

RNA Velocity using scVelo

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Data: CITEseq YS006_UW

I did pre processing in Suerat and saved object as .h5Seurat to perform RNAvelo in Python wtih scVelo

```
In [1]: # Setup
import scanpy as sc
import scvelo as scv
import anndata
import loompy
import mygene
import scipy
import seaborn as sns
import matplotlib.pyplot as plt

In [3]: h5ad_path = "/Volumes/PortableSSD/IntegratedMultimodal_CITEseq.h5ad"
adata = sc.read_h5ad(h5ad_path)
adata.obs.index = [x.split('-')[0] for x in adata.obs.index]      # TTTGGAGTCG

loom_paths = ["/Volumes/PortableSSD/YS001/possorted_genome_bam_KLW0.loom",
              "/Volumes/PortableSSD/YS002/possorted_genome_bam_PNEM4.loom",
              "/Volumes/PortableSSD/YS003/possorted_genome_bam_8Q2XV.loom",
              "/Volumes/PortableSSD/YS004/possorted_genome_bam_0XB2N.loom",
              "/Volumes/PortableSSD/YS005/possorted_genome_bam_HX08G.loom",
              "/Volumes/PortableSSD/YS006/possorted_genome_bam_751VB.loom"]
combined_loom_path = "/Volumes/PortableSSD/citeseq_combined.loom"

loompy.combine(loom_paths, combined_loom_path)
ldata = sc.read_loom(combined_loom_path)
ldata.obs.index = [x.split(':')[1] for x in ldata.obs.index]      # possorted_
ldata.obs.index = [x[:-1] for x in ldata.obs.index]

WARNING:root: └── 'batch_scan_layers' is deprecated. Use 'scan' instead
WARNING:root: └──> at /opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/loompy/loompy.py, line 471
WARNING:root: └── 'batch_scan_layers' is deprecated. Use 'scan' instead
WARNING:root: └──> at /opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/loompy/loompy.py, line 471
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```

SANITY CHECKING

- inspecting andata and ldata objects (cell and gene counts)
- checking for duplicates

```
In [4]: print("== Seurat-derived AnnData (adata) ==")
print("Cells (obs):", adata.n_obs)
print("Genes (var):", adata.n_vars)
print("\nFirst 10 cell names:", adata.obs.index[:10].tolist())
print("\nFirst 10 gene names:", adata.var.index[:10].tolist())

print("\n\n== Loom RNA velocity AnnData (ldata) ==")
print("Cells (obs):", ldata.n_obs)
print("Genes (var):", ldata.n_vars)
print("\nFirst 10 cell names:", ldata.obs.index[:10].tolist())
print("\nFirst 10 gene names:", ldata.var.index[:10].tolist())

print("\n\n== Layer shapes in ldata ==")
for layer in ["spliced", "unspliced", "ambiguous"]:
    if layer in ldata.layers:
        print(f"{layer}: {ldata.layers[layer].shape}")

# check for duplicates in adata and ldata
print("\n\n== Checking for duplicates ==")
print("Total cells in adata:", len(adata.obs.index))
print("Unique cells in adata:", len(adata.obs.index.unique()))
print("Duplicate barcodes in adata:", len(adata.obs.index) - len(adata.obs.i)

print("\nTotal cells in ldata:", len(ldata.obs.index))
print("Unique cells in ldata:", len(ldata.obs.index.unique()))
print("Duplicate barcodes in ldata:", len(ldata.obs.index) - len(ldata.obs.i

# Show some duplicates if they exist
from collections import Counter
adata_counts = Counter(adata.obs.index)
adata_duplicates = {bc: count for bc, count in adata_counts.items() if count > 1}
print(f"\nNumber of duplicated barcodes: {len(adata_duplicates)}")
if adata_duplicates:
    print("Examples:", list(adata_duplicates.items())[:5])
```

```
==== Seurat-derived AnnData (adata) ====
Cells (obs): 35317
Genes (var): 30481
```

```
First 10 cell names: ['AAACCCAAGAACAGGA', 'AAACCCAAGTCTCTGA', 'AAACGAAAGACTA
CGG', 'AAACGAAAGATTGCT', 'AAACGAAAGCTCACTA', 'AAACGAACAGCTGTAT', 'AAACGAACA
TCCTTGC', 'AAACGAAGTACTGACT', 'AAACGAAGTGTATCCA', 'AAACGAAGTTGCTCGG']
```

```
First 10 gene names: ['Xkr4', 'Gm19938', 'Sox17', 'Mrpl15', 'Lypla1', 'Tcea
1', 'Rgs20', 'Atp6v1h', 'Npbwr1', '4732440D04Rik']
```

```
==== Loom RNA velocity AnnData (ldata) ====
Cells (obs): 45487
Genes (var): 33696
```

```
First 10 cell names: ['AAAGGGCAGTCATACC', 'AAAGGTATCGGAAGGT', 'AAATGGACACTCA
AGT', 'AAAGAACCATGTTG', 'AAACGAACATCCTGC', 'AAAGGATTCCAATCTT', 'AAATGGAAG
TGGAAAG', 'AAAGTCCAGAACCGGT', 'AAAGTGAGTCACGTGC', 'AAAGGGCGTTGTCTAG']
```

```
First 10 gene names: ['ENSMUSG00000079800', 'ENSMUSG00000095092', 'ENSMUSG00
00079794', 'ENSMUSG00000079192', 'ENSMUSG00000094799', 'ENSMUSG0000009525
0', 'ENSMUSG00000095787', 'ENSMUSG00000095672', 'ENSMUSG00000094514', 'ENSMU
SG00000096100']
```

```
==== Layer shapes in ldata ====
spliced: (45487, 33696)
```

```
unsliced: (45487, 33696)
```

```
ambiguous: (45487, 33696)
```

```
==== Checking for duplicates ===
Total cells in adata: 35317
Unique cells in adata: 35173
Duplicate barcodes in adata: 144
```

```
Total cells in ldata: 45487
```

```
Unique cells in ldata: 45252
```

```
Duplicate barcodes in ldata: 235
```

```
Number of duplicated barcodes: 143
```

```
Examples: [('AAACGAAAGATTGCT', 2), ('AAACGAATCTCAACCC', 2), ('AACAAAGAGTACCT
AGT', 2), ('AAGCATCTCGTCAGAT', 2), ('AATGGCTTCGCTAATG', 2)]
```

```
In [5]: # Remove duplicates from both datasets (keep first occurrence)
```

```
print("\n==== Removing duplicates ===")
```

```
adata = adata[~adata.obs.index.duplicated(keep='first'), :]
ldata = ldata[~ldata.obs.index.duplicated(keep='first'), :]
```

```
print(f"adata after dedup: {adata.shape}")
print(f"ldata after dedup: {ldata.shape}")
```

```
# Find common barcodes
```

```
common_barcodes = set(adata.obs.index).intersection(set(ldata.obs.index))
print(f"\n==== Common cells ===")
```

```

print(f"Cells in common: {len(common_barcodes)}")

# Subset both datasets to only common cells
# Convert to list and sort for reproducibility
common_barcodes = sorted(list(common_barcodes))

adata = adata[common_barcodes, :]
ldata = ldata[common_barcodes, :]

print(f"\n==== Final datasets ===")
print(f"Final adata shape: {adata.shape}")
print(f"Final ldata shape: {ldata.shape}")
print(f"Indices match: {all(adata.obs.index == ldata.obs.index)}")

```

==== Removing duplicates ===
adata after dedup: (35173, 30481)
ldata after dedup: (45252, 33696)

==== Common cells ===
Cells in common: 34946

==== Final datasets ===
Final adata shape: (34946, 30481)
Final ldata shape: (34946, 33696)
Indices match: True

In [6]: # converting the loom's ENSEMBL ID's to match

```

mg = mygene.MyGeneInfo()
gene_index = ldata.var.index.to_series()

# only grab the genes that have ENSEMBL IDs
is_ensembl = gene_index.str.startswith("ENSMUSG")

print("Total genes:", gene_index.shape[0])
print("ENSEMBL-like genes:", is_ensembl.sum())
print("Already-symbol-like genes:", (~is_ensembl).sum(), '\n')

# only query the ENSMUSG IDs
ens_ids = gene_index[is_ensembl].unique().tolist()

results = mg.querymany(
    ens_ids,
    scopes="ensemblgene",   # or "ensembl.gene" depending on mygene version,
    fields="symbol",
    species="mouse",
    as_dataframe=False
)

# Build mapping dict ENSMUSG -> symbol
ens_to_symbol = {r["query"]る: r.get("symbol", None) for r in results}

# QC on mapping
total_ens = len(ens_ids)
mapped_ens = sum(v is not None for v in ens_to_symbol.values())
print("Total ENSMUSG IDs:", total_ens)

```

```
print("Mapped ENSMUSG → symbol:", mapped_ens)
print("Unmapped ENSMUSG:", total_ens - mapped_ens)

# 4) Create a 'symbol' column:
# default: keep existing name
ldata.var["symbol"] = gene_index.copy()

# replace ENSMUSG entries with their symbol
for g in gene_index[is_ensembl]:
    sym = ens_to_symbol.get(g, None)
    if sym is not None:
        ldata.var.at[g, "symbol"] = sym

ldata.var.index = ldata.var["symbol"]

gene_index = ldata.var.index.to_series()
is_ensembl = gene_index.str.startswith("ENSMUSG")

print("Total ENSMUSG IDs after mapping: ", is_ensembl.sum())
```

```
WARNING:biothings.client:Input sequence provided is already in string format. No operation performed
WARNING:biothings.client:Input sequence provided is already in string format. No operation performed
INFO:biothings.client:querying 1-248 ...
Total genes: 33696
ENSEMBL-like genes: 248
Already-symbol-like genes: 33448
```

```
INFO:biothings.client:Finished.  
WARNING:biothings.client:13 input query terms found no hit:      ['ENSMUSG000  
00095742', 'ENSMUSG00000095728', 'ENSMUSG00000095076', 'ENSMUSG0000121317',  
'ENSMUSG000  
INFO:biothings.client:Pass "returnall=True" to return complete lists of dupl  
icate or missing query terms.  
Total ENSMUSG IDs: 248  
Mapped ENSMUSG → symbol: 203  
Unmapped ENSMUSG: 45  
Total ENSMUSG IDs after mapping: 45
```

```
In [7]: # Intersect with Seurat genes
ladata.var_names_make_unique() # This handles any duplicate gene symbol

print("Total number of var names: ", len(adata.var_names))

# Now find common genes between the two datasets
common_genes = adata.var.index.intersection(ladata.var.index)
print("Overlapping genes after symbol mapping:", len(common_genes))

# Subset both datasets to common genes
adata_subset = adata[:, common_genes].copy()
ladata_subset = ladata[:, common_genes].copy()

print(f"adata shape: {adata_subset.shape}")
print(f"ladata shape: {ladata_subset.shape}")
```

Total number of var names: 30481
 Overlapping genes after symbol mapping: 29365
 adata shape: (34946, 29365)
 ladata shape: (34946, 29365)

```
In [8]: # Sanity Checks

# Verify cell barcodes match
print("Cells match:", (adata_subset.obs.index == ladata_subset.obs.index).all)

# If they don't match, reorder ladata_subset to match adata_subset:
ladata_subset = ladata_subset[adata_subset.obs.index, :].copy()

# Double-check genes are in same order
print("Genes match:", (adata_subset.var.index == ladata_subset.var.index).all)
```

Cells match: True
 Genes match: True

Integrating Loom and OWHA object

```
In [9]: # Add velocity layers to adata_subset
adata_subset.layers["spliced"] = ladata_subset.layers["spliced"].copy()
adata_subset.layers["unspliced"] = ladata_subset.layers["unspliced"].copy()
adata_subset.layers["ambiguous"] = ladata_subset.layers["ambiguous"].copy()

# Verify they were added
print("Layers in adata_subset:", list(adata_subset.layers.keys()))
print("Spliced shape:", adata_subset.layers["spliced"].shape)
print("Unspliced shape:", adata_subset.layers["unspliced"].shape)
```

Layers in adata_subset: ['spliced', 'unspliced', 'ambiguous']
 Spliced shape: (34946, 29365)
 Unspliced shape: (34946, 29365)

```
In [10]: adata_subset.obs
```

Out[10]:

	orig.ident	nCount_RNA	nFeature_RNA	timepo
AAACCCAAGAACAGGA	UW_CITEseq_1	4246.0	1723	Unwound
AAACCCAAGATAACAGT	D30PW_CITEseq_1	5424.0	2026	Wounded_D30P
AAACCCAAGCAATAGT	D15PW_CITEseq_2	5251.0	1547	Wounded_D15P
AAACCCAAGCGCCTAC	D15PW_CITEseq_2	6564.0	2217	Wounded_D15P
AAACCCAAGCTAGTTC	D15PW_CITEseq_1	6762.0	2138	Wounded_D15P
...
TTTGTGTCAGTCC	D30PW_CITEseq_1	16213.0	3284	Wounded_D30P
TTTGTGTCAGTCC	D30PW_CITEseq_2	15672.0	4255	Wounded_D30P
TTTGTGTCAGTCC	D15PW_CITEseq_2	14728.0	3067	Wounded_D15P
TTTGTGTCAGTCC	D15PW_CITEseq_1	5141.0	1741	Wounded_D15P
TTTGTGTCAGTCC	D15PW_CITEseq_1	3119.0	1472	Wounded_D15P

34946 rows × 28 columns

In [11]: `adata_subset.layers.keys()`

Out[11]: KeysView(Layers with keys: spliced, unspliced, ambiguous)

Starting RNA Velocity!

Now that we have combined the .loom file with our exported Seurat object (now AnnData object) we can explore with RNA Velocity

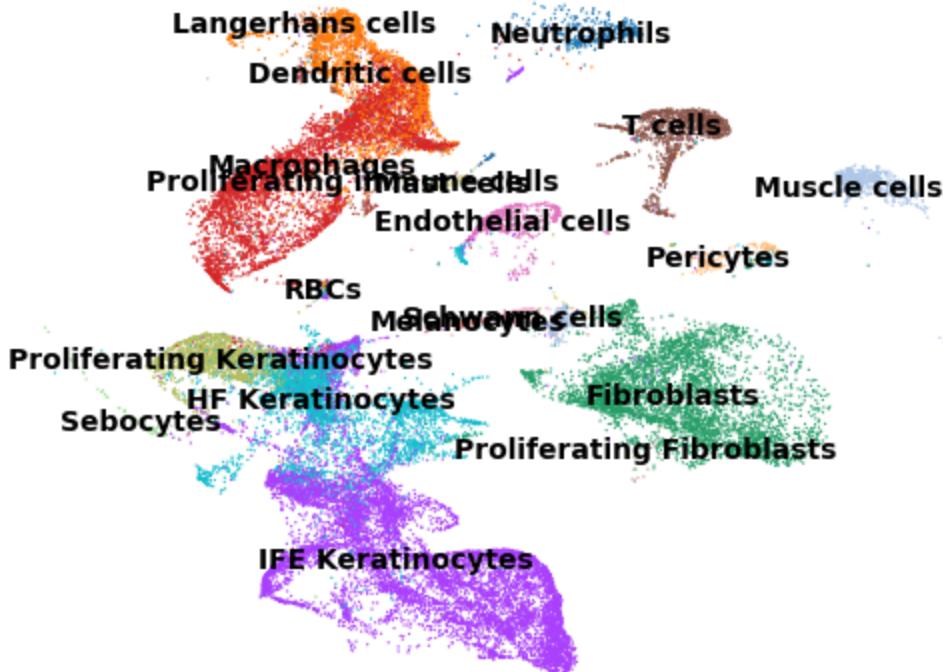
In [12]: `adata_subset.obsm.keys()`

Out[12]: KeysView(AxisArrays with keys: X_cca, X_harmony, X_pca, X_rpca, X_umap.cca, X_umap.harmony, X_umap.pca, X_umap.rpc)

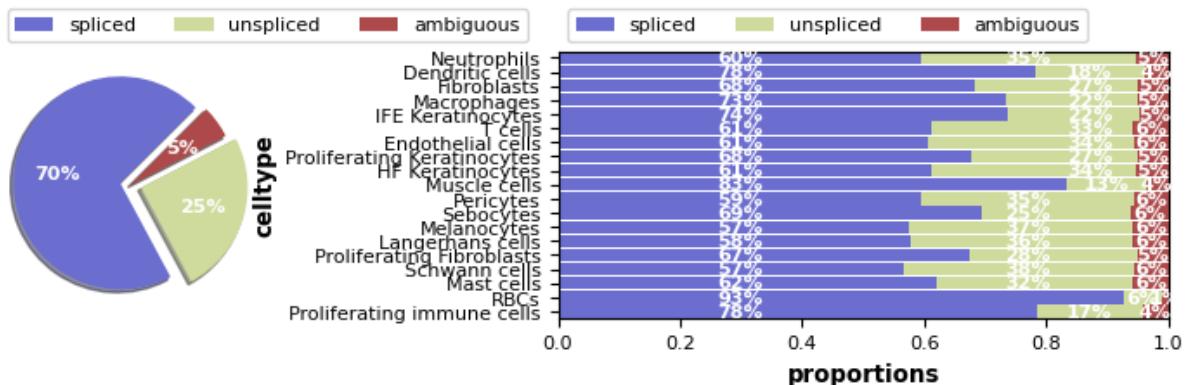
```
# plot umap to check
adata_subset.obsm["X_umap"] = adata_subset.obsm["X_umap.rpc"].copy()

sc.pl.umap(adata_subset, color="celltype", frameon=False,
           legend_loc='on data', title='', save='_celltypes_rpc.pdf')
```

WARNING: saving figure to file figures/umap_celltypes_rpc.pdf



```
In [14]: scv.pl.proportions(adata_subset, groupby='celltype')
```



```
In [15]: adata_subset.obsm["X_pca"].shape
```

```
Out[15]: (34946, 50)
```

```
In [16]: # pre-process
scv.pp.filter_and_normalize(adata_subset, min_shared_counts=20)
sc.pp.highly_variable_genes(adata_subset, n_top_genes=2000)

# subset to HVGs
adata_hvg = adata_subset[:, adata_subset.var['highly_variable']].copy()

# recompute neighbors on HVG PCA
scv.pp.moments(adata_hvg, n_pcs=30, n_neighbors=30)
```

```
Filtered out 17472 genes that are detected 20 counts (shared).
WARNING: Did not normalize X as it looks processed already. To enforce normalization, set `enforce=True`.
Normalized count data: spliced, unspliced.

/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/preprocessing/utils.py:705: DeprecationWarning: `log1p` is deprecated since scVelo v 0.3.0 and will be removed in a future version. Please use `log1p` from `scipy.stats` instead.
    log1p(adata)
Logarithmized X.

/var/folders/hq/r4mfshzj3dq22fq4382_72qw0000gp/T/ipykernel_20518/1409331441.py:9: DeprecationWarning: Automatic neighbor calculation is deprecated since scvelo==0.4.0 and will be removed in a future version of scVelo. Please compute neighbors first with Scanpy.
    scv.pp.moments(adata_hvg, n_pcs=30, n_neighbors=30)
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/preprocessing/moments.py:71: DeprecationWarning: `neighbors` is deprecated since scv elo==0.4.0 and will be removed in a future version of scVelo. Please compute neighbors with Scanpy.
    neighbors(
computing neighbors
    finished (0:00:13) --> added
    'distances' and 'connectivities', weighted adjacency matrices (adata.obs)
computing moments based on connectivities
    finished (0:00:02) --> added
    'Ms' and 'Mu', moments of un/spliced abundances (adata.layers)
```

```
In [17]: # compute velocity ( this step will take a while )
try:
    scv.tl.recover_dynamics(adata_hvg, n_top_genes=500)
    scv.tl.velocity(adata_hvg, mode='dynamical')
    scv.tl.velocity_graph(adata_hvg)
except Exception as e:
    print("Error while fitting dynamics:", e)
    print("Current gene index:", adata_hvg.var_names[adata_hvg.var['fit_alpha']])

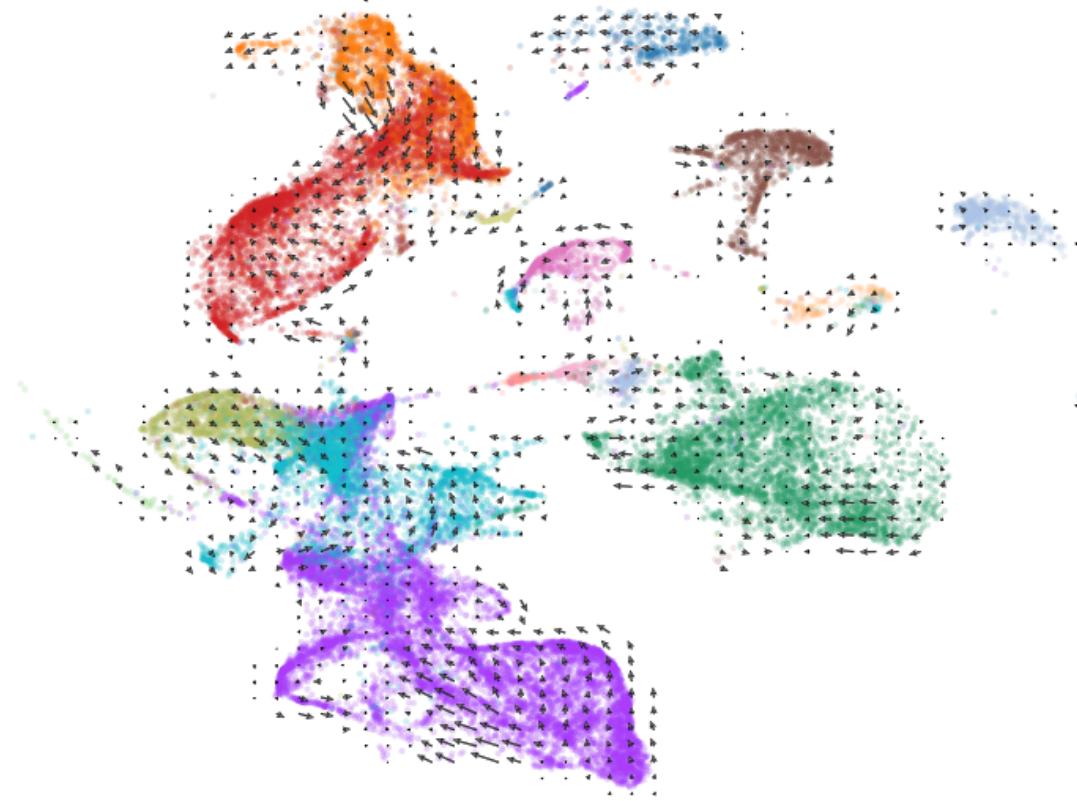
recovering dynamics (using 1/16 cores)
WARNING: Unable to create progress bar. Consider installing `tqdm` as `pip install tqdm` and `ipywidgets` as `pip install ipywidgets`, or disable the progress bar using `show_progress_bar=False`.
    finished (0:11:37) --> added
    'fit_pars', fitted parameters for splicing dynamics (adata.var)
computing velocities
    finished (0:00:04) --> added
    'velocity', velocity vectors for each individual cell (adata.layers)
computing velocity graph (using 1/16 cores)
    finished (0:00:11) --> added
    'velocity_graph', sparse matrix with cosine correlations (adata.uns)
```

```
In [18]: scv.pl.velocity_embedding_grid(
    adata_hvg,
    basis='X_umap',
    color='celltype',
    dpi=150
)
```

```
computing velocity embedding
    finished (0:00:01) --> added
    'velocity_umap', embedded velocity vectors (adata.obs['velocity_umap'])

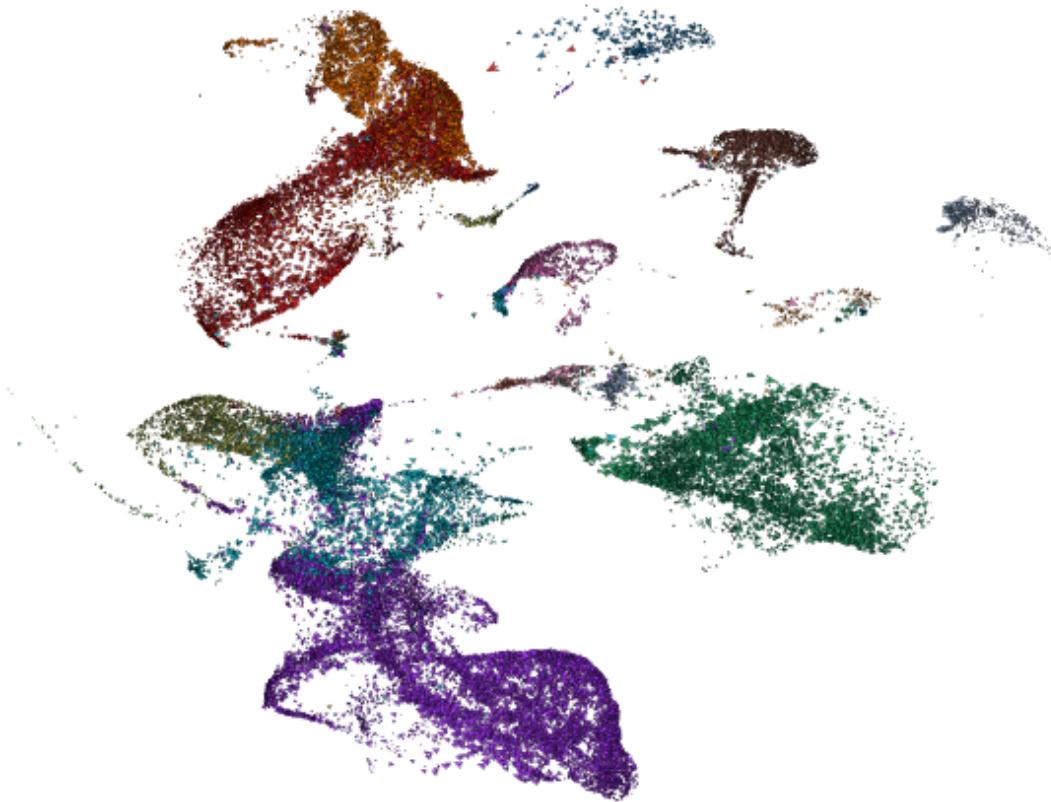
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
```

celltype



```
In [19]: scv.pl.velocity_embedding(adata_hvg,  
                                 arrow_length=3,  
                                 arrow_size=2,  
                                 color='celltype',  
                                 dpi=120)
```


celltype



```
In [20]: scv.tl.rank_velocity_genes(adata_hvg, groupby='celltype')
df = scv.get_df(adata_hvg.uns['rank_velocity_genes']['names'])
df.head()
```

ranking velocity genes

```
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/tools/ut
ils.py:463: DeprecationWarning: Please import `rankdata` from the `scipy.stats` namespace; the `scipy.stats.stats` namespace is deprecated and will be r
emoved in SciPy 2.0.0.
```

```
    from scipy.stats.stats import rankdata
    finished (0:00:06) --> added
    'rank_velocity_genes', sorted scores by group ids (adata.uns)
    'spearmans_score', spearmans correlation scores (adata.var)
```

```
/var/folders/hq/r4mfshzj3dq22fq4382_72qw0000gp/T/ipykernel_20518/2477482753.
py:2: DeprecationWarning: `get_df` is deprecated since scvelo==0.4.0 and wil
l be removed in a future version of scVelo. Please `AnnData::get_df` or Scan
py's `scanpy.get.obs_df` or `scanpy.get.var_df`.
```

```
df = scv.get_df(adata_hvg.uns['rank_velocity_genes']['names'])
```

Out[20]:

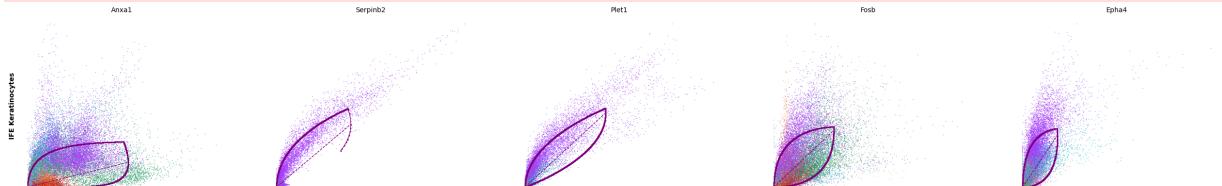
	Neutrophils	Dendritic cells	Fibroblasts	Macrophages	Keratinocytes	IFE	T cells	Endothel ce
0	Laptm5	Basp1	Ugdh	Basp1	Anxa1	Lcp2	Mef	
1	Crem	Plek	Dst	Itgb2	Serpina2	Tm6sf1	Ni	
2	Tm6sf1	Crem	Fgfr1	Emilin2	Plet1	Sp100	R	
3	Epsti1	Itgb2	Fstl1	Slc15a3	Fosb	Samhd1	E	
4	Soat1	Lilrb4b	Dab2	Lgals3	Epha4	Ms4a4c	Cd	

In [21]: `for cluster in ['IFE Keratinocytes', 'Fibroblasts', 'Proliferating immune ce
scv.pl.scatter(adata_hvg, df[cluster][:5], ylabel=cluster, frameon=False)`


```

e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plotting/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and will be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
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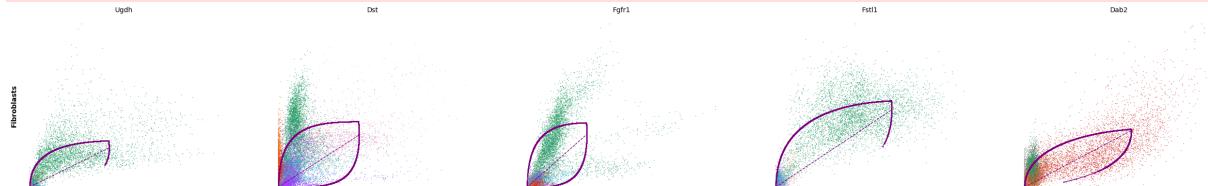
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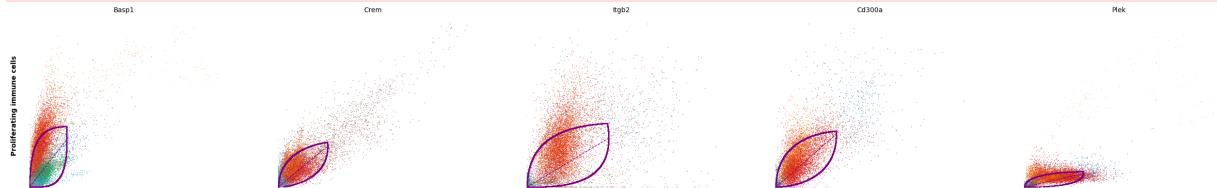
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/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plotting/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and will be removed in a future version. Use isinstance(dtype, pd.CategoricalDtype) instead

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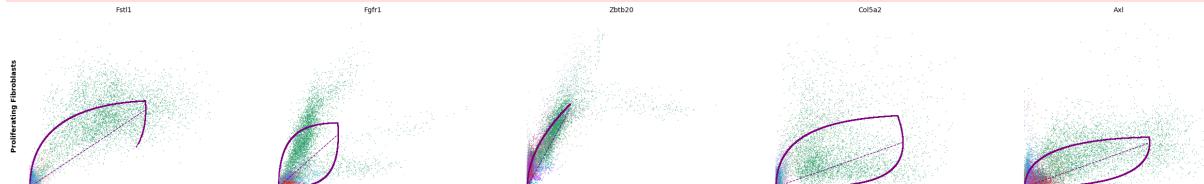
```




```

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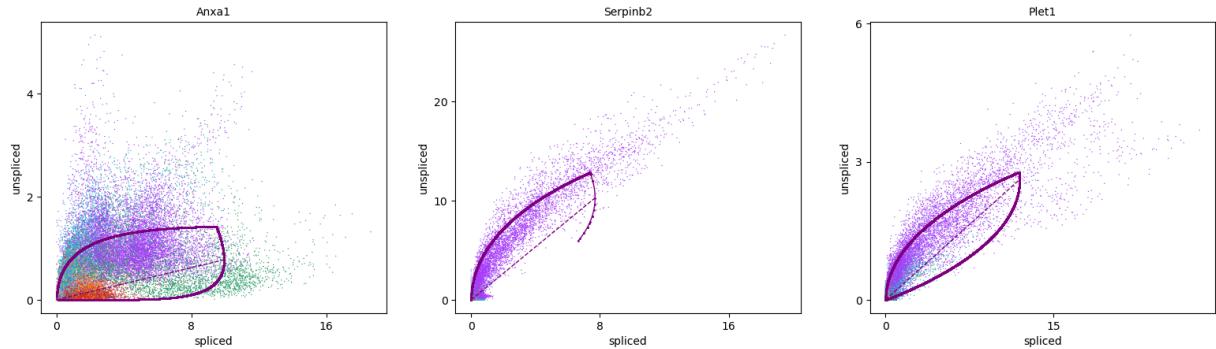
```



```
In [23]: scv.pl.scatter(adata_hvg, color='celltype', basis=adata_hvg.uns["rank_velocity"])
```

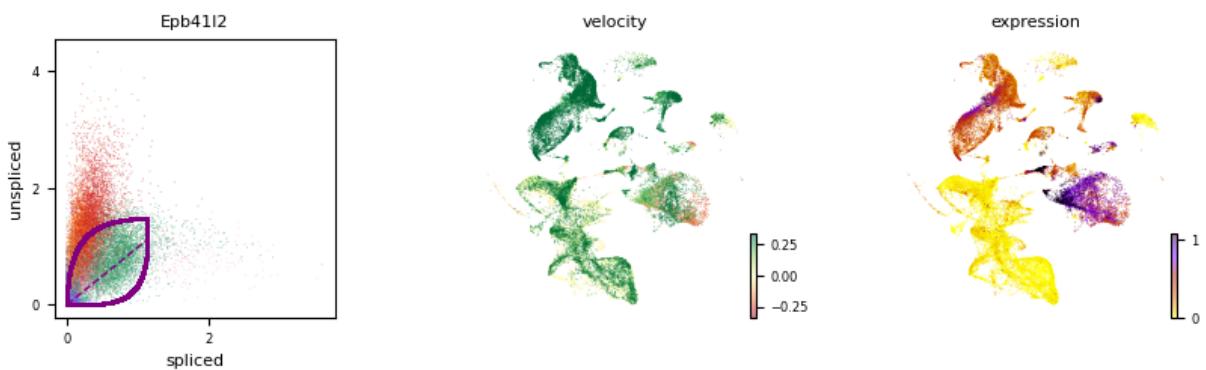


```
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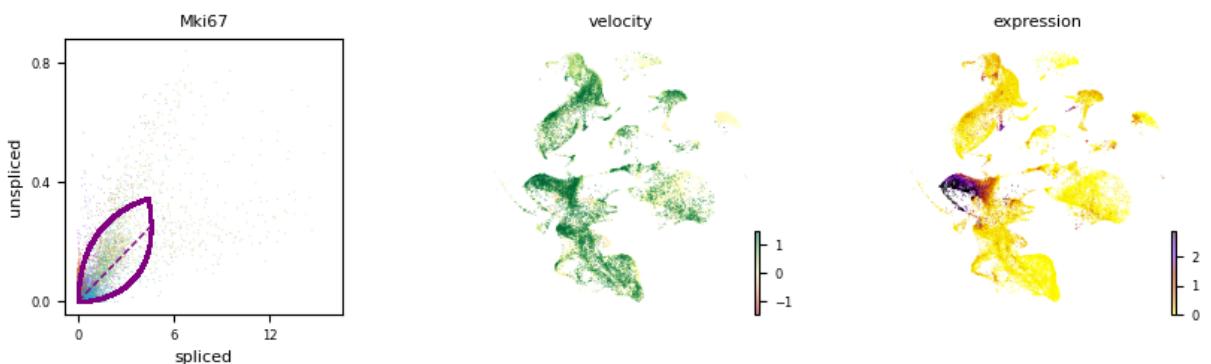
```
In [24]: scv.pl.velocity(adata_hvg, ['Epb41l2'], color='celltype')
```

```
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
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```



```
In [25]: scv.pl.velocity(adata_hvg, ['Mki67'], color='celltype') # Proliferating Keratocytes
```

```
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
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```



```
In [26]: scv.tl.latent_time(adata_hvg)
        scv.pl.scatter(adata_hvg, color='latent_time', color_map='gnuplot', size=80)
```

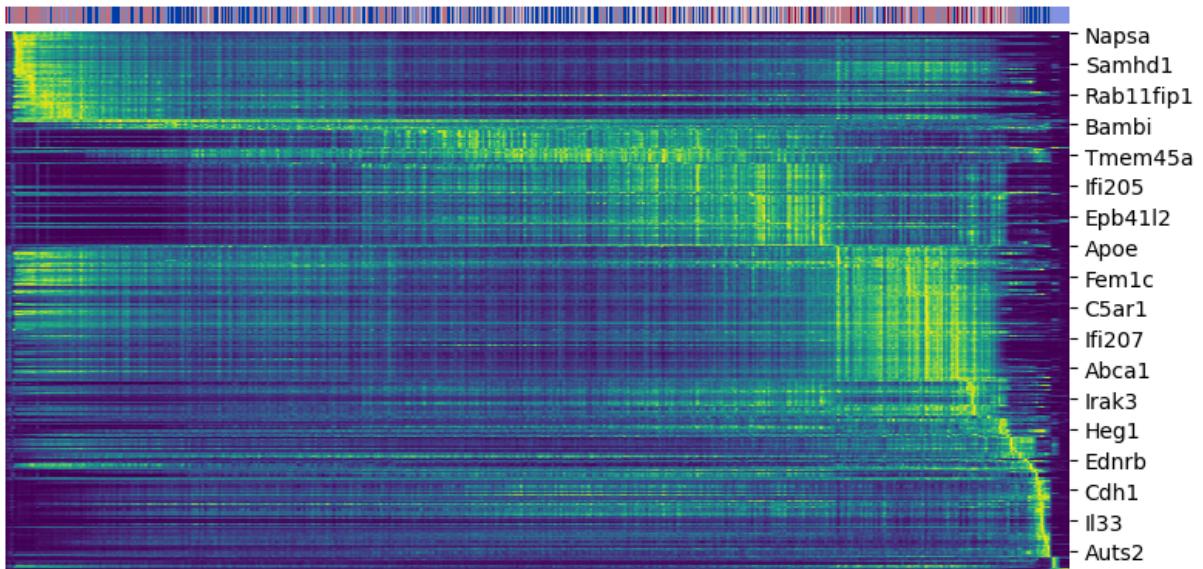
```
computing terminal states
    identified 9 regions of root cells and 2 regions of end points .
    finished (0:00:10) --> added
    'root_cells', root cells of Markov diffusion process (adata.obs)
    'end_points', end points of Markov diffusion process (adata.obs)
computing latent time using root_cells as prior
    finished (0:00:03) --> added
    'latent_time', shared time (adata.obs)

/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
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    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
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ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
```

latent time



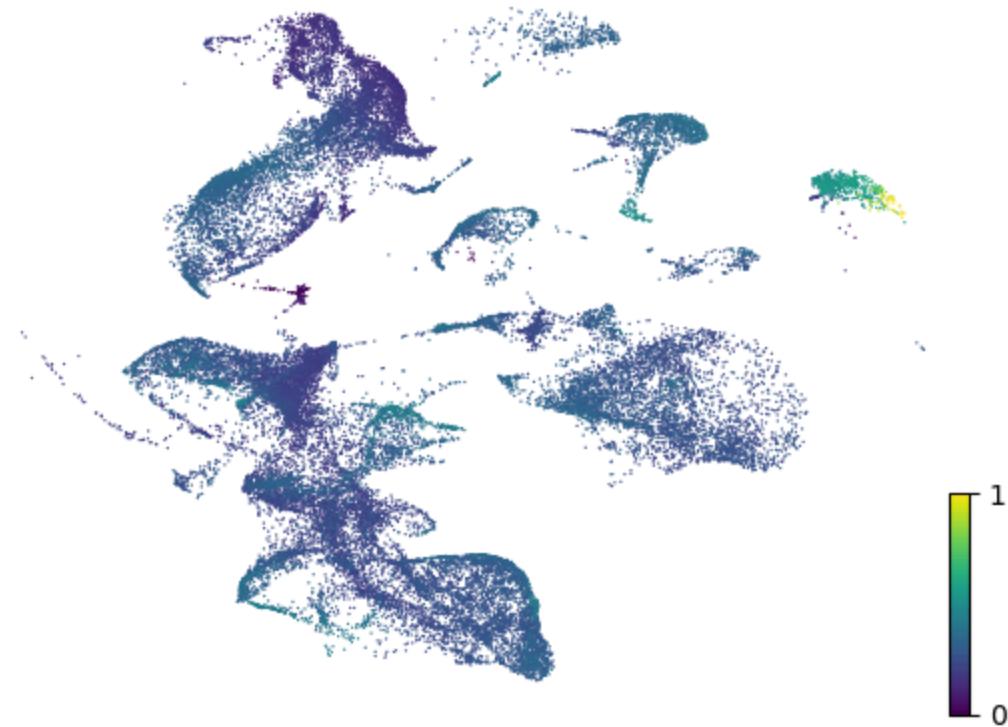
```
In [27]: top_genes = adata_hvg.var['fit_likelihood'].sort_values(ascending=False).inc  
scv.pl.heatmap(adata_hvg, var_names=top_genes, sortby='latent_time', col_col  
  
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin  
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi  
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/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin  
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi  
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp  
e) instead  
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
```



```
In [33]: scv.pl.scatter(adata_hvg, color='latent_time', color_map='viridis')
```

```
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
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e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
```

latent time

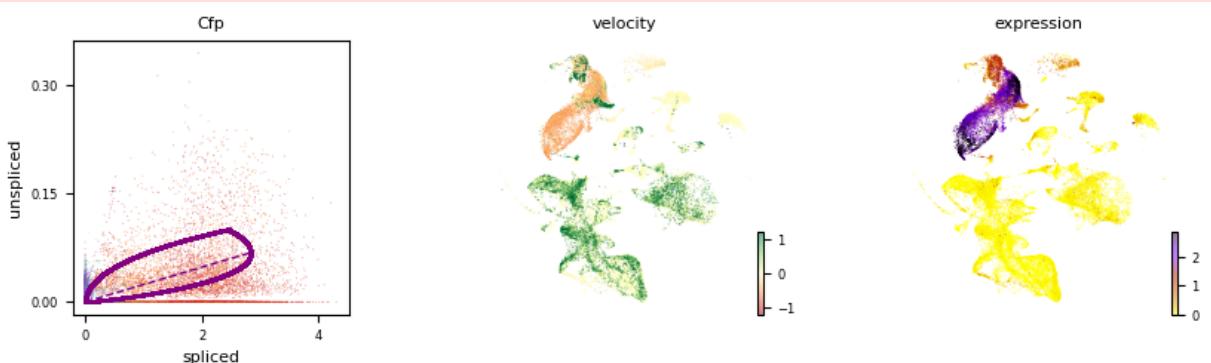


```
In [28]: top_genes
```

```
Out[28]: Index(['Dmkn', 'Cytip', 'Perp', 'Hmox1', 'Zeb2', 'Fstl1', 'F13a1', 'Serpina5',
      'Ifi205', 'Oasl1',
      ...
      'Nlrp3', 'Slc7a8', 'Slfn2', 'Actb', 'Igf1', 'Emilin2', 'Tnfaip6',
      'Kitl', 'Col6a2', 'Fcgrt'],
     dtype='object', length=300)
```

```
In [29]: scv.pl.velocity(adata_hvg, ['Cfp'], color='celltype')
```

```
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plottin
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
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ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead
    return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
```



```
In [30]: top_genes = adata_hvg.var['fit_likelihood'].sort_values(ascending=False).inc  
scv.pl.scatter(adata_hvg, basis=top_genes[:15], ncols=5, frameon=False, colc
```


ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp
e) instead

```
return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])  
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plotting/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and will be removed in a future version. Use isinstance(dtype, pd.CategoricalDtype) instead
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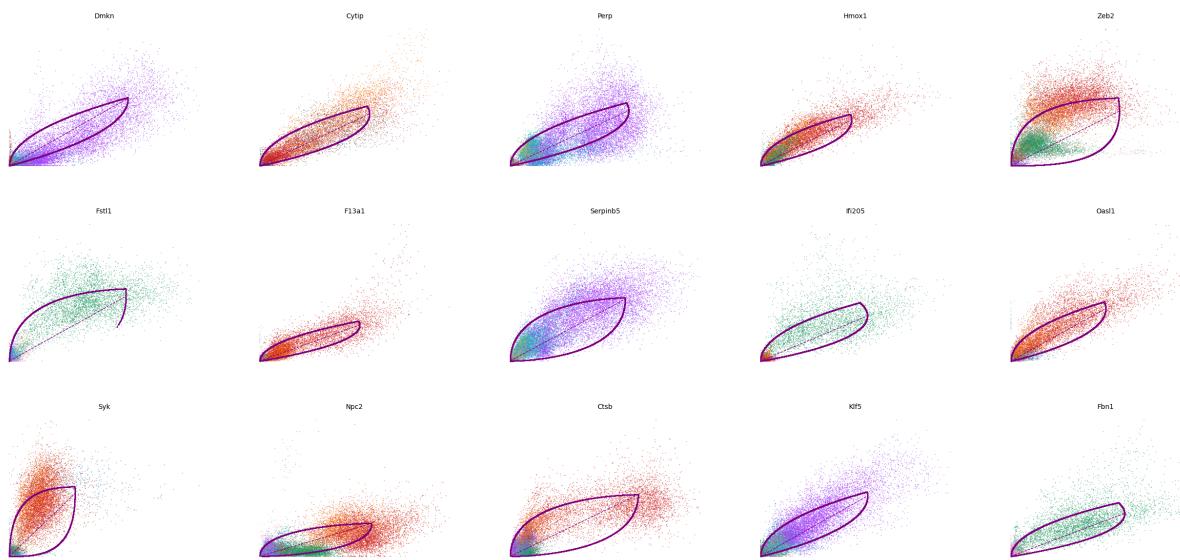
return isinstance(c, str) and c in data.obs.keys() and cat(data.obs[c])
/opt/miniconda3/envs/scvelo_env/lib/python3.10/site-packages/scvelo/plotting/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and will be removed in a future version. Use isinstance(dtype, pd.CategoricalDtype) instead

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```



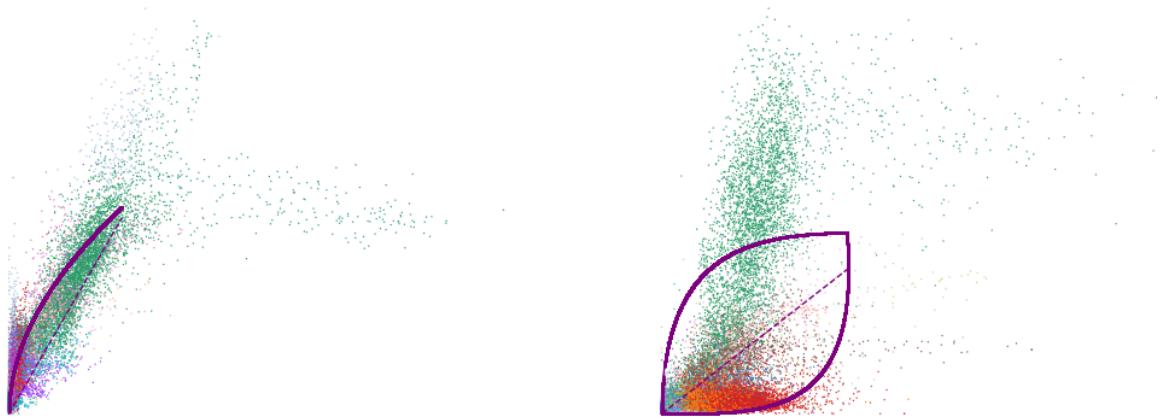
```
In [31]: var_names = ['Zbtb20', 'Celf2']
scv.pl.scatter(adata_hvg, var_names, frameon=False, color='celltype')
scv.pl.scatter(adata_hvg, x='latent_time', y=var_names, frameon=False, color
```



```
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi  
ll be removed in a future version. Use isinstance(dtype, pd.CategoricalDtyp  
e) instead  
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```

Zbtb20

Celf2




```
g/utils.py:68: DeprecationWarning: is_categorical_dtype is deprecated and wi  
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```

Zbtb20

Celf2

