Filling out incomplete coloc groupings

I found some groupings that were incomplete; that is a certain groupings were defined only by a number of pairwise relationships that is less than nC2 (n choose 2) relaionships for a grouping of n stain types. AG confirmed or amended these groupings and here I will stitch togeter the final set and begin asking some our more interesting coloc questions discussed in the previous notebook.

In [1]:
```
import numpy as np
import pandas as pd
import matplotlib.pyplot as plt
import seaborn as sns
import ast
import sys
import statsmodels.api as sm
from statsmodels.formula.api import ols

# loading some functions we wrote before
sys.path.append("/Users/jonathanramos/Desktop/LRI/Image ROI Data Wrangling/")
from clean import *
from norm import *
from count import *
```

```
/var/folders/b2/3h2lpxx14kgb12pp_7pltxnc0000gn/T/ipykernel_99650/543169497.py:2: DeprecationWarning:
Pyarrow will become a required dependency of pandas in the next major release of pandas (pandas 3.
0),
(to allow more performant data types, such as the Arrow string type, and better interoperability wit
h other libraries)
but was not found to be installed on your system.
If this would cause problems for you,
please provide us feedback at https://github.com/pandas-dev/pandas/issues/54466 (https://github.com/
pandas-dev/pandas/issues/54466)

  import pandas as pd
```

Loading in sets

We want to merge these sets so that all cells have fully updated groups (sets of unique cell roi ids) that they are associated with. Then we can build new dummy cols and start digging into the data.

We also have the following updated unpaired doubles:

- KET-10-12_PFC_3.9_C: expected the following missing pairs: [('0-FFF-00061_Npas4', '0-005-00025_cFos')] YES
- KET-10-3_PFC_3.5_C: expected the following missing pairs: [('0-FFF-00064_Npas4', '0-FFF-00070_cFos')] YES
- KET-10-3_PFC_3.7_D: expected the following missing pairs: [('0-FFF-00048_Npas4', '0-005-00024_cFos')] YES
- KET-8-2_PFC_4.0_A: expected the following missing pairs: [('0-FFF-00103_Npas4', '0-005-00015_cFos')] YES
- PE-11-1_PFC_3.6_B: expected the following missing pairs: [('0-200-00007_Npas4', '0-005-00026_cFos')] NO
- PE-11-3_PFC_3.5_C: expected the following missing pairs: [('0-200-00000_Npas4', '0-FFF-00033_cFos')] NO
- PE-11-5_PFC_3.7_D: expected the following missing pairs: [('0-FFF-00022_Npas4', '0-FFF-00004_cFos')] YES

Full cleaned set from previous notebook

In [2]:
```
df_coloc = pd.read_csv('KET-VR5_COLOC_FULL_DB.csv')

# let's take a look
print(df_coloc.shape)
print(df_coloc.columns)
df_coloc
```

```
(22798, 19)
Index(['Unnamed: 0', 'roi_id', 'coloc_w/_PV', 'coloc_w/_cFos',
       'coloc_w/_Npas4', 'coloc_w/_WFA', 'CoM_x', 'CoM_y', 'background',
       'mean_intensity', 'filename', 'rat_n', 'treatment', 'stain_type',
       'image_name', 'dummy_PV', 'dummy_cFos', 'dummy_Npas4', 'dummy_WFA'],
      dtype='object')
```

Out[2]:

	Unnamed: 0	roi_id	coloc_w/_PV	coloc_w/_cFos	coloc_w/_Npas4	coloc_w/_WFA	CoM_x	CoM_y	background	mean_intensity
0	0	0-000-00000_PV	-	-	0-FFF-00045_Npas4	0-FFF-00004_WFA	297.86	425.97	238.8715	536.8331
1	1	0-000-00001_PV	-	0-FFF-00070_cFos	0-FFF-00012_Npas4	-	340.47	43.89	238.8715	314.9278
2	2	0-000-00002_PV	-	-	0-FFF-00044_Npas4	0-FFF-00005_WFA	154.85	476.15	238.8715	324.0556
3	3	0-000-00003_PV	-	-	0-FFF-00082_Npas4	0-FFF-00003_WFA	310.10	308.22	238.8715	346.0313
4	4	0-000-00004_PV	-	-	-	-	44.35	323.64	238.8715	429.6127
...	...	...	...	...	...	...	...	...	...	...
22793	0	-	-	-	-	-	177.27	68.60	93.9887	88.2249
22794	8	-	-	-	-	-	164.01	117.08	111.9724	146.2031
22795	9	-	-	-	-	-	197.94	107.45	111.9724	123.3614
22796	10	-	-	-	-	-	229.32	96.22	111.9724	108.3670
22797	11	-	-	-	-	-	261.34	98.06	111.9724	106.3260

22798 rows × 19 columns

Amended groupings, confirmed by visual inspection of images

In [3]:

```
# reading in our set of amended groupings
paired_quads = pd.read_csv('PAIRED_unpaired_triples_quads/unpaired_quads.csv')
paired_triples = pd.read_csv('PAIRED_unpaired_triples_quads/unpaired_triples.csv')

# from the text above, building a df of amended doubles
doubles = [
    ('KET-10-12_PFC_3.9_C', ('0-FFF-00061_Npas4', '0-005-00025_cFos'), True), #YES
    ('KET-10-3_PFC_3.5_C', ('0-FFF-00064_Npas4', '0-FFF-00070_cFos'), True), #YES
    ('KET-10-3_PFC_3.7_D', ('0-FFF-00048_Npas4', '0-005-00024_cFos'), True), #YES
    ('KET-8-2_PFC_4.0_A', ('0-FFF-00103_Npas4', '0-005-00015_cFos'), True), #YES
    ('PE-11-1_PFC_3.6_B', ('0-200-00007_Npas4', '0-005-00026_cFos'), False), #NO
    ('PE-11-3_PFC_3.5_C', ('0-200-00000_Npas4', '0-FFF-00033_cFos'), False), #NO
    ('PE-11-5_PFC_3.7_D', ('0-FFF-00022_Npas4', '0-FFF-00004_cFos'), True) #YES
    ]

paired_doubles = pd.DataFrame(doubles, columns = ['image_name', 'grouping', 'correct_grouping'])

# standardizing "Correct grouping? (yes/no)" col name
paired_triples['correct_grouping'] = paired_triples['Correct grouping? (yes/no)']\
    .apply(lambda x: True if x.lower() == 'yes' else False)
paired_quads['correct_grouping'] = paired_quads['Correct grouping? (yes/no)']\
    .apply(lambda x: True if x.lower() == 'yes' else False)

# interpretting grouping string as python object
sort_order = {'PV': 0, 'cFos': 1, 'Npas4': 2, 'WFA': 3}
paired_quads['grouping'] = paired_quads.grouping.apply(
    lambda x: tuple(sorted(list(ast.literal_eval(x)),\
                           key=lambda y: sort_order[y.split('_')[-1]])))

paired_triples['grouping'] = paired_triples.grouping.apply(
    lambda x: tuple(sorted(list(ast.literal_eval(x)),\
                           key=lambda y: sort_order[y.split('_')[-1]])))

# converting amended grouping cols to tuples from strings
for col in [c for c in paired_triples.columns if 'amended' in c]:
    paired_triples[col] = paired_triples[col].apply(
        lambda x: tuple(sorted(
            x.replace("'", "").replace(' ', '').strip(',').split(','),\
                  key=lambda y: sort_order[y.split('_')[-1]]
            )) if type(x) == str else x)

for col in [c for c in paired_quads.columns if 'amended' in c]:
    paired_quads[col] = paired_quads[col].apply(
        lambda x: tuple(sorted(
            x.replace("'", "").replace(' ', '').strip(',').split(','),\
                  key=lambda y: sort_order[y.split('_')[-1]]
            )) if type(x) == str else x)

# building new roi_id col by exploding out grouping col
paired_doubles['roi_id'] = paired_doubles.grouping
paired_doubles = paired_doubles.explode('roi_id').reset_index()

paired_triples['roi_id'] = paired_triples.grouping
paired_triples = paired_triples.explode('roi_id').reset_index()

paired_quads['roi_id'] = paired_quads.grouping
paired_quads = paired_quads.explode('roi_id').reset_index()

# aggregating amended grouping columns into one
def agg_amend_dbls(x):
    if x.correct_grouping:
        return x.grouping
    else:
        return tuple([x.roi_id])

def agg_amend(x):
    # first check if correct grouping; if it is, return grouping
    if x.correct_grouping:
        return x.grouping

    # else, get grouping from amended grouping col where roi_id occurs
    elif not x.correct_grouping:
        roi_id = x.roi_id
        grps = x[[i for i in x.index if 'amended' in i]].values
        grps = grps[~pd.isna(grps)]
        amended_grp = [grp for grp in grps if roi_id in grp]
```

```
77
78          # for debugging
79          if len(amended_grp) != 1:
80              print(x)
81
82          return amended_grp[0]
83
84  # apply aggregation function
85  paired_doubles['true_grouping'] = paired_doubles.apply(agg_amend_dbls, axis=1)
86  paired_triples['true_grouping'] = paired_triples.apply(agg_amend, axis=1)
87  paired_quads['true_grouping'] = paired_quads.apply(agg_amend, axis=1)
88
89  # let's take a look!
90  print(paired_quads.shape)
91  paired_quads.head()
```

```
(484, 13)
```

Out[3]:

	index	Unnamed: 0	image_name	grouping	Correct grouping? (yes/no)	amended grouping_1	amended grouping_2	amended grouping_3	ameded grouping_4	notes	correct_grouping
0	0	0	KET-10-12_PFC_3.7_A	(0-000-00012_PV, 0-005-00036_cFos, 0-FFF-00009...	yes	NaN	NaN	NaN	NaN	NaN	True
1	0	0	KET-10-12_PFC_3.7_A	(0-000-00012_PV, 0-005-00036_cFos, 0-FFF-00009...	yes	NaN	NaN	NaN	NaN	NaN	True
2	0	0	KET-10-12_PFC_3.7_A	(0-000-00012_PV, 0-005-00036_cFos, 0-FFF-00009...	yes	NaN	NaN	NaN	NaN	NaN	True
3	0	0	KET-10-12_PFC_3.7_A	(0-000-00012_PV, 0-005-00036_cFos, 0-FFF-00009...	yes	NaN	NaN	NaN	NaN	NaN	True
4	1	1	KET-10-12_PFC_3.7_A	(0-000-00000_PV, 0-005-00015_cFos, 0-FFF-00045...	no	(0-000-00000_PV, 0-FFF-00045_Npas4, 0-FFF-0000...	(0-005-00015_cFos,)	NaN	NaN	NaN	False

selecting only the necessary cols, aggregating across amended quads/triples/doubles

In [4]:
```
paired_quads = paired_quads[['image_name', 'roi_id','true_grouping']]
paired_triples = paired_triples[['image_name', 'roi_id','true_grouping']]
paired_doubles = paired_doubles[['image_name', 'roi_id','true_grouping']]


df_amended = pd.concat([paired_quads, paired_triples, paired_doubles])
df_amended = df_amended.groupby(by=['image_name', 'roi_id'])[['image_name', 'roi_id', 'true_group
    .apply(lambda x: sorted(list(x.true_grouping.values), key=len)[0])\
    .reset_index(name='true_grouping')

df_amended
```

Out[4]:

	image_name	roi_id	true_grouping
0	KET-10-12_PFC_3.7_A	0-000-00000_PV	(0-000-00000_PV, 0-FFF-00045_Npas4, 0-FFF-0000...
1	KET-10-12_PFC_3.7_A	0-000-00001_PV	(0-000-00001_PV, 0-FFF-00012_Npas4)
2	KET-10-12_PFC_3.7_A	0-000-00012_PV	(0-000-00012_PV, 0-005-00036_cFos, 0-FFF-00009...
3	KET-10-12_PFC_3.7_A	0-000-00013_PV	(0-000-00013_PV, 0-005-00019_cFos)
4	KET-10-12_PFC_3.7_A	0-005-00015_cFos	(0-005-00015_cFos,)
...	...	...	...
879	PE-13-9_PFC_3.3_C	0-FFF-00057_Npas4	(0-000-00004_PV, 0-FFF-00057_Npas4, 0-FFF-0000...
880	PE-13-9_PFC_3.5_E	0-000-00001_PV	(0-000-00001_PV, 0-FFF-00034_cFos, 0-FFF-00005...
881	PE-13-9_PFC_3.5_E	0-FFF-00005_WFA	(0-000-00001_PV, 0-FFF-00034_cFos, 0-FFF-00005...
882	PE-13-9_PFC_3.5_E	0-FFF-00034_cFos	(0-000-00001_PV, 0-FFF-00034_cFos, 0-FFF-00005...
883	PE-13-9_PFC_3.5_E	0-FFF-00040_Npas4	(0-FFF-00040_Npas4,)

884 rows × 3 columns

Replacing old groupings with new amended groupings

Building out a grouping col in our full df

In [5]:

```
sort_order = {'PV': 0, 'cFos': 1, 'Npas4': 2, 'WFA': 3}

images = []
for image in df_coloc.image_name.unique():

    # slice out image
    df_image = df_coloc.query(f'image_name == "{image}"').copy().reset_index().drop('index', axis=

    # build new grouping col of sorted tuples
    df_image['grouping'] = df_image.apply(\
        lambda x: tuple(sorted([\
            y for y in [x.roi_id, x['coloc_w/_PV'], x['coloc_w/_cFos'], x['coloc_w/_Npas4'], x['c
                key=lambda y: sort_order[y.split('_')[-1]]))
    ), axis=1)

    # drop old dummy cols (these will be updated once i recieve updated coloc data from AG)
    df_image = df_image[[col for col in df_image.columns if not 'dummy' in col]]
    images.append(df_image)

images[0]
```

Out[5]:

	Unnamed: 0	roi_id	coloc_w/_PV	coloc_w/_cFos	coloc_w/_Npas4	coloc_w/_WFA	CoM_x	CoM_y	background	mean_intensity	
0	0	0-000-00000_PV	-	-	0-FFF-00045_Npas4	0-FFF-00004_WFA	297.86	425.97	238.8715	536.8331	1
1	1	0-000-00001_PV	-	0-FFF-00070_cFos	0-FFF-00012_Npas4	-	340.47	43.89	238.8715	314.9278	1
2	2	0-000-00002_PV	-	-	0-FFF-00044_Npas4	0-FFF-00005_WFA	154.85	476.15	238.8715	324.0556	1
3	3	0-000-00003_PV	-	-	0-FFF-00082_Npas4	0-FFF-00003_WFA	310.10	308.22	238.8715	346.0313	1
4	4	0-000-00004_PV	-	-	-	-	44.35	323.64	238.8715	429.6127	1
...	...	...	...	...	...	...	...	...	...	...	
204	204	0-FFF-00002_WFA	0-000-00008_PV	0-005-00017_cFos	0-FFF-00062_Npas4	-	172.04	315.17	115.7134	126.9843	1
205	205	0-FFF-00003_WFA	0-000-00003_PV	-	0-FFF-00082_Npas4	-	311.54	305.09	115.7134	110.1349	1
206	206	0-FFF-00004_WFA	0-000-00000_PV	0-005-00015_cFos	0-FFF-00045_Npas4	-	297.09	427.72	115.7134	122.0008	1
207	207	0-FFF-00005_WFA	0-000-00002_PV	-	0-FFF-00044_Npas4	-	157.10	478.13	115.7134	115.3184	1
208	208	0-FFF-00006_WFA	0-000-00005_PV	-	0-FFF-00041_Npas4	-	47.44	466.73	115.7134	114.0730	1

209 rows × 16 columns

Replace with amended groupings

In [6]:
```
def replace_amended_groupings(x):
    iid = x.image_name
    rid = x.roi_id
    q = df_amended.query(f'image_name == "{iid}" and roi_id == "{rid}"')

    if not q.empty:
        # for debuggin (we expect len of exactly 1 here)
        if len(q) > 1:
            print(q)
        return q.true_grouping.values[0]

    elif q.empty:
        return x.grouping

df_coloc = pd.concat(images)
df_coloc['true_grouping'] = df_coloc.apply(replace_amended_groupings, axis=1)
df_coloc
```

Out[6]:

	Unnamed: 0	roi_id	coloc_w/_PV	coloc_w/_cFos	coloc_w/_Npas4	coloc_w/_WFA	CoM_x	CoM_y	background	mean_intensity	
0	0	0-000-00000_PV	-	-	0-FFF-00045_Npas4	0-FFF-00004_WFA	297.86	425.97	238.8715	536.8331	1
1	1	0-000-00001_PV	-	0-FFF-00070_cFos	0-FFF-00012_Npas4	-	340.47	43.89	238.8715	314.9278	1
2	2	0-000-00002_PV	-	-	0-FFF-00044_Npas4	0-FFF-00005_WFA	154.85	476.15	238.8715	324.0556	1
3	3	0-000-00003_PV	-	-	0-FFF-00082_Npas4	0-FFF-00003_WFA	310.10	308.22	238.8715	346.0313	1
4	4	0-000-00004_PV	-	-	-	-	44.35	323.64	238.8715	429.6127	1
...	...	...	...	...	...	...	...	...	...	...	
118	22777	0-FFF-00008_WFA	-	-	-	-	385.12	235.30	109.6097	119.2211	
119	22778	0-FFF-00009_WFA	0-000-00002_PV	-	-	-	409.79	230.66	109.6097	128.8761	
120	22779	0-FFF-00010_WFA	-	-	-	-	300.24	100.38	109.6097	123.8280	
121	22780	0-FFF-00011_WFA	0-000-00005_PV	-	-	-	414.17	44.01	109.6097	120.4709	
122	22781	0-FFF-00012_WFA	0-000-00000_PV	-	0-FFF-00002_Npas4	-	203.68	49.91	109.6097	112.7066	

22798 rows × 17 columns

building new dummy cols

In [7]:
```
def get_dummies(x):
    groupings = [rid.split('_')[-1] for rid in x]

    dummy_PV = False
    dummy_cFos = False
    dummy_Npas4 = False
    dummy_WFA = False

    if 'PV' in groupings:
        dummy_PV = True
    if 'cFos' in groupings:
        dummy_cFos = True
    if 'Npas4' in groupings:
        dummy_Npas4 = True
    if 'WFA' in groupings:
        dummy_WFA = True

    return dummy_PV, dummy_cFos, dummy_Npas4, dummy_WFA

df_coloc['dummy'] = df_coloc.true_grouping.apply(get_dummies)
df_coloc['dummy_PV'], df_coloc['dummy_cFos'], df_coloc['dummy_Npas4'], df_coloc['dummy_WFA'] = zip
```

Some interesting timeit tests

In [8]:
```
%timeit df_coloc['dummy_PV'], df_coloc['dummy_cFos'], df_coloc['dummy_Npas4'], df_coloc['dummy_WF
%timeit df_coloc.assign(**dict(zip(['dummy_PV', 'dummy_cFos', 'dummy_Npas4', 'dummy_WFA'], zip(*d

```

```
8.42 ms ± 212 µs per loop (mean ± std. dev. of 7 runs, 100 loops each)
14.5 ms ± 348 µs per loop (mean ± std. dev. of 7 runs, 100 loops each)
```

Moving on to analyses

just doing some more preliminary cleaning

In [9]:
```
df_coloc = df_coloc[['filename', 'image_name', 'roi_id', 'true_grouping', \
                     'dummy_PV', 'dummy_cFos', 'dummy_Npas4', 'dummy_WFA',\
                     'CoM_x', 'CoM_y', 'background', 'mean_intensity', \
                     'stain_type', 'filename', 'rat_n', 'treatment']]\
                     .copy(deep=True).reset_index()

df_coloc['group_name'] = df_coloc.rat_n.apply(lambda x: '-'.join(x.split('-')[:2]))

# drop everything from group PE-11
df_coloc = df_coloc.query('group_name != "PE-11"')

print(df_coloc.shape)
df_coloc
```

```
(18632, 18)
```

Out[9]:

	index	filename	image_name	roi_id	true_grouping	dummy_PV	dummy_cFos	dummy_Npas4	dummy_WFA	CoM_x
0	0	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00000_PV	(0-000-00000_PV, 0-FFF-00045_Npas4, 0-FFF-0000...	True	False	True	True	297.86
1	1	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00001_PV	(0-000-00001_PV, 0-FFF-00012_Npas4)	True	False	True	False	340.47
2	2	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00002_PV	(0-000-00002_PV, 0-FFF-00044_Npas4, 0-FFF-0000...	True	False	True	True	154.85
3	3	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00003_PV	(0-000-00003_PV, 0-FFF-00082_Npas4, 0-FFF-0000...	True	False	True	True	310.10
4	4	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00004_PV	(0-000-00004_PV,)	True	False	False	False	44.35
...	...	...	...	...	...	...	...	...	...	...
22793	118	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00008_WFA	(0-FFF-00008_WFA,)	False	False	False	True	385.12
22794	119	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00009_WFA	(0-000-00002_PV, 0-FFF-00009_WFA)	True	False	False	True	409.79
22795	120	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00010_WFA	(0-FFF-00010_WFA,)	False	False	False	True	300.24
22796	121	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00011_WFA	(0-000-00005_PV, 0-FFF-00011_WFA)	True	False	False	True	414.17
22797	122	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00012_WFA	(0-000-00000_PV, 0-FFF-00002_Npas4, 0-FFF-0001...	True	False	True	True	203.68

18632 rows × 18 columns

SNR Threshold

For each stain type we determined the following SNR thresholds:

- PV : 0.8
- cFos : 0

- Npas4 : 0.8
- WFA : 0.85

We did this already, but given that a whole group of animals was removed, we should repeat this step. Given that the data we loaded has already been snr thresholded, we expect the same number of cells before and after filtering

In [10]:

```
def get_snr(df):
    '''
    '''
    df['snr'] = df['mean_intensity'].astype('f') / df['background'].astype('f')

    return df

def filter_snr(df, snr_cutoff):
    '''
    '''
    df_threshold = pd.concat([df.query(f'stain_type == "{stain}"').query(f'snr > {snr_cutoff[stai

    return df_threshold

# build dict of snr thresholds
snr_threshold = {
    'PV': 0.8,
    'cFos': 0,
    'Npas4': 0.8,
    'WFA': 0.85
}

df_coloc = filter_snr(get_snr(df_coloc), snr_threshold)

print(df_coloc.shape)
df_coloc
```

```
(18632, 19)
```

Out[10]:

	index	filename	image_name	roi_id	true_grouping	dummy_PV	dummy_cFos	dummy_Npas4	dummy_WFA	CoM_x
0	0	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00000_PV	(0-000-00000_PV, 0-FFF-00045_Npas4, 0-FFF-0000...	True	False	True	True	297.86
1	1	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00001_PV	(0-000-00001_PV, 0-FFF-00012_Npas4)	True	False	True	False	340.47
2	2	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00002_PV	(0-000-00002_PV, 0-FFF-00044_Npas4, 0-FFF-0000...	True	False	True	True	154.85
3	3	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00003_PV	(0-000-00003_PV, 0-FFF-00082_Npas4, 0-FFF-0000...	True	False	True	True	310.10
4	4	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00004_PV	(0-000-00004_PV,)	True	False	False	False	44.35
...	...	...	...	...	...	...	...	...	...	...
22793	118	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00008_WFA	(0-FFF-00008_WFA,)	False	False	False	True	385.12
22794	119	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00009_WFA	(0-000-00002_PV, 0-FFF-00009_WFA)	True	False	False	True	409.79
22795	120	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00010_WFA	(0-FFF-00010_WFA,)	False	False	False	True	300.24
22796	121	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00011_WFA	(0-000-00005_PV, 0-FFF-00011_WFA)	True	False	False	True	414.17
22797	122	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00012_WFA	(0-000-00000_PV, 0-FFF-00002_Npas4, 0-FFF-0001...	True	False	True	True	203.68

18632 rows × 19 columns

Drop nans, check for duplicates

In [11]:
```
# which cols have nans, how many?
print('Nan per col:')
print(df_coloc.isna().sum())

# how many duplicated rows do we have?
print('\nTotal n of duplicated rows:')

# it's best to count duplicates on the numeric data to avoid false negatives due to
# errors in spelling or systematic labeling
print(df_coloc.duplicated(subset=['filename', 'image_name', 'CoM_x', 'CoM_y',
       'background', 'mean_intensity', 'stain_type', 'filename', 'rat_n',
       'treatment', 'group_name', 'snr']).sum())
```

```
Nan per col:
index             0
filename          0
image_name        0
roi_id            0
true_grouping     0
dummy_PV          0
dummy_cFos        0
dummy_Npas4       0
dummy_WFA         0
CoM_x             0
CoM_y             0
background        0
mean_intensity    0
stain_type        0
filename          0
rat_n             0
treatment         0
group_name        0
snr               0
dtype: int64

Total n of duplicated rows:
0
```

Adjusting mean-background

Just to be sure that adjusting the mean-background with out PE-11, I inspected the original csv called "VR5_COLOC_FULL_DB.csv" and for each of the stain types, I have the minimum observed mean_intensity occurring in the following images:

- PV: 0-FFF-00006_PV from KET-10-1_PFC_3.7_B_2.tif
- cFos: 0-FFF-00008_cFos from PE-13-11_PFC_3.7_A_3.tif
- Npas4: 0-FFF-00001_Npas4 from KET-9-5_PFC_3.5_B_4.tif
- WFA: 0-FFF-00001_WFA from PE-13-11_PFC_3.7_A_5.tif

Since none of the dimmest cells came from in PE-11, the adjustment calculation is exactly the same as before.

In [12]:

```
def adjust_mmbg(df):
    '''
    '''
    # remove unnecesary index col
    if 'index' in df.columns:
        df = df.drop('index', axis=1)

    # add minimum observed intensity
    adjusted_mmbg = []
    for stain in df.stain_type.unique():
        # separate by stain
        df_stain = df.query(f'stain_type == "{stain}"').reset_index()

        # compute new mean-background col
        df_stain['mean-background'] = df_stain.loc[: ,'mean_intensity'].astype('f') - df_stain.lo

        # get min mean-background (some negative number)
        min_mmbg = df_stain['mean-background'].min()

        # add the absolute value of min to all other cells of the same stain
        df_stain['adjusted_mean-background'] = df_stain['mean-background'] + abs(min_mmbg)

        # toss back into list
        adjusted_mmbg.append(df_stain)

    df_adjusted = pd.concat(adjusted_mmbg)

    return df_adjusted

df_adjusted = adjust_mmbg(df_coloc)
print(df_adjusted.shape)
df_adjusted.head()
```

```
(18632, 21)
```

Out[12]:

	index	filename	image_name	roi_id	true_grouping	dummy_PV	dummy_cFos	dummy_Npas4	dummy_WFA	CoM_x	...
0	0	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00000_PV	(0-000-00000_PV, 0-FFF-00045_Npas4, 0-FFF-0000...	True	False	True	True	297.86	...
1	1	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00001_PV	(0-000-00001_PV, 0-FFF-00012_Npas4)	True	False	True	False	340.47	...
2	2	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00002_PV	(0-000-00002_PV, 0-FFF-00044_Npas4, 0-FFF-0000...	True	False	True	True	154.85	...
3	3	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00003_PV	(0-000-00003_PV, 0-FFF-00082_Npas4, 0-FFF-0000...	True	False	True	True	310.10	...
4	4	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00004_PV	(0-000-00004_PV,)	True	False	False	False	44.35	...

5 rows × 21 columns

building coloc_stain_type col

In [13]:
```
def coloc_staintype(x):
    self_stain = x.stain_type
    coloc = np.array([x.dummy_PV, x.dummy_cFos, x.dummy_Npas4, x.dummy_WFA])
    stains = np.array(['PV', 'cFos', 'Npas4', 'WFA'])[coloc]
    stains = np.delete(stains, np.argwhere(stains == self_stain))

    assert len(stains) < 4

    if len(stains) == 0:
        coloc_stain_type = f'lonely_{self_stain}'
    if len(stains) == 1:
        coloc_stain_type = f'{self_stain}_coloc_w_{stains[0]}'
    if len(stains) == 2:
        coloc_stain_type = f'{self_stain}_coloc_w_{stains[0]},{stains[1]}'
    if len(stains) == 3:
        coloc_stain_type = f'quad_{self_stain}'

    return coloc_stain_type

df_adjusted['coloc_stain_type'] = df_adjusted.apply(coloc_staintype, axis=1)

df_adjusted
```

Out[13]:

	index	filename	image_name	roi_id	true_grouping	dummy_PV	dummy_cFos	dummy_Npas4	dummy_WFA	CoM_x
0	0	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00000_PV	(0-000-00000_PV, 0-FFF-00045_Npas4, 0-FFF-0000...	True	False	True	True	297.86
1	1	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00001_PV	(0-000-00001_PV, 0-FFF-00012_Npas4)	True	False	True	False	340.47
2	2	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00002_PV	(0-000-00002_PV, 0-FFF-00044_Npas4, 0-FFF-0000...	True	False	True	True	154.85
3	3	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00003_PV	(0-000-00003_PV, 0-FFF-00082_Npas4, 0-FFF-0000...	True	False	True	True	310.10
4	4	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00004_PV	(0-000-00004_PV,)	True	False	False	False	44.35
...	...	...	...	...	...	...	...	...	...	...
898	22793	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00008_WFA	(0-FFF-00008_WFA,)	False	False	False	True	385.12
899	22794	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00009_WFA	(0-000-00002_PV, 0-FFF-00009_WFA)	True	False	False	True	409.79
900	22795	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00010_WFA	(0-FFF-00010_WFA,)	False	False	False	True	300.24
901	22796	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00011_WFA	(0-000-00005_PV, 0-FFF-00011_WFA)	True	False	False	True	414.17
902	22797	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00012_WFA	(0-000-00000_PV, 0-FFF-00002_Npas4, 0-FFF-0001...	True	False	True	True	203.68

18632 rows × 22 columns

Filling in some dummy_WFA entries

In [14]:
```
WFA_bool_sum = np.array(df_adjusted.stain_type == 'WFA') + np.array(df_adjusted.dummy_WFA)
df_adjusted['dummy_WFA'] = WFA_bool_sum

df_adjusted[df_adjusted.dummy_WFA]
```

Out[14]:

	index	filename	image_name	roi_id	true_grouping	dummy_PV	dummy_cFos	dummy_Npas4	dummy_WFA	CoM_x
0	0	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00000_PV	(0-000-00000_PV, 0-FFF-00045_Npas4, 0-FFF-0000...	True	False	True	True	297.86
2	2	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00002_PV	(0-000-00002_PV, 0-FFF-00044_Npas4, 0-FFF-0000...	True	False	True	True	154.85
3	3	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00003_PV	(0-000-00003_PV, 0-FFF-00082_Npas4, 0-FFF-0000...	True	False	True	True	310.10
5	5	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00005_PV	(0-000-00005_PV, 0-FFF-00041_Npas4, 0-FFF-0000...	True	False	True	True	37.02
7	7	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00008_PV	(0-000-00008_PV, 0-005-00017_cFos, 0-FFF-00062...	True	True	True	True	172.96
...	...	...	...	...	...	...	...	...	...	...
898	22793	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00008_WFA	(0-FFF-00008_WFA,)	False	False	False	True	385.12
899	22794	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00009_WFA	(0-000-00002_PV, 0-FFF-00009_WFA)	True	False	False	True	409.79
900	22795	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00010_WFA	(0-FFF-00010_WFA,)	False	False	False	True	300.24
901	22796	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00011_WFA	(0-000-00005_PV, 0-FFF-00011_WFA)	True	False	False	True	414.17
902	22797	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00012_WFA	(0-000-00000_PV, 0-FFF-00002_Npas4, 0-FFF-0001...	True	False	True	True	203.68

2099 rows × 22 columns

In [15]:
```
import itertools
stains = np.array(['PV', 'cFos', 'Npas4', 'WFA'])

coloc_stain_types = ['PV', 'cFos', 'Npas4', 'WFA',\
                     'quad_PV', 'quad_cFos', 'quad_Npas4', 'quad_WFA']
coloc_stain_types = [tuple([s]) for s in coloc_stain_types]
for stain in stains:
    self = stain
    nonself = np.delete(stains, np.argwhere(stains == self))

    # double combinations
    dbl_comb = list(itertools.product([self],itertools.combinations(nonself, r=1)))

    # triple combinations
    trp_comb = list(itertools.product([self],itertools.combinations(nonself, r=2)))

    coloc_stain_types += dbl_comb
    coloc_stain_types += trp_comb

coloc_stain_types
```

Out[15]:
```
[('PV',),
 ('cFos',),
 ('Npas4',),
 ('WFA',),
 ('quad_PV',),
 ('quad_cFos',),
 ('quad_Npas4',),
 ('quad_WFA',),
 ('PV', ('cFos',)),
 ('PV', ('Npas4',)),
 ('PV', ('WFA',)),
 ('PV', ('cFos', 'Npas4')),
 ('PV', ('cFos', 'WFA')),
 ('PV', ('Npas4', 'WFA')),
 ('cFos', ('PV',)),
 ('cFos', ('Npas4',)),
 ('cFos', ('WFA',)),
 ('cFos', ('PV', 'Npas4')),
 ('cFos', ('PV', 'WFA')),
 ('cFos', ('Npas4', 'WFA')),
 ('Npas4', ('PV',)),
 ('Npas4', ('cFos',)),
 ('Npas4', ('WFA',)),
 ('Npas4', ('PV', 'cFos')),
 ('Npas4', ('PV', 'WFA')),
 ('Npas4', ('cFos', 'WFA')),
 ('WFA', ('PV',)),
 ('WFA', ('cFos',)),
 ('WFA', ('Npas4',)),
 ('WFA', ('PV', 'cFos')),
 ('WFA', ('PV', 'Npas4')),
 ('WFA', ('cFos', 'Npas4'))]
```

In [16]:

```
bool_combs = list(itertools.product([True, False], repeat=4))[:-1]
for bcomb in bool_combs:
    stains = np.array(['PV', 'cFos', 'Npas4', 'WFA'])
    coloc_PV, coloc_cFos, coloc_Npas4, coloc_WFA = [*bcomb]

    q = []
    if coloc_PV:
        q.append(f'dummy_PV == {coloc_PV}')
    if coloc_cFos:
        q.append(f'dummy_cFos == {coloc_cFos}')
    if coloc_Npas4:
        q.append(f'dummy_Npas4 == {coloc_Npas4}')
    if coloc_WFA:
        q.append(f'dummy_WFA == {coloc_WFA}')

    query = ' and '.join(q)
    df_subset = df_adjusted.query(query)
    print(len(df_subset), query)

    for stain in df_subset.stain_type.unique():
        self = stain
        stains_filtered = stains.copy()[np.array(bcomb)]
        nonself = np.delete(stains_filtered, np.argwhere(stains_filtered == self))

        df_stain = df_subset.query(f'stain_type == "{self}"')

        if len(nonself) == 0:
            coloc_stain_type = f'single_{self}'

        if len(nonself) == 1:
            coloc_stain_type = f'{self}_coloc_w_{nonself[0]}'

        if len(nonself) == 2:
            coloc_stain_type = f'{self}_coloc_w_{nonself[0]},{nonself[1]}'

        if len(nonself) == 3:
            coloc_stain_type = f'quad_{self}'


        # normalize to FR1_SAL
        df_norm = normalize_intensity(df_stain, norm_condition='FR1_SAL', col='adjusted_mean-backg
        #df_norm.to_csv(f'{group}_{stain}_NORM.csv')

        # reorganize into cols for prism
        df_prism = prism_reorg(df_norm, col='norm_adjusted_mean-background')
        #df_prism.to_csv(f'{group}_{coloc_stain_type}_PRISM.csv')
```

```
687 dummy_PV == True and dummy_cFos == True and dummy_Npas4 == True and dummy_WFA == True
1204 dummy_PV == True and dummy_cFos == True and dummy_Npas4 == True
981 dummy_PV == True and dummy_cFos == True and dummy_WFA == True
1747 dummy_PV == True and dummy_cFos == True
927 dummy_PV == True and dummy_Npas4 == True and dummy_WFA == True
1633 dummy_PV == True and dummy_Npas4 == True
1419 dummy_PV == True and dummy_WFA == True
2802 dummy_PV == True
837 dummy_cFos == True and dummy_Npas4 == True and dummy_WFA == True
8249 dummy_cFos == True and dummy_Npas4 == True
1317 dummy_cFos == True and dummy_WFA == True
12819 dummy_cFos == True
1132 dummy_Npas4 == True and dummy_WFA == True
13147 dummy_Npas4 == True
2099 dummy_WFA == True
```

Normalizing adjusted mean-background, counting mean cell ns

Normalizing adjusted mean-background

In [17]:

```
def normalize_intensity(df, norm_condition, col='mean-background'):
    '''
    computes the mean of rows of the norm_condition and divides mean-background by this mean,
    normalizing all data to the mean of the norm_condition. sets normalized value into new
    column called "norm mean-background" and returns new dataframe containing normalized intensity
    '''
    df_norm = df[df.treatment == norm_condition]
    norm_mean = df_norm[col].astype('f').mean()

    df_norm = df.copy(deep=True)
    df_norm[f'norm_{col}'] = df[col].astype('f') / norm_mean

    # quickly check that the mean of the norm condition is set to about 1.00000
    # this is never exatly 1 due to small rounding errors from floating point operations
    assert round(df_norm[df_norm.treatment == norm_condition][f'norm_{col}'].mean(), 5) == 1

    return df_norm

def prism_reorg(df, col='norm_mean-background'):
    '''
    Takes just the norm_mean-background intensity col per rat, groups by treatment
    and
    '''
    treatments = np.unique(df.treatment)
    reorg = []

    for t in treatments:
        df_treat = df[df.treatment == t]
        norm_int_ratn = []
        treatment_ratns = np.unique(df_treat.rat_n)

        for rat in treatment_ratns:
            norm_int = df_treat[df_treat.rat_n == rat][col]
            df_normint = pd.DataFrame({t: norm_int}).reset_index(drop=True)
            norm_int_ratn.append(df_normint)

        # concat "vertically"
        df_ratn_cols = pd.concat(norm_int_ratn, axis=0).reset_index(drop=True)

        # write csv to disk
        reorg.append(df_ratn_cols)

    # concat "horizontally"
    df_prism_reorg = pd.concat(reorg, axis=1)

    return df_prism_reorg
```

Counting mean cell ns

In [18]:

```
def count_imgs(df, sid, iid):
    '''
    takes a dataframe and counts the number of unique strings that occur in the
    "image_name" col for each rat in "rat_n" col
    args:
        df: pd.core.frame.DataFrame(n, m)
            n: the number of rows,
            m: the number of features
        sid: str, denoting the name of the col containing unique subject ids
        iid: str, denoting the name of the col containing unique image ids
    return:
        df_imgn: pd.core.frame.DataFrame(n=|sid|), m=2)
            n: the number of rows, equal to the cardinality of the sid set
            (the number of unique ID strings in sid)
            this df contains 2 cols: a sid col, and an iid col containing counts
    '''
    assert iid in df.columns

    df_imgn = df.groupby([sid])[[sid, iid]]\
        .apply(lambda x: len(np.unique(x[iid])))\
        .reset_index(name='image_n')

    return df_imgn

def count_cells(df, cols):
    '''
    takes a df and counts the number of instances each distinct row
    (created by unique combinations of labels from columns indicated
    by cols arg); counts are reported in a new col called "cell_counts"
    args:
        df: pd.core.frame.DataFrame(N, M); N: the number of rows, M: the
            number of cols (assumed to have already been split by stain_type)
        cols: list(n), n: the number of cols over which to count distinct rows
    return:
        df_counts: pd.core.frame.DataFrame(N,M+1)
    '''
    df_counts = df.value_counts(cols)\
        .reset_index(name='cell_counts')\
        .sort_values(by=cols)

    return df_counts

def sum_cells(df, cols, iid):
    '''
    takes cell count df, groups by cols denoted in cols list and computes sum
    of cell_counts col for each group. Adds new column "cell_count_sums"
    containing sums.
    args:
        df: pd.core.frame.DataFrame(N, M), N: the number of rows (N=|id_col|),
            M: the number of cols, must contain col called "cell_counts"
        cols: list(M-2), list containing col name strings that define each group
            for group by and reduction (in this case summing)
        iid: str, denotes
    return:
        df_sums: pd.core.frame.DataFrame; dataframe containing summed cell
            counts per subject id.
    '''
    # remove image id col (we want to sum counts across all images per rat)
    reduce_cols = list(filter(lambda x: x != iid, cols))

    if 'scaled_counts' in df.columns:
            # group by, reduce
        df_sums = df.groupby(by=reduce_cols)[cols]\
            .apply(lambda x: np.sum(x.scaled_counts))\
            .reset_index(name='cell_count_sums')

    else:
        # group by, reduce
        df_sums = df.groupby(by=reduce_cols)[df.columns]\
            .apply(lambda x: np.sum(x.cell_counts))\
            .reset_index(name='cell_count_sums')

    return df_sums

def average_counts(df_sums, df_ns, cols, sid, iid):
    '''
```

```
    takes df of cell count sums and df of image ns, and computes the mean cell
    n (divides cell count sums by the number of images) for each subject.
    args:
        df_sums: pd.core.frame.DataFrame(ni, mi), ni: the number of rows
            (ni=|sid|), mi: the number of cols (mi = |cols|); must
            contain a col "cell_count_sums".
        df_ns: pd.core.frame.DataFrame(nj, mj), nj: the number of rows
            (nj=|sid|), mj: the number of cols (mj=2); must contain a col
            "image_n"
        cols: list(n), n: the number of cols (contains all cols necessary to
            create every unique group combination)
        sid: str, denoting the name of the col containing unique subject ids
        iid: str, denoting the name of the col containing unique image ids
    return:
        mean_cell_ns: pd.core.frame.DataFrame(N,M), N: the number of rows (N=
        |sid|), M: the number of cols (M=|cols|+2)

    '''
    # list of cols with out image id, since it was removed during the reduction step
    reduce_cols = list(filter(lambda x: x != iid, cols))

    # compute mean cell n
    mean_cell_ns = df_sums.join(df_ns.set_index(sid), on=sid, how='inner')\
        .sort_values(by=reduce_cols)
    mean_cell_ns['mean_cell_n'] = mean_cell_ns.cell_count_sums / mean_cell_ns.image_n

    # reorder so that subject id is the first col
    col_reorder = [sid] + list(filter(lambda x: x != sid, list(mean_cell_ns.columns)))
    mean_cell_ns = mean_cell_ns[col_reorder]

    return mean_cell_ns

def mean_cell_n(df_stain, df_full, cols, sid, iid, return_counts=False):
    '''
    wrapper function to compute mean cell ns; magnification/zoom factor
    is assuemd to be equal across all images. NOTE that we count total image
    ns based on full cleaned dataset: it may be the case the not every image
    contains every stain type combination, and we must still count images
    with 0 cells of a particular stain type towards the total number of images.
    args:
        df_stain: pd.core.frame.DataFrame; df containing data for a given stain type
        df_full: pd.core.frame.DataFrame; df containing data for full (cleaned) set
        cols: list, contains str denoting col names for grouping
        sid: str, col name denoting col containing unique subject ids
        iid: str, col name denoting col containing unique image ids
        return_counts: bool, flag for added utility during debugging
    return:
        mean_cell_ns: pd.core.frame.DataFrame; df containing final mean cell ns
        cell_counts: pd.core.frame. DataFram; df containing cell counts per
            image (for debugging)

    '''
    # count n of unique image names per subject
    img_ns = count_imgs(df_full, sid, iid)

    # count n of cells per image for each subject
    cell_counts = count_cells(df_stain, cols)

    # sum cell counts across all images for each subject
    cell_sums = sum_cells(cell_counts, cols, iid)

    # compute mean cell count per image for each subject
    mean_cell_ns = average_counts(cell_sums, img_ns, cols, sid, iid)

    if not return_counts:
        return mean_cell_ns

    return (cell_counts, mean_cell_ns)
```

Time to run it

Normalize, write to disk

In [19]:

```
group = 'KET-VR5'
normalized = []
bool_combs = list(itertools.product([True, False], repeat=4))[:-1]

for bcomb in bool_combs:
    stains = np.array(['PV', 'cFos', 'Npas4', 'WFA'])
    coloc_PV, coloc_cFos, coloc_Npas4, coloc_WFA = [*bcomb]

    q = []
    if coloc_PV:
        q.append(f'dummy_PV == {coloc_PV}')
    if coloc_cFos:
        q.append(f'dummy_cFos == {coloc_cFos}')
    if coloc_Npas4:
        q.append(f'dummy_Npas4 == {coloc_Npas4}')
    if coloc_WFA:
        q.append(f'dummy_WFA == {coloc_WFA}')

    query = ' and '.join(q)
    df_subset = df_adjusted.query(query)
    print(len(df_subset), query)

    for stain in df_subset.stain_type.unique():
        self = stain
        stains_filtered = stains.copy()[np.array(bcomb)]
        nonself = np.delete(stains_filtered, np.argwhere(stains_filtered == self))

        df_stain = df_subset.query(f'stain_type == "{self}"')

        if len(nonself) == 0:
            coloc_stain_type = f'single_{self}'

        if len(nonself) == 1:
            coloc_stain_type = f'{self}_coloc_w_{nonself[0]}'

        if len(nonself) == 2:
            coloc_stain_type = f'{self}_coloc_w_{nonself[0]},{nonself[1]}'

        if len(nonself) == 3:
            coloc_stain_type = f'quad_{self}'


        # normalize to FR1_SAL
        df_norm = normalize_intensity(df_stain, norm_condition='FR1_SAL', col='adjusted_mean-backg
        #df_norm.to_csv(f'{group}_{coloc_stain_type}_NORM.csv')

        # reorganize into cols for prism
        df_prism = prism_reorg(df_norm, col='norm_adjusted_mean-background')
        #df_prism.to_csv(f'{group}_{coloc_stain_type}_PRISM.csv')

# let's take a look at one of our final output dataframes, organized for entry into prism
print(stain)
df_prism
```

```
687 dummy_PV == True and dummy_cFos == True and dummy_Npas4 == True and dummy_WFA == True
1204 dummy_PV == True and dummy_cFos == True and dummy_Npas4 == True
981 dummy_PV == True and dummy_cFos == True and dummy_WFA == True
1747 dummy_PV == True and dummy_cFos == True
927 dummy_PV == True and dummy_Npas4 == True and dummy_WFA == True
1633 dummy_PV == True and dummy_Npas4 == True
1419 dummy_PV == True and dummy_WFA == True
2802 dummy_PV == True
837 dummy_cFos == True and dummy_Npas4 == True and dummy_WFA == True
8249 dummy_cFos == True and dummy_Npas4 == True
1317 dummy_cFos == True and dummy_WFA == True
12819 dummy_cFos == True
1132 dummy_Npas4 == True and dummy_WFA == True
13147 dummy_Npas4 == True
2099 dummy_WFA == True
WFA
```

Out[19]:

	FR1_KET	FR1_SAL	VR5_KET	VR5_SAL
0	0.331213	0.120762	0.297620	0.403577
1	1.077244	0.260485	0.846265	2.245242
2	0.443616	0.555018	0.266051	0.707602
3	0.889838	0.647347	0.376870	0.590796
4	0.638543	0.125722	0.099910	0.714153
...	...	...	...	...
266	NaN	0.960938	NaN	NaN
267	NaN	0.229340	NaN	NaN
268	NaN	0.456116	NaN	NaN
269	NaN	0.541623	NaN	NaN
270	NaN	0.014046	NaN	NaN

271 rows × 4 columns

Count mean cell ns, write to disk

In [20]:

```
# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', sid, iid]
group = 'KET-VR5'

# getting every combination of four booleans (position matters)
# except the last one (all False) which would mean the cell had 0 stain types
bool_combs = list(itertools.product([True, False], repeat=4))[:-1]

for bcomb in bool_combs:
    # build array of stains
    stains = np.array(['PV', 'cFos', 'Npas4', 'WFA'])

    # unpack booleans
    coloc_PV, coloc_cFos, coloc_Npas4, coloc_WFA = [*bcomb]

    # filter query statements into
    q = []
    if coloc_PV:
        q.append(f'dummy_PV == {coloc_PV}')
    if coloc_cFos:
        q.append(f'dummy_cFos == {coloc_cFos}')
    if coloc_Npas4:
        q.append(f'dummy_Npas4 == {coloc_Npas4}')
    if coloc_WFA:
        q.append(f'dummy_WFA == {coloc_WFA}')

    # build larger query
    query = ' and '.join(q)
    df_subset = df_adjusted.query(query)

    # check len
    print(len(df_subset), query)

    # in the filtered coloc set, analyze each individual stain type
    # for example, if we filter for PV, WFA and cFos, then query this set
    # for all PV cells, we've found all triple labeled PV (PV coloc w WFA and cFos)
    for stain in df_subset.stain_type.unique():
        # keep track of self
        self = stain

        # filter stains arr
        stains_filtered = stains.copy()[np.array(bcomb)]

        # remove self from filtered arr
        nonself = np.delete(stains_filtered, np.argwhere(stains_filtered == self))

        # query the coloc subset for a given individual stain
        df_stain = df_subset.query(f'stain_type == "{self}"')

        # build coloc_stain_type
        if len(nonself) == 0:
            coloc_stain_type = f'single_{self}'

        if len(nonself) == 1:
            coloc_stain_type = f'{self}_coloc_w_{nonself[0]}'

        if len(nonself) == 2:
            coloc_stain_type = f'{self}_coloc_w_{nonself[0]},{nonself[1]}'

        if len(nonself) == 3:
            coloc_stain_type = f'quad_{self}'

        # compute mean cell ns
        df_means = mean_cell_n(df_stain, df_coloc, cols, sid, iid)
        df_means['coloc_stain_type'] = coloc_stain_type

        # write to disk
        #df_means.to_csv(f'{group}_{coloc_stain_type}_mean_cell_ns.csv')

# let's take a look at one of our final output dataframes
print(stain)
df_means
```

```
687 dummy_PV == True and dummy_cFos == True and dummy_Npas4 == True and dummy_WFA == True
1204 dummy_PV == True and dummy_cFos == True and dummy_Npas4 == True
981 dummy_PV == True and dummy_cFos == True and dummy_WFA == True
1747 dummy_PV == True and dummy_cFos == True
927 dummy_PV == True and dummy_Npas4 == True and dummy_WFA == True
1633 dummy_PV == True and dummy_Npas4 == True
1419 dummy_PV == True and dummy_WFA == True
2802 dummy_PV == True
837 dummy_cFos == True and dummy_Npas4 == True and dummy_WFA == True
8249 dummy_cFos == True and dummy_Npas4 == True
1317 dummy_cFos == True and dummy_WFA == True
12819 dummy_cFos == True
1132 dummy_Npas4 == True and dummy_WFA == True
13147 dummy_Npas4 == True
2099 dummy_WFA == True
WFA
```

Out[20]:

	rat_n	treatment	stain_type	cell_count_sums	image_n	mean_cell_n	coloc_stain_type
0	KET-10-12	FR1_KET	WFA	32	5	6.40	single_WFA
1	KET-9-1	FR1_KET	WFA	29	4	7.25	single_WFA
2	PE-12-1	FR1_KET	WFA	26	5	5.20	single_WFA
3	PE-12-2	FR1_KET	WFA	38	5	7.60	single_WFA
4	PE-12-7	FR1_KET	WFA	47	5	9.40	single_WFA
5	KET-10-1	FR1_SAL	WFA	32	5	6.40	single_WFA
6	KET-10-5	FR1_SAL	WFA	33	5	6.60	single_WFA
7	KET-8-2	FR1_SAL	WFA	33	5	6.60	single_WFA
8	KET-9-2	FR1_SAL	WFA	47	5	9.40	single_WFA
9	KET-9-4	FR1_SAL	WFA	45	5	9.00	single_WFA
10	KET-9-5	FR1_SAL	WFA	44	5	8.80	single_WFA
11	KET-9-6	FR1_SAL	WFA	37	5	7.40	single_WFA
12	KET-10-14	VR5_KET	WFA	38	5	7.60	single_WFA
13	KET-8-7	VR5_KET	WFA	30	4	7.50	single_WFA
14	PE-13-2	VR5_KET	WFA	39	5	7.80	single_WFA
15	PE-13-3	VR5_KET	WFA	42	5	8.40	single_WFA
16	PE-13-6	VR5_KET	WFA	54	5	10.80	single_WFA
17	KET-10-2	VR5_SAL	WFA	31	5	6.20	single_WFA
18	KET-10-3	VR5_SAL	WFA	36	5	7.20	single_WFA
19	KET-10-4	VR5_SAL	WFA	26	5	5.20	single_WFA
20	PE-13-1	VR5_SAL	WFA	59	5	11.80	single_WFA
21	PE-13-11	VR5_SAL	WFA	58	5	11.60	single_WFA
22	PE-13-9	VR5_SAL	WFA	47	5	9.40	single_WFA

Finally getting to the good stuff

Now that our dataset is clean and has all the features we need, we can finally start digging into the questions we are interested in. Specifically:

- Does treatment/react change the distribution (ratio) of high/low intensity cFos in triple labled cFos/PV/WFA cells?
  - is PV/WFA intensity different in high/low intensity cFos?
- Does treatment/react change the distribution (ratio) of high/low intensity Npas4 in triple labled Npas4/PV/WFA cells?
  - is PV/WFA intensity different in high/low intensity cFos?
- Does cFos intensity differ betwween PV vs Non-PV cells?
- Does Npas4 intensity differ between PV vs Non-PV cells?
- Is Npas4/cFos intentensiy different in PV cells with vs without WFA?

In [21]:
```
# actually this coloc stain type col i made is not completely informative becuase originally,
# coloc stain types were all nested
#df_adjusted = df_adjusted.drop('coloc_stain_type', axis=1)
#df_adjusted.to_csv('KET-VR5_FULL_SET.csv')

df_adjusted
```

Out[21]:

	index	filename	image_name	roi_id	true_grouping	dummy_PV	dummy_cFos	dummy_Npas4	dummy_WFA	CoM_x
0	0	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00000_PV	(0-000-00000_PV, 0-FFF-00045_Npas4, 0-FFF-0000...	True	False	True	True	297.86
1	1	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00001_PV	(0-000-00001_PV, 0-FFF-00012_Npas4)	True	False	True	False	340.47
2	2	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00002_PV	(0-000-00002_PV, 0-FFF-00044_Npas4, 0-FFF-0000...	True	False	True	True	154.85
3	3	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00003_PV	(0-000-00003_PV, 0-FFF-00082_Npas4, 0-FFF-0000...	True	False	True	True	310.10
4	4	KET-10-12_PFC_3.7_A_2.tif	KET-10-12_PFC_3.7_A	0-000-00004_PV	(0-000-00004_PV,)	True	False	False	False	44.35
...	...	...	...	...	...	...	...	...	...	...
898	22793	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00008_WFA	(0-FFF-00008_WFA,)	False	False	False	True	385.12
899	22794	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00009_WFA	(0-000-00002_PV, 0-FFF-00009_WFA)	True	False	False	True	409.79
900	22795	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00010_WFA	(0-FFF-00010_WFA,)	False	False	False	True	300.24
901	22796	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00011_WFA	(0-000-00005_PV, 0-FFF-00011_WFA)	True	False	False	True	414.17
902	22797	PE-13-9_PFC_4.0_B_5.tif	PE-13-9_PFC_4.0_B	0-FFF-00012_WFA	(0-000-00000_PV, 0-FFF-00002_Npas4, 0-FFF-0001...	True	False	True	True	203.68

18632 rows × 22 columns

Binned cFos

High/Low cFos counts by treatment/react

In [22]:
```
df_cfos = df_adjusted.query('stain_type == "cFos"').copy(deep=True).drop('index', axis=1).reset_ir

# build cfos bin label
cfos_median = df_cfos.mean_intensity.median()
df_cfos['cfos_bin'] = df_cfos.mean_intensity.apply(lambda x: 'cfos_high' if x > cfos_median else

# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', 'cfos_bin', sid, iid]
group = 'KET-VR5'

# wrapper fn calls
for stain in df_cfos.cfos_bin.unique():

    # split by stain type
    df_stain = df_cfos[df_cfos.cfos_bin == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, df_cfos, cols, sid, iid)

    # write to disk
    #df_means.to_csv(f'{group}_{stain}_mean_cell_ns.csv')
    #df_means.groupby(['treatment', 'cfos_bin'])['mean_cell_n'].describe().to_csv(f'{group}_{stai

    # let's just try and get some intuition about these data
    print(df_means.groupby(['treatment', 'cfos_bin'])['mean_cell_n'].describe())

# let's take a look at one of our final output dataframes
print(stain)
df_means
```

```
                     count       mean        std    min    25%   50%   75%  \
treatment cfos_bin
FR1_KET   cfos_high    5.0  48.030000  27.626066   6.75  43.60  50.6  55.6
FR1_SAL   cfos_high    7.0  24.057143  15.240610   3.80  12.80  29.2  30.5
VR5_KET   cfos_high    5.0  33.850000  17.891688  17.80  21.25  23.6  52.2
VR5_SAL   cfos_high    6.0  37.933333  15.856187  13.40  30.40  38.7  50.6

                      max
treatment cfos_bin
FR1_KET   cfos_high  83.6
FR1_SAL   cfos_high  48.8
VR5_KET   cfos_high  54.4
VR5_SAL   cfos_high  54.6
                    count       mean        std   min    25%   50%    75%  \
treatment cfos_bin
FR1_KET   cfos_low    5.0  29.330000   6.442011  21.8  25.60  27.2  35.25
FR1_SAL   cfos_low    7.0  44.285714  13.230700  23.4  36.20  48.2  53.90
VR5_KET   cfos_low    5.0  39.470000   8.801960  30.0  33.60  36.2  47.80
VR5_SAL   cfos_low    6.0  27.166667  11.022462  17.0  19.15  22.8  35.15

                      max
treatment cfos_bin
FR1_KET   cfos_low  36.80
FR1_SAL   cfos_low  58.20
VR5_KET   cfos_low  49.75
VR5_SAL   cfos_low  43.20
cfos_low
```

Out[22]:

	rat_n	treatment	stain_type	cfos_bin	cell_count_sums	image_n	mean_cell_n
0	KET-10-12	FR1_KET	cFos	cfos_low	128	5	25.60
1	KET-9-1	FR1_KET	cFos	cfos_low	141	4	35.25
2	PE-12-1	FR1_KET	cFos	cfos_low	184	5	36.80
3	PE-12-2	FR1_KET	cFos	cfos_low	136	5	27.20
4	PE-12-7	FR1_KET	cFos	cfos_low	109	5	21.80
5	KET-10-1	FR1_SAL	cFos	cfos_low	148	5	29.60
6	KET-10-5	FR1_SAL	cFos	cfos_low	214	5	42.80
7	KET-8-2	FR1_SAL	cFos	cfos_low	241	5	48.20
8	KET-9-2	FR1_SAL	cFos	cfos_low	117	5	23.40
9	KET-9-4	FR1_SAL	cFos	cfos_low	264	5	52.80
10	KET-9-5	FR1_SAL	cFos	cfos_low	291	5	58.20
11	KET-9-6	FR1_SAL	cFos	cfos_low	275	5	55.00
12	KET-10-14	VR5_KET	cFos	cfos_low	239	5	47.80
13	KET-8-7	VR5_KET	cFos	cfos_low	199	4	49.75
14	PE-13-2	VR5_KET	cFos	cfos_low	181	5	36.20
15	PE-13-3	VR5_KET	cFos	cfos_low	150	5	30.00
16	PE-13-6	VR5_KET	cFos	cfos_low	168	5	33.60
17	KET-10-2	VR5_SAL	cFos	cfos_low	191	5	38.20
18	KET-10-3	VR5_SAL	cFos	cfos_low	216	5	43.20
19	KET-10-4	VR5_SAL	cFos	cfos_low	98	5	19.60
20	PE-13-1	VR5_SAL	cFos	cfos_low	95	5	19.00
21	PE-13-11	VR5_SAL	cFos	cfos_low	130	5	26.00
22	PE-13-9	VR5_SAL	cFos	cfos_low	85	5	17.00

High/low cFos counts in only triple PV/WFA/cFos by treatment/react

In [23]:
```
# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', 'cfos_bin', sid, iid]
group = 'KET-VR5'

q = df_cfos.query('dummy_PV == True and dummy_cFos == True and dummy_WFA == True')

df_cfos_image_ns = pd.DataFrame({
    'rat_n': ['KET-10-12','KET-9-1','PE-12-1','PE-12-2','PE-12-7','KET-10-1','KET-10-5','KET-8-2',
    'treatment': ['FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_SAL','FR1_SAL','FR1_SAL',
    'image_n': [5,4,5,5,5,5,5,5,5,5,5,4,5,5,5,5,5,5,5,5]
})

# wrapper fn calls
for stain in q.cfos_bin.unique():

    # split by stain type
    df_stain = q[q.cfos_bin == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, q, cols, sid, iid)

    # ##### not every image had a triple PV/WFA/cFos high/low cFos cell observed
    # ##### and so here I will be building a new image_n col by hand and recalculating means.
    df_means = df_cfos_image_ns.merge(df_means, how='left', on=['rat_n', 'treatment'])
    df_means['image_n'] = df_means.image_n_x
    df_means = df_means.drop(['image_n_x', 'image_n_y', 'mean_cell_n'], axis=1)
    df_means['cell_count_sums'] = df_means.cell_count_sums.fillna(0).astype(int)
    df_means['mean_cell_n'] = df_means.cell_count_sums / df_means.image_n

    # write to disk
    #df_means.to_csv(f'{group}_{stain}_triple_cFos_coloc_w_PV,WFA_mean_cell_ns.csv')
    #df_means.groupby(['treatment', 'cfos_bin'])['mean_cell_n'].describe().to_csv(f'{group}_{stai

    # let's just try and get some intuition about these data
    print(df_means.groupby(['treatment', 'cfos_bin'])['mean_cell_n'].describe())
```

```
                     count      mean       std  min   25%  50%   75%  max
treatment cfos_bin
FR1_KET   cfos_high    5.0  1.770000  0.783901  1.0  1.25  1.4  2.40  2.8
FR1_SAL   cfos_high    6.0  1.166667  0.662319  0.6  0.70  1.1  1.20  2.4
VR5_KET   cfos_high    5.0  1.270000  0.547266  0.6  0.80  1.4  1.75  1.8
VR5_SAL   cfos_high    6.0  1.500000  0.756307  0.6  1.05  1.3  2.00  2.6
                     count      mean       std  min   25%  50%   75%  max
treatment cfos_bin
FR1_KET   cfos_low     5.0  0.870000  0.233452  0.6  0.75  0.8  1.00  1.2
FR1_SAL   cfos_low     7.0  1.257143  0.250713  1.0  1.10  1.2  1.40  1.6
VR5_KET   cfos_low     4.0  1.700000  0.930949  0.8  1.25  1.5  1.95  3.0
VR5_SAL   cfos_low     5.0  0.880000  0.303315  0.4  0.80  1.0  1.00  1.2
```

High/low cFos counts in only double cFos/WFA by treatment/react

In [24]:
```
# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', 'cfos_bin', sid, iid]
group = 'KET-VR5'

q = df_cfos.query('dummy_cFos == True and dummy_WFA == True')

df_cfos_image_ns = pd.DataFrame({
    'rat_n': ['KET-10-12','KET-9-1','PE-12-1','PE-12-2','PE-12-7','KET-10-1','KET-10-5','KET-8-2',
    'treatment': ['FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_SAL','FR1_SAL','FR1_SAL',
    'image_n': [5,4,5,5,5,5,5,5,5,5,5,4,5,5,5,5,5,5,5,5]
})

# wrapper fn calls
for stain in q.cfos_bin.unique():

    # split by stain type
    df_stain = q[q.cfos_bin == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, q, cols, sid, iid)

    # ##### not every image had a triple PV/WFA/cFos high/low cFos cell observed
    # ##### and so here I will be building a new image_n col by hand and recalculating means.
    df_means = df_cfos_image_ns.merge(df_means, how='left', on=['rat_n', 'treatment'])
    df_means['image_n'] = df_means.image_n_x
    df_means = df_means.drop(['image_n_x', 'image_n_y', 'mean_cell_n'], axis=1)
    df_means['cell_count_sums'] = df_means.cell_count_sums.fillna(0).astype(int)
    df_means['mean_cell_n'] = df_means.cell_count_sums / df_means.image_n

    # write to disk
    #df_means.to_csv(f'{group}_{stain}_double_cfos_coloc_w_WFA_mean_cell_ns.csv')
    #df_means.groupby(['treatment', 'cfos_bin'])['mean_cell_n'].describe().to_csv(f'{group}_{stai

    # let's just try and get some intuition about these data
    print(df_means.groupby(['treatment', 'cfos_bin'])['mean_cell_n'].describe())
```

```
                     count      mean       std   min  25%  50%   75%  max
treatment cfos_bin
FR1_KET   cfos_high    5.0  2.330000  0.992220  1.25  1.4  2.6  2.80  3.6
FR1_SAL   cfos_high    7.0  1.628571  0.867399  0.20  1.2  1.6  2.20  2.8
VR5_KET   cfos_high    5.0  1.920000  0.228035  1.60  1.8  2.0  2.00  2.2
VR5_SAL   cfos_high    6.0  1.900000  0.485798  1.40  1.6  1.7  2.25  2.6
                    count      mean       std  min   25%  50%   75%   max
treatment cfos_bin
FR1_KET   cfos_low    5.0  1.430000  0.399375  0.8  1.40  1.4  1.75  1.80
FR1_SAL   cfos_low    7.0  2.400000  1.296148  1.2  1.50  2.0  3.00  4.60
VR5_KET   cfos_low    5.0  1.890000  1.079583  0.6  1.00  2.2  2.40  3.25
VR5_SAL   cfos_low    6.0  1.233333  0.612100  0.4  0.85  1.2  1.70  2.00
```

High/low cFos counts in only double cFos/PV by treatment/react

In [25]:
```
# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', 'cfos_bin', sid, iid]
group = 'KET-VR5'

q = df_cfos.query('dummy_PV == True and dummy_cFos == True')

df_cfos_image_ns = pd.DataFrame({
    'rat_n': ['KET-10-12','KET-9-1','PE-12-1','PE-12-2','PE-12-7','KET-10-1','KET-10-5','KET-8-2',
    'treatment': ['FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_SAL','FR1_SAL','FR1_SAL',
    'image_n': [5,4,5,5,5,5,5,5,5,5,5,4,5,5,5,5,5,5,5,5]
})

# wrapper fn calls
for stain in q.cfos_bin.unique():

    # split by stain type
    df_stain = q[q.cfos_bin == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, q, cols, sid, iid)

    # ##### not every image had a triple PV/WFA/cFos high/low cFos cell observed
    # ##### and so here I will be building a new image_n col by hand and recalculating means.
    df_means = df_cfos_image_ns.merge(df_means, how='left', on=['rat_n', 'treatment'])
    df_means['image_n'] = df_means.image_n_x
    df_means = df_means.drop(['image_n_x', 'image_n_y', 'mean_cell_n'], axis=1)
    df_means['cell_count_sums'] = df_means.cell_count_sums.fillna(0).astype(int)
    df_means['mean_cell_n'] = df_means.cell_count_sums / df_means.image_n

    # write to disk
    #df_means.to_csv(f'{group}_{stain}_double_cfos_coloc_w_PV_mean_cell_ns.csv')
    #df_means.groupby(['treatment', 'cfos_bin'])['mean_cell_n'].describe().to_csv(f'{group}_{stai

    # let's just try and get some intuition about these data
    print(df_means.groupby(['treatment', 'cfos_bin'])['mean_cell_n'].describe())
```

```
                     count      mean       std   min  25%  50%  75%  max
treatment cfos_bin
FR1_KET   cfos_high    5.0  3.250000  1.928082  1.25  2.0  2.4  5.0  5.6
FR1_SAL   cfos_high    7.0  1.914286  0.958173  0.20  1.7  1.8  2.4  3.2
VR5_KET   cfos_high    5.0  2.680000  2.022869  1.00  1.2  2.2  3.0  6.0
VR5_SAL   cfos_high    6.0  3.500000  1.946279  1.20  2.7  3.1  3.8  7.0
                     count      mean       std  min   25%  50%   75%  max
treatment cfos_bin
FR1_KET   cfos_low    5.0  1.710000  0.574891  1.0  1.60  1.6  1.75  2.6
FR1_SAL   cfos_low    7.0  2.828571  0.615668  1.8  2.60  3.0  3.00  3.8
VR5_KET   cfos_low    5.0  3.080000  3.431035  0.2  1.40  2.4  2.40  9.0
VR5_SAL   cfos_low    6.0  1.700000  1.001998  0.6  1.05  1.4  2.35  3.2
```

High/low Npas4 counts by treatment/react

In [26]:
```
df_Npas4 = df_adjusted.query('stain_type == "Npas4"').copy(deep=True).drop('index', axis=1).reset_

# build Npas4 bin label
Npas4_median = df_Npas4.mean_intensity.median()
df_Npas4['Npas4_bin'] = df_Npas4.mean_intensity.apply(lambda x: 'Npas4_high' if x > Npas4_median 

# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', 'Npas4_bin', sid, iid]
group = 'KET-VR5'

# wrapper fn calls
for stain in df_Npas4.Npas4_bin.unique():

    # split by stain type
    df_stain = df_Npas4[df_Npas4.Npas4_bin == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, df_Npas4, cols, sid, iid)

    # write to disk
    #df_means.to_csv(f'{group}_{stain}_mean_cell_ns.csv')
    #df_means.groupby(['treatment', 'Npas4_bin'])['mean_cell_n'].describe().to_csv(f'{group}_{sta

    # let's just try and get some intuition about these data
    print(df_means.groupby(['treatment', 'Npas4_bin'])['mean_cell_n'].describe())

# let's take a look at one of our final output dataframes
print(stain)
df_means
```

```
                       count       mean        std   min    25%   50%    75%  \
treatment Npas4_bin
FR1_KET   Npas4_high     4.0  50.250000  21.333151  30.8  39.95  44.8  55.10
FR1_SAL   Npas4_high     6.0  15.100000  14.468034   1.2   4.25  10.5  26.05
VR5_KET   Npas4_high     5.0  41.930000  13.869733  22.2  36.25  45.4  46.00
VR5_SAL   Npas4_high     6.0  58.733333  26.277189  14.2  50.85  60.2  77.05

                        max
treatment Npas4_bin
FR1_KET   Npas4_high  80.6
FR1_SAL   Npas4_high  35.0
VR5_KET   Npas4_high  59.8
VR5_SAL   Npas4_high  87.4
                      count       mean        std   min   25%   50%   75%   max
treatment Npas4_bin
FR1_KET   Npas4_low     4.0  37.100000  12.227292  25.4  28.1  35.5  44.5  52.0
FR1_SAL   Npas4_low     7.0  63.571429  17.250093  42.4  50.2  63.8  75.9  86.6
VR5_KET   Npas4_low     5.0  34.780000  37.563573   3.4   4.6  19.0  57.5  89.4
VR5_SAL   Npas4_low     5.0  19.800000   5.770615  11.8  18.4  19.0  22.2  27.6
Npas4_low
```

Out[26]:

	rat_n	treatment	stain_type	Npas4_bin	cell_count_sums	image_n	mean_cell_n
0	KET-9-1	FR1_KET	Npas4	Npas4_low	168	4	42.0
1	PE-12-1	FR1_KET	Npas4	Npas4_low	145	5	29.0
2	PE-12-2	FR1_KET	Npas4	Npas4_low	127	5	25.4
3	PE-12-7	FR1_KET	Npas4	Npas4_low	260	5	52.0
4	KET-10-1	FR1_SAL	Npas4	Npas4_low	226	5	45.2
5	KET-10-5	FR1_SAL	Npas4	Npas4_low	414	5	82.8
6	KET-8-2	FR1_SAL	Npas4	Npas4_low	319	5	63.8
7	KET-9-2	FR1_SAL	Npas4	Npas4_low	212	5	42.4
8	KET-9-4	FR1_SAL	Npas4	Npas4_low	345	5	69.0
9	KET-9-5	FR1_SAL	Npas4	Npas4_low	276	5	55.2
10	KET-9-6	FR1_SAL	Npas4	Npas4_low	433	5	86.6
11	KET-10-14	VR5_KET	Npas4	Npas4_low	447	5	89.4
12	KET-8-7	VR5_KET	Npas4	Npas4_low	230	4	57.5
13	PE-13-2	VR5_KET	Npas4	Npas4_low	17	5	3.4
14	PE-13-3	VR5_KET	Npas4	Npas4_low	95	5	19.0
15	PE-13-6	VR5_KET	Npas4	Npas4_low	23	5	4.6
16	KET-10-2	VR5_SAL	Npas4	Npas4_low	59	5	11.8
17	KET-10-3	VR5_SAL	Npas4	Npas4_low	138	5	27.6
18	KET-10-4	VR5_SAL	Npas4	Npas4_low	95	5	19.0
19	PE-13-11	VR5_SAL	Npas4	Npas4_low	111	5	22.2
20	PE-13-9	VR5_SAL	Npas4	Npas4_low	92	5	18.4

High/low Npas4 counts in only triple Npas4/PV/WFA cells by treatment/react

In [27]:

```
# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', 'Npas4_bin', sid, iid]
group = 'KET-VR5'

q = df_Npas4.query('dummy_PV == True and dummy_Npas4 == True and dummy_WFA == True')

df_Npas4_image_ns = pd.DataFrame({
    'rat_n': ['KET-10-12','KET-9-1','PE-12-1','PE-12-2','PE-12-7','KET-10-1','KET-10-5','KET-8-2',
    'treatment': ['FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_SAL','FR1_SAL','FR1_SAL',
    'image_n': [5,4,5,5,5,5,5,5,5,5,5,4,5,5,5,5,5,5,5,5]
})

# wrapper fn calls
for stain in q.Npas4_bin.unique():

    # split by stain type
    df_stain = q[q.Npas4_bin == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, q, cols, sid, iid)

    # ##### not every image had a triple PV/WFA/Npas4 high/low Npas4 cell observed
    # ##### and so here I will be building a new image_n col by hand and recalculating means.
    df_means = df_Npas4_image_ns.merge(df_means, how='left', on=['rat_n', 'treatment'])
    df_means['image_n'] = df_means.image_n_x
    df_means = df_means.drop(['image_n_x', 'image_n_y', 'mean_cell_n'], axis=1)
    df_means['cell_count_sums'] = df_means.cell_count_sums.fillna(0).astype(int)
    df_means['mean_cell_n'] = df_means.cell_count_sums / df_means.image_n

    # write to disk
    #df_means.to_csv(f'{group}_{stain}_triple_Npas4_coloc_w_PV,WFA_mean_cell_ns.csv')
    #df_means.groupby(['treatment', 'Npas4_bin'])['mean_cell_n'].describe().to_csv(f'{group}_{sta

    # let's just try and get some intuition about these data
    print(df_means.groupby(['treatment', 'Npas4_bin'])['mean_cell_n'].describe())
```

```
                      count  mean       std  min   25%  50%   75%  max
treatment Npas4_bin
FR1_KET   Npas4_high    4.0  1.90  1.183216  1.0  1.15  1.5  2.25  3.6
FR1_SAL   Npas4_high    4.0  0.75  0.251661  0.4  0.70  0.8  0.85  1.0
VR5_KET   Npas4_high    5.0  1.32  0.460435  0.8  1.00  1.2  1.80  1.8
VR5_SAL   Npas4_high    6.0  1.60  1.193315  0.4  0.85  1.1  2.55  3.2
                     count      mean       std  min   25%  50%     75%   max
treatment Npas4_bin
FR1_KET   Npas4_low    4.0  1.237500  1.012731  0.6  0.75  0.8  1.2875  2.75
FR1_SAL   Npas4_low    7.0  2.057143  0.977850  1.4  1.50  1.8  2.0000  4.20
VR5_KET   Npas4_low    4.0  0.912500  0.396600  0.4  0.70  1.0  1.2125  1.25
VR5_SAL   Npas4_low    4.0  0.450000  0.251661  0.2  0.35  0.4  0.5000  0.80
```

High/low Npas4 counts in only double Npas4/WFA cells by treatment/react

In [28]:

```
# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', 'Npas4_bin', sid, iid]
group = 'KET-VR5'

q = df_Npas4.query('dummy_Npas4 == True and dummy_WFA == True')

df_Npas4_image_ns = pd.DataFrame({
    'rat_n': ['KET-10-12','KET-9-1','PE-12-1','PE-12-2','PE-12-7','KET-10-1','KET-10-5','KET-8-2',
    'treatment': ['FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_SAL','FR1_SAL','FR1_SAL',
    'image_n': [5,4,5,5,5,5,5,5,5,5,5,4,5,5,5,5,5,5,5,5]
})

# wrapper fn calls
for stain in q.Npas4_bin.unique():

    # split by stain type
    df_stain = q[q.Npas4_bin == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, q, cols, sid, iid)

    # ##### not every image had a triple PV/WFA/Npas4 high/low Npas4 cell observed
    # ##### and so here I will be building a new image_n col by hand and recalculating means.
    df_means = df_Npas4_image_ns.merge(df_means, how='left', on=['rat_n', 'treatment'])
    df_means['image_n'] = df_means.image_n_x
    df_means = df_means.drop(['image_n_x', 'image_n_y', 'mean_cell_n'], axis=1)
    df_means['cell_count_sums'] = df_means.cell_count_sums.fillna(0).astype(int)
    df_means['mean_cell_n'] = df_means.cell_count_sums / df_means.image_n

    # write to disk
    #df_means.to_csv(f'{group}_{stain}_double_Npas4_coloc_w_WFA_mean_cell_ns.csv')
    #df_means.groupby(['treatment', 'Npas4_bin'])['mean_cell_n'].describe().to_csv(f'{group}_{sta

    # let's just try and get some intuition about these data
    print(df_means.groupby(['treatment', 'Npas4_bin'])['mean_cell_n'].describe())
```

```
                      count  mean       std  min   25%  50%   75%  max
treatment Npas4_bin
FR1_KET   Npas4_high    4.0  2.25  1.147461  1.2  1.50  2.0  2.75  3.8
FR1_SAL   Npas4_high    5.0  0.68  0.268328  0.4  0.40  0.8  0.80  1.0
VR5_KET   Npas4_high    5.0  1.69  0.600417  1.2  1.25  1.4  2.00  2.6
VR5_SAL   Npas4_high    6.0  1.90  1.366748  0.6  0.85  1.5  2.75  4.0
                     count    mean       std  min   25%    50%     75%   max
treatment Npas4_bin
FR1_KET   Npas4_low    4.0  1.7875  1.390668  0.8  0.80  1.300  2.2875  3.75
FR1_SAL   Npas4_low    7.0  3.0000  1.089342  1.6  2.00  3.600  3.8000  4.20
VR5_KET   Npas4_low    4.0  1.1625  0.415080  0.6  1.05  1.225  1.3375  1.60
VR5_SAL   Npas4_low    5.0  0.4800  0.334664  0.2  0.20  0.400  0.6000  1.00
```

High/low Npas4 counts in only double Npas4/PV cells by treatment/react

In [29]:

```
# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', 'Npas4_bin', sid, iid]
group = 'KET-VR5'

q = df_Npas4.query('dummy_Npas4 == True and dummy_PV == True')

df_Npas4_image_ns = pd.DataFrame({
    'rat_n': ['KET-10-12','KET-9-1','PE-12-1','PE-12-2','PE-12-7','KET-10-1','KET-10-5','KET-8-2',
    'treatment': ['FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_KET','FR1_SAL','FR1_SAL','FR1_SAL',
    'image_n': [5,4,5,5,5,5,5,5,5,5,5,4,5,5,5,5,5,5,5,5]
})

# wrapper fn calls
for stain in q.Npas4_bin.unique():

    # split by stain type
    df_stain = q[q.Npas4_bin == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, q, cols, sid, iid)

    # ##### not every image had a triple PV/PV/Npas4 high/low Npas4 cell observed
    # ##### and so here I will be building a new image_n col by hand and recalculating means.
    df_means = df_Npas4_image_ns.merge(df_means, how='left', on=['rat_n', 'treatment'])
    df_means['image_n'] = df_means.image_n_x
    df_means = df_means.drop(['image_n_x', 'image_n_y', 'mean_cell_n'], axis=1)
    df_means['cell_count_sums'] = df_means.cell_count_sums.fillna(0).astype(int)
    df_means['mean_cell_n'] = df_means.cell_count_sums / df_means.image_n

    # write to disk
    #df_means.to_csv(f'{group}_{stain}_double_Npas4_coloc_w_PV_mean_cell_ns.csv')
    #df_means.groupby(['treatment', 'Npas4_bin'])['mean_cell_n'].describe().to_csv(f'{group}_{sta

    # let's just try and get some intuition about these data
    print(df_means.groupby(['treatment', 'Npas4_bin'])['mean_cell_n'].describe())
```

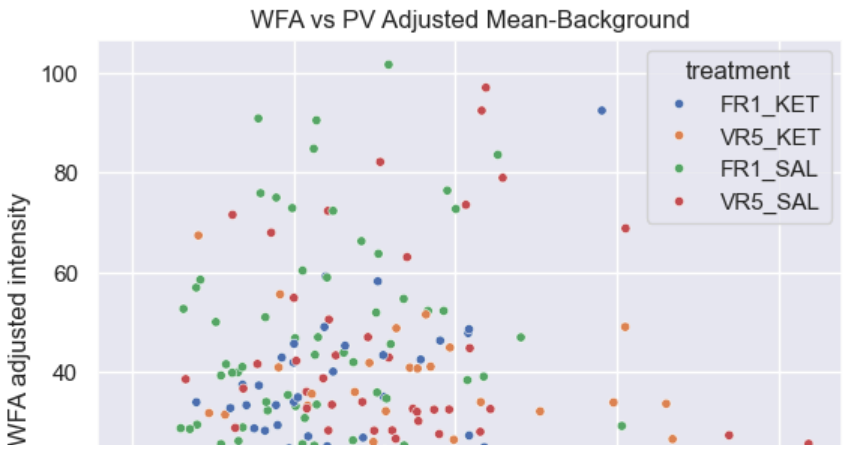
```
                       count      mean       std  min   25%  50%   75%   max
treatment Npas4_bin
FR1_KET   Npas4_high    4.0  3.150000  2.317326  1.6  2.05  2.2  3.30  6.60
FR1_SAL   Npas4_high    6.0  1.333333  1.462418  0.2  0.40  0.7  1.75  4.00
VR5_KET   Npas4_high    5.0  2.770000  2.048048  1.2  1.40  2.2  2.80  6.25
VR5_SAL   Npas4_high    6.0  4.000000  3.064637  0.4  1.55  3.8  6.65  7.60
                      count      mean       std  min  25%  50%    75%   max
treatment Npas4_bin
FR1_KET   Npas4_low    4.0  2.325000  1.687947  0.8  1.1  2.0  3.225  4.50
FR1_SAL   Npas4_low    7.0  3.542857  0.780720  3.0  3.1  3.2  3.600  5.20
VR5_KET   Npas4_low    5.0  2.050000  2.197157  0.2  0.4  1.0  3.400  5.25
VR5_SAL   Npas4_low    5.0  0.800000  0.374166  0.2  0.8  0.8  1.000  1.20
```

Pairwise plots

if any of these plots look interesting i would get regression lines for each group then probably do like an F-test to see if the linear models are different from each other.

In [30]:
```
def intensity_scatter_plot(df_data, stain_X, stain_Y, query=False):
    if not query:
        q = df_data.query(f'dummy_{stain_X} == {True} and dummy_{stain_Y} == {True}\
                          and (stain_type == "{stain_X}" or stain_type == "{stain_Y}")')
    elif query:
        q = df_data.query(query)
    df_X = q.query(f'stain_type == "{stain_X}"')[
        ['image_name', 'true_grouping', 'treatment', 'adjusted_mean-background']
        ].rename(columns={'adjusted_mean-background': f'{stain_X} adjusted intensity'})
    df_Y = q.query(f'stain_type == "{stain_Y}"')[
        ['image_name', 'true_grouping', 'treatment', 'adjusted_mean-background']
        ].rename(columns={'adjusted_mean-background': f'{stain_Y} adjusted intensity'})

    df_X['id'] = list(zip(df_X.image_name.values, df_X.true_grouping.values))
    df_Y['id'] = list(zip(df_Y.image_name.values, df_Y.true_grouping.values))

    df_X = df_X.drop(['image_name', 'true_grouping'], axis=1)
    df_Y = df_Y.drop(['image_name', 'true_grouping'], axis=1)

    df_merge = df_X.merge(df_Y, on='id').rename(columns={'treatment_x': 'treatment'})
    print(df_merge.shape)

    sns.set_theme()
    sns.scatterplot(x=f'{stain_X} adjusted intensity', y=f'{stain_Y} adjusted intensity', hue='tre
    plt.title(f'{stain_Y} vs {stain_X} Adjusted Mean-Background')
    plt.show()

intensity_scatter_plot(df_adjusted, 'PV', 'WFA')
intensity_scatter_plot(df_adjusted, 'cFos', 'PV')
intensity_scatter_plot(df_adjusted, 'cFos', 'WFA')
intensity_scatter_plot(df_adjusted, 'Npas4', 'cFos')
intensity_scatter_plot(df_adjusted, 'Npas4', 'PV')
intensity_scatter_plot(df_adjusted, 'Npas4', 'WFA')
```

```
(445, 5)
```



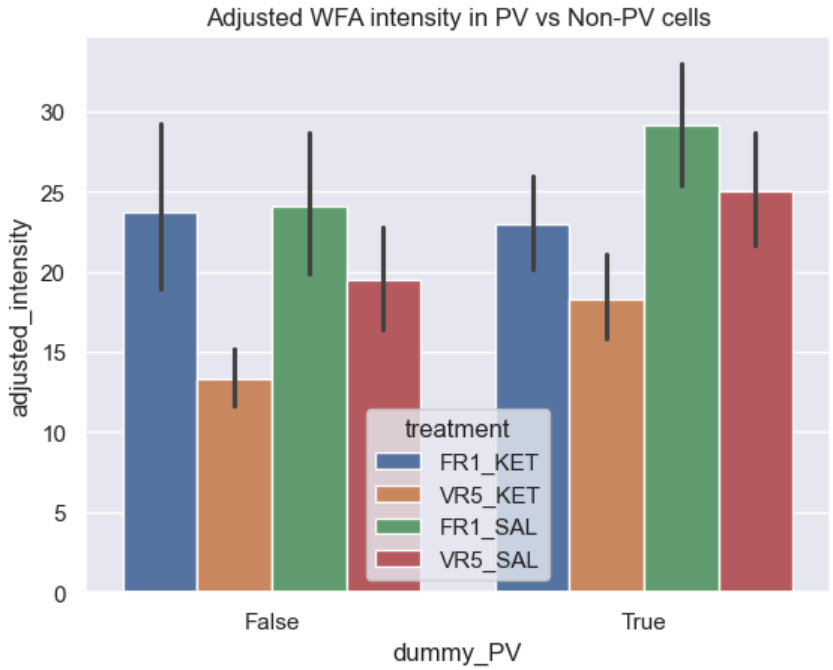
Some barplots and ANOVA

Is adjusted WFA intensity different in PV vs Non-PV cells across treatments?

No, but there is a simple main effect of treatment, react and PV (+/-) (that is, each effect does not depend on the level of another)

In [31]:
```
q = df_adjusted.query('stain_type == "WFA"').rename(columns={'adjusted_mean-background': 'adjuste
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])
sns.barplot(x='dummy_PV', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted WFA intensity in PV vs Non-PV cells')

# building model with all interaction terms
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_PV)\
             + C(treat):C(react) + C(react):C(dummy_PV) + C(dummy_PV):C(treat)\
             + C(treat):C(react):C(dummy_PV)',
            data=q).fit()
# perform 3 way ANOVA
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

	sum_sq	df	F	PR(>F)
C(treat)	7124.460285	1.0	18.395097	0.000020
C(react)	5357.884741	1.0	13.833864	0.000212
C(dummy_PV)	3707.644630	1.0	9.573004	0.002036
C(treat):C(react)	540.721974	1.0	1.396124	0.237687
C(react):C(dummy_PV)	497.943335	1.0	1.285672	0.257151
C(dummy_PV):C(treat)	385.890740	1.0	0.996356	0.318464
C(treat):C(react):C(dummy_PV)	363.260381	1.0	0.937925	0.333074
Residual	346635.400430	895.0	NaN	NaN

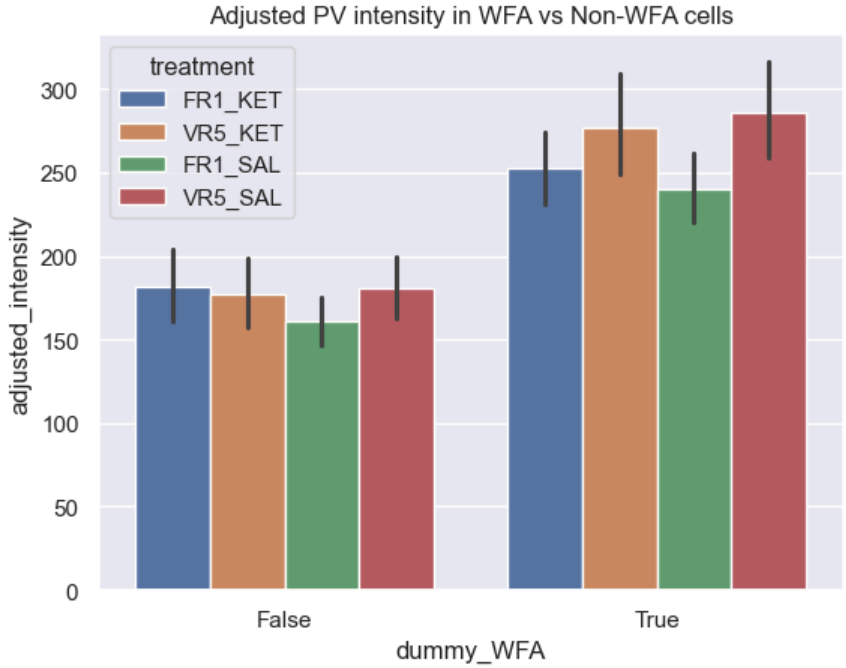


Is adjusted PV intensity different in WFA vs Non-WFA cells across treatments?

Yes, all interaction effects are significant. Basically, a three-way interaction means that one, or more, two-way interactions differ across the levels of a third variable. Something along the lines of "the interaction effect between treatment and react depends on whether or not a WFA net was present."

In [32]:
```
q = df_adjusted.query('stain_type == "PV"').rename(columns={'adjusted_mean-background': 'adjusted_
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_WFA', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted PV intensity in WFA vs Non-WFA cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_WFA)\
             + C(treat):C(react) + C(react):C(dummy_WFA) + C(dummy_WFA):C(treat)\
             + C(treat):C(react):C(dummy_WFA)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

	sum_sq	df	F	PR(>F)
C(treat)	1.138042e+05	1.0	6.085853	1.375929e-02
C(react)	9.355445e+03	1.0	0.500296	4.795009e-01
C(dummy_WFA)	2.322149e+06	1.0	124.180403	1.431371e-27
C(treat):C(react)	4.084862e+04	1.0	2.184441	1.396619e-01
C(react):C(dummy_WFA)	3.394226e+03	1.0	0.181511	6.701505e-01
C(dummy_WFA):C(treat)	5.494608e+04	1.0	2.938324	8.674676e-02
C(treat):C(react):C(dummy_WFA)	2.342998e+02	1.0	0.012530	9.108925e-01
Residual	2.352435e+07	1258.0	NaN	NaN

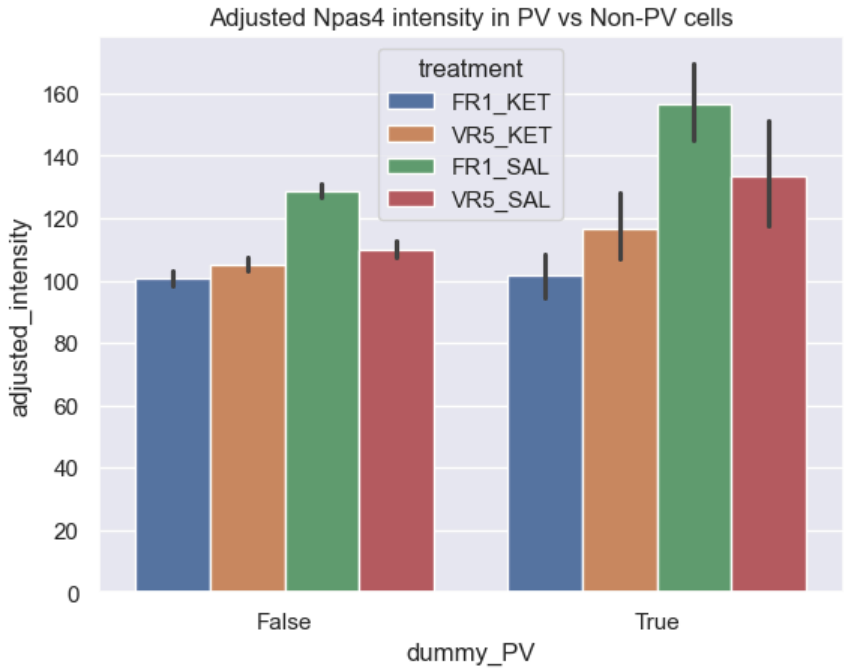


Is adjusted Npas4 intensity different in PV vs Non-PV cells across treatments?

No, we do not have a significant three way interaction effect so in this case it would be better reduce the model and go with a 2-way ANOVA only. It looks like we will probably see a significant treatment by react effect.

In [33]:
```
q = df_adjusted.query('stain_type == "Npas4"').rename(columns={'adjusted_mean-background': 'adjus
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_PV', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted Npas4 intensity in PV vs Non-PV cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_PV)\
             + C(treat):C(react) + C(react):C(dummy_PV) + C(dummy_PV):C(treat)\
             + C(treat):C(react):C(dummy_PV)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

	sum_sq	df	F	PR(>F)
C(treat)	1.731267e+05	1.0	56.428112	6.422989e-14
C(react)	6.491810e+05	1.0	211.591031	2.299029e-47
C(dummy_PV)	1.540213e+05	1.0	50.200992	1.499079e-12
C(treat):C(react)	3.025261e+05	1.0	98.603951	4.125731e-23
C(react):C(dummy_PV)	4.685034e+04	1.0	15.270181	9.390251e-05
C(dummy_PV):C(treat)	5.202496e+02	1.0	0.169568	6.805067e-01
C(treat):C(react):C(dummy_PV)	6.167826e+03	1.0	2.010313	1.562700e-01
Residual	2.594379e+07	8456.0	NaN	NaN

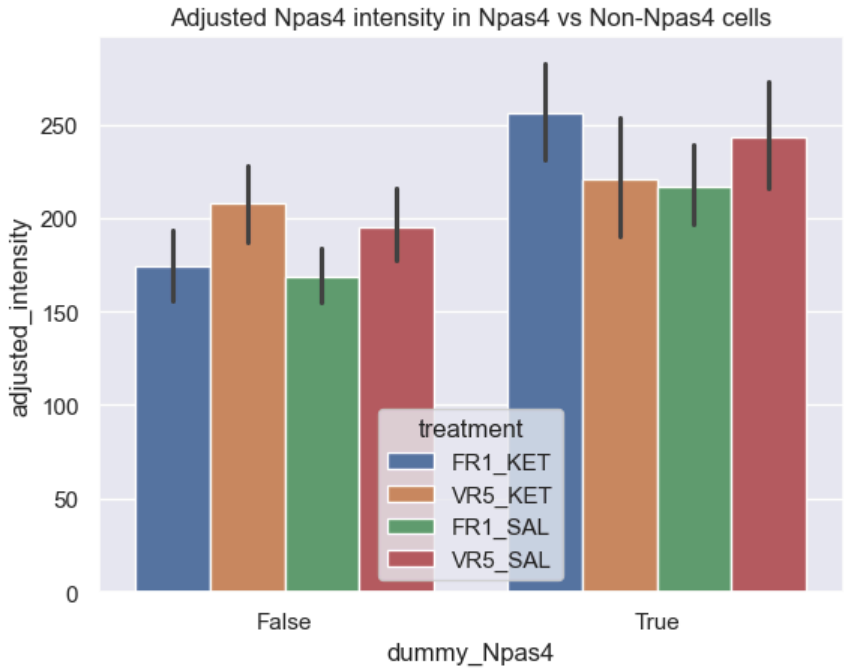


Is adjusted PV intensity different in Npas4 vs Non-Npas4 cells across treatments? (PV+/Npas4+ vs PV+/Npas-)

Probably! We do see a three way interaction effeect and so we now need to investigate which of the 2 way effects is dependent on the the level of the third variable. Just from eyeballing the bar graph below, it could be that the interaction of treatment and reactivation depends on whether or not the PV cell had Npas4.

In [34]:
```
q = df_adjusted.query('stain_type == "PV"').rename(columns={'adjusted_mean-background': 'adjusted_
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_Npas4', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted Npas4 intensity in Npas4 vs Non-Npas4 cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_Npas4)\
             + C(treat):C(react) + C(react):C(dummy_Npas4) + C(dummy_Npas4):C(treat)\
             + C(treat):C(react):C(dummy_Npas4)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

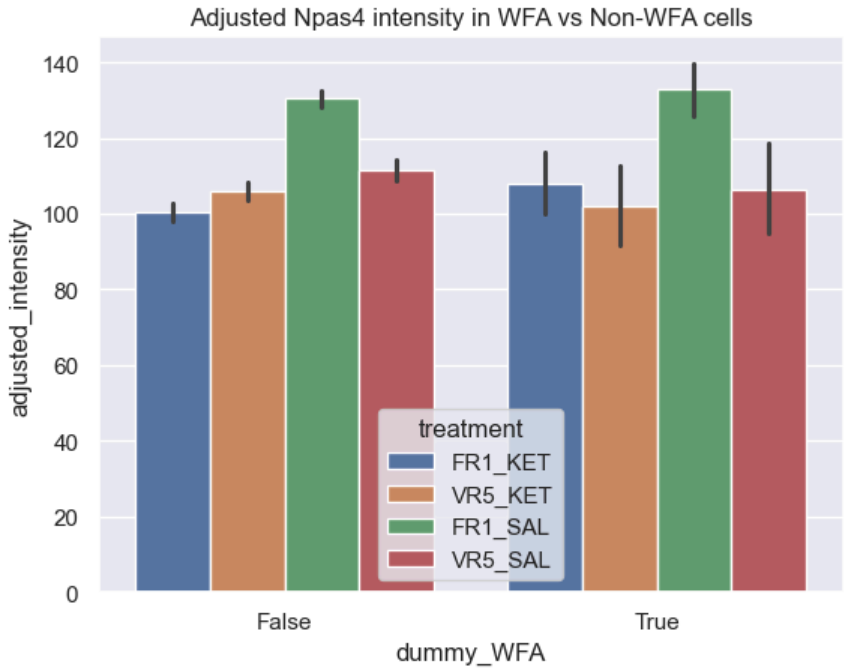
```
                                    sum_sq      df          F  \
C(treat)                      1.015755e+05     1.0   5.093936
C(react)                      2.702966e+04     1.0   1.355518
C(dummy_Npas4)                6.641316e+05     1.0  33.305727
C(treat):C(react)             3.178882e+04     1.0   1.594187
C(react):C(dummy_Npas4)       2.372515e+02     1.0   0.011898
C(dummy_Npas4):C(treat)       6.634346e+04     1.0   3.327077
C(treat):C(react):C(dummy_Npas4)  8.783783e+04     1.0   4.405005
Residual                      2.508510e+07  1258.0        NaN

                                    PR(>F)
C(treat)                      2.418061e-02
C(react)                      2.445365e-01
C(dummy_Npas4)                9.906904e-09
C(treat):C(react)             2.069630e-01
C(react):C(dummy_Npas4)       9.131581e-01
C(dummy_Npas4):C(treat)       6.838502e-02
C(treat):C(react):C(dummy_Npas4)  3.603255e-02
Residual                            NaN
```



Is adjusted Npas4 intensity different in WFA vs Non-WFA cells across combinations of react/treatment? (NO.)

In [35]:
```
q = df_adjusted.query('stain_type == "Npas4"').rename(columns={'adjusted_mean-background': 'adjus
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_WFA', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted Npas4 intensity in WFA vs Non-WFA cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_WFA)\
             + C(treat):C(react) + C(react):C(dummy_WFA) + C(dummy_WFA):C(treat)\
             + C(treat):C(react):C(dummy_WFA)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

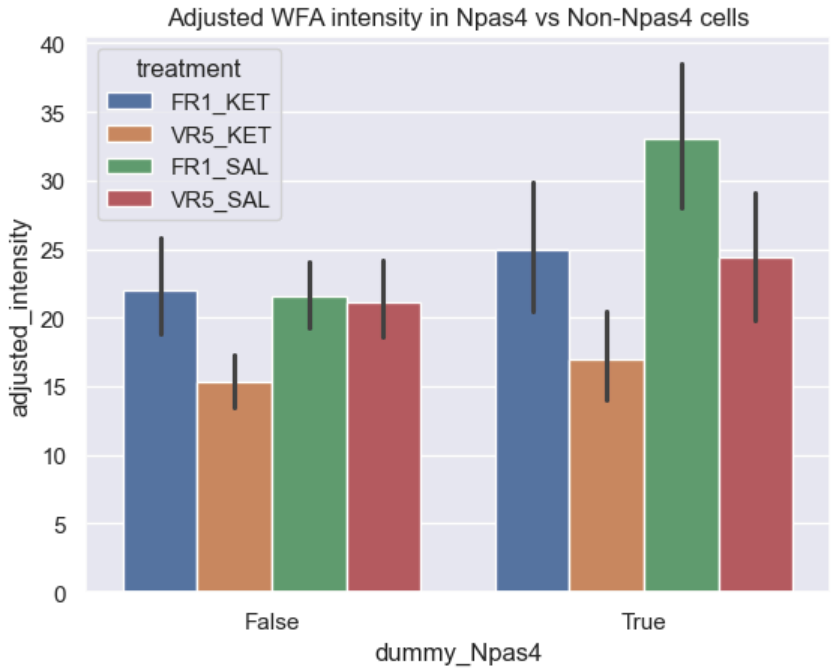
	sum_sq	df	F	PR(>F)
C(treat)	1.721251e+05	1.0	55.673121	9.406142e-14
C(react)	6.497684e+05	1.0	210.164821	4.625151e-47
C(dummy_WFA)	2.590144e+02	1.0	0.083777	7.722497e-01
C(treat):C(react)	3.027674e+05	1.0	97.928820	5.779170e-23
C(react):C(dummy_WFA)	8.104489e+02	1.0	0.262136	6.086697e-01
C(dummy_WFA):C(treat)	6.514152e+03	1.0	2.106975	1.466660e-01
C(treat):C(react):C(dummy_WFA)	2.649805e+02	1.0	0.085707	7.697149e-01
Residual	2.614349e+07	8456.0	NaN	NaN



Is adjusted WFA intensity different in Npas4 vs Non-Npas4 cells across combinations of react/treatment? (NO.)

In [36]:
```
q = df_adjusted.query('stain_type == "WFA"').rename(columns={'adjusted_mean-background': 'adjuste
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_Npas4', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted WFA intensity in Npas4 vs Non-Npas4 cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_Npas4)\
             + C(treat):C(react) + C(react):C(dummy_Npas4) + C(dummy_Npas4):C(treat)\
             + C(treat):C(react):C(dummy_Npas4)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

```
                                 sum_sq     df          F    PR(>F)
C(treat)                   5134.437216    1.0  13.453051  0.000259
C(react)                   5106.416587    1.0  13.379632  0.000269
C(dummy_Npas4)             6354.283192    1.0  16.649244  0.000049
C(treat):C(react)           740.744029    1.0   1.940868  0.163920
C(react):C(dummy_Npas4)    1377.507479    1.0   3.609291  0.057779
C(dummy_Npas4):C(treat)    1424.534954    1.0   3.732511  0.053678
C(treat):C(react):C(dummy_Npas4)  595.067648    1.0   1.559173  0.212113
Residual                 341582.091144  895.0        NaN       NaN
```

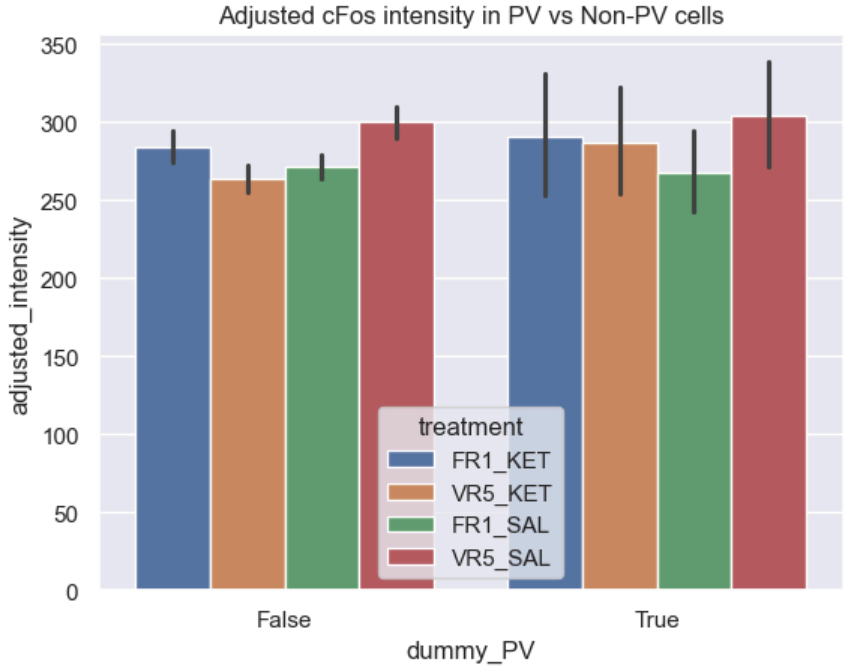


Is adjusted cFos intensity different in PV vs Non-PV cells across treatments? (cFos+/PV+ vs cFos+/PV-)

No, we only see a significant 2 way interaction between treatment and reactivation that does not depend on whether or not PV was present.

In [37]:
```
q = df_adjusted.query('stain_type == "cFos"').rename(columns={'adjusted_mean-background': 'adjuste
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_PV', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted cFos intensity in PV vs Non-PV cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_PV)\
             + C(treat):C(react) + C(react):C(dummy_PV) + C(dummy_PV):C(treat)\
             + C(treat):C(react):C(dummy_PV)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

	sum_sq	df	F	PR(>F)
C(treat)	8.848084e+04	1.0	2.153245	1.423083e-01
C(react)	1.733924e+05	1.0	4.219631	3.999132e-02
C(dummy_PV)	2.325871e+04	1.0	0.566018	4.518680e-01
C(treat):C(react)	1.127914e+06	1.0	27.448605	1.654435e-07
C(react):C(dummy_PV)	2.718370e+04	1.0	0.661535	4.160428e-01
C(dummy_PV):C(treat)	1.859282e+04	1.0	0.452470	5.011839e-01
C(treat):C(react):C(dummy_PV)	2.032199e+03	1.0	0.049455	8.240200e-01
Residual	3.283650e+08	7991.0	NaN	NaN

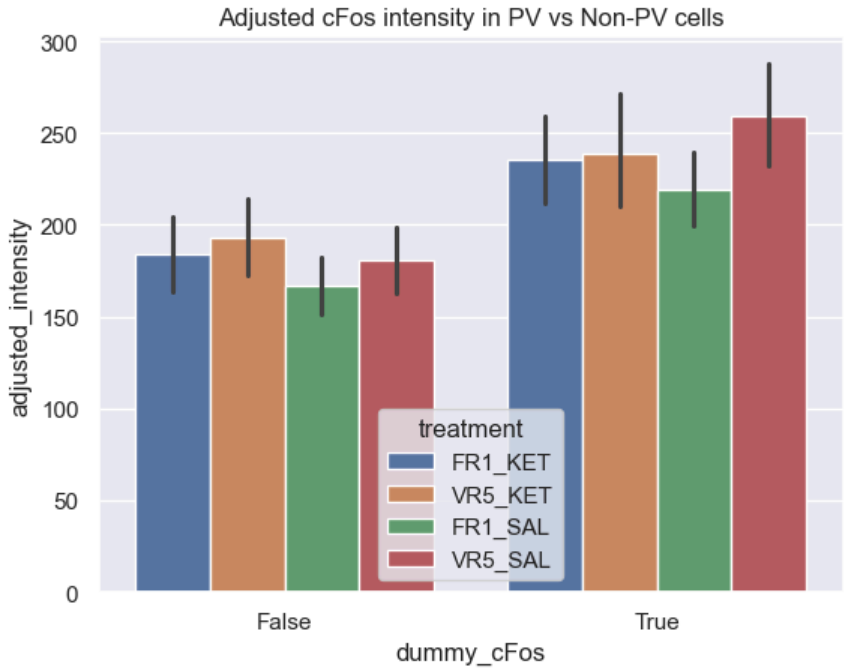


Is adjusted PV intensity different in cFos vs Non-cFos cells across treatments? (PV+/cFos+ vs PV+/cFos-)

No, we only have independent simple main effects of treatment and PV +/-.

In [38]:
```
q = df_adjusted.query('stain_type == "PV"').rename(columns={'adjusted_mean-background': 'adjusted_
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_cFos', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted cFos intensity in PV vs Non-PV cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_cFos)\
             + C(treat):C(react) + C(react):C(dummy_cFos) + C(dummy_cFos):C(treat)\
             + C(treat):C(react):C(dummy_cFos)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

	sum_sq	df	F	PR(>F)
C(treat)	9.529077e+04	1.0	4.835951	2.805373e-02
C(react)	1.391501e+04	1.0	0.706178	4.008751e-01
C(dummy_cFos)	1.061472e+06	1.0	53.869090	3.833371e-13
C(treat):C(react)	3.070918e+04	1.0	1.558473	2.121212e-01
C(react):C(dummy_cFos)	2.467093e+04	1.0	1.252035	2.633777e-01
C(dummy_cFos):C(treat)	1.246284e+04	1.0	0.632482	4.265964e-01
C(treat):C(react):C(dummy_cFos)	1.796172e+04	1.0	0.911547	3.398878e-01
Residual	2.478846e+07	1258.0	NaN	NaN



Three stain type interactions

Is Npas4 intensity different in PV cells with or without WFA nets?

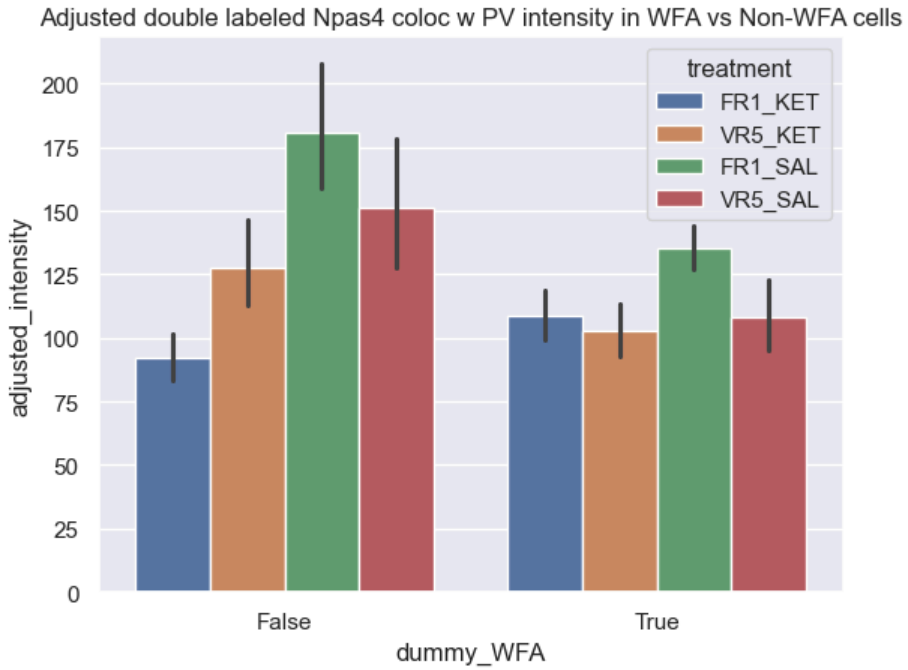
Npas4+/PV+/WFA+ vs Npas4+/PV+/WFA-

YES

we can interpret this as: the interaction between react and treatment depends on whether or not a Npas4+/PV+ cell was also WFA+.

In [39]:
```
q = df_adjusted.query('stain_type == "Npas4" and dummy_PV == True').rename(columns={'adjusted_mea
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_WFA', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted double labeled Npas4 coloc w PV intensity in WFA vs Non-WFA cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_WFA)\
             + C(treat):C(react) + C(react):C(dummy_WFA) + C(dummy_WFA):C(treat)\
             + C(treat):C(react):C(dummy_WFA)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

	sum_sq	df	F	PR(>F)
C(treat)	1.441977e+04	1.0	2.642340	1.046680e-01
C(react)	1.569748e+05	1.0	28.764722	1.241371e-07
C(dummy_WFA)	9.944630e+04	1.0	18.222962	2.343607e-05
C(treat):C(react)	5.852152e+04	1.0	10.723732	1.129554e-03
C(react):C(dummy_WFA)	5.153389e+04	1.0	9.443289	2.232568e-03
C(dummy_WFA):C(treat)	7.989842e+03	1.0	1.464093	2.268406e-01
C(treat):C(react):C(dummy_WFA)	1.492823e+04	1.0	2.735512	9.875538e-02
Residual	2.783171e+06	510.0	NaN	NaN



Is cFos intensity different in PV cells with or without WFA nets?

cFos+/PV+/WFA+ vs cFos+/PV+/WFA-

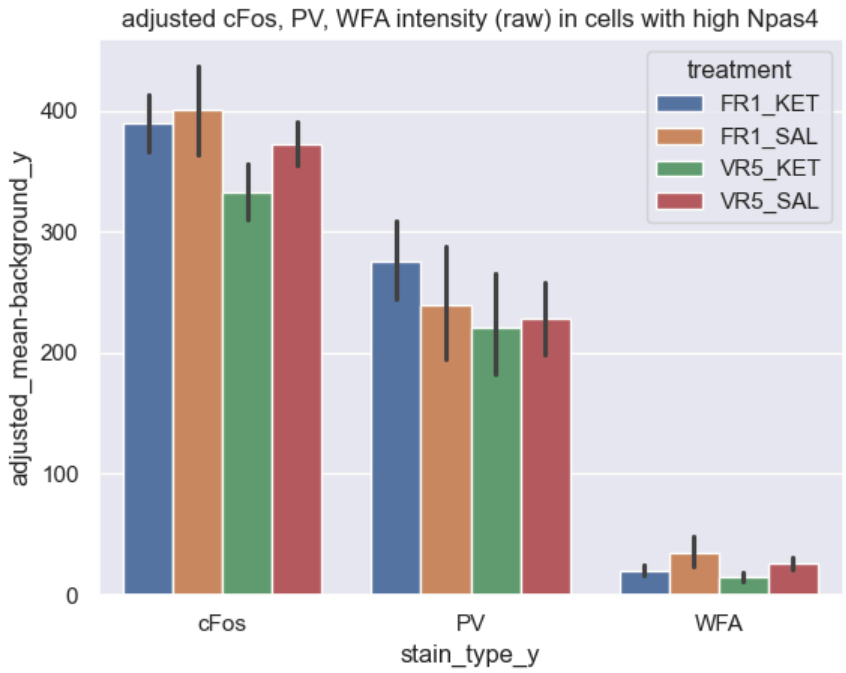
NO.

In [40]:
```
q = df_adjusted.query('stain_type == "cFos" and dummy_PV == True').rename(columns={'adjusted_mean-
q['treat'] = q.treatment.apply(lambda x: x.split('_')[0])
q['react'] = q.treatment.apply(lambda x: x.split('_')[1])

sns.barplot(x='dummy_WFA', y='adjusted_intensity', data=q, hue='treatment')
plt.title('Adjusted double labeled cFos coloc w PV intensity in WFA vs Non-WFA cells')

# performing 3way ANOVA
model = ols('adjusted_intensity ~ C(treat) + C(react) + C(dummy_WFA)\
             + C(treat):C(react) + C(react):C(dummy_WFA) + C(dummy_WFA):C(treat)\
             + C(treat):C(react):C(dummy_WFA)',
            data=q).fit()
result = sm.stats.anova_lm(model, typ=2)

# Print the result
print(result)
```

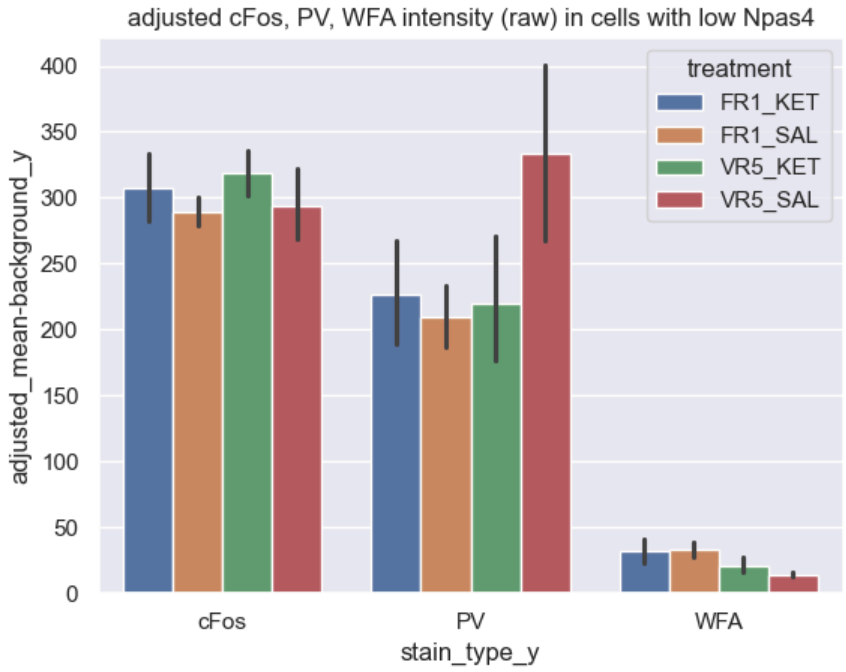
```
                                sum_sq     df         F    PR(>F)
C(treat)                  4.307610e+04    1.0  1.103221  0.294010
C(react)                  1.884221e+03    1.0  0.048257  0.826204
C(dummy_WFA)              1.080859e+05    1.0  2.768186  0.096711
C(treat):C(react)         5.209656e+04    1.0  1.334244  0.248541
C(react):C(dummy_WFA)     2.371475e+04    1.0  0.607358  0.436111
C(dummy_WFA):C(treat)     9.917844e+03    1.0  0.254006  0.614465
C(treat):C(react):C(dummy_WFA)  1.167873e+04    1.0  0.299104  0.584661
Residual                  2.202181e+07  564.0       NaN       NaN
```

In cFos/Npas4 high/low intensity cells, is PV/WFA intensity different across treatment/react groups?

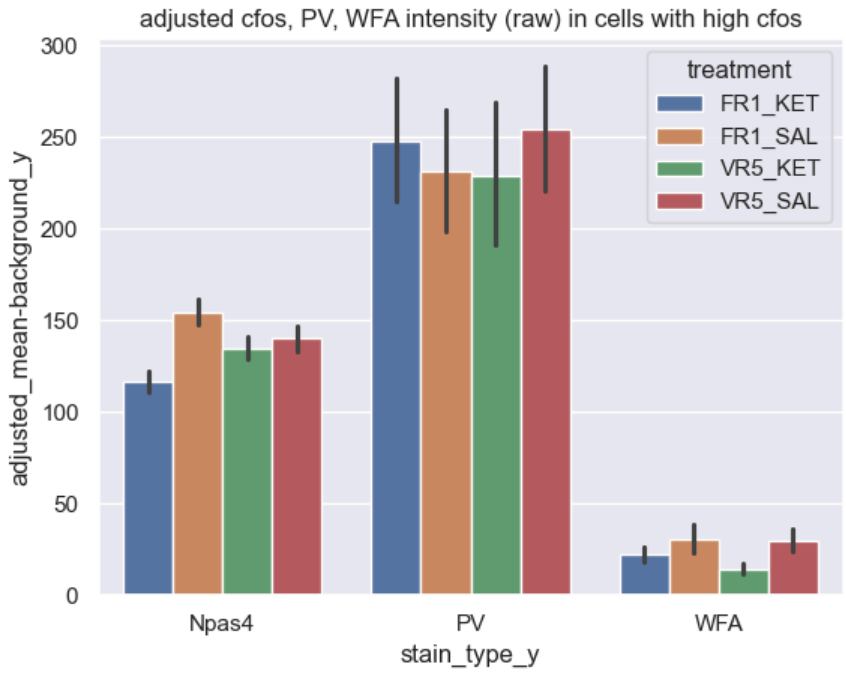
In [74]:
```
df_Npas4_merge = df_Npas4.merge(
    df_adjusted[df_adjusted.stain_type != 'Npas4'][['image_name', 'true_grouping', 'adjusted_mean-
    , on=['image_name', 'true_grouping']
    )

# wasn't sure if I should normalize to just the Npas4_high subset
# these data area double, triple and quad labeled cells: query criteria was
# any cell with Npas4_high and any other stain.
# so if i normalized, the stain

sns.barplot(x='stain_type_y', y='adjusted_mean-background_y', hue='treatment', data=df_Npas4_merge
plt.title('adjusted cFos, PV, WFA intensity (raw) in cells with high Npas4')
plt.show()

sns.barplot(x='stain_type_y', y='adjusted_mean-background_y', hue='treatment', data=df_Npas4_merge
plt.title('adjusted cFos, PV, WFA intensity (raw) in cells with low Npas4')
```



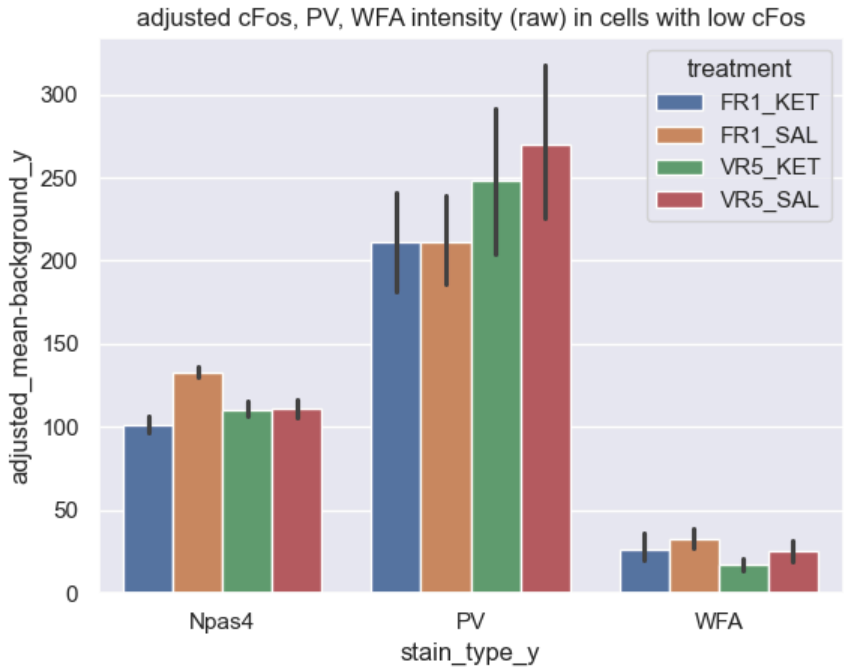
Out[74]: Text(0.5, 1.0, 'adjusted cFos, PV, WFA intensity (raw) in cells with low Npas4')



In [79]:
```
df_cfos_merge = df_cfos.merge(
    df_adjusted[df_adjusted.stain_type != 'cFos'][['image_name', 'true_grouping', 'adjusted_mean-b
    , on=['image_name', 'true_grouping']
    )

# wasn't sure if I should normalize to just the cFos_high subset
# these data area double, triple and quad labeled cells: query criteria was
# any cell with cFos_high and any other stain.
# so if i normalized, the stain

sns.barplot(x='stain_type_y', y='adjusted_mean-background_y', hue='treatment', data=df_cfos_merge
plt.title('adjusted cfos, PV, WFA intensity (raw) in cells with high cfos')
plt.show()

sns.barplot(x='stain_type_y', y='adjusted_mean-background_y', hue='treatment', data=df_cfos_merge
plt.title('adjusted cFos, PV, WFA intensity (raw) in cells with low cFos')
```



Out[79]: `Text(0.5, 1.0, 'adjusted cFos, PV, WFA intensity (raw) in cells with low cFos')`



In [77]:
```
1 df_cfos
```

Out[77]:

	index	filename	image_name	roi_id	true_grouping	dummy_PV	dummy_cFos	dummy_Npas4	dummy_WFA	CoM_x
0	0	KET-10-12_PFC_3.7_A_3.tif	KET-10-12_PFC_3.7_A	0-005-00000_cFos	(0-005-00000_cFos, 0-FFF-00006_Npas4)	False	True	True	False	127.57
1	1	KET-10-12_PFC_3.7_A_3.tif	KET-10-12_PFC_3.7_A	0-005-00001_cFos	(0-005-00001_cFos,)	False	True	False	False	364.94
2	2	KET-10-12_PFC_3.7_A_3.tif	KET-10-12_PFC_3.7_A	0-005-00002_cFos	(0-005-00002_cFos, 0-FFF-00064_Npas4)	False	True	True	False	177.76
3	3	KET-10-12_PFC_3.7_A_3.tif	KET-10-12_PFC_3.7_A	0-005-00003_cFos	(0-005-00003_cFos, 0-FFF-00038_Npas4)	False	True	True	False	332.28
4	4	KET-10-12_PFC_3.7_A_3.tif	KET-10-12_PFC_3.7_A	0-005-00004_cFos	(0-000-00010_PV, 0-005-00004_cFos, 0-FFF-00049...	True	True	True	False	418.08
...	...	...	...	...	...	...	...	...	...	...
7994	7994	PE-13-9_PFC_4.0_B_3.tif	PE-13-9_PFC_4.0_B	0-FFF-00026_cFos	(0-FFF-00026_cFos,)	False	True	False	False	82.81
7995	7995	PE-13-9_PFC_4.0_B_3.tif	PE-13-9_PFC_4.0_B	0-FFF-00027_cFos	(0-FFF-00027_cFos,)	False	True	False	False	185.73
7996	7996	PE-13-9_PFC_4.0_B_3.tif	PE-13-9_PFC_4.0_B	0-FFF-00028_cFos	(0-FFF-00028_cFos,)	False	True	False	False	343.07
7997	7997	PE-13-9_PFC_4.0_B_3.tif	PE-13-9_PFC_4.0_B	0-FFF-00029_cFos	(0-000-00004_PV, 0-FFF-00029_cFos)	True	True	False	False	8.90
7998	7998	PE-13-9_PFC_4.0_B_3.tif	PE-13-9_PFC_4.0_B	0-FFF-00030_cFos	(0-FFF-00030_cFos,)	False	True	False	False	5.37

7999 rows × 23 columns

In [ ]:
```
1
```