rows x 7 columns]
```

[44]:

```
# Compute median Prognostic_Effect for each Target_Gene within each␣
 ↪Gene_Category
gene_order = (
    df_prognostic.groupby(["Sort_Order", "Target_Gene"])["Prognostic_Effect"]
    .median()
    .reset_index()
    .sort_values(["Sort_Order", "Prognostic_Effect"], ascending=[True, True])
)

# Update Target_Gene with the new categorical order
df_prognostic["Target_Gene"] = pd.Categorical(
    df_prognostic["Target_Gene"],
    categories=gene_order["Target_Gene"],
    ordered=True
)

# Now, re-plot with ordered Target_Gene
plt.figure(figsize=(40, 8))

# Define custom palette
palette = {
    "Neutral Control": "#E69F00",
    "Gain of Representation": "#56B4E9",
    "Loss of Representation": "#CC79A7",
    "Other": "#009E73"
}

# Create the boxplot with the updated Target_Gene order
ax = sns.boxplot(
    data=df_prognostic,
    x="Target_Gene",
    y="Prognostic_Effect",
    hue="Gene_Category",
    dodge=False,
    palette=palette
)

# Add a horizontal reference line
plt.axhline(y=1.0, color="black", linestyle="dotted")
```

```
# Compute correct category boundaries
neutral_control_count = df_prognostic[df_prognostic["Gene_Category"] ==␣
↪"Neutral Control"]["Target_Gene"].nunique()
loss_of_representation_count = df_prognostic[df_prognostic["Gene_Category"] ==␣
↪"Loss of Representation"]["Target_Gene"].nunique()
gain_of_representation_count = df_prognostic[df_prognostic["Gene_Category"] ==␣
↪"Gain of Representation"]["Target_Gene"].nunique()

# Add vertical dotted lines to separate categories
plt.axvline(x=neutral_control_count - 0.5, color="gray", linestyle="dotted")
plt.axvline(x=neutral_control_count + loss_of_representation_count - 0.5,␣
↪color="gray", linestyle="dotted")
plt.axvline(x=neutral_control_count + loss_of_representation_count +␣
↪gain_of_representation_count - 0.5, color="gray", linestyle="dotted")

# Customize the plot
plt.xticks(rotation=90, fontsize=15)
plt.xlabel("Target Gene")
plt.ylabel("Prognostic Effect")
plt.title("Prognostic Effects Ordered by Neutral Control, Gain/Loss of␣
↪Representations, and Others")
plt.legend(title="Gene Category", loc="upper right", fontsize=15, frameon=True)

# Show the plot
plt.show()
```



1.8 Genotype-specific drug (predictive) effects

A vs. B <-> Treated (target gene vs NT) vs. Vehicle (target gene vs NT) * ratio of ratios * two dosages - 0.6nM and 3.5nM * three time point - T7, T10, and T13

[45]:
```
print(df_rtn["Sample_Description"].value_counts())
```

```
T10_Dox_0.6nM      2170
T10_Dox_3.5nM      2170
T10_Dox_Vehicle    2170
```

```
T13_Dox_0.6nM      2170
T13_Dox_3.5nM      2170
T13_Dox_Vehicle    2170
T4_Dox_NoTx        2170
T7_Dox_0.6nM       2170
T7_Dox_3.5nM       2170
T7_Dox_Vehicle     2170
T7_NoDox_NoTx      2170
Name: Sample_Description, dtype: int64
```

[46]:
```
# Define all possible treatment vs vehicle comparisons for predictive effect␣
 ↪calculation
timepoints = ["T7", "T10", "T13"]
dosages = ["0.6nM", "3.5nM"]

# Prepare an empty list to store results
predictive_effect_results = []

# Iterate over each combination of timepoint and dosage
for timepoint in timepoints:
    for dosage in dosages:
        # Define condition labels for treated (A) and vehicle (B)
        condition_A = f"{timepoint}_Dox_{dosage}"
        condition_B = f"{timepoint}_Dox_Vehicle"

        # Filter data for both conditions
        df_A = df_rtn[df_rtn["Sample_Description"] == condition_A][["shRNA_ID",␣
 ↪"Target_Gene", "RTN"]].rename(columns={"RTN": "RTN_A"})
        df_B = df_rtn[df_rtn["Sample_Description"] == condition_B][["shRNA_ID",␣
 ↪"Target_Gene", "RTN"]].rename(columns={"RTN": "RTN_B"})

        # Merge the two datasets on shRNA_ID and Target_Gene
        df_predictive = df_A.merge(df_B, on=["shRNA_ID", "Target_Gene"],␣
 ↪how="inner")

        # Compute the predictive effect (RTN_A / RTN_B)
        df_predictive["Predictive_Effect"] = df_predictive["RTN_A"] /␣
 ↪df_predictive["RTN_B"]

        # Add timepoint and dosage for reference
        df_predictive["Timepoint"] = timepoint
        df_predictive["Dosage"] = dosage

        # Append results
        predictive_effect_results.append(df_predictive)

# Concatenate all results into a single dataframe
```

```
df_predictive_effect = pd.concat(predictive_effect_results, ignore_index=True)
```

[47]:
```
display(df_predictive_effect)
```

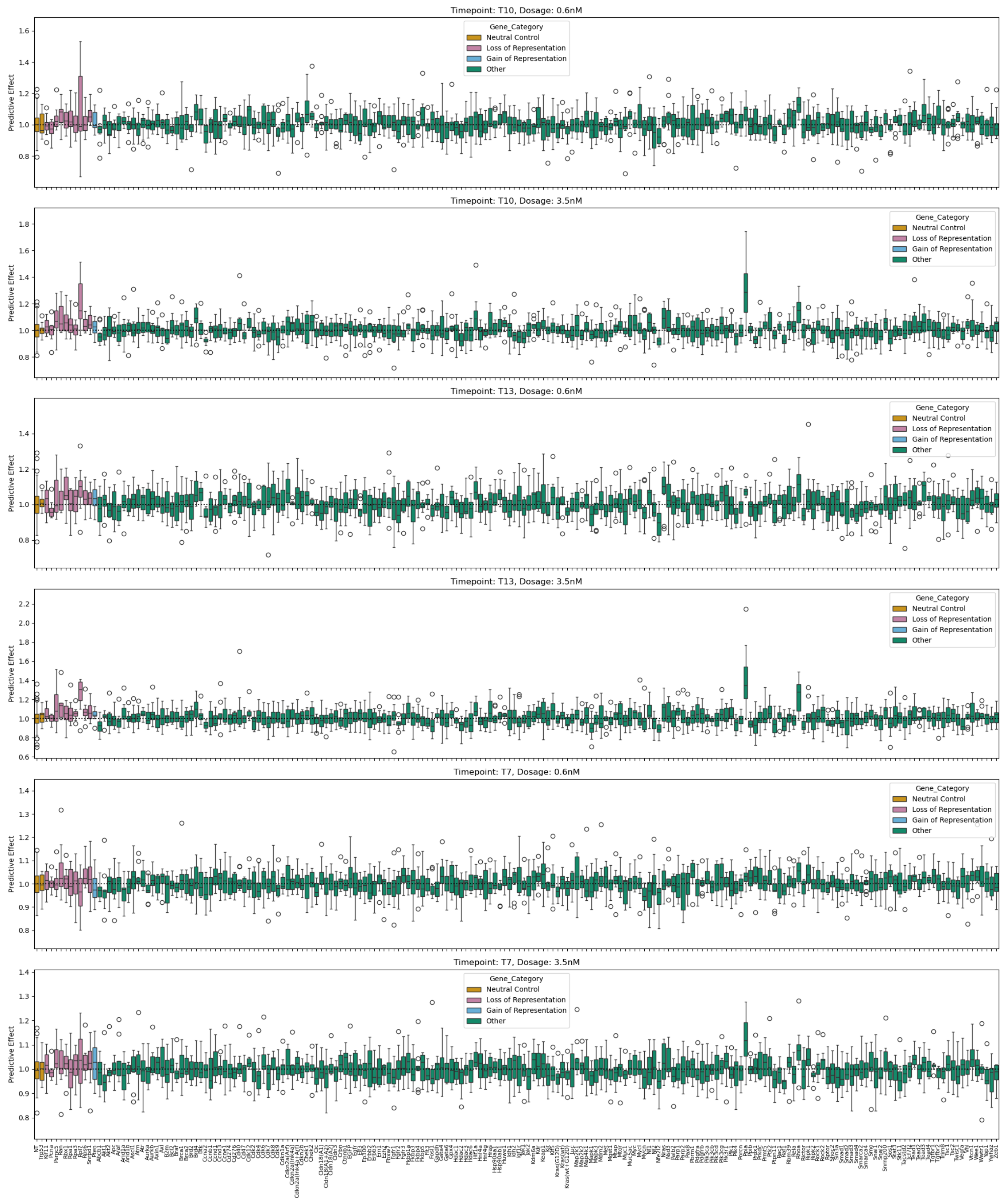
```
       shRNA_ID Target_Gene     RTN_A     RTN_B  Predictive_Effect Timepoint  \
0      Abcb1_01       Abcb1  0.002996  0.003053           0.981311        T7
1      Abcb1_02       Abcb1  0.003000  0.003185           0.942035        T7
2      Abcb1_03       Abcb1  0.002350  0.002373           0.990442        T7
3      Abcb1_04       Abcb1  0.005870  0.006089           0.964018        T7
4      Abcb1_05       Abcb1  0.006047  0.006547           0.923681        T7
…           …         …       …         …                  …       …
13015   Zeb1_06        Zeb1  0.002341  0.002152           1.087975       T13
13016   Zeb1_07        Zeb1  0.003508  0.003378           1.038571       T13
13017   Zeb1_08        Zeb1  0.002686  0.003017           0.890469       T13
13018   Zeb1_09        Zeb1  0.006507  0.006237           1.043175       T13
13019   Zeb1_10        Zeb1  0.002318  0.002172           1.067187       T13

      Dosage
0      0.6nM
1      0.6nM
2      0.6nM
3      0.6nM
4      0.6nM
…      …
13015  3.5nM
13016  3.5nM
13017  3.5nM
13018  3.5nM
13019  3.5nM

[13020 rows x 7 columns]
```

[48]:
```
# Assign categories for sorting
df_predictive_effect["Gene_Category"] = "Other"  # Default category
df_predictive_effect.loc[df_predictive_effect["Target_Gene"].
 ↪isin(neutral_control_target_genes), "Gene_Category"] = "Neutral Control"
df_predictive_effect.loc[df_predictive_effect["Target_Gene"].
 ↪isin(loss_of_representation_target_genes), "Gene_Category"] = "Loss of␣
 ↪Representation"
df_predictive_effect.loc[df_predictive_effect["Target_Gene"].
 ↪isin(gain_of_representation_target_genes), "Gene_Category"] = "Gain of␣
 ↪Representation"


# Sort Target_Gene first by category, then alphabetically within each category
df_predictive_effect["Sort_Order"] = df_predictive_effect["Gene_Category"].
 ↪map({"Neutral Control": 1,
                                                                "Loss of␣
↪Representation": 2,
                                                                "Gain of␣
↪Representation": 3,
                                                                "Other": 4})
df_predictive_effect = df_predictive_effect.sort_values(by=["Sort_Order",␣
↪"Target_Gene"])

# Get unique timepoint-dosage combinations
timepoint_dosage_combinations = df_predictive_effect[["Timepoint", "Dosage"]].
↪drop_duplicates().sort_values(by=["Timepoint", "Dosage"])

print(timepoint_dosage_combinations)
```

```
      Timepoint Dosage
5487        T10  0.6nM
7657        T10  3.5nM
9827        T13  0.6nM
11997       T13  3.5nM
1147         T7  0.6nM
3317         T7  3.5nM
```

[49]:
```
# # Compute correct category boundaries
# neutral_control_count = df_prognostic[df_prognostic["Gene_Category"] ==␣
↪"Neutral Control"]["Target_Gene"].nunique()
# loss_of_representation_count = df_prognostic[df_prognostic["Gene_Category"]␣
↪== "Loss of Representation"]["Target_Gene"].nunique()
# gain_of_representation_count = df_prognostic[df_prognostic["Gene_Category"]␣
↪== "Gain of Representation"]["Target_Gene"].nunique()

# # Add vertical dotted lines at the correct positions
# plt.axvline(x=neutral_control_count - 0.5, color="gray", linestyle="dotted") ␣
↪# End of neutral controls
# plt.axvline(x=neutral_control_count + loss_of_representation_count - 0.5,␣
↪color="gray", linestyle="dotted")  # End of loss of representation
# plt.axvline(x=neutral_control_count + loss_of_representation_count +␣
↪gain_of_representation_count - 0.5, color="gray", linestyle="dotted")

# Define grid size (rows = number of combinations, 1 column)
num_rows = len(timepoint_dosage_combinations)
num_cols = 1

# Create the grid plot with individually scaled y-axes for each subplot
fig, axes = plt.subplots(num_rows, num_cols, figsize=(20, num_rows * 4),␣
↪sharex=True)

# Ensure axes is always a list for iteration
```

```
if num_rows == 1:
    axes = [axes]

# Plot each timepoint-dosage combination in a separate row with individual␣
↪y-axis scaling
for ax, (timepoint, dosage) in zip(axes, timepoint_dosage_combinations.
↪itertuples(index=False)):
    subset = df_predictive_effect[(df_predictive_effect["Timepoint"] ==␣
↪timepoint) & (df_predictive_effect["Dosage"] == dosage)]

    sns.boxplot(data=subset, x="Target_Gene", y="Predictive_Effect",␣
↪hue="Gene_Category", dodge=False, ax=ax,
                palette=palette)

    ax.axhline(y=1.0, color="black", linestyle="dotted")  # Add reference line␣
↪at 1.0
    ax.set_title(f"Timepoint: {timepoint}, Dosage: {dosage}", fontsize=12)
    ax.set_xlabel("")
    ax.set_ylabel("Predictive Effect")
    ax.tick_params(axis='x', rotation=90, labelsize=8)

    # Adjust y-axis range dynamically based on the subset
    ax.set_ylim(subset["Predictive_Effect"].min() * 0.9,␣
↪subset["Predictive_Effect"].max() * 1.1)

# Adjust layout for better spacing
plt.tight_layout()
plt.show()
```



```
[50]: df_predictive_effect.to_csv("~/Documents/Projects/Multi_shRNA_screening_AP009/
      ↪data/predictive_effect.csv", index = False)
```