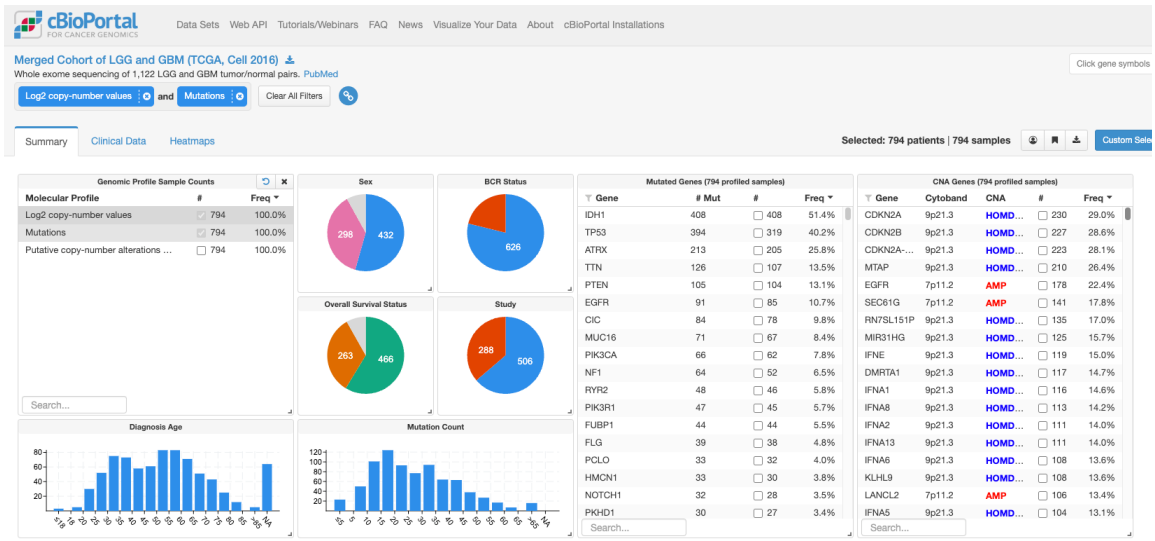
In [1]:
```
import os
import flexynesis
import torch
import numpy as np
import seaborn as sns
import pandas as pd
import random
import lightning as pl
import matplotlib.pyplot as plt

torch.set_num_threads(16)
os.environ["OMP_NUM_THREADS"] = "16"
```

```
[rank: 0] Seed set to 42
```

Finding Survival Markers in Lower Grade Glioma (LGG) and Glioblastoma Multiforme (GBM)seed



Here, we demonstrate the capabilities of `flexynesis` on a multi-omic dataset of 506 Brain Lower Grade Glioma (LGG) and 288 Glioblastoma Multiforme (GBM) samples with matching mutation and copy number alteration data downloaded from the cbioportal. The data was split into `train` (70% of the samples) and `test` (30% of the samples) data folders. The data files were processed to follow the same nomenclature.

- `cna.csv` contains "copy number alteration" data
- `mut.csv` contains "mutation" data, which is a binary matrix of genes versus samples.
- `clin.csv` contains "clinical/sample metatada", which is a table of clinical parameters such as age, sex, disease type, histological diagnosis, and overall survival time and status.

Data Download

The data can be downloaded as follows:

In [2]:
```
if not os.path.exists("lgggbm_tcga_pub_processed"):
    !wget -O lgggbm_tcga_pub_processed.tgz "https://bimsbstatic.mdc-berlin.de/akali
```

Importing Train and Test Datasets

We import train and test datasets including mutations and copy number alterations. We rank genes by Laplacian Scores and pick top 10% of the genes, while removing highly redundant genes with a correlation score threshold of 0.8 and a variance threshold of 50%. By setting `concatenate` to `False`, we will be doing an `intermediate` fusion of omic layers.

In [3]:
```
data_importer = flexynesis.DataImporter(path ='lgggbm_tcga_pub_processed',
                                        data_types = ['mut', 'cna'], log_transform=
                                        concatenate=False, top_percentile=10, min_f
                                       variance_threshold=0.5)
train_dataset, test_dataset = data_importer.import_data()
```

```
[INFO] ================= Importing Data =================
[INFO] Validating data folders...

[INFO] ----------------- Reading Data -----------------
[INFO] Importing lgggbm_tcga_pub_processed/train/clin.csv...
[INFO] Importing lgggbm_tcga_pub_processed/train/cna.csv...
[INFO] Importing lgggbm_tcga_pub_processed/train/mut.csv...

[INFO] ----------------- Reading Data -----------------
[INFO] Importing lgggbm_tcga_pub_processed/test/clin.csv...
[INFO] Importing lgggbm_tcga_pub_processed/test/cna.csv...
[INFO] Importing lgggbm_tcga_pub_processed/test/mut.csv...

[INFO] ----------------- Checking for problems with the input data -----------------
[INFO] Data structure is valid with no errors or warnings.

[INFO] ----------------- Processing Data (train) -----------------

[INFO] ----------------- Cleaning Up Data -----------------

[INFO] working on layer:  mut
[INFO] Number of NA values:  0
[INFO] DataFrame mut - Removed 5561 features.

[INFO] working on layer:  cna
[INFO] Number of NA values:  0
[INFO] DataFrame cna - Removed 12375 features.
[INFO] DataFrame mut - Removed 0 samples (0.00%).
[INFO] DataFrame cna - Removed 0 samples (0.00%).
[INFO] Implementing feature selection using laplacian score for layer: mut with  550
3 features  and  556  samples
Calculating Laplacian scores: 100%|██████████| 5503/5503 [00:00<00:00, 17718.34it/s]
Filtering redundant features: 100%|██████████| 1000/1000 [00:00<00:00, 12763.97it/s]
[INFO] Implementing feature selection using laplacian score for layer: cna with  123
71 features  and  556  samples
Calculating Laplacian scores: 100%|██████████| 12371/12371 [00:00<00:00, 19193.72it/
s]
Filtering redundant features: 100%|██████████| 1237/1237 [00:00<00:00, 217559.29it/
s]
```

```
[INFO] ----------------- Processing Data (test) -----------------

[INFO] ----------------- Cleaning Up Data -----------------

[INFO] working on layer:  mut
[INFO] Number of NA values:  0
[INFO] DataFrame mut - Removed 5627 features.

[INFO] working on layer:  cna
[INFO] Number of NA values:  0
[INFO] DataFrame cna - Removed 12382 features.
[INFO] DataFrame mut - Removed 0 samples (0.00%).
[INFO] DataFrame cna - Removed 0 samples (0.00%).

[INFO] ----------------- Harmonizing Data Sets -----------------

[INFO] ----------------- Finished Harmonizing -----------------

[INFO] ----------------- Normalizing Data -----------------

[INFO] ----------------- Normalizing Data -----------------
[INFO] Training Data Stats:  {'feature_count in: mut': 319, 'feature_count in: cna':
1237, 'sample_count': 556}
[INFO] Test Data Stats:  {'feature_count in: mut': 319, 'feature_count in: cna': 123
7, 'sample_count': 238}
[INFO] Merging Feature Logs...
[INFO] Data import successful.
```

1. Exploratory Data Analysis

Before building any machine learning models on the data, it is important to first familiarize yourself with the data you are working with. It is important to know the available data matrices, their sizes/shapes, available clinical variables and how they are distributed.

Below you are asked to do simple explorations of the available data.

1.1 Print the shapes of the available data matrices

- How many features and samples are available per data type in train/test datasets?

In [4]:
```
train_dataset.dat
```

Out[4]:
```
{'mut': tensor([[ 0.9822, -0.1485, -0.1721,  ..., -0.0601, -0.0601, -0.0601],
        [ 0.9822, -0.1485, -0.1721,  ..., -0.0601, -0.0601, -0.0601],
        [ 0.9822, -0.1485, -0.1721,  ..., -0.0601, -0.0601, -0.0601],
        ...,
        [-1.0182, -0.1485, -0.1721,  ..., -0.0601, -0.0601, -0.0601],
        [ 0.9822, -0.1485, -0.1721,  ..., -0.0601, -0.0601, -0.0601],
        [ 0.9822, -0.1485, -0.1721,  ..., -0.0601, -0.0601, -0.0601]]),
 'cna': tensor([[-0.2331, -0.7289, -0.8512,  ..., -2.0271, -0.8729, -0.8729],
        [ 1.9636,  0.9814,  0.9936,  ...,  0.4157,  0.9417,  0.9417],
        [-0.2383, -0.2835, -0.3707,  ...,  0.4020, -0.4003, -0.4003],
        ...,
        [-0.2278,  0.6431,  0.6287,  ...,  0.4020,  0.5828,  0.5828],
        [-0.2436, -0.7416, -0.8806,  ...,  0.4047, -0.9019, -0.9019],
        [-0.2489, -0.7479, -0.8716,  ..., -1.6977, -0.8930, -0.8930]])}
```

In [5]:
```
train_dataset.dat['cna'].shape, train_dataset.dat['mut'].shape
```

Out[5]:
```
(torch.Size([556, 1237]), torch.Size([556, 319]))
```

In [6]:
```
test_dataset.dat['cna'].shape, test_dataset.dat['mut'].shape
```

Out[6]:
```
(torch.Size([238, 1237]), torch.Size([238, 319]))
```

1.2 Explore sample annotations

- What are the available clinical variables? Are they available in both train and test datasets? (See .ann)

In [7]:
```
train_dataset.ann.keys()
```

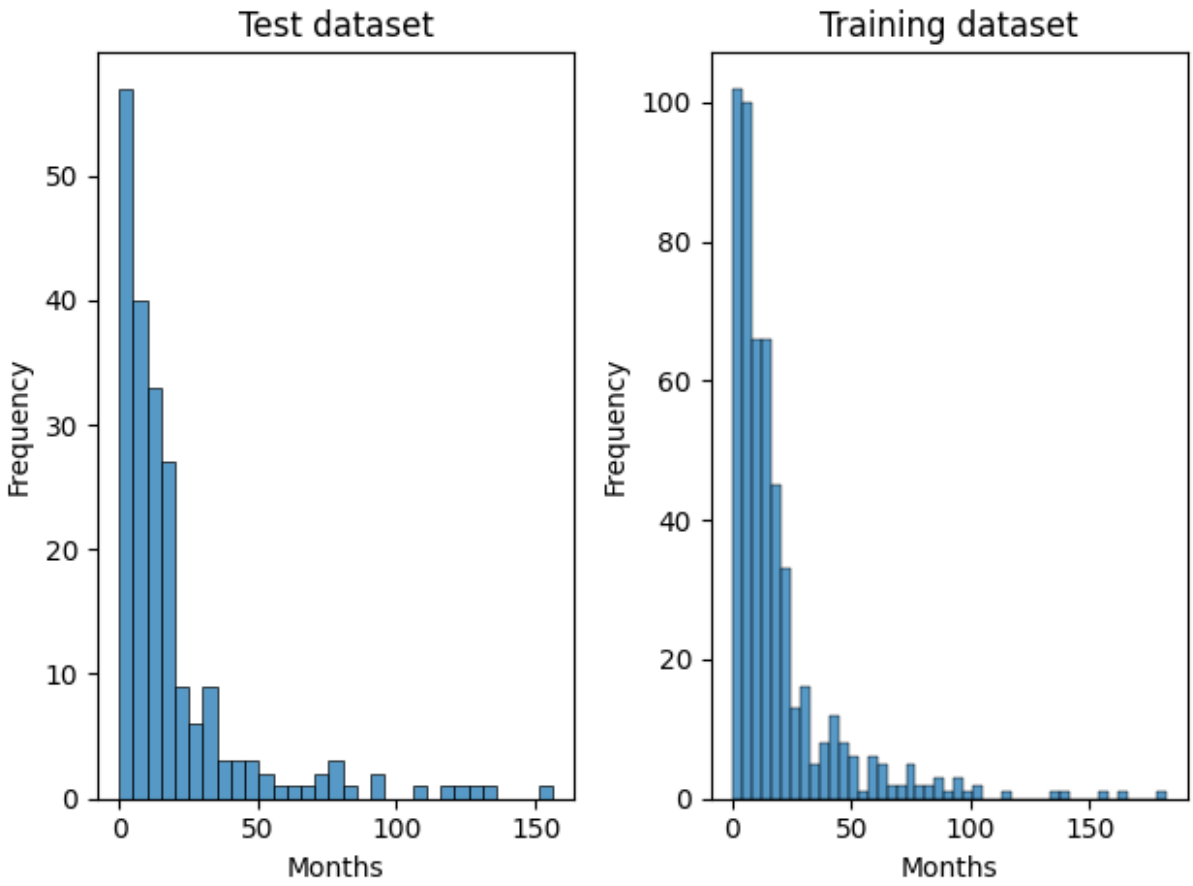
Out[7]:
```
dict_keys(['AGE', 'OS_MONTHS', 'OS_STATUS', 'KARNOFSKY_PERFORMANCE_SCORE', 'STUDY'
, 'BCR_STATUS', 'HISTOLOGICAL_DIAGNOSIS', 'SEX'])
```

In [8]:
```
test_dataset.ann.keys()
```

Out[8]:
```
dict_keys(['AGE', 'OS_MONTHS', 'OS_STATUS', 'KARNOFSKY_PERFORMANCE_SCORE', 'STUDY'
, 'BCR_STATUS', 'HISTOLOGICAL_DIAGNOSIS', 'SEX'])
```

- Make a histogram plot of the follow up times in months (OS_MONTHS) (use sns.histplot)

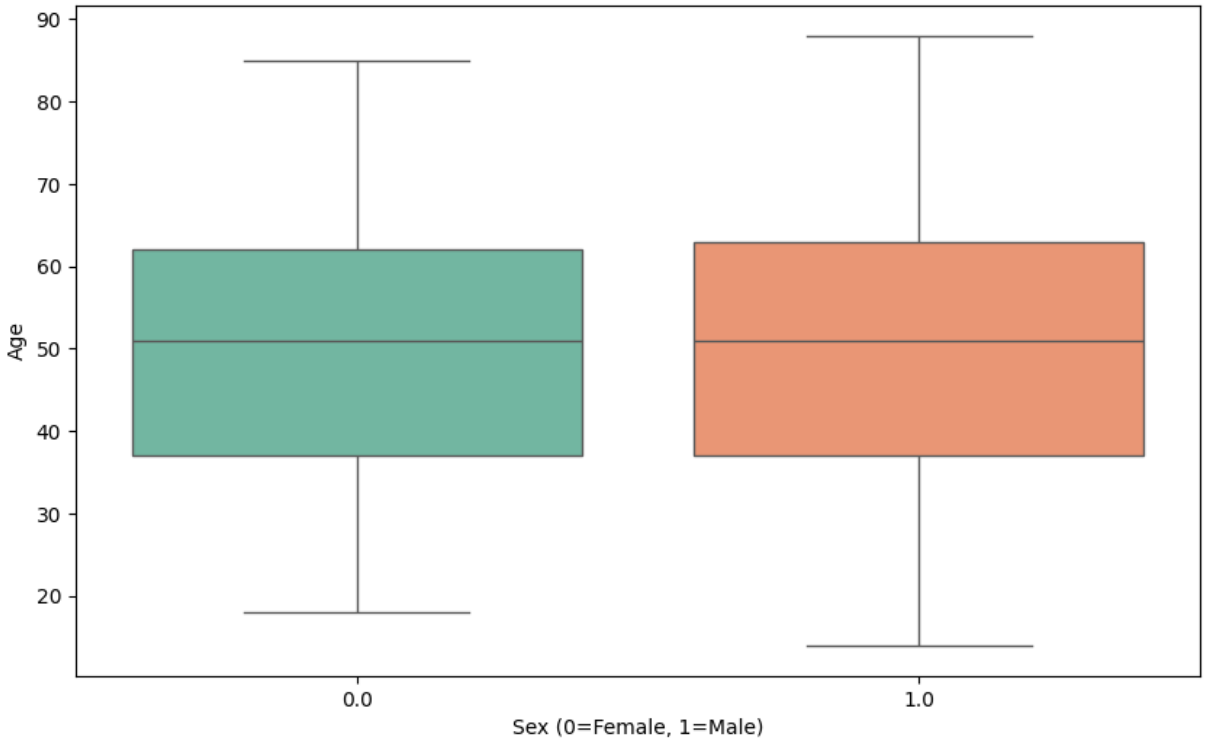
In [9]:
```
plt.subplot(1, 2, 1)
test_figure=sns.histplot(test_dataset.ann["OS_MONTHS"])
test_figure.set(xlabel='Months', ylabel='Frequency',title="Test dataset")
plt.subplot(1, 2, 2)
train_figure=sns.histplot(train_dataset.ann["OS_MONTHS"])
train_figure.set(xlabel='Months', ylabel='Frequency',title="Training dataset")
plt.tight_layout()
```



- Make a histogram of the age distribution of the patients in the training data; facet the histogram by "SEX" variable (see flexynesis.utils.plot_boxplot)

In [10]:
```
flexynesis.utils.plot_boxplot(train_dataset.ann["SEX"],train_dataset.ann["AGE"], ti
```

```
/usr/local/lib/python3.11/site-packages/flexynesis/utils.py:155: FutureWarning:

Passing `palette` without assigning `hue` is deprecated and will be removed in v0.14
.0. Assign the `x` variable to `hue` and set `legend=False` for the same effect.
```



- Make a summary of all available clinical variables (see flexynesis.print_summary_stats)

In [11]:
```
flexynesis.print_summary_stats(test_dataset)
```

```
Summary for variable: AGE
Numerical Variable Summary: Median = 52.0, Mean = 49.88516746411483
------
Summary for variable: OS_MONTHS
Numerical Variable Summary: Median = 10.9, Mean = 19.508133971291866
------
Summary for variable: OS_STATUS
Numerical Variable Summary: Median = 0.0, Mean = 0.3588516746411483
------
Summary for variable: KARNOFSKY_PERFORMANCE_SCORE
Numerical Variable Summary: Median = 90.0, Mean = 83.46456692913385
------
Summary for variable: STUDY
Categorical Variable Summary:
  Label: Brain Lower Grade Glioma, Count: 153
  Label: Glioblastoma multiforme, Count: 85
------
Summary for variable: BCR_STATUS
Categorical Variable Summary:
  Label: IGC, Count: 172
  Label: NCH, Count: 66
------
Summary for variable: HISTOLOGICAL_DIAGNOSIS
Categorical Variable Summary:
  Label: astrocytoma, Count: 52
  Label: glioblastoma, Count: 81
  Label: oligoastrocytoma, Count: 33
  Label: oligodendroglioma, Count: 43
  Label: nan, Count: 29
------
Summary for variable: SEX
Categorical Variable Summary:
  Label: Female, Count: 89
  Label: Male, Count: 120
  Label: nan, Count: 29
------
```

- Notice that the categorical variables such as "SEX", "STUDY", "HISTOLOGICAL_DIAGNOSIS" are encoded numerically in the "dataset.ann" objects. Use dataset.label_mappings to map the STUDY variable to their original labels. Print the top 10 values in dataset.ann['STUDY'] and the mapped label values.

In [12]:
```
[test_dataset.label_mappings["STUDY"][x] for x in test_dataset.ann["STUDY"].numpy()
```

Out[12]:
```
['Glioblastoma multiforme',
 'Brain Lower Grade Glioma',
 'Brain Lower Grade Glioma',
 'Brain Lower Grade Glioma',
 'Brain Lower Grade Glioma',
 'Brain Lower Grade Glioma',
 'Glioblastoma multiforme',
 'Glioblastoma multiforme',
 'Brain Lower Grade Glioma',
 'Brain Lower Grade Glioma']
```

- Now, let's explore the data matrices. Make a PCA plot of the mutation data matrix and color the samples by "HISTOLOGICAL_DIAGNOSIS". See flexynesis.plot_dim_reduced function

First create a pandas data frame with the data matrix of interest with feature and sample names

> df = pd.DataFrame(train_dataset.dat['cna'], index = train_dataset.samples, columns= train_dataset.features['cna'])

Check the data frame contents

> df.head()

Make a PCA plot of CNA values using the labels from the STUDY variable

Note: if you couldn't map the labels above, you can also use train_dataset.dat['STUDY'] as labels

In [13]:
```
df = pd.DataFrame(train_dataset.dat['cna'], index = train_dataset.samples, columns=
```

In [14]:
```
df.head()
```

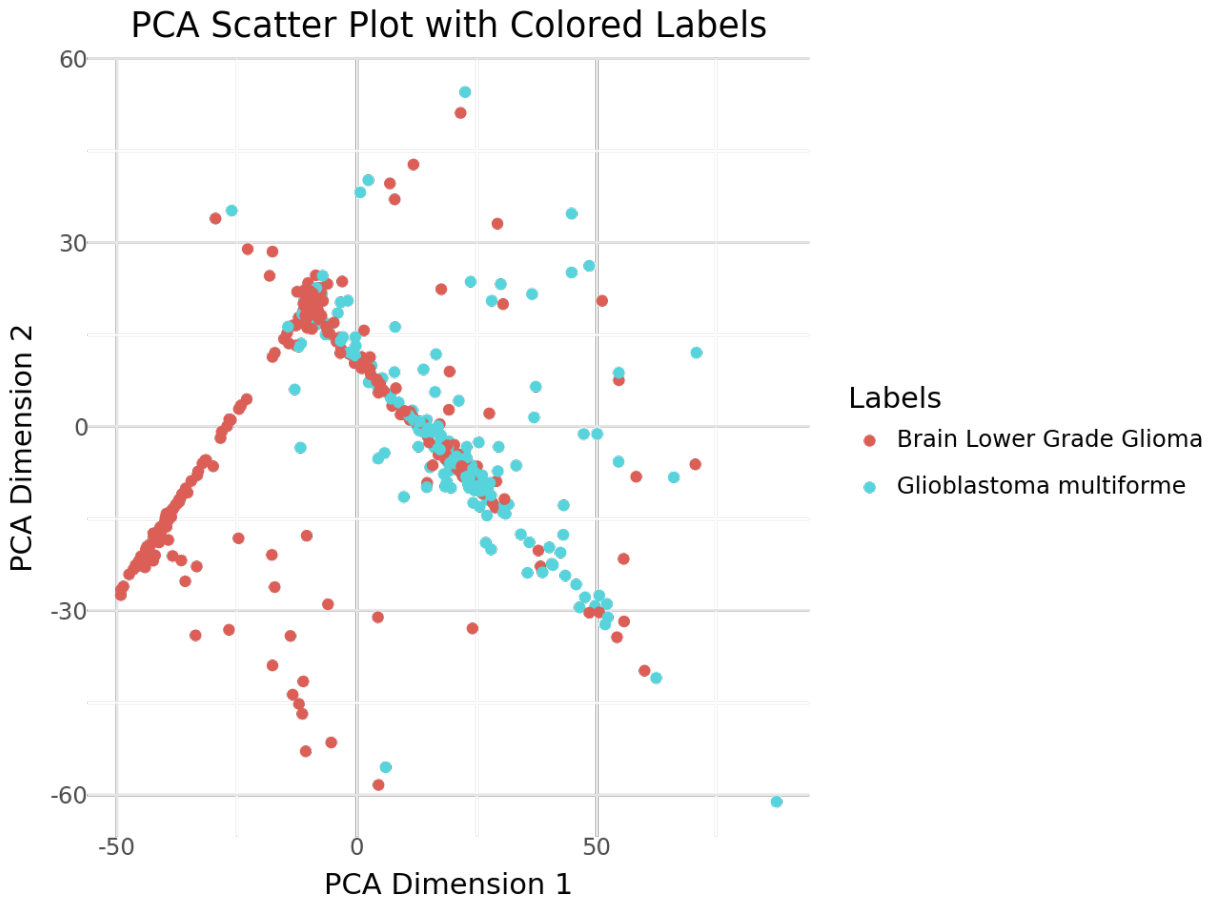
Out[14]:

	SLC30A8	ZNF273	CLEC5A	AGL	KCNA5	MIR603	SNTB1
TCGA-DB-5279	-0.233088	-0.728944	-0.851171	-2.066100	-1.377437	-0.417790	-0.241656
TCGA-TQ-A7RH	1.963561	0.981436	0.993554	0.457244	-0.108664	1.315678	1.990121
TCGA-DU-7011	-0.238350	-0.283489	-0.370727	0.375758	-0.310816	0.526311	-0.247002
TCGA-06-5415	-0.238350	0.788125	0.785059	0.381190	1.359602	-0.740713	-0.247002
TCGA-HT-A616	7.545943	0.691470	0.680812	0.375758	-0.220380	0.575646	7.661775

5 rows × 1237 columns

In [15]:
```
labels = [train_dataset.label_mappings["STUDY"][x] for x in train_dataset.ann["STUD
```

In [16]:
```
flexynesis.plot_dim_reduced(df, labels, color_type = 'categorical', method='pca')
```



- (Optional exercise ideas):
  - Make a PCA plot coloring the samples by HISTOLOGICAL_DIAGNOSIS, GENDER, or any other clinical variable
  - Repeat the same exercise on the mutation data matrix.

2. Training a single model using manually set hyperparameters

Now that we have familiarized ourselves with the dataset at hand, we can start building models.

First we will do a single model training by manually setting hyperparameters. Based on the model performance, we will try modifying individual hyperparameters and build more and more models and see if we can improve model performance.

We will need to define the following components for starting a model training:

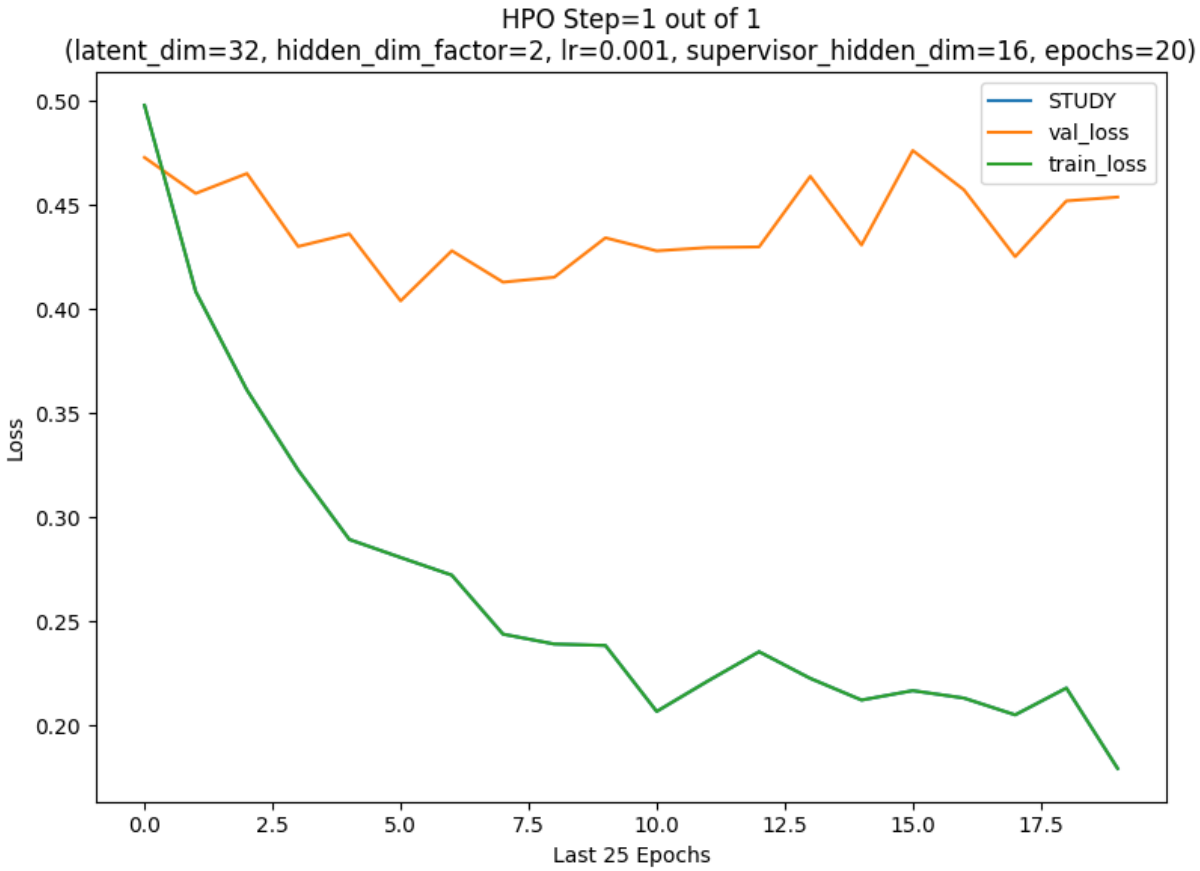
1. Split the train_dataset into train/validation components
2. Define data loaders for both train and validation splits
3. Define a pytorch-lightning trainer
4. Define a model with hyperparameters
5. Fit the model

In [17]:
```
# randomly assign 80% of samples for training, 20% for validation
train_indices = random.sample(range(0, len(train_dataset)), int(len(train_dataset)
val_indices = list(set(range(len(train_dataset))) - set(train_indices))
train_subset = train_dataset.subset(train_indices)
val_subset = train_dataset.subset(val_indices)

# define data loaders for train/validation splits
from torch.utils.data import DataLoader
train_loader = DataLoader(train_subset, batch_size=32, shuffle=True)
val_loader = DataLoader(val_subset, batch_size=32, shuffle = False)
```

Now, we need to define a model with manually set hyperparameters and a lightning-trainer fit the model.

Notice: Notice the callback we are passing to the trainer which enables us to plot the loss values as the training progresses.

In [18]:
```
# Define a model with manually set hyperparameters for the DirectPred model
myparams = {'latent_dim': 32, 'hidden_dim_factor': 2, 'lr': 0.001, 'supervisor_hidd
model = flexynesis.DirectPred(config = myparams, dataset = train_dataset, target_va
trainer = pl.Trainer(max_epochs=myparams['epochs'], default_root_dir="./", logger=F
                     callbacks=[flexynesis.LiveLossPlot(myparams, 1, 1)])
# Fit the model
trainer.fit(model, train_loader, val_loader)
```




```
`Trainer.fit` stopped: `max_epochs=20` reached.
```

While we can observe how well the model training went based on the "loss" values, we can also evaluate the model performance on test dataset

In [19]:
```
# evaluate the model performance on predicting the target variable
flexynesis.evaluate_wrapper("DirectPred", model.predict(test_dataset), test_dataset
```

Out[19]:

	method	var	variable_type	metric	value
0	DirectPred	STUDY	categorical	balanced_acc	0.801961
1	DirectPred	STUDY	categorical	f1_score	0.819087
2	DirectPred	STUDY	categorical	kappa	0.605505
3	DirectPred	STUDY	categorical	average_auroc	0.871819
4	DirectPred	STUDY	categorical	average_aupr	0.772257

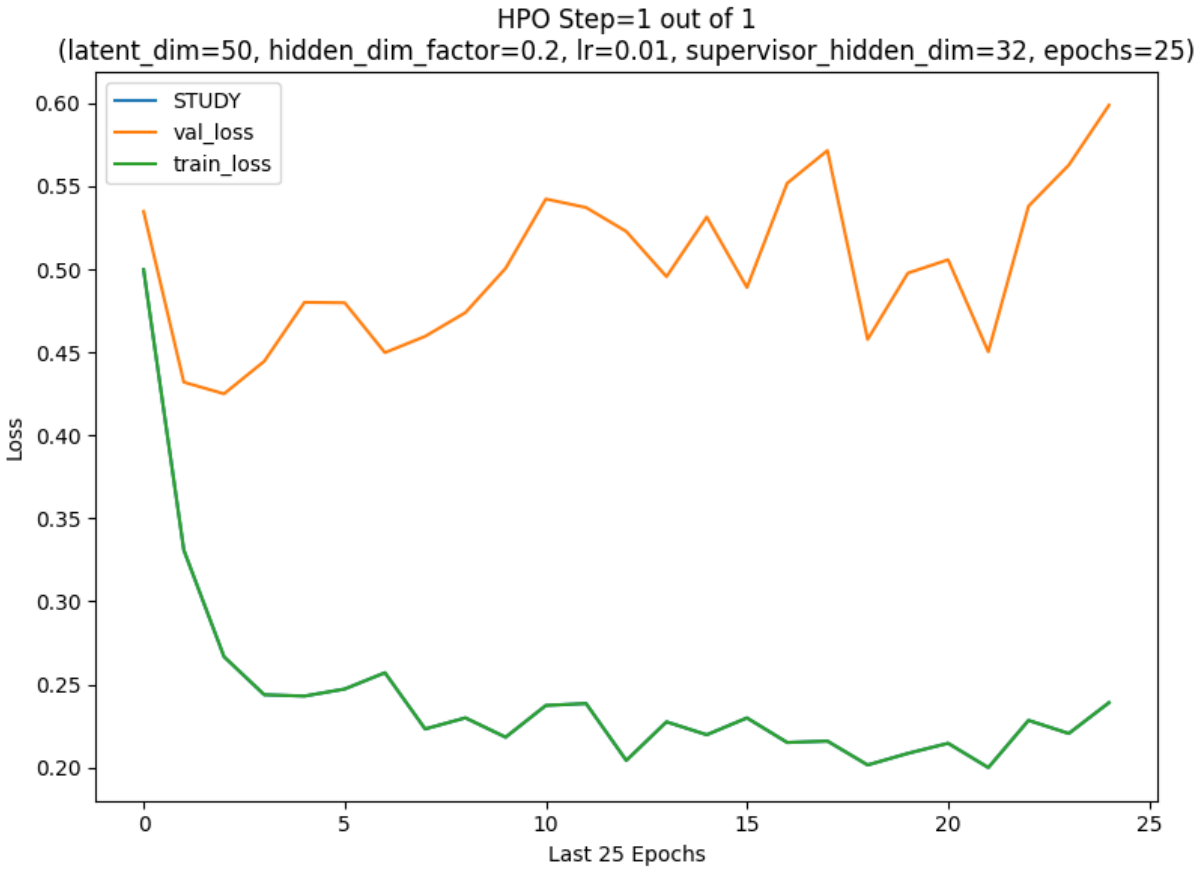
2.1 Exercise

- Now, repeat the above model training and evaluation by manually changing the hyperparameters (Try at least 5 different combinations)
- See if you can find a better hyperparameter combination that yields a better classification performance than the initial setup we provided.

- See the default hyperparameter ranges we use for Flexynesis here: https://github.com/BIMSBbioinfo/flexynesis/blob/69b92ca9370551e9fcc82a756cb42c72bef4a4b1/flexynesis/config.py#L7, but feel free to try outside these ranges too.
- Also try to observe the impact of the changing parameters on how the train/validation loss curves change.

```
myparams = {'latent_dim': XX, 'hidden_dim_factor': XX, 'lr': XX,
'supervisor_hidden_dim': XX, 'epochs': XX}

model = flexynesis.DirectPred(config = myparams, dataset =
train_dataset, target_variables=['STUDY'])

trainer = pl.Trainer(max_epochs=myparams['epochs'],
default_root_dir="./", logger=False, enable_checkpointing=False,
                     callbacks=[flexynesis.LiveLossPlot(myparams,
1, 1)])

trainer.fit(model, train_loader, val_loader)

flexynesis.evaluate_wrapper("DirectPred",
model.predict(test_dataset), test_dataset)
```

In [20]:
```
myparams = {'latent_dim': 50, 'hidden_dim_factor': 0.2, 'lr': 0.01, 'supervisor_hid
model = flexynesis.DirectPred(config = myparams, dataset = train_dataset, target_va
trainer = pl.Trainer(max_epochs=myparams['epochs'], default_root_dir="./", logger=F
                     callbacks=[flexynesis.LiveLossPlot(myparams, 1, 1)])

trainer.fit(model, train_loader, val_loader)
flexynesis.evaluate_wrapper("DirectPred", model.predict(test_dataset), test_dataset
```

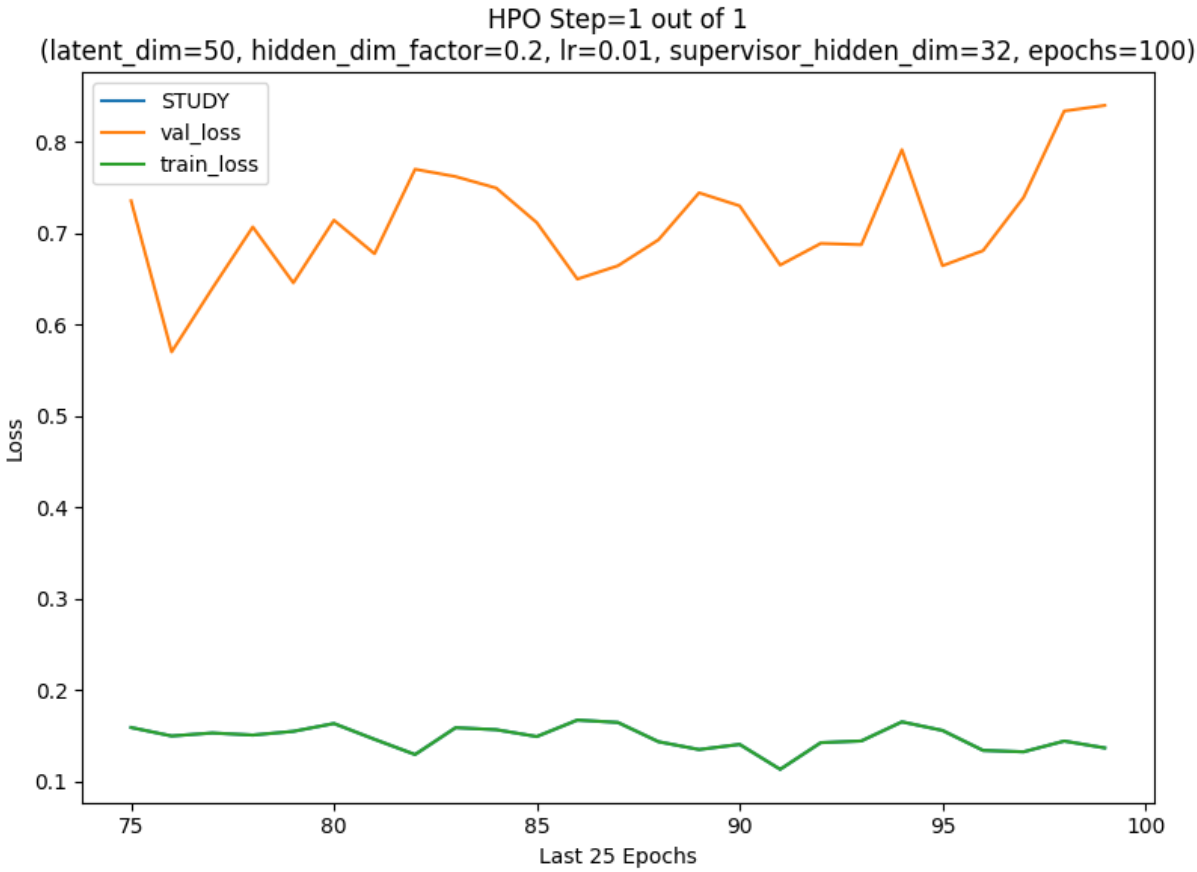



```
`Trainer.fit` stopped: `max_epochs=25` reached.
```

Out[20]:

	method	var	variable_type	metric	value
0	DirectPred	STUDY	categorical	balanced_acc	0.737908
1	DirectPred	STUDY	categorical	f1_score	0.771739
2	DirectPred	STUDY	categorical	kappa	0.495238
3	DirectPred	STUDY	categorical	average_auroc	0.839369
4	DirectPred	STUDY	categorical	average_aupr	0.697824

In [21]:
```
#same as above, but 100 epochs instead of 25
myparams = {'latent_dim': 50, 'hidden_dim_factor': 0.2, 'lr': 0.01, 'supervisor_hid
model = flexynesis.DirectPred(config = myparams, dataset = train_dataset, target_va
trainer = pl.Trainer(max_epochs=myparams['epochs'], default_root_dir="./", logger=F
                     callbacks=[flexynesis.LiveLossPlot(myparams, 1, 1)])

trainer.fit(model, train_loader, val_loader)
flexynesis.evaluate_wrapper("DirectPred", model.predict(test_dataset), test_dataset
```

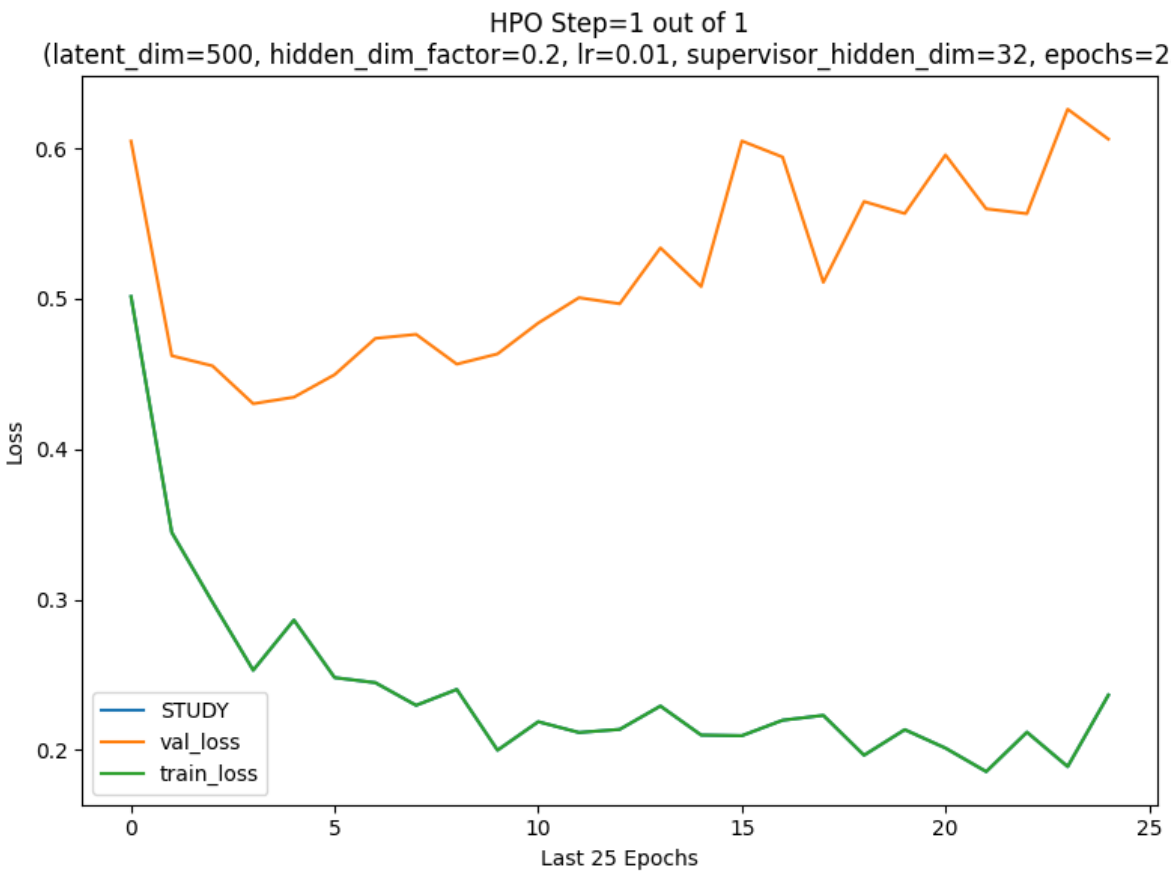



```
`Trainer.fit` stopped: `max_epochs=100` reached.
```

Out[21]:

	method	var	variable_type	metric	value
0	DirectPred	STUDY	categorical	balanced_acc	0.761438
1	DirectPred	STUDY	categorical	f1_score	0.790568
2	DirectPred	STUDY	categorical	kappa	0.538358
3	DirectPred	STUDY	categorical	average_auroc	0.868973
4	DirectPred	STUDY	categorical	average_aupr	0.752242

In [22]:
```
myparams = {'latent_dim': 500, 'hidden_dim_factor': 0.2, 'lr': 0.01, 'supervisor_hi
model = flexynesis.DirectPred(config = myparams, dataset = train_dataset, target_va
trainer = pl.Trainer(max_epochs=myparams['epochs'], default_root_dir="./", logger=F
                     callbacks=[flexynesis.LiveLossPlot(myparams, 1, 1)])

trainer.fit(model, train_loader, val_loader)
flexynesis.evaluate_wrapper("DirectPred", model.predict(test_dataset), test_dataset
```

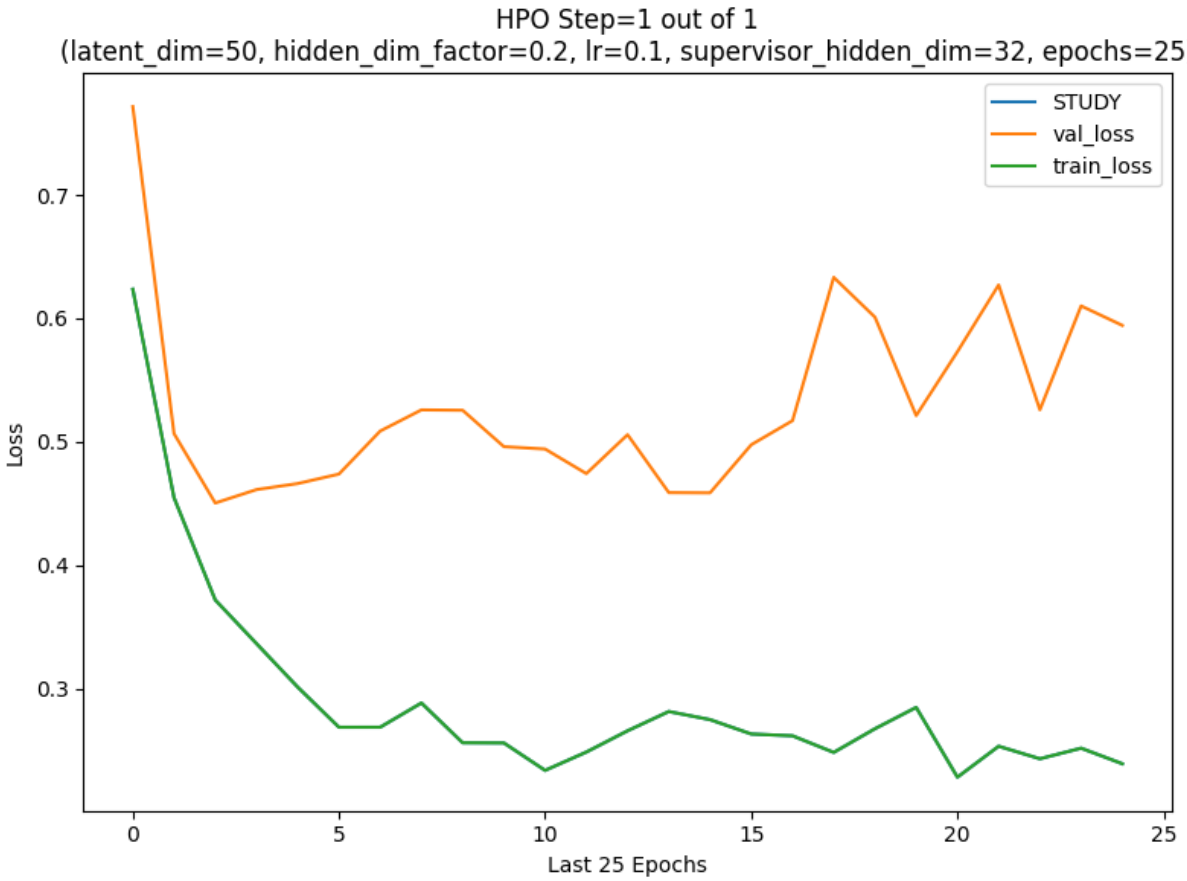



```
`Trainer.fit` stopped: `max_epochs=25` reached.
```

Out[22]:

	method	var	variable_type	metric	value
0	DirectPred	STUDY	categorical	balanced_acc	0.794118
1	DirectPred	STUDY	categorical	f1_score	0.804114
2	DirectPred	STUDY	categorical	kappa	0.577664
3	DirectPred	STUDY	categorical	average_auroc	0.858208
4	DirectPred	STUDY	categorical	average_aupr	0.727742

In [23]:
```
myparams = {'latent_dim': 50, 'hidden_dim_factor': 0.2, 'lr': 0.1, 'supervisor_hidd
model = flexynesis.DirectPred(config = myparams, dataset = train_dataset, target_va
trainer = pl.Trainer(max_epochs=myparams['epochs'], default_root_dir="./", logger=F
                     callbacks=[flexynesis.LiveLossPlot(myparams, 1, 1)])

trainer.fit(model, train_loader, val_loader)
flexynesis.evaluate_wrapper("DirectPred", model.predict(test_dataset), test_dataset
```

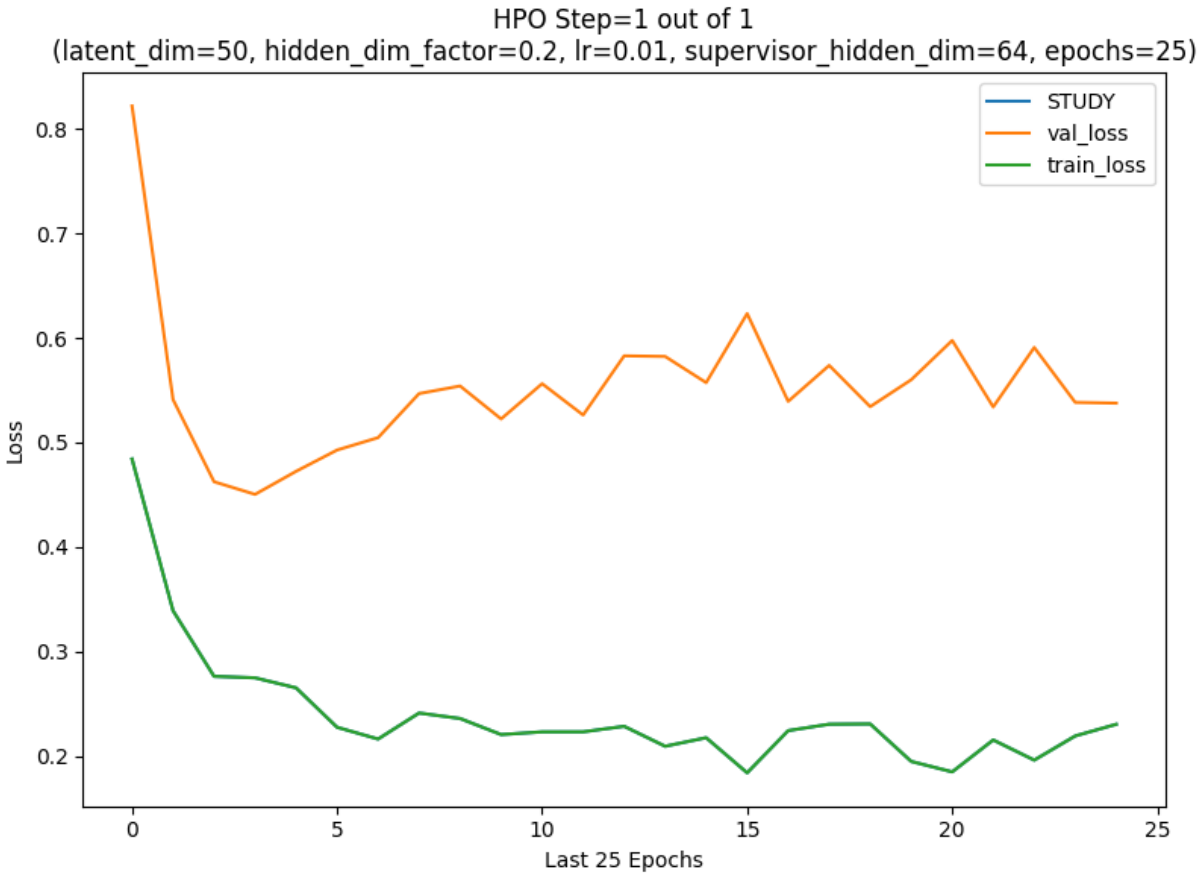



```
`Trainer.fit` stopped: `max_epochs=25` reached.
```

Out[23]:

	method	var	variable_type	metric	value
0	DirectPred	STUDY	categorical	balanced_acc	0.726144
1	DirectPred	STUDY	categorical	f1_score	0.762150
2	DirectPred	STUDY	categorical	kappa	0.473324
3	DirectPred	STUDY	categorical	average_auroc	0.838754
4	DirectPred	STUDY	categorical	average_aupr	0.706799

In [24]:
```
myparams = {'latent_dim': 50, 'hidden_dim_factor': 0.2, 'lr': 0.01, 'supervisor_hid
model = flexynesis.DirectPred(config = myparams, dataset = train_dataset, target_va
trainer = pl.Trainer(max_epochs=myparams['epochs'], default_root_dir="./", logger=F
                     callbacks=[flexynesis.LiveLossPlot(myparams, 1, 1)])

trainer.fit(model, train_loader, val_loader)
flexynesis.evaluate_wrapper("DirectPred", model.predict(test_dataset), test_dataset
```




```
`Trainer.fit` stopped: `max_epochs=25` reached.
```

Out[24]:

	method	var	variable_type	metric	value
0	DirectPred	STUDY	categorical	balanced_acc	0.760784
1	DirectPred	STUDY	categorical	f1_score	0.760608
2	DirectPred	STUDY	categorical	kappa	0.495652
3	DirectPred	STUDY	categorical	average_auroc	0.827682
4	DirectPred	STUDY	categorical	average_aupr	0.691249

Warning!!: In reality, we don't select the best models based on performance on the test dataset.

The best model is selected based on the validation loss value, where the model parameters that yields the lowest validation loss is selected to be the best model.

The validation dataset which we use to compute the validation loss is basically a subset of the training dataset.

3. Automating the Hyperparameter Optimisation Procedure

What we did in the above section was to set random hyperparameters, build a model, evaluate the model and try different hyperparameters based on our previous model performance. However, this process can be quite time consuming and arbitrary. This process can be automated using a Bayesian approach, where the model training is sequentially done for a number of hyperparameter optimisation iterations.

Now, we are ready to do a model training using hyperparameter optimisation.

- `model_class` : We pick `DirectPred` (a fully connected network) for now.
- `config_name` : We use the default/built-in hyperparameter search space for `DirectPred` class.
- `target_variables` : 'STUDY' variable contains the type of disease
- `n_iter` : We do 5 iterations of hyperparameter optimisation. For demonstration purposes, we set it to a small number.
- `plot_losses` : We want to visualize how the training progresses.
- `early_stop_patience` : If a training does not show any signs of improving the performance on the `validation` part of the `train_dataset` for at least 10 epochs, we stop the training. This not only significantly decreases the amount spent on training by avoiding unnecessary continuation of unpromising training runs, but also helps avoid over-fitting the network on the training data.

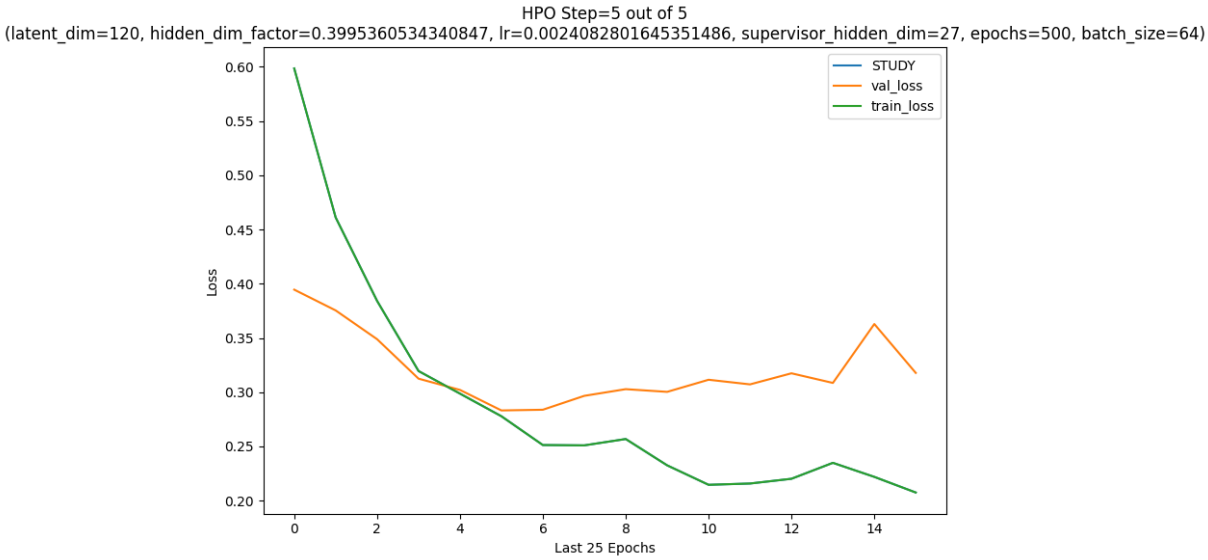
Note 1: Notice how the hyperparameters using in different HPO steps change at each iteration.

Note 2: Also notice that we are running the model for more epochs (500 by default) however, by using "early_stop_patience=10", we avoid lengthy training when validation performance is not improving.

Note 3: Try to follow the the loss curves and the used hyperparameters. See if you can spot which combination yields the lowest/best loss values.

Warning!!: In reality we need to set `n_iter` to higher values so that the optimizer can collect enough data points to learn trends in the parameter space.

In [25]:
```
# Define a tuner; See n_iter is the number of
tuner = flexynesis.HyperparameterTuning(train_dataset,
                                        model_class = flexynesis.DirectPred,
                                        config_name = "DirectPred",
                                        target_variables = ['STUDY'],
                                        n_iter=5,
                                        plot_losses=True,
                                        early_stop_patience=10)
### Perform Training
model, best_params = tuner.perform_tuning()
```




```
LOCAL_RANK: 0 - CUDA_VISIBLE_DEVICES: [0]
SLURM auto-requeueing enabled. Setting signal handlers.
Validation: |          | 0/? [00:00<?, ?it/s]
```

Validate metric	DataLoader 0
STUDY	0.317761093378067
val_loss	0.317761093378067

```
Tuning Progress: 100%|██████████| 5/5 [00:28<00:00,  5.77s/it, Iteration=5, Best Los
s=0.318]
[INFO] current best val loss: 0.317761093378067; best params: {'latent_dim': np.int6
4(120), 'hidden_dim_factor': 0.3995360534340847, 'lr': 0.0024082801645351486, 'super
visor_hidden_dim': np.int64(27), 'epochs': 500, 'batch_size': np.int64(64)} since 0
hpo iterations
```

In [26]:
```
## See which hyperparameter combination was the best
best_params
```

Out[26]:
```
{'latent_dim': np.int64(120),
 'hidden_dim_factor': 0.3995360534340847,
 'lr': 0.0024082801645351486,
 'supervisor_hidden_dim': np.int64(27),
 'epochs': 15,
 'batch_size': np.int64(64)}
```

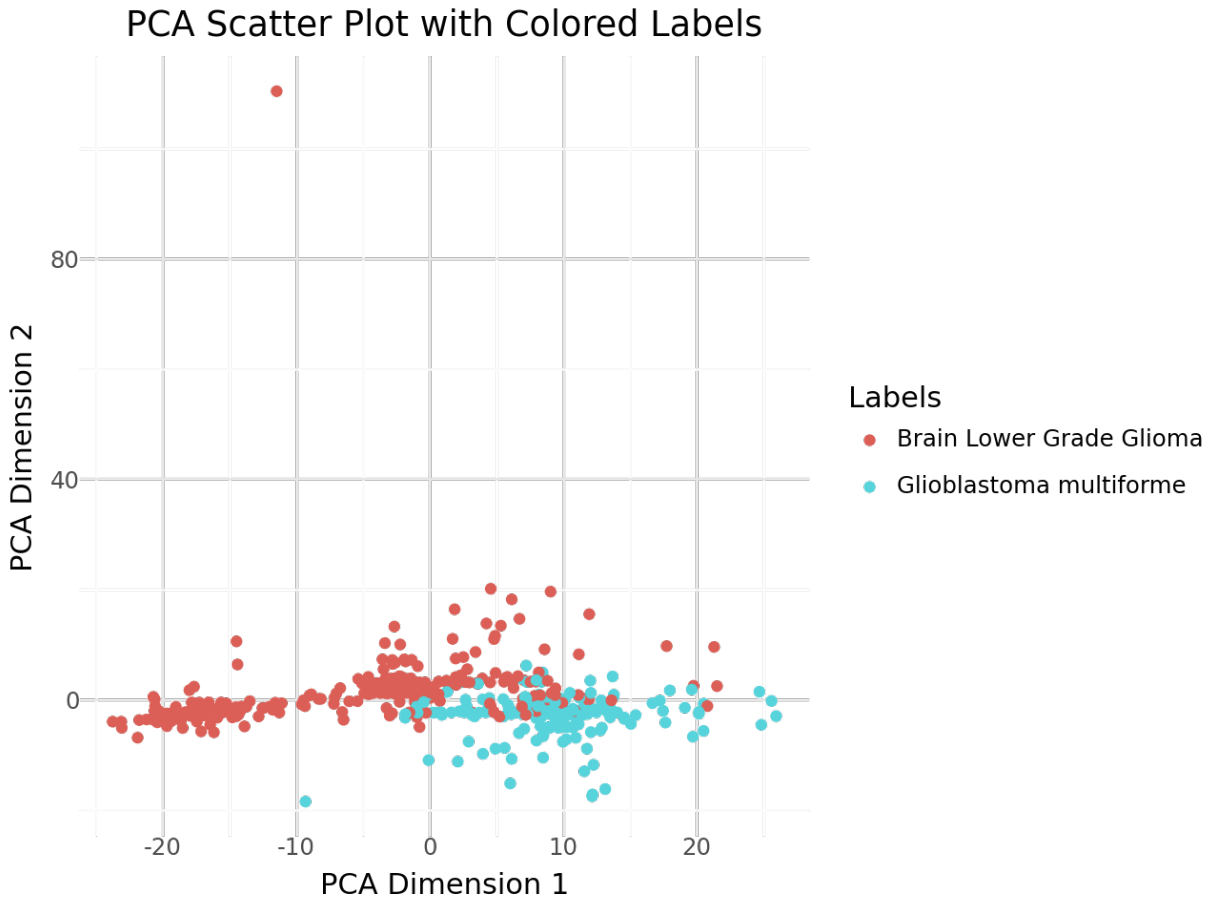
In [27]:
```
## Evaluate the model and visualising the results
flexynesis.evaluate_wrapper(method = 'DirectPred', y_pred_dict=model.predict(test_d
```

Out[27]:

	method	var	variable_type	metric	value
0	DirectPred	STUDY	categorical	balanced_acc	0.778431
1	DirectPred	STUDY	categorical	f1_score	0.801116
2	DirectPred	STUDY	categorical	kappa	0.564238
3	DirectPred	STUDY	categorical	average_auroc	0.856055
4	DirectPred	STUDY	categorical	average_aupr	0.717852

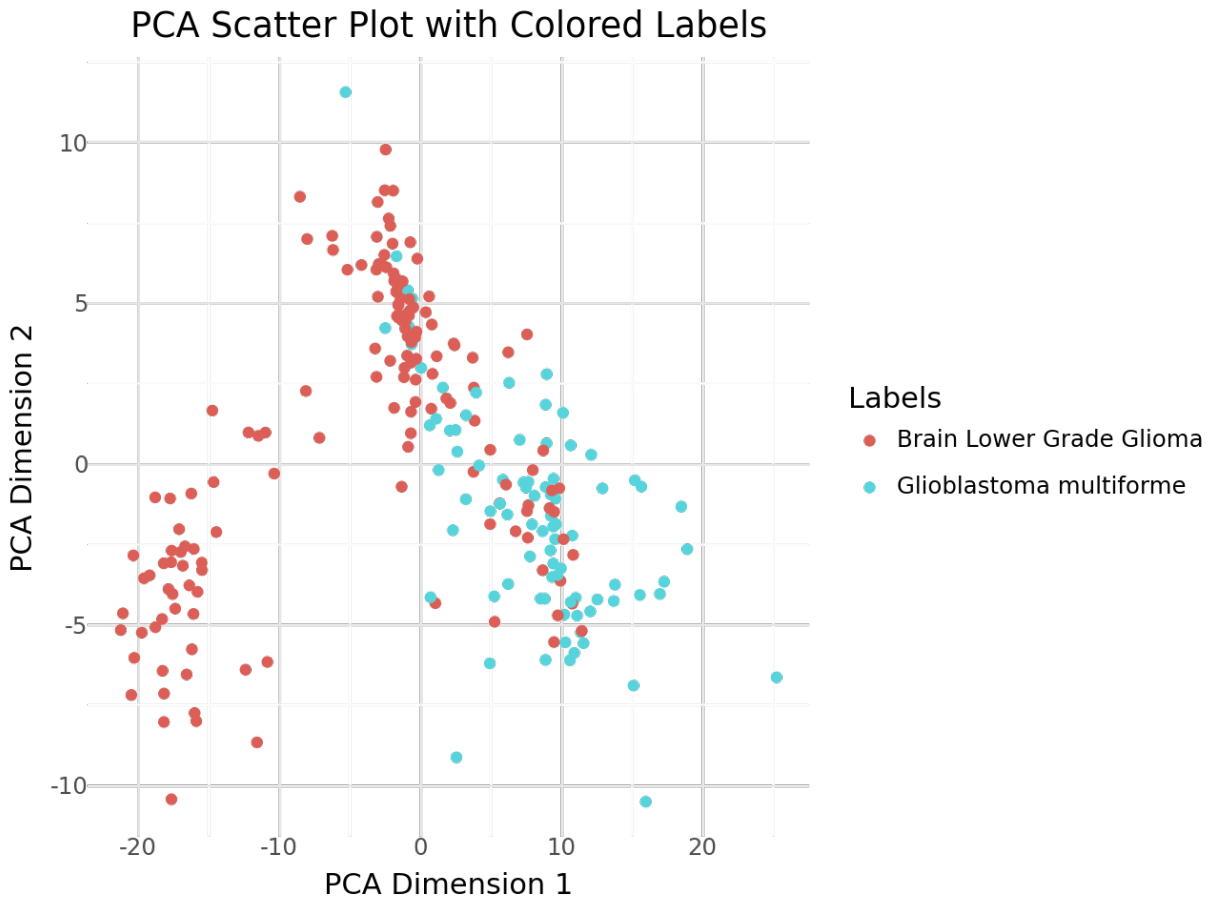
Let's extract the sample embeddings and make a PCA plot and color by the target variable

In [28]:
```
train_embeddings = model.transform(train_dataset)
# labels already set from before, changed to use those instead of study numbers
# labels = [train_dataset.label_mappings["STUDY"][x] for x in train_dataset.ann["ST
flexynesis.plot_dim_reduced(train_embeddings, labels)
```



Repeat the same for the test dataset: extract sample embeddings for test dataset samples and make a PCA plot, colored by "STUDY" variable

In [29]:
```
test_embeddings = model.transform(test_dataset)
# set labels in same way as for training set before, changed to use those instead o
labels = [test_dataset.label_mappings["STUDY"][x] for x in test_dataset.ann["STUDY"
flexynesis.plot_dim_reduced(test_embeddings, labels)
```



3.1 Exercises

Exercise 1:

Look up what Harrell's C-index means and write down a simple description of what it measures.

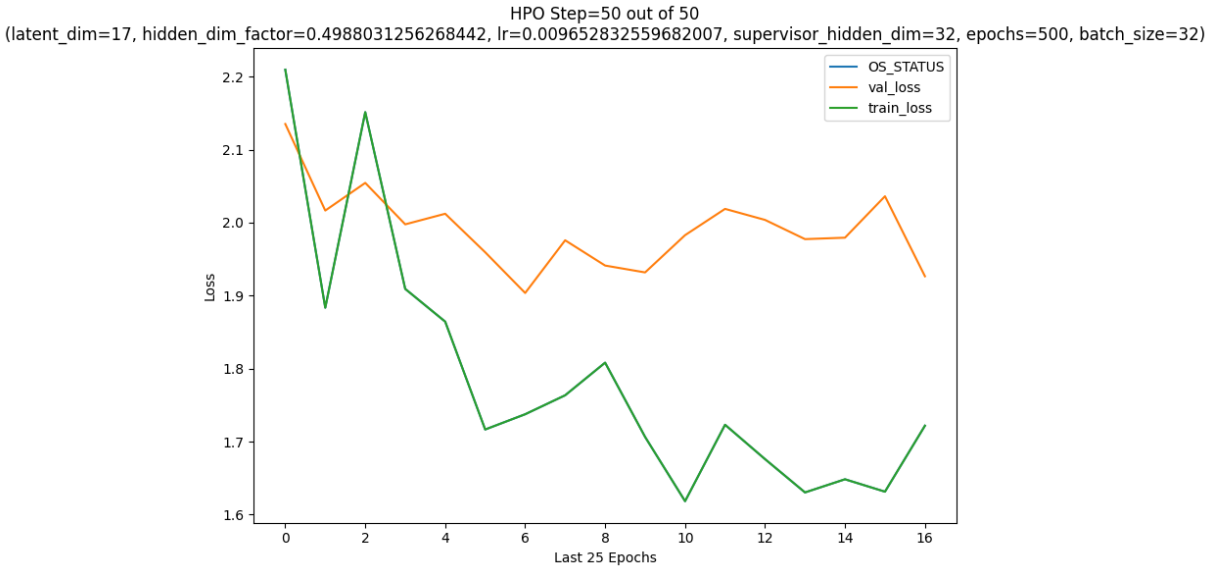
- It is a measure of how well a predictive model ranks survival time
- A higher C-index (0-1) means a better prediciton correlation (e.g. an individual with a higher predicted risk score has a lower surivial time than an individual with a lower predicted risk score)

Exercise 2:

Now, you build a model using hyperparameter tuning (run at least 10 HPO steps) to predict the survival outcomes of patients. Evaluate the final model on test dataset, which computes the "C-index".

Feel free to cheat from the tutorial available here: https://github.com/BIMSBbioinfo/flexynesis/blob/main/examples/tutorials/survival_subtypes_LGG_GBM.ipynb See how "OS_STATUS" and "OS_MONTHS" were used.

In [30]:
```
tuner = flexynesis.HyperparameterTuning(train_dataset,
                                        model_class = flexynesis.DirectPred,
                                        config_name = "DirectPred",
                                        surv_event_var="OS_STATUS",
                                        surv_time_var="OS_MONTHS",
                                        target_variables = [],
                                        n_iter=50,
                                        plot_losses=True,
                                        early_stop_patience=10)
### Perform Training
model, best_params = tuner.perform_tuning()
```




```
LOCAL_RANK: 0 - CUDA_VISIBLE_DEVICES: [0]
SLURM auto-requeueing enabled. Setting signal handlers.
Validation: |          | 0/? [00:00<?, ?it/s]
```

Validate metric	DataLoader 0
OS_STATUS	1.926513717533539
val_loss	1.926513717533539

```
Tuning Progress: 100%|██████████| 50/50 [05:21<00:00,  6.44s/it, Iteration=50, Best
Loss=1.65]
[INFO] current best val loss: 1.645938357791385; best params: {'latent_dim': np.int6
4(128), 'hidden_dim_factor': 0.2, 'lr': 0.01, 'supervisor_hidden_dim': np.int64(32),
'epochs': np.int64(500), 'batch_size': np.int64(32)} since 38 hpo iterations
```

In [31]:
```
best_params
```

Out[31]:
```
{'latent_dim': np.int64(128),
 'hidden_dim_factor': 0.2,
 'lr': 0.01,
 'supervisor_hidden_dim': np.int64(32),
 'epochs': 22,
 'batch_size': np.int64(32)}
```

In [32]:
```
flexynesis.evaluate_wrapper(method = 'DirectPred', y_pred_dict=model.predict(test_d
                            surv_event_var=model.surv_event_var, surv_time_var=mode
```

Out[32]:

	method	var	variable_type	metric	value
0	DirectPred	OS_STATUS	numerical	cindex	0.651081

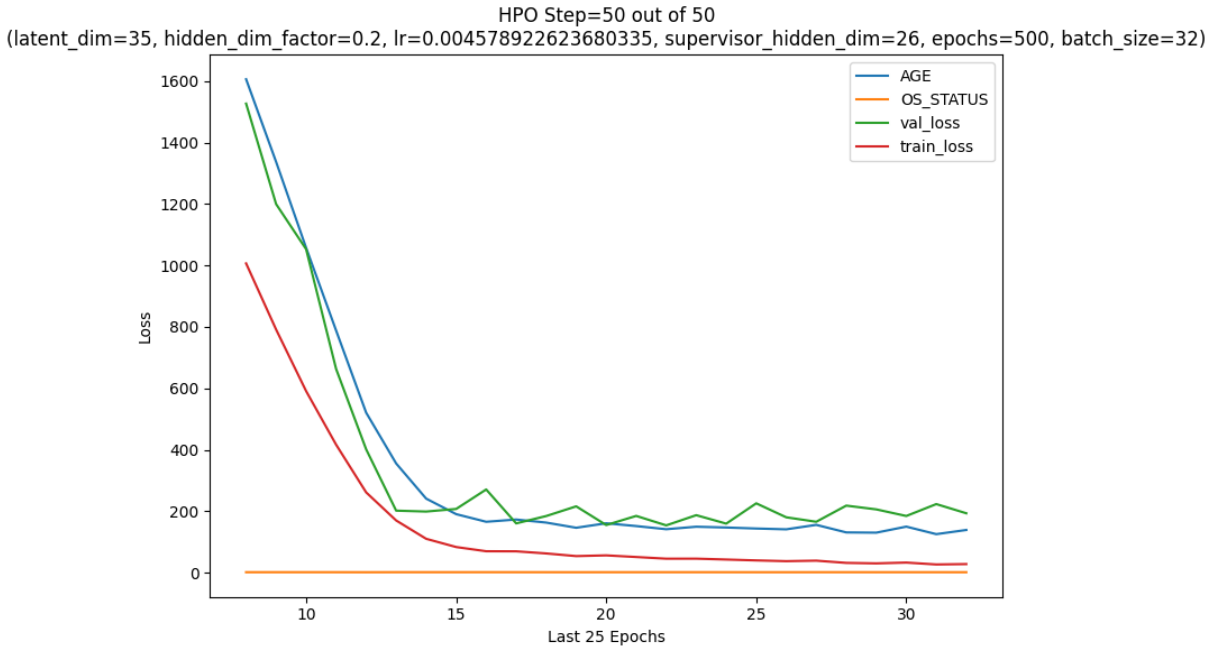
Exercise 3:

Again build a model using hyperparameter tuning to predict survival outcomes (as in Exercise 1), however, this time use additional clinical variables as targets.

```
flexynesis.HyperparameterTuning(train_dataset,
                                model_class =
flexynesis.DirectPred,
                                config_name = "DirectPred",
                                surv_event_var="OS_STATUS",
                                surv_time_var="OS_MONTHS",
                                target_variables = [], => What
other variables can you use here? Try "AGE" and/or
"HISTOLOGICAL_DIAGNOSIS" and see the model performance
                                ...
```

See if you can get a better C-index using additional target variables.

In [33]:
```
tuner_w_age = flexynesis.HyperparameterTuning(train_dataset,
                                      model_class = flexynesis.DirectPred,
                                      config_name = "DirectPred",
                                      surv_event_var="OS_STATUS",
                                      surv_time_var="OS_MONTHS",
                                      target_variables = ["AGE"],
                                      n_iter=50,
                                      plot_losses=True,
                                      early_stop_patience=10)
### Perform Training
model_w_age, best_params_w_age = tuner_w_age.perform_tuning()
```

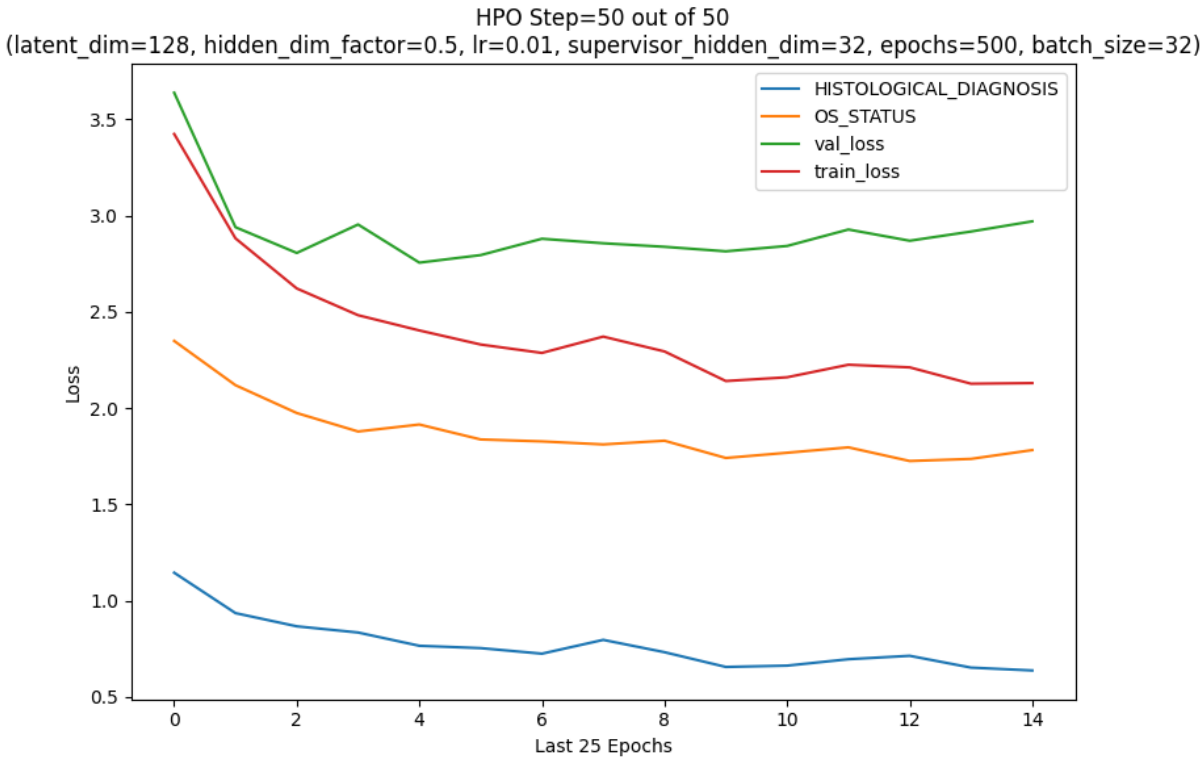



```
LOCAL_RANK: 0 - CUDA_VISIBLE_DEVICES: [0]
SLURM auto-requeueing enabled. Setting signal handlers.
Validation: |          | 0/? [00:00<?, ?it/s]
```

Validate metric	DataLoader 0
AGE	191.79644775390625
OS_STATUS	1.866253521395829
val_loss	193.6627062240985

```
Tuning Progress: 100%|██████████| 50/50 [15:07<00:00, 18.14s/it, Iteration=50, Best
Loss=127]
[INFO] current best val loss: 126.68187588817874; best params: {'latent_dim': np.int
64(29), 'hidden_dim_factor': 0.2, 'lr': 0.004321745715893343, 'supervisor_hidden_dim
': np.int64(30), 'epochs': np.int64(500), 'batch_size': np.int64(64)} since 18 hpo i
terations
```

In [34]:
```
tuner_w_hist = flexynesis.HyperparameterTuning(train_dataset,
                                               model_class = flexynesis.DirectPred,
                                               config_name = "DirectPred",
                                               surv_event_var="OS_STATUS",
                                               surv_time_var="OS_MONTHS",
                                               target_variables = ["HISTOLOGICAL_DIAGNOSIS
                                               n_iter=50,
                                               plot_losses=True,
                                               early_stop_patience=10)
### Perform Training
model_w_hist, best_params_w_hist = tuner_w_hist.perform_tuning()
```

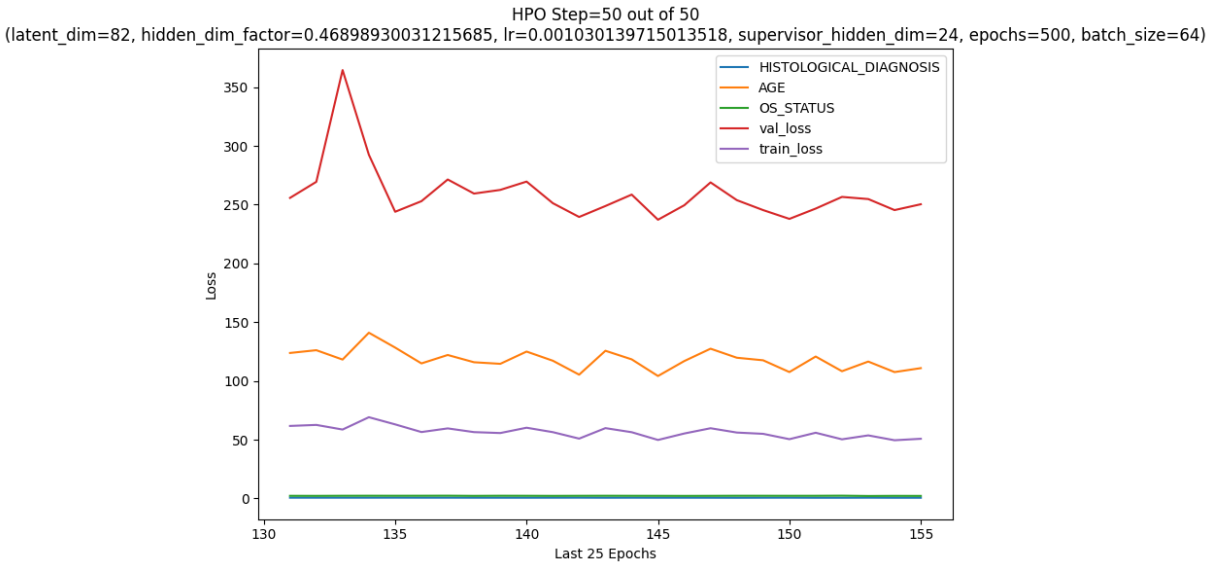



```
LOCAL_RANK: 0 - CUDA_VISIBLE_DEVICES: [0]
SLURM auto-requeueing enabled. Setting signal handlers.
Validation: |          | 0/? [00:00<?, ?it/s]
```

Validate metric	DataLoader 0
HISTOLOGICAL_DIAGNOSIS	1.0300884246826172
OS_STATUS	1.9405222978044
val_loss	2.970610682750587

```
Tuning Progress: 100%|██████████| 50/50 [06:43<00:00,  8.07s/it, Iteration=50, Best
Loss=2.58]
[INFO] current best val loss: 2.581982556300703; best params: {'latent_dim': np.int6
4(128), 'hidden_dim_factor': 0.4285174622762627, 'lr': 0.01, 'supervisor_hidden_dim'
: np.int64(32), 'epochs': np.int64(500), 'batch_size': np.int64(32)} since 6 hpo ite
rations
```

In [35]:
```
tuner_w_hist_age = flexynesis.HyperparameterTuning(train_dataset,
                                        model_class = flexynesis.DirectPred,
                                        config_name = "DirectPred",
                                        surv_event_var="OS_STATUS",
                                        surv_time_var="OS_MONTHS",
                                        target_variables = ["HISTOLOGICAL_DIAGNOSIS
                                        n_iter=50,
                                        plot_losses=True,
                                        early_stop_patience=10)
### Perform Training
model_w_hist_age, best_params_w_hist_age = tuner_w_hist_age.perform_tuning()
```




```
LOCAL_RANK: 0 - CUDA_VISIBLE_DEVICES: [0]
SLURM auto-requeueing enabled. Setting signal handlers.
Validation: |          | 0/? [00:00<?, ?it/s]
```

Validate metric	DataLoader 0
AGE	247.05078125
HISTOLOGICAL_DIAGNOSIS	0.8093999624252319
OS_STATUS	2.5288828942069146
val_loss	250.3890784321521

```
Tuning Progress: 100%|██████████| 50/50 [19:29<00:00, 23.39s/it, Iteration=50, Best
Loss=154]
[INFO] current best val loss: 154.3650991009179; best params: {'latent_dim': np.int6
4(16), 'hidden_dim_factor': 0.3372425477346168, 'lr': 0.00984039685969451, 'supervis
or_hidden_dim': np.int64(32), 'epochs': np.int64(500), 'batch_size': np.int64(64)} s
ince 27 hpo iterations
```

In [36]:
```
flexynesis.evaluate_wrapper(method = 'DirectPred', y_pred_dict=model_w_age.predict(
                            surv_event_var=model_w_age.surv_event_var, surv_time_va
```

Out[36]:

	method	var	variable_type	metric	value
0	DirectPred	AGE	numerical	mse	208.955933
1	DirectPred	AGE	numerical	r2	0.248464
2	DirectPred	AGE	numerical	pearson_corr	0.498462
3	DirectPred	OS_STATUS	numerical	cindex	0.740837

In [37]:
```
flexynesis.evaluate_wrapper(method = 'DirectPred', y_pred_dict=model_w_hist.predict
                            surv_event_var=model_w_hist.surv_event_var, surv_time_v
```

Out[37]:

	method	var	variable_type	metric	value
0	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	balanced_acc	0.472711
1	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	f1_score	0.517903
2	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	kappa	0.347106
3	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	average_auroc	NaN
4	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	average_aupr	NaN
5	DirectPred	OS_STATUS	numerical	cindex	0.697578

In [38]:
```
flexynesis.evaluate_wrapper(method = 'DirectPred', y_pred_dict=model_w_hist_age.pre
                            surv_event_var=model_w_hist_age.surv_event_var, surv_ti
```

Out[38]:

	method	var	variable_type	metric	value
0	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	balanced_acc	0.571836
1	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	f1_score	0.612964
2	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	kappa	0.509150
3	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	average_auroc	NaN
4	DirectPred	HISTOLOGICAL_DIAGNOSIS	categorical	average_aupr	NaN
5	DirectPred	AGE	numerical	mse	212.659653
6	DirectPred	AGE	numerical	r2	0.251911
7	DirectPred	AGE	numerical	pearson_corr	0.501908
8	DirectPred	OS_STATUS	numerical	cindex	0.754306

3.2 Survival-risk subtypes

Use the best model from the above exercises to inspect sample embeddings categorized by survival risk scores.

Let's group the samples by predicted survival risk scores into 2 groups and visualize the sample embeddings colored by risk subtypes.

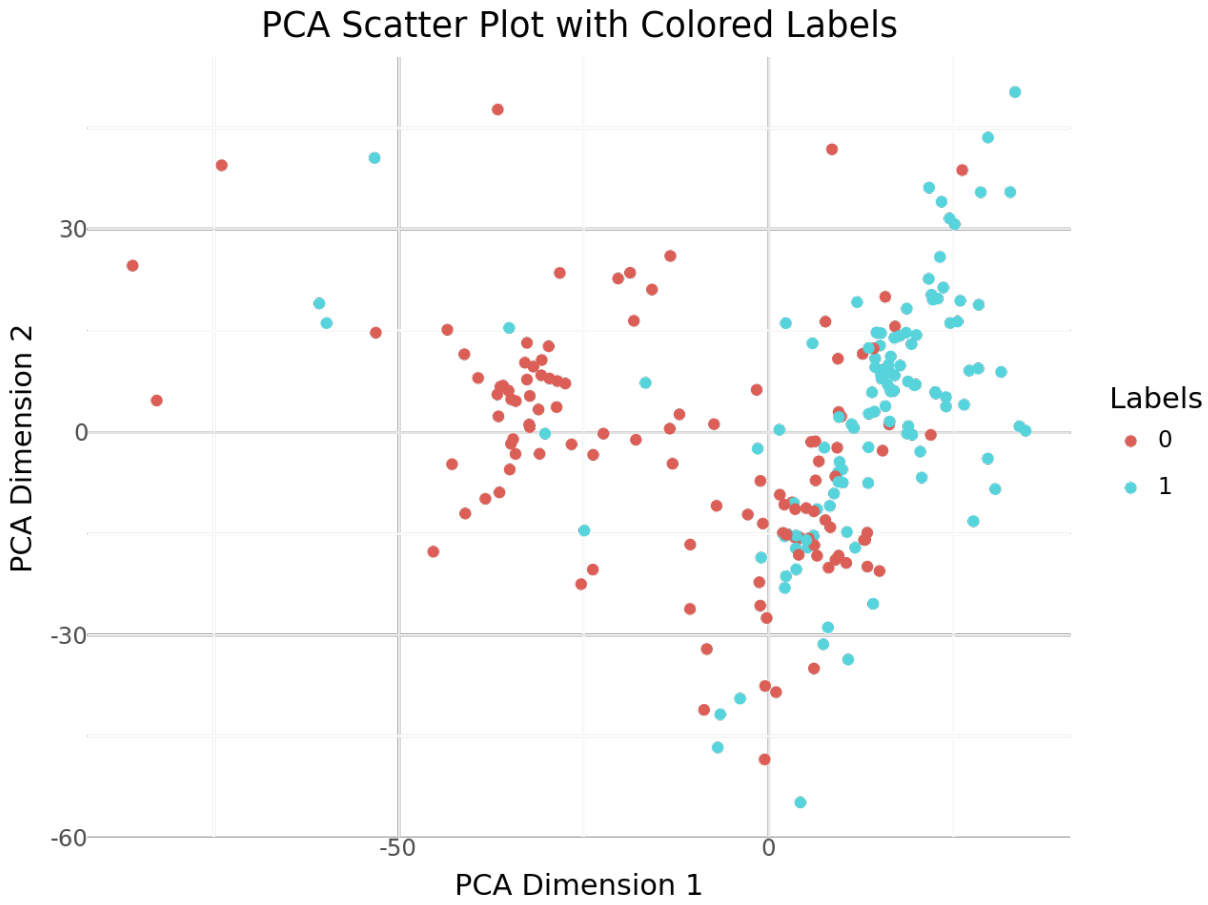
Notice: You can use the code-below to get survival risk groups, however, notice that you must have built a model with "OS_STATUS" already.

In [39]:
```
# get model outputs for survival variable
# Modle with Histology and Age had the highest C-index
outputs = model_w_hist_age.predict(test_dataset)['OS_STATUS'].flatten()
risk_scores = np.exp(outputs)
# Define quantile thresholds
quantiles = np.quantile(risk_scores, [0.5])
```

```
# Assign groups based on quantiles
groups = np.digitize(risk_scores, quantiles)
```

In [40]:
```
# Extract sample embeddings
E = model.transform(test_dataset)
```

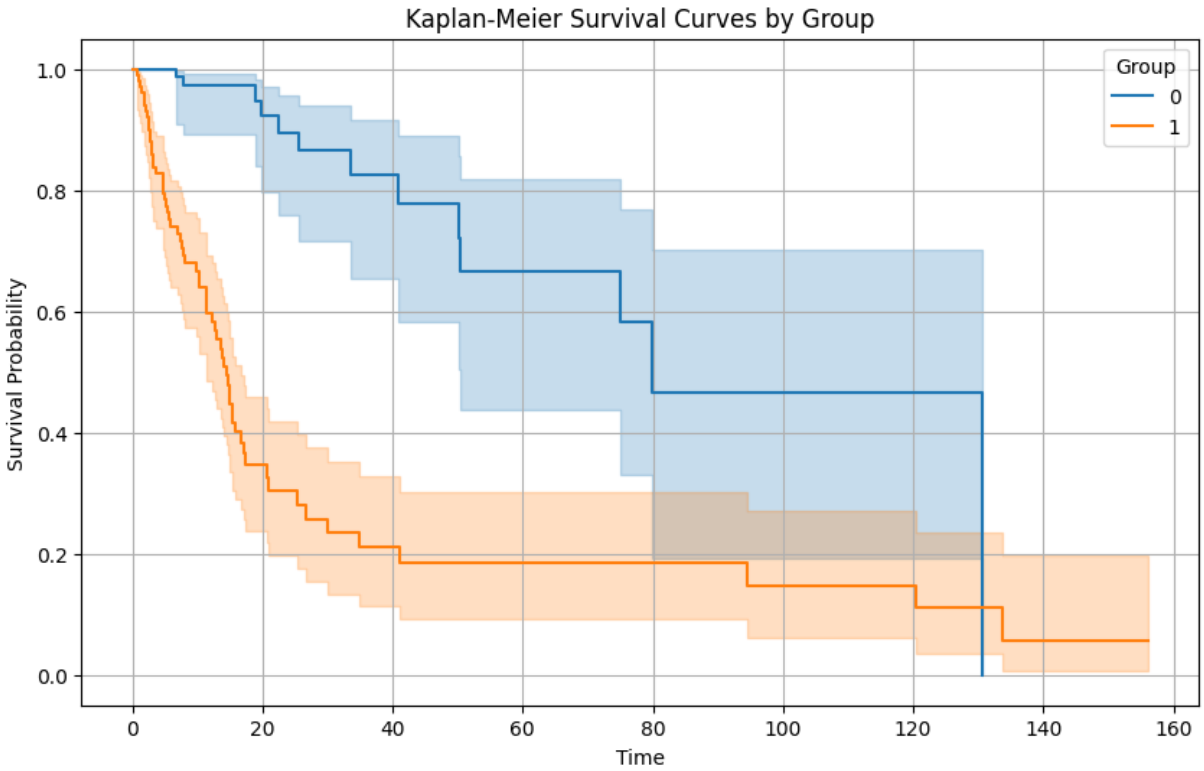
In [41]:
```
flexynesis.plot_dim_reduced(E, groups)
```



Let's also see the Kaplan Meier Curves of the risk subtypes

In [42]:
```
# remove samples with NA values first
durations = test_dataset.ann['OS_MONTHS']
events = test_dataset.ann['OS_STATUS']
valid_indices = ~torch.isnan(durations) & ~torch.isnan(events)
```

In [43]:
```
flexynesis.plot_kaplan_meier_curves(durations[valid_indices], events[valid_indices]
```



Finding survival-associated markers

We can also compute feature importance scores for prediction of overall survival.

In [44]:
```
model_w_hist_age.compute_feature_importance(train_dataset, 'OS_STATUS')
```

In [45]:
```
# get top 10 features
flexynesis.get_important_features(model_w_hist_age, var = 'OS_STATUS', top=10)
```

Out[45]:

	target_variable	target_class	target_class_label	layer	name	importance
0	OS_STATUS	0		mut	IDH1	0.623612
1	OS_STATUS	0		mut	ATRX	0.324713
2	OS_STATUS	0		mut	TP53	0.175178
3	OS_STATUS	0		mut	IDH2	0.082160
4	OS_STATUS	0		mut	TEKT4	0.046561
5	OS_STATUS	0		mut	PIK3CA	0.038255
6	OS_STATUS	0		mut	COL6A3	0.032404
7	OS_STATUS	0		mut	MUC16	0.028920
8	OS_STATUS	0		mut	SVIL	0.026944
9	OS_STATUS	0		mut	EGFR	0.025502

Comparing top markers with clinical covariates

Let's build a linear Cox-PH model including the top 5 markers and other clinical variables such as histological diagnosis, disease type (STUDY), age, and sex.

In [46]:
```
# define a data.frame with clinical covariates and top markers along with survival
vars = ['AGE', 'SEX', 'HISTOLOGICAL_DIAGNOSIS', 'STUDY', 'OS_MONTHS', 'OS_STATUS']
# read clinical variables
df_clin = pd.concat(
    [pd.DataFrame({x: train_dataset.ann[x] for x in vars}, index=train_dataset.samp
     pd.DataFrame({x: test_dataset.ann[x] for x in vars}, index=test_dataset.sample
    axis = 0)
# get top 5 survival markers and extract the input data for these markers for both
imp = flexynesis.get_important_features(model_w_hist_age, var = 'OS_STATUS', top=5)
df_imp = pd.concat([train_dataset.get_feature_subset(imp), test_dataset.get_feature

# combine markers with clinical variables
df = pd.concat([df_imp, df_clin], axis = 1)
# remove samples without survival endpoints
df = df[df['OS_STATUS'].notna()]
df
```

Out[46]:

	mut_IDH1	mut_ATRX	mut_TP53	mut_IDH2	mut_TEKT4	AGE	SEX	HISTO
TCGA-DB-5279	0.982173	-0.585658	-0.809174	-0.148522	-0.09526	59.0	1.0	
TCGA-DU-7011	0.982173	1.707482	1.235829	-0.148522	-0.09526	25.0	1.0	
TCGA-06-5415	-1.018150	-0.585658	-0.809174	-0.148522	-0.09526	60.0	1.0	
TCGA-HT-A616	0.982173	1.707482	-0.809174	-0.148522	-0.09526	36.0	0.0	
TCGA-S9-A6WH	0.982173	-0.585658	-0.809174	-0.148522	-0.09526	73.0	0.0	
...	...	...	...	...	...	...	...	
TCGA-06-A5U1	-1.018150	-0.585658	-0.809174	-0.148522	-0.09526	78.0	0.0	
TCGA-76-4934	-1.018150	-0.585658	1.235829	-0.148522	-0.09526	66.0	0.0	
TCGA-DB-A4XD	0.982173	1.707482	1.235829	-0.148522	-0.09526	32.0	1.0	
TCGA-DU-5874	0.982173	-0.585658	-0.809174	-0.148522	-0.09526	62.0	0.0	
TCGA-14-1043	-1.018150	-0.585658	-0.809174	-0.148522	-0.09526	61.0	1.0	

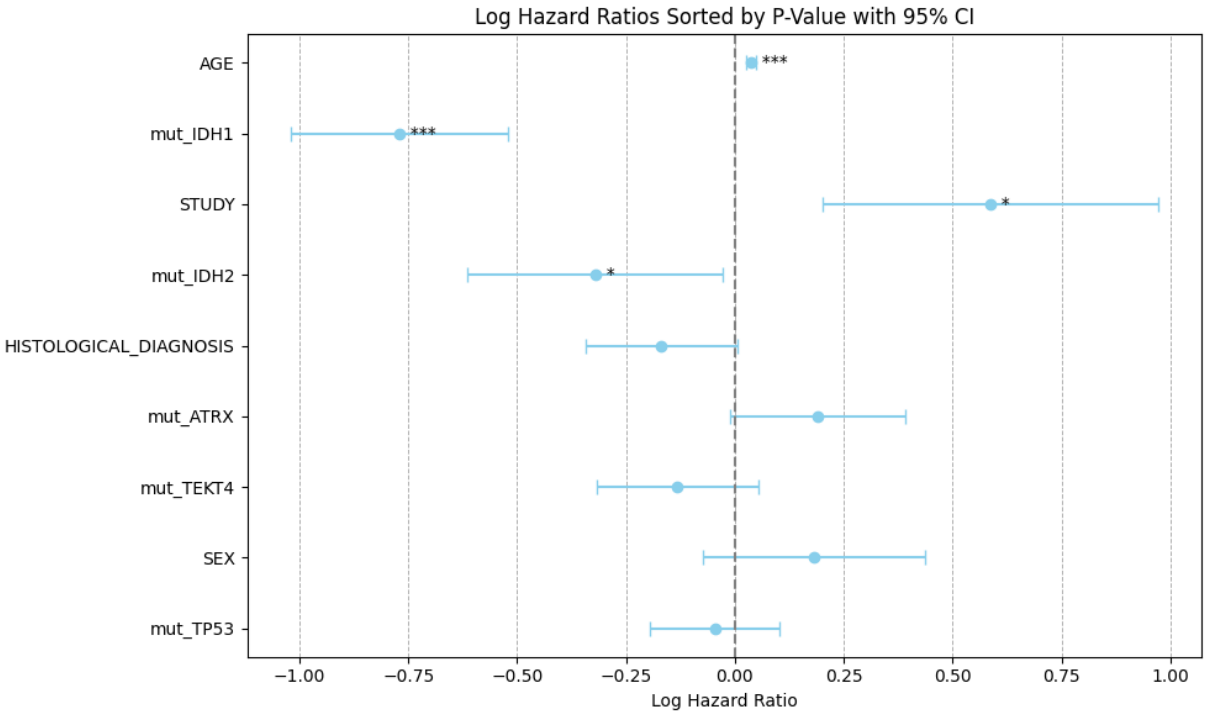
729 rows × 11 columns

In [47]:
```
# build a cox model
coxm = flexynesis.build_cox_model(df, 'OS_MONTHS', 'OS_STATUS')
```

```
No low variance features were removed based on event conditioning.
```

In [48]:
```
# visualize log-hazard ratios sorted by p-values
flexynesis.plot_hazard_ratios(coxm)
```

```
/usr/local/lib/python3.11/site-packages/flexynesis/utils.py:764: FutureWarning: Seri
es.__getitem__ treating keys as positions is deprecated. In a future version, intege
r keys will always be treated as labels (consistent with DataFrame behavior). To acc
ess a value by position, use `ser.iloc[pos]`
```



3.3 Final Exercise

- Inspect the top 10 markers from section 3.2 and see if they have been characterized in the literature as important markers for Glioma disease progression.

- Age: Yes, older age is associated with a worse prognosis
- IDH1: Yes, typically mutant IDH1 have a better prognosis
- Study: Yes, the type of Glioma is important
- IDH2: Yes, but less than IDH1
- Histology Diagnosis: Yes, morphology/classification is important
- ATRX: Yes, often co-mutated with IDH (ATRX-deficient IDH-wt are very rare)
- TEKT4: Not widely recognised as playing a role
- Sex: Few studies showing males may have worse outcomes, but not conclusive
- TP53: Possibly, TP53 mutations are common (as they are in many cancers), but little known about role in progression